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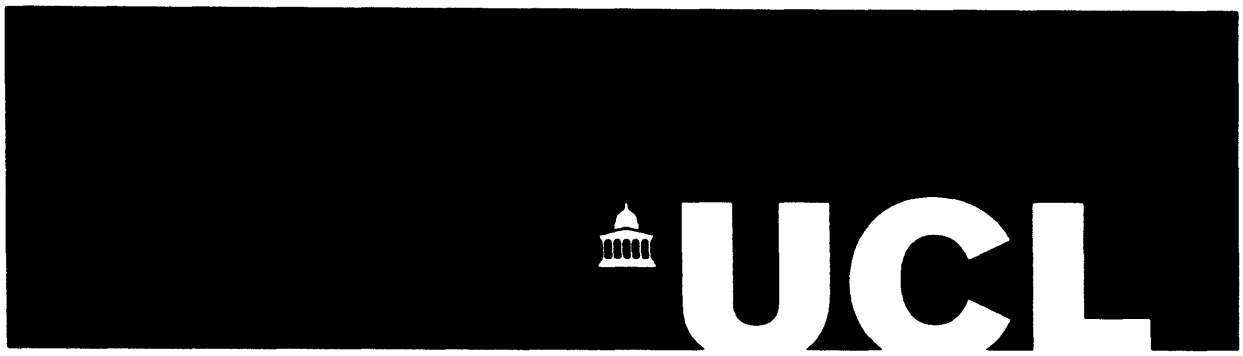

Synthesis of 4*H*-Pyran-4-ones, Other Cyclic Oxygenated Systems and Related Modelling Studies

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Signed

Date 30/4/03.

Acknowledgements

First and foremost I would like to thank my parents, Julie and Michael, for their support, patience and understanding. Without them, nothing would have been possible.

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Abstract

The aim of the project was to investigate the use of mercury mediated cyclisation reactions resulting in the generation of 2,3-dihydropyran-4*H*-one and 3(2*H*)-furanone systems.

Chapter one discusses the general synthesis of dihydropyranones and furanones. The chapter continues and focuses upon the use of a mercury-mediated cyclisation strategy in the synthesis of 2,3-dihydropyran-4-ones and 3(2*H*)-furanones. Finally the chapter describes efforts to utilise a mercury-mediated cyclisation strategy to synthesise useful synthetic intermediates.

Chapter two is focused upon protein phosphorylation inhibitors, and begins by discussing the processes of protein phosphorylation and compounds which inhibit the process. The chapter focuses on fostriecin and phospholine compounds, and discusses efforts to synthesise key fragments of the compounds using a mercury-mediated cyclisation protocol.

Chapter three describes histone deacetylase inhibitors, and their mode of action. The use of computational models to predict and rationalise inhibitor activity is discussed. A small selection of HDAC inhibitors are used to demonstrate the Autodock system in predicting inhibitory activity. A previously unknown compound is synthesized, and inhibitory properties compared to those predicted by the Autodock system.

Chapter four discusses efforts to synthesise the spiroketal subunit of (-)-calyculin A. Initially a mercury-mediated cyclisation strategy is presented, detailing the preparation of two key fragments which are combined to form a key scaffold used in the cyclisation strategy. The chapter then describes the use of auxiliary fragments in attempts to generate the spiroketal fragment.

Abbreviations

DCC	1,3-Dicyclohexylcarbodiimide
DEAD	Diethyl Azodicarboxylate
DIC	*N,N'*-Diisopropylcarbodiimide
DIPT	Diisopropyl Tartrate
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
HDAC	Histone Deacetylase
HDLP	Histone Deacetylase Like Protein
HIV	Human Immunodeficiency Virus
Ipc	*iso*-Pinocampheyl
LDA	Lithium Diisopropylamine
MMP	Matrix Metalloproteinases
NMM	*N*-Methylmorpholine
NMO	*N*-Methylmorpholine *N*-oxide
NMP	*N*-Methyl-2-Pyrrolidone
NMR	Nuclear Magnetic Resonance
p-TsOH	*para*-Toluenesulfonic acid
PDB	Protein Data Bank
PP	Protein Phosphatase
PPTS	Pyridinium *para*-Toluene Sulfonate
PTP	Protein Tyrosine Phosphatases
SAHA	Suberoylanilide Hydroxamic Acid
TBHP	*tert*-Butyl Hydroperoxide
TBS	*tert*-Butyldimethylsilyl
THF	Tetrahydrofuran
TLC	Thin-layer Chromatography
TMS	Trimethylsilyl
TSA	Trichostatin A
ZBG	Zinc Binding Group

Contents

Chapter 1

The Synthesis of Dihydropyranones and 3(2*H*)-Furanones

1.1 Introduction

The pyranoid and furanyl ring systems are present in a broad spectrum of natural products which exhibit a variety of important therapeutic properties. The stereocontrolled construction of such systems is of great synthetic interest and is the focus of this chapter, where the synthesis of 2,3-dihydro-4*H*-pyran-4-ones and 3(2*H*)-furanones are demonstrated using a mercury mediated rearrangement of epoxy alkynols and alkynone diols to give substituted dihydropyranone and furanone systems.

1.1.1 Dihydropyranones and 3(2*H*)-Furanones

The presence of dihydropyranones and furanones is to be found in a variety of natural products. Dihydropyranones, and related systems, can be seen in products such as stegobiol[1] (**3**) the sex pheromone of the drugstore beetle, and other pheromones,[2] vallartanones[3] and HIV protease inhibitors.[4,5] Compounds containing a 3-methyltetrahydropyran moiety include spiroketal compounds,[6-9] spongistatins,[10-12] milbemycins, avermectins (**1**),[13] and monensin[14,15] (**2**) a polyether antibiotic.

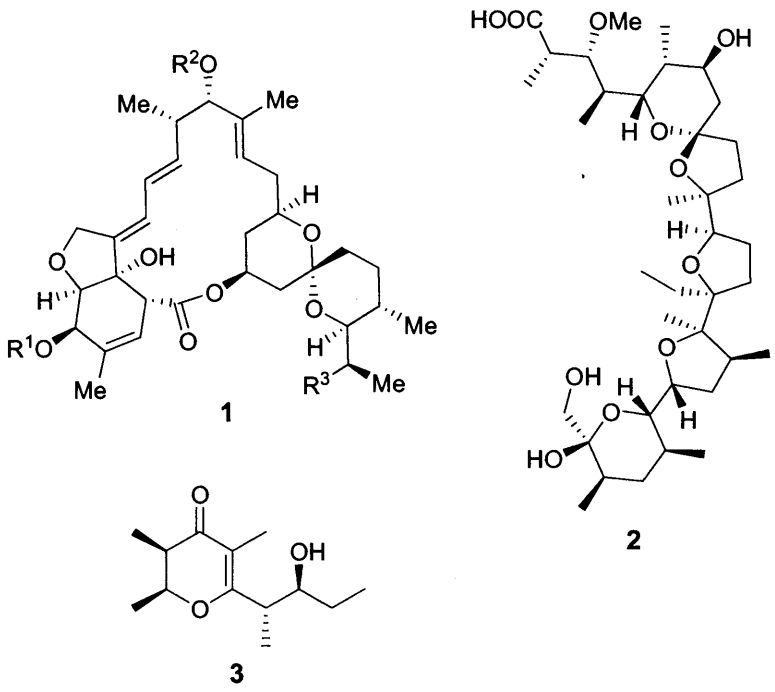


Figure 1.1.1. Examples of tetrahydropyran- and tetrahydrofuran-containing natural products. **1** Avermectin A, **2** Monensin, **3** Stegobiol. R^1 = Me, R^2 = L-oleandrosyl-L-olenandrose, R^3 = Et.

The 3(2*H*)-furanone ring system is similarly prevalent and can be found in a number of natural products exhibiting interesting pharmacological properties. These include compounds such as butenolide (**4**), tetronic acid frameworks (**5**), and the anti-tumour agent geiparvarin.[16-18]

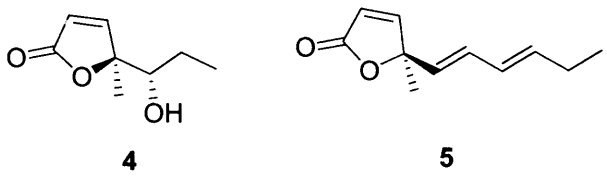
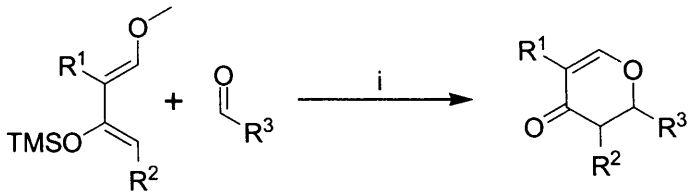


Figure 1.1.2. Butenolide (**4**) and tetronic acid frameworks (**5**).

Their variety in biological activity and potential application in therapeutic treatments has prompted the search for new methodology in the synthesis of pyranoid and furanyl systems.

1.1.2 Synthesis of Dihydropyranones and 3(2*H*)-Furanones

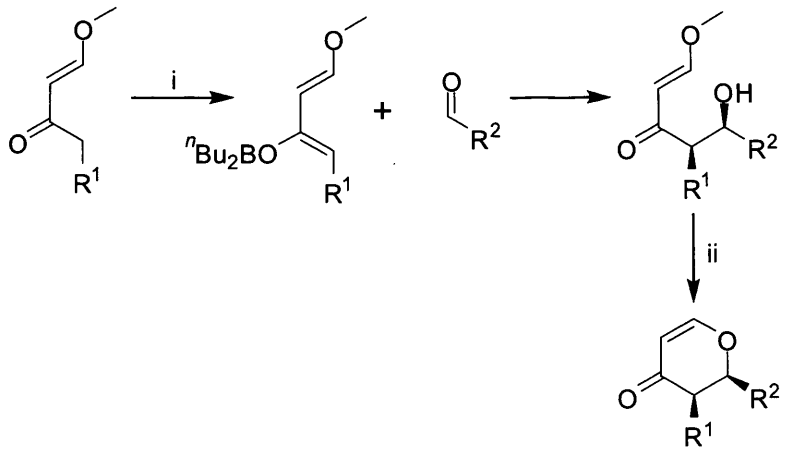
The synthesis of dihydropyranones is an important route through which it is possible to obtain a diverse range of substituted tetrahydropyrans and spiroketal systems. One of the best known routes to dihydropyranones, is a method reported and investigated by Danishefsky *et al.*[19-21] involving the Lewis acid catalysed Diels-Alder reaction of siloxy 1,3-dienes with aldehydes; a general illustration of the reaction can be seen in scheme 1.1.3.



Scheme 1.1.3. The Danishefsky synthesis of dihydropyranones. i. Lewis acid catalysis. Danishefsky's diene, R^1 and R^2 = H.

Diastereocontrol of the Danishefsky reaction is dependent upon a number of factors, including the substrate, the Lewis acid used, and the solvent.[22] Several reports detailing enhanced selectivity of the Danishefsky reaction[23-25] have been forthcoming; however, a limitation to the Danishefsky reaction exists, in that it does not allow the direct generation of C6 substituted pyranoids.

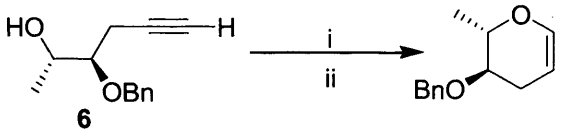
Other methods of pyranoid generation have been reported, and include the work of Paterson *et al.*[26] in aldol condensation reactions (scheme 1.1.4.). Using the chiral boron reagent (-)-$(Ipc)_2BOTf$ to generate the enolate, Paterson successfully generated material with 97% diastereoselectivity, and 80% e.e.



Scheme 1.1.4. An example of dihydropyranone synthesis using the Paterson method. i. $^{n}Bu_2BOTf$, $^{i}Pr_2NEt$; ii. Me_3SiOTf, $^{i}Pr_2NEt$.

Further reports can be found by Clarke *et al.*[27,28] who report the synthesis of tetrahydropyran-4-ones via aldol condensation of β-ketoesters and aldehydes, and Hinterding and co-workers who have described the generation of β-keto-δ-lactone through intramolecular Claisen type rearrangements.[29]

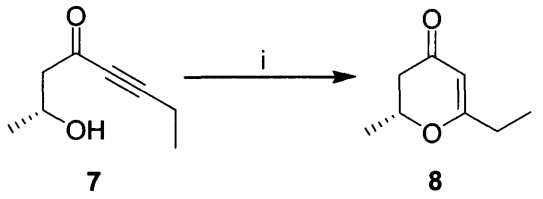
McDonald *et al.*,[30] using molybdenium and tungsten to mediate cycloisomerisation reactions, was able to generate dihydrofurans and dihydropyrans from intermediates such as **6**.



6

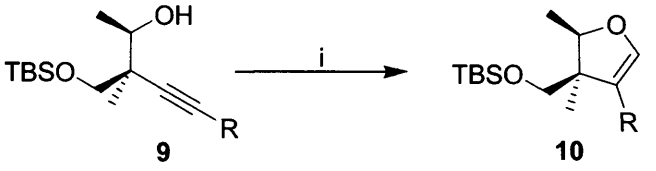
Scheme 1.1.5. Tungsten mediated cyclisation. i. $W(CO)_5$, THF; ii. Et_3N, Et_2O/THF.

Dreessen *et al.*[31] used base promoted cyclisation of β-hydroxy alkynones to give dihydropyranones. This is a one step reaction where the treatment of **7** with sodium hydride forms the dihydropyranone **8**.



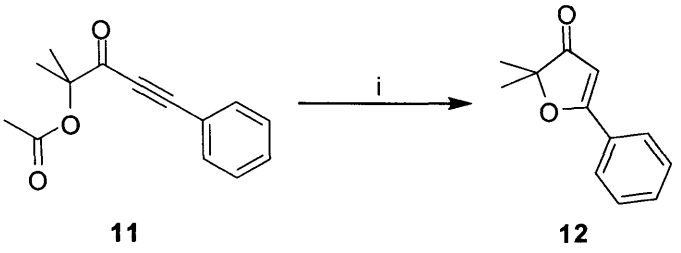
Scheme 1.1.6. Preparation of dihydropyranones according to the Dreessen procedure. i. NaH, THF.

Dihydrofuran systems have also been prepared using metal catalysis. Saito *et al.*[32] reports the synthesis of compound **10** using palladium catalysis in 91%.



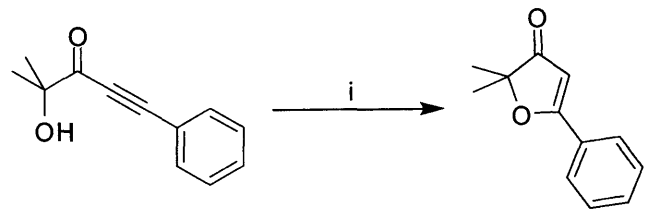
Scheme 1.1.7. Preparation of dihydrofurans using palladium (II) catalysis. i. $PdCl_2(PhCN)_2$, MeCN, 60 °C.

The synthesis of 3(2*H*)-furanone systems has been reported by Raphael *et al.*[33] in the synthesis of bullatenone **12**, through the heating of the intermediate **11** with potassium carbonate in methanol.



Scheme 1.1.8. Preparation of the 3(2*H*)-furanone **12**. i. K_2CO_3, methanol, reflux.

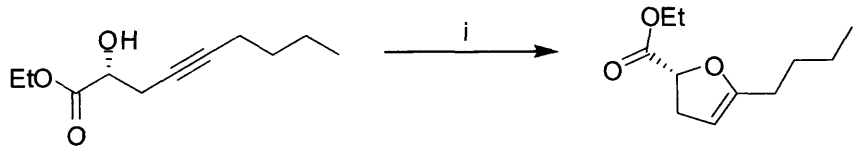
In an analogous system to that used by Raphael *et al.*,[34] Baldwin *et al.* famously used the base induced cyclisation of **13** in methanol as a test system for the 5-*endo-dig* type ring closure,[35] and again more recently in the synthesis of (-)-muscarine.[36]



13 12

Scheme 1.1.9. The Baldwin test system for 5-*endo-dig* ring closure. i. NaOMe, MeOH.

One of the more recent methods of dihydrofuran generation has been reported by Evans *et al.*[37] and references the use of palladium (II) in efforts to generate **15**.

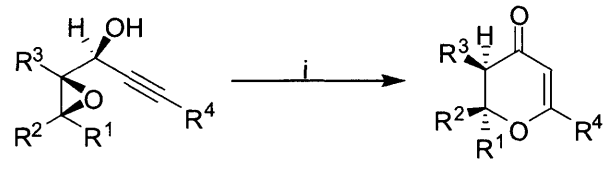


14 15

Scheme 1.1.10. Preparation of dihydrofuran **15**. i. $Pd(OAc)_2$, acetic acid, dichloromethane.

1.1.3 Mercury Catalysed Synthesis of 2,3-Dihydropyran-4-ones and 3(2*H*)-Furanones

Marson and co-workers have reported the mercury mediated and stereocontrolled rearrangement of 1-alkynyl-2,3-epoxy alcohols[38] in acidic media. This is a versatile reaction that tolerates a wide variety of functionality, and which can be modified to incorporate additional functionality into the pyranoid system.

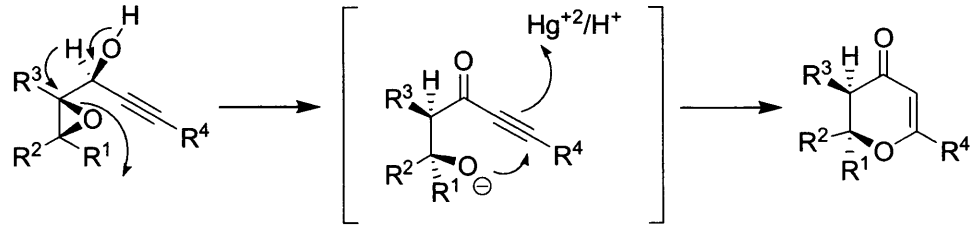


16 17

Scheme 1.1.11. General representation for the preparation of pyranoid systems using the mercury mediated cyclisation strategy. i. Hg^{+2}/H^{+}, acetone.

The configuration of the 2,3-disubstituted dihydropyranone is determined by the stereochemistry of the 1-alkynyl-2,3-epoxy alcohol (**16**), which results from asymmetric epoxidation of the chosen enynol precursor.

The reaction is believed to proceed though a semi-pinacol rearrangement of the epoxide, swiftly followed by ring closure; however, no intermediates have been found.



16 **18** **17**

Scheme 1.1.12. The rearrangement of epoxy alkynols to give 2,3-dihydro-4*H*-pyran-4-ones.

The construction of pyranoid systems using the mercury(II) mediated methodology can be applied to the synthesis of naturally occurring compounds. The methodology allows the preparation of complex pyranoid systems, which are often found as essential components of natural products. The pyranoid system can be synthesised as a small fragment, and incorporated into the synthetic procedure as a component within a larger convergent synthesis. The mercury(II) mediated methodology also permits the generation of the pyranoid system at a later point in the synthesis, this is due to the mild reaction conditions and the tolerance of a wide variety of functional groups, and results in greater flexibility in the overall synthesis of the target compound.

The use of the mercury(II) mediated cyclisation reaction to generate simple pyranoid systems are to be investigated, where R^1 to R^4 are simple alkyl or phenyl moieties. Inclusion of more complex functional groups in R^1 to R^4 would allow elaboration of the pyranoid system, and could be used to devise syntheses of natural products such as fostriecin,[39] phospholine,[40] and pironetin,[41] illustrated in figure 1.1.13.

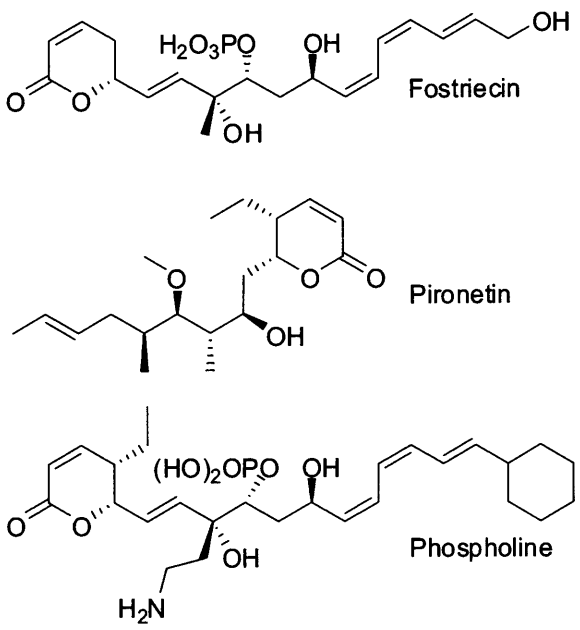
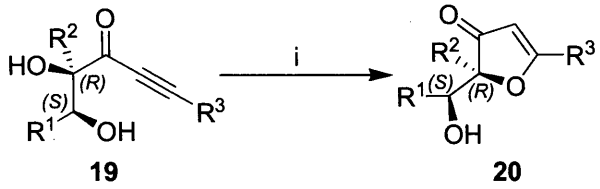


Figure 1.1.13. The natural products fostriecin (top), pironetin (middle), and phospholine (bottom).

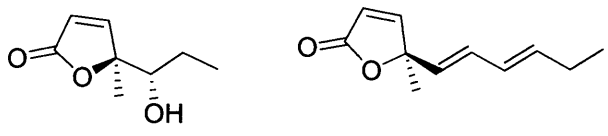
Using analogous methodology, Marson *et al.*[38] recently reported the use of the mercury mediated cyclisation to generate 3(2*H*)-furanone systems,[42] with varied functionality. Such systems are of great interest as they constitute versatile precursors in the synthesis of natural products.



19 **20**

Scheme 1.1.14. Synthesis of 3(2*H*)-furanone systems using the mercury mediated cyclisation strategy. i. Hg^{+2}/H^{+}, acetone.

The generation of tetronic acid frameworks (**5**), or butenolide (**4**) would be possible using this methodology, where the use of a carefully constructed 3(2*H*)-furanone system could be modified into the unsaturated lactones seen in figure 1.1.15.



4 **5**

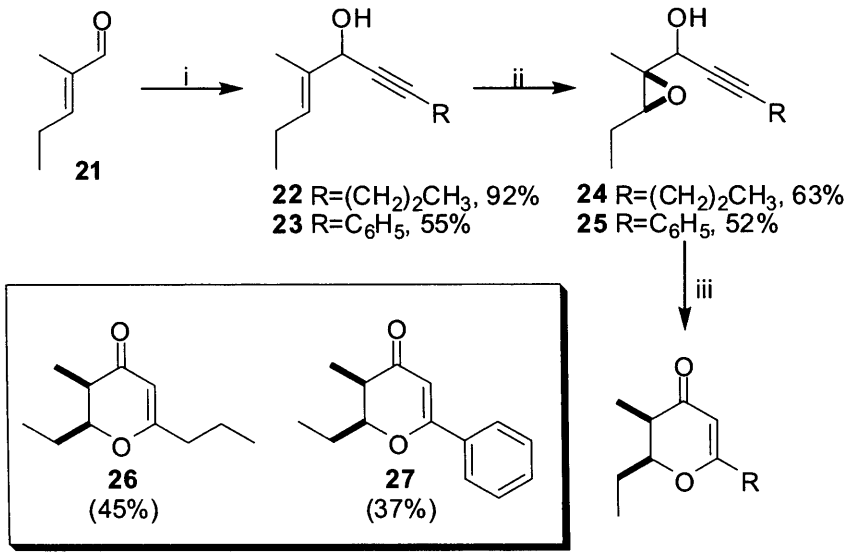
Figure 1.1.15. Butenolide (**4**) and tetronic acid frameworks (**5**).

The synthetic utility of fragment such as **20**, and dihydropyranone systems demands investigation. The Marson method[38] of 3(2*H*)-furanone and 2,3-dihydropyran-4-one generation, could be utilised in synthetic studies to greatly improve upon, and optimise the synthesis of natural products across a wide range of biologically active targets.

1.2 Results and Discussion

1.2.1 Preparation of 2,3-Disubstituted Dihydropyran-4-ones

Preparation of 2,3-disubstituted dihydropyran-4-ones was attempted using the procedure outlined in scheme 1.2.1.



Scheme 1.2.1. Preparation of 2,3-disubstituted dihydropyran-4-ones. R= $-(CH_2)_2CH_3$, $-C_6H_5$. i. *n*-BuLi, THF, HC≡C-R; ii. $VO(acac)_2$, *t*-butylhydroperoxide, benzene; iii. Hg^{+2}/H^+, acetone.

Preparation of the scaffolds **22** and **23** was accomplished by addition of lithium acetylides, prepared by the action of *n*-butyllithium upon pent-1-yne and phenyl acetylene respectively, to the enal **21**. Conversion into the epoxy alcohol was achieved using vanadyl acetylacetanoate, *tert*-butylhydroperoxide in benzene with yields of 63% and 52% for the pentyne (**24**) and phenylacetylene (**25**) derivatives respectively.

In order to conduct the cyclisation procedure upon the intermediates **24** and **25**, a mercury containing solution, comprising mercury (II) oxide yellow, water, and sulfuric acid was prepared. A small quantity of the catalytic solution (typically 0.2 mL) was added to a reaction vessel charged with analytical grade acetone, and the scaffold which was to undergo the cyclisation procedure. Reaction progress was monitored by TLC, using cerium(IV) sulfate solution as a stain to visualise the epoxide starting material. Reaction time was rapid, with the epoxide being undetectable by TLC within 10

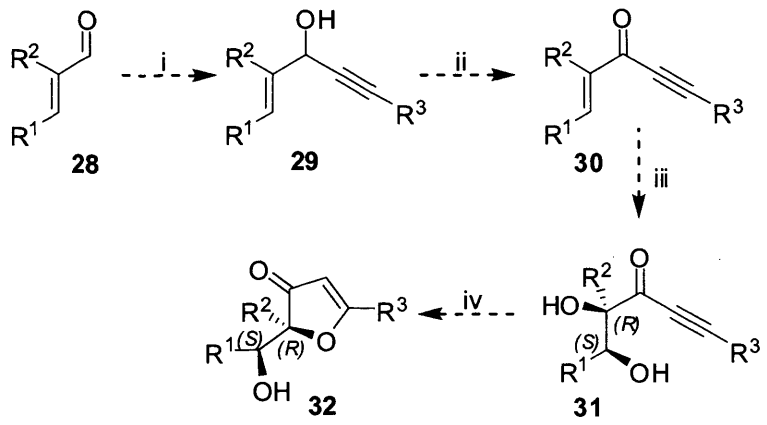
minutes. Quenching the reaction with the addition of sodium hydrogen carbonate, and stirring for 1 hour, gave the crude products after filtration and concentration. Flash column chromatography was used to obtain the pure samples **26** and **27** in 45% and 37% yield respectively. Analysis of ^{1}H and ^{13}C NMR data clearly identified the dihydropyranone products, the olefinic hydrogen and carbon being distinctive in both NMR spectra.

Use of the Marson method[38] to generate dihydropyranone systems is synthetically attractive, stereocontrol and the mild conditions enable the use of complex fragments in the cyclisation stages, simplifying the synthetic complexity of target synthesis.

Use of an acetylene such as ethoxyacetylene in the initial addition to enal **21** would result in the generation of a pyranoid system that could be used to derive unsaturated lactone species, by reduction of the carbonyl followed by dehydration. The synthetic methodology could be enhanced by the use of asymmetric reactions such as the powerful Sharpless asymmetric epoxidation reaction,[43] generation of stereochemically enriched intermediates would provide a route to stereochemically pure pyranoid systems, and greatly enhance the utility of the methodology. This would allow the generation of products and their various isoforms without significant alteration of the synthetic pathway.

1.2.2 Preparation of Furanones

The proposed synthetic route for the generation of furanone species using the mercury(II)-mediated cyclisation strategy can be seen in scheme 1.2.2.



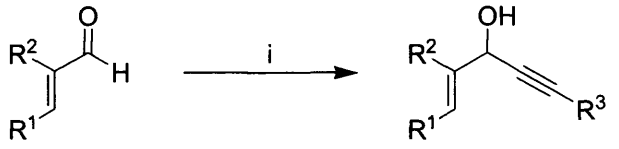
Scheme 1.2.2. The synthetic route to furanone compounds using the mercury(II)-mediated cyclisation strategy. i. *n*-BuLi, THF, HC≡C-R; ii. MnO_2, dichloromethane; iii. Sharpless asymmetric dihydroxylation; iv. Hg^{+2}/H^{+}, acetone.

Alkynylation of enal **28** affords the central scaffold of the furanone fragment **29**. Oxidation to the enynone **30**, followed by dihydroxylation allows the formation of the diol **31**. Finally, treatment of the diol with the catalytic mercury mediated cyclisation conditions yields the furanone species **32**.

The route is particularly convenient in that it enables the use of a variety of starting materials, acetylenes, and can be modified to take advantage of sterochemically controlled dihydroxylation reactions to specifically target desired furanone species.

A small number of substrates were prepared in order to examine the applicability of the synthetic route. Scaffold fragments were prepared according to the general reaction outlined in scheme 1.2.3.

The general reaction to prepare the enynol scaffold was conducted in dry tetrahydrofuran, at -78 °C. Lithium acetylides were prepared by the action of *n*-butyllithium upon suitable acetylenes. Once prepared, the acetylides were transferred by cannula to a vessel containing the appropriate conjugated aldehyde substrate, where the coupling reaction took place. After a short period, using thin layer chromatography to monitor the reaction, disappearance of the carbonyl compound would be total and the reaction would be considered complete. Work-up, followed by purification using flash column chromatography gave the required products, which can be seen as summarised in table 1.2.4.



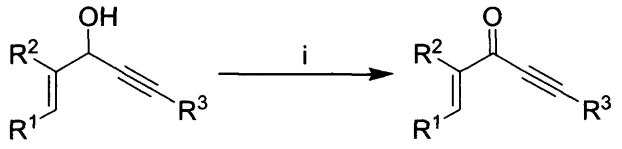
Scheme 1.2.3. Assembly of the carbon framework. i. *n*-BuLi, THF, HC≡C-R.

Compound	R^1	R^2	R^3	Yield
32	-H	$-CH_3$	$-(CH_2)_2CH_3$	68%
33	$-(CH_2)_2CH_3$	-H	$-OCH_2CH_3$	80%
34	$-(CH_2)_2CH_3$	-H	-OTBS	45%
35	$-(CH_2)_3CH_3$	-H	$-(CH_2)_4CH_3$	54%
36	$-C_6H_5$	-H	$-OCH_2CH_3$	55%

Table 1.2.4. Preparation of the Compounds **32** to **36**.

Oxidation of the enynol to an enynone was found to be easily accomplished using manganese dioxide in dichloromethane, using a method outlined by Jacobi *et al.*[44] in their oxidation of enynol substrates.

The enynol was typically added in one portion to a rapidly stirring suspension of activated manganese dioxide in dichloromethane under nitrogen. Stirring the mixture vigorously at room temperature afforded the enynone after 12-24 hours. Filtration allowed isolation of the enynone product after concentration. The reaction was found to be greatly dependent upon a variety of factors, and although simple to conduct, was unreliable due to inconsistent oxidative yields; however, the mild reaction conditions were considered to be of greater importance than the overall product yield and it was used in preference to other oxidative techniques. The enynones generated are detailed in table 1.2.6.



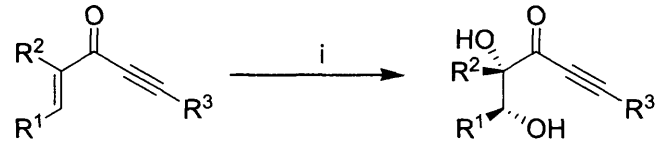
Scheme 1.2.5. Oxidation of enynols to enynones. i. MnO_2, dichloromethane, 12-24 h.

Compound	R^1	R^2	R^3	Yield
37	-H	$-CH_3$	$-(CH_2)_2CH_3$	83%
38	$-(CH_2)_2CH_3$	-H	$-OCH_2CH_3$	18%
39	$-(CH_2)_2CH_3$	-H	-OTBS	84%
40	$-(CH_2)_3CH_3$	-H	$-(CH_2)_4CH_3$	69%
41	$-C_6H_5$	-H	$-OCH_2CH_3$	51%

Table 1.2.6. Preparation of the Compounds **37** to **41**.

Sharpless asymmetric dihydroxylation according to the general scheme 1.2.7 was applied to the enynones.

The intermediates were dihydroxylated using fortified AD-mix-β (1 mol % of $K_2OsO_2(OH)_4$ and 1 mol % of ligand) and additional sodium hydrogen carbonate according to a procedure reported by Sharpless *et al.*[45] A solution containing 1:1 *tert*-butyl alcohol:water, AD-mix-β, 3 equivalents of sodium hydrogen carbonate, and 1 equivalent of methanesulfonamide was prepared, and cooled to 0 °C. The substrate was added to the vessel, and stirred vigorously until the reaction was complete by TLC. Warming the reaction to room temperature, followed by extraction of products into ethyl acetate gave the crude diols. Purification using column chromatography then allowed the isolation of the pure diol. The outcome of the dihydroxylations can be seen in table 1.2.8.



Scheme 1.2.7. Sharpless asymmetric dihydroxylation of enynones. i. Modified AD-mix-β, *t*-butyl alcohol, water, methanesulfonamide, $NaHCO_3$, 0 °C.

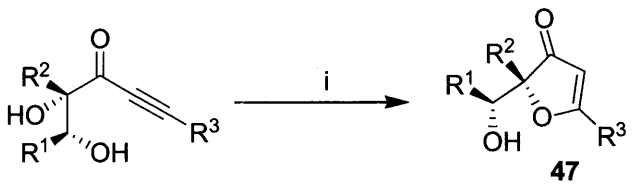
Compound	R^1	R^2	R^3	Yield
42	-H	$-CH_3$	$-(CH_2)_2CH_3$	0%
43	$-(CH_2)_2CH_3$	-H	$-OCH_2CH_3$	24%
44	$-(CH_2)_2CH_3$	-H	-OTBS	0%
45	$-(CH_2)_3CH_3$	-H	$-(CH_2)_4CH_3$	54%
46	$-C_6H_5$	-H	$-OCH_2CH_3$	0%

Table 1.2.8. Preparation of the compounds **42** to **46** by Sharpless asymmetric dihydroxylation.

Dihydroxylation of the enynone intermediates was found to be lengthy, with reaction times far longer then anticipated, even while using fortified AD-mix-β and methanesulfonamide. Reactions designed to produce the compounds **42**, and **46**, resulted in the recovery of starting material, with no diol products. The compound **39** did not generate the required diol, but produced unwanted side products. It was considered that **46** would spontaneously cyclise to the furanone species, due to the electron donating effect of the ethoxy R^3 moiety; however, this was found to be incorrect, and neither the diol nor furanone species had been formed.

38 and **40** were successful in generating the diol species **43** and **45** respectively, albeit in low yields. The intermediate **43** degraded rapidly upon standing, and prevented accurate measurement of e.e.

The e.e. of **45** was measured using a chiral column chromatography (Chiracel OD) to be 6%, an apparent lack of facial specificity in the dihydroxylation reaction.



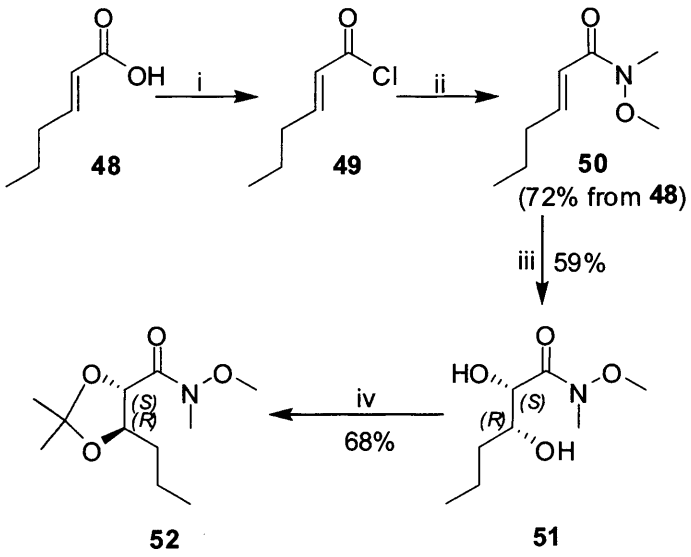
Scheme 1.2.9. Preparation of the furanone species. i. Hg^{+2}/H^{+}, acetone.

Material obtained from the dihydroxylation of the enynones was rapidly taken into the mercury mediated cyclisation. **43** and **45** were placed into a vessel containing acetone, and a small quantity (0.2 mL) of a freshly prepared mercury containing stock solution was added. The reaction was monitored by TLC, using anisaldehyde stain to visualise starting material and products. Once starting material was consumed, the reaction was quenched, and column chromatography used to separate and extract reaction products. Isolation and analysis of the reaction mixtures was unable to identify any of the desired furanone material.

The failure of the synthetic route to generate any furanone material required further investigation; however, the generation of intermediates in sufficient quantities appeared to be problematic due to the ineffective dihydroxylation of enynones. A secondary route was established to derive the intermediates required for mercury-mediated cyclisation.

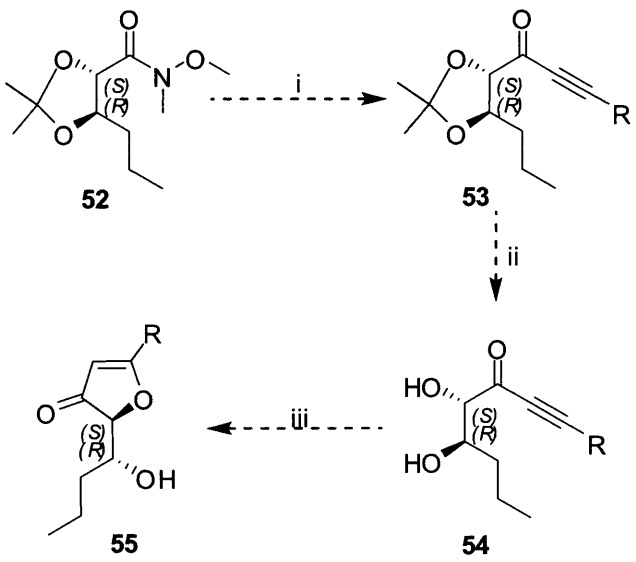
1.2.3 Preparation of Furanones Using Weinreb Amides

In previous routes to generate diol intermediates for the mercury-mediated cyclisation, the main failure was due to unsuccessful asymmetric dihydroxylation. Poor performance of the Sharpless asymmetric dihydroxylation upon electron deficient olefins is known to occur.[45] It was considered that it may be advantageous to re-sequence the dihydroxylation and acetylene coupling reactions to prevent unfavourable electronic effects upon the olefin, and to increase the level of catalytic turnover and facial specificity in the Sharpless dihydroxylation reaction.



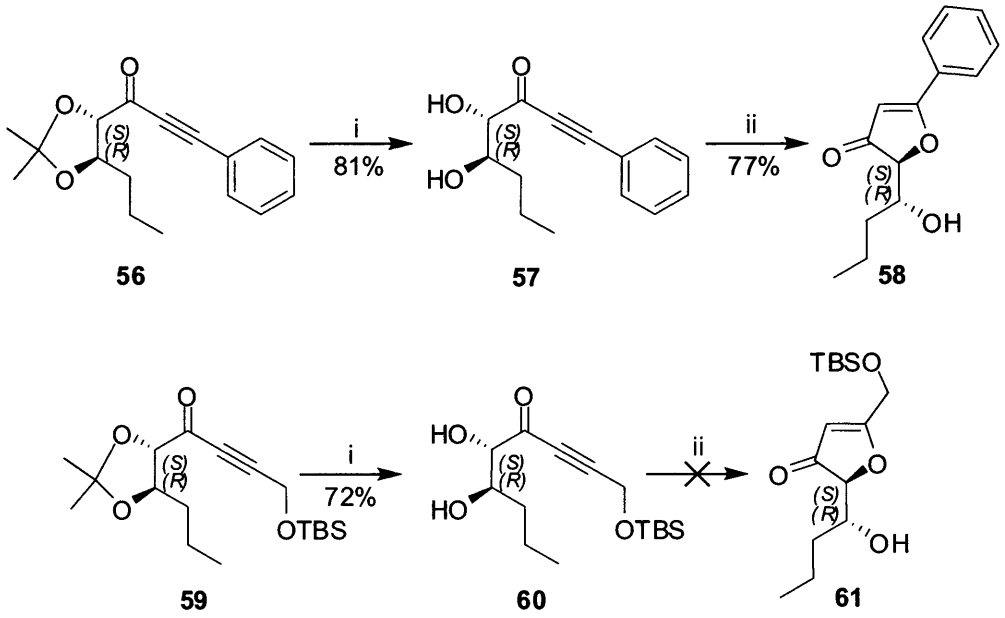
Scheme 1.2.10. The synthetic route to the dihydroxylated Weinreb amide. i. $SOCl_2$, benzene, reflux; ii. HN(OMe)Me.HCl, Et_3N, dichloromethane; iii. Modified AD-mix-β, 1:1 *t*-butyl alchol:water, $NaHCO_3$, $MeSO_2NH_2$; iv. Acetone, 2,2-dimethoxypropane, *p*-toluenesulfonic acid.

The Weinreb amide **50** was generated from the acid **48** in 72% over two steps. Sharpless asymmetric dihydroxylation gave the diol **51**, which was then quickly protected to give the Weinreb intermediate **52**. Coupling of acetylenes to the fragment could proceed according to previously established procedures, to give the protected fragment **53**. Continuation to the furanone fragment could then proceed. The synthetic route can be seen in scheme 1.2.11.



Scheme 1.2.11. Preparation of the furanone using the modified synthetic sequence. R = phenyl acetylene, *tert*-butyldimethyl(prop-2-ynyloxy)silane. i. HC≡C-R, *n*-BuLi, THF; ii. 2 M HCl, methanol; iii. Hg^{+2}/H^{+}, acetone.

The Weinreb amide fragment **52** was alkynylated according to procedures previously established in the preparation of enynols. Two fragments were constructed, one from phenylacetylene, and an additional fragment from *tert*-butyldimethyl(prop-2-ynyloxy)silane (**56** and **59** respectively, scheme 1.2.12).



Scheme 1.2.12. Construction of the furanone fragments **58** and **61**. i. 2 M HCl, methanol; ii. Hg^{+2}/H^{+}, acetone.

Attempts to use the acidic media of the mercury mediated cyclisation procedure to facilitate concomitant deprotection and cyclisation were unsuccessful. The sequential deprotection, isolation of the diol, and introduction of the intermediates to the mercury containing solution was therefore necessary.

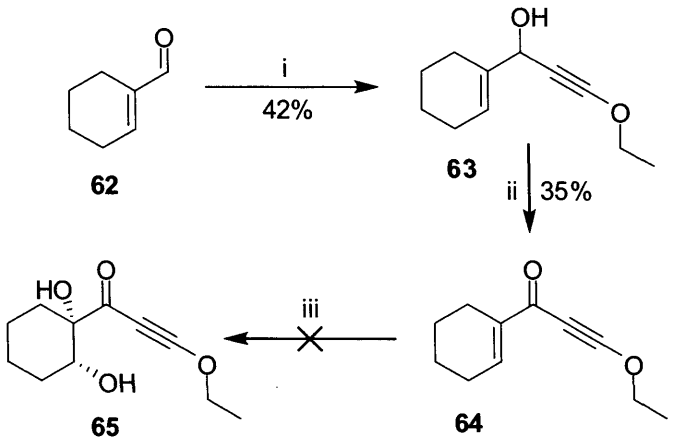
The deprotection of the diols proceeded in good yields of 81% and 72% (**57** and **60** respectively), in methanol and 2 M hydrochloric acid. The diols were isolated, followed by application of the mercury containing solution in acetone. The cyclisation reactions were conveniently monitored by TLC, using anisaldehyde stain to visualise products.

Diol **60** failed to generate any furanone material. Analysis of material from the reaction after work up, indicated that TBS protecting group cleavage had occurred and complex reaction products had been formed. Application of the mercury-containing solution to diol **57** was successful, and resulted in the generation of **58** in 77%. Analysis of the e.e of the diol, and fuanone systems using chiral column chromatography, determined the diol to possess an e.e. of 82%, and the mercury cyclised material to have an e.e. of 66%.

1.2.4 Preparation of Spiro Compounds

The generation of spiro compounds using the mercury mediated cyclisation reaction is of great synthetic potential. Application of the mercury mediated cyclisation strategy using cyclic diol intermediates was undertaken.

In the first instance, a preliminary investigation into [5.4] spiro systems was initiated. Scheme 1.2.13 illustrates attempts to prepare the diol intermediate **65** required for mercury mediated cyclisation.

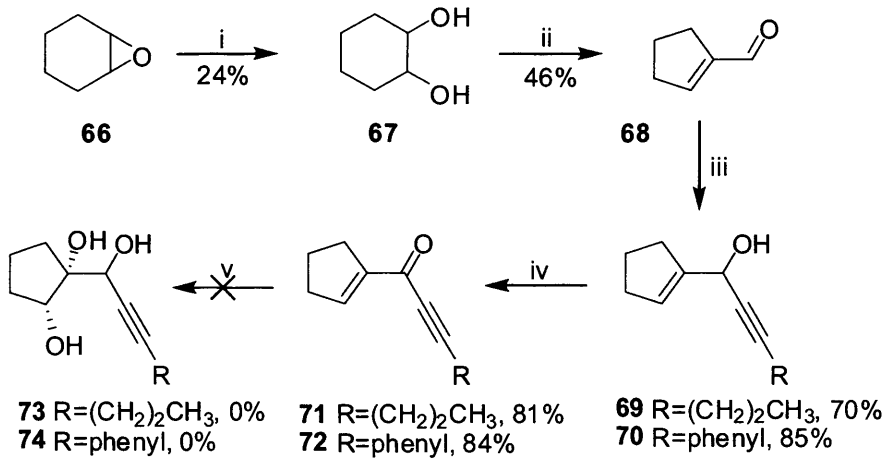


Scheme 1.2.13. Preparation of the diol **65**. i. *n*-BuLi, THF, ethoxyacetylene, -78 °C; ii. MnO_2, dichloromethane; iii. Modified AD-mix-β, 1:1 *t*-BuOH:water, $NaHCO_3$, $MeSO_2NH_2$.

Cyclohexene-1-carboxaldehyde was treated with ethoxyacetylene and *n*-butyllithium in tetrahydrofuran, generating the enynol **63** (42% yield). The choice of α,β-unsaturated aldehyde was based upon the previous reports of Sharpless *et al.*[45] and their efforts to successfully dihydroxylate 1-acetylcyclohexene using modified AD-mix reactants. It was believed that the dihydroxylation of the enynone **64**, generated by MnO_2 oxidation, would undergo dihydroxylation similarly; however, enynone **64** using fortified AD-mix-β failed to generate any dihydroxylated material or [4.5] spiro material from spontaneous cyclisation.

An alternate system was attempted, altering both the α,β-unsaturated aldehyde and the acetylene moiety in the intermediates. 1-Cyclopentene-1-carboxaldehyde (**68**) was

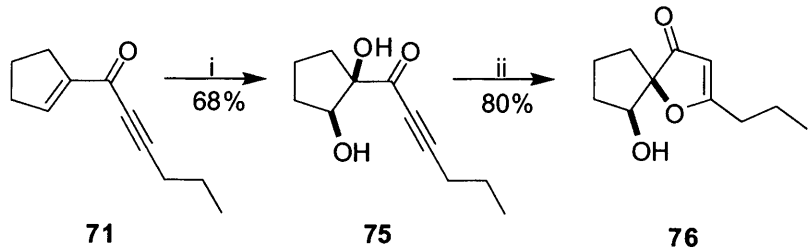
prepared from the diol **67** using sodium periodate followed by base.[46] Alkynylation using *n*-pentyne and MnO_2 oxidation yielded the intermediate **71**, acetylation using phenyl acetylene and oxidation yielded **72**.



Scheme 1.2.14. Preparation of the diol intermediates for spiro target generation. i. 10% H_2SO_4, 12 h; ii. $NaIO_4$, water, 20% KOH; iii. HC≡C-R (R = phenylacetylene, *n*-pentyne), *n*-BuLi, THF; iv. MnO_2, dichloromethane; v. Modified AD-mix-β, 1:1 *t*-BuOH:water, $NaHCO_3$.

Dihydroxylation of the enynones **71** and **72** failed to afford products, hydroxylated or otherwise.

The inability to perform asymmetric dihydroxylation reactions severely limits the scope of the synthetic utility of the method; however, in order to assess the cause of intermediate inactivity, the enynone **71** was dihydroxylated according to the Upjohn process.[47a]



Scheme 1.2.15. Preparation of the [4.4] spiro compound **76**. i. Potassium osmate, *N*-methylmorpholine (monohydrate), *t*-BuOH, acetone, water; ii. Hg^{+2}/H^{+}, acetone.

Using the Upjohn process it was possible to dihydroxylate **71** to **75** in 68%, isolating the diol as a pale oil. Treatment of the diol with the catalytic mercury solution afforded the [4.4] spiro compound **76** (80%), identified by ^{1}H and ^{13}C NMR. In particular, the C=O

carbon at 206.3 ppm corresponded to the strained carbonyl group in the five-membered ring, rather than that in the isomeric 4*H*-pyran-4-one ring. Additionally, the central spiro carbon was distinctive at 96.6 ppm, and the *alpha*-hydrogen of the furanone ring could be clearly seen at 5.41 ppm in the ^{1}H spectra.

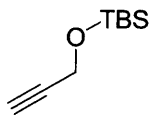
1.3 Conclusion

The synthesis of 2,3-dihydropyran-4-ones, 3(2*H*)-furanones and related spiro compounds has been demonstrated. The synthetic utility of the mercury mediated cyclisation procedure is currently limited when attempting to generate furanyl species, due to the inability to generate the required diol intermediates stereoselectively. Modification of the Sharpless asymmetric epoxidation, possibly through the use of alternate chiral ligands, could provide a means to obtain material of acceptable stereochemical purity. Alternatively, all methodology would have to pass though an intermediate subjected to asymmetric dihydroxylation prior to the construction of the central framework, as was demonstrated through Weinreb amide intermediates. The generation of furanone systems, if possible by this method, remains an interesting possibility and warrants further investigation, particularly with respect to stereoselective intermediate generation.

1.4 Experimental

Thin-layer chromatography (TLC) analyses were performed on Merck 0.2 mm aluminium-backed silica gel 60 F_{254} plates and components were visualized by illumination with UV light or through the use of with anisaldehyde stain. Flash column chromatography was performed using Merck 0.040 to 0.063 mm, 230 to 400 mesh silica gel. ^{1}H NMR spectra were recorded on a 300 MHz Bruker AC300 spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from internal tetramethylsilane (TMS), using residual chloroform (7.27 ppm) as an integral standard. The following abbreviations are used to describe NMR signals: δ, chemical shift; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad. Coupling constants *J* are given in Hertz (Hz). ^{13}C NMR were recorded on a 300 (75 MHz) Bruker AC300 spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from TMS, using the middle resonance of $CDCl_3$ (77.0 ppm) as an integral standard. Mass spectra were obtained by using a VG7070H mass spectrometer with Finigan Incos II operating in electron impact (EI) mode, unless otherwise specified in the text. Starting materials were purchased from the Aldrich or Lancaster chemical companies. Tetrahydrofuran (THF) was distilled from sodium/benzophenone prior to use. Ether refers to diethyl ether. All glassware was oven-dried, assembled hot and cooled under a stream of nitrogen gas before use. Reactions with air-sensitive materials were carried out by standard syringe techniques. Temperatures of – 78 °C were obtained by the addition of dry ice to acetone; -10 °C by the addition of acetone to ice.

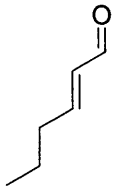
tert-Butyldimethyl(prop-2-ynyloxy)silane[47b]



To a stirring solution of propargyl alcohol (1.86g, 33.2 mmol), imidazole (4.25 g, 66.4 mmol) in DMF (100 mL), TBS-Cl (5 g, 33.2 mmol) was added in one portion. The mixture was stirred at room temperature for 12 h. Ethyl acetate (50 mL) and water (50 mL) was added. Organic fractions were separated, and washed with water (3 x 20 mL). The organic material was dried ($MgSO_4$) and concentrated under reduced pressure to give the crude product. Purification by column chromatography using an eluant of 9:1 hexane:ethyl acetate gave the required product as an oil, 5.39 g, 95%. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 4.28 (s, 2H, HC≡CH_2-), 2.39 (s, 1H,

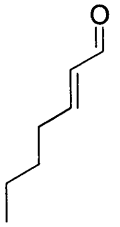
HC≡$\underline{CH_2}$-O), 0.88 (s, 9H, O-$\underline{TBS}$), 0.11 (s, 6H, O-$\underline{TBS}$). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 82.4, 72.8, 51.5, 25.7, 18.3, -3.6.

(*E*)-Hex-2-enal[47c]



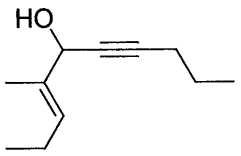
To *trans*-hexen-1-ol (4 g, 39.9 mmol) in dichloromethane (200 mL), celite (17.2 g), and PCC (2 eq, 17.2 g, 79.8 mmol) was added. The suspension was stirred at room temperature for 3 h. The reaction mixture was then filtered through a short silica plug, and the filtrate concentrated under reduced pressure to give the product which required no further purification, 3.83 g, 98%. ^1H NMR (300MHz, $CDCl_3$), δ_H 9.47 (d, J=8.0 Hz, 1H, CH=CH-$\underline{CHO}$), 6.82 (dt, J=16.0, 7.0 Hz, 1H, CH_2-$\underline{CH}$=CH), 6.08 (dd, J=16.0, 8.0 Hz, 1H, CH=$\underline{CH}$-CHO), 2.28 (m, 2H, CH_2-$\underline{CH_2}$-CH), 1.51 (m, 2H, CH_3-$\underline{CH_2}$-CH_2), 0.93 (t, J=7.3 Hz, 3H, $\underline{CH_3}$-CH_2-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 194.1, 158.7, 133.1, 34.6, 21.1, 13.6.

(*E*)-Hept-2-enal[47d]



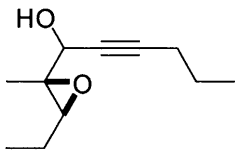
To a stirring suspension of PCC (6.5 g, 30 mmol) and celite (6.5 g) in dichloromethane (60 mL), (*E*)-hept-2-en-1-ol (2 g, 20.0 mmol) was added in one portion. The mixture was stirred, and monitored by TLC. Upon completion, the reaction mixture was filtered through a silica plug. Concentration of the filtrate under reduced pressure gave the required material as an oil, which required no further purification, 1.32 g, 59%. ^1H NMR (300MHz, $CDCl_3$), δ_H 9.5 (d, J=7.9 Hz, 1H CH=CH-$\underline{CHO}$), 6.8 (dt, J=15.6, 6.8 Hz, 1H, CH_2-$\underline{CH}$=CH), 6.1 (dd, J=15.6, 7.9 Hz, 1H, CH=$\underline{CH}$-CHO), 2.27 (dt, J=7.4, 6.8 Hz, 2H, CH-$\underline{CH_2}$-CH_2), 1.67-1.20 (m, 4H, CH_3-$\underline{CH_2}$-CH_2, CH_2-$\underline{CH_2}$-CH_2), 0.93 (t, J=7.4 Hz, 3H, $\underline{CH_3}$-CH_2-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 194.1, 158.7, 133.1, 34.7, 31.1, 21.0, 13.6. HRMS calcd. for $C_7H_{12}O$ (M+H) 113.0966, found 113.0965

(*E*)-4-Methyldec-3-en-6-yn-5-ol (22)



To a stirring solution of pentyne (3.55 g, 52.1 mmol) in 100 mL of THF at 0 °C, *n*-butyllithium was added (2.5 M in hexanes, 22.5 mL, 56.3 mmol). The mixture was stirred for 1 h. 2-Methylpent-2-enal (4.65 g, 47.4 mmol) in 30 mL of THF was added dropwise to the reaction mixture, and maintained at 0 °C. The reaction was stirred for 1 h at 0 °C, then allowed to warm to ambient temperature and stirred for 12 h. The reaction was quenched with saturated ammonium chloride solution (50 mL), and products extracted into dichloromethane (3 x 30 mL). The organic fractions were combined, dried ($NaSO_4$), and concentrated under reduced pressure to give the crude product as a light yellow oil. Purification by column chromatography gave the desired product as a colourless oil, 7.22g, 92%. IR ν_{max} (KBr, cm^{-1}) 3364 (OH), 2228 (C≡C), 1624 (C=C). ^1H NMR (300MHz, $CDCl_3$), δ_H 5.55 (t, *J*=7.0 Hz, 1H, CH_2-CH=C), 4.36 (m, 1H, CH-OH), 2.22-2.17 (m, 4H, CH_3-CH_2-CH, C≡CH_2-CH_2), 1.72 (s, 3H, CH_3-C), 1.56-1.49 (m, 2H, CH_2-CH_2-CH_3), 1.24 (t, *J*=7.1 Hz, 3H, CH_3-CH_2-CH), 0.96 (t, *J*=7.5 Hz, 3H, CH_3-CH_2-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 134.1, 129.6, 86.6, 79.7, 68.3, 22.1, 21.0, 20.8, 13.8, 13.5, 12.0. HRMS calcd. for $C_{11}H_{18}O$ (M-H) 165.1279, found 165.1289.

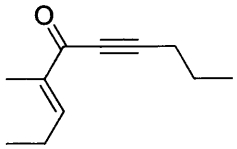
1-(3-Ethyl-2-methyl-oxiranyl)-hex-2-yn-1-ol (24)



A solution of benzene (200 mL), 4-methyldec-3-en-6-yn-5-ol (3.0 g, 18 mmol), and vanadyl acetylacetanoate (0.06 g, 0.2 mmol) was prepared and stirred for 5 min. *tert*-butylhydoperoxide (70% solution in water, 3.29 g, 25.2 mmol) was added dropwise, and was stirred at ambient temperature overnight. The mixture was then poured onto saturated sodium sulfite solution, and the organic layer extracted. The organic layer was dried using sodium sulfate, and concentrated under reduced pressure to give the crude product as an oil. Purification by column chromatography using an eluant of 9:1 hexane:ethyl acetate gave the product as a pale yellow oil, 2.08 g, 63%. IR ν_{max} (KBr, cm^{-1}) 3420 (OH), 2210 (C≡C). ^1H NMR (300MHz, $CDCl_3$), δ_H 4.37 (m, 1H, CH-OH), 3.08 (t, *J*=5.6 Hz, 1H, (O)(C)CH-CH_2), 2.18-2.16 (m, 2H, C≡CH_2-CH_2), 1.65-1.50 (m, 4H, CH_3-CH_2-CH, CH_2-CH_2-CH_3), 1.36 (s, 3H, CH_3-C), 1.01 (t, *J*=7.5 Hz, 3H, CH_3-CH_2-CH), 0.95 (t,

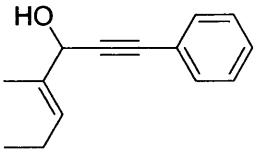
J=7.3 Hz, 3H, $\underline{CH_3}$-CH_2-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 86.9, 77.5, 66.8, 65.1, 61.4, 21.9, 21.5, 20.7, 13.7, 13.4, 10.4.

(*E*)-4-Methyldec-3-en-6-yn-5-one



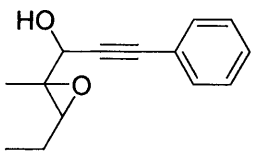
To a vigorously stirring suspension of MnO_2 (13.85 g, 320 mmol) in dichloromethane (100 mL), 4-methyldec-3-en-6-yn-5-ol (3.03 g, 19.9 mmol) was added in one portion. The reaction was stirred vigorously for 12 h, under an inert atmosphere. No reaction was found to occur.

(*E*)-4-Methyl-1-phenyl-hept-4-en-1-yn-3-ol (23)



To a stirring solution of phenylacetylene (1.35 g, 13.3 mmol) in dry THF (30 mL), *n*-butyllithium (2.5 M in hexanes, 14.3 mmol) was added dropwise at 0 °C. The reaction was stirred for 30 min. 2-Methyl-2-pentenal (1 g, 10.2 mmol) was taken into dry THF (20 mL), and added to the reaction mixture. The mixture was warmed to room temperature, and stirred for 8 h. The reaction was poured on to sat. ammonium sulfate solution (30 mL), and extracted with diethyl ether (3 x 20 mL). Combined organic fractions were dried ($MgSO_4$), and concentrated under reduced pressure to an oil. Purification by column chromatography using an eluant of 2:1 hexane:ethyl acetate gave the required product as a light orange oil, 1.12 g, 55%. IR ν_{max} (KBr, cm^{-1}) 3373 (OH), 2201 (C≡C), 1625 (C=C). ^1H NMR (300MHz, $CDCl_3$), δ_H 7.28-7.46 (m, 5H, Ar-$\underline{H}$), 5.69-5.52 (m, 1H, CH_2-$\underline{CH}$=C), 4.98 (s, 1H, $\underline{CH}$-OH), 2.15-2.04 (m, 2H, CH_3-$\underline{CH_2}$-CH), 1.82 (s, 3H, $\underline{CH_3}$-C), 1.00 (t, J=7.5 Hz, 3H, $\underline{CH_3}$-CH_2-CH). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 133.7, 131.7, 130.2, 128.3, 122.7, 88.7, 85.9, 68.5, 21.1, 13.8, 12.2.

1-(3-Ethyl-2-methyloxiran-2-yl)-3-phenylprop-2-yn-1-ol (25)



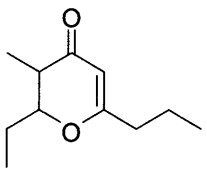
A solution of benzene (100 mL), 4-methyl-1-phenyl-hept-4-en-1-yn-3-ol (1.12 g, 5.6 mmol), and vanadyl acetylacetanoate (0.02 g, 0.07 mmol) was prepared and stirred for 5 min. *Tert*-butylhydroperoxide (70% solution in water, 1.3 g, 9.96 mmol) was added dropwise, and was stirred at ambient temperature for 18 h. The reaction was then poured onto saturated sodium sulfite solution, and the organic layer

extracted. The extracted organic layer was dried using sodium sulfate, and concentrated under reduced pressure to give the crude product as an oil. Purification by column chromatography using an eluant of 6:1 hexane:ethyl acetate gave the product as a pale yellow oil, 0.634 g, 52%. IR ν_{max} (KBr, cm^{-1}) 3416 (OH), 2231 (C≡C). ^1H NMR (300MHz, $CDCl_3$), δ_H 7.46-7.26 (m, 5H, Ar-H), 4.54 (m, 1H, CH-OH), 3.18 (t, J=6.4 Hz, 1H, (O)(C)CH-CH_2), 1.65-1.57 (m, 2H, CH_3-CH_2-CH), 1.45 (s, 3H, CH_3-C), 1.24 (t, J=7.1 Hz, 3H, CH_3-CH_2-CH). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 131.8, 128.3, 122.3, 86.6, 85.9, 67.0, 65.6, 62.0, 21.5, 13.8, 10.4. HRMS calcd. for $C_{14}H_{16}O_2$ 216.1150, found 216.1149.

Catalytic Mercury Solution

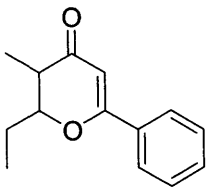
To a rapidly stirred mixture of water (1.2 mL) and concentrated sulfuric acid (0.125 mL), mercury (II) oxide yellow (0.11 g, 0.5 mmol) was added. Upon dissolution, 3.7 mL of water was added, forming the catalytic mercury solution.

2-Ethyl-3-methyl-6-propyl-2,3-dihydro-4*H*-pyran-4-one (26)



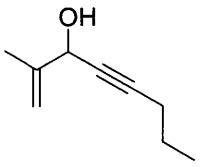
To a stirring solution of 1-(3-ethyl-2-methyl-oxiranyl)-hex-2-yn-1-ol (0.1 g, 0.55 mmol) in HPLC grade acetone (10 mL), 0.1 mL of freshly prepared mercury stock solution was added to the reaction mixture. The mixture was stirred for 20 min and observed by TLC. Sodium hydrogen carbonate (0.1 g) was added, and the reaction stirred for 1.5 h. Ethyl acetate (10 mL) was added, and the reaction mixture filtered. The filtrate was concentrated under reduced pressure, to an oil. Purification by column chromatography using an eluant of 10:1 hexane:ethyl acetate, gave the product **26** as an oil, 46 mg, 45%. IR ν_{max} (KBr, cm^{-1}) 1680 (C=O), 1615 (C=C). ^1H NMR (300MHz, $CDCl_3$), δ_H 5.23 (s, 1H, C=CH-CO), 4.21-4.14 (m, 1H, CH2-CH-(O)(CH)), 2.34-2.18 (m, 3H, CH_3-CH, C-CH_2-CH_2), 1.93-1.72 (m, 2H, CH_3-CH_2-CH), 1.63-1.55 (m, 2H, CH_2-CH_2-CH_3), 1.04-0.97 (m, 6H, CH_3-CH_2-CH, CH_3-CH), 0.94 (t, J=7.1 Hz, 3H, CH_3-CH_2-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 198.1, 178.0, 102.7, 83.2, 42.5, 36.5, 23.4, 19.8, 13.5, 9.8, 9.5. HRMS calcd. for $C_{11}H_{18}O_2$ 182.1307, found 182.1310.

2-Ethyl-3-methyl-6-phenyl-2,3-dihydro-4*H*-pyran-4-one (27)



To a stirring solution of 1-(3-ethyl-2-methyl-oxiranyl)-3-phenyl-prop-2-yn-1-ol (0.1 g, 0.46 mmol) in HPLC grade acetone (10 mL), 0.1 mL of freshly prepared mercury stock solution was added to the reaction mixture. The mixture was stirred for 20 min, and observed by TLC. Sodium hydrogen carbonate (0.1 g) was added, and the reaction stirred for 1.5 h. Ethyl acetate (10 mL) was added, and the reaction mixture filtered. The filtrate was concentrated under reduced pressure, to an oil. Purification by column chromatography using an eluant of 10:1 hexane:ethyl acetate, gave **27** as an oil (38 mg, 37%). IR ν_{max} (KBr, cm^{-1}) 1662 (C=O), 1605 (C=C). ^1H NMR (300MHz, $CDCl_3$), δ_H 8.03-7.73 (m, 5H, Ar-H), 5.94 (s, 1H, C=CH-CO), 4.45-4.39 (m, 1H, CH_2-CH-(O)(CH)), 2.47-2.42 (m, 1H, CH_3-CH), 2.10-1.95 (m, 1H, CH_3-CHH-CH), 1.75-1.66 (m, 1H, CH_3-CHH-CH), 1.44-1.09 (m, 3H, CH_3-CH), 0.87-0.82 (m, 3H, CH_3-CH_2-CH). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 198.3, 169.5, 132.8, 131.6, 128.7, 126.5, 100.6, 83.6, 43.0, 23.6, 10.1, 9.5. HRMS calcd. for $C_{14}H_{16}O_2$ 216.1150, found 216.1151.

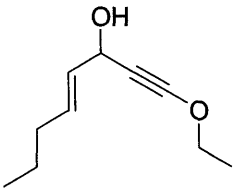
2-Methyloct-1-en-4-yn-3-ol (32)



To a stirring solution of pentyne (18.6 mmol, 1.27 g) in 50 mL of tetrahydrofuran, was added *n*-butyllithium (2.5 M in hexanes, 19.5 mmol) dropwise at room temperature. The mixture was left to stir for 30 min, and then methacrylaldehyde (1 g, 14.3 mmol) was added to the reaction in THF (20 mL). The mixture was stirred for 3 h, and then quenched with the addition of saturated ammonium carbonate solution. Extraction of organic material by washing the reaction mixture with dichloromethane (3 x 30 mL) yielded the crude product after combination, drying (magnesium sulfate), and concentration under reduced pressure. Isolation through the use of column chromatography, using an eluant of 4:1 hexane:ethyl acetate, gave the desired product as a colourless oil, 1.34 g, 68%. IR ν_{max} (KBr, cm^{-1}) 3350 (OH), 2208 (C≡C), 1650 (C=C). ^1H NMR (300MHz, $CDCl_3$), δ_H 5.14 (m, 1H, HHC=C), 4.89 (m, 1H, HHC=C), 4.77 (s, 1H, CH-OH), 2.28-2.15 (m, 2H, CH_2-CH_2-CH_3), 2.05-1.92 (br, 1H, OH), 1.84 (s, 3H, CH_3-C), 1.53 (qt, J=7.4, 7.2 Hz, 2H, CH_3-CH_2-CH_2), 0.97 (t, J=7.4 Hz, 3H, CH_3-CH_2-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C

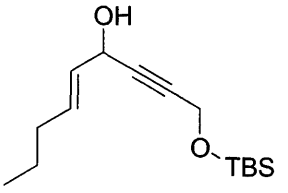
144.7, 111.9, 86.6, 79.4, 66.5, 22.0, 20.7, 18.0, 13.5. HRMS calcd. for $C_9H_{14}O$ 138.1045, found 138.1044.

(*E*)-1-Ethoxyoct-4-en-1-yn-3-ol (33)



To a stirring solution of ethoxyacetylene (21 mg, 3 mmol) in dry THF (20 mL), *n*-butyllithium (2.5 M in hexanes, 3.04 mmol) was added dropwise at 0 °C. The reaction was stirred for 10 min, followed by the dropwise addition of (*E*)-hex-2-enal (221 mg, 2.25 mmol). The reaction was stirred at 0 °C for 30 min, then warmed to room temperature. The reaction was followed by TLC, and quenched with the addition of saturated ammonium chloride solution (10 mL). Ethyl acetate (20 mL) was added, and organic material separated from the reaction mixture. The organic fraction was washed with water (1 x 5 mL) and brine (1 x 5 mL), and dried ($MgSO_4$). Concentration under reduced pressure gave the crude product. Purification by column chromatography using an eluant of 4:1 hexane:ethyl acetate gave the product as an oil, 320 mg, 80%. IR ν_{max} (KBr, cm^{-1}) 3426 (OH), 2264 (C≡C), 1625 (C=C).^{1}H NMR (300MHz, $CDCl_3$), δ_H 5.78 (dt, *J*=15.2, 6.7 Hz, 1H, CH_2-CH=CH), 5.56 (dd, *J*=15.2, 6.0 Hz, 1H, CH=CH-CH(OH)), 4.90-4.81 (m, 1H, CH-OH), 4.09 (q, *J*=6.1 Hz, 2H, O-CH_2-CH_3), 2.08-1.90 (m, 2H, CH-CH_2-CH_2), 1.50-1.27 (m, 2H, CH_2-CH_2-CH_3), 1.23 (t, *J*=6.1 Hz, 3H, O-CH_2-CH_3), 0.88 (t, *J*=7.3 Hz, 3H, CH_2-CH_2-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 132.5, 130.5, 94.7, 74.6, 62.9, 38.4, 33.9, 22.1, 14.3, 13.7. HRMS calcd. for $C_{10}H_{16}O_2$ 168.1150, found 168.1154.

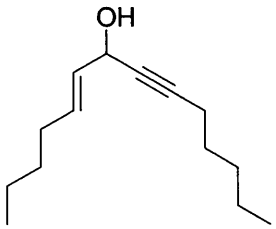
(*E*)-1-(*tert*-Butyldimethylsilyloxy)non-5-en-2-yn-4-ol (34)



To a stirring solution of *tert*-butyldimethyl(prop-2-ynyloxy)silane (0.4 g, 2.3 mmol) in 20 mL of tetrahydrofuran, was added *n*-butyllithium (2.2 M in hexanes, 2.32 mmol) dropwise at room temperature. The reaction was left to stir for 30 minutes, and then (*E*)-hex-2-enal (0.17 g, 1.73 mmol) was added to the reaction in 5 mL of tetrahydrofuran. The reaction was stirred for 3 h, and then quenched with the addition of saturated ammonium carbonate solution. Extraction of organic material into dichloromethane (3 x 20 mL) yielded the crude product after combination, drying (magnesium sulfate), and concentration under reduced pressure. Isolation through the use of column chromatography, with an eluant of 9:1 hexane:ethyl acetate, gave the

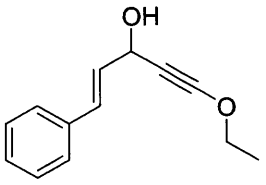
desired product as a colourless oil, 0.280 g, 45%. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 5.95-5.75 (m, 1H, CH_2-CH=CH), 5.57 (dd, *J*=6.1, 15.3 Hz, 1H, CH=CH-CH), 4.84 (d, *J*=6.1 Hz, 1H, CH-CH(OH)(C)), 4.36 (s, 2H, C-CH_2-O), 2.05-1.95 (m, 2H, CH_2-CH_2-CH), 1.50-1.10 (m, 2H, CH_3-CH_2-CH_2), 0.93-0.87 (m, 12H, O-TBS, CH_3-CH_2-CH_2), 0.11 (s, 6H, O-TBS). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 133.8, 129.0, 84.5, 84.3, 63.0, 51.8, 34.0, 25.8, 23.3, 18.3, 14.1. HRMS calcd. for $C_{15}H_{28}O_2Si$ 268.1859, found 268.1863.

(*E*)-Tetradec-5-en-8-yn-7-ol (35)



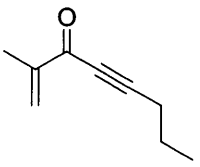
To a stirring solution of heptyne (1.14 g, 11.87 mmol) in dry THF (20 mL), *n*-butyllithium (13.06 mmol) was added dropwise. The reaction mixture was then added to a flask containing (*E*)-hept-2-enal (1 g, 8.9 mmol) in dry THF (10 mL) *via* cannula. The reaction was stirred for 1 h, and quenched with the addition of saturated ammonium carbonate solution. Extraction of organic material by washing the reaction mixture with dichloromethane (3 x 20 mL) yielded the crude product after combination, drying ($MgSO_4$), and concentration under reduced pressure. Column chromatography, using an eluant of 9:1 hexane:ethyl acetate, gave the desired product (1.34 g, 54%) as a colourless oil. IR ν_{max} (KBr, cm^{-1}) 3330 (OH), 2204 (C≡C), 1610 (C=C). ^{1}H NMR (300MHz, $CDCl_3$), δ_H 5.81 (dt, *J*= 8.0, 15.7 Hz, 1H, CH_2-CH=CH), 5.57 (dd, *J*= 4.4, 15.7 Hz, 1H, CH=CH-CH(OH)), 4.80 (d, *J*=4.4 Hz, 1H, CH-OH), 2.20 (m, 2H, CH-CH_2-CH_2), 2.00-1.90 (m, 2H, C-CH_2-CH_2), 1.47-1.30 (m, 10H, -CH_2-), 1.24 (t, *J*=7.2 Hz, 3H, CH_3-CH_2), 0.90 (t, *J*=4.8 Hz, 3H, CH_3-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 133.2, 129.8, 86.8, 79.7, 63.2, 34.0, 31.1, 28.3, 22.2, 22.1, 21.0, 18.7, 13.9, 13.7.

5-Ethoxy-1-phenyl-pent-1-en-4-yn-3-ol (36)



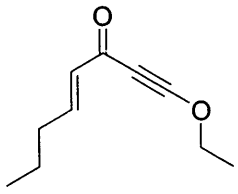
To a stirring solution of ethoxyacetylene (0.44 g, 6.3 mmol) in 100 mL of THF at 0 °C, *n*-butyllithium (2.5 M in hexanes, 6.6 mmol) was added. The mixture was stirred for 1 h. Cinnamaldehyde (0.621 g, 4.7 mmol) in THF (5 mL) was added dropwise to the reaction mixture, and maintained at 0 °C. The reaction was stirred for 1 h at 0 °C, then allowed to warm to ambient temperature and stirred for 12 h. The reaction was quenched with saturated ammonium chloride solution (20 mL), and products extracted into dichloromethane (3 x 30 mL). Organic fractions were combined, dried ($NaSO_4$), and concentrated under reduced pressure to give the crude product as a light yellow oil. Purification by column chromatography gave the desired product as a colourless oil, 0.52, 55%. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 7.52-7.21 (m, 5H, Ar-H), 6.73 (d, *J*=15.6 Hz, 1H, Ph-CH=CH), 6.30 (dd, *J*=15.6, 5.7 Hz, 1H, CH=CH-CH(OH)), 5.15-5.02 (m, 1H, CH-CH(OH)(C)), 4.15 (q, *J*=7.1 Hz, 2H, O-CH_2-CH_3), 2.05 (d, *J*=5.7 Hz, 1H, CH-OH), 1.40 (t, *J*=7.1 Hz, 3H, CH_3-CH_2-O). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 136.4, 130.8, 129.7, 128.6, 126.7, 126.5, 95.2, 74.8, 62.9, 37.9, 14.4. HRMS calcd. for $C_{13}H_{14}O_2$ 202.0994, found 202.0997.

2-Methyloct-1-en-4-yn-3-one (37)



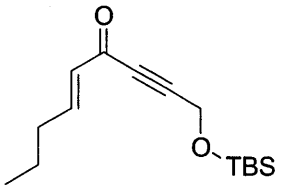
To a stirring suspension of MnO_2 (8.2 g, 116 mmol) in dichloromethane (35 mL), 2-methyloct-1-en-4-yn-3-ol (1 g, 7.2 mmol) was added, and stirred for 12 h under an inert atmosphere. The reaction was filtered through celite, and the filtrate concentrated under reduced pressure to give the crude product. Column chromatography using an eluant of 19:1 hexane:ethyl acetate gave the product as an oil, 820 mg, 83%. IR ν_{max} (KBr, cm^{-1}) 2204 (C≡C), 1652 (C=O), 1615 (C=C). ^{1}H NMR (300MHz, $CDCl_3$), δ 6.39 (m, 1H, HHC=C), 5.95 (m, 1H, HHC=C), 2.35 (t, *J*=7.0 Hz, 2H, CH_2-CH_2-C), 1.86 (s, 3H, CH_3-C), 1.64-1.62 (m, 2H, CH_2-CH_2-CH_3), 1.01 (t, *J*=6.4 Hz, 3H, CH_2-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ 180.3, 145.1, 130.3, 94.6, 78.9, 21.3, 20.9, 16.1, 13.5. HRMS calcd. for $C_9H_{13}O$ (M+H) 137.0966, found 137.0971.

(*E*)-1-Ethoxyoct-4-en-1-yn-3-one (38)



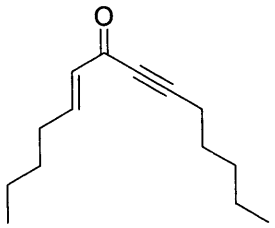
To a stirring suspension of MnO_2 (2.5 g, 28.8 mmol) in dichloromethane (20 mL), (*E*)-1-ethoxyoct-4-en-1-yn-3-ol (0.32 g, 1.8 mmol) was added, and stirred for 12 h under an inert atmosphere. The reaction was filtered through celite, and the filtrate concentrated under reduced pressure to give the crude product. Column chromatography using an eluant of 9:1 hexane:ethyl acetate gave the product as an oil, 55 mg, 18%. IR ν_{max} (KBr, cm^{-1}) 2245 (C≡C), 1685 (C=O), 1559 (C=C). ^1H NMR (300MHz, $CDCl_3$), δ_H 6.97 (dt, *J*=15.7, 6.9 Hz, 1H, CH_2-CH=CH), 6.07 (d, *J*=15.7 Hz, 1H, CH=CH-C), 4.32 (q, *J*=7.1 Hz, 2H, C-CH_2-CH_3), 2.31-2.15 (m, 2H, CH_2-CH_2-CH), 1.59-1.38 (m, 2H, CH_3-CH_2-CH_2), 1.20 (t, *J*=7.1 Hz, 3H, O-CH_2-CH_3), 0.82 (t, *J*=6.2 Hz, 3H, CH_2-CH_2-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 178.9, 151.6, 132.5, 102.3, 77.5, 34.1, 29.0, 22.6, 14.2, 14.0. HRMS calcd. for $C_{10}H_{14}O_2$ 166.0994, found 166.0994.

(*E*)-1-(*tert*-Butyldimethylsilyloxy)non-5-en-2-yn-4-one (39)



To a stirring suspension of MnO_2 (16 eq, 1.45 g, 16.64 mmol) in dichloromethane (15 mL), (*E*)-1-(*tert*-butyldimethylsilyloxy)non-5-en-2-yn-4-ol (0.28 g, 1.04 mmol) was added, and stirred for 12 h under an inert atmosphere. The reaction was filtered through celite, and the filtrate concentrated under reduced pressure to give the crude product. Column chromatography using an eluant of 9:1 hexane:ethyl acetate gave the product as an oil, 230 mg, 84%. ^1H NMR (300MHz, $CDCl_3$), δ_H 7.17 (dt, *J*=15.8, 6.9 Hz, 1H, CH=CH-CH_2), 6.14 (dt, *J*=15.8, 1.5 Hz, 1H, C-CH=CH), 4.73 (s, 2H, C-CH_2-O), 2.31-2.18 (m, 2H, CH-CH_2-CH_2), 1.61-1.43 (m, 2H, CH_2-CH_2-CH_3), 0.92 (s, 9H, O-TBS), 0.83 (t, *J*=4.8 Hz, 3H, CH_3-CH_2), 0.13 (s, 6H, O-TBS). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 178.2, 154.6, 132.2, 90.7, 82.1, 51.6, 36.0, 25.7, 22.6, 18.2, 13.7, -5.7. HRMS calcd. for $C_{15}H_{27}O_2Si$ (M+H) 267.1780, found 267.1777.

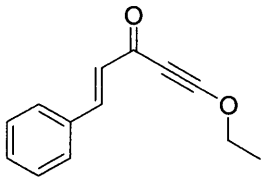
(*E*)-Tetradec-5-en-8-yn-7-one (40)



To a stirring suspension of PCC (1.55 g, 7.2 mmol) and celite (1.5 g) in dichloromethane (20 mL), (*E*)-tetradec-5-en-8-yn-7-ol (1 g, 4.8 mmol) was added in one portion. The mixture was stirred, and monitored by TLC. Upon completion, the reaction mixture was filtered through a silica plug. Concentration of the filtrate under reduced pressure gave the required material as an oil. Purification using column chromatography using an eluant of 4:1 hexane:ethyl acetate gave the required material as a pale oil, 0.69 g, 69%. IR ν_{max} (KBr, cm^{-1}) 2210 (C≡C), 1645 (C=O), 1622 (C=C). ^1H NMR (300MHz, $CDCl_3$), δ_H 7.13 (dt, *J*=15.8, 6.9 Hz, 1H, CH=C<u>H</u>-CH_2), 6.12 (d, *J*=15.8 Hz, 1H, C-C<u>H</u>=CH), 2.38 (t, *J*=7.1 Hz, 2H, C-<u>CH_2</u>-CH_2), 2.32-2.16 (m, 2H, CH-<u>CH_2</u>-CH_2), 1.81-1.49 (m, 4H, C-CH_2-<u>CH_2</u>-CH_2, CH-CH_2-<u>CH_2</u>-CH_2), 1.48-1.19 (m, 6H, -<u>CH_2</u>-), 0.97-0.87 (m, 6H, -<u>CH_3</u>). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 179.0, 153.7, 132.5, 94.6, 79.1, 34.4, 31.1, 27.5, 22.2, 22.1, 21.2, 19.8, 13.9, 13.7.

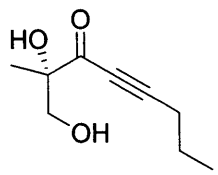
HRMS calcd. for $C_{14}H_{23}O$ (M+H) 207.1749, found 207.1750.

5-Ethoxy-1-phenylpent-1-en-4-yn-3-one (41)



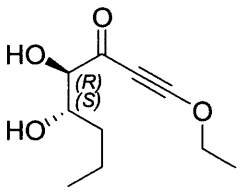
To a stirring suspension of MnO_2 (3.64 g, 41.9 mmol) in dichloromethane (15 mL), 5-ethoxy-1-phenyl-pent-1-en-4-yn-3-ol (0.55 g, 2.6 mmol) was added, and stirred for 12 h under an inert atmosphere. The mixture was filtered through celite, and the filtrate concentrated under reduced pressure to give the crude product. Column chromatography using an eluant of 6:1 hexane:ethyl acetate gave the product as an oil, 265 mg, 51%. The material was used immediately in subsequent stages.

(S)-1,2-Dihydroxy-2-methyloct-4-yn-3-one (42)



A solution of AD-mix-β (1.4 g, modified to contain 1 mol% of potassium osmate dehydrate, and 1 mol% of ligand) with sodium hydrogen carbonate (3 mmol) in water (5 mL) and *tert*-butanol (5 mL) was prepared. Cooled to 0 °C, the resulting slurry was stirred vigorously for 30 min. Methanesulfonamide (95 mg, 1 mmol), was added and stirred for 5 min. 2-Methyl-oct-1-en-4-yn-3-one (136 mg, 1 mmol) was added in one portion, and the mixture stirred vigorously at 0 °C. The reaction was monitored by TLC. Upon completion, the reaction was quenched at 0 °C with the addition of sodium sulfite (1.5 g), warmed to room temperature, and stirred for 1 hour. The reaction mixture was extracted with ethyl acetate (3 x 10 mL), organic fractions combined and washed with 2M KOH (5 mL), and dried ($MgSO_4$). Concentration gave the crude product 145 mg. No products were identified which corresponded to **42**.

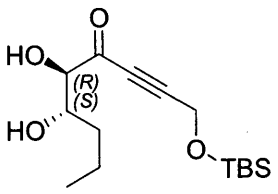
(4*R*,5*S*)-1-Ethoxy-4,5-dihydroxyoct-1-yn-3-one (43)



A solution of AD-mix-β (0.37 g, modified to contain 1 mol% of potassium osmate dihydrate, and 1 mol% of ligand) with sodium hydrogen carbonate (1 mmol) in water (3 mL) and *tert*-butanol (3 mL) was prepared. Cooled to 0 °C, the resulting slurry was stirred vigorously for 30 min. Methanesulfonamide (19 mg, 0.2 mmol), was added and stirred for 5 min. (*E*)-1-ethoxyoct-4-en-1-yn-3-one (55 mg, 0.33 mmol) was added in one portion, and the mixture stirred vigorously at 0 °C. The reaction was monitored by TLC. Upon completion, the reaction was quenched at 0 °C with the addition of sodium sulfite (0.65 g), warmed to room temperature, and stirred for 1 h. The reaction mixture was extracted with ethyl acetate (3 x 10 mL), organic fractions combined and washed with KOH (2M , 5 mL) and dried ($MgSO_4$). Concentration gave the crude product, which was purified by column chromatography using an eluant of 1:9 hexane:ethyl acetate to give the product as an oil, 15 mg, 24%. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 4.63-4.52 (m, 1H, HO-C<u>H</u>(C)(CH)), 4.28 (q, J=7.1 Hz, 2H, O-C<u>H</u>$_2$-CH$_3$), 4.20-4.06 (m, 1H, HO-C<u>H</u>(CH)CH$_2$)), 1.71-1.48 (m, 4H, CH-C<u>H</u>$_2$-C<u>H</u>$_2$-CH$_3$), 1.46 (t, J=7.1 Hz, 3H, O-CH$_2$-C<u>H</u>$_3$), 0.95 (t, J=7.2 Hz, 3H, CH$_2$-CH$_2$-C<u>H</u>$_3$). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 185.5,

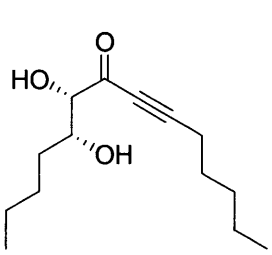
88.2, 81.3, 69.8, 68.1, 43.4, 34.7, 18.8, 14.2, 13.8. HRMS calcd. for $C_{10}H_{16}O_4$ found 200.1049, found 200.1045.

(5*R*,6*S*)-1-(*tert*-Butyldimethylsilyloxy)-5,6-dihydroxynon-2-yn-4-one (44)



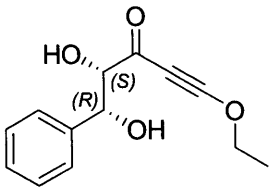
A solution of AD-mix-β (0.52 g, modified to contain 1 mol% of potassium osmate dihydrate, and 1 mol% of ligand) with sodium hydrogen carbonate (1.1 mmol) in water (4 mL) and *tert*-butanol (4 mL) was prepared. Cooled to 0 °C, the resulting slurry was stirred vigorously for 30 min. Methanesulfonamide (35 mg, 0.37 mmol), was added and stirred for 5 minutes (*E*)-1-(*tert*-butyldimethylsilyloxy)non-5-en-2-yn-4-one (100 mg, 0.37 mmol) was added in one portion, and the reaction stirred vigorously at 0 °C. The reaction was monitored by TLC. Upon completion, the reaction was quenched at 0 °C with the addition of sodium sulfite (0.70 g), warmed to room temperature, and stirred for 1 h. The reaction mixture was extracted with ethyl acetate (3 x 10 mL), organic fractions combined and washed with 2M KOH (5 mL) and dried ($MgSO_4$). Concentration gave the crude product, which was purified by column chromatography using an eluant of 4:1 hexane:ethyl acetate to give material as an oil which did not correlate to **44**.

(5*R*,6*S*)-5,6-Dihydroxytetradec-8-yn-7-one (45)

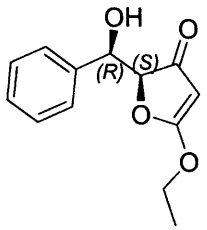


A solution of AD-mix-β (1.4 g, modified to contain 1 mol% of potassium osmate dihydrate, and 1 mol% of ligand) with sodium hydrogen carbonate (1.1 mmol) in water (5 mL) and *tert*-butanol (5 mL) was prepared and cooled to 0 °C. The resulting slurry was stirred vigorously for 30 min. Methanesulfonamide (95 mg) was added and stirred for 5 min, (*E*)-tetradec-5-en-8-yn-7-one (206 mg, 1 mmol) was added in one portion, and the reaction stirred vigorously at 0 °C. The reaction was monitored by TLC. Upon completion, the reaction was quenched at 0 °C with the addition of sodium sulfite (1 g), warmed to room temperature, and stirred for 1 h. The reaction mixture was extracted with ethyl acetate (3 x 15 mL), organic fractions combined and washed with 2M KOH (5 mL) and dried ($MgSO_4$). Concentration gave the crude material, which was purified by column chromatography, using an eluant of 4:1 hexane:ethyl acetate. Analysis of the material indicated that **45** was not formed.

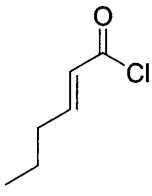
(4*S*,5*R*)-1-Ethoxy-4,5-dihydroxy-5-phenylpent-1-yn-3-one (46)



A solution of AD-mix-β (0.70 g, modified to contain 1 mol% of potassium osmate dihydrate, and 1 mol% of ligand) with Sodium hydrogen carbonate (1.4 mmol) in water (2.5 mL) and *tert*-butanol (2.5 mL) was prepared and cooled to 0 °C. The resulting slurry was stirred vigorously for 30 min. Methanesulfonamide (46 mg, 0.48 mmol), was added and stirred for 5 min. 5-Ethoxy-1-phenyl-pent-1-en-4-yn-3-one (97 mg, 0.48 mmol) was added in one portion, and the mixture stirred vigorously at 0 °C. The reaction was monitored by TLC. Upon completion, the reaction was quenched at 0 °C with the addition of sodium sulfite (0.75 g), warmed to room temperature, and stirred for 1 h. The reaction mixture was extracted with ethyl acetate (3 x 10 mL), organic fractions combined and washed with 2M KOH (5 mL), and dried ($MgSO_4$). Concentration gave the crude product, which was purified by column chromatography using an eluant of 2:1 hexane:ethyl acetate to give the product as an oil. Analysis of the material indicated that the diol product had not been isolated, and spontaneous rearrangement to the furanone had not occurred.

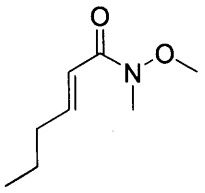


(*E*)-Hex-2-enoyl chloride (49)



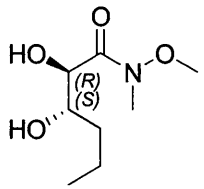
To a vessel containing thionyl chloride (10.4 g, 87.6 mmol) and 2 drops of *N*,*N*-dimethylformamide in benzene (50 mL), (*E*)-hex-2-enoic acid (5 g, 43.8 mmol) was added. The vessel was then heated to reflux for 2 h. The reaction was allowed to cool to room temperature, and benzene removed under reduced pressure. The crude material was used without further purification in later stages.

(*E*)-*N*-Methoxy-*N*-methylhex-2-enamide (50)



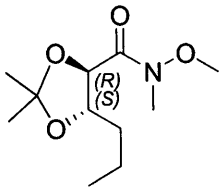
To a flask containing triethylamine (13.3 g, 109.5 mmol), dichloromethane (45 mL), and NH(OMe)Me.HCl (5.13 g, 52.6 mmol), crude (*E*)-hex-2-enoyl chloride (43.8 mmol) in 50 mL of dichloromethane was added slowly. The mixture was stirred at room temperature for 1 h, then extracted with 1 M potassium hydroxide (20 mL), 1 M hydrochloric acid (20 mL), water (20 mL), and brine. Combined organic extracts were dried with $MgSO_4$, and concentrated under reduced pressure. Column chromatography using an eluant of 4:1 hexane:ethyl acetate gave the desired product as a yellow oil, 4.97 g, 72% over two steps from the corresponding acid. IR ν_{max} (KBr, cm^{-1}) 1740 (C=O), 1608 (C=C). ^1H NMR (300MHz, $CDCl_3$), δ_H 6.96 (dt, J=15.4, 5.2 Hz, 1H, CH=C<u>H</u>-CH_2), 6.34 (d, J=15.4 Hz, 1H, C-<u>C</u>H=CH), 3.64 (s, 3H, O-<u>CH_3</u>), 3.18 (s, 3H, N-CH_3), 2.23-2.11 (m, 2H, CH-<u>CH_2</u>-CH_2), 1.54-1.38 (m, 2H, CH_2-<u>CH_2</u>-CH_3), 0.89 (t, J=7.4 Hz, 3H, CH_2-CH_2-<u>CH_3</u>). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 167.0, 147.6, 118.8, 61.6, 34.5, 32.3, 21.5, 13.6. HRMS calcd. for $C_8H_{15}NO_2$ 157.1103, found 157.1101.

(2*R*,3*S*)-2,3-Dihydroxy-*N*-methoxy-*N*-methylhexanamide (51)



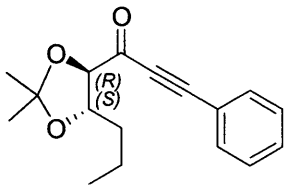
A solution of AD-mix-β (1.4 g, modified to contain 1 mol% of potassium osmate dehydrate, and 1 mol% of ligand) with sodium hydrogen carbonate (120 mg) in water (5 mL) and *tert*-butanol (5 mL) was prepared and cooled to 0 °C. The resulting slurry was stirred vigorously for 30 min. Methanesulfonamide (40 mg), was added and stirred for 5 min. (*E*)-*N*-methoxy-*N*-methylpent-2-enamide (157 mg, 1 mmol) was added in one portion, and the mixture stirred vigorously at 0 °C. The reaction was monitored by TLC. Upon completion, the reaction was quenched at 0 °C with the addition of sodium sulfite (1.5 g), warmed to room temperature, and stirred for 1 h. The reaction mixture was extracted with ethyl acetate (3 x 10 mL), organic fractions combined and washed with 2M KOH (5 mL), and dried (Na_2SO_4). Concentration gave the product **51**, 112 mg, 59%. ^1H NMR (300MHz, $CDCl_3$), δ_H 4.32 (m, 1H, C-<u>C</u>H(OH)-CH), 3.84 (m, 1H, CH-<u>C</u>H(OH)-CH_2), 3.70 (s, 3H, O-<u>CH_3</u>), 3.24 (s, 3H, N-<u>CH_3</u>), 1.71-1.24 (m, 4H, -<u>CH_2</u>-), 0.92 (t, J=7.2 Hz, 3H, CH_2-<u>CH_3</u>). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 173.2, 71.5, 70.6, 61.2, 36.1, 32.5, 18.9, 13.9. HRMS calcd. for $C_8H_{17}NO_4$ 191.1158, found 191.1159.

(4*R*,5*S*)-*N*-Methoxy-*N*-2,2-trimethyl-5-propyl-1,3-dioxolane-4-carboxamide (52)



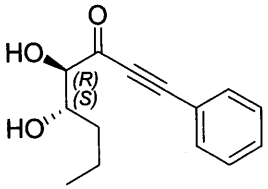
To a vessel containing (2*R*,3*S*)-2,3-dihydroxy-*N*-methoxy-*N*-methylhexanamide (3.69 g, 19.3 mmol), (+/-)-camphorsulfonic acid (0.18 g, 0.8 mmol), and acetone (60 mL), 2,2-dimethoxy propane (19.3 mL, 157 mmol) was added. The mixture was stirred and monitored by TLC. Upon completion, the reaction mixture was concentrated under reduced pressure, and purified using column chromatography with an eluant of 19:1 hexane:ethyl actate to give the desired product as a yellow oil, 3.04 g, 68%. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 4.50-4.21 (m, 2H, C-CH(OH)-CH, CH-CH(OH)-CH_2), 3.71 (s, 3H, O-CH_3), 3.20 (s, 3H, N-CH_3), 1.72-1.52 (m, 2H, CH-CH_2-CH_2), 1.49-1.24 (m, 8H, CH_2-CH_2-CH_3, C-(CH_3)$_2$), 0.91 (t, *J*=7.2 Hz, 3H, CH_2-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 170.5, 110.3, 78.4, 76.8, 61.6, 35.1, 27.4, 26.1, 19.0, 14.0. HRMS calcd. for $C_{11}H_{21}NO_4$ 231.1471, found 213.1468.

1-((4*R*,5*S*)-2,2-Dimethyl-5-propyl-1,3-dioxolan-4-yl)-3-phenylprop-2-yn-1-one (56)



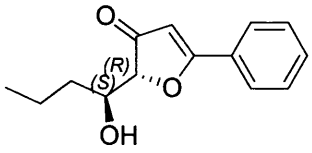
To a stirring solution of phenylacetylene (88 mg, 0.9 mmol) in 3 mL of dry THF at -78 °C, *n*-butyllithium (1.6 M in hexanes, 0.9 mmol) was added. The reaction was stirred for 1 h. (4*R*,5*S*)-*N*-methoxy-*N*-,2,2-trimethyl-5-propyl-1,3-dioxolane-4-carboxamide (0.2 g, 0.9 mmol) in 2 mL of THF was added dropwise to the reaction mixture, and maintained at -78 °C. The reaction was warmed to 0 °C, stirred for 1 h, then allowed to warm to ambient temperature. The reaction was quenched with saturated ammonium chloride solution (20 mL), and products extracted into dichloromethane (3 x 30 mL). Organic fractions were combined, dried (Na_2SO_4), and concentrated under reduced pressure to give the crude product as a light yellow oil. Purification by column chromatography gave the desired product as a colourless oil, 188 mg, 80%. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 7.70-7.30 (m, 5H, Ar-H), 4.28-4.08 (m, 2H, C-CH(OH)-CH, CH-CH(OH)-CH_2), 1.89-1.65 (m, 2H, CH-CH_2-CH_2), 1.61-1.34 (m, 8H, CH_2-CH_2-CH_3, C-(CH_3)$_2$), 0.96 (t, *J*=7.3 Hz, 3H, CH_2-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 186.6, 133.3, 131.2, 128.7, 119.7, 111.2, 95.3, 86.0, 78.5, 35.5, 27.4, 26.3, 19.0, 14.0. HRMS calcd. for $C_{17}H_{20}O_3$ 302.3760, found 302.3758.

(4*R*,5*S*)-4,5-Dihydroxy-1-phenyloct-1-yn-3-one (57)



To a stirring solution of 1-((4*R*,5*S*)-2,2-dimethyl-5-propyl-1,3-dioxolan-4-yl)-3-phenylprop-2-yn-1-one (100 mg, 0.37 mmol) in methanol (5 mL), hydrochloric acid (2 M, 3 mL) was added. The reaction was stirred at room temperature, and monitored by TLC. Upon completion, material was extracted into dichloromethane (3 x 5 mL) and dried ($MgSO_4$). Concentration under reduced pressure, followed by purification by column chromatography gave the required product as a yellow oil, 69 mg, 81%. Chiral HPLC analysis using a Chiralcel OD column and an eluant of 1:9 *iso*-propanol:hexane with a flow rate of 0.7 mL.min^{-1}, determined the e.e. to be 82%. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 7.70-7.31 (m, 5H, Ar-H), 4.47-4.20 (m, 2H, C-CH(OH)-CH, CH-CH(OH)-CH_2), 3.60 (br, OH), 2.00 (br, OH), 1.86-1.33 (m, 4H, -CH_2-), 1.00 (t, *J*=7.3 Hz, 3H, CH_2-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 187.7, 133.2, 131.4, 128.8, 119.3, 98.0, 85.0, 80.6, 71.8, 36.5, 19.0, 13.9. HRMS calcd. for $C_{14}H_{16}O_3$ 232.1099, found 232.1104.

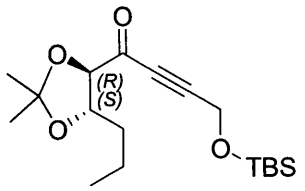
(*R*)-2-((*S*)-1-Hydroxybutyl)-5-phenylfuran-3(2H)-one (58)



To a stirring solution of (4*R*,5*S*)-4,5-dihydroxy-1-phenyloct-1-yn-3-one (69 mg, 0.30 mmol) in acetone (5 mL), was added 0.2 mL of a mercury containing solution. The reaction was then allowed to stir, until complete by TLC (approximately 10 min). Sodium hydrogen carbonate (50 mg) was added and allowed to stir for 20 min. Filtration of solid material from the reaction mixture, followed by extraction into ethyl acetate (15 mL), drying with sodium sulfate, and concentration yielded the crude furanone. Purification by column chromatography (with an eluant of 2:1 hexane:ethyl acetate) yielded the desired furanone as a colourless oil, 53 mg, 77%. Chiral HPLC analysis using a Chiralcel OD column and an eluant of 1:9 *iso*-propanol:hexane with a flow rate of 0.7 mL.min^{-1}, determined the e.e. to be 66%. IR ν_{max} (KBr, cm^{-1}) 3410 (OH), 2262 (C=O), 1676 (C=C). ^{1}H NMR (400MHz, $CDCl_3$), δ 7.96-7.57 (m, 5H, Ar-H), 6.36 (s, 1H, C-CH=C), 4.90 (d, *J*= 6.8 Hz, 1H, C-CH(O)(CH)), 4.69 (m, 1H, CH-OH), 3.89 (m, 1H, C-CH(OH)-CH_2), 1.67 (dt, *J*= 7.6, 6.8 Hz, 2H, CH_2-CH_2-CH), 1.56-1.29 (m, 2H, CH_3-CH_2-CH_2), 0.92 (t, *J*= 7.4 Hz, 3H, CH_2-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$), δ 202.5,

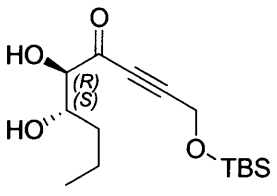
185.1, 132.6, 128.9, 128.8, 127.0, 101.8, 88.4, 69.4, 35.6, 18.4, 13.8. HRMS calcd. for $C_{14}H_{16}O_3$ 232.1099, found 232.1102.

4-(*tert*-Butyldimethylsilyloxy)-1-((4*R*,5*S*)-2,2-dimethyl-5-propyl-1,3-dioxolan-4-yl)but-2-yn-1-one (59)



To a stirring solution of *tert*-butyldimethyl(prop-2-ynyloxy)silane (93.7 mg, 0.55 mmol) in dry THF (5 mL) at -78 °C, *n*-butyllithium (1.6 M in hexanes, 0.6 mmol) was added. The mixture was stirred for 1 h. (4*R*,5*S*)-*N*-methoxy-*N*-2,2-trimethyl-5-propyl-1,3-dioxolane-4-carboxamide (0.112 g, 0.5 mmol) in THF (5 mL) was added dropwise to the reaction mixture, and maintained at -78 °C. The mixture was warmed to 0 °C, stirred for 1 h, and allowed to warm to ambient temperature. The reaction was quenched with saturated ammonium chloride solution (20 mL), and products extracted into dichloromethane (3 x 30 mL). Organic fractions were combined, dried (Na_2SO_4), and concentrated under reduced pressure to give the crude product as a light yellow oil. Purification by column chromatography using an eluant of 19:1 hexane:ethyl acetate gave the desired product as a colourless oil, 129 mg, 76%. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 4.91-4.73 (m, 1H, C-CH(OH)-CH), 4.74 (s, 2H, C-CH_2-O), 4.20-4.00 (m, 1H, CH_2-CH(OH)-CH), 1.80-1.60 (m, 2H, CH-CH_2-CH_2), 1.59 -1.36 (m, 8H, CH_2-CH_2-CH_3, $(CH_3)_2$-C), 1.06-0.74 (m, 12H, O-TBS, CH_2-CH_3), 0.12 (s, 6H, O-TBS). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 185.3, 111.2, 85.8, 82.0, 78.2, 60.3, 51.5, 35.6, 26.1, 25.7, 18.9, 18.1, 13.9. HRMS calcd. for $C_{18}H_{33}O_4Si$ (M+H) 341.2148, found 341.2147.

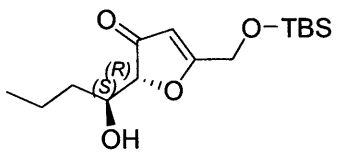
(5*R*,6*S*)-1-(*tert*-butyldimethylsilyloxy)-5,6-dihydroxynon-2-yn-4-one (60)



To a stirring solution of 14-(*tert*-butyldimethylsilyloxy)-1-((4*R*,5*S*)-2,2-dimethyl-5-propyl-1,3-dioxolan-4-yl)but-2-yn-1-one (100 mg, 0.29 mmol) in methanol (5 mL), hydrochloric acid (2 M, 3 mL) was added. The mixture was stirred at room temperature, and monitored by TLC. Upon completion, material was extracted into dichloromethane (3 x 5 mL) and dried ($MgSO_4$). Concentration under reduced pressure, followed by purification by column chromatography gave the required product as a colourless oil, 63 mg, 72%. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 4.46 (s, 2H, C-CH_2-O), 4.33-4.11 (m, 2H, CH_2-CH(OH)-CH, C-CH(OH)-CH), 1.77-1.30 (m, 4H, -CH_2-), 0.97 (t, J= 7.2 Hz, 3H, CH_2-CH_3), 0.92

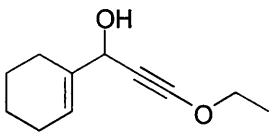
(s, 9H, O-TBS), 0.13 (s, 6H, O-TBS). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 187.9, 96.3, 81.6, 80.5, 71.8, 50.7, 36.0, 25.7, 19.0, 18.2, 13.9. HRMS calcd. for $C_{15}H_{28}O_4Si$ 300.1757, found 300.1756.

(*R*)-5-((*tert*-Butyldimethylsilyloxy)methyl)-2-((*S*)-1-hydroxybutyl)furan-3(2*H*)-one (61)



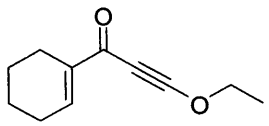
To a stirring solution of ((5*R*,6*S*)-1-(*tert*-butyldimethylsilyloxy)-5,6-dihydroxynon-2-yn-4-one (50 mg, 0.17 mmol) in acetone (5 mL), was added 0.2 mL of a mercury containing solution. The mixture was then allowed to stir, until complete by TLC (approximately 10 min). Sodium hydrogen carbonate (50 mg) was added and allowed to stir for 20 min. Filtration of solid material from the reaction mixture, followed by extraction into ethyl acetate (15 mL), drying with sodium sulfate, and concentration yielded the crude furanone. Purification by column chromatography (with an eluant of 2:1 hexane:ethyl acetate) yielded complex material that did not correlate to **61**.

1-Cyclohex-1-enyl-3-ethoxyprop-2-yn-1-ol (63)



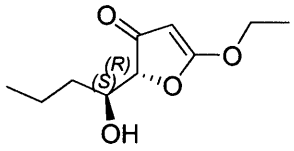
To a stirring solution of ethoxyacetylene (5.7 mmol, 0.4 g) in 50 mL of tetrahydrofuran, was added *n*-butyllithium (2.5 M in hexanes, 6.0 mmol, 2.4 mL) dropwise at room temperature. The mixture was left to stir for 30 min, and then cyclohex-1-enecarboxaldehyde (4.3 mmol, 0.474 g, 0.49 mL) was added to the reaction in 20 mL of tetrahydrofuran. The reaction was stirred for 3 h, and then quenched with the addition of saturated ammonium carbonate solution. Extraction of organic material by washing the reaction mixture with dichloromethane (3 x 30 mL) yielded the crude product after combination, drying (magnesium sulfate), and concentration under reduced pressure. Isolation through the use of column chromatography, using an eluant of 4:1 hexane:ethyl acetate, gave the desired product as a colourless oil, 0.484 g, 42%. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 4.75 (m, 1H, CH_2-CH=C), 4.54 (s, 1H, C-CH(OH)-C), 4.11 (q, J=7.0 Hz, 2H, O-CH_2-CH_3), 2.23-2.03 (m, 4H, -CH_2-), 1.67-1.55 (m, 4H, -CH_2-), 1.36 (t, J=7.0 Hz, 3H, CH_2-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 138.1, 123.9, 94.6, 74.6, 66.8, 38.0, 25.0, 24.0, 22.6, 22.3, 14.4. HRMS calcd. for $C_{11}H_{12}O$ 180.1150, found 180.1152.

1-Cyclohex-1-enyl-3-ethoxypropynone (64)



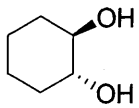
To a stirring suspension of MnO_2 (3.76 g, 43.2 mmol) in dichloromethane (35 mL), 1-cyclohex-1-enyl-3-ethoxyprop-2-yn-1-ol (0.48 g, 2.7 mmol) was added, and stirred for 12 h under an inert atmosphere. The mixture was filtered through celite, and the filtrate concentrated under reduced pressure to give the crude product. Column chromatography using an eluant of 19:1 hexane:ethyl acetate gave the product as an oil, 167 mg, 35%. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 6.79 (m, 1H, CH_2-CH=C), 4.15 (q, J=7.1 Hz, 2H, O-CH_2-CH_3), 2.38-2.16 (m, 4H, -CH_2-), 1.75-1.50 (m, 4H, -CH_2-), 1.25 (t, J=7.1 Hz, 3H, CH_2-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 168.1, 151.4, 144.8, 101.9, 76.8, 41.8, 26.7, 25.7, 22.0, 21.7, 14.1.

(*R*)-5-Ethoxy-2-((*S*)-1-hydroxybutyl)furan-3(2*H*)-one



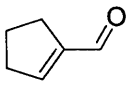
To a stirring solution of ((4*R*,5*S*)-1-ethoxy-4,5-dihydroxyoct-1-yn-3-one (50 mg, 0.25 mmol) in acetone (5 mL), was added 0.2 mL of a mercury containing solution. The mixture was then allowed to stir, until complete by TLC (approximately 10 min). Sodium hydrogen carbonate (50 mg) was added and allowed to stir for 20 min. Filtration of solid material from the reaction mixture, followed by extraction into ethyl acetate (15 mL), drying with sodium sulfate, and concentration yielded the crude material. No products were found in the reaction products, rapid decomposition into intractable mixtures was observed.

(1*R*,2*R*)-Cyclohexane-1,2-diol (67)



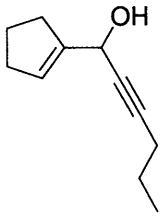
To a flask containing 10% sulfuric acid (150 mL), cyclohexene oxide (15 g, 153 mmol) was added. The reaction was stirred overnight, followed by extraction of the products into dichloromethane (3 x 30 mL). Concentration, followed by recrystallisation from petroleum ether(40-60 °C):ethyl acetate (approx. 19:1) gave the product as colourless crystals, 4.25 g, 24%. Material correlated with an authentic sample provided by the Aldrich chemical company.

Cyclopent-1-enecarboxaldehyde (68)



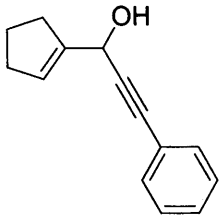
To a stirring solution of sodium metaperiodate (6.78 g, 31.7 mmol) in water (80 mL), *trans*-cyclohexane-1,2-diol (3 g, 25.8 mmol) was added. The reaction was stirred for 30 min, followed by the addition of 20% potassium hydroxide (10 mL) and diethyl ether (15 mL). The reaction was stirred vigorously for 10 min. Extraction of products into diethyl ether (3 x 30 mL), followed by drying ($MgSO_4$), and concentration under reduced pressure to give the crude material. Column chromatography afforded the required material as a colourless oil, 1.14 g, 46%. Material correlated with an authentic sample from the Aldrich chemical company. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 9.75 (s, 1H, C-CHO), 6.84 (m, 1H, CH_2-CH=C), 2.65-2.50 (m, 4H, -CH_2-), 2.05-1.85 (m, 2H, CH_2-CH_2-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 189.8, 153.1, 147.9, 33.6, 28.3, 22.9.

1-Cyclopentenylhex-2-yn-1-ol (69)



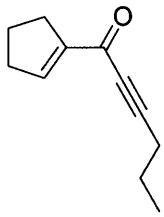
To a flask containing dry THF (20 mL) and *n*-pentyne (2.46 mL, 25.0 mmol) at -78 °C, *n*-butyllithium (1.6 M in hexanes, 27.5 mmol) was added dropwise. The reaction mixture was stirred for 10 min, then transferred via cannula to a vessel charged with THF (10 mL), and cyclopent-1-enecarbaldehyde (2 g, 20.8 mmol) at -78 °C. The mixture was stirred for 30 min, and allowed to warm to 0 °C. The reaction was quenched with the addition of saturated ammonium carbonate solution. Extraction of organic material by washing the reaction mixture with dichloromethane (3 x 20 mL) yielded the crude product after combination, drying ($MgSO_4$), and concentration under reduced pressure. Isolation through the use of column chromatography, using an eluant of 19:1 hexane:ethyl acetate, gave the desired product as a pale oil, 2.04 g, 70%. IR ν_{max} (KBr, cm^{-1}) 3335 (OH), 2215 (C≡C), 1612 (C=C). ^{1}H NMR (300MHz, $CDCl_3$), δ_H 5.75 (m, 1H, CH_2-CH=C), 4.93 (s, 1H, C-CH(OH)-C), 2.40-2.20 (m, 4H, -CH_2-), 2.19-2.16 (m, 2H, -CH_2-), 1.92 (t, J=7.2 Hz, 2H, C-CH_2-CH_2), 1.52 (qt, J=7.2, 7.3 Hz, 2H, CH_2-CH_2-CH_3), 0.97 (t, J=7.3 Hz, 3H, CH_2-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 144.0, 127.2, 85.9, 79.6, 61.7, 32.3, 31.4, 23.4, 22.1, 20.7, 13.5. HRMS calcd. for $C_{11}H_{16}O$ 164.1201, found 164.1204.

1-Cyclopentenyl-3-phenylprop-2-yn-1-ol (70)



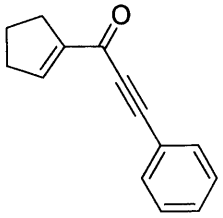
To a flask containing dry THF (15 mL) and phenylacetylene (0.57 mL, 5.2 mmol) at -78 °C, *n*-butyllithium (1.6 M in hexanes, 5.2 mmol) was added dropwise. The reaction mixture was stirred for 10 min, then transferred via cannula to a vessel charged with THF (10 mL), and cyclopent-1-enecarbaldehyde (0.5 g, 5.2 mmol) at -78 °C. The reaction was stirred for 30 min, then allowed to warm to 0 °C. The reaction was quenched with the addition of saturated ammonium carbonate solution. Extraction of organic material by washing the reaction mixture with dichloromethane (3 x 20 mL) yielded the crude product after combination, drying ($MgSO_4$), and concentration under reduced pressure. Isolation through the use of column chromatography, using an eluant of 4:1 hexane:ethyl acetate, gave the desired product as a pale oil, 0.87 g, 85%. IR ν_{max} (KBr, cm^{-1}) 3375 (OH), 2204 (C≡C), 1615 (C=C). ^1H NMR (300MHz, $CDCl_3$), δ_H 7.46-7.26 (m, 5H, Ar-H), 5.88 (m, 1H, CH_2-CH=C), 5.19 (s, 1H, C-CH(OH)-C), 2.59-2.20 (m, 4H, -CH_2-), 2.11-1.95 (m, 2H, CH_2-CH_2-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 143.3, 131.7, 128.4, 128.3, 127.9, 122.6, 88.4, 85.3, 62.1, 32.3, 31.5, 23.4. HRMS calcd. for $C_{14}H_{14}O$ 198.1045, found 198.1040.

1-Cyclopentenylhex-2-yn-1-one (71)



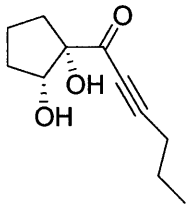
To a stirring solution of 1-cyclopentenylhex-2-yn-1-ol (2 g, 12.2 mmol) in dichloromethane (100 mL), MnO_2 (9.86 g, 113.5 mmol) was added. The mixture was stirred for 12 h at room temperature. Upon completion, the mixture was filtered through Celite, and concentrated under reduced pressure to a pale yellow oil that required no further purification, 1.7 g, 88%. IR ν_{max} (KBr, cm^{-1}) 2213 (C≡C), 1645 (C=O), 1625 (C=C). 1H NMR (300MHz, $CDCl_3$), δ_H 7.02 (m, 1H, CH_2-CH=C), 2.63-2.28 (m, 6H, -CH_2-), 1.95 (t, J=7.5 Hz, 2H, C-CH_2-CH_2), 1.70-1.53 (m, 2H, CH_2-CH_2-CH_3), 1.00 (t, J=7.5 Hz, 3H, CH_2-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 176.1, 149.8, 147.3, 92.9, 79.8, 33.8, 29.7, 23.2, 21.4, 20.9, 13.5. HRMS calcd. for $C_{11}H_{14}O$ 162.1045, found 162.1043.

1-Cyclopentenyl-3-phenylprop-2-yn-1-one (72)



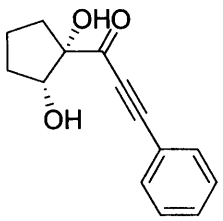
To a stirring solution of 1-cyclopentenyl-3-phenylprop-2-yn-1-ol (0.5 g, 2.5 mmol) in dichloromethane (25 mL), MnO_2 (1.86 g, 21.4 mmol) was added. The reaction was stirred for 12 h at room temperature. Upon completion, the reaction was filtered through celite, and concentrated under reduced pressure to a pale yellow oil that required no further purification, 0.42 g, 84%. IR ν_{max} (KBr, cm^{-1}) 2202 (C≡C), 1655 (C=O), 1624 (C=C). ^1H NMR (300MHz, $CDCl_3$), δ_H 7.60-7.18 (m, 5H, Ar-H), 6.77 (s, 1H, CH_2-CH=C), 2.67-2.61 (m, 4H, -CH_2-), 2.07-1.97 (m, 2H, CH_2-CH_2-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 189.0, 149.0, 146.3, 132.8, 130.4, 128.6, 120.5, 93.2, 80.5, 34.0, 29.9, 23.2. HRMS calcd. for $C_{14}H_{12}O$ (M+H) 197.0966, found 197.0960.

1-((1*S*,2*R*)-1,2-Dihydroxycyclopentyl)hex-2-yn-1-one (73)



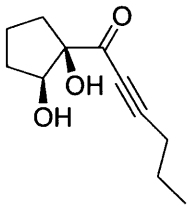
A solution of AD-mix-β (1.68 g, modified to contain 1 mol% of potassium osmate dihydrate, and 1 mol% of ligand) with sodium hydrogen carbonate (258 mg) in water (5 mL) and *tert*-butanol (5 mL) was prepared. Cooled to 0 °C, the resulting slurry was stirred vigorously for 30 min. Methanesulfonamide (114 mg, 1.2 mmol), was added and stirred for 5 min 1-cyclopentenylhex-2-yn-1-one (200 mg, 1.2 mmol) was added in one portion, and the reaction stirred vigorously at 0 °C. The reaction was monitored by TLC. Upon completion, the reaction was quenched at 0 °C with the addition of sodium sulfite (1.8 g), warmed to room temperature, and stirred for 1 hour. The reaction mixture was extracted with ethyl acetate (3 x 10 mL), organic fractions combined and washed with 2M KOH (5 mL) and dried ($MgSO_4$). Analysis indicated that **73** was not formed in the reaction.

1-((1*S*,2*R*)-1,2-Dihydroxycyclopentyl)-3-phenylprop-2-yn-1-one (74)



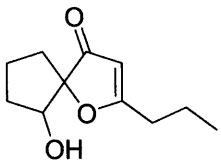
A solution of AD-mix-β (0.71 g, modified to contain 1 mol% of potassium osmate dihydrate, and 1 mol% of ligand) with sodium hydrogen carbonate (1.5 mmol) in water (2.5 mL) and *tert*-butanol (2.5 mL) was prepared. Cooled to 0 °C, the resulting slurry was stirred vigorously for 30 min. Methanesulfonamide (49 mg, 0.51 mmol), was added and stirred for 5 min 1-cyclopentenyl-3-phenylprop-2-yn-1-one (100 mg, 0.51 mmol) was added in one portion, and the mixture stirred vigorously at 0 °C. The reaction was monitored by TLC. Upon completion, the reaction was quenched at 0 °C with the addition of sodium sulfite (0.70 g), warmed to room temperature, and stirred for 1 h. The reaction mixture was extracted with ethyl acetate (3 x 10 mL), organic fractions combined and washed with 2M KOH (5 mL) and dried ($MgSO_4$). Analysis indicated that **74** was not formed in the reaction.

1-(1,2-Dihydroxycyclopentyl)hex-2-yn-1-one (75)



To a flask containing potassium osmate (2.9 mg, 7.8 x 10^{-6} moles), acetone (2.7 mL), water (0.6 mL), *tert*-butyl alcohol, and *N*-methylmorpholine (monohydrate, 89 mg, 0.66 mmol), 1-cyclopentenylhex-2-yn-1-one (100 mg, 0.6 mmol) was added. The reaction was monitored by TLC, and extracted using ethyl acetate (3 x 5 mL) upon completion. Combination of the organic fraction, followed by drying (Na_2SO_4), and concentration under reduced pressure gave the crude product. Column chromatography using an eluant of 11:1 petroleum ether (40-60 °C):ethyl acetate gave the product as a viscous pale oil, 81 mg, 68%. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 3.76-3.50 (m, 1H, CH_2-CH(OH)-C), 2.58-2.15 (m, 4H, -CH_2-), 2.00-1.43 (m, 6H, -CH_2-), 1.04 (t, J=7.3 Hz, 3H, CH_2-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 186, 97.5, 96.8, 89.5, 75.4, 36.5, 27.8, 21.3, 21.0, 16.9, 13.6. HRMS calcd. for $C_{11}H_{16}O_3$ 196.1099, found 196.1102.

6-Hydroxy-2-propyl-1-oxa-spiro[4.4]non-2-en-4-one (76)



To a flask containing analytical grade acetone (10 mL), and 1-(1,2-dihydroxycyclopentyl)hex-2-yn-1-one (50 mg, 0.25 mmol), 0.2 mL of a mercury containing solution was added. The reaction was monitored by TLC, and upon completion (approximately 5 min), sodium hydrogen carbonate (50 mg) was added and allowed to stir for 40 min. Filtration of solid material from the reaction mixture, followed by an extraction into ethyl acetate (15 mL), drying with sodium sulfate, and concentration yielded the crude product. Purification by column chromatography (with an eluant of 2:1 hexane:ethyl acetate) yielded the desired product as an oil, 39 mg, 80%. IR ν_{max} (KBr, cm^{-1}) 3408 (OH), 2254 (C=O), 1680 (C=C). ^1H NMR (300MHz, $CDCl_3$), δ_H 5.49 (s, 1H, C-CH=C), 4.01 (m, 1H, CH_2-CH(OH)-C), 3.57 (s, 1H), 2.48 (t, J=7.7 Hz, 2H, C-CH_2-CH_2), 2.28-2.23 (m, 1H, CH_2-CHH-CH_2), 2.10-1.98 (m, 2H, CH-CH_2-CH_2), 1.98-1.87 (m, 2H, C-CH_2-CH_2), 1.86-1.76 (m, 1H, CH_2-CHH-CH_2), 1.67 (tq, J=7.3, 7.7 Hz, 2H, CH_2-CH_2-CH_3), 0.99 (t, J=7.3 Hz, 3H, CH_2-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 206.3, 193.6, 103.6, 96.6, 78.6, 33.9, 33.3, 32.8, 21.7, 19.6, 13.6. HRMS calcd. for $C_{11}H_{16}O_3$ 196.1099, found 196.1095.

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Chapter 2

Enantioselective Synthesis of the Phospholine Lactone Ring

2.1 Introduction

Protein phosphorylation is a complex cellular process, and employs two categories of enzymes, the phosphatases and the protein kinases. Phosphatases remove phosphates from proteins, and the kinases are concerned with the addition of phosphates. The action of protein phosphorylation has important roles in many aspects of cell regulation, and is a crucial component in cell signal transduction pathways. If one considers that most diseases have some form of signalling malfunction associated with them, modification of the phosphorylation process has become an important strategy for obtaining therapeutic effects.

Protein kinases have been considered for some time as viable targets for therapeutic agents, and much work has been reported detailing novel compounds which interact in this way. In contrast, there is substantially less known about protein phosphatases with regard to the cellular processes and control, but that presents an equally exciting and varied area with many opportunities for therapeutic advances and remains largely unexploited.

2.1.1 Protein Phosphatases

There are many types of protein phosphatases that have been identified; enzymes which act upon serine, tyrosine, and threonine residues are considered to be within their own respective classes.[48,49]

Of the serine/threonine class of phosphatases, these can be classified into two sub-classes according to catalytic subunit homology. These are the PhosphoProtein Phosphatase (PPP) enzymes, and Metallo-PhosPhatases (PPM). Enzymes which act

upon tyrosine residues are known as Protein Tyrosine Phosphatases (PTPs). There exists within the PTP class a subset of enzymes, the dual-specificity enzymes, which can act upon all residues.
Within each class the catalytic portion is a highly conserved region; functional diversity between the members of the class is governed by regulatory domains, targeting domains or subunits.

Focusing upon the serine and threonine phosphatases, the major contributors to these families are PP1, PP2A, and PP2B for the PPP class, and PP2C for the PPM class. It is important to note that while these are the major components of the PPP and PPM families *in vivo*, there are many other members of the class that exist at low concentrations and contribute to the dephosphorylation process.

PP1, PP2A and PP2B are homologous within a catalytic domain of 280 residues, and are distinguished by their associated regulatory subunits, forming a large number of diverse holoenzymes.
PP1 has functions which include the regulation of glycogen metabolism, cell cycle progression, neuronal activities, and RNA. Differentiation between the processes is controlled by PP1 holoenzymes which bind to different regulatory and targeting subunits.

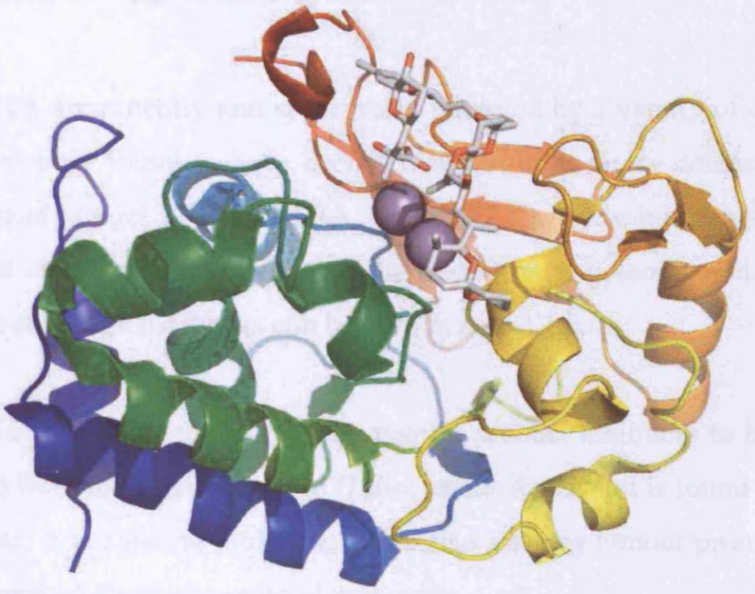


Fig. 2.1.1. Representation of the inhibitor okadaic acid bound to the PP1 enzyme, derived from the PDB database model 1JK7.

PP2A functions are very diverse and not yet fully characterized, but regulate processes such as metabolism, cell signaling, cell cycles, and telomerase activity. As with PP1, the PP2A class is defined by PP2A holoenzymes.

PP2B is identified by its dependence on Ca^{2+} ions, and plays an important part in the Ca^{2+} signaling cascade of T-cells.

The PPM family share no main sequence homology with the PPP class. The main component, PP2C, is present in all mammalian cells and its activity is dependent upon the concentration of Mg^{2+} ions. It is monomeric, and no regulatory proteins or mechanisms have been reported. Specific roles for PP2C are unclear due to significant overlapping with the PPP class, however, it has been observed that they play a particular role in connection with cellular stress.

2.1.2 Protein Phosphatase Inhibitors

PP1 and PP2A are potently and specifically inhibited by a variety of natural products,[50] and are commonly found to have been utilized as a predatory defence mechanism and appear to mainly target PP1 and PP2A, but not PP2B. These inhibitors are known as the okadaic acid class of compounds, and their selectivity, potency, and origin is of great diversity. Some of the inhibitors can be seen in Fig. 2.1.2.

Okadaic acid[51] (**77**) was the first of the natural product inhibitors to be reported. It was first isolated from the marine sponge *Halicondria okadai* and is found to be produced by dinoflagellate. It is a potent inhibitor, but is also a strong tumour promoter and is one of the main agents of diarrhetic seafood poisoning.

Tautomycin[52,53] (**78**) is an inhibitor that was the first to display 5-fold selectivity of PP1 over PP2A.

Calyculin[54] (**79**) was isolated from the sponge *Discodermia calyx* and is a potent inhibitor of both PP1 and PP2A.

Fostriecin[55] (**80**)is a small, novel antibiotic isolated from *Streptomyces pulveraceus*. It is one of the most selective small molecule inhibitor known, and exhibits a 30000 fold selectivity of PP2A over PP1.

Cantharidin[56] (**81**) was isolated from over 1500 species of blister beetle found throughout the world. It is a moderate inhibitor of PP1, PP2A, and a weak inhibitor of PP2C.

Nodularin[57] (**82**), Motuporin,[58] and Microcystins[59] are a variety identified by their cyclic peptide structures. First isolated from blue-green algae, they are varied in their potency and inhibition.

In this chapter we shall focus on just one type inhibitor, fostriecin and its analogues.

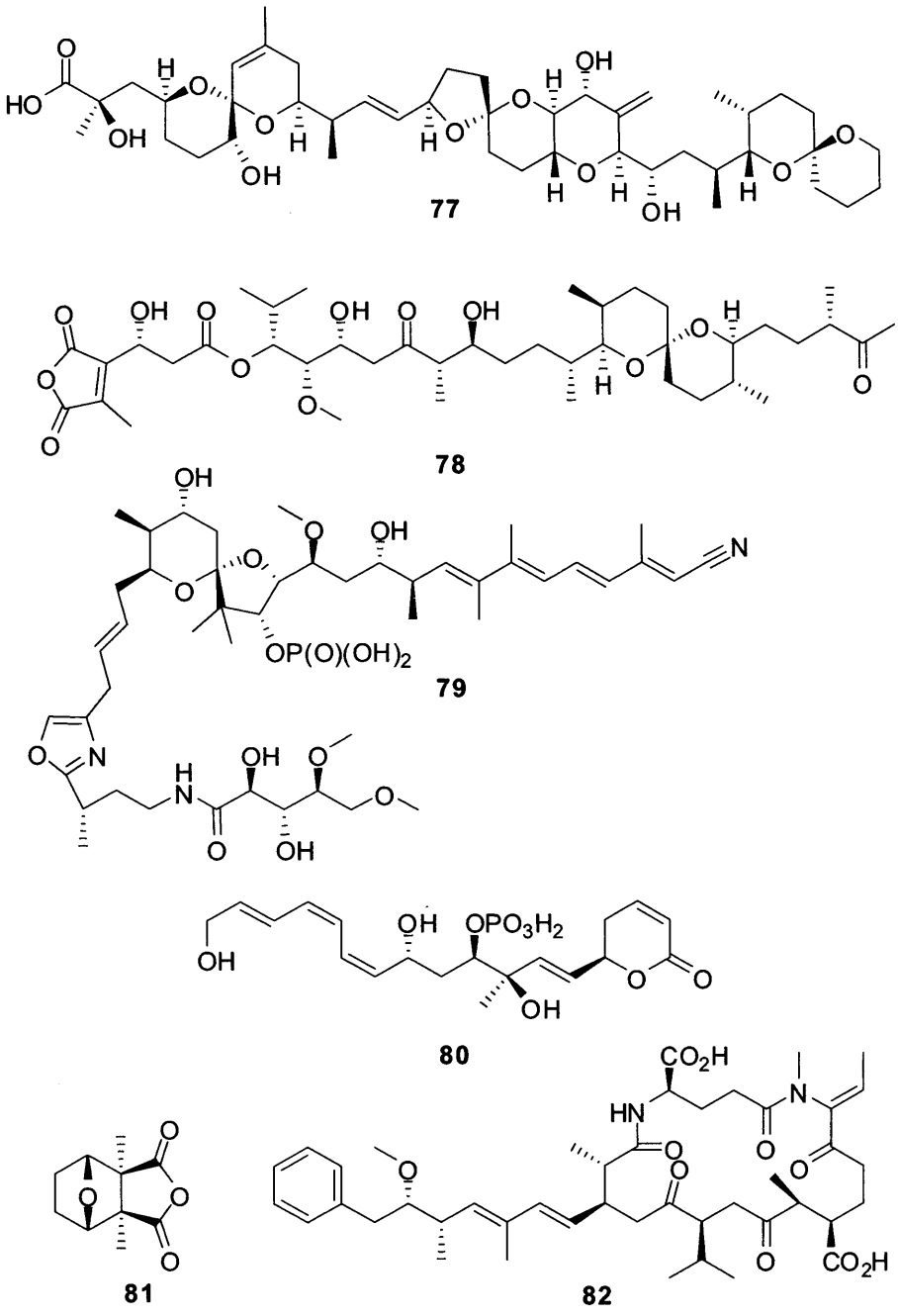


Fig. 2.1.2. PPP Phosphatase inhibitors. **77**. Okadaic acid; **78**. Tautomycin; **79**. Calyculin; **80**. Fostriecin; **81**. Cantharidin; **82**. Nodularin.

2.1.3 Fostriecin

Fostriecin, phospholine, and phoslactomycins are structurally related compounds exhibiting potent, highly selective inhibition of protein phosphatases (Fig. 2.1.3).

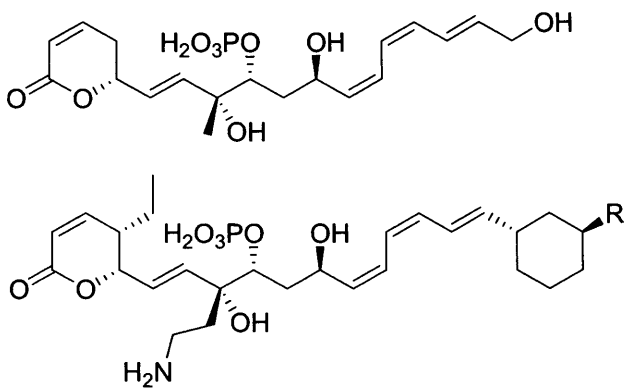


Fig. 2.1.3. Fostriecin (top) and Phospholine (below, R=H).

Fostriecin, isolated from *Strepomyces pulveraceus*, was first reported by Tunac.[60] Investigations found that in general, there was anti-tumour activity rather than tumour promoting activity as is seen with okadaic acid type compounds. This has spurred a great deal of interest, and its chemistry, and biology have been the subject of numerous studies.

Fostriecin has been shown to inhibit selectively protein phosphatases PP1, PP2A, and PP4 with an IC_{50} of 45 μM, 1.5 nM, and 3.0 nM respectively. It is known to be active *in vitro* against leukaemia (L1210), lung, breast, and ovarian cancers, is also known to be active *in vivo* against L1210, P338 leukaemia,[61] and has also been seen to radio-sensitise tumour cells.[62]

Little regarding the mechanism of inhibition is known, however it was believed that it was a topoisomerase II catalytic inhibitor,[63] this is now known to be false. It is now considered that fostriecin interferes with the mitosis entry checkpoint. Mitosis is a heavily controlled process and requires complete and undamaged DNA to succeed. Fostriecin forces cycling cells to enter mitosis prematurely[64,65] and prevents progression through the latter stages.

The ability of fostriecin to inhibit mitosis, and the inhibition of protein phosphatases are novel and desirable properties for anti-tumour compounds. Hence, fostriecin was advanced to phase I clinical trials by the NCI with initial success, and was found to possess significant tumour growth inhibition *in vitro* short of the maximum tolerated dose. Studies, however, were unfortunately halted due to concerns with the purity and the storage of the naturally occurring material. It was found that fostriecin, placed in

incubated culture medium loses activity within 30 minutes. Similar half-lives were also observed in patient plasma shortly after administration.[63] While being a setback, this has not stifled interest into fostriecin as an anti-tumour therapeutic.

Fostriecin has found a useful role as a biological tool even after the failure of the therapeutic trials. Fostriecin is highly selective to PP2A enzymes compared to PP1, and has been found to be particularly useful in aiding the elucidation of cell dephosphorylation pathways.

Fostriecin remains an interesting and promising natural product, and there has been much reported regarding its synthesis and biological action. Studies investigating the features of fostriecin in comparison to a pharmacophore derived from a PP2A homology model and a non-specific PP1 inhibitor have shown interesting relationships between the two. It was postulated that fostriecin can be considered to have three main sections. The first was the presence of a phosphate that binds to metal ions; secondly, a methyl group proximal to the phosphate; and finally, a hydrophobic section which mimics hydrophobic residues found in substrates normally bound to the enzyme.

It was surprising that the unsaturated lactone of fostriecin, one of the most significant features of the compound, was not represented in the pharmacophore. It was proposed that the lactone was the reason for PP2A selectivity. SAR studies conducted by the Boger group,[66] in which fostriecin analogues were synthesized and tested for PP2A and PP1 activity, indicated that the unsaturated lactone increased the potency of PP2A inhibition by a factor of 200. However, removal of the lactone portion did not negate the inhibition of protein phosphatases completely, such compounds still exhibited strong and selective PP2A inhibition. It was believed that the critical electrophile interacts with C269 of PP2A, which is not present in PP1 and accounts for the selectivity. This was later reported to be the case in PP2A inhibition studies of phoslactomycin.[67]
Other functional groups found to be important for protein phosphatase activity were the phosphate group, which was found to be essential, and the C11 alcohol, which was also seen to contribute significantly to the efficacy of the compound.
A representation of fostriecin and the moieties contributing to overall activity can be seen in scheme 2.1.4.

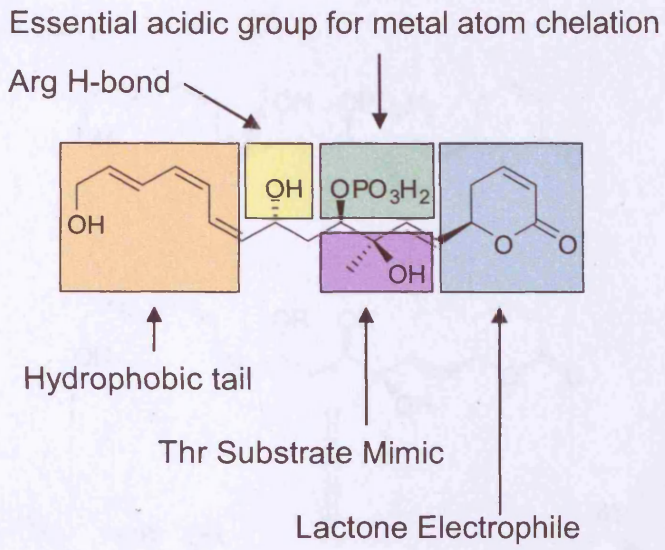
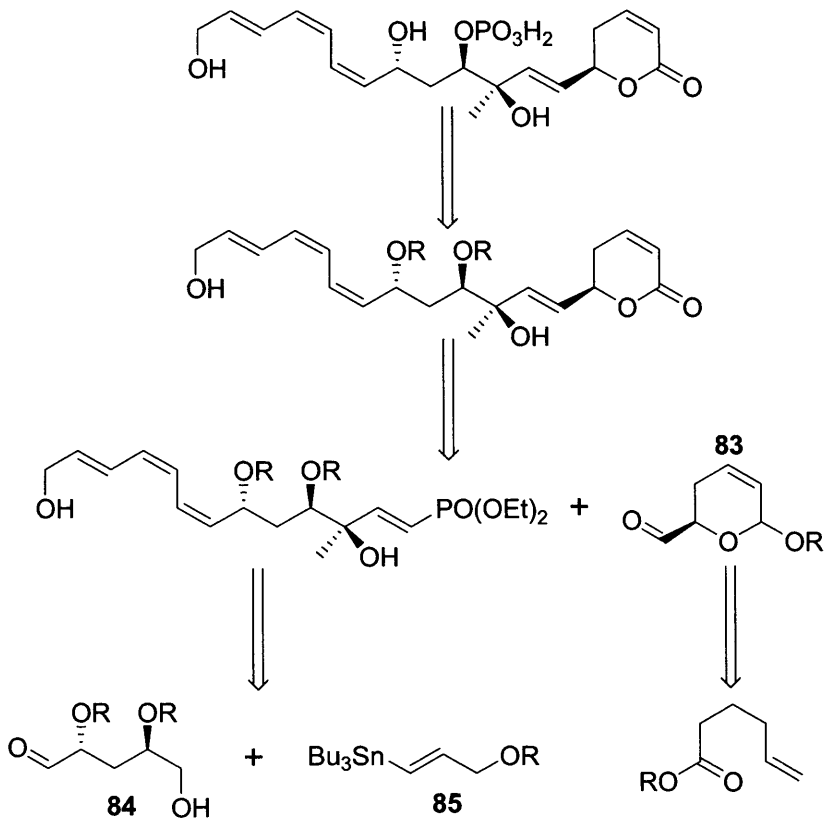


Fig. 2.1.4. The major components of fostriecin that contribute to protein phosphatase inhibition.

2.1.4 Synthesis of Fostriecin

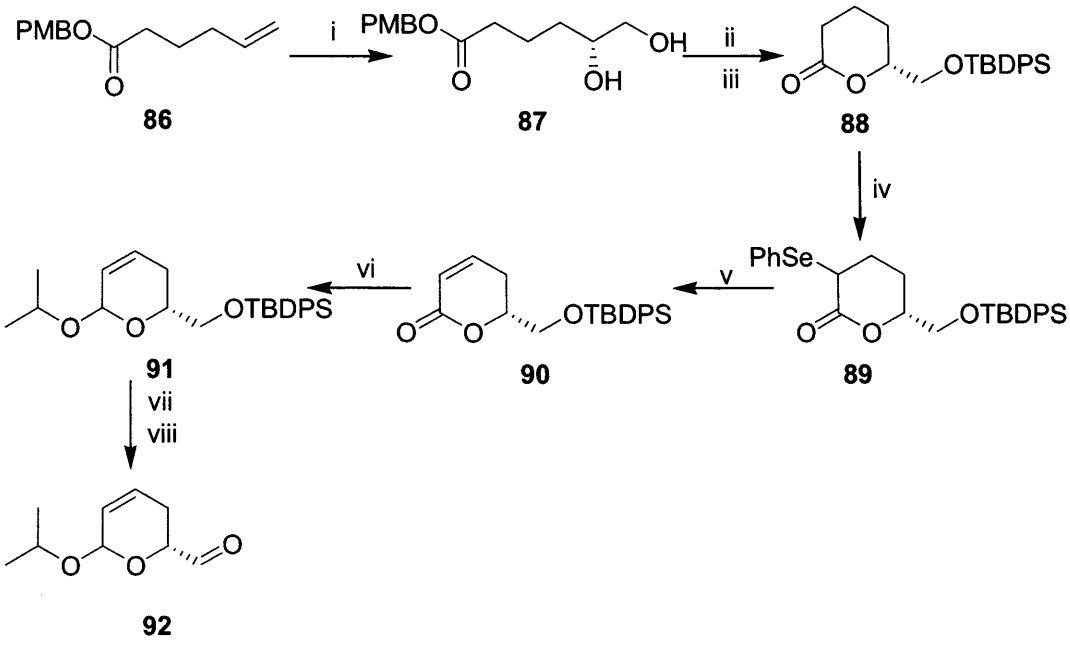
The structure of fostriecin was first eluded to by French,[68] who conducted very limited research into the compound. However, further more conclusive research was finally reported several years later by Boger,[69] who elucidated the structure by means of degradation and other synthetic methodology. Once the structure was conclusively defined, fostriecin was rapidly made the subject of several synthetic attempts. The first total synthesis was reported by Boger,[55] shortly after their publication of structural information.

Boger's approach to the synthesis of fostriecin, was to prepare 3 main synthetic fragments. The lactone fragment **83** (C1 to C6), a central fragment containing 2 chiral centres **84** (C8 to C12), and a final fragment **85** providing synthetic access to the triene within the target molecule (C16 to C18). A retrosynthetic analysis, described by Boger, can be seen in scheme 2.1.5.



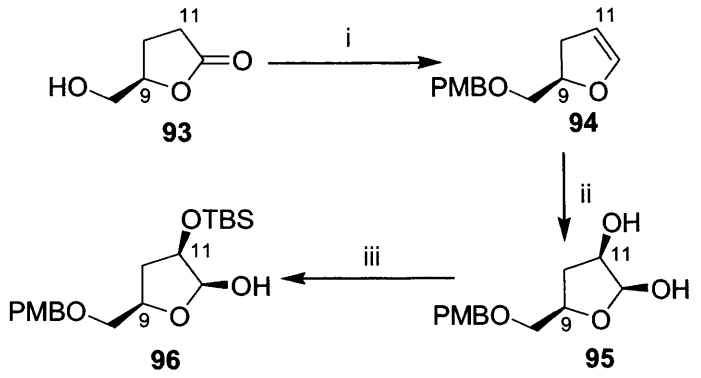
Scheme 2.1.5. Boger's retrosynthetic analysis of fostriecin.

Synthesis of the C1 to C6 fragment began with the formation of a *p*-methoxybenzyl ester derived directly from the corresponding acid, followed by Sharpless asymmetric dihydroxylation to introduce the C5 chiral configuration of fostriecin. Enrichment to >98% e.e. was possible by recrystallising from diethyl ether.[69] Protection of the primary alcohol was conducted with *tert*-butyl diphenylsilyl chloride, and followed by acid catalysed lactonisation to give **88**. The α-phenylselenyl lactone was generated, followed by oxidation to the selenoxide with hydrogen peroxide and *in situ* elimination to give the unsaturated lactone **90**. Reduction to the lactol, and protection gave **91**. Deprotection of the primary alcohol and oxidation to the aldehyde generated the first key portion of Boger's synthesis, the C1 to C6 fragment **92**.



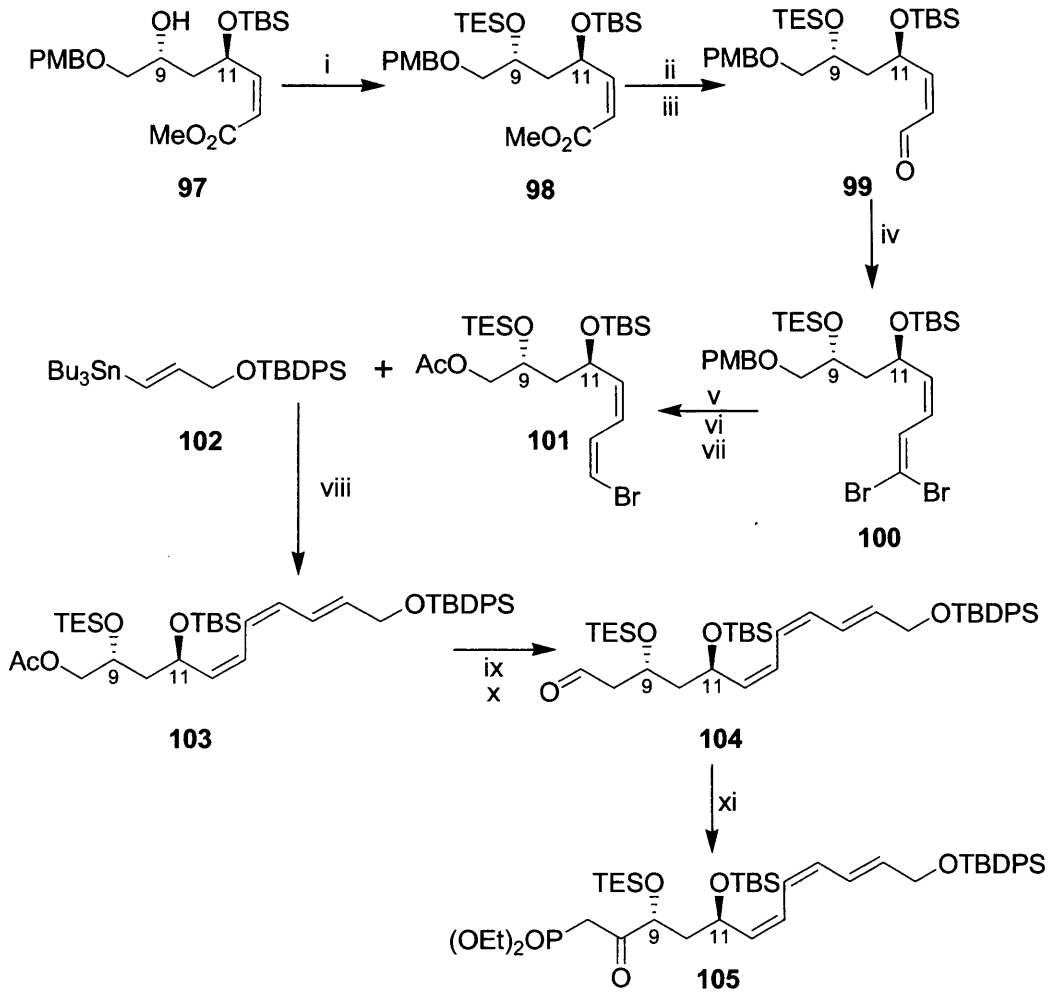
Scheme 2.1.6. The construction of the C1 to C6 fragment. i. $K_3Fe(CN)_6$, K_2CO_3, $NaHCO_3$, $(DHQD)_2$-AQN, *t*-BuOH, H_2O; ii. TBDPSCl, imidazole; iii. TFA, anisole; iv. LDA, PhSeBr; v. H_2O_2; vi. DIBAL-H, PPTS, *i*-PrOH; vii. Bu_4NF, AcOH, THF; viii. TPAP-NMO.

Synthesis of the C7 to C18 segment was conducted in two sections. The first was the C8 to C12 fragment, incorporating important stereocentres at C9 and C11. An outline of its synthesis can be seen in scheme 2.1.7. The starting material **93** was obtained from *D*-glutamic acid in 2 steps, and provided the C9 stereocentre. Protection of the free alcohol, followed by reduction to the lactol and elimination gave **94** in 69% from **93**. Introduction of the C11 centre was accomplished through Sharpless asymmetric dihydroxylation giving the diol **95** in 100% with a >10:1 ratio of diastereoisomers. Selective protection of the C11 alcohol using TBSOTf at a low temperature concluded the synthesis of the fragment in 56% yield from **93**.



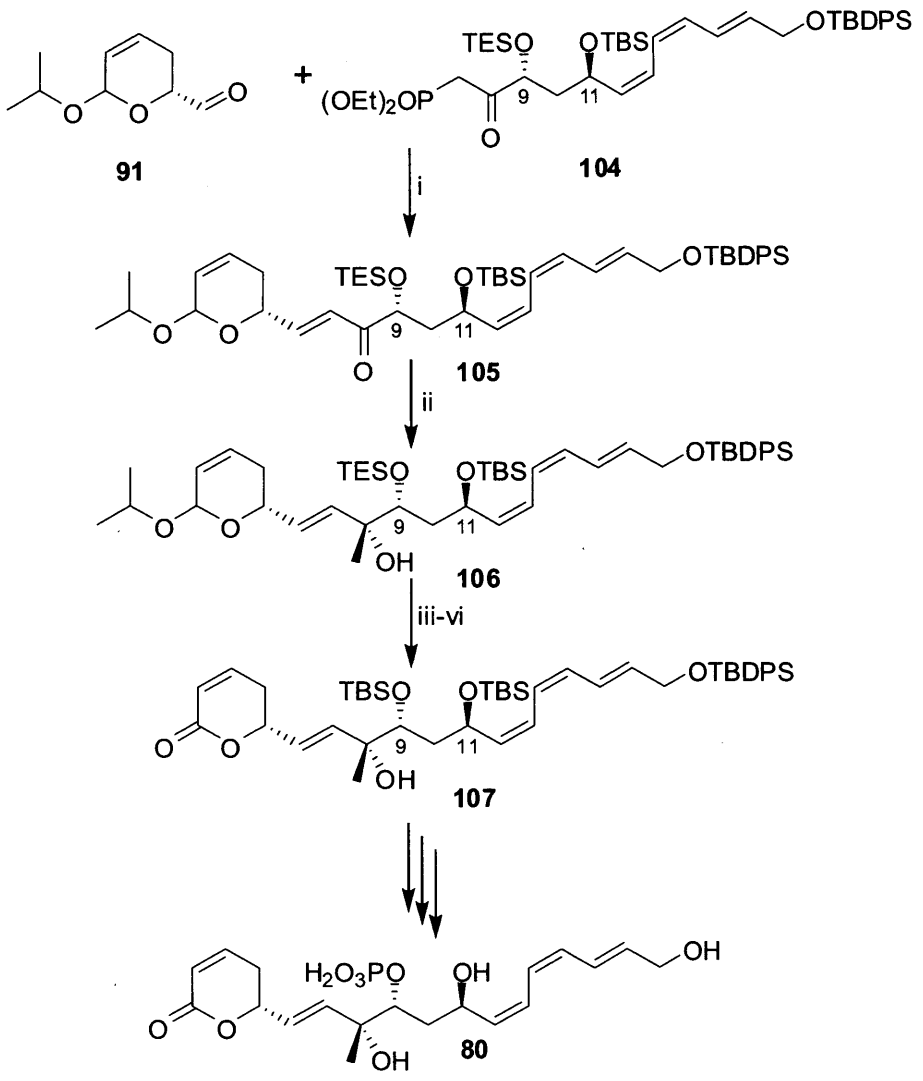
Scheme 2.1.7. Preparation of the C8 to C12 fragment incorporating the C9 and C11 stereocentres. i. PMB protection, followed by DIBAL-H, MsCl, Et_3N; ii. Sharpless Asymmetric Dihydroxylation using $(DHDQ)_2AQN$; iii. TBSOTf, Et_3N.

The final segment of the compound was prepared by building the fragile triene portion onto the already existing C8-C12 fragment. Condensation of **96** with the Still-Gennari phosphonate[70] gave the first cis olefin of the (*Z*,*Z*,*E*)-triene in 88%. Protection of the secondary alcohol with a silyl protecting group, reduction of the unsaturated ester to the alcohol, followed by oxidation to the conjugated aldehyde gave **99**. A Corey-Fuchs[71] reaction gave the vinyl dibromide **100**, followed by selective reduction to the vinyl bromide in 84%. Stille coupling[72] then furnished the triene fragment in 82% from **101**.



Scheme 2.1.8. Synthesis of the C13 to C18 fragment. i. Et_3SiOTf; ii. Dibal-H; iii. Dess-Martin; iv. CBr_4, PPh_3; v.DDQ; vi. Ac_2O, DMAP, *i*-Pr_2NEt; vii, Bu_3SnH-$(Ph_3P)_4Pd$; viii. $(CH_2CN)_2PdCl_2$, NMP, *i*-Pr_2NEt; ix. DIBAL-H; x. Dess-Martin; xi. $(EtO)_2POCH_2Li$.

The C8 to C18 fragment **103** was converted into the aldehyde, and then to the ketophosphonate **105** completing the C7 to C18 fragment. Wadsworth-Horner-Emmons methodology was then used to couple the two portions together, completing the synthesis of the fostriecin carbon scaffold. Methylation at C8 using $MeLi/CeCl_3$ and introduction of the phosphate yielded the completed fostriecin molecule (**80**) after the removal of protecting groups.



Scheme 2.1.9. Final Synthetic stages in the preparation of fostriecin. i. *t*-BuOK, toluene; ii. MeLi-$CeCl_3$, toluene-THF; iii. PPTS, EtOH; iv. TBSOTf; v. aq. HCl; vi. Ag_2CO_3.

Boger's synthetic strategy of constructing the molecule in three distinct sections was so effective, that it was easily possible to synthesise fostriecin analogues. Boger then went on to produce several variations, modifying and exploring the function the lactone moiety in particular.[66]

Shortly after Boger's announcement, several other syntheses of fostriecin were reported utilising a similar retrosynthetic approach to the construction of the central carbon scaffold. Synthetic publications include a total synthesis by Imanishi[73] and co-workers *via* a highly convergent route using a three-segment coupling procedure, in which they report the use of a vinyl iodide intermediate which was instrumental in the synthesis of the (*Z*,*Z*,*E*)-triene. The vinyl iodide intermediate has since been the focus of many

reported formal syntheses.[74-76] The use of alkene metathesis by Kobayashi[77] and Cossy[78] to generate the lactone fragment in the latter stages of the synthesis. Shibasaki,[79] who reported a catalytic asymmetric synthesis using catalysis to construct all four chiral centres. Ramachandran[80] and co-workers, who reported the synthesis of the C1 to C11 fragment of 8-*epi*-fostriecin.

2.1.5 Fostriecin and Structurally Related Compounds

Several compounds, related structurally to fostriecin, are potent inhibitors of protein phosphatases and occur naturally. Some of the members can be seen in scheme 2.1.10. These include the phoslactomycins, cytostatin, sultreicin, and the leustroducsins. The compounds while clearly similar to fostriecin, possess varied therapeutic effects. The phoslactomycins and leustroducins are only weakly cytotoxic, but possess strong broad-spectrum antifungal activity, whereas compounds such as cytostatin exhibit potent cytotoxic activity towards leukaemia and melanoma cancer cell lines.[81,82]

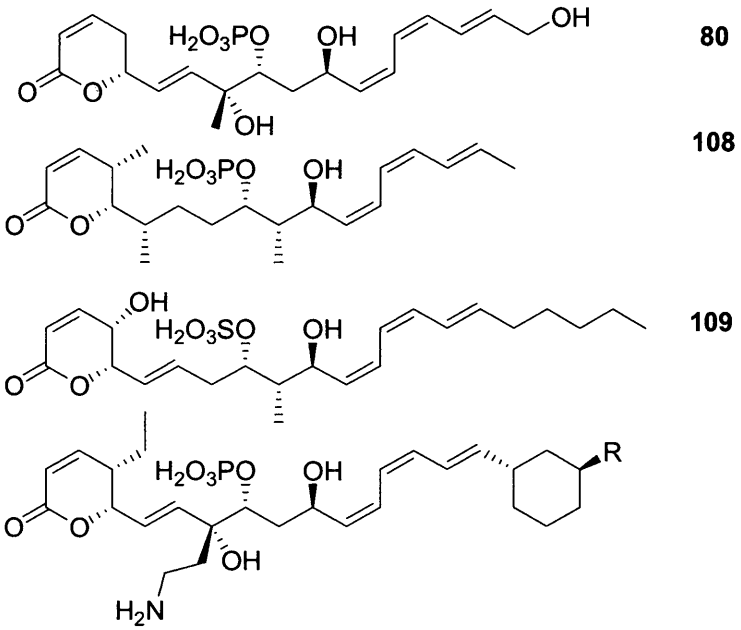


Fig. 2.1.10. Fostriecin (**80**), Cytostatin (**108**), Sultreicin (**109**). R = H, Phospholine (**110**). R = OH, Leustroducsin (**111**). R = OCOR, Phoslactomycins.

Synthesis of the compounds has been reported frequently, and due to their structural similarity to fostriecin, they have been constructed using similar methodology. A total synthesis constructing leustroducsin B, a compound closely related to phospholine,

was reported by Fukuyama[83]in 2003. A synthesis of phospholine,[84,85] also known as phoslactomycin B, by Kobayashi[86] in 2006 as was a total synthesis of cytostatin and several analogues by Waldmann,[87-90] and more recently by Marshall[91] and Boger.[92]

The Boger SAR study[66] prior to the onset of clinical fostriecin trials, highlighted a major flaw in the use of fostriecin therapeutically. During the study, two metabolites, dephosphofostriecin, and the lactone hydrolysed analogue were shown to be rapidly generated in culture medium, and both compounds were shown to have a significant decrease in activity. There considerable interest in the generation of fostriecin analogues which do not degrade or metabolise as rapidly as fostriecin.
Learning from data obtained from the previous clinical study, there are two obvious areas for development of fostriecin: modification of the metal chelating phosphate group, and modification of the lactone moiety.

The reported synthesises of fostriecin and the analogous compounds, being somewhat modular, can therefore be used to obtain new compounds of greater therapeutic potential building on the established synthetic methodology. Synthesis of alternative lactone fragments, or the inclusion of alternative acidic or metal chelating groups in the position of the phosphate can be easily accommodated.

2.1.6 Modification of the Lactone Fragment

Modification of the fostriecin lactone fragment provides an enticing synthetic opportunity in the development of new, potent, and novel anti-tumour compounds. Lactone fragment modification has been explored,[66] but the use of a fostriecin analogue with a lactone fragment mimicking those found within the phoslactomycins or cytostatin are of particular interest.

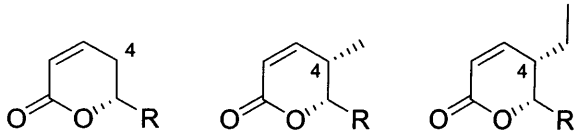
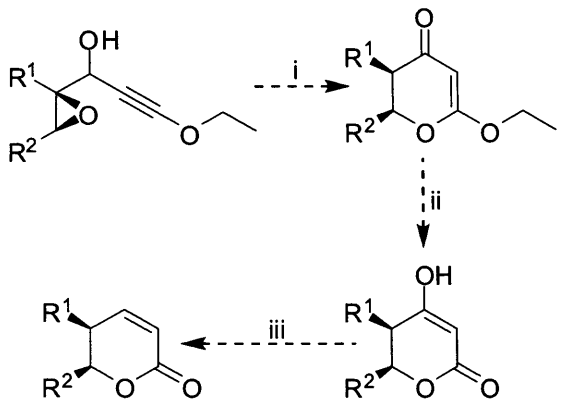


Fig. 2.1.11. The lactone fragments of fostriecin, cytostatin, and phospholine respectively.

Phospholine, is less active than fostriecin, but is also a selective inhibitor of protein phosphatases and substantially more stable. It has been used as a biological tool to aid in the analysis of PP2A inhibition and the dephosphorylation pathways, due to its specificity in PP2A inhibition.

The lactone fragment of phospholine differs only in the inclusion of an ethyl group at the C4 position. Elaboration of the lactone moiety in fostriecin to mimic that found in phospholine, gives the potential for the resulting hybrid to be not only as potent as fostriecin, but far longer lived in culture medium. This would present a truly exciting development in the search for potent anti-tumour compounds.

As most of the reported synthesises of fostriecin use a modular approach to incorporate the lactone fragment, it is possible to modify the procedure to include a modified lactone moiety. Continuation of the synthesis will then lead to the completed fostriecin analogue. It is believed that a mercury-mediated cyclisation methodology reported by Marson *et al.*[93] could provide a route to the generation of the lactone species, and could be easily extended to generate a variety of analogues enantioselectively.

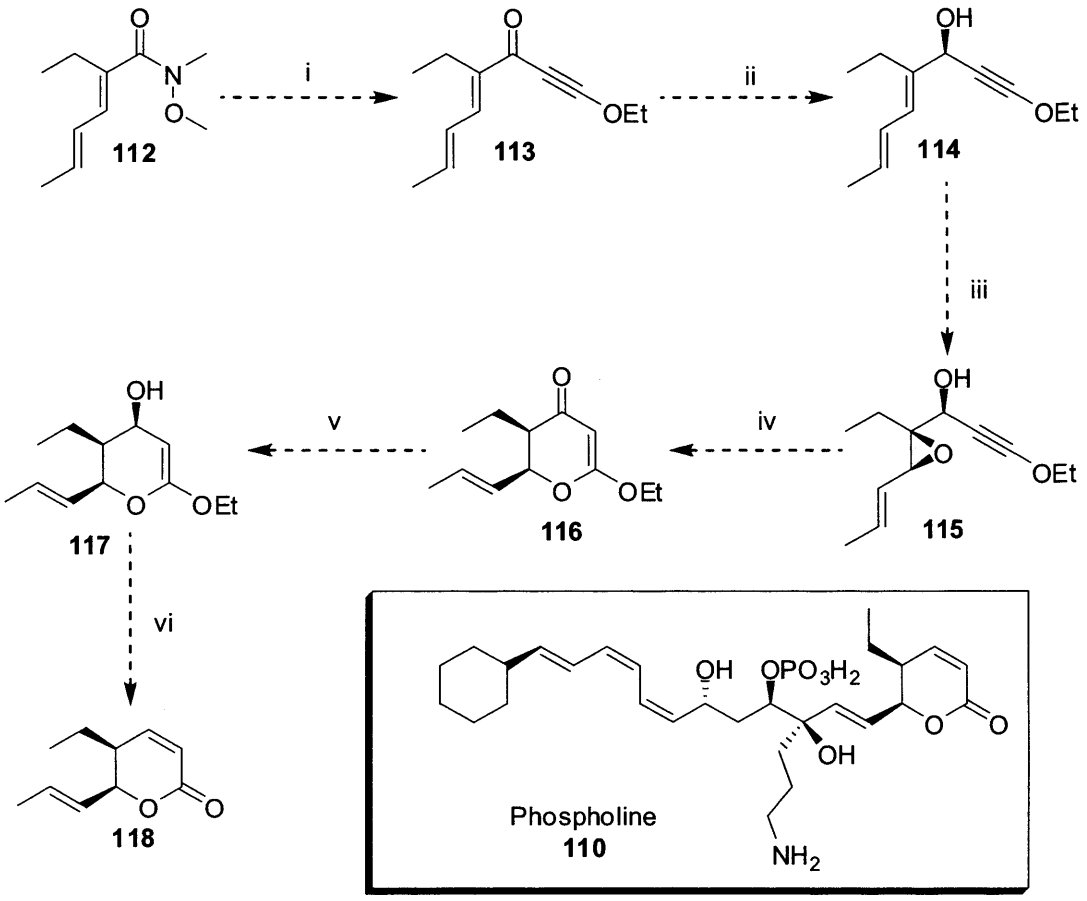


Scheme 2.1.12. The proposed Hg mediated cyclisation. i. Hg^{+2}, H_2SO_4; ii. $NaBH_4$, $CeCl_3$; iii. *p*-TsOH.

The dihydropyranone derived from the mercury-mediated cyclisation of a specifically prepared precursor, could then be selectively reduced and dehydrated to give the desired lactone. The advantages of this particular method, are that the lactone can be constructed with a high degree of enantiospecificity, combined with high yield and purity. The generated lactone fragment could then be introduced into an already existing successful synthetic route to generate the fostriecin analogue. The route, due to the enantioselectivity of the procedure, would also provide access to a variety of stereoisomers, and would allow exploration of other non-natural lactone fragments upon the fostriecin scaffold.

2.2 Results and Discussion

Synthesis of the phospholine lactone ring using a mercury-mediated cyclisation strategy was envisioned to proceed as indicated within scheme 2.2.1.



Scheme 2.2.1. The proposed synthesis of the phospholine lactone ring. i. *n*-BuLi, ethoxyacetylene; ii. (*R*)-Alpine-Borane; iii. TBHP, $Ti(O^iPr)_4$, (+)-DET; iv. 1 mM Hg^{2+} in 0.5 mM H_2SO_4; v. $NaBH_4$, $CeCl_3$; vi. *p*-TsOH.

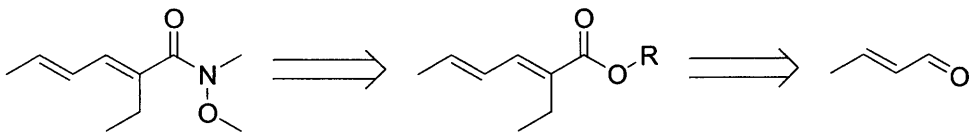
Weinreb amide **112** is alkynylated using standard procedures and yields **113**, the central carbon scaffold. Reduction of enynone **113** using (*R*)-Alpine borane would yield the enynol **114** in high e.e.[94] and would provide access to the key intermediate **115** through Sharpless asymmetric epoxidation.[95] At this point it would be possible for the fragment to spontaneously cyclise to **116**, however, the fragment would give **116** through the mercury (II) rearrangement as indicated in scheme 2.2.1. Luche reduction would then

give the alcohol **117**, and subsequent dehydration would produce **118**, the lactone terminal fragment.

2.2.1 Synthesis of the Weinreb amide

Synthesis initially focused upon the preparation of the Weinreb amide **112**. In order to ensure the stereochemistry of the resulting lactone is correct, and to mimic the corresponding (*E*)-alkene moiety found in phospholine, **112** would have to be of the (*E*,*E*)-configuration.

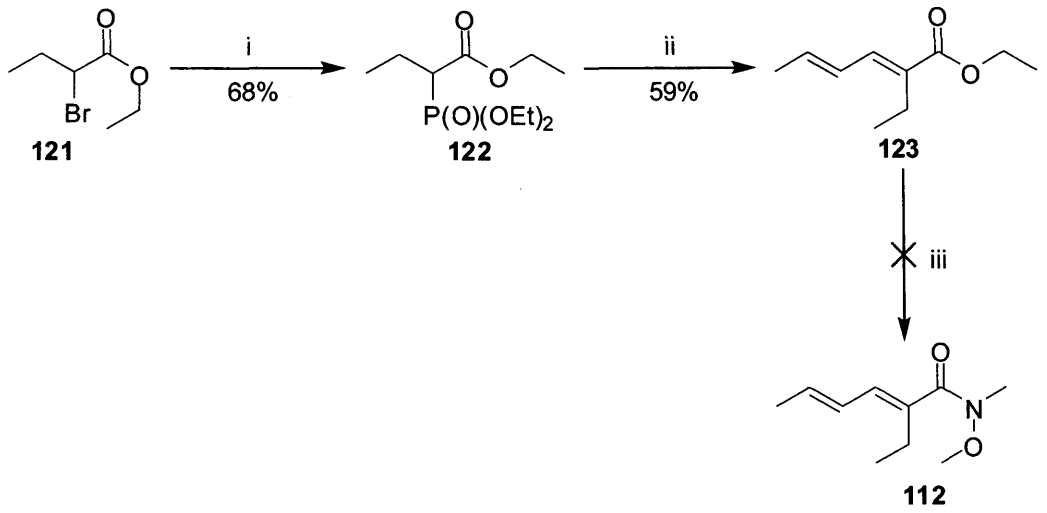
A simple retro-synthetic analysis of **112** can be seen in scheme 2.2.2.



112 **119** **120**

Scheme 2.2.2. Retro-synthetic analysis of the Weinreb amide **112**.

It was postulated that the Werinreb amide **112** could be derived rapidly from ester **119** using standard coupling procedures, which are extensively reported in the literature using a variety of reagents.[96-102] In turn, **119** could be easily derived through the use of Wittig methodology with the easily obtainable, isomerically pure starting material, crotonaldehyde (**120**). The use of Wittig methodology was thought to be sufficient to give the required (*E*,*E*)-configuration in high yield or exclusively,[103] aiding the precipitation of favourable outcomes from asymmetric procedures later in the synthesis. Formulation into a synthetically viable method gave the following synthesis shown in scheme 2.2.3.



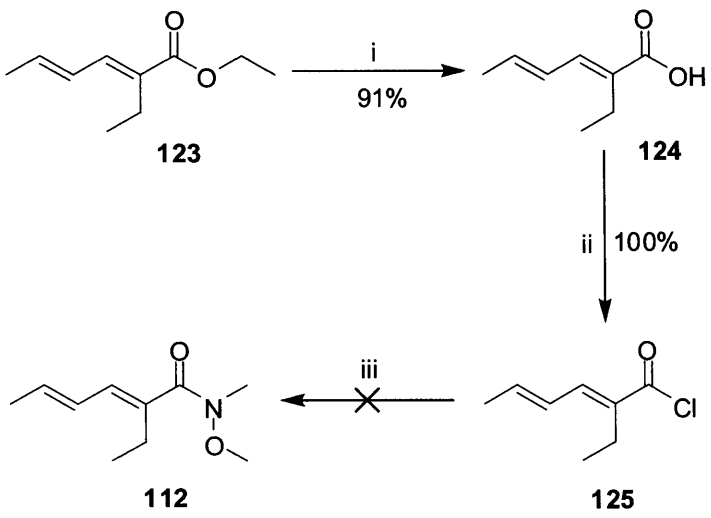
Scheme 2.2.3. i. triethyl orthophosphate, reflux; ii. NaH, THF, *trans*-crotonaldehyde; iii. $AlMe_3$, HCl.NH(OMe)Me, toluene, 0 °C.

Synthesis of the phosphonate ester **122** was achieved by introducing ethyl-2-bromobutanoate (**121**) to a gently refluxing flask of triethylphosphite, and heating at reflux for 6 hours. During the reaction, bromoethane was evolved, and allowed to escape from the vessel. Once all the ester had been consumed, the reaction was cooled, and gave crude material containing the required product and unreacted triethylphosphite. Distillation then gave the pure phosphonate ester **122** using conditions reported by Isbell.[104]

Coupling of the phosphonate ester to crotonaldehyde using Wittig methodology gave the fragment **123** with a yield of 59%. After purification, the material was then used to explore the generation of **112**, the key fragment required to begin the phospholine lactone synthesis.

Weinreb generation was initially attempted using an analogous method reported by Evans[105] and Franklin,[106] involving the use of trimethylaluminium directly from the ester **123**. It was found after several attempts that this method would not be successful. An alternative method was sought in the use of the Merck protocol as used by Williams[107] and Enders.[108] It was found that this method was also unsuccessful.

Alternative routes to the Weinreb intermediate were considered. Reports in the literature describe many procedures that use an acid intermediate combined with the coupling agents or through the corresponding acid chloride. Conversion into the already available ester **123** to the acid provided a convenient and rapid means of exploring this route.



Scheme 2.2.4. i. LiOH, MeOH; ii. sulfonyl chloride, toluene, 80 °C; iii. HCl.HN(OMe)Me, Et_3N, CH_2Cl_2.

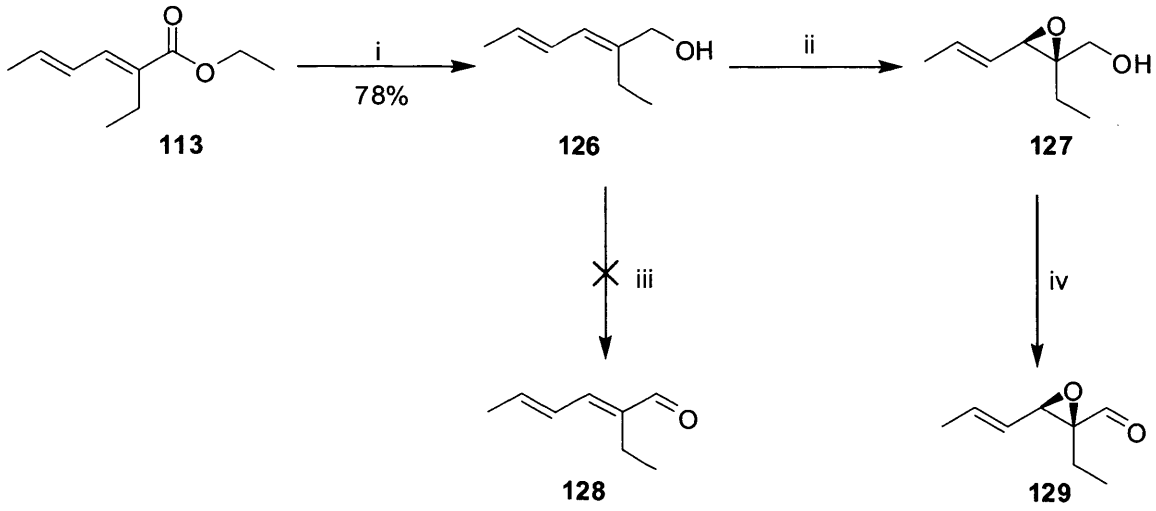
Saponification of **123** using LiOH in methanol[109] gave the acid **124** in high yield (91%). Conversion to the acid was found to increase substantially the purity after purification, and had the added advantage of generating isomerically pure material in the required (*E*,*E*)-configuration. The acid was then converted into the acid chloride by combining 2 equivalents of sulfonyl chloride with **123** in toluene and heating to 80 °C, which gave the desired product in high yield after concentration of the reaction mixture.

Attempts were then made to generate the Weinreb intermediate **112** from **125**. This was accomplished by stirring the acid chloride **125** in dichloromethane with triethylamine, followed by the addition of the *N*,*O*-dimethyl hydroxylamine at 0 °C. It was found that the generation of the Weinreb amide **112** was unsuccessful, or so low yielding that it would be synthetically unviable. Exploration of other routes found that for a variety of coupling methods, methods that were successful in converting sorbic acid to its corresponding Weinreb amide, were unsuccessful in generating **112**. It is believed that in this case, there is sufficient steric hindrance from the ethyl group α to the carbonyl which is sufficient to prevent the reaction from occurring.[110]

In failing to generate **112**, it was concluded that the Weinreb amide was inhibiting progression of the synthesis, and examination of alternative routes to the carbon scaffold of the precursors **113**, **114** and **115** was initiated. Routes which did not pass though a Weinreb intermediate such as **112**, were given particular consideration.

It was considered that in order to generate the precursors correctly, it would be necessary for the alkynylation of a suitable aldehyde fragment. The fragment would, depending on nature of the fragment, it could generate either precursor **114** or **115**, and the synthesis could continue along the earlier proposed synthetic route.

From the starting ester **113**, the conjugated aldehyde **128** and the epoxy aldehyde **129** could be derived. It would then be possible for them to undergo alkynylation to derive the central scaffolds **114** and **115** respectively.



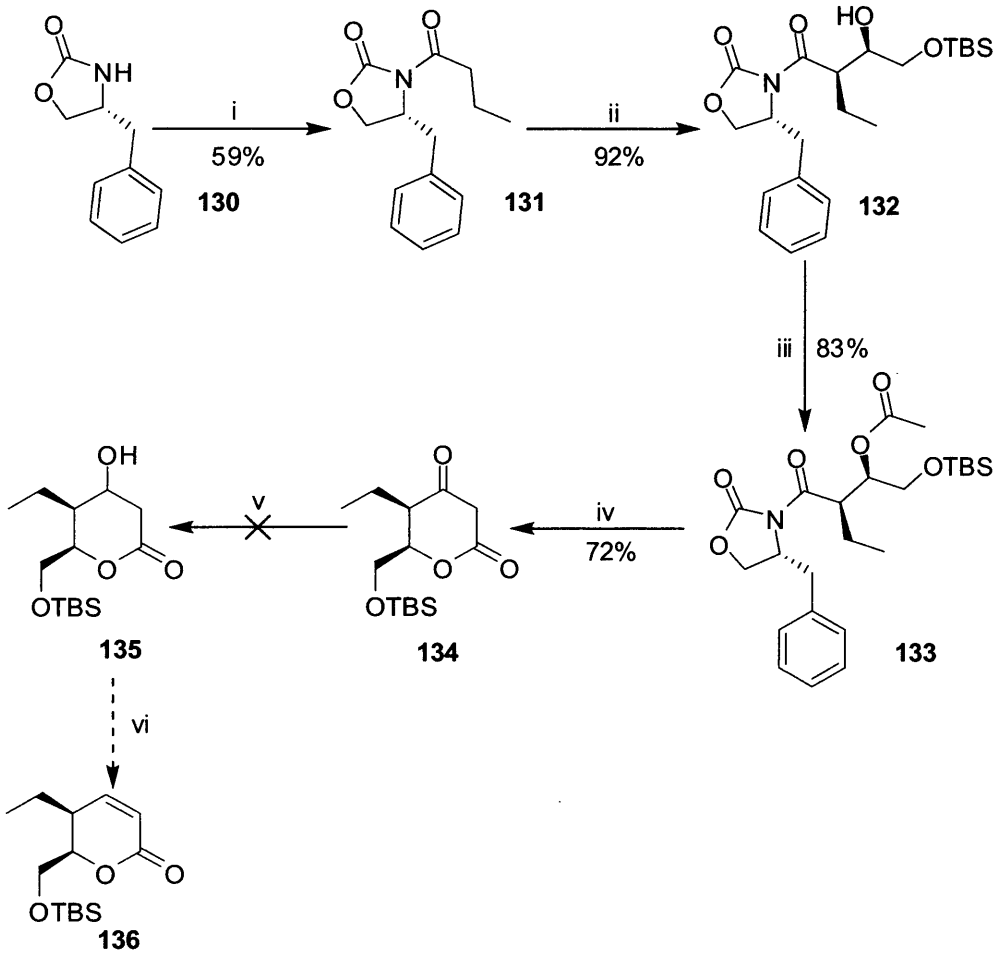
Scheme 2.2.5. i. $LiAlH_4$, Et_2O, 0 °C; ii. TBHP, $Ti(O^iPR)_4$, (+)-DET; iii. DMSO, $Py.SO_3$; iv. Swern oxidation.

Conversion of **113** to the corresponding alcohol **126** was achieved with the use of $LiAlH_4$ in diethyl ether at 0 °C and proceeded in 78% yield, following the methods reported by Davidson.[111] Oxidation of **126** to the conjugated dienal **128** failed to generate any synthetically useful quantities of material using either PCC[112-115] or $PySO_3$ complex.[116-118] Sharpless epoxidation[119] of **126** to **127** revealed such significant levels of scrambling of the diene, that the oxidized aldehyde **129** was no longer suitable for use in the synthesis. This route was then abandoned, and further alternative routes that deviated from the use of the Hg^{+2} methodology were explored.

2.2.2 Hinterding Methodology

Literature was explored for an alternative to the Hg^{+2} strategy. It was found that Hinterding reported the use of an intramolecular Claisen-like condensation/reduction procedure to generate chiral polyketide units.[120] Cleavage of an Evans-oxazolidinone using an acetate enolate yielded β-keto-δ-lactones (*cf.* **134** from **133**), reduction then furnished β-hydroxy-δ-lactones (*cf.* **135**) which were ring opened to yield the polyketide material.

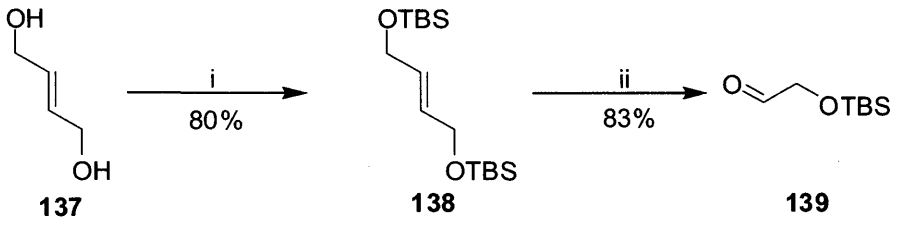
Rather than furnish polyketide material, it was believed that it would be possible to dehydrate the β-keto-δ-lactone to the required pyranone system, in one step. The proposed synthetic route using the Hinterding methodology can be seen in scheme 2.2.6.



Scheme 2.2.6. i. butyroyl chloride, Et_3N, CH_2Cl_2, 0 °C – r.t.; ii. CH_2Cl_2, $TiCl_4$, (-)-sparteine, NMP **139**; iii. DMAP, CH_2Cl_2,Ac_2O, Et_3N, 0 °C; iv. LiHMDS, THF, -78 °C; v. *t*-$BuNH_2$-BH_3, citric acid, MeOH, -5 °C; vi. *p*-TsOH.

Preparation of the Evans auxiliary was accomplished by the reduction of phenylalanine to phenylalinol using sodium borohydride and iodine in methanol.[121] The material was converted into the Evans auxiliary by carefully heating phenylalaninol with diethyl carbonate and potassium carbonate at 130-140 °C, and allowing ethanol to distil from the reaction mixture. The auxiliary could then be recovered from the residue, and after crystallization yielded the pure auxiliary **130** in good yield.[122]

The auxiliary was coupled to butyroyl chloride using procedures reported by Crimmins[123] by combining in dichloromethane and triethylamine at 0 °C to give **131** in 59% yield.



137 **138** **139**

Scheme 2.2.7. i. TBS-Cl, imidazole, CH_2Cl_2, 0 °C-r.t., 12 h; ii. O_3, CH_2Cl_2, PPh_3, -78 °C.

The preparation of the TBS aldehyde **139** began with the protection of *trans*-2-butene-1,4-diol (**137**) with *tert*-butyldimethylsilyl groups. Ozonolysis of the alkene **138,** using methods and procedures described by Lafontaine,[124] gave the aldehyde **139** in 83% yield.

Coupling aldehyde **139** and **131** together in an aldol reaction, using procedures reported by Evans[122] gave **132** in good yield, and in high e.e. Acetylation of **132** using acetic anhydride with DMAP proceeded in high yields, and gave **133**. This material was then treated with LiHMDS in accordance with the Hinterding procedure. Our findings revealed that the reaction was unsuccessful, and attempts using NaHMDS also failed. Treatment of **133** with KHMDS did, however, provide the required β-keto-δ-lactone **134** in 72% yield. Treatment of **134** with sodium borohydride in methanol, sodium borohydride in ethanol, and using a reduction method reported to be successful by Hinterding using citric acid, all failed to reduce **134** to **135**.

Analysis of the material generated from the attempts to generate **134** revealed that the major product was an 11 membered cyclic system. This was consistent with the findings of Brandänges,[125] who with norephedrine derived oxazolidinones observed predominant attack at the *endo*-carbonyl moiety forming 11 membered systems. Deviation from the Hinterding pathway which should have formed the β-keto-δ-lactone, appears to be due to the effects of the substituents upon the auxiliary. Unfortunately, this is not restricted to the functional groups contained upon the auxiliary itself, but to all fragments attached to the auxiliary including the desired chiral portion. As Hinterding himself describes, "subtle effects of substituents upon the oxazolidinone auxiliary govern the outcome of the reaction".[120] Samples of the material were recrystallized using hexane:ethyl acetate and submitted for X-ray analysis to confirm the interpreted NMR data, an illustration of the X-ray results can be seen in fig. 2.2.8.

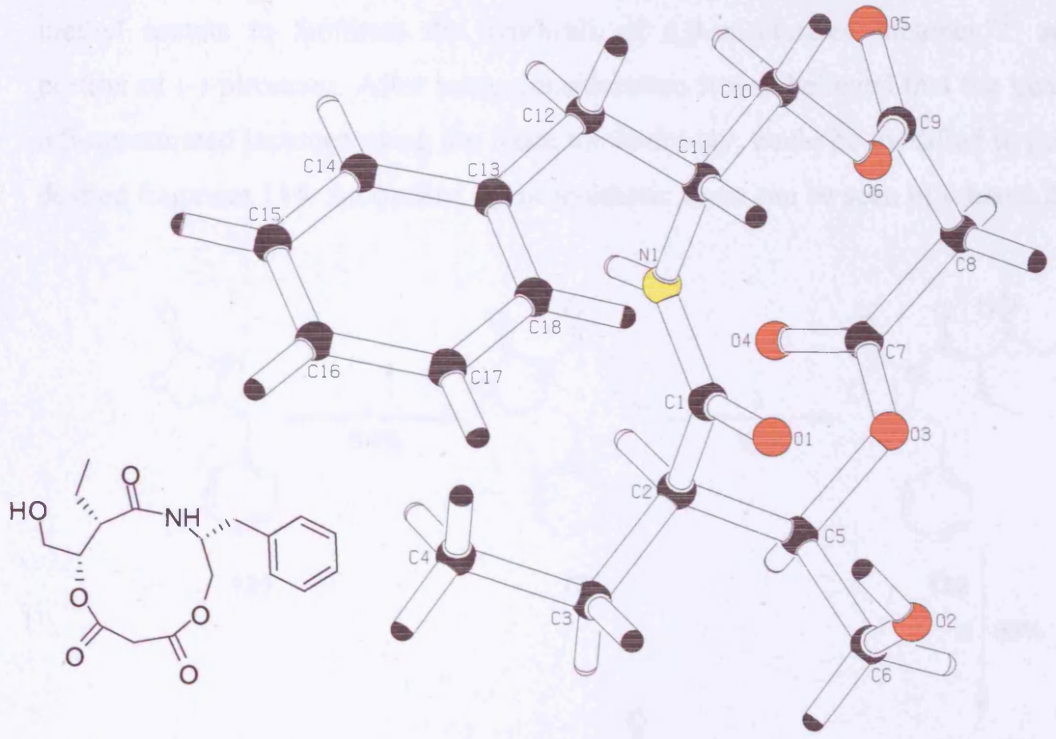
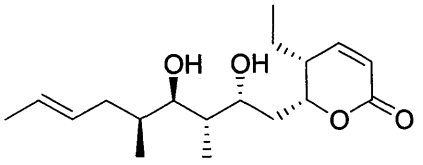


Fig. 2.2.8. Illustration of the 11 membered system derived from X-ray diffraction data.

2.2.3 The Keck Approach to the Lactone Fragment

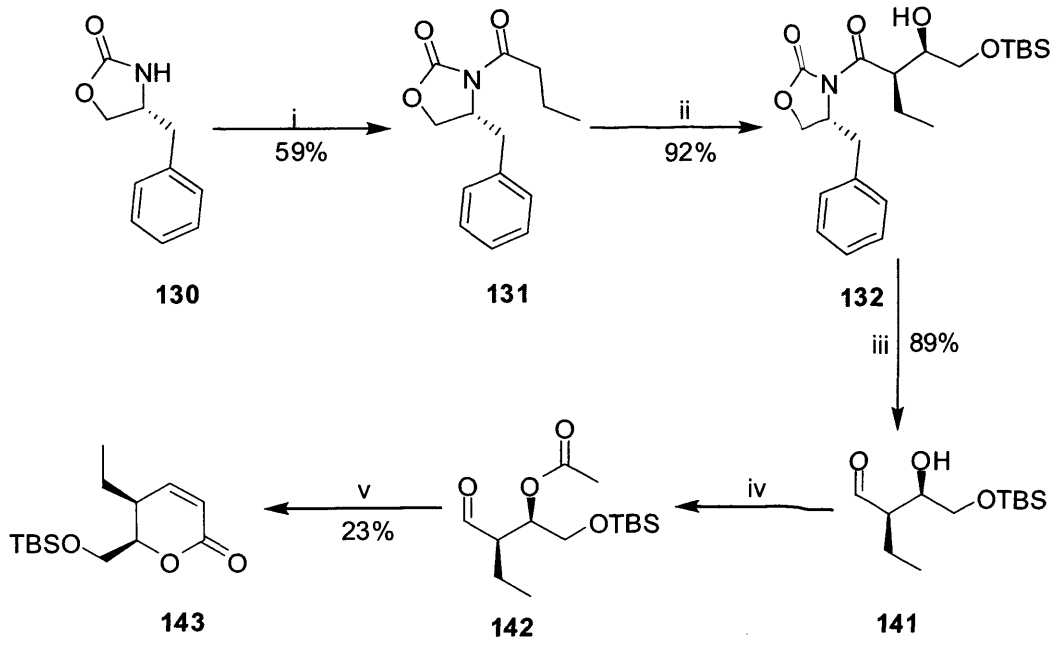
An alternative synthetic route to the lactone terminus was believed to be found in a recently reported synthesis of (-)-pironetin by Keck.[126]



140

Fig. 2.2.9. (-)-pironetin

Within the report he details the use of β-acetoxy aldehydes and the lithium enolate of methyl acetate to facilitate the synthesis of α,β-unsaturated lactones,[127] an integral portion of (-)-pironetin. After some consideration it was believed that the generation of α,β-unsaturated lactones using the Keck methodology, could be modified to generate the desired fragment **118**. An outline of the synthetic route can be seen in scheme 2.2.10.



Scheme 2.2.10. i. butyroyl chloride, Et_3N, CH_2Cl_2, 0 °C – r.t.; ii. CH_2Cl_2, $TiCl_4$, (-)-sparteine, NMP **139**; iii. Red-Al, THF, -60 °C; iv. DMAP, CH_2Cl_2,Ac_2O, Et_3N, 0 °C; v. LiHMDS, THF, -78 °C.

The synthesis using the Keck methodology began with the previously prepared Hinterding fragment **132.** Reductive cleavage of the auxiliary from the required fragment was achieved with the use of Red-Al, using methodology reported by Cane[128] in 89% yield to give **141**. The material was quickly purified by flash column chromatography, and used directly in the next stage of the synthesis. It was found that once cleaved from the chiral auxiliary, the material began to degrade substantially.

The fragment **141** was quickly acetylated to **142**, isolated, and rapidly treated with the lithium enolate of methyl acetate, generated from the action of freshly prepared lithium diisopropylamide on methyl acetate in tetrahydrofuran, as reported by Keck.[127] After work up and purification, **143** was isolated in 23% yield.

2.3 Conclusion

The construction of the phospholine lactone fragment was successful using the Keck methodology, but not by the original proposed Hg^{+2} mediated strategy. It was not possible to explore the creation of a fostriecin analogue owing to time constraints.

In the first instance, the use of the mercury-mediated cyclisation could not be assessed for the construction of the lactone, owing to the inability to generate the required Weinreb amide precursor necessary for the cyclisation to occur. It is believed that the presence of an ethyl group α to the carbonyl, inhibits or greatly reduces the generation of the Weinreb precursor through unfavourable steric interactions.

Attempts to generate an advanced precursor as an alternative to the use of a Weinreb amide also failed. In this case the inability to generate synthetically viable quantities of conjugated aldehydes and epoxy aldehydes capable of undergoing alkynylation prevented continuation to the cyclisation stage.

Deviation from the use of the mercury-mediated cyclisation strategy to the Hinterding procedure resulted in failure. Steric effects, are in this case, responsible in part for the inability to generate the required target.

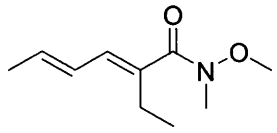
Future work may benefit from a paper published by Dake *et al.*[110] who reports the generation of Weinreb amides using acyl mesylate intermediates, and enables the synthesis of sterically hindered Werinreb amides. Using the Dake method of Weinreb generation would allow the continuation of the initially proposed methodology, and overcome unanticipated steric difficulties that could occur with further modification of the lactone fragment.

2.4 Experimental

The general experimental section can be found in chapter 1.4.

(2*E*,4*E*)-2-Ethyl-*N*-methoxy-*N*-methylhexa-2,4-dienamide (112)

Method 1:[107]



To a slurry of 2-ethyl-hexa-2,4-dienoic acid ethyl ester (0.5 g, 3 mmol), and *N*,*O*-dimethylhydroxylamine (0.44 g, 4.5 mmol) in THF (4 mL) at -10 °C, ^{i}PrMgCl (9 mmol) was added dropwise over 15 minutes. During addition, it was ensured that the temperature did not exceed -5 °C. The mixture was stirred for 20 minutes at -10 °C. The reaction was then quenched with the addition of 25% w/w NH_4Cl solution. The mixture was taken into ethyl acetate (5 mL), followed by drying ($MgSO_4$) to yield the crude product. After column chromatography and analysis, it was found that **112** had not been formed.

Method 2:[105,106]

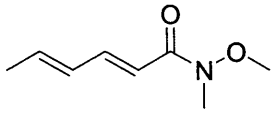
To a a stirring suspension of *N*,*O*-dimethylhydroxylamine (0.23 g, 2.36 mmol) in dry tetrahydrofuran (5 mL) at 0 °C, Me_3Al (2.1 mmol) was added slowly. The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The mixture was re-cooled to 0 °C, and 2-ethylhexa-2,4-dienoic acid ethyl ester (0.1 g, 0.6 mmol) was added dropwise. The mixture was allowed to return to room temperature, and stirred for a further 30 min. The reaction was carefully quenched with the addition of 1 M HCl, followed by the addition of dichloromethane (10 mL). Extraction of the organic fraction, followed by drying ($MgSO_4$), yielded the crude material. Purification and analysis indicated that that **112** had not been formed.

Method 3:

To (2*E*,4*E*)-2-ethylhexa-2,4-dienoyl chloride (0.57 g, 3.6 mmol) in toluene (3 mL), *N*,*O*-dimethylhydroxylamine (0.39 g, 3.96 mmol) was added, and the reaction cooled to 0 °C. Triethylamine (0.80 g, 7.92 mmol) was added, and the mixture allowed to stir for 1 h. TLC analysis indicated consumption of starting material. Dichloromethane (10 mL) was added, the reaction mixture extracted with water, and brine (10 mL, and 3 mL

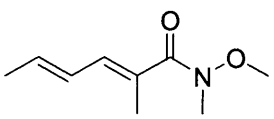
respectively). Organic fractions were dried ($MgSO_4$) and concentrated under reduced pressure. Analysis indicated that that **112** had not been formed.

Hexa-2,4-dienoic acid methoxy-methyl-amide[129]



Sorbic acid (1.0 g, 8.9 mmol) was added to a mixing solution of dry dichloromethane (8 mL) and *N*-methyl morpholine (2.45 mL, 22.25 mmol). The reaction mixture was cooled to -78 °C using dry ice and acetone. Isobutyl chloroformate (1.40 mL, 10.9 mmol) was added dropwise to the reaction mixture, and stirred for 30 min. A solution of *N,O*-dimethylhydroxyamine (1.02 mL, 10.9 mmol) and *N*-methyl morpholine (1.17 mL, 10.9 mmol), in dry dichloromethane (2 mL) was added to the reaction mixture, and stirred for 30 min. The mixture was then allowed to warm to room temperature. Upon completion, 10% sulfuric acid (10 mL) was added to the reaction mixture, followed by ethyl acetate (10 mL) and was extracted. The organic layer was washed with 10% sulfuric acid (10 mL), water (10 mL), and brine (10 mL) respectively. The organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to a pale yellow oil. Purification by column chromatography using an eluant of 4:1 hexane:ethyl acetate, product containing fractions were concentrated under reduced pressure to give the desired product as a colourless oil (0.79 g, 57%). ^{1}H NMR (300MHz, $CDCl_3$), δ_H 7.43 - 7.35 (m, 1H, CH-CH=CH), 6.42-6.38 (m, 1H, CH_3-CH=CH), 6.29 - 6.12 (m, 2H, CH_3-CH, CH=CH-C), 3.79 (s, 3H, O-CH_3), 3.33 (s, 3H, N-CH_3), 1.93 (d, *J*=6.1 Hz, 3H, CH_3-CH). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 167.9, 144.0, 138.7, 130.7, 117.2, 62.0, 32.8, 18.9.

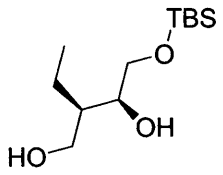
(2*E*,4*E*)-*N*-Methoxy-*N*,2-dimethylhexa-2,4-dienamide



To a flask containing 2-methylhexa-2,4-dienoic acid (0.20 g, 1.6 mmol) and *N*-methyl morpholine (0.44 mL, 4 mmol) in dry dichloromethane (5 mL) at –78 °C under argon, isobutylchloroformate (1.92 mmol, 0.26 g) was added dropwise. The mixture was then stirred for 10 min, after which a solution of *N,O*-dimethylhydroxylamine hydrochloride (3.2 mmol, 0.31 g) and *N*-methyl morpholine (0.35 mL, 3.2 mmol) in 5 mL of dry dichloromethane was added dropwise. The mixture was then stirred for 30 min at -78 °C, and allowed to warm to room temperature. The reaction was quenched with the addition of 10% H_2SO_4 (1 mL) dropwise. The reaction mixture was then washed with 10% H_2SO_4 (5 mL),

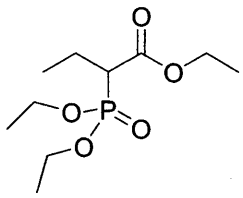
distilled water (5 mL), and brine (2 mL) respectively. Concentration of the organic fractions after drying ($MgSO_4$), yielded the crude product. Purification by column chromatography using an eluant of 4:1 hexane:ethyl acetate gave the product as a pale yellow oil (0.22 g, 81%). ^{1}H NMR (300MHz, $CDCl_3$), δ_H 7.37-7.29 (m, 1H, CH-CH=C), 6.53-6.44 (m, 1H, CH=CH-CH), 6.36-6.22 (m, 1H, CH_3-CH=CH), 3.79 (s, 3H, O-CH_3), 3.23 (s, 3H, N-CH_3), 2.04 (s, 3H, CH_3-C), 1.99 (d, J=6.7 Hz, 3H, CH_3-CH=CH). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 173.8, 140.6, 138.8, 127.4, 123.9, 61.5, 35.6, 18.9, 12.0.

(2*R*,3*S*)-4-(*tert*-Butyldimethylsilyloxy)-2-ethylbutane-1,3-diol



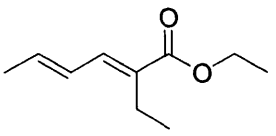
To a flask containing 4-benzyl-3-[4-(*tert*-butyldimethylsilanyloxy)-2-ethyl-3-hydroxy-butyroyl]-oxazolidin-2-one (0.1 g, 0.24 mmol) in dry diethyl ether (5 mL), $LiAlH_4$ (8 mg, 0.2 mmol) was carefully added. The mixture was stirred at room temperature for 1 h, and assessed by TLC. 1 M HCl (3 mL) was added to quench the reaction, and product extracted into dichloromethane (5 mL). Drying ($MgSO_4$), and concentration under reduced pressure yielded complex reaction materials that gave no products after purification.

Ethyl 2-(diethoxyphosphoryl)butanoate[104,130] (122)



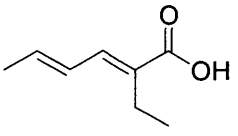
A flask containing triethyl phosphite (10.19 g, 61.3 mmol), was heated to reflux. Ethyl 2-bromobutyrate (10.0 g, 51.1 mmol) was added portion wise to the vessel, over a period of 2 h. The mixture was heated to reflux for 5 h, and was then allowed to cool. Distillation of excess triethyl phosphite, and ethyl 2-bromobutyrate from the reaction mixture afforded the crude product, which was contained within the residue. Further distillation at 136-138 °C, 1mm Hg (lit. 117-118, 0.6 mm Hg)[104] **122** as an oil (8.8 g, 68%). ^{1}H NMR (300MHz, $CDCl_3$), δ_H 4.14-4.00 (m, 6H, CH_3-CH_2-O), 2.79 (ddd, J=4.3, 8.4 Hz, J_{HP}=23.4 Hz, 1H, CH_2-CH(P)-C), 1.93-1.84 (m, 2H, CH_3-CH_2-CH), 1.26-1.20 (m, 9H, O-CH_2-CH_3), 0.89 (t, J=7.0 Hz, 3H, CH-CH_2-CH_3).

2-Ethylhexa-2,4-dienoic acid ethyl ester (123)



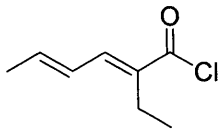
Sodium hydride (0.34 g, 14 mmol) in dry THF (20 mL) was stirred at 0 °C. 2-(diethoxy-phosphoryl)-butyric acid ethyl ester (3.53 g, 14 mmol) was added dropwise over 5 minutes. The mixture was allowed to warm to room temperature, and was stirred for 10 min. The reaction vessel was re-cooled to 0 °C, followed by the dropwise addition of crotonaldehyde (1.0 g, 14 mmol). The mixture was warmed to room temperature and stirred for 24 h. The reaction was reduced to a minimum volume under light vacuum, ethyl acetate was added, and the reaction mixture washed with water and brine respectively. Organic fractions were dried ($MgSO_4$), and concentrated under reduced pressure to an oil. The crude product was washed through a small silica plug to remove any trace baseline impurities, giving **123** as a colourless oil (1.38 g, 59%). ^{1}H NMR (300MHz, $CDCl_3$), δ_H 7.06 (d, J=11.3 Hz, 1H, CH-CH=C), 6.40-6.30 (m, 1H, CH=CH-CH), 6.11-6.01 (m, 1H, CH_3-CH=CH), 4.18 (q, J=6.9 Hz, 2H, O-CH_2-CH_3), 2.39 (q, J=7.3 Hz, 2H, CH_3-CH_2-C), 1.85 (d, J=6.9 Hz, 3H, CH_3-CH_2-O), 1.28 (t, J=7.3 Hz, 3H, CH_3-CH_2-C), 1.02 (m, 3H, CH_3-CH). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 168.2, 138.0, 137.7, 131.7, 127.1, 60.3, 20.2, 18.1, 14.3, 14.2. HRMS calcd. for $C_{10}H_{16}O_2$ 168.1150, found 168.1157.

(2*E*,4*E*)-2-Ethylhexa-2,4-dienoic acid (124)



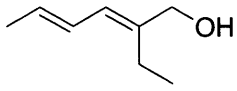
A flask was charged with tetrahydrofuran:MeOH:water (3:1:1, 30 mL), and stirred. 2-Ethyl-hexa-2,4-dienoic acid ethyl ester (3.73 g, 22.2 mmol) followed by LiOH (1.06 g, 44.4 mmol) were added to the vessel, and the mixture stirred. The reaction was monitored by TLC. Upon completion, the reaction mixture was extracted with HCl (1 M, 30 mL), brine (10 mL), and dried ($MgSO_4$). Concentration yielded the crude product as a yellow oil. Column chromatography using an eluant of 4:1 hexane:ethyl acetate gave **124** as an oil (2.82 g, 91%). ^{1}H NMR (300MHz, $CDCl_3$), δ_H 7.23 (d, J=10.0 Hz, 1H, CH-CH=C), 6.42-6.30 (m, 1H, CH=CH-CH), 6.21-6.09 (m, 1H, CH_3-CH=CH), 2.39 (q, J=7.5 Hz, 2H, CH_3-CH_2-C), 3.76 (d, J=6.7 Hz, 3H, CH_3-CH=CH), 1.04 (t, J=7.5 Hz, 3H, CH_3-CH_2-C). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 174.0, 140.6, 139.5, 130.3, 127.1, 19.9, 19.0, 14.2.

(2*E*,4*E*)-2-Ethylhexa-2,4-dienoyl chloride[131] (125)



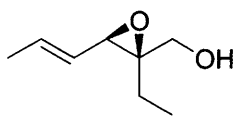
(2*E*,4*E*)-2-Ethylhexa-2,4-dienoic acid (0.5 g, 3.6 mmol) was placed into a flask containing toluene (3 mL). Thionyl chloride (0.86 g, 0.52 mL) was added, and the reaction stirred at 80 °C. The reaction was monitored by TLC. Upon completion, the reaction mixture was concentrated under reduced pressure to give **125** as an oil (0.57 g, 100%). The material was used without further purification.

2-Ethylhexa-2,4-dien-1-ol (126)



To a suspension of lithium aluminum hydride (0.23 g, 6.15 mmol) in dry diethyl ether (6 mL) cooled to 0 °C, was added 2-ethylhexa-2,4-dienoic acid ethyl ester (1.38 g, 8.2 mmol). After 2 h, the mixture was filtered through celite, and the filtrate concentrated under reduced pressure to a minimum. Ethyl acetate was added, and the solution washed with 10% sulfuric acid and brine respectively. Concentration of product containing fractions gave the crude product as a colourless oil. Purification by column chromatography using an eluant of 4:1 hexane:ethyl acetate gave **126** as a colourless oil (0.42 g, 41%). ^{1}H NMR (300MHz, $CDCl_3$), δ_H 6.31-6.26 (m, 1H, CH=C<u>H</u>-CH), 5.96 (d, *J*=10.9 Hz, 1H, CH-C<u>H</u>=C), 5.75-5.54 (m, 1H, CH_3-C<u>H</u>=CH), 4.07 (s, 2H, HO-C<u>H_2</u>-C), 2.20 (q, *J*=7.6 Hz, 2H, C-C<u>H_2</u>-CH_3), 1.56 (d, *J*=6.1 Hz, 3H, C<u>H_3</u>-CH=CH), 1.02 (t, *J*=7.6 Hz, 3H, C<u>H_3</u>-CH_2-C). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 140.5, 129.6, 126.9, 124.9, 66.6, 21.5, 18.3, 13.5.

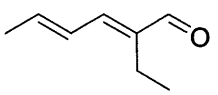
((2*R*,3*R*)-2-Ethyl-3-((*E*)-prop-1-enyl)oxiran-2-yl)methanol[119] (127)



To a flask containing dichloromethane (3 mL) at -10 °C, Ti(O^iPr)$_4$ (23 mg, 0.08 mmol) and (-)-DIPT (22 mg, 0.1 mmol) was added. The mixture was stirred for 5 min, then 2-ethylhexa-2,4-dien-1-ol (0.2 g, 1.6 mmol) was added. The mixture was allowed to age at -10 °C for 10 minutes, and *t*-butyl hydroperoxide (4.5 M, 3.2 mmol) was added dropwise. The mixture was stirred for 8 h at -10 °C, and quenched upon completion by TLC. Water (1 mL) was added and vigorously stirred for 60 min. 30% NaOH/saturated ammonium chloride solution (1 mL) was added and the reaction stirred for 60 min. The reaction mixture was then filtered, and the filtrate extracted with dichloromethane (2 x 5 mL). Drying

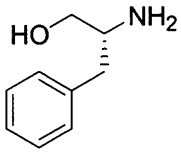
(anhydrous Na_2SO_4) and concentration gave the crude product as an oil. No material was found to corrispond to **127**.

(2*E*,4*E*)-2-Ethyl hexa-2,4-dienal (128)



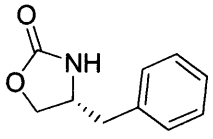
To a flask containing dimethyl sulfoxide (15 mL), triethylamine (1.88g, 18.6 mmol), and Py.SO_3 complex (2.17 g, 13.6 mmol) at -10 °C, the alcohol 2-ethylhexa-2,4-dien-1-ol (1.57g, 12.4 mmol) was added in one portion. The mixture was then stirred at 0 °C and monitored by TLC. As the reaction progressed, complex products were seen to be formed.

(*R*)-2-Amino-3-phenyl-propan-1-ol, (*R*)-phenylalaninol[132a]



To a stirring solution of sodium borohydride (125.8 mmol, 4.67 g) in 100 mL of dry tetrahydrofuran, $_D$-phenylalanine was added in one portion. The reaction vessel was purged with argon, and cooled to 0 °C. Iodine (52.4 mmol, 13.4g) in tetrahydrofuran (25 mL) was added dropwise over 30 min. The reaction was allowed to warm to room temperature, and finally heated to reflux overnight. Cooling of the mixture, followed by careful quenching with methanol until a clear solution was obtained. Solvents were then removed under reduced pressure, yielding a white paste. 100 mL of 20% potassium hydroxide solution was added and stirred for 4 hours. Extraction with 3 x 100 mL of dichloromethane, followed by drying ($NaSO_4$), and concentration under reduced pressure yielded the crude material. Recrystallisation from toluene gave the product as a white crystalline solid, 3.63g, 46%. ^1H, and ^{13}C NMR identical to previously reported data.[132]

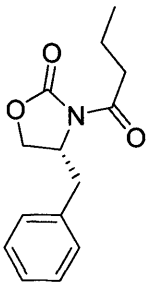
(4*R*)-4-(Phenylmethyl)-2-oxazolidinone[122] (130)



A dry flask was prepared containing *R*-phenylalaninol (3.63 g, 24 mmol), diethyl carbonate (5.67 g, 48 mmol), and potassium carbonate (0.34 g, 2.4 mmol). The mixture was heated carefully to 135-140 °C, and ethanol was distilled as it was formed. When no more ethanol could be distilled, the mixture was cooled, and diluted with dichloromethane (10 mL), and filtered. The filtrate was washed with 1 M sodium bicarbonate solution, and organic fractions were

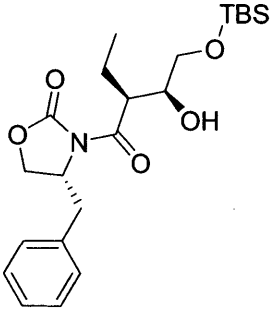
concentrated under reduced pressure to give **130** as an off-white solid (3.01 g, 71%). ^{1}H, and ^{13}C NMR identical to previously reported data.[122]

(*R*)-4-Benzyl-3-butyryl-oxazolidin-2-one (131)



To a flask containing (4*R*)-4-(phenylmethyl)-2-oxazolidinone (3 g, 17 mmol) in 70 mL of dry tetrahydrofuran at -78 °C, *n*-butyllithium (17 mmol) was added dropwise and stirred for 20 min. Butyroyl chloride was added to the reaction mixture dropwise via syringe, stirred for 15 min, and warmed to room temperature followed by stirring for 30 min. The reaction was quenched with 10% potassium carbonate solution. Extraction with dichloromethane (3 x 50 mL), followed by drying ($MgSO_4$), and concentration under reduced pressure yielded the product as a colourless oil. Purification by column chromatography using an eluant of 5:1 petroleum spirit (40-60 °C):ethyl acetate, gave the product as a colourless oil, 2.54 g, 59%. IR ν_{max} (KBr, cm^{-1}) 1781 (C=O), 1700 (NC=O). ^{1}H NMR (300MHz, $CDCl_3$), δ_H 7.33-7.19 (m, 5H, Ar-H), 4.69-4.65 (m, 1H, Ph-CH_2-CH), 4.16-4.12 (m, 2H, O-CH_2-CH), 2.92-2.88 (m, 2H, Ph-CH_2-CH), 2.80-2.76 (m, 2H, CH_2-CH_2-C), 2.02-1.68 (m, 2H, CH_3-CH_2-CH_2), 1.03-0.97 (m, 3H, CH_3-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 173.2, 153.5, 135.3, 129.4, 129.0, 127.3, 66.2, 55.1, 37.9, 37.4, 17.7, 13.7.

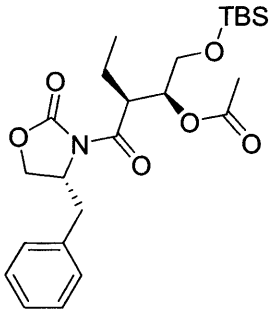
(*R*)-4-Benzyl-3-((2*S*,3*S*)-4-(tert-butyldimethylsilyloxy)-2-ethyl-3-hydroxybutanoyl)oxazolidin-2-one (132)



To a stirring solution of 4-benzyl-3-butyroyl-oxazolidin-2-one (0.22 g, 0.89 mmol) in dry dichloromethane (6 mL) at 0 °C, $TiCl_4$ (1 M in dichloromethane, 0.84 mmol) was added slowly and stirred for 5 min, generating a yellow solution. (-)-Sparteine (0.80 mmol, 0.188 g) was added dropwise and stirred for 20 min, generating a deep red solution. Cooling to -78 °C, followed by the addition of *N*-methyl-2-pyrrolidinone (0.80 mmol, 0.080 g). After stirring for 10 min, (*tert*-butyldimethylsilyloxy)-acetaldehyde (0.88 mmol, 0.154 g) was added dropwise, and stirred for 1 h, followed by warming to 0 °C and stirring for a further 30 min. The reaction was quenched with half saturated ammonium chloride solution (3 mL), and extracted using dichloromethane (3 x 5 mL). Combination of

organic fractions, drying (Na_2SO_4), followed by concentration under reduced pressure gave the crude product as an oil. Purification using column chromatography with an eluant of 10% ethyl acetate/petroleum ether (40-60 °C) gave the product as a colourless oil, 0.35 g, 92%. IR ν_{max} (KBr, cm^{-1}) 3468 (OH), 1780 (C=O), 1692 (NC=O). ^1H NMR (300MHz, $CDCl_3$), δ_H 7.36-7.21 (m, 5H Ar-H), 4.72-4.68 (m, 1H, Ph-CH_2-CH), 4.15-4.07 (m, 3H, O-CH_2-CH, C-CH(OH)-CH_2), 3.96-3.95 (m, 1H, TBS-O-CHH), 3.70 (m, 1H, TBS-O-CHH), 3.60-3.32 (m, 2H, Ph-CH_2-CH), 2.69 (m, 1H, CH_3-CH_2-CH), 2.0-1.7 (m, 2H, CH_3-CH_2-CH), 0.98 (t, *J*=7.4 Hz, 3H, CH_3-CH_2-CH), 0.90 (s, 9H, O-TBS), 0.04 (s, 6H, O-TBS). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 174.9, 153.1, 135.3, 129.4, 129.0, 127.4, 71.8, 65.9, 64.9, 55.5, 46.0, 38.1, 25.8, 21.2, 11.2. HRMS calcd. for $C_{22}H_{35}NO_5Si$ 421.2248, found 421.2251.

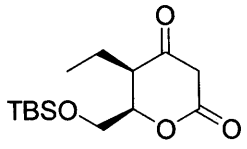
(2*S*,3*S*)-3-((*R*)-4-Benzyl-2-oxooxazolidine-3-carbonyl)-1-(*tert*-butyldimethylsilyloxy)pentan-2-yl acetate (133)



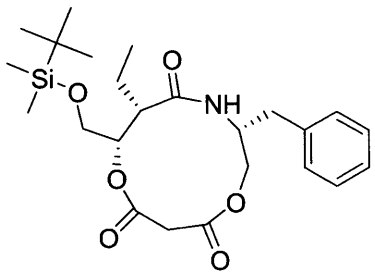
To a stirred solution of 4-benzyl-3-[4-(*tert*-butyldimethyl-silyloxy)-2-ethyl-3-hydroxy-butyryl]-oxazolidin-2-one (1 g, 2.4 mmol) in dry dichloromethane (10 mL), triethylamine (0.61 mL, 3.84 mmol), dimethylaminopyridine (0.098 g, 0.8 mmol), and acetic anhydride (0.34 mL, 3.6 mmol) was added. The mixture was stirred and monitored by thin layer chromatography to determine reaction progress. Upon completion, the reaction was quenched with the addition of saturated aqueous sodium hydrogen carbonate solution. Extraction of the reaction mixture with dichloromethane, followed by combination of product containing fractions, drying ($MgSO_4$), and concentration under reduced pressure, gave the crude product as a white solid. Recrystallisation from hexane/ethyl acetate gave the desired product as a colourless oil, 0.97 g, 83% yield. ^1H NMR (300MHz, $CDCl_3$), δ_H 7.34-7.19 (m, 5H, Ar-H), 4.62-4.61 (m, 1H, Ph-CH_2-CH), 4.23-4.10 (m, 3H, O-CH_2-CH, CH-CH(OH)-CH_2), 4.00-3.97 (m, 1H, TBS-O-CHH), 3.62-3.58 (m, 2H, Ph-CH_2-CH), 3.29 (m, 1H, TBS-O-CHH), 2.74 (m, 1H, CH_3-CH_2-CH), 2.00 (s, 3H, CH_3-C(O)-O), 1.88-1.81 (m, 2H, CH_3-CH_2-CH), 0.94 (t, *J*=7.2 Hz, 3H, CH_3-CH_2-CH), 0.86 (s, 9H, O-TBS), 0.01 (s, 6H, O-TBS). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 173.3, 170.5, 153.2, 135.4, 129.4, 128.9, 127.3, 74.0, 66.0, 63.2, 55.7, 44.8, 38.1, 25.2, 21.0, 20.6, 18.2, 14.2, 11.3. HRMS calcd. for $C_{24}H_{37}NO_6Si$ 463.2390, found 463.2396.

(5*R*,6*R*)-6-((*tert*-butyldimethylsilyloxy)methyl)-5-ethyl-dihydro-3*H*-pyran-2,4-dione[120] (134)

Method 1:



To a stirring solution of acetic acid 2-(4-benzyl-2-oxo-oxazolidine-3-carbonyl)-1-(*tert*-butyldimethylsilyloxymethyl)-butyl ester (0.1 g, 0.22 mmol) in dry tetrahydrofuran (15 mL), at -78 °C, LiHMDS (1 M in tetrahydrofuran, 0.66 mmol) was added dropwise. The mixture was stirred for 2 h. Analysis of the reaction mixture by TLC indicated that the reaction had progressed, and was quenched with the addition of saturated ammonium chloride:water:MeOH 1:1:1 (50 mL) at -78 °C. Ethyl acetate (50 mL) and water (15 mL) was added and the organic material extracted. The aqueous washings were taken, and acidified to pH 2-3 using 0.5 M HCl. The acidic aqueous material was then extracted with dichloromethane (3 x 20 mL). Drying ($MgSO_4$), and concentration under reduced pressure yielded the crude material. Column chromatography afforded a lactone as a white solid, Mp 216 - 217 °C, 34 mg. NMR analysis indicated that the required product had not been formed. Further analysis indicated that the product was an 11 membered cyclic structure, generated from an intramolecular cyclisation and the loss of the silyl protecting group. $C_{24}H_{37}NO_6Si$, 34mg, 0.07 mmol, 31% yield. Elemental; calculated (loss of TBS group) [C: 61.88, H: 6.64, N: 4.01], found [C: 61.49, H: 6.66, N: 3.92]. X-ray data can be found in appendix A. HRMS calcd. for $C_{18}H_{23}NO_6$ (loss of TBS) 349.1525, found 349.1546.



Method 2:

To a stirring solution of acetic acid 2-(4-benzyl-2-oxo-oxazolidine-3-carbonyl)-1-(*tert*-butyl-dimethylsilyloxymethyl)-butyl ester (0.1 g, 0.22 mmol) in dry tetrahydrofuran (15 mL), at -78 °C, NaHMDS (1 M in tetrahydrofuran, 0.66 mmol) was added dropwise. The reaction was stirred for 2 h. Analysis of the reaction mixture by TLC indicated that the reaction had progressed, and was quenched with the addition of saturated ammonium chloride:water:MeOH 1:1:1 (50 mL) at -78 °C. Ethyl acetate (50 mL) and water (15 mL) was added and the organic material extracted. The aqueous washings were taken, and acidified to pH 2-3 using 0.5 M HCl. The acidic aqueous material was then extracted with dichloromethane (3 x 20 mL). Drying ($MgSO_4$), and concentration under reduced

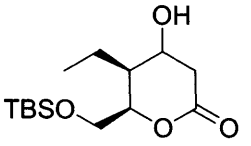
pressure yielded the crude material. NMR analysis indicated that the required product had not been formed.

Method 3:

To a stirring solution of acetic acid 2-(4-benzyl-2-oxo-oxazolidine-3-carbonyl)-1-(*tert*-butyldimethylsilanyloxymethyl)-butyl ester (0.1 g, 0.22 mmol) in dry tetrahydrofuran (5 mL), at -78 °C, KHMDS (0.5 M in toluene, 0.66 mmol) was added dropwise. The mixture was stirred for 2 h. Analysis of the reaction mixture by TLC indicated that the reaction had progressed, and was quenched with the addition of saturated ammonium chloride:water:MeOH 1:1:1 (20 mL) at -78 °C. Ethyl acetate (20 mL) and water (5 mL) was added and the organic material extracted. The aqueous washings were taken, and acidified to pH 2-3 using 0.5 M HCl. The acidic aqueous material was then extracted with dichloromethane (3 x 5 mL). Drying ($MgSO_4$), and concentration under reduced pressure yielded the crude material. Purification by column chromatography gave **134** (45 mg, 72%). ^{1}H NMR (300MHz, $CDCl_3$), δ_H 4.66-4.63 (m, 1H, CH_2-CH(O)(CH)), 3.93 (dd, *J*=11.4, 2.2 Hz, 1H, TBS-O-CHH), 3.74 (dd, *J*=11.4, 1.53 Hz, 1H, TBS-O-CHH), 3.48 (d, *J*=20.7 Hz, 1H, C(O)-CHH-C(O)), 3.29 (dd, *J*=20.7, 1.1 Hz, 1H, C(O)-CHH-C(O)), 2.57 (m, 1H, CH_3-CH_2-CH), 2.04 (m, 1H, CH_3-CHH-CH), 1.28 (m, 1H, CH_3-CHH-CH), 1.04 (t, *J*=7.5 Hz, 3H, CH_3-CH_2), 0.84 (s, 9H, O-TBS), 0.02 (s, 6H, O-TBS). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 200.0, 168.5, 79.1, 62.2, 49.8, 45.6, 25.7, 18.4, 17.9, 12.1. HRMS calcd. for $C_{14}H_{26}SiO_4$ 286.1600, found 286.1613.

(5*S*,6*R*)-6-((*tert*-butyldimethylsilyloxy)methyl)-5-ethyl-4-hydroxy-tetrahydropyran-2-one[120] (135)

Method 1:



To a stirring solution of (5*R*,6*R*)-6-((*tert*-butyldimethylsilyloxy)methyl)-5-ethyl-dihydro-3*H*-pyran-2,4-dione (45 mg, 0.16 mmol) and dry MeOH (1 mL) at 0 °C, $NaBH_4$ (1 mg, 0.26 mmol) was added. The mixture was stirred at 0 °C and monitored my TLC. No recoverable product was formed in the reaction.

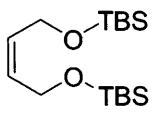
Method 2:

To a stirring solution of (5*R*,6*R*)-6-((*tert*-butyldimethylsilyloxy)methyl)-5-ethyl-dihydro-3*H*-pyran-2,4-dione (50 mg, 0.18 mmol) and dry ethanol (1.5 mL) at 0 °C, $NaBH_4$ (1 mg, 0.26 mmol) was added. The mixture was stirred at 0 °C and monitored my TLC. No recoverable product was formed in the reaction.

Method 3:

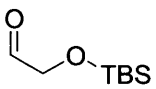
To a stirring solution of (5*R*,6*R*)-6-((*tert*-butyldimethylsilyloxy)methyl)-5-ethyl-dihydro-3*H*-pyran-2,4-dione (50 mg, 0.18 mmol) and dry ethanol (1.5 mL) at – 5 °C, a solution of *t*-$BuNH_2$-BH_3 (51 mg, 0.55 mmol) in MeOH (2 mL) was added, followed immediately by a 1 M solution of citric acid in water (1.5 mL).The reaction was stirred for 4 h at -5 °C. dichloromethane (15 mL) was and water (5 mL) were added. Organic layers were extracted, and the aqueous layer extracted with dichloromethane (3 x 10 mL). Drying ($MgSO_4$) and concentration under reduced pressure yielded crude material. Analysis indicated that no required material had been formed in the reaction.

1,4-Bis-(*tert*-butyldimethylsilyloxy)-but-2-ene[132b] (138)



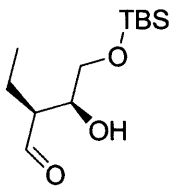
To but-2-ene-1,4-diol (2.5 g, 56.8 mmol) in 100 mL of dimethyl formamide at 0 °C, imidazole (114 mmol, 7.73 g) and *tert*-butyldimethylsilyl chloride (56.8 mmol, 8.55 g) was added. The mixture was stirred at ambient temperature for 12 h. The reaction mixture was then extracted using diethyl ether (3 x 50 mL) and water (200 mL). Combination, drying (Na_2SO_4), and concentration under reduced pressure gave the product as a colourless oil. The crude material was purified by column chromatography using an eluant of 10% ethyl acetate/petroleum ether (40-60 °C) giving the product as a colourless oil 14.4 g, 80%. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 5.56-5.50 (m, 2H, CH=CH-CH_2), 4.23-4.22 (m, 4H, CH-CH_2-O), 0.89 (s, 18H, O-TBS), 0.06 (s, 12H, O-TBS). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 130.2, 59.6, 25.9, 18.3.

(*tert*-Butyldimethylsilyloxy)-acetaldehyde[132c] (139)



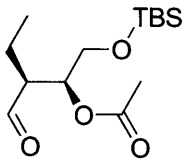
To a flask containing dichloromethane (60 mL) and 1,4-bis-(*tert*-butyldimethylsilyloxy)-but-2-ene (3 g, 9.47 mmol) at -78 °C, O_3 was added to the reaction mixture until the mixture appeared blue in colour. Excess O_3 was then purged from the reaction mixture, and triphenylphosphine (7.45 g, 28.4 mmol) was added. The mixture was then allowed to warm to room temperature slowly, and was stirred for 12 h. The reaction mixture was then concentrated under reduced pressure, and dichloromethane was added. The solution was passed through a short silica plug, and concentrated under reduced pressure. The crude material was then purified using column chromatography using an eluant of 5% ethyl acetate/petroleum ether (40-60 °C), product containing fractions were concentrated under reduced pressure to a colourless oil to give **139** (2.75 g, 83%). ^{1}H NMR (300MHz, $CDCl_3$), δ_H 9.74 (s, 1H, CHO-CH_2), 4.22 (s, 2H, CHO-CH_2-O), 0.93 (s, 9H, O-TBS), 0.11 (s, 6H, O-TBS). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 202.4, 69.6, 25.8, 11.4.

(2*S*,3*S*)-4-(*tert*-Butyldimethylsilyloxy)-2-ethyl-3-hydroxybutanal (141)



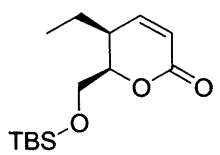
To a stirring solution of Red-Al (0.35 mmol) in tetrahydrofuran (5 mL) at -78 °C, 4-benzyl-3-[4-(*tert*-butyldimethylsilyloxy)-2-ethyl-3-hydroxy-butyryl]-oxazolidin-2-one (0.133 g, 0.32 mmol) in 3 mL of tetrahydrofuran was added dropwise and stirred for 15 min. The mixture was then warmed to -60 °C, and stirred for 1 h. The reaction was cooled to -78 °C and quenched using a 4:1 ethyl acetate:methanol solution (1 mL), and stirred for 15 min. Warming the mixture to -20 °C, 1:2 ethyl acetate:5% HCl mixture was added (6 mL), and stirred for 15 min. Organic material was decanted from the solid aqueous fractions, which were washed quickly with diethyl ether. Combination of the organic extracts, drying (Na_2SO_4), and concentration under reduced pressure gave the crude product as an oil. Purification by column chromatography using an eluant of 5% ethyl acetate/petroleum spirit (40-60 °C) gave the product as a colourless oil, 0.072 g, 89%. The material was used immediately in further stages.

(2*S*,3*S*)-1-(*tert*-Butyldimethylsilyloxy)-3-formylpentan-2-yl acetate (142)



To a stirring solution of 4-(*tert*-butyldimethylsilyloxy)-2-ethyl-3-hydroxy-butyraldehyde (0.32 mmol) in dichloromethane (5 mL) at 0 °C, acetic anhydride (27 mg, 0.44 mmol), DMAP (0.012 g, 0.1 mmol), and triethylamine (0.46 mmol) was added sequentially. The mixture was stirred until completion by TLC analysis. Water was then added (5 mL), and the reaction mixture extracted with dichloromethane (3 x 5 mL). Combined organic fractions were dried (Na_2SO_4), and concentrated under reduced pressure to an oil. The product was used rapidly as crude material in further stages.

(5*S*,6*R*)-6-((*tert*-butyldimethylsilyloxy)methyl)-5-ethyl-5,6-dihydro-2H-pyran-2-one[127] (143)



To tetrahydrofuran (10 mL) and diisopropylamine (267 mg, 2.64 mmol) at -78 °C was added *n*-butyllithium (2.5 M in hexanes, 2.4 mmol). The mixture was warmed to 0 °C, stirred for 30 min and re-cooled to -78 °C. Methyl acetate (196 mg, 2.64 mmol) was added and stirred for 30 min. Acetic acid 1-(*tert*-butyldimethylsilyloxymethyl)-2-formyl-butyl ester (649 mg, 2.4 mmol) was added and stirred for 1 h. The mixture was warmed to -20 °C, and the reaction left for a further 1 h .The reaction was quenched with the addition of saturated ammonium chloride solution. Product containing material was extracted with dichloromethane (3 x 5 mL), dried ($MgSO_4$), and concentrated under reduced pressure to an oil. Purification by column chromatography using an eluant of 4:1 hexane:ethyl acetate gave the desired product as a colourless oil, 0.145g, 23%. IR ν_{max} (KBr, cm^{-1}) 1728 (C=O). ^1H NMR (300MHz, $CDCl_3$), δ_H 7.02 (dd, *J*=6.0, 9.7 Hz, 1H, CH-CH=CH), 6.02 (d, *J*=9.7 Hz, 1H, C(O)-CH=CH), 4.52-4.46 (m, 1H, CH-CH(O)-CH_2), 3.89-3.77 (m, 2H, TBS-O-CH_2), 2.49-2.45 (m, 1H, CH-CH(CH_3)-CH), 1.71-1.27 (m, 2H, CH_3-CH_2-CH), 0.96 (t, *J*=7.4 Hz, 3H, CH_3-CH_2), 0.98 (s, 9H, O-TBS), 0.05 (s, 6H, O-TBS). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 164.0, 150.5, 120.8, 79.7, 61.4, 34.0, 25.8, 20.4, 18.2, 11.0. HRMS calc. for $C_{14}H_{26}SiO_3$ 270.1651, found 270.1660.

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Chapter 3

Studies Towards Histone Deacetylase Inhibition

3.1 Introduction

The origin, and appropriate forms of treatment for cancer, is an often discussed topic throughout the scientific literature. The range and breadth of the area is staggering, and generates a multitude of publications spanning from the discovery of natural products with favourable therapeutic effects, to the study of cellular processes underlying the disease.

Recently, there have been many reports indicating the importance of epigenetic processes within cancer development. These are processes which affect the transcription of DNA, silencing some genes and activating others, without altering the nucleotide sequence itself. Two main processes are thought to be regulating gene transcription epigenetically, histone acetylation, and DNA methylation.

This chapter will focus upon histone interactions, and in particular upon the role of histone deacetylase in the activation and silencing of genes through histone acetylation and deacetylation.

3.1.1 Chromatin

Deoxyribonucleic acid, or DNA, is a long polymer consisting of nucleic acids. The length of DNA varies from organism to organism, but can be short as tens of kilobases, to hundreds of megabases. Within the sequence of nucleotides, DNA contains genetic instructions known as genes, relating to all aspects of development and function in living organisms. Transcription of these genes into ribonucleic acid, or RNA, allows the cell to orchestrate various cellular processes such as mitosis and cellular death.

In eukaryotic cells, DNA is found in large linear portions, and is collectively known as the chromosome. The helical structure of DNA, originating from the discoveries of

Watson and Crick,[133] is widely recognised. The helical DNA is held mostly within the nucleus, and is tightly packaged in higher order structures known as collectively as chromatin.

The term chromatin originates from the ease at which the material can be stained, and means quite literally, coloured material. Chromatin is varied in its packing configuration within the nucleus, at the very highest level of compression is metaphase chromatin, it is physically robust and is responsible for the 'X' shaped appearance of the chromosome. At the very lowest, the DNA is held in a form known as the nucleosome, and is the fundamental repeating unit of chromatin.

The nucleosome is built around a core of 8 histone proteins, H2A, H2B, H3 and H4, 2 of each being present in the histone octamer. DNA is wrapped around the histone core in two superhelical turns, and contains 145 base pairs in total. The histones contain a terminal amino acid tail, rich in lysine residues, which is free of the central histone octamer. An illustration of the nucleosome can be seen in figures 3.1.1, and 3.1.2, showing the construction of the nucleosome, the DNA, and the lysine rich tail portions free of the histone core.

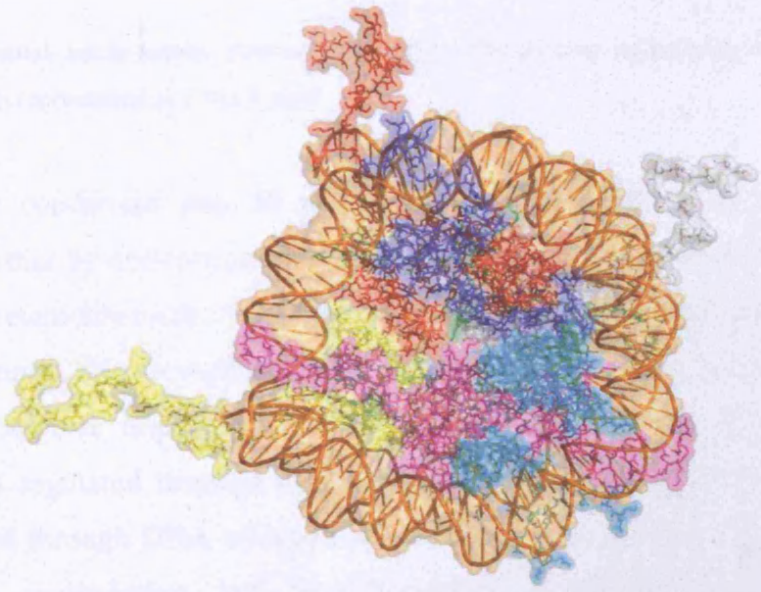


Fig. 3.1.1. A representation of a nucleosome showing a helical DNA sequence (orange) wrapping round a histone octamer core. Derived from the PDB database data 1KX5.

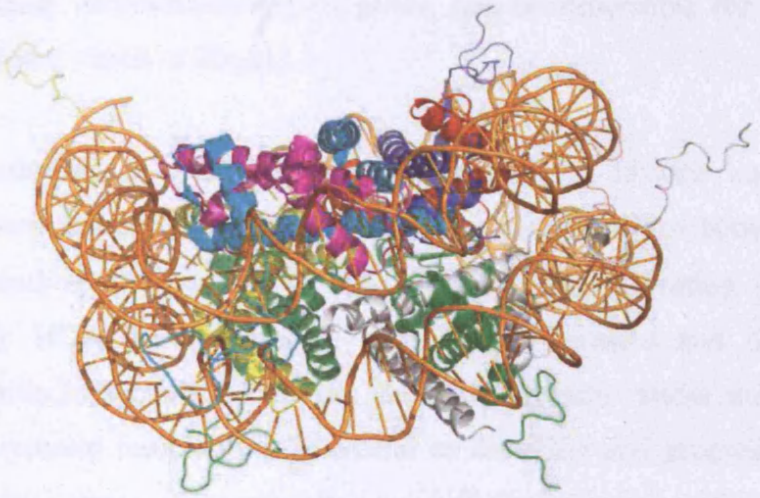


Fig. 3.1.2. A side-on representation of a nucleosome showing a helical DNA sequence (orange) wrapping round a histone octamer core. Derived from the PDB database data 1KX5.

Repeating units of nucleosomes on the DNA strand gives the so called "beads-on-a-string" appearance (figure 3.1.3).

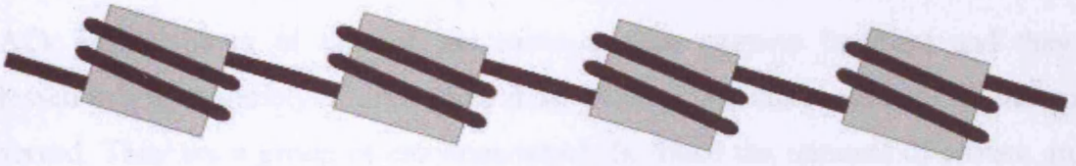


Fig. 3.1.3. Sequential nucleosomes forming chromatin. The histone octamer is represented by grey rectangles, DNA is represented as a black cord.

The beads are condensed into 30 nm fibres with the addition of histone H1, and compressed further by conversion into higher order chromatin such as euchromatin, and higher still to heterochromatin. As the DNA is compressed into the more densely packed forms of chromatin, the accessibility of the DNA to proteins with which it can interact lessens, and therefore impacts on transcriptional activity.[134] The level of chromatin compression is regulated through the modification of the lysine rich filaments which pass around and through DNA wrapped around the histone octamer. Modifications such as acetylation, methylation, and phosphorylation mediate the level of chromatin compression, and is a prominent method within the cell by which it can regulate gene expression. This epigenetic process, one which influences the cell without altering the DNA, is heritable from one cell through to its decedents, is considered to provide a mechanism for the cell to respond to environmental changes through cell progression and differentiation. Recent evidence has shown that the perturbation of chromatin

epigenetic processes causes silencing of genes, and is responsible for the underlying pathogenesis of many types of disease.

Chromatin remodelling is regulated by the activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs). There is a constant flux between acetylation by HATs, expanding the chromatin and allowing the activation of genes, and deacetylation by HDACs, compressing chromatin to silence and deactivate gene sequences. Recently, associations between aberrant epigenetic states and tumours have revealed that chromatin remodelling is crucial to the onset and progression of cancer, particularly in initial stages of tumourigenesis.[135,136] The prevention of gene silencing by modulating epigenetic processes has therefore prompted considerable interest as a means to obtain therapeutic anti-cancer effects.

3.1.2 Histone Deacetylase

HDACs are members of one of the more ancient enzyme families, and they are expressed in a wide variety of organisms from bacteria to humans, and are evolutionarily conserved. They are a group of enzymes which facilitate the removal of acetate groups from histone lysine rich tails, as well as the cytoskeletal protein tubulin[137] and transcription factors such as p53.[138] HDACs have been considered to act mostly upon histones, however it is becoming apparent that acetylation and deacetylation may play a larger role, and may be as prominent as phosphorylation and dephosphorylation.[139,140]

Four classes of HDAC have been identified in mammalian cells, and are classified according to their structural similarity to yeast HDAC enzymes, localization, and activities.

Class I is comprised of HDAC 1, HDAC 2, HDAC 3, and HDAC 8. They are homologous to yeast RPD3 protein, are Zn^{+2} ion dependent, and are found generally in the cell nucleus, and expressed in most human cell lines. Class II enzymes are also Zn^{+2} dependent, share homology with yeast Hda1 protein, and can move freely between the nucleus and cytoplasm. Enzymes contained within class II are HDAC 4, HDAC 5, HDAC 6, HDAC 7, HDAC 9, and HDAC 10. Class III HDAC enzymes are homologues of the protein Sir2, and are NAD dependent. Class IV is occupied by HDAC 11, and

resembles class I and class II HDACs in sharing sequence similarity within the catalytic core region, however, it is placed within a class of its own as it does not share any sufficient identity with the other HDAC classes.

3.1.3 Histone Deacetylase Active Site

Until very recently, the only source of structural data on HDAC enzymes was the X-ray structure of Histone Deacetylase Like Protein (HDLP, PDB database ID: 1C3P), a HDAC analogue from *Aquifex Aeolius*. The function of HDLP is unknown, but the enzyme can deacetylate histones *in vitro* with a specific activity of about 7.5% of FLAG tagged HDAC 1. HDLP has been used successfully in inhibition assays and computational studies, and has proved to be useful in determining the nature of the catalytic process underlying class I and class II HDAC deacetylation.

The ability of class I and II HDACs to deacetylate substrates stems from a central catalytic area, built around a Zn^{+2} ion contained within an angled cavity. The cavity consists of a surface pocket, containing mostly hydrophobic residues, and descending 11 Å into the body of the enzyme. The bottom of the pocket contains the central Zn^{+2} ion, and a series of chelating residues important for the catalytic deacetylation process to occur. Deeper into the enzyme, the catalytic site extends a further 14 Å and forms an internal cavity. A representation of the catalytic are can be seen in figure 3.1.4.

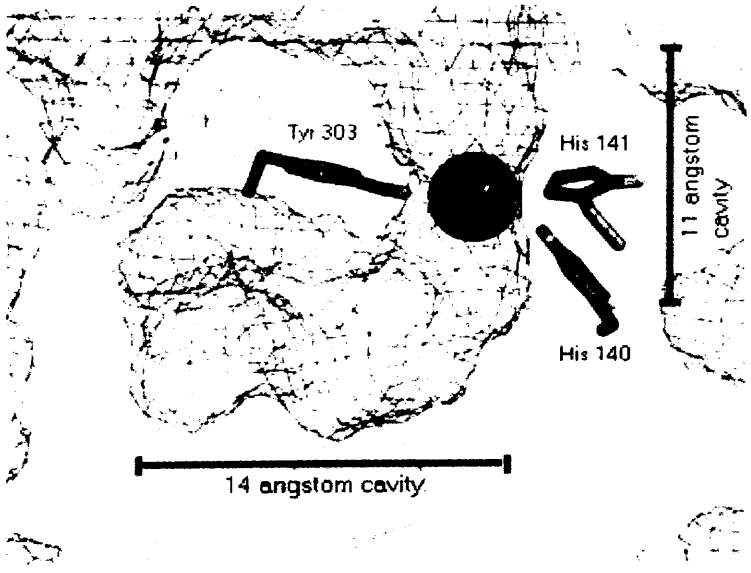


Fig. 3.1.4 A representation of the HDAC 1 11 Å catalytic channel, and 14 Å internal cavity.

Surrounding the Zn^{+2} ion are three residues, Tyr 303 (HDLP: Tyr 297), His 141 (HDLP: His 132), and His 140 (HDLP: His 131), which constitute the main structural features of the catalytic deacetylation area. Mechanistic studies of the catalytic site, conducted through mutagenesis studies of mammalian HDACs and HDLP, indicated that His 140, and its associated His-Asp charge relay system was essential for catalytic function. Modification of His 140 or the charge relay associated Asp 174 abolishes activity. Alteration of His 141 and its associated Asp 191 charge relay system reduces activity, but does not remove it completely. This indicated that His 141 is beneficial, but not essential to the catalytic functioning of the enzyme. Furthermore, it was found Tyr 303 is essential to catalytic activity, and mutagenesis to phenylalanine indicated that removal would eliminate activity completely.[141]

Ab initio studies of the HDLP catalytic region[142] have shown that the area is sufficiently basic around His 140 to deprotonate water. The presence of this 'hydroxide' ion in the catalytic area is believed to be essential for deacetylation to occur, and is associated with Tyr 303.[143] An illustration of the deacetylation mechanism in class I HDACs can be seen in figure 3.1.5.

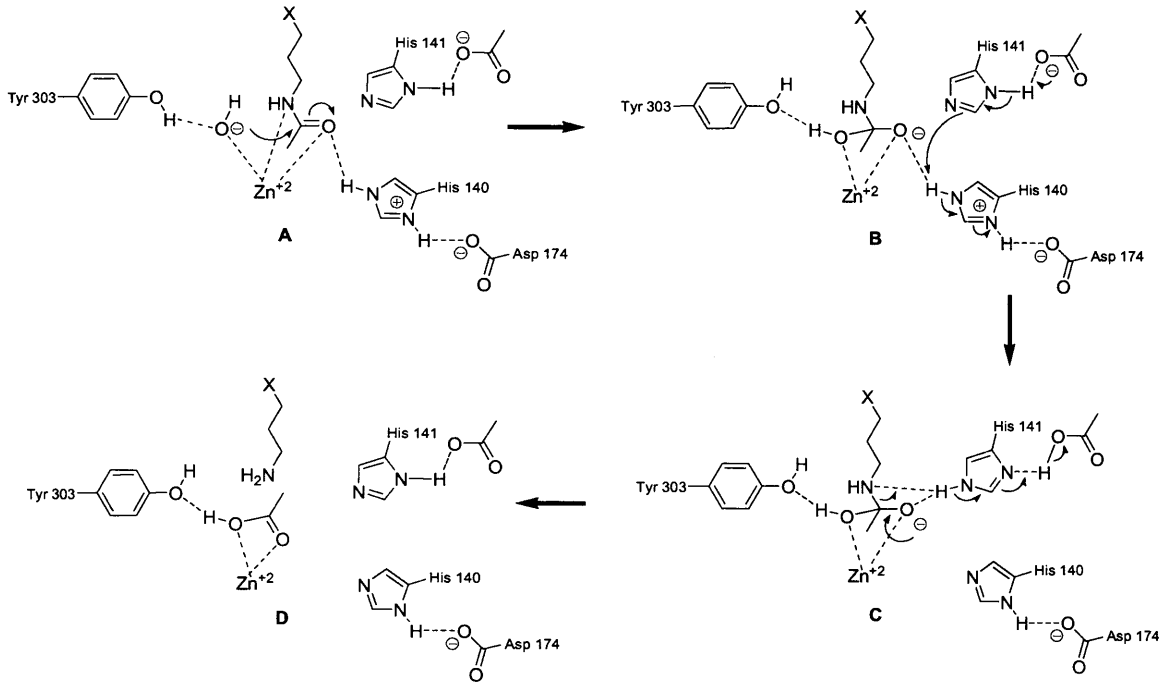


Fig. 3.1.5. Illustration of the HDAC catalytic deacetylation mechanism. X represents the lysine rich histone protein.[143]

The deacetylation process begins with the chelation of the acetylated residue to the Zn^{+2} ion. The hydroxide ion, near to Tyr 303, attacks the carbonyl group of acetate in a manner analogous to that observed in carbonic anhydrase. Proton transfer from His 140 to His 141 occurs, and the tetrahedral transition state formed in **B** interacts with the newly transferred proton of His 141, illustrated in **C**. Finally, the tetrahedral transition state collapses, resulting in cleavage of the amide bond, and liberation of acetate and the free amine (**D**). The acetate is then passed into the 14 Å cavity,[144] and a new water molecule binds to the site to restart the catalytic cycle.

3.1.4 Histone Deacetylase Inhibitors

HDAC inhibitors have the ability to selectively induce apoptosis in tumour cells at low toxicity levels, typically at levels 10 times less than the toxic dose for normal cells. Activity has been observed for a wide variety of cancer cell lines, including leukaemia, lymphoma, breast, bladder, ovarian, prostate and lung. Activity of HDAC inhibitors has

been postulated to be due to the interaction of the inhibitor with the Zn^{+2} catalytic site, inhibiting deacetylation, and thus affecting chromatin remodelling and transcription. It has been shown, however, that HDAC inhibition can also affect other molecular processes including mitosis, DNA replication, and DNA repair.[145] It is becoming apparent that HDAC inhibitors may have a larger effect on cellular processes than was originally believed to be the case. Furthermore, the variation in HDAC activities, and the many HDAC variations in different tumour types, makes determination of the true inhibitory mechanism a challenging prospect.

One of the first HDAC inhibitors to be reported is trichostatin A (TSA, **144**), isolated from *Streptomyces platensis*,[146,147] and originally developed as an antifungal agent. It was found that at extremely low concentrations (nM), TSA could induce Friend leukaemia cell differentiation, and additionally inhibition of the cell cycle in G1 and G2 phases. *In vivo* it was found to increase the accumulation of acetylated histones, and inhibit strongly the activity of partially purified histone deacetylase *in vitro*. It has not progressed into clinical trials, but has since been used as a useful tool in studying histone acetylation and mechanisms of cell proliferation and differentiation.

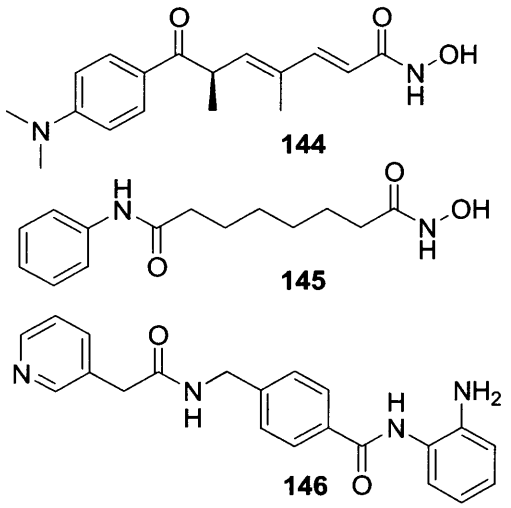


Fig. 3.1.6. HDAC inhibitors. **144**. Trichostatin A (TSA); **145**. Suberoylanilide hydroxamic acid (SAHA, Vorinostat); **146**. MS-275.

TSA is a member of a class of HDAC inhibitors known as hydroxamic acids, which strongly chelate to Zn^{+2} within the HDAC catalytic site. The hydroxamic acids are the largest class of HDAC inhibitors currently known, but other types exist, such as the benzamides (MS-275, **146**), short-chain fatty acids (butyrate, valproic acid), and cyclic

tetrapeptides (apicidin, depsipeptide). All HDAC inhibitors can be generally described to contain a chelating group, which binds to the Zn^{+2} ion in the core of the HDAC enzyme, a cap-group which interacts at the top of the catalytic pocket, and a lipophilic linker joining the two together and spanning the distance into the enzyme between the surface and the Zn^{+2} ion containing catalytic core.

TSA was first synthesised by Mori and Koseki,[148] and is considered to be an acetyl-lysine mimic. Modification of TSA, alteration of stereochemistry, or addition of alternate groups and functionality, generally generate compounds that are less active then the original natural product; however, it does provide the basic structural template for the whole of the hydroxamic class of inhibitors. One such inhibitor that can be seen to be adhering to the TSA pharmacophore is suberoylanilide hydroxamic acid (SAHA, **145**). SAHA is currently one of the most important reverse amide hydroxamic acid HDAC inhibitors undergoing testing. SAHA has been shown to bind into the catalytic ion-containing region in the same manner as that of TSA,[141] and has shown promising activity in T-cell lymphoma, myeloma, and breast cancer cells. SAHA is currently one of the most advanced HDAC inhibitors, and has entered phase III clinical trials for the treatment of both solid and haematological tumours.

Clinical trials have shown that most HDAC inhibitors are well tolerated by normal cells and have good clinical activity. The inhibitors are a subject of great interest, and their potential to provide therapeutic effects not only in the treatment of cancer, but for the use of antimalarial, antiviral, and antiprotozoan chemotherapy, has been the focus of many research groups in the effort to generate an ever more potent compound. There has been a multitude of publications detailing analogue synthesis, *in vivo* and *in vitro* studies, and more recently studies of HDAC inhibitors *in silico*.[141,149-151]

In silico analysis represents one of the more modern methods of scientific analysis. The use of computers to correctly model and emulate a large biological system accurately, such as an enzyme, is only possible with the more powerful processing power of modern computers. Computational analysis has developed from a highly specialist, extremely expensive undertaking, to an efficient cost effective method of structural analysis and lead compound optimisation.

3.2 Results and Discussion

The success of molecular modelling depends greatly upon the preparation of the model used to describe the ligands, and the enzyme or macromolecule. The model itself can vary in a many ways, from the physical location of atoms within the model, to how the physical properties of atoms are described. The choice of enzyme model and method of calculation are of great importance in ensuring that information recovered from the analysis is viable and accurately predicts experimentally determined data.

Of the many examples of computational software available, Autodock 3[152] was chosen as the method to compute HDAC-ligand interactions, based upon its speed, accessibility, and demonstrably robust and superior docking of ligands into metalloproteins.[153]

The overall aim of the project is to create a working computational model to aid in the analysis and synthesis of novel HDAC inhibitors.

3.2.1 Preparation of the HDAC Enzyme

The first consideration was to determine the sequence and structure of the HDAC enzyme to be used in the analysis. In previously reported analyses, the use of X-ray diffraction data are used extensively to aid development of the model. The use of experimental crystallographic data in computational studies can be highly desirable, as models can be validated by comparing computer calculated predictions with known interactions from experimentally derived data. In the case of mammalian HDAC enzymes, only the structure of HDAC 8 (PDB database id: 1W22) has been experimentally confirmed and reported.[154,155]

A HDAC 1 homology model, originally constructed and reported by Wiest[149] was chosen as the basis of the enzyme model. The model was constructed by taking X-ray crystal structure data of HDLP (PDB database ID: 1C3P), a HDAC homologue from *Aquifex Aeolicus*. A sequence alignment of HDAC and HDLP shows a 35.2% sequence identity, in addition to conservation of the residues around the important Zn^{+2} binding site and within the 11Å channel. Substantial similarity between the two proteins allowed

Wiest to use the positional data of the HDLP protein to construct a HDAC 1 homology model, using the catalytic site and conserved features common to both as a template.[150]

```
HDAC 1  MAQTQGTRRKVCYYYDGDVGNYYYGQGHPMKPHRIRMTHNLLLNYGLYRK
HDLP    -------MKKVKLIGTLDYGKYRYPKNHPLKIPRVSLLLRFKDAMNLIDE
                :**      * *:* * :.**:*  *: :  .:    .*  :

HDAC 1  MEIYRPHKANAEEMTKYHSDDYIKFLRSIRPDNMSEYSKQMQRFNVGEDC
HDLP    KELIKSRPATKEELLLFHTEDYINTLMEAERCQCVPKGAREKYNIGGYEN
         *: :.: *. **:  :*::***: * . .  :    . : :    * :

HDAC 1  PVFDGLFEFCQLSTGGSVASAVKLNKQQTDIAVNWAGGLHHAKKSEASGF
HDLP    PVSYAMFTGSSLATGSTVQAIEEFLKG--NVAFNPAGGMHHAFKSRANGF
        **  .:*  ..*:**.:* :  :: *   ::*.* ***:*** **.*.**

HDAC 1  CYVNDIVLAILELLKY-HQRVLYIDIDIHHGDGVEEAFYTTDRVMTVSFH
HDLP    CYINNPAVGIEYLRKKGFKRILYIDLDAHHCDGVQEAFYDTDQVFVLSLH
        **:*: .:.*  * *  .:*:****:* ** ***:**** **:*:.:*:*

HDAC 1  KYGEYFP--GTGDLRDIGAGKGKYYAVNYPLRDGIDDESYEAIFKPVMSK
HDLP    QSPEYAFPFEKGFLEEIGEGKGKGYNLNIPLPKGLNDNEFLFALEKSLEI
        :  **     .* *.:** **** * :* ** .*::*:.:   ::  :.

HDAC 1  VMEMFQPSAVVLQCGSDSLSGDRLGCFNLTIKGHAKCVEFVKSFNLPMLM
HDLP    VKEVFEPEVYLLQLGTDPLLEDYLSKFNLSNVAFLKAFNIVREVFGEGVY
        * *:*:*.. :** *:*.*  * *. ***:  .. *..::*:..    :

HDAC 1  LGGGGYTIRNVARCWTYETAVALDTEIPNELPYNDYFEYFGPDFKLHISP
HDLP    LGGGGYHPYALARAWTLIWCELSGREVPEKLNN----------------
        ******    :**.**   .   . *:*::*

HDAC 1  SNMTNQNTNEYLEKIKQRLFENLRMLPHAPGVQMQAIPEDAIPEESGDED
HDLP    ------KAKELLKSIDFEEFD----------------------------
              :::* *:.*. . *:

HDAC 1  EDDPDKRISICSSDKRIACEEEFSDSEEEGEGGRKNSSNFKKAKRVKTED
HDLP    -DEVDR-----------------------------------------SYML
         *: *:                                          .

HDAC 1  EKEKDPEEKKEVTEEEKTKEEKPEAKGVKEEVKLA
HDLP    ETLKDPWRGGEVRKEVKDTLEKAKASS--------
        *. *** .  ** :* * . **.:*..
```

Scheme 3.2.1. Sequence alignment of HDAC 1 and HDLP produced by ClustalX.

'*' -indicates positions which have a single, fully conserved residue

':' -indicates that one of the following 'strong' groups is fully conserved

'.' -indicates that one of the following 'weaker' groups is fully conserved

Fig. 3.2.2. Image of the homology model of HDAC 1 (left) and HDLP (right) proteins. Generated from PBD database entry 13CP and a HDAC 1 homology model reported by Weist.[149]

In order to generate a functioning computational model, the structural data for the HDAC 1 homology model provided by Weist, was extracted and imported into Autodock Tools.[156] This eliminated the need to generate new homology models and enabled an already validated HDAC 1 homology model to be used as a basis of our own studies.

All bonds in the model were reconstructed by distance, and all hydrogen atoms were added. The model was checked for missing atoms, and repaired where appropriate. The construction of the catalytic Zn^{+2} domain was examined visually to ensure the correct conformation. All non-polar hydrogens were removed, and Kollman charges[157] were added to the model, followed by solvation parameters.

With the basis of the model prepared, attention was focused upon the catalytic core and the protonation states of the residues localised within the area. Through previous *ab initio* studies of HDLP, it was shown that His 131, analogous to His 140 in HDAC 1, deprotonated water when minimized within the active site.[142] This is in accordance with the proposed catalytic mechanism reported by Finnin *et al.*[141] and indicates that the presence of any ligand in the catalytic area would also have the potential to be deprotonated.[151]

The histidine residues in the model were protonated, and edited to reflect the configuration adopted when binding to a ligand. His 140 was defined as being protonated at both nitrogens (HE2 and HD1), and was intended to simulate the action of

deprotonation in the catalytic area. His 178 was protonated only on HE2, and all other histidine residues were protonated on HD1.

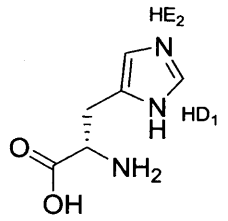


Fig. 3.2.3. Histidine image showing the HD1 and HE2 nitrogens.

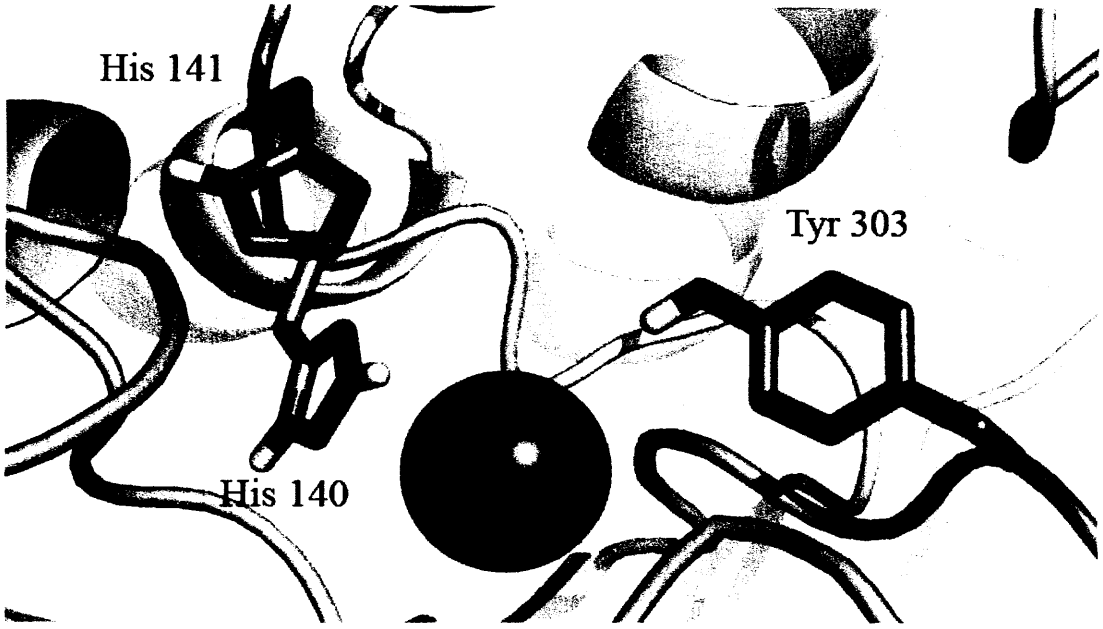


Fig. 3.2.4. Image of the catalytic site in the HDAC 1 model, showing the protonation of the histidines 140 and 141.

In accordance with the required Autodock 3 preparation procedure, it was essential to have the Zn^{+2} ion in the centre of the catalytic core accurately defined. Zn^{+2} ions in computational studies have typically been problematic, coordination changes, problems accounting for charge, and radius are all variables that have to be accurately described in order to generate a working model. This is not restricted to zinc ions specifically, but to all metal ions in general.

The zinc parameters were derived from work reported by Stote and Karplus,[158] and have been used successfully in other molecular dynamics studies concerning matrix metalloproteinases.[159,160] Wiest *et al.*[144,149] reports the successful use of the non-bonded

Zinc parameters in the generation of the HDAC 1 homology model, and successfully in HDLP docking studies where they have shown excellent agreement with calculated and experimental binding affinities.

The values used within the HDAC 1 homology model to define the zinc ion are; σ_{ij} = 1.95 Å, ε_{ij} = 0.25 kcal/mol, with a charge of +2 e.

With the HDAC 1 enzyme defined, it was possible to generate energy grid maps. These were generated by the Autodock package, within a 60 x 60 x 60 Å cube centered around the top of the 11 Å channel with a resolution of 0.375 Å. An illustration of the oxygen grid map can be seen in figure 3.2.5.

Fig. 3.2.5. An illustration showing the generated energy grid maps. This example shows the surface of the HDAC 1 enzyme in white, and the oxygen affinity areas cross-hatched.

3.2.2 Ligand Preparation

Ligands were initially prepared using ChemDraw,[161] and geometry minimized using modified version of Allinger's MM2 force field. Geometry minimised ligands were saved in PDB format. Ligands were then converted to SYBYL MOL2 files, with the addition of Gasteiger charges[162,163] using OpenBabel.[164]

All hydroxamic acids were prepared in the deprotonated hydroxamate form. Ligand torsions were defined in the Autodock Tools software package, finally giving the fully prepared ligands. Prepared ligands can be seen in figure 3.2.6.

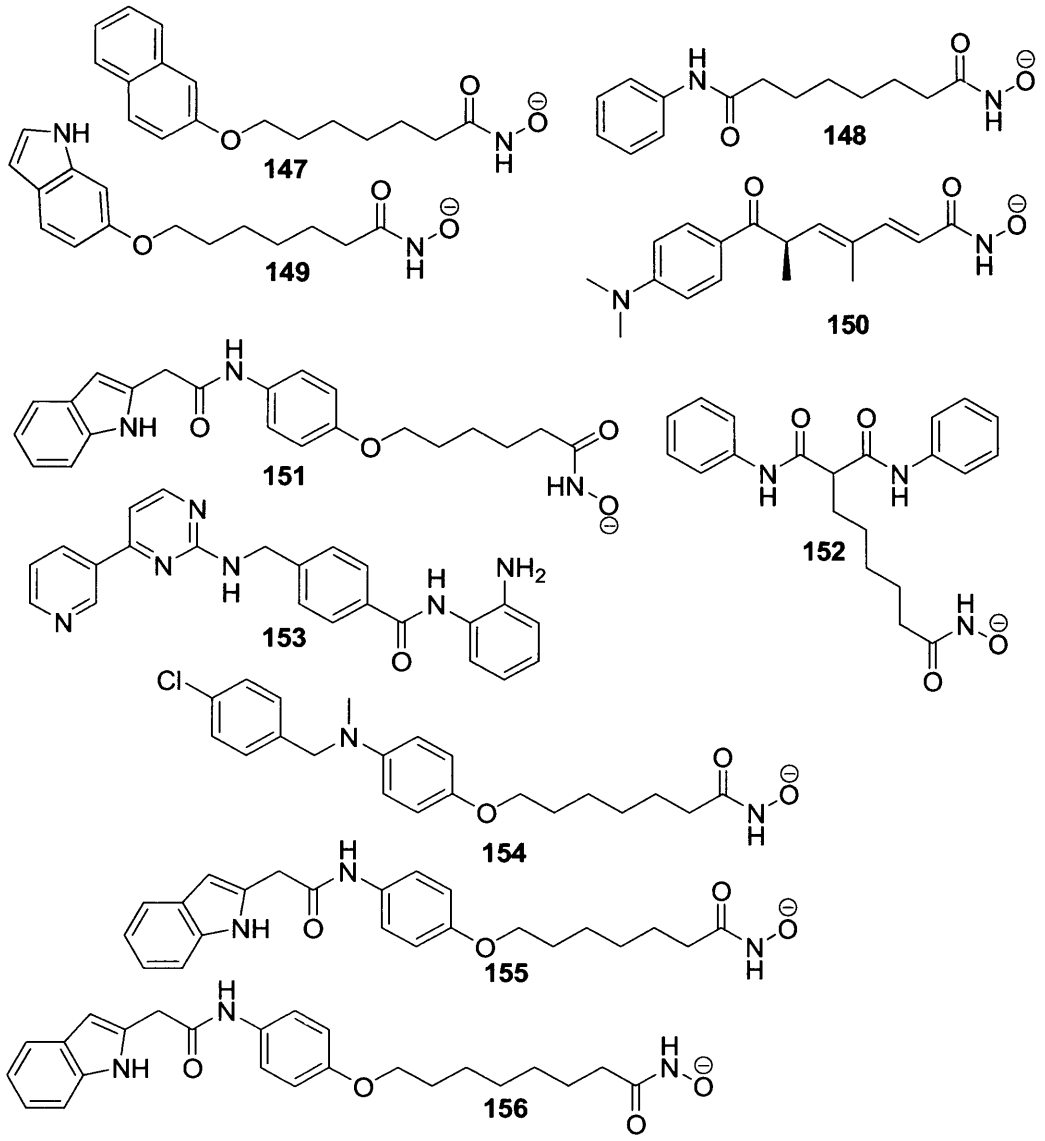


Fig. 3.2.6. Ligands prepared for input into the computational model. **147**. UCL159; **148**. SAHA; **149**. UCL171; **150**. TSA; **151**. UCL173; **152**. UCL2200; **153**. MGCD; **154**. UCL158; **155**. UCL169; **156**. UCL174.

The computations were run using a Lamarckian Genetic Algorithm,[152] with a maximum of 100 runs and a population of 50 per run. The maximum number of evaluations was defined as 2.5 x 10^6, and the maximum number of generations to be 2.7 x 10^5.

3.2.3 Analysis of the Computational Data

The integrity and ability of the system to generate useful data was first established. It was necessary to determine if the model could return data that was representative of the binding conformations of each ligand. Concerns were focused upon the binding of the hydroxamate to the zinc metal atom in the core of the catalytic site.

In order to determine if the non-bonded zinc parameters were resulting in correct ligand binding predictions, a model was prepared from an existing HDLP crystal structure. The HDLP crystal structure was obtained from the PDB databank (PDB database ID: 1C3P) and contained a co-crystallized molecule of TSA. The model was edited, and the bound TSA molecule was removed. The non-bonded Zinc parameters were added, and a new model was formed. A TSA ligand was prepared with Autodock Tools, and a docking against the new HDLP model was initiated.

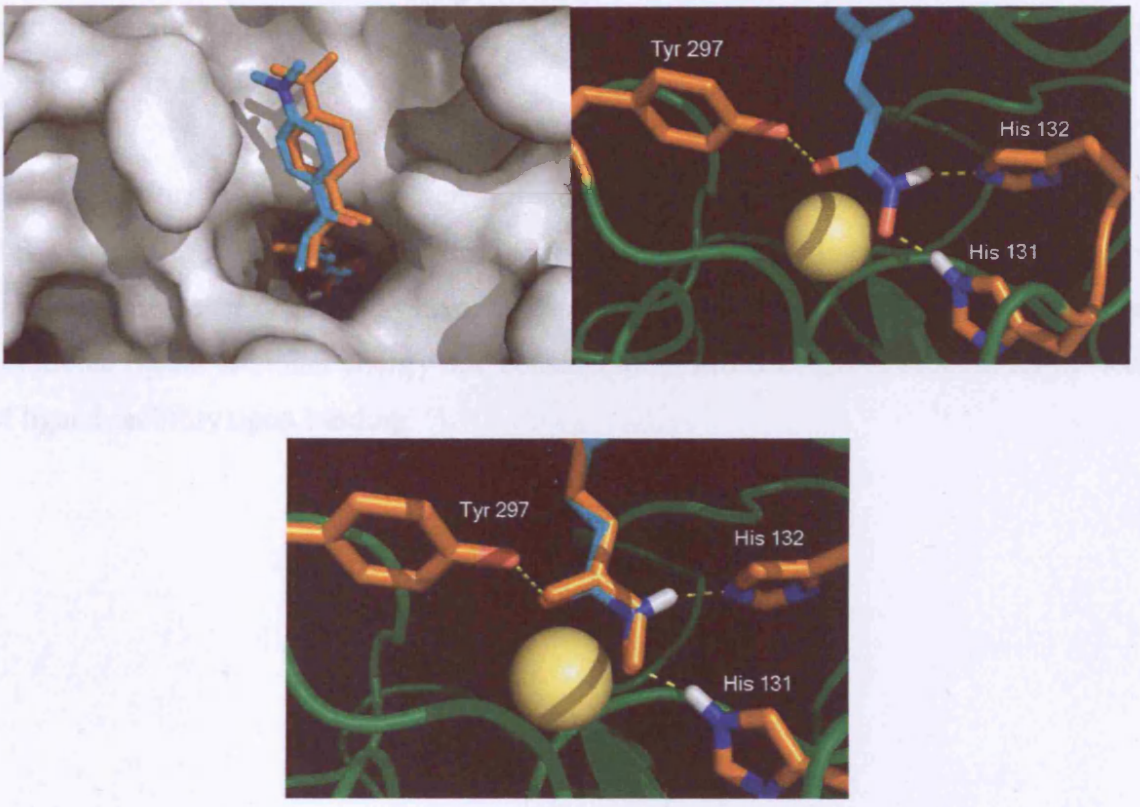


Fig. 3.2.7. Illustrations showing the HDLP model. TSA models shown in blue are derived from computational studies. TSA models shown in orange are derived from published experimental crystal structure data (PDB database entry 1C3R). Overlay of the computational and experimentally derived TSA structures indicates a good correlation between the model and the true binding mode of the ligand.

Results from the HDLP model indicated that the simulation was accurately defining the environment around the catalytic Zn^{+2} site, and would generate accurate data with respect to the hydroxamic acid and catalytic area. Figure 3.2.7 illustrates that the computed and experimentally determined positions of the hydroxamic acid moiety are almost identical, and replicates the important H-bonds from Tyr 297, His 132, and His 131 accurately.

Having demonstrated that the HDLP model around the catalytic site could be accurately defined and used in the docking model. The HDLP model parameters in the HDAC 1 model could be used to generate HDAC 1-ligand docking information that would be accurate around the catalytic site with a high degree of confidence. Ligands were processed using a Lamarckian Genetic Algorithm, sorted in the order of decreasing energy conformation, and finally clustered according to a Root Mean Squared Deviation (RMSD) of 1.25 Å. Inspection of an energy histogram then gave the most favorable

binding modes for each ligand, as low energy RMSD clusters. The lowest energy and correctly binding cluster was used in the study. The results from the computational study can be seen in table 3.2.8.

In vitro testing of compounds were measured against deacetylation of histone H4 by HeLa extract,[165-168] and was used to derive IC_{50} values. Docking energy represents the calculated free energy of the system; binding energy takes the energy of the system and additional ligand torsional energy into consideration, and attempts to account for the loss of ligand mobility upon binding.

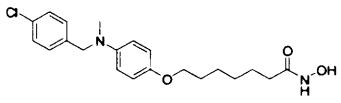
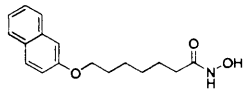
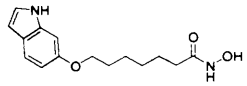
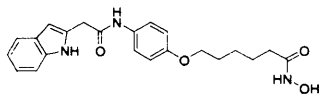
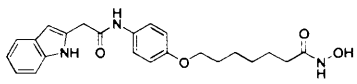
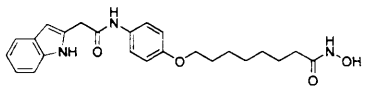
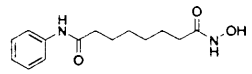
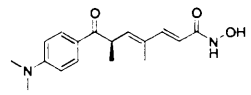
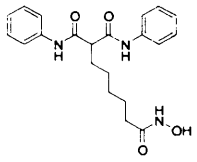
Structure	ID	HDAC IC_{50} (nM)	Binding Energy (kcal/mol)	Docking Energy (kcal/mol)
	UCL158	2.0 (± 1.0)	-9.76	-13.51
	UCL159	0.9 (± 0.3)	-10.05	-12.7
	UCL171	3.6 (± 1.8)	-9.71	-12.73
	UCL173	50 (± 21)	-10.61	-14.61
	UCL169	12.8 (± 5.2)	-10.36	-14.45
	UCL174	22.4 (± 8.9)	-10.46	-14.68
	SAHA	13	-9.86	-13.02
	TSA	5	-10.18	-12.1
	UCL2200	30	-13.21	-16.97

Table 3.2.8. Binding and docking energies of the prepared ligands. TSA and SAHA IC_{50} derived from PC-3 cell lines.[169]

One of the most heavily studied HDAC inhibitors is TSA. It is widely used as a comparator for the assessment of other HDAC inhibitors, and was chosen to serve the same purpose in this study. Calculations predicted that TSA would have a docking energy of -12.1 kcal/mol and a binding energy of -10.18 kcal/mol. This compares well with the data of Wiest *et al.*[149] with the reported calculated free energy of binding at -

10.1 kcal/mol, and an experimental free energy of binding at -10.3 kcal/mol.[169] The binding conformation of TSA also correlates with that of the established paradigm, and places the cap portion of TSA towards Glu 98 and Pro 29. The hydroxamic acid moiety is also found to be binding correctly with an orientation indicating hydrogen bonding to Tyr 303, His 140, and His 141, mimicking the docking conformation found in the previous HDLP model. Computation results from SAHA indicate that the ligand is a weaker binder to HDAC 1 than TSA with a binding energy of -9.86 kcal/mol, consistent with reported experimental data.[170] Thus far, the model can be said to be predicting effectively, as it can correctly predict the binding mode, and approximate binding affinities of well known HDAC inhibitors.

UCL173, UCL169, and UCL174 are analogues differing only in the length of their linker portions. It would be expected that these ligands would conform to the general conclusions derived from QSAR studies,[169,171] that is that there is an optimal length to the linker fragment, and deviation from that length will result in decreased efficacy. This can be rationalized by considering the two possible situations arising from suboptimal linker length. If the linker is too short, steric clashes within and around the catalytic site will make any Zn^{+2} binding conformation unfavorable. Should the linker be too long, then entropic considerations, in addition to steric clashes within the cap region and crowding within the catalytic channel, would be unfavorable to ligand-enzyme interaction.

The calculated binding energies for UCL173, UCL169 and UCL174 differ within a narrow range of only -0.25 kcal/mol. Experimentally, the activity of the ligands is quite distinct, with UCL169 being the best binding molecule of the three. The experimental values correlate with previous studies concerning linker length, and that is that the optimal length for the unsaturated linker is around 6 atoms in length. Disappointing as it is for the model to have failed to predict this, the inability of the model to mirror the experimental data can be rationalized by considering the size, and flexibility of the molecule. As the ligand under investigation is being processed, the bonds previously identified as being rotatable are randomly modified, and a series of energy calculations are performed. As the molecule becomes larger, and the number of rotatable bonds increases, the number individual rotations required to manipulate the molecule into an acceptable conformation increases dramatically. Furthermore, it is necessary for the complete computation to be of sufficient size to allow the exploration of all possible

rotations and conformations. Should the computation be insufficient to allow complete exploration of ligand conformations, the model will not be able to provide accurate information, and hence may be unable to differentiate between ligands bearing substantial similarity. It is this that appears to have befallen the UCL174, UCL169 and UCL173 ligands.

Observation of the docking orientation (figure 3.2.9), shows the molecules all in a similar conformation. The hydroxamic acid and linker are all located in reasonable positions, and conform to the commonly accepted hydroxamate binding mode within the catalytic region.

The region the linker inhabits can be loosely described as a tube, and due to the multitude of steric clashes and strongly charged zinc atom, there are few minima the computation has to explore to obtain a reasonable conformation in this area. The molecules rapidly obtain a good binding conformation within the catalytic site, as many rotations are energetically unfavourable and discounted from the calculation. The model therefore predicts the binding conformation of the ligands correctly within the Zn^{+2} binding catalytic area.

In comparison to the catalytic site, the cap region of the ligand is open, with an opportunity for ligand mobility across the surface. Coupled with the large number of rotatable bonds UCL169, UCL173 and UCL174 contain in the cap region, there is an increased computational overhead that was not satisfied by the constraints placed upon the model. Analysis of the data produced by the calculations indicate significant movement around the cap region. Insufficient calculation time can be confirmed within RMSD clusters of binding results, which show large numbers of widely spaced clusters, as opposed to highly populated singular clusters of low energy binding modes. Analysis of the conformations contained within the clusters reveals that each cluster represents a conformation with typical Zn^{+2} binding, but with seemingly unresolved cap region binding (figure 3.2.9 implies convergence of the modelling result; however, as there is significant deviation within each cluster, it cannot be concluded that the conformations seen are accurate).

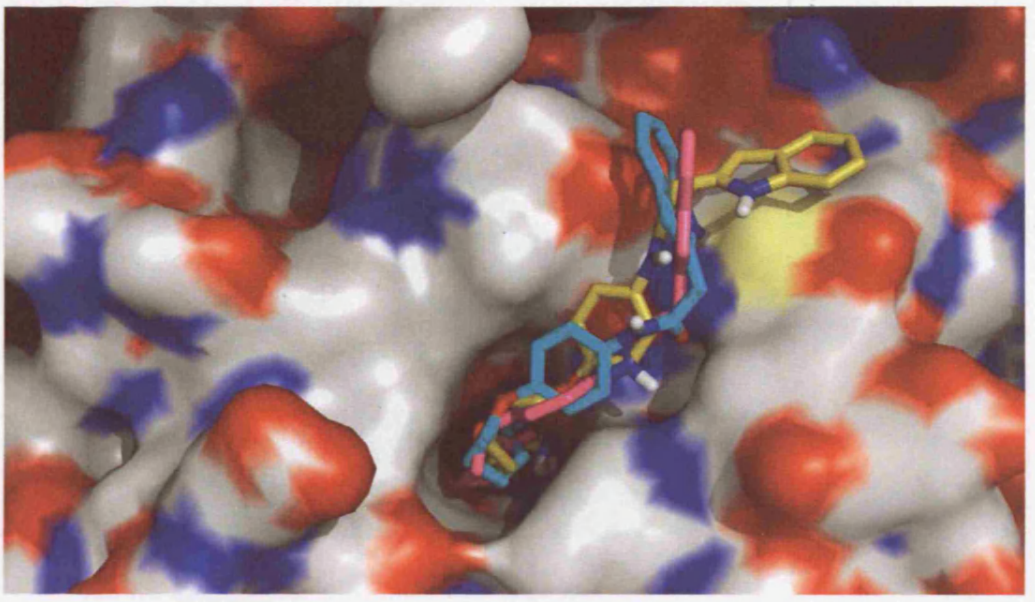


Fig. 3.2.9. Binding positions of UCL169 (**155**, light blue), UCL173 (**151**, magenta) and UCL174 (**156**, yellow).

Analysis of the binding energies also highlights further discrepancies in respect to these molecules. The calculated binding energies are seemingly comparable to that of TSA in magnitude, yet experimentally the compounds are far less potent. This indicates that in regard to these compounds, there is a failure of the model to correctly predict their inhibitory properties.

UCL158, UCL159 and UCL171 all correlate their binding energies to the experimentally determined IC_{50}. UCL159 and UCL171 both contain similarity to SAHA and TSA, and can be seen to occupy the same binding area in the computation. An illustration of the ligands with TSA can be seen in figure 3.2.10.

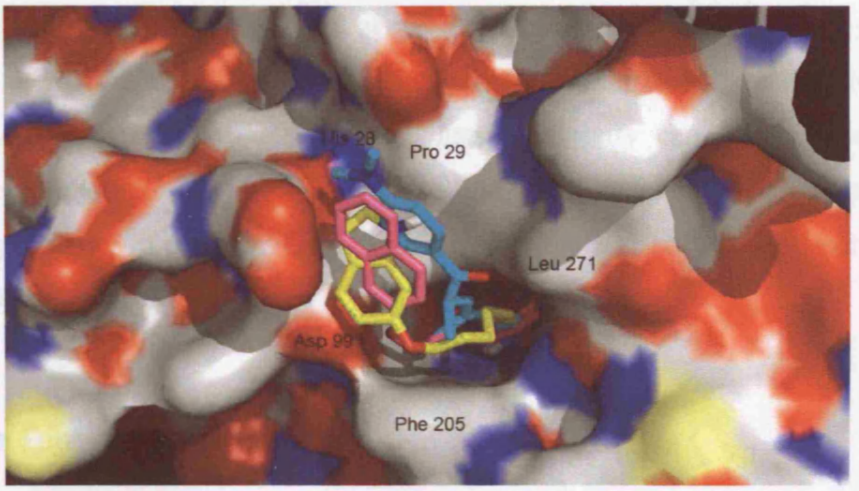


Fig. 3.2.10. An illustration of the binding modes of TSA (**150**, blue), UCL159 (**147**, magenta) and UCL171 (**149**, yellow).

The planar rings can be seen to be occupying a flat hydrophobic area within the cap region, much like that observed with TSA and SAHA.

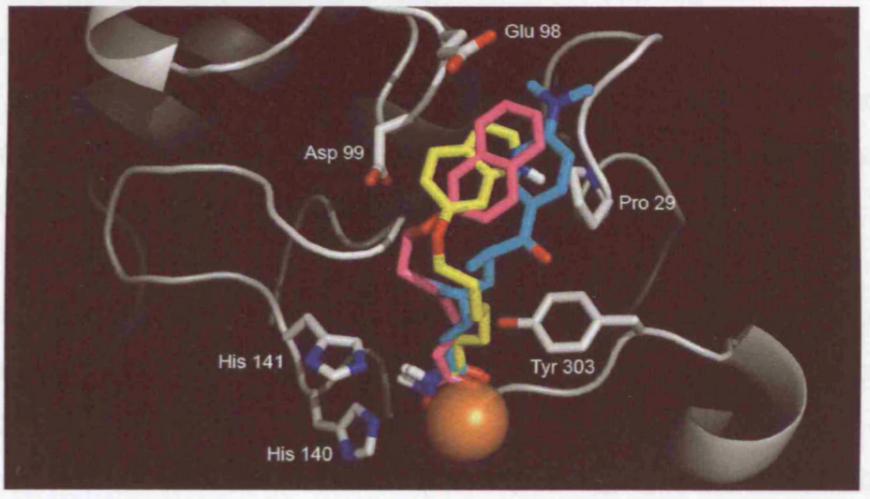


Figure 3.2.11. Illustration of ligand binding in the catalytic region of TSA (**150**, blue), UCL159 (**147**, magenta), and UCL171 (**149**, yellow).

Observation of the catalytic site, also shows a good comparison with the established TSA binding mode. UCL159 and UCL171 can both be seen to be in the correct conformation, with H-bonding to the important residues His 140, His 141 and Tyr 303.

UCL2200 is predicted to be a very potent binder to the HDAC 1 enzyme, with a binding energy of -13.21 kcal/mol, greater than that of TSA, one of the strongest binding ligands

known. Although the calculated potency of the compound is excellent, the model fails to predict the strength of the compound accurately. Analysis of the docking logs indicate that while there is a degree of rotation in the cap region, there is a definite docking conformation, and it is unlikely that the loss in activity can be accounted for by incomplete computations. This may indicate that other factors may be influencing the inhibitory properties of the HDAC inhibitors, factors that are external, and not accounted for within the model.

3.2.6 The Dimer Hypothesis

The computational model is unable to accurately predict the properties of certain ligands. Small ligands such as TSA, and SAHA, appear to be correctly defined and tolerated by the model and correlate to previous studies.[144,149] Larger ligands, however, do not correlate experimental and predicted values. Other studies have found good agreement with *in silico* methods; however, many have found that for some compounds there is deviation from *in vitro* enzyme assay values.[144,149,169,171] This appears to indicate a more wide-scale problem in predicting HDAC inhibition, namely that there is another important factor in HDAC inhibition that is not being considered in the model. As a hypothesis, we propose that species involving HDAC dimers (with and without ligand) may account for some of the observed discrepancies.

It is known that mammalian HDACs associate not only with themselves to form homo-oligomers, but with other HDACs to form hetero-oligomers. Seiser *et al.*[172] observed the ability of HDAC 1 to self associate *in vitro*, and Hessig *et al.*[173] confirmed the ability of HDAC 1 to form hetero-oligomers, through the immunoprecipitation of HeLa cells extract which coimmunoprecipitates HDAC 1 and HDAC 2. HDAC 1, in addition to association with other HDAC enzymes, can also be found associated with transcription related enzymes, such as CHD chromatin proteins, Sin3A/Sin3B, and RbAp48.[166,172]

Association of enzymes to HDAC can be attributed to a particular domain, a series of 60 residues localized on the N-terminus of the HDAC enzyme. Removal of the residues results in reduction of deacetylation, presumably due to the inability to associate with enzymes such as RbAp48, and a reduction in the ability to dimerize.[172] A representation

of HDLP bound to TSA in a dimeric form can be seen in figure 3.2.12, derived from X-ray crystallographic data; further dimeric examples can be found for HDAC 8 (PDB database ID. 1W22).

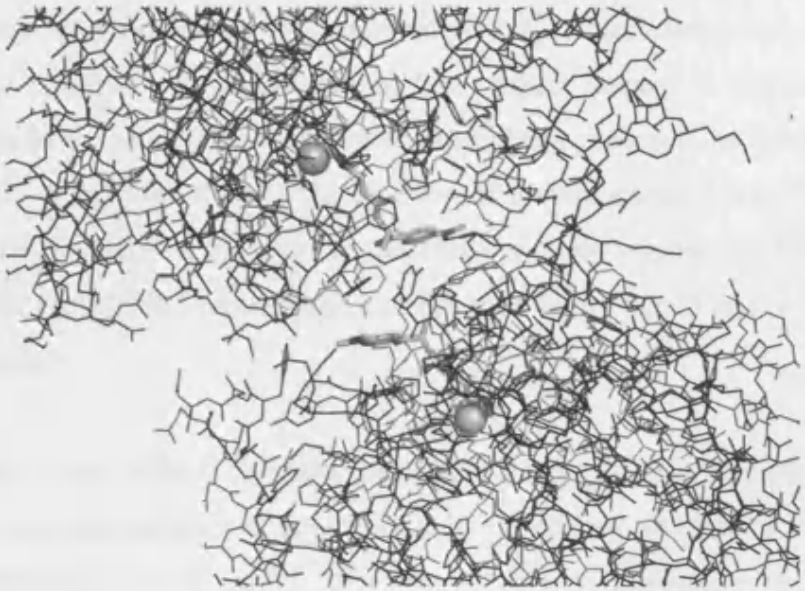


Fig. 3.2.12. Image of the HDLP-TSA dimer, taken from the PDB database (ID: 13CR).

A vastly simplified representation of the process that occurs as HDAC deacetylates histones can be seen in figure 3.2.13. For simplicity, only homodimeric HDAC species are considered; however, heterodimeric species are known have important biological roles.

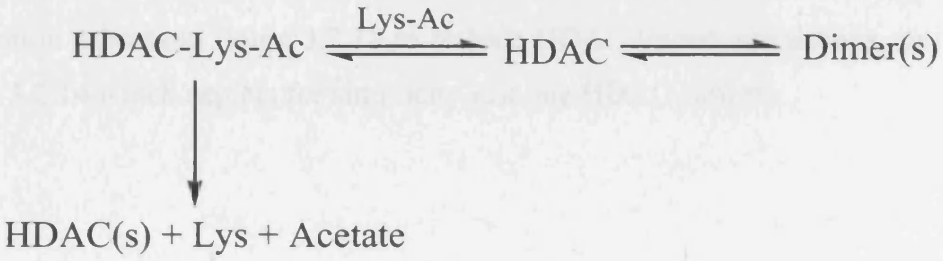


Fig. 3.2.13. A simplified representation of HDAC deacetylation processes.

The HDAC enzyme exists as the monomeric form, as illustrated in the center of figure 3.2.13. An equilibrium exists between the self association with another HDAC enzyme to form a dimer, and association with other classes of enzyme which will allow the HDAC enzyme to perform in deacetylation processes. Inhibition of the HDAC enzyme

with an inhibitor would therefore decrease deacetylation, but not necessarily inhibit the formation of HDAC complexes through self association or otherwise.

Currently, the computational model only attempts correlation of ligand interaction with ligand inhibitory activity. Should the ligand only inhibit deacetylation and the formation of complexes required for deacetylation, one would expect a linear, and easily predictable model to result. This representation has been proven to be inaccurate, as the model fails in correctly predicting the potency of certain compounds. The secondary process, alluded to earlier in the chapter and believed to be responsible for inaccuracies in the model, is represented on the right hand side of figure 3.2.13 and is the formation of HDAC dimers.

Should the use of an HDAC inhibitor affect the ability of HDAC enzymes to form dimers, the monomers-to-dimers equilibria will be shifted so that more monomeric enzyme is available, which would therefore be able to participate in deacetylation processes. If one extrapolates and considers how this would effect the relationship between the predicted inhibitory activity from the computational model, and the experimentally derived activity, a theorem as to the nature of the discrepancy can be derived. If the inhibition of dimer formation is high, then more HDAC enzyme monomer would be available, and inhibition would be less than predicted. Conversely, should the inhibitor not effect the formation of the dimeric species, correlation between the calculated activity and the experimental activity will only rely on the effect of the ligand on deacetylation directly, and should be relatively accurate. Modification of the deacetylation process in figure 3.2.13 to include HDAC-ligand interactions, can be seen in figure 3.2.14 which depicts for simplicity just one HDAC isoform.

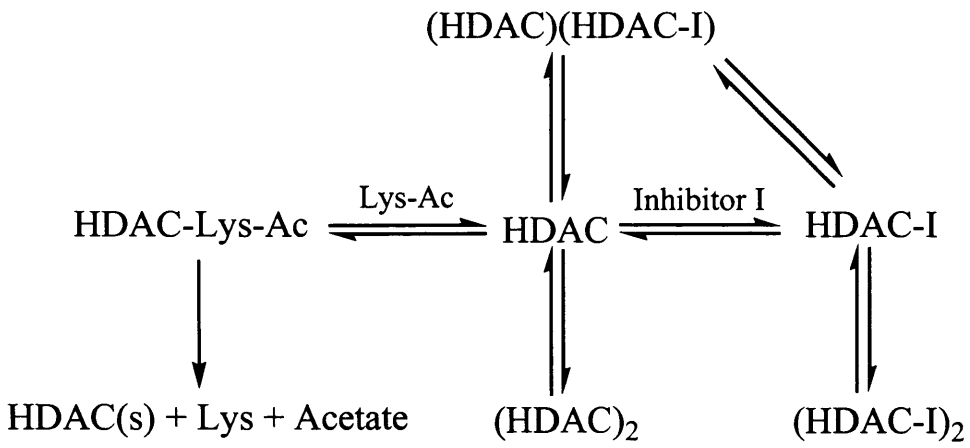


Fig. 3.2.14. Representation of processes occurring during HDAC inhibition, showing HDAC oligomers.

Analysis of the processes to account for the various HDAC-ligand interactions and dimer generation leads to the following conclusions with respect to the model:

I. Destabilization of the HDAC dimer, either of the form of one HDAC enzyme and a HDAC-ligand complex (represented as (HDAC)(HDAC-I)), or two HDAC-ligand complexes (represented as $(HDAC\text{-}I)_2$), will result in an increase of HDAC and more deacetylation. The single-molecule HDAC modelling will therefore predict the ligand to be better than experimentally determined.

II. Stabilization of any HDAC dimer will result in a decrease of HDAC, and hence an increase in effective inhibition through the reduction of HDAC monomer, which is assumed to be the most potent/or most significant molecule in HDAC inhibition. Thus the effective inhibition produced by the molecule will be greater than predicted by single-molecule HDAC modelling.

III. Where dimers are not significant, or their concentrations are unaffected by the introduction of an inhibitor, the single-molecule modelling will be accurate.

Relating the observations to the compounds studied, UCL169, UCL171, UCL173, and UCL2200 are thought to be destabilizing to one or more dimeric species, and therefore are not as potent as predicted by the model. They are thus examples of Type I. This could be attributed to the size of the ligands, the inability of the dimer to accommodate them within the cavity, or the blocking of favourable enzyme-enzyme interactions.

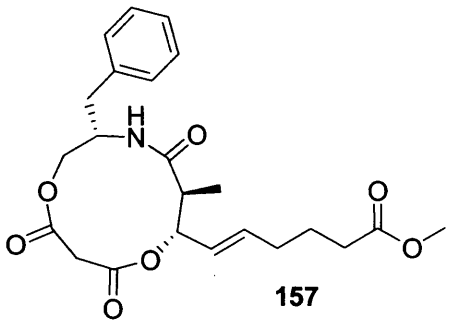
Examples of Type II, involving the stabilization of one or more HDAC dimers, could be found in the cyclic tetrapeptides, including Apicidin B (IC_{50} of 0.7 nM vs.HDAC 1). Cyclic tetrapeptides are far more potent than predicted by computational models. The increased activity could be due to the stabilization of the HDAC dimers arising from the extended plateau of the ligand, which could permit H-bonding and lipophilic interactions with the neighbouring HDAC enzyme in the dimer complex. It is also possible that cyclic depsipeptides, such as FK228, are very potent for similar reasons. In these cases, where a disulfide bridge is cleaved to reveal the HDAC inhibitor, the resulting thiol may subsequently form a new disulfide bridge with a cystine of the second enzyme within the HDAC dimer complex.

Examples of type III appear to be TSA, SAHA, UCL158, UCL159, and correlate well with the model.

Correction of the single-molecule model and accounting for the shift in potency due to additional favourable or unfavourable interactions could be achieved by creating an additional model based upon a (HDAC)(HDAC-I) dimer structure. The strength of interaction with the new HDAC dimer model could contribute to HDAC-I dimer stability information, and used in conjunction with the single-molecule binding energies to account for inhibitor-HDAC dimer effects.

3.2.7 A New HDAC Inhibitor

The construction of a new HDAC inhibitor was proposed, based upon the concept of the cyclic tetrapeptide class of inhibitors. Consisting of a Zinc Binding Group (ZBG), a large peptidic cap region moiety, coupled together with a linker, compounds such as these are known to form some of the most potent HDAC inhibitors currently reported.



157

Fig. 3.2.15. Structure of the new HDAC inhibitor.

Typically, the ZBG would be in the form of a hydroxamic acid; however, many other groups are known to chelate to the Zn^{+2} ion, including thiols, esters, and ketones. The choice of an ester was considered to be sufficient to obtain a reasonable degree of chelation, while maintaining ease of synthesis.

The cap region moiety consists of a cyclic 11 membered system, and is formed using methodology reported by Hinterding *et al.*[174] The system is believed to occupy much of the space immediately above the catalytic cavity, forming H-bonds with exposed residues in close proximity, and hoped to be as effective as the cyclic peptide class of compounds.

The ZBG and cap region moiety are linked together with a short six-atom carbon chain, incorporating one unsaturated bond close to the cap region.

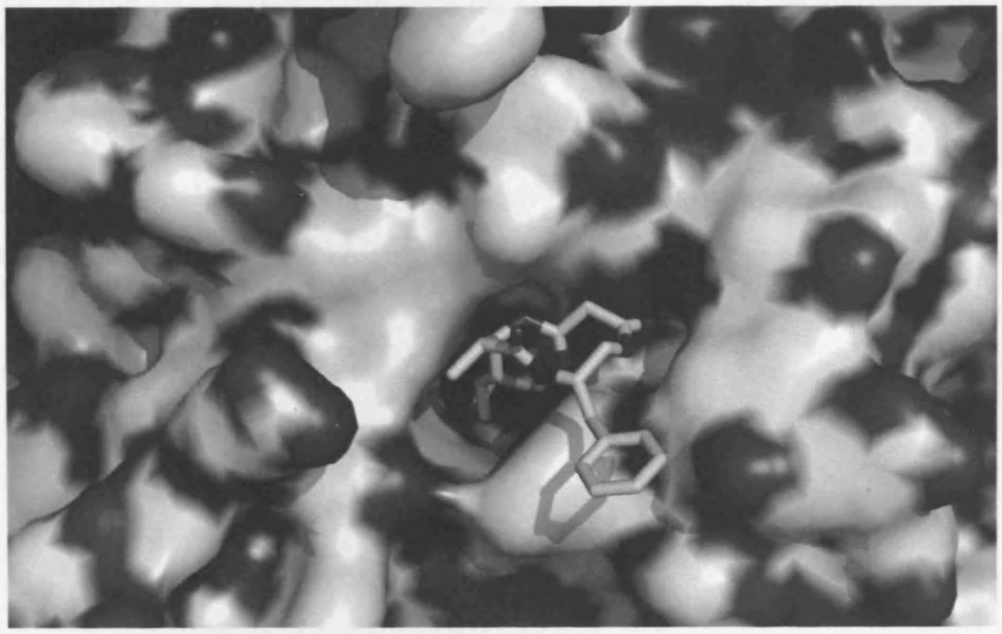


Fig. 3.2.16. Proposed binding conformation of the compound.

Modeling studies using an HDAC 1 model, indicate that the new HDAC inhibitor will bind in a similar orientation to that of UCL173, UCL174, and UCL169, rather than extending towards Pro 29 as in TSA and SAHA. Binding energies are predicted to be -9.79 kcal/mol, and a docking energy of -12.37 kcal/mol. If the prediction of the model is accurate, this would place the inhibitor within the same range of activity as SAHA with an IC_{50} of 10-15 nM; however, as the calculated binding conformation parallels the position of UCL173, UCL174 and UCL169, one would expect the *in vitro* inhibition values to be less that those calculated *in silico*, which could be thought of being attributable to the dimer hypothesis.

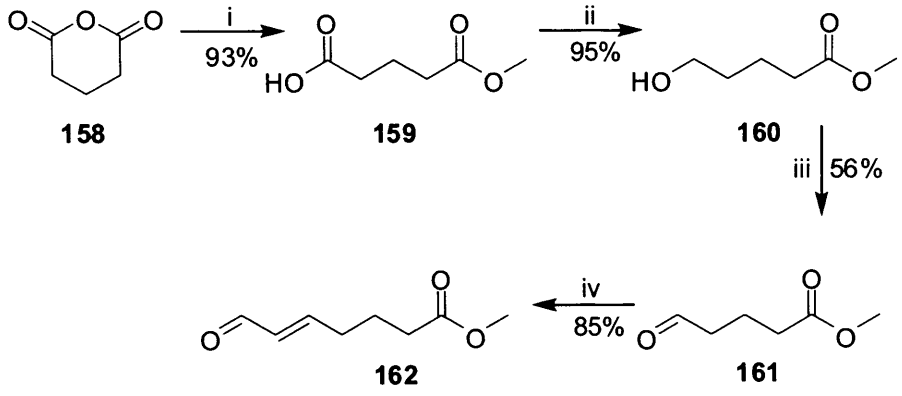
3.2.8 Synthesis of an HDAC inhibitor

Construction of a HDAC inhibitor bearing similarities to the cyclopeptidic class of HDAC inhibitors was attempted.

Methodology to generate eleven-atom cyclic structures was previously reported by Hinterding[174] as a by-product in an attempt to generate chiral polyketide units from an acetate enolate cleavage of an Evans oxazolidinone. It was found that the use of

LiHMDS or NaHMDS in place of KHMDS when generating the acetate enolate, increased the yield of the eleven-membered cyclic structure. It was this approach combined with the construction of a suitable fragment that was proposed.

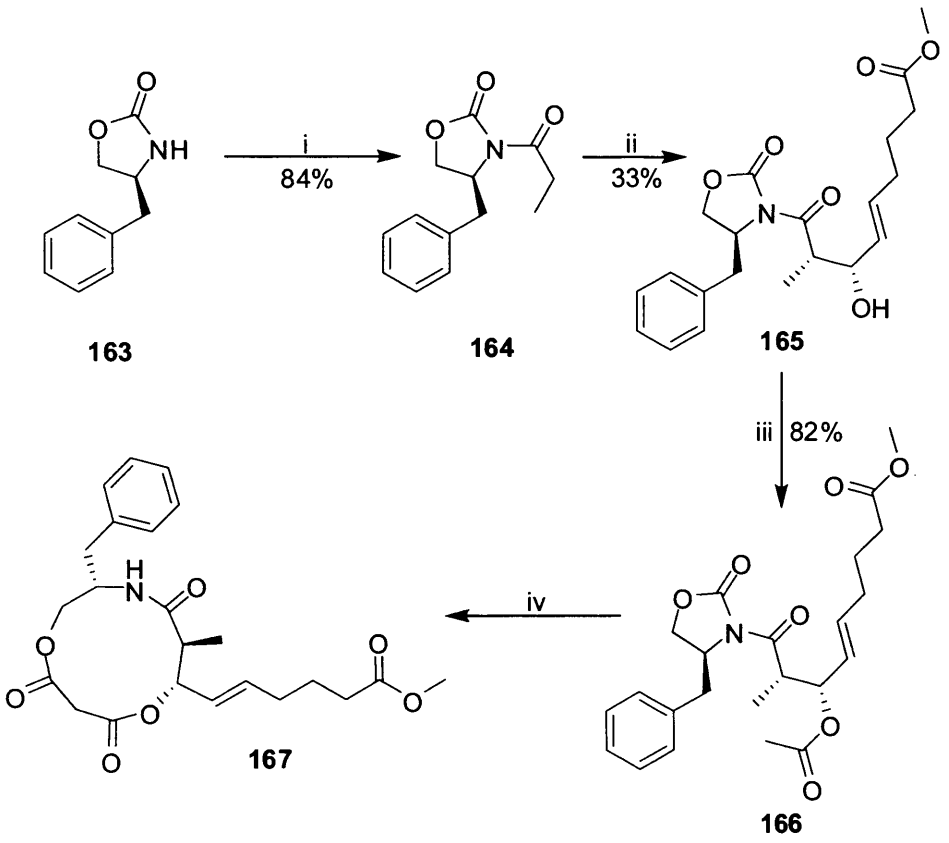
The synthesis began with the preparation of the aldehyde **162**, required for an Evans aldol addition to the Evans auxiliary fragment. The aldehyde fragment would contain the linker, and the important Zinc binding moiety.



Scheme 2.3.17. i. Dry methanol, NaOMe; ii. $BH_3.Me_2S$, THF, -10 °C; iii. PCC, DCM, Celite; iv. Ph_3P=CHCHO, benzene, reflux.

Mono-methyl glutarate (**159**) was prepared from glutaric anhydride and sodium methoxide in dry methanol.[175] Reduction to methyl 5-hydroxypentanoate (**160**) was conducted according to the procedure reported by Heathcock[176] with a yield of 95%. Oxidation to aldehyde **161** was achieved with a 56% yield using PCC in dichloromethane. The unsaturated aldehyde **162** was prepared with (*E*)-selectivity by using the procedure of Gung,[177] using the stabilised ylid (formylmethylene)triphenylphosphorane and generated the required conjugated aldehyde system.

The synthetic route used to couple the aldehyde fragment to the auxiliary, and continuation onto **167**, can be seen in scheme 2.3.18.



Scheme 2.3.18. Final stages in the synthesis of the HDAC inhibitor. i. Propionyl chloride, *n*-BuLi, THF; ii. (-)-sparteine, NMP, DCM, $TiCl_4$, **162**, -78 °C – 0 °C; iii. Ac_2O, DMAP, CH_2Cl_2, Et_3N; iv. LiHMDS, THF, -78 °C.

The Evans auxiliary was first coupled with propionyl chloride using *n*-BuLi in THF with 84% yield. The auxiliary fragment then underwent Evans aldol addition with the previously prepared aldehyde **162**. Coupling was not efficient, and only yielded 33% of the aldol product after purification. This was due to E/Z isomerization of the terminal conjugated aldehyde moiety in **162**, resulting in a reduced yield of the required (*E*)-isomer, which could be isolated using column chromatography. Acetylation of **165** was conducted using acetic anhydride, DMAP, triethylamine in dichloromethane, and gave **166** in 82% as a colourless oil. Preparation of compound **167** was attempted by the dropwise addition of LiHMDS to **166** in THF at -78 °C. After quenching and extraction, the material was passed through a silica column to remove impurities. Further purification using flash column chromatography using an eluant of 5% methanol/dichloromethane, gave an intractable mixture of compounds. NMR analysis of the gave results that were consistent with product that contains **167**, in addition to other complex material.

Material obtained containing **167** was tested for HDAC inhibitory activity, and was found to have little, or no effect on the nM scale. While not consistent with the computational model predictions, this may be expected of compounds that have been predicted to bind in the manner shown in figure 3.2.16. The large cap region portion of the ligand may be inhibiting the formation of HDAC homo- and hetero-dimers. The unsaturated bond within the linker portion may also be introducing rigidity in the inhibitor too near the top of the catalytic channel. This could be preventing the ligand from adopting a relaxed conformation onto the top of the cap region, and instead could be being forced vertically outward. Destabilization of the HDAC dimers would thereby increase the level of HDAC within the assay, and the molecule would therefore have an effective inhibition far less than that predicted by the computational model due to increased HDAC deacetylation.

3.3 Conclusion

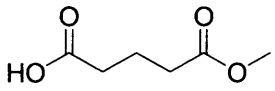
A computational model that can predict HDAC-ligand interactions has been described. The model is successful for small, TSA and SAHA like hydroxamate inhibitors; however, larger ligands are found to be problematic, as was discovered with the correlation between experimental and *in silico* analysis of a new cyclic inhibitor.

Alleviation of the large ligand discrepancy could be achieved through the construction of a model based upon the HDAC dimer hypothesis. A model consisting of a HDAC dimers may be used to study ligand-HDAC dimer interactions, and used to adjust the single-molecule model and accounting for perturbation of HDAC dimers. This should be explored both *in silico*, and through the construction of appropriate analytical experiments to determine the nature of the HDAC dimers.

3.4 Experimental

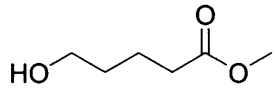
The general experimental section can be found in chapter 1.4.

5-Methoxy-5-oxopentanoic acid[175] (159)



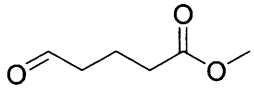
Glutaric anhydride (6.0 g, 52.6 mmol), dry methanol (75 mL), and NaOMe (57 mg, 1.06 mmol) were stirred overnight at room temperature. Methanol was removed under reduced pressure, and the residue diluted with water (50 mL). Extraction with ethyl acetate (3 x 50 mL), drying ($MgSO_4$), and concentration under reduced pressure gave the crude material. Column chromatography with an eluant of 1:1 hexane:ethyl acetate gave the required product, 7.13 g, 93%. NMR data correlated to that found in the literature.[175] ^1H NMR (300MHz, $CDCl_3$), δ_H 3.61 (s, 3H, O-$\underline{CH_3}$), 2.40-2.25 (m, 2H, HOOC-$\underline{CH_2}$-CH_2, CH_2-$\underline{CH_2}$-$CHOOCH_3$), 1.85 (m, 2H, CH_2-$\underline{CH_2}$-CH_2).

Methyl 5-hydroxypentanoate[176] (160)



To a flask containing 5-methoxy-5-oxopentanoic acid (5 g, 34.2 mmol), and dry THF (50 mL) cooled to -10°C, $BH_3.Me_2S$ complex (37.6 mmol) was added dropwise. The mixture was then allowed to warm to room temperature, and left to stir overnight. Water was added (50 mL) at 0°C, and the reaction concentrated under reduced pressure. Material was then extracted using ethyl acetate (3 x 50 mL), and yielded material upon drying ($MgSO_4$) and concentration. Column chromatography yielded the required alcohol as a pale oil, 4.28 g, 95%. Spectral data correlated to that found in the literature.

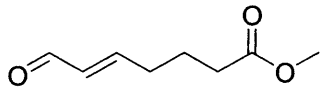
Methyl 5-oxopentanoate[177] (161)



To a stirring suspension of PCC (9.8 g, 45.5 mmol) and Celite (9.8 g) in dry dichloromethane (50 mL), methyl 5-hydroxypentanoate (4 g, 30.3 mmol) was added dropwise at 0 °C. The mixture was stirred and allowed to warm to room temperature. TLC analysis indicated the progress of the reaction. Upon completion, the reaction was diluted with ethyl acetate (100 mL) and filtered though a silica plug,

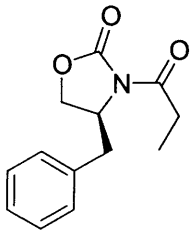
washing with ethyl acetate to remove any remaining material. Concentration of the filtrate gave **161** (2.22 g, 56%) as an oil that required no further purification. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 9.74 (s, 1H, CHO-CH_2), 3.64 (s, 3H, CH_3-O), 2.51 (t, J=7.2 Hz, 2H, OOC-CH_2-CH_2), 2.35 (m, 2H, CHO-CH_2-CH_2), 1.87 (m, 2H, CH_2-CH_2-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 201.6, 173.4, 51.6, 42.9, 32.9, 17.3.

(*E*)-Methyl 7-oxohept-5-enoate (162)



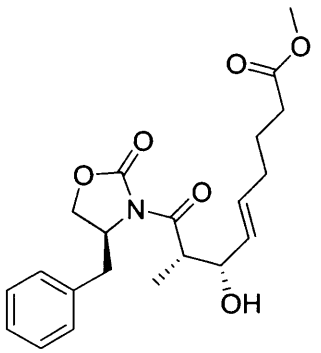
(Formylmethylene)triphenylphosphorane (3.18 g, 10.45 mmol) was dissolved in benzene (15 mL), and methyl 5-oxopentanoate (1.13 g, 8.7 mmol) was added in one portion. The reaction mixture was heated to reflux, and monitored by TLC. Upon completion, the reaction was concentrated under reduced pressure, and the residue filtered through a silica plug with an eluant of 4:1 petroleum ether(40:60):ethyl acetate. The filtrate was concentrated under reduced pressure to give **162** (1.15 g, 85%) as a yellow oil. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 9.53 (d, J=13.0 Hz, 1H, CHO-CH), 6.80 (dt, J=15.7, 6.7 Hz, 1H, CH=CH-CH_2), 6.11 (dd, J=15.7, 13.0 Hz, 1H, CHO-CH=CH), 3.65 (s, 3H, O-CH_3), 2.41-2.33 (m, 2H, -CH_2-), 1.94-1.64 (m, 4H, -CH_2-). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 193.9, 173.4, 157.1, 133.5, 51.7, 33.1, 31.9, 21.4.

(*S*)-4-Benzyl-3-propionyloxazolidin-2-one[178] (164)



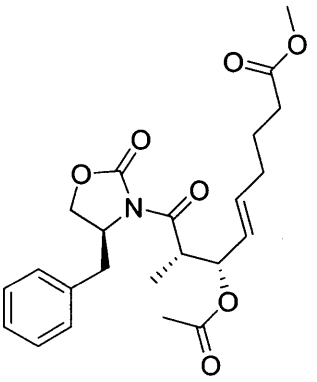
To a stirring solution of (*S*)-4-benzyloxazolidin-2-one (5.8 g, 33 mmol) in THF (50 mL) at -78 °C, *n*-BuLi (1 eq, 33 mmol) was added. The reaction was stirred for 10 minutes, and propionyl chloride (1.2 eq, 3.66g, 39.6 mmol) was added slowly. The mixture was stirred for 1 hour, then warmed to room temperature. The reaction was quenched with the addition of sat. ammonium chloride, and concentrated. The residue was washed with dichloromethane (3 x 50 mL), extracts dried ($MgSO_4$), and concentrated under reduced pressure to give **164** (6.46 g, 84%) as a colourless crystalline solid. Spectral data were identical to that found in the literature.[178] ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 173.2, 153.5, 135.3, 129.4, 129.0, 127.3, 66.2, 55.1, 37.9, 37.4, 17.7, 13.7

(7*S*,8*S*,*E*)-Methyl 9-((*S*)-4-benzyl-2-oxooxazolidin-3-yl)-7-hydroxy-8-methyl-9-oxonon-5-enoate (165)



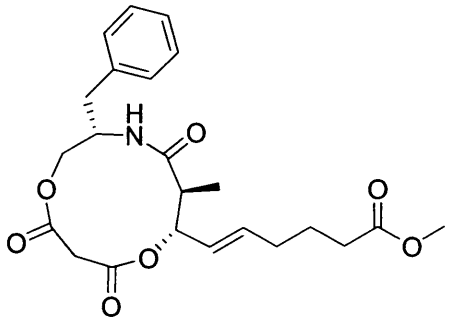
To a stirring solution of (*S*)-4-benzyl-3-propionyloxazolidin-2-one (0.2 g, 0.9 mmol) in dry dichloromethane (7 ml) at 0 °C, $TiCl_4$ (1 M in dichloromethane, 0.9 mmol) was added slowly and stirred for 5 min, generating a yellow solution. (-)-Sparteine (0.2 g, 0.9 mmol) was added dropwise and stirred for 20 min, generating a deep red solution. Cooling to -78 °C, followed by the addition of *N*-methylpyrrolidin-2-one (0.85 g, 0.9 mmol). After stirring for 10 min, (*E*)-methyl 7-oxohept-5-enoate (0.148 g, 0.95 mmol) was added dropwise, and stirred for 1 h, followed by warming to 0 °C and stirring for a further 30 min. The reaction was quenched with half saturated ammonium chloride solution (3 mL), and extracted using dichloromethane (3 x 5 mL). The combined organic fractions were dried (Na_2SO_4), followed by concentration under reduced pressure gave the crude product as an oil. Purification using column chromatography with an eluant of 2:1 hexane:ethyl acetate gave **165** (0.13 g, 37%) as a colourless oil. IR ν_{max} (KBr, cm^{-1}) 3465 (OH), 1782 (C=O), 1738 (C=O), 1690 (NC=O), 1639 (C=C). ^1H NMR (300MHz, $CDCl_3$), δ_H 7.36-7.18 (m, 1H, Ar-H), 5.77 (dt, *J*=16.2, 7.0 Hz, 1H, CH=CH-CH_2), 5.29 (dd, *J*=6.1, 16.2 Hz, 1H, CH-CH=CH), 4.70-4.69 (m, 1H, CH_2-CH(CH_2)-N), 4.45-4.42 (m, 1H, CH-OH), 4.23-4.17 (m, 3H, OH, O-CH_2-CH), 3.87-3.83 (m, 1H, C(O)-CH(CH_3)-CH), 3.65 (s, 3H, CH_3-O), 3.24 (dd, *J*=13.4, 3.2 Hz, 1H, Ph-CHH-CH), 2.78 (dd, *J*=13.4, 9.4 Hz, 1H, Ph-CHH-CH), 2.30 (t, *J*=7.4 Hz, 2H, CH_2-CH_2-CHOO), 2.09 (dt, *J*=7.6, 7.0 Hz, 2H, CH-CH_2-CH_2), 1.72 (tt, *J*=7.6, 7.4 Hz, 2H, CH_2-CH_2-CH_2), 1.24 (d, *J*=7.0 Hz, 3H, CH_3-CH). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 176.6, 174.0, 153.2, 135.1, 132.1, 130.1, 129.4, 129.0, 127.5, 72.6, 66.2, 55.2, 51.5, 42.8, 37.8, 33.3, 31.6, 24.3, 11.4. HRMS calcd. for $C_{21}H_{27}NO_6$ 389.1838, found 389.1845.

(7*S*,8*S*,*E*)-methyl 7-acetoxy-9-((*S*)-4-benzyl-2-oxooxazolidin-3-yl)-8-methyl-9-oxonon-5-enoate (166)



To a stirring solution of (7*S*,8*S*,*E*)-methyl 9-((*S*)-4-benzyl-2-oxooxazolidin-3-yl)-7-hydroxy-8-methyl-9-oxonon-5-enoate (0.11 g, 0.29 mmol) in dichloromethane (2 mL) at 0 °C, acetic anhydride (44 mg, 0.44 mmol), DMAP (12 mg, 0.1 mmol), and triethylamine (0.46 mmol) was added sequentially. The mixture was stirred until completion by TLC. Water was added (5 ml), and the reaction mixture extracted with dichloromethane (3 x 5 ml). Combined organic fractions were dried (Na_2SO_4), and concentrated under reduced pressure to an oil. Column chromatography using an eluant of 2:1 hexane:ethyl acetate gave **166** (0.10 g, 82%) as a colourless oil. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 7.34-7.18 (m, 5H, Ar-H), 5.76 (dt, J=16.9, 7.7 Hz, 1H, CH-CH=CH), 5.59-5.45 (m, 2H, CH=CH-CH_2), 4.61-4.59 (m, 1H, CH_2-CH(CH_2)-N), 4.24-4.04 (m, 3H, O-CH_2-CH, CH-CH(O)-CH=CH), 3.65 (s, 3H, O-CH_3), 3.25 (dd, J=13.3, 3.2 Hz, 1H, Ph_CHH-CH), 2.76 (dd, J=13.3, 9.8 Hz, 1H, Ph-CHH-CH), 2.28 (t, J=7.4 Hz, 2H, OOC-CH_2-CH_2), 2.11-2.05 (m, 2H, CH-CH_2-CH_2), 2.04 (s, 3H, CH_3-C(O)), 1.70 (tt, J=7.4, 7.3 Hz, 2H, CH_2-CH_2-CH_2), 1.20 (d, J=6.9 Hz, 3H, CH_3-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 173.9, 173.6, 170.2, 153.5, 135.3, 133.9, 129.5, 128.9, 127.4, 126.8, 74.2, 66.3, 55.6, 51.5, 42.0, 37.9, 33.2, 31.4, 24.0, 21.0, 11.5. HRMS calcd. for $C_{23}H_{29}NO_7$ 431.1944, found 431.1941.

(*E*)-Methyl-6-((3*S*,6*S*,7*S*)-3-benzyl-6-methyl-5,9,11-trioxo-1,8-dioxa-4-azacycloundecan-7-yl)hex-5-enoate (167)



To a stirring solution of (7*S*,8*S*,*E*)-methyl 7-acetoxy-9-((*S*)-4-benzyl-2-oxooxazolidin-3-yl)-8-methyl-9-oxonon-5-enoate (100 mg, 0.23 mmol) in THF (15 mL) at -78 °C, LiHMDS (1 M in THF, 0.69 mmol) was added dropwise. The reaction was stirred for 2 h. Addition of 1:1:1 ammonium sulfate:water:methanol quenched the reaction. Products were extracted into dichloromethane (3 x 10 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Column chromatography using an eluant of 5% methanol/dichloromethane yielded 59.4 mg of a yellow oil. Analysis was inconclusive due to the purity of the material obtained.

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Chapter 4

Towards the synthesis of the (-)-Calyculin A Spiroketal Fragment

4.1 Introduction

In this chapter the novel marine phosphatase inhibitor, (-)-calyculin A, is examined. In particular, the chapter focuses upon the synthesis of the spiroketal core of the molecule, and the application of a novel mercury mediated cyclisation strategy in the synthesis of the spiroketal fragment.

4.1.1 Calyculin

The calyculins represent a group of natural marine compounds from the sponge *Discodermia calx*. First isolated from a sponge collected in the Gulf of Sagami, Japan, by Fusetani *et al.*[179] The metabolites were found to exhibit strong activity in the starfish egg assay,[180] and L1210 cells where the compounds were shown to possess strong cytotoxic effects. Isolation and structural elucidation of one of the major active components successfully identified calyculin A (figure 4.1.1), a novel compound containing a spiroketal, phosphate, and oxazole functionalities.

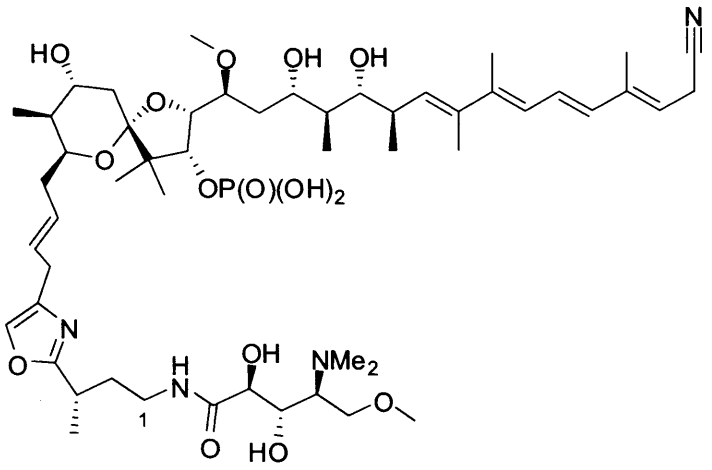


Figure 4.1.1. (-)-Calyculin A

Several other members of the calyculin family, were isolated and characterised by Fusetani *et al.*[181,182] and termed calyculins B through to H. Calyculins A, B, E, and F are all isomeric in the tetraene portion, and interconvert through exposure to light. Calyculins C, D, G, and H incorporate an extra methyl group at carbon 32, and are also interconverted around the tetraene portion through light exposure.

The calyculins have been shown to possess a wide range of biological activity. They have been shown to inhibit protein phosphatases PP1 and PP2A in nanomolar concentrations,[183,184] possess a high level of membrane permeability, exhibit strong tumour promoting effects,[185] and are cytotoxic with respect to leukaemia cell lines.[182]

Calyculin, due to its impressive inhibitory properties, has found growing use in the exploration of intracellular phosphorylation. Its cytotoxic effects have also prompted considerable interest in the synthesis of the natural and unnatural forms.

4.1.2 The Synthesis of the Spiroketal Unit of Calyculin

Calyculin has been the focus of synthetic attempts and studies[186] for several years since the initial discovery and characterisation of the molecule.[179] Confirmation of the absolute configuration by Shioiri *et al.*[187] was swiftly followed by the first total synthesis of the unnatural (+)-calyculin A by Evans *et al.*[188]

In their strategy to synthesise (+)-calyculin A, Evans chose to separate the molecule into two fragments. A C26 to C37 fragment, and a C1 to C25 fragment, which could be easily combined to generate the entire scaffold of the molecule though a Wittig type process (figure 4.1.2).

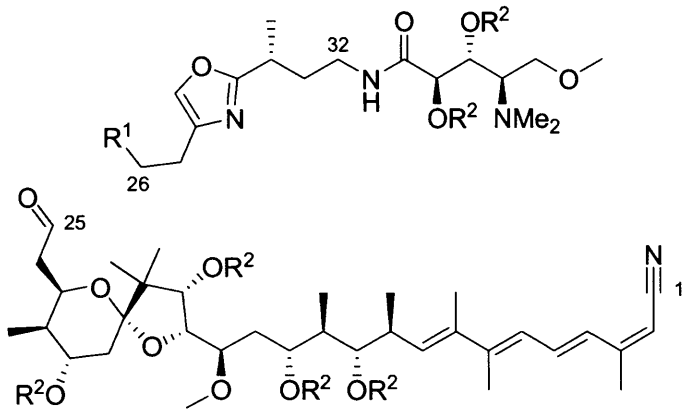
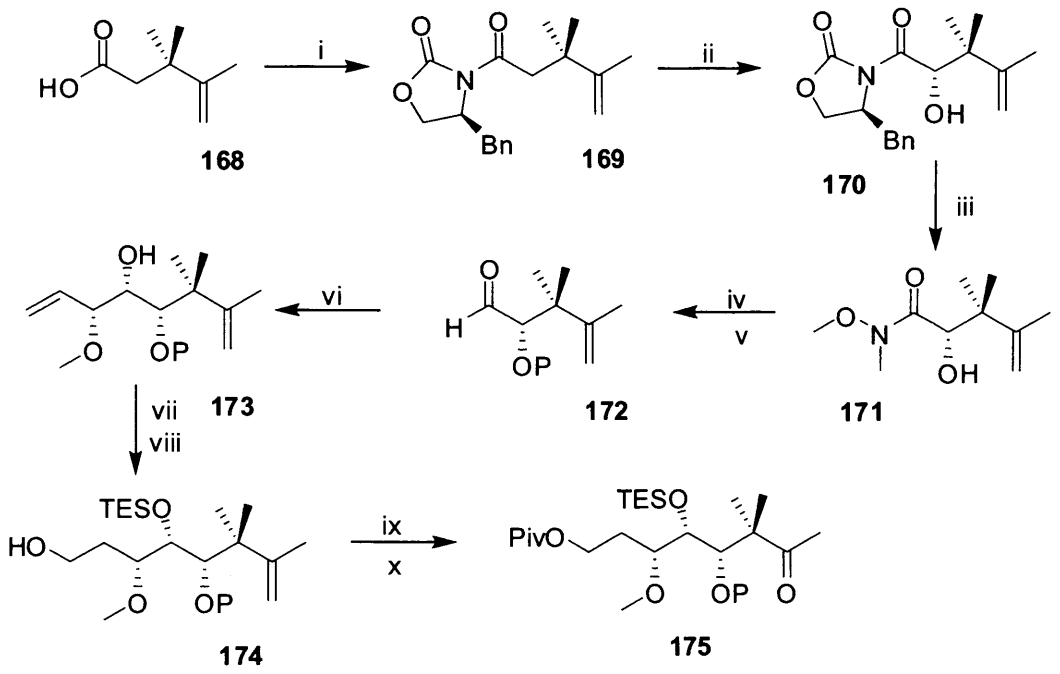


Figure 4.1.2. Fragments C1-C25 and C26-C37 of the Evans (+)-calyculin A synthesis. R^1 represents a phosphorous containing moiety suitable for Wittig type reactions. R^2 represents protecting groups.

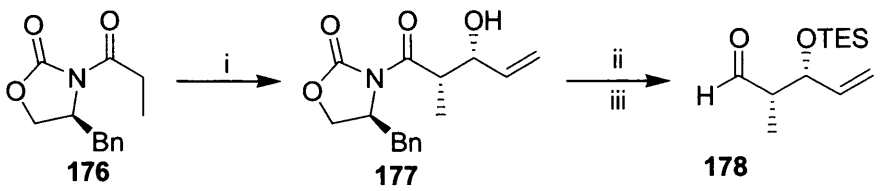
The Evans synthesis began with the preparation of the spiroketal moiety of calyculin, and utilised oxazolidinone auxiliaries in those very first stages to introduce the stereochemical centres of the spiroketal core. The route used by Evans involved the synthesis of two fragments which were combined to generate the spiroketal core. The synthesis of the first fragment can be seen in scheme 4.1.3.



Scheme 4.1.3. Preparation of the first fragment in the Evans preparation of the calyculin spiroketal core. i. (4*S*)-4-benzyl-2-oxazolodinone, THF, pivaloyl chloride, Et_3N, Et_2O, -78 °C to 0 °C; ii. 2-(phenylsulfonyl)-3-phenyloxaziridine, THF, -78 °C, NaHMDS; iii. $AlMe_3$, MeNH(OMe).HCl, dichloromethane, reflux; iv. NaH, *p*-methoxybenzyl bromide, THF/DMF, 0 °C; v. DIBAl-H, toluene, -78 °C; vi. $MgBr_2$, dichloromethane, -40 °C, tributyl(3-methoxyallyl)stannane; vii. TES-OTf, 2,6-lutidine, dichloromethane, 0 °C; viii. $Rh(PPh_3)_3Cl$, catecholborane, THF; H_2O_2; $NaBH_4$, EtOH; ix. Pivaloyl chloride, DMAP, pyridine, dichloromethane; x. OsO_4, NMO, *tert*-butyl alcohol/THF/water, $NaIO_4$.
P = *para*-methoxybenzyl protecting group.

Preparation of the compound **170** by auxiliary controlled hydroxylation generated the first stereochemical centre at C17 and proceeded with an 88% yield with the production of one single diastereoisomer. Removal of the auxiliary by trimethyl alumnimium-mediated conversion to the Weinreb amide gave **171**. Protection of the free alcohol as a *p*-methoxy benzyl grouping, allowed the unhindered reduction of the Weinreb to the aldehyde **172** using diisobutylaluminium hydride. Treatment of **172** with tributyl(3-methoxyallyl)stannane and magnesium bromide etherate gave **173** in 71% yield. Protection of the alcohol **173** using a triethyl silane protecting group, and subsequent rhodium catalysed hydroboration gave **174**. Formation of the pivaloyl ester, osmylation and periodate cleavage generated the required fragment **175** in 88% over the two steps.

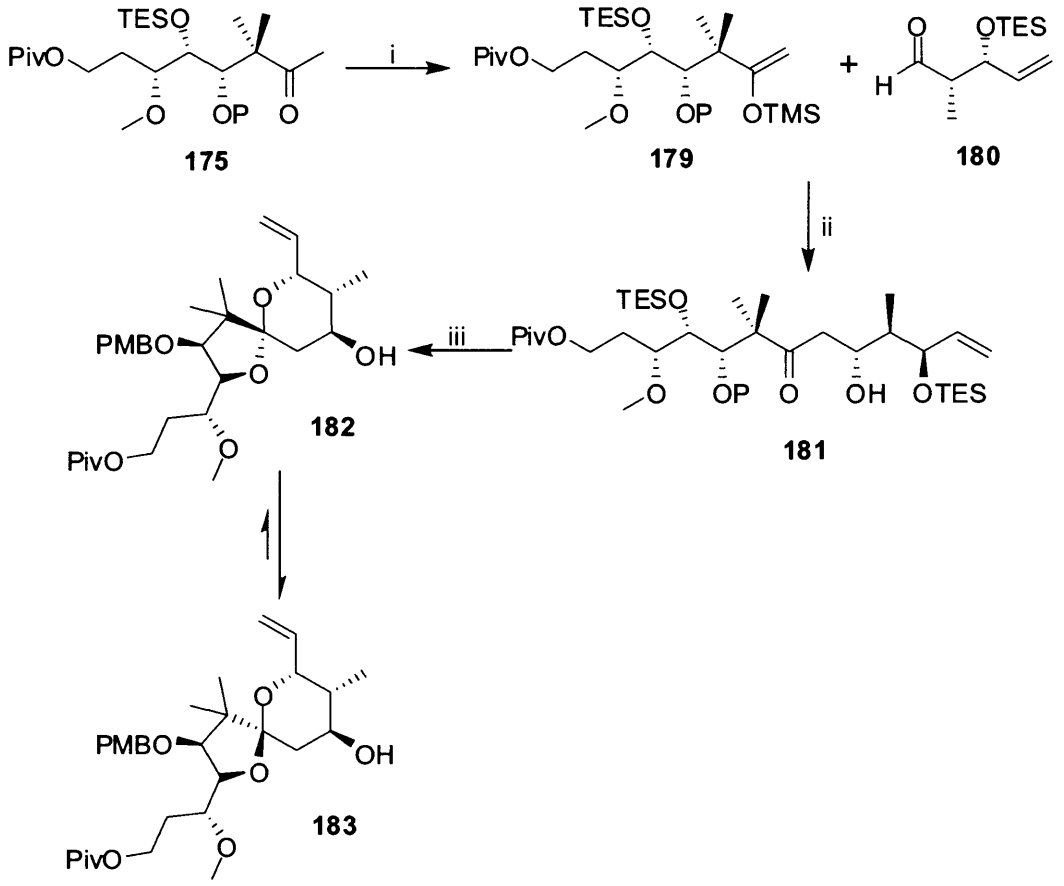
The preparation of the second fragment can be seen in scheme 4.1.4.



Scheme 4.1.4. Preparation of the second fragment in the Evans preparation of the calyculin spiroketal core. i. *n*-Bu_2BOTf, *i*-Pr_2NEt, acrolein, dichloromethane, -78 °C; ii. MeNH(OMe).HCl, $AlMe_3$, THF; TES-Cl, Et_3N, dichloromethane. iii. DIBAL-H, THF, -90 °C.

The oxazolidinone **176** underwent aldol addition to give **177** in 83%. Conversion into the Weinreb amide using trimethylaluminium in tetrahydrofuran, silyl protection of the alcohol, and reduction using diisobutylalumnium hydride gave the aldehyde **178**.

The method used to couple the two fragments generating the spiroketal fragment can be seen in scheme 4.1.5.



Scheme 4.1.5. Coupling of the two fragments in the Evans preparation of the calyculin core. i. TMS-OTf, Et_3N, dichloromethane, 0 °C; ii. $BF_3.OEt_2$, dichloromethane, -78 °C; iii. 48% aq. HF, acetonitrile, water. P = *para*-methoxybenzyl protecting group

Conversion of **175** into the silyl enol ether **179**, followed by aldol addition to **180** gave the carbon scaffold of the spiroketal fragment **181** in 80% yield as a single diastereoisomer. Treatment with HF/acetonitrile/water at room temperature gave the diasteromeric spiroketals **182** and **183** in a 1:5 ratio. This completed spiroketal fragment was advanced by Evans and ultimately to the first total synthesis of (+)-calyculin A.

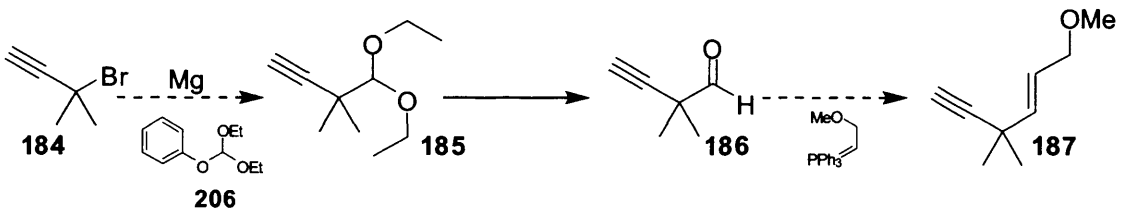
The Evans approach to the spiroketal core has since been used in other syntheses of the calyculin family, albeit modified to take advantage of new or alternative synthetic methodology. These can be seen in the work of Masamune *et al.*[189] in their total synthesis of the naturally occurring (-)-calyculin A, and in the work of Shioiri *et al.*[190] in their synthesis of the calyculin A C9 to C37 fragment. More recently the reports of Armstrong and Ogawa[191] in their published synthesis of calyculin C, and in reports from Smith *et al.*[192,193] in their total synthesis of (+)-calyculin A and (-)-calyculin B. Also of

note are the recently reported formal total synthesis of (+)-calyculin A by Anderson *et al.*,[194] and the use of chiral pool and rhodium catalysed cyclisation approach by Trost and Flygare.[195]

4.1.3 Synthesis of the (-)-Calyculin A Spiroketal Core

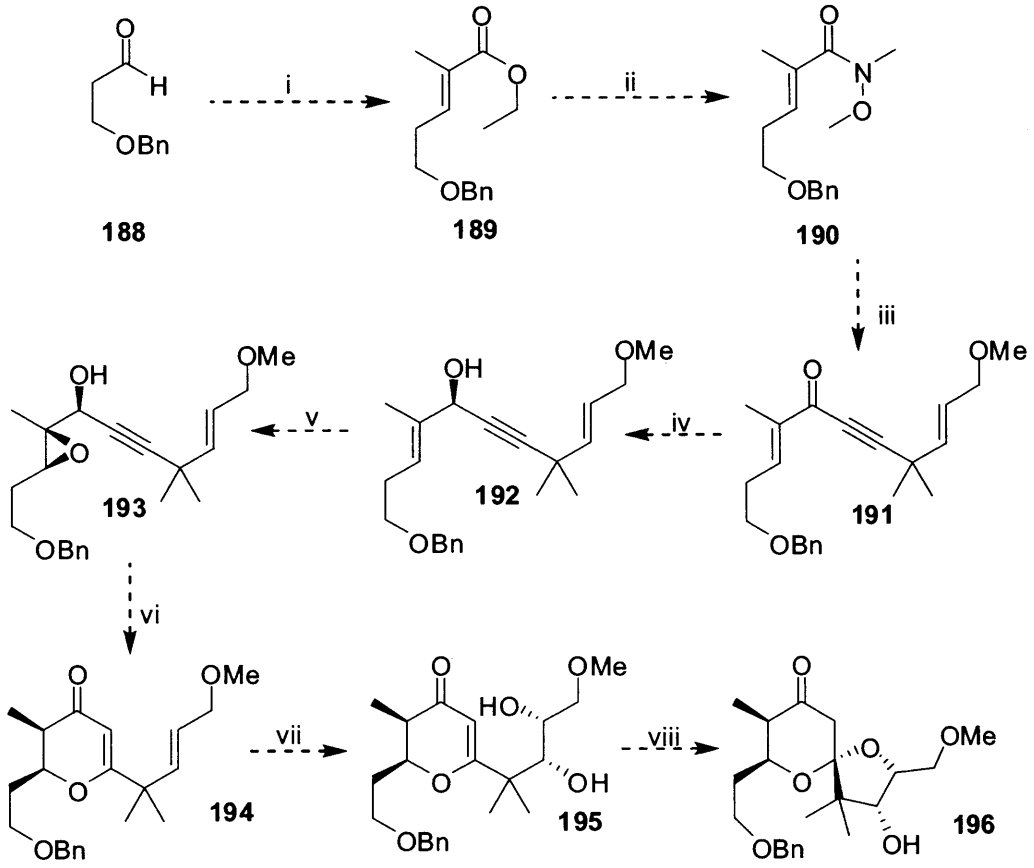
The spiroketal core of (-)-calyculin A is a potential synthetic target which would demonstrate the synthetic utility of the mercury mediated cyclisation strategy. Using a convergent approach requiring the synthesis of two precursors, the synthesis of the (-)-calyculin A spiroketal core was considered, with Hg^{+2} mediated cyclisation being an integral step.

The synthesis of the first precursor in the synthesis can be seen in scheme 4.1.6.



Scheme 4.1.6. Preparation of the alkyne fragment.

The first precursor **187** would be prepared starting from inexpensive 2-methylbut-3-yn-2-ol (**184**). Conversion to **185** using chemistry reported by Barbot and Miginiac,[196] was followed by acid catalysed conversion of the acetal to the aldehyde to give **186**. Wittig chemistry could then be used to provide **187**, the first fragment.



Scheme 4.1.7. Preparation of the spiroketal scaffold of (-)-calyculin A. i. Wittig preparation of the olefin; ii. MeNH(OMe).HCl, Me_3Al, iii. **187**, *n*-BuLi; iv. (*R*)-alpine Borane reduction; v. Sharpless asymmetric epoxidation; vi. HgO, dil. H_2SO_4; vii. Sharpless asymmetric dihydroxylation; viii. cat. *p*-TsOH.

The second fragment (**190**) is a Weinreb amide prepared from the allylic ester **189**, which is in turn easily synthesised in a Wittig reaction from the aldehyde **188**.

The two fragments, **190** and **187** would then be coupled to give the completed carbon scaffold **191**. Asymmetric reduction, using (*R*)-alpine borane, followed by Sharpless asymmetric epoxidation is expected to give **193**. Mercury mediated cyclisation according to the procedures reported by Marson *et al.*[197] would give the key dihydropyranone **194**. Sharpless asymmetric dihydroxylation to give **195**, followed by dilute acid to catalyse the formation of the spiroketal, should furnish **196** cleanly from **195**.

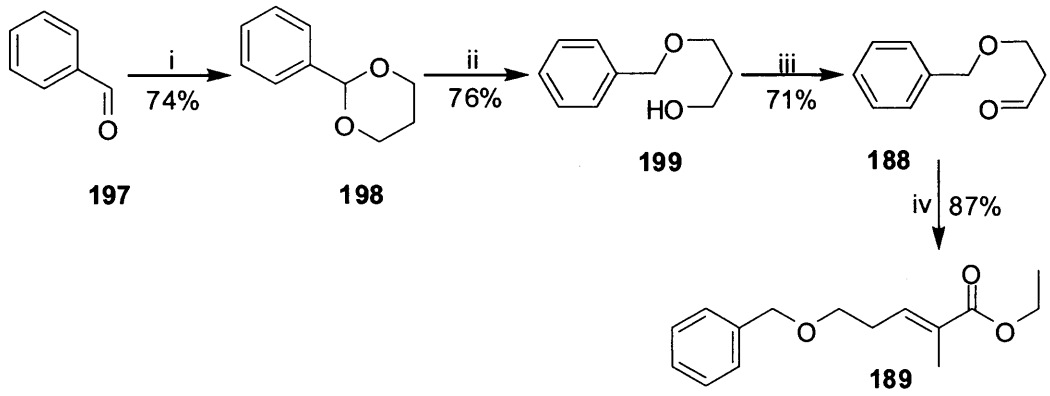
This approach to the (-)-calyculin A core is flexible, and would allow other alkynes to be used in the synthesis; thus the generation of quite different spiroketals using this route is possible. It is also a concise synthesis, incorporating stereocentres using well known and

established methodology and required only a few synthetic steps, making the route desirable in comparison to some of the longer reported syntheses.

4.2 Results and Discussion

4.2.1 Synthesis of the Weinreb Amide Fragment

Synthesis of the Weinreb amide fragment began with the preparation of **189**, the central scaffold of the molecule. The synthetic route used to obtain **189** can be seen in scheme 4.2.1. Reported syntheses of the alcohol **199**, including benzylation of propane-1,3-diol with benzyl chloride,[198] benzyl alcohol with acrolein,[199] were discounted, and the synthesis focused upon a reductive ring opening of the acetal **198** to furnish the required intermediate. Conventional synthetic methodology to effect the reductive ring opening, such as using DIBAL-H as reported by Martinelli[200] and Takano *et al.*,[201] or the use of borane-containing reagents as reported by Lewis *et al.*[202] were disfavoured due to the large quantities of material required from this particular stage. Instead, the use of lithium aluminium hydride modified with aluminium chloride as reported by Eliel *et al.*[203] was considered as an alternative. Oxidation, followed by Wittig homologation would then furnish **189**.

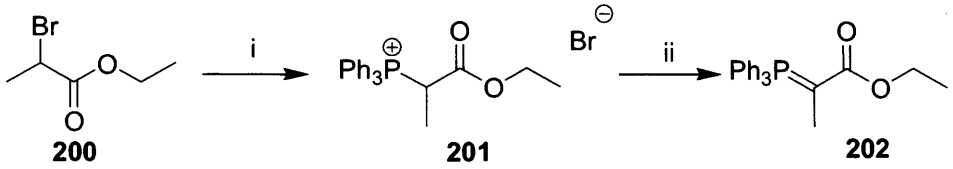


Scheme 4.2.1. synthesis of **189**. i. propane-1,3-diol, toluene, *p*-toluenesulfonic acid; ii. $AlCl_3$, $LiAlH_4$, Et_2O; iii. PCC, celite, dichloromethane; iv. **202**, toluene.

The protection of benzaldehyde with propane-1,3-diol was conducted by refluxing toluene using Dean-Stark apparatus with a small quantity of catalytic *p*-toluenesulfonic acid.[201] As the reaction progressed, water was removed as it was generated, and the volume of water produced was used to monitor the progress of the reaction. Once complete, concentration of the reaction mixture under reduced pressure gave the material

198 as a colourless crystalline solid achieved in 74% yield. Lewis acid mediated reductive ring opening of the crude acetal using a 4:1 ratio of aluminium trichloride and lithium aluminium hydride in dry diethyl ether at 0 °C gave the crude alcohol **199**. Distillation at approximately 2 torr between 105 and 110 °C, gave **199** as a colourless oil in 76% yield.

Oxidation of the alcohol **199** to the aldehyde **188** was achieved by adding the alcohol to a suspension of Celite and pyridinium chlorochromate (PCC) in dichloromethane, and stirring for 4 hours.[204-207] Filtration of the reaction mixture through a small silica plug removed all Celite and chromium material. The aldehyde **188** was contained within the filtrate, and concentration under reduced pressure yielded the required material in high purity; however, short path flash chromatography was used to increase the purity of the sample, and to ensure no chromium impurities were present prior to storage.

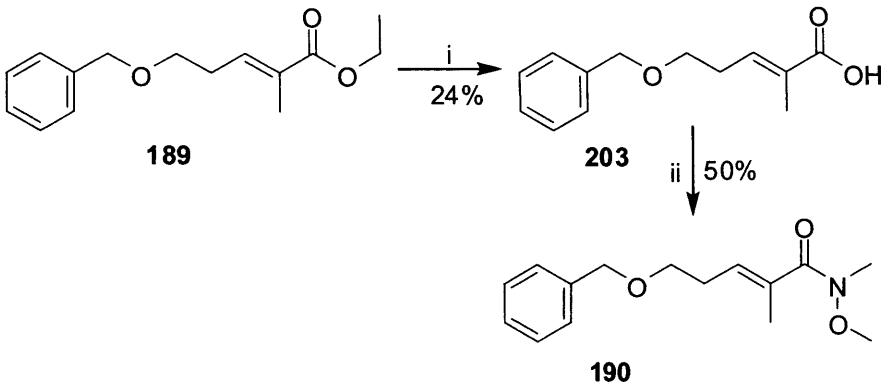


200 **201** **202**

Scheme 4.2.2. Preparation of **202**. i. PPh_3, toluene; ii. 1 M NaOH.

The stabilised ylid **202** was prepared, which was required for the synthesis of **189**. Triphenylphosphine was dissolved in toluene at 0 °C. Ethyl-2-bromopropionate was added, and the reaction allowed to warm up. After stirring for 12 hours at room temperature, a white precipitate could be easily removed from the reaction mixture, and after drying gave the phosphonium salt **201**. Treatment with base gave the stabilised phosphonium ylide **202** as bright yellow crystals (mp. 161-163 °C).[208]

Synthesis of **189** was prepared by a procedure similar to that described by Kodama *et al.*[209] The Wittig coupling of **188** and **202** furnished **189** in 87% yield as a pale yellow oil.[210,211]

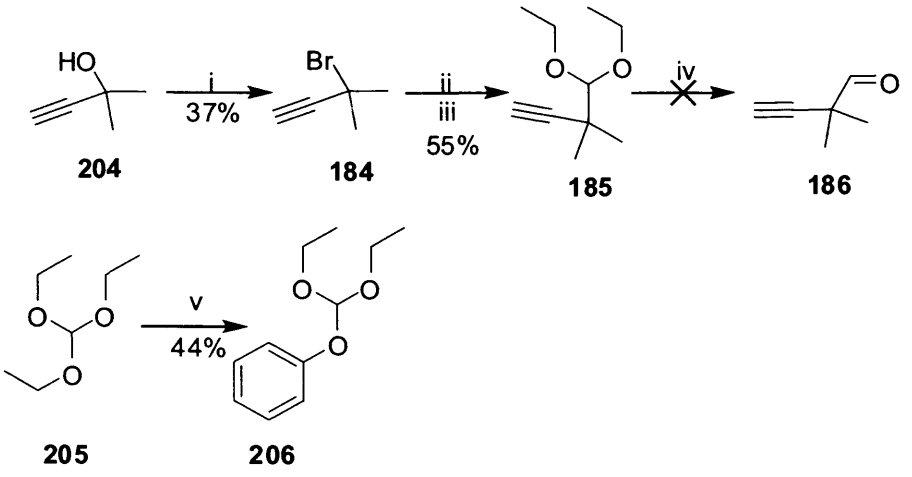


Scheme 4.2.3. Preparation of the Weinreb amide **190**. i. LiOH, THF:water:methanol; ii. ^{i}PrMgCl, MeNH(OMe).HCl, THF.

Saponification of the ester **189** proceeded with lithium hydroxide in a 1:1:1 mixture of THF:water:methanol at room temperature giving the acid in a surprisingly low yield. Conversion into the Weinreb amide was accomplished using the Merck protocol,[212,213] using isopropylmagnesium chloride and *N,N*-dimethylhydroxylamine hydrochloride in dry THF. After quenching and workup, **190** was recovered in 50% yield from the acid **203**.

4.2.2 Synthesis of the Second Fragment

Preparation of the acetylenic fragment was initially believed to be possible using the procedure illustrated in scheme 4.2.4.



Scheme 4.2.4. Preparation of the acetylenic fragment. i. PBr_3; ii. Mg, Et_2O; iii. (diethoxymethyl)benzene; iv. Acid catalysis; v. Phenol, *p*-toluenesulfonic acid.

From the readily available and inexpensive starting material, 2-methyl-3-yn-2-ol, synthesis of the bromide **184** was easily completed by the careful addition of phosphorus tribromide following a procedure described by Brückner and Rank.[214] Distillation of the reaction mixture at 100 torr, at 40 °C gave the product as a colourless lachrymatory oil in 37% yield.

Preparation of **206** was conducted using a procedure reported by Miginiac *et al.*[215] whereby triethyl orthoformate is heated in the presence of phenol, and a catalytic quantity of *p*-toluenesulfonic acid. Ethanol, produced in the reaction, is distilled from the reaction mixture and gives the desired product **206** after a basic work up and distillation under high vacuum.

The bromide **184** was converted into a the Grignard reagent using magnesium and a small quantity of iodine. **206** was added to the reaction and the mixture stirred overnight. Quenching, and work up gave the desired material in 55% yield.

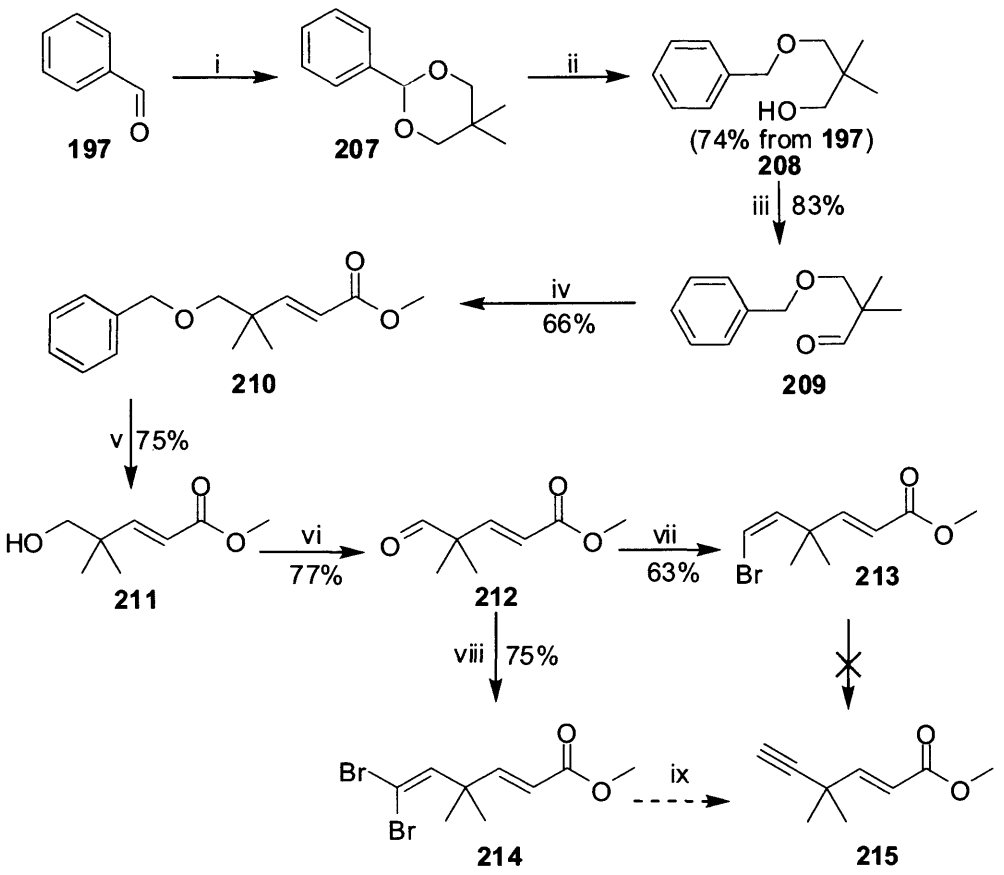
Attempts to generate the acetylenic aldehyde **186** through hydrolysis of the acetal **185** was unsuccessful. Several methods were conducted, oxalic acid, formic acid, and acetic acid catalysed hydrolysis all failed, generating inseparable mixtures.[196,216] Trapping of any aldehyde formed in solution was also attempted using Wittig chemistry, using (triphenyl-λ^5-phosphanylidene)-acetic acid ethyl ester. No products were found to be

formed in the reaction. Due to low yielding reactions, and failure of the synthetic route to generate the aldehyde **186**, alternative methods of synthesising the required fragment was explored.

4.2.3 An Alternative Acetylene Synthesis

An alternative to the synthesis to the acetylene fragment was sought in an analogous synthetic procedure to the one used in the construction of the Weinreb amide fragment.

Starting from benzaldehyde and using 2,2-dimethyl-1,3-propanediol as the basis for the core of the acetylenic fragment, a synthetic procedure was devised to generate **215,** as illustrated in scheme 4.2.5.



Scheme 4.2.5. Preparation of the acetylene fragment. i. 2,2-dimethyl propane-1,3-diol, toluene, *p*-toluenesulfonic acid; ii. $AlCl_3$, $LiAlH_4$, Et_2O; iii. $Py.SO_3$, DMSO, dichloromethane, Et_3N; iv. triphenylphosphanylidene acetic acid methyl ester, toluene, reflux; v. $AlCl_3$, dichloromethane; vi. PCC, Celite, dichloromethane; vii. bromomethyltriphenylphosphonium bromide, toluene; viii. CBr_4, PPh_3, dichloromethane, -15 °C; ix. *n*-BuLi, THF.

Benzaldehyde was converted into the acetal **207** using 2,2-dimethyl propane-1,3-diol in toluene and a catalytic quantity of acid. A Dean-Stark apparatus was used to remove water from the reaction, thus driving it to completion. After approximately 4 hours, the reaction mixture was concentrated, and material recovered from the reaction used crude in the next synthetic step. Aluminium chloride and lithium aluminium hydride were used in diethyl ether to reductively ring open the acetal, to give the benzyloxy protected core of the acetylenic fragment **208** in 74% yield overall.[203]

Introduction of the alkene moiety to the fragment was accomplished by first oxidising the alcohol **208** to the aldehyde using the mild, and highly successful Parikh-Doering procedure,[217] followed by Wittig coupling to generate an α,β-unsaturated ester. This

furnished the material **210** containing a benzyl ether protected alcohol, intended for subsequent deprotection and modification to generate the acetylene, and an alkene which is to undergo future modification in the synthesis of the spiroketal core.

In order to generate the acetylene moiety of the molecule, it was necessary to remove the benzyloxy methyl protecting group. Most traditional methods of removal, the use of palladium, Raney nickel, or Birch reduction are not suitable as they would interfere with the ester or the alkene portions of the molecule. An aluminium chloride mediated deprotection, previously reported by Akita *et al.*[218,219] and a similar method by Ozaki *et al.*[220] were chosen due to the mild conditions, the rapidity, and the compatibility of the reaction to olefin, and ester functionality. Deprotection of **210** was conducted in dichloromethane with 5 equivalents of aluminium chloride and a small quantity of *m*-xylene. The reaction progressed rapidly at 0 °C, and was quenched after 10 minutes by pouring the reaction mixture into a stirred mixture of ice and concentrated hydrochloric acid. Extraction of organic material gave the alcohol **211** in 75% yield. Successfully deprotected, the fragment could now be modified to incorporate the acetylene moiety.

Conversion into the acetylenic fragment was attempted through the aldehyde **212**, conveniently prepared by PCC oxidation of **211** in 77% yield. Initial synthetic attempts involved were based upon reports by Corey, involving the base mediated dehydro-halogenation of a vinyl halide.[221] Preparation of a suitable vinyl alkene was accomplished using bromomethyltriphenylphosphonium bromide,[222-224] in a Wittig reaction. Unfortunately, treatment of **213** with base failed to generate any of the required acetylenic material.

Other methods of acetylenic homologation were considered; of particular note was the use of Seyferth-Gilbert reagent[225-227] in an Ohira-Bestmann[228,229] reaction. The Ohira-Bestmann reaction, however, was rejected in favour of the two stage Corey-Fuchs reaction,[230-233] due to concerns regarding the diazo Seyferth-Gilbert reagent. The Corey-Fuchs reaction was also advantageous in that over the two stages of acetylene generation, it would be convenient for the removal of the α,β-unsaturated ester which would be incompatible with later synthetic stages.

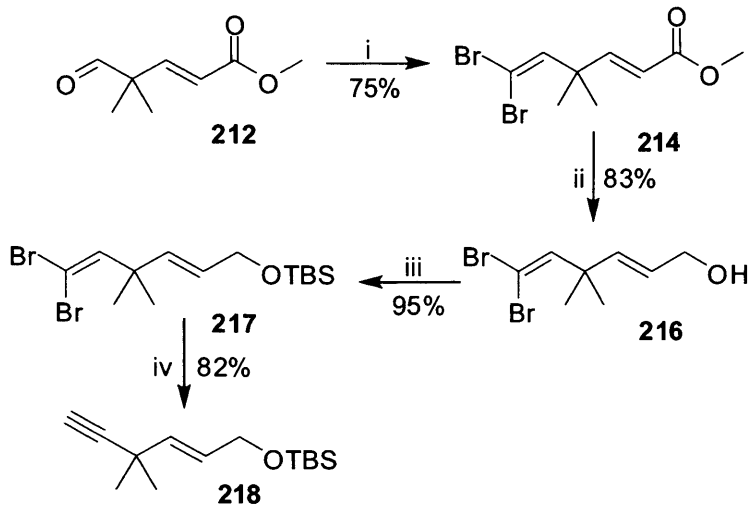
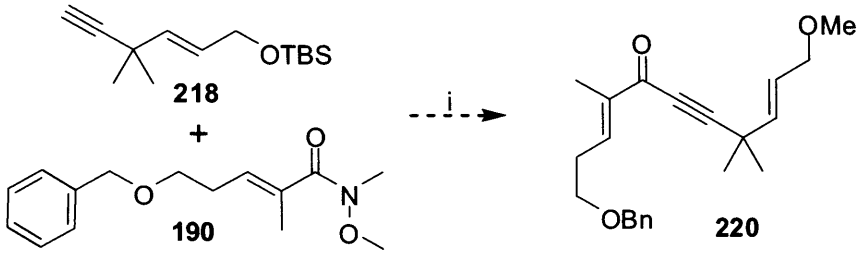


Figure 4.2.6. Synthesis of the acetylene – modification of the ester moiety. i. CBr_4, PPh_3, dichloromethane, -15 °C; ii. DIBAL-H, Et_2O, -78 °C; iii. TBS-Cl, imidazole, DMF; iv. *n*-BuLi, THF.

The first stage of the Corey-Fuchs reaction was conducted in a solution of dry dichloromethane at -15 °C, into which triphenylphosphine and carbon tetrabromide was added, followed shortly after by the aldehyde **212**. The reaction was stirred for 1 hour, and yielded the dibromoolefin **214** in 75% yield. At this stage it was concluded that it would be convenient for the conversion of the α,β-unsaturated ester into a less reactive moiety, and was rapidly reduced to the alcohol **216** in 83% with the use of diisobutyl aluminium hydride. The resulting primary alcohol was transformed into the *tert*-butyldimethylsilyl ether, using *tert*-butyldimethylsilyl chloride, and imidazole in dimethyl formamide with a yield of 95%. Finally, the acetylenic fragment was completed by treatment of **217** with 2 equivalents of *n*-butyllithium in tetrahydrofuran at -78 °C to give **218** in 82% yield.

4.2.4 Coupling of the Fragments

With the completion of the two fragments, attention was focused upon combining them to generate the central scaffold of the spiroketal fragment.

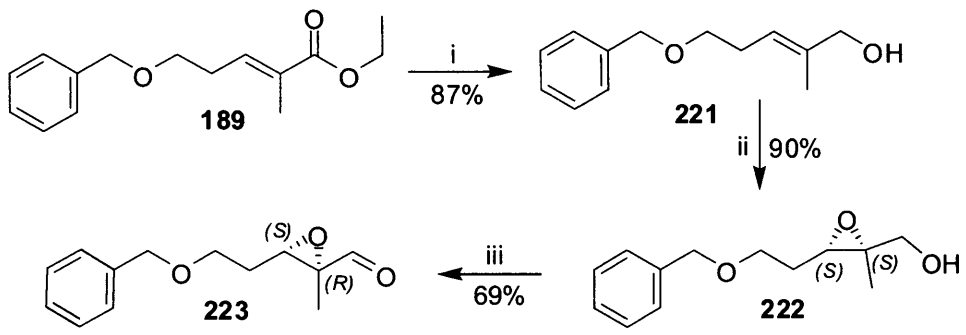


Scheme 4.2.7. Coupling of the two fragments. i. *n*-BuLi, THF, -78 °C to 0 °C.

Examination of the coupling of the Weinreb amide **190** to the alkyne **218** and subsequent procedures thereafter, indicated that the route could be suboptimal, and that it may not be advantageous to couple the two fragments prematurely. As opposed to conducting Alpine borane reduction[234] and Sharpless asymmetric epoxidation reactions upon the fully constructed carbon scaffold, it would be preferable to conduct the Sharpless epoxidation on the precursor **221** prior to coupling. Oxidation to the aldehyde would then enable the coupling to proceed, and would obviate the need for Alpine borane reduction.

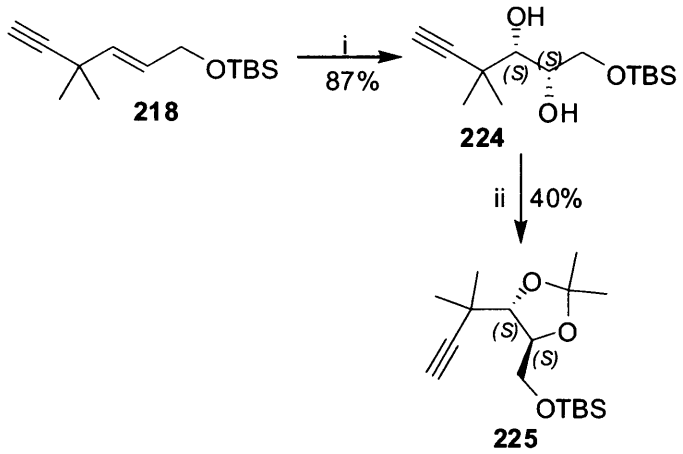
Reduction of the ester **189** to the alcohol **221** was conducted using a suspension of lithium aluminium hydride in dry diethyl ether at 0 °C, with a yield of 87% after quenching with hydrochloric acid and work up. Sharpless asymmetric epoxidation[235-238] of **221** using *tert*-butyl hydroperoxide and titanium isopropoxide according to a procedure reported by Marshall *et al.*[210] gave the material **222** in 90% yield. Derivitisation to the Mosher ester[239,240] and analysis of the ^{1}H NMR spectrum quantified the e.e. to be 84%.

Oxidation using Parikh-Doering[217] conditions gave the epoxy aldehyde **223** in 69% yield.



Scheme 4.2.8. Preparation of the epoxy aldehyde **223**. i. $LiAlH_4$, Et_2O, 0 °C; ii. Sharpless asymmetric expoxidation; iii. $Py.SO_3$, Et_3N, DMSO, dichloromethane.

In preparation for the coupling of the two fragments, concerns developed around the compatibility of the unsaturated pyranone ring after cyclisation, and a future Sharpless asymmetric dihydroxylation required to generate the final spiroketal core. In order to address the potential conflict, a second modified acetylene fragment was prepared. The new acetylene fragment would undergo dihydroxylation prior to coupling, and would therefore negate any future incompatibilies. The modification of the acetylene can be seen in scheme 4.2.9.

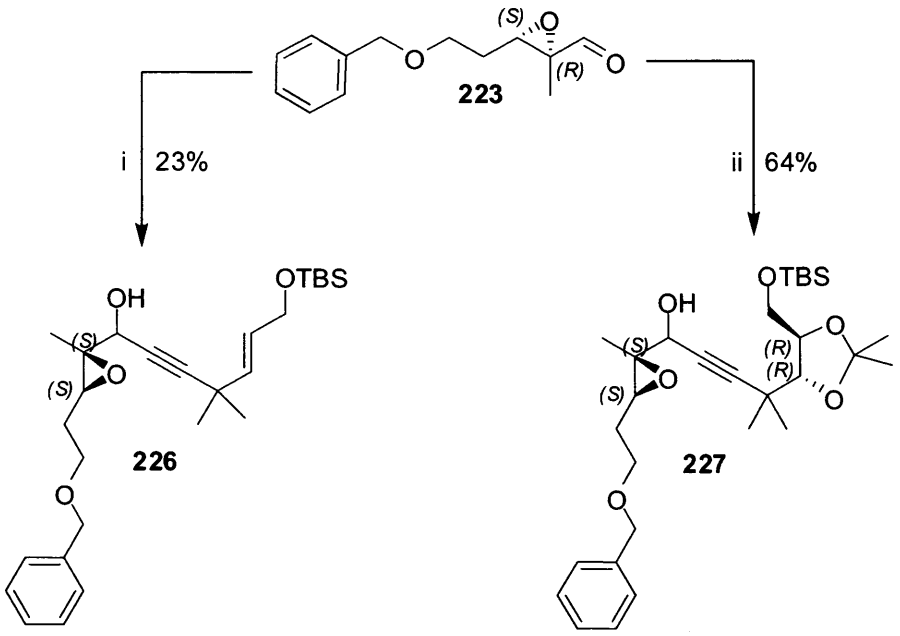


Scheme 4.2.9. Modification of the acetylene fragment. i. Sharpless asymmetric dihydroxylation; ii. 2,2-dimethoxypropane, (+/-)-CSA, acetone.

The acetylene **218** was dihydroxylated using AD-mix β, in a 1:1 mixture of *tert*-butyl alcohol and water according to a general procedure reported by Sharpless *et al.*[241] The diol was then protected as the acetal, giving **225** in a low yield of 40%. This is believed

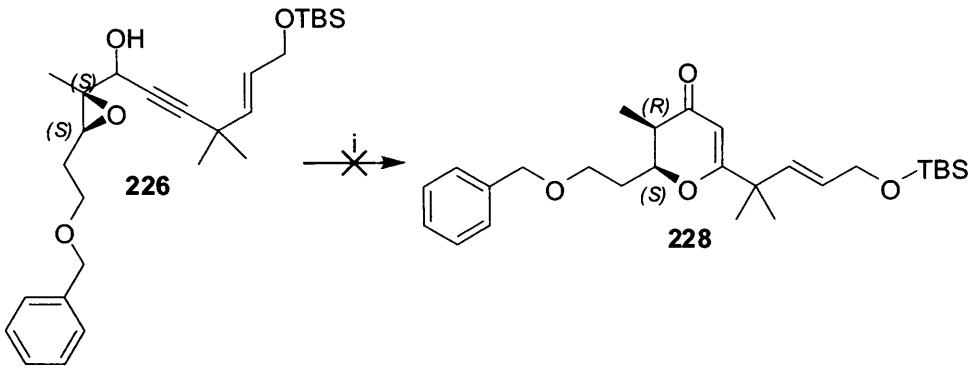
to be due to the cleavage of the protecting group during purification, generating the diol which was recovered after collection of **225**.

Coupling of the acetylenes to the epoxy aldehyde **223** proceeded in THF, using *n*-butyllithium to generate the lithium acetylide. Addition of the epoxy aldehyde then furnished the corresponding scaffold fragment **226** or **227**.



Scheme 4.1.10. Coupling of the aceylenes **225** and **218** to the epoxy aldehyde **223**. i. *n*-BuLi, THF, **218**; ii. *n*-BuLi, THF, **225**.

Having assembled the carbon framework, the compounds were ready to be subjected to the mercury mediated cyclisation conditions to generate 2,3-dihydropyran-4-one systems. An illustration of the reactions, and the expected products can be seen in schemes 4.2.11 and 4.2.12.



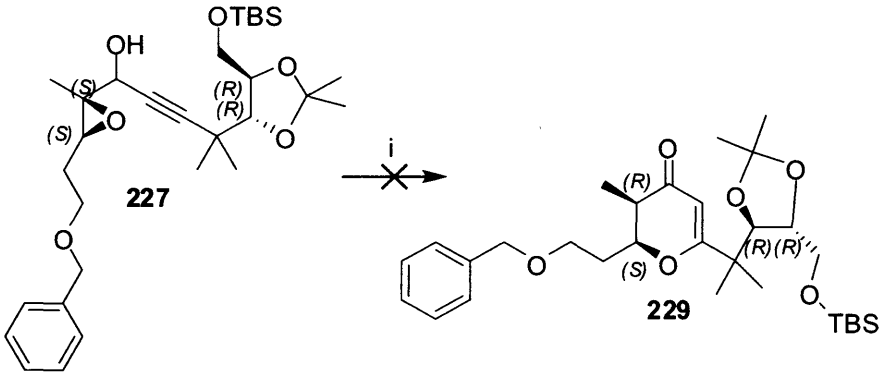
Scheme 4.2.11. The mercury catalysed cyclisation of **226**. i. HgO, dilute acid, acetone.

The material **226** (scheme 4.2.11) was taken and placed into a vessel containing analytical grade acetone. A solution of mercury(II) in dilute acid was freshly prepared by dissolving yellow mercury(II) oxide into dilute sulphuric acid. After ensuring that the solution was clear and no precipitates had formed, a small quantity was added to the reaction mixture. TLC samples were taken and run at two minute intervals to assess the progress of the reaction. As the reaction progressed, using cerium(IV) sulfate to visualise the epoxide, it was possible to clearly see the consumption of the starting material. Visualisation using anisaldehyde solution revealed the generation of products on the TLC plate. Once all starting epoxide had been consumed, sodium hydrogen carbonate was added to quench the reaction, and the mixture stirred for 1 hour. The reaction mixture, once quenched was filtered to remove solids, and was purified using column chromatography. Material that was recovered was analysed using ^{1}H and ^{13}C NMR spectroscopy.

Analysis of the reaction products indicated that the required pyranone system had not been formed. ^{1}H and ^{13}C NMR spectra showed quite clearly that the protecting silyl group had been cleaved from the molecule. The acetylene moiety was not present, indicating that some form of cyclisation had occurred; however, the NMR spectra also indicated that the pyranone system was not present, and indicated the formation of aldehyde functionality. This is tentatively attributed to a 1,2-acetylenic rearrangement forming an aldehyde, followed by subsequent mercury mediated cyclisation forming a furanone system. Further studies will be required to confirm this conclusively.

The second constructed scaffold (**227**) was placed into the mercury mediated cyclisation conditions. TLC samples were taken at 2 minute intervals, and developed using ceric

sulphate solution. Once all starting epoxide had been consumed, sodium hydrogen carbonate was added, and the mixture stirred for 1 hour. Upon work-up, the material was purified using column chromatography, and analysed by ^{1}H and ^{13}C NMR spectroscopy.

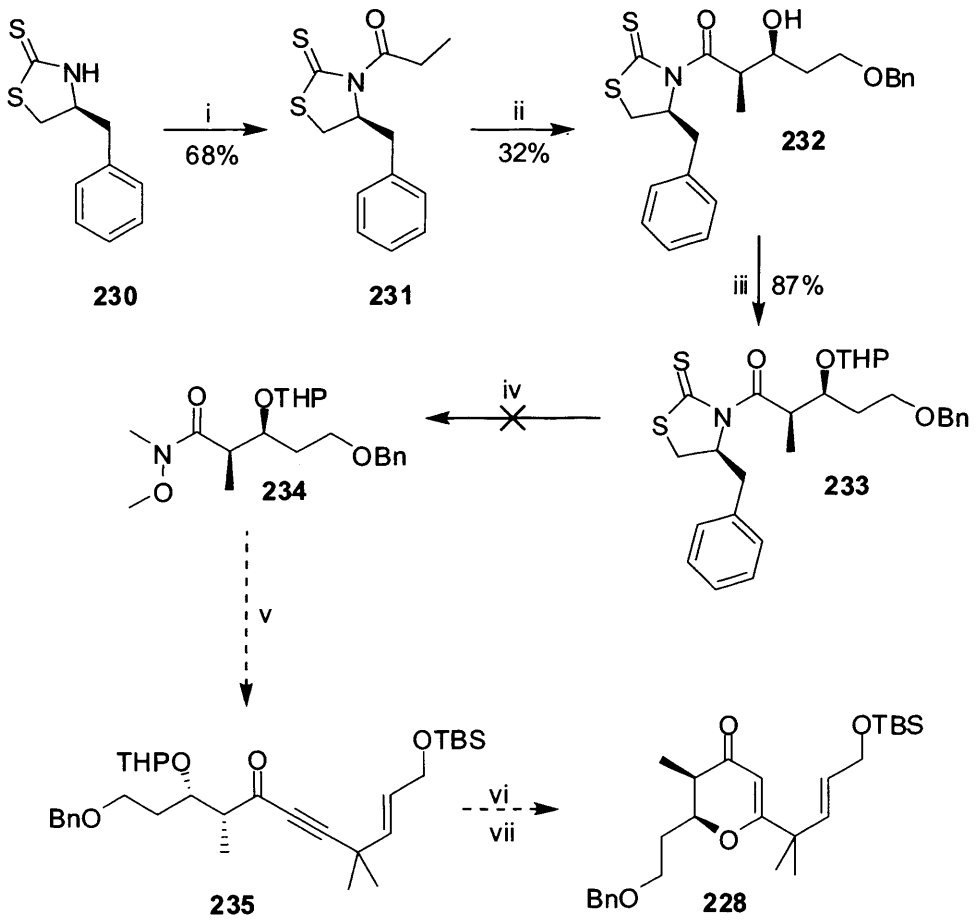


Scheme 4.2.12. The mercury catalysed cyclisation of **227**. i. HgO, dilute acid, acetone.

In this case, analysis revealed again that again the pyranone system had not been formed. It was hoped that the expected cyclisation, followed by acid catalysed deprotection of the acetal protected diol would lead to the required spiroketal product directly. NMR spectra show, in contrast to the previous olefinic adduct **226**, that the silyl protecting group was present and intact, as was the acetal protected diol. Surprisingly, the benzyloxy protecting group was found to be absent, and it can be concluded that it was cleaved from the molecule during the course of the reaction.

4.2.5 The Alternative Evans Auxiliary Route to the Spiroketal Core

The failure of the two scaffolds to cyclise in the manner that would have furnished the required intermediates to the spiroketal core prompted the investigation of simpler systems that could undergo a similar mercury mediated cyclisation. A method was devised to synthesise the (+)-calyculin A spiroketal fragment similar to that described by Smith *et al.*,[192,193] to allow comparison of published spectral data to that which would hopefully be obtained from a correctly constructed fragment. An illustration of the synthetic route can be seen in scheme 4.2.13.



Scheme 4.2.13. Preparation of the (+)-calyculin A core. i. Propionyl chloride, Et_3N, dichloromethane; ii. 3-benzyloxypropionaldehyde, $TiCl_4$, (-)-sparteine, dichloromethane -78 °C; iii. 3,4-dihydro-2*H*-pyran, THF, *p*-toluenesulfonic acid, 0 °C; iv. MeNH(MeO).HCl, Et_3N; v. **218**, *n*-BuLi, THF, -78 °C; vi. cat. *p*-toluenesulfonic acid; vii. HgO, acetone.

Construction of the compound **232** by Evans aldol addition to **231** would prepare the two key stereocentres for the correct formation of the pyranone ring. Protection of the alcohol **232** as a tetrahydropyran moiety, followed by metal acetylide addition to the Weinreb amide **234** would form the scaffold **235**. Deprotection, and Hg^{+2} catalysed cyclisation should yield **236**. Through simplification of the cyclisation and removal of the epoxide, it was hoped that the precursor **235** would cyclise cleanly, and without further complications that have plagued the previous epoxide scaffolds.

Preparation of the thiazolidinethione auxiliary was conducted according to a procedure reported by Delaunay *et al.*[242] whereby (*S*)-phenylalaninol was heated to reflux in the presence of 1 M potassium hydroxide and carbon disulfide. **231** was prepared according to methodology reported by Crimmins *et al.*[243,244] yielding a bright yellow crystalline material that could be easily purified through recrystallization from hexane. Formation

of the enolate at 0 °C using 1 equivalent of (-)-sparteine, and titanium tetrachloride in dry dichloromethane, gave the *anti*-Evans aldol addition product **232**.[244] Undesired material was easily separated from the required diasteroisomer using column chromatography, the compound being bright yellow in appearance, could be visually identified as it passed through the column and could be preferentially collected. THP protection of the aldol adduct, yielded **233**, and attempts were conducted to convert the fragment to the Weinreb amide **234**. It has been reported that thiozolidinethione auxiliary can be easily displaced by nucleophiles, thus increasing the synthetic utility of the auxiliary in general. It was this property that underlied the selection of the auxiliary over the more traditional Evans auxiliaries, and it was expected that the conversion would be straightforward, with high yielding results. In practice, the generation of the Weinreb was not possible, and attempts to generate the Weinreb amide, using a variety of bases failed.

It is believed that this approach to the spiroketal fragment would be successful, perhaps through conversion to the aldehyde with DIBAL-H, addition of the acetylene portion, and oxidation to the ketone **235**. It would also be possible to remove the thiozolidinethione auxiliary and utilise traditional Evans oxazolidinones in an effort to generate **234**; however, time constraints limited the ability to explore the area fully and investigate this fragment.

4.3 Conclusions

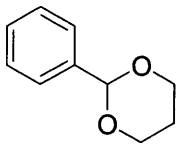
The construction of the (-)-calyculin A spiroketal fragment using the epoxy alkynols **226** and **227** failed. Premature cleavage of the protecting groups is one explanation for the failure of the cyclisation; however, the cleavage is clearly not the only adverse process that occurs to the precursors. Future investigation into the effects of mercury mediated cyclisation on large substituted systems would be beneficial, and may aid in the choice of protecting group to one which is unaffected by the Hg^{+2} cyclisation conditions.

Use of the Evans aldol fragments is a promising alternative to the epoxy alkynols approach, and the simplified system would generate the required pyranone systems. Future work focused upon producing fragments such as **234**, should be successful in achieving the synthesis of the calyculin spiroketal fragments.

4.4 Experimental

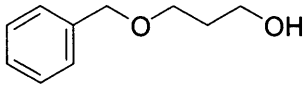
The general experimental section can be found in chapter 1.4.

2-Phenyl-[1,3]dioxane[203] (198)



Benzaldehyde (27.9 g, 263 mmol), *p*-toluenesulfonic acid (0.08 g, catalyst), and propane-1,3-diol (20 g, 263 mmol), in toluene (80 mL), was heated to reflux in a Dean-Stark apparatus for 4 h. The mixture was then allowed to cool. The reaction mixture was then concentrated to a minimum under reduced pressure. Upon standing, crystallisation occurred, yielding **198** (32.0 g, 74.1%) as a colourless crystalline solid. Analysis correlates to previously described material. ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 138.8, 128.8, 128.3, 126.0, 101.7, 67.4, 25.8.

3-Benzyloxy-propan-1-ol[203,245,246] (199)

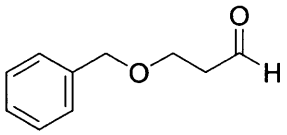


Diethyl ether (100 mL) was placed in a dry round bottomed flask, and cooled to 0 °C. $AlCl_3$ (7.52 g, 131 mmol) was added portionwise, cautiously. The mixture was stirred for 30 min at 0 °C as dissolution occurred. $LiAlH_4$ (1.25 g, 32.9 mmol) was added to the reaction mixture portionwise, and the mixture kept at 0 °C after addition for a further 30 min. 2-Phenyl-[1,3]-dioxane (10.788 g, 65.7 mmol) was diluted into ether (100 mL) and added to the reaction vessel dropwise through an addition funnel. The reaction was allowed to warm to room temperature, and was stirred for 2 h. The mixture was quenched through the addition of 10% sulfuric acid (100 mL, dropwise at first), and the resulting suspension filtered through celite. The organic fractions were separated, dried (Na_2SO_4), and concentrated to a pale yellow oil. Distillation of the crude oil (approx 2 torr, 105-110 °C) gave **199** as a colourless oil (8.300 g, 76%). ^1H NMR (300MHz, $CDCl_3$), δ_H 7.48-7.36 (m, 5H, Ar-H), 4.63 (s, 2H, Ph-CH_2-O), 3.87 (t, J=5.7 Hz, 2H, HO-CH_2-CH_2), 3.76 (t, J=5.8 Hz, 2H, O-CH_2-CH_2), 2.63 (br, 1H, OH), 2.01 - 1.93 (m, 2H, CH_2-CH_2-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 138.6, 128.8, 128.1, 128.0, 73.6, 69.3, 61.6, 32.7. Analysis was consistent with that of previously described material.

Pyridinium Chlorochromate (PCC)

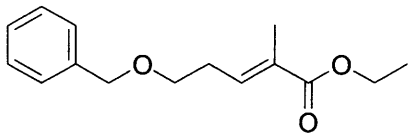
Chromium trioxide (10 g, 100 mmol) was dissolved in HCl (6M, 20 mL) and cooled to 0 °C. Pyridine (8.06 mL, 100 mmol) was introduced to the reaction mixture dropwise over a 10 min. The mixture was re-cooled to 0 °C resulting in the generation of a bright orange precipitate. The solid was obtained by vacuum filtration and was dried in a desiccator, to give 19.0 g, 88%, of the product as a bright orange solid.

3-Benzyloxy-propionaldehyde[245-247] (188)



To a stirring suspension of PCC (2.59 g, 12 mmol), celite (2.6 g), and dichloromethane (20 mL), the alcohol 3-benzyloxy-propan-1-ol (0.5 g, 3 mmol) was added in one portion. The mixture was stirred at room temperature for 4 h. The mixture was filtered through a silica plug, and the filtrate concentrated to an oil. Chromatography on silica gel (4:1 hexane:ethyl acetate) afforded **188** (0.35 g, 71%) as a colourless oil. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 9.83 (s, 1H, CHO-CH_2), 7.63 - 7.51 (m, 5H, Ar-H), 4.78 (s, 2H, Ph-CH_2-O), 4.06 (t, J=6.1 Hz, 2H, O-CH_2-CH_2), 2.92 (t, J=6.1 Hz, 2H, CH_2-CH_2-CHO). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 201.3, 138.3, 128.8, 128.2, 128.1, 73.6, 64.3, 44.3, 35.6, 29.5. Analysis was consistent with that of previously described material.

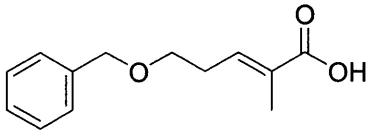
(2*E*)-5-Benzyloxy-2-methylpent-2-enoic acid ethyl ester[210] (189)



3-Benzyloxypropionaldehyde (2.38 g, 1.45 mmol), 2-(triphenyl-λ5-phosphanylidene)-propionic acid ethyl ester (0.525 g, 1.45 mmol), and dry dichloromethane (5 mL) were combined in a vessel purged with an inert atmosphere. The reaction mixture was heated to reflux until all staring material had been consumed by TLC using an eluant of 4:1 hexane:ethyl acetate. The reaction mixture was concentrated under reduced pressure to an oil. Ethyl acetate was added (10 mL) and the resulting solution passed through a short silica plug. The product containing solution was concentrated under reduced pressure to an oil, whereupon hexane was added. Filtration of the solution followed by concentration under reduced pressure gave **189** (0.318 g, 87%) as a pale yellow oil. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 7.55 - 7.46 (m, 5H, Ar-H), 6.99 (t, J=7.1 Hz,

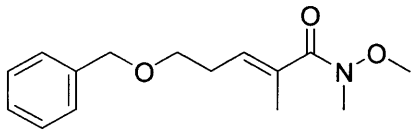
1H, CH_2-CH=C), 4.73 (s, 2H, Ph-CH_2-O), 4.36 (q, J=7.1 Hz, 2H, O-CH_2-CH_3), 3.77 (t, J=6.7 Hz, 2H, O-CH_2-CH_2), 2.73 - 2.66 (m, 2H, CH_2-CH_2-CH), 2.06 (s, 3H, CH_3-C), 1.47 (t, J=7.1 Hz, 3H, CH_3-CH_2-O). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 168.0, 138.3, 129.5, 128.4, 127.7, 73.0, 68.6, 60.5, 29.4, 14.3, 12.6. Analysis correlated with previously reported data.[211]

5-Benzyloxy-pent-2-enoic acid (203)



5-Benzyloxy-2-methylpent-2-enoic acid ethyl ester (1.0 g, 4 mmol) and lithium hydroxide (0.192 g, 8 mmol) were dissolved in a 3:1:1 mixture of THF, methanol, and water. The mixture was stirred for 2 h, followed by TLC using an eluant of 4:1 hexane:ethyl acetate. Upon completion, the mixture was diluted with ethyl acetate and was extracted with 2M hydrochloric acid and brine respectively. Organic fractions were dried ($MgSO_4$), and concentrated under reduced pressure to an oil. Chromatographic purification using an eluant of 4:1 hexane:ethyl acetate gave **203** as a white solid (0.194 g, 24%). ^{1}H NMR (300MHz, $CDCl_3$), δ_H 7.64 - 7.49 (m, 5H, Ar-H), 7.19 (t, J=6.2 Hz, 1H, CH_2-CH=C), 4.77 (s, 2H, Ph-CH_2-O), 3.82 (t, J=6.6 Hz, 2H, O-CH_2-CH_2), 2.79 - 2.72 (m, 2H, CH_2-CH_2-CH), 2.10 (s, 3H, CH_3-C). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 173.6, 138.6, 132.5, 129.2, 128.8, 128.1, 73.6, 68.9, 30.1, 12.7.

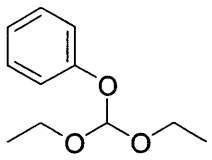
5-Benzyloxy-pent-2-enoic acid methoxymethylamide (190)



To a slurry of 5-benzyloxy-2-methylpent-2-enoic acid ethyl ester (0.1 g, 0.4 mmol), *N*,*N*-dimethylhydroxylamine hydrochloride (0.059 g, 0.6 mmol), in dry THF (5 mL) was added isopropylmagnesium chloride (1.2 mmol) over 15 min at -10 °C. After aging at -10 °C for 30 min, 25% w/w ammonium chloride solution (3 mL) was added to quench the reaction. Ethyl acetate was added (5 mL), and the mixture separated. Organic fractions were dried ($MgSO_4$), and concentrated under reduced pressure to an oil. Purification by flash column chromatography using an eluant of 2:1 hexane:ethyl acetate gave **190** as a colourless oil (49 mg, 50%). ^{1}H NMR (300MHz, $CDCl_3$), δ_H 7.34 - 7.22 (m, 5H, Ar-H), 5.87 (t, J=7.1 Hz, 1H, CH_2-CH=C), 4.47 (s, 2H, Ph-CH_2-O), 3.58 (s, 3H, CH_3-O), 3.53 (t, J=6.7 Hz, 2H, O-CH_2-CH_2), 3.18

(s, 3H, N-$\underline{CH_3}$), 2.46-2.39 (m, 2H, CH_2-$\underline{CH_2}$-CH_2), 2.14 (s, 3H, $\underline{CH_3}$-C). HRMS calcd. for $C_{15}H_{21}NO_3$ 263.1521, found 263.1525.

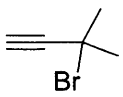
(Diethoxymethyl)benzene[215] (206)



Phenol (5 g, 53 mmol), triethyl orthoformate (7.86g, 53 mmol), and *p*-toluene sulfonic acid (0.05 g) were placed into distillation apparatus and heated to 40 °C at approx. 150 torr, removing ethanol from the mixture. The crude product was washed with 5M sodium hydroxide and water respectively. Organic extracts were dried ($NaCO_3$), filtered, and distilled at 75-80 °C under high vacuum to give **206** as a colourless oil (4.5 g, 44%). Analysis is consistent with data previously reported.

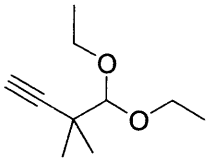
^{1}H NMR (300MHz, $CDCl_3$), δ_H 7.31-7.03 (m, 5H, Ar-$\underline{H}$), 5.72 (s, 1H, O-$\underline{CH}$(O)-O), 3.81 - 3.71 (m, 4H, CH_3-$\underline{CH_2}$-O), 1.26 (t, J=7.1 Hz, 6H, $\underline{CH_3}$-CH_2-O).

3-Bromo-3-methyl-but-1-yne[214] (184)



A vessel was charged with 2-methyl-3-yn-2-ol (0.5 g, 5.9 mmol), to which was added PBr_3 (0.22 mL, 2.36 mmol) dropwise. The mixture was stirred for 30 min at room temperature. The reaction mixture was distilled at approx. 100 torr, 40 °C (lit. 30 °C, 80 torr). **184** was retrieved as a colourless oil (0.316 g, 37%). Analysis correlates to data previously reported. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 2.62 (s, 1H, $\underline{HC}$≡C), 1.93 (s, 6H, C($\underline{CH_3}$)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 36.5, 47.3, 73.0, 88.0.

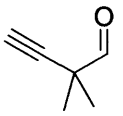
4,4-Diethoxy-3,3-dimethylbut-1-yne[214] (185)



An argon purged vessel containing activated magnesium turnings (0.1 g) was charged with dry THF (15 mL) and stirred. 3-Bromo-3-methyl-but-1-yne (0.5 g, 3.4 mmol) was added to the vessel, and the mixture stirred at room temperature for 1 h. (Diethoxymethyl)benzene (0.667 g, 3.4 mmol) was added, and the mixture stirred overnight. The reaction was quenched with the addition of sat. ammonium chloride solution (5 mL) at 0 °C. Dichloromethane (15 mL) was added, and the reaction extracted. The organic fraction was then washed with 5M NaOH (10 mL), brine (5 mL), and dried (Na_2CO_3), and concentrated to an oil to give **185** (0.318 g, 55%). Analysis correlated with previously reported data.

2,2-dimethylbut-3-ynal (186)

Method 1:



To a flask containing 4,4-diethoxy-3,3-dimethylbut-1-yne (0.1 g, 0.6 mmol), and diethyl ether (2 mL), oxalic acid dihydrate (0.074 g, 0.6 mmol) was added. The mixture was stirred and monitored using TLC. Upon the disappearance of starting material, the reaction mixture was washed with water (1 mL). The extracted organic fractions were combined, and dried with $MgSO_4$. Concentration gave crude material that did not contain **186**.

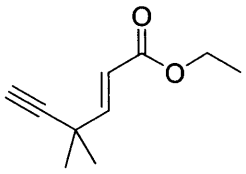
Method 2:

To a flask containing 4,4-diethoxy-3,3-dimethylbut-1-yne (0.1 g, 0.6 mmol), and pentane (2 mL), formic acid (1 mL) was added. The mixture was stirred and monitored using TLC. Upon the disappearance of starting material, the reaction mixture was washed with water (1 mL). The extracted organic fractions were combined, and dried with $MgSO_4$. Concentration gave crude material that did not contain **186**.

Method 3:

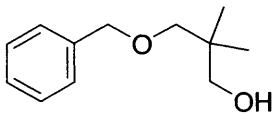
To a flask containing 4,4-diethoxy-3,3-dimethylbut-1-yne (0.1 g, 0.6 mmol), and pentane (2 mL), acetic acid (1 mL) was added. The mixture was stirred and monitored using TLC. Upon the disappearance of starting material, the reaction mixture was washed with water (1 mL). The extracted organic fractions were combined, and dried with $MgSO_4$. Concentration gave crude material that did not contain **186**.

(*E*)-Ethyl 4,4-dimethylhex-2-en-5-ynoate



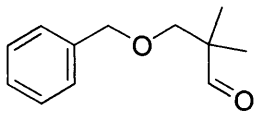
To a flask containing 4,4-diethoxy-3,3-dimethylbut-1-yne (0.1 g, 0.6 mmol), and toluene (2 mL), (triphenyl-λ^5-phosphanylidene)-acetic acid ethyl ester (0.42 g, 1.2 mmol) was added and the mixture warmed to 50 °C and stirred. The reaction was left for 12 h, and tested by TLC. (*E*)-Ethyl 4,4-dimethylhex-2-en-5-ynoate was not found to be formed.

3-Benzyloxy-2,2-dimethylpropan-1-ol (208)



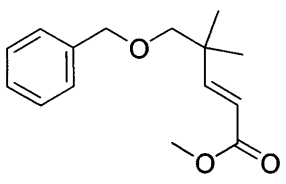
A mixture of benzaldehyde (20.38 g, 192 mmol), 2,2-dimethylpropane-1,3-diol (20 g, 192 mmol), and 80 mg of *p*-toluene sulfonic acid in 80 mL of toluene was heated in Dean-Stark apparatus to reflux. Azeotropic removal of water was continued until no more water could be removed from the reaction. The reaction was then concentrated under reduced pressure reducing the volume to approx. one tenth. Aluminium chloride (25.6 g, 192 mmol) was dissolved into 100 mL of dry diethyl ether at 0 °C under argon, and stirred for 30 min. Lithium aluminium hydride (3.64 g, 96 mmol) was then added, and stirred for a further 30 min at 0 °C. The crude reside was dissolved in 100 mL of dry diethyl ether, and added dropwise to the reaction mixture. Care was taken to maintain the temperature, and to prevent vigorous foaming during addition. The reaction was stirred at 0 °C for 3 h, and quenched with the addition of 5% sulfuric acid solution. The silver solution was filtered through celite, the residue washed with diethyl ether (3 x 20 mL), organic fractions combined and concentrated under reduced pressure to an oil. Distillation afforded **208** (27.5 g, 74%) as a colourless oil. IR ν_{max} (KBr, cm^{-1}) 3345 (OH). ^1H NMR (300MHz, $CDCl_3$), δ_H 7.34 - 7.26 (m, 5H, Ar-H), 4.49 (s, 2H, Ph-CH_2-O), 3.44 (s, 2H, HO-CH_2-C), 3.22 (s, 2H, O-CH_2-C), 0.93 (s, 6H, -$(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 138.2, 128.5, 127.7, 127.5, 79.4, 73.5, 71.7, 36.3, 21.9. HRMS calcd. for $C_{12}H_{18}O_2$ 194.1307, found 194.1308.

3-Benzyloxy-2,2-dimethylpropionaldehyde (209)



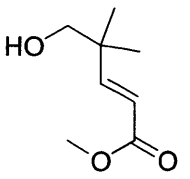
A solution of 3-benzyloxy-2,2-dimethylpropan-1-ol (14.2 g, 73.3 mmol), in dichloromethane (70 mL) and dimethylsulfoxide (14 mL), was prepared and stirred at 0 °C. Triethylamine (4.65 mL, 293 mmol) was added, followed by $Py.SO_3$ complex (23.3 g, 147 mmol). The reaction was stirred for 2 hours, and allowed to warm to room temperature. Ethyl acetate was added (100 mL), and the organic components washed with ice cold water (150 mL), saturated ammonium chloride (150 mL), and finally brine (20mL). Organic fractions were dried ($MgSO_4$), and concentrated under reduced pressure to an oil. Crude material was purified by column chromatography using an eluant of 2:1 hexane:ethyl acetate giving **209** (13.2 g, 83%) as a colourless oil. IR ν_{max} (KBr, cm^{-1}) 1729 (C=O). ^1H NMR (300MHz, $CDCl_3$), δ_H 9.57 (s, 1H, CHO-C), 7.37 - 7.05 (m, 5H, Ar-H), 4.47 (s, 2H, Ph-CH_2-O), 3.39 (s, 2H, O-CH_2-C), 1.09 (s, 6H-(CH_3)$_2$).

(2*E*)-5-Benzyloxy-4,4-dimethylpent-2-enoic acid methyl ester (210)



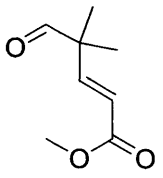
To 3-benzyloxy-2,2-dimethylpropionaldehyde (5 g, 26 mmol) in 100 mL of toluene was added triphenylphosphanylidene acetic acid methyl ester (8.7 g, 26 mmol) and heated to reflux for 5 h. After cooling, the majority of toluene was removed under reduced pressure, 40 mL of 4:1 hexane:ethyl acetate was added resulting in the precipitation of a white solid. Filtration through a plug of silica, followed by concentration of the filtrate afforded **210** (4.26g, 66%) as a colourless oil. IR ν_{max} (KBr, cm^{-1}) 1704 (C=O), 1610 (C=C). ^1H NMR (300MHz, $CDCl_3$), δ_H 7.38 - 7.25 (m, 5H, Ar-H), 7.03 (d, *J*=16.1 Hz, 1H, C-CH=CH), 5.82 (d, *J*=16.1 Hz, 1H, CH=CH-C(O)), 4.51 (s, 2H, Ph-CH_2-O), 3.70 (s, 3H, CH_3-O), 3.26 (s, 2H, O-CH_2-C), 1.10 (s, 6H, -(CH_3)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 167.4, 156.0, 138.4, 128.3, 127.5, 127.4, 118.4, 78.2, 73.3, 51.4, 38.3, 23.9. HRMS calcd. for $C_{15}H_{20}O_3$ 248.1412, found 248.1410.

(2*E*)-5-Hydroxy-4,4-dimethylpent-2-enoic acid methyl ester (211)



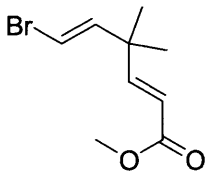
To aluminium chloride (0.267 g, 2 mmol) in 3 mL of dichloromethane at 0 °C, was added 0.1g (0.4 mmol) of 5-benzyloxy-4,4-dimethylpent-2-enoic acid methyl ester in xylene (0.63 mL). The mixture was stirred for 10 min. The reaction was then poured into a stirred mixture of ice (10 g) and concentrated HCl (1 mL). The aqueous mixture was then extracted with diethyl ether (3 x 10 mL), organic fractions were collected, washed with brine (10 mL), dried ($MgSO_4$), and then concentrated under reduced pressure to an oil. Purification by column chromatography (2:1 petroleum ether (40-60):ethyl acetate) gave **211** (0.047 g, 75%) as a colourless oil. Rf. 0.2 (2:1 petroleum ether (40-60):ethyl acetate). IR ν_{max} (KBr, cm^{-1}) 3350 (OH), 1705 (C=O), 1614 (C=C). ^1H NMR (300MHz, $CDCl_3$), δ_H 6.88 (d, J=15.8 Hz, 1H, C-CH=CH), 5.72 (d, J=15.8 Hz, 1H, CH=CH-C(O)), 3.58 (s, 3H, CH_3-O), 3.29 (s, 2H, HO-CH_2-C), 0.94 (s, 6H, -(CH_3)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 167.3, 155.4, 119.3, 70.9, 51.5, 39.2, 23.1. HRMS calcd. for $C_8H_{14}O_3$ 158.0943, found 158.0946.

(2*E*)-4,4-Dimethyl-5-oxo-pent-2-enoic acid methyl ester (212)



4,4-Dimethyl-5-oxo-pent-2-enoic acid methyl ester (1.75 g, 11.1 mmol) in 2 mL of dichloromethane was added to a stirring suspension of dichloromethane (25 mL), celite (4.79 g), and PCC (4.79 g, 2.22 mmol). The mixture was stirred at room temperature until complete by TLC. The reaction was filtered through silica, and the filtrate concentrated under reduced pressure, yielding **212** (1.34 g, 77%) as a colourless oil requiring no further purification. IR ν_{max} (KBr, cm^{-1}) 1731 (C=O), 1707 (C=O), 1610 (C=C). ^1H NMR (300MHz, $CDCl_3$), δ_H 9.55 (s, 1H, CHO-C), 6.97 (d, J=16.1 Hz, 1H, C-CH=CH), 5.88 (d, J=16.1 Hz, 1H, CH=CH-C(O)), 3.75 (s, 3H, CH_3-O), 1.26 (s, 6H, -(CH_3)$_2$).

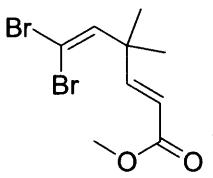
(2*E*,5*E*)-6-Bromo-4,4-dimethylhexa-2,5-dienoic acid methyl ester (213)



(Bromomethyl)-triphenylphosphonium bromide (0.1 g, 0.23 mmol) in 3 mL of dry THF was cooled to -78 °C. *n*-Butyllithium (0.21 mmol) was added to the dropwise, resulting in the formation of a yellow solution. The mixture was allowed to stir for 15 min. 0.034 g (0.21 mmol) of 4,4-dimethyl-5-oxo-pent-2-enoic acid methyl ester in 0.5 mL of dry THF was then added to the reaction mixture, while maintaining the temperature at -78 °C. An off-white precipitate was seen to be formed during the progress of the reaction. The reaction mixture was warmed to room temperature, and concentrated under reduced pressure. 2:1 petroleum ether:ethyl acetate was added, and the resulting suspension filtered through a short silica plug, the residue was washed, and the filtrate concentrated under reduced pressure to give **213** (31 mg, 63%) a light yellow oil.

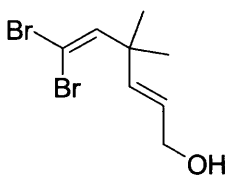
^{1}H NMR (300MHz, $CDCl_3$), δ_H 6.99 (d, J=15.5, 1H, CH=CH-COO), 5.82 (d, J=9.7, 1H, Br-CH=CH), 5.75 (d, J=15.5, 1H, CH=CH-COO), 4.97 (d, J=9.7, 1H, Br-CH=CH), 3.73 (s, 3H, CH_3-O), 1.16 (s, 6H, -(CH_3)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 167.5, 156.5, 144.7, 117.7, 112.4, 51.5, 39.7, 29.2.

(2*E*)-6,6-Dibromo-4,4-dimethylhexa-2,5-dienoic acid methyl ester (214)



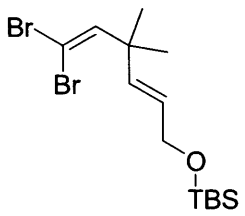
To a cold (-15 °C) stirred solution of triphenyl phosphine (12.6 mmol, 3.29 g) in 10 mL of dry dichloromethane, carbon tetrabromide (2.08 g, 6.28 mmol) in 6 mL of dry dichloromethane was added, and continuously stirred for 30 min. 4,4-dimethyl-5-oxo-pent-2-enoic acid methyl ester (0.419 g, 3.14 mmol) in 5 mL of dichloromethane was added slowly, and the reaction stirred for 1 h. The mixture then filtered through a short slug of silica using an eluant of initially hexane, then 5% ethyl acetate/hexane gave **214** (0.73 g, 75%) as a colourless oil. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 6.99 (d, J=16.0 Hz, 1H, CH=CH-COO), 6.70 (s, 1H, Br_2-C=CH), 5.87 (d, J=16.0 Hz, 1H, CH=CH-COO), 3.73 (s, 3H, CH_3-C), 1.30 (s, 6H, -(CH_3)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 167.2, 153.2, 143.3, 118.8, 89.3, 51.6, 41.2, 27.0. HRMS calcd. for $C_9H_{12}Br_2O_2$ 309.9204, found 309.9207.

(2*E*)-6,6-Dibromo-4,4-dimethylhexa-2,5-dien-1-ol (216)



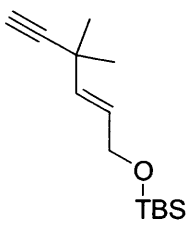
To a stirred solution of 6,6-dibromo-4,4-dimethyl-hexa-2,5-dienoic acid methyl ester (2.5 g, 8.0 mmol) in dichloromethane (36 mL) at -78 °C, DIBAL-H (2.5 eq, 20% wt.% in toluene, 20.0 mmol) was added dropwise and the reaction stirred for 30 minutes. The reaction mixture was quenched with the addition of saturated NH_4Cl solution at -78 °C. The quenched reaction was then warmed to 0 °C and 20 mL of 1 M HCl was added, and stirred for 15 minutes. The mixture was filtered through a small celite plug, which was then washed with 2 x 10 mL portions of diethyl ether. The combined mixture was dried ($MgSO_4$) and concentrated under reduced pressure to a colourless oil. Purification by column chromatography using an eluant of 5% ethyl acetate/petroleum spirit (40-60 °C), gave **216** (1.871 g, 83%) as a colourless oil. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 6.31 (s, 1H, Br_2-C=CH), 5.76 (d, J=13.1 Hz, 1H, C-CH=CH), 5.65 (dt, J=13.1, 5.4 Hz, 1H, CH=CH-CH_2), 4.14 (d, J=5.4 Hz, 2H, CH-CH_2-OH), 1.75-1.63 (br, OH), 1.26 (s, 6H, -$(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 145.0, 137.8, 126.7, 87.6, 63.7, 40.6, 27.5.

(2*E*)-*tert*-Butyl-(6,6-dibromo-4,4-dimethylhexa-2,5-dienyloxy)-dimethylsilane (217)



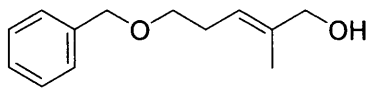
To 59 mg, 0.21 mmol, of 6,6-dibromo-4,4-dimethylhexa-2,5-dien-1-ol in 1 mL of dimethyl formamide at 0 °C, imidazole (19 mg, 0.27 mmol), and *tert*-butyldimethylsilyl chloride (35 mg, 0.23 mmol) were added. The mixture was stirred at ambient temperature for 12 h. The reaction mixture was extracted between diethyl ether and water, the product containing organic fractions were combined, dried ($MgSO_4$), and concentrated under reduced pressure to an oil. Column chromatography using an eluant of 10% ethyl acetate/petroleum ether (40-60 °C) gave **217** (80 mg, 95%). ^{1}H NMR (300MHz, $CDCl_3$), δ_H 6.58 (s, 1H, Br_2-C=CH), 5.73 (d, J=15.4 Hz, 1H, C-CH=CH), 5.53 (dt, J=15.4, 4.9 Hz, 1H, CH=CH-CH_2), 4.18 (d, J=4.9 Hz, 2H, CH-CH_2-O), 1.26 (s, 6H, -$(CH_3)_2$), 0.90 (s, 9H, O-TBS), 0.06 (s, 6H, O-TBS). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 145.2, 135.7, 127.0, 87.2, 63.8, 40.5, 27.5, 26.0, 18.5, 1.0. HRMS calcd. for $C_{14}H_{26}Br_2SiO$ 396.0120, found 369.0122.

(2*E*)-*tert*-Butyl-(4,4-dimethylhex-2-en-5-ynyloxy)-dimethylsilane (218)



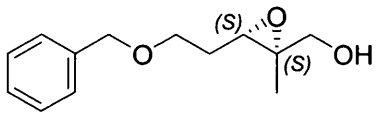
To a stirred,-78 °C solution of *tert*-butyl-(6,6-dibromo-4,4-dimethylhexa-2,5-dienyloxy)-dimethylsilane (0.576 g, 1.8 mmol) in tetrahydrofuran (3 mL), *n*-butyllithium (3.6 mmol, 2.5 M in hexanes) was added dropwise. The mixture was stirred for 1 h, and then warmed to 0 °C. Saturated ammonium chloride was then added to quench the reaction, and subsequently extracted with dichloromethane. Organic fractions were combined, and dried ($MgSO_4$). Concentration under reduced pressure yielded the crude product as a colourless oil. Purification by column chromatography using an eluant of 10% diethyl ether/petroleum ether (40-60 °C) gave **218** (224 mg, 82%) as a colourless oil. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 5.76 (dt, *J*=15.3, 4.8 Hz, 1H, C-CH=CH), 5.54 (dt, *J*=15.3, 1.6 Hz, 1H, CH_2-CH=CH), 4.19 - 4.14 (m, 2H, CH-CH_2-O), 2.28 (s, 1H, HC≡C-C), 1.24 (s, 6H, -$(CH_3)_2$), 0.84 (s, 9H, O-TBS), 0.06 (s, 6H, O-TBS). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 136.2, 127.4, 90.3, 69.8, 63.9, 33.4, 30.0, 26.3, 18.8, -4.7. HRMS calcd. for $C_{14}H_{26}SiO$ 238.1753, found 238.1760.

(2*E*)-5-Benzyloxypent-2-en-1-ol[210] (221)



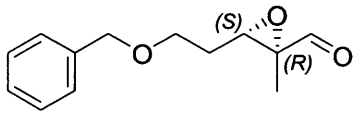
5-Benzyloxy-2-methyl-pent-2-enoic acid ethyl ester (3.5 g, 14.1 mmol) was added dropwise to a rapidly stirring suspension of lithium aluminum hydride (0.40 g, 10.6 mmol), in dry diethyl ether (35 mL) at 0 °C and stirred for 1 h. A solution of 2 M hydrochloric acid (40 mL, careful dropwise addition initially) was added and the mixture stirred for 30 min. Extraction with ethyl acetate (3 x 30 mL), combination of product containing fractions, drying ($MgSO_4$), and concentration under reduced pressure gave the crude product. Purification by column chromatography 4:1 hexane:ethyl acetate to 3:1 hexane:ethyl acetate gave **221** (2.53 g, 87%) as a colourless oil. Analysis correlates with previously reported examples. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 7.37 - 7.26 (m, 5H, Ar-H), 5.45 (t, *J*=5.8 Hz, 1H, CH_2-CH=C), 4.52 (s, 2H, Ph-CH_2-O), 3.99 (s, 2H, HO-CH_2-C), 3.49 (t, *J*=6.9 Hz, 2H, O-CH_2-CH_2), 2.41-2.36 (m, 2H, CH_2-CH_2-CH), 1.68 (s, 3H, CH_3-C). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 138.4, 136.8, 128.4, 127.7, 127.6, 122.0, 72.9, 69.7, 68.7, 28.3, 13.8.

((2*S*,3*S*)-3-(2-(Benzyloxy)ethyl)-2-methyloxiran-2-yl)methanol[210] (222)



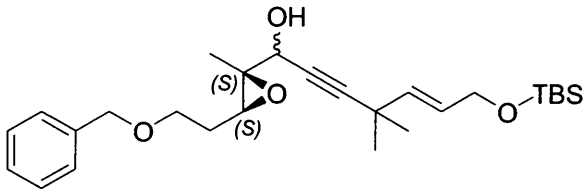
(+)-Diisopropyl tartrate (0.108 g, 0.46 mmol), Ti(^{i}PrO)$_4$ (0.108 g, 0.38 mmol), *tert*-butyl hydroperoxide (5.2 mL, 2.5 M, 16.6 mmol) and 4Å molecular sieves (300 mg) were cooled in dichloromethane (80 mL) to -20 °C in an inert atmosphere. The mixture was aged for 30 min. The mixture was cooled to -28 °C and benzyloxypent-2-en-1-ol (1.5 g, 7.3 mmol) was added slowly. The reaction stirred at -28 °C for 12 h. The reaction was monitored by TLC 2:1 hexane:ethyl acetate. Upon completion, water (12 mL) was added and vigorously stirred for 60 min. 30% NaOH/sat. brine (3 mL) was added and the mixture stirred for 60 min. The reaction mixture was then filtered, and the filtrate extracted with dichloromethane (2 x 30 mL). Drying (anhydrous Na_2SO_4) and concentration yielded the crude product as an oil. Purification by column chromatography using an eluant of 2:1 hexane:ethyl acetate gave **222** (1.47 g, 90%) as a colourless oil. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 7.38 - 7.25 (m, 5H, Ar-H), 4.53 (s, 2H, Ph-CH_2-O), 3.69 - 3.52 (m, 2H, HO-CH_2-C), 3.64 (t, J=5.7 Hz, 2H, O-CH_2-CH_2), 3.18 (t, J=6.3 Hz, 1H, CH_2-CH(O)-CH), 2.04 - 1.79 (m, 2H, CH_2-CH_2-CH), 1.28 (s, 3H, CH_3-C). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 138.2, 128.4, 127.6, 73.1, 67.4, 65.4, 60.9, 57.9, 29.0, 14.4. Analysis correlates with previously reported material. ^{1}H analysis of the Mosher ester[239,240] indicated an e.e. of 84%.

3-(2-Benzyloxyethyl)-2-methyl-oxirane-2-carboxaldehyde (223)



To a 0 °C stirred mixture of dimethyl sulfoxide (1.85 mL), triethyl amine (1.46 mL, 9.2 mmol), [3-(2-benzyloxyethyl)-oxiranyl]-methanol (300 mg, 1.3 mmol), and dichloromethane (3.2 mL), pyridine SO_3 complex dissolved in dimethyl sulfoxide (1 mL) was added dropwise. The mixture was stirred for 2 h at 0 °C, after which TLC analysis (eluant of 3:1 hexane:ethyl acetate) indicated that the reaction was complete. Addition of 10 mL of 1:1 hexane:diethyl ether to the solution, was followed by extraction with ice cold water (15 mL). The aqueous washings were extracted with 2 x 15 mL of diethyl ether. The organic fractions were combined, then washed once again with ice-cold water(10 mL). The organic fractions were then dried (Na_2SO_4) and concentrated under reduced pressure to an oil. Purification by column chromatography (3:1 hexane:ethyl acetate), gave **223** (0.191 g, 69%) as a colourless oil. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 8.86 (s, 1H, CHO-C), 7.47 - 7.26 (m, 5H, Ar-H), 4.54 (s, 2H, Ph-CH_2-O), 3.66 (t, J=6.3 Hz, 2H, O-CH_2-CH_2), 3.33 (t, J=5.7 Hz, 1H, CH_2-CH(O)-C), 2.04 - 1.86 (m, 2H, CH_2-CH_2-CH), 1.41 (s, 3H, CH_3-C). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 200.0, 138.0, 128.5, 127.8, 127.6, 73.2, 66.9, 62.1, 57.9, 28.8, 10.2. HRMS (positive ion FAB) calcd. for $C_{13}H_{16}O_3$ (M+Na) 243.0997, found 243.0981.

(*E*)-1-((2*S*,3*S*)-3-(2-(Benzyloxy)ethyl)-2-methyloxiran-2-yl)-7-(*tert*-butyldimethylsilyloxy)-4,4-dimethylhept-5-en-2-yn-1-ol (226)



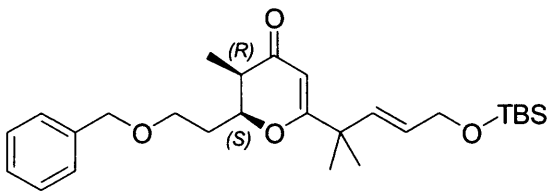
To a stirring solution of dry tetrahydrofuran (3 mL) and *tert*-butyl-(4,4-dimethylhex-2-en-5-ynyloxy)-dimethylsilane (100 mg, 0.7 mmol) at -78 °C, *n*-butyllithium (2 M in hexanes, 0.8 mmol) was added dropwise. The mixture was stirred for 30 min, and the reaction mixture transferred via cannula to a vessel charged with dry tetrahydrofuran (3 mL), and 3-(2-benzyloxyethyl)-2-methyl-oxirane-2-carboxaldehyde (185 mg, 0.84 mmol) at -78 °C. The reaction was stirred for 2 h, and allowed to warm to 0 °C. The reaction was quenched with the addition of saturated ammonium chloride solution, and extracted with dichloromethane (3 x 5 mL). The organic fractions were combined, dried ($MgSO_4$), and

concentrated under reduced pressure to give the crude material. Purification by column chromatography using an eluant of 4:1 hexane:ethyl acetate gave **226** (75 mg, 23%) as a pale oil. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 7.37-7.26 (m, 5H, Ar-H), 5.81-5.75 (m, 1H, CH=CH-CH_2), 5.60 (d, J=15.3 Hz, 1H, C-CH=CH), 4.69 (br, 3H, C-CH(OH)-C, Ph-CH_2-O), 4.17-4.15 (m, 2H, CH-CH_2-O), 3.65 (t, J=7.7 Hz, 2H, O-CH_2-CH_2), 3.24-3.22 (m, 1H, C-CH(O)-CH_2), 2.04-1.83 (m, 2H, CH_2-CH_2-CH), 1.40 (s, 3H, CH_3-C), 1.29 (s, 6H, C-(CH_3)$_2$), 0.90 (s, 9H, O-TBS), 0.07 (s, 6H, O-TBS). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 138.2, 136.0, 128.4, 127.6, 126.9, 82.7, 79.8, 73.2, 67.4, 66.2, 63.4, 62.7, 58.3, 33.2, 29.7, 29.0, 26.0, 18.4, 13.0. HRMS calcd. for $C_{27}H_{42}O_4Si$ (M+H) 459.2931, found 459.2933.

Catalytic Mercury Solution

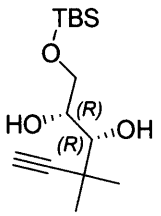
To a rapidly stirred mixture of water (1.2 mL) and concentrated sulfuric acid (0.125 mL), mercury (II) oxide yellow (0.11 g, 0.5 mmol) was added. Upon dissolution, 3.7 mL of water was added, forming the catalytic mercury solution.

(2*S*,3*R*,*E*)-2-(2-(Benzyloxy)ethyl)-6-(5-(*tert*-butyldimethylsilyloxy)-2-methylpent-3-en-2-yl)-3-methyl-2,3-dihydropyran-4-one (228)



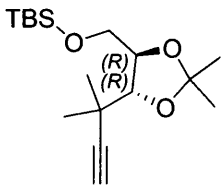
To a stirring solution of 1-[3-(2-benzyloxyethyl)-2-methyl-oxiranyl]-7-(*tert*-butyldimethylsilyloxy)-4,4-dimethylhept-5-en-2-yn-1-ol (50 mg, 0.1 mmol) in acetone (5 mL), 0.05 mL of a Hg^{2+} stock solution was added. The reaction was continued until complete by TLC. Sodium hydrogen carbonate (100 mg) was added and the reaction stirred for 1 h. The reaction was filtered and concentrated under reduced pressure to give crude material. Purification and analysis indicated that **228** had not been formed.

1-(*tert*-Butyl-dimethylsilyloxy)-4,4-dimethylhex-5-yne-2,3-diol (224)



To a stirring mixture of *tert*-butyl alcohol (5 mL) and water (5 mL) AD-mix β (1.4 g) was added at room temperature. Methane sulfonamide (95 mg, 1 mmol) was added, and the mixture cooled to 0 °C. The alkene, *tert*-butyl-(4,4-dimethylhex-2-en-5-ynyloxy)-dimethylsilane (1 mmol, 0.238 g) was added and the mixture stirred until all starting material was consumed, as indicated by TLC. Sodium sulfite was added to quench the reaction, and was stirred while allowing to warm to room temperature for 30 min. The reaction mixture was extracted with dichloromethane (3 x 10 mL) and organic fractions washed with 2M KOH solution. Organic material was combined, dried ($MgSO_4$) and concentrated under reduced pressure to an oil. Purification by column chromatography using an eluant of 4:1 hexane:ethyl acetate gave **224** (237 mg, 87%) as a colourless oil. ^{1}H NMR (300MHz, $CDCl_3$), δ_H 4.35-4.04 (m, 2H, O-C<u>H</u>$_2$-CH), 3.68-3.64 (m, 1H, CH_2-C<u>H</u>(OH)-CH), 3.32 (m, 1H, CH-C<u>H</u>(OH)-C), 2.03-2.02 (m, 1H, <u>H</u>C≡C), 1.27 (s, 6H, -(C<u>H</u>$_3$)$_2$), 0.89 (s, 9H, O-<u>TBS</u>), 0.08 (s, 6H, O-<u>TBS</u>). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 90.0, 75.8, 70.2, 69.6, 66.2, 29.0, 26.1, 24.4, 18.7.

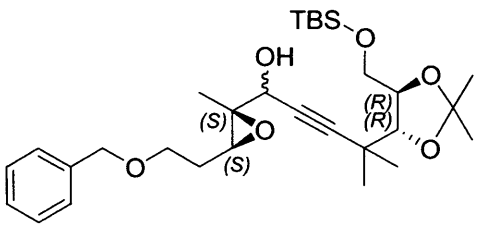
***tert*-Butyl-[5-(1,1-dimethylprop-2-ynyl)-2,2-dimethyl-[1,3]dioxolan-4-ylmethoxy]-dimethylsilane (225)**



A stirring solution of acetone (3.5 mL), 2,2-dimethoxypropane (1.5 mL), and camphor sulfonic acid (12 mg, 0.05 mmol) was prepared and stirred for 5 min. The diol, 1-(*tert*-butyldimethylsilyloxy)-4,4-dimethylhex-5-yne-2,3-diol (1 mmol, 272 mg) was added in one portion, and the mixture stirred at room temperature. Upon completion by TLC, a small quantity of sodium hydrogen carbonate was added, and the reaction concentrated under reduced pressure. Purification by column chromatography using an eluant of 9:1 hexane:ethyl acetate, and concentration of product containing fractions gave **225** (128 mg, 41%). ^{1}H NMR (300MHz, $CDCl_3$), δ_H 4.10-4.04 (m, 1H, CH_2-C<u>H</u>(O)-CH), 3.88 (dd, *J*=11.2, 3.1 Hz, 1H, TBS-O-C<u>H</u>H-CH), 3.81 (d, *J*=7.2 Hz, 1H, C-C<u>H</u>(O)-CH), 3.73 (dd, *J*=11.2, 4.5 Hz, 1H, TBS-O-CH<u>H</u>-CH), 2.13 (s, 1H, <u>H</u>C≡C), 1.43 (s, 3H, C<u>H</u>$_3$-acetal), 1.39 (s, 3H, C<u>H</u>$_3$-acetal), 1.28 (s, 3H, C-CH_3), 1.23 (s, 3H, C-C<u>H</u>$_3$), 0.90 (s, 9H, O-<u>TBS</u>), 0.08 (s, 6H, O-<u>TBS</u>). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 109.1, 88.9, 82.5, 79.7, 69.7, 65.0,

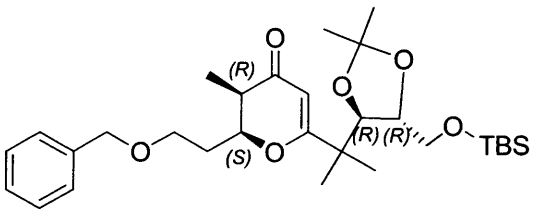
33.8, 27.5, 27.4, 26.7, 26.0, 24.9, 18.5. HRMS calcd. for $C_{17}H_{32}O_3Si$ 312.2121, found 312.2126.

1-((2*S*,3*S*)-3-(2-(Benzyloxy)ethyl)-2-methyloxiran-2-yl)-4-((4*R*,5*R*)-5-((*tert*-butyldimethylsilyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-4-methylpent-2-yn-1-ol (227)



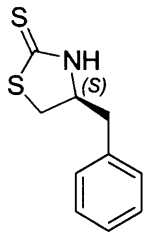
To a stirring solution of *tert*-butyl-[5-(1,1-dimethylprop-2-ynyl)-2,2-dimethyl-[1,3]dioxolan-4-ylmethoxy]-dimethylsilane (128 mg, 0.4 mmol) in dry tetrahydrofuran (1 mL) at -78 °C, *n*-butyllithium (2.5 M in hexanes, 0.44 mmol) was added dropwise and the reaction stirred for 30 min. The solution was then added dropwise to a separate flask, charged with tetrahydrofuran (1 mL) and 3-(2-benzyloxyethyl)-2-methyl-oxirane-2-carboxaldehyde (86 mg, 0.39 mmol) at -78 °C. The reaction was stirred for 2 h, and warmed to 0 °C. Saturated ammonium chloride solution was added, and the reaction mixture extracted with ethyl acetate (3 x 5 mL). Organic fractions were combined, dried ($MgSO_4$), and concentrated under reduced pressure to give the crude product. Column chromatography using an eluant of 4:1 hexane:ethyl acetate gave the **227** as an oil (133 mg, 64%). ^1H NMR (300MHz, $CDCl_3$), δ_H 7.47-7.24 (m, 5H, Ar-H), 4.53 (s, 2H, Ph-CH_2-O), 4.15-4.04 (m, 2H, O-CH_2-CH_2), 3.84-3.59 (m, 5H, TBS-O-CH_2-CH, CH(epoxide)-CH(OH)-C), 3.22-3.20 (m, 1H, CH_2-CH(epoxide)), 2.03-1.91 (m, 2H, CH_2-CH_2-CH), 1.42 (s, 3H, CH_3-C(O)), 1.39 (s, 3H, CH_3-acetal), 1.38 (s, 3H, CH_3-acetal), 1.27 (s, 3H, C-CH_3), 1.20 (s, 3H, C-CH_3), 0.90 (s, 9H, O-TBS), 0.07 (s, 6H, O-TBS). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 138.2, 128.4, 127.6, 109.2, 82.7, 79.8, 73.1, 67.3, 66.1, 65.4, 64.9, 62.5, 60.4, 58.1, 28.9, 27.5, 26.6, 26.0, 25.3, 18.4, 14.2. HRMS calcd. for $C_{30}H_{48}O_6Si$ (M+H) 533.3298, found 533.3304.

(2*S*,3*R*)-2-(2-(benzyloxy)ethyl)-6-(2-((4*S*,5*S*)-5-((*tert*-butyldimethylsilyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)propan-2-yl)-3-methyl-2,3-dihydropyran-4-one (229)



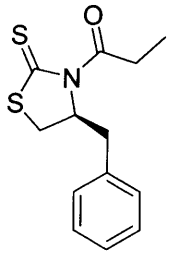
To a stirred solution of 1-[3-(2-benzyloxyethyl)-2-methyl-oxiranyl]-4-[5-(*tert*-butyldimethylsilyloxymethyl)-2,2-dimethyl-[1,3]dioxolan-4-yl]-4-methyl-pent-2-yn-1-ol (50 mg, 0.1 mmol), in acetone (5 mL), 0.05 mL of a Hg^{+2} solution was added. The mixture was stirred until all epoxide material was consumed by TLC (visualisation using cerium(IV) sulphate stain). Column chromatography using an eluant of 4:1 hexane:ethyl acetate recovered 36 mg of material as an oil. Analysis indicated that **229** had not been formed.

(*S*)-4-Benzylthiazolidine-2-thione[242] (230)



To a stirring solution of (*S*)-phenylalaninol (5 g, 38 mmol) in 150 mL of 1 M potassium hydroxide, CS_2 (12.6 g, 9.92 mL, 165 mmol) was added and the reaction heated to reflux for 12 h. After cooling, the reaction was extracted with dichloromethane (3 x 100 mL), organic fractions combined, dried (Na_2SO_4), and concentrated under reduced pressure to give the crude product. Purification by column chromatography using an eluant of 4:1 hexane:ethyl acetate (0.5% acetic acid) gave **230** (5.06 g, 74%) as a bright yellow solid. Analysis of a recrystallised sample (ethanol) correlates spectral data with that of previously reported material. HRMS (CI) calcd. for $C_{10}H_{11}NS_2$ (M+H) 210.0411, found 210.0397.

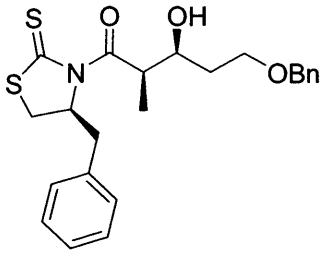
(*S*)-1-(4-Benzyl-2-thioxothiazolidin-3-yl)propan-1-one[243,244] (231)



To a stirring solution of (*S*)-4-benzylthiazolidine-2-thione (4 g, 19.1 mmol), triethylamine (6.66 mL, 47.75 mmol), in dichloromethane (120 mL) at 0 °C, propionyl chloride (2.49 mL, 28.65 mmol) was added slowly. The mixture was allowed to warm to room temperature, and stirred for 12 h. The reaction mixture was washed with water (2 x 40 mL),

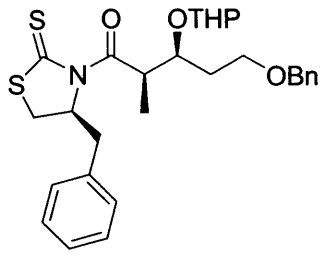
dried ($MgSO_4$), and concentrated under reduced pressure to give the crude material. Recrystallisation from hexane afforded **231** as bright yellow crystals (3.43 g, 68%). Analysis was consistent with that of previously reported data.

(2*R*,3*S*)-1-((*S*)-4-Benzyl-2-thioxothiazolidin-3-yl)-6-(benzyloxy)-3-hydroxy-2-methylhexan-1-one (232)



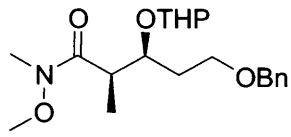
To a 0 °C solution of (*S*)-1-(4-benzyl-2-thioxothiazolidin-3-yl)propan-1-one (1.5 g, 5.6 mmol), in dry dichloromethane (30 mL), $TiCl_4$ (1 M in dichloromethane, 5.6 mmol) was added dropwise. The mixture was stirred for 5 min, and (-)-sparteine (1.29 mL, 5.6 mmol) was added and stirred for a further 20 min. The reaction was cooled to -78 °C and 3-benzyloxypropionaldehyde (1.01 g, 6.16 mmol) was added dropwise. The reaction was stirred at -78 °C for 1 h, and allowed to warm to 0 °C. The reaction was quenched with 1:1 saturated ammonium chloride:water. Organic material was extracted, dried (Na_2SO_4), and concentrated under reduced pressure. Column chromatography using an eluant of 4:1 hexane:ethyl acetate gave **232** (0.78 g, 32%) as a yellow oil. 0.86 g of unreacted auxiliary was recovered. ^1H NMR (300MHz, $CDCl_3$), δ_H 7.59-7.26 (m, 10H, Ar-H), 5.40-5.36 (m, 1H, N-CH(CH_2)-CH_2), 4.53 (s, 2H, Ph-CH_2-O), 4.29-4.15 (m, 1H, CH-CH(OH)-CH_2), 3.71-3.65 (m, 2H, O-CH_2-CH_2), 3.38-2.85 (m, 4H, S-CH_2-CH, Ph-CH_2-CH), 2.03-1.73 (m, 3H, CH_3-CH, CH(OH)-CH_2-CH_2), 1.23 (d, J=7.3 Hz, 3H, CH_3-CH). ^{13}C NMR (75 MHz, $CDCl_3$), δ_C 201.4, 177.6, 138.1, 137.9, 129.5, 128.9, 128.4, 127.7, 127.5, 127.3, 73.4, 71.6, 64.9, 43.8, 43.2, 38.3, 36.8, 21.1, 11.3.

(2*R*,3*S*)-1-((*S*)-4-Benzyl-2-thioxothiazolidin-3-yl)-6-(benzyloxy)-2-methyl-3-(tetrahydro-2*H*-pyran-2-yloxy)hexan-1-one (233)



To a solution of (2*R*,3*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-6-(benzyloxy)-3-hydroxy-2-methylhexan-1-one (500 mg, 1.1 mmol) in dry tetrahydrofuran (5 mL), *p*-toluene sulfonic acid (5 mg), and 3,4-dihydro-2*H*-pyran (91 mg, 1.08 mmol) were added at 0 °C. The mixture was stirred and allowed to warm to room temperature. The reaction was concentrated under reduced pressure, and purified by column chromatography using an eluant of 9:1 hexane:ethyl acetate to give the desired material as an oil, 415 mg, 87%. The material was immediately transferred into the next synthetic stage. HRMS calcd. for $C_{28}H_{35}NO_4Si$ (M+H) 514.2086, found 514.2064.

(2*R*,3*S*)-6-(Benzyloxy)-*N*-methoxy-*N*,2-dimethyl-3-(tetrahydro-2*H*-pyran-2-yloxy)hexanamide (234)



To a stirring solution of (2*R*,3*S*)-1-((*S*)-4-benzyl-2-thioxothiazolidin-3-yl)-6-(benzyloxy)-2-methyl-3-(tetrahydro-2*H*-pyran-2-yloxy)hexan-1-one (200 mg, 0.39 mmol) in dichloromethane (10 mL), a solution consisting of dichloromethane (5 mL), triethylamine (0.47 mmol, 47 mg), and *N*,*O*-dimethyl hydroxylamine hydrochloride (0.43 mmol, 42 mg), was added slowly. The reaction was stirred for 12 h. Upon work up, no products were found to have been formed.

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Appendix A

Table 1. Crystal data and structure refinement.

Identification code	**04src1124 (139JM)**	
Empirical formula	$C_{18}H_{23}NO_6$	
Formula weight	349.37	
Temperature	120(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 8.713(4) Å	α = 90°
	b = 6.582(4) Å	β = 101.70(5)°
	c = 15.739(7) Å	γ = 90°
Volume	883.9(8) Å^3	
Z	2	
Density (calculated)	1.313 Mg / m^3	
Absorption coefficient	0.099 mm^{-1}	
F(000)	372	
Crystal	Blade; colourless	
Crystal size	0.60 × 0.06 × 0.01 mm^3	
θ range for data collection	3.37 – 27.50°	
Index ranges	$-11 \le h \le 11$, $-8 \le k \le 8$, $-20 \le l \le 20$	
Reflections collected	3834	
Independent reflections	3834 [R_{int} = 0.0000]	
Completeness to θ = 27.50°	98.6 %	
Absorption correction	Semi–empirical from equivalents	
Max. and min. transmission	0.9990 and 0.9432	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3834 / 1 / 233	
Goodness-of-fit on F^2	1.380	
Final R indices [$F^2 > 2\sigma(F^2)$]	$R1$ = 0.0570, $wR2$ = 0.0983	
R indices (all data)	$R1$ = 0.0811, $wR2$ = 0.1043	
Absolute structure parameter	0.1(13)	
Extinction coefficient	0.059(5)	
Largest diff. peak and hole	0.239 and –0.210 e Å^{-3}	

Diffractometer: *Nonius KappaCCD* area detector (ϕ scans and ω scans to fill *asymmetric unit* sphere). **Cell determination:** DirAx (Duisenberg, A.J.M.(1992). J. Appl. Cryst. 25, 92-96.) **Data collection:** Collect (Collect: Data collection software, R. Hooft, Nonius B.V., 1998). **Data reduction and cell refinement**: *Denzo* (Z. Otwinowski & W. Minor, *Methods in Enzymology* (1997) Vol. **276**: *Macromolecular Crystallography*, part A, pp. 307–326; C. W. Carter, Jr. & R. M. Sweet, Eds., Academic Press). **Absorption correction**: *SORTAV* (R. H. Blessing, Acta Cryst. A**51** (1995) 33–37; R. H. Blessing, J. Appl. Cryst. **30** (1997) 421–426). **Structure solution**: *SHELXS97* (G. M. Sheldrick, Acta Cryst. (1990) A**46** 467–473). **Structure refinement**: *SHELXL97* (G. M. Sheldrick (1997), University of Göttingen, Germany). **Graphics:** Cameron - A Molecular Graphics Package. (D. M. Watkin, L. Pearce and C. K. Prout, Chemical Crystallography Laboratory, University of Oxford, 1993).

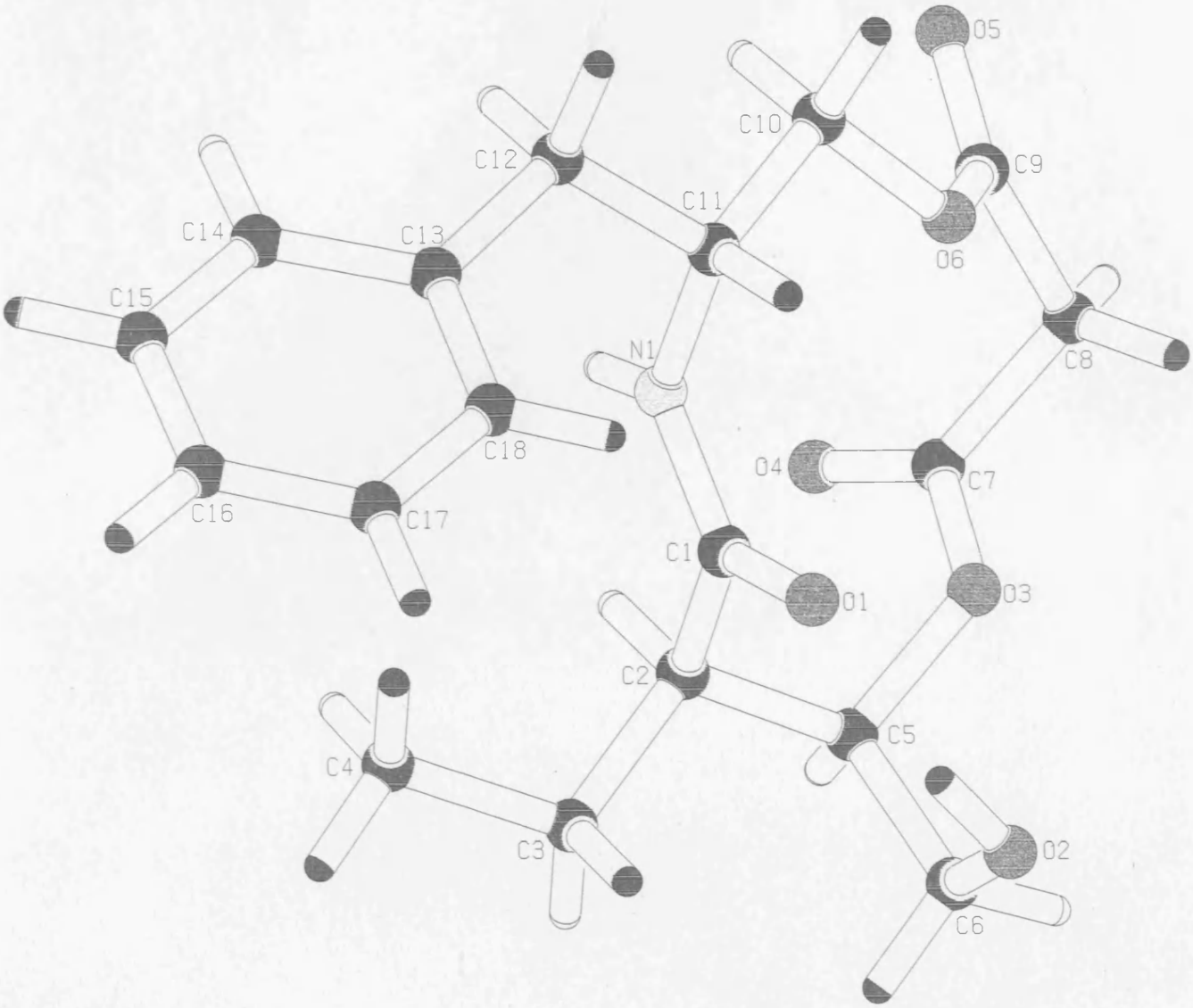
Special details:

Table 2. Atomic coordinates [$\times 10^4$], equivalent isotropic displacement parameters [$Å^2 \times 10^3$] and site occupancy factors. U_{eq} is defined as one third of the trace of the orthogonalized U^{ij} tensor.

Atom	x	y	z	U_{eq}	$S.o.f.$
C1	2646(3)	2188(4)	2649(2)	29(1)	1
C2	914(3)	2235(4)	2218(2)	29(1)	1
C3	700(3)	2788(4)	1253(2)	35(1)	1
C4	1262(4)	1103(5)	738(2)	47(1)	1
C5	−2(3)	3658(4)	2711(2)	29(1)	1
C6	32(3)	5912(4)	2530(2)	35(1)	1
C7	176(3)	1729(4)	4012(2)	30(1)	1
C8	958(3)	1620(4)	4969(2)	34(1)	1
C9	2311(3)	165(4)	5050(2)	33(1)	1
C10	4502(3)	−486(4)	4408(2)	35(1)	1
C11	4736(3)	134(4)	3509(2)	29(1)	1
C12	5683(3)	−1503(4)	3157(2)	33(1)	1
C13	5914(3)	−1166(4)	2245(2)	33(1)	1
C14	5938(3)	−2822(5)	1706(2)	39(1)	1
C15	6245(3)	−2573(5)	880(2)	46(1)	1
C16	6509(3)	−668(5)	580(2)	46(1)	1
C17	6452(3)	996(5)	1107(2)	44(1)	1
C18	6157(3)	750(4)	1934(2)	37(1)	1
N1	3203(2)	392(3)	2942(1)	29(1)	1
O1	3463(2)	3744(3)	2714(1)	37(1)	1
O2	1549(2)	6783(3)	2708(2)	47(1)	1
O3	571(2)	3434(3)	3649(1)	29(1)	1
O4	−631(2)	409(3)	3634(1)	33(1)	1
O5	2399(2)	−1468(3)	5401(1)	43(1)	1
O6	3389(2)	915(3)	4643(1)	33(1)	1

Table 3. Anisotropic displacement parameters [$Å^2 \times 10^3$]. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U^{11} + \cdots + 2\,h\,k\,a^*\,b^*\,U^{12}]$.

Atom	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C1	26(1)	28(2)	36(2)	0(1)	12(1)	−2(1)
C2	27(1)	27(1)	32(2)	5(1)	7(1)	−3(1)
C3	32(2)	39(2)	35(2)	2(1)	7(1)	0(1)
C4	54(2)	51(2)	38(2)	0(1)	14(1)	−1(2)
C5	23(1)	31(1)	31(2)	2(1)	1(1)	−5(1)
C6	30(1)	28(1)	47(2)	5(1)	8(1)	−1(1)
C7	26(1)	29(1)	36(2)	−1(1)	10(1)	−1(1)
C8	34(2)	36(2)	33(2)	−2(1)	8(1)	−1(1)
C9	31(1)	37(2)	31(2)	−3(1)	3(1)	−2(1)
C10	29(1)	31(2)	45(2)	1(1)	6(1)	4(1)
C11	25(1)	24(1)	38(2)	2(1)	7(1)	−1(1)
C12	25(1)	31(1)	43(2)	3(1)	5(1)	2(1)
C13	22(1)	35(2)	41(2)	0(1)	6(1)	3(1)
C14	34(2)	35(2)	45(2)	−1(1)	7(1)	−3(1)
C15	42(2)	51(2)	45(2)	−9(2)	8(1)	2(2)
C16	40(2)	56(2)	43(2)	2(1)	10(1)	4(1)
C17	37(2)	40(2)	56(2)	6(2)	14(1)	−3(1)
C18	31(1)	35(2)	46(2)	1(1)	11(1)	−1(1)
N1	23(1)	25(1)	39(1)	1(1)	6(1)	0(1)
O1	29(1)	26(1)	56(1)	3(1)	7(1)	−4(1)
O2	32(1)	25(1)	83(2)	1(1)	11(1)	−3(1)
O3	29(1)	27(1)	32(1)	−1(1)	7(1)	−4(1)
O4	35(1)	28(1)	37(1)	0(1)	9(1)	−4(1)
O5	37(1)	42(1)	53(1)	15(1)	12(1)	4(1)
O6	30(1)	30(1)	41(1)	0(1)	12(1)	2(1)


O5
C10
C12
C9
C11
C14
C13
O6
C15
N1
C8
C18
O4
C7
C16
C17
C1
O1
O3
C2
C5
C4
C3
O2
C6