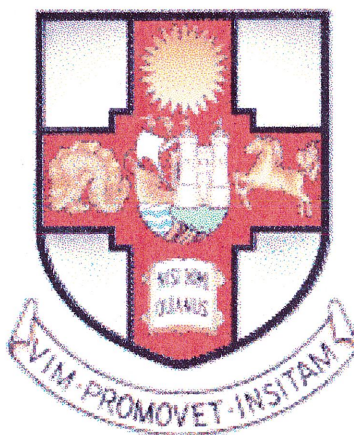


هَدِيَّة GIFT

Synthetic and Biosynthetic Studies on Squalestatin S1.

Awatef Mahdi Hassan.



A thesis submitted to the University of Bristol in
partial fulfilment of the requirements for
the degree of Doctor of Philosophy
in the Faculty of Science.

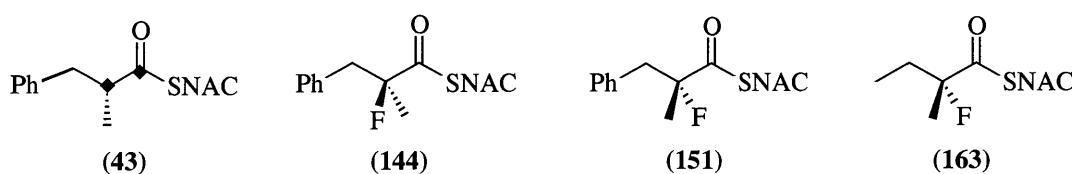
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October 1997.

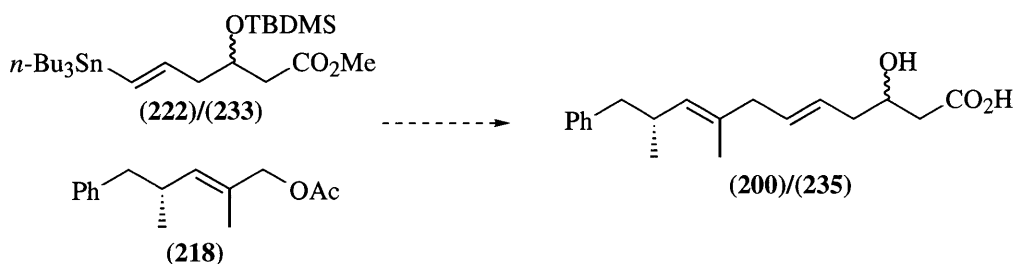
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Abstract.

Cardiovascular diseases (CVD) remains the largest cause of human death in industrialised nations. Since the discovery of the relation between CVD and hypercholesterolemia, extensive efforts have been focused on finding drugs that reduce serum cholesterol level by controlling key enzymes in the biosynthetic pathway of cholesterol. Squalostatins are a family of novel fungal metabolites which possess potent inhibitory action against squalene synthase and hence cholesterol biosynthesis. Therefore, squalostatins could provide a method for treatment of hypercholesterolemia. The aim of the project is to elucidate the biosynthetic pathway to squalestatin S1.



Efficient methods for the synthesis of ^{13}C - and fluorine-labelled analogues (43), (144), (151) and (163) of the putative diketide precursor to squalostatins have been developed. The introduction of the fluorine α to the carbonyl was to try to prevent *in vivo* β -oxidation of the diketide precursor which resulted in the degradation of (43). The fluorine was introduced stereoselectively using Evans' oxazolidinone chiral auxiliaries and an electrophilic fluorine source. Synthetic routes to the (*E,E*)-diastereomers of putative hexaketide intermediates (200) and (235) was developed. The key step in the synthesis has been the palladium-catalysed coupling of the vinylstannane (222) or (233), and the allylic acetate (218).



Studies on the incorporation of $[1,2-^{13}\text{C}_2]$ acetate gave a level of incorporation up to 5.6% using pulse feeding to *Phoma* sp C2932 cultures. Incorporation studies were also undertaken with the fluorinated diketide analogues, however no incorporation was detected. $[1,2-^{13}\text{C}_2]$ Acetate has been successfully incorporated into S1 using another S1 producer, strain ATCC 20986. On the other hand feeding (43) to either active fermentation or resting cell preparation of strain ATCC 20986 resulted in degradation of the precursor and random incorporation of the ^{13}C label into S1.

To

my mum and husband

who helped make it possible

Fatima and Hassan

who helped make it worthwhile

Acknowledgments.

I would like to express my sincere thanks to my supervisor Prof. Tom Simpson for his guidance and encouragement, and to Dr Chris Willis for her interest in the project and useful discussions. I wish to express my thanks to University of Bahrain for scholarship and the support provided throughout the duration of this project.

My thanks also go to Dr Ken McNeil for mass spectrometry service and to Dr Martin Murry, Dr Torren Peakman and Rose Sylvester for NMR service and training.

A big thank you to all my lab mates who were happy to see me leave the lab to have the music on!!! KumYul is also thanked for helping me to slow down and take care of myself in many occasions. Mairi, Tim and Lee are thanked for reading my thesis.

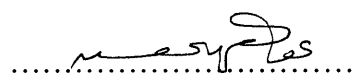
Special thank you must go to my wonderful friend Eiman and her mother with whom I shared very precious times and memories that I will cherish forever. I must also thank my friends Safaa and Mabrooka for always being there for me to make me listen to their problems but never talk about mine!!!

A huge thanks to my family especially my mother for her invaluable love and care for my kids. To my son and daughter, thank you very much for having a big mind and patience. I am indebted to my husband for his love and encouragement which inspire me at all times.

Last but not least, I am obliged to thank a great father who lead me towards my dream but sadly did not wait to see it fulfilled... thank you for all the strength and determination that you put into me.

Declaration.

The work described in this thesis was performed in the School of Chemistry, University of Bristol under the supervision of Prof. T. J. Simpson between October 1994 and September 1997. Except where indicated by reference, the work is original and has not been submitted for any other degree. The views expressed in this thesis are those of the author and not of the university.

A handwritten signature in black ink, appearing to read 'Awatef Mahdi Hassan', is written over a horizontal dotted line.

Awatef Mahdi Hassan

October 1997

Abbreviations.

Ac	Acetyl
ACP	Acyl carrier protein
α_D	optical rotation
AIBN	Azo(bis) <i>isobutyronitrile</i>
br	Broad
BuLi	Butyllithium
<i>c</i>	Concentration
°C	Degree centigrade
CHD	Coronary heart disease
CoA	Coenzyme A
COSY	Correlation spectroscopy
d	doublet
dd	double doublet
ddd	double double doublet
ddq	double doublet of quartets
ddqn	double doublet of quintets
dt	doublet of triplets
dq	doublet of quartets
dqd	double quartet of doublets
dqnd	double quintet of doublets
dsp	doublet of septets
dsx	doublet of sextets
DAST	Diethylaminosulfur trifluoride
dba	Dibenzylethane acetone
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	1, 3-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DDQ	2, 3-Dichloro-5, 6-dicyano-1, 4-benzoquinone
d.e	Diastereomeric excess
DH	Dehydratase
DIBAL-H	<i>Diisobutylaluminium hydride</i>
δ_H	Chemical shift
DMAP	4-Dimethylaminopyridine
DMF	<i>N,N</i> -Dimethylformamide
DMPU	1,3-Dimethyl-3,3,5,6-tetrahydro-2(1 <i>H</i>)-pyrimidinone

DMSO	Dimethyl sulfoxide
EDCI	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
E.I	Electron impact
eq	Equivalent
ER	Enolreductase
FAB	Fast atom bombardment
FPP	Farnesyl pyrophosphate
g	Gram(s)
h	Hour(s)
HMG	Hydroxymethylglutaryl
HMPA	Hexamethylphosphoramide
HPLC	High performance liquid chromatography
Hz	Hertz
IC ₅₀	50% Inhibitory concentration
IPP	<i>iso</i> -Pentenyl pyrophosphate
IR	Infrared
<i>J</i>	Coupling constant
KS	Ketosynthase
KR	Ketoreductase
L	Ligand
LDA	Lithium <i>diisopropyl</i> amine
lit	Literature
m	Multiplet
<i>m</i>	<i>meta</i>
M	Metal
M ⁺	Molecular ion
mmol	Millimole
mp	Melting point
MPLC	Medium pressure liquid chromatography
Ms	Methanesulfonyl
MT	Methyl transferase
m/z	Mass to charge ratio
<i>n</i>	Normal
NAC	<i>N</i> -Acetylcysteamine
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NaHMDS	Sodium hexamethyldisilazide
NFBS	<i>N</i> -Fluorobenzenedisulfonimide
NMR	Nuclear magnetic resonance
nOe	Nuclear <i>Overhauser</i> effect

<i>o</i>	<i>ortho</i>
oct	octet
<i>p</i>	<i>para</i>
PAL	Phenyl ammonia lyase
PDC	Pyridinium dichromate
PKS	Polyketide synthase
PMB	<i>para</i> -Methoxybenzyl
ppm	Parts per million
PSPP	Presqualene pyrophosphate
q	Quartet
qd	Quartet of doublets
rpm	Revolutions per minute
s	Singlet
S1	Squalestatin S1
SAM	<i>S</i> -Adenosyl methionine
spd	Septet of doublets
SS	Squalene synthase
sxd	Sextet of doublets
<i>t</i>	<i>tertiary</i>
t	Triplet
tt	Triplet of triplets
TBDMS	<i>tertiary</i> -Butyldimethylsilyl
Tf	Trifluoromethanesulfonyl
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TLC	Thin layer chromatography
TMS	Trimethylsilyl
Ts	<i>para</i> -Toluenesulfonyl
UV	Ultraviolet
$\nu_{\max}$	Frequency

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Chapter 1

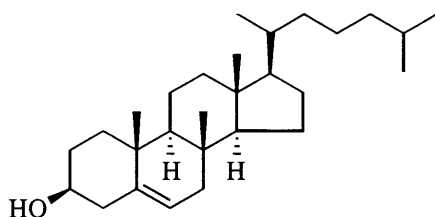
Introduction.

1.1 Hypercholesterolemia and the Discovery of the Squalostatins.

Cardiovascular disease (CVD) remains the largest cause of death in industrialised nations. In the USA in 1992, 915,000 deaths were recorded as resulting from CVD, compared with 521,000 from all types of cancer combined and 23,000 from AIDS. Therefore, even though the relative incidence of CVD has almost halved since the 1950's, there remains keen interest in the pharmaceutical industry in developing better drugs for the treatment of CVD.¹

The majority of CVD mortalities (79% in 1992) are due to coronary heart disease (CHD) or coronary artery disease (CAD). The main cause of both CHD and CAD is atherosclerosis, which is characterised by the formation of atherosclerotic plaques. This is composed of cholesterol esters, lipids and other cellular debris on the inner walls of arteries. Atherosclerosis causes hardening of the artery wall and reduction of blood flow. Atherosclerosis is also implicated in other mortalities and illnesses, owing to the resulting reduced blood flow to the brain, kidneys and intestine. Atherosclerosis can be treated in some cases by bypassing the damaged area, by opening the lumen by balloon angioplasty, or by removing the offending plaques by surgical andarectomy. However, in a 10 year study completed in 1984, the Lipid Research Clinic Program² showed that by lowering serum cholesterol levels, the incidence of fatal heart disease could be decreased significantly. This study established high serum cholesterol level as a major factor in heart disease.

Cholesterol (**1**) is a sterol which is vital for many biological processes such as maintaining the fluidity of membranes of eukaryotic cells, acting as a precursor to the steroid hormones such as progesterone, testosterone, eostradiol and cortisol, and to bile acids which are necessary for the absorption and degradation of dietary lipids.

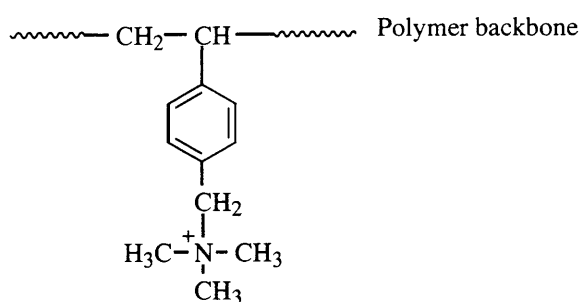


Cholesterol (**1**).

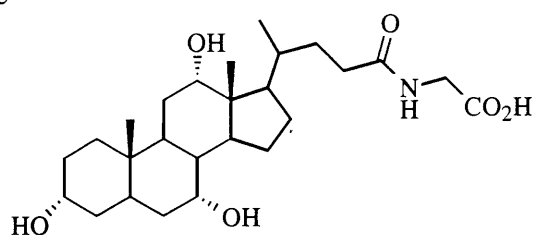
The human body takes about half of its requirement for cholesterol (**1**) by endogenous biosynthesis which occurs mainly in the liver. The remaining amount is obtained from the diet. Consequently, one effective way of reducing serum cholesterol level is to reduce the dietary intake of cholesterol.³

In many instances dietary regulation is impractical or insufficient. Consequently, extensive efforts have been focused on finding effective therapeutic agents for lowering serum cholesterol levels by controlling certain key steps in the cholesterol biosynthetic pathway.⁴ Those currently available can be divided into two main classes: bile acid sequestrants and HMG-CoA reductase inhibitors.

Bile acid sequestrants (for example cholestyramine⁵ (2)) are basic amine anion exchange resins. They work by sequestering sterol-derived bile acid anions such as glycocholic acid (3) in the intestine as an insoluble complex which is excreted. Although it is often an effective treatment, large doses are required, socially unpleasant side effects arise, and the patient response and compliance is often poor.

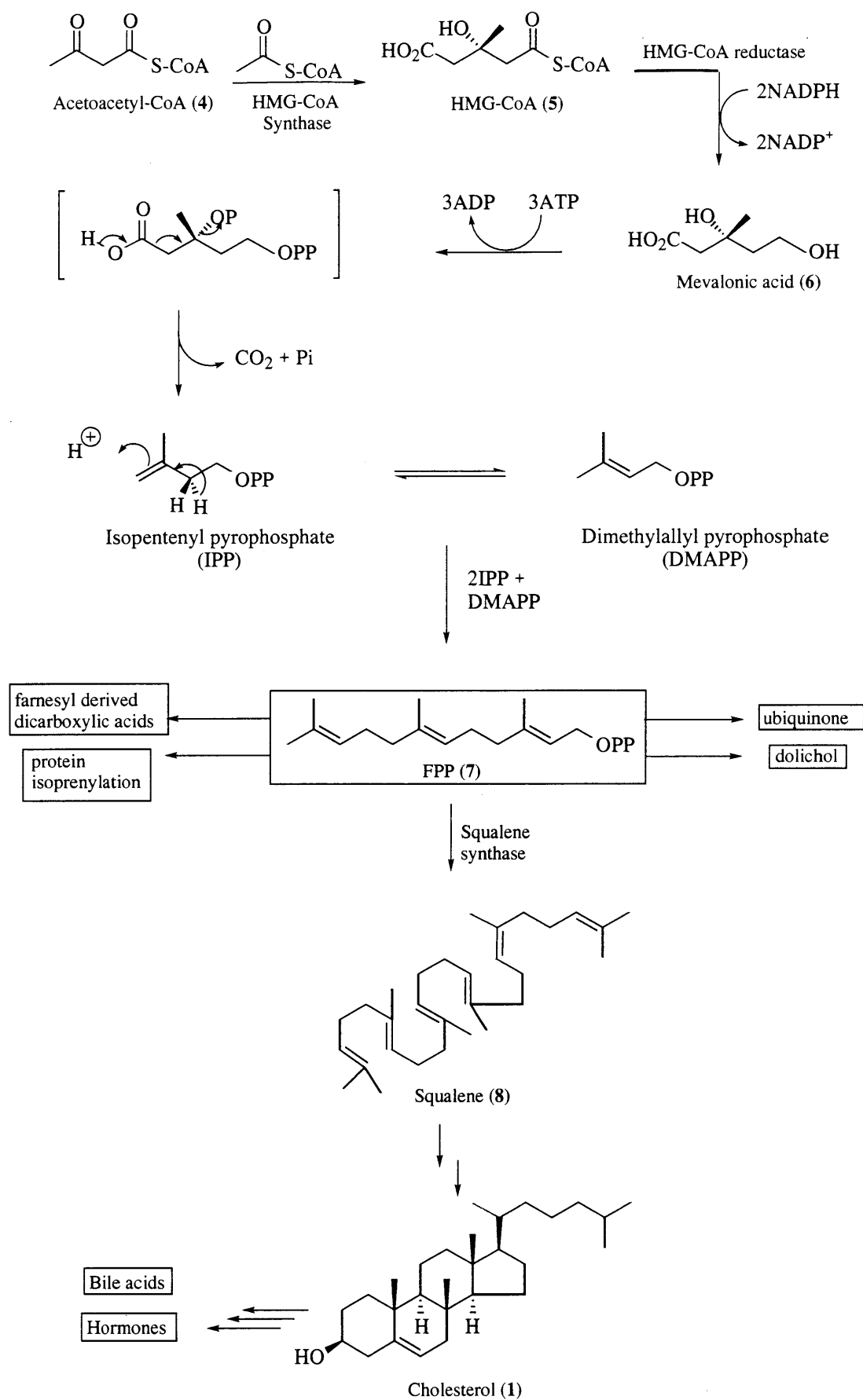


Cholestyramine (2)



Glycocholic acid (3)

The biosynthetic pathway of cholesterol (Scheme 1.1), starts with acetyl CoA and acetoacetyl CoA (4) (also obtained from acetyl CoA) which are joined together by the enzyme hydroxymethylglutaryl CoA (HMG-CoA) synthase to form HMG-CoA (5). The latter is then reduced by HMG-CoA reductase to give mevalonate (6). A number of steps then convert mevalonic acid into farnesyl pyrophosphate (FPP) (7). FPP is the last common intermediate for the synthesis of all the end products of the sterol biosynthetic pathways. In addition to its role in cholesterol biosynthesis, FPP is used in farnesylation and isoprenylation of certain proteins and in the synthesis of ubiquinone. In cholesterol biosynthesis, squalene synthase (SS) catalyses the head-to-head dimerization of FPP to give squalene (8). A series of enzyme-catalysed reactions finally produces cholesterol (1).



Scheme 1.1 Sterol biosynthetic pathway.

Since HMG-CoA reductase inhibitors have been shown to affect post-mevalonate processes other than sterol biosynthesis, several screening campaigns have been initiated to find inhibitors of other enzymes in the cholesterol biosynthetic pathway. These investigations led recently to the discovery of a class of natural products named zaragozic acids or squalostatins, which act as potent inhibitors of squalene synthase (SS). Inhibition at this stage in the biosynthetic pathway would be preferred to inhibition at HMG-CoA reductase catalysed step since SS catalyses the first specific step to sterol biosynthesis (Scheme 1.1) and hence other post mevalonate processes should not be affected.

1.2 Structure and Biological Activity of the Squalostatins .

In 1991 and 1992 three groups independently and almost simultaneously reported the isolation of zaragozic acids (squalestatins). Merck isolated zaragozic acid A (**9**) from an unidentified fungus, strain ATCC 20986, isolated from filtrates of the Jalon river in the province of Zaragoza in Spain.⁸ Glaxo isolated the same compound (as squalestatin S1) from a different fungus, *Phoma* sp C2932, found in soil from Portugal.^{9, 10} A Japanese group (at Tokyo Noko University /Mitsubishi Kasei Corporation) isolated it from yet another fungus, *Setosphaeria khartoumensis*.¹¹

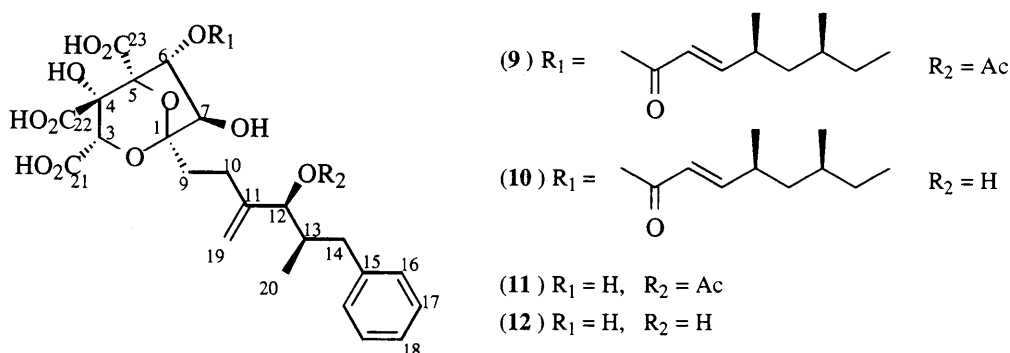


Figure 1.2 The structure of squalestatins.

In addition to the major metabolite squalestatin S1 (**9**) a number of squalestatins have also been isolated such as S2 (**10**), H1 (**11**) and H2 (**12**) (Figure 1.2). They all possess a unique highly oxygenated 2,8-dioxabicyclo[3.2.1]octane-3,4,5-tricarboxylic acid "core" with two lipophilic "arms" attached either as an acyl group on the C-6 hydroxyl, or as an alkyl group on the C-1 carbon.

Zaragozic acid A is identical to squalestatin S1 (**9**). The structures of other zaragozic acids are shown in Figure 1.3, along with their producing organisms.^{12, 13}

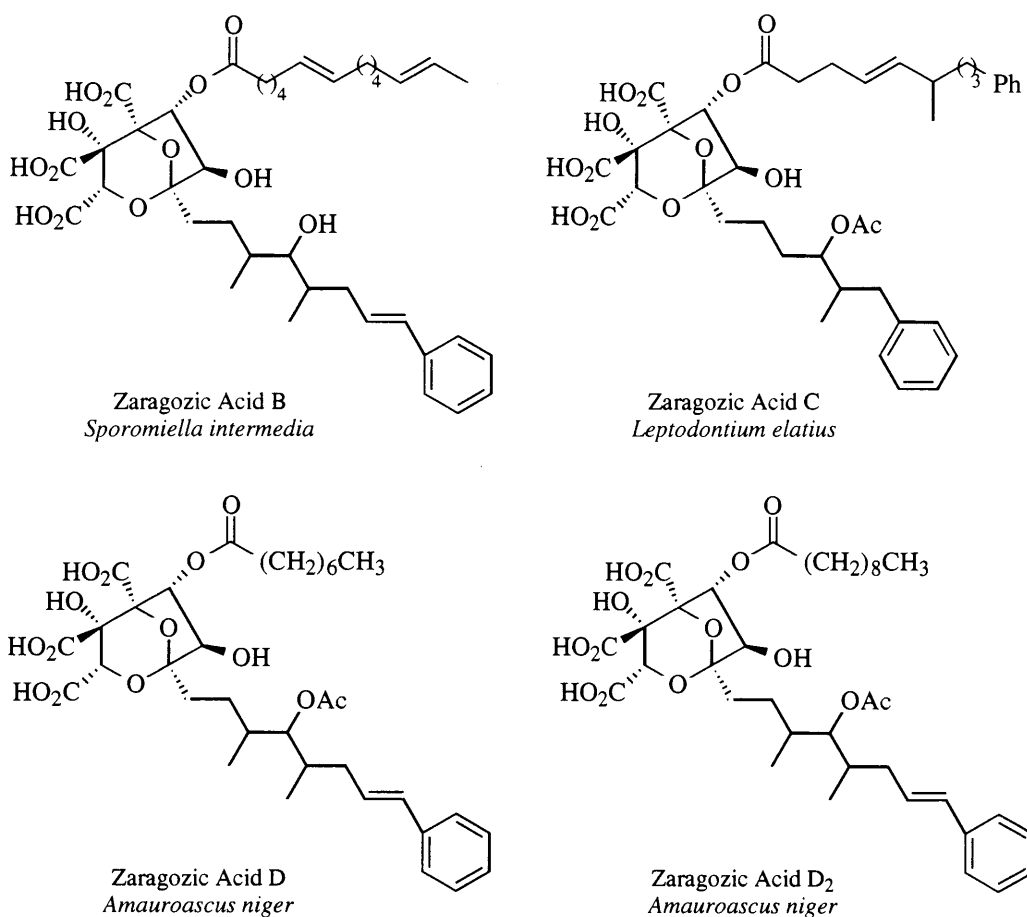


Figure 1.3 The structures of the zaragozic acids.

The structure of squalestatin S1 was determined by a standard combination of NMR spectroscopy, mass spectrometry and X-ray crystal structure analysis and has also been confirmed by three total syntheses.¹⁴⁻¹⁷

The squalostatins are the most potent inhibitors of mammalian squalene synthase known to date. *In vitro* assays with rat microsomal squalene synthase, Hep-G2 (a human liver cell line) microsomes, and isolated rat hepatocytes (tissue functionally equivalent to liver) showed that the squalostatins and the zaragozic acids were potent inhibitors.¹

In vivo studies, conducted by Glaxo, showed that when rats were dosed intravenously with S1 at levels of 0.1 mg per Kg of body weight, cholesterol biosynthesis was reduced by 50%. When marmosets (which have a similar lipoprotein profile to man) were dosed orally with 10 mg per kg per day of S1, serum cholesterol levels fell by up to 75% for eight weeks, with no attenuation of response.¹⁸

Recently, other naturally occurring, fungal-derived SS inhibitors have been reported (Figure 1.4). The viridifungins (**13**), (**14**) and (**15**) were isolated by Merck from *Trichoderma viride* (ATCC 74084)¹⁹, and CP-225 917 (**16**) has been isolated by

Pfizer from an uncharacterised fungus (ATCC 74256).²⁰ The viridiofungins are less potent than squalostatins and the potency of CP-225 917 has not been reported.

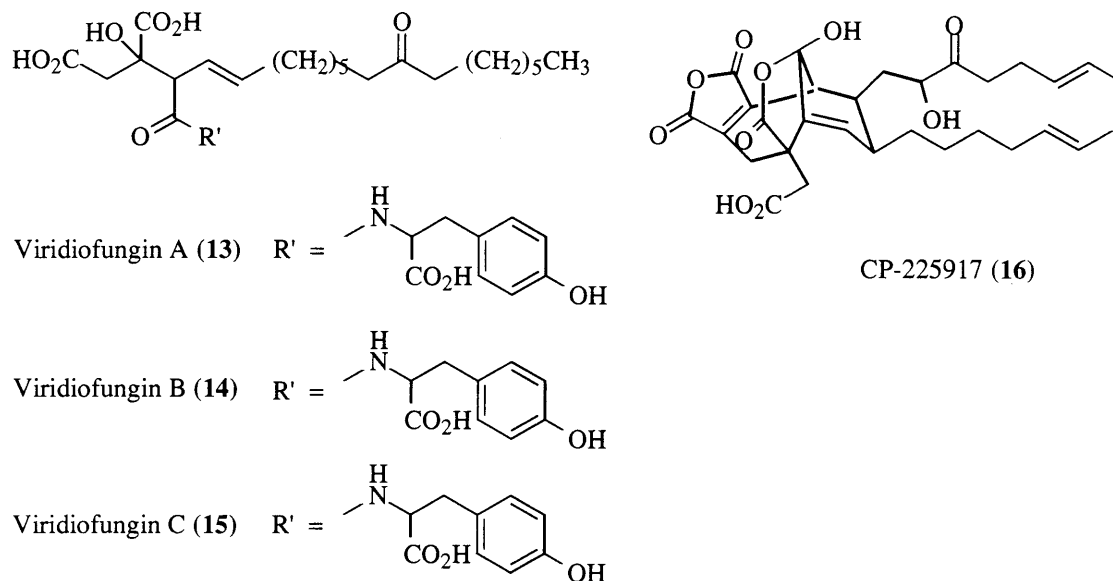
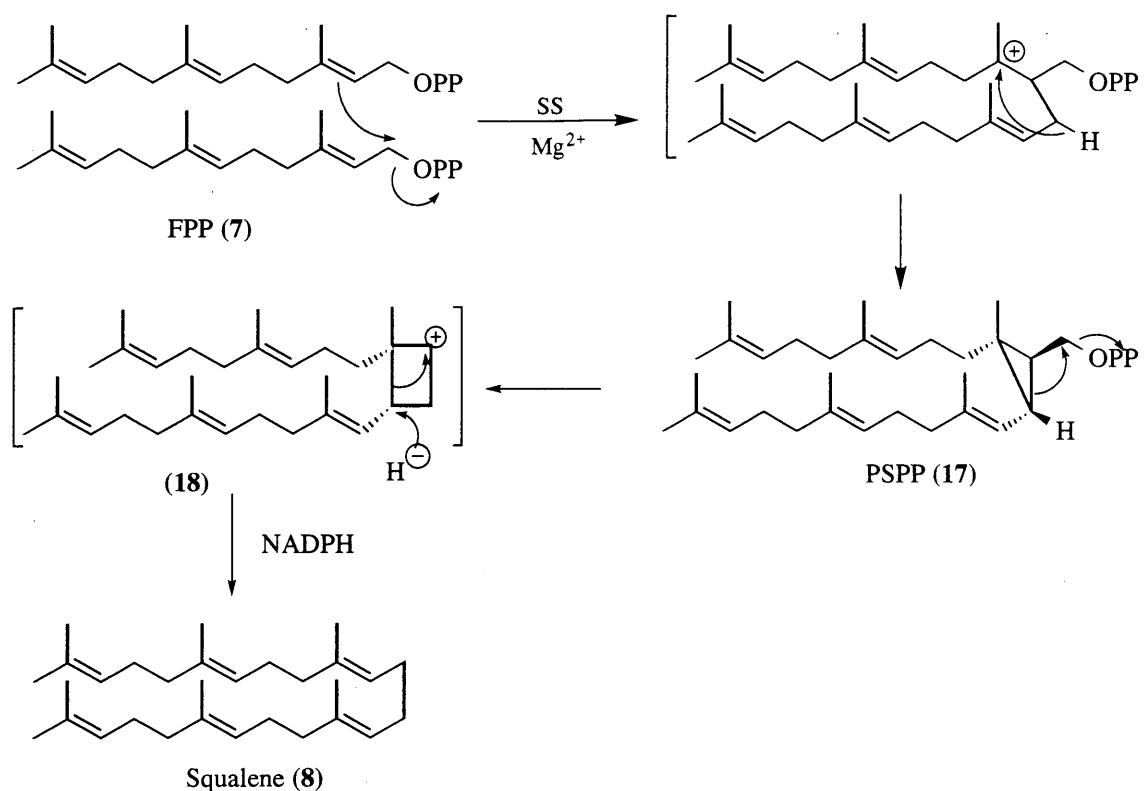


Figure 1.4 *Squalene synthase inhibitors: viridiofungins and cp-225917.*

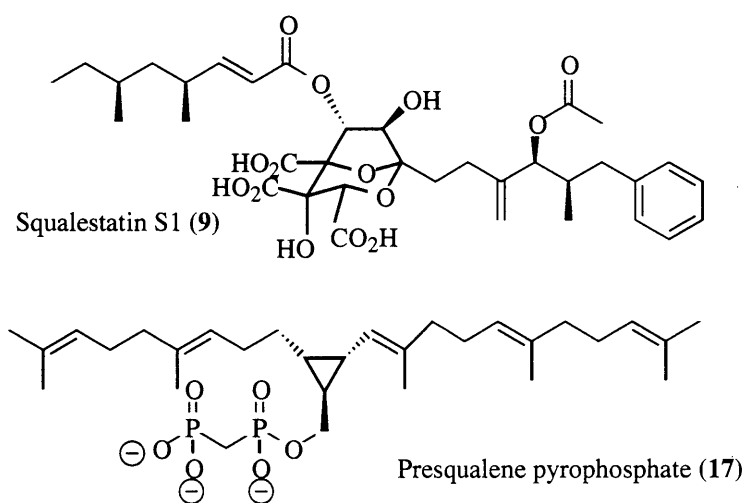
It is interesting to compare the structural similarities of the viridiofungins and CP-225917 with those of the squalostatins. Both squalestatin S1 (9) and CP-225917 (16) have two lipophilic side chains flanking a polar bicyclic core. Squalestatin H1 (11) contains only one side chain attached to the tricarboxylic acid core, and the viridiofungins contain a similar tricarboxylate function attached to a lipophilic tail.

1.3 Mechanism of Action of the Squalostatins.

Squalene synthase converts two molecules of farnesyl pyrophosphate (7) into one molecule of squalene (Scheme 1.2). SS first catalyses the head to head condensation of two farnesyl pyrophosphate molecules to give the cyclopropyl carbinyl diphosphate, (PSPP) (17). Then, at a second site within the active centre of the enzyme, the PSPP undergoes ring expansion to the cyclobutyl carbocation (18) followed by reduction to give squalene (8).^{21, 22}

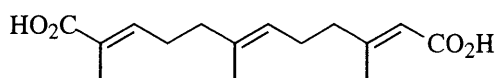
Scheme 1.2 *Formation of squalene catalysed by squalene synthase.*

A mechanism for the inactivation of SS by squalestatin S1 was proposed in the original publication by the Merck group.²³ They concluded that squalestatins are competitive inhibitors of SS, which work by mimicking the role of PSPP due to the similarity in structure (Figure 1.5). As indicated both contain a highly polar central core with attached lipophilic side chains.

Figure 1.5 *Structural analogy between squalestatin S1 and presqualene pyrophosphate.*

A recent study conducted by Harwood and Lindsey at Pfizer²⁴ suggested that the inactivation of the enzyme results from competitive inhibition followed by mechanism-based irreversible inactivation. In other words, S1 acts as a suicide substrate that binds covalently to the enzyme rendering it inactive. It was suggested that the α,β -unsaturated ester of the C-6 side chain was the likely site of the reaction, presumably by conjugate addition of a nucleophilic residue in the active site. This hypothesis was supported by detailed analysis of the kinetics of inhibition of SS by two synthetic analogues. It should be noted however, that zaragozic acid B and C and other squalostatins do not possess an α,β -unsaturated ester. This suggests the possibility that S1 is a mechanism based irreversible inhibitor of SS, but that other members of the family are competitive inhibitors.

Despite their promising cholesterol lowering activity, the use of squalostatins as therapeutic agents has been limited by their toxicity. S1 was found to be toxic to both rats and dogs.^{21, 22} One possible consequence of blocking squalene synthase is that FPP accumulates since it cannot be processed through the cholesterol pathway. The excess levels of FPP appear to be rapidly catabolised in the liver to a range of farnesyl-derived dicarboxylic acids (FDDCAs) such as (19) which are excreted in the urine. It was suggested that the observed toxicity was a result of acidosis. In contrast, rhesus monkeys dosed orally with S1 showed 50-60% lower cholesterol levels and no increase in the FDDCAs. The reasons for this difference are not clear.



(19)

The production of analogues which would retain the inhibitory action of squalostatins but not their toxicity has been the focus of a great deal of research. Synthetic approaches to and biological testing of compounds containing modifications in the C-1 chain (20)²⁵, at the C-3 (23) and (24)²⁶ and the C-6,C-7 (25)^{27, 28} as well as preparation of monocyclic (21) and (22)²⁹ and acyclic analogues (26)³⁰ have been published. Novel squalostatins have been also obtained *via* biotransformation of the C6 side chain of S1.³¹ The structural analogues are shown in Figure 1.6 along with their inhibitory concentrations (IC₅₀) against rat squalene synthase compared with that of S1 (IC₅₀ 12 nM).

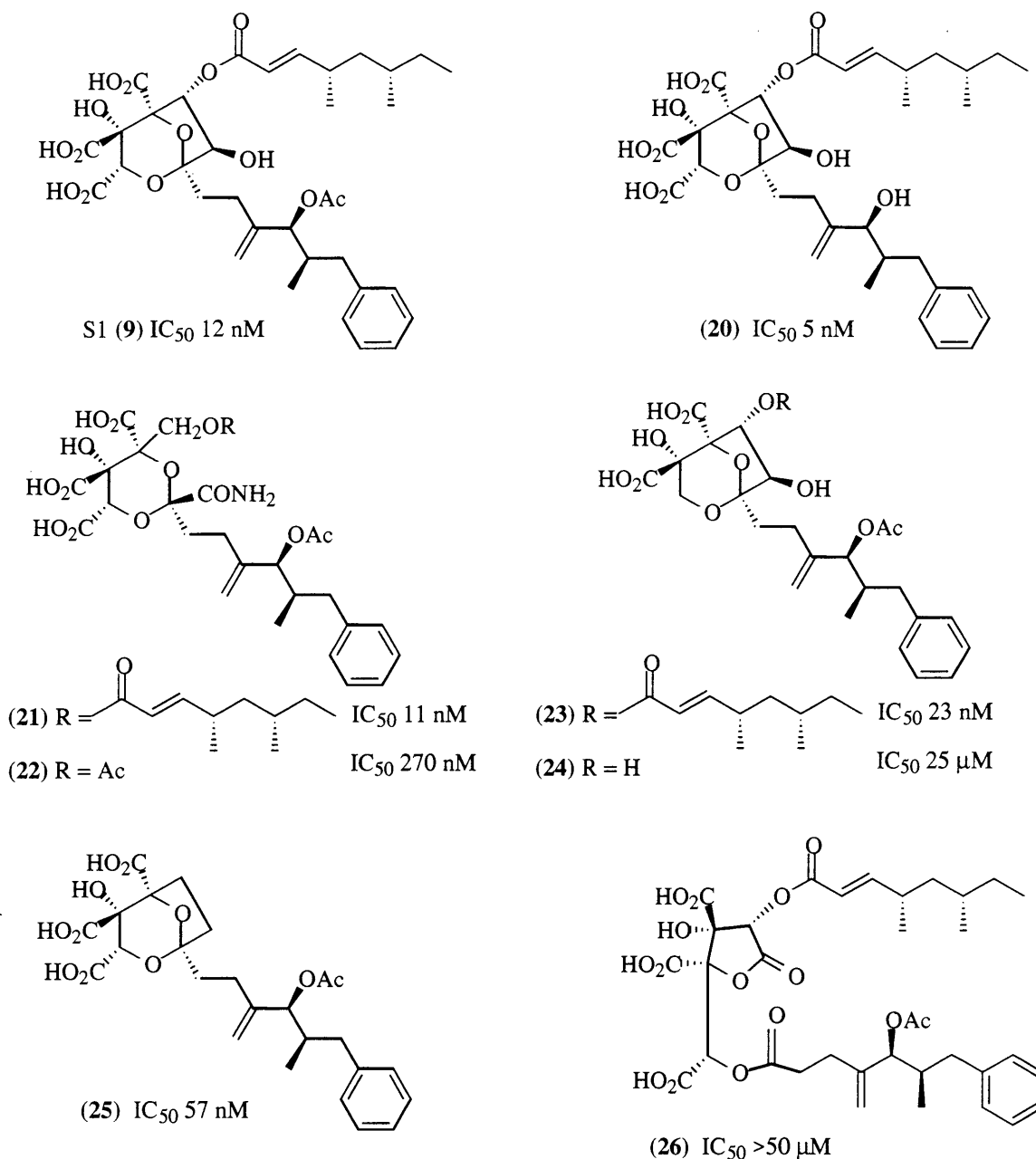


Figure 1.6 *Squalestatin S1 (9) and its structural analogues with their inhibitory concentrations (IC_{50}) against rat squalene synthase.*

The unique structure of the squalestatins has stimulated the interest of synthetic chemists, leading to studies on synthetic approaches toward these metabolites. The most notable of these are the total syntheses of S1 by Nicolaou *et al.*³² and of zaragozic acid C by Carreira and Dubois,^{15,16} and by Evans *et al.*¹⁷ The details of these syntheses will not be discussed here, as our main interest lay in the area of squalestatins biosynthesis (Section 1.5).

1.4 Structure Activity Relationship of the Squalostatins.

A large body of information on structure activity relationships has been collected by testing analogues obtained from the natural product, biotransformation and total synthesis. The details of these studies have been the subject of a thorough review by Procopiou and Watson. The main findings from assays with rat microsomal squalene synthase are outlined in Figure 1.7.¹

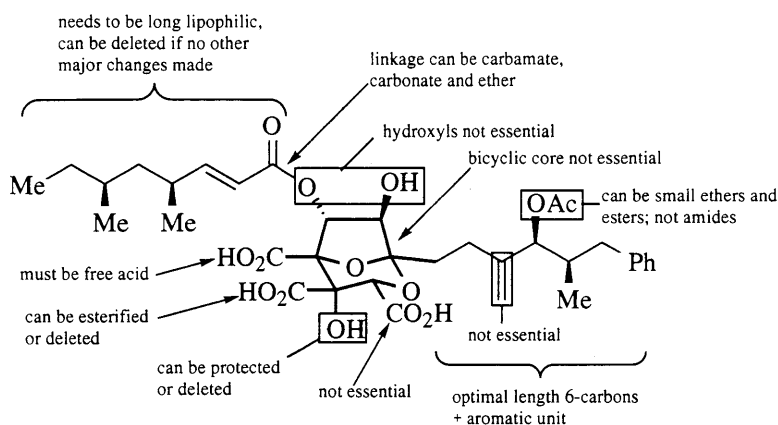
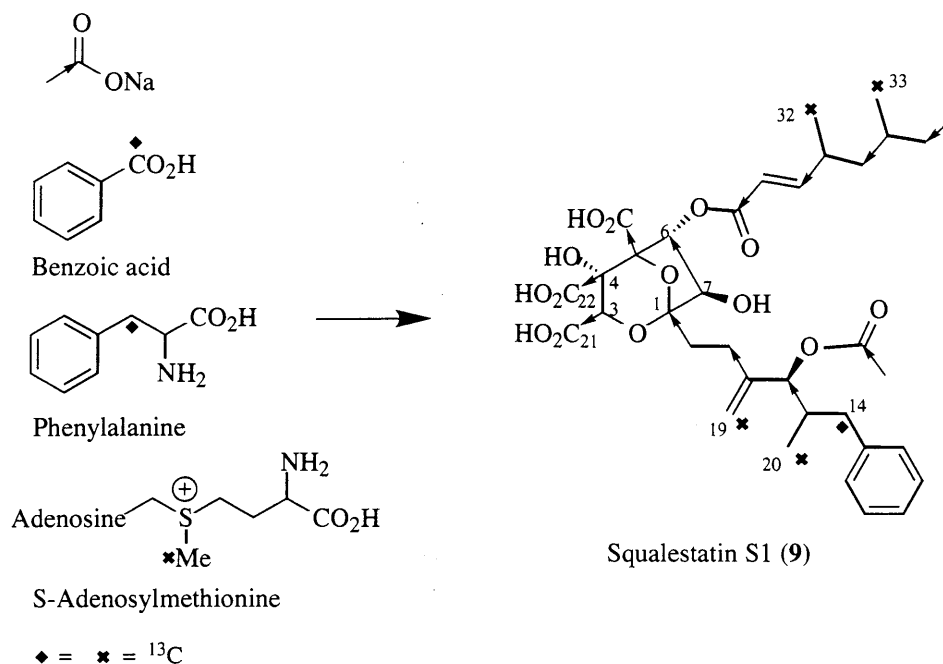


Figure 1.7 Important structure-activity relationships of squalostatins.

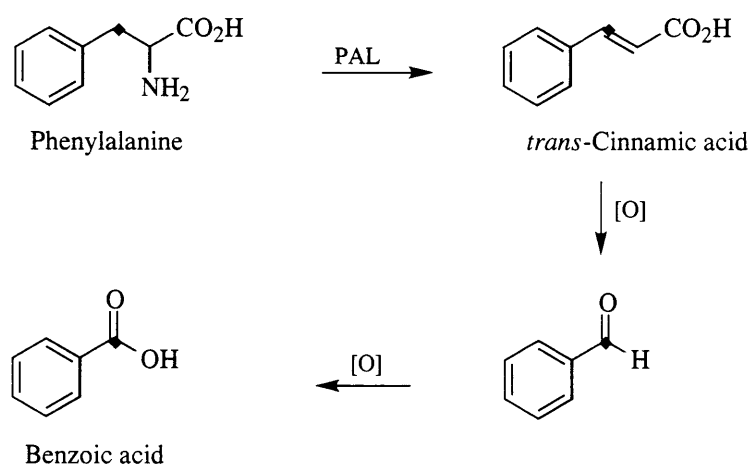
1.5 Biosynthesis of the Squalostatins.

The biosynthetic origin of S1/zaragozic acid A (**9**) was established independently by studies at both Merck and Glaxo.^{33, 34} In whole cell feeding studies, [1-¹³C] and [2-¹³C]acetate was incorporated into S1(2) at levels ranging from 1.8-7.0%. [1,2-¹³C₂]Acetate was incorporated at a level of ~0.5% into the positions indicated in Scheme 1.3. However with carbon pairs (3,21) and (4,22) the observed level of incorporation was lower, at ~0.2%. This indicated that the [1,2-¹³C₂]acetate had been metabolised *via* the Krebs cycle to a four carbon unit with consequent label dilution before incorporation at this site. This hypothesis was supported by the intact incorporation of [2,3-¹³C₂]succinate at carbons 3 and 4, at a level of 1.3%.



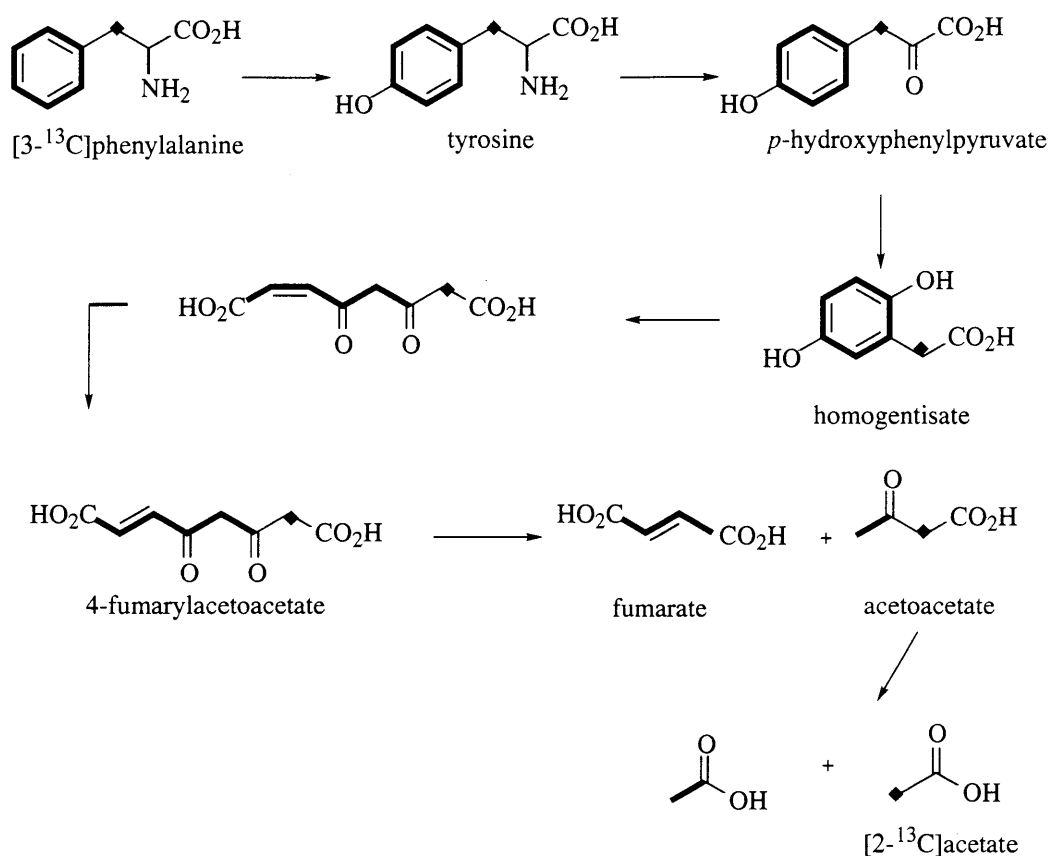
Scheme 1.3 Incorporation of ¹³C-labelled acetate, benzoic acid, phenylalanine, and S-adenosyl methionine into squalestatin S1.

The methyl groups (carbon 20, 32, 33) and the methylene group (carbon 19) were labelled by L-[methyl-¹³C]methionine which implicates S-adenosyl methionine as the origin of these atoms. The isotopic labels from [carboxy-¹³C]benzoic acid and D,L-[3-¹³C]phenylalanine were incorporated at C-14 at levels of 62% and 47% respectively. This result suggested that, prior to incorporation, phenylalanine is converted into benzoic acid as shown in Scheme 1.4.



Scheme 1.4 The formation of benzoic acid from phenylalanine.

The pattern of enrichment observed previously with $[2-^{13}\text{C}]$ acetate was also observed in the phenylalanine feed. $[3-^{13}\text{C}]$ Phenylalanine could form $[2-^{13}\text{C}]$ acetate via the catabolic pathway shown in Scheme 1.5.

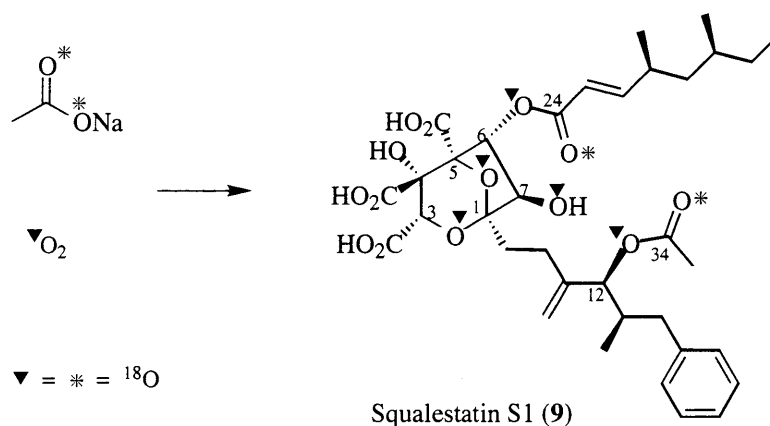


Scheme 1.5 Catabolism of $[3-^{13}\text{C}]$ phenylalanine to $[2-^{13}\text{C}]$ acetate.

Feeding of $[aromatic-^{13}\text{C}_6]$ benzoic acid resulted in strong enhancement (30% incorporation) of the aromatic ring signals. In addition, there were lower levels of incorporation at other sites of the metabolite. The signals due to carbons 3, 4, 21 and 22 were complex multiplets implying incorporation of a $^{13}\text{C}_4$ unit. This could have occurred via degradation of $[aromatic-^{13}\text{C}_6]$ benzoate to uniformly labelled $[^{13}\text{C}_4]$ succinate and $[1,2-^{13}\text{C}_2]$ acetate. This was supported by the fact that the enhanced signals and carbon-carbon coupling corresponding to the incorporation of $[1,2-^{13}\text{C}_2]$ acetate were also observed in the ^{13}C NMR spectrum of S1 from this feed.

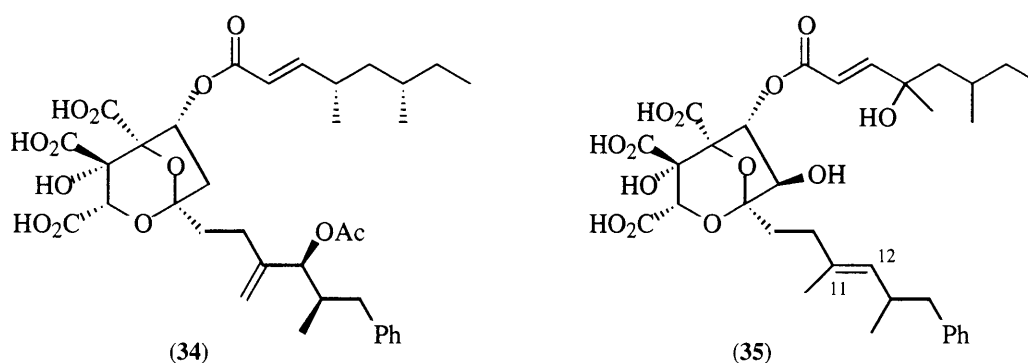
On feeding of $[1-^{13}\text{C}, ^{18}\text{O}_2]$ acetate to *Phoma* cultures, the S1 produced contained labelled carbon-oxygen bonds at positions 24 and 34 indicating that these oxygen atoms are derived from acetate. Growth of the organism under an $^{18}\text{O}_2$ atmosphere resulted in incorporation of ^{18}O into the molecule at carbons 6, 24, 5, 1, 3, 7, 12 and 34 (Scheme 1.6). In both of these experiments the presence of ^{18}O in the metabolite was indicated by upfield α -isotope shift in the ^{13}C NMR spectra. The results of these incorporation

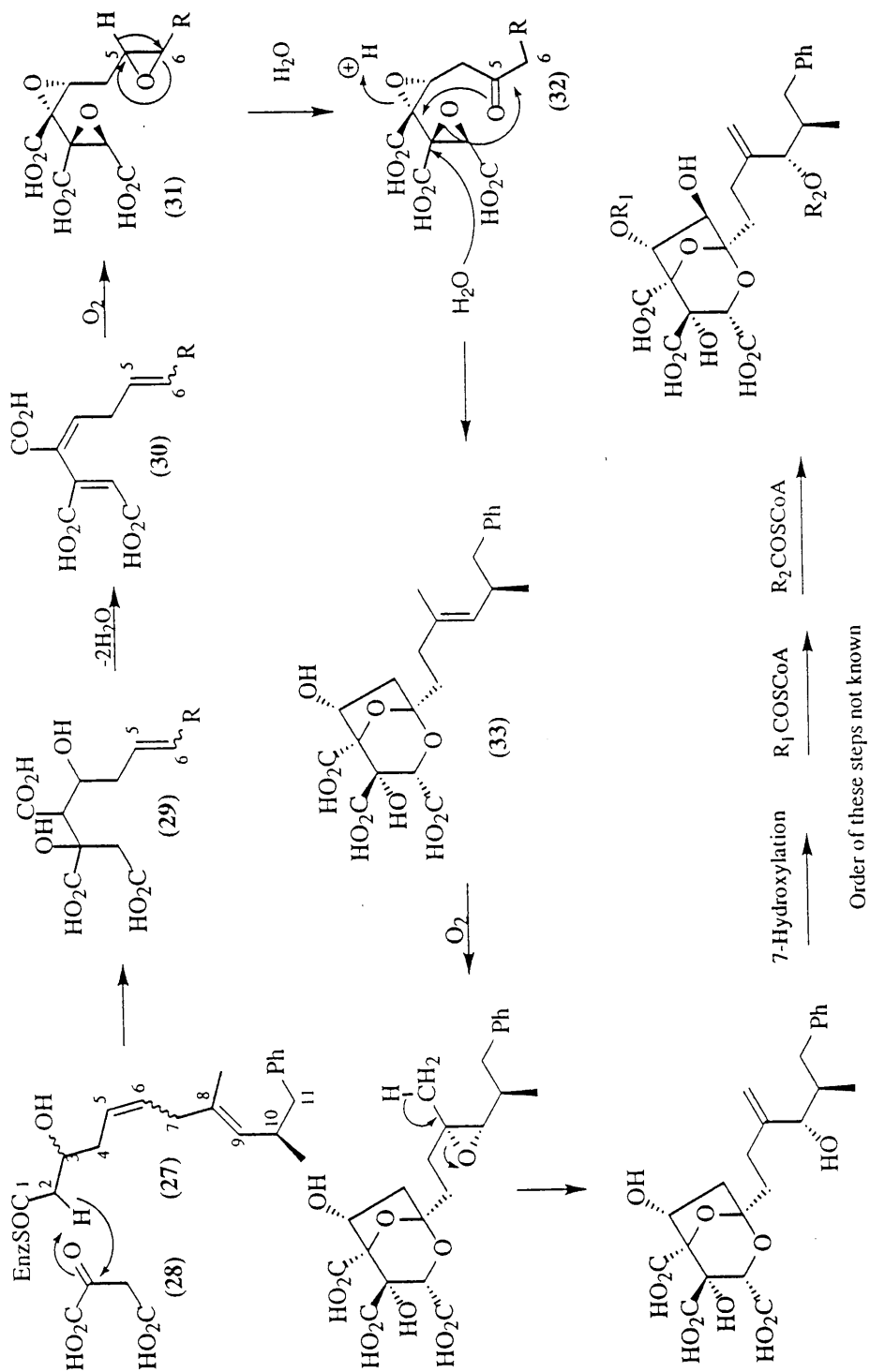
studies suggested that S1 is derived from a mixed pathway involving a polyketide derived intermediate and a Krebs cycle intermediate (Scheme 1.7).



Scheme 1.6 The incorporation of ^{18}O into the squalestatin.

A key step in the pathway is the condensation of the hexaketide intermediate (27) with oxaloacetate (28) to give the tricarboxylic acid (29) (Scheme 1.7). Formation of the bicyclic system with the observed origin of oxygen atoms led to the proposal of a *tris*-epoxide intermediate (31). Dehydration of (29) to give the triene (30), followed by epoxidation with molecular oxygen would give (31). Rearrangement of the 5,6-epoxide to the ketone (32) precedes an attack by water to give the 2,8-dioxabicyclo[3.2.1]octane core lacking oxygen at C-7 (33). Subsequent modification of the C-1 side chain involves epoxidation of the double bond by molecular oxygen; oxidation at C-7, and esterification at C-6 and C-12, in unknown order, would then give the squalestatin structure. Support for these latter stages of the squalestatin biosynthesis has been obtained in the form of minor metabolites isolated from *Phoma* cultures, e.g 7-deoxy squalestatin S1 (34) and a metabolite known as squalestatin V5 (35) which contains the (*E*)-11,12 olefin.

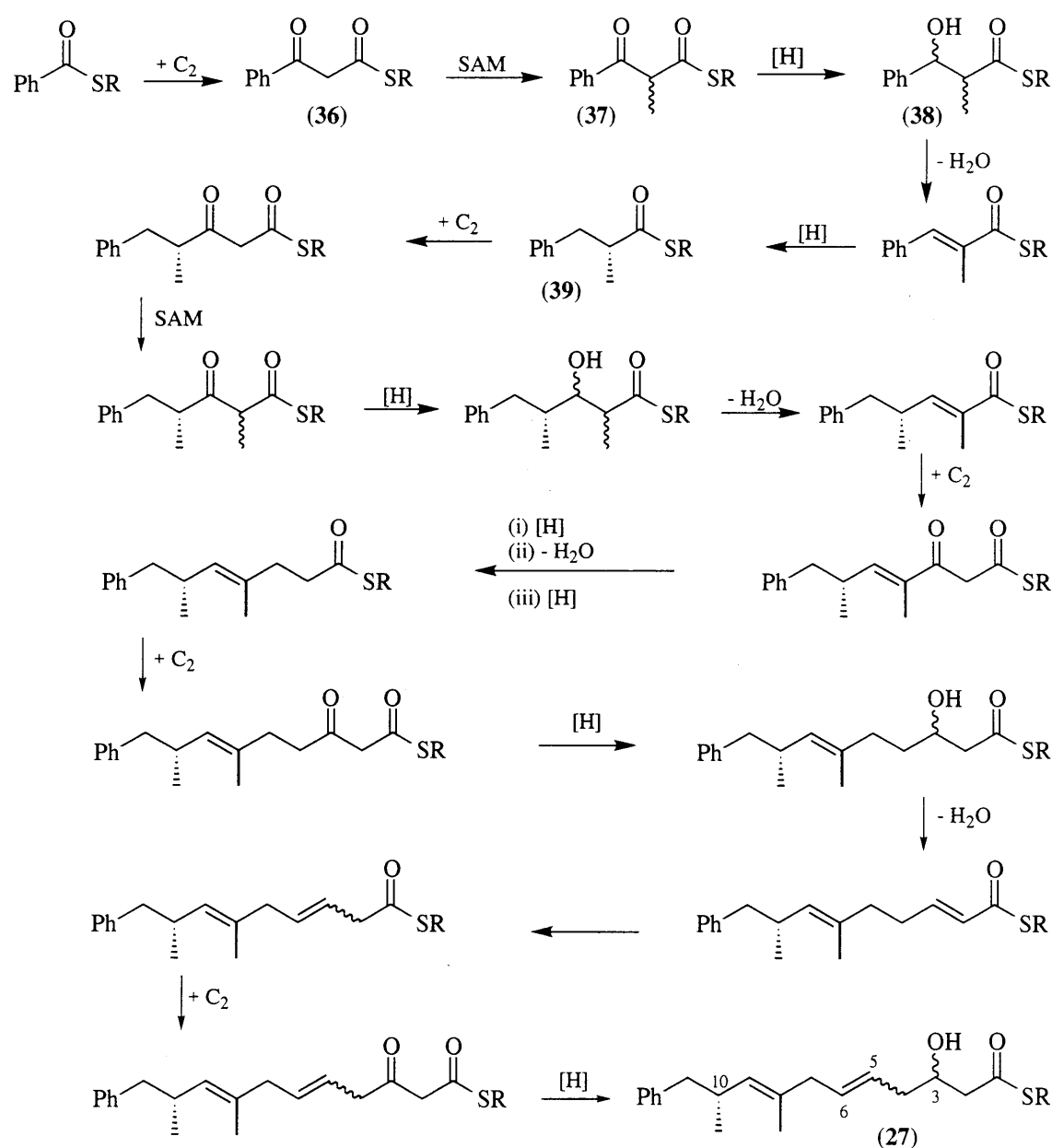




Scheme 1.7 Proposed biosynthetic pathway to the squalenestains.

1.6 Biosynthesis of the hexaketide.

A biosynthetic pathway to the putative hexaketide intermediate (**27**), based on the results of feeding labelled precursors to the squalstatin producer, *Phoma* sp C2932, was proposed (Scheme 1.8). This pathway involves the condensation of an enzyme bound benzoate starter unit with malonyl CoA, followed by methylation of the resulting β -ketothioester (**36**) by *S*-adenosyl methionine (SAM) to give (**37**). Reduction by NADPH then gives the 3-hydroxyl-2-methyl thioester intermediate (**38**). Dehydration and further reduction results in the formation of the saturated diketide (**39**). Further malonate condensations and reductive modifications then lead to the hexaketide (**27**).



Scheme 1.8 The proposed biosynthesis of the hexaketide intermediate.

To investigate whether the proposed hexaketide (**27**) is produced *via* this processive pathway, it was necessary to prepare labelled putative intermediates to (**27**) in the form of *N*-acetylcysteamine (NAC) thioesters and to carry out feeding studies.

Feeding intermediates in the form of NAC thioesters has been employed in studies on the biosynthesis of several polyketide natural products, such as tylactone (**40**)³⁵, erythromycin³⁶, avermectin³⁷ and tetronasin^{38, 39}. It has been proposed that the *N*-acetylcysteamine unit (**41**) aids the transfer of the precursor onto the polyketide synthase (PKS) by mimicking the phosphopantetheine side chain of coenzyme A (CoA) and of the acyl carrier protein (ACP) component of the PKS which is responsible for this process *in vivo*. The structural similarity between NAC and the phosphopantetheine side chain in CoA and ACP is illustrated in Figure 1.8.

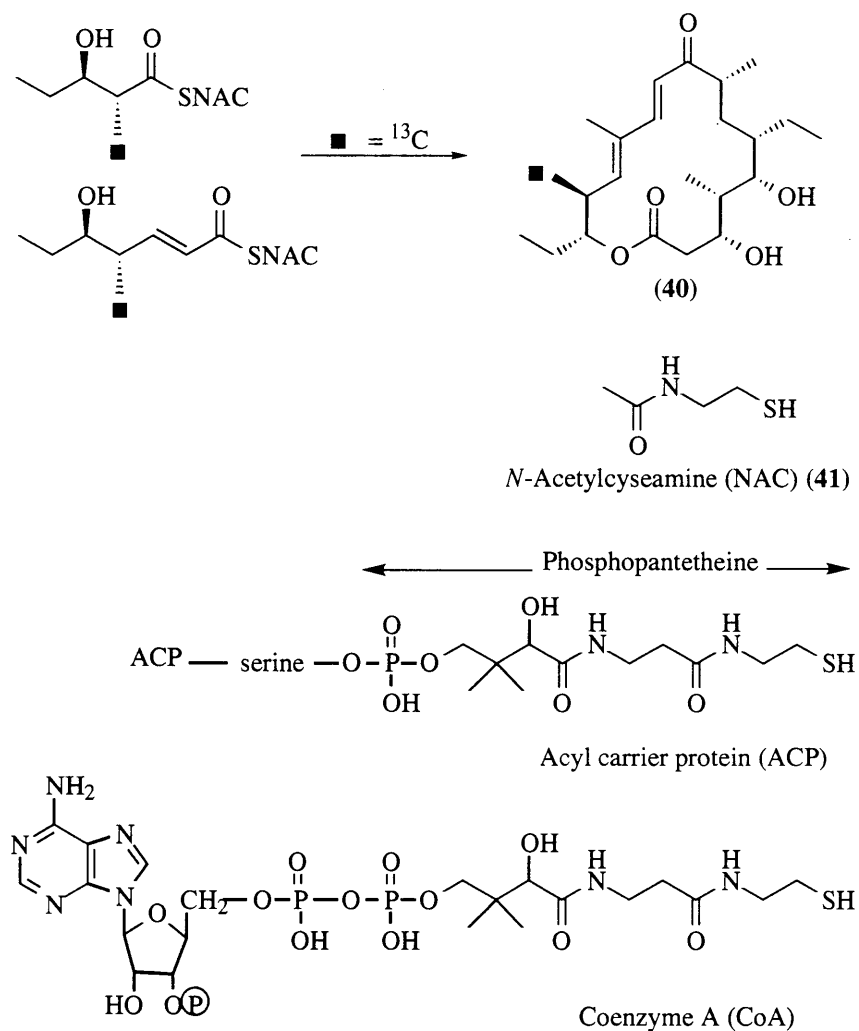
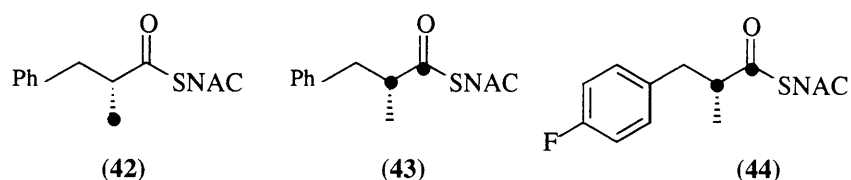


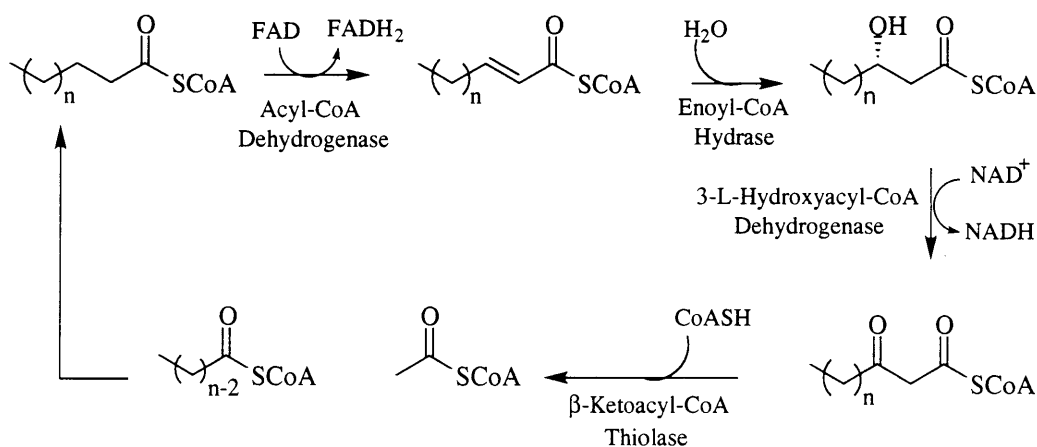
Figure 1.8 *Structural similarities between N-acetylcysteamine (NAC) and pantetheine chain in ACP and coenzyme A (CoA).*

Different isotopomeric analogues of the α -methyl diketide intermediate (**39**) were prepared as their NAC thioesters and were fed to *Phoma*.⁴⁰ Intact incorporation of (**42**) would result in enhancement of the C-20 signal in the ^{13}C -NMR spectrum of S1. However no significant increase in the intensity of this signal or any other signal was observed. The ^{13}C -NMR spectrum of the metabolite isolated from cultures supplemented with (**43**) contained no ^{13}C - ^{13}C coupled satellites in the C-12 and C-13 signals. These results indicated that no specific incorporation of (**42**) and (**43**) into S1 (**9**) had occurred. However, analysis by mass spectroscopy showed that ^{13}C label had been incorporated into the metabolite above natural abundance levels. This could occur through indirect incorporation *via* degradation of the precursor, which indicated that the metabolite had been transported in to the cell and metabolised although it was not specifically incorporated into S1.



Further evidence for the degradation of the diketide to benzoate and acetate came from feeding the fluorodiketide analogue (**44**). The ^{13}C -NMR analysis of the isolated metabolite showed no intact incorporation, while the ^1H -NMR spectrum revealed the incorporation of the fluorobenzoate part of the molecule at a level of 41%.

Long chain fatty acids are catabolised *in vivo* by a process known as β -oxidation (Scheme 1.9). This process might be responsible for the degradation of the diketide to give benzoate and propionate; propionate could then be further degraded to acetate and incorporated in S1. Methods for combating β -oxidation are described in the next section.



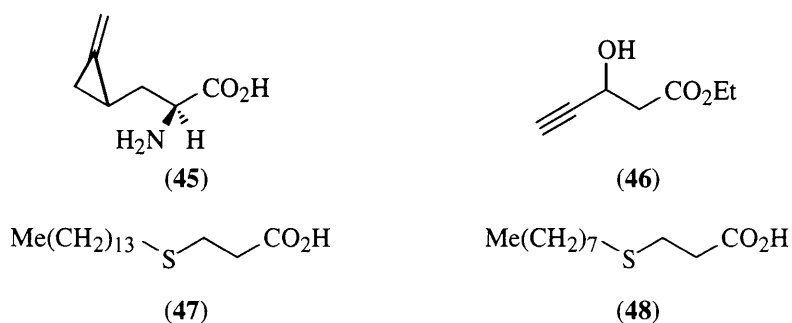
Scheme 1.9 β -Oxidation of fatty acids to acetate and propionate.

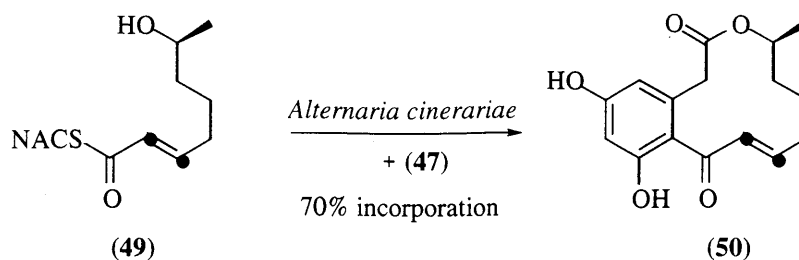
Our aim was to study the proposed biosynthetic pathway to (27). To do this it was necessary to develop means to avoid the enzymatic degradation of the diketide precursors. The preparation of α -fluorodiketide intermediates in the form of *N*-acetylcysteamine thioesters is discussed in chapter 2. The results of feeding studies with these compounds, the ^{13}C -labelled diketide (43) and labelled starter units to cultures of two S1 producing strains, *Phoma* and ATCC 20986, is then described in chapter 4.

As it is also evident from Scheme 1.8, the stereochemistry at C-10 of the hexaketide (27) is defined to be (*R*) because this stereogenic centre is present in the final structure of the metabolite. The 8,9-double bond is also known to have (*E*)-geometry as the minor metabolite (35) possesses this structural feature. The proposed path to the formation of the 2,8-dioxabicyclo[3.2.1]octane core *via* the *tris*-epoxidation of the triene (30) (Scheme 1.7) requires the C-3 hydroxyl and the C-5, C-6 olefin to be present, but the stereochemistry of these latter two groups is not known as they are lost in subsequent steps. Therefore, the preparation of all four possible isomers in labelled form is needed to study the biosynthetic route to the intermediate (27). The preparation of some of the putative hexaketide intermediate (27) *via* convergent approaches from two fragments will be discussed in chapter 3. Means of linking these two fragments required preparation of organometallic species and transition metal catalysis, this subject is described in Section 1.9.

1.7 Combating Enzymatic Degradation.

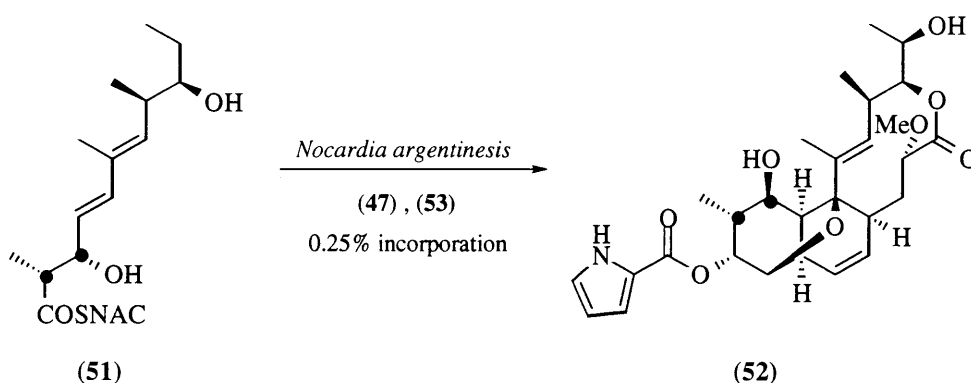
The degradation of administered advanced intermediates has been reported in a number of studies of polyketide biosynthetic pathways.⁴¹ The co-feeding of β -oxidation inhibitors was one approach used to tackle the problem. Vederas and co-workers⁴² have described the use of a number of β -oxidation inhibitors such as hypoglycin (45), ethyl-3-hydroxypentynoate (46), 3-(tetradecylthio)propanoic acid (47) and 3-(octylthio)propanoic acid (48). The most effective in his work on incorporating the labelled tetraketide (49) into dehydrocurvularin (50) was found to be (47) which suppressed precursor degradation and enhanced its incorporation ten fold (Scheme 1.10).





Scheme 1.10 Incorporation of tetraketide intermediate (49) into dehydrocurvularin (50).

Similarly, a combination of the β -oxidation inhibitors (47) and 4-pentynoic acid (53) has been used by Cane and Luo⁴³ in their work on incorporating pentaketide (51) into nargenicin (52) (Scheme 1.11).



Scheme 1.11 Incorporation of pentaketide intermediate (51) into nargenicin (52).

4-Pentynoic acid (53) is thought to act *via* propiolic acid (54), formed by fatty acid degradation of (53) (Figure 1.9). (54) is a potent inhibitor of fatty acid acyl CoA dehydrogenase, the first enzyme in the degradation pathway. Interestingly, the co-administration of 4-pentynoic acid to *Phoma* cultures during the investigation of diketide (43) incorporation resulted only in lowering the production level of S1 (9).⁴⁰

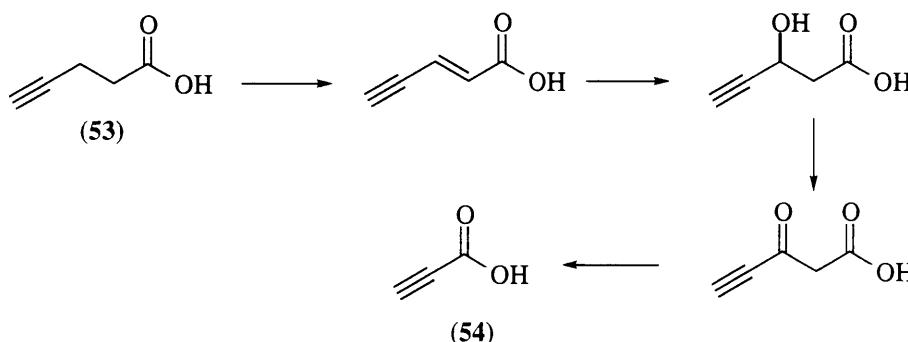
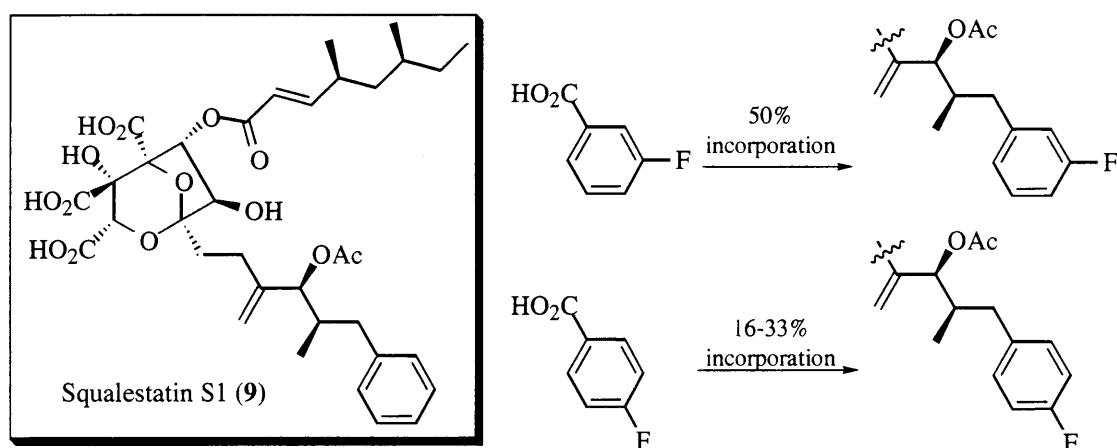


Figure 1.9 The degradation of 4-pentynoic acid (53) to propiolic acid.

Another strategy for inhibition of unwanted β -oxidation involves structural modification of the precursors. Since the first step in the fatty acid degradation process (Scheme 1.9) is thought to be enzymatic dehydrogenation of the substrate to give an α,β -unsaturated intermediate, it was proposed that if the α -hydrogen in the diketide precursor was replaced by an alternative atom then this first step of β -oxidation process would be blocked. Fluorine is an ideal substitute for hydrogen because the similar size of the atoms (Van der Waals radii of 1.35 Å and 1.2 Å respectively) results in only a minimal change in the steric requirements of the molecule.⁴⁴ Moreover the carbon-fluorine bond is at least 14 kcal/mol stronger than the carbon-hydrogen bond and as a consequence, the introduction of fluorine makes the substrate relatively resistant to metabolic transformations. Therefore, it was anticipated that replacement of the α -hydrogen atom on the diketide precursor by a fluorine atom would result in the substrate being resistant to β -oxidation.

Many fluorinated analogues have been shown to follow the metabolic pathways of the parent protio compounds. Examples of fluorinated precursor analogues undergoing bioconversion to fluorinated metabolites have been reported in work on both fatty acid^{45, 46} and polyketide biosynthesis.

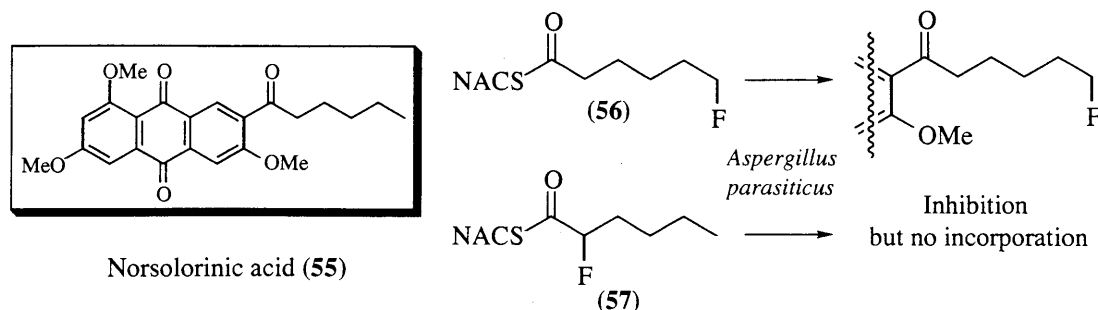
For example in an attempt to generate novel squalestatins, fluorinated analogues of the benzoate starter unit were fed to *Phoma* cultures.⁴⁷ Benzoic acid fluorinated at positions 3 and 4 gave the most efficient incorporation, at levels of 50% and 16-33% respectively (Scheme 1.12). The *N*-acetylcysteamine thioesters of 3- and 4-fluorobenzoic acid were incorporated into S1 at levels of approximately 30%.⁴⁰



Scheme 1.12 Incorporation of 3- and 4-fluorobenzoates into squalestatin S1 (9).

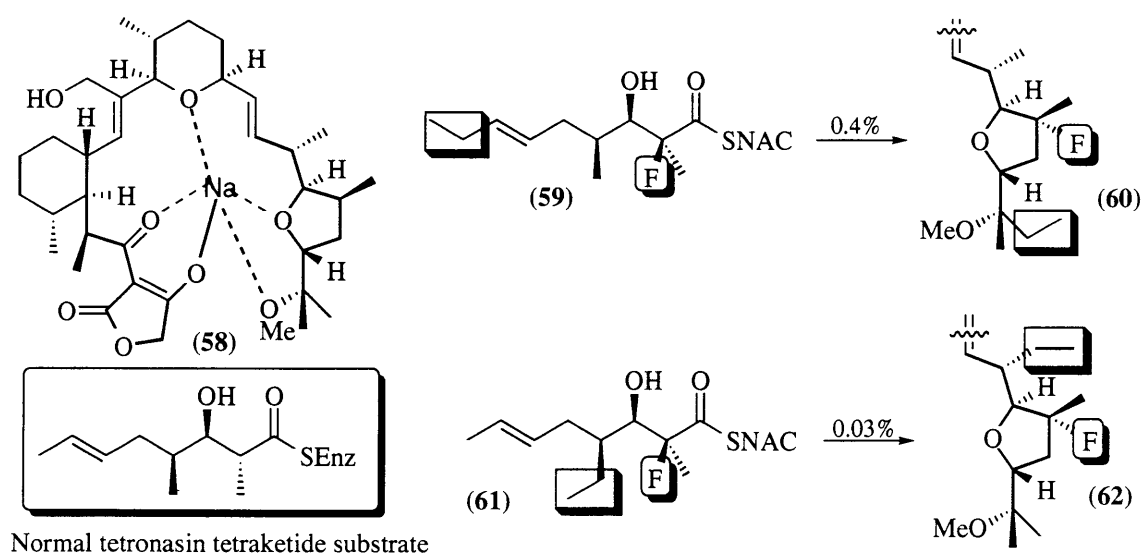
Norsolorinic acid (55) has been shown to incorporate [1-¹³C]hexanoic acid as a starter unit. To examine norsolorinic acid synthase substrate flexibility for the starter units a variety of deuterium labelled substrates including butyrate, pentanoate, heptanoate

and octanoate were fed to the norsolorinic acid producer, *Aspergillus parasiticus*. Only the pentanoate was incorporated. The NAC thioesters of 2-fluoro and 6-fluorohexanoate, (**57**) and (**56**) respectively, were also fed and only the latter was incorporated at a level of 36%⁴⁸ (Scheme 1.13).



Scheme 1.13 Incorporation of fluorinated precursors into norsolorinic acid.

In the study of the biosynthesis of tetronasin (**58**), various di-, tri- and tetraketide analogues were fed as their NAC thioesters to *Streptomyces longisporoflavus*.⁴⁹ The analogues were different in that one of the natural pendant or methyl terminal groups was replaced by an ethyl, *iso*-propyl or benzyl moiety. In addition to this, the α -hydrogen was replaced by fluorine in several of the substrates. The very low level of incorporation of some of these intermediates was detected by high resolution mass spectroscopy. Two of the modified tetraketide precursors (**59**) and (**61**) and their biotransformation into the novel tetronasins (**60**) and (**62**) are shown in Scheme 1.14.

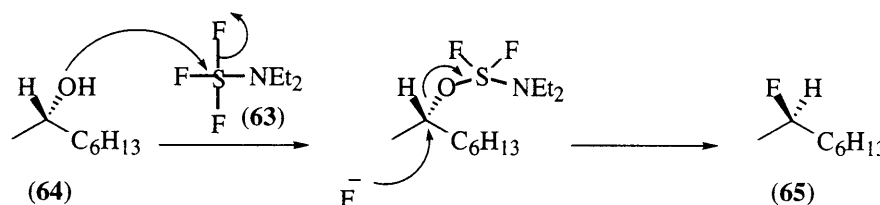


Scheme 1.14 Incorporation of fluorinated tetraketide analogues into tetronasin.

1.8 Methods for Introducing Fluorine Label.

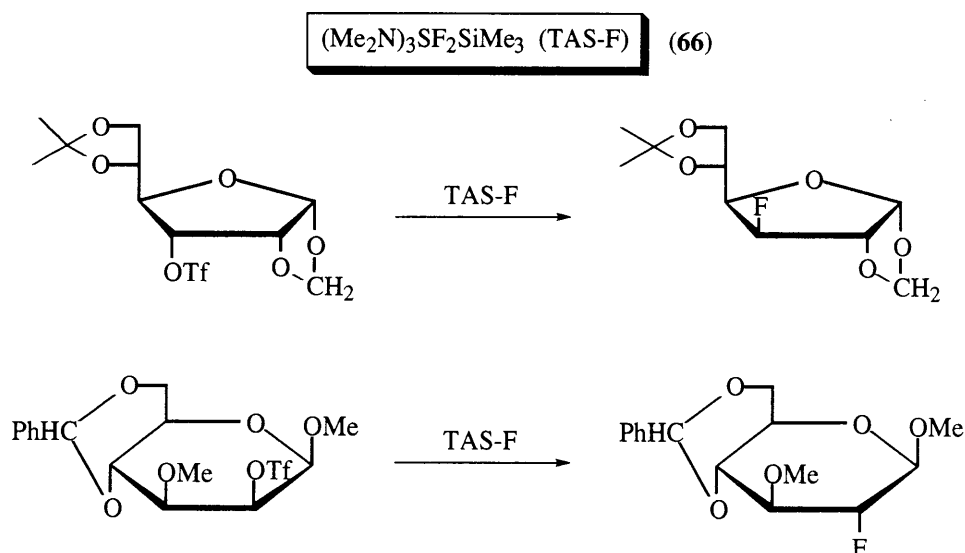
1.8.1 Nucleophilic Fluorination.

(i) Dialkylaminotrifluoro sulfuranes e.g. diethylaminosulfur trifluoride (DAST) (**63**) substitute a fluorine for a hydroxyl group. The reaction involves nucleophilic displacement of fluorine from sulfur by the oxygen of the hydroxyl group, to give hydrogen fluoride and an alkoxydiethylaminosulfur difluoride intermediate. A good leaving group is thus generated at the alcohol carbon and intramolecular attack of fluoride ion at this site leads to the desired alkyl fluoride. With most substrates an S_N2 process is preferred and hydroxyl replacement by fluorine is often claimed to occur with inversion of configuration. (*S*)-Octan-2-ol (**64**) gave (*R*)-2-fluorooctane (**65**) of 97.6% optical purity (Scheme 1.15).⁵⁰



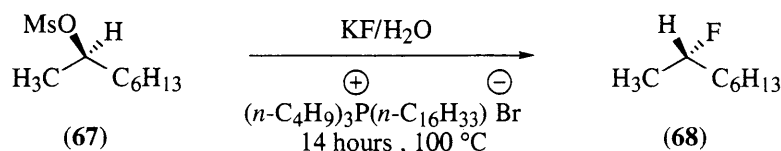
Scheme 1.15 Preparation of (*2R*)-fluorooctane using DAST.

(ii) Tris(dimethylamino)sulfur (trimethylsilyl) difluoride (TAS-F) (**66**) displaces triflates with inversion of configuration on furanose and pyranose derivatives (Scheme 1.16).⁵¹



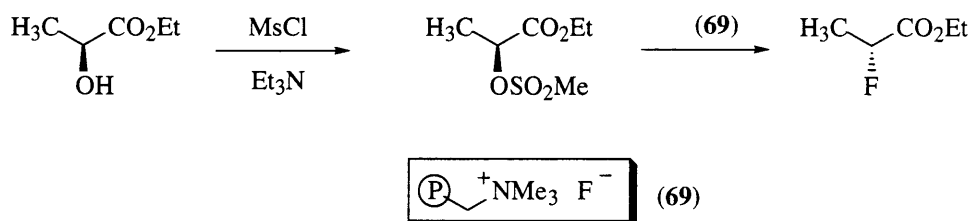
Scheme 1.16 The use of TAS-F (**66**) in preparing fluorodeoxy sugars.

(iii) Alkali metal fluorides such as cesium fluoride, sodium fluoride and most commonly potassium fluoride are classical sources of fluoride ions. Stereospecific fluorodehydroxylation was achieved by reacting (*R*)-2-octylmethylsulfonate (**67**) with an aqueous solution of potassium fluoride, in the presence of catalytic amounts of phase transfer agent in a heterogeneous aqueous-organic system, to give (*S*)-2-octylfluoride (**68**) (Scheme 1.17). The reaction occurs normally *via* an S_N2 mechanism with inversion of configuration.⁵²



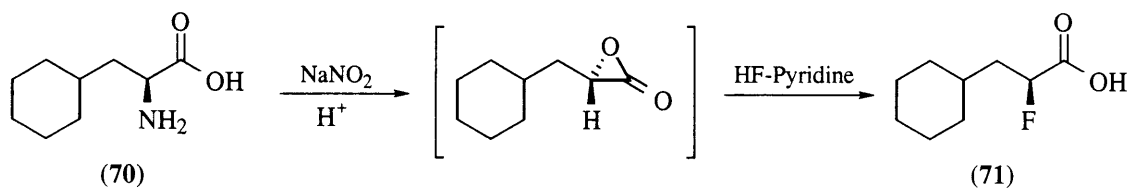
Scheme 1.17 Asymmetric fluorination using potassium fluoride.

(iv) Polymer supported fluoride salts (**69**) was used in the fluorodehydroxylation of simple chiral secondary alcohols. The reaction proceeded with high stereospecificity and inversion of configuration (S_N2 process) (Scheme 1.18).⁵³



Scheme 1.18 Polymer supported fluoride as fluorinating agent.

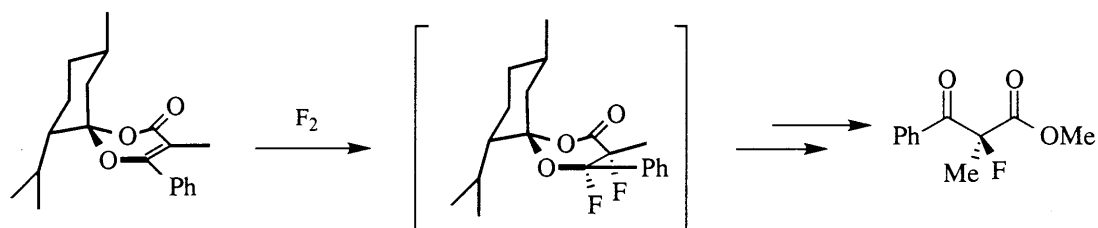
(v) Hydrogen fluoride is a cheap fluorinating agent but due to its corrosive properties and high toxicity is rarely used. The reaction of α -amino acid (**70**) with excess sodium nitrite in pyridinium polyhydrogen fluoride afforded α -fluorocarboxylic acid (**71**) with retention of configuration (Scheme 1.19).⁵⁴ The stereochemical course was ascribed to the formation of an α -lactone intermediate.⁵⁵



Scheme 1.19 Preparation of α -fluorocarboxylic acid (**71**) using HF-pyridine.

1.8.2 Electrophilic Fluorination.

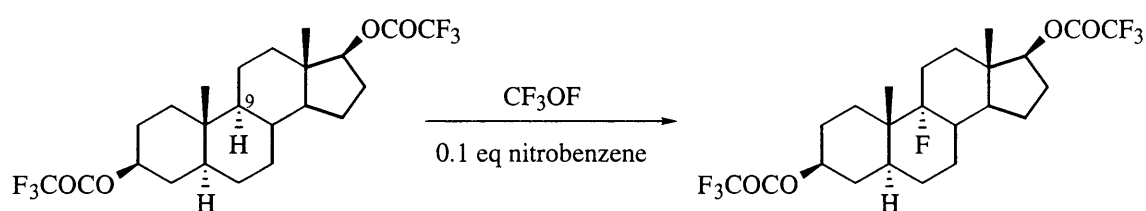
(i) Elemental fluorine, despite its high reactivity, has been employed effectively (diluted with nitrogen) for the fluorination of cyclic α -substituted β -dicarbonyls attached to a chiral auxiliary with high stereoselectivity. α -Fluorinated β -ketoacids were prepared enantioselectively *via* F_2 fluorination of 1,3-dioxin-4-ones having L-menthone as chiral auxiliary. An example is illustrated in Scheme 1.20.⁵⁶



Scheme 1.20 Stereoselective fluorination using F_2 .

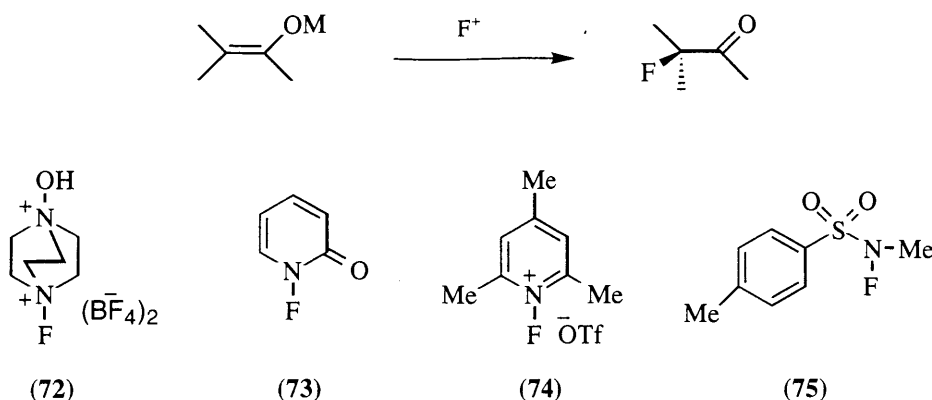
F_2 is also employed in the preparation of other electrophilic fluorinating agents in excellent yields as will be described later in Scheme 1.25.

(ii) Fluoroxy reagents are much more tractable and selective fluorinating agents than elemental fluorine. Fluoroxy-trifluoromethane (CF_3OF) effects the fluorination of tertiary saturated carbon. In the presence of a radical inhibitor and in $CFCl_3/CHCl_3$ solvent mixture it replaces the hydrogen at C-9 of some steroids with retention of configuration (Scheme 21).⁵⁷

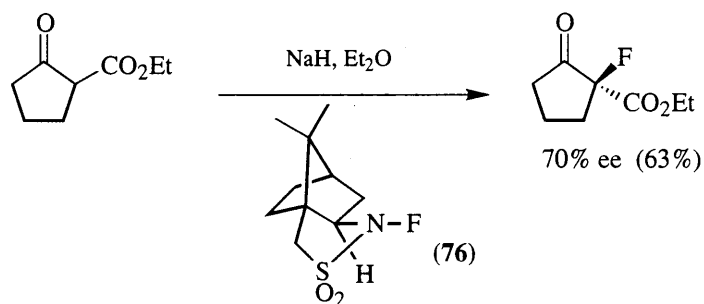


Scheme 1.21 The use of a fluoroxy reagent in preparation of fluoro steroids.

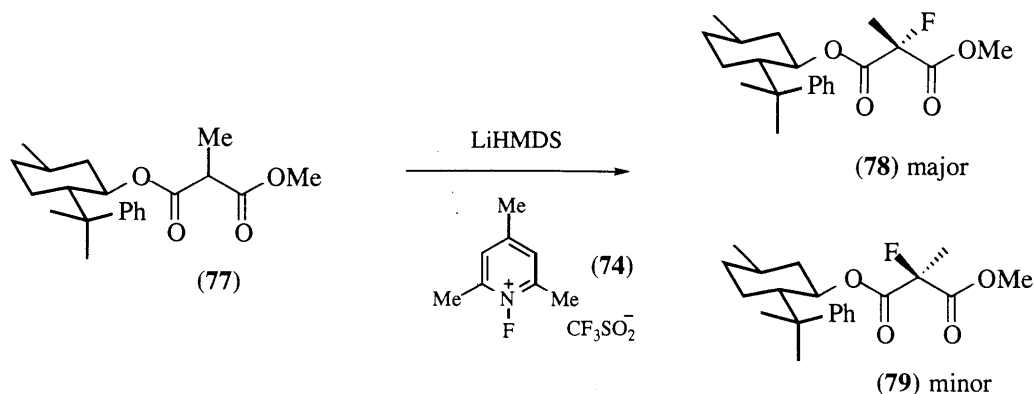
(iii) Efforts to find new, mild and selective fluorinating reagents led to the development of *N*-fluoro compounds as substitutes for the potentially hazardous fluoroxy compounds for the fluorination of carbanions. Thus 1-fluoro-4-hydroxy-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) or Accufluor (**72**),⁵⁸ *N*-fluoro-2-pyridone (**73**),⁵⁹ *N*-fluoropyridinium triflates (**74**)⁶⁰ as well as *N*-fluoro sulfonylimides (**75**),⁶¹ are all effective in fluorinating enolates to give α -fluorocarbonyl compounds (Scheme 1.22). They are all regioselective but none is stereoselective.

Scheme 1.22 *N*-Fluoro reagents effective in fluorinating enolates.

The first asymmetric electrophilic fluorinating reagent, *N*-fluorocamphor sultam (**76**), was prepared by Differding and Lang in 1988.⁶² *N*-Fluorocamphor sultams were used to synthesise α -fluorocarbonyl compounds from the corresponding metal enolate. In one example the e.e reached 70% (Scheme 1.23), but with other enolates the e.es and yields were much poorer.

Scheme 1.23 *N*-Fluorocamphor sultam in asymmetric fluorination.

Another method for the enantioselective preparation of α -fluoro quaternary centres, using *N*-fluoropyridinium triflate (**74**), utilised chiral auxiliaries to direct fluoro addition to stabilised (malonate or benzylic) α -alkylenolates. Ihara *et al.* reported that diastereoselective fluorination of 8-phenylmenthyl methylmalonate (**77**) gave (**78**) and (**79**) with 58% d.e. (Scheme 1.24).⁶³

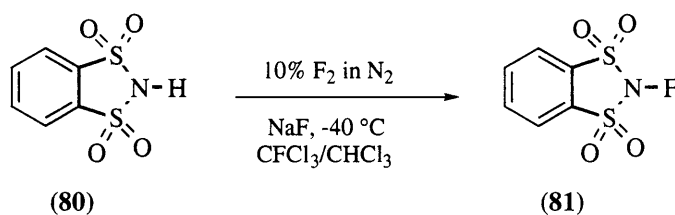


Scheme 1.24 Stereoselective fluorination using *N*-fluoropyridinium triflate (74).

It is important to notice that the alternative sequence, i.e. the methylation of 8-phenylmenthyl fluoromalonate, gave better diastereoselectivity.⁶⁴

There was still a need for the development of a general method (without the need for a β -carbonyl functionality) for the enantioselective preparation of α -fluoro quaternary centres. There is a considerable current interest in the development of efficient methodology for the asymmetric synthesis of organofluorine compounds, particularly of α -fluoro carbonyl compounds. These often have potent biological activities, and have found important applications in the study of enzyme mechanisms.⁴⁴ α -Fluorocarbonyl oxazolidinones have been synthesised *via* electrophilic fluorination of the corresponding acyl oxazolidinone enolates.

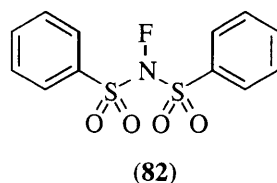
Davis and Han^{65,66} have described the preparation of *N*-fluoro-*O*-benzenedisulfonimide (81) in a greater than 90% yield by the treatment of *O*-benzenedisulfonimide (80) with 10% molecular fluorine in nitrogen gas (Scheme 1.25).



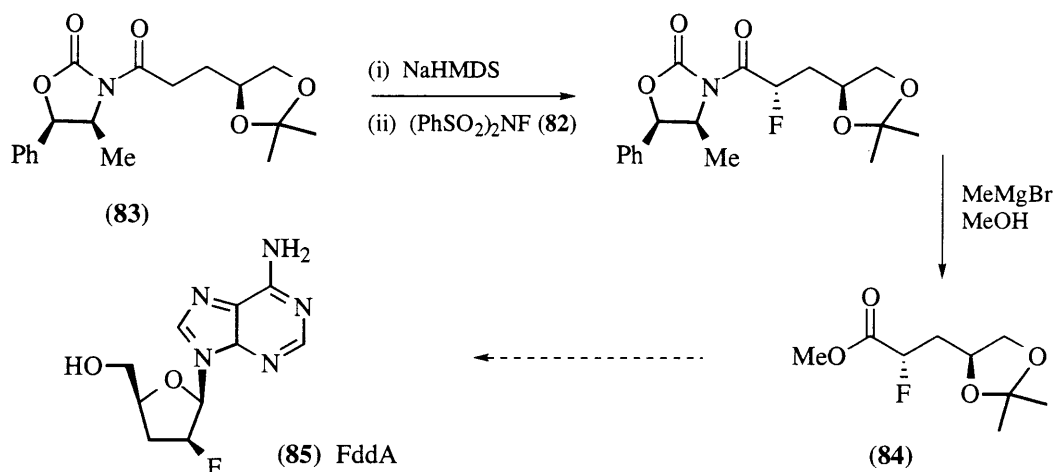
Scheme 1.25 Preparation of *N*-fluoro-*O*-benzenedisulfonimide (81).

This reagent was used in achiral α -fluorination of several ketones and esters in good to excellent yields. Davis⁶⁶ later reported the use of (81) in diastereoselective fluorination of a series of acyl oxazolidinone enolates in (80-88%) yields and around 95% d.e. Differding *et al.*⁶⁷ reported the preparation and use of a similar reagent, *N*-fluorodibenzenesulfonimide (82) in achiral α -fluorination of neutral and carbanionic

nucleophiles ranging from slightly activated aromatic compounds to highly reactive aryl- and vinyl lithium derivatives in good yield.

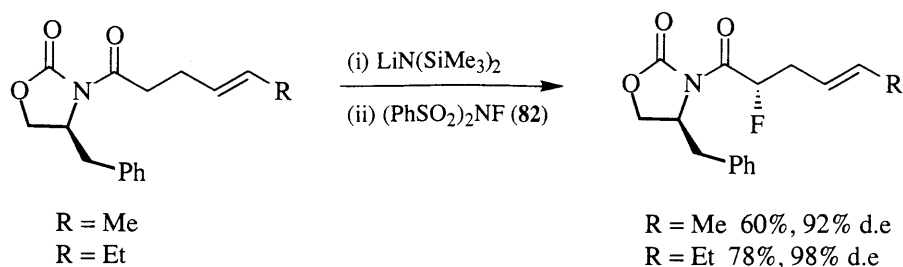


The latter reagent (**82**) was used in the fluorination of acyl oxazolidinone (**83**) enolate with complete diastereoselectivity (>99 d.e.). (*S,S*)- α -Fluoro-2,2-dimethyl-1,3-dioxolane-4-propionic acid methyl ester (**84**) is a key intermediate for the preparation of anti-HIV active compound FddA (**85**) (Scheme 1.26).⁶⁸



Scheme 1.26 The use of (**82**) in preparation of an intermediate in the synthesis of FddA (**85**).

N-Fluorodibenzenesulfonimide (**82**) was also used in fluorinating the acyl oxazolidinone of putative triketide biosynthetic precursors of tetronasin (**58**) (Scheme 1.27).^{69, 70} The reaction proceeded by treatment of acyloxazolidinones with lithium hexamethyldisilazide followed by 1.2 equivalents of (**82**) to give the α -fluorinated acyl oxazolidinones in good yield and excellent diastereoselectivity.



Scheme 1.27 Preparation of fluorotriketide analogues of tetronasin biosynthetic intermediates using *N*-fluorodibenzenesulfonimide (82).

In conclusion fluoroorganic bioactive compounds in enantiomerically pure form especially α -fluoro carbonyl compounds, have found important applications as enzyme inhibitors in studies of enzyme mechanisms. They are also employed in the synthesis and biosynthesis of bioactive natural product analogues. In chapter 2, the syntheses of some α -fluoro diketide analogues in the biosynthetic pathway of squalestatin S1 (**9**) are described.

1.9 Coupling Reactions of Organometallic Reagents with Organic Electrophiles.

Organometallic reagents have been used extensively in reactions that generate carbon-carbon bonds which is a critical operation in the synthesis of organic molecules. This section is not a thorough review of organometallic reagents and their use in organic synthesis, but aims to highlight the scopes and limitations of some of these reagents in the light of our aim in linking two fragments (an allylic electrophile and a vinylic nucleophile) in the synthesis of the putative hexaketide intermediate (**26**) (see chapter 3).

The polarisation of the carbon-metal bond allows the organometallic reagent, RM , to be regarded as equivalent to R^- with a metal cation M^+ and hence the carbanion R^- could react with various organic electrophiles to make new carbon-carbon bonds. A number of organometallic reagents have been utilised in coupling reactions with varying success.

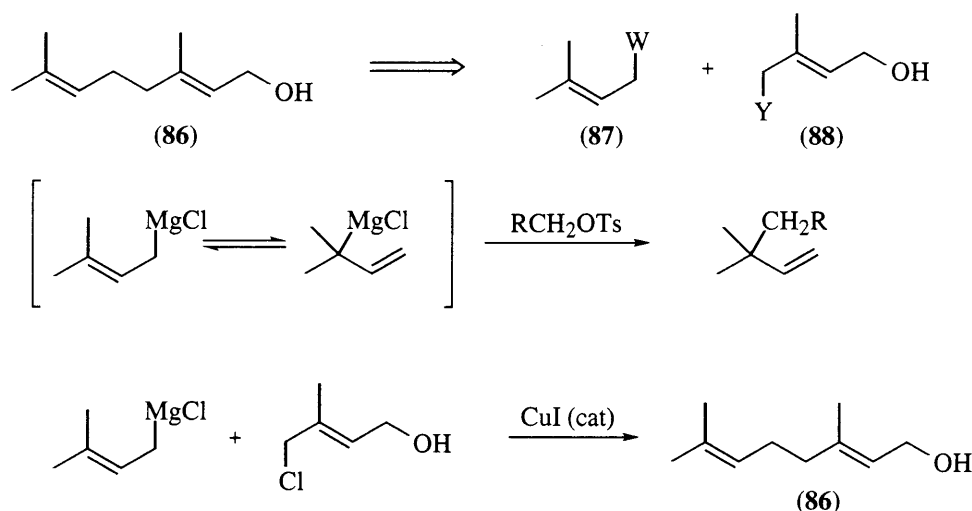


The organometallic compounds of group IA and IIA metals are strongly basic and nucleophilic, however their use in alkylation reactions is somewhat limited in scope. Alkyl lithium and magnesium reagents do not react cleanly with alkyl halides, as the required nucleophilic substitution reaction is accompanied by elimination, metal halogen exchange and often homocoupling of the organic halide.⁷¹ Tosylates and mesylates may be used instead of halides to avoid metal-halogen exchange, however the competing

elimination reaction can still be a problem. The reaction of alkyl lithium reagents with benzyl and allyl halides works well since β -elimination is impossible in these cases. Another limitation for using organolithium and magnesium in coupling reactions is that neither will tolerate a wide variety of functional groups on either coupling partner.⁷²

Organocopper promoted coupling reactions are more successful than their lithium or magnesium counterparts. Elimination is not a serious competing process and the reaction tolerates the presence of free hydroxyl groups which would be deprotonated by the more basic lithium and magnesium reagents.

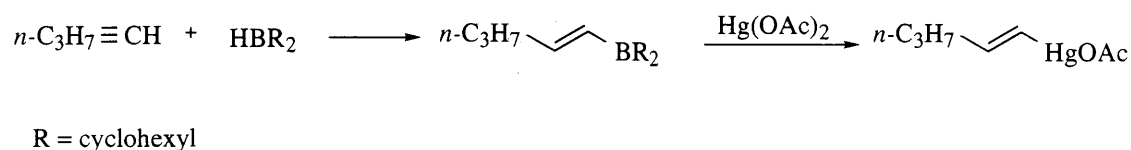
Nucleophilic substitution of a vinylic halide, which is not possible with organolithium and magnesium reagents, is readily achieved with cuprates. It occurs with complete retention of stereochemistry of the olefin.⁷³ Benzylic halides react in a similar manner.⁷⁴ Copper promoted coupling of an allylic electrophile with an allylic nucleophile was demonstrated in the synthesis of geraniol (**86**), a perfume constituent and a food flavour. Successful construction of geraniol (**86**) has been reported from nucleophilic (**87**) and electrophilic (**88**) (Scheme 1.28). When (**87**) is a Grignard reagent, an equilibrium is set up between two isomeric Grignard olefins. In contrast, when the Grignard reagent is treated with catalytic amount of cuprous iodide, coupling with the alkyl electrophile occur rapidly and exclusively at the primary carbon atom to generate geraniol (**86**).⁷⁵



Scheme 1.28 Synthesis of geraniol (**86**).

Cuprate couplings often however result in extensive homocoupling.⁷⁶ An additional problem is that the method of synthesis of the organocuprate, which involves organolithium, does not allow the presence of reactive functional groups on the organocopper partner.

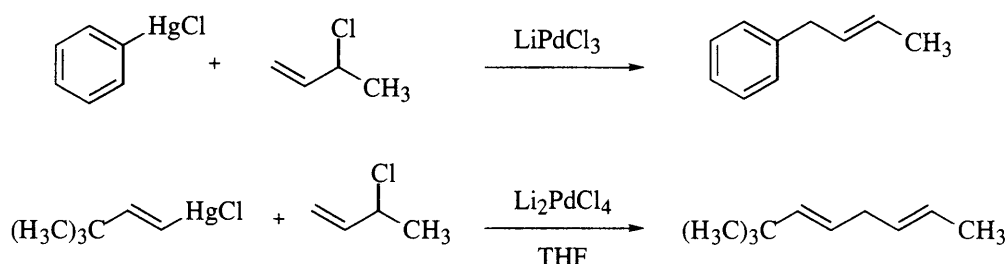
Organomercurials also tolerate functionality, but are somewhat limited by the methods of their synthesis. Direct synthesis of organomercurials from organic halides and metallic mercury or mercury amalgams is rather limited.⁷⁷ Organomercurials are most commonly prepared from the corresponding organolithium or magnesium compounds. However, the limitations on functionality and the already numerous synthetic applications of these reactive organometallics severely restricts the synthetic utility of the resulting organomercury compounds. The mercuration of organoboranes offers a valuable method for the synthesis of organomercurials containing a variety of functional groups.^{78, 79} Vinylmercury reagents can be obtained by hydroboration-mercuration of alkynes (Scheme 1.29).^{80, 81}



Scheme 1.29 Preparation of vinylmercury by hydroboration-mercuration of alkynes.

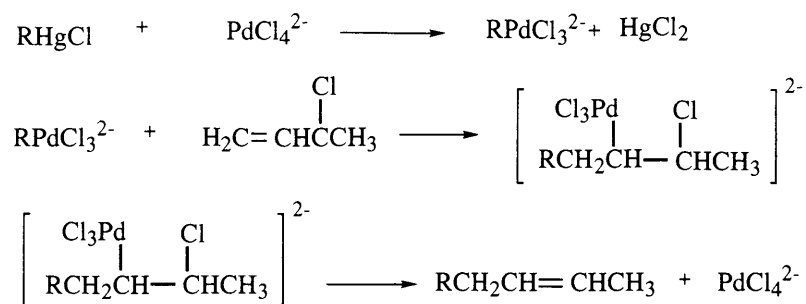
Organomercury compounds are quite inert toward alkyl halides in their reactions with alkylating reagents, however transition metal reagents can be used to promote alkylation. Both aryl- and vinylmercury reagents undergo a smooth coupling with allylic halides when palladium chloride plus lithium chloride is employed as a catalyst.⁷⁷

The palladium-catalysed coupling of arylmercuric halides with organic halides in good yields was reported by Heck in 1968⁸² (Scheme 1.30). A similar reaction involving vinylmercuric chlorides was developed by Larock (Scheme 1.30).⁷⁷



Scheme 1.30 The palladium-catalysed coupling of aryl- and vinylmercurials with organic halides.

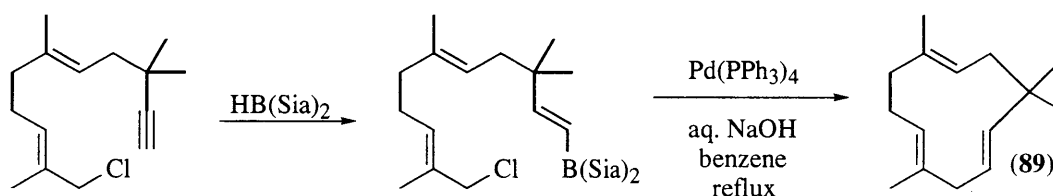
The above two reactions tolerate a number of functional groups and result in transposition of the double bond of the allylic halide. These reactions apparently proceeds as indicated below in Scheme 1.31.



Scheme 1.31 *The mechanism of palladium-catalysed coupling of organomercurials with allylic halides.*

The palladium-catalysed cross-coupling reaction of organometallic nucleophiles is one of the most versatile and efficient methods for the formation of carbon-carbon bonds.⁸³ In addition to the mediation of reactions involving organomercury compounds discussed earlier, a great variety of organic electrophiles have been shown to react with mild organometallic nucleophiles such as tetraorganostannanes⁸⁴ and organoboranes⁸⁵ in the presence of palladium.

Hydroboration of alkynes with disiamylborane followed by cross-coupling with allylic halides in the presence of palladium tetrakis(triphenylphosphine) and aqueous sodium hydroxide produces 1,4-dienes. The latter coupling was developed by Suzuki⁸⁵ and has been applied in a short step synthesis of humulene (**89**) (Scheme 1.32).⁸⁶

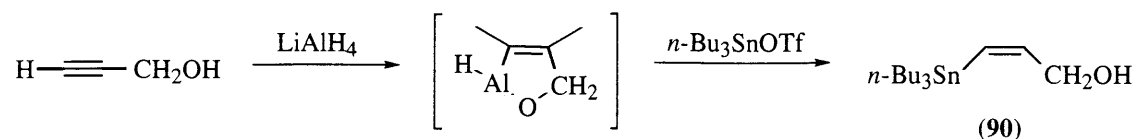


Scheme 1.32 *Preparation of humulene by Suzuki reaction.*

Organotin compounds containing a variety of reactive functional groups can be prepared by a number of routes.⁸⁷ Although the reaction of triorganotin halides with organometallic compounds is widely used its scope of application is limited since the organometallic compound (usually lithium or magnesium) does not allow a large variety of functional group to be introduced into the organotin product.

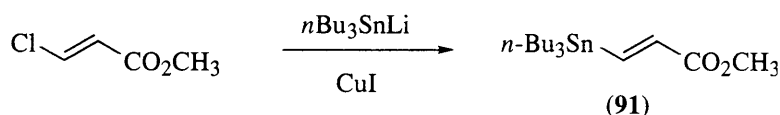
Propargyl alcohols react with lithium aluminium hydride followed by tributyltin triflates to give the (*Z*)-vinyltin reagent (**90**).⁸⁸ The basic lithium aluminium hydride first deprotonates the alcohol so that the aluminium becomes attached to the oxygen atom. Intramolecular delivery of the aluminium to the acetylene then leads to the cyclic product. The transmetallation step between the aluminium and tin reagent then proceeds with

retention of double bond geometry (Scheme 1.33). Cuprates also undergo *cis*-addition to acetylenes, generating vinylmetal compounds which react with tributyl triflate to yield (*Z*)-vinyltin compounds.⁸⁸



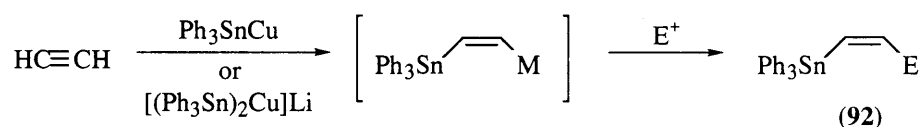
Scheme 1.33 Preparation of (*Z*)-vinylstannane.

The reaction of lithium tributylstannane with *Z*- β -chlorostyrene occurs with retention of geometry of the double bond. This reaction provides a method of synthesis of pure *E*- or *Z*-tributyltin acrylates (**91**) from the corresponding *E*- or *Z*- β -chloroacrylates (Scheme 1.34).⁸⁹



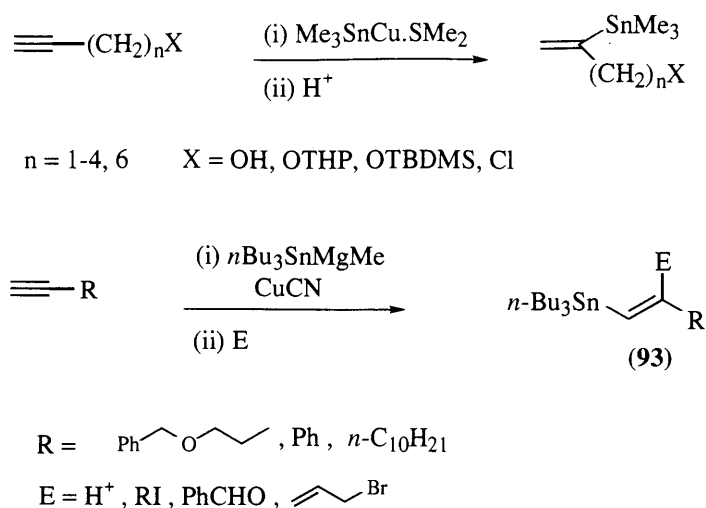
Scheme 1.34 Preparation of β -chloroacrylates via vinylstannane.

The stereospecific addition of a complex containing a copper-tin bond across a triple bond is very useful in generating *E*- and *Z*-vinyltin reagents. The stereochemistry of the addition depends on the reagent and the reaction conditions. Acetylenes undergo a *cis* addition with the copper(I) reagents, Ph_3SnCu and $[(\text{Ph}_3\text{Sn})_2\text{Cu}]\text{Li}$.⁹⁰ The initially formed anion can be trapped by different electrophiles to yield a *Z*-substituted vinyltin reagent (**92**) (Scheme 1.35).



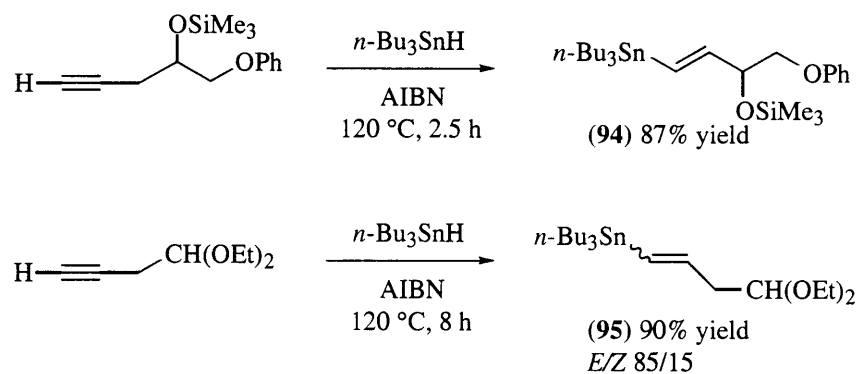
Scheme 1.35 Use of copper-tin complexes in cross-coupling reactions.

When $\text{Me}_3\text{SnCu} \cdot \text{SMe}_2$ is used the regiochemistry of addition to a monosubstituted alkyne is that expected for an ionic addition.⁹¹ Methylmagnesium tributylstannane in the presence of Cu(I) cyanide, however, yields product (**93**) of opposite regiochemistry (Scheme 1.36).⁹²



Scheme 1.36 Regioselectivity of the addition of copper-tin complexes to alkynes.

The hydrostannation of acetylenes is the simplest and most direct route to vinylstannanes. Radical addition of triorganotin hydride to monosubstituted alkynes is regioselective. The *E*-vinylstannane is favoured, although an *E/Z* mixture is obtained in some cases. Only *E*-vinylstannane (**94**) was reported by Tolstikov *et al.* from hydrostannation of the corresponding terminal alkyne in the presence of a radical initiator (AIBN) and high temperature.⁹³ Under the same conditions 85/15 mixture of *E/Z* vinyl stannane (**95**) was obtained (Scheme 1.37).⁹⁴



Scheme 1.37 Stereoselectivity of hydrostannation.

1.10 Palladium-Catalysed Coupling Reactions of Organotin Reagents with Organic Electrophiles.

The coupling reaction of organotin reagents with organic electrophiles catalysed by palladium takes place under mild conditions in high yields. A wide variety of organic electrophiles and organotin compounds can be coupled either directly or in the presence of

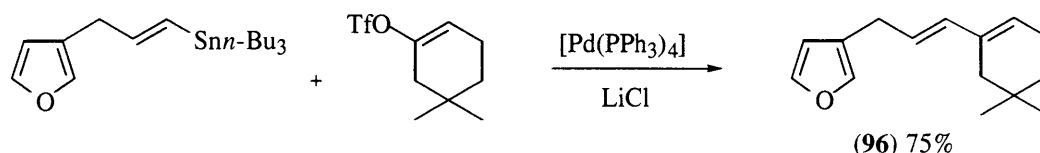
carbon monoxide (to yield ketones). A wide variety of functional groups are tolerated on either partner.

The palladium catalysed reaction of allyl chlorides and bromides with vinyl or aryl tin reagents gave cross coupling products in high yields (Table 1.1, entry 1).⁹⁵ The reaction proceeds with retention of double bond geometry in the vinyltin partner. In the allylic partner, coupling shows regioselectivity for the primary allylic carbon rather than secondary (entry 2). When the reaction is carried out under a moderate carbon monoxide pressure, a product resulting from the cross-coupling accompanied by carbon monoxide insertion is obtained (entry 3).

Table 1.1 *Palladium-catalysed coupling of allyl halides and organotin.*

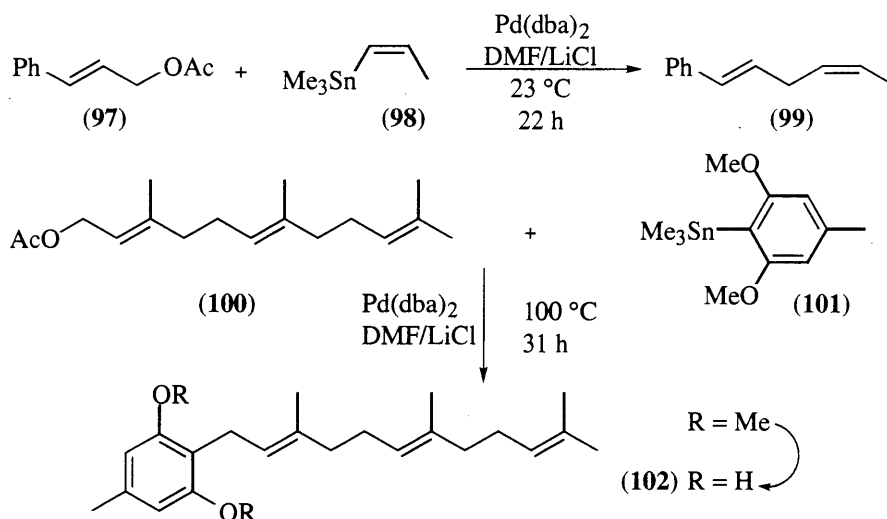
	Allyl halide	Organotin	Product	
1				86
2				87
3				75

Vinyl triflates undergo palladium-catalysed coupling with organostannanes provided lithium chloride is added to the reaction.⁹⁶ Apparently the vinyl palladium chloride complex is required for the transmetalation reaction, since the palladium triflate complex will not undergo transmetalation with vinyl tin compound. This reaction has been employed in a short synthesis of pleraplysillin (**96**) (Scheme 1.38).⁹⁶



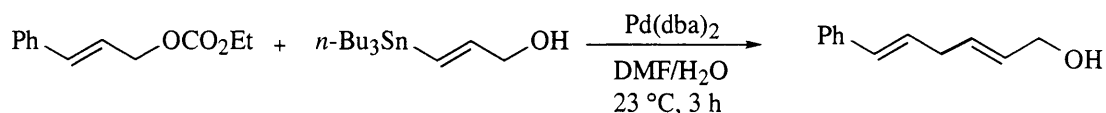
Scheme 1.38 *Palladium-catalysed coupling of vinyltriflates with organostannanes.*

Allylic acetates are generally unreactive under the standard conditions used for allylic halides, which involve heating equimolar amounts of halide and tin reagents in THF in the presence of a catalytic amount of Pd(0) and triphenylphosphine. However a study by Stille and co-workers revealed that simple allyl acetates were reactive toward aryl and vinyltin reagents, if DMF was used as solvent in the presence of lithium chloride to assist transmetallation and catalytic amount of Pd(0) in the absence of phosphine. In the example shown in Scheme 1.39, the geometry of the olefin in both the allylic substrate (**97**) and the vinyltin reagent (**98**) was retained in the coupling product (**99**). The potential for use of this coupling in organic synthesis was illustrated by the clean coupling of (*E,E*)-farnesyl acetate (**100**) to 3,5-dimethoxy-4-trimethylstannyl)toluene (**101**) to produce a precursor to the antibiotic grifolin (**102**)⁹⁷, in excellent yield and in a single step (Scheme 1.39).



Scheme 1.39 The palladium-catalysed coupling of allylic acetate with vinyl- and arylstannane.

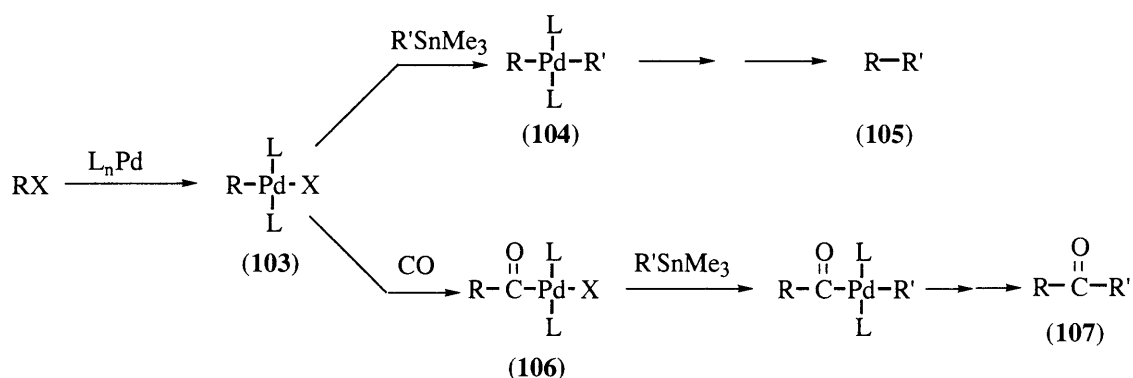
The use of allylic carbonates has been described recently.⁹⁸ In this case, the coupling reaction took place in shorter times and generally with higher yields than the corresponding acetates (Scheme 1.40). Importantly, this palladium-catalysed coupling reaction can also be carried out with water as the solvent at ambient temperature.



Scheme 1.40 The palladium-catalysed coupling of allylic carbonate with vinylstannane.

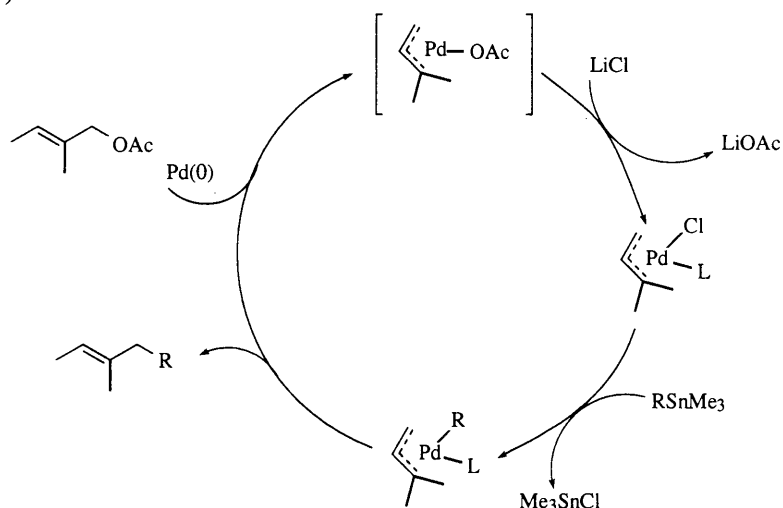
1.10.1 Mechanism of Palladium Catalysed Cross-Coupling.

The sequence of catalytic events includes oxidative addition of the allylic halide to the Pd(0) catalyst to give an allyl palladium (II) halide (**103**).⁹⁹ This undergoes transmetalation with the organotin reagent to yield the diorganopalladium complex (**104**) that undergoes reductive elimination,¹⁰⁰⁻¹⁰³ generating the coupled product (**105**). If carbon monoxide is present, CO insertion takes place with (**103**) to yield acyl palladium species (**106**) prior to transmetalation and reductive elimination, which will eventually lead to the ketone product (**107**) (Scheme 1.41).



Scheme 1.41 Mechanism of direct and carbonylative palladium-catalysed coupling.

In the particular palladium catalysed coupling of allylic acetate with organotin, the exchange of acetate for chloride facilitates the transmetalation from tin to palladium¹⁰⁴ (Scheme 1.42).



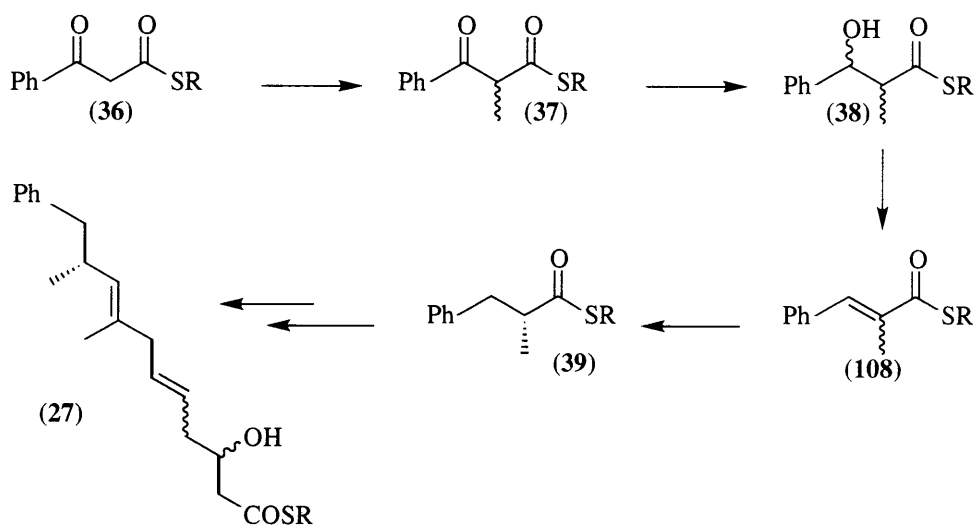
Scheme 1.42 The palladium catalytic cycle for the coupling of allylic acetate with organostannane.

Chapter 2

Synthesis of Diketide Precursors.

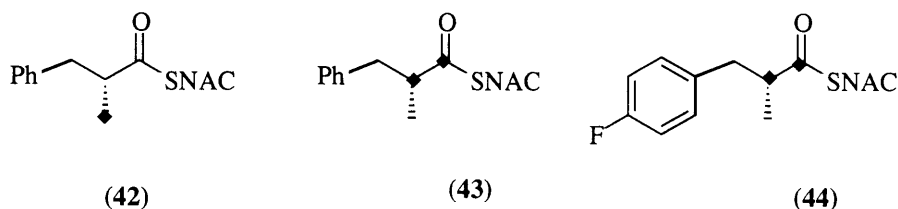
2.1 Introduction.

In the proposed biosynthetic pathway to the putative hexaketide intermediate (27) shown in Scheme 2.1, there are several possible diketides that could be involved in the assembly of the hexaketide (27). These are β -keto thioester (36), β -keto- α -methyl thioester (37), four possible diastereomers of β -hydroxy- α -methyl thioester (38), two geometric isomers of α -methyl- α,β -unsaturated thioester (108) and the α -methyl thioester (39).



Scheme 2.1 Putative intermediates in the biosynthetic route to hexaketide (27).

The α -methyl diketide (39) in the form of *N*-acetylcysteamine thioester was our first target compound as its carbon skeleton is present in the final structure of squalestatin S1 (9).



Different ^{13}C -labelled isotopomers of the *N*-acetylcysteamine thioester of (*R*)-2-benzylpropionic acid, (42) and (43), and the fluorinated analogue (44) have been prepared previously in our laboratories.⁴⁰ These were fed to S1 producer, *Phoma* sp C2932 and the incorporation of the carbon-13 label into the final structure of S1(9) was monitored by ^1H - and ^{13}C -NMR spectroscopy. However, they showed no intact incorporation. Mass spectrometric analysis of squalestatin S1 trimethyl ester revealed

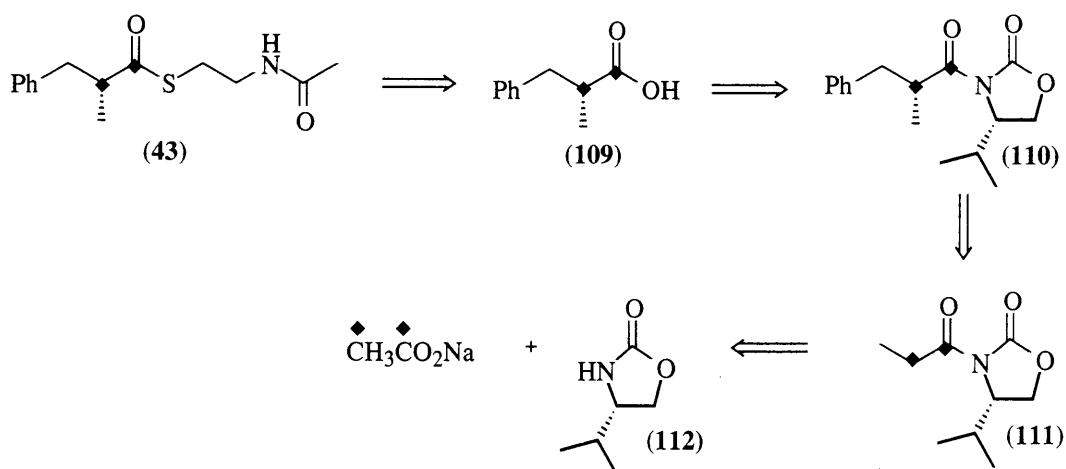
random incorporation of the ^{13}C label. The method used to calculate the random incorporation of the label allows for the natural abundance of ^{13}C , ^2H and oxygen isotopes. The random incorporation of the ^{13}C label was rationalised by catabolism of labelled diketide *via* β -oxidation, to benzoate and propionate which would be further degraded to acetate and incorporated into squalstatin S1 (**9**). Interestingly the use of the β -oxidase inhibitor, 4-pentynoic acid, to minimise diketide degradation resulted only in a decrease in S1 production.

Two other ways to overcome this problem remained to be investigated. One approach would involve feeding the labelled precursors to a different squalstatin producer. The other approach would be to carry out the synthesis and feeding studies of α -fluorinated diketide analogues which could not be degraded *via* β -oxidation. Hence it was necessary to repeat the synthesis of *N*-acetylcysteamine thioester of (*R*)-2-[1,2- $^{13}\text{C}_2$]-benzylpropionic acid (**43**) (section 2.2.1) in order to carry out feeding studies using the zaragozic acid producing strain MF5453 (ATCC74256) and to prepare α -fluorinated diketide analogues (Sections 2.2.2 and 2.2.3).

2.2 Results and Discussion.

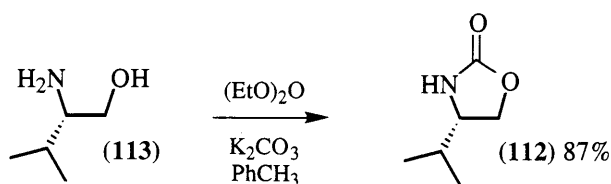
2.2.1 Preparation of (*R*)-2-[1,2- $^{13}\text{C}_2$]-Benzylpropionic Acid *N*-Acetylcysteamine Thioester (**43**).

A retrosynthetic analysis for the preparation of the labelled diketide precursor using Evans' chiral auxiliary to establish the (*R*)-stereochemistry at C-2 is shown in Scheme 2.2. The thioester (**43**) is obtained from coupling *N*-acetylcysteamine to acid (**109**) prepared using Evans' asymmetric alkylation methodology. The label is to be introduced *via* sodium [1,2- $^{13}\text{C}_2$]acetate.



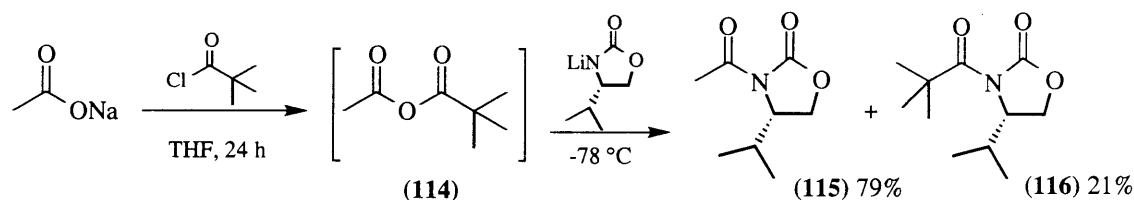
Scheme 2.2 Retrosynthetic analysis of α -methyl diketide thioester.

The chiral auxiliary, (*S*)-4-isopropyl-2-oxazolidinone (**112**), was prepared from (*S*)-valinol (**113**) (Scheme 2.3).¹⁰⁵



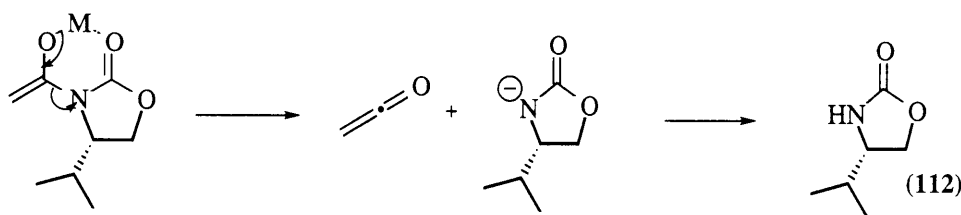
Scheme 2.3 Preparation of (*S*)-4-isopropyl-2-oxazolidinone (**112**).

Our ultimate goal was to introduce the ^{13}C label at the next stage of the synthesis using $[1,2-^{13}\text{C}_2]\text{acetate}$. Before proceeding with labelled material the synthesis of unlabelled thioester (**124**) was investigated. Sodium acetate was activated towards nucleophilic attack by formation of the mixed anhydride (**114**) prepared by reacting sodium acetate with pivaloyl chloride in THF at room temperature for 24 hours (Scheme 2.4). The oxazolidinone anion was prepared by treating oxazolidinone (**112**) with *n*-butyl lithium at $-78\text{ }^\circ\text{C}$ in THF. Reaction of oxazolidinone anion with mixed anhydride (**114**) gave the required acetyl oxazolidinone (**115**) in 79% yield. Pivaloyl oxazolidinone (**116**) was produced in minor quantity (21%).



Scheme 2.4 Preparation of acetyl oxazolidinone (**115**) from sodium acetate via pivaloyl mixed anhydride.

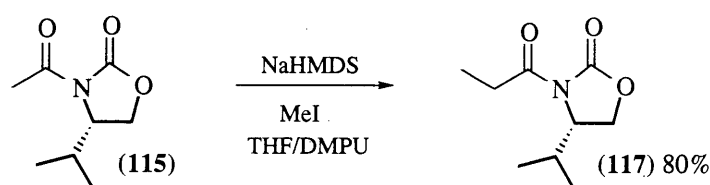
The next step was the methylation of acetyl oxazolidinone (**115**) with methyl iodide. Treatment of (**115**) with sodium bis(trimethylsilyl)amide gave the sodium enolate which was unstable even at $-78\text{ }^\circ\text{C}$, undergoing substantial decomposition possibly *via* the ketene pathway shown in Scheme 2.5.



Scheme 2.5 Decomposition of oxazolidinone enolate.

Optimisation of the yield to 85% was reported⁴⁰ using two equivalents of hexamethylphosphoramide (HMPA) as a cosolvent. In our hands the use of HMPA improve the yield only to 66%.

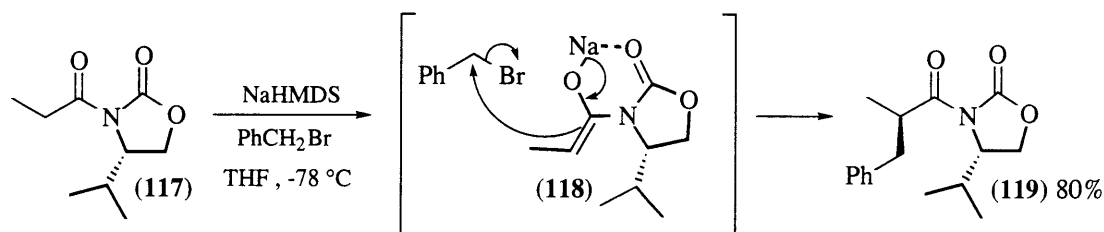
N,N'-Dimethyl-*N,N'*-propylene urea (DMPU) has been used successfully as a safe substitute for carcinogenic HMPA as a cosolvent within diverse types of reactions.¹⁰⁶ Moreover, unlike HMPA, DMPU is miscible with water at any ratio. It can therefore be removed from the reaction mixture by washing with water. Using two equivalents of DMPU as a cosolvent in the methylation of acetyl oxazolidinone (**115**) the product was obtained only in 25% yield. However, when used in 1:3 ratio with THF the yield was improved significantly to 80% (Scheme 2.6).



Scheme 2.6 Methylation of acetyl oxazolidinone (**115**).

The next step was the key asymmetric benzylation of propionyl oxazolidinone (**117**). Treatment of (**117**) with sodium bis(trimethylsilyl)amide at -78 °C followed by the addition of two equivalents of benzyl bromide yielded the desired benzylpropionyl oxazolidinone (**119**) in 80% yield after three hours.

The stereochemical-outcome of this step was controlled by the chiral auxiliary *via* the *Z*-enolate (**118**) which is formed in preference to the *E*-enolate in a ratio > 100:1.¹⁰⁷ The locked conformation of the enolate and the stereochemistry at C-4 of the oxazolidinone ring, direct the approach of the electrophile (Scheme 2.7).

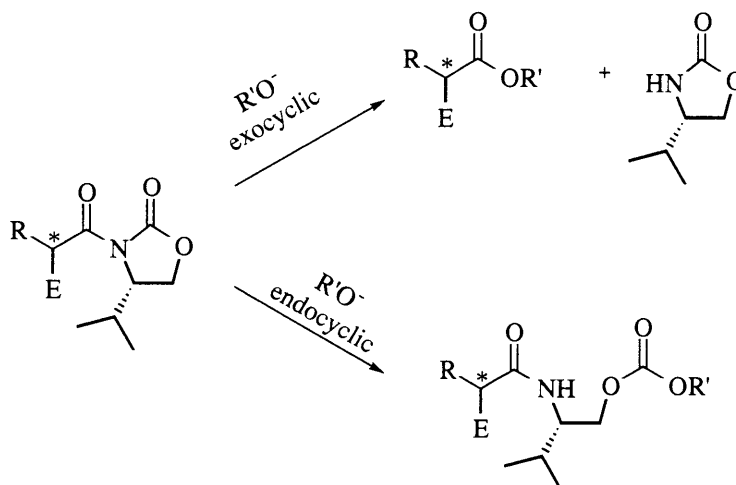


Scheme 2.7 Benzylation of propionyl oxazolidinone.

The use of the Evans' chiral auxiliaries is also advantageous due to their facile removal which is needed in the next step of the synthesis. Base hydrolysis with methanolic potassium hydroxide¹⁰⁸ or lithium hydroperoxide,¹⁰⁹ reduction with lithium

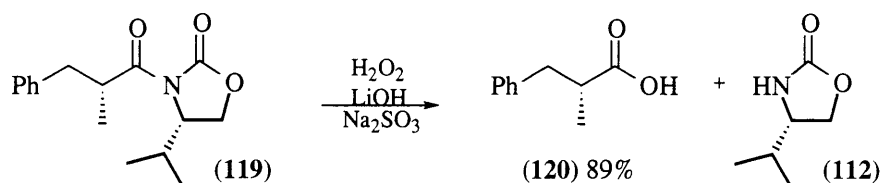
aluminium hydride¹⁰⁷ or replacement with lithium benzyl oxide¹⁰⁷ are some of the methods available for the cleavage of the oxazolidinone in intact form.

The site of nucleophilic cleavage of *N*-acyloxazolidinone is subject to both steric and electronic factors. As illustrated in Scheme 2.8, in the absence of significant steric crowding in the vicinity of the exocyclic carbonyl function, electronic factors direct hydrolysis or esterification in the desired fashion affording a carboxylic acid along with recovered chiral auxiliary. However, as the steric bulk of the R group increases, competition from an undesired reaction involving reagent attack at the endocyclic auxiliary carbonyl may be observed. It was found that lithium hydroperoxide displays the greatest exocyclic cleavage regioselectivity amongst all other reagents.¹⁰⁹



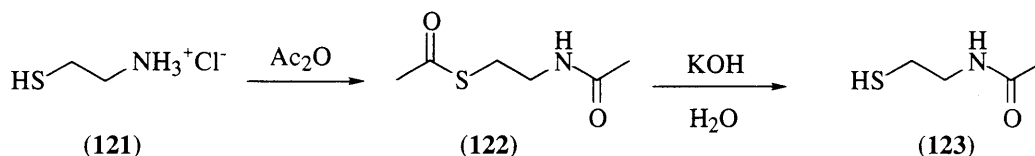
Scheme 2.8 Possible sites of nucleophilic attack on *N*-acyl oxazolidinones.

For the synthesis of (*R*)-2-benzylpropionic acid (**120**) the auxiliary was removed by reaction of (**119**) with lithium hydroperoxide formed *in situ* from lithium hydroxide and hydrogen peroxide to give the acid in 89% yield (Scheme 2.9). The optical rotation of the resulting acid (**120**) (-29.8 in chloroform) was in agreement with the published value (-25.4, neat).¹¹⁰



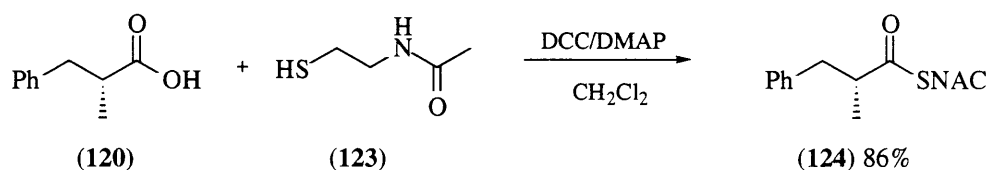
Scheme 2.9 Preparation of (*R*)-2-benzylpropionic acid (**119**) via hydrolysis of the chiral auxiliary.

N-acetylcysteamine (**123**) has been prepared in good yield by the acetylation of 2-mercaptoethylamine hydrochloride (**121**) followed by partial base hydrolysis of the resulting *N,S*-diacetylcysteamine (**122**) following a literature procedure (Scheme 2.10).¹¹¹ *N*-acetylcysteamine should be used shortly after preparation as it tends to dimerise *via* the formation of disulfide bond. It can however be stored under nitrogen in a freezer for several days.



Scheme 2.10 Synthesis of *N*-acetylcysteamine.

Dicyclohexylcarbodiimide (DCC)/*N,N*-dimethyl-4-aminopyridine (DMAP) mediated coupling¹¹² of acid (**120**) with *N*-acetylcysteamine then gave the thioester (**124**) in 86% yield (Scheme 2.11). DCC is used in the coupling reaction in order to activate the acid towards nucleophilic attack.

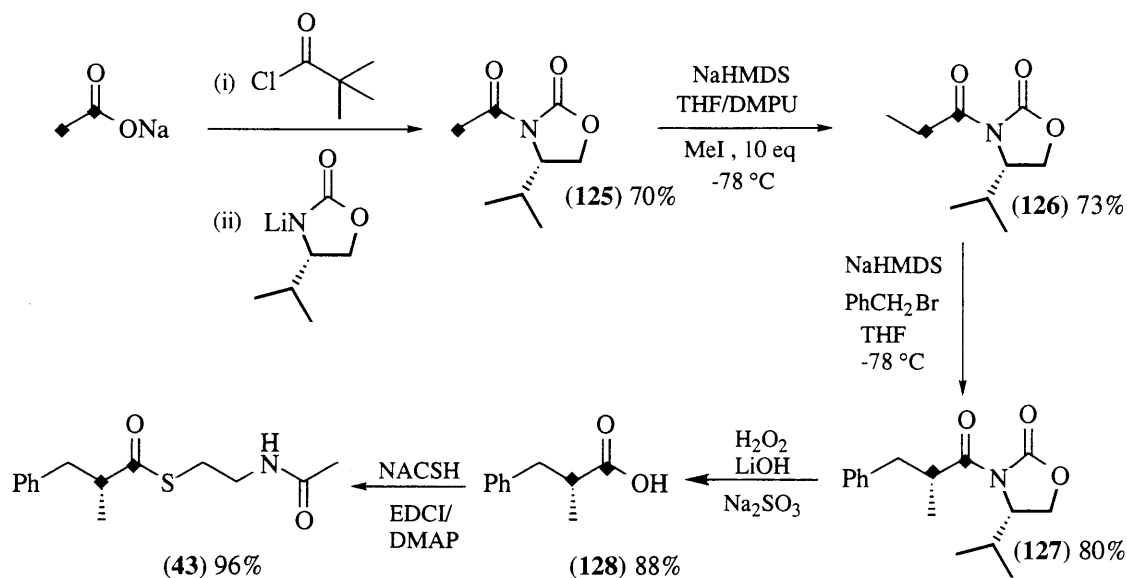


Scheme 2.11 Synthesis of *N*-acetylcysteamine thioester of (*R*)-2-benzylpropionic acid (**124**).

Having established a route for the synthesis of *N*-acetylcysteamine thioester of (2*R*)-benzylpropionic acid (**124**) in 39% yield over 5 steps from sodium acetate, the synthesis of [1,2-¹³C₂]thioester (**43**) was examined. [1,2-¹³C₂]Acetate was used in the preparation of [1,2-¹³C₂]acetyl oxazolidinone (**125**) *via* the mixed anhydride (Scheme 2.11). Methylation of (**125**) gave [1,2-¹³C₂]propionyl oxazolidinone (**126**) which was then benzylated with benzyl bromide to furnish (**127**) in 80% yield. The chiral auxiliary was then hydrolysed by lithium hydroperoxide to give acid (**128**) in excellent yield.

Although DCC gave an excellent yield in the preparation of thioester (**124**), it was not used in the synthesis of the labelled thioester (**43**). One problem associated with using DCC as a coupling agent is the formation of dicyclohexyl urea (DCU) which is often difficult to completely remove. Filtration through Celite, followed by column chromatography removes most of the DCU but some traces often remain. Another coupling agent is 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (EDCI) which forms

the corresponding water soluble urea. This reagent is commonly used on small scale reactions but could prove expensive for large scale reactions. EDCI was used in the coupling of acid (**128**) and *N*-acetylcysteamine (**123**) to give the desired [1,2- $^{13}\text{C}_2$]thioester (**43**) in 96% yield (Scheme 2.12).

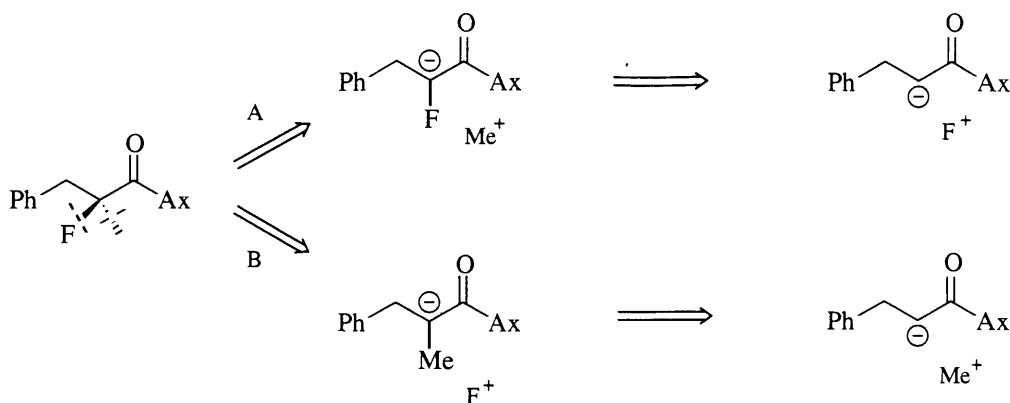


Scheme 2.12 Preparation of the *N*-acetylcysteamine thioester of (R)-2-[1,2- $^{13}\text{C}_2$]benzylpropionic acid.

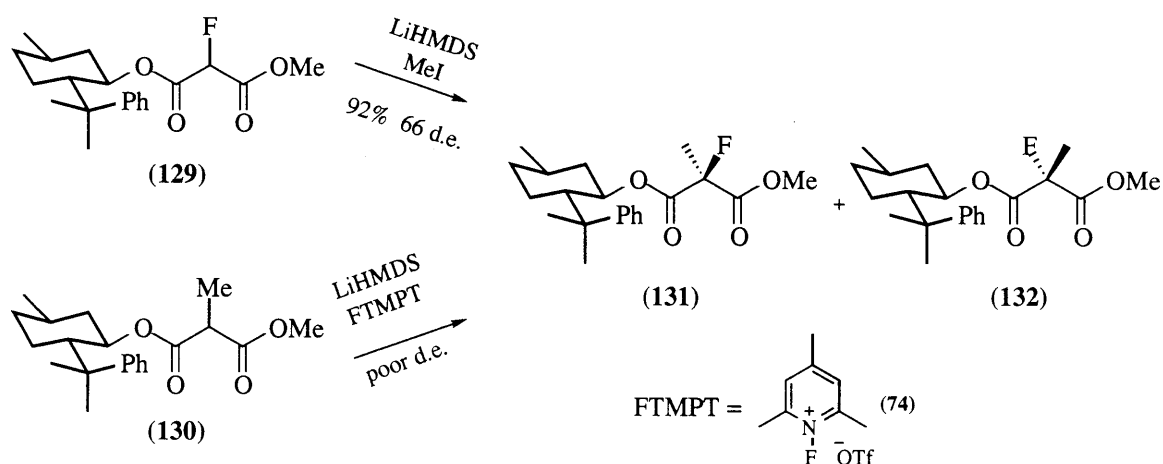
The ^1H -NMR spectra of ^{13}C labelled compounds (**125**), (**126**), (**127**), (**128**) and (**43**) exhibited characteristic ^{13}C - ^1H couplings. For example the signal arising from the proton attached to the labelled carbon in (**126**) appeared as a doublet of doublet of quintets due to one bond coupling to ^{13}C of 130 Hz, geminal proton coupling of 14.7 Hz as well as the vicinal coupling to the methyl protons and a two bond ^{13}C - ^1H coupling of 7.3 Hz.

2.2.2 Preparation of Fluorodiketide Analogues (**144**) and (**151**).

In order to try to combat enzymatic degradation of the diketide precursors it was decided to introduce a fluorine atom in the place of α -hydrogen into a selection of diketide analogues. The retrosynthetic analysis for the preparation of the α -fluorinated diketide analogues is shown in Scheme 2.13. The two strategies (A and B) required the use of an electrophilic fluorinating agent but differ in the sequence of introducing the fluoro and the methyl groups onto the acyl oxazolidinone enolate.

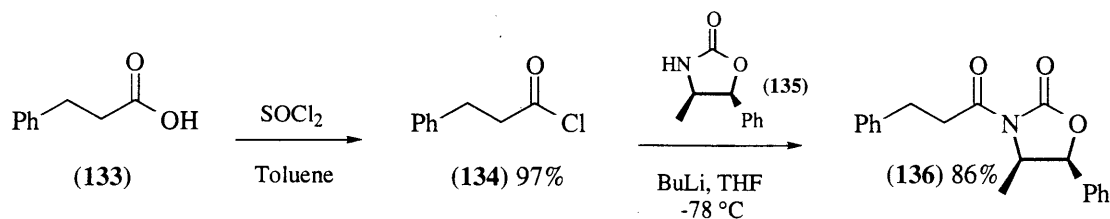
Scheme 2.13 Retrosynthetic analysis of α -fluorinated diketide.

Choosing between the two strategies, it was decided to introduce the fluorine first (strategy A). The reason was that since fluorine (similar in size to hydrogen) is much smaller than the methyl group, it was less likely to interfere with the formation of the *E*-enolate required for stereoselective addition. Furthermore, the deprotonation of a hindered dialkylated α -position may be difficult. Ihara *et al.*⁶⁴ reported the fluorination of (1*R*, 3*R*, 4*S*)-8-phenylmenthyl methylmalonate (**130**), using a number of electrophilic fluorinating agents, gave (**131**) and (**132**) in very low stereoselectivity (Scheme 2.14). However, better diastereoselectivity was obtained by methylation of (1*R*, 3*R*, 4*S*)-8-phenylmenthyl fluoromalonate (**129**). The dianion was formed by reaction of (**129**) with excess LiHMDS then reacted with methyl iodide in THF at $-78\text{ }^{\circ}\text{C}$ to ambient temperature to provide the 5:1 mixture of (**131**) and (**132**) in 92% yield.

Scheme 2.14 Fluorination of (1*R*, 3*R*, 4*S*)-8-phenylmenthyl methylmalonate (**130**) vs methylation of (1*R*, 3*R*, 4*S*)-8-phenylmenthyl fluoromalonate (**129**).

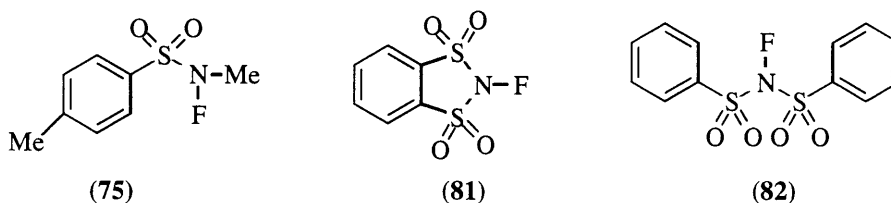
The starting material, 3-phenylpropionyl chloride (**134**), is commercially available. It was however easily prepared from 3-phenylpropionic acid (**133**) by

reaction with thionyl chloride (Scheme 2.15). Oxazolidinone (**135**), was treated with *n*-butyl lithium at $-78\text{ }^{\circ}\text{C}$ and reacted with (**134**) to yield 3-phenylpropionyl oxazolidinone (**136**) in 86% yield.



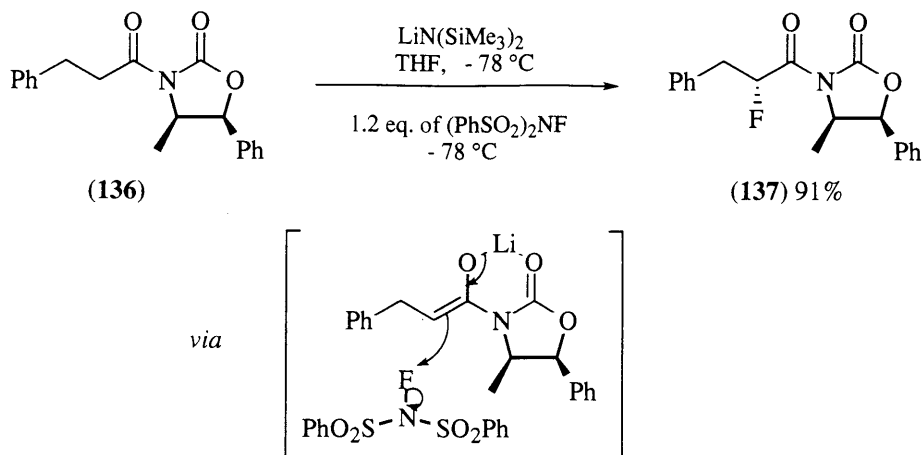
Scheme 2.15 Preparation of 3-phenylpropionyl oxazolidinone (**136**).

The next step of the synthesis would involve electrophilic fluorination of the acyl chain. A number of reagents that can act as a source of electrophilic fluorine have been reported (see Section 1.8). The reaction of *N*-fluoro-*N*-methyl sulfonimide (**75**)⁶¹ with an acyl oxazolidinone enolate was unsuccessful due to elimination of hydrogen fluoride from the sulfonimide (**75**) occurring in preference to electrophilic attack on the enolate.¹¹³ Davis *et al.*^{65, 66} have described the diastereoselective fluorination of an acyl oxazolidinone enolate in good yield and diastereoselectivity using the reagent *N*-fluoro-*O*-benzenedisulfonimide (**81**). Unfortunately, this reagent is not commercially available but may be prepared by fluorination of the corresponding imide with fluorine gas. This was deemed to be too hazardous. Differding *et al.*⁶⁷ reported the use of a similar reagent, *N*-fluorodibenzenesulfonimide (**82**), for achiral α -fluorinations of ketones and esters. The use of this fluorinating reagent which is commercially available, was later reported by Marquez *et al.* in which the α -fluoro derivative of acyloxazolidinone (**83**) was obtained in 56% yield and $> 99\%$ d.e. (Scheme 1.26). *N*-Fluorodibenzenesulfonimide (**82**) has also been used in the preparation of fluorinated triketide analogues to tetronasin (**58**) (Scheme 1.27).



The fluorination step was thus performed by converting acyl oxazolidinone (**136**) to the corresponding lithium enolate with lithium bis(trimethylsilyl)amide, at $-78\text{ }^{\circ}\text{C}$ which was then reacted with *N*-fluorodibenzenesulfonimide (**82**) at the same temperature. Both the yield and stereoselectivity of the required product (**137**) were excellent (Scheme 2.16). The stereocontrol was derived from preferential formation of

the Z-enolate followed by selective attack by the F^+ reagent on the least hindered face of the chiral enolate.



Scheme 2.16 Electrophilic fluorination of an acyl oxazolidinone (136).

Only one diastereomer was detected by ^1H - and ^{19}F -NMR spectroscopy. The ^1H -NMR signals due to the methine proton geminal to fluorine at 6.17 ppm appears as a doublet of doublet of doublets with a ^1H - ^{19}F coupling of 49.4 Hz and coupling of 8.7 Hz and 3.5 Hz to vicinal methylene protons. The 282 MHz ^{19}F -NMR spectrum of (137) showed one signal as a broad doublet of doublet of doublet signal at -190.8 ppm (Figure 2.1). The effect of fluorine is also pronounced in the ^{13}C -NMR spectrum of (137) where the signal due to the carbon attached to the fluorine appears as a doublet with ^{13}C - ^{19}F coupling of 182 Hz. The signals assigned to the acyl carbonyl and the benzylic carbon also show a small two bond coupling to the fluorine.

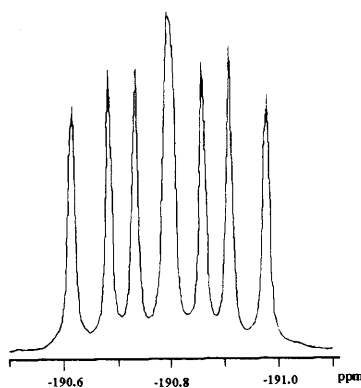
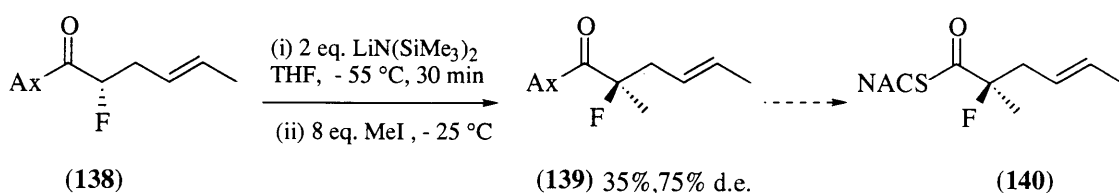


Figure 2.1 ^{19}F -NMR Spectrum of α -fluorocinnamoyl oxazolidinone.

The next stage was the most crucial step in the synthesis, namely the setting up of the asymmetric quaternary centre. The stereoselective methylation reaction was to be

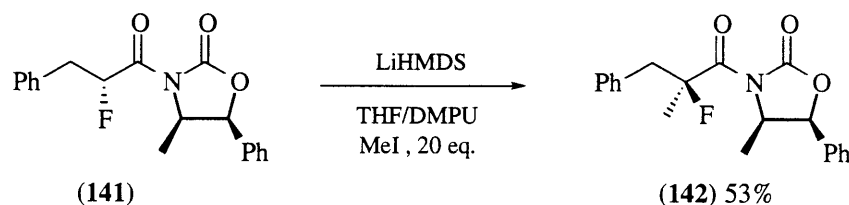
carried out *via* the *E*-enolate of α -fluoroacyl oxazolidinone and then reacting it with methyl iodide. A similar reaction has provided some difficulty in the synthesis of triketide analogue precursor of tetronasin (**140**) due to the instability of the enolate.⁴⁹ The optimised conditions for the preparation of triketide analogue (**140**) involved forming the α -fluoroenolate at $-55\text{ }^{\circ}\text{C}$ by the addition of two equivalents of LiHMDS to (**138**) and carrying out the stereoselective methylation at $-25\text{ }^{\circ}\text{C}$ using excess of methyl iodide to give (**139**) in 35% yield and 75 d.e. (Scheme 2.17). The authors⁶⁹ claim that maintaining the temperature at $-25\text{ }^{\circ}\text{C}$ was crucial for the methylation.



Scheme 2.17 Stereoselective methylation of α -fluorotriketide analogue (**138**).

The same conditions were examined for the synthesis of the α -fluoromethylacyl oxazolidinone (**142**) using 20 equivalents of methyl iodide. The reaction gave similar yield (37%) and excellent diastereoselectivity. The excellent diastereoselectivity ($>98\%$), as compared to (**139**), could be attributed to the higher selectivity of *E*-enolate with the more bulky phenyl group in (**141**). The use of a different base, sodium bis(trimethylsilyl)amide, was investigated but it did not improve the yield.

The methylation step gave low yields even in the nonfluorinated system and the problem was solved by increasing the polarity of the solvent. Indeed addition of DMPU in a 1:3 ratio with THF gave slight improvement in the yield (53%). An appreciable amount of the starting material appeared to have degraded to free oxazolidinone (**135**) as observed by TLC (Scheme 2.18). In the ¹H-NMR spectrum the signal due to the methyl group geminal to the fluorine appeared as a doublet with a ¹H-¹⁹F coupling of 22 Hz.



Scheme 2.18 Stereoselective methylation of α -fluorocinnamoyl oxazolidinone (**141**).

The standard conditions for methylation of an acyl oxazolidinone, i.e. 1.3 equivalents of base and 10 equivalents of methyl iodide at $-78\text{ }^{\circ}\text{C}$, were also examined in the presence of DMPU, however, no product was formed and the starting material

decomposed completely, which indicate that the enolate had been formed at $-78\text{ }^{\circ}\text{C}$ but the methylation required a higher temperature. It is also thought that the electronic effect of the fluorine substituent promoted cleavage of the enolate-oxazolidinone bond.¹¹⁴ A possible mechanism for the cleavage of the oxazolidinone auxiliary from the enolate is shown in Figure 2.2.

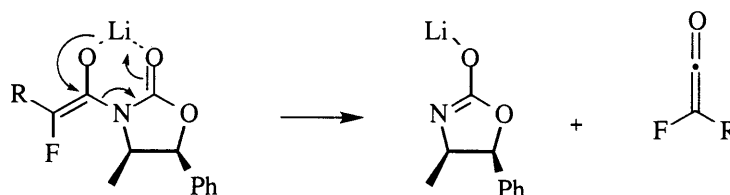
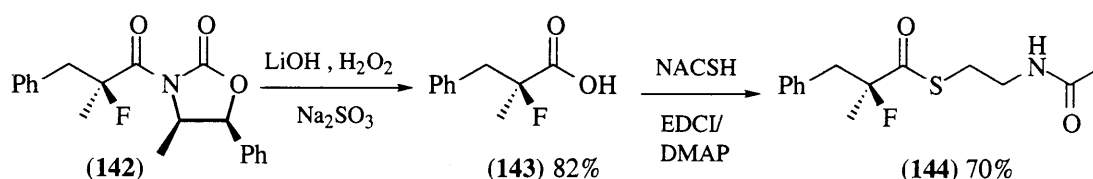


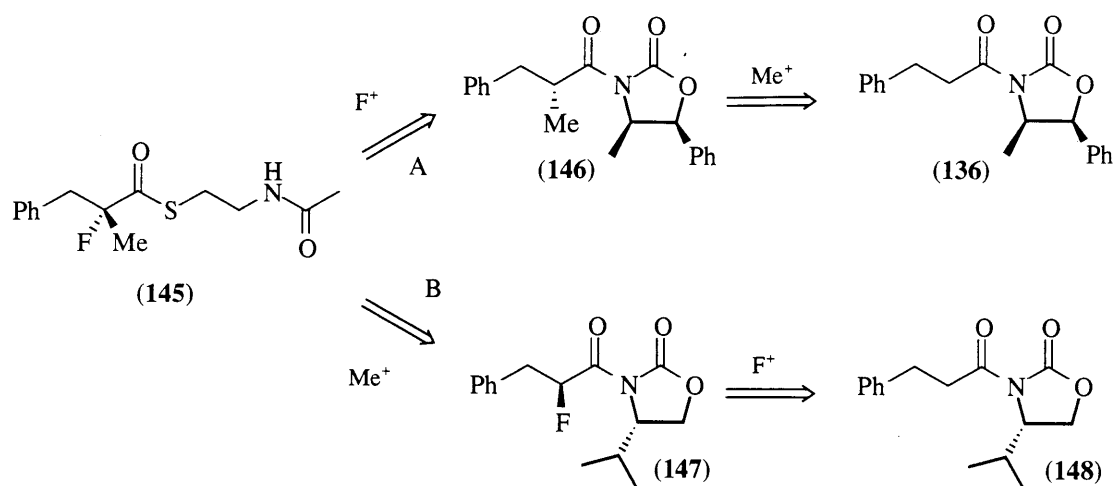
Figure 2.2 A proposed mechanism for the breakdown of the enolate.

The fluorinated derivative (**142**) was then treated with lithium hydroperoxide to give α -fluoroacid (**143**) in 82% yield which was coupled to *N*-acetylcysteamine using EDCI/DMAP to furnish α -fluorodiketide thioester analogue (**144**) in 70% yield (Scheme 2.19).



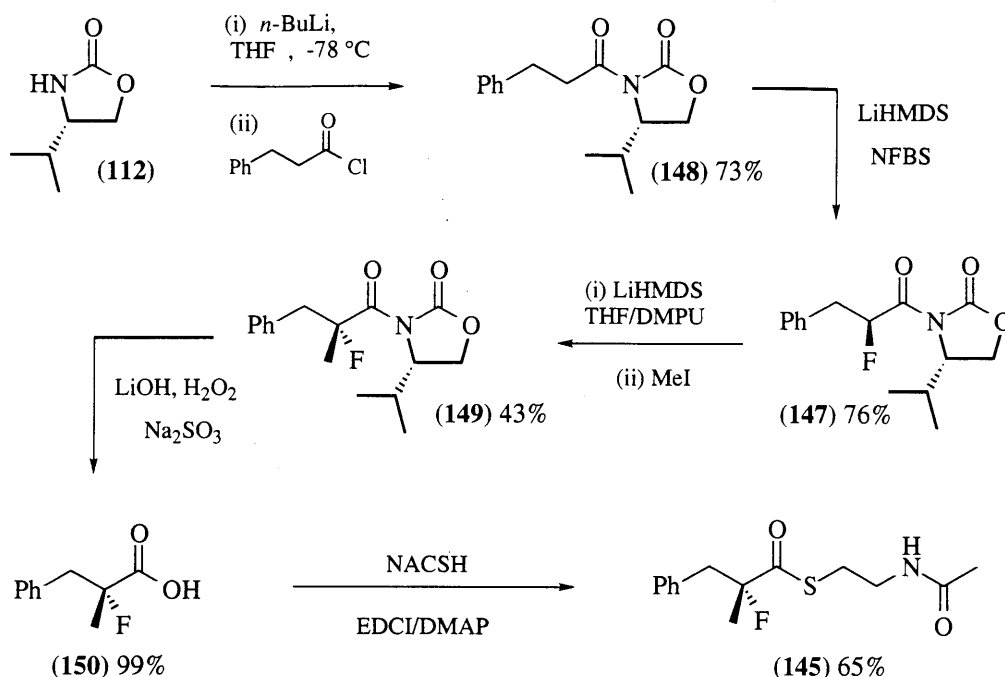
Scheme 2.19 Preparation of *N*-acetylcysteamine thioester (**144**).

The enantiomer (2*R*)-*N*-acetylcysteamine thioester (**145**) was also to be prepared using a chiral oxazolidinone. The opposite stereochemistry at α -carbonyl position could be constructed in two ways illustrated in Scheme 2.20. Route A utilises the same acyl oxazolidinone intermediate (**136**) synthesised for (**144**) but reversing the order of fluorination and methylation steps. Route B involves using a different acyl oxazolidinone (**148**) followed by fluorination to get (**147**) then carry out the methylation step. The former was disfavoured because of the reported difficulty in the fluorination of alkylated enolate.



Scheme 2.20 Retrosynthetic analysis of fluorodiketide analogue (145).

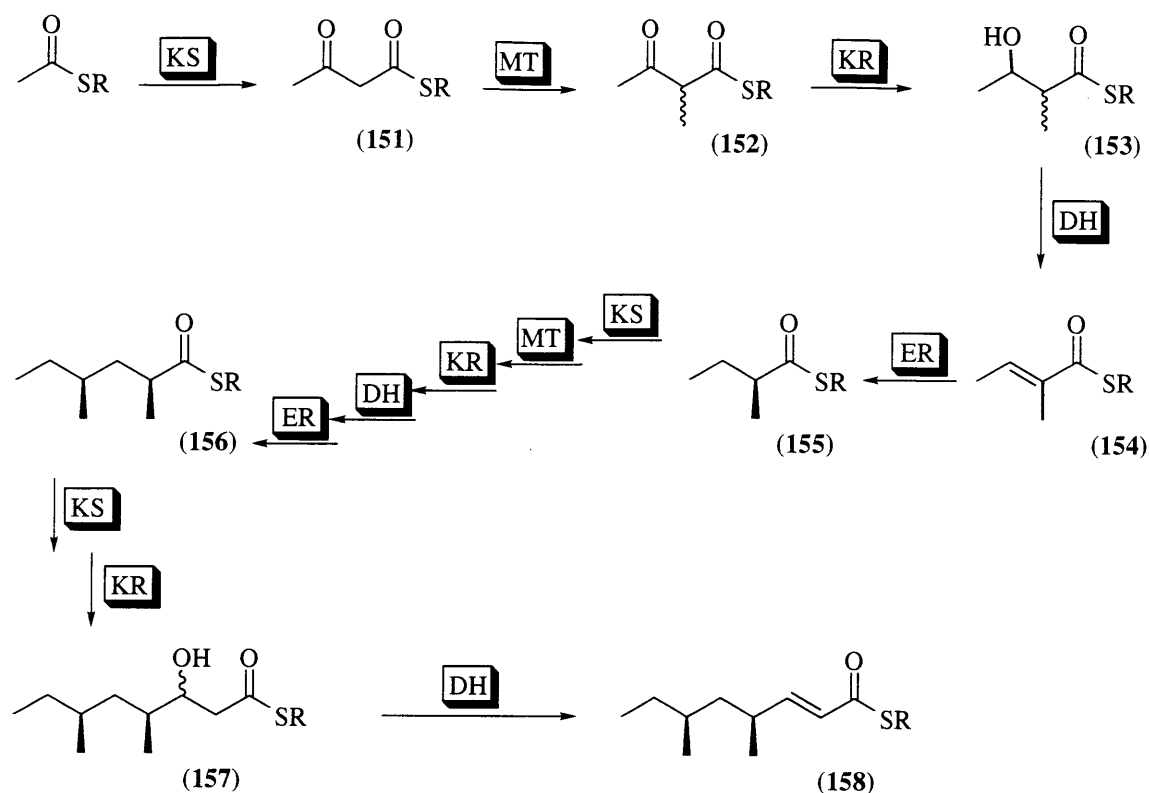
3-Phenylpropionyl chloride was reacted with lithiated (4*S*)-isopropyl oxazolidinone (112) to give acyl oxazolidinone (148) in excellent yield (Scheme 2.21). Fluorination of acyl oxazolidinone (148) using *N*-fluorodibenzene sulfonimide gave α -fluoroacyloxazolidinone (147) and then methylation of lithium enolate of (147) proceeded in 1:3 DMPU/THF as the solvent to give α -fluoromethyloxazolidinone (149). The auxiliary was removed under the standard conditions using lithium hydroperoxide and coupling of the resulting acid (150) and *N*-acetylcysteamine was successfully achieved using EDCI/DMAP to give the thioester (145) in 65% yield. As expected the $^1\text{H-NMR}$ spectra of acid (150) and thioester (145) were identical to their respective (*S*)-enantiomers (143) and (144). The values for the optical rotations were opposite in sign and similar in value indicating the opposite enantiomer had been formed. Thioester (145) was thus synthesised in 15% over 5 steps from 3-phenylpropionyl chloride (Scheme 2.21).



Scheme 2.21 Synthesis of N-acetylcysteine thioester of (R)-2-benzyl-2-fluoropropionic acid (**145**).

2.2.3 Preparation of the NAC thioester of (R)-(2-Fluoro-2-Methyl)-Butanoic Acid (**163**).

Feeding labelled acetate to the squalstatin producer, *Phoma* sp C2932, showed that the C-6 acyl chain in squalstatin S1 (**9**) originates from a tetraketide (**157**). A proposed biosynthetic pathway to tetraketide (**157**) is shown in Scheme 2.22. The pathway involves the condensation of an enzyme bound acetate starter unit with malonyl CoA followed by methylation of the resulting β -keto thioester (**151**) by *S*-adenosyl methionine (SAM) to give (**152**). Reduction of (**152**) gives 3-hydroxyl-2-methyl thioester (**153**). Dehydration, and further reduction of (**154**) results in the formation of the saturated 2-methyl diketide (**155**). Another cycle of malonyl CoA condensation, methylation, reduction, dehydration and reduction give the saturated triketide (**156**). Further malonate condensation, reduction and dehydration of the resulting (**157**) then lead to tetraketide (**158**).

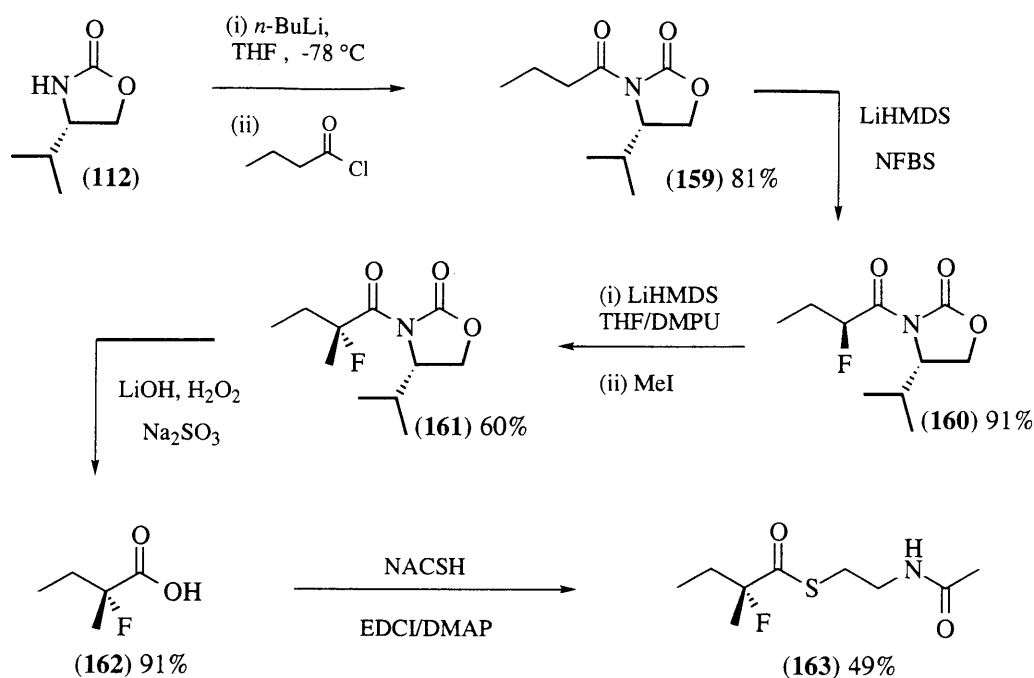


Scheme 2.22 Proposed biosynthetic pathway to tetraketide (158).

The *N*-acetylcysteamine thioester of diketide (155) was chosen to be synthesised as its fluorine analogue to feed to *Phoma*. The synthetic route utilises the methodology developed for the synthesis of α -fluorodiketide analogues (144) and (145).

Butyryl chloride was reacted with lithiated oxazolidinone (112) to give butyryl oxazolidinone (159) in 81% yield (Scheme 2.23). Butyryl oxazolidinone (159) was previously reported but no optical rotation data was given.¹⁰⁷ (159) gave an optical rotation value of $[\alpha]_D^{22} +89$ (*c* 2.76 in CHCl_3). Oxazolidinone (159) was treated with lithium bis(trimethylsilyl)amide and the resulting enolate was fluorinated with *N*-fluorodibenzenesulfonimide to give α -fluorobutyryloxazolidinone (160). Methylation of (160) was carried out in 1:3 DMPU/THF as the solvent using the base lithium bis(trimethylsilyl) amide and methyl iodide to obtain 2-fluoro-2-methyl acyloxazolidinone (161) in 60% yield in addition to some free oxazolidinone resulting from decomposition of the enolate.

The standard procedure was followed to remove the oxazolidinone by lithium hydroperoxide. Coupling of the resulting acid (162) with *N*-acetylcysteamine was successful using EDCI/DMAP to give the desired diketide thioester analogue (163) in 49% yield.



Scheme 2.23 Synthesis of *N*-acetylcysteamine thioester of (R)-(2-fluoro-2-methyl)-butanoic acid (163).

2.3 Conclusion.

The *N*-acetylcysteamine thioester of [1,2-¹³C₂]benzylpropionic acid was synthesised in 34.5% yield over 5 steps from sodium acetate after experimenting with unlabelled material which gave slightly higher overall yield of 39%. The feeding of the labelled thioester (43) to squalestatin S1 producer, ATCC 74256, is described in Chapter 4.

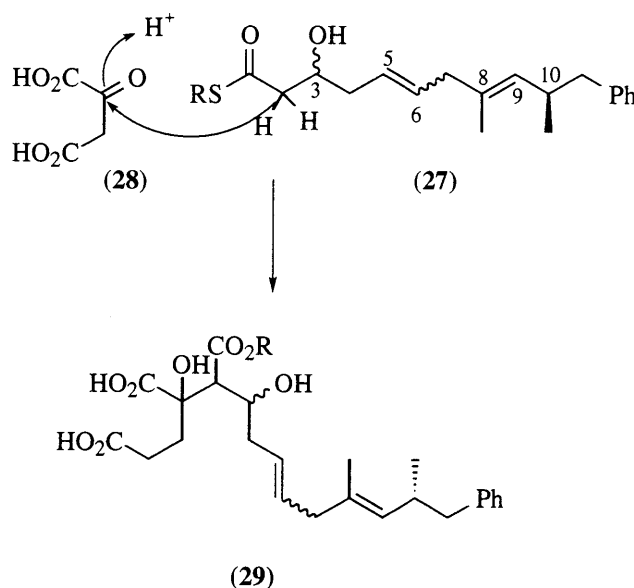
Novel fluorinated diketide precursor analogues (144), (145) and (163) have been synthesised in good yield and excellent enantioselectivity utilising Evans' chiral auxiliaries to control the stereochemistry at the α -centre and *N*-fluorodibenzene sulfonimide as the fluorinating agent. The feeding of the fluorinated diketide analogues to *Phoma* sp C2932 is described in Chapter 4.

Chapter 3

Synthesis of Hexaketide.

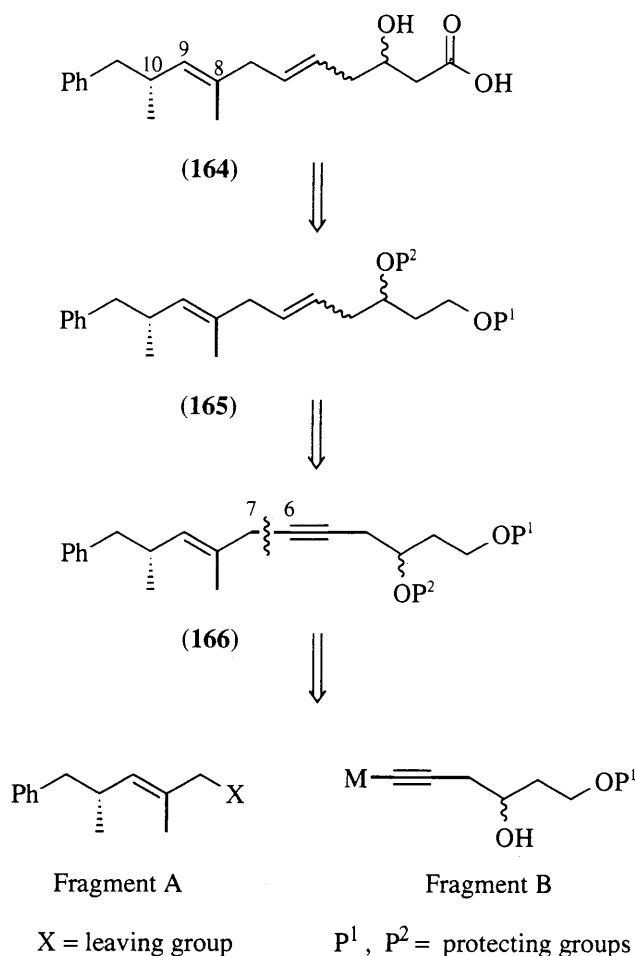
3.1 Introduction.

As described in Chapter 1, one of the key steps in the proposed biosynthetic pathway to squalestatin S1 (**9**) is the aldol condensation of the putative hexaketide intermediate (**27**) with a four carbon dicarboxylic acid (**28**), derived from the Krebs cycle (Scheme 3.1).



Scheme 3.1 Aldol condensation of the hexaketide (**27**) with oxaloacetate (**28**).

It was proposed that hexaketide (**27**) should be synthesised in isotopically labelled form for use in feeding studies to test for its intact incorporation into squalestatin S1 (**9**). Since both the geometry of the 5,6-double bond and the absolute configuration at C-3 are unknown due to the later modifications required to produce the final metabolite, it was necessary to design a synthetic route which would give access to all four possible isomers. The retrosynthetic analysis shown in Scheme 3.2 was therefore proposed. Acid (**164**) is obtained after functional group manipulation on the protected primary alcohol (**165**) which is in turn derived from acetylene (**166**). Disconnection of the C-6, C-7 bond gives two fragments A and B. The use of an acetylene was desirable since it was anticipated that it should be possible to prepare both (*Z*)- and (*E*)-olefins by appropriate choice of conditions for the reduction of the triple bond in alkyne (**166**).



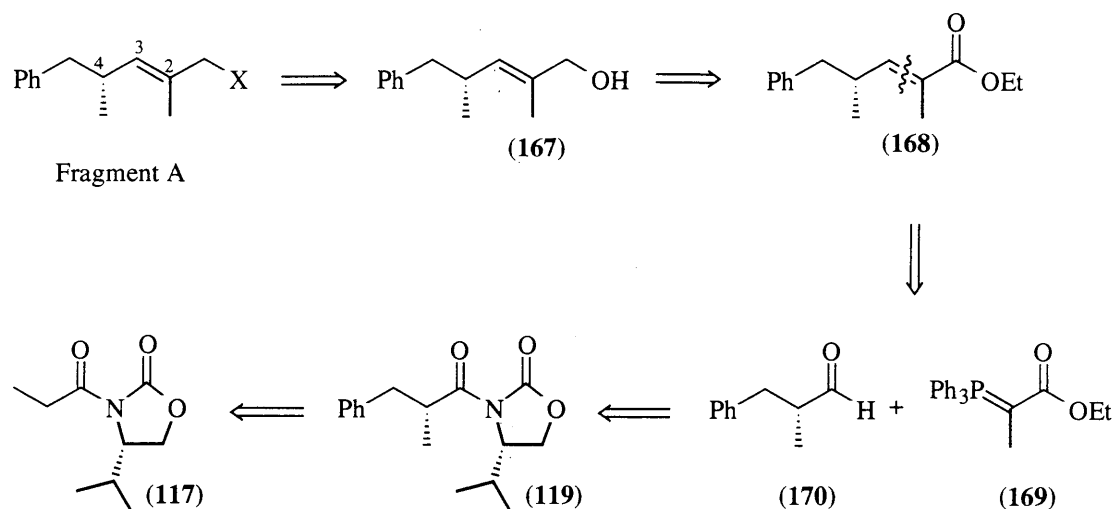
Scheme 3.2 Retrosynthetic analysis of hexaketide intermediate.

The synthesis of fragment A using the chiral oxazolidinone auxiliaries was completed by Westaway¹¹⁵ and is described in section 3.1.1. The preparations of fragment B from malic acid and (5*Z*, 8*E*)-hexaketide were carried out by Smith¹¹⁵ in our laboratories and will be described in sections 3.1.2 and 3.1.3.

3.1.1 Synthesis of Fragment A of the Hexaketide.

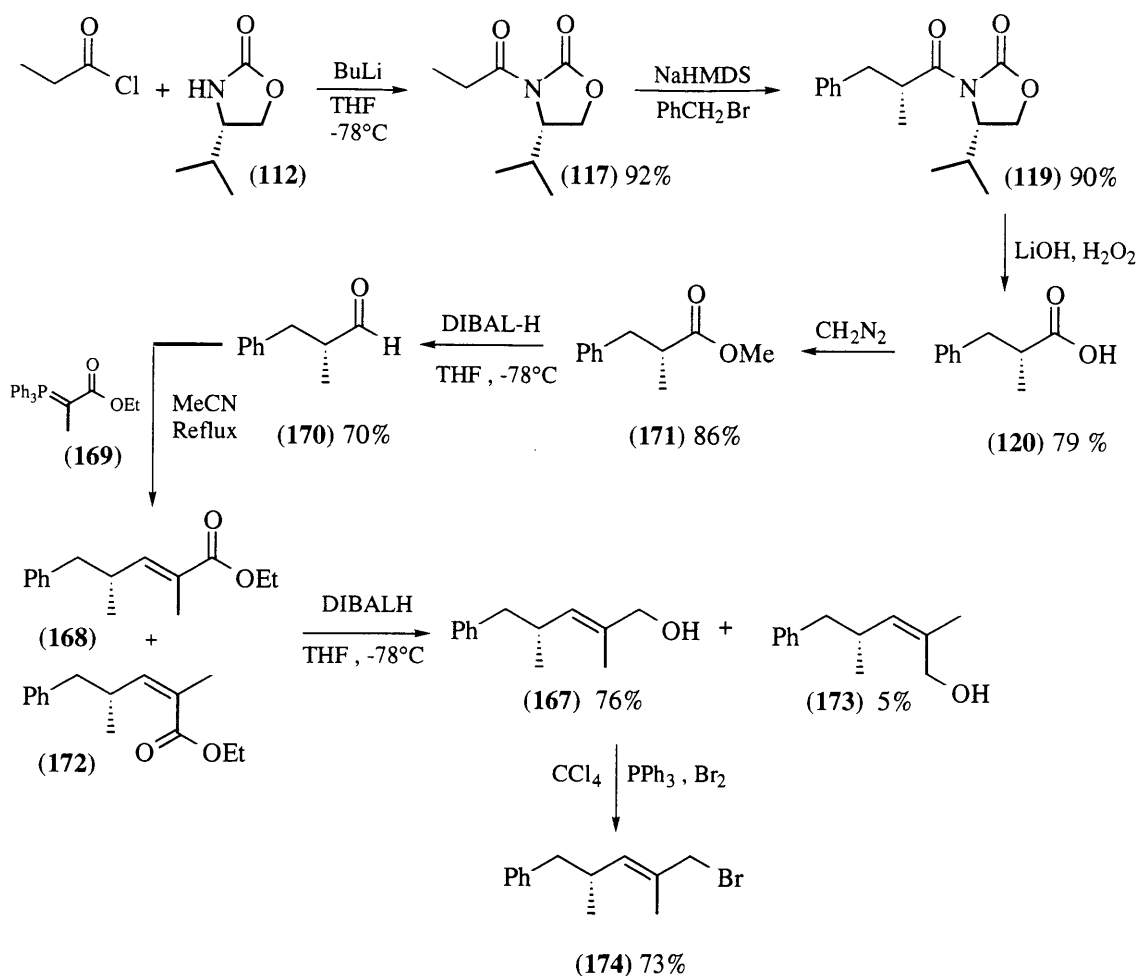
The stereogenic centre C-10 of hexaketide (27) is known to have the *R* configuration since this is retained in the final structure of squalestatin S1. It was also assumed that the 8,9-double bond adopts *E*-geometry, since a minor metabolite, squalestatin V5 (35), has been isolated containing this structural feature.¹¹⁶ A retrosynthetic analysis of (4*R*, 2*E*)-fragment A is shown in Scheme 3.3. The enantioselective route was based on the use of Evans' chiral oxazolidinone auxiliary to control the stereochemistry at C-4, and on Wittig chemistry to introduce the 2,3 double

bond. A key advantage of this approach was that both "halves" of fragment A, (170) and (169) could be made doubly ^{13}C -labelled.



Scheme 3.3 Retrosynthetic analysis of fragment A.

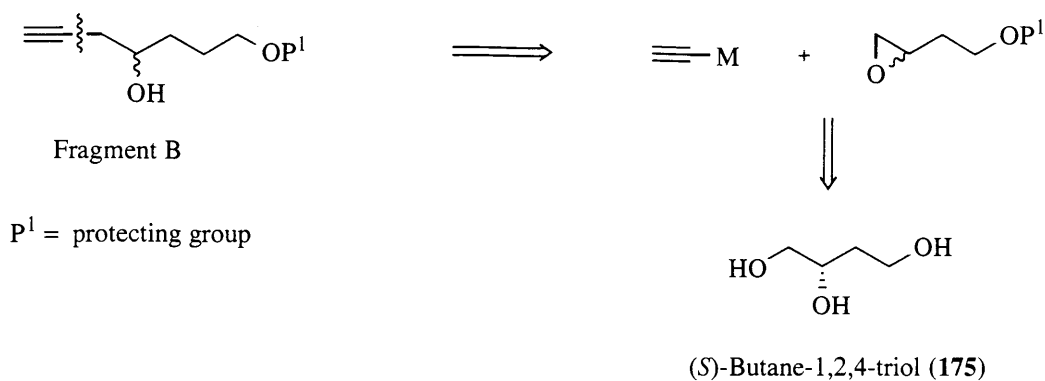
The optimised synthetic route to fragment A is shown in Scheme 3.4. Propionyl oxazolidinone (117) was obtained from propionyl chloride and chiral oxazolidinone auxiliary (112) in excellent yield. Alkylation of the sodium enolate of the propionyloxazolidinone (117) with benzyl bromide followed by the removal of the chiral auxiliary gave the acid (120) in 72% yield over the two steps. Acid (120) was converted to the methyl ester (171) using diazomethane and subsequent reduction with DIBAL-H provided aldehyde (170) in 70% yield. The key step was the use of the Wittig reaction to form the (*E*)-olefin (168) from aldehyde (170) and the commercially available carbethoxyethylidene triphenylphosphorane (169). The coupling reaction gave a mixture of (*E*)- and (*Z*)- α,β -unsaturated esters (168) and (172) which were inseparable by either flash or medium pressure chromatography (mplc). Reduction of the mixture of esters with DIBALH furnished the corresponding allylic alcohols (167) and (173) in a ratio of 15:1. The alcohols were separated by MPLC and the *nOe* studies confirmed that the required (*E*)-isomer was the major product. Signal enhancement was observed for the olefinic proton on irradiation of the allylic methyl protons in the (*Z*)-compound but not in the (*E*)-compound. Treatment of (167) with bromine and triphenylphosphine gave allylic bromide (174) in 73% yield. Hence, fragment A was synthesised over 8 steps in 14% yield from the chiral auxiliary (112).



Scheme 3.4 Preparation of fragment A .

3.1.2 Synthesis of Fragment B.

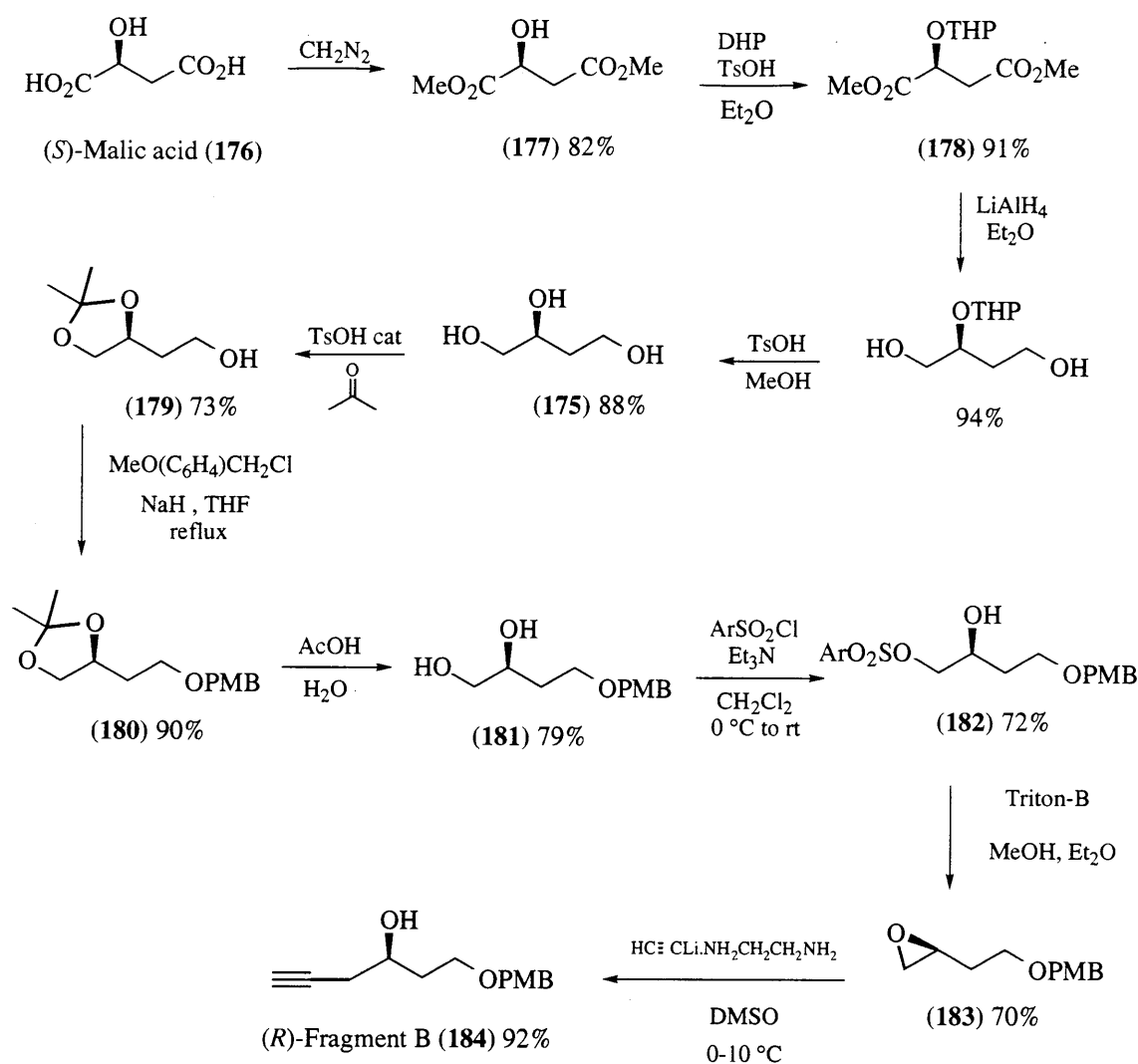
Since the stereochemistry at C-3 in hexaketide (**27**) is lost during the biotransformation into squalestatin S1, it was necessary to synthesise fragment B of the hexaketide in both (*R*)- and (*S*)-enantiomeric forms (**184**) and (**189**) respectively, as it was not known which was the precursor. A retrosynthetic analysis of fragment B is shown in Scheme 3.5. The carbon framework of fragment B could be formed *via* nucleophilic attack of an acetylide anion on the epoxide which could in turn be derived from commercially available (*S*)-butane-1,2,4-triol (**175**).



Scheme 3.5 Retrosynthetic analysis of fragment B.

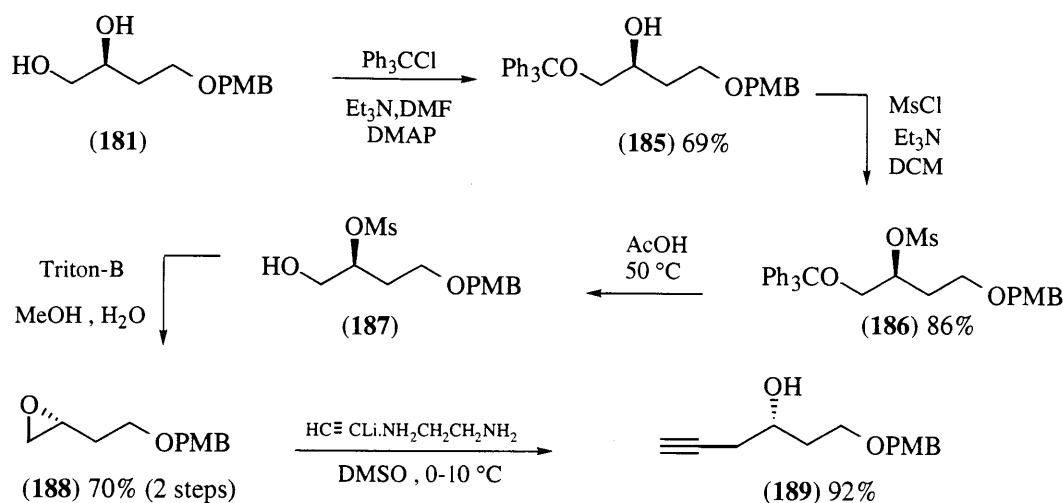
The synthetic route to the (*R*)-enantiomer (**184**) is shown in Scheme 3.6. Although the chiral starting material, (*S*)-butane-1,2,4-triol (**175**) is commercially available, it was easily and more cheaply prepared from (*S*)-malic acid (**176**). Acid (**176**) was converted to dimethyl ester (**177**) which could then be directly reduced with lithium aluminium hydride to triol (**175**) in 30-50% yield. However, the triol could also be prepared from ester (**177**) in better yield *via* the THP ether (**178**). Selective protection of the 1,2-diol moiety as acetonide (**179**) was achieved in 73% yield. The primary alcohol was then protected as the 4-methoxybenzyl (PMB) ether (**180**). Removal of the acetonide was followed by selective activation of the primary alcohol as its 2,4,6-triisopropylbenzenesulfonate ester (**182**). Treatment of (**182**) with the base benzyltrimethylammonium hydroxide (Triton-B) effected ring closure to give (*S*)-epoxide (**183**) in 70% yield. The epoxide was opened with lithium acetylide-ethylenediamine complex in DMSO to furnish the (*R*)-enantiomer of fragment B (**184**) in an overall 9% yield over 12 steps from (*S*)-malic acid (**176**).

The final stages of the hexaketide synthesis would involve selective deprotection of the primary alcohol followed by its oxidation to the carboxylic acid. It was necessary to avoid protecting groups which could only be removed by hydrogenolysis because of the presence of the two olefins. The PMB protecting group was therefore chosen, as it can be removed under oxidative conditions using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).

Scheme 3.6 Preparation of (*R*)-enantiomer of fragment B (**184**).

The same chemistry could have been used to obtain the (*R*)-enantiomer of fragment B (**189**) from (*R*)-butane-1,2,4-triol. However, the latter is considerably more expensive than its (*S*)-enantiomer. A route to (**189**) from (*S*)-triol (**175**) was therefore devised (Scheme 3.7). The key (*R*)-epoxide (**188**) was prepared from PMB protected triol (**181**) by $\text{S}_{\text{N}}2$ attack of the primary alcohol on the activated secondary alcohol resulting in an inversion of the stereochemistry at the 3-position. It was necessary to first protect the primary alcohol in order to selectively activate the secondary alcohol. The triphenylmethyl group is selective for primary alcohols for steric reasons and was used successfully to give (**185**). The secondary alcohol was then activated as the mesylate (**187**). Removal of the trityl group gave the hydroxymesylate (**187**) which on treatment with Triton-B gave the desired (*R*)-epoxide (**188**) in good yield. Treatment of

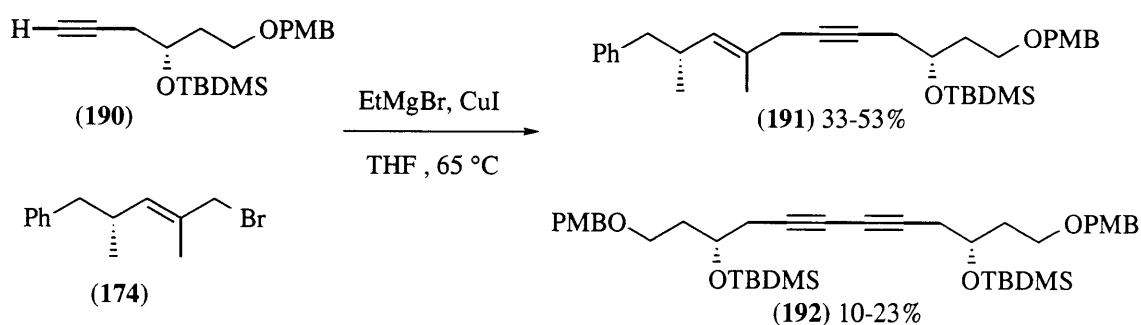
epoxide (**188**) with lithium acetylide ethylenediamine complex gave (*S*)-alkynol (**189**) in excellent yield.



Scheme 3.7 Preparation of the (*S*)-enantiomer of fragment B (**189**).

3.1.3 Coupling of Fragment A and B.

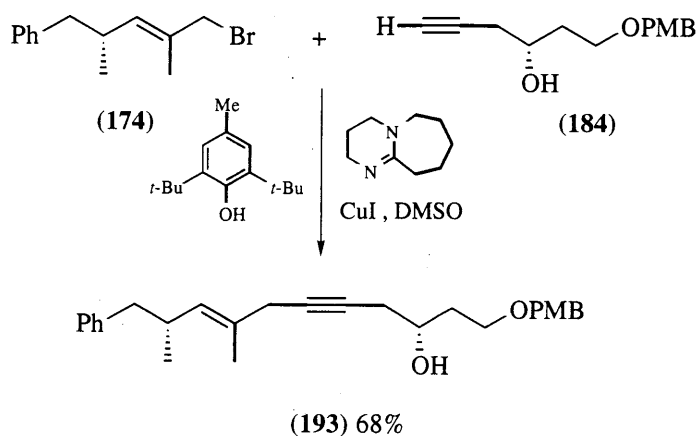
Having established routes to fragment A and the two enantiomeric forms of fragment B, the construction of the hexaketide was investigated. The initial investigation into the coupling process reacted alkynol (**190**) with ethylmagnesium bromide,¹¹⁷ followed by catalytic amount of copper(I) iodide and allylic bromide (**174**). Yields of the desired coupling product (**191**) using this procedure were low and an appreciable amount of the homocoupling product (**192**) was isolated (Scheme 3.8).



Scheme 3.8 Coupling of allylic bromide (**174**) and (*R*)-fragment B (**190**) using ethylmagnesium bromide.

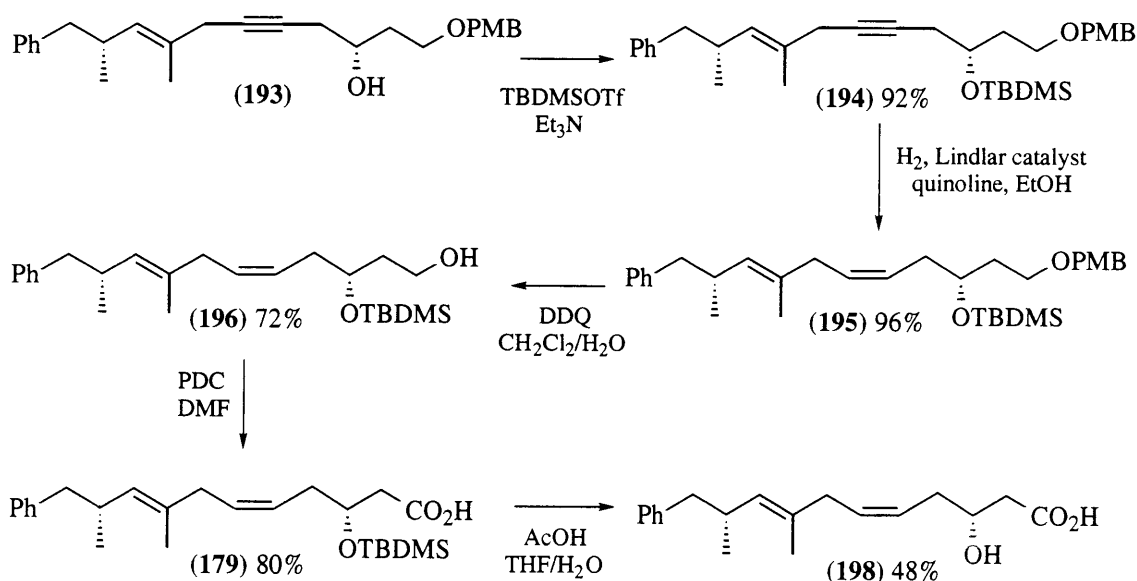
An improved result in the coupling reaction was achieved by the use of 1,8-diazabicyclo[5.4.0]undec-7-ene - copper(I) iodide complex to form the acetylide anion of

(184)¹¹⁸ followed by reaction with 1.2 equivalents of bromide (174) in the presence of a radical scavenger, 2,6-di-*t*-butyl-4-methylphenol, as shown in Scheme 3.9.



Scheme 3.9 Coupling of allylic bromide (174) and (R)-fragment B (184) using DBU- CuI complex.

A series of functional group manipulations were then used to convert the alkyne (193) to hexaketide (198) (Scheme 3.10). The protection of the 3-hydroxyl as the *t*-butyldimethylsilyl ether (194) was followed by partial hydrogenation in the presence of Lindlar catalyst¹¹⁹ gave *cis*-alkene (195) in excellent yield. The PMB group was removed under oxidative conditions with DDQ¹²⁰, followed by oxidation of the resulting primary alcohol (196) to the carboxylic acid (197) using pyridinium dichromate in DMF.¹²¹ Finally, removal of the TBDMS protective group by mild acid hydrolysis¹²² gave the desired hexaketide (198) in 4% yield over 12 steps from (*S*)-butane-1,2,4-triol (175).



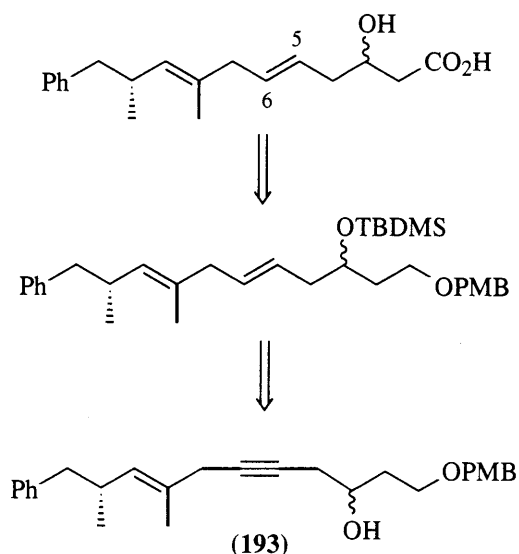
Scheme 3.10 Preparation of (3R,5Z,8E,10R)-hexaketide (198).

In conclusion, a synthetic route to the two (*Z*)-diastereomers of the putative hexaketide intermediate (**27**) has been developed. The route followed was convergent and was anticipated to allow the preparation of the two (*E*)-diastereomers. It would also allow the incorporation of ^{13}C labels *via* bromide (**174**) which can be synthesised with ^{13}C -label at C-3 and C-4 using $[1,2-^{13}\text{C}_2]$ -acetate, or at the 4-methyl using $[^{13}\text{C}]$ methyl iodide, thus providing valuable compounds for biosynthetic studies.

3.2 Results and discussion.

3.2.1 Preparation of (3*R*,5*E*)-Hexaketide: Alkyne Reduction Approach.

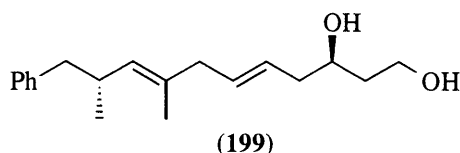
As described in section 3.1.3, alkyne (**193**) could give access to both (5*E*)- and (5*Z*)-isomers of the hexaketide by appropriate choice of reducing agents. Partial hydrogenation with Lindlar catalyst gave the (5*Z*)-isomer. It was envisaged that use of lithium aluminium hydride as the reducing agent would furnish the (5*E*)-diastereomers (Scheme 3.11).



Scheme 3.11 Retrosynthetic analysis of (5*E*)-hexaketide.

Lithium aluminium hydride has been widely used to reduce alkynes to (*E*)-alkenes. This reduction is greatly accelerated by a nearby hydroxyl group. Typically, propargylic alcohols react in ether or THF over a period of several hours,^{123, 124} whereas forcing conditions are required for β -hydroxyalkynes. The latter requires a large excess of lithium aluminium hydride at 140 °C for 2-3 days in a mixture of diglyme and THF.¹²⁵

Alkyne (**193**) was prepared in 55% yield using the DBU-copper(I) iodide method described in Section 3.4. Our attempts to reduce (**193**) with 10 equivalents of lithium aluminium hydride in THF at 50 °C were not successful. When the reaction was carried out in a mixture of diglyme and THF at 140 °C for three days it gave, amongst other products, the dienediol (**199**) in 22% yield. Although the primary alcohol could be protected again with the PMB group, the low yield led us to investigate other routes to the (*E*)-hexaketide.

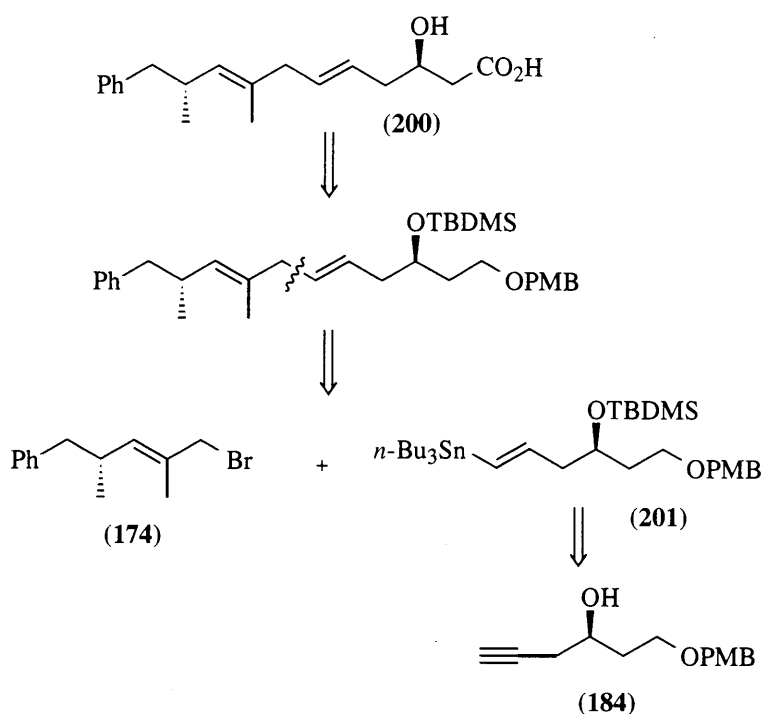


Another way to achieve the *E*-geometry of the 5,6-olefin was to isomerise the (*Z*)-isomer using known methods of isomerisation. The 3-hydroxyl of alkyne (**193**) was protected as *t*-butyldimethylsilyl ether (**194**) using TBDMS triflate/triethylamine¹²⁶ and the (5*Z*)-alkene (**195**) was obtained in quantitative yield by partial hydrogenation of the alkyne using Lindlar catalyst. Heating (*Z*)-alkene (**195**) to reflux in iodine/toluene¹²⁷ did not affect the double bond geometry and starting material was returned. Isomerisation was also attempted by irradiating the (*Z*)-alkene (**195**) with UV-light.¹²⁸ Short time exposure of up to two hours returned starting material but longer reaction times resulted in decomposition of the starting material.

As the reduction of alkyne (**193**) using lithium aluminium hydride proved to be unsatisfactory and attempts to interconvert the (5*Z*)-isomer (obtained from partial hydrogenation using Lindlar catalyst) to the desired (5*E*)-isomer failed, the alkyne reduction approach to (5*E*)-hexaketide was abandoned.

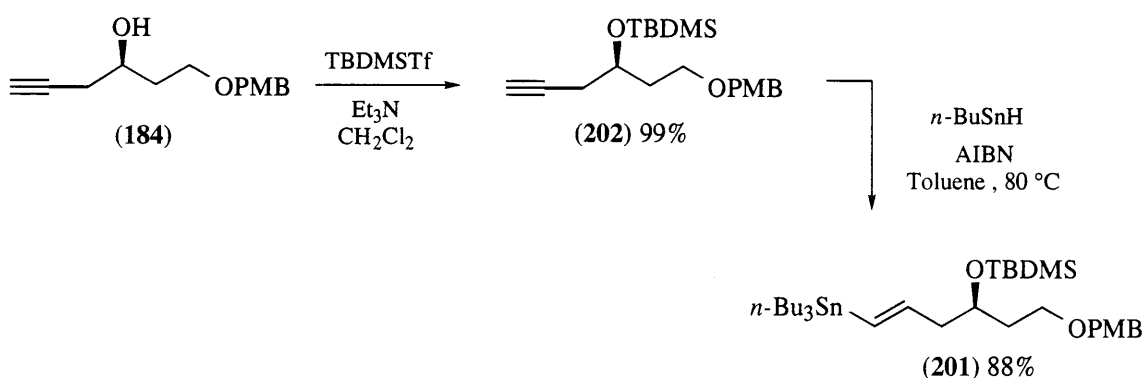
3.2.2 Preparation of (3*R*,5*E*)-Hexaketide: Coupling of Vinylstannane and Allylic Halide Approach.

An alternative approach to the (5*E*)-hexaketide (**200**) was proposed (Scheme 3.12). Disconnection of the 6,7-bond gave allylic bromide (**174**) and a vinyl stannane (**201**) which in turn derived from alkyne (**184**). The key step in this assembly of the carbon framework is the Stille coupling of allylic bromide (**174**) with (*E*)-vinylstannane (**201**). The geometry of the double bond is defined before the coupling and retained throughout. This approach had the attraction that it still utilised the available fragments A and B.



Scheme 3.12 Revised retrosynthetic analysis of (5E)-hexaketide.

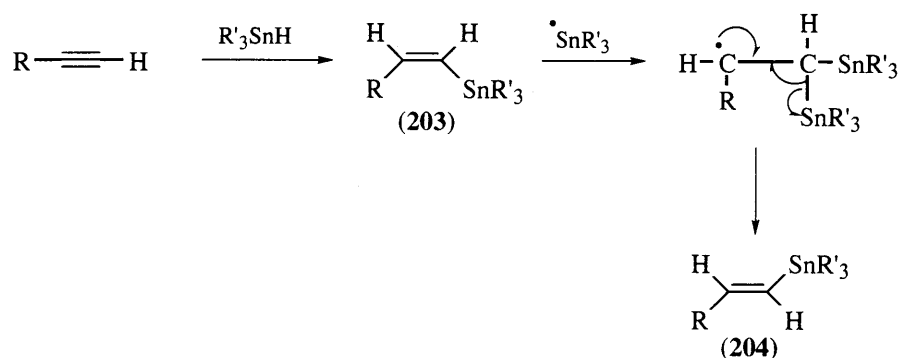
The 3-hydroxy group of (*R*)-fragment B (184) was protected as its TBDMS ether and the resulting silyl ether (202) was treated with 1.1 equivalents tributyltin hydride in the presence of catalytic amounts of azobis(isobutyronitrile) (AIBN)¹²⁹ in toluene at 80 °C for 3 hours to give (*E*)-vinylstannane (201) in 88% yield (Scheme 3.13). Vinyl stannane (201) is moisture and air stable and withstood chromatography on silica gel.

Scheme 3.13 Hydrostannation of (*R*)-fragment B TBDMS ether (202).

Only one isomer of the vinylstannane was observed in the ¹H-NMR spectrum using these reaction conditions. The stereochemistry of the vinylstannane was difficult to assign due to the overlap of the signals due to the two vinyl protons in the ¹H-NMR

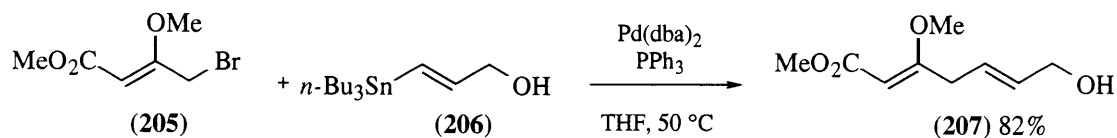
spectrum. Miftakhov *et al.*¹²⁹ showed that in the presence of excess tin hydride, radical initiator and high temperature, the kinetically formed (*Z*)-vinylstannane would isomerise to the (*E*)-isomer which is favoured for steric reasons.⁸⁴ We therefore assumed that the isomer we obtained was of (*E*)-configuration.

The proposed mechanism for the isomerisation of (*Z*)- to (*E*)-vinylstannane is shown in Scheme 3.14. It is believed that the (*Z*)-alkene (**203**) is the primary product, which then rearranges to the (*E*)-alkene. Attack of stannyl radicals on the resulting double bond produces a radical with two stannyl groups. Elimination of one of these groups leads to the formation of the isomerised product (**204**).⁸⁷



Scheme 3.14 Isomerisation of (*Z*)-vinylstannane.

The next step in the synthesis of (**200**) was the palladium-catalysed coupling of vinylstannane (**201**) with allyl bromide (**174**). Stille and co-workers reported similar couplings which proceeded under very mild conditions in good to excellent yields (see section 1.9.1). One such example⁹⁹ is shown in Scheme 3.15 in which allylic bromide (**205**) was coupled to vinylstannane (**206**) using bis(dibenzylideneacetone)palladium(0) [Pd(dba)₂] as the catalyst in the presence of triphenylphosphine in THF to give the cross-coupled product (**207**) in 82% yield with retention of double bond geometry of the vinylstannane partner. It is worth noticing that the mild reaction conditions tolerated the ester functionality in the allyl halide and the alcohol in the tin reagent.



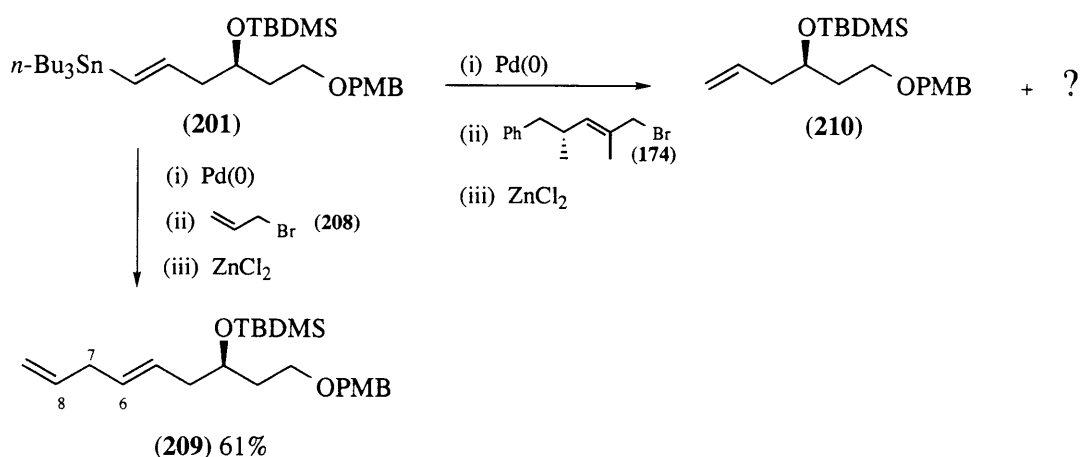
Scheme 3.15 Palladium-catalysed coupling of an allylic bromide (**205**) with a vinylstannane (**206**).⁹⁹

Coupling of allylic bromide (**174**) and vinylstannane (**201**) was examined in the presence of palladium(0) and triphenylphosphine in THF at 50 °C for 5 days. However reaction did not occur and starting materials were recovered. The palladium(0) catalyst used for the coupling reaction was either a pre-formed complex, namely bis(dibenzylideneacetone)palladium(0), or formed *in situ* by DIBAL-H reduction of bis(triphenylphosphine)palladium(II) chloride.

A model reaction, using allyl bromide (**208**) and vinylstannane (**201**) was investigated. Allyl bromide (**208**) was treated with 5 mol% palladium(0) followed by vinylstannane (**201**). No reaction was observed after 12 hours. Zinc chloride is known to assist the coupling reaction, but its role is unclear.¹³⁰ It may serve as a Lewis acid, generating an allylic cation from the allylic bromide, but the generation of vinyl zinc by exchange with vinylstannane is also possible.¹³¹

Zinc chloride was therefore added to the reaction mixture of the model system. In a few hours a new spot was observed by TLC, which was then proved to be the desired diene (**209**). The structure of diene (**209**) was confirmed by ¹H-¹H COSY NMR which revealed couplings between the multiplet at 2.7 ppm assigned to the 7-methylene protons and the two multiplets assigned to the vinylic protons at positions 6 and 8 at 5.4 ppm and 5.8 ppm respectively.

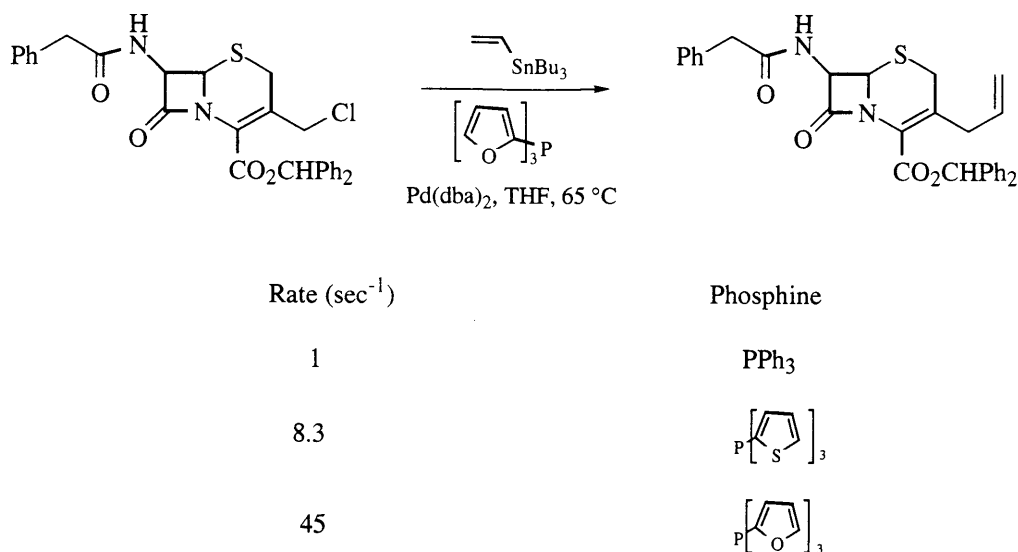
However, when identical conditions were applied to the coupling of allylic bromide (**174**) with vinylstannane (**201**) the coupling was not successful and decomposition of the starting material was observed on TLC. One of the products identified from the reaction mixture was the terminal alkene (**210**) (Scheme 3.16).



Scheme 3.16 Palladium-catalysed coupling of vinylstannane (**201**) with (**208**) and (**174**).

The nature of the ligand has been shown to have an effect on the rate of the reaction. Farina *et al.* showed that in the palladium-catalysed coupling of 3-

chloromethylcephems and unsaturated stannanes, the use of tri(2-furyl)phosphine enhanced the rate 45 times over triphenylphosphine¹³⁰ as shown in Scheme 3.17.



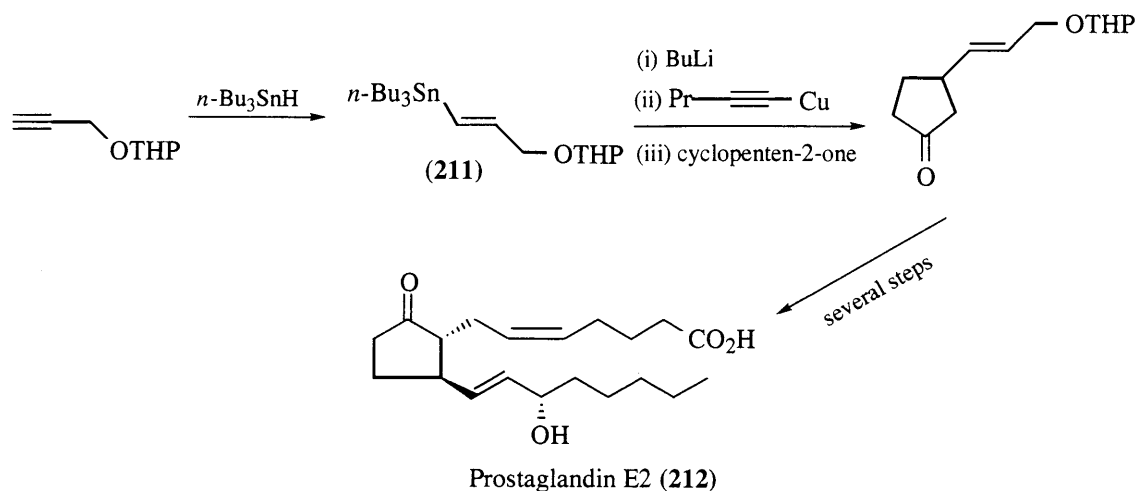
Scheme 3.17 Use of 2-furyl phosphine as a ligand in palladium-catalysed coupling reaction.¹³⁰

Farina *et al.* proposed that tri(2-furyl)phosphine enhanced the rate of coupling by rendering the allyl-Pd(II) intermediate more electrophilic and therefore more reactive in the transmetallation step, which is thought to be rate limiting in palladium-catalysed couplings involving organostannanes.¹³² Tri(2-furyl)phosphine was therefore used in the attempted coupling of allylic bromide (**174**) and vinylstannane (**201**), but was unsuccessful. The reaction returned many decomposition products amongst which was terminal alkene (**210**).

3.2.3 Preparation of (3*R*,5*E*)-Hexaketide: Coupling of Vinyl Lithium and Allylic Halide.

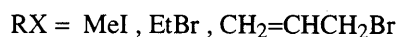
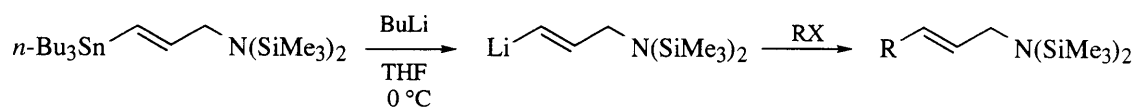
Different approaches to join the two fragments of the hexaketide were then investigated. It is known that the vinylstannane moiety allows the transfer of a functionalised carbon unit by undergoing a transmetallation to an intermediate vinyl lithium reagent¹³³ in addition to the palladium-catalysed direct coupling reaction with organic halides. It has been shown that the translithiation of vinylstannane occurs with retention of geometric configuration of the double bond.¹³⁴ This property has been extensively used as a consequence of easy access to vinylstannane compounds with fixed configuration.¹³⁵ In the synthesis of β -chain of prostaglandin E₂ (**212**), Corey and

coworkers reported the use of a vinyl lithium reagent, produced by transmetallation of vinylstannane (**211**) (Scheme 3.18).¹³⁶



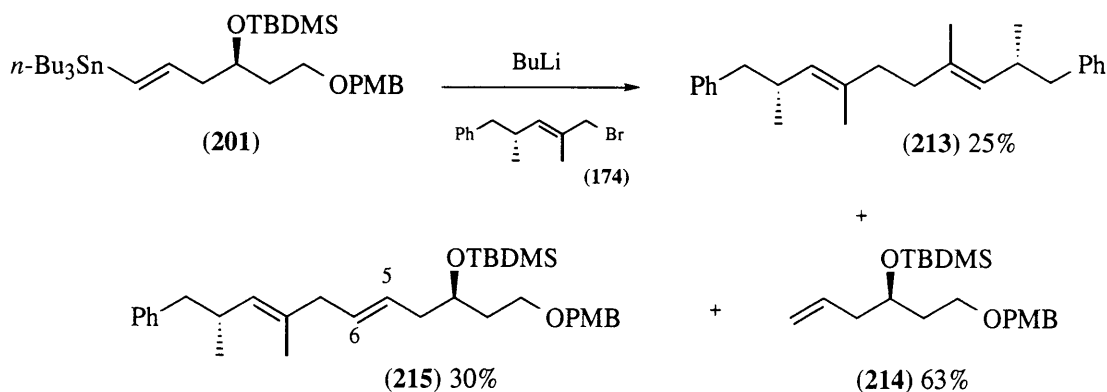
Scheme 3.18 Use of organolithium in the synthesis of prostaglandin E2 (**212**).

The stereochemistry of the vinyl lithium is also retained during its reaction with organic halides. Corriu *et al.* used this property to make a series of (*E*)-allylic amines by coupling functionalised vinyl lithium species with alkyl halides (Scheme 3.19).¹³⁷



Scheme 3.19 Coupling of organolithium reagents with alkyl halides.

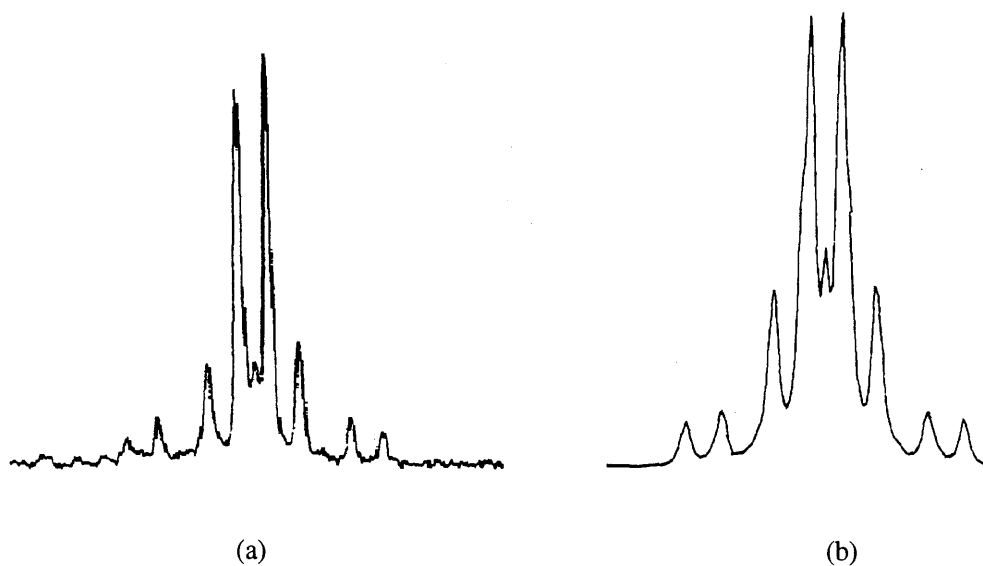
The transmetallation method used by Corriu was adapted to couple vinylstannane (**201**) with allylic bromide (**174**). The reaction was successful and gave the desired (*E,E*)-diene (**215**) in moderate yield (Scheme 3.20).



Scheme 3.20 Coupling of vinyl lithium reagent with allylic bromide (**174**).

It was necessary to confirm the geometry of the newly formed double bond in (**215**). The ^1H -NMR signals of the two olefinic protons, 5-H and 6-H, in deuteriochloroform overlapped giving a complicated second order pattern (Figure 3.1). Initially the signal of the two olefinic protons of the *E*-double bond in (**215**) was simulated with the Loacoön program.¹³⁸ By using a value for a *trans* coupling constant of 15 Hz, an identical pattern shown in Figure 3.1 was obtained. Conclusive evidence for an *E*-double bond was obtained by recording the ^1H -NMR spectrum in deuterobenzene. In this solvent the two olefinic hydrogen signals were slightly better resolved and selective irradiation of 4- H_2 and 7- H_2 collapsed 5-H and 6-H signals respectively into second order doublets with a coupling constant of 15.4 Hz typical of an (*E*)-double bond.

It was worthwhile to compare these NMR data with those obtained for the (*Z*)-compound (**195**). The ^1H -NMR signals due to the two olefinic protons appeared as a complicated multiplet. However selective irradiation of 4- H_2 and 7- H_2 again allowed an olefinic coupling constant of 10.9 Hz to be determined confirming the *Z*-geometry.



(a) The ^1H (400MHz, CDCl_3) signal of the olefinic protons at 5.35 ppm

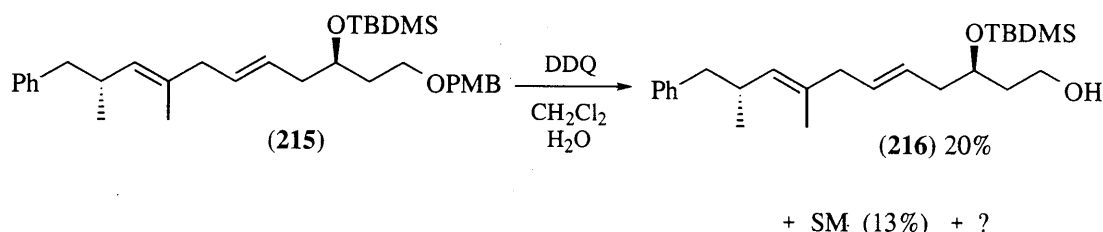
(b) The simulated signal for the olefinic protons using Lacoön Program.

Figure 3.1 Comparison of the ^1H -NMR signals assigned to 5-H and 6-H and simulated signals for *E*-(**215**).

In addition to the desired coupling product (**215**), the vinyl lithium coupling reaction gave a significant amounts of homocoupling product (**213**), and a protonated vinyl lithium product (**214**). Homocoupling product could arise from the coupling of

radical intermediates. Several mechanistic studies of the reaction between simple alkyl lithium compounds and alkyl halides have led to the conclusion that radicals are involved.¹³⁹ Radicals were detected during the organolithium coupling reaction by electron paramagnetic resonance (EPR)¹⁴⁰ and chemically induced dynamic nuclear polarisation (CIDNP)¹⁴¹ techniques.

The basic framework of the desired (5*E*)-hexaketide was now present but required functional group manipulations in order to generate the hydroxy acid (**200**). The PMB protecting group of (**215**) was cleaved by DDQ following a literature procedure,¹²⁰ to give primary alcohol (**216**) but in low yield. Some of the starting material was also recovered (Scheme 3.21).



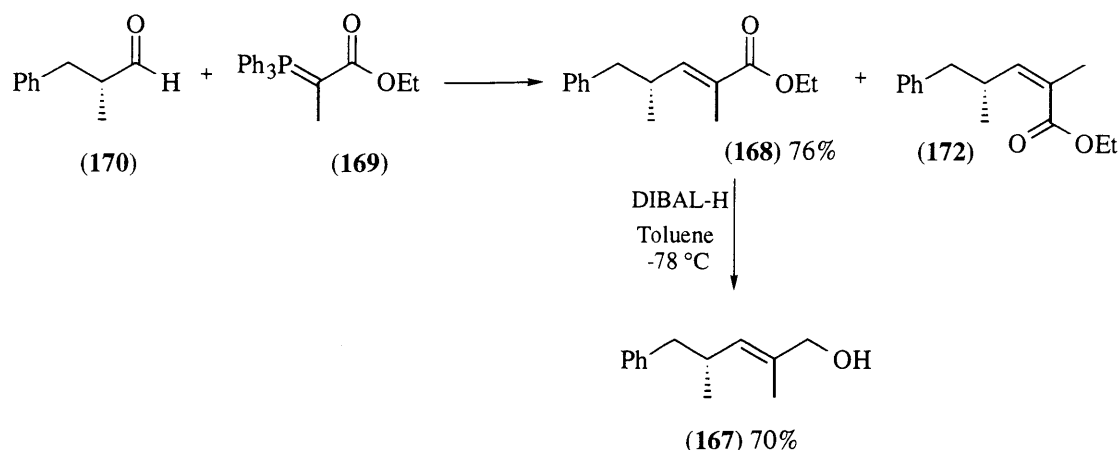
Scheme 3.21 Removal of PMB protecting group using DDQ.

Since both the coupling step and the PMB group cleavage were problematic, a modified approach was clearly required.

3.2.4 Preparation of (3*R*,5*E*)-Hexaketide: Coupling of Vinylstannane and Allylic Acetate Approach.

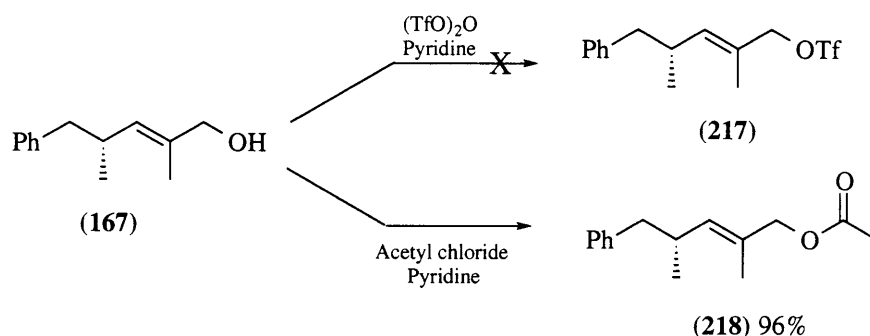
It is known that the electrophile in the Stille coupling method can be varied. Use of a triflate¹⁴², acetate¹⁰⁴ or carbonate⁹⁸ instead of the halide have been reported. All of these could be derived from a common allylic alcohol (**167**). Preparation of derivatives of (**167**) suitable for coupling with the vinylstannane (**201**) was then undertaken.

Aldehyde (**170**) was available in our laboratories from previous work.⁴⁰ It was coupled with commercially available phosphorane (**169**) to give a mixture of the (*Z*)- and (*E*)- α,β -unsaturated esters (**172**) and (**168**) respectively in 1:7.3 ratio (Scheme 3.22). The ratio of the geometric isomers was based on the ¹H-NMR spectrum of the mixture. The signal for the olefinic proton in the major *E*-alkene appeared as doublet of quartet (*J* 9.7, 1.4) at 6.6 ppm whereas the olefinic proton signal for the minor isomer was shifted upfield by 0.4 ppm. The *E*-geometry of the alkene was confirmed by nOe studies. On irradiation of the methyl group at C-2, no signal enhancement for the olefinic proton in (**167**) was observed while 8.1% enhancement was observed for the allylic proton at C-4.



Scheme 3.22 Preparation of allylic alcohol (167).

Careful separation by flash column chromatography gave pure (168) which was then reduced with DIBAL-H at -78 °C to give (*E*)-allylic alcohol (167) in 70% yield. Attempted preparation of the allylic triflate (217) using triflic anhydride was unsuccessful with no identifiable material being recovered. However, reaction of allylic alcohol (167) with acetyl chloride returned the allylic acetate (218) in excellent yield (Scheme 3.23).

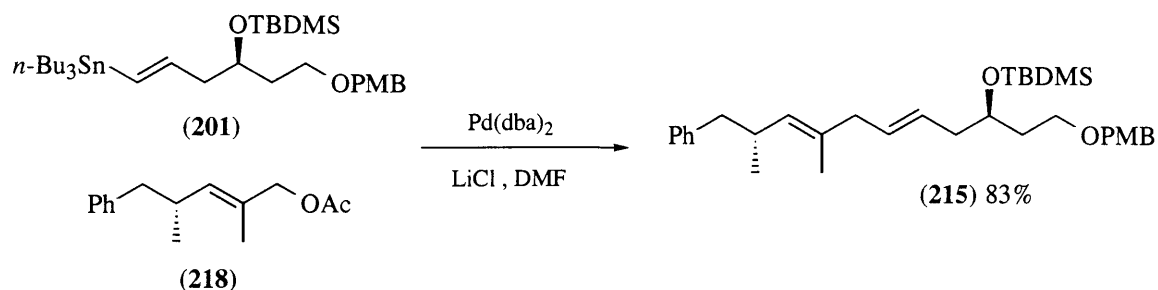


Scheme 3.23 Derivatisation of allylic alcohol (167).

Coupling of allylic acetate (218) with vinylstannane (201) was then explored. Del Valle *et al.*¹⁰⁴ stated that most allylic acetates failed to undergo the palladium-catalysed coupling with vinyl stannanes under the standard conditions used for coupling of vinylstannane with alkyl bromides. However, their study revealed that with the use of DMF as the solvent, 3 equivalents of lithium chloride and 3 mol% Pd(dba)₂ in the absence of added phosphine, simple allylic acetates were generally reactive towards variety of aryl- and vinylstannane reagents. The mechanism of the reaction is typical of other reactions involving allylic substrates and transmetallation from stannane. Oxidative addition of the allyl acetate to palladium(0) produces an allyl palladium acetate. Exchange of acetate for chloride facilitates the transmetallation from tin to palladium, and reductive

elimination produces the coupling product and regenerates the palladium(0) complex (see Scheme 1.42).

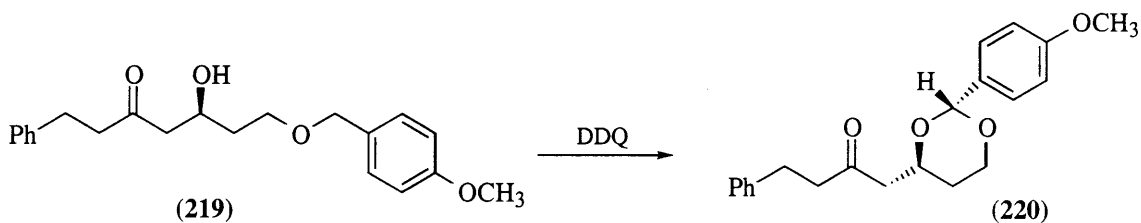
Allylic acetate (**218**) was thus reacted with vinyl stannane (**201**) using Del Valle conditions and the coupling product (**215**) was obtained as one isomer in excellent yield (Scheme 3.24).



Scheme 3.24 Palladium-catalysed coupling of vinylstannane (**201**) with allylic acetate (**218**).

Although the excellent yield of the coupling step would enhance the overall yield of the final hexaketide, the yield of the next step which involved the removal of the PMB groups was not satisfactory. Oxidative removal of the PMB group is a well known procedure using DDQ, or cerium(IV) ammonium nitrate (CAN).¹⁴³ However, neither of these methods gave a satisfactory yield of primary alcohol (**216**) when used to cleave the PMB protecting group. Some starting material and decomposition products were also returned.

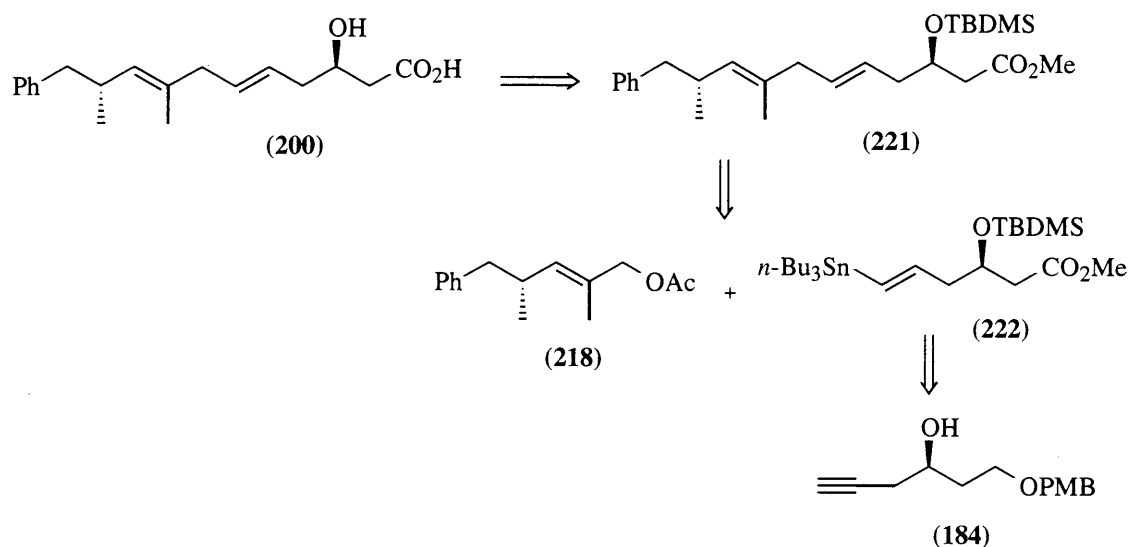
Schicker,¹⁴⁴ in our laboratories reported that attempts at the removal of the PMB protecting group from (**219**) with DDQ gave a 6-membered ring compound (**220**) where the PMB group appears to have reacted with the free hydroxyl group (Scheme 3.25). The 3-hydroxyl group in (**219**) was thus protected as the acetate but the removal of the PMB group at this stage using DDQ or CAN did not give the desired primary alcohol. Instead starting material or decomposition products were returned.



Scheme 3.25 Attempted removal of PMB group from (**219**) using DDQ.

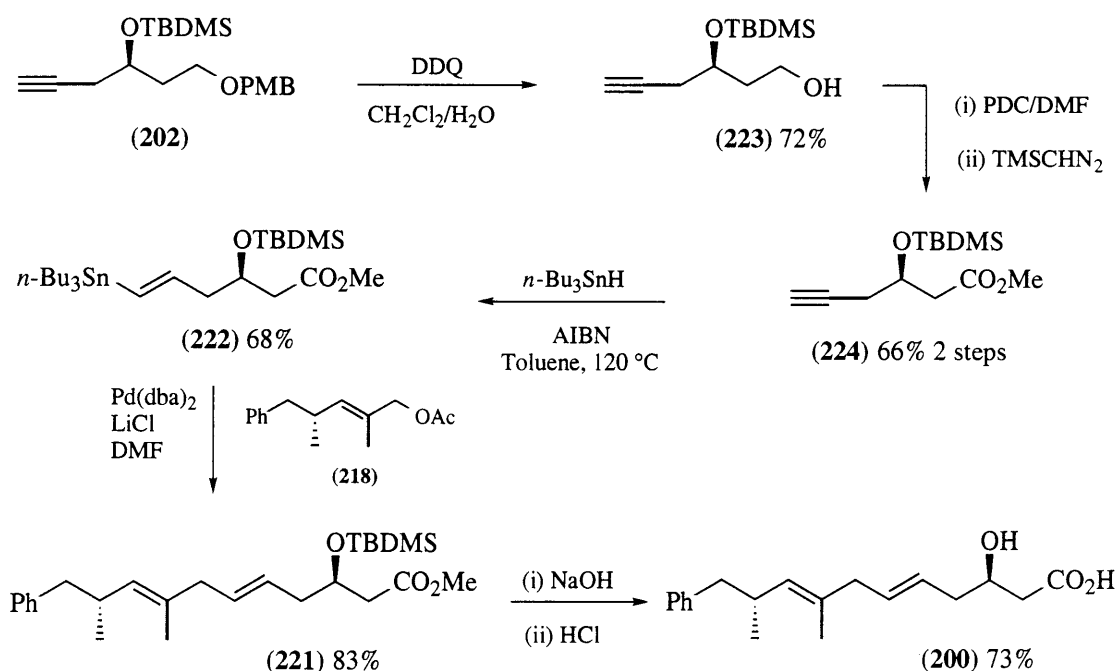
Following the problems with the removal of the PMB group at this stage of our synthesis of the hexaketide, the retrosynthesis of the (*E,E*)-hexaketide was revised as

shown in Scheme 3.26. Changes in the retrosynthetic analysis were aimed at minimising the number of steps after introducing the label *via* fragment A in future work.



Scheme 3.26 Revised retrosynthetic analysis of (3R,5E)-hexaketide (200).

Since the palladium-catalysed coupling of organostannane with organic electrophiles tolerates ester functionality on the organostannane partner, the PMB group could be removed from fragment B and the resulting primary alcohol oxidised to the carboxylic acid and converted to the methyl ester before converting it to the vinylstannane (222) (Scheme 3.27). Palladium-catalysed coupling of (222) and (218) will give the protected hexaketide (221).



Scheme 3.27 Preparation of (3R,5E)-hexaketide (200).

The PMB protecting group was oxidatively cleaved from alkyne (**202**) by DDQ in dichloromethane/water. The reaction was clean and gave alcohol (**223**) in 73% yield. Primary alcohol (**223**) was oxidised to the corresponding carboxylic acid using PDC in DMF and the crude acid was methylated with trimethylsilyl diazomethane in a 1:1 mixture of toluene and methanol, giving the methyl ester (**224**) in 66% yield over the two steps. Hydrostannation of the methyl ester returned vinylstannane (**222**) in good yield. Coupling of vinylstannane (**222**) with the allylic acetate (**218**) using the previously optimised conditions gave the desired (*E,E*)-diene in 83% yield. All that remained was to hydrolyse the methyl ester and remove the TBDMS protecting group. This was done in one pot: the ester was hydrolysed first with sodium hydroxide, and the reaction mixture was then acidified with hydrochloric acid to pH 2 and left stirring until the TBDMS group was hydrolysed to give the target (3*R*,5*E*,8*E*,10*R*)-hexaketide (**200**) in 73% yield. The optimised route to (**200**) is shown in Scheme 3.27. This route provided (**200**) in an overall 19% yield from TBDMS ether of (*R*)-fragment B (**202**).

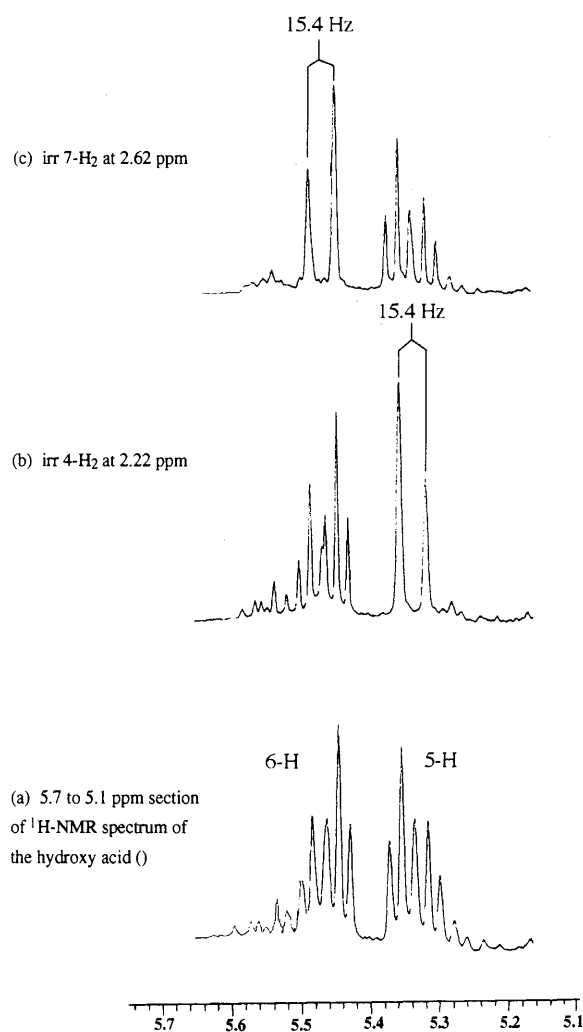
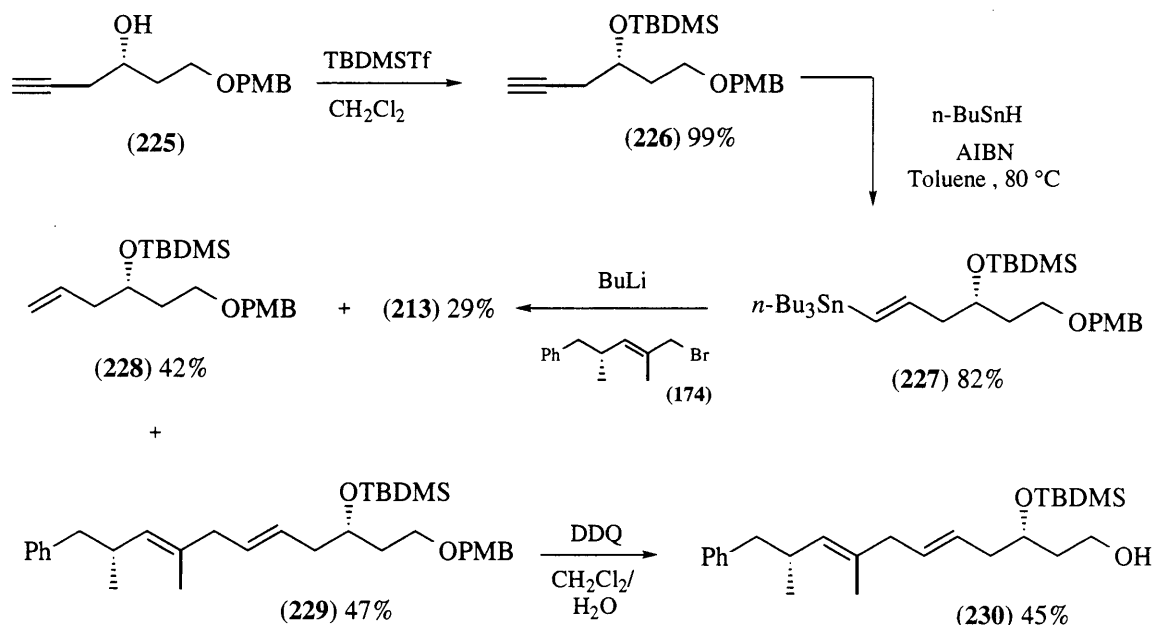


Figure 3.2 Section of the ¹H-NMR spectrum of hydroxy acid (**200**) in CDCl₃.

The ^1H -NMR signals assigned to the vinylic protons at position 5 (at 5.32 ppm) and 6 (at 5.46 ppm) were well resolved in the final hexaketide (Figure 3.5.2). Irradiation of the 4-methylene at 2.22 ppm and the 7-methylene at 2.62 ppm permitted the calculation of a large coupling (15.4 Hz) between the two olefinic protons and confirmed the (*E*)-configuration of the 5,6-double bond in the final hexaketide structure (**200**).

3.2.5 Preparation of (3*S*,5*E*)-Hexaketide.

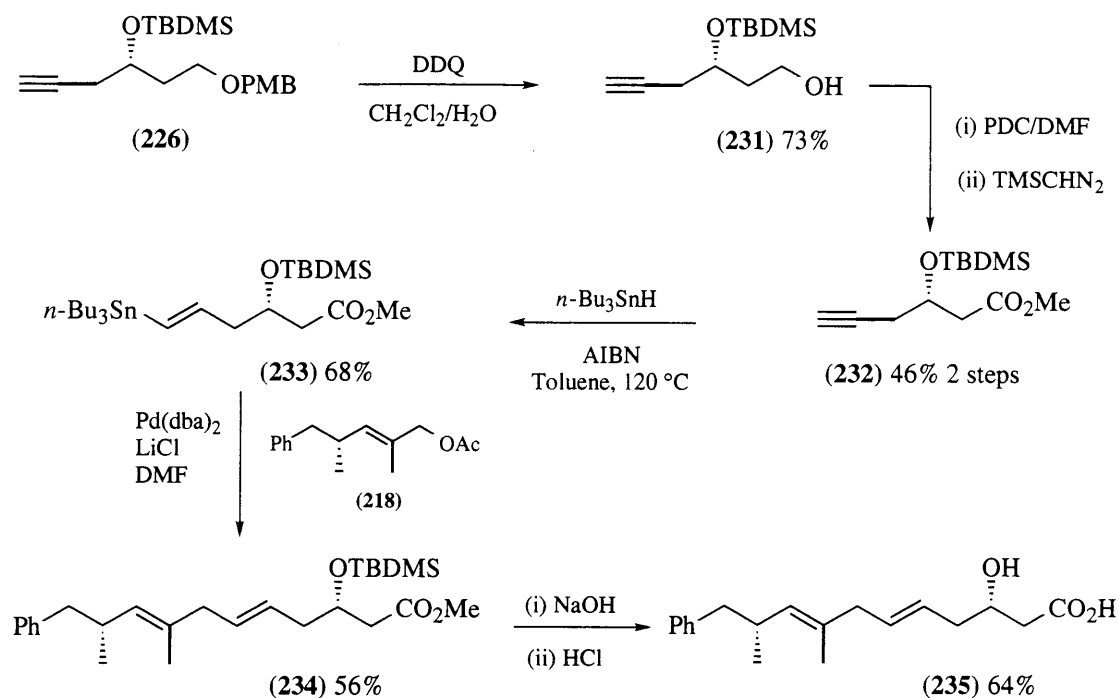
The synthesis of (3*S*,5*E*,8*E*,10*R*)-hexaketide was investigated using the organolithium approach from fragment A and (*S*)-fragment B (Scheme 3.28). The 3-hydroxyl group of alkyne (**225**) was protected as the *t*-butyldimethylsilyl ether (**226**) in near quantitative yield. Hydrostannation of TBDMS ether (**226**) yielded vinylstannane (**227**) which was then lithiated with *n*-butyl lithium at $-78\text{ }^\circ\text{C}$ and reacted with allyl bromide (**174**) to give the protected hexaketide (**229**) in 47% yield along with side products (**213**) and (**228**). Oxidative removal of the PMB group with DDQ gave the primary alcohol (**230**) in 45% yield.



Scheme 3.28 Towards the synthesis of (3*S*,5*E*)-hexaketide - organolithium approach.

As for (3*R*,5*E*,8*E*,10*R*)-hexaketide (**200**), the optimised route to (3*S*,5*E*,8*E*,10*R*)-hexaketide (**235**) used a Stille coupling approach as shown in Scheme 3.29. First, the PMB protecting group was cleaved by DDQ from TBDMS ether of (3*S*)-fragment B (**226**) to furnish the primary alcohol (**231**) in 73% yield. Primary alcohol (**231**) was oxidized with PDC in DMF and the crude acid was reacted with trimethyl silyldiazomethane to give ester (**232**). Reaction of ester (**232**) with excess tri-butyl tin

hydride in the presence of catalytic amounts of AIBN at 120 °C for 6 hours gave (*E*)-vinylstannane (**233**). The palladium-catalysed coupling of vinylstannane (**233**) with allylic acetate (**218**) in the presence of 3 equivalents of lithium chloride in DMF proceeded to give (**234**). Transformation of (**234**) to the final hexaketide (**235**) was achieved by saponification of the methyl ester followed by acid hydrolysis of TBDMS group in 64% yield over the two steps.



Scheme 3.29 Preparation of (*3S,5E*)-hexaketide (**235**) - palladium coupling approach.

In conclusion, hexaketide (**235**) has been synthesised in 8 steps and 8% overall yield from (*S*)-fragment B. The route followed to prepare both (**200**) and (**235**) from fragment A and either (*S*)- or (*R*)-enantiomer of fragment B should allow incorporation of ^{13}C labels *via* fragment A. Along with the (*5Z*)-diastereomers (**198**) and (**236**) prepared previously, (**200**) and (**235**) completed the set of four isomers required for biosynthetic studies of squalastatin S1 (Figure 3.3).

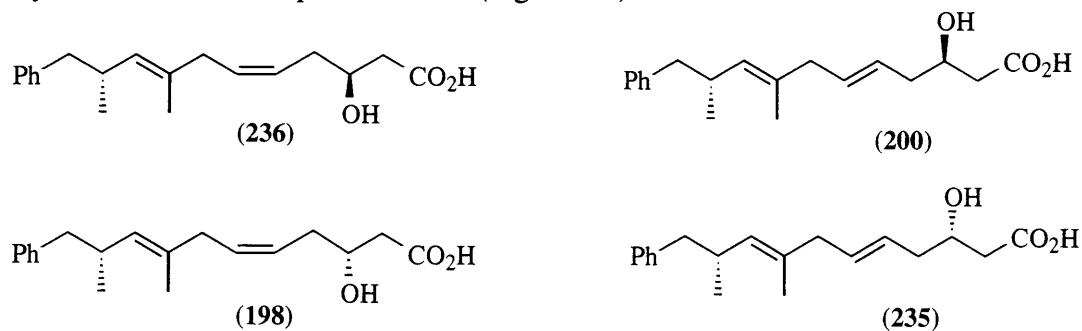


Figure 3.3 The four isomers of the putative hexaketide intermediate in the biosynthesis of squalastatin S1 (**9**).

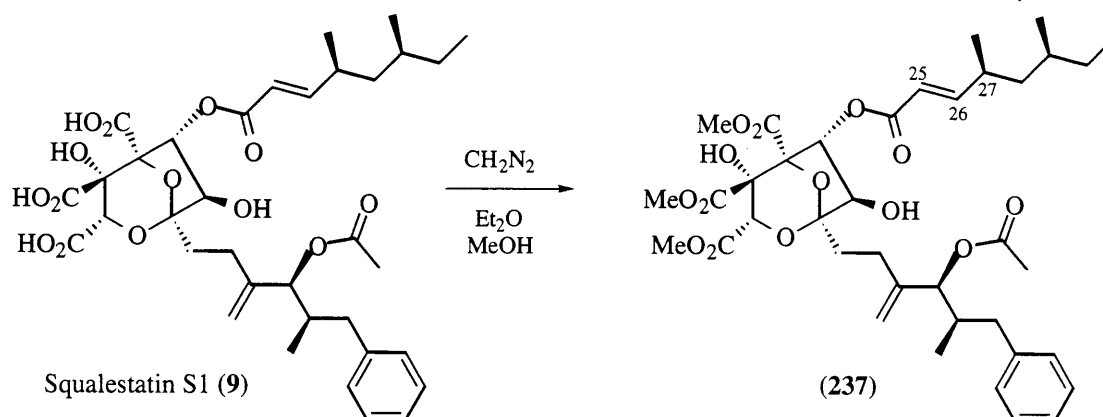
Chapter 4

Feeding Studies on Squalestatin S1.

4.1 Growth Conditions for *Phoma* sp C2932.

Following the synthesis of the α -fluorinated diketide analogues (Section 2.2.2 and 2.2.3) we then intended to carry out feeding studies with the squalestatin producing *Phoma* sp C2932 strain in order to try to establish intact incorporation after blocking β -oxidative degradation of the diketide precursors. Intact incorporation of the saturated fluorinated diketide analogues would provide evidence for the processive assembly of the hexaketide (**27**) and the tetraketide (**158**), and would generate novel fluorinated squalestatins.

Strain PA13 of *Phoma* sp C2932 was stored as a spore suspension in 5% w/v glycerol in liquid nitrogen. Once thawed, this 1 ml suspension was used to inoculate a seed flask containing 50 ml of medium which consisted of lactose, cotton seed flour and Junlon PW110 in water. The Junlon was added to seed medium to prevent aggregation of the mycelium. After 3 days the seed culture was used to inoculate flasks containing the production medium (SC5) which consisted of lactose and cotton seed flour in water. Junlon was not added to the production medium since it had been shown that its presence at this stage decreased the production of squalestatin S1 significantly.⁴⁰ Both the seed and the production flasks were shaken at 250 rpm at 25 °C. The production of S1 was determined by harvesting the production cultures, filtering the biomass and extracting the metabolites in ethyl acetate. Purification of squalestatin S1 at this stage would be difficult due to its extreme polarity. S1 was thus converted to its trimethyl ester (**237**) using ethereal diazomethane (Scheme 4.1) followed by flash chromatography on silica gel.



Scheme 4.1 Methylation of squalestatin S1 using diazomethane.

A typical growth production curve which was obtained by harvesting three production flasks every 24 hours over ten days of growth is shown in Figure 4.1. Squalestatin production began 3 days after inoculation and was accompanied by the

organism changing in colour from brown to dark green. Levels of S1 rose sharply from day 3 to 6 then started to level out.

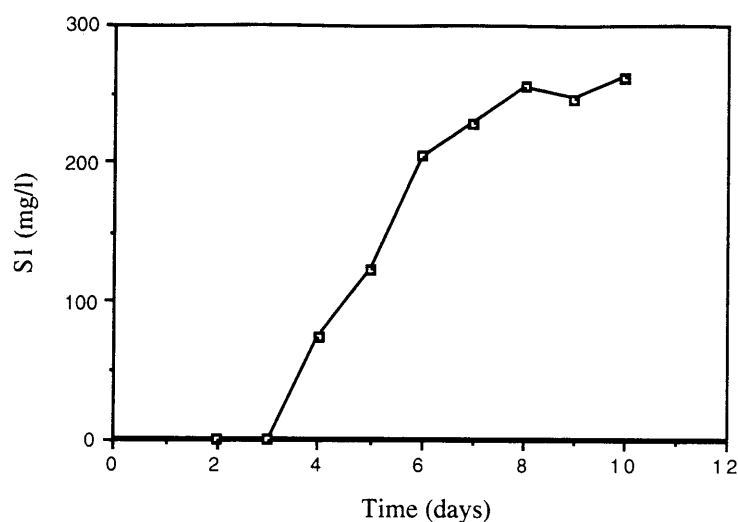


Figure 4.1 Growth-production curve for S1 production by *Phoma* sp C2932.

4.2 Incorporation Studies with [1,2- $^{13}\text{C}_2$]Acetate.

Previous studies on the biosynthetic origins of squalostatins had shown that [1,2- $^{13}\text{C}_2$]acetate was incorporated into squalostatin S1 at levels of *ca.* 0.5%.³⁴ The feeding of [1,2- $^{13}\text{C}_2$]acetate in SC5 optimal medium was investigated to optimise conditions for the feeding of advanced intermediates.

The production cultures were prepared using the procedure described above and the [1,2- $^{13}\text{C}_2$]acetate was administered as an aqueous solution at a level of 12 mmol per litre of culture after 3 days. S1 was isolated in the usual manner, converted to its trimethyl ester and analysed by ^{13}C -NMR spectrometry. The ^{13}C -NMR spectrum showed intact incorporation of [1,2- $^{13}\text{C}_2$]acetate unit into S1 as indicated by the presence of satellites flanking the natural abundance ^{13}C signals. Analysis of the ^{13}C -NMR spectrum revealed incorporation level of 0.3-2.0%. The level of incorporation was measured from the height of the satellites relative to the natural abundance signal. A slight improvement in the level of incorporation of labelled acetate was achieved by pulse feeding the precursor to shake cultures at a level of 4 mmol per litre of culture per day after 3, 4 and 5 days of growth. The S1 was then isolated after 7 days and shown to contain 0.3 to 2.2 % incorporation of the ^{13}C label. The results are summarised in Table 4.1.

Carbon	$\delta^{13}\text{C}$ (ppm)	%incorporation ^{13}C <i>a</i>	%incorporation ^{13}C <i>b</i>
1	105.9	0.3	1.3
3	75.6	0.5	1.3
4	74.5	0.3	1.3
5	88.8	0.4	2.4
6	81.5	*	*
7	81.8	*	*
9	34.1	1.4	4.8
10	25.3	0.8	5.6
11	145.5	0.5	3.0
12	79.0	0.7	2.3
13	36.8	1.6	4.4
21	166.9	0.3	3.0
22	169.6	0.5	0.8
23	165.4	1.2	4.0
24	166.9	0.3	3.0
25	118.0	0.8	3.5
26	157.9	1.6	3.6
27	34.5	0.4	2.8
28	42.2	0.7	2.8
29	31.9	0.9	3.0
30	29.7	0.9	3.6
31	11.1	1.2	4.2
34	170.2	1.4	5.2
35	21.1	2.2	5.0

a Results of [1,2- $^{13}\text{C}_2$]acetate incorporation.

b Results of [1,2- $^{13}\text{C}_2$]acetate diluted with unlabelled acetate incorporation.

* C-6 and C-7 showed a complex AB system.

Table 4.1 *Incorporation of [1,2- $^{13}\text{C}_2$]acetate into squalestatin S1.*

Complex splitting patterns were seen in the ^{13}C -NMR of the S1Me₃ due to incorporation of adjacent labelled acetate units in the same S1 molecule. This is illustrated for C-26 in Figure 4.2 which shows the intra-acetate coupling to C-27 of 33 Hz and a further splitting due to inter-acetate coupling to C-25 (69 Hz). To circumvent

this problem which interferes with accurate calculations of the level of incorporation of the ^{13}C -label, unlabelled acetate was used to dilute $[1,2-^{13}\text{C}_2]\text{acetate}$ as 1:1 mixture before feeding to *Phoma* cultures. The ^{13}C -NMR spectrum of the resulting S1 showed the incorporation levels of 0.8-5.6%, Table 4.1.

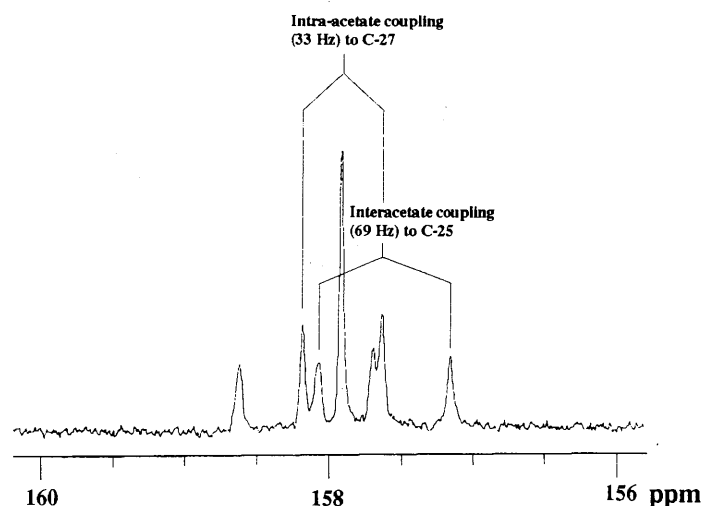
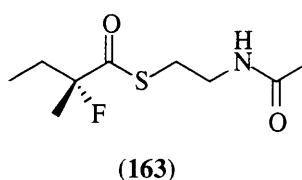
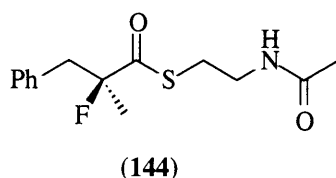


Figure 4.2 Signal for C-26 in the 75.45 MHz ^{13}C -NMR of trimethyl S1 (237) labelled by $[1,2-^{13}\text{C}_2]\text{acetate}$.

4.3 Incorporation Studies with Fluorinated Diketide Intermediates.

The incorporation of α -fluorinated tri- and tetraketides precursors into tetronasin (58) had provided a powerful means of combating oxidative degradation of the precursors and a convenient way of making metabolite analogues (Scheme 1.14).

α -Fluoromethyl diketide analogues (144) and (163) contained the same structural features as the C-12 to C-14 and C-28 to C-31 regions of the squalastatin S1 respectively. Moreover both contained α -fluorine in the place of α -hydrogen that should make them resistant to β -oxidase degradation.

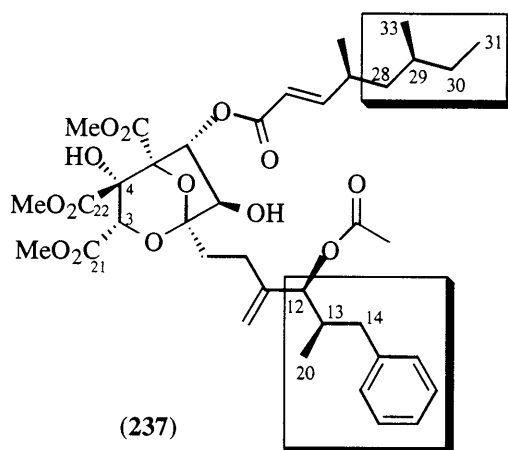


The precursors were pulse fed as ethanolic solution on days 3 and 4 at a level of *ca* 0.7 mmol per litre per day. The cultures were harvested after 7 days of growth, S1

was derivatised to the trimethyl ester and purified by column chromatography. The level of S1 produced by the fed culture was similar to that in a control culture (*ca.* 160 mg/l).

Intact incorporation of (**144**) would result in the presence of a characteristic doublet for the 20-methyl group coupled to the vicinal fluorine substituent in the ^1H NMR spectrum, a signal in the ^{19}F -NMR spectrum and a $M+18$ peak in the mass spectrum. However, ^1H NMR and mass spectra of the S1 produced by the cultures supplemented with (**144**) did not indicate any incorporation. Both FAB^+ and electrospray mass spectrometry analysis were performed for the metabolite isolated from feed cultures and a control culture. Unfortunately the spectra were identical.

The diketide analogue thioester (**163**) was also fed to *Phoma* cultures using the same procedure. Intact incorporation of (**163**) would test the processive mode assembly of the tetraketide chain (**158**). Analysis of the isolated metabolite by ^1H -NMR, ^{19}F -NMR and mass spectrometry again revealed no sign of incorporation.



It was concluded that the microorganism did not accept the diketide analogues as a substituent for the natural diketide. Although fluorine is an ideal substitute for hydrogen because of similar size of the two atoms which will result in minimal change in the steric requirements of the molecule, it is however the most electronegative element. Accordingly, it alters the electron density, basicity and acidity of neighbouring groups. The chemical and enzymatic reactivity of surrounding functionalities are affected and the conformation of the whole molecule may be changed due to dipole interactions. In previous work in our group⁴⁸ 6-fluorohexanoic acid NAC thioester (**56**) had been incorporated into norsolorinic acid (**55**) at a level of 33-42% (Scheme 1.13) while the corresponding 2- and 3-fluorohexanoic acid were not incorporated. This might indicate that when the fluorine substituent is near to the site of enzymatic action, the fluorinated substrate is rejected by the enzyme.

In diketide analogue (**144**) and (**163**) the fluorine substituent is located α - to the carbonyl functionality which would be transesterified by the acyltransferase. It is

therefore a strong possibility that it is not recognised by this enzyme or later enzymes in the biosynthetic pathway. It is however important to state that from the negative result of the experiment it is impossible to conclude that the substrates were taken up by the cells.

In conclusion, feeding studies carried out with *Phoma* provided reasonable level of incorporation of primary precursors such as acetate. It was however difficult to obtain any observable incorporation at the diketide level. Feeding ^{13}C labelled diketide (**43**) with and without β -oxidase inhibitors failed to show intact incorporation into S1. The α -fluorinated diketide analogues (**144**) and (**163**), which could not be degraded *via* β -oxidation, were fed but they were either not accepted as a substitute for the natural diketide precursors or not taken by the microorganism. Therefore studies on the incorporation of diketide precursor (**43**) into S1, described in the next section were undertaken with another zaragozic acid A (S1) producing fungus (strain ATCC 20986).

4.4 Growth Conditions for Strain ATCC 20986.

The organism was stored as frozen mycelia in seed medium containing 10% glycerol in liquid nitrogen. The frozen aliquot (1 ml) was used to inoculate a seed flask containing 50 ml of medium which consisted of tomato paste, corn steep liquor, glucose, oat flour and trace mineral mixture in water at pH 6.8. After 3 days the seed culture was used to inoculate production flasks containing the same seed medium. Both the seed and production cultures were shaken at 220 rpm at 25 °C. It was noticed that using the frozen aliquot to inoculate production flasks resulted in reduction in the level of squalstatin S1 production.

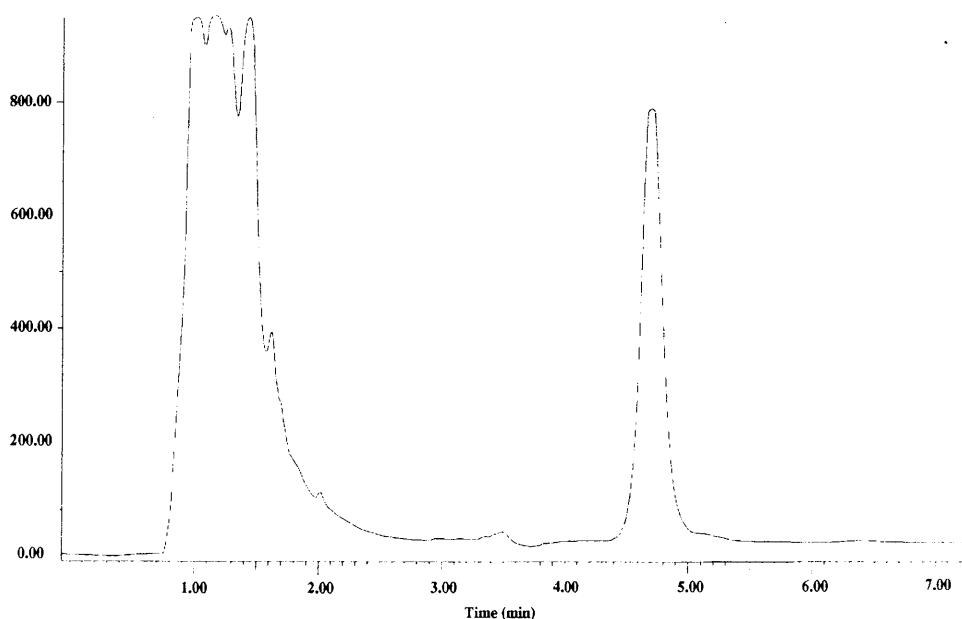


Figure 4.3 An HPLC trace of ATCC 20986 culture broth at day 6.
squalstatin S1 eluted at ~4.7 min.

The production of S1 was determined by HPLC. Aliquots (1.0 ml) were taken from a production flask every 24 hours from day 2 to 8. An equal volume of acidified acetonitrile was added to each sample which was then centrifuged. The supernatant was analysed by reverse phase HPLC under isocratic conditions using 60% acetonitrile in water as the solvent. An HPLC trace of a sample taken from a culture at day 6 is shown in Figure 4.3.

Squalestatin S1 (**9**) eluted after approximately 4.7 minutes. The growth production curve obtained by monitoring the production of S1 for 7 days is shown in Figure 4.4. The production of S1 (**9**) began 3 days after inoculation accompanied by the organism changing colour from brown to dark green. Levels of S1 rose sharply from day 3 to day 5 then started to decline.

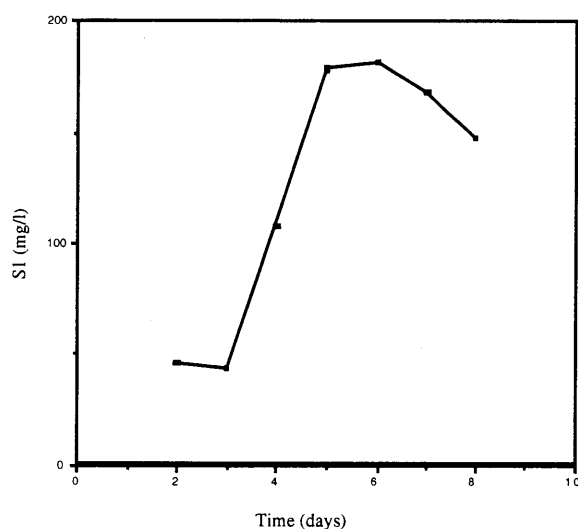


Figure 4.4 Growth production curve of ATCC 20986.

The feeding of [1,2- $^{13}\text{C}_2$]acetate to ATCC 20986 was investigated to optimise conditions for the feeding of advanced intermediates. The production cultures were prepared using the procedure described above and the labelled acetate was administered as an aqueous solution at a level of 1 mg per ml of culture twice a day on days 3, 4 and 5 of growth. The ^{13}C -NMR spectrum of the isolated metabolite showed intact incorporation of $^{13}\text{C}_2$ units at levels of 0.6-1.3%.

Following the good level of incorporation of ^{13}C -labelled acetate, the incorporation of [1,2- $^{13}\text{C}_2$]diketide thioester (**43**) into S1 was investigated. The diketide precursor (**43**) was fed at level of 2 mmol per litre of culture on days 3, 4 and 5 after inoculation. ^{13}C -NMR showed that the diketide thioester was degraded to [1,2- $^{13}\text{C}_2$]acetate prior to incorporation. This was indicated by the presence of satellites

flanking the natural abundance signals throughout the ^{13}C -NMR spectrum. The most enriched positions were C-21, C-3 and C-22 and C-4 pairs which represent the four carbon dicarboxylic acid derived from the Krebs cycle as illustrated in Figure 4.5.

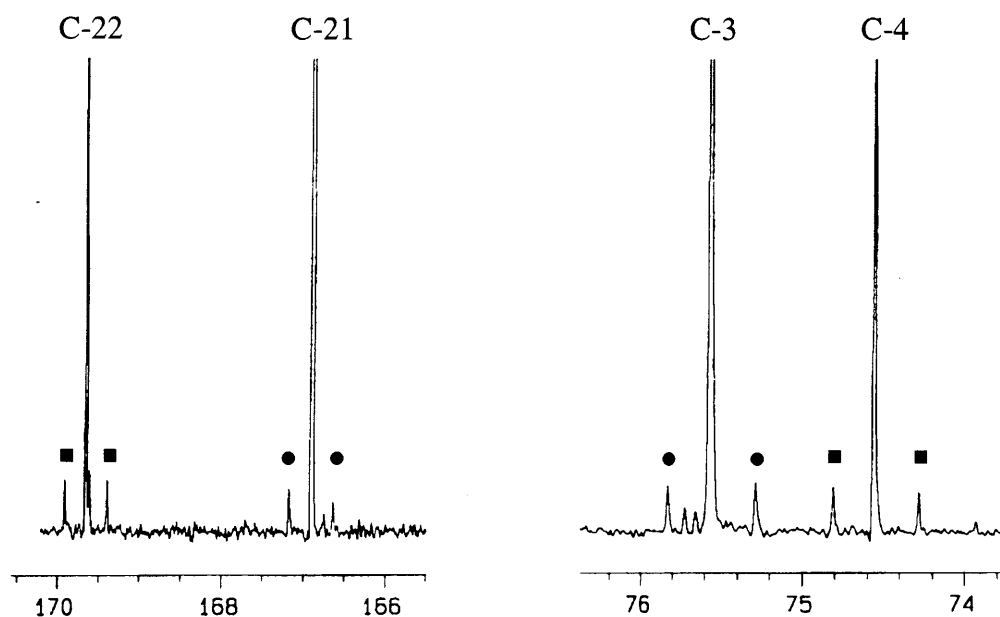
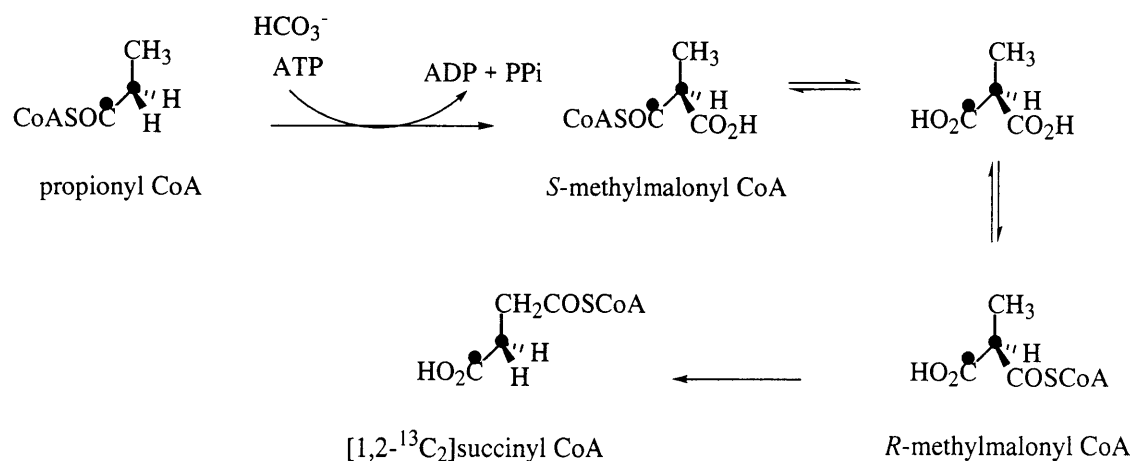


Figure 4.5 Signals for C-21, C-3, C-24 and C-4 in the 125 MHz ^{13}C -NMR spectrum of S1. Satellites show the incorporation of $[1,2-^{13}\text{C}_2]\text{succinate}$.

■ = $J_{21,3} = 68.3$ Hz, ● = $J_{22,4} = 65.7$ Hz.

The fact that the degradation product $[1,2-^{13}\text{C}_2]\text{propionate}$ is efficiently incorporated into these positions attributes to an especially interesting pathway which converts propionyl CoA to succinyl CoA (Scheme 4.3). Propionyl CoA is carboxylated at the expense of an ATP to yield the *S*-isomer of methylmalonyl CoA. This reaction is catalysed by a biotin enzyme, propionyl CoA carboxylase. The *S*-isomer is then converted to the *R*-isomer possibly *via* methylmalonic acid. *R*-methylmalonyl CoA is the substrate for the mutase enzyme that converts it into succinyl CoA.¹⁴⁵



Scheme 4.3 Conversion of propionyl CoA to succinyl CoA.

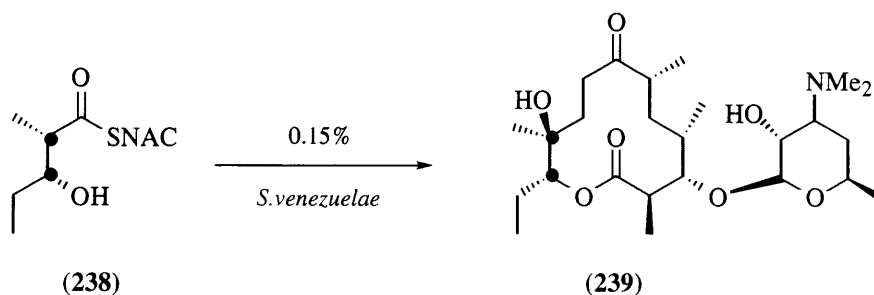
Succinyl CoA is formed from *R*-methylmalonyl CoA by intramolecular rearrangement. The COSCoA group migrate from C-2 to C-3 in exchange for a hydrogen atom. This very unusual isomerisation is catalysed by methylmalonyl CoA mutase which contain a derivative of vitamin B12 (cobalamin) as a co-enzyme. [1,2- $^{13}\text{C}_2$]succinate is then incorporated into the highly oxygenated core of S1. Due to the symmetry in the succinate molecule the label is equally distributed between the C-21, C-3 pair and C-22, C-4 pair. Further metabolism of the labelled succinate through the Krebs cycle would generate [1,2- $^{13}\text{C}_2$]acetate which was incorporated into S1 at varying levels.

In a further attempt to overcome the problem of precursor degradation, the incorporation of the diketide precursor (**43**) into squalastatin S1 by washed cell suspensions of strain ATCC 20986 was investigated as described in the following section.

4.5 Washed Cells of Strain ATCC 20986.

The use of washed cell preparations has been shown to be a successful method for obtaining intact incorporation of putative biosynthetic precursors into a number of polyketide derived natural product, e.g. dehydrocurvarin¹⁴⁶ and methymycin (**239**).⁴¹

Attempts at intact incorporation of *N*-acetylcysteamine thioester of (2*S*,3*R*)-2-methyl-3-hydroxy pentanoate (**238**) into methymycin (**239**) resulted in degradation of the precursor. However, successful incorporation of (**238**) was achieved by feeding the precursor to a resuspended cultures of *Streptomyces venezuelae* in a replacement medium.



Scheme 4.2 Incorporation of triketide intermediate (**238**) into methymycin.

Washed cell preparations of zaragozic acid producer ATCC 20986 had already been successfully used in the feeding studies of ^{13}C labelled primary precursors such as acetate and methionine. The feeding were carried out in a replacement medium rich in sucrose. We decided therefore to use the same medium for the feeding studies of $^{13}\text{C}_2$ -labelled diketide (**43**).

Fermentations of ATCC 20986 were grown under the usual conditions except that after 48 or 72 hours the mycelium was filtered and washed with sterile water and resuspended in a replacement medium containing sucrose (30 g/l) and MES buffer (20 mM). Squalstatin S1 production before and after resuspension was monitored by HPLC (Figure 4.3).

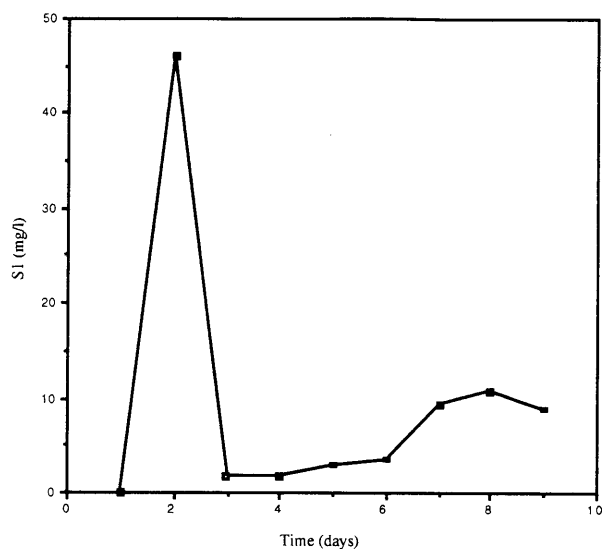


Figure 4.3 Growth production of resting cell cultures of ATCC 20986.

The growth production curve showed that after resuspension of the cells in the replacement sucrose medium at 48 hours the levels of S1 remained low for one day then started to rise. When the cell resuspension was carried out after 72 hours the *de novo* production of S1 was negligible. Although the maximum levels of S1 in cultures resuspended after 48 hours did not reach the S1 level produced by active fermentation, incorporation studies with *N*-acetylcysteamine thioesters of [^{13}C -carboxy]benzoic acid, 3- and 4-fluoro benzoic acid were undertaken.

The precursors were each fed to 10 flasks of cultures twice per day on day 2, 3 and 4 after resuspension. The metabolites were isolated on day 6 in the usual manner. The results of the feed are given in Table 4.2.

Precursor	% incorporation
Ph ^{13}C OSNAC	2.5
3-F(C $_6$ H $_4$)COSNAC	-
4-F(C $_6$ H $_4$)COSNAC	-

Table 4.2 Results of feeding studies with ^{13}C - and F- labelled benzoate starter units.

The ^{13}C -NMR spectrum showed that the ^{13}C label had been incorporated into the benzylic position at a level of 2.5% as indicated by an enhancement of the signal at δ_{C} 39.9 ppm due to C-14. This level of incorporation is lower than that obtained in feeding the same starter unit to resting cell cultures of *Phoma* where a level of 17% was obtained.⁴⁰

Incorporation of the [^{13}C -carboxy]benzoate thioester could be ascribed to the fact that the medium does not contain any source of aromatic amino acids which are the precursors for the benzoate starter unit of S1.⁴⁷ So the microorganism obtained some of the benzoate it requires from the added precursors. The fluorinated benzoate thioesters were not incorporated into the final metabolite. Similarly 4-fluorobenzoic acid thioester was not incorporated into S1 when fed to a resting cell preparation of *Phoma* although it was incorporated when the feeding was performed with active fermentations.⁴⁰

The good level of incorporation of ^{13}C labelled benzoate led us to investigate the incorporation of [1,2- $^{13}\text{C}_2$]labelled thioester (**43**) into S1 by this cell preparation. It was proposed that the high level of sucrose (converted to glucose) in the replacement medium should suppress β -oxidation since the organism does not need to obtain the acetate it requires from fatty acids. Unfortunately administration of the diketide thioester precursor (**43**) at a level of 1.6 mmol per litre of culture as an ethanolic solution lowers the production of S1 considerably. ^{13}C -NMR spectrum indicated that the isolated S1 was coeluted with some of the diketide precursor and an unidentifiable ^{13}C -enriched metabolite.

4.6 Conclusions and Future work.

In our feeding studies we have shown that using *Phoma*, conditions could be established to obtain good levels of incorporation of [1,2- $^{13}\text{C}_2$]acetate into squalastatin S1. There was small increase in these levels when the acetate was pulse fed compared to one dose feed. Multilabel incorporation in the same S1 molecule resulted in complex ^{13}C -NMR signals and the problem was overcome by diluting the label before feeding.

Studies on the incorporation of α -fluorodiketide analogues (**144**) and (**163**) into squalastatin S1 were unsuccessful. It is not known whether the analogues were not recognised by the enzymes in the biosynthetic pathway of S1 as a substitute for the natural diketide or due to problems with their transportation into the cells.

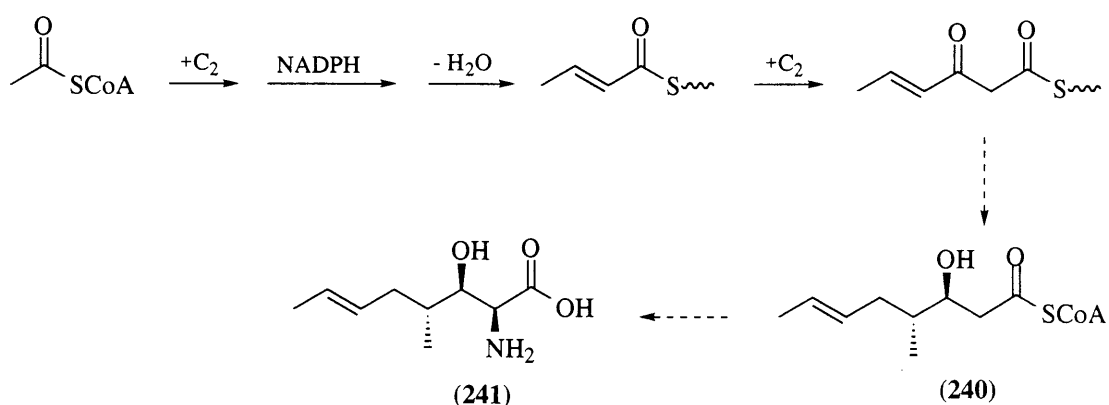
Another squalastatin S1 producer, strain ATCC 20986 had been chosen for further feeding studies. ^{13}C labelled acetate has been successfully incorporated into S1. Studies on the incorporation of [1,2- $^{13}\text{C}_2$]labelled diketide (**43**) did not show intact incorporation. Random incorporation of labelled acetate as a result of the degradation of the diketide was observed.

A resting cell preparation of ATCC 20986 has been also developed. ^{13}C -labelled benzoate precursor was incorporated into S1 using this cell system, nevertheless it has not been possible to obtain intact incorporation of the $^{13}\text{C}_2$ -labelled putative diketide intermediate (43).

Feeding labelled substrates to intact cell fermentations is an attractive approach to the study of biosynthetic pathways because the whole enzyme machinery and all co-factors are preserved. Nonetheless it is not the only means. Further studies on the incorporation of advanced intermediate into S1 should be aimed to develop an active cell free system to investigate the incorporation of the available labelled diketide precursors.

Another possibility is to trace the presence of low levels of intermediates in the fermentations at different stages of growth by HPLC or HPLC-MS. Several methods could be used to monitor the presence of the minor quantities of the intermediates such as UV, fluorescence and radioactivity detection.

Such an approach was adopted in the study of biosynthesis of the unusual amino acid (4*R*, 2*E*)-4-(but-2-enyl)-4-methyl-L-threonine (Bmt) (241) of cyclosporin A¹⁴⁷ produced by fungus *Tolypocladium niveum*. The nine carbon backbone of Bmt namely, (3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-oct-6-enoic acid (240) is biosynthesised as a coenzyme A thioester *via* a polyketide pathway (Scheme 4.3). The study showed that after attachment of the activated building units to Bmt polyketide synthase the intermediates remained enzyme bound throughout the cycle. Premature cutoff of biosynthesis led to the release of the intermediates from the enzyme usually as CoA thioesters. The analysis of the reaction mixture was carried out by HPLC separation and detection of all CoA thioesters by diode array detector, monitoring the absorbance at 260 nm. The carboxylic acids obtained after alkaline treatment of the reaction product were converted to 4-bromomethyl-6,7-dimethoxy coumarin (Br-DMC) derivatives, separated by HPLC and detected by fluorescence (excitation 343 nm, emission 423 nm). The intermediate compounds were identified by a comparison with a synthetic reference sample and/or by mass determination.



Scheme 4. Polyketide pathway for the biosynthesis of Bmt (241).

The first elongation cycle was shown to follow a processive mechanism. The activated intermediates of this processive cycle could be introduced into the biosynthetic pathway at the respective step *via* cell free assay in the presence of *S*-[methyl-¹⁴C]adenosyl methionine and the products of the *in vitro* reaction were analysed by TLC radioactivity detector.

This work demonstrated that enzymatic *in vitro* studies are valuable tool to analyse the individual steps of a polyketide pathway. For our purposes the synthesised hexaketide and diketide intermediates could serve as the internal standards in these type of experiments.

Chapter 5

Experimental.

5.1 General Experimental.

Nuclear Magnetic Resonance (NMR) spectra were recorded as solutions in deuteriochloroform using tetramethylsilane as internal standards. The spectra were recorded on Joel GX270, GX400 or Lamda300 spectrometers. The chemical shift values for all spectra are given in parts per million with the coupling constants in Hertz (Hz).

Infrared (IR) Spectra were recorded on a Perkin-Elmer 881 infrared spectrometer as liquid film or Nujol mull.

Low and high resolution mass spectra were recorded on a Fisons Autospec spectrometer. Spectra were obtained using electronic impact (EI) (at potential of 70 eV), chemical ionisation (CI) and fast atom bombardment (FAB) techniques.

Melting points were measured on a Gallenkamp Melting Point apparatus and are uncorrected.

Optical rotations were measured using the sodium-D line on a Perkin-Elmer 241MC polarimeter as solutions in chloroform or dichloromethane.

Flash column chromatography was carried out according to the procedure of Still *et al.*¹⁴⁸ using Merck silica gel 60 as the solid support. Compounds were loaded neat or adsorbed on Celite and eluted with mixtures of ethyl acetate and 40-60 petroleum ether of varying polarity.

Thin layer chromatography was carried out using Merck Kieselgel 60 HF₂₅₄ aluminium backed TLC plates eluting with ethyl acetate and 40-60 petroleum ether mixtures. Visualisation of the plates was achieved using one of three methods:

- (i) fluorescence on exposure to short wave ultraviolet light.
- (ii) developing in an aqueous potassium permanganate (0.5%) and sodium hydrogen carbonate (2.5%) solution followed by heating with a hot air gun.
- (iii) developing in aqueous ammonium molybdate (5%) and cerium sulfate (0.2%) in sulfuric acid (5%) followed by heating with a hot air gun.

High Performance Liquid Chromatography (HPLC) was carried out using a Gilson apparatus and a 12.5 cm Whatman Partisphere C-18 column. The mobile phase was 60% acetonitrile/40% water acidified with sulfuric acid at 150 ml/l. A flow rate of 1 ml/minute was used and the peaks were detected at 210 nm.

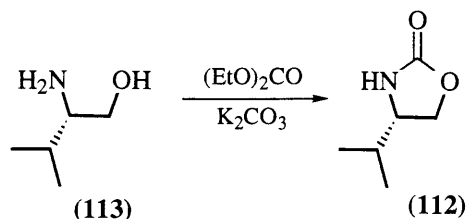
Analytical grade ethyl acetate and petroleum ether supplied by Fisher were used for column chromatography. Spectroscopic grade acetonitrile supplied by Ruthburn chemicals was used for HPLC. For reactions under anhydrous conditions, the solvents used were supplied by Fluka over molecular sieves.

N, N'-dimethyl-*N, N'*-propylene urea (DMPU) was purified by distillation from calcium hydride under reduced pressure and stored in a sealed flask under nitrogen atmosphere over molecular sieves.

Diazomethane was prepared from Diazald following the published procedure by Lombardi¹⁴⁹ and was used immediately after preparation.

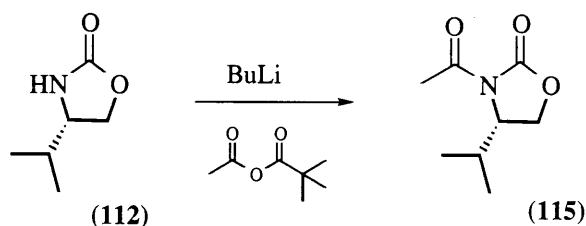
5.2 Experimental.

(*S*)-4-(1-Methylethyl)-2-oxazolidinone (112).



To a round-bottomed flask fitted with a Vigreux column and distillation apparatus was added (*S*)-2-amino-3-methylbutan-1-ol (**113**) (1.68 ml, 15.08 mmol), diethyl carbonate (3.68 ml, 30.4 mmol, 2 eq), toluene (0.4 ml) and potassium carbonate (21 mg, 0.15 mmol). The reaction was stirred in an oil bath at 125-130 °C. After 1 h ethanol and toluene began to distil over. After a further hour, when the reaction was complete, the reaction mixture was cooled to 70 °C and poured into chloroform (15 ml). The solvent was removed *in vacuo* to give a white solid which was recrystallised from ethyl acetate and 60-80 petroleum ether to give the product (**112**) as a fine white crystalline solid (1.63 g, 87%); mp 70-71 °C (lit.¹⁰⁸ 71-72 °C); $[\alpha]_D^{22} +14.2$ (*c* 3.4 in CHCl₃) [lit.¹⁰⁸ +14.8 (*c* 7.0 in CHCl₃)]; δ_H (300 MHz) 5.96 (1H, br s, NH), 4.45 (1H, t, *J* 8.7, CHHO), 4.11 (1H, dd, *J* 8.7, 6.2, CHHO), 3.60 (1H, m, CHN), 1.73 (1H, oct, *J* 6.7, CH(CH₃)₂), 0.97 (3H, d, *J* 6.7, CHCH₃), 0.90 (3H, d, *J* 6.7, CHCH₃); *m/z* (CI) 130 (MH⁺, 70%), 114 (12), 89 (100), 85 (85) and 58 (35).

(*S*)-3-Acetyl-4-(1-methylethyl)-2-oxazolidinone (115).



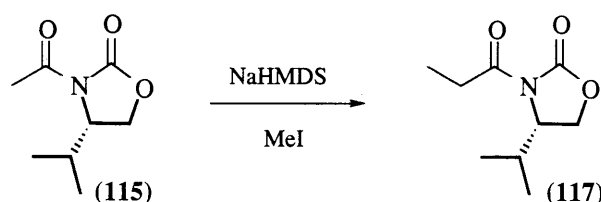
To a solution of sodium acetate (1.0 g, 12.2 mmol) in anhydrous THF (20 ml) under nitrogen atmosphere was added trimethylacetyl chloride (1.65 ml, 13.42 mmol, 1.1 eq) and the mixture stirred at room temperature for 24 h then cooled to -78 °C.

To a solution of 2-oxazolidinone (**112**) (1.73 g, 13.42 mmol, 1.1 eq) in anhydrous THF (40 ml) at -78 °C under nitrogen atmosphere was added *n*-butyllithium (6.97 ml, 2.1 M solution in hexanes, 14.64 mmol, 1.2 eq) dropwise and the resulting

white suspension stirred for 20 minutes. This suspension was then transferred to the cooled mixed anhydride solution and the reaction stirred at $-78\text{ }^{\circ}\text{C}$ for 3 h. Saturated ammonium chloride solution (60 ml) was added and the reaction allowed to warm to room temperature. The mixture was acidified to pH 2 with 2 M HCl and extracted with ethyl acetate (4 x 100 ml). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate, dried over anhydrous sodium sulfate and concentrated *in vacuo* to give the crude product as a yellow oil which was further purified by flash column chromatography.

Elution with 5% ethyl acetate in 40-60 petroleum ether returned the trimethylacetyl oxazolidinone as a yellow oil (563 mg, 21%). Further elution with 10% ethyl acetate in 40-60 petroleum ether returned acetyl oxazolidinone (**115**) as colourless oil (1.64 g, 78.6%); $[\alpha]_{\text{D}}^{22} +94.8$ (c 6.0 in CH_2Cl_2) [lit.,¹⁰⁸ $+98.4$ (c 2.6 in CH_2Cl_2)]; $\nu_{\text{max}}(\text{film})/\text{cm}^{-1}$ 1786 (C=O oxaz), 1705 (C=O); δ_{H} (300 MHz) 4.42 (1H, m, CHN), 4.25 (2H, m, CH_2O), 2.54 (3H, s, 2'-H₃), 2.39 (1H, m, $\text{CH}(\text{CH}_3)_2$), 0.92 (3H, d, J 7.0, CHCH_3), 0.88 (3H, d, J 7.0, CHCH_3); m/z (EI) 171 (M^+ , 20%), 149 (30), 139 (30), 89 (100), 130 (95) and 86 (100).

(S)-4-(1-Methylethyl)-3-(1'-oxopropyl)-2-oxazolidinone (117).



Typical procedure:

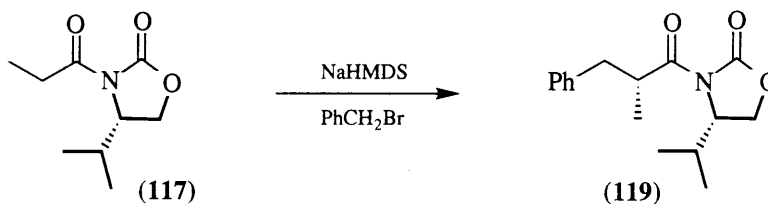
To a solution of sodium bis(trimethylsilyl)amide in THF at $-78\text{ }^{\circ}\text{C}$ under nitrogen atmosphere was added a solution of acetyl oxazolidinone (**115**) in THF and the mixture stirred for 20 minutes at $-78\text{ }^{\circ}\text{C}$. In reaction (1) hexamethylphosphoramide (HMPA) was added dropwise followed immediately by dropwise addition of methyl iodide. Where DMPU was used, it was added at this stage and stirred for 10 minutes before the alkylating agent was added. All reactions were stirred at $-78\text{ }^{\circ}\text{C}$ and quenched by addition of an equal volume of saturated aqueous ammonium sulfate then allowed to warm to room temperature and acidified to pH 2 with 2 M HCl. The reaction mixture was extracted with ethyl acetate, the combined organic extracts dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude product was purified by flash column chromatography.

Elution with 5% ethyl acetate in 40-60 petroleum ether returned propionyl oxazolidinone (**117**) as colourless oil; $[\alpha]_D^{22} +96.5$ (c 5.2 in CH_2Cl_2) [lit.,¹⁰⁸ $+96.8$ (c 8.7 in CH_2Cl_2)]; $\nu_{\text{max}}(\text{film})/\text{cm}^{-1}$ 1782 (C=O oxaz), 1705 (C=O); δ_{H} (300 MHz) 4.43 (1H, m, CHN), 4.28 (1H, t, J 9.0, CHHO), 4.21 (1H, dd, J 9.0, 3.3, CHHO), 2.95 (2H, m, 2'-H₂), 2.38 (1H, spd, J 7.0, 4.0, CH(CH₃)₂), 1.17 (3H, t, J 7.3, 3'-H₃), 0.92 (3H, d, J 7.0, CHCH₃), 0.88 (3H, d, J 7.0, CHCH₃); m/z (CI) 186 (MH⁺, 41%), 158 (18), 130(100) and 83(65).

Reaction Number	Amount of Base	Amount of Reagent	Amount of MeI	Reaction Time	Yield
1	1 eq.	2 eq. HMPA	10 eq.	4 hours	66.0%
2	1 eq.	2 eq. DMPU	10 eq.	4 hours	25.0%
3	1.3 eq.	1:3 DMPU in THF	10 eq.	3 hours	80.5%

Table: Reaction conditions for the methylation of acetyl oxazolidinone.

(4S,2'R)-3-[2'-Benzyl-1'-oxopropyl]-4-(1-methylethyl)-2-oxazolidinone (119).

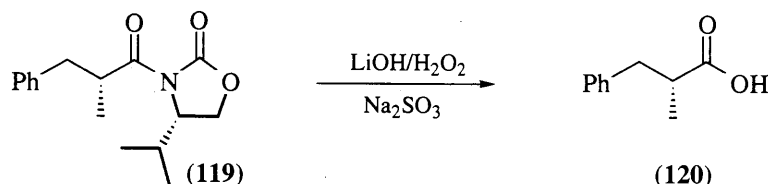


To a solution of sodium bis(trimethylsilyl)amide (1.40 ml, 1 M solution in THF, 1.4 mmol, 1.3 eq) in THF (8 ml) at -78°C under nitrogen atmosphere was added dropwise a solution of propionyl oxazolidinone (**117**) (200 mg, 1.08 mmol) in THF (4 ml) and the resulting yellow solution stirred for 20 minutes. Benzyl bromide (0.26 ml, 2.16 mmol, 2 eq) was then added and the reaction stirred for 3 h. Saturated aqueous ammonium chloride (12 ml) was added and after warming to room temperature, the mixture was acidified to pH 2 with 2 M HCl and extracted with ethyl acetate (3 x 20 ml). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo* to give the crude product which was purified by dry flash chromatography.

Elution with 40-60 petroleum ether removed excess benzyl bromide. Further elution with 5% ethyl acetate in 40-60 petroleum ether gave the desired product (**119**) as a colourless oil (238 mg, 80%); $[\alpha]_D^{20} +9.13$ (c 1.20 in CH_2Cl_2) [lit.,¹⁰⁷ $+9.42$ (c 2.06 in CH_2Cl_2)]; $\nu_{\text{max}}(\text{film})/\text{cm}^{-1}$ 1777 (C=O oxaz), 1700 (C=O), 1496 (C=C); δ_{H} (300 MHz) 7.19 (5H, m, Ph), 4.42 (1H, td, J 8.5, 3.5, CHN), 4.23 (1H, t, J 8.5, CHHO),

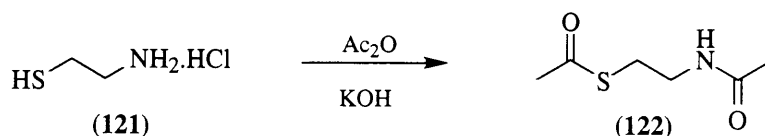
4.14 (1H, dd, J 8.5, 3.1, CHHO), 4.17 (1H, m, 2'-H), 3.12 (1H, dd, J 13.1, 7.5, PhCHH), 2.62 (1H, dd, J 13.1, 7.5, PhCHH), 2.14 (1H, spd, J 7.0, 3.5, CH(CH₃)₂), 1.15 (3H, d, J 6.8, 3'-H₃), 0.83 (3H, d, J 7.0, CHCH₃), 0.59 (3H, d, J 7.0, CHCH₃); m/z (CI) 276 (MH⁺, 81%), 158 (21), 147 (46), 130 (100), 119 (40), 91 (15) and 83 (18).

(*R*)-2-Benzylpropionic acid (120).



To a solution of the oxazolidinone (**119**) (300 mg, 1.1 mmol) in 4:1 THF/H₂O mixture (10 ml) cooled to 0 °C, was added hydrogen peroxide (0.45 ml, 30% w/w solution in water, 3.96 mmol, 3.6 eq) and a solution of lithium hydroxide monohydrate (64.7 mg, 1.54 mmol, 1.4 eq) in water (4 ml) and the reaction stirred for 1 h at 0 °C. A solution of sodium sulfite (0.5 g, 3.96 mmol, 3.6 eq) in water (6 ml) was added and the solvent was removed *in vacuo*. The remaining aqueous solution was extracted with dichloromethane (4 x 15 ml), acidified to pH 2 with 2 M HCl and then extracted with ethyl acetate (6 x 20 ml). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo* to give the acid (**120**) as colourless oil (160 mg, 89%); $[\alpha]_D^{23}$ -29.8 (c 3.62 in CHCl₃) [lit.,⁴⁰ -27.1 (c 1.65 in CHCl₃)]; $\nu_{\max}(\text{film})/\text{cm}^{-1}$ 3285 (OH), 1748 (C=O), 1515 (C=C); δ_{H} (300 MHz) 7.24 (5H, m, Ph), 3.08 (1H, dd, J 13.0, 7.0, PhCHH), 2.75 (1H, sextet, J 7.0, 2-H), 2.66 (1H, dd, J 13.0, 7.0, PhCHH), 1.18 (3H, d, J 7.0, 3-H₃); m/z (CI) 165 (MH⁺, 40%), 147 (43), 119 (100), 91 (27) and 83 (50).

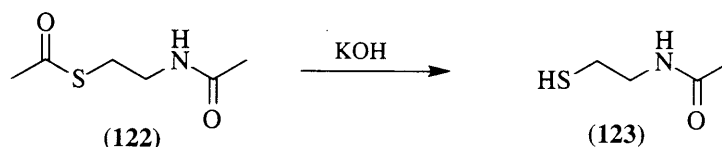
Ethanethioic acid *S*-[2-(acetylamino)ethyl] ester - (*N,S*-diacetyl-cysteamine) (122**).¹¹¹**



A solution of 2-aminoethanethiol hydrochloride (**121**) (5.75 g, 50.6 mmol) in water (40 ml), cooled to 0 °C, was adjusted to pH 8 with 8 M potassium hydroxide solution. Acetic anhydride (14.5 ml, 153.6 mmol, 3 eq) was added dropwise with further 8 M potassium hydroxide solution so that the solution remained at pH 8. Dilute

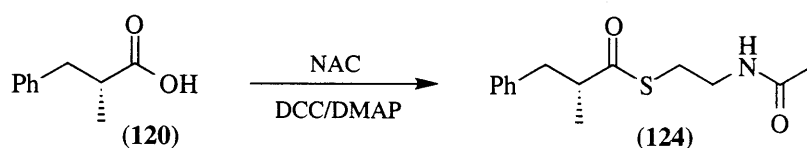
HCl was added to neutralise the solution and the reaction stirred at 0 °C for 1 h. The reaction mixture was saturated with sodium chloride and extracted with dichloromethane (6 x 50 ml). The combined organic fractions were dried over sodium sulfate and concentrated *in vacuo* to give *N, S*-diacetylcysteamine (**122**) as a colourless oil (8.19 g, 99.8%); δ_{H} (270 MHz) 6.53 (1H, br s, NH), 3.42 (2H, q, J 6.5, CH_2N), 3.03 (2H, t, J 6.5, CH_2S), 2.36 (3H, s, CH_3COS), 1.99 (3H, s, CH_3CON).

***N*-(2-Mercaptoethyl)acetamide - (*N*-acetylcysteamine) (**123**).¹¹¹**



To a solution of *N, S*-diacetylcysteamine (**122**) (1.0 g, 6.2 mmol) in water (32 ml) was added potassium hydroxide (0.97 g, 17.4 mmol, 2.8 eq) and the reaction stirred at room temperature for 45 minutes. The reaction mixture was then acidified to pH 6 with 2 M hydrochloric acid, saturated with sodium chloride and extracted with dichloromethane (6 x 50 ml). The combined organic extracts were dried over magnesium sulfate and concentrated *in vacuo* to give *N*-acetylcysteamine (**123**) as a colourless oil (0.70 g, 95%); ν_{max} (film)/ cm^{-1} 1640 (C=O); δ_{H} (270 MHz) 6.46 (1H, br s, NH), 3.43 (2H, q, J 6.5, CH_2N), 2.68 (2H, dt, J 8.5, 6.5, SCH_2), 2.02 (3H, s, CH_3CO), 1.42 (1H, t, J 8.5, SH).

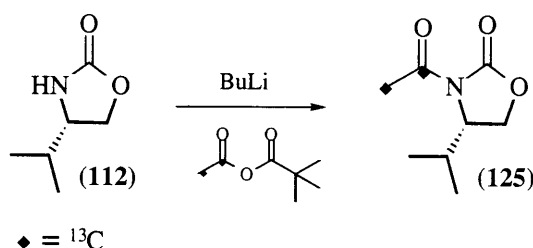
(*R*)-2-Benzylpropionic acid *N*-acetylcysteamine thioester (124**).⁴⁰**



To a solution of (*R*)-2-benzylpropionic acid (**120**) (200 mg, 1.2 mmol) in dichloromethane (5 ml) was added a solution of *N*-acetylcysteamine (160 mg, 1.3 mmol, 1.1 eq) in dichloromethane (3 ml) and the stirred mixture was cooled to 0 °C. To this solution was added dropwise a solution of 1,3-dicyclohexylcarbodiimide (352 mg, 1.7 mmol, 1.4 eq) and 4-dimethylaminopyridine (8 mg, 0.06 mmol, 0.05 eq) in dichloromethane (2 ml) and the reaction stirred at room temperature for 18 h. Half of the solvent was removed *in vacuo* and the white precipitate of DCU was filtered. The solid was washed with diethyl ether (2 x 20 ml), the filtrate and washings were combined and concentrated *in vacuo* to give the crude product as a yellow oil which was purified by flash column chromatography.

Elution with 50% ethyl acetate in 40-60 petroleum ether gave the thioester (**124**) as a colourless oil (263 mg, 83%); $[\alpha]_D^{22}$ -78.0 (c 1.6 in CHCl_3) [lit.,⁴⁰ - 73.2 (c 0.36 in CHCl_3)]; $\nu_{\text{max}}(\text{film})/\text{cm}^{-1}$ 3449, 3250 (N-H), 1651 (C=O thioester), 1625 (C=O amide), 1523 (C=O amide), 1497 (C=C); δ_{H} (300 MHz) 7.24 (5H, m, Ph), 5.62 (1H, br s, NH), 3.46-2.67 (7H, m, CH_2N , SCH_2 , PhCH_2 , 2-H), 1.91 (3H, s, CH_3CONH), 1.20 (3H, d, J 6.6, 3- H_3); m/z (CI) 266 (MH^+ , 80), 224 (10), 206 (10), 147 (30), 119 (100) and 91 (45).

(S)-(1',2'- $^{13}\text{C}_2$)-3-Acetyl-4-(1-methylethyl)-2-oxazolidinone (125).

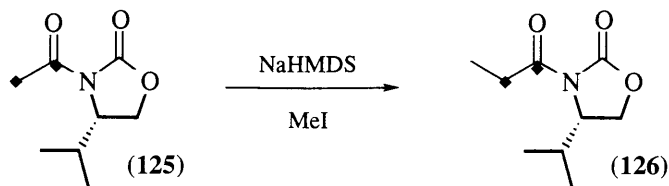


To a solution of [1,2- $^{13}\text{C}_2$]sodium acetate (2.5 g, 29.8 mmol) in anhydrous THF (40 ml) under nitrogen atmosphere was added trimethylacetyl chloride (4.0 ml, 32.7 mmol, 1.1 eq) and the mixture stirred at room temperature for 24 h then cooled to -78 °C.

To a solution of 2-oxazolidinone (**112**) (4.22 g, 32.7 mmol, 1.1 eq) in anhydrous THF (100 ml) at -78 °C under nitrogen atmosphere was added *n*-butyllithium (20.8 ml, 2.0 M solution in hexanes, 41.7 mmol, 1.4 eq) dropwise and the resulting white suspension stirred for 20 minutes. This suspension was then added to the cooled mixed anhydride solution and the reaction stirred at -78 °C for 3 h. Saturated ammonium chloride solution (100 ml) was added and the reaction allowed to warm to room temperature. The mixture was acidified to pH 2 with 2 M HCl and extracted with ethyl acetate (4 x 100 ml). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate, dried over anhydrous sodium sulfate and concentrated *in vacuo* to give the crude product as a yellow oil which was further purified by flash column chromatography.

Elution with 5% ethyl acetate in 40-60 petroleum ether returned the trimethylacetyl oxazolidinone as a yellow oil (0.6 g, 9%). Further elution with 10% ethyl acetate in 40-60 petroleum ether returned acetyl oxazolidinone (**125**) as a colourless oil (3.6 g, 70%); δ_{H} (300 MHz) 4.37 (1H, ddd, J 9.0, 4.0, 3.3, CHN), 4.19 (1H, t, J 9.0, CHHO), 4.06 (1H, dd, J 9.0, 3.3, CHHO), 2.48 (3H, dd, J 130.6, 6.8, 2'- H_3), 2.32 (1H, spd, J 7.4, 4.0, $\text{CH}(\text{CH}_3)_2$), 0.85 (3H, d, J 7.0, CHCH_3), 0.81 (3H, d, J 7.0, CHCH_3); m/z (CI) 174 (MH^+ , 78%), 158 (28), 144 (8), 130 (75) and 83 (100).

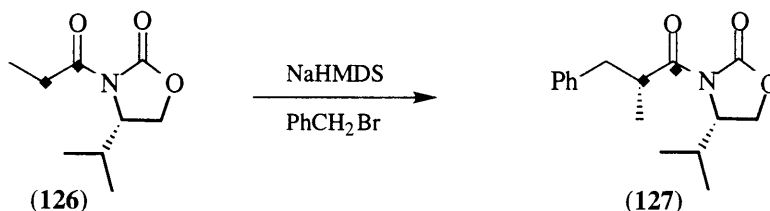
(S)-(1',2'-¹³C₂)-4-(1-Methylethyl)-3-(1'-oxopropyl)-2-oxazolidinone (126).



To a solution of sodium bis(trimethylsilyl)amide (20.0 ml, 1 M in THF, 20.0 mmol, 1.3 eq) in THF (90 ml) at -78 °C under nitrogen atmosphere was added a solution of acetyl oxazolidinone (**125**) (2.66 g, 15.4 mmol) in THF (10 ml) and the mixture stirred for 20 minutes at -78 °C. DMPU (30 ml) was added and stirring continued for 10 minutes then methyl iodide (9.34 ml, 154 mmol, 10 eq) was added and the mixture stirred at -78 °C for 4 h. The reaction was quenched by addition of an equal volume of saturated aqueous ammonium sulfate then allowed to warm to room temperature and acidified to pH 2 with 2 M HCl. The reaction mixture was extracted with ethyl acetate, the combined organic extracts dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude product was purified by flash column chromatography.

Elution with 5% ethyl acetate in 40-60 petroleum ether returned propionyl oxazolidinone (**126**) as colourless oil (2.1 g, 73%); δ_{H} (300 MHz) 4.44 (1H, ddd, J 9.0, 4.0, 3.3, CHN), 4.28 (1H, t, J 9.0, CHHO), 4.22 (1H, dd, J 9.0, 3.3, CHHO), 2.89 (1H, ddqn, J 129.9, 14.7, 7.3, 2'-H), 2.91 (1H, ddqn, J 129.9, 14.7, 7.3, 2'-H), 2.39 (1H, spd, J 7.0, 4.0, CH(CH₃)₂), 1.15 (3H, tdd, J 7.3, 5.5, 4.6, 3'-H₃), 0.92 (3H, d, J 7.0, CHCH₃), 0.88 (3H, d, J 7.0, CHCH₃); m/z (EI) 187 (M⁺, 65%), 173 (10), 157 (7), 144 (28), 130 (15) and 83 (100); ¹³C Incorporation, 78.8% with 2 x ¹³C, 5.4% with 1 x ¹³C.

(4S, 2'R)-(1',2'-¹³C₂)-3-(2'-Benzyl-1'-oxopropyl)-4-(1-methylethyl)-2-oxazolidinone (127).

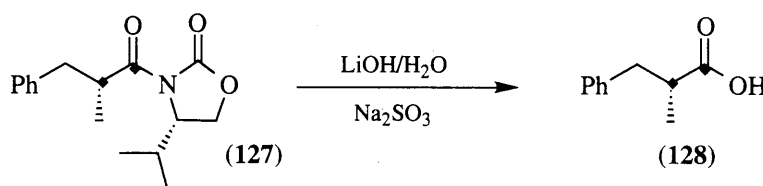


To a solution of sodium bis(trimethylsilyl)amide (10.3 ml, 1 M solution in THF, 10.3 mmol, 1.2 eq) in THF (15 ml) at -78 °C under nitrogen atmosphere was added dropwise a solution of propionyl oxazolidinone (**126**) (1.6 g, 8.6 mmol) in THF (5 ml)

and the resulting yellow solution stirred for 20 minutes. Benzyl bromide (2.0 ml, 17.2 mmol, 2 eq) was then added and the reaction stirred for 3 h. Saturated aqueous ammonium chloride (20 ml) was added and after warming to room temperature, the mixture was acidified to pH 2 with 2 M HCl and extracted with ethyl acetate (3 x 20 ml). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo* to give the crude product which was purified by dry flash chromatography.

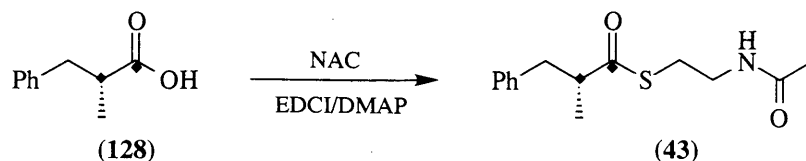
Elution with 40-60 petroleum ether removed excess benzyl bromide. Further elution with 5% ethyl acetate in 40-60 petroleum ether gave the product (**127**) as a colourless oil (1.9 g, 80%); δ_{H} (300 MHz) 7.20 (5H, m, Ph), 4.45 (1H, m, CHN), 4.24 (1H, t, J 8.7, CHHO), 4.14 (1H, dd, J 8.7, 3.3, CHHO), 4.12 (1H, dm, J 135.7, 2'-H), 3.12 (1H, m, PhCHH), 2.63 (1H, m, PhCHH), 2.16 (1H, spd, J 7.0, 3.1, CH(CH₃)₂), 1.15 (3H, m, 3'-H₃), 0.83 (3H, d, J 7.0, CHCH₃), 0.60 (3H, d, J 7.0, CHCH₃); m/z (EI) 277 (M⁺, 37%), 166 (10), 149 (9), 148 (22), 147 (4), 133 (5), 131 (5), 130 (54), 120 (28), 119 (100), 118 (20) and 91 (76).

(*R*)-(1,2-¹³C₂)-2-Benzylpropionic acid (128**).**



To a solution of the oxazolidinone (**127**) (1.18 g, 4.3 mmol) in 4:1 THF/H₂O mixture (25 ml) cooled to 0 °C, was added hydrogen peroxide (1.74 ml, 30% w/w solution in water, 15.4 mmol, 3.6 eq) and a solution of lithium hydroxide monohydrate (215 mg, 5.13 mmol, 1.4 eq) in water (3 ml) and the reaction stirred for 1 h at 0 °C. A solution of sodium sulfite (1.94 g, 15.4 mmol, 3.6 eq) in water (8 ml) was added and the solvent was removed *in vacuo*. The remaining aqueous solution was extracted with dichloromethane (4 x 15 ml), acidified to pH 2 with 2 M HCl and then extracted with ethyl acetate (6 x 20 ml). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo* to give the acid (**128**) as colourless oil (625.2 mg, 88%); δ_{H} (300 MHz) 7.26 (5H, m, Ph), 3.08 (1H, m, PhCHH), 2.69 (1H, m, PhCHH), 2.72 (1H, dsp, J 131.7, 7.0, 2-H), 1.18 (3H, dt, J 7.0, 5.0, 3-H₃); m/z (EI) 166 (M⁺, 70%), 151 (15), 133 (15), 119 (30), 92 (30), 91 (100), 83 (32) and 78(7); ¹³C Incorporation, 94.9% with 2 x ¹³C, 2.14% with 1 x ¹³C.

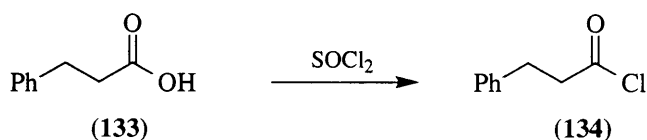
(*R*)-(1,2-¹³C₂)-2-Benzylpropionic acid *N*-acetylcysteamine thioester (43).⁴⁰



To a solution of acid (**128**) (625 mg, 3.8 mmol) in dichloromethane (20 ml) was added a solution of *N*-acetylcysteamine (492.7 mg, 4.1 mmol, 1.1 eq) in dichloromethane (3 ml) and the stirred mixture was cooled to 0 °C. To this solution was then added 4-dimethylaminopyridine (22 mg, 0.19 mmol, 0.05 eq) and (EDCI) (1.0 g, 5.28 mmol, 1.4 eq) and the reaction left stirring at room temperature for 18 h. The reaction mixture was then washed with water (3 x 25 ml), the organic layer dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude product was purified by flash column chromatography.

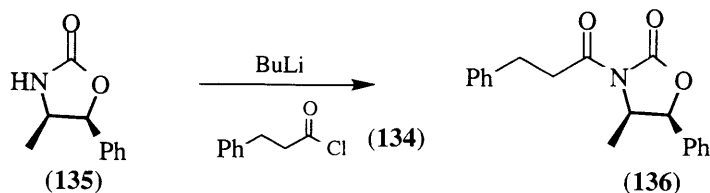
Elution with 50% ethyl acetate in 40-60 petroleum ether returned the *N*-acetyl cysteamine thioester (**43**) as a colourless oil (970 mg, 96%); $[\alpha]_D^{22}$ -75.6 (*c* 2.1 in CHCl₃); δ_H (300 MHz) 7.25 (5H, m, Ph), 5.68 (1H, br s, NH), 3.36 (2H, m, CH₂N), 3.08-2.65 (4H, m, SCH₂, PhCH₂), 2.95 (1H, dm, *J* 140, 2-H), 1.91 (3H, s, CH₃CONH), 1.20 (3H, m, 3-H₃); *m/z* (EI) 267 (M⁺, 70), 149 (15), 120 (90), 92 (25), 91 (100) and 86 (5), ¹³C Incorporation, 98.3% with 2 x ¹³C, 1.7% with 1 x ¹³C.

3-Phenylpropionyl chloride (134).



To a solution of 3-phenylpropionic acid (**133**) (1.0 g, 6.7 mmol) in toluene (15 ml) was added one drop of pyridine and thionyl chloride (1 ml, 13.2 mmol, 2 eq). The reaction was heated to reflux for 2 h. The toluene was then removed *in vacuo* to give the desired product (**134**) as a yellowish oil (1.1 g, 97%); $\nu_{\text{max}}(\text{film})/\text{cm}^{-1}$ 1803 (C=O), 958 (C-Cl); δ_H (300 MHz) 7.25 (5H, m, Ph), 3.20 (2H, t, *J* 7.8, 3-H₂), 3.00 (2H, t, *J* 7.8, 2-H₂); *m/z* (CI) 168 (M⁺, 15%), 150 (73), 133 (56), 105 (100), 91 (37).

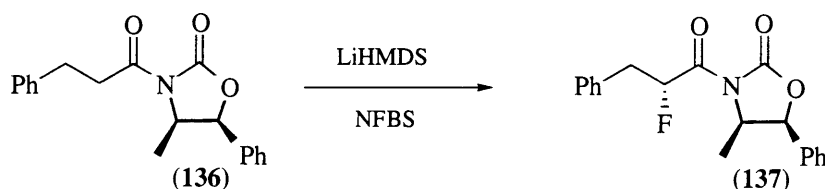
(4*R*,5*S*)-4-Methyl-3-(1'-oxo-3'phenylpropy)-5-phenyl-2-oxazolidinone (136).



To a solution of oxazolidinone (**135**) (4.0 g, 22.6 mmol) in THF (100 ml) at -78 °C under nitrogen atmosphere was added *n*-butyllithium (9.94 ml, 2.5 M solution in hexanes, 24.8 mmol, 1.1 eq) dropwise and the mixture stirred for 0.5 h. 3-Phenylpropionyl chloride (**134**) (3.36 ml, 22.6 mmol) was added dropwise and the resulting yellow solution stirred at -78 °C for 2 h. Water (100 ml) was added and the mixture was acidified to pH 2 with 2 M HCl. The mixture was then extracted with ethyl acetate (5 x 100 ml) and the combined ethyl acetate extracts were washed with saturated aqueous sodium hydrogen carbonate (100 ml), saturated aqueous sodium chloride (100 ml) and dried over anhydrous magnesium sulfate. After concentrating *in vacuo*, the crystalline crude product was purified by flash chromatography.

Elution with 5% ethyl acetate in 40-60 petroleum ether returned the acylated oxazolidinone (**136**) as a white crystalline solid which was recrystallised from ethyl acetate and petroleum ether (6.0 g, 86%); mp 97.5-98.9 °C; (Found: C, 74.0; H, 6.4; N, 4.2. C₁₉H₁₉NO₃ requires C, 73.8; H, 6.2; N, 4.5%); [α]_D²² +28.8 (*c* 3.2 in CH₂Cl₂) [lit.,¹⁵⁰ +28.7 (*c* 0.45 in CH₂Cl₂)]; ν_{max} (mull)/cm⁻¹ 1774 (C=O oxaz), 1698 (C=O); δ_{H} (300 MHz) 7.41 (5H, m, Ph), 7.27 (5H, m, Ph), 5.63 (1H, d, *J* 7.0, CHO), 4.75 (1H, quintet, *J* 7.0, CHN), 3.29 (2H, m, 3'-H₂), 3.01 (2H, t, *J* 7.3, 2'-H₂), 0.88 (3H, d, *J* 7.0, CH₃); *m/z* (CI) 310 (MH⁺, 100%), 266 (57), 178 (30), 134 (24), 105 (7), 91 (5); [Found: (CI) MH⁺, 310.1443. C₁₉H₁₉NO₃ requires *MH*, 310.1443].

(4*R*,5*S*,2'*R*)-3-(2'-Fluoro-1'-oxo-3'phenyl-propy)-4-methyl-5-phenyl-2-oxazolidinone (137).

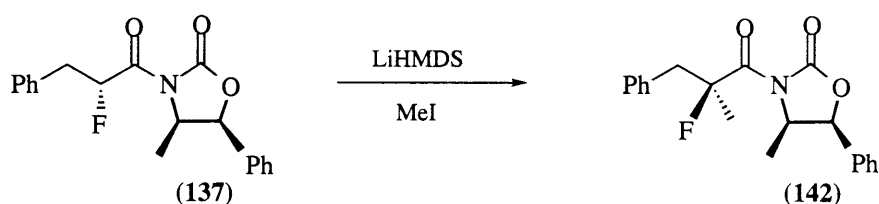


To a solution of the oxazolidinone (**136**) (2.0 g, 6.5 mmol) in THF (50 ml) at -78 °C under nitrogen atmosphere was added dropwise lithium bis(trimethylsilyl)amide (8.5 ml, 1 M solution in THF, 8.5 mmol, 1.3 eq) and the resulting yellow solution stirred

at -78 °C for 0.5 h. A solution of *N*-fluorobenzenesulfonimide (2.46 g, 7.8 mmol, 1.2 eq) in THF (12 ml) was added dropwise and the mixture stirred for 1 h. The reaction was quenched by addition of saturated aqueous ammonium chloride (100 ml) then allowed to warm to room temperature and acidified to pH 2 by addition of 2 M HCl. The mixture was then extracted with ethyl acetate (4 x 100 ml) and the combined organic extracts were washed with saturated aqueous sodium chloride (2 x 50 ml), dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The byproduct (PhSO₂)₂NH was insoluble in ethyl acetate and was separated from the product by filtration and the precipitate was washed with generous amount of ethyl acetate. The combined filtrate and washings were concentrated *in vacuo* to give the crude product which was purified by flash chromatography.

Elution with 5% ethyl acetate in 40-60 petroleum ether yielded α -fluoroacyl oxazolidinone (**137**) as white crystalline solid which was recrystallised from ethyl acetate and petroleum ether (1.97 g, 91%); mp 114.8-115.9 °C; $[\alpha]_D^{23} +11.2$ (*c* 1.47 in CH₂Cl₂) [lit.,⁶⁶ no value given]; (Found: C, 69.8; H, 5.9; N, 4.0. C₁₉H₁₈NO₃F requires C, 69.7; H, 5.5; N, 4.3%); $\nu_{\max}(\text{mull})/\text{cm}^{-1}$ 1793 (C=O oxaz), 1705 (C=O); δ_{H} (300 MHz) 7.35 (10H, m, Ph), 6.17 (1H, ddd, *J* 49.4, 8.7, 3.5, 2'-H), 5.62 (1H, d, *J* 7.0, CHO), 4.69 (1H, quintet, *J* 7.0, CHN), 3.27 (1H, ddd, *J* 32.9, 14.3, 3.5, 3'-HH), 3.14 (1H, ddd, *J* 19.4, 14.3, 8.7, 3'-HH), 0.96 (3H, d, *J* 7.0, CH₃); *m/z* (CI) 328 (MH⁺, 80%), 284 (100), 157 (80), 134 (34) and 91 (15); [Found: (CI) MH⁺, 328.1349. C₁₉H₁₈FNO₃ requires *MH* 328.1349].

(4*R*,5*S*,2'*S*)-3-[2'-Benzyl-2'-fluoro-1'-oxopropyl]-4-methyl-5-phenyl-2-oxazolidinone (142).

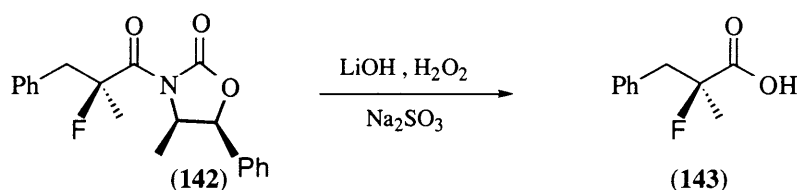


To a solution of the oxazolidinone (**137**) (1.0 g, 3.06 mmol) in THF (30 ml) at -78 °C under nitrogen atmosphere was added lithium bis(trimethylsilyl)amide (9.17 ml, 1 M solution in THF, 9.17 mmol, 3 eq) dropwise and the resulting yellow mixture stirred for 0.5 h. DMPU (10 ml) was then added dropwise and the temperature was allowed to rise to -60 °C over 10 minutes. Methyl iodide (3.8 ml, 61.1 mmol, 20 eq) was added and the reaction stirred for 2 h at -25 °C before quenching with saturated aqueous ammonium chloride (50 ml). The mixture was allowed to warm up to room temperature then acidified to pH 2 with 2 M HCl and extracted with ethyl acetate (4 x 100 ml). The

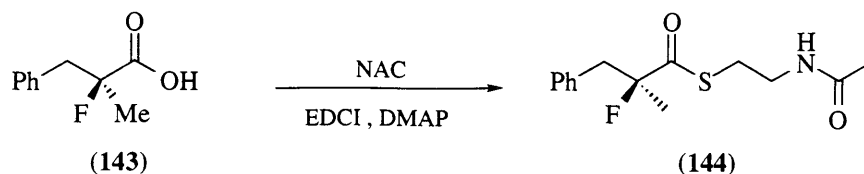
organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo*. DMPU was removed from the product by dry flash chromatography. Elution with 5% ethyl acetate in 40-60 petroleum ether gave the product which was further purified by flash column chromatography.

Elution with 5% ethyl acetate in 40-60 petroleum ether yielded α -fluoro- α -methyl acyloxazolidinone (**142**) as white crystalline solid which was recrystallised from ethyl acetate and petroleum ether (559 mg, 53%); mp 118.7-119.6 °C; $[\alpha]_D^{22}$ -43.5 (*c* 1.72 in CH₂Cl₂); (Found: C, 70.5; H, 6.4; N, 3.7. C₂₀H₂₀FNO₃ requires C, 70.4; H, 5.9; N, 4.1%); δ_H (300 MHz) 7.40 (10H, m, Ph), 5.63 (1H, d, *J* 6.8, CHO), 4.63 (1H, quintet, *J* 6.8, CHN), 3.70 (1H, dd, *J* 31.0, 14.6, PhCHH), 3.30 (1H, dd, *J* 24.2, 14.6, PhCHH), 1.79 (3H, d, *J* 22.0, 3'-H₃), 0.65 (3H, d, *J* 6.8, CH₃CHN); *m/z* (CI) 342 (MH⁺, 100%), 322 (82), 298 (86), 145 (54), 134 (30), 91 (20); [Found: (CI) MH⁺, 342.1511. C₂₀H₂₀FNO₃ requires *MH*, 342.1505].

(S)-2-Benzyl-2-fluoropropionic acid (143).

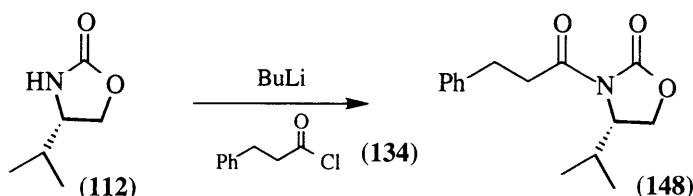


To a solution of the oxazolidinone (**142**) (212 mg, 0.62 mmol) in 4:1 THF/H₂O (10 ml) at 0 °C was added hydrogen peroxide (0.25 ml, 30% w/w solution in water, 2.24 mmol, 3.6 eq) and a solution of lithium hydroxide monohydrate (36.5 mg, 0.87 mmol, 1.4 eq) in water (3 ml) and the reaction stirred for 1 h at 0 °C. A solution of sodium sulfite (282 mg, 2.24 mmol, 3.6 eq) in water (5 ml) was added and the organic solvent was evaporated *in vacuo*. The remaining aqueous solution was extracted with dichloromethane (3 x 20 ml), acidified to pH 1 with 2 M HCl and extracted with ethyl acetate (6 x 20 ml). The ethyl acetate extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo* to give the α -fluoropropionic acid (**143**) as white crystalline solid (92.7 mg, 82%); mp 61.0-63.0 °C; $[\alpha]_D^{23}$ -30.9 (*c* 1.6 in CH₂Cl₂); (Found: C, 65.9; H, 6.3. C₁₀H₁₁FO₂ requires C, 65.9; H, 6.1%); δ_H (300 MHz) 7.29 (5H, m, Ph), 3.26 (1H, dd, *J* 26.6, 14.5, PhCHH), 3.11 (1H, dd, *J* 21.4, 14.5, PhCHH), 1.61 (3H, d, *J* 21.1, 3-H₃); δ_C (75 MHz) 176.0 (1-C), 134.0, 130.3, 128.4, 127.4 (Ph), 95.0 (2-C, d, *J* 177.5), 43.7 (3-C, d, *J* 21.7), 23.5 (2-CH₃, d, *J* 24.2); *m/z* (CI) 183 (MH⁺, 27%), 163 (72), 145 (63), 137 (100), 119 (14), 91 (22); [Found: (CI) MH⁺, 183.0812. C₁₀H₁₁FO₂ requires *MH*, 183.0821].

(S)-2-Benzyl-2-fluoropropionic acid *N*-acetylcysteamine thioester (144).

To a solution of the acid (143) (240 mg, 1.32 mmol) in dichloromethane (10 ml) at 0 °C was added *N*-acetylcysteamine (166.6 mg, 1.4 mmol, 1.1 eq). To the stirring solution was then added 4-dimethylaminopyridine (8.0 mg, 0.066 mmol, 0.05 eq) and (EDCI) (353.9 mg, 1.84 mmol, 1.4 eq) and the reaction left stirring at room temperature for 48 h. The reaction mixture was then washed with water (3 x 15 ml), the organic layer dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude product was purified by flash column chromatography.

Elution with 50% ethyl acetate in 40-60 petroleum ether returned the *N*-acetyl cysteamine thioester (144) as a white solid which was recrystallised from ethyl acetate and petroleum ether (260 mg, 70%); mp 71-72 °C ; Found: C, 59.3; H, 6.8; N, 4.8%. $\text{C}_{14}\text{H}_{18}\text{FNO}_2\text{S}$ requires C, 59.3; H, 6.4; N, 4.9%. $[\alpha]_D^{22}$ -38.9 (*c* 1.9 in CHCl_3); $\nu_{\text{max}}(\text{mull})/\text{cm}^{-1}$ 1682 (C=O), 1642 (C=O), 1561 (C=O); δ_{H} (300 MHz) 7.25 (5H, m, Ph), 5.30 (1H, br, NH), 3.43 (1H, dq, 14.0, 5.8, PhCHH), 3.21 (1H, t, 14.0, PhCHH), 3.17-2.80 (4H, m, CH_2S , CH_2N), 1.9 (3H, s, CH_3CON), 1.50 (3H, d, *J* 21.2, 3- H_3); δ_{C} (75 MHz) 202.4 (1-C, d, *J* 32.0), 170.0 (amide C=O), 134.3, 130.7, 128.2, 127.1 (Ph), 101.0 (2-C, d, *J* 188.3), 43.4 (3-C, d, *J* 21.1), 38.7, 28.1 (S- CH_2 , N- CH_2), 23.3 (2- CH_3 , d, *J* 24.2), 23.2 (amide CH_3); *m/z* (CI) 284 (MH^+ , 100%), 264 (7), 242 (7), 145 (52), 120 (82); [Found: (CI) MH^+ , 284.1120. $\text{C}_{14}\text{H}_{18}\text{FNO}_2\text{S}$ requires *MH*, 284.1120].

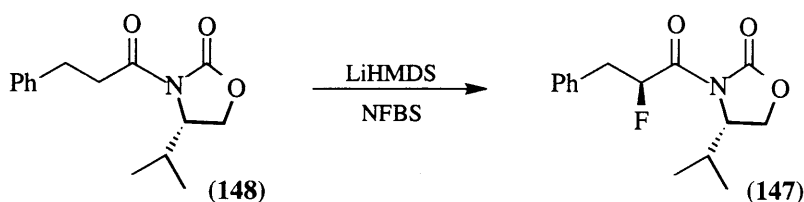
(S)-3-Dihydrocinnamoyl-4-(1-methylethyl)-2-oxazolidinone (148).

To a solution of 2-oxazolidinone (112) (2.13 g, 16.5 mmol) in THF (100 ml) at -78 °C under a nitrogen atmosphere was added *n*-butyllithium (8.5 ml, 2.3 M solution in hexanes, 19.5 mmol, 1.2 eq.) dropwise and the resulting mixture stirred for 0.5 h. 3-Phenylpropionyl chloride (2.75 g, 16.3 mmol) was added and the reaction stirred for 2 h. The reaction was quenched by the addition of water (100 ml), allowed to warm up to

room temperature and acidified to pH 2 with 2 M HCl and extracted with ethyl acetate (4 x 100 ml). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate, dried over anhydrous sodium sulfate and concentrated *in vacuo* to give the crude product which was purified by flash chromatography.

Elution with 10% ethyl acetate in 40-60 petroleum ether gave the acyl oxazolidinone (**148**) as a white crystalline solid which was recrystallised from ethyl acetate and petroleum ether (3.1 g, 73%); mp 68.7-70.0 °C; $[\alpha]_D^{22} +72.8$ (c 2.6 in CH_2Cl_2) [lit.,¹⁵⁰ +71 (c 4.6 in CH_2Cl_2)]; (Found: C, 68.8; H, 7.5; N, 5.4. $\text{C}_{15}\text{H}_{19}\text{NO}_3$ requires C, 68.9; H, 7.3; N, 5.4%); $\nu_{\text{max}}(\text{mull})/\text{cm}^{-1}$ 1793 (C=O oxaz), 1706 (C=O); δ_{H} (300 MHz) 7.14 (5H, m, Ph), 4.40 (1H, ddd, J 7.7, 7.5, 3.8, CHN), 4.19 (2H, m, CH_2O), 3.32 (1H, ddd, J 16.8, 8.4, 6.9, 3'-HH), 3.21 (1H, dt, J 16.8, 7.5, 3'-HH), 2.99 (2H, m, 2'-H₂), 2.34 (1H, spd J 7.0, 3.8, $\text{CH}(\text{CH}_3)_2$), 0.89 (3H, d, J 7.0, CH_3), 0.83 (3H, d, J , 7.0, CH_3); m/z (CI) 262 (MH^+ , 58%), 130 (100), 105 (20), 91 (16) and 83 (48); [Found: (CI) MH^+ , 262.1447. $\text{C}_{15}\text{H}_{19}\text{NO}_3$ requires MH , 262.1443].

(4S,2'S)-3-(2'Fluoro-1'-oxo-3'-phenylpropyl)-4-(1-methylethyl)-2-oxazolidinone (147).

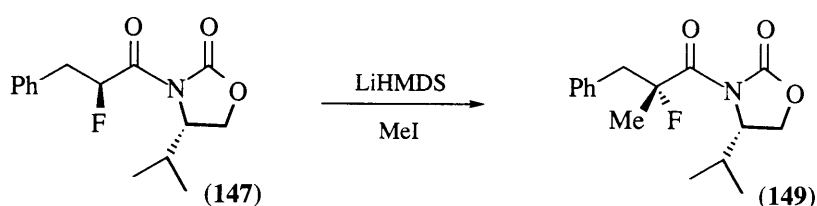


To a solution of the oxazolidinone (**148**) (540 mg, 2.07 mmol) in THF (30 ml) at -78 °C under nitrogen atmosphere was added lithium bis(trimethylsilyl)amide (2.7 ml, 1 M solution in THF, 2.7 mmol, 1.3 eq) dropwise. The slightly yellow solution was stirred for 0.5 h then a solution of *N*-fluorobenzenesulfonimide (NFBS) (783 mg, 2.5 mmol, 1.2 eq) in THF (20 ml) was added dropwise and the reaction stirred for further 2 h at -78 °C. Saturated aqueous ammonium sulfate (50 ml) was added and the reaction allowed to warm to room temperature before acidifying to pH 2 with 2 M HCl. The mixture was extracted with ethyl acetate (3 x 100 ml), the organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo*. Ethyl acetate was added to the crude product and the insoluble benzenesulfonimide was filtered and washed with generous amounts of ethyl acetate. The combined filtrate and washings were concentrated *in vacuo* to give the crude product which was further purified by flash column chromatography.

Elution with 10% ethyl acetate in 40-60 petroleum ether yielded pure α -fluoroacyl oxazolidinone (**147**) as white crystalline solid which was recrystallised from ethyl acetate

and petroleum ether (440 mg, 76%); mp 103.0-105.8 °C; $[\alpha]_D^{23} +0.6$ (c 2.04 in CH₂Cl₂); $\nu_{\text{max}}(\text{mull})/\text{cm}^{-1}$ 1780 (C=O), 1580 (C=O); δ_{H} (300 MHz) 7.30 (5H, m, Ph), 6.15 (1H, ddd, J 49.4, 8.6, 3.5, 2'-H), 4.36 (1H, m, CHN), 4.26 (2H, m, CH₂O), 3.22 (1H, ddd, J 33.5, 14.3, 3.5, 3'-HH), 3.09 (1H, ddd, J 19.6, 14.3, 8.6, 3'-HH), 2.48 (1H, spd, J 7.0, 3.7, CH(CH₃)₂), 0.94 (3H, d, J 7.0, CH₃), 0.89 (3H, d, J 7.0, CH₃); m/z (CI) 280 (MH⁺, 22%), 259 (100), 130 (100), 91 (18) and 83 (30); [Found: (CI) MH⁺, 280.1354. C₁₅H₁₈FNO₃ requires MH , 280.1349].

(4S,2'R)-3-(2'-Benzyl-2'-fluoro-1'-oxopropyl)-4-(1-methylethyl)-2-oxazolidinone (149).



Typical procedure:

Reaction Number	Amount of Base	DMPU / THF	Amount of MeI	Temp. of enolate	Temp. of methyl'n	Time of Reaction	Yield
1	2 eq.	0	20 eq.	-55 °C	-25 °C	4 h	37%
2	3 eq.	0	20 eq.	-55 °C	-25 °C	6 h	34%
3	3 eq.	1:3	20 eq.	-55 °C	-25 °C	1 h	43%
4	1.3 eq.	1:3	10 eq.	-78 °C	-78 °C	3 h	decomp'n

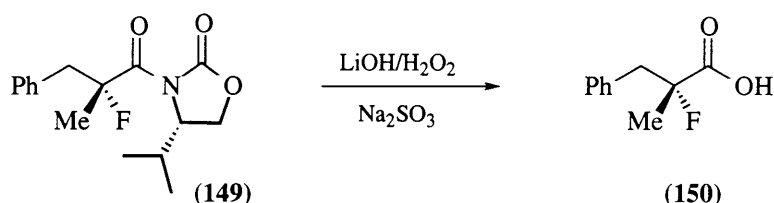
Table: Reaction Conditions for the Methylation of α -fluoro-acyl Oxazolidinone (149).

To a solution of the oxazolidinone (147) in THF at -78 °C under nitrogen atmosphere was added dropwise lithium bis(trimethylsilyl)amide and the temperature was kept at -55 °C for 0.5 h. In reactions where DMPU was used it was added and the mixture stirred for 10 minutes before the addition of methyl iodide. In the other reactions only the alkylating agent was added at this stage. The temperature was then raised to -25 °C, the reactions were stirred for the indicated time and quenched by addition of an equal volume of saturated aqueous ammonium sulfate then allowed to warm to room temperature and acidified to pH 2 with 2 M HCl. The reaction mixture was extracted with ethyl acetate, the combined organic extracts dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude product was purified by flash column chromatography.

Elution with 5% ethyl acetate in 40-60 petroleum ether returned α -fluoro- α -methyl acyl oxazolidinone (**149**) as a white crystalline solid; mp 105.2-106.8 °C; $[\alpha]_D^{22} +76.0$ (c 1.2 in CH_2Cl_2); (Found: C, 66.0; H, 6.9; N, 4.6. $\text{C}_{16}\text{H}_{20}\text{FNO}_3$ requires C, 65.5; H, 6.9; N, 4.8%); $\nu_{\text{max}}(\text{mull})/\text{cm}^{-1}$ 1789 (C=O), 1685 (C=O); δ_{H} (300 MHz) 7.28 (5H, m, Ph), 4.38 (1H, ddd, 7.9, 4.0, 3.0, CHN), 4.27 (1H, dd, J 8.9, 7.9, CHHO), 4.19 (1H, dd, J 8.9, 3.0, CHHO), 3.65 (1H, dd, J 26.9, 14.5, PhCHH), 3.32 (1H, dd, J 26.6, 14.5, PhCHH), 2.16 (1H, spd, J 7.0, 4.0, CH(CH₃)₂), 1.69 (3H, d, J 22.2, 3'-H₃), 0.85 (3H, d, J 7.0, CHCH₃), 0.75 (3H, d, J 7.0, CHCH₃); m/z (CI) 294 (MH⁺, 65%), 274 (100), 145 (96), 137 (7), 130 (26) and 83 (20); [Found: (CI) MH⁺, 294.1504. $\text{C}_{16}\text{H}_{20}\text{FNO}_3$ requires MH, 294.1505].

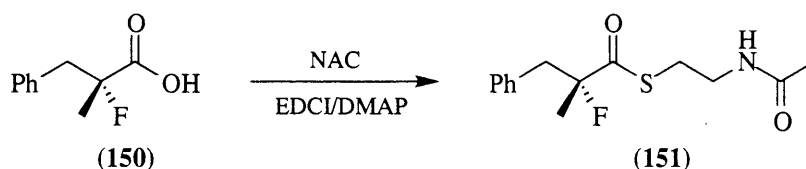
The remaining starting material was eluted with 10% ethyl acetate in 40-60 petroleum ether.

(*R*)-2-Benzyl-2-fluoropropionic acid (150).



To a solution of the oxazolidinone (**149**) (277 mg, 0.95 mmol) in 4:1 THF/H₂O mixture (10 ml) cooled to 0 °C was added hydrogen peroxide (0.29 ml, 30% w/w solution in water, 2.54 mmol, 3.6 eq) and a solution of lithium hydroxide monohydrate (41.4 mg, 0.99 mmol, 1.4 eq) in water (4 ml) and the reaction stirred for 1 h at 0 °C. A solution of sodium sulfite (302 mg, 2.54 mmol, 3.6 eq) in water (6 ml) was added and the solvent was removed *in vacuo*. The remaining aqueous phase was extracted with dichloromethane (4 x 20 ml), acidified to pH 1 with 2 M HCl and then extracted with ethyl acetate (6 x 20 ml). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo* to give α -fluoro acid (**150**) as a white crystalline solid which was recrystallised from ethyl acetate and petroleum ether (127 mg, 74%); $[\alpha]_D^{23} +34.8$ (c 2.53 in CH_2Cl_2); all other data were consistent with (*S*)-2-benzyl-2-fluoropropionic acid (**143**).

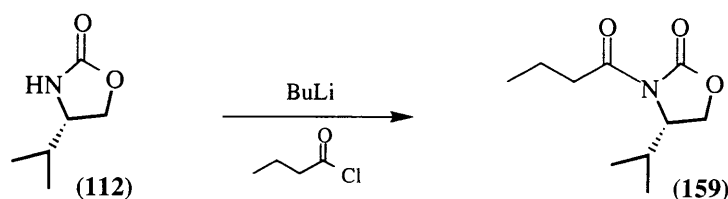
(*R*)-2-Benzyl-2-fluoropropionic acid *N*-acetylcysteamine thioester (151).



To a solution of the acid (**150**) (27.5 mg, 0.15 mmol) in dichloromethane (10 ml) at 0 °C was added *N*-acetylcysteamine (19 mg, 0.16 mmol, 1.1 eq). To the stirring solution was then added 4-dimethylaminopyridine (0.9 mg, 0.008 mmol, 0.05 eq) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (40.3 mg, 0.21 mmol, 1.4 eq) and the reaction left stirring at room temperature for 48 h. The reaction mixture was then washed with water (3 x 15 ml), the organic layer dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude product was purified by flash column chromatography.

Elution with 50% ethyl acetate in 40-60 petroleum ether returned the product (**151**) as white crystals (27.6 mg, 65%); $[\alpha]_D^{22} +40.0$ (*c* 2.1 in CHCl₃); all data other were consistent with (*S*)-2-benzyl-2-fluoropropionic acid *N*-acetyl cysteamine thioester (**144**).

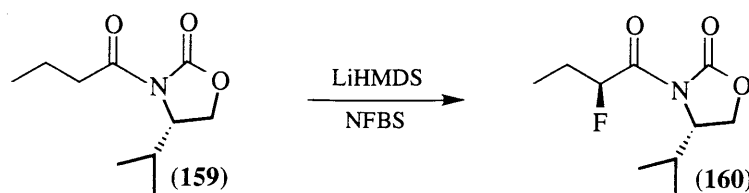
(*S*)-4-(1-Methylethyl)-3-(1'-oxobutyl)-2-oxazolidinone (159**).**



To a solution of 2-oxazolidinone (**112**) (4 g, 31.0 mmol) in THF (140 ml) at -78 °C under a nitrogen atmosphere was added *n*-butyllithium (13.6 ml, 2.5 M solution in hexanes, 34.1 mmol, 1.1 eq) and the mixture stirred for 0.5 h. Butyryl chloride (3.22 ml, 31.0 mmol) was added dropwise and the reaction was stirred at -78 °C for 2 h. Water (100 ml) was added and the mixture was acidified to pH 2 with 2 M HCl and extracted with ethyl acetate (5 x 100 ml). The combined organic extracts were washed with saturated aqueous sodium chloride solution (100 ml), dried over anhydrous magnesium sulfate, concentrated *in vacuo* and purified by flash chromatography.

Elution with 5% ethyl acetate in 40-60 petroleum ether gave the acyl oxazolidinone (**159**) as colourless oil (5.0 g, 81%); $[\alpha]_D^{22} +88.91$ (*c* 2.76 in CHCl₃) [lit.,¹⁰⁷ (no data given)]; $\nu_{\max}(\text{film})/\text{cm}^{-1}$ 1781 (C=O oxaz), 1705 (C=O); δ_{H} (300 MHz) 4.44 (1H, dt, *J* 8.0, 3.8, CHN), 4.27 (1H, t, *J* 8.0, CHHO), 4.20 (1H, dd, *J* 8.0, 3.8, CHHO), 2.97 (1H, ddd, *J* 14.8, 7.9, 7.0, 2'-HH), 2.83 (1H, ddd, *J* 14.8, 7.9, 7.0, 2'-HH), 2.38 (1H, spd, *J* 7.0, 3.8, CH(CH₃)₂), 1.70 (2H, m, 3'-H₂), 0.99 (3H, t, *J* 8.0, 4'-H₃), 0.92 (3H, d, *J* 7.0, CH₃CH), 0.88 (3H, d, *J* 7.0, CH₃CH); *m/z* (CI) 200 (MH⁺, 67%), 158 (32), 130 (52), 83 (100) and 71 (32); [Found: (CI) MH⁺, 200.1290. C₁₀H₁₇NO₃ requires *MH*, 200.1287].

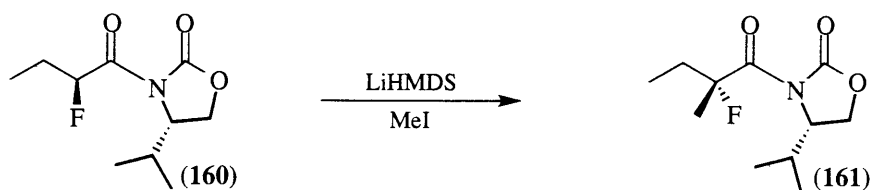
(4*S*,2'*S*)-3-(2'-Fluoro-1'-oxobutyl)-4-(1-methylethyl)-2-oxazolidinone (160).



To a solution of the oxazolidinone (**159**) (2.0 g, 10.0 mmol) in anhydrous THF (50 ml) at $-78\text{ }^{\circ}\text{C}$ under nitrogen atmosphere was added lithium bis(trimethylsilyl)amide (13.0 ml, 1 M solution in THF, 13.0 mmol, 1.3 eq) and the resulting intense yellow solution stirred for 0.5 h. A solution of *N*-fluorobenzenesulfonimide (3.78 g, 12.0 mmol, 1.2 eq) in THF (15 ml) was added and the reaction stirred at $-78\text{ }^{\circ}\text{C}$ for 1 h. The reaction was quenched by addition of saturated aqueous ammonium chloride solution (100 ml), warmed to room temperature, acidified to pH 2 with 2 M HCl and extracted with ethyl acetate (4 x 100 ml). The combined organic extracts were washed with saturated aqueous sodium chloride (2 x 50 ml), dried over anhydrous magnesium sulfate and concentrated *in vacuo* to give the crude product as yellow oil. The byproduct $(\text{PhSO}_2)_2\text{NH}$ was insoluble in ethyl acetate and was removed by filtration.

The non-crystalline product was purified by flash column chromatography. Elution with 5% ethyl acetate in 40-60 petroleum ether returned the α -fluoroacyl oxazolidinone (**160**) as a colourless oil (1.97 g, 91%); $[\alpha]_{\text{D}}^{22} +68.4$ (*c* 1.69 in CH_2Cl_2); $\nu_{\text{max}}(\text{film})/\text{cm}^{-1}$ 1781 (C=O oxaz), 1721 (C=O); $\delta_{\text{H}}(300\text{ MHz})$ 5.87 (1H, ddd, *J* 50.0, 7.9, 3.3, 2'-H), 4.43 (1H, dt, *J* 8.4, 3.6, CHN), 4.35 (1H, t, *J* 8.4, CHHO), 4.29 (1H, dd, *J* 8.4, 3.6, CHHO), 2.50 (1H, spd, *J* 7.0, 3.6, CH(CH₃)₂), 1.90 (2H, m, 3'-H₂), 1.09 (3H, t, *J* 7.3, 4'-H₃), 0.94 (3H, d, *J* 7.1, CHCH₃), 0.90 (3H, d, *J* 7.0, CHCH₃); *m/z* (CI) 218 (MH⁺, 100%), 174 (5), 156 (10) and 130 (62); [Found: (CI) MH⁺, 218.1193. C₁₀H₁₆FNO₃ requires *MH*, 218.1192].

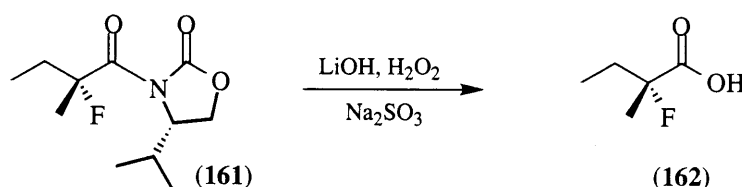
(4*S*,2'*R*)-3-(2'-Fluoro-2'-methyl-1'-oxobutyl)-4-(1-methylethyl)-2-oxazolidinone (161).



To a solution of the oxazolidinone (**160**) (1.0 g, 4.6 mmol) in anhydrous THF (30 ml) at -78 °C under nitrogen atmosphere was added lithium bis(trimethylsilyl)amide (13.8 ml, 1 M solution in THF, 13.8 mmol, 3 eq) dropwise and the resulting yellow solution stirred for 0.5 h. N,N'-Dimethyl-N,N'-propylene urea (DMPU) (7 ml) was then added dropwise and the temperature allowed to rise to -60 °C over 10 minutes. Methyl iodide (5.75 ml, 92.1 mmol, 20 eq) was added and the reaction stirred for 2 h at -25 °C. Saturated aqueous ammonium chloride solution (50 ml) was added and on warming to room temperature the mixture was acidified to pH 2 with 2 M HCl and extracted with ethyl acetate (4 x 100 ml). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo*. DMPU was removed by dry flash chromatography. Elution with 5% ethyl acetate in 40-60 petroleum ether gave the crude product which was further purified by flash column chromatography.

Elution with 5% ethyl acetate in 40-60 petroleum ether returned α -fluoro- α -methylacyl oxazolidinone (**161**) as a colourless oil (639 mg, 60%); $[\alpha]_D^{22} +104.6$ (*c* 2.05 in CHCl₃); $\nu_{\max}(\text{film})/\text{cm}^{-1}$ 1787 (C=O oxaz), 1705 (C=O); δ_{H} (300 MHz) 4.47 (1H, dt, *J* 8.5, 3.0, CHN), 4.33 (1H, t, *J* 8.5, CHHO), 4.24 (1H, dd, *J* 8.5, 3.0, CHHO), 2.53 (2H, m, 3'-H₂), 2.01 (1H, spd, *J* 7.0, 3.0, CH(CH₃)₂), 1.73 (3H, d, *J* 22.1, 2'-CH₃), 0.97 (3H, t, *J* 7.5, 4'-CH₃), 0.94 (3H, d, *J* 7.0, CHCH₃), 0.92 (3H, d, *J* 7.0, CHCH₃); *m/z* (CI) 232 (MH⁺, 84%), 212 (100), 156 (22), 130 (27) and 83 (65); [Found: (CI) MH⁺, 232.1356. C₁₁H₁₈FNO₃ requires *MH*, 232.1349].

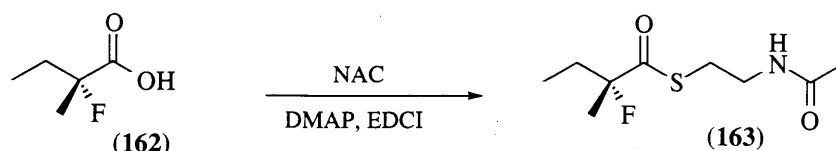
(R)-2-Fluoro-2-methylbutanoic acid (162).



To a solution of the oxazolidinone (**161**) (638 mg, 2.76 mmol) in 4:1 THF/H₂O mixture (10 ml) cooled to 0 °C was added hydrogen peroxide (1.13 ml, 30% w/w solution in water, 9.95 mmol, 3.6 eq) and a solution of lithium hydroxide monohydrate (162.1 mg, 3.86 mmol, 1.4 eq) in water (3 ml) and the reaction stirred for 1 h at 0 °C. A solution of sodium sulfite (1.25 g, 9.95 mmol, 3.6 eq) in water (5 ml) was added and the organic solvent was removed *in vacuo*. The remaining aqueous solution was extracted with dichloromethane (3 x 20 ml), acidified to pH 1 with 2 M HCl and then extracted with ethyl acetate (6 x 20 ml). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo* to give the α -fluoro acid (**162**) as colourless oil (300 mg, 91%); $[\alpha]_D^{22} +3.54$ (*c* 0.6 in CHCl₃); δ_{H} (300 MHz) 6.58 (1H, br s, OH), 1.94 (2H, m, 3-H₂), 1.60 (3H, d, *J* 21.2, 2-CH₃), 1.01 (3H, t, *J* 7.4, 4-H₃); δ_{C} (75

MHz) 176.0 (1-C), 95.2 (2-C, d, J 184.0), 31.2 (3-C, d, J 22.9), 23.1 (2-CH₃, d, J 24.2), 7.5 (4-C); m/z (CI) 121 (MH⁺, 10%), 101 (55), 91 (7) and 75 (30); [Found: (CI) MH⁺, 121.0663. C₅H₉FO₂ requires MH , 121.0665].

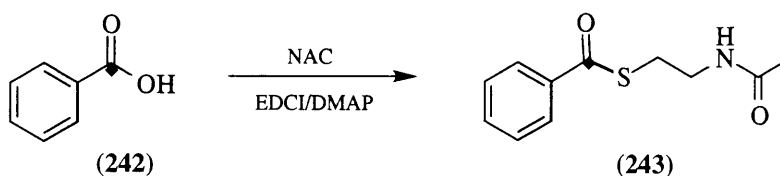
(*R*)-2-Fluoro-2-methylbutanoic acid *N*-acetylcysteamine thioester (163).



To a solution of the acid (**162**) (300 mg, 2.5 mmol) in dichloromethane (10 ml) at 0 °C was added *N*-acetylcysteamine (329.6 mg, 2.8 mmol, 1.1 eq). To the stirring solution was then added 4-dimethylaminopyridine (15.4 mg, 0.126 mmol, 0.05 eq) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) (676.7 mg, 3.53 mmol, 1.4 eq) and the reaction left stirring at room temperature for 48 h. The reaction mixture was then washed with water (3 x 15 ml), the organic layer dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude product was purified by flash column chromatography.

Elution with 50% ethyl acetate in 40-60 petroleum ether returned the *N*-acetylcysteamine thioester (**163**) as colourless oil (270 mg, 49%); [α]_D²³+36.4 (c 4.11 in CH₂Cl₂); $\nu_{\max}$ (film)/cm⁻¹ 3300 (N-H), 1686 (C=O thioester), 1650 (C=O amide), 1551 (C=O amide); δ_{H} (300 MHz) 5.88 (1H, br s, NH), 3.45 (2H, q, J 6.2, CH₂N), 3.04 (2H, t, J 6.2, CH₂S), 1.98 (3H, s, CH₃CON), 1.83 (2H, m, 3-H₂), 1.50 (3H, d, J 21.63, 2-CH₃), 0.95 (3H, t, J 7.3, 4-H₃); δ_{C} (75 MHz) 204.2 (1-C), 170.3 (amide C=O), 101.0 (2-C, d, J 187.0), 39.4, 27.8 (S-CH₂, N-CH₂), 31.2 (3-C, d, J 22.3), 23.2 (2-CH₃, d, J 24.2), 23.0 (amide CH₃), 7.4 (4-C); m/z (CI) 221 (M⁺, 95%), 202 (16), 180 (17), 120 (100), 104 (7), 83 (57) and 75 (13); [Found: (CI) MH⁺, 222.0959. C₉H₁₆FNO₂S requires MH , 222.0964].

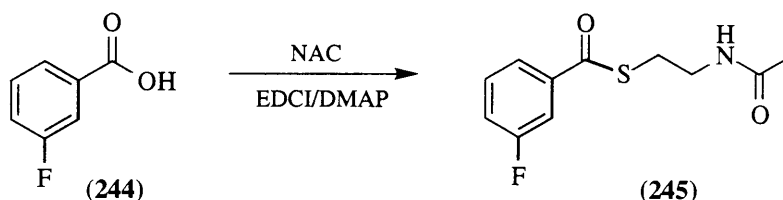
[Carboxy-¹³C]-Benzoic acid *N*-acetylcysteamine thioester (243).



To a solution of [carboxy-¹³C]benzoic acid (**242**) (1.0 g, 8.1 mmol) in dichloromethane (10 ml) was added a solution of *N*-acetylcysteamine (1.06 g, 8.93 mmol, 1.1 eq) in dichloromethane (10 ml) and the stirred mixture was cooled to 0 °C. A

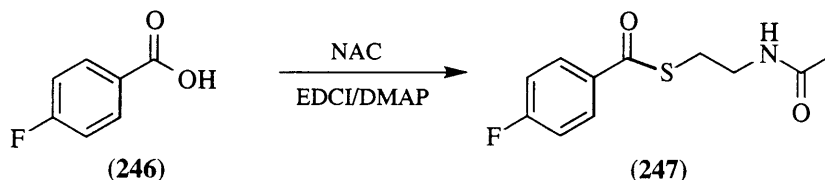
solution of EDCI (2.25 g, 11.7 mmol, 1.4 eq) and DMAP (51 mg, 0.42 mmol) in dichloromethane (10 ml) was added dropwise to the benzoic acid solution at 0 °C and the reaction stirred at room temperature for 16 h. The reaction mixture was washed with water (50 ml) and the aqueous layer was extracted with dichloromethane (2 x 30 ml). The combined organic layers were washed with water (30 ml), dried over anhydrous sodium sulfate and concentrated *in vacuo*. Recrystallisation from ethyl acetate and 60-80 petroleum ether gave the title compound (**243**) (1.05 g, 58%) as white crystalline solid; mp 87-89 °C (lit.,⁴⁰ 89-90 °C) (Found: C, 59.5; H, 5.9; N, 6.0. C₁₁H₁₃NO₂S requires C, 59.2; H, 5.9, N; 6.3%); δ_{H} (300 MHz) 7.97 (2H, m, *o*-H₂), 7.61 (1H, m, *p*-H), 7.47 (2H, m, *m*-H₂), 5.92 (1H, br s, NH), 3.54 (2H, q, *J* 6.0, CH₂N), 3.24 (2H, dt, *J* 6.0, 5.1, CH₂S), 1.98 (3H, s, CH₃CON), *m/z* (CI) 225 (MH⁺, 80%), 207 (20), 165 (20), and 106 (100).

3-Fluorobenzoic acid *N*-acetylcysteamine thioester (**245**).



The above reaction was repeated using 3-fluorobenzoic acid (**244**) (800 mg, 5.7 mmol) and gave the title compound (**245**) (960 mg, 70%) as a white crystalline solid, mp 90-92 °C (lit.,⁴⁰ 89-90 °C); (Found: C, 55.0; H, 5.2; N, 5.6. C₁₁H₁₂FNO₂S requires C, 54.8; H, 5.0, N, 5.8%); δ_{H} (300 MHz) 7.77 (1H, m, 6-H), 7.64 (1H, m, 4-H), 7.45 (1H, m, 5-H), 7.30 (1H, m, 2-H), 5.87 (1H, br s, NH), 3.54 (2H, q, *J* 6.0, CH₂N), 3.25 (2H, t, *J* 6.0, CH₂S), 1.99 (3H, s, CH₃CO), *m/z* (CI) 242 (MH⁺, 70%), 222 (8), 182 (10), 123 (100), 118 (8) and 83 (50).

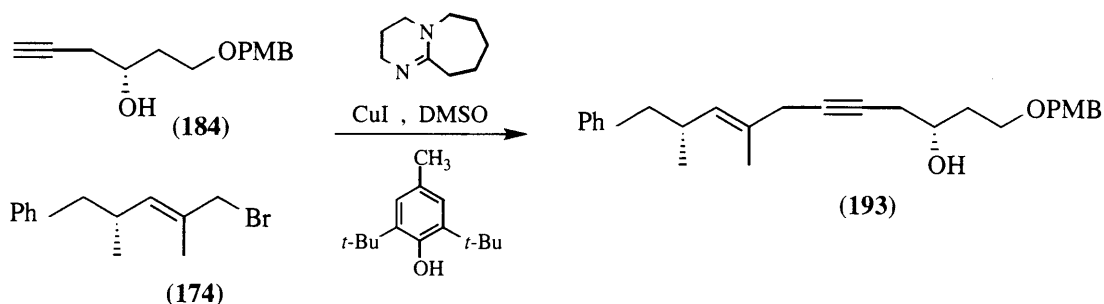
4-Fluorobenzoic acid *N*-acetylcysteamine thioester (**247**).



The above reaction was repeated using 4-fluorobenzoic acid (**246**) (800 mg, 5.7 mmol) and gave the title compound (**247**) (1.18 g, 86%) as a white crystalline solid, mp 98-100 °C (lit.,⁴⁰ 99-100 °C); (Found: C, 54.8; H, 4.9; N, 6.0. C₁₁H₁₂FNO₂S requires C, 54.8; H, 5.0; N, 5.8%); δ_{H} (300 MHz) 8.00 (2H, m, 2-H, 6-H), 7.15 (2H, m, 3-H,

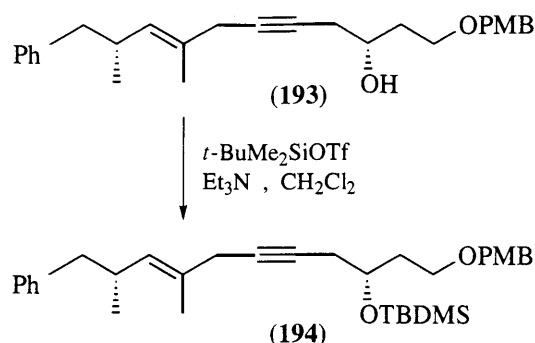
5-H), 5.88 (1H, br s, NH), 3.54 (2H, q, J 6.0, CH_2N), 3.24 (2H, t, J 6.0, CH_2S), 1.98 (3H, s, CH_3CON), m/z (CI) 242 (MH^+ , 65%), 222 (16), 182 (12), 123 (100) and 83 (23).

(3*R*,8*E*,10*R*)-1-[(4-Methoxybenzyl)oxy]-8,10-dimethyl-11-phenylundec-8-en-5-yn-3-ol (193).



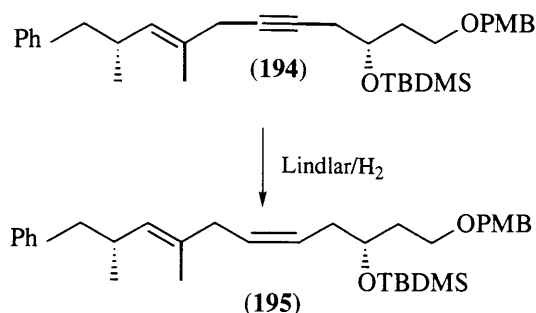
To a stirred suspension of copper(I) iodide (275 mg, 1.4 mmol) in dry dimethyl sulfoxide (DMSO) (0.66 ml) at room temperature was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.45 ml, 2.97 mmol, 2 eq). The suspension turned green as complexation proceeded. After 20 minutes 2,6-di-*t*-butyl-4-methylphenol (10 mg, 0.05 mmol, 0.035 eq) was added followed by the alkyne (**184**) (336 mg, 1.43 mmol) in dimethyl sulfoxide (0.66 ml). After a further 0.5 h (*4R*, *2E*)-1-bromo-2,4-dimethyl-5-phenylpent-2-ene (**174**) (508 mg, 2.01 mmol, 1.4 eq.) was added and stirring was continued for a further 0.5 h. Diethyl ether (20 ml) and brine (20 ml) were added with vigorous stirring and the two layers were separated. The aqueous layer was further extracted with ether (2 x 20 ml). The combined organic extracts were washed with cold hydrochloric acid (2 M, 2 x 20 ml) and brine (2 x 20 ml) then dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The crude product was purified by flash column chromatography.

Elution with 50% diethyl ether in petroleum ether yielded the title compound (**193**) as a colourless oil (322 mg, 55%); $[\alpha]_D^{22}$ -32.5 (c 0.8 in CHCl_3); ν_{max} (film)/ cm^{-1} 3469 (OH), 2060w (CC), 1612 (C=C), 1513 (C=C), 1248 (C-O); δ_{H} (300 MHz) 7.20 (7H, m, Ar), 6.87 (2H, br d, J 8.6, Ar), 5.23 (1H, dsxtet, J 8.8, 1.4, 9-H), 4.46 (2H, s, ArCH_2O), 3.93 (1H, m, 3-H), 3.80 (3H, s, OCH_3), 3.66 (2H, m, 1-H₂), 3.04 (1H, br s, OH), 2.80 (2H, d, J 1.4, 7-H₂), 2.67-2.49 (3H, m, 10-H, 11-H₂), 2.40 (2H, m, 4-H₂), 1.79 (2H, m, 2-H₂), 1.46 (3H, d, J 1.4, 8-CH₃), 0.94 (3H, d, J 6.2, 10-CH₃); m/z (EI) 406 (M^+ , 44%), 389 (18), 299 (25) and 121 (100); [Found (EI): M^+ , 406.2493. $\text{C}_{27}\text{H}_{34}\text{O}_3$ requires M , 406.2508].



120

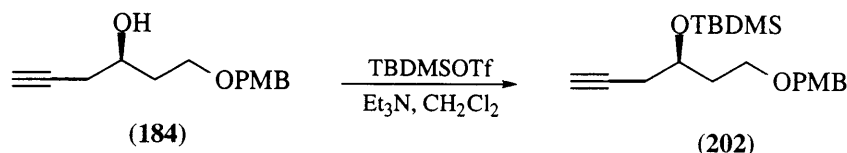
(3*R*,5*Z*,8*E*,10*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-1-[(4-methoxybenzyl)oxy]-8,10-dimethyl-11-phenylundeca-5,8-diene (195).



To a solution of the alkyne (**194**) (137 mg, 0.26 mmol) in ethanol (7 ml) was added Lindlar catalyst (13.7 mg) followed by quinoline (1.9 μ l). The mixture was stirred at room temperature under hydrogen (1 atmosphere) for 10 h. The catalyst was removed by filtration through Celite and the filtrate was concentrated *in vacuo*. The crude product was purified by flash column chromatography.

Elution with 9% ether in 40-60 petroleum ether yielded the required diene (**195**) as a colourless oil (135 mg, 99%); $[\alpha]_D^{20}$ -38.2 (*c* 0.84 in CHCl₃); $\nu_{\max}$ (film)/cm⁻¹ 1612 (C=C), 1513 (C=C), 1250 (C-O); δ_H (400 MHz) 7.21 (7H, m, Ar), 6.85 (2H, br d, *J* 8.8, Ar), 5.71-5.25 (2H, m, 5-H, 6-H), 5.12 (1H, dq, *J* 9.3, 1.2, 9-H), 4.42 (1H, d, *J* 11.5, ArCHHO), 4.37 (1H, d, *J* 11.5, ArCHHO), 4.06 (1H, m, 3-H), 3.58 (2H, m, 1-H₂), 3.34 (3H, s, OCH₃), 2.74-2.48 (5H, m, 7-H₂, 10-H, 11-H₂), 2.34 (2H, t, *J* 6.5, 4-H₂), 1.89 (2H, m, 2-H₂), 1.44 (3H, d, *J* 1.2, 8-CH₃), 1.05 (9H, s, *t*-BuSi), 1.01 (3H, d, *J* 6.4, 10-CH₃), 0.08 (3H, s, SiCH₃), 0.06 (3H, s, SiCH₃); *m/z* (CI) 521 [(M-H)⁺, 5%], 391 (10), 309 (40), 189 (24), 173 (10), 121 (100) [Found: (CI) (M-H)⁺, 521.3459. C₃₃H₅₀O₃Si requires *M-H*, 521.3451].

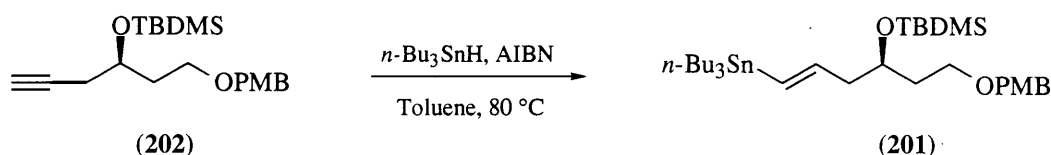
(*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-1-[(4-methoxybenzyl)oxy]hex-5-yne (202).



To a solution of the alcohol (**184**) (1.00 g, 4.27 mmol) in dry dichloromethane (5 ml) at 0 °C was added triethylamine (1.2 ml, 8.55 mmol, 2 eq) followed by *tert*-butyldimethylsilyl trifluoromethanesulfonate (1.5 ml, 6.4 mmol, 1.5 eq). The mixture was allowed to warm to room temperature and stirred for 1 h. Ether (30 ml) was added and the solution was washed with brine, dried over anhydrous sodium sulfate and

concentrated *in vacuo* to give the *title compound* (**202**) as a colourless oil (1.47 g, 99%); $[\alpha]_D^{20}$ -21.8 (*c* 1.6 in CHCl_3); $\nu_{\text{max}}(\text{neat})/\text{cm}^{-1}$ 3309 (H-CC), 2100w (CC), 1248 (C-O); δ_{H} (300 MHz) 7.27 (2H, br d, *J* 9.0, Ar), 6.88 (2H, br d, *J* 9.0, Ar), 4.43 (1H, d, *J* 12.0, OCHHAr), 4.40 (1H, d, *J* 12.0, OCHHAr), 3.98 (1H, m, 3-H), 3.80 (3H, s, OCH₃), 3.53 (2H, dd, *J* 8.0, 6.0, 1-H₂), 2.35 (2H, dd, *J* 6.0, 3.0, 4-H₂), 1.97 (1H, t, *J* 3.0, 6-H), 1.95 (1H, m, 2-HH), 1.81 (1H, m, 2-HH), 0.88 (9H, s, *t*-BuSi), 0.08 (3H, s, SiCH₃), 0.07 (3H, s, SiCH₃); δ_{C} (75 MHz) 158.0, 130.6, 129.3, 113.9 (Ar), 81.6 (5-C), 72.6 (Ar-CH₂O), 70.1 (6-C), 67.9, 66.3 (1-C, 3-C), 55.3 (Ar-OCH₃), 36.6 (2-C), 27.7 (4-C), 25.8 (SiC(CH₃)₃), 18.0 (SiC(CH₃)₃), -4.5, -4.8 (Si(CH₃)₂); *m/z* (CI) 347 [(M-H)⁺, 10%], 309 (27), 231 (12), 225 (20), 215 (18), 211 (25), 137 (402), 123 (100) and 79 (15) [Found: (CI) MH⁺ 349.2198. C₂₀H₃₂O₃Si requires *MH*, 349.2199].

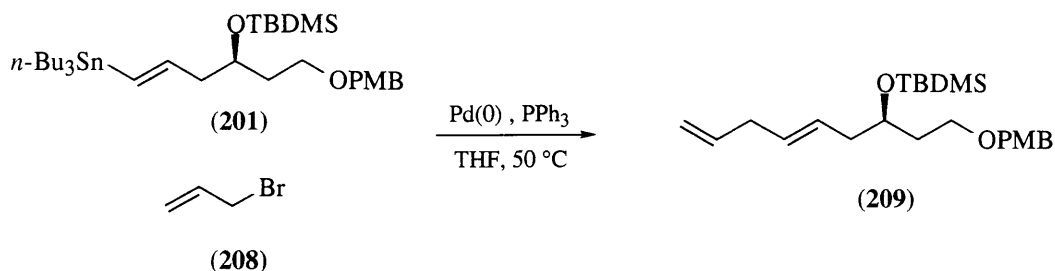
(3*R*,5*E*)-3-[(*tert*-Butyldimethylsilyl)oxy]-1-[(4-methoxybenzyl)oxy]-6-(tri-*n*-butylstannyl)hex-5-ene (201).



To a solution of the alkyne (**202**) (552 mg, 1.59 mmol) in toluene (4 ml) under nitrogen atmosphere at room temperature was added tri-*n*-butyltin hydride (0.47 ml, 1.75 mmol, 1.1 eq.) and a solution of azobis(isobutyronitrile) (1.5 mg, 0.0095 mmol, 0.006 eq) in toluene (0.5 ml). The mixture was stirred at 80 °C for 3h then concentrated *in vacuo* to give the crude product which was then purified by flash column chromatography.

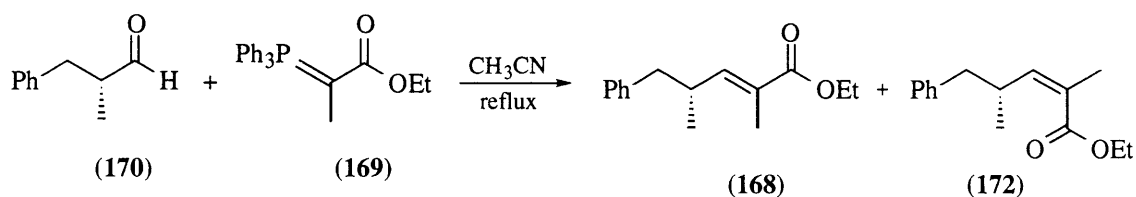
Elution with 1% ethyl acetate in 40-60 petroleum ether gave the *title compound* (**201**) as a colourless oil (890 mg, 87.6%); $[\alpha]_D^{20}$ -8.2 (*c* 0.94 in CH_2Cl_2); δ_{H} (300 MHz) 7.26 (2H, br d, *J* 8.6, Ar), 6.88 (2H, br d, *J* 8.6, Ar), 5.95-5.85 (2H, m, 5-H and 6-H), 4.44 (1H, d, *J* 11.4, OCHHAr), 4.39 (1H, d, *J* 11.4, OCHHAr), 3.89 (1H, m, 3-H), 3.81 (3H, s, OCH₃), 3.51 (2H, t, *J* 6.7, 1-H₂), 2.32 (2H, m, 4-H₂), 1.52 (20H, m, 2-H₂, (CH₃CH₂CH₂CH₂)₃Sn), 0.89 (9H, t, *J* 7.2, (CH₃CH₂CH₂CH₂)₃Sn), 0.88 (9H, s, *t*-BuSi), 0.05 (3H, s, SiCH₃), 0.03 (3H, s, SiCH₃); δ_{C} (75 MHz) 159.0, 145.5, 130.7 (5-C, 6-C), 129.2, 113.7 (Ar), 72.5 (Ar-CH₂O), 69.1 (3-C), 66.8 (1-C), 55.2 (Ar-OCH₃), 46.4, 36.8 (2-C, 4-C), 29.3, 27.8, 13.7, 9.3 (*n*-BuSn), 25.8 (SiC(CH₃)₃), 18.1 (SiC(CH₃)₃), -4.3, -4.7 (Si(CH₃)₂); *m/z* (CI) 639 [(M-H)⁺, 40%], 583 (50), 291 (25) and 121 (100) [Found: (CI) (M-H)⁺, 639.2367. C₃₂H₆₀O₃SiSn requires *M-H*, 639.3255].

(3*R*,5*E*)-3-[(*tert*-Butyldimethylsilyl)oxy]-1-[(4-methoxybenzyl)oxy]nona-5,8-diene (209).



To a yellow solution of bis(triphenylphosphine)palladium(II) chloride in THF (0.2 ml) was added DIBAL-H (1.3 μ l, 1 M solution in hexane, 0.0014 mmol, 0.04 eq). The colour of the solution turned into deep red as palladium(0) formed. Allyl bromide (208) (3 μ l, 0.034 mmol) was added followed by the vinylstannane (201) (22 mg, 0.034 mmol) and zinc chloride (0.2 ml, 1 M solution in ether, 0.2 mmol). The reaction mixture was then stirred at 60 $^\circ\text{C}$ for 3 days. The reaction was cooled, partitioned between diethyl ether (10 ml) and one-third saturated solution of sodium fluoride (10 ml), and the mixture was vigorously stirred for 15 min. The gelatinous precipitate of tri-*n*-butylstannyl fluoride was removed by gravity filtration. The organic layer was separated, washed with concentrated sodium chloride, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. Purification by flash column chromatography with 0.5% ethyl acetate in 40-60 petroleum ether yielded the *title compound* (209) as a colourless oil (8 mg, 61%); $[\alpha]_D^{23}$ -10.5 (*c* 0.2 in CHCl_3); δ_{H} (300 MHz) 7.25 (2H, br d, *J* 8.6, Ar), 6.87 (2H, br d, *J* 8.6, Ar), 5.80 (1H, m, 8-H), 5.50-5.42 (2H, m, 5-H and 6-H), 4.99 (2H, m, 9-H₂), 4.42 (1H, d, *J* 11.4, OCHHAr), 4.40 (1H, d, *J* 11.4, OCHHAr), 3.84 (1H, m, 3-H), 3.80 (3H, s, OCH₃), 3.50 (2H, t, *J* 6.7, 1-H₂), 2.75 (2H, m, 7-H₂), 2.18 (2H, m, 4-H₂), 1.71 (2H, m, 2-H₂), 0.87 (9H, s, *t*-BuSi), 0.05 (3H, s, SiCH₃), 0.03 (3H, s, SiCH₃); *m/z* (CI) 389 [(*M*-H)⁺, 34%], 323 (12), 173 (5), 121 (100) and 84 (34) [Found: (CI) (*M*-H)⁺, 389.2516. C₂₃H₃₈O₃Si requires *M*-H, 389.2512].

(2*E*,4*R*)-2,4-Dimethyl-5-phenylpent-2-enoic acid ethyl ester (168).

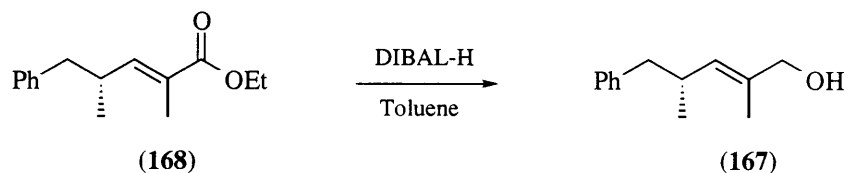


To a solution of the aldehyde (170) (3.0 g, 20.3 mmol) in dry acetonitrile (50 ml) was added (carboethoxyethylidene)triphenylphosphorane (169) (8.1 g, 22.3 mmol,

1.1 eq) and the mixture was heated at reflux for 4 h. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography.

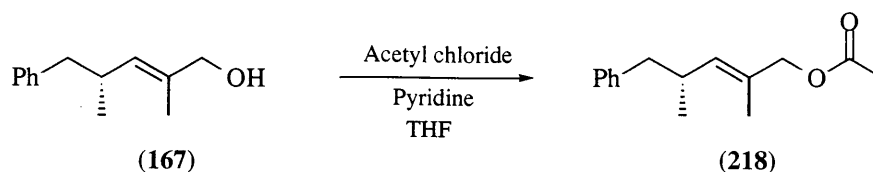
Elution with 1% ethyl acetate in 40-60 petroleum ether returned the minor *2Z*-isomer (**172**) (0.52 g, 11%) (yield based on ^1H NMR) as a colourless oil then *2E*-ethyl ester (**168**) as a colourless oil (3.8 g, 80.7%); $[\alpha]_{\text{D}}^{21} -51.35$ (*c* 5.4 in CHCl_3); $\nu_{\text{max}}(\text{neat})/\text{cm}^{-1}$ 1710 (C=O), 1649 (C=C), 1495 (C=C), 1248 (C-O); $\delta_{\text{H}}(300 \text{ MHz})$ 7.30-7.13 (5H, m, Ph), 6.62 (1H, dq, *J* 9.7, 1.4, 3-H), 4.18 (2H, q, *J* 7.2, CH_2O), 2.76 (1H, m, 4-H), 2.67 (1H, dd, *J* 13.2, 6.7, 5-H), 2.61 (1H, dd, *J* 13.2, 7.6, 5-H), 1.66 (3H, d, *J* 1.4, 2- CH_3), 1.29 (3H, t, *J* 7.2, CH_2CH_3), 1.03 (3H, d, *J* 6.6, 4- CH_3); *m/z* (CI) 233 (MH^+ , 44%), 187 (60) and 84 (100) [Found: (CI) MH^+ 233.1539. $\text{C}_{15}\text{H}_{20}\text{O}_2$ requires *MH*; 233.1542].

(2*E*,4*R*)-2,4-Dimethyl-5-phenylpent-2-en-1-ol (167).

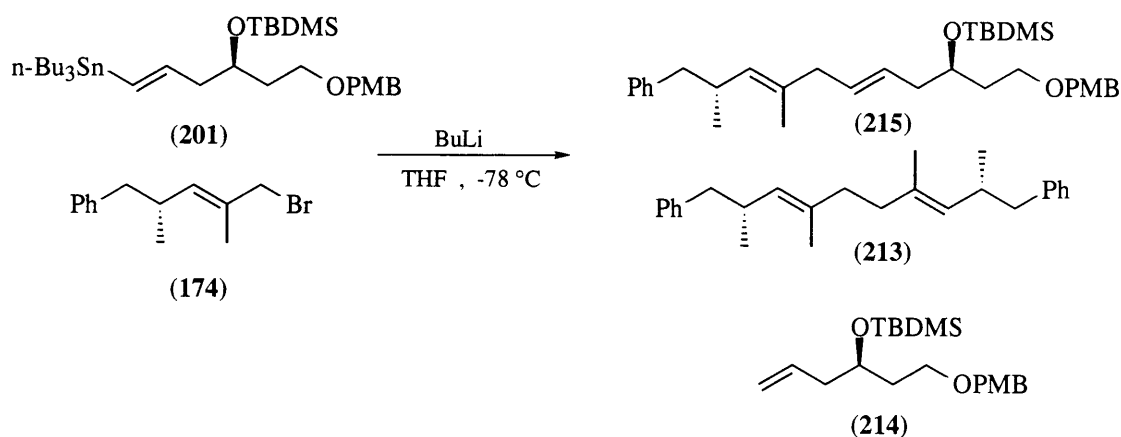


To a solution of the α,β-unsaturated ester (**168**) (3.5 g, 15.1 mmol) in dry toluene (100 ml) at -78 °C was added diisobutylaluminium hydride (33.2 ml, 1 M solution in hexane, 33.2 mmol, 2.2 eq) and the mixture stirred for 4 h at -78 °C then left to warm up to room temperature overnight. A few drops of water was added carefully and stirring continued for 0.5 h then anhydrous sodium sulfate was added to the reaction mixture and filtered over Celite. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography.

Elution with 5% ethyl acetate in 40-60 petroleum ether gave the title compound (**167**) as a colourless liquid (2.0 g, 70%); $[\alpha]_{\text{D}}^{22} -37.0$ (*c* 1.1 in CCl_4) [lit.⁴⁰ -36.8 (*c* 0.6 in CCl_4)]; $\nu_{\text{max}}(\text{film})/\text{cm}^{-1}$ 3368 (OH), 1604 (C=C), 1452 (C=C), 1217 (C-O); $\delta_{\text{H}}(300 \text{ MHz})$ 7.21 (5H, m, Ph), 5.26 (1H, dq, *J* 9.4, 1.3, 3-H), 3.96 (1H, d, *J* 6.0, 1-*HH*), 3.95 (1H, d, *J* 6.0, 1-*HH*), 2.66 (1H, m, 4-H), 2.57 (2H, d, *J* 7.2, 5- H_2), 1.48 (3H, d, *J* 1.3, 2- CH_3), 0.98 (3H, d, *J* 6.4, 4- CH_3); *m/z* (CI) 189 [$(\text{M}-\text{H})^+$, 30%], 171 (20), 159 (18), 131 (25), 119 (28), 91 (68) and 84 (100).

(2E,4R)-1-Acetoxy-2,4-dimethyl-5-phenylpent-2-ene (218).

To an ice cooled solution of the alcohol **(167)** (207 mg, 1.1 mmol) in THF (4 ml) was added pyridine (105 μ l, 1.3 mmol, 1.2 eq) followed by acetyl chloride (93 μ l, 1.3 mmol, 1.2 eq) and the mixture was stirred at 0 °C for 1 h. Ice cold water (5 ml) was added and the reaction mixture was extracted with ethyl acetate (4 x 15 ml). The organic extracts were washed with saturated sodium chloride, dried over anhydrous sodium sulfate and concentrated *in vacuo* to give the *acetate* **(218)** as a colourless oil (242.7 mg, 96%); $[\alpha]_D^{23}$ -29.6 (*c* 2.8 in CHCl_3); $\nu_{\text{max}}(\text{neat})/\text{cm}^{-1}$ 1740 (C=O), 1604 (C=C), 1494 (C=C), 1231 (C-O); δ_{H} (300 MHz) 7.18 (5H, m, Ph), 5.30 (1H, dsxtet, *J* 9.1, 1.2, 3-H), 4.43 (1H, dd, *J* 11.9, 1.2, 1-H), 4.39 (1H, dd, *J* 11.9, 1.2, 1-H), 2.66 (1H, m, 4-H), 2.57 (2H, m, 5-H₂), 2.07 (3H, s, C(O)CH₃), 1.44 (3H, d, *J* 1.2, 2-CH₃), 0.98 (3H, d, *J* 6.6, 4-CH₃); δ_{C} (75 MHz) 170.9 (C=O), 140.5, 134.9, 129.3, 129.2, 128.3, 125.7 (Ar, 2-C, 3-C), 70.1 (1-C), 43.5 (5-C), 34.4 (4-C), 20.3 (2-CH₃), 13.9 (4-CH₃); *m/z* (CI) 233 (MH⁺, 2%), 189 (12), 173 (12), 149 (45), 91 (40) and 83 (100) [Found: (CI) MH⁺ 233.1538 and C₁₅H₂₀O₂ requires *MH*, 233.1541].

(3R,5E,8E,10R)-3-[(*tert*-Butyldimethylsilyl)oxy]-1-[(4-methoxybenzyl)oxy]-8,10-dimethyl-11-phenylundeca-5,8-diene (215).**(a) Organolithium coupling with allylic bromide (174).**

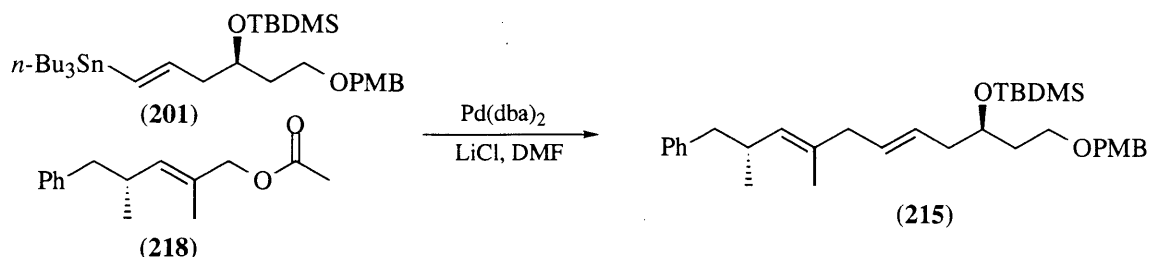
To a solution of the vinylstannane **(201)** (619.7 mg, 0.97 mmol) in anhydrous THF (3 ml) at -78 °C was added dropwise *n*-butyllithium (0.63 ml of 2.0 M solution in

THF, 1.26 mmol, 1.3 eq) and the mixture stirred for 1 h. A solution of (2*E*,4*R*)-1-bromo-2,4-dimethyl-5-phenylpent-2-ene (**174**) (245.5 mg, 0.97 mmol) in THF (1.0 ml) was added dropwise and stirring continued for a further 4 h. A saturated aqueous solution of ammonium chloride (5 ml) was added to the mixture and extracted with ethyl acetate (4 x 15 ml). The combined organic extracts were washed with saturated sodium chloride, dried over anhydrous sodium sulfate and concentrated *in vacuo* to give the crude product which was purified with flash column chromatography.

Elution with 40-60 petroleum ether yielded (2*R*,3*E*,7*E*,9*R*)-2,4,7,9-tetramethyl-1,10-diphenyldeca-3,7-diene (**213**) as a colourless oil (42.0 mg, 25%); $[\alpha]_D^{23}$ -43.8 (*c* 4.1 in CHCl₃); δ_H (300 MHz) 7.20 (10H, m, Ph), 4.97 (2H, br d, *J* 8.4, 3-H, 8-H), 2.67-2.45 (6H, m, 1-H₂, 2-H, 9-H, 10-H₂), 2.02 (4H, s, 5-H₂, 6-H₂), 1.41 (6H, d, *J* 1.5, 4-CH₃, 7-CH₃), 0.92 (6H, d, *J* 6.3, 2-CH₃, 9-CH₃); *m/z* (CI) 345 [(*M*-H)⁺, 75%], 255 (60), 227 (7), 187 (20), 137 (75), 159 (100), 119 (80) and 91 (30) [Found: (CI) (*M*-H)⁺ 345.2586. C₂₆H₃₄ requires *M*-H, 345.2582].

Elution with 0.5% ethyl acetate in 40-60 petroleum ether returned first (*R*)-3-[(*tert*-butyldimethylsilyl)oxy]-1-[(4-methoxybenzyl)oxy]hex-5-ene (**214**) as a colourless oil (214.5 mg, 63.2%); $[\alpha]_D^{20}$ -20.2 (*c* 0.60 in CH₂Cl₂); δ_H (300 MHz) 7.27 (2H, br d, *J* 8.7, Ar), 6.68 (2H, br d, *J* 8.7, Ar), 5.81 (1H, m, 5-H), 5.01 (1H, m, 6-H), 5.05 (1H, m, 6-H), 4.43 (1H, d, *J* 11.6, CHHAr), 4.40 (1H, d, *J* 11.6, CHHAr), 3.88 (1H, m, 3-H), 3.81 (3H, s, OCH₃), 3.51 (2H, dd, *J* 7.0, 6.1, 1-H₂), 2.27 (2H, m, 4-H₂), 1.73 (2H, m, 2-H₂), 0.88 (9H, s, *t*-BuSi), 0.05 (3H, s, SiCH₃), 0.04 (3H, s, SiCH₃); δ_C (75 MHz) 159.0, 134.9, 130.7, 129.3, 113.7 (Ar, 5-C), 116.9 (6-C), 72.6 (Ar-CH₂O), 68.9 (3-C), 66.8 (1-C), 55.3 (Ar-OCH₃), 42.3, 36.7 (2-C, 4-C), 25.9 (SiC(CH₃)₃), 18.1 (SiC(CH₃)₃), -4.3, -4.7 (Si(CH₃)₂); *m/z* (CI) 349 [(*M*-H)⁺, 10%], 173 (20) and 121 (100) [Found: (CI) (*M*-H)⁺, 349.2207. C₂₀H₃₄O₃Si requires *M*-H, 349.2199] followed by the *title compound* (**215**) as a colourless oil (153 mg, 30.3%); $[\alpha]_D^{22}$ -26.8 (*c* 1.0 in CHCl₃); $\nu_{\max}$ (neat)/cm⁻¹ 1612 (C=C), 1513 (C=C) and 1249 (C-O); δ_H (300 MHz) 7.20 (7H, m, Ar), 6.88 (2H, br d, *J* 8.6, Ar), 5.40-5.29 (2H, m, 5-H and 6-H), 4.99 (1H, dq, *J* 9.6, 1.4, 9-H), 4.44 (1H, d, *J* 11.5, OCHHAr), 4.41 (1H, d, *J* 11.5, OCHHAr), 3.87 (1H, m, 3-H), 3.80 (3H, s, OCH₃), 3.51 (2H, t, *J* 6.6, 1-H₂), 2.63-2.48 (5H, m, 7-H₂, 10-H, 11-H₂), 2.17 (2H, m, 4-H₂), 1.73 (2H, m, 2-H₂), 1.39 (3H, d, *J* 1.4, 8-CH₃), 0.93 (3H, d, *J* 6.4, 10-CH₃), 0.88 (9H, s, *t*-BuSi), 0.05 (3H, s, SiCH₃), 0.04 (3H, s, SiCH₃); δ_C (75 MHz) 159.1, 141.1, 134.9, 133.1, 130.8, 129.3, 127.9, 127.4, 125.5, 116.9, 113.7 (Ar, 5-C, 6-C, 8-C, 9-C), 69.4 (3-C), 66.8 (1-C), 55.3 (OCH₃), 43.9, 43.0, 42.3, 41.1, 34.6 (2-C, 4-C, 7-C, 10-C, 11-C), 25.9 (SiC(CH₃)₃), 20.7 (8-CH₃), 18.1 (SiC(CH₃)₃), 16.1 (10-CH₃), -4.3, -4.7 (Si(CH₃)₂); *m/z* (CI) 521 [(*M*-H)⁺, 35%], 465 (10), 415 (40), 401 (10), 391 (35) and 121 (100) [Found: (CI) (*M*-H)⁺ 521.3438. C₃₃H₅₀O₃Si requires *M*-H, 521.3451].

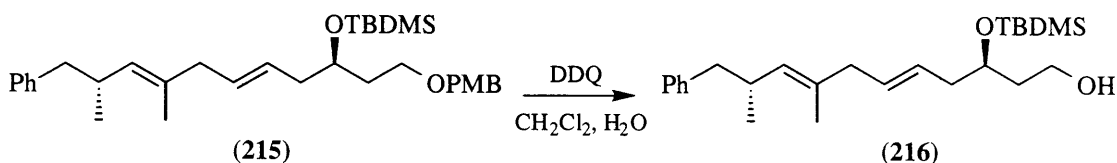
(b) Palladium-catalysed coupling of vinylstannane (201) and allylic acetate (218).



To a mixture of acetate (218) (260.8 mg, 1.12 mmol), vinylstannane (201) (718.0 mg, 1.12 mmol) and lithium chloride (143 mg, 3.37 mmol, 3 eq) in DMF (8 ml) was added bis(dibenzylideneacetone)palladium(0) ($\text{Pd}(\text{dba})_2$) (23.3 mg, 0.06 mmol, 5 mol%). The mixture was stirred at 23 °C for 40 h then partitioned between water (20 ml) and ether (3 x 25 ml). The combined ether extracts were washed with saturated aqueous sodium chloride, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude compound was purified by thin layer chromatography.

The mixture was applied on silica plates and developed in 1% ether in 40-60 petroleum ether. The desired band was eluted from the silica with ether and the solvent removed *in vacuo* to give the required diene (215) as a colourless oil (485 mg, 83%), data reported earlier.

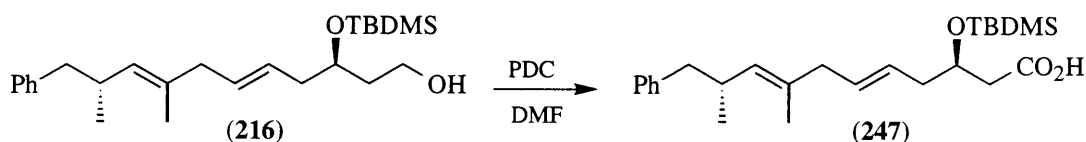
(3*R*,5*E*,8*E*,10*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-8,10-dimethyl-11-phenylundeca-5,8-dien-1-ol (216).



To a stirred solution of the 4-methoxybenzyl ether (215) (301.4 mg, 0.58 mmol) in dichloromethane (6 ml) was added water (480 μl) followed by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (225.5 mg, 0.99 mmol, 1.5 eq). The mixture was stirred at room temperature for 1 h. Saturated aqueous sodium hydrogen carbonate (10 ml) was added and the mixture was extracted with ether (4 x 20 ml). The combined organic extracts were washed with saturated sodium carbonate (10 ml) and brine (10 ml) then dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude mixture was purified by thin layer chromatography using 20% ethyl acetate in 40-60 petroleum ether as the eluent. The desired band was extracted with ethyl acetate and concentrated *in*

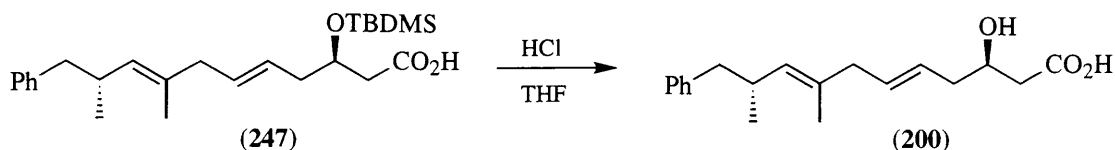
vacuo to give the desired *alcohol* (**216**) as a colourless oil (52 mg, 22%); $[\alpha]_D^{22}$ -40.9 (*c* 1.40 in CHCl_3); $\nu_{\text{max}}(\text{film})/\text{cm}^{-1}$ 3684 (Free OH), 3411 (Bonded OH), 1602 (C=C), 1520 (C=C), 1255 (C-O); $\delta_{\text{H}}(300 \text{ MHz})$ 7.20 (5H, m, Ph), 5.49-5.28 (2H, m, 5-H and 6-H), 5.00 (1H, dq, *J* 9.0, 1.3, 9-H), 3.95 (1H, m, 3-H), 3.84 (1H, m, 1-H), 3.72 (1H, m, 1-H), 2.67 (1H, m, 10-H), 2.60 (2H, m, 7-H₂), 2.53 (2H, m, 11-H₂), 2.29 (2H, m, 4-H₂), 1.80 (1H, m, 2-H), 1.65 (1H, m, 2-H), 1.4 (3H, d, *J* 1.3, 8-CH₃), 0.95 (3H, d, *J* 6.4, 10-CH₃), 0.91 (9H, s, *t*-BuSi), 0.11 (3H, s, SiCH₃), 0.10 (3H, s, CH₃Si); *m/z* (CI) 401 [(M-H)⁺, 20%], 269 (70), 159 (40), 119 (55), 91 (94) and 57 (100) [Found: (CI) (M-H)⁺, 401.2880. C₂₅H₄₂O₂Si requires *M-H*, 401.2876].

(3*R*,5*E*,8*E*,10*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-8,10-dimethyl-11-phenylundeca-5,8-dienoic acid (247**).**



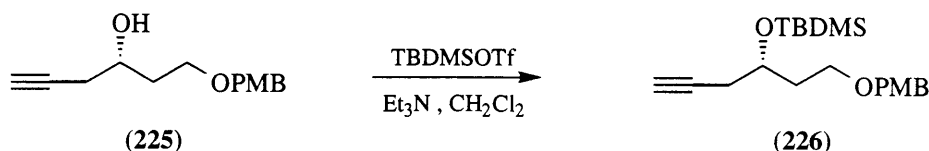
To a solution of the alcohol (**216**) (61 mg, 0.15 mmol) in DMF (0.4 ml) at room temperature was added pyridinium dichromate (266 mg, 0.71 mmol). Stirring was maintained for 18 h then water (15 ml) was added and the mixture was extracted with ether (4 x 25 ml). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude mixture was purified by thin layer chromatography using 20% ethyl acetate in 40-60 petroleum ether as the eluent. The desired band was eluted from the silica with ethyl acetate and the solvent was removed *in vacuo* to give the *dienoic acid* (**247**) as a colourless oil (47 mg, 75%); $[\alpha]_D^{20}$ -20.3 (*c* 1.1 in CHCl_3); $\nu_{\text{max}}(\text{film})/\text{cm}^{-1}$ 3680 (Free OH), 3400 (Bonded OH), 1711 (C=O), 1492 (C=C), 1430 (C=C), 1255 (C-O); $\delta_{\text{H}}(300 \text{ MHz})$ 7.19 (5H, m, Ph), 5.51-5.30 (2H, m, 5-H, 6-H), 4.98 (1H, dq, *J* 8.0, 1.3, 9-H), 4.12 (1H, m, 3-H), 2.65-2.41 (7H, m, 4-H₂, 7-H₂, 10-H, 11-H₂), 2.25 (2H, m, 2-H₂), 1.60 (3H, d, *J* 1.3, 8-CH₃), 0.94 (3H, d, *J* 8.0, 10-CH₃), 0.89 (9H, s, *t*-BuSi), 0.10 (3H, s, SiCH₃), 0.08 (3H, s, SiCH₃); *m/z* (CI) 417 (MH⁺, 10%), 399 (55), 373 (12), 359 (60), 301 (12), 159 (50), 57 (100) [Found: (CI) MH⁺, 417.2829. C₂₅H₄₀O₃Si requires *MH*, 417.2825].

(3*R*,5*E*,8*E*,10*R*)-3-Hydroxy-8,10-dimethyl-11-phenylundeca-5,8-dienoic acid (200).



A solution of the silyl ether (**247**) (10 mg, 0.024 mmol) in THF (1 ml) was acidified by HCl (2 M) to pH 2 and stirred for 16 h at 23 °C. Water (5 ml) was added and the mixture was extracted with ethyl acetate (5 x 10 ml). The combined ethyl acetate extracts were washed with saturated sodium chloride (10 ml), dried over anhydrous sodium sulfate and concentrated *in vacuo*. The product was purified by thin layer chromatography using 10% methanol in dichloromethane as the eluent. The desired band was eluted from the silica with ethyl acetate (0.05% acetic acid) and the solvent was removed *in vacuo* to give the *hydroxy acid* (**200**) as a colourless oil (5.5 mg, 76%); $[\alpha]_D^{21}$ -31.09 (*c* 5.5 in CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ 3423 (Bonded OH), 1710 (C=O), 1641 (C=C), 1419 (C=C), 1215 (C-O); δ_{H} (300 MHz) 7.19 (5H, m, Ph), 5.47 (1H, dt, *J* 15.2, 6.5, 6-H), 5.34 (1H, dt, *J* 15.2, 6.8, 5-H), 4.99 (1H, dd, *J* 9.0, 1.2, 9-H), 4.06 (1H, m, 3-H), 2.68-2.43 (7H, m, 2H₂, 7-H₂, 10-H, 11-H₂), 2.25 (2H, t, *J* 6.8, 4-H₂), 1.38 (3H, d, *J* 1.2, 8-CH₃), 0.95 (3H, d, *J* 8.0, 10-CH₃); δ_{C} (75 MHz) 177.5 (1-C), 141.0, 133.1, 131.3, 129.3, 128.0, 125.8, 125.6 (Ph, 5-C, 6-C, 9-C), 132.6 (8-C), 67.5 (3-C), 43.9, 42.9, 40.4, 39.7 (2-C, 4-C, 7-C, 11-C), 34.6 (10-C), 20.8 (8-CH₃), 16.1 (10-CH₃); *m/z* (CI) 301 [(M-H)⁺, 42%], 257 (10), 239 (35), 159 (65), 131 (30), 119 (42) and 91 (100) [Found: (CI) MH⁺, 303.1955. C₁₉H₂₆O₃ requires *MH*, 303.1960].

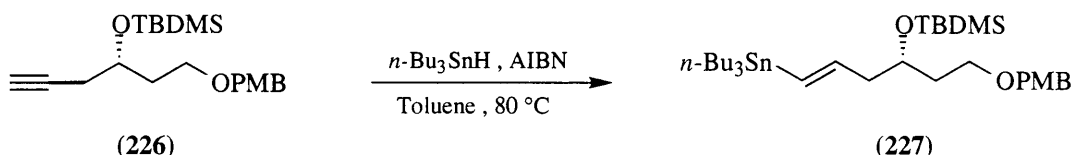
(*S*)-3-[(*tert*-Butyldimethylsilyl)oxy]-1-[(4-methoxybenzyl)oxy]hex-5-yne (226).



To a solution of (*S*)-3-hydroxy-1-[(4-methoxybenzyl)oxy]hex-5-yne (**225**) (240 mg, 1.02 mmol) in dry dichloromethane (1.5 ml) at 0 °C was added triethylamine (0.28 ml, 2.05 mmol, 2 eq) followed by *tert*-butyldimethylsilyltrifluoromethanesulfonate (0.35 ml, 1.54 mmol, 1.5 eq). The mixture was allowed to warm to room temperature and stirred for 1 h. Ether (10 ml) was added and the solution was washed with brine, dried

over anhydrous sodium sulfate and concentrated *in vacuo* to give the *silyl ether* (**226**) as a colourless oil (350 mg, 98.6%); $[\alpha]_D^{22} + 21.52$ (*c* 1.7 in CHCl_3) all other data were consistent with (*R*)-3-[(*tert*-butyldimethylsilyl)oxy]-1-[(4-methoxybenzyl)oxy]hex-5-yne (**202**).

(3*S*,5*E*)-3-[(*tert*-Butyldimethylsilyl)oxy]-1-[(4-methoxybenzyl)oxy]-6-[tri-*n*-butylstannyl]hex-5-ene (227**).**

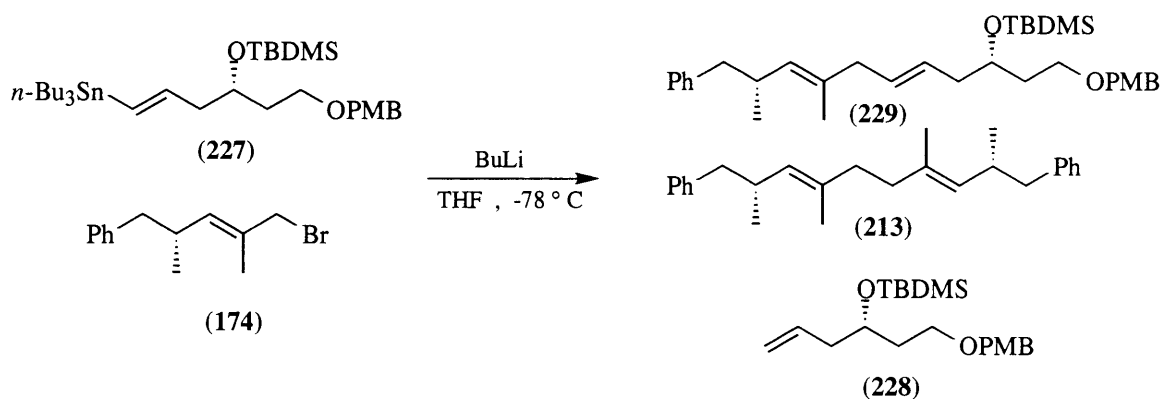


To a solution of alkyne (**226**) (400 mg, 1.15 mmol) in toluene (4 ml) under nitrogen atmosphere at room temperature was added tributyltin hydride (0.34 ml, 1.26 mmol, 1.1 eq) and a solution of azobis(isobutyronitrile) (1.1 mg, 6.9 μmol) in toluene (0.5 ml). The mixture was stirred at 80 $^\circ\text{C}$ for 3 h then concentrated *in vacuo* to give the crude product which was then purified by flash column chromatography.

Elution with 1% ethyl acetate in 40-60 petroleum ether gave the *vinyl stannane* (**227**) as a colourless oil (599.4 mg, 81.6%); $[\alpha]_D^{20} + 10.3$ (*c* 0.93 in CHCl_3) all other data were consistent with (3*R*,5*E*)-3-[(*tert*-butyldimethylsilyl)oxy]-1-[(4-methoxybenzyl)oxy]-6-[*n*-tributylstannyl]hex-5-ene (**201**).

(3*S*,5*E*,8*E*,10*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-1-[(4-methoxybenzyl)oxy]-8,10-dimethyl-11-phenylundeca-5,8-diene (229**).**

(a) Organolithium coupling with allylic bromide (**174**).



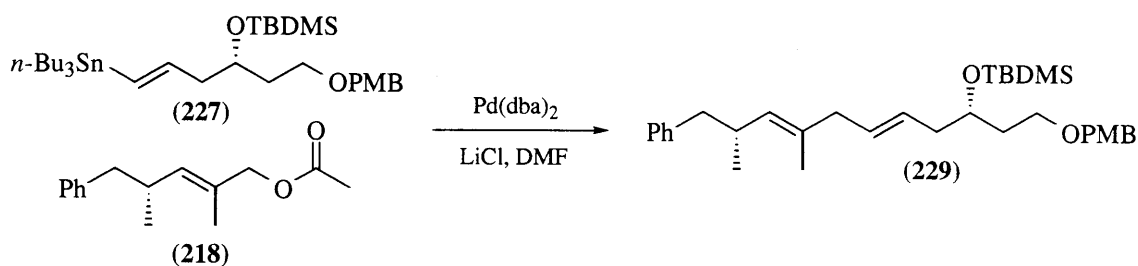
To a solution of the vinylstannane (**227**) (924 mg, 1.44 mmol) in anhydrous THF (7.0 ml) at -78°C was added dropwise *n*-butyllithium (0.64 ml of 2.5 M solution in

THF, 1.59 mmol, 1.1 eq) and the mixture stirred for 1 h. (4*R*,2*E*)-1-bromo-2,4-dimethyl-5-phenylpent-2-ene (**174**) (366 mg, 1.44 mmol) was added and stirring continued for a further 2 h then left to warm up to room temperature over 0.5 h. Water (10 ml) was added to the mixture and extracted with ether (4 x 15 ml). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo* to give the crude product which was purified with flash column chromatography.

Elution with 40-60 petroleum ether yielded (2*R*,9*R*,3*E*,7*E*)-2,4,7,9-tetramethyl-1,10-diphenyldeca-3,7-diene (**213**) as a colourless oil (72.8 mg, 29%); (data reported earlier)

Elution with 0.5% ethyl acetate in 40-60 petroleum ether returned (3*S*)-3-[(*tert*-butyldimethylsilyl)oxy]-1-[(4-methoxybenzyl)oxy]hex-5-ene (**228**) as a colourless oil (214.5 mg, 42%); $[\alpha]_D^{20} +21.0$ (*c* 0.48 in CH₂Cl₂); followed by the required diene (**229**) as a colourless oil (357 mg, 47%); $[\alpha]_D^{22} +6.5$ (*c* 2.2 in CHCl₃); δ_H (300 MHz) 7.28-7.12 (7H, m, Ar), 6.88 (2H, br d, *J* 8.6, Ar), 5.44-5.29 (2H, m, 5-H, 6-H), 4.99 (1H, dq, *J* 8.8, 1.1, 9-H), 4.42 (1H, d, *J* 11.3, OCHHAr), 4.40 (1H, d, *J* 11.3, OCHHAr), 3.83 (1H, m, 3-H), 3.80 (3H, s, OCH₃), 3.51 (2H, t, *J*, 6.6, 1-H₂), 2.64-2.49 (5H, m, 7-H₂, 10-H, 11-H₂), 2.17 (2H, m, 4-H₂), 1.70 (2H, m, 2-H₂), 1.39 (3H, d, *J* 1.1, 8-CH₃), 0.93 (3H, d, *J* 6.3, 10-CH₃), 0.88 (9H, s, *t*-BuSi), 0.05 (3H, s, SiCH₃), 0.04 (3H, s, SiCH₃); δ_C (75 MHz) as for (**215**); *m/z* (CI) 521 [(*M*-H)⁺, 35%], 415 (50), 401 (10), 389 (30), 385 (20) and 121 (100) [Found: (CI) (*M*-H)⁺, 521.3445. C₃₃H₅₀O₃Si requires *M*-H, 521.3451].

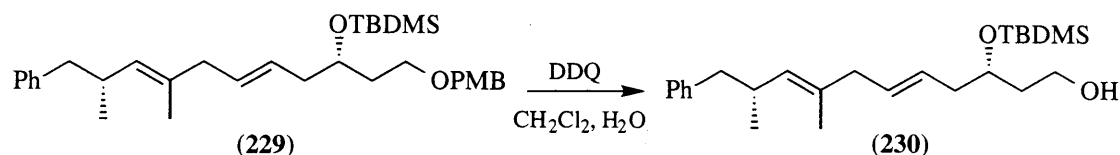
(b) Palladium-catalysed cross coupling of vinylstannane (227) and allylic acetate (218).



To a mixture of acetate (**218**) (59.9 mg, 0.26 mmol), vinylstannane (**227**) (155.6 mg, 0.24 mmol), and lithium chloride (31 mg, 0.73 mmol, 3 eq) in DMF (1.5 ml) was added bis(dibenzylideneacetone)palladium (0) (Pd(dba)₂) (7 mg, 0.01 mmol, 5 mol%). The mixture was stirred at 23 °C for 40 h then partitioned between water (10 ml) and ether (3 x 15 ml). The combined ether extracts were washed with saturated aqueous sodium chloride, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude compound was purified by thin layer chromatography using 1% ether in 40-60 petroleum ether as the eluent. The desired band was eluted from the silica with ether and

the solvent was removed *in vacuo* to give *title compound* (**229**) as a colourless oil (87.7 mg, 70%), data reported earlier.

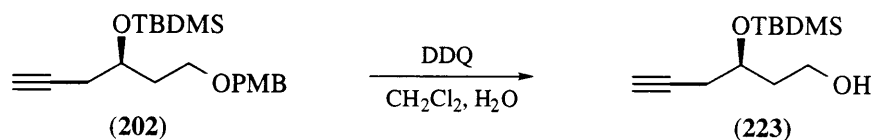
(3*S*,5*E*,8*E*,10*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-8,10-dimethyl-11-phenylundeca-5,8-dien-1-ol (230**).**



To a stirred solution of diene (**229**) (63 mg, 0.12 mmol) in dichloromethane (2.5 ml) was added water (150 μ l) followed by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (41 mg, 0.19 mmol). The mixture was stirred at room temperature for 35 minutes. Saturated aqueous sodium hydrogen carbonate (15 ml) was added and the mixture was extracted with ether (3 x 20 ml). The combined organic extracts were washed with saturated sodium carbonate (2 x 15 ml) and brine (2 x 12 ml) then dried over anhydrous magnesium sulfate and concentrated *in vacuo*.

Purification by flash chromatography eluting with 5% ether in 40-60 petroleum ether yielded the required *alcohol* (**230**) as a colourless oil (22 mg, 45%); $[\alpha]_D^{23} +29.9$ (c 1.0 in CHCl_3); δ_{H} (300 MHz) 7.21 (5H, m, Ph), 5.47-5.41 (2H, m, 5-H, 6-H), 4.99 (1H, dq, J 7.5, 1.4, 9-H), 3.95 (1H, m, 3-H), 3.83 (1H, m, 1-HH), 3.72 (1H, m, 1-HH), 2.67-2.51 (5H, m, 7-H₂, 10-H, 11-H₂), 2.29 (2H, m, 4-H₂), 1.73 (2H, m, 2-H₂), 1.40 (3H, d, J 1.4, 8-CH₃), 0.94 (3H, d, J 6.4, 10-CH₃), 0.90 (9H, s, *t*-BuSi), 0.10 (6H, s, Si(CH₃)₂); m/z (CI) 401 [(*M*-H)⁺, 15%], 385 (18), 285 (42), 269 (68), 119 (55), 91 (92) and 75 (100) [Found: (CI) (*M*-H)⁺, 401.2882. C₂₅H₄₂O₂Si requires *M*-H, 401.2876].

(*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]hex-5-yn-1-ol (223**).**

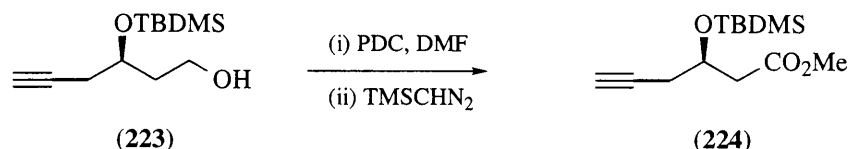


To a stirred solution of alkyne (**202**) (1.5 g, 4.31 mmol) in dichloromethane (15 ml) was added water (0.9 ml) followed by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (1.5 g, 6.5 mmol). The mixture was stirred at room temperature for 0.5 h. Saturated aqueous sodium hydrogen carbonate (20 ml) was added and the mixture was extracted with ether (4 x 30 ml). The combined organic extracts were washed with saturated sodium carbonate (20 ml) and brine (20 ml) then dried over anhydrous sodium sulfate

and concentrated *in vacuo*. The crude mixture was purified by flash column chromatography.

Elution with 10% ether in 40-60 petroleum ether yielded the required *alcohol* (**223**) as a colourless oil (706 mg, 72%); $[\alpha]_D^{23}$ -33.9 (*c* 4.4 in CHCl_3); $\nu_{\text{max}}(\text{neat})/\text{cm}^{-1}$ 3684 (Free OH), 3400 (Bonded OH), 3307 (H-CC), 1711 (C=O), 1255 (C-O); δ_{H} (300 MHz) 4.08 (1H, qd, *J* 6.7, 3.8, 3-H), 3.78 (2H, m, 1-H₂), 2.31 (2H, dd, *J* 6.7, 2.7, 4-H₂), 2.01 (1H, t, *J* 2.7, 6-H), 1.91 (1H, m, 2-H), 1.71 (1H, m, 2-H), 0.90 (9H, s, *t*-BuSi), 0.12 (6H, s, $\text{Si}(\text{CH}_3)_2$); δ_{C} (75 MHz) 80.9 (5-C), 70.4 (6-C), 70.0 (3-C), 59.9 (1-C), 37.7 (2-C), 27.0 (4-C), 25.8 ($\text{SiC}(\text{CH}_3)_3$), 17.9 ($\text{SiC}(\text{CH}_3)_3$), -4.5, -4.8 ($\text{Si}(\text{CH}_3)_2$); *m/z* (CI) 229 (MH^+ , 18%), 211 (30), 171 (58), 145 (50), 115 (25), 97 (32) and 57 (100) [Found: (CI) MH^+ , 229.1618. $\text{C}_{12}\text{H}_{24}\text{O}_2\text{Si}$ requires *MH*, 229.1625].

(*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]hex-5-ynoic acid methyl ester (224**).**

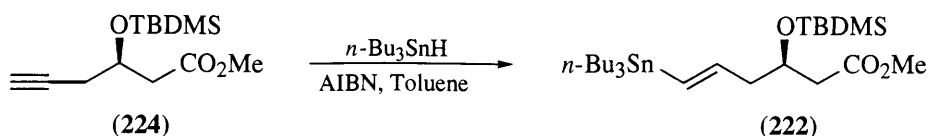


To a solution of the alcohol (**223**) (653 mg, 2.9 mmol) in DMF (10 ml) at room temperature was added pyridinium dichromate (6.5 g, 17.2 mmol, 6 eq). Stirring was maintained for 18 h then water (50 ml) was added and the mixture was extracted with ether (4 x 50 ml). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo*.

To a stirred solution of crude product in methanol/toluene mixture (6 ml, 1:1) at room temperature was added trimethylsilyldiazomethane (2.15 ml, 2 M solution in hexane, 4.3 mmol) and the stirring was maintained for 2 h. The solvent was evaporated *in vacuo* and the crude product was purified by flash column chromatography.

Elution with 2% ether in 40-60 petroleum ether gave the *methyl ester* (**224**) as a colourless oil (486 mg, 66%); $[\alpha]_D^{24}$ -40.3 (*c* 2.65 in CHCl_3); $\nu_{\text{max}}(\text{neat})/\text{cm}^{-1}$ 3308 (H-CC), 1734 (C=O), 1256 (C-O); δ_{H} (300 MHz) 4.23 (1H, m, 3-H), 3.63 (3H, s, OCH_3), 2.67 (1H, dd, *J* 14.7, 4.4, 2-H), 2.49 (1H, dd, *J* 14.7, 7.9, 2-H), 2.37 (2H, m, 4-H₂), 1.97 (1H, t, *J* 2.7, 6-H), 0.81 (9H, s, *t*-BuSi), 0.12 (3H, s, SiCH_3), 0.06 (3H, s, SiCH_3); δ_{C} (75 MHz) 171.8 (1-C), 80.5 (5-C), 70.7 (6-C), 68.1 (3-C), 51.5 (OCH_3), 41.8 (2-C), 27.5 (4-C), 25.7 ($\text{SiC}(\text{CH}_3)_3$), 17.9 ($\text{SiC}(\text{CH}_3)_3$), -4.5, -5.0 ($\text{Si}(\text{CH}_3)_2$); *m/z* (CI) 257 (MH^+ , 16%), 255 (87), 199 (75), 183 (55), 171 (76), 145 (26), 115 (20) and 83 (100) [Found: (CI) MH^+ , 257.1578. $\text{C}_{13}\text{H}_{24}\text{O}_3\text{Si}$ requires *MH*, 257.1573].

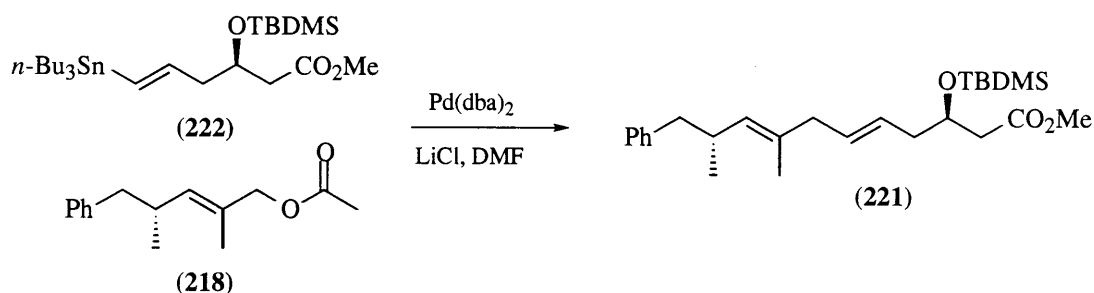
(3*R*,5*E*)-3-[(*tert*-Butyldimethylsilyl)oxy]-6-(tri-*n*-butylstannyl)hex-5-enoic acid methyl ester (222).



To a solution of alkyne (**224**) (51 mg, 0.20 mmol) in toluene (1 ml) under nitrogen atmosphere at room temperature was added tributyltin hydride (0.06 ml, 0.22 mmol, 1.1 eq) and a solution of azobis(isobutyronitrile) (0.2 mg, 1.2 μmol) in toluene (0.2 ml). The mixture was stirred at 120 °C for 4 h then concentrated *in vacuo* to give the crude product which was purified by flash column chromatography.

Elution with 1% ethyl acetate in 40-60 petroleum ether gave vinylstannane (**222**) as a colourless oil (74.3 mg, 68%); $[\alpha]_D^{23}$ -20.4 (*c* 2.2 in CHCl_3); $\nu_{\text{max}}(\text{neat})/\text{cm}^{-1}$ 1731 (C=O), 1599 (C=C), 1254 (C-O); δ_{H} (300 MHz) 5.97 (2H, m, 5-H, 6-H), 4.19 (1H, m, 3-H), 3.67 (3H, s, OCH_3), 2.45 (2H, m, 2- H_2), 2.35 (2H, m, 4- H_2), 1.54-1.24 (18H, m, $(\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2)_3\text{Sn}$), 0.89 (9H, t, *J* 7.4, $(\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2)_3\text{Sn}$), 0.86 (9H, s, *t*-BuSi), 0.07 (3H, s, SiCH_3), 0.04 (3H, s, SiCH_3); δ_{C} (75 MHz) 173.0 (1-C), 144.4, 131.9 (5-C, 6-C), 69.1 (3-C), 51.4 (OCH_3), 46.3, 42.1 (2-C, 4-C), 29.2, 26.8, 25.7, 17.5, 13.7, 9.3, -4.4, -4.9 (TBDMS, *n*- Bu_3Sn); *m/z* (CI) 546 [(*M*-H)⁺, 1%], 255 (87), 491 (86), 489 (64), 291 (74), 232 (20), 115 (18), 83 (100) and 57 (45) [Found: (CI) (*M*-H)⁺, 547.2665. $\text{C}_{25}\text{H}_{52}\text{O}_3\text{SiSn}$ requires *M*-H, 547.2629].

(3*R*,5*E*,8*E*,10*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-8,10-dimethyl-11-phenylundeca-5,8-dienoic acid methyl ester (221).

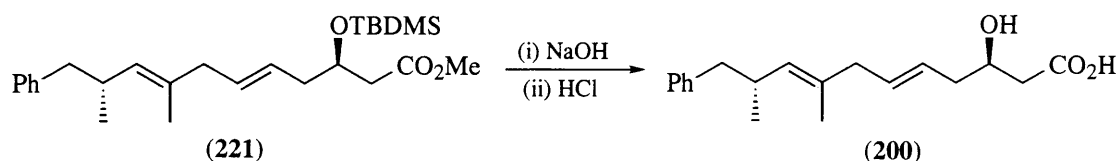


To a mixture of unsaturated acetate (**218**) (85 mg, 0.36 mmol), vinylstannane (**222**) (200 mg, 0.36 mmol), and lithium chloride (46.5 mg, 1.1 mmol, 3 eq) in DMF (2 ml) was added bis(dibenzylideneacetone)palladium(0) (Pd(dba)_2) (10.5 mg, 0.02 mmol, 5 mol%). The mixture was stirred at 23 °C for 48 h then partitioned between water (10 ml) and ethyl acetate (3 x 15 ml). The combined organic extracts were washed with saturated aqueous sodium chloride, dried over anhydrous sodium sulfate and

concentrated *in vacuo*. The crude compound was purified by flash column chromatography.

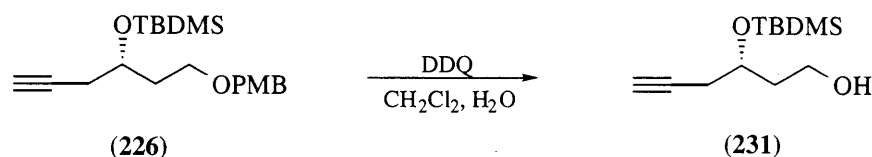
Elution with 1% ether in 40-60 petroleum ether yielded the required *diene* (**221**) as a colourless oil (130 mg, 83%); $[\alpha]_D^{21}$ -22.1 (*c* 1.8 in CHCl_3); ν_{max} (film)/ cm^{-1} 1730 (C=O), 1648 (C=C), 1437 (C=C), 1255 (C-O); δ_{H} (300 MHz) 7.18 (5H, m, Ph), 5.39-5.35 (2H, m, 5-H, 6-H), 4.99 (1H, dq, *J* 8.9, 1.3, 9-H), 4.16 (1H, m, 3-H), 3.67 (3H, s, OCH₃), 2.64-2.51 (5H, m, 7-H₂, 10-H, 11-H₂), 2.42 (2H, m, 2-H₂), 2.20 (2H, m, 4-H₂), 1.38 (3H, d, *J* 1.3, 8-CH₃), 0.94 (3H, d, *J* 6.5, 10-CH₃), 0.86 (9H, s, *t*-BuSi), 0.06 (3H, s, SiCH₃), 0.03 (3H, s, SiCH₃); δ_{C} (75 MHz) 172.4 (1-C), 141.1, 132.9, 132.0, 131.0, 129.3, 127.9, 126.5, 125.5 (Ar, 5-C, 6-C, 8-C, 9-C), 69.5 (3-C), 51.4 (OCH₃), 43.9, 42.9, 42.0, 40.9, 34.6 (2-C, 4-C, 7-C, 11-C), 34.6 (10-C), 25.7 (SiC(CH₃)₃), 20.7 (8-CH₃), 17.9 (SiC(CH₃)₃), 16.1 (10-CH₃), -4.4, -4.9 (Si(CH₃)₂); *m/z* (CI) 431 (MH⁺, 8%), 399 (6), 373 (50), 299 (4), 183 (36), 171 (100), 159 (34) and 131 (34) [Found: (CI) MH⁺, 431.2977. C₂₆H₄₂O₃Si requires *MH*, 431.2981].

(3*R*,5*E*,8*E*,10*R*)-3-Hydroxy-8,10-dimethyl-11-phenylundeca-5,8-dienoic acid (200).



To a solution of methyl ester (**221**) (110 mg, 0.25 mmol) in methanol (6 ml) was added sodium hydroxide (0.25 ml, 2 M solution in water) and the mixture stirred for 48 h at room temperature. The solvent was then removed *in vacuo* and the mixture was redissolved in THF (6 ml) and acidified with HCl (2 M) to pH 2. The reaction mixture was stirred for a further 48 h, water (5 ml) was added and extracted with ethyl acetate (5 x 20 ml). The organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo* to give the crude product which was purified by thin layer chromatography using 10% methanol in dichloromethane as the eluent and the desired band was eluted with ethyl acetate (0.05% acetic acid). The solvent was removed *in vacuo* to give the *hydroxy acid* (**200**) (55.1 mg, 73%); data reported earlier.

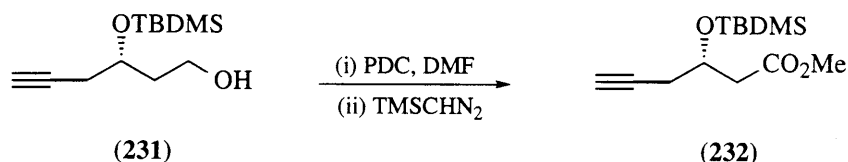
(*S*)-3-[(*tert*-Butyldimethylsilyl)oxy]hex-5-yn-1-ol (231).



To a stirred solution of alkyne (**226**) (800 mg, 2.30 mmol) in dichloromethane (8 ml) was added water (480 μ l) followed by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (783 mg, 3.45 mmol). The mixture was stirred at room temperature for 0.5 h. Saturated aqueous sodium hydrogen carbonate (15 ml) was added and the mixture was extracted with ether (4 x 25 ml). The combined organic extracts were washed with saturated sodium carbonate (15 ml) and brine (15 ml) then dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude mixture was purified by flash column chromatography.

Elution with 10% ether in 40-60 petroleum ether yielded the required alcohol (**231**) as a colourless oil (383 mg, 73%); $[\alpha]_D^{24} +30.6$ (*c* 1.76 in CHCl_3) all other data were consistent with (*R*)-3-[(*tert*-butyldimethylsilyl)oxy]hex-5-yn-1-ol (**223**)

(*S*)-3-[(*tert*-Butyldimethylsilyl)oxy]hex-5-ynoic acid methyl ester (232**).**

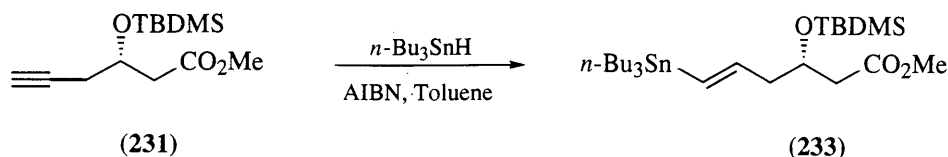


To a solution of the alcohol (**231**) (320 mg, 1.4 mmol) in DMF (5 ml) at room temperature was added pyridinium dichromate (3.17 g, 8.42 mmol, 6 eq). Stirring was maintained for 18 h then water (20 ml) was added and the mixture was extracted with ether (4 x 30 ml). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo*.

To a stirred solution of crude product in methanol/toluene mixture (4 ml, 1:1) at room temperature was added trimethylsilyldiazomethane (1.05 ml, 2.1 mmol, 2 M solution in hexane) and the stirring was maintained for 2 h. The solvent was evaporated *in vacuo* and the crude product was purified by flash column chromatography.

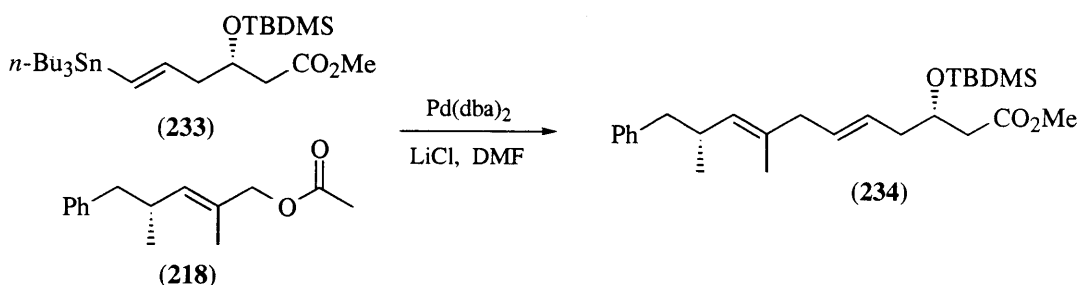
Elution with 2% ether in 40-60 petroleum ether gave the desired *methyl ester* (**232**) as a colourless oil (246 mg, 69%); $[\alpha]_D^{24} +38.2$ (*c* 1.43 in CHCl_3) and all other data were consistent with (*R*)-3-[(*tert*-butyldimethylsilyl)oxy]hex-5-ynoic acid methyl ester (**224**).

(3*S*,5*E*)-3-[(*tert*-Butyldimethylsilyl)oxy]-6-(tri-*n*-butylstannyl)hex-5-enoic acid methyl ester (233).



To a solution of alkyne **(231)** (224 mg, 0.87 mmol) in toluene (2 ml) under nitrogen atmosphere at room temperature was added tributyltin hydride (0.26 ml, 0.96 mmol, 1.1 eq) and a solution of azobis(isobutyronitrile) (0.86 mg, 5.2 μ mol) in toluene (0.2 ml). The mixture was stirred at 120 °C for 4 h then concentrated *in vacuo* to give the crude product which was purified by thin layer chromatography using 2% ethyl acetate in 40-60 petroleum ether as the eluent. The desired band was eluted with ethyl acetate and the solvent was removed *in vacuo* to give the *vinylstannane* **(233)** as a colourless oil (325 mg, 68%); $[\alpha]_D^{21} +21.5$ (*c* 1.0 in CHCl_3) and all other data were consistent with (3*R*,5*E*)-3-[(*tert*-butyldimethylsilyl)oxy]-6-(tri-*n*-butylstannyl)hex-5-enoic acid methyl ester **(222)**.

(3*S*,5*E*,8*E*,10*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-8,10-dimethyl-11-phenylundeca-5,8-dienoic acid methyl ester (234).



To a mixture of unsaturated acetate **(218)** (51 mg, 0.22 mmol), vinylstannane **(233)** (120 mg, 0.22 mmol), and lithium chloride (28 mg, 0.66 mmol, 3 eq) in DMF (1 ml) was added bis(dibenzylideneacetone)palladium(0) ($\text{Pd}(\text{dba})_2$) (6.3 mg, 0.01 mmol, 5 mol%). The mixture was stirred at 23 °C for 48 h then partitioned between water (10 ml) and ethyl acetate (3 x 15 ml). The combined organic extracts were washed with saturated aqueous sodium chloride, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude compound was purified by flash column chromatography.

Elution with 1% ether in 40-60 petroleum ether yielded the required *diene* **(234)** as a colourless oil (53 mg, 56.4%); $[\alpha]_D^{21} +5.5$ (*c* 2.5 in CHCl_3); δ_{H} (300 MHz) 7.21 (5H, m, Ph), 5.46-5.30 (2H, m, 5-H, 6-H), 4.99 (1H, dq, *J* 8.9, 1.2, 9-H), 4.15 (1H,

The crude product was applied on a silica plate, developed in 10% methanol in dichloromethane and the desired band was eluted with ethyl acetate (0.05% acetic acid). The solvent was removed *in vacuo* to give the *hydroxy acid* (**235**) as a colourless oil (18.4 mg, 64%); $[\alpha]_D^{21}$ -6.0 (*c* 1.8 in CHCl_3); $\nu_{\text{max}}(\text{film})/\text{cm}^{-1}$ 3684 (Free OH), 3404 (Bonded OH), 1710 (C=O), 1516 (C=C), 1422 (C=C), 1215 (C-O); δ_{H} (300 MHz) 7.20 (5H, m, Ph), 5.48 (1H, dt, *J* 15.0, 6.6, 6-H), 5.35 (1H, dt, *J* 15.0, 6.6, 5-H), 4.99 (1H, dq, *J* 9.2, 1.3, 9-H), 4.08 (1H, m, 3-H), 2.68-2.43 (7H, m, 2-H₂, 7-H₂, 10-H, 11-H₂), 2.25 (2H, t, *J* 6.6, 4-H₂), 1.38 (3H, d, *J* 1.3, 8-CH₃), 0.95 (3H, d, *J* 6.4, 10-CH₃); δ_{C} (75 MHz) as for (**200**); *m/z* (CI) 301 [(M-H)⁺, 26%], 283 (46), 255 (10), 199 (10), 159 (50), 131 (30), 119 (48) and 91 (100) [Found: (CI) MH⁺, 303.1971. C₁₉H₂₆O₃ requires *MH*, 303.1960].

Biological Experimental.

Culture conditions.

Seed cultures.

Phoma sp C2932 (strain PA13) was stored as a spore suspension (5% w/v, 1.5 ml) in glycerol in liquid nitrogen. Each thawed suspension was used to inoculate a seed flask containing 50 ml of SC4 medium. The medium consisted of 100 g lactose, 20 g cotton seed flour and 200 ml of a 0.5% w/v solution of Junlon PW110 in 1000 ml of distilled water and was autoclaved at 15 psi and 121 °C for 20 minutes. The seed flask was incubated on a rotary shaker (250 rpm) at 25 °C for 3 days.

Production cultures.

Aliquots (1 ml) of seed culture were used to inoculate production flasks containing 50 ml of SC5 medium. The medium consisted of 100 g of lactose and 20 g of cotton seed flour in 1000 ml of deionised water. The medium was autoclaved at 15 psi and 121 °C for 20 minutes. The production cultures were incubated on a rotary shaker (250 rpm) at 25 °C.

Incorporation studies.

The test compounds were administered in the minimum amount of ethanol (except sodium acetate which was dissolved in sterile water) to five flasks of the production cultures on days 3, 4 and 5 at a total concentration of 2 mmol/l. The cultures were harvested and the metabolites isolated on day 7.

Growth production studies.

Three flasks of the production cultures were harvested every 24 h from day 2 until day 10 and the metabolites were isolated, methylated and purified (as described below).

Culture conditions for ATCC 20986.

Seed culture.

Zaragozic acid A producing culture, MF5453 (ATCC20986), was stored as frozen mycelia in a seed medium containing 10% glycerol. The seed medium consisted of tomato paste, 40 g/l; corn steep liquor, 5 g/l; D-glucose, 10 g/l; oat flour, 10 g/l; and 10 ml/l of a trace mineral mixture composed of FeSO₄·7H₂O, 1.0g; MnSO₄·H₂O, 1.0 g; CuCl₂·2H₂O, 0.025 g; CaCl₂·2H₂O, 0.1 g; H₃BO₃, 0.056 g; (NH₄)₆Mo₇O₂₄·4H₂O, 0.019 g; and ZnSO₄·7H₂O, 0.2 g, dissolved in 1.0 l of 0.6 M HCl, and adjusted to pH 6.8 before autoclaving. Growth was initiated by inoculating 50 ml of seed medium in a

250 ml non-baffled Erlenmeyer flask with 1.5 ml of the frozen cells and incubating the culture at 220 rpm and 25 °C for 3 days.

Production culture.

Production flasks each containing 50 ml of the seed medium were inoculated with 1 ml of the seed culture and incubated at 220 rpm and 25 °C.

Washed cells incubations.

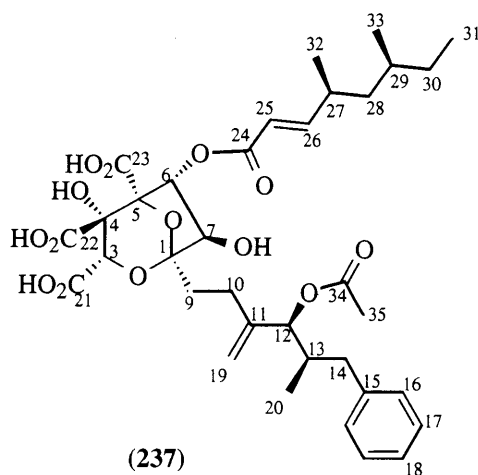
After 48-96 h of growth of the production cultures, the mycelia were separated from the growth medium by centrifugation (9000 g, 15 minutes) or filtration, resuspended to the original volume in sterilised deionised water, and homogenised with a homogeniser until large clumps disappeared (ca. 20 s, low speed). The mycelia were filtered again and resuspended to the original volume in water. 15 ml of the resuspended cells was added to an equal volume of incubation buffer in a 250 ml Erlenmeyer flask. The incubation buffer contained 30 g/l sucrose and 20 mM of MES buffer, pH 6.1.

Isolation procedure.

To each culture flask was added acidified acetonitrile (150 µl H₂SO₄/l) (50 ml) and left to stand for 0.5 h. The mycelia were removed by filtration and washed with distilled water. The acetonitrile was removed from the filtrate under reduced pressure, the remaining aqueous phase was acidified to pH 1-2 with concentrated sulfuric acid and extracted with ethyl acetate. The combined organic extracts were washed with 5% aqueous sodium hydrogen carbonate solution. The combined base washes were then acidified to pH 1 with concentrated sulfuric acid and extracted with ethyl acetate, the combined organic extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude extract was dissolved in methanol and ethereal diazomethane was added dropwise until nitrogen evolution ceased. Excess diazomethane was removed by bubbling nitrogen through the solution. The solution was then concentrated *in vacuo* and the crude product was purified by flash chromatography.

Elution with 40% ethyl acetate/petroleum ether returned S1 trimethyl ester⁴⁰ as a colourless gum. δ_{H} (300 MHz), 7.21 (5H, m, 16-H₂, 17-H₂, 18-H), 6.84 (1H, dd, *J* 16.0, 8.5, 26-H), 5.82 (1H, d, *J* 2.0, 6-H), 5.75 (1H, d, *J* 15.5, 25-H), 5.27 (1H, s, 3-H), 5.10 (1H, d, *J* 5.0, 12-H), 5.00 (1H, s, 19-H), 4.98 (1H, s, 19-H), 4.05 (1H, d, *J* 2.0, 7-H), 3.93 (3H, s, 22-OCH₃), 3.81 (3H, s, 23-OCH₃), 3.76 (3H, s, 21-OCH₃), 2.70 (1H, dd, *J* 13.5, 5.0, 14-H), 2.40-2.32 (4H, m, 10-H₂, 14-H, 27-H), 2.20-2.12 (3H, m, 9-H₂, 13-H), 2.05 (3H, s, 35-H₃), 1.40-1.22 (3H, m, 28-H, 29-H, 30-H), 1.18-1.10 (2H, m, 28-H, 30-H), 1.04 (3H, d, *J* 6.5, 32-H₃), 0.85 (9H, m, 20-H₃, 31-H₃, 33-H₃); δ_{C} (75 MHz) 170.2 (34-C), 169.6 (22-C), 166.9 (22-C, 24-C), 165.4 (23-C), 157.9 (26-C), 145.5 (11-C), 140.4 (15-C), 129.1 (16-C₂), 128.3 (17-C₂), 125.9

(18-C), 118.0 (25-C), 111.7 (19-C), 105.9 (1-C), 88.8 (5-C), 81.8 (7-C), 81.5 (6-C), 79.0 (12-C), 75.6 (3-C), 74.5 (4-C), 53.8 (22-OC), 52.9 (23-OC), 52.6 (21-OC), 43.2 (28-C), 39.9 (14-C), 36.8 (13-C), 34.5 (27-C), 34.1 (9-C), 31.9 (29-C), 29.7 (30-C), 25.3 (10-C), 21.1 (35-C), 20.3 (32-C), 18.9 (33-C), 13.8 (20-C), 11.1 (31-C); m/z (EI) 732 (M^+ , 1%), 690 (2), 672 (2), 623 (1), 581 (10), 325 (10), 235 (50) and 91 (100).



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