Investigations of the Biosynthesis and Biomimetic Synthesis of Bioactive Natural Products

by

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Declaration

Experimental work contained in this thesis is original research carried out by the author, unless otherwise stated, in the Department of Chemistry at the University of Warwick, between October 2004 and October 2007. No material contained herein has been submitted for any other degree, or at any other institution.

Results from other authors are referenced in the usual manner throughout the text.

Laura J. Montgomery

Date: 24/6/08

Abstract

This thesis describes work towards the biomimetic synthesis and understanding the biosynthesis of two families of natural products: prodiginines and quartromicins.

Prodiginines are a large family of red pigmented tripyrrole antibiotics. Although they have not been used clinically, the promising anti-cancer, immunosuppressive and anti-malarial activity they display at non-toxic doses has generated renewed interest in their utilisation. The synthesis of an analogue of the proposed pyrrole-2-carboxyl-RedO intermediate in prodiginine biosynthesis has been achieved. The resulting NAC thioester and analogues of it have been used to investigate the prodiginine biosynthetic pathway in *Streptomyces coelicolor*, and to examine the production of prodiginine analogues by mutasynthesis.

Quartromicins, novel anti-viral antibiotics, are a structurally unique group of spirotetronate natural products produced by *Amycolatopsis* species. They are unusual symmetric macrocyclic compounds which possess a 32-membered carbocyclic structure with four spirotetronic acid units connected by enone or dienone linkers in a head-to-tail fashion. These macrocyclic compounds are intriguing because they have alternating *endo-* and *exo-* spirotetronic acid units, with the opposite "corners" being identical. Although the quartromicins have therapeutic potential, very little is known about their biosynthesis. In this research a biosynthetic pathway to the quartromicins has been proposed based on hypothetical pathways to related natural products. The synthesis of the two putative key intermediates in quartromicin biosynthesis has been achieved. An improved method for the synthesis of exomethylene tetronates has been developed, and

novel rearrangements have been discovered. The two putative key intermediates have been used to investigate the biomimetic synthesis of the carbon skeleton of the quartromicin algycone, and mass spectrometric evidence for formation of homo- and heterodimers, and a heterotetramer of the key intermediates has been obtained.

List of Abbreviations

1,3-BPG	1,3-bisphosphoglycerate
6DeB	6-deoxyerythronolide B
A	adenylation
ACP	acyl carrier protein
AD	asymmetric dihydroxylation
AIBN	2,2'-azobisisobutyronitrile
AIDS	acquired immune deficiency syndrome
AMV	avian myeloblastosis virus
AT	acyltransferase
ATP	adenosine triphosphate
AZT	3-azido-2',3'-dideoxythymidine
B.C.	before christ
BLAST	basic local alignment search tool
BOC	*tert*-butyloxycarbonyl
BuLi	butyl lithium
C	condensation
CD^{4+}	T cells expressing CD4 protein
CoA	coenzyme A
COSY	correlation spectroscopy
CPE	cytopathic effect
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DCE	1,2-dichloroethane

DCM	dichloromethane
DEBS	6-deoxylerythronolide B synthase
DH	dehydratase
DIBAL-H	diisobutyl aluminum hydride
DMAP	4-dimethylaminopyridine
DMF	dimethyl formamide
DMF-DMA	dimethyl formamide dimethyl acetyl
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dppe	1,2-bis(diphenylphosphino)ethane
E	epimerisation
EDCI	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
ER	enoylreductase
FAB-MS	fast atom bombardment mass spectroscopy
FAD	flavin adenine dinucleotide
FAS	fatty acid synthase
FMN	flavin mononucleotide
HIV	human immunodeficiency virus
HMBC	heteronuclear multiple bond connectivity
HMDS	hexamethyldisilazide
HMPA	hexamethylphosphoramide
HMQC	heteronuclear multiple quantum coherence
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HSV-1	herpes simplex virus type 1

HTLV	human T-cell lymphotrophic virus
IL-2	inter-leukin-2
IMDA	intramolecular Diels-Alder
IR	infra-red
JAK-3	Janus Kinase 3
KAPA	7-keto-8-aminopelargonic acid
KR	ketoreductase
KS	β-ketoacyl synthase
LC-MS	liquid chromatography mass spectrometry
LDA	lithium diisopropylamide
LNKS	lovastatin nonaketide synthase
LTMP	lithium tetramethylpiperidine
M	molar
MBC	4-methoxy-2,-2'-bipyrrole-5-carboxaldehyde
MDCK	Madin-Darby canine kidney
Min	minutes
MM	molecular mechanics
MOM	methoxymethyl
MPS	macrophomate synthase
MS	mass spectrometry
Ms	mesityl
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NMR	nuclear magnetic resonance
NRPS	non-ribosomal peptide synthetase

OAS	oxoamine synthase
PCP	peptidyl carrier protein
PEPS	phosphoenol pyruvate synthase
PKS	polyketide synthetase
PLA_2	phospholipase A2
PLP	pyridoxal 5'-phosphate
PPDK	phosphate pyruvate dikinase
PPTase	phosphopantophenyl transferase
PPTS	pyridinium *para*-toluene sulfonate
QM	quantum mechanics
r.t.	room termperature
SAM	S-Adenosylmethionine
SAR	structure activity relationships
Sat.	saturated
sp.	species
TASF	tris(dimethylamino)sulfonium difluorotrimethyl silicate
TBAF	tetra-*n*-butylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TE	thioesterase
t-	tertiary
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMPH	2,2,6,6-tetramethylpiperidine

TNF-α and -β	tumour necrosis factor
Tr	trityl
UP	2-undecylpyrrole

Chapter 1: General Introduction

1.1: Natural Products

Natural products are chemical compounds or substances that are produced by living organisms such as plants, animals and micro-organisms. Many of these abundant natural products are produced by secondary metabolism and often exhibit biological or pharmacological activities. For example, penicillin G is an antibiotic produced by the fungus *Penicillium notatum* which can be used to treat streptococcal, pneumococcal, gonococcal and meningococcal infections.[1] An amazing variety and number of natural products are found in nature and more than 100,000 secondary metabolites have been characterised to date. Natural products fall into several main categories including complex polyketides, aromatic polyketides, peptides, terpenes and aminoglycosides and alkaloids (Figure 1).

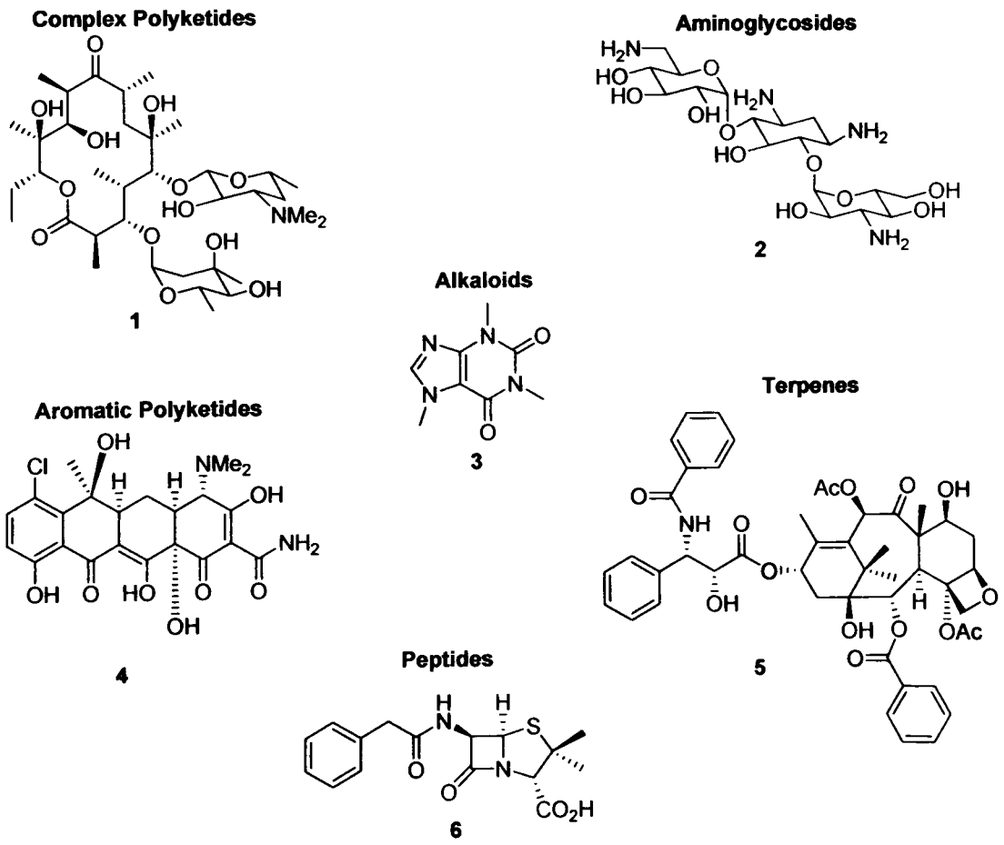

Figure 1: Natural products classes

including complex polyketides: erythromycin A **1**, aminoglycosides: kanamycin **2**, alkaloids: caffine **3**, aromatic polyketides: chlorotetracycline **4**, terpenes: taxol **5**, and peptides: benzylpenicillin **6**

Erythromycin A **1** (Figure 2), first isolated in 1952 from *Saccharopolyspora erythraea,* belongs to the class of complex polyketides. Members of this family, which are characterised by macrocycles containing 12, 14 or 16 atoms, show an intriguing common structural and stereochemical relationship. Erythromycin A is used clinically against infections caused by Gram-positive bacteria, pulmonary infections such as legionnaires disease and as an alternative for patients allergic to penicillin.[2] It is biosynthesised by a polyketide synthase (PKS) which catalyses the sequential condensation of one unit of propionyl-CoA and six units of methylmalonyl-CoA to give 6-deoxyerythronolide B (6DeB), then it is elaborated by a series of tailoring enzymes which include regiospecific hydroxylases, glycosyl transferases and methyl transferases.[2]

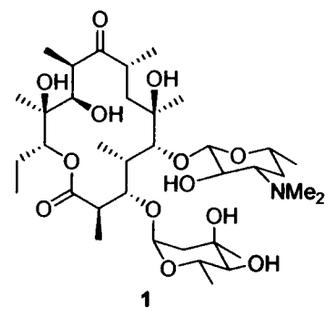

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Figure 2: Erythromycin A

Gramicidin S **7** was first isolated in 1942 from *Bacillus brevis.* Gramicidin is toxic internally to the human body inducing haemolysis at lower concentrations than bacterial cell death. It acts on bacterial membranes, increasing permeability resulting in a loss of barrier function. It is a mixture of cyclic peptides biosynthesised by a non-ribosomal peptide synthetase (NRPS) system, which constructs a pentapeptide by activating and condensing in turn L-phenylalanine (which undergoes epimerization to D-phenyalanine after activation), L-proline, L-valine, L-ornithine and L-leucine, and finally catalysing cyclodimerisation.[1,3]

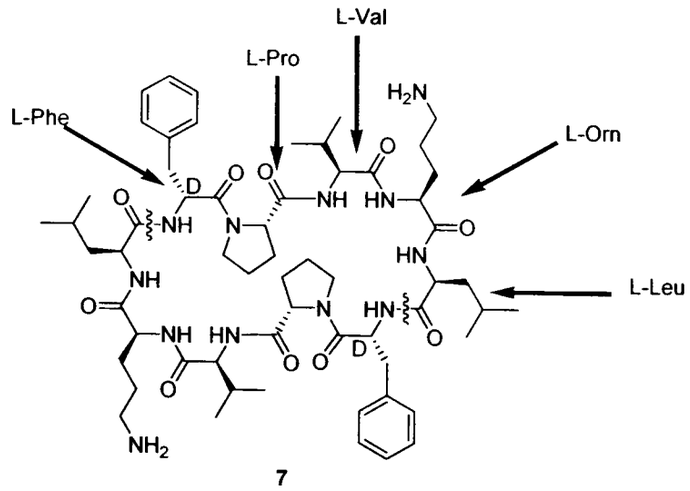

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Figure 3: Gramicidin S

1.2: Biosynthesis of Natural Products

The most important building blocks employed in the biosynthesis of secondary metabolites can be derived from intermediates from the acetyl coenzyme A, shikimic acid, mevalonic acid and 1-deoxyxylulose-5-phosphate pathways as well as amino acids.

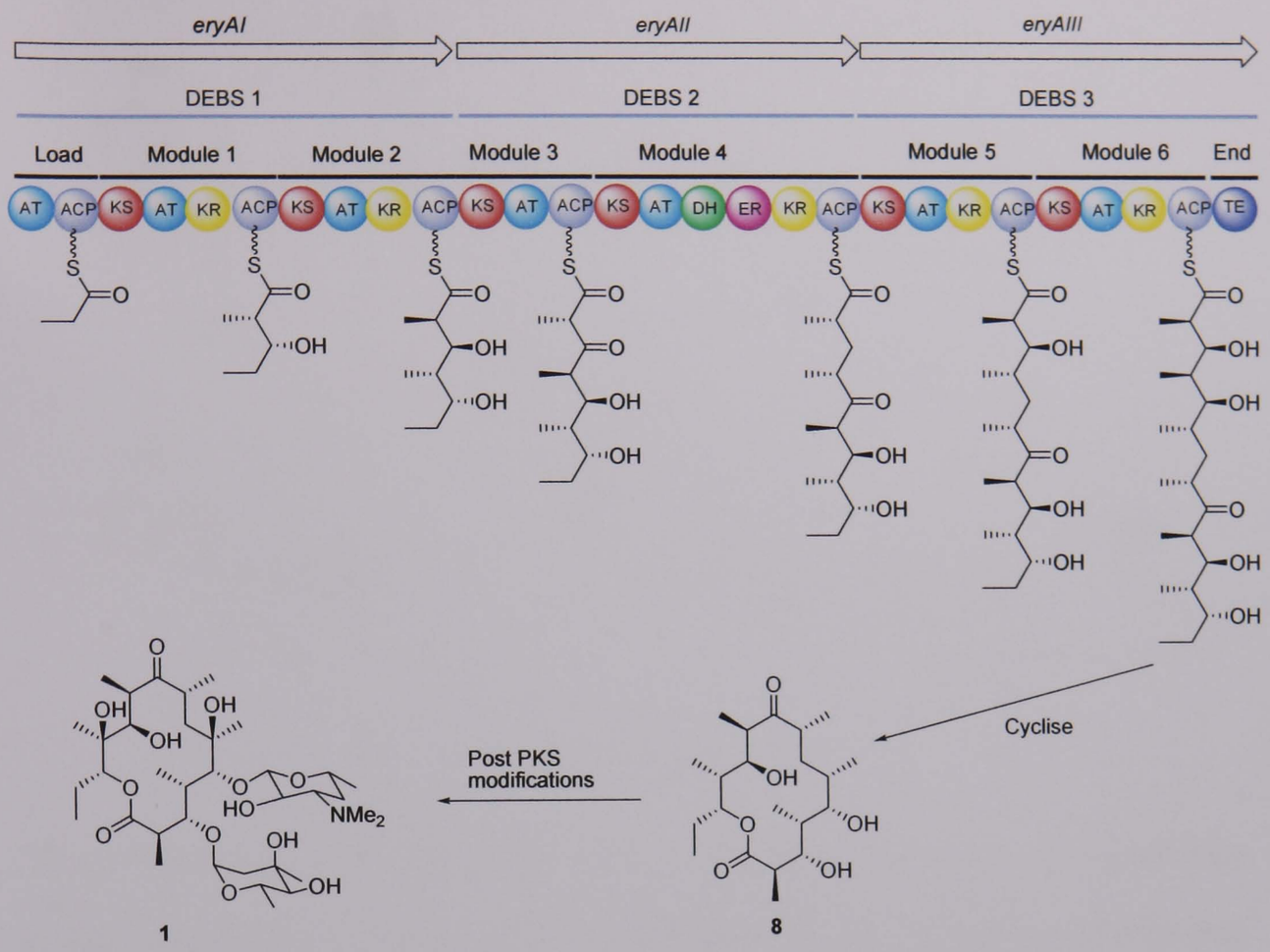
Natural products utilise a broad range of enzymes in biosynthesis including nonribosomal peptide synthetases, siderophore synthetases, fatty acid synthases, polyketide synthases, non-heme iron-dependent and flavin-dependent oxygenases, acyl transferases, pyridoxal 5'-phosphate (PLP)-dependent α-oxoamine synthases and decarboxylases, hydrolases, and terpene synthases. Two important types of natural product biosynthetic systems are described below.

1.2.1: Polyketide Synthases (PKSs)

Detailed studies of amino acid sequences and mechanistic similarities in various polyketides synthase enzymes have led to three types of PKSs being distinguished.

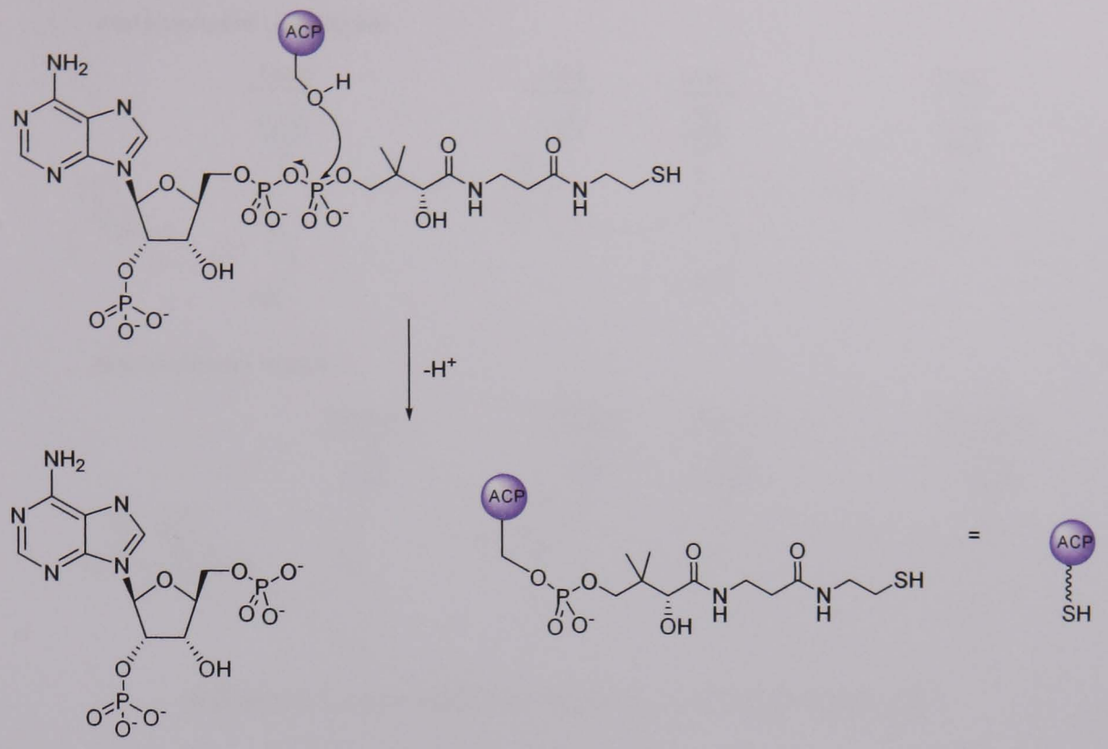
Type I enzymes consist of one or more large multifunctional proteins that possess a distinct active site for every enzyme-catalysed step. Type II enzymes consist of multi-enzyme complexes that carry out a set of repeating reactions. Type III PKSs, leading to natural products such as quinolines and flavonoids, involve a homodimeric condensing enzyme that utilises coenzyme A esters rather than the acyl carrier proteins used by type I and type II enzymes. They also employ a single active site to perform a series of decarboxylation, condensation, cyclisation and aromatisation reactions. Usual starter units for polyketide biosynthesis are propionyl-CoA or acetyl-CoA and the main extender units are malonyl-CoA and methylmalonyl-CoA.[1]

6-Deoxyerythronolide B synthase (DEBS) is a modular type I PKS involved in erythromycin biosynthesis (Scheme 1) which consists of three large multi-enzymes (DEBS-1, 2, and 3) each encoded for by a giant gene (*eryA*-I, II, and III). It has a linear organisation of six modules, each of which contains the activities for one cycle of chain extension. These modules are broken down into domains. A minimal module contains β-ketoacyl synthase (KS), acyltransferase (AT) and acyl carrier protein (ACP) domains. These would catalyse a two carbon chain extension reactions. After each condensation reaction the oxidation state of the β-carbon is determined by the presence of β-ketoacyl reductase (KR), dehydratase (DH) and enoylreductase (ER) domains. The sequence is finally terminated by a thioesterase (TE) domain, which releases the polyketide from the enzyme. In the case of erythromycin, a propionyl-CoA starter unit is condensed with successive methylmalonyl-CoA units to give a 14-carbon chain which can then be cyclised, giving 6-deoxyerythronolide B **8** (6DeB).[2]



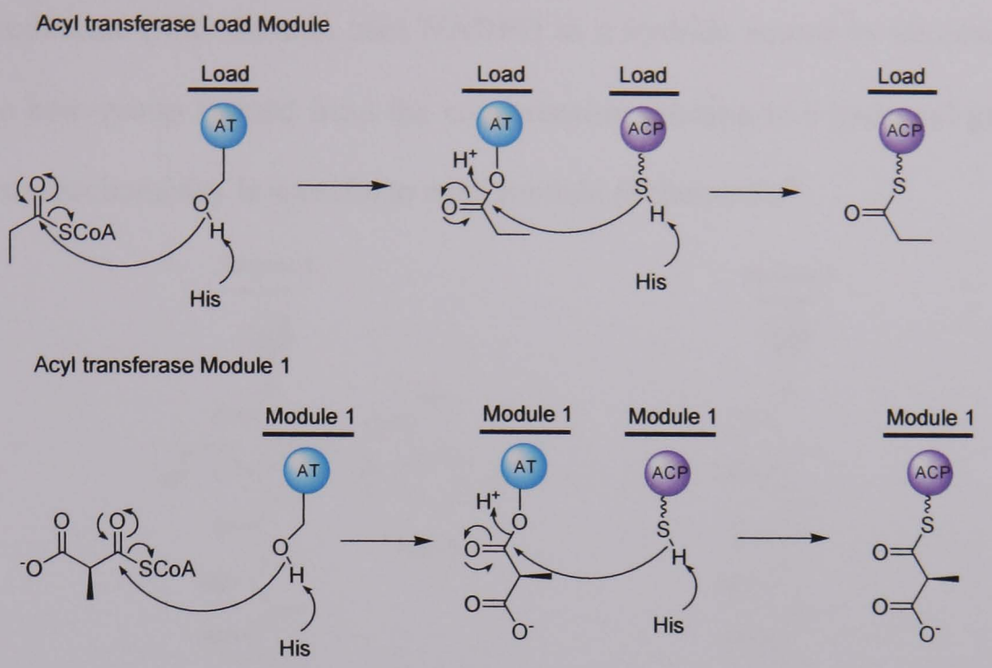
Scheme 1: Erythromycin **1** biosynthesis[2]

Another important enzyme is phosphopantetheinyl transferase (PPTase) which is required to make the ACP domain active (Scheme 2). The conserved serine residue on the ACP is not able to reach all the domains present in each module to allow chain extension and modification. The phosphopantetheinyl arm allows an extension of approximately 20 Å in length and can deliver the substrates to all the different parts of the protein.[1]



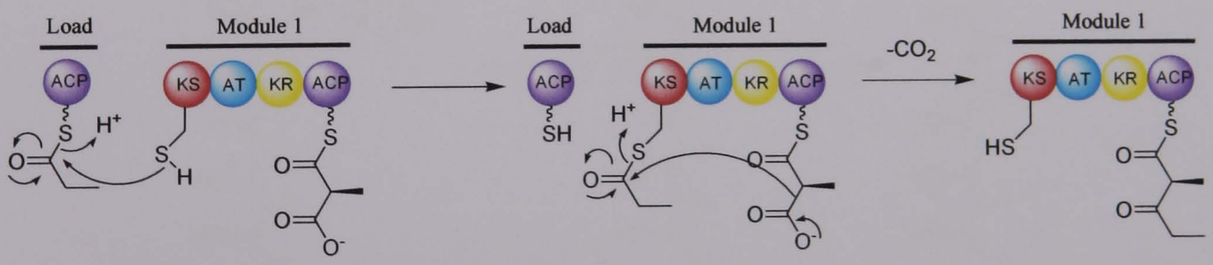
Scheme 2: PPTase activity

The acyltransferase (AT) domain has an active site serine residue which is acylated by an acyl-CoA starter or extender unit. The resulting AT-ester then reacts with the free thiol on the adjacent ACP domain to form the ACP-thioester as shown in Scheme 3. In erythromycin biosynthesis the AT domain in the loading module recognises and loads a propionyl-CoA starter unit followed by transacylation onto the ACP domain. The remaining AT domains catalyse the transfer of methylmalonyl-CoA onto the corresponding ACP domains of the remaining six chain extension modules.



Scheme 3: Load module and Module 1 acyltransferase activity

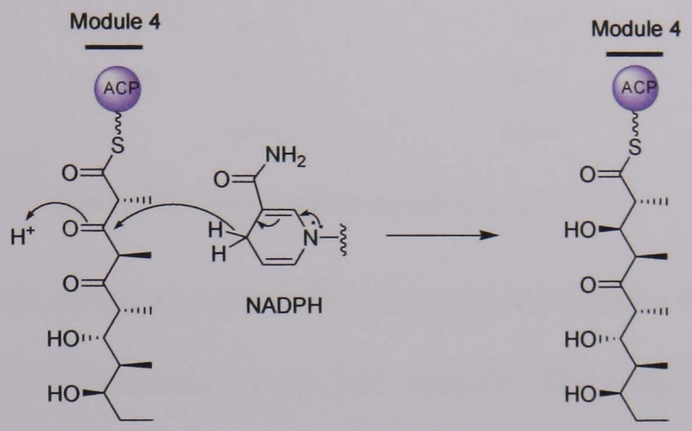
Once the ACP-thioester has been formed, condensation can occur. The ketosynthase (KS) domain catalyses transfer of the acyl portion of the thioester from the ACP domain onto the KS domain. Decarboxylation of methylmalonyl ACP followed by nucleophilic attack on the carbonyl carbon of the KS-thioester, results in the release of the KS-thiol giving a two carbon chain extension (Scheme 4).



Scheme 4: Activity of the ketosynthase domain

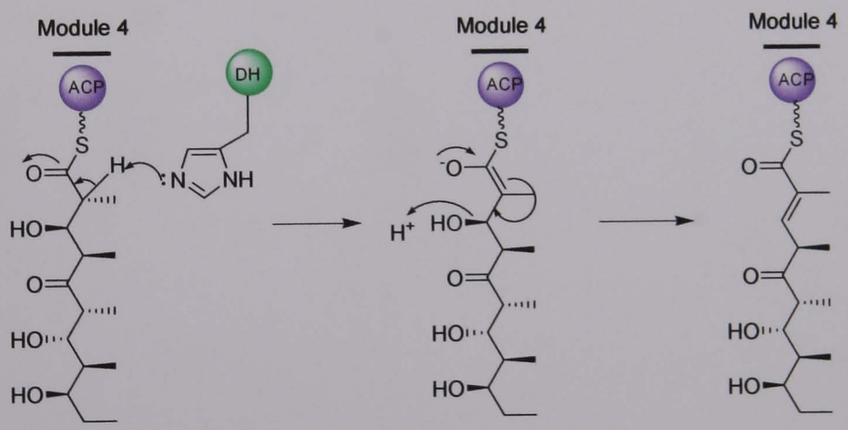
After each condensation, the oxidation state of the β-carbon is determined by the presence of KR, KR + DH, or KR + DH + ER domains. For example in module one only a KR domain is present, but in module four all three domains are present.

The ketoreductase (KR) domain uses NADPH as a hydride source to stereoselectively reduce the keto-group formed from the condensation reaction to a hydroxyl group. The resulting stereochemistry is specific to each module (Scheme 5).[4]



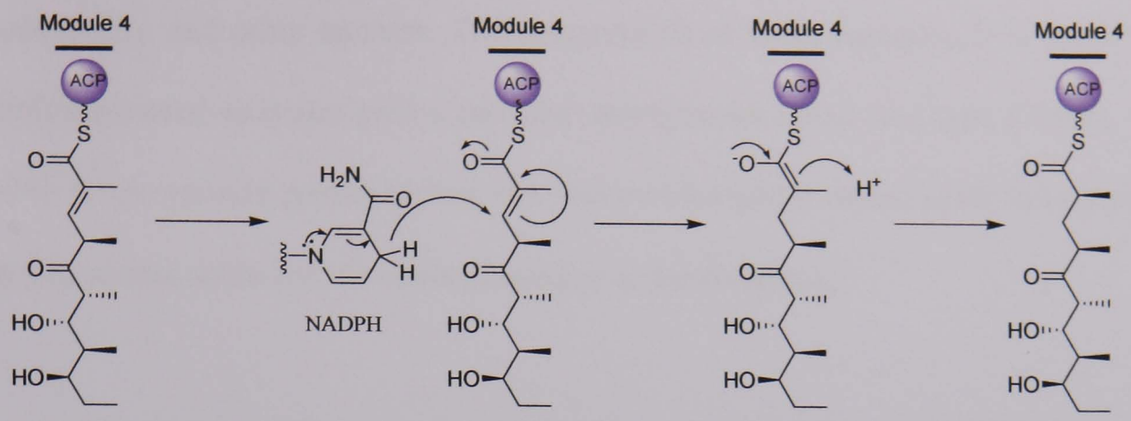
Scheme 5: Activity of the ketoreductase domain

The dehydratase (DH) domain uses an active site histidine residue as a base, catalysing the elimination of water across the β-hydroxy thioester, to give a double bond (Scheme 6).



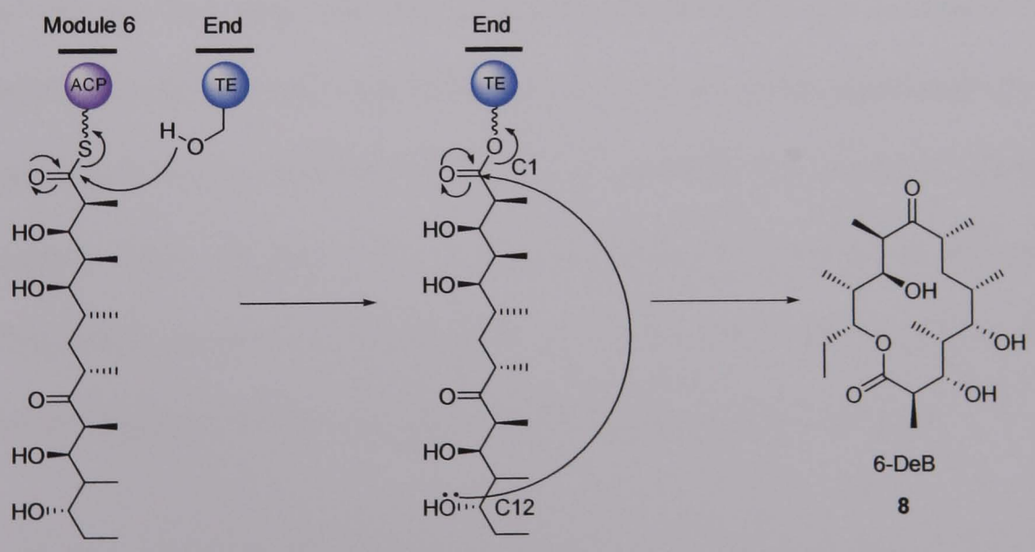
Scheme 6: Activity of the dehydratase domain

The α,β-unsaturated double bond is reduced by the enoyl reductase (ER) domain using another molecule of NADPH to give a saturated thioester chain (Scheme 7).



Scheme 7: Activity of the enoylreductase domain

The final step in the biosynthesis of 6-DeB is the macrocyclisation of the linear chain to release it from the ACP domain in the last module. This step is catalysed by the thioesterase (TE) domain. An active serine residue in the TE domain is acylated with the 14-carbon chain attached to the ACP domain in module six. The C-12 hydroxyl group then attacks the C-1 ester assisted by an active site histidine residue that acts as a general base, resulting in release of the chain from the PKS and formation of the macrocycle **8** (Scheme 8).



Scheme 8: Thioesterase-catalysed cyclisation and release of the fully assembled polyketide chain

1.2.2:Non-Ribosomal Peptide Synthetases (NRPS)

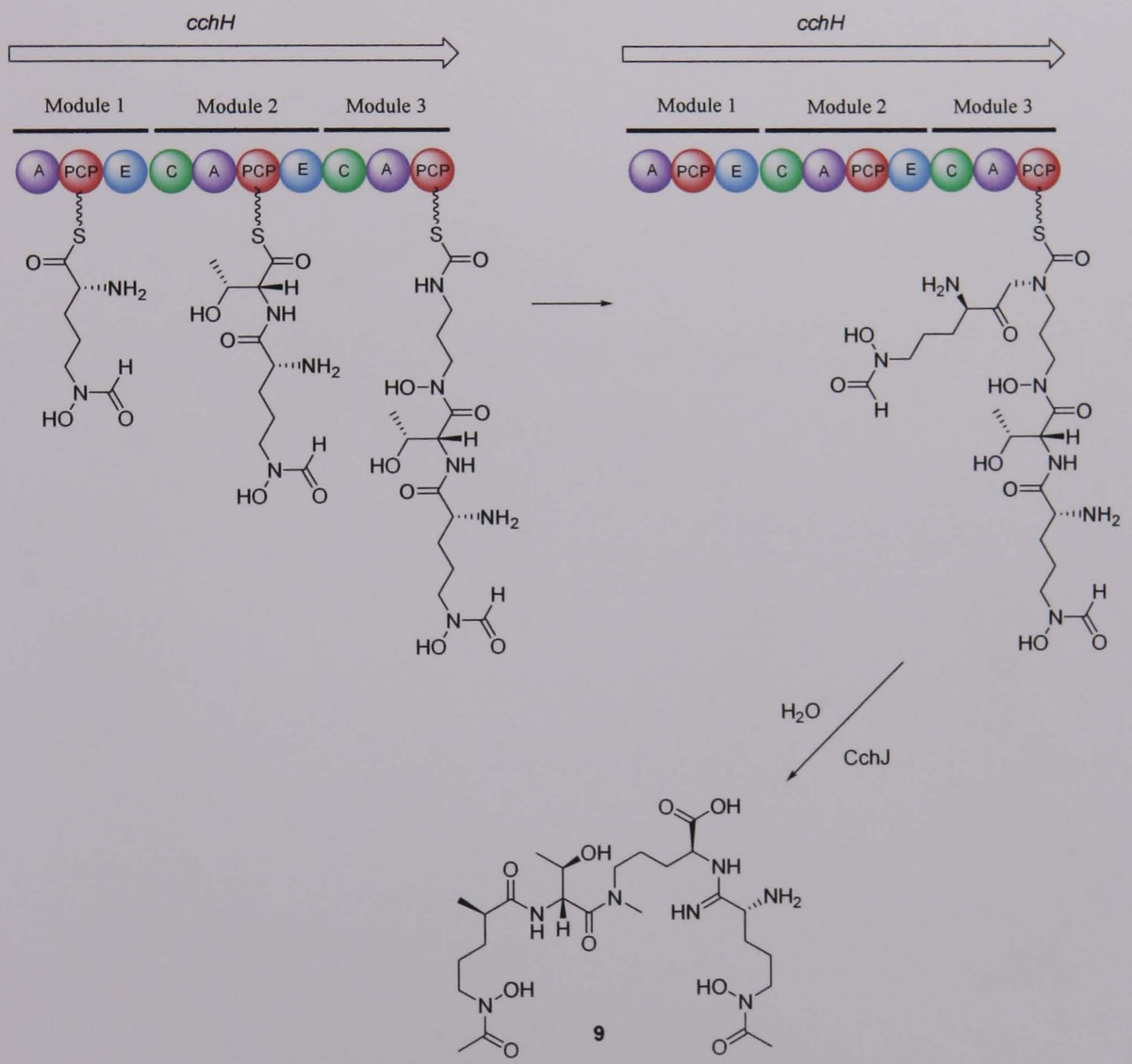
Non-ribosomal peptide synthetases are responsible for the biosynthesis of peptide antibiotics, peptide toxins, many siderophores and modified peptides such as penicillins,

cephalosporins and other lactams. The biosynthesis of these compounds is carried out by multifunctional enzymes with a modular arrangement rather like type I PKSs. They are able to incorporate proteinogenic and non-proteinogenic amino acids into peptides, as well as amino acids with D-stereochemistry at the α-carbon.[1]

The linear sequence of modules usually corresponds to the generated amino acid sequence in the peptide product. A typical module consists of an adenylation (A) domain, a peptidyl carrier protein (PCP) domain and a condensation (C) domain. The A domain activates a specific amino acid as an aminoacyl adenylate which is transferred to the PCP domain forming an aminoacyl thioester. This A domain requires a co-factor ATP for activation of the substrates carbonyl group. Nucleophilic attack by the amino group of a downstream aminoacyl thioester on the PCP bound amino acid is catalysed by the C domain and results in amide bond formation. The PCP is responsible for "holding" the growing chain and uses its phosphopantetheinyl arm to deliver the chain to the various domains. Additional domains, for example an epimerisation (E) domain for epimerising L-amino acids to D-amino acids (probably *via* an enolate intermediate) in the peptide chain, can also occur. A terminal thioesterase domain is responsible for terminating chain assembly and release of the peptide from the enzyme. Cyclisation, hydrolysis or oligomerisation-cyclisation can be catalysed by this domain.[1]

One example of a natural product assembled by an NRPS is the novel siderophore coelichelin **9**, recently isolated and characterised from *Streptomyces coelicolor*.[5,6] *In silico* sequence analysis of the *cch* cluster, which has been shown to encode all of the proteins required for the assembly of the coelichelin molecule, predicted that the CchH NRPS enzyme contains three modules suggesting that coelichelin is a tripeptide, with

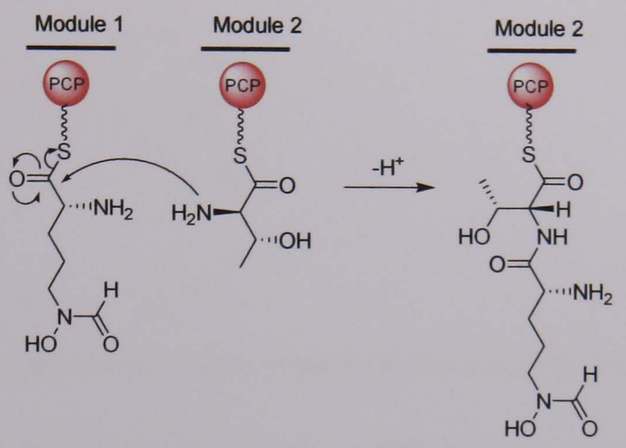
modules one, two and three predicted to incorporate L-δ-*N*-formyl-δ-*N*-hydroxyornithine, L-threonine and L-δ-*N*-hydroxyornithine respectively. However, structural characterisation of coelichelin revealed that it is in fact a tetrapeptide.[6] This is unusual as there is no literature precedent for the formation of a tetrapeptide by a trimodular NRPS. This structure probably arises from iterative use of module one as well as the C domain of module two or three.



Scheme 9: Module organisation of the CchH NRPS catalysing coelichelin **9** biosynthesis

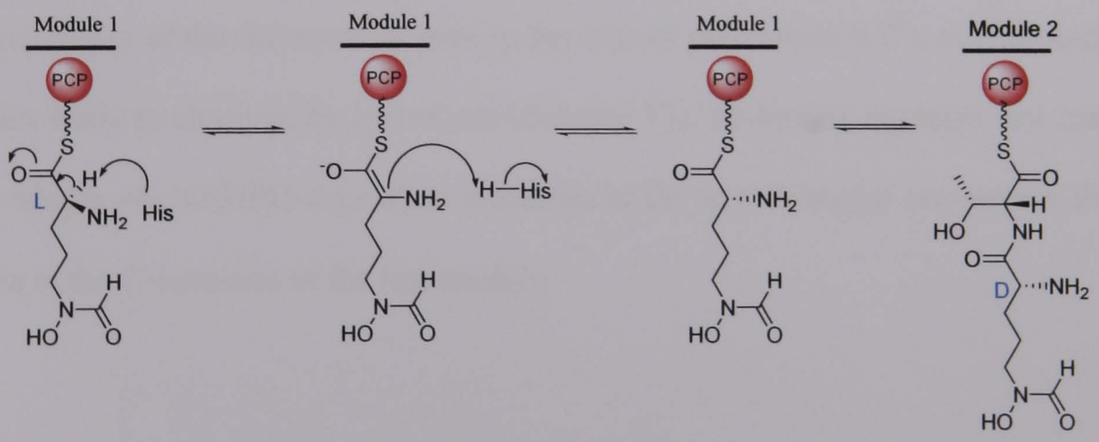
Initially, the required amino acids are selectively recognised by the A domains and activated by reaction with ATP followed by attachment of the phosphopantetheinyl arm of the adjacent PCP domains, which again allows delivery of the thioester to other domains in the module analogous to the PPTase in PKSs (Scheme 2).

The C domains assemble the peptide by catalysing the condensation of the amino acids attached to PCP domains in adjacent modules (Scheme 10).



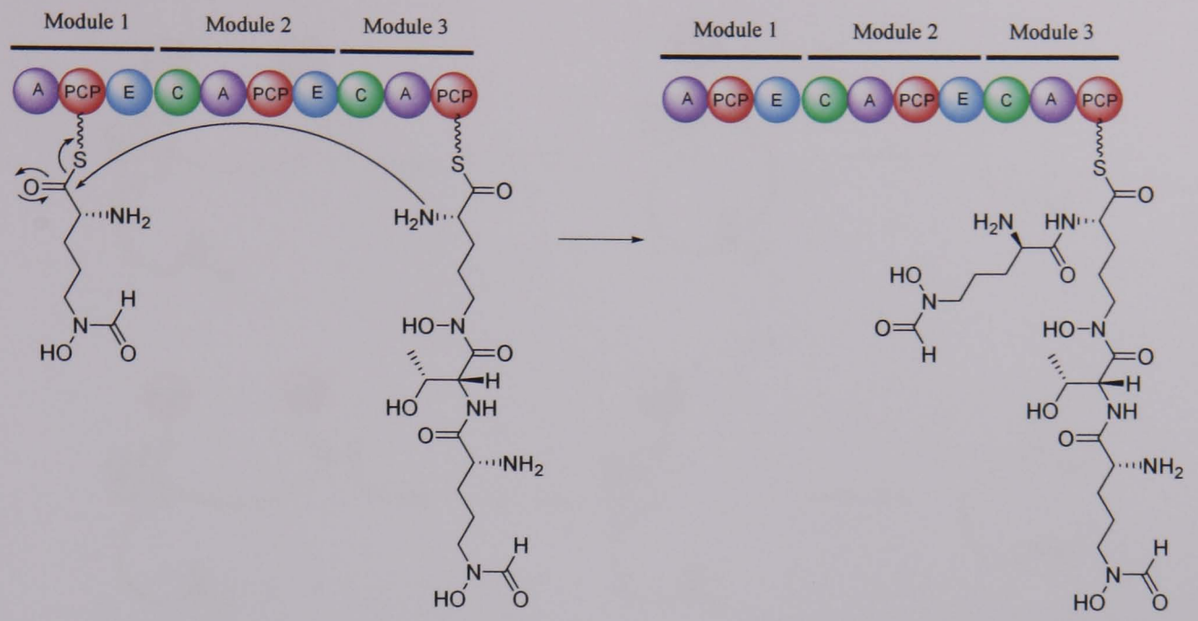
Scheme 10: Condensation of PCP bound amino acids catalysed by the C domain

The epimerisation domain (E) is able to catalyse the racemisation of the L-amino acids *via* an enolate intermediate. The D-epimer is kinetically selected from the mixture by the downstream C domain (Scheme 11).[7]



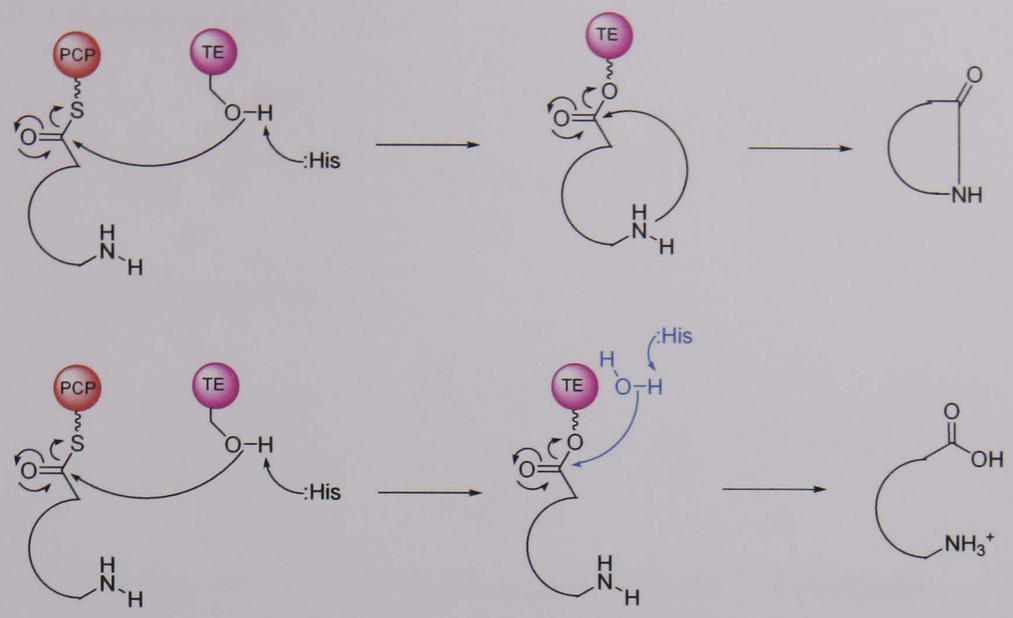
Scheme 11: Epimerisation domain

The experimentally characterised tetrapeptide structure of coelichelin is consistent with the iterative use of the A, PCP and E domains of module one and either the C domain of module two or three (Scheme 12). The alternative possibility of a second NRPS has been ruled out by expressing the *cch* cluster in an heterologous host (*Streptomyces fungicidicus*).[6]



Scheme 12: Iterative domain use and domain skipping believed to occur in coelichelin biosynthesis

In the majority of NRPS systems a thioesterase (TE) domain catalyses the release of the assembled peptide chain from the NRPS, either by cyclisation or hydrolysis.[1] The thioesterase is usually located at the C-terminus of the final module. Transacylation of the active thioester to the serine residue of the thioesterase domain, followed by deprotonation of an amino or hydroxyl group facilitates cyclisation by attack on the carbonyl group of the thioester (as seen in the case of gramicidin S **7**[8]), or deprotonation of water leads to cleavage by hydrolysis (Scheme 13). Reductase domains that catalyse chain release *via* NADPH-dependent reduction of the acyl thioester can replace the TE domain at the C-terminus of the last module.[1]



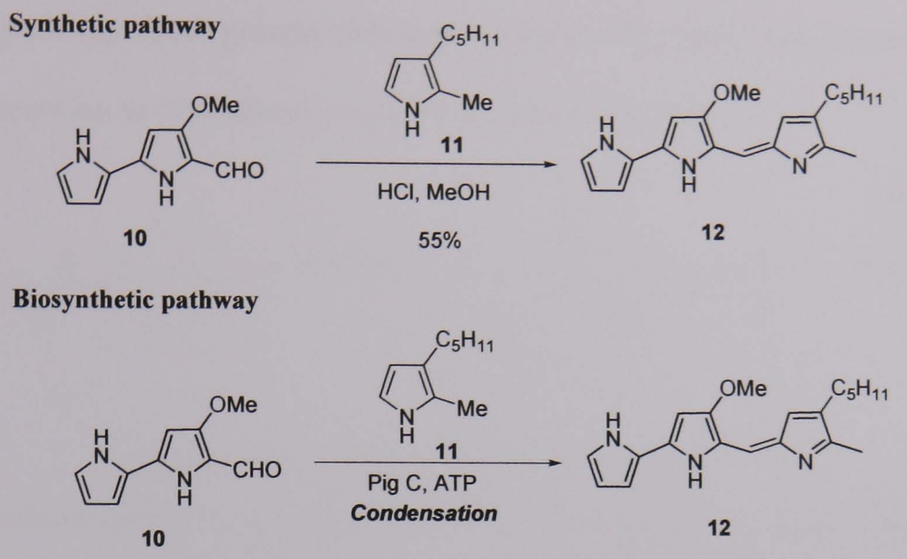
Scheme 13: Chain release by cyclisation or hydrolysis catalysed by the thioesterase domain

However, another unusual feature in coelichelin biosynthesis is that CchH does not contain a thioesterase or a reductase domain at the C-terminus of the final module (Scheme 9) to catalyse cleavage of the thioester bond between the fully assembled peptide and the last PCP domain. In this case, it is predicted that another protein CchJ catalyses the hydrolysis of the peptide to form coelichelin (Scheme 9).[6,9]

1.3: Biomimetic Synthesis

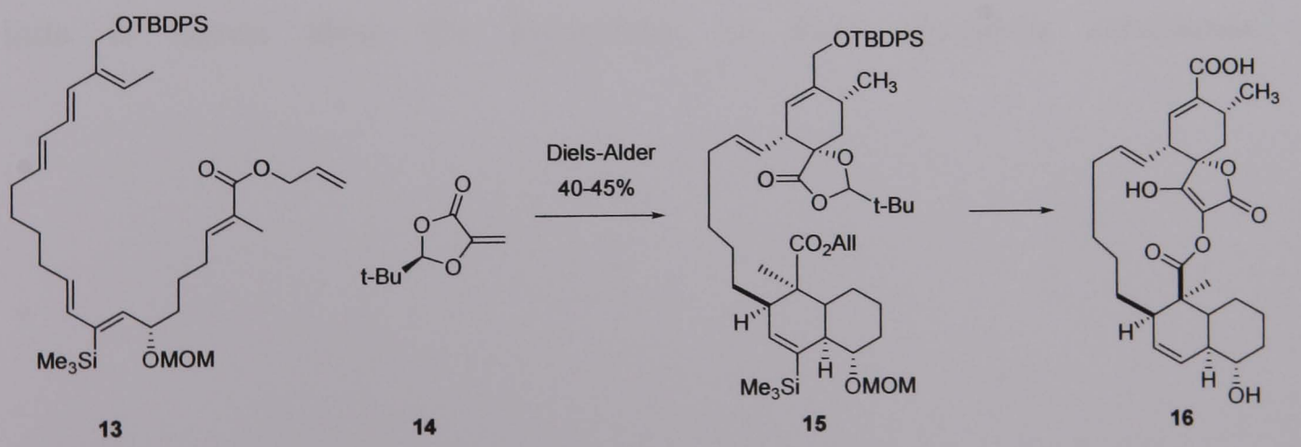
The biomimetic synthesis of a natural product is where one or more steps of a chemical route mimic the equivalent steps in the biosynthetic pathway. Biomimetic synthesis also allows a postulated biosynthetic route to be probed by looking into the chemical feasibility of a key step or steps *in vitro.*

Rapoport and Holden carried out a biomimetic synthesis of prodigiosin **12**.[10] In the final step of their total synthesis there is an acid catalysed condensation of 2-methyl-3-pentylpyrrole **11** with aldehyde **10**, which directly mimics the final proposed condensation step in the biosynthesis (Scheme 14).[11]



Scheme 14: Biomimetic[10] and biosynthetic assembly [11] of prodigiosin

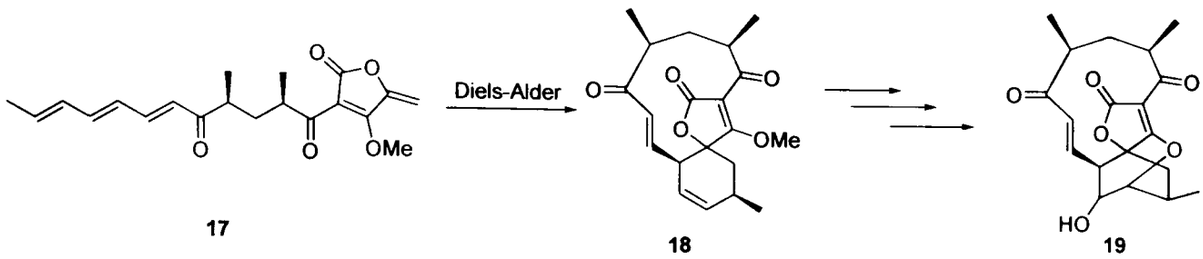
Roush and co-workers reported a biomimetic intra and intermolecular Diels-Alder reaction to complete the synthesis of chlorothricolide **16**, the algycone of chlorothricin.[12,13] Based on the knowledge of the relative reactivity of the alkene components, they chose **13** and **14** as their key synthetic intermediates. The Diels-Alder reaction of **13** and **14** took place at 120°C in toluene with high diastereoselectivity. This provides strong support for the biosynthetic proposal that spirotetronates are synthesised by Diels-Alder reactions (Scheme 15).



Scheme 15: Biomimetic synthesis of chlorothricolide **16**

More recently, both Sorensen and Snider have successfully completed biomimetic syntheses of abyssomicin C **19** (Scheme 16), another spirotetronate natural product.[14,15] It is proposed that this spirotetronate is formed *via* a Diels-Alder cyclisation in the

biosynthetic pathway. Both groups managed to chemically mimic this diastereoselective Diels-Alder reaction to give direct access to the natural product.



17 18 19

Scheme 16: Biomimetic synthesis of abyssomicin C **19**

Diels-Alder conditions; Snider – chloroform, 70°C, 2 days; Sorensen – toluene, 100°C, 4 h

1.4: Aims of the Research Reported in this Thesis

The research reported in this thesis focused on the biosynthesis and biomimetic synthesis of natural products. Initially investigations into the biosynthetic pathway of the prodiginines were undertaken. By understanding the biosynthetic pathway it may be possible to produce analogues of the prodiginines allowing more potent and less toxic drugs to be developed. Secondly, the biosynthetic and biomimetic synthesis of the quartromicins was examined. To date no total synthesis has been accomplished and little is known about the biosynthesis of these fascinating compounds.

Chapter 2: Introduction to Prodiginine Alkaloids

2.1: Introduction to *Streptomyces coelicolor*

Streptomyces coelicolor is a Streptomycete, which is a group of GC-rich gram-positive actinobacteria that reside in the soil. Streptomycetes are well known for producing a large variety of antibiotics. Around two-thirds of the known antibiotics produced by micro-organisms are biosynthesised by actinomycetes and approximately 80% of these are produced by *Streptomyces* spp.[16] Some examples of natural products produced by *S. coelicolor* are shown in Figure 4, giving an indication of the wide variety of natural products that can be produced by one micro-organism.

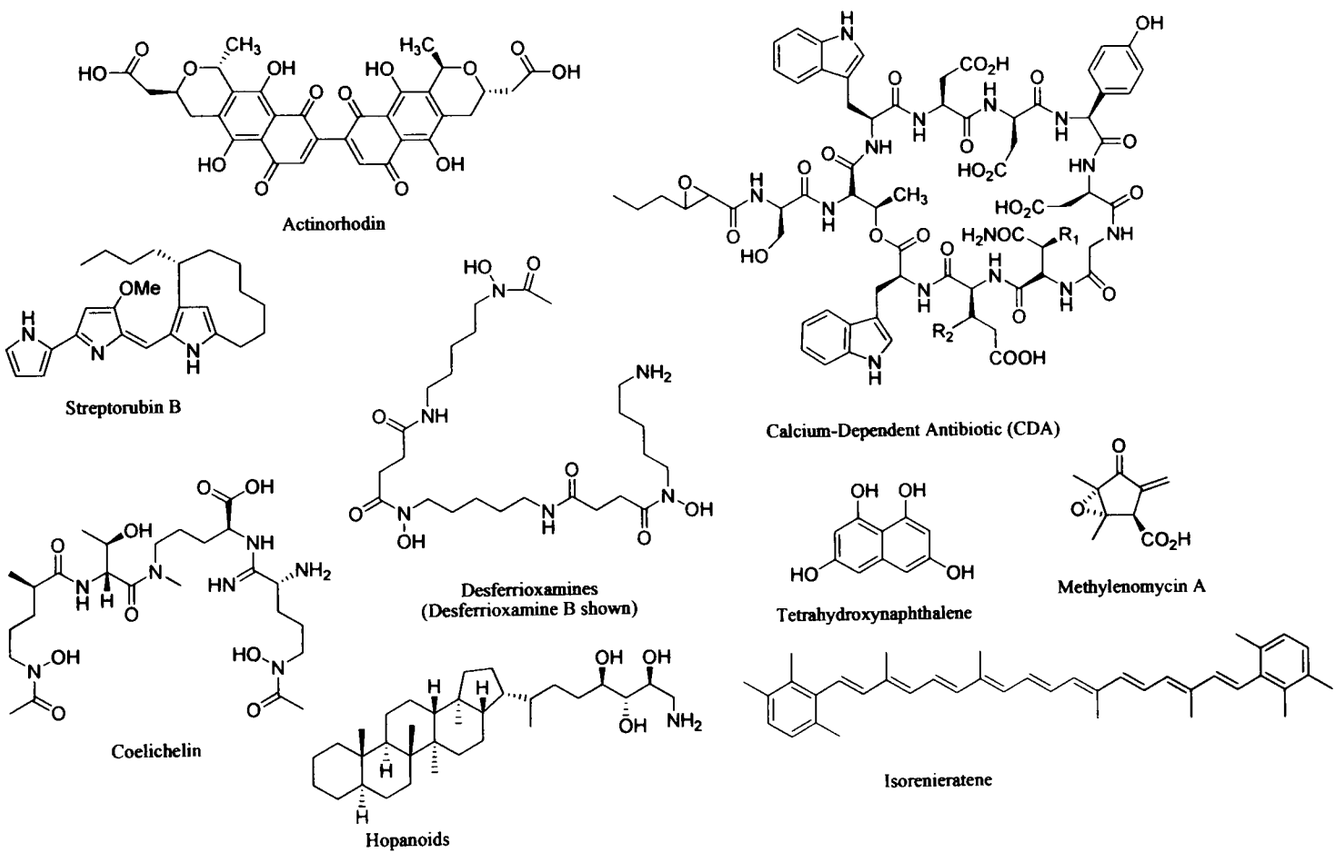


Figure 4: Examples of the number of diverse natural products produced by *S. coelicolor*

2.2: Isolation and Structures of Prodiginines

Prodiginines are a large family of red pigmented antibiotics sharing the same unique structural core as prodigiosin **12**, which are produced by several actinomycetes and other eubacteria. The major pigment of *Serratia marcescens,* originally named prodigiosine, now known as prodigiosin was first isolated in 1902. Many methods have been used for its isolation.[17,18] However, its structure was only determined in 1960 through total and partial synthesis.[10] This discovery led to many other prodigiosin related pigments being isolated.

Prodigiosin is a typical secondary metabolite and its intense red colour is due to the highly conjugated planar pyrrolylpyrromethene chromophore.[19] The prodigiosin pyrrolylpyrromethene skeleton consists of three pyrrole rings of which two are directly linked, generating a bipyrrole unit (pyrrole ring A and the methyoxypyrrole ring B) and the third, a monopyrrole unit (ring C), joined *via* a methene bridge **20** (Figure 5). Gerber suggested molecules with this tripyrrole aromatic moiety should be known as prodiginines.[20]

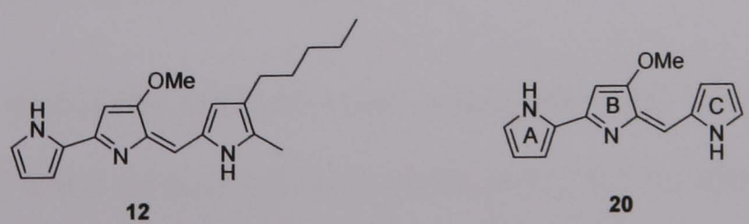


Figure 5: Prodigiosin **12** and the prodigiosin pyrrolylpyrromethene skeleton **20**

There are many structural variations within the prodiginine family. Several of these are produced by actinomycetes, including the linear prodiginine (undecylprodiginine) **21**

originally isolated from *Streptomyces sp.* Y-42, and several cyclic derivatives; butyl-meta-cycloheptylprodiginine (Streptorubin B) **22**, ethyl-meta-cyclononylprodiginine (Streptorubin A) **23**, and methylcyclodecylprodigine **24**. Some more significant structural differences are also seen for example in the anti-tumour antibiotics BE18591 **25** and roseophilin **26**, first isolated in 1998 from *Streptomyces sp. BA18591* and *Streptomyces griseoviridis* respectively,[21,22] which are also considered members of the prodiginine family.[23] The latter of these contains a methoxyfuran in place of the methoxypyrrole ring (Figure 6).

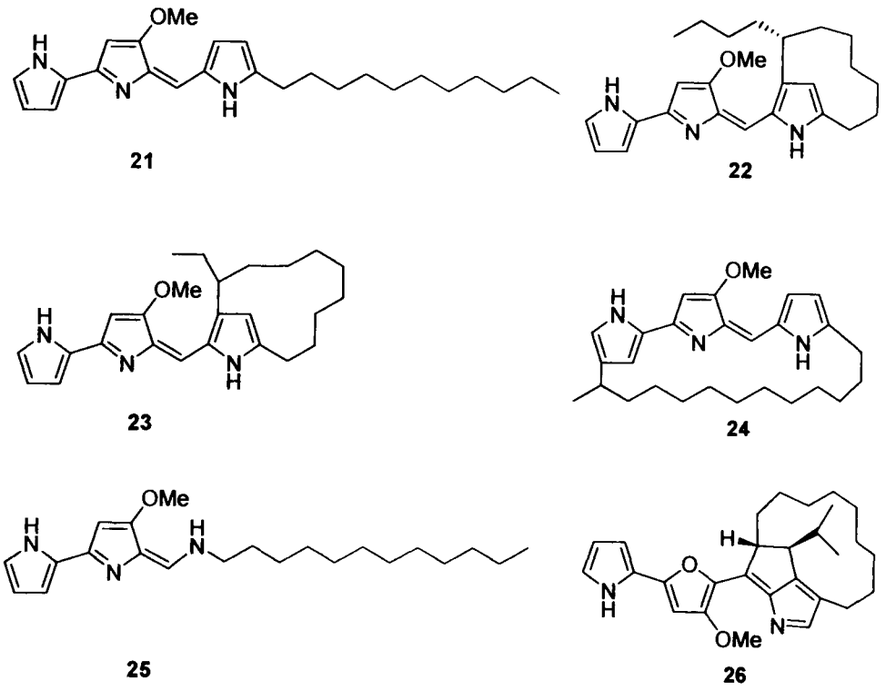


Figure 6: Members of the prodiginine family

2.3: History of the Prodiginine Pigments

The story of the prodiginine pigments began a long time ago. Accounts from Europe recording "blood" found on food date as far back as 322 B.C to Alexander the Great's siege on the city of Tyre. Macedonian troops were disturbed when trickles of "blood" were seen on broken bread and they believed it to be a sign of terrible things to come. A soothsayer Aristander predicted it as a good omen demonstrating the blood to be shed in the besieged town inflicted by Alexander's men, and was rewarded for his prophecy.[24]

Many reports exist of red pigments observed on bread and wafers used for Eucharistic liturgy and gradually there became a link with religious and superstitious beliefs for Jews and Christians. The most famous example, now referred to as the miracle of Bolsena, occurred in 1263. A priest who was struggling with loss of faith noticed "blood" as he celebrated mass on a pilgrimage to Rome. He declared the miracle should be celebrated as Corpus Christi, and it is still celebrated today. However, many blamed maleficent spirits and witchcraft for these events.[24,25]

In the 19th century, Sette and Bizio separately investigated these apparent blood-like traces which led to significant understanding of their formation and massive advances in the understanding of microbiology. Although both Bizio and Sette both made the same mistake and thought a fungus was responsible, they were the first to suggest that a living organism produced the red pigment.[24]

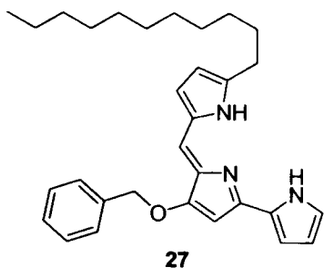
2.4: Biological Activities of the Prodiginine Family

Although the prodiginine family have an extremely broad range of antibiotic activities being active against Gram-positive bacteria, protozoa, and pathogenic fungi they have not been used clinically owing to their toxicity at therapeutic doses. Despite attempts to optimise their application profile by studies of the structure activity relationships (SAR), it appears that the therapeutic window is too narrow for clinical development in this area.[26,27] Therefore the interest in these compounds died out after initial investigations early last century. However, today there is renewed interest mainly due to the finding that they are potent immunosuppressants. Extensive work has been carried out on their pharmacological activity.

Prodiginines have been demonstrated to have anti-malarial activity making them an attractive target for modern genetic and chemical studies.[28-31] Recently prodigiosin has been shown to kill the parasites that cause Chagas' disease and is thought to be more potent against *Trypanosoma* than the current treatment of benznidazole.[31] Prodiginines increased the survival time of malaria infected mice, with cyclic derivatives, for example streptorubin A **23**, proving most potent.[30]

One remarkable property of the prodiginine family is their immunosuppressant activity which derives from their ability to inhibit blastogenesis of T-cells and suppress T-cell dependent antibody responses without damaging the lymphoid organs.[32,33] There are only three immunosuppressants currently recognised in medicinal practice. FK506, rapamycin and cyclosporin A. Cyclosporin A is used as an immunosuppressant for organ transplants and to treat some autoimmune diseases. However, due to its deleterious side effects, particularly renal toxicity, new immunosuppressants are required.[34,35] Prodiginines have demonstrated immunosuppressant activity on T-cells by blocking inter-leukin-2 (IL-2) dependant proliferation through the down regulation of the IL-2Rα receptor[32,36] and therefore improving animal survival in mouse graft-versus-host disease.[37] The chemical structures of prodiginines are small enough that minor structural alterations are likely to significantly reduce toxicity and increase pharmacological activity.[34,38] Despite potential side effects observed *in vivo,* it is likely that these compounds will be useful immunosuppressants. In fact, their ability to interfere with IL-2 by inhibiting phosphorylation and hence activation of Janus Kinase 3 (JAK-3) has led to the development of PNU-156804 (**27**, Figure 7) a synthetic analogue of undecylprodiginine, which is vastly more potent than the natural product. *In vivo* studies have demonstrated that oral administration of PNU-156804 suppresses heart

allograft rejection in rats, and a promising synergistic effect was noticed when PNU-156804 was administered with cyclosporin A.[39]



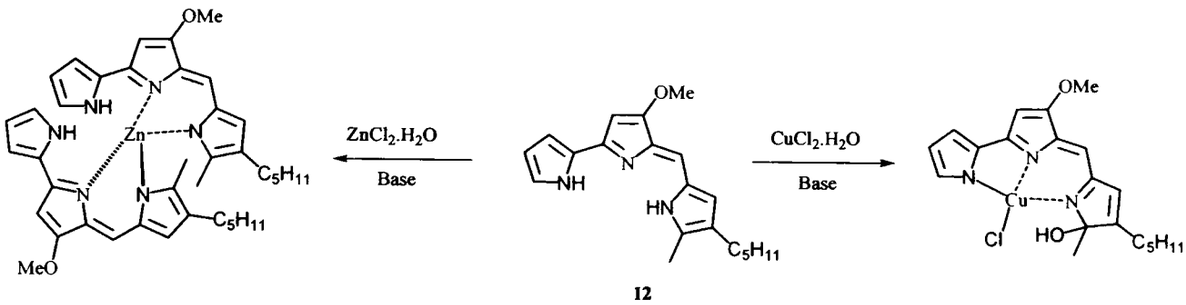
27

Figure 7: Synthetic analogue PNU-156804, **27** of undecylprodiginine

Prodiginines and their synthetic analogues are also considered potential chemotherapy agents. In 1888 Dr William B. Coley, a New York surgeon, stumbled across one of the most intriguing findings ever made in cancer research. Having combined cultures of *Streptococcus sp.* and *Bacillus prodsious* (*Serratia marcescens*) and sterilised them by heat, he made a mixed bacterial vaccine (today called Coley's toxin). This therapy, which is likely to have contained prodigiosins, was used to treat tumours. Since then, prodiginines have been shown to induce apoptosis in many different human cancer cell lines with minimal effect on non-malignant cells.[40-44] Promising results have been obtained against P388 mouse leukaemia cells,[27,45] human melanoma, lung and colon cancer cell lines and hepatocellular carcinomas for which there is currently no effective treatment.[40,41] In 2007 undecylprodiginine was shown to induce apoptosis in human breast carcinoma cell lines.[46] Furthermore, prodigiosin was demonstrated to effectively inhibit tumour metastasis and migration *in vitro* and *in vivo*.[47]

The mechanism of action of prodiginines *in vivo* is not very clear. There is substantial evidence for potent DNA damaging properties arising from their ability to co-ordinate metal ions, in particular copper (II), which leads to efficient single and double strand cleavage.[48,49,50] The analogous zinc (II) complex co-ordinates two molecules of

prodigiosin around the metal ion.[51] Cleavage was not initiated in the presence of iron (III), nickel (II) and zinc (II) (Scheme 17).[51,52]

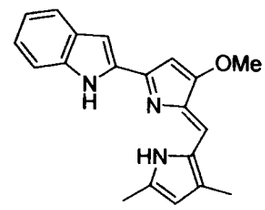


12

Scheme 17: The binding of copper (II) and zinc (II) with prodigiosin **12**

It is suggested that these complexes intercalate in the minor groove with a preference for AT rich sites. The metal oxidises the electron rich pyrrolylpyrromethene moiety of prodigiosin. Redox cycling between Cu(I) and Cu(II) is likely to facilitate prodiginine oxidation, converting O_2 to H_2O_2, triggering DNA cleavage.[51,53] This double stranded cleavage is likely to be important therapeutically, as cancerous cells have more copper present than non-cancerous ones, making the development of these anti-cancer agents advantageous.[54] Structure activity relationship (SAR) and electrochemical investigations show that structural modification which interferes with cation binding diminishes potency.[53,55] Specifically the A-ring, the lone pairs of electrons in conjugation with the tricyclic frame work and the B-ring methoxy groups are critical for cytotoxicity (Figure 5).[27,45,51] Replacement of any of the pyrrole rings with another heteroaromatic ring results in a decrease in potency. Surprisingly, roseophilin **26**, which has a methoxyfuran ring in place of the methoxypyrrole B-ring, exhibits cytotoxcity against K562 human erythroid leukaemia and KB human epidermoid carcinoma cell likes. This appears to result from a different mechanism of action as roseophilin does not cause DNA damage.[55] The proton affinity of the pyrromethene chromophore which is strongly influenced by the A-ring, also plays a critical role in its biological activity, and inhibition of cell proliferation.[50]

New synthetic analogues are being developed in the hope of finding compounds less toxic than the natural products.[56] Currently the synthetic prodigiosin analogue GX15-070 **28** (Figure 8) is in phase 1 and 2 oncology clinical trials directed against multiple solid tumour and haematological malignancies. GX15-070 is of interest both as a combination therapy and as a single agent. It is an antagonist of the BH3-binding groove of the Bcl-2 family of proteins. GX15-070 induces apoptosis in several cancer cell lines including multiple myeloma[57] and mantle cell lymphoma by inhibiting the interaction between pro- and anti-apoptotic proteins.[58]



28

Figure 8: GX015-070, **28**

Another mode of action results from the ability of prodigiosin to reversibly disrupt the pH gradient between various cellular compartments (generated by ATPase) by functioning as H^+/Cl^- symporters, resulting in the acidification of the cytoplasm and in some cases apoptosis.[59,60] This activity is thought to derive from the ability of the protonated product to electrostatically interact with Cl^- resulting in the formation of a lipophilic ion pair which facilitates proton coupled transmembrane transport of halides.[50] This mode of action may allow prodigiosins to be developed as anti-tumour agents.

2.5: The Biosynthesis of Prodiginines in *S. coelicolor* A3(2)

S. coelicolor A3(2) is a known producer of prodiginine antibiotics that has been studied for a number of years. Previous investigations and the recent sequencing of the genome

meant that major advances in the understanding of prodiginine biosynthesis have occurred.[61-63] This allowed functions to be assigned to the genes involved in prodiginine biosynthesis, based on sequence similarities, in 2001.[23,64]

The biosynthesis of prodigiosin was originally studied in *S. marcescens* and several actinomycete prodiginines have been examined by the incorporation of labelled precursors.[65-68] Early studies showed that prodigiosin, despite being a pyrrolic compound, was not biosynthetically related to porphyrins.[69] Studies in *Streptomyces longispororuber* showed that undecylprodiginine is biosynthesised from one unit of proline, one unit of serine, one unit of glycine and eight units of acetate. These units were proposed to make up two putative precursors: 2-undecylpyrrole (UP) **29** and 4-methoxy-2,-2'-bipyrrole-5-carboxaldehyde (MBC) **10**, which are condensed to give undecylprodiginine **21**.

The undecylpyrrole moiety **29** is made up of seven acetates condensed in a head to tail fashion with the remaining carbon and nitrogen atoms deriving from glycine. Based on these studies Gerber *et al.* concluded that 2-undecylpyrrole **29** is biosynthesised *via* condensation of β-ketomyristoyl coenzyme A and glycine with loss of carbon dioxide.[70] Further studies on *S. longispororuber* and other actinomycetes have shown that the monosubstituted A-ring of undecylprodiginine is derived from the pyrrolidine ring of proline. The final step in the biosynthetic pathway has been proposed to be a condensation of MBC **21** and UP **29.** This gives a completely conjugated double bond network responsible for the red colour of the pigments. Macrocylic prodiginines (**22**, **23**, and **24**) are proposed to be formed *via* a two-electron oxidative cyclisation of undecylprodiginine, **21** (Figure 9).[23]

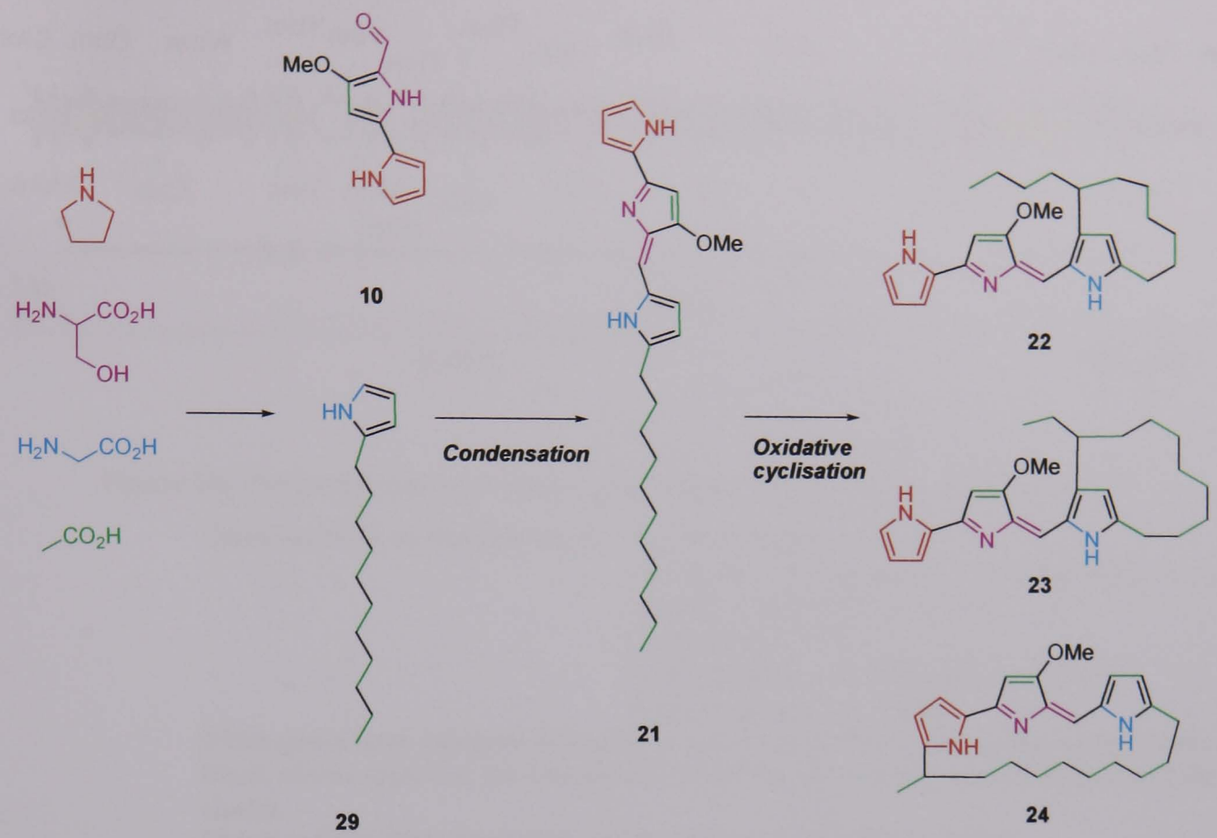


Figure 9: Proposed condensation and oxidative cyclisation reactions in prodiginine biosynthesis

Since the initial study of the gene cluster in 2001, extensive work carried out by Challis and co-workers has allowed the function of several of the genes involved in the prodiginine gene cluster to be reassigned. Some of these functions were predicted by comparison of the genes in the *red* cluster with those in the *Serratia pig* cluster that directs prodigiosin biosynthesis.[64, 11]

The gene cluster responsible for prodiginine biosynthesis in *S. coelicolor* is known as the *red* cluster. It contains twenty-three genes organised into four transcription units, (Figure 10). The left limit of the *red* cluster is defined by the *trkA* operon that encodes the proteins required for the potassium uptake system.

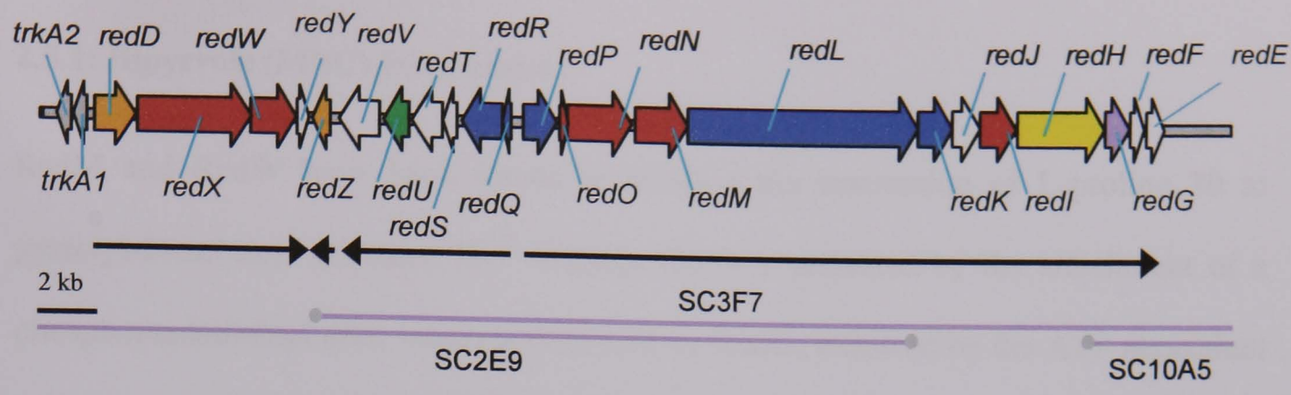


Figure 10: The prodiginine biosynthetic gene cluster in *Streptomyces coelicolor A3*(2)

Genes involved in biosynthesis of 2-undecylpyrrole, 5: blue
4-methoxy-2,2'-bipyrrole-5-carboxaldehyde, 6: red
Oxidative cyclisation, 1: purple
Condensation, 1: Yellow
Posttranslational modification, 1: green
Regulatory genes, 2: orange
White genes have unknown functions or are not required for prodiginine biosynthesis, 7
Black arrows represent the 4 molecules of mRNA generated by the transcription of the cluster.
The grey lines show the regions of the cluster as spanned by cosmids.

To date all but seven (white) of the twenty-three genes have been assigned functions, four of these, *redY*, *redF*, *redE* and *redJ*, have been shown not to be required for prodiginine biosynthesis in *S. coelicolor* (Figure 10).[71] *redD* and *redZ* have been shown to encode pathway regulators (orange). Six genes are assigned to the biosynthesis of MBC (red) and five to UP biosynthesis (blue). One of the genes *redH* has been shown to be involved in condensation of the intermediates **10** and **29**. The protein encoded by *redG* shows homology to a family of Rieske non-heme, iron-dependent oxygenase enzymes and has been shown to be involved in the oxidative cyclisation to form the cyclic derivative of undecylprodiginine, streptorubin B. Mechanisms for the biosynthesis of these two precursors have been proposed based on genetic and biochemical studies.[11,23,72-76]

2.5.1: Bipyrrole (MBC) Biosynthesis

RedM and RedW have been shown to catalyse the conversion of L-proline **30** to pyrrolyl-2-carboxyl thioester **32**.[73] Initially RedO is activated by the attachment of a phosphopantetheinyl arm, which is catalysed by RedU, followed by the ATP dependent acylation of RedO with L-proline catalysed by RedM. Subsequent FAD dependent oxidation of **31** catalysed by RedW gives the thioester **32** (Figure 11).[73] It is proposed that the thioester attached to the PCP RedO **32** is transferred to the KS^C domain of RedX **35**. Subsequent condensation with a malonyl-ACP thioester attached to one of the domains of RedN **34** would give the β-keto-acyl thioester **36**, which is cleaved in a PLP-dependent reaction with serine catalysed by the C-terminal α-oxoamine synthase (OAS) domain. The PLP-assisted deprotonation of serine leads to nucleophilic addition to the acyl thioester, followed by decarboxylation, PLP imine hydrolysis, cyclisation and dehydration to give the dihydroxybipyrrole **37**. Oxidation of **37** which is proposed to be catalysed by RedV[11] to give **38**, and subsequent methylation catalysed by RedI[77] yields the bipyrrole **10**.

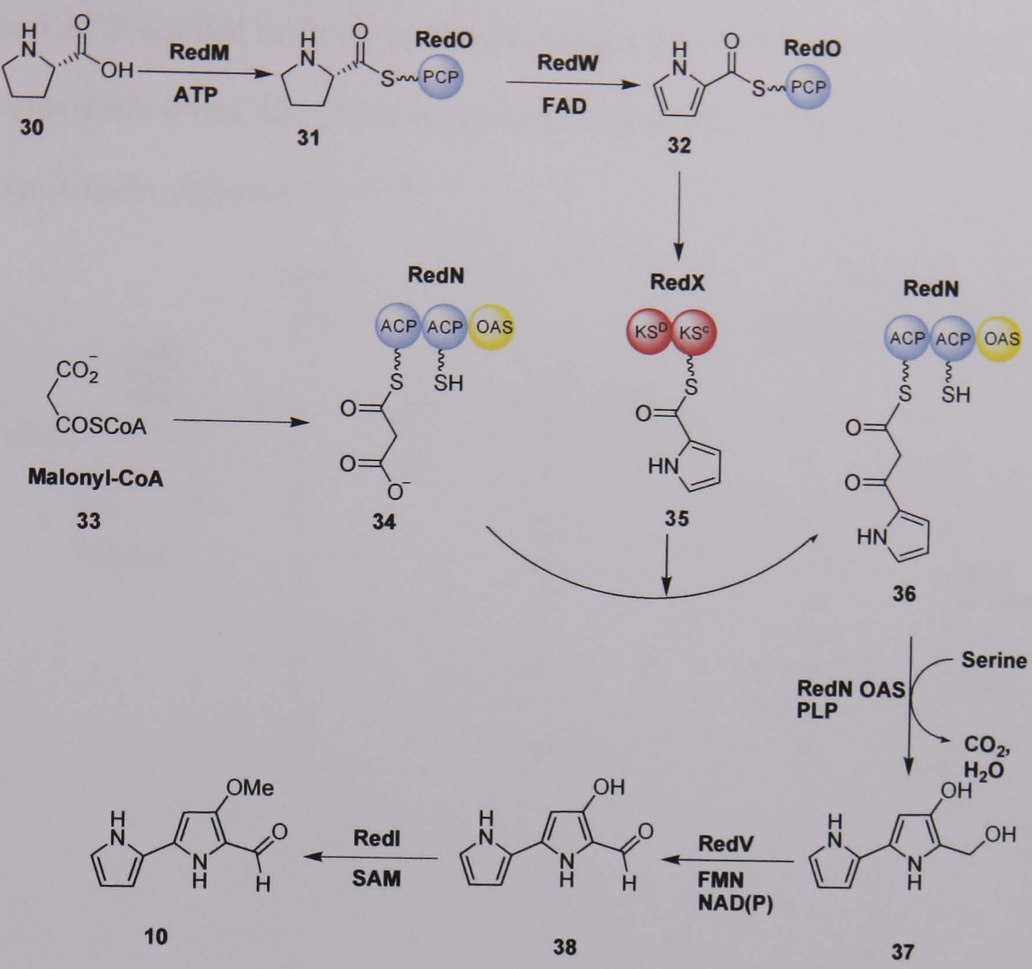


Figure 11: Proposed biosynthetic pathway of the MBC **10**

2.5.2: Biosynthesis of 2-undecylpyrrole (UP) 29

The monopyrrole fragment is biosynthesised from seven acetate units and one glycine unit.[65] Recent genetic studies show that 2-undecylpyrrolin-4-one **43** is assembled by fatty acid synthase (FAS) and PKS enzymes (Figure 12).[74] RedP is thought to generate an acetoacetyl thioester on the ACP RedQ. Subsequent reduction and dehydration by the ketoreductase, dehydratase and enoylreductase enzymes of the *S. coelicolor* FAS yields butyryl-ACP. Chain extension by RedR and continued reduction and dehydration by FAS reductive enzymes would result in the production of dodecanoyl-RedQ **39**. The dodecanoyl-RedQ thioester **39** is believed to be hydrolysed by an as yet unidentified enzyme to give **40,** which is loaded onto the first RedL ACP domain to give the corresponding thioester **41** where it undergoes one further chain extension with malonyl-RedL **33** catalysed by the KS domain to give **42**. This thioester is cleaved from

the second ACP domain of RedL by the PLP-dependant OAS domain of RedL to yield 2-undecylpyrrolin-4-one **43,** which is reduced by RedK in a NADH dependent reaction to produce 2-undecylpyrrole **29**. [74,78]

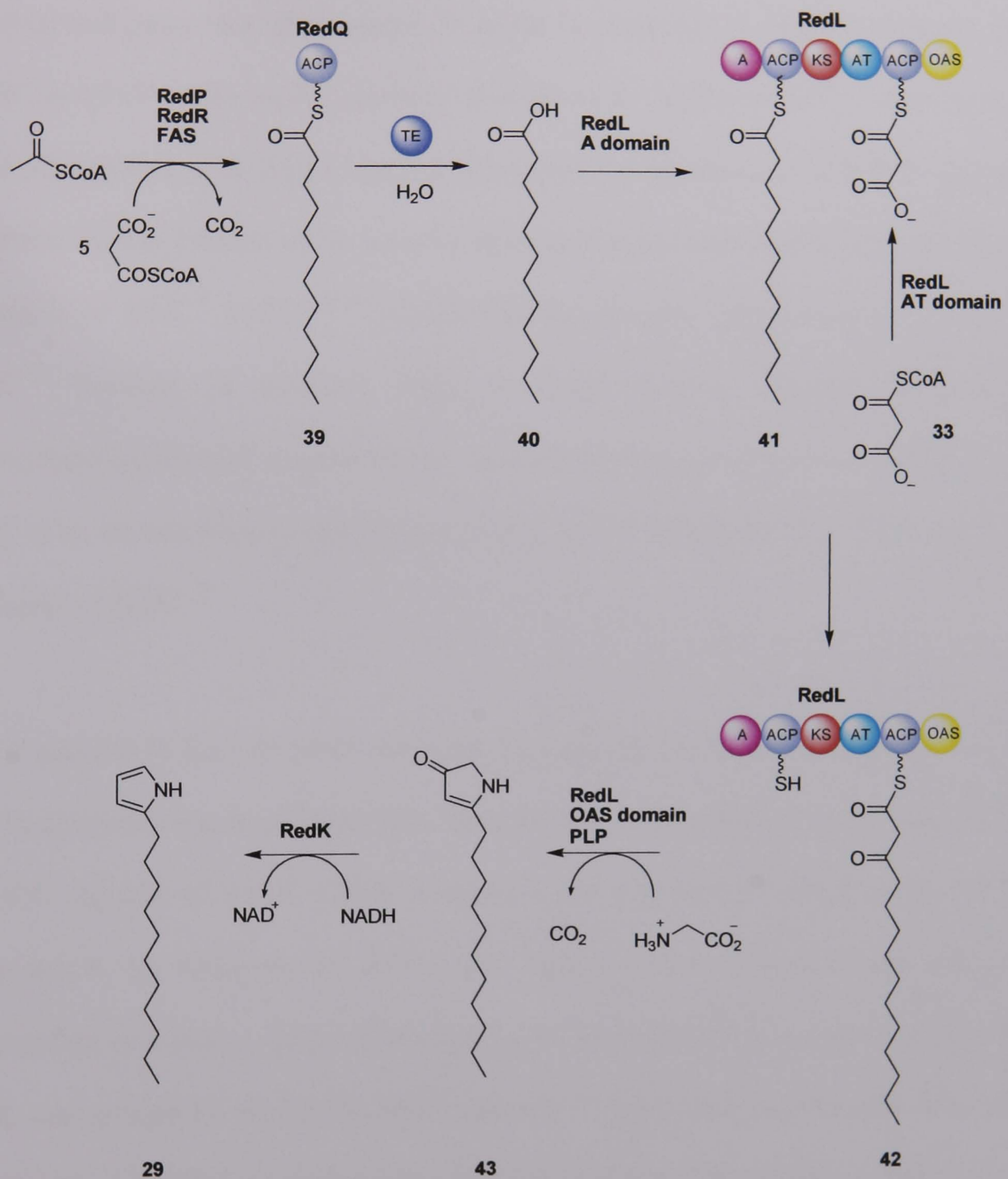


Figure 12: Proposed biosynthetic pathway of the UP **29**

2.5.3: The Coupling of UP 29 and MBC 10

MBC and UP have been proposed as intermediates in the biosynthesis of undecylprodiginine and streptorubin B in *Streptomyces* species[23,65] although direct evidence for this has been lacking until now.[72,74]

RedM and RedW are proposed to be involved in MBC biosynthesis[23] and gene replacement mutants were created to inactivate each of these genes.[72] Neither undecylprodiginine nor streptorubin B could be detected in organic extracts of the *redM::aac(3)IV* or the *redW::aac(3)IV* mutants of *S. coelicolor* M511 when grown on solid R5 medium or in liquid supplemented minimal medium. LC-MS/MS analysis of acidified organic extracts of the mycelia of these mutants and comparison with synthetic standards of MBC[79] and UP[80,81] showed that the mutants both accumulate UP, but not MBC.[72] Feeding of synthetic MBC to these mutants restored production of undecylprodiginine and streptorubin B, whereas feeding of UP did not, confirming that MBC is an intermediate in their biosynthesis and that RedM and RedW are required for assembly of MBC.[72]

Initial analysis of the *red* cluster led to the proposal that the dodecanyl chain involved in UP biosynthesis was transferred after assembly by the RedPQR proteins from RedQ to the KS^C domain of RedX. Chain elongation and subsequent cyclisation to UP were proposed to be catalysed by RedX and RedN. Ketone reduction and subsequent elimination of water to give UP was proposed to be catalysed by RedF and RedH.[23] RedL was assigned a role in the MBC pathway.[23] Subsequent experiments have shown that RedX and RedN are required for MBC biosynthesis,[72]since both a *redX::aac(3)IV* mutant and a Δ*redN* mutant[23] have been shown to accumulate UP and not MBC by LC-MS/MS analysis of acidified organic extracts of their mycelia. Furthermore, feeding of MBC but not UP restored the production of prodiginines in these mutants. Comparison of the *red* cluster of *S. coelicolor* with the *pig* cluster of *Serratia marcescens* also revealed no *redL* homologue in the prodigiosin biosynthetic gene cluster.[82] This would

suggest that RedL therefore plays a role in UP biosynthesis. Until recently UP had only been observed in mutants blocked in the biosynthesis of MBC, implicating it as an intermediate or a shunt product in the biosynthetic pathway of undecylprodiginine and streptorubin B.[72] The observation that *redL::oriT-aac(3)IV* mutant does not produce detectable quantities of undecylprodiginine or streptorubin B, but can be complemented with chemically synthesised UP provides clear evidence for its role in UP biosynthesis. It also provides direct evidence that UP is an intermediate in the biosynthesis of undecylprodiginine and streptorubin B.[74]

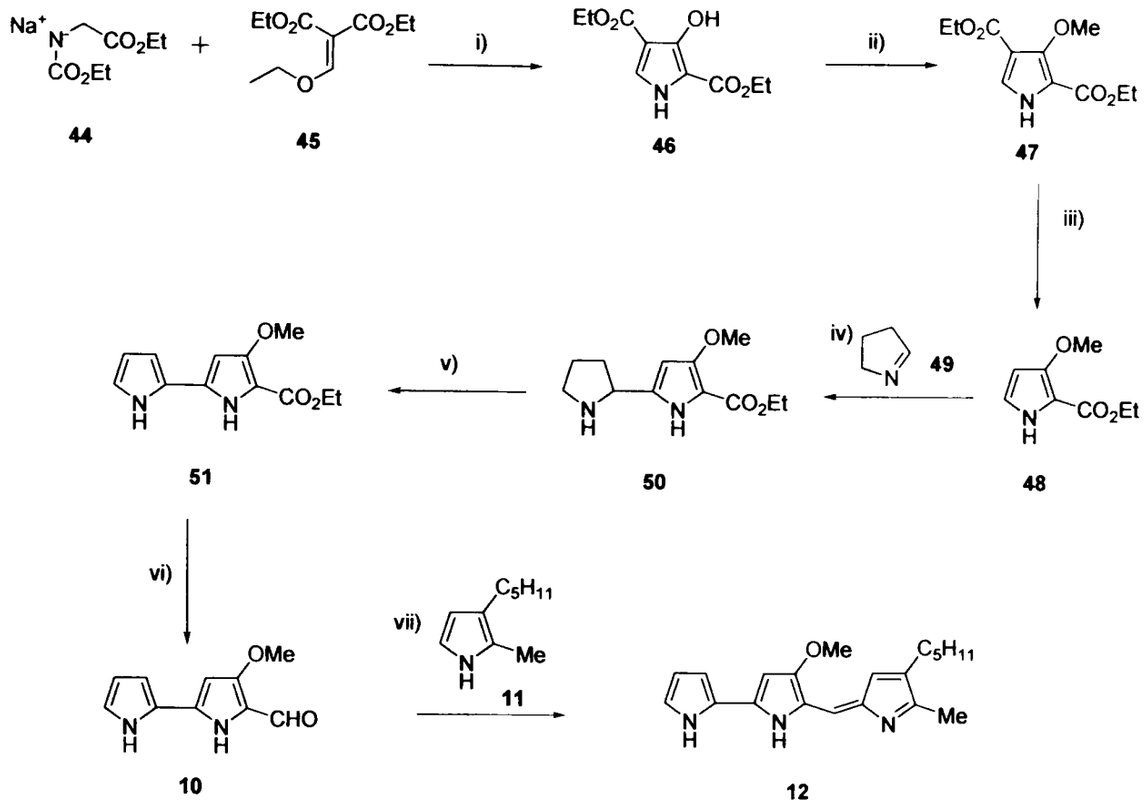
RedH has been proposed to catalyse the condensation of UP and MBC to yield undecylprodiginine[11] but this hypothesis had not been experimentally examined. Recently experimental evidence for the role of RedH has been obtained. The *redH* gene was replaced on the chromosome of *S. coelicolor* M511 with a "cassette" containing *oriT* and the *acc(3)IV* gene conferring apramycin resistance to create the *S. coelicolor* mutant W33. When grown on agar no red pigment was visible indicating that *redH* is required for prodiginine production. LC-MS/MS analysis showed small quantities of undecylprodiginine suggesting that MBC and UP can, to an extent, spontaneously condense.[83] However, control experiments indicated that this is likely to be an artefact of the isolation procedure. Further investigations where pPKS1 (containing *redH* under the control of the *ermE* promoter) had been integrated into the chromosome of *Streptomyces venezuelae* ATCC10712 to express the RedH protein in a heterologous host were carried out. LC-MS/MS analysis of wild type *S. venezuelae* showed that prodiginines are not produced.[83] Feeding of MBC and UP to wild type *S. venezualae* did not result in visible red-pigment production and only small traces of undecylprodiginine, relative to UP, could be seen by LC-MS/MS analysis of acidified

extracts of mycelia, probably resulting from the isolation procedure.[83] In contrast, feeding MBC and UP to the *S. venezuelae* strain with *redH* expressed resulted in substantial quantities of undecylprodiginine production relative to UP. These data provide strong evidence that RedH catalyses the condensation of MBC and UP to form undecylprodiginine.[83]

2.6: Synthetic Approaches to the Prodiginines

There are many approaches to the synthesis of prodigiosin and its analogues but very few reports exist for the synthesis of the key biosynthetic intermediate bipyrrole **10**.[10,27,84-88]

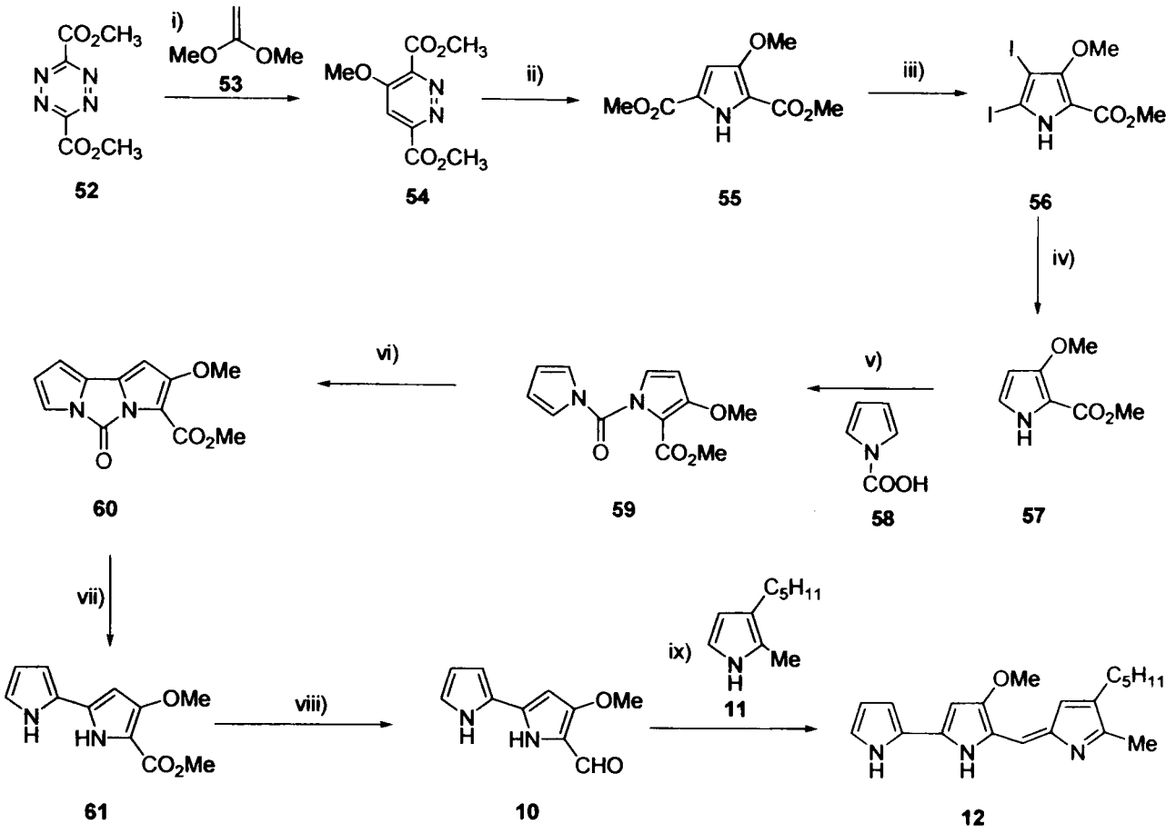
Rapoport and Holden proved the structure of prodigiosin in 1962 after extensive studies by the synthesis of MBC **10** and condensation with 2-methyl-3-amylpyrrole **11**.[10] Their synthesis of **10** is outlined in Scheme 18. Despite all the problems with this synthetic route, it was the first total synthesis and was the first definitive proof of the structure of prodigiosin.



Scheme 18: Synthesis of MBC **10** and elaboration to prodigiosin **12**[10]

i) Xylene, benzene, 30%; ii) CH_2N_2, MeOH, 44%; iii) 1) H_2SO_4; 2) heat, 42%; iv) **49**, 13%; v) Pd/C, *p*-cymene, 82%; vi) 1) H_2NNH_2, 2) TsCl, pyridine, 3) Na_2CO_3, heat, 32%; vii) **11**, HCl, MeOH, 55%

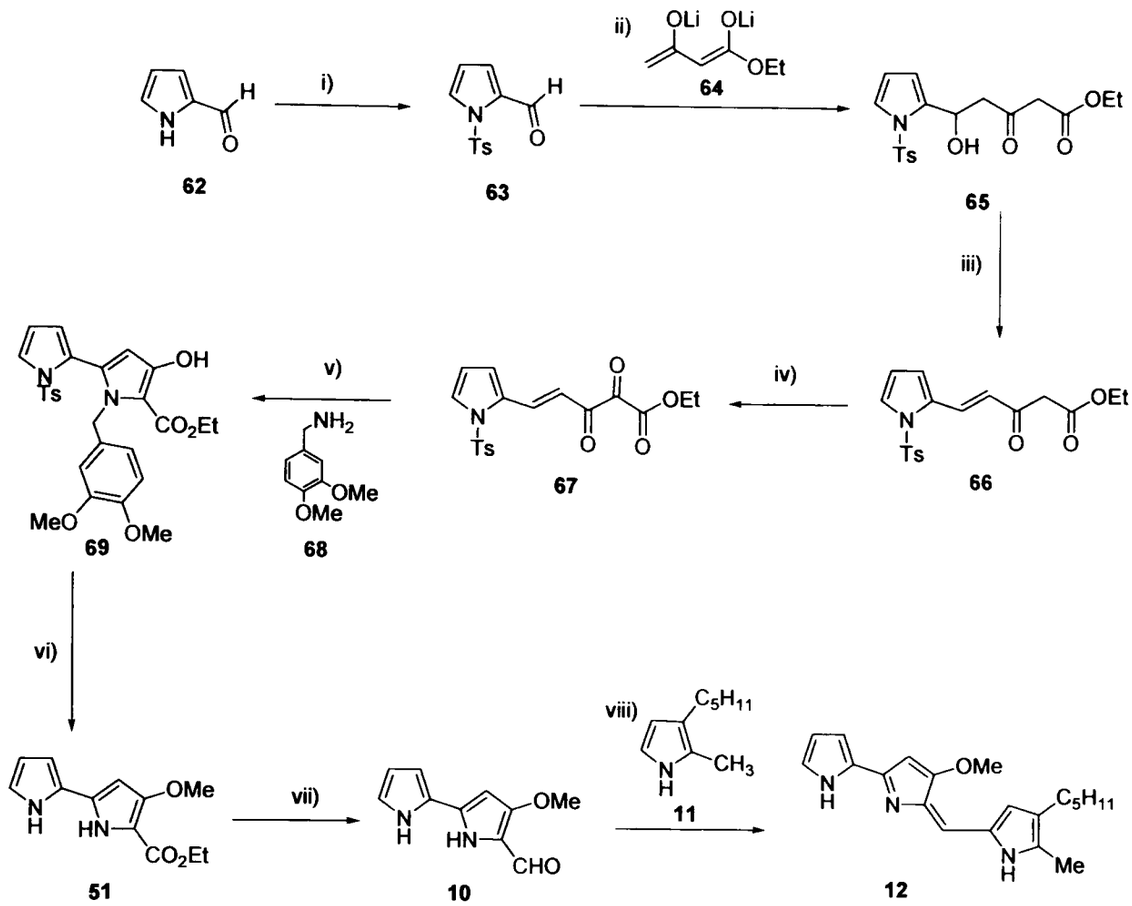
Boger and Patel based their approach to the synthesis of prodigiosin on the application of inverse electron demand Diels-Alder reaction of **52** for preparation of the pyrrole ring B **55** (Figure 5 and Scheme 19), and subsequent implementation of an effective intramolecular palladium(II) promoted 2,2'-diaryl coupling for the construction of the prodigiosin 2,2'-bipyrrole AB ring system. Boger used the conditions developed by Rapoport and Holden for the final steps to generate prodigiosin **12** (Scheme 19). This route was utilised to synthesise prodigiosene and 2-methyl-3-pentylprodigiosene.[27,45]



Scheme 19: The synthesis of prodigiosin as detailed by Boger and Patel[45]

i) **53**, 94%; ii) Zn, HOAc, 68%; iii) 1) LiOH, 91% 2) NaI, I_2, $NaHCO_3$, 89%; iv) Pd/C, H_2, K_2CO_3, 96%; v) 1) NaH 2) **58**, $(COCl)_2$, DMF, 89%; vi) polymer supported $Pd(OAc)_2$, CH_3CO_2H, 96%; vii) $LiOCH_3$, 88%; viii) 1) H_2NNH_2, 2) TsCl, pyridine, 3) Na_2CO_3, heat, 34% ix) **11**, HCl, MeOH, 59%

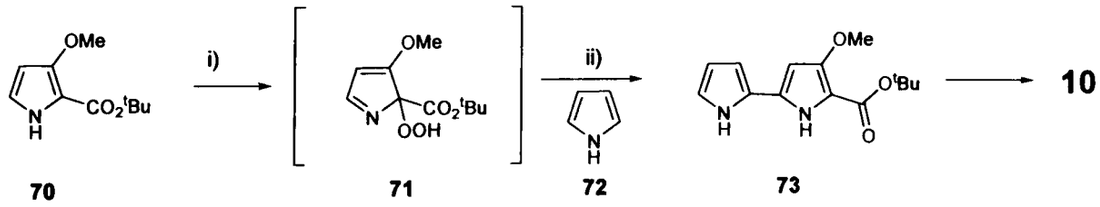
Wasserman and co-workers developed two different syntheses to the bipyrrole precursor **10**. The first strategy was based on the cyclisation of vinyl vicinal tricarbonyls **67** to generate 3-hydroxypyrrole-2-carboxylates **69** (Scheme 20). The conjugated double bond in **67** can be used in a Michael-type addition to form substituted α,α'-bipyrroles **69**, leading to the formation of key intermediate **10** which can be transformed by acid catalysis to prodigiosin **12** or other members of the prodiginine family.[86]



Scheme 20: Synthesis of prodigiosin as detailed by Wasserman[86]

i) NaH, *p*-TsCl, 93%; ii) **64**, 75%; iii) HCl, 58%; iv) *p*-Me_2N-C_6H_4-NO, NaOH, 70%; v)HOAc, **68**, 23%; vi) 1) NaH, Me_2SO_4, 2) 5% H_2SO_4, TFA, anisole, 3) NaOH, EtOH, heat, 31%; vii) 1) H_2NNH_2, 2) TsCl, pyridine, 3) Na_2CO_3, heat, 25%; viii) **11**, HBr (cat.), 50%

The other method developed by Wasserman and co-workers involved pyrrole-singlet oxygen reactions leading to α,α'-bipyrroles. Singlet oxygen oxidation of the *tert*-butyl ester of 3-methoxy-2-pyrrolecarboxylic acid **70** activated the pyrrole ring to give intermediate imino hydroperoxide **71**, which can be used to form substituted pyrrole derivatives **73** (Scheme 21). [89-91]

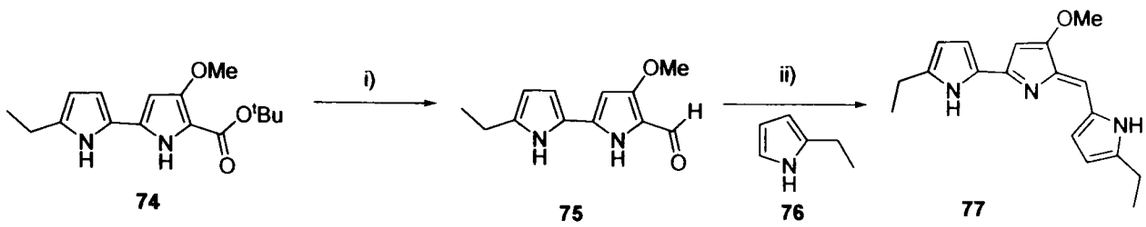


Scheme 21: Synthesis of substituted pyrrole derivatives **73** *via* proton-singlet oxygen reaction as reported by Wasserman and co-workers[90]

i) $^1O_2$; ii) **72**; 42% over 2 steps

Bipyrroles such as **73** and **74** can be elaborated by the McFayden-Stevens reduction and condensed with a substituted pyrrole as seen in the previous routes by Rapoport

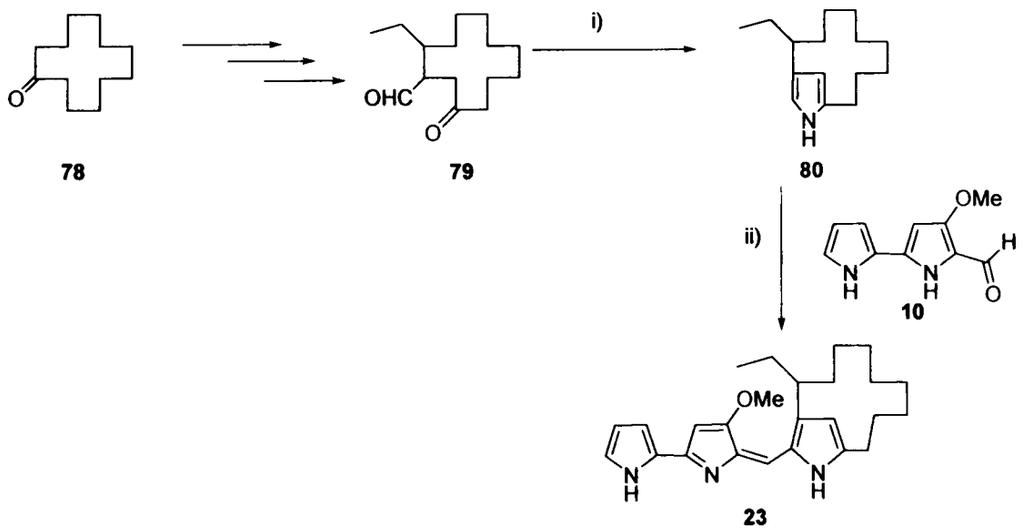
(Scheme 18) and Boger (Scheme 19) to produce prodiginine analogues. Ring A analogues which had not been produced earlier are easily accessible by this route. This pathway also works with substituted pyrroles in place of pyrrole. (Scheme 22).[91,92]



Scheme 22: McFayden-Stevens reaction to transform **74** to an analogue of the key intermediate in the prodigiosin pathway **75** and elaboration to a prodigiosin analogue **77**[91]

i) 1) H_2NNH_2, 2) TsCl, pyridine, 3) Na_2CO_3, heat, 40%; ii) **76**, HCl, MeOH, 41%

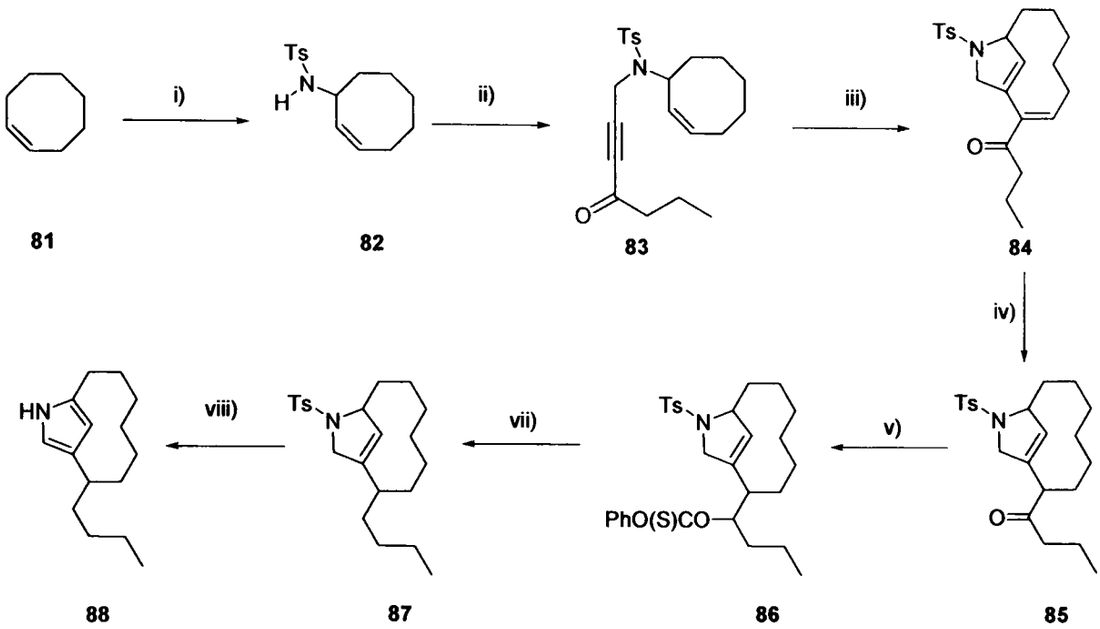
Synthesis of cyclic prodiginines, namely metacycloprodigiosin (streptorubin A) **23** and streptorubin B **22**, is difficult due to the limited success of synthesising the corresponding building blocks. However, Wasserman and co-workers completed the first synthesis of highly strained streptorubin A **23**. In this synthesis the pyrrole ring was formed by functionalisation of cyclododecanone followed by a Paal-Knorr condensation of **79** with ammonium bicarbonate. This method however has a large number of steps and is low yielding (Scheme 23).[93]



Scheme 23: Synthesis of metacycloprodigiosin (Streptorubin A), **23**[93]

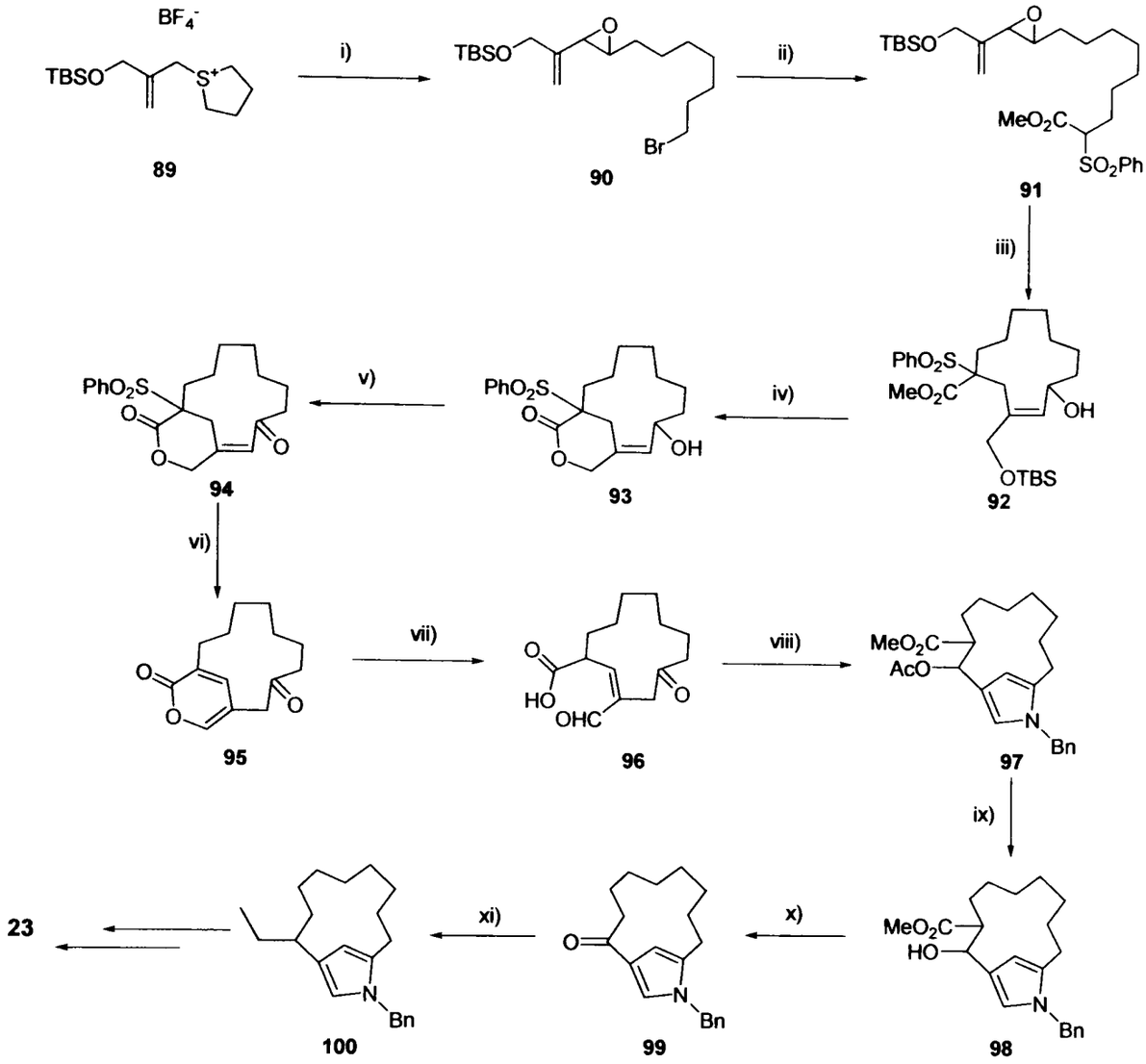
i) $(NH_4)_2CO_3$, H_2O, DMF, 115°C; ii) **10**, HCl, EtOH, 90%

Complementary syntheses of streptorubin B **22** (Scheme 24) and metacycloprodigiosin (streptorubin A) **23** (Scheme 25) have also been developed by Fürstner and co-workers.[94,95] These methods rely on transition metal catalysed C-C bond formation.



Scheme 24: Synthesis of streptorubin B **22** core by Fürstner and co-workers[94]

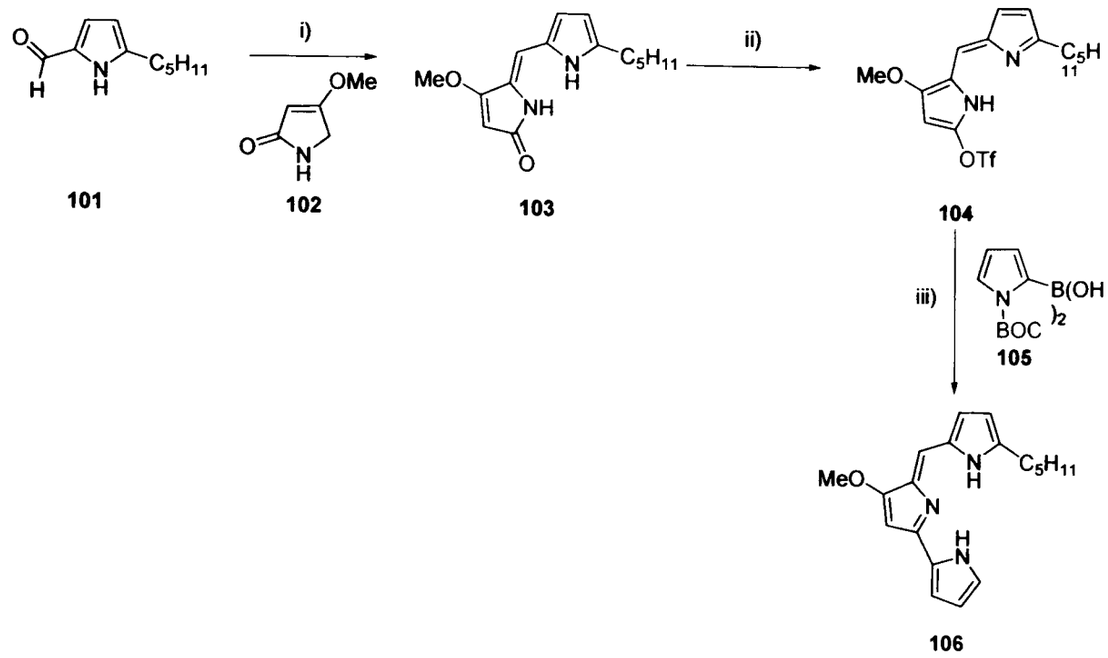
i) $[TsNCl]^-Na^+$, Se, 75%; ii) 1) NaH, propargyl bromide, 92%, 2) *n*-BuLi, then $ZnCl_2$; 3) butanoyl chloride, 82% over two steps; iii) $PtCl_2$ (5% mol), toluene, 79%; iv) Bu_3SnH, Pd^0 cat, HBF_4, 94%; v) 1) $LiAlH_4$, 96%, 2) PhOC(S)Cl, pyridine, 95%; vii) Bu_3SnH, AIBN, 64%; viii) 1) KAPA, 2) H_2O, 55% over 2 steps



Scheme 25: Synthesis of metacycloprodiginine **23** developed by Fürstner and co-workers[95]

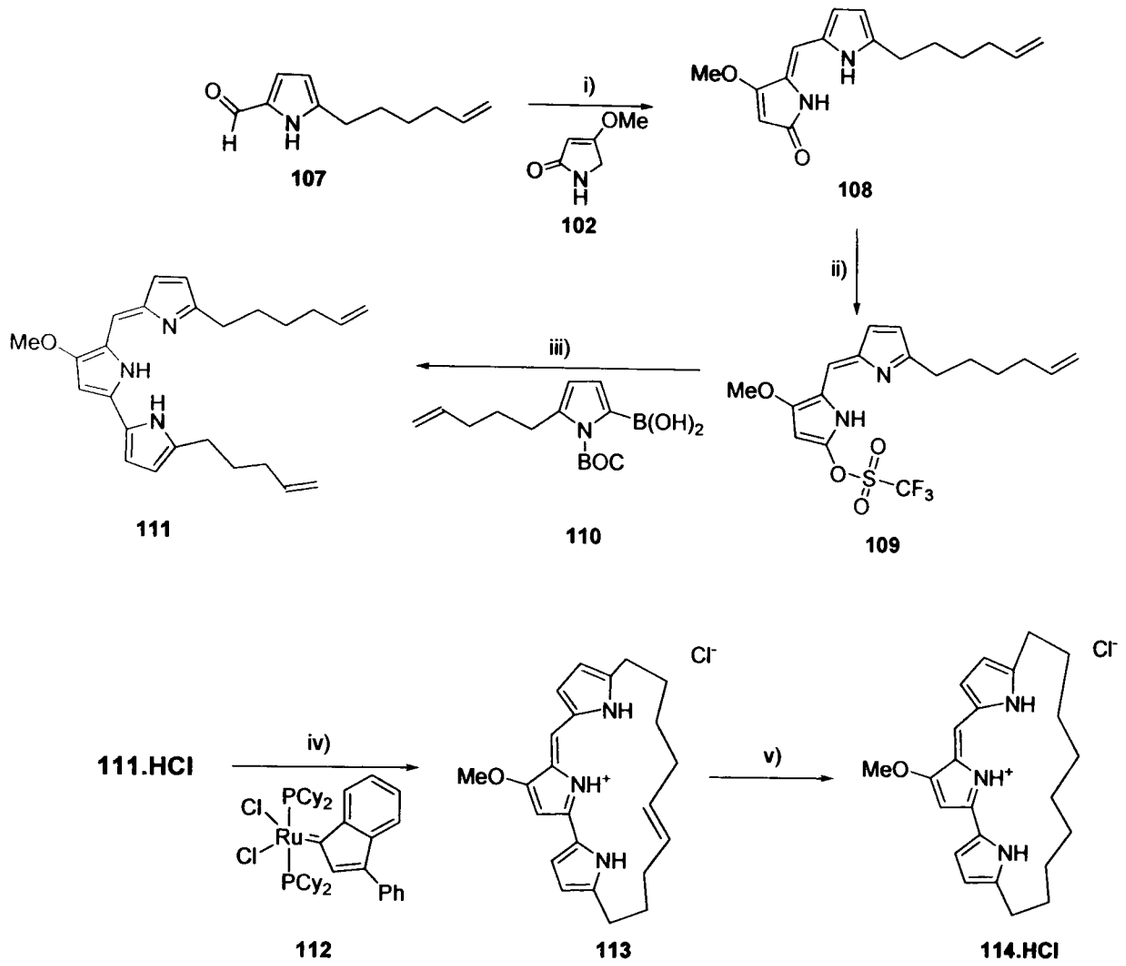
i) 1) *t*-BuLi; 2) 8-bromooctanal; 67-73% ii) PhO_2SCH_2COOMe, KH, DMF, 78%; iii) $[Pd(Ph_3)_4]$cat. dppe cat. THF, 77%; iv) TASF, 55%; v) Dess-Martin, 74%; vi) DBU, 79%; vii) NaOH, MeOH, 78%; viii) $BnNH_2$, HOAc, 45%; ix) NaOMe, 91%, x) 1) Pr_4NRuO_4, 63%; 2) NaCl, aq. DMSO, 180°C, 91%; xi) Ph_3P=CHMe, 73%; 2) H_2, Crabtree catalyst, 89%

D'Alessio and co-workers developed a total synthesis of undecylprodiginine. This route relies on a Suzuki cross coupling reaction for the formation of the bipyrrole as shown in Scheme 26.[96,97]



Scheme 26: Synthesis of undecylprodigiosin **106** by D'Alessio and co-workers[96,97]
i) **102**, NaOH, DMSO, 88%, ii) Tf_2O, 71%, iii) **105**, $[Pd(PPh_3)_4]$ cat. 73%

Nonylprodigiosin **114** and some other large ring analogues have been synthesised by Fürstner and co-workers, using Suzuki cross coupling reactions of an easily accessible pyrrolyl triflate with aryl boronic acids, the resulting diene was then subject to a ring closing metathesis or metathesis dimerisation (Scheme 27). This methodology was adapted from the synthesis of undecylprodiginine developed previously by D'Alessio and co-workers, and was also used to produce macrocyclic prodigiosin analogues. [88,98]



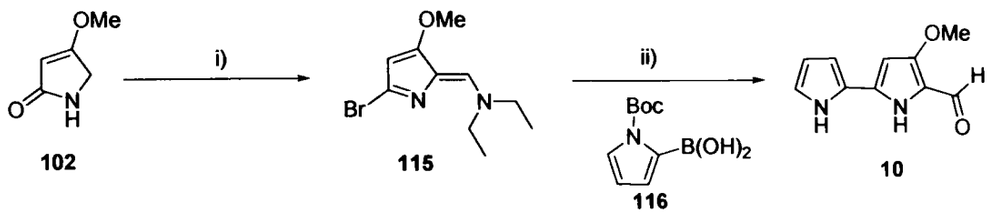
Scheme 27: Synthesis of nonylprodigiosin **114** by Fürstner and co-workers[88]

i) **102**, NaOH, DMSO, 94%, ii) Tf_2O, 93%, iii) **110**, $[Pd(PPh_3)_4]$ cat. 57%; iv) **112** cat.; 65%, v) H_2, [Rh] cat. 90%

Fürstner also completed a total synthesis of roseophilin, which is a related natural product. In this synthesis the key element is formation of the macrocyclic ring moiety which is accomplished with palladium coupling. The heterocyclic rings are formed in the presence of the macrocycle before coupling to the methoxyfuran-pyrrole.[99] Roseophilin has also been synthesised by a number of other groups.[100-102]

Several of these synthetic routes are limited owing to the low yielding McFayden-Stevens reduction. A more recent approach developed by Tripathy and Lavallée describe an efficient two step synthesis of bipyrrole **10**, which allows easy access to prodiginines in good yields (Scheme 28). This methodology treats commercially available 4-methoxy-3-pyrolin-2-one **102** with the Vilsmeier-Haack reagent derived

from diethylformamide to produce the bromo pyrrole enamine **115**. This can then be used in a Suzuki cross coupling reaction with *N*-BOC-pyrrole-2-boronic acid **116**, leading to the bipyrrole **10**.[79,103] This synthetic approach, combined with the condensation step reported previously by Rapoport and Holden[10] allowed access to a number of prodiginine analogues.



Scheme 28: Methodology developed to bipyrrole developed by Tripathy and Lavallée[79,103]
i) $POBr_3$, diethylformamide, chloroform, 70%; ii) **116**, $Pd(PPh_3)_4$, dioxane, H_2O, $NaCO_3$, 95%

This methodology has allowed the Challis Group to synthesise bipyrrole **10** analogues (Figure 13), which have been used to probe the substrate specificity of the RedH enzyme in undecylprodiginine biosynthesis.[83]

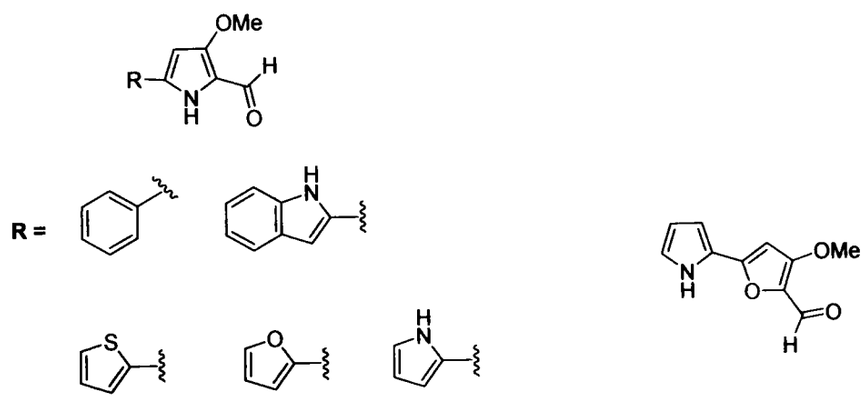


Figure 13: Analogues synthesised by Challis group

2.7: Aims and Objectives – Prodiginines

The aim of this project was to synthesise the mimic **117** (Figure 14) of a key putative intermediate in the biosynthesis of MBC **10** to investigate the biosynthetic pathway of the prodiginines produced by *S. coelicolor* A3(2). The role of *redM* in MBC biosynthesis was then analysed by feeding **117** to a *redM* deficient mutant unable to

produce MBC to see whether prodiginine production was restored. Analogues of **117** were then synthesised to examine whether novel "natural" products could be produced *via* incorporation of the analogues.

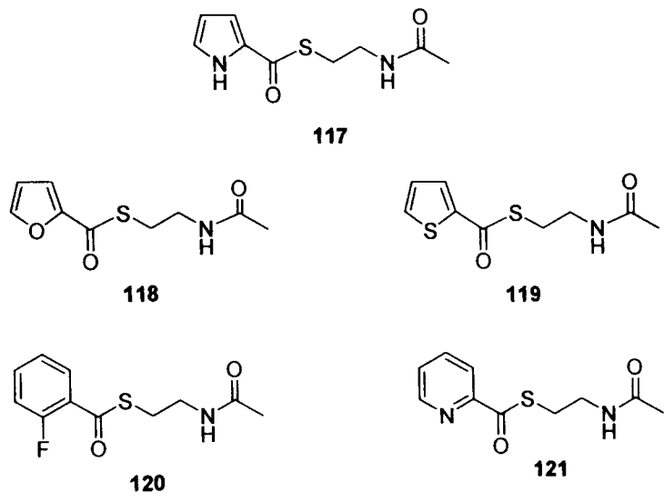


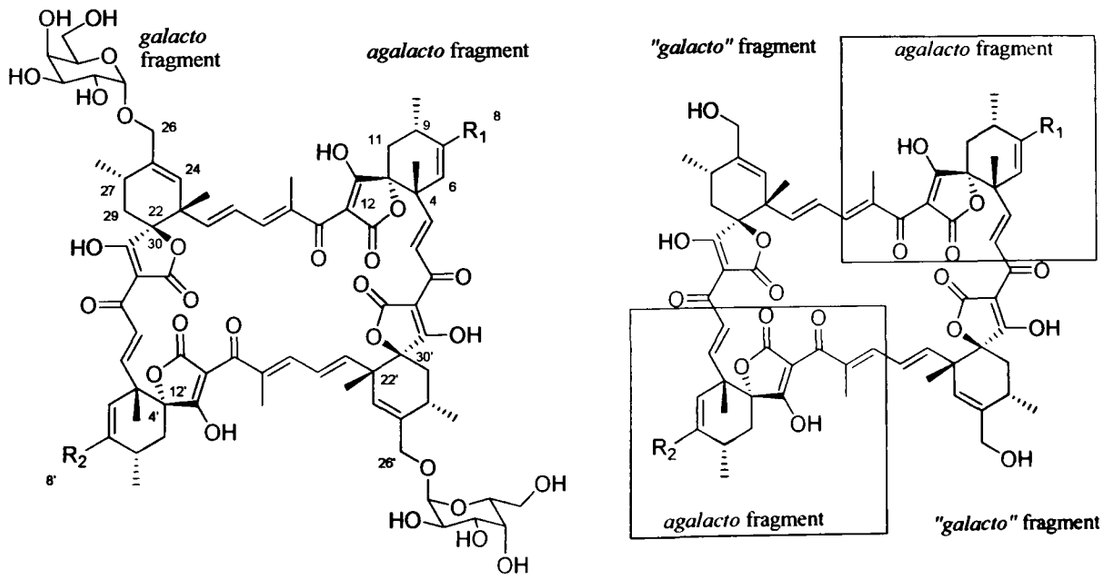
Figure 14: Mimic of the proposed precursor **117** and structures of analogues

Chapter 3: Introduction to Spirotetronate and Exomethylene Tetronate Antibiotics

3.1: Isolation and Structure Elucidation of Quartromicins

3.1.1: Determination of Planar Structures

Quartromicins, first isolated in 1991, are a structurally unique group of spirotetronate natural products produced by *Amycolatopsis* species.[104-106] A new actinomycete strain was isolated from a soil sample collected in Maharastra state in India during a search for novel bioactivities. This strain, identified as *Amycolatopsis orientalis,* No. Q427-8 (ATCC 53884), was shown to produce novel anti-viral antibiotics, the quartromicins, as a complex termed BU3889V.[106] This antibiotic complex was shown to consist of six bioactive components: A_1, A_2, A_3, D_1, D_2 and D_3 which were isolated after filtration using a non-ionic porous polymer resin and column chromatography.[106] Quartromicins A_1 **122a**, A_2 **122b**, and A_3 **122c** (Figure 15), were isolated as weakly acidic pale-yellow amorphous powders.[107] Aglycons quartromicins D_1 **122d**, D_2 **122e** and D_3 **122f** (Figure 15), were also isolated as only minor metabolites.



122a: Quartromicin A_1 R_1=R_2=CHO
122b: Quartromicin A_2 R_1 = CHO, R_2= CH_2OH
122c: Quartromicin A_3 R_1= R_2 = CH_2OH

122d: Quartromicin D_1 R_1 = R_2 =CHO
122e: Quartromicin D_2 R_1 = CHO, R_2=CH_2OH
122f: Quartromicin D_3 R_1 = R_2 = CH_2OH

Figure 15: Quartromicins A_1-A_3 and D_1-D_3 **(122a-f)**

The structures of these bioactive compounds were investigated. Using negative ion FAB-mass spectrometry quartromicin A_1 and A_3 were found to have the molecular formula $C_{78}H_{88}O_{30}$, m/z 1525 $(M-2+Na)^-$ 1541 $(M-2+K)^-$ and $C_{78}H_{92}O_{30}$, m/z 1545 $(M-2+K)^-$ respectively.[104] ^1H and ^{13}C NMR spectra suggested the presence of a sugar which was determined to be D-galactose after isolation from acid hydrolysis of the natural products. The ^{13}C NMR spectra of quartromicin A_1 and A_3 each exhibit thirty-nine well defined carbon signals hence showing they both possess symmetrical dimeric structures. 2D NMR data led to partial structures accounting for thirty-three of thirty-nine carbon signals. The remaining six signals were accounted for in two sets of carbonyl and olefin groups.[104] Simultaneously, structure elucidation was carried out on quartromicin D_1, D_2 and D_3, and it was shown by ^{13}C NMR spectroscopy that they contained thirty-three carbon atoms in comparison to the thirty nine carbon atoms present in quartromicin A_1, A_2 and A_3. It was determined that quartromicin A_1 afforded quartromicin D_1 and methyl *D*-galactoside upon mild acid methanolysis. In a similar way quartromicin A_2 yielded quartromicin D_2, and quartromicin A_3 yielded quartromicin D_3. The difference between quartromicins A_1 and D_1 of 324 mass units corresponds to two equivalents of galactose, suggesting that A_1 is a dimer of two thirty-nine carbon monomers. Hence A_1 is a galactoside derivative of D_1. This led to the proposed structure **122c** for quartromicin A_3, a unique thirty-two-membered carbocyclic ring possessing four spirotetronic acid moieties connected by enone or dienone linkers in a head to tail fashion (although no stereochemistry had been assigned at this stage). A synthetic model of a spirotetronate was produced which possessed intense IR signals at 1630 cm^{-1} and 1450 cm^{-1} as well as similar NMR signals to the natural product (Figure 16). This allowed the structures of **122a** and **122b** to be deduced, and hence **122d, 122e** and **122f** (Figure 15).[107]

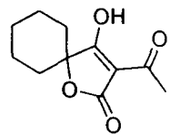


Figure 16: Synthetic model of a spirotetronate

Quartromicins are the largest members of a family of antibiotics whose macrocyclic framework consists of only C-C linkages. These compounds are intriguing because they have alternating *endo-* and *exo*-spirotetronic acid units, with opposite "corners" being identical. **122a** and **122c** are C-2 symmetric and contain two distinct spirotetronate units. Two of the units have α-galactopyranosyl residues appended, which are known as the *galacto* fragments (Figure 15). **122d** and **122f** are also C-2 symmetric but the "*galacto*" fragments are *des-D*-galactosyl analogues. The remaining members of the quartromicin group, **122b** and **122e**, differ from the C-2 symmetric members, in that the agalacto fragments have different oxidation states at the C-8 and C-8' carbons. It has been confirmed that all members of the family have the same carbon skeleton by reducing quartromicins A_1, A_2, D_1 and D_2 with sodium borohydride to A_3 and D_3.[108]

There have been several other macrocyclic antibiotics reported containing tetronic acid units (Figure 17), including tetrocarcin A **123**,[109,110]pyrroindomycins A and B **124a** and **124b**,[111] pyrrolosporin A **125**,[112,113] chlorothricin **126**,[114] kijanimicin **127**,[115,116] tetronothiodin **128**[117] and MM46115 **129**.[118] These differ from quartromicin **122** in having only one tetronic acid functional group in the molecule and being asymmetric. Quartromicins **122** and kijanimicin **127**[115] are both known to chelate metal ions (Na^+, K^+ and Ca^+) strongly[104] as has been reported for other 2-acyl tetronic acids, for example tetronasin.[119]

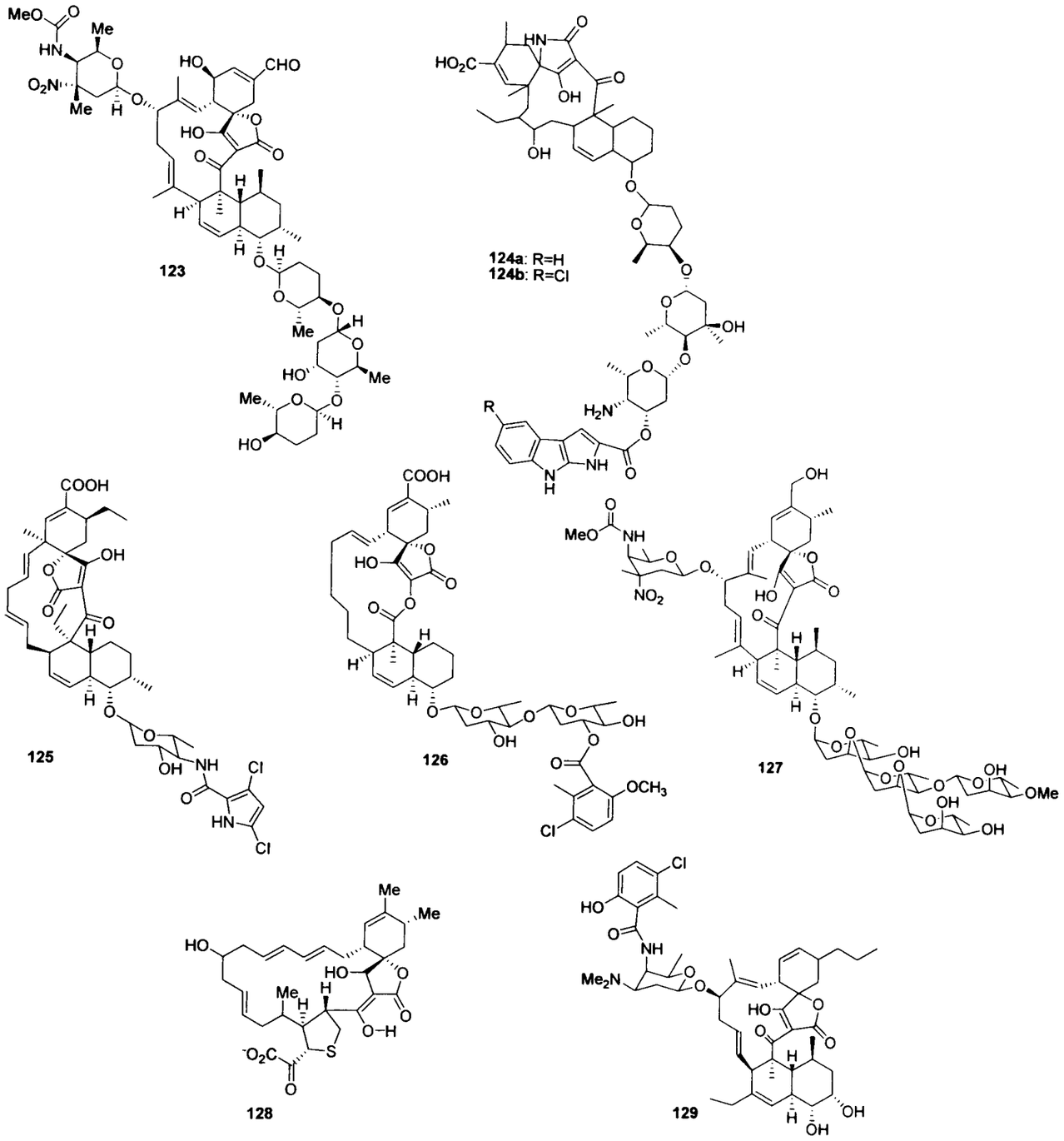
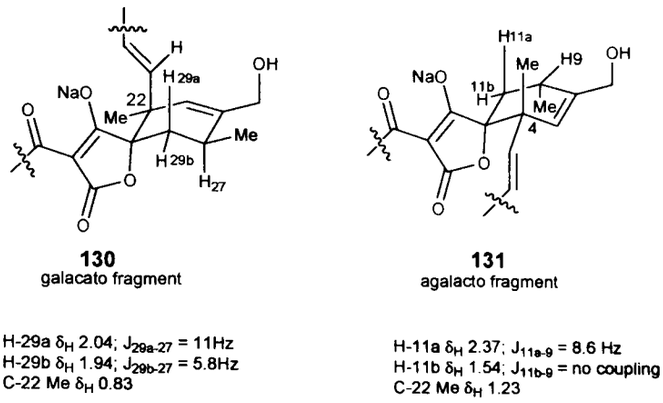


Figure 17: Other members of the spirotetronic acid family

3.1.2: Stereochemical Assignment of Quartromicin A_3 and D_3

^{1}H NMR data for quartromicins A_3 and D_3 revealed that the two spirotetronic fragments had strikingly different properties.[104,107] The methyl groups attached to C-22 and C-4 in the *galacto* and *agalacto* fragments were in very different environments, indicated by the large difference in chemical shifts (δ=0.83 and δ=1.23 respectively). However, the most diagnostic feature was the different appearances of the ABX pattern of signals for

the methylene groups at C-29 and C-11. In the *galacto* fragment both H-29a and H-29b exhibit coupling to H-27 (J = 11 Hz and 5.8 Hz respectively) whereas in the *agalacto* fragment the coupling is only seen between H-11a and H-9 (J = 8.6 Hz); no coupling is seen between H-11b and H-9 (Figure 18).[108]



130
galacato fragment

H-29a δ_H 2.04; $J_{29a\text{-}27}$ = 11Hz
H-29b δ_H 1.94; $J_{29b\text{-}27}$ = 5.8Hz
C-22 Me δ_H 0.83

131
agalacto fragment

H-11a δ_H 2.37; $J_{11a\text{-}9}$ = 8.6 Hz
H-11b δ_H 1.54; $J_{11b\text{-}9}$ = no coupling
C-22 Me δ_H 1.23

Figure 18: NMR data showing the difference in chemical environment of the *galacto* and *agalacto* fragments in quartromicin[108]

This data was similar to that reported for the *exo-* and *endo-* spirotetronates prepared in connection with the synthesis of chlorothricolide and kijanolide, the aglycones of the natural products chlorothricin **126** and kijanimicin **127** respectively.[108]

3.1.3: Synthesis and NMR Studies of the Possible Four Diastereoisomers of the Quartromicin Spirotetronate Subunits

Roush and co-workers have attempted to verify the stereochemical assignments discussed above by developing a synthesis for all four possible diastereoisomers of the core spirotetronate structure and ultimately hope to develop a total synthesis of the quartromicins.[108]

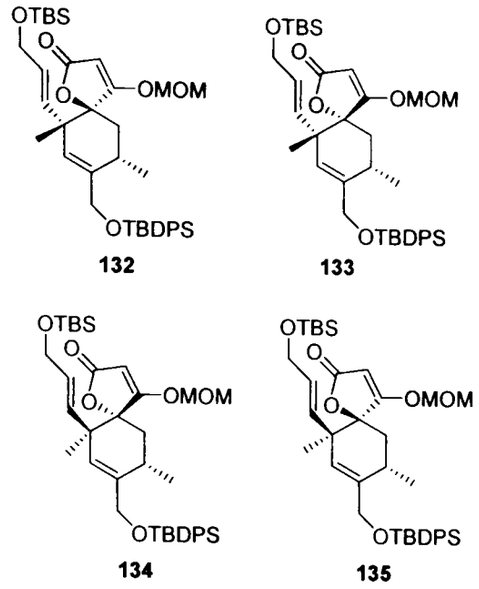


Figure 19: The four stereodivergent spirotetronate moieties that could correspond to the spirotetronates in quartromicin

In order to synthesise the four stereodivergent spirotetronate moieties (Figure 19), **132** and **133**, which possess the stereochemistry assigned for the *galacto* and *agalacto* fragments of quartromicin, and **134** and **135**, synthesised for comparative studies, two issues had to be addressed. Firstly, up until this point, no stereochemistry had been assigned to the quartromicin structures. By comparing the NMR data of quartromicin D_3 **122f** and the synthetic spirotetronates corresponding to those in chlorothricin **126**[120] and kijanimicin **127**,[121] it was suggested that the stereochemistry of the *galacto* fragment in the quartromicins was *endo-* (i.e. stereochemically analogous to the spirotetronate pyrrolosporin A **125**),[112] and that in the *agalacto* fragment it was *exo-* (i.e. stereochemically analogous to the spirotetronate of chlorothricin).[122] This assignment could also be compared with other natural products containing spirotetronate units, for example PA-46101 A[123] (*exo*-spirotetronate) and A88696 C and F[124] (*endo*-spirotetronates).

The second issue was the installation of a methyl group onto one of the quaternary centres of **132**, **133**, **134** and **135**. It was thought that this extra methyl group would

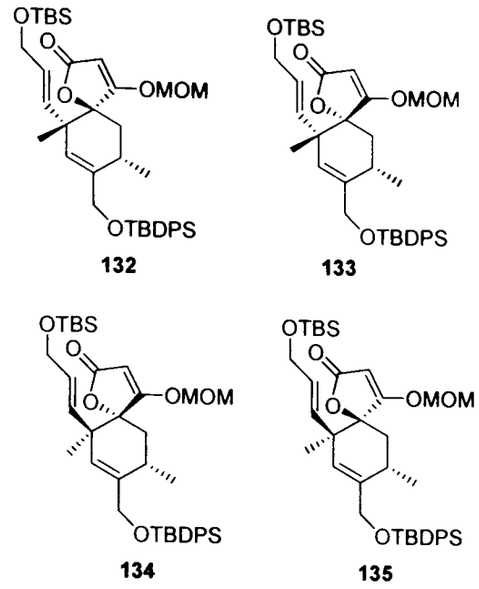


Figure 19: The four stereodivergent spirotetronate moieties that could correspond to the spirotetronates in quartromicin

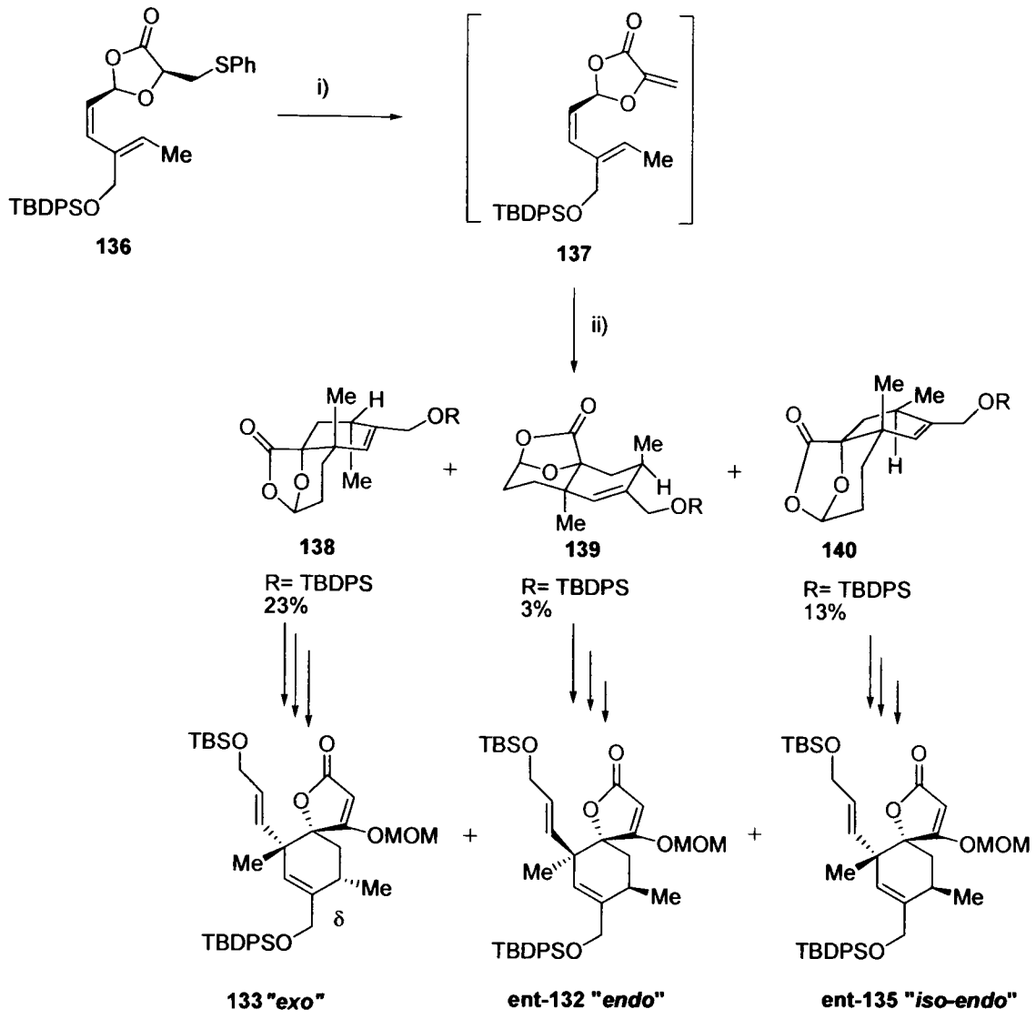
In order to synthesise the four stereodivergent spirotetronate moieties (Figure 19), **132** and **133**, which possess the stereochemistry assigned for the *galacto* and *agalacto* fragments of quartromicin, and **134** and **135**, synthesised for comparative studies, two issues had to be addressed. Firstly, up until this point, no stereochemistry had been assigned to the quartromicin structures. By comparing the NMR data of quartromicin D_3 **122f** and the synthetic spirotetronates corresponding to those in chlorothricin **126**[120] and kijanimicin **127**,[121] it was suggested that the stereochemistry of the *galacto* fragment in the quartromicins was *endo*- (i.e. stereochemically analogous to the spirotetronate pyrrolosporin A **125**),[112] and that in the *agalacto* fragment it was *exo*- (i.e. stereochemically analogous to the spirotetronate of chlorothricin).[122] This assignment could also be compared with other natural products containing spirotetronate units, for example PA-46101 A[123] (*exo*-spirotetronate) and A88696 C and F[124] (*endo*-spirotetronates).

The second issue was the installation of a methyl group onto one of the quaternary centres of **132**, **133**, **134** and **135**. It was thought that this extra methyl group would

hinder the bimolecular Diels-Alder approach used for the synthesis of chlorothricolide and kijanolide spirotetronates. Roush and co-workers decided to pursue the synthesis of *exo/endo-* spirotetronates *via* an intramolecular Diels-Alder (IMDA) reaction of **137** generated from **136** (Scheme 29). It was anticipated that this reaction would lead to a mixture of *exo/endo*-cycloadducts hence leading to two of the four spirotetronates needed for the stereochemical assignment of the quartromicins.[122]

This first generation strategy was used to produce **137** and hence **138** and **139** (Scheme 29). However, in practice, the IMDA reaction of **137** produced a mixture of three products **138**, **139** and **140**, in yields of 23%, 3% and 13%, respectively (Scheme 29). While **138** and **139** derive from **137** *via endo-* and *exo-* transition states respectively, it is clear that **140** must arise by a pathway involving diene isomerisation prior to IMDA cyclisation.[122]

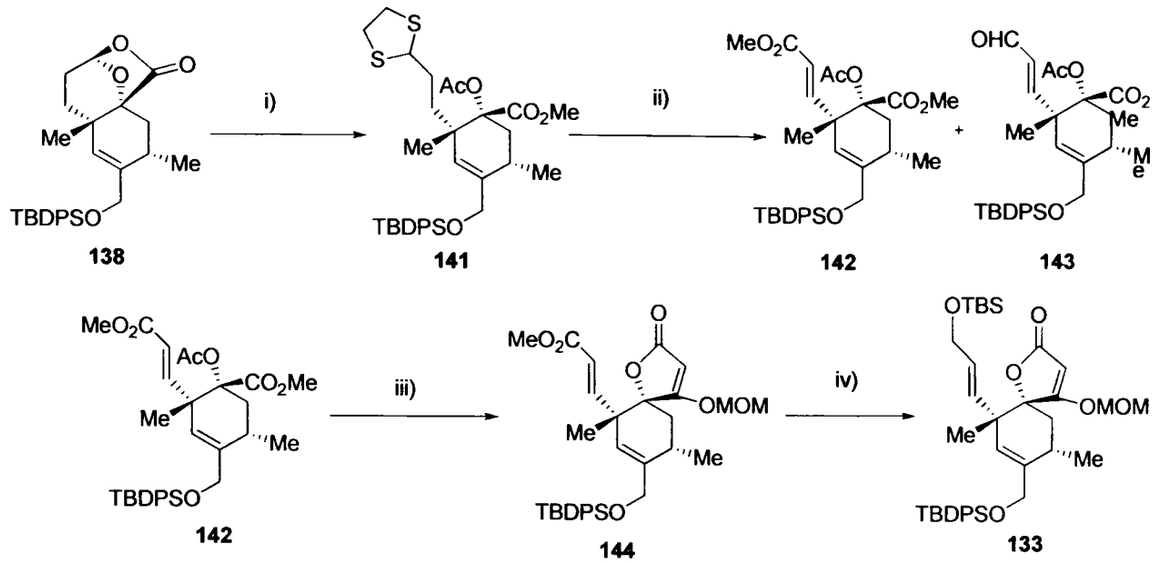
The Diels-Alder adducts were elaborated to spirotetronates **133**, ent-**132** and ent-**135** (Scheme 29). Spirotetronate **133** corresponding to the *agalacto* fragment of the quartromicins, was first prepared from *exo*-Diels-Alder adduct **138** and hence was called the *exo*-spirotetronate. Ent-**132** was referred to as the *endo*-spirotetronate and ent-**135** as the *iso-endo*-spirotetronate (Scheme 29).[122]



Scheme 29: First generation strategy which produced spirotetronates **133**, **ent-132** and **ent-135**[122]

i) 1) *m*CPBA, CH_2Cl_2, -78°C; 2) PhH, $P(OEt)_3$, 210°C, 20 h; ii) 1) TBAF, THF; 2) HPLC separation; 3) TBDPSCl, imidazole, DMF

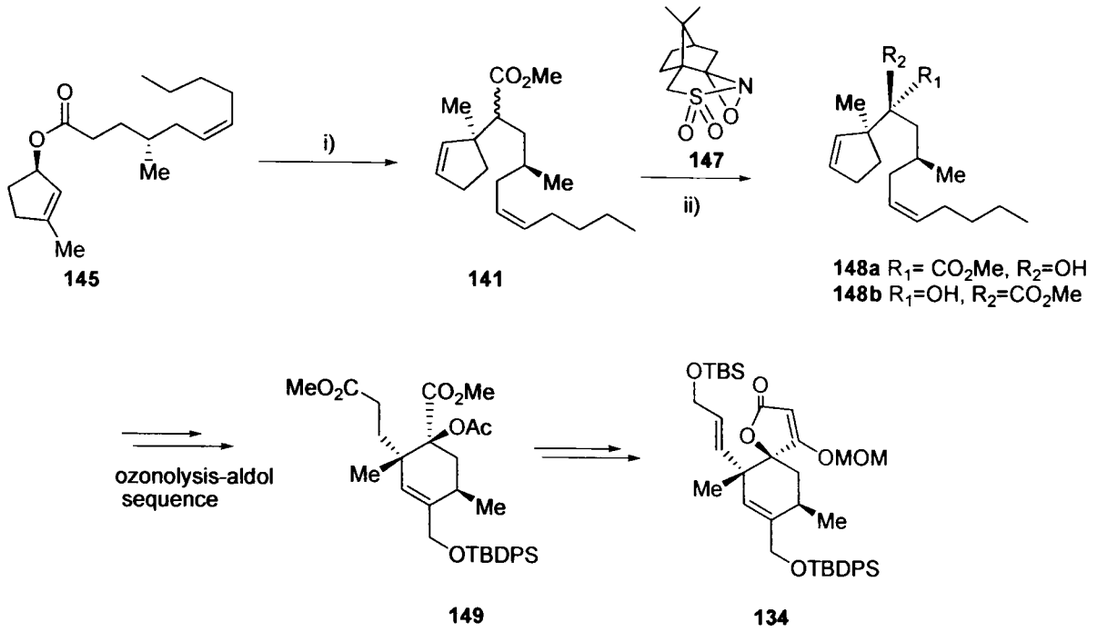
The intramolecular Diels-Alder reaction of **137** proved less selective than anticipated at the outset, owing to a competing olefin isomerisation that led to cycloadduct **140** (Scheme 29). This route was clearly too inefficient, both in terms of chemical yield and stereoselectivity, for use in a hypothetical total synthesis of the quartromicins. Nevertheless, three of the four required spirotetronates had been synthesised and elaborated to contain part of the unsaturated side chains present in quartromicins (Scheme 30).[122]



Scheme 30: Elaboration of spirotetronate intermediate **138** to the *exo*-spirotetronate **133**. A similar procedure was used to produce ent-**132** and ent-**135**[122]

i) 1)$HS(CH_2)_2SH$, $BF_3.Et_2O$; 2) Ac_2O, pyridine; 3) $TMSCHN_2$ THF/MeOH; ii) 1) $HgCl_2$, $CaCO_3$; 2) piperidine, $CaCl_2$; 3) PhSeCl, -78°C; 4) H_2O_2; 5) $TMSCHN_2$; iii) 1) LiHMDS, THF -78 to 0°C; 2) MOMCl, HMPA; iv) 1) DIBAL, THF, -100 to -78°C, 2) TBS-OTf

Roush and co-workers developed a second generation route to synthesise the fourth spirotetronate **134** and to improve the yield of **132** in order to allow the introduction of the unsaturated side chain (Scheme 31). This route allowed the stereochemistry of the two methyl centres in the spirotetronate subunits to be controlled in early intermediates. The enolate Ireland Claisen rearrangement of **145** allowed the correct stereochemistry to be produced (Scheme 31). However, subsequent enolate hydroxylation proceeded with poor selectivity. An ozonolysis-intramolecular aldol sequence from **148a** provided **149**, which could be elaborated to produce the desired fourth spirotetronate diastereomer the "*iso-exo*" isomer **134** (Scheme 31).[125]

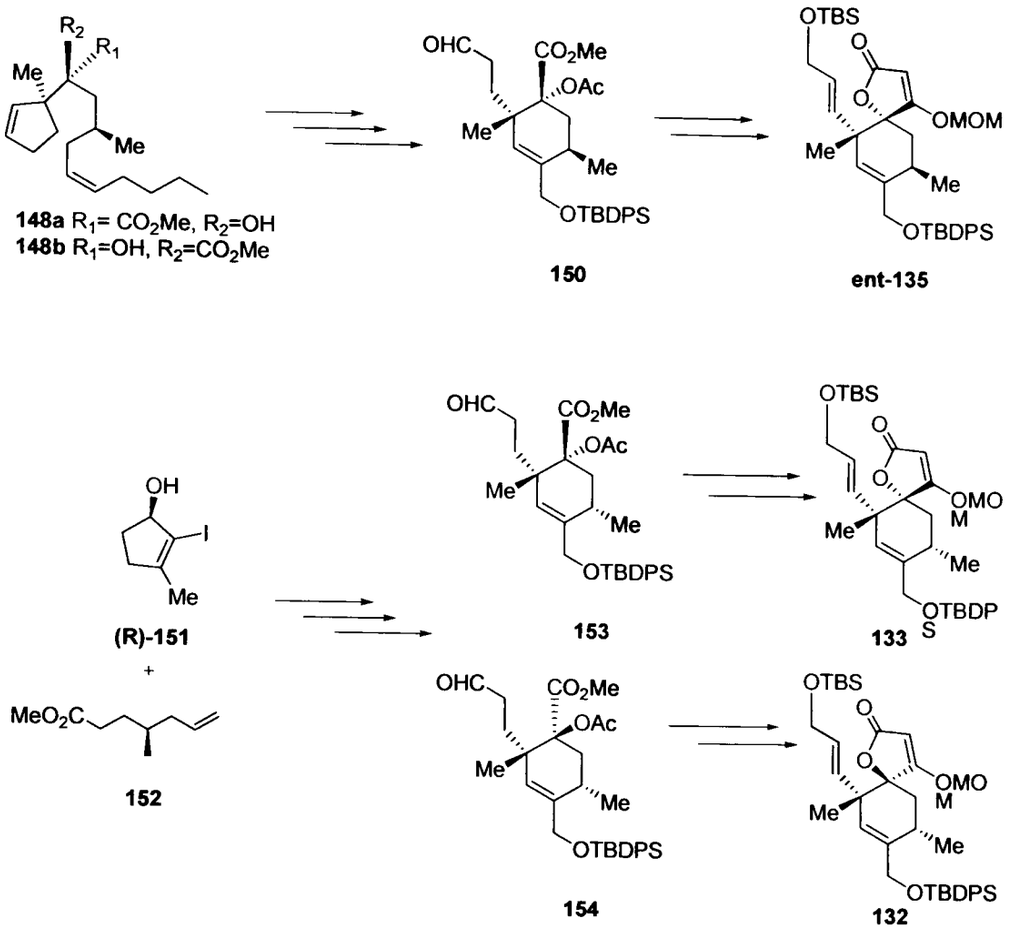


145 **141** **148a** R_1= CO_2Me, R_2=OH **148b** R_1=OH, R_2=CO_2Me **149** **134**

Scheme 31: Second generation strategy which allowed the synthesis of the "*iso-exo*" isomer **134**[125]

i) 1) LDA, THF, TBS-Cl, HMPA; 2) $TMSCHN_2$, 96%; ii) **147**, $LiNEt_2$, THF, -78°C, 61%

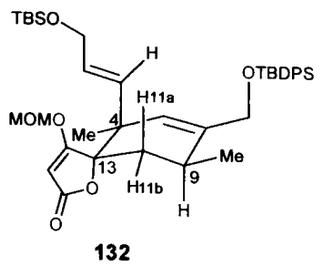
Although the original goal of total stereo-control was unsuccessful this route allowed the synthesis of all four spirotetronates and hence was superior to the previous route. Cyclopentene **148b** was further modified into **150** (an intermediate in the synthesis of ent-**135**) by the sequence in Scheme 32. By choosing (R)-**151** and **152** as starting materials, **153** and **154** were prepared as intermediates in the synthesis of **133** and **132** respectively (Scheme 32).



Scheme 32: Second generation strategy to the spirotetronates **133**, **ent-135** and **132**[125]

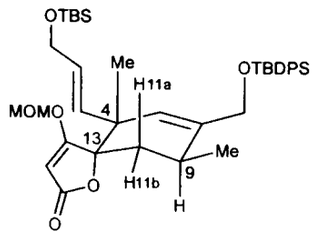
^{1}H NMR studies of the model spirotetronate diastereomers revealed that all four spirotetronates adopt conformations where the two methyl groups are in equatorial positions. This was verified by the coupling constants for H-9 and H-11, which in all cases were in the range of *J* 8.3-10.8 Hz and *J* 6.3-6.8 Hz, respectively (Figure 20). This data supported the idea that the methyl group is shielded by the tetronate unit in the equatorial position. It also verifies that none of these spirotetronates match the NMR data for the fourth *agalacto* fragment **131** of quartromicins **122** (Figure 18) in which the secondary methyl group is in an axial position.[108] Roush presumed that conformational constraints caused the *agalacto* fragment to occur in the natural product and adopt a different conformation to **133**. This could of course mean that all four spirotetronates have the same stereochemistry in quartromicins such that it exists with two *galacto* fragments in dimethyl equatorial conformations and the *agalacto* fragments in "chair

inverted" conformations. However, extensive molecular modelling studies showed this to be unlikely.[108]



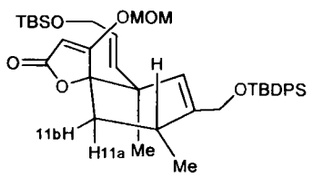
132

H11a: δ_H=1.99; $J_{11a\text{-}9}$=10.8 Hz
H11b: δ_H=1.73; $J_{11b\text{-}9}$=6.3 Hz
C-4-Me: δ_H=1.07



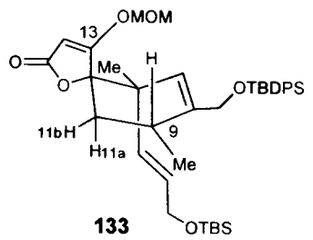
134

H11a: δ_H=2.00; $J_{11a\text{-}9}$=10.3 Hz
H11b: δ_H=1.84; $J_{11b\text{-}9}$=6.6 Hz
C-4-Me: δ_H=1.27



135

H11a: δ_H=1.95; $J_{11a\text{-}9}$=8.3 Hz
H11b: δ_H=2.08; $J_{11b\text{-}9}$=6.8 Hz
C-4-Me: δ_H=1.19



133

H11a: δ_H=2.04; $J_{11a\text{-}9}$=10.4 Hz
H11b: δ_H=1.83; $J_{11b\text{-}9}$=6.6 Hz
C-4-Me: δ_H=1.05

Figure 20: NMR analysis of the synthetic spirotetronates[108]

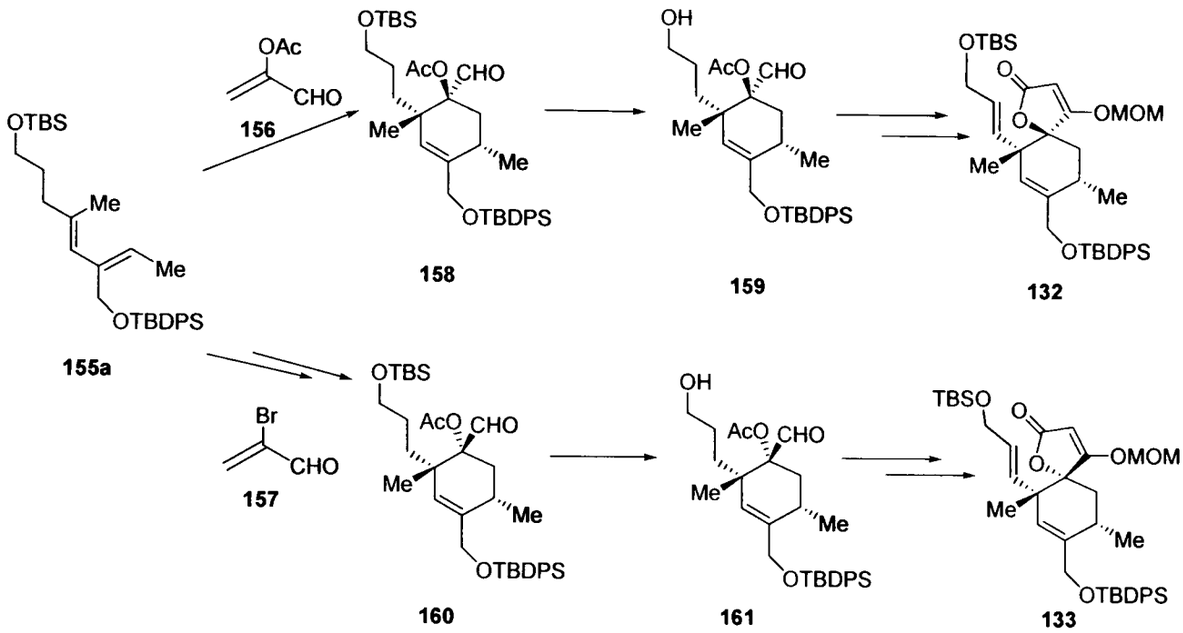
3.2: Studies Towards the Total Synthesis of Quartromicins

Elucidation of the stereochemical features of the quartromicins has set the stage for their total synthesis. Studies towards this goal are described in the following subsections.

3.2.1: Diastereoselective Synthesis of Spirotetronates 132 and 133 *via* Diels-Alder Reactions of Acyclic (*Z,E*)-1,3-diene, by Roush and Co-workers

A third generation synthesis of **132** and **133** was carried out by Roush and co-workers.[126] In principle, the most straightforward approach to the synthesis of spirotetronates **132** and **133** involves Diels-Alder reactions of an acrylic (*Z*)-substituted 1,3-diene such as **155a** and an appropriate α-acetoxy acrylate dienophile (Scheme 33). If this reaction could be induced to produce the *endo-* and *exo-* Diels-Alder adducts with good selectivity, then **132** and **133** would be easily accessible, and the isomeric diene **155b** could serve as a viable Diels-Alder reaction substrate to the isomeric pair **134** and

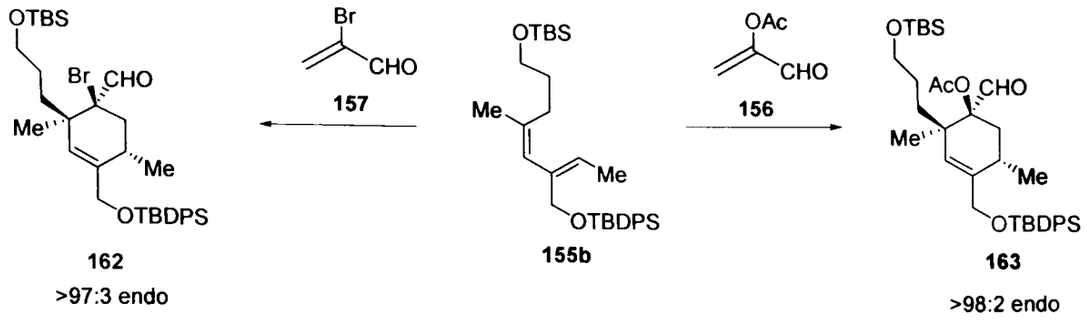
135 (Scheme 34). Attempts to perform Diels-Alder reactions of **155a** with acrylate dienophiles using Lewis acid catalysts were unsuccessful; therefore the reaction with α-acetoxy acrylate was not explored. However, treatment of **155a** with four equivalents of α-acetoxy acrolein **156**, and one equivalent of $MeAlCl_2$ in toluene provided the Diels-Alder adduct **158**. This compound was deprotected to form **159**, which was further elaborated to the desired spirotetronate **132** (Scheme 33).[126]



Scheme 33: Conversion of diene **155a** into spirotetronates **132** and **133**[126]

Lewis acid-catalysed Diels-Alder reactions of **155a** with other dienophiles were also investigated in order to obtain routes to formal *exo*-cycloadduct **160**. It was determined that α-bromoacrolein **157** was a good dienophile for the production of **160**, which in turn could be deprotected to form **161** and further elaborated to produce the desired spirotetronate **133** (Scheme 33).[126]

In order to demonstrate the generality of the Lewis acid catalysed Diels-Alder reaction, Roush *et al.* synthesised the isomeric diene **155b** and were able to produce cycloadducts **163** and **162** with ≥97:3 diastereoselectivity and in 84-88% yield (Scheme 34).[108]



Scheme 34: Conversion of diene **155b** into cycloadducts **162** and **163**[108]

Elaboration of the racemic hydroxyesters **159** and **161** to the quartromicin *endo-* and *exo-* spirotetronates **132** and **133** has been accomplished. However, the use of these in a total synthesis of quartromicin D_3 requires them to be prepared as single enantiomers to avoid production of racemates / diastereomers in the late stage coupling sequences. Therefore, further reactions of **155a** with chiral dienophiles were investigated.[108] Thus all four spirotetronates have been successfully synthesised by Roush and co-workers.

3.2.2: Assembly of Bis-spirotetronate Quartromicin Fragments by Roush and Co-workers

In 2006 Roush and co-workers published the synthesis of both the vertical **164** and horizontal **165** bis-spirotetronate precursors to quartromicin D_3 (Figure 21).[127]

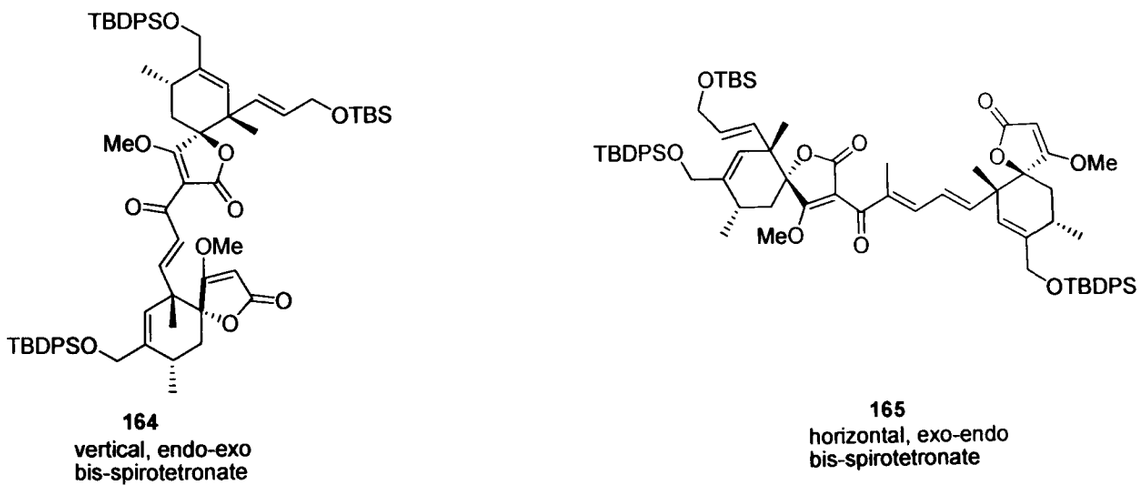
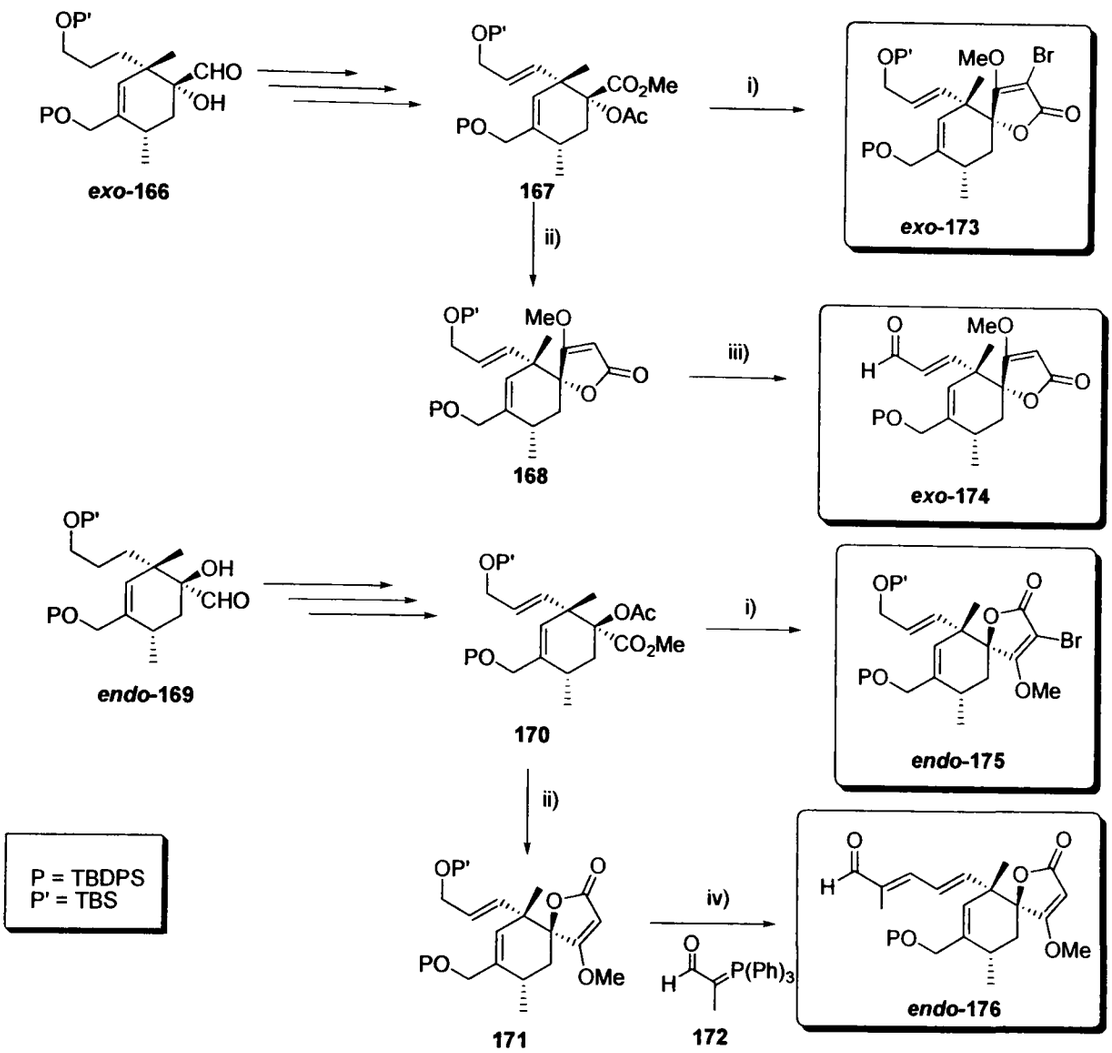


Figure 21 : Vertical *endo-exo bis*-spirotetronate **164** and horizontal *exo-endo bis*-spirotetronate **165**

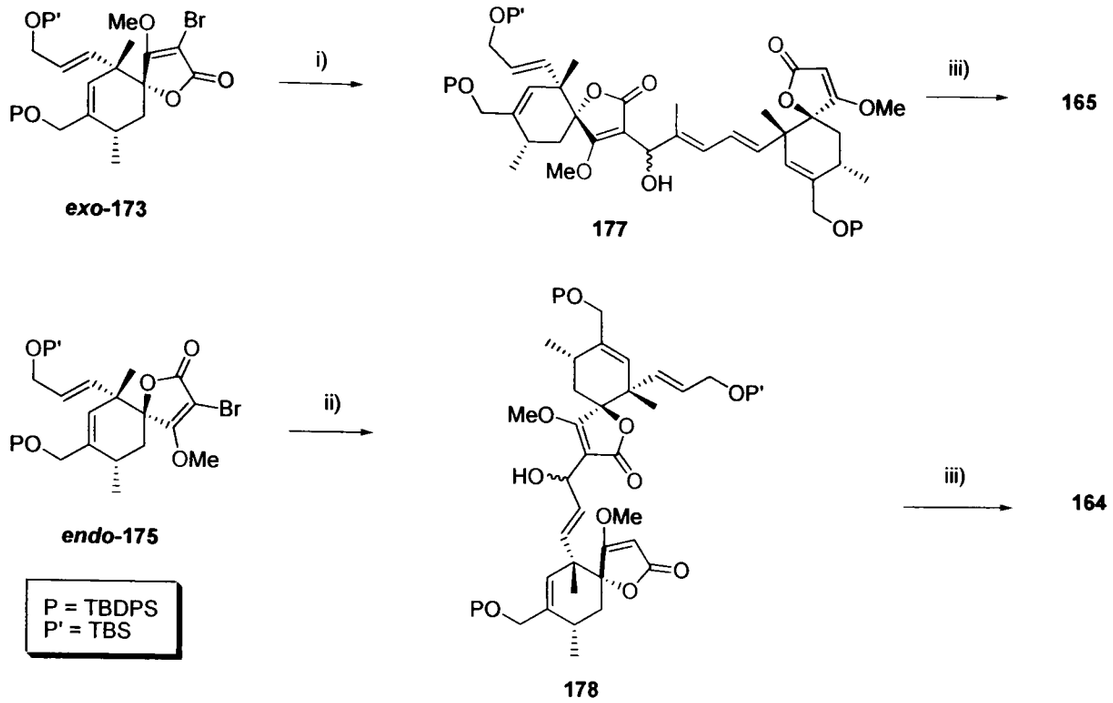
Roush and co-workers have also recently synthesised *endo*-**169** and *exo*-**166** (Scheme 35). These were then further modified to the corresponding 2-bromo spirotetronates *exo*-**173** and *endo*-**175** and the unsaturated aldehydes *exo*-**174** and *endo*-**176** (Scheme 35) which were required for coupling experiments to access the vertical and horizontal fragments of quartromicin.[127]



Scheme 35: Synthesis of *exo*-**173** *exo*-**174**, *endo*-**175** and *endo*-**176**[127]

i) 1) LiHMDS, THF/HMPA, -78°C, NBS; 2) CH_2N_2, 58-86%; ii) 1) LiHMDS, THF/HMPA, -78°C, 69-78%; 2) CH_2N_2; iii) 1) PPTS, MeOH; 2) SO_3.Pyridine, 71%; iv) 1) PPTS, MeOH; 2) SO_3.Pyridine; 3) **172**, 110°C, 43%

Both the vertical **164** and horizontal **165** fragments of quartromicins A_3 and D_3 could then be accessed *via* the 1,2-addition of organocerium intermediates derived from **173** and **175** onto the required enals **176** and **174**, respectively, followed by oxidation of the resulting alcohols to ketones (Scheme 36). **164** and **165** are proposed to be used in a total synthesis of quartromicins **122**.[127]

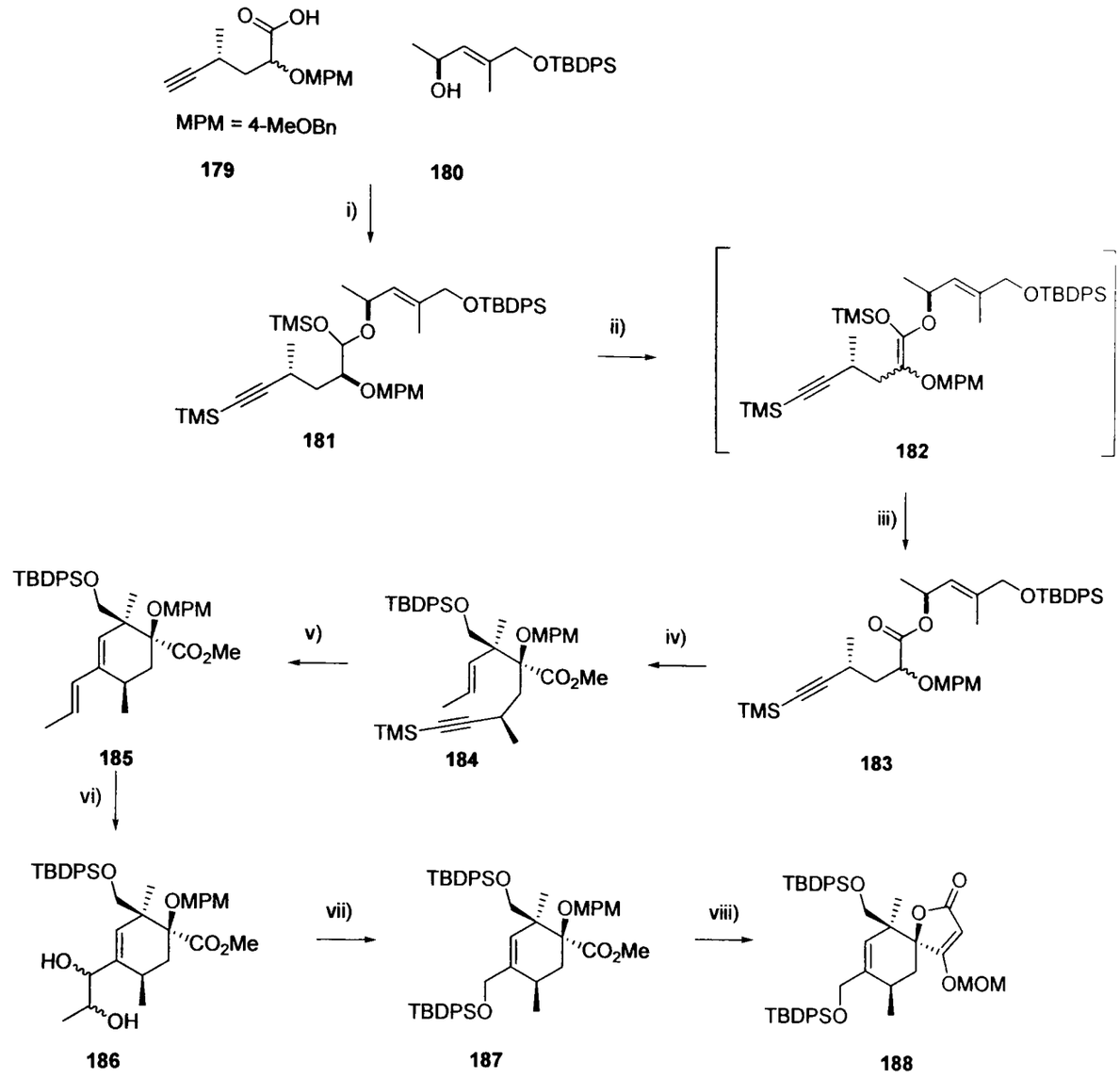


Scheme 36: Synthesis of vertical *endo-exo bis*-spirotetronate **164** and horizontal *exo-endo bis*-spirotetronate **165**

i) 1) THF, 4Å sieves, -78°C, *n*-BuLi. 2) Activated $CeCl_3$ THF solution. 3) ***endo*-176**, 58%; ii) 1) THF, 4Å sieves, -78°C, *n*-BuLi. 2) Activated $CeCl_3$ THF solution. 3) ***exo*-174**, 65% iii) MnO_2, ether 0°C to 23°C, 58-78%

3.2.3: Synthetic Approaches of Bedel and Co-workers to Quartromicins

Recently Bedel and co-workers have also published a synthetic route to access the *agalacto*-spirotetronate subunit of quartromicins (Scheme 37). The key steps in an approach are the Ireland-Claisen rearrangement followed by an ene-yne metathesis ring closure to give **185** from **184**. The *galacto* subunit can also be prepared in this manner using a modified alcohol.[128]



Scheme 37: Synthesis of the *agalacto* subunit **188** developed by Bedel and co-workers[128]

i) DCC, DMAP, CH_2Cl_2, 0°C; ii) LiHMDS/TMSCl, -78°C; iii) AcOH, THF, -78°C, 69% over three steps; iv) 1) KHMDS, Toluene, TMSCl, -78°C to rt; 2) H_2O then CH_2N_2; v) 1) K_2CO_3, MeOH; 2) Grubbs II, toluene, 80°C, 73% over 2 steps; vi) AD mix β, $MeSO_2NH_2$, *t*-BuOH, H_2O; vii) 1) $NaIO_4$, rt; 2) $NaBH_4$, 0°C, THF/H_2O, 48% over 2 steps; viii) 1) PhSH, $AlCl_3$, CH_2Cl_2; 2) Ac_2O, $Sc(OTf)_3$, MeCN; 3) LiHMDS, MOMCl, THF, 94%

3.3: Biological Properties of Quartromicins and Related Spirotetronates

Quartromicins have a range of biological properties including activity against several important viral targets, such as herpes simplex virus type 1 (HSV-1),[107] influenza A virus[107] and Human Immunodeficiency Virus (HIV).[105] Cytopathic effect (CPE) reduction was used to evaluate the antiviral activity of quartromicins against HSV-1

infection in Vero cells and influenza virus A infection in Madin-Darby canine kidney (MDCK) cells *in vitro*. Quartromicins A_1, A_2 and A_3 exhibited potent antiviral activity against HSV-1 infection (ID_{50}: 11 μg/mL), but little or no antiviral activity against influenza virus infection. Quartromicin D_1, D_2 and D_3 showed activity against influenza virus (ID_{50}: 6.8~34 μg/mL) but less anti-HSV-1 activity, without cytotoxicity against respective host cells.[107]

It has also been shown that quartromicins A_1 and D_1 significantly inhibit (HIV)-induced cytopathic effect and virus specific antigen expression at concentrations of 25-100 μg/mL in MT-4 cells, which were infected with HIV-III_B.[105] The reverse transcriptase activity of disrupted HTLV-IIIB particles, recombinant HIV-1 enzyme and purified avian myeloblastosis virus (AMV) enzymes were also inhibited. The combined antiviral effect of quartromicin A_1 and 3-azido-2',3'-dideoxythymidine (AZT) (the first drug licensed for treatment of certain HIV-related disorders) on the replication of HIV in MT-4 cells showed that quartromicin synergistically enhanced the inhibitory effect of AZT. As a result of immune defects, almost all patients with AIDS also suffer from opportunistic infections such as *Pneumocystis carinii pneumonia,* HSV and malignancies like Kaposi's sarcomas. Micro-organisms such as HSV enhance the transcription of HIV. Similarly cytokines such as TNF-α and –β stimulate transcription of HIV. It seems that various stimuli that activate cells lead to the production of virus resulting from loss of $CD4^+$ cells and appearance of immunodeficiency. Thus quartromicins also have an inhibitory effect against HSV-1 and influenza virus A infection. This means that quartromicin A_1 might not only be useful for suppressing the causative agent of AIDS but also opportunistic infections, which may be important for the pathogenesis of AIDS.[105]

A study by Berry and co-workers investigated the use of quartromicins A_1, A_2, A_3, D_1, D_2 and D_3 as phospholipase A2 inhibitors. It was determined that quartromicin D_2 inhibits phospholipase A2 (PLA_2) activity.[106,108] PLA_2 is a human enzyme that catalyses the hydrolysis of membrane phospholipids in the biosynthesis of eicosanoids, an important step in the inflammatory response associated with arthritis, psoriasis, asthma, and atherosclerosis. It is thought that the other quartromicins may also be active phospholipase A2 inhibitors.

3.4: Biosynthetic Studies of Tetronate-Containing Natural Products

While there have been no studies on the biosynthesis of quartromicins **122**, the biosynthesis of several related acyltetronic acid-containing natural products has been investigated.

3.4.1: Investigation of the Biosynthetic Origin of Tetronic Acids by Feeding Studies

Many tetronic acid-containing natural products are known and as a result there have been several independent biosynthetic investigations of such metabolites over the last twenty years. Although the biosynthesis of the α-acyltetronic acid moieties in caloric acid and protoanemonin has been reported to involve C_4 compounds from the Krebs cycle (succinnate and α-ketoglutarate, respectively), most α-acyltetronic acid moieties appear to be biosynthesised from three-carbon glycerol-derived units. [129-131]

Feeding studies using ^{13}C labelled precursors have been carried out on tetronic acids and it has been determined that acaterin **189**, isolated from *Pseudomonas sp.* A92, and the agglomerin A **190**, isolated from *Enterobacter agglomerans* PB-6042, both

incorporate a three carbon unit derived from glycerol into the tetronic acid moieties. In both cases it is thought that the direct precursor of the three carbon unit is 1,3-biphosphoglycerate (Figure 22).[132-135]

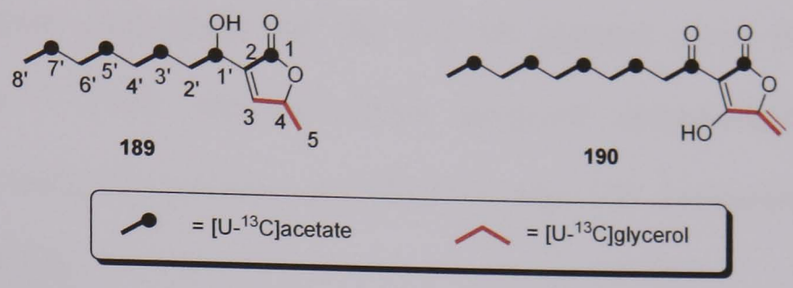


Figure 22: Biosynthetic origins of acaterin **189** and agglomerin A **190**

Although these two natural products do not possess the same spirotetronic acid moiety as quartromicin **122** or chlorothricin **126**, it is has been shown that spirotetronic acids such as chlorothricin **126** also incorporate a glycerol derived unit. Early biosynthetic studies established the origin of most of the carbon framework of chlorothricin **126** (Figure 23). The modified 6-methylsalicylic acid unit is derived from four acetate units *via* a polyketide pathway; the additional *O*-methyl group is derived from methionine; the two 2,6-dideoxyhexose moieties are derived directly from glucose; and the aglycon (chlorothricolide) is biosynthesised primarily *via* a polyketide pathway from ten acetate and two propionate units that account for all but three of the carbon atoms of chlorothricolide (C-22, C-23 and C-24), which are not labelled by acetate or propionate (Figure 23).[136,137] Large numbers of compounds were tested in order to identify the missing precursor of these three carbon atoms.[114] While [*U*-^{14}C]pyruvic acid and [1-^{14}C]lactic acid were not found to be specifically or efficiently incorporated, it was determined that [2-^{14}C]glycerol was an efficient precursor of chlorothricin. To test if glycerol was specifically incorporated into C-22, C-23 and C-24 of chlorothricolide as an intact three-carbon unit, [*U*-$^{13}C_3$]glycerol was synthesised and fed to *S. antibioticus* Tü 99.[114] NMR analysis showed that glycerol was incorporated intact into these three carbons (Figure 23). In order to determine the orientation of the 3-carbon moiety,

glycerol was stereospecifically labelled at C-1 (the *pro*-R hydroxymethyl group which undergoes phosphorylation during metabolism) giving rise to C-3 of triose phosphates, 3-phosphoglyceric acid and phosphoenolpyruvate. Incorporation experiments with this labelled precursor determined that the C-1 of glycerol gives rise to C-22 of chlorothricolide.[114] Floss and co-workers proposed phosphoenolpyruvate as a mechanistically attractive precursor to C-22, C-23 and C-24, consistent with the above results (Scheme 38).

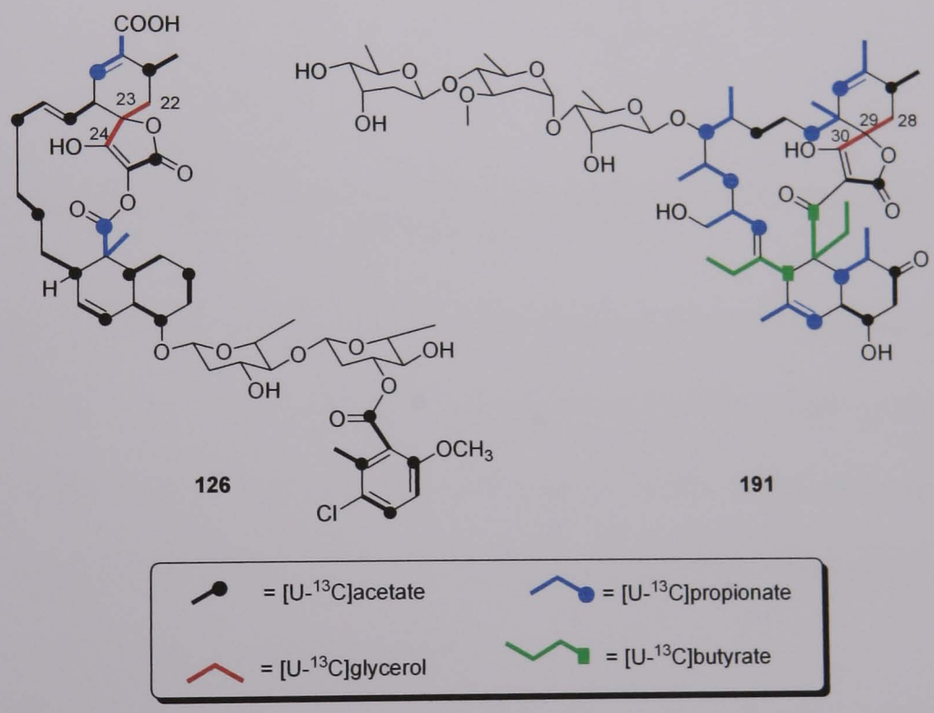
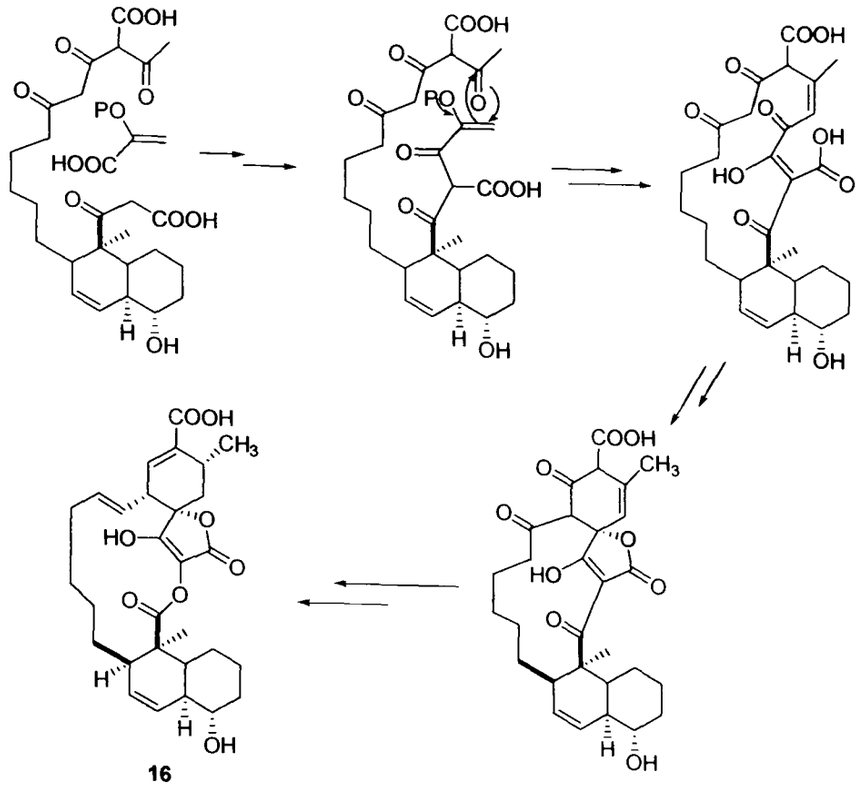


Figure 23: Biosynthetic origins of chlorothricin **126** and versipelostatin **191**



Scheme 38: Mechanism for formation of the spirotetronic acid moiety in chlorothricin proposed by Floss and co-workers. $P=PO_3^{2-}$

Versipelostatin **191** has been shown to be biosynthesised *via* condensation of a linear polyketide chain and a C_3 branching unit. Feeding experiments established that C-28, C-29 and C-30 are derived from an intact glycerol molecule rather than pyruvic acid or succinic acid (Figure 23).[138] It seems likely that chlorothricin **126** and versipelostatin **191** share a common biosynthetic pathway.

3.4.2: Cloning, Sequence and Analysis of Gene Clusters that Direct Spirotetronate Biosynthesis

The gene clusters that direct chlorothricin **126** and kijanimicin **127** biosynthesis were published in 2006 and 2007, respectively (Figure 24 and Figure 25).[139,140] Gene sequencing, preparation of mutant strains and BLAST searching has allowed the assignment of putative functions to the genes in both clusters and the genes that putatively direct assembly of the spirotetronate moiety to be identified. DNA sequencing and genetic studies of chlorothricin **126** biosynthesis led to the proposal that

ChlD1, ChlD2 and ChlD3, which are homologues of KijC, KijD, the N-terminal domain of KijE, respectively, are involved in the biosynthesis of the glycerol-derived three carbon unit.[139,140]

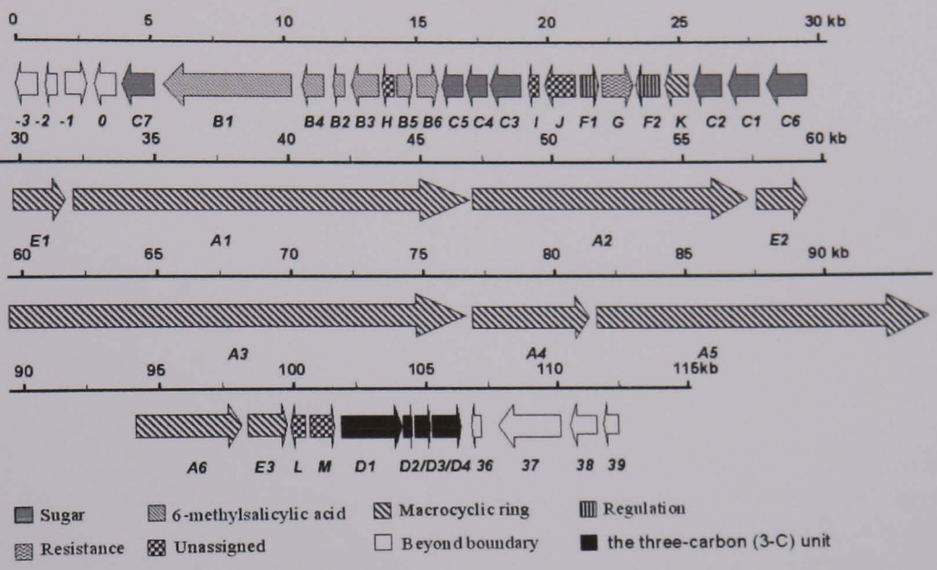


Figure 24: Chlorothricin **126** gene cluster[139]

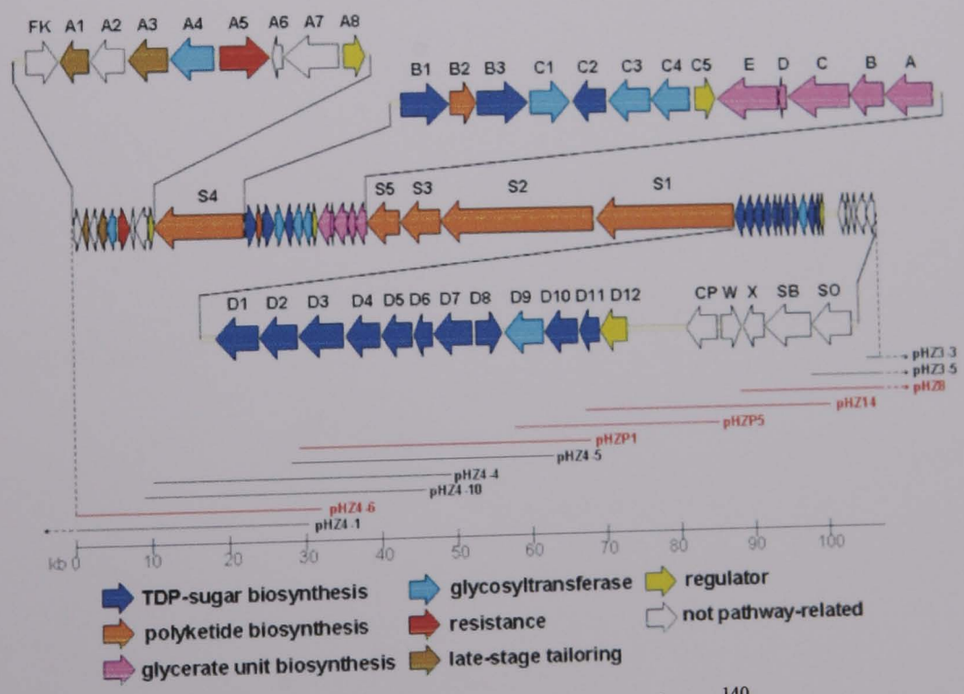


Figure 25: Kijanimicin **127** gene cluster[140]

This led Liu and co-workers to predict that the five proteins encoded by *kijABCDE* are involved in activation of the glycerol-derived three carbon unit, its attachment to the

polyketide chain, and subsequent intramolecular Diels-Alder cyclisation required to generate the spirotetronate ring system **192** as shown in Figure 26.[140]

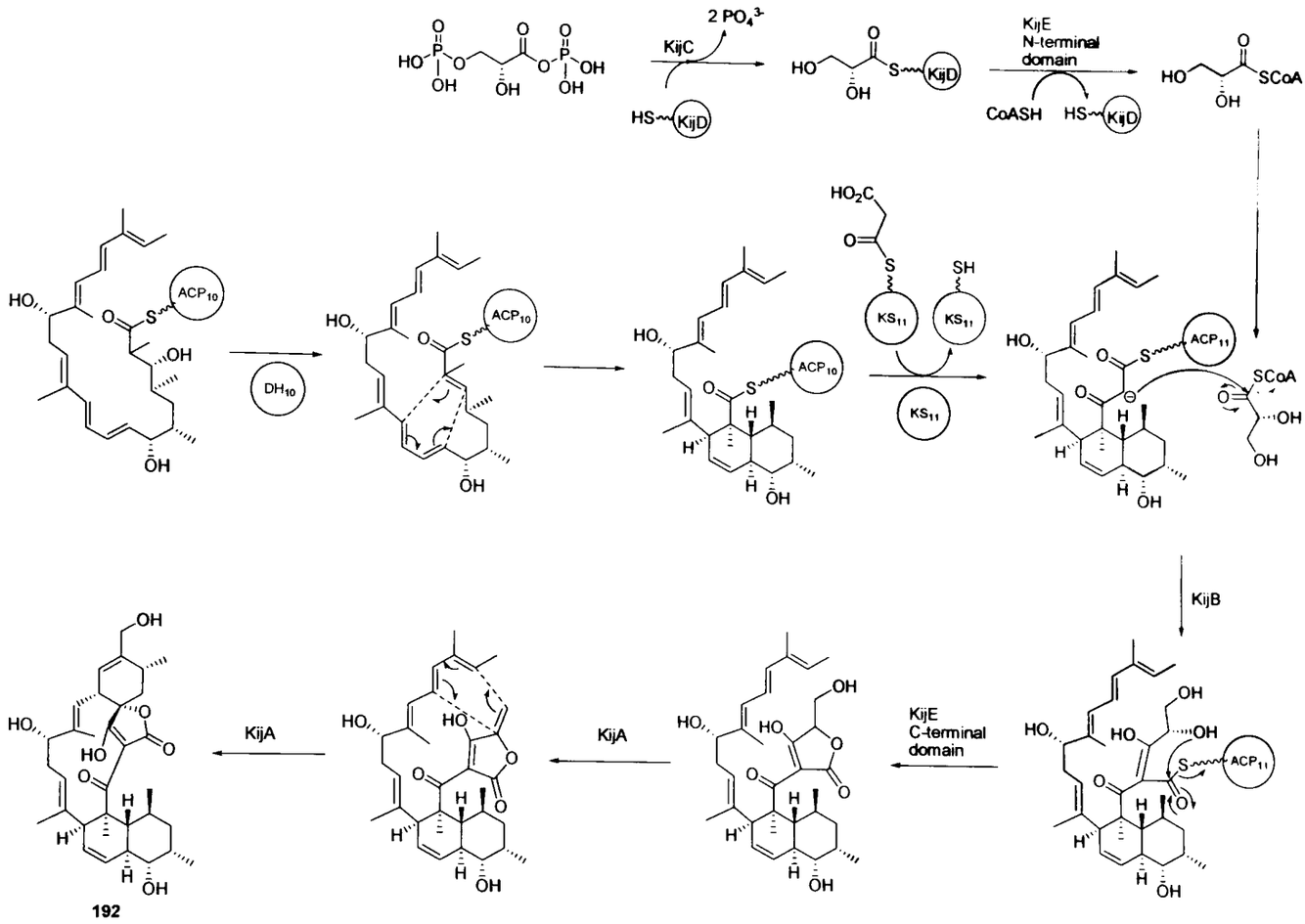


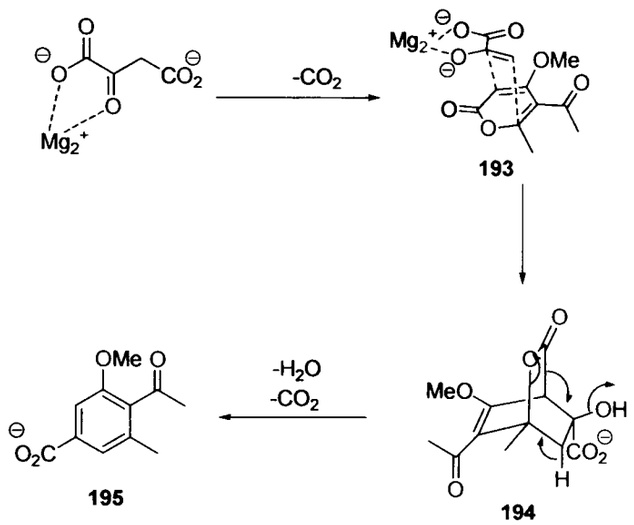
Figure 26: Proposed pathway for generation of spirotetronate **192** in kijanimicin **127**[140]

3.5: Diels-Alderase Enzymes

Despite the belief that many natural products are biosynthesised by Diels-Alder reactions (including those containing spirotetronates), there are only three known Diels-Alderase enzymes of which only one has been structurally characterised. Solanapyrone synthase,[141,142] lovastatin nonaketide synthase[143] and macrophomate synthase (MPS)[144] catalyse not only Diels–Alder reactions but also oxidation, polyketide chain formation and decarboxylation, respectively. These enzymes convert their corresponding substrates into reactive Diels–Alder precursors, forcing them into reactive conformations to undergo cycloaddition and releasing them from the active site.

3.5.1: Macrophomate Synthase

The phytopathogenic fungus *Macrophoma commelinae* transforms 2-pyrone derivatives **193** into the corresponding benzoate **195**; this reaction is catalysed by the MPS enzyme (Scheme 39).[144,145]



Scheme 39: Proposed reaction catalysed by MPS[144]

The active site of MPS is at the C-terminal end of the β-barrel, which is covered by the long loop from the three-fold-related chain. At one side of this catalytic cavity, Mg(II) is located in an octahedral coordination. Based on the crystal structure (Figure 27) a binding model was produced (Figure 28). In this binding model, the 2-pyrone molecule is likely to be fixed in place through two hydrogen bonds between the carbonyl oxygen of the 2-pyrone and Arg 101, and the C5-acyl oxygen of the pyrone and Tyr 169. Tyr 169 is in turn placed in the proper orientation through π-π stacking with Phe 149. The flexible loop (residues 139–170) with hydrophobic side-chains (Phe 149, Pro 151 and Trp 152) from the three-fold related protomer shields this transition state from the solvent.

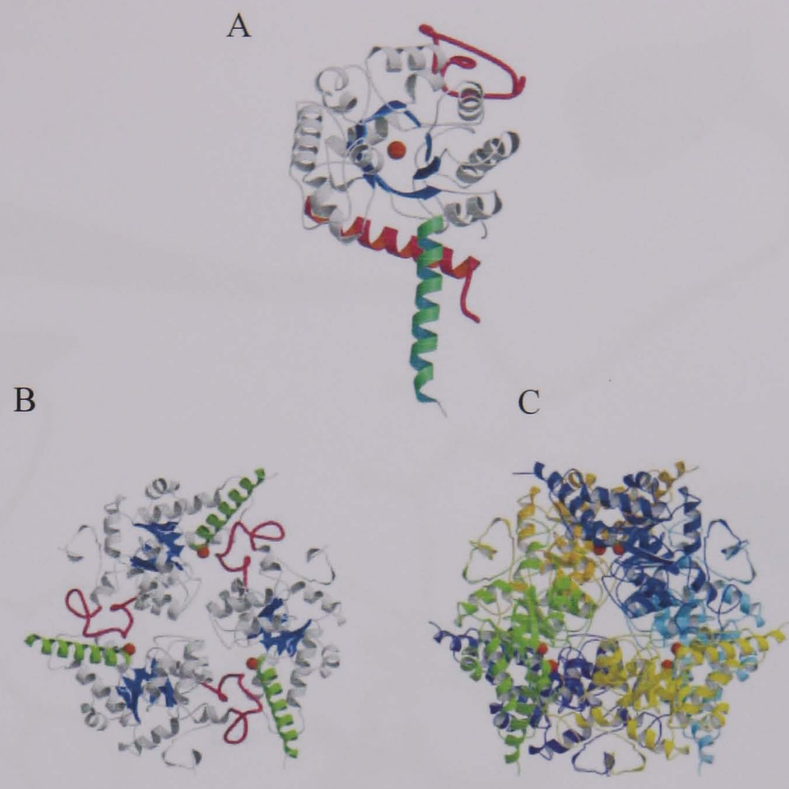


Figure 27: Overall structure of MPS

a) Protomer structure of MPS showing an α-helix swapped $(\beta/\alpha)_8$ barrel fold. The β-barrel core (blue) is surrounded by 11 α-helices. The long α-helix (magenta) belongs to a neighbouring protomer related by the two fold axis and joins the β-barrel to form the $(\beta/\alpha)_8$ barrel. The divalent magnesium ion (red) is located at the C-terminal region of the β-barrel. A long loop (magenta) opposite to the swapped helix interacts with an adjacent protomer by the three fold axis – shown in b); b) Three protomers related by the three fold axis, each loop joins the active site to the next protomer. c) The functional unit of MPS[144]

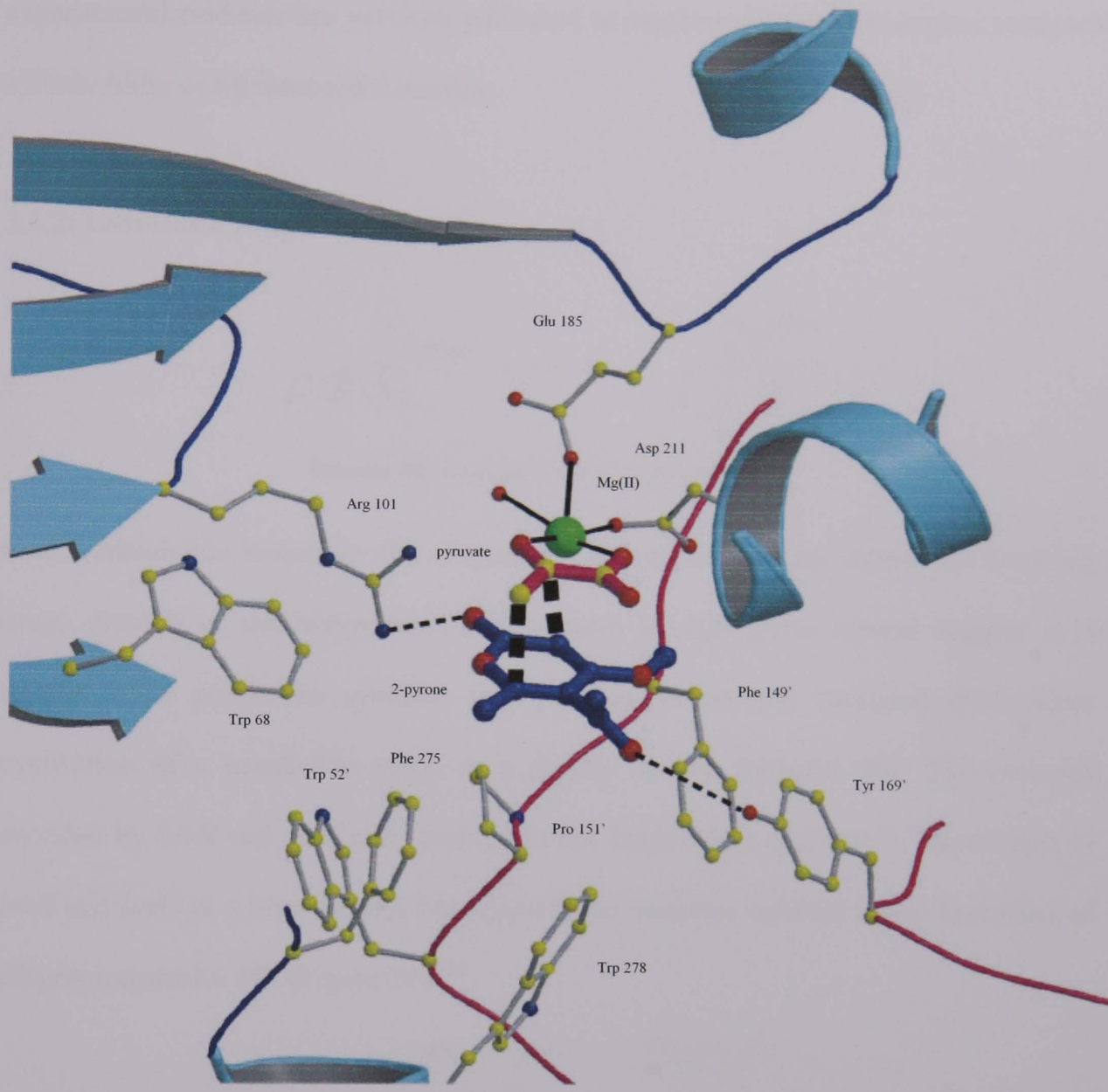
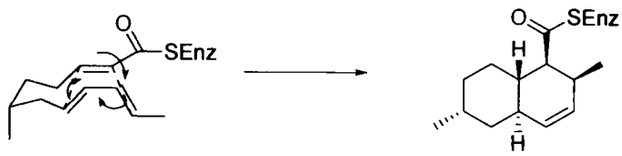


Figure 28: The residues in the active site pocket of MPS and proposed model for the early transition state of the Diels-Alder reaction catalysed by this enzyme[144]

However, more recently Jorgensen and co-workers have suggested that this enzyme may not catalyse a Diels-Alder reaction, but a Michael-aldol reaction. They carried out mixed quantum and molecular mechanics (QM/MM) combined with Monte Carlo simulations and free energy calculations to investigate the relative stabilities of the transition states for both reaction mechanisms. The results of these studies indicated that the Diels-Alder transition states are less stable than the Michael-aldol transitions states and therefore that MPS may not be a Diels-Alderase enzyme.[146] However, no

experimental evidence has yet been published to confirm whether the enzyme catalyses a Diels-Alder or Michael aldol reaction.

3.5.2: Lovastatin Nonaketide Synthase



Scheme 40: Lovastatin Diels-Alder cyclisation

Fungal metabolite lovastatin **196** (Figure 29) and derivatives are cholesterol lowering drugs. Studies of the biosynthesis of lovastatin in *Aspergillus terreus* suggest it is formed by a polyketide synthase incorporating an enzyme catalysed Diels-Alder cyclisation of a hexaketide triene to a decalin system (Scheme 40). The enzymes encoded by *lovB* and *lovC* are involved in the Diels-Alder cyclisation. Expression of *lovB* and *lovC* in a heterologous host *Aspergillus nidulans* resulted in the formation of dihydromonacolin **197** (Figure 29).[143]

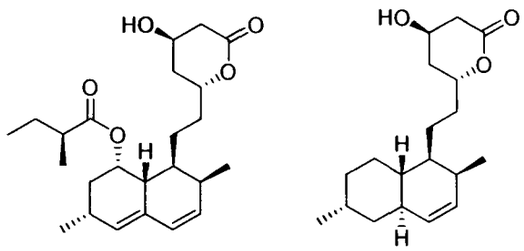


Figure 29: Lovastatin **196**, and dihydromonacolin **197**

However, expression of *lovB* without *lovC* resulted in pyrone formation and no Diels-Alder cyclisation reaction. To ascertain whether lovastatin nonaketides (LNKS, encoded by *lovB*) could catalyse Diels-Alder reactions, the 335kD enzyme was purified from *A. nidulans* and added to **198** (Figure 30). It was determined that, in the absence of LNKS, cyclisation occurred spontaneously to give a mixture of *exo* and *endo* products (1:1 ratio **200:201**, **202** and **199** were not observed). The *endo* product **202** is produced in the

presence of LNKS (the stereochemistry of which corresponds to the lovastatin precursor **197**) as well as **200** and **201** (in a ratio of 15:15:1 **200:201:202**, again **199** was not observed).[143] When LNKS was inactivated production of **202** was no longer seen. It is assumed that a key function of LNKS is to bind to the substrate in a conformation resembling the *endo* stereochemistry that leads to **202** in which the branching methyl group is pseudo-axial (unfavourable). Hence the product is formed when the enzyme is present.[143] Although the enzyme encoded by *lovC* has not yet been purified, preliminary experiments suggest that it binds to LNKS along with the usual co-factors and substrates.

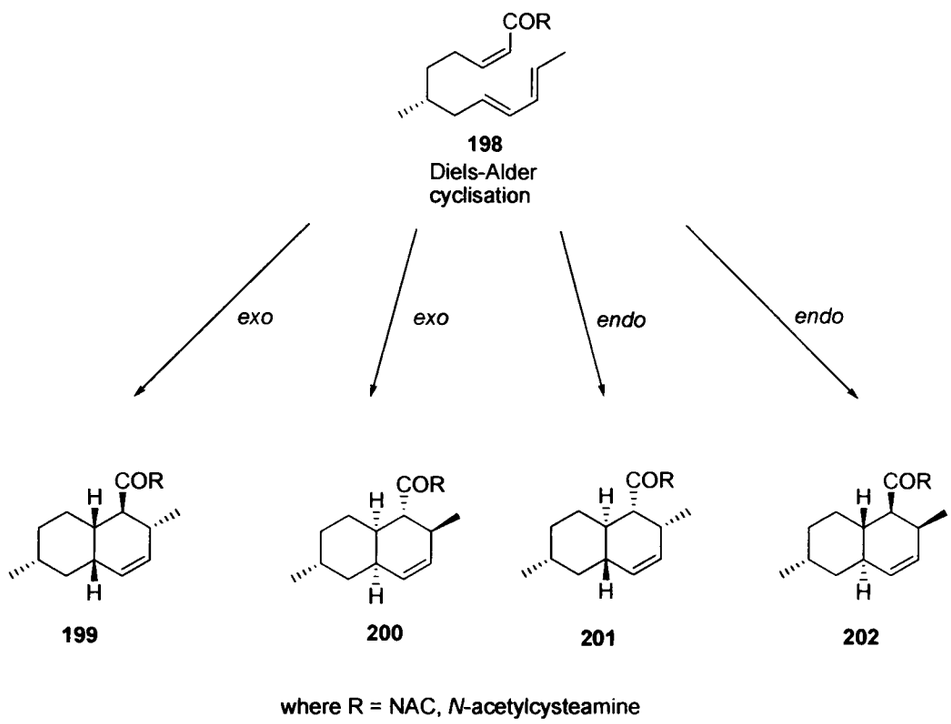
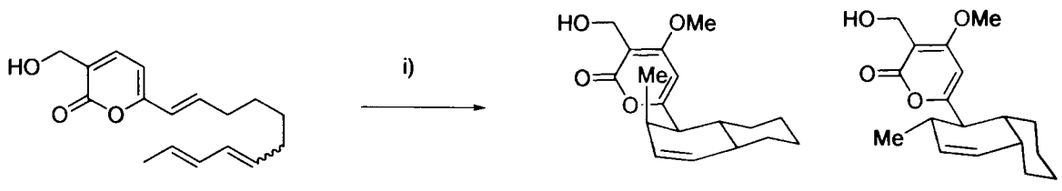


Figure 30: Intramolecular Diels-Alder cyclisation reactions of **198**. Product **202** is only observed in the presence of LNKS[143]

3.5.3: Solanpyrone synthase

Feeding experiments have demonstrated that the phytotoxins salanopyrones produced by *Altermaria solani* are biosynthesised by a [4+2] cycloaddition which proceeds with high *exo*-specificity. Oikawa and co-workers have partially purified the enzyme salanopyrone synthase, and shown that this converts synthetic substrate **203** into **204**

and **205** in the presence of molecular oxygen, on a semi-preparative scale without loss of enantioselectivity or diastereoselectivity (Scheme 41).[141,142]



203 **204** **205**

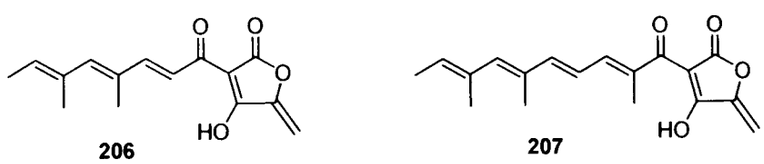
51% (>98% ee)

Scheme 41: Enzymatic Diels-Alder reaction of a synthetic prosolanopyrone

i) Partially purified the enzyme salanopyrone synthase

3.6: Proposed Biosynthetic Pathway to Quartromicins

Although little has been determined about the biosynthetic pathway of the quartromicins, a plausible biosynthetic pathway to the carbon skeleton of **122a-f** has been proposed by Challis and co-workers, which could proceed *via* the intermediates **206** and **207** (Figure 31). The pathway is initially proposed to involve a type I modular polyketide synthase-mediated assembly of two enzyme bound β-ketothioesters. The type I PKS is likely to contain five modules (Figure 32). Both of the two enzyme bound β-ketothioesters could be produced by the same PKS, although the assembly of **206** would require "skipping" of module four.



206 **207**

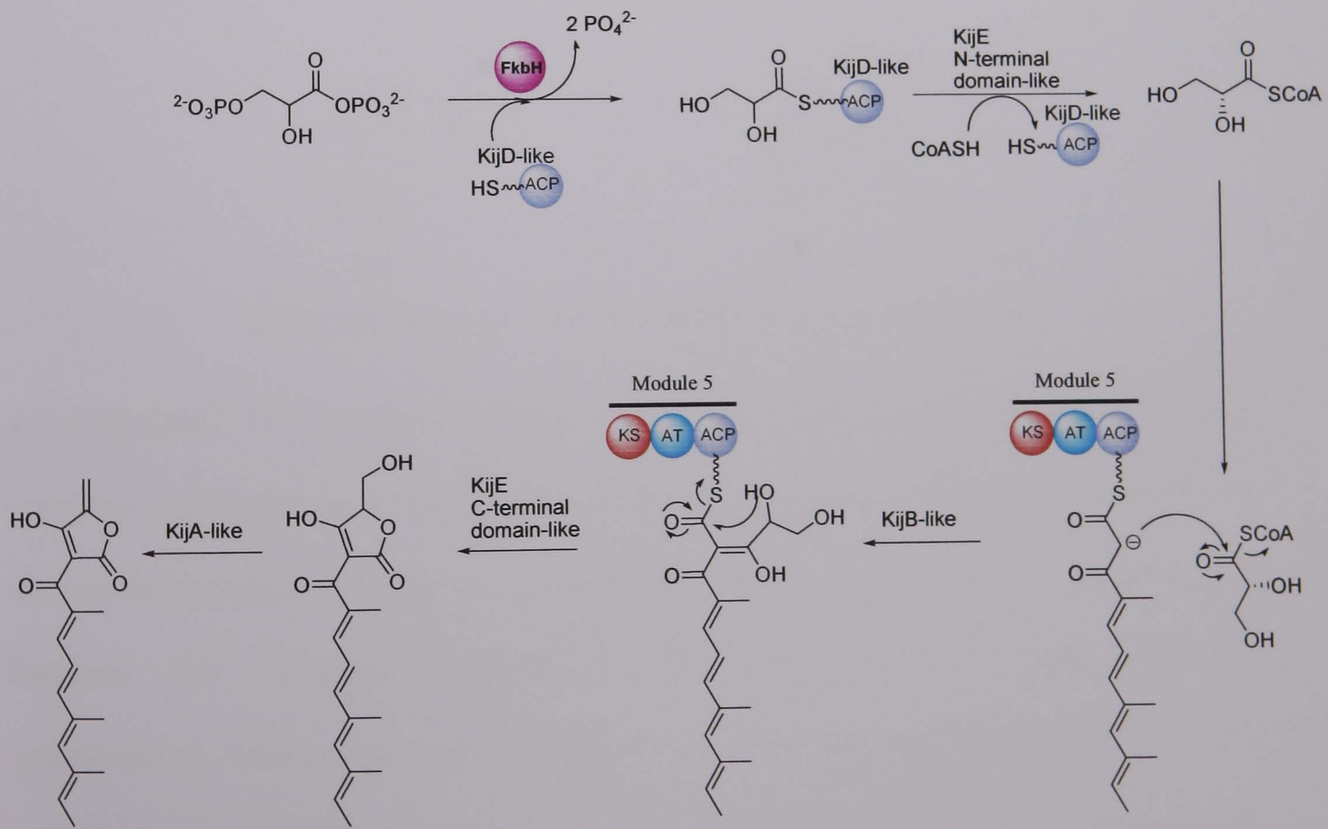
Figure 31: Proposed intermediates **206** and **207** in the quartromicin biosynthetic pathway

Figure 32: Proposed module organisation of the PKS catalysing the assembly of linear polyketide chains in putative intermediates **206** and **207**

Module "skipping" occurs in several other PKSs, for example the pikromycin PKS.[147-149] Pikromycin is a 14-membered macrolide produced by *Streptomyces venezuelae.* This bacterium also produces several other macrolides, including the 12-membered macrolide methymycin.[150] Methymycin is produced by the same PKS as pikromycin, except the final module is "skipped" hence a shorter chain is formed.[147-149] It is proposed that the rate of transfer of the polyketide chain from module five to the thioesterase of module six is similar to the rate of loading of the final extension unit onto the ACP domain of module six. Thus the final condensation can be skipped and the TE catalysed cyclisation can occur to form the smaller 12-membered ring. In quartromicin biosynthesis, the same situation could apply if the loading of the malonyl-CoA extender unit onto the ACP domain of module four occurs at a similar

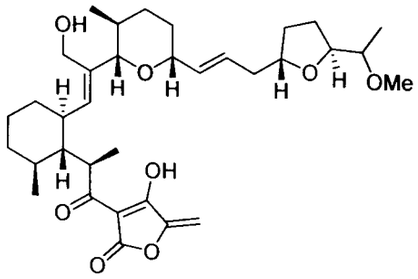
rate to the transfer of the β-ketothioester from module three to module five. Module four would then be skipped and the shorter chain of precursor **206** would be produced.

A FkbH-like stand alone protein would then load 1,3-biphosphoglycerate on to a KijD-like stand alone ACP and catalyse phosphate hydrolysis to give the glyceryl-ACP. An enzyme similar to the N-terminal domain of KijE would convert the glyceryl-ACP into glycerol-CoA, and a KijB-like enzyme would condense the β-ketothioester chains attached to module five of the PKS with glyceryl-CoA. An enzyme similar to the C-terminal domain of KijE would catalyse lactonisation and release of the polyketide chain from the ACP of module five and a KijA-like protein catalyses dehydration of the resulting 2-acyl-4-hydroxy-methyl tetronic acid, which would then undergo Diels-Alder cycloadditions.



Scheme 42: Proposed biosynthetic pathway to quartromicin intermediates **206** and **207**

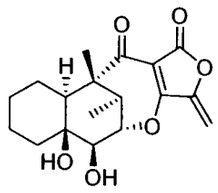
Analogous enzymes to those proposed to assemble the putative exomethylene tetronic acid intermediates in kijanimicin / chlororthricin biosynthesis have been found encoded within the tetronomycin **208** biosynthetic gene cluster (Figure 33).[151] A recent publication shows that enzymes from the tetronomycin biosynthetic gene cluster divert glyceryl units from glycolysis into the tetronate ring formation.[152]



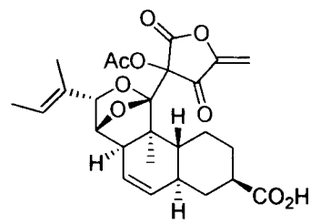
208

Figure 33: Tetronomycin **208**

Other exomethylene tetronic acids such as tetrodecamycin **209**[153] and FR-900109[154] **210** are known and are probably biosynthesised by similar PKS systems (Figure 34).



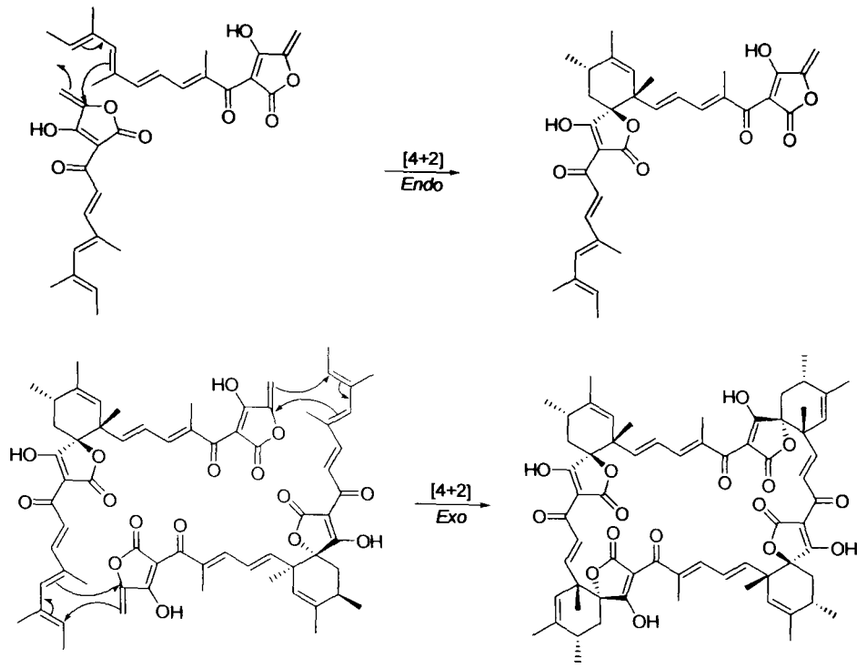
209



210

Figure 34: Structures of tetrodecamycin **209** and FR-900109 **210**

An *endo*-Diels-Alder reaction between the two acyl tetronic acids **206** and **207** would yield the corresponding heterodimer. This dimer could then undergo homodimerisation *via exo*-Diels-Alder cyclisations to yield the carbon skeleton of the quartromicins (Scheme 43). Subsequent oxidations and galactosylations would yield the quartromicins. Similar biosynthetic pathways, all involving elimination of water from a tetronic or tetramic acid followed by IMDA cyclisation, have been proposed for the other members of the spirotetronic acid family of natural products.



Scheme 43: Diels-Alder cyclisation of proposed intermediates **206** and **207** to produce carbon skeleton of quartromicin **122**

3.7: Aims and Objectives

The aim of this project was to develop a concise and versatile synthetic route to 2-enoyltetronic acids. Such tetronic acids include the proposed precursors **206** and **207** of the quartromicin antibiotics (Figure 35).

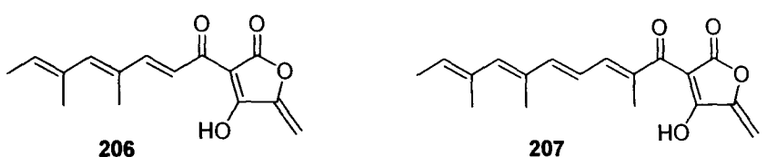


Figure 35: Proposed precursors in quartromicin biosynthesis **206** and **207**

Once a synthetic route had been developed to these two precursors it was proposed to investigate the biomimetic synthesis of the carbon skeleton of the quartromicin algycone from the precursors.

Chapter 4: Synthesis and Feeding of Analogues of a Key Intermediate in Prodiginine Biosynthesis

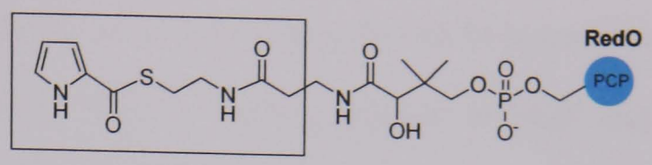
4.1: Aims of the Work Described in this Chapter

Chemically-synthesised analogues of proposed biosynthetic intermediates can be used in conjunction with mutants containing knockouts in individual biosynthetic genes to investigate natural product biosynthetic pathways. In an effort to investigate the biosynthetic pathway of the prodiginines produced by *S. coelicolor* A3(2), a synthetic route was developed to produce a mimic **117** of a proposed key intermediate **32** in the biosynthesis of the bipyrrole **10**, which is in turn a key intermediate in prodiginine biosynthesis. Further analogues of intermediate **32** were also synthesised and the ability of mimic **117** and its analogues to direct production of natural prodiginines and analogues modified in the A-ring by feeding to bipyrrole-deficient mutants of *S. coelicolor* was explored.

4.2: Preparation of a Mimic 117 of a Proposed Key Intermediate 32 in the Biosynthesis of Bipyrrole 10

In order to establish the sequence of biochemical events in the biosynthesis of several natural products, *N*-acetylcysteamine (NAC) thioester analogues of putative carrier protein bound intermediates have been used.[73,155-161] Therefore, it was thought that the NAC thioester of pyrrole-2-carboxylic acid **117** would be a suitable mimic for the key intermediate **32** in the biosynthesis of the bipyrrole **10** (Figure 36).

Pyrrole-2-carboxyl-S-RedO, **32**



Pyrrole-2-carboxyl-thioester-NAC, **117**

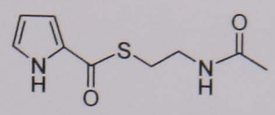
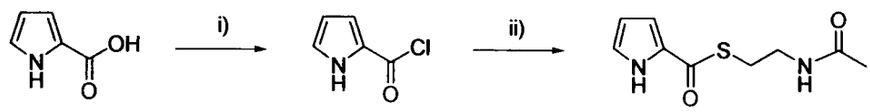


Figure 36: A mimic **117** of a proposed key intermediate **32** in the biosynthesis of bipyrrole **10**

The preparation of pyrrole-2-carboxyl-*N*-acetylcysteamine thioester **117** from pyrrole-2-carboxylic acid **211** and *N*-acetylcysteamine was attempted initially using the coupling reagents DMAP and EDCI. The reaction was investigated using a variety of solvents, including anhydrous DCM, DCE and toluene, at different temperatures. However, these attempts were unsuccessful and multiple products were formed in each of these reactions. This is likely to be because of the low electrophilicity of the carbonyl carbon of the activated ester derivatives of pyrrole-2-carboxylic acid, resulting from the donation of electron density from the electron rich pyrrole ring into the carbonyl π* orbital. Owing to the failure of these reactions, alternative procedures were sought. Conversion of pyrrole-2-carboxylic acid **211** to the acid chloride **212** followed by coupling with *N*-acetylcysteamine was thus examined.

A procedure to synthesise compound **117** has been previously reported by Thomas *et al.*[73] who converted pyrrole-2-carboxylic acid **211** to the corresponding acid chloride **212** using excess oxalyl chloride, then coupled the acid chloride and *N*-acetylcysteamine by heating to reflux overnight in DCE, yielding the product **117**. This method was attempted but none of the desired **117** was obtained. It appeared that the acid chloride had not formed in the reaction; several other possible methods are available for preparing the acid chloride, **212**. One method reported by Bourguignon and co-workers involves heating pyrrole-2-carboxylic acid with thionyl chloride at 80°C in benzene.[162] However, when this was attempted, a brown solid was produced which could not be

dissolved in any solvent or combination of solvents, probably due to polymerisation of the starting material. Other methods utilised a catalytic amount of DMF and oxalyl chloride to aid the conversion of the carboxylic acid to the acid chloride probably *via* formation of the Vilsmeier salt.[163-166] Using this modification of Thomas and co-workers procedure resulted in the desired acid chloride product, as determined by IR spectroscopy which showed a significant change in the frequency of absorption of the carbonyl group (pyrrole-2-carboxylic acid 1651 cm^{-1} and pyrrole-2-carbonyl chloride 1718 cm^{-1}) (Scheme 44).



211 **212** **118**

Scheme 44: Preparation of pyrrole-2-carboxyl-*N*-acetylcysteamine thioester **117**
i) Oxalyl chloride, DMF, reflux, 1h. ii) *N*-acetylcysteamine, DMAP, reflux, 14h, DCE, 75% over 2 steps

Coupling of acid chloride **212** with *N*-acetylcysteamine was initially attempted by heating to reflux the acid chloride and *N*-acetylcysteamine in DCE, without success. It was thought that the *N*-acetylcysteamine may have dimerised *via* disulfide formation after exposure to the air. However, a ^1H NMR spectrum of the *N*-acetylcysteamine showed the signal for the SH proton at 1.4 ppm. The *N*-acetylcysteamine was then dissolved in deuterated methanol. The solvent was removed *in vacuo* and a further ^1H NMR spectrum was recorded showing that the signal corresponding to the SH proton at 1.4 ppm was no longer present due to the acidic thiol hydrogen exchanging with deuterium. This confirmed that no *N*-acetylcysteamine dimer was present, because if a dimer of the starting material was present no SH proton would have been seen in the original ^1H NMR spectrum, i.e. both spectra would have been identical.

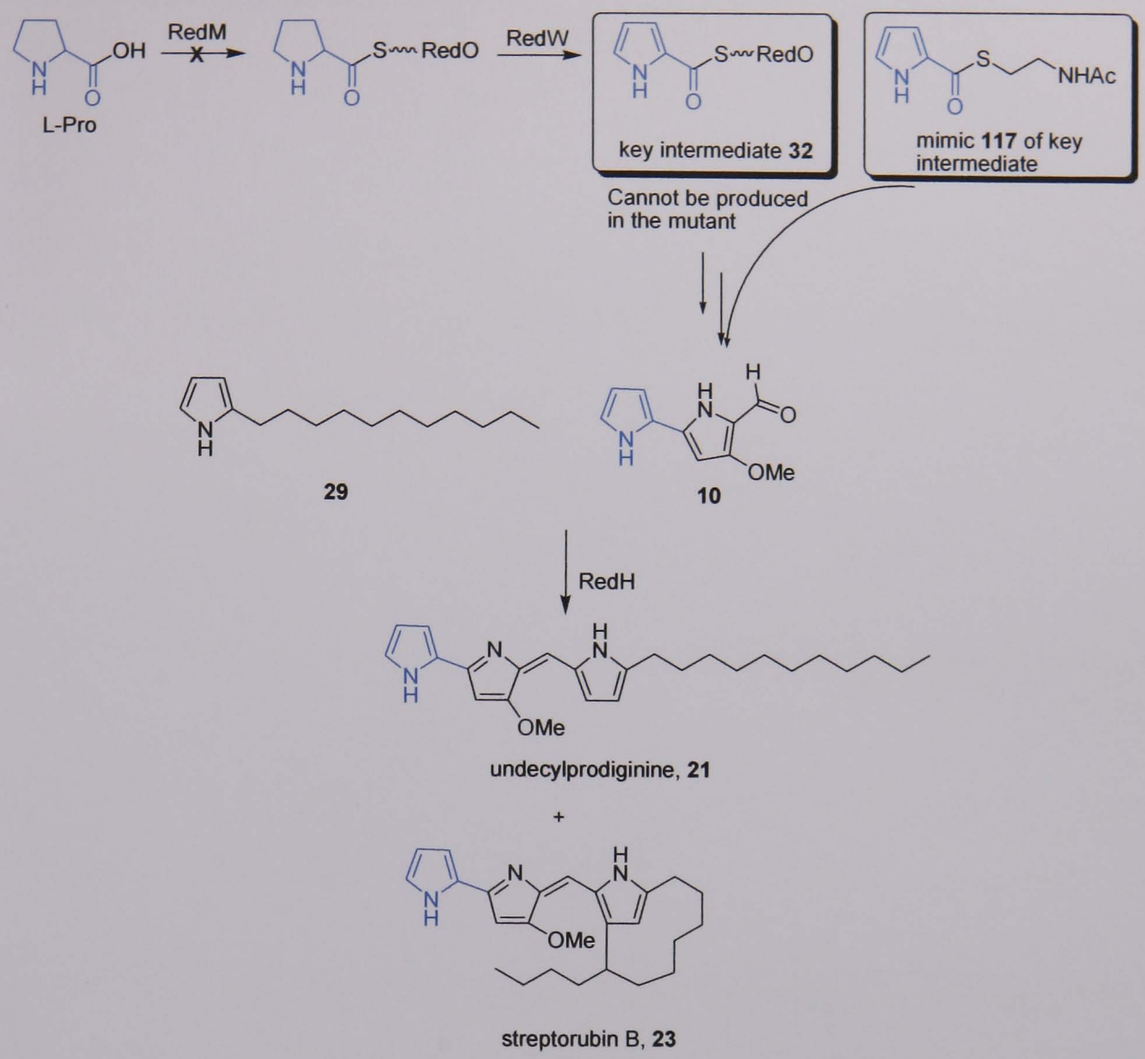
Having determined that oxidative dimerisation of *N*-acetylcysteamine was not causing the problem, the reaction was attempted again with the addition of DMAP as a

nucleophilic catalyst. The reaction was successful and the desired synthetic analogue **117** was produced in a good yield (75%). It was determined that the reaction proceeded better utilising DCE as solvent, as opposed to DCM, as this allowed the reaction to be carried out at a higher temperature, thus increasing the rate of reaction. The harsh conditions required for the synthesis of acid chloride **212** and its conversion to thioester **117** reinforce the hypothesis that the carboxyl group in pyrrole-2-carboxylic acid and its derivatives are unreactive towards nucleophiles.

Purification of compound **117** was undertaken by passing the pyrrole-2-carboxyl-*N*-acetylcysteamine thioester through a plug of silica impregnated with copper sulphate. The excess *N*-acetylcysteamine was removed by the formation of a copper complex. The silica impregnated with copper sulphate changed from blue to yellow as the complex formed and remained at the top of the column.[167]

4.3: Feeding of Pyrrole-2-carboxyl-*N*-acetylcysteamine thioester 117 to *redM::aac(3)IV* Mutant of *S. coelicolor* M511

A mutant of *Streptomyces coelicolor* M511 (*redM::aac(3)IV*) has been prepared by Stanley (a former PhD student in the Challis group) by replacing *redM* on the chromosome of *S. coelicolor* M511 with a "cassette" containing *oriT* and the *acc(3)IV* apramycin resistance gene.[72,168] Neither undecylprodiginine **21** nor streptorubin B **23** could be detected in organic extracts of the *redM::aac(3)IV* mutant. However, 2-undecylpyrrole **29** was seen by LC-MS analysis and LC-MS/MS comparison with a synthetic standard of **29** (Scheme 45 and Figure 37). No accumulation of the bipyrrole **10** was seen by LC-MS/MS comparisons to a chemically synthesised authentic standard (Scheme 45 and Figure 37).[72]



Scheme 45: Proposed roles of RedM, RedW and RedO in assembly of bipyrrole intermediates in the biosynthesis of prodiginines

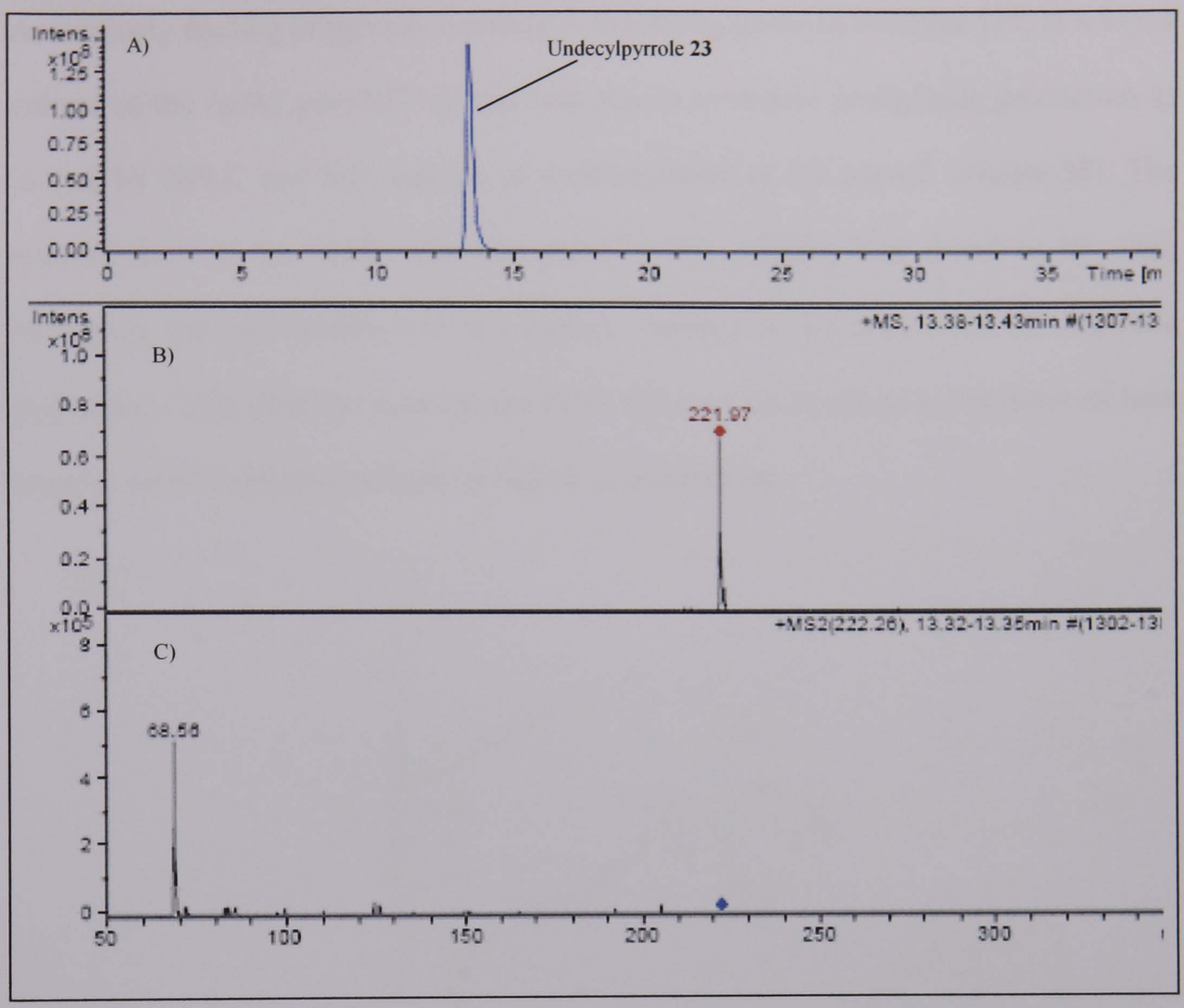
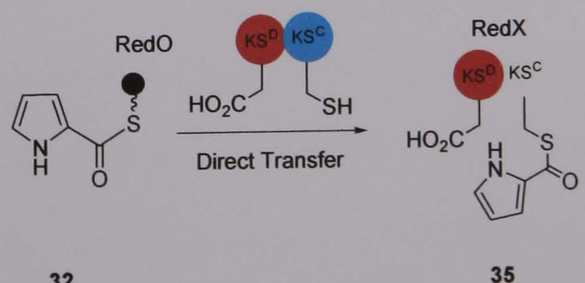


Figure 37: LC-MS/MS analysis of *redM::acc(3)IV* mutant of *S. coelicolor* showing **A)** accumulation of UP in this mutant **B)** EIC showing m/z 222 corresponding to the mass of UP **C)** Fragmentation pattern of 2-undecylpyrrole

Direct transfer of the pyrrole-2-carboxyl group between the RedO PCP and the RedX enzyme is proposed to occur in the assembly of MBC **10** (Scheme 46).[72,76] Thus feeding of the synthetic mimic **117** of pyrrole-2-carboxyl-RedO should restore production of the prodiginines in the *redM::aac*(3)IV mutant of *S. coelicolor*.



Scheme 46: Proposed pathway for the transfer of the pyrrole-2-carboxylic group from RedO to RedX

Accordingly feeding of pyrrole-2-carboxyl-*N*-acetylcysteamine thioester **117**, to a liquid culture of the *redM::aac(3)IV* mutant was shown to restore prodiginine production as judged by HPLC and MS analysis at concentrations of 0.1 mg/mL (Figure 38). The concentration of the NAC thioester added to the cultures was found to be vital; increasing the concentration to 0.5 mg/mL resulted in no restoration of antibiotic production. This could be because the NAC thioester could act as an inhibitor of later steps in the biosynthetic pathway at higher concentrations.

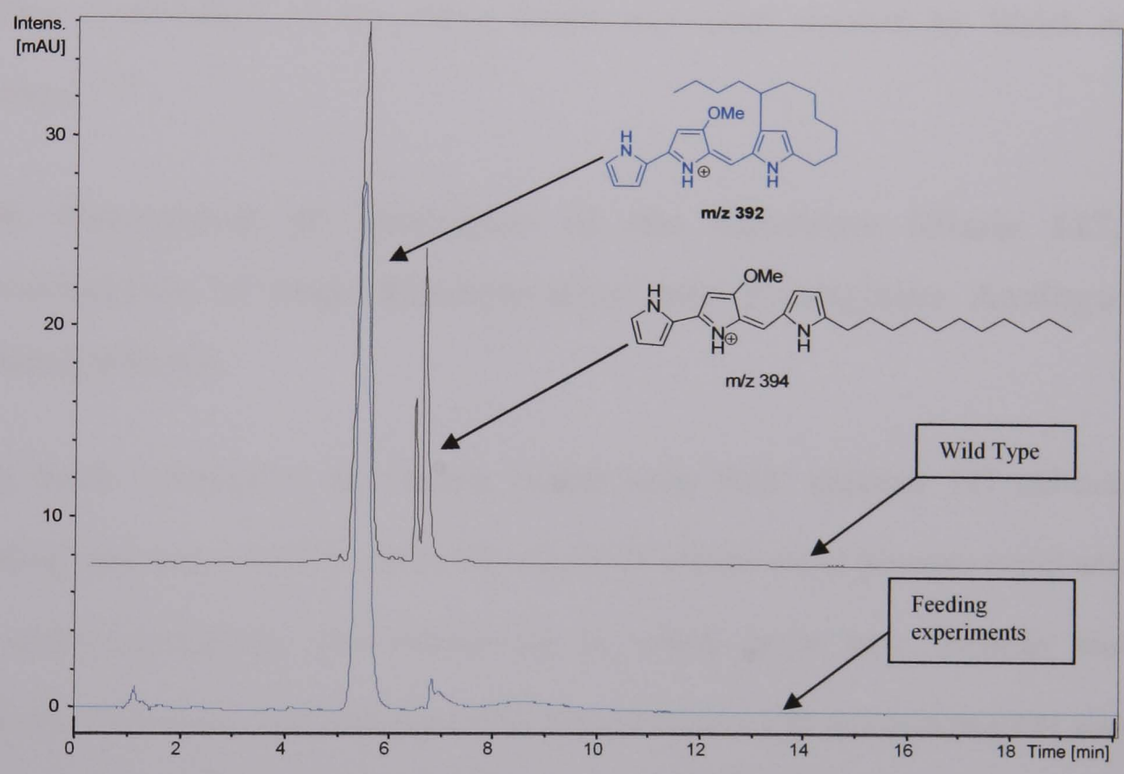


Figure 38: UV chromatogram of the wild type *Streptomyces coelicolor* (black) and *redM::acc(3) IV* mutant (blue) mycelia after prodiginine production had been restored

Ion extraction from the LC-MS chromatogram revealed that the large peak with retention time of 5.5 minutes corresponded to streptorubin B **22** (m/z 392.3) and the small peak at a retention time of 7.0 minutes corresponded to undecylprodiginine **21** (m/z 394.3). Interestingly, the purity of the NAC thioester was not an important factor in the success of this experiment. Both crude and purified samples of the NAC thioester were fed and both restored production of prodiginines in the *S. coelicolor*

redM::aac(3)IV mutant. These studies are consistent with the hypothesis that direct transfer of the pyrrole-2-carboxyl group from the RedO PCP to the Red-X enzyme occurs.[72,76]

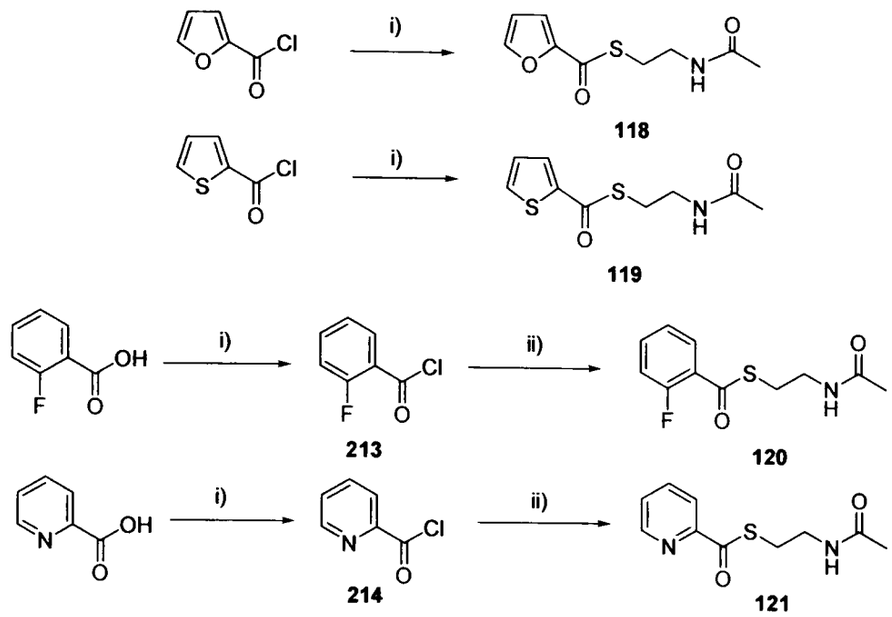
Further feeding studies of analogue **117** with bipyrrole-deficient *redM::aac(3)IV* and *redW::aac(3)IV* mutants (Scheme 45) were carried out by Stanley.[168] When **117** was fed to the *redW::aac(3)IV* mutant, prodiginine production was also restored, providing further confirmation of the above results and those reported by Walsh and co-workers.[73,76]

4.4: Generation of Analogues of the Substrate Mimic 117, and Investigation of their Incorporation into Prodiginine Analogues by Mutasynthesis

The results obtained in the feeding studies using NAC thioester **117** indicated that feeding analogues of **117** to the *redM::acc(3) IV* mutant could generate novel analogues of undecylprodiginine and streptorubin B, which might have superior biological activity. Thus furan **118**, thiophene **119**, fluorobenzene **120** and pyridine **121** analogues of **117** were synthesised from commercially available materials using the procedure outlined in Scheme 47, and fed to the *redM::acc(3)IV* mutant.

Unfortunately, feeding of these analogues did not lead to the production of novel prodiginines by mutasynthesis. This may be because the NAC thioesters do not penetrate *S. coelicolor* cells, or because the substrate analogues are not substrates of RedX. If the latter is the case, then perhaps another analogue more similar in structure to NAC thioester **117**, for example **215**, would successfully lead to prodiginine analogues (Figure 39). However, it is possible that one or more of the enzymes involved

in prodiginine biosynthesis are very specific for their substrates, thus precluding the production of prodiginine analogues by mutasynthesis.



Scheme 47: Preparation of pyrrole-2-carboxylic acid-NAC thioester **117** analogues

i) Oxalyl chloride, DMF, reflux, 1h. ii) *N*-acetylcysteamine, DMAP, reflux, 14h, DCE, **118** 75%, **119** 58%, **120** 73%, **121** 69%

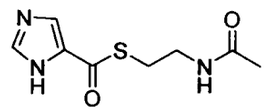


Figure 39: Another potential analogue of **117** that could be used in feeding studies **215**

4.5: Conclusion

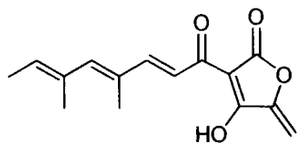
The synthesis of pyrrole-2-carboxyl-*N*-acetylcysteamine thioester **117** using modified literature conditions was achieved. Feeding of this analogue of the proposed pyrrole-2-carboxyl-RedO intermediate in prodiginine biosynthesis to a *redM::acc(3)IV* mutant of *S. coelicolor* restored prodiginine production, consistent with the hypothesis that direct transfer of the pyrrole-2-carboxyl group from the RedO-PCP to the Red-X enzyme occurs.

Furan **118**, thiophene **119**, fluorobenzene **120** and pyridine **121** analogues of **117** were also synthesised and fed to the *redM::acc(3)IV* mutant. This did not lead to the production of novel prodiginines by mutasynthesis. However, in work carried out by the Challis group, bipyrrole **10** analogues have been synthesised (Figure 13) and used to probe the substrate specificity of the RedH enzyme in undecylprodiginine biosynthesis. These analogues were incorporated to produce novel prodiginines by mutasynthesis.[83] This would suggest that either NAC thioesters **118**, **119**, **120** and **121** did not penetrate *S. coelicolor* cells, or that one or more of the enzymes involved in prodiginine biosynthesis subsequent to RedM are very specific for their substrates.

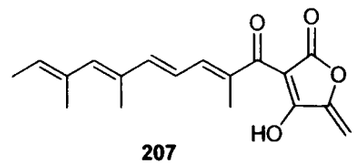
In the future, in order to demonstrate whether or not analogues of **117** could be incorporated, a deuterated analogue could be synthesised and fed to determine whether or not it is incorporated into the corresponding prodiginine analogue. If this was successful some more similar analogues could also be generated such as substituted pyrroles to determine whether or not the pathway is specific for pyrrole substrates.

Chapter 5: First Generation Synthetic Approaches to Putative Key Intermediates in Quartromicin Biosynthesis

In order to probe the biosynthetic pathway to quartromicins the two precursors **206** and **207** (Figure 40) would need to be synthesised in labelled form to ultimately be used in feeding studies aimed at investigating their incorporation into the natural product. Thus, any synthetic route to these would need to incorporate stable isotope labels at a late stage.



206

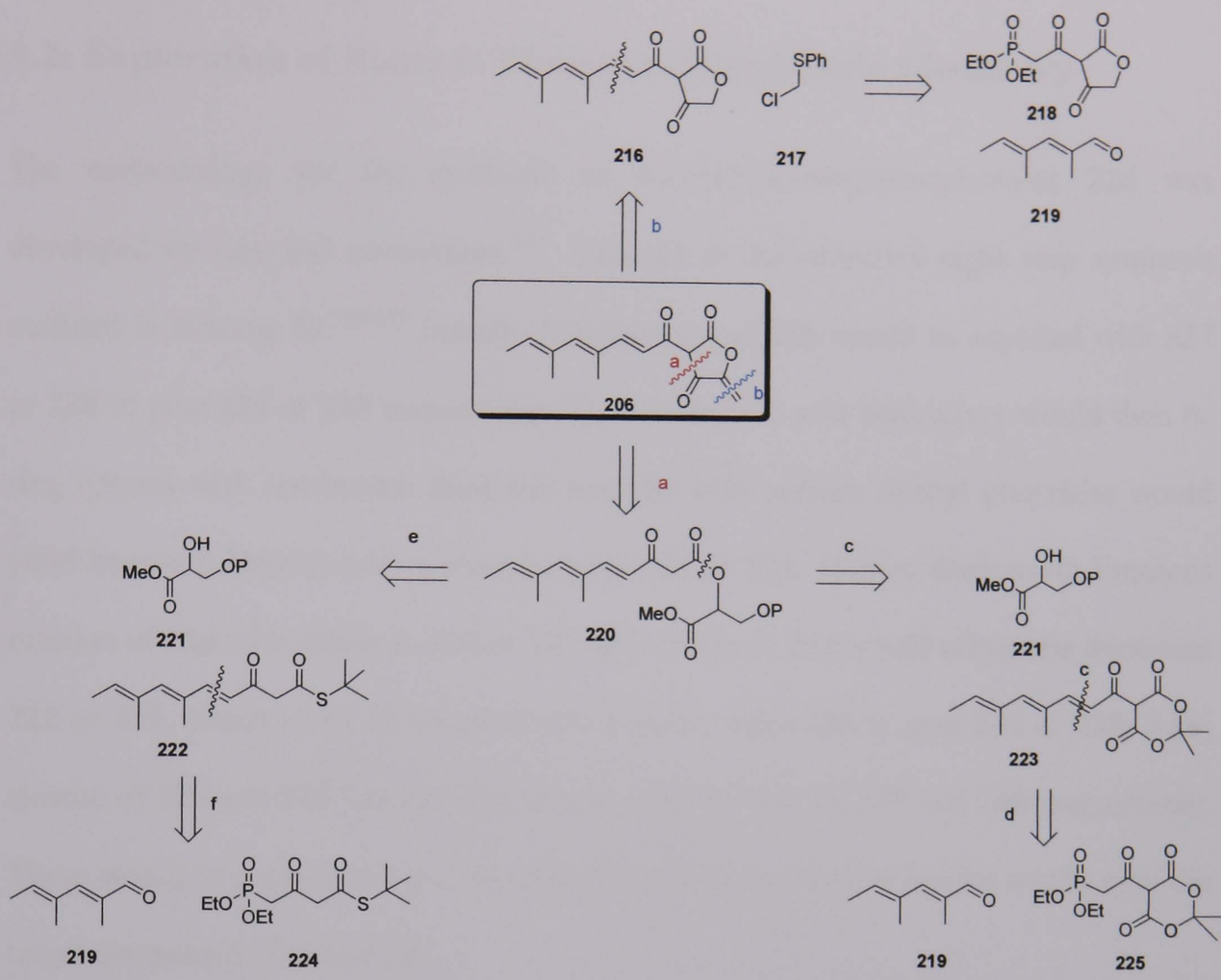


207

Figure 40: Proposed precursors in quartromicin biosynthesis **206** and **207**

5.1: Initial Retrosynthetic Analysis

A review of the literature revealed that targets **206** and **207** have not previously been synthesised. Therefore a first generation synthetic approach was based on the retrosynthetic analysis of target **206** which is shown in Scheme 48.

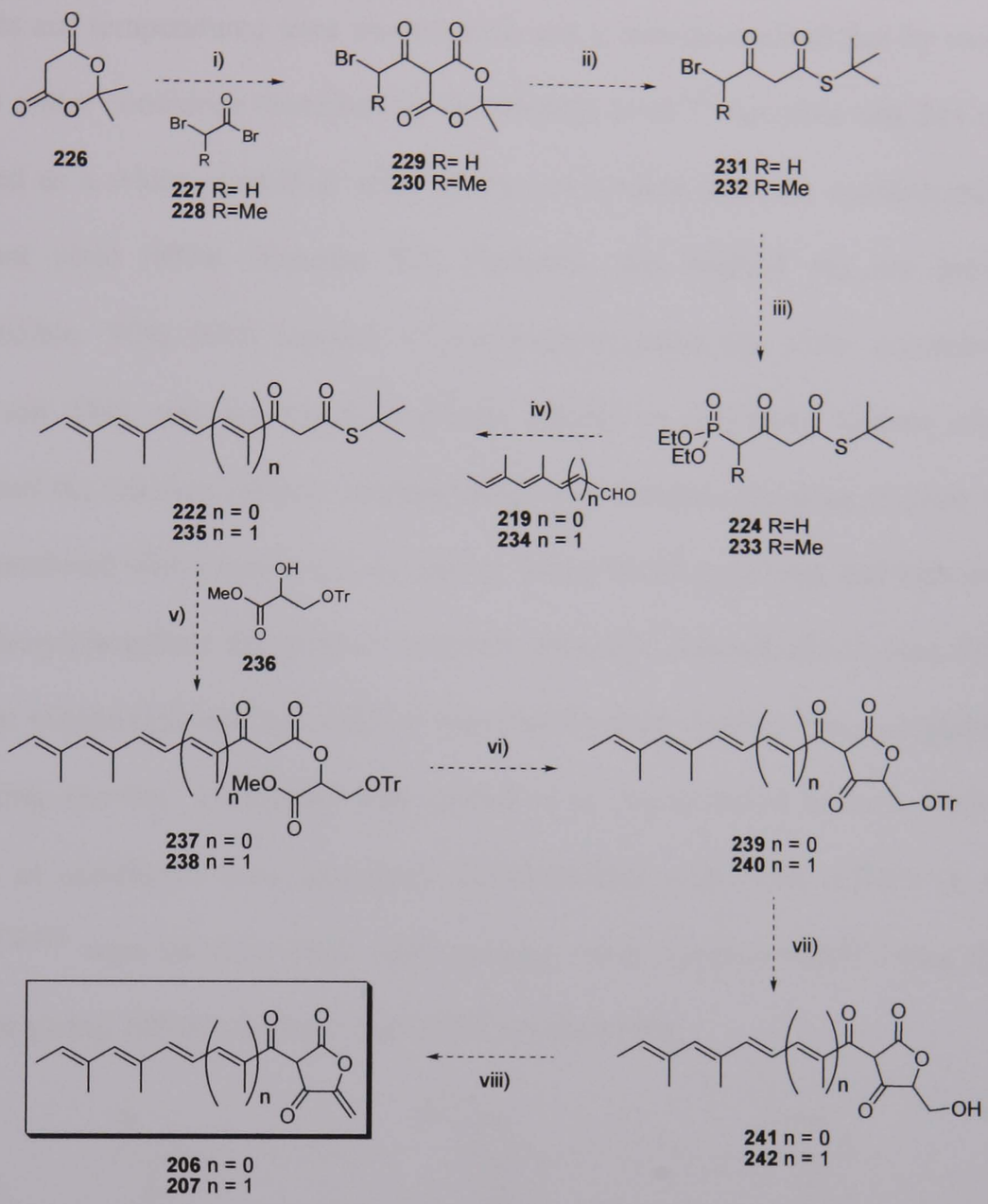


Scheme 48: Three possible retrosyntheses of **206**

Retrosynthetic analysis of **206** identified two main initial disconnections **a** and **b**. Disconnection **a** leads back to two different possible precursors; the known β-ketothioester-γ-phosphonate **224**[169] and the Meldrum's acid derivative **225**, as well as a common precursor, dienal **219**. Disconnection **b** leads back to the tetronic acid derivative **218** and dienal **219**. It was envisaged that similar chemistry to that used for the synthesis of **224** could be used for the preparation of the Meldrum's acid derived phosphonate **225** and the tetronic acid derived phosphonate **218**, both of which represent attractive precursors for the synthesis of **207**.

5.2: Exploration of Route 1: Thioester Phosphonate Chemistry

The methodology for the synthesis of β-ketothioester-γ-phosphonates **224** was developed by Ley and co-workers.[169] This led to the proposed eight step synthesis outlined in Scheme 49.[169-171] Initially Meldrum's acid **226** would be acylated with **227** or **228** to give **229** or **230** respectively. The Meldrum's acid derivatives would then be ring opened with *tert*-butane thiol and reaction with sodium diethyl phosphite would yield known β-ketothioester-γ-phosphonates **224** or **233**. Horner-Wadsworth-Emmons reaction of **224** with aldehyde **219** or **233** with aldehyde **234** would afford the thioesters **222** or **235**, which could be coupled with hydroxy ester **236** to give **237** or **238**. Ring closure of **237** and **238** would yield tetronic acid derivatives **239** and **240** respectively. These would be deprotected and the mesylation followed by elimination would give the target compounds **206** and **207**.

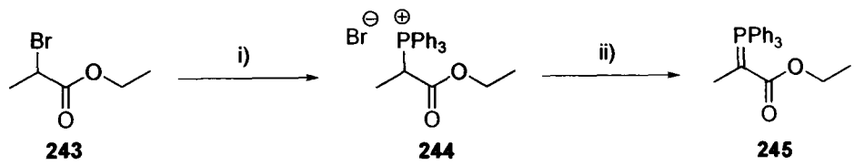


Scheme 49: Proposed synthetic route to **206** and **207**

i) **227** or **228**, pyridine, DCM, 0°C; ii) HS^{t}Bu, toluene, reflux; iii) NaP(O)(OEt)$_2$, NaH, THF, 0°C to rt; iv) NaH, THF, **219** or **234**, 0°C to rt ; v) **236**, CF_3CO_2Ag, THF, rt; vi) TBAF, rt; vii) TFA, rt; viii) 1) MsCl, pyridine; 2) NaOH

This route required access to dienal **219** and trienal **234** to synthesise **206** and **207** respectively. Initially **219** was synthesised using the literature chemistry outlined in Schemes 50 and 51.[172] However, several steps required significant optimisation. Conditions for the production of ylide **245** were screened. A one-pot reaction with triphenylphosphine and **243** in toluene followed by treatment with *n*-BuLi proved very low yielding due to aggregation of the ylide salt **244**.[173] Crushing the intermediate before the addition of *n*-BuLi did not increase the yield significantly. A variety of

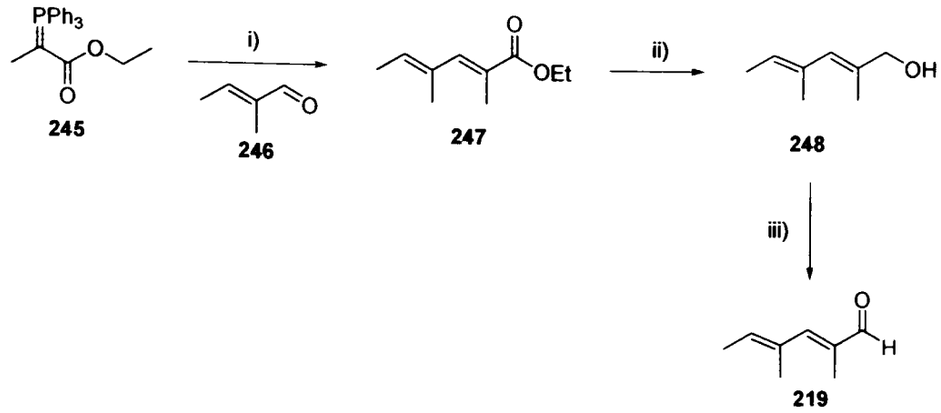
solvents and temperatures were investigated, and it was determined that by using ethyl acetate under conditions described by Werkhoven *et al.*[174] the ylide salt **244** could be obtained as a white crystalline solid which precipitated from the reaction mixture in excellent yield (88%) (Scheme 50). However, this method did not prove very reproducible. With fresh supplies of triphenylphosphine the white crystalline solid (ylide salt **244**), was no longer produced. Instead an extremely viscous oil, which prevented the reaction mixture stirring freely, was obtained resulting in ylide salt **244** being produced with lower yield and purity. Using DCM as solvent and with an excess of triphenylphosphine, the product could be obtained, although not in pure form. The remnant triphenylphosphine could be removed by flash column chromatography after the Wittig reaction. Compound **244** needed to be deprotonated to form ylide **245**; a variety of conditions were examined. Deprotonation with NaH, *n*-BuLi or aqueous NaOH[173-175] were all successful, with washing using aqueous NaOH being the most efficient giving **245** in excellent yield (90%) (Scheme 50).



Scheme 50: Preparation of ylide **245**

i) PPh_3, EtOAc, reflux, 24 hrs, 88%; ii) 2 equivalents of NaOH(aq), DCM, rt, 90%

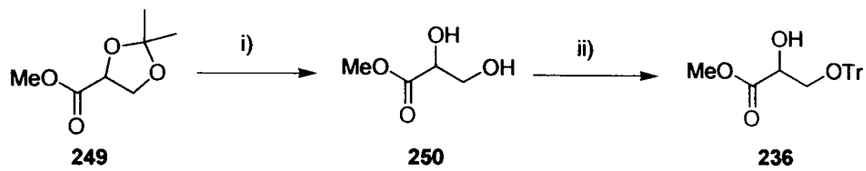
Once ylide **245** had been formed a Wittig reaction with tiglic aldehyde **246** was carried out. This reaction proceeded smoothly in good yield (80%), affording ethyl ester **247** which was then reduced with DIBAL-H to produce primary alcohol **248** in excellent yield (85%) (Scheme 51). Subsequent oxidation conditions were investigated to produce aldehyde **219**, including Swern,[176] Dess-Martin[177] and manganese dioxide oxidations,[172] of which manganese dioxide proved to be the most efficient giving **219** in excellent yield (96%) (Scheme 51).



Scheme 51: Synthesis of dienal aldehyde **219**

i) **246**, toluene, reflux, 80%; ii) DIBAL-H, Et_2O, 0°C, 85%; iii) MnO_2, DCM, rt, 96%.

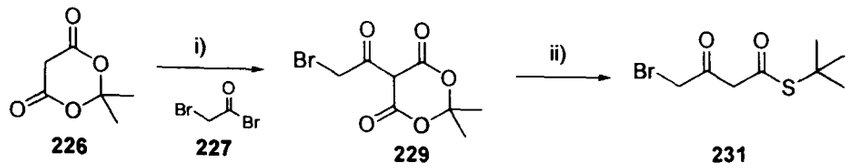
Another key building block required for this synthetic route was ester **236.** The chemistry used for the synthesis of **236** from commercially available **249**, is outlined in Scheme 52.[178] Removal of the acetonide group in **249** gave dihydroxy ester **250** and subsequent selective protection of the primary alcohol with a trityl group gave α-hydroxy ester **236** in excellent yield (79% over 2 steps).



Scheme 52: Synthesis of ester **236**

i) MeOH, 1N HCl, rt, 86%; ii) Ph_3CCl, DMAP, DCM, rt, 93%

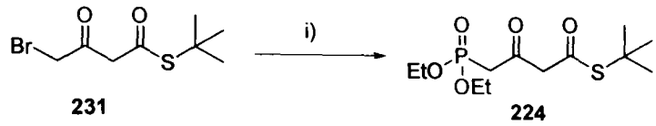
The final building block to be synthesised was β-ketothioester-γ-phosphonate **224** (Scheme 54). Acylation of Meldrum's acid **226** with bromoacetyl bromide **227** was carried out following the procedure developed by Haynes, affording **229** in good yield (65%) (Scheme 53).[169,179] Unfortunately, compound **229** was extremely air and moisture sensitive. Upon contact with air it forms an insoluble black substance - probably a polymeric material. However, its direct use without further purification allowed ring opening by *tert*-butanethiol to be carried out producing **231**. Initially toluene was used as a solvent, but competing side reactions meant low yields were obtained. The use of 1,2-dichloroethane as a solvent circumvented this problem giving much improved yields, 57% over the two steps (Scheme 53).



Scheme 53: Synthesis of **231** from Meldrum's acid

i) **227**, pyridine, DCM, 0°C to rt; ii) HS^{t}Bu, DCE, reflux, 57% over 2 steps

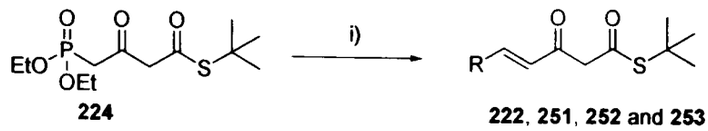
Compound **231** was relatively stable at low temperatures under argon, but rapidly decomposed at room temperature, again forming a black insoluble solid. Reaction of **231** with sodium diethylphosphite proceeded smoothly giving the β-ketothioester-γ-phosphonate **224** in excellent yield (85%) as an orange oil, which was stable at low temperatures (Scheme 54).



Scheme 54: Conversion of **231** to the β-ketothioester-γ-phosphonate **224**

i) NaH, THF, $NaP(O)(EtO)_2$, -10°C to rt, 85%

Initially, in model reactions, phosphonate **224** was coupled with a variety of commercially available aldehydes in Horner-Emmons reactions to give a range of *E*-alkenes in good to excellent yields (Scheme 55 and Table 1). Horner-Emmons reaction of phosphonate **224** and aldehyde **219** was successful and gave **222** in good yield (64%), although some minor geometrical isomerisation was observed (Scheme 55 and Table 1).



224 **222, 251, 252 and 253**

Scheme 55: General procedure for synthesis of thioesters **222, 251, 252** and **253**

i)NaH, RCHO, THF, 0°C to rt

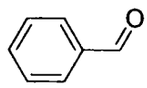
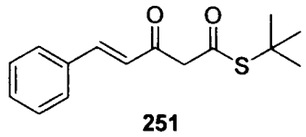
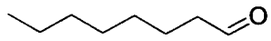
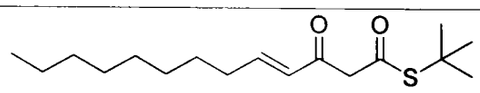
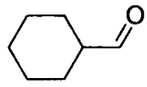
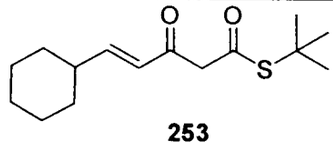
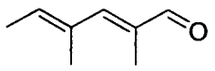
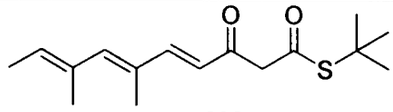
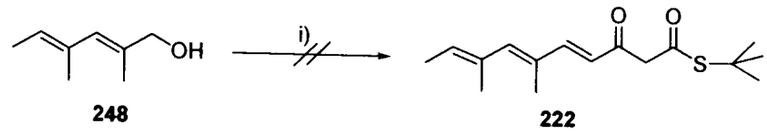
RCHO	Product	Yield	Length of reaction
	 **251**	83%	16 hours
	 **252**	70%	48 hours
	 **253**	98%	16 hours
	 **222a**	64%	48 hours

Table 1: Synthesis of thioesters **222, 251, 252** and **253**

Taylor and co-workers have developed one-pot tandem alcohol oxidation-Horner-Wadsworth-Emmons reactions, using manganese dioxide as the oxidising agent.[180] Both lithium hydroxide and guanidine bases were found to be very efficient in the process giving rise to high yields. The literature also stated that pre-existing double bond geometry remained unchanged and that *E*-alkene products were predominant under the standard conditions used.[180,181] In an effort to make the synthesis of **206** and **207** more efficient this methodology was employed and although the alcohol **248** was oxidised to the aldehyde **219** during the reaction, none of the desired *E*-alkene **222** was produced (Scheme 56). This was not completely unexpected as the dianion of **224** is required for the Horner-Wadsworth-Emmons reaction to occur. NaOH and guanidine are not strong

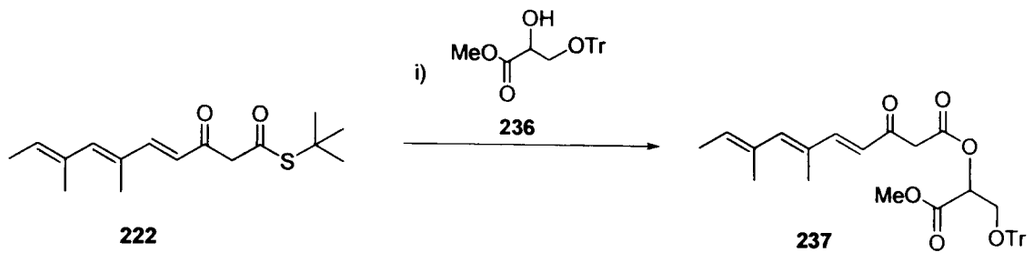
enough bases to form the dianion of **224** which is why NaH was used in the original reaction conditions.



Scheme 56: Attempted preparation of **222** one pot reaction[180,181]

i) MnO_2, **248**, LiOH, molecular sieves, THF

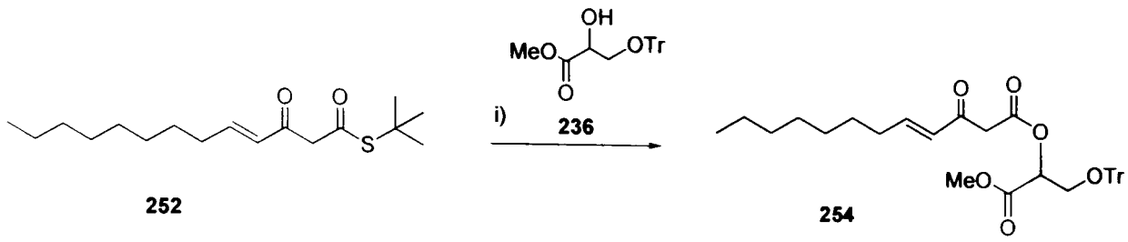
The β-ketoester **222** was condensed with hydroxyl ester **236** by silver salt promoted condensation to give **237** (Scheme 57). This reaction proved problematic and low yields ranging from 11-23% of product **237** were obtained.



Scheme 57: Silver mediated coupling reactions to synthesise **237**

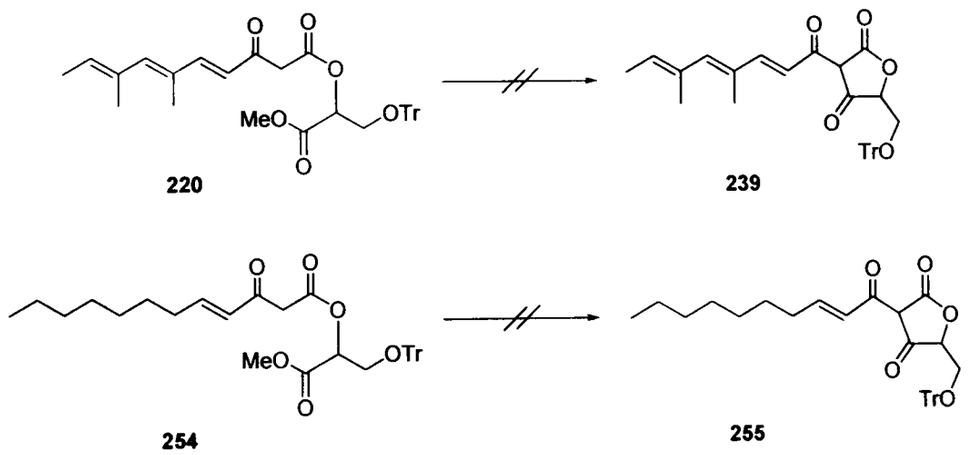
i) **236**, CF_3CO_2Ag, THF, shielded from the light, 23%

It was discovered that silver trifluoroacetate was readily hydrolyses in air, meaning trifluoroacetic acid (TFA) was present in the reaction mixture. The acidity of the reaction mixture caused removal of the trityl group. To overcome this problem, triethylamine was added to basify the reaction before the substrate was added.[182] Although this prevented deprotection, the yield of the reaction did not improve significantly (27%). To see whether or not the polyene chain was causing any difficulties during the reaction, silver salt promoted condensation of the hydroxy ester **236** and thioester **252** was also attempted. Compound **254** was obtained (Scheme 58), but the yields could not be improved, suggesting the presence of the polyene does not affect the outcome of the reaction.



Scheme 58: Silver mediated coupling reactions to synthesise **254**
i) **236**, silver trifluoroacetate, THF, shielded from the light, rt, 16 h, 16%

Dieckmann cyclisation of **237** and **254** was investigated under a variety of conditions using different bases but with little success (Scheme 59). The use of sodium methoxide resulted in trans-esterification. Sodium hydride appeared to afford the desired tetronic acid **239** together with some undesired by-products; however, the desired product could not be purified. Tetra-*n*-butylammonium fluoride (TBAF) was reported as the base of choice for such cyclisations,[170,171,178,183] but despite numerous attempts under a variety of conditions none of the desired material could be synthesised. It is possible that the use of anhydrous TBAF generated *in situ* may be required for this chemistry to work. This would allow the impact of water in commercially-available samples of this reagent to be assessed upon the cyclisation reaction.[184]



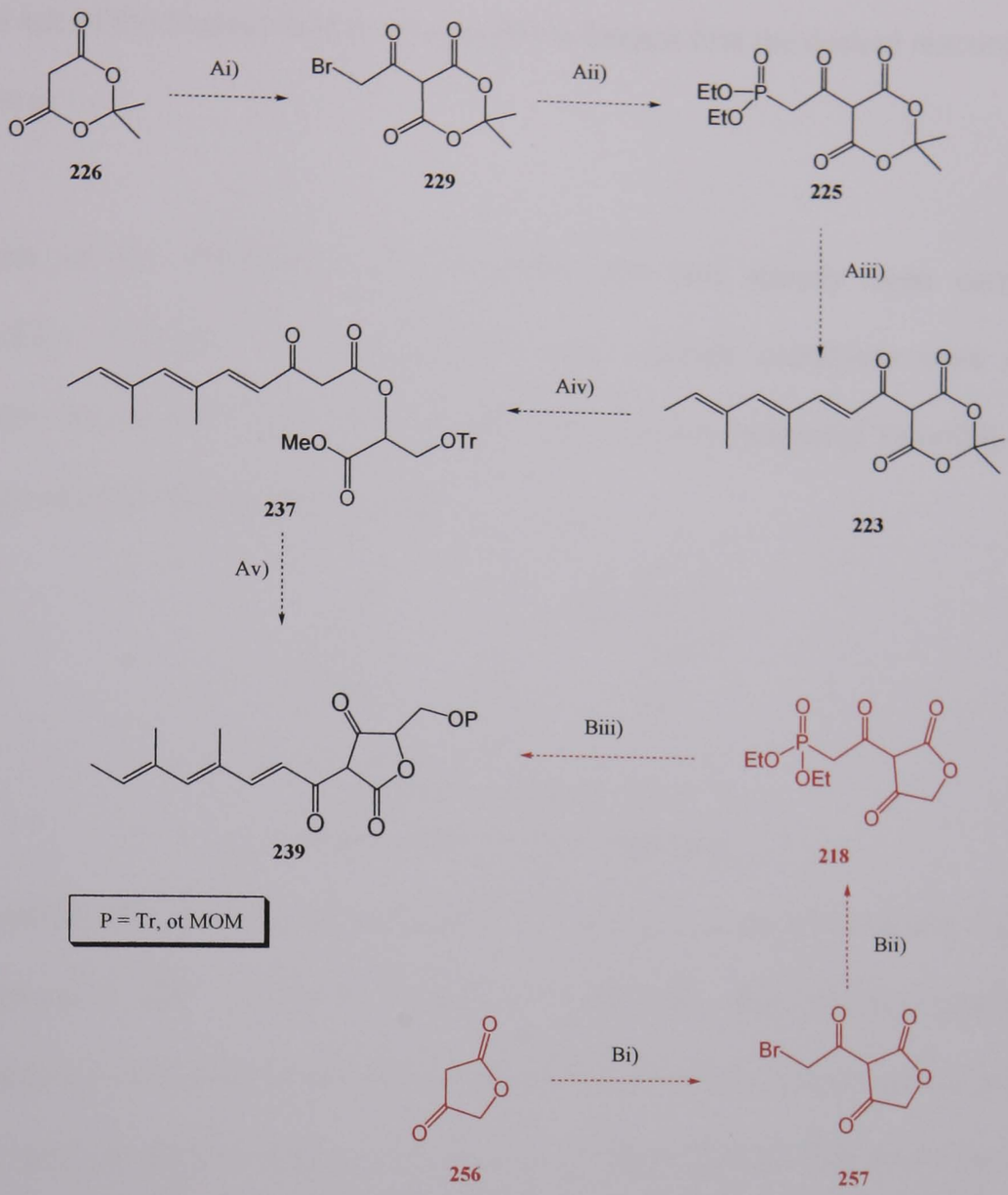
Scheme 59: Attempted synthesis of **239** and **255**

Two major problems were encountered in this synthetic route; the first being the low yields of the silver trifluoroacetate coupling reaction and the second being the Dieckmann cyclisation to afford tetronic acids derivatives. For this reason alternative synthetic routes were examined while these conditions were being investigated further.

Once it was established that the route described in Chapter 6 was successful, no further investigations were carried out on this synthetic route.

5.3: Exploration of Route 2: Meldrum's Acid and Tetronic Acid-derived Phosphonates

As an alternative strategy, it was thought that a more direct route to the desired precursors **206** and **207** could involve acylation of Meldrum's acid **226** or tetronic acid **256** with bromoacetyl bromide **227**, followed by conversion of the resulting products to the corresponding phosphonium salts or phosphonates. The resulting compounds could be coupled in Wittig or Horner-Emmons reactions with aldehyde **219** (Scheme 60).



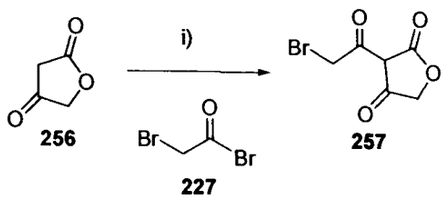
Scheme 60: Proposed route 2 to desired compounds **206** and **207**

Ai) **227**, pyridine, DCM 0°C; Aii) NaH, THF, $NaP(O)(OEt)_2$, 0°C to rt; Aiii) **219**, NaH, THF; Aiv) **236**, heat; Av) TBAF; Bi) **227**, pyridine, DCM 0°C; Bii) NaH, THF, $NaP(O)(OEt)_2$, 0°C to rt; Biii)1) **219**, NaH, THF, 2) *n*-BuLi then MOMCl

Initially this route was investigated by Haynes, but was unsuccessful; neither **225** nor **218** could be synthesised.[179] However, the Arbuzov-like reaction used for the preparation of phosphonate **224** following the procedure of Ley and co-workers[170] (Scheme 55) used two equivalents of sodium hydride. It was thought that two equivalents of sodium hydride might be required to react diethyl phosphate with 2-bromoacetyl tetronic acid **257** and Meldrum's acid derivative **229** because they contain highly acidic protons that are likely to be removed by sodium diethyl phosphate. If the

sodium salt of the tetronic acid derivative **257** is formed first the desired reaction should take place.

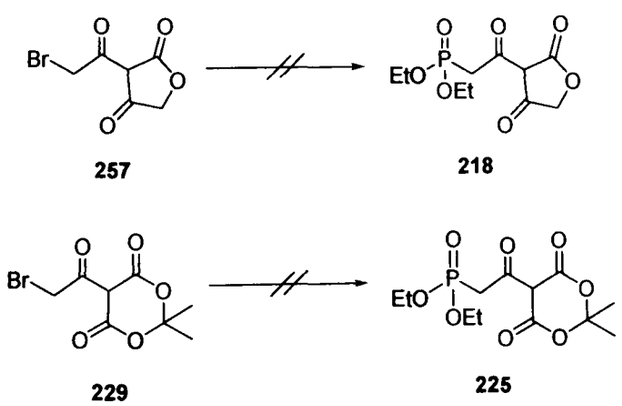
Synthesis of the Meldrum's acid derivative **229** had already been carried out successfully (Scheme 53). Therefore the same reaction conditions were used to synthesise the tetronic acid derivative **257**. This reaction proceeded smoothly to give **257** in good yield (66%) (Scheme 61).



Scheme 61: Synthesis of **257**

i) **227**, pyridine, DCM 0°C to rt, 66%

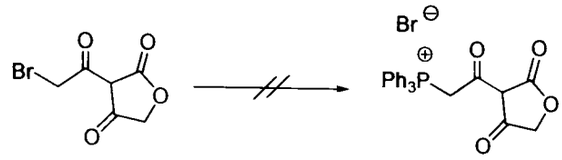
Unfortunately the tetronic acid derivative **257** was just as unstable as the Meldrum's acid derivative **229**, making it difficult to work with. The Arbuzov reaction was attempted at a variety of temperatures with both the Meldrum's acid derivative and the tetronic acid derivative, none of the desired products **225** or **218** could be isolated (Scheme 62). Instead a mixture of inseparable compounds was produced. This may have been because tetronic acid and Meldrum's acid derivatives are susceptible to nucleophilic attack at C-1, which would give rise to competing side reactions.



Scheme 62: Attempted synthesis of **218** and **225**

i) **257** or **229**, NaH, THF, $NaP(O)(OEt)_2$, -10°C to rt

Having found a method for the preparation of ylide **245** (Scheme 50) it was thought that reaction of **257** with triphenylphosphine may lead to the production of the tetronic acid ylide (Scheme 63). Although initial attempts under these conditions looked promising, by mass spectrometry none of **258** could be detected in the reaction mixture.



257 **258**

Scheme 63: Attempted synthesis of **258**

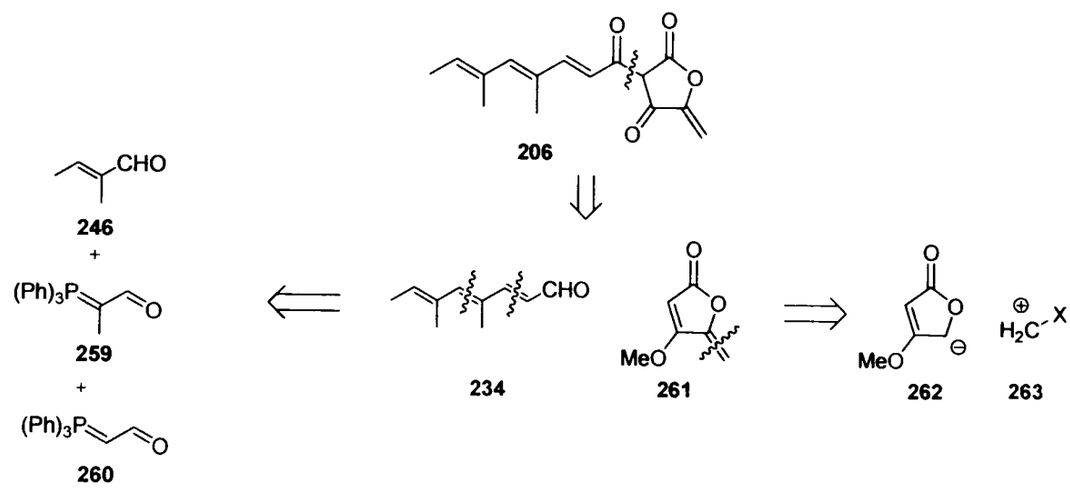
i) PPh_3, DCM, rt

5.4: Conclusion

Neither of the above two synthetic routes investigated proved to be very successful. Route one looked promising at first, but owing to the low yields of the silver trifluoroacetate reaction and the failure of the Dieckmann cyclisation, this method was not suitable for developing an efficient and concise route to **206** and **207**. If route two had been successful a very concise synthesis would have been developed. However, because suitable conditions for the synthesis of **225**, **218** and **258** were not found, this route could not be developed further.

Chapter 6: Second Generation Approach to Key Putative Intermediates in Quartromicin Biosynthesis and Investigations of Biomimetic Diels-Alder Reactions

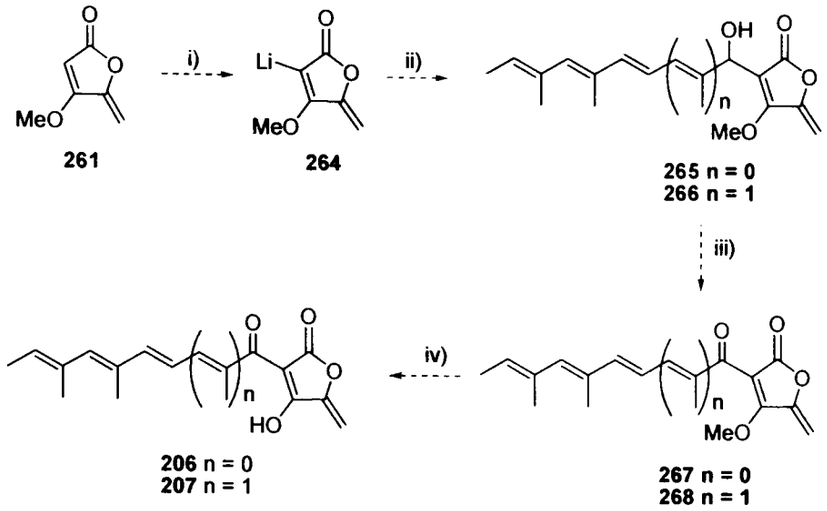
Because the previously described attempts to synthesis **206** and **207** did not meet with much success, a new route was investigated. Retrosynthetic analysis of desired compound **206** identifies aldehyde **234** as a starting material, which can be synthesised using precedented synthetic procedures and known methyl protected exomethylene tetronate **261** (Scheme 64).



Scheme 64: Retrosynthetic analysis of **206**

This alternative approach to the synthesis of acyl tetronic acids developed by Yoshii and co-workers in 1987 utilises the lithiated derivative of the methyl protected exomethylene tetronate **261** (Scheme 65).[185] Several total and partial syntheses of tetronate natural products have utilised these lithium derivatives, for example tetrodecamycin and abyssomicins C and D.[14,15,186-188] With this methodology in mind the following synthetic route was proposed (Scheme 65). First, the lithiated derivative of the methyl protected exomethylene tetronate **261** could be generated using lithium diisopropylamide (LDA) as the base, followed by the addition of an appropriate aldehyde, RCHO, to generate the secondary alcohols **265** and **266**. Oxidation of the

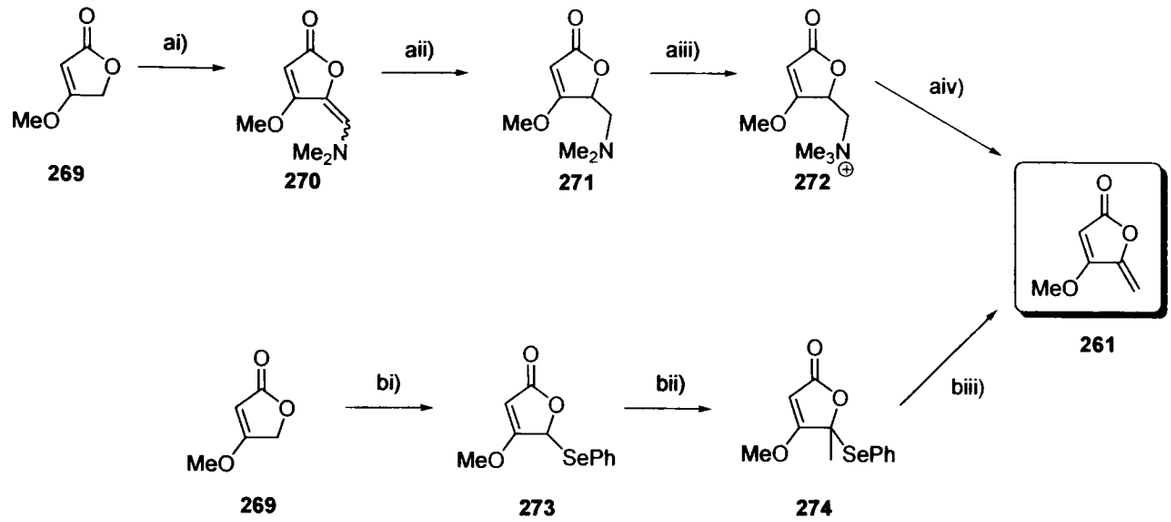
alcohols **265** and **266** to ketones **267** and **268** followed by deprotection would give the desired compounds **206** and **207**.



Scheme 65: Proposed route to **206** and **207** based on methodology developed by Yoshii and co-workers[185]

i) LDA, -78°C; ii) RCHO, -78°C; iii) MnO_2, DCM, rt; iv) LiCl, DMSO, 50°C

Both Yoshii and Paintner describe methods for the synthesis of the methyl protected exomethylene tetronate **261** (Scheme 66).[185,186] The first method involves (dimethylamino)methylenation at C(5) by reaction of the commercially available 4-methoxy-2(5*H*)-furanone **269** with dimethylformamide dimethylacetal (DMF-DMA) to give **270**. Subsequent reduction of **270** with sodium cyanoborohydride to give **271**, followed by quaternisation of the resulting 5-(dimethylamino)methyl tetronate with methyl iodide and treatment with aqueous $NaHCO_3$ to yield **261** (Scheme 66).[185] The latter method involves the preparation of 4-methoxy-5-phenylseleno-2(5*H*)-furanone **273** from 4-methoxy-2(5*H*)-furanone **269** followed by alkylation with methyl iodide to give **274** and subsequent oxidative removal of the phenylseleno-group using *m*CPBA to yield **261** (Scheme 66).[186]

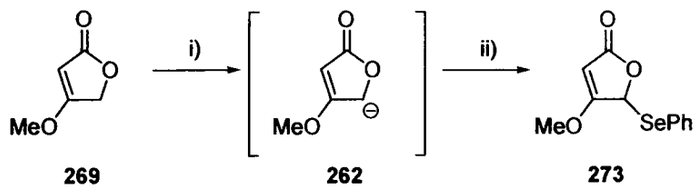


Scheme 66: Two literature routes for the synthesis of the exomethylene tetronate **261**

ai) $Me_2NCH(OMe)_2$; aii) $NaCNBH_3$; aiii) MeI; aiv) $NaHCO_3$ bi) *n*-BuLi, PhSeCl; bii) *t*-BuLi, MeI; biii) *m*CPBA

6.1: Synthesis of Exomethylene Tetronate 261 *via* the Phenylselenide 273

As the route to **261** described by Painter is more concise this route was initially investigated. Commercially available 4-methoxy-2(5*H*)-furanone **269** was deprotonated with *n*-BuLi at C(5) to form the corresponding anion **262** which was reacted with phenylselenyl chloride. This reaction proved successful on a small scale (5 mmol) giving the desired product **273** in moderate yield (43%) (Scheme 67).

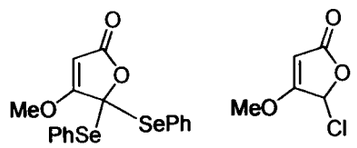


Scheme 67: Synthesis of **273**

i) *n*-BuLi, -78°C; ii) PhSeCl, -78°C to rt, 16 h, 43%

The reaction was attempted on larger scales, however significant amounts of the disubstituted selenyl-compound **275** (Figure 41) were formed during the reaction and the two compounds were difficult to separate by flash column chromatography. Attempts were made to prevent this by-product from forming. Originally the anion was formed by the addition of *n*-BuLi to a solution of 4-methoxy-2(5*H*)-furanone **269** at

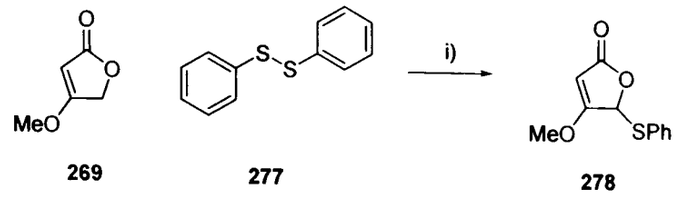
-78°C, then a solution of phenylselenyl chloride was added dropwise. This approach resulted in a large quantity of the undesired disubstituted compound **275**; it was proposed that formation of **275** occurs *via* deprotonation of **273** by **262** and reaction with a second molecule of phenylselenyl chloride. It was thought that the formation of **275** would be avoided if anion **262** was added to a solution of the phenylselenyl chloride thus reducing the concentration of **262** in the solution. These conditions were attempted on a small scale (5 mmol) resulting in yields of approximately 52% of **273**. Unfortunately, when these reaction conditions were scaled up (10 mmol) a second by-product was isolated that had the same retention time as the desired product **273** making it extremely difficult to separate the two compounds. The structure of this by-product was determined by ^{1}H NMR spectroscopy and mass spectrometry to be the chlorinated species **276** (Figure 41).



275 **276**

Figure 41: By-products isolated **275** and **276**

In an attempt to increase the yield of **273** a different approach was taken in which the methyl protected tetronate **269** was reacted with *n*-BuLi followed by diphenyldiselenide. Similar methodology has been reported by Pelter and co-workers where **269** was reacted with *n*-BuLi followed by diphenyldisulfide **277** resulting in a high yield of the monosubstituted phenylsulfide **278** (Scheme 68).[189]

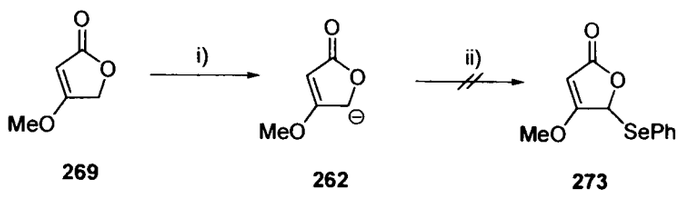


269 **277** **278**

Scheme 68: Synthesis of **278**[189]

i) *n*-BuLi, -78°C; ii) PhSSPh, -78°C to -60°C, 90%

However, when this reaction was attempted with diphenyldiselenide using the conditions reported by Pelter only starting material was isolated (Scheme 69).[189] Extensive investigations into these conditions would be required to establish why no reaction occurred, thus this was not pursued further.

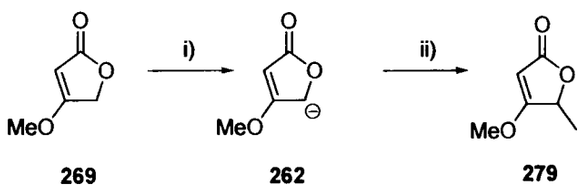


269 **262** **273**

Scheme 69: Alternative approach to the synthesis of **273**

i) *n*-BuLi, -78°C; ii) PhSeSePh, -78°C to rt, 24 h

As an alternative it was thought that it would be possible to alkylate **269** with methyl iodide first to give **279** (Scheme 70), then react **279** with *n*-BuLi and phenylselenyl chloride to afford **274** i.e. reverse steps one and two in the proposed synthesis (Scheme 66). However, the low yields of **279** obtained meant that this methodology was not investigated further.



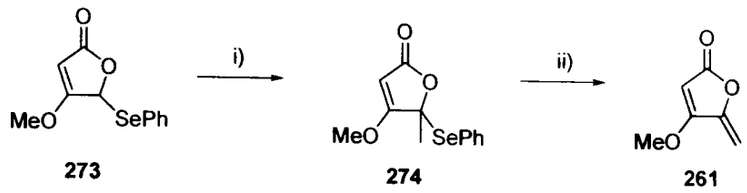
269 **262** **279**

Scheme 70: Synthesis of **279**

i) *n*-BuLi, -78°C; ii) MeI, -78°C to rt, 24 h, 29%

Due to the inability to increase the yield of **273**, attention was turned to the remaining two steps in the synthesis of **261**. Alkylation of **273** with methyl iodide proceeded smoothly to give **274** in excellent yield (78%). This reaction was carried out using *t*-BuLi rather than *n*-BuLi due to reports that generating the anion with *n*-BuLi resulted in

the cleavage of the selenyl-carbon bond to considerable extent resulting in the formation of **279**.[186] Subsequent oxidation of **274** with *m*CPBA gave the desired product **261** in excellent yield (74%) (Scheme 71).



273 **274** **261**

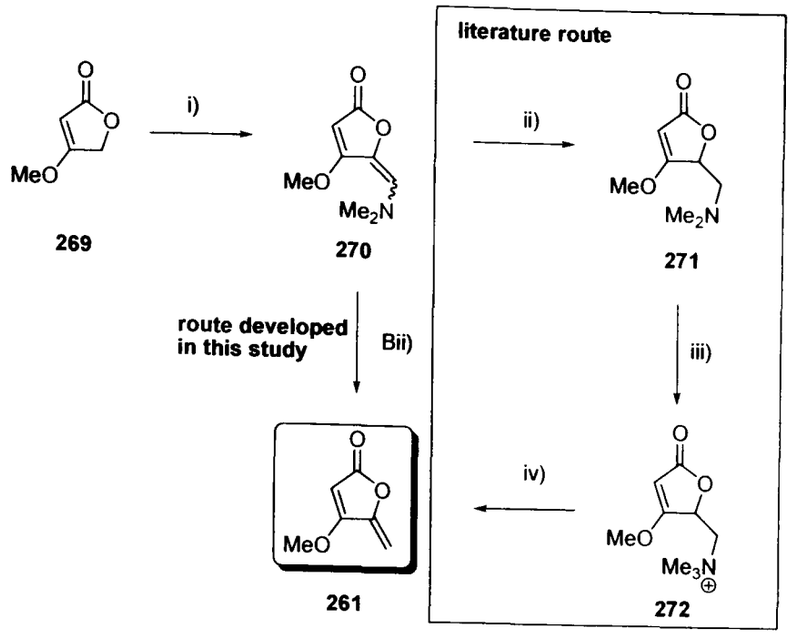
Scheme 71: Synthesis of the exomethylene tetronate **261**

i) 1) *t*-BuLi, MeI, -78°C to rt, 16 h, 78%; ii) *m*CPBA, DCM, 0°C, 74%

6.2: Synthesis of Exomethylene Methyl Tetronate 261 *via* Enamine 270

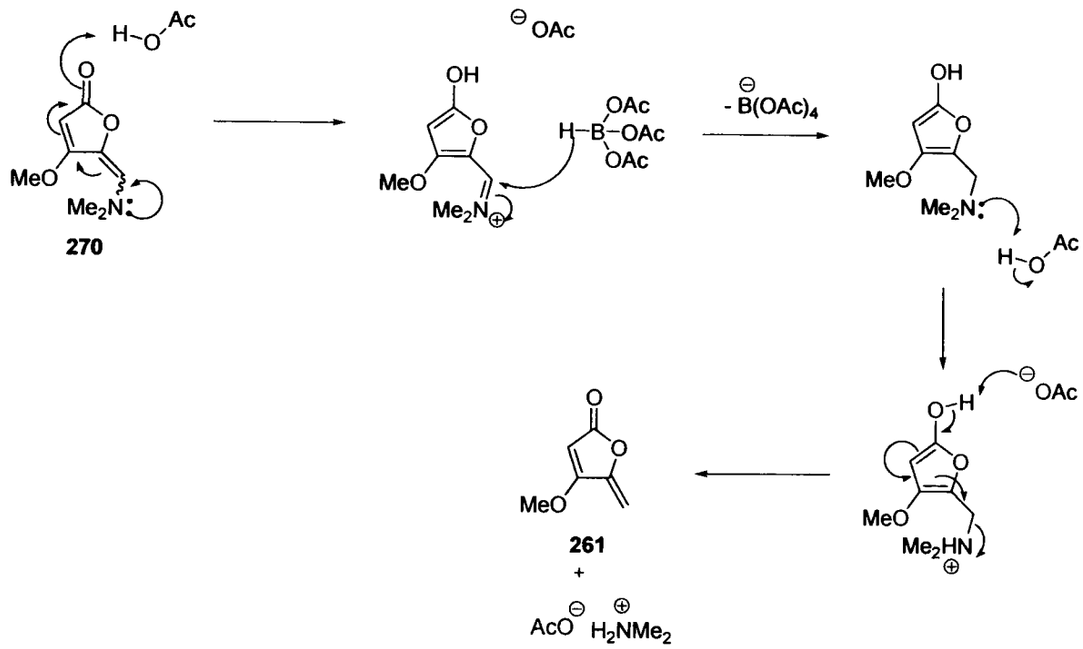
As the phenylselenide based method one was low yielding in the first step and required painstaking separation of the products a second alternative approach was considered for the synthesis of **261**. This would in turn allow the most efficient synthesis of **206** and **207** to be designed.

Related research carried out by the Challis group resulted in the synthesis of enamine **270** in excellent yield *via* a literature synthesis (94%).[189] The literature route involves reduction of **270** to **271** with sodium cyanoborohydride (Scheme 72). However, sodium triacetoxyborohydride in acetic acid was used for this transformation instead.[190-192] This resulted in an *in situ* protonation of the amino group in the initially formed intermediate followed by elimination of dimethylamine affording **261** directly in excellent yield (88%) (Scheme 73). This methodology improves on both literature methods for the synthesis of **261** as it involves fewer steps and results in high yields of the desired product.



Scheme 72: Alternative literature synthesis of the exomethylene tetronate **261** and the new consise route developed by this study

i) $Me_2NCH(OMe)_2$ 94%; ii) $NaCNBH_3$; iii) MeI; iv) $NaHCO_3$ Bii) $NaBH(OAc)_3$, AcOH, DCE, 88%

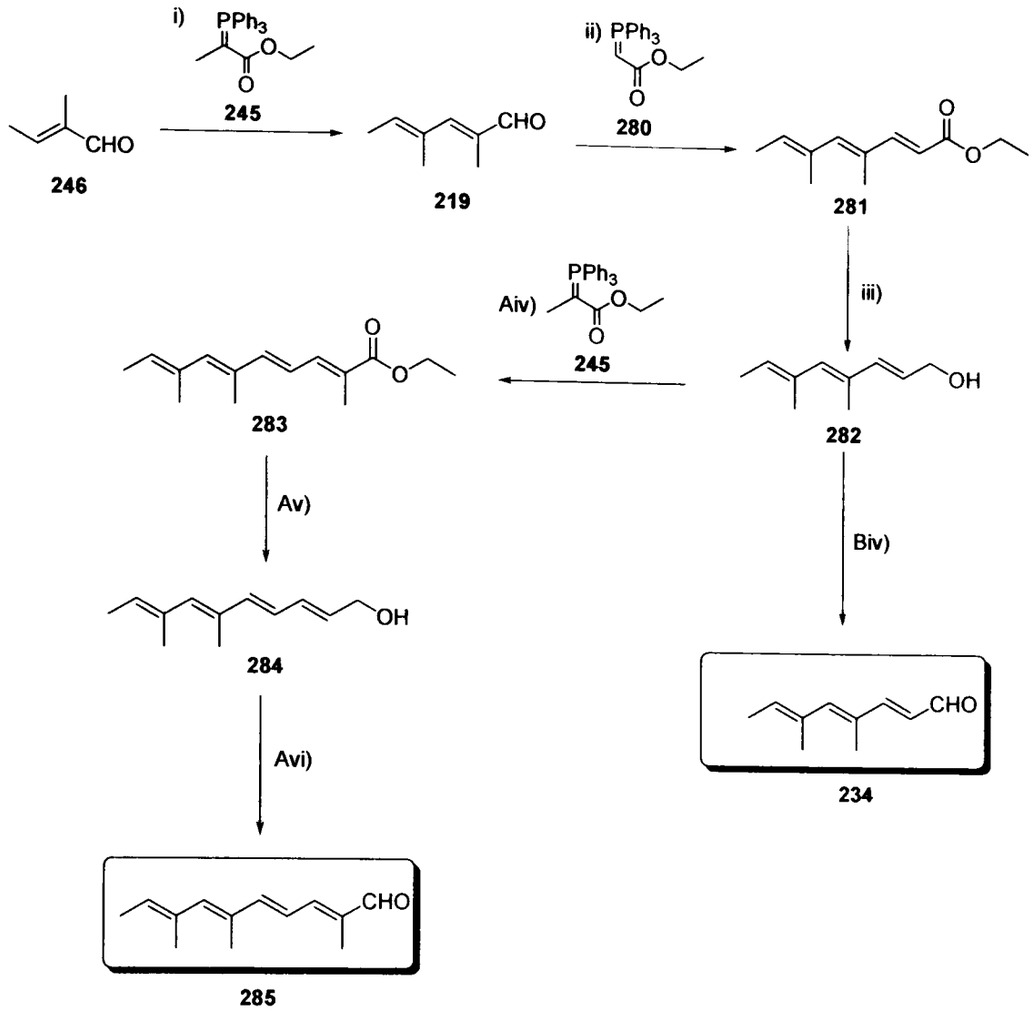


Scheme 73: Possible mechanism for direct conversion of **270** to **261** by $NaBH(OAc)_3$/AcOH

6.3: Synthesis of Trienal 234 and Tetraenal 285

Having synthesised tetronate **261** attention turned to the synthesis of aldehydes **234** and **285**, which were synthesised from **219** (Scheme 74; the synthesis of dienal **219** is described in chapter 5). Chain extension of **219** was achieved using a Wittig reaction with phosphorane **280** to give ester **281** (67%), which was reduced using DIBAL-H to

give the primary alcohol **282** in excellent yield (79%). Alcohol **282** could either be oxidised with manganese dioxide giving desired aldehyde **234** in excellent yield (96%) (step Biv, Scheme 74), or chain extended further in a one-pot oxidation-Wittig reaction (step Aiv Scheme 74) to give ethyl ester **283** in good yield (60%), using methodology developed by Taylor and co-workers.[180,193-195] DIBAL-H reduction and subsequent oxidation with manganese dioxide yielded the desired aldehyde **285**. Although these final two steps were successful, albeit in low yield (25% over two steps), they proved to be problematic. DIBAL-H reduction under the conditions established for reduction of **281** to **282** was not possible, only degradation products were observed. When the reaction was carried out at lower temperature (-78°C) it was possible to isolate the desired alcohol, although small quantities of geometrical isomers were also observed. Oxidation of the crude alcohol **284** gave aldehyde **285**, along with geometrical isomers and degradation products (~ 6% determined by ^{1}H NMR spectroscopy on the crude product) (Scheme 74). With good synthetic routes to both aldehydes **234** and **285** and the exomethylene tetronate **261** in hand, coupling conditions were investigated next.

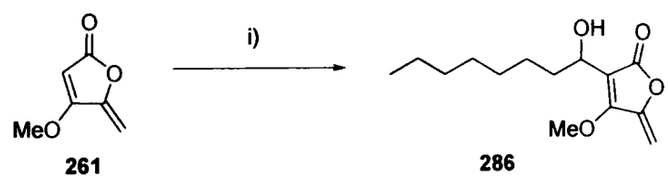


Scheme 74: Synthesis of desired aldehydes **234** and **285**

i) 1) **245**, toluene, reflux, 72 h; 2) DIBAL-H, Et_2O, 0°C, 3 h; 3) MnO_2, DCM, rt, 66% over 3 steps; ii) **280**, toluene, reflux, 72 h, 67%; iii) DIBAL-H, Et_2O, 0°C, 3 h, 79%; Biv) MnO_2, DCM, 96%; Aiv) **245**, MnO_2, DCE, reflux, 72 h, 60%; Av) DIBAL-H, Et_2O, -78°C, 3 h; Avi) MnO_2, DCM, rt, 25% over 2 steps

6.4: Addition of Lithiated Exomethylene Tetronate 261 to Aldehydes

Attempts were made to add lithiated **261** to octanal in a model reaction. This reaction proceeded smoothly giving the desired product **286** in good yield (57%) (Scheme 75).

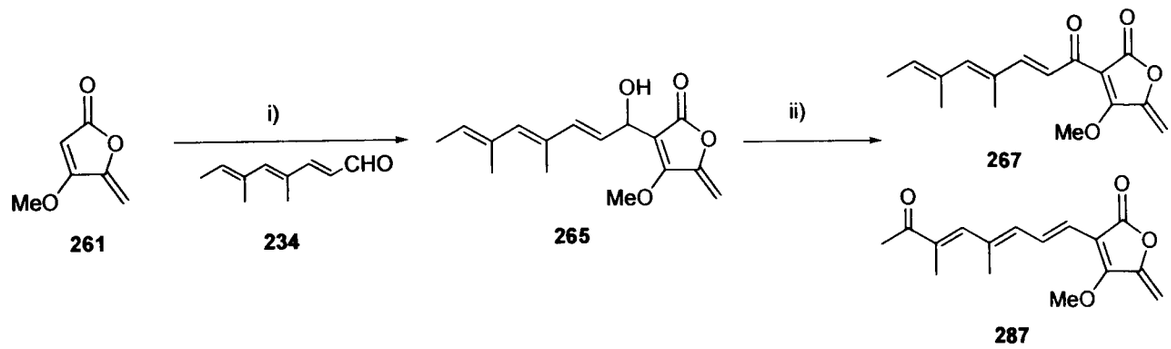


Scheme 75: Model reaction; synthesis of **286**

i) LDA, then octanal, THF, -78°C to -60°C, 57%

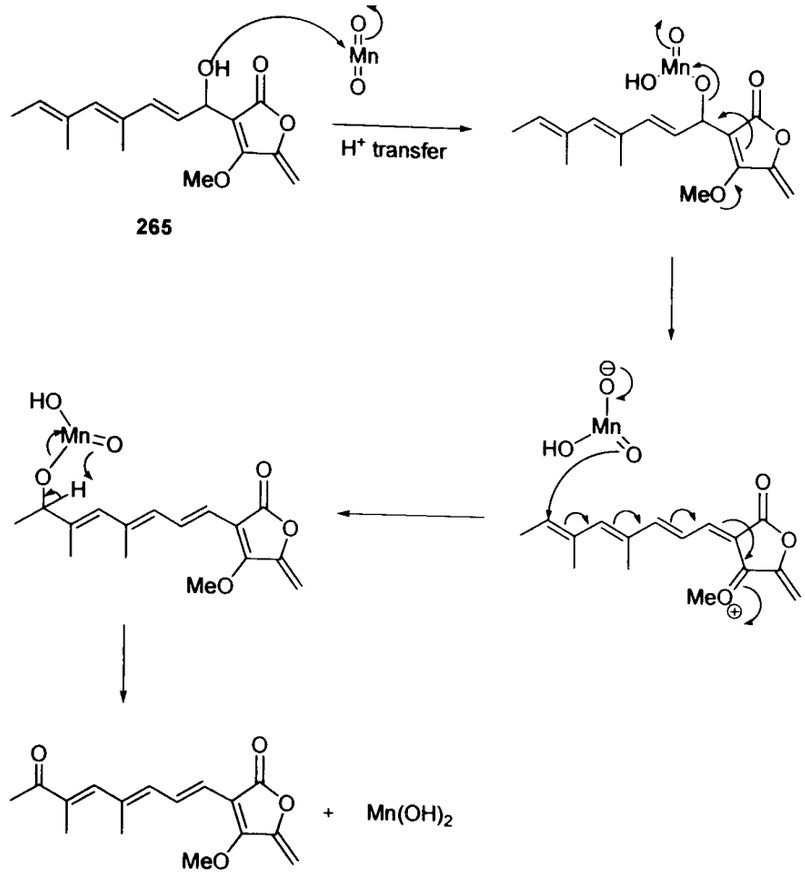
Formation of the lithiated derivative of the methyl protected exomethylene tetronate **261** with LDA followed by the addition of the synthesised aldehyde **234** gave **265** in

moderate yield (35%) (Scheme 76). Both commercially available and freshly prepared LDA solutions were investigated as bases. The freshly prepared LDA gave **265** in approximately 10% higher yield than the commercial LDA. Oxidation of the product **265** to **267** was attempted under Swern conditions but none of the desired product could be isolated and it appeared that the starting material had degraded under these conditions. However, oxidation with Dess-Martin periodinane afforded **267** but in low yield (15%). A rearrangement product **287** resulting from a competing reaction was also isolated (Schemes 76 and 77) as well as many degradation products. The assignment of structure to the rearrangement product **287** was based on HRMS, and 1- and 2-D NMR analysis. HMBC correlation observed for **287** are shown in Figure 42. Similar results were obtained when manganese dioxide was used instead of Dess-Martin periodinane.



Scheme 76: Synthesis of **265** and subsequent oxidation to give **267** and **287**

i) LDA, then **234**, THF, -78°C to -60°C, 35%; ii) Dess-Martin periodinane, DCM, rt, **267** 15%, **287** 10%



Scheme 77: Possible mechanism for formation of rearrangement product **287** by reaction of **265** with manganese dioxide

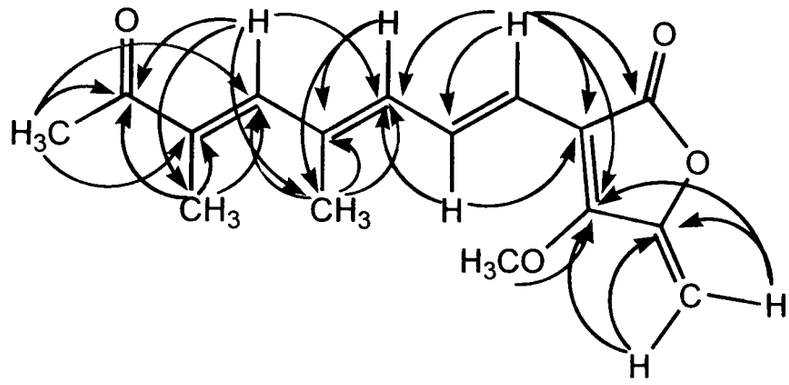
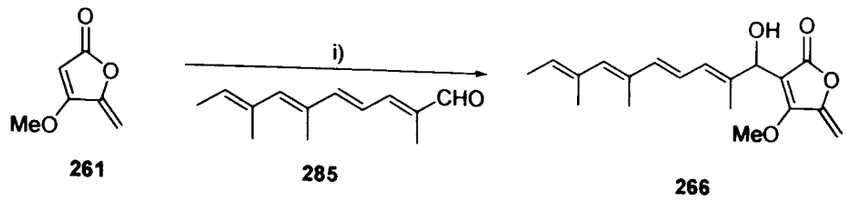


Figure 42: HMBC correlations observed for compound **287**

Attempts were also made to carry out the coupling reaction with tetraene aldehyde **285** (Scheme 78), but although the desired product **266** could be observed by mass spectrometry of the crude product, the ^{1}H NMR spectrum revealed that many degradation products and isomers had also been formed during the reaction.



261 **285** **266**

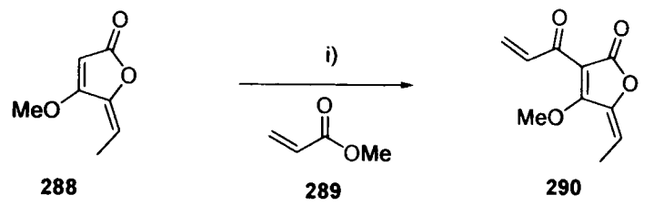
Scheme 78: Synthesis of **266**

i) LDA, then **285**, THF, -78°C to -60°C, 11% impure

6.5: Alternative Strategies for Coupling Tetronate 261 with Triene and Tetraene Fragments

The failure to adapt the literature procedures for alkylation of **261** for our purposes prompted the exploration of other strategies for coupling of **261** with triene and tetraene fragments.

A literature survey revealed a report of a coupling reaction between methyl tetronate **288** and methyl acrylate **289** (Scheme 79).[196]



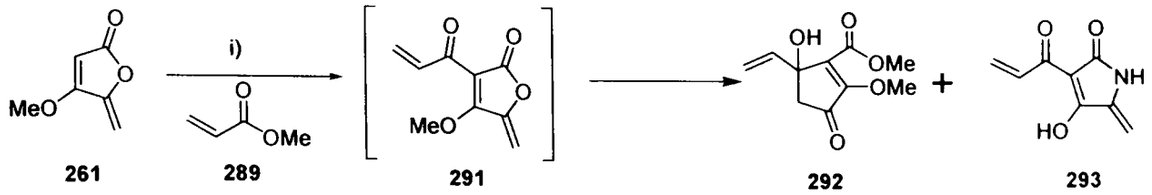
288 **289** **290**

Scheme 79: Coupling reaction between **288** and methyl acrylate **289**[196]

i) LDA, then **289**, THF, -78°C to rt, $NH_4Cl_{(sat.)}$ quench, 50%

It was reasoned that if the previously synthesised ethyl esters **281** and **283** could be coupled with **261** rather than the aldehydes, then fewer steps would be required in the overall synthesis of the two precursors **206** and **207**, resulting in a more concise synthesis. This methodology would also circumvent the problematic low yields and the by-product formation of the oxidation step. Initially, as a model reaction, coupling of the methyl-protected exomethylene tetronate **261** to methyl acrylate **289** was attempted using the procedure reported by Buck *et al.*[196] This involves lithiation of **261** using LDA at -78°C followed by the addition of three equivalents of methyl acrylate to the reaction,

before allowing the mixture to warm to room temperature. The reaction was quenched with saturated ammonium chloride. Unfortunately none of the expected product **291** was isolated and instead a mixture of two other products **292** and **293** was obtained (Scheme 80). This was probably due to the *in-situ* rearrangement of the desired product.

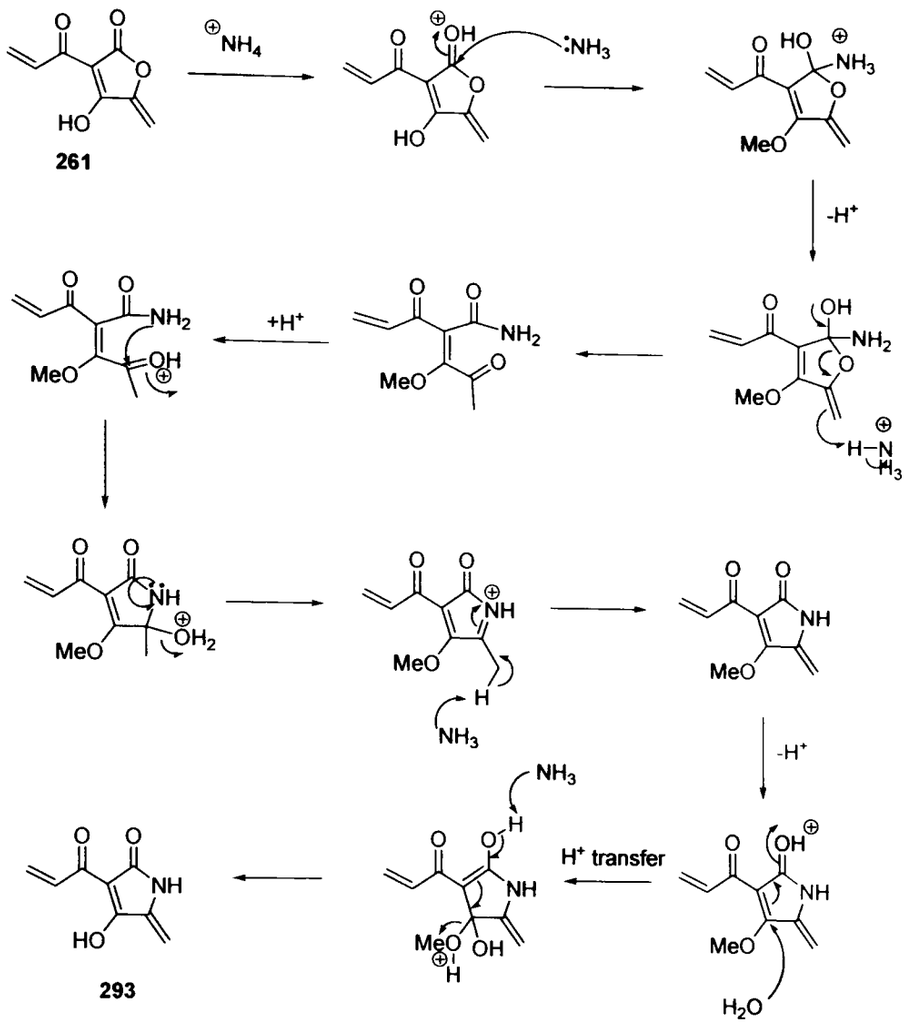


261 **289** **291** **292** **293**

Scheme 80: Attempted synthesis of **291**

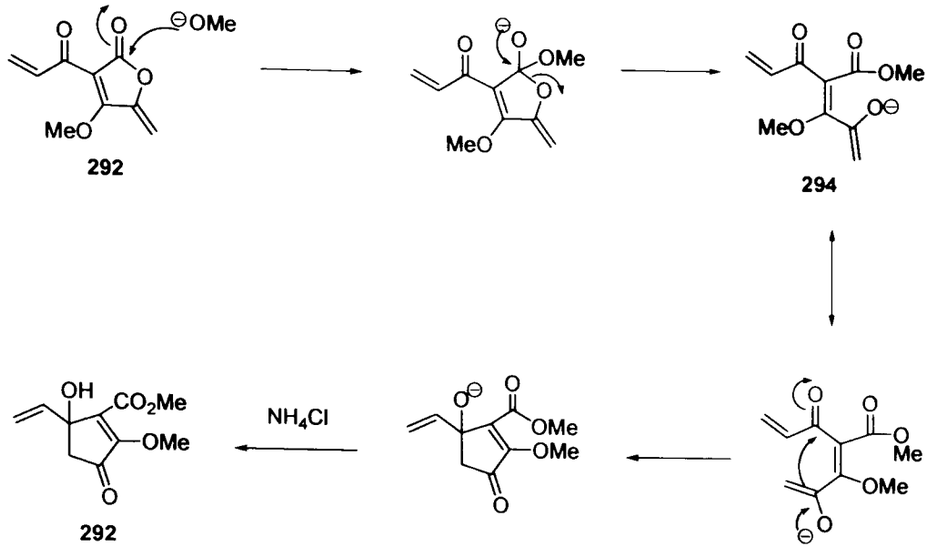
i) LDA, -78°C, then **289**, THF, -78°C to rt, $NH_4Cl_{(sat.)}$ quench

Previous research by Farina *et al.* showed that the presence of ammonia in a solution of tetronic acid derivatives converted them to the corresponding tetramic acid derivatives.[197] It is therefore likely that quenching the reaction with ammonium chloride led to some ammonia being present in the reaction work-up allowing this conversion to occur, giving **293** in low yield (11%) (Scheme 81).



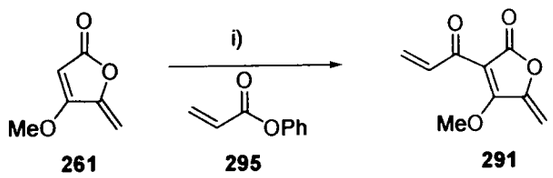
Scheme 81: Possible mechanism for formation of **293**

On the other hand compound **292** appeared to have been formed *via* a novel rearrangement during the coupling reaction. The methoxy anion generated in the reaction could attack the carbonyl carbon of the tetronic acid, resulting in ring opening and the formation of ester **294**. Ring closure after attack of the enolate on the other carbonyl group gives a highly functionalized novel compound **292** (Scheme 82).



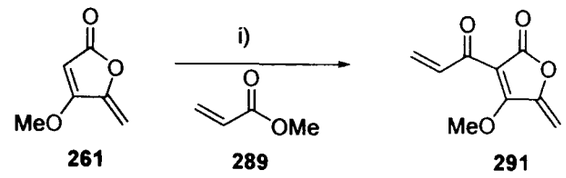
Scheme 82: Proposed mechanism for formation of **292**

Different conditions were screened to avoid the rearrangements occurring during the above reaction. Quenching the reaction with saturated citric acid rather than with ammonium chloride meant that the conversion to the tetramic acid derivative was avoided (Scheme 83). It was thought that by using phenyl acrylate **295** these rearrangements could be prevented, because the phenoxy anion generated would be more bulky and more stable than the methoxy anion, and thus less nucleophilic. The reaction was successful using phenyl acrylate **295**, and keeping the temperature at -78°C, giving **291** in low yields (~ 5%), but the product was extremely volatile thus isolation proved difficult (Scheme 83).



Scheme 83: Synthesis of **291** using phenyl acrylate **295** and citric acid to quench the reaction
i) 1) LDA, -78°C; then **295**, THF, -78°C, 16 h, citric $acid_{(sat.)}$, 5%

Further attempts employing either methyl acrylate **289** or phenyl acrylate **295** afforded the target compound **291** in improved yields. Removing the solvent *in vacuo* without heating helped to minimize loss of the volatile product **291**. Under these new conditions it was possible to isolate the desired product **291** in 18% yield (Scheme 84).

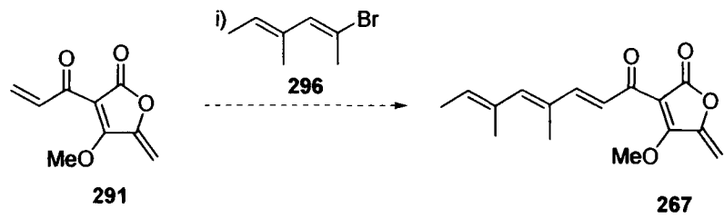


261 289 291

Scheme 84: Synthesis of **291** using methyl acrylate **289** and citric acid to quench the reaction

i) LDA, then **289**, THF, -78°C, 16 h, citric $acid_{(sat.)}$, 18%

The synthesised **291** could allow the development of a new synthetic route to the putative key intermediates in quartromicin biosynthesis. This method would utilise Heck coupling with a vinyl bromide allowing the polyene chain to be installed at a later stage in the synthesis (Scheme 85). This was not explored due to time constraints.

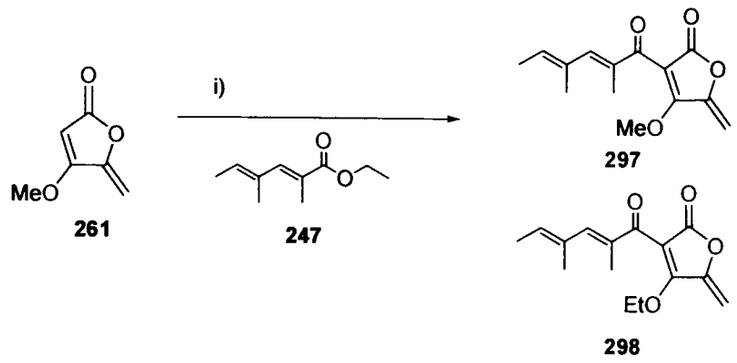


291 267

Scheme 85: Proposed Heck reaction

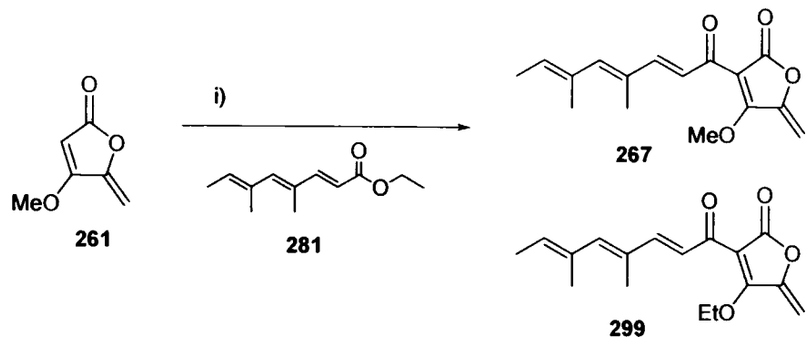
i) $Pd(OAc)_2$

The success of the model reaction to form **291** led to attempts to couple the diene ethyl ester **247** and triene ethyl ester **281** with the methyl protected exomethylene tetronate **261** (Scheme 86 and 87 respectively). In both cases two products were isolated; the desired compounds **297** and **267** in low yields (11% and 14% respectively) and their ethyl protected analogues, **298** and **299**. It was thought that both **298** and **299** were formed by transesterification *via* nucleophilic addition of the ethoxy anion generated in the reaction to the carbon bearing the methoxy group followed by elimination of the methoxy group.



Scheme 86: Synthesis of **297**

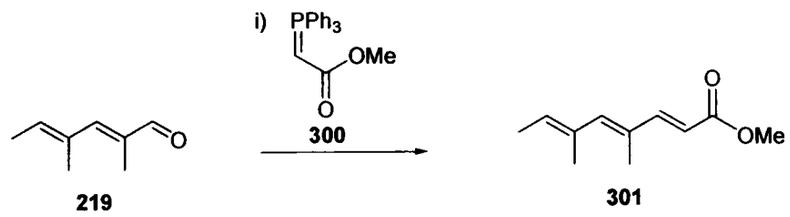
i) LDA, then **247**, THF, -78°C, 24 h, citric $acid_{(sat.)}$ quench, 11% of **297** and 9% of **298**



Scheme 87: Synthesis of **267**

i) LDA, then **213**, THF, -78°C to -60°C, citric $acid_{(sat.)}$ quench, **267** 14%, and **299** 14%

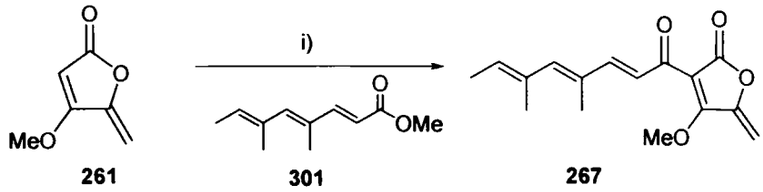
In order to circumvent the problem of transesterification the methyl ester equivalent of **281** was synthesised (Scheme 88). Chain extension of **219** with Wittig chemistry using phosphorane **300** proceeded smoothly to give the desired compound **301** in excellent yield (83%).



Scheme 88: Synthesis of **301**

i) **300**, toluene, reflux, 72 hrs, 83%

Ester **301** could then be coupled with the methyl protected exomethylene tetronate **261** to give the product **267** in low yield as shown in Scheme 89.

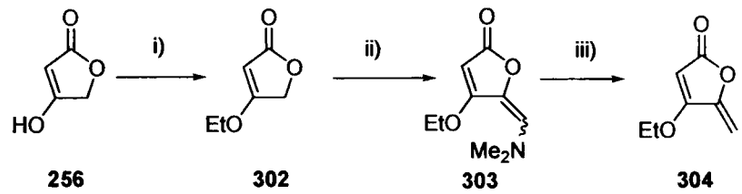


261 **301** **267**

Scheme 89: Synthesis of **267** avoiding transesterification

i) TMP, then **301**, THF, -78°C, 24 h, citric $acid_{(sat.)}$ quench, 17%

Another possibility to prevent transesterification is to use the ethyl-protected exomethylene tetronate **304**, which was synthesised as shown in Scheme 90 using the methodology developed previously (Scheme 72). Reaction of tetronic acid and ethanol in toluene under Dean-Stark conditions gave the ethyl protected tetronate **302** in excellent yield (86%).[198] (Dimethylamino)methylenation at C(5) followed by reduction and elimination gave the ethyl protected exomethylene tetronate **304** in excellent yield (79% over the two steps) (Scheme 90).

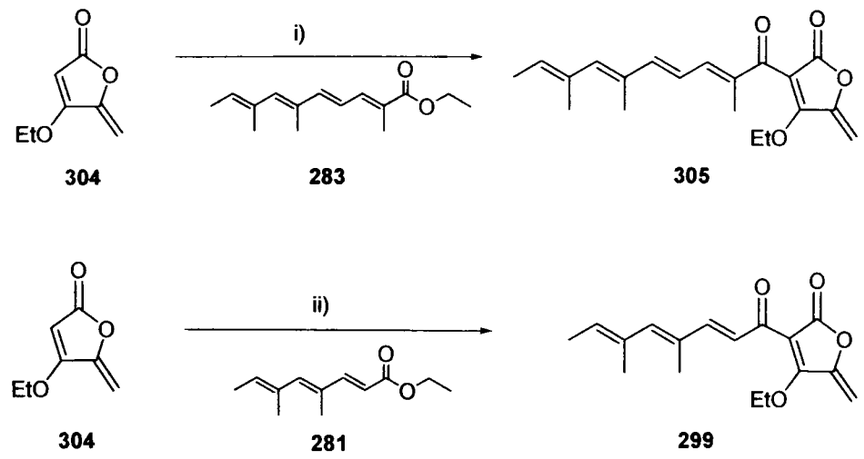


256 **302** **303** **304**

Scheme 90: Synthesis of ethyl-protected tetronate **304**

i) EtOH, Toluene, reflux, 86%; ii) $Me_2NCH(OMe)_2$ 89%; iii) $NaBH(OAc)_3$, AcOH, DCE, 89%

Coupling of the tetraene ethyl ester **283** and the ethyl protected exomethylene tetronic acid **304** gave compound **305** in low yield (27%) (Scheme 91). A similar reaction was carried out with the triene ethyl ester **281** to give the ethyl protected product **299** in low yield (25%), an improvement on the coupling reaction between the methyl ester **301** and tetronate **261**

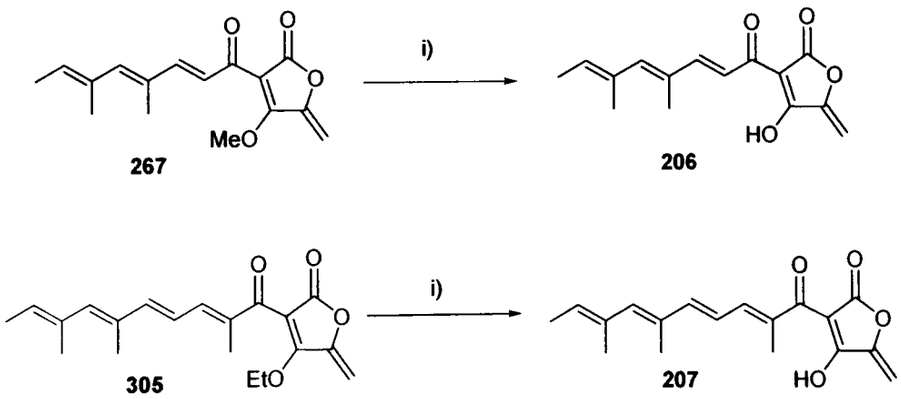


Scheme 91: Synthesis of **305** and **299**

i) TMP, then **283** or **281**, -78°C, 24 h, citric $acid_{(sat.)}$ quench, 27%, and 25% respectively

In order to optimise the reaction, four different bases LDA, Lithium-2,2,6,6-tetramethylpiperidide (Li-TMP), lithium-dicyclohexylamide and lithium-hexamethyldisilazide (Li-HMDS) and a variety of reagent concentrations were investigated. Using 1.25 equivalents of Li-TMP to one equivalent of **261** was found to give the cleanest reaction and highest yields (~30%).

Deprotection of compounds **267** and **305** was carried out using LiCl (Scheme 92). Deprotection of **267** was carried out in DMSO at 50°C giving **206** as the sole product (100% conversion as determined by ^{1}H NMR). The end point of the reaction was established by following the reaction by ^{1}H NMR spectroscopy. Purification of **206** was carried out by HPLC on two milligrams of the crude product.

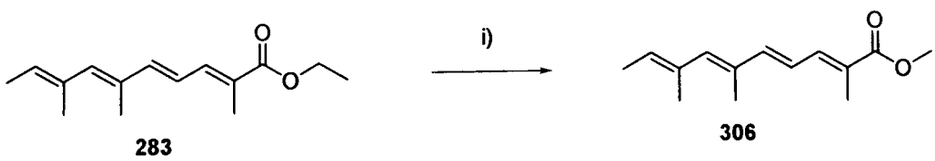


Scheme 92: Deprotection of **267** and **305** to give **206** and **207**, respectively

i) LiCl, $DMSO(d_6)$, 50°C, 16 h, 100% conversion to **206**, 95% conversion to **207**

However, when using the same conditions to deprotect the ethyl protected tetraene derivative **305** the reaction proceeded at a much slower rate (after 24 hours only 5% deprotection had occurred). This is possibly because the boiling point of the methyl chloride by-product of the reaction is a lot lower than that of the ethyl chloride by product (-24°C compared to 12.5°C) so at the lower temperature the ethyl chloride is lost from the reaction at a lower rate but more likely to be because of the increased steric bulk of the ethyl group in comparison to the methyl group. The temperature was increased to 65°C and, again following the reaction by ^{1}H NMR spectroscopy, the end point was established after seventy-two hours. Although only 95% of the starting material had been converted to product after this time no further deprotection was observed over a subsequent three hours by ^{1}H NMR spectroscopy, and geometrical isomers of the tetraene product **207** also began to appear. The product was only partially purified by HPLC. Thus the synthesis of the methyl ester analogue **306** of the ethyl ester **283** was explored.

The methyl phosphorane required for the synthesis of the tetraene methyl ester **306** was not available commercially. A survey of the literature showed that methods exist for the synthesis of this phosphorane[174,199] but first it was decided to attempt to convert the ethyl ester **283** to methyl ester **306** by a transesterification reaction using sodium methoxide in methanol. This reaction proceeded smoothly to give the desired methyl ester **306** in good yield (74%) (Scheme 93).

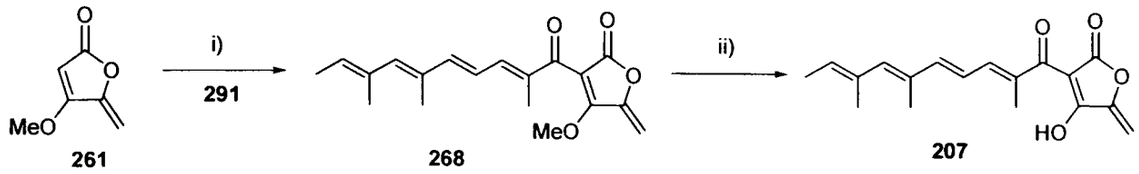


283 **306**

Scheme 93: Synthesis of **306**

i) NaOMe, MeOH, rt, 3 h, 74%

Coupling of the methyl-protected exomethylene tetronate **261** and methyl ester **306** proceeded smoothly to give **268** in low yield (23%) (Scheme 94). The deprotection of ester **268** with LiCl / DMSO gave a clean ^{1}H NMR spectrum for the crude product **207** (with minor geometrical isomers of the tetraene observed), which was purified by HPLC.

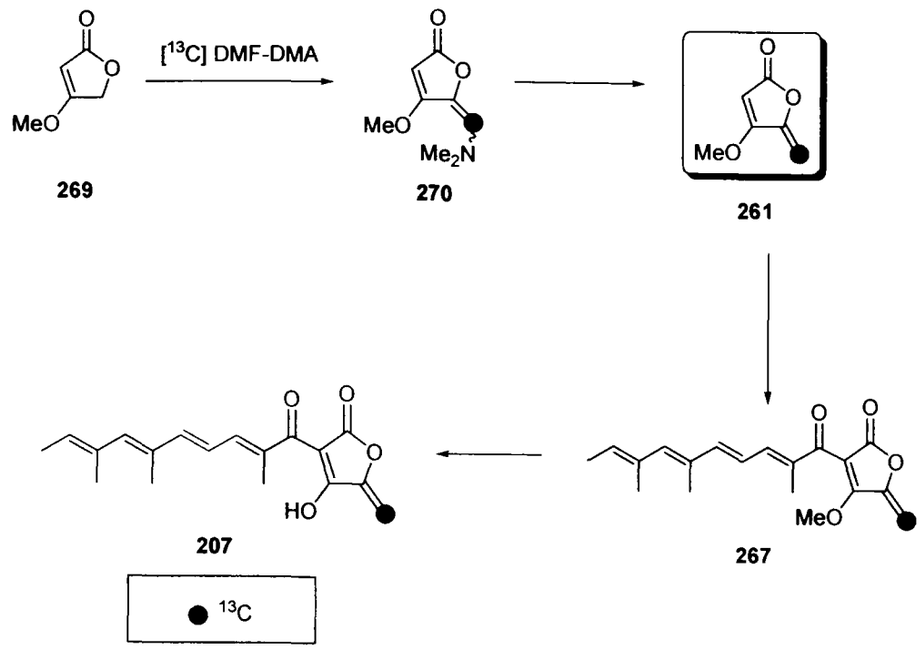


Scheme 94: Coupling and deprotection reaction to afford **207**

i) LTMP, then ester **306**, -78°C , 24 h, citric $acid_{(sat.)}$ quench, 23%, ii) LiCl, $DMSO(d_6)$, 50°C, 16 h, 100%

Compounds **206** and **207** will be utilised in labelled form for feeding experiments in the bacteria that produce quartromicins to help investigate the biosynthetic pathway. It would be possible to prepare these two compounds easily *via* the procedure described using labelled DMF-DMA. This reagent could be prepared from *N,N*-dimethylform-^{13}C-amide (which is commercially available), NaOMe, and Me_2SO_4 in methanol.[200]

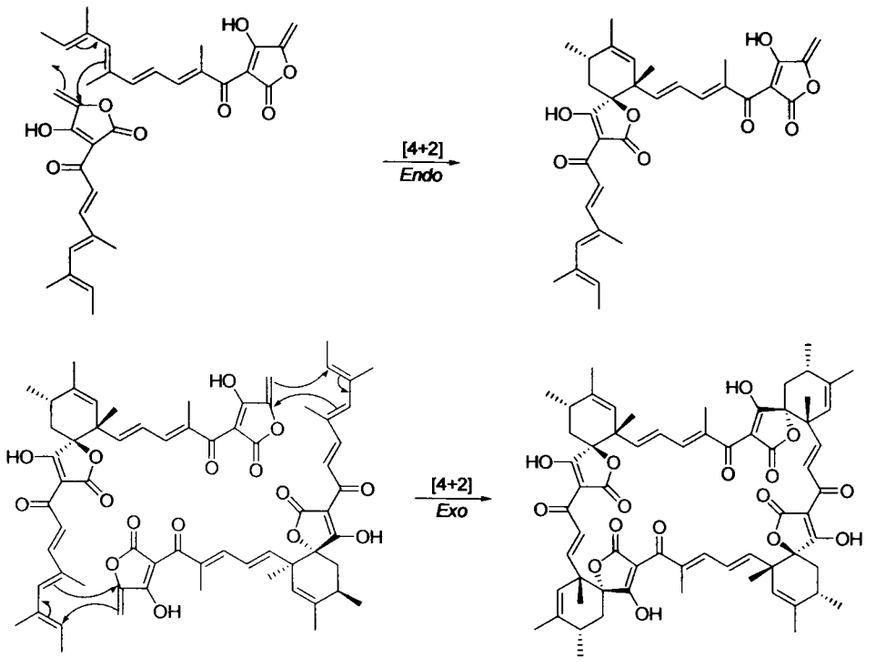
The exomethylene group is a good place to incorporate an isotope label as there are only two steps to make the methyl protected exomethylene tetronic acid **261** from [^{13}C] DMF-DMA (both of which are high yielding). Two further steps from **261**, the coupling reaction and deprotection, would yield the desired compounds **206** and **207** in labelled form (Scheme 95).



Scheme 95: Synthesis of ^{13}C labelled putative key intermediates in quartromicin biosynthesis

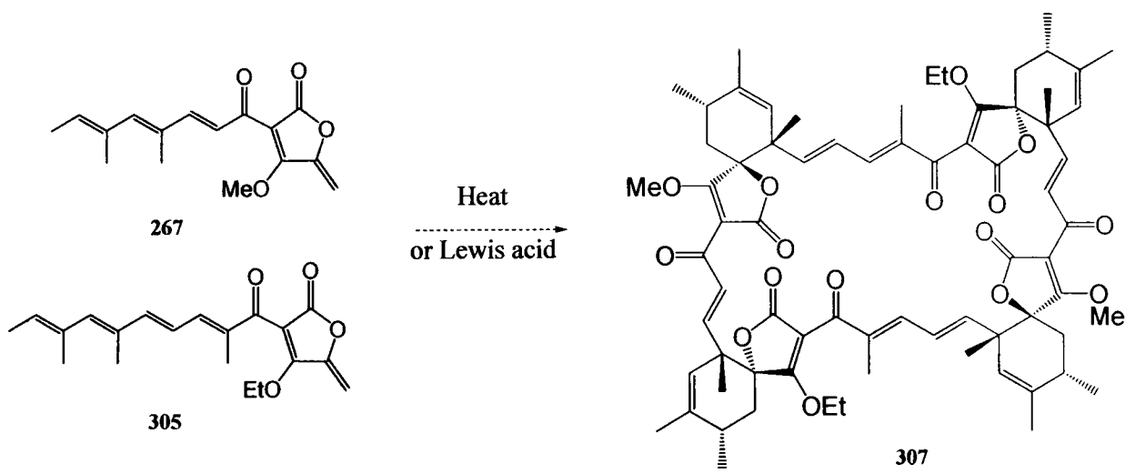
6.6: Investigation of Biomimetic Diels-Alder Reactions of Putative Key Intermediates 267 and 305

Quartromicins **122a-f** fall into the spirotetronate family of natural products that are proposed to be biosynthesised *via* Diels-Alder reactions (Scheme 96). The most intriguing aspect of the quartromicins is the alternating *exo-* and *endo-* stereochemistry that arises from the Diels-Alder reactions, with the opposite corners being stereochemically identical. As no total synthesis has been accomplished and the biosynthetic pathway has yet to be established, the quartromicins are attractive targets for biomimetic synthesis investigations.



Scheme 96: Proposed biosynthetic Diels-Alder cyclisations between **206** and **207** to generate the quartromicin carbon skeleton

It was therefore proposed to investigate the biomimetic synthesis of the quartromicins by heating the two synthesised precursors **267** and **305** to determine if the cyclo-pseudo-tetrameric carbon skeleton of the quartromicin aglycone **307** could be formed (Scheme 97).



Scheme 97: Proposed biomimetic experiment: Diels-Alder reaction of **267** and **305** to give the quartromicin carbon skeleton **307**

Initially the Diels-Alder reaction was carried out at 100°C in toluene (10 mg in 3 mL); conditions used by Sorenson and co-workers in the biomimetic studies of abyssomicins.[15] However, after forty-eight hours no change was observed by LC-MS and the starting material remained. The reaction was then attempted in a sealed tube,

initially at 100°C for three days, as this would increase the pressure in the reaction vessel. It was hoped that the increased pressure would speed up any reaction that would take place. 100 µl aliquots were analysed by LC-MS: After forty-eight hours, ions with m/z corresponding to a homodimer of the triene **308a-d** (m/z 549) and a heterodimer of the tetraene and the triene **3094a-j** (m/z 603) were observed (Figure 43).

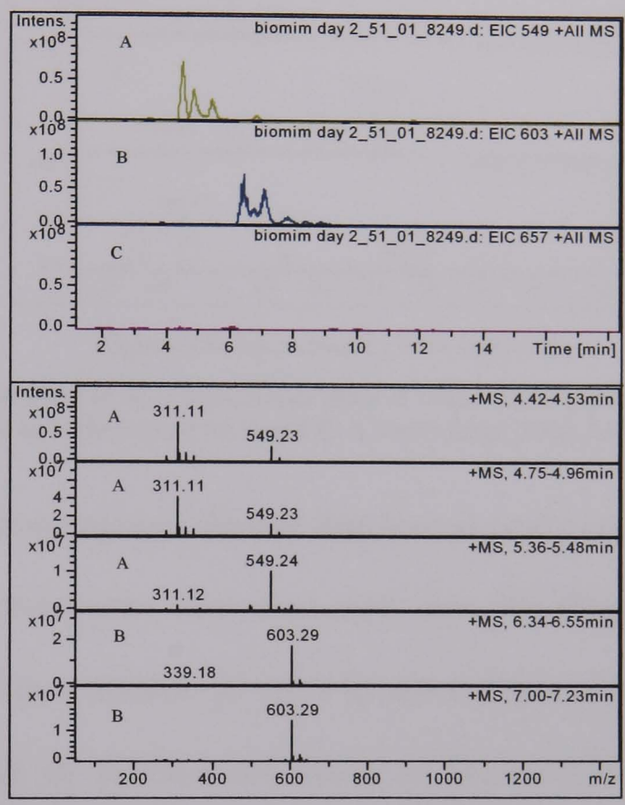


Figure 43: Extracted ion chromatogram (EIC) from LC-MS analysis

Showing masses corresponding to **A**: a homodimer of the triene (m/z 549), **B**: a heterodimer of the tetraene and the triene (m/z 603) and **C**: a homodimer of the tetraene

After another twenty-four hours no further change was observed, so the temperature was increased to 125°C. 100 µL aliquots were analysed after seven, eleven, fourteen and eighteen days and peaks were observed that corresponded to homodimers of the tetraene **310a-f** (m/z 657), as well homodimers of the triene **308a-d** and the heterodimers **309a-j** previously observed suggesting these compounds had again been formed (Figure 44).

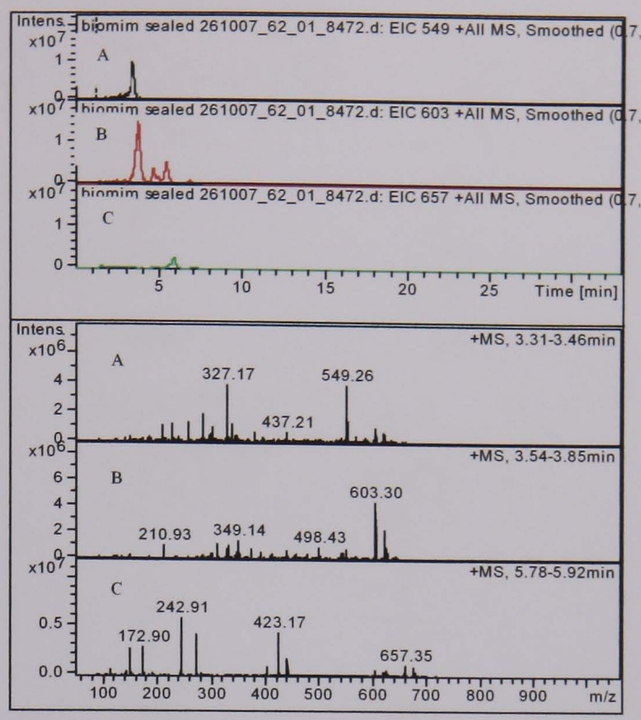
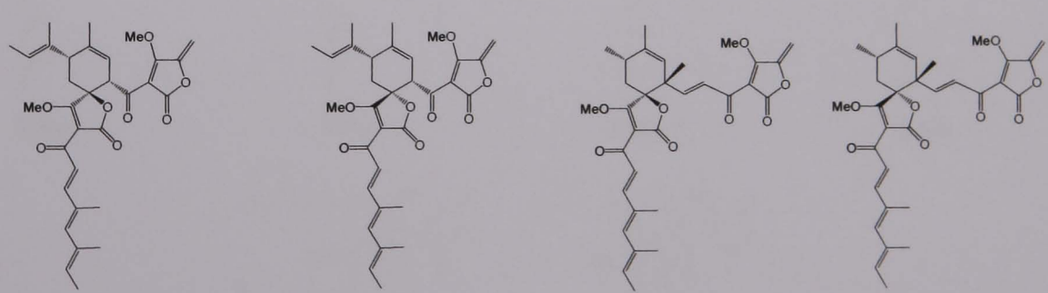


Figure 44: EICs from LC-MS analysis

Showing masses corresponding to **A**: a homodimer **308a-d** of the triene (m/z 549) and **B**: a heterodimer **309a-j** of the tetraene and triene (m/z 603) and **C**: a homodimer **310a-f** of the tetraene (m/z 657)

The extracted ion chromatograms showed that several peaks corresponded to the same mass for example three peaks were seen with m/z 549 (Figure 43). This was not unexpected as there are a number of ways in which Diels-Alder reactions can occur within each molecule to produce the homo or heterodimers; some of these are represented in Figures 45, 46 and 47. The enantiomers of each dimer can also be produced but for clarity only one is shown.



308a **308b** **308c** **308d**

Figure 45: Some possible homodimers **308a-d** of the triene **267**

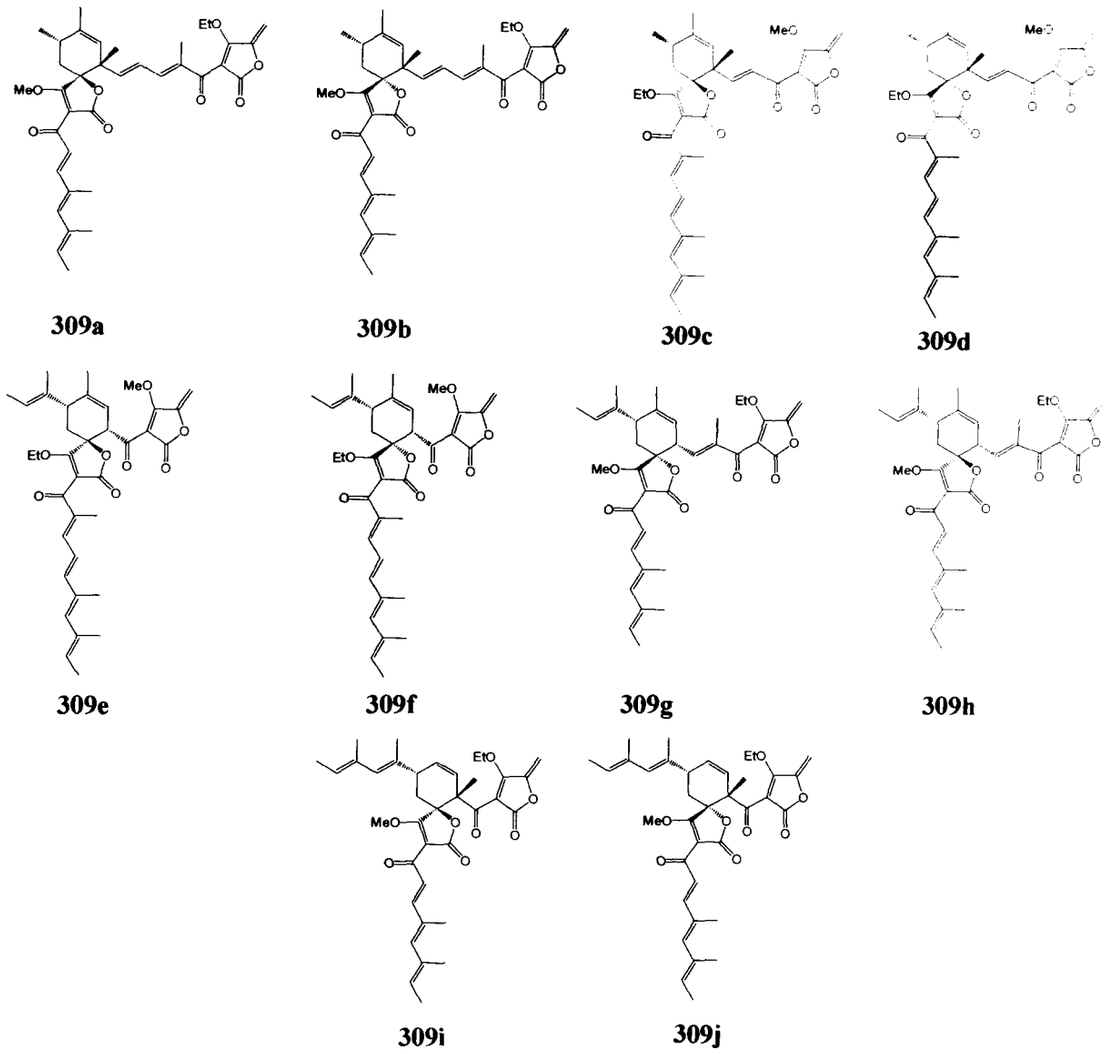


Figure 46: Some possible heterodimers **309a-j** formed by reaction of triene **267** and tetraene **305**

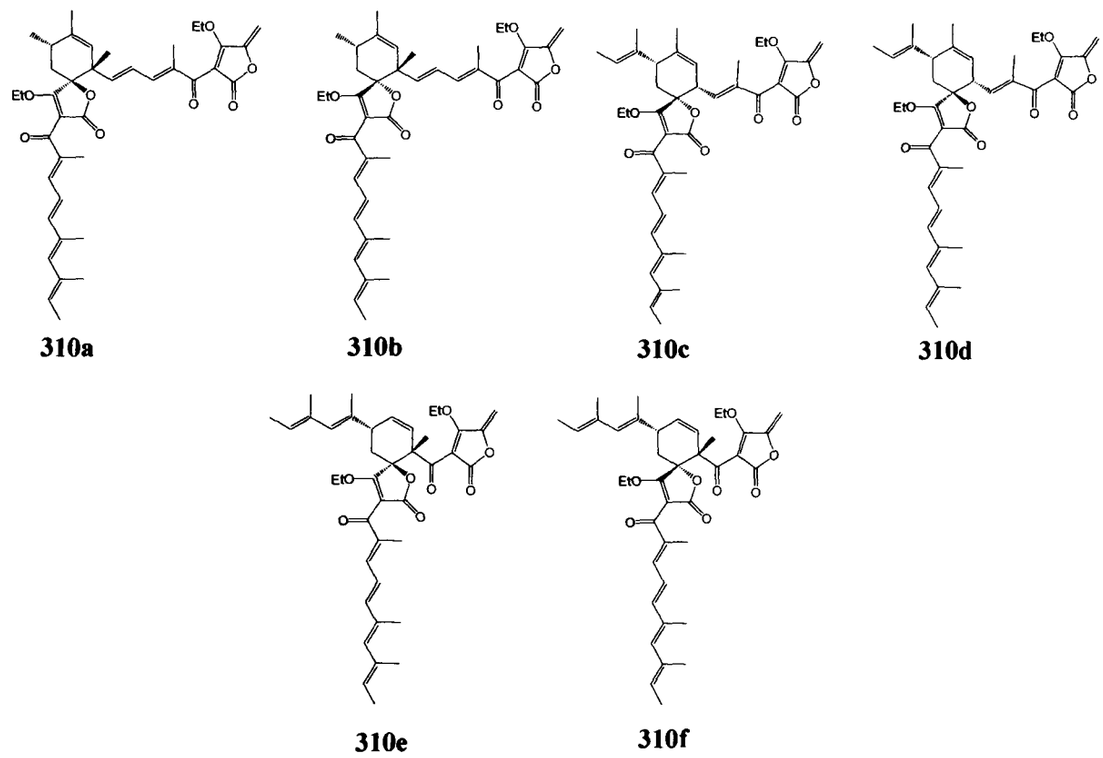


Figure 47: Some possible homodimers **310a-f** of the tetraene **305**

Comparison of the EIC for day two and day eighteen of the biomimetic experiment in Figure 48 shows that over a period of time the three mass ion peaks corresponding to m/z 549 became one signal with a different retention time. It is possible that the Diels-Alder reaction is reversible and therefore a more thermodynamically stable compound is produced over a period of time. However it is also possible that some degradation of the dimer is occurring. As can be seen in Figure 48, the intensity of the peak after eighteen days is approximately 10 fold lower than after two days which could also indicate degradation of the dimer.

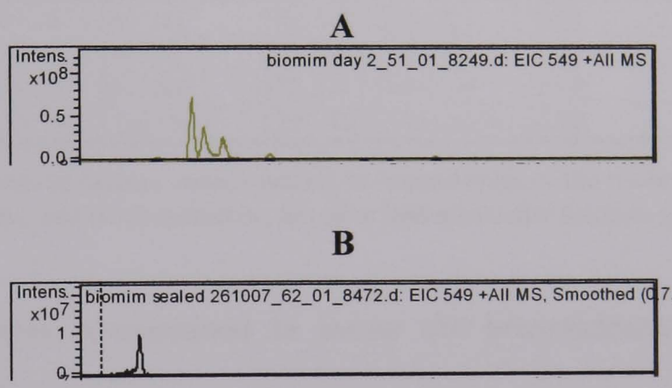


Figure 48: ECI showing **A**: Day 2 three mass ion peaks corresponding to m/z549 and **B**: Day 18 one mass ion peak corresponding to m/z549

It is also apparent that the tetraene monomer **305** is either less reactive then the triene monomer **267**, perhaps because the ethyl group in the tetraene derivative **305** was sterically blocking the exomethylene preventing it from reacting readily at this position; compounds with m/z corresponding to the dimer of the tetraene required a longer period of heating before they formed, i.e. dimers are not observed after forty-eight hours but are observed after eighteen days.

After three days of heating in a sealed tube a 20 μL aliquot from the reaction was separated by LC-MS and fractions were collected every thirty seconds for analysis by ESI-TOF-MS. The data from these analyses (Table 2) suggest that a species with a ESI-TOF-MS molecular formula corresponding to the cyclo-pseudo-tetrameric carbon

skeleton of the quartromicin aglycone had been generated during the reaction (m/z 1227 $[M+ Na]^+$). It was not surprising that only the sodiated mass ion signal was seen, because quartromicin and the precursors have oxygen atoms in a 1,3-diketone configuration and are thus strong metal chelators. It has been reported that the quartromicins bind strongly to sodium, potassium, calcium and magnesium.[104] Other mass ion signals correspond to the molecular formula of the homodimer of the tetraene and triene species as well as the homotrimer of the triene (Table 2).

Sample	m/z observed	m/z calculated	Error [ppm]	Molecular Formula
Homodimer triene	549.2478 $[M+H]^+$	549.2483 $[M+H]^+$	0.83	$C_{23}H_{27}O_8$
Homotrimer triene	823.3654 $[M+H]^+$	823.3688 $[M+H]^+$	4.15	$C_{48}H_{55}O_{12}$
Heterodimer	625.2772 $[M+Na]^+$	625.2777 $[M+Na]^+$	2.98	$C_{36}H_{42}O_8Na$
Heterotetramer	1227.5602 $[M+Na]^+$	1227.5652 $[M+Na]^+$	4.00	$C_{72}H_{84}O_{16}Na$

Table 2: HRMS for observed masses corresponding to homodimers of the triene, tetraene, homotrimer of the triene, and heterotetramer, the error and molecular formula generated

The LC-MS data was re-examined to locate the heterotetramer species; however, although there were some small signals corresponding to protonated molecular ion (m/z 1205) and the sodiated molecular ion (m/z 1227), the intensity of these signals was very low. This could be for a number of reasons: The quartromicin aglycone may be produced in such small quantities in comparison to other species it is not visible because of the noise. The species may be doubly-charged and therefore a signal would be seen at m/z 603, but because of the strong signal at m/z 603 due to the heterodimer, the heterotetramer signal could be masked. Also the m/z 1227 signal may not in fact correspond to the heterotetramer at all, but merely a mass spectrometry artefact, i.e. from two heterodimers that have chelated a sodium ion.

In an attempt to increase the rate and specificity of the Diels-Alder reaction it was carried out in a microwave reactor. Several different reaction times and solvents were

tested to see if this increased or decreased the rate of the Diels–Alder reactions (Table 3). Four solvents were tested: toluene, nonane, isopropanol and a toluene/water mixture.

Solvents can greatly affect the rate of Diels-Alder reactions. Hydrocarbons, which are non-polar, are generally used because they dissolve the reactants easily; hence toluene and nonane are good choices. However, in a microwave reactor these solvents do not couple with the microwave energy, hence the temperature of the reaction increases more slowly. Isopropanol on the other hand is polar, so is not such a good choice for a Diels-Alder reaction, but it couples well with microwave energy. Water surprisingly accelerates Diels-Alder reactions and it is thought that the non-soluble reactants are pushed together in oily drops formed in the water and are therefore forced into close proximity to allow the reaction to occur.

Solvent	Time[c]	m/z	Time[c]	m/z	Time[c]	m/z	Time[c]	m/z
Toluene[a]	0.5	549, 603	1.5	549, 603	3.5	549, 603, 657	7.5	549, 603, 657
Nonane[b]	0.5	549, 589, 629	1.5	549, 589, 629	3.5	549, 589, 629	7.5	n/a[d]
Iso-propanol[b]	0.5	n/d	1.5	n/d[e]	3.5	n/a[d]	7.5	n/a[d]
Toluene/ water mix[b]	0.5	549, 589, 629	1.5	549, 589, 629	3.5	549, 589, 629	7.5	n/a[d]

Table 3: Dimers observed by LCMS from reactions in various solvents and reaction times in a microwave

[a]Uses ethyl protected tetraene **305** and methyl protected triene **267**.[b] Uses methyl protected tetraene **268** and methyl protected triene **267**. [c] time in hours. [d] not attempted; [e]none detected

The reaction using toluene as the solvent showed that using the microwave considerably increased the rate of reaction; the mass corresponding to the tetraene homodimer **310a-f** was observed after three and a half hours. However, when the reaction was carried out in nonane or the toluene water mixture, the masses corresponding to the homodimer of the tetraene were seen after thirty minutes. This could be because these solvents improve the rate of reaction, but more likely because the methyl protecting group on the

tetraene **268** used in this reaction does not sterically block the exomethylene group as much as the ethyl protecting group on **305** used in the reaction with toluene, allowing it to react more readily. As can be seen from Table 3, none of the desired masses were observed when isopropanol was used as solvent.

Preliminary biomimetic transformations of these precursors to the cyclo-pseudo-tetrameric carbon skeleton of the quartromicin aglycone suggest that Diels-Alder reactions occur to give both hetero and homodimers of the two intermediates. HRMS evidence suggesting the presence of a heterotetramer has also been obtained.

6.7: Conclusions and Future Work

Overall, the initial aims of this project were achieved. A concise and reasonably efficient synthesis of both putative quartromicin precursors **206** and **207** was completed. However, the acylation of the lithiated methyl-protected exomethylene tetronate **261** required further optimisation to improve yields. Preliminary biomimetic studies were carried out to ascertain whether the cyclo-pseudo-tetrameric carbon skeleton of the quartromicin aglycone could be produced and test the hypothesis that spirotetronates such as the quartromicins are biosynthesised by Diels-Alder reactions. HRMS and LC-MS data provide evidence that homo and heterodimers of the putative precursors have been formed and suggest that a compound with a molecular formula corresponding to the quartromicin aglycone has been produced.

In the future, both compounds **206** and **207** should be prepared in labelled form, for use in feeding studies to help elucidate the biosynthetic pathway to the quartromicins. Optimisation of quartromicin production by the producing *Amycolatopsis* strains will be

required prior to undertaking these experiments. Malek Kourdi-Zerikly, a PhD student in the Challis Group, has identified the putative quartromicin biosynthetic gene cluster in two *Amycolatopsis* species. Thus compounds **206** and **207** will be useful as standards for *in vivo* and *in vitro* experiments designed to probe the mechanism of quartromicin biosynthesis.

The biomimetic synthesis experiments will also be further investigated, by developing better conditions to generate the carbon skeleton of the quartromicin aglycone, which would allow the reactions to be carried out on a larger scale. This in turn would allow the structures of the homo and heterodimers produced to be fully characterised. Using the deprotected precursors **206** and **207** may also change the electronics sufficiently to allow greater specificity to occur during the biomimetic reactions. Another possible approach would be to isolate and characterise the heterodimers, and use the heterodimer with the appropriate stereochemistry in further biomimetic experiments aimed at producing the quartromicin carbon skeleton.

Chapter 7: Experimental

7.1: General Experimental

Dry toluene was obtained by evaporation of the toluene/water azeotrope *in vacuo* followed by distillation from calcium hydride under argon, and stored over 4 Å molecular sieves. Dry dichloromethane, methanol and acetonitrile were produced in the same way. Dry tetrahydrofuran was pre-dried over sodium wire and dried in a still over potassium for three days before use. Dry triethylamine and diisopropylamine were obtained by distillation from sodium hydroxide pellets under argon and stored over sodium hydroxide pellets. All other reagents and solvents were used as supplied. Petroleum ether refers to the fraction of light petroleum boiling between 40°C and 60°C. Solvents were evaporated using a Buchi Rotavapor R-200 equipped with a Buchi Vacuubrand pump.

Flash column chromatography was conducted on Fluka Silica Gel (40-63 μm, 60 Å), or basic aluminum oxide (activated basic Brockman 1 standard grade 150 mesh) or florisil (100-200 mesh). TLC was performed on aluminium backed plates pre-coated with Merck silica gel 60 F_{254}, visualised by UV radiation. Preparative TLC was performed on glass plates pre-coated with Merck silica gel 60 F_{254}, visualised by UV radiation. Phosphomolybdic acid, potassium permanganate, and vanillin were also used for visualisation of TLC plates.

IR spectra were recorded on solid compounds using a Perkin-Elmer Avatar 320 Fourier Transformation spectrometer. Only selected absorptions are reported, in units of wavenumbers (cm^{-1}), using the following abbreviations: w, weak; m, medium; s, strong; br, broad.

^{1}H NMR spectra were recorded at 300, 400 or 700 MHz using Bruker DPX300, DPX400 or AV700 spectrometers respectively. Chemical shifts (δ_H) are quoted in ppm with reference to the residual solvent peak. The data in parentheses follow the order (i) number of equivalent protons, (ii) multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet, (iii) coupling constant (*J*): in Hz to the nearest 0.5 Hz, (iv) assignment. COSY was used in selected cases to aid assignment.

^{13}C NMR spectra were recorded on Bruker DPX300, DPX400 and AV700 spectrometers at 75, 100 or 175 MHz. HMBC and HMQC spectra were recorded to aid assignments. Chemical shifts (δ_C) are quoted in ppm with reference to the residual solvent peak. The assignment is given in parentheses.

CHN analysis was performed by Warwick Analytical Services using an Exeter Analytical CE 440.

Low resolution EI and CI mass spectra were recorded using a Micromass Autospec spectrometer. Low resolution ESI mass spectra were recorded using a Bruker Esquire 2000 spectrometer. Only molecular ions and major fragments are reported with intensities quoted as percentages of the base peak. High resolution mass spectra were recorded on a Micromass Autospec spectrometer equipped with EI or CI source, or on a Bruker microTOF spectrometer equipped with an ESI source.

Melting points were determined (uncorrected) on a Stuart Scientific SMP10 machine to the nearest degree.

LC-MS was carried out on an Agilent 1100 HPLC instrument with the outflow connected *via* a splitter (10% to mass spectrometer, 90% to waste) to a Bruker Daltonics esquire HCT plus mass spectrometer equipped with an ESI source.

Bacteria were grown on solid medium in an incubator GenLAB 2216e or in liquid medium in a New Brunswich Scientific shaker, model Innova 4330, at 30°C.

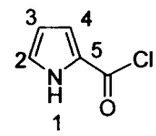
Microwave reactions were carried out in a CEM Discover microwave.

7.2: Experimental - Results and Discussion Chapter 4

General procedure for preparation of acid chlorides

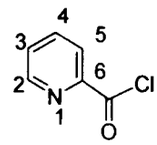
To a solution of oxalyl chloride (0.30 mL, 3.75 mmol) in dry DCM (50 mL) was added a catalytic amount of DMF. After the evolution of gas had finished, pyrrole-2-carboxylic acid, picolinic acid, or 2-fluorobenzoic acid (1.35 mmol) was added, and the mixture was heated to reflux for one hour under nitrogen. The solvent was removed *in vacuo*. The product was analysed by IR spectroscopy and ^{1}H NMR spectroscopy, and used in the preparation of the *N*-acetyl-cysteamine thioesters without further purification.

1-pyrrole-2-carbonyl chloride 212[73]



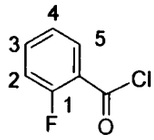
Yielded 0.14 g (1.05 mmol, 77%). δ_H (400 MHz, $CDCl_3$), 6.30 (1H, dd, *J* 6.0, 6.0, H-3), 7.10-7.15 (2H, m, H-2 and H-4), 9.20 (1H, br s, N*H*), hydrogen coupling confirmed by COSY. ν_{max}/cm^{-1} 3353 (N-H), 2900 (C-H), 1718 (C=O), 1658, 1531 (C=C-N), 754 (C-Cl).

Picolinoyl chloride 214



Yielded 0.13 g (0.94 mmol, 69%) δ_H (400 MHz, $CDCl_3$), 7.51 (1H, ddd, *J* 1.0, 4.5, 8.0, H-3), 7.86 (1H, ddd, *J* 1.5, 8.0, 8.0, H-4), 7.91 (1H, d, *J* 8.0, H-5), 8.64 (1H, d, *J* 4.5, H-2), ν_{max}/cm^{-1} 2900 (C-H), 1735 (C=O), 1655, 1613, 1590 (C=C and C=N aromatic), 793 (C-Cl).

2-fluorobenzoyl chloride 213

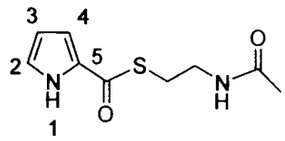


Yielded 0.16 g (1.00 mmol, 74%) δ_H (400 MHz, $CDCl_3$), 7.18 (1H, ddd, *J* 11.0, 8.0, 1.0, H-2), 7.23 (1H, ddd, *J* 7.5, 7.5, 1.0, H-4), 7.58 (1H, m, H-3), 7.84 (1H, ddd, *J* 7.5, 7.5, 1.0, H-5), ν_{max}/cm^{-1} 2900 (C-H), 1782 (C=O), 1670, 1605, 1576 (C=C aromatic), 879 (C-F), 733 (C-Cl).

General method for preparation of NAC thioester analogues

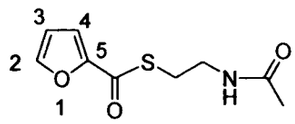
To a solution of acid chloride (pyrrole-2-carbonyl chloride **212**, furan-2-carbonyl chloride, thiophene-2-carbonyl chloride, 1-fluorobenzene-2-carbonyl chloride **213** or pyridine-2-carbonyl chloride **214**) (1.05 mmol) in DCE (40 mL) was added DMAP (0.30 g, 2.50 mmol), followed by *N*-acetylcysteamine (0.20 mL, 0.24 g, 2.50 mmol). The resulting mixture was heated to reflux overnight. The solvent was removed *in vacuo* and the crude residue was analysed by ^1H NMR spectroscopy. The product was purified using a short column of silica impregnated with $CuSO_4$ to give the desired product **117**, **118**, **119**, **120** or **121**.

Pyrrole-2-carboxyl-*N*-acetylcysteamie thioester 117[73]



The product (0.13 g, 0.63 mmol, 60%) was a yellow-white crystalline solid. δ_H (400 MHz, $CDCl_3$), 2.04 (3H, s, Me), 3.10 (2H, t, *J* 6.0, $SC\underline{H_2}CH_2$), 3.52 (2H, dt, *J* 6.0, 6.0, $SCH_2C\underline{H_2}$), 5.95 (1H, br s, N*H*), 6.29 (1H, dd, *J* 3.5, 3.5, H-3), 7.03 (2H, m, H-2 and H-4), 9.28 (1H, br s, N*H*(CO)), hydrogen couplings were confirmed by a COSY experiment. δ_C (100 MHz, $CDCl_3$), 23.3 (Me), 27.8 ($S\underline{C}H_2CH_2$), 40.1 ($SCH_2\underline{C}H_2$), 111.0 (C-3), 115.7 (C-4), 124.1 (C-2), 129.8 (C-5), 170.7 ($NH\underline{C}(O)CH_3$), 181.7 ($\underline{C}(O)SCH_2$). ***m/z (EI)*** 235 ($[M + Na]^+$, 35%), 185 (30), 149 (6), 123 (100). ν_{max}/cm^{-1} 3353 (N-H), 2931 (C-H), 1691 (C=O), 1536 (C=C aromatic). ***mp:*** 108-110°C. **HRMS** Calculated for $[C_9H_{12}N_2O_2SNa]^+$: 235.0513, observed: 235.0512.

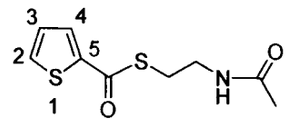
S-2-acetamidoethyl furan-2-carbothioate 118



The product (0.18 g, 0.79 mmol, 75%) was a white crystalline solid. δ_H (400 MHz, $CDCl_3$), 1.98 (3H, s, $C\underline{H_3}$), 3.21 (2H, t, *J* 6.0, $SC\underline{H_2}CH_2$), 3.53 (2H, dt, *J* 6.0, 6.0, $C\underline{H_2}NH$), 5.90 (1H, br s, NH), 6.56 (1H, dd *J* 3.5, 1.5, H-3), 7.22 (1H, dd, *J* 3.5, 1.0, H-4), 7.60 (1H, dd, *J* 1.5, 1.0, H-2), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 23.3 ($\underline{C}H_3$), 27.8 ($S\underline{C}H_2CH_2$), 39.9 ($CH_2\underline{C}H_2NH$) 112.4 (C-3), 116.2 (C-4), 146.6 (C-2), the signal C=O was not observed in the spectrum, signal assignment confirmed by HMQC. ν_{max}/cm^{-1} 3317 (N-H), 2933 (C-H), 1641 (C=O), 1550 (C=C aromatic), ***mp***: 91-92°C **CHN** Calculated: C, 50.69; H, 5.20; N,

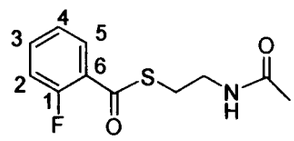
6.57; S, 15.04 found: C, 50.20; H, 5.19; N, 6.40; S,14.57. ***m/z (EI)*** 214 [M^+] (2%), 154 (44), 126 (19), 118 (35), 95 (100), 86 (23), 76 (19).

S-2-acetamidoethyl thiophene-2-carbothioate 119



The product (0.14 g, 0.61 mmol, 58%) was a white crystalline solid. δ_H (400 MHz, $CDCl_3$), 1.94 (3H, s, C*H*$_3$), 3.18 (2H, t, *J* 6.5, SC*H*$_2$CH$_2$), 3.53 (2H, td, *J* 6.5, 6.5, SCH$_2$C*H*$_2$), 6.40 (1H, br s, N*H*), 7.08 (1H, dd, *J* 5.5, 4.0, H-3), 7.60 (1H, dd, *J* 5.5, 1.0, H-4), 7.76 (1H, dd, *J* 4.0, 1.0, H-2), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 23.1 (*C*H$_3$), 28.8 (S*C*H$_2$CH$_2$), 39.8 (SCH$_2$*C*H$_2$), 128.1 (C-3), 131.6 (C-4), 133.2 (C-2), 141.6 (C-5), 170.6 (NH*C*=O), 184.1 (*C*(=O)S) Carbon assignments were confirmed by HMQC. ν_{max}/cm^{-1} 3301 (N-H), 2929 (C-H), 1643 (C=O), 1541, 1509 (C=C aromatic) ***m/z (EI)*** 229 [M^+] (1%), 170 (39), 144 (1), 128 (6), 118 (21), 111 (100), 86.0 (123), 83 (11), 76 (7), **CHN** Calculated: C, 50.69; H, 5.20; N, 6.57; S, 15.04; found: C, 50.20; H, 5.19; N, 6.40; S, 14.57.

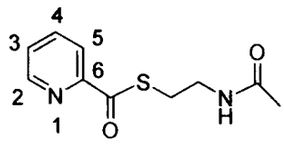
S-2-acetamidoethyl 2-(1-fluorobenzo)thioate 120



The product (0.18 g, 0.77 mmol, 73%) was a white crystalline solid. δ_H (400 MHz, $CDCl_3$), 1.98 (3H, s, C*H*$_3$), 3.23 (2H, t, *J* 6.5, SC*H*$_2$CH$_2$), 3.55 (2H, dt, *J* 6.5, 6.5 SCH$_2$C*H*$_2$), 5.88 (1H, br s, NH), 7.17 (1H, ddd, *J* 11.0, 8.5, 1.0, H-2), 7.23 (1H, ddd, *J* 7.5, 7.5, 1.5, H-4), 7.51-7.58 (1H, m, H-3), 7.86 (1H, ddd, *J* 7.5, 7.5, 1.5, H-5), hydrogen couplings were confirmed by a COSY experiment.; δ_C (100 MHz, $CDCl_3$),

23.1 ($\underline{C}H_3$), 28.9 ($S\underline{C}H_2CH_2$), 39.3 ($SCH_2\underline{C}H_2$), 116.9 (d, *J* 22, C-2), 124.3 (d, *J* 3.0, C-4), 125.2 (d, *J* 11.0, C-6), 129.7 (d, *J* 11.0, C-5), 134.6 (d, *J* 9.0, C-3), 160.3 (d, *J* 255, C-1), 170.5 ($NH\underline{C}OMe$), 190.0 ($\underline{C}(O)SCH_2CH_2$), assignments were confirmed by an HMQC experiment. ν_{max}/cm^{-1} 3299 (N-H), 2900 (C-H), 1639 (C=O), 1607, 1545 (aromatic C=C), 1101 (C-F). ***m/z (EI)*** 242 [M^+] (7%), 182 (21), 123 (100), 118 (37), 95 (44), 86 (14), 75 (21). **CHN** Calculated: C, 54.76; H, 5.01; N, 5.81; S, 13.29; F, 7.87, found: C, 54.22; H, 5.00; N, 5.70; S, 13.41; F, 7.65. ***mp*** 74-75°C.

S-2-acetamidoethyl pyridine-2-carbothioate 121



The product (0.16 g, 0.72 mmol, 69%) was a yellow oil that was not fully purified. δ_H (400 MHz, $CDCl_3$), 1.95 (3H, s, $C\underline{H_3}$), 3.16 (2H, t, *J* 6.5, $SC\underline{H_2}CH_2$), 3.48 (2H, dt, *J* 6.5, 6.5 $SCH_2C\underline{H_2}$), 6.30 (1H, br s, $N\underline{H}$), 7.50 (1H, ddd, *J* 7.5, 5.0, 1.5, H-3), 7.83 (1H, ddd, *J* 7.5, 7.5, 1.5, H-4), 7.91 (1H, d, *J* 7.5, H-5), 8.65 (1H, d, *J* 5.0, H-2), hydrogen couplings were confirmed by a COSY experiment.; ***m/z (EI)*** 224 [M^+] (1%), 165 (69), 137 (31), 118 (51), 106 (66), 86 (87), 78 (100).

Solid Media

R5

Chemical	Amount / litre
Sucrose	103.0 g
K_2SO_4	0.25 g
$MgCl_2.6H_2O$	10.12 g
Glucose	20.0 g
Difco Casaminoacids	0.10 g
Difco Yeast Extract	5.00 g
TES Buffer	5.73 g
Distilled H_2O	To 1000 ml

Table 4: R5 Medium[16]

100 mL of the above solution (Table 4) was poured into 250 mL Erlenmeyer flasks each containing 2.2 g Difco Bacto Agar. The flasks were closed and autoclaved. The medium was re-melted at the time of use in a microwave and to each flask was added a sterile solution of each of the following, (as shown in Table 5)

Chemical	Amount / 100 mL of R5 medium
KH_2PO_4 (0.5%)	1.0 mL
$CaCl_2.2H_2O$ (5M)	0.4 mL
L-proline (20%)	1.5 mL
NaOH (1N)	0.7 mL
Trace Element Solution: $ZnSO_4.7H_2O$ (0.1 g/L) $FeSO_4.7H_2O$ (0.1 g/L) $MnCl_2.4H_2O$ (0.1 g/L) $CaCl_2.6H_2O$ (0.1 g/L) NaCl (0.1 g/L)	0.2 mL

Table 5: Sterile solutions added to R5 media before use

SMM

Chemical	Amount / liter
Agar	20 g
Mannitol	20 g
Soya flour	20 g

Table 6: SFM Medium[16]

The mannitol and soy flour were dissolved in 1 litre of tap water. 100 mL portions of this solution were poured into 250 mL Erlenmeyer flasks, each containing 2.0 g of agar. The flasks were closed and autoclaved.

SMMS

SMMS medium consisted of Difco Casaminoacids 2.0 g/l, TES buffer 5.73 g/l and distilled water to balance, the pH was adjusted to 7.2 with NaOH. 200 mL portions of this solution were poured into 250 mL Erlenmeyer flasks, each containing 3.0 g of Difco Bacto agar. The flasks were closed and autoclaved. At the time of use the media was re-melted and the following sterile solutions were added:

Sterile Solution (Initial Concentration)	Amount / litre
NaH_2PO_4 (50 mM) + K_2HPO_4 (50 mM)	20 mL
$MgSO_4$ (1M)	10 mL
Glucose (50% w/v)	36 mL
Trace Element Solution As for R5 medium (Table 5)	0.2 mL

Table 7: SMMS Medium[16]

Liquid Media

SMM

Sterile solution (initial Concentration)	Amount / 100 ml
$MgSO_4.7H_2O$ (24 g/L)	2.5 mL
TES Buffer (0.25 M, pH 6.2)	10 mL
NaH_2PO_4 (6 g/L) + K_2HPO_4 (6.8 g/L)	1.0 mL
Trace Element Solution (as for R5 medium Table 5)	0.1 mL
Casaminoacids (20% w/v)	1.0 mL
Water	81.9 mL
Glucose (sterile filtered and added separately) (20% w/v)	5.0 mL

Table 8: Supplemented Minimal Medium[16]

Growth of the *redM::aac(3)IV* mutant of *Streptomyces coelicolor* M511

10 µl of a spore stock of the mutant (supplied by Stanley) were used to inoculate 100 mL of SMM liquid medium and the resulting culture was incubated for 2 days at 30°C and 200 rpm. The culture was then divided into ten 10 mL aliquots. To each 10 mL culture, a different concentration of a NAC thioester was added. The compounds and amounts added are shown in Table 9.

Compound	1: Mass added /mg	2: Mass added /mg	3: Mass added /mg
Pyrrole-2-carboxyl-*N*-acetylcysteamie thioester, **118**	1	5	10
S-2-acetamidoethyl furan-2-carbothioate, **119**	1	5	10
S-2-acetamidoethyl pyridine-2-carbothioate, **122**	1	5	10
S-2-acetamidoethyl thiophene-2-carbothioate, **120**	1	5	10
S-2-acetamidoethyl-2-(1-fluorobenzo)thioate, **121**	1	5	10

Table 9: Mass of NAC thioester added

After 4 days the mycelia were pelleted by centrifugation, washed with water and extracted into 1.0 mL of 50:50 acetonitrile/methanol (acidified using 10 µl of 20%

HCl). The extract was centrifuged at 4000 g for 10 minutes, decanted, and passed through a 0.4 micron filter. 50 µl of the filtrate was analysed by HPLC.

LC-MS analysis of prodiginines in culture extracts

The method used for analysis of undecylprodiginine and streptorubin B in the culture extracts is detailed below (Table 10).

Time (mins)	H_2O (pH3 adjusted with HCl), %	Acetonitrile, %	Flow rate (mL/ min)
0	50	50	1.0
1	50	50	1.0
4	25	75	1.3
21	20	80	1.4
23	50	50	1.0

Table 10: HPLC method for prodiginine analysis

Column: 4.6x 150 mm Agilent Xorbax C8 column

Temperature: room temperature

Detection wavelength: 533 nm

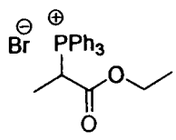
Total run time: 30 minutes

Retention time of undecylprodiginine: 5.5 minutes

Retention time of streptorubin B: 7 minutes

7.3: Experimental - Results and Discussion Chapter 5

(1-ethoxy-1-oxopropan-2-yl)triphenylphosphonium bromide 244[174]



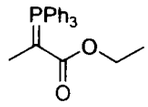
Ethyl acetate (60 mL) was dried with $MgSO_4$ and filtered into a round bottom flask to which triphenyl phospine (PPh_3) (10.48 g, 40.0 mmol) was added. After complete dissolution 2-bromopropionate ethyl ester **243** (5.20 mL, 7.24 g, 40.0 mmol) was added

and the mixture was heated to reflux for 16 hours. After this time, the product precipitated from the solution as a white solid which was removed by filtration. The precipitate was washed with ethyl acetate giving the desired product (15.61 g, 35.23 mmol, 88%) as a white crystalline solid. δ_H (400 MHz, $CDCl_3$), 0.98 (3H, t, *J* 7.0, C(O)OCH$_2$C$\underline{H}_3$), 1.67 (3H, dd, *J* 18.5, 7.5, C$\underline{H}_3$CH(PPh$_3$)(CO)), 3.96 (2H, m, C(O)OC$\underline{H}_2$CH$_3$), 6.85 (1H, dq, *J* 16.0, 7.5, CH$_3$C$\underline{H}$(PPh$_3$)(CO)), 7.66 (6H, m, aromatic), 7.76 (3H, m, aromatic), 7.97 (6H, m, aromatic), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 13.0 ($\underline{C}$H$_3$CH(CO)(PPh$_3$)), 13.7 (C(O)O$\underline{C}$H$_3$CH$_2$), 37.0 (d, *J* 50.5, CH$_3$$\underline{C}$H(CO)(PPh$_3$)), 62.9 (C(O)OCH$_3$$\underline{C}H_2$), 118.3 (d, *J* 86.5, aromatic), 130.2 (d, *J* 13.0, aromatic), 134.4 (d, *J* 10.0, aromatic), 134.9 (d, *J* 3.0, aromatic), 168.1 ($\underline{C}$=O), carbon signal assignments were confirmed by HMBC and HMQC experiments. **CHN** Calculated: C, 62.31; H, 5.46; Br, 18.02; Found: C, 62.31; H, 5.41; Br, 18.00; ν_{max}/cm^{-1} 1732 (C=O), 2981 (C-H), 1584, 1580, 1474 (C=C aromatic). ***m/z (ESI)*** 363.09 $[M+H]^+$, 335.06, 261.88, 182.78. **HRMS** Calculated for $[C_{23}H_{24}O_2P]^+$: 364.1605, observed: 364.1611. ***mp***: 128-130°C

Method 2

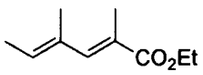
Triphenyl phosphine (22 g, 85 mmol) was dissolved in DCM. 2-bromopropionate ethyl ester **243** (5.4 ml, 42 mmol) was added. The reaction was stirred at room temperature for 72 hours. The solvent was removed in vacuo to give crude **244**, which was used without further purification.

(1-ethoxycarbonylethylidine)triphenylphosphorane 245 [174]



The ylide salt **244** (15.61 g, 35.2 mmol) was dissolved in DCM (100 mL) and washed with a solution of NaOH (2.82 g, 70.5 mmol dissolved in 70 mL of water). The organic phase changed from colourless to yellow. The organic layer was separated, dried with $MgSO_4$ and concentrated *in vacuo* to give the product (11.49 g, 31.70 mmol, 90%) as a yellow crystalline solid. δ_H (400 MHz, $CDCl_3$), 0.45 (3H, t, *J* 7.0, C(O)OCH$_2$C$\underline{H}_3$), 1.60 (3H, d, *J* 14.0, PCHC$\underline{H}_3$), 3.71 (2H, q, *J* 7.0, C(O)OC$\underline{H}_2$CH$_3$), 7.44 (6H, m, aromatic), 7.53 (3H, m, aromatic), 7.60 (6H, m, aromatic), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 13.1 (C(O)OCH$_2$$\underline{C}H_3$), 14.0 ($\underline{C}H_3$C=P(Ph$_3$)(CO)), 32.1 (CH$_3$$\underline{C}$=P(Ph$_3$)(CO)), 57.4 (C(O)O$\underline{C}H_2CH_3$), 128.5 (d, *J* 90.4, aromatic), 128.2 (d, *J* 12.0, aromatic), 131.6 (aromatic), 133.6 (d, *J* 10.0, aromatic), 170.6 ($\underline{C}$=O), carbon signal assignments were confirmed by HMQC experiments. **CHN** Calculated: C, 76.23; H, 6.40; Found: C, 75.79; H, 6.35; ν_{max}/cm^{-1} 1625 (CO), 2978 (CH), 1588, 1570, 1483 (aromatic). ***m/z (ESI)*** 363.07 $[M+H]^+$, 335.03, 261.87, 182.82. **HRMS** Calculated for $[C_{23}H_{23}O_2P]^+$: 363.1508, observed: 363.1530. ***mp***: 158-162°C literature value 159-160°C.[201,202]

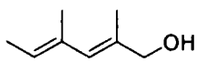
Ethyl-(2*E*,4*E*)-2,4-dimethylhexa-2,4-dienoate 247 [172]



To a solution of tiglic aldehyde **246** (9.95 g, 118.0 mmol) in dry toluene (120 ml) under argon was added ylide **245** (47.40 g, 131.0 mmol). The mixture was heated to reflux for 48 hours then allowed to cool to room temperature and concentrated under reduced pressure. Et_2O was added, and the resulting precipitated triphenylphosphine oxide was

removed by filtration. This was repeated until no precipitate was formed. The yellow filtrate was concentrated *in vacuo* and the residue purified by flash column chromatography (19:1 petroleum ether / diethyl ether), to give the product (15.87 g, 94.40 mmol, 80%) as a colourless oil. δ_H (400 MHz, $CDCl_3$), 1.28 (3H, t, *J* 7.0, C(O)OCH$_2$C$\underline{H}_3$), 1.73 (3H, d, *J* 7.0, C$\underline{H}_3$CH=C), 1.82 (3H, s, =C(C$\underline{H}_3$)CH=), 1.98 (3H, s, =C(C$\underline{H}_3$)CO), 4.17 (2H, q, *J* 7.0, C(O)OC$\underline{H}_2$CH$_3$), 5.69 (1H, q, *J* 7.0, CH$_3$C$\underline{H}$=C), 7.10 (1H, s, =C(CH$_3$)C$\underline{H}$=), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 14.4 (C$\underline{H}_3$CH=C) and (=C(C$\underline{H}_3$)CO), 14.6 (C(O)OCH$_2$C$\underline{H}_3$), 17.7 (=C(C$\underline{H}_3$)CH=), 60.5 (C(O)OC$\underline{H}_2$CH$_3$), 124.9 (=C$\underline{C}$(CH$_3$)CO), 130.8 (CH$_3$C$\underline{H}$=C), 133.1 (=C$\underline{C}$(CH$_3$)CH=), 142.9 (=C(CH$_3$)C$\underline{H}$=), 169.3 (C$\underline{C}$(O)OCH$_2$CH$_3$), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 2981 (C-H), 1702 (C=O), 1625 (C=C). ***m/z (ESI)*** 168.89 $[M+H]^+$, 140.88, 122.94, 95.15. **HRMS** Calculated for $[C_{10}H_{16}O_2Na]^+$: 191.1043, observed: 191.1044.

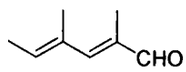
(2*E*,4*E*)- 2,4-dimethylhexa-2,4-dien-1-ol 248[172]



To a stirred solution of ester **247** (18.0 g, 107.0 mmol) in dry Et_2O (250 mL) under argon at 0°C, was added DIBAL-H (225 mL of a 1M solution in hexanes, 225.0 mmol) dropwise. After 1 hour, the reaction mixture was quenched by the cautious addition of MeOH (10 mL). The reaction mixture was diluted with ether (200 ml) and a saturated solution of sodium potassium tartrate tetrahydrate (300 mL) was added. The mixture was stirred vigorously for several hours until the organic and aqueous layers had completely separated. The organic layer was separated, washed with brine (200 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue

was purified by flash column chromatography (20:1 petroleum ether / diethyl ether) to give the product (11.47 g, 90.95 mmol, 85%) as a colourless oil. δ_H (400 MHz, $CDCl_3$), 1.65 (3H, d, *J* 7.0, C*H*$_3$CH=C), 1.72 (3H, s, =C(C*H*$_3$)CO), 1.77 (3H, s, =C(C*H*$_3$)CH=), 2.31 (1H, s, O*H*), 3.99 (2H, s, C*H*$_2$OH), 5.38 (1H, q, *J* 7.0, CH_3C*H*=C), 5.85 (1H, s, =C(CH_3)C*H*=), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 13.9 (*C*H_3CH=C), 15.5 (=C(*C*H_3)CH=), 16.7 (=C(*C*H_3)CO), 69.7 (*C*H_2OH), 124.8 (CH_3*C*H=C), 129.8 (=C(CH_3)*C*H=), 133.3, (=*C*(CH_3)CH=), 134.1 (=*C*(CH_3)CO), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 3334 (O-H), 2913, 2857, (C-H), 1651 (C=C). ***m/z (ESI)*** [M-H_2O]$^+$ 109.10, **HRMS** Calculated for $[C_8H_{14}O_1Na]^+$:149.0937 observed:149.0943.

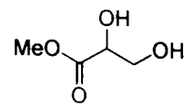
(2*E*,4*E*)-2,4-dimethylhexa-2,4-dienal 219 [172]



To a stirred solution of alcohol **248** (2.29 g, 18.1 mmol) in dry DCM (200 mL) was added MnO_2 (23.60 g, 270.0 mmol) at ambient temperature. After 72 hours the mixture was filtered through celite and concentrated *in vacuo* to give the product as a yellow oil, which was used without further purification (2.15 g, 17.36 mmol, 96%). δ_H (400 MHz, $CDCl_3$), 1.78 (3H, d, *J* 7.0, C*H*$_3$CH=C), 1.91 (3H, s, =C(C*H*$_3$)CO), 1.94 (3H, s, =C(C*H*$_3$)CH=), 5.95 (1H, q, *J* 7.0, CH_3C*H*=C), 6.69 (1H, s, =C(CH_3)C*H*=), 9.30 (1H, s, C*H*O), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 11.6 (=C(*C*H_3)CH=), 14.3 (*C*H_3CH=C), 15.5 (=C(*C*H_3)CO), 134.1 (CH_3CH=*C*(CH_3)), 135.0 (CH_3CH=C(CH_3)-CH=*C*(CH_3)-C(=O)H), 135.6 (CH_3*C*H=C), 155.0 (=C(CH_3)*C*H=), 196.1 (*C*=O), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 2920 (C-H), 1670 (C=O), 1620 (C=C). ***m/z***

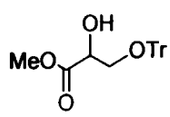
(ESI) 124.97 $[M\text{-}H_2O]^+$, 107.06, 79.36. **HRMS** Calculated for $[C_8H_{12}O_1Na]^+$: 147.0780, observed: 147.0787.

Methyl 2,3-dihydroxypropanoate 250[178]



To a solution of 2,2-dimethyl-1,3-dioxalone-4-carboxylate **249** (3.00 mL, 3.54 g, 20.0 mmol) in methanol (60 mL) was added HCl (1M, 27.0 mL, 27.0 mmol). The mixture was stirred at room temperature for 4.5 hours. The methanol was evaporated *in vacuo*, and the reaction mixture neutralised with saturated sodium bicarbonate. The product was extracted with 10% isopropanol in ethyl acetate successively (5 x 40 mL). The organic layer was dried with $MgSO_4$ and concentrated *in vacuo* to give the product (1.03 g, 8.60 mmol, 86%) as a colourless oil which was used without further purification. δ_H (400 MHz, D_2O), 3.75 (3H, s, $OC\underline{H}_3$), 3.76 (2H, dd, *J* 4.0, 1.5 $HOC\underline{H}_2CH(OH)$), 4.31 (1H, t, *J* 4.0, $HOCH_2C\underline{H}(OH)$), O-H signal not visible in D_2O, hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, D_2O), 52.7 ($O\underline{C}H_3$), 63.2 ($\underline{C}H_2$), 71.6 ($\underline{C}H$), 174.3 ($\underline{C}$=O). ν_{max}/cm^{-1} 3375 (O-H), 2956 (C-H), 1732 (C=O). ***m/z (ESI)*** 142.88 $[M+H]^+$. **HRMS** Calculated for $[C_4H_8O_4]^+$: 143.0315, observed: 143.0318.

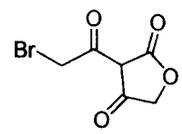
Methyl 2-hydroxy-3-(trityloxy)propanoate 236[178]



To a solution of ester **250** (1.39 g, 11.6 mmol), in dry DCM (60 mL) under argon at ambient temperature, was added triphenylchloromethane (4.20 g, 15.1 mmol), triethylamine (2.30 mL, 16.5 mmol), and *N,N*-dimethylaminopyridine (DMAP) (0.99 g,

0.81 mmol), and the mixture was stirred for 24 hours. The reaction mixture was poured onto ice-water (50 mL) and extracted into DCM (50 mL). The organic layer was washed with brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash column chromatography (DCM) to give the product (3.91 g, 10.78 mmol, 93%) as a colourless solid. δ_H (400 MHz, $CDCl_3$), 3.07 (1H, d, *J* 8.0, O*H*), 3.29 (1H, dd, *J* 9.5, 3.0, TrOC*H*$_2$CH(OH)CO), 3.40 (1H, dd, *J* 9.5, 3.0, TrOC*H*$_2$CH(OH)CO), 3.70 (1H, s, OCH_3), 4.20 (1H, dt, *J* 8.0, 3.0, $TrOCH_2CH(OH)CO$), 7.13-7.15 (15H, m, Ph), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 52.4 (OCH_3), 65.1 (CH_2), 70.6 (*C*H), 86.3 (CPh_3), 127.0 (Ph), 127.7 (Ph), 128.4 (Ph), 143.3 (Ph), 173.3 (*C*=O), carbon signal assignments were confirmed by HMQC experiments. **CHN:** Calculated: C, 76.22; H, 6.12; Found: C, 76.02; H, 6.08; ν_{max}/cm^{-1} 3537 (O-H), 2971, 2901 (C-H), 1732 (C=O). ***m/z (ESI)*** 385.1244 $[M+Na]^+$. **HRMS** Calculated for $[C_{23}H_{22}O_3Na]^+$: 385.1410, observed: 385.1410. ***mp***: 88-90°C literature value 88-90°C.[178]

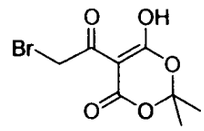
3-(2-bromoacetyl)furan-2,4(3H,5H)-dione 257[203]



To a solution of tetronic acid **256** (1.40 g, 14.0 mmol) in dry DCM (60 mL) under argon at 0°C was added dry pyridine (2.50 mL, 30.0 mmol), followed by bromoactyl bromide (1.50 mL, 15.0 mmol) dropwise over 10 minutes. The reaction was stirred at 0°C for 1 hour followed by stirring at ambient temperature for an additional 1 hour. After this time the reaction mixture was poured into 3M HCl (50 mL) in ice-water (70 g). The organic layer was separated, washed with water, dried with $MgSO_4$ and concentrated *in vacuo* to give the product (2.02 g, 9.24 mmol, 66%) as a dark red brown oil, which was

used without further purification. δ_H (400 MHz, $CDCl_3$), 4.02 (2H, s, C$\underline{H_2}$Br), 4.88 (2H, s, COC$\underline{H_2}$O), 6.04 (1H, s, C$\underline{H}$). δ_C (100 MHz, $CDCl_3$), 24.2 ($\underline{C}H_2Br$), 68.1 ($CO\underline{C}H_2O$), 101.9 ($\underline{C}H$), 162.5 ($\underline{C}O$), 168.3 ($\underline{C}O$), 171.7 ($\underline{C}O$), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 2956 (C-H), 1778, 1750, 1625 (C=O), 709 (C-Br). ***m/z (ESI)*** 242.82 [^{79}Br-M+Na]$^+$, 244.80 [^{81}Br-M+Na]$^+$. **HRMS** Calculated for [$C_6H_5O_4{}^{79}BrNa$]$^+$: 242.9263, observed: 242.9274, (1:1 isotope pattern of bromine).

5-(2-bromoacetyl)-2,2-dimethyl-1,3-dioxane-4,6-dione 229[179,203]



Synthetic procedure as for **257** using Meldrum's acid **226** (2.02 g, 14.0 mmol) in place of tetronic acid. The product was isolated as a brown oil (2.40 g, 9.10 mmol, 65%). δ_H (400 MHz, $CDCl_3$), 1.76 (6H, s, 2 x C$\underline{H_3}$), 4.67 (2H, s, BrC$\underline{H_2}$CO), O-H not visible on ^{1}H NMR spectrum; δ_C (100 MHz, $CDCl_3$), 26.0 ($\underline{C}H_2Br$), 27.0 (C($\underline{C}H_3$)$_2$), 91.9 (CO(CO)$\underline{C}$=CO), 105.7 ($\underline{C}(CH_3)_2$), 141.9 ($\underline{C}OH$), 165.0 ($\underline{C}$=O), 188.4 ($\underline{C}$=O), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 2996 (C-H), 1725, 1659 (C=O), 725 (C-Br). ***m/z (ESI)*** Molecular ion not visible **HRMS** Calculated for [$C_8H_9O_4{}^{81}BrNa$]$^+$: 286.9526 observed: 286.9521. (1:1 isotope pattern of bromine).

S-*tert*-butyl 4-bromo-3-oxobutanethioate 231[203]

To a solution of crude Meldrum's acid derivative **229** (7.92 g, 30.0 mmol) in dry DCE (120 mL) under argon was added *t*-butanethiol (3.20 mL, 30.0 mmol). The solution was

heated to reflux for 4 hours. After this time the solvent was removed *in vacuo*, to give a red brown oil which was purified by flash column chromatography (10:1 hexane / ethyl acetate). The product was isolated as a dark red oil (4.31 g, 17.10 mmol, 57% over 2 steps from 30.0 mmol of Meldrum's acid). *1:0.6 Keto / enol based on the ^1H NMR spectrum;* δ_H (400 MHz, $CDCl_3$), *Keto* 1.45 (9H, s, 3 x C$\underline{H}_3$), 3.81 (2H, s, C(O)C$\underline{H}_2$C(O)), 4.03 (2H, s, C$\underline{H}_2$Br). *Enol* 1.49 (9H, s, 3 x C$\underline{H}_3$), 3.76 (2H, s, C$\underline{H}_2$Br), 5.56 (1H, s, C$\underline{H}$), 12.67 (1H, s, O$\underline{H}$), hydrogen couplings were confirmed by a COSY experiment. δ_C (100 MHz, $CDCl_3$), *Keto* 30.0 (3 x $\underline{C}H_3$), 34.7 ($\underline{C}H_2Br$), 49.9 (S$\underline{C}$(Me)$_3$), 55.4 (C(O)$\underline{C}H_2$C(O)), 192.5 ($\underline{C}$=O), 194.3 ($\underline{C}$=O), *Enol* 30.4 (3 x $\underline{C}H_3$), 29.1 ($\underline{C}H_2Br$), 49.3 (S$\underline{C}$(Me)$_3$), 101.5 (C(O)$\underline{C}$HC(OH)), 167.8 ($\underline{C}$OH), 197.2 ($\underline{C}$=O), carbon signal assignments were confirmed by HMQC experiments. ν_{max}/cm^{-1} 2962, 2923 (C-H), 1724, 1670 (C=O), 1620 (C=C conj with C=O), 3072 (O-H), 779 (C-Br). **HRMS** Calculated for $[C_8H_{13}{}^{81}BrO_2SNa]^+$: 276.9697 observed: 276.9684 (1:1 isotope pattern of bromine).

S-*tert*-butyl 4-(diethoxyphosphoryl)-3-oxobutanethioate 224[169]

To a suspension of sodium hydride (NaH) (0.30 g, 12.65 mmol) in dry THF (75 mL), under argon, at -10°C was added thioester **231** (2.91 g, 11.50 mmol) in dry THF (10 mL) dropwise. Meanwhile to a suspension of NaH (0.33 g, 13.96 mmol) in dry THF (30 mL) under argon at -10°C, was added diethyl phosphate (1.63 mL, 12.65 mmol) dropwise. The solutions were stirred for 1 hour before the solution of sodium diethylphosphite was added dropwise *via* cannula to the solution of **231**. The reaction was stirred at 0°C for 1 hour before being allowed to warm to ambient temperature over 24 hours by removing the ice bath. The mixture was poured into a mixture of saturated ammonium chloride (200 mL), and ether (300 mL). The organic layer was separated

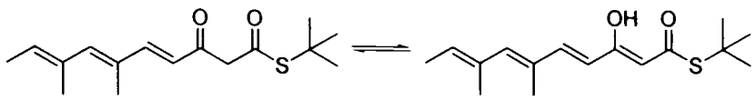
and the aqueous layer re-extracted with ether (2 x 50 mL). The combined organic layers were washed with water (2 x 100 mL), brine (100 mL), dried with $MgSO_4$. and concentrated to give a crude oil which was purified by flash column chromatography (1:1 hexane / ethyl acetate) to give the desired product (3.33 g, 10.75 mmol, **85%**) as an orange oil. *Keto / enol 3:1 based on the ^1H NMR spectrum* δ_H (400 MHz, $CDCl_3$), 1.32 (6H, m, $P(OCH_2C\underline{H}_3)_2$), 1.44 (7H, s, 3 x $C\underline{H}_3$), 1.48 (2H, s, 3 x $C\underline{H}_3$), 2.69 (0.4H, d, *J* 23.0, $PC\underline{H}_2CO$), 3.22 (1.6H, d, *J* 23.0, $PC\underline{H}_2CO$), 3.77 (1.6H, s, $PCH_2COC\underline{H}_2CO$), 4.13 (4H, m, $P(OC\underline{H}_2CH_3)_2$), 5.45 (0.4H, d, *J* 3.0, $PCH_2COC\underline{H}COH$), 12.90 (0.4H, br s, $PCH_2COCHCO\underline{H}$), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 16.5 (d, *J* 6.5, $POCH_2\underline{C}H_3$), 16.6 (d, *J* 6.5, $POCH_2\underline{C}H_3$), 29.8 (3 x $\underline{C}H_3$), 30.3 (3 x $\underline{C}H_3$), 33.8 (d, *J* 136.0, $P\underline{C}H_2CO$) 42.8 (d, *J* 126.0, $P\underline{C}H_2CO$), 48.7 ($S\underline{C}(Me)_3$), 49.4 ($S\underline{C}(Me)_3$), 58.7 ($-CH_2C(=O)\underline{C}H_2-C(=O)-S-$), 62.8 (d, *J* 6.5, $PO\underline{C}H_2CH_3$), 62.9 (d, *J* 6.5, $PO\underline{C}H_2CH_3$), 102.3 (d, *J* 8.0, $PCH_2CO\underline{C}HCOH$), 166.4 ($\underline{C}OH$), 192.5 (d, *J* 6.5, $\underline{C}=O$), 194.3 (d, *J* 6.5, $\underline{C}=O$), 196.5 ($\underline{C}=O$), carbon signal assignments were confirmed by HMQC experiments. ν_{max}/cm^{-1} 2962, 2923 (C-H), 1725, 1675 (C=O), 1257 (P=O). ***m/z (ESI)*** 333.02 $[M+Na]^+$, 242.83, 214.76, 168.72, 126.88 **HRMS** Calculated for $[C_{12}H_{23}O_5S_1P_1]^+$: 311.1077 observed: 311.1085.

General method for synthesis of β-keto thioesters[170]

To a suspension of NaH (0.10 g, 4.00 mmol) in dry THF (40 mL) under argon at 0°C, was added phosphonate **224** (0.50 g, 1.61 mmol) in dry THF (5 mL) dropwise. The mixture was stirred for 1 hour at 0°C then the corresponding aldehyde (1.30 mmol) in dry THF (2 mL) was added dropwise. The mixture was stirred for 1 hour at 0°C then allowed to warm to ambient temperature overnight. The mixture was poured into saturated ammonium chloride (40 mL) and ether (80 mL). The organic layer was

separated and the aqueous layer re-extracted with ether (2 x 50 mL). The combined organic layers were washed with water (2 x 50 mL) and brine (50 mL), dried with $MgSO_4$, and concentrated *in vacuo* to give the crude product which was purified by flash column chromatography.

S-*tert*-butyl-(4*E*,6*E*,8*E*)-6,8-dimethyl-3-oxodeca-4,6,8-trienethioate 222



The crude residue was purified by flash column chromatography (25:1 petroleum ether / diethyl ether) to give the product as a brown solid (0.23 g, 0.83 mmol, 64%). *1:1 Keto / enol based on ^1H NMR spectrum* δ_H (400 MHz, $CDCl_3$), 1.40 (4.5H, s, $SC(C\underline{H}_3)_3$), 1.53 (4.5H, s, $SC(C\underline{H}_3)_3$), 1.75 (3H, d, *J* 7.0, $C\underline{H}_3CH=C(CH_3)$), 1.83 (3H, s, $CH_3CH=C(C\underline{H}_3)CH=$), 1.94 (3H, s, $CH_3CH=C(CH_3)CH=C(C\underline{H}_3)$), 3.69 (1H, s, $COC\underline{H}_2C(O)$), 5.41 (0.5H, s, $C(OH)C\underline{H}C(=O)$), 5.62 (0.5H, q, *J* 7.0, $CH_3C\underline{H}=C(CH_3)$), 5.68 (0.5H, q, *J* 7.0, $CH_3C\underline{H}=C(CH_3)$), 5.74, (0.5H, d, *J* 15.5, $CH_3CH=C(CH_3)CH=C(CH_3)$-CH=C$\underline{H}$-C(OH)), 6.22 (0.5H, d, *J* 15.5, $CH_3CH=C(CH_3)CH=C(CH_3)$-CH=C$\underline{H}$-C(OH)), 6.23 (0.5H, s, $CH_3CH=C(CH_3)C\underline{H}=C(CH_3)$), 6.32 (0.5H, s, $CH_3CH=C(CH_3)C\underline{H}=C(CH_3)$), 7.17 (0.5H, d, *J* 15.5, $CH_3CH=C(CH_3)CH=C(CH_3)$-C$\underline{H}$=CH-C(OH)), 7.32 (0.5H, d, *J* 15.5, $CH_3CH=C(CH_3)CH=C(CH_3)$-C$\underline{H}$=CH-C(=O)), 12.60 (0.5H, s, C(O$\underline{H}$)CHC=O), hydrogen coupling was confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 13.7 ($CH_3CH=C(CH_3)CH=C(\underline{C}H_3)$), 13.8 ($CH_3CH=C(CH_3)CH=C(\underline{C}H_3)$), 14.0 ($\underline{C}H_3CH=C(CH_3)$), 14.2 ($\underline{C}H_3CH=C(CH_3)$), 16.2 ($CH_3CH=C(\underline{C}H_3)CH=$), 16.4 ($CH_3CH=C(\underline{C}H_3)CH=$), 29.6 ($SC(\underline{C}H_3)_3$), 30.20 ($SC(\underline{C}H_3)_3$), 48.2 ($S\underline{C}(CH_3)_3$), 48.89 ($S\underline{C}(CH_3)_3$), 56.7 (-CO-$\underline{C}H_2$-CO-$SC(CH_3)_3$), 101.4 (-COH-$\underline{C}$H-CO-$SC(CH_3)_3$), 119.5 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=$\underline{C}$H-COH-CH-CO-$SC(CH_3)_3$), 123.6

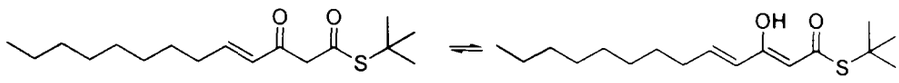
($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=$\underline{C}$H-CO-CH_2-CO-$SC(CH_3)_3$), 129.1 ($CH_3\underline{C}H=C(CH_3)$), 131.0 ($CH_3\underline{C}H=C(CH_3)$), 131.1 ($CH_3CH=C(CH_3)CH=\underline{C}(CH_3)$-CH=CH-COH), 131.4 ($CH_3CH=C(CH_3)CH=\underline{C}(CH_3)$-CH=CH-COH), 133.8 ($CH_3CH=\underline{C}(CH_3)CH=C(CH_3)$-CH=CH-COH), 142.8 ($CH_3CH=C(CH_3)\underline{C}H=C(CH_3)$-CH=CH-C(OH)-CH-CO-$SC(CH_3)_3$), 144.5 ($CH_3CH=C(CH_3)CH=C(CH_3)$-$\underline{C}$H=CH-CO-$CH_2$-CO-$SC(CH_3)_3$), 146.6 ($CH_3CH=C(CH_3)\underline{C}H=C(CH_3)$-CH=CH-COH-CH-CO-$SC(CH_3)_3$), 152.0 ($CH_3CH=C(CH_3)CH=C(CH_3)$-$\underline{C}$H=CH-CO-$CH_2$-CO-$SC(CH_3)_3$), 167.5 (-CH=CH-$\underline{C}$OH-CH-CO-$SC(CH_3)_3$), 191.6 (-CH=CH-$\underline{C}$O-$CH_2$-CO-$SC(CH_3)_3$), 193.0 (-CH=CH-CO-$CH_2$-$\underline{C}$O-$SC(CH_3)_3$), 195.9 (-CH=CH-COH-CH-$\underline{C}$O-$SC(CH_3)_3$), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/ cm^{-1} 2960, 2914 (C-H), 1775, 1749 (C=O), 1623 (C=C conj with C=O). ***m/z (ESI)*** 280.96 $[M+H]^+$, 262.94, 206.87, 164.87, 144.91 **HRMS** Calculated $[C_{16}H_{24}O_2S_1Na]^+$: 303.1389 observed: 303.1398 ***mp:*** 35-37°C.

S-*tert*-butyl-(*E*)-3-oxo-5-phenylpent-4-enethioate 251[170]

The crude residue was purified by flash column chromatography (10:1 petroleum ether / diethyl ether), to give the product as a yellow solid (0.28 g, 1.08 mmol, 83%). *8:1 enol / keto based on the ^1H NMR spectrum,* δ_H (400 MHz, $CDCl_3$), 1.39 (1H, s, $C(C\underline{H}_3)_3$), 1.45 (8H, s, $C(C\underline{H}_3)_3$), 3.71 (0.2H, s $COC\underline{H}_2CO$), 5.39 (0.8H, s, C(OH)C$\underline{H}$C(=O)), 6.21 (0.9H, dd, *J* 15.5, 1.0, PhHC=C$\underline{H}$-COH), 6.72 (0.1H, d, *J* 15.5, PhHC=C$\underline{H}$-CO), 7.21-7.38 (5.9H, m, Ph and Ph$\underline{H}$C=CH-COH), 7.52 (0.1H, d, *J* 15.5, Ph$\underline{H}$C=CH-CO), 12.60 (0.9H, d, *J* 1.0, C(O$\underline{H}$)-CH-C=O), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 29.7 ($C(\underline{C}H_3)_3$), 30.2 ($C(\underline{C}H_3)_3$), 48.8 ($\underline{C}(CH_3)_3$),

49.4 (<u>C</u>(CH$_3$)$_3$), 57.2 (CO<u>C</u>H$_2$CO), 102.1 (C(OH)<u>C</u>HC(=O), 121.7 (PhHC=<u>C</u>H-COH), 125.3 (PhHC=<u>C</u>H-CO), 127.9 (Ph), 128.9 (Ph), 129.14 (Ph), 129.3 (Ph), 129.8 (Ph), 131.2 (Ph), 134.5 (Ph), 135.7 (Ph), 138.0 (PhH<u>C</u>=CH-COH), 145.2 (PhH<u>C</u>=CH-CO), 166.7 (<u>C</u>OH), 191.8 (<u>C</u>OCH$_2$CO), 192.9 (COCH$_2$<u>C</u>O), 196.6 (C(OH)CH<u>C</u>(=O)), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 2959, 2920 (C-H), 1637 (C=O) ***m/z (ESI)*** 262.90 $[M+H]^+$, 206.80, 190.82, 172.80, 130.99 **HRMS** Calculated for $[C_{15}H_{18}SO_2Na]^+$: 285.0920 observed: 285.0923, ***mp*** 62-64°C.

S-*tert*-butyl-(*E*)-3-oxododec-4-enethioate 252[170]



The crude residue was purified by flash column chromatography (19:1 petroleum ether / diethyl ether), to give the product as a bright red oil (0.26 g, 0.7 mmol, 70%) *1:1 Keto / enol based on ^1H NMR spectrum* δ_H (400 MHz, $CDCl_3$), 0.85 (3H, t, *J* 7.0, <u>C</u>H$_3$(CH$_2$)$_4$CH$_2$CH$_2$CH=CH), 1.25 (8H, m, CH$_3$(C<u>H$_2$</u>)$_4$CH$_2$CH$_2$CH=CH), 1.42 (2H, m, CH$_3$(CH$_2$)$_4$C<u>H$_2$</u>CH$_2$CH=CH), 1.44 (4.5H, s, SC(C<u>H$_3$</u>)$_3$), 1.48 (4.5H, s, SC(C<u>H$_3$</u>)$_3$), 2.20 (2H, m, CH$_3$(CH$_2$)$_4$CH$_2$C<u>H$_2$</u>CH=CH), 3.67 (1H, s, -(CO)C<u>H$_2$</u>(CO)-), 5.27 (0.5H, s, -(COH)C<u>H</u>(CO)-), 5.64 (0.5H, dd, *J* 15.0, 1.0, CH$_3$(CH$_2$)$_4$CH$_2$CH$_2$CH=C<u>H</u>-(COH)CH(CO)), 6.12 (0.5H, dd, *J* 15.0 1.0, CH$_3$(CH$_2$)$_4$CH$_2$CH$_2$CH=C<u>H</u>-(COH)CH(CO)), 6.67 (0.5H, dt, *J* 15.0, 7.0, CH$_3$(CH$_2$)$_4$CH$_2$CH$_2$C<u>H</u>=CH-(COH)CH(CO)), 6.88 (0.5H, dt, *J* 15.0, 7.0, CH$_3$(CH$_2$)$_4$CH$_2$CH$_2$C<u>H</u>=CH-(CO)CH$_2$(CO)), 12.58 (0.5H, d, *J* 1.0, -(CO<u>H</u>)CH(CO)-), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 12.5 (<u>C</u>H$_3$), 22.6 (<u>C</u>H$_2$), 22.2 (<u>C</u>H$_2$), 27.9 (<u>C</u>H$_2$), 28.5 (<u>C</u>H$_2$,), 29.0 (<u>C</u>H$_2$), 29.1 (<u>C</u>H$_2$), 29.1 (<u>C</u>H$_2$), 29.6 (SC(<u>C</u>H$_3$)$_3$), 31.1 (SC(<u>C</u>H$_3$)$_3$), 31.7 (<u>C</u>H$_2$), 31.8 (<u>C</u>H$_2$), 32.6 (<u>C</u>H$_2$), 32.7 (<u>C</u>H$_2$), 48.2 (S<u>C</u>(CH$_3$)$_3$), 48.9 (S<u>C</u>(CH$_3$)$_3$), 56.1 (-(CO)<u>C</u>H$_2$(CO)-), 100.2 (-(COH)<u>C</u>H(CO)-), 124.1

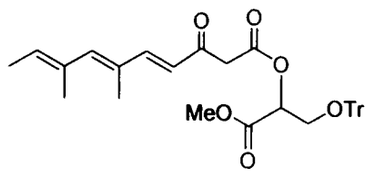
($CH_3(CH_2)_4CH_2CH_2CH$=$\underline{C}$H-(CO)CH_2(CO)), 129.6 ($CH_3(CH_2)_4CH_2CH_2CH$=$\underline{C}$H-(COH)CH(CO)), 142.7 ($CH_3CH_2(CH_2)_4CH_2\underline{C}$H=CH-(CO)$CH_2$(CO)), 150.7 ($CH_3(CH_2)_4CH_2CH_2\underline{C}$H=CH-(COH)$CH_2$(CO)), 166.8 ($CH_3(CH_2)_4CH_2CH_2CH$=CH-($\underline{C}$OH)CH(CO)), 191.8 (-($\underline{C}$O)$CH_2$(CO)-), 192.7 (-(CO)$CH_2$($\underline{C}$O)-), 196.3 (-(COH)CH($\underline{C}$O)-), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 2957, 2924, 2855 (C-H), 1655 (C=O), 1581 (C=C) ***m/z (ESI)*** 285.00 $[M+H]^+$, 228.89, 152.94, 134.98, 107.11, 93.27, 79.39 **HRMS** Calculated for $[C_{16}H_{28}NaO_2S]^+$: 307.1702 observed: 307.1707.

S-*tert*-butyl-(*E*)-5-cyclohexyl-3-oxopent-4-enethioate 253[170]

The crude residue was purified by flash column chromatography (10:1 petroleum ether / diethyl ether) to give the product as a pink oil (0.34 g, 1.27 mmol, 98%). *1:1 Keto / enol based on ^1H NMR spectrum* δ_H (400 MHz, $CDCl_3$), 1.0-1.3 (6H, m, 6 x cyclohexyl protons), 1.40 (4.5H, s, SC($C\underline{H}_3$)$_3$), 1.45 (4.5H, s, SC($C\underline{H}_3$)$_3$), 1.5-1.75 (4H, m, cyclohexyl protons), 2.0-2.2 (1H, m, CH cyclohexyl proton), 3.69 (1H, s, -CH=CH-(C=O)$C\underline{H}_2$(C=O)SC(CH_3)$_3$), 5.31 (0.5H, s, -CH=CH-(C=O)C$\underline{H}$(COH)SC(CH_3)$_3$), 5.62 (0.5H, d, *J* 15.5, -CH=C$\underline{H}$-(C=O)CH_2(C=O)SC(CH_3)$_3$), 6.10 (0.5H, d, *J* 15.5, -CH=C$\underline{H}$-(C=O)CH(COH)SC(CH_3)$_3$), 6.64 (0.5H, dd, *J* 15.5, 7.0, -C$\underline{H}$=CH-(C=O)CH_2(C=O)SC(CH_3)$_3$), 6.81 (0.5H, dd, *J* 15.5, 7.0, -C$\underline{H}$=CH-(C=O)CH(COH)SC(CH_3)$_3$), 12.62 (0.5H, s, -CH=CH-(C=O)CH(CO$\underline{H}$)SC(CH_3)$_3$), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 25.6 (Cy), 25.8 (Cy), 29.6 (SC($\underline{C}H_3$)$_3$), 30.2 (SC($\underline{C}H_3$)$_3$), 31.6 (Cy), 32.1 (Cy), 40.8 (Cy), 40.9 (Cy), 48.3 (S$\underline{C}$(CH_3)$_3$), 48.9 (S$\underline{C}$(CH_3)$_3$), 56.12 (-CH=CH-

(C=O)$\underline{C}$H$_2$(C=O)SC(CH$_3$)$_3$), 100.4 (-CH=CH-(C=O)$\underline{C}$H(COH)SC(CH$_3$)$_3$), 121.8 (-CH=$\underline{C}$H-(C=O)CH$_2$(C=O)SC(CH$_3$)$_3$), 127.1 (-CH=$\underline{C}$H-(C=O)CH(COH)SC(CH$_3$)$_3$), 147.7 (-$\underline{C}$H=CH-(C=O)CH$_2$(C=O)SC(CH$_3$)$_3$), 155.3 (-$\underline{C}$H=CH-(C=O)CH(COH)SC(CH$_3$)$_3$), 167.1 (-CH=CH-(C=O)CH($\underline{C}$OH)SC(CH$_3$)$_3$), 192.2 ($\underline{C}$=O), 192.8 ($\underline{C}$=O), 196.3 ($\underline{C}$=O), carbon signal assignments were confirmed by HMQC experiments. ***ν_{max}/cm^{-1}*** 2923, 2851 (C-H), 1650 (C=O), ***m/z (ESI)*** 268.94 [M+H]$^+$, 250.92, 232.86, 194.85, 152.88, 134.98, 119.00 **HRMS** Calculated for [C$_{15}$H$_{24}$O$_2$SNa]$^+$: 291.1389 observed: 291.1394 **CHN:** Calculated: C, 67.12; H, 9.01; S, 11.95; Found: C 67.22; H, 9.15; S, 11. 80.

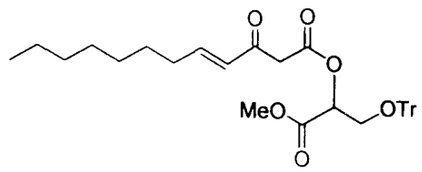
1-methoxy-1-oxo-3-(trityloxy)propan-2-yl-(4*E*,6*E*,8*E*)-6,8-dimethyl-3-oxodeca-4,6,8-trienoate 237[170]



To a solution of thioester **222** (0.51 g, 1.78 mmol), and ester **236** (0.52 g, 1.42 mmol) in dry THF (14 mL) under argon, was added silver trifluoroacetate (0.47 g, 2.20 mmol). The reaction mixture was stirred at ambient temperature for 16 hours whilst shielded from light. The mixture was diluted with ether and passed through a plug of silica and concentrated *in vacuo*. The residue was purified by flash column chromatography (20:1 petroleum ether / diethyl ether) to give the product as an off white oil (0.23 g, 038 mmol, 27%). *Enol / Keto 3:2 based on the ^{1}H NMR spectrum,* δ_H (400 MHz, CDCl$_3$), 1.68 (3H, d, *J* 7.0, C$\underline{H}_3$CH=C(CH$_3$)), 1.76 (3H, s, CH$_3$CH=C(C$\underline{H}_3$)CH=), 1.87 (3H, m, CH$_3$CH=C(CH$_3$)CH=C(C$\underline{H}_3$)), 3.41 (2H, m, CH$_3$O(C=O)-CH(-O-)-C$\underline{H}_2$OTr), 3.64 (3H, s, C$\underline{H}_3$O(C=O)-CH(-O-)-CH$_2$OTr), 3.69 (1.2H, s, -(CO)C$\underline{H}_2$(CO)-), 5.18 (0.6H, m, CH$_3$O(C=O)-C$\underline{H}$(-O-)-CH$_2$OTr), 5.20 (0.4H, s, -(COH)C$\underline{H}$(CO)-) 5.23 (0.4H, m,

$CH_3O(C{=}O)$-C<u>*H*</u>(-O-)-CH_2OTr), 5.55 (1H, m, CH_3C<u>*H*</u>$=C(CH_3)$), 5.83 (0.4H, d, *J* 15.5. $CH_3CH{=}C(CH_3)CH{=}C(CH_3)$-$CH{=}C$<u>*H*</u>-C(OH)), 6.17 (0.4H, s. $CH_3CH{=}C(CH_3)C$<u>*H*</u>$=C(CH_3)$-$CH{=}CH$-C(OH)), 6.28 (0.6H, s. $CH_3CH{=}C(CH_3)C$<u>*H*</u>$=C(CH_3)$-$CH{=}CH$-C(OH)), 7.10-7.40 (16H, m, (Ph), $CH_3CH{=}C(CH_3)CH{=}C(CH_3)$-C<u>*H*</u>$=CH$-C(OH), and 2 x $CH_3CH{=}C(CH_3)CH{=}C(CH_3)$-C<u>*H*</u>$=CH$-C(OH)), 11.55 (0.4H, s, CO<u>*H*</u>), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 13.8 (<u>*C*</u>$H_3CH{=}C(CH_3)$, 13.8 (<u>*C*</u>$H_3CH{=}C(CH_3)$, 14.1 ($CH_3CH{=}C(CH_3)CH{=}C($<u>*C*</u>$H_3)$), 14.1 ($CH_3CH{=}C(CH_3)CH{=}C($<u>*C*</u>$H_3)$), 16.2 ($CH_3CH{=}C($<u>*C*</u>$H_3)CH{=}$), 16.3 ($CH_3CH{=}C($<u>*C*</u>$H_3)CH{=}$), 47.1 (-(CO)<u>*C*</u>H_2(CO)-), 52.4 (O<u>*C*</u>H_3), 62.8 ($CH_3O(C{=}O)$-CH(-O-)-<u>*C*</u>H_2OTr), 63.3 ($CH_3O(C{=}O)$-CH(-O-)-<u>*C*</u>H_2OTr), 71.6 ($CH_3O(C{=}O)$-<u>*C*</u>H(-O-)-CH_2OTr), 72.7 ($CH_3O(C{=}O)$-<u>*C*</u>H(-O-)-CH_2OTr), 86.7 (<u>*C*</u>Ph_3), 89.9 (-(COH)<u>*C*</u>H(CO)-), 119.6 ($CH_3CH{=}C(CH_3)CH{=}C(CH_3)$-$CH{=}$<u>*C*</u>H-C(OH)), 123.6 ($CH_3CH{=}C(CH_3)CH{=}C(CH_3)$-$CH{=}$<u>*C*</u>H-CO), 127.2 (Ph), 127.0 (Ph), 128.6 (Ph), 129.1 (CH_3<u>*C*</u>$H{=}C(CH_3)CH{=}C(CH_3)$-$CH{=}CH$-C(OH)), 131.0 (CH_3<u>*C*</u>$H{=}C(CH_3)CH{=}C(CH_3)$-$CH{=}CH$-CO), 131.1 ($CH_3CH{=}C(CH_3)CH{=}$<u>*C*</u>(CH_3)-$CH{=}CH$-CO), 131.4 ($CH_3CH{=}C(CH_3)CH{=}$<u>*C*</u>(CH_3)-$CH{=}CH$-CO), 133.8 ($CH_3CH{=}$<u>*C*</u>$(CH_3)CH{=}C(CH_3)$-$CH{=}CH$), 133.8 ($CH_3CH{=}$<u>*C*</u>$(CH_3)CH{=}C(CH_3)$-$CH{=}CH$-CO), 142.7 ($CH_3CH{=}C(CH_3)$<u>*C*</u>$H{=}C(CH_3)$-$CH{=}CH$-C(OH)), 144.8 ($CH_3CH{=}C(CH_3)CH{=}C(CH_3)$-C<u>*H*</u>$=CH$-C(OH)), 146.3 ($CH_3CH{=}C(CH_3)$<u>*C*</u>$H{=}C(CH_3)$-$CH{=}CH$-CO), 151.5 ($CH_3CH{=}C(CH_3)CH{=}C(CH_3)$-<u>*C*</u>$H{=}CH$-CO), 167.1 (<u>*C*</u>OH), 168.2 (<u>*C*</u>$=$O), 168.8 (<u>*C*</u>$=$O), 171.3 (<u>*C*</u>$=$O), 171.9 (<u>*C*</u>$=$O), 191.3 (<u>*C*</u>$=$O), carbon signal assignments were confirmed by HMQC and HMBC experiments. ν_{max}/cm^{-1} 2915 (C-H), 1777, 1755 (C=O), 1667 (C=C). ***m/z (ESI)*** 575.23 $[M+Na]^+$, 331.07, 298.96, 243.95 212.89, 148.95. **HRMS** Calculated for $[C_{35}H_{36}O_6Na]^+$: 575.2404, observed: 575.2428.

1-methoxy-1-oxo-3-(trityloxy)propan-2-yl-(4*E*)-3-oxododec-4-enoate 254[170]

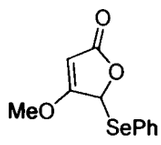


Synthesised using the same method as for **237** using thioester **252** instead of **222**. The product was purified by flash column chromatography (16:1 hexane / ethyl acetate), to give the product (0.12 g, 0.22 mmol, 16%) as a white oil. *11:5 keto / enol based on the ^1H NMR spectrum.* δ_H (400 MHz, $CDCl_3$), 0.80 (3H, t, *J* 7.0, C*H*$_3$), 1.11-1.30 (8H, m, $CH_3(C\underline{H}_2)_4CH_2CH_2CH{=}CH$), 1.35 (2H, m, $CH_3(CH_2)_4C\underline{H}_2CH_2CH{=}CH$), 2.15 (2H, m, $CH_3(CH_2)_4CH_2C\underline{H}_2CH{=}CH$), 3.40 (2H, m, $CH_3O(C{=}O)$-CH(-O-)-C*H*$_2$OTr), 3.64 (3H, s, C*H*$_3$O(C=O)-), 3.66 (0.7H, s, -(CO)C*H*$_2$(CO)-), 5.11 (0.3H, s, -(COH)C*H*(CO)-), 5.18 (0.7H, t, *J* 3.5, $CH_3O(C{=}O)$-C*H*(-O-)-CH_2OTr), 5.23 (0.3H, t, *J* 3.5, $CH_3O(C{=}O)$-C*H*(-O-)-CH_2OTr), 5.77 (0.3H, dd, *J* 15.0, 1.0, $CH_3(CH_2)_4CH_2CH_2CH{=}C\underline{H}$-(COH)CH(CO)), 6.15 (0.7H, d, *J* 15.0, $CH_3(CH_2)_4CH_2CH_2CH{=}C\underline{H}$-(CO)$CH_2$(CO)), 6.63 (0.3H, dt, *J* 15.0, 7.0, $CH_3(CH_2)_4CH_2CH_2C\underline{H}$=CH-(COH)CH(CO)), 6.88 (0.7H, dt, *J* 15.0, 7.0, $CH_3(CH_2)_4CH_2CH_2C\underline{H}$=CH-(CO)$CH_2$(CO)), 7.10-7.42 (15H, m, Ph), 11.65 (0.3H, d, *J* 1.0, -(CO)CH(CO*H*)-), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 14.4 ($\underline{C}H_3$), 23.0 ($\underline{C}H_2$), 29.0 ($\underline{C}H_2$), 29.0 ($\underline{C}H_2$), 29.4 ($\underline{C}H_2$,), 29.5 ($\underline{C}H_2$), 29.5 ($\underline{C}H_2$), 32.1 ($\underline{C}H_2$), 32.1 ($\underline{C}H_2$), 33.0 ($\underline{C}H_2$), 46.8 (-(CO)$\underline{C}H_2$(CO)-), 52.7 ($\underline{C}H_3O(C{=}O)$-CH(-O-)-CH_2OTr), 63.1 ($CH_3O(C{=}O)$-CH(-O-)-$\underline{C}H_2$OTr), 63.4 ($CH_3O(C{=}O)$-CH(-O-)-$\underline{C}H_2$OTr), 71.9 ($CH_3O(C{=}O)$-$\underline{C}$H(-O-)-CH_2OTr), 73.0 ($CH_3O(C{=}O)$-$\underline{C}$H(-O-)-CH_2OTr), 87.1 ($CH_3O(C{=}O)$-CH(-O-)-$CH_2O\underline{C}(Ph)_3$), 89.6 (-(COH)$\underline{C}$H(CO)-), 124.5 ($CH_3(CH_2)_4CH_2CH_2CH{=}\underline{C}$H-(COH)CH(CO)), 127.5 (Ph), 128.9 (Ph), 129.0 (Ph), 129.8 ($CH_3(CH_2)_4CH_2CH_2CH{=}\underline{C}$H-(CO)$CH_2$(CO)), 142.6 ($CH_3(CH_2)_4CH_2CH_2\underline{C}$H=CH-(COH)CH(CO)), 143.7 (Ph), 143.8 (Ph), 151.0

($CH_3(CH_2)_4CH_2CH_2\underline{C}H$=CH-(CO)$CH_2$(CO)), 167.2 (<u>C</u>-OH), 168.5 (<u>C</u>=O), 169.1 (<u>C</u>=O), 171.1 (<u>C</u>=O), 172.3 (<u>C</u>=O), 191.8 (<u>C</u>=O), carbon signal assignments were confirmed by HMQC and HMBC experiments. ν_{max}/cm^{-1} 2925, 2854 (C-H), 1747 (C=O), 1665 (C=C). ***m/z (ESI)*** 579.25 $[M+Na]^+$, 335.09, 303.03, 274.39, 242.91. **HRMS** Calculated for $[C_{35}H_{40}O_6Na]^+$: 579.2717, observed: 579.2717.

7.4: Experimental – Results and Discussion Chapter 6

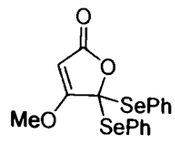
4-methoxy-5-(phenylselenyl)furan-2(5H)-one 273[186]



To a solution of *n*-BuLi (1.37M solution in hexanes, 2.75 mL, 2.75 mmol) in dry THF (20 mL) at -78°C under argon, was added a solution of 4-methoxyfuran-2(5H)-one **269** (1.14 g, 10.0 mmol) in dry THF (10 mL) dropwise *via* cannula. The reaction was stirred for 1 hour at -78°C. To this solution was added a solution of phenylselenyl chloride (1.91 g, 10.0 mmol) in dry THF (10 mL) dropwise *via* cannula. The reaction mixture was stirred at -78°C for 1 hour then allowed to warm to room temperature overnight. The reaction was quenched with saturated ammonium sulphate (20 mL), and diluted with DCM (50 mL). The organic layer was separated, and the aqueous layer was extracted with DCM (5 x 50 mL). The combined organic layers were dried with $MgSO_4$, and concentrated *in vacuo*. The product was purified by flash column chromatography (2: 1 hexane / ethyl acetate) to give the product (1.40 g, 5.20 mmol, 52%) as a yellow oil. δ_H (400 MHz, $CDCl_3$), 3.70 (3H, s, $OC\underline{H}_3$), 4.80 (1H, d, *J* 1.0, C=C<u>H</u>), 6.40 (1H, d, *J* 1.0, SeC<u>H</u>), 7.22-7.53 (5H, m, Ph); δ_C (100 MHz, $CDCl_3$), 59.5 ($O\underline{C}H_3$), 76.8 (Se<u>C</u>H), 89.9 (C=<u>C</u>H), 123.9 (Ph), 129.2 (Ph), 129.6 (Ph), 136.8 (Ph), 170.8 (<u>C</u>=O), 179.1 (<u>C</u>OMe). ν_{max}/cm^{-1} 3117, 2940 (C-H), 1770 (C=O), 1744 (O-Me) 1622, 1567, 1578

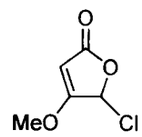
(C=C, aromatic). ***m/z (ESI)*** 292.80 $[M+H]^+$, 270.79, 224.72, 194.76, 182.72. **HRMS** Calculated for $[C_{11}H_{10}NaO_3Se]^+$: 292.9687, observed: 292.9687.

4-methoxy-5,5-bis(phenylselanyl)furan-2(5H)-one 275



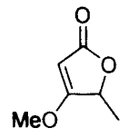
Impurity isolated during the synthesis of **273** as an orange crystalline solid. δ_H (400 MHz, $CDCl_3$), 3.60 (3H, s, OC$\underline{H}_3$), 4.52 (1H, s, C=C$\underline{H}$), 7.18-7.55 (10H, m, Ph). δ_C (100 MHz, $CDCl_3$), 59.2 (O$\underline{C}H_3$), 89.9 (C=$\underline{C}$H), 126.1 (Ph), 129.2 (Ph), 130.0 (Ph), 137.3 (Ph), 168.9 ($\underline{C}$=O), 180.1 ($\underline{C}$OMe), carbon signal assignments were confirmed by HMQC and HMBC experiments. ν_{max}/cm^{-1} 1776 (C=O), 1749 (O-Me), 1621, 1571, 1453 (aromatic) ***m/z (ESI)*** 426.84 $[M+H]^+$, 268.77, 240.74, 188.82, 240.73, 160.88 **HRMS** Calculated for $[C_{17}H_{14}O_3SeNa]^+$: 448.9169, observed: 448.9170 ***mp*** 164-165°C

5-chloro-4-methoxyfuran-2(5H)-one 276



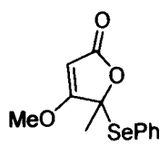
Impurity isolated during the synthesis of **273** as a yellow oil δ_H (400 MHz, $CDCl_3$), 3.93 (3H, s, OC$\underline{H}_3$), 5.21 (1H, s, C=C$\underline{H}$), 6.30 (1H, s, -O-C$\underline{H}$Cl(-COMe)). δ_C (100 MHz, $CDCl_3$) 60.4 (O$\underline{C}H_3$), 83.0 (-O-$\underline{C}$HCl(-COMe)), 89.1 (C=$\underline{C}$H), 168.9 ($\underline{C}$=O), 178.7 ($\underline{C}$OMe), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 3128, 2961 (C-H), 1781 (C=O), 1635 (C=C). ***m/z (ESI)*** 170.97 $[M+H]^+$, 148.80, 116.88, 112.96, 89.13, 85.24, 71.43 **HRMS** Calculated for $[C_5H_5O_3{}^{35}ClNa]^+$: 170.9819, observed: 170.9812, isotopic pattern for chlorine observed 3:1.

4-methoxy-5-methylfuran-2(5H)-one 279[186]



To a solution of 4-methoxyfuran-2(5H)-one **269** (0.57 g, 5.00 mmol) in dry THF (10 mL) under argon was added *n*-BuLi (1.37M solution in hexanes, 3.9 mL, 5.50 mmol) in dry THF (10 mL) at -78°C dropwise *via* syringe. The reaction was stirred for 1 hour at -78°C. To this mixture was added a solution of methyl iodide (0.78 g, 0.35 mL, 5.50 mmol) in dry THF (10 mL) dropwise *via* cannula. The reaction mixture was stirred at -78°C for 1 hour then allowed to warm to ambient temperature overnight. The reaction was quenched with saturated ammonium sulphate (20 mL), and diluted with DCM (50 ml). The organic layer was separated, and the aqueous layer was extracted with DCM (5 x 50 mL). The combined organic layers were dried with $MgSO_4$, and concentrated *in vacuo*. The product was purified by flash column chromatography (1:1 hexane/ ethyl acetate) to give the product (0.17 g, 1.45 mmol, 29%) as a white solid. δ_H (400 MHz, $CDCl_3$), 1.33 (3H, d, *J* 7.0, C$\underline{H}_3$), 3.79 (3H, s, -OC$\underline{H}_3$), 4.73 (1H, q, *J* 7.0, -O-C(COMe)$\underline{H}$-CH_3), 4.94 (1H, s, =C$\underline{H}$-(CO)); δ_C (100 MHz, $CDCl_3$), 17.8 ($\underline{C}H_3$), 59.7 (-O$\underline{C}H_3$), 75.30 (-O-$\underline{C}$H(COMe)-CH_3), 87.9 (=$\underline{C}$H-(CO)), 172.6 ($\underline{C}$=O), 183.7 ($\underline{C}$OMe) ν_{max}/cm^{-1} 3105, 2989 (C-H), 2945 (CH), 1739 (C=O), 1618 (C=C). **HRMS** Calculated for $[C_6H_8NaO_3]^+$: 151.0366 observed: 151.0371 ***mp*** 44-46°C

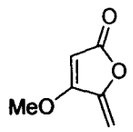
4-methoxy-5-methyl-5-(phenylselenyl)furan-2(5H)-one 274[186]



Tetronate **273** (0.63 g, 2.40 mmol) was dissolved in dry THF (30 mL) under argon, and cooled to -78°C. *t*-BuLi (1.40 mL) was added dropwise *via* syringe and the reaction

mixture stirred at -78°C for 1 hour. A solution of methyl iodide (0.41 g, 2.90 mmol) in dry THF (10 mL) was added dropwise *via* cannula, and the reaction mixture was stirred for 1 hour at -78°C then allowed to warm to ambient temperature overnight. The reaction was quenched with saturated ammonium sulphate (10 mL). The organic layer was separated, and the aqueous layer was extracted with DCM (5 x 50 mL). The combined organic layers were dried with $MgSO_4$, and concentrated *in vacuo*. The product was purified by flash column chromatography (2: 1 hexane / ethyl acetate) to give the product (0.53 g, 1.87 mmol, 78%) as a pale yellow oil which crystallised on standing at 5°C. δ_H (400 MHz, $CDCl_3$), 1.77 (3H, s, C$\underline{H}_3$), 3.69 (3H, s, OC$\underline{H}_3$), 4.63 (1H, s, C$\underline{H}$) 7.22, 7.30, 7.49 (5H, m, Ph); δ_C (100 MHz, $CDCl_3$) 24.2 (C(OMe)-$\underline{C}H_3$), 59.7 (C(O$\underline{C}H_3$)-CH_3), 88.9 ($\underline{C}$H), 126.1 (Ph), 129.5 (Ph), 130.2 (Ph), 137.8 (Ph), 170.1 ($\underline{C}$=O), 182.3 ($\underline{C}$OMe). ν_{max}/cm^{-1} 3125, 2951 (C-H), 2854 (O-Me), 1749 (C=O), 1624 (C=C). ***m/z (ESI)*** 284.91 $[M+H]^+$, 306.9 $[M+Na]^+$, 322.89, 346.04, 149.88, 167.81 **HRMS** Calculated for $[C_{12}H_{12}O_3SeNa]^+$: 306.9830, observed: 306.9844 ***mp*** 70-73°C

4-methoxy-5-methylenefuran-2(5H)-one 261



Method 1[186]

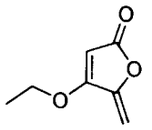
Tetronate **274** (0.43 g, 1.51 mmol) was dissolved in dry DCM (20 mL) under argon and cooled to 0°C. A solution of *m*CPBA (0.29 g, 1.65 mmol) in DCM (5 mL) was added dropwise. The solution turned bright yellow. After 1 hour the reaction had not gone to completion as judged by TLC therefore additional *m*CPBA (0.16 g, 0.90 mmol) in dry DCM (5 mL) was added and the reaction stirred at 4°C overnight. The solvent was removed *in vacuo*, the residue dissolved in ether and the white precipitate was removed

by filtration. This process was repeated until no white solid precipitated. The filtrate was concentrated *in vacuo*, and the residue was purified by flash column chromatography (5:3 hexane / ethyl acetate) to give the product as an off white solid, which was further purified by dissolving in ether and washing with saturated sodium bicarbonate. The ether layer was dried and concentrated to give the title compound (0.14 g, 1.11 mmol 74%) as a white crystalline solid.

Method 2[191]

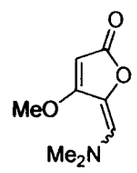
To a solution of **270** (7.00 g, 41.4 mmol) in DCE (120 mL) was added acetic acid (13.80 mL, 207 mmol) and sodium triacetoxyborohydride (43.87 g, 207 mmol). The reaction mixture was heated to reflux for 48 hours. After cooling to room temperature the reaction was quenched with a solution of NaOH (1M) and the product extracted into ether (50 ml). The organic layer was dried with $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by flash column chromatography (5:1 hexane / ethyl acetate) to give the desired product as a white crystalline solid. This solid was dissolved in Et_2O, and washed with a saturated solution of sodium bicarbonate which removed final traces of chlorobenzoic acid and gave the title compound (4.59 g, 36.43 mmol, 88%) as a crystalline white solid. δ_H (400 MHz, $CDCl_3$), 3.93 (3H, d, *J* 1.0, -OC$\underline{H}_3$), 5.03 (1H, m, C=C$\underline{H}_2$), 5.06 (1H, m, C=C$\underline{H}_2$), 5.25 (1H, d, *J* 1.0, C$\underline{H}$), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 59.3 ($\underline{C}H_3$), 89.9 ($\underline{C}H$), 92.3 ($\underline{C}H_2$), 149.7 ($\underline{C}$=CH_2), 167.0 ($\underline{C}$=O), 169.8 ($\underline{C}$OMe), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 3109 (C=CH_2), 2900 (C-H), 1760 (C=O), 1598 (C=C conj). ***mp***: 92-93°C. ***m/z (ESI)*** 149.021 $[M+Na]^+$, 126.91 $[M+H]^+$, 109.01, 81.00. **HRMS** Calculated for $[C_6H_6O_3Na]^+$: 149.0209, observed: 149.0211.

4-ethoxy-5-methylenefuran-2(5H)-one 304



Synthesised using method 2 for the synthesis of **261**. The residue was purified by flash column chromatography (5:3 hexane / ethyl acetate) to give the product (4.75 g, 33.96 mmol, 89%) as an orange crystalline solid. δ_H (400 MHz, $CDCl_3$), 1.43 (3H, t, *J* 7.0, $C\underline{H}_3CH_2O$-), 4.11 (2H, q, *J* 7.0 -$OC\underline{H}_2CH_3$), 5.01 (2H, m, $C\underline{H}_2$=C), 5.18 (1H, d, *J* 1.0, C*H*), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 14.2 ($OCH_2\underline{C}H_3$), 68.8 ($O\underline{C}H_2CH_3$), 90.1 (*C*H), 92.4 ($\underline{C}H_2$=C), 150.2 (CH_2=*C*), 168.8 (C=O), 169.0 (*C*OEt), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 3129 (C=CH_2), 2999 (C-H), 1777 (C=O), 1606 (C=C conj). **HRMS** Calculated for $[C_7H_8O_3Na]^+$:163.0366, observed: 163.0372. ***mp***: low melting point solid.

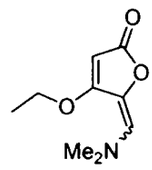
5-((dimethylamino)methylene)-4-methoxyfuran-2(5H)-one 270[189]



A solution of 4-methoxy-2(5H)furanone **269** (5.00 g, 43.9 mmol) in dimethylformamide dimethyl acetal (25 mL) was made up in a 100 mL round bottomed flask fitted with distillation apparatus. The mixture was heated in an oil-bath at 110°C overnight (temperature not in excess of 120°C) with slow, continuous distillation of methanol. The mixture was cooled to room temperature and the excess of solvent-reagent was removed under reduced pressure to leave a brown oil, which slowly crystallised. Purification by flash column chromatography (ethyl acetate) gave the desired product (7.91 g, 41.22

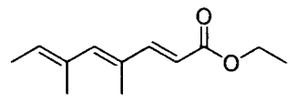
mmol, 94%) as a yellow crystalline solid. δ_H (400 MHz, $CDCl_3$), 3.06 (6H, s, -N(C$\underline{H}_3$)$_2$), 3.81 (3H, s, -OC$\underline{H}_3$), 4.85 (1H, s, -C=C$\underline{H}$-CO-), 6.00 (1H, s, -C=C$\underline{H}$-NMe_2). δ_C (100 MHz, $CDCl_3$), 42.4 (–N($\underline{C}H_3$)$_2$), 58.4 (O$\underline{C}H_3$), 80.6 (=$\underline{C}$H(CO)-), 120.1 (-$\underline{C}$=CHNMe_2), 122.8 (-C=$\underline{C}$HNMe_2), 170.0 ($\underline{C}$=O), 171.4 ($\underline{C}$OCH_3), assignments were confirmed using HMQC and HMBC experiments. ν_{max}/cm^{-1} 2901, 2990, 2822 (C-H), 1704 (C=O), 1655 (C=C). ***m/z (ESI)*** 169.87 $[M+H]^+$, 154.85, 139.89, 126.90, 110.05, 83.32. **HRMS** Calculated for $[C_8H_{11}O_3N_1Na]^+$: 192.0631, observed: 192.0624 ***mp:*** 72-73°C, literature value 59-65°C.[189]

5-((dimethylamino)methylene)-4-ethoxyfuran-2(5H)-one 303



Synthesised using the same procedure as **270**. Purified by flash column chromatography (1:1 hexane / ethyl acetate) to give the title compound (6.98 g, 38.14 mmol, 89%) as a yellow solid. δ_H (400 MHz, $CDCl_3$), 1.23 (3H, dt, *J* 7.0, 2.0, -OCH$_2$C$\underline{H}_3$), 2.93 (6H, s, -N(C$\underline{H}_3$)$_2$), 3.89 (2H, dq, *J* 7.0, 2.0, -OC$\underline{H}_2$CH$_3$), 4.66 (1H, d, *J* 2.0, -C=C$\underline{H}$-CO), 5.91 (1H, d, *J* 2.0, =C$\underline{H}$-NMe_2). δ_C (100 MHz, $CDCl_3$) 14.3 (-OCH$_2$$\underline{C}H_3$), 42.4 (–N($\underline{C}H_3$)$_2$), 67.4 (-O$\underline{C}H_2CH_3$), 80.5 (=$\underline{C}$H-(CO)), 120.3 (-$\underline{C}$=CH-$NMe_2$), 122.8 (-C=$\underline{C}$H-$NMe_2$), 170.1 ($\underline{C}$=O), 170.5 (-$\underline{C}OCH_2CH_3$), assignments were confirmed using an HMQC experiment. ν_{max}/cm^{-1} 2921 (C-H), 1734 (C=O), 1662 (C=C). ***m/z (ESI)*** 205.90 $[M+Na]^+$, 183.92 $[M+H]^+$, 155.89 **HRMS** calculated for $[C_9H_{13}O_3N_1]^+$: 184.0968 observed: 184.0972 ***mp:*** 85-86°C

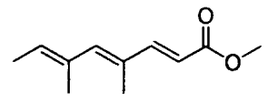
Ethyl-(2*E*,4*E*,6*E*)-4,6-dimethylocta-2,4,6-trienoate 281[172]



To a solution of (2*E*,4*E*)-2,4-dimethylhexa-2,4-dienal **219** (9.95 g, 118.0 mmol), in dry toluene (250 mL) under argon at ambient temperature, was added phosphorane **280** (47.40 g, 131.0 mmol). The mixture was heated to reflux for 48 hours then allowed to cool to room temperature and concentrated under reduced pressure. Et_2O was added, and the resulting triphenylphosphine oxide precipitate was removed by filtration. The yellow filtrate was concentrated *in vacuo* and purified by flash column chromatography (20:1 petroleum ether / diethyl ether), to give the product (15.52 g, 79.61 mmol, 67%) as a colourless oil. δ_H (400 MHz, $CDCl_3$), 1.28 (3H, t, *J* 7.0, -$OCH_2C\underline{H}_3$), 1.73 (3H, d, *J* 7.0, $C\underline{H}_3CH$=), 1.81 (3H, s, CH_3CH=$C(C\underline{H}_3)$CH=), 1.92 (3H, s, CH_3CH=$C(CH_3)$CH=$C(C\underline{H}_3)$), 4.19 (2H, q, *J* 7.0, -$OC\underline{H}_2CH_3$), 5.61 (1H, q, *J* 7.0, $CH_3C\underline{H}$=), 5.81 (1H, d, *J* 15.5, CH_3CH=$C(CH_3)$CH=$C(CH_3)$-CH=C*H*-C=O), 6.25 (1H, s, CH_3CH=$C(CH_3)C\underline{H}$=), 7.33 (1H, d, *J* 15.5, CH_3CH=$C(CH_3)$CH=$C(CH_3)$-C*H*=CH-C=O), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 13.4 (CH_3CH=$C(CH_3)$CH=$C(\underline{C}H_3)$-CH=CH-C=O), 13.8 ($\underline{C}H_3CH$=$C(CH_3)$CH=$C(CH_3)$-CH=CH-C=O), 14.1 (-$OCH_2\underline{C}H_3$), 16.0 (CH_3CH=$C(\underline{C}H_3)$CH=$C(CH_3)$-CH=CH-C=O), 59.9 (-$O\underline{C}H_2CH_3$), 115.8 (CH_3CH=$C(CH_3)$CH=$C(CH_3)$-CH=$\underline{C}$H-C=O), 129.3 ($CH_3\underline{C}H$=$C(CH_3)$CH=$C(CH_3)$-CH=CH-C=O), 130.6 (CH_3CH=$C(CH_3)$CH=$\underline{C}(CH_3)$-CH=CH-C=O), 133.3 (CH_3CH=$\underline{C}(CH_3)$CH=$C(CH_3)$-CH=CH-C=O), 143.5 (CH_3CH=$C(CH_3)\underline{C}H$=$C(CH_3)$-CH=CH-C=O), 150.6 (CH_3CH=$C(CH_3)$CH=$C(CH_3)$-$\underline{C}$H=CH-C=O), 167.3 ($\underline{C}$=O), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 2977 (C-H), 1708 (C=O) 1605 (C=C). ***m/z (ESI)*** 217.87 $[M+Na]^+$, 194.96

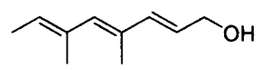
$[M+H]^+$, 121.10, 93.27 **HRMS** Calculated for $[C_{12}H_{18}O_2Na]^+$: 217.1299, observed: 217.1299.

Methyl-(2*E*,4*E*,6*E*)-4,6-dimethylocta-2,4,6-trienoate 301[172]



Synthesised as for **281** using phosphorane **300** in place of phosphorane **280**. The product was purified by flash column chromatography (20:1 petroleum ether / diethyl ether), to give the product (4.47 g, 24.7 mmol, 83%) as a colourless oil. δ_H (400 MHz, $CDCl_3$), 1.73 (3H, d, *J* 7.0, C*H*$_3$CH=), 1.82 (3H, s, $CH_3CH=C(CH_3)CH=$), 1.92 (3H, s, $CH_3CH=C(CH_3)CH=C(CH_3)CH=C(CH_3)$), 3.73 (3H, s -OC*H*$_3$), 5.61 (1H, q, *J* 7.0, $CH_3CH=$), 5.81 (1H, d, *J* 15.5, ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=C*H*-C=O), 6.25 (1H, s, $CH_3CH=C(CH_3)CH=$), 7.33 (1H, d, *J* 15.5, ($CH_3CH=C(CH_3)CH=C(CH_3)$-C*H*=CH-C=O), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 13.9 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-C=O), 14.3 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-C=O), 16.5 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-C=O), 51.7 (-OCH_3), 115.8 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-C=O), 130.0 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-C=O), 131.1 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-C=O), 133.8 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-C=O), 144.1 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-C=O), 151.4 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-C=O), 168.2 (C=O), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 2948 (C-H), 1716 (C=O) 1603 (C=C). **HRMS** Calculated for $[C_{11}H_{16}O_2]^+$: 181.1223, observed: 181.1224.

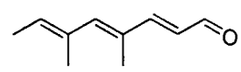
(2*E*,4*E*,6*E*)-4,6-dimethylocta-2,4,6-trien-1-ol 282[172]



To a stirred solution of ester **281** (18.0 g, 107.0 mmol) in dry Et_2O (250 mL), under argon at 0°C, was added DIBAL-H (225 mL, 1M solution in hexanes, 225.0 mmol). After 3 hours, the reaction was quenched by the cautious addition of MeOH (10 mL). The mixture was diluted with Et_2O (100 mL), and a saturated solution of sodium potassium tartrate tetrahydrate (300 mL) was added. The mixture was stirred vigorously for several hours until the organic and aqueous layers had completely separated. The organic layer was extracted, washed with brine (200 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting oil was purified by flash column chromatography (2:1 petroleum ether / diethyl ether). The product was isolated as a colourless oil (12.93 g, 84.53 mmol, 79%). δ_H (400 MHz, $CDCl_3$) 1.69 (3H, d, *J* 7.0, C*H*$_3$CH=), 1.76 (3H, s, $CH_3CH=C(CH_3)CH=$), 1.88 (3H, s, $CH_3CH=C(CH_3)CH=C(CH_3)$), 4.19 (2H, dd, *J* 5.5, 1.0, -C*H*$_2$OH), 5.45 (1H, q, *J* 7.0, $CH_3CH=$), 5.76 (1H, dt, *J* 15.5, 5.5, $CH_3CH=C(CH_3)CH=C(CH_3)$-CH=C*H*-CH_2-OH), 5.90 (1H, s, $CH_3CH=C(CH_3)CH=$), 6.26 (1H, dd, *J* 15.5, 1.0, $CH_3CH=C(CH_3)CH=C(CH_3)$-C*H*=CH-CH_2-OH), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$) 14.2 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-CH_2-OH) and ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-C=O), 17.0 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-CH_2-OH), 64.2 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-CH_2-OH), 126.4 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-CH_2-OH), 126.6 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-CH_2-OH), 132.0 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-CH_2-OH), 133.8 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-CH_2-OH), 136.6 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-CH_2-OH), 137.9 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-CH_2-OH)), carbon signal assignments were confirmed by HMBC and HMQC

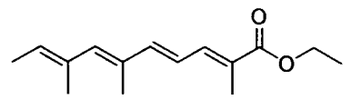
experiments. ν_{max}/cm^{-1} 3305 (O-H), 2915, 2858, (C-H) 1637 (C=C) ***m/z (ESI)*** 152.92 $[M+H]^+$, 134.99 $[M-H_2O]^+$, 111.09, 93.24, **HRMS** Calculated for $[C_{10}H_{16}O_1Na]^+$: 175.1093, observed: 175.1100.

(2*E*,4*E*,6*E*)- 4,6-dimethylocta-2,4,6-trienal 234[172]



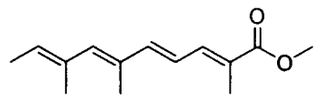
To a stirred solution of alcohol **282** (2.29 g, 18.10 mmol) in DCM (200 mL) under argon was added MnO_2 (23.60 g, 271.5 mmol) at ambient temperature. After 72 hours the mixture was filtered through celite and the filtrate concentrated *in vacuo* to give the product as a yellow oil, which was used without further purification. (2.62 g, 17.38 mmol, 96%) δ_H (400 MHz, $CDCl_3$), 1.72 (3H, d, *J* 7.0, $C\underline{H}_3CH$=), 1.82 (3H, s, CH_3CH=$C(C\underline{H}_3)$CH=), 1.93 (3H, s, CH_3CH=$C(CH_3)CH$=$C(C\underline{H}_3)$), 5.65 (1H, q, *J* 7.0, $CH_3C\underline{H}$=), 6.01 (1H, dd, *J* 15.5, 8.0, CH_3CH=$C(CH_3)CH$=$C(CH_3)$-CH=C*H*-CHO), 6.33 (1H, s, CH_3CH=$C(CH_3)C\underline{H}$=), 7.09 (1H, d, *J* 15.5, CH_3CH=$C(CH_3)CH$=$C(CH_3)$-C*H*=CH-CHO), 9.51 (1H, d, *J* 8.0, CH_3CH=$C(CH_3)CH$=$C(CH_3)$-CH=CH-C*H*O), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 13.2 (CH_3CH=$C(CH_3)CH$=$C(\underline{C}H_3)$-CH=CH-CHO), 13.6 ($\underline{C}H_3CH$=$C(CH_3)CH$=$C(CH_3)$-CH=CH-CHO), 15.5 (CH_3CH=$C(\underline{C}H_3)CH$=$C(CH_3)$-CH=CH-CHO), 126.4 (CH_3CH=$C(CH_3)CH$=$C(CH_3)$-CH=$\underline{C}$H-CHO), 130.5 (CH_3CH=$C(CH_3)CH$=$\underline{C}(CH_3)$-CH=CH-CHO), 131.0 ($CH_3\underline{C}H$=$C(CH_3)CH$=$C(CH_3)$-CH=CH-CHO), 133.1 (CH_3CH=$\underline{C}(CH_3)CH$=$C(CH_3)$-CH=CH-CHO), 145.2 (CH_3CH=$C(CH_3)\underline{C}H$=$C(CH_3)$-CH=CH-CHO), 158.7 (CH_3CH=$C(CH_3)CH$=$C(CH_3)$-$\underline{C}$H=CH-CHO), 193.3 ($\underline{C}$HO), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 2918 (C-H), 1673 (C=O), ***m/z (ESI)*** 150.88 $[M+H]^+$, 132.92, 122.96, 105.00, 83.31 **HRMS** Calculated for $[C_{10}H_{14}O_1Na]^+$: 173.0937, observed: 173.0939.

Ethyl-(2*E*,4*E*,6*E*,8*E*)-2,6,8-trimethyldeca-2,4,6,8-tetraenoate 283[172]



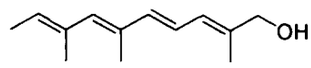
To a solution of alcohol **282** (3.10 g, 24.6 mmol) was added phosphorane **245** (13.4 g, 36.9 mmol), and MnO_2 (32.0 g, 375 mmol) in dry toluene (150 mL) at ambient temperature. The mixture was heated to reflux for 48 hours then allowed to cool to room temperature, filtered through celite and concentrated under reduced pressure. Et_2O was added, and the resulting triphenylphosphine oxide was removed by filtration. The yellow filtrate was concentrated *in vacuo* and purified by flash column chromatography (25:1 petroleum ether / diethyl ether), to give the product (3.46 g, 14.76 mmol, 60%) as a pale yellow oil. δ_H (400 MHz, $CDCl_3$), 1.28 (3H, t, *J* 7.0, C$\underline{H}_3$CH$_2$), 1.71 (3H, d, *J* 7.0, C$\underline{H}_3$-CH=C), 1.79 (3H, s, =C(C$\underline{H}_3$)CH=C(CH$_3$)-CH), 1.95 (6H, s, =C(C$\underline{H}_3$)-CH=CH-CH=C(C$\underline{H}_3$)CO), 4.18 (2H, q, *J* 7.0, OC$\underline{H}_2$CH$_3$), 5.54 (1H, q, *J* 7.0, CH$_3$C$\underline{H}$=), 6.06 (1H, s, CH$_3$CH=C(CH$_3$)-C$\underline{H}$=) 6.41 (1H, dd, *J* 15.0, 11.5, =C(CH$_3$)-CH=C$\underline{H}$-CH=C(CH$_3$)CO), 6.55 (1H, d, *J* 15.0, =C(CH$_3$)-C$\underline{H}$=CH-CH=C(CH$_3$)CO), 7.24 (1H, d, *J* 11.5, =C(CH$_3$)-CH=CH-C$\underline{H}$=C(CH$_3$)CO), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 13.0 (C=C($\underline{C}$H$_3$)C=O), 14.2 (C($\underline{C}$H$_3$)CH=CH=CH-C(CH$_3$)-CO), 14.3 ($\underline{C}$H$_3$-CH=C), 14.7 (OCH$_2\underline{C}$H$_3$), 16.8 (CH$_3$CH=C($\underline{C}$H$_3$)-CH), 60.8 (O$\underline{C}$H$_2$CH$_3$), 122.6 (C-CH=$\underline{C}$H-CH=C(CH$_3$)), 126.0 (=$\underline{C}$(CH$_3$)CO-O), 128.3 (CH$_3\underline{C}$H=CH(CH$_3$), 132.8 (CH$_3$CH=C(CH$_3$)-CH=$\underline{C}$(CH$_3$)C-), 134.1 (CH$_3$CH=$\underline{C}$(CH$_3$)C-), 139.3 (CCH=CH-$\underline{C}$H=C(CH$_3$)), 140.1 (CH$_3$CH=C(CH$_3$)-$\underline{C}$H=C(CH$_3$)C-), 146.0 (=C(CH$_3$)-$\underline{C}$H=CH-CH=C(CH$_3$)CO), 168.6 ($\underline{C}$=O), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 2954 (C-H), 1697 (C=O), 1606 (C=C). **HRMS** Calculated for $[C_{15}H_{22}O_2Na]^+$: 257.1512, observed: 257.1504

Methyl-(2*E*,4*E*,6*E*,8*E*)-2,6,8-trimethyldeca-2,4,6,8-tetraenoate 306



To a stirred solution of ester **283** (0.45 g, 1.90 mmol) in methanol (10 mL) was added sodium methoxide (1.10 g, 21.0 mmol) at room temperature. The reaction mixture was stirred for 3 hours, quenched with ammonium chloride and the aqueous layer extracted with DCM (5 x 50 mL). The organic layer was dried with $MgSO_4$ and the solvent removed *in vacuo*. The crude residue purified by flash column chromatography (25:1 petroleum ether / diethyl ether), to give the product (0.30 g, 1.47 mmol, 74%) as a pale yellow oil. δ_H (400 MHz, $CDCl_3$), 1.62 (3H, d, *J* 7.0, C$\underline{H}_3$-CH=C), 1.72 (3H, s, =C(C$\underline{H}_3$)CH=C(CH_3)-CH), 1.88 (6H, s, =C(C$\underline{H}_3$)-CH=CH-CH=C(C$\underline{H}_3$)CO), 3.65 (3H, s, OC$\underline{H}_3$), 5.46 (1H, q, *J* 7.0, CH_3-C$\underline{H}$=), 5.97 (1H, s, CH_3-CH=C(CH_3)-C$\underline{H}$=), 6.33 (1H, dd, *J* 15.0, 11.5, =C(CH_3)-CH=C$\underline{H}$-CH=C(CH_3)CO), 6.47 (1H, d, *J* 15.0, =C(CH_3)-C$\underline{H}$=CH-CH=C(CH_3)CO), 7.19 (1H, dd, *J* 11.5, 1.0, =C(CH_3)-CH=CH-C$\underline{H}$=C(CH_3)CO), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 12.7 (CH=CH=CH($\underline{C}H_3$)-CO), 14.0 (CH_3CH=C(CH_3)-CH=C($\underline{C}H_3$)), 14.2 ($\underline{C}H_3$CH=C(CH_3)-CH=C(CH_3)), 16.6 (CH_3-CH=C($\underline{C}H_3$)-CH=C(CH_3)), 51.2 (O$\underline{C}H_3$), 122.3 (=C(CH_3)-CH=$\underline{C}$H-CH=C(CH_3)CO), 125.3 (=C(CH_3)-CH=CH-CH=$\underline{C}$(CH_3)CO), 128.3 ($CH_3\underline{C}$H=C(CH_3)-CH=C(CH_3)), 132.6 (CH_3CH=C(CH_3)-CH=$\underline{C}$(CH_3)), 133.9 (CH_3CH=$\underline{C}$(CH_3)-CH=C(CH_3)), 139.4 (=C(CH_3)-CH=CH-$\underline{C}$H=C(CH_3)CO), 140.0 (CH_3-CH=C(CH_3)-$\underline{C}$H=C(CH_3)C-), 146.0 (=C(CH_3)-$\underline{C}$H=CH-CH=C(CH_3)CO), 169.0 ($\underline{C}$=O), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 2952 (C-H), 1698 (C=O), 1607 (C=C). **HRMS** Calculated for $[C_{14}H_{20}O_2Na]^+$: 243.1356, observed: 243.1360.

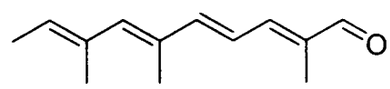
(2*E*,4*E*,6*E*,8*E*)- 2,6,8-trimethyldeca-2,4,6,8-tetraen-1-ol 284[204,205]



To a stirred solution of ester **283** (0.40 g, 1.76 mmol) in dry Et_2O (10 mL) under argon at -78°C, was added DIBAL-H (1.50 mL of a 1M solution in hexanes, 1.50 mmol). After 3 hours, the reaction was quenched by the cautious addition of methanol (2 mL). A saturated solution of sodium potassium tartrate tetrahydrate (10 mL) was added and the mixture stirred vigorously for several hours until the organic and aqueous layers had completely separated. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified on florisil (2.5:1 petroleum ether / diethyl ether) to give the product (0.08 g, 0.53 mmol, 30%) as a colourless oil. δ_H (400 MHz, $CDCl_3$) 1.64 (3H, d, *J* 7.0, C*H*$_3$-CH=C(CH$_3$)CH=C(CH$_3$)-CH=CH-CH=C(CH$_3$)-CH$_2$OH), 1.71 (3H, s, CH$_3$-CH=C(C*H*$_3$)CH=C(CH$_3$)-CH=CH-CH=C(CH$_3$)-CH$_2$OH), 1.74 (3H, s, CH$_3$-CH=C(CH$_3$)CH=C(CH$_3$)-CH=CH-CH=C(C*H*$_3$)-CH$_2$OH), 1.87 (3H, s, CH$_3$-CH=C(CH$_3$)CH=C(C*H*$_3$)-CH=CH-CH=C(CH$_3$)-CH$_2$OH), 3.99 (2H, s, CH$_3$-CH=C(CH$_3$)CH=C(CH$_3$)-CH=CH-CH=C(CH$_3$)-C*H*$_2$OH), 5.41 (1H, q, *J* 7.0, CH$_3$-C*H*=C(CH$_3$)CH=C(CH$_3$)-), 5.86 (1H, s, CH$_3$.CH=C(CH$_3$)-C*H*=C(CH$_3$)-), 6.04 (1H, dd, *J* 10.5, 1.0, CH$_3$-CH=C(CH$_3$)CH=C(CH$_3$)-CH=CH-C*H*=C(CH$_3$)-CH$_2$OH), 6.19 (1H, d, *J* 15.0, CH$_3$-CH=C(CH$_3$)CH=C(CH$_3$)-C*H*=CH-CH=C(CH$_3$)-CH$_2$OH), 6.34 (1H, dd, *J* 15.0, 10.5, CH$_3$-CH=C(CH$_3$)CH=C(CH$_3$)-CH=C*H*-CH=C(CH$_3$)-CH$_2$OH), O-H signal not visible on the ^{1}H NMR spectrum, hydrogen coupling was confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 13.9 (CH$_3$-CH=C(CH$_3$)CH=C(*C*H$_3$)-CH=CH-CH=C(CH$_3$)-CH$_2$OH), 14.0 (*C*H$_3$-CH=C(CH$_3$)CH=C(CH$_3$)-CH=CH-CH=C(CH$_3$)-CH$_2$OH), 14.3 (CH$_3$-CH=C(CH$_3$)CH=C(CH$_3$)-CH=CH-CH=C(*C*H$_3$)-CH$_2$OH), 16.7 (CH$_3$-CH=C(*C*H$_3$)CH=C(CH$_3$)-CH=CH-CH=C(CH$_3$)-CH$_2$OH), 68.8 (CH=CH-

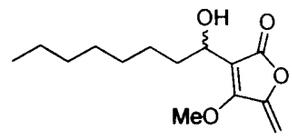
CH=C(CH_3)-$\underline{C}H_2OH$), 122.7 (CH_3-CH=C(CH_3)CH=C(CH_3)-CH=$\underline{C}$H-CH=C(CH_3)-CH_2OH), 125.9 (CH_3-CH=C(CH_3)CH=C(CH_3)-CH=CH-$\underline{C}$H=C(CH_3)-CH_2OH), 126.2 (CH_3-$\underline{C}$H=C(CH_3)CH=C(CH_3)-CH=CH-CH=C(CH_3)-CH_2OH), 132.8 (CH_3-CH=C(CH_3)-CH=$\underline{C}$(CH_3)C-), 133.7 (CH_3-CH=$\underline{C}$(CH_3)CH=C(CH_3)-CH=CH-CH=C(CH_3)-CH_2OH), 136.2 (CH_3-CH=C(CH_3)CH=C(CH_3)-CH=CH-CH=$\underline{C}$(CH_3)-CH_2OH), 136.3 (CH_3-CH=C(CH_3)-$\underline{C}$H=C(CH_3)C-), 139.0 (CH_3-CH=C(CH_3)CH=C(CH_3)-C$\underline{H}$=CH-CH=C(CH_3)-CH_2OH), carbon signal assignments were confirmed by HMBC and HMQC experiments ν_{max}/cm^{-1} 3302 (O-H), 2912, 2856 (C-H), 1620 (C=C). **HRMS** Calculated for $[M\text{-}H_20]^+$ $[C_{13}H_{19}]^+$: 175.1481, observed: 175.1482.

(2*E*,4*E*,6*E*,8*E*)- 2,6,8-trimethyldeca-2,4,6,8-tetraenal 285[172]



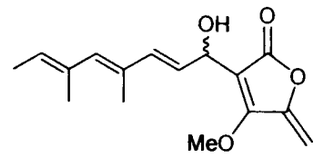
To a stirred solution of alcohol **284** (0.10 g, 0.50 mmol) in DCM (10 mL) was added MnO_2 (0.65 g, 7.50 mmol) at ambient temperature. After 72 hours the mixture was filtered through celite and the filtrate concentrated *in vacuo* to give the product as a yellow oil, which was used without further purification. (0.09 g, 0.49 mmol, 98%) δ_H (400 MHz, $CDCl_3$) 1.71 (3H, d, *J* 7.0, C$\underline{H}_3$CH=), 1.81 (3H, s, CH_3CH=C(C$\underline{H}_3$)CH=), 1.97 (3H, s, CH_3CH=C(CH_3)CH=C(C$\underline{H}_3$)), 5.61 (1H, q, *J* 7.0, $CH_3C\underline{H}$=), 6.14 (1H, s, CH_3CH=C(CH_3)C$\underline{H}$=), 6.59 (2H, m, CH_3CH=C(CH_3)CH=C(CH_3)-CH=C$\underline{H}$-CH= and CH_3CH=C(CH_3)CH=C(CH_3)-CH=CH-C$\underline{H}$=C(CH_3)-C(=O)H), 6.90 (1H, d, *J* 11.0, CH_3CH=C(CH_3)CH=C(CH_3)-C$\underline{H}$=CH-CH=), 9.40 (1H, s, CH=CH-CH=C(CH_3)-C(=O)$\underline{H}$), hydrogen couplings were confirmed by a COSY experiment. **HRMS** Calculated for $[C_{13}H_{19}O_1]^+$: 191.1430, observed: 191.1432.

3-(1-hydroxyoctyl)-4-methoxy-5-methylenefuran-2(5H)-one 286[15]



Tetronate **261** (0.10 g, 0.80 mmol) was dissolved in dry THF (20 mL) under argon and cooled to -78°C. LDA (2M, in mixture of tetrahydrofuran, heptane, and ethylbenzene, 0.45 mL, 0.88 mmol) was added dropwise *via* syringe and the reaction mixture stirred at -78°C for 1 hour. Octanal (0.12 mL, 0.80 mmol) was then added dropwise and the reaction allowed to warm to -60°C. The reaction was quenched with saturated ammonium chloride and the organic layer separated. The aqueous layer was extracted with DCM (5 x 50 mL). The combined organic layers were dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography (4:1 hexane / ethyl actetate) to give the product (0.08 g, 0.30 mmol, 57%) as a viscous white oil. δ_H (400 MHz, $CDCl_3$), 0.86 (3H, t, *J* 6.5, C*H*$_3$), 1.26 (9H, m, 4 x C*H*$_2$ and 1 x C*H*), 1.45 (1H, m, C*H*), 1.73 (1H, m, C*H*), 1.90 (1H, m, C*H*), 3.09 (1H, br s, O*H*), 4.12 (3H, s, O C*H*$_3$), 4.79 (1H, t, *J* 6.5, C*H*OH), 5.05 (2H, s, C=C*H*$_2$), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 12.5 (CH_3), 21.6 ($CH_3\underline{C}H_2$), 25.1 ($CH_3CH_2\underline{C}H_2$), 28.2 ($CH_3CH_2CH_2\underline{C}H_2$), 28.3 ($\underline{C}H_2CH_2CH_2CHOH$), 30.8 ($\underline{C}H_2CH_2CHOH$), 36.7 ($\underline{C}H_2CHOH$), 59.3 ($O\underline{C}H_3$), 65.2 ($\underline{C}HOH$), 92.2 ($C=\underline{C}H_2$), 106.3 ($C=\underline{C}(CO)$-CH), 148.4 ($\underline{C}=CH_2$), 159.9 ($\underline{C}OMe$), 168.3 ($\underline{C}=O$), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 3452 (O-H), 2924 (C-H), 2854 (O-Me), 1756 (C=O), 1622 (C=C). ***m/z (ESI)*** 531.2 $[2M+Na]^+$, 277.1 $[M+Na]^+$, 254.98, 236.92, 180.85, 166.85, 152.86, 138.87, 83.26. **HRMS** Calculated for $[C_{14}H_{22}O_4Na]^+$: 277.1410, observed: 277.1407.

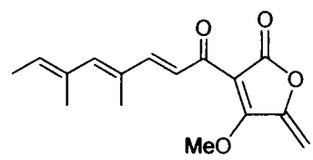
(2*E*,4*E*,6*E*)-3-(1-hydroxy-4,6-dimethylocta-2,4,6-trienyl)-4-methoxy-5-methylenefuran-2(5H)-one 265[15]



To a solution of diisopropylamine (0.70 mL) in dry THF (5.4 mL) under argon was added *n*-BuLi (3.90 mL) dropwise at -78°C. The mixture was stirred at this temperature for 15 minutes then warmed to 0°C and stirred for a further 15 minutes to give a solution of LDA. To a solution of 4-methoxy-5-methylenefuran-2(5H)-one **261** (0.25 g, 2.00 mmol) in dry THF (20 mL) was added the freshly prepared LDA (4.40 mL, 2.20 mmol) dropwise at -78°C. The mixture was stirred for 30 minutes then aldehyde **234** was added (2.00 mmol). The reaction was stirred at -78°C for 1 hour then allowed to warm to -60°C. The reaction was quenched with saturated ammonium chloride (5 mL). The organic layer was separated, and the aqueous layer was extracted with DCM (5 x 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The crude residue was purified by flash column chromatography (5:1 hexane / ethyl acetate) to give the desired product (0.19 g, 0.70 mmol, 35%) as a yellow oil. δ_H (400 MHz, $CDCl_3$) 1.64 (3H, d, *J* 7.0, C$\underline{H}_3$CH=), 1.70 (3H, s, $CH_3CH=C(C\underline{H}_3)CH=$), 1.82 (3H, s, $CH_3CH=C(CH_3)CH=C(C\underline{H}_3)$), 3.19 (1H, d, *J* 7.5, CH(O$\underline{H}$)), 4.10 (3H, s, -OC$\underline{H}_3$), 5.02 (2H, m, $\underline{C}H_2$=C(-COMe)-O), 5.38 (2H, m, $CH_3C\underline{H}$= and $CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-C$\underline{H}$(OH)), 5.81 (1H, dd, *J* 17.0, 7.5, $CH_3CH=C(CH_3)CH=C(CH_3)$-CH=C$\underline{H}$-CH(OH)), 5.87 (1H, s, $CH_3CH=C(CH_3)C\underline{H}$=), 6.17 (1H, d, *J* 17.0, ($CH_3CH=C(CH_3)CH=C(CH_3)$-C$\underline{H}$=CH-C=O), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 13.3 ($CH_3CH=C(CH_3)CH=C(\underline{C}H_3)$-CH=CH-C=O), 13.5 ($\underline{C}H_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-C=O), 15.9 ($CH_3CH=C(\underline{C}H_3)CH=C(CH_3)$-CH=CH-C=O), 60.1 (-O$\underline{C}H_3$), 66.0

(CH=CH-<u>C</u>H(OH)), 92.4 (<u>C</u>H_2=C(O)-C(COMe)), 105.1 (-CH(OH)-<u>C</u>(CO)-C(OMe)), 125.9 (CH_3CH=C(CH_3)CH=C(CH_3)-CH=<u>C</u>H-CH(OH), 126.3 (CH_3<u>C</u>H=C(CH_3)CH=C(CH_3)-CH=CH-), 130.7 (CH_3CH=<u>C</u>(CH_3)CH=C(CH_3)-CH=CH-), 136.8 and 136.9 (CH_3CH=C(CH_3)CH=<u>C</u>(CH_3)-CH=CH-CH(OH), and (CH_3CH=C(CH_3)CH=C(CH_3)-<u>C</u>H=CH-C=O), 148.9 (CH_2=<u>C</u>(O)-C(COMe)), 160.8 (<u>C</u>=O), 168.9 (<u>C</u>OMe), carbon signal assignments were confirmed by an HMQC experiment. ν_{max}/cm^{-1} 3444 (O-H) 2914, 2857 (C-H), 1760 (C=O) 1668 (C=C). **HRMS** Calculated for $[C_{16}H_{18}O_4Na]^+$: 299.1254, observed: 299.1240.

(2*E*,4*E*,6*E*)-3-(4,6-dimethylocta-2,4,6-trienoyl)-4-methoxy-5-methylenefuran-2(5H)-one 267



Method 1[15]

To a solution of **265** (0.07 g, 0.27 mmol) in DCM (5 mL) was added Dess-Martin periodinane (0.17 g, 0.40 mmol). The reaction was stirred at room temperature for 30 minutes. The solvent was removed *in vacuo* and the crude residue applied to a silica gel column. The product was eluted with 5:1 hexane / ethyl acetate to give the desired product (0.01 g, 0.05 mmol, 15%) as a yellow oil.

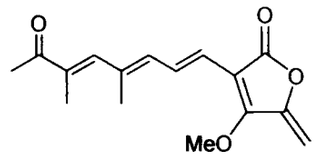
Method 2[196]

To a solution of diisopropylamine (0.70 mL), in dry THF (5.40 mL) under argon was added *n*-BuLi (3.9 mL) dropwise at -78°C. The mixture was stirred at this temperature for 15 minutes then warmed to 0°C and stirred for a further 15 minutes giving to a

freshly prepared solution of LDA. To a solution of tetronate **261** (0.25 g, 2.00 mmol) in dry THF (20 mL) was added LDA (4.40 mL, 2.20 mmol) dropwise at -78°C. The mixture was stirred for 30 minutes then the ester **281** was added (0.36 g, 2.00 mmol) in dry THF (2 mL). The reaction was stirred at -78°C for 1 hour then allowed to warm to -60°C. The reaction was quenched with saturated citric acid (5 mL), the organic layer was separated, and the aqueous layer was extracted with DCM (5 x 50 mL). The combined organic layers were dried with $MgSO_4$ and concentrated *in vacuo*. The crude residue was purified by flash column chromatography (5:1 petroleum ether / diethyl ether) to give the title compound (0.03 g, 0.11 mmol, 14%) as a viscous yellow oil. δ_H (700 MHz, $CDCl_3$), 1.76 (3H, d, *J* 7.0, CH_3CH=), 1.85 (3H, d, *J* 1.0, CH_3CH=C(CH_3)CH=), 2.01 (3H, s, CH_3CH=C(CH_3)CH=C(CH_3)), 4.13 (3H, s, -OCH_3), 5.17 (1H, d, 1.5, CH_2=C(COMe)-O-(C=O)), 5.25 (1H, d, 1.5, CH_2=C(COMe)-O-(C=O)), 5.71 (1H, q, *J* 7.0, CH_3CH=), 6.40 (1H, s, CH_3CH=C(CH_3)CH=), 6.74 (1H, d, *J* 15.5, CH_3CH=C(CH_3)CH=C(CH_3)-CH=CH-C=O), 7.33 (1H, d, *J* 15.5, CH_3CH=C(CH_3)CH=C(CH_3)-CH=CH-C=O), hydrogen couplings were confirmed by a COSY experiment; δ_C (175 MHz, $CDCl_3$), 13.9 (CH_3CH=C(CH_3)CH=C(CH_3)-CH=CH-C=O), 14.2 (CH_3CH=C(CH_3)CH=C(CH_3)-CH=CH-C=O), 16.2 (CH_3CH=C(CH_3)CH=C(CH_3)-CH=CH-C=O), 62.50 (-OCH_3), 94.0 (CH_2=C(COMe)-O-(C=O)), 104.5 (C(=O)C=(COMe)(C=O)), 124.0 (CH_3CH=C(CH_3)CH=C(CH_3)-CH=CH-C=O), 131.4 (CH_3CH=C(CH_3)CH=C(CH_3)-CH=CH-C=O), 131.5 (CH_3CH=C(CH_3)CH=C(CH_3)-CH=CH-C=O), 134.0 (CH_3CH=C(CH_3)CH=C(CH_3)-CH=CH-C=O), 147.0 (CH_3CH=C(CH_3)CH=C(CH_3)-CH=CH-C=O), 149.8 (CH_2=C(COMe)-O-(C=O)), 152.5 (CH_3CH=C(CH_3)CH=C(CH_3)-CH=CH-C=O)), 165.6 (C=O), 166.6 (COMe), 187.4 (C=O), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 2922, 2852 (C-H), 1752 (C=O), 1588

(C=C). ***m/z (ESI)*** 297.11 $[M+Na]^+$, **HRMS** Calculated for $[C_{16}H_{18}O_4Na]^+$: 297.1097, observed: 297.1103.

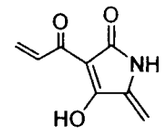
(1*E*,3*E*,5*E*)-3-(4,6-dimethyl-7-oxoocta-1,3,5-trienyl)-4-methoxy-5-methylenefuran-2(5H)-one 287



Isolated as an impurity during the synthesis of **267** - method 1. The product was eluted with 5:1 hexane / ethyl acetate, to give the title compound (0.08 g, 0.03 mmol 10%) as a yellow oil. δ_H (700 MHz, $CDCl_3$), 2.03 (3H, s, $CH_3C(=O)$-$C(C\underline{H}_3)$=CH), 2.13 (3H, s, $CH_3C(=O)$-$C(CH_3)$=CH-$C(C\underline{H}_3)$=CH-), 2.37 (3H, s, $C\underline{H}_3C(=O)$-$C(CH_3)$=CH), 4.24 (3H, s, -$OC\underline{H}_3$), 5.06 (1H, d, *J* 3.0, $C\underline{H}_2$=C-), 5.09 (1H, d, *J* 3.0, $C\underline{H}_2$=C-), 6.33 (1H, d, *J* 11.5, $CH_3C(=O)$-$C(CH_3)$=CH-$C(CH_3)$=C$\underline{H}$-CH=CH-), 6.61 (1H, d, *J* 15.0, $CH_3C(=O)$-$C(CH_3)$=CH-$C(CH_3)$=CH-CH=C$\underline{H}$-), 7.03 (1H, s, $CH_3C(=O)$-$C(CH_3)$=C$\underline{H}$-$C(CH_3)$=CH-CH=CH-), 7.69 (1H, dd, *J* 15.0, 11.5, $CH_3C(=O)$-$C(CH_3)$=CH-$C(CH_3)$=CH-C$\underline{H}$=CH), hydrogen couplings were confirmed by a COSY experiment; δ_C (175 MHz, $CDCl_3$), 13.6 ($CH_3C(=O)$-$C(\underline{C}H_3)$=CH), 17.6 ($CH_3C(=O)$-$C(CH_3)$=CH-$C(\underline{C}H_3)$=CH-), 26.1 ($\underline{C}H_3C(=O)$-$C(CH_3)$=CH), 60.8 (-$O\underline{C}H_3$), 93.6 ($\underline{C}H_2$=C-), 104.4 (C=C-$\underline{C}$(=COMe)-C(=O)), 119.0 ($CH_3C(=O)$-$C(CH_3)$=CH-$\underline{C}(CH_3)$=CH-CH=CH-), 120.3 ($CH_3C(=O)$-$C(CH_3)$=CH-$C(CH_3)$=CH-CH=$\underline{C}$H-), 129.7 ($CH_3C(=O)$-$C(CH_3)$=CH-$C(CH_3)$=CH-$\underline{C}$H=CH-), 135.6 ($CH_3C(=O)$-$C(CH_3)$=CH-$C(CH_3)$=$\underline{C}$H-CH=CH-), and ($CH_3C(=O)$-$\underline{C}(CH_3)$=CH-$C(CH_3)$=CH-CH=CH-), 142.4 ($CH_3C(=O)$-$C(CH_3)$=$\underline{C}$H-$C(CH_3)$=CH-CH=CH), 148.2 (CH_2=$\underline{C}$-), 158.4 (C=C-C(=$\underline{C}$OMe)-C(=O)), 166.4 (C=C-C(=COMe)-$\underline{C}$(=O)), 199.5 ($CH_3\underline{C}(=O)$-$C(CH_3)$=CH-$C(CH_3)$=CH-CH=CH), carbon signal assignments were confirmed by HMBC and HMQC

experiments. ν_{max}/cm^{-1} 2951 (C-H), 1762 (C=O) 1614 (C=C). **HRMS** Calculated for $[C_{16}H_{18}O_4Na]^+$: 297.1097, observed: 297.1095.

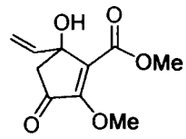
3-acryloyl-4-hydroxy-5-methylene-1H-pyrrol-2(5H)-one 293[196]



To a stirred solution of diisopropylamine (0.70 mL) in dry THF (5.40 mL) at -78°C under argon was added *n*-BuLi (1.6M in hexanes, 3.90 mL) dropwise. After 15 minutes the solution was warmed to room temperature and stirred for a further 30 minutes to give a 0.50 M solution of LDA. To a stirred solution of **261** (0.20 g, 1.59 mmol) in dry THF (20 mL) at -78°C under argon was added a freshly prepared solution of LDA (0.5M in THF, 4.40 mL, 2.20 mmol) dropwise. After stirring for 1 hour a solution of methyl acrylate **289** (0.71 g, 4.77 mmol) in dry THF (2 mL) was added dropwise. The reaction mixture was allowed to warm to ambient temperature, quenched with saturated ammonium chloride (20 mL), and the organic layer separated with DCM (5 x 50 mL). The combined organic layers were dried with $MgSO_4$ and the solvent removed *in vacuo*. The crude residue was purified by flash column chromatography (5:1 hexane / ethyl acetate) to give the title compound (0.03 g, 0.17 mmol, 11%) as a white solid. δ_H (700 MHz, Acetone) 5.17 (1H, d, *J* 3.0, C*H*$_2$=C(NH)(COH)), 5.49 (1H, d, *J* 3.0, C*H*$_2$=C(NH)(COH)), 5.59 (1H, d, *J* 10.5, C*H*$_2$=CH-CO), 6.22 (1H, d, *J* 17.0, C*H*$_2$=CH-CO), 7.38 (1H, dd, *J* 17.0, 10.5, CH_2=C*H*-CO), 8.36 (1H, br s, NH or OH), 8.94 (1H, br s, NH or OH), hydrogen couplings were confirmed by a COSY experiment; δ_C (175 MHz, Acetone) 94.0 (*C*H_2=C(NH)(COH)), 94.8 (-(C=O)-*C*(CO)(COH)-), 126.3 (*C*H_2=CH-CO), 132.9 (CH_2=*C*H-CO), 147.9 (CH_2=*C*(NH)(COH)), 162.8 (CH_2=C(NH)(*C*OH)), 166.1 (-(C=O)-C(*C*O)(COH)-), 184.7 (CH_2=CH-*C*O), carbon

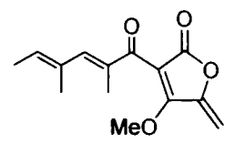
signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 3377 (N-H), 3217 (O-H), 2987 (C-H), 1735 (C=O) 1647, 1601 (C=C). ***m/z (ESI)*** 165.85 $[M+H]^+$, 155.83, 137.86 **HRMS** Calculated for $[C_8H_7O_3NNa]^+$: 188.0318 observed: 188.0318.

Methyl 5-hydroxy-2-methoxy-3-oxo-5-vinylcyclopent-1-enecarboxylate 292



Second product from method for **293**. The crude residue was purified by flash column chromatography (5:1 hexane / ethyl acetate) to give the title compound (0.02 g, 0.11 mmol, 7%) as a white solid. δ_H (700 MHz, $CDCl_3$), 2.57 (1H, d, *J* 19.0, C(O)-C*H*$_2$-CH(OH)), 2.67 (1H, d, *J* 19.0, C(O)-C*H*$_2$-C(OH)), 3.75 (1H, s, OH), 3.80 (3H, s, -C-(C=O)-OC*H*$_3$), 4.10 (3H, s, =C(-OC*H*$_3$)), 5.10 (1H, d, *J* 11.0, C*H*$_2$=CH-C(OH)), 5.20 (1H, d, *J* 17.0, C*H*$_2$=CH-C(OH)), 5.95 (1H, dd, *J* 17.0, 11.0, CH_2=C*H*-C(OH)), hydrogen couplings were confirmed by a COSY experiment; δ_C (175 MHz, $CDCl_3$), 49.0 (C(O)-*C*H_2C(OH)), 52.1 (COMe)=C-(C=O)-O*C*H_3), 59.4 (-O*C*H_3), 73.7 (CH_2=CH-*C*(OH)), 113.9 (*C*H_2=CH-C(OH)), 131.7 (COMe)=*C*-(C=O)-OCH_3), 141.2 (CH_2=*C*H-C(OH)), 156.1 (=*C*(-OCH_3)), 165.0 (C-(*C*=O)-OCH_3), 200.1 (CH_2-*C*(=O)-C(OMe)), carbon signal assignments were confirmed by HMBC and HMQC experiments. **HRMS** Calculated for $[C_{10}H_{12}O_5Na]^+$: 235.0577 and for $[C_{10}H_{11}O_4]^+$ loss of water: 195.0652, observed: 235.0575 and 195.0651 respectively.

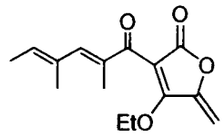
(2*E*,4*E*)-3-(2,4-dimethylhexa-2,4-dienoyl)-4-methoxy-5-methylenefuran-2(5H)-one 297[196]



To a stirred solution of dry diisopropylamine (0.70 mL) in dry THF (5.40 mL) at -78°C under argon was added *n*-BuLi (3.90 mL) dropwise. After 15 minutes the solution was warmed to room temperature and stirred for a further 30 minutes to give a 0.50 M solution of LDA. To a stirred solution of 4-methoxy-5-methylenefuran-2(5H)-one **261** (0.20 g, 1.59 mmol) in dry THF (20 mL) at -78°C under argon was added freshly prepared LDA (0.5M, in THF, 4.40 mL, 2.20 mmol) dropwise. After stirring for 1 hour a solution of ester **247** (0.71 g, 4.77 mmol) in dry THF (2 mL) was added dropwise. The reaction mixture was stirred at -78°C overnight and quenched with a solution of saturated citric acid (20 mL). The organic layer was separated, and the aqueous layer was extracted with DCM (5 x 50 mL). The combined organic layers were dried with $MgSO_4$ and the solvent removed *in vacuo*. The crude residue was purified by flash column chromatography (5:1 hexane / ethyl acetate) to give the title compound (0.04 g, 0.16 mmol, 11%) as a viscous yellow oil. δ_H (300 MHz, $CDCl_3$), 1.78 (3H, d, *J* 7.0, $C\underline{H}_3CH$=), 1.92 (3H, s, CH_3CH=C($C\underline{H}_3$)CH=), 2.07 (3H, s, CH_3CH=C(CH_3)CH=C($C\underline{H}_3$)), 3.19 (3H, s, -O$C\underline{H}_3$), 5.14 (2H, m, $C\underline{H}_2$=C(COMe)-O-(C=O)), 5.91 (1H, q, *J* 7.0, $CH_3C\underline{H}$=), 6.89 (1H, s, CH_3CH=C(CH_3)$C\underline{H}$=), hydrogen couplings were confirmed by a COSY experiment; δ_C (75 MHz, $CDCl_3$), 12.7 (CH_3CH=C(CH_3)CH=C($\underline{C}H_3$)-C=O), 14.4 ($\underline{C}H_3CH$=C(CH_3)CH=C(CH_3)-C=O), 15.8 (CH_3CH=C($\underline{C}H_3$)CH=C(CH_3)-C=O), 60.9 (-O$\underline{C}H_3$), 94.0 ($\underline{C}H_2$=C(COMe)-O-(C=O)), 105.0 ((C=O)$_2\underline{C}$=COMe), 133.8 (CH_3CH=C(CH_3)CH=$\underline{C}$(CH_3)-C=O), 134.6 (CH_3CH=$\underline{C}$(CH_3)CH=C(CH_3)-C=O), 135.9 ($CH_3\underline{C}H$=C(CH_3)CH=C(CH_3)-CH=CH-

C=O), 149.0 (CH_2=$\underline{C}$(COMe)-O-(C=O)), 151.3 (CH_3CH=C(CH_3)$\underline{C}$H=C(CH_3)-C=O), 165.2 ((C=O)$_2$C=$\underline{C}$OMe), 166.2 ($\underline{C}$=O), 192.0 (CH_3CH=C(CH_3)CH=C(CH_3)-$\underline{C}$=O)), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 2948, 2858 (C-H), 1764 (C=O) 1613 (C=C). **HRMS** Calculated for $[C_{14}H_{16}O_4Na]^+$: 271.0941, observed: 271.0937.

(2*E*,4*E*)-3-(2,4-dimethylhexa-2,4-dienoyl)-4-ethoxy-5-methylenefuran-2(5H)-one 298[196]



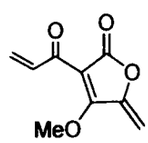
Isolated as a by-product of the reaction for the synthesis of **297.** The crude residue was purified by flash column chromatography (5:1 hexane / ethyl acetate) to give the title compound (0.03 g, 0.12 mmol, 9%) as a viscous yellow oil. δ_H (300 MHz, $CDCl_3$), 1.36 (3H, t, *J* 7.0, C$\underline{H}_3$CH$_2$O-), 1.79 (3H, d, *J* 7.0, C$\underline{H}_3$CH=), 1.93 (3H, s, CH_3CH=C(C$\underline{H}_3$)CH=), 2.07 (3H, s, CH_3CH=C(CH_3)CH=C(C$\underline{H}_3$)), 4.14 (2H, q, *J* 7.0, -OC$\underline{H}_2$CH$_3$), 5.05 (1H, d, *J* 2.5, C$\underline{H}_2$=C(COEt)-O-(C=O)), 5.15 (1H, d, *J* 2.5, C$\underline{H}_2$=C(COEt)-O-(C=O)), 5.91 (1H, q, *J* 7.0, CH_3C$\underline{H}$=), 6.91 (1H, s, CH_3CH=C(CH_3)C$\underline{H}$=), hydrogen couplings were confirmed by a COSY experiment; δ_C (75 MHz, $CDCl_3$), 12.7 (CH_3CH=C(CH_3)CH=C($\underline{C}$H$_3$)-), 14.4 ($\underline{C}$H$_3CH$=C(CH_3)CH=C(CH_3)-), 14.7 (-OCH$_2\underline{C}$H$_3$), 15.8 (CH_3CH=C($\underline{C}$H$_3$)CH=C(CH_3)), 70.0 (-O$\underline{C}$H$_2$CH$_3$), 93.9 ($\underline{C}$H$_2$=C(COMe)-O-(C=O)), 104.7 ((C=O)$_2\underline{C}$=COMe), 133.8 (CH_3CH=C(CH_3)CH=$\underline{C}$(CH_3)-), 134.3 (CH_3CH=$\underline{C}$(CH_3)CH=C(CH_3)-), 136.0 ($CH_3\underline{C}$H=C(CH_3)CH=C(CH_3)-), 149.2 (CH_2=$\underline{C}$(COEt)-O-(C=O)), 151.2 (CH_3CH=C(CH_3)$\underline{C}$H=C(CH_3)-), 164.4 ($\underline{C}$OEt), 166.4 ($\underline{C}$=O), 192.2 (CH_3CH=C(CH_3)CH=C(CH_3)-$\underline{C}$=O), carbon signal assignments were

confirmed by HMBC and HMQC experiments; ν_{max}/cm^{-1} 2949, 2856 (C-H), 1766 (C=O) 1610 (C=C). **HRMS** Calculated for $[C_{15}H_{18}O_4Na]^+$: 285.1097, observed: 285.1097.

General method for the synthesis of 291, 299, 305 and 268

To a stirred solution of 2,2,6,6-tetramethylpiperidine (TMP) (0.84 mL) in THF (5.50 mL) at -78°C under argon was added *n*-BuLi (1.6M in hexanes, 3.70 mL) dropwise. After 15 minutes the solution was warmed to room temperature and stirred for a further 30 minutes to give a 0.50 M solution of LTMP. To a stirred solution of **304** or **261**(1.4 mmol) in THF (20 mL) at -78°C under argon was added a solution of LTMP (0.5M, in THF, 4.40 mL, 2.20 mmol) dropwise. After stirring for 1 hour a solution of ester **281** or **283** or **306** or **289** (0.70 mmol) in THF (2 mL) was added dropwise. The reaction mixture was stirred at -78°C overnight and quenched with saturated citric acid (20 mL). The organic layer was separated, and the aqueous layer was extracted with DCM (5 x 50 mL). The combined organic layers were dried with $MgSO_4$ and the solvent removed *in vacuo*.

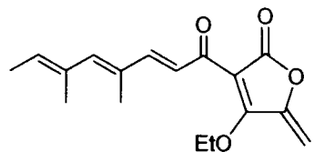
3-acryloyl-4-methyoxy-5-methylenefuran-2(5H)-one 291[196]



The product was purified by flash column chromatography (1.5:1 hexane / diethyl ether) to give the desired product (0.03 g, 0.13 mmol, 18%) as a white crystalline solid. δ_H (400 MHz, $CDCl_3$), 4.15 (3H, s, -OC$\underline{H}_3$), 5.23 (1H, d, *J* 3.0, C$\underline{H}_2$=C(COMe)-O-(C=O)), 5.28 (1H, d, *J* 3.0, C$\underline{H}_2$=C(COMe)-O-(C=O)), 5.93 (1H, d, *J* 10.5, C$\underline{H}_2$=CH-CO), 6.36 (1H, d, *J* 17.5, C$\underline{H}_2$=CH-CO), 7.04 (1H, dd, *J* 17.5, 10.5, CH_2=C$\underline{H}$-CO), hydrogen

couplings were confirmed by a COSY experiment; δ_C (175 MHz, $CDCl_3$), 63.0 (-O<u>C</u>H_3), 95.8 (<u>C</u>H_2=C(COMe)-O-(C=O)), 104.5 ((C=O)$_2$<u>C</u>=COMe), 130.8 (<u>C</u>H_2=CH-CO), 135.0 (CH_2=<u>C</u>H-CO), 148.3 (CH_2=<u>C</u>(COMe)-O-(C=O)), 166.3 (C=O), 168.6 (CH_2=C(<u>C</u>OMe)-O-(C=O)), 186.0 (CH_2=CH-<u>C</u>O), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 3014, 2974 (C-H), 1769 (C=O) 1681, 1664 (C=C). **HRMS** Calculated for $[C_9H_8O_4Na]^+$: 230.0315, observed: 203.0320. ***mp***: 69-70°C

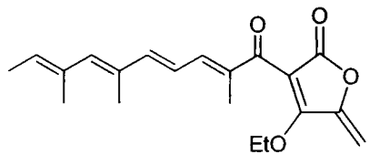
(2*E*,4*E*,6*E*)-3-(4,6-dimethylocta-2,4,6-trienoyl)-4-ethoxy-5-methylenefuran-2(5H)-one 299[196]



The crude product was purified by flash column chromatography (5:1 hexane / ethyl acetate) to give the title compound (0.05 g, 0.18 mmol, 25%) as a viscous yellow oil. δ_H (700 MHz, $CDCl_3$), 1.38 (3H, t, *J* 7.0, C<u>H</u>$_3$CH$_2$O-), 1.74 (3H, d, *J* 7.0, C<u>H</u>$_3$CH=), 1.84 (3H, s, CH_3CH=C(C<u>H</u>$_3$)CH=), 1.99 (3H, s, CH_3CH=C(CH_3)CH=C(C<u>H</u>$_3$)), 4.42 (2H, q, *J* 7.0, -OC<u>H</u>$_2$CH$_3$), 5.17 (1H, d, 1.5, C<u>H</u>$_2$=C(COMe)-O-(C=O)), 5.25 (1H, d, 1.5, C<u>H</u>$_2$=C(COMe)-O-(C=O)), 5.69 (1H, q, *J* 7.0, CH_3C<u>H</u>=), 6.38 (1H, s, CH_3CH=C(CH_3)C<u>H</u>=), 6.73 (1H, d, *J* 15.0, CH_3CH=C(CH_3)CH=C(CH_3)-CH=C<u>H</u>-CO), 7.32 (1H, d, *J* 15.0, CH_3CH=C(CH_3)CH=C(CH_3)-C<u>H</u>=CH-CO), hydrogen couplings were confirmed by a COSY experiment; δ_C (175 MHz, $CDCl_3$), 12.9 (CH_3CH=C(CH_3)CH=C(<u>C</u>H_3)-CH=CH-C=O), 13.2 (<u>C</u>H_3CH=C(CH_3)CH=C(CH_3)-CH=CH-C=O), 13.8 (-OCH$_2$<u>C</u>H_3), 15.2 (CH_3CH=C(<u>C</u>H_3)CH=C(CH_3)-CH=CH-C=O), 70.6 (-O<u>C</u>H$_2$CH$_3$), 95.5 (<u>C</u>H_2=C(COMe)-O-(C=O)), 104.5 ((C=O)$_2$<u>C</u>=COMe), 122.9 (CH_3CH=C(CH_3)CH=C(CH_3)-CH=<u>C</u>H-CO), 130.3 (CH_3CH=C(CH_3)CH=<u>C</u>(CH_3)-

CH=CH-C=O), 130.5 ($CH_3CH=\underline{C}(CH_3)CH=C(CH_3)$-CH=CH-C=O), 133.0 ($CH_3\underline{C}H=C(CH_3)CH=C(CH_3)$-CH=CH-C=O), 148.4 ($CH_2=\underline{C}$(COEt)-O-(C=O)), 145.9 ($CH_3CH=C(CH_3)\underline{C}H=C(CH_3)$-CH=CH-C=O), 151.3 ($CH_3CH=C(CH_3)CH=C(CH_3)$-$\underline{C}$H=CH-CO), 165.0 ($\underline{C}$OEt), 165.9 ($\underline{C}$=O), 185.1 ($CH_3CH=C(CH_3)CH=C(CH_3)$-CH=CH-$\underline{C}$=O)), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 2950, 2857 (C-H), 1768 (C=O) 1608 (C=C). ***m/z (ESI)*** 311.06 $[M+Na]^+$, 289.04 $[M+H]^+$, 270.96, 242.90, 214.88, 172.88 **HRMS** Calculated for $[C_{17}H_{20}O_4Na]^+$: 311.1254, observed: 311.1260.

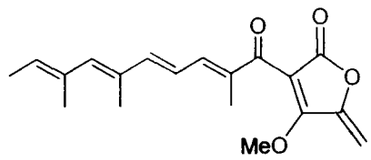
(2*E*,4*E*,6*E*,8*E*)-3-(2,6,8-trimethyldeca-2,4,6,8-tetraenoyl)-4-ethoxy-5-methylene furan-2(5H)-one 305[196]



The crude residue was purified by flash column chromatography (5:1 hexane / ethyl acetate) to give the desired product (0.07 g, 0.19 mmol, 27%) as a yellow oil. δ_H (400 MHz, $CDCl_3$), 1.34 (3H, t, *J* 7.0, $-OCH_2C\underline{H}_3$), 1.74 (3H, d, *J* 7.0, $CH_3CH=$), 1.83 (3H, s, $CH_3CH=C(C\underline{H}_3)CH=$), 1.99 (3H, s, $CH_3CH=C(CH_3)CH=C(C\underline{H}_3)$), 2.02 (3H, s, $CH_3CH=C(CH_3)CH=C(CH_3)CH=CH-CH=C(C\underline{H}_3)$-(C=O)), 4.14 (2H, q, *J* 7.0, $-OC\underline{H}_2CH_3$), 5.13 (1H, d, *J* 1.0, $C\underline{H}_2=$C(COEt)-O), 5.17 (1H, d, *J* 1.0, $C\underline{H}_2=$C(COEt)-O), 5.61 (1H, q, *J* 7.0, $CH_3C\underline{H}=$), 6.16 (1H, s, $CH_3CH=C(CH_3)C\underline{H}=$), 6.55 (1H, dd, *J* 15.0, 11.0, $=C(CH_3)CH=C\underline{H}$-CH=$C(CH_3)$-(C=O)), 6.67 (1H, d, *J* 15.0, $=C(CH_3)C\underline{H}$=CH-CH=$C(CH_3)$-(C=O)), 7.04 (1H, d, *J* 11.0, $=C(CH_3)CH=CH-C\underline{H}=C(CH_3)$-(C=O)), hydrogen couplings were confirmed by a COSY experiment; δ_C (175 MHz, $CDCl_3$), 11.6 ($CH_3CH=C(CH_3)CH=C(CH_3)CH=CH-CH=C(\underline{C}H_3)$-(C=O)), 14.0 ($CH_3CH=C(CH_3)CH=C(\underline{C}H_3)CH=CH-CH=C(CH_3)$-(C=O)), 14.2 ($OCH_2\underline{C}H_3$),

14.9 ($\underline{C}H_3CH=C(CH_3)CH=C(CH_3)CH=CH-CH=C(CH_3)-(C=O)$), 16.6 ($CH_3CH=C(\underline{C}H_3)CH=C(CH_3)CH=CH-CH=C(CH_3)-(C=O)$), 70.1 ($O\underline{C}H_2CH_3$), 93.9 ($\underline{C}H_2=C(COEt)-O-(C=O)$), 104.8 ($CH_2=\underline{C}(COMe)-O-(C=O)$), 122.4 ($CH_3CH=C(CH_3)CH=C(CH_3)CH=\underline{C}H-CH=C(CH_3)-(C=O)$), 129.6 ($CH_3\underline{C}H=C(CH_3)CH=C(CH_3)-$), 132.6 ($CH_3CH=C(CH_3)CH=\underline{C}(CH_3)-$), 134.0 ($CH_3CH=\underline{C}(CH_3)CH=C(CH_3)-$), 135.4 ($CH_3CH=C(CH_3)CH=C(CH_3)CH=CH-CH=\underline{C}(CH_3)-(C=O)$), 142.4 ($CH_3CH=C(CH_3)\underline{C}H=C(CH_3)CH=CH-CH=C(CH_3)-(C=O)$), 146.9 ($CH_3CH=C(CH_3)CH=C(CH_3)CH=CH-\underline{C}H=C(CH_3)-(C=O)$), 149.4 ($(C=O)_2\underline{C}=COEt$), 149.5 ($CH_3CH=C(CH_3)CH=C(CH_3)\underline{C}H=CH-CH=C(CH_3)-(C=O)$), 164.4 ($(C=O)_2C=\underline{C}OEt$), 166.6 ($\underline{C}=O$), 190.6 ($CH_3CH=C(CH_3)CH=C(CH_3)CH=CH-CH=C(CH_3)-(\underline{C}=O)$), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 2921 (C-H), 1768 (C=O), 1667 (C=C). **HRMS** Calculated for $[C_{20}H_{24}O_4Na]^+$: 351.1561, observed: 351.1573.

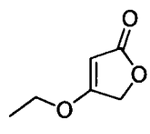
(2*E*,4*E*,6*E*,8*E*)-3-(2,6,8-trimethyldeca-2,4,6,8-tetraenoyl)-4-methoxy-5-methylene furan-2(5H)-one 268[196]



The product was purified by flash column chromatography (5:1 hexane / ethyl acetate) to give the desired product (0.05 g, 0.16 mmol, 23%) as a yellow oil. δ_H (400 MHz, $CDCl_3$), 1.67 (3H, d, *J* 7.0, $CH_3CH=$), 1.76 (3H, s, $CH_3CH=C(C\underline{H}_3)CH=$), 1.92 (3H, s, $CH_3CH=C(CH_3)CH=C(C\underline{H}_3)$), 1.95 (3H, s, $CH_3CH=C(CH_3)CH=C(CH_3)CH=CH-CH=C(C\underline{H}_3)-(C=O)$), 3.85 (3H, s $-OC\underline{H}_3$), 5.08 (2H, m, $C\underline{H}_2=C(COMe)-O-(C=O)$), 5.55 (1H, q, *J* 7.0, $CH_3C\underline{H}=$), 6.09 (1H, s, $CH_3CH=C(CH_3)C\underline{H}=$), 6.48 (1H, dd, *J* 15.0, 11.0, $=C(CH_3)CH=C\underline{H}-CH=C(CH_3)-(C=O)$), 6.61 (1H, d, *J* 15.0,

$CH_3CH=C(CH_3)CH=C(CH_3)C\underline{H}=CH-CH=C(CH_3)-(C=O)$), 7.21 (1H, d, *J* 11.0, $CH_3CH=C(CH_3)CH=C(CH_3)CH=CH-C\underline{H}=C(CH_3)-(C=O)$), hydrogen couplings were confirmed by a COSY experiment; δ_C (175 MHz, $CDCl_3$), 11.5 ($=C(CH_3)-CH=CH=CH(\underline{C}H_3)-C=O$), 13.9 ($CH_3CH=C(CH_3)CH=C(\underline{C}H_3)-CH=CH=CH(CH_3)-C=O$), 14.1 ($\underline{C}H_3CH=C(CH_3)CH=C(CH_3)-CH=CH=CH(CH_3)-C=O$), 16.4 ($CH_3CH=C(\underline{C}H_3)CH=C(CH_3)-CH=CH=CH(CH_3)-C=O$), 60.8 ($-O\underline{C}H_3$), 94.0 ($\underline{C}H_2=C(COMe)-O-(C=O)$), 104.9 ($(C=O)_2\underline{C}=COMe$), 122.2 ($CH_3CH=C(CH_3)CH=C(CH_3)CH=\underline{C}H=CH-C(CH_3)-(C=O)$), 129.5 ($CH_3\underline{C}H=C(CH_3)CH=C(CH_3)CH=CH=CH-C(CH_3)-(C=O)$), 132.4 ($CH_3CH=C(CH_3)CH=\underline{C}(CH_3)-$), 133.9 ($CH_3CH=\underline{C}(CH_3)CH=C(CH_3)-CH=CH=CH(CH_3)-CO$), 135.3 ($CH_3CH=C(CH_3)CH=C(CH_3)CH=CH-CH=\underline{C}(CH_3)-(C=O)$), 142.4 ($CH_3CH=C(CH_3)\underline{C}H=C(CH_3)-CH=CH=CH(CH_3)-CO$), 146.9 ($CH_3CH=C(CH_3)CH=C(CH_3)CH=CH=\underline{C}H-C(CH_3)-(C=O)$), 149.0 ($CH_2=\underline{C}(COMe)-O-(C=O)$), 149.4 ($CH_3CH=C(CH_3)CH=C(CH_3)\underline{C}H=CH=CH-C(CH_3)-(C=O)$), 165.0 ($\underline{C}OMe$), 166.3 ($\underline{C}=O$), 190.3 ($CH_3CH=C(CH_3)CH=C(CH_3)CH=CH=CH-C(CH_3)-(\underline{C}=O)$), carbon signal assignments were confirmed by HMBC and HMQC experiments. ν_{max}/cm^{-1} 2917, 2857 (C-H), 1766 (C=O) 1667 (C=C). **HRMS** Calculated for $[C_{19}H_{22}NaO_4]^+$: 337.1416, observed: 337.1417.

4-ethoxyfuran-2(5H)-one 302[198]



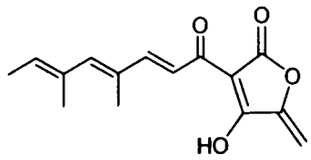
A solution of tetronic acid **256** (5.00 g, 50.0 mmol), *p*-toluenesulfonic acid (0.50 g, cat.) and ethanol (10 mL, 200 mmol) in toluene (150 mL) was heated to reflux in a flask equipped with a reflux condenser and Dean-Stark separator. The reaction was heated to

reflux for 24 hours then cooled to ambient temperature. The solvent was removed *in vacuo* and the product purified by flash column chromatography (1:1 ethyl acetate / hexane) to give the title compound (5.50 g, 42.97 mmol, 86%) as an off white oil. δ_H (400 MHz, $CDCl_3$) 1.38 (3H, t, *J* 7.0, -$OCH_2C\underline{H}_3$), 4.07 (2H, q, *J* 7.0, -$OC\underline{H}_2CH_3$), 4.58 (2H, s, -$OC\underline{H}_2C$(OEt)), 5.03 (1H, s, C=C$\underline{H}$-(C=O)O-), hydrogen couplings were confirmed by a COSY experiment; δ_C (100 MHz, $CDCl_3$), 14.2 (-$OCH_2\underline{C}H_3$), 68.1 ((C=O)O$\underline{C}H_2$C(OEt)), 68.9 (-$O\underline{C}H_2CH_3$), 88.8 (C=$\underline{C}$H-(C=O)O-), 173.9 ($\underline{C}$=O), 179.6 ($\underline{C}$OEt), carbon signal assignments were confirmed by an HMQC experiment. ν_{max}/cm^{-1} 2986 (C-H), 1772 (C=O), 1737, 1617 (C=C). ***m/z (ESI)*** 150.90 $[M+Na]^+$, 129.01 $[M+H]^+$, 168.84. **HRMS** Calculated for $[C_6H_8O_3]^+$: 129.0546, observed: 129.0544.

General method for the synthesis of 206 and 207

To a solution of tetronate **267** or **268** (15 mg, 0.06 mmol), in d_6-DMSO (0.75 μL) was added LiCl (24 mg, 0.60 mmol) and the reaction was heated at 50°C for 16 hours after which time a ^{1}H NMR spectrum confirmed the consumption of the starting material. Water (40 ml) was added and the solvent removed by freeze drying. The product was dissolved in 80:20 acetonitrile / water and purified by HPLC.

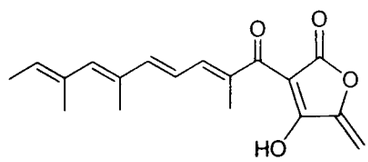
(2*E*,4*E*,6*E*)-3-(4,6-dimethylocta-2,4,6-trienoyl)-4-hydroxy-5-methylenefuran-2(5H)-one 206[15]



100% conversion judged by the ^{1}H NMR spectrum, 2 mg purified by HPLC (see Table 11). δ_H (700 MHz, $CDCl_3$), 1.82 (3H, d, *J* 7.0, $C\underline{H}_3CH$=), 1.92 (3H, s, CH_3CH=C($C\underline{H}_3$)CH=), 2.09 (3H, s, CH_3CH=C(CH_3)CH=C($C\underline{H}_3$)), 2.41 (1H, s, -OH),

5.15 (1H, s, CH_2=C-(O)-COH), 5.41 (1H, s, CH_2=C-(O)-COH), 5.90 (1H, q, *J* 7.0. $CH_3C\underline{H}$=), 6.58 (1H, s, CH_3CH=C(CH_3)C$\underline{H}$=), 7.04 (1H, d, *J* 15.5. (CH_3CH=C(CH_3)CH=C(CH_3)-CH=C$\underline{H}$-C=O), 7.78 (1H, d, *J* 15.5 CH_3CH=C(CH_3)CH=C(CH_3)-C$\underline{H}$=CH-C=O), hydrogen couplings were confirmed by a COSY experiment; δ_C (175 MHz, $CDCl_3$), 13.4 (CH_3CH=C(CH_3)CH=C($\underline{C}H_3$)-CH=CH-C=O), 13.8 ($\underline{C}H_3CH$=C(CH_3)CH=C(CH_3)-CH=CH-C=O), 15.6 (CH_3CH=C($\underline{C}H_3$)CH=C(CH_3)-CH=CH-C=O), 95.3 ($\underline{C}H_2$=C-(O)-COH), *carbon at about 104 not visable, 114.8 (CH_3CH=C(CH_3)CH=C(CH_3)-CH=$\underline{C}$H-C=O), 132.3 (CH_3CH=C(CH_3)CH=$\underline{C}$(CH_3)-CH=CH-C=O), 134.0 (CH_3CH=$\underline{C}$(CH_3)CH=C(CH_3)-CH=CH-C=O), 136.0 ($CH_3\underline{C}H$=C(CH_3)CH=C(CH_3)-CH=CH-C=O), 150.1 (CH_2=$\underline{C}$-(O)-COH)), 151.5 (CH_3CH=C(CH_3)$\underline{C}$H=C(CH_3)-CH=CH-C=O), 156.2 (CH_3CH=C(CH_3)CH=C(CH_3)-$\underline{C}$H=CH-C=O), *(C=O) not visible, 180.6 (CH_3CH=C(CH_3)CH=C(CH_3)-CH=CH-$\underline{C}$=O), 186.0 (CH_2=C-(O)-$\underline{C}$OH), carbon signal assignments were confirmed by HMBC and HMQC experiments. **HRMS** Calculated for $[C_{15}H_{16}O_4]^+$: 261.1121, observed: 261.1121.

(2*E*,4*E*,6*E*,8*E*)-3-(2,6,8-trimethyldeca-2,4,6,8-tetraenoyl)-4-hydroxy-5-methylenefuran-2(5H)-one 207[15]



100% conversion judged by ^{1}H NMR spectroscopy, 2 mg purified by HPLC (see Table 11). δ_H (700 MHz, $CDCl_3$), 1.77 (3H, d, *J* 7.0, C$\underline{H}_3CH$=C(CH_3)CH=C(CH_3)CH=CH-CH=C(CH_3)-C=O), 1.86 (3H, s, CH_3CH=C(C$\underline{H}_3$)CH=C(CH_3)CH=CH-CH=C(CH_3)-C=O), 2.03 (3H, s, CH_3CH=C(CH_3)CH=C(C$\underline{H}_3$)CH=CH-CH=C(CH_3)-C=O), 2.07 (3H, s, CH_3CH=C(CH_3)CH=C(CH_3)CH=CH-CH=C(C$\underline{H}_3$)-C=O), 5.21 (1H, br s, C$\underline{H}_2$=C(-

O)-(COH)), 5.45 (1H, br s, C$\underline{H_2}$=C(-O)-(COH)), 5.68 (1H, q, *J* 7.0, $CH_3C\underline{H}$=C(CH_3)CH=C(CH_3)-CH=CH-CH=C(CH_3)C=O), 6.28 (1H, s, CH_3CH=C(CH_3)C$\underline{H}$=C(CH_3)-CH=CH-CH=C(CH_3)C=O), 6.62 (1H, dd, *J* 14.5, 11.5, CH_3CH=C(CH_3)CH=C(CH_3)-CH=C$\underline{H}$-CH=C(CH_3)C=O), 6.89 (1H, d, *J* 14.5 CH_3CH=C(CH_3)CH=C(CH_3)-C$\underline{H}$=CH-CH=C(CH_3)C=O), 8.28 (1H, br s H_3CH=C(CH_3)CH=C(CH_3)-CH=CH-C$\underline{H}$=C(CH_3)C=O), O-H signal not viable on spectrum, hydrogen couplings were confirmed by a COSY experiment; δ_C (175 MHz, $CDCl_3$), 12.1 (CH_3CH=C(CH_3)CH=C(CH_3)CH=CH-CH=C($\underline{C}H_3$)-C=O), 13.9 (CH_3CH=C(CH_3)CH=C($\underline{C}H_3$)CH=CH-CH=), 14.3 ($\underline{C}H_3CH$=C(CH_3)CH=C(CH_3)CH=), 16.5 (CH_3CH=C($\underline{C}H_3$)CH=C(CH_3)CH=), 95.5 ($\underline{C}H_2$=C(-O)-(COH)), *Carbon at approx 105 not visible, 122.7 (CH_3CH=C(CH_3)CH=C(CH_3)CH=$\underline{C}$H-CH=C(CH_3)-C=O), 128.6 (=C(CH_3)CH=CH-CH=$\underline{C}$(CH_3)-C=O), 130.4 ($CH_3\underline{C}H$=C(CH_3)CH=C(CH_3)CH=CH-CH=C(CH_3)-C=O), 132.6 (CH_3CH=C(CH_3)CH=$\underline{C}$(CH_3)CH=CH-CH=C(CH_3)-C=O), 134.0 (CH_3CH=$\underline{C}$(CH_3)CH=C(CH_3)CH=CH-CH), 143.6 (CH_3CH=C(CH_3)$\underline{C}$H=C(CH_3)CH=CH-CH=C(CH_3)-C=O), 149.2 (CH_2=$\underline{C}$(-O)-(COH)), 149.3 (=C(CH_3)CH=CH-$\underline{C}$H=C(CH_3)-C=O), 152.1 (=C(CH_3)$\underline{C}$H=CH-CH=), 164.8 ((C=O)-C($\underline{C}$=O)(COH)), 185.8 (CH_2=C(-O)-($\underline{C}$OH)), 190.2 (-CH=CH-CH=C(CH_3)$\underline{C}$=O), carbon signal assignments were confirmed by HMBC and HMQC experiments. **HRMS** Calculated for $[C_{19}H_{18}O_4]^-$: 299.1278, observed: 299.1280.

Time (mins)	Solvent A (H_2O + 0.1% TFA), %	Solvent B (Acetonitrile + 0.1% TFA), %
0	25	75
15	20	80
20	20	80
25	0	100
30	25	75
40	25	75

Table 11: HPLC method for purification of **206** and **207**

Column: 100 x 21 mm, 5μm, Agilent Xorbax C18 column equipped with a 10 x 21 mm guard column

Flow Rate: 5.0 mL / min

Temperature: room temperature

Detection wavelength: 210 nm

Total run time: 40 minutes

Retention time 20 to 21 minutes

Biomimetic Experiments

Biomimetic conditions 1

A solution of tetraene **305** (5 mg, 0.02 mmol) and **267** (4 mg, 0.02 mmol) was heated to 100°C in dry toluene (3 mL) for 72 hours. Aliquots (100 μl) were taken after 24, 48 and 72 hours and analysed by LC-MS (LC-MS conditions are shown in Table 14). No product was observed by LC-MS over the 72 hour period.

Biomimetic conditions 2

A solution of tetraene **305** (5 mg, 0.02 mmol) and triene **267** (4 mg, 0.02 mmol) was heated in a sealed tube at 100°C in dry toluene (3 mL) for 72 hours. After this time the temperature was increased to 125°C and the reaction heated for a further 15 days. Aliquots (100 μL) were taken after 1, 2, 3, 5, 7, 11, 14 and 18 days and analysed by LC-MS. LC-MS conditions shown in Table 14, results shown in Table 12.

Time[a]	m/z
1	549, 603, 823
2	549, 603, 803
3	549, 603, 657, 823
5	549, 603, 657, 823
7	549, 603, 657, 823
11	549, 603, 657, 823
14	549, 603, 657, 823
18	549, 603, 657, 823

Table 12: Dimers observed by LC-MS in toluene at different time intervals from sealed tube reaction

[a]Time in days

Biomimetic conditions 3

A solution of tetraene **305** or **268** (5 mg, 0.02 mmol) and triene **267** (4 mg, 0.02 mmol) was heated in a microwave tube at 250°C in dry toluene (3 mL), nonane (3 mL), isopropanol (3 mL) or water/toluene (1:1, 3 mL). Aliquots (100 μL) were taken after 0.5, 1.5, 3.5 and 7.5 hours and analysed by LC-MS as shown in Table 13. LC-MS conditions shown in Table 14.

Solvent	Time[c]	m/z	Time[c]	m/z	Time[c]	m/z	Time[c]	m/z
Toluene[a]	0.5	549, 603	1.5	549, 603	3.5	549, 603, 657	7.5	549, 603, 657
Nonane[b]	0.5	549, 589, 629	1.5	549, 589, 629	3.5	549, 589, 629	7.5	n/a[d]
Iso-propanol[b]	0.5	n/d[e]	1.5	n/d[e]	3.5	n/a[d]	7.5	n/a[d]
Toluene/ water mix[b]	0.5	549, 589, 629	1.5	549, 589, 629	3.5	549, 589, 629	7.5	n/a[d]

Table 13: Dimers observed by LC-MS in various solvents at different time intervals in microwave

[a]Uses ethyl protected tetraene **305** and methyl protected triene **267**. [b] Uses methyl protected tetraene **268** and methyl protected triene **267**. [c] time in hours. [d] not attempted; [e]n/d none detected,

Time (mins)	Solvent A (H_2O + 0.1% TFA), %	Solvent B (Acetonitrile + 0.1% TFA), %
0	20	80
5	20	80
20	5	95
40	5	95
55	0	100
60	0	100
62	20	80

Table 14: LC-MS method for analysis of biomimetic experiments

Column: 4.6x 150 mm Agilent Xorbax C8 column

Flow Rate: 1.0 mL / min

Temperature: room temperature

Detection wavelength: 210 nm

Total run time: 75 minutes

Chapter 8: References

(1) Dewick, P. M. *Medicinal Natural Products*; 1st ed.; John Wiley & Sons Ltd.: Chichester, 2002.
(2) Staunton, J. *Chem. Rev.* **1997**, *97*, 2611-2629.
(3) Hori, K.; Yamamoto, Y.; Minetoki, T.; Kurotsu, T.; Kanda, M. *J. Biochem.* **1989**, 639-645.
(4) Keatinge-Clay, A. T. *Chem. Biol.* **2007**, 898-908.
(5) Challis, G. L.; Ravel, J. *FEMS Microbiol. Lett.* **2000**, *187*, 111-114.
(6) Lautru, S.; Deeth, R. J.; Bailey, L. M.; Challis, G. L. *Nat. Chem. Biol.* **2005**, *1*, 265-269.
(7) Luo, L.; Kohli, R. M.; Onishi, M.; Linne, U.; Marahiel, M. A.; Walsh, C. T. *Biochemistry* **2002**, *41*, 9184-9196.
(8) Trauger, J. W.; Kohli, R. M.; Mootz, H. D.; Marahiel, M. A.; Walsh, C. T. *Nature* **2000**, *407*, 215-218.
(9) Lautru, S.; Challis, G. L. *Microbiology* **2004**, *150*, 1629-1636.
(10) Rapoport, H.; Holden, K. G. *J. Am. Chem. Soc.* **1962**, *84*, 635-642.
(11) Williamson, N. R.; Simonsen, H. T.; Ahmed, R. A.; Goldet, G.; Slater, H.; Woodley, L.; Leeper, F. J.; Salmond, G. P. *Mol. Microbiol.* **2005**, *56*, 971-989.
(12) Roush, W. R.; Sciotti, R. J. *J. Am. Chem. Soc.* **1998**, *120*, 7411-7419.
(13) Roush, W. R.; Sciotti, R. J. *J. Org. Chem.* **1998**, *63*, 5473-5482.
(14) Snider, B. B.; Zou, Y. *Org. Lett.* **2005**, *7*, 4939-4941.
(15) Zapf, C. W.; Harrison, B. A.; Drahl, C.; Sorenson, E. J. *Angew. Chem. Int. Ed.* **2005**, *44*, 6533-6537.
(16) Kieser, T.; Chater, K. F.; Bibb, M. J.; Buttner, M. J.; Hopwood, D. A. *Practical Streptomyces Genetics;*; 1st ed.; The John Innes Foundation: Norwich, 2000.
(17) Morgan, E. N.; Tanner, E. M. *J. Chem. Soc.* **1955**, 3305-3305.
(18) Castra, A. J.; Corwin, A. H.; Waxham, F. J.; Beilby, A. L. *J. Org. Chem.* **1959**, *24*, 455-459.
(19) Hearn, W. R.; Medina-Castro, J.; Elson, M. K. *Nature* **1968**, *220*, 170-170
(20) Gerber, N. N. *CRC Crit. Rev. Microbiol.* **1975**, *3*, 469-485.
(21) Fürstner, A.; Weintritt, H. *J. Am. Chem. Soc.* **1998**, *120*, 2817-2825.
(22) Furihata, K.; Hayakawa, Y.; Kawakami, K.; Sero, H. *Tetrahedron Lett.* **1992**, *33*, 2701-2704.
(23) Cerdeno, A. M.; Bibb, M. J.; Challis, G. L. *Chem. Biol.* **2001**, *8*, 817-829.
(24) Bennett, J. W.; Bentley, R. *Ad. in Appl. Microbiol.* **2000**, *47*, 1-32.
(25) Fürstner, A. *Angew. Chem. Int. Ed.* **2003**, *42*, 3582-3603.
(26) Brown, D.; Griffiths, D.; Rider, M. E.; Smith, R. C. *J. Chem. Soc.; Pekin Trans. I* **1986**, 455-463.
(27) Boger, D. L.; Patel, M. *Tetrahedron Lett.* **1987**, *28*, 2499-2502.
(28) Hearn, W. R.; Elson, M. K.; Williams, R. H.; Medina-Castro, J. *J. Org. Chem.* **1970**, *35*, 142-146.
(29) Isaka, M.; Jaturapat, A.; Kramyu, J.; Tanticharoen, M.; Thebtaranonth, Y. *Antimicrob. Agents Chemother.* **2002**, *46*, 1112-1113.
(30) Gerber, N. N. *J. Antibiot.* **1975**, *28*, 194-199.
(31) Melo, P. d. S.; Durán, N.; Haun, M. *Toxicology Lett.* **2000**, *116*, 237-242.
(32) Han, S. B.; Kim, H. M.; Kim, Y. H.; Lee, C. W.; Jang, E.; Som, K. H.; Kim, S. U.; Kim, Y. K. *Int. J. Immunopharma.* **1998**, *20*, 1-13.

(33) Azuma, T.; Watanabe, N.; Yagisawa, H.; Hirata, H.; Iwamura, M.; Kobayashi, Y. *Immunopharm.* **2000**, *46*, 29-37.
(34) Mortellaro, A.; Songia, S.; Gnocchi, P.; Ferrari, M.; Forasiero, C.; D'Alessio, R.; Isetta, A.; Colotta, F.; Golay, J. *J. Immunol.* **1999**, *162*, 7102-7109.
(35) Blades, K.; Percy, J. M. *Tetrahedron Lett.* **1998**, *39*, 9085-9088.
(36) Han, S. B.; Park, S. H.; Jean, Y. J.; Kim, Y. K.; Kim, H. M.; Yang, K. H. *J. Pharmacol. Exp. Thera.* **2001**, *299*, 415-425.
(37) Han, S.-B.; Lee, C. W.; Yoon, Y. D.; Kang, J. S.; Lee, K. H.; Yoon, W. K.; Kim, Y. K.; Lee, K.; Park, S.-K.; Kim, H. M. *Biochemical Pharmacol.* **2005**, *70*, 1518-1526.
(38) Pandey, R.; Chander, R.; Sainis, K. B. *Int. Immunopharma.* **2003**, *3*, 1599-1607.
(39) Stepkowski, S. M.; Erwin-Cohen, R. A.; Behbod, F.; Wang, M.-E.; Qu, X.; Tejpal, N.; Nagy, Z. S.; Kahan, B. D.; Kirken, R. A. *Blood* **2002**, *99*, 680-689.
(40) Yamamoto, D.; Kiyozuka, Y.; Uemura, Y.; Yamamoto, C.; Takemoto, H.; Hirata, H.; Tanaka, K.; Hioki, K.; Tsubura, A. *J. Cancer Res. Clin. Oncol.* **2000**, *126*, 191-197.
(41) Yamamoto, C.; Takemoto, H.; Kuno, H.; Yamamoto, D.; Tsubura, A.; Kamata, K.; Hirata, H.; Yamamoto, A.; Kano, H.; Seki, T.; Inoue, K. *Hepatology* **1999**, *30*, 894-902.
(42) Diaz-Ruiz, C.; Montaner, B.; Perez-Tomas, R. *Histol. Histopathol.* **2001**, *16*, 415-421.
(43) Montaner, B.; Perez-Tomas, R. *Life Sci.* **2001**, *68*, 2025-2026.
(44) Montaner, B.; Navarro, S.; Pipué, M.; Vilasecca, M.; Martinell, M.; Giralt, E.; Gill, J.; Pérez-Thomas, R. *British J. Pharmacol.* **2000**, *131*, 585-593.
(45) Boger, D. L.; Patel, M. *J. Org. Chem.* **1988**, *53*, 1405-1415.
(46) Ho, T.-F.; Ma, C.-J.; Lu, C.-H.; Tsai, Y.-T.; Wei, Y.-H.; Chang, J.-S.; Lai, J.-K.; Cheuh, P.-J.; Yeh, C.-T.; Tang, P.-C.; Chang, J. T.; Ko, J.-L.; Liu, F.-S.; Yen, H. E.; Chang, C.-C. *Toxicol. Appl. Pharmacol.* **2007**, *225*, 318-328.
(47) Zhang, J.; Shen, Y.; Liu, J.; Wei, D. *Biochemical Pharmacol.* **2005**, *69*, 407-414.
(48) Borah, S.; Melvin, M. S.; Lindquist, N.; Manderville, R. A. *J. Am. Chem. Soc.* **1998**, *120*, 4557-4562
(49) Melvin, M.; Thomlinson, J. T.; Sulata, G. R.; Kucera, G. L.; Lindquist, N.; Maderville, R. A. *J. Am. Chem. Soc.* **2000**, *122*, 6333-6334.
(50) Melvin, M. S.; Calcutt, M. W.; Noftle, R. E.; Manderville, R. A. *Chem. Res. Toxicol.* **2002**, *15*, 742-748.
(51) Park, G.; Tomlinson, J. T.; Melvin, M. S.; Wright, M. W.; Day, C. S.; R.A. *Org. Lett.* **2003**, *5*, 113-116.
(52) Melvin, M.; Thomlinson, J. T.; Sulata, G. R.; Kucera, G. L.; Lindquist, N.; Maderville, R. A. *J. Am. Chem. Soc.* **2000**, *122*, 6333-6334
(53) Melvin, M. S.; Tominson, J. T.; Park, G.; Day, C. S.; Saluta, G. R.; Kucera, G. L.; Manderville, R. A. *Chem. Res. Toxicol.* **2002**, *15*, 734 - 741
(54) Melvin, M. S.; Wooton, K. E.; Rich, C. C.; Salutra, G. R.; Kucera, G. L.; Lindquist, N.; Manderville, R. A. *J. Inorg. Biochem.* **2001**, *87*, 129-135.
(55) Fürstner, A.; Grabowski, E. J. *ChemBioChem* **2001**, *2*, 706-709.
(56) Pérez-Tomás, R.; Montaner, B.; Llagostera, E.; Soto-Cerrato, V. *Biochemical Pharmacol.* **2003**, *66*, 1447-1452.
(57) Trudel, S.; Li, Z. H.; Rauw, J.; Tiedemann, R. E.; Wen, X. Y.; Stewart, A. K. *Blood* **2007**, *109*, 5430-5438.

(58) Pérez-Galán, P.; Roué, G.; Villamor, N.; Campo, E.; Colomer, D. *Blood* **2007** *109*, 4441-4449.
(59) Matsuya, H.; Okamoto, M.; Ochi, T.; Nishikawa, A.; Shimizu, S.; Kataoka, T.; Nagai, K.; Wasserman, H. H.; Ohkuma, S. *Biochemical Pharmacol.* **2000**, *16*, 1855-1863.
(60) Tanigaki, K.; Sasaki, S.; Ohkuma, S. *FEBS Lett.* **2003**, *537*, 79-84.
(61) Rudd, B. A. M.; Hopwood, D. A. *J. Gen Microbiol.* **1980**, *119*, 333-340.
(62) Feitelson, J. S.; Malpartida, F.; Hopwood, D. A.; J. Gen. Microbiol. 1985, 2431-2441 *J. Gen Microbiol.* **1985**, *131*, 2431-2441.
(63) Chakraburtty, R.; Bibb, M. J. *J. Bacteriol.* **1997**, *179*, 5854-5861.
(64) Bentley, S. D.; Chater, K. F.; Cerdeño-Tarranga, A. M.; Challis, G. L.; Thomson, N. P.; James, K. D.; Harris, D. E.; Quail, M. A.; Kieser, H.; Harper, D.; Bateman, A.; Brown, S.; Chandra, G.; Chen, C.; Collins, M.; Cronin, A.; Fraser, A.; Goble, A.; Hidalgo, J.; Hornsby, T.; Howarth, S.; Huang, C. H.; Kieser, T.; L. Larke, L.; Murphy, L.; Oliver, K.; O'Neil, S.; Rabbinowitsch, E.; Rajandream, M. A.; Rutherford, K.; Rutter, S.; K Seeger; Saunders, D.; Squares, R.; Taylor, K.; Warren, T.; Wietzorrek, A.; Woodward, J.; Barrel, B. G.; Parkhill, J.; Hopwood, D. A. *Nature* **2002**, *417*, 141-147.
(65) Wasserman, H. H.; Shaw, C. K.; Sykes, R. J. *Tetrahedron Lett.* **1974**, *15*, 2787-2790.
(66) Wasserman, H. H.; Synkes, R. J.; Peverada, P.; Shaw, C. K. *J. Am. Chem. Soc.* **1973**, *95*, 6874-6575.
(67) Cushley, R. J.; Anderson, D. R.; Lipsky, S. R.; Sykes, R. J.; Wasserman, H. H. *J. Am. Chem. Soc.* **1971**, *93*, 6284-6286.
(68) Gerber, N.; McInnes, A.; Smith, D.; Walter, J.; Wright, J.; Vining, L. *Can. J. Chem.* **1978**, *56*, 1155-1163.
(69) Shrimpton, D. M.; Marks, G. S.; Bogorad, L. *Biochimica Biophysica Acta* **1963**, *71*, 408-415.
(70) Gerber, N.; McInnes, A.; Smith, D.; Walter, J.; Wright, J.; Vining, L. *Can. J. Chem.* **1987**, *56*, 1155-1163.
(71) Odulate, O.; Stanley, A. E.; Challis, G. L. *Unpublished data.*
(72) Stanley, A. E.; Walton, L. J.; Zerikly, M. K.; Corre, C.; Challis, G. L. *Chem. Commun.* **2006**, 3981-3983.
(73) Thomas, M. G.; Burkart, M. D.; Walsh, C. T. *Chem. Biol.* **2002**, *9*, 171-184
(74) Mo, S.; Sydor, P. K.; Corre, C.; Alhamadsheh, M. M.; Stanley, A. E.; Haynes, S. W.; Song, L.; Reynolds, K. A.; Challis, G. L. *Chem. Biol.* **2008**, *15*, 137-148.
(75) Mo, S.; Kim, B. S.; Reynolds, K. A. *Chem. Biol.* **2005**, *12*, 191-200.
(76) Garneau-Tsodikova, S.; Dorrestein, P. C.; Kelleher, N. L.; Walsh, C. T. *J. Am. Chem. Soc.* **2006**, *128*, 12600-12601.
(77) Sydor, P. K.; Challis, G. L. *unpublished data.*
(78) Stanley, A. E.; Challis, G. L.; Zydek, P. K.; Corre, C. *unpublished data* .
(79) Dairi, K.; Tripathy, S.; Attardo, G.; Lavallée, J.-F. *Tetrahedron Lett.* **2006**, *47*, 2605-2606.
(80) Wasserman, H. H.; Rodgers, G. C.; Keith, D. D. *Tetrahedron* **1976**, *32*, 1851-1854.
(81) Wasserman, H. H.; Keith, D. D.; Rodgers, G. C. *Tetrahedron* **1976**, *32*, 1855-1861.
(82) Harris, A. K.; Williamson, N. R.; Slater, H.; Cox, A.; Abbasi, S.; Foulds, I.; Simonson, H. T.; Leeper, F. J.; Salmond, G. P. *Microbiology* **2004**, *150*, 3547-3560.

(83) Haynes, S. W.; Sydor, P. K.; Stanley, A. E.; Song, L.; Challis, G. L. *Chem. Commun.* **2008**, *in press*.
(84) Kresze, G.; Morper, M.; Bijev, A. *Tetrahedron Lett.* **1977**, *26*, 2259-2262.
(85) Castro, A. J.; Deck, J. F.; Hugo, M. T.; Lowe, E. J.; Jr, J. P. M.; Pfeiffer, R. J. *J. Org. Chem.* **1963**, *28*, 857-860.
(86) Wasserman, H. H.; Lombardo, L. J. *Tetrahedron Lett.* **1989**, *30*, 1725-1728.
(87) Wasserman, H. H.; Friedland, D. J.; Morrison, D. A. *Tetrahedron Lett.* **1968**, *6*, 641-644.
(88) Fürstner, A.; Grabowski, J.; Lehmann, C. W. *J. Org. Chem.* **1999**, *64*, 8275-8280.
(89) Wasserman, H. H.; Power, P.; Peterson, A. K. *Tetrahedron Lett.* **1996**, *37*, 6657-6660.
(90) Wasserman, H. H.; Petersen, A. K.; Xia, M.; Wang, J. *Tetrahedron Lett.* **1999**, *40*, 7587-7589.
(91) Wasserman, H. H.; Xia, M.; Wang, J.; Petersen, A. K.; Jorgensen, M. *Tetrahedron Lett.* **1999**, *40*, 6145-6148.
(92) Wasserman, H. H.; Xia, M.; Wang, J.; Petersen, A. K.; Jorgensen, M. *Tetrahedron Lett.* **1999**, *40*, 6145-6148.
(93) Wasserman, H. H.; Keith, D. D.; Nadelson, J. *Tetrahedron* **1976**, *32*, 1867-1871.
(94) Fürstner, A.; Szillat, H.; Gabor, B.; Mynott, R. *J. Am. Chem. Soc.* **1998**, *120*, 8305-8314.
(95) Fürstner, A.; Krause, H. *J. Org. Chem.* **1999**, *64*, 8281-8286.
(96) D'Allessio, R.; Rossi, A. *Synlett* **1996**, 512-541.
(97) D'Allessio, R.; Bargiotti, A.; Carlini, O.; Calotta, F.; Ferrari, M.; Gnocchi, P.; Isetta, A.; Monelli, N.; Motta, O.; Rossi, A.; Rossi, M.; Tibolla, M.; Vanotti, E. *J. Med. Chem.* **2000**, *43*, 2557-2565.
(98) Fürstner, A. *ChemBioChem* **2001**, *2*, 60-68.
(99) Fürstner, A.; Weintritt, H. *J. Am. Chem. Soc.* **1998**, *120*, 2817-2825.
(100) Boger, D. L.; Hong, J. *J. Am. Chem. Soc.* **2001**, *123*, 8515-8519.
(101) Kim, S. H.; Figueroa, I.; Fuchs, P. L. *Tetrahedron Lett.* **1997**, *38*, 2601-2604.
(102) Trost, B. M.; Doherty, G. A. *J. Am. Chem. Soc.* **2000**, *122*, 3801 - 3810.
(103) Attardo, G.; Dairi, K.; Lavallée, J.-F.; Rioux, E.; Tripathy, S. *PCT Int. Appl* **2004**, *WO 2004/106328*, 172.
(104) Kusumi, T.; Ichikawa, A.; Kakisawa, H.; Tsunakawa, M.; Konishi, M.; Oki, T. *J. Am. Chem. Soc.* **1991**, *113*, 8947 - 8948.
(105) Tanabe-Tochikura, A.; Nakashima, H.; Murakami, T.; Tenmyo, O.; Oki, T.; Yamamoto, N. *Antivir. Chem. Chemother.* **1992**, *3*, 345-349.
(106) Berry, D. R.; Dantzig, A. H.; Debono, M.; Hamilll, R.; Molloy, R. M.; Yoa, R. C., **5,451,403**, USA, **1995**,
(107) Tsunakawa, M.; Tenmyo, O.; Tomita, K.; Naruse, N.; Kotake, C.; Miyaki, T.; Konishi, M.; Oki, T. *J. Antibiot.* **1992**, *45*, 180-188.
(108) Roush, W. R.; Barda, D. A.; Limberakis, C.; Kunz, R. K. *Tetrahedron* **2002**, *58*, 6433-6454
(109) Hirayama, N.; Kasai, M.; Shirahata, K.; Ohashi, Y.; Sasada, Y. *Tetrahedron Lett.* **1980**, *21*, 2559-2560.
(110) Kyowa Hakko Kogyo Co., L., Japan **JP 83-107406 19830615**, Japan, **1985**,
(111) Zehner, S.; Kotzsch, A.; Bister, B.; Süssmuth, R. D.; Méndez, C.; Salas, J. A.; Pée, K.-H. v. *Chem. Biol.* **2005**, *12*, 445-452.
(112) Schroeder, D. R.; Colson, K. L.; Klohr, S. E.; Lee, M. S.; Matson, J. A.; Brinen, L. S.; Clardy, J. *J. Antibiot.* **1996**, *46*, 865-872.

(113) Lam KS, H. G., Gustavson DR, Berry RL, Tomita K, MacBeth JL, Ross J, Miller D, Forenza S. *J. Antibiot.* **1996**, *49*, 860-864.
(114) Lee, J. J.; Lee, J. P.; Keller, P. J.; Cottrell, C. E.; Chang, C. J.; Zaehner, H.; Floss, H. G. *J. Antibiot.* **1986**, *39*, 1123-1134.
(115) Mallams, A. K. P., Mohindar S.; Rossman, Randall R.; McPhail, Andrew T.; Macfarlane, Ronald D.; Stephens, Richard L. *J. Chem. Soc. Perkin Trans. 1* **1983**, *7*, 1497-1534.
(116) Mallams, A. K. P., Mohindar S.; Rossman, Randall R.; McPhail, Andrew T.; Macfarlane, Ronald D. *J. Am. Chem. Soc.* **1981**, *103*, 3940-3943.
(117) Ohtsuka, T.; Nakayama, N.; Itezono, Y.; Shimma, N.; Kuwahara, T.; Yokose, K.; Seto, H. *J. Antibiot.* **1993**, *46*, 18-24.
(118) Luk, K.; Readshaw, S. A. *J. Chem. Soc. Perkin Trans. 1* **1991**, *7*, 1641-1644.
(119) Martinek; Riddell, F. G.; Rutherford, T. J.; Sareth, S.; Waller, C. T. *Chem. Comm.* **1998**, 1893-1894.
(120) Roush, W. R.; Sciotti, R. J. *J. Org. Chem.* **1998**, *63*, 5473-5482.
(121) Roush, W. R.; Sciotti, R. J. *J. Org. Chem.* **1993**, *58*, 2151-2161.
(122) Roush, W. R.; Barda, D. A. *Tetrahedron Lett.* **1997**, *38*, 8781-8784.
(123) Matsumoto, M.; Kawamura, Y.; Yoshimura, Y.; Terui, Y.; Nakai, H.; Yoshida, T.; Shoji, J. *J. Antibiot.* **1990**, *43*, 739-47.
(124) Bonjouklian, R.; Mynderse, J. S.; Hunt, A. H.; Deeter, J. B. *Tetrahedron Lett.* **1993**, *34*, 7857-7860.
(125) Roush, W. R.; Barda, D. A. *Tetrahedron Lett.* **1997**, *38*, 8785-8788.
(126) Roush, W. R.; Limberakis, C.; Kunz, R. K.; Barda, D. A. *Org. Lett.* **2002**, *4*, 1543-1546.
(127) Trullinger, T. K.; Qi, J.; Roush, W. R. *J. Org. Chem.* **2006**, *71*, 6915-6922.
(128) Bedel, O.; Francais, A.; Haudrechy, A. *Synlett* **2005**, *15*, 2313-2316.
(129) Bloomer, J. L.; Kappler, F. E.; Pandey, G. N. *J. Chem. Soc., Chem. Commun.* **1972**, *4*, 243-234.
(130) Suga, T.; Hirata, T. *Bull Chem Soc Jpn* **1982**, *55*, 1584-1587.
(131) Walton, L. J.; Corre, C.; Challis, G. L. *J. Indust. Mircobiol. Biotech.* **2006**, *33*, 105 - 120.
(132) Sekiyama, Y.; Araya, H.; Hasumi, K.; Endo, A.; Fujimoto, Y. *Tetrahedron* **1998**, *39*, 6233-6236.
(133) Sekiyama, Y.; Hasumi, K.; Endo, A.; Fujimoto, Y. *Tetrahedron* **1999**, *40*, 4223-4226.
(134) Sekiyama, Y.; Fujimoto, Y.; Hasumi, K.; Endo, A. *J. Org. Chem.* **2001**, *66*, 5649-5654.
(135) Mashimo, Y.; Sekiyama, Y.; Araya, H.; Fujimoto, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 649-651.
(136) Holzbach, R.; Pape, H.; Hook, D.; Kreutzer, E. F.; Chang, C. J.; Floss, H. G. *Biochemistry* **1978**, *17*, 556-560.
(137) Mascaretti, O. A.; Chang, C. J.; Hook, D.; Otsuka, H.; Kreutzer, E. F.; Floss, H. G. *Biochemistry* **1981**, *20*, 919-924.
(138) Chijiwa, S.; Park, H.-R.; Furihata, K.; Ogata, M.; Endo, T.; Kuzuyama, T.; Hayakawa, Y.; Shin-ya, K. *Tetrahedron* **2003**, *44*, 5897-5900.
(139) Jia, X.-Y.; Tian, Z.-H.; Shao, L.; Qu, X.-D.; Zhao, Q.-F.; Tang, J.; Tang, G.-L.; Liu, W. *Chem. Biol.* **2006**, *13*, 575-585.
(140) Zhang, H.; White-Phillip, J. A.; Melcancon, C. E.; Kwon, H.-j.; Yu, W.-l.; Lei, H.-w. *J. Am. Chem. Soc.* **2007**, *129*, 14670-14683.

(141) Oikawa, H.; Katayama, K.; Suzuki, Y.; Ichihara, A. *J. Chem. Soc. Chem. Commum.* **1995**, 1321-1322.
(142) Oikawa, H.; Kobayashi, T.; Katayama, K.; Suzuki, Y.; Ichihara, A. *J. Org. Chem.* **1998**, *63*, 8748-8756.
(143) Auclair, K.; Sutherland, A.; Kennedy, J.; Witter, D. J.; Heever, J. P. V. d.; Hutchinson, C. R.; Vederas, J. C. *J. Am. Chem. Soc.* **2000**, *122*, 11519-11520.
(144) Ose, T.; Watanabe, K.; Mie, T.; Honma, M.; Watanabe, H.; Yao, M.; Oikawa, J.; Tanaka, I. *Nature* **2003**, *422*, 185-189.
(145) Watanabe, K.; Mie, T.; Ichihara, A.; Oikawa, H.; Honma, M. *J. Biol. Chem.* **2000**, *275*, 38393-38401.
(146) Guimaraes, C. R. W.; Udier-Blagovic, M.; William L, J. *J. Am. Chem. Soc.* **2005**, *127*, 3577-3588.
(147) Beck, A. K.; Hoekstra, M. S.; Seebach, D. *Tetrahedron Lett.* **1977**, *13*, 1187-1190.
(148) Tang, L.; Fu, H.; Betlach, M. C.; McDaniel, R. *Chem. Biol.* **1999**, *6*, 553-558.
(149) Xue, Y.; Sherman, D. H. *Nature* **2000**, *403*, 571-575.
(150) Betlach, M. C.; Kealey, J. T.; Betlach, M. C.; Ashley, G. W.; McDaniel, R. *Biochemistry* **1998**, *37*, 14937-14942.
(151) Leadley, P. F.; Demydchuk, Y. *personal communication*
(152) Sun, Y.; Hong, H.; Gillies, F.; Spencer, J. B.; Leadlay, P. F. *ChemBioChem* **2007**, *9*, 150-156.
(153) Tsuchida, T.; Iinuma, H.; Sawa, R.; Takahashi, Y.; Nakamura, H.; Nakamura, K. T.; Sawa, T.; Naganawa, H.; Takeuchi, T. *J. Antibiot.* **1995**, *48*, 1110-1114.
(154) Koda, S.; Morimoto, Y. *J. Antibiot.* **1983**, *36*, 1237-1238.
(155) Cane, D. E.; Prabhakaran, P. C.; Tan, W.; Ott, W. R. *Tetrahedron Lett.* **1991**, *32*, 5457-5460.
(156) Powell, A.; Borg, M.; Amir-Heidari, B.; Neary, J. M.; Thirlway, J.; Wilkinson, B.; Smith, C. P.; Micklefield, J. *J. Am. Chem. Soc.* **2007**, *129*, 15182-15191.
(157) Cane, D. E.; Lambalot, R. H.; Prabhakaran, P. C.; Ott, W. R. *J. Am. Chem. Soc.* **1993**, *115*, 522-526.
(158) Cane, D. E.; Tan, W.; Ott, W. R. *J. Am. Chem. Soc.* **1993**, *115*, 527-535.
(159) Xie, G.; Uttamchandani, M.; Chen, G. Y. J.; Bu, X.; Lin, S. S.; Wong, K. M.; Yan, W.; Yaob, S. Q.; Guoa, Z. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 989-992.
(160) Ehmann, D. E.; Trauger, J. W.; Stachelhaus, T.; Walsh, C. T. *Chem. Biol.* **2000**, *7*, 765-772.
(161) Less, S. L.; Handa, S.; Millburn, K.; Leadlay, P. F.; Dutton, C. J.; Staunton, J. *Tetrahedron Lett.* **1996**, *37*, 3515-3518.
(162) Bourguignon, J.; Moreau, M.; Quéguiner, G. *Bull. Soc. Chim. Fr.* **1975**, *11-12*, 2483-2487.
(163) Bella, A. F.; Slawin, A. M. Z.; Walton, J. C. *J. Org. Chem.* **2004**, *69*, 5926-5933.
(164) Ahrendt, K. A.; Williams, R. M. *Org. Lett.* **2004**, *6*, 4539-4541.
(165) Khasanov, A. B.; Ramirez-Weinhouse, M. M.; Webb, T. R.; Thiruvazhi, M. *J. Org. Chem.* **2004**, *69*, 5766-5769.
(166) Subasinghe, N. L.; Ali, F.; Illig, C. R.; Rudolph, M. J.; Klein, S.; Khalil, E.; Soll, R. M.; Bone, R. F.; Spurlino, J. C.; DesJarlais, R. L.; Crysler, C. S.; Cummings, M. D.; Morris, P. E.; Kilpatrick, J. M.; Babu, Y. S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3043-3047.
(167) Gilbert, I. H.; Ginty, M.; O'Neill, J. A.; Simpson, T. J.; Staunton, J.; Willis, C. L. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1587-1590.
(168) Stanley, A. E. *PhD Thesis, University of Warwick* **2007**.

(169) Ley, S. V.; Woodward, P. R. *Tetrahedron Lett.* **1987**. *28*, 345-346.
(170) Ley, S. V.; Smith, S. C.; Woodward, P. R. *Tetrahedron* **1992**, *48*. 1145-1174.
(171) Booth, P. M.; Fox, C. M. J.; Ley, S. V. *J. Chem. Soc.; Pekin Trans. I* **1987**. 121-129.
(172) Moses, J. E.; Bladwin, J. E.; Bruckner, S.; Eade, S. J.; Adlington, R. M. *Org. Biomol. Chem.* **2003**, *1*, 3670-3684.
(173) Hoshino, T.; Nakano, S.-I.; Kondo, T.; Sato, T.; Miyoshi, A. *Org. Biomol. Chem.* **2004**, 1456-1470.
(174) Werkhoven, T. M.; Nispen, R. V.; Lugtenburg, J. *Eur. J. Org. Chem.* **1999**, 2909-2914.
(175) Abdou, W. M.; Kamel, A. A. *Tetrahedron* **2000**, *56*, 7573-7580.
(176) Burke, L. T.; Dixon, D. J.; Ley, S. V.; Rodríguez, F. *Org. Biomol. Chem.* **2005**, *3*, 274-280.
(177) Venkatraman, L.; Salomon, C. E.; Sherman, D. H.; Fecik, R. A. *J. Org. Chem.* **2006**, *71*, 9853 - 9856.
(178) Sodeoka, M.; Sampe, R.; Kojima, S.; Morisaki, N.; Hashimoto, Y. *Chem. Pharm. Bull.* **2001**, *49*, 206-212.
(179) Haynes, S. *MChem Project Report, University of Warwick* **2005**.
(180) Blackburn, L.; Pei, C.; Taylor, R. J. K. *Synlett* **2001**, *2*, 215-218.
(181) Wei, X.; Taylor, R. J. K. *Tetrahedron Lett.* **1998**, *39*, 3815-3818.
(182) Longbottom, D. A.; Morrison, A. J.; Dixon, D. J.; Ley, S. V. *Tetrahedron* **2003**, *59*, 6955–6966.
(183) Sodeoka, M.; Sampe, R.; Kojima, S.; Baba, Y.; Usui, T.; Ueda, K.; Osada, H. *J. Med. Chem.* **2001**, *44*, 3216-3222.
(184) Sun, H.; DiMagno, A. G. *J. Am. Chem. Soc.* **2005**, *127*, 2050-2051.
(185) Takeda, K.; Yano, S.; Yoshii, M. *J. Org. Chem.* **1987**, *52*, 4135-4137.
(186) Paintner, F. F.; Bauschke, G.; Kestel, M. *Tetrahedron Lett.* **2000**, *41*, 9977-9980.
(187) Paintner, F. F.; Allmendinger, L.; Bauschke, G.; Berns, C.; Heisig, P. *Bioorg. Med. Chem. Lett.* **2003**, *11*, 2823-2833.
(188) Couladouros, E. A.; Bouzas, E. A.; Magos, A. D. *Tetrahedron* **2006**, *62*, 5272-5279.
(189) Pelter, A.; Al-Bayati, R. I. H.; Ayoub, M. T.; Lewis, W.; Pardasani, P.; Hansel, R. *J. Chem. Soc. Perkin Trans. 1* **1987**, 717-742.
(190) Mauduit, M.; Kouklovsky, C.; Langlois, Y.; Riche, C. *Org. Lett.* **2000**, *2*, 1053-1056.
(191) Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849-3862.
(192) Abdel-Magid, A. F.; Maryanoff, C. A.; Carson, K. G. *Tetrahedron Lett.* **1990**, *31*, 5595-5598.
(193) Wei, X.; Taylor, R. J. K. *J. Org. Chem.* **2000**, *65*, 616-620.
(194) Wei, X.; Taylor, R. J. K. *Tetrahedron Lett.* **1998**, *39*, 3815-3818.
(195) Blackburn, L.; Wei, X.; Taylor, R. J. K. *Chem. Commun.* **1999**, *1999*, 1337-1338.
(196) Buck, J.; Clemo, N. G.; Pattenden, G. *J. Chem. Soc.; Pekin Trans. I* **1985**, 2399-2405.
(197) Farina, F.; Martin, M. V.; Paredes, M. C. *Heterocycles* **1984**, *22*, 1733-1739.
(198) Martin, H.; Hoffmann, R.; Schmidt, B.; Wolff, S. *Tetrahedron* **1989**, *45*, 6113-6126.
(199) House, H. O.; Rasmusson, G. H. *J. Org. Chem.* **1961**, *26*, 4278-4281.

(200) Mesnard, D.; Miginiac, L. *J. Organomet. Chem.* **1989**, *373*, 1-10.
(201) Bestmann, H. J.; Hartung, H. *Chemische Berichte* **1966**, *99*, 1198-1207.
(202) Lang, R. W.; Hansen, H. J. *Helv. Chim. Acta* **1980**, *63*, 438-455.
(203) Prinn, R. G.; Fegley, B. F. *Earth and Planetary Sci. Lett.* **1987**, *83*, 1-15.
(204) Breit, B.; Breuninger, D. *J. Am. Chem. Soc.* **2004**, *129*, 10244-10245.
(205) Söker, U.; Sasse, F.; Kunze, B.; Höfle, G. *E. J. Org. Chem.* **2000**, 2021-2026.