SYNTHESES OF SULFUR-SUBSTITUTED 2,3-OXIDOSQUALENE ANALOGS AS INHIBITORS OF 2,3-OXIDOSQUALENE-LANOSTEROL CYCLASE

by

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Syntheses of Sulfur-Substituted 2,3- Oxidosqualene Analogs as Inhibitors of 2,3-Oxidosqualene-Lanesterol Cyclase

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Abstract

2,3-Oxidosqualene-lanosterol cyclases (OSCs) catalyze the cyclization of (3*S*)-2,3-oxidosqualene (2,3-OS) *via* a series of partially cyclized carbocationic intermediates to the tetracyclic protosterol cation. Backbone rearrangement of the latter gives lanosterol which serves as precursor of cholesterol and ergosterol in mammalian and fungal systems, respectively. This thesis focuses on the syntheses of two classes of sulfur-containing substrate analogs as mechanism-based inhibitors of OSCs. In the first series of compounds, sulfur or sulfoxide replace carbons at C-6, C-10, C-14, C-18 or C-19 in 2,3-OS which are considered to become cationic centres during the cyclization. These 2,3-OS analogs were designed to interfere with ring A, B, C or D formation by "enzyme-activated" mimicry of the presumptive cationic intermediates. In the second series of compounds, sulfur substitutes for carbons at position α (C-5, C-13 or C-20) or β (C-8 in 2,3-OS) to those carbons presumed to be cationic during the cyclization. Again, the sulfur atoms were positioned to probe the existence of monocyclic, tricyclic and tetracyclic protosterol cationic intermediates.

The syntheses of compounds in the first series were achieved by coupling the appropriate epoxy mesylates and thiols under phase-transfer conditions. Biological evaluation revealed that 18-thia-19-dehydro-2,3-OS had an IC_{50} of 0.22 nM for *Candida albicans* and 0.08 nM for rat liver OSCs, which is the best inhibitor for these OSCs to date. Enzyme kinetic analyses indicated that this compound was a competitive, time-dependent and irreversible inhibitor.

The syntheses of all *trans* vinylic sulfur-substituted 2,3-OS analogs involved two synthetic methodologies. A new methodology involving stereospecific coupling of trisubstituted vinylic anions with alkyl 4-methylbenzenthiosulfonates was developed and applied to the syntheses of 6(*E*)-

8-thia- and 14(*E*)-13-thia-2,3-OS. A modified Wittig-Horner reaction was used in the syntheses of 6(*E*)-5-thia- and 18(*E*)-20-thia-2,3-OS. Biological studies showed an IC_{50} of 0.20 μM for *C. albicans* and 0.32 μM for rat liver OSCs for (18*E*)-20-thia-2,3-OS which are the lowest values among this series of compounds.

Biological studies lead to the conclusion that modification of near C-20 of region of 2,3-OS results in more potent inhibitors of OSCs.

Dedication

This thesis is dedicated to my wife Heung Yeung

and to my late parents

Tian Ni Zheng and Wen Ying Zhang

Acknowledgment

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List of Abbreviations

br	broad (^{1}H-NMR)
n-Bu_4NF	tetrabutylammonium fluoride
CI	chemical ionization (mass spectroscopy)
Cp	cyclopentadienyl
C-S	cation-stabilizing
m-CPBA	*meta*-chloroperbenzoic acid
d	doublet (^{1}H-NMR)
dt	double triplet (^{1}H-NMR)
δ	chemical shift in ppm downfield from tetramethyl silane (NMR)
DEAE-Sephacel	diethylaminoethyl sephacel
DIBAL-H	diisobutylaluminum hydride
DMAP	4-*N,N*-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMS	dimethyl sulfide
DMSO	dimethyl sulfoxide
EI	electron impact (mass spectroscopy)
equiv.	equivalent
g	grams
GC	gas chromatography
IC_{50}	molar concentration of inhibitor required to decrease enzymatic activity by 50% (biological inhibition assays)
LDA	lithium diisopropylamide

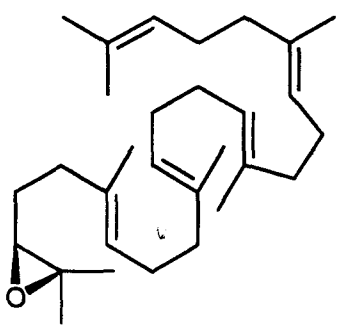
m	multiplet (^{1}H-NMR)
m/z	mass to charge ratio (mass spectroscopy)
MDBK cells	madin darbin bovine kidney cells
MIC	minimum concentration of inhibitor required to completely inhibit cell growth (biological assays)
29-MOS	29-methylidene-2,3-oxidosqualene
NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser enhancement (^{1}H-NMR)
2,3-OS	(3*S*)-2,3-oxidosqualene
OSC	2,3-oxidosqualene cyclase
OTBS	*tert*-butyldimethylsilyloxy
Oct_4NBr	tetraoctylammonium bromide
q	quartet (^{1}H-NMR)
s	singlet (^{1}H-NMR)
Sia_2BH	diisiamylborane
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
t	triplet
THF	tetrahydrofuran
TBSCl	*tert*-butyldimethylsilyl chloride
p-TsOH	*para*-toluenesulfonic acid

Chapter 1

Introduction

1.1. Overview of 2,3-oxidosqualene cyclases.

2,3-Oxidosqualene cyclases (OSCs) play important roles in sterol biosynthesis through catalysis of the cyclization of (3*S*)-2,3-oxidosqualene (**1**, 2,3-OS) to a number of tetracyclic triterpenes.[1,2] In mammalian and fungal systems, lanosterol (**8**) is the immediate cyclized product which serves as a precursor of cholesterol or ergosterol, respectively. In photosynthetic plants, cycloartenol or β-amyrin are the products. Because the enzymatic cyclization represents the most remarkable step in the biosynthesis of steroids and triterpenes, its mechanistic features have been of great interest to both chemical and biochemical communities. Woodward and Bloch first proposed a mechanism for the cyclization of squalene and rearrangement to lanosterol in 1953.[3] Although this fascinating cyclization has received forty years of intensive study, the mechanism of the cyclization of 2,3-OS to lanosterol is still a matter of debate and conjecture. The generally accepted mechanism of OSC cyclization was proposed by van Tamelen (Figure 1).[4a,b] OSCs are considered



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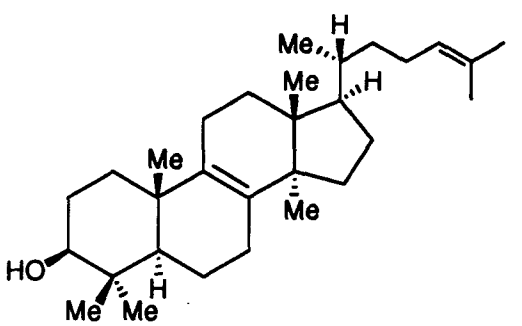

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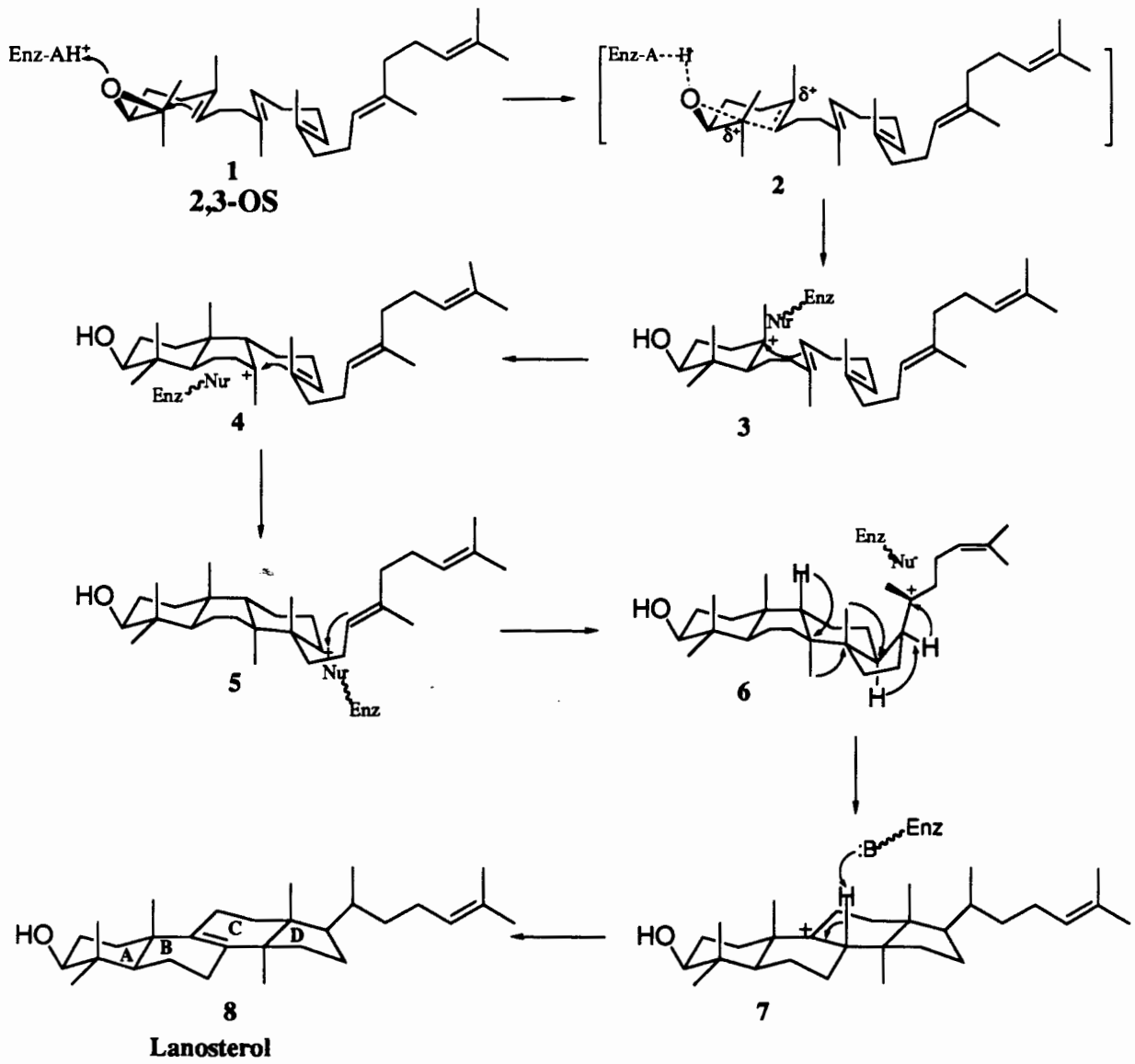


Figure 1. Proposed mechanism of OSC-mediated cyclization of 2,3-OS (**1**) to lanosterol (**8**).

to initially bind 2,3-OS in a chair-boat-chair conformation, then catalyze the sequential formation of four new C-C bonds leading *via* cations **2-5** to a tetracyclic protosterol cation **6**. Rearrangement of **6** by hydride and methyl migrations followed by elimination of a proton gives lanosterol in fungi and mammalian systems. Although early suggestions were for a "synchronous"

process,[5] current hypotheses center on a "stepwise" process,[4] proceeding through a series of discrete, conformationally rigid, partially cyclized carbocationic intermediates (**2-6**, Figure 1). The natural occurrence of monocyclic[6a] and bicyclic[6b] triterpenes, obviously derived from **1**, has been viewed as evidence for the "stepwise" mechanism. This thesis focuses on the design and syntheses of mechanism-based inhibitors for 2,3-oxidosqualene-lanosterol cyclase (OSC) based on the "stepwise" process. As well the inhibitory activities of the 2,3-OS analogs prepared are examined.

1.2. 2,3-Oxidosqualene-lanosterol cyclase.

1.2.1. Characterization of 2,3-oxidosqualene-lanosterol cyclases.

2,3-Oxidosqualene-lanosterol cyclases (OSCs), which mediate the conversion of (3*S*)-2,3-oxidosqualene to lanosterol, are membrane-bound proteins which are stabilized and solubilized in the presence of detergents. Activity of the OSCs requires both detergents and the appropriate salts. For instance, solubilized yeast OSC has been reported to be strongly inhibited in solutions of high ionic strength and to be stimulated by the neutral detergent Triton®X-100.[7] Soluble OSC from hog liver requires both anionic detergent (deoxycholate) and high salt concentration for activity.[8] Critical dependency on additives causes difficulties in purification of active forms of these enzymes. Partial purification of OSC from hog liver[9] and yeast[10,11a] have been only recently reported. Yeast OSC[10] was found to be negatively charged at pH 6 and its activity was stimulated by high concentrations of potassium phosphate buffer. Both partially purified hog liver[9] and yeast[10] OSCs were strongly inhibited by cysteine-modifying reagents such as N-ethylmaleimide suggesting that a cysteine residue is essential for their activity. Recent chemical modification of yeast OSC[11b] using 3-carboxyl-4-nitrophenyl-

dithio-1,1',2-*trisnor*squalene supports the existence of an essential thiol group within the active site of the enzyme.

In the mammalian system, a 1863-fold purification of OSC from rat liver has been reported with 28% recovery.[12] The purified rat liver cyclase displays a single band on SDS-PAGE gel with a molecular mass of 75 KDa. In separate studies, pig and rat liver OSCs were purified to homogeneity and showed single bands on SDS-PAGE gel with 75 KDa and 78 KDa molecular masses, respectively.[13a] Affinity labeling of these pig and rat liver OSCs with a mechanism-based irreversible inhibitor of OSC, (^{3}H)-29-methylidene-2,3-OS[14] (29-MOS), showed a single radioactive band at 75 KDa for pig liver cyclase and 78 KDa for rat liver cyclase.[13a] These results confirmed that these protein bands are responsible for cyclase activity. Further study of the labeled rat liver cyclase revealed that two adjacent Asp residues in the most highly conserved region (*Asp-Asp*-Thr-Ala-Gul-Ala) of the cyclase were equally labeled by (^{3}H)-29-MOS.[13b] This result suggests that the carboxyl group of one of the two adjacent Asp residues traps the 21-methylidene protosterol cation (**9**) to form an ester bond causing the irreversible inhibition (Figure 2).[13b] This result also implicates this highly conserved region in the stabilization of the protosterol cation (**6**) during the cyclization. It is noteworthy that the same affinity labeling experiment was not successful with yeast cyclases suggesting differences in the active sites of mammalian and fungal OSCs.[13a]

Yeast OSC from *Saccharomyces cerevisiae* was purified and showed a molecular mass of 26 KDa,[15] substantially lower than that of mammalian OSCs. Very recently, the *ERG7* genes encoding oxidosqualene cyclases from the yeast *Candida albicans*[16] and *S. cerevisiae*[17] have been cloned by genetic complementation of a cyclase-deficient *erg7* strain, and their DNA sequence codings have been found to be very similar. Thus, the sequence in *C. albicans*

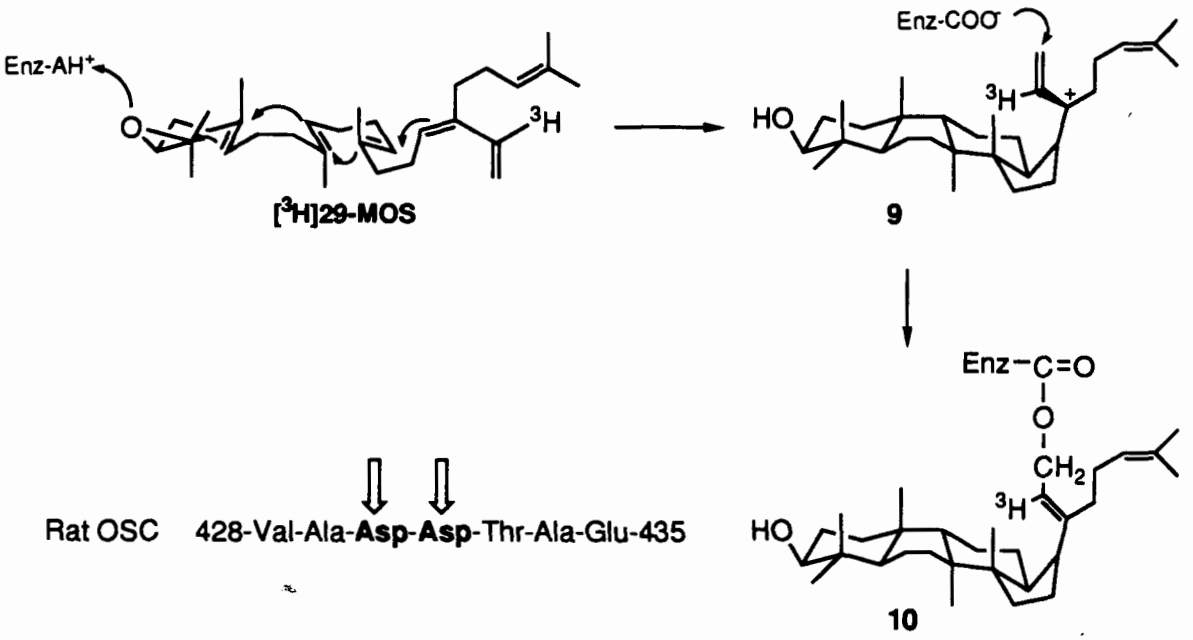


Figure 2. Proposed mechanism of irreversible inhibition of rat liver OSC by 29-MOS.

contains an open reading frame of 2187 nucleotides and encodes a predicted protein of 728 amino acids with molecular mass of 83.7 kDa,[16a] while the sequence in *S. cerevisiae* has an open reading frame of 2196 nucleotides and encodes a predicted protein of 731 amino acids with molecular mass of 83.4 KDa.[17] *S. cerevisiae* and *C. albicans* OSCs share 63% identity of amino acid sequences.[17] The unusually high abundance of tryptophan and tyrosine found in the amino acid sequences in both proteins has led to the hypothesis that the electron-rich aromatic side chains of these residues are essential features of the active sites of these cyclases.[17]

1.2.2. Mechanistic aspects of (3*S*)-2,3-oxidosqualene cyclization mediated by 2,3-oxidosqualene-lanosterol cyclase.

The OSC-mediated cyclization of 2,3-OS to lanosterol is one of the most complex reactions in nature. The enzyme-catalyzed reaction forms four rings and establishes seven stereocenters in lanosterol (**8**) with precision that is not yet approached by any known synthetic methodology. After the initial proposal by Woodward and Bloch that lanosterol could be formed by the cyclization of squalene,[3] Stork and Burgstahler proposed sequential ring formation based on polyene cyclization studies[18a] (Figure 3). Eschenmoser and coworkers proposed

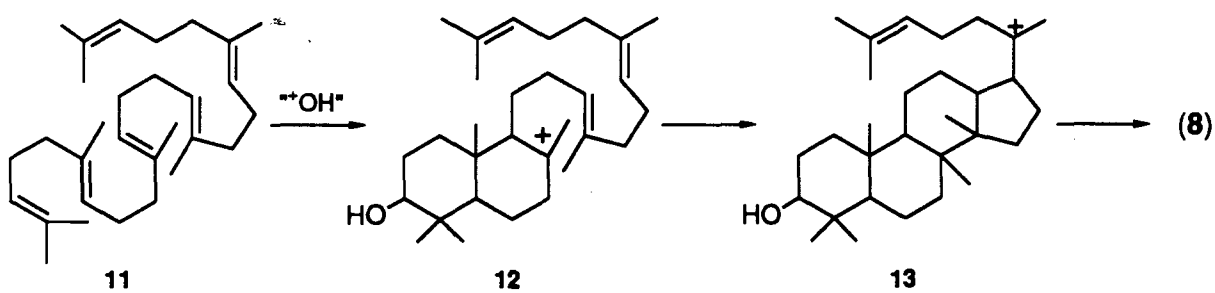


Figure 3. Proposed mechanism of cyclization of squalene (**11**) to lanosterol (**8**).

that the enzymatic cyclization of **11** to **8** could be a "concerted" single step process.[18b] The first demonstration that 2,3-oxidosqualene and not squalene (**11**) was the intermediate to undergo enzymatic cyclization was independently reported by Corey *et al.*[19a] and by van Tamelen *et al.*[19b] Cornforth[20] suggested that cyclization of 2,3-OS (**1**) by the cyclase proceeded *via* a "synchronous" process. In his proposal four rings were formed by initial acid-catalyzed opening of the terminal epoxy group to generate the protosterol cation equivalent **14**. The latter underwent rearrangement followed by elimination to give

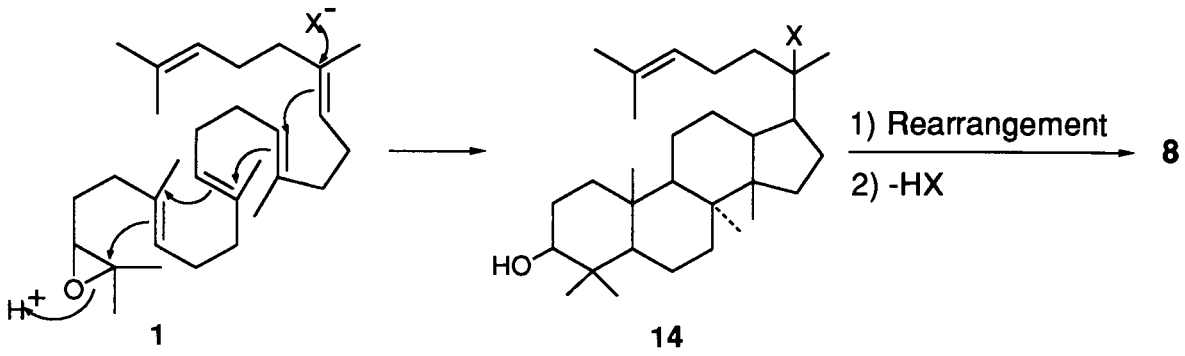
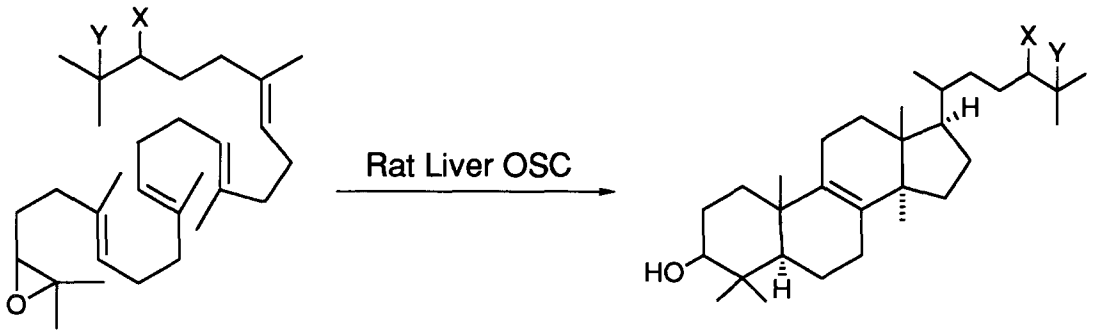


Figure 4. Proposed mechanism of "synchronous" cyclization of 2,3-OS (**1**) to lanosterol (**8**).

lanosterol (Figure 4). That only (3*S*)- and not the (3*R*)-enantiomer of oxidosqualene acted as a substrate was demonstrated by Barton and coworkers.[19c]

Early mechanistic studies concentrated on synthesis and enzymatic transformation of unnatural modified substrates to probe the enzyme mechanism. It was found that modification of the 2,3-OS skeleton has provided acceptable substrates.[21] The conversion of 2,3:22,23-dioxidosqualene (**15**) and 2,3-oxido-22,23-dihydrosqualene (**16**) to the corresponding 24,25-oxidolanosterol (**17**) and 24,25-dihydrolanosterol (**18**) respectively are good examples (Figure 5).[21e,f] This result demonstrated that the side chain subunit was not involved in the cyclization. Another significant observation was that the partially cyclized epoxide **19** was cyclized to the tetracyclic product **20**[21j] (Figure 6). This indicated that the acyclic substrate framework was not a prerequisite for enzymatic recognition. Thus, a model of full "pocket fit" does not adequately describe the substrate-enzyme interaction either for binding or catalytic activity. This result also implies that the minimal requirement for the enzymatic cyclization is the terminal epoxide with two

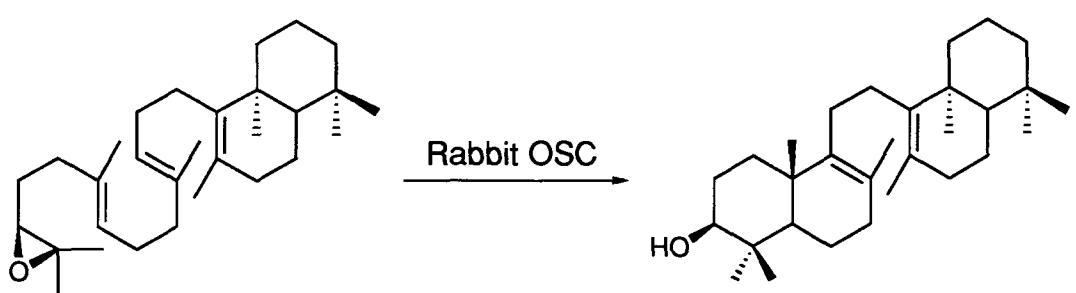


15: X - Y = O
16: X - Y = H

17: X - Y = O
18: X - Y = H

Figure 5. OSC-mediated cyclization of modified substrates **15** and **16** to their corresponding lanosterol analogs **17** and **18**.

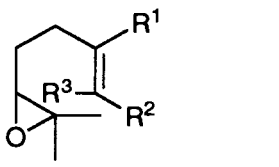
appropriately situated olefinic bonds. More importantly, this result revealed that the protosterol **14** proposed by Cornforth[20] (Figure 4) is not a requirement for the process. i.e., an X^- as stabilizing group is not necessary in the C-20 cation region. This gave the impetus to consider an alternative mechanism for the enzymatic cyclization.



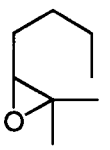
19 **20**

Figure 6. Induced cyclization of the bicarbocyclic epoxide **19** to 3β-hydroxy-β-onoceradiene (**20**).

Based on entropic considerations and experimental evidence, van Tamelen[4] suggested that the enzymatic cyclization proceeded through a series of discrete conformationally rigid, partially cyclized carbocationic intermediates (**3-6**, Figure 1) referred to as a "stepwise" process.[4] This contrasts with the earlier proposal of Cornforth of a single polycyclization transition state referred to as a "synchronous" process (Figure 4). To support the "stepwise" hypothesis, van Tamelen's group investigated chemically induced model reactions. They compared the rates of reaction of monoene epoxides **21a-c** with that of the saturated analog **22** (Figure 7). It was observed that the rates of Lewis acid catalyzed ring-opening of the unsaturated epoxides **21a-c** were much higher than that of the saturated analog **22**.[4b] Furthermore, the Lewis acid catalyzed cyclization of the deuterium-labelled epoxide **23** gave exclusively **24** (Figure 7) showing that the deuterium-labelled methyl group had maintained its stereochemical location in the 4α-position.[4b] These results provided strong evidence that ring A is formed biomimetically with a high degree of π-bond participation in an S_N2-like epoxide cleavage process. To



21a: R ^{1} = H, R ^{2} = R ^{3} = Me
b: R ^{1} = Me, R ^{2} = R ^{3} = H
c: R ^{3} = H, R ^{1} = R ^{2} = Me



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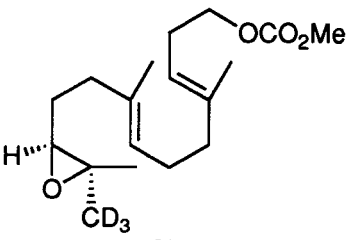

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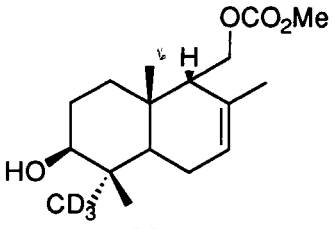

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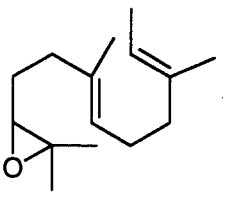

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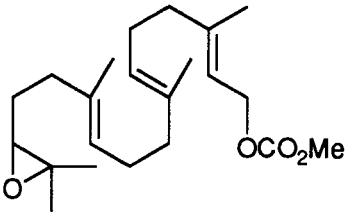


26

Figure 7. Lewis acid induced cyclization of oxido-1,5-polyenes.

evaluate the influence of additional π-bonds on the rate of polycyclization, van Tamelen *et al.* selected monoene epoxide **21c**, diene-epoxide **25** and triene epoxide **26** (Figure 7). In Lewis acid catalyzed cyclization these epoxides had half-lives of 75, 100 and 100 min, respectively, suggesting that initial epoxide opening is the slow step and that additional π-bonds do not accelerate the rate of cyclization.[4b] Based on these results, van Tamelen concluded that in the biomimetic process ring A is formed with a high degree of π-bond participation in the epoxide ring opening. He further extrapolated these results to suggest the enzymatic cyclization proceeds through a series of discrete conformationally rigid, partially cyclized cation intermediates in which formation of ring A is likely to be the slow step.

Theoretical calculations of Dewar *et al.*[22] also strongly support the "stepwise" hypothesis. They found that ring formation involving cation addition to a double bond proceeds *via* formation of a cyclic π-complex. The olefin-carbenium ion π-complex intermediates require stereospecific *trans* addition of the next double bond allowing ring formation to proceed "stepwise" rather than "synchronous".[22] This result is consistent with van Tamelen's proposal.

The isolation of naturally occurring monocyclic (**27**)[6a] and bicyclic triterpenes (**28**)[6b] provides further evidence to support the existence of cationic intermediates **3** and **4** (Figure 8). The structures and absolute stereochemistry of both **27** and **28** were postulated to be formed by "trapping" of partially cyclized intermediates **3** and **4** believed to be involved in the enzymatic cyclization of 2,3-OS (**1**). For instance, **28** with a β-methyl group attached to C-8

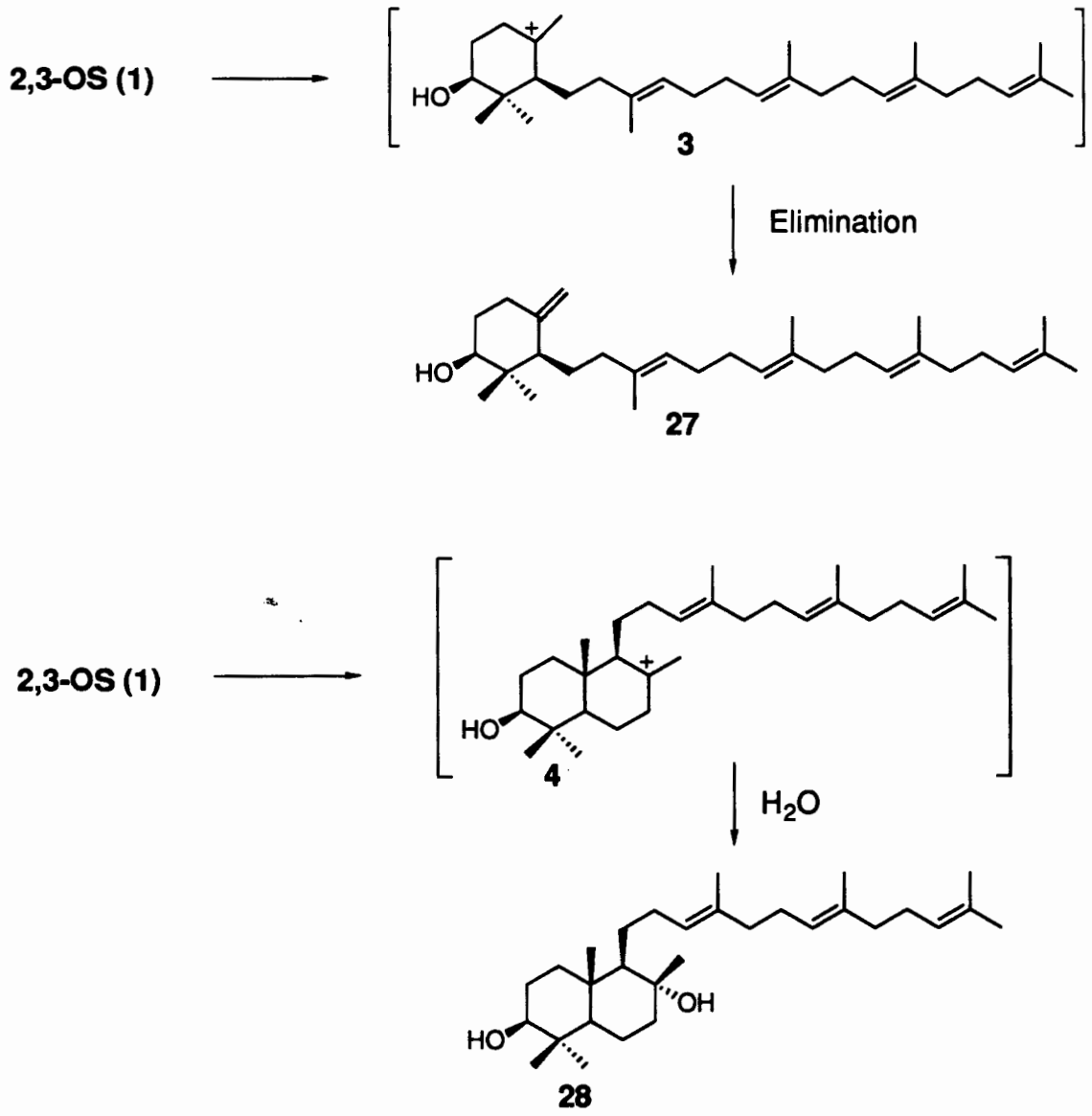
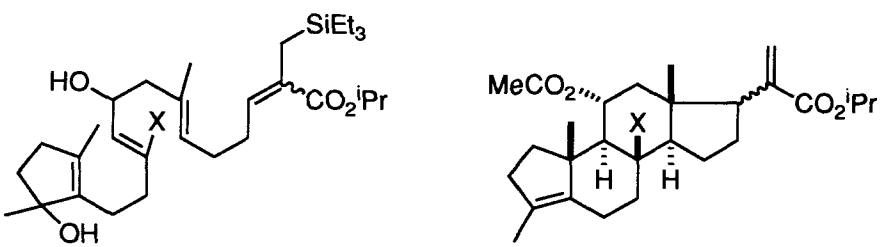


Figure 8. Monocyclic (**27**) and bicyclic triterpenes (**28**) from *Achillea odorata L.* and *Pistacia lentiscus L.*

(steroid numbering) is suggested to be the product derived from attack of water at the α-face of C-8 of **4**. Compound **27** is an elimination product of **3**. Both **27** and **28** are the first examples of monocyclic and bicyclic triterpenes isolated from partial cyclization of 2,3-OS (**1**) in nature.

Study of non-enzymatic polyene cyclizations has been used to gain insight about the enzymatic mechanism. "Biomimetic" cyclizations proceed in a "stepwise" manner.[4c,d,23] Recently, Johnson *et al.* established that cation-stabilizing (C-S) auxiliaries increase the rate of biomimetic cyclizations.[23a-e] Introduction of isobutenyl or fluoride[23b-g] as C-S groups adjacent to the *pro*-C-8 or *pro*-C-13 (steroid numbering) carbons increased the rate of cyclization and yield of the final product. For example, Lewis acid catalyzed cyclization of substrate **29a** (X = H) to **30a** (X = H) gave a yield of 20% after 24 h while the same cyclization of **29b** (X = $CH{=}CMe_2$) underwent complete reaction to **30b** (X = $CH{=}CMe_2$) in 1 h with an isolated yield of 80-83% after acetylation (Figure 9).[23a] Introduction of fluoride as a C-S auxiliary allowed a one-step conversion of 2,3-OS analog **31**

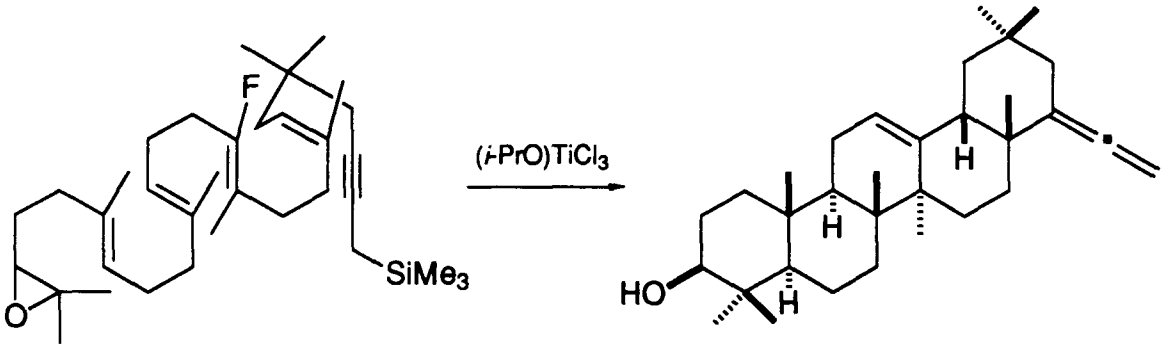


29a: X = H
29b: X = CH=CMe 2

30a: X = H
30b: X = CH =CMe 2

Figure 9: Biomimetic polyene cyclizations of substrates containing cation-stabilizing auxiliaries.

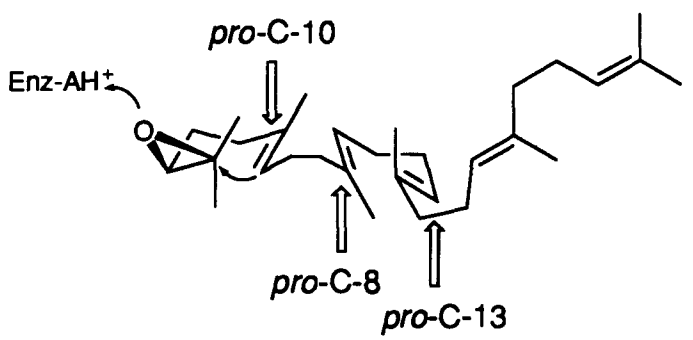
to its corresponding pentacyclic derivative **32** in 10% yield (Figure 10).[23f] Compounds such as **32** are always accompanied by partially cyclized products. These observations revealed that even assisted biomimetic cyclizations achieve limited success and are still far from the efficiency of the cyclases. Introduction of cation stabilizing auxiliaries is thought to reduce the energy of the intermediates which generally correlates with more selectivity.



31 **32**

Figure 10. Lewis acid induced biomimetic polyene pentacyclization of fluorine-substituted 2,3-OS analog.

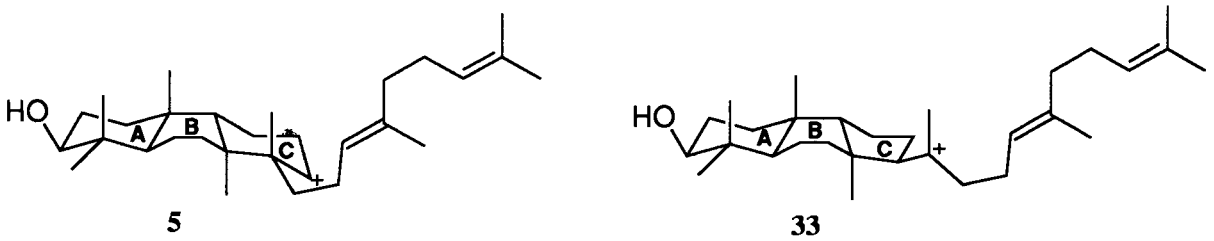
Based on his investigation of biomimetic cyclizations, Johnson recently proposed refinement in the mechanism of cyclase action. He suggested axial delivery of negative point charges by the cyclase to the individual carbons of 2,3-OS.[23a,e] He reasoned that sterospecific delivery (from the α- or β-face) of anions would stabilize the carbocationic transition-state and lower the activation energy



2,3-OS (1)

Figure 11. Stabilization of carbocation intermediates by delivery of negative point charges by OSC.

required for their conversion to subsequent cations (Figure 11). For example, closure of ring B in a boat rather than chair conformation could be promoted by delivery of a point charge to the α-face at the *pro*-C-8 (steroid numbering) carbon. Similarly, point charge delivery to the α-face of the *pro*-C-13 to give **5** instead of the *pro*-C-14 to give **33** could promote the closure of ring C in an *anti*-Markovnikov manner (Figure 12).



5 33

Figure 12. OSC-mediated formation of ring C in *anti*-Markovnikov addition generating cation **5** instead of **33**.

The non-enzymatic cyclization of racemic 2,3-OS (**1a**)[24a] and its analog **34**[24b] gives exculsively five-membered ring C products **35a-b** and **36** *via* Markovnikov addition (Figure 13). Such tricyclic systems are formed in the enzymatic transformation of 2,3-OS analogs lacking Δ^{18} (**37**)[21h] or containing a C-18(*Z*) double bond (**38**)[4e] (Figure 14). The tricyclic products **39** and **40a-b** are derived from elimination of and capture by water, respectively, of the tertiary cation intermediates. Enzymatic transformation of 2,3-OS analog **41**[25a] in which the methyl groups at C-10 and C-15 were replaced by hydrogen gave 6.6.5-tricyclic product **44** (Figure 15). The chair-chair-boat conformation of **44**

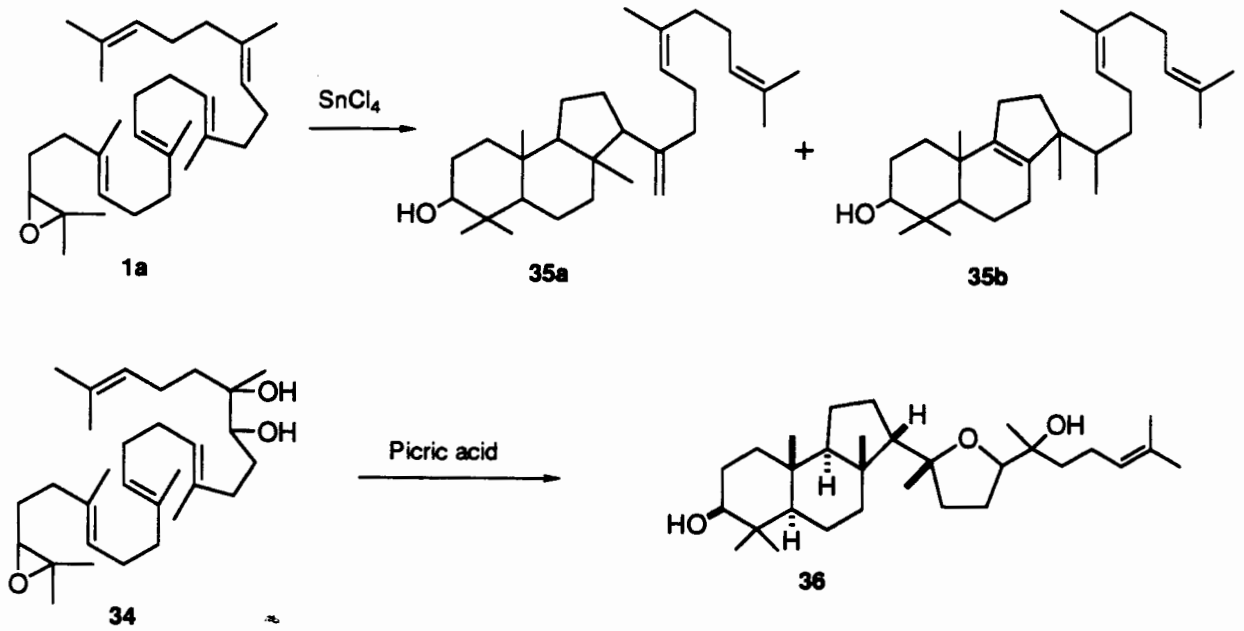


Figure 13. Lewis acid catalysis of cyclization of racemic 2,3-OS (**1a**) and its diol analog (**34**).

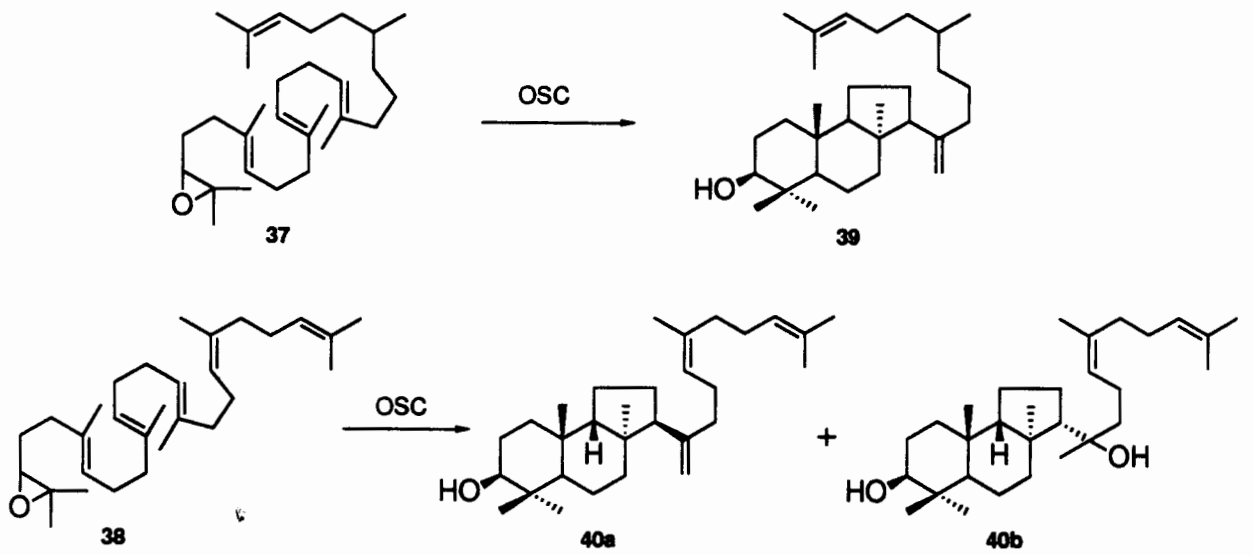


Figure 14. OSC-catalyzed cyclization of 18,19-dihydro-2,3-OS and 18(*Z*)-2,3-OS.

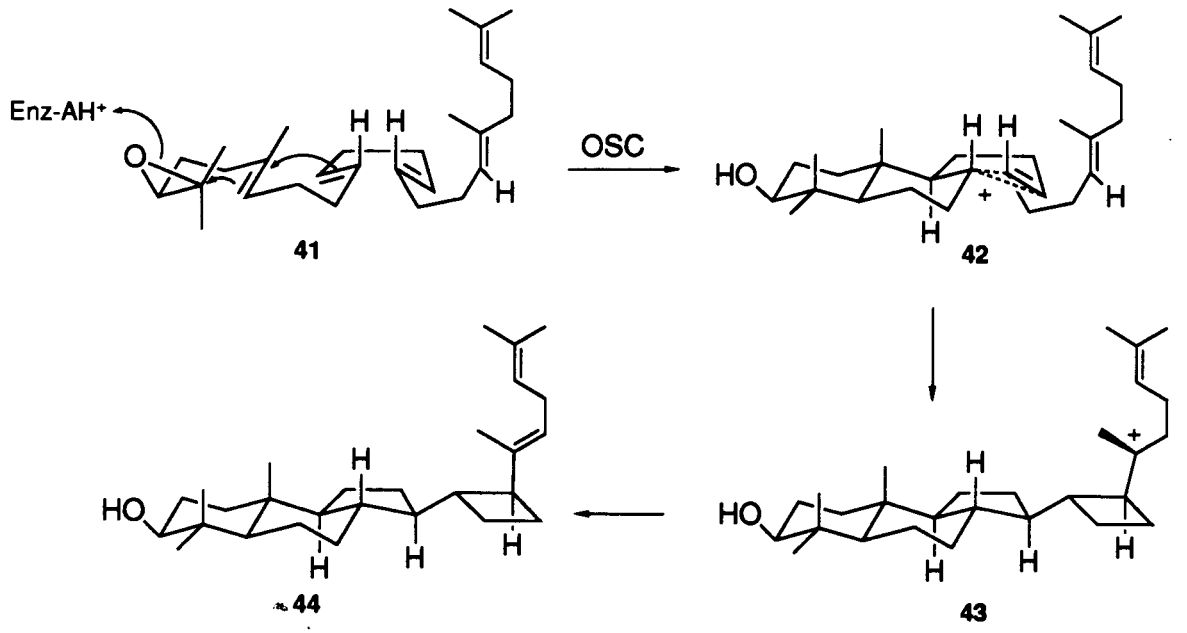


Figure 15. OSC-mediated cyclization of 10,15-didemethyl-2,3-OS (**41**).

suggests that **41** is folded by the cyclase to a conformation in which the 10,11-olefinic subunit is rotated 180° relative to the normal conformation adopted by 2,3-OS (**1**). Such folding is unfavorable for **1** because of the large steric interaction between the C-10 and C-15 methyl groups. The course of the enzymatic transformation of **41** implies that the cyclization can proceed *via* secondary cationic intermediates. By comparison the conformation of the 14,15-olefinic subunit in **41** remains the same as during enzymatic cyclization of **1** suggesting that the rigidity of this subunit is more strongly controlled by the enzyme.

The stereochemistry at C-20 of protosterol **6** has been recently examined by Corey *et al.* Transformation of 18(*E*)-20-oxa-2,3-OS[25b] and 20(*E*)-20,21-dehydro-2,3-OS[25c] to their respective protosterol derivatives established that these derivatives have a 17β-side chain instead of the previously postulated 17α-orientation.[20]

The natural occurrence of monocyclic[6a] and bicyclic[6b] triterpenes, theoretical calculations,[22] biomimetic cyclization,[4a-d,23] and enzymatic transformations of modified substrates[4e,21h,25a] all point to an enzymatic process proceeding through a series of conformationally rigid, partially cyclized carbocationic intermediates (**2-5**, Figure 1).

1.2.3. Inhibition of 2,3-oxidosqualene-lanosterol cyclase using charged heteroatom substituted analogs.

Inhibition of 2,3-oxidosqualene-lanosterol cyclase (OSC) using various strategies to probe the existence of cationic intermediates during the sterol biosynthesis has been reviewed recently.[2,26] Important strategies include the introduction of stabilizing groups at carbons considered to be cationic during the enzyme cyclization or inclusion of a heteroatom in substrate or transition state analogs to mimic the presumptive carbocationic intermediates. The use of the first strategy led to the discovery of the first mechanism-based irreversible inhibitor, 29-MOS, and its usefulness to map the active site (Figure 2).[14] Use of charged heteroatom mimics has led to several good inhibitors of the enzymes involved in sterol biosynthesis.[2,26] Indeed, this strategy has proven an effective guide in the rational design of inhibitors of sterol biosynthesis. Because it has been frequently applied to inhibitory studies of those enzymes involved in sterol[26-28] and terpenoid[29] biosynthesis and is well documented,[2] we focus here only on the target enzyme: 2,3-oxidosqualene-lanosterol cyclase.

It is well known that OSC can be inhibited either by substrate[26d,30] or transition state[31] analogs. Substrate and transition state analogs containing secondary and tertiary amine functionalities are thought to be good inhibitors because they are protonated at physiological pH and thereby resemble the structure and charge of carbocationic intermediates. Substrate analogs containing

nitrogen replacing C-2, C-10 or C-19 in the 2,3-OS skeleton[26d,30a-c] such as **45-47** (Figure 16) inhibited OSC from mammalian and fungal sources (Table 1). Compound **47** containing nitrogen in place of C-19 is considered to be

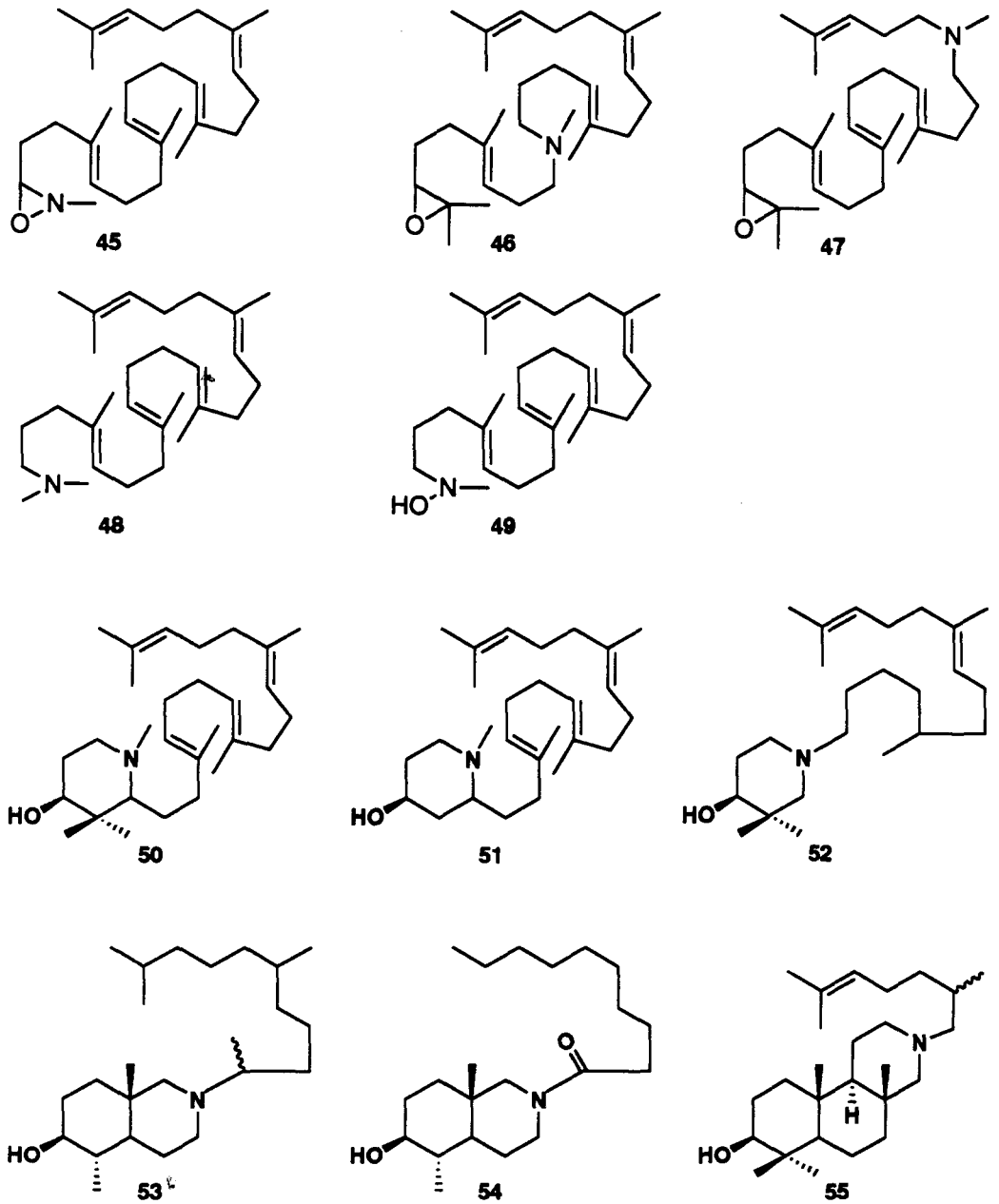


Figure 16. Nitrogen-substituted squalenes (**48-49**), 2,3-OSs (**45-47**) and transition state analogs (**50-55**) as inhibitors of OSCs.

Table 1. Inhibition of OSCs by heteroatom (N and O) substituted substrate and transition state mimics (**45-62**).

Compound	Enzyme (IC_{50}[a]: μM) Pig Liver	Rat liver	Yeast	*C. albicans*
45	ND[b]	1.5	2.5	ND[b]
46	5	4.8	5	22
47	1.5	7.5	35	ND[b]
48	ND[b]	7.5	10	ND[b]
49	5	ND[b]	ND[b]	ND[b]
50	1.4	ND[b]	ND[b]	0.23
51	0.3	ND[b]	ND[b]	0.67
52	ND[b]	20	ND[b]	ND[b]
53	ND[b]	2	ND[b]	ND[b]
54	ND[b]	0.11	ND[b]	ND[b]
55	ND[b]	> 300	ND[b]	ND[b]
56	ND[b]	21.3	ND[b]	ND[b]
57	ND[b]	13.0	ND[b]	ND[b]
58	ND[b]	NI[c]	ND[b]	ND[b]
59	ND[b]	0.11	ND[b]	ND[b]
60	ND[b]	142	ND[b]	ND[b]
61	ND[b]	80	ND[b]	ND[b]
62	ND[b]	120	ND[b]	ND[b]

[a]: mole concentration of inhibitor required to reduce enzyme activity by 50%.
[b]: Not determined. [c]: No inhibition in the 1-40 μM concentration range.

an analog of the cation formed during the OSC-mediated cyclization. It showed potent activity against mammalian cyclase (IC_{50} = 1.5 μM, pig liver). Compounds **48** and **49** are transition state analogs in which a nitrogen atom replaces C-2 of the

squalene skeleton (Figure 16).[28a,31a] In derivatives **50-55** the *pro*-C-8, *pro*-C-10 and *pro*-C-13 (steroid numbering) positons of monocyclic, bicyclic, and tricyclic compounds, respectively, are replaced by nitrogen[31b-g] (Figure 16). Except for **55**, these compounds were designed as mimics of cationic intermediates **2-5** and display a good to potent inhibitory activity against cyclases from mammalian and fungal sources (Table 1).

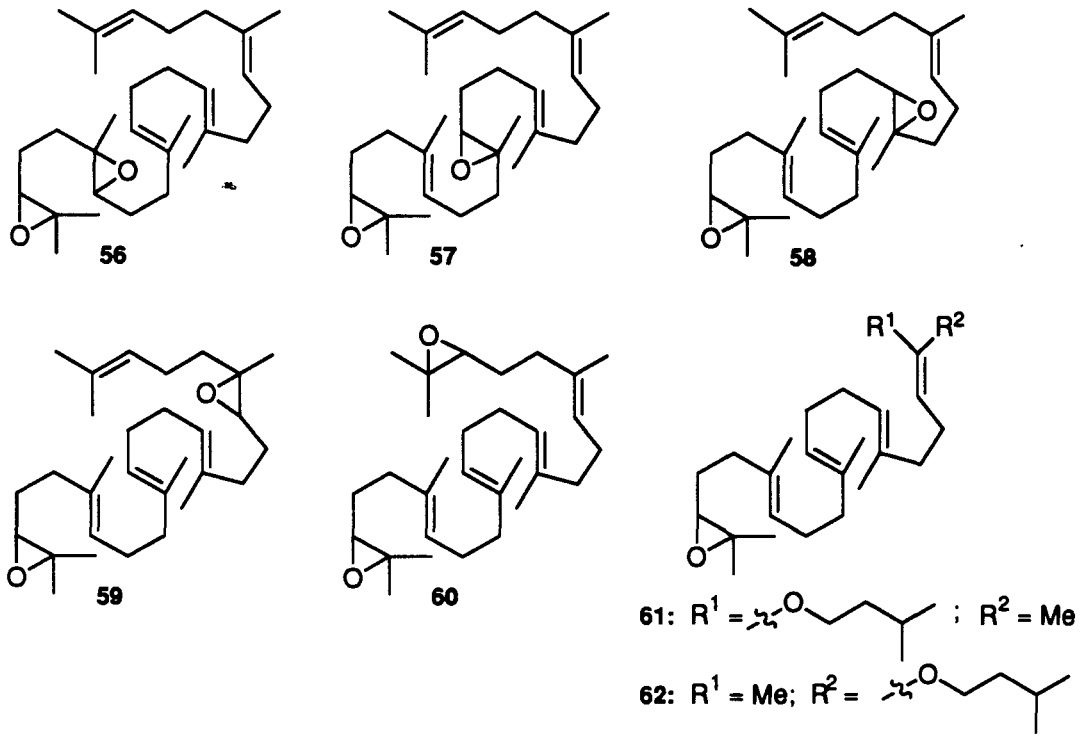


Figure 17. Oxygen-substituted 2,3-OS analogs as inhibitors of OSCs.

Inhibition of OSC by substrate analogs containing oxygen in the 2,3-OS skeleton is exemplified by dioxido analogs such as **56-60**,[30d,e] *E*-vinylic ether **61** and *Z*-vinylic ether **62** [30f](Figure 18). All dioxido analogs except **58** showed good inhibitory activity in rat liver cyclase (Table 1). Both vinylic ethers **61** and **62** were competitive inhibitors exhibiting very weak inhibition of OSC (IC_{50} = 80 μM and 120 μM, respectively, rat liver OSC). Among the heteroatom analogs, compound

59 proved to be the best OSC inhibitor (IC_{50} = 0.11 μM, rat liver OSC) and showed time-dependent, non-competitive behavior.[30d,e] These results highlight substrate analogs as attractive targets for further study.

The heteroatom-containing compounds **45-48** and **50** showed modest different inhibitory activity against cyclases from different sources (mammalian or fungal) suggesting the active sites of these cyclases could be different.

1.3 Research objective and thesis organization.

In view of the important role that charged heteroatom substrate analogs have played in the study of OSC and the observation that **59** was the most potent inhibitor of OSC,[30e] my research focused on the design and synthesis of substrate analogs containing heteroatoms at key structural points. The introduction of heteroatoms into the skeleton of **1** could lead to analogs having sufficiently flexible conformations to be recognized by the cyclases. Such analogs could cyclize *in situ* to generate heteroatom analogs of the presumptive cationic intermediates **3-6** (Figure 1). The latter could be potent inhibitors through intra- or intermolecular interaction with native nucleophilic sites. I considered that the information derived from study of heteroatom-containing 2,3-OS analogs would be of use in designing more potent inhibitors of OSCs.

In this thesis, two sets of 2,3-OS analogs have been prepared and examined for their ability to inhibit OSCs. In one set of 2,3-OS analogs sulfur or sulfoxide functions replace the olefinic carbons considered to become cationic during the enzymatic cyclization (**63-72**, Figure 18). These strategically placed heteroatoms were expected to interfere with ring A, B, C, D formation by acting as mimics of presumptive cationic intermediates **3-6**. Another set of 2,3-OS analogs contained sulfur α or β to carbons considered to become cationic during the cyclization (**73-76**, Figure 19). The underlying assumption was that these vinylic

sulfide analogs of 2,3-OS would cyclize to the point that the carbocation formed could be stabilized by an adjacent sulfur. Such stabilization could provide a sufficiently long life to the thiocarbenium ions that they might react with the nucleophilic residues involved in the stabilization of the presumptive cationic intermediates. If this occurred, it was expected that this series of 2,3-OS analogs would act as irreversible inhibitors. Methodology for the synthesis of *E*-vinylic sulfides has been developed by Dodd and Oehlschlager[31h], and two *E*-vinylic sulfide 2,3-OS analogs in which sulfur replaced carbons C-9 and C-16 of **1** have previously been synthesized.

This thesis is divided into six chapters. Chapter 2 describes the synthesis of sulfur- and sulfoxide-substituted 2,3-OS analogs **63-71** (Figure 18) and their evaluation as inhibitors of fungal and mammalian OSCs. Synthetic methodologies such as zirconium-catalyzed carboalumination, barium allylic-allylic coupling, and phase-transfer catalysis have been applied to achieve the synthetic goals. Due to the observation that **66** in which sulfur replaces C-19 of **1** is the most potent inhibitor of OSCs, the synthesis of **72** (Figure 18) in which sulfur replaces C-18 of **1** was undertaken (Chapter 3). The inhibitory activities of these two 2,3-OS analogs were compared. An enzyme kinetic analysis of the actions of **66** and **72** is also presented and discussed in Chapter 3. Chapter 4 outlines the successful synthesis of vinylic sulfide analogs **73-76**. Two synthetic strategies were used for the preparation of the required *E*-vinylic sulfides. A new methodology involving stereospecific coupling of trisubstituted vinylic anions with alkyl 4-methylbenzenthiosulfonates was developed and applied to the synthesis of **74** and **75**. An alternative method involved the modified Wittig-Horner reaction. This synthetic strategy affords **73** and **76** *via* a set of common intermediates.

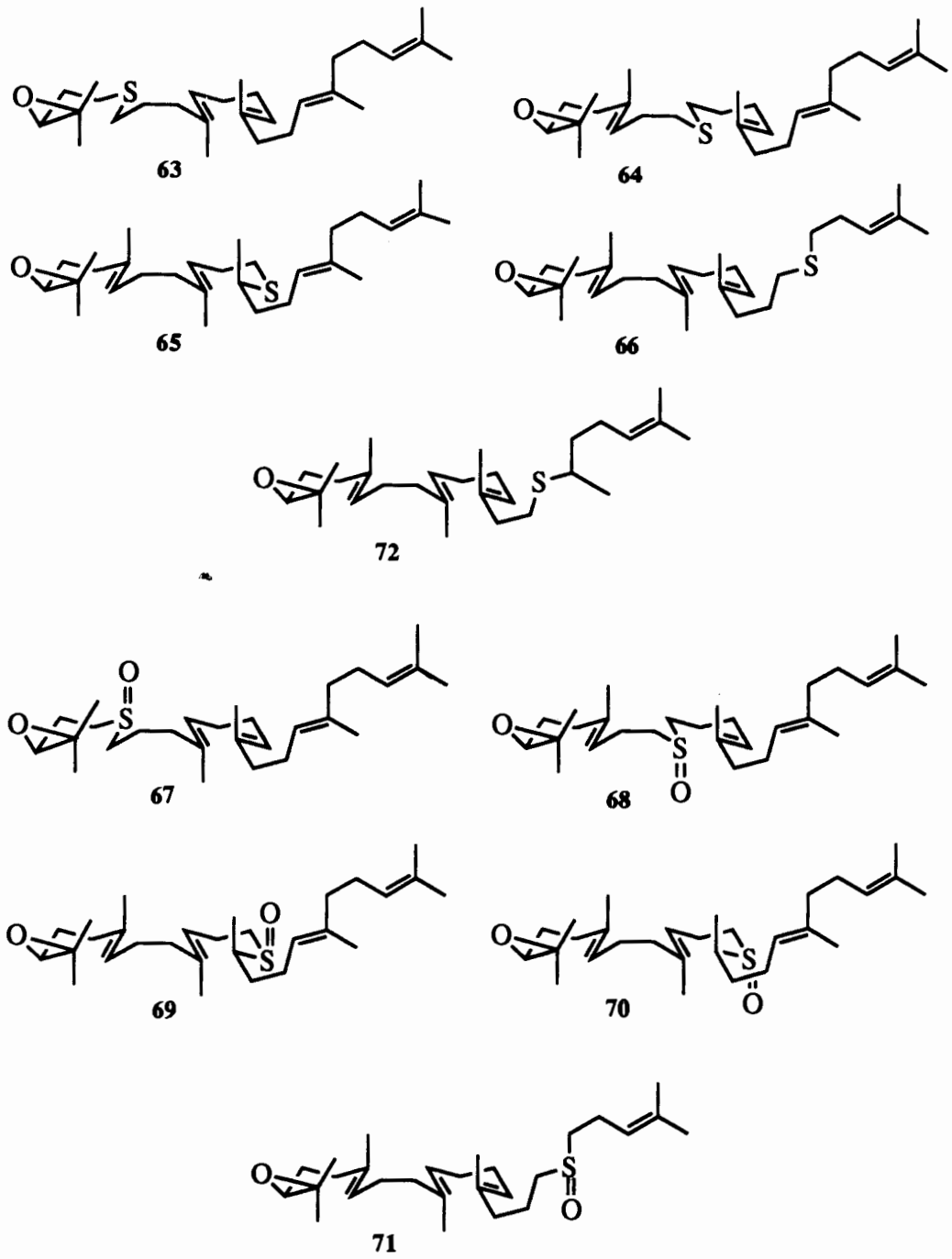


Figure 18. Sulfur- and sulfoxide-substituted 2,3-OS analogs (**63-72**) prepared as substrate mimics for OSCs.

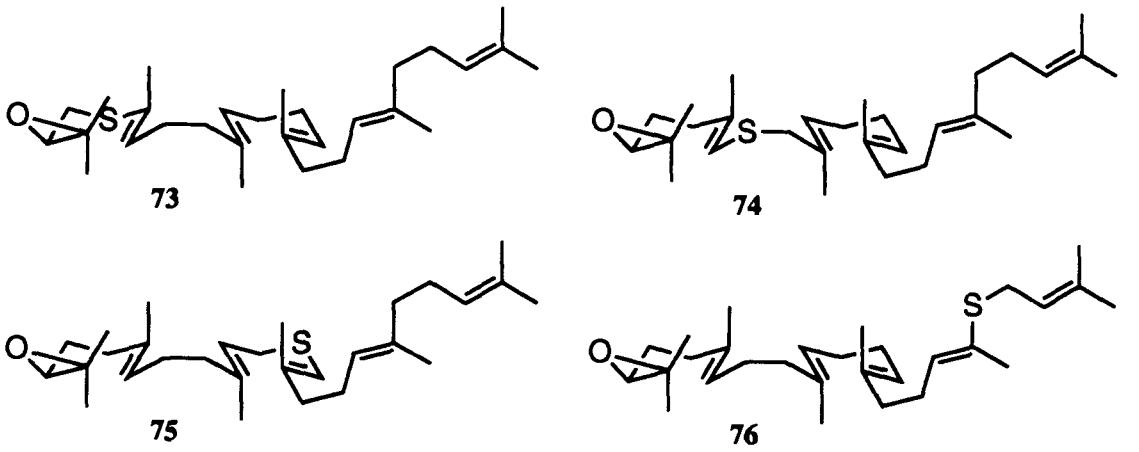


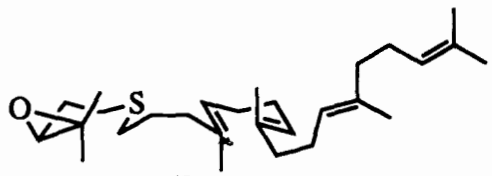
Figure 19. *E*-Vinylic sulfur-substituted 2,3-OS analogs (**73-76**) as possible mechanism-based inhibitors of OSCs.

Chapter 5 summarizes the results of the inhibition studies and outlines further possible investigations. Chapter 6 contains the experimental details.

Chapter 2

Synthesis and Biological Evaluation of Sulfur- and Sulfoxide-Substituted 2,3-Oxidosqualene Analogs 63-71

This chapter describes the synthesis and inhibitory activity of 2,3-OS analogs **63-71** in which sulfur or sulfoxide replaces the olefinic carbons C-6, C-10, C-14 and C-19 of **1** that are considered to become positively charged during OSC-mediated cyclization.



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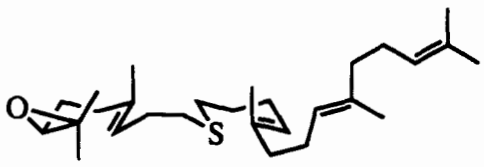

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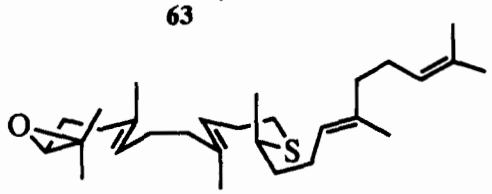

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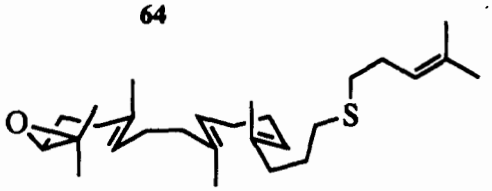

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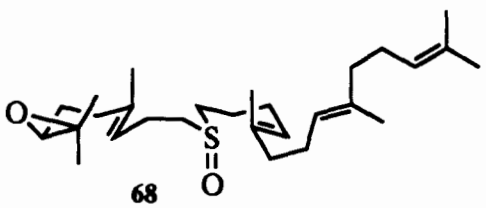

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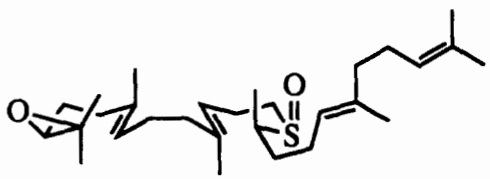

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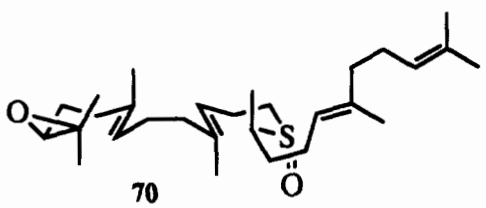

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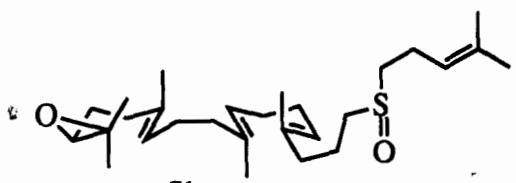


71

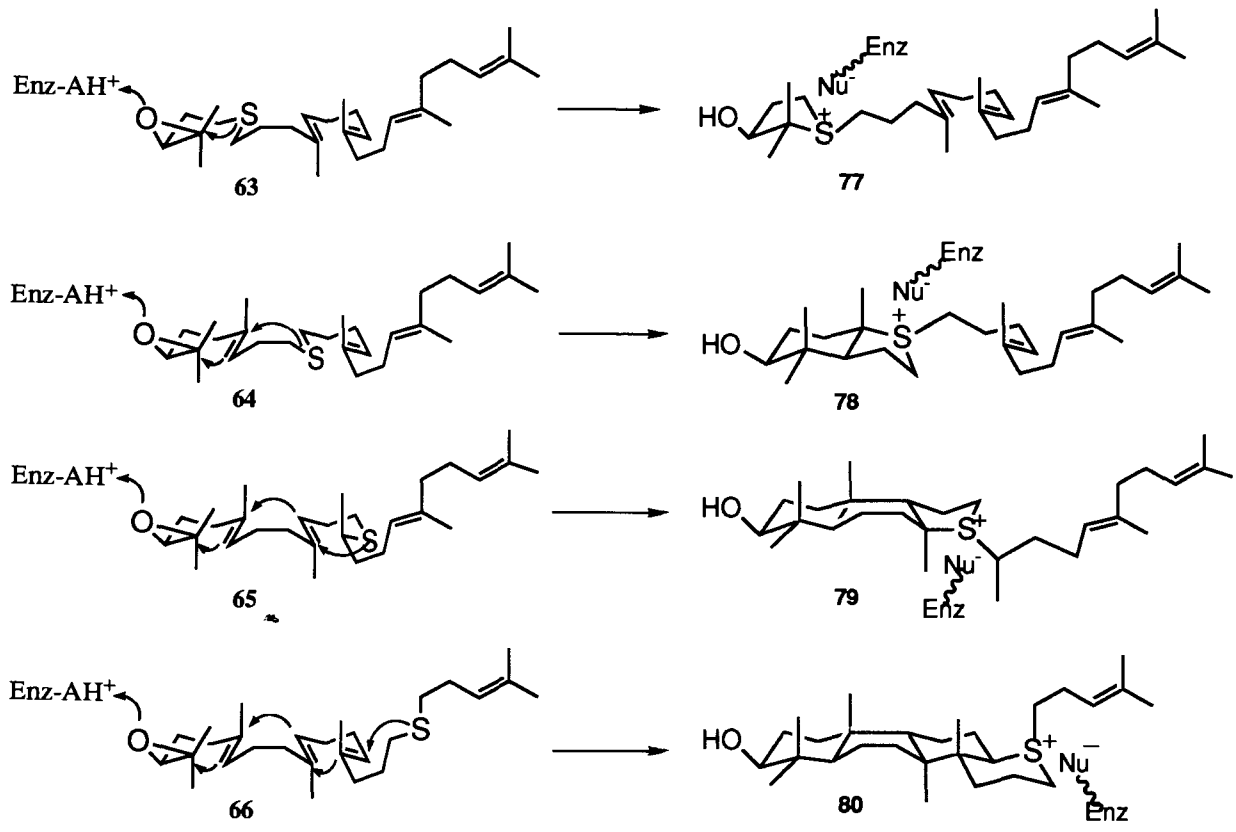
2.1. Rationale.

Our choice of sulfur-substituted 2,3-OS analogs[32] was guided by several considerations. In the native cyclization, π bonds are the intramolecular nucleophiles that react with each cation (**2-5**). The excellent nucleophilic properties of sulfur[33] compared to candidates such as nitrogen or oxygen were considered to be an advantage if **63-66** acted as substrate mimics. We reasoned that initial enzymatic interaction with **1** would be primarily with the π orbitals of the sp^2 carbons normally at the sites now occupied by sulfur and the latter could mimic these interactions.

As each ring is formed in the native cyclization, the carbon positions replaced by sulfur or sulfoxide normally become positively charged and require an enzymatic nucleophile for stabilization. The sulfoxide analogs **67-71** provide electron-deficient centers at the relevant locations which could take advantage of this interaction to inhibit OSC.

Finally, it is possible that **63-66** could bind to OSCs and cyclize to **77-80**, respectively (Scheme 1). In this event, the new sulfonium ions formed would each be positioned near OSC nucleophilic sites normally stabilizing **3-6**. Since formation of rings A[4a] and B[34] has been shown to be the rate-determining step in the biomimetic polyene cyclization of analogs of **1**, one might reasonably expect that analogs **63** and **64** leading to **77** and **78**, respectively, would be the most potent inhibitors. If cyclization of **63-66** occurred, one would expect the thioethers to be stronger inhibitors than their sulfoxide analogs **67-71** since **77-80**, the cyclized derivatives of **63-66**, should be better mimics of enzyme-intermediate complexation.

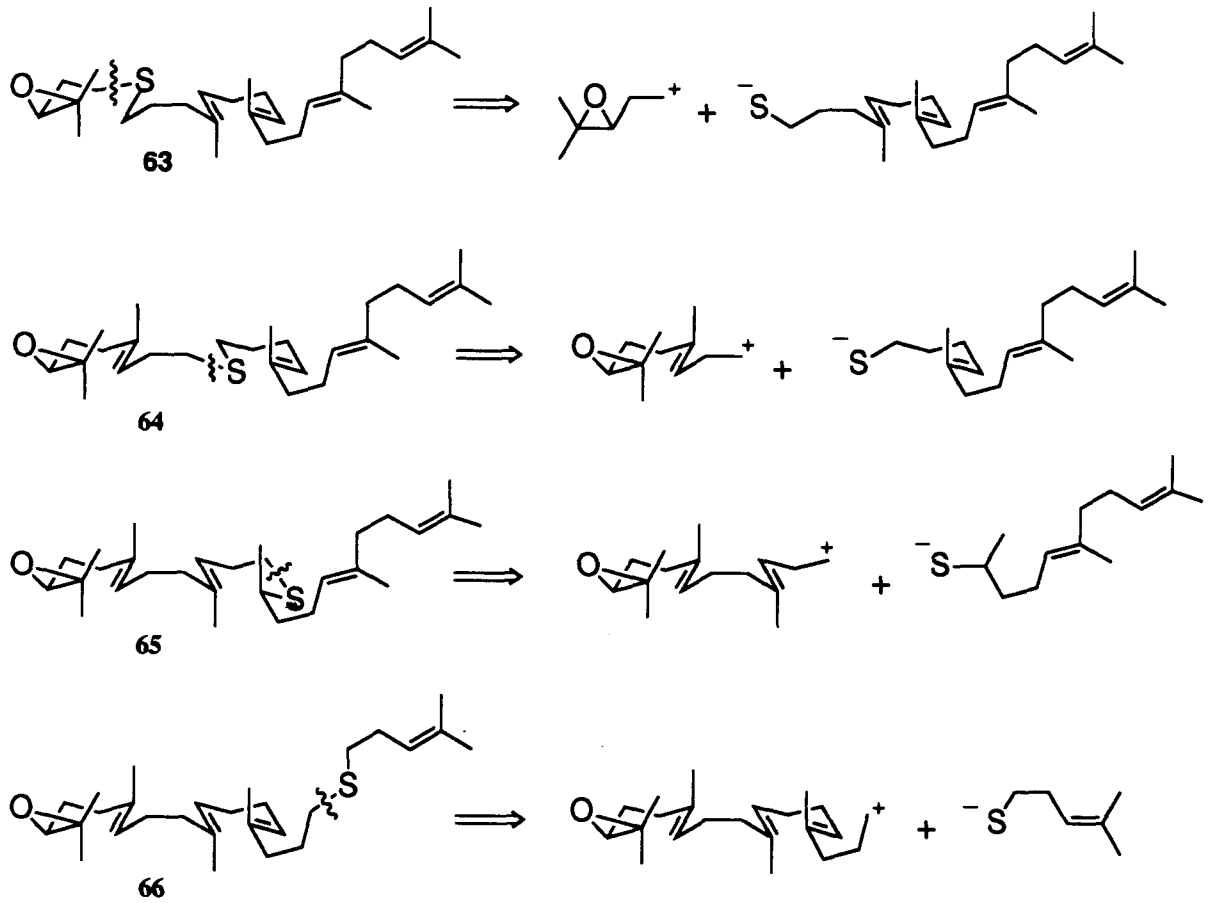
If both the thioether and sulfoxide analogs of **1** behave as substrate mimics, the sulfoxides should be the more potent inhibitors. While interactions between



Scheme 1. Hypothetical mechanism of inhibition of OSCs by sulfur-substituted 2,3-oxidosqualene analogs.

OSC and the sulfoxides mimic enzyme-intermediate complexation, the interactions between OSC and the corresponding thioethers mimic enzyme-substrate complexation which is generally weaker.

The target compounds **63-66** require construction of terminal oxirane and thioether functionalities in unsaturated backbones. Retrosynthetic analysis reveals that they can be assembled from the epoxy carbocation and thiolate anion equivalents (Scheme 2). We selected the phase-transfer catalysis reaction[35] developed by Herriott and Picker which has the advantages of high yield, mild



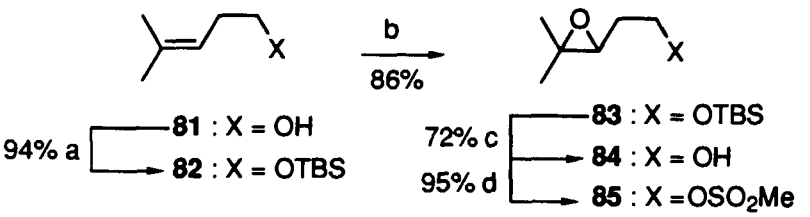
Scheme 2. Retrosynthetic analyses of **63-66**.

reaction conditions and inexpensive reagents. The application of mild phase-transfer conditions [NaOH, methyltrioctylammonium chloride, H_2O-benzene (5:4)] should prevent nucleophilic opening of the epoxy ring by the thiolate anion.[36] The epoxy-containing reactants and products would be expected to remain in the organic phase, most of the thiolate anions in the aqueous phase. Among the many oxidation methods leading to sulfoxides (**67-71**) from thioethers,[37] we selected the potassium peroxymonosulfate oxidation described by Trost *et al.*[38] This reaction is selective, tolerates olefins and proceeds rapidly with high yield and easy work-up. With these rationales in mind, I commenced the syntheses of **63-71**.

2.2. Syntheses.

2.2.1. Syntheses of 63 and 67.

The synthesis of **63** (Scheme 3) commenced with conversion of alcohol **81** to its protected form **82** in 94% yield. Epoxidation of **82** with *m*-CPBA in methylene chloride gave **83** in 86% yield. Deprotection of **83** followed by mesylation gave cation synthon **85** in 68% yield over two steps (Scheme 3). Epoxy alcohol **84** can also be prepared by direct epoxidation of alcohol **81** with *m*-CPBA in methylene chloride. However, a much lower yield of **84** (31%) was obtained in this reaction. We believe that the lower yield is due to high solubility of compound **84** in water and high volatility causing loss of the compound during work-up. Introduction of a silyl protecting group for the alcohol **81** during the epoxidation and use of less water (Bu_4NF, H_2O <5 %) to remove the silyl protecting group of **83** avoids these problems and provides a high yield of **84**.



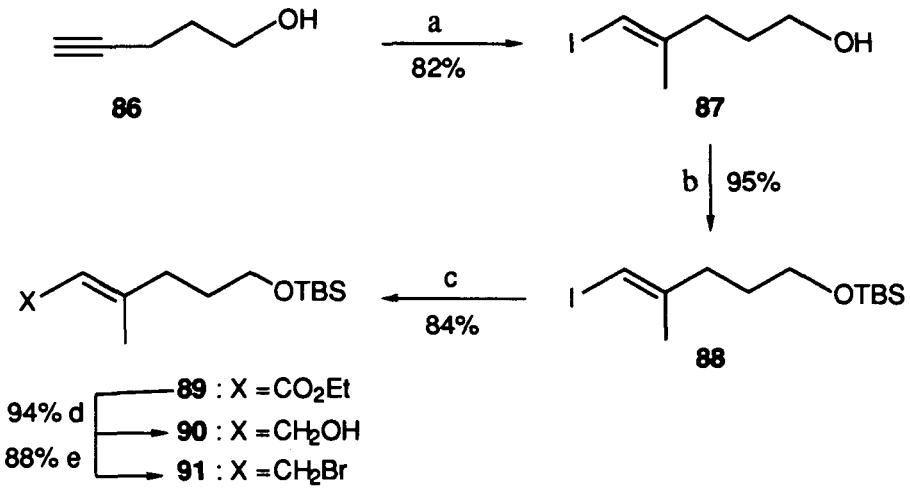
(a)TBSCl, Et_3N, DMAP, CH_2Cl_2; (b) *m*-CPBA, CH_2Cl_2; (c) Bu_4NF, THF;

(d) $MeSO_2Cl$, Et_3N, -50 °C, CH_2Cl_2.

Scheme 3. Synthesis of epoxy mesylate **85**.

The anion synthon **97**, a tetraenic thiol (Scheme 5), was prepared from the corresponding alcohol **95** which was obtained by allyl-allyl coupling of farnesyl chloride (**93**) with allylic bromide **91**.[39] The synthesis of **91** commenced with

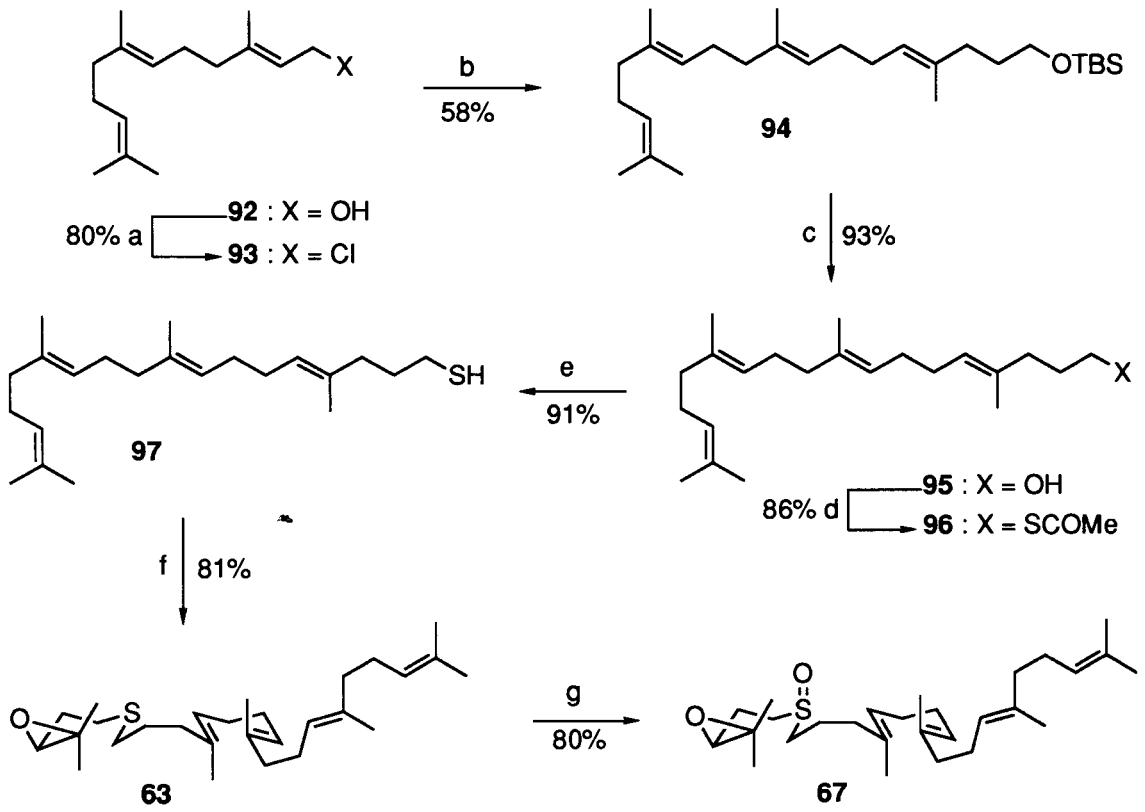
zirconium-catalyzed carboalumination of 4-pentyn-1-ol (**86**) followed by iodine trapping to give the desired *E*-iodide **87** in 82% yield [40] (Scheme 4). Capillary GC analysis of **87** revealed a single peak which contained *E*- and Z-isomers. Reaction of **87** with *tert*-butyldimethylsilyl chloride and Et_3N gave **88** in 95% yield. Analysis of **88** by GC/MS revealed two components (*E*:*Z* ratio 96:4). Conversion of **88** to its vinylic lithium derivative with 1.1 equiv. *n*-BuLi at -78°C followed by addition of ethyl chloroformate gave conjugated ester **89**. This product was then reduced with DIBAL-H to allylic alcohol **90** which was converted to allylic bromide **91** (Scheme 4).[39] Reaction of farnesol (**92**) with NCS-DMS complex[41] gave farnesyl



(a) $Zr(Cp)_2Cl_2$, $AlMe_3$, then I_2, CH_2Cl_2; (b) TBSCl, DMAP, Et_3N, CH_2Cl_2; (c) *n*-BuLi, -78 °C, THF, then $ClCO_2Et$; (d) DIBAL-H, ether; (e) $MeSO_2Cl$, Et_3N, -40 °C, LiBr, CH_2Cl_2.

Scheme 4. Synthesis of allylic bromide **91**.

chloride (**93**) in 81% yield (Scheme 5). Coupling of allylic bromide **91** with farnesyl chloride was achieved *via* the barium derivative of **93** to give the protected tetraenol **94** in 58% yield.[39] Removal



(a) NCS, DMS, CH_2Cl_2; (b) Li, Ph-Ph, 2 h, THF, then BaI_2, 0.5 h, then, **93**, 0.5 h, -78 °C, THF, then **91**, 12 h, THF; (c) Bu_4NF, THF; (d) PPh_3, i-PrO_2C-N=N-CO_2Pr-i, MeCOSH, 0 °C, THF; (e) $LiAlH_4$, 0 °C, ether; (f) 50% NaOH, **85**, Oct_4NBr, 40 °C, H_2O-Toluene; (g) $KHSO_5$, 2 min, -5 °C, MeOH.

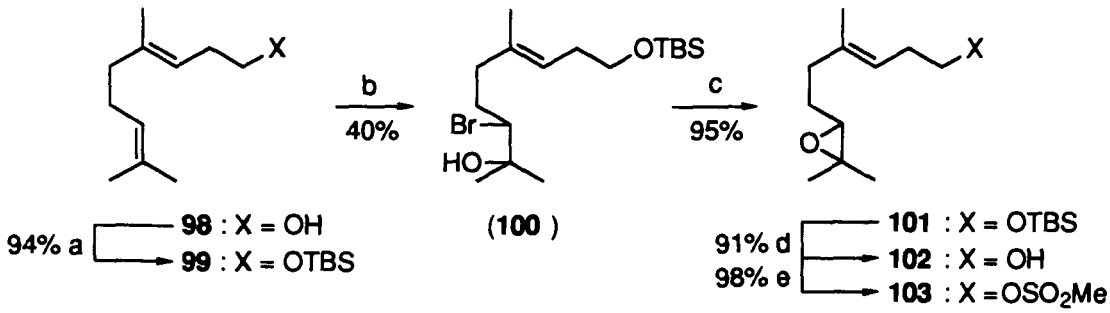
Scheme 5. Syntheses of **63** and **67**.

of the silyl protecting group of **94** followed by Mitsunobu reaction gave thioacetate **96** which was reduced to thiol **97** (73% yield over three steps).[42] Coupling of epoxy mesylate **85** and tetraenic thiol **97** to give **63** was achieved in 81% yield by treatment with 50% NaOH in toluene:H_2O (1:1) in the presence of

tetraoctylammonium bromide as a phase-transfer agent.[35] The structure of **63** was confirmed by ^{1}H-NMR which revealed a triplet at δ 2.82 (J = 6.2 Hz) for the oxirane hydrogen (C-3) and two multiplets at δ 2.52 and 2.68 for four hydrogens on C-5 and C-7 carbon. Oxidation of **63** with $KHSO_5$ in MeOH gave sulfoxide **67** in 80% yield.[38] The structure of **67** was also confirmed by ^{1}H-NMR which revealed a double doublet at δ 2.88 (J = 4.6, 7.9 Hz) for the oxirane hydrogen (C-3) and a multiplet at δ 2.68 for the four hydrogens on C-5 and C-7.

2.2.2. Syntheses of 64 and 68.

The syntheses of **64** and **68** in which the sulfur or sulfoxide replaces C-10 of 2,3-OS involved coupling of epoxy mesylate **103** with trienic thiol **109** (Scheme 7). The former was prepared from homogeraniol (**98**)[43] which was initially protected (to **99**), converted *via* NBS in THF-H_2O (3.8:1)[44] to bromohydrin **100** and hence to oxirane **101** (Scheme 6). Deprotection of **101** using Bu_4NF gave epoxy alcohol **102** which was converted to the required epoxy mesylate **103**. The preparation of

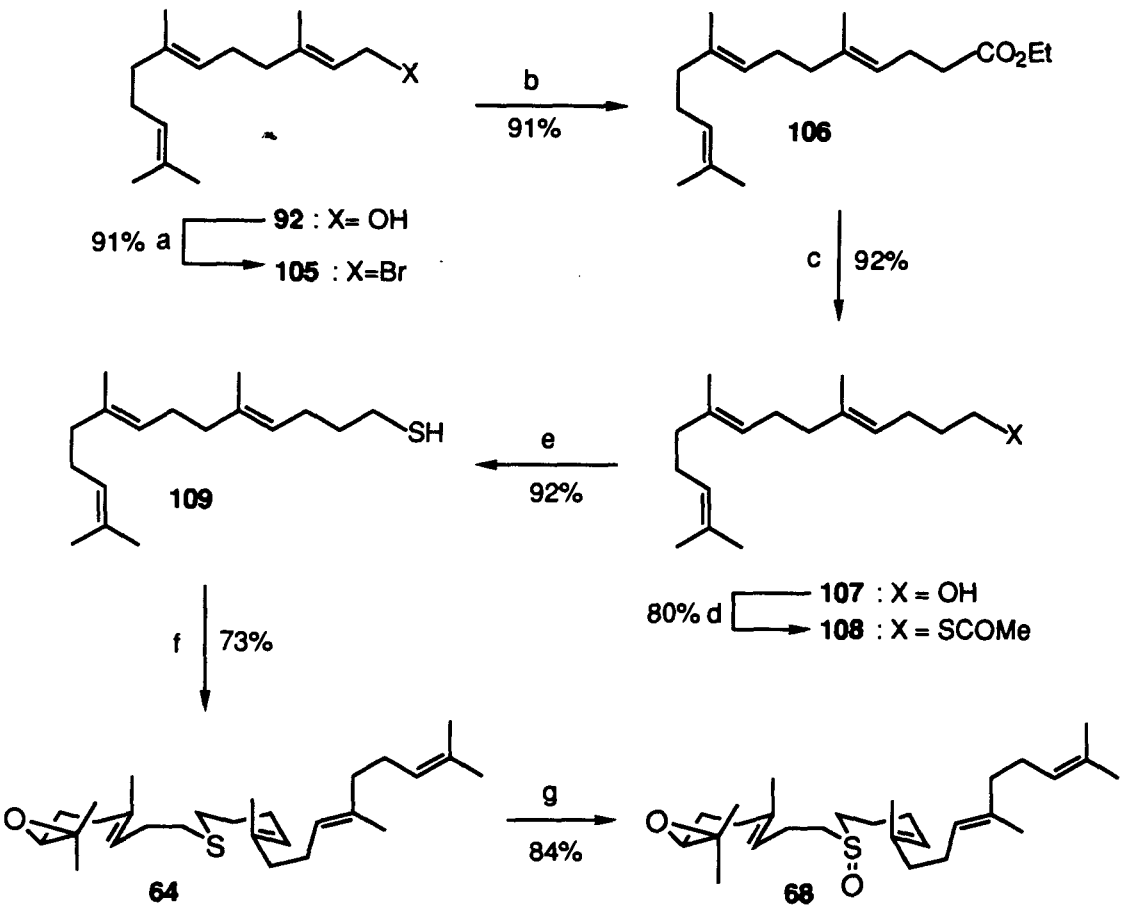


(a) TBSCl, Et_3N, DMAP, CH_2Cl_2; (b) 1.0 eq. NBS, 0 °C, THF-H_2O; (c) K_2CO_3, MeOH;

(d) Bu_4NF, THF; (e) $MeSO_2Cl$, Et_3N, -50 °C, CH_2Cl_2.

Scheme 6. Synthesis of epoxy mesylate **103**.

trienic thiol **109** commenced with the conversion of commercially available (*E*,*E*)-farnesol (**92**) to farnesyl bromide (**105**) in 91% yield (Scheme 7).[45] Alkylation of **105** with the lithium enolate of ethyl acetate in the presence of CuI at -100 °C gave **106** in 91% yield according to the procedure of Coates.[46] Reduction of **106** with $LiAlH_4$ gave the alcohol **107** in 92% yield. Mitsunobu reaction of **107** with thiolacetic acid gave thioacetate **108** which was reduced to the required trienic thiol



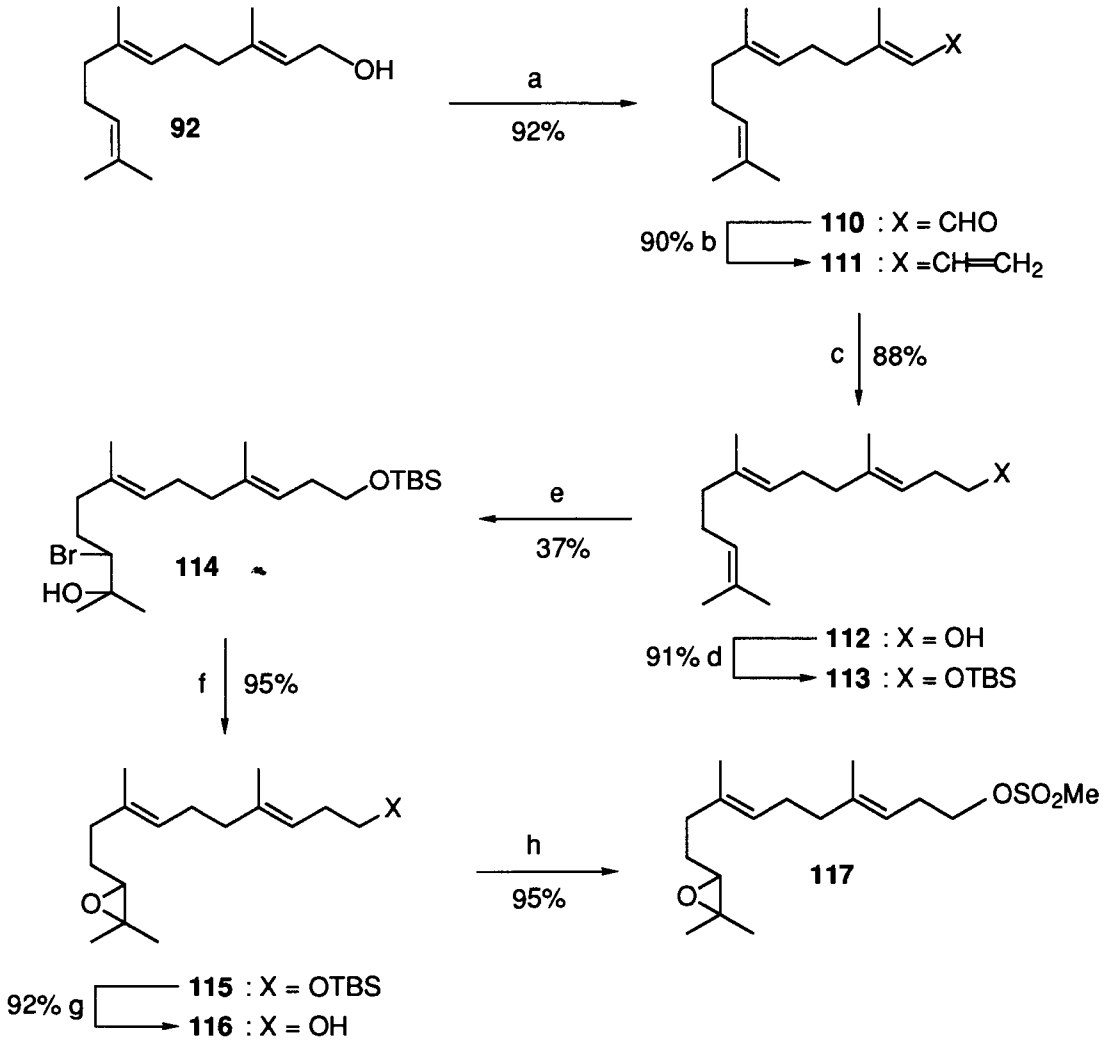
(a) PBr_3, ether; (b) $MeCO_2Et$, CuI, LDA, -100 °C, THF; (c) $LiAlH_4$, ether; (d) PPh_3, i-PrO_2C-N=N-CO_2Pr-i, MeCOSH, 0 °C, THF; (e) $LiAlH_4$, 0 °C, ether; (f) 50% NaOH, **103**, Oct_4NBr, 40 °C, H_2O-Toluene; (g) $KHSO_5$, 2 min, -5 °C, MeOH.

Scheme 7. Syntheses of **64** and **68**.

109 (74% yield over two steps).[42] Coupling of **103** and **109** with the aid of a phase-transfer agent gave **64** in 73% yield.[35] Oxidation of **64** with $KHSO_5$ in MeOH gave sulfoxide **68** in 84% yield (Scheme 7).[38] The structures of **64** and **68** were confirmed by ^1H NMR analysis. The ^1H-NMR spectrum of **64** revealed a triplet at δ 2.71 (J = 6.2 Hz) due to the oxirane hydrogen (C-3) and a triplet at δ 2.51 (J = 8.0 Hz) for the four hydrogens on C-9 and C-11 adjacent to sulfur. The ^1H-NMR spectrum of **68** revealed overlapping signals of δ 2.56-2.76 for the oxirane hydrogen (C-3) and the four hydrogens on C-9 and C-11 adjacent to sulfoxide. An ^1H-^1H COSY ^1H-NMR analysis of **68** permitted the distinction of these signals and the establishment of H-H connectivities. A double triplet at δ 2.49 (J = 7.5, 7.5 Hz), was assigned to the two hydrogens on C-8. It showed a cross-peak in the COSY spectrum at δ 2.63 and δ 2.60 which indicated that these two signals are due to the two hydrogens on the carbon adjacent to the sulfoxide (C-9). Similarly, the quintet at δ 1.81 (J = 7.2 Hz), assigned to the two hydrogens on C-12, displayed a cross-peak in the COSY spectrum at δ 2.69 and δ 2.59 which indicated that these two signals are due to the two hydrogens on C-11.

2.2.3. Syntheses of 65, 69 and 70.

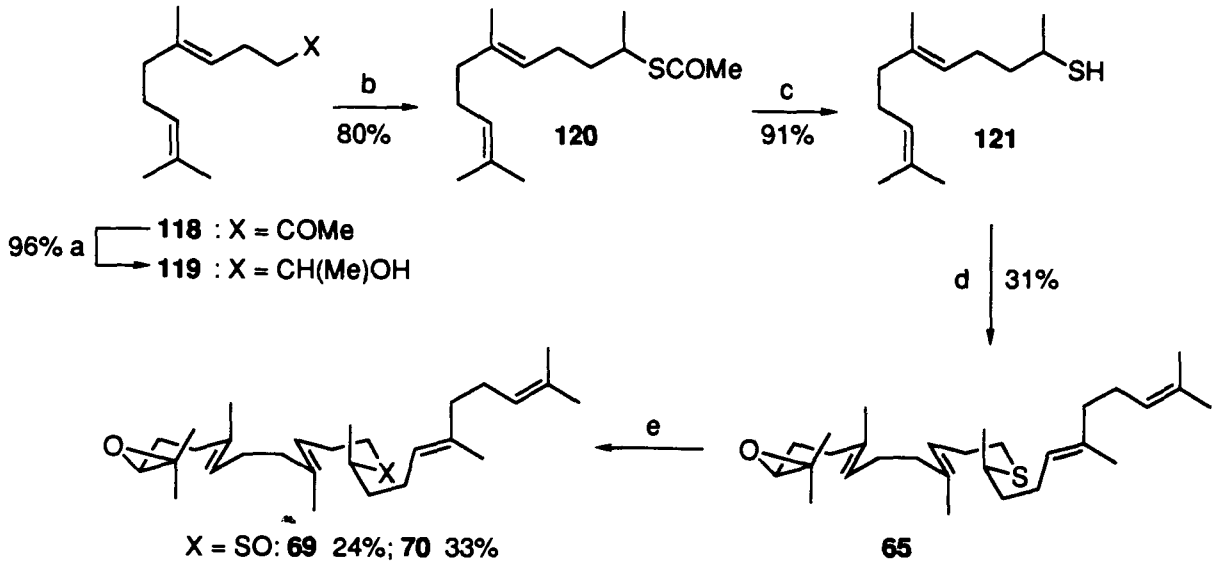
The syntheses of **65**, **69** and **70** in which sulfur or sulfoxide replaces C-14 of 2,3-OS involved coupling of epoxy mesylate **117** and dienic thiol **121** (Scheme 9). The former was prepared from homofarnesol (**112**)[47] obtained by chain extension of farnesol (**92**) in a procedure which was described for the synthesis of homogeraniol[43] (**98**, Scheme 8), and conversion of homofarnesol (**112**) to mesylate **117** followed by a sequence involving hydroxyl protection to **113**, addition of aqueous NBS to **114**, dehydrobromination to **115**, hydroxyl deprotection to **116** and mesylation to **117**. Dienic thiol **121** was prepared from geranylacetone (**118**) *via*



(a) ClCOCOCl, DMSO, -60 °C, CH_2Cl_2, then **92**, Et_3N (Swern oxidation); (b) CH_2=PPh_3, THF; (c) Sia_2BH, THF, H_2O_2, NaOH; (d) TBSCl, Et_3N, DMAP, CH_2Cl_2; (e) 1.0 eq. NBS, 0 °C, THF-H_2O; (f) K_2CO_3, MeOH; (g) Bu_4NF, THF; (h) $MeSO_2Cl$,Et_3N, -50 °C, CH_2Cl_2.

Scheme 8. Synthesis of epoxy mesylate **117**.

reduction to alcohol **119**, Mitsunobu reaction of the latter with thiolacetic acid to give thioacetate **120** and reduction. Coupling of mesylate **117** and thiol **121** under phase-transfer conditions was more difficult than for previous cases because a



(a) $LiAlH_4$, ether; (b) PPh_3, i-PrO_2C-N=N-CO_2Pr-i, MeCOSH, 0 °C, THF; (c) $LiAlH_4$, 0 °C, ether; (d) 50% NaOH, **117**, Oct_4NBr, 40 °C, H_2O-Toluene; (e) $KHSO_5$, 2 min, -5 °C, MeOH.

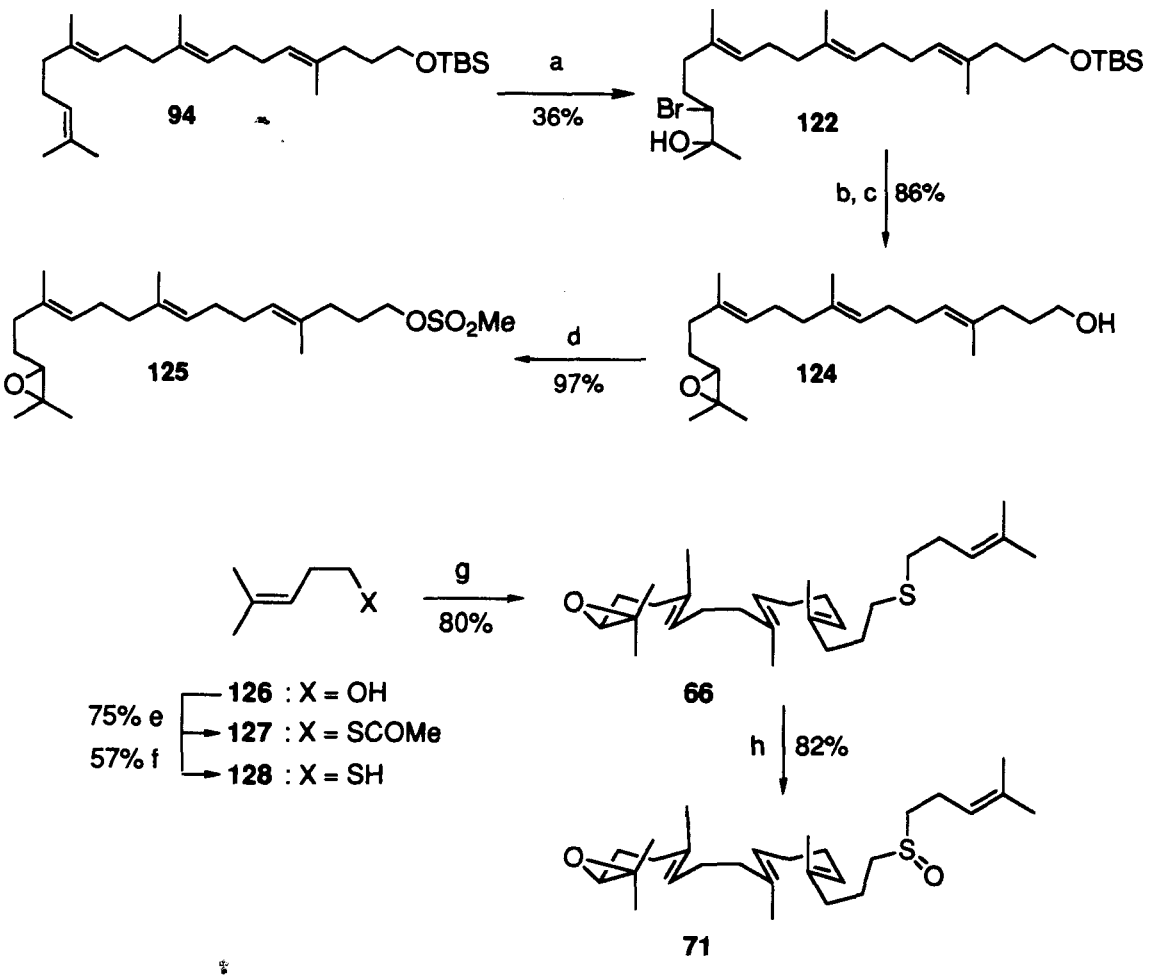
Scheme 9. Syntheses of **65, 69** and **70**.

secondary sulfide was involved; the maximum yield of **65** was 31%. The structure of **65** was confirmed by ^1H NMR which revealed a sextet at δ 2.76 (J = 6.7 Hz) for the hydrogen on C-15 and a triplet signal at δ 2.51 (J = 7.7 Hz) for the two hydrogens on C-13. Oxidation[38] of **65** with $KHSO_5$ in MeOH gave the two diastereoisomers **69** (24% yield) and **70** (33% yield) which were separated by column chromatography.[48] The ^1H-NMR spectra of **69** and **70** showed identical features except signals attributable to the methyl and hydrogen attached to C-15. The less polar (TLC) diastereoisomer **69** is assigned the *syn*-configuration with δ 1.23 (d, J = 6.9 Hz) for the CH_3 and δ 2.73 for the hydrogen attached to C-15 while the more polar diastereomer **70** is assigned the *anti*-configuration with δ 1.27 (d, J = 6.9 Hz) for the CH_3 and δ 2.67 for the hydrogen attached to this carbon. These

^{1}H-NMR assignments are consistent with those of axial and equatorial sulfoxides derived from oxidation of 5-thioglucose derivatives.[49,50]

2.2.4. Syntheses of 66 and 71.

Similar chemistry was applied to the synthesis of **66** and **71** in which sulfur or sulfoxide replaces C-19 of 2,3-OS. Thus, **66** was prepared by coupling of epoxy mesylate **125** and thiol **128** (Scheme 10). The former was prepared from **94** by



(a) 1.0 eq. NBS, 0 °C, THF-H_2O; (b) K_2CO_3, MeOH; (c) Bu_4NF, THF; (d) $MeSO_2Cl$,Et_3N, -50 °C, CH_2Cl_2 ; (e) PPh_3, i-PrO_2C-N=N-CO_2Pr-i, MeCOSH, 0 °C, THF; (f) $LiAlH_4$, 0 °C, ether; (g) 50% NaOH, **125**, Oct_4NBr, 40 °C, H_2O-Toluene; (h) $KHSO_5$, 2 min, -5 °C, MeOH.

Scheme 10. Syntheses of **66** and **71**.

addition of aqueous NBS to **122**,[44] dehydrobromination and deprotection to **124** and mesylation to **125** (Scheme 10). Thiol **128** was prepared from the corresponding alcohol **126**.[42] Coupling of **125** and **128** to **66** was achieved in 80% yield. Oxidation of **55** with $KHSO_5$ in MeOH gave sulfoxide **60** in 82% yield. The structures of **66** and **71** were confirmed by ^1H NMR. Thus, the ^1H NMR spectrum of **66** showed a triplet at δ 2.70 (J = 6.2 Hz) for the oxirane hydrogen (C-3) and two overlapping triplets at δ 2.50 and 2.48 (J = 7.7 Hz) for the four hydrogens on C-18 and C-20 while the ^1H NMR spectrum of **70** displayed a multiplet at δ 2.62 for the four hydrogens on C-18 and C-20.

2.3. Biological Studies.

2,3-Oxidosqualene analogs **63-71** were examined for their ability to inhibit *C. albicans* and rat liver OSC in cell-free extracts as well as cholesterol biosynthesis in intact MDBK cells (Table 2). These biological studies were performed by Dr. N. H. Georgopapadakou at Hoffmann-La Roche Inc. in Nutley, New Jersey and Dr. P. G. Hartman at F. Hoffmann-La Roche Ltd. in Basel, Switzerland. Comparison of activity is best measured in cell-free systems since adventitious adsorption and differing penetration into cells distorts relative activities in whole-cell systems. All 2,3-OS analogs prepared in this study possess sufficient conformational flexibility to assume conformations that should allow them to be recognized by OSCs as substrates.

The 2,3-OS analogs **63** and **67**, which situate sulfur at the position normally occupied by C-6, designed to interfere with the formation of the A ring, showed more potent inhibition in fungal and rat liver OSC [For **63**, IC_{50} = 0.069 μM, *C. albicans;* 0.0084 μM, rat liver, (Table 2)] than previously reported inhibitors.[26] It is remarkable that thioether **63** is 2.3 fold more potent in *C. albicans* and 145 fold more potent in rat liver cyclase than the sulfoxide **67**. If **63** and **67** are acting as

Table 2. Evaluation of compounds **63-71** as inhibitors of OSC and cholesterol biosynthesis.

	IC_{50}[a] (μM)		
Compounds	C. *albicans* cyclase (cell-free)	Rat liver cyclase (cell-free)	MDBK[b] (intact-cell)
63	0.069	0.0084	1.16
64	0.069	0.55	2.31
65	2.24	5.15	76.2
66	0.0023	0.00082	4.62
67	0.16	1.22	1.11
68	0.26	ND[e]	0.45
69	3.90	1.73	0.87
70	5.41	7.78	0.87
71	0.065	0.29	0.45
Keto[c]	ND[e]	ND[e]	0.94
Nafti[d]	ND[e]	ND[e]	10.4

[a]: molar concentration of inhibitor required to reduce enzyme activity by 50%.

[b]: Madin darbin bovine kidney cells.

[c]: Ketoconazole [51] (14α-demethylase inhibitor).

[d]: Naftifine [52] (squalene epoxidase inhibitor).

[e]: not determined.

unmodified substrate analogs, these relative activities reveal for **63** a strong interaction with OSC binding sites normally stabilizing the initially formed cation **3**.

The 2,3-OS analogs **64** and **68** possess sulfur at the position normally occupied by C-10 and were designed to interfere with B ring formation. Again, thioether **64** was more potent (3.7-fold in *C. albicans*) than its sulfoxide **68**. For *C. albicans* OSC, **64** was as potent as **63** which qualified it as a more potent inhibitor than any previously reported[26] (Table 2). In rat liver OSC, **64** was 65 fold less potent than **63**. Thioether **64** was the only thioether examined in this study to be more active in *C. albicans* than rat liver cyclase (~8 fold). The stronger inhibition of **63** compared to **64** in *C. albicans* OSC is consistent with stronger interaction of cation **3** compared with **4** which would be expected if ring A is formed more slowly than ring B.

The 2,3-OS analogs **65** and **69** possess sulfur at the position normally occupied by C-14 and were expected to interfere with the *anti*-Markovnikov cyclization leading to ring C. Inhibition observed for thioether **65** in the cell-free *C. albicans* OSC revealed that it was 32 fold less potent than **63** or **64** and 613 fold less potent than **63** for rat liver cyclase (Table 2). The low activity of **65** compared to **63** or **64** could be due to misplacement of the sulfur in **65**. Thus, formation of ring C could proceed *via* cyclization to a five-membered ring and formation of a tertiary carbocation followed by a 1,2 shift.[4e,21h] In this event, the sulfurs in **65** and **69** should be more effective at the position normally occupied by C-15. The van Tamelen and Krief groups have examined this question by cyclization of 2,3-OS analogs lacking Δ^{18} unsaturation and possessing *Z*-geometry of Δ^{18} (Figure 14). These derivatives cyclized to produce a five-membered ring C suggesting preferential cation formation at C-15 if one interfered with subsequent ring D formation.[4e,21h] For both *C. albicans* and rat liver cyclases, thioether **65** exhibited activity similar to the corresponding sulfoxides **69** and **70** with **69** being slightly

more inhibitory (~3 fold) than **65** in the latter. This is the only case in which a sulfoxide was more inhibitory than the corresponding thioether and is consistent with the action of both 2,3-OS analogs as unmodified substrate mimics.

The 2,3-OS analogs **66** and **71**, possessing sulfur at C-19, were designed to interfere with the formation of the protosterol cation **6** (Figure 1). Analog **66** is the most powerful inhibitor prepared in this study [IC_{50} = 0.0023 μM, *C. albicans;* 0.00082 μM, rat liver, (Table 2)]. This 2,3-OS mimic is some 30 fold more potent than **63** or **64** and 974 fold more potent than **65** in *C. albicans* and 102, 670 and 6,280 fold more active than **63**, **64** and **65**, respectively, for rat liver cyclase. It is the most powerful OSC inhibitor reported to date and suggests 2,3-OS modifications in the region of C-19 as good candidates for further investigation.

Sulfoxides were generally less active than their corresponding thioethers. In *C. albicans* cyclase, sulfoxide analogs of the thioethers were 2 to 4 fold less potent except for **71** which was 28 fold less potent than its thioether analog **66**. In rat liver cyclase, the differences between thioethers and the corresponding sulfoxides were more striking. Thioether **63** was 145 fold more active than sulfoxide **67** whereas differences between **65** and its sulfoxides **69** and **70** were less than 3-fold. The largest difference between a thioether and the corresponding sulfoxide was observed for **66** *vs* **70**. The former was 353 fold more active than the latter for the rat liver cyclase. An interesting feature of sulfoxide activity is the relative activity of diastereoisomers **69** and **70**. In rat liver cyclase, they exhibited noticeably different activities which actually bracket the activity of the corresponding thioether (0.33 and 1.5 fold differences compared with **65**). We attribute the activity of the sulfoxides to coulombic interactions of the electron deficient sulfurs with the sites normally stabilizing cationic intermediates **3-6** (Figure 1). It is noteworthy that **71**, which possesses a sulfoxide in place of C-19 of 2,3-OS, exhibited the strongest inhibitory activity of any sulfoxide in both *C. albicans* and rat liver cyclases.

The superior activity of thioethers in comparison to their sulfoxide analogs is puzzling if both types of analogs behave as unmodified substrate mimics. In the event that **63-66** are cyclized to the sulfonium ions **77-80** (Scheme 1) or close structural relatives, then one would expect the observed stronger inhibition for thioethers than for the corresponding sulfoxides. The mode of action of **66** with pig liver OSC[13a] has been probed in preliminary kinetic studies in collaboration with Professor G. D. Prestwich at State University of New York at Stony Brook. With this mammalian OSC **66** acts as a competitive inhibitor and its inhibition is reversible in contrast to 29-methylidene-2,3-OS.[14] This result suggests that **66** is recognized as a pseudo substrate and competes with the substrate for the same binding site. This issue will be discussed in the next chapter.

Activity of **63-71** in intact mammalian MDBK cells was significantly lower than in the cell-free systems. Thioethers **63-65** exhibited the same relative order of activity in this system as they did in the cell-free systems. Thus, **63** was more potent than **64** and the latter was more potent than **65**. In MDBK cells **66** was observed to be less active than both **63** and **64**. Indeed, in this system sulfoxides were either as active as (**63** *vs* **67**) or more active (**64** *vs* **68**, **65** *vs* **69** and **70**, **66** *vs* **71**) than their thioether analogs. We suspect the lower activity of **63-71** in MDBK cells is due to low permeability of the thioethers relative to the sulfoxides and possibly also to adventitious adsorption. Interestingly, none of the compounds were toxic to MDBK cells up to 100 μg/mL. Several of the compounds prepared in this study were more potent inhibitors of cholesterol synthesis in MDBK cells than Ketoconazole[51] (a 14α-demethylase inhibitor) and all except **65** were more active than Naftifine[52] (a squalene epoxidase inhibitor) (Table 2).

The 2,3-OS analogs **63-71** were examined for their antifungal activity against *C. albicans*. All exhibited MIC values over 100 μg/mL compared to an MIC of 20 μg/mL for the commercial antifungal agent, Ketoconazole. Furthermore, none

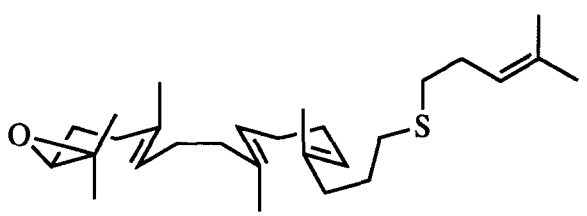
inhibited ergosterol biosynthesis in growing *C. albicans* cells up to 100 μg/mL. We suspect that the poor activity of **63-71** in growing *C. albicans* cells is due to low permeability of these compounds.[31b]

In summary, efficient routes have been developed for the preparation of 2,3-oxidosqualenes containing sulfur or sulfoxide replacing those carbons considered to develop cationic character during the OSC mediated cyclization of **1**. Thioethers **63-66** showed powerful inhibitory activity in fungal and mammalian OSCs while the corresponding sulfoxides were less potent. It is difficult to rationalize the superior inhibition of thioethers **63-66** compared to the sulfoxides **67-71** unless one assumes cyclization of the former to sulfonium ions **77-80** (Scheme 1) or structural relatives. The relative activities of **63-66** suggest that placement of heteroatoms near C-19 of 2,3-OS results in the most significant inhibition. Thioether **66** exhibits IC_{50} values of 0.0023 and 0.00082 μM for *C. albicans* and rat liver cyclase, respectively, and is the most potent OSC inhibitor reported to date. Preliminary kinetic studies of the inhibition of pig liver OSC of **66** reveal that it is a competitive inhibitor. This is consistent with its activity in an unmodified form and suggests that **66** acts as a substrate mimic. The kinetics of inhibition of **66** in comparison with **72** (in which a sulfur atom replaces C-18 of **1**) will be discussed in the next chapter.

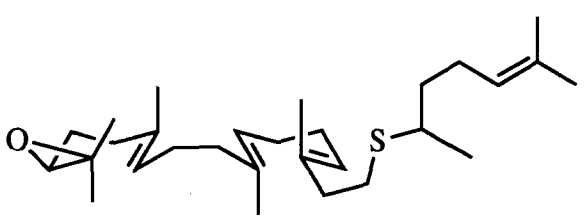
Chapter 3

Synthesis of 18-Thia-19-dehydro-2,3-oxidosqualene (72) and Kinetics of Inhibition by 66 and 72

In view of the observation that substrate analog **66** with sulfur replacing C-19 of **1** is a potent OSC inhibitor (Chapter 2), structural modification near C-19 of **1** became of interest for the development of new OSC inhibitors. An additional sulfide analog, **72**, in which sulfur replaced the olefinic carbon C-18 of **1** was prepared to examine its inhibitory power. This chapter outlines the synthesis of 18-thia-19-dehydro-2,3-OS (**72**) and the kinetics of its inhibitory action on OSC from fungal (*C. albicans*) and mammalian (rat and pig liver) sources.



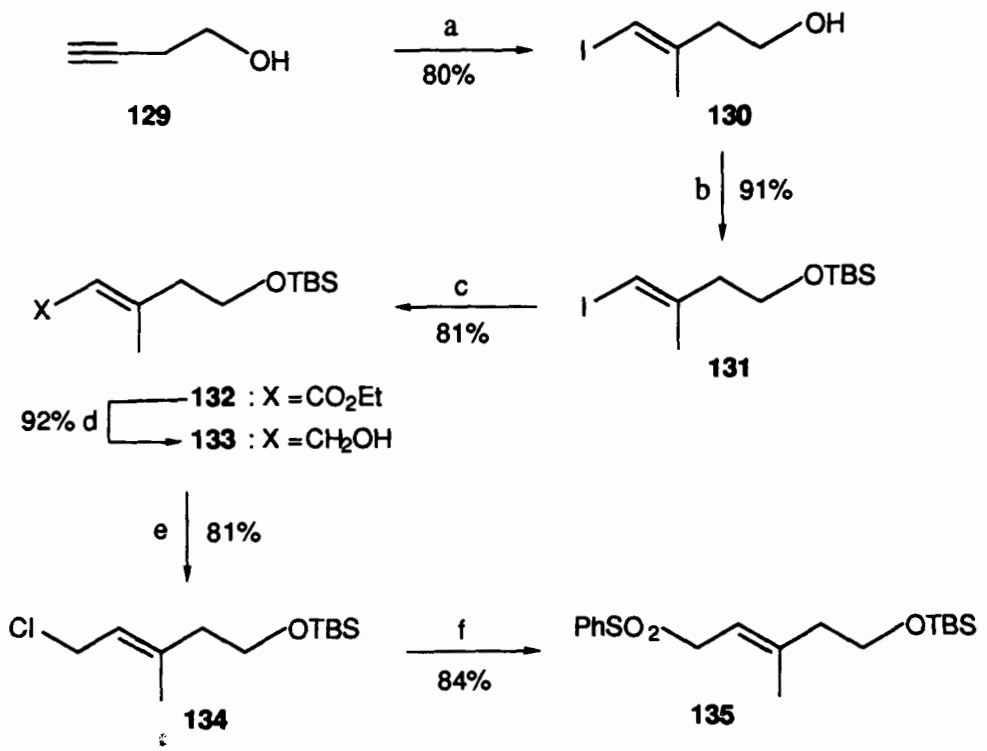
66



72

3.1. Synthesis of 72.

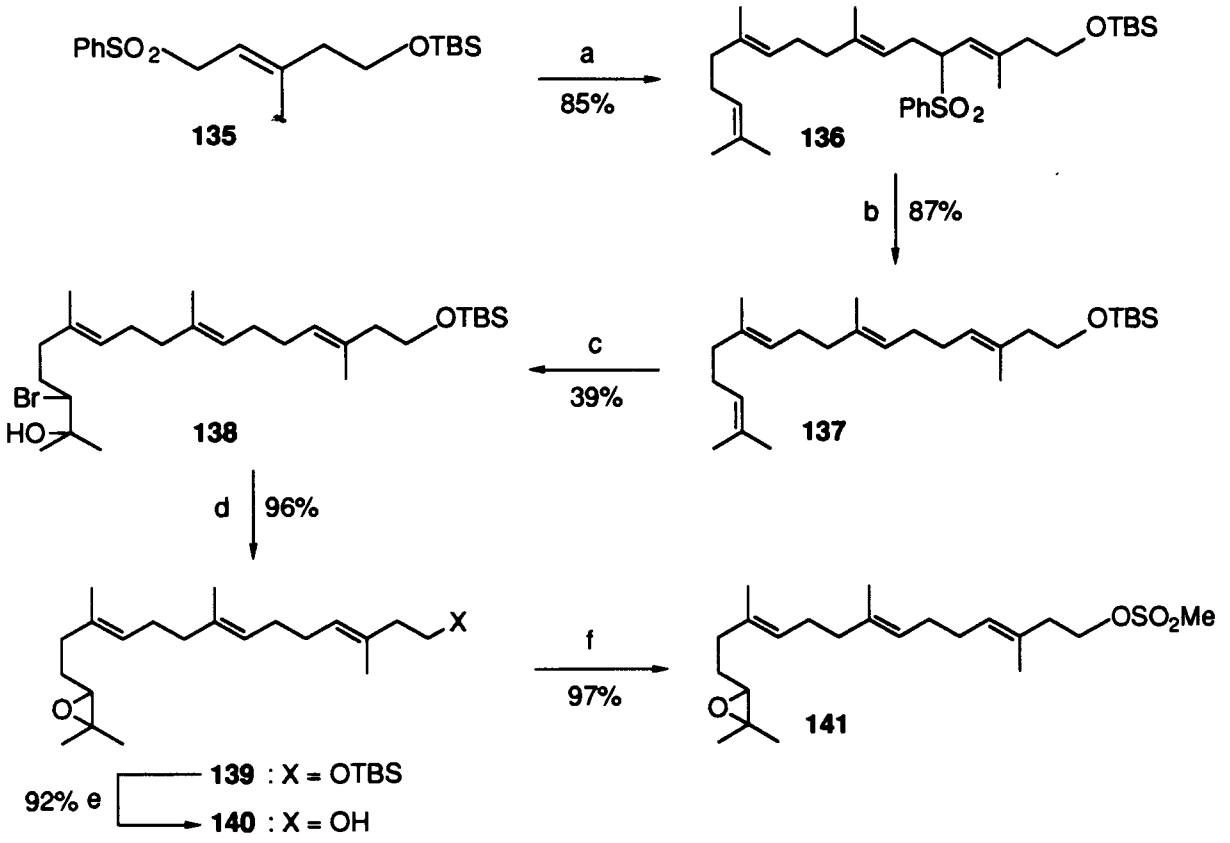
Chemistry similar to that used for the preparation of sulfides **63-66** was applied to the synthesis of **72**. Thus, analog **72** was synthesized by coupling triene mesylate **141** and thiol **144** (Scheme 13). Synthesis of mesylate **141** commenced with carboalumination of 3-butynol (**129**) followed by iodine trapping to give 4-iodo-3-methyl-3(*E*)-butenol (**130**)[40] in 80% yield (Scheme 11). Conversion of alcohol **130** to its protected form **131** followed by addition of 1.1 equiv. *n*-BuLi at -78 °C to generate the vinylic anion and addition of ethyl chloroformate gave the conjugated ester **132** in 74% yield over two steps. Reduction of ester **132**[47] with DIBAL-H gave allylic alcohol **133** which reacted with NCS-DMS[41] complex to give allylic chloride **134**. Reaction of chloride **134** with $NaSO_2Ph$ in DMF gave sulfone **135**[47]



(a) $Zr(Cp)_2Cl_2$, $AlMe_3$, then I_2, CH_2Cl_2; (b) TBSCl, DMAP, Et_3N, CH_2Cl_2; (c) *n*-BuLi, -78 °C, THF, then $ClCO_2Et$; (d) DIBAL-H, ether; (e) NCS-DMS, CH_2Cl_2; (f) $NaSO_2Ph$, DMF.

Scheme 11. Synthesis of allylic sulfone **135**.

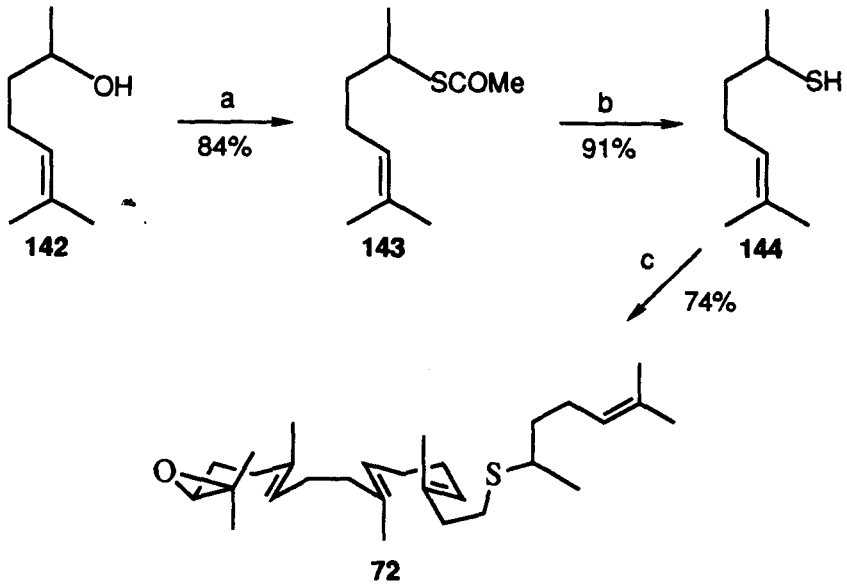
in 84% yield (Scheme 11). Metalation of **135** with 1.1 equiv. *n*-BuLi generated the allylic anion which was alkylated by farnesyl bromide (**105**) to give tetraene-sulfone **136** in 85% yield[47] (Scheme 12). Reductive elimination of the benzenesulfonyl group of sulfone **136** with Li in $EtNH_2$ at -78 °C gave the required protected alcohol **137**[47] in 87% yield. Reaction of **137** with 1 equiv. NBS in THF-H_2O (3.8:1)[44] gave terminal bromohydrin **138** which was converted to the terminal oxirane **139** by K_2CO_3 in MeOH in 37% yield over two steps. Removal of the silyl protecting group



(a) *n*-BuLi, -78 °C, THF, then **105**; (b) Li, $EtNH_2$, THF; (c) 1.0 eq. NBS, 0 °C, THF-H_2O; (d) K_2CO_3, MeOH; (e) Bu_4NF, THF; (f) $MeSO_2Cl$, Et_3N, -50 °C, CH_2Cl_2.

Scheme 12. Synthesis of mesylate **141**.

of **139** followed by mesylation of the resulting epoxy alcohol **140** provided the corresponding mesylate **141** (Scheme 12). The required thiol **144** was prepared in two steps in an overall 76% yield from thioacetate **143** which was obtained from alcohol **142** by the Mitsunobu reaction[42] (Scheme 13). Synthesis of **72** involved coupling of **141** and **144** in 74% yield (Scheme 13) under phase-transfer conditions[35] as described for the syntheses of **63-66** in Chapter 2.



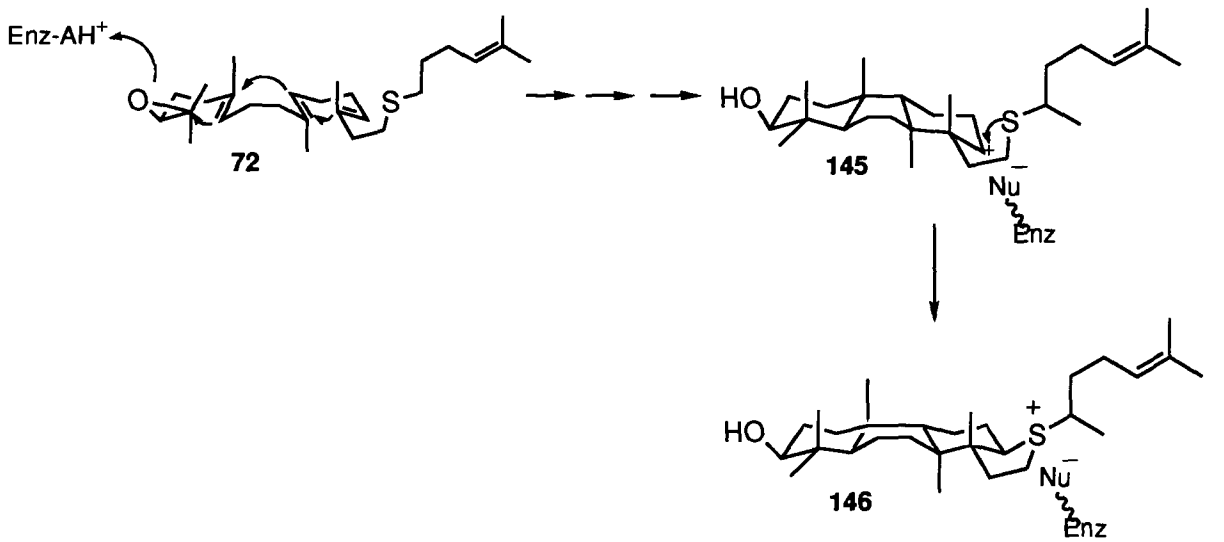
(a) PPh_3, i-PrO_2C-N=N-CO_2Pr-i, MeCOSH, 0 °C, THF; (b) $LiAlH_4$, 0 °C, ether; (c) 50% NaOH, **141**, Oct_4NBr, 40 °C, H_2O-Toluene;

Scheme 13. Synthesis of **72**.

3.2: Enzyme kinetics of inhibition by 66 and 72.

The 2,3-OS analog **72** was tested for its ability to inhibit *C. albicans* and rat liver OSC in cell-free extracts and showed superior activity against these cyclases (biological tests were conducted by Dr. P. G. Hartman at F. Hoffmann-La Roche Ltd. in Basel, Switzerland.). In *C. albicans*, **72** displayed an IC_{50} value of 0.00022 μM which is nearly 10 fold more potent than the previously known best inhibitor **66**

(IC_{50} = 0.0023 μM, Chapter 2, Table 2) for the cyclase. Similarly, analog **72** showed an IC_{50} value of 0.00008 μM which is 10 fold more potent than **66** (IC_{50} = 0.00082 μM) in rat liver cyclase. These biological results confirm that situating sulfur near C-18 and C-19 of the 2,3-OS skeleton yields analogs with significant inhibitory power and suggest future guidelines for the development of new substrate analog inhibitors of OSC. Because both **66** and **72** were the most potent inhibitors reported to date, we undertook a kinetic study. The initially proposed mode of action was that either or both **66** and **72** bound to OSC and were cyclized by the enzyme to form sulfonium ions which complexed with the enzyme (Schemes 1 and 14). Alternatively, **66** and **72** could act as unmodified substrate analogs. If the first mechanism prevailed, we expected that both **66** and **72** would



Scheme 14. Proposed mechanism of inhibition of OSC by substrate analog **72**.

exhibit time-dependent[53] inhibition. With this in mind, we collaborated with Dr. G. D. Prestwich at State University of New York at Stony Brook, NY, in a study of the inhibition kinetics[14,54] of **66** and **72**. In purified pig liver cyclase, both **66** and **72** have comparable IC_{50} values of 1.0 μM and 2.3 μM, respectively. Both **66** and **72** showed time-dependent inactivation of the cyclase (Figure 20-21) exhibiting rate constants of inactivation (k_{inact}) of 0.00014 min^{-1} and 0.06 min^{-1}, respectively.[14,53] This results reveals that **72** has a much faster inactivation rate than **66**. Preliminary kinetic studies of the effect of **72** on purified pig liver OSC revealed that **72** is a competitive inhibitor (Figure 22). Similar kinetic behavior was found for **66** (figure not shown). The irreversible inhibition of pig liver OSC by **66** and **72** was studied in comparison with the known irreversible inhibitor

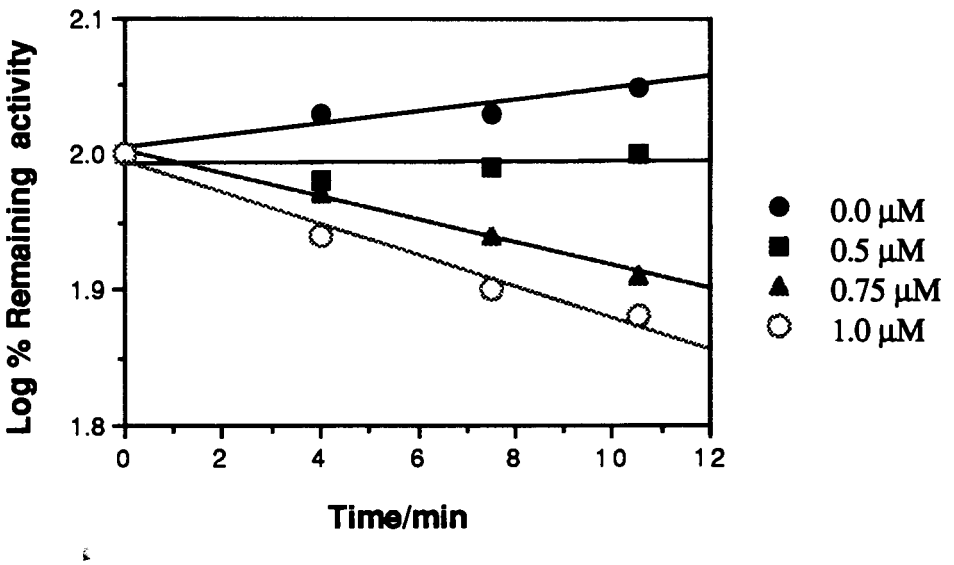


Figure 20. Time dependency of inhibition by **66**.

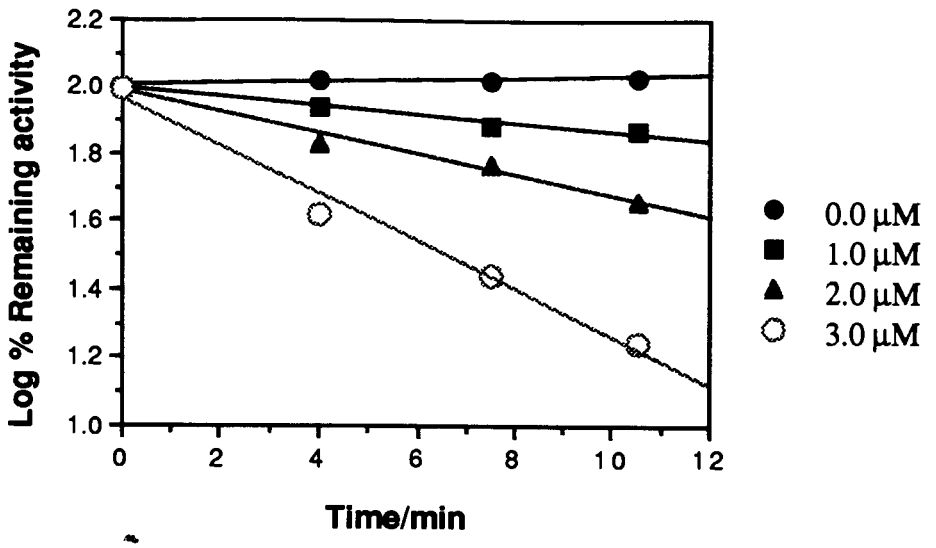


Figure 21. Time dependency of inhibition by **72**.

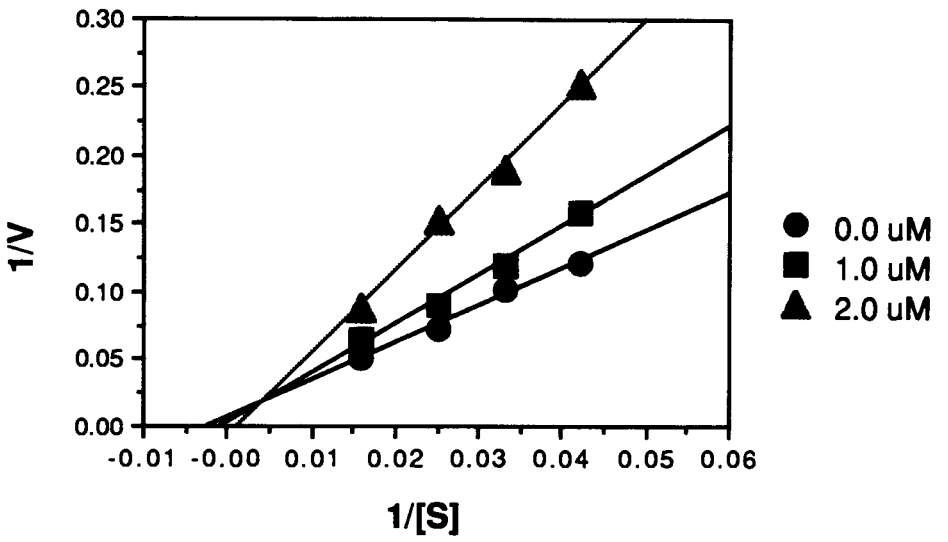


Figure 22. Lineweaver-Burk plot of **72** for competitive inhibition.

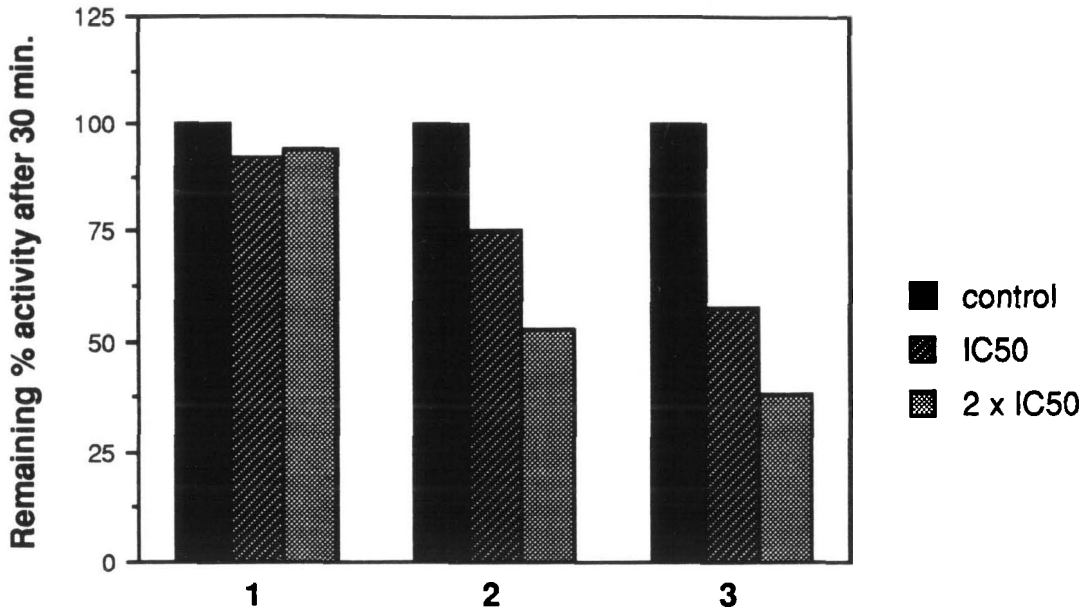


Figure 23. Irreversible inhibitory study of pig liver OSC by **66, 72** and 29-MOS.

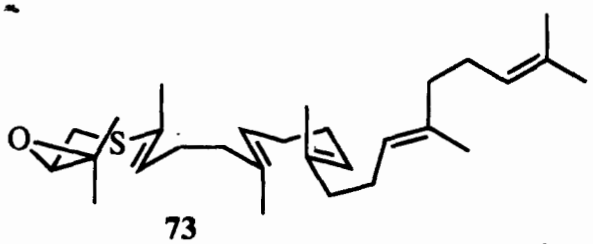
29-MOS.[13a] Thus, inhibitors **66**, **72** and 29-MOS (at the three concentrations 0, 1 x IC_{50}, and 2 x IC_{50}) were incubated with pig liver OSC at 37 °C for 30 min and the non-bound inhibitor was removed by adsorption of protein on a DEAE-sephacel column.[55] The remaining enzymatic activity of eluted protein for pig liver OSC was re-assayed using [^{14}C]-2,3-OS. In this study (Figure 23), **72** and 29-MOS showed significant ability to decrease the activity of pig liver OSC while **66** showed no evidence of irreversible inhibition. With the information that **72** displayed time-dependent inhibition and **66** showed very weak time-dependent inhibition (Figures 20-21), we reasoned that **72** may act as a mechanism-based inhibitor and **66** as an unmodified substrate analog. Incubation of radio-labelled **66** and **72** with OSC

would establish covalent binding to the enzyme and the formation of released cyclic product. Indeed, the synthesis of ^{3}H-labeled **72** is underway.

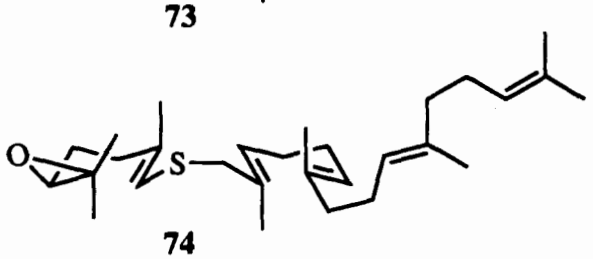
Chapter 4

Syntheses of sulfur substituted 2,3-oxidosqualene analogs 73-76 and evaluation of their inhibitory activities

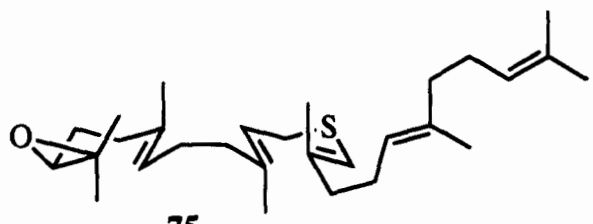
This chapter describes the syntheses and inhibitory activity of 2,3-OS analogs **73-76** in which sulfur replaces carbons C-5, C-8, C-13 or C-20 in **1**. A new methodology was developed for the preparation of **74** and **75**. Analogs **73-76** were expected to be mechanism-based inhibitors of OSC.



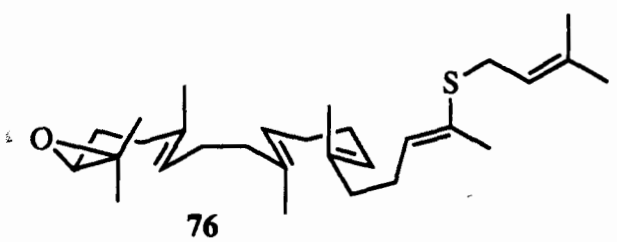
73



74



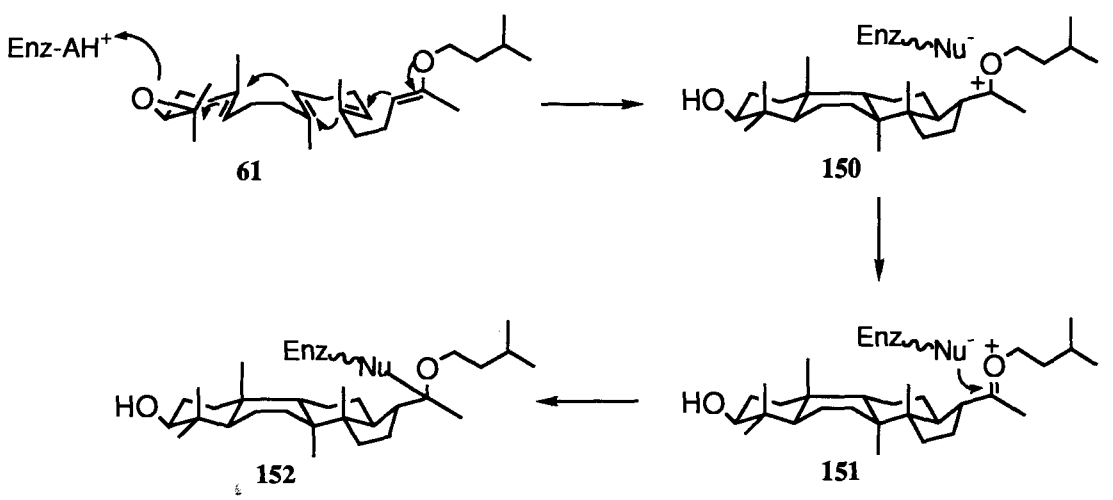
75



76

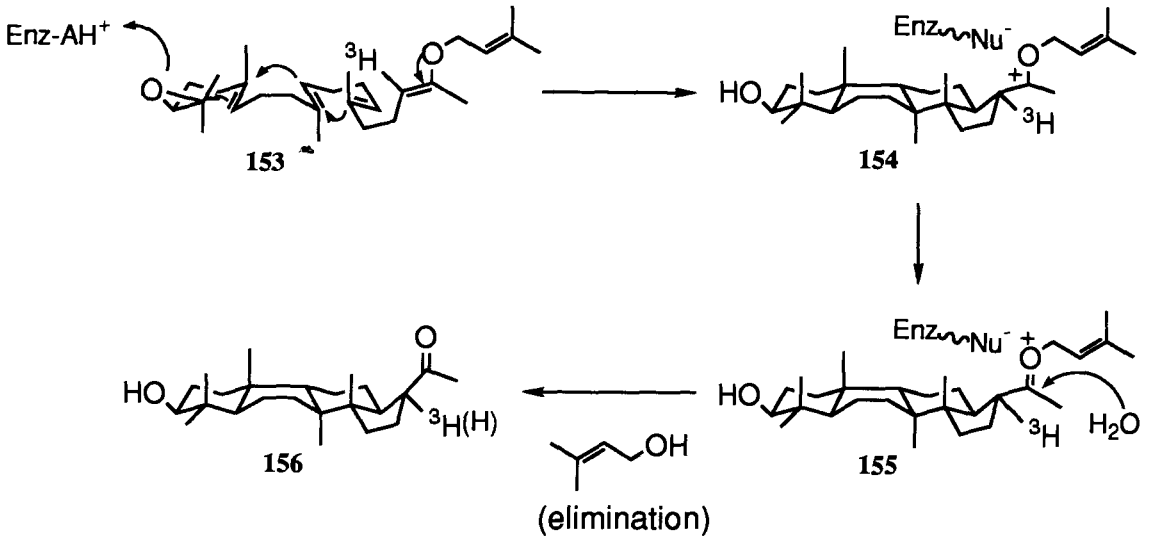
4.1. Rationale.

The use of vinylic sulfides **73-76** as possible mechanism-based inhibitors of OSC arises from work of Ceruti *et al.* on a vinylic ether analog of 2,3-OS[30f] and of Xiao and Prestwich on (^{3}H)-29-methylidene-2,3-OS.[14] 22,23-Dihydro-18(*E*)-20-*oxa*-2,3-oxidosqualene (**61**), a vinylic ether with oxygen replacing C-20 of **1**, was synthesized by Ceruti *et al.* as a mechanism-based inhibitor of OSC to probe the existence of protosterol **6**. It was originally thought that **61** would be recognized and cyclized by OSC to the C-20 cation intermediate **150** which is stabilized by resonance with an oxocarbenium ion **151** (Scheme 15). Cation **151** was expected to undergo nucleophilic attack by the amino acid residue which is responsible for stabilization of the C-20 protosterol cation **6** and to form a covalent bond within the active site of the enzyme leading to irreversible inhibition. However, **61** was a very weak competitive inhibitor of rat liver OSC but not, as expected, an irreversible



Scheme 15. Hypothetical mechanism of inhibition of OSC by 22,23-dihydro-18(*E*)-20-oxa-2,3-OS (**61**).

inhibitor. This result led to the postulation that the presumed oxocarbenium ion **151** may undergo hydrolysis faster than nucleophilic attack by an amino acid. If this were the case, one would expect to obtain the hydrolysis product, the corresponding tetracyclic ketone. Ceruti and his coworkers did not provide evidence for such a cyclized product. Corey and Virgil recently demonstrated cyclization of a radiolabelled derivative of **153** to protosterol **156**.[25c] The ^{3}H-label



Scheme 16. OSC mediated cyclization of (18*E*)-20-oxa-2,3-oxidosqualene (**153**) to its corresponding tetracyclic ketone protosterol (**156**).

of **153** allowed Corey to locate the cyclization product. Thus, the existence of a ^{3}H-labelled protosterol tetracyclic ketone **156** was confirmed as a product of yeast OSC mediated cyclization of **153** (Scheme 16). This result implied that oxocarbenium ion **155** did occur as an intermediate in the reaction and this cation could be attacked by water producing a hemiacetal which released isopentenyl alcohol to give tetracyclic ketone **156**. The stereochemistry of **156** revealed that the side chain at C-17 (steroid numbering) of **6** is β–oriented.

In a separate study, Xiao and Prestwich[14] introduced an olefinic unit adjacent to C-19 (*pro*-C-20 cation, steroid numbering) of **1** (Figure 9, Chapter 1) to stabilize the presumptive protosterol cation **6**. The cyclization of (^{3}H)-29-methylidene-2,3-OS (29-MOS) by rat liver OSC yielded initially 21-methylene protosterol cation **9** (Figure 2, Chapter 1) and subsequently the vinylic lanosterol **157** (Figure 24) through the usual series of 1,2-hydride/ methyl migrations and proton loss, or cation **9** was stabilized by reaction with an active site nucleophile of the enzyme (Figure 2, Chapter 1).[13b] In the case of the latter process, species **10** was formed by covalent bonding, causing the irreversible inhibition of rat liver OSC.[13b]

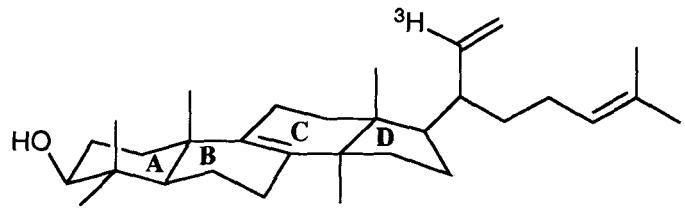


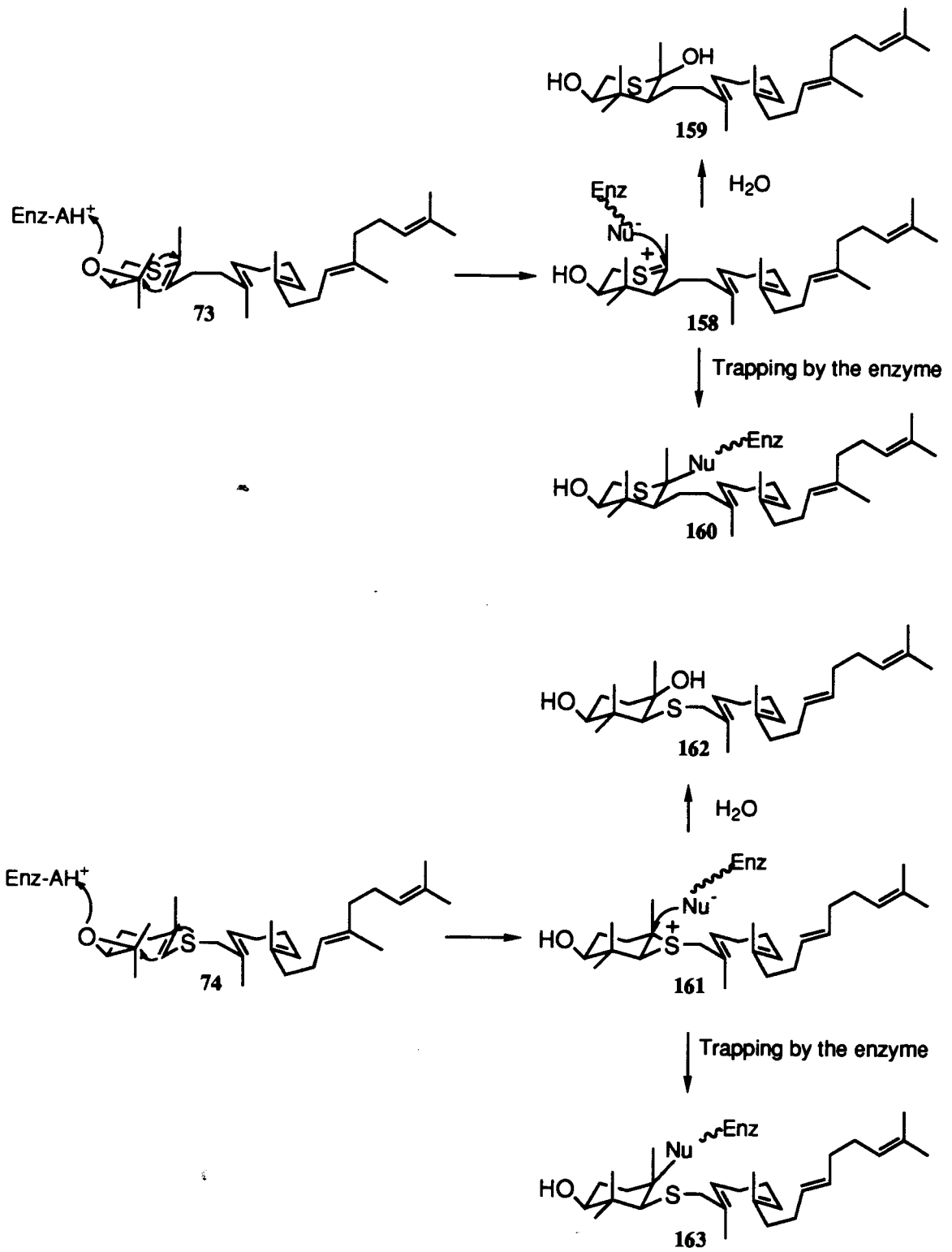
Figure 24. Vinylic lanosterol analog **157** formed *via* OSC-catalyzed cyclization of 29-MOS.

In the present study, sulfur was selected for its ability to stabilize the carbocation.[56-65] Theoretical studies have shown that sulfur is a better π- and σ-donor than oxygen to electron deficient α-carbon.[56,57] Thus, we prepared four analogs of 2,3-OS (**73-76**) in which sulfur was located at a position α or β to those carbons presumed to possess a positive charge during the normal cyclization of **1**. Our presumption was that these 2,3-OS analogs would cyclize to the point of formation of resonance-stabilized thiocarbenium ions.[59] We reasoned that this stabilization would decrease the reactivity of the cation and interrupt further

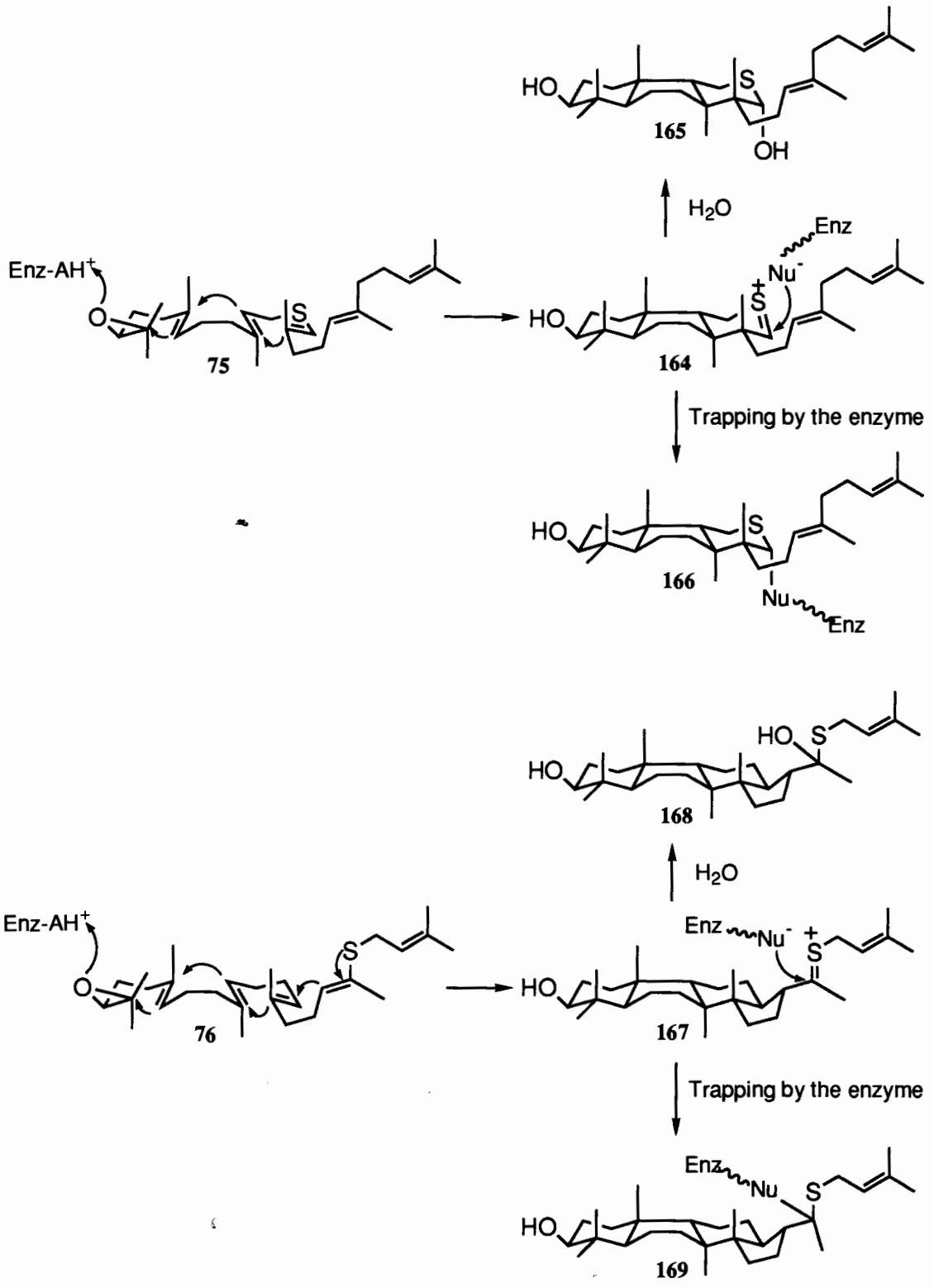
cyclization. Also, thiocarbenium ions are more stable to hydrolysis [63,65] than the corresponding oxocarbenium ions derived from vinylic ethers. This might provide a sufficiently long life to the thiocarbenium ions to allow their attack by nucleophilic amino acids in the active site. Our hope was that the presumptive thiocarbenium ions would either react with nucleophilic amino acids to irreversibly bond to OSC as in the case of 21-methylene protosterol cation **9** or be captured by water to form an alcohol (Scheme 17-18). Both pathways could provide opportunities to gain further insight into the involvement of carbocationic intermediates in the OSC-mediated cyclization of **1**.

Analogs **73** and **74** in which sulfur replaces C-5 and C-8 of **1**, respectively, were designed to interfere with ring B formation (Scheme 17). Analogs **75** and **76** in which sulfur replaces C-13 and C-20 of **1**, respectively, were designed to interrupt ring D formation or the rerrangement of protosterol cation **6** (Scheme 18).

The syntheses of **73-76** required construction of trisubstituted (*E*)-vinylic sulfides using conditions which tolerate the sensitive epoxy group and a highly unsaturated backbone. Previous methodologies for preparation of vinylic sulfides have usually produced mixtures of *E* and *Z* stereoisomers.[66-68] Stereospecific methodologies such as catalytic hydroboration-coupling[69] or metal-catalyzed sulfenylation of alkenyl halides[70,71] are not applicable to the present synthesis because of the highly unsaturated backbone of the target molecule. A stereospecific method to prepare *Z*-divinylic sulfides involves cross-coupling of cuprous thiolates with alkenyl halides but requires high temperature and gives relatively low yields.[72] Two synthetic methods are preferred for the



Scheme 17. Hypothetical inhibition of OSC by 6(*E*)-5-thia-2,3-OS (**73**) and 6(*E*)-8-thia-2,3-OS (**74**).



Scheme 18. Hypothetical inhibition of OSC by14(*E*)-13-thia-2,3-OS (**75**) and 18(*E*)-20-thia-2,3-OS (**76**).

present synthetic targets. One involves the modified Wittig-Horner reaction (see, for example, Scheme 25) that has been developed by Dodd in this laboratory.[31h] This method originated from the work of Ceruti *et al.*[30f] and later Corey *et al.*[25b] for the synthesis of *E*-vinylic ether analogs of 2,3-OS. Its disadvantage is that condensation of Wittig-Horner reagents with aldehydes generally produces mixtures of *syn* and *anti* diastereomers. Only the *syn* isomer can be transformed to the required *E*-vinylic sulfides. Although the syntheses of **73** and **76** have been carried out by this method and proven to be satisfactory (section 4.3), we sought an improvement. Thus, a new methodology for the stereospecific coupling of trisubsitituted vinylic anions with alkyl 4-methylbenzenethiosulfonates has been developed in the present study. The reaction was first studied by Scholz[73] who used it for preparation of β-keto sulfides by reaction of enolate anions with alkyl 4-methylbenzenethiosulfonates. We reasoned that by preparation of an *E*-vinylic halide, its conversion to the corresponding vinylic anion and reaction with an appropriate alkyl 4-methylbenzenethiosulfonate well-defined *E*-vinylic sulfides could be produced. The following two sections describe the use of this new coupling as well as the Wittig-Horner method for the syntheses of **74-76**.

4.2. Syntheses of 74 and 75.

Retrosynthetic analysis revealed that both **74** and **75** can be assembled from allylic sulfonium ion equivalents and vinylic anion synthons (Figure 25). The synthesis of **74** commenced with conversion of available alcohol **95** (Chapter 2) by Swern oxidation[74] to aldehyde **170** in 93% yield (Scheme 19). Aldehyde **170** was then treated with (carbethoxyethylidene)triphenyl phosphorane in refluxing methylene chloride to generate the desired (*E*)-α,β-unsaturated ester **171** in 90% yield (*E*-isomer > 97%). The *E*-geometry of **171** was confirmed by ^{1}H NMR which

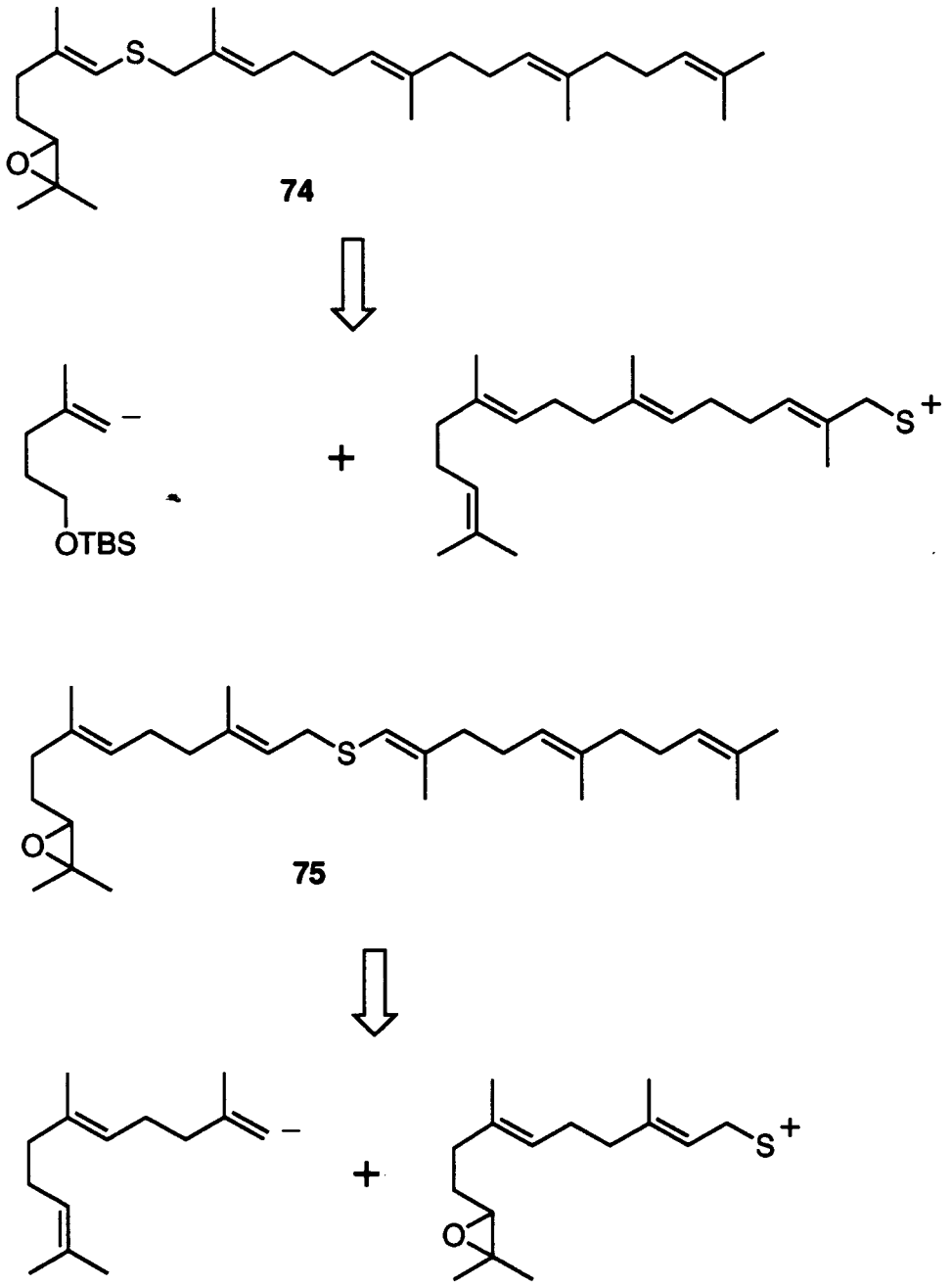
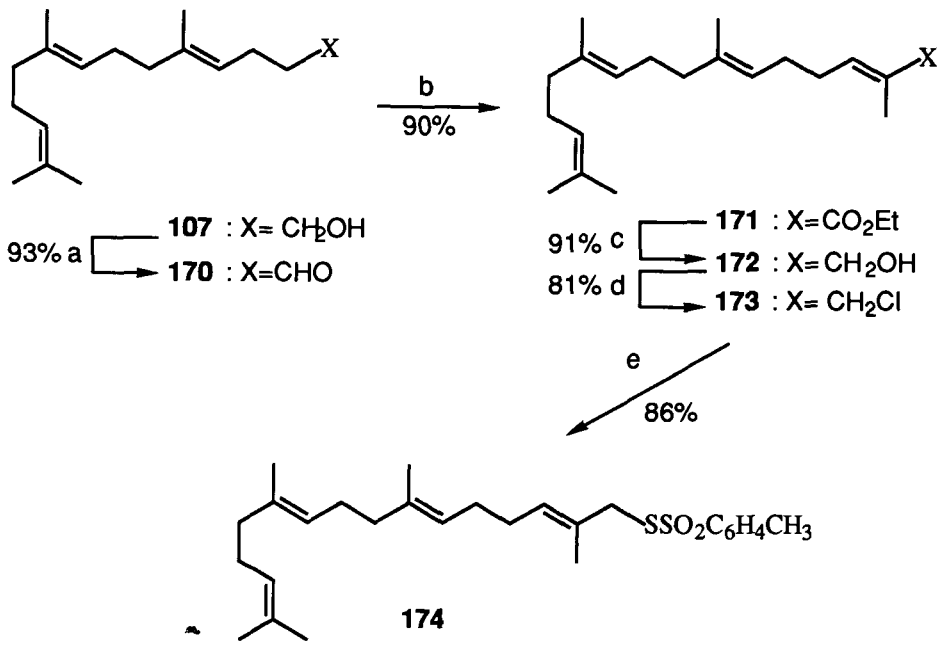


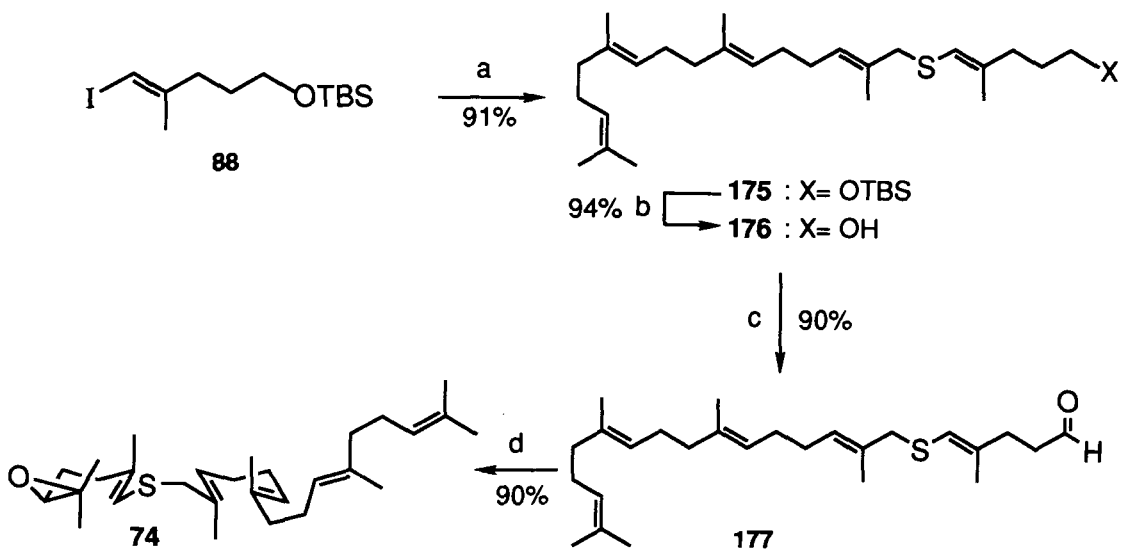
Figure 25. Retrosynthetic analyses of **74** and **75**.



(a) Swern oxidation; (b) $Ph_3P=C(CH_3)CO_2Et$, CH_2Cl_2; (c) DIBAL-H, Et_2O;

(d) NCS, DMS, CH_2Cl_2; (e) $KSSO_2C_6H_4CH_3$, DMF.

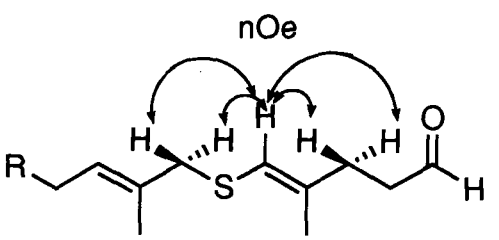
Scheme 19. Synthesis of thiosulfonate **174**.



(a) *n*-BuLi, -78 °C, then **174**, THF; (b) Bu_4NF, H_2O, THF; (c) Swern oxidation; (d) $Ph_2S(i\text{-}Pr)BF_4$, *t*-BuLi, THF.

Scheme 20. Synthesis of 6(*E*)-8-thia-2,3-oxidosqualene (**74**).

revealed a triplet of quartets at δ 6.76 (J = 7.3, 1.4 Hz) for the hydrogen attached to C-3. Reduction of **171** with DIBAL-H gave an allylic alcohol **172** which was converted with NCS-DMS[41] to allylic chloride **173** in 74% yield over two steps. The latter was converted to **174** in excellent yield by reaction with potassium *p*-toluenethiosulfonate in DMF.[73a] The required vinylic anion synthon **88** was prepared by zirconium-catalyzed carboalumination of 4-pentyn-1-ol (**86**) followed by iodine trapping and alcohol protection as described in Chapter 2. Conversion of **88** to **175** (91%) was effected *via* the vinylic lithium intermediate (with *n*-BuLi in THF at -78°C) followed by addition of **174** (Scheme 20). This new coupling reaction is the key step in the generation of the *E*-vinylic sulfide **74**. The structure of **175** was confirmed by ^{1}H NMR which revealed a quartet at δ 5.58 (J = 1.0 Hz) for the vinylic sulfide hydrogen (C-5) and a singlet at δ 3.19 for the two hydrogens on the methylene carbon (C-7) adjacent to sulfur. Assignments were confirmed by decoupling experiments. Deprotection of **175** with Bu_4NF in methylene chloride followed by Swern oxidation[74] gave **177** in 85% yield over the two steps. The ^{1}H NMR nOe difference spectrum of **177** confirmed the stereochemistry of the vinylic sulfide. When the C-5 vinylic hydrogen (δ 5.62, J = 1.0 Hz) was irradiated, no enhancement of the methyl protons attached to C-4



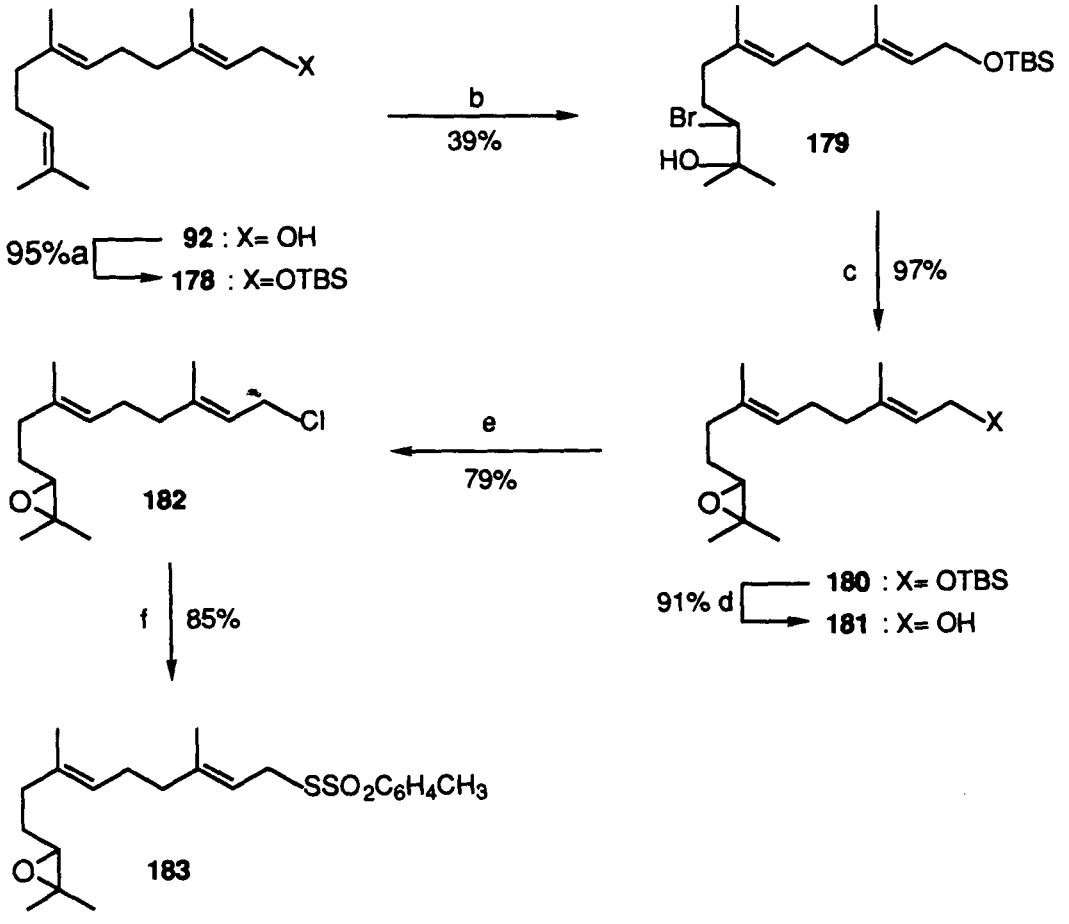
R= (*E*),(*E*)-Farnesyl chain

(**177**)

Figure 26. nOe experiment on **177**.

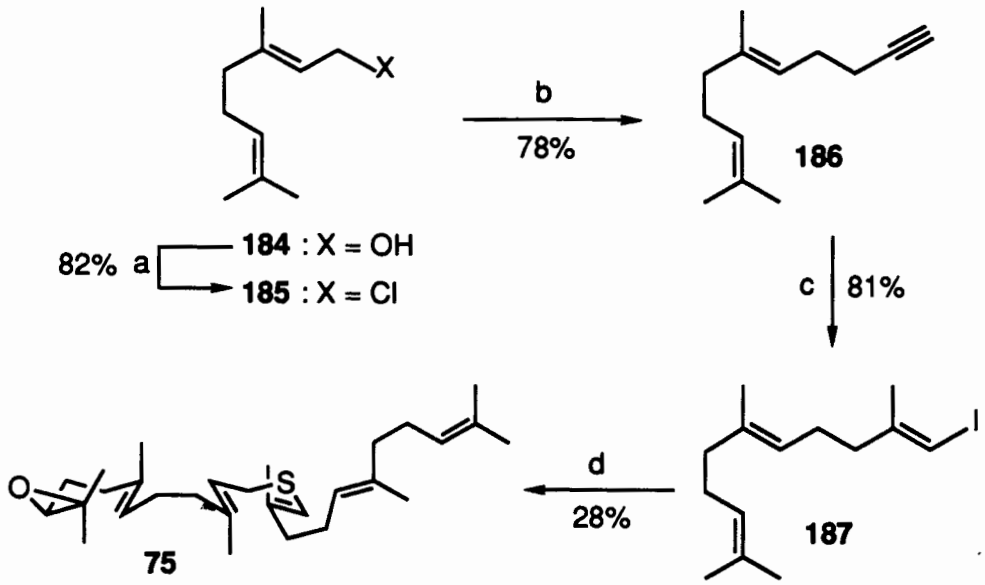
(δ 1.72, J = 1.0 Hz) was observed. In this experiment enhancement of the signal attributed to the C-3 hydrogens (δ 2.37, J = 7.4 Hz) was observed (Figure 26). These results indicate that the methyl attached to C-4 and the C-5 vinylic hydrogen are *trans* to each other while the C-5 vinylic hydrogen and the C-3 hydrogens are *cis*.[75] Generation of the sulfonium ylide of $Ph_2S(i\text{-}Pr)BF_4$ by treatment with *t*-BuLi in THF at -78°C under argon followed by reaction with **177** gave **74** in 90% yield.[76] Preparation of **74** from **95** in 9 steps proceeded with an overall yield of 37%.

The synthesis of **75** commenced with conversion of commercially available (*E,E*)-farnesol (**92**) to **178** in 95% yield. Reaction of the latter with NBS in THF-H_2O[44] followed by treatment with K_2CO_3 in methanol gave **180** in 37% yield over two steps (Scheme 21). Deprotection of **180** with Bu_4NF in THF gave **181** which was converted to the allylic chloride **182** by reaction with NCS-DMS complex[41] (72% over two steps). Reaction of **182** with potassium *p*-toluenethiosulfonate in DMF gave epoxy thiosulfonate **183** in 85% yield.[73a] Reaction of geraniol (**184**) with NCS-DMS complex [41] gave geranyl chloride (**185**)[77] in 80% yield. The latter was then converted to **186** in 78% yield according to the procedure of Hooz.[78] Treatment of **186** with $AlMe_3$ in the presence of $Zr(Cp)_2Cl_2$ followed by treatment with iodine gave **187** in 81% yield[40] (Scheme 22). Reaction of **187** with *n*-BuLi in THF at -78°C under argon followed by addition of **183** gave **75** in 28% yield (7 steps from **92**, overall yield 6.1 %). We believe that the lower yield in this coupling reaction is due to epoxide cleavage by the vinylic anion.[79] The *E*-geometry of vinylic sulfide **75** was again confirmed by an nOe difference experiment. Irradiation of the C-14 vinylic hydrogen (δ 5.64) enhanced the signal of the hydrogens attached to C-16 (δ 2.07) while no enhancement of the signal due to the methyl attached to C-15 (δ 1.73) was observed (Figure 27).[75]



(a) TBSCl, DAMP, Et_3N, CH_2Cl_2; (b) NBS, H_2O, THF, O °C; (c) K_2CO_3, CH_3OH; (d) Bu_4NF, H_2O, THF; (e) NCS, DMS, CH_2Cl_2, -20 °C; (f) $KSSO_2C_6H_4CH_3$, DMF.

Scheme 21. Synthesis of epoxy thiosulfonate **183**.



(a) NCS, DMS, CH_2Cl_2, -20 °C; (b) $CH_2=C=CH_2$, *n*-BuLi, Et_2O; (c) $Zr(Cp)_2Cl_2$, $AlMe_3$, then I_2, CH_2Cl_2; (d) *n*-BuLi, -78 °C, then **183**, THF.

Scheme 22. Synthesis of 14(*E*)-13-thia-2,3-oxidosqualene (**75**).

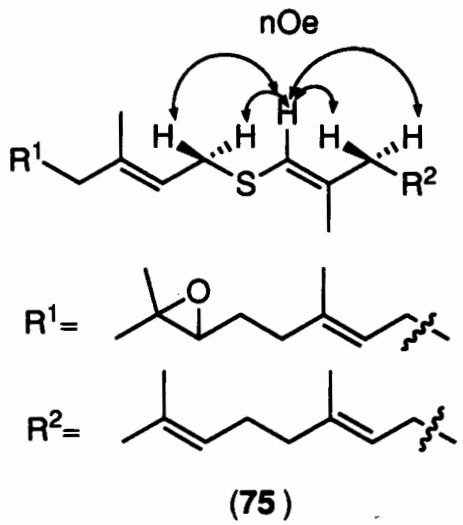


Figure 27. nOe experiment on **75**.

In summary, we achieved the total syntheses of 6(*E*)-8-thia and 14(*E*)-13-thia-2,3-oxidosqualenes (**74**) and (**75**), respectively. The key to the syntheses was a new methodology for the generation of *E*-vinylic sulfides involving stereospecific coupling of tri-substituted vinylic anions with appropriate alkyl 4-methylbenzenethiosulfonates. The commercial availability of potassium *p*-toluenethiosulfonate and the convenient generation of vinylic anions by reaction of vinylic iodides with alkyl lithiums make this a useful method.

4.3. Syntheses of 73 and 76.

Syntheses of 2,3-OS analogs **73** and **76** containing sulfur at C-5 and C-20 of **1** involved Wittig-Horner methodology developed by Cattel[30f] and Corey[25b] for the synthesis of 18(*E*)-20-*oxa*-2,3-OS analogs and by Dodd for the syntheses of 10(*E*)-9-thia- and 14(*E*)-16-thia-2,3-OS analogs in this laboratory.[31h] This method was effective for the synthesis of compounds **73** and **76** because it can utilize a set of common intermediates. Retrosynthetic analyses of **73** and **76** reveal that both can be derived from **193** (Figure 28).

Synthesis of **73** in which sulfur replaces C-5 of 2,3-OS commenced with construction of key intermediate **193**[31b] (Scheme 23). In comparison with the previously reported procedure,[31b] the synthetic route to **193** was shortened by two steps. Reaction of geranyl chloride (**185**) with $NaSO_2Ph$ in DMF gave sulfone **188** in 85% yield.[80] Selective epoxidation of **188** gave epoxide **189** in 93% yield and the latter was oxidatively cleaved to **190** which was converted to acetal **191**. The latter was subjected to metalation-alkylation to furnish the tetraene sulfone **192**. Reductive elimination of the sulfone moiety gave the key tetraene acetal **193** (Scheme 23). The required 1-(diphenylphosphinoyl)-ethanethiol (**194**)[81] was prepared by a procedure similar to that used for the synthesis of

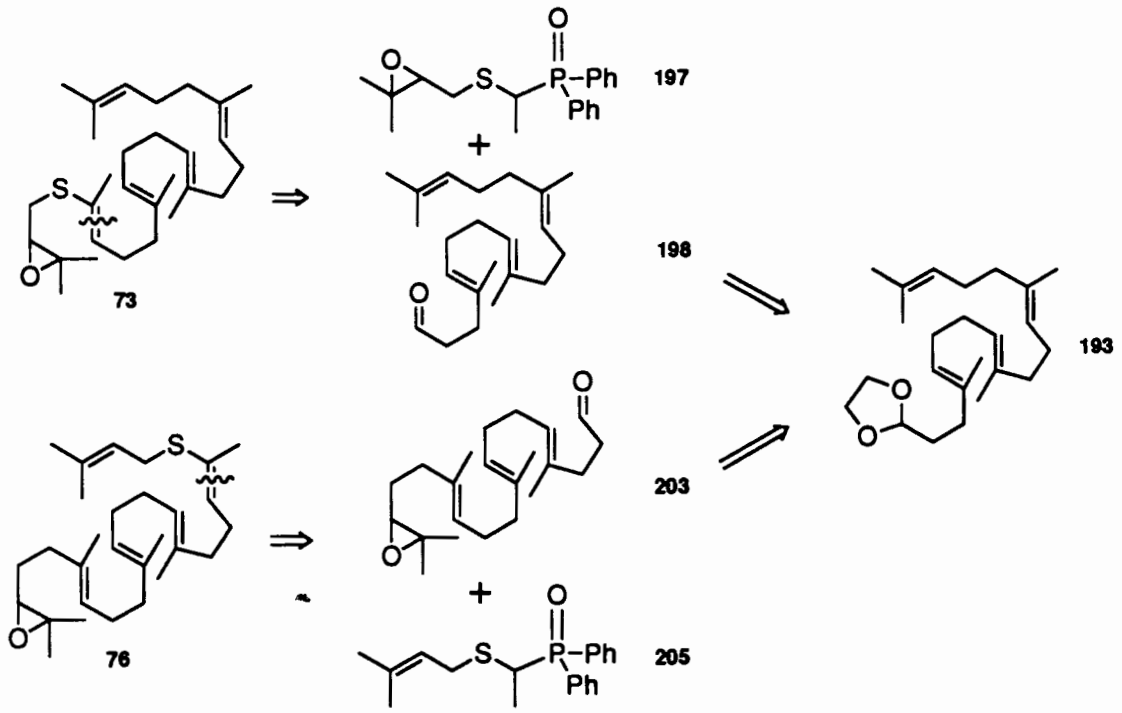
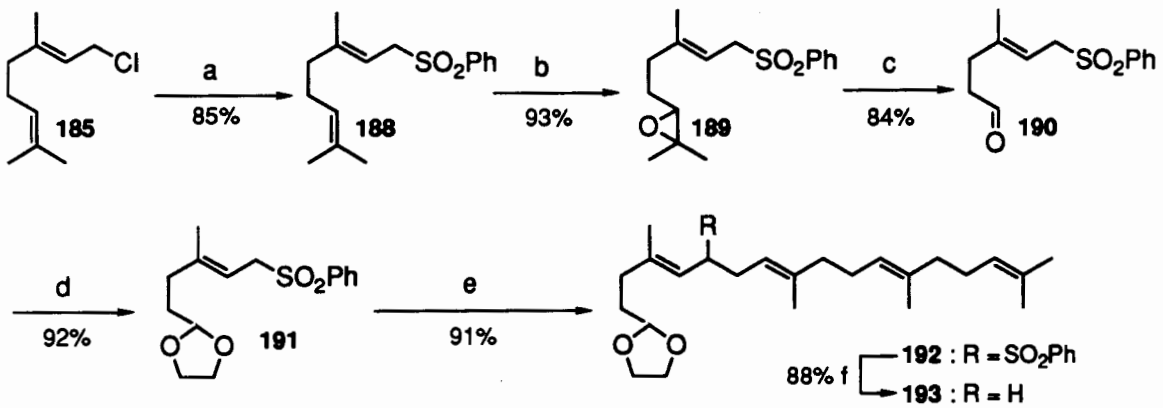
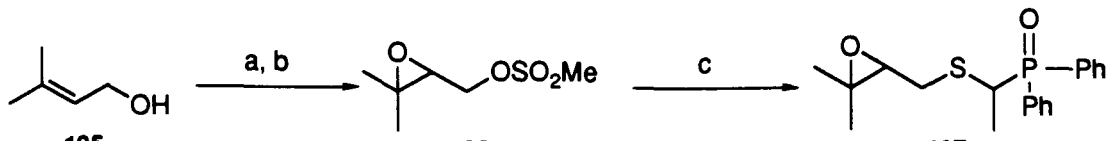


Figure 28. Retrosynthetic analyses of **73** and **76**.



(a) $NaSO_2Ph$, DMF; (b) *m*-CPBA, NaOAc, CH_2Cl_2; (c) HIO_4-H_2O, THF-Et_2O; (d) $(CH_2OH)_2$, TsOH, toluene; (e) *n*-BuLi, -78 °C, farnesyl bromide (**105**), (f) Li, $EtNH_2$,-78 °C.

Scheme 23. Synthesis of tetraene acetal **193**.



195 196 197

23% yield

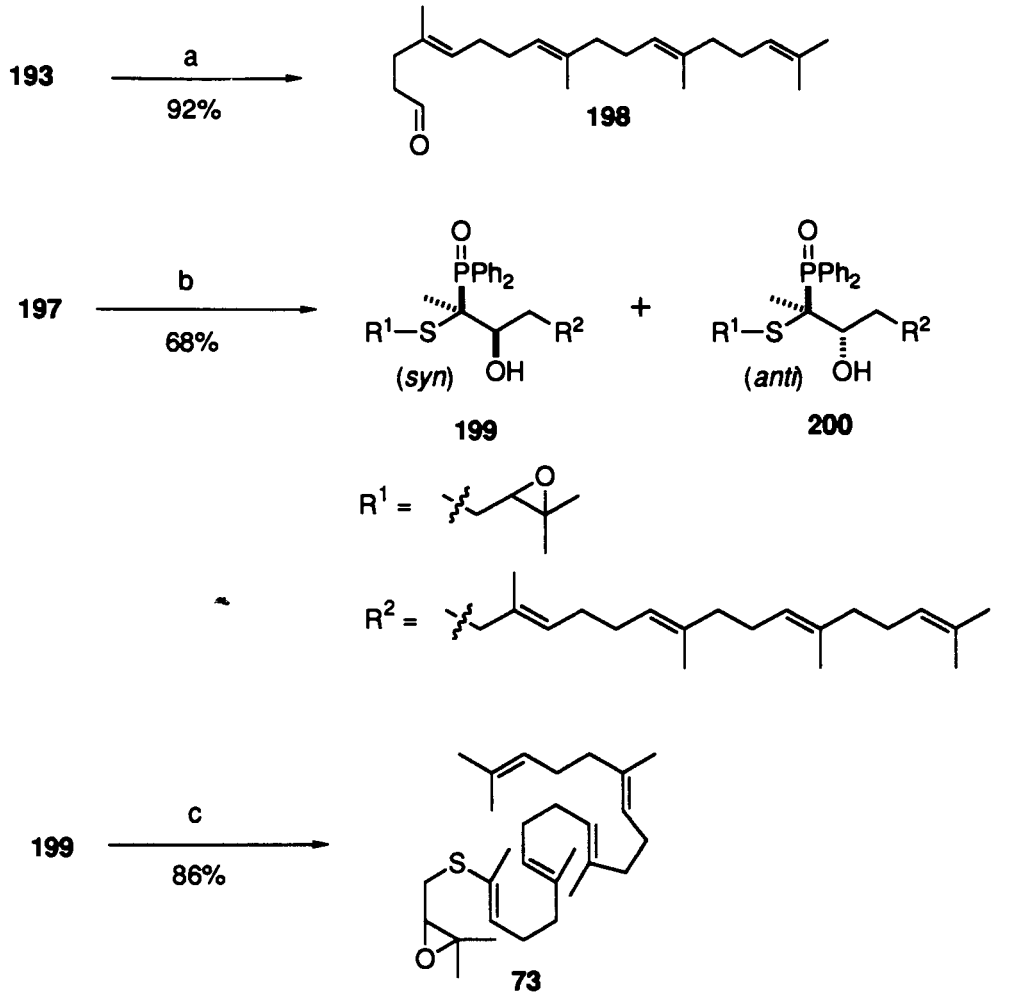
over three steps

(a) *m*-CPBA, CH_2Cl_2,-50 °C; (b) $MeSO_2Cl$, Et_3N, CH_2Cl_2; (c) 50% NaOH, Oct_4NBr, **194**, toluene-H_2O (1:1).

Scheme 24. Synthesis of sulfur-substituted Wittig-Horner agent **197**.

diethylphosphorylthiol.[66] Conversion of commerically available alcohol **195** to the corresponding epoxy mesylate **196** was executed by epoxidation followed by mesylation (Scheme 24). The required Wittig-Horner agent **197** was obtained in 23% overall yield by coupling available thiol **194** with mesylate **196** under phase-transfer conditions[35] (Scheme 24).

Deacetalization of **193** in aqueous acetone generated **198** in 92% yield (Scheme 25). Metalation of **197** followed by addition of aldehyde **198** gave a mixture of *syn* and *anti* α-hydroxydiphenylphosphinoyl isomers **199** and **200** (~60/40; by ^{1}H-NMR analysis) (Scheme 25). Analysis of this mixture by thin layer chromatography revealed two overlapping spots. Repeated flash column chromatography partially separated the two isomers to give pure **199** (*syn*) in 31% yield. The *anti* isomer **200** was contaminated with ~15% of **199**. Reaction of **199** with NaH in THF gave isomerically pure (6*E*)-5-thia-2,3-OS (**73**) in 86% yield (Scheme 25). ^{1}H-NMR of **73** revealed a triplet of quartets at δ 5.55 (J = 7.1, 1.21 Hz) for the vinylic hydrogen attached to C-7. The nOe difference ^{1}H-NMR spectrum of **73** confirmed the *E*-stereochemistry of the vinylic sulfide. A nOe enhancement was observed between the C-7 vinylic hydrogen (δ 5.55, J = 7.1, 1.2

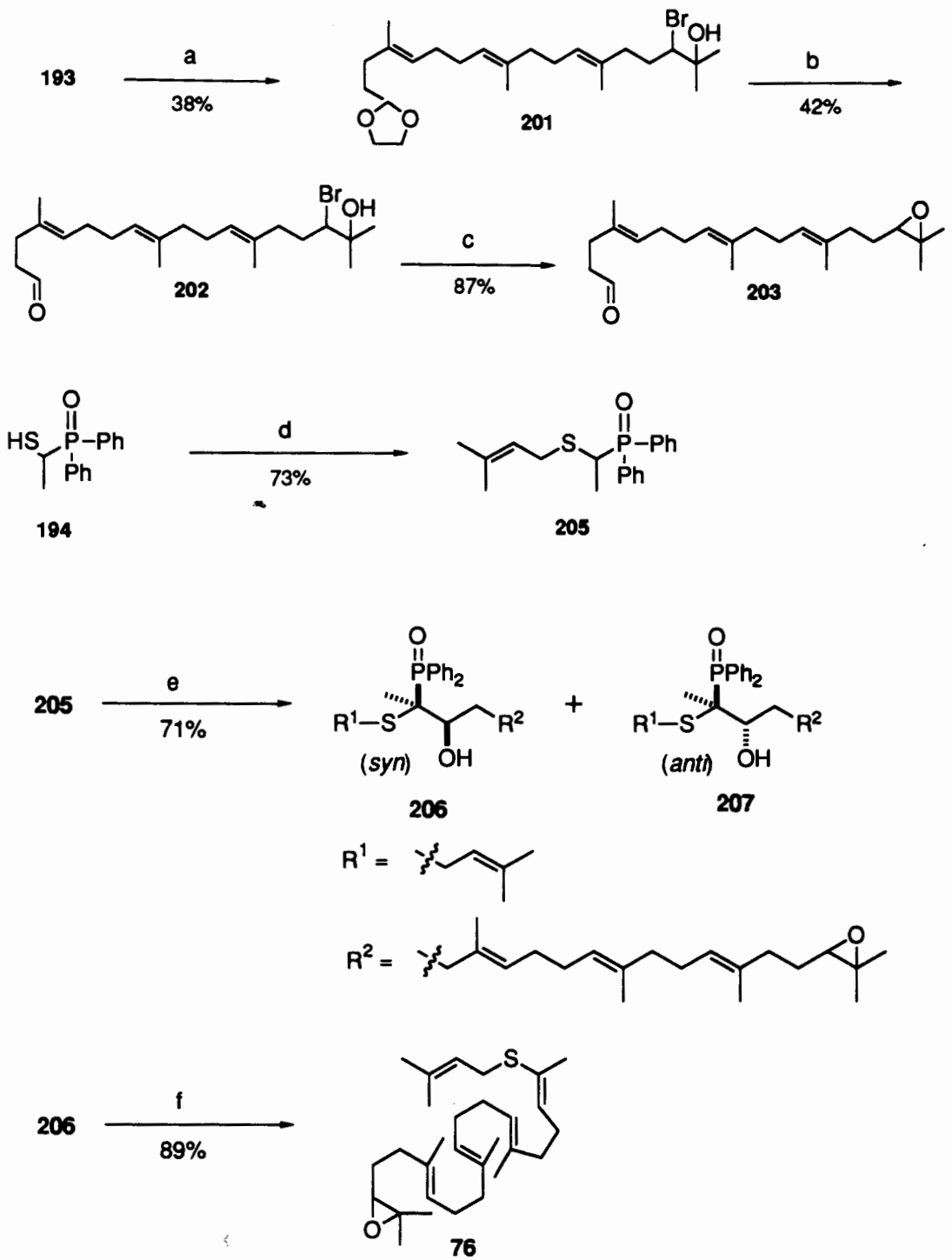


(a) TsOH, acetone-H_2O (85:15), reflux, 92%; (b) LDA, -100 °C, **198**, THF, and (c) NaH, THF (modified Wittig-Horner reaction).

Scheme 25. Synthesis of 6(*E*)-5-thia-2,3-oxidosqualene (**73**).

Hz) and C-4 hydrogens (δ 2.92), but not between the C-7 vinylic hydrogen and the methyl attached to C-6. This indicates that the C-7 vinylic hydrogen and the C-6 methyl are *trans* to each other.[25c,82]

The synthesis of **76** in which sulfur replaces C-20 of 2,3-OS commenced with conversion of **193** to terminal bromohydrin **201** in 38% yield.[44] Hydrolysis of the acetal followed by treatment with K_2CO_3 in methanol gave epoxy aldehyde **203**[25c] in 37% yield over two steps (Scheme 26). Coupling of thiol **194** with allylic



(a) 1.0 eq. NBS, THF-H_2O, 0 °C; (b) TsOH, acetone-H_2O, reflux; (c) K_2CO_3, MeOH, 0 °C; (d) 50% NaOH, Oct_4NBr, $Me_2CH{=}CH{-}CH_2Cl$,**204**, toluene-H_2O (1:1); (e) LDA, -100 °C, then **203**, THF; (f) NaH, THF.

Scheme 26. Synthesis of 18(*E*)-20-thia-2,3-oxidosqualene (**76**).

chloride **204** under phase transfer conditions[35] gave the required Wittig-Horner agent **205** in 73% yield. Reaction of **203** with the anion of **205** at -100 °C in THF followed by addition of acetic acid gave a mixture of *syn* and *anti* (~65/35; by ^{1}H NMR analysis) α-hydroxydiphenylphosphinoyl isomers (**206** and **207**) in 71% yield (Scheme 26). Partial separation using flash column chromatography gave pure **206** (*syn*) in 33% yield. The *anti* isomer **207** was contaminated with ~15% of **206**. Treatment of **206** with NaH/THF gave isomerically pure 18(*E*)-20-thia-2,3-OS (**76**) in 89% yield (Scheme 26). The structure of **76** was confirmed by ^{1}H NMR analysis, which revealed a triplet of quartets at δ 5.35 (J = 7.0, 1.2 Hz) for the vinylic hydrogen attached to C-18. The *E*-geometry of the vinylic sulfide was confirmed by observation of a nOe enhancement between the C-18 vinylic and the C-21 hydrogen.[25c,82]

4.4. Biological results.

All four sulfur 2,3-OS analogs (**73-76**) were found to be inhibitors of OSC from fungal (*C. albicans*) and mammalian (rat liver cyclase) sources (biological results were provided by Dr. P. G. Hartman, F. Hoffmann-La Roche Ltd., Basel, Switzerland.). Both **73** and **76** inhibited cell-free OSC of *C. albicans* with IC_{50} values of 47 and 0.2 μM, respectively. Sulfide **76** is 235 fold more potent than **73** in *C. albicans* cyclase. With rat liver OSC, **73** displayed an IC_{50} value of 7.7 μM and **76** showed an IC_{50} value of 0.32 μM. i.e., **76** is 24 fold more potent than **73** (Table 3). **73** is over 69 fold less potent than **74** toward *C. albicans* OSC and showed comparable activity with **75**, while **76** has the best inhibitory activity being 3.4 fold more potent than **74** and 225 fold more potent than **75**. With rat liver OSC, **73** displayed good activity being 4.4 fold and nearly 8 fold more potent than **74** and **75**, respectively. In this system, **76** again showed the best inhibitory activity being 106

Table 3. Evaluation of compounds **73** -**76** as inhibitors of OSC.

	IC_{50}[a] (μM)	
compound	*C. albicans* cyclase (cell-free)	Rat liver cyclase (cell-free)
73	47	7.7
74	0.68	34
75	45	61
76	0.2	0.32

[a] : molar concentration of inhibitor required to reduce enzyme activity by 50%.

fold more potent than **74** and nearly 191 fold more potent than **75**. 2,3-OS analog **76** which contains a sulfur in place of C-20 displayed the highest activity among the vinylic sulfides tested in both *C. albicans* and rat liver cyclases. This result is consistent with the observation for sulfur-substituted 2,3-OS analog **72** (Chapter 3, section 3.2) and suggests that modification of the C-20 region of **1** leads to the most potent inhibitors of OSC. All vinylic sulfides showed good inhibitory activity in agreement with the hypothesis that partial cyclization (Scheme 17) may occur and that the α-thiocarbenium ions generated might bind to the enzyme. It is worth noting that vinylic sulfide **76** showed superior activity (IC_{50} = 0.32 μM, rat liver) in comparison to the corresponding oxygen analog 22,23-dihydro-20-*oxa*-2,3-OS (**61**)[30f] (IC_{50} = 80 μM, rat liver). This result may be interpreted as being due to the greater stability of the presumptive α-thiocarbenium ion compared to the corresponding oxocarbenium ion. Considering only the IC_{50} values of this series, it is difficult to rationalize the mode of action of these inhibitors (see Scheme 17). The modest inhibitory power of these vinylic sulfides leads us to speculate that they act as competitive inhibitors of OSC similar to the oxygen-substituted 2,3-OS

analog **61** and the related sulfur-substituted analogs **66** and **72** (Chapter 3). Lack of evidence for sulfur-containing cyclized products and of kinetic data limits the further discussion. The IC_{50} value of **76** (0.2 μM, *C. albicans*; 0.32 μM, rat liver) is comparable with that of 2,3-iminosqualene[83,84] (0.15 μM, *C. albicans*; 0.4 μM, rat liver), a potent inhibitor of OSC.

Chapter 5

Concluding Remarks

We achieved the total syntheses of two classes of sulfur-containing 2,3-OS analogs (**63-72** and **73-76** ,respectively) as inhibitors of OSC. The strategic substitution of sulfur in the skeleton of **1** allowed us to probe the existence of carbocationic intermediates **2-6**, which are presumed to be formed during OSC-mediated cyclization of **1** to **8** according to the "step-wise" hypothesis and led to the preparation of the best inhibitor for both mammalian and fungal OSCs to date.

All compounds of the first series (**63-72**) were found to be good to potent inhibitors of OSCs. Sulfides **63**, **64**, **66**, **72** and sulfoxide **71** displayed IC_{50} values of 69, 69, 2.3, 0.23 and 65 nM, respectively, for OSC from fungal *C. albicans* which are the lowest values reported for inhibitors of this enzyme. These compounds were more powerful than the previously reported most potent inhibitor, 2,3-iminosqualene[83,84] (IC_{50} = 0.15 μM, *C. albicans*). Similarly, with rat liver OSC, sulfides **63**, **66** and **72** showed IC_{50} values of 8.4, 0.82, 0.08 nM, respectively, which are the best inhibitors known for this enzyme. These compounds were 13, 134 and 1375 fold, respectively, more potent than the previously known best rat liver OSC inhibitors such as 2,3:18,19-dioxidosqualene (**59**)[30d,e] (IC_{50} = 0.11 μM, rat liver OSC) and N-(1-oxododecyl)-4α-10-dimethyl-8-aza-*trans*-decal-3β-ol (**54**)[31f] (IC_{50} = 0.11 μM, rat liver OSC). Sulfides **66** and **72** displayed the first reported nanomolar IC_{50} values for both mammalian (rat liver) and fungal (*C. albicans*) OSC. These results highlight the potency of sulfur-containing 2,3-OS analogs as inhibitors of OSCs. It is noteworthy that the most potent inhibitors **66** and **72** have sulfur in

place of C-19 or C-18 in the skeleton of **1**. This suggests that substrate analogs with modification in the region of C-18 and C-19 of **1** have high affinity for the active site of OSCs. It implies that nucleophilic residues of OSCs which are responsible for the stabilization of protosterol C-20 cation **6** develop a stronger interaction with the cation than those responsible for stabilization of cationic intermediates **2-5**. Kinetic analysis of the inhibition by **66** and **72** of pig liver OSC was carried out to reveal the inhibition mechanism. Both **66** and **72** were competitive inhibitors of pig liver OSC while **72** displayed strong time-dependency. We suspect that **72** irreversibly deactivates pig liver OSC with the same facility as 29-MOS, a known irreversible inhibitor of OSC (Figure 23, Chapter 3). In the same study, **66** showed no evidence of irreversible inhibition (Figure 23, Chapter 3). It seems appropriate to conclude that **72** acts as an irreversible mechanism-based inhibitor of OSC while **66** acts as an unmodified substrate analog, reversibly binding to OSC. The preparation of ^{3}H-labeled **72** is underway to probe the active site of pig liver OSC. Incubation of ^{3}H-labeled **72** with OSC will hopefully reveal which amino acid residue is responsible for stabilization of the protosterol cation in pig liver OSC.

The biological study of sulfur-substituted 2,3-OS analogs **73-76** showed they were all good inhibitors of OSCs (Table 3, Chapter 4). Analog **76** in which sulfur replaced C-20 of **1** showed the strongest activity among the vinylic sulfides against both *C. albicans* (IC_{50} = 0.20 μM) and rat liver (IC_{50} = 0.32 μM) OSCs. This suggests that modification of C-20 of **1** causes the most significant inhibition of OSC by interrupting formation of the C-20 protosterol cation **6**. Analogs **73-76** have sufficient conformational flexibility to be cyclized by OSC. Generation of thiocarbenium ions **158**, **161**, **164** and **167** (Scheme 17-18) should lead to rather long-lived cationic species that would allow nucleophilic

attack from amino acid residues of OSC. We expect to observe the covalent modification of OSC by analogs **73-76** according to Schemes 17-18. Thus, preparation of ^{3}H-labeled vinylic sulfide analogs of 2,3-OS to allow the isolation of cyclized products or to conduct affinity labelling experiments is suggested.

Chapter 6

Experimental

6.1. Instrumentation for spectroscopic analyses.

^{1}H and ^{13}C-NMR spectra were recorded on a Bruker AMX-400 spectrometer. ^{1}H and ^{13}C chemical shift are reported in parts per million (ppm, δ) and relative to $CHCl_3$ (7.26 ppm) and (77.0 ppm) respectively. Mass spectra were obtained on a Hewlett-Packard 5985B GC/MS equipped with a DB-1 capillary column (30 m x 0.25 mm ID; with 0.25 μM film) operating at 70 ev for electron impact (EI) ionization. Chemical ionization (CI) was performed using isobutane as the proton source. High resolution mass spectra were performed on a Kratos/AEI MS 50 spectrometer at the University of British Columbia. IR spectra were recorded on a Perkin-Elmer Model FT 1605 spectrophotometer. Elemental analyses were performed using a Carlo Erba Model 1106 Elemental Analyzer. Gas chromatographic analyses were recorded on a Hewlett-Packard 5890 instrument equipped with a DB-1 capillary column (15 m x 0.25 mm ID; with 0.25 μM film) and flame-ionization detector.

6.2. General chemical procedures.

Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium and benzophenone-ketyl. Triethylamine (Et_3N) and dichloromethane (CH_2Cl_2) were freshly distilled from CaH_2 prior to use. Diisopropylamine [$(i\text{-}Pr)_2NH$] was freshly distilled from sodium prior to use. N,N-Dimethylformamide (DMF) was dried over 4A molecular sieves. Iodine was purified by sublimation. N-Bromosuccinimide and N-chlorosuccinimide were recrystallized from glacial acetic acid, washed with ice-water, and dried under high vacuum prior to use. Triphenylphosphine was dried over phosphorus pentoxide under high vacuum for 4 h in a drying pistol using

acetone as solvent. Anhydrous BaI_2 was prepared by drying $BaI_2 \cdot H_2O$ (Aldrich, 95%) at 160°C for 2 h under high vacuum (<5 torr). Ethyl chloroformate (Sigma) was freshly distilled under argon prior to use. Other chemicals obtained from commercial sources were used without further purification. All moisture- and air-sensitive reactions were conducted under argon in vacuum-dried glassware. A nitrogen glovebag was used to weigh all the moisture-sensitive compounds. Syringes and cannulas were used to transfer reagents. Unless otherwise stated, standard work-up refers to the combined organic extracts being washed with ice-cold brine, dried over anhydrous $MgSO_4$, filtered, and the filtrate being concentrated *in vacuo*. Thin layer chromatography (TLC) was the usual analysis method and was performed on aluminum plates precoated with Merck silica gel 60 F-254 as the adsorbent. The TLC plates were developed in the specified solvent and spots were visualized by iodine or sprayed with a solution of $Ce(SO_4)_2$ (1%) and molybdic acid (1.5%) and gently heated on a hot plate. Flash column chromatography was performed on silica gel 60 (E. Merck No. 9385, 230-400 mesh) as described by Still and coworkers.[85]

6.3. Biological methods.

Cell culturing, *in vitro* antifungal activity and mammalian cell toxicity measurements, and cholesterol biosynthesis inhibition assays of MBDK cells were carried out by Dr. N. H. Georgopapadakou at Hoffmann-La Roche Inc. (Nutley, New Jersey) as described in reference 32. Cell-free enzyme inhibition assays of rat liver and *C. albicans* OSCs were performed by Dr. Peter G. Hartman and Ms. Petra Scheliga at F. Hoffmann-La Roche Ltd. (Basel, Switzerland) as described in references 32 and 84, and enzyme kinetic and inhibition studies on pig liver OSC by Dr. I. Abe and Professor G. D. Prestwich (Department of Chemistry, State University of New York at Stony Brook, NY) as described in references 14 and 55a.

6.4. Experimental procedures and spectral data.

6.4.1. Syntheses discussed in Chapter 2.

4-Methyl-3-pentenyl *tert*-butyldimethylsilyl ether (82): To a solution of 4-methyl-3-penten-1-ol (**81**) (0.45 g, 4.5 mmol) in CH_2Cl_2 (30 mL) and Et_3N (0.485 g, 4.8 mmol) at 0 °C was added TBSCl (0.694 g, 4.6 mmol) and DMAP (0.02 g). This was stirred at 0°C for 0.5 h and at room temperature for 6 h. The reaction mixture was poured into water (20 mL). The organic phase was separated and the aqueous layer was extracted with diethyl ether (4 X 30 mL). Standard work-up followed by flash column chromotography using diethyl ether:pentane (1:9) as the eluant gave **82** (0.91 g, 94% yield) as colourless oil: IR (film) 2957, 2930, 2859, 1672, 1473, 1257 and 1100 cm^{-1}; CIMS *m/z* (rel. intensity) 215 (M^++1, 1.0), 157 (6.2), 95 (2.9), 91 (2.4), 89 (2.2), 85 (9.1), 84 (7.4), 83 (100), 81 (9.0); ^1H NMR ($CDCl_3$, ppm) 5.12-5.08 (m, 1H), 3.57 (t, J =7.2 Hz, 2H), 2.21 (dt, J = 7.2, 7.2 Hz, 2H), 1.69 (s, 3H), 1.59 (s, 3H), 0.89 (s, 9H), 0.05 (s, 6H). Anal. Calcd for $C_{12}H_{26}OSi$: C, 67.22; H, 12.22. Found: C, 67.36; H, 12.30.

4-Methyl-3,4-epoxy-pentyl *tert*-butyldimethylsilyl ether (83): To a stirred solution of **82** (0.91 g, 4.25 mmol) in CH_2Cl_2 (35 mL) at -40 °C was added *m*-CPBA (1.08 g, 5.0 mmol, 80% pure) in one portion. The reaction mixture was stirred for 0.5 h at this temperature and warmed to 0 °C over 1 h. The mixture was poured into saturated aqueous $Na_2S_2O_3$ solution (10 mL) and the organic phase was separated. The aqueous phase was extracted with CH_2Cl_2 (3 X 20 mL) and the combined organic phase was washed with saturated $NaHCO_3$ (2 X 20 mL). Standard work-up followed by flash column chromatography using diethyl ether:pentane (3:7) as the

eluant gave pure **83** (0.843 g, 86% yield): IR (film) 2957, 2929, 2858, 1775, 1472, 1378, 1256, 1097, 1005, 939 and 836 cm^{-1}; CIMS *m/z* (rel. intensity) 231 (M^++1, 100), 213 (44), 173 (44.3), 145 (32.6),133 (4.1),115 (2.2), 99 (79.2), 89 (23.4); ^1H NMR ($CDCl_3$, ppm) 3.79-3.75 (m, 2H), 2.84 (t, J = 6.2 Hz, 1H), 1.86-1.68 (m, 2H), 1.31(s, 3H), 1.27 (s, 3H), 0.89 (s, 9H), 0.06 (s, 6H). Anal. Calcd for $C_{12}H_{26}O_2Si$: C, 62.55; H, 11.37. Found: C, 62.68; H, 11.45.

4-Methyl-3,4-epoxy-pentanol (84): To a solution of **83** (0.81 g, 3.52 mmol) in THF (10 mL) at room temperature was added tetrabutylammonium fluoride (10 mL, 1 M solution in THF, 10 mmol). This was stirred for 8 h at room temperature. Then water (10 mL) was added and the mixture was extracted with ether (4 X 20 mL). Standard work-up followed by flash chromatography using diethyl ether:pentane (9:1) as the eluant afforded **84** (0.295 g, 72% yield; GC purity 91%): IR (film) 3428, 2950, 2929, 2870, 1459, 1379 and 1064 cm^{-1}; CIMS *m/z* (rel. intensity) 117 (M^++1,100), 99 (43), 85 (4.2), 81 (4.0) MS, *m/z* (rel. intensity) 116 (M^+, trace), 101 (4.0), 85 (100),73 (4.6), 71 (4.2), 59 (97), 57 (25.5), 45 (15.5),43 (40.6), 42 (20.3), 41 (47.2); ^1H NMR ($CDCl_3$, ppm) 3.88-3.79 (m, 2H), 2.90 (dd, J = 4.7, 7.8 Hz, 1H), 1.92-1.84 (m, 2H),1.74-1.66 (m, 1H), 1.32 (s, 3H), 1.29 (s, 3H).

3,4-Epoxy-4-methyl-pentyl methanesulfonate (85): To a solution of **84** (0.174 g, 1.5 mmol) in CH_2Cl_2 (20 mL) at -50 °C was added triethylamine (0.202 g, 2.0 mmol) and methanesulfonyl chloride (0.208 g, 1.8 mmol). This was stirred at -50 °C for 10 min, warmed to 0 °C over 1 h then poured into water (10 mL) and the organic phase separated. The aqueous phase was extracted with ether (3 X 20 mL). Standard work-up followed by high vacuum drying gave **85** (0.276 g, 95% yield) of high purity (GC purity >99%). CIMS *m/z* (rel. intensity) 195 (M^++1, 100), 177 (10.2);

^{1}H NMR ($CDCl_3$, ppm) 4.43 -4.32 (m, 2H), 3.03 (s, 3H), 2.86 (dd, J = 4.9, 7.4 Hz, 1H), 2.13-2.05 (m, 1H), 1.91-1.81(m, 1H), 1.33 (s, 3H), 1.29 (s, 3H).

5-Iodo-4-methyl-4(*E*)-pentenol (87): To a slurry of $ZrCp_2Cl_2$ (2.54 g, 8.75 mmol) in dry CH_2Cl_2 (100 mL) at -20 °C under argon was added $AlMe_3$ (10.38 mL, 105 mmol) dropwise over 5 min. 4-Pentynol (**86**) (3.01 g, 35 mmol) in CH_2Cl_2 (5 mL) was then added dropwise. The mixture was warmed to room temperature and stirred for 15 h, then cooled to -30 °C and iodine (10.15 g, 40 mmol) in THF (50 mL) was added slowly. Twenty minutes after addition of iodine, excess $AlMe_3$ was destroyed (caution!!) by the addition of 5 mL of distilled water under argon at 0°C. The slurry was diluted with 100 mL of hexane and the precipitated salt was removed by filtration through a pad of Celite. The pad was rinsed with 50 mL of hexane. Standard work-up of the filtrate followed by flash chromatography using ethyl acetate:hexane (3:7) as the eluant gave pure **87** (6.49 g, 82% yield): IR (film) 3347, 1617, 1062 and 769 cm^{-1}; ^{1}H NMR ($CDCl_3$, ppm) 5.91 (s, 1H), 3.61 (t, J = 6.4 Hz, 2H), 2.28 (t, J = 7.5 Hz, 2H), 1.83 (s, 3H),1.80-1.60 (m, 3H); ^{13}C NMR ($CDCl_3$, ppm) 147.46, 74.90, 62.04, 35.78, 30.61, 23.84; Anal. Calcd for $C_6H_{11}IO$: C, 31.86; H, 4.91. Found : C, 32.13; H, 5.04 .

5-Iodo-4-methyl-4(*E*)-pentenyl-*tert*-butyldimethylsilyl ether (88): To a solution of TBSCl (4.04 g, 26 mmol) in CH_2Cl_2 (80 mL) and Et_3N (27.3 g, 27 mmol) at 0 °C was added **87** (5.65 g, 25 mmol) and DMAP (0.05 g). This was stirred at room temperature for 6 h and the mixture was poured into water (20 mL). The organic phase was separated and the aqueous layer was extracted with diethyl ether (3 X 30 mL). Standard work-up followed by flash column chromatography using ethyl acetate:hexane (5:95) as the eluant gave **88** (8.08 g, 95% yield) as

colourless liquid: IR (film) 1618, 1105, 836 and 775 cm^{-1}; CIMS *m/z* (rel. intensity) 341 (M^++1, 28.0), 283 (27.6), 251 (3.6), 210 (7.2), 209 (100), 123 (10.4); ^1H NMR ($CDCl_3$, ppm) 5.88 (q, J = 1.10 Hz, 1H), 3.58 (t, J = 6.27 Hz, 2H), 2.26 (t, J = 7.65 Hz, 2H), 1.83 (d, J = 1.10 Hz, 3H), 1.68-1.60 (m, 2H), 0.89 (s,9H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 147.79, 74.60, 62.15, 35.85, 30.84, 25.93, 23.87, 18.33, -5.32.

Ethyl 6-[*tert*-Butyldimethylsilyloxy]-3-methyl-2(*E*)-hexenoate (89): To a stirred solution of **88** (0.681g, 2.0 mmol) in THF (30 mL) under argon at -78 °C was added dropwise *n*-BuLi (0.8 mL, 2.0 mmol, 2.5 M solution in hexane). The reaction was stirred for 20 min. Freshly distilled ethyl chloroformate (0.21 mL, 2.2 mmol) was added dropwise and the mixture was allowed to stand at -78 °C for 1 h. After warming to room temperature over 3 h, it was quenched by pouring into water (10 mL) and extracted with diethyl ether (3 X 20 mL). Standard work-up followed by flash colunm chromatography using ethyl acetate:hexane(1:9) as the eluant gave **89** (0.48 g, 84% yield): IR (film) 2953, 2931, 2858, 1719, 1649, 1472, 1384, 1256, 1223, 1148, 1105, 837 and 776 cm^{-1}; CIMS *m/z* (rel. intensity) 288 (M^++2, 22.1), 287 (M^++1, 100), 242 (3.6), 230 (2.2), 229 (13.3), 155 (16.8); ^1H NMR ($CDCl_3$, ppm) 5.65 (s, 1H), 4.12 (q, J = 7.1 Hz, 2H), 3.60 (t, J = 6.2 Hz, 2H), 2.19 (t, J = 7.7 Hz, 2H), 2.15 (s, 3H), 1.71-1.64 (m, 2H), 1.26 (t, J = 7.1 Hz, 3H), 0.89 (s, 9H), 0.045 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 166.75, 159.73, 115.71, 62.31, 59.38, 37.26, 30.60, 25.90, 18.75, 18.22, 14.30, -5.37. Anal. Calcd for $C_{15}H_{30}O_3Si$: C, 62.89; H, 10.56. Found: C, 63.01; H, 10.75.

6-[*tert*-butyldimethylsilyloxy]-3-methyl-2(*E*)-hexenol (90): To a solution of **89** (0.45 g,1.57 mmol) in diethyl ether (20 mL) at -78 °C under argon was added diisobutylaluminum hydride (DIBAL-H) (4 mL, 4 mmol, 1 M solution in hexane). The

mixture was warmed to 0 °C and stirred for 1.5 h, then excess DIBAL-H was destroyed by addtion of water (1 mL) and the resulting mixture was poured into an ice-cold 5% aqueous solution of tartaric acid (10 mL). The mixture was extracted with ether (3 X 30 mL) and the combined organic phase was washed with $NaHCO_3$ solution. Standard work-up followed by flash column chromatography using ethyl acetate:hexane(1:4) as the eluant gave **90** (0.36 g, 94% yield) as colourless liquid: IR (film) 3344, 2965, 2929, 2857, 1669, 1471, 1387, 1255, 1101, 836 and 775 cm^{-1}; CIMS *m/z* (rel. intensity) 245 (M^++1, 3.8), 227 (100), 228 (17.9), 133 (2.0); ^1H NMR ($CDCl_3$, ppm) 5.41 (t, J = 7.0 Hz, 1H), 4.14 (d, J = 6.8 Hz, 2H), 3.59 (t, J = 6.5 Hz, 2H), 2.05 (t, J = 7.5 Hz, 2H), 1.67 (s, 3H), 1.65-1.60 (m, 2H), 0.89 (s, 9H), 0.045 (s, 6H). Anal. Calcd for $C_{13}H_{28}O_2Si$: C, 63.88; H, 11.55. Found: C, 64.05; H, 11.70.

6-Bromo-4-methyl-(4*E*)-hexenyl *tert*-butyldimethylsilyl ether (91): To a solution of **90** (0.244 g, 1.0 mmol) in CH_2Cl_2 (15 mL) at -50 °C under argon was added Et_3N (0.19 mL, 1.36 mmol) and methanesulfonyl chloride (0.138 g, 1.2 mmol). This was stirred at -50 °C for 30 min. A solution of LiBr (0.217 g, 2.5 mmol) in THF (5 mL) was added into the mixture. The mixture was warmed to 0 °C and stirred for 1 h. Water (5 mL) was added and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (2 X 20 mL). Standard work-up gave **91** (0.27 g, 88% yield) as a liquid: IR (film) 2975, 2929, 2857, 1663, 1472, 1387, 1255, 1101, 836 and 661 cm^{-1}; CIMS *m/z* (rel. intensity) 309/307 (M^++1, 7.8, 8.5), 263 (13.4), 261 (16), 193 (6.7),191 (7.4); ^1H NMR ($CDCl_3$, ppm) 5.45 (t, J = 8.0 Hz, 1H), 4.09 (d, J = 8.0 Hz, 2H), 3.59 (t, J = 6.4 Hz, 2H), 2.09 (t, J = 7.7 Hz, 2H), 1.72 (s, 3H), 1.68-1.58 (m, 2H), 0.89 (s, 9H), 0.045 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 142.60, 120.39, 62.51,41.04, 35.67, 30.75, 29.69, 25.95, 18.31, 16.05.

1-Chloro-3,7,11-trimethyldodeca-2(*E*),6(*E*),10-triene (farnesyl chloride) (93): To a stirred solution of NCS (1.22 g, 9.1 mmol) in dry CH_2Cl_2 (50 mL) at 0 °C under argon was added dropwise DMS (0.73 mL, 10.0 mmol). The mixture was cooled to -20 °C and (*E*,*E*)-farnesol (**92**) (1.112 g, 5.0 mmol) in CH_2Cl_2 (5.0 mL) was added dropwise over 5 min. The mixture was warmed to 0 °C and stirred for 3 h, then poured into ice-cold distilled water (20 mL) and extracted with diethyl ether (4 X 30 ml). Standard work-up followed by flash column chromatography using ethyl acetate:hexane(1:99) gave **93** (0.946 g, 80%) as an oil: IR (film) 2965, 2922, 2850, 1662, 1448, 1382, 1253, 838 and 668 cm^{-1}; ^1H NMR ($CDCl_3$, ppm) 5.44 (t, J = 8.0 Hz, 1H), 5.10-5.06 (m, 2H), 4.10 (d, J = 8.0 Hz, 2H), 2.15-2.02 (m, 6H), 2.00-1.94 (m, 2H), 1.73 (s, 3H), 1.68 (s, 3H), 1.60 (s, 3H).

4,9,13,17-Tetramethyloctadeca-4(*E*),8(*E*),12(*E*),16-tetraenyl *tert*-butyl dimethylsilyl ether (94): This was prepared by coupling farnesyl barium and **91** according to the procedure of Corey *et al.*[39] To a freshly cut piece of lithium (41 mg, 5.9 mmol) under argon was added a solution of biphenyl (0.924 g, 6.0 mmol) in THF (18 mL). This was stirred at room temperature for 2.5 h at which time a greenish-blue solution of lithium biphenylide was observed. To a suspension of anhydrous BaI_2 (1.15 g, 2.94 mmol) in THF (5 mL), at room temperature, was added the lithium biphenylide solution through a cannula. The reaction mixture was stirred for further 45 min. at room temperature at which time a brown suspension of barium powder was formed. To this under argon at -78°C was added dropwise a solution of **93** (0.708 g, 2.94 mmol) in THF (5 mL) and the mixture was stirred for 45 min forming a red solution. Into this red solution at -78 °C was added *via* cannula a solution of **91** (0.354 g, 1.15 mmol) in THF (5 ml). The mixture was stirred at -78°C for 2 h, at room temperature for 14 h, then poured into the ice-cold saturated

aqueous NH_4Cl solution and extracted with diethyl ether (4 X 30 ml). The organic phase was washed with dilute $Na_2S_2O_3$ solution. Standard work-up followed by flash column chromatography using ethyl acetate:hexane (1:20) as the eluant gave **94** (0.289 g, 58% yield) as an oil: IR (film) 2950, 2928, 2856, 1668, 1462, 1383, 1255, 1101, 836 and 775 cm^{-1}; CIMS *m/z* (rel. intensity) 433 (M^++1, 16.1),432 (M^+, 3.5), 375 (12.0), 302 (23.2), 301 (100), 219 (24.8), 191 (24.1), 177 (28.0), 163 (25.0), 151 (29.2), 137 (60.3), 123 (18.5); ^1H NMR ($CDCl_3$, ppm) 5.18-5.08 (m, 4H), 3.58 (t, J = 6.6 Hz, 2H), 2.10-1.95 (m, 14H), 1.68 (s, 3H), 1.63-1.58 (m, 2H), 1.60 (s, 12H), 0.89 (s, 9H), 0.043 (s, 6H). IR and ^1H NMR spectra are in agreement with those reported in reference 25c.

4,9,13,17-Tetramethyloctadeca-4(*E*),8(*E*),12(*E*),16-tetraen-1-ol (95): This was prepared from **94** in 93% yield by the procedure described for the preparation of **84**. **95**: IR (film) 3330, 2958, 2923, 2855, 1669, 1447, 1382, 1058 and 839 cm^{-1}; CIMS *m/z* (rel. intensity) 319 (M^++1, 100), 318 (M^+,11.1), 301 (10.8), 263 (7.7), 249 (10.4), 237 (26.2), 219 (21.4), 193 (20.5), 163 (19.1); ^1H NMR ($CDCl_3$, ppm) 5.21-5.16 (m, 1H), 5.13-5.08 (m, 3H), 3.64 (dt, J = 5.9, 6.2 Hz, 2H), 2.10-1.95 (m, 14H), 1.74-1.64 (m, 2H), 1.68 (s, 3H), 1.62 (s, 3H), 1.60 (s, 9H), 1.29 (t, J = 5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, ppm) 135.27, 134.92, 134.75, 131.22, 124.89, 124.44, 124.29, 124.18, 62.86, 39.74, 36.02, 30.84, 28.25, 28.19, 26.81, 26.67, 25.65, 17.65, 16.04, 15.99, 15.89. Anal.Calcd for $C_{22}H_{38}O$: C, 82.95; H, 12.02. Found: C, 82.85; H, 12.05.

4,9,13,17-Tetramethyloctadeca-4(*E*),8(*E*),12(*E*),16-tetraenyl thioacetate (96): To a solution of triphenylphosphine (0.81 g, 3.0 mmol) in THF (30 mL) was added diisopropyl azodicarboxylate (0.63 g, 3.0 mmol) at -20 °C. The mixture was

stirred efficiently and warmed to 0 °C for 1 h by which time a thick white precipitate formed. Then a solution of thioacetic acid (0.23 g, 3.0 mmol) and **95** (0.48 g, 1.51 mmol) in THF (5 mL) was added dropwise over 20 min at -20 °C. The mixture was warmed to 0 °C over a period of 0.5 h, allowed to stand at 0 °C for 1 h, then stirred overnight at room temperature, poured into water (10 mL) and extracted with diethyl ether (3 X 40 mL). Standard work-up followed by flash colunm chromatography using ethyl acetate:hexane (5:95) as the eluant gave **96** (0.49 g, 86% yield) as an oil: IR (film) 2964, 2922, 2854, 1695, 1447, 1382, 1135, 1107, 954, 836 and 626 cm^{-1}; CIMS *m/z* (rel. intensity) 377 (M^++1, 100), 335 (45.2), 293 (15.1), 267 (12.6), 253 (37.4), 225 (15.9), 213 (28.3),199 (13.1), 185 (36.2), 171 (12.8), 151 (22.4), 137 (42.8), 123 (17.7); ^1H NMR ($CDCl_3$, ppm) 5.19-5.06 (m, 4H), 2.82 (t, J = 7.3 Hz, 2H), 2.32 (s, 3H), 2.10-1.93 (m, 14H), 1.70-1.63 (m, 2H), 1.68 (s, 3H),1.60 (s, 6H), 1.58 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 196.00, 135.24, 134.91, 133.78, 131.21, 125.42, 124.46, 124.31, 124.21, 39.75, 38.69, 30.59, 28.67, 28.26, 28.18, 27.75, 26.82, 26.69, 25.65, 17.66, 16.05, 15.80. Anal.Calcd for $C_{24}H_{40}OS$: C, 76.54; H, 10.71. Found: C, 76.38; H, 10.56.

4,9,13,17-Tetramethyl-4(*E*),8(*E*),12(*E*),16-octadecatetraen-1-thiol (97): A solution of **96** (0.475 g, 1.26 mmol) in dry ether (5 mL) was slowly added to a stirred suspension of $LiAlH_4$ (0.303 g, 8.0 mmol) in dry ether (40 mL) under argon at 0 °C. After 0.5 h at 0 °C, excess $LiAlH_4$ was destroyed at -30 °C by slow addition of water (1.0 g). The reaction mixture was filtered and the white precipatate was washed with diethyl ether (3 X 20 mL). Standard work-up followed by flash colunm chromatography using ethyl acetate:hexane (1:20) as eluant gave **97** (0.381 g, 91% yield) as an oil: IR (film) 2950, 2926, 2853, 1666, 1448, 1382, 1151, 1107 and 838 cm^{-1}; CIMS *m/z* (rel. intensity) 335 (M^++1, 100), 334 (M^+, 8.5), 333 (10.6), 265

(8.9), 257 (3.0), 211 (2.0); ^{1}H NMR ($CDCl_3$, ppm) 5.18-5.06 (m, 4H), 2.48 (dt, J = 7.1, 7.6 Hz, 2H), 2.10-1.95 (m, 14H), 1.74-1.66 (m, 2H), 1.68 (s, 3H), 1.60(s, 9H),1.59 (s, 3H), 1.33 (t, J = 7.9 Hz, 1H). Anal.Calcd for $C_{22}H_{38}S$: C, 78.98; H, 11.46. Found: C, 79.10; H, 11.62.

6-Thia-7-dehydro-2,3-oxidosqualene (63): To a solution of NaOH (2.5 g, 62.5 mol) in H_2O (10 mL) and toluene (10 mL) was added tetraoctylammonium bromide (0.05 g), **85** (0.194 g, 1.0 mmol) and **97** (0.244 g, 0.73 mmol) at room temperature. This mixture was warmed to 40 °C, stirred for 10 h and then extracted with ether (3 X 20 mL). Standard work-up followed by chromatography using ethyl acetate:hexane(1:20) as the eluant gave pure **63** (0.255 g, 81% yield) as a colourless oil: IR(film) 2950, 2924, 2854, 1667, 1448, 1378, 1249 and 1123 cm^{-1}; CIMS *m/z* (rel. intensity) 433 (M^++1, 100), 432 (M^+, 9.0), 391 (1.30), 363 (1.8), 335 (4.0), 333 (4.2), 301 (1.9), 295 (8.3), 257 (2.3); ^{1}H NMR ($CDCl_3$, ppm) 5.18-5.06 (m, 4H), 2.82 (t, J = 6.2 Hz, 1H), 2.78-2.58 (m, 2H), 2.56-2.48 (m, 2H), 2.10-1.93 (m, 14H), 1.86-1.77 (m, 2H), 1.76-1.66 (m, 2H), 1.68 (s, 3H), 1.59 (s, 9H), 1.58 (s, 3H), 1.32 (s, 3H), 1.28 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 135.22, 134.92, 134.08, 131.02, 125.16, 124.44, 124.28, 124.21, 63.25, 58.50, 39.74, 38.75, 31.85, 29.30, 29.01, 28.23, 27.91, 26.80, 26.68, 25.66, 24.76, 18.66, 17.73, 16.05, 15.89. Anal. Calcd for $C_{28}H_{48}OS$: C, 77.71; H, 11.18. Found: C, 77.50; H, 10.95.

6-Sulfinyl-7-dehydro-2,3-oxidosqualene (67): This was prepared by oxidation of **63** according to the procedure of Trost *et al.*[38] for the oxidation of thioanisole to its corresponding sulfoxide except the reaction time was 2 min and the temperature was -5°C. Chromatography using ethyl acetate:hexane (7:3) as the eluant gave **67** in 80% yield: IR (film) 2963, 2923, 2850, 1444, 1378 and 1056 cm^{-1};

CIMS *m/z* (rel. intensity) 449 (M^++1, 78), 448 (M^+, 5.0), 391 (44.5), 351 (82.7), 299 (39), 149 (100); ^{1}H NMR ($CDCl_3$, ppm) 5.21-5.16 (m, 1H), 5.15-5.06 (m, 3H), 2.88 (dd, J = 4.6, 7.9 Hz, 1H), 2.85-2.52 (m, 4H), 2.22-2.10 (m, 4H), 2.10-1.8 (m, 14H), 1.68 (s, 3H), 1.60 (s, 9H), 1.56 (s, 3H), 1.33 (s, 3H), 1.31 (s, 3H). Anal. Calcd for $C_{28}H_{48}O_2S$: C, 74.94; H, 10.79. Found: C, 74.86; H, 10.67.

4,8-Dimethyl-3(*E*),7-nonadienyl *tert*-butyldimethylsilyl ether (99): This was prepared in 94% yield by the procedure described for the preparationof **82. 99**: IR (film) 2956, 2928, 2857, 1663, 1463, 1382, 1255, 1103 and 836 cm^{-1}; CIMS *m/z* (rel. intensity) 284 (M^++2, 2.4), 283 (M^++1, 10.3), 225 (27.2) 151 (100), 149 (9.6), 137 (4.0), 123 (4.9); ^{1}H NMR ($CDCl_3$, ppm) 5.12-5.09 (m, 2H), 3.58 (t, J = 7.2 Hz, 2H), 2.23 (dt, J = 7.2, 7.1 Hz, 2H), 2.08-1.98 (m, 4H), 1.68 (s, 3H), 1.61 (s, 3H), 1.60 (s, 3H), 0.892 (s, 9H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 136.95, 131.27, 124.35, 120.35, 63.12, 39.76, 31.87, 26.71, 25.96, 25.62, 18.35, 17.61, 16.13, -5.26. Anal. Calcd for $C_{17}H_{34}OSi$: C, 72.27; H, 12.13. Found: C, 72.16; H, 12.29.

7-Bromo-8-hydroxy-4,8-dimethyl-3(*E*)-nonenyl *tert*-butyldimethylsilyl ether (100): To a vigorously stirred solution of **99** (1.58 g, 5.60 mmol) in THF (106 mL) and water (28 mL) at 0 °C was added dropwise over 30 min a solution of NBS (1.0 g, 5.61 mmol) in THF (16 mL) and water (4.9 mL). The mixture was stirred for 1 h at 0°C, the THF was removed *in vacuo*, and the aqueous phase was extracted with diethyl ether (3 X 30 mL). Standard work-up followed by flash column chromatography gave recovered starting material **99** (0.42 g) and pure **100** (0.85 g, 40% yield) as a colourless oil: IR (film): 3443, 2929, 2857, 1461, 1384, 1255, 1103 and 836 cm^{-1}; CIMS *m/z* (rel. intensity) 379 (M^++1, 31.8), 321 (8.3), 249 (76.5), 247 (73.8), 229 (17.1), 167 (31.9), 149 (100); ^{1}H NMR ($CDCl_3$, ppm) 5.21 (t, J = 7.2

Hz, 1H), 3.98 (dd, J = 1.9, 11.3 Hz, 1H), 3.59 (t, J = 7.0 Hz, 2H), 2.34 (m, 1H), 2.23 (dt, J = 7.1, 7.2 Hz, 2H), 2.13-2.07 (m, 1H), 2.01-1.93 (m, 2H), 1.86-1.74 (m, 1H), 1.61 (s, 3H), 1.59 (s, 3H), 1.34 (s, 3H), 1.32 (s, 3H), 0.89 (s, 9H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 135.13, 122.08, 72.45, 70.84, 62.97, 38.29, 31.84, 26.65, 25.85, 25.65, 18.39, 16.07, -5.24. Anal. Calcd for $C_{17}H_{35}BrO_2Si$: C, 53.81; H, 9.30. Found: C, 53.63; H, 9.34.

7,8-Epoxy-4,8-dimethyl-3(*E*)-nonenyl *tert*-butyldimethylsilyl ether (101): To a solution of **100** (0.5 g, 1.32 mmol) in methanol (25 mL) was added K_2CO_3 (0.365 g, 2.64 mmol) at room temperature. This mixture was stirred for 1 h, then most methanol was removed *in vacuo*. The slurry was diluted with water (20 mL) and the aqueous phase was extracted with diethyl ether (3 X 20 mL). Standard work-up followed by flash column chromatography using ethyl acetate:hexane (1:9) as the eluant gave pure **101** (0.373 g, 95% yield) as a colourless oil: IR (film): 2957, 2923, 2857, 1670, 1472, 1463, 1378, 1254, 1098 and 836 cm^{-1}; CIMS *m/z* (rel. intensity) 300 (M^++2, 6.2), 299 (M^++1, 27.2), 281 (25.4), 241 (12.4), 167 (63.3), 149 (100); ^1H NMR ($CDCl_3$, ppm) 5.17 (t, J =7.2 Hz, 1H), 3.58 (t, J = 7.1 Hz, 2H), 2.70 (t, J = 6.3 Hz, 1H), 2.23 (dt, J = 7.2 Hz, 7.2 Hz, 2H), 2.18-2.04 (m, 2H), 1.71-1.64 (m, 1H), 1.63 (s, 3H), 1.62-1.56 (m, 1H), 1.29 (s, 3H), 1.25 (s, 3H), 0.89 (s, 9H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 136.06, 121.13, 64.15, 63.03, 58.24, 36.36, 31.89, 27.49, 25.98, 24.89, 18.75, 18.38, 16.17, -5.23. Anal. Calcd for $C_{17}H_{34}O_2Si$: C, 68.40; H, 11.49. Found: C, 68.38; H, 11.53.

7,8-Epoxy-4,8-dimethyl-3(*E*)-nonen-1-ol (102): This was prepared by the same procedure as described for the preparation of **84**. Flash chromatography using hexane:ethyl acetate (2:3) as the eluant gave **102** in 91% yield: IR (film) 3424,

2960, 2827, 1668, 1451, 1379, 1122, 1049 and 874 cm^{-1}; CIMS *m/z* (rel. intensity) 186 (M^++2, 15.9), 185 (M^++1, 68.6), 167 (100), 149 (24.4), 123 (18.6), 109 (8.4); ^1H NMR ($CDCl_3$, ppm) 5.20 (t, *J* = 7.2 Hz, 1H), 3.62 (dt, *J* = 2.3, 6.3 Hz, 2H), 2.69 (dd, *J* = 5.3, 7.0 Hz, 1H), 2.36-2.11 (m, 5H), 1.74-1.61 (m, 2H), 1.66 (s, 3H), 1.29 (s, 3H), 1.25 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 137.79, 120.97, 64.34, 62.52, 58.21, 36.78, 31.66, 27.37, 24.92, 18.86, 16.27. Anal. Calcd for $C_{11}H_{20}O_2$: C, 71.70; H, 10.94. Found: C, 71.60; H, 10.82.

7.8-Epoxy-4,8-dimethyl-3(*E*)-nonenyl methanesulfonate (103): This was prepared in 98% yield by the procedure described for the preparation of **85**. Compound **103** was obtained sufficiently pure to be used for the next reaction without purification. **103**: ^1H NMR ($CDCl_3$, ppm) 5.17 (t, *J*=7.2 Hz, 1H), 4.18 (t, *J* = 7.0 Hz, 2H), 2.99 (s, 3H), 2.69 (t, *J*=6.2 Hz, 1H), 2.46 (dt, *J* = 7.1,7.0 Hz, 2H), 2.23-2.10 (m, 2H), 1.65-1.58 (m, 2H), 1.66 (s, 3H), 1.30 (s, 3H), 1.26 (s, 3H).

1-Bromo-3,7,11-trimethyl-2(*E*),6(*E*),10-dodecatriene (farnesyl bromide) (105): This was prepared according to the procedure of Katzenellenbogen *et al.*[45] in 91% yield. ^1H NMR ($CDCl_3$, ppm) 5.55 (t of partially resolved m, 1H), 5.25-4.91 (m, 2H), 4.05 (d, *J* = 8.0 Hz, 2H), 2.28-1.90 (m, 8H), 1.74 (d, *J* = 1.3 Hz, 3H), 1.69 (s, 3H), 1.61 (s, 6H).

Ethyl 5,9,13-trimethyl-4(*E*),8(*E*),12-tetradecatrienoate (106): This was prepared according to the procedure of Coates *et al.*[46] To a slurry of CuI (5.33 g, 56 mmol) and ethyl acetate (2.75 mL, 28 mmol, freshly distilled from CaH_2) in THF (120 mL) at -100 °C under argon was added dropwise a solution of LDA (28 mmol), prepared from *n*-BuLi (11.2 mL, 28 mmol, 2.5 M in hexane) and diisopropyl amine

(3.95 mL, 28.1 mmol) in THF (30 mL) at -78 °C. This was stirred for 1 h at -100 °C. A solution of farnesyl bromide (**105**) (4.0 g, 14 mmol) in THF (20 mL) was added slowly to the reaction mixture. The reaction mixture was allowed to warm to 0 °C over a period of 5 h. A solution of saturated NH_4Cl (120 mL) was added, the mixture was stirred open to air for 0.5 h and then extracted with diethyl ether (4 X 60 mL). Standard workup followed by chromatography using ethyl acetate:hexane (15:85) as the eluant gave **106** (3.74 g, 91% yield) as an oil : IR (film) 2977, 2924, 1738, 1447, 1375, 1179, and 1098 cm^{-1}; CIMS *m/z* (rel. intensity) 293 (M^++1, 100), 292 (M^+,9.3), 247 (45.6), 225 (22.1), 211 (56.6), 197 (27.8), 183 (30), 169 (49), 157 (29), 137 (71), 123 (33); ^1H NMR ($CDCl_3$, ppm) 5.08 (m, 3H), 4.12 (q, *J* = 7.6 Hz, 2H), 2.30 (m, 4H), 2.04 (m, 4H), 1.97 (m, 4H), 1.67 (s, 3H), 1.61 (s, 3H), 1.59 (s, 3H), 1.58 (s, 3H), 1.25 (t, J = 7.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, ppm) 173.3, 136.6, 135.0, 131.2, 124.4, 124.1, 122.4, 60.1, 39.7, 39.6, 34.5, 26.8, 26.7, 25.6, 23.6, 17.6, 14.2. The ^1H NMR spectrum is in agreement with that reported in reference 46.

5,9,13-Trimethyl-4(*E*),8(*E*),12-tetradecatrienol (107): To a solution of $LiAlH_4$ (2 g, 50 mmol) in dry ether (50 mL) at 0 °C under argon was added dropwise a solution of **106** (7.32 g, 25 mmol) in ether (20 mL). The mixture was warmed to room temperature and stirred for 3 h. Excess $LiAlH_4$ was destroyed at -20 °C by slow addition of water (1 mL) followed by 15% NaOH (1 mL). The reaction mixture was filtered and the white precipitate was washed with ether (3 X 20 mL). Standard work-up followed by flash chromatography using ethyl acetate:hexane (3:7) as the eluant gave **107** (5.75 g, 92% yield): IR (film) 3346, 2963, 2927, 2850, 1448, 1381, 1058 and 833 cm^{-1}; CIMS *m/z* (rel. intensity) 251 (M^++1, 62), 250 (M^+, 9), 233 (18), 205 (4), 181(22), 169 (35), 137 (100), 123 (52); ^1H NMR ($CDCl_3$, ppm) 5.17-5.04 (m, 3H), 3.63 (t, *J* = 5.88 Hz, 2H), 2.10-1.94 (m, 10H), 1.67 (s, 3H), 1.61 (m,

2H), 1.60 (s, 3H), 1.59 (s, 6H); ^{13}C NMR (CDCl3, ppm) 135.8, 135.0, 131.2, 124.4, 124.1, 123.8, 62.7, 39.7, 32.8, 26.8, 26.6, 25.6, 24.3, 17.6, 16.0.

5,9,13-Trimethyl-4(*E*),8(*E*),12-tetradecatrienyl thioacetate (108): This was prepared in 80% yield by the procedure described for the preparationof **96**. **108**: IR (film): 2965, 2923, 2854, 1695, 1446, 1382, 1353, 1134, 1108, 936 and 835 cm^{-1}; MS *m/z* (rel intensity): 309 (M^++1, 1.3) 308 (M^+, 5.2), 265 (14.1), 197 (9.3), 136 (27.0), 129 (37.4), 121 (11.1); ^1H NMR ($CDCl_3$, ppm) 5.10-5.09 (m, 3H), 2.86 (t, J = 7.20 Hz, 3H), 2.32 (s, 3H), 2.09-1.96 (m, 10H), 1.68 (s, 3H), 1.62 (m, 2H), 1.60 (s, 9H); ^{13}C NMR ($CDCl_3$, ppm) 195.89, 136.33, 135.11, 131.30, 124.53, 124.25, 123.19, 39.81, 30.67, 29.68, 28.84, 27.15, 26.88, 26.69, 25.74, 17.75, 16.14, 16.09. Anal. Calcd for $C_{19}H_{32}OS$: C, 73.97; H, 10.45. Found: C, 73.83; H, 10.34.

5,9,13-Trimethyl-4(*E*),8(*E*),12-tetradecatrienthiol (109): This was prepared in 92% yield by the procedure described for the preparation of **97**. **109**: IR (film) 2964, 2924, 2855, 1442, 1377, 1259, 1222 and 834 cm^{-1}; CIMS *m/z* (rel. intensity) 267 (M^++1, 100), 266 (M^+, 10.3), 195 (7.5), 177 (10.9), 137 (2.2), 136 (1.2), 123 (1.1); ^1H NMR (CDCl3, ppm) 5.11-5.07 (m, 3H), 2.51 (dt, J = 7.3, 7.2 Hz, 2H), 2.12-1.95 (m, 10H), 1.68 (s, 3H), 1.65 (m, 2H), 1.61 (s, 3H), 1.60 (s, 6H), 1.32 (t, J = 7.82 Hz, 1H). Anal. calcd for $C_{17}H_{30}S$: C, 76.62; H, 11.36. Found: C, 76.55; H, 11.50.

10-Thia-11-dehydro-2,3-oxidosqualene (64): This was prepared in 73% yield by the procedure described for the preparation of **63**. **64**: IR (film) 2961, 2920, 2854, 1681, 1448, 1377, 1249, 1122, 1043 and 834 cm^{-1}; CIMS *m/z* (rel. intensity) 435 (M^++3, 9.6), 434 (M^++2, 30.6), 433 (M^++1, 100), 415 (9.9), 283 (6.9), 201 (11.3), 167 (14.9), 151 (1.6), 149 (14.7), 137 (4.9), 123 (3.4); ^1H NMR ($CDCl_3$, ppm)

5.21 (t, J = 7.0 Hz, 1H), 5.12-5.07 (m, 3H), 2.71 (t, J = 6.2 Hz, 1H), 2.51 (t, J = 8.0 Hz, 4H), 2.28 (dt, J = 7.4, 7.4 Hz, 2H), 2.17-1.95 (m, 14H), 1.72-1.62 (m, 2H), 1.68 (s, 3H), 1.63 (s, 3H), 1.60 (s, 9H), 1.30 (s, 3H), 1.26 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 135.95, 135.71, 134.99, 131.25, 124.42, 124.18, 123.49, 123.22, 64.07, 58.25, 39.72, 36.30, 32.17, 31.78, 29.84, 28.44, 27.41, 27.12, 26.79, 26.62, 25.64, 24.86, 18.74, 17.65, 16.06. Anal. Calcd. for $C_{28}H_{48}OS$: C, 77.71; H, 11.18. Found: C, 77.80; H, 11.14.

10-Sulfinyl-11-dehydro-2,3-oxidosqualene (68): This was prepared in 84% yield by oxidation of **64** using the same procedure described for the preparation of **67**. **68**: IR (film) 2961, 2922, 2860, 1665, 1449, 1377 and 1047 cm^{-1}; CIMS *m/z* (rel. intensity) 449 (M^++1, 14.5), 283 (100), 265 (55), 167 (59), 149 (76); ^1H NMR ($CDCl_3$, ppm) 5.20 (t, J = 7.0 Hz, 1H), 5.12-5.05 (m, 3H), 2.76-2.56 (m, 5H), 2.49 (dt, J = 7.5, 7.5 Hz, 2H), 2.25-1.93 (m, 14H), 1.81 (quintet, J = 7.2 Hz, 2H), 1.67 (s, 6H), 1.59 (s, 9H), 1.30 (s, 3H), 1.26 (s, 3H). Anal. Calcd. for $C_{28}H_{48}O_2S$: C, 74.94; H, 10.79. Found: C, 74.90; H, 10.75.

3,7,11-Trimethyl-2(*E*),6(*E*), 10-dodecatrienal (farnesal) (110): This was prepared by Swern oxidation of farnesol **92** according to the method of Dodd and Oehlschlager.[47] Chromatography using ethyl acetate:hexane (1:9) as the eluant gave **110** in 93% yield: IR (film) 2966, 2917.7, 2854, 1676, 1632, 1443, 1381, 1194, 1120 and 832 cm^{-1}; MS, *m/z* (rel intensity) 220 (M^+, 1.5), 191 (2.7), 177 (4.9), 151 (2.8), 136 (9.8), 123 (6.4), 121 (8.3), 84 (49.5), 69 (100); ^1H NMR ($CDCl_3$, ppm) 9.99 (d, J = 8.11 Hz, 1H), 5.88 (dq, J = 1.16, 8.1 Hz, 1H), 5.10-5.04 (m, 2H), 2.23-2.20 (m, 4H), 2.17 (d, J = 1.27 Hz, 3H), 2.06-2.03 (m, 2H), 2.00-1.95 (m, 2H), 1.67 (s, 3H), 1.60 (s, 3H), 1.59 (3, 3H); ^{13}C NMR ($CDCl_3$, ppm) 191.15, 163.62, 136.59,

131.45, 127.45, 124.15, 122.49, 40.63, 39.63, 26.65, 25.71, 25.64, 17.65, 17.58, 16.04.

4,8,12-Trimethyl-1,3(*E*),7(*E*),11-tridecatetraene (111): This was also prepared according to the method of Dodd and Oehlschlager[47] in 90% yield. **111**: IR (film) 3084, 2966, 2924, 2855, 1651, 1598, 1447, 1379, 986 and 896 cm^{-1}; CIMS, *m/z* (rel. intensity) 219 (M^++1, 100), 218 (M^+, 17.4), 205 (19.5), 191 (9.1), 177 (7.1), 163 (23.7), 149 (26.4), 137 (37.8), 123(19.4), 121(14.2); ^1H NMR ($CDCl_3$, ppm) 6.55 (ddd, J = 10.4, 10.5, 16.8 Hz, 1H), 5.87 (d, J = 10.7 Hz, 1H), 5.14-5.07 (m, 3H), 4.98 (dd, J =1.70, 10.2 Hz, 1H), 2.19-2.04 (m, 6H), 1.98 (t, J = 8.2 Hz, 2H), 1.77 (s, 3H), 1.68 (s, 3H), 1.61 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 139.44, 135.33, 133.44, 131.24, 125.53, 124.39, 123.89, 114.48, 39.86, 39.71, 26.77, 26.45, 25.65, 17.65, 16.65.

4,8,12-Trimethyl-3(*E*),7(*E*),11-tridecatrienol (112): Hydroboration of **111** (3.20 g, 14.7 mmol) with disiamylborane followed by oxidation with H_2O_2/NaOH gave **112** in 88% yield according to the method of Dodd and Oehlschlager.[47] **112**: IR (film) 3348, 2965, 2926, 2854, 1668, 1448, 1377, 1104, 1048 and 835 cm^{-1}; mass spectrum *m/z* (rel intensity) 236 (M^+, 1.6), 193 (3.0), 152 (2.7), 149 (2.1), 136 (15.2), 123 (13.0), 121 (8.5), 81 (42.0), 69 (100); ^1H NMR ($CDCl_3$, ppm) 5.11-5.06 (m, 3H), 3.60 (t, J = 6.5 Hz, 2H), 2.27 (dt, J = 6.6, 6.8 Hz, 2H), 2.10-1.96 (m, 8H), 1.67 (s, 3H), 1.64 (s, 3H), 1.59 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 138.78, 135.24, 131.24, 124.34, 123.98, 119.88, 62.41, 39.78, 39.69, 31.51, 26.74, 26.49, 25.64, 17.63, 16.18, 15.99.

4,8,12-Trimethyl-3(*E*),7(*E*),11-tridecatrienyl *tert*-butyldimethylsilyl ether (113): This was prepared in 91% yield by the procedure described for the

preparation of **82**. **113**: IR (film) 2965, 2928, 2857, 1669, 1462, 1382, 1255, 1103, 836 and 775 cm^{-1}; MS *m/z* (rel. intensity) 350 (M^+,1.8), 293 (22.1), 217 (12.0), 191 (18.1), 157 (10.1), 135 (10.3), 123 (7.1), 121 (8.3), 109 (12.7); ^1H NMR ($CDCl_3$, ppm) 5.16-5.04 (m, 3H), 3.57 (t, *J* = 6.3 Hz, 2H), 2.23 (dt, *J* = 7.1, 7.1 Hz, 2H), 2.09-1.95 (m, 8H), 1.68 (s, 3H), 1.62 (s, 3H), 1.59 (s, 6H), 0.89 (s, 9H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 137.12, 135.08, 131.31, 124.52, 124.31, 120.41, 63.73, 39.86, 31.99, 26.89, 26.72, 26.07, 25.74, 17.74, 16.25, 16.07, -5.14. Anal. Calcd. for $C_{22}H_{42}OSi$: C, 75.36; H, 12.07. Found: C, 75.58; H, 12.30.

11-Bromo-12-hydroxy-4,8,12-trimethyl-3(*E*),7(*E*)-tridecadienyl *tert*-butyldimethylsilyl ether (114): This was prepared in 37% yield by the same procedure as described for the preparation of **100**. **114**: IR (film) 3450, 2954, 2928, 2856, 1667, 1471, 1446, 1384, 1101 and 836 cm^{-1}; CIMS *m/z* (rel. intensity) 447 (M^++1, 7.9), 429 (1.2), 315 (33.3), 299 (22.4), 297 (24.8), 235 (25.3), 217 (100); ^1H NMR ($CDCl_3$, ppm) 5.20 (t, *J* = 6.4 Hz, 1H), 5.13 (t, *J* = 7.2 Hz, 1H), 3.98 (dd, *J* = 1.8, 11.9 Hz, 1H), 3.58 (t, *J* = 7.2 Hz, 2H), 2.35-2.28 (m, 1H), 2.23 (dt, *J* = 7.2, 7.2 Hz, 2H), 2.14-2.06 (m, 4H), 2.02-1.92 (m, 2H), 1.83-1.73 (m, 1H), 1.62 (s, 3H), 1.59 (s, 3H), 0.89 (s, 9H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 136.77, 133.09, 125.93, 120.52, 72.44, 70.87, 63.11, 39.65, 38.18, 32.21, 31.88, 26.67, 26.60, 25.97, 25.85, 18.36, 16.14, 15.83, -5.23. Anal. Calcd for $C_{22}H_{43}O_2BrSi$: C, 59.04; H, 9.68. Found: C, 58.98; H, 9.59.

11,12-Epoxy-4,8,12-trimethyl-3(*E*),7(*E*)-tridecadienyl *tert*-butyldimethylsilyl ether (115): This was prepared in 95% yield by the same procedure as described for the preparation of **101**. **115:** IR (film) 2956, 2928, 2857, 1667, 1472, 1378, 1254, 1102, 836 and 776 cm^{-1}; CIMS *m/z* (rel. intensity) 367 (M^++1, 12), 349 (3.4), 309 (5.0), 235 (76.1), 218 (16.7), 217 (100), 191 (22.1); ^1H NMR ($CDCl_3$, ppm)

5.17-5.10 (m, 2H), 3.57 (t, J = 7.2 Hz, 2H), 2.70 (t, J = 6.2 Hz, 1H), 2.22 (dt, J = 7.1, 7.2 Hz, 2H), 2.17-2.00 (m, 6H), 1.69-1.60 (m, 2H), 1.61 (s,6H), 1.30 (s, 3H), 1.26 (s,3H), 0.89 (s, 9H), 0.045 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 136.87, 134.10, 124.87, 120.44, 64.18, 63.12, 58.23, 39.69, 36.32, 31.88, 27.52, 26.64, 25.98, 24.98, 18.75, 18.37, 16.16, 15.98, -5.23; Anal. Calcd for $C_{22}H_{42}O_2Si$: C, 72.07; H, 11.56. Found: C, 72.03; H, 11.44.

11,12-Epoxy-4,8,12-trimethyl-3(*E*),7(*E*)-tridecadienol (116): This was prepared in 92% yield by same procedure as described for the preparation of **102**. **116**: IR (film) 3439, 2960, 2823, 2856, 1669, 1449, 1378, 1122, 1049 and 874 cm^{-1}; CIMS *m/z* (rel. intensity) 253 (M^++1, 85.5), 235 (100), 217 (33.1), 191 (21.1), 167 (14.0), 153 (39.9),149 (18.7), 135 (31.1), 123 (17.2), 121 (13.4); ^1H NMR ($CDCl_3$, ppm) 5.15-5.10 (m, 2H), 3.61(dt, J = 6.3, 6.1 Hz, 2H), 2.70 (t, J = 6.3 Hz, 1H), 2.28 (dt, J = 6.6, 6.7 Hz, 2H), 2.18-2.02 (m, 6H), 1.72-1.61 (m, 2H), 1.64 (s, 3H), 1.61 (s, 3H), 1.48 (t, J = 5.84 Hz, 1H), 1.29 (s, 3H), 1.25 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 138.52, 134.34, 124.69, 120.12, 64.16, 62.43, 58.29, 39.71, 36.31, 31.54, 27.46, 26.48, 24.88, 18.74, 16.19, 16.00. Anal. Calcd for $C_{16}H_{28}O_2$: C, 76.14; H, 11.18. Found: C, 76.30; H, 11.23.

11,12-Epoxy-4,8,12-trimethyl-3(*E*),7(*E*)-tridecadienyl methanesulfonate (117): This was prepared in 95% yield by the procedure described for the preparation of **85**. Compound **117** was used for the next reaction without further purification: ^1H NMR ($CDCl_3$, ppm) 5.26-5.00 (m, 2H), 4.19 (t, J = 7.0 Hz, 2H), 3.01 (s, 3H), 2.71 (t, J = 6.4 Hz, 1H), 2.47 (dt, J = 7.3, 7.2 Hz, 2H), 2.21-2.00 (m, 6H), 1.72-1.59 (m, 2H), 1.63 (s, 6H), 1.31 (s, 3H), 1.27 (s, 3H).

6,10-Dimethyl-5(*E*),9-undecadien-2-ol (119): A solution of **118** (2.23 g, 11.5 mmol) in dry ether (10 mL) was slowly added to a stirred suspension of $LiAlH_4$ (0.95 g, 23.8 mmol) in dry ether (40 mL) at 0 °C. After 0.5 h at 0 °C, excess $LiAlH_4$ was destroyed at -30 °C by slow addition of water (1 mL) followed by 15% NaOH (1 mL). The resulting mixture was filtered and the white precipitate was washed with ether (2 X 10 mL). Standard work-up followed by flash colunm chromatography using ethyl acetate:hexane (3:7) as the eluant gave **119** (2.16 g, 96% yield) as a colourless liquid: IR (film) 3346, 2966, 2925, 2856, 1450, 1376, 1128, 1084 and 833 cm^{-1}; MS *m/z* (rel intensity) 196 (M^+, 1.4),153 (96), 135 (52.9), 123 (20.0), 109 (100), 81 (23.8), 69 (66.4); ^1H NMR ($CDCl_3$, ppm) 5.14 (m, 1H), 5.08 (m, 1H), 3.82-3.76 (m, 1H), 2.10-1.98 (m, 6H), 1.67 (s, 3H), 1.62 (s, 3H), 1.59 (s, 3H), 1.52-1.46 (m, 2H), 1.19 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, ppm) 135.76, 131.47, 124.38, 124.06, 68.04, 39.81, 39.32, 26.77, 25.73, 24.47, 23.56, 17.75, 16.06. Anal. Calcd for $C_{13}H_{24}O$: C, 79.52; H, 12.33. Found: C, 79.40; H, 12.29.

6,10-Dimethyl-5(*E*),9-undecadien-2-yl thioacetate (120): This was prepared in 80% yield by the procedure described for the preparation of **96**. **120**: IR (film) 2965, 2923, 2855, 1692, 1449, 1376, 1352, 1113 and 952 cm^{-1}; CIMS *m/z* (rel. intensity) 255 (M^++1, 26.6), 214 (15.3), 213 (100), 211 (13.4), 179 (11.7), 173 (5.3), 131 (12.7); ^1H NMR ($CDCl_3$, ppm) 5.11-5.06 (m, 2H), 3.59-3.50 (m, 1H), 2.30 (s, 3H), 2.09-2.03 (m, 6H), 1.69 (s, 3H), 1.61-1.54 (m, 2H), 1.60 (s, 3H), 1.59 (s, 3H), 1.30 (d, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, ppm) 135.89, 131.31, 124.32, 123.29, 39.69, 39.35, 36.43, 30.73, 26.69, 25.64, 25.51, 23.80, 21.38, 17.66, 15.97. Anal. Calcd for $C_{15}H_{26}OS$: C, 70.81; H, 10.30. Found: C, 70.94; H, 10.41.

6,10-Dimethyl-5(*E*),9-undecadien-2-thiol (121): This was prepared in 91% yield by the procedure described for the preparation of **97**. **121**: IR (film) 2965, 2922, 2854, 1668, 1449, 1376, 1109 and 833 cm^{-1}; CIMS, *m/z* (rel intensity) 212 (M^+, 7.6), 169 (4.3), 143 (11.5), 141 (14.4), 109 (21.2), 101 (30.7), 81 (21.0), 69 (100); ^1H NMR ($CDCl_3$, ppm) 5.10-5.05 (m, 2H), 2.97-2.87 (m, 1H), 2.14-2.04 (m, 4H), 2.00-1.96 (m, 2H), 1.68 (s, 3H), 1.62 (s, 3H), 1.60 (s, 3H), 1.59-1.52 (m, 2H), 1.47 (d, J = 6.3 Hz, 1H), 1.33 (d, J = 6.7 Hz, 3H). Anal. Cacld for $C_{13}H_{24}S$: C, 73.52; H, 11.39. Found: C, 73.60; H, 11.31.

14-Thia-15-dehydro-2,3-oxidosqualene (65): This was prepared in 31% yield by the procedure described for the preparation of **63**. **65**: IR (film) 2961, 2918, 2871, 1668, 1449, 1376, 1248, 1122 and 874 cm^{-1}; CIMS *m/z* (rel. intensity) 448 (M^++2, 34.5), 447 (M^++1, 100),446 (M^+, 5.5),429 (11.6), 377 (2.6), 269 (3.1), 217 (3.7); ^1H NMR ($CDCl_3$, ppm) 5.18-5.14 (m, 2H), 5.11-5.07 (m, 2H), 2.76 (sextet, J = 6.7 Hz, 1H), 2.70 (t, J = 6.2 Hz, 1H), 2.51 (t, J = 7.7 Hz, 2H), 2.26 (dt, J = 7.4, 7.6 Hz, 2H), 2.15-1.97 (m, 14H), 1.68 (s, 3H), 1.61 (s, 9H),1.60 (s, 3H), 1.45-1.51 (m, 2H), 1.30 (s, 3H), 1.27 (d, J = 6.7 Hz, 3H), 1.26 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 136.42, 135.61, 134.17, 131.30, 124.80, 124.36, 123.81, 122.80, 64.14, 58.24, 39.74, 38.64, 39.60, 37.15, 36.33, 30.35, 28.75, 27.53, 26.74, 26.60, 25.66, 25.49, 24.90, 23.37, 21.45, 18.76, 17.67, 16.15, 16.07. Anal. Calcd for $C_{29}H_{50}OS$: C, 77.97; H, 11.20. Found: C, 78.07; H, 11.40.

14-Sulfinyl-15-dehydro-2,3-oxidosqualenes (69) and (70) These were prepared by the procedure described for the preparation of **67**. Chromatography using ethyl acetate:hexanes (7:3) as the eluant gave the two diastereomers **69** and **70** in 24% and 33% yields, respectively. **69**: R_f 0.44 (silica, ethyl acetate:hexanes

7:3); IR (film) 2963, 2922, 2856, 1666, 1450,1377, 1116 and 1047 cm^{-1}; CIMS *m/z* (rel. intensity) 463 (M^++1, 6.5), 285 (10), 269 (14), 229 (100), 217 (30.6); ^1H NMR ($CDCl_3$, ppm) 5.20-5.11 (m, 2H), 5.11-5.03 (m, 2H), 2.73 (m, 1H), 2.70 (t, *J* = 6.3 Hz, 1H), 2.61-2.53 (m, 2H), 2.53-2.44 (m, 2H), 2.28-1.94 (m, 14H), 1.62-1.70 (m, 2H), 1.68 (s, 3H), 1.65 (s,3H), 1.61 (s, 3H), 1.60 (s, 3H), 1.57 (s, 3H), 1.30 (s, 3H), 1.26 (s, 3H), 1.23 (d, *J* = 6.9 Hz, 3H). Anal.Calcd for $C_{29}H_{50}O_2S$: C, 75.27; H, 10.90. Found: C, 75.26; H, 11.04. **70**: R_f 0.35 (ethyl acetate:hexanes 7:3); IR (film) 2962, 2922, 2850, 1666, 1450,1377, 1117 and 1047 cm^{-1}; CIMS *m/z* (rel. intensity) 463 (M^++1, 11.6), 285 (16.3), 269 (40), 229 (100), 217 (27.7); ^1H NMR ($CDCl_3$, ppm) 5.20-5.11 (m, 2H), 5.11-5.03 (m, 2H), 2.70 (t, *J* = 6.3 Hz, 1H), 2.67 (m, 1H), 2.61-2.53 (m, 2H), 2.53-2.44 (m, 2H), 2.27-1.93 (m, 14H), 1.62-1.70 (m, 2H), 1.67 (s, 3H), 1.65 (s, 3H), 1.61 (s, 3H), 1.60 (s, 3H), 1.57 (s, 3H), 1.30 (s, 3H), 1.27 (d, *J* = 6.9 Hz, 3H), 1.26 (s, 3H). Anal.Calcd for $C_{29}H_{50}O_2S$: C, 75.27; H, 10.90. Found: C, 75.20; H, 11.05.

16-Bromo-17-hydroxy-4,9,13,17-tetramethyl-4(*E*),8(*E*),12(*E*)-octadecatrienyl *tert*-butyldimethylsilyl ether (122): This was prepared in 36% yield form **94** by the procedure described for the preparation of **100**. **122**: IR (film) 3456, 2958, 2856, 1462, 1384, 1254, 1101, 836 and 775 cm^{-1}; ^1H NMR ($CDCl_3$, ppm) 5.20 (t, *J* = 6.5 Hz, 1H), 5.17-5.10 (m, 2H), 3.98 (dd, *J* = 1.9, 11.4 Hz, 3.58 (t, *J* = 6.6 Hz, 2H), 2.33-2.28 (m, 1H), 2.13-2.06 (m, 4H), 2.01-1.90 (m, 10H), 1.83-1.74 (m, 1H), 1.64-1.61 (m, 1H), 1.60 (s, 9H), 1.34 (s, 3H), 1.33 (s, 3H), 0.89 (s, 9H), 0.05 (s, 6H). IR and ^1H NMR spectra are in agreement with those reported in reference 25c.

16,17-Epoxy-4,9,13,17-tetramethyl-4(*E*),8(*E*),12(*E*)-octadecatrienol (124): To a solution of **122** (0.456 g, 0.86 mmol) in methanol (20 mL) was added K_2CO_3

(0.276 g, 2.0 mmol) at room temperature. This mixture was stirred for 1 h after which time most of methanol was removed *in vacuo*. The resulting slurry was diluted with water (20 mL) and the aqueous phase was extracted with diethyl ether (3 X 20 mL). Standard work-up gave the protected epoxy alcohol **123** (0.364 g). To a solution of **123** (0.364 g, 0.81 mmol) in THF (20 mL) at room temperature was added tetrabutylammonium flouride (8 mL, 1 M solution in THF, 8 mmol). The resulting mixture was stirred overnight at room temperature. Water (10 mL) was added and the mixture was extracted with diethyl ether (3 X 20 mL). Standard work-up followed by flash column chromatography using ethyl acetate:hexane (4:6) as the eluant gave **124** (0.255 g, 89% yield, over two steps): IR (film) 3440, 2957, 2925, 2856, 1667, 1448, 1379, 1058 and 871 cm^{-1}; ^1H NMR ($CDCl_3$, ppm) 5.20-5.08 (m, 3H), 3.63 (dt, J = 6.4, 6.4 Hz, 2H),2.70 (t, J = 6.3 Hz, 1H), 2.18-1.97 (m, 13H), 1.69-1.97 (m, 4H), 1.69-1.64 (m, 4H), 1.61 (s, 6H), 1.56 (s, 3H), 1.30 (s, 3H), 1.26(s, 3H). IR and ^1H NMR spectra were in agreement with those reported in reference 25c.

16,17-Epoxy-4,9,13,17-tetramethyl-4(*E*),8(*E*),12(*E*)-octadecatrienyl methanesulfonate (125): This was prepared in 97% yield by the procedure described for the preparation of **85**. Compound **125** was used in the next reaction without further purification:^1H NMR ($CDCl_3$, ppm) 5.20-5.10 (m, 3H), 4.20 (t, J = 6.5 Hz, 2H), 3.00 (s, 3H), 2.70 (t, J = 6.2 Hz, 1H), 2.18-1.96 (m, 12H), 1.90-1.80 (m, 2H),1.76-1.60 (m, 2H), 1.61(s, 3H), 1.60 (s, 3H), 1.57 (s, 3H), 1.30 (s, 3H), 1.25 (s, 3H).

4-Methyl-3-pentenyl thioacetate (127): This was prepared in 75% yield by the procedure described for the preparation of **96** except that the solvent was carefully distilled off at atmospheric pressure using 20-cm Vigreux column. The

residue was purified by flash chromatography using diethyl ether:pentane (2:3) as eluant and the solvent was removed again through a Vigreux column to give **127**: GC purity 96%; IR (film) 2965, 2920, 2853, 1691, 1450, 1374 and 1113 cm^{-1}; MS, *m/z* (rel. intensity) 159 (M^++1, 5.5), 158 (M^+, 59.9), 115 (2.9), 101 (2.0), 82 (100), 69 (22.8), 67 (29.1); ^1H NMR ($CDCl_3$, ppm) 5.14-5.06 (m, 1H), 2.86 (t, J = 7.4 Hz, 2H), 2.32 (s, 3H), 2.25 (dt, J = 7.2,7.4 Hz, 2H), 1.70 (s, 3H), 1.62 (s, 3H).

4-Methyl-3-pentene-1-thiol (128): This was prepared in 57% yield by the procedure described for the preparation of **97** except that the solvent was carefully distilled off at atmospheric pressure using Vigreux column to give **128**: GC purity 94%; IR (film) 2962, 2920, 2855, 1666,1450, 1378 and 832 cm^{-1}; MS *m/z* (rel. intensity) 117 (M^++1, 1.0), 116 (M^+, 12.4), 101 (100), 83 (2.6), 69 (92.0), 67 (33.2), 55 (15.1), 53 (14.2), 47 (14.0), 41 (69.0); ^1H NMR ($CDCl_3$, ppm) 5.12-5.08 (m, 1H), 2.52 (dt, J = 7.2, 7.4 Hz, 2H), 2.30 (dt, J =7.1, 7.2 Hz, 2H), 1.72 (s, 3H), 1.63 (s, 3H), 1.40 (t, J = 7.7 Hz, 1H).

18-Dehydro-19-thia-2,3-oxidosqualene (66): This was prepared in 80% yield by the procedure described for the preparation of **63**. **66**: IR (film) 2965, 2924, 2863, 1666, 1449, 1377, 1281, 1122 and 875 cm^{-1}; CIMS *m/z* (rel. intensity) 433 (M^++1, 100), 432 (M^+, 5.0), 415 (33.8), 391 (7.5), 377 (6.3), 351 (2.0), 279 (5.8), 185 (4.2), 143 (11.1), 127 (2.3); ^1H NMR ($CDCl_3$, ppm) 5.20-5.10 (m, 4H), 2.70 (t, J = 6.2 Hz, 1H), 2.50, 2.48 (overlap of two triplets, J = 7.7, 7.7 Hz, 4H), 2.26 (dt, J = 7.2, 7.5 Hz, 2H), 2.16-1.96 (m, 14H), 1.70 (s, 3H), 1.67-1.62 (m, 2H), 1.62 (s, 6H), 1.59 (s, 6H), 1.30 (s, 3H), 1.25 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 135.07, 134.23, 134.03, 132.99, 125.02, 124.95, 124.33, 122.74, 64.18, 58.23, 39.68, 38.81, 36.33, 32.28, 31.73, 28.58, 28.26, 28.24, 28.02, 27.53, 26.70, 25.65, 24.89, 18.75, 17.78, 16.04,16.00.

Anal.Calcd for $C_{28}H_{48}OS$: C, 77.71; H, 11.18. Found: C, 77.78; H, 11.04.

18-Dehydro-19-sulfinyl-2,3-oxidosqualene (71): This was prepared in 82% yield by the procedure described for the preparation of **67**. **71**: IR (film) 2963, 2924, 2850, 1667, 1449, 1378 and 1048 cm^{-1}; CIMS *m/z* (rel. intensity) 449 (M^++1, 54.4), 448 (M^+, 52.5), 367 (67.7), 349 (68.8), 317 (10.6), 133 (100); ^1H NMR ($CDCl_3$, ppm) 5.20-5.08 (m, 4H), 2.70 (t, J = 6.3 Hz, 1H), 2.70-2.54 (m, 4H), 2.45 (dt, J = 7.2, 7.3 Hz, 2H), 2.20-1.95 (m, 14H), 1.92-1.80 (m, 2H), 1.71 (s, 3H), 1.65 (s, 3H), 1.61 (s, 3H), 1.60 (s, 3H), 1.59 (s, 3H), 1.30 (s, 3H), 1.25 (s, 3H). Anal. Calcd for $C_{28}H_{48}O_2S$: C, 74.94; H, 10.79. Found: C, 74.92; H, 10.73.

6.4.2. Syntheses discussed in Chapter 3.

4-Iodo-3-methyl-3(*E*)-butenyl *tert*-butyldimethylsilyl ether (131): This was prepared by the procedure of Negishi *et al.*[40] To a slurry of $ZrCp_2Cl_2$ (1.68 g, 5.8 mmol) in dry CH_2Cl_2 (50 mL) at -20 °C under argon was added $AlMe_3$ (6.88 mL, 69.6 mmol) dropwise over 5 min. A solution of 3-butynol (**129**) (1.62 g, 23.2 mmol) in CH_2Cl_2 (3 mL) was then added dropwise. The mixture was warmed to room temperature, stirred for 12 h, then cooled to -30 °C and iodine (7.11 g, 28 mmol) in THF (50 mL) was added slowly. After iodine addition (20 min), excess $AlMe_3$ was destroyed (caution!!) by the addition of 5 mL of distilled water under argon at 0°C. The slurry was diluted with 80 mL of hexane and the precipitated salt was filtered through a pad of Celite. The pad was rinsed with 50 mL of hexane. The organic phase was dried over anhydrous Na_2SO_4. After evaporation of the solvents, crude 4-iodo-3-methyl-3(*E*)-butenol (**130**) (3.95 g, 80% yield) was obtained.

To a solution of **130** (3.82 g, 18 mmol) in dry CH_2Cl_2 (50 mL) at 0 °C was

added Et_3N (1.87 g, 18.5 mmol), TBSCl (2.83 g, 18.2 mmol) and DMAP (0.05 g). The reaction mixture was warmed to room temperature and stirred for 12 h. Water (20 mL) was then added and the organic phase was separated. The aqueous phase was extracted with diethyl ether (3 X 30 mL). Standard work-up followed by flash column chromatography using ethyl acetate:hexane (5:95) as eluant gave **131** (5.33 g, 91% yield) as an oil: IR (film) 2953, 2928, 2857, 1618, 1472, 1380, 1256, 1103 and 835 cm^{-1}; ^1H NMR ($CDCl_3$, ppm) 5.93 (q, J =1.1 Hz, 1H), 3.68 (t, J = 6.6 Hz, 2H), 2.41 (t, J = 6.6 Hz, 2H), 1.85 (d, J = 1.1 Hz, 3H), 0.89 (s, 9H), 0.043 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 145.26, 76.30, 61.4, 42.70, 25.96, 24.33, 18.31, -5.26. IR, ^1H NMR and ^{13}C NMR spectra of **131** are in agreement with those reported in reference 40.

Ethyl 5-[*tert*-butyldimethylsilyloxy]-3-methyl-2(*E*)-pentenoate (132): This was prepared by the procedure as described for the synthesis of **89**. **132**: IR (film) 2955, 2930, 2858, 1718, 1652, 1472 and 1100 cm^{-1}; CIMS *m/z* (rel. intensity) 273 (M^++1, 100), 257 (1.9), 215 (26.5), 141 (49.3); ^1H NMR ($CDCl_3$, ppm) 5.67 (s, 1H), 4.13 (q, J = 7.1 Hz, 2H), 3.73 (t, J = 6.9 Hz, 2H), 2.33 (t, J = 6.9 Hz, 2H), 2.17 (d, J = 1.3 Hz, 3H), 1.26 (t, J = 7.1 Hz, 3H), 0.87 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 166.61, 156.90, 117.26, 61.32, 59.40, 44.01, 25.97, 25.86, 19.10, 14.31, -5.40. IR, ^1H NMR and ^{13}C NMR spectra of **132** are in agreement with those reported in reference 47.

5-[*tert*-Butyldimethylsilyloxy]-3-methyl-2(*E*)-pentenol (133): This was prepared by the same procedure as described for the synthesis of **90**. **133**: IR (film) 3352, 2929, 2858, 1669, 1472, 1254 and 1097 cm^{-1}; CIMS, *m/z* (rel. intensity) 231 (M^++1, 1.6), 213 (100), 173 (5.2), 141 (43.4); ^1H NMR ($CDCl_3$, ppm) 5.42 (t, J = 6.9

Hz, 1H), 4.13 (d, J = 6.9 Hz, 2H), 3.69 (t, J = 7.0 Hz, 2H), 2.23 (t, J = 7.0 Hz, 2H), 1.68 (s, 3H), 0.87 (s, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 136.90, 125.28, 62.07, 59.29, 42.78, 25.91, 18.29, 16.64, -5.32. IR, ^1H NMR and ^{13}C NMR spectra of **133** are in agreement with those reported in reference 47.

1-Chloro-5-[*tert*-butyldimethylsilyloxy]-3-methyl-2(*E*)-pentene (134): This was prepared by the method of Dodd *et al.* [47] **134**: IR (film) 2955, 2929, 2857, 1663, 1472, 1255 and 1099 cm^{-1}; CIMS, *m/z* (rel. intensity) 249 (M^++1, 20.3), 213 (100), 191 (10.7), 155(16.9), 145 (94.5), 123 (12); ^1H NMR ($CDCl_3$, ppm) 5.48 (t, J = 8.0 Hz, 1H), 4.09 (d, J = 6.9 Hz, 2H), 3.69 (t, J = 6.8 Hz, 2H), 2.25 (t, J = 6.8 Hz, 2H), 1.74 (d, J = 1.1 Hz, 3H), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 140.02, 122.06, 61.75, 42.67, 40.81, 25.90, 16.45, -5.35. IR, ^1H NMR and ^{13}C NMR spectra of **134** are in agreement with those reported in reference 47.

1-(Benzenesulfonyl)-5-[*tert*-butyldimethylsilyloxy]-3-methyl-2(*E*)-pentene (135): This was prepared by the method of Dodd *et al.* [47] **135**: IR (film) 2929, 2857, 1663, 1308 and 1086 cm^{-1}; CIMS, *m/z* (rel. intensity) 355 (M^++1, 100), 325 (14.4), 223 (16.0), 217 (24), 143 (34.3); ^1H NMR ($CDCl_3$, ppm) 7.87 (dd, J =1.3, 7.3 Hz, 2H), 7.64 (t, J = 7.4 Hz, 1H), 7.53 (t, J = 7.4 Hz, 2H), 3.80 (d, J = 8.0 Hz, 2H), 3.59 (t, J = 6.9 Hz, 2H), 2.20 (t, J = 6.9 Hz, 2H), 1.32 (s, 3H), 0.87 (s, 9H), 0.04 (s, 6H). IR and ^1H NMR spectra of **135** are in agreement with those reported in reference 47.

5-(Benzenesulfonyl)-1-[*tert*-butyldimethylsilyloxy]-3,8,12,16-tetramethyl-3(*E*),7(*E*),11(*E*),15-heptadecatetraene (136): This was prepared by the method of Dodd *et al.* [47] IR (film) 2953, 2928, 2856, 1665, 1447, 1384, 1305, 1253, 1147 and 1086 cm^{-1}; CIMS, *m/z* (rel. intensity) 559 (M^++1, 5.4), 419 (2.5), 417 (26.5), 285

(100), 259 (18.9), 257 (46.3), 217 (15.9), 143 (36.3); ^{1}H NMR ($CDCl_3$, ppm) 7.84 (d, J = 7.9 Hz, 2H), 7.61 (t, J = 7.3 Hz, 1H),7.50 (t, J = 7.9 Hz, 2H), 4.90-5.10 (m, 4H), 3.71 (dt, J = 3.3, 8.9 Hz, 1H), 3.53 (dt, J = 1.9, 7.2 Hz, 2H), 2.89 (ddd, J = 3.6, 7.0, 14 Hz, 1H), 2.35 (ddd, J =3.6, 7.0, 14 Hz, 1H), 2.16 (t, J = 7.2 Hz, 2H), 1.90-2.10 (m, 8H), 1.67 (s, 3H0, 1.60 (s, 3H), 1.58 (s, 3H), 1.56 (s, 3H), 1.20 (s, 3H), 0.87 (s, 9H), 0.04 (s, 6H). IR and ^{1}H NMR spectra of **136** are in agreement with those reported in reference 47.

3,8,12,16-Tetramethyl-3(*E*),7(*E*),11(*E*)-15-heptadecatetraenyl *tert*-butyldimethylsilyl ether (137): This was prepared by the method of Dodd *et al.*[47] **137**: IR (film) 2928, 2857, 1668, 1447, 1383, 1255, 1096 and 836 cm^{-1}; CIMS, *m/z* (rel. intensity) 419 (M^++1, 12), 418 (M^+, 1.3), 361 (12.6), 287 (100), 261 (33), 231(13.2), 205 (35), 177 (20.7), 149 (25.2); ^{1}H NMR ($CDCl_3$, ppm) 5.05-5.20 (m, 4H), 3.65 (t, J = 7.2 Hz, 2H), 2.19 (t, J =7.2 Hz, 2H), 2.10-1.92 (m, 12H), 1.68 (s, 3H), 1.61 (s, 3H), 1.60 (s, 9H), 0.89 (s, 9H), 0.04 (s, 6H). IR and ^{1}H NMR spectra of **137** are in agreement with those reported in reference 47.

3-Bromo-17-[*tert*-butyldimethylsilyloxy]-2,6,10,15-tetramethyl-6(*E*), 10(*E*),14(*E*)-heptadecatrien-2-ol (138): This was prepared in 39% yield from **137** by the procedure as described for the preparation of **100**. **138**: IR (film) 3454, 1666, 1462, 1383, 1255 and 1098 cm^{-1}; CIMS *m/z* (rel. intensity) 517/515 (M^++1, 9.7/10.2), 385 (33.5), 383 (37.4), 367 (38.4), 365 (40.4), 303 (47.2), 285 (100), 231 (14.3), 229 (11), 205 (11.9), 203 (16.6), 191 (13.4), 189 (8.1), 135 (30.2), 133 (42.4); ^{1}H NMR ($CDCl_3$) δ 5.22-5.10 (m, 3H), 3.98 (dd, J = 11.3, 1.6 Hz, 1H), 3.65 (t, J = 7.2 Hz, 2H), 2.36-2.28 (m, 1H), 2.19 (t, J = 7.2 Hz, 2H), 2.16-1.90 (m, 10H), 1.84-1.73 (m, 1H), 1.68 (m, 1H), 1.61 (s, 3H), 1.59 (s, 6H), 1.34 (s, 3H), 1.32 (s, 3H), 0.88 (s,

9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 134.96, 133.06, 132.27, 126.24, 126.03, 124.47, 72.45, 70.84, 62.60, 43.13, 39.66, 38.22, 32.29, 28.33, 28.20, 26.71, 25.97, 25.92, 18.33, 16.45, 16.00, 15.87, -5.25; Anal. Calcd for $C_{27}H_{51}BrO_2Si$: C, 63.00; H, 9.99. Found: C, 63.16; H, 10.10.

15,16-Epoxy-3,8,12,16-tetramethyl-3(*E*),7(*E*),11(*E*)-heptadecatrienyl *tert*-butyldimethylsilyl ether (139): This was prepared in 96% yield by the procedure described for the preparation of **101**. **139**: IR (film) 2957, 2928, 2856, 1666, 1462, 1378, 1252, 1096 and 836 cm^{-1}; CIMS *m/z* (rel. intensity) 435 (M^++1, 6.3), 418 (6.3), 303 (62.9), 285 (100), 259 (12.6), 191 (28.0), 163 (37.2), 155 (22.7), 149 (30.5), 135 (38.2), 123 (16.3); ^1H NMR ($CDCl_3$) δ 5.20-5.10 (m, 3H), 3.65 (t, *J* = 7.2 Hz, 2H), 2.70 (t, *J* = 6.3 Hz, 1H), 2.19 (t, *J* = 7.2 Hz, 2H), 2.16-1.95 (m, 12H), 1.61 (s, 6H), 1.59 (s, 3H), 1.30 (s, 3H), 1.26 (s, 3H), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 135.28, 134.26, 132.48, 126.48, 125.20, 124.60, 64.41, 62.82, 58.45, 43.36, 39.92, 36.57, 28.56, 28.42, 27.78, 26.96, 26.20, 25.12, 18.98, 18.57, 16.68, 16.23, -5.02; Anal. Calcd for $C_{27}H_{50}O_2Si$: C, 74.59; H, 11.60. Found: C, 74.66; H, 11.78.

15,16-Epoxy-3,8,12,16-tetramethyl-3(*E*),7(*E*),11(*E*)-heptadecatrienol (140): This was prepared in 92% yield by the procedure described for the preparation of **102**. **140**: IR (film) 3418, 2955, 2927, 2852, 1667, 1448, 1379, 1250, 1049 and 875 cm^{-1}; CIMS *m/z* (rel. intensity) 321 (M^++1, 61.1), 303 (100), 285 (15.6), 221 (11.7), 217 (10.6), 207 (12.6), 191 (37.1), 163 (27.5), 153 (50.3), 149 (30.4); ^1H NMR ($CDCl_3$) δ 5.24 (dt, *J* = 1.2, 6.3 Hz, 1H), 5.18-5.08 (m, 2H), 3.64 (dt, *J* = 5.9, 6.0 Hz, 2H), 2.70 (t, *J* = 6.3 Hz, 1H), 2.24 (t, *J* = 6.0 Hz, 2H), 2.20-1.95 (m, 12H), 1.63 (d, *J* = 1.2 Hz, 3H), 1.61 (s, 3H), 1.59 (s, 3H), 1.30 (s, 3H), 1.25 (s, 3H); ^{13}C NMR ($CDCl_3$)

δ 135.40, 134.07, 131.43, 127.76, 124.91, 122.50, 64.18, 60.19, 58.21, 42.77, 39.64, 36.34, 28.26, 28.10, 27.57, 26.65, 24.86, 21.01, 18.74, 16.03, 15.98, 15.8; Anal. Calcd for $C_{21}H_{36}O_2$: C, 78.68; H, 11.33. Found: C, 78.66; H, 11.17.

15,16-Epoxy-3,8,12,16-tetramethyl-3(*E*),7(*E*),11(*E*)-heptadecatrienyl methanesulfonate (141): This was prepared in 97% yield by the procedure described for the preparation of **85**. Compound **141** was used for the next reaction immediately without further purification: ^{1}H NMR ($CDCl_3$) δ 5.30-5.00 (m, 3H), 4.27 (t, *J* = 6.8 Hz, 2H), 3.00 (s, 3H), 2.70 (t, *J* = 6.3 Hz, 1H), 2.42 (t, *J* = 6.8 Hz, 2H), 2.25-1.86 (m, 12H), 1.66 (s, 3H), 1.58 (s, 6H), 1.30 (s, 3H), 1.26 (s, 3H).

1,5-Dimethyl-4-hexenyl thioacetate (143): This was prepared in 84% yield by the procedure described for the preparation of **96**. **143**: IR (film) 2965, 2924, 2856, 1692, 1450, 1378, 1353, 1115 and 952 cm^{-1}; CIMS *m/z* (rel. intensity) 187 (M^++1, 13.4), 186 (M^+, 2.3), 146 (10.3), 145 (100), 101 (3.9); ^{1}H NMR ($CDCl_3$) δ 5.07 (tq, *J* = 7.2, 1.3 Hz, 1H), 3.54 (sextet, *J* = 6.9 Hz, 1H), 2.30 (s, 3H), 2.04 (dt, *J* = 7.4, 7.4 Hz, 2H), 1.68 (s, 3H), 1.59 (s, 3H), 1.61-1.50 (m, 2H), 1.29 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 195.60, 132.14, 123.41, 39.26, 36.42, 30.63, 25.59, 23.92, 21.36, 17.58; Anal. Calcd for $C_{10}H_{18}OS$: C, 64.48; H, 9.75. Found: C, 64.60; H, 9.75.

1,5-Dimethyl-4-hexenethiol (144): This was prepared in 91% yield by the procedure described for the preparation of **97**. **144**: IR (film) 2966, 2921, 2850, 1667, 1449, 1376, 1115 and 826 cm^{-1}; CIMS *m/z* (rel. intensity) 147/145 (M^++1, 5.6/100), 111 (12.2), 109 (1.4), 103 (4.5), 101 (19.8); ^{1}H NMR ($CDCl_3$) δ 5.07 (tq, *J* = 7.2, 1.3 Hz, 1H), 2.92 (heptaplet, *J* = 6.7 Hz, 1H), 2.09 (dt, *J* = 7.2, 7.2 Hz, 2H), 1.68 (s, 3H),

1.62 (s, 3H), 1.59-1.49 (m, 2H), 1.47 (d, J = 6.3 Hz, 1H), 1.33 (d, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 131.93, 123.42, 40.91, 34.96, 25.85, 25.54, 21.98, 17.57; Anal. Calcd for $C_8H_{16}S$: C, 66.60; H, 11.18. Found: C, 66.73; H, 11.30.

18-Thia-19-dehydro-2,3-oxidosqualene (72): This was prepared in 74% yield by coupling of **141** and **144** according to the procedure described for the preparation of **63**. **72**: IR (film) 2960, 2922, 2855, 1667, 1449, 1376, 1248, 1119 and 826 cm^{-1}; CIMS *m/z* (rel. intensity) 447 (M^++1, 100), 429 (48.8), 335 (11.4), 293 (26.8), 285 (14.6), 145 (9.2); ^1H NMR ($CDCl_3$) δ 5.22-5.05 (m, 4H), 2.75 (sextet, J = 6.7 Hz, 1H), 2.70 (t, J = 6.3 Hz, 1H), 2.57 (t, J = 7.3 Hz, 2H), 2.23 (t, J = 7.3 Hz, 2H), 2.20-1.95 (m, 12H), 1.68 (s, 3H), 1.65-1.58 (m, 2H), 1.61 (s, 9H), 1.59 (s, 3H), 1.51-1.44 (m, 2H), 1.29 (s, 3H), 1.27 (d, J = 6.8 Hz, 3H), 1.25 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 135.12, 134.05, 133.80, 131.90, 125.69, 124.97, 124.29, 123.98, 64.16, 58.16, 40.16, 39.68, 39.63, 37.19, 36.35, 29.11, 28.27, 28.13, 27.58, 26.74, 25.63, 24.87, 21.41, 18.75, 17.68, 16.04, 15.99, 15.90; Anal. Calcd for $C_{29}H_{50}OS$: C, 77.96; H, 11.29. Found: C, 78.10; H, 11.44.

6.4.3: Syntheses discussed in Chapter 4

5,9,13-Trimethyl-4(*E*),8(*E*),12-tetradecatrienal (170): To a vigorously stirred solution of oxalyl chloride (2 mL, 22 mmol) in CH_2Cl_2 (60 mL) at -60 °C under argon was added dimethylsulfoxide (3.4 mL, 44 mmol). The mixture was allowed to stir for 5 min. A solution of **107** (5.0 g, 20 mmol) in CH_2Cl_2 (10 mL) was added over 5 min. After 30 min stirring, triethylamine (14 mL, 100 mmol) was added over 10 min and the mixture was allowed to warm to room temperature. Water (100 mL) was then added and the organic phase was separated. The aqueous layer was extracted with

CH_2Cl_2 (4 X 30 mL). Standard work-up followed by flash column chromatography using ethyl acetate:hexane (1:9) as eluant gave **170** (4.61 g, 93% yield): IR (film) 2965, 2922, 2857, 1727, 1448, 1383, 1108 and 835 cm^{-1}; CIMS, *m/z* (rel. intensity) 249 (M^++1, 46), 231(85), 205 (7.7), 167 (41), 149 (44), 137 (100), 123 (32); ^1H NMR ($CDCl_3$, ppm) 9.75 (t, J = 1.6 Hz, 1H), 5.18-5.00 (m, 3H), 2.58-2.41 (m, 2H), 2.25-1.98 (m, 8H), 1.67 (s, 3H), 1.63 (s, 3H), 1.61 (s, 3H), 1.59 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 202.50, 136.79, 135.12, 131.28, 124.29, 123.88, 122.01, 43.87, 39.69, 39.65, 26.70, 26.41, 25.57, 20.78, 17.58, 16.02. The ^1H NMR spectrum of **170** is in agreement with that reported in reference 46.

Ethyl 2,7,11,15-tetramethyl-2(*E*),6(*E*),10(*E*),14-hexadecatetraenoate (171): To powdered (carbethoxyethylidene)triphenylphosphorane (3.90 g, 10.1 mmol) under argon was added a solution of **170** (2.49 g, 10 mmol) in CH_2Cl_2 (100 mL) and the mixture was refluxed under argon for 12 h. Water (40 mL) was added to the cooled mixture and the organic phase was separated. The aqueous phase was extracted with CH_2Cl_2 (3 X 40 mL). Standard work-up followed by flash column chromatography using ethyl acetate:hexane (5:95) as eluant gave pure **171** (2.90 g, 90% yield): IR (film) 1711, 1649 cm^{-1}; CIMS *m/z* (rel. intensity) 333 (M^++1, 100), 287 (24.3), 259 (43.1), 251 (53.5), 237 (21.9), 223 (30), 209 (38.3), 205 (23.8), 197 (37.0), 193 (20.6), 183 (31.7), 177 (24.2), 149 (31.8), 137 (73.0), 123 (41.1); ^1H NMR ($CDCl_3$, ppm) 6.76 (tq, J = 7.3, 1.4 Hz, 1H), 5.16-5.05 (m, 3H), 4.18 (q, J = 7.1 Hz, 2H), 2.25-1.91 (m, 12H), 1.81 (s, 3H), 1.67 (s, 3H), 1.61 (s, 3H), 1.58 (s, 6H), 1.28 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, ppm) 168.14, 141.76, 136.16, 134.93, 131.11, 127.87, 124.36, 124.08, 123.13, 60.25, 39.66, 28.96, 26.92, 26.73, 25.60, 17.58, 15.97, 15.91, 14.23, 12.23. Anal. Calcd for $C_{22}H_{36}O_2$: C, 79.46; H, 10.91. Found: C, 79.59; H, 10.68.

2,7,11,15-Tetramethyl-2(*E*),6(*E*),10(*E*),14-hexadecatetraenol (172): To a solution of **171** (2.50 g, 7.53 mmol) in anhydrous diethyl ether (80 mL) at -78 °C under argon was added DIBAL-H (19 mL, 19 mmol, 1 M solution in THF). The mixture was allowed to warm to 0 °C and stirred for 2 h. Excess DIBAL-H was destroyed by addition of distilled water (2 mL) and the mixture was poured into an ice-cold 5% aqueous solution of tartaric acid (20 mL). The mixture was extracted with ether (3 X 40 mL) and the combined organic phase was washed with $NaHCO_3$ solution. Standard work-up followed by flash column chromatography using ethyl acetate:hexane (2:8) as the eluant gave **172** (1.98 g, 91% yield): CIMS *m/z* (rel. intensity) 291 (M^++1, 8.7), 290 (M^+, 4.3), 273 (8.7), 217 (19), 205 (41), 191 (36), 177 (26.2), 163 (35.5), 149 (71.1), 137 (100), 123 (76); ^1H NMR ($CDCl_3$, ppm) 5.45-5.37 (m, 1H), 5.18-5.06 (m, 3H), 3.98 (s, 2H), 2.12-1.92 (m, 12H), 1.68 (s, 3H), 1.66 (s, 3H), 1.60 (s, 3H), 1.35 (br s, 1H); ^{13}C NMR ($CDCl_3$, ppm) 135.50, 134.95, 134.91, 131.20, 126.15, 124.44, 124.24, 123.95, 69.03, 39.72, 27.95, 27.88, 26.80, 26.66, 25.61, 17.62, 16.03, 15.99, 13.64. Anal. Calcd for $C_{20}H_{34}O$: C, 82.69; H, 11.80. Found: C, 82.47; H, 11.69.

1-Chloro-2,7,11,15-tetramethyl-2(*E*),6(*E*),10(*E*),14-hexadecatetraene (173): To a solution of NCS (0.735 g, 5.5 mmol) in anhydrous CH_2Cl_2 (30 mL) at 0 °C under argon was added dropwise DMS (0.45 mL, 6.0 mmol). The mixture was cooled to -20°C and **172** (1.39 g, 4.79 mmol) in CH_2Cl_2 (3 mL) was added over 5 min. The mixture was allowed to warm to 0°C, stirred for 1 h and then poured into ice-cold brine. The organic phase was separated and the aqueous phase was extracted with diethyl ether (3 X 30 mL). Standard work-up followed by flash column chromatography using ethyl acetate:hexane (1:9) as the eluant gave pure **173** (1.19

g, 81% yield): IR (film) 1667, 1264 and 685 cm^{-1}; CIMS *m/z* (rel. intensity) 309 (M^++1, 10.3), 308 (M^+, 3.1), 273 (35.2), 217 (14.2), 205 (34.3), 191 (29.0), 177 (18.1), 163 (23.6), 149 (53.0), 137 (100), 123 (72.8); ^1H NMR ($CDCl_3$, ppm) 5.57-5.50 (m, 1H), 5.16-5.04 (m, 3H), 4.02 (s, 2H), 2.24-1.92 (m, 12H), 1.73 (s, 3H), 1.68 (s, 3H), 1.60 (s, 9H); ^{13}C NMR ($CDCl_3$, ppm) 135.84, 134.99, 131.82, 131.21, 130.68, 124.45, 124.23, 123.57, 52.48, 39.74, 28.36, 27.53, 26.83, 26.65, 25.63, 17.65, 16.06, 16.00, 14.12; Anal. Calcd for $C_{20}H_{33}Cl$: C, 77.76; H, 10.77. Found: C, 77.48; H, 10.69.

2,7,11,15-Tetramethyl-2(*E*),6(*E*),10(*E*),14-hexadecatetraenyl 4'-methylbenzenethiosulfonate (174): To a solution of potassium 4-methylbenzenethiosulfonate (0.90 g, 3.8 mmol) in DMF (20.0 mL) was added a solution of **173** (1.11 g, 3.6 mmol) in DMF (5 mL). The mixture was stirred at room temperature for 24 h and then poured into ice-cold water (30 mL). The mixture was extracted with diethyl ether (4 X 30 mL) and the ethereal solution was washed with saturated $NaHCO_3$ solution (20 mL). Standard work-up followed by flash column chromatography using ethyl acetate:hexane (15:85) as the eluant gave pure **174** (1.42 g, 86% yield): IR (film) 1666, 1595, 1329, 1142 and 812 cm^{-1}; CIMS *m/z* (rel. intensity) 461 (M^++1, 2.2), 305 (14.0), 295 (8.7), 279 (18.0), 273 (17.2), 157 (100), 156 (2.9), 139 (16.2), 123 (4.5); ^1H NMR ($CDCl_3$, ppm) 7.78-7.32 (AA'BB', 4H), 5.38-5.30 (m, 1H), 5.12-5.00 (m, 3H), 3.65 (s, 2H), 2.44 (s, 3H), 2.10-1.88 (m, 12H), 1.67 (s, 3H), 1.59 (s, 6H), 1.57 (s, 3H), 1.51 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 144.40, 142.62, 135.81, 135.00, 131.42, 131.21, 129.63 (2C), 127.71, 127.06 (2C), 124.42, 124.19, 123.50, 45.69, 39.73, 28.48, 27.50, 26.81, 26.65, 25.64, 21.57, 17.66, 16.06, 16.00, 15.00; Anal. Calcd for $C_{27}H_{40}O_2S_2$: C, 70.39; H, 8.76. Found: C, 70.11; H, 8.88.

6-Thia-4,8,13,17,21-pentamethyl-4(*E*),8(*E*),12(*E*),16(*E*),20-docosapentenyl *tert*-butyldimethylsilyl ether (175): To a stirred solution of **88** (0.558 g, 1.64 mmol) in dry THF (40 mL) at -78 °C under argon was added dropwise *n*-BuLi (0.66 mL, 1.65 mmol, 2.5 M solution in hexane) and the mixture was stirred for 20 min. To this mixture, at -78 °C under argon, was added dropwise a solution of **174** (0.75 g, 1.63 mmol) in THF (3 mL) over 3 min. The mixture was stirred at -78 °C for 15 min and water (10 mL) was added. The mixture was warmed to room temperature and extracted with diethyl ether (4 X 30 mL). Standard work-up followed by flash column chromatography using ethyl acetate:hexane (5:95) as eluant gave pure **175** (0.767 g, 91% yield): IR (film) 1668, 1104 and 836 cm^{-1}; CIMS *m/z* (rel. intensity) 519 (M^++1, 100), 518 (M^+,7.0), 461 (2.1), 387 (3.8), 305 (11.1), 303 (51.8), 273 (42.3), 247 (18.0), 231 (20.0), 191 (16.2), 149 (15.0), 137 (33.0), 123 (23); ^1H NMR ($CDCl_3$, ppm) 5.58 (q, J = 1.0 Hz, 1H), 5.35-5.28 (m, 1H), 5.17-5.06 (m, 3H), 3.57 (t, J = 6.5 Hz, 2H), 3.19 (s, 2H), 2.12-1.94 (m, 14H), 1.72 (d, J = 1.0 Hz, 3H), 1.69 (s, 3H), 1.67 (s, 3H), 1.64-1.56 (m, 2H), 1.60 (s, 3H), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 136.38, 135.40, 134.88, 131.43, 131.10, 128.22, 124.46, 124.27, 123.96, 117.75, 62.59, 43.33, 39.75, 35.53, 31.12, 28.47, 28.06, 26.69, 26.59, 26.04, 25.87, 25.84, 18.29, 18.05, 17.64, 16.05, 15.99, 14.90, -5.27; Anal. Calcd for $C_{32}H_{58}SiOS$: C, 74.07; H, 11.28. Found: C, 74.25; H, 11.49.

6-Thia-4,8,13,17,21-pentamethyl-4(*E*),8(*E*),12(*E*),16(*E*),20-docosapentaen-1-ol (176): To a solution of **175** (0.67 g, 1.29 mmol) in THF (15.0 mL) at room temperature was added tetrabutylammonium fluoride (5 mL, 5 mmol, 1 M solution in THF). The mixture was stirred at room temperature for 10 h, then poured into ice-cold water (5 mL) and extracted with diethyl ether (3 X 30 mL). Standard work-up

followed by flash column chromatography using ethyl acetate:hexane (3:7) as eluant gave pure **176** (0.49 g, 94% yield): IR (film) 3348, 1666 and 1063 cm^{-1}; CIMS *m/z* (rel. intensity) 405 (M^++1, 100), 404 (M^+, 6.3), 307 (14.4), 273 (38.3), 205 (6.7), 191 (12.1), 173 (16.5), 149 (12.2), 137 (19.9), 123 (12.9); ^1H NMR ($CDCl_3$, ppm) 5.61 (q, J = 1.0 Hz, 1H), 5.35-5.28 (m, 1H), 5.18-5.06 (m, 3H), 3.61 (t, J = 6.5 Hz, 2H), 3.20 (s, 2H), 2.15-1.93 (m, 14H), 1.73 (d, J = 1.0 Hz, 3H), 1.70-1.63 (m, 2H), 1.69 (s, 3H), 1.66 (s, 3H), 1.59 (s, 9H), 1.42 (br s, 1H); ^{13}C NMR ($CDCl_3$, ppm) 136.09, 135.45, 134.91, 131.40, 131.11, 128.25, 124.41, 124.22, 123.88, 118.19, 62.45, 43.30, 39.69, 35.53, 30.79, 28.40, 27.99, 26.78, 26.65, 25.56, 17.90, 17.57, 15.99, 14.84; Anal. Calcd for $C_{26}H_{44}OS$: C, 77.17; H, 10.97. Found: C, 77.48; H, 11.20.

6-Thia-4,8,13,17,21-pentamethyl-4(*E*),8(*E*),12(*E*),16(*E*),20-docosapentenal (177): To a vigorously stirred solution of oxalyl chloride (0.10 mL, 1.15 mmol) in CH_2Cl_2 (15 mL) at -60 °C under argon was added dimethyl sulfoxide (0.16 mL, 2.25 mmol). The mixture was stirred for 5 min at -60 °C, then a solution of **176** (0.37 g, 0.91 mmol) in CH_2Cl_2 (2 mL) was added. After stirring for 30 min, triethylamine (0.84 mL, 6.0 mmol) was added over 2 min and the mixture was allowed to warm to room temperature. Water (10 mL) was added and the organic phase was separated. The aqueous layer was extracted with CH_2Cl_2 (4 X 20 mL). Standard work-up followed by flash column chromatography using ethyl acetate:hexane (1:9) as eluant gave **177** (0.33 g, 90% yield): IR (film) 1727, 1667 and 1069 cm^{-1}; CIMS *m/z* (rel. intensity) 403 (M^++1, 100), 402 (M^+, 5.1), 273 (47.7), 205 (6.4), 191 (11.0), 171 (12.2), 149 (12.3), 137 (12.0); ^1H NMR ($CDCl_3$, ppm) 9.74 (t, J = 1.7 Hz, 1H), 5.62 (q, J = 1.0 Hz, 1H), 5.35-5.27 (m, 1H), 5.17-5.05 (m, 3H), 3.20 (s, 2H), 2.55-2.49 (m, 2H), 2.37 (t, J = 7.4, 2H), 2.10-1.92 (m, 12H), 1.72 (d, J = 1.0 Hz, 3H), 1.68 (s, 3H), 1.67 (s, 3H), 1.59 (s, 9H); ^{13}C NMR ($CDCl_3$, ppm) 201.66, 135.53, 134.96, 133.71,

131.22, 128.54, 124.41, 124.20, 123.84, 119.31, 43.21, 42.04, 39.72, 31.48, 28.42, 28.01, 26.79, 26.65, 25.64, 18.07, 17.64, 16.05, 15.98, 14.84; Anal. Calcd for $C_{26}H_{42}OS$: C, 77.55; H, 10.51. Found: C, 77.28; H, 10.74.

6(*E*)-8-Thia-2,3-oxidosqualene (74): To a stirred solution of diphenylisopropylsulfonium fluoroborate (0.167 g, 0.53 mmol) in dry THF (15 mL) at -78°C, under argon, was added dropwise *t*-BuLi (0.31 mL, 0.53 mmol, 1.7 M in hexane). The mixture was stirred at -78 °C under argon for 1 h, and a solution of **177** (0.209 g, 0.52 mmol) in THF (3 mL) was added dropwise. The mixture was maintained at -70 °C for 1 h and between -70 °C and -50 °C for 1 h, then treated with distilled water (10 mL) and extracted with diethyl ether (3 X 30 mL). Standard work-up followed by flash column chromatography using ethyl acetate:hexane (5:95) as eluant gave **74** (0.208 g, 90% yield): IR (film) 1669, 1247 and 1122 cm^{-1}; CIMS *m/z* (rel. intensity) 445 (M^++1, 65.5), 444 (M^+, 3.9), 403 (35.1), 305 (19.5), 273 (45.2), 191 (12.3), 173 (12.5), 149 (11.7), 139 (100), 123 (12.0); ^1H NMR ($CDCl_3$, ppm) 5.63 (s, 1H), 5.35-5.27 (m, 1H), 5.16-5.03 (m, 3H), 3.20 (s, 2H), 2.68 (t, J = 6.2 Hz, 1H), 2.28-2.11 (m, 2H), 2.10-1.92 (m, 12H), 1.73 (s, 3H), 1.69 (s, 3H), 1.68 (s, 3H), 1.66-1.61 (m, 2H), 1.60 (s, 9H), 1.30 (s, 3H), 1.25 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 135.51, 135.25, 134.97, 131.35, 128.38, 124.42, 124.23, 123.87, 118.53, 63.90, 58.29, 43.29, 39.74, 36.01, 28.45, 28.04, 27.49, 26.81, 26.68, 25.64, 24.84, 18.72, 18.07, 17.65, 16.00, 14.99; Anal. Calcd for $C_{29}H_{48}OS$: C, 78.32; H, 10.89. Found: C, 78.22; H, 11.02.

3,7,11-Trimethyl-2(*E*),6(*E*),10-dodecatrienyl *tert*-butyldimethylsilyl ether (178): This was prepared in 95% yield by the procedure described for the preparation of **88**. **178**: IR (film) 1669, 1110 and 1065 cm^{-1}; ^1H NMR ($CDCl_3$, ppm)

5.31 (t, J = 6.2 Hz, 1H), 5.10 (t, J = 6.0 Hz, 2H), 4.19 (d, J = 6.5 Hz, 2H), 2.04 (m, 8H), 1.68 (s, 3H), 1.62 (s, 3H), 1.59 (s, 6H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 136.89, 135.16, 131.23, 124.47, 124.40, 124.01, 60.35, 39.72, 39.56, 26.78, 26.35, 26.03, 25.65, 18.41, 17.65, 16.35, 15.98, -5.04.

10-Bromo-11-hydroxy-3,7,11-trimethyl-2(*E*),6(*E*)-dodecadienyl *tert*-butyldimethylsilyl ether (179): This was prepared in 39% yield by the procedure described for the preparation of **100**. **179**: IR (film) 3452, 1668 and 1064 cm^{-1}; ^1H NMR ($CDCl_3$, ppm) 5.30 (t, J = 6.1 Hz, 1H), 5.20 (t, J = 6.0 Hz, 1H), 4.19 (d, J = 6.5 Hz, 2H), 3.96 (dd, J = 10.0 Hz, 1.5 Hz, 1H), 2.03 (m, 8H),1.62 (s, 3H), 1.58 (s, 3H), 1.34 (s, 3H), 1,31 (s, 3H), 0.90 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 136.63, 133.29, 125.73, 124.63, 72.45, 70.79, 60.35, 39.41, 38.17, 32.17, 26.63, 26.30, 26.03, 25.89, 18.43, 16.35, 15.85, -5.03.

10,11-Epoxy-3,7,11-trimethyl-2(*E*),6(*E*)-dodecadienyl *tert*-butyldimethylsilyl ether (180): This was prepared in 97% yield by the procedure described for the preparation of **101**. **180**: IR (film) 1668, 1253, 1110, 1065 and 835 cm^{-1}; ^1H NMR ($CDCl_3$, ppm) 5.30 (t, J = 6.0 Hz, 1H), 5.15 (t, J = 6.0 Hz, 1H), 4.13 (d, J = 7.4 Hz, 2H), 2.68 (t, J = 6.2 Hz, 1H), 2.09 (m, 6H),1.61 (m, 2H), 1.60 (s, 6H), 1.29 (s, 3H), 1,25 (s, 3H), 0.90 (s, 9H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, ppm) 136.70, 134.27, 124.64, 124.55, 64.16, 60.30, 58.23, 39.44, 36.30, 27.48, 26.31, 26.01, 24.86, 18.73, 18.40, 16.33, 15.97, -5.06.

10,11-Epoxy-3,7,11-trimethyl-2(*E*),6(*E*)-dodecadienol (181): This was prepared by the procedure described for the preparation of **176**. Flash column chromatography using ethyl acetate:hexane (6:4) as eluant gave **181** in 91% yield as

a colourless liquid: IR (film) 3424, 1642, 1249, 1120 and 836 cm^{-1}; CIMS *m/z* (rel. intensity) 239 (M^++1, 1.6), 221 (100.0), 203 (59.3), 186 (18.0), 153 (45.9), 135 (35.1); ^1H NMR ($CDCl_3$, ppm) 5.40 (t, *J* = 6.0 Hz, 1H), 5.15 (t, *J* = 6.0 Hz, 1H), 4.13 (d, *J* = 7.4 Hz, 2H), 2.68 (t, *J* = 6.2 Hz, 1H), 2.08 (m, 6H), 1.66 (s, 3H), 1.62 (m, 2H), 1.60 (s, 3H), 1.29 (s, 3H), 1.25 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 139.17, 134.36, 124.55, 123.77, 64.15, 59.29, 58.30, 39.40, 36.33, 27.34, 26.20, 24.83, 18.75, 16.20, 15.96.

1-Chloro-10,11-epoxy-3,7,11-trimethyl-2(*E*),6(*E*)-dodecadiene (182): This was obtained by the procedure described for the preparation of **173**. Flash column chromatography using ethyl acetate:hexane (15:85) as eluant gave **182** in 79% yield: IR (film) 1662, 1252, 874 and 678 cm^{-1}; CIMS *m/z* (rel. intensity) 257 (M^++1, 88), 239 (53.2), 221 (100), 203 (47.3), 153 (43.2), 135 (36.9); ^1H NMR ($CDCl_3$, ppm) 5.43 (t, *J* = 8.0 Hz, 1H), 5.13 (t, *J* = 6.1 Hz, 1H), 4.08(d, *J* = 8.0 Hz, 2H), 2.69 (t, *J* = 6.2 Hz, 1H), 2.10 (m, 6H), 1.71 s, 3H), 1.61 (s, 3H), 1.60 (m, 2H), 1.29 (s, 3H), 1,25 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 142.51, 134.76, 124.09, 120.52, 64.12, 58.20, 41.01, 39.34, 36.31, 27.49, 26.11, 24.88, 18.75, 16.04; HRMS *m/z* calcd for $C_{15}H_{25}ClO$: 256.1594, found: 256.1598.

10,11-Epoxy-3,7,11-trimethyl-2(*E*),6(*E*)-dodecadienyl 4'-methylbenzene-thiosulfonate (183): This was prepared in 85% yield by the procedure described for the preparation of **174**. **183**: IR (film) 1659, 1594, 1326, 1142 and 813 cm^{-1}; CIMS *m/z* (rel. intensity) 409 (M^++1, 3.5), 222 (6.7), 201 (3.1), 157 (100), 155 (6.1), 141 (15.8), 127 (10.5); ^1H NMR ($CDCl_3$, ppm) 7.32-7.84 (AA'BB', 4H), 5.07 (m, 2H), 3.67 (d, *J* = 7.9 Hz, 2H), 2.68 (t, *J* = 6.2 Hz, 1H), 2.44 (s, 3H), 2.03 (m, 6H), 1.61 (m, 2H), 1.59 (s, 3H), 1.58 (s, 3H), 1.29 (s, 3H), 1,25 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm)

144.51, 143.32, 142.44, 134.78, 129.73, 127.04, 124.00, 115.47, 64.10, 58.20, 39.34, 36.30, 34.22, 27.52, 26.07, 24.88, 21.57, 18.76, 16.24, 16.01; HRMS *m/z* calcd for $C_{22}H_{32}O_3S_2$: 408.1792, found: 408.1786. Anal. Calcd for $C_{22}H_{32}O_3S_2$: C, 64.67; H, 7.89. Found: C, 64.84; H, 7.78.

1-Chloro-3,7-dimethyl-2(*E*),6-octadiene (geranyl chloride) (185): This was prepared in 80% yield by the procedure described for the synthesis of **173**. **185** :IR (film) 2968, 2927, 2856, 1662, 1450, 1377, 1253, 1109 and 839 cm-1; ^{1}H NMR ($CDCl_3$, ppm) 5.45 (m, 1H), 5.25-4.91 (m, 1H), 4.10 (d, *J* = 8 Hz, 2H), 2.28-1.98 (m, 4H), 1.73 (d, J = 1.4 Hz, 3H), 1.69 (s, 3H), 1.61 (s, 3H). IR and ^{1}H NMR spectra are in agreement with those reported in reference 77.

1-Iodo-2,6,10-trimethyl-1(*E*),5(*E*),9-undecatriene (187): To a solution of $AlMe_3$ (0.241 g, 0.32 mL, 3.3 mmol) and $ZrCp_2Cl_2$ (0.122 g, 0.41 mmol) in CH_2Cl_2 (10 mL) at -10 °C under argon was added dropwise a solution of **186** (0.287 g, 1.63 mmol) in CH_2Cl_2 (5 mL). After stirring for 20 h at room temperature, the reaction mixture was cooled to -30 °C and a solution of iodine (0.508 g, 2 mmol) in THF (10 mL) was added dropwise over 20 min. The mixture was allowed to warm to 0 °C and a saturated aqueous solution of K_2CO_3 (10 mL) was added to destroy the excess $AlMe_3$ under argon. The slurry was diluted with 40 mL of hexane and the precipitated salt was removed by filtration through a pad of Celite. The pad was rinsed with ether (3 x 30 mL). Standard workup followed by flash column chromatography using ethyl acetate:hexane (1:9) as eluant gave **187** (0.421 g, 81% yield): CIMS *m/z* (rel. intensity) 319 (M^++1, 3.9), 263 (2.6), 249 (1.9), 235 (2.4), 192 (16.2), 191 (100), 178 (1.6) 163 (1.8); ^{1}H NMR ($CDCl_3$, ppm) 5.87 (s, 1H), 5.07 (m, 2H), 2.11 (m, 8H), 1.84 (s, 3H), 1.68 (s, 3H), 1.64 (s, 3H), 1.59 (s, 3H); ^{13}C NMR

($CDCl_3$, ppm) 147.81, 136.07, 131.37, 124.26, 123.00, 74.65, 39.68, 39.50, 26.73, 26.31, 25.66, 23.94, 17.68, 16.00.

14(*E*)-13-Thia-2,3-oxidosqualene (75): This was prepared in 28% yield by the procedure described for the preparation of **175**. **75**: IR (film) 1663, 1248 and 1122 cm^{-1}; CIMS *m/z* (rel. intensity) 445 (M^++1, 48.4), 237 (11.5), 221 (100), 204 (10.3), 191 (44.6), 153 (28.5), 135 (22.6); ^1H NMR ($CDCl_3$, ppm) 5.64 (s, 1H), 5.28 (t,*J* = 7.8 Hz, 1H), 5.15 (t, *J* = 6.8 Hz, 1H), 5.09 (m, 2H), 3.27 (d, *J* = 7.8 Hz, 2H), 2.70 (t, *J* = 6.2 Hz, 1H), 2.07 (m, 14H), 1.73 (s, 3H), 1.68 (s, 6H), 1.61 (s, 3H), 1.60 (m, 2H), 1.59 (s, 3H), 1.58 (s, 3H), 1.29 (s, 3H), 1.25 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 138.71, 137.38, 135.44, 134.42, 131.26, 124.52, 124.38, 123.77, 120.57, 117.62, 64.15, 58.20, 39.72, 39.56, 39.38, 36.33, 31.50, 27.53, 26.81, 26.54, 25.63, 24.88, 18.75, 18.07, 17.65, 16.17, 16.00; HRMS. Calcd for $C_{29}H_{48}OS$: 444.3426, found 444.3425. Anal. Calcd for $C_{29}H_{48}OS$: C, 78.32; H, 10.88. Found: C,78.28; H, 10.79.

3,7-Dimethyl-1-(benzenesulfonyl)-2(*E*),6-octadiene (188): To a solution of geranyl chloride (**185**) (3.66 g, 21.2 mmol) in DMF (40 mL) at 0 °C was added $NaSO_2Ph$ (3.78 g, 23 mmol). The reaction mixture was stirred for 12 h. Water (60 mL) was added and the aqueous layer extracted with diethyl ether (4 x 40 mL). Standard work-up followed by chromatography using ethyl acetate:hexane (4:6) as the eluant gave the sulfone **188** (5.03 g, 85%) as an oil: IR (film) 1663, 1447, 1307, 1150, 1085 and 742 cm^{-1}; CIMS *m/z*(rel. intensity) 279 (M^++1, 2.9), 185 (1.0), 143 (11.2), 137 (100); ^1H NMR ($CDCl_3$, ppm) 7.87 (d, *J* = 7.0 Hz, 2H), 7.62 (t, *J* = 7.0 Hz, 1H), 7.52 (t, *J* = 7.0 Hz, 2H), 5.18 (t, *J* = 7.3 Hz, 1H), 5.03 (m, 1H), 3.80 (d, *J* = 8.0 Hz, 2H), 1.99 (s, 4H), 1.68 (s, 3H), 1.58 (s, 3H), 1.30 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm)

146.33, 138.83, 133.47, 132.07, 128.93, 128.57, 123.47, 110.38, 56.16, 39.69, 26.22, 25.65, 17.66, 16.18; Anal. calcd. for $C_{16}H_{22}O_2S$: C, 69.03; H, 7.96. Found: C, 68.92; H, 7.89.

6,7-Epoxy-3,7-dimethyl-1-(benzenesulfonyl)-2(*E*)-octene (189): To a solution of **188** (4.7 g, 16.9 mmol) and NaOAc (1.48 g, 18 mmol) in CH_2Cl_2 (50 mL) at -40 °C to -20 °C was added *m*-CPBA (3.66 g, 85% pure, 18 mmol) in three portions over 1 h. The mixture was warmed to -10 °C and stirred for an additional 1.5 h. Saturated $NaHCO_3$ solution (50 mL) was added to the mixture and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (3 x 30 mL) and the combined organic layer was washed with ice-cold 1N NaOH. Standard work-up followed by chromatography using ethyl acetate:hexane (1:1) as the eluant gave the epoxy sulfone **189** (4.63 g, 93%) as an oil: IR (film) 1664, 1585, 1447, 1306, 1248, 1151, 1085 and 739 cm^{-1}; CIMS *m/z* (rel. intensity) 295 (M^++1, 89), 277 (39), 153 (100), 143 (40.6), 135 (60); ^1H NMR ($CDCl_3$, ppm) 7.85 (d, J = 7.0 Hz, 2H), 7.64 (t, J = 7.0 Hz, 1H), 7.54 (t, J = 7.0 Hz, 2H), 5.21 (t, J = 7.9 Hz, 1H), 3.81 (d, J = 7.9 Hz, 2H), 2.66 (t, J = 6.3 Hz, 1H), 2.25-2.05 (m, 2H), 1.62-1.45 (m, 2H), 1.36 (s, 3H), 1.30 (s, 3H), 1.25 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 145.55, 138.92, 133.57, 129.03, 128.50, 110.96, 63.62, 58.32, 56.09, 36.42, 27.17, 24.83, 18.72, 16.26; Anal. calcd. for $C_{16}H_{22}O_3S$: C, 65.28; H, 7.53. Found: C, 65.36; H, 7.70.

1-(Benzenesulfonyl)-(2*E*)-hexen-6-al (190): To a stirred solution of **189** (4.5 g, 15.3 mmol) in diethyl ether (80 mL) at 0 °C was added dropwise a solution of $HIO_4 \cdot 2H_2O$ (3.76 g, 16.5 mmol) in THF (35 mL) over 1 h. The mixture was stirred for an additional 0.5 h and water (50 mL) was added. The organic phase was separated and the aqueous phase was extracted with diethyl ether (4 x 30 mL). The combined organic extract was washed with saturated $NaHCO_3$ solution (2 x 30 mL). Standard

work-up followed by chromatography using ethyl acetate:hexane (6:4) as the eluant gave aldehyde **190** (3.25 g, 84%) as an oil: IR (film) 1722, 1447, 1305, 1150, 1085 and 742 cm^{-1}; CIMS *m/z* (rel. intensity) 253 (M^++1, 100), 235 (63), 143 (15.5); ^1H NMR ($CDCl_3$, ppm) 9.74 (t, *J* = 1.4 Hz, 1H), 7.84 (d, *J* = 7.0 Hz, 2H), 7.64 (t, *J* = 7.0 Hz, 1H), 7.53 (t, *J* = 7.0 Hz, 2H), 5.21 (t, *J* = 8.0 Hz, 1H), 3.81 (d, *J* = 8.0 Hz, 2H), 2.49 (dt, *J* = 1.4, 7.4 Hz, 2H), 2.32 (t, *J* = 7.4 Hz, 2H), 1.34 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 200.94, 144.27, 138.76, 133.60, 129.04, 128.43, 111.45, 55.95, 41.53, 31.56, 16.28; Anal. calcd. for $C_{13}H_{16}O_3S$: C, 61.88; H, 6.39. Found: C, 61.80; H, 6.38.

6-(Ethylenedioxy)-1-(benzenesulfonyl)-3-methyl-2(*E*)-hexene (191): A mixture of **190** (3.0 g, 11.9 mmol), $(CH_2OH)_2$ (1.49 g, 24 mmol) and *p*-TsOH (50 mg) in toluene (50 mL) was refluxed for 4 h under nitrogen using a Dean-Stark trap to remove the water. Saturated $NaHCO_3$ solution (30 mL) was added to the cold mixture and the organic phase was separated. The aqueous phase was extracted with ether (3 x 30 mL). Standard work-up followed by chromatography using ethyl acetate:hexane (6:4) as the eluant gave acetal **191** (3.25 g, 92%) as an oil: IR (film) 2955, 2885, 1664, 1586, 1447, 1306, 1240, 1150, 1085, 1034 and 741 cm^{-1}; CIMS *m/z* (rel. intensity) 297 (M^++1, 100), 235 (19), 155 (16.7), 143 (8.6); ^1H NMR ($CDCl_3$, ppm) 7.84 (d, *J* = 7.0 Hz, 2H), 7.64 (t, *J* = 7.0 Hz, 1H), 7.53 (t, *J* =7.0 Hz, 2H), 5.21 (tq, *J* =1.3 , 7.96 Hz, 1H), 4.80 (t, *J* = 4.7 Hz, 1H), 3.95 (m, 2H), 3.84 (m, 2H), 3.80 (d, *J* = 7.96 Hz, 2H), 2.10 (t, *J* = 7.8 Hz, 2H), 1.70-1.58 (m, 2H), 1.30 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 145.61, 138.81, 133.51, 128.97, 128.53, 110.76, 103.84, 64.93, 56.10, 33.76, 31.94, 16.15. IR and ^1H NMR spectra of **191** are in agreement of those reported in reference 31b.

1-(Ethylenedioxy)-6-(benzenesulfonyl)-4,9,13,17-tetramethyl-4(*E*),8(*E*), 12(*E*),16-octadecatetraene (192): This was prepared in 91% yield according to the procedure described in reference 31b. **192**: 2926, 1664, 1446, 1304, 1145, 1108 and 734 cm^{-1}; CIMS *m/z* (rel. intensity) 501 (M^++1, 100), 455 (0.9) 415 (1.3), 359 (16.1), 297 (16.6), 283 (5.4), 143 (100), 126 (15); ^1H NMR ($CDCl_3$, ppm) 7.86 (d, *J* = 7.0 Hz, 2H), 7.62 (t, *J* = 7.0 Hz, 1H), 7.51 (t, *J* = 7.0 Hz, 2H), 5.10-4.98 (m, 3H), 4.95 (t, *J* = 7.3 Hz, 1H), 4.78 (t, *J* = 4.7 Hz, 1H), 3.98-3.92 (m, 2H), 3.86-3.82 (m, 2H), 3.72 (dt, *J* = 3.2, 10.6 Hz, 1H), 2.87 (ddd, *J* = 3.1, 7.2, 14.1 Hz, 1H), 2.34 (ddd, *J* = 3.1, 7.2, 14.1 Hz, 1H), 2.08-1.90 (m, 10H), 1.66 (s, 3H), 1.65-1.60 (m, 2H), 1.59 (s, 3H), 1.58 (s, 3H), 1.56 (s, 3H), 1.19 (d, *J* = 1.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, ppm) 144.31, 138.75, 138.26, 135.21, 133.31, 131.26, 129.13, 128.72, 124.33, 123.88, 118.55, 117.54, 103.86, 64.91, 39.72, 33.81, 32.02, 26.78, 26.60, 26.50, 25.65, 17.66, 16.48, 16.37, 15.95. IR and ^1H NMR spectra of **192** are in agreement of those reported in reference 31b.

1-(Ethylenedioxy)-4,9,13,17-tetramethyl-4(*E*),8(*E*),12(*E*),16-octadeca-tetraene (193): This was prepared in 88% yield according to the procedure described in ref 31b. **193**: IR (film) 2922, 2853, 1665, 1445, 1381, 1140, 1041 and 745 cm^{-1}; CIMS *m/z* (rel. intensity) 361 (M^++1, 52.5), 300 (22.7), 299 (100), 217 (29.8), 175 (15.7), 163 (13.3), 149 (31.8); ^1H NMR ($CDCl_3$, ppm) 5.20-5.05 (m, 4H), 4.84 (t, J = 4.8 Hz, 1H), 3.97 (m, 2H), 3.85 (m, 2H), 2.12-1.93 (m, 14 H0, 1.78-1.72 (m, 2H), 1.68 (s, 3H), 1.60 (s, 3H), 1.59 (s, 9H); ^{13}C NMR ($CDCl_3$, ppm) 135.19, 134.92, 134.28, 131.23, 124.60, 124.46, 124.31, 124.24, 104.44, 64.88, 39.77, 33.94, 32.52, 28.28, 28.19, 26.81, 26.70, 25.68, 17.68, 16.03.

Diphenylphosphinoyl ethanethiol (194): This was prepared in 80% yield according to the procedure described by Dodd[31h] and Mikolajczyk[66] for the synthesis of diethyl (1-mercaptoethyl)phosphonate. **194**: IR (KBr) 3055, 1619, 1185, 1120, 1070, 1029, 745 and 698 cm^{-1}; ^1H NMR ($CDCl_3$, ppm) 7.90 (m, 4H), 7.50 (m, 6H), 3.45 (m, 1H), 2.07 (dd, J_{H-H} = 7.0 Hz; J_{P-H} = 10 Hz, 1H), 1.50 (dd, J_{H-H} = 7.5 Hz; J_{P-H} = 15.0 Hz, 3H). IR and ^1H NMR spectra of **194** are in agreement of those reported in reference 31h.

Diphenylphosphinoyl 1-(3-methyl-2,3-oxido-butylthio)ethane (197): To a solution of **195** (1.82 g, 21.1 mmol) in CH_2Cl_2 (60 ml) at -40 °C was added *m*-CPBA (4.88 g, 85% pure, 24 mmol). The reaction mixture was stirred at -40 °C for 1 h and warmed to -20 °C for an additional 1 h. Excess *m*-CPBA was destroyed by addition of saturated $Na_2S_2O_3$ solution (20 mL) and the organic phase was washed with saturated $NaHCO_3$ solution (2 X 30 mL). The aqueous layer was extracted with CH_2Cl_2 (3 X 20 mL) and the combined organic layer was washed with ice-cold 1N NaOH. Standard work-up gave epoxy alcohol (0.705 g, ~33% yield): GC purity: 100%; IR (film) 3429, 1119 and 1064 cm^{-1}; CIMS *m/z* (rel. intensity) 103 (M^++1, 100), 97 (2.3), 95 (2.2), 93 (2.9), 91 (4.2); ^1H NMR ($CDCl_3$, ppm) 3.76 (m, 2H), 3.0 (dd, *J* = 5.1, 6.4 Hz, 1H), 2.6 (m, 1H), 1.36 (s, 3H), 1.31 (s, 3H). The epoxy alcohol was converted to the mesylate **196** by reaction with $MeSO_2Cl$ in the presence of Et_3N at -50 °C (96% yield) as described for the preparation of **85**. Mesylate **196** was sufficiently pure (GC purity >98%) for use in the subsequent reaction. For **196**: CIMS *m/z* (rel. intensity) 181 (M^++1, 100); ^1H NMR ($CDCl_3$, ppm) 4.43 (dd, *J* = 11.7, 4.2 Hz, 1H), 4.23 (dd, *J* = 11.7, 4.2 Hz, 1H), 3.08 (s, 3H), 3.07 (m, 1H), 1.36 (s, 3H), 1.33 (s, 3H).

To a solution of NaOH (5.0 g, 125 mmol) in H_2O (10 mL) and toluene (10 mL)

was added tetraoctylammonium bromide (0.05 g), **194** (0.472 g, 1.8 mmol) and **196** (0.413 g, 2.3 mmol) at room temperature. The reaction mixture was stirred for 12 h and then extracted with ether (3 x 30 mL). Standard work-up followed by chromatography using ethyl acetate:hexane (7:3) as the eluant gave **197** (0.45 g, 72%) as an oil: IR (film) 3055, 1630, 1183, 1118, 1072, 723 and 695 cm^{-1}; CIMS *m/z* (rel. intensity) 347 (M^++1, 100), 346 (M^+, 9.0), 331 (1.3), 329 (7.5), 275 (1.4), 261 (12); ^1H NMR ($CDCl_3$, ppm) 7.96-7.75 (m, 4H), 7.60-7.40 (m, 6H), 3.59 (dq, J_{H-H} = 7.4 Hz, J_{P-H} = 16.0 Hz, 1H), 2.90 (m, 2H), 2.77(m, 1H), 1.56 (dd, J_{H-H} = 7.5 Hz, J_{P-H} = 15.0 Hz, 1H), 1.28 (s, 3H), 1.23 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 131.93-128.50 (12C, m), 63.46, 58.38, 37.28, 36.80, 24.40, 18.65, 16.20; Anal. calcd. for $C_{19}H_{23}PO_2S$: C, 65.88; H, 6.69. Found: C, 65.60; H, 6.56.

4,9,13,17-Tetramethyl-4(*E*),8(*E*),12(*E*),16-octadecatetraenal (198): This was prepared in 92% yield according to the procedure described in ref 31b. **198**: IR (film) 2964, 2923, 2855, 1728, 1446, 1383 and 1108 cm^{-1}; CIMS *m/z* (rel. intensity) 317 (M^++1, 100), 299 (88), 235 (20), 217 (56.7), 193 (54.7), 179 (15.4), 165 (15.6), 149 (30.7), 137 (66.2); ^1H NMR ($CDCl_3$, ppm) 9.74 (t, J = 1.8 Hz, 1H), 5.21-5.04 (m, 4H), 2.51 (dt, J = 1.8, 7.5 Hz, 2H), 2.31 (t, J = 7.5 Hz, 2H), 2.12-1.90 (m, 12H), 1.67 (s, 3H), 1.61 (s, 3H), 1.59 (s, 9H); ^{13}C NMR ($CDCl_3$, ppm) 202.51, 135.39, 134.94, 133.06, 131.23, 125.51, 124.43, 124.26, 124.01, 42.20, 39.74, 31.91, 28.22, 28.06, 26.81, 26.67, 25.65, 17.66, 16.10, 16.04. IR and ^1H NMR spectra of **198** are in agreement of those reported in reference 31b.

6(*E*)-5-Thia-2,3-oxidosqualene (73): To a solution of **197** (0.173 g, 0.5 mmol) in THF (10 mL) was added dropwise a solution of LDA [0.5 mmol, prepared from diisopropyl amine (0.07 mL, 0.5 mmol) and *n*-BuLi (0.2 mL, 0.5 mmol, 2.5 M in

hexanes at -78 °C] in THF (5 mL) under argon, at -100 °C. After 20 min, aldehyde **198** (0.159 g, 0.5 mmol) in THF (5.0 mL) was added dropwise to the orange-coloured solution. 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 removed *in vacuo* and the resulting slurry filtered through a small column of silica gel using ethyl acetate:hexanes (7:3) as the eluant to give **199** and **200** (~6:4) (0.225 g, 68% yield). Thin layer chromatographic analysis on silica gel revealed two overlapping components. The major, faster eluting isomer **199** (*syn*) 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 **199** (0.103 g, 31%) and a mixture of **199** and **200**. Major isomer **199** (*syn*): IR (film) 3322, 2960, 2924, 2854, 1725, 1665, 1590, 1437, 1377, 1319, 1249, 1159, 1111, 1072, 850, 746 and 696 cm^{-1}; ^1H NMR ($CDCl_3$, ppm) 8.35 (m, 2H), 8.07 (m, 2H), 7.50 (m, 6H), 5.53 (bm, 1H), 5.17 (m, 1H), 5.10 (m, 3H), 4.07 (t, J = 8.8 Hz, 1H), 2.68 (m, 2H), 2.43 (dd, J = 11.0, 6.6 Hz, 1H), 2.28 (m, 1H), 2.10-1.90 (m, 15H), 1.67 (s, 3H), 1.64 (s, 3H), 1.59 (s, 6H), 1.56 (s, 3H), 1.40 (d, J_{P-H} = 16.0 Hz, 3H), 1.27 (s, 3H), 1.24 (s, 3H); Anal. calcd. for $C_{41}H_{59}O_3PS$: C, 74.28; H, 8.97. Found: C, 74.56; H, 9.06.

To a solution of **199** (90 mg, 0.136 mmol) in THF (10 mL) was added NaH (pre-washed with hexanes, 15 mg, 0.625 mmol). This mixture was stirred for 3 h under argon and the reaction was quenched by addition of water (0.1 mL). The solvent was removed *in vacuo*, and column chromatography using ethyl acetate:hexanes (5:95) as the eluant gave pure **73** (52 mg, 86%) as an oil: IR (film) 2963, 2921, 2854, 1717, 1666, 1630, 1449, 1377, 1249, 1124 and 838 cm^{-1}; CIMS *m/z* (rel. intensity) 445 (M^++1, 6.4), 359 (11.9), 327 (3.1), 295 (3.0), 217 (2.8), 191(5.0), 189 (5.4), 177 (11), 171 (100) 163 (7.5), 161 (5.9); ^1H NMR ($CDCl_3$, ppm) 5.55 (tq, J = 7.1, 1.20 Hz, 1H), 5.12 (m, 4H), 2.92 (m, 2H), 2.67 (dd, J = 2.2, 8.8 Hz,

1H), 2.18 (dt, J = 7.3, 7.4 Hz, 2H), 2.0 (14 H, bm), 1.92 (d, J = 1.20 Hz, 3H), 1.68 (s, 3H), 1.60 (s, 12H), 1.32 (s, 3H), 1.29 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 138.61, 135.27, 134.45, 132.87, 131.19, 129.45, 125.01, 124.90, 124.47, 124.39, 63.20, 59.01, 39.82, 39.21, 39.15, 30.90, 30.32, 28.53, 27.94, 26.87, 26.78, 25.51, 24.50, 24.20, 18.81, 18.16, 17.62, 16.10, 16.02; Anal. calcd. for $C_{29}H_{48}OS$: C, 78.32; H, 10.89. Found: C, 78.23; H, 10.95.

1-(Ethylenedioxy)-4,9,13,17-tetramethyl-16-bromo-17-hydroxy-4(*E*), 8(*E*), 12(*E*)-octadecatriene (201): To a vigorously stirred solution of **193** (2.15 g, 6.0 mmol) in THF (100 mL) and water (30 mL) at 0 °C was added dropwise a solution of NBS (1.07 g, 6.0 mmol) in THF (15 mL) and water (5.0 mL) over 30 min. The mixture was stirred for 1 h at 0 °C, the THF was removed *in vacuo* and the aqueous layer was extracted with diethyl ether (4 x 30 mL). Standard work-up followed by flash column chromatography using ethyl acetate:hexane (65:35) as the eluant gave pure **201** (1.04 g, 38%) as an oil: IR (film) 3477, 1667, 1630, 1446, 1384, 1141 and 1037 cm^{-1}; CIMS *m/z* (rel. intensity) 459/457 (M^++1, 42.7/45.5), 441/439 (M^++1-H_2O, 17.4/18.2), 397/395 (100/97.9) 379/377 (52.2/92.7), 359 (51.5), 315 (97.1), 297 (77.3), 243 (18.3), 205 (22.6); ^1H NMR ($CDCl_3$, ppm) 5.22-5.10 (m, 3H), 4.85 (t, J = 4.82 Hz, 1H), 3.98 (dd, J = 1.8, 11.3 Hz, 1H), 3.96 (m, 2H), 3.85 (m, 2H), 2.38-2.25 (m, 1H), 2.22-1.92 (m, 12H), 1.82-1.72 (m, 3H), 1.60 (s, 3H), 1.59 (s, 6H), 1.34 (s, 3H), 1.32 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 134.93, 134.28, 133.00, 125.98, 124.52, 124.38, 104.41, 72.43, 70.87, 64.84, 39.62, 38.17, 33.89, 32.49, 32.21, 28.22, 28.15, 26.64, 25.84, 16.03, 15.84; Anal. calcd. for $C_{24}H_{41}BrO_3$: C, 63.13; H, 9.06. Found: C, 63.30; H, 9.09.

4,9,13,17-Tetramethyl-16-bromo-17-hydroxy-4(*E*),8(*E*),12(*E*)-octadecatrienal (202): This was prepared in 42% yield by the procedure described for the preparation of **198**. **202**: IR (film) 3440, 2933, 2850, 1725, 1445, 1383, 1178 and 907 cm^{-1}; CIMS *m/z* (rel. intensity) 415/413 (M^++1, 0.86/0.74), 397/395 (8.8/7.4), 379/377 (4.5/4.6), 333 (75.6), 315 (100), 297 (21.9), 191 (24), 149 (19.5), 135 (27.3); ^1H NMR ($CDCl_3$, ppm) 9.74 (t, *J* =1.7 Hz, 1H), 5.25-5.06 (m, 3H), 3.98 (dd, *J* = 1.8, 11.4 Hz, 1H), 2.51 (dt, *J* =1.8, 7.5 Hz, 2H), 2.32 (t, *J* =7.5 Hz, 2H), 2.38-2.25 (m, 1H), 2.20-1.92 (m, 10H), 1.82-1.72 (m, 1H), 1.61 (s, 3H), 1.59 (s, 6H), 1.34 (s, 3H), 1.32 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 202.55, 135.17, 133.10, 126.00, 125.49, 124.20, 72.47, 70.90, 42.19, 39.65, 38.20, 32.21, 31.92, 28.22, 28.06, 26.67, 25.88, 16.13, 16.03, 15.86. ^1H-NMR and IR spectra of **202** are in agreement with those reported in reference 30f.

4,9,13,17-Tetramethyl-16,17-epoxy-4(*E*),8(*E*),12(*E*)-octadecatrienal (203): This was prepared in 87% yield by ring closure of bromohydrin **202** in a procedure similar to that described for the synthesis of **101**. **203**: IR (film) 2978, 2926, 2870, 1727, 1667, 1448, 1378 and 1123 cm^{-1}; CIMS *m/z* (rel. intensity) 333 (M^++1, 60.8), 316 (22.3), 315 (100), 297 (20.2), 191 (24.4), 175 (15.1), 153 (24.1), 149 (19.4), 109 (17.9); ^1H NMR ($CDCl_3$, ppm) 9.74 (t, *J* = 1.8 Hz, 1H), 5.20-5.05 (m, 3H), 2.70 (t, *J* = 6.3 Hz, 1H), 2.51 (dt, *J* = 1.8, 7.5 Hz, 2H), 2.32 (t, *J* =7.5 Hz, 2H), 2.20-1.93 (m 12H), 1.61 (s, 6H), 1.59 (s, 3H), 1.30 (s, 3H), 1.25 (s, 3H). ^1H-NMR and IR spectra of **203** are in agreement with those reported in reference 25c.

Diphenylphosphinoyl 1-(prenylthio)ethane (205): This was prepared in 73% yield by coupling **194** and 1-chloro-3-methyl-2-butene (**204**) under phase-transfer conditions as described for the preparation of **197**. **205**: IR (film) 3055, 1667,

1186, 1118, 1072, 722 and 697 cm^{-1}; CIMS *m/z* (rel. intensity) 333/331 (M^++1, 7.8/100), 263 (2.0), 231 (5.8), 230 (11.7), 229 (4.6); ^1H NMR ($CDCl_3$, ppm) 7.95-7.75 (m, 4H), 7.58-7.40 (m, 6H), 5.07 (t, J = 7.7 Hz, 1H), 3.27 (dq, J_{H-H} = 7.4 Hz, J_{P-H} = 14.7 Hz, 1H), 3.12 (dd, J = 3.3, 7.7 Hz, 2H), 1.71 (s, 3H, 1.58 (s, 3H), 1.55 (dd, J_{H-H} = 7.4 Hz, J_{P-H} = 15.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, ppm) 136.90, 132.58, 132.15, 131.94, 131.86, 131.62, 131.53, 130.61, 128.50, 128.40, 128.29, 119.49, 36.50, 30.03, 25.68, 17.73, 16.14; Anal. calcd. for $C_{19}H_{23}POS$: C, 69.07; H, 7.02. Found: C, 68.92; H, 6.89.

18(*E*)-20-Thia-2,3-oxidosqualene (76): This was prepared by the procedure described for the synthesis of **73**. A mixture of *syn* and *anti* isomers **206** and **207** (~65:35) was obtained by condensation of **194** with **203**. Flash column chromatography partially separated this mixture to give the pure *syn* isomer **206** in 33% yield: IR (film) 3315, 2960, 2927, 2854, 1730, 1666, 1590, 1438, 1377, 1319, 1156, 1112, 745 and 699 cm^{-1}; ^1H NMR ($CDCl_3$, ppm) 8.40 (m, 2H), 8.10 (m, 2H), 7.54 (m, 6H), 5.66 (bm, 1H), 5.51 (m, 3H), 4.96 (t, J = 8.0 Hz, 1H), 4.05 (t, J = 8.8 Hz, 1H), 2.84 (dd, J = 8.3, 10.9 Hz, 1H), 2.70 (t, J = 6.3 Hz, 1H), 2.41 (dd, J = 8.0, 10.9 Hz, 1H), 2.34 (m, 1H), 2.20-1.90 (m, 15H), 1.67 (s, 3H), 1.61 (s, 3H), 1.59 (s, 6H), 1.54 (s, 3H), 1.42 (d, J_{P-H} = 16.2 Hz, 3H), 1.29 (s, 3H), 1.25 (s, 3H); Anal. calcd. for $C_{41}H_{59}O_3PS$: C, 74.28; H, 8.97. Found: C, 74.04; H, 9.02.

For **76**: IR (film) 2960, 2923, 2858, 1663, 1630, 1448, 1376, 1247 and 1120 cm^{-1}; CIMS *m/z* (rel. intensity) 445 (M^++1, 90), 427 (28.5), 375 (44.4), 343 (100), 325 (32.3); ^1H NMR ($CDCl_3$, ppm) 5.35 (tq, J = 7.1, 1.20 Hz, 1H), 5.23 (tm, J = 7.4 Hz, 1H), 5.19-5.10 (m, 3H), 3.30 (d, J = 7.4 Hz, 2H), 2.70 (t, J = 6.3 Hz, 1H), 2.20-1.94 (bm, 16H), 1.87 (s, 3H), 1.72 (s, 3H), 1.68 (s, 3H), 1.61 (s, 3H), 1.60 (s, 6H), 1.30 (s, 3H), 1.26 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm) 138.20, 135.08, 134.60, 131.06,

129.56, 126.91, 125.00, 124.59, 124.38, 119.73, 64.18, 58.19, 39.70, 39.40, 36.35, 29.95, 29.28, 28.30, 27.65, 27.58, 26.75, 25.62, 24.89, 24.04, 18.76, 18.09, 17.75, 16.10, 16.00; Anal. calcd. for $C_{29}H_{48}OS$: C, 78.32; H, 10.89. Found: C, 78.41; H, 11.01.

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