
The Chemoenzymatic and Enantioselective Synthesis of Biologically Active Resorcylic Acid Lactones (RALs)

*A thesis submitted for the degree of Doctor of Philosophy
of The Australian National University*

By

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Declaration

I declare that, to the best of my knowledge, the material presented in this thesis represents the result of original work carried out by the author during the period 2007-2010 and has not been presented for examination for any other degree. This thesis is less than 100,000 words in length. Established methodologies have been acknowledged, wherever possible, by citation of the original publications from which they derive.

A handwritten signature in black ink, consisting of a large, stylized capital 'A' followed by a cursive 'L' and a small flourish.

Andrew Lin
January 2012

“I have not failed. I’ve just found 10,000 ways that won’t work”

- Thomas A. Edison

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Publications and Presentations

The following list details the publications, patents and presentations that have resulted from research performed during the candidature of the Doctor of Philosophy.

Publications:

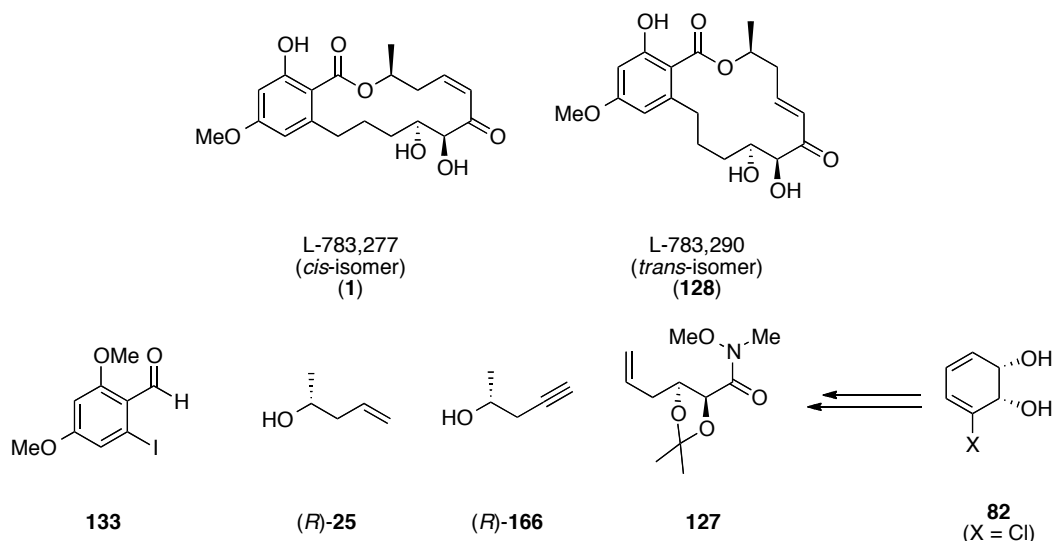
- I. Lin, A; Banwell, M. G.; Willis, A. C., A chemoenzymatic and enantioselective total synthesis of the resorcylic acid lactone L-783,290, the *trans*-isomer of L-783,277, *Tetrahedron Lett.*, **2010**, 51, 1044.
- II. Lin, A; Banwell, M. G.; Willis, A. C., An enantioselective synthesis of the resorcylic acid lactone L-783,277 *via* addition of an acetylide anion to tethered Weinreb amide, *Heterocycles*, **2010**, 82, 313.

Presentations:

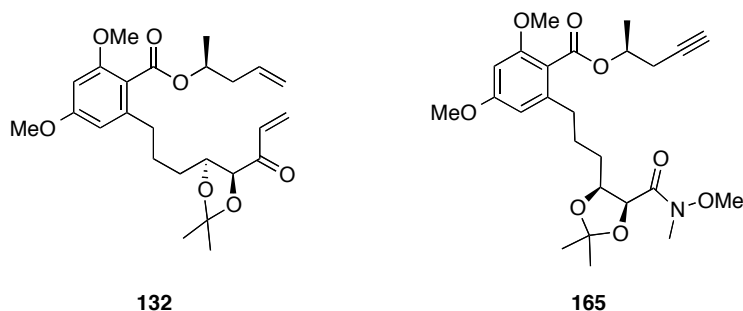
- I. 18th International Conference on Organic Synthesis. Bergen, Norway. 1st – 6th August 2010. Poster Presentation: “*The Chemoenzymatic and Enantioselective Total Synthesis of L-783,277*”
- II. 29th Royal Australian Chemical Institute, One-Day Organic Chemistry Symposium. Sydney, Australia. 2nd December 2009. Oral Presentation: “*A Chemoenzymatic and Enantioselective Total Synthesis of the Resorcylic Acid Lactone L-783,290, the trans-Isomer of L-783,277.*”
- III. 23rd Royal Australian Chemical Institute, Organic Division Conference. Tasmania, Australia. 7th – 12th August 2010. Poster Presentation: “*Towards the Chemoenzymatic Total Synthesis of Resorcylic Acid Lactone, L-783,277.*”

Abstract

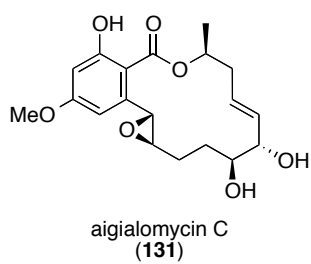
Modular total syntheses of the biologically active resorcylic acid lactones (RALs) L-783,277 (**1**) and L-783,290 (**128**) are reported. The three key building blocks employed for this purpose were the aromatic **133**, the enantiopure alcohol (*R*)-**25** or (*R*)-**166** and the protected diol **127**. The building blocks (*R*)-**25**, (*R*)-**166** and **127** were prepared by chemoenzymatic methods with the last of these being derived from the *cis*-1,2-dihydrocatechol **82** (X = Cl) which is itself obtained through the whole-cell biotransformation of chlorobenzene.



These building blocks have been linked to one another using Heck and Mitsunobu chemistries and the fourteen-membered macrolide rings formed using either a RCM reaction (as applied to compound **132**) or through intramolecular addition of an acetylide anion to a tethered Weinreb amide (as applied to compound **165**).



Attempts to extend the RCM-based route to the RAL aigialomycin C (**131**) are also described.



Abbreviations

The following abbreviations have been used throughout this Thesis.

Å	Angstrom(s)
δ	chemical shift (parts per million)
μg	microgram(s)
μL	microlitre(s)
μwave	microwave
λ	wavelength (nm)
ν _{max}	infrared absorption maxima (cm ⁻¹)
Ac	acetyl
AcOH	acetic acid
AIBN	azobisisobutyronitrile
a.k.a	also known as
[α] _D	optical rotation at the sodium D-line, <i>i.e.</i> at λ=589 nm (10 ⁻¹ .deg.cm ² .g ⁻¹)
aq.	aqueous
Ar	unspecified aryl group
atm	atmosphere(s)
ATP	adenosine triphosphate
9-BBN	9-borabicyclo[3.3.1]nonane
BHT	butylated hydroxytoluene
Bn	benzyl
br	broad
<i>t</i> -Bu	<i>tertiary</i> -butyl
<i>ca.</i>	<i>circa</i> (around)
CAN	ceric ammonium nitrate
cat.	catalytic
CDK	cyclin dependent kinase(s)
cm	centimetre(s)
conc.	concentrated
COSY	¹ H- ¹ H correlation spectroscopy
CSA	(1 <i>S</i>)-(+)-10-camphor sulfonic acid
d	doublet

DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
<i>c</i> DHC	<i>cis</i> -1,2-dihydrocatechols
DIAD	diisopropyl azodicarboxylate
DIBAL-H	di- <i>isobutyl</i> aluminium hydride
DMAP	4-(dimethylamino)pyridine
DME	1,2-dimethoxyethane
DMEDA	N,N'-dimethylethylenediamine
DMF	dimethylformamide
DMP	Dess-Martin periodinane
DMS	dimethyl sulfide
<i>E</i>	<i>entgegen</i> (opposite)
ee	enantiomeric excess
e.g.	<i>exempli gratia</i> (for example)
EI	electron impact (mass spectrometry)
EOM	ethoxymethyl
equiv.	equivalents
ESI	electrospray ionisation (mass spectrometry)
<i>et al.</i>	<i>et alia</i> (and others)
Et	ethyl
EtOAc	ethyl acetate
eV	electron volts
FGI	functional group interconversions(s)
FT	Fourier transform
g	grams(s)
GC	gas chromatography
gen.	generation
h	hour(s)
HMBC	heteronuclear multiple-bond correlation
HMPA	hexamethyl phosphoramide
HPLC	high pressure liquid chromatography
HMQC	heteronuclear multiple-quantum coherence
HRMS	high-resolution mass spectrometry
<i>hν</i>	light
HWE	Horner-Wadsworth-Emmons
Hz	Hertz
IBX	2-iodoxybenzoic acid
<i>i</i>	iso

<i>i.e.</i>	<i>id est</i> (that is)
IR	infrared
<i>J</i>	coupling constant (Hz)
KHMDS	potassium bis(trimethylsilyl)amide
L	litre(s)
<i>l</i>	path length (cm)
LDA	lithium diisopropylamide
lit.	literature value
LRMS	low resolution mass spectrometry
m	multiplet
M	molar
MAPK	mitogen activating protein kinase
<i>m</i> -CPBA	<i>meta</i> -chloroperbenzoic acid
Me	methyl
MeCN	acetonitrile
MHz	mega-Hertz
min	minute(s)
mL	millilitre(s)
mmol	millimole(s)
mol	mole(s)
MOM	methoxymethyl
m.p.	melting point (°C)
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MS	molecular sieves
Ms	methanesulfonyl
<i>m/z</i>	mass-to-charge ratio
nm	nanometre(s)
NBS	<i>N</i> -bromosuccinimide
NMO	<i>N</i> -methylmorpholine- <i>N</i> -oxide
NMP	<i>N</i> -methy-2-pyrrolidone
NMR	nuclear magnetic resonance
<i>o</i>	ortho
ORTEP	Oak Ridge Thermal Ellipsoid Plot
<i>p</i>	para
PCC	pyridinium chlorochromate
PG	unspecified protecting group
Ph	phenyl

ppm	parts per million
<i>i</i> -PrMgCl	<i>iso</i> -propyl magnesium chloride
q	quartet
quant.	quantitative
ref.	reference
R	unspecified alkyl group
RAL	resorcylic acid lactone(s)
RCM	ring closing metathesis
R _f	thin layer chromatography retardation factor
R _t	HPLC retardation time
s	singlet
SAE	Sharpless asymmetric epoxidation
t	triplet
tlc	thin layer chromatography
TBAB	tetra- <i>n</i> -butylammonium bromide
TBAF	tetra- <i>n</i> -butylammonium fluoride
TBAI	tetra- <i>n</i> -butylammonium iodide
TBDPS	<i>tert</i> -butyldiphenylsilyl
TBS	<i>tert</i> -butyldimethylsilyl
TDO	toluene dioxygenase
TFA	trifluoroacetic acid
TFP	tris(2-furyl)phosphine
THF	tetrahydrofuran
TMS	trimethylsilyl
TMSE	trimethylsilyl ethanol
TPAP	tetrapropylammonium perruthenate
Ts	<i>p</i> -toluenesulfonyl
<i>p</i> -TSA	<i>p</i> -toluenesulfonic acid
TsCl	<i>p</i> -toluenesulfonyl chloride
TsOH	toluenesulfonic acid
UV	ultra violet (spectroscopy)
viz.	videlicet (namely)
vs.	versus
v/v	unit volume per unit volume (ratio)
v/v/v	unit volume per unit volume per unit volume (ratio)
w/w	unit weight per unit weight (%)
Z	<i>zusammen</i> (together)

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Chapter One

Introduction

The Resorcylic Acid Lactones (RALs)

1.1 Introductory Comments

Resorcylic acid lactones (RALs) represent a fascinating class of fungal metabolites (mycotoxins) that has attracted the attention of pharmaceutical companies and synthetic groups alike due to their broad range of biological activities. The intense interest in these polyketides has resulted in the development of numerous total syntheses, the generation of significant analogue libraries and the establishment of comprehensive structure activity relationship profiles for the class.¹⁻³

RALs are comprised of a β -resorcylic acid unit (highlighted in red) that is annulated to a fourteen-membered macrolactone (highlighted in blue) incorporating an *S*-configured stereogenic centre at C10' that invariably bears a methyl group (Figure 1.1).

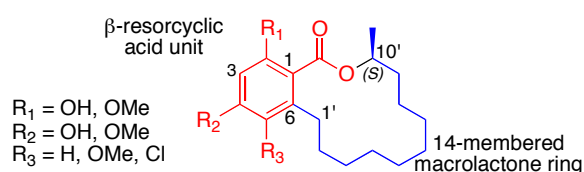


Figure 1.1: General structural features associated with the resorcylic acid lactones (RALs).

To date, a large number of RALs have been isolated and these compounds differ in their embodied functionality that includes epoxides, as well as variations in the degree of unsaturation and the geometry of the olefinic residues incorporated within the macrolactone ring. Variations are also encountered in the nature of substituents associated with the aromatic ring, which may include methyl, chlorine or oxygen-based groups (Figure 1.2).

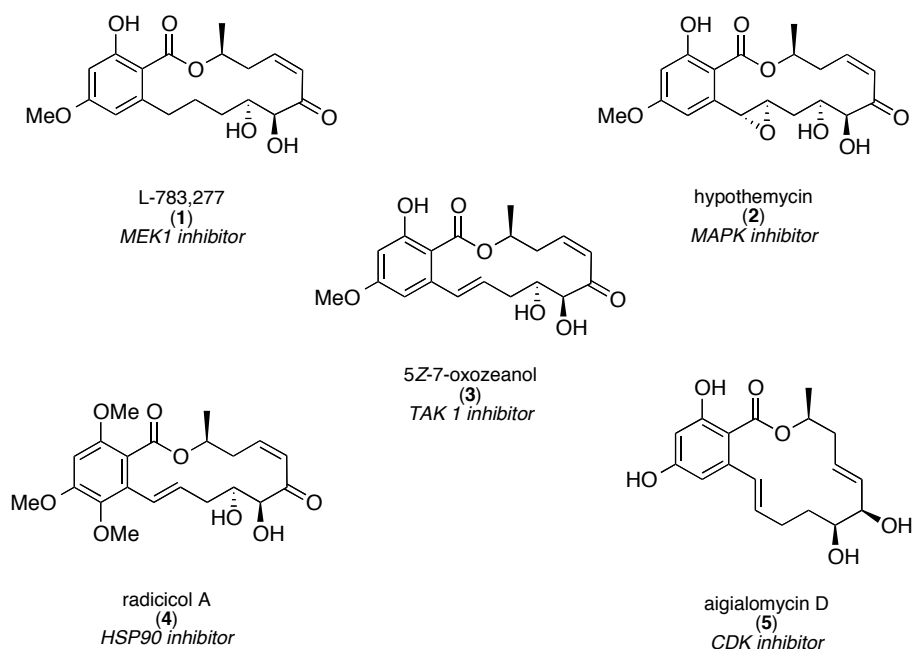


Figure 1.2: Selected members of the RAL family that inhibit kinases.

1.2 Biological Activity of RALs

1.2.1 Overview

The RAL-type natural products have been screened for a diverse range of biological activities and shown to display anti-bacterial,⁴ anti-malarial,⁵ anti-inflammatory⁶ and estrogenic agonist activities.⁷ However, it is their anti-tumour activity that has generated the most attention and this arises from the capacity of the compounds to suppress cellular replication and differentiation through the inhibition of kinases and growth factors.

Kinases, also known as phosphotransferases, are enzymes that catalyse the phosphorylation of proteins associated with receptors and enzymes by transferring a molecule of phosphate from adenosine triphosphate (ATP) onto either a tyrosine, serine or threonine amino acid residue.^{8,9} Once a protein is phosphorylated, it often undergoes changes in its conformation, cellular location and activity or selectively phosphorylates the next kinase in a multi-step cascade.⁸ While kinases facilitate and regulate many biological processes, their main role is to act as a messenger for the transmission of intracellular signals. The protein kinases are an important subclass and their role is to control, upon extracellular stimulation, the intra-cellular signalling of critical biological processes such as mitosis (cellular replication), apoptosis (programmed cell death), homeostasis and cell movement.¹⁰

A pivotal type of protein kinase, and one which is involved in a number of important cellular processes, is the mitogen-activating protein kinase (MAPK). These mediate gene regulation which may lead to apoptosis or may activate a separate group of kinases called the cyclin-dependent kinases (CDKs) which lead to cell proliferation (Figure 1.3).^{11,12} Tumour growth can occur when kinase signalling favours mitosis over apoptosis.¹³

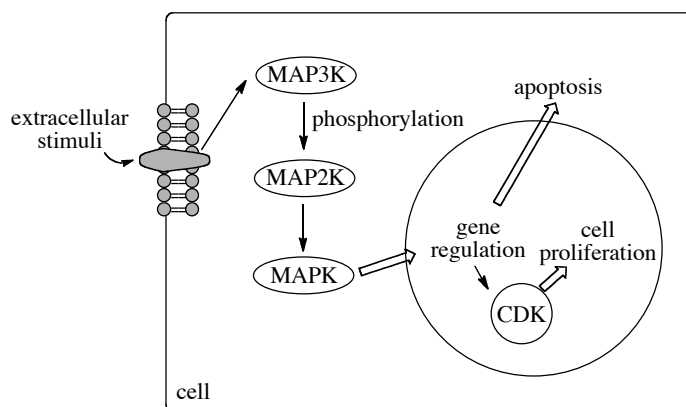


Figure 1.3: MAPK cellular signalling pathway.

Through the application of various biological assays, it was shown that the RALs described in this thesis target either MAPKs or CDKs. As such, these RALs are attractive agents for targeting cancer at the signalling level, for probing kinase activity and for understanding the involvement of kinases in a number of diseases. On the basis of various studies, it is believed that the mechanism of kinase inhibition exerted by RALs involves their binding in the ATP active site of the enzyme and so preventing it from phosphorylating the relevant amino acid residue.¹⁴ The following section provides a brief summary of RALs that have been found to inhibit MAPKs and CDKs.

1.2.1.1 Mitogen-Activating Protein Kinases (MAPKs)

The pivotal involvement of MAPKs in a number of critical cellular processes suggests that they are a suitable target for treating patients with disorders regulated by this kinase and thus the RALs that inhibit these are potentially useful therapeutic agents.

The capacity of RALs to inhibit kinases was first reported in 1998 by Zhao *et al.*,¹⁵ who showed that L-783,277 (**1**) and hypothemycin (**2**) were potent inhibitors of MEK1 (a type of MAPK) displaying IC₅₀ values of 15 and 4 nM, respectively (MEK1 participates in the *Erk* cascade, a process that regulates several important cellular events such as mitosis, apoptosis and cellular differentiation).^{11,12} The inhibitory mechanism of these compounds is believed to involve a conjugate addition of the thiol moiety of a cysteine residue within the ATP active site to the α,β -unsaturated ketone residue within these biologically active RALs.^{15,16,17}

A study by Matsumoto *et al.* revealed that the RAL 5Z-7-oxozeanol (**3**, a.k.a LL-Z1640-2) was a potent inhibitor of TAK1 (IC₅₀ of 8 nM) but a weak inhibitor of MEK 1 (IC₅₀ of 411 nM), both of which are types of MAPKs, and thus indicating that there can be selectivity in the interaction of these macrolides with different MAPKs.⁶

Radical A (**4**) is a *cis*-configured enone RAL discovered by the workers at Sandoz who found that it caused the degradation of the mRNA associated with interleukin 1 beta (IL1 β), a protein responsible for certain inflammatory effects.¹⁸ The biological activity exhibited by RAL **4** led to an evaluation of this compound against a kinase panel by Winssinger *et al.* where it was found to be capable of inhibiting several kinases (at micro- and nano-molar concentrations) involved in the MAPK cascade.¹⁹ Consequently, Winssinger *et al.* postulated that the cause of IL1 β 's mRNA degradation was the deactivation of these MAPKs by radical A (**4**).¹⁹ This observation highlights the importance of MAPKs in protein translation through stabilisation of mRNA.

1.2.1.2 Cyclin-Dependent Kinases (CDKs)

CDKs are critical enzymes that regulate and facilitate various stages of the cell division and differentiation cycle. A particular kinase of this class that has the potential for suppressing tumour growth is CDK1, an enzyme that regulates the division of a mother cell into two daughter cells (a.k.a mitosis).^{8,20,21} Aigialomycin D (**5**) is a RAL that has attracted a significant amount of interest since its isolation by Isaka *et al.* in 2002 due to its potent inhibitory activity against CDK1 and 5 in Winssinger's kinase screening panel, with IC₅₀ values of 5.7 and 5.8 μ M, respectively, being observed.^{1,5,22}

1.3 Synthetic Approaches to RALs

1.3.1 Overview

The abovementioned biological activity highlights the importance of RALs as potential therapeutic agents. As such, a significant number of total and formal syntheses of various RALs have been reported and an assessment of these reveals a common strategy for their assembly, involving construction of the C6–C1', C7'–C8' and macrolactone bonds. The fragments required in such an approach are the aromatic (blue), secondary alcohol (green) and diol (red) units shown in Figure 1.4. A brief description of the means for preparing each of these fragments is presented in the following sections.

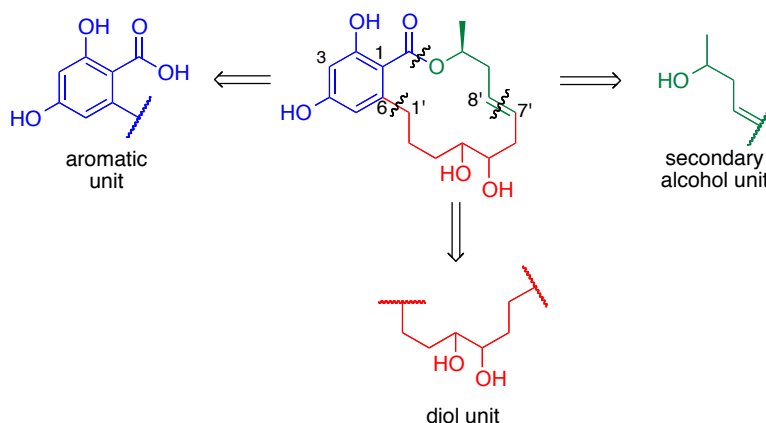
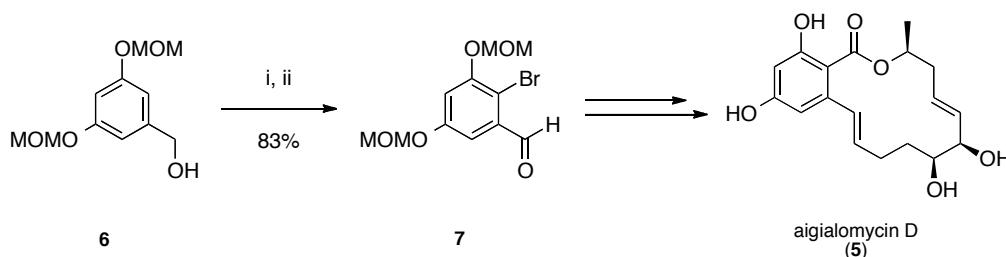


Figure 1.4: Common disconnections associated with many RAL syntheses.

1.3.2 Synthesis of Fragments

1.3.2.1 Synthesis of the Aromatic Unit

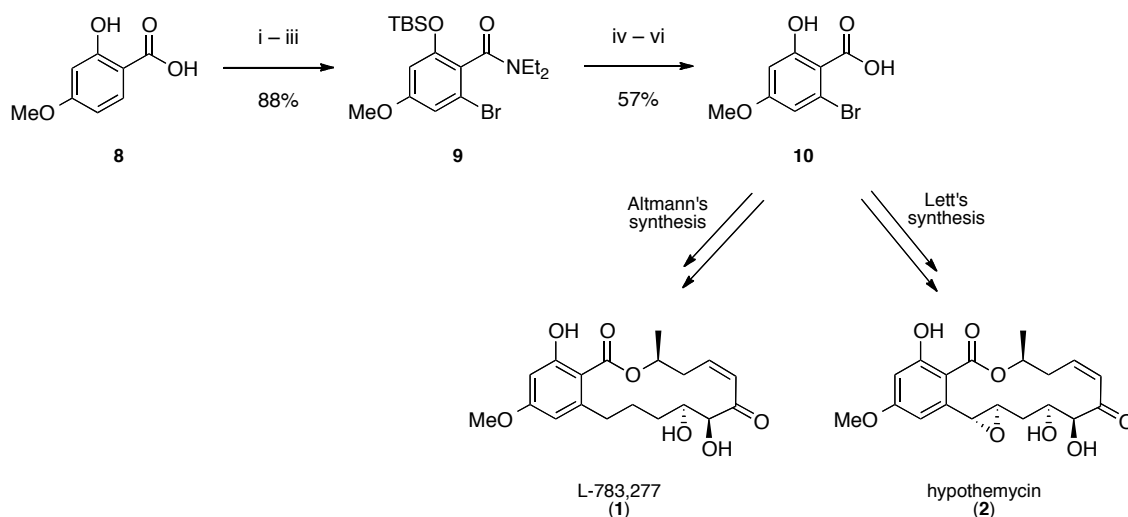
With the exception of the Barrett and Danishefsky groups' syntheses of RALs, both of which are detailed in Section 1.4, the aromatic fragment of each RAL was prepared from resorcinol derivatives that contained "handles" for the installation of the secondary alcohol and/or the diol moieties associated with the macrolactone ring. As a result, the functionality present on the resorcinol unit is introduced at an early stage and examples of RAL syntheses involving this approach are now presented. In most of these cases a substituent (usually bromine) is incorporated on the aromatic ring at the position which corresponds to the desired point for the attachment of the secondary alcohol or diol fragment. For example, Pan *et al.* (Scheme 1.1) efficiently assembled the required aromatic fragment **7** from the MOM-protected resorcinol derivative **6** through an electrophilic aromatic substitution reaction using *N*-bromosuccinimide as the bromine source.²³ The bromide **7** so-formed was used to introduce the carboxylic acid functionality, *via* a carboxylation involving sequential treatment with *n*-BuLi and carbon dioxide, for the attachment of the secondary alcohol residue.



Reagents and conditions: (i) NBS, CHCl₃, rt, 0.5 h; (ii) PCC, NaOAc, CH₂Cl₂, rt, 4 h.

Scheme 1.1: Synthesis of aigialomycin D (**5**) by Pan *et al.* from resorcinol **6**.²³

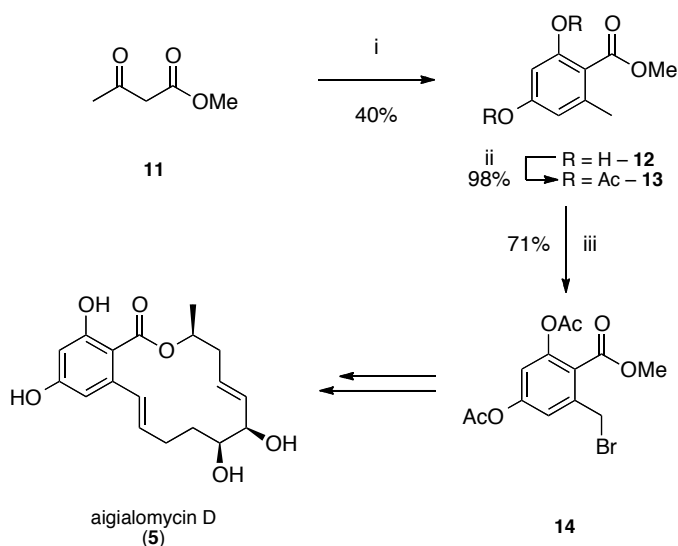
In the two examples shown in Scheme 1.2, Lett *et al.* and Altmann *et al.* prepared the required aromatic fragment **10** by generating bromide **9** from precursor **8** using directed-metallation followed by bromination. Several straight-forward steps were then used to elaborate compound **9** to key building block **10**. Both Altmann *et al.* and Lett *et al.* employed this same functionalised aromatic fragment, namely compound **10**, in their syntheses of targets **1** and **2**, respectively, and thus highlighting the potential of a common aromatic fragment for assembling several different RALs.



Reagents and conditions: (i) TBSCl, *i*-Pr₂NEt, DMF, rt, 2 h; (ii) (a) (COCl)₂, DMF, CH₂Cl₂, -10 °C → rt, overnight, (b) Et₂NH, 1 h, rt; (iii) (a) *t*-BuLi, Et₂O, -78 °C, 0.2 h (b) Br₂; (iv) Me₃O⁺BF₄⁻, CH₂Cl₂, rt, overnight, then evaporation; (v) aq. saturated Na₂CO₃/MeOH v/v 1:1, rt, 6 h; (vi) conc. NaOH/DME v/v 1:1, reflux, overnight.

Scheme 1.2: Altmann's synthesis of L-783,277 (**1**) and Lett's synthesis of hypothemycin (**2**) from aromatic fragment **10**.²⁴⁻²⁶

The aromatic fragment can also be generated in a biomimetic fashion as seen in Harvey's synthesis of aigialomycin D (**5**) (Scheme 1.3) wherein the arene **12** was prepared by cyclodimerisation reaction of methyl acetoacetate (**11**).²⁷ The readily derived bromide **14** was then used as the building block to which the diol unit associated with the macrocycle was attached.



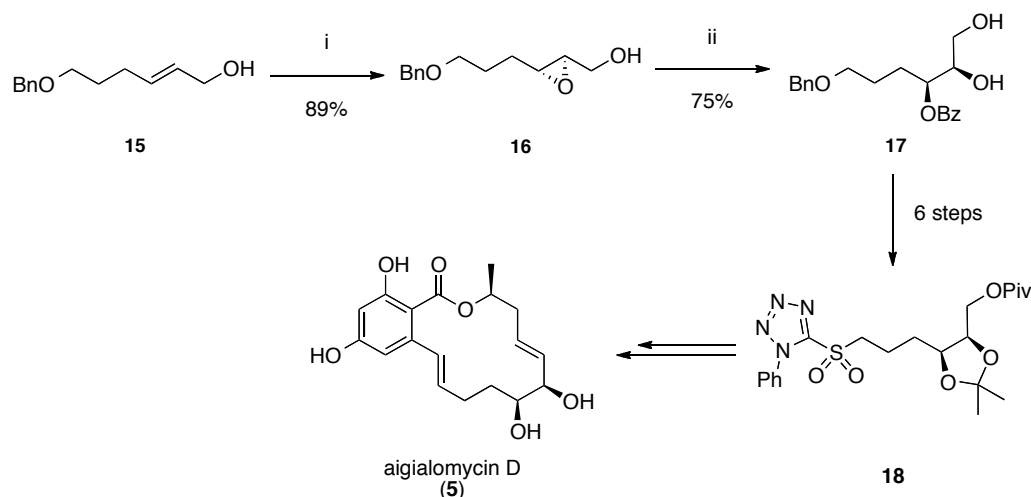
Reagents and conditions: (i) (a) NaH, THF, 0 °C $\rightarrow$ rt, (b) *n*-BuLi, THF, -78 °C $\rightarrow$ rt, 12 h, (c) HCl, H₂O, rt, 12 h; (ii) Ac₂O, NEt₃, CH₂Cl₂, rt, 12 h; (iii) NBS, [PhC(O)O₂]₂, CCl₄, reflux $\rightarrow$ rt, 6 h.

Scheme 1.3: Biomimetic synthesis of the aromatic fragment **14** associated with Harvey's synthesis of aigialomycin D (**5**).²⁷

1.3.2.2 Synthesis of the Diol Unit

The diol unit is commonly prepared by one of two methods, namely through a regioselective ring-opening of a chiral epoxide or through the manipulation of a monosaccharide precursor.

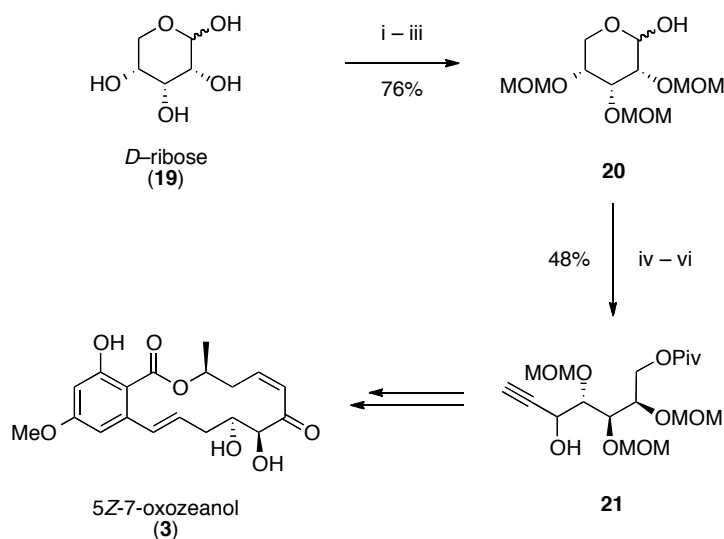
The ring-opening strategy was exploited in Pan's synthesis of aigialomycin D (**5**) (Scheme 1.4) wherein the protected diol fragment **18** was prepared by a titanium-assisted regioselective epoxide ring-opening of compound **16** (itself obtained by a SAE of allylic alcohol **15**) with benzoic acid to afford the benzoate **17**. Compound **17** was then converted into the target diol fragment **18** in a further six steps.²³



Reagents and conditions: (i) $\text{Ti}(\text{O-}i\text{Pr})_4$, TBHP, $(-)\text{-DIPT}$, CaH_2 , CH_2Cl_2 , $-25\text{ }^\circ\text{C}$, 12 h; (ii) $\text{Ti}(\text{O-}i\text{Pr})_4$, PhCO_2H , CH_2Cl_2 , rt, 0.25 h.

Scheme 1.4: Pan's synthesis of aigialomycin D (5) from allylic alcohol 15 using Sharpless asymmetric epoxidation and epoxide ring-opening protocols.²³

The second method for generating the diol fragment involves the manipulation of monosaccharides that possess the diol motif in the desired configuration. Such an approach was exploited in Tatsuta's synthesis of 5Z-7-oxozeanol (3) wherein *D*-ribose (19) was converted into the diol fragment 21 in six steps (Scheme 1.5).²⁸

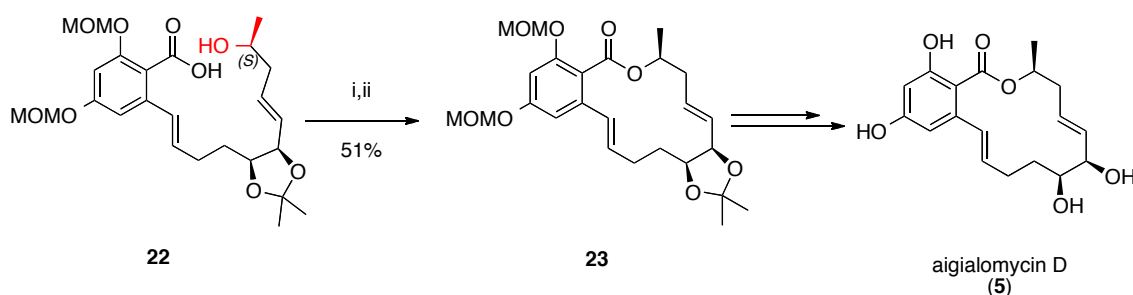


Reagents and conditions: (i) CSA, BnOH , $80\text{ }^\circ\text{C}$; (ii) MOMCl , $i\text{-Pr}_2\text{NEt}$, MeCN , $50\text{ }^\circ\text{C}$, 4 h; (iii) H_2 , $\text{Pd}(\text{OH})_2$, EtOH , 3 h; (iv) TMS-acetylene, $n\text{-BuLi}$, $\text{BF}_3\cdot\text{Et}_2\text{O}$, THF , $-78\text{ }^\circ\text{C} \rightarrow \text{rt}$; (v) PivCl , pyridine, $0\text{ }^\circ\text{C}$, 1 h; (vi) TBAF, AcOH , THF , 2 h.

Scheme 1.5: Construction of the protected diol fragment 21 associated with Tatsuta's synthesis of 5Z-7-oxozeanol (3) from *D*-ribose (19).²⁸

1.3.2.3 Synthesis of the Secondary Alcohol Unit

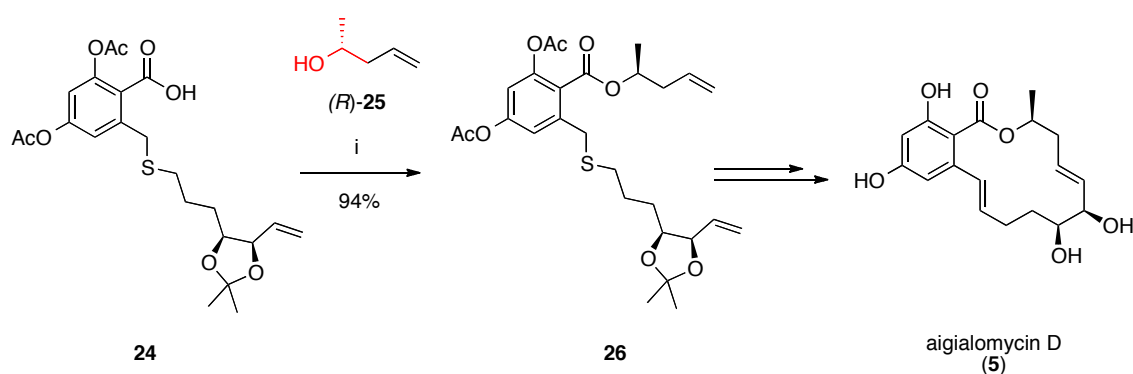
The precise choice of the secondary alcohol is dependent on the coupling strategy planned for linking this unit to the aromatic fragment. If the coupling strategy results in retention of stereochemistry, by virtue of using a Mukaiyama or Yamaguichi esterification or macrolactonisation protocol, then the *S*-configured alcohol is used, as exemplified in Pan's synthesis of aigialomycin D (**5**) (Scheme 1.6).²³



Reagents and conditions: (i) 2,4,6-trichlorobenzoyl chloride, Et₃N, THF, rt, 2 h; (ii) DMAP, PhMe, reflux, 36 h.

Scheme 1.6: Synthesis of aigialomycin D (**5**) utilising a Yamaguchi macrolactonisation as employed by Pan et al.²³

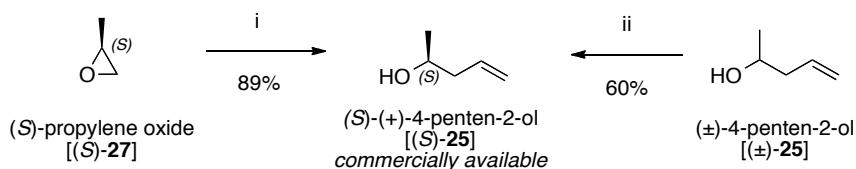
Conversely, if an inversion of the stereochemistry is involved, as would be the case, for example, when Mitsunobu conditions are employed, then the *R*-configured alcohol is used. Harvey's synthesis of aigialomycin D (**5**) (Scheme 1.7) demonstrates this approach.²⁷



Reagents and conditions: (i) DIAD, PPh₃, THF.

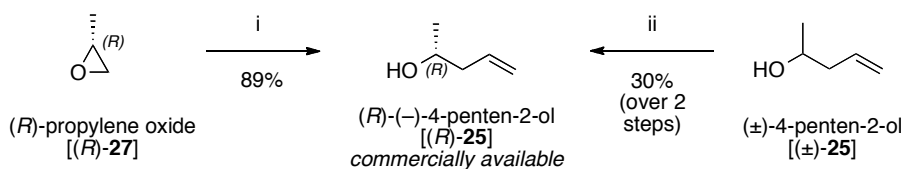
Scheme 1.7: Synthesis of aigialomycin D (**5**) using a Mitsunobu esterification by Harvey et al.²⁷

In many previous syntheses, the commercially available *R*- and *S*- enantiomers of 4-penten-2-ol (**25**) were used as the secondary alcohol unit due to the capacity for the terminal olefin to engage in a subsequent ring-closing metathesis (RCM) process as a means for constructing the macrolactone ring. This alcohol fragment can also be readily prepared by chemical means (e.g. through the nucleophilic ring-opening of the appropriate enantiomer of propylene oxide **27**)^{29,30} or using enzymatic techniques^{31,32} [in particular, through the resolution of the corresponding racemate ($\pm$)-**25** (Scheme 1.8 and Scheme 1.9), as described in Chapter 2].



Reagent and conditions: (i) vinylmagnesium bromide, THF, CuI, $-20\text{ }^{\circ}\text{C}$; (ii) (a) *C. antarctica* lipase B, vinyl acetate, $30\text{ }^{\circ}\text{C}$, 1 h.

Scheme 1.8: Methods for the generation of (*S*)-(+)-4-penten-2-ol [(*S*)-**25**].^{29,30,31,32}



Reagent and conditions: (i) vinylmagnesium bromide, THF, CuI, $-20\text{ }^{\circ}\text{C}$; (ii) (a) *C. antarctica* lipase B, vinyl acetate, $30\text{ }^{\circ}\text{C}$, 1 h; (b) *C. antarctica* lipase B, phosphate buffer (pH = 7.3), $30\text{ }^{\circ}\text{C}$, 10 h.

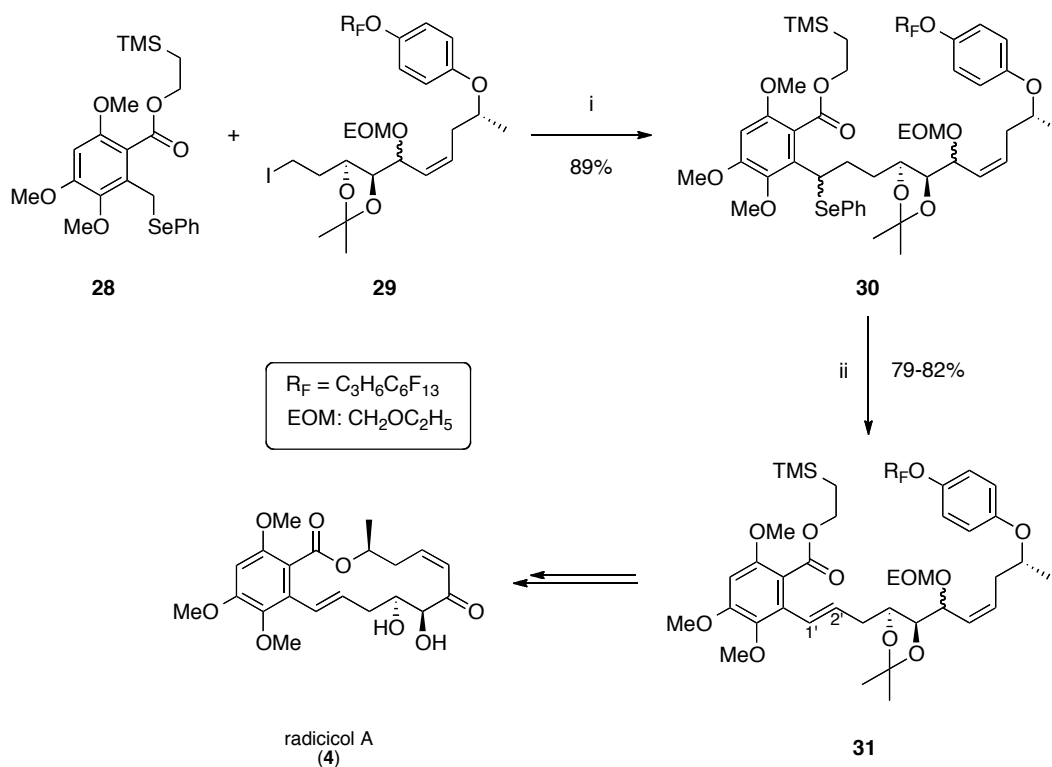
Scheme 1.9: Methods for the generation of (*R*)-(-)-4-penten-2-ol [(*R*)-**25**].^{29,30,31,32}

1.3.3 Strategies for Fragment Coupling

An assessment of the RAL syntheses reported to date reveals that several reaction types have been used repeatedly to couple the abovementioned fragments due to their reliability in achieving the desired outcomes. These reactions are discussed below together with a review of the details of several recently reported and significant syntheses of enone-containing RALs and aigialomycin D (**5**).

1.3.3.1 Union of Aromatic and Diol Units

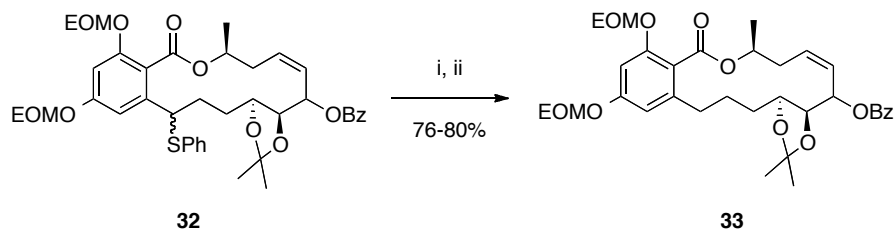
Winssinger's synthesis of radicicol A (**4**), wherein the α -anion of selenide **28** was alkylated by iodide **29** (Scheme 1.10), highlights one method for the coupling of the aromatic and diol units, namely through a nucleophilic substitution reaction. The C1'-C2' double bond of target alkene **31** was then revealed through an oxidation and elimination of the phenylselenenyl moiety within product **30**. As such, this strategy can be used to prepare RALs incorporating unsaturation between C1' and C2'.



Reagents and conditions: (i) LDA, THF/HMPA v/v 10:1, -78 °C, 0.3 h; (ii) H₂O₂, THF, 20 °C, 2 h.

Scheme 1.10: Coupling of aromatic fragment **28** and protected diol fragment **29** associated with Winssinger's synthesis of radical A (**4**).³³

That having been said, the application of the nucleophilic substitution strategy has also been extended to the preparation of RALs, such as **33**, which are saturated at this position – a state that can be achieved through reductive desulfurisation of lactones such as **32** (Scheme 1.11) to give compound **33**.³⁴

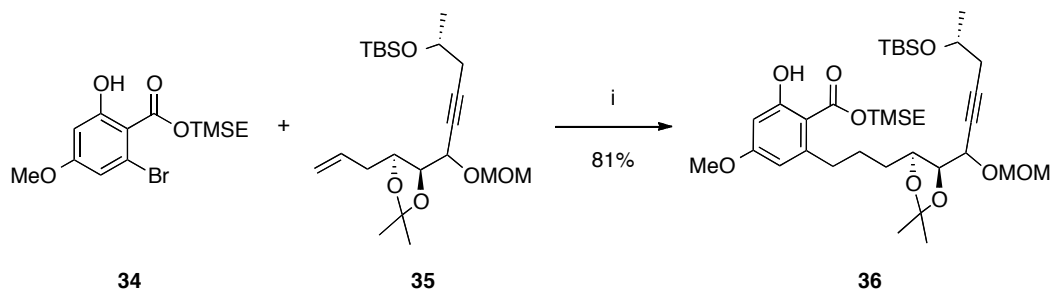


Reagents and conditions: (i) tris(1H,1H,2H,2H-perfluorooctyl)tin hydride, AIBN, PhMe, 150 °C, μ wave, 0.2 h; (ii) 1% NaOH/MeOH, 23 °C, 12 h.

Scheme 1.11: Access to saturated C1'-C2' RALs as achieved by Winssinger et al.³⁴

* Winssinger *et al.* employed fluororous tags in their synthesis to simplify the isolation of products using chromatography.³³

Another strategy used to link the title fragments involves Suzuki-Miyaura or Sonogashira cross-coupling reactions. An example of the former approach is seen in Altmann's synthesis of L-783,277 (**1**) wherein the alkene residue of protected diol fragment **35** was hydroborated with 9-BBN and the resulting borane cross-coupled with the aryl bromide **34** to afford compound **36** in 81% yield (Scheme 1.12).²⁴

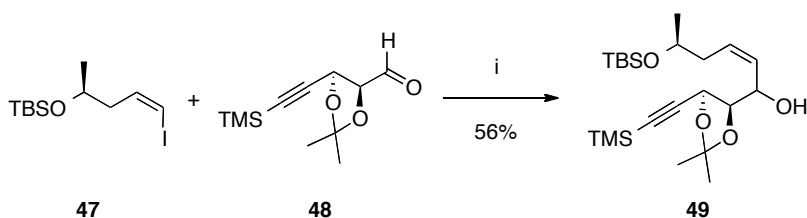


Reagents and conditions: (i) (a) **35**, 9-BBN, THF, rt, 2 h, (b) 2 M K₃PO₄, [Pd(OAc)₂ + 4TFP], DME, reflux, 5.5 h.

Scheme 1.12: Suzuki-Miyaura cross-coupling reaction employed in Altmann's synthesis of L-783,277 (**1**).²⁴

1.3.3.2 Union of the Secondary Alcohol and Diol Units

The union of the secondary alcohol and diol fragments, prior to the attachment to the aromatic unit, is a strategy that is employed heavily in the syntheses of *cis*-configured enone RALs. This approach often involves a nucleophilic addition of an organolithium (generated from a reaction between the secondary alcohol fragment and a butyl lithium) to an aldehyde residue on the diol unit. An example of this strategy is seen in Lett's synthesis of 5*Z*-7-oxozeanol (**3**), wherein the organolithium, prepared from the reaction of alkyl iodide **47** and *t*-BuLi, undergoes an addition to the aldehyde moiety within compound **48** (Scheme 1.13).²⁶ Compounds **29** (Scheme 1.10) and **35** (Scheme 1.12) were also prepared in a similar manner and the details of their syntheses can be found in Section 1.4.1.



Reagents and conditions: (i) (a) **47**, Et₂O, -78 → 0 °C, (b) *t*-BuLi, pentane, 0.25 h, (c) **48**, pentane, -78 → 0 °C.

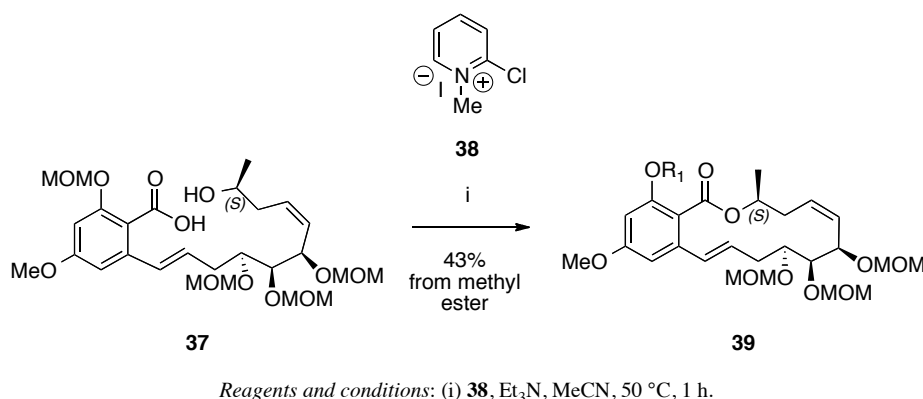
Scheme 1.13: Assembly of the secondary alcohol **47** and the protected diol fragment **48** in Lett's synthesis of 5*Z*-7-oxozeanol (**3**).²⁶

1.3.4 Modes of Macrocycle Preparation

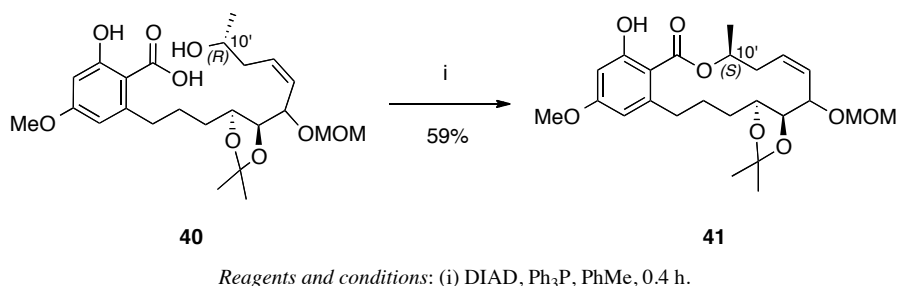
Analysis of previous syntheses of RALs indicates that there have been two major approaches employed for the construction of the macrolactone moiety. The first is a coupling between the secondary alcohol unit and the aromatic carboxylic acid fragment of the open-chain precursor, and the second being a reaction between the secondary alcohol unit and the diol fragment.

1.3.4.1 Macrolactonisation Between the Termini of the Secondary Alcohol and Aromatic Units

In the first instance, as mentioned earlier, the precise choice of conditions required for the macrolactonisation between the secondary alcohol and aromatic carboxylic acid fragments is dependent on the stereochemistry of the secondary alcohol used and the mode of ring-closure. For example, macrolactonisation has been achieved using compound **37** under Yamaguchi or Mukaiyama protocols (Scheme 1.14), or with the related *R*-enantiomer **40** by employing Mitsunobu conditions (Scheme 1.15) to invert the stereochemistry of the C10' methyl group.



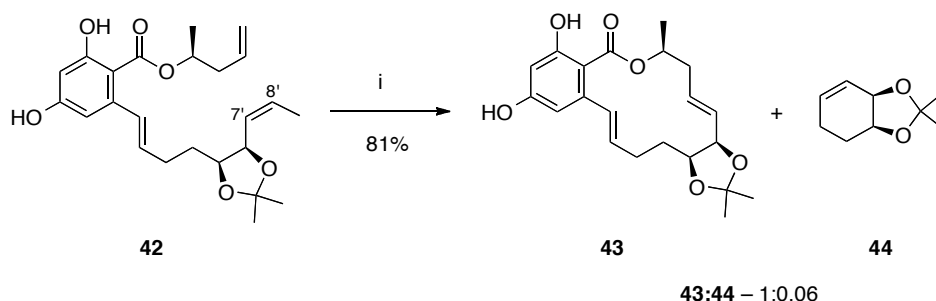
Scheme 1.14: Mukaiyama macrolactonisation protocol employed in Tatsuta's synthesis of 5*Z*-7-oxozeanol (**3**).²⁸



Scheme 1.15: Mitsunobu macrolactonisation protocol employed in Altmann's synthesis of L-783,277 (**1**).²⁴

1.3.4.2 Ring-closing Metathesis Reaction Between the Termini of the Secondary Alcohol and Diol Units

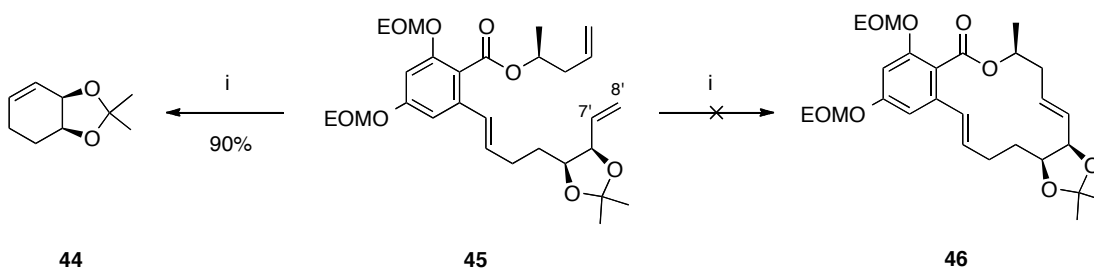
The second approach to creating the macrolactone moiety, involving a linking of the secondary alcohol and diol units, is a RCM reaction between the unsaturated termini associated with the respective units. This method is common amongst the aigialomycin D (**5**) syntheses (Scheme 1.16 and 1.17).



Reagents and conditions: (i) Grubbs-Hoveyda 2nd generation catalyst, CH₂Cl₂, μ wave, 85 °C, 3 h.

Scheme 1.16: RCM reaction employed in Barrett's synthesis of aigialomycin D (**5**).³⁵

However, difficulties are sometimes encountered in using this reaction due to the potential for formation of a cyclohexene by-product, such as compound **44**, (Scheme 1.17) in substrates such as **42** and **45** incorporating three alkene moieties.



Reagents and conditions: (i) Grubbs' 2nd generation catalyst, PhMe, 80 °C, 12 h.

Scheme 1.17: Cyclohexene-containing by-product **44** obtained in Winssinger's synthesis of aigialomycin D (**5**).¹

The difference in the outcomes of the RCM reactions shown in Scheme 1.16 and Scheme 1.17 may be due to the RCM precursor **42** (Scheme 1.16) containing a single mono-substituted terminal olefin with the second terminal olefin replaced with a 1,2-disubstituted alkene (at C7'-C8' position). As discussed in more detail in Chapter Four, the two now quite distinct olefinic residues allow the catalyst to differentiate between double bonds and initiate metathesis at the more reactive olefin, namely the sterically less hindered mono-substituted alkene. In contrast, the presence of two very similar mono-substituted olefinic termini in RCM substrate **45** (Scheme 1.17), may result in initiation of the metathesis process at either end and where commencement of the process at the C7'-C8' residue may produce the presumably kinetically favoured, but undesired, by-product **44**.

1.4 Significant RAL Syntheses

The large number of RAL syntheses reported to date reflects the increased interest, especially by pharmaceutical companies, in their biological activity. Detailed summaries of several significant syntheses of enone-containing RALs and aigialomycin D (**5**) are presented herein.

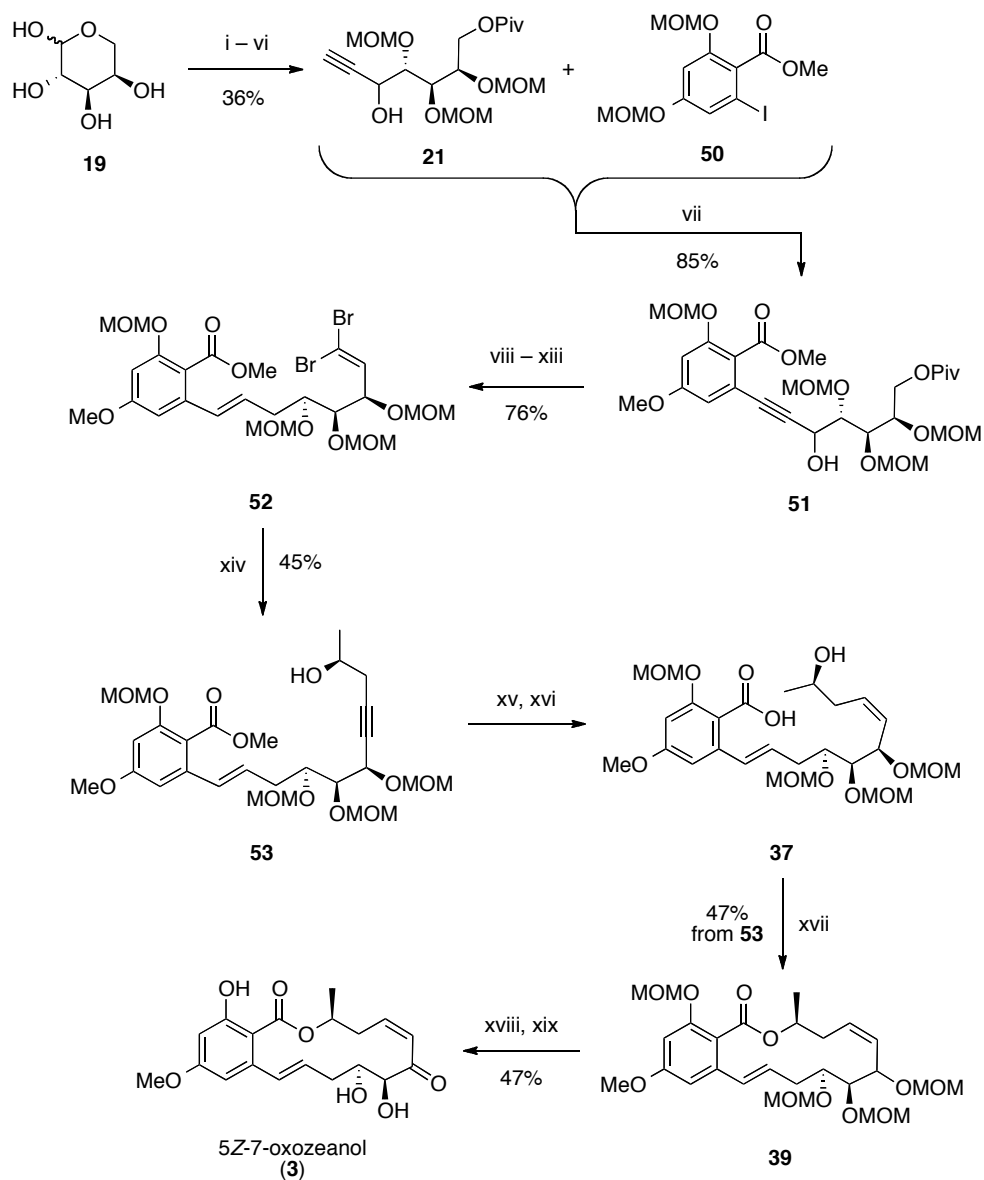
1.4.1 Syntheses of Enone-containing RALs

The synthesis of *cis*-configured enone-containing RALs is not a trivial task as the enone functionality is susceptible to nucleophilic addition/elimination reactions and, thereby, isomerisation to the more stable *trans*-isomer. Consequently, most syntheses establish this sensitive moiety towards the end of the reaction sequence. Three noteworthy syntheses of biologically significant, *cis*-enone-containing RALs that exemplify this approach are presented below.

Tatsuta's Synthesis of 5Z-7-oxozeanol (3, LL-Z1640-2, 2001)²⁸

Tatsuta *et al.* completed the first synthesis of 5Z-7-oxozeanol (**3**) in 2001 by employing *D*-ribose (**19**) (Scheme 1.18) to prepare the protected diol **21** in six steps. Fragment **21** was coupled to the aromatic fragment **50** *via* a Sonogashira reaction to give alkyne **51**. Compound **51** was then subjected to a Lindlar reduction and a Tsuji hydrogenolysis to convert the alkyne into the *E*-alkene while the pivolyl-protected hydroxyl group was deprotected, oxidised to an aldehyde (under Swern conditions) and then olefinated under Corey-Fuchs conditions to afford di-bromoolefin **52**. Installation of the secondary alcohol was accomplished by a ring-opening of *S*-propylene oxide [(*S*)-**27**] with an acetylide anion generated using a Corey-Fuchs reaction of the dibromo-olefin **52** to give alkyne **53** which was, in turn, subjected to a Lindlar reduction to furnish the *Z*-configured alkene. A base-promoted hydrolysis, followed by acidic work up, of the methyl ester provided acid **37**.[†] Compound **37** was subjected to a Mukaiyama macrolactonisation reaction to give macrocycle **39** which, in turn, was globally deprotected and then the so formed allylic alcohol was oxidised with Dess-Martin periodinane (DMP) to deliver 5Z-7-oxozeanol (**3**).

[†] Results obtained from the work completed in Chapter Three indicate that the alkyne can be incorporated into the macrocycle due to the large ring size.

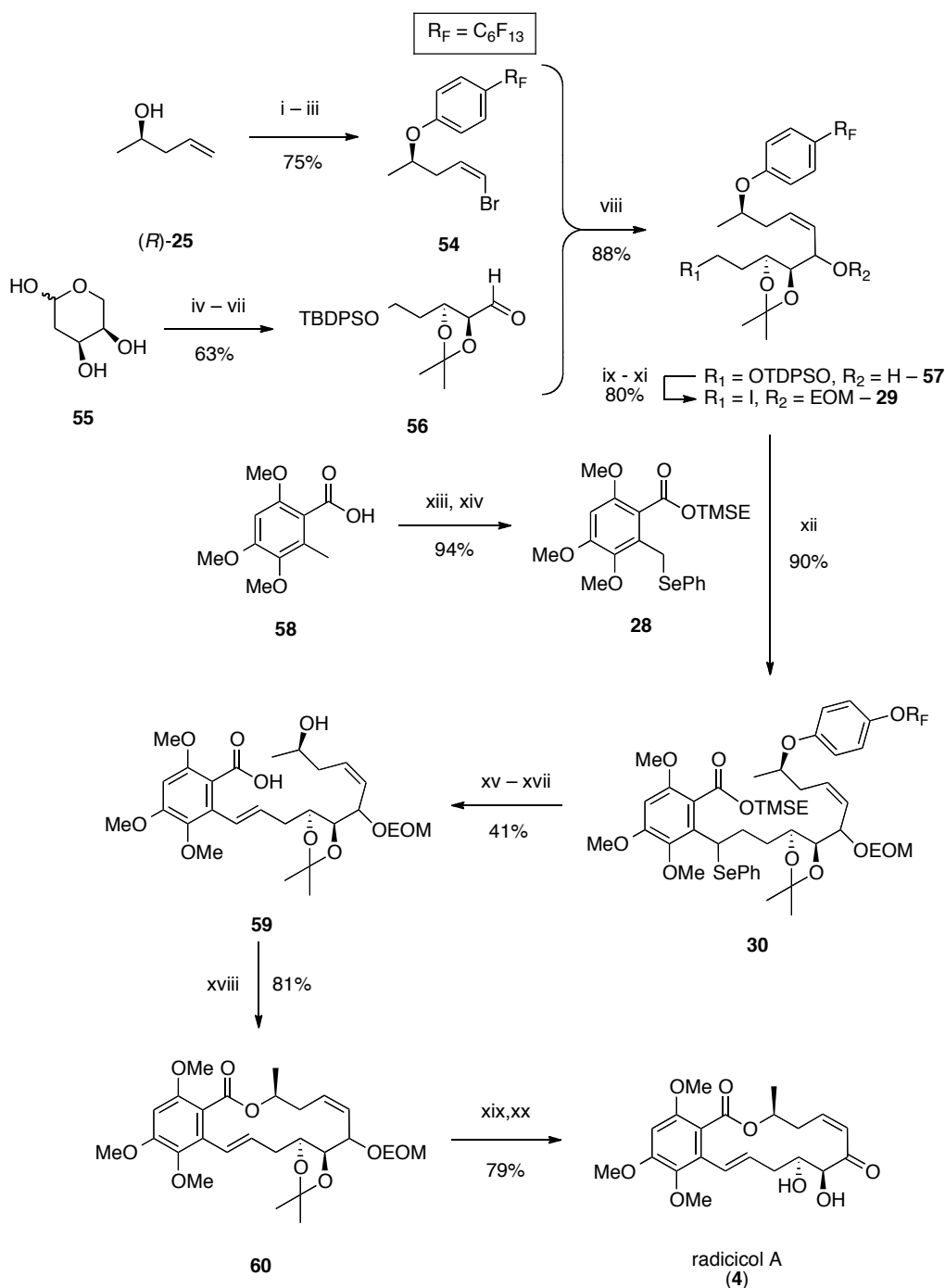


Reagents and conditions: (i) CSA, BnOH; (ii) MOMCl, *i*-Pr₂NEt; (iii) H₂, Pd(OH)₂; (iv) TMS-acetylene, *n*-BuLi, BF₃•Et₂O; (v) PivCl, pyridine; (vi) TBAF, AcOH; (vii) Pd(OAc)₂, CuI, Ph₃P, Et₃N; (viii) ClC(O)OEt, pyridine; (ix) H₂, Pd on BaCO₃, quinoline, EtOH; (x) Pd(dba)₂CHCl₃, *n*-Bu₃P, HCOONH₄; (xi) MeONa; (xii) (COCl)₂, DMSO, Et₃N; (xiii) CBr₄, Ph₃P; (xiv) (*S*)-propylene oxide, *n*-BuLi, BF₃•Et₂O; (xv) H₂, Pd on BaCO₃, quinoline; (xvi) 2 M NaOH; (xvii) 2-chloro-1-methyl-pyridinium iodide (**38**), Et₃N; (xviii) 5% HCl; (xix) DMP.

Scheme 1.18: Tatsuta's synthesis of 5Z-7-oxozeanol (**3**).²⁸

Winssinger's Synthesis of Radicol A (4) (2007)³³

In 2007 Winssinger *et al.* completed an enantioselective total synthesis of radicol A (**4**) (Scheme 1.19). The preparation of this target commenced with the secondary alcohol (*R*)-(-)-4-penten-2-ol [(*R*)-**25**] which was converted into the *Z*-configured and 1,2-disubstituted olefin **54** by cross-metathesis with vinyl pinacolborolane followed by a regioselective bromination. The organolithium generated from olefin **54** participated in a nucleophilic addition to the aldehyde functionality of protected diol fragment **56** [synthesised from 2-deoxy-*D*-ribose (**55**)]. The resulting alcohol, **57**, was protected as the EOM ether, while the TBDPS-protected alcohol was liberated and converted into the alkyl halide **29**. Fragment **29** was subsequently coupled to the aromatic unit **28** (prepared from the β -resorcylic acid derivative **58** in two steps) *via* a nucleophilic substitution reaction to afford selenide **30**. Intermediate **30** was itself subjected to an oxidation and elimination of the selenide functionality to reveal the C1'-C2' olefin which underwent a subsequent desilylation to deliver acid **59**. Compound **59** was subjected to a Mitsunobu macrolactonisation reaction to provide the fourteen-membered macrocyclic ring **60** in 81% yield. The protecting groups within compound **60** were carefully removed with BCl₃ and the allylic alcohol so revealed was oxidised with IBX to furnish radicol A (**4**).

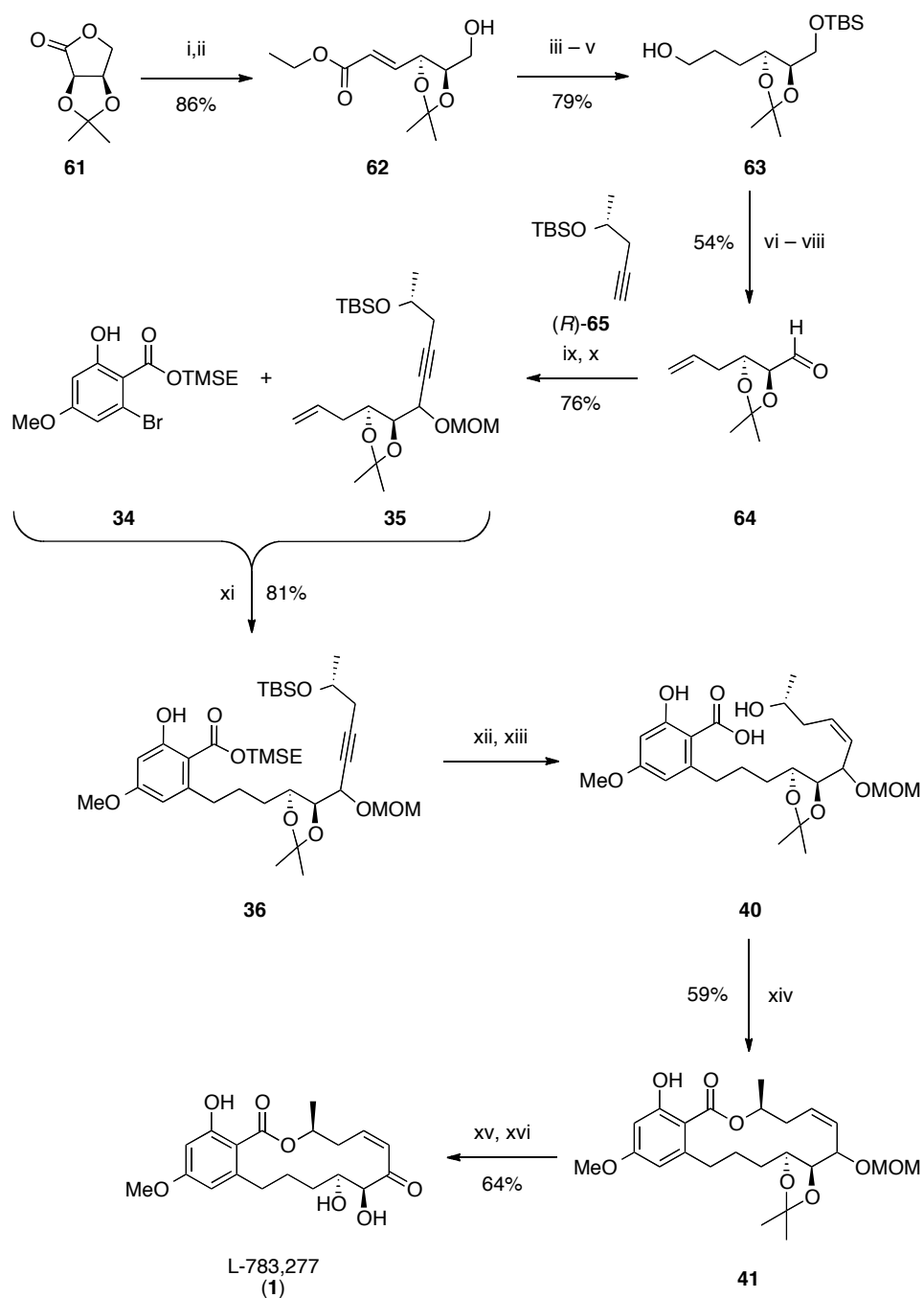


Reagents and conditions: (i) p -($\text{C}_6\text{F}_{13}\text{C}_3\text{H}_6\text{O}$) $\text{BnO}(\text{NH}=\text{CCl}_3)$, CSA; (ii) vinyl pinacolborolane, Grubbs' 2nd generation catalyst; (iii) Br_2 , NaOMe; (iv) 2-methoxypropene, p -TSA; (v) LiAlH_4 ; (vi) TBDPSCl, imidazole; (vii) polystyrene bound IBX; (viii) (a) **54**, t -BuLi, (b) **56**; (ix) EOMCl, i -Pr₂NEt, TBAI; (x) TBAF; (xi) Ph_3P , I_2 , imidazole; (xii) **28**, LDA; (xiii) $(\text{COCl})_2$, DMF then 2-(trimethylsilyl)ethanol, Et₃N, DMAP; (xiv) LDA, $(\text{PhSe})_2$; (xv) H_2O_2 ; (xvi) DDQ; (xvii) TBAF; (xviii) $\text{R}^F\text{-Ph}_3\text{P}$, $\text{R}^F\text{-DEAD}$; (xix) BCl_3 ; (xx) polystyrene bound IBX.

Scheme 1.19: Synthesis of radicicol A (**4**) by Winssinger et al.³³

*Altmann's Synthesis of L-783,277 (1) (2008)*²⁴

In 2008 Altmann *et al.* reported the first enantioselective synthesis of L-783,277 (**1**) (Scheme 1.20). The preparation of this RAL commenced with the manipulation of isopropylidene-*D*-erythrono-1,4-lactone (**61**) which was ring-opened and olefinated, under Wittig conditions, to afford α,β -unsaturated ethyl ester **62**. The associated double bond was hydrogenated and the primary alcohol was protected as the silyl ether before the ethyl ester was reduced to give the alcohol **63**. The alcohol residue within compound **63** was dehydrated under Grieco conditions to provide the corresponding alkene while removal of the silyl ether and oxidation of the resulting primary alcohol afforded fragment **64**. The organolithium generated from secondary alcohol (*R*)-**65** then participated in a nucleophilic addition to the aldehyde functionality of fragment **64** and the resulting alcohol was protected as the MOM ether to afford compound **35**. Hydroboration of olefin **35** with 9-BBN followed by a Suzuki-Miyaura reaction with the aromatic fragment **34** provided intermediate **36** that was subjected to a Lindlar reduction to convert the alkyne into the *Z*-configured alkene and a global desilylation of this material using TBAF then gave acid **40**. Compound **40** participated in a Mitsunobu macrolactonisation reaction to provide the fourteen-membered ring product **41** which was globally deprotected and the allylic alcohol unit so revealed was subsequently oxidised with IBX to afford L-783,277 (**1**).



Reagents and conditions: (i) DIBAL-H; (ii) $\text{Ph}_3\text{PCHCOOEt}$; (iii) H_2 , Pd on C; (iv) TBSOTf; (v) DIBAL-H; (vi) (a) $o\text{-NO}_2\text{PhSeCN}$, $n\text{-Bu}_3\text{P}$, (b) NaHCO_3 , H_2O_2 ; (vii) TBAF; (viii) $(\text{COCl})_2$, DMSO, Et_3N ; (ix) (R)-65, $n\text{-BuLi}$; (x) MOM-Cl, $i\text{Pr}_2\text{NEt}_2$, TBAI; (xi) (a) 35, 9-BBN, (b) 2M K_3PO_4 , $[\text{Pd}(\text{OAc})_2 + 4\text{TFP}]$; (xii) (a) H_2 , Lindlar catalyst; (xiii) TBAF; (xiv) DIAD, Ph_3P , PhMe ; (xv) sulfonic acid resin; (xvi) polymer bound IBX.

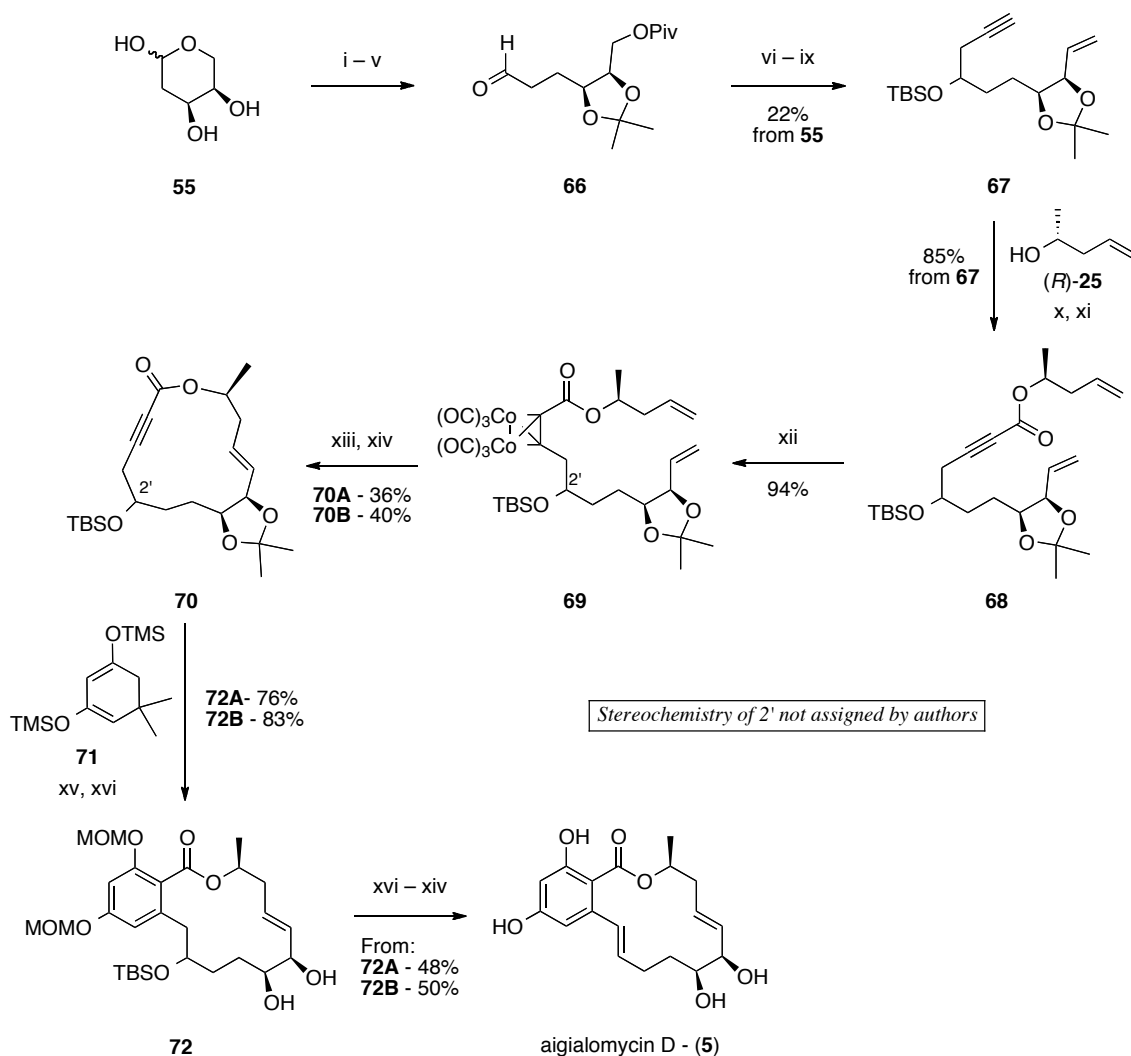
Scheme 1.20: Synthesis of L-783,277 (**1**) by Altmann et al.²⁴

1.4.2 Previous Syntheses of Aigialomycin D (5)

Another biologically significant RAL is aigialomycin D (**5**) due to its potent inhibition of CDKs 1 and 5 which could, therefore, serve as new therapeutic agents. Numerous syntheses of RAL **5** have been reported to date and some of these, most notably those due to Danishefsky *et al.* and Barrett *et al.*, possess unique features. In particular, an aromatic fragment is not employed at the outset with this functionality being installed towards the end of the sequence. These two syntheses are outlined below, together with a more recent one and they all serve to further emphasise the utility of the common reactions discussed in Section 1.3.

*Danishefsky Synthesis of Aigialomycin D (5) (2004)*³⁶

The first enantioselective total synthesis of aigialomycin D (**5**) (Scheme 1.21) was accomplished by Danishefsky *et al.* in 2004 by employing a Diels-Alder cycloaddition reaction between cyclohexadiene **71** and ynolide **70** to establish the aromatic functionality. Thus, the preparation of aigialomycin D (**5**) commenced with 2-deoxy-*D*-ribose (**55**) which was converted into the protected diol fragment **66** through several standard functional group manipulations. The required alkyne and allylic ether functionalities were installed in three steps from protected diol **66**, *via* a Barbier-type reaction and an oxidation-Wittig olefination reaction sequence, to give enyne **67**. The secondary alcohol fragment (*R*)-**25** was coupled to the carboxylated derivative of compound **67** (obtained through reaction of compound **67** with *n*-butyl lithium and carbon dioxide) under Mitsunobu conditions to furnish compound **68**. To prevent RCM precursor **68** from undergoing an yne-ene metathesis reaction, the alkyne functionality was protected as the cobalt complex **69** before being subjected to Grubbs' 2nd generation catalyst.³⁷ A ceric ammonium nitrate (CAN) deprotection step then afforded ynolide **70**. The aromatic functionality was introduced through the aforementioned Diels-Alder cycloaddition reaction of the propargylic ketone **70** with cyclohexadiene **71** which resulted in the loss of isobutene (*via* a retro-Diels-Alder reaction) and the trimethylsilyl ethers (during flash chromatography on silica). With the phenols now protected as MOM ethers, the TBS group within compound **72** was cleaved and the resulting C2' alcohol was dehydrated with Martin's sulfurane to establish the C1'-C2' olefin. This material was subsequently deprotected to complete the synthesis of aigialomycin D (**5**).



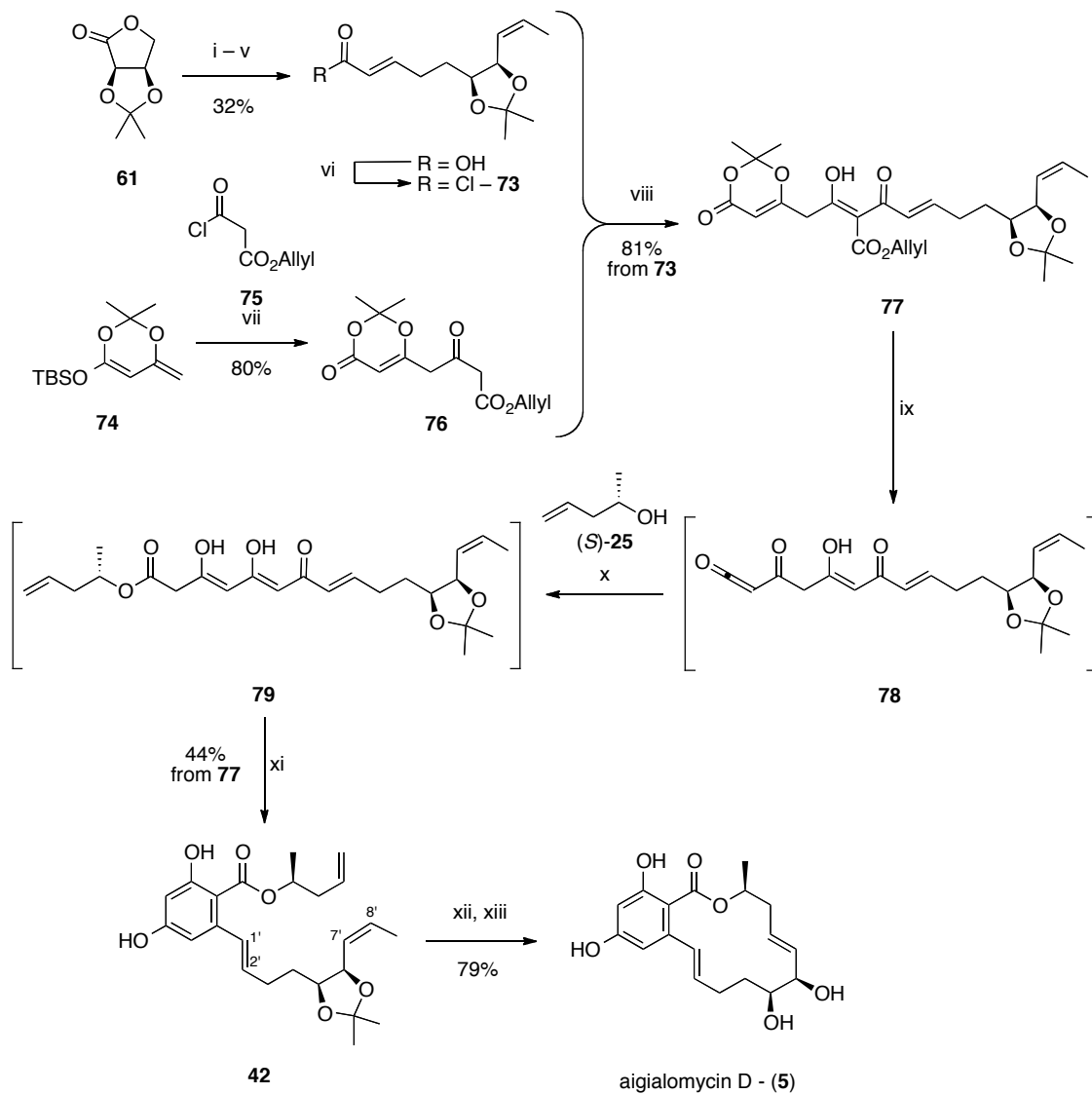
Reagents and conditions: (i) 2-methoxypropene, *p*-TSA; (ii) KHMDS, CH₃PPh₃I; (iii) PivCl, Et₃N, DMAP; (iv) (a) 9-BBN, (b) NaOH, H₂O₂; (v) SO₃•pyridine, DMSO, Et₃N; (vi) (a) propargyl bromide, Zn; (b) TBSOTf; (vii) MeONa; (viii) SO₃•pyridine, DMSO, Et₃N; (ix) KHMDS, CH₃PPh₃I; (x) *n*-BuLi, dry ice; (xi) (*R*)-25, Ph₃P, DIAD; (xii) (Co)₂(CO)₈; (xiii) Grubbs' 2nd generation catalyst; (xiv) CAN; (xv) **71**, neat; (xvi) MOMCl, *i*-Pr₂NEt; (xvii) HF•pyridine; (xviii) [PhC(CF₃)₂O]₂SPh₂; (xix) 0.5 N HCl.

Scheme 1.21: Synthesis of aigialomycin D (5) by Danishefsky et al.³⁶

Barrett's Synthesis of Aigialomycin D (5) (2009)³⁵

In 2009 Barrett *et al.* completed a synthesis of aigialomycin D (**5**) (Scheme 1.22) that revolved around a particularly elegant ketene-generation-trapping-aromatization sequence to introduce the β -resorcylic functionality and establish the RCM precursor **42** in one pot from compound **77**. Thus, the preparation of RAL **5** commenced with *O*-isopropylidene-*D*-erythrone (61) which was converted into the protected diol fragment **73** via several functional group interconversions. Compound **73** was itself coupled with the enolate generated by treatment of fragment **76** (dioxine **76** was prepared from reaction of compound **74** with acyl chloride **75**) with pyridine and MgCl_2 . With the crucial intermediate **77** synthesised, it was heated in the presence of $\text{Pd}(\text{OAc})_2$ and morpholine to initiate a retro-Diels-Alder reaction to afford ketene **78** that was trapped *in situ* with (*S*)-(+)-4-penten-2-ol [(*S*)-**25**] to afford ester **79**. Compound **79** was aromatised by treatment with caesium acetate and then acetic acid to furnish triene **42**. Compound **42** itself was subjected to a RCM reaction with Hoveyda-Grubbs 2nd generation catalyst³⁸ to furnish a macrolactone that was then globally deprotected to provide aigialomycin D (**5**).[‡]

[‡] For this RCM reaction, protection of the C1'-C2' alkene moiety of **42** was not required to obtain the desired product due to catalyst selectivity for the olefin associated with the homoallylic ester over that associated with the C7'-C8' fragment.

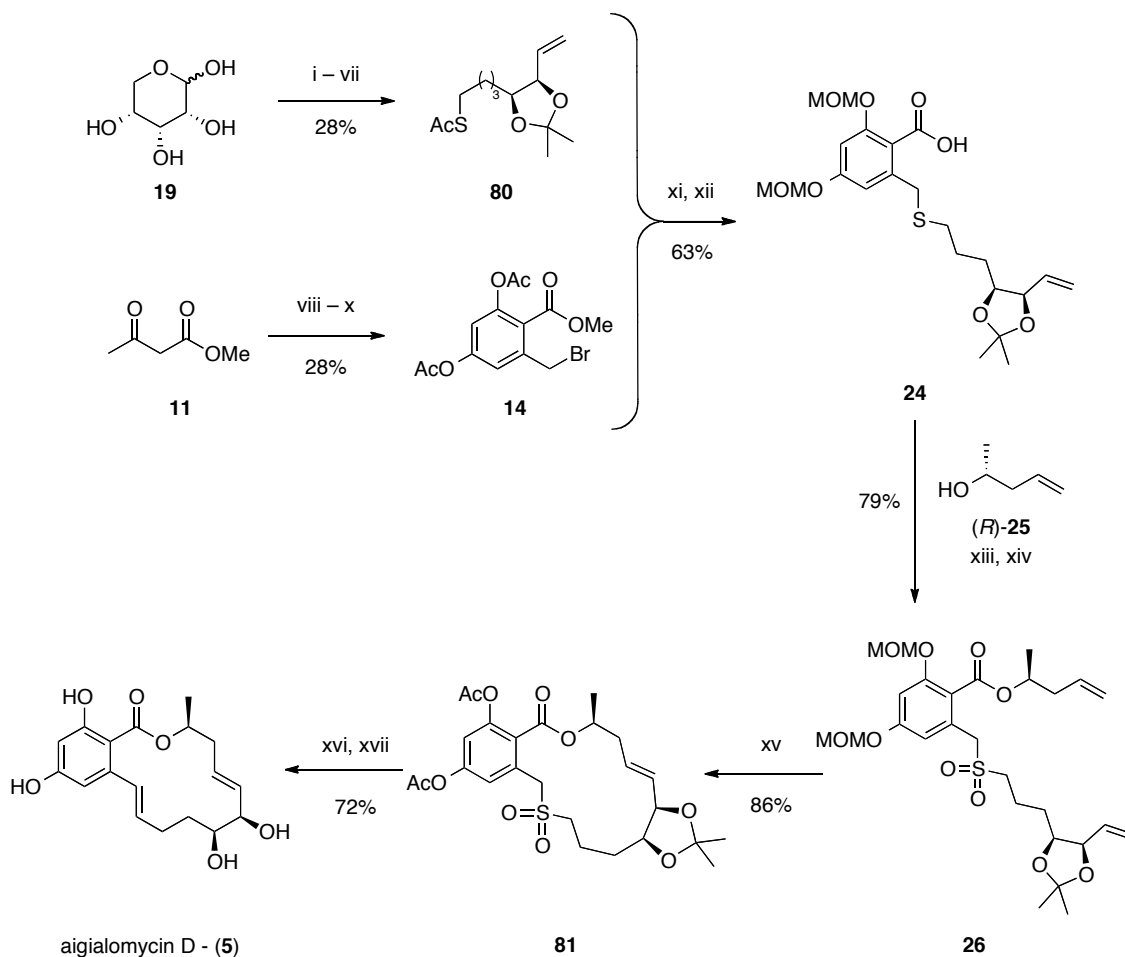


Reagents and conditions: (i) DIBAL-H; (ii) methyl (triphenylphosphoranylidene)acetate; (iii) (a) DMP, (b) CH₃CH=PPh₃; (iv) (a) DIBAL-H, (b) Ph₃P=CHCO₂Me; (v) LiOH; (vi) (COCl)₂; (vii) **74**, Bi(OTf)₃; (viii) (a) **76**, MgCl₂, pyridine, (b) **73**; (ix) morpholine, Pd(PPh₃)₄; (x) (S)-**25**; (xi) CsOAc, AcOH; (xii) Hoveyda-Grubbs 2nd generation catalyst; (xiii) aq. 1 M HCl/MeOH v/v 1:1.

Scheme 1.22: Synthesis of aigialomycin D (**5**) by Barrett et al.³⁵

Harvey's Synthesis of Aigialomycin D (5) (2009)²⁷

A recent synthesis of aigialomycin D (**5**) completed by Harvey *et al.* (Scheme 1.23) is also representative of an approach utilising the common reactions described in Section 1.3. A significant feature of Harvey's synthesis is the masking of the C1'-C2' alkene as a sulfone to prevent unwanted cyclohexene formation during the RCM reaction of diene **26**. The olefin was then unmasked using a Ramberg-Bäcklund reaction. Thus, the synthesis of target **5** commenced with *D*-ribose (**19**) which was converted into the protected diol fragment **80** in seven steps. Compound **80** was itself coupled to the aryl bromide **14** *via* a nucleophilic substitution reaction followed by an immediate re-protection of the phenols as MOM ethers to afford sulfide **24**. Compound **24** was, in turn, coupled to (*R*)-(-)-4-penten-2-ol [(*R*)-**25**] under Mitsunobu conditions and subsequently oxidised with *m*-CPBA to the sulfone **26**. Compound **26** was treated with Grubbs' 2nd generation catalyst³⁷ to deliver macrocycle **81** which was, in turn, subjected to a Ramberg-Bäcklund reaction to reveal the C1'-C2' olefin. Global deprotection of the four hydroxyl groups associated with the target then provided aigialomycin D (**5**).



Reagents and condions: (i) MeOH, HCl; (ii) TsCl, pyridine; (iii) NaI, methyl ethyl ketone; (iv) (a) Zn, AcOH, (b) $\text{Ph}_3\text{P}=\text{CHCO}_2\text{CH}_3$; (v) NaBH_4 , CuCl, cyclohexene; (vi) LiAlH_4 (vii) (a) MsCl, NEt_3 , DMAP; (b) KSAc; (viii) (a) **11**, NaH, (b) *n*-BuLi, (c) HCl; (ix) Ac_2O , NEt_3 ; (x) NBS, $[\text{PhC}(\text{O})\text{O}_2]_2$; (xi) K_2CO_3 ; (xii) (a) NaH, MOMCl, (b) KOH; (xiii) (*R*)-**25**, DIAD, PPh_3 ; (xiv) *m*-CPBA; (xv) Grubbs' 2nd generation catalyst; (xvi) KOH, *t*-BuOH; (xvii) HCl.

Scheme 1.23: Harvey's synthesis of aigialomycin D (5).²⁷

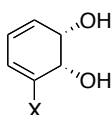
1.4.3 Overview of Previous Synthetic Efforts

The foregoing commentary should serve to highlight the continuing popularity of RALs as synthetic targets. Despite this attention, there remains a need for shorter and more flexible approaches which would deliver collections of analogues for drug development. Given the great interest in RALs and the Banwell group's successes in synthesising related macrolides using enantiopure *cis*-1,2-dihydrocatechols as starting materials, the use of these novel metabolites in the preparation of the title compounds formed the basis of the research reported in this thesis. Accordingly, it is appropriate to now provide some background on the generation and synthetic utility of *cis*-1,2-dihydrocatechols.

1.5 *cis*-1,2-Dihydrocatechols as Starting Materials for the Enantiospecific Synthesis of Macrolides

1.5.1 Overview

In an approach that differs from those outlined previously, it was anticipated that the diol unit of the RALs could be generated from the enzymatically-derived and readily available metabolite, *cis*-1,2-dihydrocatechol (*c*DHC) **82** (Figure 1.5).



82

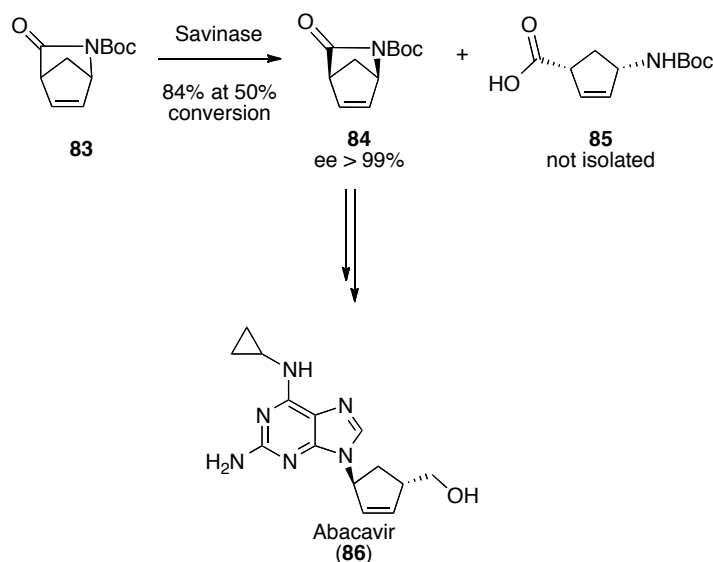
X = Me, Br, Cl, I, CN, CF₃, CO₂H, CH=CH₂, etc

Figure 1.5: Commonly available *cis*-1,2-dihydrocatechols (*c*DHCs, **82**).

These synthetically valuable metabolites have been employed previously in various total syntheses, including those of macrolides such as (+)-aspicilin.³⁹ Accordingly, it was anticipated that the use of *c*DHCs in the synthesis of RALs would be ideal due to the correlation of diol stereochemistry in the *c*DHCs with its counterparts present in the majority of RALs. Furthermore, by using a *c*DHC as a starting material, it was envisaged that the number of steps required to generate the diol unit could be reduced when compared to previous RAL syntheses. Before detailing this work, the biosynthesis, reactivity and use of such starting material in macrolide preparations is described.

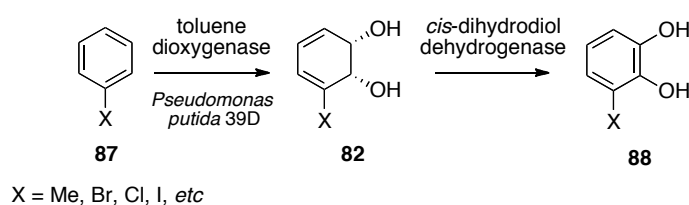
1.5.2 Enzymatic Production of *cis*-1,2-Dihydrocatechols from Arenes

Since Pasteur's discovery of whole-cell alcoholic fermentation,⁴⁰ the use of enzyme-mediated transformations has become a particularly useful tool in chemical synthesis. Some significant uses of enzymes in organic chemistry include the kinetic resolution of racemates and, thereby, the preparation of enantiopure compounds. These enzymatic transformations are not confined to laboratory-scale syntheses and an example of their use on an industrial scale is seen in the GlaxoSmithKline synthesis of the reverse transcriptase inhibitor Abacavir (**86**) (ZiagenTM) (Scheme 1.24).^{41,42}



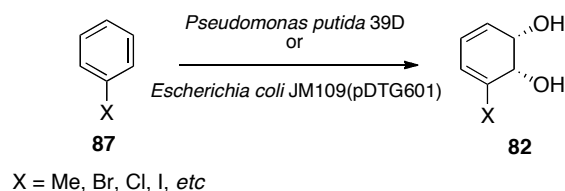
Scheme 1.24: Industrial synthesis of Abacavir (**86**) by GlaxoSmithKline using enzymes.^{41,42}

The enzymatic pathway associated with the biosynthesis of *c*DHCs, namely microbial aromatic metabolism, was first discovered by Strömer *et al.*, when they demonstrated that the bacterium *Bacillus hexacarovorum* consumed toluene or xylene as a source of carbon.⁴⁰ In 1968, studies carried out by Gibson *et al.* led to the identification of a possible pathway for the metabolism of arenes by the soil bacterium *Pseudomonas putida* (Scheme 1.25). It was proposed that the first stage of the process involves a stereoselective dihydroxylation and dearomatisation of aromatic substrates **87** with the enzyme toluene dioxygenase (TDO) to afford the *c*DHC metabolites **82**.^{40,43,44} These primary metabolites were themselves converted into the corresponding catechol **88** by the enzyme dihydrodiol dehydrogenase.^{40,43,44}



Scheme 1.25: The microbial aromatic oxidation pathway of *P. putida* elucidated by Gibson *et al.*^{40,43,44}

Gibson *et al.* later sequenced and over-expressed the genes encoding for TDO in host organisms, such as *Escherichia coli* JM109, to afford clones, such as JM109(pDTG601), lacking the dihydrodiol dehydrogenase and thus allowing for the accumulation of significant amounts (up to 25 g per litre of fermentation broth) of enantiopure *c*DHC [$> 99\%$ enantiomeric excess (e.e.)] that could then be isolated and subjected to the normal modes of chemical characterisation (Scheme 1.26).⁴⁵



Scheme 1.26: Whole-cell biotransformation used for generating *c*DHCs **82** as developed by Gibson *et al.*⁴³⁻⁴⁵

The versatility of these enzymes is highlighted by the fact that at least 400 such metabolites have now been isolated (Figure 1.6).⁴⁶

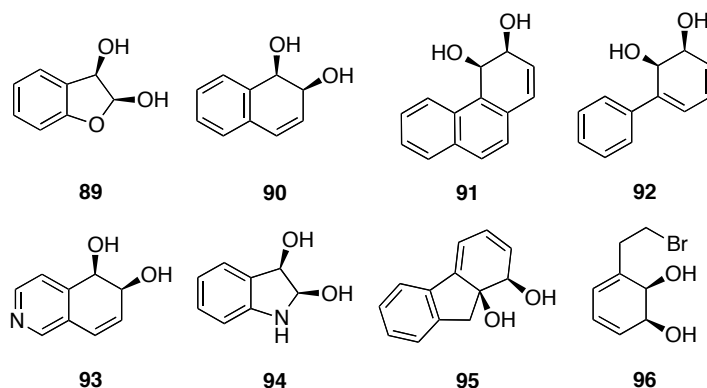
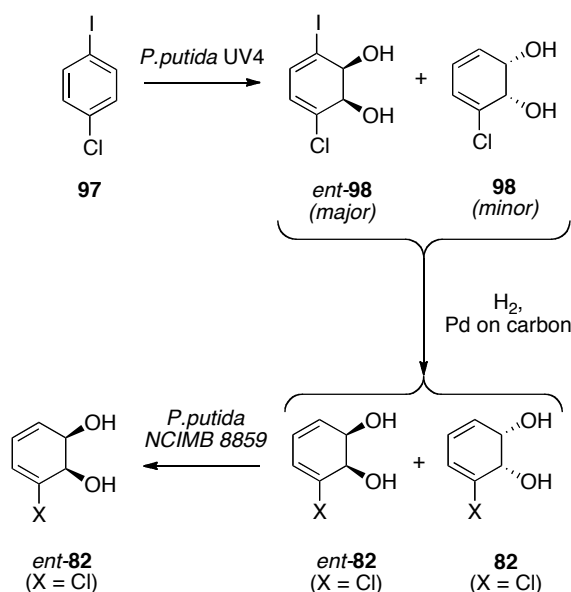


Figure 1.6: Various metabolites prepared by whole-cell biotransformation of the corresponding aromatic.⁴⁶

1.6 Acquisition of Either Enantiomer of *c*DHC

A limitation of using *c*DHCs in the synthesis of non-racemic chiral natural products is the seeming inability to access its enantiomer, *viz ent-c*DHC [*ent*-**82** (X = Cl)]. To overcome this difficulty, Boyd *et al.* developed a process called ‘enantiomeric switching’ which involved exposing *p*-iodochlorobenzene (**97**) to TDO and thus generating a mixture of metabolites **98** and *ent*-**98** (with the latter being the major product) (Scheme 1.27).⁴⁷ The observed preference for *ent*-**98** arises from the larger iodo-substituent directing the enzymatic dihydroxylation process. The iodo-group within compounds **98** and *ent*-**98** was then reductively cleaved to afford the corresponding mixture of *c*DHC **82** (X = Cl) and *ent-c*DHC *ent*-**82** (X = Cl). By subjecting this mixture to *P. putida* NCIMB 8859 (dihydrodiol dehydrogenase) the undesired enantiomer

82 ($X = \text{Cl}$) was selectively aromatised to provide a chromatographically separable mixture of the catechol **88** ($X = \text{Cl}$) and the enantiomerically pure *ent*-*c*DHC *ent*-**82** ($X = \text{Cl}$). This chemoenzymatic process could be applied to various *p*-disubstituted benzene substrates and so allowing access to either enantiomeric form of the desired *c*DHC.



Scheme 1.27: Access to *ent*-*c*DHC [*ent*-**82** ($X = \text{Cl}$)] through “enantiomeric switching”.⁴⁷

1.7 Synthetic Utility of Halogenated *c*DHC

The enantiopure halogenated *c*DHCs of the general form **82** represent important “building blocks” because they can be subjected to an array of regio- and/or stereo-selective chemical transformations including electrophilic addition, [4+2] cycloaddition, oxidative cleavage and cross-coupling reactions to give a range of useful products (Figure 1.7). The following sections provide a summary of these types of transformations.

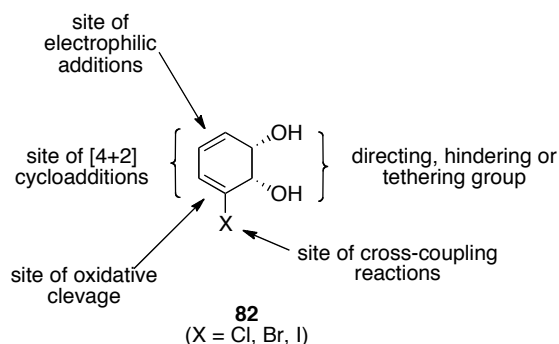
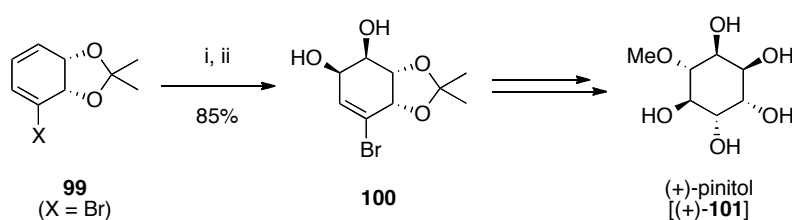


Figure 1.7: Sites of reactions within *c*DHC **82** and their derivatives.

1.7.1.1 *cis*-Configured Diols

The two *cis*-related hydroxyl groups embedded within the halogenated cDHCs (**82**) allow, through chelation with the relevant reagents, for the regioselective functionalisation of the C3-C4 and C5-C6 olefins on the α -face. Conversely, if the hydroxyl groups are derivatised using bulky groups (such as acetonides for example), the functionalisation of the two olefins will occur on the β -face due to steric effects. This feature has been exploited in Hudlicky's synthesis of (+)-pinitol [(+)-**101**],⁴⁸ wherein the non-halogenated olefin within cDHC acetonide derivative **99** (X = Br) was dihydroxylated on the opposite face to the protected diol to give product **100** (Scheme 1.28).⁴⁸

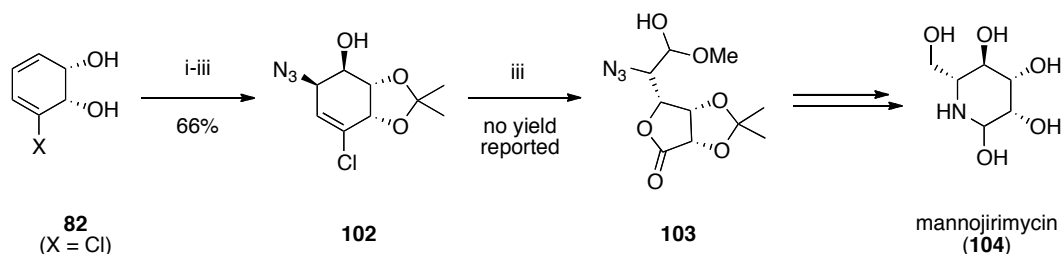


Reagents and conditions: (i) 2,2-dimethoxypropane, *p*-TSA; (ii) OsO₄, NMO, acetone, H₂O.

Scheme 1.28: Synthesis of (+)-pinitol [(+)-**101**] by Hudlicky et al.⁴⁸

1.7.1.2 Diene Moiety

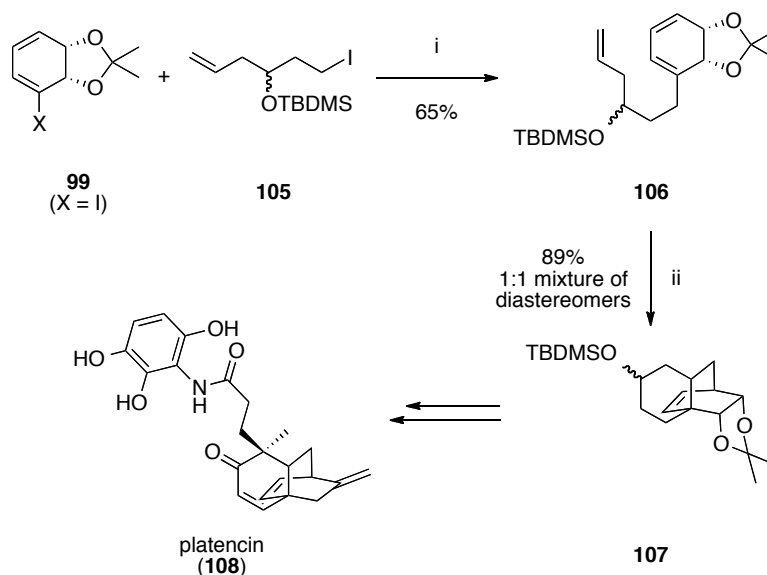
As observed in the above example (Scheme 1.28), the diene moiety of the cDHCs contains two non-equivalent alkenes due to the presence of a halogen at the C3 position which results in a more electron-deficient C3-C4 olefin. As a consequence, the C5-C6 olefin is more susceptible to electrophilic addition reactions such as epoxidation, dihydroxylation and aziridination. With the non-halogenated olefin removed, the remaining olefin can undergo an oxidative cleavage under ozonolytic conditions to provide, depending on the reaction conditions used, ring expanded, contracted and/or acyclic products, with the stereochemistry of the diols remaining intact. The differentiation between the two olefins was exploited in Hudlicky's synthesis of mannojirimycin (**104**) (Scheme 1.29) wherein the C5-C6 olefin was first aziridinated and the remaining, halogenated, olefin then oxidatively cleaved to afford lactone **103**⁴⁹ (the strategy of cleaving the C3-C4 olefin with ozone has been used in the preparation of the diol fragment described in this thesis).



Reagents and conditions: (i) 2,2-dimethoxypropane, *p*-TSA; (ii) *m*-CPBA, CH_2Cl_2 ; (iii) LiCl, ethyl acetoacetate, NaN_3 ; (iv) (a) O_3 , MeOH, NaHCO_3 , -78°C .

Scheme 1.29: Hudlicky's synthesis of mannojirimycin (**104**).⁴⁹

The presence of the diene moiety within the *c*DHCs also allows for various regioselective [4+2] Diels-Alder cycloaddition reactions to be carried out. This strategy was employed by Banwell *et al.* in their formal total synthesis of the anti-bacterial platencin (**108**) (Scheme 1.30) wherein an intramolecular Diels-Alder cycloaddition reaction was undertaken with compound **106**, and leading, *via* the initial adduct **107**, to the rapid assembly of the tricyclic core of the target natural product.⁵⁰

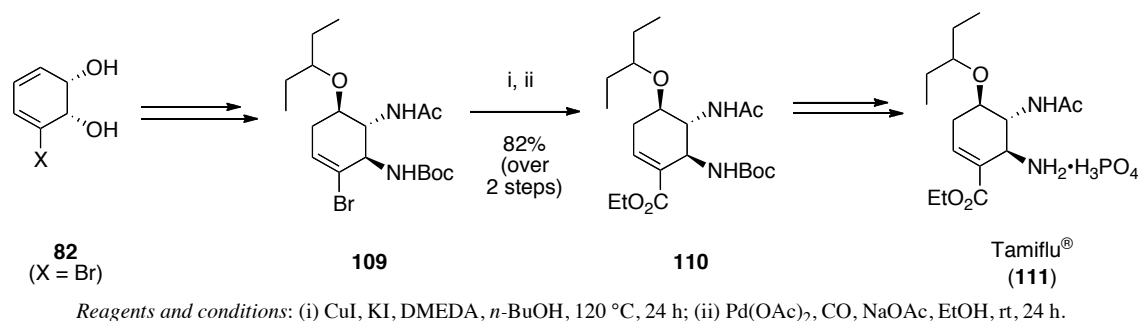


Reagents and conditions: (i) (a) *t*-BuLi, ZnI_2 , $\text{Et}_2\text{O}/\text{THF}$, $-78 \rightarrow 18^\circ\text{C}$, 1 h, (b) $\text{Pd(PPh}_3)_4$, THF, 18°C , 3 h; (ii) BHT, PhMe, 110°C , 16 h.

Scheme 1.30: Banwell's synthesis of platencin (**108**).⁵⁰

1.7.1.3 Halogen Substituent

The halogen substituent at C3 in the *c*DHCs provides a handle for radical-based and metal-based cross-coupling reactions. The platencin synthesis shown in Scheme 1.30 provides one example of this. Another is seen in Fang's synthesis of Tamiflu® (**111**) wherein the bromine of *c*DHC **82** (X = Br) was converted into the reactive iodide analogue and then subjected to a palladium-catalysed carbonylation reaction to afford the ethyl ester **110** (Scheme 1.31).⁵¹



Scheme 1.31: Synthesis of Tamiflu® (**111**) by Fang et al.⁵¹

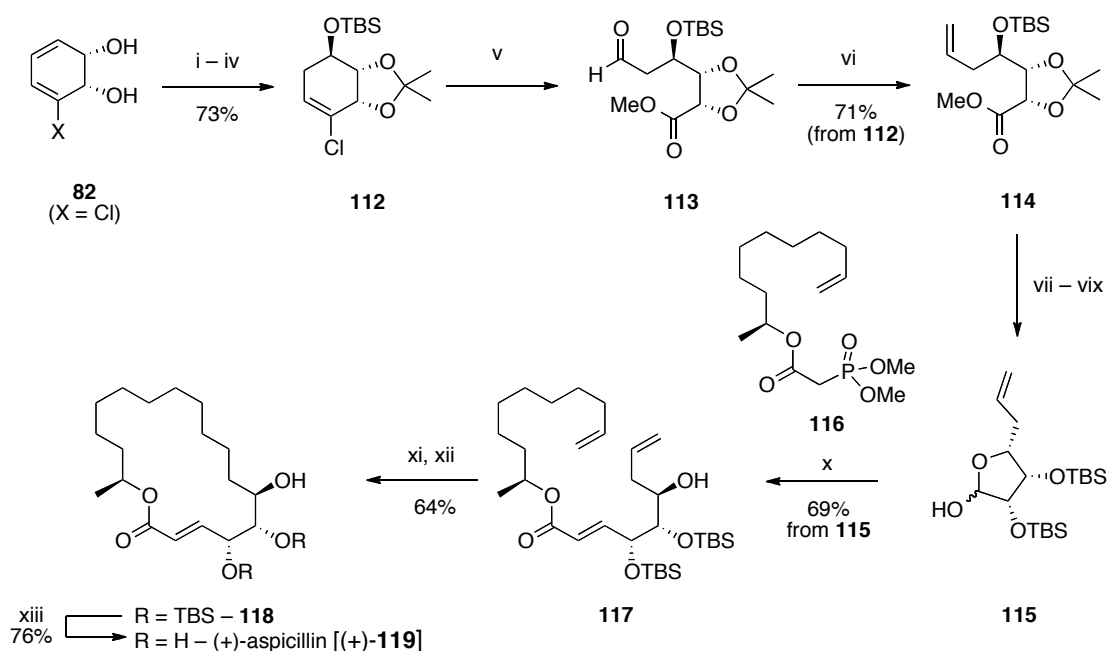
1.8 Oxidative Cleavage of *cis*-1,2-Dihydrocatechols

It is evident from the examples shown above that halogenated *c*DHCs serve as valuable synthons for accessing natural products that contain six-membered rings bearing the same diol stereochemistry or related functionality. However, by engaging the C3-C4 double bond in an oxidative cleavage reaction, the synthetic utility of these halogenated metabolites can be expanded to the preparation of both acyclic and heterocyclic systems. The ability to access linear and open-chain compounds incorporating the intact diol unit from the *c*DHCs provides a vehicle for preparing macrolides. Two syntheses that highlight the potential of such processes, are those leading to (+)-aspicilin and (–)-cladospolide A. These are detailed below.

*Banwell's Synthesis of (+)-Aspicilin [(+)-119] (2000)*³⁹

Banwell *et al.* reported the synthesis of (+)-aspicilin [(+)-**119**] (Scheme 1.32) from chlorobenzene-derived *c*DHC **82** (X = Cl) by employing the oxidative cleavage strategy to deliver a fragment incorporating the requisite triol with the correct stereochemistry. The synthesis began with the protection of the diol functionality of *c*DHC **82** (X = Cl) with an acetonide which was itself converted into the TBS ether **112** in four steps. Cyclohexene **112** was oxidatively cleaved with ozone and a reductive workup using NaBH₄ gave the unstable aldehyde **113**. Compound **113** was immediately subjected to a Wittig olefination reaction to afford the terminal alkene **114**. The acetonide functionality within compound **114** was

hydrolysed and the resulting diol re-protected as the *bis*-silyl ether to give lactol **115**.[§] Compound **115** was coupled to the phosphonate fragment **116** under Horner Wadsworth Emmons (HWE) conditions to furnish the RCM precursor **117**. Treatment of triene **117** with Grubbs' 1st generation catalyst,^{52,53} followed by a selective reduction of the newly formed alkene then afforded macrolactone **118**. Finally, a global deprotection of macrocycle **118** provided the macrolide (+)-aspicilin [(+)-**119**].



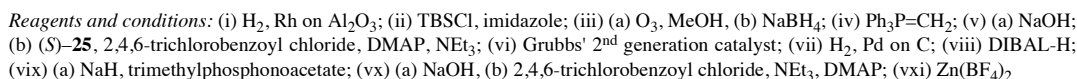
(i) 2,2-dimethoxypropane, *p*-TSA; (ii) *m*-CPBA; (iii) DIBAL-H; (iv) TBSCl, imidazole; (v) (a) O₃, MeOH, (b) NaBH₄; (vi) Ph₃P=CH₂; (vii) HCl; (viii) TBSCl, imidazole; (vix) DIBAL-H; (x) (a) **116**, NaH, (b) **115**; (xi) Grubbs' 1st generation catalyst; (xii) H₂, Pd on BaSO₄; (xiii) HCl.

Scheme 1.32: Banwell's synthesis of (+)-aspicilin [(+)-**119**].³⁹

Banwell's Synthesis of (–)-Cladospolide A [(–)-**126**] (2002)^{31,32}

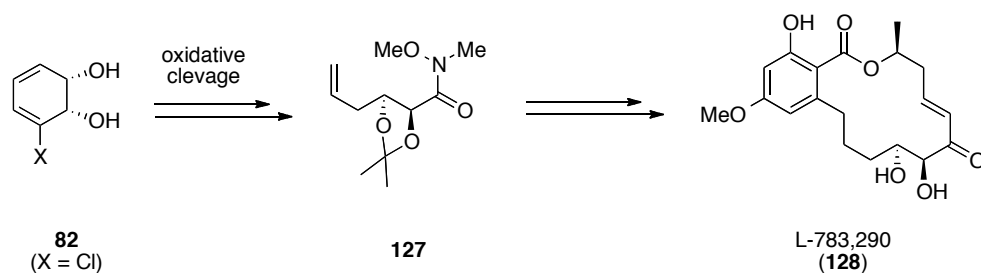
Following the completion of the synthesis of (+)-aspicilin [(+)-**119**] by Banwell *et al.*,³⁹ a similar modular strategy was employed to prepare (–)-cladospolide A [(–)-**126**] (Scheme 1.33) – a fungal metabolite that was isolated from various *Cladosporium* species and found to inhibit shoot elongation in rice and lettuce seedlings.^{31,32} The synthesis commenced with the conversion, in two steps, of the chlorobenzene-derived *c*DHC (X = Cl) **82** into the TBS ether-protected compound **120**. The latter material was subjected to an oxidative cleavage and a reductive workup to furnish the unstable aldehyde **121** which was immediately subjected to a Wittig olefination reaction to give olefin **122**. The methyl ester moiety present in compound **122** was hydrolysed and the ensuing acid was coupled to (*S*)-(–)-4-penten-2-ol [(*S*)-**25**] under

[§] This swapping of protecting groups was required because when the acetonide group was retained the planned late-stage macrolide-forming RCM reaction failed.



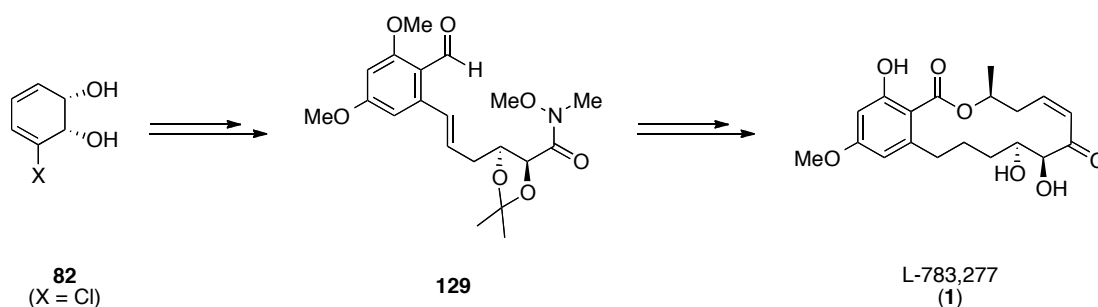
Scheme 1.33: *Banwell's synthesis of (–)-cladospolide A [(–)-126].*^{31,32}

The intense interest in the biologically active RALs coupled with the need for quicker and more efficient routes to these natural products and their analogues prompted an investigation of the capacity of the enantiopure *c*DHC to serve as starting materials for RAL synthesis. Thus, Chapter Two describes the first enantioselective synthesis of the enone-containing RAL L-783,290 (**128**) from the chlorobenzene-derived *c*DHC **82** (X = Cl). A key step in the eventually successful synthesis involved a coupling of the *c*DHC-derived alkene **127** with the relevant aromatic bromide to produce a key precursor to target **128** (Scheme 1.34).



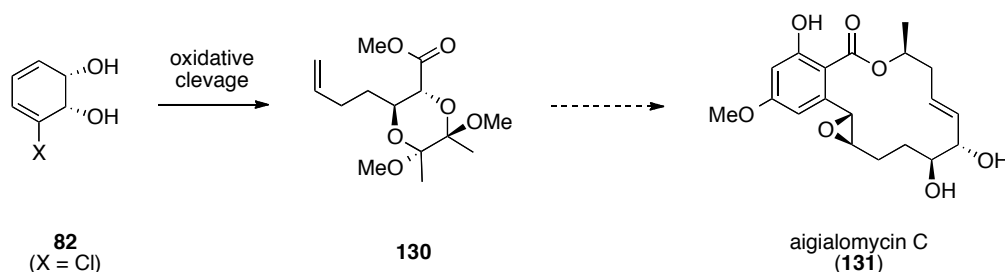
Scheme 1.34: Synthesis of L-783,290 (**128**) from cDHC **82** ($X = \text{Cl}$).

Chapter Three describes an efficient synthesis of the RAL L-783,277 (**1**) (Scheme 1.35), using the advanced intermediate, **129**, generated during the course of the work described in Chapter Two. In this approach, an unusual intramolecular nucleophilic addition of an acetylide anion onto a tethered Weinreb amide was used to generate the macrocycle.



Scheme 1.35: Synthesis of L-783,277 (**1**) from intermediate **129**.

Chapter Four describes efforts directed towards the synthesis of aigialomycin C (**131**) (Scheme 1.36). The approach draws upon the modular strategy described in Chapter Two, while demonstrating the synthetic usefulness of the C1'-C2' double bond obtained from the Heck reaction between aromatic and diol units.



Scheme 1.36: Efforts towards the synthesis of aigialomycin C (**131**) from cDHC **82** ($X = \text{Cl}$).

Chapter Five outlines the potential for the extension of the research described in Chapters Two to Four to syntheses of other biologically active RALs.

1.10 References

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Chapter Two

The Total Synthesis of L-783,290 (*trans*-isomer)

2.1 Introduction

2.1.1 Isolation and Characterisation

In 1999 Zhao *et al.* reported on the bioassay-guided isolation of the enone resorcyllide L-783,290 (**128**), its *cis*-isomer, (L-783,277) (**1**), and hypothemycin (**2**) (Figure 2.1) from the fruiting bodies of the mushroom *Helvella acetabulum*.¹

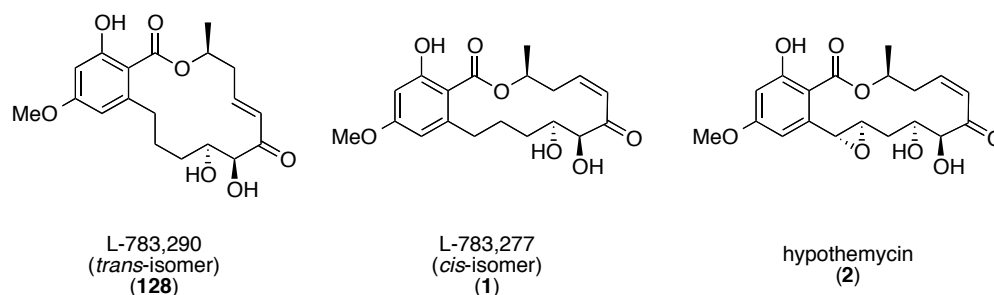


Figure 2.1 The structures of L-783,290 (**128**), L-783,277 (**1**) and hypothemycin (**2**) isolated from the fruiting bodies of *Helvella acetabulum*.

The structures of compounds **128** and **1** were determined using ¹H-¹H COSY, HMQC and ¹H-¹³C HMBC NMR techniques.¹

2.1.2 Biological activity

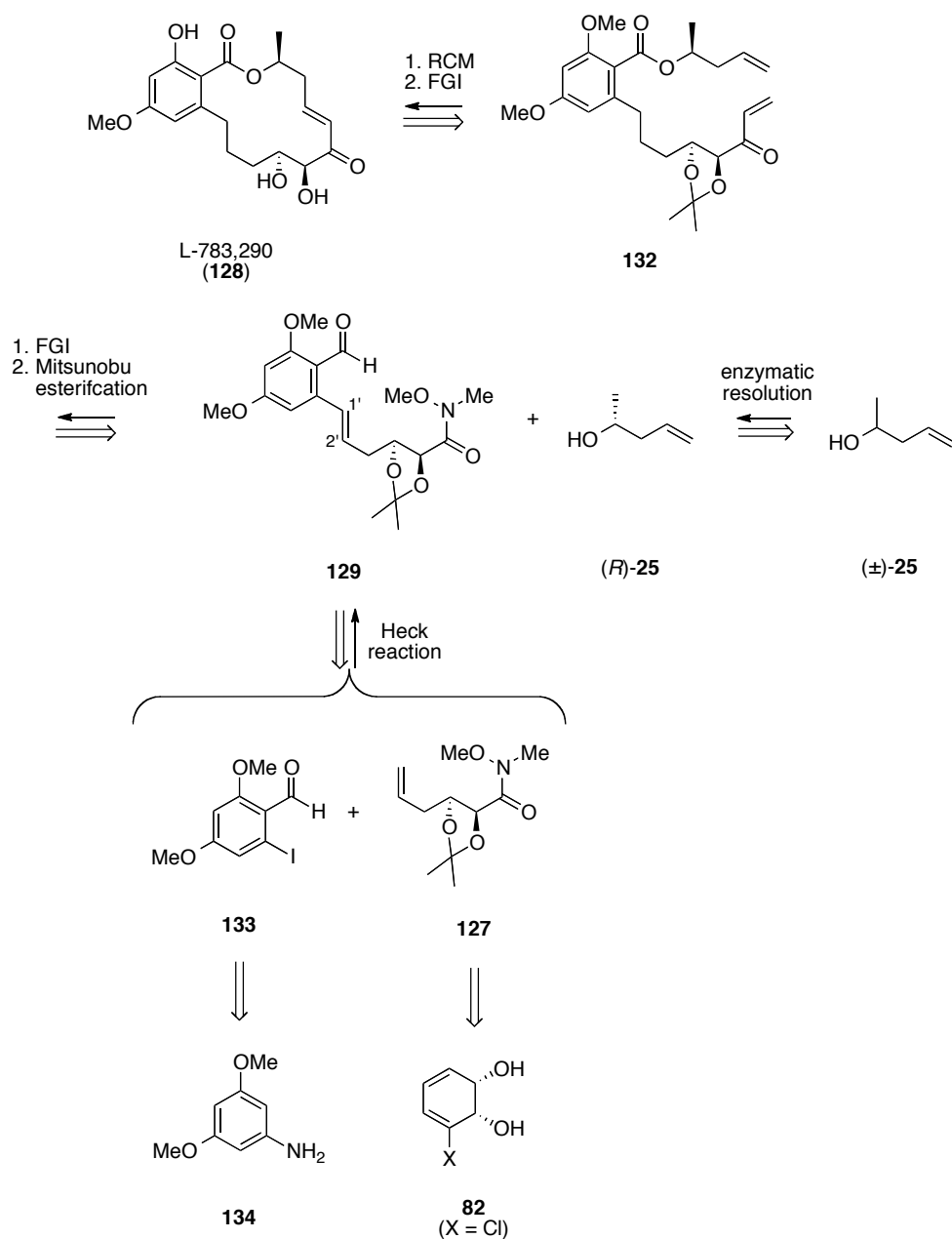
Both compounds **128** and **1** were found to be competitive inhibitors of the ATP active site of the kinase MEK, with L-783,290 (**128**) (IC₅₀ = 300 nM) exhibiting a weaker inhibitory activity than congener **1** (IC₅₀ = 4 nM).¹ They also acted as weak and reversible inhibitors of LCK kinase.¹ The biological activities of compounds **1** and **128** most likely arise from their capacities to engage in 1,4-conjugate addition reactions with the thiol moiety of a cysteine residue within

the kinase active site. The significant difference in potency between the two compounds is believed to be due to the superior Michael acceptor properties of the *cis*-enone residue within compound **1**.

2.2 Retrosynthetic Analysis of L-783,290 (**128**)

Given the Banwell group's successful modular syntheses of various macrolides, including (+)-aspicilin [(+)-(**119**)] and certain members of the cladospolide family of natural products,²⁻⁶ it was anticipated that L-783,290 (**128**) could be prepared using a similar approach.

A retrosynthetic analysis of L-783,290 (**128**) based on such thinking is presented in Scheme 2.1 and this highlights the use of a ring-closing metathesis (RCM) reaction of diene **132** to establish the fourteen-membered macrocyclic ring of this RAL. The substrate **132** required for this process would be assembled from three fragments *via* two crucial reactions, these being, in order of execution, a Heck reaction between the olefin **127** and the aryl iodide **133**, and a Mitsunobu esterification to effect coupling of enantiopure alcohol (*R*)-**25** with the acid derived from the oxidation of aldehyde **129**.



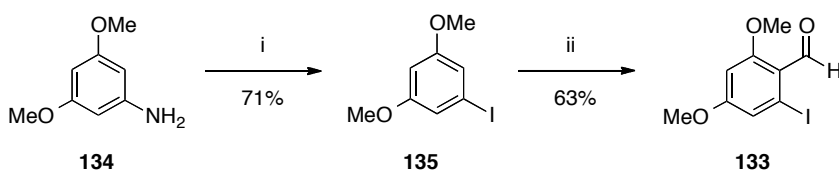
Scheme 2.1: Retrosynthetic analysis of L-783,290 (trans-isomer) (**128**).

In this synthesis, the C1'-C2' alkene residue associated with compound **129** would be reduced to the corresponding alkane. In other instances, however, this double bond could be retained in order to provide other RALs incorporating functionality at these positions.

Compound **127** would, in turn, be derived from enantiopure *c*DHC **82** (X = Cl) in a manner similar to that employed by Banwell, McRae and Loong in their syntheses of (+)-aspicilin [(+)-**119**] and certain members of the cladospolide family.²⁻⁶ The enantiopure alcohol [(*R*)-**25**] would be prepared using a well-established double enzymatic kinetic resolution of racemate [(±)-**25**].^{2,3}

2.3 Synthesis of the Aromatic Fragment 133

The synthesis of the title fragment (**133**) was readily achieved following a protocol originally reported by Abe *et al.* (Scheme 2.2).⁷ Thus, the amine group within 3,5-dimethoxyaniline (**134**) was converted into the corresponding iodide **135** via a Sandmeyer reaction. This involved formation of the diazonium salt followed by its displacement with an iodide anion. As a result, the aryl halide **135** was obtained in 71% yield. The EI mass spectrum of this material displayed the expected molecular ion at m/z 264 and an accurate mass measurement on this species established that it had the anticipated molecular composition, viz C₈H₉IO₂. All the other spectral data obtained on this compound were in accord with the assigned structure and matched those reported earlier.⁸



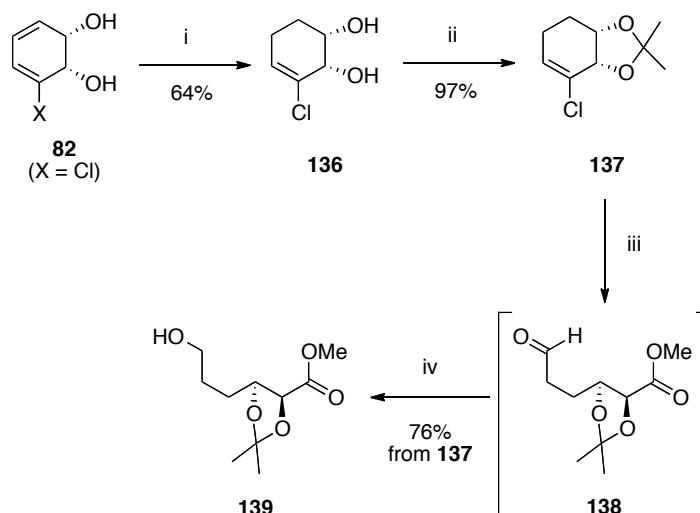
Reagents and conditions: (i) conc. H₂SO₄, H₂O, NaNO₂, -5 °C, 0.5 h then KI, H₂O, -5 °C, 3 h; (ii) POCl₃, DMF, 100 °C, 19 h.

Scheme 2.2: Synthesis of the aromatic fragment **133**.

Regioselective formylation of the aryl iodide **135** was achieved under Vilsmeier-Haack conditions, and thus delivered the target aromatic fragment **133** in 63% yield (Scheme 2.2). The ¹H NMR spectrum of compound **133** showed a one-proton singlet at δ 10.14 arising from the newly introduced aldehyde proton, while the corresponding EI mass spectrum revealed a molecular ion at m/z 292 and an accurate mass measurement on this species established that it was of the expected molecular composition, viz C₈H₉IO₃.

2.4 Synthesis of the (Protected) Diol Fragment 127

With the synthesis of the aromatic unit **133** complete, attention was directed towards the synthesis of the protected diol unit **127** (Scheme 2.3) from the chlorobenzene-derived *c*DHC **82** (X = Cl). This was accomplished using protocols established earlier.^{2-6,9,10} Thus, the synthesis commenced with a regioselective reduction of the less sterically hindered and more electron rich double bond in *c*DHC **82** (X = Cl) using dihydrogen in the presence of catalytic amounts of rhodium on alumina (Scheme 2.3).¹⁰ By such means, cyclohexene **136** was obtained in 64% yield. Small amounts (10 – 15%) of the corresponding fully saturated congener of compound **136** were also obtained but these were readily removed by column chromatography. All the spectral data obtained on compound **136** were completely in accord with the assigned structure and consistent with these reported earlier.¹⁰ Diol **136** was then protected, under standard conditions,¹¹ as the corresponding acetonide **137**.

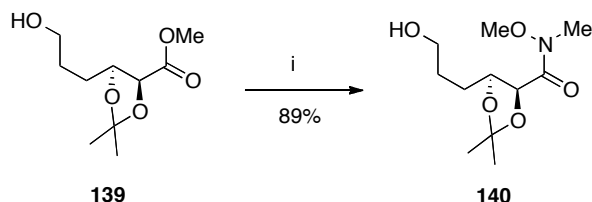


Reagents and conditions: (i) H₂, 5% Rh on Al₂O₃, EtOH, 18 °C, 1 h; (ii) 2,2-dimethoxypropane, *p*-TSA, 0 → 18 °C, 19 h; (iii) (a) O₃, MeOH, pyridine, −78 °C, 0.3 h; (b) Me₂S, −78 → 0 °C, 2 h; (iv) NaBH₄, 0 °C, 1 h.

Scheme 2.3: Synthesis of the primary alcohol **139**, via oxidative cleavage of **137**.

With the cyclohexene **137** in hand, ozonolytic cleavage of the associated alkene residue was undertaken (Scheme 2.3). Once again, protocols established earlier were employed for this purpose.^{2-6,10} Thus, a methanolic solution of cyclohexene **137** maintained at −78 °C was subjected to a stream of ozone. Reductive workup with dimethylsulfide and sodium borohydride then afforded, presumably *via* the intermediate aldehyde **138**, the primary alcohol **139** in 76% yield. Monitoring of reaction temperature and time were crucial, especially during the reductive workup step, to ensure maximum yields of product.^{12,13} While it has been shown that prolonged exposure of peroxidic intermediates to sodium borohydride furnishes primary alcohols,^{14,15} when a direct reduction of the product(s) of the ozonolysis of alkene **137** with sodium borohydride was attempted, the yield of **139** obtained was lower (40 – 50%) than when a combination of dimethylsulfide and sodium borohydride was employed. The IR spectrum of compound **139** exhibited a broad OH absorption band at 3452 cm^{−1} while examination of the corresponding ¹H NMR spectrum revealed the two oxymethine protons resonating as a doublet (at δ 4.60) and a doublet of triplets (at δ 4.36). A three-proton singlet, due to the three protons on the methyl ester group, appeared at δ 3.75. The ¹³C NMR spectrum of compound **139** displayed the expected ten carbon resonances with the most downfield signal, at δ 171.0, being assigned to the ester-carbonyl carbon and the signal due to the carbon of the primary alcohol appearing at δ 62.2.

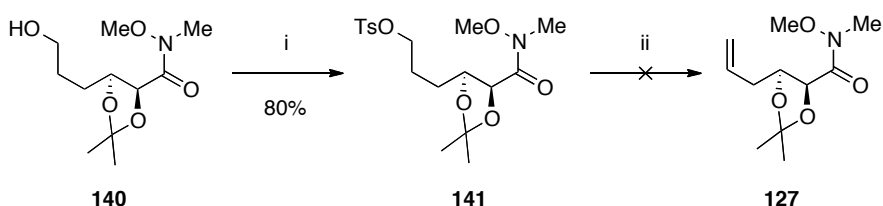
Following a procedure reported by Williams *et al.*,¹⁶ the methyl ester **139** was converted into the corresponding Weinreb amide **140** (89% yield) using *N,O*-dimethylhydroxylamine hydrochloride and *iso*-propylmagnesium chloride (Scheme 2.4). The ¹H NMR spectrum of amide **140** displayed two, three-proton, singlets at δ 3.14 and 3.66, corresponding to the protons of the *N*-methyl and *N*-methoxy groups, respectively. The ESI mass spectrum of the amide **140** exhibited ions at m/z 248 and 270 that are associated with its protonated and sodiated derivatives, respectively.



Reagents and conditions: (i) MeNH(OMe)•HCl, *i*-PrMgCl, THF, −15 °C, 1 h.

Scheme 2.4: Synthesis of Weinreb amide **140** from methyl ester **139**.

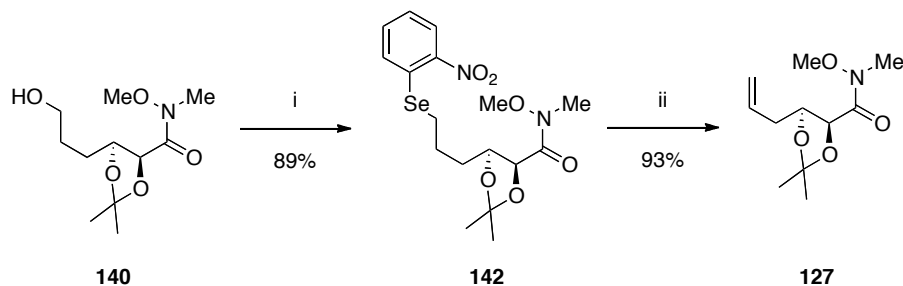
It was anticipated that the dehydration of the primary alcohol **140** could be accomplished *via* tosylation of the alcohol followed by its elimination. Accordingly, alcohol **140** was converted into the tosylate **141** (80% yield) using *p*-toluenesulfonyl chloride and *n*-triethylamine (Scheme 2.5). Unfortunately, exposure of tosylate **141** to DBU failed to effect the desired elimination and thereby generate target alkene **127**. Only decomposition of the starting material was observed under these conditions.



Reagents and conditions: (i) TsCl, Et₃N, THF, 18 °C; (ii) DBU, DMF, 80 °C.

Scheme 2.5: First attempt at dehydrating primary alcohol **140**.

As a result, an alternate method for dehydration of the primary alcohol **140** was sought. To such ends, and with a view to employing protocols first established by Grieco *et al.*,¹⁷ the primary alcohol **140** was smoothly converted into the selenide **142** using *o*-nitrophenyl selenocyanate and *n*-butyl phosphine (Scheme 2.6). The spectral data obtained on selenide **142**, which was obtained in 89% yield, were in complete accord with the assigned structure.



Reagents and conditions: (i) $o\text{-O}_2\text{NC}_6\text{H}_4\text{SeCN}$, $n\text{-Bu}_3\text{P}$, THF, 18 °C, 1 h; (ii) (a) $m\text{-CPBA}$, CH_2Cl_2 , $-78 \rightarrow 18$ °C, 1 h; (b) $(\text{CH}_3\text{CH}_2)_2\text{NH}$, O_2 , 18 °C, 4 h.

Scheme 2.6: Synthesis of the protected diol fragment **127** via, a Grieco elimination of primary alcohol **140**.

Oxidation of the selenide **142** with $m\text{-CPBA}$ proceeded efficiently to give a labile selenoxide that underwent smooth elimination on treatment with diethylamine to deliver the desired crystalline alkene **127** in 93% yield. The ^1H NMR spectrum of this compound (Figure 2.2) not only lacked the aromatic resonances that were associated with the precursor selenide, **142**, but also displayed a doublet of doublet of triplets at δ 5.81 and a complex multiplet at δ 5.08 that correspond to the protons of the newly installed olefinic residue. A single-crystal X-ray analysis of this material was undertaken and the derived ORTEP is shown in Figure 2.3.

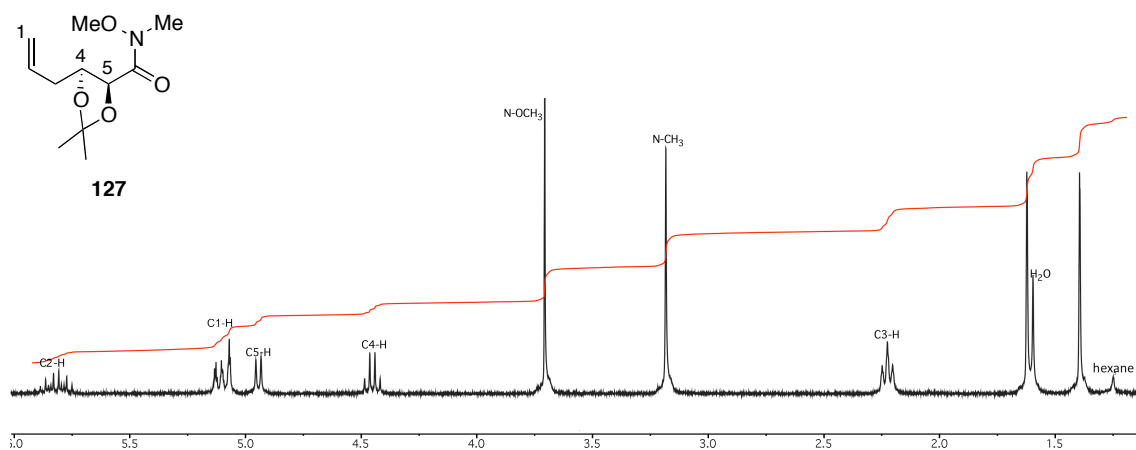


Figure 2.2: 300 MHz ^1H NMR spectrum of diol unit **127** (recorded in CDCl_3).

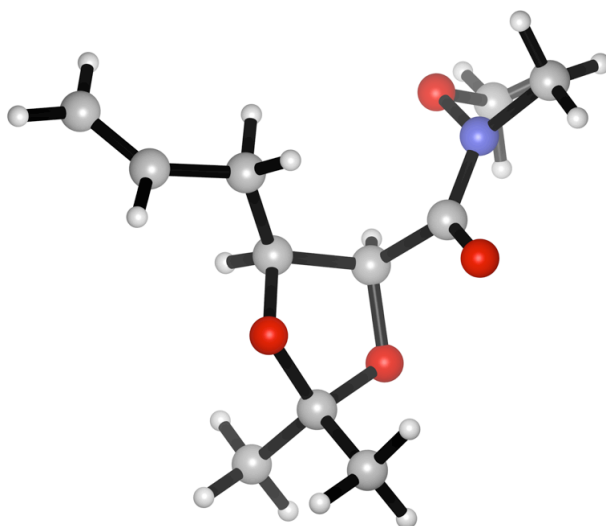
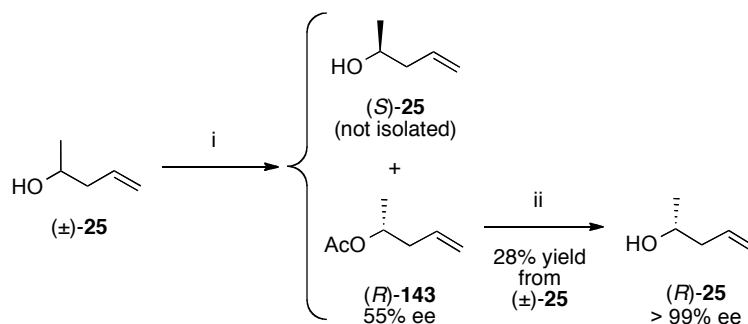


Figure 2.3: Ball and stick model of the protected diol fragment **127** generated using the data derived from a single-crystal X-ray analysis.

2.5 Synthesis of the Alcohol Fragment [(*R*)-**25**]

With the aromatic **133** and protected diol **127** fragments in hand, the remaining fragment required for the synthesis of target L-783,290 (**128**) was the enantiopure homoallylic alcohol (*R*)-(-)-4-penten-2-ol [(*R*)-**25**]. A common method for preparing this known alcohol involves the nucleophilic epoxide ring-opening of *R*-propylene oxide [(*R*)-**27**] with vinyl lithium, vinyl cuprate or a Grignard reagent.¹⁸⁻²⁰ The required *R*-propylene oxide [(*R*)-**27**] can, in turn, be obtained by a hydrokinetic resolution of racemic propylene oxide [(±)-**27**] using the (*S,S*)-form of Jacobsen's catalyst.¹⁸⁻²⁰ Another method for generating alcohol (*R*)-**25** involves the enzymatic kinetic resolution of the corresponding racemate, viz. (±)-4-penten-2-ol [(±)-**25**].^{2,3} In particular, it was discovered, through an extensive study conducted by Banwell and Loong, that *Candida antarctica* Lipase B (CALB) could also be used to generate the desired alcohol (*R*)-**25** in > 99% ee through a double kinetic resolution sequence.^{2,3}

Although both of the abovementioned methods could yield the desired alcohol (*R*)-**25** with exceptional ee's, the double kinetic resolution protocol was considered more attractive because it follows a major theme being pursued in the Banwell Group, namely the use of enzymes in synthesis. Accordingly, the racemic alcohol (±)-**25** was treated with CALB in vinyl acetate (Scheme 2.7) which served as both a reaction medium and an acyl donor, to furnish a mixture of enantio-enriched acetate (*R*)-**143** and unreacted alcohol (*S*)-**25** (not isolated). The two compounds were readily separated by flash chromatography.



Reagents and conditions: (a) CALB, vinyl acetate, 30 °C, 1 h; (ii) CALB, phosphate buffer (pH = 7.3), 30 °C, 10 h.

Scheme 2.7: Synthesis of enantiopure secondary alcohol (*R*)-**25**.^{2,3}

The acetate (*R*)-**143** was then hydrolysed using CALB, which resulted in the recovery of alcohol (*R*)-**25** in 28% yield [from the racemic alcohol ($\pm$)-**25**] and > 99% ee (as determined by chiral GC analysis of the derived acetate). The modest recovery of the alcohol (*R*)-**25** was attributed to its high volatility (b.p. 115 °C) and water solubility. Nevertheless, this procedure could be carried out on a multi-gram scale with no reduction in ee.

2.6 Assembly of Macrocycle Through the Union of Fragments **127**, **133** and (*R*)-**25**

2.6.1 The Heck Reaction

With all fragments synthesised, the stage was set for the assembly of the target macrolactone from them. The first fragment coupling to be undertaken was that between the aryl iodide **133** and alkene **127**. Before describing the Heck coupling of fragments **127** and **133** a general discussion of the utility of this reaction and its mechanism (Figure 2.4) are presented. It is appropriate to do so because the conversion of fragment **127** and **133** into product **129** is a pivotal aspect of the synthetic plan leading to the target RAL.

The catalytic variant of the Heck reaction involves the initial oxidative insertion of palladium(0) (**146**) into the carbon-halogen bond of aryl iodide **133** to afford the Pd(II) complex **147**. This complex then co-ordinates with the target alkene **127** to give the π -palladium(II) complex **148** which, in turn, engages in a *syn*-addition reaction with the double bond to provide σ -palladium(II) complex **149**. Compound **149** itself undergoes a β -hydride elimination reaction to give complex **150** that immediately collapses to give the “Heck product **129**” and hydridopalladium(II) halide **151**. The final step of the catalytic cycle involves the regeneration of the catalytically active palladium(0) species (**146**) from compound **151** via a reductive elimination process effected by the added base.

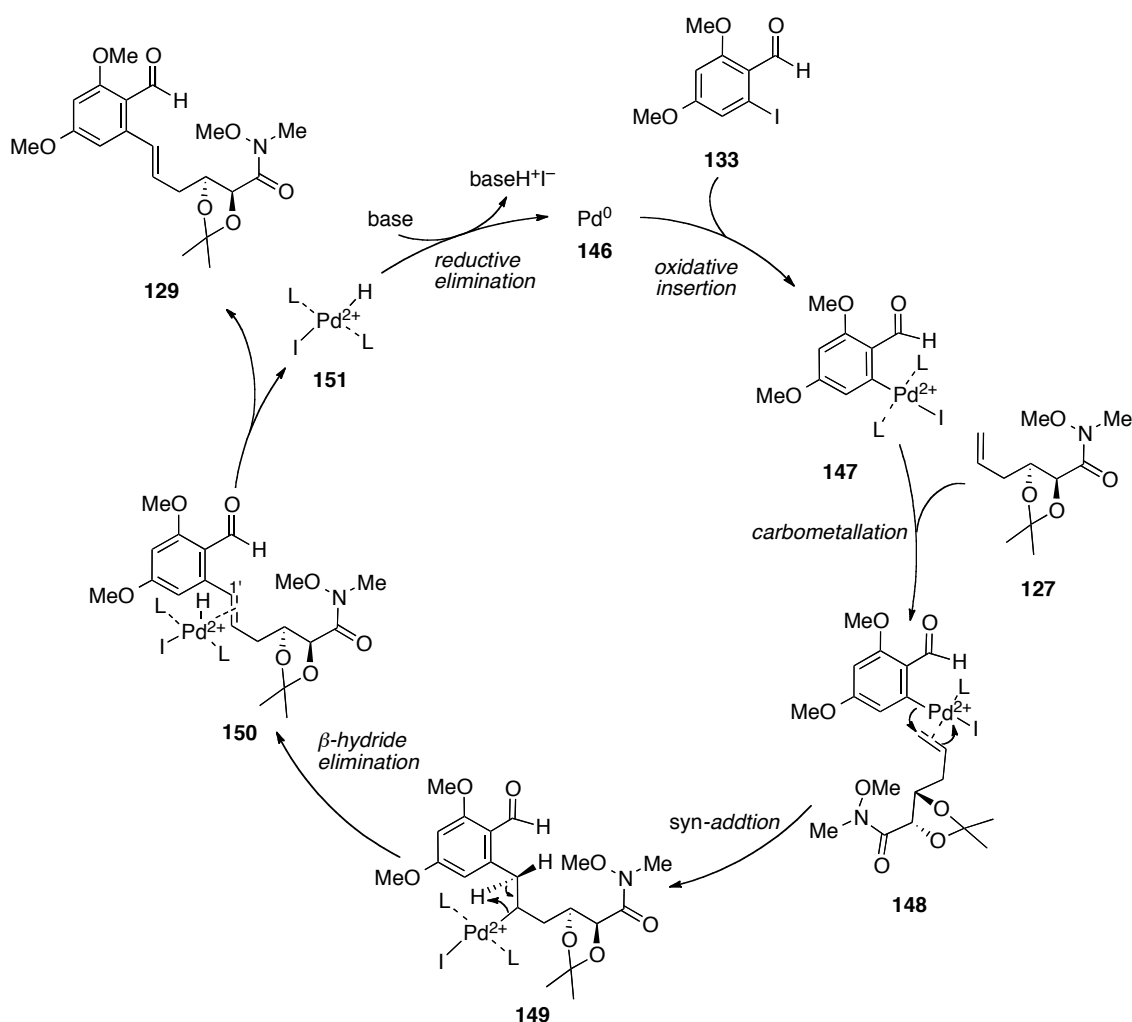
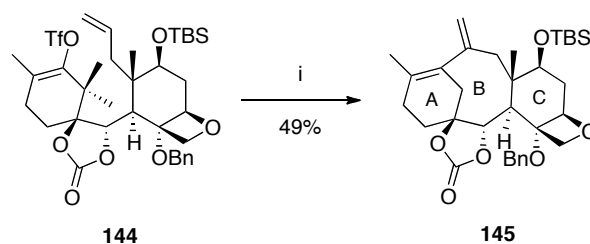


Figure 2.4: The catalytic cycle associated with the Heck reaction.

The Heck reaction is powerful and versatile in that it links an alkene with an aryl halide in a C–C bond-forming process. Today, this reaction is extensively employed in both laboratory scale syntheses as well as on the tonne scale in industry.^{21,22} Since Heck's seminal work on the coupling of stoichiometric amounts of organopalladium halide with various alkenes,²³ the reaction has matured to become a catalytic process that provides a powerful tool for creating pivotal C–C bonds including those embodied in complex ring systems. An example of the Heck reaction being applied in a complex natural product synthesis is seen in Danishefsky and co-worker's elegant construction of the B-ring of Taxol™ *via* the conversion of compound **144** into the pentacyclic derivative **145** as shown in Scheme 2.8.²⁴



Reagents and conditions: (i) Pd(PPh₃)₄, K₂CO₃, CH₃CN, 85 °C, 49%.

Scheme 2.8: Construction of the B-ring of Taxol *via* a Heck-mediated ring closure.²⁴

2.6.2 Investigation of Conditions for the Heck Cross-coupling Between the Aromatic **133** and Protected Diol **127** Units

While various palladium-catalysed cross-coupling reactions, especially the Suzuki-Miyaura and Sonogashira processes, have been used in several syntheses of RALs, the Heck reaction has not. Nevertheless, it was confidently anticipated that the aryl iodide **133** and the alkene moiety within compound **127** could be coupled by such means so as to deliver product **129**. However, the coupling of these two fragments was considered likely to be challenging since each contains functionalities that might degrade the catalyst or interfere with its activity. As a result, a comprehensive examination of the various conditions used for effecting Heck reactions was undertaken and the outcomes of these are presented in Table 2.1. All reactions were conducted for 19 hours since after this time yields of target **129** were observed to decline.

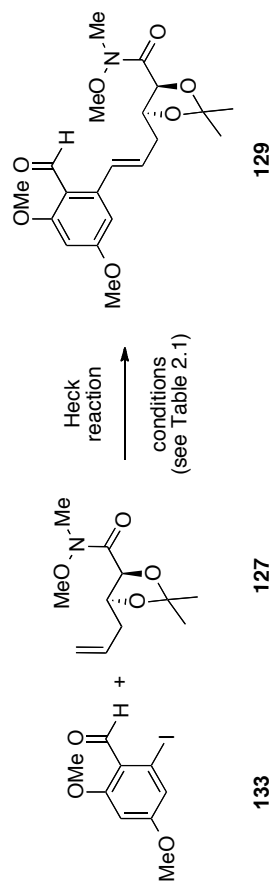
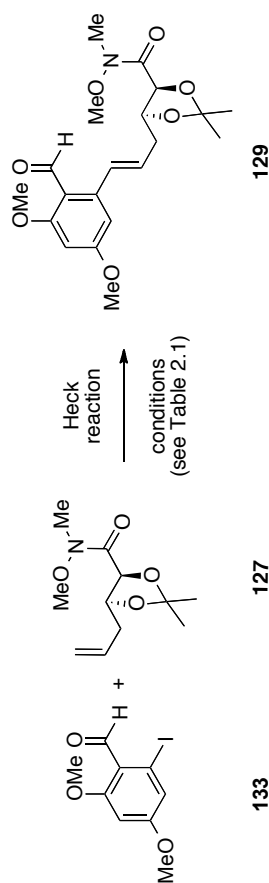
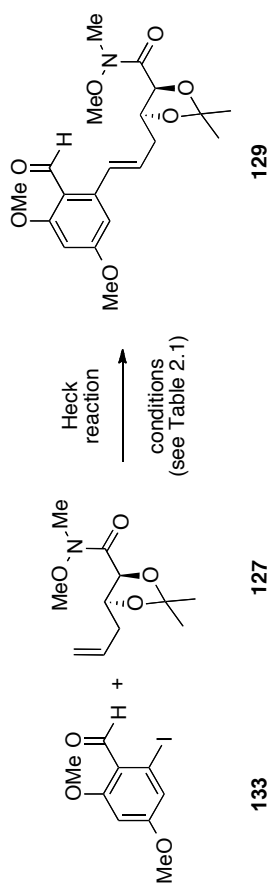


Table 2.1: Outcomes of attempts to effect coupling of compounds **127** and **133** under Heck-type conditions.

	Palladium Source	Cat. loading (%)	Ligands	Temp. (°C)	Base	Additive	Solvent	Yield (%)	Outcome/Comments
1	Pd(OAc) ₂	10	PPh ₃	80	NaHCO ₃	None	DMF	30 – 40	127 recovered (<10%).
2	Pd(OAc) ₂	10	PPh ₃	100	NEt ₃	None	NEt ₃	ca. 10	Completed in a sealed tube. Predominantly 127 recovered.
3	Pd ₂ (<i>o</i> -tolyl) ₂	5	–	100	NaOAc	TBAB	DMF	ca. 10	Predominantly 127 recovered.
4	10% Pd on carbon	5	None	140	NMP	None	DMA	None	Coupled product 129 was obtained however acetone was cleaved. Product was unstable on SiO ₂ and decomposed on isolation.
5	10% Pd on carbon	5	None	140	K ₂ CO ₃	None	DMA	None	No reaction.
6	Pd(dba) ₂	10	PPh ₃	80	K ₂ CO ₃	None	10:1 v/v DMF/H ₂ O	None	No Reaction.

Table 2.1: Outcomes of attempts to effect coupling of compound **127** and **133** under Heck-type conditions. (cont.)

	Palladium Source	Cat. loading (%)	Ligands	Temp. (°C)	Base	Additive	Solvent	Yield (%)	Outcome/Comments
7	Pd(dba) ₂	10	PPh ₃	80	K ₂ CO ₃	TBAB	10:1 v/v DMF/H ₂ O	None	No Reaction.
8	Pd(<i>t</i> -Bu ₃ P) ₂	10	PPh ₃	80	K ₂ CO ₃	TBAB	10:1 v/v DMF/H ₂ O	<10	Predominantly 127 and dehalogenated 133 recovered.
9.	Pd(<i>t</i> -Bu ₃ P) ₂	5	PPh ₃	80	K ₂ CO ₃	TBAB	10:1 v/v DMF/H ₂ O	ca. 10	Attempted in a microwave (80 °C, 220 W, 200 psi). Predominantly 127 and dehalogenated 133 recovered.
10	Pd(OAc) ₂	5	PPh ₃	80	K ₂ CO ₃	AgOAc	10:1 v/v DMF/H ₂ O	None	No Reaction.
11	Pd(OAc) ₂	5	PPh ₃	80	K ₂ CO ₃	TBAI	10:1 v/v DMF/H ₂ O	ca. 10	Predominantly 127 and dehalogenated 133 recovered.
12	Pd(OAc) ₂	5	PPh ₃	80	K ₂ CO ₃	TBAB	10:1 v/v DMF/H ₂ O	40 – 50	ca. 10% of 127 recovered.
13	Pd(OAc) ₂	10	None	80	K ₂ CO ₃	TBAB	10:1 v/v DMF/H ₂ O	45 – 50	ca. 10% of 127 recovered.

Table 2.1: Outcomes of attempts to effect coupling of compound **127** and **133** under Heck-type conditions. (cont.)

	Palladium Source	Cat. loading (%)	Ligands	Temp. (°C)	Base	Additive	Solvent	Yield (%)	Outcome/Comments
14	Pd(OAc) ₂	20	None	80	K ₂ CO ₃	TBAB	10:1 v/v DMF/H ₂ O	50 – 60	ca. 10% of 127 recovered.
15	Pd(OAc) ₂	30	None	80	K ₂ CO ₃	TBAB	10:1 v/v DMF/H ₂ O	20 – 30	ca. 10% of 127 recovered.
16	Pd(OAc) ₂	5	PPh ₃	60 → 80	K ₂ CO ₃	TBAB	10:1 v/v DMF/H ₂ O	50 – 65	ca. 10% of 127 recovered.
17	Pd(OAc) ₂	10	None	80	NaHCO ₃	TBAB	10:1 v/v DMF/H ₂ O	20 – 35	ca. 10% of 127 recovered.
18	Pd(OAc) ₂	10	PPh ₃	80	K ₂ CO ₃	TBAB	10:1 v/v DMF/H ₂ O	ca. 10	Attempted in a microwave (80 °C, 220 W, 200 psi). Predominantly 127 and dehalogenated 133 recovered.
19	Pd(OAc) ₂	10	PPh ₃	80	K ₂ CO ₃	TBAB	4:1 v/v THF/H ₂ O	ca. 10	Attempted in a microwave (80 °C, 220 W, 200 psi). Predominantly 127 and dehalogenated 133 recovered.

As a result of the studies summarised in Table 2.1, it became clear that very specific conditions were required in order to ensure a useful outcome. Thus, the optimum conditions established for effecting the Heck reaction between the aryl halide **133** and alkene **127** involved using 20 mol% palladium(II) acetate, tetra-*n*-butylammonium bromide (TBAB) as additive, potassium carbonate as base and 10:1 *v/v* DMF/water as solvent. Although the yield for the Heck reaction was, even after much optimisation, a moderate 50 – 65%, these conditions provided a reproducible outcome and reliably delivered compound **129**. The ^1H NMR spectrum of this material established that it was composed of a *ca.* 9:1 mixture of *E*- and *Z*- isomers. The *E*-configured alkene could be obtained in pure form after flash chromatography with the spectrum of this material (Figure 2.5) showing two mutually-coupled proton resonances at δ 7.41 and 6.20. The magnitude of this coupling ($J = 16.0$ Hz) suggested a *trans*-relationship between them and, therefore, an *E*-configuration about the associated double bond. A molecular associated ion observed at m/z 416 in the ESI mass spectrum corresponded to the sodiated derivative of compound **129** and an accurate mass measurement on this species established it was of the expected composition, *viz* $\text{C}_{20}\text{H}_{27}\text{NNaO}_7$.

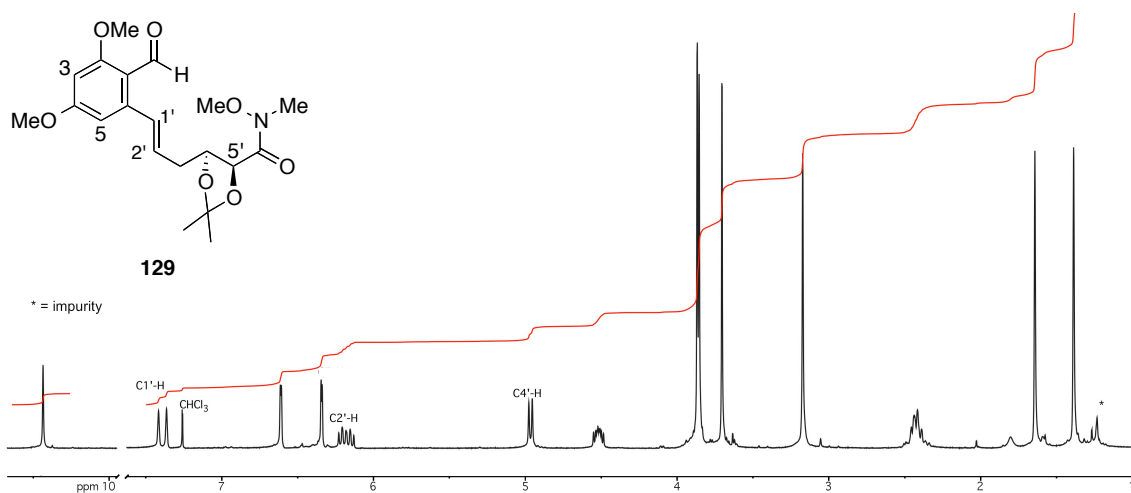
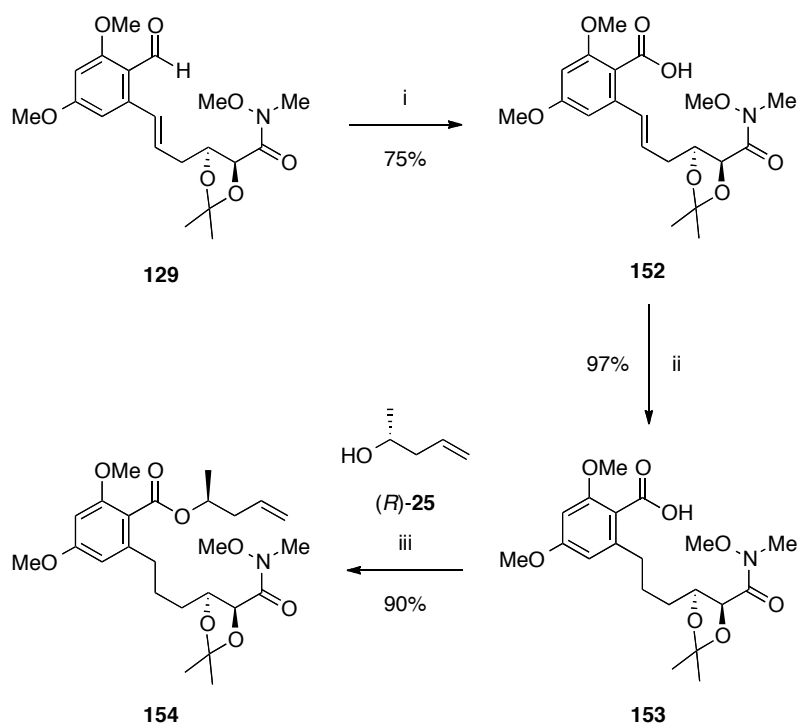


Figure 2.5: 300 MHz ^1H NMR spectrum of compound **129** (recorded in CDCl_3).

2.6.3 Union of the Final Fragment: Formation of the Diastereomerically Pure Ester **154**

With compound **129** in hand, attention could be focused on its elaboration to diene **132**, the substrate required for the pivotal macrolide-forming RCM reaction. To such ends, benzaldehyde **129** was oxidised, under Pinnick conditions (Scheme 2.9),²⁵ to the corresponding acid **152** which was obtained in 75% yield. The absence of a one-proton singlet at δ 10.45 in the ^1H NMR spectrum of compound **152** and the presence of an ion at m/z 408 in the negative ESI mass spectrum (corresponding to its conjugate base) clearly indicated that the desired oxidation reaction had taken place.

The C1'-C2' alkene residue associated with compound **152** was reduced by hydrogenation in the presence of catalytic amounts of palladium on charcoal (Scheme 2.9) to give the corresponding alkane **153** in 97% yield. The absence of the olefinic resonances in the ^1H NMR spectrum of this product and the lack of a C=C absorption band (as seen at 1578 cm^{-1} in the analogous spectrum of the precursor) in its IR spectrum clearly suggests that the desired alkane had been formed.



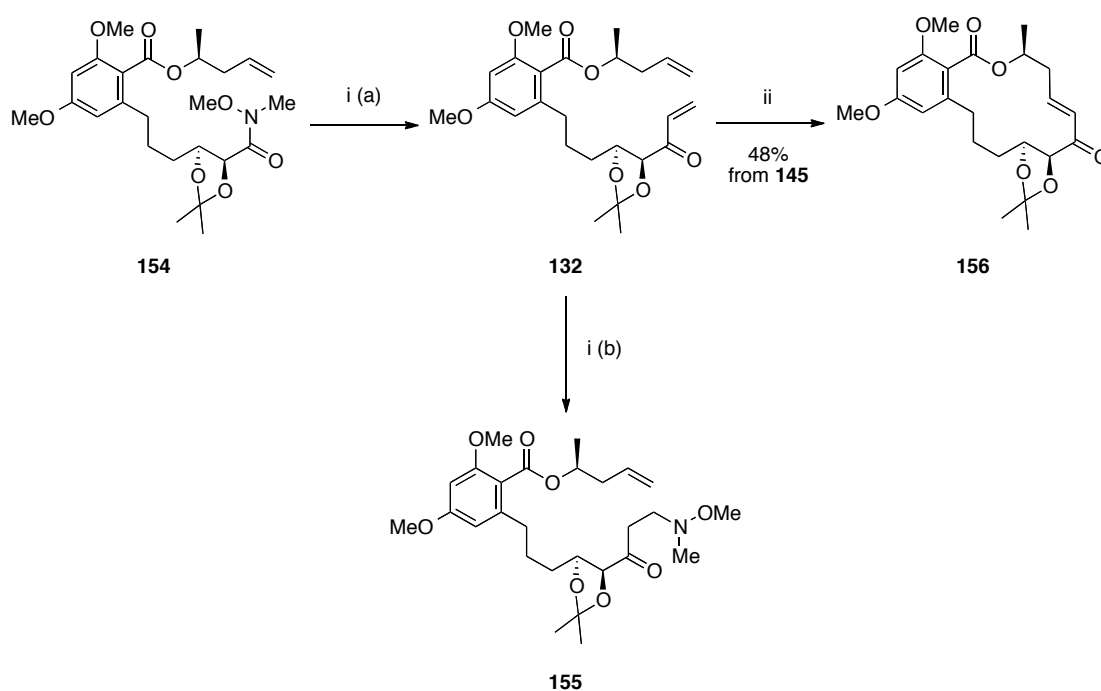
Reagents and conditions: (i) 2-methyl-2-butene, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, NaClO_2 , *t*-BuOH, H_2O , $18\text{ }^\circ\text{C}$, 1 h; (ii) H_2 (50 psi), 10% Pd on carbon, EtOH, $18\text{ }^\circ\text{C}$, 24 h; (iii) (*R*)-**25**, DIAD, PPh_3 , PhMe, $0 \rightarrow 18\text{ }^\circ\text{C}$, 0.5 h.

Scheme 2.9: Synthesis of ester **154**.

The alcohol (*R*)-**25** was coupled to the acid **153** via a Mitsunobu esterification reaction (Scheme 2.9) to give the desired ester **154** (90% yield) possessing the illustrated *S*-configuration at C10' as a result of the expected inversion process. The ^1H NMR spectrum of ester **154** featured a two-proton multiplet at δ 5.10 and a one-proton multiplet at δ 5.82, which are attributed to the hydrogens attached to the terminal alkene residue of the homoallylic ester. The resonance appearing at δ 167.8 in the ^{13}C NMR spectrum is assigned to the carbonyl carbon of the newly introduced ester moiety. In addition, the ions appearing at m/z 480 and 502 in the ESI spectrum of compound **154** are assigned to the protonated and sodiated molecular ions, respectively. Accurate mass measurements on these species established their molecular formulae as $\text{C}_{25}\text{H}_{38}\text{NO}_8$ and $\text{C}_{25}\text{H}_{37}\text{NNaO}_8$, respectively.

2.6.4 Completion of Synthesis using a RCM Protocol

With all three fragments assembled into compound **154**, attention was now focused on closing the macrocycle using RCM techniques. To that end, diene **132** was required and the preparation of this involved converting Weinreb amide **154** into the vinyl ketone **132** (Scheme 2.10). However, initial attempts to do so resulted in low yields, an outcome attributed to the conjugate addition of the displaced *N,O*-dimethylhydroxylamine to the initially formed product **132** and so generating compound **155**. The formation of similar by-products by analogous processes has been reported previously.²⁶⁻²⁹ Support for the structure of compound **155** came from the observation of two three-proton singlets at δ 3.48 and 2.57 in the ^1H NMR spectrum of this material. These are assigned to the *N*-methyl and *N*-methoxy groups, respectively, of the nitrogen centred residue incorporated in this by-product. The ESI mass spectrum of this material displayed a molecular associated ion at m/z 530 ($\text{M}+\text{Na}^+$)⁺.



Reagents and conditions: (i) vinylmagnesium bromide, THF, $-15\text{ }^{\circ}\text{C}$, (a) 0.1 h (b) 1 h; (ii) Grubbs' 2nd generation catalyst, CH_2Cl_2 , $45\text{ }^{\circ}\text{C}$, 19 h.

Scheme 2.10: Synthesis of macrolide **156** via RCM of precursor **132**.

Through careful monitoring of reaction time as well as by quenching the reaction mixture with 5:1 *v/v* acetic acid/water, the vinyl ketone **132** was obtained with minimal concomitant formation of by-product **155**. Since the possibility of conjugate addition by adventitious nucleophiles to the vinyl ketone **132** was a serious concern, the manipulation of this sensitive material was kept to a minimum. Accordingly, once obtained diene **132** was immediately subjected to a RCM reaction with Grubbs' 2nd generation catalyst³⁰ (Scheme 2.10) to give the *E*-configured enone **156** in 48% yield from Weinreb amide **154**. Evidence for the exclusive

generation of the *E*-configured enone came from the presence of a mutual coupling between the olefinic protons in the ^1H NMR spectrum (Figure 2.6), at δ 6.96 (ddd, $J = 15.8, 12.8$ and 6.8 Hz) and δ 6.64 (d, $J = 15.8$ Hz), which is indicative of the assigned configuration. Confirmation of the presence of the α,β -unsaturated ketone moiety followed from the appearance of a resonance at δ 197.4 in the ^{13}C NMR spectrum, which is assigned to the carbonyl carbon of the enone moiety. Absorption bands observed at 1627 and 1604 cm^{-1} in the IR spectrum are assigned to C=C and C=O stretching bands. The ESI mass spectrum revealed molecular associated ions at m/z 419 and 441 and accurate mass measurements on these established that they were of the expected compositions, viz $\text{C}_{23}\text{H}_{31}\text{O}_7$ and $\text{C}_{23}\text{H}_{30}\text{NaO}_7$, respectively.

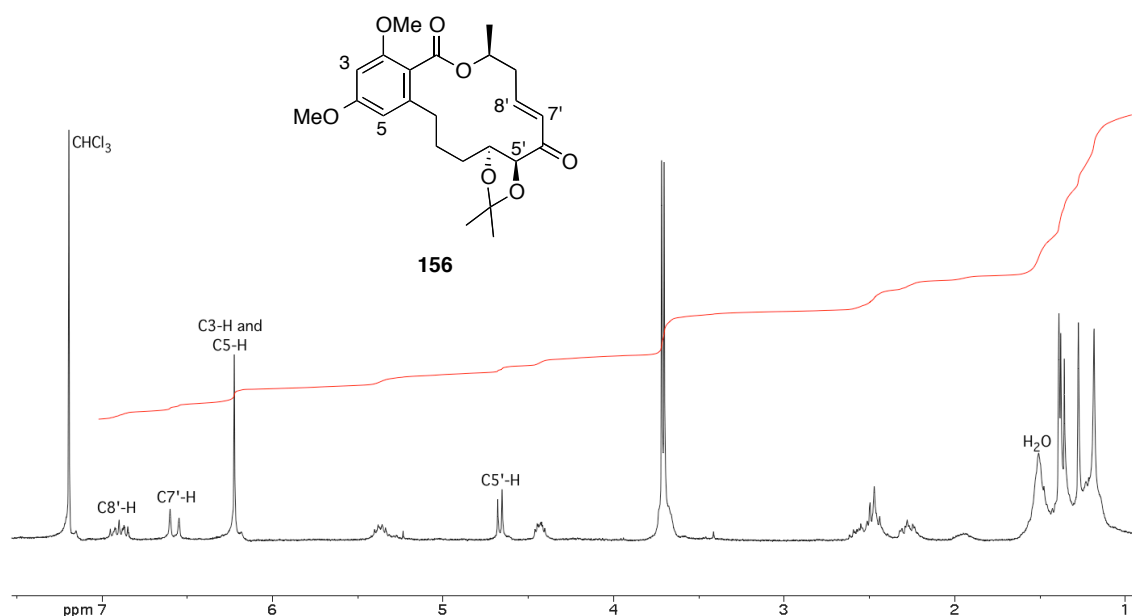
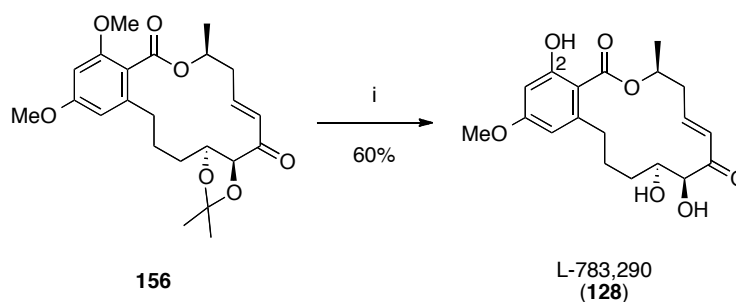


Figure 2.6: 500 MHz ^1H NMR spectrum of macrolactone **156** (recorded in CDCl_3).

The acquisition of macrolactone **156** provided the protected form of target L-783,290 (**128**) so attention was focused on the final deprotection step so as to provide the natural product **128** itself. In the event, careful treatment of macrolactone **156** with BCl_3 (Scheme 2.11) led to the selective cleavage of the methyl ether adjacent to the carbonyl group of the macrolactone ring to reveal the C2 phenol. Upon quenching the reaction mixture with water, HCl was generated and this effected cleavage of the acetonide residue to afford the natural product, L-783,290 (**128**), as an off-white powder in 60% yield.



Reagents and conditions: (i) BCl_3 , CH_2Cl_2 , -78°C , 1 h.

Scheme 2.11: Deprotection to give L-783,290 (**128**).

The 800 MHz ^1H NMR spectrum of product **128** (Figure 2.7) displayed a one-proton singlet at δ 11.83 which is attributed to an intramolecularly hydrogen-bonded (to the adjacent lactone $\text{C}=\text{O}$) phenolic proton and lacked two three-proton singlets associated with the two methyl groups on the acetonide of the precursor. The remaining resonances in the spectrum were in complete accord with the assigned structure. The ^{13}C NMR spectrum of this material (Figure 2.8) displayed the expected nineteen resonances, with the most downfield signal, at δ 197.0, being assigned to the enone-carbonyl carbon. The EI mass spectrum displayed an ion at m/z 364 and an accurate mass measurement of this species established the composition $\text{C}_{19}\text{H}_{24}\text{O}_7$. All in all, the spectral data collected on this material were entirely consistent with the assigned structure, however final confirmation of this was secured through a single-crystal X-ray analysis. The derived ORTEP plot is shown in Figure 2.9 while further details of this analysis are presented in the Experimental Section.

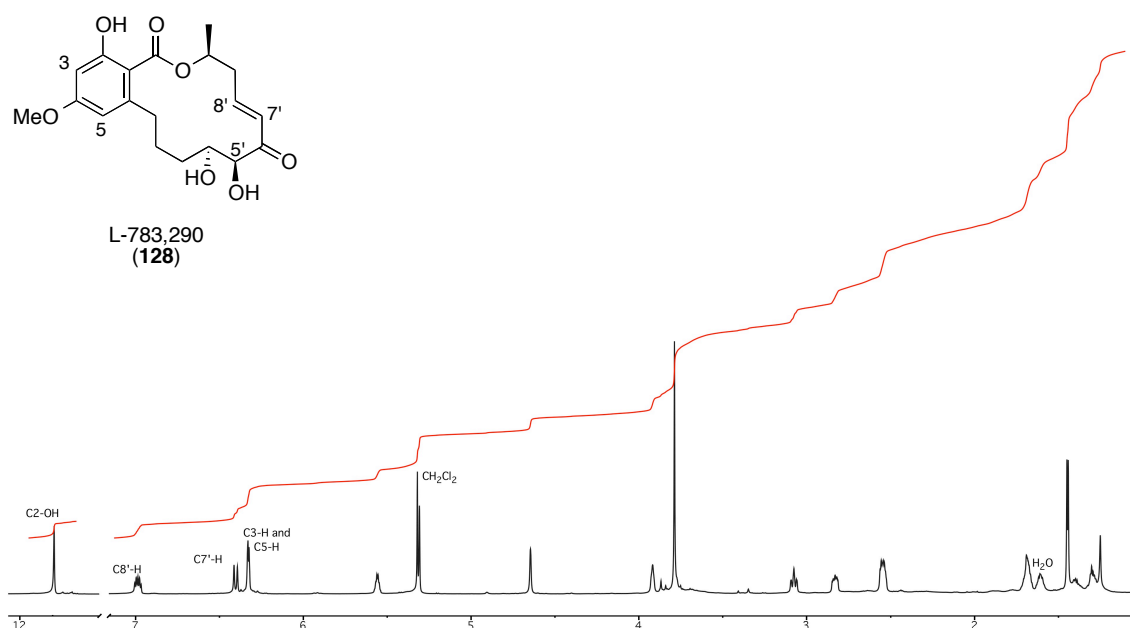


Figure 2.7: 800 MHz ^1H NMR spectrum of L-783,290 (**128**) (recorded in CD_2Cl_2).

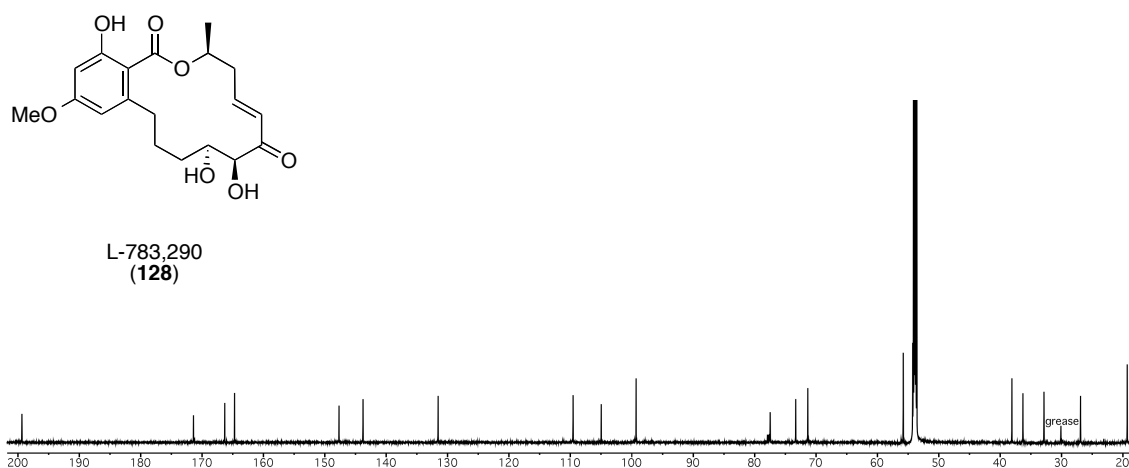


Figure 2.8: 200 MHz ^{13}C NMR spectrum of L-783,290 (**128**) (recorded in CD_2Cl_2).

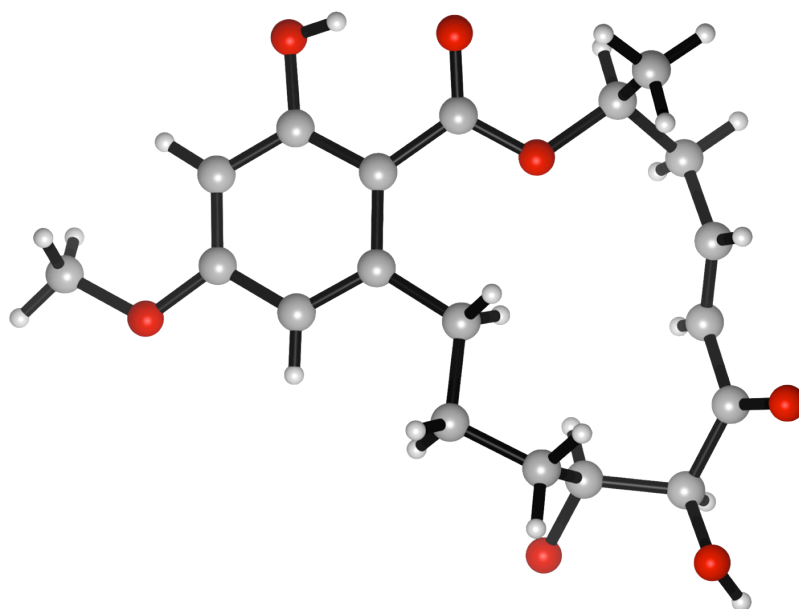


Figure 2.9: Ball and stick model of L-783,290 (**128**) generated using the data derived from a single-crystal X-ray analysis.

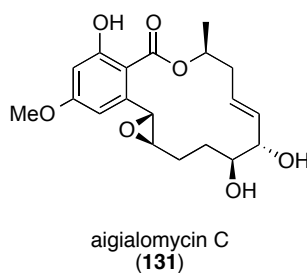
Unfortunately, no spectral data derived from the natural product have been published that would allow for comparison with the synthetic product obtained as described above. Efforts to secure these data through email correspondence with the investigators at Merck have been unsuccessful. As a result, relevant comparisons between spectral data for the synthetic L-783,290 (**128**) and the natural product were not possible.

2.7 Conclusion

In the work detailed above, the RAL L-783,290 (**128**) has been synthesised in 16 steps from the biocatalytically-derived starting material *c*DHC **82** ($\text{X} = \text{Cl}$). Two of the stereocentres in L-783,290 (**128**) were derived from the *c*DHC **82** while the final one originated from alcohol (*R*)-

25 which was itself established using enzymatic resolution techniques. Target **128** was assembled in a modular and completely diastereoselective fashion.

A study of the Heck reaction between the aromatic **133** and the protected diol **127** units was undertaken and the coupled product derived from this contained a double bond that was removed in the latter stages of the synthesis. The option to retain the C1'-C2' alkene derived from the Heck reaction offers the potential to synthesise natural products that contain this or related functionality at the homo-benzylic position such as is seen in aigialomycin C (**131**). Work directed towards these latter targets is now under way in the Banwell laboratories. Biological testing of the synthetic sample of L-783,290 (**128**) will be carried out in the near future.



2.8 References

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Chapter Three

The Total Synthesis of L-783,277 (*cis*-isomer)

3.1 Introduction

Three syntheses of L-783,277 (**1**) (Figure 3.1) have been reported thus far, with two of these surfacing during the course of the work described in this chapter. The first was reported by Altmann *et al.* in 2008 and this was followed by Winssinger's in 2009.^{1,2} The final synthesis was published in 2010 by Sim *et al.*³ and essentially at the same time as the one detailed here appeared in print.⁴ A comparison of these three syntheses together with the one reported here is presented at the end of this chapter.

After completing an enantioselective synthesis of L-783,290 (**128**) using the microbially-derived chlorobenzene building block *c*DHC **82** ($X = \text{Cl}$), it was anticipated, based on earlier work in the group, that a synthesis of compound L-783,277 [**1**], the *cis*-isomer of L-783,290 (**128**) could be achieved directly from the *trans*-isomer **128** through a late-stage photoisomerisation reaction. Details associated with this approach are discussed in the following section.

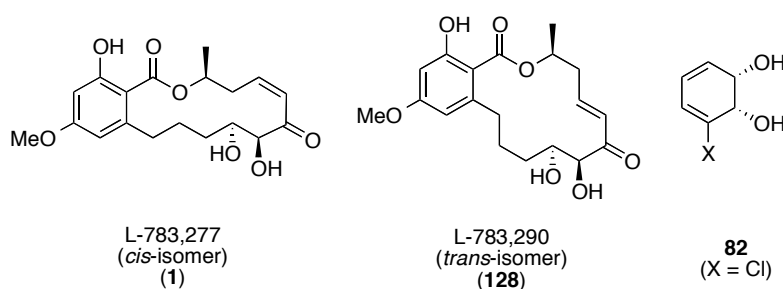
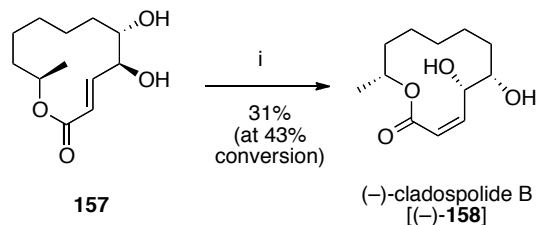


Figure 3.1: Structures of L-783,277 (**1**), L-783,290 (**128**) and *c*DHC **82** ($X = \text{Cl}$).

3.2 Attempted Synthesis of L-783,277 (1) From L-783,290 (128) via Photoisomerisation

3.2.1 Related *trans-cis* Photoisomerisation Processes

In 2005 Banwell and Loong reported the photoisomerisation reaction of the *E*-configured α,β -unsaturated macrolactone **157** to its *Z*-configured isomer (–)-**158** and thereby completing a total synthesis of (–)-cladospolide B [(–)-**158**] (Scheme 3.1).⁵

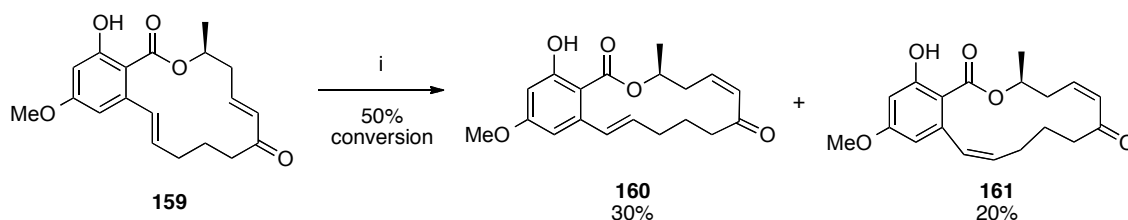


Reagents and conditions: (i) $h\nu$ (300 nm), C_6H_6 .

Scheme 3.1: Banwell and Loong's synthesis of (–)-cladospolide B [(–)-**158**] via photoisomerisation reaction of compound **157**.⁵

This conversion involved irradiating a benzene solution of compound **157** at 300 nm (Scheme 3.1) and by such means (–)-cladospolide B [(–)-**158**] was obtained, albeit in just 31% yield (at 43% conversion).⁵

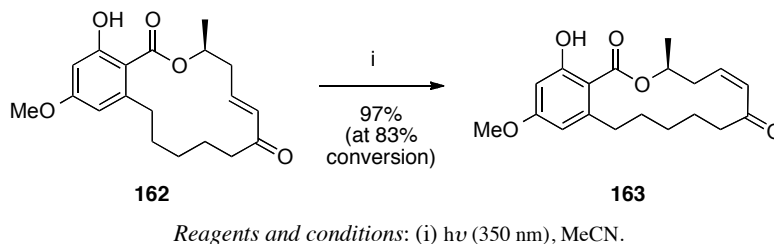
In related work, Murphy *et al.* were able to effect the photoisomerisation of the *E*-configured enone **159** to its *Z*-isomers **160** (30%) and **161** (20%) by irradiation of the former compound with 350 nm wavelength light (Scheme 3.2).⁶



Reagents and conditions: (i) $h\nu$ (350 nm), MeCN.

Scheme 3.2: Photochemically-promoted conversion of diene **159** to isomers **160** and **161** reported by Murphy *et al.*⁶

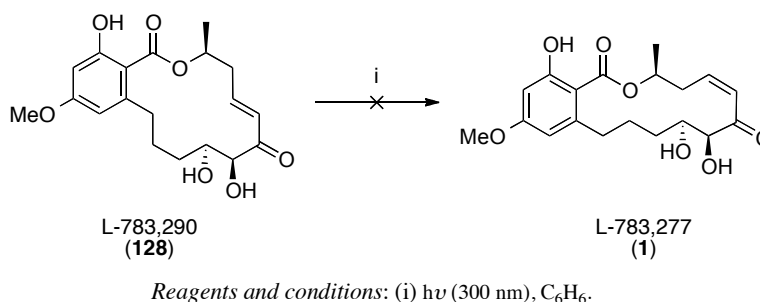
Similarly, the dihydro-analogue of **159**, namely enone **162**, was efficiently converted into its *Z*-isomer **163** (97% at 83% conversion) under the same conditions (Scheme 3.3).⁶



Scheme 3.3: Photoisomerisation reaction of RAL **162** to **163** reported by Murphy *et al.*⁶

3.2.2 Attempted Photoisomerisation Reaction of L-783,290 (**128**) to L-783,277 (**1**)

On the basis of the work described above, a benzene solution of the *trans*-compound **128** was irradiated at 300 nm (Scheme 3.4). However, after 3 hours of irradiation only decomposition of the starting material was observed. Unfortunately, the conditions reported by Murphy *et al.* for affecting the photoisomerisation reaction of compound **162** into congener **163** (Scheme 3.3) could not be applied to substrate **128** due to a lack of additional material.

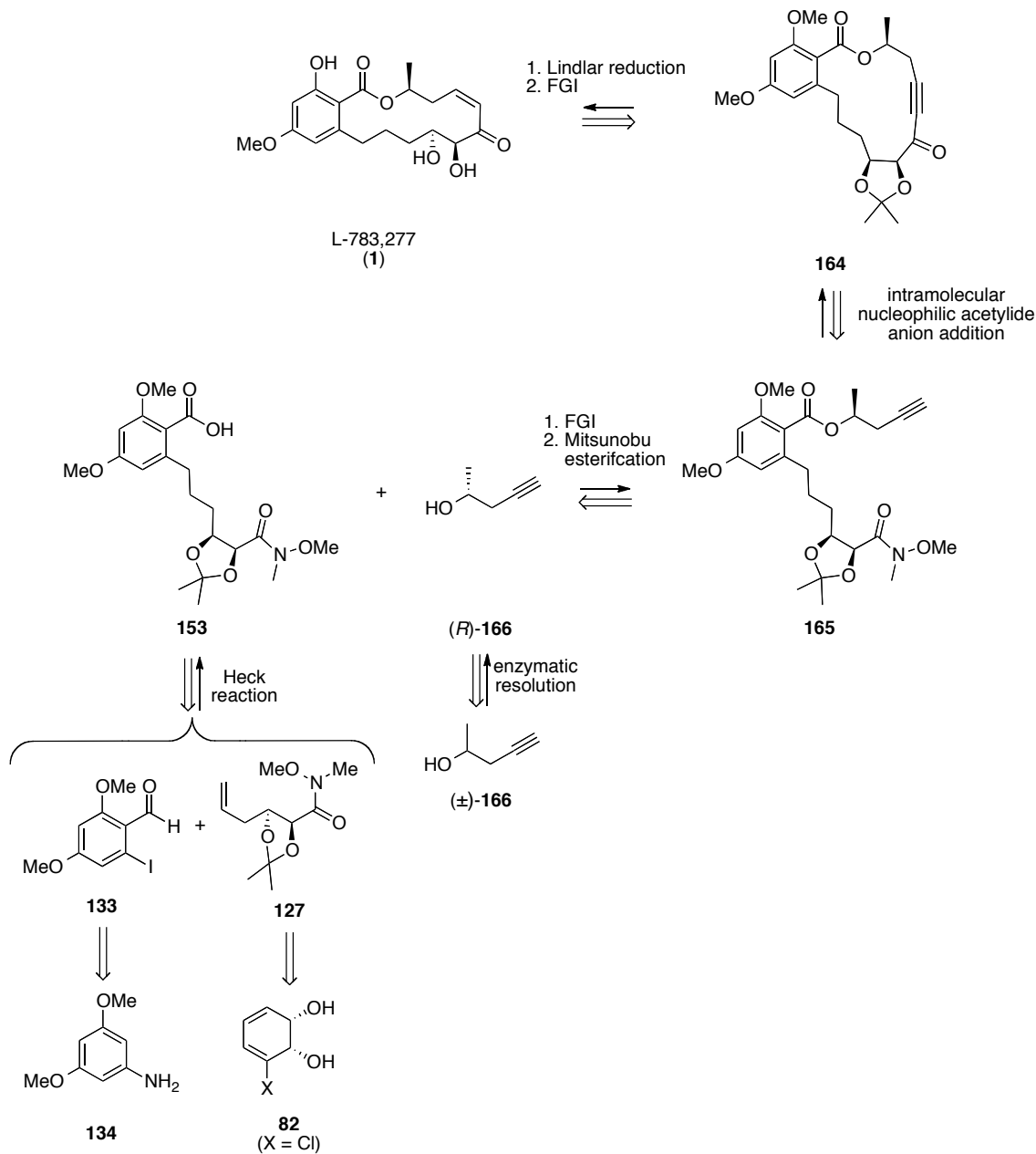


Scheme 3.4: Attempt to photoisomerise L-783,290 (**128**) to L-783,277 (**1**).

3.3 Revised Retrosynthetic Analysis of L-783,277 (**1**)

Given the difficulties encountered during attempts to prepare L-783,277 (**1**) from the corresponding *trans*-isomer (**128**) through a photoisomerisation reaction, a new approach was pursued. In particular, it was anticipated that the *Z*-configured enone in L-783,277 (**1**) could be prepared through a Lindlar reduction (Scheme 3.5) of the corresponding propargylic ketone **164**. The synthesis of such an alkyne was thought achievable through an intramolecular nucleophilic addition of an acetylide anion onto the tethered Weinreb amide such as encountered in compound **165**. A review of the relevant literature clearly established that this ring-forming process has not been exploited in any previous RAL syntheses and it was believed that the incorporation of the alkyne functionality into the target macrocycle by this means was realistic due to the length of the tether linking the two reaching centres (and which should,

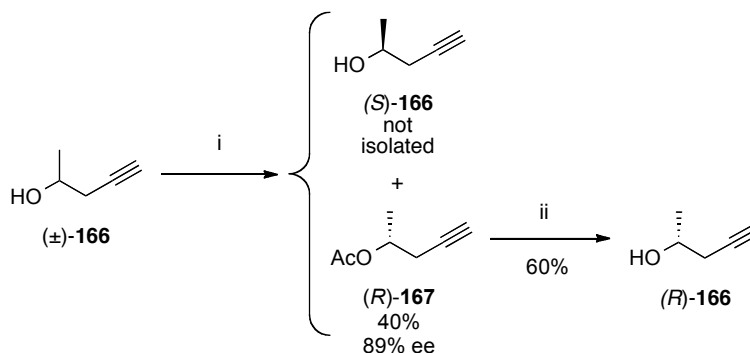
therefore, allow the required reaction trajectory to be adopted with relative ease). The substrate required for the formation of the alkyne-containing macrocycle would come from a Mitsunobu esterification of the relevant-enantiopure homo-propargylic alcohol, *viz* (*R*)-(-)-4-pentyn-2-ol [(*R*)-**166**], with the benzoic acid **153** obtained during the course of the work described in Chapter Two. The alcohol (*R*)-**166** should be accessible through a double kinetic enzymatic resolution of the corresponding racemate ($\pm$)-**166** using protocols analogous to those described in Chapter Two for the preparation of compound (*R*)-**25**.



Scheme 3.5: Retrosynthetic analysis of **L-783,277 (1)**.

3.4 Synthesis of Enantiopure Propargylic Alcohol [(*R*)-166] via the Lipase-catalysed Resolution of Racemate ($\pm$)-166

With the required benzoic acid **153** available through the protocols described in Chapter Two, the early stages of efforts associated with implementing the plan defined in Scheme 3.5 focused on preparing the chiral alcohol (*R*)-**166**. Like its congener (*R*)-(-)-4-penten-2-ol [(*R*)-**25**], compound (*R*)-**166** could be generated by either chemical or enzymatic means. A previous chemical synthesis of this fragment [(*R*)-**166**] involved the nucleophilic epoxide ring-opening of (*R*)-propylene oxide [(*R*)-**27**] with lithium acetylide,^{1,7} while a second approach involved an enzymatic kinetic resolution of the racemate ($\pm$)-**166**. The first reported enzymatic resolution of alcohol (*R*)-**166** was undertaken by Takano *et al.*, who achieved a 91.2% ee by using a double kinetic resolution protocol (with the lipase Amano PS) similar to that described in Chapter Two.⁸ It was anticipated that the ee associated with the enzymatic resolution of alcohol ($\pm$)-**166** to give the *R*-enantiomer could be improved by drawing directly upon the protocols described in Chapter 2. Accordingly, racemic 4-pentyn-2-ol [($\pm$)-**166**] was exposed to CALB using vinyl acetate as both solvent and acyl donor (Scheme 3.6) to afford a chromatographically separable mixture of unreacted *S*-configured alcohol (*S*)-**166** (not isolated) and the corresponding acetate now enriched with the *R*-enantiomer. By such means, the acetate (*R*)-**167** was obtained in 40% yield and 89% ee (as determined by chiral GC analysis).



Reagents and conditions: (i) CALB, vinyl acetate, 30 °C, 1 h; (ii) CALB, phosphate buffer (pH = 7.3), 30 °C, 10 h.

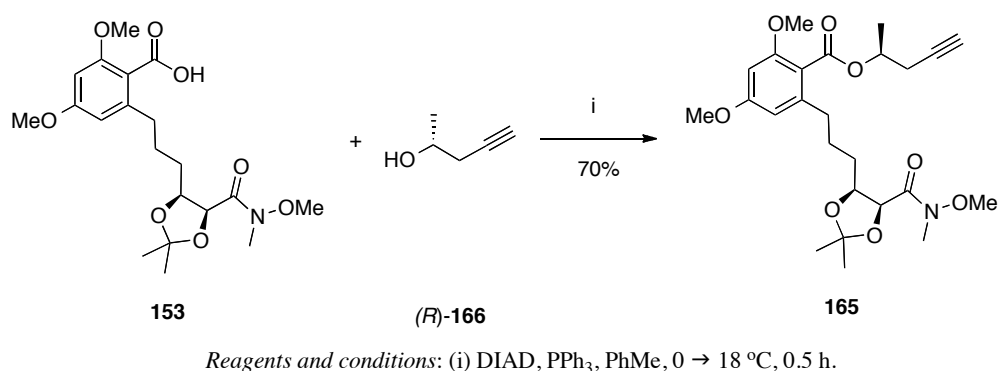
Scheme 3.6: Synthesis of (*R*)-(-)-4-pentyn-2-ol [(*R*)-**166**].

The second stage of the resolution process involved the hydrolysis of the acetate (*R*)-**167** with CALB in phosphate buffer (Scheme 3.6) to afford the target alcohol (*R*)-**166** (60% yield) with an ee of 98.9% (as determined by chiral GC of the acetate derivative). The optical rotation recorded for this sample of (*R*)-**166** was found to be levorotary $\{[\alpha]_{\text{D}} = -16.3$ (*c* 0.46, CHCl₃) $\}$ which matched that reported for the compound prepared by Dimitriadis *et al.* $\{[\alpha]_{\text{D}} = -17.7$ (*c* 0.13, CHCl₃) $\}$.⁹

The successful resolution of (*R*)-(-)-4-penten-2-ol [(*R*)-**25**] (Section 2.5) and its dehydro-analogue (*R*)-(-)-4-pentyn-2-ol [(*R*)-**166**] as described here highlights the utility of the CALB-based method for resolving racemic mixtures of various secondary alcohols.

3.5 Synthesis of Substrate for the Formation of Macrocycle **164**

With the homopropargyl alcohol (*R*)-**166** successfully obtained in high ee, it was coupled to acid **153** under the same (Mitsunobu) conditions used in preparing ester **154** (Scheme 2.9). By such means ester **165** was obtained in 70% yield (Scheme 3.7).



Scheme 3.7: Synthesis of substrate **165** for the formation of macrocycle **164**.

The ¹³C NMR spectrum of compound **165** (Figure 3.2) displayed a resonance at δ 167.5 which is assigned to the newly introduced ester functionality. The ¹H NMR spectrum obtained on compound **165** was in full agreement with the assigned structure. The ESI mass spectrum revealed two molecular associated ions at *m/z* 478 and 500 that corresponded to the protonated and sodiated derivatives of compound **165**, respectively. Accurate mass measurements on these species established that they were of the expected composition, viz C₂₅H₃₆O₈ and C₂₅H₃₅NaO₈, respectively.

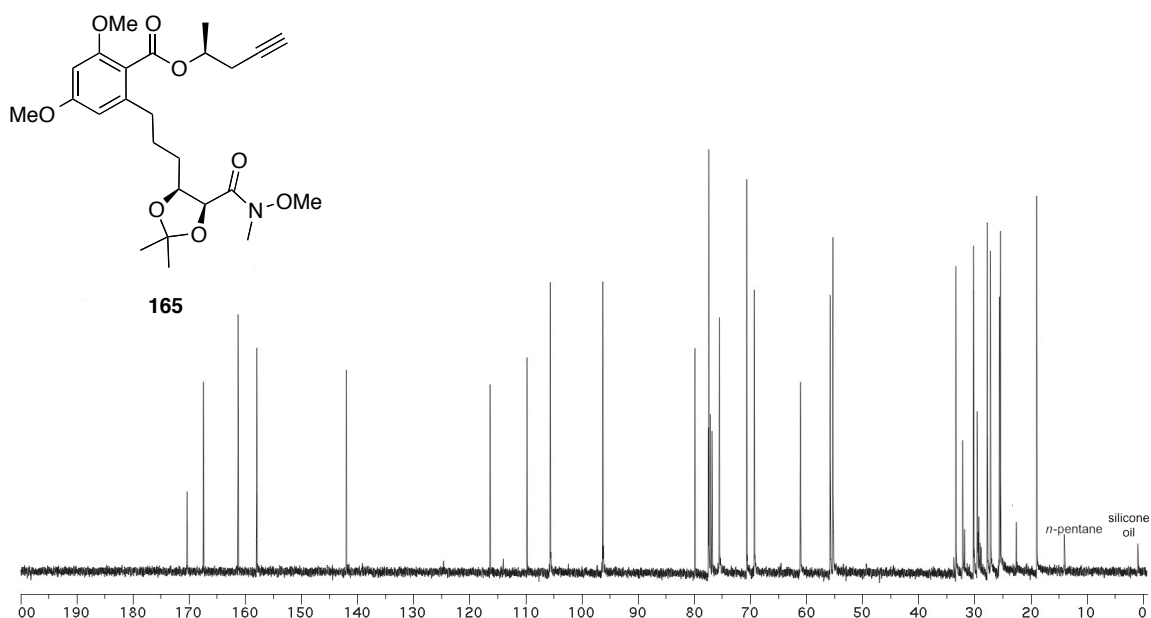
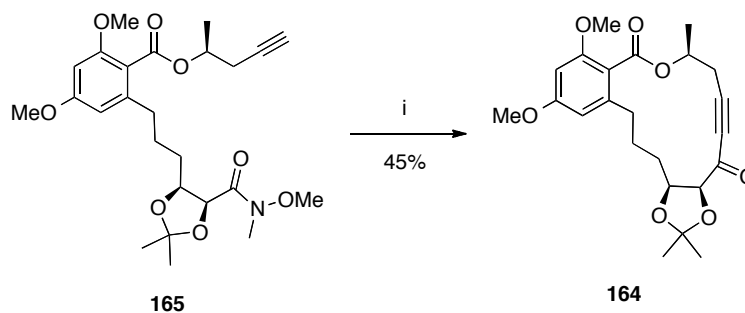


Figure 3.2: 75 MHz ^{13}C NMR spectrum of compound **165** (recorded in CDCl_3).

3.6 Macrocyclisation of Compound **165**

With the macrocyclisation substrate **165** in hand, the stage was set for the key intramolecular acetylide anion addition onto the tethered Weinreb amide and thereby generating the macrolactone ring incorporating a propargylic ketone. Success was achieved when a pre-cooled ($-35\text{ }^\circ\text{C}$) and dilute solution of excess lithium hexamethyldisilazide (LiHMDS) was treated, dropwise, with a dilute solution of the terminal alkyne **165** (Scheme 3.8). By such means, the crystalline propargylic ketone **164** was obtained in 45% yield.



Reagents and conditions: (i) LiHMDS, THF, $-35 \rightarrow 18\text{ }^\circ\text{C}$, 0.1 h.

Scheme 3.8: Macrocyclisation of substrate **165** using an intramolecular acetylide anion addition.

The ^1H NMR spectrum of compound **164** lacked any resonances due to a terminal alkyne proton (as seen at δ 2.04 in precursor **165**) or the $\text{N}(\text{Me})\text{OMe}$ residue associated with the Weinreb amide. The ^{13}C NMR of this material (Figure 3.3) displayed the expected 23 carbon resonances, with the most downfield signal at δ 184.7 being assigned to the ynone-carbonyl carbon. The ESI

mass spectrum of product **164** displayed the protonated and sodiated associated molecular ions at m/z 417 and 439, respectively, and accurate mass measurements on these established that they were of the expected composition, *viz* $C_{23}H_{29}O_7$ and $C_{23}H_{28}NaO_7$. The IR spectrum of compound **164** displayed a weak $C\equiv C$ stretching band at 2215 cm^{-1} . While all of the data were completely consistent with the assigned structure final confirmation of this followed from a single-crystal X-ray analysis. The derived ORTEP is shown in Figure 3.4 while further details are presented in the Experimental Section.

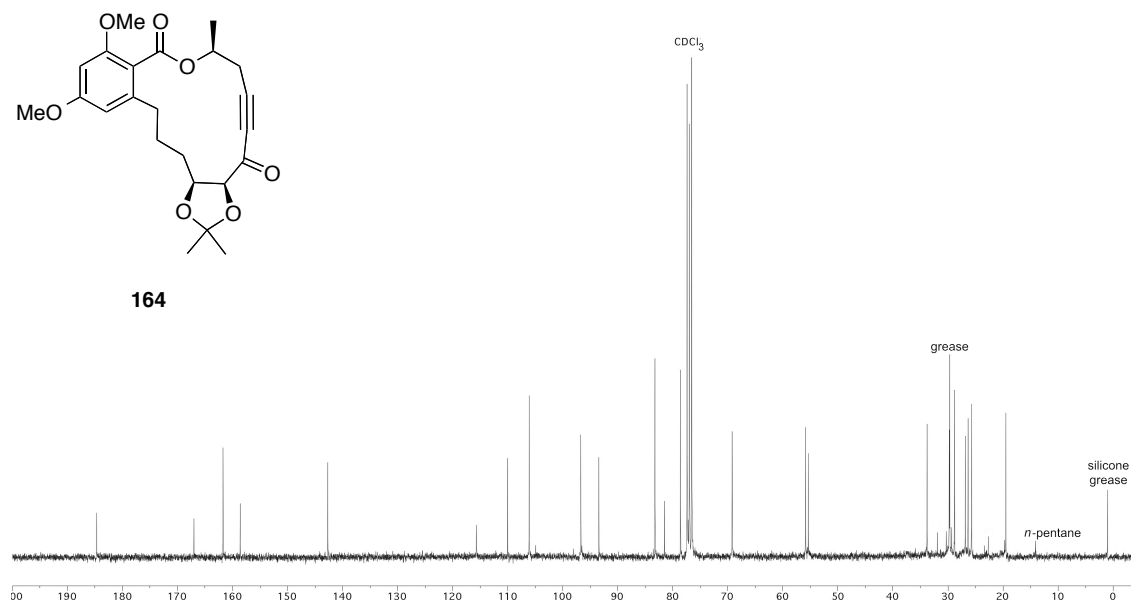


Figure 3.3: 75 MHz ^{13}C NMR spectrum of macrolide **164** (recorded in $CDCl_3$).

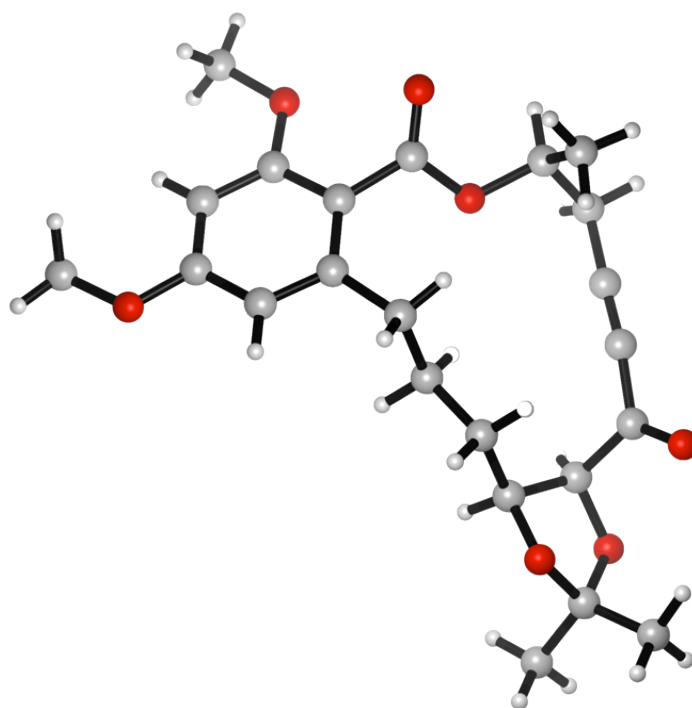
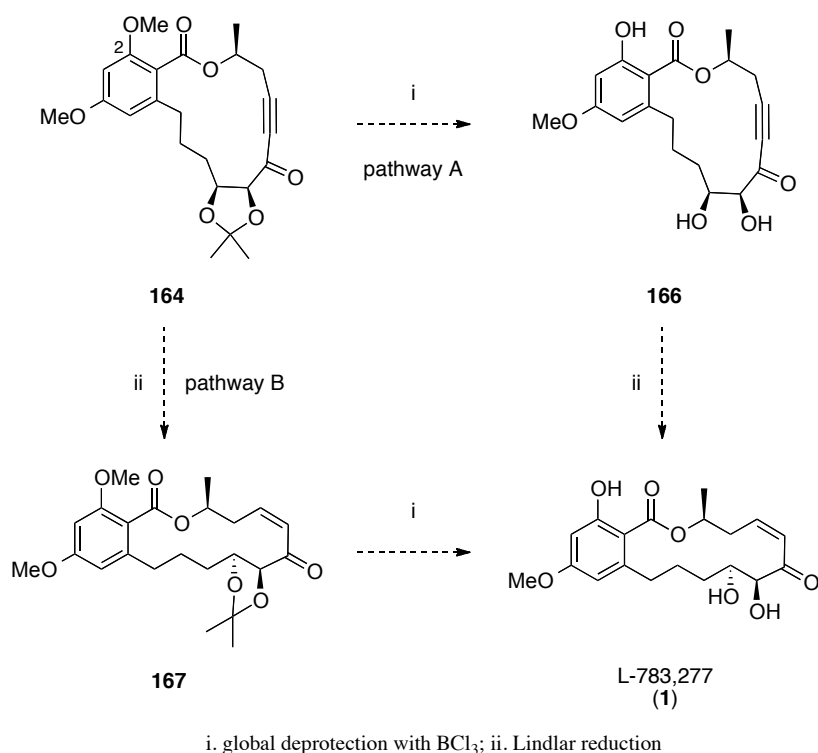


Figure 3.4: Ball and stick model of macrolactone **164** generated from the data derived from a single-crystal X-ray analysis.

3.7 Completion of the Synthesis of L-783,277 (**1**)

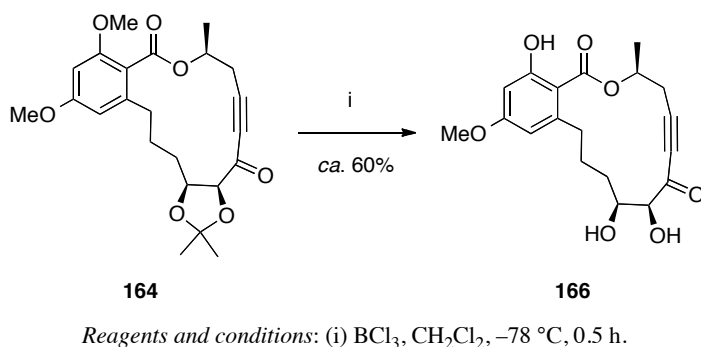
With the macrocycle **164** in hand, completion of a synthesis of L-783,277 (**1**) could be pursued. The conversion of compound **164** into target **1** necessarily involves the stereoselective (Lindlar) reduction of the alkyne **164** (Scheme 3.9) to the *Z*-configured enone, cleavage of the C2 methyl ether and accompanying hydrolysis of the acetonide group. Clearly there are two distinct ways in which these transformations could be carried out. The first involves the (Lindlar) reduction of macrocycle **164** followed by a BCl₃ deprotection step (and hydrolysis of the acetonide residue by the HCl generated on quenching the reaction mixture with water). The alternate sequence would involve carrying out the ether and acetonide cleavage processes prior to the Lindlar reduction. Both approaches were examined and the outcomes of these are detailed below.



Scheme 3.9: The two possible end game sequences for completing the synthesis of L-783,277 (1).

3.7.1 First Approach to the Completion of the Synthesis of L-783,277 (1)

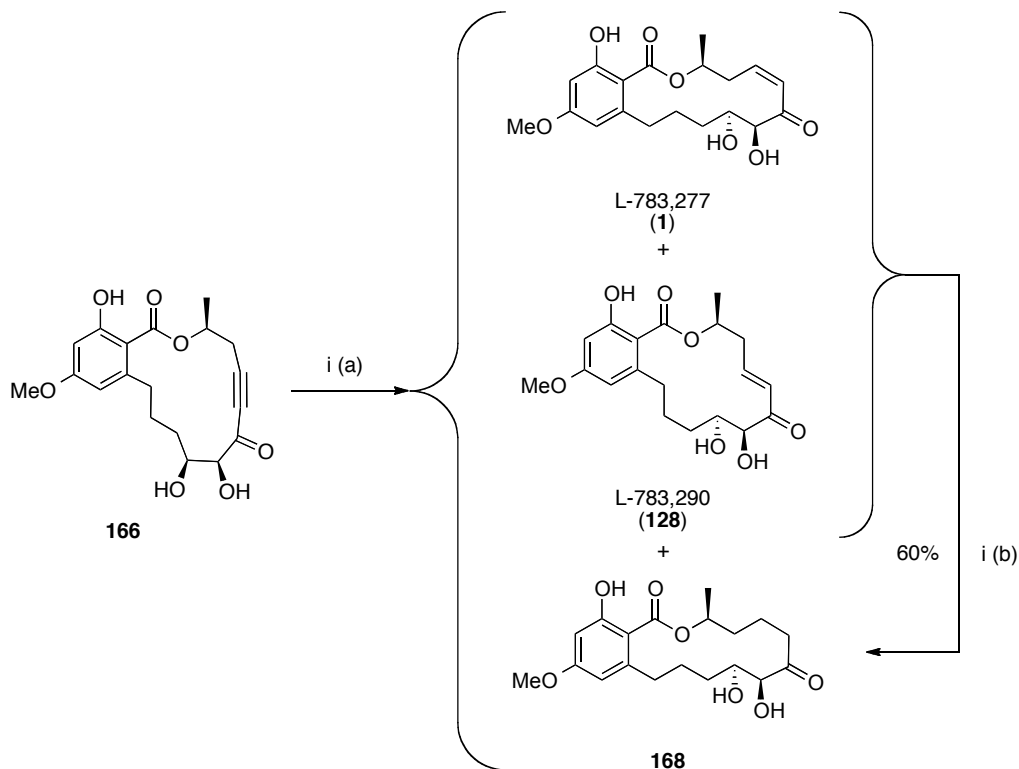
In the first study undertaken to complete the synthesis of the target L-783,277 (1), macrocycle **164** was treated with BCl_3 (Scheme 3.10) and thus affording compound **166** which proved to be unstable to flash chromatography.



Scheme 3.10: Deprotection of macrolactone **164** with BCl_3 .

As a consequence, it was immediately subjected to the Lindlar reduction using dihydrogen in the presence of catalytic quantities of palladium on calcium carbonate poisoned by lead and pyridine (Scheme 3.11). A mixture of products was thus obtained. The ESI mass spectrum of the crude reaction mixture displayed two distinct molecular associated ions m/z 387 and 389. The first was attributed to the sodiated derivative of the expected enone (E/Z ratio not

determined) while the second is presumed to be due to the sodiated derivative of the “over-reduced” macrocycle **168**.



Reagents and conditions: (i) H_2 (1 atm), pyridine, 5% Pd on CaCO_3 poisoned with Pb, PhMe, 18 °C, (a) 2 h; (b) 13 h.

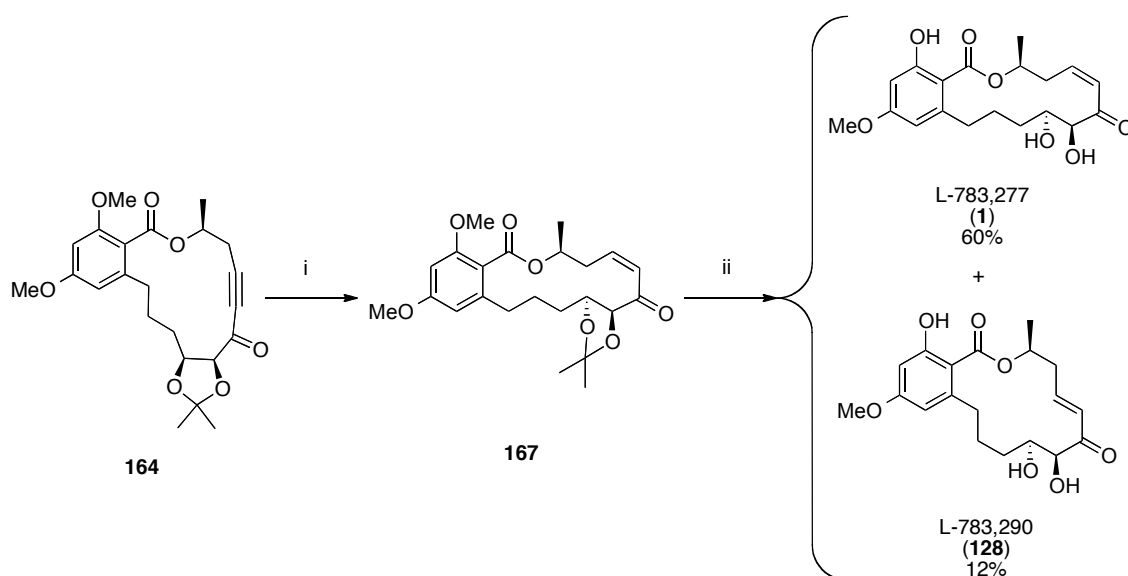
Scheme 3.11: Lindlar reduction of compound **166**.

Since these products could not be separated from one another by conventional chromatography, the reaction mixture was subjected to reverse phase HPLC. Two of the three compounds had very similar retention times ($t_R = 38.0$ and 38.5 min) with the less mobile of the two being identified as the over-reduced macrocycle **168** and the more mobile being L-783,277 (**1**). The remaining compound was the *trans*-isomer **128**, the identity of which was confirmed by comparison with the authentic sample prepared by the route described in Chapter Two.

The confirmation of the structure of macrolide **168** followed from its comparison with an authentic sample which was produced (in 60% yield) using the protocol shown in Scheme 3.11, involving a prolonged Lindlar reduction (13 hours). The ^1H NMR spectrum of this material lacked any olefinic resonances due to the protons associated with an enone moiety. When a sample of compound **168** was subjected to HPLC analysis, its retention time matched that of the less mobile product (38.5 min) derived from the initial reduction mixture [Scheme 3.11 – i(a)], and so confirming its identity as the over-reduced macrocycle. Despite exhaustive investigations, it proved impossible to prevent the over-reduction of target **1** to its dihydro-congener **168**.

3.7.2 Second and Successful Approach to L-783,277 (1)

Given the inability to access a pure sample of L-783,277 (**1**) by the means outlined above, pathway B shown in Scheme 3.9 was pursued in an effort to achieve such ends. Accordingly, compound **164** was subjected to the Lindlar reduction with dihydrogen in the presence of catalytic quantities of palladium on calcium carbonate poisoned by lead and pyridine (Scheme 3.12) to furnish the *cis*-configured enone **167**.



Reagents and conditions: (i) H_2 (1 atm), pyridine, 5% Pd on CaCO_3 poisoned with Pb, PhMe, 18 °C, 2 h; (ii) BCl_3 , CH_2Cl_2 , -78 °C, 0.5 h.

Scheme 3.12: Completion of a synthesis of L-783,277 (**1**) from ynone (**164**).

Without purification, the crude product obtained from this reaction was treated carefully with BCl_3 then subjected to an aqueous work-up. By such means, a 5:1 mixture of compounds **1** and **128** was obtained. The mixture was subjected to semi-preparative reverse phase HPLC and thus affording compounds **1** and **128** in pure form and 60% and 12% yield, respectively.

The ^1H NMR spectral data obtained on synthetic L-783,277 (**1**) (Figure 3.5) were in full accord with those reported for the natural product by the researchers at Merck.¹⁰ Furthermore, the resonances for each carbon of the synthetic sample observed in the corresponding ^{13}C NMR spectrum (Figure 3.6) compared favourably with those reported for the natural product, with the exception of the carbon bearing the phenolic group. This discrepancy can be attributed to the differences in concentration, solvent and pH. The ESI mass spectrum of compound **1** revealed a molecular associated ion at m/z 387 which is attributed to the sodiated form of the target compound. An accurate mass measurement on this species established it was of the expected composition, viz $\text{C}_{13}\text{H}_{24}\text{NaO}_7$.

Such comparison were complicated by the differing solvents used in recording the NMR spectrum of the various samples of L-783,277 (**1**) (Table 3.1).

	^1H NMR spectral solvent	^{13}C NMR spectral solvent
Natural product ¹⁰	CD_2Cl_2	CD_2Cl_2
Altmann group ¹	d_6 DMSO	d_6 DMSO
Winssinger group ²	CDCl_3	Not Reported
Sim group ³	CD_2Cl_2 and CDCl_3	CDCl_3
Banwell group ⁴	CD_2Cl_2	CD_2Cl_2

Table 3.1: The different NMR solvents used to analyse L-783,277 (**1**).

Comparison of the ^{13}C NMR Spectral Data

A comparison of the relevant sets of ^{13}C NMR spectra data is shown in Figure 3.7 (Author's synthesis in blue and Sim's synthesis in red). At first glance, the carbon resonances associated with Sim's synthetic sample consistently deviates from the baseline by *ca.* 0.3 ppm. This is attributed to the sample being analysed in CDCl_3 as rather than CD_2Cl_2 .

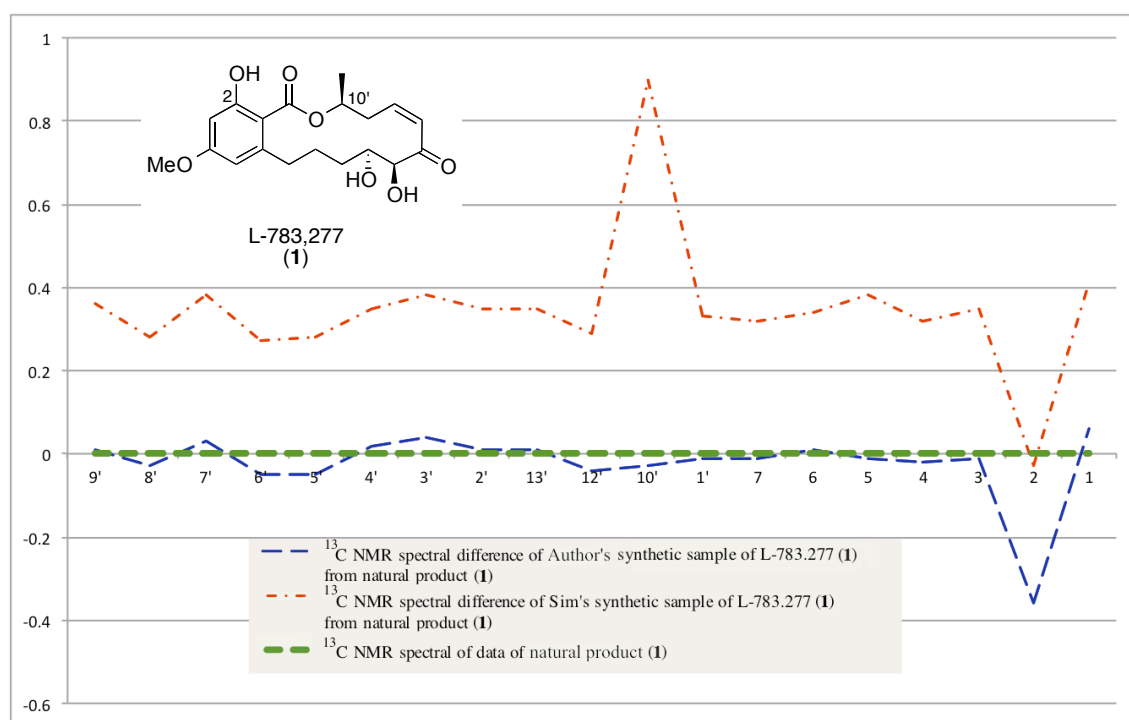


Figure 3.7: Comparison of the ^{13}C NMR spectral data derived from various samples of compound **1**.

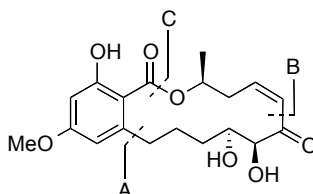
Nevertheless, even making allowance for this, there are “worrying” carbon chemical shift differences associated with Sim's sample of RAL **1**. In particular, the chemical shift attributed to the C10' (adjacent to the macrolactone functionality bearing the methyl group), differs

significantly which suggests that Sim's assignment of the *S*-stereochemistry at C10' may be incorrect. An inversion in the C10' stereochemistry would also explain the difference in the observed optical rotation between Sim's product $[+8.8 (c\ 0.5, \text{CHCl}_3)]^3$ and the Author's sample of L-783,277 (**1**) $[-15.3 (c\ 0.5, \text{CHCl}_3)]$ (no other optical rotation data have been reported for **1**). Efforts to resolve this discrepancy through email correspondence with Sim *et al.* have been unsuccessful.

The greater consistency observed between the spectral data recorded on the Author's sample of L-783,277 (**1**) and the natural product suggests they are one and the same. Therefore, the specific rotation for L-783,277 (**1**) reported here, is more likely to represent the specific rotation that would be recorded for the naturally isolated material.

3.8 Conclusion

In the work detailed above the versatile building block *c*DHC **82** ($X = \text{Cl}$) derived from microbial oxidation of chlorobenzene was converted into the natural product L-783,277 (**1**) over 16 steps. This synthetic sequence is shorter than those reported by Altmann *et al.* (17 steps),¹ Winssinger *et al.* (21 steps)² and Sim *et al.* (18 steps).³ A comparison of the assembly of the fragments involved in all the reported syntheses of L-783,277 (**1**) is described in Table 3.2.



Lead Author	Date Reported	Number of Steps	Overall Yield	Sequence of key bond forming events
Altmann ¹	2008	17	9.1%	B → A → C
Winssinger ²	2009	21	2.7%	B → A → C
Sim ³	2010	18	0.1%	A → B → C
Present Work ⁴	2010	16	2.1%	A → C → B

Table 3.2: Comparison of the four reported syntheses of L-783,277 (**1**).

The present synthesis exploits the many features that were used to prepare L-783,290 (**128**), namely the use of the benzoic acid synthon **153**, constructed from a Heck reaction between the aromatic **133** and diol **127** units, and the use of a double kinetic enzymatic resolution to generate substantial quantities of enantiomerically pure (*R*)-(-)-4-pentyn-2-ol [(*R*)-**166**].

In the present synthesis, a novel mode of macrocyclisation, *viz* one involving an intramolecular nucleophilic addition of an acetylide anion onto a tethered Weinreb amide, was used to access macrolactone **164** incorporating a propargylic ketone. The application of this type of cyclisation, followed by a Lindlar reduction, should provide a useful means for obtaining other *Z*-configured α,β -unsaturated ketone-containing RALs. A discussion of other possible applications of this approach is presented in Chapter Five.

3.9 References

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Towards The Total Synthesis of Aigialomycin C

4.1 Introduction

4.1.1 Overview and Isolation

The aigialomycins (A to G) are a new family of RALs that were isolated, together with hypothemycin (**2**) (Figure 4.1), from the extracts of a marine mangrove fungus *Aigialus parvus* BCC531 by Isaka *et al.* using activity-guided fractionation techniques.^{1,2}

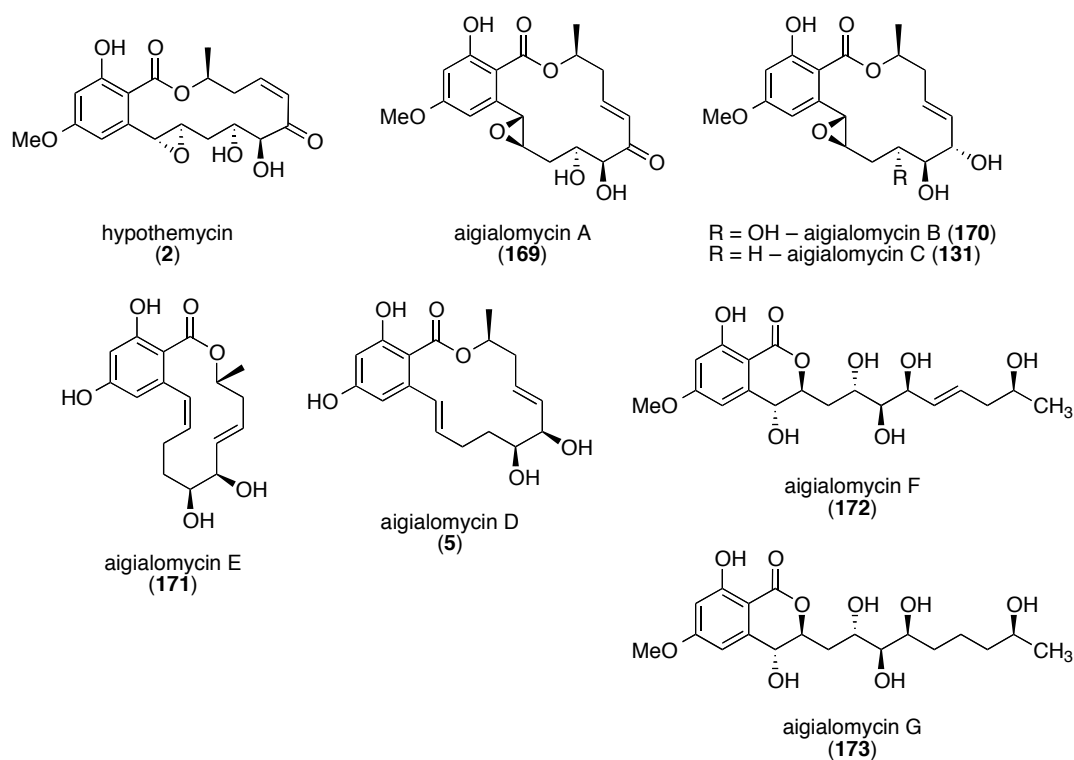


Figure 4.1: Structures of *aigialomycins A to G* and *hypothemycin (2)*, isolated from the mangrove ascomycete, *Aigialus parvus* BCC531.^{1,2}

The structures assigned to aigialomycins A to G were determined using a combination of chemical correlation, X-ray crystallographic and spectroscopic (NMR, MS, IR and UV) techniques.^{1,2}

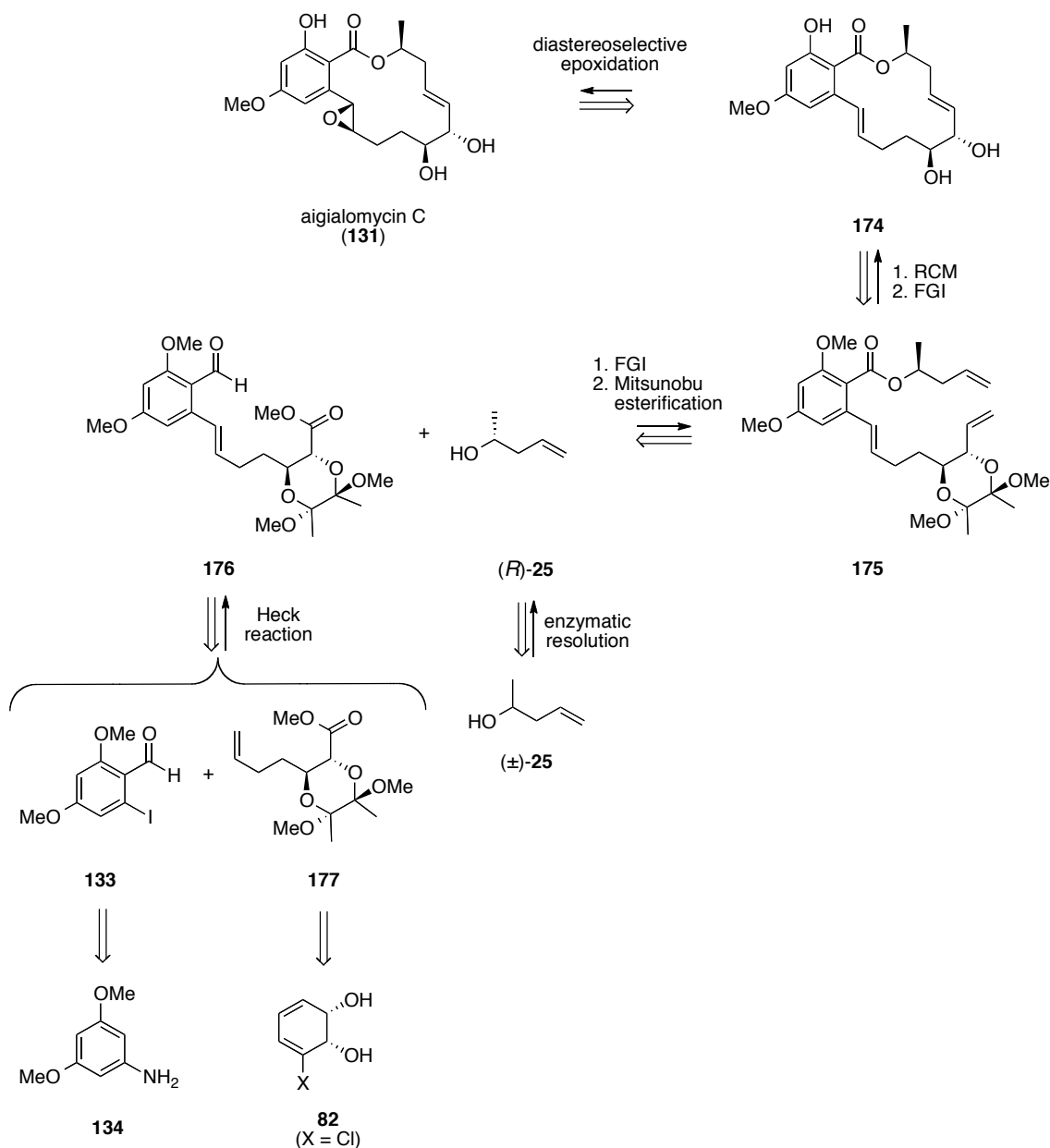
4.1.2 Biological Activity

Initial screening of aigialomycins A to E for biological activity by Isaka *et al.* revealed that only aigialomycin D (**5**) exhibited anti-malarial activity ($IC_{50} = 6.6 \mu\text{g/mL}$).¹ Further testing of this RAL against a kinase panel by Winssinger *et al.* revealed that it was a potent inhibitor of CDKs 1 and 5 ($IC_{50} = 5.7$ and $5.8 \mu\text{M}$, respectively).³ Unfortunately, no other aigialomycin analogues were screened by Winssinger *et al.* and, thus, the kinase inhibitory activities of the remaining members of the class have yet to be determined.

4.2 Retrosynthetic Analysis of Aigialomycin C (**131**)

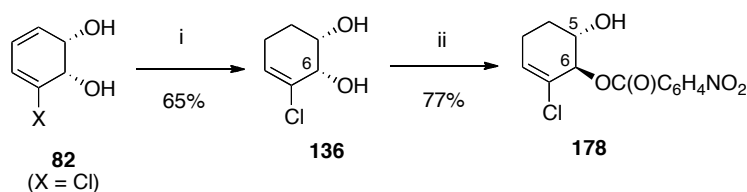
Despite showing no biological activity in the initial screening,¹ it was anticipated that any progress made towards the synthesis of aigialomycin C (**131**) could provide access to the other members of the aigialomycin family, as well as analogues, for the purpose of establishing their kinase inhibitory activities.

Given the successful modular synthesis of L-783,290 (**128**) as described in Chapter Two, it was envisaged that natural product **131** could be prepared using a similar approach. Thus, it was anticipated this could be achieved (Scheme 4.1) through regio- and diastereo-selective epoxidation of macrolactone **174**, a compound that can be obtained from the RCM reaction of substrate **175**. Triene **175** would itself be assembled from three fragments in a similar sequence to that presented in Chapter Two these being, in order of execution, a Heck coupling between the aryl halide **133** and olefin **177** and a Mitsunobu esterification to couple the enantiopure alcohol (*R*)-**25** to the acid derived from an oxidation reaction of aldehyde **176**. While the aromatic fragment **133** and homoallylic alcohol (*R*)-**25** are readily available as a result of the work described in Chapter Two, olefin **177** (a known compound) would be prepared in the same manner as reported by Banwell *et al.* in their synthesis of *ent*-cladospolide C from the enantiopure *c*DHC **82** ($X = \text{Cl}$).^{4,5,6}

Scheme 4.1: Retrosynthetic analysis of aigialomycin C (**131**).

4.3 Synthesis of the (Protected) Diol Fragment **177**

With the aromatic **133** and alcohol (*R*)-**25** fragments readily available, the remaining unit required for the proposed synthesis of RAL **131** was the protected diol **177**. Following the sequence reported by Banwell *et al.*,^{4,5,6} the electron-rich double bond within the chlorobenzene-derived *c*DHC **82** (X = Cl) was selectively reduced (Scheme 4.2) using the same conditions described earlier to give cyclohexene **136** in 65% yield. All the spectral data derived from this material were consistent with those reported previously.^{4,6}

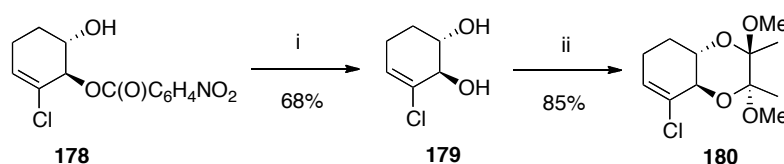


Reagents and conditions: (i) 5% Rh on Al₂O₃, H₂, EtOH, 18 °C, 0.75 h; (ii) *p*-nitrobenzoic acid, DIAD, PPh₃, PhMe, 0 → 18 °C, 12 h.

Scheme 4.2: Synthesis of nitrobenzoate ester **178** from cDHC **82** ($X = \text{Cl}$).^{4,6}

The C6 hydroxyl group within diol **136** selectively participated in a Mitsunobu esterification with *p*-nitrobenzoic acid to furnish the *R*-configured C6 *p*-nitrobenzoate **178** (77% yield). The ¹H NMR spectrum obtained on this material was in complete accord with the assigned structure and the EI mass spectrum of compound **178** displayed molecular ions at *m/z* 297 and 295 in the ratio (3:1) expected for a mono chlorinated material. Accurate mass measurements of this species established that they were of the expected molecular composition, *viz* C₁₃H₁₂³⁷ClNO₅ and C₁₃H₁₂³⁵ClNO₅.

Hydrolysis of the *p*-nitrobenzoate ester **178** with potassium carbonate (Scheme 4.3) provided the *trans*-configured diol **179** in 68% yield. The lack of any aromatic resonances in the ¹H NMR spectrum of this compound together with the observation of molecular ions at *m/z* 150 and 148 in the EI mass spectrum suggested the *trans*-configured diol **179** had been obtained.



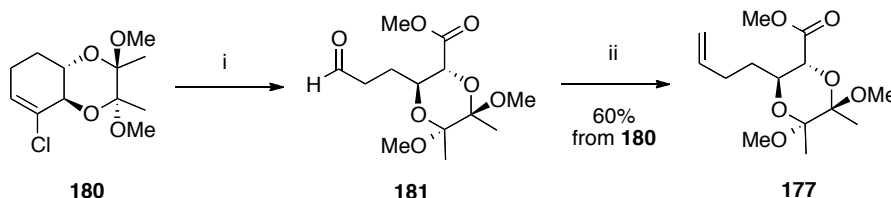
Reagents and conditions: (i) K₂CO₃, MeOH, 18 °C, 24 h. (ii) [CH₃C(OMe)₂]₂, CH(OMe)₃, MeOH, CSA, 80 °C, 20 h.

Scheme 4.3: Synthesis of cyclohexene **180** from benzoate **178**.

Diol **179** was protected as the corresponding *bis*-acetal **180** using the Ley/Frost protocol,^{5,7,8} which was chosen for its capacity to protect *trans*-related vicinal diols and the greater tolerance of the resulting protecting group to acidic conditions.

With the required cyclohexene, **180**, in hand ozonolytic cleavage of the associated alkene residue was undertaken by employing the protocols reported earlier (Scheme 4.4). Thus, a methanolic solution of cyclohexene **180** maintained at −78 °C was subjected to a stream of ozone. Reductive workup with dimethyl sulfide afforded the unstable aldehyde **181** which was immediately subjected to a Wittig olefination reaction to furnish alkene **177**. This was obtained in 60% yield from cyclohexene **180**. The ¹H NMR spectrum of compound **177** (Figure 4.2) displayed a doublet of doublet of triplets at δ 5.58 and a complex multiplet at δ 4.99 as would be

expected for the compound embodying a terminal olefinic residue. A three-proton singlet, due to the three protons on the methyl ester group, appeared at δ 3.76. The EI mass spectrum revealed a fragment ion at m/z 257, which is associated with the loss of a methoxy radical from the molecular ion. All of the remaining spectral data acquired on this material were consistent with the assigned structure and matched those reported previously.^{4,5}



Reagents and conditions: (i) (a) O_3 , pyridine, MeOH, $-78^\circ C$, 0.2 h; (b) Me_2S , $0^\circ C$, 2 h. (ii) CH_3PPh_3Br , KHMDS, THF, $0 \rightarrow 18^\circ C$, 1 h.

Scheme 4.4: Synthesis of protected diol fragment **177**.

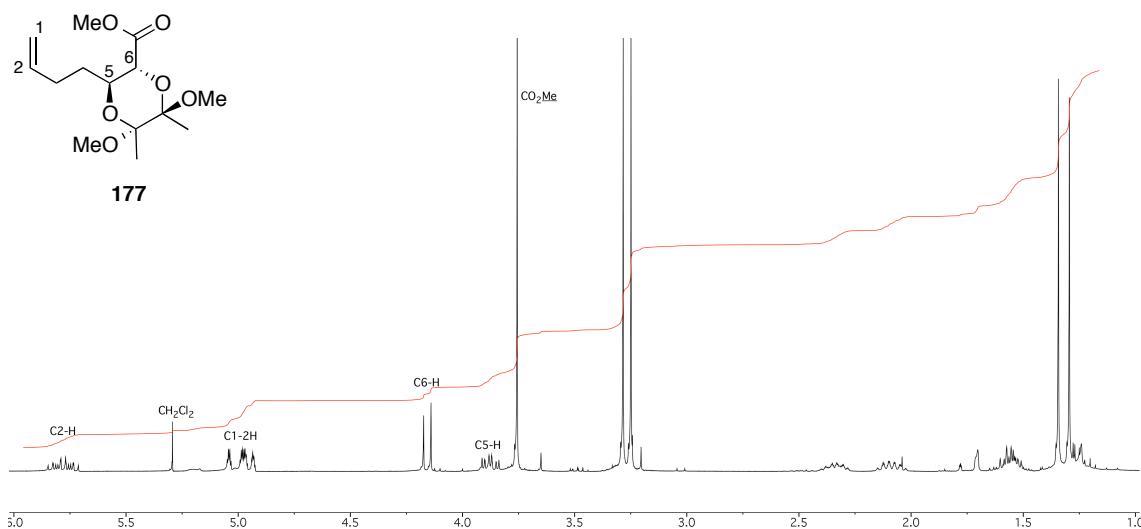


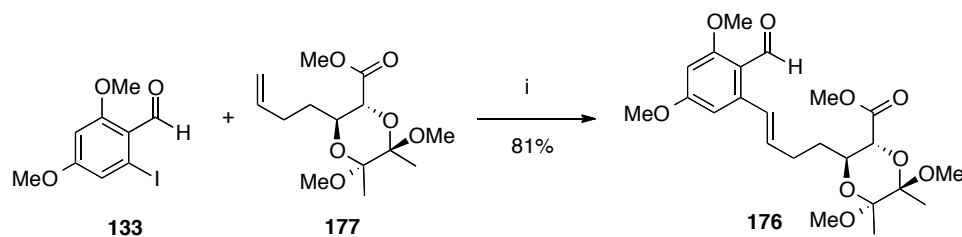
Figure 4.2: 300 MHz 1H NMR spectrum of the diol fragment **177** (recorded in $CDCl_3$).

4.4 Assembly of the RCM Precursor **175**

4.4.1 Heck Cross-coupling of Aryl Iodide **133** and Alkene **177**

With all three of the required fragments to hand, the stage was set for the assembly of the target macrolactone by coupling of them. The first such coupling was that between the aryl iodide **133** and alkene **177** by way of a Heck reaction (Scheme 4.5). This was accomplished using the optimised conditions described earlier (Chapter Two) to give the *E*-configured alkene **176** in 81% yield. The 1H NMR spectrum (Figure 4.3) of this material showed two mutually-coupled resonances at δ 7.36 and 5.11 and the magnitude of this ($J = 15.6$ Hz) suggested that these protons were *trans*-related and, thus, the compound embodied an *E*-configured C1'-C2' olefin.

A molecular ion observed at m/z 475 in the ESI mass spectrum corresponded to the sodiated derivative of alkene **176** and an accurate mass measurement on this species established it was of the expected composition, viz $C_{23}H_{32}NaO_9$.



Reagents and conditions: (i) $Pd(OAc)_2$, TBAB, K_2CO_3 , 10:1 v/v DMF/ H_2O , 80 °C, 14 h.

Scheme 4.5: Heck reaction between the aryl iodide **133** and alkene **177**.

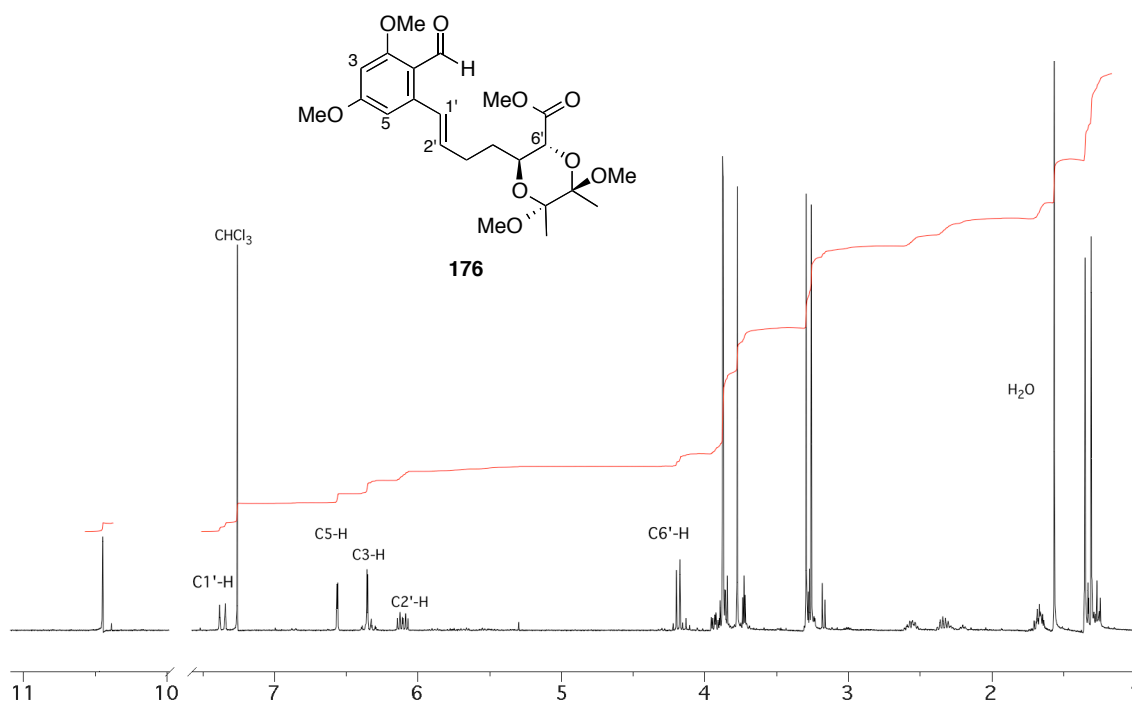
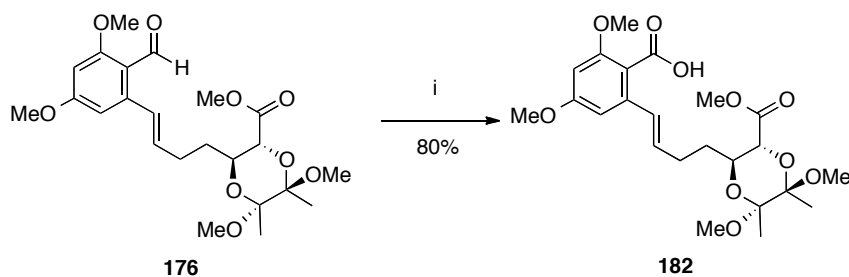


Figure 4.3: 400 MHz 1H NMR spectrum of compound **176** derived from the Heck reaction between aromatic and diol fragments **133** and **177**, respectively (recorded in $CDCl_3$).

The acid **182**, required for the coupling to the secondary alcohol (R)-**25**, was obtained in 80% yield via a Pinnick oxidation (Scheme 4.6) of the aldehyde **176**.⁹ The absence of a signal due to an aldehyde proton resonance in the 1H NMR spectrum of compound **182** and the observation of a molecular ion m/z 467 in the negative ESI mass spectrum (corresponding to the conjugate base of the acid **182**) confirmed that the desired oxidation reaction had taken place.

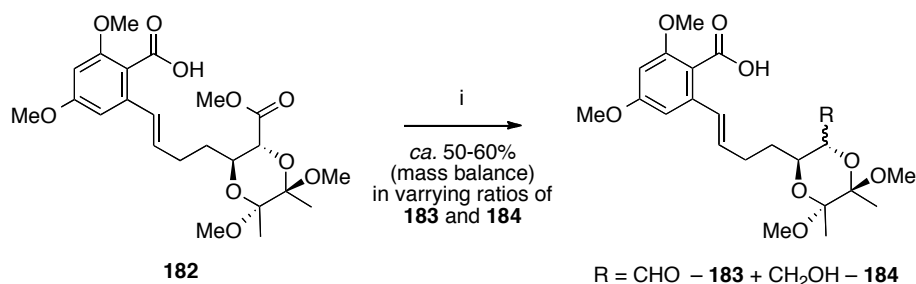


Reagents and conditions: (i) 2-methyl-2-butene, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, NaClO_2 , *t*-BuOH, H_2O , 18 °C, 1 h.

Scheme 4.6: Oxidation of benzaldehyde **176** to benzoic acid **182**.

4.4.2 First Approach To RCM Precursor **175**

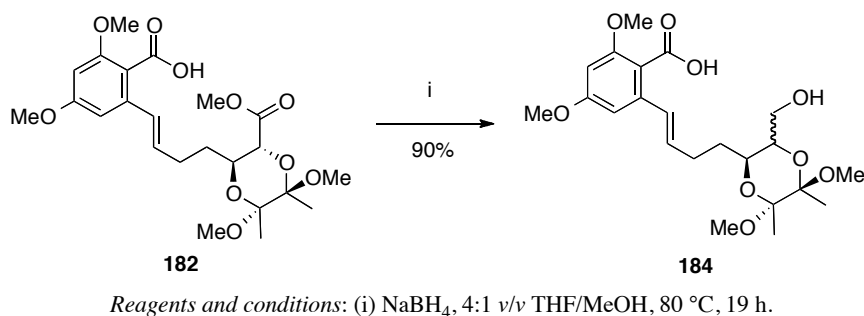
With acid **182** to hand, attention could now be focused on its elaboration to triene **175**, the substrate required for the pivotal RCM reaction. It was decided that manipulation of the methyl ester residue within compound **182** should occur before the Mitsunobu esterification with alcohol (*R*)-**25**, due to the difficulties that would be created in distinguishing between the newly installed homo-allylic and methyl ester residues. To such ends, methyl ester **182** (Scheme 4.7) was subjected to the reduction conditions reported by Ley *et al.* involving the use of DIBAL-H.^{10,11} This afforded an inseparable mixture of aldehyde **183** and primary alcohol **184** in every instance, so it became clear that extensive optimisation of the required partial reduction process was needed.



Reagents and conditions: (i) DIBAL-H, CH_2Cl_2 , -78 °C, 0.04 h.

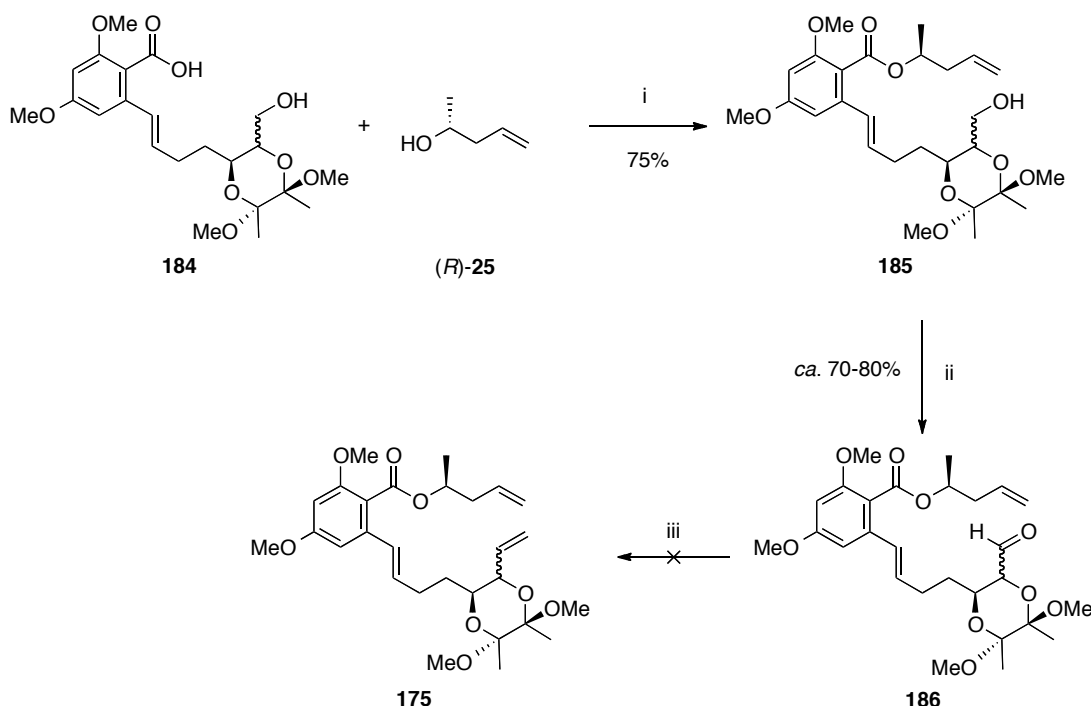
Scheme 4.7: Outcome of the reduction of methyl ester **182**.

Given this situation it was thought that a simpler, albeit slightly longer, route to aldehyde **183** would involve a reduction of the methyl ester **182** to the alcohol **184** followed by an oxidation of the latter to the aldehyde. Accordingly, methyl ester **182** was subjected to a reaction with sodium borohydride (Scheme 4.8). This gave the primary alcohol **184** as a mixture of epimers (*trans*:*cis* – 1:0.06) in 90% combined yield. The lack of a three-proton singlet associated with the precursor methyl ester **182** in the ¹H NMR spectrum of the reduction product and the presence of a molecular ion *m/z* 467 in the negative ESI mass spectrum (corresponding to the conjugate base of **184**) confirmed that the desired reduction had taken place.



Scheme 4.8: NaBH₄ mediated reduction of methyl ester **182** to alcohol **184**.

Unfortunately, treatment of alcohol **184** with a variety of oxidants failed to generate target **183**. Only decomposition of the starting material was observed under all the conditions attempted. It was presumed that the benzoic acid residue within compound **184** was responsible for these failures. Accordingly, this was esterified with the homoallylic alcohol (*R*)-**25** under Mitsunobu conditions (Scheme 4.9) to afford ester **185** in 75% yield. All spectral data derived from this material were in accord with the assigned structure.



Reagents and conditions: (i) DIAD, PPh₃, PhMe, 0 → 18 °C, 0.5 h. (ii) (a) DMP, CH₂Cl₂, 2 h, 18 °C; or (b) TPAP, NMO, 4 Å MS, CH₂Cl₂, 0.5 h, 18 °C; (iii) CH₃PPh₃Br, *n*-BuLi, ether, −78 °C.

Scheme 4.9: Alternate synthesis of triene **175** from primary alcohol **184**.

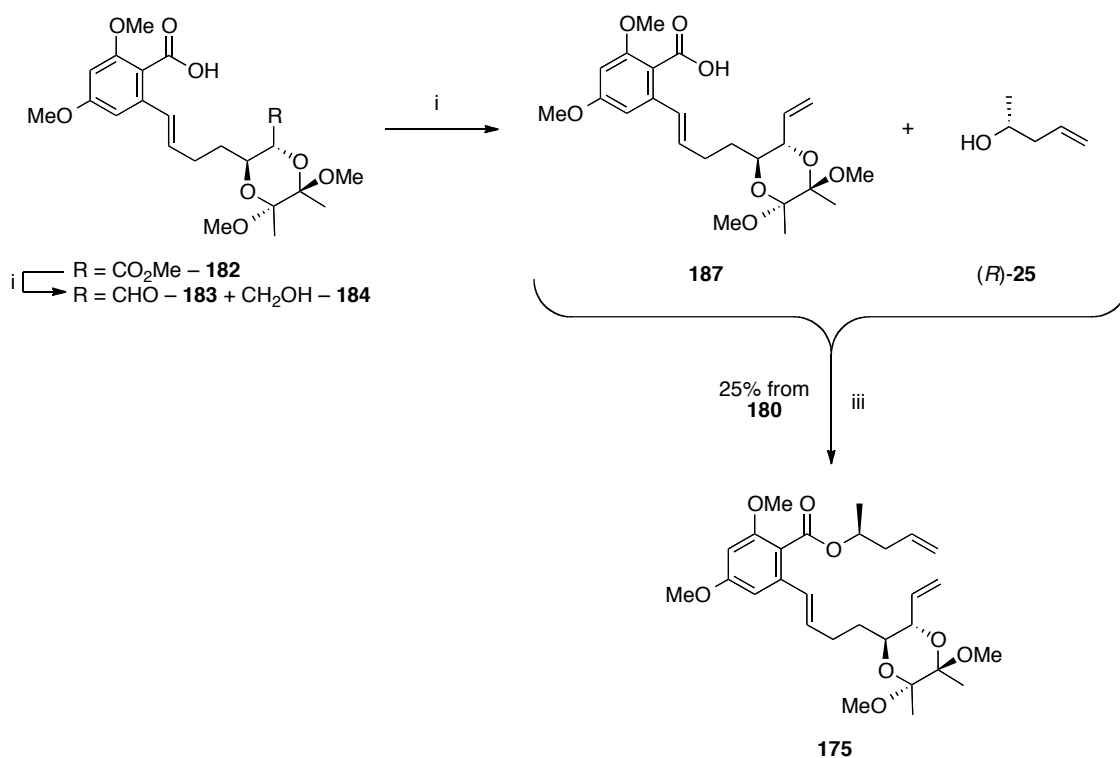
With the benzoic acid residue no longer present, the primary alcohol **185** was successfully oxidised with Dess-Martin Periodinane (Scheme 4.9) to afford aldehyde **186** that was contaminated with the by-products from the oxidant. Since compound **186** was unstable to chromatography on silica gel, it could not be obtained in pure form. Accordingly, a cleaner

oxidation process was sought and it was found that using tetrapropylammonium perruthenate (TPAP) in conjunction with *N*-methyilmorpholine-*N*-oxide (NMO) afforded a pure sample of aldehyde **186** after workup.

With aldehyde **186** in hand, this material was immediately subjected to a Wittig olefination reaction (Scheme 4.9) with the ylide prepared from the reaction of methylenetriphenylphosphonium bromide and *n*-butyl lithium. Disappointingly, this resulted in the decomposition of the starting material. As a result, various other olefination protocols were pursued but none of these gave the required triene **175**.

4.4.3 Second Attempt at Synthesising RCM Precursor 175

In light of the difficulties encountered in the above-mentioned approach to compound **175** via aldehyde **186**, an attempt was made to introduce the C7'-C8' olefin while the free benzoic acid residue was still present. Unfortunately, attempts to effect the clean reduction of methyl ester **182** to aldehyde **184** with DIBAL-H (Scheme 4.10) were unsuccessful as the formation of alcohol **184** could not be avoided. Despite the presence of alcohol **184**, the reaction mixture from the reduction was subjected to the abovementioned Wittig olefination reaction (Scheme 4.10) and the alkene **187** thereby obtained in yields varying from 30 – 50%. The ¹H NMR spectrum of this material, displayed a one-proton doublet of doublet of doublets at δ 5.32 and a complex two-proton multiplet at δ 5.78, which are attributed to the hydrogens attached to the newly installed terminal alkene residue.



Reagents and conditions: (i) DIBAL-H, CH_2Cl_2 , -78°C , 0.5 h; (ii) $\text{CH}_3\text{PPh}_3\text{Br}$, $n\text{-BuLi}$, Et_2O , $-78 \rightarrow 18^\circ\text{C}$, 19 h; (iii) DIAD, PPh_3 , PhMe , $0 \rightarrow 18^\circ\text{C}$, 0.5 h.

Scheme 4.10: Synthesis of triene **175** from methyl ester **182**.

Since compound **187** seemed to be unstable it was immediately subjected to a Mitsunobu esterification reaction with alcohol $(R)\text{-25}$ (Scheme 4.10). By such means, an impure sample of triene **175** was obtained in *ca.* 25% overall yield from methyl ester **182**. The ^1H NMR spectrum of compound **175** (Figure 4.4) confirmed the successful installation of the ester with the presence of a one-proton doublet of doublet of triplet at δ 5.84 and a two-proton complex multiplet at δ 5.10 being taken as indicative of the presence of the alkene residue of the homo-allylic ester. The remainder of the ^1H NMR spectrum was consistent with the assigned structure. The ^{13}C NMR spectrum of this material displayed the 28 resonances expected for compound **182** while the ESI mass spectrum displayed a molecular-associated ion at m/z 527 which corresponds to the sodiated derivative of compound **175**. An accurate mass measurement on this species established the expected composition, *viz* $\text{C}_{28}\text{H}_{28}\text{NaO}_8$.

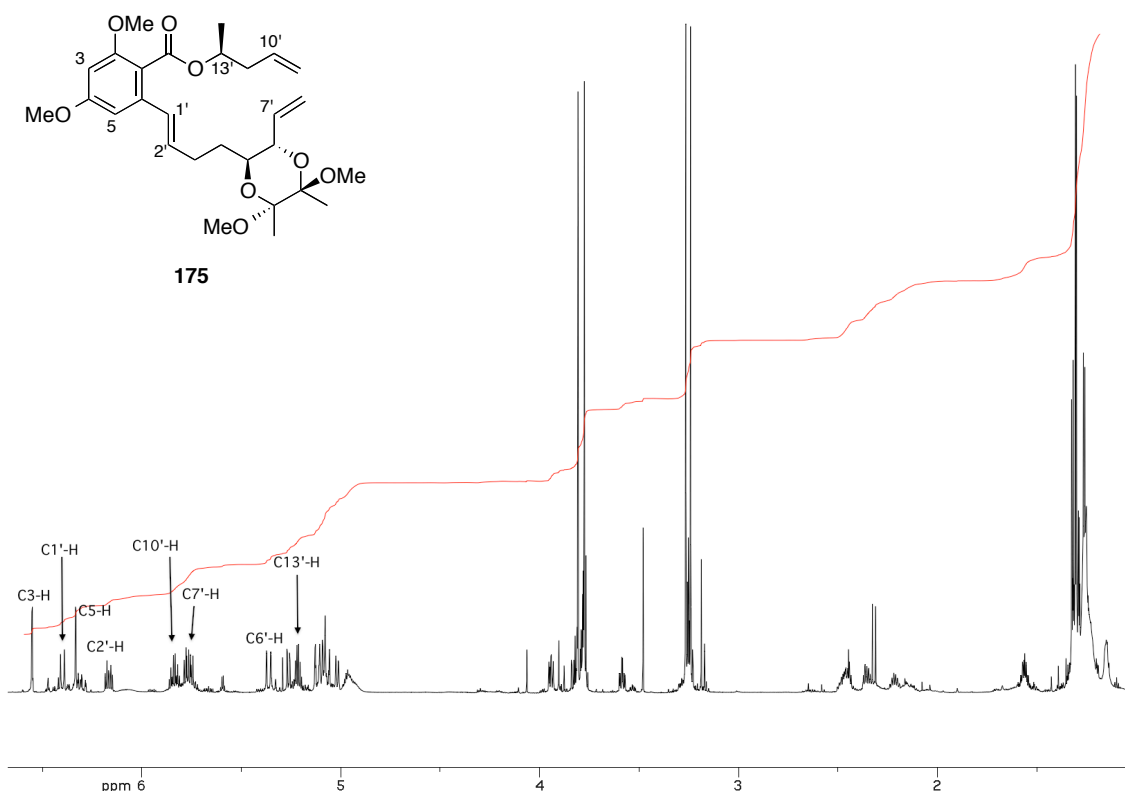
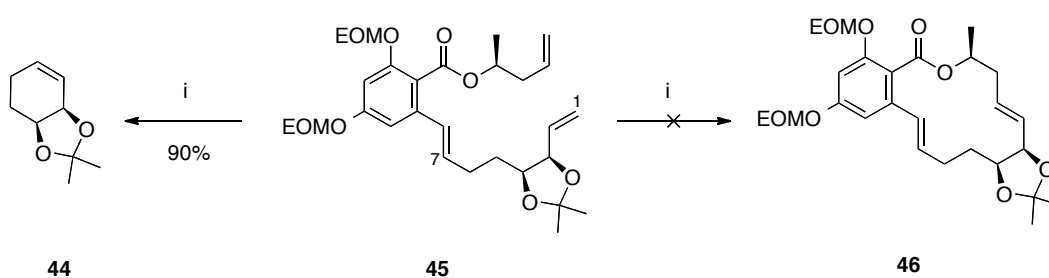


Figure 4.4: 800 MHz ^1H NMR spectrum of triene **175** (recorded in CDCl_3).

4.5 RCM Reaction of Triene **175**

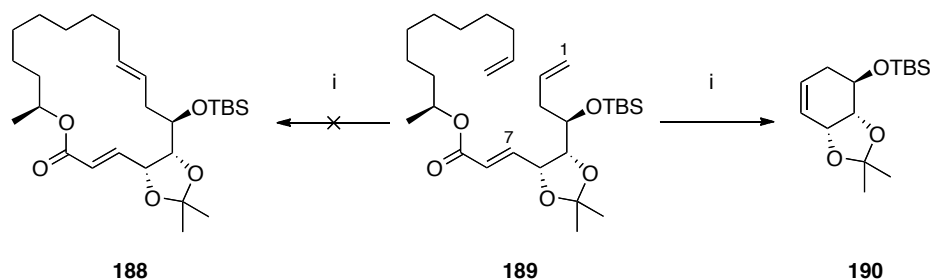
4.5.1 Previous Use of RCM Processes in Macrolide Syntheses

As discussed in Chapter One, many syntheses of aigialomycin D (**5**) employed a RCM reaction to construct the fourteen-membered macrolactone ring. However, a common problem associated with this strategy for constructing large (>10-membered) rings is the potential for formation of kinetically favoured by-products such as cyclohexenes **44** and **190**, as encountered during Winssinger's synthesis of aigialomycin D (Scheme 4.11) and Banwell's synthesis of (+)-aspicilin (Scheme 4.12), respectively.¹²⁻¹⁴



Reagents and conditions: (i) Grubbs' 2nd generation catalyst, PhMe, 80 °C, 12 h.

Scheme 4.11: Formation of cyclohexene **44** observed by Winssinger et al.¹⁵

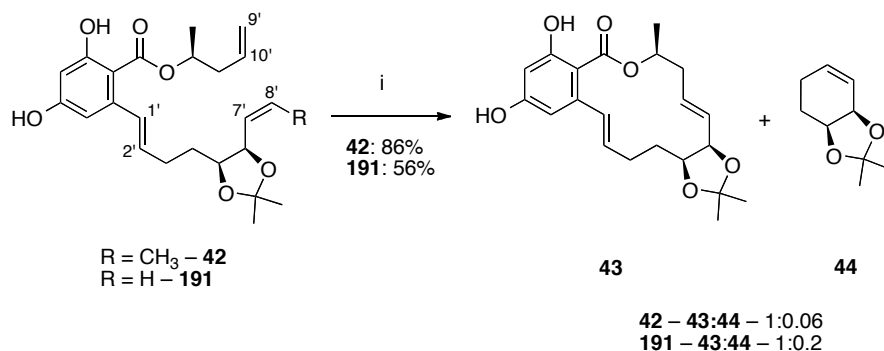


Reagents and conditions: (i) Grubbs' 1st generation catalyst, CH₂Cl₂, 18 °C, 4 h.

Scheme 4.12: Formation of cyclohexene **190** encountered by Banwell et al.¹⁴

As such, the presence of the C1'-C2' alkene residue found in aigialomycin D (**5**) adds another level of complexity to its synthesis *via* an RCM approach.^{3,15,16}

In order to overcome the type of difficulty mentioned above, most syntheses mask the C1'-C2' alkene residue as a selenide,³ sulfone,¹⁷ ketone or silyl ether,¹⁸ which is then revealed after the RCM reaction. However, Barrett *et al.* recently demonstrated (Scheme 4.13) that the masking isn't always necessary since they observed that the RCM reaction of substrate **42** favoured the formation of macrocycle **43** over cyclohexene **44** (86% yield, **43:44** – 1:0.06 ratio).¹⁶ This result represents a remarkable improvement over the equivalent transformation involving compound **191** which gave the desired macrocycle in significantly lower yield (56%) and with greater quantities of the cyclohexene by-product being formed (**43:44** – 1:0.2 ratio).¹⁶



Reagents and conditions: (i) Hoveyda-Grubbs' 2nd generation catalyst, CH₂Cl₂, μ wave, 85 °C, 3 h.

Scheme 4.13: Barrett's RCM reactions of trienes **191** and **42**.¹⁶

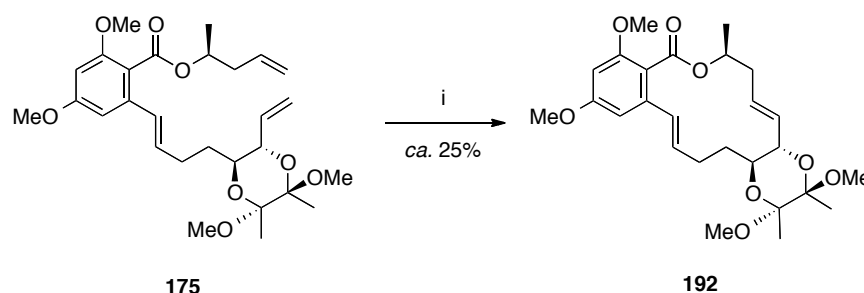
It can be argued that by replacing the C7'-C8' terminal olefin in triene **191** with a 1,2-disubstituted olefin, as encountered in triene **42**, then the substrate will react more slowly with the catalyst. As a consequence, olefin metathesis is initiated preferentially at the C9'-C10' olefin and, therefore, provides the desired macrocycle **43**. The formation of some cyclohexene by-product (**44**) could be attributed to the ability of the C7'-C8' 1,2-disubstituted olefin of

compound **42** to participate in the RCM reaction, albeit more slowly when compared to the corresponding terminal olefin of substrate **191**.

Although triene **175** lacks the 1,2-disubstituted olefin found in triene **42**, it was anticipated that the presence of a *trans*-configured diol instead of the *cis*-configured one, as encountered in the previous examples (see Scheme 4.11 and Scheme 4.13) could deter the catalysts from terminating the RCM reaction at the C1'-C2' double bond due to the greater conformational rigidity around this bond. Therefore, it was envisaged that initiation at either terminal olefin might still give the desired macrocycle **192**.

4.5.2 RCM Reaction of Triene **175**

With triene **175** in hand, the stage was set for an examination of the pivotal RCM reaction. To such ends, a dilute solution of triene **175** was exposed to Grubbs' 2nd generation catalyst¹⁹ (Scheme 4.14) and this afforded the target macrocycle **192**, albeit in *ca.* 25% yield.



Reagents and conditions: (i) Grubbs' 2nd generation catalyst, CH₂Cl₂, 40 °C, 12 h.

Scheme 4.14: RCM reaction of triene **175** with Grubbs' 2nd generation catalyst.

The ¹H NMR spectrum of compound **192** (Figure 4.5) features a complex one-proton multiplet at δ 5.50 and a one-proton doublet of doublet at δ 5.68 which correspond to the hydrogens associated with the newly formed (C7'-C8') alkene. The C1'-C2' alkene gives rise to a one proton doublet at δ 6.37 and a one proton multiplet at δ 5.99. The ESI mass spectrum of this material revealed a molecular associated ion at m/z 499, which corresponds to the sodiated derivative of **192**. An accurate mass measurement on this species established that it possessed the expected molecular formula, *viz* C₂₆H₃₆NaO₈. Despite these promising data, full and rigorous characterisation on compound **192** could not be achieved with the small and impure quantities of material available. Nevertheless, there are some encouraging precedents in the literature suggesting that the conversion of triene **175** into the fourteen-membered macrocyclic ring **192** has been successful.

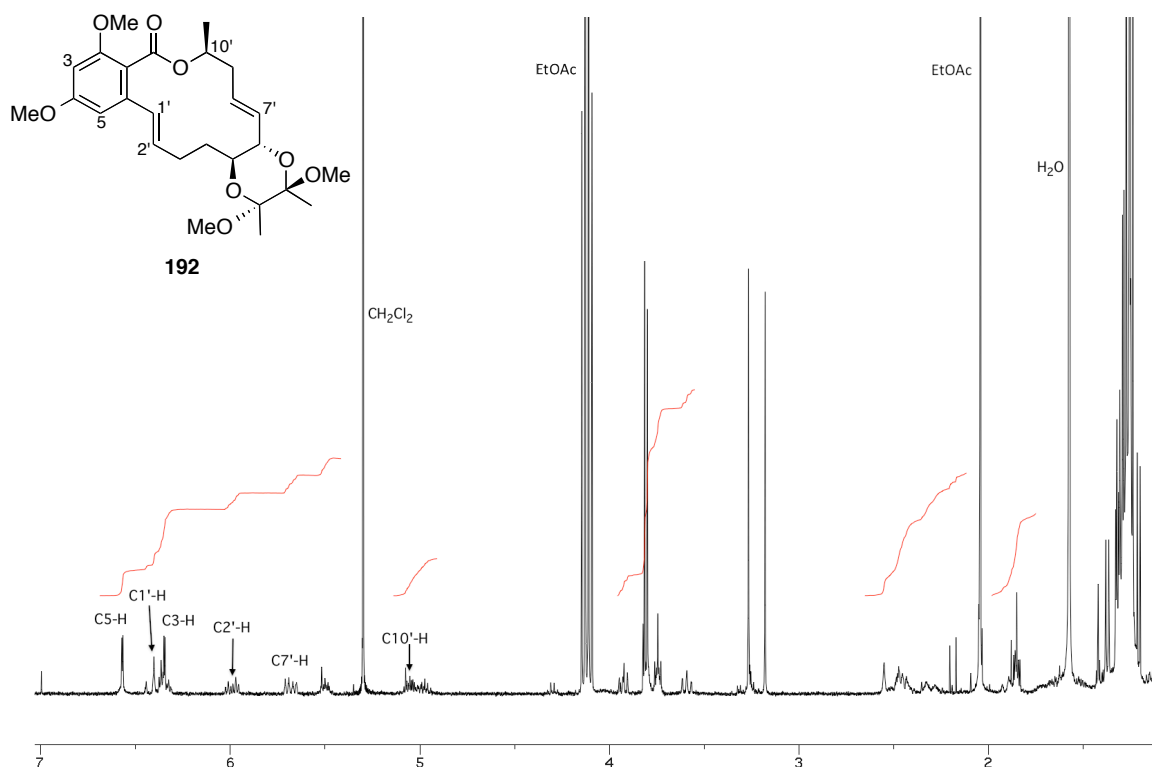
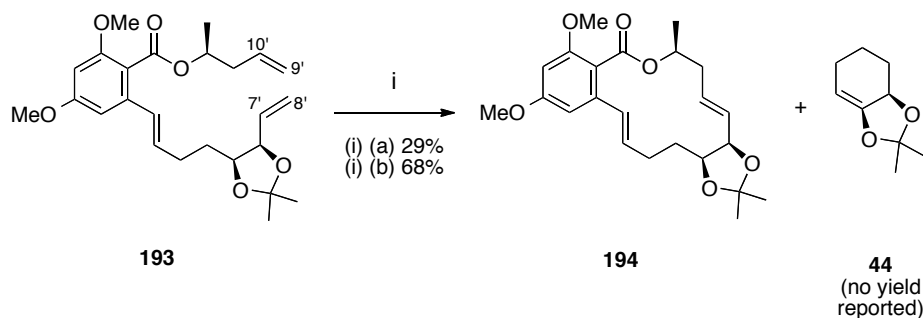


Figure 4.5: 400 MHz ^1H NMR spectrum of macrocycle **192** (recorded in CDCl_3).

So, after attempting the RCM reaction with triene **175** (Scheme 4.14), Takahashi *et al.* reported a similar outcome when the closely related substrate **193** was subjected to an RCM reaction (Scheme 4.15).²⁰



Reagents and conditions: (i) (a) Grubbs' 2nd generation catalyst, CH_2Cl_2 , rt, 24 h; (b) Grubbs' 1st generation catalyst, CH_2Cl_2 , rt, 24 h.

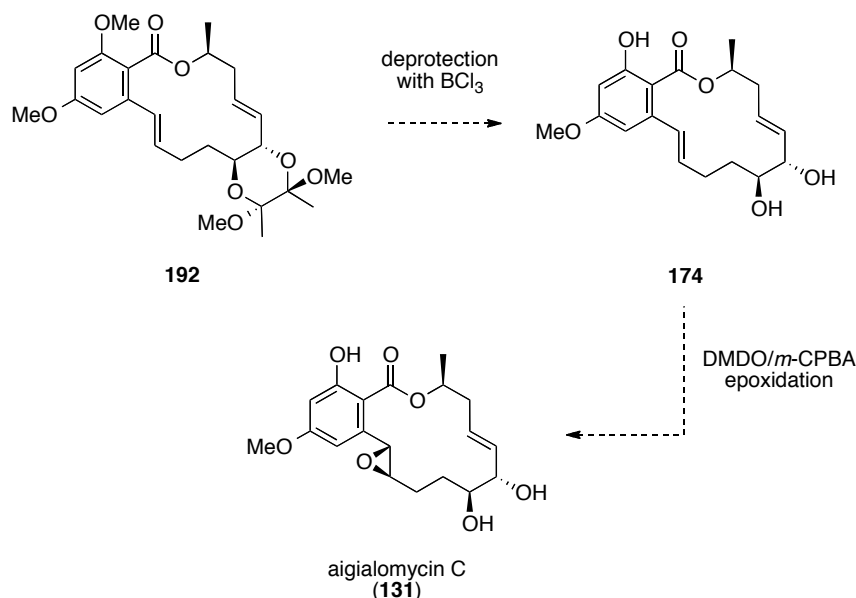
Scheme 4.15: Takahashi's RCM reaction of substrate **193**.²⁰

The workers attributed the modest yield of the desired macrocycle (**194**) to the competing generation of cyclohexene **44** although no yield was reported for this by-product.²⁰ In light of this, the similarities of the trienes **175** and **193** and the yields of the desired RCM products, it seems reasonable to suggest that a cyclohexene by-product was produced in the RCM of the former substrate even if this could not be isolated from the reaction mixture.

When Takahashi *et al.* repeated the RCM reaction on substrate **193** with a less active catalyst (Scheme 4.15), namely Grubbs' 1st generation system,^{21,22} the yield of the macrocycle improved significantly (to 68%) although they did not indicate if the cyclohexene by-product **44** was produced on this occasion.²⁰ A possible explanation for this improvement in the yield may be that the metathesis initiates at the C9'-C10' olefin and, thus leading to a greater yield of desired macrocycle **195**. Unfortunately, insufficient material was available to examine the effect of subjecting compound **175** to reaction with Grubbs' 1st generation catalyst.

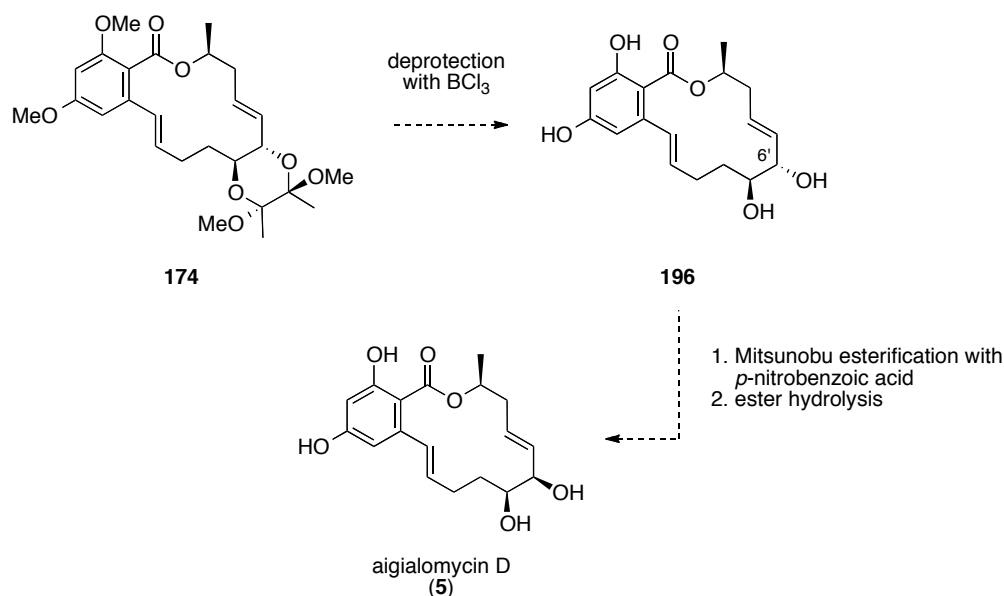
4.6 Ongoing Studies Directed Towards the Synthesis of Aigialomycin C (131)

Further work is now being undertaken within the Banwell group to complete the synthesis of aigialomycin C (**131**). In principle, all that remains in this synthesis is a global deprotection of compound **192** followed by a regio- and diastereo-selective epoxidation of triol **174** (Scheme 4.16) using the conditions reported by Lett *et al.* or Winssinger *et al.* in their synthesis of hypothemycin (**2**).^{23,24}



Scheme 4.16: Conversion of macrocycle **192** into aigialomycin C (**131**).

An ability to access synthetically useful quantities of macrocycle **174**, would also provide an opportunity to synthesise aigialomycin D (**5**) (Scheme 4.17) through an inversion of the C6' alcohol moiety within compound **195** *via* a regioselective Mitsunobu esterification reaction involving the allylic hydroxyl residue.



Scheme 4.17: Proposed synthesis of aigialomycin D (5).

4.7 Conclusion

In the work described above, the biocatalytically-derived starting material *c*DHC **82** ($\text{X} = \text{Cl}$) has been converted into the macrolactone ring **192**. The latter compound represents an advanced intermediate in a projected synthesis of aigialomycin C (**131**). As such completion of this target **131** seems likely in the near future. This synthesis builds upon the protocols established during the preparation of L-783,290 (**128**), namely the chemoenzymatic resolution of homoallylic alcohol (*R*)-**25** and the use of the Heck reaction to couple the aromatic and diol fragments, **133** and **177**, respectively.

This approach also demonstrates the usefulness of the Heck reaction in RAL synthesis, as the alkene derived from the coupling of the aryl halide and olefin of the diol unit directly corresponds to the alkene present in 5*Z*-7-oxozeanol (**3**) and aigialomycin D (**5**). but also provides a handle for further functionalisation, such as generation of an epoxide, as seen in hypothemycin (**2**) and aigialomycins A to C.

4.8 References

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5.1 Introduction

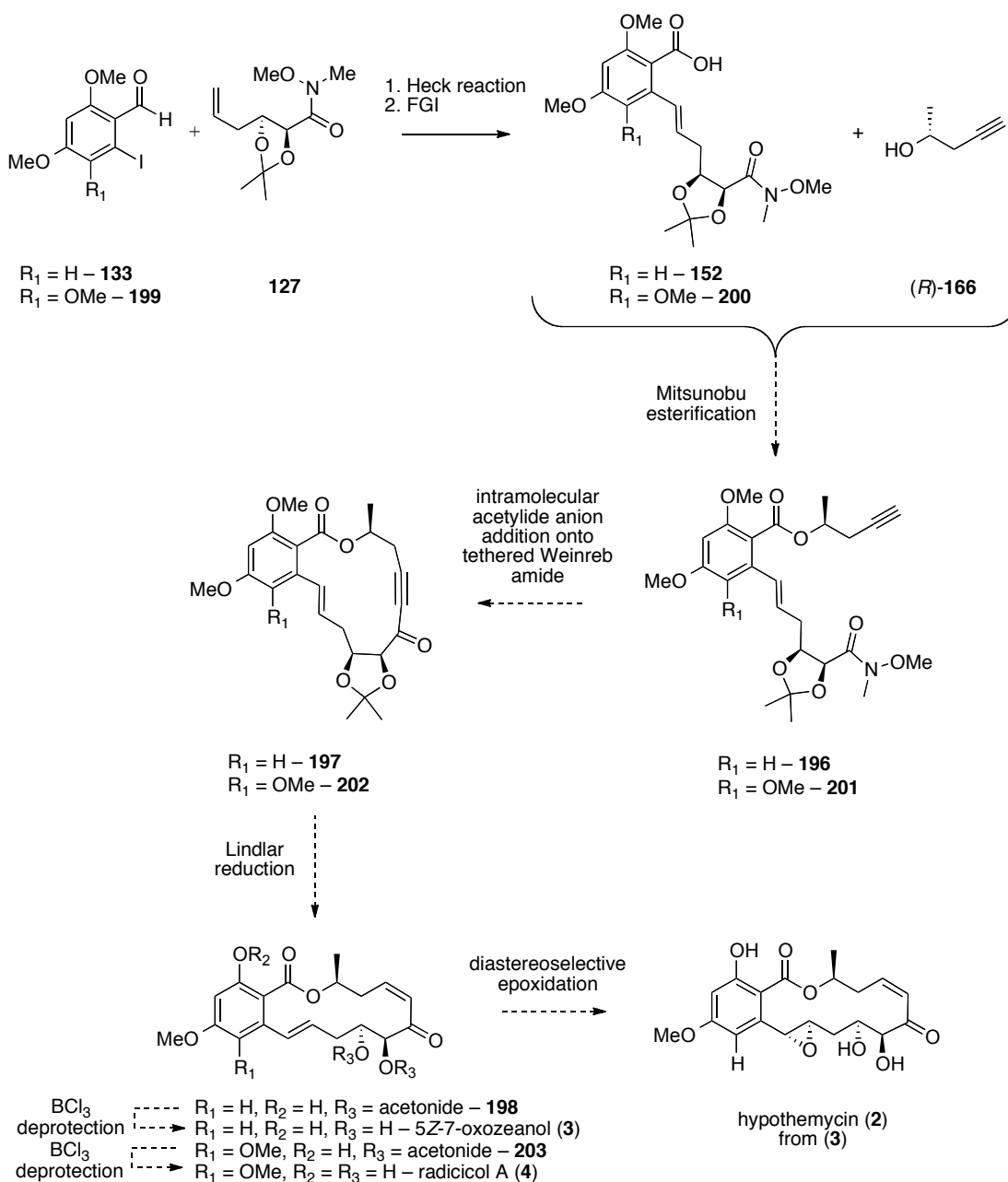
Chapters Two, Three and Four of this thesis describe modular and versatile approaches to *cis*- and *trans*-configured enone-containing RALs, as well as members of the aigialomycin family. The use of the Heck reaction for the coupling of the aromatic and diol fragments affords a product bearing a synthetically useful alkene at the C1'-C2' position. Although this was subsequently reduced to the corresponding alkane in the preparation of L-783,290 (**128**) and L-783,277 (**1**), its retention would provide an opportunity to access RALs that contain this alkene or related functionality (e.g. epoxides) within their frameworks. To emphasise such opportunities, several possible pathways to RALs containing such functionality are detailed below. These are categorised according to the proposed mode of macrocyclisation, namely those involving the intramolecular nucleophilic addition reaction (as described in Chapter Three) or those employing the RCM reaction (as presented in Chapters Two and Four).

5.2 Proposed Syntheses of Additional Biologically Active RALs

5.2.1 Syntheses of RAL Derivatives by Employing an Intramolecular Acetylide Anion Addition to a Tethered Weinreb Amide

It is envisaged that the strategy employed in the synthesis of L-783,277 (**1**), as described in Chapter Three, could be used in the preparation of 5Z-7-oxozeanol (**3**) and hypothemycin (**2**). Both RALs are potent inhibitors of MAPKs and, thus, present themselves as interesting targets. The proposed syntheses exploit the advanced intermediate **153** obtained during the course of the synthesis of L-783,290 (**128**). So, subjection of compound **152** to a Mitsunobu esterification reaction with (*R*)-(-)-4-pentyn-2-ol [(*R*)-**166**] would be expected to afford ester **196** (Scheme 5.1) which should, in turn, yield macrocycle **197** bearing the C1'-C2' alkene and an alkyne after treatment with LiHMDS. It is anticipated that the synthesis of 5Z-7-oxozeanol (**3**) could be completed through a Lindlar reduction of the embodied alkyne to the corresponding *Z*-

configured enone (as shown in compound **198**), followed by a step-wise deprotection with BCl_3 . The successful acquisition of 5Z-7-oxozeanol (**3**) by this means also constitutes a formal total synthesis of hypothemycin (**2**) as this has been obtained by Lett *et al.* and Winssinger *et al.* through diastereoselective epoxidation of compound **3**.^{1,2}



Scheme 5.1: Proposed synthesis of hypothemycin (**2**), 5Z-7-oxozeanol (**3**) and radicicol A (**4**).

Replacement of aryl iodide **133** in the above-mentioned sequence with 2-iodo-3,4,6-trimethoxybenzaldehyde (**199**) (Scheme 5.1) also provides a plausible approach to radicicol A (**4**).

5.2.2 Syntheses of RAL Derivatives by Employing RCM Reaction

Cochliomycins (A to C, Figure 5.1) are a new class of RALs recently isolated from the fungus *Cochliobolous lunatus*. Interestingly, they have been shown to exhibit potent anti-fouling activity.³

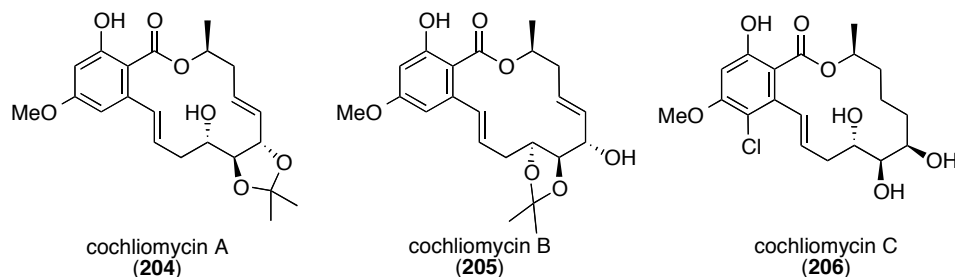
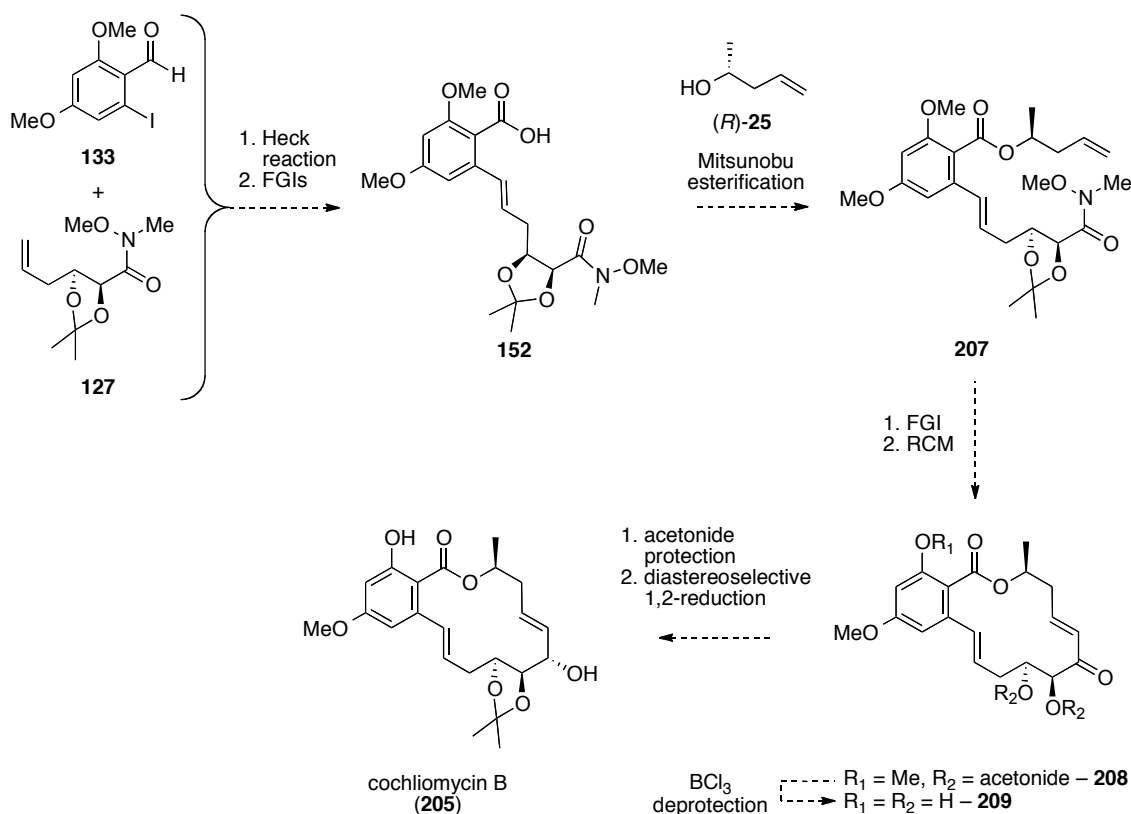


Figure 5.1: Cochliomycins A – C isolated from *C. lunatus*.³

By retaining the C1'–C2' alkene derived from the Heck reaction of fragments **127** and **133**, a divergent synthesis of cochliomycin B (**205**) could be achieved by employing the same approach as used for the preparation of L-783,290 (**128**) (Chapter Two). Careful deprotection of macrocycle **208** with BCl_3 should provide triol **209** (Scheme 5.2) which could, in turn, be converted into cochliomycin B (**205**) after “protection” of the diol as an acetonide followed by a diastereoselective 1,2-reduction of the enone residue.



Scheme 5.2: Proposed synthesis of cochliomycin B (**205**).

5.3 Conclusion

Based on the total syntheses of L-783,290 (**128**) and L-783,277 (**1**), and the significant advances towards aigialomycin C (**131**) – as described in Chapters Two, Three and Four, respectively– a unified approach for the preparation of other biologically active RALs should be able to be realised. The use of the Heck reaction not only serves as a strategy for the coupling of the aromatic and diol fragments, but it also provides the potential for accessing several RALs containing functionality at the C1'-C2' position. In all the syntheses achieved thus far it has been shown that a combination of the microbial oxidation, Heck coupling, Mitsunobu esterification and one of the two presented modes of macrocyclisation, namely RCM reaction or intramolecular acetylide anion addition onto a tethered Weinreb amide, represent significant contributions to the preparation of RALs.

5.4 References

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Experimental Procedures Associated with Work Described in Chapters Two to Four

6.1 General Procedure

Starting materials and reagents were obtained from the Sigma-Aldrich, Merck, TCI or Lancaster Chemical Companies and used as supplied or, occasionally, recrystallised or distilled. Inorganic salts were purchased from the Sigma-Aldrich, Alfa Aesar, AJAX, BDH or Unilab Chemical Companies. The *cis*-1,2-dihydrocatechols **82** (X = Cl) was purchased from Professor Derek Boyd of The Queen's University of Belfast (Belfast, UK). (*R*)-(-)-4-penten-2-ol, [(*R*)-**25**], was generously provided by Dr Jens Renner of BASF (Germany). CALB was obtained, as a gift, from Novo Nordisk (Denmark).

THF, DMF, dichloromethane and toluene were dried using a Glass Contour™ solvent purification system that is based upon a technology originally described by Grubbs *et al.*¹ Solvents collected from the purification system were stored over 4 Å molecular sieves that had previously been dried in a conventional microwave oven, then cooled under high vacuum and stored under anhydrous nitrogen or argon.

Glassware was rinsed with acetone, dried then soaked in a base bath (Pyronex® in water) before being rinsed with distilled water and oven-dried at 120 °C. Assembled apparatus was evacuated (< 0.1 mmHg) and flushed three times with dry nitrogen prior to use. Reaction mixtures were manipulated under nitrogen using standard Schlenk techniques.

Ambient temperature was assumed to be *ca.* 18 °C. Temperatures higher than ambient were attained using oil baths heated on a thermostated hot-plate stirrer. To attain temperatures lower than ambient, appropriate cooling baths were used (ice/water slurry, 0 °C; dry ice/acetonitrile, –40 °C; dry ice/acetone, –78 °C).

Organic solutions (extracts) obtained from the work-up of reaction mixtures were dried with anhydrous sodium sulfate (Na_2SO_4) or magnesium sulfate (MgSO_4) before being filtered and concentrated under reduced pressure on a rotary evaporator with a water bath temperature generally not exceeding 25 °C.

Analytical thin layer chromatography (TLC) was performed on aluminium-backed 0.2 mm thick silica gel 60 F₂₅₄ plates as supplied by Merck. Eluted plates were visualised using a 254 nm UV lamp and/or by treatment with a suitable dip followed by heating. These dips included: a) potassium permanganate : potassium carbonate : 5% sodium hydroxide aqueous solution : water (3 g : 20 g : 5 mL : 300 mL) and b) anisaldehyde : sulfuric acid (conc.) : ethanol (3ml : 4.5 mL : 200 mL).

Melting points were measured on a Stanford Research Systems Optimelt – Automated Melting Point System and are uncorrected.

Unless specified otherwise, proton (^1H) and carbon (^{13}C) NMR spectra were recorded at 20 °C on either a Varian Mercury 300 spectrometer (operating at 300 MHz for proton and 75 MHz for carbon nuclei) or a Varian MR400 spectrometer (operating at 400 MHz for proton and 100 MHz carbon nuclei) using CDCl_3 that had been filtered through basic alumina. In certain cases, a Varian Inova 500 spectrometer (operating at 500 MHz for proton and 125 MHz for carbon nuclei) or a Bruker AV800 spectrometer (operating at 800 MHz for proton and 200 MHz for carbon nuclei) was used. Signals arising from the residual protio-forms of the solvent were used as the internal standard. Chemical shifts are recorded as δ values in parts per million (ppm). ^1H NMR data are recorded as follows: chemical shift (δ) [multiplicity, coupling constant(s) J (Hz), relative integral] where multiplicity is defined as: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet or combinations of the above. For ^{13}C NMR spectra, the chemical shifts are reference against with the central peak (δ 77.0) of the CDCl_3 “triplet”.

Infrared spectra (ν_{max}) were recorded on a Perkin-Elmer 1800 Series FTIR Spectrometer. Samples were analysed as KBr discs (for solids) or as thin films on KBr plates (for liquids/oils).

Mass spectrometry was performed by the Australian National University’s Mass Spectrometric Services Unit located in the Research School of Chemistry, Canberra, Australia. Low- and high-resolution electron impact (EI) spectra were obtained on a VG Fisions AutoSpec M series three-sector (E/B/E) double-focusing mass spectrometer. Low and high resolution electrospray (ES) mass spectra were recorded on a Micromass-Waters LC-ZMD single quadrupole liquid chromatograph-MS or a VG Quattro II triple quadrupole MS instrument operating in positive or negative ionisation mode. Gas chromatographic analyses (GC) were performed using Agilent 7890A instrument fitted with a 25 m $\times$ 0.22 mm permethylated β -cyclodextrin (SGE Cydex B, wall coated to 0.25 microns)

capillary column. Peaks were detected using a flame ionisation detector operating at 200 °C. Hydrogen served as the carrier gas (flow rate *ca.* 28.6 cm.s⁻¹) and a gradient temperature program ranging from 50 °C (5 min) to 200 °C (20 min) with a heating rate of 10 °C.min⁻¹ was used. High performance liquid chromatography (HPLC) were performed on a Waters system consisting of a 600E quaternary pump and 2996 diode detector system.

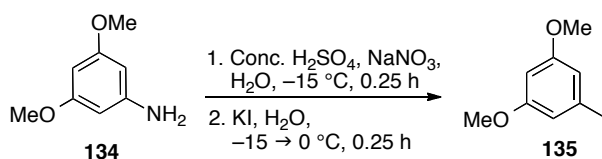
Optical rotations were measured between 17 to 20 °C on a Perkin-Elmer 241 polarimeter at the sodium-D line ($\lambda = 589$ nm) and the concentrations (*c*) (g/100 mL) indicated using spectroscopic grade chloroform (CHCl₃) as solvent. The measurements were carried out in a cell with a path length (*l*) of 1 dm. Specific rotations $[\alpha]_D$ were calculated using the equation $[\alpha]_D = (100 \times \alpha) / (c \times l)$ and are given in 10⁻¹.deg.cm².g⁻¹.

Ozonolyses were performed using a Model 500 Fischer portable ozone-generator with the luteinizing power and flow rate adjusted to 80 V and 50 L/h, respectively.

Flash chromatography² was performed using analytical grade solvents and silica gel 60 (230 – 400 mesh, 0.040 – 0.0063 mm) as supplied by Merck.

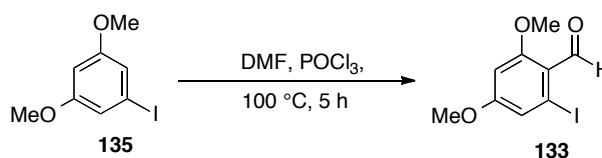
6.2 Experimental Procedures Associated with Work Described in Chapter Two

1-Iodo-3,5-dimethoxybenzene (**135**)



A vigorously stirred suspension of 3,5-dimethoxyaniline (**134**) (3.25 g, 19.50 mmol) in water (11 mL) was rapidly cooled to -15 °C and immediately treated, dropwise, with sulfuric acid (879 μ L of 95 – 97% by weight material). After 0.2 h the reaction mixture was treated, dropwise, with a solution of sodium nitrite (680 mg, 9.79 mmol) dissolved in a minimum volume of water. After being stirred for a further 0.25 h at -15 °C, the reaction mixture was treated with diethyl ether (20 mL) then treated, dropwise, with a solution of potassium iodide (3.25 g, 19.57 mmol) in water (2 mL) (*Caution*: vigorous nitrogen evolution). After leaving the reaction mixture to stir for 3 h at 0 °C, the aqueous phase was separated and extracted with diethyl ether (3 $\times$ 20 mL). The combined organic phases were washed with sodium thiosulfate (2 $\times$ 20 mL of a 10% w/v aqueous solution), sodium bicarbonate (1 $\times$ 20 mL of a saturated aqueous solution), water (1 $\times$ 20 mL) and brine (1 $\times$ 20 mL) before being dried (MgSO₄), filtered and concentrated under reduced pressure to give an orange oil. This material was placed on the top of a pad of TLC-grade silica gel that was then eluted with ethyl acetate/hexane (300 mL of a 1:9 v/v mixture). Concentration of the filtrate under reduced pressure gave the title compound **135**³ (1.23 g, 71%) as light-yellow crystalline masses, m.p. = 72–74 °C (lit.³ m.p. = 74–75 °C). The spectroscopic data correlated with those previously reported.³

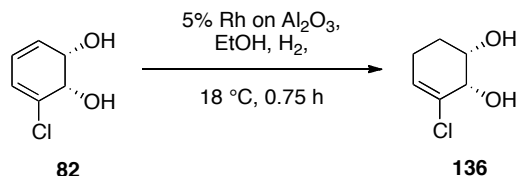
2-Iodo-4,6-dimethoxybenzaldehyde (**133**)



A magnetically stirred solution of aryl iodide **135** (500 mg, 1.01 mmol) in dry DMF (3 mL) maintained under a nitrogen atmosphere at 0 °C was treated, dropwise, with freshly distilled POCl₃ (433 μ L, 4.73 mmol). After 0.5 h the reaction mixture was warmed to 18 °C, kept at this temperature for 0.5 h, then heated to 100 °C. After 5 h the reaction was cooled to 18 °C and poured, with stirring, into ice/water (500 mL). After 3 h the mixture was filtered to give a light-brown solid that was then subjected to flash chromatography (silica, 2:3 v/v ethyl acetate/hexane elution). Concentration of the relevant fractions (R_f = 0.2) afforded the title compound **133**⁴

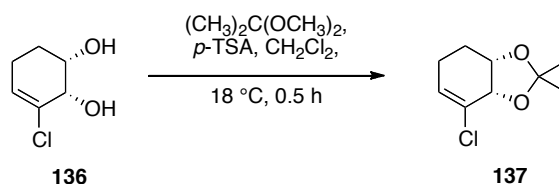
(350 mg, 63%) as light-yellow needles, m.p. = 81–82 °C (lit.⁴ m.p. = 80–80.5 °C). The spectroscopic data correlated with those previously reported.⁴

(1*S*,2*S*)-3-Chlorocyclohex-3-ene-1,2-diol (136**)**

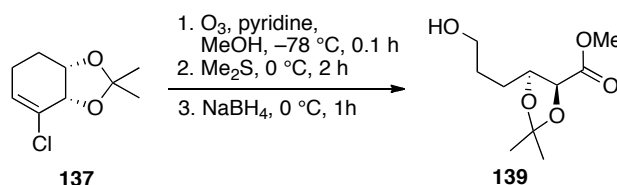


Following a procedure reported by Banwell and Loong,⁵ a magnetically stirred solution of diene **82** (2.00 g, 14.00 mmol) in absolute ethanol (170 mL) maintained at 18 °C was treated with 5% rhodium on alumina (200 mg of 10% w/w material, *ex. Alfa Aesar*). The resulting black slurry was evacuated and refilled with dihydrogen three times prior to the contents being stirred at 18 °C under a dihydrogen atmosphere for 0.75 h. The reaction mixture was then filtered through a pad of CeliteTM that was washed with ethanol (3 × 50 mL). The combined filtrates were concentrated under reduced pressure to give a grey solid that was subjected to flash chromatography (silica, 1:5:0.1 → 2:3:0.1 v/v/v ethyl acetate/hexane/isopropanol gradient elution). Concentration of the relevant fractions (R_f = 0.25 in 1:2 v/v ethyl acetate/hexane) then gave the title compound **136**⁵ (1.36 g, 65%) as a white, crystalline solid, m.p. = 113.6–114.1 °C (lit.⁵ m.p. = 115–116 °C). This material was identical, in all aspects, with an authentic sample.⁵

(3*aS*,7*aS*)-7-Chloro-3*a*,4,5,7*a*-tetrahydro-2,2-dimethylbenzo[*d*][1,3]dioxole (137**)**



A magnetically stirred solution of diol **136** (1.36 g, 9.15 mmol) in 2,2-dimethoxypropane (3 mL) maintained at 18 °C under nitrogen was treated with *p*-toluenesulfonic acid (0.16 g, 0.92 mmol). After 1 h the reaction mixture was quenched with NaHCO₃ (1 × 20 mL of a saturated aqueous solution) and extracted with dichloromethane (4 × 5 mL). The combined organics phases were washed with brine (1 × 20 mL) then dried (MgSO₄), filtered and concentrated under reduced pressure to give a brown oil. Subjection of this material to flash chromatography (silica, 1:9 → 3:7 v/v diethyl ether/pentane gradient elution) and concentration of the relevant fractions (R_f = 0.3 in 1:9 v/v ethyl acetate/hexane) then afforded the title compound **137**⁶ (1.68 g, 97%) as a clear, colourless oil. This material was identical, in all aspects, with an authentic sample.⁶

(4S,5S)-Methyl 5-(3-hydroxypropyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylate (139)


A magnetically stirred solution of alkene **137** (500 mg, 2.7 mmol) and pyridine (2.14 mL, 27.0 mmol) in methanol (50 mL) was cooled to $-78\text{ }^\circ\text{C}$ and then treated with a stream of ozone until a persistent blue colour was observed. After a further 0.1 h the excess ozone was removed with a stream of nitrogen and the resulting clear, colourless solution was treated with dimethyl sulfide (7.84 mL, 106.0 mmol) and allowed to warm to $0\text{ }^\circ\text{C}$. After 2 h the reaction mixture was treated, portion-wise over 0.5 h, with sodium borohydride (670 mg 17.85 mmol). After an additional 0.5 h the reaction mixture was treated with NH_4Cl (30 mL of a saturated aqueous solution) and extracted with ethyl acetate ($9 \times 30\text{ mL}$). The combined organic phases were washed with brine ($1 \times 150\text{ mL}$) then dried (MgSO_4), filtered and concentrated under reduced pressure to give a light-yellow oil. Subjection of this material to flash chromatography (silica, 2:3:0.1 $\rightarrow$ 3:2:0.1 v/v/v ethyl acetate/hexane/isopropanol gradient elution) and concentration of the relevant fractions ($R_f = 0.3$ in 2:3 v/v ethyl acetate/hexane) gave the *title compound* **139** (440 mg, 76%) as a light-yellow oil.

^1H NMR (CDCl_3 , 300 MHz) δ 4.60 (d, $J = 6.8\text{ Hz}$, 1H), 4.36 (m, 1H), 3.75 (s, 3H), 3.66 (m, 2H), 1.80–1.65 (complex m, 4H), 1.61 (s, 3H), 1.45 (m, 1H), 1.38 (s, 3H).

^{13}C NMR (CDCl_3 , 75 MHz) δ 171.0, 110.7, 77.8, 77.5, 62.2, 52.1, 29.7, 27.1, 26.9, 25.8.

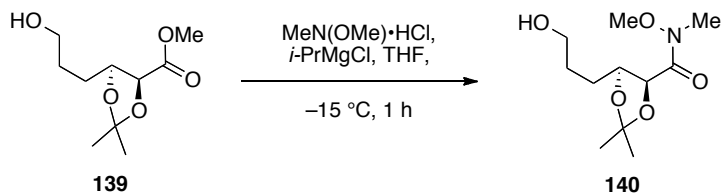
IR ν_{max} (KBr) 3452, 2939, 1757, 1439, 1382, 1161, 1095 cm^{-1} .

Mass Spectrum (ESI, +ve) m/z 241 $[(\text{M} + \text{Na})^+]$, 101 (87).

HRESIMS Found: $(\text{M} + \text{Na})^+$, 241.1048. $\text{C}_{10}\text{H}_{18}\text{O}_5$ requires $(\text{M} + \text{Na})^+$, 241.1052.

Specific Rotation: $[\alpha]_D = +0.8$ (c 0.45, CHCl_3).

(4*S*,5*S*)-5-(3-Hydroxypropyl)-*N*-methoxy-*N*,2,2-trimethyl-1,3-dioxolane-4-carboxamide
(140)



A magnetically stirred solution of ester **139** (490 mg, 1.83 mmol) in THF (3.67 mL) maintained under a nitrogen atmosphere was treated with *N,O*-dimethylhydroxylamine hydrochloride (360 mg, 3.65 mmol). The resulting mixture was cooled to $-15\text{ }^{\circ}\text{C}$ then treated, dropwise over 0.3 h, with *i*-propylmagnesium chloride (4.58 mL of a 2.0 M solution in THF, 9.16 mmol). After 1 h at $-15\text{ }^{\circ}\text{C}$ the reaction mixture was treated with phosphate buffer (10 mL of a 1 M aqueous solution, pH = 4) and extracted with ethyl acetate ($10 \times 30\text{ mL}$). The combined organic phases were washed with brine ($1 \times 150\text{ mL}$) before being dried (MgSO_4), filtered and concentrated under reduced pressure to give a yellow oil. Subjection of this material to flash chromatography (silica, 3:2:0.1 $\rightarrow$ 9:0:1 v/v/v ethyl acetate/hexane/isopropanol gradient elution) and concentration of the relevant fractions ($R_f = 0.25$ in ethyl acetate) then afforded the *title compound* **140** (410 mg, 89%) as a white, crystalline solid, m.p. = $88\text{--}90\text{ }^{\circ}\text{C}$.

^1H NMR (CDCl_3 , 300 MHz) δ 4.91 (d, $J = 6.8\text{ Hz}$, 1H), 4.37 (m, 1H), 3.66 (s, 3H), 3.59 (t, $J = 6.2\text{ Hz}$, 2H), 3.15 (s, 3H), 2.32 (broad s, 1H), 1.80–1.35 (complex m, 4H), 1.57 (s, 3H), 1.35 (s, 3H).

^{13}C NMR (CDCl_3 , 75 MHz) δ 170.4, 110.2, 77.5, 75.6, 62.6, 61.4, 32.5, 29.5, 27.5, 27.3, 25.9.

IR ν_{max} (KBr) 3453, 2982, 2937, 1685, 1459, 1380, 1330, 1248, 1217, 1165, 1067, 1017, 988, 884 cm^{-1} .

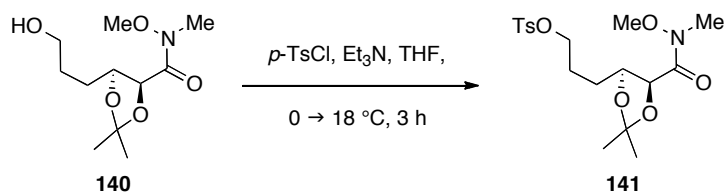
Mass Spectrum (ESI, +ve) m/z 241 $[(\text{M} + \text{Na})^+]$, 101 (87).

HRESIMS Found: $(\text{M} + \text{Na})^+$, 270.1316. $\text{C}_{11}\text{H}_{21}\text{NO}_5$ requires $(\text{M} + \text{Na})^+$, 270.1317.

Specific Rotation: $[\alpha]_{\text{D}} = -60.7$ (c 0.24, CHCl_3).

This compound has been subjected to a single-crystal X-ray analysis. Details are presented in Appendix A1.

3-((4*S*,5*S*)-5-(Methoxy(methyl)carbamoyl)-2,2-dimethyl-1,3-dioxolan-4-yl)propyl 4-methylbenzenesulfonate (141**)**

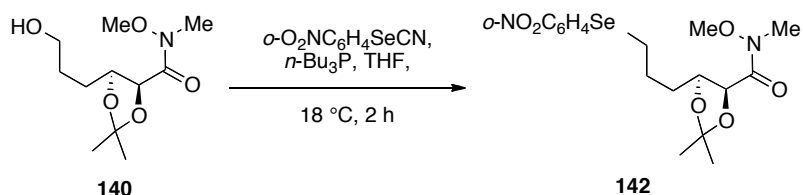


A magnetically stirred solution of alcohol **140** (10 mg, 0.4 mmol) in dry dichloromethane (5 mL) maintained under a nitrogen atmosphere was cooled to 0 °C then treated with triethylamine (0.14 mL, 1.0 mmol) and *p*-toluenesulfonyl chloride (190.7 mg, 1.0 mmol). After 3 h the reaction was treated with water (1 × 10 mL) and extracted with dichloromethane (5 × 5 mL). The combined organic phases were washed with brine (1 × 10 mL) before being dried (MgSO₄), filtered and concentrated under reduced pressure to give a yellow oil.

¹H NMR (CDCl₃, 300 MHz) δ 7.77 (d, *J* = 8.3 Hz, 2H), 7.34 (d, *J* = 8.3 Hz, 2H), 4.95 (d, *J* = 6.9 Hz, 1H), 4.33 (m, 1H), 3.92–4.13 (complex m, 4H), 3.69 (s, 3H), 3.15 (s, 3H), 2.45 (s, 3H), 2.01–1.67 (complex m, 2H), 1.55 (s, 3H), 1.35 (s, 3H).

Due to the ready decomposition of this material and its lack of synthetic utility the compound was not fully characterised.

(4*S*,5*S*)-5-(3-(2-Nitrophenylselenenyl)propyl)-*N*-methoxy-*N*,2,2-trimethyl-1,3-dioxolane-4-carboxamide (142**)**



A magnetically stirred solution of alcohol **140** (390 mg, 1.56 mmol) in THF (5.2 mL) maintained at 18 °C under a nitrogen atmosphere was treated with *o*-nitrophenylselenenyl cyanate (710 mg, 3.12 mmol, *ex.* Aldrich) then *n*-tributylphosphine (0.77 mL, 3.12 mmol) was added dropwise. The ensuing mixture was stirred at 18 °C for 2 h before being concentrated under reduced pressure to give a dark-brown solid. Subjection of this material to flash chromatography (silica, 3:7:0.1 → 1:1:0.1 *v/v/v* ethyl acetate/hexane/isopropanol gradient elution) and concentration of the relevant fractions (*R*_f = 0.25 in 7:3 *v/v* ethyl acetate/hexane) afforded the *title compound* **142** (600 mg, 89%) as a brown, crystalline solid, m.p. = 81–83 °C.

^1H NMR (CDCl_3 , 300 MHz) δ 8.28 (m, 1H), 7.51 (m, 2H), 7.30 (m, 1H), 4.95 (d, J = 6.8 Hz, 1H), 4.40 (m, 1H), 3.70 (s, 3H), 3.19 (s, 3H), 2.90 (m, 2H), 2.02 (m, 2H), 1.82 (m, 2H), 1.61 (s, 3H), 1.39 (s, 3H).

^{13}C NMR (CDCl_3 , 75 MHz) δ 170.1, 146.8, 133.6, 133.5, 129.0, 126.5, 125.3, 110.0, 76.9, 75.7, 61.2, 32.3, 30.8, 27.4, 25.7 (two signals overlapping), 25.3.

IR ν_{max} (KBr) 2981, 2937, 1684, 1590, 1565, 1513, 1453, 1379, 1332, 1304, 1249, 1218, 1169, 1097, 1071, 1037, 987, 874, 852, 785, 731 cm^{-1} .

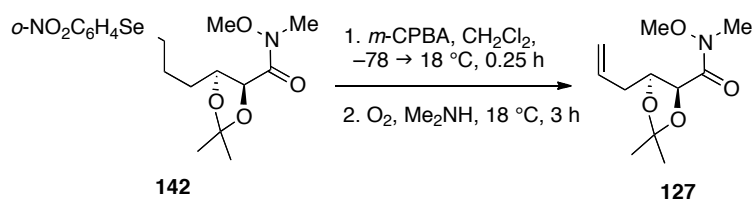
Mass Spectrum (ESI, +ve) m/z 455 $[(\text{M} + \text{Na})^+]$, 100%, 453 (53), 375 (55), 219 (80).

HRESIMS Found: $(\text{M} + \text{Na})^+$, 455.0703. $\text{C}_{17}\text{H}_{24}\text{N}_2\text{O}_6\text{Se}$ requires $(\text{M} + \text{Na})^+$, 455.0697.

Specific Rotation: $[\alpha]_{\text{D}} = -58.0$ (c 0.56, CHCl_3).

This compound has been subjected to a single-crystal X-ray analysis. Details are presented in Appendix A2.

(4*S*,5*S*)-5-Allyl-*N*-methoxy-*N*,2,2-trimethyl-1,3-dioxolane-4-carboxamide (127**)**



A magnetically stirred solution of selenide **142** (360 mg, 0.84 mmol) in dry dichloromethane (11 mL) maintained under a nitrogen atmosphere was cooled to -78°C then treated, dropwise, with a solution of *m*-CPBA (210 mg of *ca.* 77% material, 0.93 mmol) in dichloromethane (11 mL). After 0.25 h the reaction mixture was allowed to warm to 18°C in the presence of atmospheric oxygen. At the end of this period it was treated with dimethylamine (0.35 mL, 3.37 mmol) and after a further 3 h with NaHCO_3 (10 mL of a saturated aqueous solution) then diluted with ethyl acetate (1×15 mL). The separated aqueous phase was extracted with ethyl acetate (3×10 mL) and the combined organic phases were then washed with brine (1×20 mL) before being dried (MgSO_4), filtered and concentrated under reduced pressure to give a dark-brown oil. Subjection of this material to flash chromatography (silica, 3:7:0.1 $\rightarrow$ 3:2:0.1 $v/v/v$ ethyl acetate/hexane/isopropanol gradient elution) and concentration of the relevant fractions (R_f = 0.25 in 2:3 v/v ethyl acetate/hexane) gave the *title compound* **127** (190 mg, 93%) as a white, crystalline solid, m.p. = $92\text{--}93^\circ\text{C}$.

¹H NMR (CDCl₃, 300 MHz) δ 5.81 (m, 1H), 5.13-5.03 (complex m, 2H), 4.93 (d, *J* = 6.8 Hz, 1H), 4.45 (q, *J* = 6.8 Hz, 1H), 3.71 (s, 3H), 3.18 (s, 3H), 2.23 (m, 2H), 1.62 (s, 3H), 1.39 (s, 3H).

¹³C NMR (CDCl₃, 75 MHz) δ 169.9, 134.1, 117.2, 109.9, 77.0, 75.4, 61.2, 35.3, 32.2, 27.3, 25.6.

IR ν_{max}(KBr) 2988, 2941, 1662, 1463, 1431, 1376, 1363, 1254, 1219, 1166, 1096, 1068, 996, 981, 922, 897, 736 cm⁻¹.

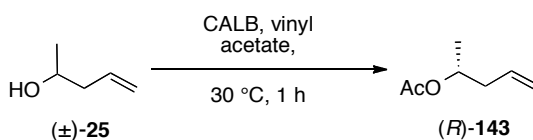
Mass Spectrum (ESI, +ve) *m/z* 252 [(M + Na)⁺, 100%], 172 (11).

HRESIMS Found: (M + Na)⁺, 252.1208. C₁₁H₁₉NO₄ requires (M + Na)⁺, 252.1212.

Specific Rotation: [α]_D = −97.8 (*c* 0.28, CHCl₃).

This compound has been subjected to a single-crystal X-ray analysis. Details are presented in Appendix A3.

(*R*)-(-)-4-Penten-2-yl acetate [(*R*)-143]



Following a procedure reported by Banwell and Loong,^{7,8} a slurry of 4-penten-2-ol [(±)-**25**] (2 g, 23.0 mmol) and vinyl acetate (2.71 mL, 0.87 mmol) was treated with CALB (200 mg of 10% *w/w* material, *ex. Norvo Nordisk*) then agitated vigorously at 30 °C (water bath) for 1 h. The cooled slurry was filtered through a glass frit to remove immobilised enzyme particles which were washed with diethyl ether (2 × 20 mL). The combined filtrates were concentrated under reduced pressure (200 mbar) at 0 °C to provide a yellow oil. Subjection of this material to flash chromatography (silica, 1:9 → 3:7 *v/v* diethyl ether/pentane gradient elution) and concentration of the relevant fractions (*R_f* = 0.8 in 1:9 *v/v* diethyl ether/pentane) under reduced pressure (200 mbar) at 0 °C gave the title compound (*R*)-**143** as an ethereal solution.^{7,8} This material was identical, in all aspects, with an authentic sample and used immediately in the next reaction.

× 10 mL). The combined organic extracts were washed with brine (1 × 20 mL) before being dried (MgSO₄), filtered and concentrated under reduced pressure to give a brown oil. Subjection of this material to flash chromatography (silica, 3:7:0.1 → 3:2:0.1 v/v/v ethyl acetate/hexane/isopropanol gradient elution) afforded two fractions, A and B.

Concentration of fraction A (*R*_f = 0.25 in 2:3 v/v ethyl acetate/hexane) afforded the starting amide **127** (4.5 mg, 15% recovery) as a clear, colourless solid. This material was identical, in all aspects, with an authentic sample.

Concentration of fraction B (*R*_f = 0.25 in 3:2 v/v ethyl acetate/hexane) afforded the *title compound* **129** (26 mg, 56% at 85% conversion) as a cloudy, yellow oil

¹H NMR (CDCl₃, 300 MHz) δ 10.45 (s, 1H), 7.41 (d, *J* = 16.0 Hz, 1H), 6.63 (d, *J* = 2.0 Hz, 1H), 6.36 (d, *J* = 2.0 Hz, 1H), 6.20 (ddd, *J* = 16, 12.6 and 7.1 Hz, 1H), 4.98 (d, *J* = 7.0 Hz, 1H), 4.53 (m, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 3.72 (s, 3H), 3.19 (s, 3H), 2.44 (m, 2H), 1.66 (s, 3H), 1.40 (s, 3H).

¹³C NMR (CDCl₃, 75 MHz) δ 190.8, 170.3, 165.0, 164.8, 143.2, 131.3, 130.5, 116.1, 110.4, 104.5, 97.2, 77.7, 75.7, 61.4, 56.1, 55.8, 34.6, 32.5, 27.5, 26.0.

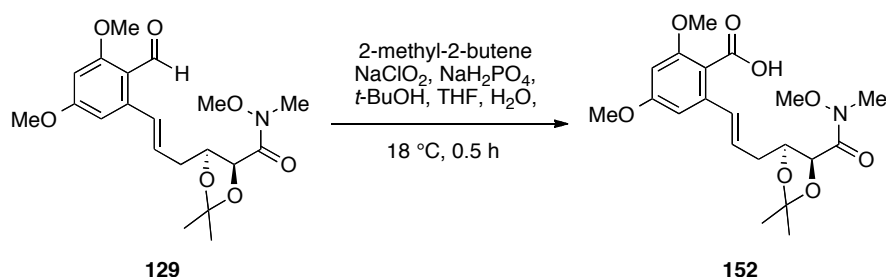
IR ν_{max}(KBr) 2981, 2938, 1677, 1595, 1567, 1459, 1429, 1410, 1379, 1339, 1285, 1238, 1205, 1153, 1073, 987, 831, 731 cm⁻¹.

Mass Spectrum (ESI, +ve) *m/z* 416 [(M + Na)⁺, 100%], 336 (25), 229 (20).

HRESIMS Found: (M + Na)⁺, 416.1685. C₂₀H₂₇NO₇ requires (M + Na)⁺, 416.1685.

Specific Rotation [α]_D = −72.5 (*c* 0.64, CHCl₃).

2,4-Dimethoxy-6-((*E*)-3-((4*S*,5*S*)-5-(methoxy(methyl)carbamoyl)-2,2-dimethyl-1,3-dioxolan-4-yl)prop-1-en-1-yl)benzoic acid (152**)**



A magnetically stirred solution of aldehyde **129** (80 mg, 0.22 mmol) in *t*-butanol (3 mL) maintained at 18 °C under a nitrogen atmosphere was treated with sodium dihydrogen phosphate (0.6 mL of a 2.5 M aqueous solution, 1.51 mmol) and 2-methyl-2-butene (2.2 mL of

a 2.0 M THF solution, 4.31 mmol). The ensuing mixture was then treated, dropwise, with sodium chlorite (0.6 mL of a 3.5 M aqueous solution, 1.51 mmol) and a further 0.5 h after the completion of this addition the reaction mixture was diluted with phosphate buffer (20 mL of a 1.0 M aqueous solution, pH = 4.0 M) and extracted with ethyl acetate (9×10 mL). The combined organic phases were then washed with brine (1×50 mL) before being dried (MgSO_4), filtered and concentrated under reduced pressure to give a light-yellow oil. Subjection of this material to flash chromatography (silica, 2:5:0.1 $\rightarrow$ 7:3:0.1 v/v/v ethyl acetate/hexane/acetic acid gradient elution) and concentration of the relevant fractions ($R_f = 0.25$ in 2:1:0.1 v/v/v ethyl acetate/hexane/acetic acid) afforded the *title compound* **152** (50 mg, 75%) as a clear, colourless oil.

^1H NMR (CDCl_3 , 300 MHz) δ 6.89 (d, $J = 16.0$ Hz, 1H), 6.69 (d, $J = 2.0$ Hz, 1H), 6.38 (d, $J = 2.0$ Hz, 1H), 6.16 (ddd, $J = 16.0$, 12.6 and 7.1 Hz, 1H), 4.96 (d, $J = 7.0$ Hz, 1H), 4.51 (m, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 3.69 (s, 3H), 3.19 (s, 3H), 2.40 (m, 2H), 1.63 (s, 3H), 1.38 (s, 3H) (signal due to carboxylic acid group proton not observed).

^{13}C NMR (CDCl_3 , 75 MHz) δ 169.9, 169.2, 162.0, 158.7, 140.8, 130.4, 129.1, 112.4, 110.0, 103.6, 97.6, 77.2, 76.5, 75.3, 56.2, 55.4, 34.3, 32.2, 27.2, 25.6.

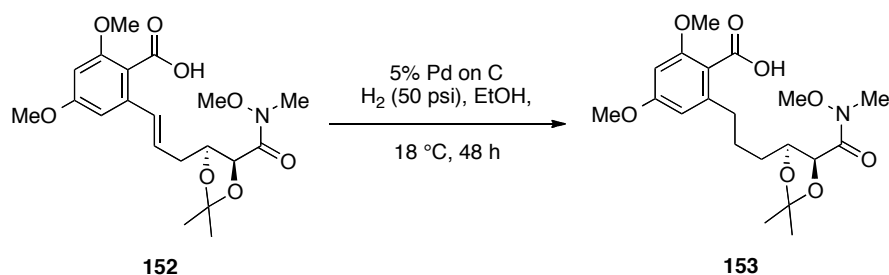
IR ν_{max} (KBr) 2984, 2940, 1724, 1682, 1600, 1578, 1459, 1425, 1380, 1325, 1204, 1161, 1075, 986, 732 cm^{-1} .

Mass Spectrum (ESI, -ve) m/z 442 (11%), $[(M - H)^-]$, 408 (100), 276 (3), 258 (3), 219 (32).

HRESIMS Found: $(M - H)^-$, 408.1660. $\text{C}_{20}\text{H}_{27}\text{NO}_8$ requires $(M - H)^-$, 408.1658.

Specific Rotation $[\alpha]_D = -60.3$ (c 0.27, CHCl_3).

2,4-Dimethoxy-6-(3-((4*S*,5*S*)-5-(methoxy(methyl)carbamoyl)-2,2-dimethyl-1,3-dioxolan-4-yl)propyl)benzoic acid (153**)**



A solution of carboxylic acid **152** (100 mg, 0.24 mmol) in ethanol (5 mL) maintained at 18 °C in a Parr hydrogenator was treated with palladium on charcoal (10 mg of 10% w/w material). The reaction vessel was then evacuated and refilled with dihydrogen three times prior to the contents being shaken under a dihydrogen atmosphere (50 psi) at 18 °C for 48 h. The reaction

mixture was then filtered through a pad of Celite™ that was washed with ethanol (2 × 10 mL). The combined filtrates were concentrated under reduced pressure to give a light-yellow oil that was subjected to flash chromatography (silica, 2:5:0.1 → 7:3:0.1 v/v/v ethyl acetate/hexane/acetic acid gradient elution). Concentration of the relevant fractions ($R_f = 0.25$ in 2:1:0.1 v/v/v ethyl acetate/hexane/acetic acid) gave the *title acid* **153** (100 mg, 97%) as a clear, colourless oil.

^1H NMR (CDCl_3 , 300 MHz) δ 6.43 (d, $J = 2.0$ Hz, 1H), 6.37 (d, $J = 2.0$ Hz, 1H), 4.93 (d, $J = 7.0$ Hz, 1H), 4.39 (m, 1H), 3.92 (s, 3H), 3.83 (s, 3H), 3.68 (s, 3H), 3.12 (s, 3H), 2.99 (m, 1H), 2.80 (m, 1H), 1.75 (m, 3H), 1.53 (s, 3H), 1.50 (m, 2H), 1.37 (s, 3H).

^{13}C NMR (CDCl_3 , 75 MHz) δ 170.5, 170.2, 161.9, 158.8, 145.2, 114.0, 109.9, 107.1, 96.5, 77.6, 75.5, 61.1, 56.2, 55.4, 34.1, 32.2, 30.1, 27.9, 27.2, 25.7.

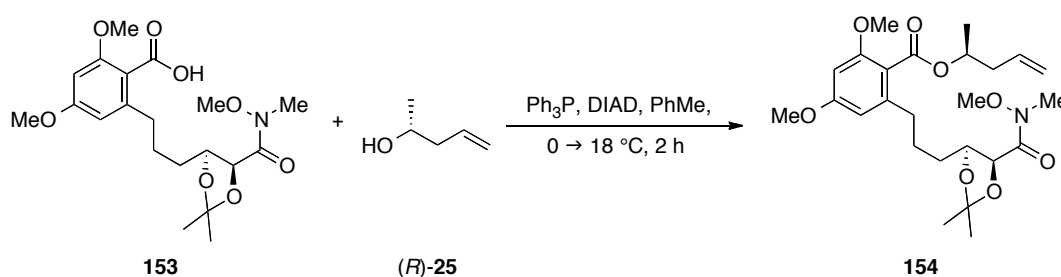
IR ν_{max} (KBr) 3174, 2982, 2940, 1725, 1682, 1603, 1460, 1380, 1328, 1204, 1160, 1091, 1073, 987, 731 cm^{-1} .

Mass Spectrum (ESI, -ve) m/z 410 $[(\text{M} - \text{H})^-]$, 100%, 278 (11), 260 (13), 219 (12).

HRESIMS Found: $(\text{M} - \text{H})^-$, 410.1816. $\text{C}_{20}\text{H}_{29}\text{NO}_8$ requires $(\text{M} - \text{H})^-$, 410.1815.

Specific Rotation $[\alpha]_{\text{D}} = -20.7$ (c 0.44, CHCl_3).

(S)-Pent-4-en-2-yl 2,4-dimethoxy-6-(3-((4S,5S)-5-(methoxy(methyl)carbamoyl)-2,2-dimethyl-1,3-dioxolan-4-yl)propyl)benzoate (154)



A magnetically stirred solution of carboxylic acid **153** (50 mg, 0.1 mmol) in dry toluene (2 mL) maintained at $18\text{ }^\circ\text{C}$ under a nitrogen atmosphere was treated with triphenylphosphine (50 mg, 0.2 mmol) and (R) -**25** (0.02 mL, 0.2 mmol) then cooled to $0\text{ }^\circ\text{C}$. After 0.2 h the reaction mixture was treated, dropwise, with diisopropyl azodicarboxylate (0.04 mL, 0.2 mmol) and then the reaction mixture was warmed to $18\text{ }^\circ\text{C}$. After a further 2 h the reaction mixture was concentrated under reduced pressure to give a yellow resin that was subjected to flash chromatography (silica, 3:7 → 7:3 v/v ethyl acetate/hexane gradient elution). Concentration of the relevant fractions ($R_f = 0.4$ in 2:3 v/v ethyl acetate/hexane) then gave the *title compound* **154** (50 mg, 90%) as a clear, light-yellow oil.

^1H NMR (CDCl_3 , 300 MHz) δ 6.31 (m, 2H), 5.83 (m, 1H), 5.23–5.05 (complex m, 3H), 4.91 (d, $J = 7.0$ Hz, 1H), 4.36 (m, 1H), 3.88 (s, 3H), 3.77 (s, 3H), 3.67 (s, 3H), 3.16 (s, 3H), 2.57 (t, $J = 7.0$ Hz, 2H), 2.50–2.30 (complex m, 2H), 1.83 (m, 1H), 1.61 (s, 3H), 1.46 (m, 2H), 1.37 (s, 3H), 1.32 (d, $J = 7.0$ Hz, 3H), 1.25 (m, 1H).

^{13}C NMR (CDCl_3 , 75 MHz) δ 170.4, 167.8, 161.2, 157.8, 141.8, 133.8, 117.6, 116.8, 109.8, 105.6, 96.3, 77.5, 76.8, 75.6, 70.9, 55.8, 55.3, 40.2, 33.4, 32.2, 30.2, 27.8, 27.3, 25.7, 19.4.

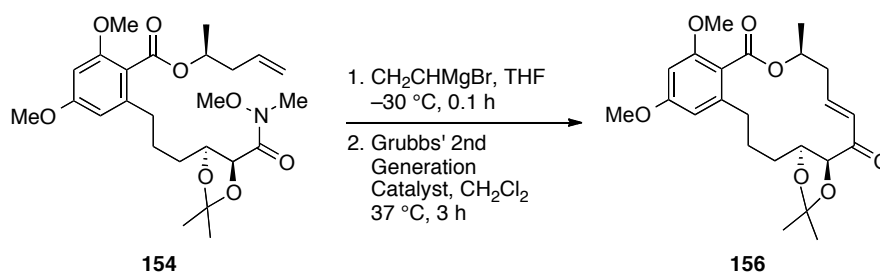
IR ν_{max} (KBr) 2979, 2938, 1720, 1687, 1604, 1586, 1459, 1379, 1341, 1262, 1204, 1160, 1099, 1073, 991 cm^{-1} .

Mass Spectrum (ESI, +ve) m/z 502 $[(\text{M} + \text{Na})^+]$, 100%, 394 (22), 336 (42).

HRESIMS Found: $(\text{M} + \text{Na})^+$, 502.2417. $\text{C}_{25}\text{H}_{37}\text{NO}_8$ requires $(\text{M} + \text{Na})^+$, 502.2417.

Specific Rotation $[\alpha]_{\text{D}} = -32.0$ (c 0.24, CHCl_3).

(3a*S*,8*R*,17a*S*,*E*)-11,13-Dimethoxy-2,2,8-trimethyl-7,8,15,16,17,17a-hexahydro-3a*H*-benzo[*c*][1,3]dioxolo[4,5-*h*][1]oxacyclotetradecine-4,10-dione (156)



A magnetically stirred solution of amide **154** (40 mg, 0.075 mmol) in dry THF (0.8 mL) maintained under a nitrogen atmosphere was cooled to -30°C and then treated, dropwise, with vinylmagnesium bromide (0.30 mL of a 1.0 M solution in THF, 0.3 mmol, *ex* Aldrich). After a further 0.1 h the reaction mixture was treated with acetic acid/water (5 mL of a 5:1 *v/v* mixture) then warmed to 18°C , treated with NH_4Cl (5 mL of a saturated aqueous solution) and extracted with diethyl ether (4×10 mL). The combined organic phases were then dried (Na_2SO_4), filtered and concentrated under reduced pressure to afford a light-yellow oil presumed to contain the *enone* **132**. This oil was dissolved in dry dichloromethane (37 mL) and the reaction vessel containing the resulting solution was evacuated then filled with nitrogen. This process was repeated twice more before Grubbs' 2nd generation catalyst¹⁰ (4 mg, 0.001 mmol, *ex* Aldrich) was added and the ensuing mixture heated at reflux for 19 h. The cooled reaction mixture was concentrated under reduced pressure to give a light-brown oil that was subjected to flash chromatography (silica, 1:4:0.1 $\rightarrow$ 1:1:0.1 *v/v/v* ethyl acetate/hexane/isopropanol gradient

elution). Concentration of the appropriate fractions ($R_f = 0.45$ in 2:1 v/v ethyl acetate/hexane) then gave the *title compound* **156** (20 mg, 48% from **154**) as a clear, colourless oil.

^1H NMR (CDCl_3 , 300 MHz) δ 6.95 (ddd, $J = 15.8, 12.8$ and 6.8 Hz, 1H), 6.64 (d, $J = 15.8$ Hz, 1H), 6.29 (s, 2H), 5.43 (m, 1H), 4.73 (d, $J = 4.6$ Hz, 1H), 4.49 (m, 1H), 3.80 (s, 3H), 3.78 (s, 3H), 2.63 (m, 1H), 2.60–2.50 (complex m, 2H), 2.35 (m, 1H), 1.58 (m, 3H), 1.45 (s, 3H), 1.43 (d, $J = 6.0$ Hz, 3H), 1.34 (s, 3H), 1.25 (m, 1H).

^{13}C NMR (CDCl_3 , 75 MHz) δ 197.4, 168.1, 161.7, 158.4, 144.2, 143.0, 129.1, 116.6, 109.9, 106.1, 96.8, 81.5, 77.7, 69.9, 56.0, 55.6, 39.9, 33.2, 30.6, 27.7, 26.9, 25.1, 21.0.

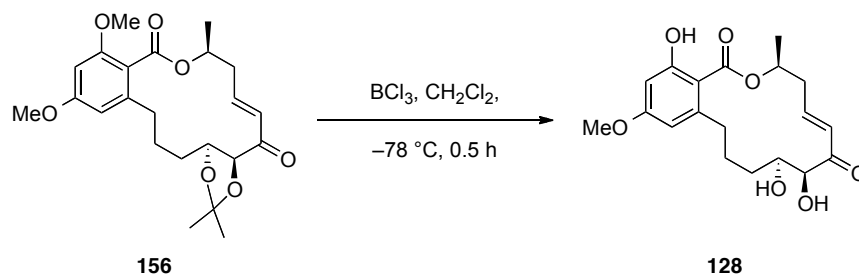
IR ν_{max} (KBr) 2935, 1717, 1690, 1627, 1604, 1457, 1422, 1380, 1320, 1263, 1203, 1160, 1099 cm^{-1} .

Mass Spectrum (ESI, +ve) m/z 441 $[(M + \text{Na})^+]$, 98%, 307 (100).

HRESIMS Found: $(M + \text{Na})^+$, 441.1878. $\text{C}_{23}\text{H}_{30}\text{O}_7$ requires $(M + \text{Na})^+$, 441.1889.

Specific Rotation $[\alpha]_{\text{D}} = -43.5$ (c 0.14, CHCl_3).

L-783,290 (**128**)



A magnetically stirred solution of macrolide **156** (30 mg, 0.06 mmol) in dry dichloromethane (6.2 mL) maintained under nitrogen was cooled to -78°C then treated, dropwise, with boron trichloride (0.25 mL of a 1.0 M solution in dichloromethane, 0.25 mmol). After 0.5 h the reaction mixture was treated, dropwise, with isopropanol (5 mL) and phosphate buffer (5 mL of 1M aqueous solution, pH = 6.0) before being slowly warmed to 18°C then diluted with dichloromethane (10 mL). The separated aqueous phase was extracted with dichloromethane (10×5 mL) and the combined organic phases were then dried (MgSO_4), filtered and concentrated under reduced pressure to yield a light-yellow solid that was subjected to semi-preparative HPLC (22×250 mm 5 μm C18 Alltima column, 0.1:100:150 v/v/v acetic acid/water/methanol as eluent, flow rate of 11.5 mL/min). Concentration of the relevant fractions ($R_t = 22.57$ min) then gave the *title compound* **128**¹¹ (16 mg, 60%) as a white, crystalline solid, m.p. = $127.5\text{--}136.5^\circ\text{C}$.

¹H NMR (CD₂Cl₂, 800 MHz) δ 11.83 (s, 1H), 7.00 (dt, *J* = 15.9 and 8.0 Hz, 1H), 6.40 (d, *J* = 15.9 Hz, 1H), 6.33 (d, *J* = 5.3 Hz, 2H), 5.57 (m, 1H), 4.68 (s, 1H), 3.96 (s, 1H), 3.80 (s, 3H), 3.06 (t, *J* = 12.8 Hz, 1H), 2.84 (broad s, 1H), 2.54 (m, 2H), 1.71 (broad s, 2H), 1.62 (s, 1H), 1.45 (d, *J* = 6.1 Hz, 3H), 1.30 (m, 1H) (signals due to two protons not observed).

¹³C NMR (CD₂Cl₂, 200 MHz) δ 199.3, 171.4, 166.3, 164.7, 147.7, 143.8, 131.5, 109.5, 104.9, 99.3, 77.4, 73.3, 71.3, 55.7, 38.0, 36.2, 32.8, 26.9, 19.2.

IR ν_{max}(KBr) 3347, 2938, 1695, 1642, 1615, 1353, 1316, 1250, 1202, 1161, 1131, 1111, 1089, 1045, 995 cm⁻¹.

Mass Spectrum (EI, 70 eV) *m/z* 364 (M⁺, 17%), 221 (80), 202 (43), 193 (58), 192 (100), 177 (48), 164 (75).

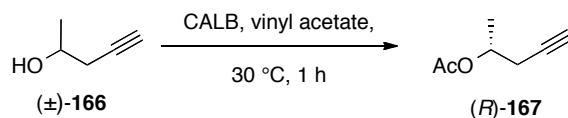
HRESIMS Found: M⁺, 364.1532. C₁₉H₂₄O₇ requires M⁺, 364.1522.

Specific Rotation: [α]_D = + 9.7 (*c* 0.23, CHCl₃).

This compound has been subjected to a single-crystal X-ray analysis. Details are presented in Appendix A4.

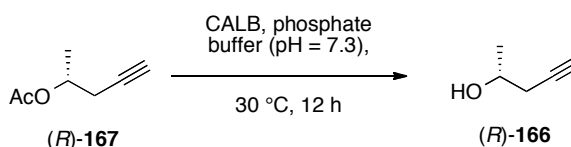
6.3 Experimental Procedures Associated with Work Described in Chapter Three

(R)-(-)-4-Pentyn-2-yl acetate [(R)-167]



A modification of a procedure reported by Banwell and Loong was used.^{7,8} Thus, a slurry of 4-pentyn-2-ol [(±)-166] (2 g, 26.75 mmol) and CALB (0.2 g of 10% w/w material, ex. Norvo Nordisk) in vinyl acetate (2.4 mL, 23.11 mmol) was vigorously agitated at 30 °C (water bath) for 1 h. The cooled reaction mixture was filtered through a pad of Celite™ and the immobilised enzyme particles thus retained were washed with diethyl ether (3 × 20 mL). The combined filtrates were concentrated under reduced pressure (200 mbar) at 0 °C to provide a yellow oil. Subjection of this material to flash chromatography (silica, 1:9 → 3:7 v/v diethyl ether/pentane gradient elution) and concentration of the relevant fraction ($R_f = 0.25$ in 1:9 v/v diethyl ether/pentane) under reduced pressure (200 mbar) at 0 °C gave a light-yellow oil. Distillation (1 atm) of this material then gave the title compound (R)-167¹² (0.96 g, 60%) as a clear, colourless oil, b.p. 110–115 °C (1 atm). GLC analysis of the derived acetate established that this was of 89% ee. This material was identical, in all aspects, with an authentic sample.¹²

R-(-)-4-Pentyn-2-ol [(R)-166]



A modification of a procedure reported by Banwell and Loong was used.^{7,8} Thus, a slurry of CALB (703 mg) and (R)-167 acetate (5.9 g, 0.14 mmol, 89% ee) in phosphate buffer (15 mL of a 0.5 M aqueous solution, pH = 7.3) maintained at 30 °C (water bath) was vigorously agitated for 12 h then cooled to 18 °C and filtered through a pad of Celite™. The filter cake was washed with diethyl ether (5 × 5 mL) and the separated aqueous layer associated with the filtrate was extracted with diethyl ether (10 × 30 mL). The combined organic fractions were washed with NaHCO₃ (2 × 100 mL of a saturated aqueous solution) and brine (1 × 200 mL) before being dried (MgSO₄), filtered and concentrated under reduced pressure (200 mbar) at 0 °C to give a yellow oil. Subjection of this material to flash column chromatography (1:3 v/v diethyl ether/pentane elution) and concentration of the relevant fractions ($R_f = 0.3$ in 1:9 v/v diethyl ether/pentane) under reduced pressure (200 mmbar) at 0 °C afforded a light-yellow oil.

Distillation of this material (1 atm) then gave the title compound [(*R*)-**166**]^{13,14} (3.81 g, 60%), as clear, colourless oil, b.p 127–128 °C (1 atm) (lit.¹⁴ b.p. = 126 °C). GLC analysis of the derived acetate established it was of 98.9% ee. This material was identical, in all aspects, with an authentic sample.^{12,14}

¹H NMR (CDCl₃, 300 MHz) δ 3.97 (m, 1H), 2.45–2.28 (complex m, 2H), 2.08–2.05 (complex m, 1H), 1.93 (s, 1H), 1.27 (d, *J* = 6.2 Hz, 3H).

¹³C NMR (CDCl₃, 75 MHz) δ 80.8, 70.7, 66.1, 28.9, 22.2.

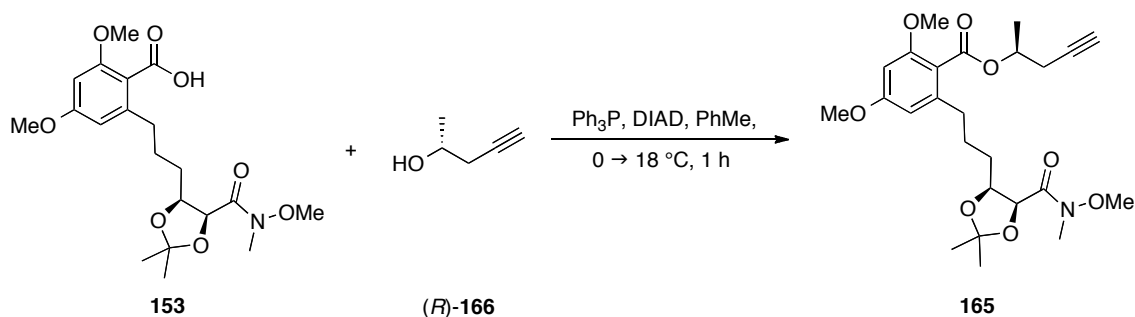
IR ν_{max}(KBr) 3921, 3299, 2973, 2932, 2914, 2590, 2119, 1718, 1640, 1458, 1421, 1377, 1350, 1311, 1267, 1216, 1116, 1087, 1067, 958, 936, 913, 886, 829, 793 cm⁻¹.

Mass Spectrum (EI, 70 eV) *m/z* 84 (M⁺, <1%), 69 (11), 45 (100), 39 (36).

HRESIMS Found: M⁺, 84.0570. C₅H₈O requires M⁺, 84.0575.

Specific Rotation: [α]_D = –16.3 (0.46, CHCl₃) {lit.¹⁴ [α]_D = –17.7 (0.13, CHCl₃)}.

(*S*)-Pent-4-yn-2-yl 2,4-dimethoxy-6-(3-((4*S*,5*S*)-5-(methoxy(methyl)carbamoyl)-2,2-dimethyl-1,3-dioxolan-4-yl)propyl)benzoate (165**)**



A magnetically stirred solution of carboxylic acid **153** (200 mg, 0.48 mmol) in dry toluene (49 mL) maintained at 18 °C under a nitrogen atmosphere was treated with triphenylphosphine (252 mg, 0.96 mmol) and (*R*)-(-)-4-pentyn-2-ol [(*R*)-**166**] (0.02 mL, 0.2 mmol). The ensuing mixture was cooled to 0 °C and after 0.2 h the reaction mixture was treated, dropwise, with diisopropyl azodicarboxylate (0.19 mL, 0.96 mmol) then warmed to 18 °C. After 1 h the reaction mixture was concentrated under reduced pressure to give a yellow resin. Subjection of this material to flash chromatography (silica, 3:7 → 7:3 v/v ethyl acetate/hexane gradient elution) and concentration of the relevant fractions (*R*_f = 0.4 in 2:3 v/v ethyl acetate/hexane) afforded the *title compound* **165** (162 mg, 70%) as a clear, light-yellow oil.

¹H NMR (CDCl₃, 800 MHz): δ 6.32 (d, *J* = 2.2 Hz, 1H), 6.30 (d, *J* = 2.2 Hz, 1H), 5.25 (m, 1H), 4.91 (d, *J* = 16.4 Hz, 1H), 4.36 (ddd, *J* = 9.6, 6.7 and 3.7 Hz, 1H), 3.80 (s, 3H), 3.78 (s, 3H),

3.68 (s, 3H), 3.17 (s, 3H), 2.65–2.48 (complex m, 4H), 2.04 (t, $J = 2.7$ Hz, 1H), 1.85 (m, 1H), 1.64 (m, 1H), 1.61 (s, 3H), 1.43 (d, $J = 6.2$ Hz, 3H), 1.40–1.50 (complex m, 2H), 1.37 (s, 3H).

^{13}C NMR (CDCl_3 , 100 MHz): δ 170.4, 167.5, 161.3, 158.0, 142.0, 116.4, 109.8, 105.7, 96.3, 79.9, 77.4, 75.6, 70.7, 69.3, 61.1, 55.8, 55.3, 33.4, 32.2, 30.3, 27.8, 27.2, 25.6, 25.5, 19.0.

IR ν_{max} (KBr) 2979, 2938, 1720, 1687, 1604, 1586, 1459, 1379, 1341, 1262, 1204, 1160, 1099, 1073, 991 cm^{-1} .

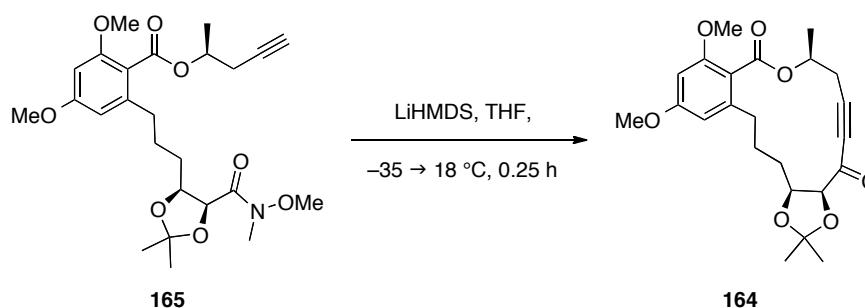
Mass Spectrum (ESI, +ve), m/z 500 $[(\text{M} + \text{Na})^+]$, 58%], 497 (13), 478 $[(\text{M} + \text{H})^+]$, 9%], 394 (62), 376 (11), 336 (100).

HRESIMS Found: $(\text{M} + \text{H})^+$, 478.2446. $\text{C}_{25}\text{H}_{35}\text{NO}_7$ requires $(\text{M} + \text{H})^+$, 478.2441.

Found: $(\text{M} + \text{Na})^+$, 500.2262. $\text{C}_{25}\text{H}_{35}\text{NO}_7$ requires $(\text{M} + \text{Na})^+$, 500.2260.

Specific Rotation: $[\alpha]_{\text{D}} = -20.2$ (c 0.4, CDCl_3).

(3a*S*,8*S*,17a*S*)-11,13-Dimethoxy-2,2,8-trimethyl-5,6-didehydro-7,8,15,16,17,17a-hexahydro-3a*H*-benzo[*c*][1,3]dioxolo[4,5-*h*][1]oxacyclotetradecine-4,10-dione (164)



A magnetically stirred solution of lithium bis(trimethylsilyl)amide (1.63 mL of a 1.0 M solution in THF, 1.63 mmol) in dry THF (2.17 mL) maintained at $-35\text{ }^\circ\text{C}$ under a nitrogen atmosphere was treated, dropwise, with a solution of ester **165** (27 mg, 0.06 mmol) in dry THF (33 mL). After completion of the addition the reaction mixture was warmed to and then maintained at $18\text{ }^\circ\text{C}$ for 0.25 h. At the end of this period it was treated with acetic acid/water (2 mL of 5:1 v/v mixture), phosphate buffer (1 $\times$ 100 mL of a 1 M aqueous solution, pH = 6.0) and extracted with diethyl ether (3 $\times$ 50 mL). The combined organic extracts were washed with water (3 $\times$ 100 mL) and brine (2 $\times$ 100 mL) before being dried (Na_2SO_4), filtered and concentrated under pressure at $18\text{ }^\circ\text{C}$ to give a light-yellow oil. Subjection of this material to flash chromatography (silica, 1:1 $\rightarrow$ 3:1 v/v diethyl ether/pentane gradient elution) and concentration of the relevant fractions ($R_{\text{f}} = 0.8$ in 0.9:1:0.1 v/v/v ethyl acetate/hexane/acetic acid) afforded the *title compound* **164** (9 mg, 45%) as a white, crystalline solid, m.p. = $125.6\text{--}132.1\text{ }^\circ\text{C}$.

¹H NMR (CDCl₃, 800 MHz): δ 6.33 (s, 2H), 5.30 (m, 1H), 4.68 (d, J = 6.4 Hz, 1H), 4.31 (m, 1H), 3.82 (s, 3H), 3.80 (s, 3H), 2.82 (dd, J = 17.4 and 3.3 Hz, 1H), 2.74–2.70 (complex m, 1H), 2.71 (dd, J = 17.4 and 3.3 Hz, 2 H), 2.64 (m, 1H), 1.81–1.78 (complex m, 3H), 1.57 (s, 3H), 1.48 (d, J = 6.2 Hz, 3H), 1.36 (s, 3H).

¹³C NMR (CDCl₃, 75 MHz) δ 184.7, 167.0, 161.7, 158.6, 142.7, 115.7, 110.0, 106.1, 96.7, 93.4, 83.2, 81.5, 78.6, 69.2, 55.9, 55.4, 33.8, 29.7, 28.8, 26.8, 26.4, 25.7, 19.5

IR ν_{max} (KBr) 2260, 2925, 2853, 2215, 1721, 1683, 1604, 1586, 1458, 1422, 1380, 1370, 1341, 13419, 1260, 1223, 1203, 1160, 1095, 1044, 831, 733

Mass Spectrum (ESI, +ve), m/z 440 [(M + Na)⁺, 100%], 418 [(M + H)⁺, 44%], 359 (31), 341 (13), 315 (6), 235 (14), 207 (7), 177 (8), 85 (25).

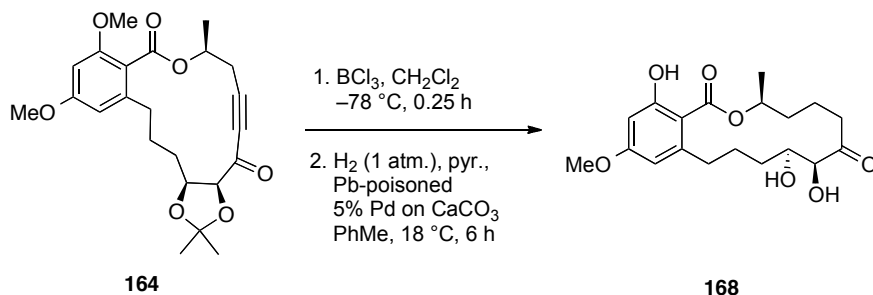
HRESIMS Found: (M + H)⁺, 417.1913. C₂₃H₂₈O₇ requires (M + H)⁺, 417.1914.

Found: (M + Na)⁺, 439.1732. C₂₃H₂₈O₇ requires (M + Na)⁺, 439.1733.

Specific Rotation: $[\alpha]_{\text{D}} = +103.6$ (c 0.05, CHCl₃).

This compound has been subjected to a single-crystal X-ray analysis. Details are presented in Appendix A5.

(3*S*,8*S*,9*S*)-8,9,16-Trihydroxy-14-methoxy-3-methyl-3,4,5,6,9,10,11,12-octahydro-1*H*-benzo[*c*][1]oxacyclotetradecine-1,7(8*H*)-dione (168)



A magnetically stirred solution of macro lactone **164** (8 mg, 0.014 mmol) in dry dichloromethane (2 mL) maintained at $-78\text{ }^\circ\text{C}$ under nitrogen atmosphere was treated, dropwise, with boron trichloride (0.07 mL of a 1.0 M solution in dichloromethane, 0.07 mmol). After 0.5 h the reaction mixture was treated, dropwise, with isopropanol (2 mL) and phosphate buffer (10 mL of a 1 M aqueous solution, pH = 6.0) before being warmed to $18\text{ }^\circ\text{C}$. The separated aqueous was extracted with dichloromethane ($3 \times 5\text{ mL}$) and the combined organic phases were then dried (Na₂SO₄), filtered and concentrated under reduced pressure at $18\text{ }^\circ\text{C}$ to afford a pale-yellow solid that was presumed to be the deprotected material. This solid was dissolved in dry toluene (2.5 mL) and the resulting solution treated with freshly distilled

pyridine (0.02 mL, 0.22 mmol) and 5% palladium on calcium carbonate; poisoned with lead (1 mg of 10% w/w material, *ex. Aldrich*). The resulting black slurry was evacuated and refilled with dihydrogen three times prior to the contents being stirred under a hydrogen atmosphere (1 atm) at 18 °C for 10 h. The reaction was then filtered through a pad of Celite™ that was washed with acetonitrile (2 × 5 mL). The combined filtrates were concentrated under reduced pressure at 18 °C to give a light-yellow solid that was subjected to flash chromatography (silica, 6.9:3:0.1 v/v/v ethyl acetate/hexane/isopropanol). Concentration of the relevant fractions (R_f = 0.2 in 1:1 v/v ethyl acetate/hexane) gave the *title compound* **168** (4 mg, 60%) as a white crystalline, solid, m.p. = 113–133.0 °C.

^1H NMR (CD_2Cl_2 , 800 MHz) δ 12.20 (s, 1H), 6.35 (d, J = 2.7 Hz, 1H), 6.32 (d, J = 2.7 Hz, 1H), 5.22–5.18 (complex m, 1H), 4.39 (dd, J = 5.1 and 2.5 Hz, 1H), 3.97 (s, 1H), 3.82 (s, 2H), 3.62 (d, J = 5.3 Hz, 1H), 3.21 (td, J = 12.3 and 3.0 Hz, 1H), 2.87 (m, 1H), 2.53 (td, J = 12.4 and 6.3 Hz, 1H), 2.36–2.33 (complex m, 1H), 1.86–1.77 (complex m, 3H), 1.76–1.71 (complex m, 5H), 1.37 (d, J = 6.2 Hz, 3H), 1.15 (ddt, J = 14.9, 11.3 and 3.9 Hz, 1H).

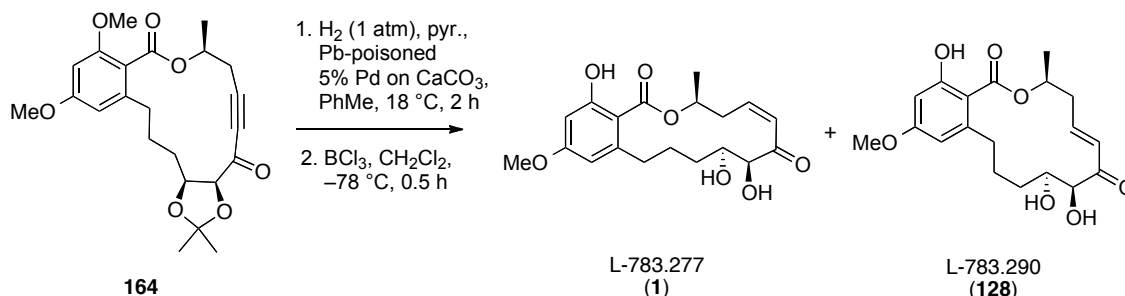
^{13}C NMR (CD_2Cl_2 , 75 MHz) δ 210.2, 172.3, 167.1, 164.7, 148.2, 128.9, 110.6, 99.7, 80.8, 74.0, 73.6, 55.9, 39.8, 37.6, 35.2, 34.1, 30.8, 22.0, 21.2.

IR ν_{max} (KBr) 3346, 2927, 2852, 1699, 1644, 1611, 1576, 1462, 1381, 1353, 1313, 1254, 1205, 1160, 1117, 1054, 976, 956, 872, 809, 738, 710 cm^{-1} .

Mass Spectrum (ESI, +ve), m/z 389 [$(\text{M}+\text{Na})^+$, 100%], 331 (9), 301 (6), 287 (7), 279 (6), 235 (9), 221 (13), 193 (9), 177 (17), 149 (11).

Specific Rotation: $[\alpha]_{\text{D}} = +103.6$ (c 0.05, CHCl_3).

L-783,277 (**1**) and L-783,290 (**128**)



A magnetically stirred solution of macro lactone **164** (9 mg, 0.06 mmol) in dry toluene (6 mL) maintained at 18 °C was treated with pyridine (0.03 mL, 0.35 mmol) and 5% palladium on calcium carbonate, poisoned with lead (1 mg of 10% w/w material, *ex. Aldrich*). The ensuing black slurry was evacuated and refilled with dihydrogen three times prior to the contents being stirred under a hydrogen atmosphere (1 atm) at 18 °C for 2 h. The reaction mixture was filtered

through a pad of Celite™ that was then washed with acetonitrile (2×10 mL). The combined filtrates were concentrated under reduced pressure at $18\text{ }^{\circ}\text{C}$ to give a light-yellow oil presumed to contain the *cis*-configured enone **167**. This oil was dissolved in dry dichloromethane (6 mL) and the reaction vessel containing the resulting solution was cooled to $-78\text{ }^{\circ}\text{C}$ under nitrogen atmosphere and treated, dropwise, with boron trichloride (0.24 mL of a 1.0 M solution in dichloromethane, 0.24 mmol). After a further 0.5 h the reaction mixture was treated, dropwise, with isopropanol (5 mL) and phosphate buffer (20 mL of a 1.0 M aqueous solution, pH = 6.0) before being warmed to $18\text{ }^{\circ}\text{C}$. The separated aqueous was extracted with dichloromethane (3×10 mL) and the combined organic phases were dried (Na_2SO_4), filtered and concentrated under reduced pressure to afford a light-yellow solid that was subjected to preparative HPLC (22 x 250 mm 5 μm C18 Alltima column, 0.05:65:35 v/v/v acetic acid/water/acetonitrile as eluent, flow rate of 11.5 mL/min) and thus affording two major fractions, A and B.

Fraction A ($R_t = 29.95$ min) had the same retention time as an authentic sample of L-783,290 (**128**¹¹) and was, therefore, presumed to be this compound.

Concentration of fraction B ($R_t = 38.50$ min) followed by freeze-drying afforded L-783,277 (**1**¹¹) (6 mg, 40% over 2 steps) as an off-white solid, m.p. = $110.1\text{--}133.1\text{ }^{\circ}\text{C}$.

¹H NMR (CD_2Cl_2 , 800 MHz) δ 12.21 (s, 1H), 6.46 (ddd, $J = 11.2, 3.2$ and 0.8 Hz, 1H), 6.39 (d, $J = 2.4$ Hz, 1H), 6.36 (d, $J = 2.4$ Hz, 1H), 6.32 (td, $J = 11.2$ and 2.4 Hz, 1H), 5.48 (m, 1H), 4.57 (d, $J = 2.4$ Hz, 1H), 3.87 (m, 1H), 3.86 (s, 3H), 3.41 (m, 1H), 3.03 (m, 1H), 2.62 (dm, $J = 17.6$ Hz, 1H), 2.55 (m, 1H), 1.79 (m, 1H), 1.59 (m, 2H), 1.49 (d, $J = 5.6$ Hz, 3H) (signals due to aliphatic hydroxyl groups not observed).

¹³C NMR (CD_2Cl_2 , 100 MHz) δ 200.4, 172.2, 167.0, 164.8, 148.0, 146.7, 126.7, 109.7, 104.9, 99.4, 81.6, 74.0, 73.6, 55.9, 37.6, 37.0, 33.5, 29.4, 21.2.

IR ν_{max} (KBr) 3315, 2921, 2850, 1727, 1683, 1648, 1615, 1568, 1461, 1420, 1379, 1350, 1313, 1291, 1252, 1220, 1203, 1053, 1038, 1016, 902, 870, 838, 795, 748, 712 cm^{-1} .

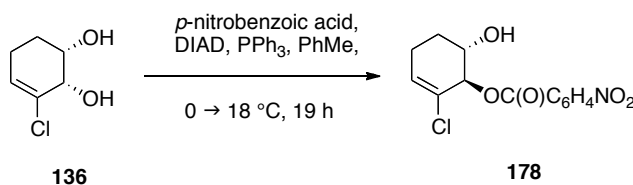
Mass Spectrum (ESI, +ve), m/z 387 [$(\text{M} + \text{Na})^+$, 38%], 369 (13), 301 (6), 221 (5), 149 (10), 79 (100).

HRESIMS Found: $(\text{M} + \text{Na})^+$, 387.1418. $\text{C}_{19}\text{H}_{24}\text{O}_7$ requires $(\text{M} + \text{Na})^+$, 387.1418.

Specific Rotation $[\alpha]_{\text{D}} = +103.6$ (c 0.05, CHCl_3).

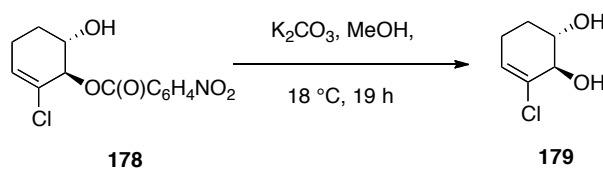
6.4 Experimental Procedures Associated with Work Described in Chapter Four

(1*R*,6*S*)-2-Chloro-6-hydroxycyclohex-2-en-1-yl 4-nitrobenzoate (**178**)



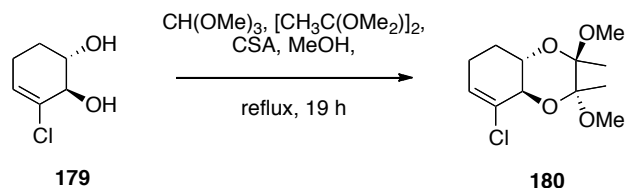
Following a procedure reported by Banwell and Loong,⁵ a magnetically stirred slurry of diol **136** (2.64 g, 17.7 mmol) in dry toluene (250 mL) maintained under a nitrogen atmosphere at 0 °C was treated with 4-nitrobenzoic acid (9.90 g, 53.24 mmol) and triphenylphosphine (6.98 g, 26.62 mmol). After 0.2 h the reaction mixture was treated, dropwise, with diisopropyl azodicarboxylate (4.55 mL, 24.85 mmol) then warmed to 18 °C over 19 h and concentrated under reduced pressure to give a yellow paste. Subjection of this material to flash chromatography (silica, 1:9 → 3:5 v/v ethyl acetate/hexane gradient elution) and concentration of the relevant fractions (R_f = 0.2 in 1:1 v/v ethyl acetate/hexane) gave the title compound **178**⁵ (4.13 g, 77%) as a yellow resin. This material was identical, in all aspects, with an authentic sample.⁵

(1*S*,2*R*)-3-Chlorocyclohex-3-ene-1,2-diol (**179**)



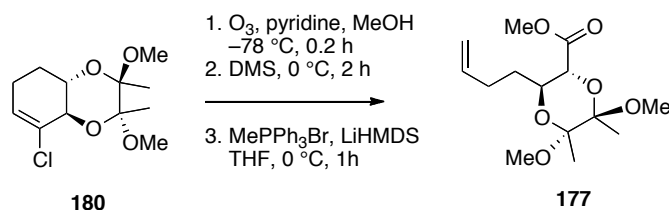
Following a procedure reported by Banwell and Loong,⁵ a magnetically stirred solution of nitrobenzoate **178** (4.12 g, 13.56 mmol) in methanol (215 mL) maintained at 18 °C was treated with potassium carbonate (7.49 g, 54.24 mmol). After 19 h the reaction mixture was treated with silica gel (8 g, type 60) and the mixture so-formed was concentrated under reduced pressure to give a free-flowing yellow powder. This powder was added to the top of a flash chromatography column (silica, 7:13 → 4:1 v/v ethyl acetate/hexane gradient elution) and concentration of the relevant fractions (R_f = 0.3 in diethyl ether) afforded the title compound **179**⁵ (1.4 g, 68%) as a white, crystalline solid, m.p. = 69.7–71.4 °C (lit.⁵ m.p. = 71–71.5 °C). This material was identical, in all aspects, with an authentic sample.⁵

(2*R*,3*R*,4*aS*,8*aR*)-8-Chloro-2,3-dimethoxy-2,3-dimethyl-2,3,4*a*,5,6,8*a*-hexahydrobenzo[*b*][1,4]dioxine (180).



Following a procedure reported by Banwell and Loong,¹⁵ a magnetically stirred solution of *trans*-diol **179** (1.46 g, 9.85 mmol) in methanol (73 mL) maintained at 18 °C under a nitrogen atmosphere was treated with trimethyl orthoformate (4.3 mL, 39.38 mmol), $[\text{CH}_3\text{C(OMe)}_2]_2$ (2.11 g, 11.82 mmol) and (+)-camphorsulfonic acid (0.14 g, 0.59 mmol) then heated at reflux for 19 h. The cooled reaction mixture was quenched with NaHCO_3 (1 × 200 mL of a saturated aqueous solution) and extracted with diethyl ether (3 × 200 mL). The combined organic phases were washed with brine (1 × 200 mL) then dried (MgSO_4), filtered and concentrated under reduced pressure to give a brown oil. Subjection of this material to flash chromatography (silica, 0:100 → 1:9 v/v ethyl acetate/hexane gradient elution) and concentration of the relevant fractions ($R_f = 0.4$ in 1:9 v/v ethyl acetate/hexane) gave the title compound **180**¹⁵ (2.19 g, 85%) as a white, crystalline solid, m.p. = 74.5–76.1 °C (lit.¹⁵ m.p. = 78–81 °C). This material was identical, in all aspects, with an authentic sample.¹⁵

(2*R*,3*S*,5*R*,6*R*)-Methyl 3-(but-3-en-1-yl)-5,6-dimethoxy-5,6-dimethyl-1,4-dioxane-2-carboxylate (177)

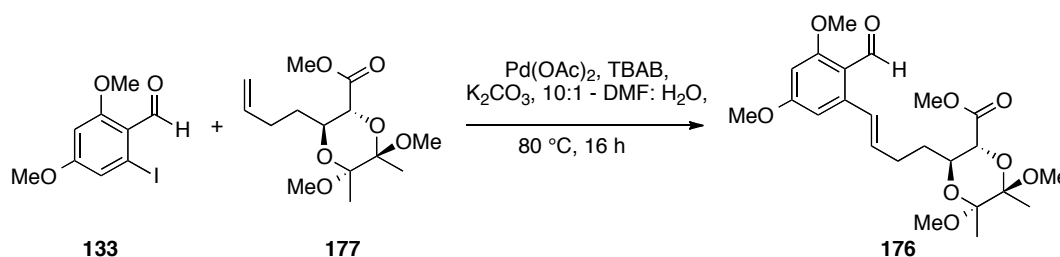


Following a procedure established by Banwell and Loong,¹⁵ a magnetically stirred solution of alkene **180** (1.2 g, 4.58 mmol) in methanol (200 mL) maintained at $-78\text{ }^\circ\text{C}$ was treated with pyridine (3.69 mL, 45.8 mmol) and then with a stream of ozone until a persistent blue colour was observed. After a further 0.4 h the excess ozone was removed with a stream of nitrogen and the resulting colourless solution was treated with dimethyl sulfide (6.8 mL, 91.6 mmol) and allowed to warm to $0\text{ }^\circ\text{C}$. After 2 h the reaction mixture was warmed to $18\text{ }^\circ\text{C}$ then diluted with water (1 × 250 mL) and the separated aqueous layer was extracted with ethyl acetate (6 × 100 mL). The combined organic extracts were washed with water (2 × 100 mL) and brine (1 × 200 mL) before being dried (MgSO_4), filtered and concentrated under reduced pressure to give

aldehyde **181** as an unstable, yellow oil. This material was used immediately in the next step of the reaction sequence.

A magnetically stirred solution of methyltriphenylphosphonium bromide (2.4 g, 6.87 mmol) in dry THF (30 mL) maintained at 0 °C under a nitrogen atmosphere was treated with potassium bis(trimethylsilyl)amide (13.73 mL of a 0.5 M solution in toluene, 6.4 mmol). The resulting yellow mixture was allowed to warm to 18 °C over 0.5 h then re-cooled to 0 °C and treated, *via* cannula, with a solution of aldehyde **181** in THF (30 mL). After 19 h the reaction mixture was treated with NH₄Cl (1 × 150 mL of a saturated aqueous solution) and the separated aqueous phase was extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed with water (2 × 100 mL) and brine (1 × 100 mL) before being dried (MgSO₄), filtered and concentrated under reduced pressure to give a yellow oil. Subjection of this material to flash chromatography (silica, 1:20 → 1:9 v/v ethyl acetate/hexane gradient elution) and concentration of the relevant fractions (R_f = 0.5 in 1:3 v/v ethyl acetate/hexane) gave the title compound **177**¹⁵ (0.79 g, 60%) as a clear, light-yellow oil. This material was identical, in all aspects, with an authentic sample.¹⁵

(2*R*,3*S*,5*R*,6*R*)-Methyl 3-((*E*)-4-(2-formyl-3,5-dimethoxyphenyl)but-3-en-1-yl)-5,6-dimethoxy-5,6-dimethyl-1,4-dioxane-2-carboxylate (176**)**



A magnetically stirred solution of aryl iodide **133** (80 mg, 0.27 mmol) and alkene **177** (60 mg, 0.021 mmol) in DMF/water (2 mL of 10:1 v/v mixture) maintained at 18 °C was treated with tetra-*n*-butylammonium bromide (74 mg, 0.23 mmol) and potassium carbonate (72 mg, 0.53 mmol). The ensuing mixture was evacuated and filled with argon three times before being treated with palladium acetate (9 mg, 0.02 mmol) in three portions over 0.5 h and then heated to 80 °C for 16 h. The reaction mixture was then cooled and filtered through a pad of Celite™ that was washed with ethyl acetate (3 × 20 mL). The combined filtrates were washed with HCl (1 × 15 mL of a 1 M aqueous solution) and the separated aqueous phase extracted with ethyl acetate (4 × 10 mL). The combined organic extracts were washed with brine (1 × 20 mL) before being dried (MgSO₄), filtered and concentrated under reduced pressure to give a brown oil. Subjection of this material to flash chromatography (silica, 3:7:0.1 → 3:2:0.1 v/v/v ethyl acetate/hexane/isopropanol gradient elution) and concentration of the relevant fractions (R_f =

0.25 in 1:3 v/v ethyl acetate/hexane) afforded the *title compound 176* (76 mg, 81%) as a light-yellow oil.

^1H NMR (CDCl_3 , 300 MHz) δ 10.45 (s, 1H), 7.36 (dd, $J = 15.7$ and 1.2 Hz, 1H), 6.56 (d, $J = 2.3$ Hz, 1H), 6.35 (d, $J = 2.3$ Hz, 1H), 6.10 (ddd, $J = 15.7$, 12.6 and 7.1 Hz, 1H), 4.19 (d, $J = 9.9$ Hz, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 3.78 (s, 3H), 3.73 (t, $J = 3.5$ Hz, 1H), 3.30 (s, 3H), 3.26 (s, 3H), 2.64–2.52 (complex m, 1H), 2.36–2.28 (complex m, 1H), 1.71–1.64 (complex m, 2H), 1.35 (s, 3H), 1.31 (s, 3H).

^{13}C NMR (CDCl_3 , 75 MHz) δ 190.4, 169.3, 164.7, 143.2, 134.1, 129.3, 115.7, 104.0, 98.9, 98.6, 96.7, 72.7, 67.9, 55.8, 55.4, 52.3 (2 overlapping peaks), 48.3, 48.0, 30.1, 28.5, 17.7, 17.4

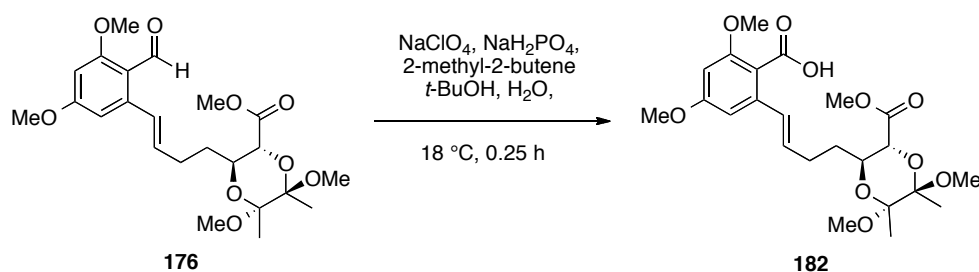
IR ν_{max} (KBr) 3307, 2993, 2950, 2837, 2785, 2332, 1748, 1675, 1595, 1567, 1455, 1431, 1411, 1378, 1327, 1304, 1281, 1235, 1205, 1151, 1142, 1119, 1091, 1051, 1037, 888, 872, 851, 827, 808, 734, 693, 660 cm^{-1} .

Mass Spectrum (ESI, +ve) m/z 475 $[(\text{M} + \text{Na})^+]$, 100%, 389 (53).

HRESIMS Found: $(\text{M} + \text{Na})^+$, 475.1934. $\text{C}_{23}\text{H}_{32}\text{O}_9$ requires $(\text{M} + \text{Na})^+$, 475.1944.

Specific Rotation: $[\alpha]_{\text{D}} = -8.3$ (c 0.09, CHCl_3).

2-((*E*)-4-((2*S*,3*R*,5*R*,6*R*)-5,6-Dimethoxy-3-(methoxycarbonyl)-5,6-dimethyl-1,4-dioxan-2-yl)but-1-en-1-yl)-4,6-dimethoxybenzoic acid (182**)**



A magnetically stirred solution of aldehyde **176** (94 mg, 0.2 mmol) in *t*-butanol (10 mL) maintained at 18 °C under a nitrogen atmosphere was treated with sodium dihydrogen phosphate (1 mL of a 1.4 M aqueous solution, 1.4 mmol) and 2-methyl-2-butene (4 mL of a 2.0 M THF solution, 4.00 mmol). The ensuing mixture was treated, dropwise, with sodium chlorite (1 mL of a 2.0 M aqueous solution, 2.0 mmol). After a further 0.25 h the reaction mixture was treated with phosphate buffer (20 mL of a 1.0 M aqueous solution, pH = 4.0) and extracted with ethyl acetate (9×10 mL). The combined organic phases were then washed with brine (1×50 mL) before being dried (MgSO_4), filtered and concentrated under reduced pressure to give a light-yellow oil. Subjection of this material to flash chromatography (silica, 2:5:0.1 $\rightarrow$ 7:3:0.1 v/v/v ethyl acetate/hexane/acetic acid gradient elution) and concentration of the relevant

fractions ($R_f = 0.25$ in 0.9:1:0.1 v/v/v ethyl acetate/hexane/acetic acid) afforded the *title compound* **182** (80 mg, 80%) as a cloudy, yellow oil.

^1H NMR (CDCl_3 , 400 MHz) δ 9.36 (broad s, 1H), 6.58 (d, $J = 1.4$ Hz, 1H), 6.34 (d, $J = 15.6$ Hz, 1H), 6.36 (d, $J = 1.4$ Hz, 1H), 6.10 (ddd, $J = 15.6, 12.6$ and 7.1 Hz, 1H), 3.91 (complex m, 1H), 3.84 (s, 3H), 3.82 (s, 3H), 3.75 (s, 3H), 3.27 (s, 3H), 3.23 (s, 3H), 2.82 (m, 1H), 2.45 (m, 1H), 2.30 (m, 1H), 1.64 (m, 2H), 1.32 (s, 3H), 1.28 (s, 3H).

^{13}C NMR (CDCl_3 , 75 MHz) δ 177.5, 169.8, 162.3, 158.8, 140.8, 133.6, 128.7, 103.5, 99.3, 99.0, 97.8, 73.0, 68.2, 56.6, 55.8, 52.7, 48.6, 30.3 (2 overlapping peaks), 28.7, 18.0, 17.7.

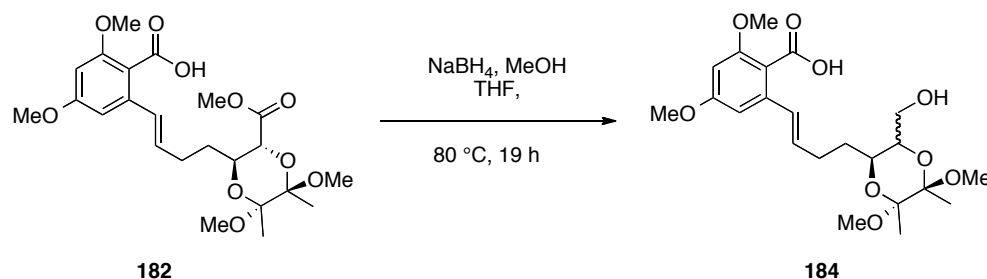
IR ν_{max} (KBr) 2950, 1748, 1675, 1595, 1567, 1455, 1431, 1378, 1327, 1281, 1205, 1151, 1119, 1037, 888, 851 cm^{-1} .

Mass Spectrum (ESI, -ve) m/z 467 [$(\text{M} - \text{H})^-$, 25%], 391 (4), 359 (100).

HRESIMS Found: $(\text{M} - \text{H})^-$, 467.1917. $\text{C}_{23}\text{H}_{31}\text{O}_{10}$ requires $(\text{M} - \text{H})^-$, 467.1917.

Specific Rotation: $[\alpha]_{\text{D}} = -70.9$ (c 0.02, CHCl_3).

2-((*E*)-4-((2*S*,5*R*,6*R*)-3-(Hydroxymethyl)-5,6-dimethoxy-5,6-dimethyl-1,4-dioxan-2-yl)but-1-en-1-yl)-4,6-dimethoxybenzoic acid (184**)**



A magnetically stirred solution of methyl ester **182** (67.5 mg, 0.16 mmol) in dry THF (0.25 mL) maintained at $18\text{ }^\circ\text{C}$ under nitrogen was treated with sodium borohydride (18 mg, 0.48 mmol), and methanol (0.1 mL, 2.88 mmol) and then heated at $80\text{ }^\circ\text{C}$ for 19 h. The cooled reaction mixture was quenched with phosphate buffer (1×20 mL of a 1.0 M aqueous solution, pH = 4.0) and extracted with ethyl acetate (4×10 mL). The combined organic extracts were washed with brine (1×30 mL) before being dried (MgSO_4), filtered and concentrated under reduced pressure to give a yellow oil. Subjection of this material to flash chromatography (silica, 2:5:0.1 $\rightarrow$ 7:3:0.1 v/v/v ethyl acetate/hexane/acetic acid gradient elution) and concentration of relevant

fractions ($R_f = 0.6$ in 0.9:1:0.1 v/v/v ethyl acetate/hexane/acetic acid) afforded the *title compound 184*** (64.1 mg, 90%) as a clear, colourless oil.

^1H NMR (CDCl_3 , 400 MHz) δ 6.82 (d, $J = 15.7$ Hz, 1H), 6.50 (d, $J = 2.3$ Hz, 1H), 6.39 (d, $J = 2.3$ Hz, 1H), 6.08 (ddd, $J = 15.7$, 6.0 and 7.0 Hz, 1H), 3.88 (s, 3H), 3.83 (s, 3H), 3.71 (m, 2H), 3.58 (m, 1H), 3.27 (s, 3H), 3.25 (s, 3H), 2.40 (m, 2H), 2.40 (broad s, 1H), 1.63 (m, 2H), 1.31 (s, 3H), 1.31 (s, 3H) (signal due to carboxylic acid group proton not observed).

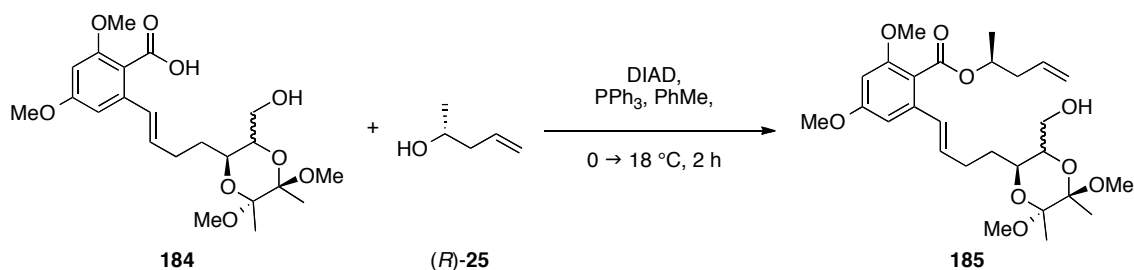
^{13}C NMR (CDCl_3 , 75 MHz) δ 169.3, 161.8, 158.4, 140.8, 134.0, 129.0, 104.0, 98.9, 98.8, 97.6, 72.4, 66.7, 62.1, 56.4, 55.6, 48.1, 48.1, 30.5, 29.8, 28.1, 17.9, 17.7.

IR ν_{max} (KBr) 3185, 2995, 2952, 2838, 2639, 2086, 1901, 1736, 1600, 1580, 1457, 1438, 1378, 1326, 1288, 1204, 1161, 1141, 1120, 1037, 965, 888, 851, 735, 701, 661, 626, 600, 573 cm^{-1} .

Mass Spectrum (ESI, -ve) m/z 439 [$(\text{M} - \text{H})^-$, 100%].

HRESIMS Found: $(\text{M} - \text{H})^-$, 439.1968. $\text{C}_{22}\text{H}_{31}\text{O}_9$ requires $(\text{M} - \text{H})^-$, 439.1968.

(S)-Pent-4-en-2-yl 2-((E)-4-((2S,5R,6R)-3-(hydroxymethyl)-5,6-dimethoxy-5,6-dimethyl-1,4-dioxan-2-yl)but-1-en-1-yl)-4,6-dimethoxybenzoate (185)



A magnetically stirred solution of carboxylic acid **184** (5 mg, 0.02 mmol) in dry toluene (2.7 mL) maintained at 18 °C under a nitrogen atmosphere was treated with triphenylphosphine (14 mg, 0.05 mmol) and (*R*)-(-)-4-penten-2-ol [(*R*)-**25**] (0.03 mL, 0.27 mmol) then cooled to 0 °C. After 0.2 h the reaction mixture was treated, dropwise, with diisopropyl azodicarboxylate (0.01 mL, 0.05 mmol) and after a further 0.25 h the reaction mixture was re-warmed to 18 °C. After 2 h the reaction mixture was concentrated under reduced pressure to give a yellow resin. Subjection of this material to flash chromatography (silica, 1:4 $\rightarrow$ 1:2 v/v ethyl acetate/hexane gradient elution) and concentration of the relevant fractions ($R_f = 0.4$ in 2:3 v/v ethyl acetate/hexane) gave the *title compound 185* (7 mg, 70%) as a clear, light-yellow oil.

** This material was contaminated with *ca.* 10% of the corresponding C6' epimer.

^1H NMR (CDCl_3 , 400 MHz) δ 6.56 (d, $J = 2.2$ Hz, 1H), 6.42 (m, 1H), 6.34 (d, $J = 2.2$ Hz, 1H), 6.18 (dt, $J = 15.6$ and 6.9 Hz, 1H), 5.84 (m, 1H), 5.22 (m, 1H), 5.11 (m, 2H), 3.81 (s, 3H), 3.78 (s, 3H), 3.73 (m, 1H), 3.65–3.60 (complex m, 2H), 3.26 (s, 3H), 3.22 (s, 3H), 2.50–2.32 (complex m, 3H), 2.25 (m, 1H), 2.09 (m, 1H), 1.69–1.47 (complex m, 2H), 1.32 (d, $J = 10.0$ Hz, 3H), 1.31 (s, 3H), 1.31 (s, 3H) (signal due to hydroxyl group proton not observed).

^{13}C NMR (CDCl_3 , 75 MHz) δ 167.7, 161.3, 158.1, 137.5, 143.0, 133.4, 127.2, 117.7, 116.2, 101.4, 98.9, 98.8, 97.7, 72.4, 71.3, 67.0, 62.3, 56.0, 55.6, 48.1, 40.3, 30.3, 29.8, 28.8, 19.7, 17.9, 17.7.

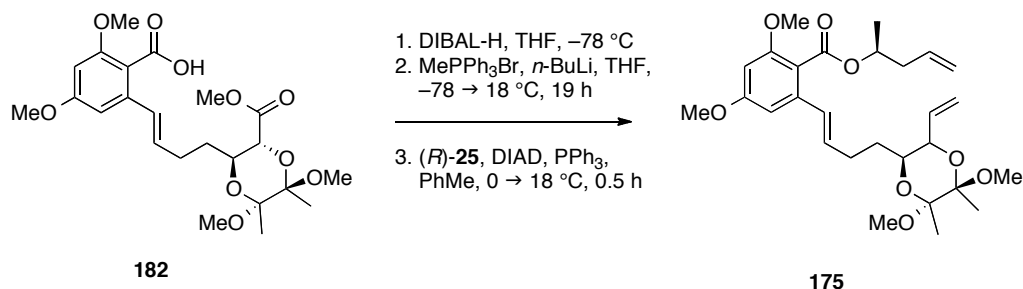
IR ν_{max} (KBr) 3413, 2925, 2851, 1720, 1641, 1601, 1579, 1457, 1374, 1346, 1228, 1262, 1233, 1203, 1159, 1124, 1099, 1047, 964, 917, 873 cm^{-1} .

Mass Spectrum (ESI, +ve) m/z 531 $[(\text{M} + \text{Na})^+, 100\%]$.

HRESIMS Found: $(\text{M} + \text{Na})^+$, 531.2571. $\text{C}_{27}\text{H}_{40}\text{O}_9$ requires $(\text{M} + \text{Na})^+$, 531.2570.

Specific Rotation: $[\alpha]_{\text{D}} = -106.0$ (c 0.05, CHCl_3).

(*S*)-Pent-4-en-2-yl 2-((*E*)-4-((2*S*,5*R*,6*R*)-5,6-dimethoxy-5,6-dimethyl-3-vinyl-1,4-dioxan-2-yl)but-1-en-1-yl)-4,6-dimethoxybenzoate (175**)**



A magnetically stirred solution of methyl ester **182** (15 mg, 0.03 mmol) in dry dichloromethane (3 mL) maintained at -78°C under a nitrogen atmosphere was treated dropwise with DIBAL-H (0.08 mL of a 1.0 M solution in hexane, 0.08 mmol). After an additional 0.03 h the reaction mixture was treated with methanol (0.4 mL) and HCl (1 mL of a 1.0 M aqueous solution) then allowed to warm to 18°C . The separated aqueous phase was extracted with dichloromethane (3×10 mL) and the combined organic fractions were washed with brine (2×10 mL) before being dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the *aldehyde 183* as an unstable yellow oil. This material was used immediately in the next step of the reaction sequence.

A magnetically stirred solution of methyltriphenylphosphonium bromide (16 mg, 0.21 mmol) in dry diethyl ether (0.4 mL) maintained at 0°C under nitrogen was treated with *n*-butyllithium (0.21 mL of a 1.0 M solution in hexane, 0.21 mmol). The resulting yellow mixture was allowed

to warm to 18 °C over 0.5 h then re-cooled to 0 °C and treated, *via* cannula, with a solution of aldehyde **183** in dry diethyl ether (0.6 mL) and the resulting mixture allowed to stir at 18 °C for 19 h. The resulting suspension was treated with phosphate buffer (20 mL of a 1.0 M aqueous solution, pH = 4.0) then extracted with ethyl acetate (5 × 10 mL). The combined organic extracts were washed with brine (1 × 20 mL) before being dried (Na₂SO₄), filtered and concentrated under reduced pressure to afford a yellow, cloudy oil presumed to contain the *olefin* **187**. This material was used immediately in the next step of the reaction sequence.

A magnetically stirred solution of olefin **187**, obtained as described above, in dry toluene (1 mL) maintained at 0 °C under a nitrogen atmosphere was treated with triphenylphosphine (26 mg, 0.1 mmol) and (*R*)-(-)-4-penten-2-ol [(*R*)-**25**] (0.04 mL, 0.4 mmol). After 0.25 h the ensuing mixture was treated, dropwise, with diisopropyl azodicarboxylate (0.02 mL, 0.4 mmol) and then the reaction mixture was warmed to 18 °C. After 2 h at this temperature the reaction mixture was concentrated under reduced pressure to give a yellow resin. Subjection of this material to flash chromatography (silica, 3:7 → 7:3 *v/v* ethyl acetate/hexane gradient elution) and concentration of the relevant fractions (*R_f* = 0.4 in 2:3 *v/v* ethyl acetate/hexane) gave the *title compound* **175** (10 mg, *ca.* 25% from methyl ester **182**) as a clear, light-yellow oil.

¹H NMR (CDCl₃, 800 MHz) δ 6.55 (t, *J* = 2.3 Hz, 1H), 6.40 (dt, *J* = 15.6 and 1.4 Hz, 1H), 6.34 (d, *J* = 2.3 Hz, 1H), 6.17 (ddd, *J* = 15.6, 12.6 and 7.1 Hz, 1H), 5.83 (m, 1H), 5.76 (m, 2H), 5.36 (m, 1H), 5.26 (m, 1H), 5.22 (m, 1H), 5.12 (m, 2H), 5.09-5.05 (complex m, 2H), 5.02 (dt, *J* = 10.2 and 0.9 Hz, 1H), 3.94 (m, 1H), 3.80 (m, 3H), 3.76 (m, 3H), 3.58 (m, 1H), 3.27 (m, 3H), 3.24 (m, 3H), 2.49-2.43 (complex m, 3H), 2.37-2.33 (complex m, 2H), 2.22 (m, 2H), 1.57 (m, 3H), 1.33 (d, *J* = 6.3 Hz, 3H), 1.31 (s, 3H), 1.30 (s, 3H) (certain of these signals are due to the presence of unidentified impurities present in the same).

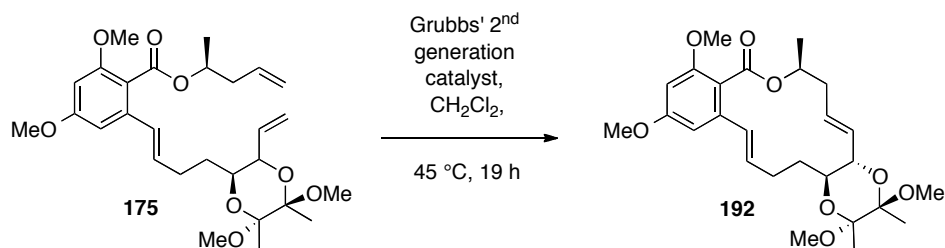
¹³C NMR (CDCl₃, 75 MHz) δ 167.7, 161.3, 158.1, 137.5, 134.4, 134.0, 133.6, 127.0, 120.1, 117.7, 101.4, 98.8, 97.6, 74.6, 71.2, 70.3 (2 overlapping peaks), 55.7, 55.5, 48.1 (2 overlapping peaks), 40.3, 30.3, 29.0 (2 overlapping peaks), 19.7, 17.9, 17.8.

IR *v*_{max}(KBr) 3883, 3747, 3359, 2923, 2852, 1724, 1644, 1600, 1579, 1458, 1375, 1325, 1261, 1203 1158, 1120, 1049, 930, 875, 848, 800 cm⁻¹.

Mass Spectrum (ESI, +ve) *m/z* 528 [(M + Na)⁺, 100%].

HRESIMS Found: (M + Na)⁺, 527.2621. C₂₈H₄₀O₈ requires (M + Na)⁺, 527.2621.

Specific Rotation: [α]_D = -38.4 (*c* 0.05, CHCl₃).

(2R,3R,4aS,5E,8S,15E,18aS)-2,3,11,13-Tetramethoxy-2,3,8-trimethyl-4a,7,8,17,18,18a-hexahydro-2H-benzo[*c*][1,4]dioxino[2,3-*i*][1]oxacyclotetradecin-10(3H)-one (192)

A magnetically stirred solution of triene **175** (10 mg, 0.02 mmol) in dry dichloromethane (13.2 mL) maintained under a nitrogen atmosphere at 18 °C was evacuated then filled with nitrogen. This process was repeated twice more before Grubbs' 2nd generation catalyst¹⁰ (2 mg, 0.002 mmol) was added and the ensuing mixture was then heated at reflux for 12 h. The cooled reaction mixture was concentrated under reduced pressure to give a light-brown oil that was subjected to flash chromatography (silica, 1:4 → 1:1 *v/v* ethyl acetate/hexane gradient elution). Concentration of the appropriate fractions (R_f = 0.45 in 2:1 *v/v* ethyl acetate/hexane) then gave the *title compound* **192** (6 mg, *ca.* 25%) as a clear, colourless oil.

¹H NMR (CDCl₃, 400 MHz) δ 6.57 (d, J = 2.2 Hz, 1H), 6.35 (d, J = 2.2 Hz, 1H), 5.99 (m, 1H), 5.68 (m, 1H), 5.64–5.72 (complex m, 1H), 4.93–5.09 (complex m, 2H), 3.93 (m, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.75 (complex m, 1H), 3.27 (s, 3H), 3.18 (s, 3H) (due the presence of aliphatic impurities the resonances appearing below δ 2.00 could not be assigned).

Mass Spectrum (ESI, +ve) m/z 499 [(M + Na)⁺, 100%], 446 (11), 327 (14), 313 (76), 309 (14), 227 (14), 193 (43), 121 (13), 102 (13), 77 (49).

HRESIMS Found: (M + Na)⁺, 499.2307. C₂₆H₃₆O₈ requires (M + Na)⁺, 499.2308.

No other spectral data could be obtained on this material because of significant amounts of contamination by aliphatic impurities.

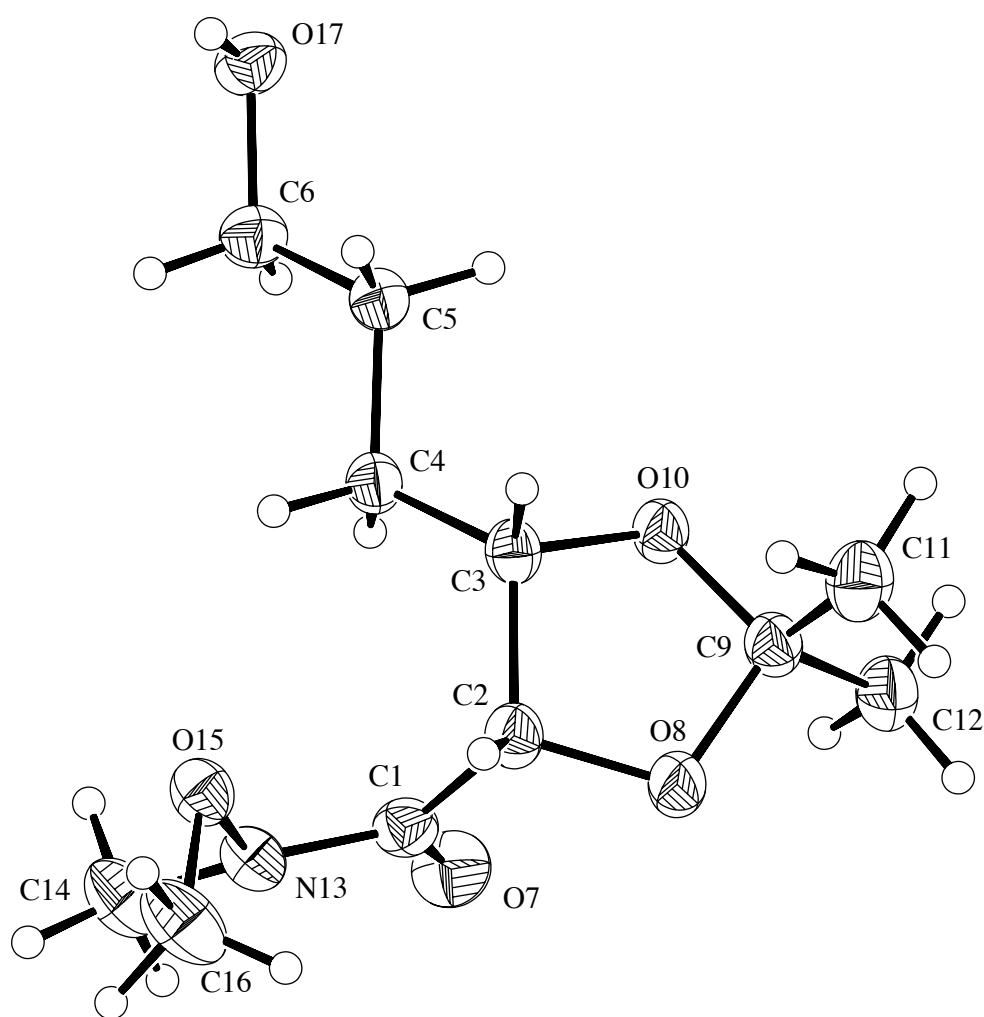
6.5 References

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X-ray Crystal Structure Report for Compound 140

A full X-ray crystallographic report for compound **140** (as compiled by Anthony C. Willis of the Australian National University) is provided in PDF-format on the compact disc found on the inside back cover of this thesis.

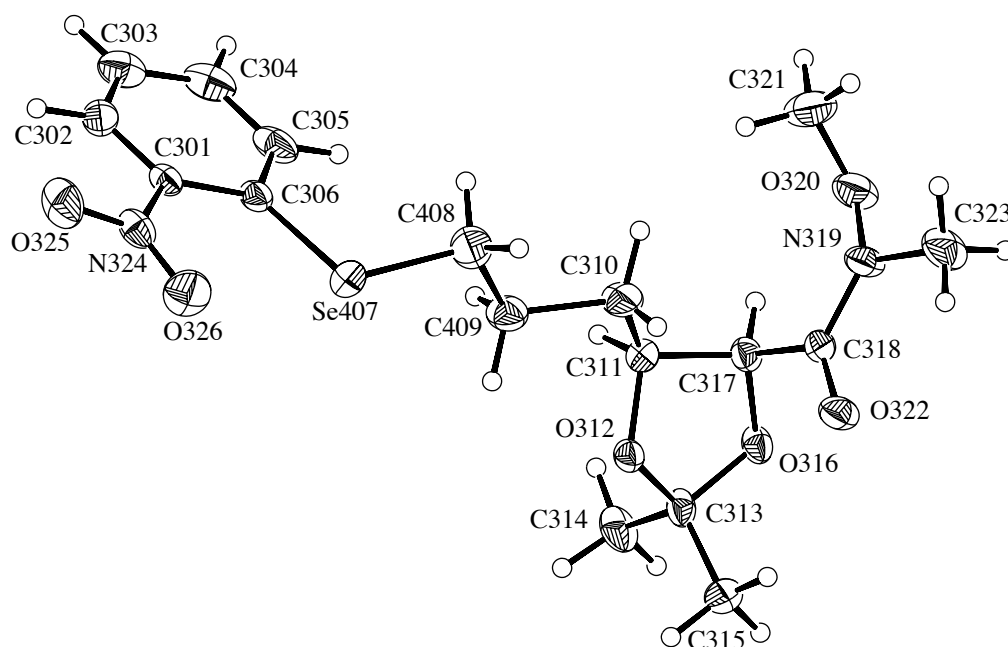
X-ray report 140.pdf



X-ray Crystal Structure Report for Compound 142

A full X-ray crystallographic report for compound **142** (as compiled by Anthony C. Willis of the Australian National University) is provided in PDF-format on the compact disc found on the inside back cover of this thesis.

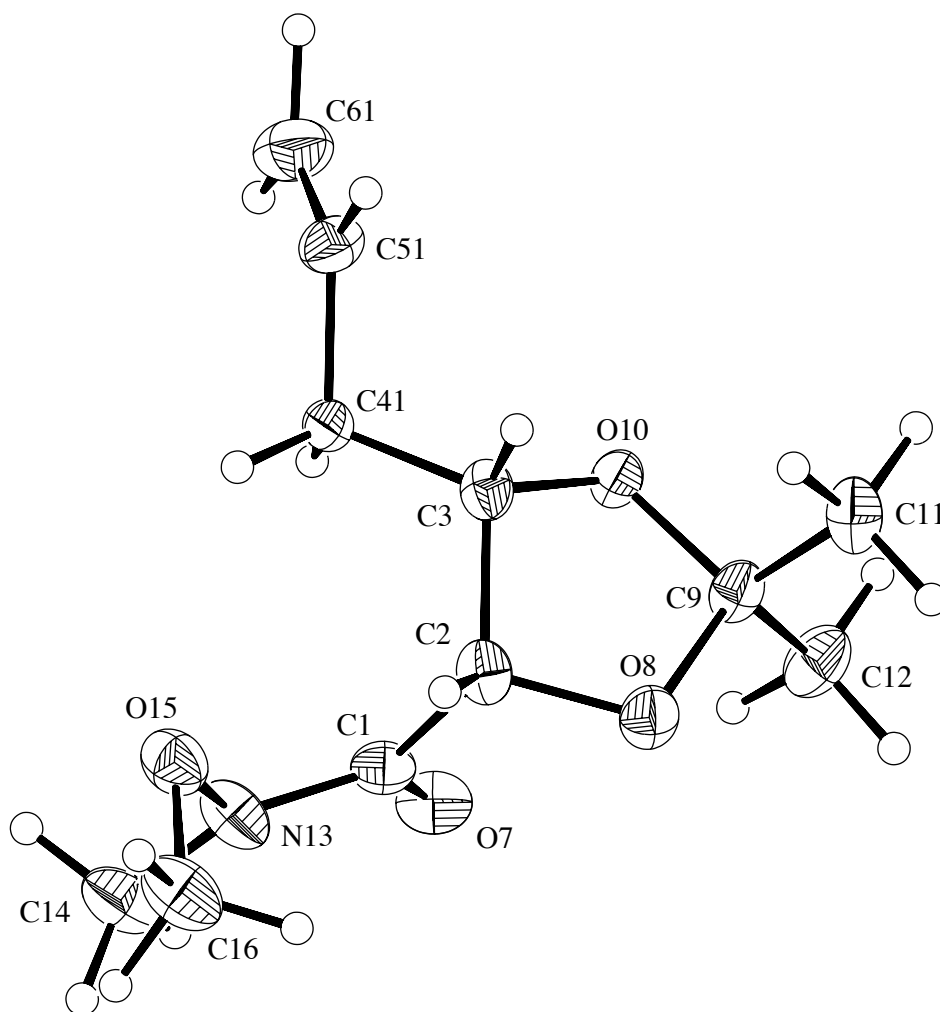
X-ray report 142.pdf



X-ray Crystal Structure Report for Compound 127

A full X-ray crystallographic report for compound **127** (as compiled by Anthony C. Willis of the Australian National University) is provided in PDF-format on the compact disc found on the inside back cover of this thesis.

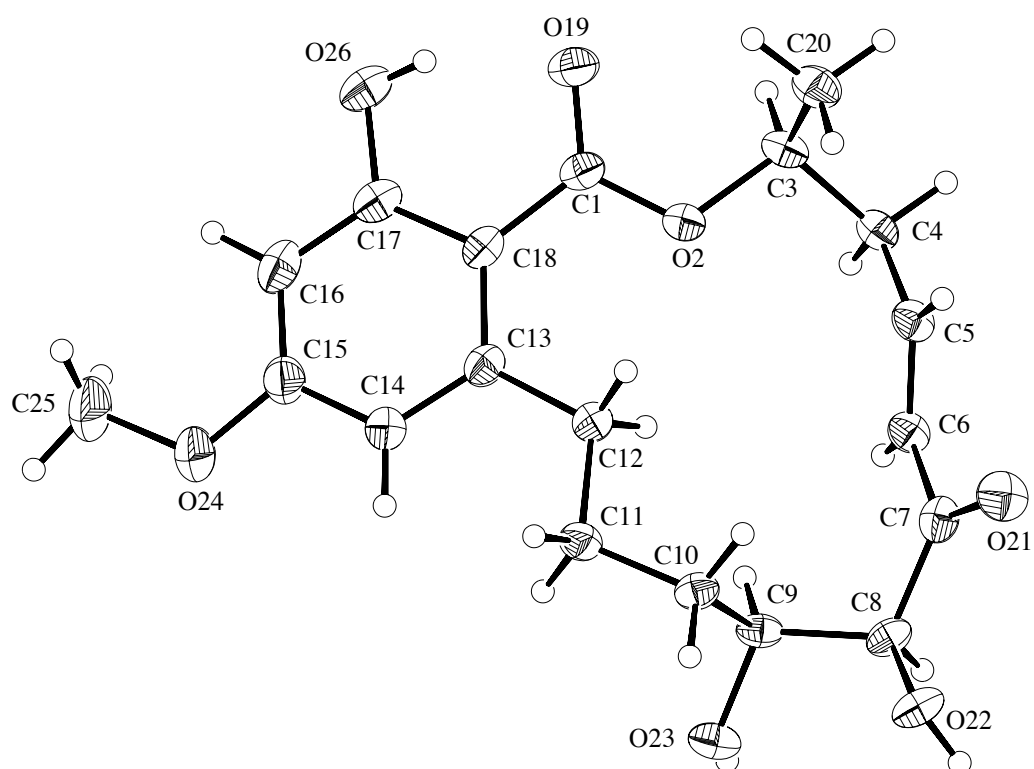
X-ray report 127.pdf



X-ray Crystal Structure Report for Compound 128

A full X-ray crystallographic report for compound **128** (as compiled by Anthony C. Willis of the Australian National University) is provided in PDF-format on the compact disc found on the inside back cover of this thesis.

X-ray report 128.pdf



X-ray Crystal Structure Report for Compound 164

A full X-ray crystallographic report for compound **164** (as compiled by Anthony C. Willis of the Australian National University) is provided in PDF-format on the compact disc found on the inside back cover of this thesis.

X-ray report 164.pdf

