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During the last three decades a very wide range of oxazole and thiazole containing secondary metabolites have been isolated and characterised from Nature. Furthermore, several of these metabolites have been shown to exhibit profoundly useful biological properties. Amongst the more commonly known natural oxazoles are calyculin A, isolated from the sponge *Discoderma calix* [1], the antibiotic substance virginiamycin produced by *Streptogramin sp* [2], and rhizoxin, a phytotoxin isolated from *Rhizopus chinensis* and the causal agent of rice seedling blight [3].

Althiomycin is a novel thiazole-containing peptide antibiotic which is active against gram-positive organisms, and also shows anticoccidial and antiherpes activity [4]. The *bis*-thiazole myxothiazol is an interesting antifungal antibiotic isolated from the myxobacterium *Myxococcus fulvus* [5]; it binds strongly to the cytochrome bc [1] segment of the respiratory chain, and is now used as a biochemical tool for studying electron transport through the cytochrome bc [1] complex. Finally, mirabazole B and tantazole A are representative examples of two novel thiazole-thiazoline-oxazole containing natural products isolated recently from the blue-green alga *Scytonema mirabile* [6]; tantazole A has been found to exhibit murine solid tumour selective cytotoxicity.

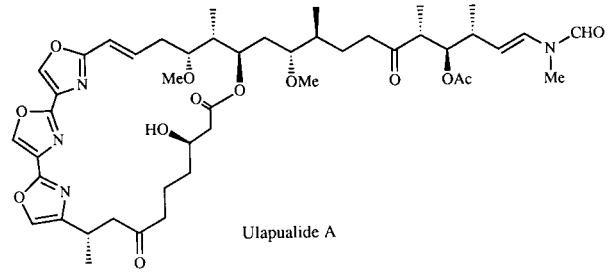
Three oxazole/thiazole-ring containing natural products that have been of special interest to our group in Nottingham over the past three or more years are:

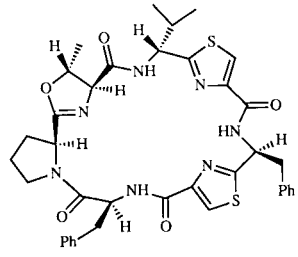
(i) the family of *tris*-oxazole macrolides, designated ulapualides, which are produced by marine nudibranchs and sponges.

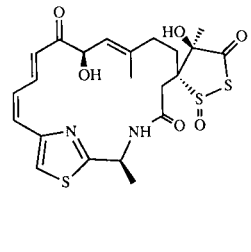
(ii) the thiazole-thiazoline-oxazolidine containing cyclic peptides called lissoclinamides produced by tunicates, and

(iii) the *Streptomyces* metabolite leinamycin, which not only features a thiazole containing 18-ring lactam but also a novel and unusual 1,3-dioxo-1,2-dithiolane residue.

Individually and collectively these oxazole/thiazole-ring containing macrocyclic natural products have structural features which are special and unique to their number. The three molecules also have well







defined biological profiles, but it is not immediately apparent which of their varying structural features are responsible for these special activities. Intuitively, we expect the oxazole/thiazole rings to play an important role, but the overall conformations of the molecules are also probably important. With a view to constructing models for the biological activities of the ulapualides, lissoclinamides and leinamycin, and at the same time engaging our interests in organic synthesis, molecular recognition, metal chelation and sulphur chemistry, we have embarked on the synthesis of these three oxazole/thiazole ring containing molecules. This presentation summarises the progress we have made so far.

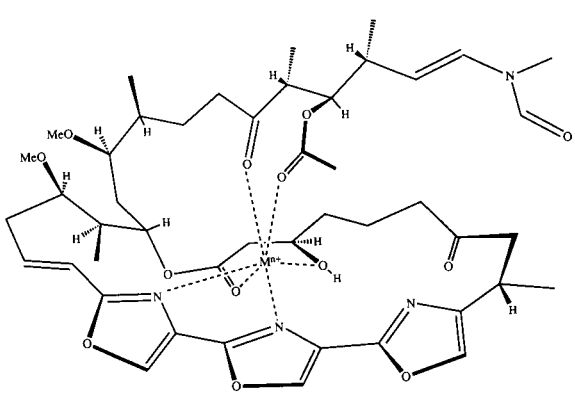
Ulapualides

The ulapualides (also known as kabiramides, halichondramides and mycalolides) are a family of secondary metabolites which were first isolated from egg masses of marine nudibranchs, *i.e.* sea slugs. The molecules show structures based on a 25-membered macrocyclic lactone which incorporates a novel *tris*-oxazole unit, and to which is attached a C_{11}-oxygenated side chain terminating in an unusual formyl enamine residue. The brilliantly coloured nudibranch *Hexabranchus sanguineus*, deposits its striking red egg masses on ledges in underwater caves,

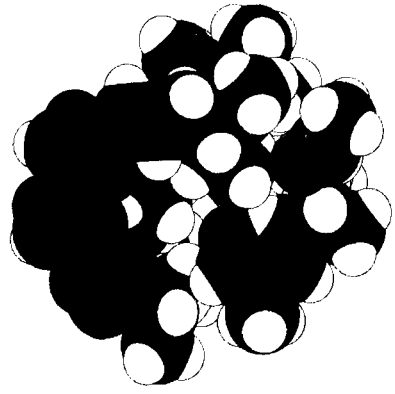
and though exposed and vulnerable these egg masses have no known predators. Ulapualides A and B, together with kabiramide C were first reported in 1986 from *H. sanguineus* [7,8]. Later Faulkner *et al.* [9] isolated the related kabiramides A, B, D and E and also a family of "halichondramides" from two species of the sponge *Halichondria*, on which *H. sanguineus* is known to feed. Even more recently the "mycalolides", which are essentially hybrids of the ulapualides and halichondramides, have been isolated from the sponge *Mycale* [10]. These molecules differ from each other only according to the number of oxy and methyl substituents found in the aliphatic portions of their structures.

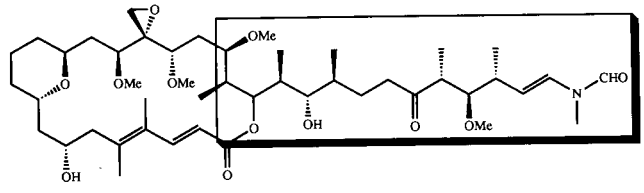
This novel family of marine metabolites shows a range of interesting and unusual biological activities. For example, all the metabolites show pronounced antifungal activity, and the ulapualides A and B together with the kabiramides and dihydrohalichondramide inhibit leukaemia cell proliferation. In addition, kabiramide C and some halichondramides have shown ichthyotoxic properties, and all the metabolites inhibit cell division in the fertilised sea urchin egg assay.

It is possible that the diverse range of biological activity shown by the ulapualides is in part associated with their capacity to sequester and transport metal and other cations *in vivo* using the several nitrogen and oxygen ligand binding sites present in their structures. Indeed, molecular graphics analysis has clearly demonstrated this capability of the molecules, and energy minimisation data obtained from conformational analysis of metal-chelated ulapualides have even permitted us to assign the relative stereochemistry shown in the formula for ulapualide A, based on this supposition. This outcome is interesting, since the relative stereochemistry of the groups along the side-chain in ulapualide A correlate precisely with the same centres in the related metabolite scytophycin C [11].



Our initial design for a total synthesis of ulapualide A, which is probably the simplest member of the family, accommodating only ten asymmetric centres, is shown below. The plan was to elaborate the doubly functionalised *tris*-oxazole unit together with a C_8 β-hydroxy acid right-hand side unit, the chiral C_6 top unit and the chiral C_{11} side chain residue - and then to assemble these units in a stepwise manner. The order of assemblage of these units will depend on such factors as: (i) sensitivity of protecting groups needed in the various synthetic operations, (ii) whether or not the macrolide would be formed before or after elaboration of the C_{11}-side chain together with the acid sensitive formyl enamine terminus is elaborated.

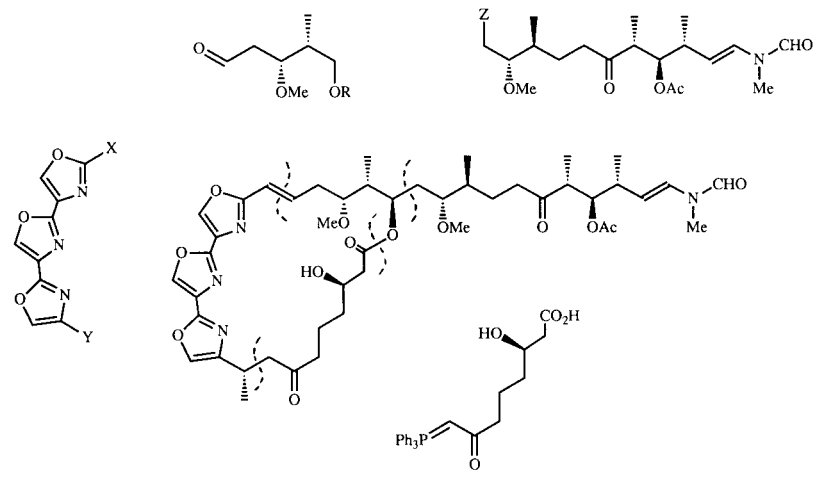




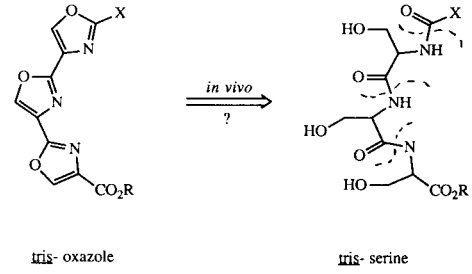
Scytophycin C

Thus, a biogenetically patterned synthesis of the *tris*-oxazole portion of the ulapualides was first developed based on the sequential oxazole amide-serine cyclisations followed by oxidations of intermediate oxazolines, summarised in Scheme 1. A considerable improvement in this overall strategy was seen when the method of Hamada and Shiori, using silver triflate to cyclise N-chloroethyl amides to oxazolines, was incorporated [12].

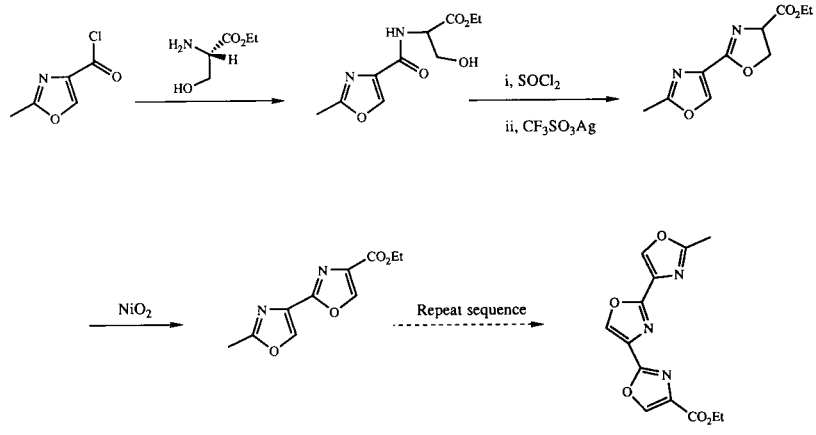
The protected C_8 β-hydroxy carboxylic acid derivative required for a Wittig reaction coupling to the *tris*-oxazole aldehyde was next smoothly prepared, in eight steps, starting with methyl acetoacetate (MAA) as outlined in Scheme 2. Wittig coupling then produced the required enone intermediate, for projected incorporation of the equatorial oriented β-methyl group in the macrolide by Michael addition of lithium dimethylcuprate.



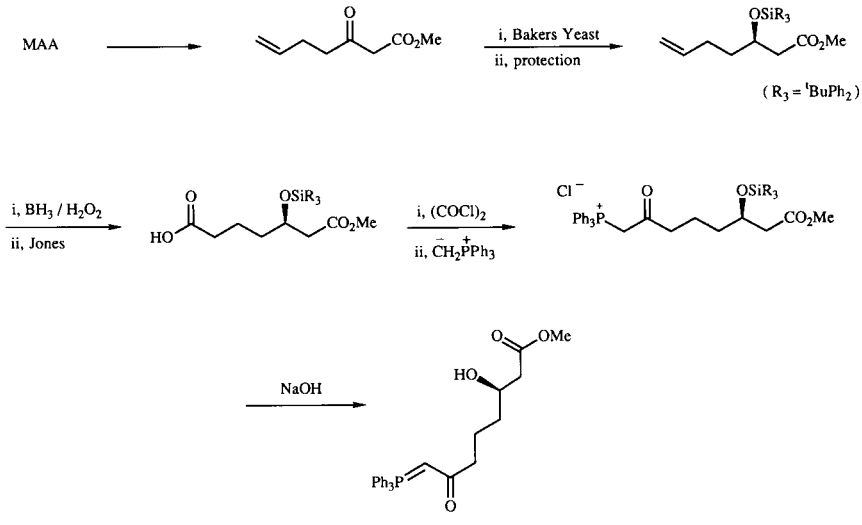
A second Wittig reaction strategy was then used to fuse the chiral C_6 unit making up the remainder of the carbon centres in the macrolide portion of ulapualide A. This sequence required the synthesis of the necessary C_6-aldehyde and the *tris*-oxazole phosphonium salt, and the details are collected in Scheme 3. The Wittig coupling proceeded smoothly and led to the required *E*-alkene product, which could then be linked in a repeat, second Wittig reaction sequence to produce an advanced precursor to ulapualide A. Work is now in progress to convert this advanced precursor to the macrolide portion of ulapualide A with simultaneous introduction of the equatorial secondary methyl substituent.

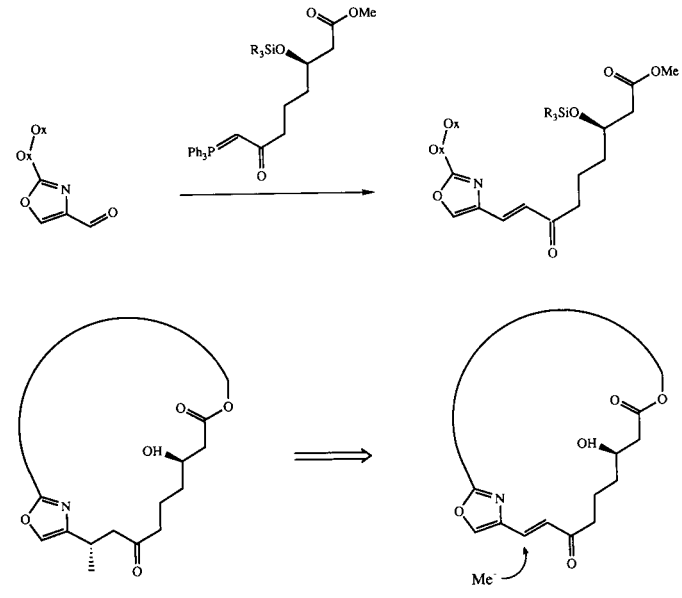


Scheme 1

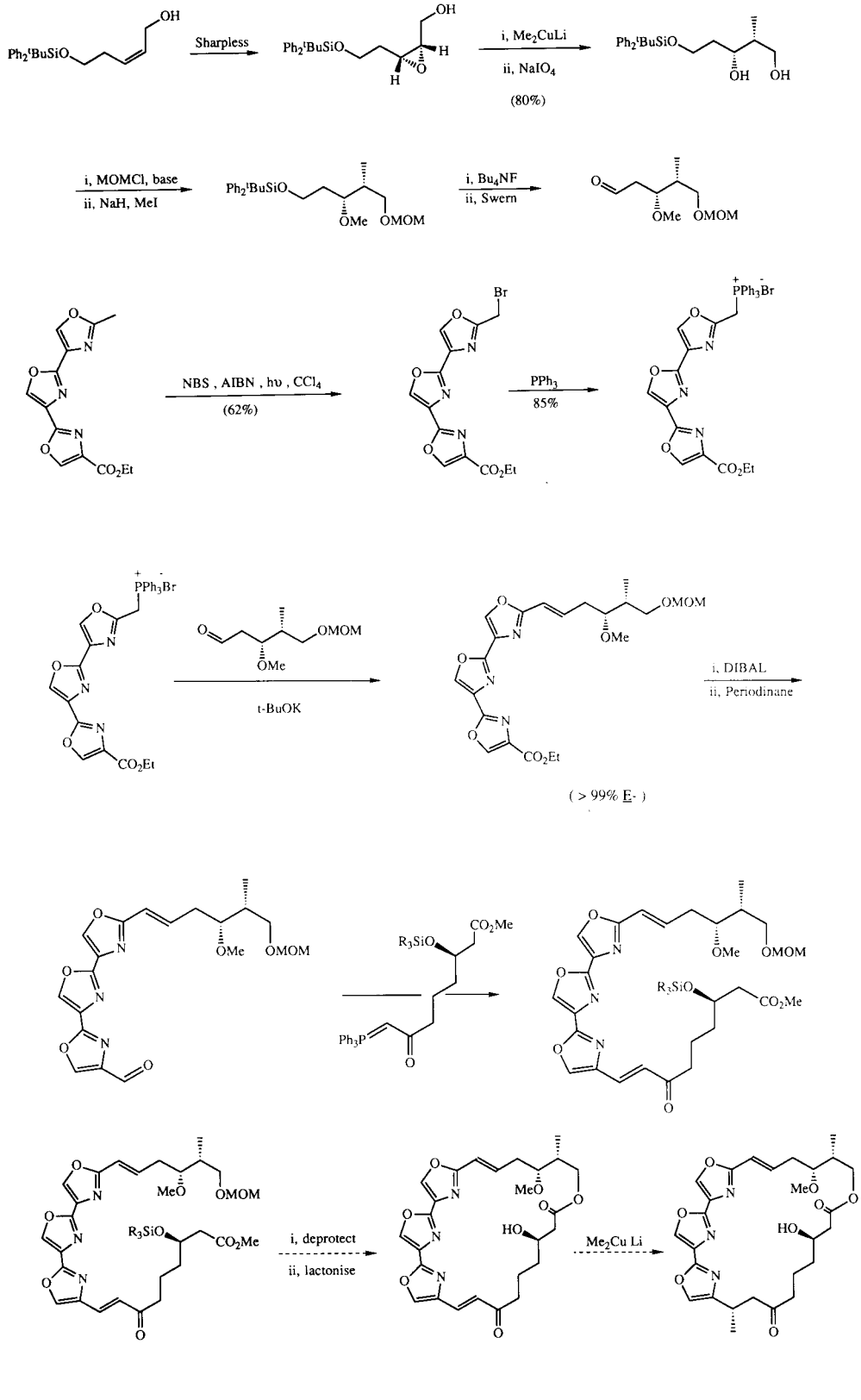


Scheme 2

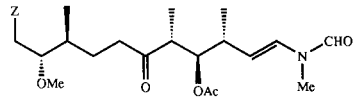

MAA
CO2Me
i, Bakers Yeast
ii, protection
OSiR3
CO2Me
(R3 = tBuPh2)
i, BH3 / H2O2
ii, Jones
HO
i, (COCl)2
ii, CH2PPh3
Cl−
Ph3P
NaOH
OMe
Ph3P


R3SiO
Ox
N
OH
Me

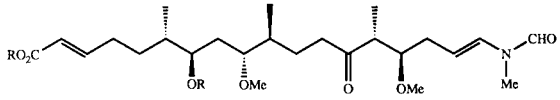
Scheme 3


Ph2tBuSiO
OH
Sharpless
i, Me2CuLi
ii, NaIO4
(80%)
OH
OH
i, MOMCl, base
ii, NaH, MeI
OMe
OMOM
i, Bu4NF
ii, Swern
NBS , AIBN , hυ , CCl4
(62%)
Br
PPh3
85%
PPh3Br
CO2Et
t-BuOK
i, DIBAL
ii, Periodinane
(> 99% E-)
R3SiO
CO2Me
Ph3P
MeO
i, deprotect
ii, lactonise
HO
Me2Cu Li

In other investigations towards the ulapualides we have examined a number of complementary routes to the chiral side chains (*i.e.* non macrolide portions) in both ulapualide A and the halichondramides.

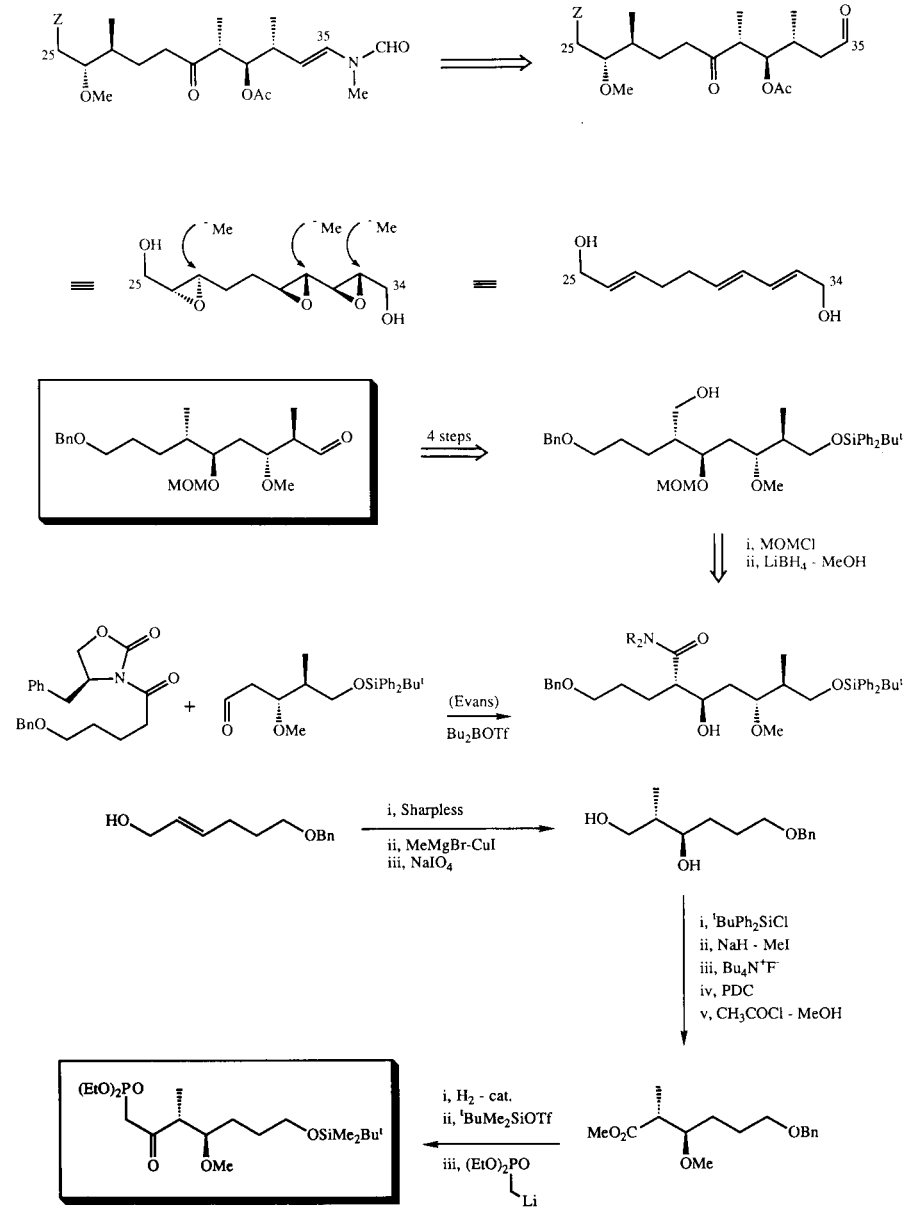


Ulapualide side chain



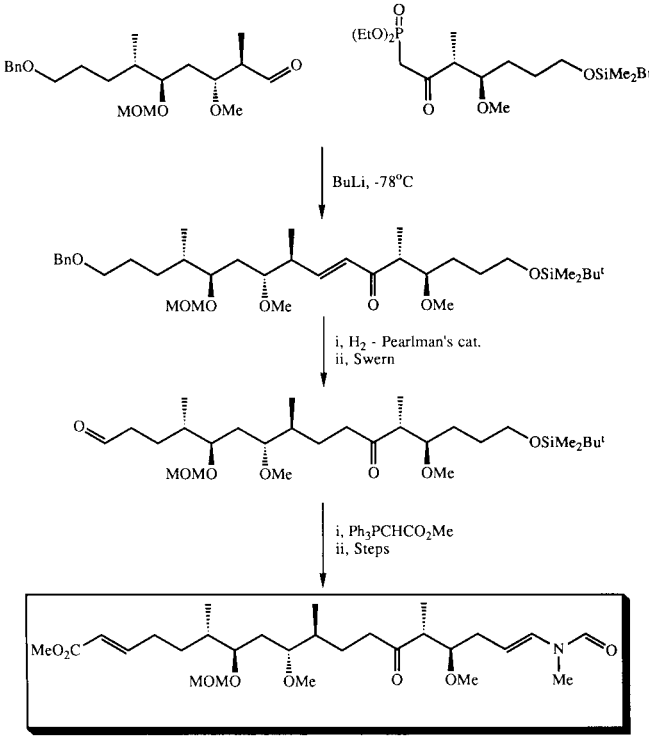
Halichondramide side chain

Thus in an approach to the chiral side chain in ulapualide A (carbon numbering C-25 to C-35) we first disconnected at C-35 revealing the C-35 aldehyde intermediate shown. Model work has demostrated that the formyl enamine residue in the ulapualides can be incorporated efficiently and conveniently by acid-catalysed condensation between methyl formamide and this aldehyde leading to the required *E*-double bond. Our aim then was to use the ubiquitous Sharpless epoxidation of allylic alcohols protocol, followed by methyl nucleophile ring opening of the epoxy-alcohol intermediates, to elaborate the whole of this side chain in ulapualide A. This strategy however, was only partially successful, and floundered when only poor



selectivity was observed during the nucleophilic ring openings of the variously substituted epoxy-alcohol intermediates.

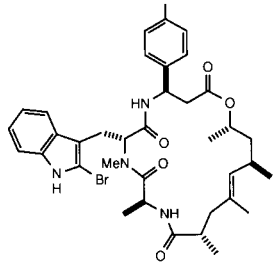
In a second approach - this time to the side chain in halichondramide - we have applied Evans aldol protocol in combination with the elaboration of key intermediates *via* ring opening of simple chiral epoxy alcohol precursors in the presence of lithium dimethylcuprate. In this manner we secured precursors for the Wadsworth-Emmons olefination sequence illustrated. Functional group manipulation, as shown, has then led to the C_{18}-residue, accommodating six chiral centres, found in halichondramide.



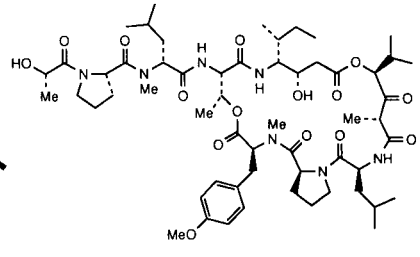
Having established satisfactory methodology for elaborating all of the key structural units in the ulapualide framework, we are now poised to put these units in place and complete the first syntheses of these molecules and some of their hybrids. In tandem with the synthetic studies, interactive molecular graphics together with metal chelation studies have been used to probe the preferred conformations of the ulapualides, their precursors and analogues, both in 'free' form and also when the molecules are associated with selected metal ions. These studies will help us to construct appropriate models for the biological activities of these fascinating *tris*-oxazole macrolides.

Lissoclinamide

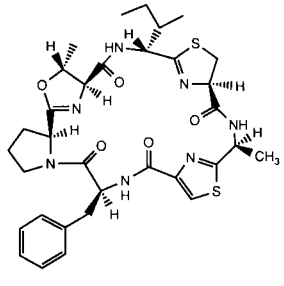
A very wide range of structurally novel and biologically interesting cyclic peptides *e.g.* jasplakinolide and didemnin B, have been isolated and characterised from marine organisms. Many of these small peptides display useful antimicrobial or neurophysiological properties, whereas others exhibit antileukaemic activity. Indeed some members show great promise as potential antineoplastic agents, and didemnin B from the tunicate *Tridedemnum solidum*, for example, is now in phase II clinical trials [13]. A particularly intriguing family of cyclic peptides produced by tunicates is the lissoclinamides [14]. These secondary metabolites contain at least one thiazole or thiazoline and usually an oxazoline amino acid residue *e.g.* lissoclinamide-3 and lissoclinamide-5. Although it is known that both the oxazoline and the thiazoline rings play an important role in the biological activity of these cyclic peptides, their modes of action have not been determined. Clearly the overall conformations of the molecules are important, and several factors including the presence of metal ions could influence these molecular arrangements. Indeed molecular modelling studies on the lissoclinamides and other related peptides based on molecular dynamics, nmr spectroscopy and X-ray structure data suggest that these peptides could be involved in a significant way in metal chelation and metal transport phenomena *in vivo* [15].



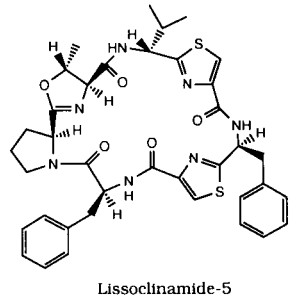
Jasplakinolide



Didemnin B

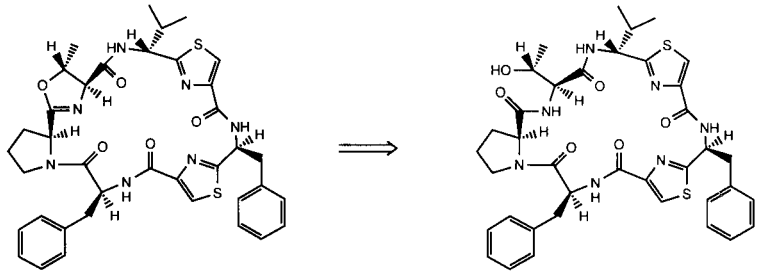


Lissoclinamide-3



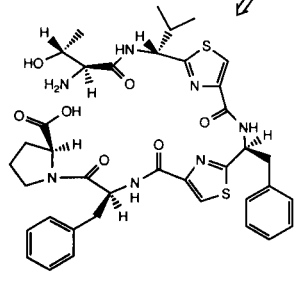
Lissoclinamide-5

With a view to constructing a model for the biological activity of the lissoclinamides, in addition to investigating their ionophore properties, we have embarked on a program towards the synthesis of certain of their number. Here we describe concise syntheses of thiazole containing amino acids suitably substituted for elaboration to pre-lissoclinamide-5.



Lissoclinamide-5

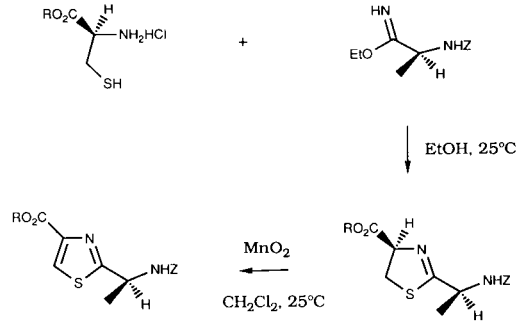
Pre-lissoclinamide-5



Pre-pre-lissoclinamide-5

Our strategy for the final stages in the synthesis of pre-lissoclinamide-5 was based on: (i) macrolactam bond formation adjacent to the proline residue in a "pre-pre-lissoclinamide" precursor, and (ii) elaboration of pre-pre-lissoclinamide from the thiazole-containing tetrapeptide and tripeptide units shown below.

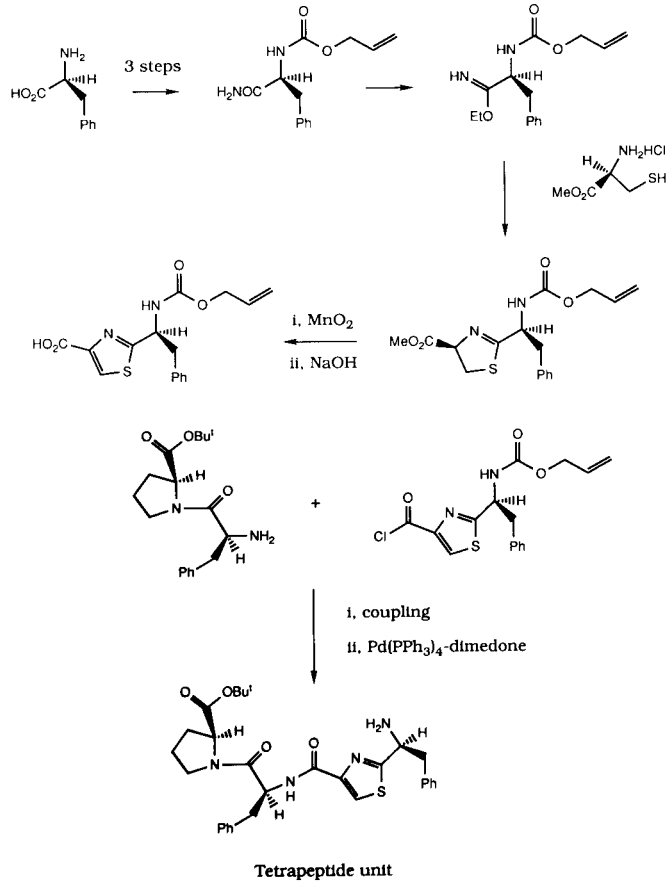
After much experimentation, we have found that thiazole containing amino acids can be obtained efficiently and in optically pure form from simple condensation reactions between cysteine esters and N-protected (Z=CO.OCH$_2$CH=CH$_2$, COOCH$_2$CCl$_3$, COOBu^t) imino ethers derived from chiral amino acids [16].



Z = $CO_2CH_2CH{=}CH_2$; $CO_2CH_2CCl_3$; $CO_2{}^tBu$

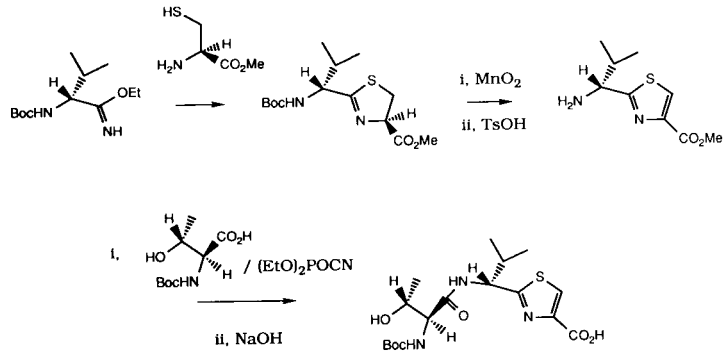
R = Me; $CHPh_2$

A coupling reaction between the allyloxyphenylalanine-cysteine derived thiazole carboxylic acid and (*S*)-Phe-(*S*)-Pro-^{t}Bu followed by removal of the allyloxy residue then produced the tetrapeptide unit.



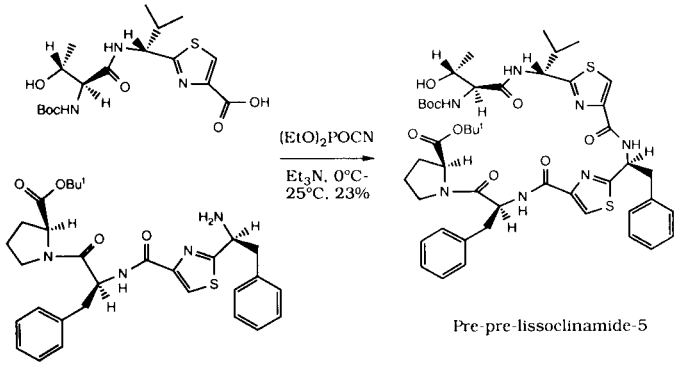
Tetrapeptide unit

In a similar manner, the tripeptide unit for the pre-lissoclinamide synthesis was derived from BOC protected valine imino ether cysteine and BOC-threonine as shown.

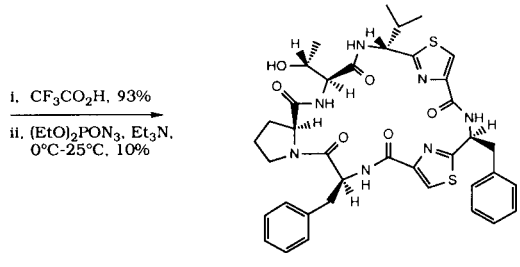


Tripeptide unit

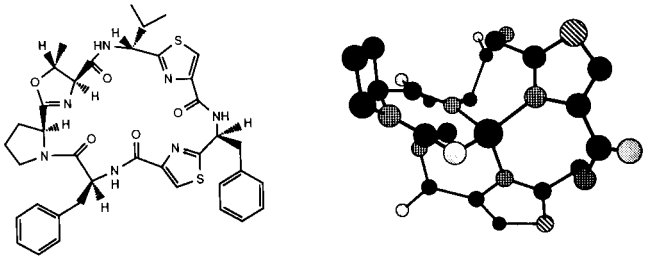
A coupling reaction between the tri- and tetra-peptide units in the presence of diethyl cyanophosphonate then gave pre-pre-lissoclinamide in 10-40% yields. Removal of the protecting groups in the presence of acid followed by macrolactamisation using diphenylphosphoranyl azide in triethylamine-dimethylformamide then led to pre-lissoclinamide-5 (~10%).



Pre-pre-lissoclinamide-5



Pre-lissoclinamide-5

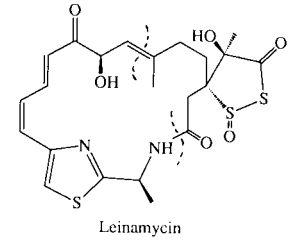


Lissoclinamide-5 + ion complex

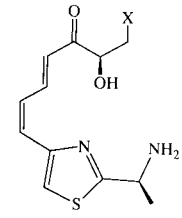
Work is now in progress to optimise the yields in the crucial amide bond forming reactions between the tri- and tetra-peptide units and pre-lissoclinamide, and to evaluate the metal chelating capacities of the synthetic lissoclinamides and their precursors.

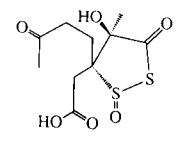
Leinamycin

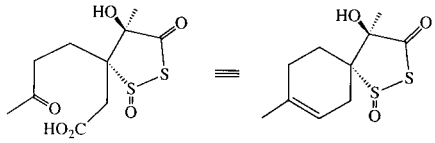
Leinamycin is a new and potent anti-tumour antibiotic substance which has been isolated from *Streptomyces sp* [17]. It shows a structure based on a novel and unusual 1,3-dioxo-1,2-dithiolane residue which is spiro-fused to a thiazole containing 18-ring lactam. Although 1,2-dithiolanes, *e.g.* α-lipoic acid, guinesine A, and their S-oxides, *e.g.* asparagusic acid S-oxide, brugierol [18], are not uncommon in Nature, the 1,3-dioxo-1,2-dithiolane residue is unique to leinamycin. Furthermore, it would not be surprising to find that this residue has an important and determining role in the biological activity of leinamycin. As part of a program towards a total synthesis of leinamycin, based on the disconnection shown we present a concise synthetic route to the model 5,5-dimethyl and cyclohexene spiro-fused 1,2-dithiolane systems illustrated.

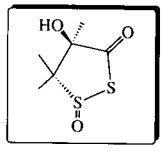


Leinamycin

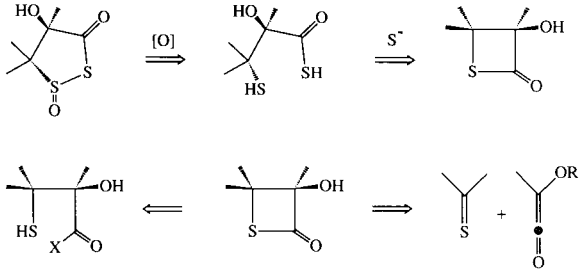




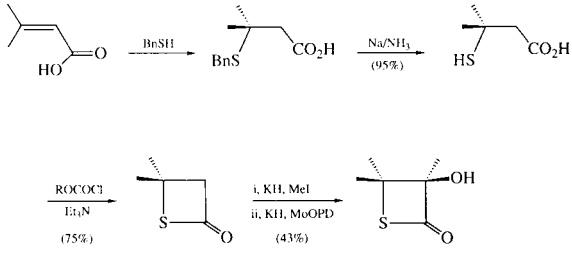


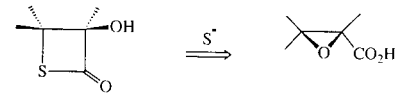


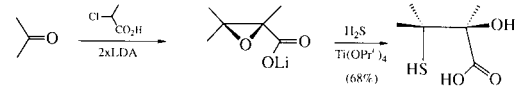
Our general strategy for the synthesis of the model 5,5-dimethyl substituted 1,3-dioxo-1,2-dithiolane system relied on access to an intermediate β-thiolactone which we planned to open and then close to the target molecule using sulphide ion followed by ferric salt oxidation. Two conceptually distinct approaches to the β-thiolactone intermediate were designed; the first was based on elaboration of a thiol acid derivative whereas the second approach hinged on a [2+2]-cycloaddition reaction with thiones.



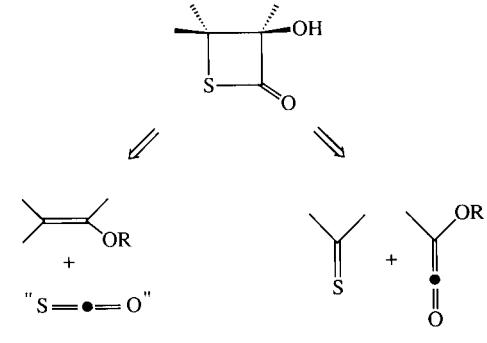
Thus, deprotection of the thiobenzyl ether produced from 3-methylbut-2-enoic acid and benzylthiol, using sodium in ammonia, first produced a thiol acid, which was then cyclised in the presence of *i*BuOCOCl-Et_3N [19] leading to the corresponding thiolactone. α-Methylation of this intermediate, followed by α-oxygenation next provided the 3-hydroxy-3-methyl-4,4-dimethyl substituted β-thiolactone. The same substituted β-thiolactone could be obtained more conveniently following reaction between hydrogen sulphide and the lithium glycidate derived from acetone and α-chloroacetic acid, then thiolactone formation in the presence of diethyl cyanophosphonate.





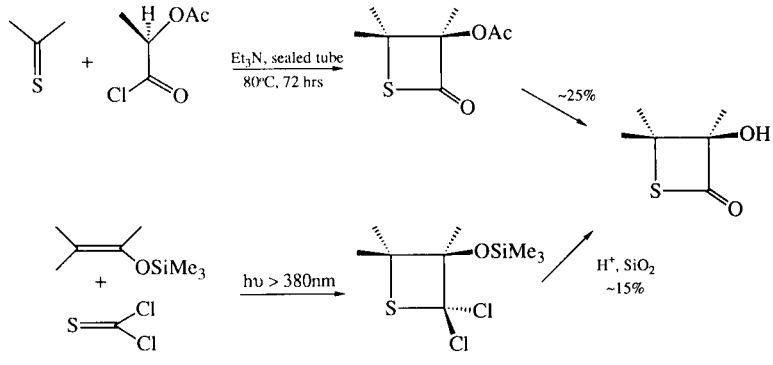


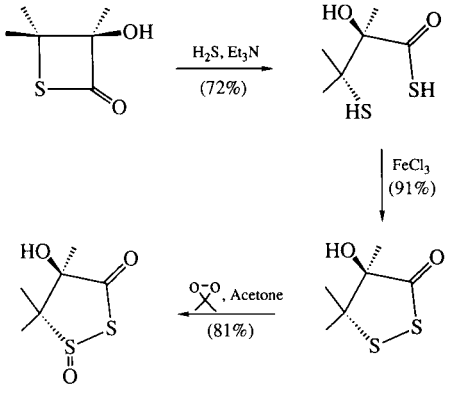
Finally, two synthetic approaches to the substituted β-thiolactone based on [2+2]-cycloaddition reactions with thiones were examined. Thus, in one approach, triethylamine was introduced to a solution of thioacetone and 2-acetoxypropionyl chloride and the mixture was sealed and heated at 80°C for 24h [20]. Work up then gave the thiolactone in a modest 25% yield. In a second, photochemical, approach to the same thiolactone, a solution of the trimethylsilyl enol ether derived from 3-methylbutan-2-one was irradiated at >380nm in the presence of thiophosgene [21]. The resulting α,α-dichlorothietan was then treated with acid washed silica affording the α-hydroxythiolactone.



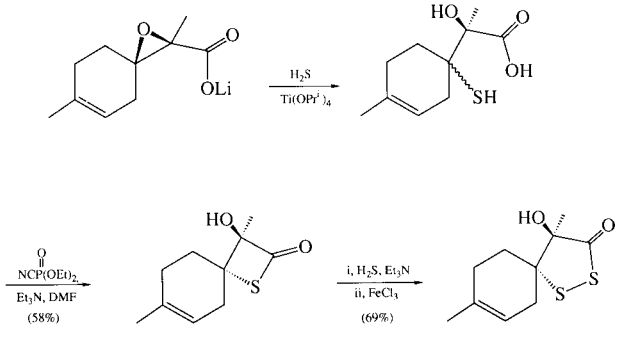
When a solution of the 3-hydroxy-3,4,4-trimethyl substituted β-thiolactone in carbon tetrachloride saturated with H_2S at -78°C was treated dropwise with triethylamine, and then left at -78°C overnight in an atmosphere of H_2S, work up gave the thiol thioacid shown [22]. Oxidative cyclisation of the thio thioacid in the presence of ferric chloride [23] then produced the 1,2-dithiolane 1-oxide which underwent smooth oxidation in the presence of dimethyldioxirane giving the target 1,3-dioxo-1,2-dithiolane.

The aforementioned reaction sequence was translated to the glycidate derived from 4-methylcyclohex-3-enone and α-chloroacetic acid, leading to satisfying syntheses of key intermediates en route to the spiro-





fused 1,2-dithiolane intermediates required in our projected synthesis of leinamycin.



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