Synthesis of β-lactone, γ-lactam 20S proteasome inhibitors

By

Ross L. Goodyear

A doctoral thesis

Submitted in partial fulfilment of the requirements for the award of

Doctor of philosophy

At

The University of East Anglia

Preface

The research in this thesis is, to the best of my knowledge, original and my own work except where due reference has been made. Neither the thesis nor the original work contained therein has been submitted to any other institution for a degree.

Ross L. Goodyear

Febuary 2019

Abstract

The 20S proteasome is a large protein complex, primarily responsible for the breakdown of ubiquitinated proteins. It is therefore a vital component in the ubiquitin proteasome pathway, disruptions of which can induce cell death. Such an effect could be targeted for cancer therapeutics. Some β-lactone γ-lactam scaffolds, such as the natural product omuralide (the biologically active form of lactacystin) have been found to inhibit the 20S proteasome.

The discovery of several new natural 20S proteasome inhibitors such as the salinosporamides and cinnabaramides, have shown potential for the development of new inhibitors with increased potency and specificity. Development of synthetic routes to produce these targets and their analogues is key to developing new proteasome inhibitors with greater potency and reduced side effects.

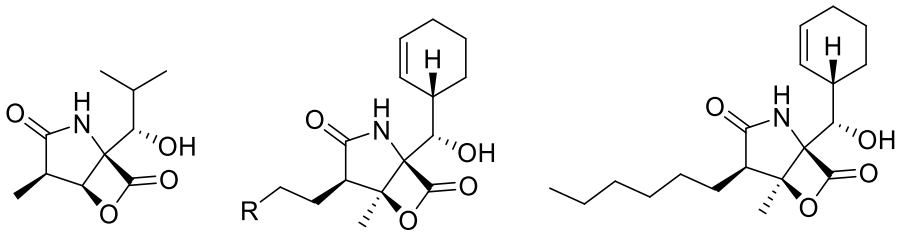


Figure 1. Several natural products with 20S proteasome inhibitory activity

Our methodology involves the incorporation of amino acids into the γ-lactam core. Leucine and serine have been used to produce a new formal synthesis of 9-deoxy omuralide and omuralide respectively. Key steps include a diastereoselective acylation with Mander's reagent and desulfurization. This methodology offers a flexible route allowing rapid generation of omuralide analogues in order to produce known and novel 20S proteasome inhibitors for biological testing.

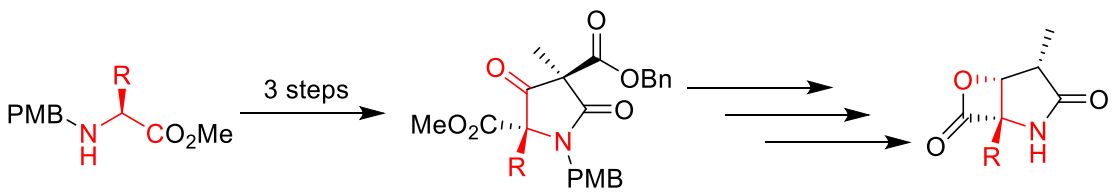


Figure 2. An overview of our methodology

In addition to our work incorporating amino acids into γ-lactam cores, we have also developed a new route to synthesize either diastereomer of hydroxy leucine. We hope that this valuable intermediate could be incorporated into the γ-lactam using our devised methodology quickly forming an advanced intermediate of omuralide.

Acknowledgements

I'd like thank Prof. Phil Page for his supervision and friendship throughout my PhD, I'm forever grateful for the opportunity he gave me and have enjoyed my time at UEA and in his group immensely. In addition, I would like to thank my secondary supervisor Prof. Andy Cammidge for his advice and guidance, along with Dr Tom Storr and Dr Yohan Chan. Prof. Alexandra Slawin is also gratefully acknowledged for obtaining all X-ray crystal structures contained within this thesis. The University of East Anglia and Charnwood Molecular Ltd. are appreciated for the funding of this work.

I'm extremely grateful to the Page group, especially those who have worked on this project before me, and the rest of my friends from chemistry and pharmacy, particularly those whom I have been friends with since the first day; Greg Hughes, Thomas Bridge, Gerald Keil and Yannick Gama.

To Yun-en, without a doubt, the highlight of my time in Norwich has been meeting you. I can't wait to see where our lives now take us. See you soon!

To my sisters and parents, I absolutely wouldn't be able to be here without your love, help and support. I am so thankful for all you have done for me and love you all so much.

List of abbreviations

AD	Asymmetric dihydroxylation
AIBN	Azobisisobutyronitrile
Aq	Aqueous
Bz	Benzoyl
BIAB	(Diacetoxyiodo)benzene
Binol	1,1’-Bi-2-naphthol
BOPCl	Bis(2-oxo-3-oxazolidinyl)phosphinic chloride
CAN	Ceric ammonium nitrate
Cat	Catalytic
CSA	Camphorsulfonic acid
CT	Chymotrypsin
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	N,N'-Dicyclohexylcarbodiimide
DCM	Dichloromethane
$(DHQD)_2PHAL$	Hydroquinidine 1,4-phthalazinediyl diether
$(DHQ)_2PHAL$	Hydroquinine 1,4-phthalazinediyl diether
DIBAL	Diisobutylaluminum hydride
DIEA	N,N-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DME	1,2-Dimethoxyethane
DMF	NN-Dimethyl formamide
DMP	Dess–Martin periodinane
DMPU	N,N’-Dimethylpropyleneurea
DMSO	Dimethyl sulphoxide
dr	Diastereomeric ratio
EDAC.HCl	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl
ee	Enantiomeric excess
GSH	Glutathione
h	Hour/s

HOTT	S-(1-Oxido-2-pyridinyl)-1,1,3,3-tetramethyl-thiouronium Hexafluorophosphate
HMPA	Hexamethylphosphoramide
HPLC	High performance liquid chromatography
IPA	Isopropyl alcohol
Ipc	Isopinocampheyl
i-pr	*Iso*-propyl
LAH	Lithium aluminium hydride
LDA	Lithium diisopropylamide
LiHMDS	Lithium bis(trimethylsilyl)amide
m	Minute/s
M/mol	Molar
MSH	*O*-Mesitylenesulfonylhydroxylamine
NCS	N-Chlorosuccinimide
NFT	Neurotrophic factors
N-MM	N-Methylmorpholine
NMO	N-Methylmorpholine N-oxide
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser effect spectroscopy
P	Product
PEG	Polyethylene glycol
Piv	Pivaloyl
PLE	Porcine liver esterase
PLP	Piridoxal phosphate
PMB	*para*-Methoxy benzyl
PPTS	Pyridinium *p*-toluenesulfonate
p-TSA	*para*-Toluene sulphonic acid
Quant	Quantitative yield
Red-Al®	Sodium bis(2-methoxyethoxy)aluminium hydride
rt	Room temperature
SAR	Structure activity relationship

Sm	Starting material
TBAF	Tetra-N-butylammonium fluoride
TBS	*tert*-Butyldimethylsilyl
TBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate
TEA	Triethylamine
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIPS	Triisopropyl silyl
TLC	Thin layer chromatography
Tom	2-O-Triisopropylsilyloxymethyl
TTMS	Tris(trimethylsilyl)silane
UPP	Ubiquitin proteasome pathway
UV	Ultraviolet
WHO	World health organization

Contents

1.0 Introduction

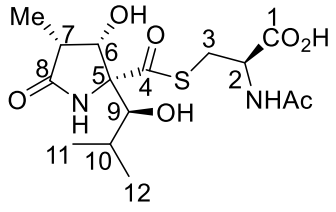
1.1 Natural products and medicines

The search for new drugs and biologically active molecules has sought inspiration from natural products or analogues thereof[1] since the start of the study of medicine.[2] Perhaps the most famous example is the discovery of penicillin by Alexander Fleming. As research into molecules derived from natural sources increased, the common and easily accessible drug compounds were soon discovered. This increased the difficulty of discovering new drugs and druglike molecules against an ever-increasing demand for new medicines.

1.2 The discovery and early work on lactacystin

1.2.1 The discovery of lactacystin

In response to this problem, one method for the discovery of new bioactive compounds was to screen the cultured broths of numerous microbes against an assay. In 1991 Ōmura *et al.* used this method to find new compounds with neurotrophic factors (**NTFs**) by testing the broths of soil isolates.[3,4] NTFs are essential for the growth and survival of neurones. A broth from the strain of *Streptomyces sp* OM-6519 was found to induce differentiation of a mouse neuroblastoma cell line 2A. Isolated from this broth, the active compound was characterized and designated lactacystin **1**. Ōmura later was jointly awarded the 2015 Nobel prize for medicine.



Lactacystin 1

Figure 1: Lactacystin, discovered by Ōmura in 1991

Lactacystin was the first microbial metabolite with neurotrophic activity; the hope of developing drugs to treat neurodegenerative disorders prompted several early synthetic efforts. The first was completed by Corey *et al.* in 1992,[5] which was followed shortly after by an alternative route from Ōmura *et al.*[6] The first insights into a mode of action for lactacystin were proposed by Schreiber *et al.*[7] in an early structure activity relationship (**SAR**) study, it

was found that the most potent analogues of lactacystin had the potential to form β-lactones from the cysteine-derived thioester and the C6 hydroxyl group, forming compound **2**, later named omuralide. Further modification to the cysteine had little effect on activity. Analogues that were unable to form the β-lactone such as the acid **3** or 6-deoxy lactacystin **4** had no biological activity.

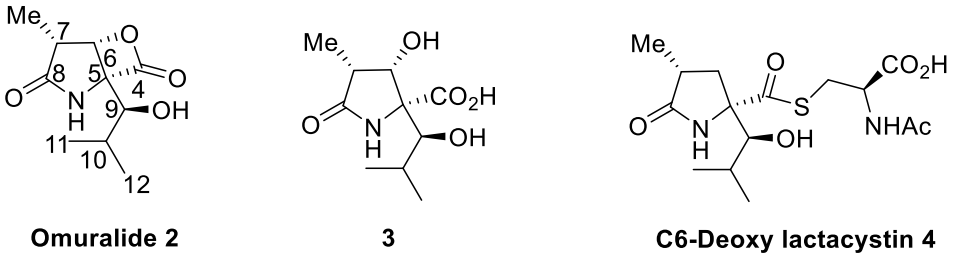


Figure 2: Omuralide and inactive derivatives

In addition, the epimers at C9, C6, and an analogue with the hydroxylated *iso*-butyl moiety removed, resulted in little or no activity. Furthermore, this initial SAR study led to the discovery that lactacystin **1** and active analogues were able to inhibit cell cycle progression beyond the G1 phase in MG-63 human osteosarcoma cells. Like neurite outgrowth, the most active analogues of lactacystin had the structural requirements for formation of β-lactones. The observations of the necessity of β-lactone formation in the active compounds led Schreiber to hypothesize that acylation of the cellular target may occur, that is, a nucleophilic group on the target attacks the carbonyl of the β-lactone during the mechanism of action.

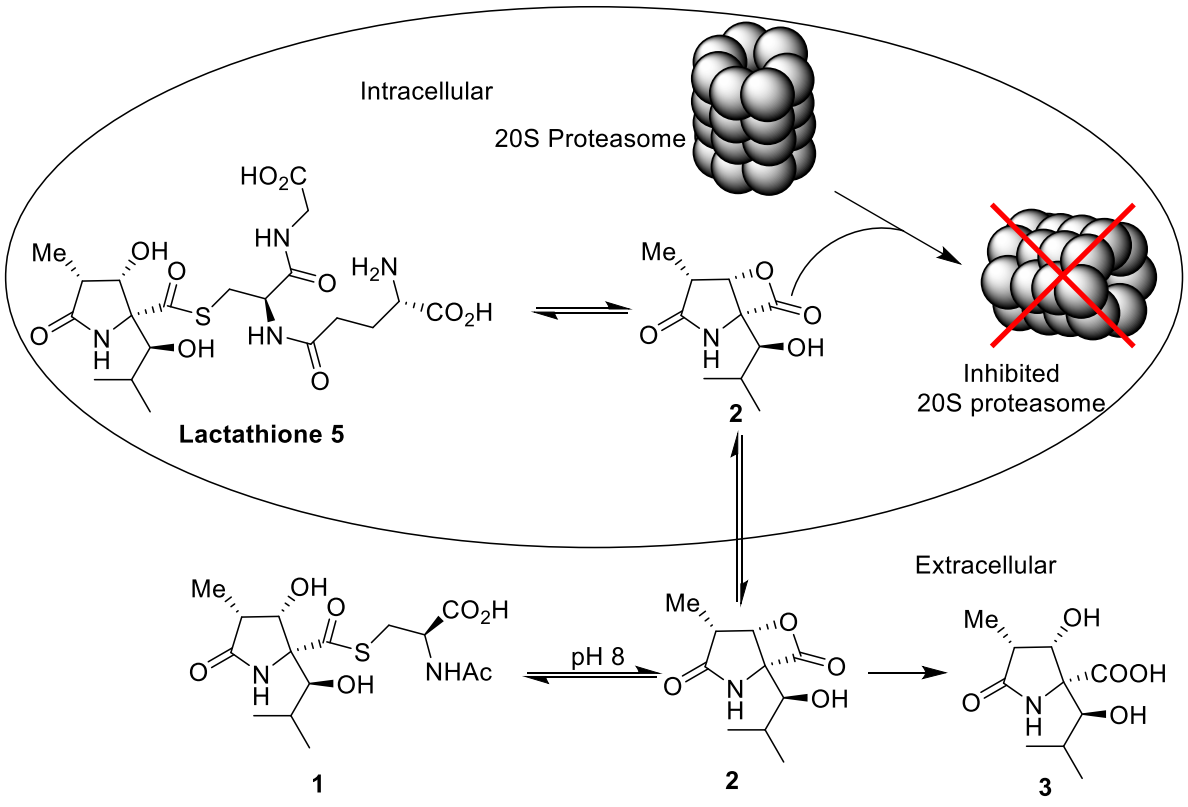
1.3 The target and mode of action of lactacystin

1.3.1 Discovery of the target

The cellular target of lactacystin **1** was discovered in later work by Schreiber *et al.* in 1995, using tritium labelled forms of lactacystin and analogues.[8] The target was found to be the 20S proteasome, for which lactacystin could inhibit specific sites at different rates. This work also confirmed the previous structure observations, through the testing of several analogues including omuralide. Omuralide associated with the 20S proteasome far faster than lactacystin. Several other analogues were tested, such as 6-deoxy lactacystin **4**, which showed no inhibition.

1.3.2 Mode of action of lactacystin

In 1996 Dick *et al.* reported the central role omuralide **2** plays in the inhibition of the 20S proteasome.[9] These studies showed that lactacystin **1** was not the proteasome inhibitor *per se*, but only a precursor to the sole inhibitory complex omuralide **2** in a pro-drug-like form. Lactacystin **1**, at pH 8 in an aqueous solution, undergoes reversible cyclization to omuralide **2**, then hydrolysis to the inactive compound **3**. Under these basic conditions, 20S proteasome inhibition occurred. At a pH of 6.3, however, where cyclization of lactacystin **1** to omuralide does not occur, no inhibition was observed, whereas omuralide retained inhibitory activity under the same conditions. Dick expanded these results in a further paper[10] with *in vitro* studies on mammalian cells. Lactacystin **1** is unable to enter cells until lactonization to omuralide **2** has occurred. In the cell omuralide can either inhibit the proteasome or form lactathione **5**, a compound analogous to lactacystin **1,** through a reversible reaction with glutathione (**GSH**). Lactathione **5** was presumed to act as a reservoir of omuralide **2**, concentrating the inhibitor in the cell and allowing prolonged release (**Scheme 1**).



Scheme 1: The mode of action and relationship between **1** and **2**

1.3.3 The 20S proteasome

The 20S proteasome is a large (around 700 KD) cylindrical multisubunit enzyme complex. The crystal structure of the yeast proteasome was obtained by Groll *et al.* in 1997.[11] The shape of the proteasome is comparable to a barrel with a hollow core (**Figure 3**).

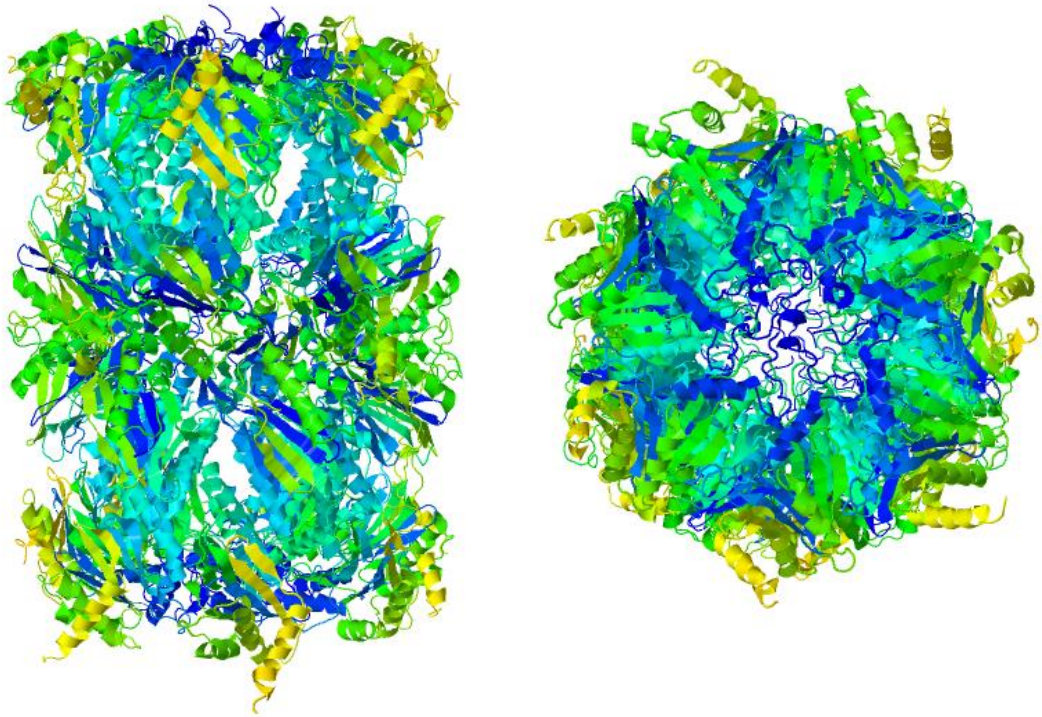


Figure 3: The crystal structure of the 20S proteasome of yeast

The 20S proteasome is a vital component of the ubiquitin proteasome pathway, the process by which proteins are labelled for degradation by ubiquitination and then destroyed. The proteasome is key in the second part of this process. Ubiquitinated proteins are fed into the proteasome and digested into short peptide chains ranging from 2-25 amino acids in length.[12]

The structure is comprised of 4 rings, two outer α-rings and two inner β-rings, each comprised of 7 subunits. The quaternary structure therefore can be described as α7β7β7α7.[13] The 20S proteasome can be further "capped" to with one or two 19S proteasomes, thus forming the 26S proteasome. The 19S proteasomes function is to control access to the proteasome core by recognising ubiquitinated proteins and denaturing their structure. The denatured proteins are fed into the narrow core created by the α-ring subunits to be degraded by the catalytic β-rings. Of the 7 subunits in each of the 2 β-rings, 3 of these subunits contain active sites to

degrade proteins (**Figure 4**). These 3 subunits, called post-glutamyl peptidyl hydrolytic-like, trypsin-like and chymotrypsin-like are known to be selective to hydrolysing the peptide bond following from specific amino acids.

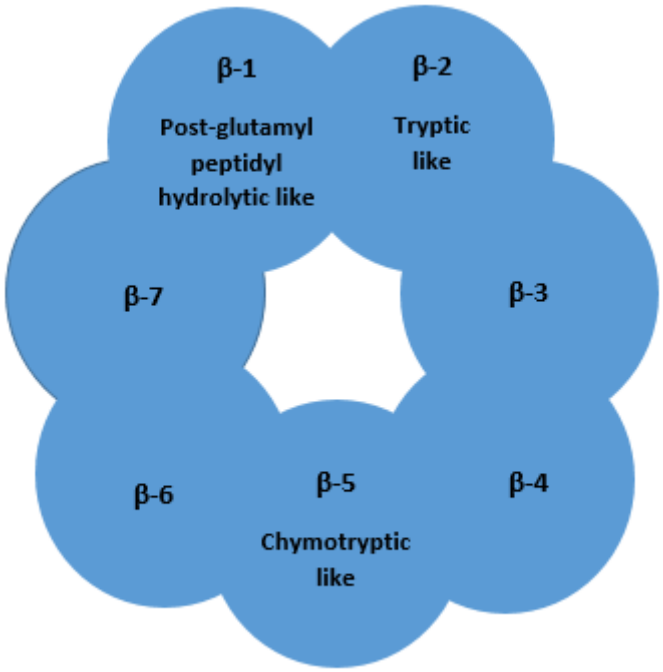


Figure 4. A diagram showing a cross section of a 20S proteasome β ring

Post-glutamyl: Acidic, a preference for peptide bonds following amino acids such as aspartic acid and glutamic acid

Tryptic: Basic, a preference for peptide bonds following amino acids such as arginine or lysine

Chymotryptic: Hydrophobic, a preference for peptide bonds following amino acids such as tyrosine and phenylalanine

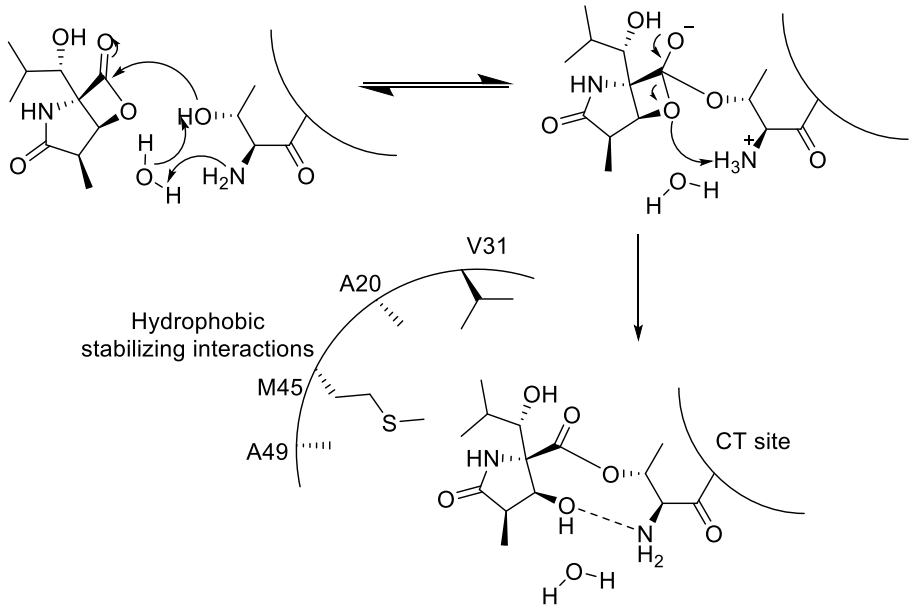
Tabe 1 describes the rates of association between lactacystin or omuralide and the 3 catalytic sites of the 20S proteasome. Schreiber identified lactacystin and omuralide as inhibiting all active sites of the proteasome – albeit at different concentrations.[8] Both active compounds, however, were found to be far more specific for the chymotrypsin like active site. Chymotrypsin (**CT**) and trypsin active sites were thought to bind irreversibly, whereas the post-glutamyl peptidyl hydrolytic site (the slowest inhibited site) was bound reversibly.

$K_{assoc} = K_{obs}/[I]$ (M^{-1} S^{-1})			
Compound and concentration	**Chymotrypsin like**	**Trypsin like**	**Post-glutamyl peptidyl hydrolytic like**
1 (10 µM)	194 ± 15	10.1 ± 1.8	
1 (100 µM)			4.2 ± 0.6
2 (1 µM)	3059 ± 478		
2 (5 µM)		208 ± 21	
2 (50 µM)			59 ± 17

Table 1: Association rates of **1** and **2** on the 20S proteasome catalytic sites

1.3.4 Mechanism of the inhibition of the 20S proteasome by omuralide

The mechanism for inhibition of the proteasome shown in **scheme 2** was proposed by Groll *et al.*, supported by crystal structures of omuralide associated with the chymotrypsin-like active site.[14,15]



Scheme 2: Mechanism of inhibition of the chymotrypsin site by omuralide

As predicted by Schreiber,[7] inhibition of the proteasome occurs due to nucleophilic attack to the β-lactone by a threonine residue, ordinarily the amino acid responsible for the catalytic

hydrolysis of peptide bonds. Various interactions then stabilize the ligand, increasing binding affinity, primarily the C5 moiety, which fits into the hydrophobic S1 pocket of the β5 subunit, prolonging retention time and allowing nucleophilic attack from the threonine Thr1O$^{\gamma}$ residue.

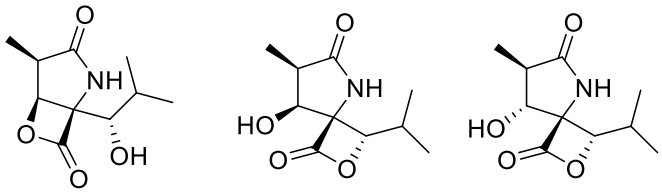


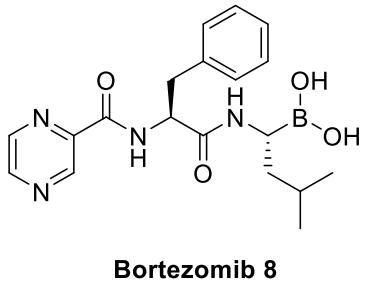
Figure 5: Omuralide and spiro cyclic analogues synthesized by Jacobsen

A more precise nature of the critical role of the C6 hydroxyl group was reported by Groll *et al.*[14] through the testing of a spiro-lactone derivative of omuralide and its C6 epimer synthesized by Jacobsen and Balskus.[16] The spiro-lactone form of omuralide **6** had comparable inhibition potency to omuralide, but the spiro-lactone C6 epimer **7** potency was greatly decreased. This result indicated that the function of the C6 hydroxyl group was not solely to allow formation of the β-lactone. Comparing the crystal structures of each of these compounds revealed that in addition to the stabilizing hydrophobic interactions between the C5 isobutyl group and the S1 pocket of the CT site, the C6 hydroxyl group interacts with the active threonine terminal amine group, further increasing stability. Another crucial role of the hydroxyl group was also reported; the hydroxyl group in omuralide and **6** points out along the path that would be required for hydrolysis of the ester linkage, blocking attack and subsequent dissociation of the omuralide-enzyme complex. It is therefore clear that omuralide is well designed for purpose.

1.4 The ubiquitin proteasome pathway in cancer therapy

The ubiquitin proteasome pathway (UPP) is critical for the survival and development of both healthy and cancerous cells, disruption of which can induce cell death. A number of studies have been undertaken to disrupt this pathway at any of the key stages.[17,18] One of the most studied methods to disrupt the UPP is 20S proteasome inhibition.[19,15,20,21,22] Tumour cells are thought to be more susceptible to cell death through this mechanism. It has been found that some healthy cells can survive with up to 80% inhibition of the CT site, whereas tumour cells

can only withstand 25% inhibition.[23] The first drug to target the UPP was a 20S proteasome inhibitor developed by Takeda called bortezomib **8**,[24] and was approved for clinical use in 2003 for treatment of multiple myeloma.



Bortezomib 8

Figure 6: The structure of bortezomib

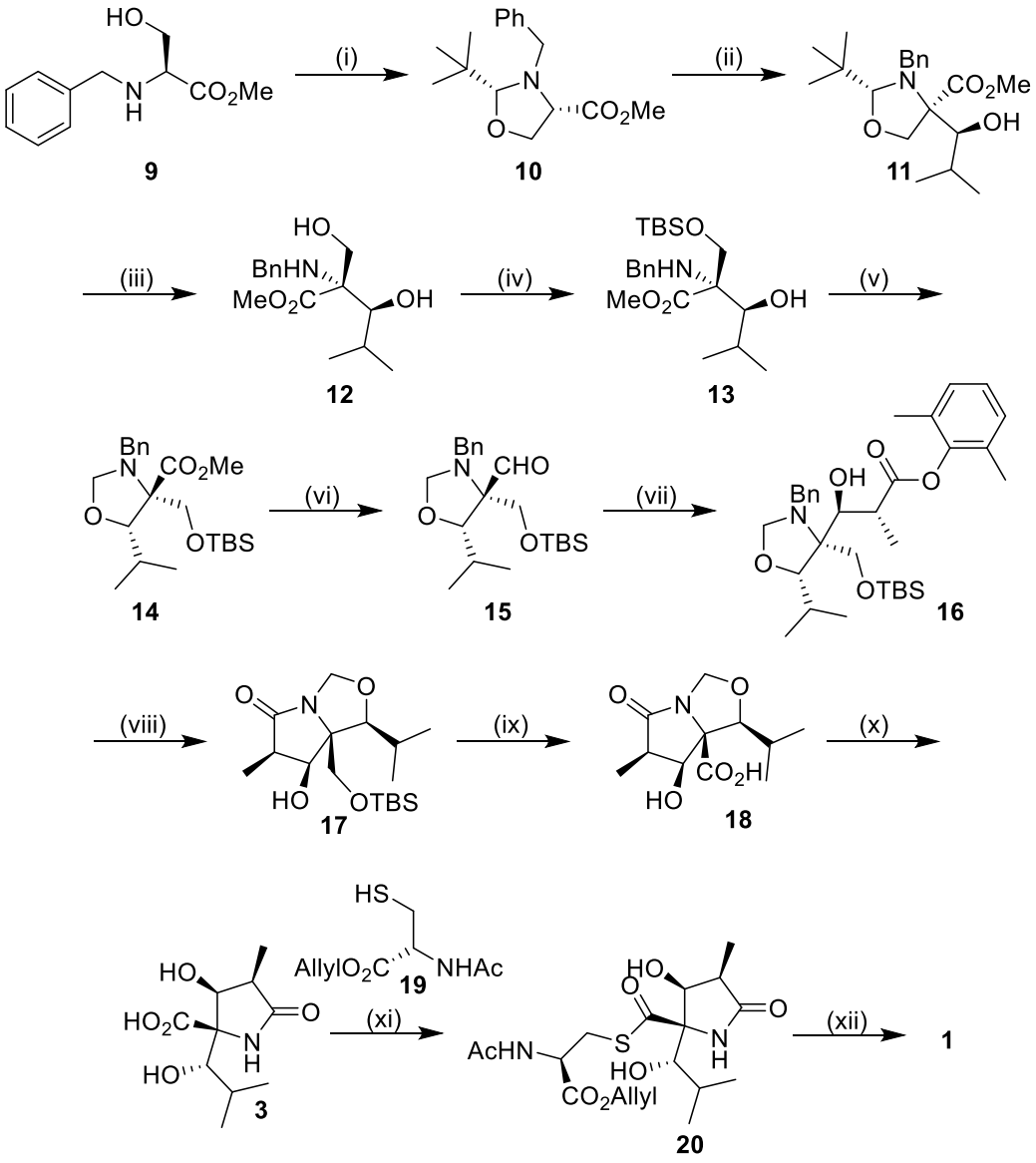
The mechanism for inhibition of the 20S proteasome occurs through binding of the active threonine to the boronic acid, blocking the active site in a similar manner to omuralide.[15] One of the mechanisms for apoptosis after inhibition of the proteasome is thought to be based on the proteins usually responsible for inducing cell death being retained by the cell instead of being destroyed by the proteasome.[19] Bortezomib may also cause disruption to proteasome-dependent pathways, which increase a cell's resistance to radiation and other drugs.[19] This makes bortezomib particularly potent when combined with radiotherapy or other chemotherapy drugs. For this reason, the discovery of new proteasome inhibitors and the modification of known inhibitors to increase selectivity and reduce toxic side effects plays a vital role in research for cancer therapies. An important role of this research is the synthesis of various inhibitors and their derivatives for further understanding of the SAR and mode of action in the hope of producing inhibitors with a greater potential to become future drug candidates.

1.5 Omuralide and lactacystin as targets for total synthesis

1.5.1 Corey's strategy for lactacystin

1.5.1.1 The first synthesis of lactacystin

The first total synthesis of lactacystin **1** was completed by Corey and Reichard in 1992.[5] The total yield was 4.6%. The total number of steps was 15 (**Scheme 3**).



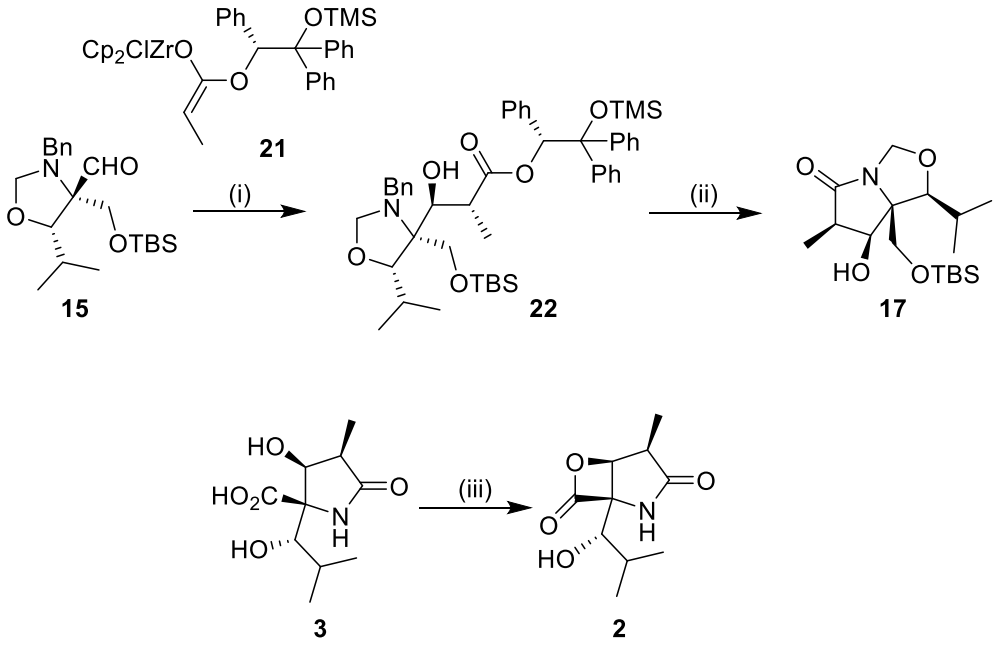
Reagents and conditions: (i) $(CH_3)_3CCHO$, *p*-TsOH, PhMe; (ii) LDA, LiBr, isobutyraldehyde, THF, -78 °C 8 h 51%; (iii) MeOH, TfOH, 80 °C, 6.5 h, 91%; (iv) TBSCl, imidazole, DMF, 23 °C, 3.5 h, 97%; (v) TsOH, CH_2O, C_6H_6, 30 m, 96%; (vi) 1. $LiBH_4$/THF, MeOH, 23 °C, 24 h, 2. DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, -78 °C, 85%; (vii) LDA, THF, -78 °C, 2,6-dimethylphenylpropionate, 48%; (viii) H_2, Pd/C, EtOH, 23 °C, 1 d, 87%; (ix) 1. 5% HF/CH_3CN, 23 °C, 9 h, 90%; 2. DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, -78 °C, 2 h, 73%; 3. $NaClO_2$, NaH_2PO_4, *t*-BuOH, 2-methyl-2-butene, 23 °C, 15 m, >95%; (x) 1,3-propanedithiol, 2% HCl in CF_3CH_2OH, 50 °C, 6 h, >95%; (xi) *N*-acetylcystine allyl ester, BOPCl, Et_3N, CH_2Cl_2, 1 h, 23 °C, 79%; (xii) $Pd(Ph_3P)_4$ HCO_2H, Et_3N, THF, 23 °C, 1 h, 84%.

Scheme 3: The first synthesis of lactacystin, by Corey

The route is remarkable by being the first synthesis of lactacystin but also by providing much of the material used in the early biological testing of lactacystin **1**. The route also contained only a few instances of column chromatography. The N-benzyl serine methyl ester **9** was cyclized to oxazolidine **10** with pivaldehyde in acidic conditions. An aldol reaction with *iso*-butyraldehyde afforded **11** in excellent diastereoisomeric purity (>98%); after a recrystallization from pentane, **11** was diastereoisomerically and enantiomerically pure. Acid cleavage of the oxazolidine ring produced **12**; following a silyl-based hydroxyl protection to **13**, a second oxazolidine **14** was formed through a formaldehyde bridge in an excellent yield of 96%. Reduction of the methyl ester and subsequent Swern oxidation afforded aldehyde **15**, a key intermediate. An *anti*-aldol reaction using 2,6 dimethylphenyl propionate under Pirrung-Heathcock conditions[25] gave the desired isomer **16,** but the yield was low at 48%. Hydrogenolysis of the ester produced the γ-lactam ring found in lactacystin in a bicyclic system with an oxazolidine (**17)**. A deprotection of the silyl group and an oxidation of the resulting alcohol to a carboxylic acid yielded **18,** which, on opening of the oxazolidine ring, afforded the dihydroxy acid **3.** Addition of an allyl protected cysteine **19** and its subsequent deprotection yielded lactacystin **1**.

1.5.1.2 Corey's improvement to the aldol reaction

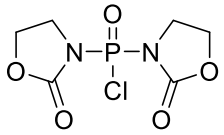
Corey later revisited this synthesis (**Scheme 4**),[26] primarily improving the *anti*-aldol reaction of **15** using a chiral zirconium enolate **21**.[27]



Reagents and conditions: (i) **21**, THF, -20 °C, 24 h, 80%; (ii) 1) H_2, Pd/C, 2) CH_2N_2; 3) MeOH; (iii) BOPCl, Et_3N, CD_2Cl_2, Et_3N, 20 min, 23 °C, >95%.

Scheme 4: Corey's first improvement to the *anti*-aldol step

The overall yield of the desired diastereoisomer was 80% after column chromatography. Conversion of **22** to the bicyclic γ-lactam intermediate **17**, however, was less efficient, proceeding in 64% yield over 3 steps. The overall yield was, however, increased. In addition to the improvement of the aldol reaction, Corey reported a procedure allowing the acid **3** to be converted to omuralide in one step using the peptide coupling reagent bis(2-oxo-3-oxazolidinyl)phosphinic chloride (**BOPCl**).



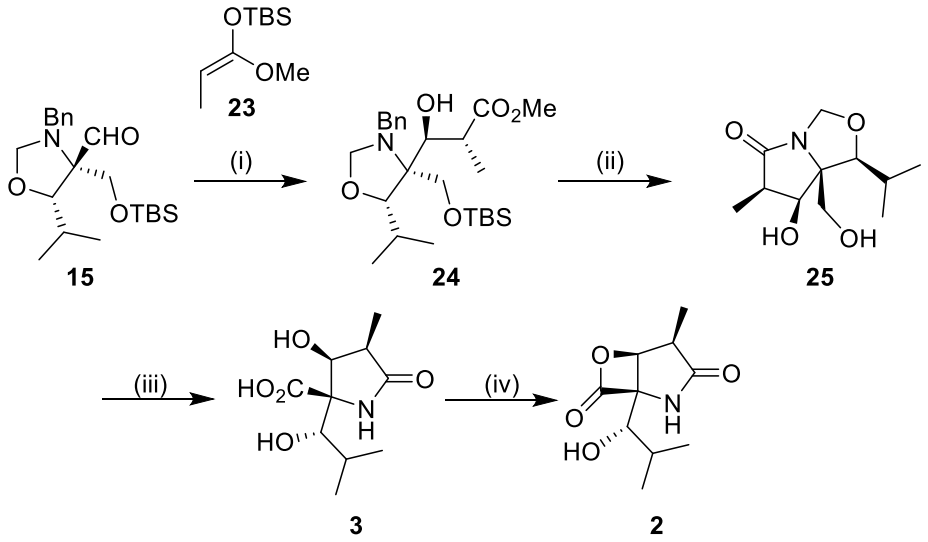
BOPCl

Figure 7: The peptide coupling reagent BOPCl

1.5.1.3 Further improvement to the aldol reaction

In a final improvement to the aldol reaction (**Scheme 5**), Corey used addition of a simple achiral silyl enol ether **23** catalysed by magnesium iodide to provide the desired intermediate

24 in 77% yield.[28] This simple intermediate could be converted to the deprotected bicyclic lactam **25** in 76% yield over 3 steps.

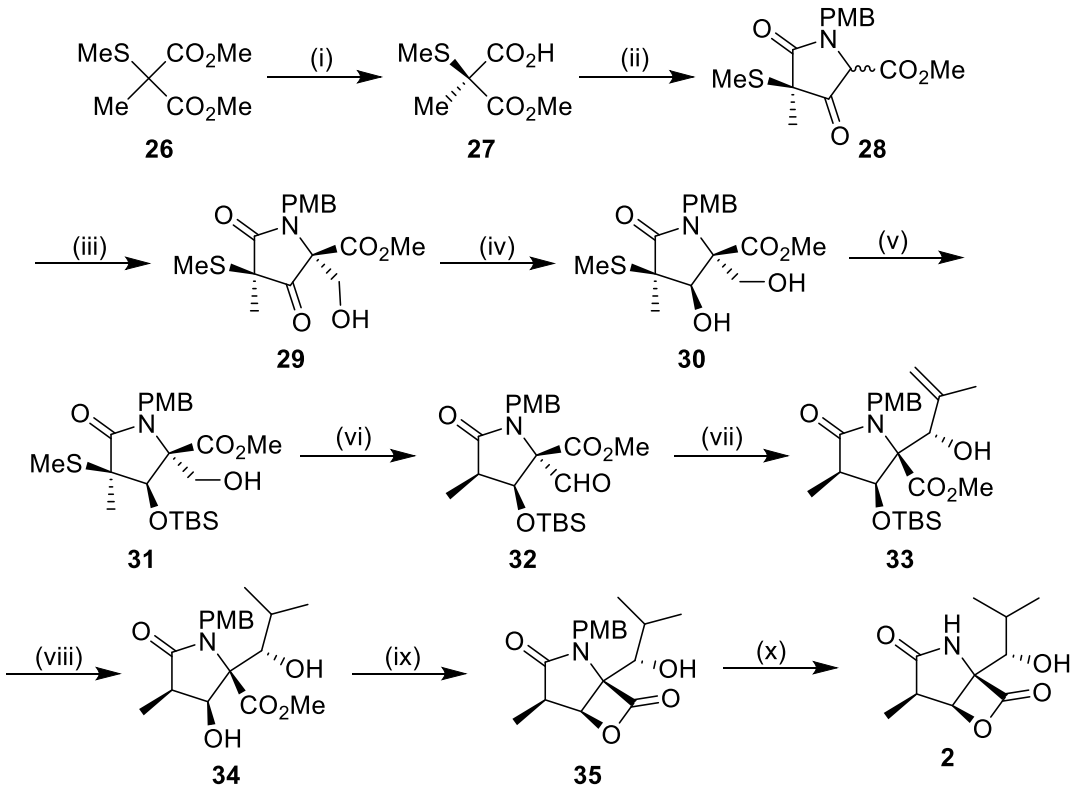


Reagents and conditions: (i) MgI_2, CH_2Cl_2, 0 °C, 20 min, -10 °C, **23**, 0 °C 2 h, 77%; (ii) 1) H_2, Pd/C, EtOH, (*i*-pr)$_2$NEt, 23 °C, 30 h, 2) MeOH 55 °C, 20 h, 3) 5%, HF-MeCN, 23 °C, 24 h 76%; (iii) 1) Et_3N, DMSO-$(COCl)_2$, CH_2Cl_2, -78 °C, 2) $NaClO_2$, NaH_2PO_4, *t*-BuOH, 23 °C, 3) $HS(CH_2)_3SH$, HCl, CF_3CH_2OH, 55 °C, 8 h, 77%; (iv) BOPCl, Et_3N, CD_2Cl_2, Et_3N, 1 h, 23 °C, 93%.

Scheme 5: Corey's second improvement to the anti-aldol step

1.5.1.4 Corey's second strategy to omuralide

In 1998 Corey *et al.* reported a radically different approach to omuralide and lactacystin (**Scheme 6**).[29] The methylsulfanyl derivative **26** was converted to the chiral monoester **27** in 97% yield and a 95% enantiomeric excess (**ee**) through hydrolysis with porcine liver esterase (**PLE**). Formation of the quinine salt and recrystallization from aqueous ethanol provided **27** in 95% ee. Treatment with oxalyl chloride provided the acid chloride, which was coupled to a *para*-methoxy benzyl (**PMB**)-protected glycine methyl ester. A lithium diisopropylamide (**LDA**)-induced Dieckmann cyclization formed lactam **28** in a 1:1 mix of diastereoisomers. Hydroxymethylation occurred using formaldehyde in a highly stereoselective (9:1) reaction, formaldehyde addition favouring the face opposite the SMe group to yield **29**.



Reagents and conditions: (i) PLE, H_2O, pH 7.3, 23 °C, 24 h, 97%; (ii) 1) $(COCl)_2$, DMF (cat), 23 °C, 1 h, 2) PMB-NHCHCO$_2$Me, Et_3N, CH_2Cl_2, 23 °C, 1 h, 3) LDA, THF, -78 °C, 2 h, 93%; (iii) DBU, THF, -78 °C, then aq. CH_2O, -78 °C, 30 m, 90%; (iv) $NaBH(OAc)_3$, HOAc, 23 °C, 1 h, 95%; (v) 1) PivCl, pyridine, 23 °C, 10 h, 85%, 2) TBSOTf, 2,6-lutidine, 23 °C, 12 h, 98%, 3) NaOMe, MeOH, 23 °C, 92%; (vi) 1) Raney Ni, EtOH, 0 °C, 1 h, 82%, 2) Dess-Martin reagent, CH_2Cl_2 23 °C, 1 h, 95%; (vii) H_2C=C(Me)MgBr, TMSCl, THF, -40 °C, 0.5 h, 97%; (viii) 1) H_2, Pd/C, EtOH, 23 °C, 12 h, 99%, 2) TFA/H_2O 4:1, 50 °C, 4 h, 87%; (ix) 1) LiOH, THF/H_2O 1:1, 23 °C, 0.5 h, 2) BOPCl, Et_3N, CH_2Cl_2, Et_3N, 0.5 h, 23 °C, 90%; (x) CAN, CH_3CN/H_2O 3:1, rt, 1 h, 62%.

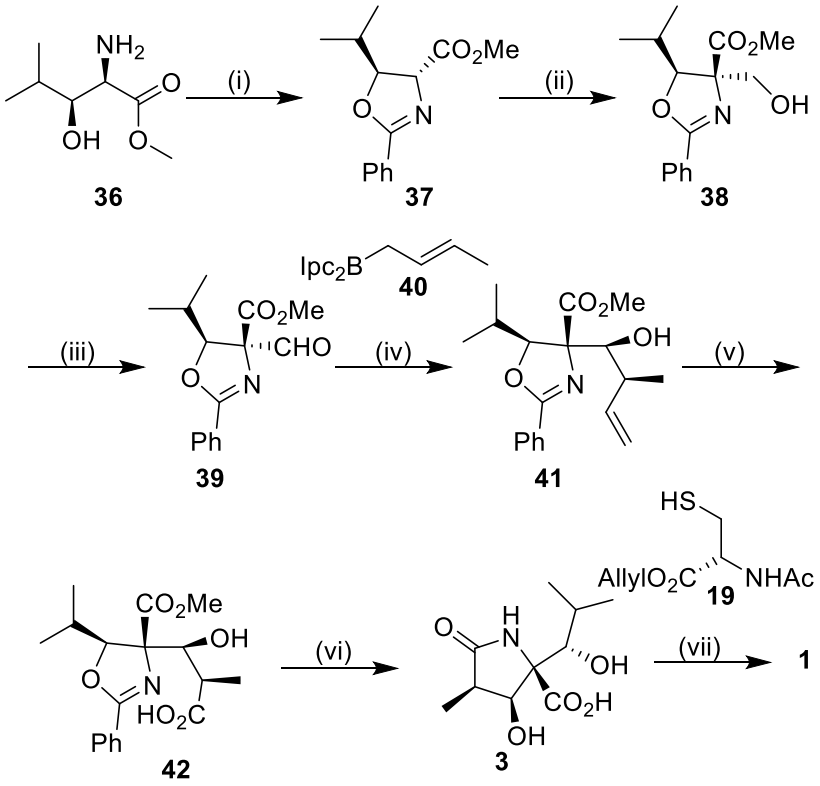
Scheme 6: Corey's alternative route to omuralide

A stereoselective reduction with $NaH(OAc)_3$ formed the C6 hydroxyl group in the desired chirality for omuralide; again, attack occurred opposite to the SMe moiety to form **30**. The primary hydroxyl group was protected with a pivaloyl protecting group allowing a *tert*-Butyldimethylsilyl (**TBS**) protection of the C6 hydroxyl position. Hydrolysis of the pivaloyl (**Piv**) protecting group provided **31**. An unusually stereoselective (10:1) desulphurization with Raney® Nickel provided the C7 methyl group with the desired stereochemistry (*cis* to the hydroxyl group). Subsequent oxidation of the primary alcohol to the aldehyde with Dess-Martin periodinane[30] yielded the advanced intermediate **32**. Treatment of the aldehyde with

a prop-2-enyl Grignard reagent incorporated the isobutyl moiety found in omuralide. Reduction of the alkene, formation of the β-lactone, and final protecting group removals furnished omuralide in a total of 18 steps from **26**.

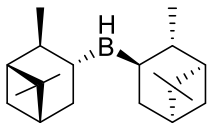
1.5.2 The Ōmura synthesis of lactacystin

Shortly after Corey's seminal synthesis, Ōmura *et al.* published their route to lactacystin (**Scheme 7**).[6] (2*R*,3*S*) Hydroxy leucine methyl ester **36** was cyclized to oxazoline **37** by treatment with methyl benzimidate. Hydroformylation through a lithium enolate formed **38** in excellent diastereoselectivity (>98%). A Moffatt oxidation formed the aldehyde **39**, which was subjected to an allylboration with **40**, a method reported by Brown utilizing camphor derived borane **43** (**Figure 7)**.[31]



Reagents: (i) Ph(MeO)C=NH, 72%; (ii) LiHMDS, HCHO, 85%; (iii) DMSO, DCC, Pyridine, TFA, Benzene, rt, overnight; (iv) (E)-crotyl(diisopinocampheyl)borane **40**, 70% (over 2 steps); (v) 1) O_3, DMS; 2) $NaClO_2$, NaH_2PO_4, 56%; (vi) 1) Pd, HCO_2NH_4 2) 0.1 N NaOH, 82%; (vii) 1) *N*-acetylcystine allyl ester **19**, BOPCl, Et_3N, 79%; 2) $Pd(Ph_3P)_4$, 81%.

Scheme 7: Ōmura's strategy for lactacystin

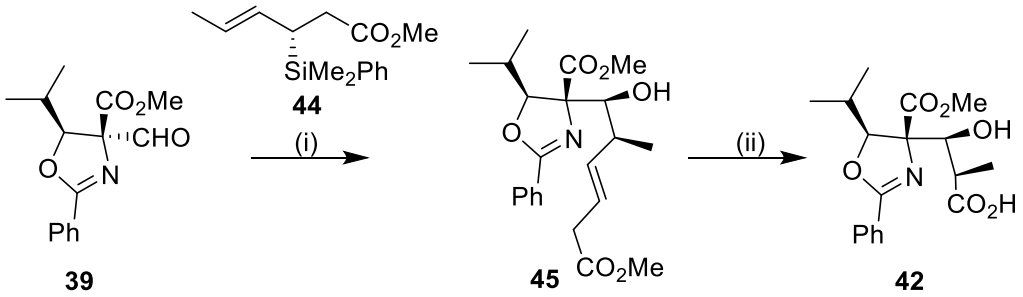


43

Figure 8: The camphor derived borane **43**

The alkene was converted to the aldehyde after treatment with ozone. The resulting aldehyde was oxidized to provide **42**. Hydrogenation formed the γ-lactam ring, which, after ester hydrolysis, gave **3**, which was converted to lactacystin by methods identical to Corey's synthesis. From **36** the overall number of steps was 10 and lactacystin was obtained in 13% overall yield.

1.5.3 The Panek synthesis of lactacystin



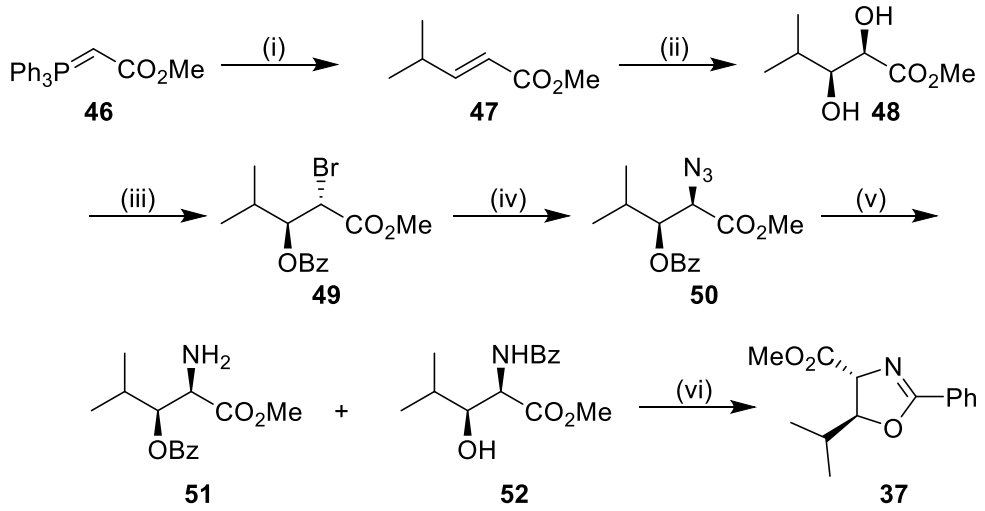
Reagents and conditions: (i) **44**, $TiCl_4$, -78 °C - -35 °C, 60% (ii) 1) O_3, DMS 2) $NaClO_2$, NaH_2PO_4 90% (over the 2 steps).

Scheme 8: Panek's improvement to the Ōmura synthesis

In 1999 Panek *et al*.[32] made several improvements to the Ōmura synthesis, firstly to the synthesis of the starting material (*R,S*) hydroxy leucine **36** in a reduced number of steps (**See chapter 2.5.1.4.4.3**). In addition, improvements were made to the incorporation of the C7 methyl and C6 hydroxyl groups to produce **42** (**Scheme 8**). Panek's strategy involved a crotylation with $TiCl_4$ providing the *anti* diasteroisomer **45** in >30:1 ratio to the *syn*. Ozonolysis of the alkene and oxidation of the resulting aldehyde formed the common intermediate **42**. The synthesis was then completed in an analogous fashion to Ōmura.

1.5.4 The Adams synthesis of omuralide

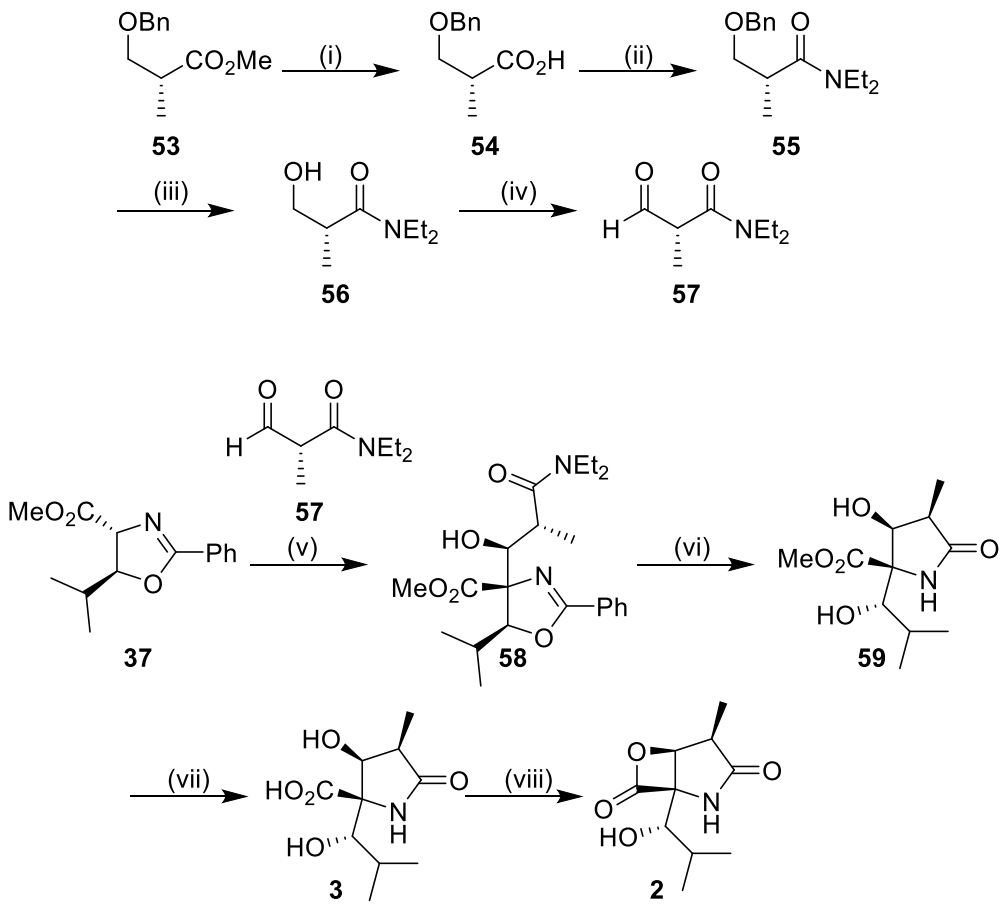
The shortest and most high yielding route to omuralide to date was completed by Adams *et al*. in 1999,[33] starting in a similar manner to Ōmura using a (*R*,*S*) hydroxy leucine derivative. Enone **47** was synthesized using a Wittig reaction from ylide **46**. Sharpless dihydroxylation of the enone produced **48** in good yield and >99% ee after recrystallization. Methodology Developed by Sharpless[34] was used to brominate the alpha position of the ester; displacement by sodium azide gave **50**. Reduction of the azide produced a mixture of the benzyl ester **51** and benzyl amide **52**, but, on treatment with *p*-TsOH only the Ōmura intermediate **37** was formed from the mixture (**Scheme 9**).



Reagents and conditions: (i) *iso*-butyraldehyde, DCM, 93%; (ii) $DHQ_2(PHAL)$, $K_2OsO_2(OH)_4$, NMO, *t*-BuOH:H_2O 1:1, 60%; (iii) $PhC(OMe)_3$, $BF_3.OEt_2$, DCM, then CH_3COBr, NEt_3, 0 °C, 93%; (iv) NaN_3, DMSO, 85%; (v) H_2, $Pd(OH)_2/C$, 85%; (vi) *p*-TsOH, 89%.

Scheme 9: The Adams synthesis of omuralide

A chiral aldehyde, **57**, was formed from **53**. Saponification of **53** and subsequent construction of the amide with diethyl amine formed **55**. Deprotection of the alcohol, followed by oxidation, formed **57** (**Scheme 10**). Treating the Ōmura intermediate **37** to an aldol reaction with a chiral aldehyde **57**, with the correct stereochemistry of the methyl unit already incorporated, furnished intermediate **58**. Hydrogenation and subsequent cyclization provided the ester **59**. Saponification and formation of the β-lactone ring formed omuralide (**Scheme 10**).



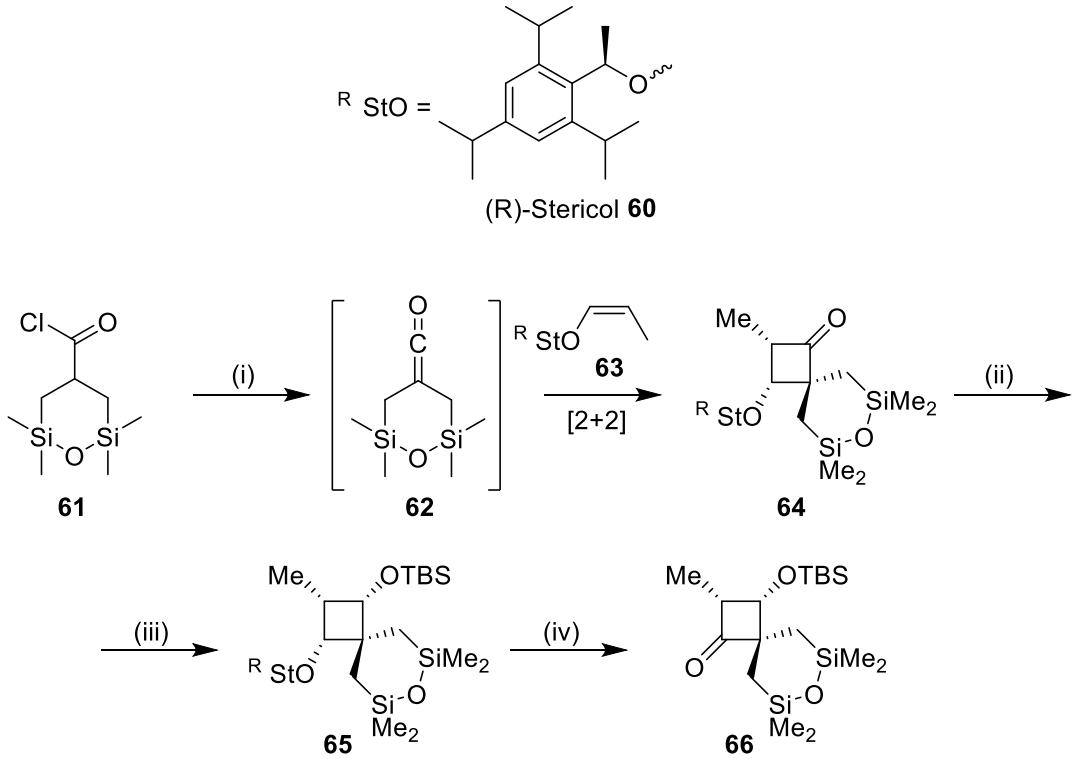
Reagents: (i) LiOH (ii) Et_2NH, DIEA, TBTU, 85% (over the 2 steps); (iii) H_2, $Pd(OH)_2/C$, 100%; (iv) Dess-Martin oxidation, 88%; (v) LiHMDS, Me_2AlCl, **57**; (vi) H_2, $Pd(OH)_2/C$, 67% (over the 2 steps); (vii) $NaOH/H_2O$, 93%; (viii) isopropenyl chloroformate, NEt_3, 79%.

Scheme 10: Synthesis of the chiral aldehyde **57** and its use to synthesize omuralide

1.5.5 The Poisson synthesis of omuralide

To date, the most recent total synthesis of omuralide was completed by Poisson *et al.*, leading on from their work on highly diastereoselective [2+2] cycloadditions. Using stericol as a chiral auxiliary, the methodology[35] was applied to the synthesis of omuralide.[36] Treatment of **61** with triethylamine formed ketene **62** *in situ*, which underwent a [2+2] cycloaddition with alkene **63** to give **64**. The chiral auxiliary stericol **60** was able to produce diastereoselectivities of >98:2 in **64**. Reduction of the carbonyl group occurred in again excellent diastereoselectivities (>98:2) to produce a hydroxyl group which was protected as a TBS ether.

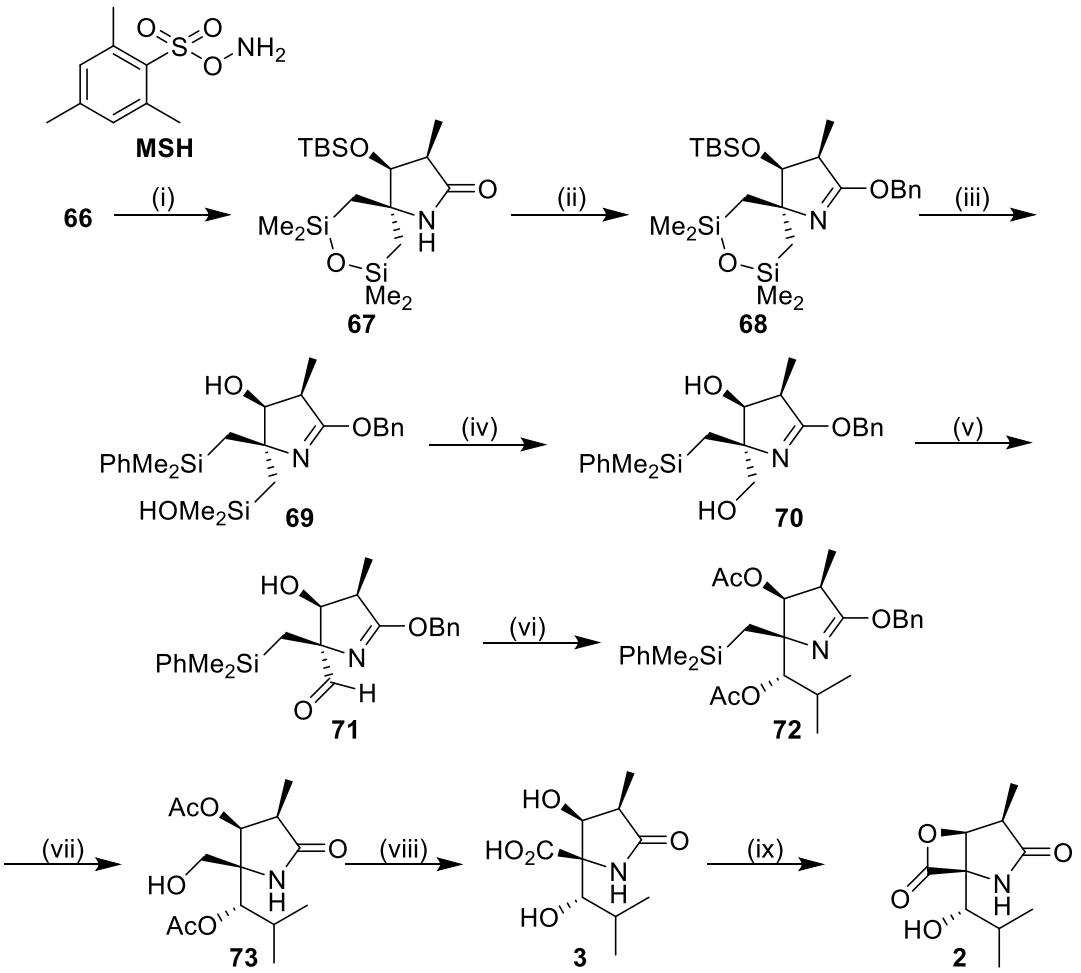
Removal of the stericol chiral auxiliary **60** allowed the hydroxyl group to be oxidized to **66** (**Scheme 11**).



Reagents: (i) Et_3N, **62**, Toluene 70 °C, 97%; (ii) 1) DIBAL-H 2) TBSOTf, lutidine, 95%; (iii) 1) TFA 2) DMP, 75%.

Scheme 11: Part 1 of the Poisson synthesis of omuralide

An *O*-mesitylenesulfonylhydroxylamine (**MSH**) mediated Beckmann transposition formed the γ-lactam found in omuralide, and protection of the amide was carried out with benzyl bromide and sodium hydride to form **68** (**Scheme 12**). Removal of the TBS ether allowed selective opening of the oxidisilinane to a silanol and a dimethylphenylsilane. The silanol was oxidized using Tamao oxidation conditions, allowing addition of the *iso*-propyl group through the corresponding organolithium species. Subsequent protection of the free hydroxyl groups produced **72**. Deprotection of the amide and oxidation of the silane produced the alcohol **73**, which could be further oxidized to the acid **3**, in turn cyclized to **2** using Corey's methodology.[26]



Reagents: (i) MSH, 58%; (ii) NaH, BnBr, 92% (iii) 1) TBAF, 2) PhLi (iv) KF, H_2O_2, 54% (over the 3 steps); (v) TEMPO, NCS, 64%; (vi) 1) *iso*-propylLi 2) Ac_2O, Py, 51%; (vii) 1) H_2 Pd/C 2) KBr, AcOOH, 80%; (viii) 1) Jones oxidation; 2) NaOH; (ix) BOPCl, Et_3N, 90%.

Scheme 12: Part 2 of the Poisson synthesis of omuralide

1.6 SAR studies of omuralide

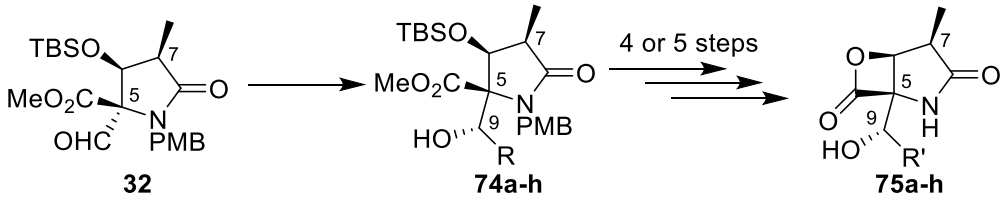
1.6.1 Introduction to the SAR of omuralide

Total synthesis of natural products is always a worthwhile pursuit for the exploration and understanding of molecules and reactivity, but when a bioactive natural product is the target, the most prized routes are those where the final compound can be easily modified to produce analogues of the natural product. This can often provide critical information about the mechanism of action in these compounds and occasionally improve the bioactivity, or create more stable versions, allowing a greater chance of using the molecule as a drug candidate.

The largest contribution to date on the SAR studies of omuralide was made by Corey.[37] Simple, mid to late stage modifications of two of his total syntheses was able to produce a range of C5, C9 and C7 analogues.

1.6.2 Modifications of the C5 and C9 position

The overwhelming majority of C5 and C9 analogues Corey was able to produce were from simply exchanging the vinyl Gringard reagent or using organochromium reagents. Taking the product through methodology analogous (reduction of the alkene step was removed for **74d**,**f**,**h**) to the original route discussed in **Chapter 1.4.1.4** was sufficient to produce a wide variety of C9 analogues. Each were tested in their inhibition rates of the chymotrypsin-like activity of the 20S proteasome (**Table 2**).

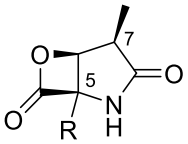


32 **74a-h** **75a-h**

Analogue	R' =	K_{assoc} (M^{-1} S^{-1})
Omuralide 2	$CH(CH_3)_2$	3059 ± 478
75a	H	9.7 ± 6.2
75b	C_6H_5	No inhibition
75c	C_2H_5	290 ± 12
75d	$CH=CH_2$	188 ± 11
75e	$CH_2CH_2CH_3$	192 ± 35
75f	$CH_2CH=CH_2$	255 ± 40
75g	$CH_2CH(CH_3)_2$	17.4 ± 2.4
75h	$CH_2CH(CH_3)=CH_2$	64.7 ± 2.2

Table 2: Association rates of C9 analogues on the 20S proteasome CT site

Corey was unable to improve on activity with any of the C9 analogues. Further modification of **32** allowed a library of C5 analogues to be produced primarily involving modifications to the C5 hydroxyl group (**Table 3**).



76a-d

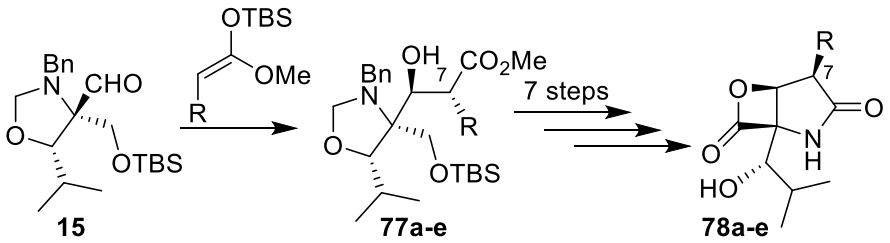
Analogue	R =	K_{assoc} (M^{-1} S^{-1})
Omuralide 2	(*S*) $CHOHCH(CH_3)_2$	3059 ± 478
76a	(*R*) $CHOHCH(CH_3)_2$	No inhibition
76b	$COCH(CH_3)_2$	65 ± 9.6
76c	$CH_2CH(CH_3)_2$	235 ± 16
76d	$CH{=}C(CH_3)_2$	98 ± 5

Table 3: Association rates of C5 analogues on the 20S proteasome CT site

Again, activity was not improved past that of omuralide. All modifications made to either the C9 or C5 position drastically reduced activity. It seemed the hydroxylated *iso*-butyl moiety was optimal for proteasome inhibition.

1.6.3 Modifications to the C7 position

To analyse the position at C7, Corey modified his improved original route to omuralide. Not only did Corey's improved *anti*-aldol reaction discussed in **chapter 1.5.1.3** improve yields of the omuralide synthesis, but the reaction was also able to tolerate various other alkyl groups into the C7 position to produce intermediates **77a-e**. The remainder of the synthesis was completed in an analogous way to the original route. The C7 epimer **78f** (derived from the by-product of the *anti*-aldol reaction) was also tested. Also included in **table 4** is an analogue synthesized by Adams *et al*.[33] Like Corey's synthesis, modification during the aldol reaction (in this case through the use of a different chiral aldehyde) was able to provide an *n*-propyl analogue. Analogue **78g** was then elaborated to the omuralide derivative in an analogous way to the original Adams route discussed in **chapter 1.5.4**.



Analouge	R =	K_{assoc} (M^{-1} S^{-1})
Omuralide 2	CH_3	3059 ± 478
78a	H	450 ± 77
78b	CH_2CH_3	6679 ± 553
78c	$(CH_2)_3CH_3$	7275 ± 466
78d	$CH(CH_3)_2$	8465 ± 1572
78e	$CH_2C_5H_6$	2227 ± 180
78f	CH_3 (epimer)	1250 ± 180
PS – 519 78g	$CH_2CH_2CH_3$	7127

Table 4: Association rates of C7 analogues on the 20S proteasome CT site

Modifications to the C7 site were not only well tolerated but in many cases also vastly increased rates of association. In general, larger groups improved rates of inhibition. The most improved analogue tested was an *iso*-propyl group derivative. However, if the group size was increased vastly, such as the benzyl group in **78e**, inhibition would decrease compared to omuralide.

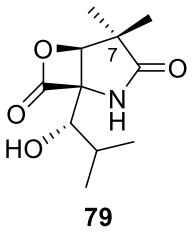


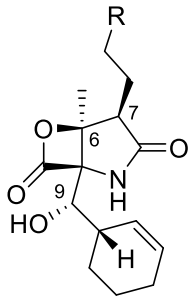
Figure 9: The potent dimethylated Corey analogue

Another important C7 analogue is the *gem*-dimethyl omuralide analogue **79**. Despite slightly lower activities than omuralide, the loss of one chiral centre has made the analogue a more attractive target for practical purposes. Corey has undertaken several syntheses of this target by increasingly efficient methods.[38,39,40]

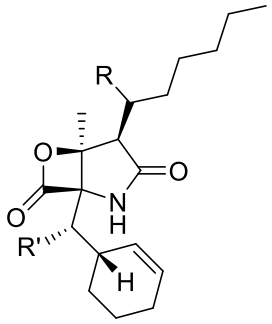
It was thought by Corey that at this point omuralide and related β-lactone, γ-lactam proteasome inhibitors could be improved no further, concluding these investigations.

1.7 The salinosporamides and cinnabaramides

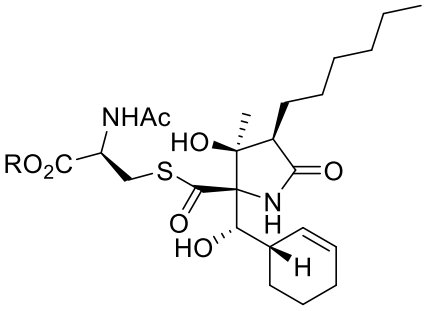
1.7.1 A new generation of β-lactone, γ-lactam proteasome inhibitors



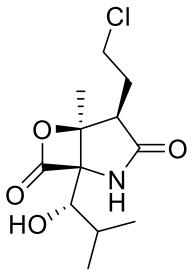
R = Cl Salinosporamide A **80**
R = H Salinosporamide B **81**



R' = H R = OH Cinnabaramide A **82**
R' = OH R = OH Cinnabaramide B **83**
R' = H R = H Cinnabaramide C **84**



R = H Cinnabaramide F **85**
R = CH_3 Cinnabaramide G **86**



Antiprotealide **87**

Figure 10: New β-lactone, γ-lactam proteasome inhibitors

Contrary to the belief that the β-lactone, γ-lactam core could be improved no further, in 2003 Fenical *et al.* reported the discovery of a new β-lactone, γ-lactam proteasome inhibitor.[41] Salinosporamide A; isolated from the newly discovered marine actinomycete salinispora tropica. Salinosporamide contained the same β-lactone, γ-lactam core as omuralide, but this scaffold contained increasingly advanced substructures. Most unusual was the modification to the C7 position, which now contained a chloroethyl chain. Further modifications to the structure included a cyclohexene moiety at C5, and a methyl at C6. When tested against omuralide for proteasome inhibition activity at the chymotrypsin-like site, salinosporamide

showed remarkable activity; approximately 35x more potent. Later work[42,43] looking at salinispora tropica uncovered several more members of the salinosporamide family of compounds, including salinosporamide B - a dechlorinated derivative of salinosporamide A. The majority of these salinosporamide derivatives were found to be considerably more active than omuralide.

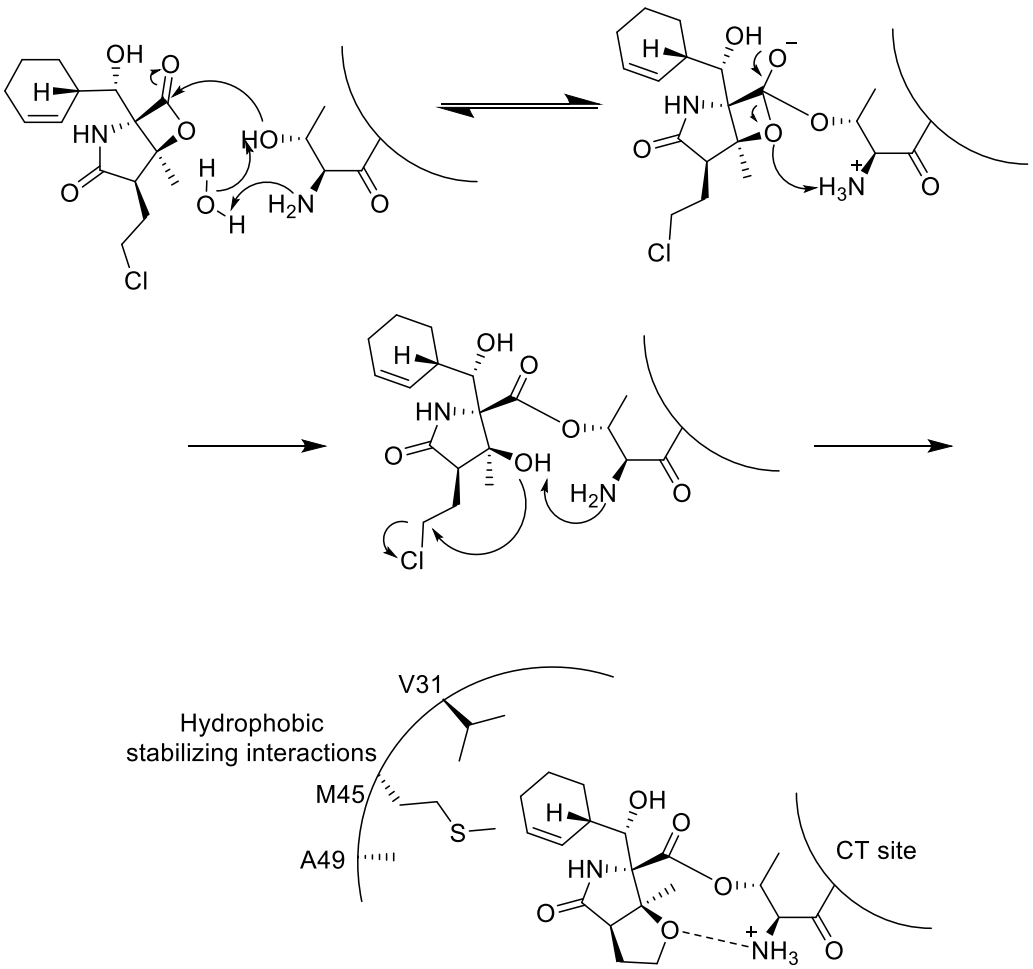
Furthermore, in 2007 the discovery of the cinnabaramides A-G was reported.[44] Containing the same β-lactone, γ-lactam core as omuralide, and an identical cyclohexene moiety as found in the salinosporamides. The cinnabaramides also displayed activities far greater than lactacystin. Interestingly the thioester derivatives **85** and **86**, unlike lactacystin, show similar, even increased activities compared to their β-lactone counterpart (**Table 5**).

Compound	IC_{50} [nm] inhibition of human proteasome
Lactacystin 1	259
Cinnabaramide A 82	1
Cinnabaramide B 83	245
Cinnabaramide C 84	12
Cinnabaramide F 85	6
Cinnabaramide G 86	0.6

Table 5: Inhibition activities of the cinnabaramides

1.7.2 Salinosporamide A's mechanism of action

In 2006 Groll *et al.* reported a crystal structure of salinosporamide A in the 20S proteasome.[45] A key difference to omuralide was the formation of a cyclic ether between the chloroethyl and C6 hydroxyl group. Groll suggested this blocked hydrolysis of the ester, increasing stability of the salinosporamide A-proteasome complex. The cyclization was also entropically and enthalpically favourable. The observations led Groll to propose the mechanism of 20S proteasome inhibition by salinosporamide A, shown below (**Scheme 13**).



Scheme 13: The mechanism of salinosporamide A inhibiting the CT site of the 20S proteasome

Unlike the cinnabaramides, lactacystin-like thioester derivatives of salinosporamide have not been discovered in nature. It is likely that this is because this could induce premature cyclization of the cyclic ether due to the free hydroxyl group, reducing the inhibitory potential of salinosporamide A.[46]

1.7.3 SAR of the salinosporamides

Extensive work has been reported on the SAR studies of salinosporamide, and reviewed.[46,47] Unlike omuralide, these studies have been unable to produce dramatic changes in the potency of salinosporamide, however, analogues have been produced with complementary inhibitory potential as salinosporamide A, and have shown the importance of several salinosporamide functionalities (**Table 6**). This provides potentially useful structural information for future development of β-lactone, γ-lactam 20S proteasome inhibitors.

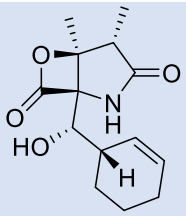
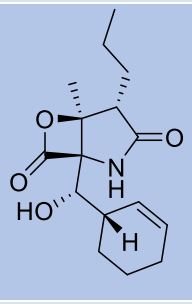
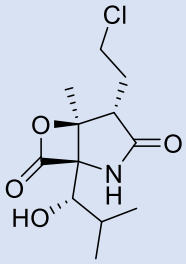
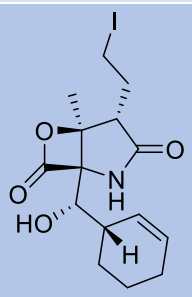
Name	Structure	IC_{50} Value CT site nM
Omuralide	**2**	57 ± 6
Salinosporamide A 80	**80**	2.6 ± 0.2
Salinosporamide B 81	**81**	27 ± 4
Salinosporamide D 88		7.7 ± 3
Salinosporamide E 89		24 ± 5
Antiprotealide 87		31 ± 5
Iodosalinosporamide 90		2.8 ± 0.5

Table 6: SAR activity of salinosporamide analogues

Although changes to the structure of salinosporamide A generally provide less potent analogues. Many of these derivatives still possess activity greater than omuralide. These results seem to suggest the greatest modification from omuralide to salinosporamide A for proteasome inhibition, is the addition of the cyclohexene unit. This can be seen clearly in the comparison of omuralide with salinosporamide D **88**, where addition of the C6 methyl and C5 cyclohexene ring have increased potency from omuralide by about 7x. Furthermore, replacement of the cyclohexene ring in salinosporamide A with an omuralide like *iso*-propyl

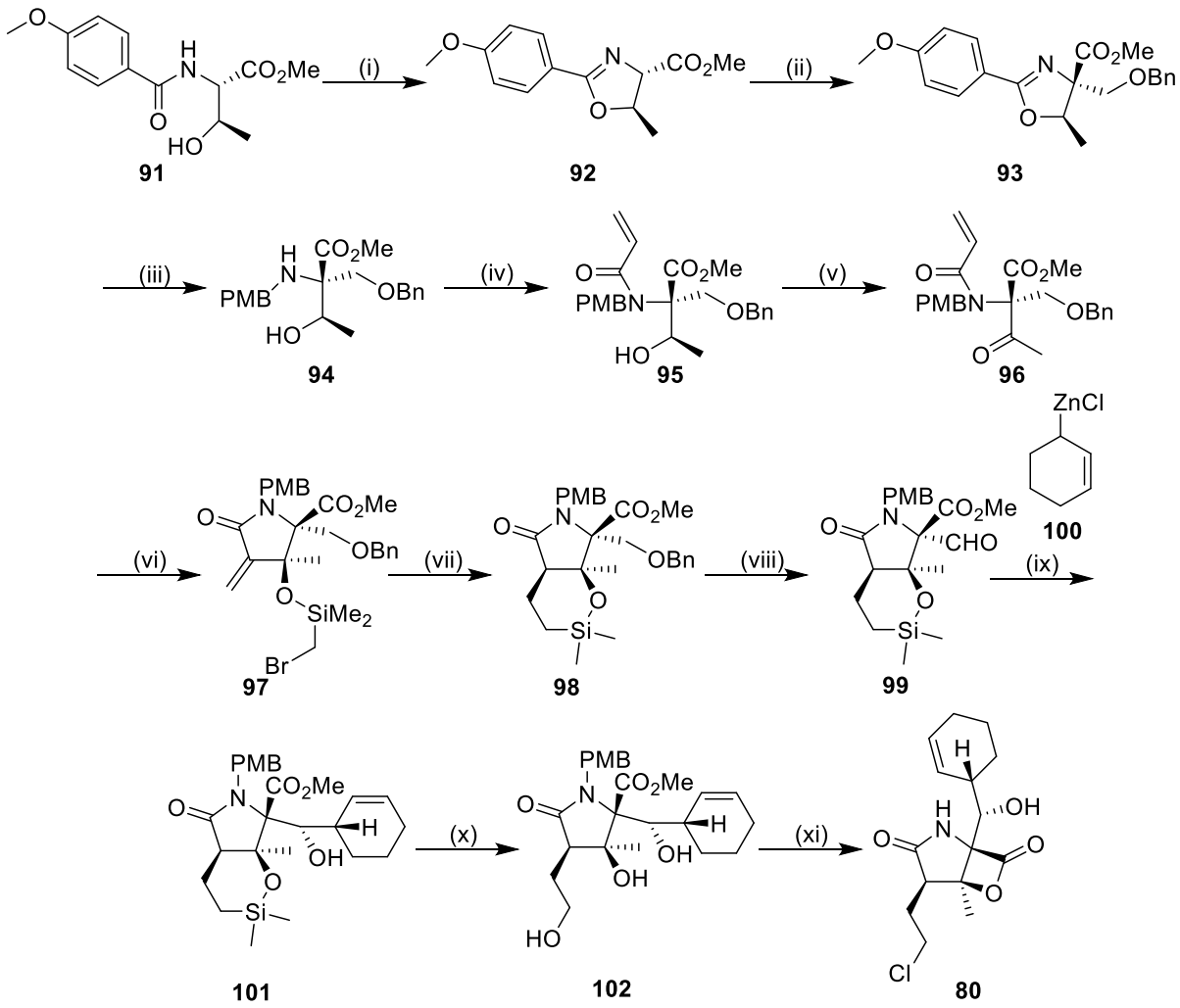
group (antiprotealide **87**) decreases potency by 12x. Antiprotealide was first synthesized by Corey[48] as a hybrid of omuralide and salinosporamide A then, later, found also to be a natural product.[49] Interestingly, unlike omuralide analogues, salinosporamide B appears not to benefit from extension of the C7 alkyl group (salinosporamide E **89**), potency even increased on shortening the ethyl chain to a methyl, as in salinosporamide D **88**.

Where the most promising results have been found however, is modification of the chlorine on the C7 alkyl chain with better leaving groups. Although IC_{50} values have remained relatively unchanged, cytotoxicity has been found to increase slightly.[47]

Like omuralide, key to understanding the full activity of salinosporamide A has been aided by a number of syntheses, first of which was completed by Corey *et al.*[50]

1.7.4 Total synthesis of salinosporamide A

1.7.4.1 The first total synthesis of salinosporamide A



Reagents and conditions: (i) *p*-TsOH, toluene, reflux, 12 h, 80%; (ii) LDA, THF, HMPA, -78 °C, $ClCH_2OBn$, 4 h, 69%; (iii) $NaCNBH_3$, AcOH, 40 °C, 12 h, 90%; (iv) 1) TMSCl, Et_2O, 23 °C, 12 h, 2) Acrylyl chloride, *i*-Pr_2NEt, CH_2Cl_2, 1 h, 0 °C, then H^+ Et_2O, 23 °C, 1 h, 96%; (v) Dess-Martin periodinane, 96%; (vi) Quinuclidine, DME, 0 °C, 7 d, 90%, 2) $BrCH_2Si(CH_3)_2Cl$, DMAP, CH_2Cl_2, 0 °C, 30 m, 95%; (vii) Bu_3SnH, AIBN, benzene, reflux, 8 h, 89%; (viii) 1) Pd/C, EtOH, H_2, 18 h, 95%, 2) Dess-Martin periodinane, 23 °C, 1 h, 95%; (ix) **100**, -78 °C, 5 h, 88%; (x) KF, $KHCO_3$, H_2O_2, THF-MeOH (1:1), 23 °C, 18 h, 92%; (xi) 1) CAN, MeCN-H_2O (3:1), 0 °C, 1 h, 83%, 2) 3 M, LiOH-THF (3:1), 5 °C, 4 d, 3) BOPCl, Pyridine, CH_2Cl_2, 23 °C, 1 h, 4) Ph_3PCl_2, Pyridine, MeCN, 23 °C, 1 h, (65% over the 3 steps).

Scheme 14: Corey's synthesis of salinosporamide A

The threonine amide derivative **91** was cyclized to **92** with *p*-TsOH allowing a completely diastereoselective alkylation with a chloro methyl benzyl ether (**Scheme 14**). Reduction with $NaBH_3CN$ provided the quaternary amino acid derivative **94**. Protection of the threonine alcohol with a trimethyl silyl (**TMS**) protecting group allowed clean acylation of the amine, subsequent deprotection of the alcohol provided **95** in 96% yield over the whole sequence. The threonine alcohol was then oxidized with Dess-Martin periodinane. Cyclization to the γ-lactam proceeded through a Baylis-Hillman aldol with quinuclidine in a highly diastereoselective manner (9:1). Protection of the hydroxyl group with a bromomethyldimethylsilyl chloride to **97** allowed a radical cyclization to **98**. Cleavage of the benzyl ether and oxidation of the resulting alcohol proceeded in excellent yield to **99**. The resulting aldehyde was used to incorporate the cyclohexene unit found in salinosporamide using 2-cyclohexene zinc chloride **100** in excellent diasteroselectivity (20:1). Tamo-Fleming oxidation provided **102** in excellent yield (92%). Removal of the PMB protecting group and saponification of the methyl ester was followed by formation of the β-lactone. Finally, chlorination of the alcohol provided salinosporamide A.

1.7.4.2 The Romo synthesis of salinosporamide A and (±)-cinnabaramide A

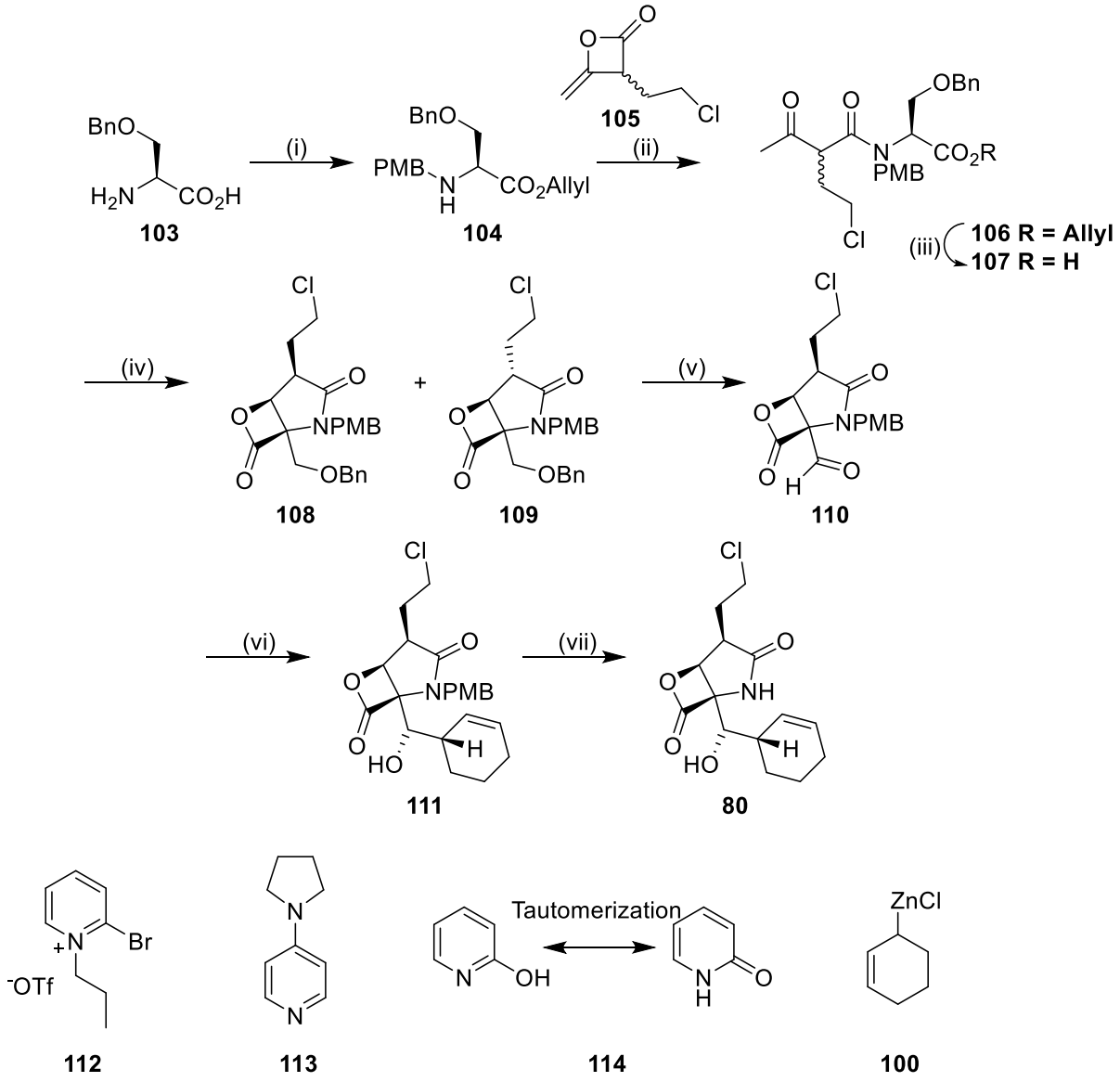
1.7.4.2.1 Introduction

Romo *et al.* utilized methodology previously devised in the group[51] which allowed a 1-pot production of β-lactone, γ-lactam scaffolds to great effect, applying this to both the synthesis of salinosporamide A and cinnabaramide A.[52]

1.7.4.2.2 Romo's synthesis of (±)-salinosporamide A

Benzyl protected serine **103** was protected with PMB on the amine and an allyl protecting group for the carboxylic acid to product **104**. The β-Lactone **105** was coupled to the protected serine derivative **104** in good yield (80%) using **115**. The pyridine derivative **114** opens the lactone **105** through attack of the carbonyl, forming an activated ester which is attacked by amine **104**. Amide **106** was deprotected to produce the cyclization precursor **107**. The *bis*-cyclization (**Scheme 16**) proceeded in low yields (up to 35%) and produced 2 diasteroisomers in up to a 3:1 ratio. Removal of the benzyl ether protecting group and oxidation of the resulting aldehyde provided **110**. Using Corey's methodology to install the cyclohexene ring (**Scheme 14**), which was surprisingly tolerant to the β-lactone, produced a 3.5:1 ratio of

diastereoisomers which, after PMB removal by ceric ammonium nitrate (**CAN**), produced salinosporamide A (**Scheme 15**).

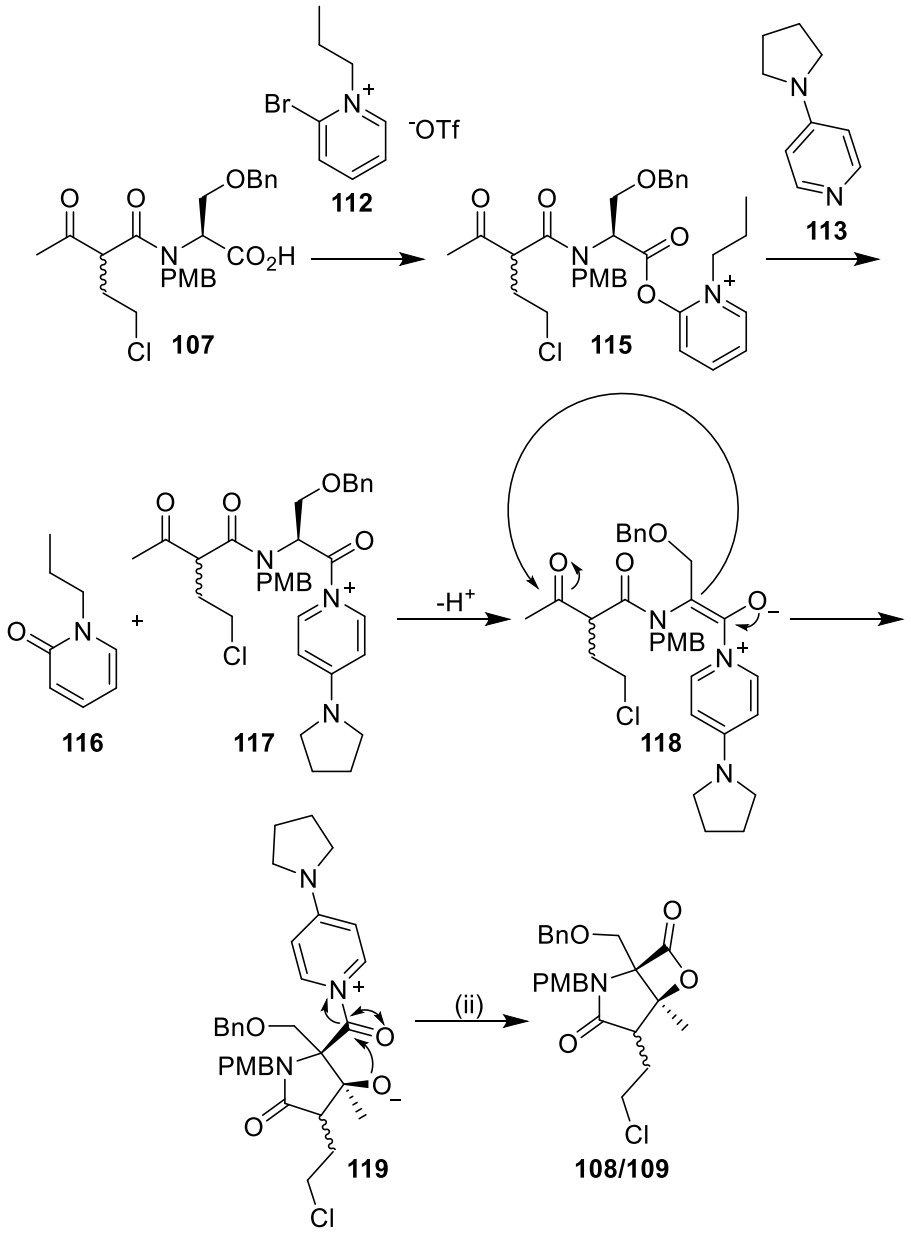


Reagents and conditions: (i) 1) *p*-methoxy benzaldehyde, MeOH, $NaBH_4$, 2) *p*-TsOH, allyl alcohol, (2 steps, 74%); (ii) **105**, **112**, THF, 60 °C, 36 h, 80%; (iii) ($Pd(PPh_3)_4$, morpholine, 75%; (iv) **114**, *i*-Pr_2NEt, **113**, CH_2Cl_2, -10 °C, 6 h, up to 35%; (v) 1) H_2 Pd/C, THF, 25 °C, 98%; 2) EDAC.HCl, DMSO, Cl_2CHCO_2H; (vi) **100**, THF, -78 °C, 33% over the 2 steps; (vii) CAN, $MeCN/H_2O$, 49%.

Scheme 15: Romo's synthesis of salinosporamide A

1.7.4.2.3 The key *bis*-cyclization in Romo's synthesis of salinosporamide A

The *bis*-cyclization reported by Romo first requires activation of the acid **107** by **112**. Substitution of this by the 4-dimethylaminopyridine (**DMAP**)-like derivative **113** further activated the acid, providing the intermediate **117** with an excellent leaving group. Deprotonation to the pyridinium enolate allows an aldol reaction, to form the γ-lactam (**119**). Attack from the newly formed alkoxylate on the pyridinium-activated-carbonyl forms the β-lactone (**Scheme 16**).

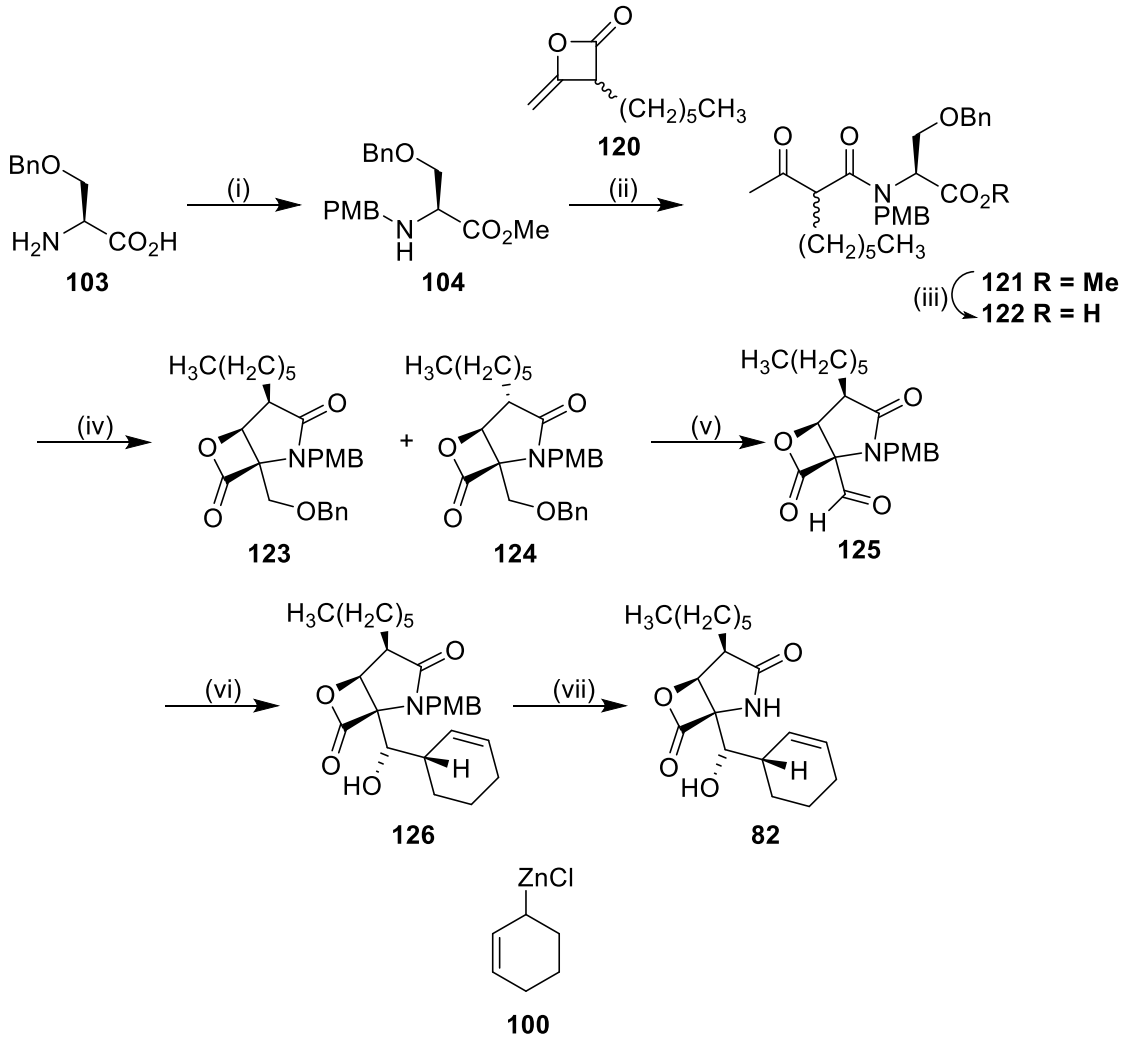


Scheme 16: The mechanism for the bis-cyclization reported by Romo

Despite several steps with decreased yields, Romo synthesized salinosporamide A in a remarkably short number of steps. In addition, modification of the lactone **105** allowed the methodology to be expanded further.

1.7.4.2.4 The first synthesis of (±)-cinnabaramide A by Romo

The methodology designed by Romo was then applied to cinnabaramide A (**Scheme 17**). Like the route to salinosporamide A, benzyl protected serine **103** was used, and protected with a PMB group and a methyl ester. Coupling of this to **120** produced the *bis*-cyclization precursor after deprotection of the ester to the acid with trimethyltin hydroxide. The cyclization proceeded in slightly better yield than the route to salinosporamide A, and better diastereoselectivity - (4.7:1) **123:124**. The final steps occurred in an analogous fashion to Romo's synthesis of salinosporamide A.



Reagents and conditions: (i) 1) *p*-methoxy benzaldehyde, MeOH, $NaBH_4$, 2) $TMSCHN_2$, $MeOH/Et_2O$, (2 steps, 58%); (ii) **120**, **114**, THF, 50 °C, 48 h, 85%; (iii) Me_3SnOH, 69%; (iv) **112**, *i*-Pr_2NEt, **113**, CH_2Cl_2, 0 °C, up to 45%; (v) 1) H_2 Pd/C, THF, 25 °C, 79%; 2) SO_3.pyridine, DMSO; vi) **100**, THF, -78 °C, 57% over the 2 steps; (vii) CAN, $MeCN/H_2O$, 48%.

Scheme 17: Romo's synthesis of cinnabaramide A

Later variants of this synthesis, primarily the separation of the **107** diastereoisomers, allowed an enantioselective route to salinosporamide A, including the production of new analogues of this valuable compound.[53]

1.8 Similar γ-lactam cores in other natural products

Similar γ-lactam cores are found in other natural products which are currently not known to have proteasome inhibiting potential. Although our primary focus is omuralide and related

derivatives, such compounds further highlight the importance of developing methodology to access these biologically relevant cores.

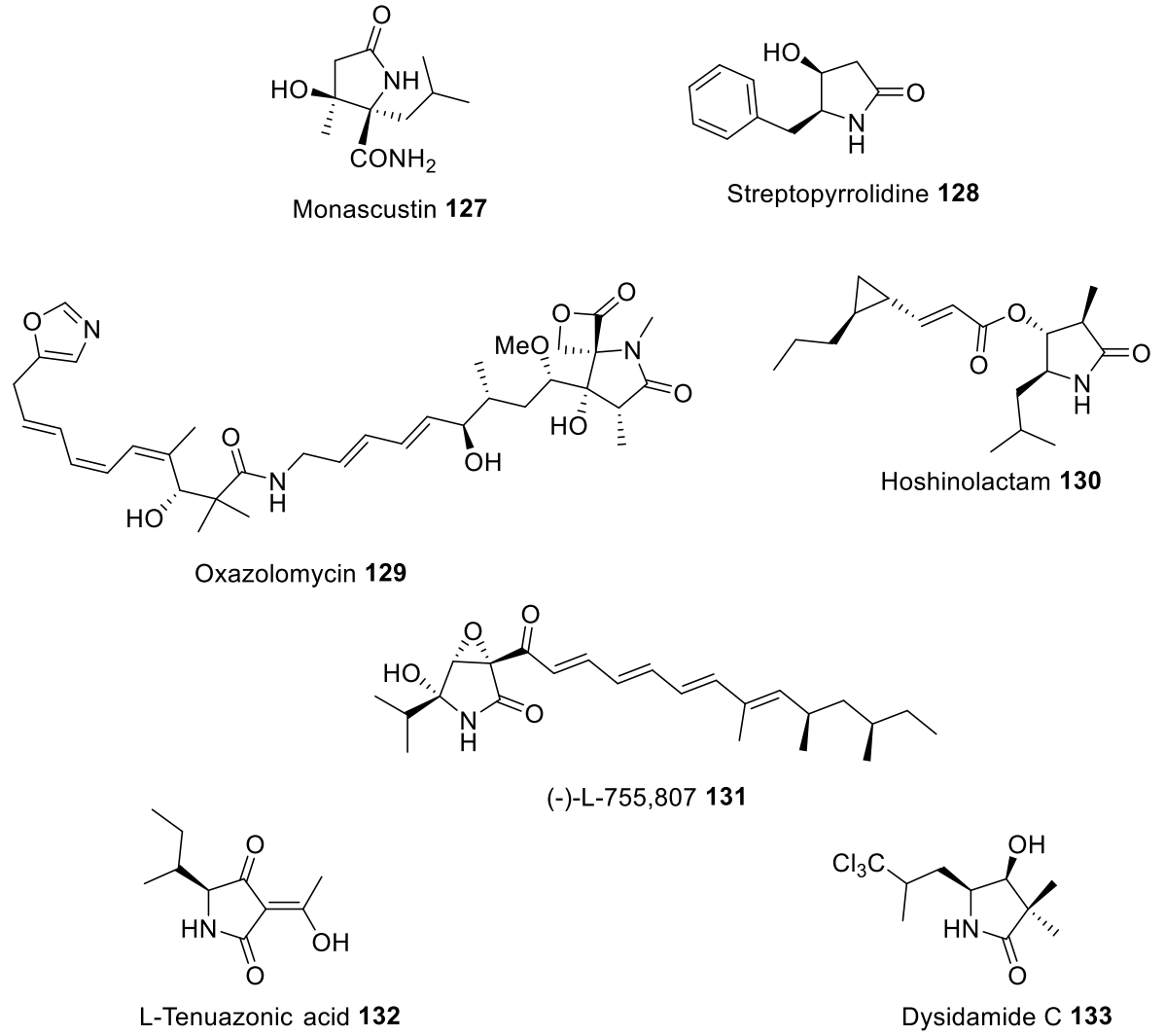


Figure 11: A selection of γ-lactam natural products

Monascutin **127** was discovered in 2016 after being isolated from red yeast rice.[54] Streptopyrrolidine **128** was discovered in 2008 after isolation from deep sea sediment.[55] Interest in this molecule stems from its ability to inhibit angiogenesis, which could provide promising anti-tumour drugs. Oxazolomycin **129** is particularly interesting, not only due to its γ-lactam core but also the spiro β-lactone ring.[56] Oxazolomycin **129** exhibits antibacterial activity and is under investigation by several groups.[57,58] The lajollamycins are another related family of compounds which share similar structural features to oxazolomycin.[59] Hoshinolactam **130** was recently discovered as an isolate from a marine cyanobacterium which displays potent anti-parasitic properties.[60] (-)-L-755,807 **131** was originally discovered

by Merk,[61] interestingly, under investigation for its ability to induce neurite outgrowth, which studies into omuralide have shown can be a consequence of proteasome inhibition. The absolute configuration was later reported using total synthesis by Kogen *et al.*[62] Dysidamide C **132** belongs to a family of compounds isolated from marine sponges.[63] Finally, L-tenuazonic acid[64] **133** was one of the first tetramic acid natural product to be isolated, since discovery, numerous additions to this class of natural products have been isolated, characterized and synthesized.[65,66] Such compounds have been found to exhibit a wide range of biological activities, such as anti-microbial, anti-viral, and anti-tumour properties.

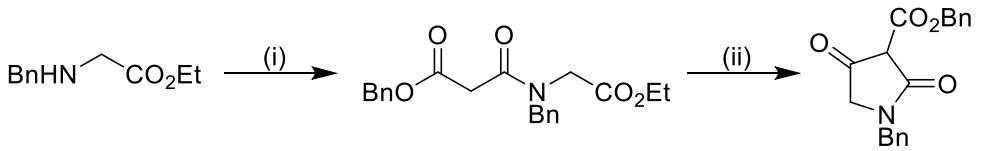
1.9 Previous work in the Page group

1.9.1 Previous work using glycine as the starting material

1.9.1.1 Synthesis of an advanced intermediate from glycine

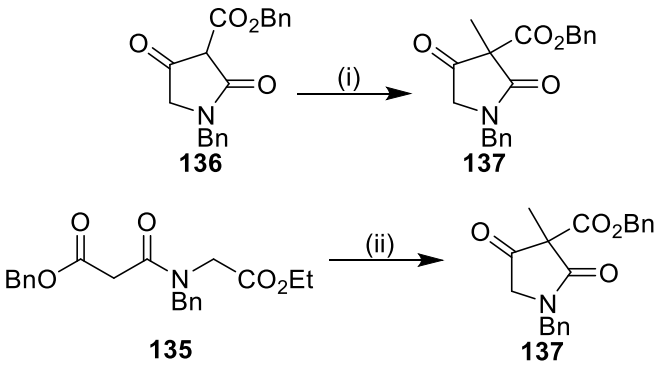
Initial work in the Page group focussed of the synthesis of functionalized lactam cores related to omuralide.[67] The lactam core was derived from glycine, with no original chirality, the cores were racemic.

The key step was the Dieckmann cyclization (**Scheme 18**), allowing formation of the lactam core **136**, then the alkylation of the tetramic acid like position with tetra-N-butylammonium fluoride (**TBAF**) and MeI. Further optimization of this step allowed a 1 pot procedure to be developed, where TBAF induced cyclization of **135**, then, after MeI addition, facilitated alkylation (**Scheme 19**).



Reagents and conditions: (i) $BnOCOCH_2COCl$, py, DMAP, CH_2Cl_2, rt, 24 h, 83%; (ii) NaH, PhH, 6 h, 63%.

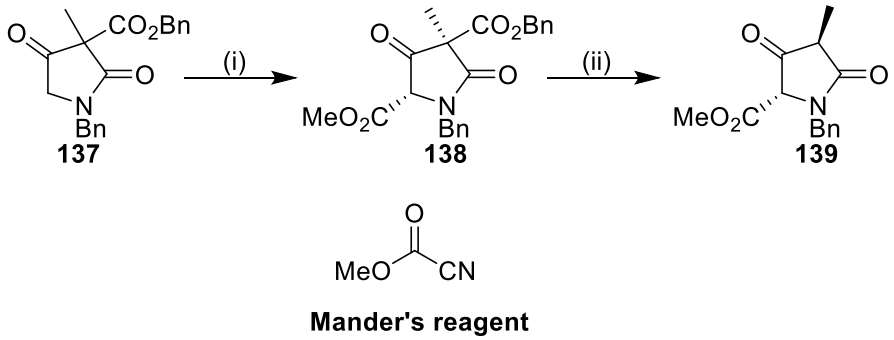
Scheme 18: Cyclization of glycine to a functionalized γ-lactam core



Reagents and conditions: (i) TBAF, MeI, THF, 24 h, 70%; (ii) TBAF, Et_2O, rt, MeI, THF, 24 h, 53%.

Scheme 19: Alkylation of the γ-lactam core and the 1-pot cyclization/alkylation

A further key step in the synthesis of the functionalized lactam core, was the addition of a methyl ester using Mander's reagent.[68] This allowed acetylation at the carbon, with no competing *O*-acetylation, in a diastereoselective manner (5:1). Finally, removal of the benzyl ester produced the advanced intermediate of omuralide (**Scheme 20**).



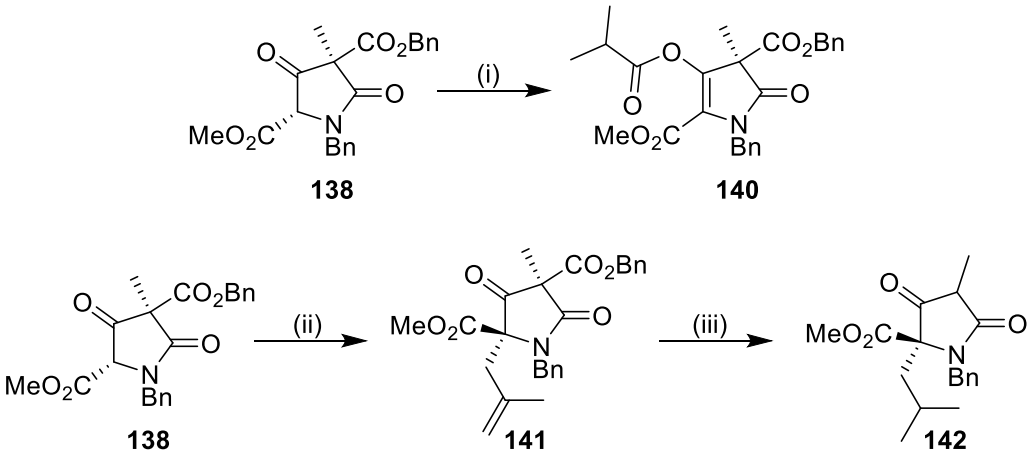
Reagents and conditions: (i) LiHMDS, $NCCO_2Me$, DMPU, THF, -78 °C, 2 h, 75%; (ii) H_2, $Pd(OH)_2$, THF, rt, 20 min, 95%.

Scheme 20: Acylation of **137** leading to the advanced intermediate of omuralide **139**

1.9.1.2 Synthesis of the full carbon skeleton of omuralide from glycine

Later work by the Page group built on this, allowing elaboration of intermediate **138** to the full carbon skeleton of omuralide.[69] Initial work to install the C9 hydroxyl group with the *iso*-butyl moiety through an acylation, was thwarted by formation of the *O*- acetylated product **140**. However, alkylation at this position was successful, forming the full carbon skeleton.

Reduction of the alkene and hydrogenolysis of the benzyl ester was achieved in one step, providing the advanced intermediate of C9-deoxy omuralide **142** (**Scheme 21**).



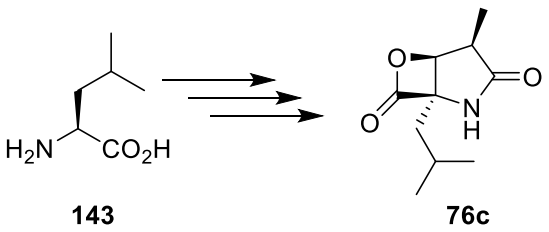
Reagents and conditions: (i) $(CH_3)_2CHCOCl$, py, CH_2Cl_2 24 h, 68%; (ii) NaH, DMF, $CH_2{=}CCH_3CH_2Br$, rt, 24 h, 75%; (iii) H_2, Pd/C (cat), 90% (1:1 mix of diastereoisomers by NMR).

Scheme 21: Elaboration of **138** to the full carbon skeleton of omuralide

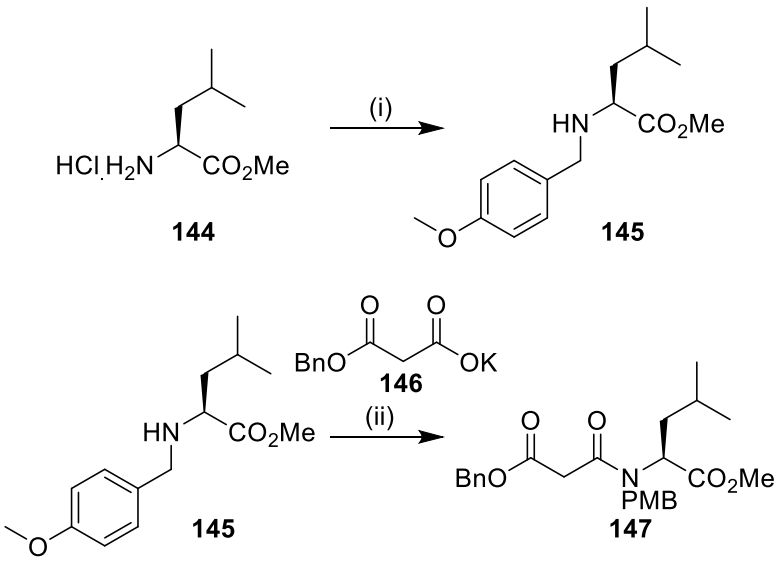
1.9.2 Studies towards the synthesis of C9-deoxy omuralide from L-leucine

1.9.2.1 Synthesis of the γ-lactam core

Later work in the Page group has concentrated on the use of chiral amino acids as starting materials.[70] Not only does this allow the carbon skeleton of omuralide to be synthesized in a much more efficient manner (the *iso*-butyl does not need to be incorporated later), but the initial chirality should also be able to direct the synthesis, providing a route to enantiopure compounds. Varying the amino acid starting material should also be able to produce new analogues of omuralide. Currently, the majority of this work has focused on leucine, which, once fully elaborated, could produce C9-deoxy omuralide (**Scheme 22**).



Scheme 22: Using leucine to form an omuralide analogue



Reagents and conditions (i) 1) *para*-Methoxy benzaldehyde, acetic acid, toluene, reflux 2) $NaBH_3CN$, acetic acid, MeOH, 93%. (ii) **146**, EDAC.HCl, DMAP, *N*-methyl morpholine, CH_2Cl_2, 72%.

Scheme 23: Synthesis of a leucine derived Dieckmann cyclization precursor **147**

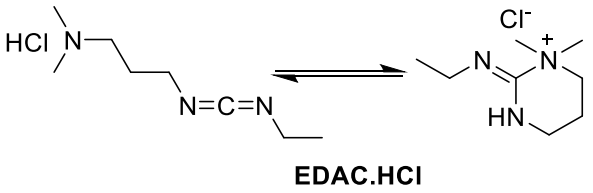
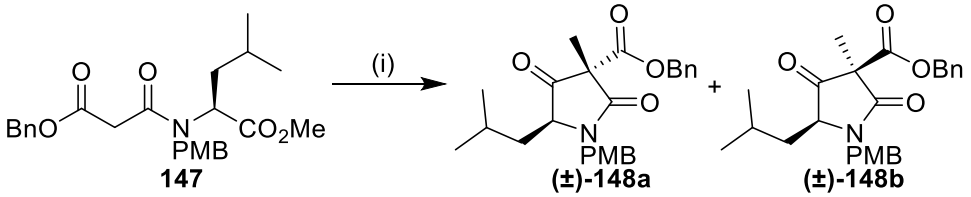


Figure 12: EDAC.HCl in straight chain and cyclized form

Leucine methyl ester **144** was protected with PMB using an acid catalysed reductive amination procedure. Coupling to **146** with peptide coupling reagent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl **(EDAC.HCl)** provided a Dieckmann cyclization precursor **147** similar to **135** (**Scheme 23**).



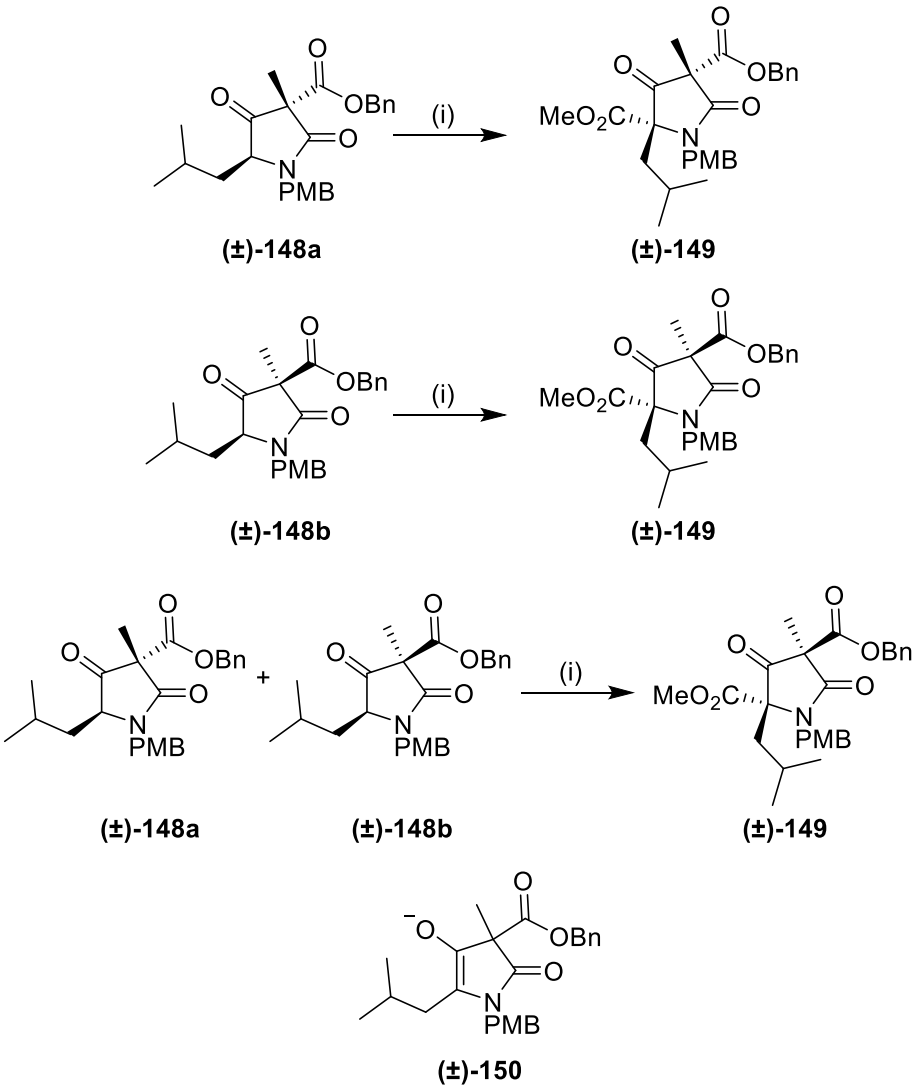
Reagents and conditions: (i) TBAF (1M in THF), THF, MeI, rt, overnight, 59%.

Scheme 24: The Dieckmann cyclization incorporation leucine into the γ-lactam core

Dieckmann cyclization precursor **147** was treated to a comparable reaction developed for the glycine route, producing a 1:2 mixture of diastereoisomers. Unfortunately, however, the material was found to be racemic (**Scheme 24**). This was assumed to have occurred during the Dieckmann cyclization by fluoride mediated deprotonation of the α-ketone position.

1.9.2.2 The acylation using Mander's reagent

Each of the diastereoisomers were found to undergo acetylation diastereoselectivly using Mander's reagent (**Scheme 25**).



Reagents and conditions: (i) LiHMDS, DMPU, methyl cyanoformate, THF, -78 °C, using (±)-**148a**: 73%, using (±)-**148b** 79%, using (±)-**148a** and (±)-**148b** 86%.

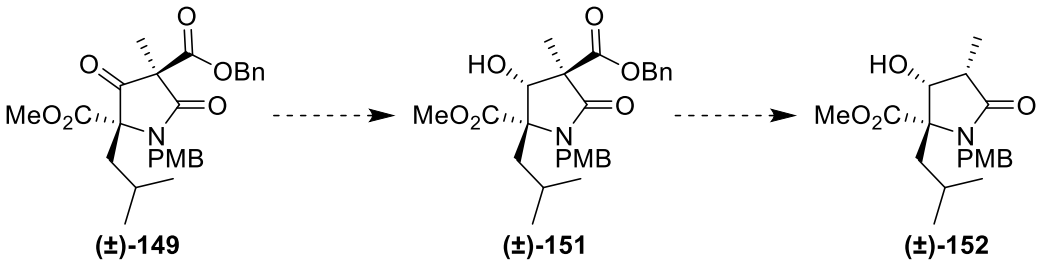
Scheme 25: The acylation using Mander's reagent of the diasteroisomers (±)-**148a** and (±)-**148b**

Addition of the methyl ester occurred opposite the bulky benzyl ester. Because both diastereoisomers were racemic, and the reaction was diastereoselective, separation of the diasteroisomers (±)-**148a** and (±)-**148b** was not necessary, as both would go through the same racemic intermediate (±)-**150**.

1.9.2.3 Elaboration of the γ-lactam core to C9-deoxyomuralide

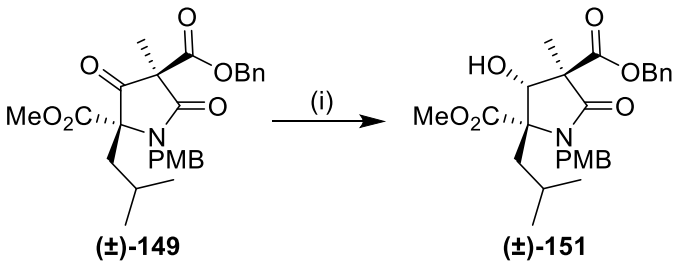
1.9.2.3.1 Strategy and reduction of the C6 ketone

Following from the success of the construction of the γ-lactam core, work focused on the functional group manipulations required for the synthesis of C9-deoxy omuralide. The strategy developed involved reduction of the C6 ketone, followed by removal of the benzyl ester (**Scheme 26**).



Scheme 26: Strategy consisting of reduction and removal of the benzyl ester to produce advanced intermediate (±)-**152**

This should produce the advanced intermediate (±)-**152**. Reduction of (±)-**149** using sodium borohydride occurred in surprisingly high diastereoselectivity with the hydride adding on the same face as both the *iso*-butyl and the benzyl ester (**Scheme 27**).



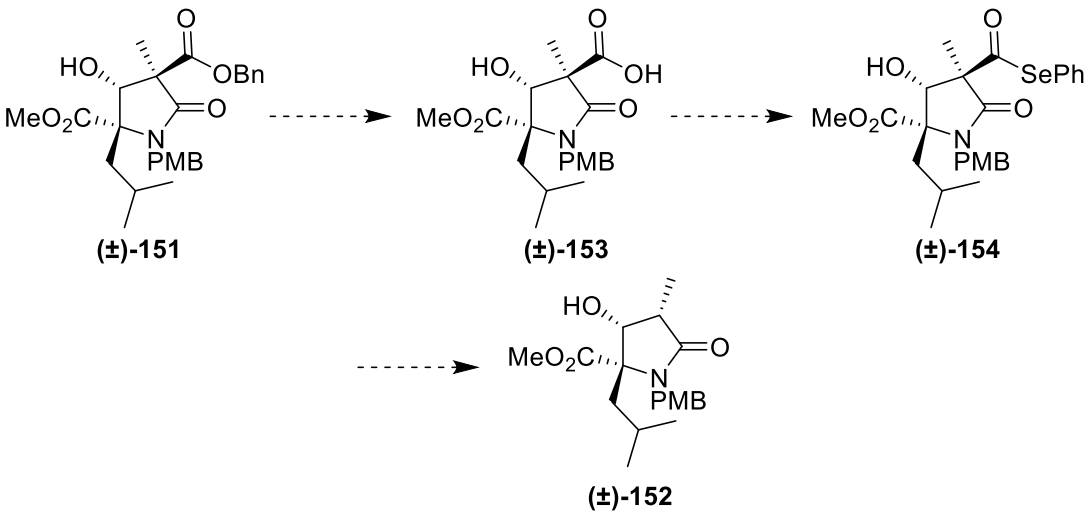
Reagents and conditions: (i) $NaBH_4$, EtOH, 0 °C to rt, 30 min, up to 57%.

Scheme 27: Reduction of (±)-**152** with $NaBH_4$ producing (±)-**151**

1.9.2.3.2 Attempts at removal of the benzyl ester

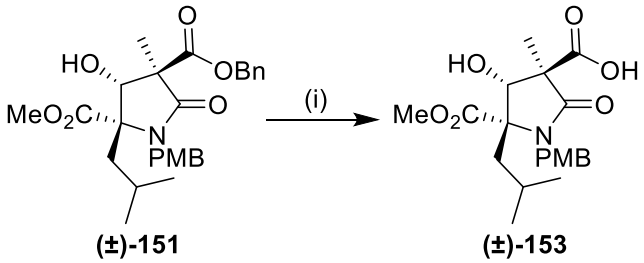
1.9.2.3.2.1 Removal using an acyl selenium decarboxylation

The first decarboxylation strategy attempted was *via* the acyl selenium (±)-**154** through a radical mediated pathway (**Scheme 28**).



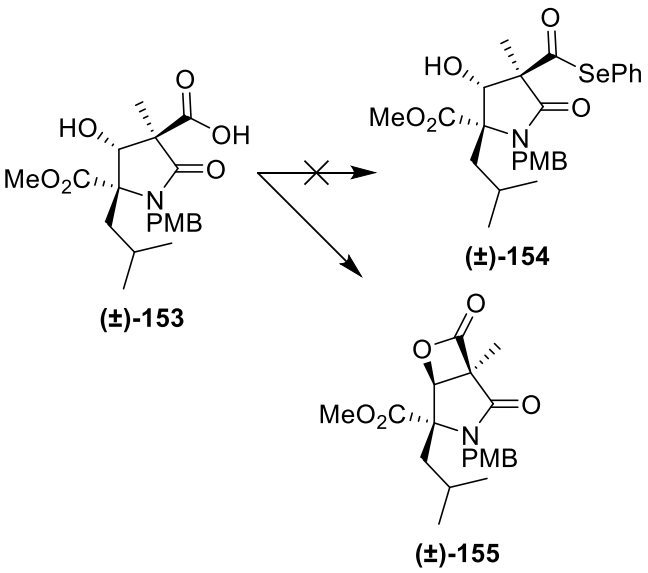
Scheme 28: Strategy for the formation of an acyl selenium derivative (±)-**154** for decarboxylation

An example by Allin *et al.*[71] shows decarboxylation can occur in similar functionalities. Removal of the benzyl ester proceeded in good yield, providing the acid (±)-**153** (**Scheme 29**). Formation of the acyl selenide however failed, instead producing the β-lactone (±)-**155** (**scheme 30**). Various methods to protect the C6 hydroxyl group were then attempted, however, this was surprisingly difficult. The most effective group was found to be a trifluoroacetate.



Reagents and conditions: (i) H_2, $Pd(OH)_2$, THF, quant.

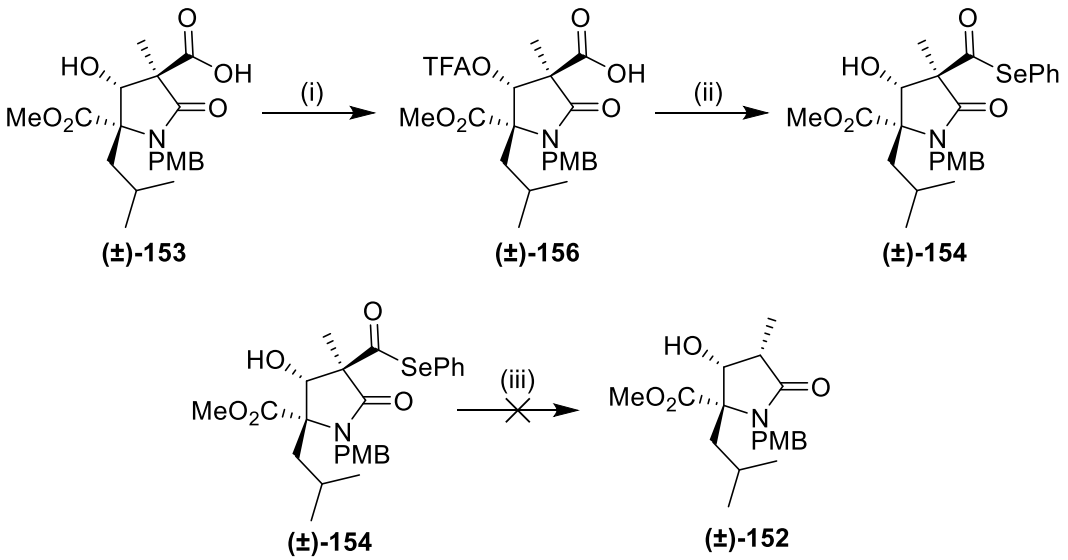
Scheme 29: Removal of the benzyl providing acid (±)-**153**



Reagents and conditions: (i) Bu_3P, $(PhSe)_2$, CH_2Cl_2, 24 h, 66%.

Scheme 30: Attempt at forming the acyl selenide (±)-**154**

Trifluoroacetate protection proceeded in good yield allowing the acyl selenide (±)-**154** to be formed, unfortunately however, subjecting this to the conditions required for decarboxylation resulted in a complicated mixture of products (**Scheme 31**).

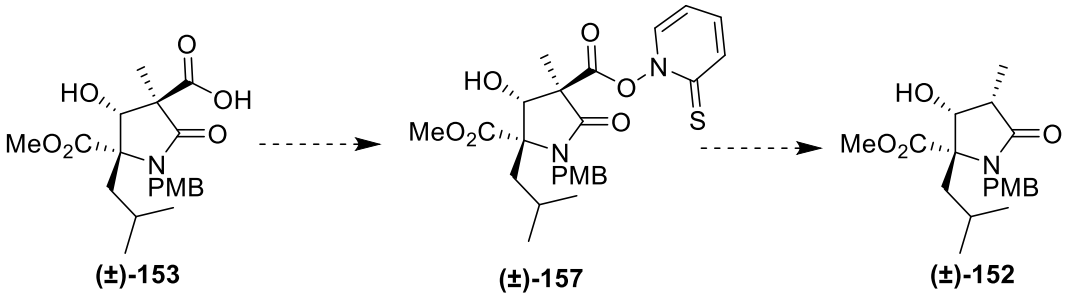


Reagents and conditions: (i) $(CF_3CO)_2O$, pyridine, Et_2O, 81%; (ii) Bu_3P, $(PhSe)_2$, CH_2Cl_2, 24 h, 31%; (iii) *n*-Bu_3SnH, AIBN, toluene, 80 °C, 2 h.

Scheme 31: Formation of acyl selenide (±)-**154** and attempts at decarboxylation

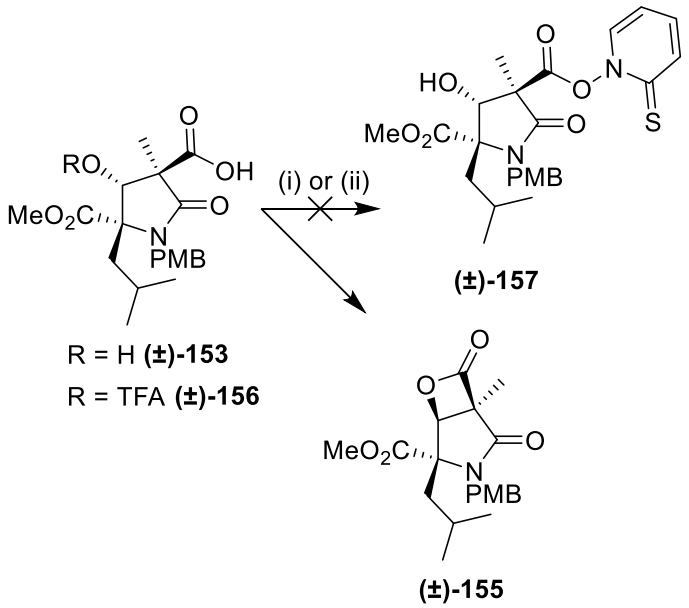
1.9.2.3.2.2 Removal using Barton decarboxylation

Following on from the failed decarboxylation attempts with the acyl selenide. Formation of a Barton ester was pursued. In the correct conditions, this should undergo Barton decarboxylation.



Scheme 32: Decarboxylation strategy *via* Barton ester (±)-**160**

Unfortunately, attempts to form the Barton ester (±)-**157** all resulted in the β-lactone by-product (±)-**155** (**Scheme 33**). Both (±)-**153** and the trifluoroacetic acid (**TFA**) ester protected derivative (±)-**156** were treated with coupling conditions, using peptide coupling EDAC.HCl and 2-mercaptopyridine N-oxide sodium salt. (±)-**157** was also attempted to be synthesized through an acyl chloride (**Scheme 33**) but again this resulted in β-lactone (±)-**155**.



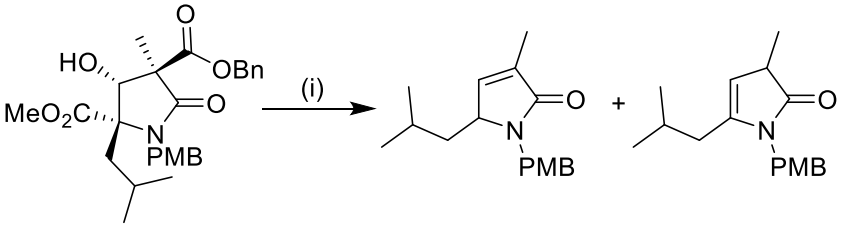
Reagents and conditions: (i) (±)-**153**, oxalyl chloride, and 2-mercaptopyridine N-oxide sodium salt, DMF, $CHCl_3$; (ii) (±)-**153** or (±)-**156**, EDAC.HCl, N-MM, DMAP, 2-mercaptopyridine N-oxide sodium salt, THF.

Scheme 33: Attempts to form the Barton ester

1.9.2.3.2.3 Removal using a Krapcho decarboxylation

A final attempt at decarboxylation was attempted using Krapcho[72] conditions, unfortunately this resulted in removal of both the methyl ester and the benzyl ester (**Scheme 34**).

A potential approach to future work on decarboxylation strategies of (±)-**153** may find success using the reagent by Garner[73] S-(1-Oxido-2-pyridinyl)-1,1,3,3-tetramethyl-thiouronium Hexafluorophosphate (**HOTT**). Which has been found to decarboxylate particularly hindered esters.



(±)-151 **(±)-158** **(±)-159**

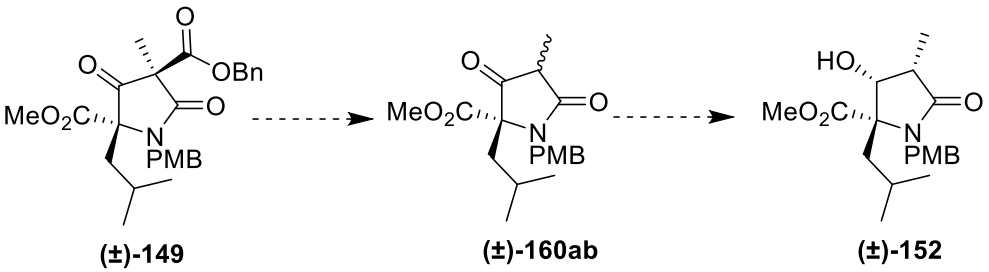
Reagents and conditions: LiCl, DMF, 135 °C, 4 h, (±)-**158** 49% and (±)-**159** 38%.

Scheme 34: Treatment of (±)-**151** with Krapcho decarboxylation conditions

With all strategies for decarboxylation failing, a new approach was undertaken.

1.9.2.3.3 New strategy to reach intermediate (±)-152

The new strategy involved first removing the benzyl ester, then reducing the C6 carbonyl (**Scheme 35**).

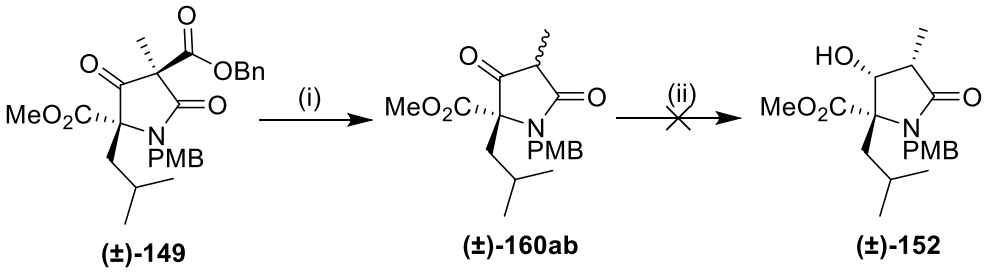


(±)-149 **(±)-160ab** **(±)-152**

Scheme 35: New strategy to (±)-**152** involving removal of the benzyl ester followed by reduction

Removal of the benzyl ester was achieved using hydrogenolysis conditions (**Scheme 36**). Despite a number of methods attempted however (**Table 7**), (±)-**152** was unable to be

synthesized (**Scheme 36**). This is perhaps due to the readily enolizable nature of the ketone, a new strategy was devised based on a procedure used by Pattenden[74,74] where a thiomethyl group would be installed, the ketone reduced, then the thiomethylated compound desulfurized. This strategy is explained in more detail in chapter **2.2.3.1**.



Reagents and conditions: (i) H_2, $Pd(OH)_2/C$, THF, 30 °C, > 90%; (ii) See **table 7**.

Scheme 36: Hydrogenolysis followed by reduction strategy

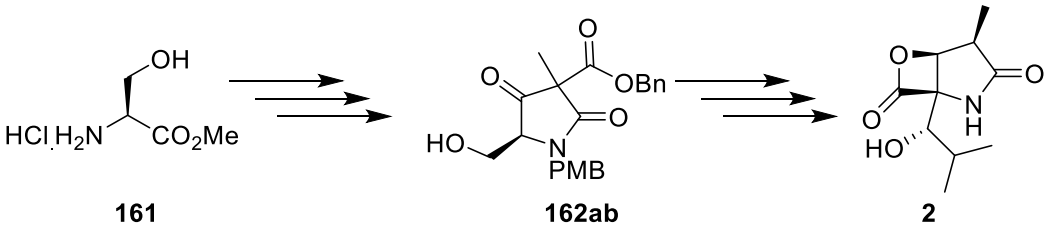
Reducing reagent	Conditions	Result
$NaBH_4$	EtOH 0 °C, 30 m	Complex mixture
$NaBH_3CN$	MeOH 0 °C - rt, 2 h	SM
$NaBH(OAc)_3$	Acetic acid, rt, 1 h	Unidentified compound
LAH	THF, 0 °C, 2 h	Decomposition
LAH	THF, -78 °C, 1-2 h	Complex mixture
DIBAL	THF, 0 °C, 2 h	Complex mixture
DIBAL	THF, -78 °C, 1-2 h	Complex mixture
Red-Al®	DCM, -78 °C, 1-2 h	Complex mixture
Noyori catalyst	IPA/THF 5:1, 3-6 days	Unidentified compound

Table 7: Reduction conditions attempted for formation of (±)-**152**

1.9.3 Studies towards the synthesis of omuralide from L-serine

1.9.3.1 Overview of route using serine

Previous work in the group has also focussed on incorporating serine into the γ-lactam core. Using the previously developed Dieckmann cyclization/alkylation methodology. Once incorporated, with the C5 hydroxyl group in place, elaboration to omuralide should be achievable (**Scheme 37**).

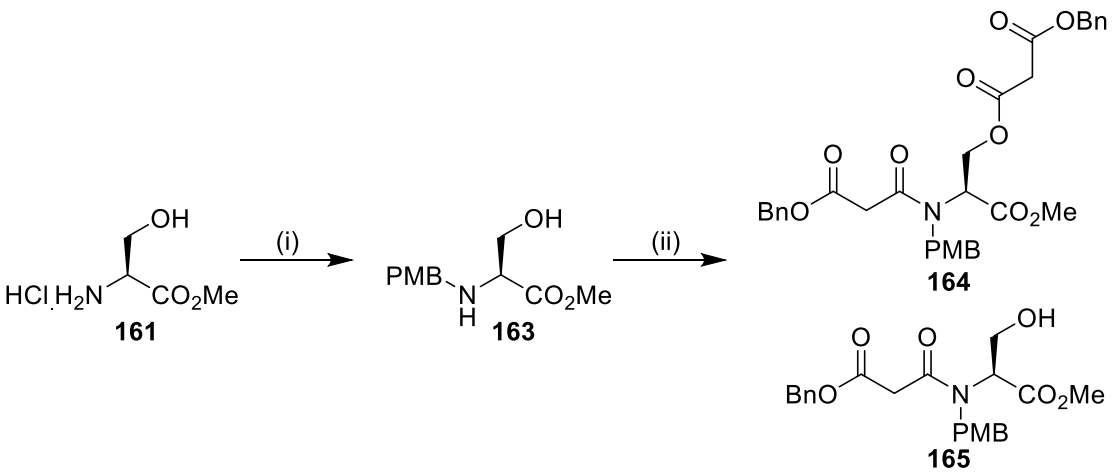


161 **162ab** **2**

Scheme 37: Incorporation of **161** into the lactam core and elaboration to omuralide

1.9.3.2 Studies toward the synthesis of omuralide from serine

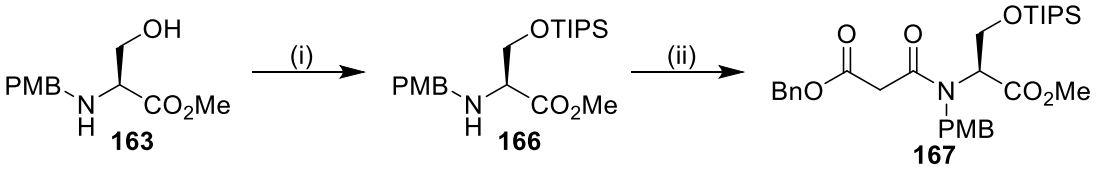
Protection of serine proved more difficult compared to the leucine route. Methods previously developed failed (**Scheme 23**), however formation of the imine, and its subsequent reduction under an atmosphere of hydrogen, afforded **163** in fair yield. Unfortunately, however, coupling to **146** under the standard conditions produced only a small quantity of desired product **165**. This is primarily down to the disubstituted by-product **164** being formed (**Scheme 38**).



Reagents and conditions: (i) *para*-methoxybenzaldehyde, triethylamine, H_2, Pd/C, MeOH, 64%; (ii) **146**, EDAC.HCl, DMAP, *N*-methyl morpholine, CH_2Cl_2, **165:** 14%, **164:** 31 %.

Scheme 38: PMB protection of serine and coupling of the product to **168**

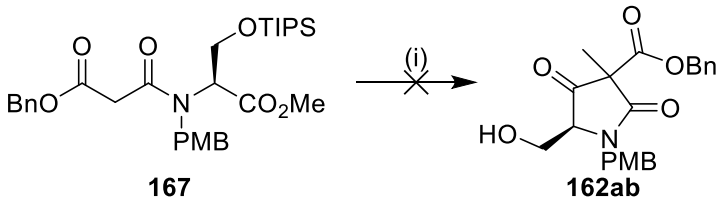
Due to these problems, a protection strategy was devised, protecting the hydroxyl group with protecting group triisopropyl silyl (**TIPS**). The Dieckmann cyclization precursor **167** was then obtained in excellent yields (**Scheme 39**).



Reagents and conditions: TIPSCl, imidazole, DMF, reflux, 90%; (ii) **146**, EDAC.HCl, DMAP, *N*-methyl morpholine, CH_2Cl_2, 91%.

Scheme 39: The formation of the TIPS protected cyclization precursor **167**

Unfortunately, however, when treated to the cyclization/alkylation conditions the γ-lactam **162ab** was not isolated. It was thought there could be too many competing reactions between cyclization and TBAF induced deprotection of the TIPS group (**Scheme 40**).



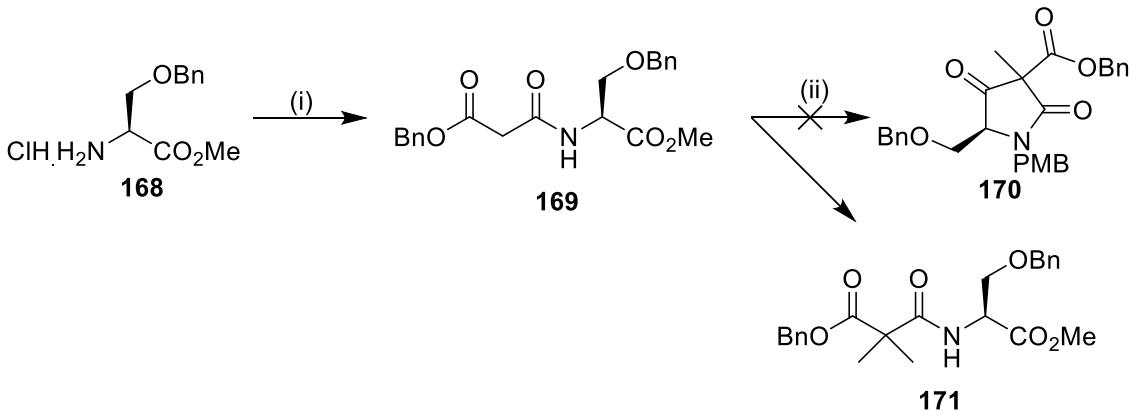
167 **162ab**

Reagents and conditions: TBAF, MeI, THF, overnight.

Scheme 40: Treatment of **167** with the Dieckmann cyclization/alkylation conditions

1.9.3.3 Starting from a benzyl ether serine derivative

To avoid these problems, a benzyl ether protected version of serine was acquired (**168**). Protection of the amine again proved difficult and so **168** was coupled to **146** forming the Dieckmann cyclization precursor **169** as a secondary amide. Treating this to the Dieckmann cyclization conditions, however, failed to cyclize **169** to the lactam **170**, instead, alkylating the malonic position twice forming **171** (**Scheme 41**).



168 **169** **170** **171**

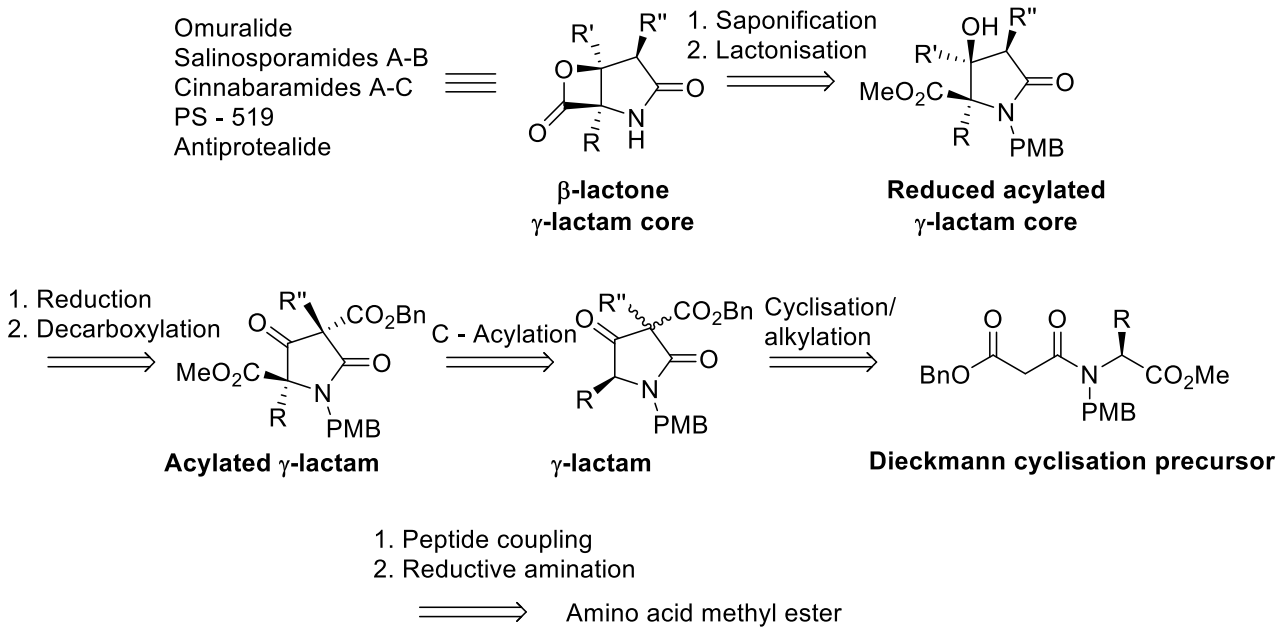
Reagents and conditions: 146, EDAC.HCl, DMAP, *N*-methyl morpholine, CH_2Cl_2, 69%; (ii) TBAF, MeI, THF, overnight, 30%.

Scheme 41: Attempted cyclization of **170** with the Dieckmann cyclization/alkylation conditions

2.0 Results and discussion

2.1 Retrosynthetic analysis of omuralide and related γ-lactam materials

We aim to develop a flexible route to a highly functionalized γ-lactam core. This route, modified appropriately, should be able to produce known natural products such as omuralide and the salinosporamides, and known and novel analogues of these compounds. **Scheme 1** shows the general retrosynthetic analysis used to produce the β-lactone, γ-lactam proteasome inhibitors and analogues. The **β-lactone γ-lactam core** could be formed through saponification of the corresponding methyl ester to the acid, followed by lactonization to the **C6 reduced, acylated β-lactam core**. The C6 alcohol, which would also become the β-lactone, could be formed either through hydride reduction or alkylation of the ketone, depending on the analogue required. Previous work in the group had shown that the methyl ester could be inserted diastereo- and chemoselectivly using Mander's reagent directed by a benzyl ester leading to the **acylated γ lactam core**. The lactam core would be produced through a tandem Dieckmann cyclization/alkylation leading to the **Dieckmann cyclization precursor**. Modification of the alkylating agent should produce lactam cores with various C7 alkyl groups, allowing further modification of the route. The Dieckmann precursor could be coupled to a half malonic benzyl ester through a peptide coupling, leading to the N-protected amino acid methyl ester. Modification of the amino acid starting material provides a final degree of flexibility allowing various different moieties at the C9 position. Amino acids were used as the starting material due to their inherent chirality, allowing the synthesis to be stereochemically directed.



Scheme 1. Retrosynthetic analysis of the β-lactone, γ-lactam core

The work presented here describes our synthetic route from L-leucine. Fully elaborated, L-leucine would form C9-deoxy-omuralide, which possesses the full carbon skeleton of omuralide. Also discussed is our work using L-serine as starting material, with the C9 hydroxyl group in place from the beginning. Fully elaborated, this could form omuralide. Modification of the L-serine route could also be used to produce other related natural products such as the cinnabaramides, salinosporamides and antiprotealide.

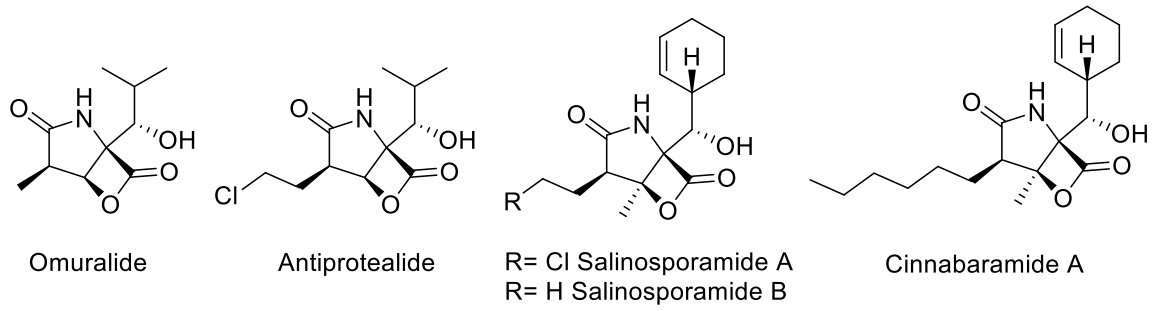


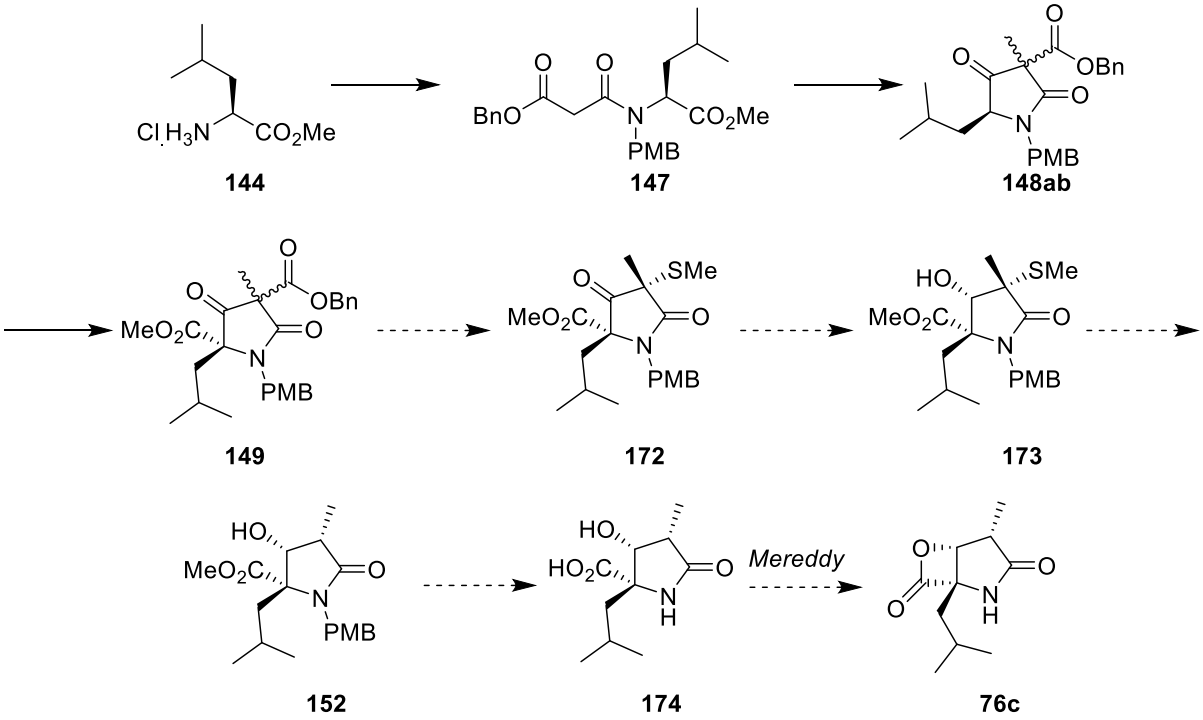
Figure 1. Various β-lactone, γ-lactam natural products

2.2 Elaboration of L-Leucine methyl ester HCl to (+)-C9-deoxy-omuralide

2.2.1 Outline of the general procedure

The proposed route to C9-deoxy-omuralide (**Scheme 2**) followed the outline of the route in **Scheme 1.** Building on previous work in the Page group,[67,69,75] leading to **149**, following

removal of the benzyl ester, the thiomethyl blocking group strategy first used by Corey[29] and later by Pattenden[76,74] would be followed to allow reduction of the C6 ketone (**173**). Desulphurization followed by protecting group removal should provide the substituted pyroglutamate **174,** which has been cyclized to C9-deoxy-omuralide by Mereddy *et al*.[77]



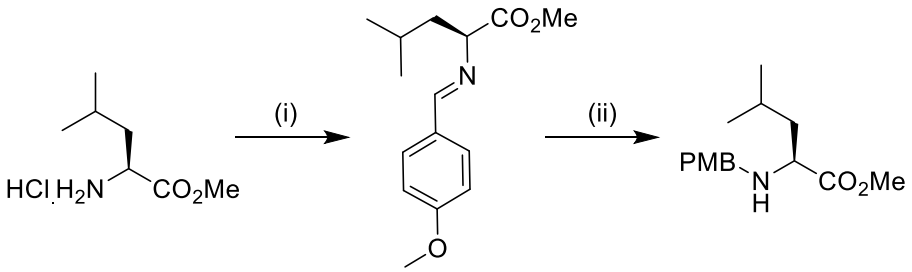
Scheme 2: Plan for the synthesis of 9-deoxy-Omuralide from L-Leucine

2.2.2 Formation of the full carbon skeleton of omuralide

2.2.2.1 N-Protection of leucine

Corey had shown in previous syntheses that the PMB protecting group could be removed from late stage intermediates and analogues of omuralide and lactacystin. This result has led PMB to be a common protecting group in lactacystin synthesize and hence PMB was utilized in our route.

Para-methoxy benzaldehyde and L-leucine were heated under reflux in acidic conditions using a Dean-Stark apparatus to form the corresponding imine **175**. The resulting imine could be reduced with sodium cyanoborohydride to afford the amine **145** in excellent yield (**Scheme 3**).

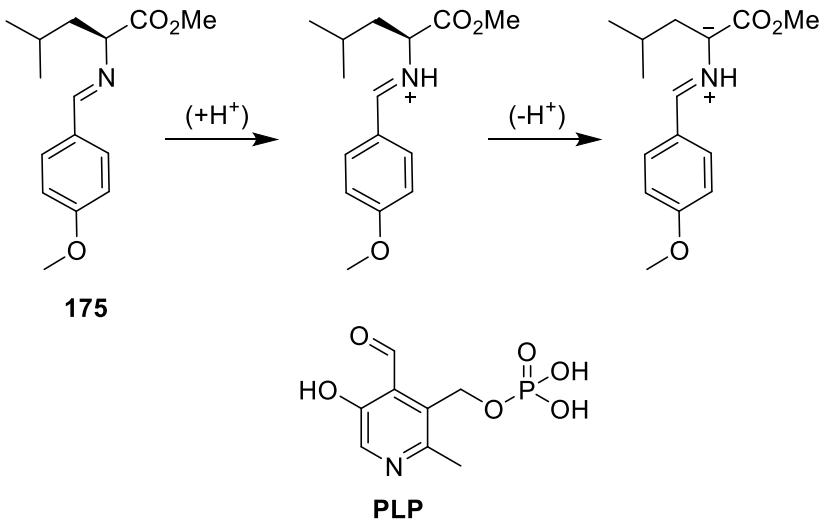


144 **175** **145**

Reagents and conditions: i) 4-methoxybenzaldehyde (1.1 equiv), acetic acid (0.6 equiv), toluene, reflux, quant; ii) $NaBH_3CN$ (2 equiv), acetic acid (0.9 equiv), MeOH, 96%.

Scheme 3: Two step, one pot reductive amination

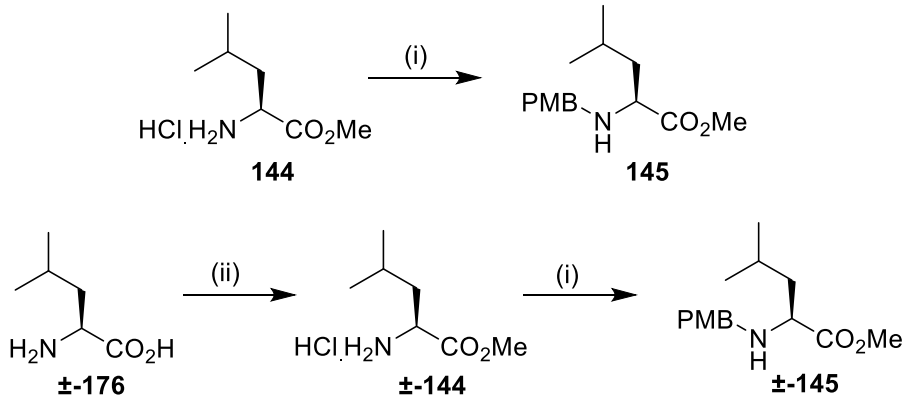
However, during further characterization, discrepancies were found between the optical rotation value of the amine and the literature value.[78] To our surprise, it was found that the leucine chirality had been completely lost under the reaction conditions. Although previous work in the Page group had observed racemization in the synthetic route, this was assumed to have been much later in the synthesis. Very similar findings have been observed in the comprehensive study by Yamada *et al*.,[79] where various amino acids were racemized at high temperatures in the presence of aldehydes and acetic acid due to the formation of a Schiff base, lowering the pKa of the alpha ester proton (**Scheme 4**). A similar racemization involving piridoxal phosphate (**PLP**) (the active form of vitamin B_6) is a common occurrence in metabolic pathways,[80] one key use being the formation of D-serine from L-serine in conjunction with serine racemase.[81]



175 **PLP**

Scheme 4: Racemization of leucine and the structure of PLP

Due to the racemization occurring in the acidic conditions during imine formation the procedure was modified to a 1-pot reaction in basic conditions. A similar racemization has also been observed under basic conditions,[82] therefore, DL-Leucine (**176**) was also acquired, esterified and subject to the same conditions. The reductive amination proceeded with good yield in both cases, but, more importantly, comparison of the known racemate and the N-protected amino acid from L-leucine using high performance liquid chromatography (**HPLC**) with a chiral stationary phase, showed there had been no observable racemization using the new procedure (**Scheme 5**).

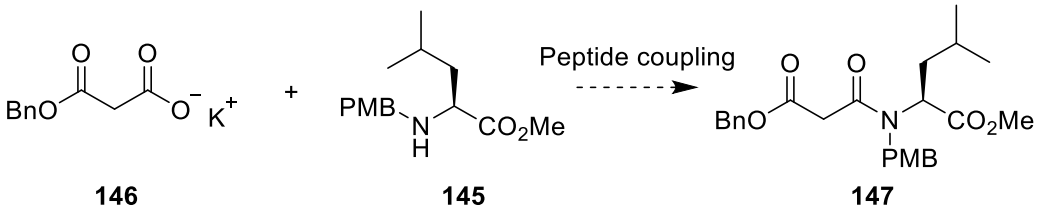


Reagents and conditions: i) *p*-methoxybenzaldehyde (1.1 equiv), Et_3N (1 equiv), MeOH, $NaBH_4$ (1.9 equiv), 0 °C to rt, 76% from L-leucine, 68% from DL-leucine; ii) acetyl chloride (3 equiv), MeOH, 0 °C – reflux, 16 h, quant.

Scheme 5: Esterification and one step reductive amination

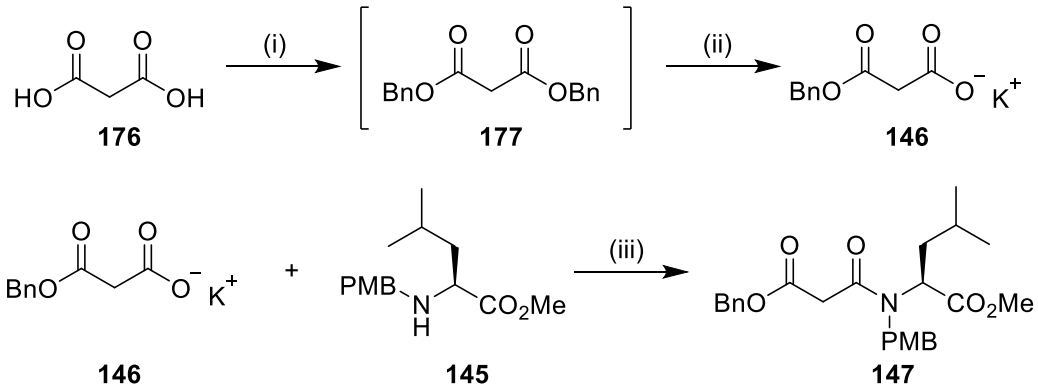
2.2.2.2 Synthesis of 146 and coupling to the N-protected leucine ester

The Dieckmann cyclization precursor **147** was synthesized from the corresponding acid **146** and the PMB protected leucine ester **145** (**Scheme 6**)



Scheme 6: Formation of the Dieckmann cyclization precursor

Synthesis of the half malonic ester was achieved by forming the dibenzyl malonic ester through acidic (*para*-toluene sulphonic acid monohydrate) (***p*-TSOH**) esterification of malonic acid **146** with benzyl alcohol (**BnOH**) in a Dean-Stark apparatus (**Scheme 7**). Mono-saponification of the di-ester **177** induced precipitation of the potassium salt in excellent yield. EDAC.HCl, a common peptide coupling reagent, was used to form the peptide bond along with catalytic quantities of DMAP and *N*-methyl morpholine (**N-MM**). After purification by column chromatography, the Dieckmann cyclization precursor **147** was observed as a pair of rotamers in ^{13}C and ^{1}H nuclear magnetic resonance (**NMR**) experiments in an approximately 4:1 ratio. Variable temperature experiments in dimethyl sulphoxide (**DMSO**) found that peak coalescence occurred at 100 °C. Analysis of **147** by chiral stationary phase HPLC in comparison to a synthesized racemate showed that no observable racemization had occurred at this point.



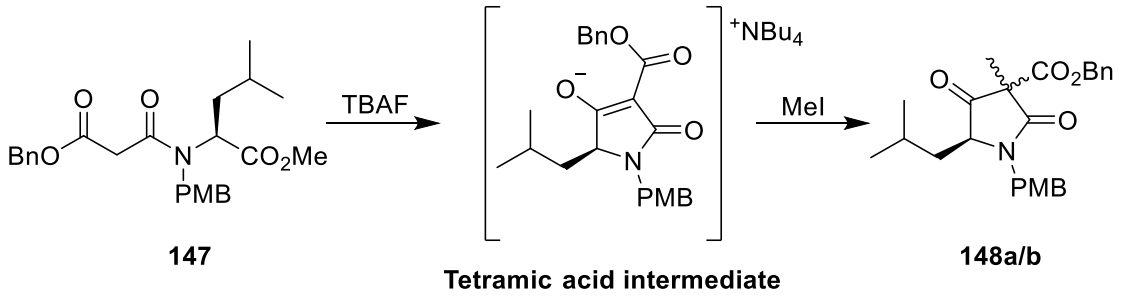
Reagents and conditions: i) Benzyl alcohol (2.1 equiv), *p*-TsOH (0.01 equiv), toluene, reflux (Dean-Stark), 16 h; ii) KOH in BnOH, 74%; iii) EDAC.HCl (2.6 equiv), DMAP (0.25 equiv), *N*-MM (2.3 equiv), DCM, 93%.

Scheme 7: Formation of **146** and its coupling to **145**

2.2.2.3.1 The Dieckmann cyclization/alkylation

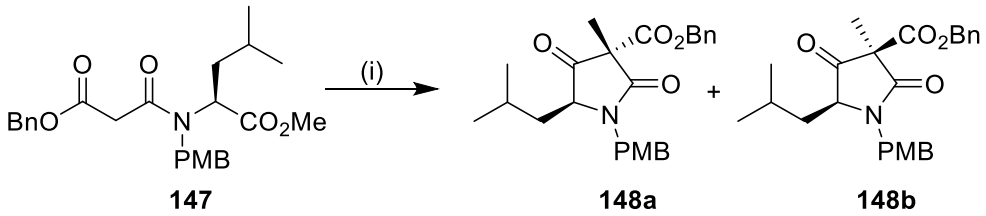
The previously developed cyclization/alkylation occurs through deprotonation of the malonic position in the Dieckmann precursor **147** using TBAF as the base. Nucleophilic attack of the malonate at the leucine ester group forms the gamma lactam as a tetramic acid. A second deprotonation at the C7 position forms a highly stabilized enolate; addition of iodomethane *in situ* then provides the alkylated gamma lactam with the methyl group required for omuralide. The tetramic acid intermediate was assumed to be planar and therefore we hoped

to see diastereoselective control with the methyl iodide adding opposite the leucine's bulky *iso*-butyl group (**Scheme 8**).



Scheme 8: The first and second steps of the cyclization/alkylation

Compound **147** was dissolved in tetrahydrofuran (**THF**) and treated with TBAF. After 30 m, iodomethane was added at 0 °C. After work-up and purification by column chromatography, separable diastereoisomers **148a** and **148b** were isolated in a 1:2 ratio (**Scheme 9**).



Reagents and conditions: TBAF in THF (3.6 equiv), THF, rt, 0.5 h, then MeI (4 equiv), 0 °C to rt, 16 h, 57 %, Ratio 2:1 by crude NMR analysis

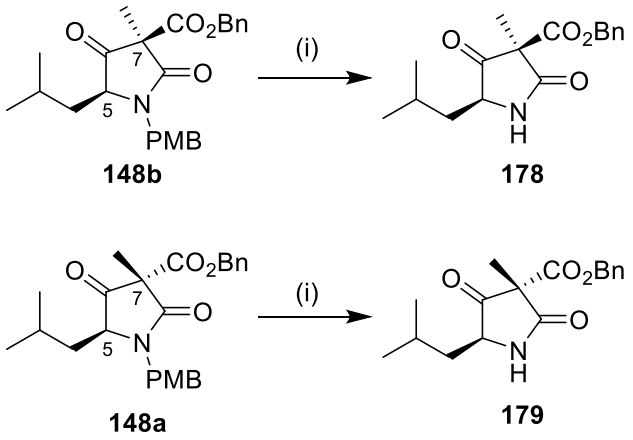
Scheme 9: The tandem Dieckmann cyclization/alkylation

Unfortunately, analysis of each diastereoisomer and a synthesized racemate by HPLC using a chiral stationary phase showed that a loss of enantiopurity had occurred in both diastereoisomers, providing the first eluting diastereoisomer in 9% ee and the second in 79% ee.

2.2.2.3.2 Confirmation of the relative stereochemistry

Each diastereoisomer was subject to analysis by nuclear Overhauser effect spectroscopy (**NOESY**) NMR experiments. It was hoped that interactions would be seen between the *iso*-butyl and the C7 methyl group in one of the diastereoisomers and interactions between the methyl and C5 proton in the other. Perhaps similar to the interactions seen on **page 66** in

compounds **172ab**. Unfortunately however, no clear interactions could be seen that could suggest which diastereoisomer was isolated in excess.



148b **178**

148a **179**

Reagents and conditions: (i) CAN (5 equiv), 3:1 MeCN/Water, 63% for **148b**, 84% for **148a**.

Scheme 10: Removal of the PMB group in compounds **148a** and **148b**

Both diastereoisomers **148a** and **148b** were isolated as oils, and we were therefore unable to obtain a single crystal X-ray structure. Removal of the PMB protecting group in the major second eluting fraction **148b** with CAN provided the deprotected lactam as an oil, but PMB removal from the minor, first eluting, diastereoisomer **148a** provided **179** as a colourless crystalline solid of which a crystal of racemic material was obtained (**Scheme 10**).

The crystal structure (**Figure 2**) shows the methyl group *cis* to the bulky *iso*-butyl group, meaning that our major diastereoisomer **148b** has the methyl group *trans*. Although the diastereoselectivity was not as high as hoped, we expected this to be the major diastereoisomer due to the steric hindrance of the *iso*-butyl moiety.

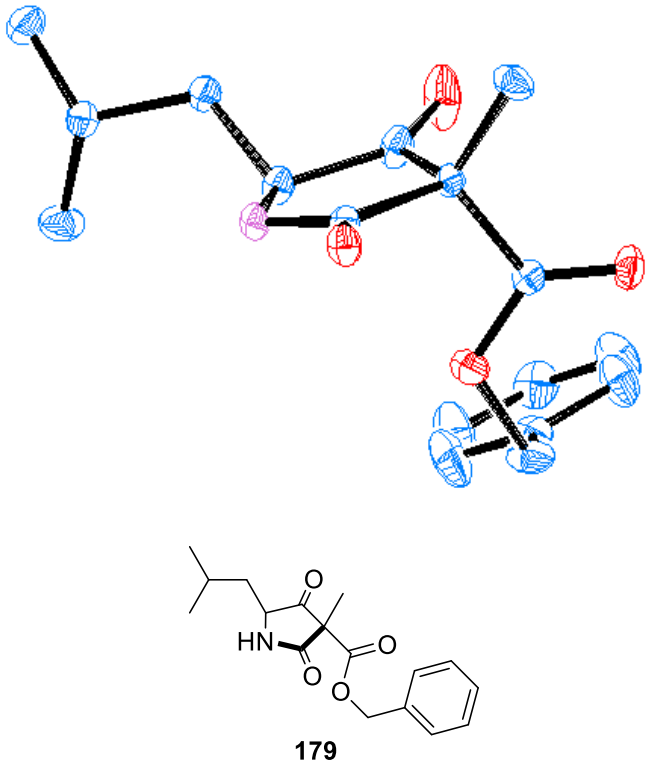
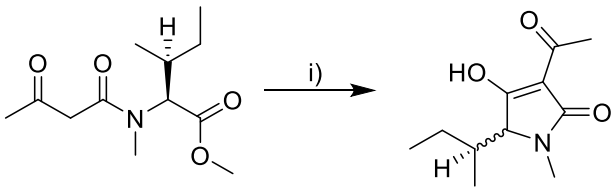


Figure 2: The x-ray crystal structure of (±)-**179** (hydrogen atoms and the two other structure conformations removed for clarity)

2.2.2.3.3 Loss of enantiopurity

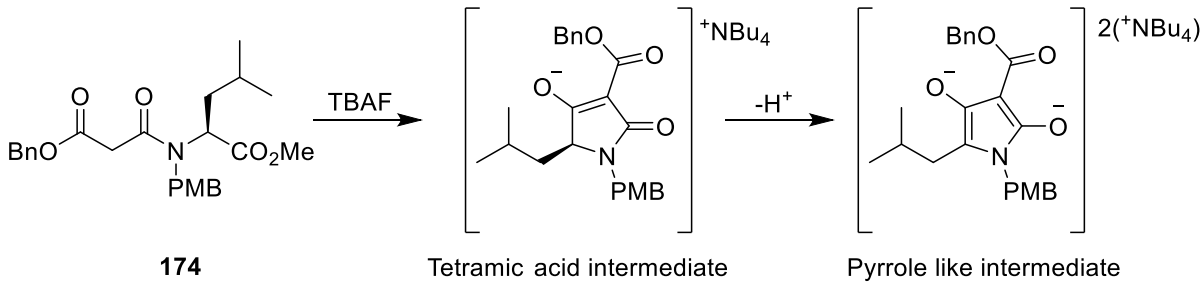
Loss of enantiopurity has been observed before in similar cyclizations. In 1990 Poncet *et al.*[83] studied C5 epimerization rates in various tetramic acid derivatives in sodium methoxide and methanol under reflux (**Scheme 11**).



Reagents and conditions: MeONa, MeOH, reflux, 2 h, 97%.

Scheme 11: Poncet's Dieckmann cyclization epimerization

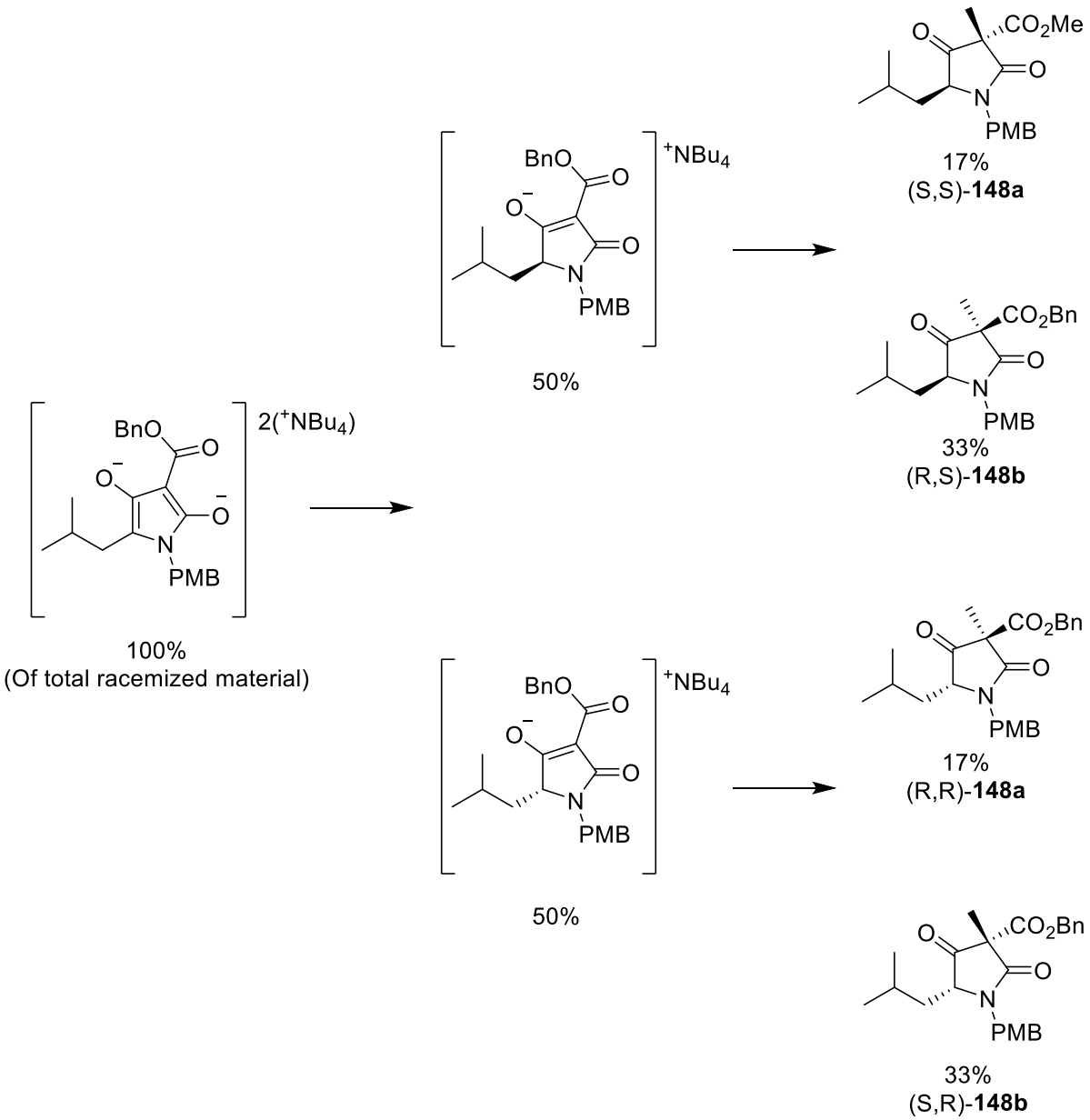
A mechanism where epimerization occurs before alkylation seems likely as double deprotonation of the tetramic acid core could produce a stabilized, aromatic, pyrrole-like intermediate (**Scheme 12**).



174 Tetramic acid intermediate Pyrrole like intermediate

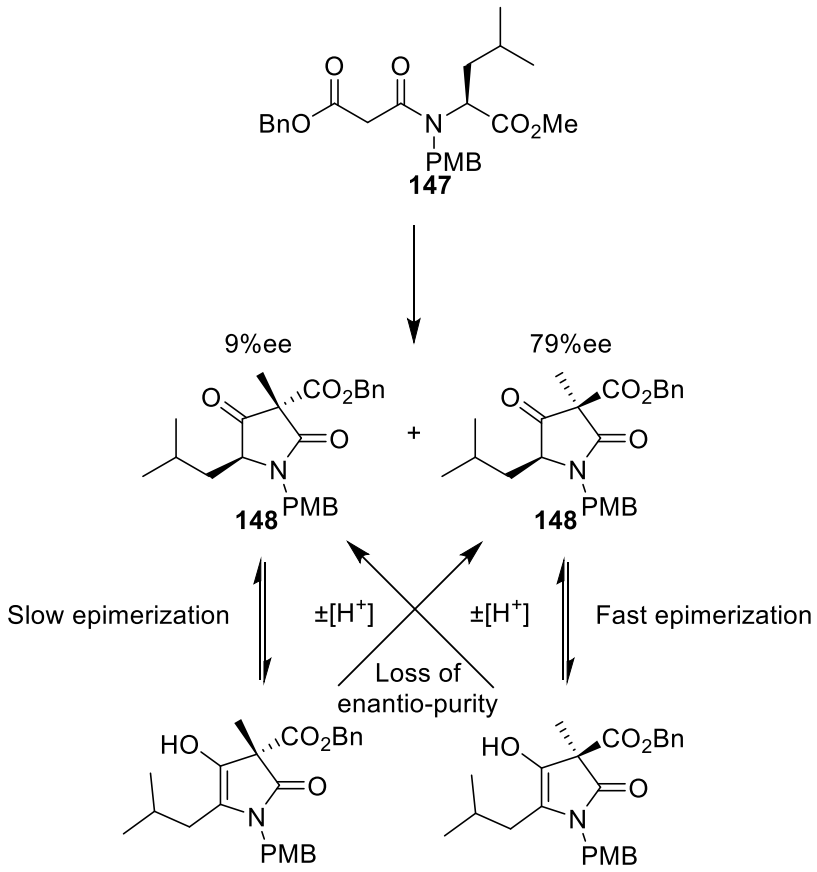
Scheme 12: Potential driving force for epimerization before alkylation

However, in-depth analysis of the reaction mixture shows this cannot be the case, or at least not the sole reason. The intermediate necessary for racemization to occur before alkylation would be achiral. This intermediate would, therefore, once protonated for alkylation to occur, be a 50/50 mix of enantiomers. The enolate would now undergo the diastereoselective alkylation. Our reaction has shown that the alkylation is diastereoselective, the methyl preferentially adding opposite the isobutyl group in a 1:2 ratio. **Scheme 13** shows the outcome of this mode of racemization assuming the diastereoselectivity is 1:2, favouring the methyl group adding opposite the *iso*-butyl group. This model should produce 34% of the racemic material in diastereoisomer **148a** and 66% of the racemic material in diastereoisomer **148b,** but we find 81% of the racemic material in diastereoisomer **148a** and 19% in diastereoisomer **148b**. For a mechanism where epimerization occurs before alkylation, the ratio between the diastereoisomers in the racemic material should match the total diastereoselectivity of the reaction. However, not only did the diastereoisomer ratio not match, more racemic material was found in the unfavoured diastereoisomer **148a**. We can therefore assume the material must be racemizing predominantly after alkylation.



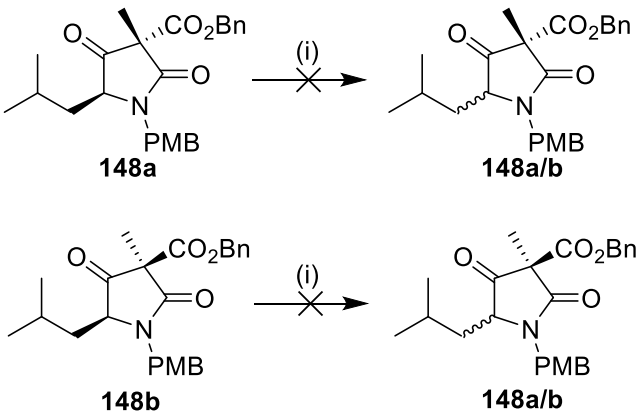
Scheme 13: Diastereoisomeric outcome assuming an epimerization before alkylation

Because of this discrepancy we believe the primary cause of loss in enantiopurity is an epimerization of the diastereoisomers **148a** and **148b**, scrambling the stereochemistry. This could also explain why the first eluting diastereoisomer has a lower ee. The second eluting diastereoisomer has the *iso*-butyl and the benzyl ester on the same side. Both are quite bulky groups, and would presumably produce considerable steric interaction. The release of this steric strain by enolate formation could mean **148b** is more susceptible to epimerization than the more thermodynamically stable **148a** (**Scheme 14**).



Scheme 14: Possible explanation for the partial racemization of **148a** and **148b**

To provide some evidence for this hypothesis, each diastereoisomer was stirred in TBAF in an attempt to induce epimerization, potentially showing **148b** epimerized to **148a** faster than **148a** to **148b.** Unfortunately, however, both decomposed to a complex mixture of products on addition of TBAF (**Scheme 15**). Nevertheless the major and more enantiopure (79% ee) diastereoisomer **148b** was used in the next step.

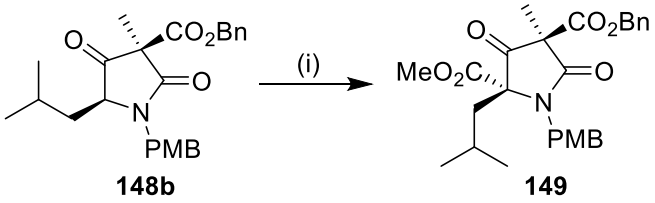


Reagents and conditions: (i) TBAF (1 M in THF) (3.4 equiv), rt, THF.

Scheme 15: Epimerization studies on **148a** and **148b**

2.2.2.4 Acylation of C5 using Mander's reagent

To provide the methyl ester which would later form the β-lactone found in omuralide, an acylation was required at the C5 α-keto position. Mander's reagent was chosen due to its high chemo-selectivity for C-acylation of enolates over addition at the O nucleophile.[68]



Reagents and conditions: LiHMDS (2.1 equiv), DMPU (2.2 equiv), THF, −78 °C, 0.5 h, then $NCCO_2Me$ (3.2 equiv), −78 °C, 4 h, 70%.

Scheme 16: Acylation using Mander's reagent

Enolate formation followed by addition of Mander's reagent proceeded with good yield (**Scheme 16**). No competing O-acylation was seen and furthermore the reaction was highly diastereoselective, with no minor diastereoisomer observed in NMR. The acylation had occurred opposite the benzyl ester as expected due to the bulky nature of the group forming **149**.

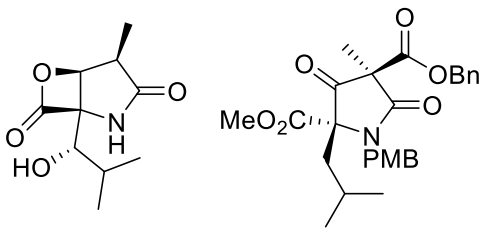


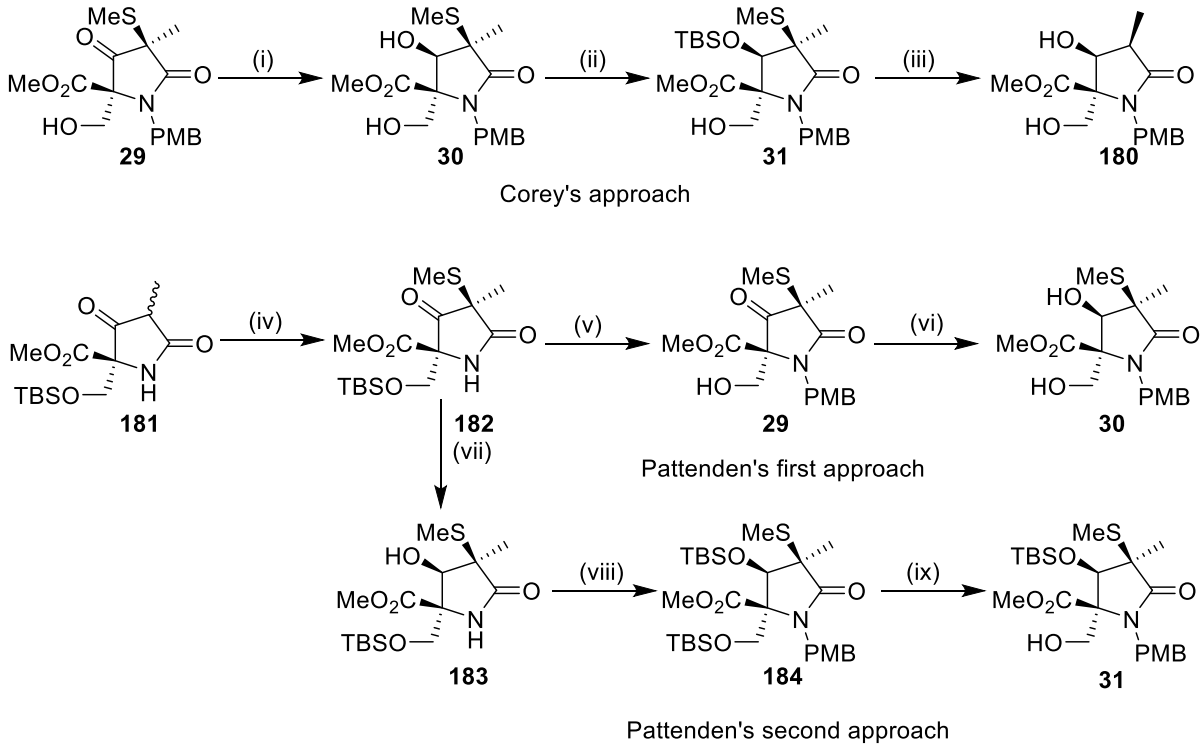
Figure 3: The acylated lactam core **149** and omuralide

Although at this stage we have the correct relative stereochemistry for omuralide analogues, the absolute stereochemistry is that of the opposite enantiomer to natural omuralide (**Figure 3**). Completing the synthetic scheme with the unnatural enantiomer of leucine (D) should therefore produce the correct enantiomer for omuralide analogues. Although commercially available, the unnatural enantiomer is far more expensive, and therefore we decided not to complete the synthesis with this starting material unless biological testing was required. Comparison with a synthesized racemate of **149** by chiral phase HPLC showed no further racemization had taken place. With the full carbon skeleton of omuralide completed we started the functional group manipulations required to produce C9-deoxy-omuralide.

2.2.3 Manipulation of the C7 and C6 stereogenic centres

2.2.3.1 Strategy for the construction of the C7 and C6 stereogenic centres

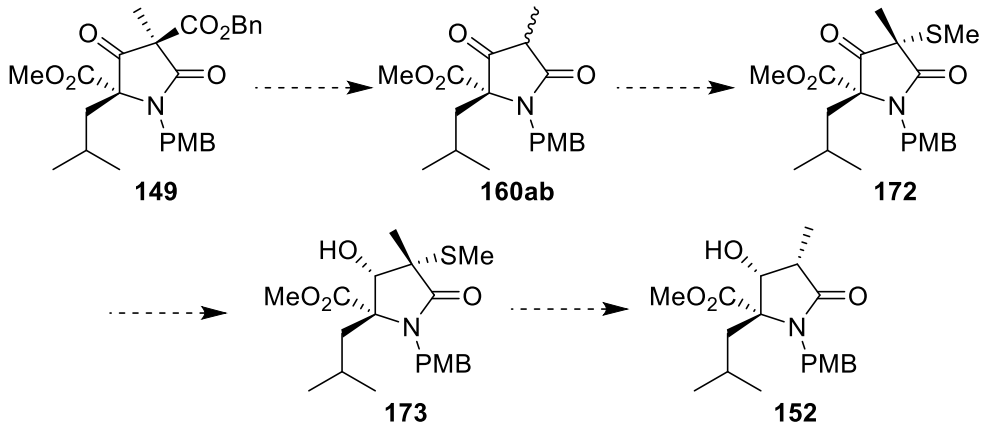
Our strategy for the construction of the *cis* C6 hydroxyl and C7 methyl was based on a blocking group tactic originally used by Corey[29] and later by Pattenden[76,74] in their respective syntheses of omuralide. Corey's approach incorporated a thiomethyl group from the beginning of the route. The C6 ketone could be reduced with complete diastereoselectivity using $NaBH(OAc)_3$. Removal of the thiomethyl group with Raney® nickel occurred with high diastereoselectivity (10:1), leaving the methyl group in the position *cis* to the alcohol as required for omuralide (**180**). Pattenden installed the thiomethyl later using a thiosulphonate reagent with good diastereoselectivity (**182**), where addition favoured the face opposite the TBS group, and then reduced the C6 ketone in a similar fashion, also using $NaBH(OAc)_3$ or $Zn(BH_4)_2$ to provide the sulphated Corey intermediates **30** and **183** (**Scheme 17**).



Reagents and conditions: (i) $NaBH(OAc)_3$, AcOH, 23 °C, 1 h, recrystallization, 95%; (ii) 1) PivCl, Pyridine, 23 °C, 10 h 85%; 2) TBSOTf, 2,6-lutidine, 23 °C, 12 h, 98%; 3) NaOMe, MeOH, 23 °C, 5 h, 92%; (iii) Raney-Nickel, EtOH, 0 °C, 1 h, 82%; (iv) $MePhSO_2SMe$, Et_3N, DCM, rt, 78%; (v) PMBBr, NaH, DMF, THF, 0 °C to rt; 2) HF, pyridine, THF, rt, 40% (two steps); (vi) $NaBH(OAc)_3$, AcOH, rt, 90%; (vii) $Zn(BH_4)_2$ (4.4 M in THF), THF, 0 ℃, 79%; viii) 1) TBSOTf, 2,6-lutidine, DCM, 0 °C to rt, 80%; 2) PMBBr, NaH, DMF, 0 ℃ to rt, 73%; viii) HF‑pyridine, pyridine, THF, rt - 40 ℃, 89%.

Scheme 17: The Corey and Pattenden approach to C6 reduction

We decided to follow a similar strategy to Pattenden; removal of the benzyl ester would provide **160ab**, a similar intermediate to **181,** allowing addition of the thiomethyl group (**Scheme 18**). We hoped that the bulky nature of the *iso*-butyl group could direct the addition opposite the lactam ring. With the thiomethyl blocking group in place we envisaged a similarly diastereoselective reduction of the C6 ketone analogous to both Corey and Pattenden. Diastereoselective desulphurization of the thiomethyl group would then provide all the stereogenic centres required to produce the C9-deoxy-omuralide analogue.

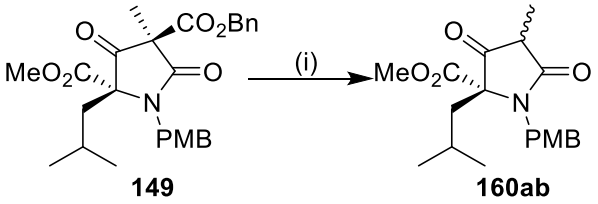


Scheme 18: Planned approach to the functional group manipulations to provide **7**

2.2.3.2 Removal of the benzyl ester and addition of the thiomethyl group

2.2.3.2.1 Hydrogenolysis of the benzyl ester

Removal of the benzyl ester was achieved through a hydrogenolysis using Pearlman's catalyst[84] ($Pd(OH)_2/C$) and hydrogen in THF at 35 °C (**Scheme 19**). Unfortunately, proper characterization of this compound could not be achieved due to decomposition on silica gel, presumably due to the readily enolizable centre. Although the no starting material could be seen on thin layer chromatography (**TLC**), a mixture of compounds was seen in ^1H NMR spectrum. In the most abundant compounds, however, a doublet was seen in place of the singlet that corresponded to the methyl group, indicating that the benzyl ester had been removed. As it could be possible for **160ab** to be in equilibrium with each of the C7 diastereoisomers and the ketone and lactam enolates, we decided to use the compound the next step without further purification.

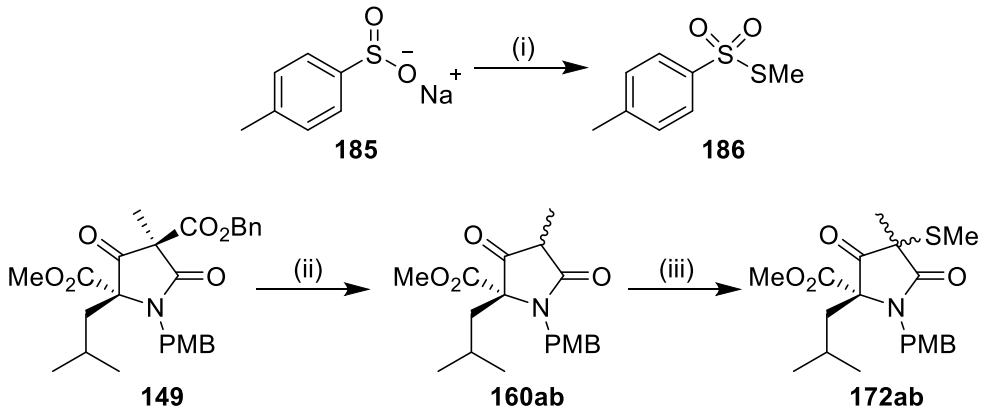


Reagents and conditions: (i) $Pd(OH)_2/C$ (cat), THF, 35 °C, overnight, quant.

Scheme 19: Removal of the benzyl ester

2.2.3.2.2 Synthesis of the thiosulphonate reagent and use with lactam 160ab

Like Pattenden, we decided to synthesize **186** as an electrophilic source of thiomethyl through the use of *p*-toluene sulphinate leaving group ability. The method used for synthesizing the reagent was developed by Fujiki,[85] who produced thiosulphonates with differing R groups in good yield with a wide substrate scope through oxidation of the sulphinate with iodine. (**Scheme 20**).



Reagents and conditions: (i) $(MeS)_2$ (1 equiv), I_2 (2 equiv), CH_2Cl_2, rt, 76%, (ii) $Pd(OH)_2/C$ (cat), THF, 35 °C, overnight, (iii) $MePhSO_2SMe$ (1.7 equiv), Et_3N (1.2 equiv), CH_2Cl_2, rt, 6 h mixture of **172ab**, 68% over the 2 steps.

Scheme 20: Synthesis of the thiomethylating reagent **186** and its use with lactam **160ab**

The conditions used by Pattenden (**Table 1 entry 3**) gave a disappointing yield of 48%, a short optimization was carried out, changing reaction time and temperature, increasing the yield to 68%. The isomers were obtained after column chromatography as a 1:2 mixture of inseparable diastereoisomers (ratio obtained by ^1H NMR spectroscopy).

	Time	Temp °C	Yield %*
1	36 h	16-18	33
2	Overnight	0	34
3	Overnight	25	48
4	Overnight	35	38
5	Overnight	20	54
6	6 h	25	52
7	6 h	20	68

Table 1: Optimization of the thiomethyl addition (step (iii) **scheme 20**).

*Yields shown over the 2 steps (ii) and (iii) of scheme 20.

Unfortunately, NOESY NMR experiments on the inseparable mixture showed that the isomer in excess was the undesired **172b**, showing that the thiomethyl group preferentially adds opposite the methyl ester. Although we hoped the *iso*-butyl would be the predominant directing group, it is possible that the bulky head sits too far from the lactam ring to effectively direct attack of the thiomethyl unit, whereas the methyl ester is in closer proximity to the ring (**Figure 4**).

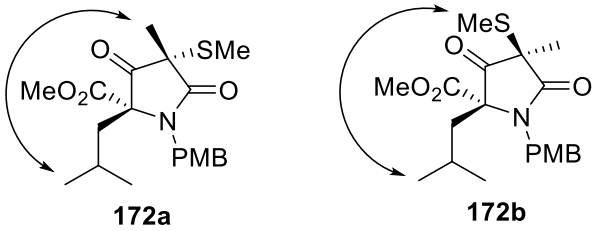
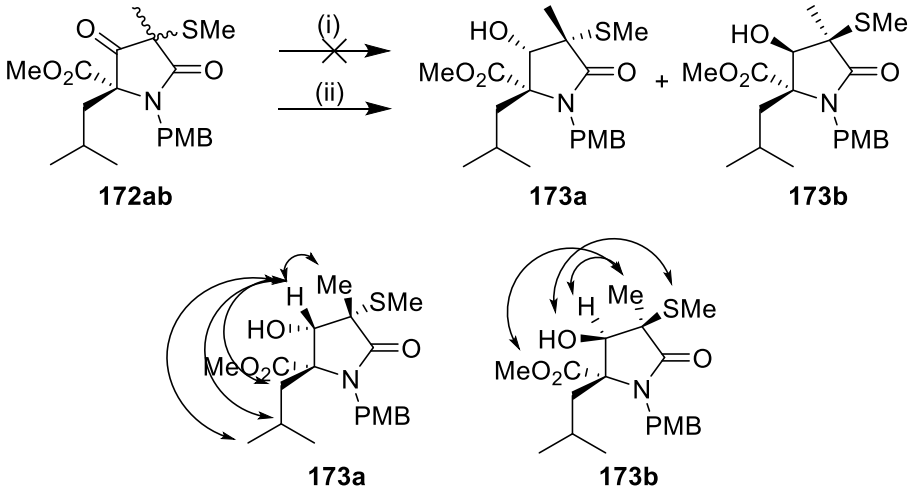


Figure 4: NOESY correlations of **172ab**

2.2.3.3 Reduction of the C6 ketone

Following Corey's strategy and Pattenden's experimental conditions, reduction of the C6 ketone on was attempted with $NaBH(OAc)_3$ using the mixture of diastereoisomers **172ab**. Unfortunately, no reaction was seen and only starting material could be isolated. It is possible that the increased size of the boron complex decreases accessibility to the ketone in our system. Fortunately, however, the stronger reducing agent sodium borohydride was able to reduce the ketone, and, like Corey's reaction, was found to be stereoselective. Hydride attack occurred opposite the thiomethyl group. Due to the poor diastereoselectivity of the

thiomethyl addition, our desired isomer **173a** could only be isolated in 17% yield. Recovered starting material indicated that the ratio of diastereoisomers had changed from 2:1 to 6:1, showing that the desired diastereoisomer reacted much faster than the major one. This potentially shows again that the ester is effectively the bulkier group, slowing down hydride attack more than the *iso*-butyl group. Both isomers were analysed by NOESY experiments, showing that hydride attack had occurred opposite the thioether, and again indicating that the major product was our undesired diastereoisomer (**Scheme 21**).



Reagents and conditions: (i) $NaBH(OAc)_3$ (2 equiv), AcOH, 40 °C, 16 h; (ii) $NaBH_4$ (0.6 equiv), EtOH, 30 m, -10 °C, **173a** 17 %, **173b** 46%.

Scheme 21: Reduction of **173ab**

2.2.3.4 Desulphurization of C7

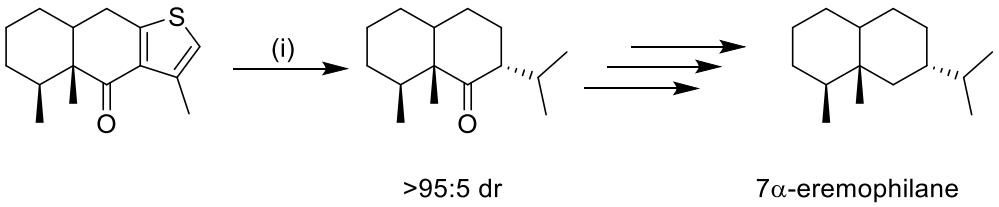
2.2.3.4.1 Raney® Nickel and uses

Raney® nickel was developed by Murry Raney in 1926 as a method of hydrogenation of vegetable oils. The catalyst is formed by sodium hydroxide dissolution of aluminium from a nickel/aluminium amalgam, providing nickel with a large surface area.[86] Raney® nickel's use in desulphurization was observed in 1939,[87] and a more comprehensive analysis was carried out in 1943[88] with a wide variety of aliphatic and aromatic compounds. Perhaps the most famous use of Raney® nickel's desulphurizing ability is the Mozingo reduction, whereby a thioketal is formed from a carbonyl and then hydrogenolysed to an alkane through treatment with Raney® nickel. Despite its wide use, however, the mechanism of action of Raney® nickel desulphurization is under debate. The prevailing theory involves a free radical mechanism,

where sulphur is chemisorbed on the surface of the nickel inducing homolytic bond fission. Hydrogen associated with the catalyst surface can then add to the radical species. Evidence for this theory includes the observation of dimers[89] and the racemization of chiral sulphides.[90] Although beyond the scope of this chapter, more evidence pointing to a radical mechanism and the counter-theories can be found in the literature.[91]

2.2.3.4.2 Diastereoselective uses of Raney® nickel

Despite the unpredictability of Raney® nickel desulphurizations, diastereoselective desulphurizations have been used efficiently in the synthesis of several natural products. One example is the synthesis of 7α-eremophilane, where reduction/desulphurization of the thiophene yields the alpha anomer with greater than 95% diastereoselectivity (**scheme 22**).



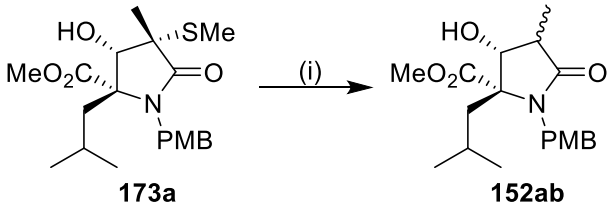
Reagents and conditions: (i) W-7 Raney Nickel, hexanes, 85%.

Scheme 22: Synthesis of 7α-eremophilane using a diastereoselective desulphurization

Not only has Raney® nickel been used for diastereoselective reactions, but modification of the catalyst surface originally by glucose[92] and later tartaric acid[93] has provided enantioselective reductions.

2.2.3.4.3 Desulphurization of the thiomethyl group with Raney® nickel

Treatment of compound **173a** with Raney® nickel under Corey's conditions (0 °C) only provided starting material, as did a room temperature reaction. Heating to reflux, however, did produce the desulphurized compound as a 3:1 mixture of diastereoisomers, but, in a very disappointing yield of 7% (**Scheme 23**). These decreased yields meant we were unable at this stage to properly analyse the product to conclude whether the desired diastereoisomer was in excess. This result combined with the low yields and poor stereoselectivity of the previous two steps led us to abandon our work with the thiomethyl derivative.



173a **152ab**

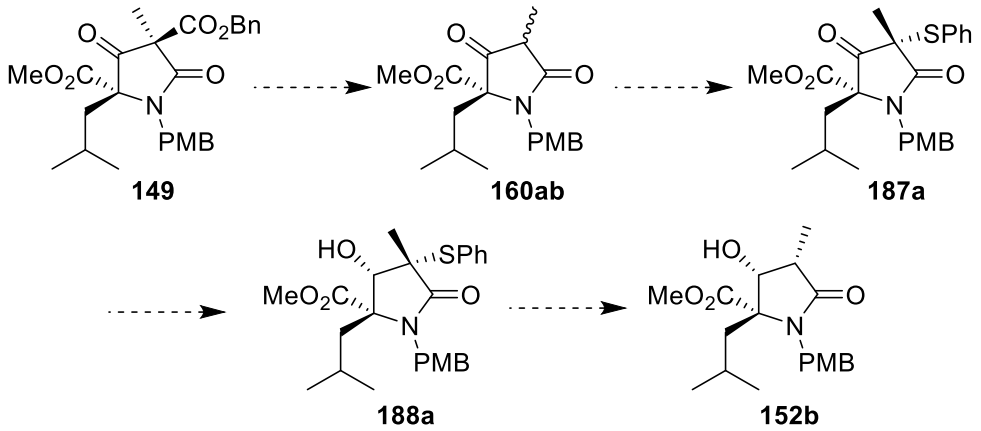
Reagents and conditions: (i) Raney® nickel, ethanol, reflux, 4 h, **152ab** (3:1), 7%.

Scheme 23: Raney® nickel desulphurization of **173a**

2.2.3.4.4 Replacement of the thiomethyl with a thiophenyl group

2.2.3.4.4.1 Strategy

We decided to exchange the thiomethyl group for a thiophenyl in the hope the thiophenyl would be more labile (**Scheme 24**). Although we were unable to find examples in the literature of the use Raney® nickel to remove thiophenyl more efficiently than thiomethyl, examples showing this with azobisisobutyronitrile (**AIBN**)-initiated radical-based removals have been well studied.[94,95] Because the mechanism for Raney® nickel desulphurization was potentially also radical based, we thought this approach worth attempting. The bulkier phenyl group would also mean that desulphurization could be favoured by the release of strain in the system.

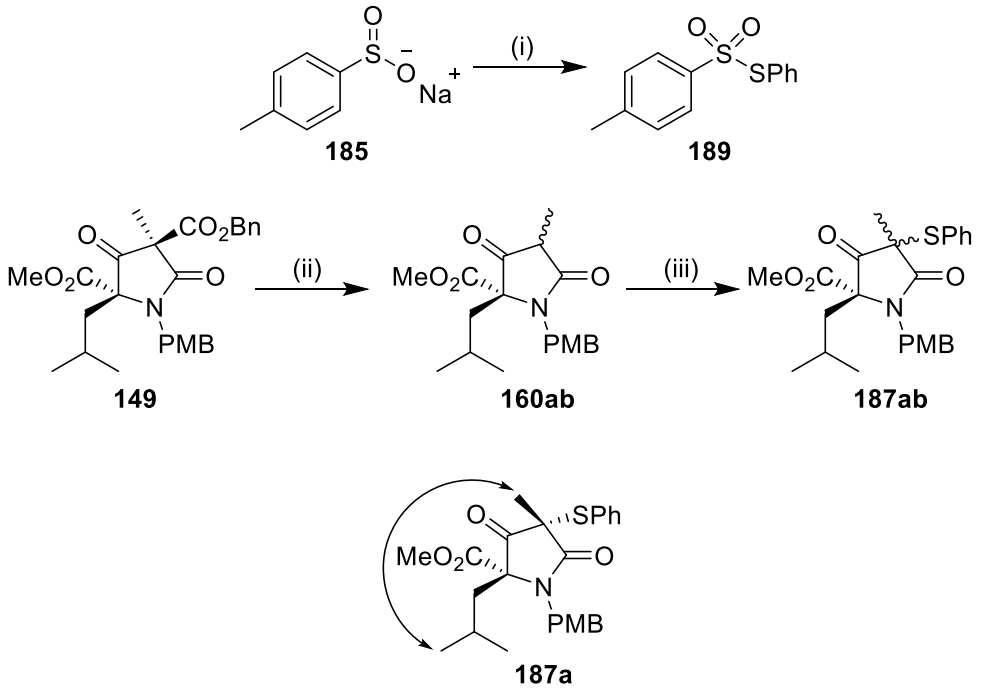


149 **160ab** **187a** **188a** **152b**

Scheme 24: Strategy for the incorporation of thiophenyl and its removal

2.2.3.4.4.2 Hydrogenolysis and insertion of the thiophenyl

The thiophenylating reagent was synthesized in an analogous way to the thiomethylating reagent, and could be used without purification.[96,85]



Reagents and conditions: (i) $(PhS)_2$ (0.5 equiv), I_2 (0.5 equiv), CH_2Cl_2, rt, overnight, 67%, (ii) $Pd(OH)_2/C$, THF, 35 °C, overnight, (iii) $MePhSO_2SPh$ **189** (2.9 equiv), Et_3N (1.6 equiv), CH_2Cl_2, rt, mixture of **187ab**, 67% over the 2 steps.

Scheme 25: Synthesis of the thiophenylating reagent **189** and its incorporation into the lactam core

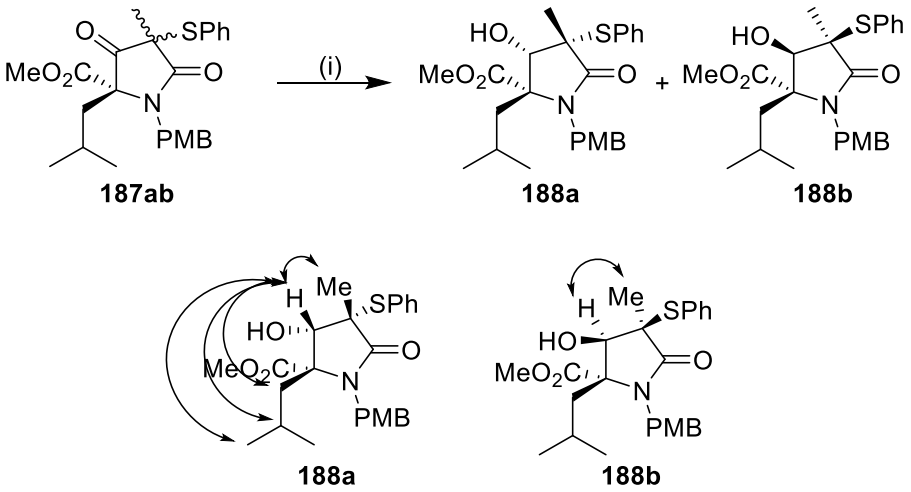
After hydrogenolysis, the lactam **160ab** was treated with reagent **189** (**Scheme 25**). Using Pattenden's conditions gave only moderate yields, but a slight improvement was seen using our optimized conditions (**Table 2**, **entry 2**) and reducing the time further provided a good yield of 67%. Furthermore, the diastereoselectivity was now found to be 1:0.9 and although this was still disappointingly low, as the thiomethyl analogue had provided the unwanted diastereoisomer in 2:1 excess, this was a vast improvement. Although the diastereoisomers were inseparable, NOESY analysis gave evidence suggesting the unwanted diastereoisomer was still in excess, albeit only moderately.

Experiment	Time	Temp °C	Yield
1	overnight	25	49%
2	6 h	20	56%
3	6 h	17-18	57%
4	5 h	20	67%

Table 2: Optimization of the thiophenylation of **160ab**

2.2.3.4.4.3 C6 reduction of compound 187ab

The thiophenyl analogue was reduced using the same conditions as the thiomethyl and the reaction occurred with comparable yields and selectivity (**Scheme 26**). The diastereoisomers were easily separable by column chromatography. Led by NOESY analysis, we concluded, as before, that the first eluting diastereoisomer was the required stereochemistry, with the C6 hydroxyl *cis* to the methyl ester. Analysis of **188a** by chiral stationary phase HPLC indicated that the ee had decreased slightly from 79% to 77%. We believe this to be due to trace amounts of the unwanted diastereoisomer being produced in the acylation with Mander's reagent. This would form trace amounts of the opposite enantiomer and therefore lower the ee.



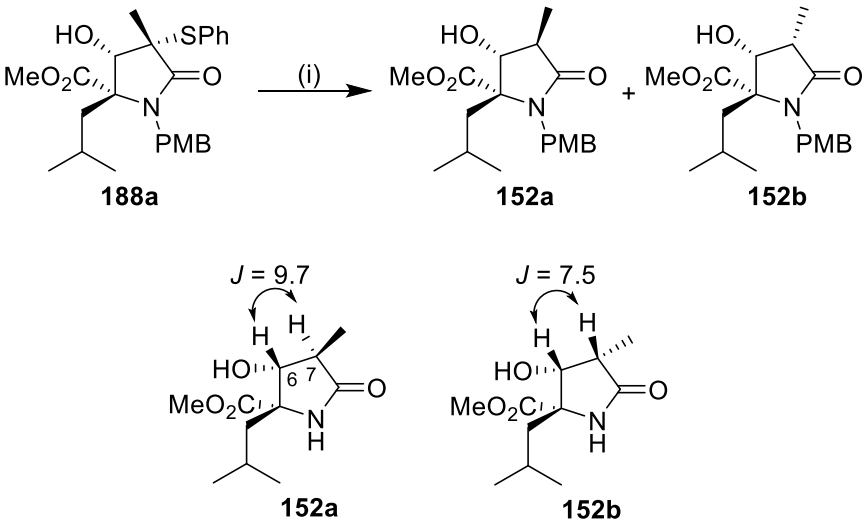
187ab **188a** **188b** **188a** **188b**

Reagents and conditions: (i) $NaBH_4$ (0.7 equiv), EtOH, −10 °C, 0.5 h, 30% **188a** and 35% **188b**.

Scheme 26: Reduction of the C6 position of **187ab**

2.2.3.4.5 Raney nickel desulphurization with thiophenyl analogue 188a

Compound **188a** was treated with Corey's conditions using Raney® nickel in ethanol at 0 °C, however once again an NMR of the crude material showed no product was formed. Increasing the temperature gave a trace of desired product but not enough to isolate. When increasing the temperature to 50 °C we were pleased to see that the same mixture of diastereoisomers could be isolated in 37% yield. The same diastereoisomer was in excess, however the ratio was now 2:1. Finally, heating to reflux as previously used for the thiomethyl analogue **173a** provided the mixture of diastereoisomers in a 3:1 ratio and a 42% yield allowing us to separate and properly analyse each diastereoisomer. Separation allowed each of the diastereoisomers to be more accurately assigned. The first and major eluting diastereoisomer was found to have a *J* value of 9.7 Hz between the C7 and C6 protons (**Scheme 27**). Our minor second eluting diastereoisomer, however, had a value of 7.5 Hz between the same positions. The higher J value of our first eluting diastereoisomer suggests that the protons are *trans* to each other, and therefore the undesired diastereoisomer was in excess. Although our yield was still low, we had succeeded in dramatically increasing the yield by replacement of the thiomethyl group by the thiophenyl group. However, due to the incorrect diastereoisomer being formed in excess, we decided to abandon our work using Raney® nickel for the desulphurization.



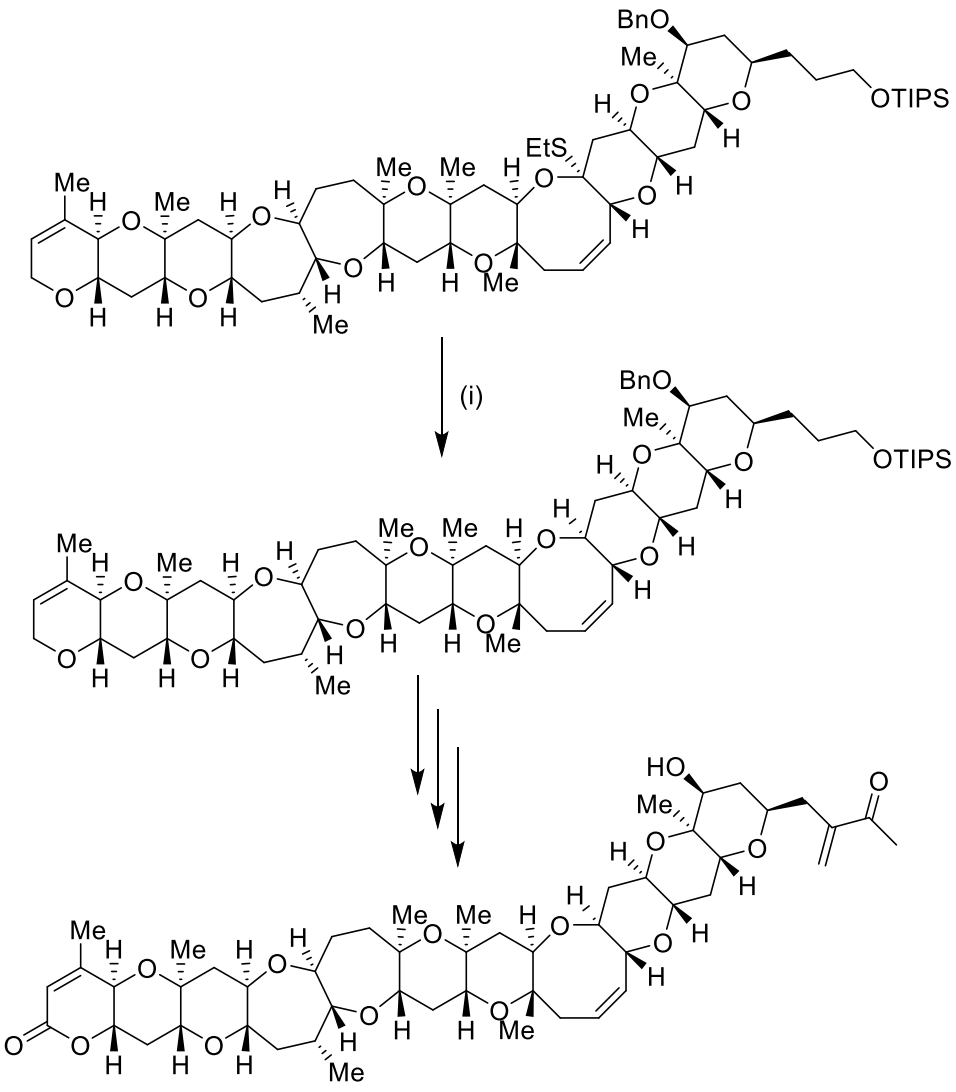
Reagents and conditions: (i) Raney nickel, ethanol, reflux, 4 h, **152ab** (3:1), 42%.

Scheme 27: Treatment of **188a** with Raney® nickel and assignment of the diastereoisomers through *J* values

2.2.3.4.6 AIBN initiated radical desulphurizations

2.2.3.4.6.1 Introduction to radical mediated desulphurizations

Along with Raney® nickel, one of the more common methods of desulfurization is through use of an organotin hydride radical propagator and AIBN as a radical initiator. Such reactions have been comprehensively reviewed[97] and have found use in natural product synthesis, most notably in Nicolaou's synthesis of brevitoxin B (**scheme 28**).[98]



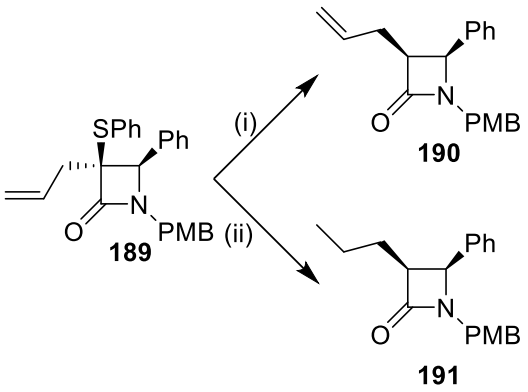
Reagents and conditions: (i) Ph_3SnH, AIBN, toluene, 100 °C, 2 h, 100%

Scheme 28: Use of an organotin hydride desulphurization in the synthesis of brevitoxn B

Study of the literature revealed examples where desulphurizations using Raney® nickel had been compared to AIBN initiated, organotin hydride/organosilane propagated desulphurizations. These examples ranged from Raney® nickel providing:

1) **The same stereochemical outcome as an organo-tin hydride**:

During the preparation of functionalized β-lactams (**Scheme 29**), both Raney® nickel and tributyltin hydride were used to desulphurize thiophenyl groups. β-Lactam **189** provided the propyl/propenyl *cis* to the phenyl in both experiments (albeit with the propyl reduced in the Raney® nickel experiment to provide **191**.)[99]

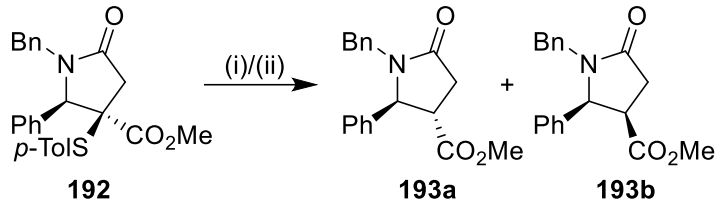


Reagents and conditions: (i) *n*-Butyl tin hydride, AIBN, toluene, reflux, 50%; (ii) Raney® nickel, acetone, reflux, 45%.

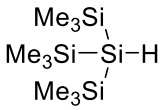
Scheme 29: A comparison of the stereochemical outcome between Raney® nickel and *n*-butyltin-hydride mediated desulphurization

2) **No stereochemical preference**:

During the preparation of a library of functionalized γ-lactams (**Scheme 30**), Raney® nickel desulphurization of **192** was found to be completely unselective, providing a 50:50 mix of diastereoisomers **193a** and **193b.** An AIBN, tris(trimethylsilyl)silane (**TTMS**) radical-propagated desulphurization, however, was highly selective (>95:5). As before, the AIBN-initiated desulphurized position was left *cis* to the bulky phenyl group.[100]



192 **193a** **193b**



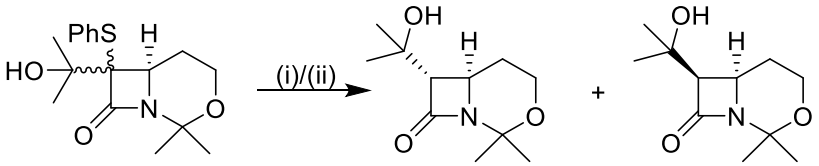
TTMS

Reagents and conditions: (i) TTMS, AIBN, toluene, 90 °C, 93% (>95:5); (ii) Raney® nickel, THF/Ethanol (1:2), rt, 96%, (1:1 ratio of diastereoisomers).

Scheme 30: A comparison of the stereochemical outcome between Raney® nickel and TTMS mediated desulphurization

3) The opposite stereochemical outcome:

Most importantly for our scenario, the opposite selectivity has also been observed (**Scheme 31**). During the preparation of carbapenem antibiotic analogues, switching the method of desulphurization completely changed the observed diastereoselectivity between **195** and **196**. Previous examples have shown that the AIBN-mediated method gave the more thermodynamically stable product, while Raney® nickel favoured the opposite diastereoisomer (**scheme 31**).[95]



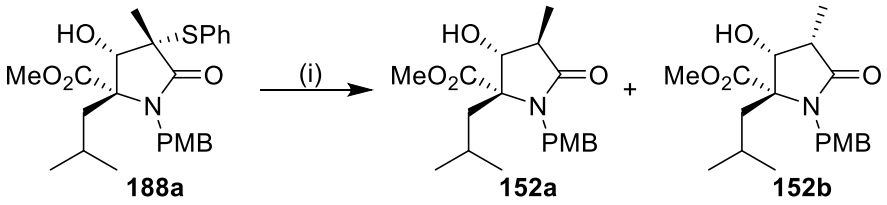
194 **195** **196**

Reagents and conditions: (i) *n*-butyl tin hydride, AIBN, acetone, reflux, 16 h, **195**-22% **196**-72%; (ii) Raney® nickel, acetone, reflux, 2 m, **195**-47%, **196**-21%.

Scheme 31: A comparison of the stereochemical outcome between Raney® nickel and *n*-butyltin-hydride mediated desulphurization.

2.2.3.4.6.2 Tributyltin hydride desulphurization of 188a

Due to this final positive finding, **188a** was heated to reflux with AIBN and tributyltin hydride overnight. Crude NMR spectroscopic analysis proved very difficult due to the tin residues left in the reaction mixture. Following the work by Harrowven, we decided to purify this complex mixture using a mixed silica stationary phase. Harrowven found that 10% finely ground potassium fluoride mixed with silica could remove tin residues to below 30 ppm.[101] Later work found that 10% ground potassium carbonate in silica could reduce levels to as low as 15 ppm.[102] We decided to use the potassium carbonate method due to the increased effectiveness. To our delight, after purification by column chromatography, we found that the organotin method vastly increased yields to 93% providing **152ab** in a 1:2 ratio of diastereoisomers. Most importantly however, the desired diastereoisomer was now the major product (**Scheme 32**).



188a 152a 152b

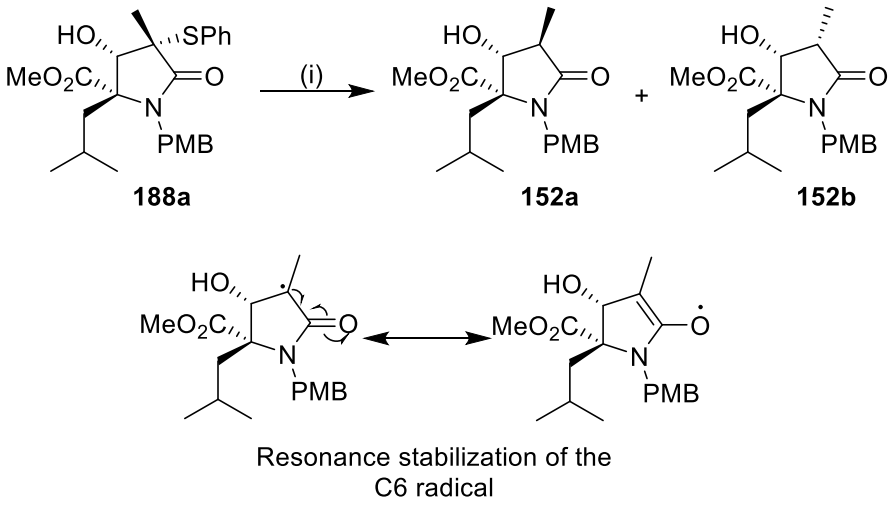
Reagents and conditions: Tributyltin hydride (3.4 equiv), AIBN (0.2 equiv), acetone, reflux, 16 h, **152a/152b** (1:2), 93%.

Scheme 32: Desulphurization of **188a** with tributyltin hydride

2.2.3.4.6.3 Exchange of the organotin radical propagator to TTMS

Although the desulphurization had been greatly improved from our initial reactions, we decided to modify the reaction to see if the diastereoselectivity could be further improved. We theorized that a bulkier radical propagator could increase the selectivity of our desired diastereoisomer. The bulkiest radical propagator we could find commonly available was TTMS. Si-H bonds are not common radical propagators due to the relatively high bond dissociation energy, however, due to the steric repulsion of the TMS groups, bond dissociation enthalpy falls about 17 kJ/Mol per TMS bond replacement from $(Me)_3SiH$. The use and scope of TTMS have been recently reviewed.[103]

When applied to our system we were delighted to find that the diastereoselectivities increased greatly from 1:2 to 1:5 solely on exchange of the radical propagator and our desired diastereoisomer was still the major product. The reaction also took place in an excellent yield of 88%. Part of the reason for this high yield is the stabilization of our radical intermediate (**Scheme 33**).

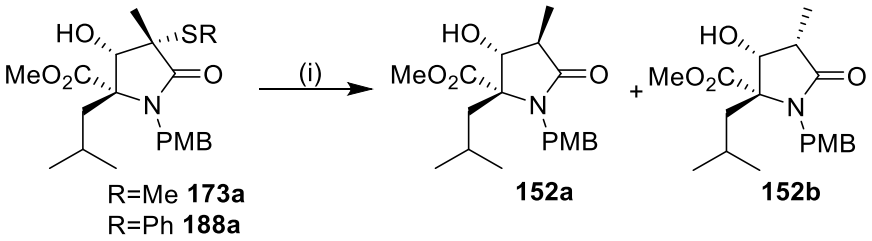


Reagents and conditions: TTMS (3.2 equiv), AIBN (0.3 equiv), acetone, reflux, 16 h, **152a**-14 %, **152b**-74%.

Scheme 33: Desulphurization of **188a** with TTMS and the resonance stabilized radical intermediate

2.2.3.4.7 Summary of the desulphurization conditions

For clarity all the desulphurization optimizations are presented in the following **table 3**.



R=Me **173a**
R=Ph **188a**

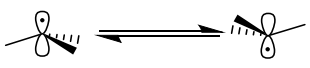
152a **152b**

Entry	R =	Method	Temperature °C	Yield %	Diastereoselectivity 7a:7b
1	Me	Raney® nickel	0	0	N/a
2	Me	Raney® nickel	18	0	N/a
3	Me	Raney® nickel	Reflux	7	3:1
4	Ph	Raney® nickel	0	0	N/a
5	Ph	Raney® nickel	18	Trace	N/a
6	Ph	Raney® nickel	50	37	2:1
7	Ph	Raney® nickel	Reflux	42	3:1
8	Ph	AIBN, acetone, Bu_3SnH	Reflux	93	1:2
9	Ph	AIBN, acetone, TTMS	Reflux	88	1:5

Table 3: Summary of the desulphurization experiments

2.2.3.4.8 The origin of the desulphurization diastereoselectivity

Radical intermediates are not planar *per se*. The unpaired electron is held in a p orbital. The conformation of the intermediate adopts an orientation somewhere between an sp^2 trigonal planar orientation and an sp^3 pyramidal; this conformation is known as a shallow based pyramid (**scheme 34**).



Shallow based pyramid

Scheme 34: Representation of the conformation of a carbon centred radical

The energy required to invert this is so small, that rapid interconversion between the two states takes place. The radical species can therefore be considered planar, like an sp^2 centre.

Radical insertion will therefore take place equally at the top or bottom face in achiral intermediates. If the radical has another stereocenter however, attack will usually occur from the least hindered face.

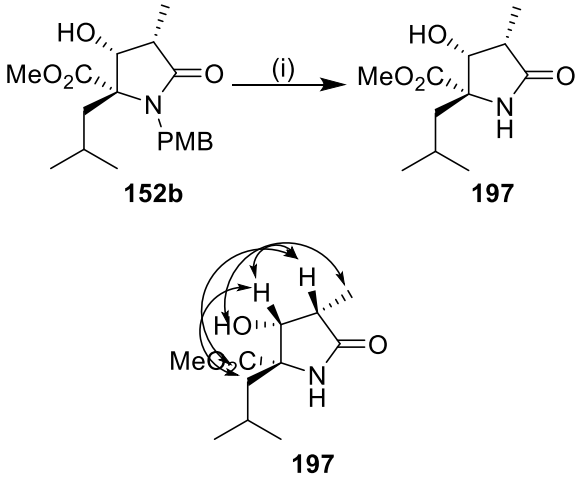
Insertion of the thioether groups (**scheme 20 and 25**) favoured the face opposite the methyl ester in each case rather than the *iso*-butyl. It seems reasonable therefore to assume that the methyl ester is the primary directing moiety in our system. This could explain the selectivity observed if hydrogen is inserted from the face opposite the methyl ester in our AIBN-initiated reactions.

The factors involved in Raney® nickel desulphurization are far more complex as shown in the previous examples (**schemes 29, 30 and 31**). Although general trends tend to show that hydrogen will insert from the least hindered face, the stereochemical outcome is far less predictable. Polar groups have been shown to disrupt selectivity[104] potentially by coordination to the catalyst surface. Both the C5 ester and the C6 hydroxyl could contribute to this.

In Corey's intermediate, the TBS group and the absence of an *iso*-butyl means that intermediate **31** has a far more well defined sterically hindered face. The TBS group could also block potentially competing coordination effects. This could contribute to the high diastereoselectivity Corey achieved. Interestingly, during our Raney® nickel thiophenyl removal, the selectivity changed from 2:1 to 3:1 on an increase in temperature. Corey's reaction occurred at 0 °C when he obtained the desired diastereoisomer. If our analogue underwent epimerization at the α-amide position due to elevated temperatures, this could produce the more thermodynamically stable diastereoisomer (preventing hindrance from the C7 methyl and the C5 methyl ester) and explain the change of diastereoselectivity at different temperatures. Raney® nickel desulphurization can also produce metal salts which could act as Lewis acids potentially catalysing this process. Nevertheless, with our advanced intermediate **152b** in hand we started on the final deprotection steps to produce the omuralide analogue.

2.2.4 Removal of the PMB and methyl ester groups

Various methods are available for the removal of PMB groups. CAN is however the most commonly used in previous omuralide syntheses. The removal proceeded in fair yield (**Scheme 35**), and NOESY correlations seemed to confirm our previous experiments had provided the desired diastereoisomer.



Reagents and conditions: CAN (5.2 equiv), MeCN/H_2O (3:1), rt, 62%.

Scheme 35: Removal of the PMB protecting group

With all the stereocentres in place and due to the crystalline nature of the product, we subjected compound **197** to recrystallization. We successfully obtained a diffractable crystal from our racemic series, synthesized for HPLC traces (**Figure 5**).

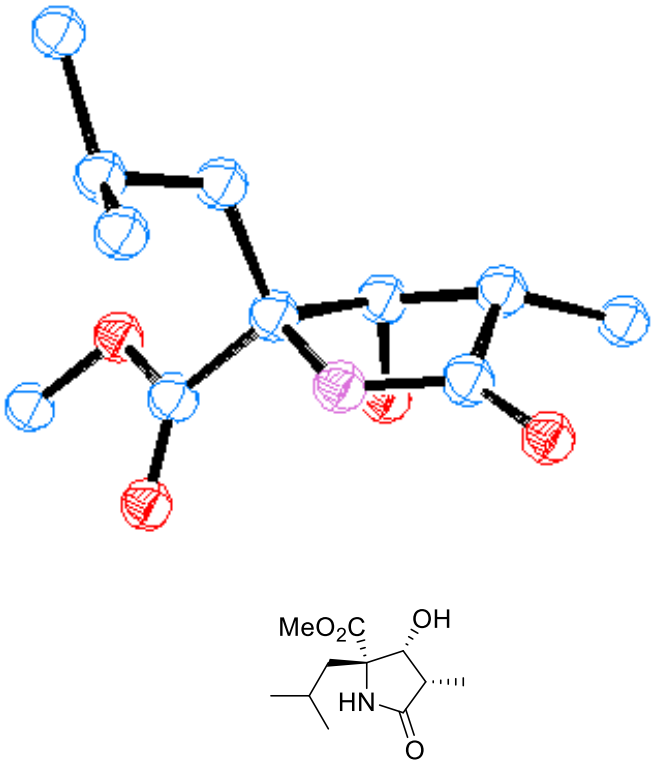
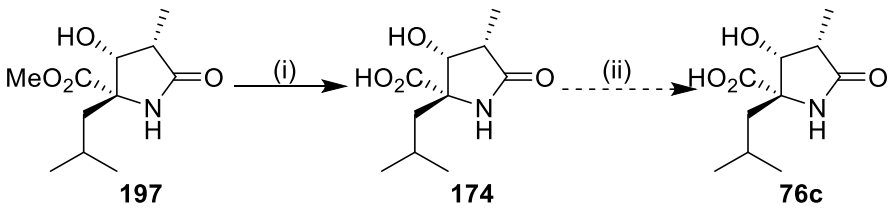


Figure 5: The X-ray crystal structure of (±)-**197** (hydrogen atoms omitted for clarity)

We were pleased to confirm that our NOESY analysis throughout the synthesis had been correct. As in omuralide, the C5 ester, C6 hydroxyl, and the C7 methyl are all *cis* to each other. Our final step involved saponification of the methyl ester to the acid with NaOH. The acid was obtained in good yield, and NMR data matched the data from Mereddy,[77] confirming that we had made the pyroglutamate **174**. This had previously been cyclized to 9-deoxy-omuralide **76c** by Mereddy in one step, thus completing a formal synthesis (**Scheme 36**).



197 **174** **76c**

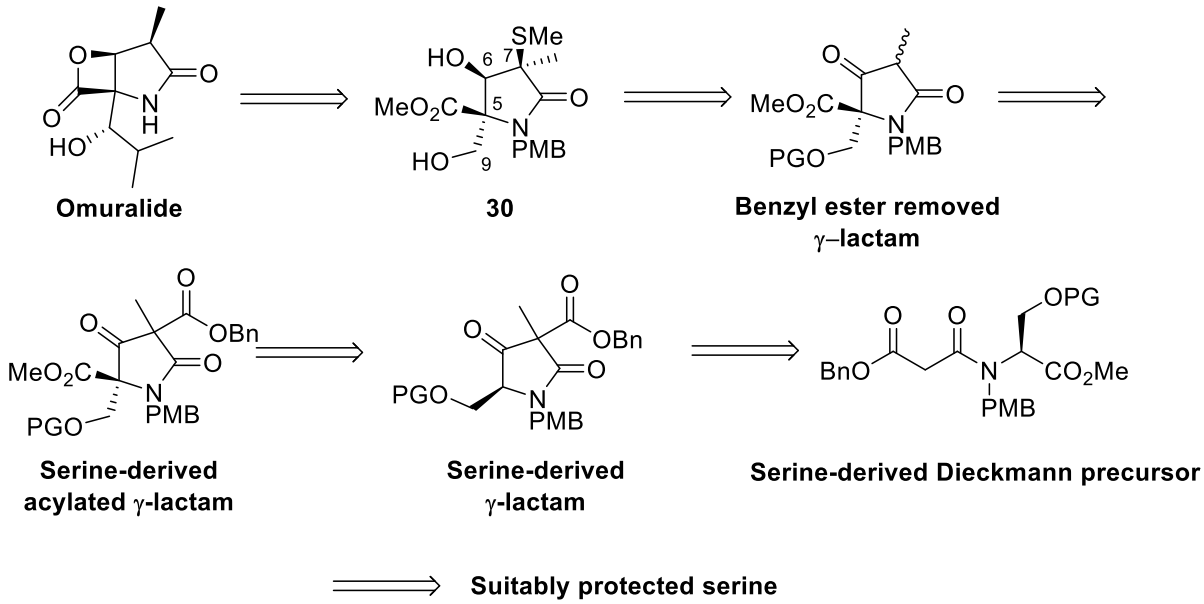
Reagents and conditions: (i) NaOH (0.5 M), 0-5 °C, 86%; (ii) BOPCl, Et_3N, DCM, 40%.

Scheme 36: Saponification of the methyl ester and Mereddy's formation of the β-lactone

2.3 Elaboration of L-serine methyl ester hydrochloride to omuralide

2.3.1 Choice of amino acid starting material and retrosynthetic analysis

For our second route we decided to start with L-serine. The primary reason for this is that the hydroxyl group will be in place from the beginning of the synthesis; the serine hydroxyl group will end up in the C9 position required for elaboration to omuralide (**Scheme 37**).



Scheme 37: Retrosynthetic analysis of omuralide

Our target molecule would be Corey's advanced intermediate **30**.[29] Not only was this the most direct way to confirm that our previously developed methodology could incorporate serine, but also this intermediate had previously been used to produce a wide range of C9 omuralide analogues.[105,37] A suitably protected serine derivative would be coupled to a half malonic benzyl ester to form the **serine-derived Dieckmann precursor**. After the tandem cyclization/alkylation procedure to form the **serine-derived γ-lactam core**, Mander's reagent would again be used to insert the methyl ester to form the **serine-derived acylated γ-lactam core**. Removal of the benzyl ester would allow insertion of the thiomethyl group which along with a stereoselective reduction and protecting group removal would provide the advanced intermediate **30**.

2.3.2 Choice of serine *O* protecting group

Following the general reaction scheme from our previous work to synthesize 9-deoxy omuralide, we had to consider that any protecting group used would have to be stable to basic conditions from the acylation with Mander's reagent, to fluoride sources from the TBAF mediated Dieckmann cyclization and potentially to conditions used for the benzyl ester hydrogenolysis. Because of these restrictions we decided that an acid-labile protecting group would be most suitable. In addition to stability, we also had to consider the steric bulk of the protecting group. Our synthesis is based on the amino acid directing the stereochemical outcome of the reaction. Serine has very little steric bulk in its structure, and we had already found that leucine did not direct our stereochemical outcome as much as we had hoped. We therefore aimed to use as bulky protecting group as possible. Two initial protecting groups were considered. The first was a tetrahydropyranyl ether. Although it fit all the criteria required for our synthesis, the protecting group would insert a second chiral centre. This would make full characterization of subsequent intermediates by NMR spectroscopy extremely difficult. We settled on a *tert*-butyl ether protecting strategy. Not only is the *tert*-butyl protected serine methyl ester hydrochloride salt commercially available, it is also available as the unnatural enantiomer (**Figure 6**).

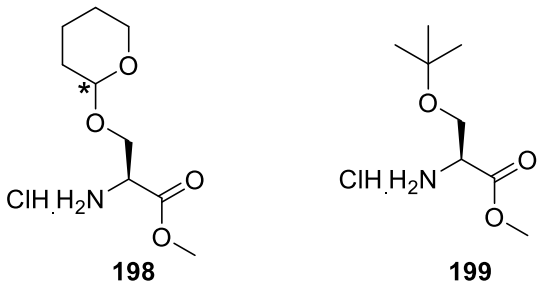
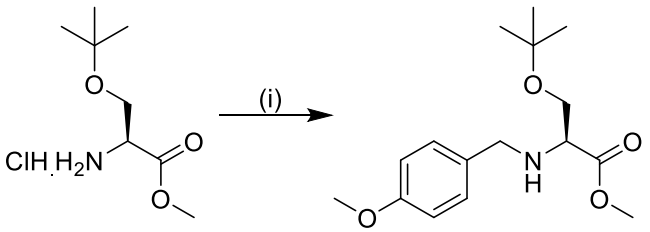


Figure 6: Suitable groups for protection of the hydroxyl group

2.3.3 Synthesis of the serine-derived Dieckmann cyclization precursor

2.3.3.1 PMB protection of the *tert*-butyl protected serine

PMB was once again used for the nitrogen protection. An extensive optimization was carried out to find acceptable conditions (**Table 4**).



199 200

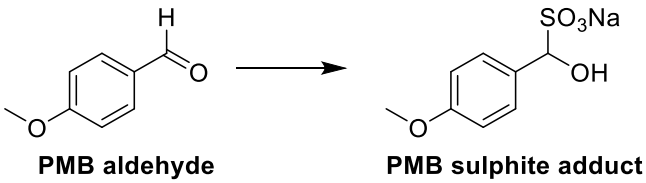
Entry	Reagents and conditions	Yield %
1	1) PMB aldehyde (1.5 equiv), TEA (1.2 equiv), DCM 2) $NaBH_4$ (1.6 equiv), EtOH	71
2	*p*-TSA (1 equiv), PMB aldehyde (10 equiv), $NaBH_4$ (1.1 equiv)	N/a
3	PMB, TEA (1 equiv), $NaBH_4$ (1.2 equiv), MeOH	18
4	PMB sulphite adduct (1.5 equiv), $NaBH_3CN$ (2.7 equiv), MeOH	28
5	PMB sulphite adduct (3.3 equiv), $NaBH_3CN$ (22 equiv), TEA (1 equiv), EtOH	58
6	PMB sulphite adduct (1.6 equiv), $NaBH_4$ (1 equiv), TEA (1 equiv), MeOH	imine
7	PMB sulphite adduct (1.6 equiv), $NaBH_4$ (1.7 equiv), TEA (1 equiv), EtOH	imine
8	PMB sulphite adduct (1.6 equiv), $NaBH_4$ (1.6 equiv), TEA (1 equiv), IPA	N/a
9	PMB sulphite adduct (1.2 equiv), $NaBH_3CN$ (6.3 equiv), pyridine (10 equiv), MeOH	24
10	PMB sulphite adduct (1.5 equiv), $NaBH_3CN$ (6.2 equiv), TEA (1 equiv), MeOH	67
11	PMB sulphite adduct (3.2 equiv), $NaBH_3CN$ (13 equiv), TEA (1 equiv), MeOH	85

Table 4: Optimization of the PMB protection of **199**

Our first reaction (**Entry 1**) involved formation of the imine, with triethylamine (**TEA**) in dichloromethane (**DCM**), and after evaporation a sodium borohydride reduction. Despite good yields, we decided to find an alternative method due to the possibility of racemization during imine formation. Reduction of the imine *in situ* should limit the potential for

racemization. A solvent-free reaction was attempted based on similar conditions reported by Cho *et al.*[106], unfortunately, however, no reaction was observed. **Entry 3** shows the yields obtained when using conditions analogous to the leucine route, unfortunately however the yield was very low. It was possible $NaBH_4$ was reducing the aldehyde, stopping imine formation.

A promising method was found in the paper '*Direct reductive alkylation of amine hydrochlorides with aldehyde bisulphite adducts*'[107] where the methyl ester hydrochloride of phenyl alanine was protected with the bisulphite adduct of PMB in 95% yield. No racemization was mentioned, so we decided to attempt this method (**Scheme 38**).



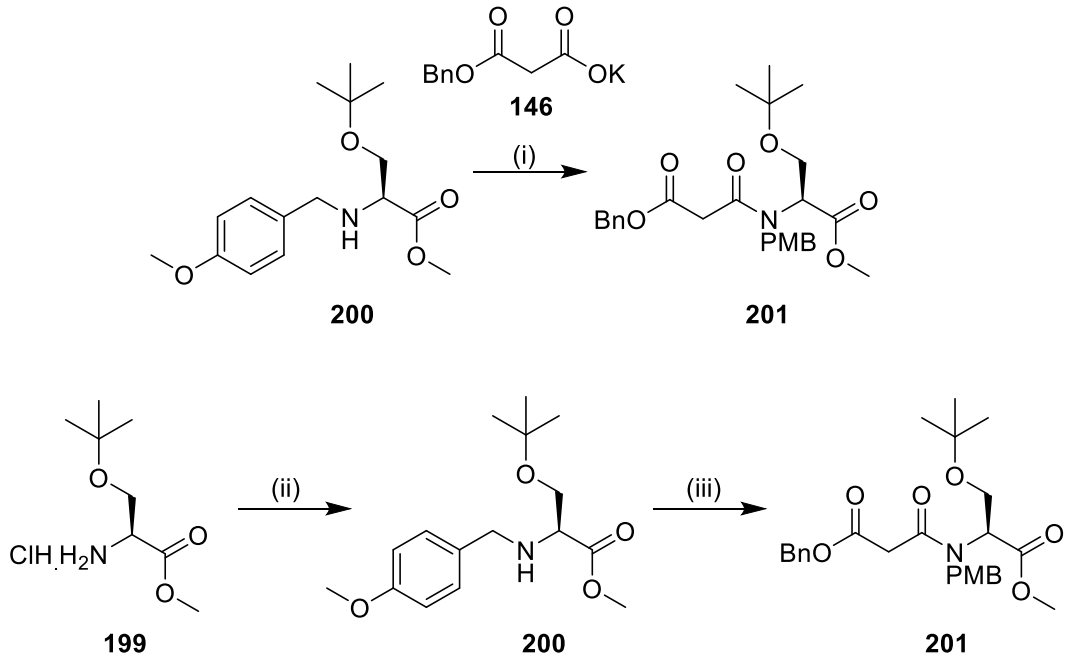
Reagents and conditions: Na_2SO_3, EtOH, rt, 16 h, 81%.

Scheme 38: Formation of the sodium bisulphite adduct of PMB

The sodium bisulphite adduct of PMB was prepared and isolated in good yield to be used in our reductive amination. Unfortunately, in our hands using standard conditions we were only able to isolate 28% of the protected amine (**Entry 4**). Yields were found to drastically increase when triethylamine was added. With ethanol as the solvent, yields of 58% were achieved. Switching the reducing agent to sodium borohydride did not produce any of the desired product. Only the imine could be observed by NMR spectroscopy of the crude material when the reaction was carried out in methanol or ethanol, and neither the amine or imine could be seen if the reaction was run in *iso*-propyl alcohol (**IPA**) (**Entries 6,7, and 8**). With sodium cyanoborohydride as the reducing agent, pyridine was used to catalyse imine formation as it has been found to induce much less racemization than triethylamine. Unfortunately, yields were greatly reduced (**entry 9**). Returning to triethylamine but switching the solvent to methanol vastly increased yields to 67%, and finally increasing the proportions of the aldehyde and reducing agent provided us with the protected amino acid in good yields of 85%. Unfortunately, we were unable to achieve separation of a synthesized racemate of this compound on HPLC using a chiral stationary phase.

2.3.3.2 Coupling to the half malonic benzyl ester

Coupling of the half malonic benzyl ester **146** proceeded in good yield (**Scheme 39**) in a reaction analogous to the leucine route.



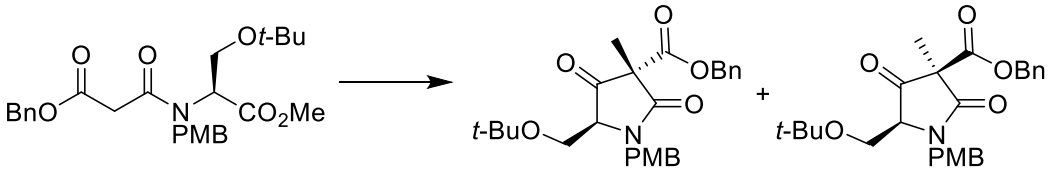
Reagents and conditions: i) **146** (2.5 equiv), EDAC.HCl (2.6 equiv), N-MM (2.5 equiv), DMAP (0.2 equiv), DCM, 16 h; 73%. ii) **146** (1.6 equiv), $NaBH_3CN$ (6.1 equiv), Et_3N (0.9 equiv), MeOH, 0 °C, 16 h (iii) **146** (2.5 equiv), EDAC.HCl (2.7 equiv), N-MM (2.5 equiv), DMAP (0.2 equiv), DCM, 16 h; 49% over the 2 steps.

Scheme 39: Peptide coupling of **146** to **200**

Once again, the Dieckmann cyclization precursor was found to be a pair of rotamers in a 1:5 ratio. Full coalescence was observed at 100 °C. Analysis on a HPLC using a chiral stationary phase in comparison to a synthesized racemic compound found **201** to be 94% ee. It was found that there was a degree of variability in this value depending on the room temperature at the time of the reductive amination. In light of this, we decided to modify the reductive amination to ensure that a cooler temperature was maintained. The crude product from this reaction was pure enough to be used in the peptide coupling step without purification by column chromatography. The Dieckmann cyclization precursor **201** could now be isolated in good yield over the two steps in 49% yield and 97% ee.

2.3.4 The Dieckmann cyclization of the serine derived analogue

2.3.4.1 Optimization of the Dieckmann cyclization of 201



201 **202a** **202b**

Entry	Conditions	Approx. Scale (g)	Yield %	Ratio of 202a:202b	ee of (202a)
1	TBAF (3.4 equiv), THF 0.5 h then MeI (4.5 equiv) 16 h, rt	0.1	65	10:1	-
2	TBAF (4 equiv), THF 0.5 h then MeI (4 equiv) 16 h	2.5	31	10:1	-
3	TBAF (3.7 equiv) THF 2 h then MeI (4.1 equiv) 2 h	0.2	62	10:1	44
4	TBAF (3.6 equiv), THF 0.5 h, MeI (3.9 equiv), 0 °C to rt over 16 h	0.2	42	10:1	60
5	TBAF (3.7 equiv), THF 0.5 h, MeI (3.9 equiv) 1 h -10 °C	0.1	Trace	-	76
6	TBAF (3.4 equiv), 0.5 h THF MeI (4.1 equiv), -15 °C, 64 h	0.3	30	3:1	76
7	TBAF (2 equiv), Ether, 5 m, THF MeI (4.3 equiv), -12 °C, 64 h	0.2	56	3:1	83
8	TBAF (2.1 equiv), Ether, 5 m, THF MeI 10 equiv), -12 °C, 64 h	1.6	66	3:1	79

Table 5: Optimization of the cyclization

Treating the serine-derived Dieckmann precursor **201** under conditions analogous to our leucine route provided the isomers **202a** and **202b** with excellent diastereoselectivity and in good yield (**Table 5, entry 1**). Unfortunately, we found increasing the scale of this reaction resulted in a large drop in yield. We were also surprised to see that the major diastereoisomer

produced was that with the methyl and *tert*-butyl groups *cis* to each other. We assumed that, as we had theorised in the leucine route, the thermodynamically more unstable diastereoisomer (**202b**) was epimerizing to the more stable one (**202a**). Due to this resulting in racemization during the leucine route, we decided to analyse our reaction further using HPLC with a chiral stationary phase.

Two strategies were considered in an attempt to stop the epimerization:

1) Epimerization could be reduced with shorter reaction times
2) Cooling the reaction could stop the epimerization from occurring

Decreased reaction times provided **202ab** in good yield, but the diastereoselectivity remained unchanged. The ee was analysed and found to be only 44%, indicating that a similar epimerization to our leucine route was occurring. Cooling the reaction to 0 °C led to a slightly increased ee of 60%. Cooling to -10 °C increased the ee to 76% but only trace a amount of the product was isolated (**entry 5**). Separation of the cyclization and alkylation steps was also attempted along with longer reaction times and cooler temperatures. The diastereoselectivity then dropped to 3:1 indicating that the majority of the epimerization had been prevented. This was reflected in the ee, which was now 76%. The ee was not improved further at -15 °C, leading us to our optimal conditions. Long reaction times and cooling to -12 °C provided a yield of 53% and 83% ee. Once again, scaling up the reaction with these conditions decreased the yields. Yields could however be increased if a large excess of MeI (10 equiv) was used - which provided acceptable yields of up to 66% and with a comparable ee of 79% (**Entry 7**). Unfortunately, the second eluting, minor diastereoisomer **202b** could not be successfully separated from the major diastereoisomer. Overlapping peaks in the HPLC chromatograph meant accurate information about the enantiopurity could not be obtained.

The ee could be improved further if necessary by a recrystallization from IPA, providing racemic solid and a filtrate of 98% ee. Although we had been able to stop the epimerization to a large degree, we were still surprised to find that the major diastereoisomer formed was still the diastereoisomer where the methyl and benzyl ester are *cis* to each other. This result is surprising because the expected diastereoselectivity was for the methyl iodide to add opposite the bulky amino acid-derived *tert*-butoxy group. The leucine analogue route had shown that the methyl group adds opposite the bulky *iso*-butyl moiety of the leucine, and we expected a similar outcome in our serine derivative. It is possible that the serine *tert*-butyl

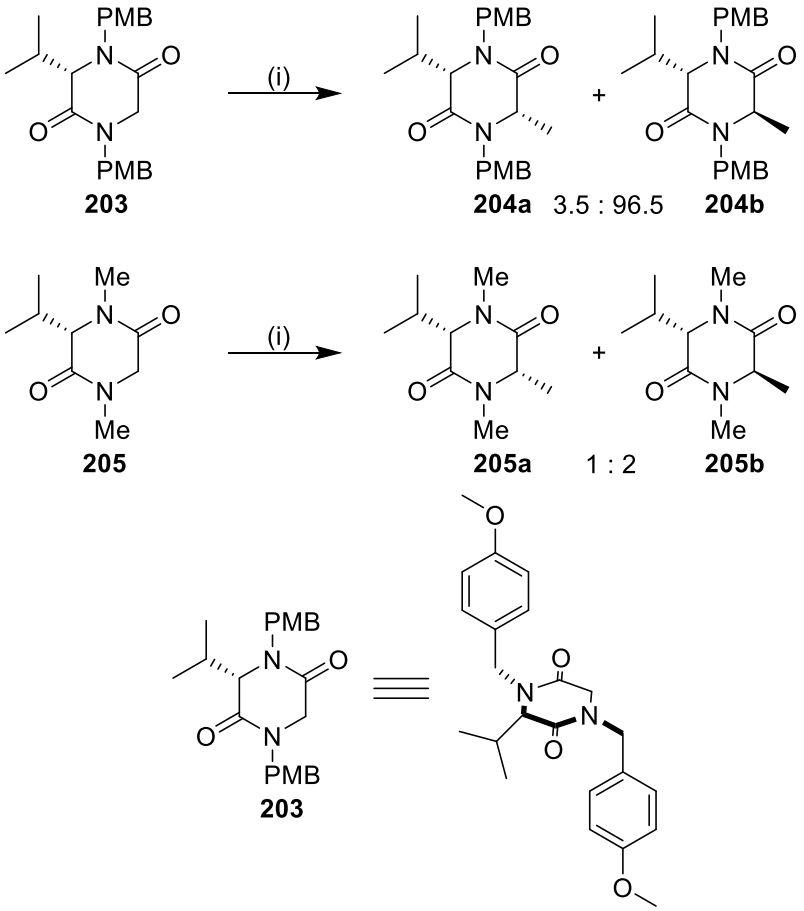
group sits too far from the ring to block the top face effectively, unlike the shorter chain *iso*-butyl group in leucine, allowing other factors to come into play. Further investigation of the cyclization was carried out in order to find a reason for this surprising diastereoselectivity.

2.3.4.2 Investigation of the unexpected diastereoselectivity

2.3.4.2.1 Investigation of a chiral relay effect

2.3.4.2.1.1 Introduction to the chiral relay effect

In 1998 Davies *et al.*[108] showed that PMB groups, despite not being chiral themselves, were capable of relaying chiral information from a valine-derived *iso*-propyl group around a diketopiperazine ring, due to the rotation around the PMB methylene carbon (**203**). This effect could then be used to increase the diastereoselectivity of an alkylation compared to using increasingly more rigid methyl groups (**205**) (**Scheme 40**).



Reagents and conditions: (i) LiHMDS, THF, -78 °C, MeI (10 equiv).

Scheme 40: Davies investigation of a chiral relay effect to increase diastereoselectivity

It seemed reasonable that similar phenomenon might be occurring in our system. The bulky *tert*-butoxy group could potentially be repelling the PMB group to the opposite face of the ring. The methoxy benzyl could now be competing with the *tert*-butyl group, directing the methyl to the same face of the ring as the *tert*-butoxy group. (**Figure 7**)

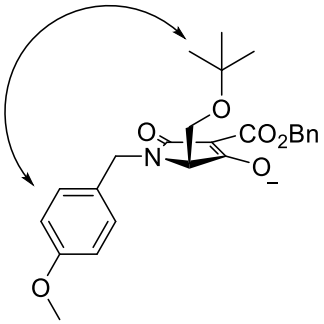
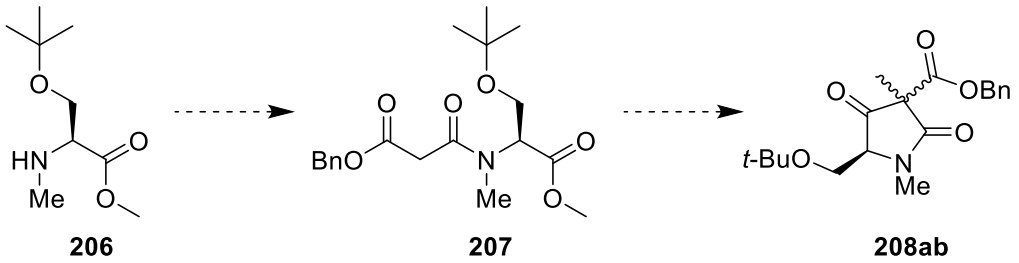


Figure 7: A conformation of our enolate which could potentially exhibit a chiral relay effect

In order to investigate this, we decided to replace the PMB group with a methyl. The less flexible nature of the methyl group would be unable to relay any of the chirality from the serine *tert*-butyl group. We would then expect if this was contributing to our diastereoselectivity that the iodomethane might favour addition to the face opposite the *tert*-butyl. (**Scheme 41**)

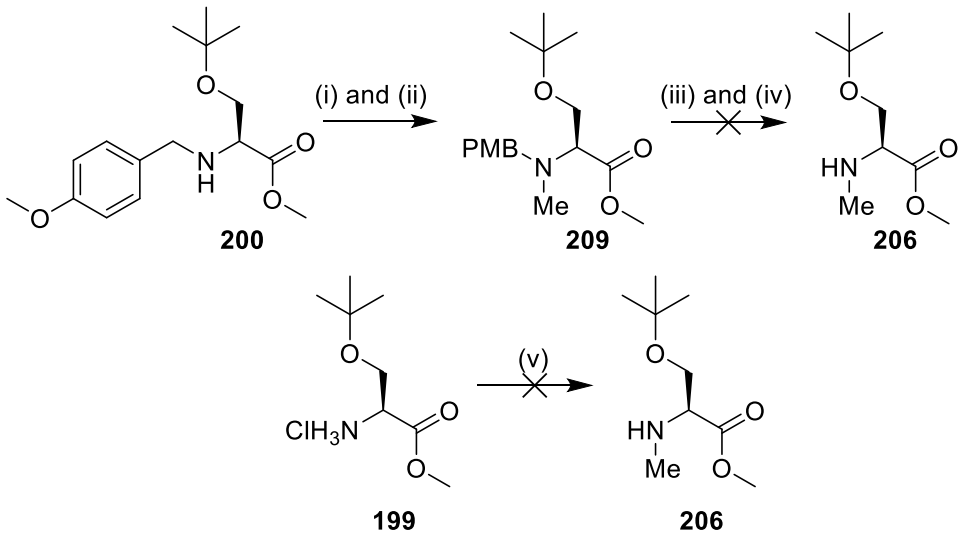


Scheme 41: A planned synthetic scheme to investigate a possible chiral relay effect

2.3.4.2.1.2 Attempts to synthesize an N-Me derivative

Our N-protected serine derivative **206** was methylated using potassium carbonate and MeI achieving the product in fair yield. Basic conditions could potentially racemize the amino acid therefore analysis of the ee was attempted using HPLC with a chiral stationary phase. Unfortunately, we were unable to achieve separation of enantiomers in a synthesized racemate. We therefore decided it would be safer to find a non-basic method of methylation. Using a modified procedure from Konopelski *et al.,*[109] formaldehyde was used to form an

imine, which underwent reduction with sodium cyanoborohydride. Once again, the tertiary amine **209** was isolated in good yield; furthermore after work up no further purification was necessary. Attempts were then made to cleave the PMB group leaving the methylated serine derivative (**Scheme 42**).



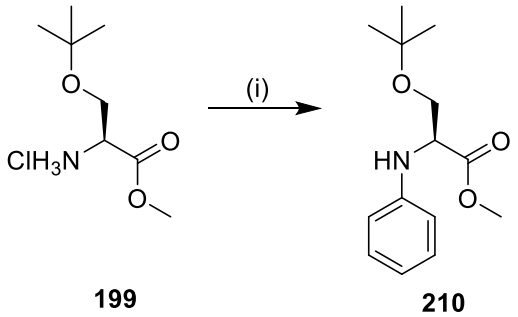
Reagents and conditions: (i) MeI, K_2CO_3, DMF, rt, 1 h, 62%; (ii) *para*-Formaldehyde (14 equiv) in MeOH, after 5 h $NaBH_3CN$ (2.2 equiv), rt, 16 h, 74%; (iii) CAN (5.3 equiv), 3:1 ($MeCN/H_2O$), 2 h; (iv) Pd/C, H_2, MeOH, 16 h; (v) paraformaldehyde (14 equiv), MeOH, $NaBH_3CN$, 2.2 equiv, rt, 16 h.

Scheme 42: Attempts to form the N-methyl serine derivative **206**

Our previous PMB removals had been effective with CAN; unfortunately, however, only traces of material could be recovered. Although the NMR spectrum showed promising signals, the material was heavily contaminated. We felt the problem could lie in the work-up procedure due to the basicity or solubility of the product, and we therefore modified the procedure to remove the PMB group with hydrogenolysis conditions, this would only require filtration and evaporation. After subjecting **209** to standard benzyl removal conditions however, we were still unable to obtain any of the desired product. Whether this is because the product is highly volatile or undergoes decomposition is unknown. Reductive amination of the primary amine **199** also failed to produce the methylated derivative. With the methylated derivative proving surprisingly difficult, we decided to exchange the methyl group for a phenyl. This would have a similar effect acting as a rigid group, but we hoped would be more stable allowing for easier purification.

2.3.4.2.1.3 Synthesis of the N-phenylated serine derivative

There are two common methods for the formation of N-Ar bonds. The Buchwald[110]-Hartwig[111] method is perhaps the most utilized, but along with potentially expensive ligands, a strong base is often required. Due to the possibility of racemization, we decided to avoid this and instead use a Chan-Lam coupling (**Scheme 43**).[112]



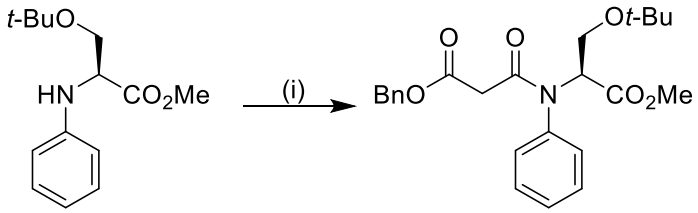
199 **210**

Reagents and conditions: (i) $PhB(OH)_2$ (2 equiv), Et_3N (2 equiv), $Cu(OAc)_2$ (1.1 equiv), O_2 balloon, DCM, rt, 3 days, 22%.

Scheme 43: The Chan-Lam coupling to produce **210**

Coupling the amine hydrochloride salt **199** to benzyl boronic acid produced **210** in poor yield (**Scheme 43**). As only a small amount of material was needed, however, no further optimization was attempted. Due to the weakly basic conditions used we decided to check for racemization using HPLC with a chiral stationary phase in comparison to a separately synthesized racemate. We found that some racemization had occurred at this point to 72% ee, but fortunately however, the product could be recrystalized from petroleum ether providing crystals of 95% ee and a racemic supernatant.

2.3.4.2.1.4 Coupling of 210 to 146 and its cyclization/alkylation

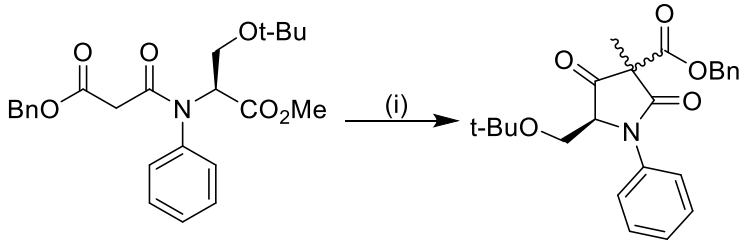


210 **211**

Reagents and conditions: **146** (2.2 equiv), EDAC.HCl (2.7 equiv), N-MM (2.3 equiv), DMAP (0.19 equiv), DCM, 16 h, rt, 63%.

Scheme 44: Peptide coupling to form compound **211**

Coupling of **146** to the phenylated serine derivative **211** provided the Dieckmann cyclization precursor in fair yield (**Scheme 44**). The cyclization precursor this time did not appear as a set of rotamers in the ^{1}H NMR spectrum, presumably due to the increase in rigidity we had introduced in the system compared to the PMB analogues. Analysis of **210** against a synthesized racemate HPLC using a chiral stationary phase showed that no further racemization had occurred at this point. The phenyl derivative **211** was treated under similar conditions to **201** in order to induce the Dieckmann cyclization/alkylation (**Scheme 45**).



211 **212**

Reagents and conditions: TBAF (2.1 equiv), Ether, 5 m, THF, MeI (4.7 equiv), -12 °C, 64 h, 8%.

Scheme 45: The Dieckmann cyclization of **211**

Analysis of the ^{1}H NMR spectrum of the crude material showed that poor levels of alkylation were observed. There did appear to be a set of diastereoisomers, however, which were observed in a 1:5 ratio. Purification by column chromatography allowed isolation of the major diastereoisomer as a colourless crystalline solid. Analysis by HPLC using a chiral stationary phase in comparison with a synthesized racemate showed that minor amounts of

epimerization had occurred during the cyclization, providing **212a** with an ee of 87%. NOESY experiments were inconclusive as to which diastereoisomer this was, fortunately a crystal was able to be obtained of **212a** suitable for analysis by X-ray diffraction (**Figure 8**).

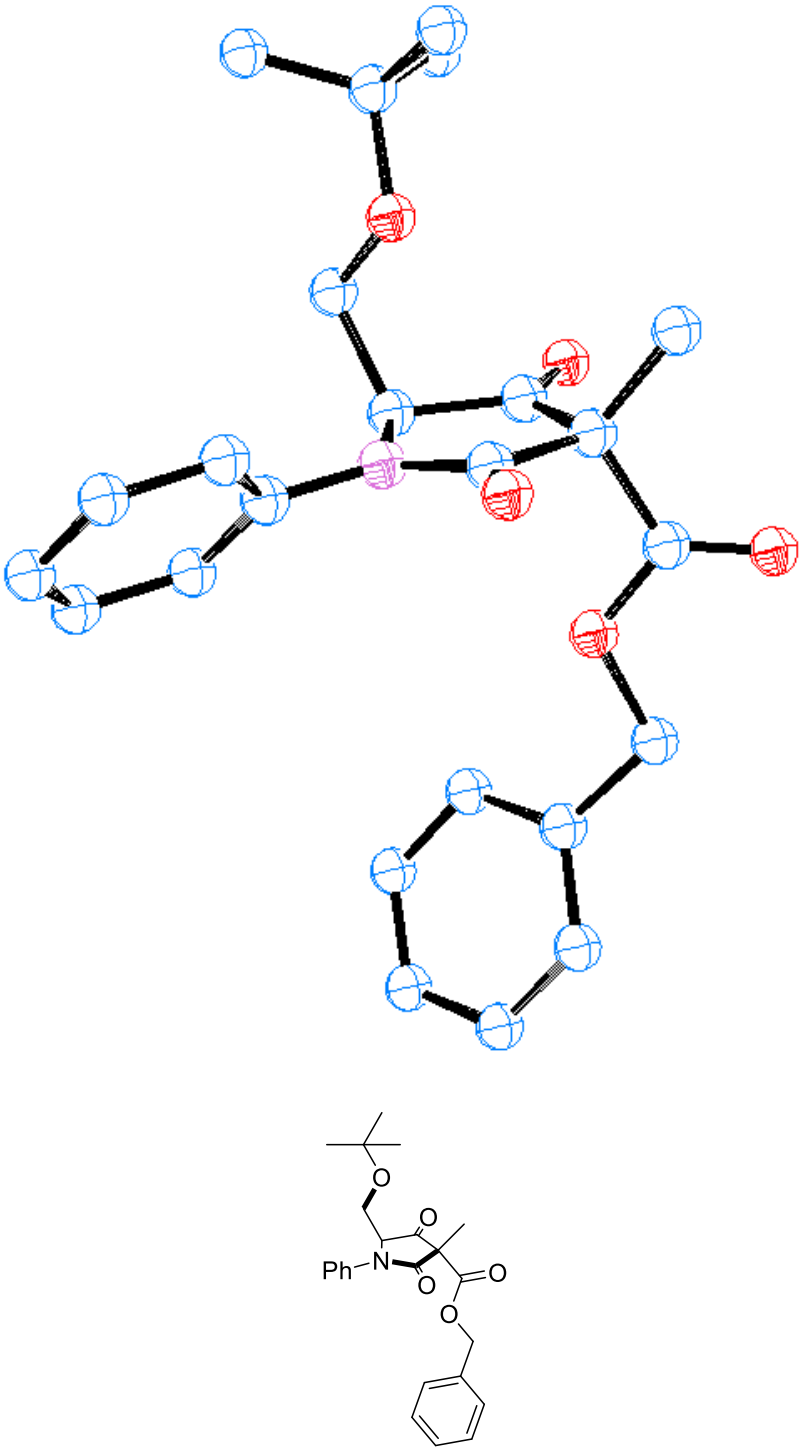


Figure 8: The X-ray structure of **212a** (hydrogens omitted for clarity)

To our surprise, however, we found that once again the major product was the diastereoisomer with the methyl and *tert*-butoxy group *cis*. This showed that the PMB group was not causing a chiral relay as we had expected. Furthermore, the diastereoisomeric ratio had even increased.

2.3.4.2.1.5 Investigation into the effect of the benzyl ester

Our observations suggested that the PMB group was not the primary reason for the observed diastereoselectivity. A chiral relay effect could still be the cause, but instead of the PMB group rotating away from the *tert*-butoxy group, it could be the benzyl ester. This could then hinder attack of the methyl iodide at the face opposite the *tert*-butoxy group accounting for the unexpected diastereoselectivity (**Figure 9**).

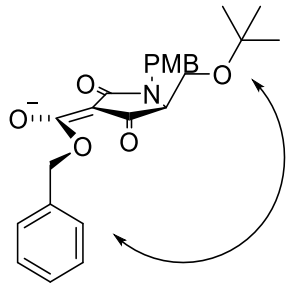
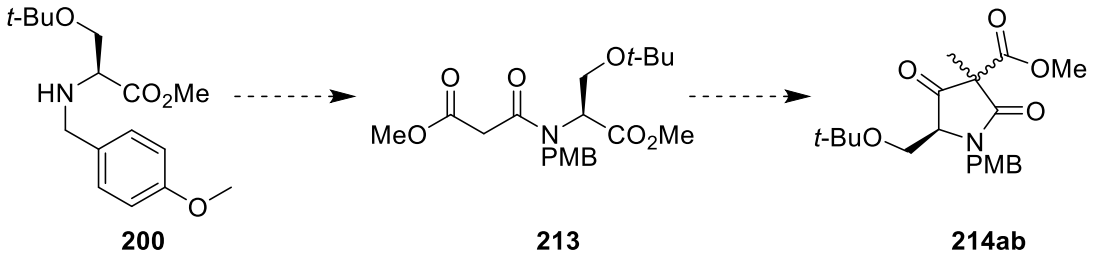


Figure 9: A conformation of the benzyl ester which could hinder attack of the methyl iodide on the face opposite the *tert*-butyl group

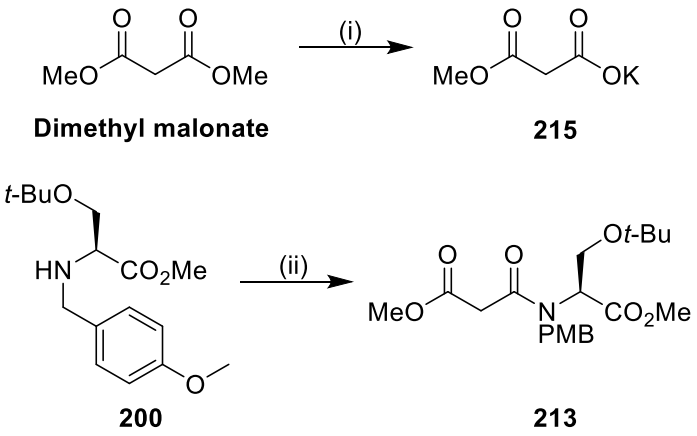
In order to test this, we decided to once again to replace the potentially rotatable group due to the methylene with a rigid group. We chose a methyl group due to this being the smallest possible group to limit other potential steric effects (**Scheme 46**).



Scheme 46: Planned route for investigation of the effect of the benzyl ester on diastereoselectivity

2.3.4.2.1.6 Synthesis of a methyl ester Dieckmann cyclization precursor

The mono potassium salt **215** of dimalonic methyl ester was formed through a saponification with potassium hydroxide (**Scheme 47**).



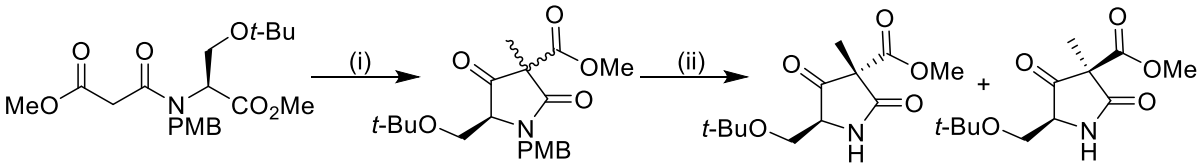
Reagents and conditions: (i) KOH (1.2 equiv), MeOH, rt, 51%; (ii) **215** (2.4 equiv), EDAC.HCl (2.9 equiv), DMAP (0.18 equiv), N-MM (2.4 equiv), rt, 24 h, 73%.

Scheme 47: Synthesis of the half malonic methyl ester salt and its coupling to amine **200**

Using once again EDAC.HCl to couple the salt to the secondary amine, the Dieckmann cyclization precursor **213** was produced, appearing on ^{1}H NMR spectroscopy as a set of rotamers. Unfortunately, we were unable to check for possible epimerization as we were unable to separate the enantiomers of a synthesized racemate of **213** by HPLC using a chiral stationary phase. However, because we had previously not observed racemization during the coupling, we assumed that **213** was unlikely to have lost any enantiopurity and remained at 97% ee.

2.3.4.2.1.7 The Dieckmann cyclization and identification of products

Treatment of Dieckmann precursor **213** under our optimized conditions provided a 3:1 mixture of diastereoisomers as observed in ^{1}H NMR spectroscopic analysis of the crude material (**Scheme 48**).



213 **214ab** **216a** **216b**

Reagents and conditions: (i) TBAF (1 M in THF), ether, 5 m, then MeI, THF, 58 h, -12 °C, 87% 3:1 mixture of diastereoisomers (by crude ^{1}H NMR analysis); (ii) CAN (5 equiv), 3:1 ($MeCN/H_2O$), 1.5h, 57%.

Scheme 48: The cyclization of **213** and removal of the PMB groups

We found, however, that upon column chromatography the diastereoisomers **214ab** could degrade into a complicated mixture of products. Furthermore the diastereoisomers were obtained as an inseparable oil from which no NOESY correlations could be used to assign relative stereochemistry. We found that acceptable levels of purification could be achieved by pouring the crude reaction mixture onto a silica gel column and rapidly washing through with ethyl acetate until the product stopped eluting. This technique provided the inseparable mix of diastereoisomers in acceptable levels of purity. Removal of the PMB group with CAN seemed to increase stability, allowing for the major isomer **216a** to be partially separated from the minor isomer **216b**. The major diastereoisomer was isolated as a solid from which a crystal could be obtained, allowing for structure determination through X-ray diffraction (**Figure 10**).

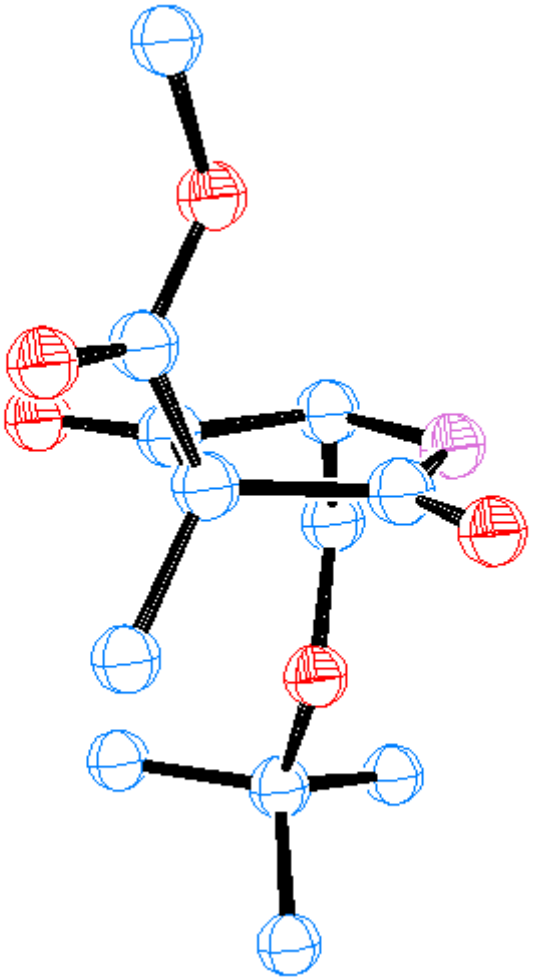


Figure 10: X-ray crystal structure of **216a**

Once again, the methyl group was introduced at the face opposite the *tert*-butoxy. It appeared therefore that rotation around the methylene carbon of the benzyl ester was also not a cause of the unusual diastereoselectivity. However, the increased yield of our cyclization step does indicate that the benzyl ester hinders attack to an extent.

2.3.4.2.2 Pyramidalization of the amide

Our original stereochemical reasoning, where methyl iodide would add opposite the bulky *tert*-butoxy group was based on the assumption that the gamma lactam intermediate (**figure 11**) would be planar. This seemed plausible due to the sp^2 hybridized nature of the C6 ketone

and C8 carbonyl groups inducing planarity in the nitrogen in the form of an amide. Furthermore, once the C7 position was deprotonated to the enolate, four out of the five atoms of the lactam ring would be planar.

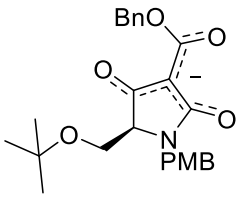
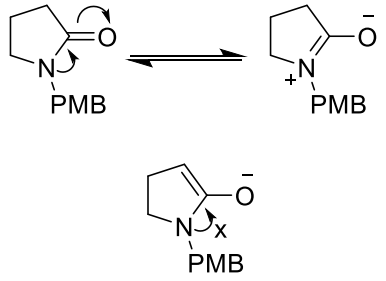


Figure 11: The pre-alkylated cyclization intermediate

However, on further contemplation we felt it was possible that this would not always be the case. Amides are planar due to the resonance of the nitrogen lone pair with the carbonyl, forming the imidic acid form (or lactim in a lactam). Once C7 deprotonation had occurred however, the amide would be partially in the enolate form, meaning that the nitrogen lone pair is less available to be involved in resonance (**scheme 49**). The result of this could be that the nitrogen becomes partially pyramidal. This has been observed computationally and in crystal structures of amide enol ethers.[113]



Scheme 49: Restricted resonance in amide enolates

The increase in flexibility in the lactam ring could allow the PMB group on the nitrogen to orientate away from the *tert*-butoxy group to release steric strain, again, hindering the face opposite the *tert*-butoxy group (**figure 12**). This could explain not only the outcome for the major diastereoisomer but also the increase in diastereoselectivity when the PMB group was replaced with a phenyl.

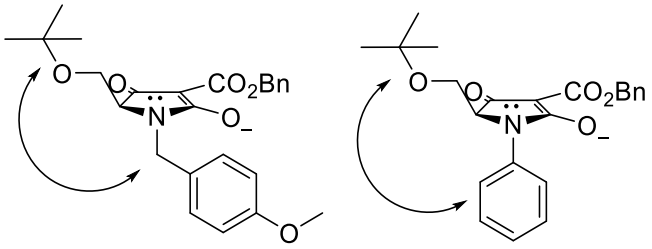


Figure 12: Potential conformations of the pre-alkylated cyclized intermediate from **201** and **211** showing a pyramidalized amide

Similar systems have been studied both computationally and experimentally.[114,115] Calculations performed by Mayers *et al.* on simple pyrrolidone enolates have shown that pyramidalization of the nitrogen does occur in enolate formation, and that 1,2 interaction from the methyl substituent favours the second conformation shown in **Figure 13**, directing the N substituent away from the methyl group. Although Mayers used this to rationalize the unusually high *trans* diastereoselectivity observed in enolate alkylation, it is possible that the increased size of our nitrogen substituent could overpower the electronic effect observed by Mayers.

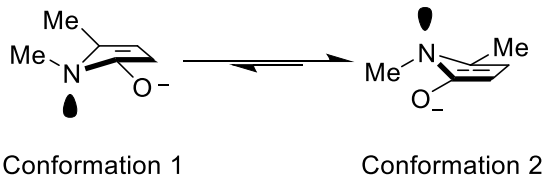
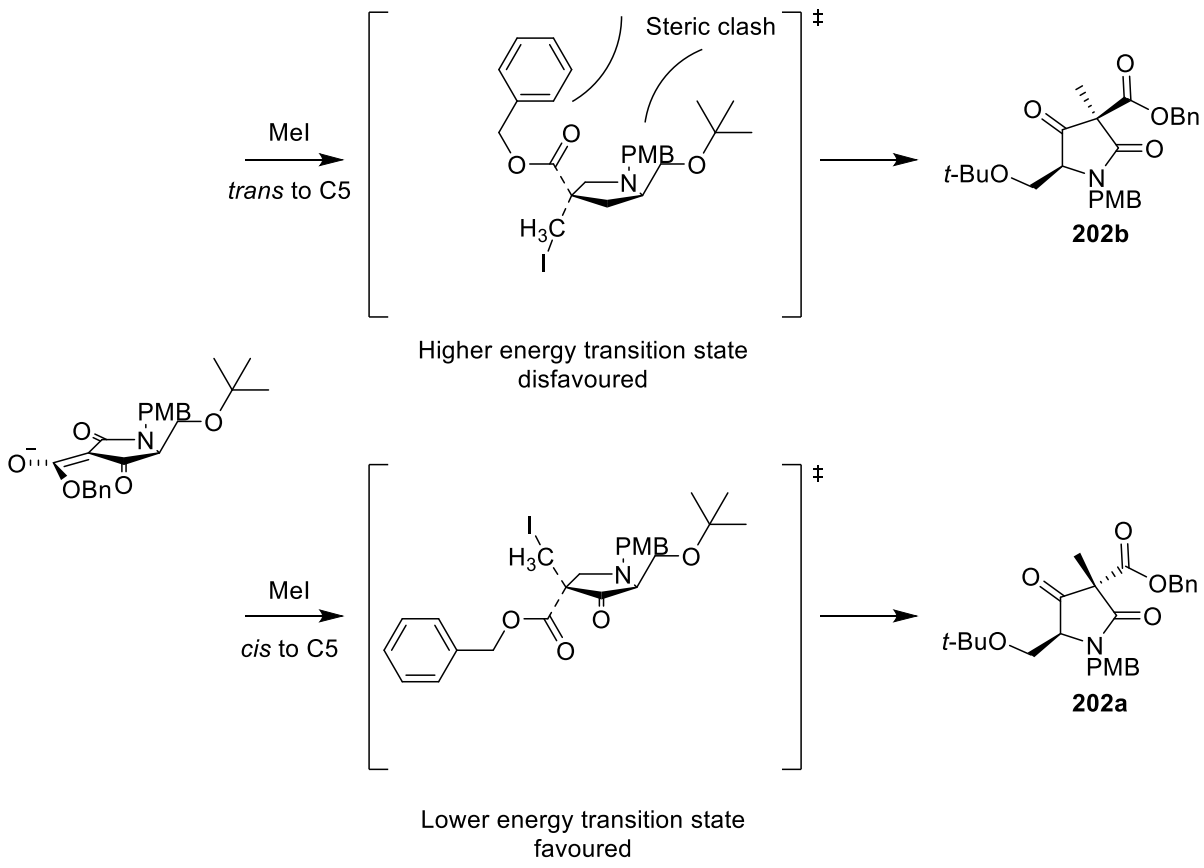


Figure 13: Favourable enolate conformations in a simple substituted pyrrolidone

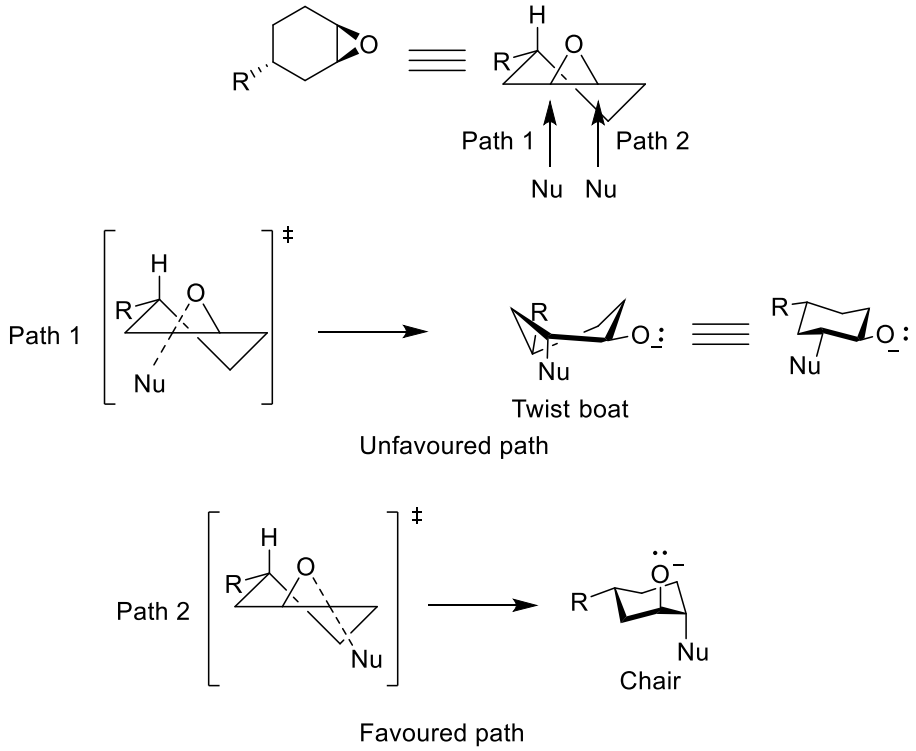
2.3.4.2.3 Diastereoselectivity reasoning due to transition state

Another potential cause could be based on the transition states in the approach of the methyl iodide from the top or bottom face. An approach from the bottom face could potentially form a higher energy transition state compared to a top face approach due to an unfavourable clash between the *tert*-butyl group and the benzyl ester (**Scheme 50**).



Scheme 50: Higher energy transition states affecting diastereoselectivity

Similar phenomena avoiding high energy transition states are well known, for instance in the Fürst-Plattner rule (or trans-diaxial effect) (**Scheme 51**).[116]

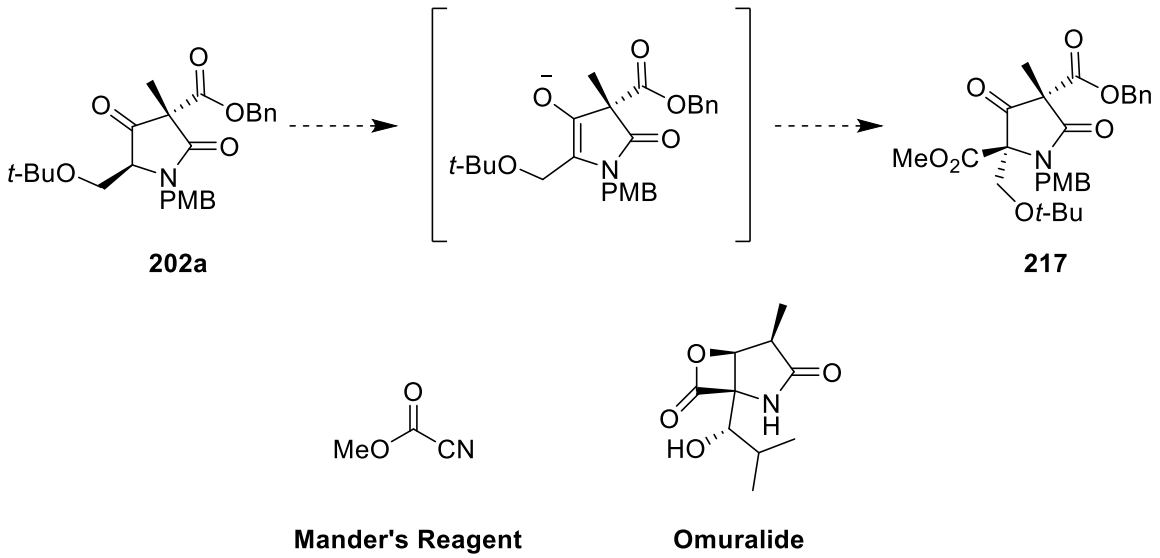


Scheme 51: The Fürst Plattner effect

The nucleophile could attack either the C3 or C4 position. However, attack at position 3 shown by path 1 would mean that the transition state would adopt an unfavourable twist boat conformation. Path 2 is therefore dominant.

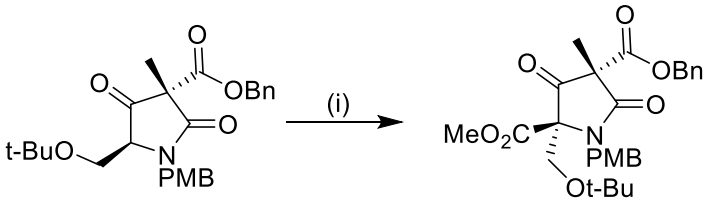
2.3.4.2.4 Summary of the investigation into observed diastereoselectivity

Although we were not able to find a single reason for the unusual diastereoselectivity observed, we believe there are several possible plausible explanations and have been able to exclude some of the more well-known reasons. Fortunately, however, this unplanned stereoselectivity works as an advantage in our synthesis. Whereas in our leucine route we synthesized the opposite enantiomer of the analogue with respect to omuralide, the benzyl ester should now direct acylation with Mander's reagent to the face opposite, providing the correct stereochemistry (**Scheme 52**).



Scheme 52: Prediction of absolute stereochemistry after acylation with Mander's reagent

2.3.5 Acylation with Mander's reagent



202a **217**

Reagents and conditions: LiHMDS (2.2 equiv), DMPU (3 equiv), THF.

Scheme 53: Acylation of **202a** with Mander's reagent

Entry	Equiv of Methyl cyanoformate	Time h	Temperature °C	Yield %
1	3	4	-78	1.7:1 Sm:P
2	5	4	-78	1.7:1 Sm:P
3	5	2	-78	1.5:1 Sm:P
4	5	4	-40	54%
5	4.5	3	-40	49%
6	4.5	3	-40	69% (new work up)

Table 6: Optimization of the acylation of **202a** with Mander's reagent

With the lactam core formed, the next step was to insert the methyl ester moiety, we hoped using the benzyl ester to direct the stereochemical course of the reaction (**Scheme 53**). Treatment of lactam **202a** under analogous conditions to the leucine route provided a complex mixture of products. Analysis of the ^{1}H NMR spectrum of the crude material showed the presence of both diastereoisomers **202a** and **202b** from the previous step along with a new compound that we assumed to be the desired acylated lactam **217,** in an approximately 1.7:1 mixture of starting material to product. The observation of an approximately equal quantity of the two diastereoisomers despite only starting with one suggested that the lithium bis(trimethylsilyl)amide (**LiHMDS**) was successfully forming the enolate, the problem appeared to be with enolate attack at the methyl cyanoformate. Attempted purification of crude material was not able to separate the product from the starting material. Increase of

the equivalents of methyl cyanoformate from three to five (**Table 6**, **entry 2**) provided a cleaner crude ^{1}H NMR spectrum of the product mixture, but, the ratio of starting material to product was still approximately the same (1.7:1). Retaining the increased number of equivalents and reducing the reaction time also led to a cleaner crude ^{1}H NMR spectrum and slightly increased the ratio of product (now at 1.5:1). Although still no pure material could be recovered, this indicated that the product could be decomposing with longer reaction times (**Entry 3**).

Running the reaction at increased temperatures (-40 °C) (**entry 4**) showed complete consumption of starting material, allowing the product to be purified and characterized in moderate yield. Further optimization of the reaction and modification of the workup procedure by removing an evaporation of the quenched reaction mixture led to good yields of **217** (69%) (**entry 6**). The ee of the material was measured at this stage against a synthesized racemic compound by HPLC using a chiral stationary phase. We found that a slight drop in enantiopurity had occurred during the acylation. We do not believe this is due to racemization of the product but rather impurities of the cyclization diastereoisomer **202b** in our starting material. When using the enriched recrystallized material from the cyclization, an ee of 84% was obtained (from 98% ee). Using the non-recrystallized material from the cyclization, the ee was 69% (from 79% ee). The product was isolated as a crystalline solid, and therefore we attempted to obtain a crystal suitable for X-ray analysis. Even with enantio-enriched material, racemic crystals would be preferentially produced over enantiopure crystals. The material could therefore be recrystallized from IPA to remove racemic material leading to the supernatant containing material of up to 93% ee. The recovery from this was, however, poor, but it did allow us to obtain an enantiopure crystal for X-ray analysis.

Racemic crystals are sometimes more stable and can have an increased density over their enantiopure counterparts (Wallach's rule).[117] Our crystals follow this observation due to the preferential formation of racemic crystals, and analysis of both crystal structures show that the racemate volume is about 3% smaller than that of the enantiopure crystal. This phenomenon was found not to be due to any specific interaction between the enantiomers of molecules but rather due the increased number of space groups available to racemic molecules.[118]

Our enantiopure crystal (**Figure 14**) confirmed our findings that the methyl group adds to the same face as the *tert*-butoxy group. The methyl ester once again adds opposite the benzyl ester, providing the C5 stereochemistry as that required for the natural enantiomer of omuralide.

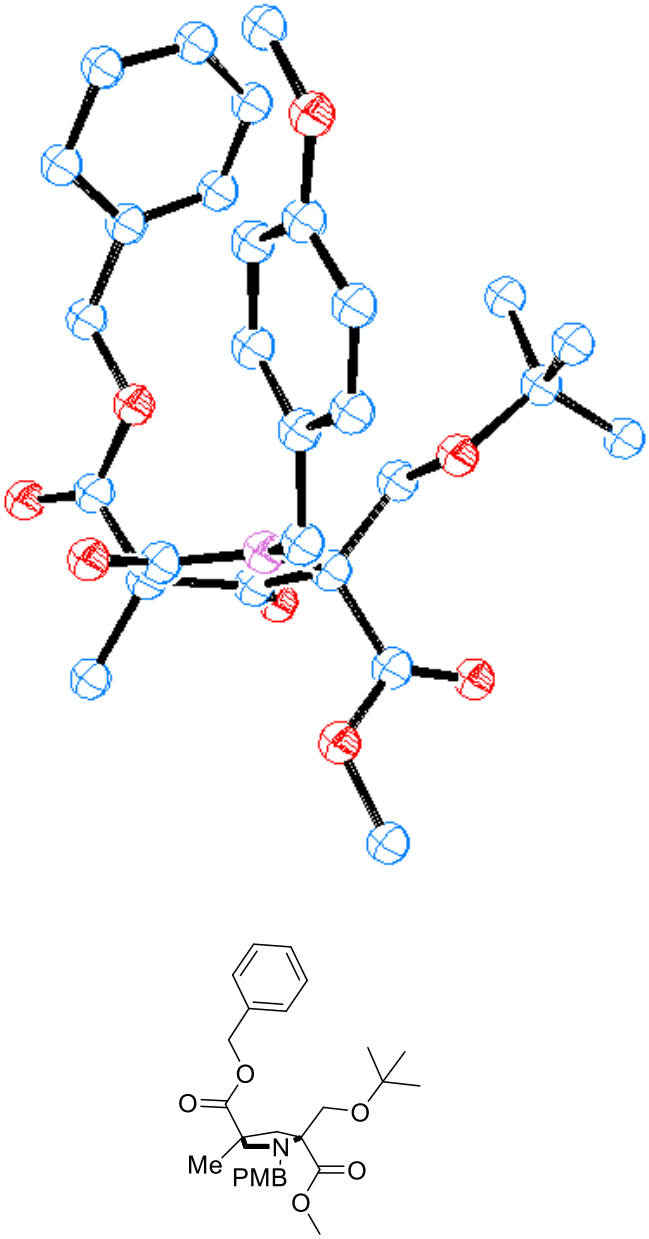
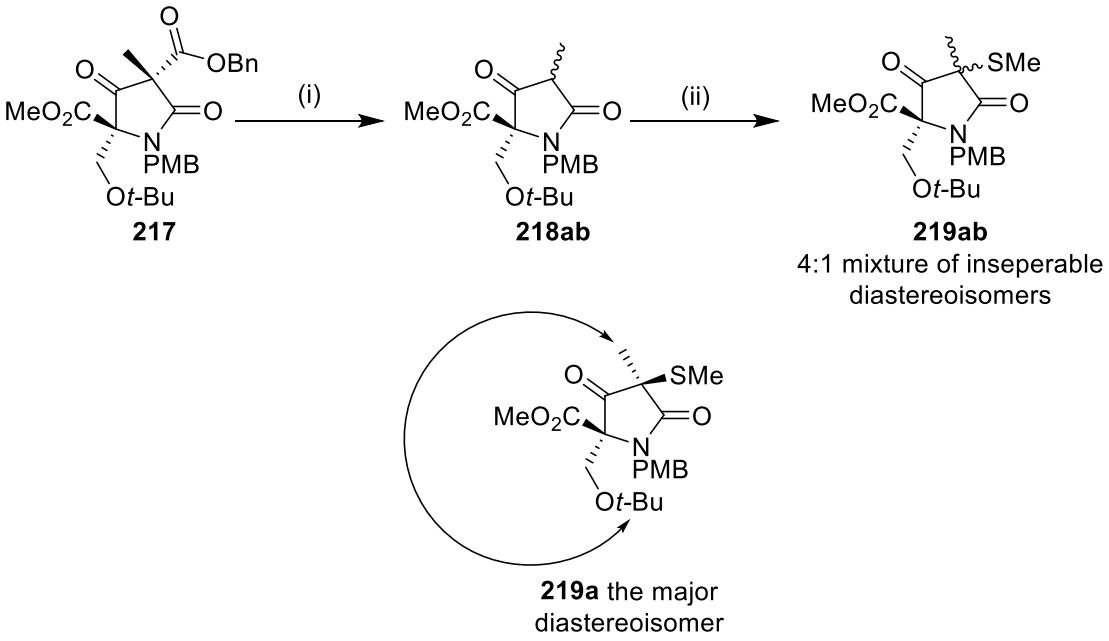


Figure 14: X-ray crystal structure of **217** hydrogen atoms removed for clarity (carbonyls removed for clarity in chemdraw structure)

2.3.6 Hydrogenolysis and thiomethylation of 217

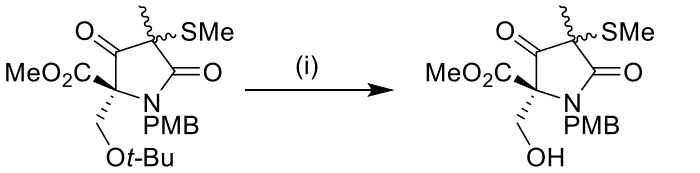
The acylated product **217** was hydrogenolysed in an identical way to the leucine derivative to remove the benzyl ester. Once again, the product was isolated as a mixture of isomers **218ab** and therefore subjected to the thiomethylation conditions optimized in the leucine route without purification (**Scheme 54**). The inseparable mixture of diastereoisomers **219ab** were isolated in a 4:1 mixture. Fortunately, the bulkier residue on the amino acid directed the thiomethylation far more effectively than in our leucine derivative. NOESY analysis provided evidence that the major diastereoisomer was the one required to elaborate to Corey's intermediate **29a**.



Reagents and conditions: (i) 1) $Pd(OH)_2/C$ (cat), H_2, 35 °C, 16 h; ii) **186** (1.7 equiv), Et_3N (1.1 equiv), DCM, 4 h (70% over 2 steps).

Scheme 54: Benzyl ester removal and thiomethylation of **219ab**

2.3.7 Removal of the *tert*-butyl protecting group from compound 219ab



219ab 4:1
Mixture of inseperable
diastereoisomers

29ab 2:1
Mixture of inseperable
diastereoisomers

Reagents and conditions: TFA/DCM, 1:1, 1.5h, 76%.

Scheme 55: Removal of the *tert*-butyl group

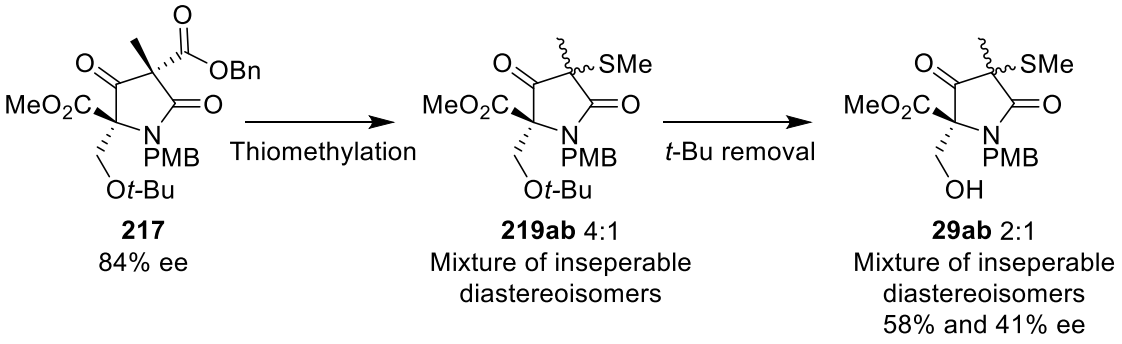
The mixture of diastereoisomers was treated with $ZnBr_2$, a methodology reported by Wu *et al.,*[119] chosen due to an example given of *tert*-butyl-*O*-threonine being deprotected. Also attempted was a method reported by Li *et al.*[120] using 85% phosphoric acid. This method had also been successful for a deprotection of threonine, and other examples where methyl esters were unchanged. Unfortunately, both methods resulted in a complex mixture of products for our compound **219ab**. Treatment of the mixture of diastereoisomers **219ab** with a 1:1 mixture of TFA:DCM however resulted in the desired Corey intermediate and diastereoisomers **29ab** in excellent yield (**Scheme 55**). To our surprise, the product was isolated after column chromatography in a 2:1 mixture of diastereoisomers rather than the original 4:1 ratio. Interestingly, the crude material before column chromatography appeared to still be in a 4:1 ratio.

2.3.8 Epimerization of the Corey intermediate 29ab

2.3.8.1 Introduction to the investigation of the epimerization of 29ab

The most likely reasons for the drop in diastereoisomeric ratio seemed to be either one diastereoisomer reacting faster than the other or, one diastereoisomer undergoing decomposition faster than the other. An alternative possibility is one or both diastereoisomers epimerizing, but this seemed unlikely as both chiral centres were quaternary and no obvious mechanism for epimerization could be postulated. It is perhaps important to note that despite Corey and Pattenden synthesizing the same compound, neither mentioned observing a similar result. Analysis of the 2:1 mixture in comparison with a synthesized racemate however showed partial racemization had occurred, the ee dropping

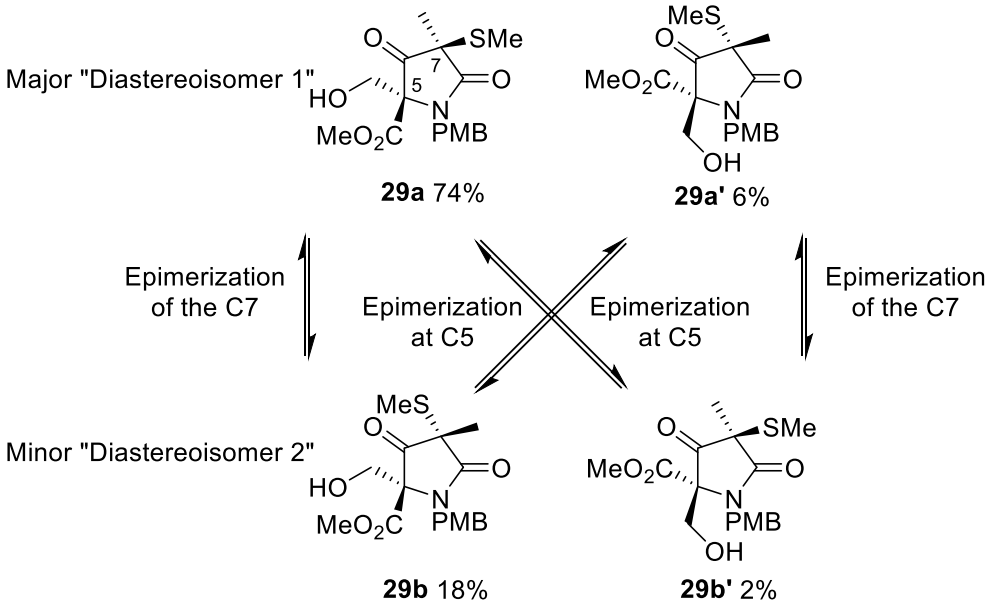
from 84% to 58% (major) and 41% (minor) for the two diastereoisomers **29ab** respectively. This suggested that an epimerization of one of the chiral centres was the reason behind the change in diastereoisomer ratio (**Scheme 56**).



Scheme 56: Summary of the drop in ee

2.3.8.2 Logical identification of the epimerizing chiral centre

An epimerization as a mix of diastereoisomers would not necessarily result in a drop in ee. It would depend on which of the chiral centres was epimerizing (**Scheme 57**). This can be seen more clearly in the following scheme. Based on the 4:1 ratio of diastereoisomers and the original ee of 84%, each isomer has been calculated a % abundance in the diastereoisomeric mixture.



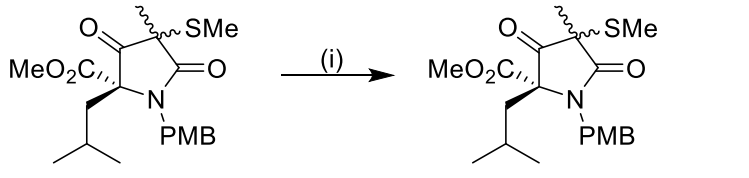
Scheme 57: The result of a C5 or C7 epimerization

If epimerization were occurring at C7, the diastereoisomeric ratio would change, but ees would not change. Both enantiomers would epimerize at the same rate, therefore the ratio of enantiomers would stay constant in each mixture, i.e. the minor enantiomer of diastereoisomer 1 **29a'** would mix with the minor enantiomer of diastereoisomer 2 **29b'** and the major enantiomer of diastereoisomer 1 **29a** would mix with the major enantiomer of diastereoisomer 2 **29b** the ee would therefore remain unchanged. If C5 were undergoing epimerization, however, the minor enantiomer of diastereoisomer 1 **29a'** would mix with the major enantiomer of diastereoisomer 2 **29b** and the major enantiomer of diastereoisomer 1 **29a** would mix with the minor enantiomer of diastereoisomer 2 **29b'**. This would scramble the enantiopurity. Using this reasoning we theorised that the C5 centre was epimerizing.

2.3.8.3 Experimental identification and reasoning of the epimerizing centre

2.3.8.3.1 Epimerization studies on 172ab

Not only did we want to confirm which centre was epimerizing, but also which functional groups were required for the epimerization to occur, to help elucidate a mechanism. For evidence that the hydroxyl group was needed for the epimerization and not the thiomethyl we treated our thiomethylated leucine analogue **172ab** under identical conditions to our *tert*-butoxy deprotection (**scheme 58**) and purified the reaction mixture using column chromatography. Both the ratio of diastereoisomers and the starting material remained unchanged as expected. This gave us evidence that any mechanism would probably require the hydroxyl group.



172ab **172ab**

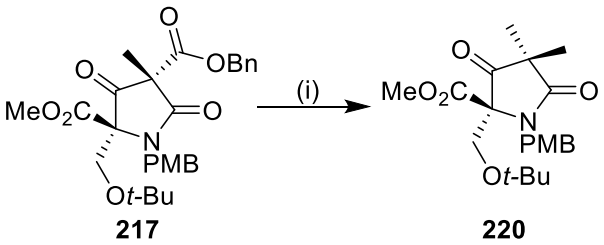
No change in diastereomer ratio

Reagents and conditions: (i) TFA:DCM (1:1), 4 h.

Scheme 58: Attempt to induce a similar epimerization in our leucine series

2.3.8.3.2 Synthesis of a *gem*-dimethylated analogue

For a final confirmation that the thiomethyl group is not required for the epimerization but the hydroxyl group is, a derivative was synthesized by replacing the thiomethylating reagent **186** with iodomethane. This produced the di-methylated analogue **220** in 46% yield over the 2 steps in 86% ee, as observed by HPLC using a chiral stationary phase (**Scheme 59**).

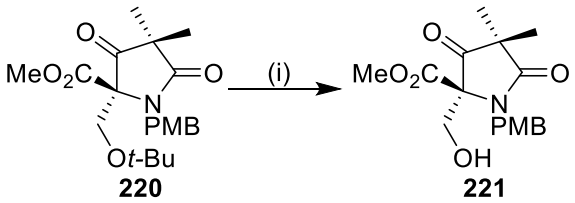


217 **220**

Reagents and conditions: (i) 1) $Pd(OH)_2/C$ (cat), H_2, 35 °C, 16 h 2) MeI (4 equiv), Et_3N (1.8 equiv), DCM, 4 h (46% over 2 steps).

Scheme 59: Synthesis of the *gem*-dimethylated analogue **220**

If **220** racemizes under the *tert*-butyl deprotection conditions, with only one chiral centre at C5, the epimerization at C7 can be ruled out completely, furthermore we can exclude the possibility of the thiomethyl group being involved with any possible mechanism.



220 **221**

Reagents and conditions: TFA:DCM, 1:1, 1.5h, 67%.

Scheme 60: TFA-mediated deprotection of **220**

As expected, treatment of **220** with the TFA:DCM mixture led to **220** in completely racemic form (**Scheme 60**) after purification of column chromatography. Our original experiment with the thiomethyl analogues **219ab** had shown that the crude material before column chromatography had retained the original 4:1 ratio, we therefore decided that the epimerization must not be occurring under the reaction conditions but, surprisingly, upon the purification. Further evidence of this was found when, instead of using a dry-load technique to purify **221** (dissolving the crude material in DCM, adding silica gel and evaporating,

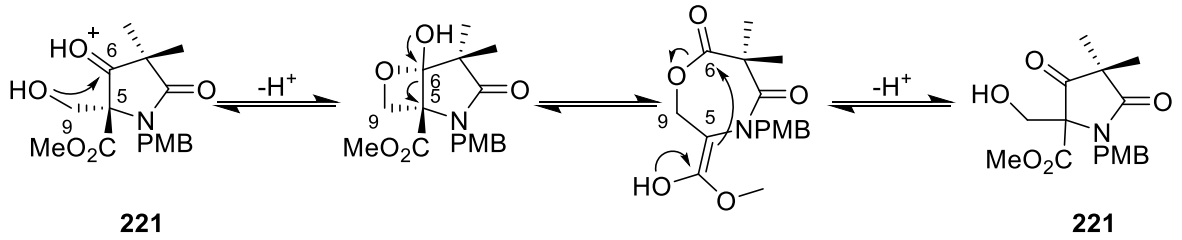
adsorbing the material onto silica gel), a wet technique was used (dissolving the crude material in the petroleum ether/ethyl acetate solvent system and applying the solution to the column). The wet technique resulted in no epimerization of the C5 centre, analysis on HPLC using a chiral stationary phase showing the ee from compound **221** to be retained.

With the cause, epimerizing centre, and functional groups involved known, two mechanisms were postulated.

2.3.8.3.3 Potential mechanism for C5 epimerization

2.3.8.3.3.1 Mechanism 1

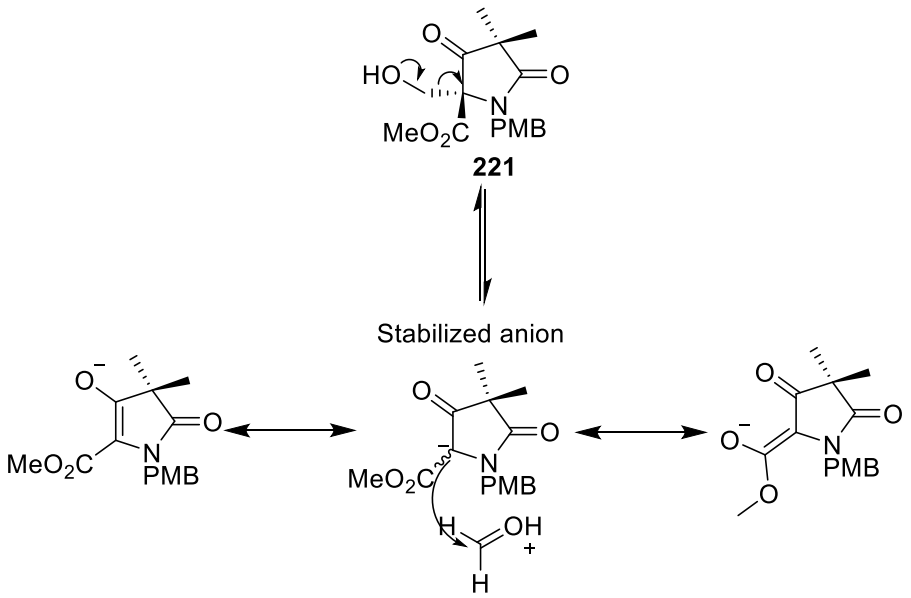
Mechanism one (**Scheme 61**) involves protonation of the C6 ketone, inducing nucleophilic attack from the now deprotected C9 hydroxyl. Reformation of the ketone could break the C6-C5 bond due to the stabilized nature of the anion through the ester, thus losing the chirality. The ester enolate could then attack the newly formed ester, reforming the original compound. Both ring-forming reactions would be plausible according to Baldwin rules,[121] the first being 4-exo-trig and the second 4-enolexo-exo-trig.



Scheme 61: 1st Postulated mechanism for C5 epimerization

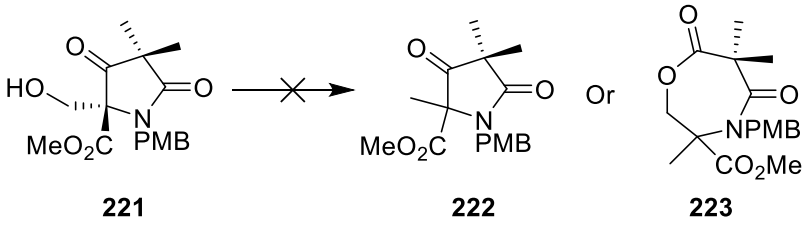
2.3.8.3.3.2 Mechanism 2

The second mechanism (**Scheme 62**) involves a retro-aldol type reaction. The removal of the hydroxyl group as a formaldehyde cation forms a highly stabilized anion, and thus the chirality is lost. Attack of the malonate back at the formaldehyde cation reforms the original compound.



Scheme 62: 2nd Postulated mechanism for C5 epimerization

It should be noted, however that the precise role of silica gel and why DCM would induce epimerization but not ethyl acetate/petrol is unknown. Both mechanisms postulated would seem equally likely under the strong acidic conditions of the TFA:DCM mixture, if not more so than under the relative weak acidic conditions of silica gel. However, it is possible the epimerization requires a Lewis acid to occur. Trapping experiments with iodomethane were carried out (**Scheme 63**) in an attempt to isolate a possible intermediate of the reaction, such as **222** or **223**, but unfortunately only starting material was observed.

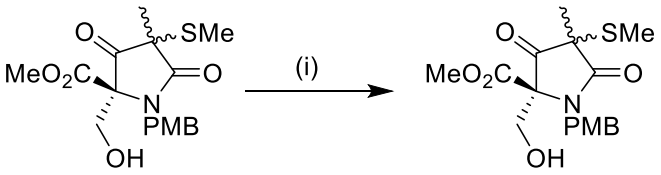


Reagents and conditions: MeI, DCM, Silica gel, rt, overnight

Scheme 63: Attempt at trapping an intermediate with MeI

2.3.8.3.4 NMR experiments of the mixture in the presence of silica

Returning to the original thiomethylated compounds, we sought further confirmation that silica gel was the cause of the epimerization. A ^{1}H NMR spectrum was taken of the crude deprotected **29ab** mixture as a control showing the 4:1 ratio of diastereoisomers. A microspatula of silica gel was added, and the NMR experiment ran immediately and after 16 h. The ^{1}H NMR spectrum after treatment with silica gel showed epimerization from 4:1 to 3:1.3 (<5 mins). Upon being left for 16 h compound continued epimerizing, reaching a 3:2 ratio. On further inspection, the 16 h experiment also contained a minor signal at δ = 9.73 ppm which was absent in the control and the initial addition of silica gel experiment. Although this by no means confirms the mechanism, this does provide evidence for our second mechanism as the signal is consistent with formaldehyde. Interestingly, Dixon *et al.*[122] and Massa *et al.*[123] both reported similar observations. Both groups observed decomposition of a range of β-hydroxy malonates during chromatographic separation. It is perhaps surprising therefore that we were able to isolate the product at all.



29ab 4:1
Mixture of inseparable
diastereoisomers

29ab 3:2
Mixture of inseparable
diastereoisomers

Reagents and conditions: (i) Silica gel, chloroform, 16 h.

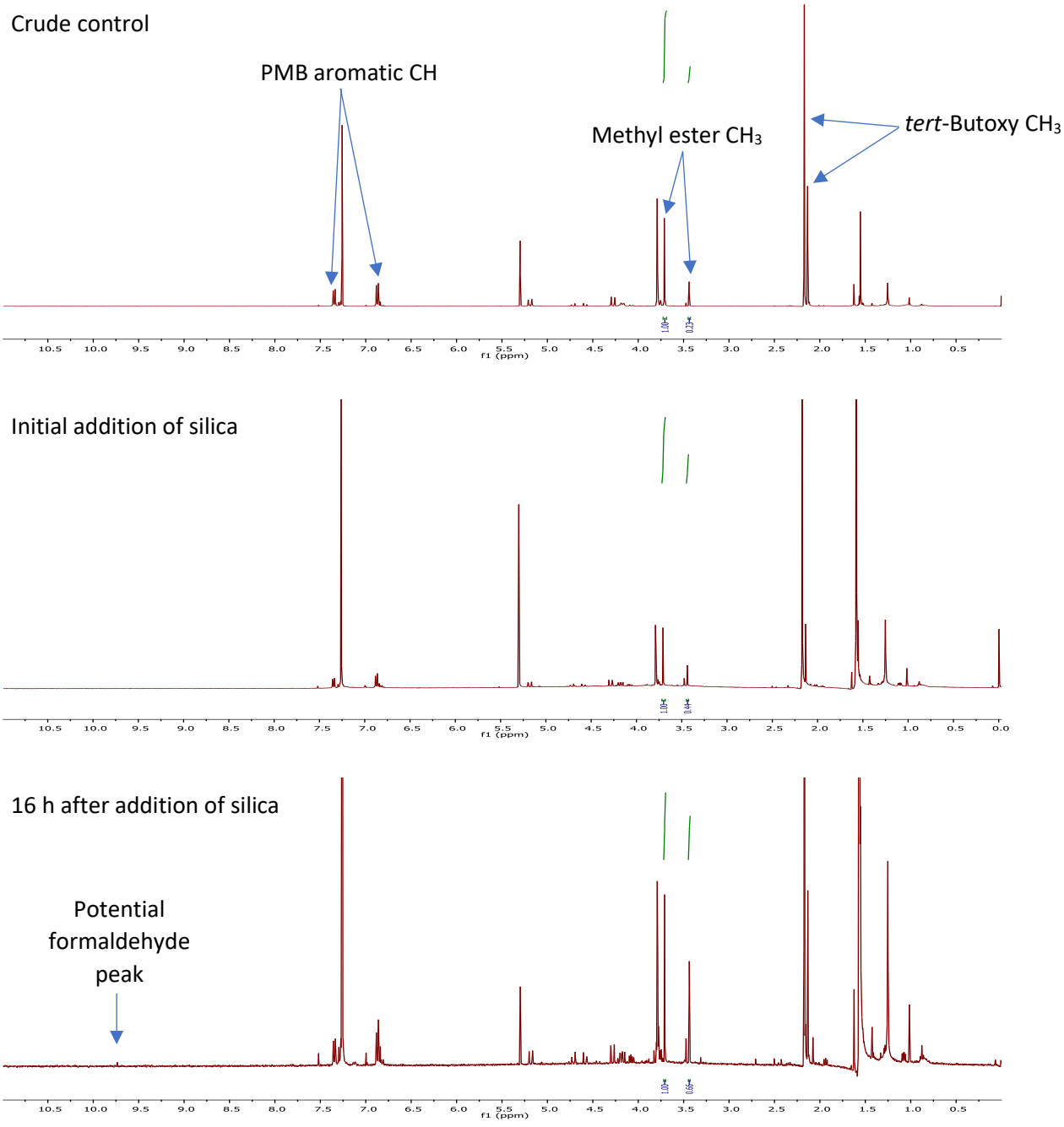


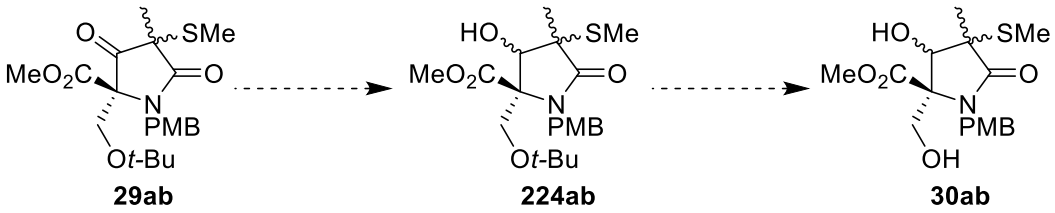
Figure 15: Inducing of the epimerization with silica and the analysis with NMR

2.3.9 Ketone reduction followed by *tert*-butyl deprotection strategy

2.3.9.1 Reduction of the C6 ketone

With the diastereoisomers of Corey's intermediate **29ab** proving inseparable, we decided to attempt an alternative route. Rather than removal of the *tert*-butyl group followed by reduction, we decided to reduce the mixture of diastereoisomers then use our *tert*-butyl removal procedure. Where the previous experiments had been carried out with the recrystallized material from the Dieckmann cyclization/alkylation (**202a**), the following procedures were carried out with the un-recrystalized material (69% ee) in the hope we could enrich the ee of this material only once at a later stage intermediate.

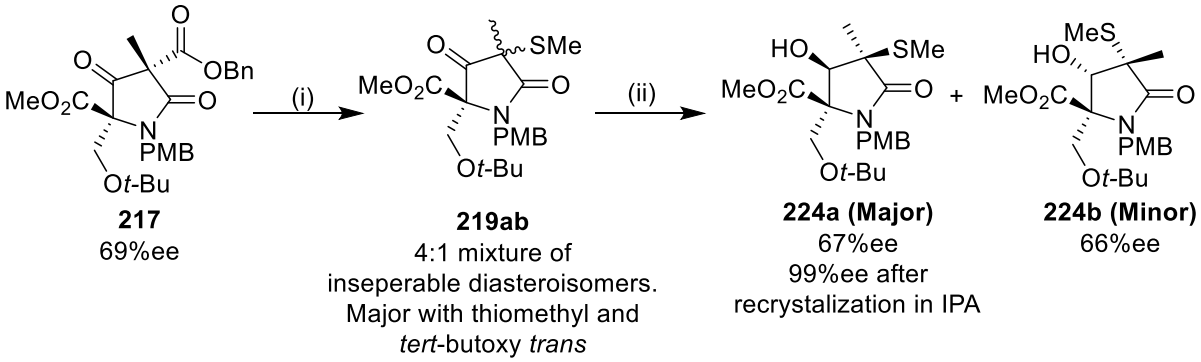
Following either postulated mechanism for the epimerization, reduction of the C6 centre before deprotection of the hydroxyl group should prevent the epimerization (**Scheme 64**).



Scheme 64: Rearrangement of the reduction and *tert*-butyl removal steps

Our primary concern with the new route was that the bulky *tert*-butoxy group would direct the reduction more than the thiomethyl group. This would decrease the yield of the desired diastereoisomer. Reduction with sodium borohydride produced two separable diastereoisomers **224a** and **224b** in an approximately 1:4 ratio along with a small amount of starting material. NOESY experiments were carried out for the minor diastereoisomer confirming our stereochemical predictions that the hydride would add preferentially to the face opposite the thiomethyl group. Unfortunately, due to overlapping ^{1}H NMR signals, the necessary interactions could not be seen for the major diastereoisomer. Analysis of both diastereoisomers on HPLC using a chiral stationary phase compared to a synthesized racemate showed a small drop in ee from 69 to 67% ee. We believe this to be due to minor quantities of the unwanted diastereoisomer being formed during the acylation with Mander's reagent.

The major diastereoisomer **224a** was isolated as a waxy oil (**Scheme 65**). To our delight, recrystallization from IPA produced a solid of 12% ee and a filtrate of 99% ee, as confirmed by analysis by HPLC using a chiral stationary phase compared to a synthesized racemate.

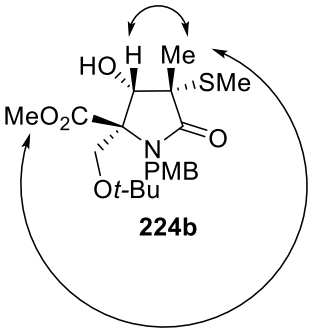


217
69%ee

219ab
4:1 mixture of inseperable diasteroisomers. Major with thiomethyl and *tert*-butoxy *trans*

224a (Major)
67%ee
99%ee after recrystalization in IPA

224b (Minor)
66%ee

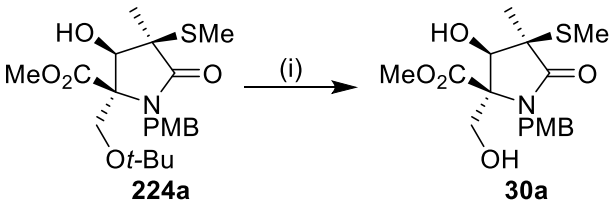


224b

Reagents and conditions: (i) 1) $Pd(OH)_2/C$ (cat), H_2, 35 °C, 16 h 2) **186**, Et_3N, DCM, 4 h (70% over 2 steps) ii) $NaBH_4$ (0.6 equiv), EtOH, 30 m 0 °C, **224a** 54%, **224b** 13%, **219ab** 4%.

Scheme 65: Thiomethylation and reduction of compound **217**

2.3.9.2 Deprotection of the *tert*-butyl group



224a **30a**

Reagents and conditions: TFA/DCM 1:1, 1.5h, 75%.

Scheme 66: Deprotection of **224a** to produce the Corey intermediate **30a**

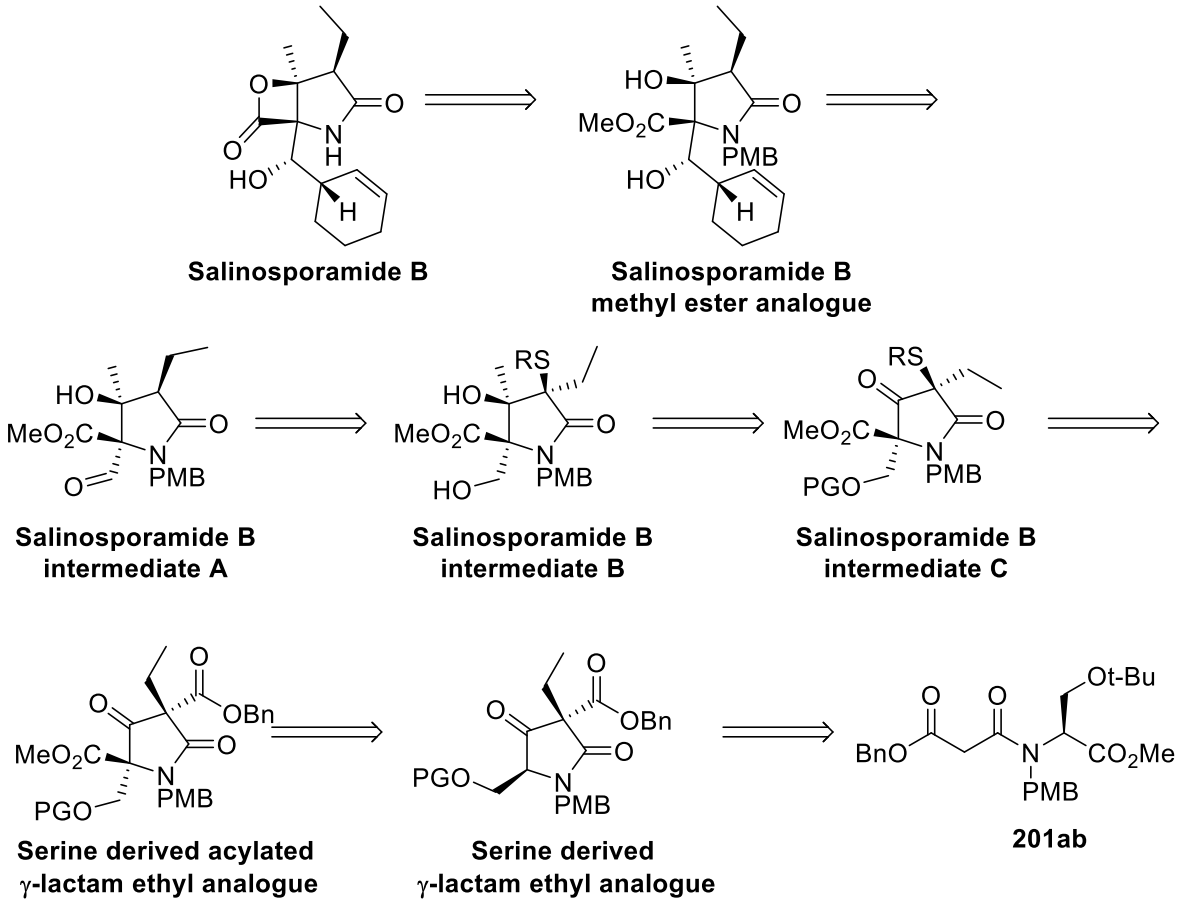
Treatment of **224a** under the same deprotection conditions as before provided the Corey intermediate **30a** in excellent yield (**Scheme 66**). The compound was purified with column chromatography by the usual dry load method which previously epimerized the compound,

however with C6 now reduced, no epimerization was observed, providing the Corey intermediate **30a** in 99% ee as confirmed by HPLC with a chiral stationary phase compared with a synthesized racemate. The intermediate synthesized is important in omuralide analogue synthesis and has been elaborated by Corey to a wide variety of C5 analogues.[37]

2.4 Towards the total synthesis of salinosporamide B

2.4.1 Strategy to salinosporamide B

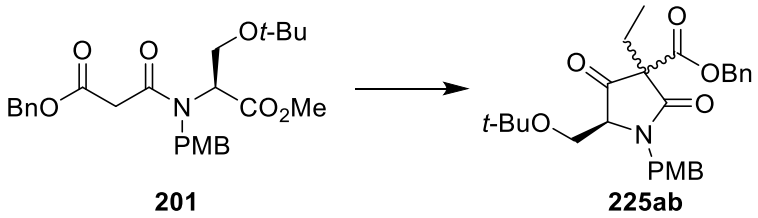
With a formal synthesis of omuralide completed and the successful incorporation of 2 amino acids into the lactam core using our methodology, we turned our attention to modification of the C7 carbon. Our aim was to incorporate longer carbon chains into the lactam core. Our target would be to incorporate an ethyl group into the C7 position and then elaborate the product to salinosporamide B.[42] Although salinosporamide A[41] is a more potent proteasome inhibitor, we decided to start on the simpler version of the salinosporamide family; there is currently no synthesis of salinosporamide B in the literature.



Scheme 67: Retro-synthetic analysis of salinosporamide B

Our serine-derived Dieckmann cyclization precursor **201ab** would be treated under similar conditions to our previous cyclization/alkylations to produce the **serine-derived γ-lactam ethyl analogue** (**Scheme 67**). An acylation using Mander's reagent would be used to incorporate the methyl ester forming the **acylated serine-derived γ-lactam ethyl analogue**. Removal of the benzyl ester would allow incorporation of the thioether blocking group to give **salinosporamide B intermediate C**, attempts would then be made to reduce the ketone diastereoselectively with methyl Grignard reagent, incorporating the C6 methyl group to produce **salinosporamide B intermediate B**. Diastereoselective desulphurization and oxidation of the C9 hydroxyl group forming **salinosporamide B intermediate A** could allow for incorporation of the cyclohexene ring using 2-cyclohexyenylzinc chloride, a strategy developed by Corey,[50] but also used successfully by Danishefsky,[124] Hatakeyama,[125] Pattenden[126,127] and Romo[128] in their respective syntheses. Removal of protecting groups and cyclization to the β-lactone could then form **salinosporamide B**.

2.4.2 The Dieckmann cyclization/alkylation using EtI



201 **225ab**

Entry	Conditions	Approx. Scale g	Equiv of EtI	Yield %	225a:b Ratio
1	TBAF (3.4 equiv), THF 30 m then EtI 16 h, rt	0.5	4	19 (with inseparable by-product)	-
2	TBAF (2 equiv), THF, 30 m removal of volatiles, THF, EtI 16 h, rt	0.1	2.2	65	1:0.72
3	"	1.4	2.2	26	-
4	TBAF (2 equiv), THF, 5 m removal of volatiles, THF, EtI 20 h, rt	1.5	2.2 added at 0 h, 2.2 added at 16 h	43	1:0.4
5	TBAF (2 equiv), THF, 5 m removal of volatiles, THF, EtI 20 h, 0 °C to rt	0.6	9	36	1:0.65

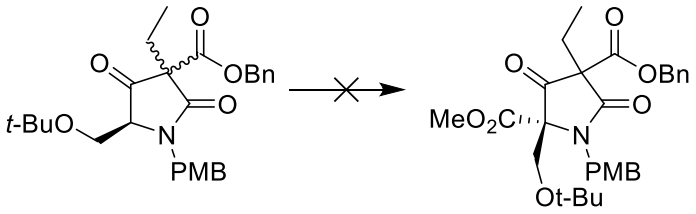
Table 7: Optimization of the Dieckmann cyclization/alkylation using EtI

Using similar conditions to our leucine cyclization/alkylation procedure (**Table 7**, **Entry 1**) provided **225ab** as a mixture of inseparable diastereoisomers along with another inseparable unknown by-product. As our yield for this step was therefore less than 19%, a modification of the procedure was required. Using a procedure similar to the one used in our formal synthesis of omuralide, where the cyclization and alkylation were separated, provided the mixture of diastereoisomers in good yield (**Entry 2**). Unfortunately, scale up of this reaction to just 1.4 g

greatly decreased the yield (**Entry 3**). Increasing the equivalents of EtI improved yields slightly (**Entry 4**). Cooling the reaction decreased the diastereoselectivity, presumably, as in our previous cyclization due to the cooler temperatures reducing the amount of product from epimerization, however, yields dropped to 36% (**Entry 5**). Unfortunately, at this stage we were unable to check to what degree our material had racemized due to the inseparable nature of the diastereoisomers. Furthermore, NOESY experiments were inconclusive as to which diastereoisomer was in excess.

2.4.3 Attempted acylation 225ab using Mander's reagent

Treatment of the mixture of diastereoisomers under our acylation conditions resulted in a complex mixture from which no product could be isolated cleanly (**Scheme 68**).



225ab **226**

Reagents and conditions: LiHMDS (2.1 equiv), DMPU (3.1 equiv), THF, -78 °C, 0.5 h, then $NCCO_2Me$ (5.1 equiv), (-78 °C, -40 °C, -15 °C), 4 h.

Scheme 68: Attempted acylation of **225ab** with Mander's reagent

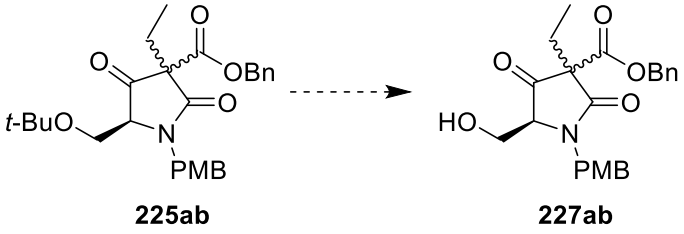
The high levels of diastereoselectivity we had seen in our previous routes indicated that bulky groups in the C7 position are highly effective at blocking addition of the Mander's reagent. The exchange of the methyl group for the ethyl in our starting material could also be hindering attack of the methyl cyanoformate, resulting in our complex mixture. Warming the reaction, which had helped in our omuralide route, also resulted in complex mixtures. We therefore decided to attempt a different strategy to the acylation.

2.4.4 Acylation following removal of the *tert*-butyl group

2.4.4.1 Revised strategy for acylation

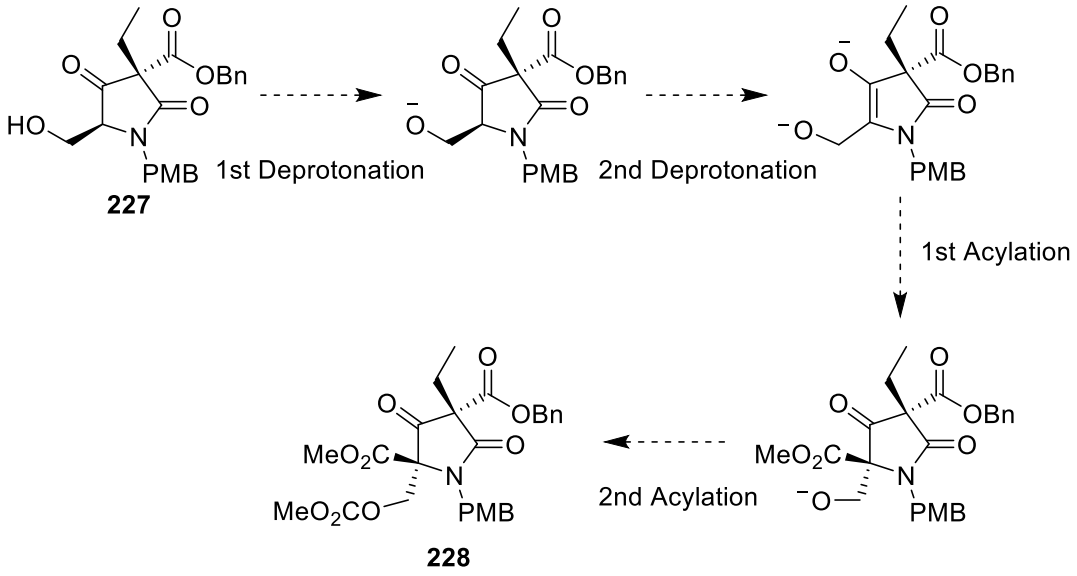
Both the ethyl alkyl chain and the benzyl ester group were necessary in our synthesis and therefore could not be removed, but we felt that removal of the *tert*-butyl group would potentially reduce some of the steric hindrance around the C5 position, allowing insertion of

the methyl ester. We had previously experienced similar problems when switching from the leucine analogue to the *tert*-butoxy analogue, and we therefore felt that removal could be beneficial for the reaction (**Scheme 69**).



Scheme 69: Removal of the *tert*-butyl protecting group

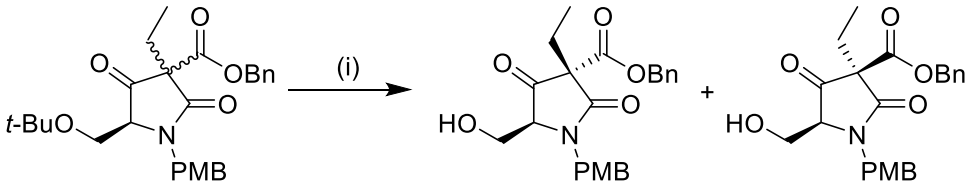
After removal of the *tert*-butyl group our new strategy (**Scheme 70**) would involve use of an excess of base inducing double deprotonation of both the C5 position, forming the enolate, and the hydroxylate group. We theorized (**Scheme 70**) that addition of Mander's reagent would add first at the most nucleophilic position (the C5 enolate) then at the hydroxyl group, re-protecting the hydroxyl group of the newly acylated compound with a methyl carbamate protecting group to give **228**.



Scheme 70: Removal of the *tert*-butyl group

2.4.4.2 Removal of the *tert*-butyl group

Both the inseparable mixture of diastereoisomers from the room temperature cyclization (**Table 7, entry 4**) and the 0 °C cyclization (**Entry 5**) were treated with TFA under the *tert*-butyl ether deprotection conditions previously used to produce **227a** and **227b** (**Scheme 71**).



225ab **227a** **227b**

Reagents and conditions: (i) TFA/DCM 1:1, rt.

Scheme 71: Removal of the *tert*-butyl group

Deprotection proceeded in good yield and provided a separable mixture of diastereoisomers after column chromatography, furthermore, analysis using HPLC with a chiral stationary phase allowed ees to be determined (**Table 8**). Each diastereoisomer was examined against a synthesized racemate. Unfortunately, however, we were still unable through either NOSEY experiments or X-ray crystallography to determine which diastereoisomer was in excess. Our analysis had, however, shown that cooler temperatures during the cyclization had, as expected, improved ees.

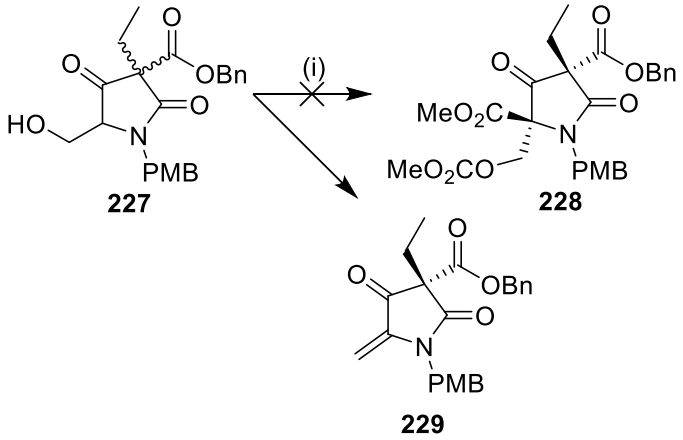
Starting material	Diastereoisomeric ratio of starting material	Yield and ee of the first eluting fraction %	Yield and ee of the second eluting fraction %
Material from table 7 entry 4	1:0.4	18 66 ee	55 45 ee
Material from table 7 entry 5	1:0.65	29 78 ee	46 60 ee

Table 8: Analysis of the deprotected material **227a** and **227b**

2.4.4.3 Acylation with Mander's reagent using the deprotected 227ab

With the *tert*-butyl ether removed, **227ab** was treated with a modified version of our original Mander's acylation (**Scheme 72**). Firstly, the quantity of LiHMDS and N,N'-

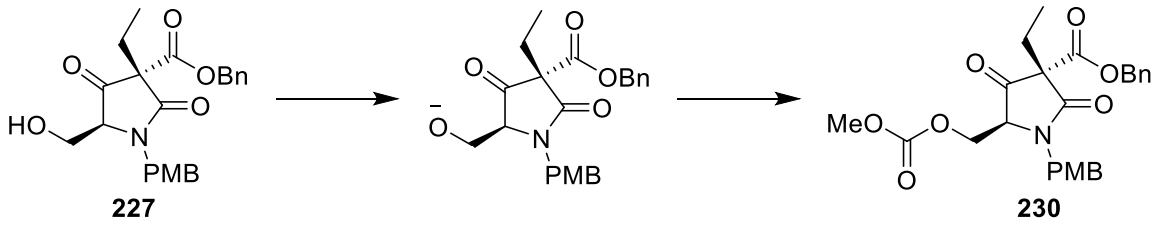
dimethylpropyleneurea (**DMPU**) were increased due to the need for two deprotonations to occur; secondly, the reaction required an increase in equivalents of Mander's reagent because the methyl ester was now required to add twice.



Reagents and conditions: (i) LiHMDS (3 equiv), DMPU (30 equiv), THF, −78 °C, 0.5 h, then $NCCO_2Me$ (4.6 equiv), −78 °C, 3 h, 27%.

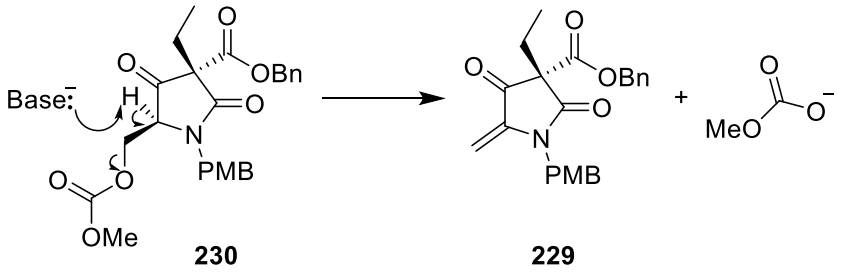
Scheme 72: Attempted acylation of **227ab**

^{1}H NMR spectrum of the crude material after work-up showed a complicated mixture of compounds. However, one compound was observed in excess and was isolated by column chromatography. Unfortunately, ^{1}H NMR spectroscopic analysis only showed one OMe peak corresponding to the *para*-methoxy benzyl group. Our desired product should have contained three. Further analysis by ^{1}H NMR spectroscopy showed a doublet with a *J* value of 2.3 Hz. The chemical shift of this peak (δ-4.53) and its coupling partner (δ-5.21) combined with the small *J* value, pointed towards a terminal alkene **229**. We were able to postulate a reason to explain the observed elimination. Lactam **227a** is likely to have undergone an initial deprotonation from the LiHMDS at the hydroxyl position (**Scheme 73**).



Scheme 73: Mono-deprotonation and acylation of **227a**

If a second deprotonation does not occur before addition of Mander's reagent, however, the hydroxyl group will become acylated to the carbonate **230** on addition of the reagent. This activates the hydroxyl group into a good leaving group, a second deprotonation at the C5 position therefore would induce an elimination before the second acylation could take place **(Scheme 74)**, hence the formation of the enone.

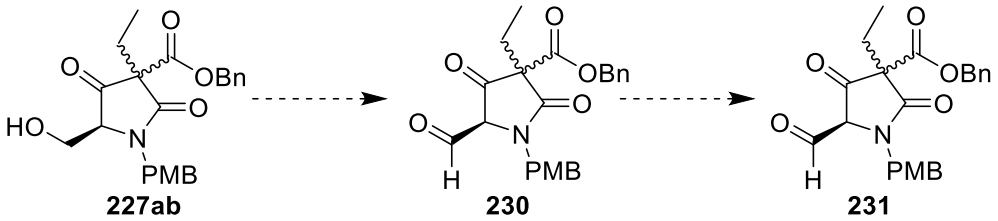


Scheme 74: Mechanism for the formation of **230** from **229**

An analogous reaction was attempted at -40 °C in an attempt to encourage the initial double deprotonation but unfortunately decomposition of the starting material occurred.

2.4.5 Attempted oxidation of the C9 hydroxyl group

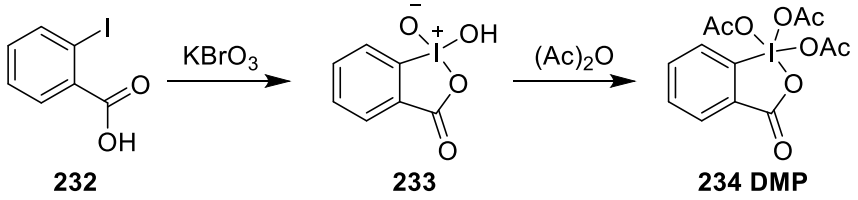
After our failure to incorporate the methyl ester with the hydroxyl group at C9, we devised a different approach. Oxidation of the C9 position to the aldehyde would form a malonic carbon centre at C5. This would lower the pKa of the C5 proton, potentially allowing a wider variety of acylation techniques to be used along with weaker bases, which could be less likely cause of decomposition of the product (**Scheme 75**). The resulting aldehyde could then either be protected or utilized to insert the cyclohexene ring earlier than originally intended (**Scheme 67**).



Scheme 75: Revised strategy for the insertion of the methyl ester via the malonic intermediate **230**

For the oxidation, we chose to use Dess-Martin periodinane (**DMP**). DMP **234** is a hypervalent iodine species which can be bought commercially or prepared from 2-iodobenzoic acid **232** (**Scheme 76**).[129]

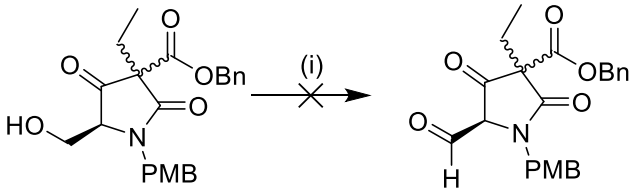
In 1983 Dess and Martin prepared this reagent and reported its use[30] on a range of primary and secondary alcohols, oxidising them to aldehydes and ketones respectively.



232 **233** **234 DMP**

Scheme 76: Preparation of DMP **234** from **232**

DMP has been shown to not over-oxidize alcohols past the aldehyde and furthermore had been used on similar substrates in Corey's synthesis of omuralide to great effect.[29]



227ab **230**

Reagents and conditions: DMP (2 equiv), DCM, 16 h, rt.

Scheme 77: Attempted oxidation of **230ab**

Unfortunately, however, after work up, no compound could be observed in the crude ^{1}H NMR spectrum or seen on TLC. It is perhaps the case that the malonic product was too unstable and therefore underwent decomposition. Due to the significant problems from the beginning of this route and time constraints we decided to stop working towards the total synthesis of salinosporamide B.

2.5 Toward the total synthesis of omuralide and analogues from hydroxy-leucine

2.5.1 Introduction to β-hydroxy, α-amino acids

2.5.1.1 β-hydroxy, α-amino acids in natural products

Of the DNA-encoded 20 amino acids, only two are β-hydroxy, α-amino acids; serine and threonine. More unusual β-hydroxy amino acid backbones, however, are found regularly in natural products.

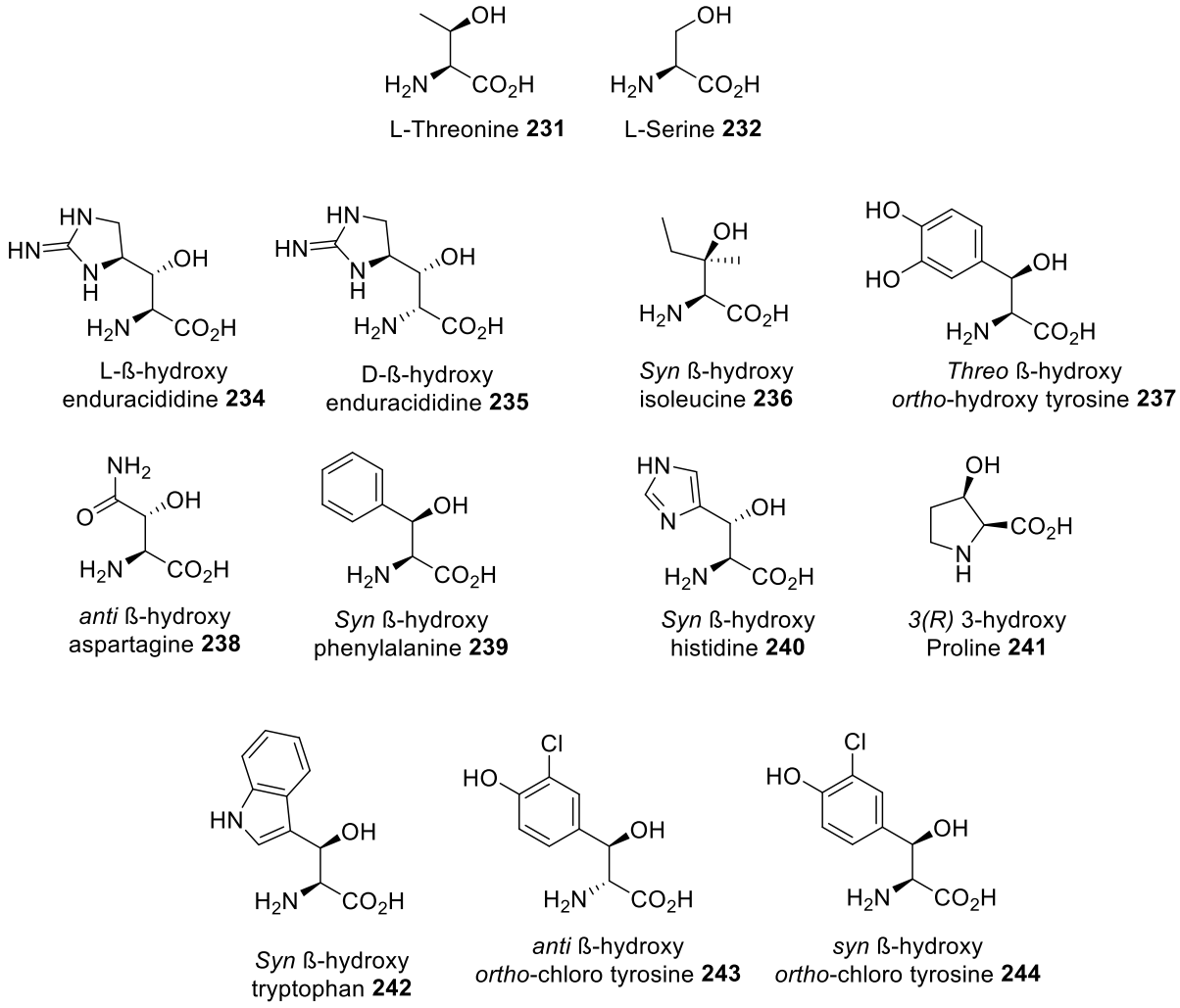
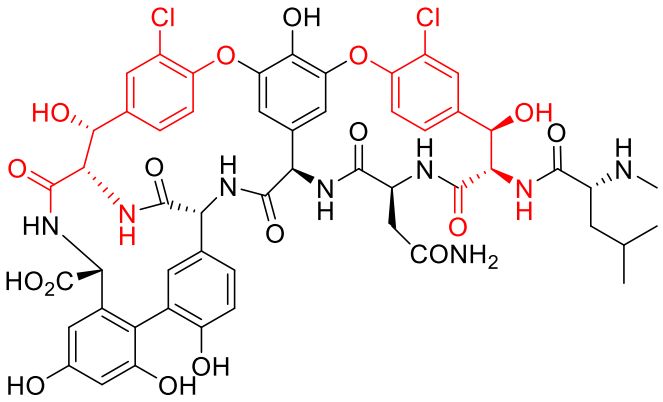


Figure 16: An array of β-hydroxy, α-amino acids

The recently reviewed guanidine-containing amino acid enduracididine has a β-hydroxy amino acid form **234** and its epimer, **235**.[130] Both are found in a group of glycopeptides called mannopeptimycins discovered in 2002.[130] Mannopeptimycins α-ε were found to be active against gram-positive bacteria. Synthesis and a structural revision of the aglycone was

reported in 2014.[131] Hydroxylated forms of isoleucine **236** and tyrosine **237** have also been found in the cyclic peptides ustiloxins[132] isolated from false smut balls found on rice,[133] and were found to have antimitotic properties. β-Hydroxylated forms of asparagine **238**, phenylalanine **239** and leucine were found in the potent antibiotic lysobactin, discovered in 1988.[134] Full elucidation of the structure of lysobactin was completed in 1989.[135] The anti-cancer drug bleomycin[136] contains a substituted β-hydroxy histidine **240** moiety.[137] A hydroxylated proline derivative **241** is found in the DNA gyrase inhibitor cyclothialidine, both discovered[138] and structurally determined[139] in 1993. Substituted hydroxy-tryptophan derivatives **242** were also found in the cyclomarins A-C,[140] which display significant anti-inflammatory properties. The cyclomarins also contained a β-methoxylated phenylalanine. Perhaps most famous, however are the chlorohydroxy tyrosine derivatives **243** and **244**, due to their occurrence in the potent antibiotic vancomycin. Discovered in 1956[141] the aglycone **245** was first synthesized by Evans *et al.*[142] after full structure elucidation.[143,144]

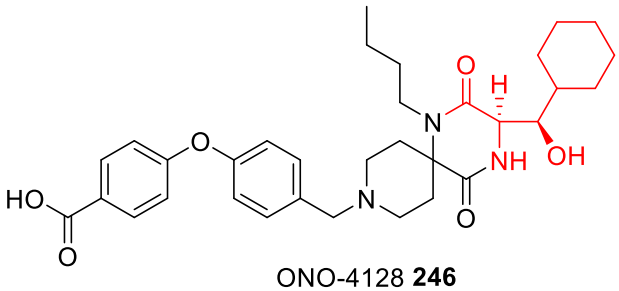


245

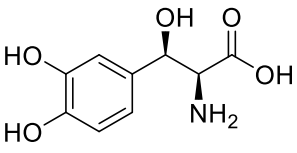
Figure 17: The aglycone of vancomycin, β-hydroxy, α -amino acids highlighted

Vancomycin has become the last line of defence in the fight against antibiotic resistance,[145,146] and is listed on the world health organization's (**WHO**) list[147] of essential medicines. Several structural modifications over the years has retained vancomycin at the forefront of attention.[148]

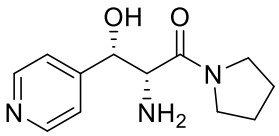
2.5.1.2 β-Hydroxy, α-amino acids in pharmaceuticals



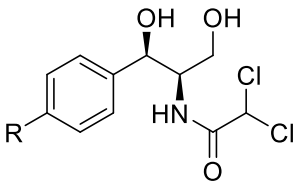
ONO-4128 **246**



Droxidopa **247**



BMS drug candidate **248**

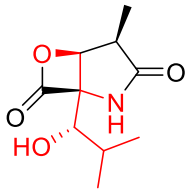


R= NO_2 Chloramphenicol **249**
R= SO_2Me Thiamphenicol **250**

Figure 18: β-Hydroxy, α-amino acids in pharmaceuticals

In addition to natural products, β-hydroxy amino acids and their derivatives also are prevalent in drug molecules (**Figure 18**). ONO-4128 **246** was developed by GSK for treatment of HIV.[149] The hypotension drug droxidopa **247**[149] developed by Sumitomo pharmaceuticals is used worldwide, and the antibiotic chloramphenicol **249** which is used to treat a wide variety of diseases including the plague. Extracted from bacteria, chloramphenicol (discovered in 1947[150] and structure elucidated[151] in 1949) was the first antibiotic to be synthesized.[152] Later the more potent analogue thiamphenicol **250** was synthesized after replacement of the nitro group with a methyl sulphone. Both could be derived from β-hydroxy amino esters after reduction.

2.5.1.3 Hydroxy leucine in natural products



Omuralide

Figure 19: Omuralide, with the motif for hydroxy-leucine highlighted

The hydroxy leucine motif is found in omuralide. (**Figure 19**) The β-hydroxy amino acid and its derivatives have been identified in a plethora of natural products, usually with anti-bacterial properties. These include; azinothricin[153], A83586C[154], citropeptin[155], the papuamides A-D[156], verucopeptin,[157] kettapeptin,[158] polyoxypeptins A and B,[159] piplamycin,[160] GE3,[161] JBIR – 78,[162] JBIR – 95,[162] telemycin,[163] HV-toxin M,[164] leucinostatin,[165] scytonemin A,[166] and lysobactin.[134]

2.5.1.4 Strategies for the synthesis of β-hydroxy, α-amino acids

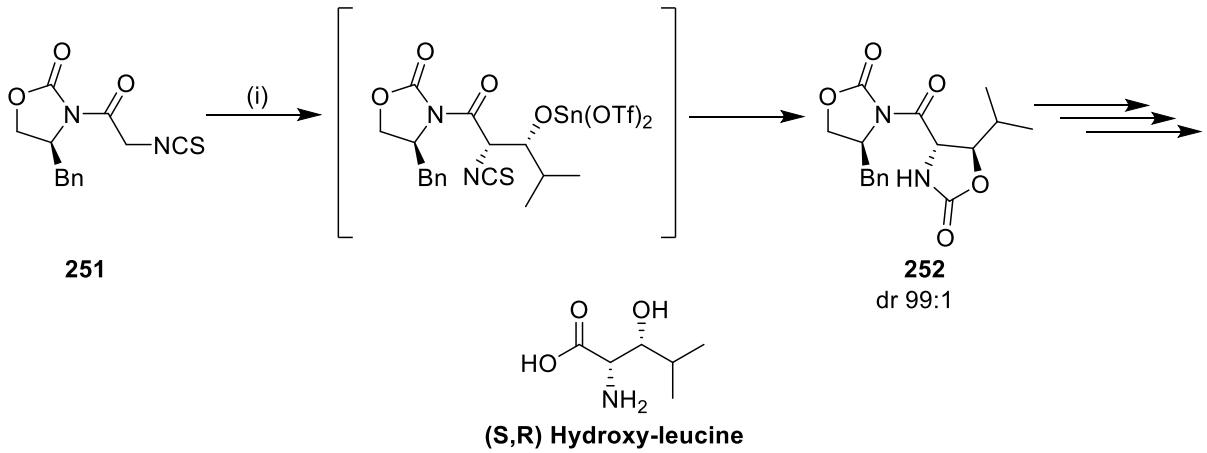
2.5.1.4.1 Introduction to β-hydroxy, α-amino acid synthesis

With the widespread occurrence of the β-hydroxy, α-amino acids backbone and its derivatives in natural and synthetic compounds. Methods for the synthesis of these valuable structures are important.

2.5.1.4.2 Synthesis of hydroxy leucine utilizing an aldol reaction

2.5.1.4.2.1 An aldol reaction employing a chiral auxiliary

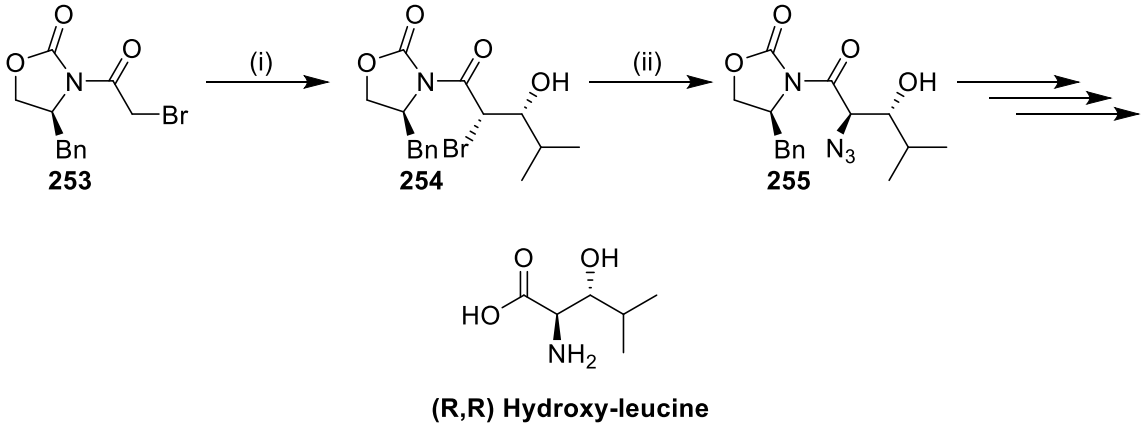
Early work in the asymmetric synthesis of β-hydroxy, α-amino acids was pioneered by Evans. Using an oxazolidinone chiral auxiliary attached to halo acetate or glycine derivatives, Evans was able to produce *anti* or *syn* β-hydroxy, α-amino acids or their derivatives. Evans' approach to *syn* amino acids used an isocyanate attached to a chiral oxazolidinone *via* an amide bond (**251**).[167] Using an aldehyde and stannous triflate as the Lewis acid, several β-hydroxy, α-amino acid precursors were synthesized in excellent diastereoselectivity including a precursor to hydroxy leucine **252** which was synthesized in 92% yield with a diastereoisomeric ratio (**dr**) of 99:1 (**Scheme 78**).



Reagents and conditions: (i) $Sn(OTf)_2$, *iso*-Butyraldehyde, N-ethyl piperidine THF, -78 °C, 92%.

Scheme 78: Evans approach to *syn* β-hydroxy, α-amino acids

Complementary work by Evans[168] showed that using an oxazolidinone bonded to a halo acetate **253** could produce bromo-hydrins such as **254**, which, when bromine was displaced with sodium azide (**255**), could produce anti-β-hydroxy, α-amino acids. Again, hydroxy leucine was one of several amino acids synthesized in the work (**Scheme 79**).



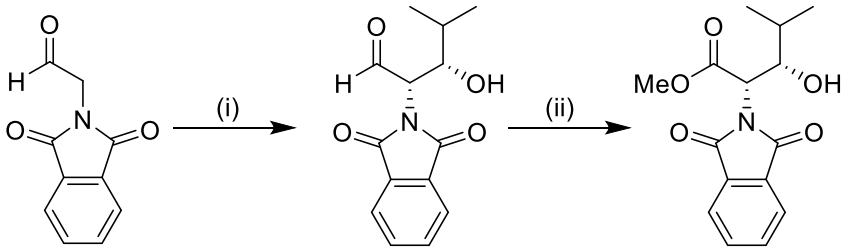
Reagents and conditions: (i) Bu_2BOTf, *iso*-Butyraldehyde, NEt_3, DCM, -78 °C – 0 °C, 63% (ii) NaN_3, DMSO.

Scheme 79: Evans approach to *anti* β-hydroxy, α-amino acids

2.5.1.4.2.2 An aldol reaction using an organocatalyst

Barbas[169] showed in 2004 that proline could be used to catalyse aldol reactions between phthalimidoacetaldehyde **256** and various aldehydes. An extensive optimization using *iso*-

butyraldehyde was carried out that produced **257** in 93% yield and >99% ee and >100:1 dr, which could be elaborated to a derivative of (*S*,*S*) hydroxy-leucine **258** (**scheme 80**).



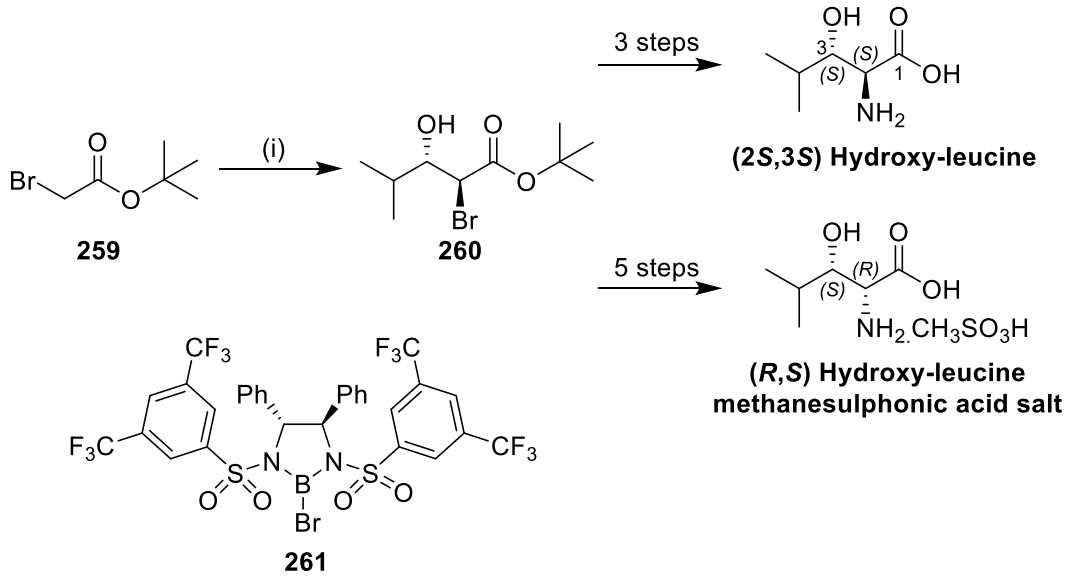
256 **257** **258**

Reagents and conditions: (i) *iso*-Butyraldehyde, L-proline, NMP, 4 °C, 6 days, 93%; (ii) 1) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, *t*BuOH-H_2O, rt; 2) $TMSCHN_2$, MeOH-toluene-hexane -20 °C, 73% over the 3 steps.

Scheme 80: Strategy by Barbas to synthesize β-hydroxy, α-amino acids

2.5.1.4.2.3 An aldol using a chiral Lewis acid

Corey was able to produce bromohydrin[170] **260** using chiral Lewis acid **261**. The bromohydrin **260** (90% yield, 92% ee) was then elaborated to both diastereoisomers of hydroxy leucine (**scheme 81**).



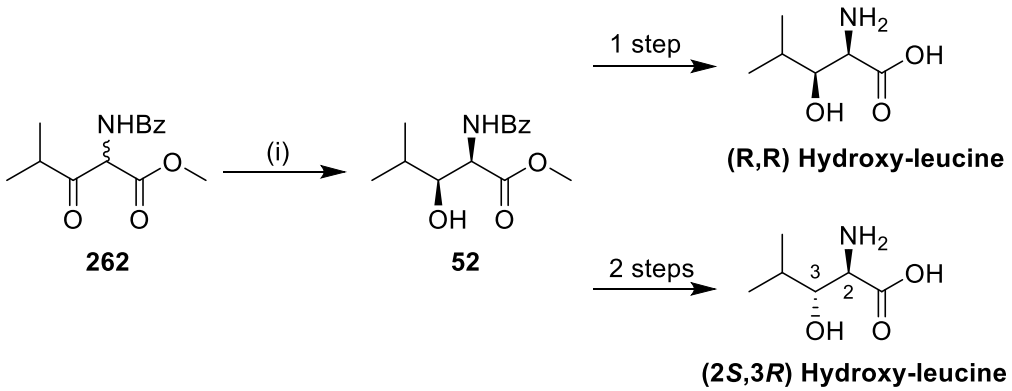
259 **260** **261**

Reagents and conditions: 261, DCM, *iso*-butyraldehyde, NEt_3 -78 °C, 10 h, 90%.

Scheme 81: Corey's strategy to produce either hydroxy-leucine diastereoisomer

2.5.1.4.3 Synthesis using asymmetric hydrogenation

The groups of Genet[171] and Noyori[172] each published asymmetric dynamic kinetic resolutions through the hydrogenation of α-amino, β-keto esters using a ruthenium catalyst. This laid the foundation for Hamada[173] to synthesize all diastereoisomers of hydroxy leucine through hydrogenation, producing the *anti*-diastereoisomer derivative **52,** then inverting the β-hydroxy to produce the *syn*. Genet[174] was later able to report methodology allowing the formation of *syn* or *anti*-products depending on the nature of the starting material (**Scheme 82**).



Reagents and conditions: H_2 (100 atm), $RuCl_2$[(*S*)-binap](dmf)n, DCM, 50 °C, 48 h, 100%.

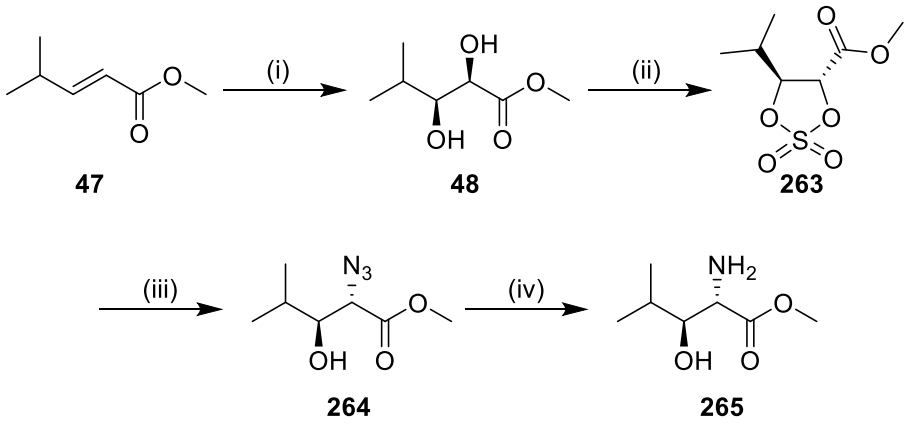
Scheme 82: Hamada's strategy for the synthesis of all stereoisomers of hydroxy leucine

Later work by Genet[175] and Hamada[176] developed the methodology further, allowing asymmetric hydrogenation of the primary amine salts as opposed to further substituted counterparts.

2.5.1.4.4 Synthesis using Sharpless methodology

2.5.1.4.4.1 Asymmetric dihydroxylation

In 1988 Sharpless reported a useful activation of 1,2 diols using a cyclic sulphate. The strategy activated the diol much like an epoxide, but the sulphate could undergo displacement by nucleophiles far more readily.[177] Corey later used this methodology, in combination with the Sharpless dihydroxylation reaction, to synthesize the methyl ester of (*S*,*S*) hydroxy leucine **265** (**Scheme 83**).[39]

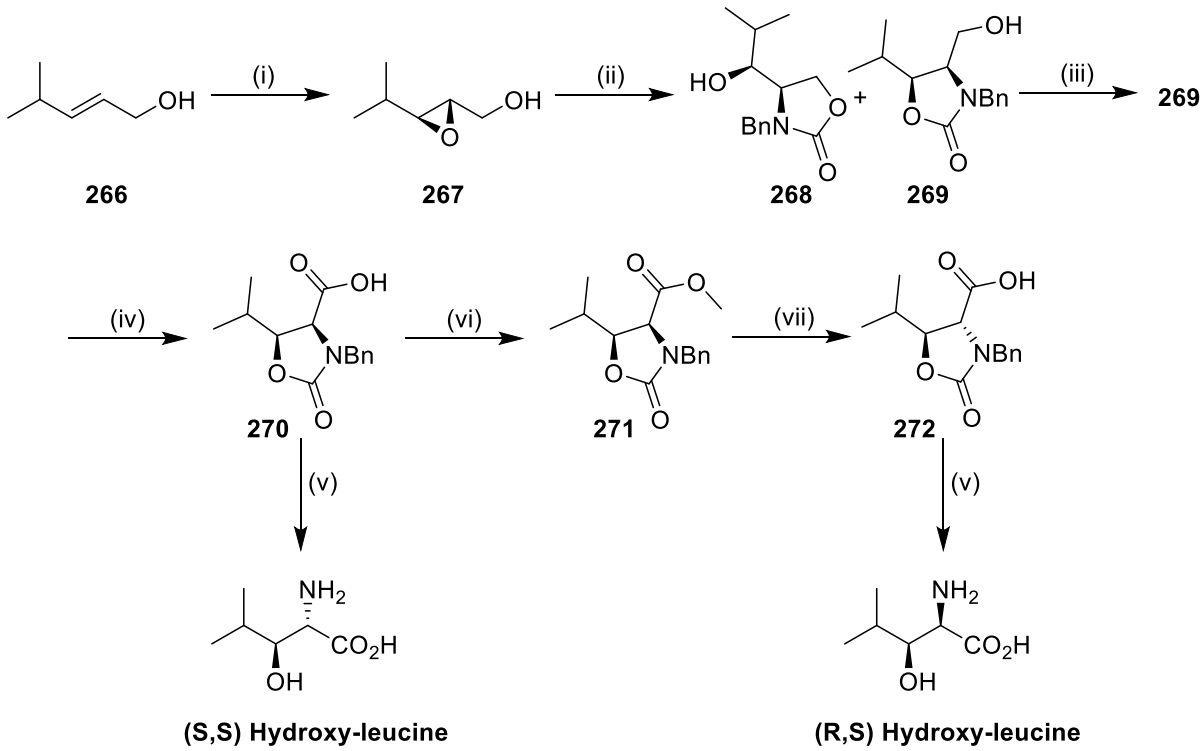


Reagents and conditions: (i) $(DHQ)_2PHAL$, K_2OsO_4, *t*-BuOH-H_2O, 0 °C, 3 days, 92%; (ii) 1) $SOCl_2$, Py, CH_2Cl_2, 0 °C, 10 m, H_2SO_4; 2) $NaIO_4$, $RuCl_3$, MeCN-CCl_4, 97% over 2 steps; (iii) NaN_3, Acetone, rt, 30 m then H^+ (iv) H_2, Pd/C, EtOH/EtOAc, 89% over 2 steps.

Scheme 83: Corey's synthesis of **265** *via* a cyclic sulphate

2.5.1.4.4.2 Asymmetric epoxidation

Ōmura[178] used an asymmetric epoxidation[179] to form **266** which after conversion to the cyclic carbamate **267** and oxidation to the acid **270**, could be converted to (*S*,*S*)-hydroxy-leucine. Intermediate **270** could also be esterified and epimerized to **271**, which could then be converted to (*R*,*S*)-hydroxy leucine (**scheme 84**).

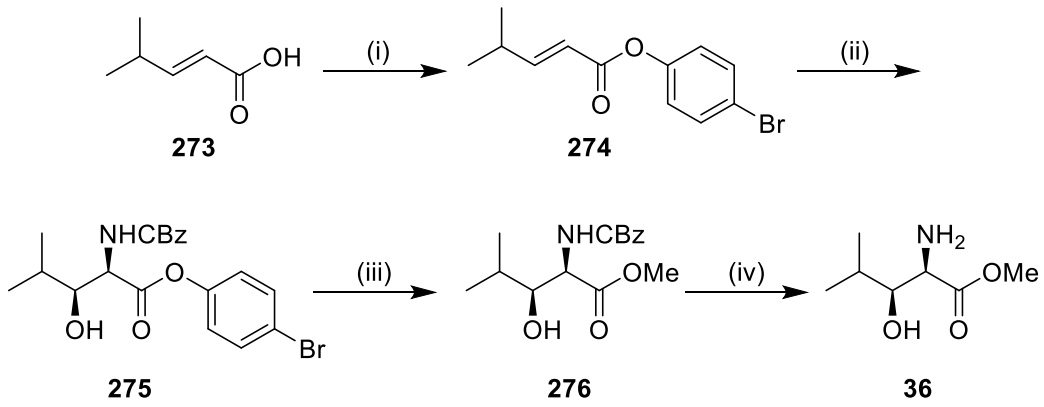


Reagents and conditions: (i) 1) $PhC(Me)_2O_2H$, $Ti(O\text{-}i\text{-}Pr)_4$, (+)-DIPT, 82%; (ii) NaH, BnNCO, THF; (iii) NaH, THF, 75%; (iv) CrO_3, Acetone, 100%; (v) 1) KOH (2 M), 2) H_2, Pd/C, 98%; (vi) CH_2N_2, Et_2O, 86%; (vii) KOH, EtOH, 97%.

Scheme 84: Ōmura's synthesis of the hydroxy-leucine using a Sharpless epoxidation

2.5.1.4.4.3 Asymmetric amino hydroxylation

Another method developed by Sharpless that has been utilized in the synthesis of β-hydroxy, α-amino acids is the amino-hydroxylation, where a double bond can be simultaneously hydroxylated and nitrated.



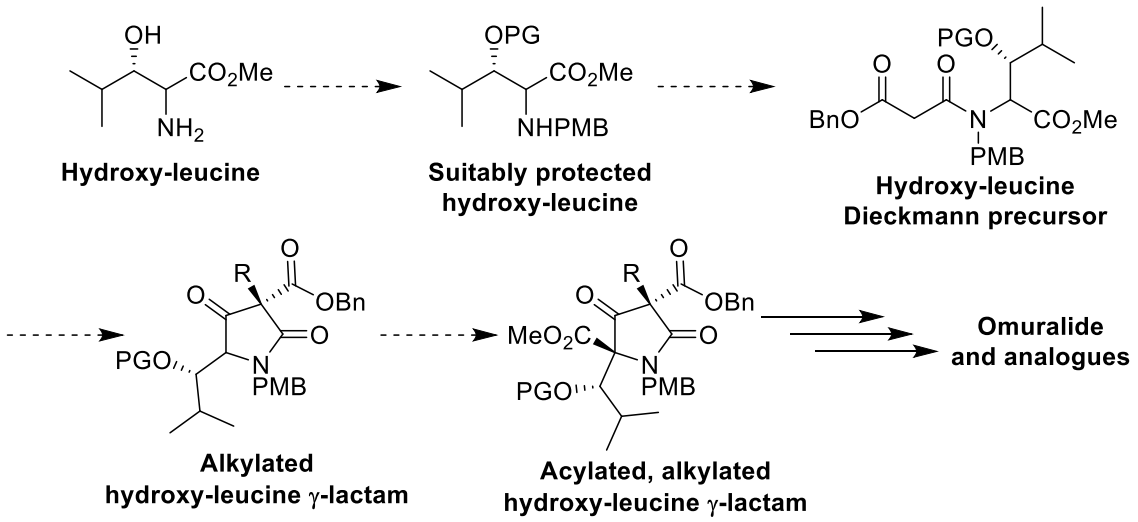
Reagents and conditions: (i) *p*-Bromophenol, DCC, DMAP, CH_2Cl_2, rt, 12 h 80%; (ii) $(DHQ)_2$-AQN, K_2OsO_4, H_2NCBz, NaOH, *t*-BuOCl, *n*-propanol, 4 h, rt, 60%. (iii) Ti(*n*-PrO)$_4$, MeOH, 2 h, 100%; (iv) H_2, Pd/C, MeOH, 12 h, 99 %.

Scheme 85: Panek's synthesis of **36**

Substrate **274** was found to produce the best regio- and enantioselectivity, allowing **275** to be produced in 87% ee, which could be recrystallized to the single enantiomer.[32] Exchange of the bromo-phenyl to the methyl and protecting group removal then afforded **36** in excellent yield.

2.5.2 Strategy of the synthesis of omuralide from hydroxy leucine

Although our Corey analogue **19a** allowed late stage modification of the C9 position for omuralide, the C7 methyl group was installed early on in the synthesis. Ideally, diversification of the structure for the synthesis of analogues should be as late in the synthesis as possible to reduce the time taken and number of steps required. We therefore decided to design a route based around using hydroxy leucine as the starting amino acid. This would install the C5 moiety early on in the synthesis, providing a rapid route to omuralide. Furthermore, by incorporating much of the stereochemistry and carbon skeleton from the beginning, this could allow late stage diversification of the C7 position (**Scheme 86**).

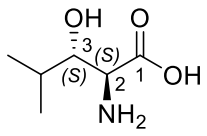


Scheme 86: Outline of a route starting with hydroxy-leucine

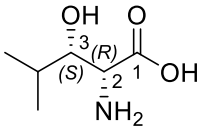
2.5.3 Diastereoisomer of hydroxy leucine required for the synthesis

Although the synthesis of hydroxy leucine has been well studied, we felt a method where either diastereoisomer could be produced from a late stage intermediate would be a valuable addition to the literature. We also sought a method which would avoid expensive or unusual ligands and proceeded in as few steps as possible, preferably using column chromatography to purify intermediates as little as possible.

Hydroxy leucine is commercially available, however, we were presented with two problems. Firstly, hydroxy leucine is very expensive, and we would need multi-gram quantities in order to complete our synthesis. Secondly, we were unable to predict which stereochemical course our route would take, as the diastereoselectivity during the Dieckmann cyclization had switched between the leucine and serine routes. We did know, however, that we required the hydroxyl group to be in the *S* configuration, as in omuralide.



- (*S*,*S*) Hydroxy-leucine
- Anti
- "Serine route" stereochemical course
- 1 g = £1180.00*

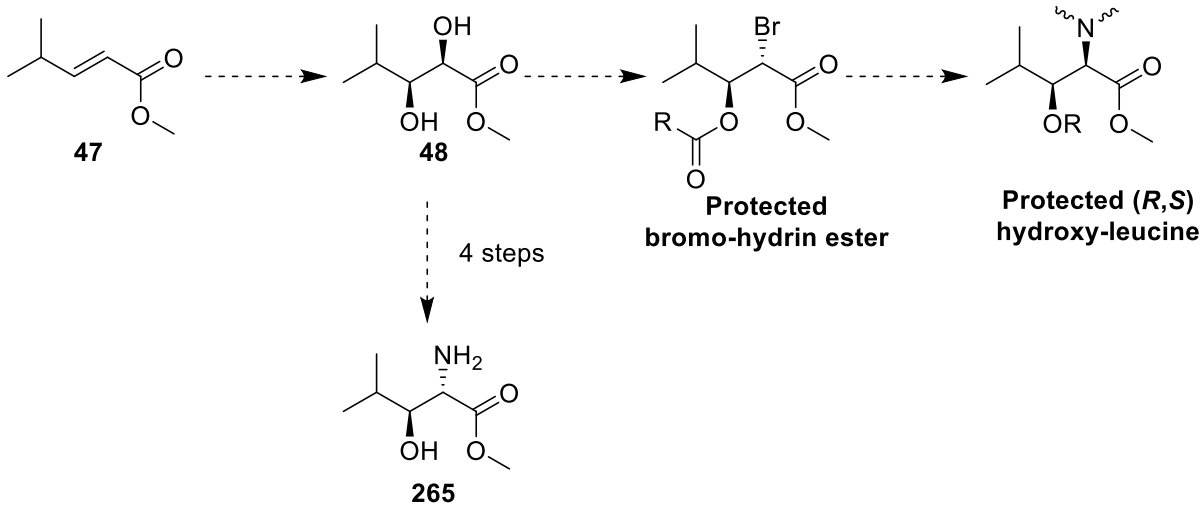


- (*R*,*S*) Hydroxy-leucine
- Syn
- "Leucine route" stereochemical course
- 1 g = £18500.00*
- Used as an inhibitor of serine protease

*Costs shown are approximate values from Santa Cruz Biotechnology https://www.scbt.com/scbt

We therefore decided to synthesize both of the diastereoisomers of hydroxy leucine.

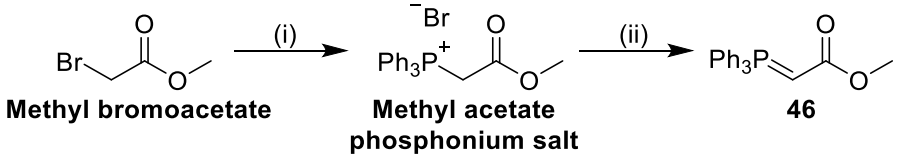
Our aim was to synthesize the enone **47** by a Wittig reaction, then use the Sharpless dihydroxylation procedure to produce diol **48**. The *syn* diastereoisomer could be synthesized utilizing the Sharpless methodology for producing β-acetoxy, α-bromo esters. We would then attempt to displace the bromine with a nitrogen-based nucleophile in an S_N2 fashion, inverting the stereocentre producing the *syn* diastereoisomer (**Scheme 87**). The *anti*-diastereoisomer could be synthesized using Corey's methodology as previously described (**Scheme 83**).



Scheme 87: Our planned methodology to synthesize both diastereoisomers of hydroxy leucine

2.5.4 Synthesis of the enone precursor through a Wittig reaction

We planned to synthesize the isobutyl enone by a Wittig reation. The **methyl acetate phosphonium salt** was prepared by triphenylphosphine substitution of methyl bromoacetate. Deprotonation of the salt with sodium hydroxide produced the ylide **46** in excellent yields (**scheme 88**).

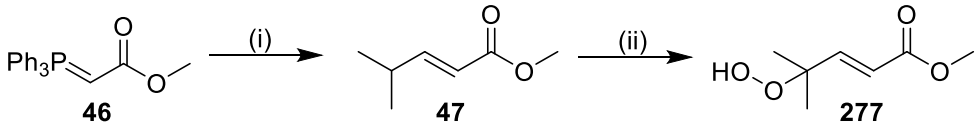


Methyl bromoacetate — **Methyl acetate phosphonium salt** — **46**

Reagents and conditions: (i) PPh_3, EtOAc, 24 h, rt, 80% (ii) DCM, 1 M NaOH Sol, 99%.

Scheme 88: Synthesis of the Wittig ylide **46**

The α, β-unsaturated ester was synthesized through treatment of the ylide with isobutyl-aldehyde. The stabilized nature of the ylide provided the product as the *E* isomer only and purification could be completed by Kugelrohr distillation, avoiding the use of column chromatography. The ester slowly oxidized upon storage to the γ-peroxidized form through an auto-oxidation pathway. Storage under argon and protecting the glassware from light did, however, reduce conversion to the peroxidized product (**Scheme 89**).



46 — **47** — **277**

Reagents and conditions: (i) *iso*-butyraldehyde, DCM, 20 h, rt, 63%; (ii) air, *hv*.

Scheme 89: Synthesis of enone **47** and its oxidation to **277**

2.5.5 Sharpless asymmetric dihydroxylation

2.5.5.1 Introduction to the Sharpless asymmetric dihydroxylation

The reaction between osmium tetroxide and olefins was discovered in 1908.[180] Later work by Criegee *et al.* [181] found that reaction rates could be increased with the addition of pyridine. This work laid the foundation for Sharpless in 1980 to publish the first asymmetric dihydroxylation using chinchona alkaloid ligands.[182] Later work by Sharpless *et al.*[183] developed this asymmetric procedure into a catalytic system, re-oxidizing the osmium with

tertiary amine N-oxides, a technique originally discovered by the Upjohn company.[184] The reaction has since undergone substantial development of both the conditions and ligands used. The most important improvement from the previous pseudo-enantiomer ligands, quinine and quinidine (providing either enantiomer of the substrate), being the avoidance of using stoichiometric ligand through the development of the phthalazine class of ligands (**Figure 20**)[185] hydroquinine 1,4-phthalazinediyl diether (**$(DHQ)_2PHAL$**) and hydroquinidine 1,4-phthalazinediyl diether (**$(DHQD)_2PHAL$**), again respectively quinine- and quinidine-derived, and now the most widely used. These commercially available ligands can be bought as pre-mixed powders containing potassium carbonate, potassium osmate dihydrate, the oxidant potassium ferricyanide and either ligand depending on the enantiomer required.

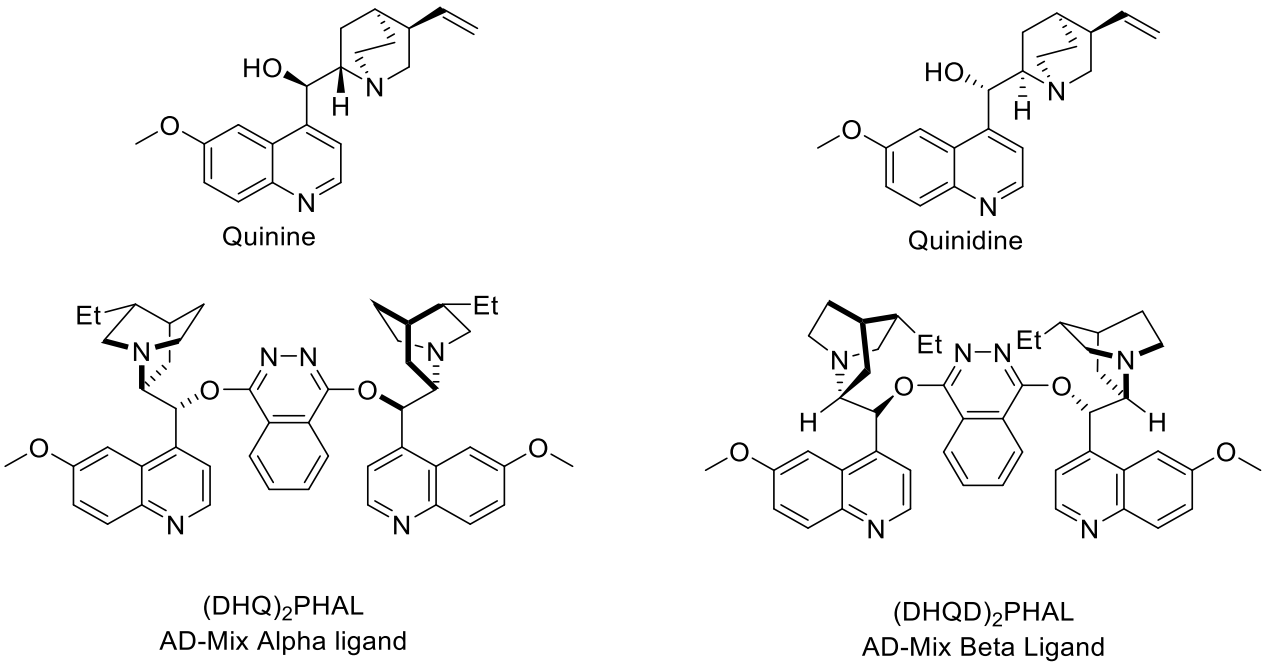


Figure 20: Ligands used in asymmetric dihydroxylation

2.5.5.2 Ligand required for the dihydroxylation

Sharpless also developed a mnemonic allowing identification of the ligand needed depending on the enantiomer of the substrate required. We subjected our substrate to the analysis. As mentioned (**Chapter 2.5.3**), the β-enantiomer would need to have the *S* stereochemistry as found in the C9 position of omuralide. The substrate is orientated so the largest moiety (the methyl ester) points to the "southwest" corner and the second largest (the *iso*-propyl) to the "northeast" corner. Once in this position, Sharpless has shown that $(DHQD)_2PHAL$ would add

the hydroxyl groups from the top face and $(DHQ)_2PHAL$ from the bottom face (**Figure 21**). This would provide us with *S*,*R* or *R*,*S* respectively, allowing us to conclude that the $(DHQ)_2PHAL$ ligand found in AD-Mix α would be required.

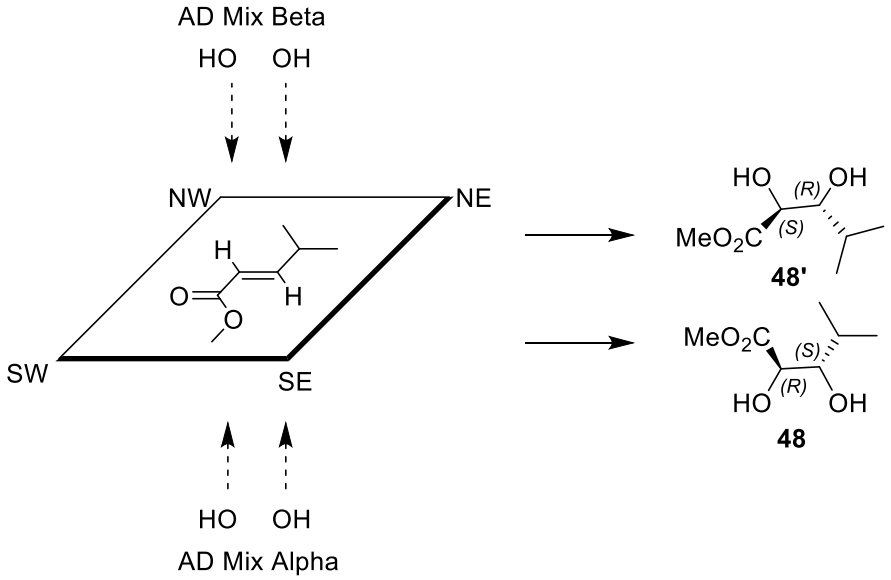
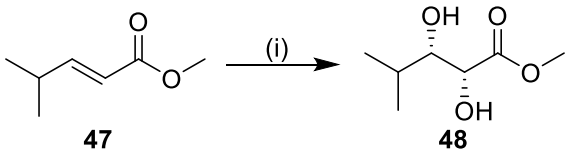


Figure 21: Sharpless mnemonic for choice of ligand required

2.5.5.3 Asymmetric dihydroxylation of 47

Enone **47** was treated under standard conditions for asymmetric dihydroxylation. This provided **48** in 90% ee, which after recrystallization from petroleum ether/ethyl acetate was enriched to 96% ee (**Scheme 90**).

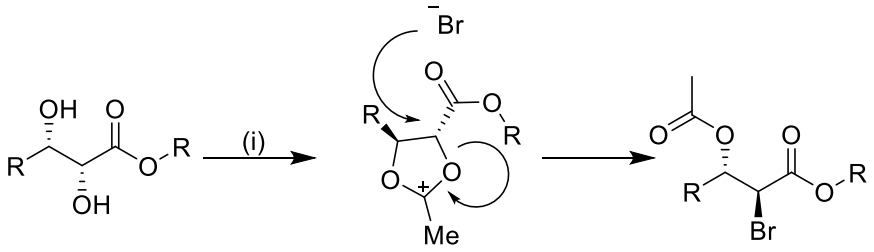


Reagents and conditions: AD-Mix α, *t*-BuOH/H_2O 1:1, 68% (90% ee). Recrystallization in petroleum ether/ethyl acetate (50% overall yield, 96% ee).

Scheme 90: The dihydroxylation of **47**

2.5.6 Tandem bromination/esterification of diol 48

In 1991, Sharpless published "Selective Transformations of *threo* -2,3-dihydroxy esters", which contained several useful reactions for the transformation of dihydroxylated esters.[34] One of the reported transformations was an α-bromination, β-esterification of α,β diol esters. The reaction proceeded through the formation of a cyclic oxocarbenium ion and subsequent opening with bromine in an S_N2 fashion (**Scheme 91**).

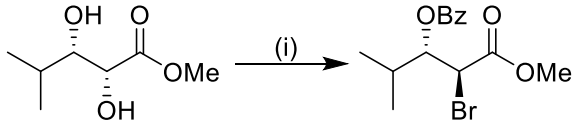


Cyclic acetoxonium ion

Reagents and conditions: (i) HBr, AcOH, 40 °C.

Scheme 91: Regioselective bromination of α,β diol esters

A modified version of this methodology developed by Adams *et al*.[33] was used for the bromination of our diol. A benzoyl cyclic orthoester was formed, catalysed by $BF_3.OEt_2$. Opening was induced by acetyl bromide, brominating the α-position and forming the benzoyl ester on the β-hydroxyl group (**Scheme 92**).

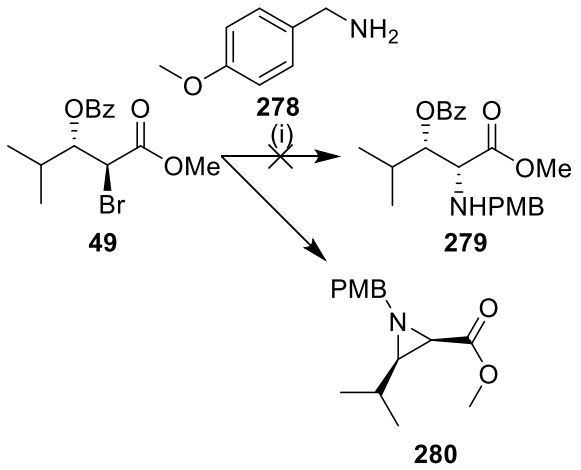


48 **49**

Reagents and conditions: (i) $PhC(MeO)_3$ (1.3 equiv), $BF_3.OEt_2$ (cat), Et_3N (cat), DCM 2 h then AcBr (1 equiv), 3 h, rt, 91%.

Scheme 92: Regioselective bromination of **48**

Bromo ester **49** was used without further purification. We decided to attempt to use *para*-methoxy benzylamine **278** to displace the bromine. Not only would this provide the amine necessary for the amino acid, but also install the PMB protecting group (**279**), which would be required for our omuralide synthesis (**Scheme 93**).

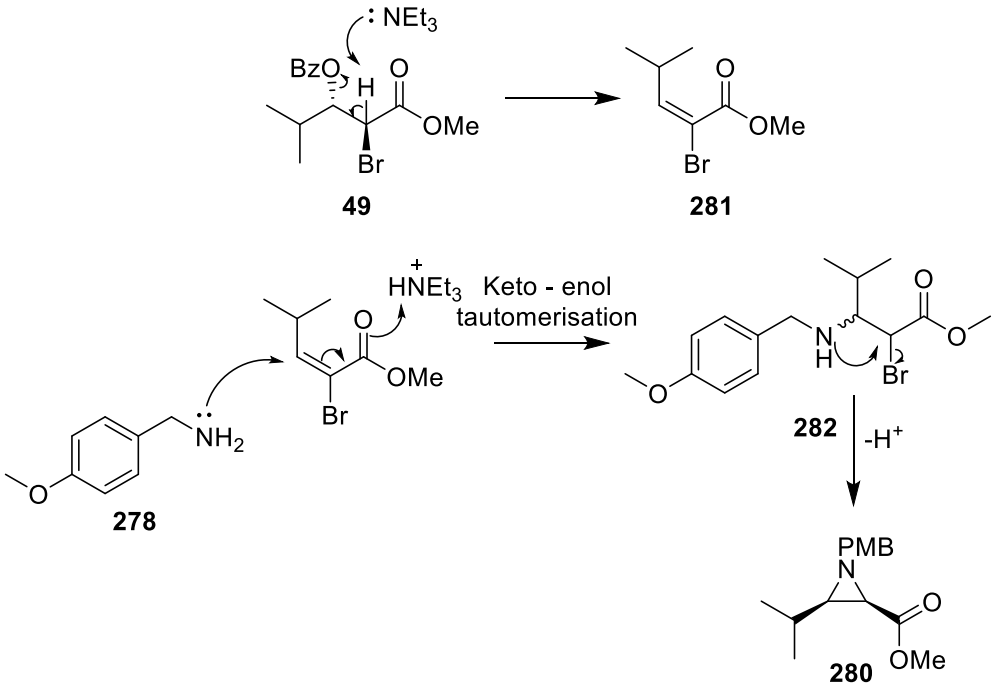


Reagents and conditions: $PMBNH_2$, THF, -78 °C to rt, 64 h, 25%.

Scheme 93: Attempted displacement of bromine by **278**

To our surprise however, analysis of the ^1H NMR spectrum of our product suggested that it was aziridine **280** instead of our desired compound.

A reaction involving α-halo, α,β-unsaturated esters and amines to form aziridines has been observed before and is known as the Gabriel-Cromwell reaction.[186] The proposed mechanism is shown in **scheme 94**.



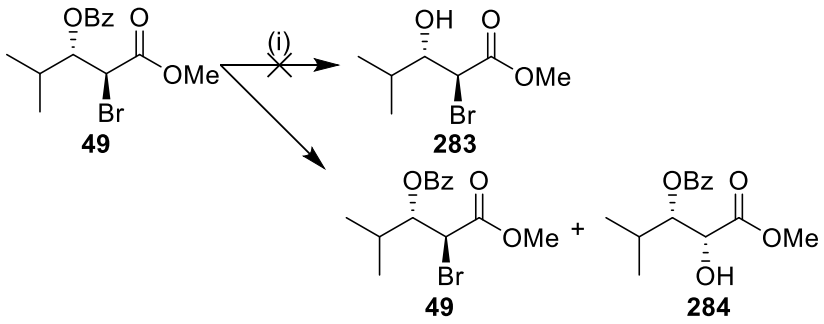
Scheme 94: A Gabriel-Cromwell style reaction to produce by-product **280**

Triethylamine deprotonates the acidic alpha proton and the benzoyl ester is eliminated. A Michael type addition from **278** onto the α,β-unsaturated ester forms a secondary amine, which can *inter*-molecularly undergo nucleophilic substitution with the bromine, forming the aziridine **280**. Although we were only able to isolate the *cis* diastereoisomer, Gabriel-Cromwell reactions usually provide an unequal mixture of diastereoisomers[187] due to the free rotation possible after keto – enol tautomerization.

2.5.7 Transesterification of the β-ester

2.5.7.1 Transesterification of the benzoyl ester

Although substitution of the bromine with $PMBNH_2$ **278** had failed, we felt a similar reaction was worth pursuing. If we were able to remove the β-ester, leaving the unprotected alcohol as in **283**, the original elimination shown in **scheme 94** would presumably not occur, perhaps resulting in the desired substitution. Usually esters are hydrolysed under basic conditions; but in our system this would probably lead to the elimination product again. We therefore decided to attempt to hydrolyse the ester under acidic conditions. Methanol was chosen as the solvent so as not to interfere with the methyl ester in **49**. Attempts at removing the benzyl ester through a methanolic transesterification failed with (±)-camphorsulphonic acid ((±)-**CSA**) at room temperature, (**Scheme 95, table 9**), providing only starting material after 24 h.



Scheme 95: Attempt at transesterification of the benzoyl ester **133**

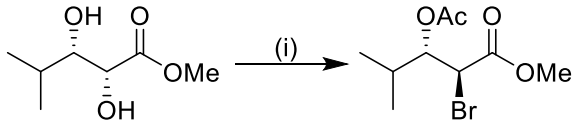
Entry	Conditions	Product and yields %
1	(±)-CSA (1.4 equiv), MeOH, rt, 24 h	**49** 92%
2	*p*-TSOH (1.1 equiv), rt, overnight	**49**
3	*p*-TSOH (1.1 equiv), reflux, 5 h	**49** 32% **284** 39%.

Table 9: Attempted acid hydrolysis of **49**

Increasing the acidity of the organic acid by using *p*-TsOH also provided only starting material at room temperature. Heating this to reflux overnight provided starting material along with hydrolysed material, but the hydrolysis had occurred not at the ester but at the bromine, providing compound **284**. Because of these problems, we decided to modify the method, exchanging the benzoate for an acetate in the hope that the decreased size of the ester would increase the likelihood of transesterification.

2.5.7.2 Transesterification of the α-bromo, β-acetoxy ester

The α-bromo β-acetoxy ester **285** was synthesized in an analogous way to **49** by the exchange of trimethoxybenzoate with trimethoxy acetate (**Scheme 96**).

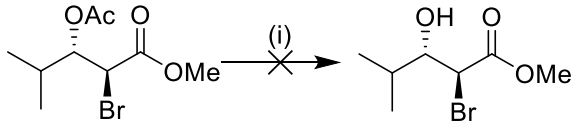


48 **285**

Reagents and conditions: $MeC(MeO)_3$ (1.7 equiv), $BF_3.OEt_2$ (cat), Et_3N (cat), DCM 2 h then AcBr (3 equiv), 3 h, 91%.

Scheme 96: Formation of the α-bromo, β-acetoxy ester **285**

Although the compound could be isolated in crude form from the reaction mixture, under high vacuum, evaporation appeared to occur, causing purification to be difficult. Because of these problems **285** was taken onto the deprotection conditions without further purification. Unfortunately stirring with (±)-CSA in methanol also failed to provide the desired bromohydrin **283**, only starting material being isolated.



285 **283**

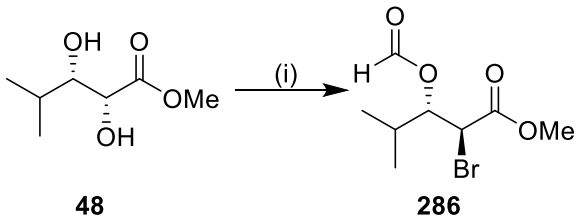
Reagents and conditions: i) (±)-CSA, MeOH, rt.

Scheme 97: Attempt at transesterification of the benzoyl ester **283**

Due to the volatile nature of **285,** we decided not to try further deprotection methods for the acetoxy group.

2.5.7.3 Transesterification of the α-bromo β-formate ester

One of the most labile ester based protecting groups is a formate ester.[188] Due to the difficulty in removing our previous esters, we decided to synthesize the α-bromo β-formate ester **286**. Once again, the reaction proceeded cleanly, and furthermore, no loss of mass was observed after extended periods under vacuum (**Scheme 98**).

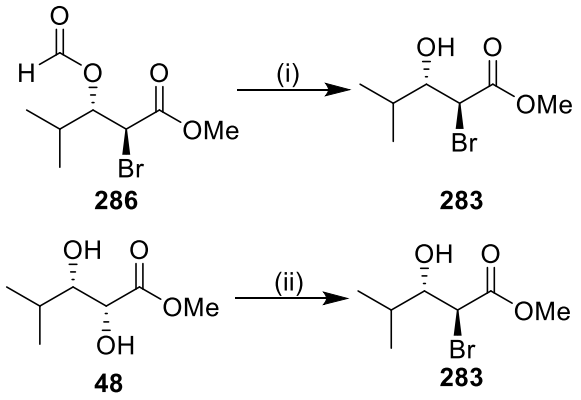


48 **286**

Reagents and conditions: $HC(MeO)_3$ (1.4 equiv), $BF_3.OEt_2$ (cat), Et_3N (cat), DCM then AcBr (1.2 equiv), 91%.

Scheme 98: Formation of the α-bromo, β-formate ester **286**

The α-bromo β-formate ester was treated with the acid hydrolysis conditions at room temperature, and to our delight this produced the desired bromohydrin **283** in excellent yields. Because both our bromination and transesterification appeared to proceed well based on crude ^1H NMR analysis, a one pot procedure was developed. After bromination, methanol and (±)-CSA were added, and the solution stirred vigorously (**Scheme 99**). The development of this one pot procedure only decreased overall yields slightly but removed a step from the synthesis.



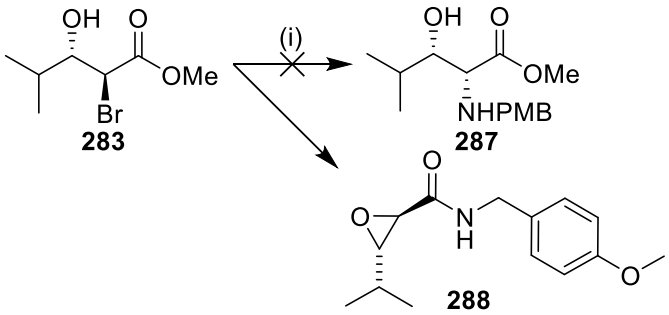
Reagents and conditions: (i) (±)-CSA (1.7 equiv), MeOH, rt 89%. (ii) $HC(MeO)_3$ (1.4 equiv), $BF_3.OEt_2$ (cat), Et_3N (cat), DCM then AcBr (1.1 equiv), MeOH, (±)-CSA (2.6 equiv), 67%.

Scheme 99: Deprotection of the formate ester **286** and the one pot α-bromination of diol **48**

Although only minor impurities were present, the bromohydrin **283** required purification by column chromatography at this stage. However, this was the first column chromotography required in our (*R,S*) hydroxy leucine synthesis.

2.5.8 Displacement of bromine with *para*-methoxy benzylamine 278

Under similar reaction conditions to our previous *para*-methoxy benzylamine substitution attempt (**Scheme 93**), the bromine displacement reaction was run until full consumption of the starting material had occurred (**Scheme 100**).



Reagents and conditions: $PMBNH_2$ (2 equiv), Et_3N (0.9 equiv), THF, overnight, rt, 67%.

Scheme 100: Attempt to displace the α-bromine position with **278**

One major product was observed in the crude reaction mixture. The major compound was active under ultraviolet (**UV**) light, which indicated the $PMBNH_2$ had been added to the molecule, but unfortunately, after purification by column chromatography, analysis of the ^1H

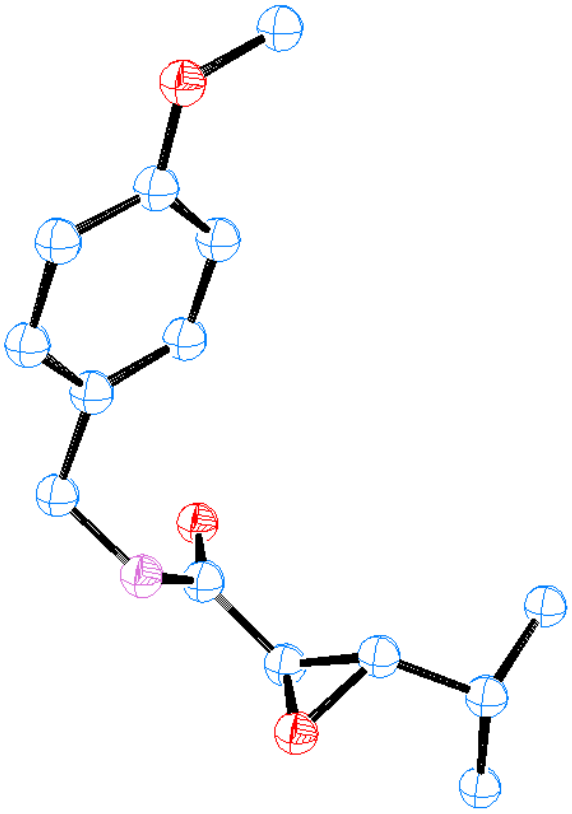
NMR spectrum indicated that the colourless crystalline compound isolated was not our desired product. Crucially, only one OMe peak could be observed and a characteristic amide peak could be seen. NMR analysis pointed toward α,β-epoxy amide **288**. Triethylamine could induce a rapid intramolecular cyclization to the epoxide. Substitution at the methyl ester from **278** would then form the by-product **288**.

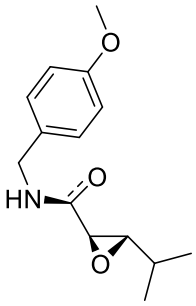
Although not our desired product, **288** proved useful in confirming the absolute and relative stereochemistry.

Firstly, the UV active nature of **288** allows measurement by HPLC, due to the method of detection used by the instrument. We were able to confirm the ee as 96% using HPLC with a chiral stationary phase in comparison with a synthesized racemate.

Secondly, a crystal structure of the enantiopure product confirmed that the correct dihydroxylation ligand had been used, providing our β-hydroxyl group as the *S* stereocentre.

Finally, a crystal structure was obtained, which showed the substituents had a *trans* relationship (**Figure 22**). This provided evidence that the bromohydrin starting material had an *anti*-conformation between the bromine and the hydroxyl group.





288

Figure 22: X-ray crystal structure of **288**, hydrogen atoms removed for clarity

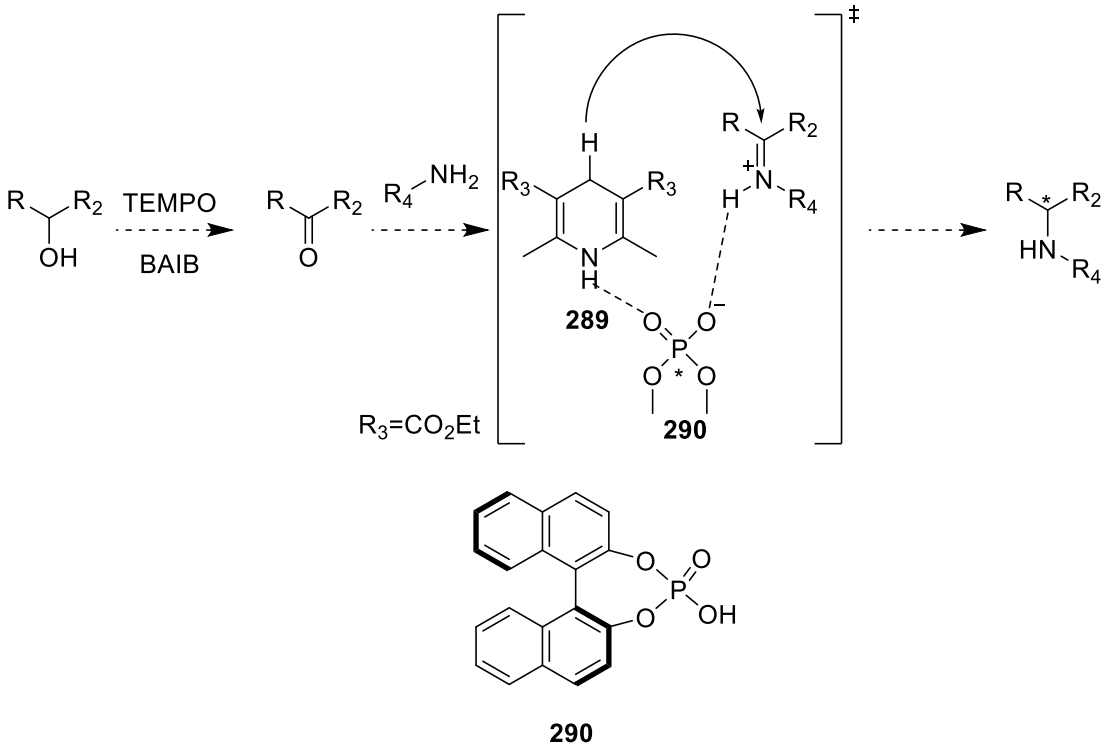
2.5.9 Asymmetric reductive amination

2.5.9.1 Introduction to asymmetric reductive amination

With our methods to incorporate the PMB group utilizing an S_N2 reaction failing, we decided to attempt a different approach. Secondary amines are commonly formed through reductive amination. In the last few decades, considerable progress has been made in the formation of chiral amines using asymmetric reduction of the imine. In our pursuit of finding a rapid route to the synthesis of hydroxy leucine we decided to utilize a method by Saxena *et al.*,[189] who developed a one pot oxidation, reductive amination of alcohols. The method utilizes the

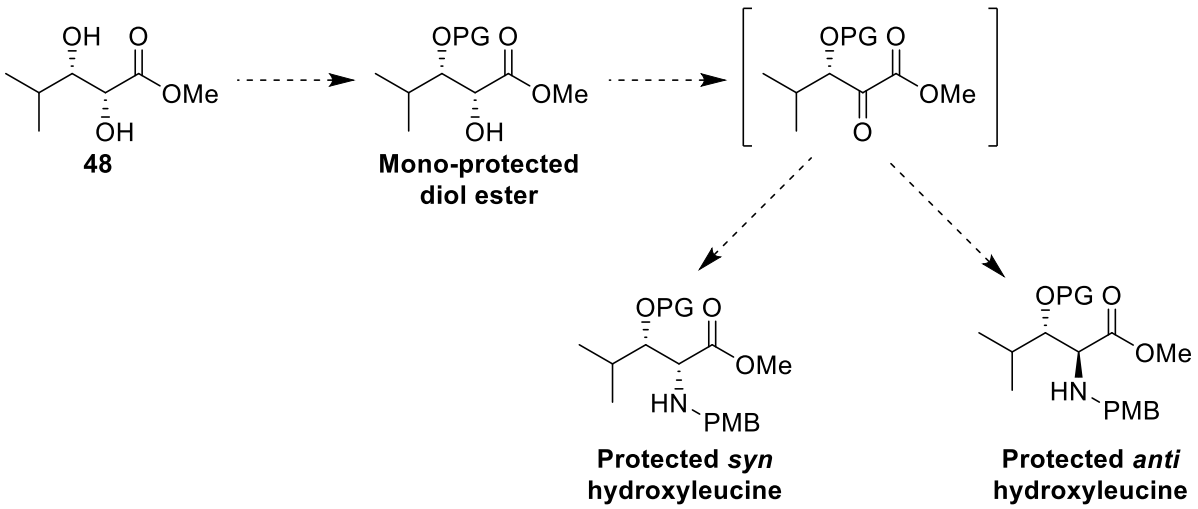
2,2,6,6-tetramethyl-1-piperidinyloxy (**TEMPO**)/(diacetoxyiodo)benzene (**BAIB**) oxidation developed by Piancatelli.[190] The oxidation was chosen by Saxena in part due to the release of acetic acid, catalysing the second step of imine formation. TEMPO-based oxidations have been reviewed.[191]

After oxidation, the imine can be formed with the required primary amine. A chiral Brönsted-acid, in this case the binol-derived phosphoric acid catalysts such as **290**, pioneered by Akiyama[192] and Terada,[193] co-ordinates to the imine creating an environment where the bio-mimetic reducing agent, Hantzsch ester (**289**), is able to reduce the imine asymmetrically (**Scheme 101**). Work on asymmetric reductive aminations with similar Brönsted acid-catalysed systems was initiated by Macmillan.[194]



Scheme 101: One-pot asymmetric synthesis of amines from alcohols

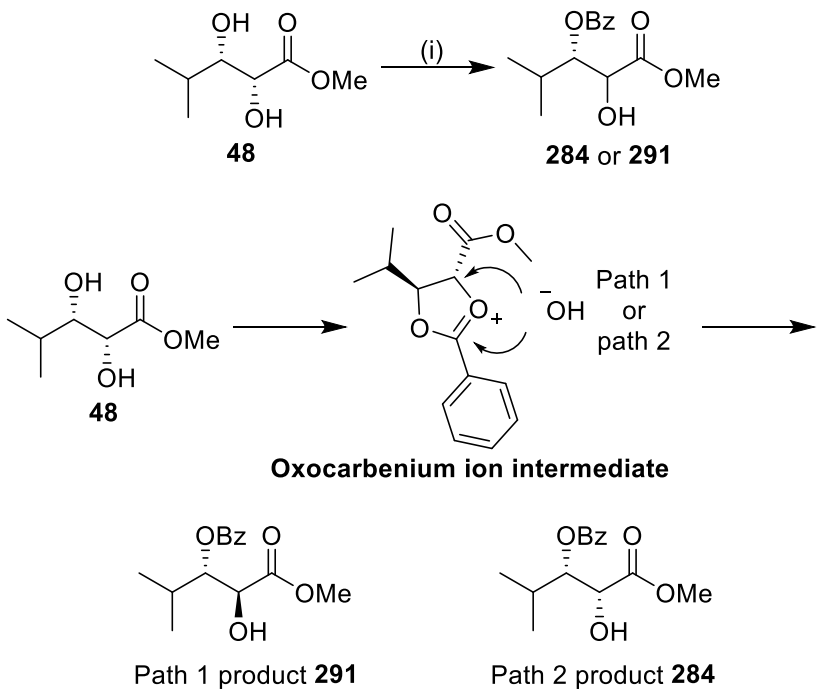
We hoped with correct choice of chiral Brönsted acid, either diastereoisomer could be synthesized (**Scheme 102**); however, we needed first to develop a method allowing regioselective protection of the β-hydroxyl group in the diol ester **48**.



Scheme 102: Strategy for the synthesis of protected hydroxy-leucine from **48**

2.5.9.2 Reigioselective protection of the β-alcohol

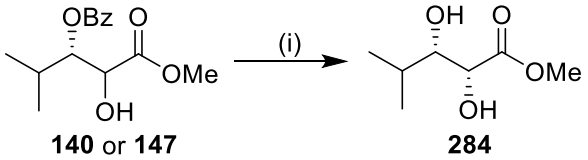
A procedure based on the original bromination from **scheme 92** was developed to regioselectivly mono-benzoylate the β-hydroxyl group. Addition of water instead of acetyl bromide after formation of the oxocarbenium ion opened the ring, providing a free hydroxyl group alpha to the methyl ester and leaving the β-position protected with a benzoyl ester. Depending on the mechanism, the α-hydroxyl group could now be *anti* or *syn* to the β position. Path 1, where the hydroxyl attacks at the α-position of the methyl ester would produce the *anti*-isomer **291**, whereas the *syn*-isomer **284** would be produced by attack at the benzoyl carbonyl (path 2) (**Scheme 103**).



Reagents and conditions: (i) $PhC(MeO)_3$ (1.4 equiv), $BF_3.OEt_2$ (cat), Et_3N (cat), DCM 2 h then H_2O, rt, 70%.

Scheme 103: Hydrolysis of the oxocarbenium ion intermediate and potential products formed

Although our next step would oxidize the α-hydroxyl group making its configuration irrelevant, we decided to confirm the stereochemistry as the compound was novel. Removal of the benzoyl with potassium *tert*-butoxide in methanol provided our original *syn*-dihydroxylated ester **48** (**Scheme 104**).

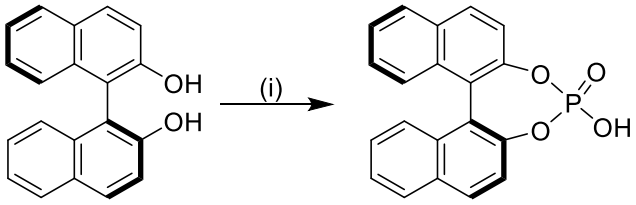


Reagents and conditions: KO*t*-Bu, MeOH, 0 °C, 84%.

Scheme 104: Removal of the benzoyl ester allowing identification of **284**

This result confirmed the stereochemistry to be *syn* and the product from **scheme 103** to be **284**, meaning the reaction proceeds by path 2, with the hydroxide opening the ring by attacking the benzoyl carbonyl.

2.5.9.3 Synthesis of the reagents for asymmetric reductive amination



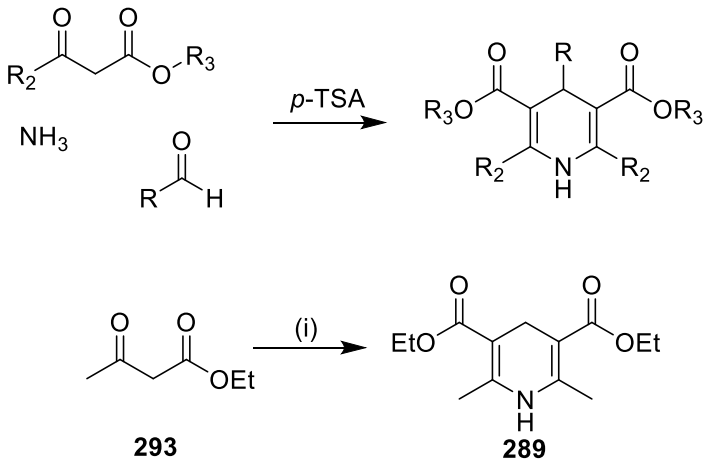
292 **290**

Reagents and conditions: (i) $POCl_3$, Py, 75 °C, 5 h, then H_2O, 2 h, rt, 46%.

Scheme 105: Synthesis of the chiral Brönsted acid **290**

Following a procedure by Zhou *et al.*[195] (S)-binol was converted into the phosphoric acid analogue by reacting **292** with phosphoryl chloride. Quenching the mixture provided the chiral Brönsted acid **290** after purification by column chromatography (**Scheme 105**).

Hantzsch's ester was synthesised following a procedure by Kumar *et al.*[196] based on the Hantzsch dihydropyridine synthesis. This multi-component reaction can be applied to a wide variety of aldehydes and malonic keto esters to provide various dihydropyridine derivatives (**Scheme 106**).



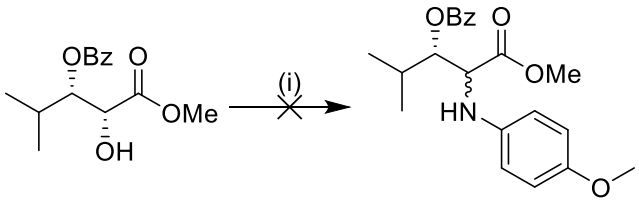
293 **289**

Reagents and conditions: (i) Formaldehyde (aq) (1.1 equiv), $CH_3CO_2NH_4$ (1 equiv), *p*-TSA (cat), 16 h, rt, 23%.

Scheme 106: The Hantzsch dihydropyridine synthesis to **293**

2.5.9.4 Asymmetric reductive amination applied to compound 284

Our mono-protected diol was treated under the oxidation conditions provided from the literature[189] until TLC showed that consumption of the starting material had occurred. The PMB amine **278** was then added along with **289** and **290** and again left until consumption of the starting material had occurred (**Scheme 107**). Unfortunately, upon work-up we found that both by TLC and analysis of the crude ^{1}H NMR spectrum, a complex mixture of products was formed. It is possible that the alpha ester ketone is too hindered, the bulky nature of the benzoyl ester protecting group in **284** preventing imine formation. Because of this, we decided to synthesize a less hindered analogue in the hope the imine could now be formed successfully.



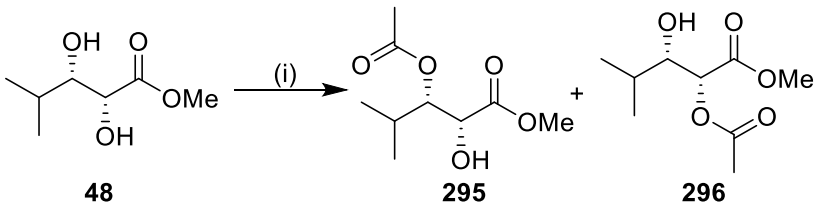
284 **294**

Reagents and conditions: (i) BAIB (1.2 equiv), TEMPO (0.2 equiv), DCM, 16 h then **278** (2.3 equiv), **289** (1.9 equiv), **290** (0.18 equiv), 2.5 h, rt.

Scheme 107: Attempt at asymmetric reductive amination of **284**

2.5.9.5 Mono-acetate protection of the diol 48

Diol **48** was treated under an analogous procedure to our mono-benzoate protection (**Scheme 108**) to produce the acetoxy analogue **295**. Unfortunately, however, the reaction provided two products, the major being our desired β-protected alcohol **295**, but significant amounts of the α-protected alcohol was also isolated after column chromatography **296**.

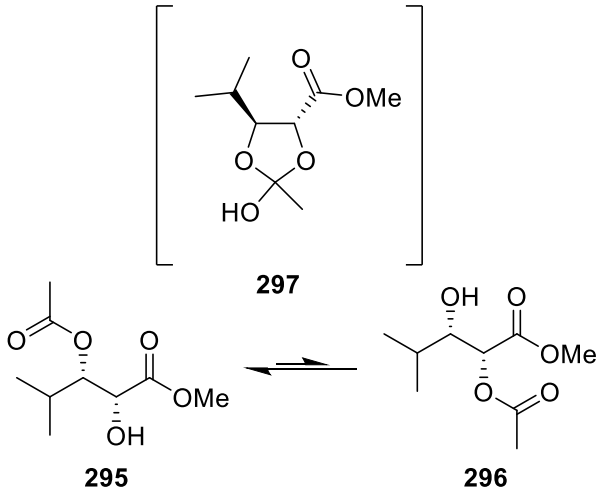


48 **295** **296**

Reagents and conditions: $MeC(MeO)_3$ (1.3 equiv), $BF_3.OEt_2$ (cat), Et_3N (cat), DCM 2 h then H_2O, rt, **150** 31% **151** 11%.

Scheme 108: Mono-acetate protection of diol **48**

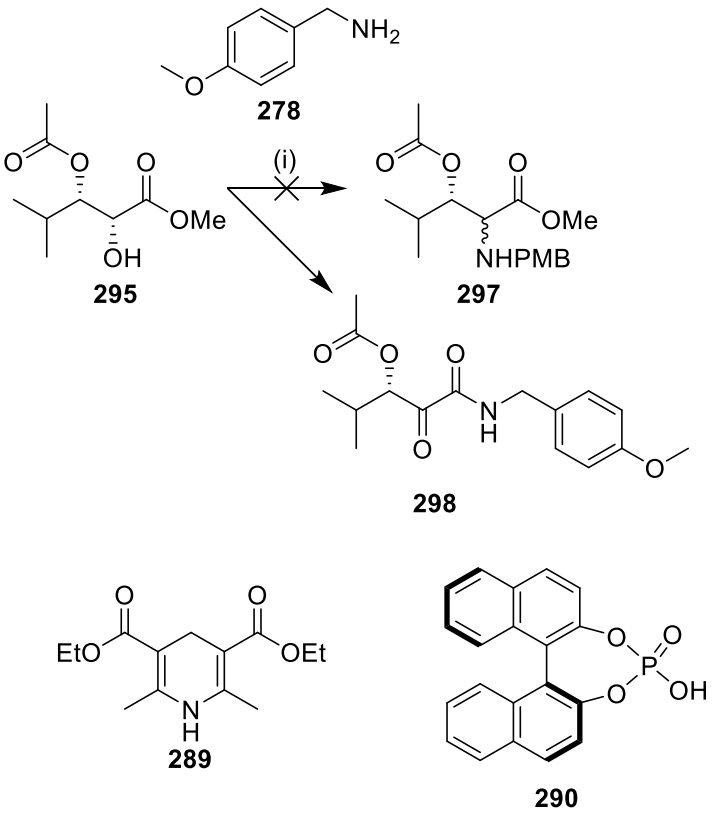
During further characterization of the α-protected diol-ester, we found that the acetate would migrate to the β-position upon standing in chloroform (**Scheme 109**), presumably to the more stable regioisomer *via* **297** this migration is potentially due to the acidic content of the chloroform. Analysis of the ^{1}H NMR spectrum showed after 16 h a 0.07:1 (**295**:**296**) mixture would convert to a 3.55:1 mixture of regioisomers. In comparison, conversion of **295** to **296** was almost negligible, with a 1:0.01 (**295**:**296**) mixture of regioisomers converting to a 1:0.03 mixture after 16 h.



Scheme 109: Equilibrium of **295** and **296**

2.5.9.6 Asymmetric reductive amination applied to compound 295

The less hindered mono-protected diol ester **295** was treated under similar conditions to our previous benzoylated analogue **284** (**Scheme 107**). Unfortunately, once again a complex mixture of products was formed (**Scheme 110**). A major product was partially isolated, but it could not be fully characterized. It appeared that the PMB group had been incorporated, but not in the desired fashion. A peak in the ^{1}H NMR spectrum appeared to correspond to an amide, which could correspond to a substitution of the methyl ester, suggesting **298**. We had seen similar reactions occur previously during our attempts to displace the bromine in **scheme 100**.

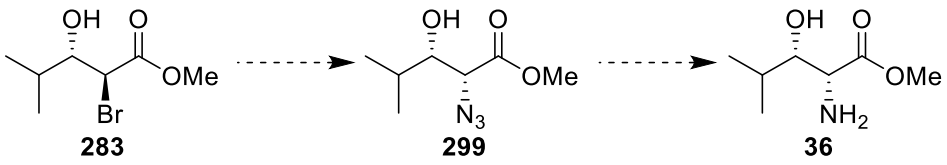


Reagents and conditions: (i) BAIB (1.1 equiv), TEMPO (0.2 equiv), DCM, 16 h, **278** (2 equiv), **289** (1.2 equiv), **290** (0.15 equiv), 2.5 h, rt.

Scheme 110: Oxidation/amide formation of **298**

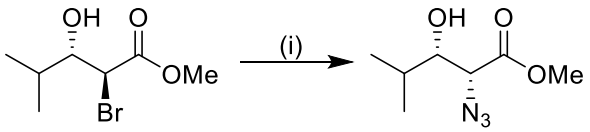
2.5.10 Displacement of bromine with sodium azide

It seemed that in general $PMBNH_2$ was quite ineffective at displacing bromine and would often act as a base rather than a nucleophile. We therefore decided to switch to sodium azide which would form **299**. Reduction of the azide **299** would then produce the primary amine **36**, providing us with the amino acid for use in our synthesis (**Scheme 111**).



Scheme 111: Revised strategy for the synthesis of hydroxy-leucine

To our delight, sodium azide was able to displace the bromine in *N*,*N*-Dimethyl formamide (**DMF**). The obtained product appeared pure by analysis of the ^1H NMR spectrum and therefore **299** could be used in the next step without further purification.



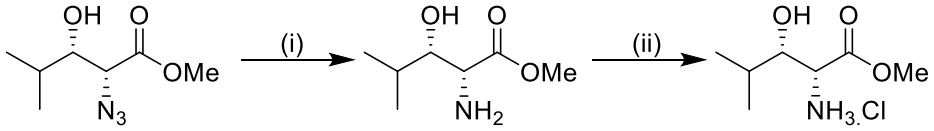
283 **299**

Reagents and conditions: (i) NaN_3 (3.4 equiv), DMF, 20 h, 80%.

Scheme 112: Displacement of bromine in **283** by sodium azide

2.5.11 Reduction of azide 137 and amine HCl salt formation

Reduction of the azide to **36** appeared to proceed as observed by TLC. Unfortunately, however, analysis of the crude ^1H NMR spectrum showed a mixture of compounds. We theorized that co-ordination between the amine and various impurities (potentially from the celite) were forming complexes, thereby increasing the number of signals present in the ^1H spectrum. To prevent this, the amine hydrochloride salt **300** was synthesized with methanolic HCl (**Scheme 113**).



299 **36** **300**

Reagents and conditions: (i) H_2, Pd/C, MeOH (quant) (ii) Methanolic HCl (quant)

Scheme 113: Reduction of the azide

Clarity of the ^1H spectrum increased dramatically, allowing proper characterization of the salt. In addition to the enantiomeric product, a racemic series has also been synthesized, (±)-**300** was obtained as crystalline solid from which a crystal was obtained for analysis by X-ray diffraction.

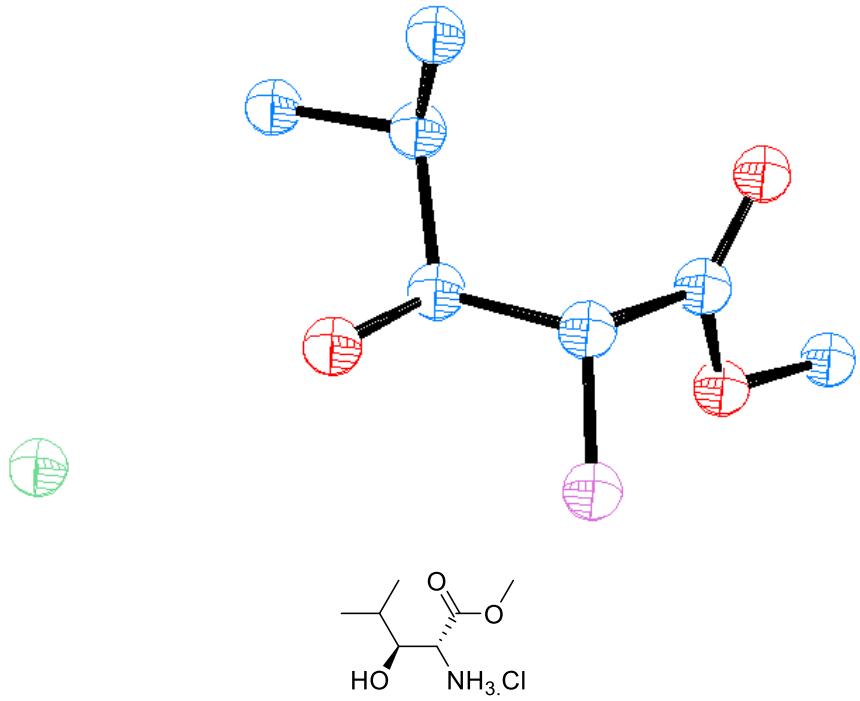
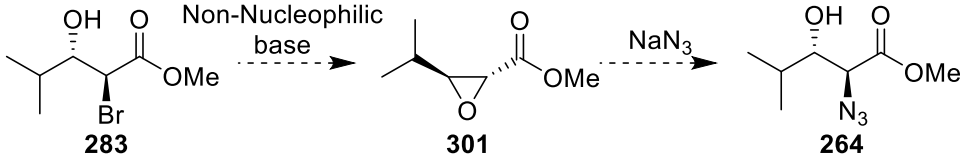


Figure 23: X-ray crystal structure of **300**, hydrogen atoms removed for clarity

2.5.12 Synthesis of *anti* hydroxy leucine from 283 *via* an epoxide

2.5.12.1 Introduction to strategy

With the success of our route to produce the *syn* amino acid ester, we decided to modify the route to form the *anti*-amino acid ester. Ideally, the routes to the hydroxy leucine diastereoisomer would be identical until a late stage modification which would allow either diastereoisomer required to be formed. The route planned involved bromohydrin **283**, which would be subjected to conditions which would form the epoxide. Then, sodium azide would be added *in situ* in an attempt to open the epoxide. This strategy would involve 2 S_N2 inversions of the stereochemistry at the alpha position, overall retaining the original *anti* relationship in the final compound **264**.

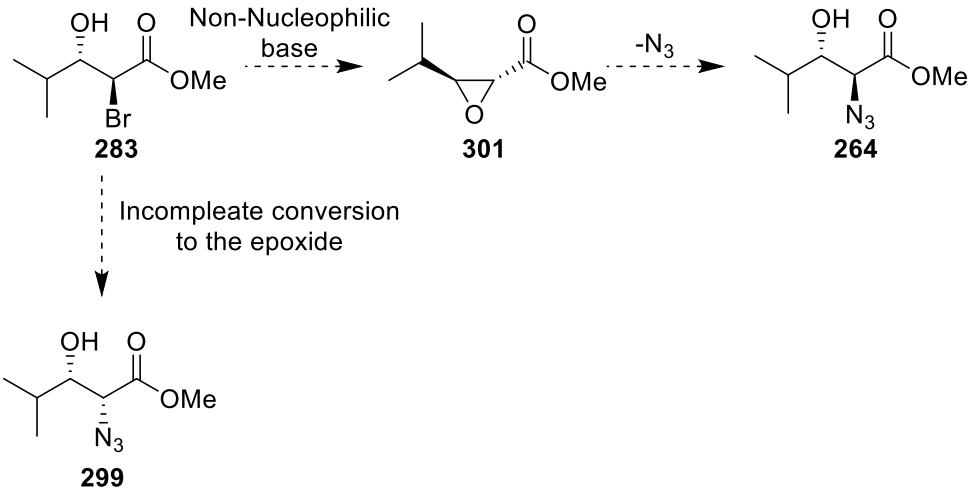


Scheme 114: Strategy for the substitution of bromine by sodium azide *via* the epoxide

Although the reaction sequence appeared to be plausible, there were several possible outcomes that could potentially occur which we would have to control.

Problem 1: Full epoxide conversion not achieved

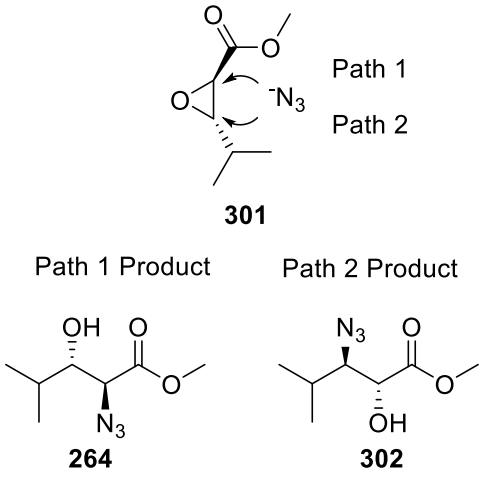
If sodium azide was added before full conversion to the epoxide, the azide would displace the bromine as we have already shown (**Scheme 112**). To control this, epoxide formation would be followed by TLC and excess time would be provided for epoxide formation to occur (**Scheme 115**).



Scheme 115: Potential side reactions if incomplete conversion to the epoxide occurred

Problem 2: Regioselectivity

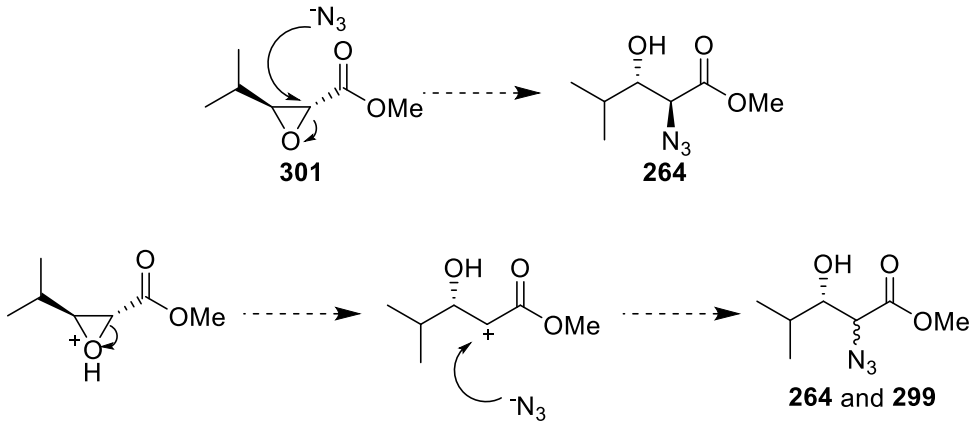
After epoxide formation, the azide could potentially attack the epoxide in either the α or β position (**Scheme 116**). Both the *iso*-propyl and the methyl ester are approximately equal in size, suggesting that steric bulk is unlikely to be a factor in the regioselectivity of the reaction. We do envisage, however, that the electron-withdrawing properties of the ester will increase the δ^+ charge on the α position, providing a favourable reaction at the desired α position.



Scheme 116: Potential regioselectivity problems

Problem 3: Mechanistic path of epoxide opening

A final potential problem is that the reaction may proceed mechanistically as an S_N1 reaction. If the epoxide opened first to produce a carbocation which the azide could attack, we would be unlikely to see any diastereoselectivity, producing a mixture of diastereoisomers **264** and **299** (**Scheme 117**).

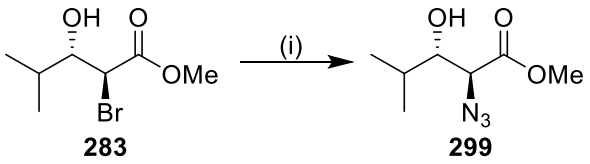


Scheme 117: Outcome of an S_N1 or S_N2 mechanism

An S_N2 reaction however would be diastereospecific, providing us with only the desired diastereoisomer **299**. An S_N2 reaction is fortunately the most likely route for this reaction. The S_N1 mechanism would produce an unstable carbocation intermediate due to the electron-withdrawing nature of the ester.

2.5.12.2 Epoxide formation and azide opening experiments

Epoxide formation was originally achieved by use of 1,8-diazabicyclo[5.4.0]undec-7-ene (**DBU**) as a non-nucleophilic base. DBU was found to achieve full epoxide formation in about 1-2 h in most solvents used. Later experiments used triethylamine, which was also found to work well, but had the added benefit of volatility, allowing easier extraction from the crude material. Efficiency of epoxide formation between the two bases was equivalent.

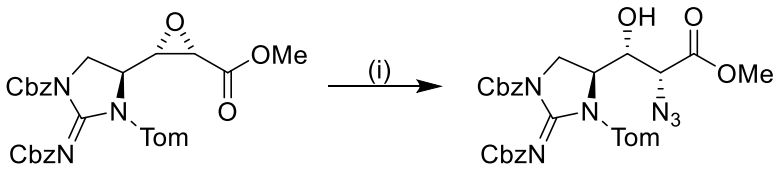


283 **299**

Entry	Reagents and Conditions (i)	Analysis of the ^{1}H NMR Spectrum	Yield of azide
1	DBU (2 equiv), DMF 2 h, NaN_3 (2.5 equiv), 16 h	Only epoxide observed	-
2	DBU (1.9 equiv), DMF 2 h, NaN_3 (2.2 equiv), 60 °C 16 h	Complex mixture	-
3	PEG 400, Et_3N (1.3 equiv) 1 h NaN_3 (3.4 equiv) 16 h	10:1 epoxide to azide ratio	trace
4	PEG 400, Et_3N (1.4 equiv) 1 h NaN_3 (4.1 equiv) 40 h	0.5:1 epoxide to azide ratio	9%
5	DMF, Et_3N (1.3 equiv) 4 h then PPTS (1.9 equiv), NaN_3 (3.3 equiv), 16 h	1:0.25 epoxide to azide ratio	-
6	DMF, Et_3N (1.3 equiv) then NH_4Cl (2.4 equiv), NaN_3, 16 h	Azide observed in mixture of compounds	Azide unable to be isolated pure from by-products
7	H_2O/MeCN (1:9), Et_3N (1.2 equiv) then $CeCl_3$ (0.5 equiv), NaN_3 (1.9 equiv), reflux 3 h	Mostly epoxide, trace azide observed	-
8	H_2O/MeCN (1:9), Et_3N (1.1 equiv) then oxone®(1 equiv) , NaN_3 (2.3 equiv)	-	-
9	H_2O/MeCN (1:9), Et_3N (1.1 equiv) then $CeCl_3$, NaN_3 (3 equiv), reflux overnight	Mostly azide observed	19%

Table 9: *In situ* epoxide forming/opening reactions.

Treatment of the epoxide with sodium azide provided **301** only (**Table 1**, **entry 1**). Heating an equivalent reaction resulted in a complex mixture from which nothing could be identified or isolated (**Entry 2**), potentially indicating that the epoxide was unstable at higher temperatures. With these two results it became clear that the epoxide would probably need activation in order to ring open. We had originally sought to avoid activation of the epoxide in case it induced an S_N1 epoxide opening mechanism. In 2006 Das *et al.* published "Catalyst-free highly regio- and stereoselective ring opening of epoxides and aziridines with sodium azide using poly(ethylene glycol) as an efficient reaction medium".[197] The authors suggest that hydrogen bonding between polyethylene glycol (**PEG**) and the reactants induces epoxide opening; furthermore the epoxides seemed to be shown to open diastereospecifically. After reacting the epoxide overnight (**Entry 3**), analysis of the ^{1}H NMR spectrum of the crude mixture showed mostly the epoxide present, along with a small amount of the azido alcohol (**Entry 3**). Purification by column chromatography, however, provided only trace levels of material. Increasing reaction times and the amount of sodium azide provided a yield of 9% despite conversion looking positive according to the ^{1}H NMR spectrum of the crude material (**Entry 4**). Although this result was encouraging, we decided to attempt alternative methods due to the low yields so attained. In 2010 Van Nieuwenhze published a synthesis of ß-hydroxy enduracididine in which pyridinium *para*-toluene sulphonate (**PPTS**) is used to activate the epoxide and induce ring opening (**Scheme 118**).[198]



303 **304**

Reagents and conditions: NaN_3, DMF, PPTS, 3d, 44%.

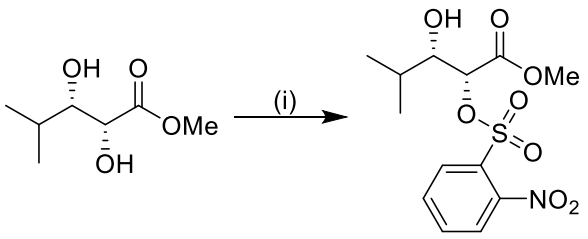
Scheme 118: Opening of epoxide **303** with sodium azide and PPTS

Unfortunately, with our substrate, we failed to isolate any product (**Entry 5**). Switching to ammonium chloride as the salt also failed to produce any of the desired product cleanly enough to isolate (**Entry 6**). In 2002, Sabitha *et al.* published a procedure for opening epoxides activated by the weakly acidic nature of oxone®.[199] Although attempted, in our system no product was observed on TLC after 64 h (**Entry 8**). Finally, substoichiometric cerium chloride

heptahydrate was used to activate the epoxide following a procedure once again by Sabitha *et al.*[200] The 3 h reflux unfortunately only provided trace amounts of the azido alcohol. Leaving the reaction overnight, however, produced the desired product, but unfortunately in poor yield (**Entries 7** and **9**). We believe that the epoxide is too unstable to be effectively opened by the azide, usually producing a complex mixture rather than our desired product, if reacting at all. We therefore decided to attempt an alternative procedure.

2.5.13 Nosylation of the α-hydroxyl group

Also developed by Sharpless, was a method for activating the alpha hydroxyl group of α,β dihydroxy esters with *ortho*-nosyl chloride.[34] A range of diol esters were monosylated in the alpha position with nosyl chloride and triethylamine in DCM. The surprisingly high regioselectivity of this reaction could be due to the greater acidity of the alpha alcohol due to the electron withdrawing properties of the ester. The steric bulk of the sulphonate ester could be the reason the reaction stops at the mono-substituted product and does not react further to the *bis*. This methodology has been utilized in the synthesis of a hydroxy tyrosine derivative[201] and ß-hydroxy enduracididine.[198] Following treatment of **48** with nosyl chloride, the desired product **305** was obtained in fair yield (**Scheme 119**).



48 **305**

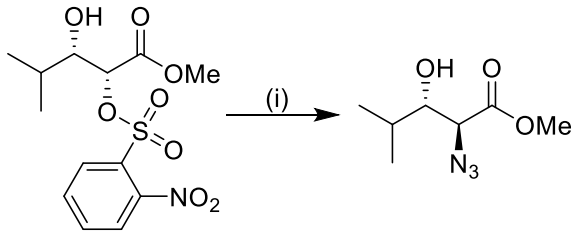
Reagents and conditions: (i) *ortho*-Nosyl chloride (1.3 equiv), NEt_3 (1 equiv), DCM, 3 h, rt, 62%.

Scheme 119: *ortho*-Nosylation of the α-position in **48**

Column chromatography was necessary in order to separate the excess nosyl chloride. Analysis by HPLC with a chiral stationary phase was used to ensure that no racemization had occurred; **305** was found to have consistent ee with by-product **288** (**Scheme 100**) at 96%. With the alpha position now activated, sodium azide should be able to efficiently displace the nosyl group.

2.5.14 Sodium azide displacement of the nosyl group

Stirring the monosylated diol ester **305** with sodium azide produced a clean displacement. Analysis of the ^{1}H NMR spectrum after work up showed that no by-products had been formed (**Scheme 120**). The ^{1}H NMR spectra of the azide matched the ^{1}H NMR spectra of Corey's (**Scheme 83**), completing our route to the valuable intermediate **264**.[39]



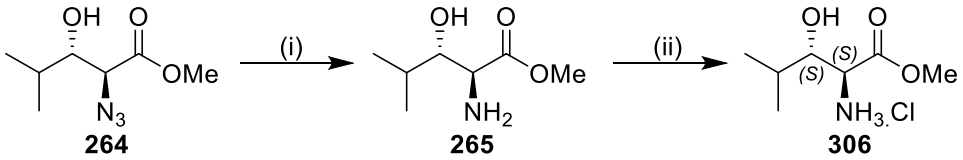
305 **264**

Reagents and conditions: NaN_3 (2.9 equiv), DMF, 20 h, rt, 95%

Scheme 120: Displacement of the nosyl group with sodium azide

2.5.15 Azide reduction and formation of the hydrochloride salt

Although we had been able to synthesize an advanced known intermediate of *anti*-hydroxy leucine, we desired a crystal structure of the final compound for completion. Reduction of the azide with Pd/C produced the free amine as an oil. The hydrochloride salt was also formed in the hope of forming a solid, but unfortunately we were unable to produce crystallizable material.



264 **265** **306**

Reagents and conditions: (i) H_2, Pd/C, MeOH, 41%; (ii) Methanolic HCl (quant).

Scheme 121: Reduction of azide **264** and formation of the amine hydrochloride salt **306**

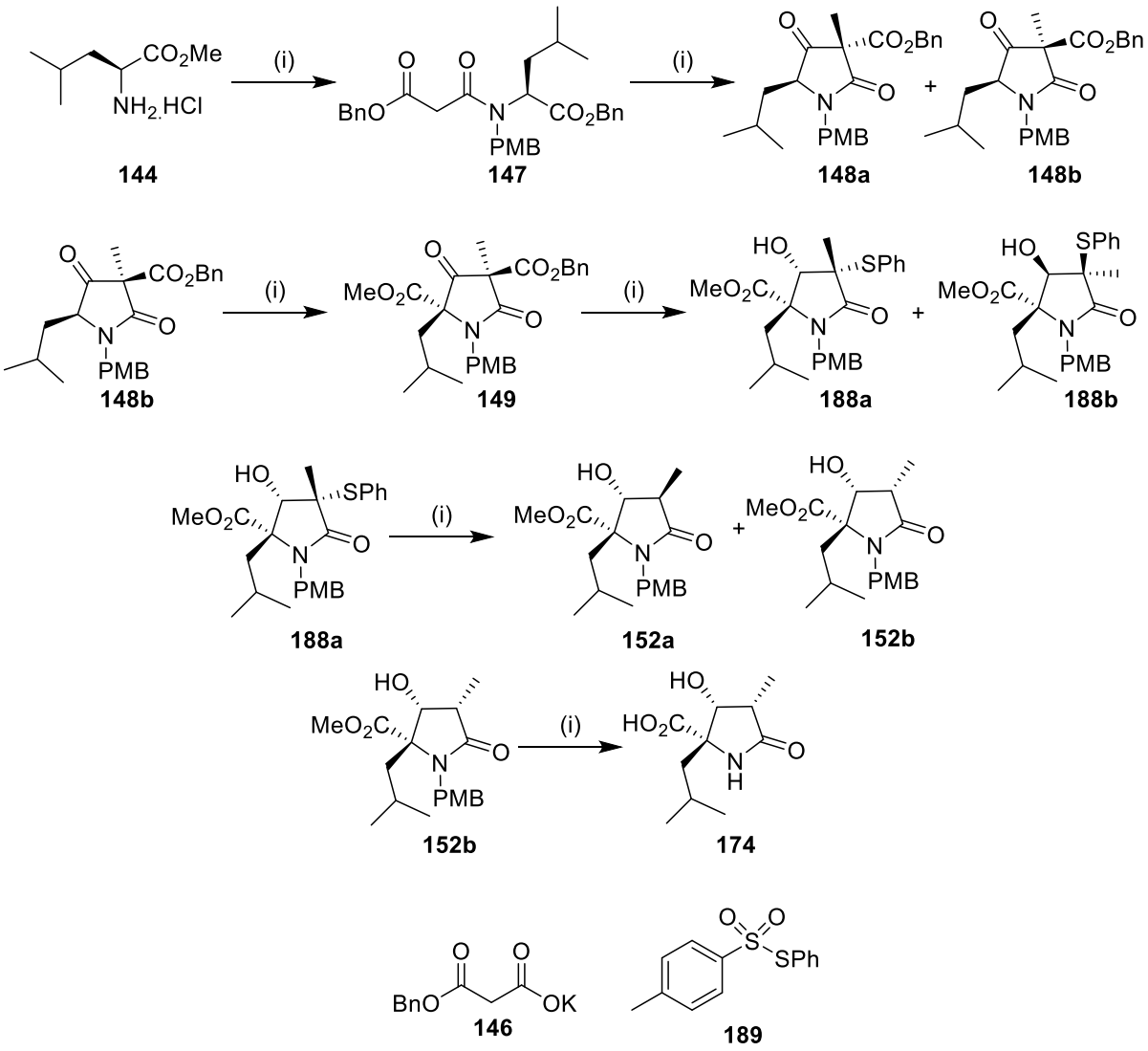
Due to time constraints we were unable to take the amino acids through our methodology to synthesize omuralide and analogues. Nevertheless, we had been able to synthesize both of the diastereoisomers of hydroxy leucine methyl ester through a short and simple method.

2.6 Conclusion and future work

2.6.1 L-Leucine as a starting material

We have successfully been able to incorporate leucine into a γ-lactam core, producing to date the shortest route to the full carbon skeleton of omuralide (4 steps). This lactam has been elaborated to provide a formal synthesis of the omuralide analogue C9-deoxyomuralide in the shortest non-racemic route to date (**Scheme 122**). The previous enantioselective route was completed by Corey in a total of 19 steps and overall yield of 10%, our synthesis took only 11 steps, unfortunately however, the yield was lower at 0.6%. During this route, a diastereoselective desulphurization to **152b** has been extensively studied and optimized. We believe this desulphurization will be an important step in our future work on the synthesis of omuralide and analogues.

Although vast improvements have been made to the enantioselectivity of this route, our final ee of 77% could potentially be improved by either further optimization of the Dieckmann cyclization step, or recrystallization to enrich ee. Further improvements to our synthesis which could be addressed include the production of unwanted diastereoisomers such as **148a** and the thiophenylated analogue, resulting in large quantities of **188b** being isolated.

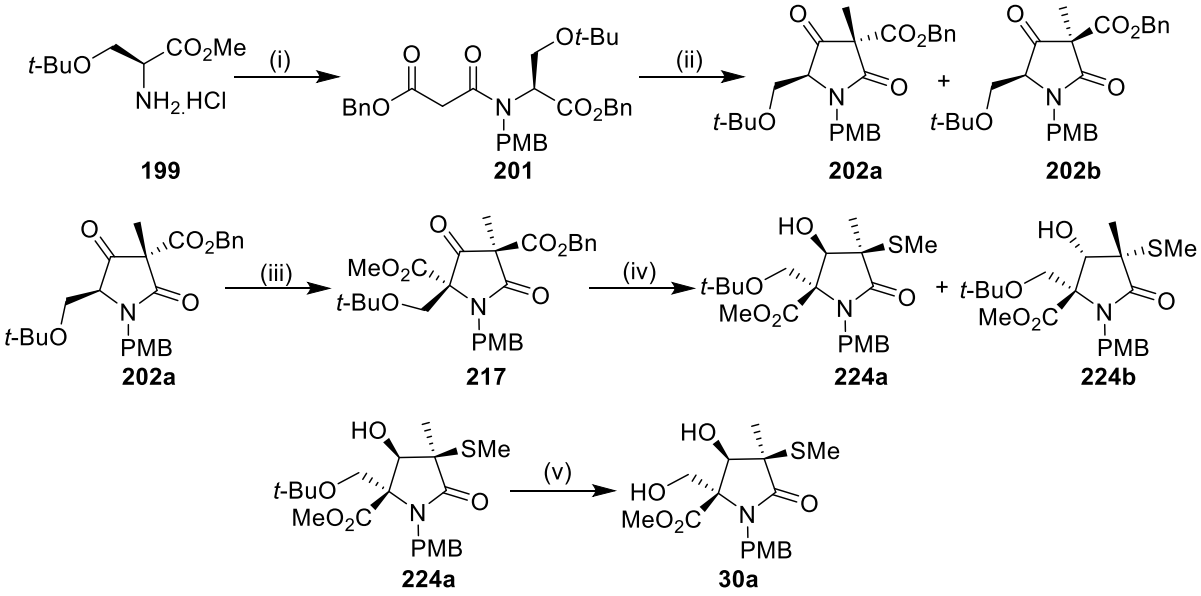


Reagents and conditions: (i) 1) *p*-Methoxybenzaldehyde (1.1 equiv), Et_3N (1 equiv), MeOH, $NaBH_4$ (1.9 equiv), 0 °C to rt, 76%; 2) **146**, EDAC.HCl (2.6 equiv), DMAP (0.25 equiv), *N*-MM (2.3 equiv), DCM, 93%; (ii) TBAF in THF (3.6 equiv), THF, rt, 0.5 h, then MeI (4 equiv), 0 °C to rt, 16 h, 57 %, Ratio 1:2 by crude NMR analysis; (iii) LiHMDS (2.1 equiv), DMPU (2.2 equiv), THF, −78 °C, 0.5 h, then $NCCO_2Me$ (3.2 equiv), −78 °C, 4 h, 70%; (iv) 1) H_2, $Pd(OH)_2/C$, THF, 30 °C, 12 h; 2) **189** (2.9 equiv), Et_3N (1.6 equiv), CH_2Cl_2, rt, mixture of **187ab**, 67% over the 2 steps. 3) $NaBH_4$ (0.7 equiv), EtOH, −10 °C, 0.5 h, **188a**-30% and **188b**-35%; (v) TTMS (3.2 equiv), AIBN (0.3 equiv), acetone, reflux, 16 h, **152a**-14 %, **152b**-74%; (vi) 1) CAN (5.2 equiv), $MeCN/H_2O$ (3:1), rt, 62%; 2) NaOH (0.5 M), 0-5 °C, 86%.

Scheme 122: Our final route to the formal synthesis of C9-deoxy omuralide

2.6.2 L-Serine as a starting material

In addition to L-leucine, L-serine has been incorporated into the lactam core in an analogous fashion (**Scheme 123**). Although some changes to our methodology were required, the overall procedures remained the same. Diasteroselectivity during the Dieckmann cyclization step was investigated due to a surprising change from our leucine route. This change, however, worked to our advantage, allowing the natural enantiomer of omuralide to be synthesized.



Reagents and conditions: (i) 1) **PMB sulphite adduct** (1.6 equiv), $NaBH_3CN$ (6.1 equiv), Et_3N (0.9 equiv), MeOH, 0 °C, 16 h 2) **146** (2.5 equiv), EDAC.HCl (2.7 equiv), N-MM (2.5 equiv), DMAP (0.2 equiv), DCM, 16 h; 49% over the 2 steps. (ii) TBAF (2.1 equiv), Ether, 5 m, THF, MeI (10 equiv), -12 °C, 64 h 66%, ratio 3:1 by crude NMR analysis; (iii) LiHMDS (2.2 equiv), DMPU (3 equiv), THF, -40 °C 30 m then $NCCO_2Me$ (4.5 equiv), 3 h, 69%; (iv) 1) $Pd(OH)_2/C$ (cat), H_2, 35 °C, 16 h; 2) **186** (1.7 equiv), Et_3N (1.1 equiv), DCM, 4 h (70% over 2 steps); 3) $NaBH_4$ (0.6 equiv), EtOH, 30 m 0 °C, **224a** 54%, **224b** 13%, **219ab** 4%. (v) TFA/DCM 1:1, 1.5 h, 75%.

Scheme 123: Our final route to the formal synthesis of omuralide

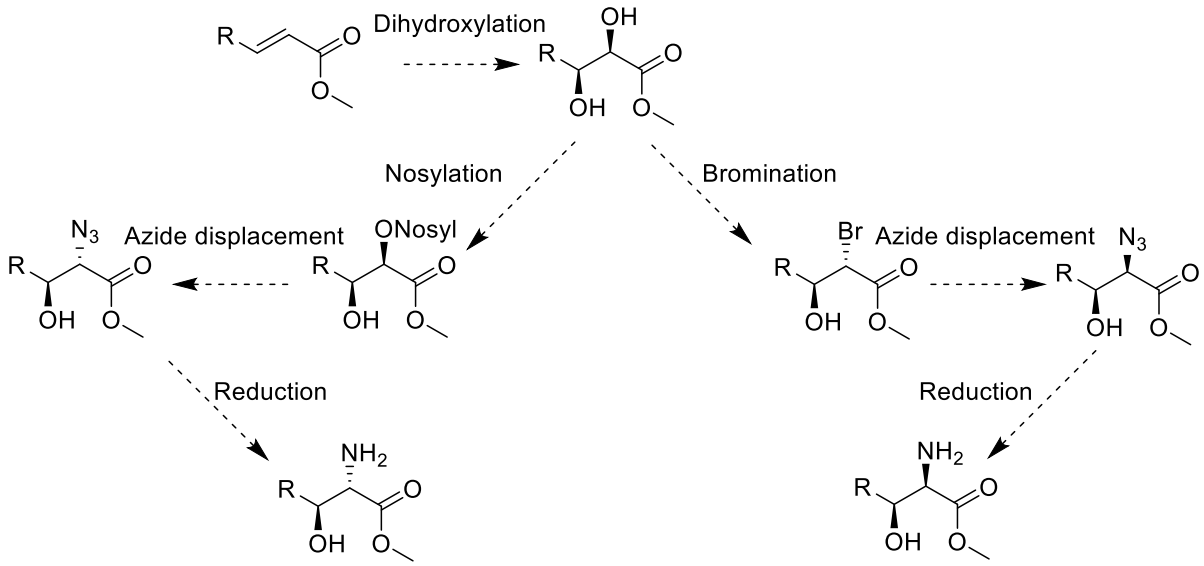
The lactam core was elaborated to an advanced intermediate of the synthesis of Corey, completing a formal synthesis. Although synthesized in an equal number of steps (8 steps),[29] our route's initial chirality is derived from cheap amino acid precursors. Corey required an enzymatic reaction with PLE, potentially providing our method with a considerable cost advantage. Corey's route however was higher yielding with 47% overall yield compared to

our 5%. The analogue produced has been elaborated by Corey to a library of C5 and C9 analogues.[37] In recent years, this position has received renewed interest due to the discovery of the salinosporamides[41] and cinnabaramides.[44]

Work has also been initiated towards the synthesis of salinosporamide B; unfortunately it appeared that our lactam core was too sterically congested to allow acylation at the C5 position, leading us to abandon our attempts. Future work should concentrate on modification of the serine protecting group, potentially allowing access to this position.

2.6.3 The synthesis of hydroxy leucine

We have also developed a new method to provide either diastereoisomer of hydroxy leucine. Correct choice of ligand during the dihydroxylation should also allow the opposite enantiomers to be synthesized. In the future, work should concentrate on the incorporation of these into the lactam core, potentially providing a rapid route to omuralide. We hope that this methodology could also be applied to other α,β unsaturated esters to synthesize other β-hydroxy amino acids (**Scheme 124**) for incorporation into the lactam to produce a wider array of analogues.



Scheme 124: Potential method to synthesize various β-hydroxy, α-amino acids.

2.6.4 Application of our methodology to new natural product targets

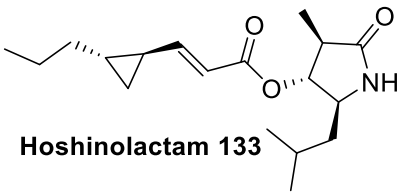
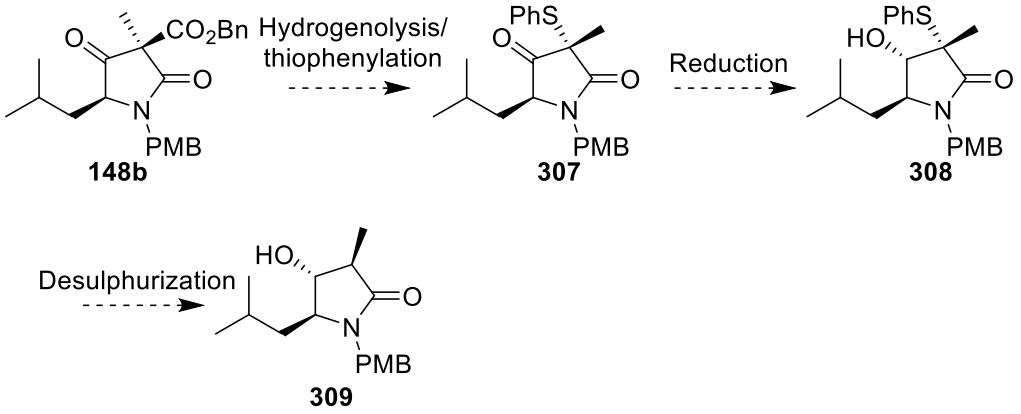


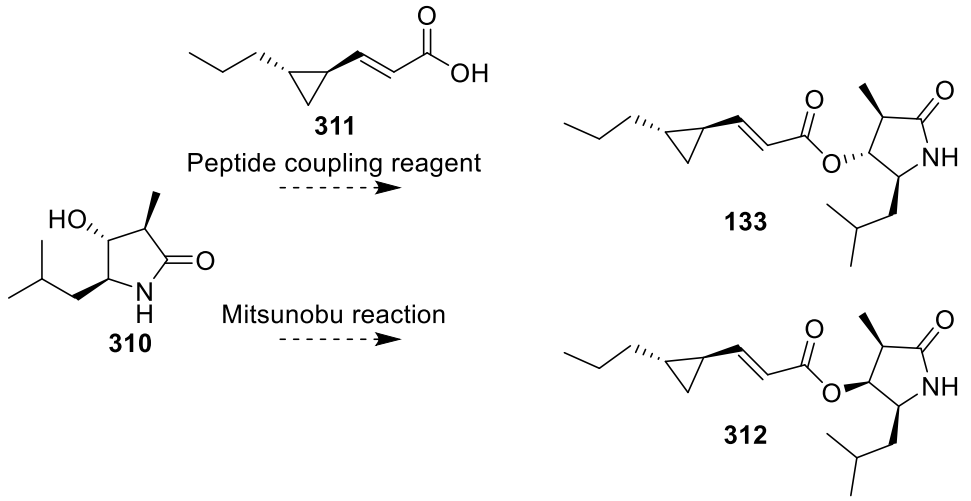
Figure 24: The structure of Hoshinolactam

We believe our methodology is not only useful in the synthesis of omuralide analogues but could also be applied to other γ-lactam natural products. In 2007, the discovery and structure elucidation by total synthesis of hoshinolactam **133** was reported.[139] Hoshinolactam was found to have potent antitrypanosomal activity without cytotoxicity against human fetal lung MRC-5 cells. Despite these promising results, however, a SAR study has currently not yet been reported nor the cellular target of hoshinolactam identified. Structurally, hoshinolactam has a strikingly similar core to many of our intermediates, particularly in our route to C9-deoxyomuralide. For example, intermediate **148b** has the potential to be elaborated into the lactam core of hoshinolactam in four steps.



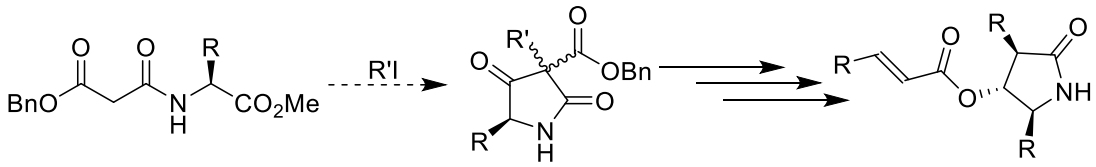
Scheme 125: Synthesis of the lactam core of hoshinolactam from **148b**

Depending on the method used, coupling this core to the known acid **311** could produce either hoshinolactam or the un-synthesized epimer **312**.



Scheme 126: Potential synthesis of hoshinolactam from lactam **310**

Modification of the amino acid starting material, and the alkylation agent during the Dieckmann cyclisation, should produce a wide array of analogues which could be used to further study the interesting properties of hoshinolactam (**Scheme 127**).



Scheme 127: Potential route to a library of hoshinolactam analogues

To summarize, our studies have developed methodology allowing amino acids to be incorporated into γ-lactam rings. This procedure has been used to produce formal syntheses of C9-deoxyomuralide and omuralide. The reactions developed in this not only are useful in the synthesis of omuralide analogues but are also applicable in other γ-lactam natural products. When combined with the methodology developed to produce β-hydroxy, α-amino acids, this should allow access to a wide variety of biologically relevant natural product cores and their derivatives.

3.0 Experimental

3.1 General experimental

3.1.1 Preparation of glassware, solvents and reagents

Reactions requiring anhydrous conditions were carried out under a nitrogen atmosphere, unless otherwise stated, using flame-dried glassware. Reaction solvents were dried using the following methods: dichloromethane was distilled over calcium hydride, tetrahydrofuran and diethyl ether were distilled under an argon atmosphere from the sodium/benzophenone ketyl radical and if anhydrous acetone was required, an unopened bottle was used.

3.1.2 Analysis of compounds

The infrared spectra was recorded using, a PerkinElmer Spectrum 100 IR spectrophotometer and the sample ran as a thin film of their evaporated solution from CH_2Cl_2 or $CHCl_3$ on sodium chloride plates. A PerkinElmer spectrum 2 was used if the data states the sample was ran as a solid. ^{1}H and ^{13}C NMR spectra were measured respectively at 500 and 126 MHz using a Bruker Ascend 500 or at 400 and 100 MHz using a Bruker Ultrashield 400 Plus instrument. The solvent used for NMR spectroscopy was deuteriated chloroform unless stated otherwise, using tetramethylsilane as the internal reference or the residual deuterated solvent peak. For ^{13}C experiments run in deuterium oxide, methanol was added and the resulting peak at δc 49.50 was used as the reference. Chemical shifts are given in parts per million, and *J* values are given in hertz. Mass spectra were recorded by the EPSRC Mass Spectrometry Service at the University of Swansea; the ionization and detection technique is stated alongside the data. HPLC was carried out on a VWR Elite Lachrom instrument. Separation was achieved by either an AD-H Chiralpak column (4.6 mm × 250 mm 5 μm) or Eurocel 01 Knauer (4.6 mm × 250 mm 5 μm) under the stated conditions. Melting points were recorded using a Büchi B-545 melting point instrument. Optical rotation values were measured with a Bellingham and Stanley ADP-440 instrument, operating at λ = 589 nm, corresponding to the sodium D line, at the temperatures indicated. Spectrophotometric grade chloroform was used for these measurements unless otherwise stated; solutions for these measurements were prepared in a 1 mL volumetric flask for maximum accuracy of the solvent volume used.

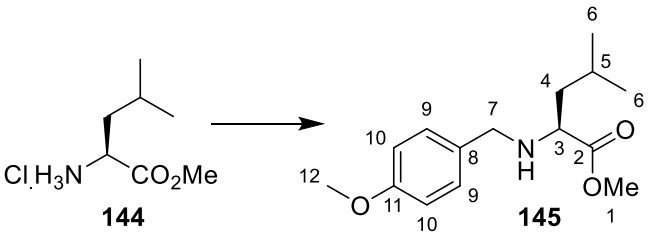
3.1.3 Chromatographic techniques

All chromatographic manipulations used silica gel as the adsorbent. Reactions were monitored using thin layer chromatography (TLC) on aluminum with Merck Kieselgel 60 F254 silica gel. TLC plates were visualized by UV radiation at a wavelength of 254 nm or stained by exposure to an ethanolic solution of phosphomolybdic acid or aqueous potassium permanganate, followed by heating at 220 °C. Purification by column chromatography used Material Harvest silica gel 60.

3.2 Individual experimental procedures and characterization

3.2.1 Experimental for procedures starting from L-leucine

2-(4-Methoxy-benzylamino)-4-methyl-pentanoic acid methyl ester 145[78,75]



Unintentional, racemic synthesis using the Dean-Stark apparatus:

Leucine methyl ester hydrochloride **144** (9.96 g, 55 mmol) and *p*-methoxybenzaldehyde (7.4 mL, 61 mmol, 1.1 equiv) were dissolved in toluene (100 mL). Acetic acid (2 mL, 35 mmol) was added and the solution heated to vigorous reflux with a well-insulated Dean−Stark apparatus overnight. The resulting solution was evaporated to dryness to produce a thick red/brown oil, which was dissolved in methanol (130 mL). Acetic acid was added (2.8 mL, 50 mmol, 0.9 equiv) and the reaction mixture cooled to 0 °C. Sodium cyanoborohydride (6.9 g, 110 mmol, 2 equiv) was added in small portions and the reaction stirred for 30 m. The solution was allowed to reach room temperature and stirred for a further 5 h. A few drops of water were added, and the solution was evaporated to dryness. The resulting oil was dissolved in dichloromethane (150 mL) and washed twice each with equal amounts of water, brine, and aqueous sodium carbonate. The organic layer was dried over magnesium sulphate, filtered, and evaporated to dryness to produce compound **145** as a red-brown oil (14 g, 96%), which was used without further purification.

Non-racemizing synthesis:

Leucine methyl ester hydrochloride **144** (0.19 g, 1.05 mmol) was dissolved in methanol (10 mL). To this solution was added Et_3N (0.15 mL, 1.0 mmol), and *p*-methoxybenzaldehyde (0.15 mL, 1.2 mmol). The mixture was stirred for 90 m, cooled to 0 °C, and sodium borohydride (0.82 g, 2.0 mmol) was added in portions over 30 m. The mixture was stirred for a further 30 m. The solvents were removed under reduced pressure and the resulting oil was dissolved in ethyl acetate (approximately 50 mL). The organic solution was washed twice each with equal

amounts of water, brine, and aqueous sodium carbonate. The aqueous layer was extracted with ethyl acetate, and the organic fractions were combined, dried over magnesium sulphate, filtered and the solvents removed under reduced pressure. The resulting residue was purified by column chromatography on silica gel using petroleum ether (40-60 °C)/ethyl acetate (2:1) as the eluent to afforded **145** as a colourless oil (0.21 g, 76%). $[\alpha]^{D\ 25}$ = −40.3 (*c* 1.3, $CHCl_3$), (lit[78] $[\alpha]^{D\ 27}$ = −31.2 (*c* 1.5, $CHCl_3$)); ν_{max} (neat)/cm^{-1}: 3331 N-H (b), 2997 C-H (s), 2955 C-H (s), 1736 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.23 (d, *J* = 8.5 Hz, 2H, H10), 6.84 (d, *J* = 8.6 Hz, 2H, H9), 3.78 (s, 3H, H12), 3.73 (d, *J* = 12.7 Hz, 1H, H7), 3.71 (s, 3H, H1), 3.54 (d, *J* = 12.7 Hz, 1H, H7), 3.29 (t, *J* = 7.3 Hz, 1H, H3), 1.81 – 1.69 (m, 2H, H3), 1.46 (td, *J* = 7.3, 2.0 Hz, 2H, H4), 0.90 (d, *J* = 6.6 Hz, 3H, H6), 0.84 (d, *J* = 6.6 Hz, 3H, H6); ^{13}C NMR (126 MHz, $CDCl_3$) δC 176.6 C2, 158.8 C11, 129.6 C10, 113.9 C9, 59.2 C3, 55.4, 51.7 C7, 51.7 C1, 42.9 C4, 25.0 C5, 22.9 C6, 22.3 C6; one peak unobserved in ^{13}C NMR spectrum. Determined by HPLC to be >99.5% ee. Determination of ee was found by HPLC using an AD-H Chiralpak column 95:5 hexane/IPA, 230 nm, 0.8 mL/min, 25 °C.

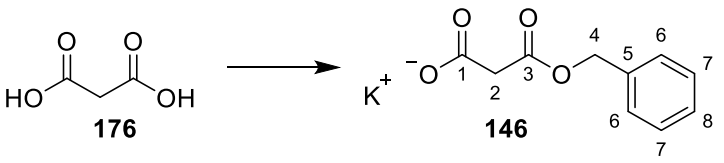
Intentional racemic synthesis:

Acetyl chloride (3.3 mL, 46.41 mmol, 3 equiv) was added to methanol (60 mL) at 0 °C and the solution left for 40 m. L-D leucine (2.003 g, 15.26 mmol) was added in one portion. The solution was allowed to stir at 0 °C for a further 30 m, then heated to reflux overnight. The solvents were evaporated under reduced pressure to provide L-D leucine methyl ester hydrochloride **144** (2.748 g, quant) which was used without further purification.

LD - Leucine methyl ester hydrochloride **144** (1.819 g, 10.01 mmol) was dissolved in methanol (71 mL). To this solution was added Et_3N (1.4 mL, 9.96 mmol, 1 equiv), and *p*-methoxybenzaldehyde (1.3 mL, 10.65 mmol, 1.1 equiv). The mixture was stirred for 90 m, cooled to 0 °C, and sodium borohydride (0.751 g, 19.85 mmol, 2 equiv) was added in portions over 30 m. The mixture was stirred for a further 30 m. The solvents were removed under reduced pressure and the resulting oil was dissolved in ethyl acetate (approximately 250 mL). The organic solution was washed twice each with equal amounts of water, brine, and aqueous sodium carbonate. The aqueous layer was extracted with ethyl acetate, and the organic fractions were combined, dried (magnesium sulphate), filtered, and the solvents removed under reduced pressure. The resulting residue was purified by column chromatography on

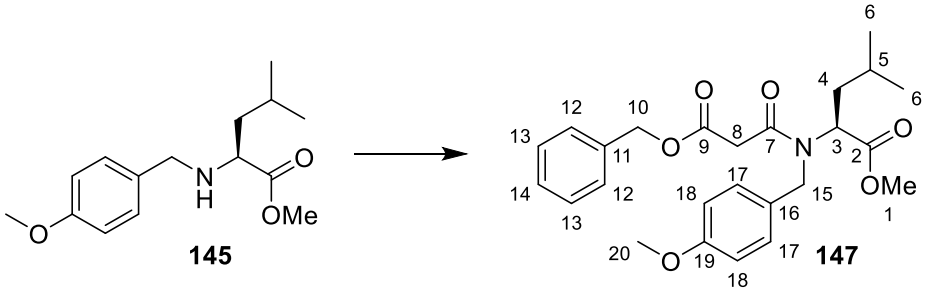
silica gel using petroleum ether (40-60 °C)/ethyl acetate (2:1) as eluent to afforded **145** as a colourless oil (1.806 g, 68%).

Potassium benzyloxycarbonyl acetate 146[67]



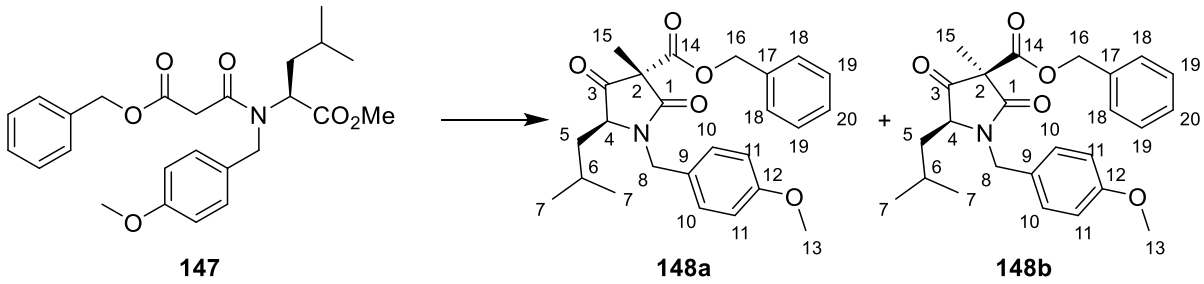
Malonic acid **176** (24.9 g, 0.24 mol, 1 equiv), benzyl alcohol (53 mL, 0.5 mol 2.1 equiv) and *p*-TsOH (0.475 g, 2.75 mmol, 0.01 equiv) were dissolved in toluene (250 mL) and heated to reflux using a Dean-Stark apparatus overnight. The resulting solution was evaporated to produce an orange oil. A solution of KOH in benzyl alcohol (1 M) (240 mL) was added forming a pale-yellow solid, which was collected by vacuum filtration. The solid was washed with ether and transferred to a vacuum oven to produce **146** as a white solid. (41.2 g, 74%). Mp 197-199 °C (lit[2b] 201 °C); ν_{max} (solid)/cm^{-1} 3035 CH unsaturated (s), 1721 C=O (s), 1599, 1370; ^{1}H NMR (500 MHz, D_2O) δH 7.51 – 7.40 (m, 5H), 5.22 (s, 2H), 3.36 (s, 2H); ^{13}C NMR (126 MHz, D_2O) δC 173.9 C1, 171.1 C2, 135.8 Ar, 128.9 Ar, 128.6 Ar, 128.3 Ar, 67.3 C4, 44.7 C2.

2-{(4-Methoxy-benzyl)-[2-(benzyloxycarbonyl)-acetyl]-amino}-4-methyl-pentanoic acid methyl ester 147[75]



Compound **145** (1.38 g, 5.19 mmol), *N*-methylmorpholine (1.3 mL, 11.82 mmol, 2.3 equiv), benzyl malonic half ester **146** (2.54 g, 10.95 mmol, 2.1 equiv), EDAC.HCl (2.63 g, 13.75 mmol, 2.6 equiv) and 4-dimethylaminopyridine (0.16 g, 1.3 mmol, 0.25 equiv) were dissolved in anhydrous dichloromethane (40 mL). The mixture was stirred for 20 h under a nitrogen atmosphere. Aqueous HCl (1 M, 1.5 mL) was added, and the reaction mixture stirred for a further 5 m, then washed with water (2 x 50 mL), and the organic layer dried (magnesium sulphate), filtered, and evaporated under reduced pressure. The resulting oil residue was purified by column chromatography using petroleum ether (40-60 °C)/ethyl acetate as the eluent, to provide the product **147** as a yellow oil (2.128 g, 93%). $[\alpha]^{D\,26} = -46.42$ (*c* 1.12, $CHCl_3$) ν_{max} (neat)/cm^{-1}: 3472, 2956 CH (s), 1740 C=O (s), 1651 C=O (s, Amide); ^{1}H NMR (500 MHz, $CDCl_3$) Major rotamer: δH 7.40 – 7.31 (m, 5H, H12, 13, 14), 7.15 (d, *J* = 8.7 Hz, 2H, H18), 6.85 (d, *J* = 8.7 Hz, 2H, H17), 5.16 (d, *J* = 2.4 Hz, 2H, H10), 4.83 (t, *J* = 6.9 Hz, 1H, H8), 4.55 (d, *J* = 17.2 Hz, 1H, H15), 4.42 (d, *J* = 17.2 Hz, 1H, H15), 3.79 (s, 3H, H1), 3.58 (s, 3H, H3), 3.48 (d, *J* = 3.6 Hz, 2H, H3), 1.90 – 1.77 (m, 1H, H4), 1.62 – 1.49 (m, 2H, H5), 0.88 (d, *J* = 6.4 Hz, 3H, H6), 0.80 (d, *J* = 6.4 Hz, 3H, H6); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.8, 167.3, 167.3, 159.3, 135.5, 128.7, 128.5, 128.5, 128.1, 114.3, 67.3, 56.2, 55.5, 52.2, 50.1, 41.8, 38.4, 25.2, 22.7, 22.4; one peak unobserved in ^{13}C NMR spectrum. Determined by HPLC to be >99.5% ee. Determination of ee was found by HPLC using an AD-H Chiralpak column 90:10 hexane/IPA, 230 nm, 0.8 mL/min, 25 °C.

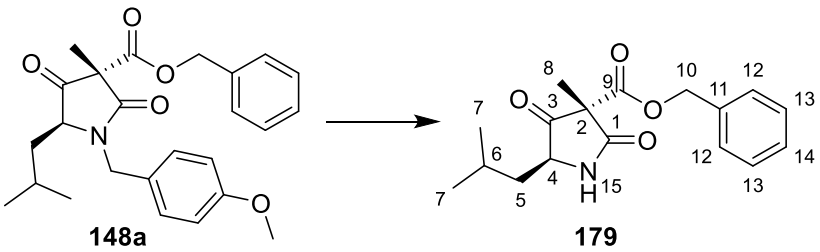
(3S,5S)-N-(4'-Methoxybenzyl)-3-methyl-5-(2'-methylpropyl)-pyrrolidin-2,4-dione-3-carboxylic acid benzyl ester 148a[2a] **and (3R,5S)-N-(4'-methoxybenzyl)-3-methyl-5-(2'-methylpropyl)-pyrrolidin-2,4-dione-3-carboxylic acid benzyl ester 148b**[75]



Tetrabutylammonium fluoride (1 M solution in THF, 11.5 mL, 11.5 mmol, 3.6 equiv) was added to a solution of **147** (1.442 g, 3.2 mmol) in THF (72 mL) at room temperature under a nitrogen atmosphere. The mixture was stirred for 30 m and cooled to 0 °C using an ice bath. Iodomethane (0.81 mL, 13 mmol, 4 equiv) was added and the reaction mixture stirred overnight being allowed to reach room temperature. Water (4 mL) was added, and the solvents were removed under reduced pressure. The resulting residue was dissolved in dichloromethane (50 mL) and washed with water (50 mL). The organic layer was dried over magnesium sulphate and the solvents removed under reduced pressure. The resulting residue was purified and partially separated by column chromatography, using petroleum ether (40−60 °C)/ethyl acetate (9:1) as the eluent to afford the diastereoisomers **148ab** in a 1:2 ratio (0.78 g, 57%). **Data for the minor, first eluting diasteroisomer 148a:** (Yield 16%), $[\alpha]^{D\ 26}$ = +14.54 (*c* 0.44, $CHCl_3$) (9% ee); ν_{max} (thin film)/cm^{-1} 2959 C-H (s), 1777 C=O (s), 1747 C=O (s), 1696 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.37 – 7.34 (m, 3H, H18, 20), 7.24−7.21 (m, 2H, H19), 7.01 (d, *J* = 8.7 Hz, 2H, H11), 6.59 (d, *J* = 8.7 Hz, 2H, H10), 5.42 (d, *J* = 15.0 Hz, 1H, H16 or 8), 5.19 (d, *J* = 12.3 Hz, 1H, H16 or 8), 5.10 (d, *J* = 12.3 Hz, 1H, H16 or 8), 3.84 (dd, *J* = 7.8, 3.9 Hz, 1H, H4), 3.80 (d, *J* = 15.0 Hz, 1H, H16 or 8), 3.73 (s, 3H, H13), 1.81−1.80 (m, 1H, H6), 1.66−1.61 (m, 1H, H5), 1.58 (s, 3H, H15), 1.54−1.50 (m, 1H, H5), 0.87 (d, *J* = 6.7 Hz, 3H, H7), 0.76 (d, *J* = 6.5 Hz, 3H, H7); ^{13}C NMR (126 MHz, $CDCl_3$) δC 206.1 C3, 169.7 C14, 165.7 C1, 159.4 Ar, 134.9 Ar, 129.5 Ar, 128.8 Ar, 128.7 Ar, 128.3 Ar, 126.3 Ar, 114.2 Ar, 68.3 C16, 62.5, 58.8, 55.4 C13, 43.4 C8, 38.0 C5, 24.7 C6, 23.4 C7, 22.5 C7, 16.2 C15. Determined by HPLC to be 9% ee. **Data for the major, second eluting diastereoisomer 148b:** (Yield 41%), $[\alpha]^{D\ 26}$ = −17.14 (c 0.98, $CHCl_3$) (79% ee); ν_{max} (thin film)/cm^{-1} 2958 C-H (s), 1775 C=O (s), 1746 C=O (s), 1696 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.30−7.18 (m, 5H, H18-20), 7.05 (d, *J* = 8.7 Hz, 2H, H11), 6.76

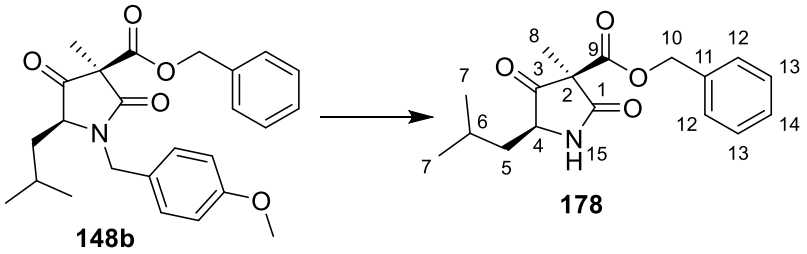
(d, *J* = 8.7 Hz, 2H, H10), 5.18 (d, *J* = 12.1 Hz, 1H, H16 or 8), 5.11 (d, *J* = 14.9 Hz, 1H, H16 or 8), 5.01 (d, *J* = 12.1 Hz, 1H, H16 or 8), 3.94 (d, *J* = 14.9 Hz, 1H, H16 or 8), 3.71 (s, 3H, H13), 3.60 (t, *J* = 6.9 Hz, 1H, H4), 1.71−1.63 (m, 1H, H6), 1.46 (s, 3H, H15), 1.42 (t, *J* = 6.7 Hz, 2H, H5), 0.65 (d, *J* = 6.6 Hz, 3H, H7), 0.62 (d, *J* = 6.6 Hz, 3H, H7); ^{13}C NMR (126 MHz, $CDCl_3$) δC 205.7 C3, 169.4 C14 or 1, 165.7 C14 or 1, 159.5 Ar, 134.8 Ar, 129.5 Ar, 128.8 Ar, 128.7 Ar, 128.5 Ar, 127.2 Ar, 114.4 Ar, 68.3, 62.5, 58.7, 55.4 C13, 43.6 C8, 39.0 C5, 24.5 C6, 23.1 C7, 22.0 C7, 16.7 C15. Determined by HPLC to be 79% ee. Determination of ee was found by HPLC using an AD-H Chiralpak column 95:5 hexane/IPA, 230 nm, 0.8 mL/min, 25 °C.

Benzyl (3S,5S)-3-methyl-5-(2-methylpropyl)-2,4-dioxopyrrolidine-3-carboxylate 179



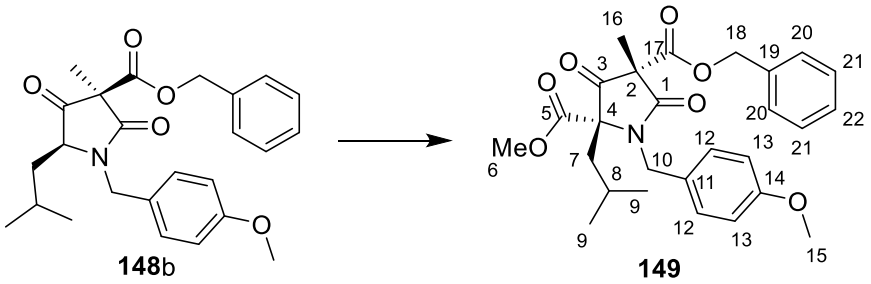
Lactam **148a** (1.2023 g, 2.83 mmol) was dissolved in a MeCN/water mixture (3:1, 27.6 mL). CAN (8.3 g, 14.22 mmol, 5 equiv) was added and the solution stirred until complete consumption of the starting material had occurred (approx. 2 h). The solution was diluted with water (150 mL) and extracted with ethyl acetate (150 mL x 3). The organic layers were combined and washed with brine (100 mL x 2), dried (sodium sulphate), filtered, and the solvents were removed under reduced pressure. The resulting residue was purified by column chromatography using petroleum ether (40-60 °C)/ethyl acetate (8:2) as the eluent to produce **179** as a white crystalline solid. (0.725 g, 84%). Mp 118-124 °C; $[\alpha]^{D\ 24}$ = +11.6 (*c* 2.3, $CHCl_3$) (9% ee); ν_{max} (thin film)/cm^{-1}: 3209, 2960 CH (s), 1781 C=O (s), 1748 C=O (s), 1705 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.38-7.30 (m, 3H, H14, 12), 7.29−7.23 (m, 2H, H13), 7.02 – 6.60 (m, 1H, H15), 5.24 - 511 (m, 2H, H10), 4.18 (dd, *J* = 9.5, 3.5 Hz, 1H, H4), 1.78−1.68 (m, 2H, H5), 1.53 (s, 3H, H8), 1.43 (m, 1H, H7), 0.96 (d, *J* = 6.2 Hz, 3H, H7), 0.94 (d, *J* = 6.2 Hz, 3H, H7); ^{13}C NMR (126 MHz, $CDCl_3$) δC 206.7, 171.9, 165.5, 135.0, 128.8, 128.6, 127.9, 68.1 C10, 61.4, 58.6, 41.3 C5, 25.2 C6, 23.2 C7, 21.5 C7, 15.9 C8; HRMS (NSI-FTMS) m/z $[M + NH_4]^+$ calcd for $[C_{17}H_{25}N_2O_4]^+$ 321.1809, found 321.1812.

Benzyl (3R,5S)-3-methyl-5-(2-methylpropyl)-2,4-dioxopyrrolidine-3-carboxylate 178



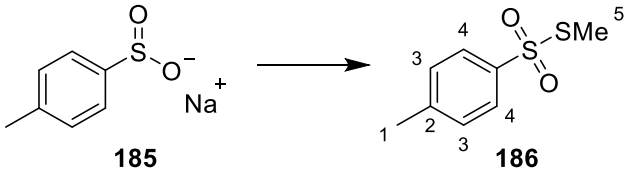
Lactam **148b** (0.1174 g, 0.277 mmol) was dissolved in a MeCN/water mixture (3:1, 2.8 mL). CAN (0.1634 g, 0.29 mmol, 1 equiv) was added and the solution stirred for 3 h. A further portion of CAN was added (0.6482 g, 1.18, 4.3 equiv) and the solution stirred until complete consumption of the starting material had occurred. The solution was diluted with water (50 mL) and extracted with ethyl acetate (50 mL x 3). The organic layers were combined and washed with brine (50 mL), dried (magnesium sulphate), filtered and the solvents were removed under reduced pressure. The resulting residue was purified by column chromatography using petroleum ether (40-60 °C)/ethyl acetate (9:1 to 2:1) as the eluent to produce **178** as a grey oil (0.0526 g, 63%). $[\alpha]^{D\ 22}$ = -34.3 (c 0.7, $CHCl_3$) (79% ee); ν_{max} (thin film)/cm^{-1}: 3215, 2959 C-H (s), 1779 C=O (s), 1748 C=O (s), 1703 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.37-7.31 (m, 3H, H14, 12), 7.28−7.25 (m, 2H, H13), 6.67 (d, *J* = 38.5, 1H, H15), 5.22 (d, *J* = 12.2, 1H, H10), 5.10 (d, *J* = 12.2, 1H, H10), 4.00 (dd, *J* = 9.7, 4.4 Hz, 1H, H4), 1.70−1.56 (m, 2H, H5), 1.55 (s, 3H, H8), 1.47 (m, 1H, H6), 0.87 – 0.83 (m, 6H, H7); ^{13}C NMR (126 MHz, $CDCl_3$) δC 206.8, 171.5, 165.5, 134.8, 128.8, 128.8, 128.4, 68.4 C10, 61.1 C2 or 4, 58.2 C2 or 4, 41.7 C5, 25.2 C6, 23.0 C7, 21.5 C7, 16.3 C8; HRMS (NSI-FTMS) m/z $[M + Na]^+$ calcd for $[C_{17}H_{21}N_1O_4Na]^+$ 326.1363, found 326.1363.

(3R,5R)-N-(4'-Methoxybenzyl)-3-methyl-5-(2'-methylpropyl)pyrrolidin-2,4-dione-3,5 dicarboxylic Acid 3-Benzyl Ester 5-Methyl Ester 149[75]



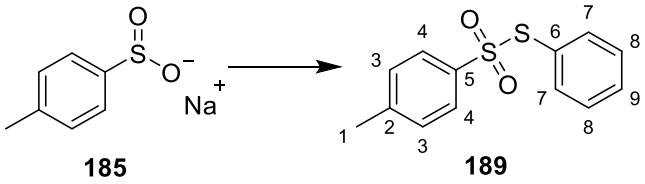
Compound **148b** (1.747 g, 4.125 mmol) was dissolved in anhydrous THF (70 mL) in a flame dried flask and the solution cooled to −78 °C. DMPU (1.5 mL, 9.2 mmol) was added followed by LiHMDS (1 M in THF, 8.5 mL, 8.5 mmol) and the mixture stirred at −78 °C for 30 m under an atmosphere of nitrogen. Methyl cyanoformate (1.1 mL, 13 mmol) was added and the mixture stirred for a further 4 h at −78 °C. Saturated aqueous ammonium chloride was added (2 mL) at −78 °C, the mixture was allowed to warm to room temperature, and the organic solvents were removed under reduced pressure. The residue was dissolved in ethyl acetate and the solution washed with water (2 × 100 mL) and brine (2 × 100 mL). The organic layer was dried (sodium sulphate), filtered, and evaporated under reduced pressure. The residue was purified by column chromatography using petroleum ether (40−60 °C)/ethyl acetate (4:1) as the eluent to yield **149** as a waxy solid (1.985 g, 70%). $[\alpha]^{D\ 21}$ = −20.35 (*c* 1.12, $CHCl_3$) (79% ee); ν_{max} (thin film)/cm^{-1} 2959 C-H (s), 1783 C=O (s), 1752 C=O (s), 1698 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.38 – 7.28 (m, 5H, H20, 21, 22), 7.19 (d, *J* = 8.7 Hz, 2H, H13), 6.77 (d, *J* = 8.7 Hz, 2H, H12), 5.19 (d, *J* = 12.1 Hz, 1H, H18), 5.14 (d, *J* = 12.1 Hz, 1H, H18), 4.89 (d, *J* = 15.1 Hz, 1H, H10), 4.15 (d, *J* = 15.1 Hz, 1H, H10), 3.77 (s, 3H, H15), 3.23 (s, 3H, H6), 2.14 (dd, *J* = 15.2, 5.5 Hz, 1H, H7), 1.86 (dd, *J* = 15.2, 6.3 Hz, 1H, H7), 1.72 (s, 3H, H16), 1.44 (d, *J* = 6.5 Hz, 1H, H8), 0.64 (d, *J* = 6.6 Hz, 3H, H9), 0.52 (d, *J* = 6.6 Hz, 3H, H9); ^{13}C NMR (126 MHz, $CDCl_3$) δC 201.9, 170.9, 167.7, 165.3 Ar, 159.3 Ar, 134.6 Ar, 130.4 Ar, 128.8 Ar, 128.7 Ar, 128.7 Ar, 127.7 Ar, 113.9 Ar, 76.2 C18, 68.6, 58.5, 55.4 C15, 53.1 C6, 44.0 C10, 38.8 C7, 24.3 C8, 23.5 C9, 23.1 C9, 19.1 C16.

S-Methyl 4-methylbenzene-1-sulfonothioate 186[85]



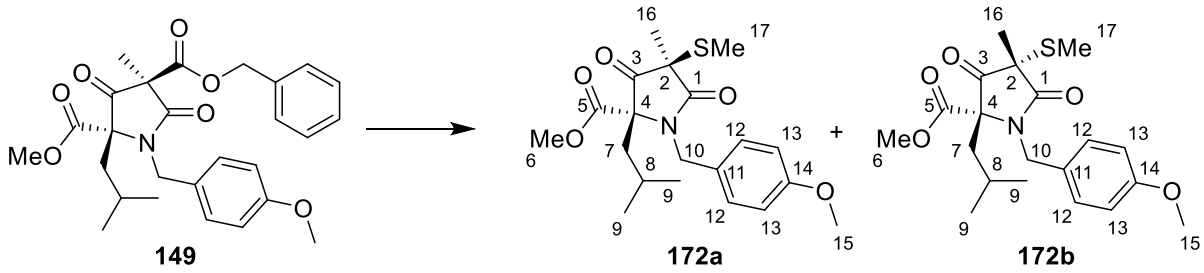
Sodium *p*-toluenesulphinate **185** (9.930 g, 55.7 mmol, 3.1 equiv) was dissolved in DCM, $(SMe)_2$ (1.6 mL, 17.7 mmol, 1 equiv) was added with iodine (8.988 g, 35 mmol, 2 equiv) and the reaction stirred vigorously. The reaction was left stirring overnight at room temperature. Saturated sodium thiosulphate solution was added until the iodine colour was removed. The reaction was them washed with an equal amount of water and the organic layer was dried (sodium sulphate), filtered and evaporated. Sulphonothioate **186** was obtained as a yellow crystalline solid which could be used without further purification (5.47 g, 76%). Mp 56−57 °C, (lit[85] 54-55 °C); ν_{max} (thin film)/cm^{-1} 2996 C-H (s), 2926 1593; ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.81 (d, *J* = 8.4 Hz, 2H, H3), 7.35 (d, *J* = 8.0 Hz, 2H, H4), 2.50 (s, 3H, H5), 2.46 (s, 3H, H1); ^{13}C NMR (126 MHz, $CDCl_3$) δC 144.9 Ar, 141.1 Ar, 130.0 Ar, 127.3 Ar, 21.8 C1, 18.2 C5.

S-Phenyl 4-Methylbenzene-1-sulfonothioate 189[96]



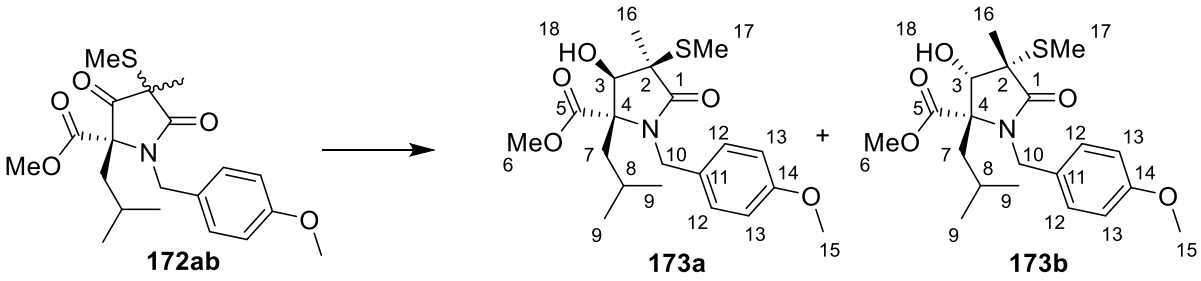
Sodium *p*-toluenesulfinate **185** (1.20 g, 6.7 mmol, 1.2 equiv) and iodine (0.74 g, 2.9 mmol, 0.5 equiv) were dissolved in DCM (15 mL). Diphenyl disulfide (0.55 g, 2.9 mmol, 0.5 equiv) was added with vigorous stirring, and the mixture stirred overnight. Aqueous sodium thiosulfate (1 M) was added until the iodine colour was removed. The mixture was washed with water (100 mL x 2), dried (sodium sulphate), filtered, and the solvents were removed under reduced pressure to give an oil that crystallized on cooling, to afford **189** as a colourless solid (1.0 g, 67%). Mp 71−75 °C, (lit[202] 74–75 °C); ν_{max} (thin film)/cm^{-1} 3063, 1594; ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.50−7.30 (m, 7H, H9, 8, 7, 3), 7.20 (d, *J* = 8.0 Hz, 2H, H4), 2.42 (s, 3H, H1); ^{13}C NMR (126 MHz, $CDCl_3$) δC 144.8 Ar, 140.5 Ar, 136.7 Ar, 131.4 Ar, 129.5 Ar, 129.5 Ar, 128.2 Ar, 127.8 Ar, 21.8 C1. The product was sufficiently pure to be used without further purification.

(2S,4S)-Methyl 2-Isobutyl-1-(4-methoxybenzyl)-4-methyl-3,5-dioxo-4-(phenylthio)pyrrolidine-2-carboxylate 172a[75] **and (2S,4R)-Methyl 2-Isobutyl-1-(4-methoxybenzyl)-4-methyl-3,5-dioxo-4-(phenylthio)pyrrolidine-2-carboxylate 172b**[75]



Lactam **149** (0.4377 g, 0.53 mmol) was dissolved in THF (1.3 mL). A catalytic amount of $Pd(OH)_2/C$ was added, the mixture was stirred overnight under a static pressure of hydrogen at 35 °C and filtered through celite. The solvents were removed under reduced pressure. The crude material was dissolved in anhydrous dichloromethane (1.8 mL) and heated to at 20 °C under a nitrogen atmosphere. Triethylamine (0.09 mL, 0.64 mmol, 1.2 equiv) and S-methyl *p*-toluenethiosulfonate **186** (0.18 g, 0.89 mmol, 1.7 equiv) were added. The mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the resulting residue purified by column chromatography on silica gel using petroleum ether (40−60 °C)/ethyl acetate (9:1) as the eluent, to yield diastereoisomers **172ab** as an inseparable mixture in a 1:2 ratio (0.142 g, 68%). **Data for the minor diastereoisomer 172b:** ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.26 (d, *J* = 8.5 Hz, 2H, H13), 6.83 (d, *J* = 8.0 Hz, 2H, H12), 4.79 (d, *J* = 15.1 Hz, 1H, H10), 4.42 (d, *J* = 15.5 Hz, 1H, H10), 3.77 (s, 3H, H15), 3.44 (s, 3H, H6), 2.24 (dd, *J* = 15.5, 7.0 Hz, 1H, H7), 2.14 (s, 3H, H17), 1.95 (dd, *J* = 15.5, 6.5 Hz, 1H, H7), 1.57 (s, 3H, H16), 1.35− 1.29 (m, 1H, H8), 0.74 (d, *J* = 4.5 Hz, 3H, H9), 0.73 (d, *J* = 4.5 Hz, 3H, H9); ^{13}C NMR (126 MHz, $CDCl_3$) δ 200.4, 171.8, 168.2, 159.2, 130.0, 128.7, 113.9, 75.5, 55.4, 53.0, 49.4, 45.0, 40.1, 24.0, 23.9, 23.6, 17.0, 12.4. **Data for the major diastereoisomer 172a:** ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.23 (d, *J* = 8.7 Hz, 2H, H13), 6.81 (d, *J* = 8.6 Hz, 2H, H12), 4.95 (d, *J* = 14.9 Hz, 1H, H10), 4.02 (d, *J* = 14.9 Hz, 1H, H10), 3.77 (s, 3H, H15), 3.15 (s, 3H, H6), 2.27 (s, 3H, H17), 2.23 (dd, *J* = 15.2, 5.4 Hz, 1H, H7), 1.97 (dd, *J* = 15.2, 6.2 Hz, 1H, H1), 1.76- 1.71 (m, 1H, H8), 1.66 (s, 3H, H16), 0.92 (d, *J* = 6.6 Hz, 3H, H9), 0.86 (d, *J* = 6.7 Hz, 3H, H9); ^{13}C NMR (126 MHz, $CDCl_3$) δC 202.7, 172.5, 168.1, 159.4, 130.8, 127.6, 113.9, 75.3, 55.4, 52.9, 48.3, 43.8, 38.4, 24.7, 24.0, 23.8, 18.6, 11.7. **Data for the mixture 172ab**: ν_{max} (thin film)/cm^{-1} 2957 C-H (s), 1767 C=O (s), 1745 C=O (s), 1698 C=O (s).

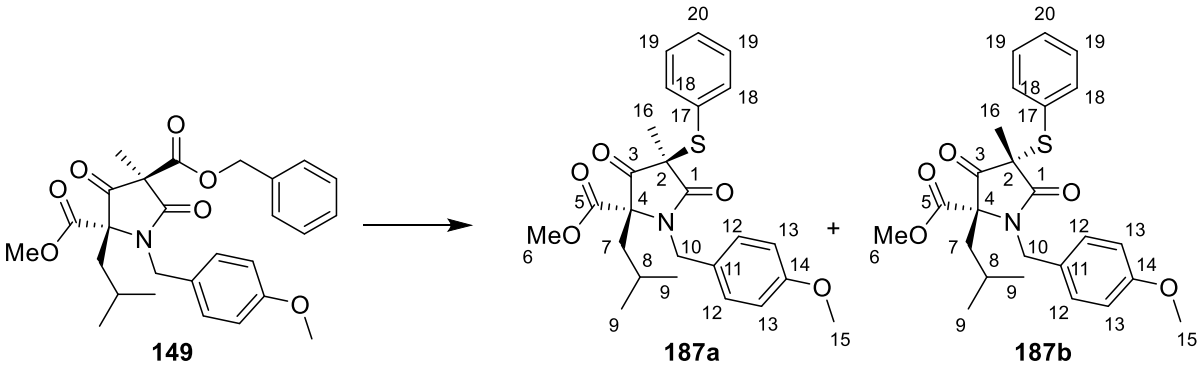
(3S,4S,5R)-N-(4'-Methoxybenzyl)-3-methyl-3-(methylsulfanyl)-4-hydroxy-5-(2'-methylpropyl)pyrrolidin-2-one-5-carboxylic Acid Methyl Ester 173a[75] **and (3R,4R,5R)-N-(4'-Methoxybenzyl)-3-methyl-3-(methylsulfanyl)-4-hydroxy-5-(2'-methylpropyl)pyrrolidin-2-one-5-carboxylic Acid Methyl Ester 173b**[75]



The mixture of diastereoisomers **172ab** (0.4842, 1.23 mmol) was dissolved in ethanol (25 mL) and the solution stirred at -10 °C. $NaBH_4$ was added (0.026, 0.68 mmol, 0.55 equiv) and the mixture stirred for 30 m. The solvents were removed under reduced pressure and the residue dissolved in ethyl acetate (100 mL). The organic solution was washed with water (50 mL x 2) and brine (50 mL x 2), dried (sodium sulphate), filtered, and the solvents removed under reduced pressure. The resulting pale yellow residue was purified by column chromatography using petroleum ether (40-60 °C)/ethyl acetate (3:1) as the eluent to provide **173a** as the first eluting diastereomer (0.0815 g, 17%) and **173b** as the second (0.222 g, 46%), each as colourless oils**. Data for the minor, first eluting diastereoisomer 173a**: $[\alpha]^{D\ 22}$ = -2.1 (*c* 1.6, $CHCl_3$) (77% ee); ν_{max} (thin film)/cm^{-1} 3425 O-H (b), 2957 C-H (s), 2927, 1739 C=O (s), 1697 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.15 (d, *J* = 8.7 Hz, 2H, H13), 6.82 (d, *J* = 8.7 Hz, 2H, H12), 4.83 (d, *J* = 15.9 Hz, 1H, H10), 4.46 (s, 1H, H18), 4.36 (d, *J* = 15.9 Hz, 1H, H10), 4.03 (s, 1H, H3) 3.78 (s, 3H, H15), 3.65 (s, 3H, H6), 2.15 (s, 3H, H17), 1.84 (dd, *J* = 14.5, 6.0 Hz, 1H, H7), 1.74 (d, *J* = 6.5 Hz, 1H, H7), 1.66−1.63 (m, 1H, H8), 1.62 (s, 3H, H16), 0.81 (d, J = 6.5 Hz, 3H, H9), 0.70 (d, *J* = 6.6 Hz, 3H, H9); ^{13}C NMR (126 MHz, $CDCl_3$) δC 173.3, 173.2, 158.6 Ar, 130.7 Ar, 128.3 Ar, 113.9 Ar, 76.1 C3, 71.7 C2 or 4, 57.6 C2 or 4, 55.4 C15, 52.5 C6, 45.4 C10, 40.8 C7, 24.3 C9, 24.3 C9, 23.6 C8, 22.1 C16, 12.8 C17. Determined by HPLC to be 77% ee. Determination of ee was found by HPLC using an AD-H Chiralpak column 95:5 hexane/IPA, 230 nm, 0.8 mL/min, 20 °C. **Data for the major, second eluting diastereoisomer 173b**: $[\alpha]^{D\ 26}$ = −17.3 (*c* 1.1, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3386 O-H (s), 2957 C-H (s), 1736 C=O (s), 1679 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.14 (d, *J* = 8.7 Hz, 2H, H13), 6.81 (d, *J* = 8.7 Hz, 2H, H12), 4.88 (d, *J* = 16.0 Hz, 1H, H10), 4.46 (s, 1H, H3), 4.43 (d, *J* = 16.0 Hz, 1H, H10), 3.77 (s, 3H, H15), 3.69 (s, 3H, H6), 3.24 (s,

1H, H18), 2.15 (s, 3H, H17), 1.88 (dd, *J* = 13.7, 6.4 Hz, 1H, H7), 1.83 – 1.67 (m, 2H, H8), 1.49 (s, 3H, H16), 0.90 (d, *J* = 6.6 Hz, 3H, H9), 0.76 (d, *J* = 6.5 Hz, 3H, H9); ^{13}C NMR (126 MHz, $CDCl_3$) δC 174.7, 173.2, 158.8, 130.3, 128.8, 113.9, 81.4 C3, 67.5 C2 or 4, 55.4 C2 or C4, 52.8 C15, 51.6 C6, 44.9 C10, 44.1 C7, 24.5 C9, 24.0 C9, 23.6 C8, 21.9 C16, 11.6 C17.

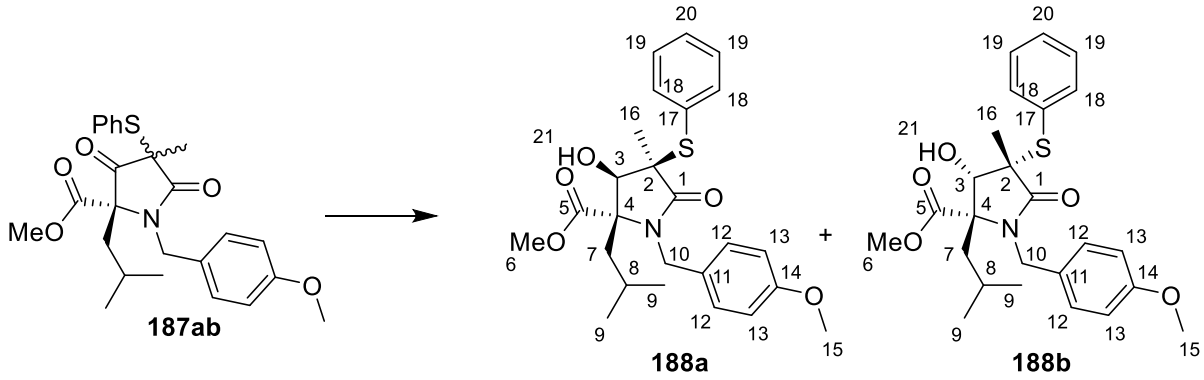
(2S,4S)-Methyl 2-Isobutyl-1-(4-methoxybenzyl)-4-methyl-3,5-dioxo-4(phenylthio)pyrrolidine-2-carboxylate 187a and (2S,4R)-Methyl 2-Isobutyl-1-(4-methoxybenzyl)-4-methyl-3,5-dioxo-4-(phenylthio)pyrrolidine-2-carboxylate 187b



Lactam **149** (0.4377 g, 0.70 mmol) was dissolved in THF (2.2 mL). A catalytic amount of $Pd(OH)_2/C$ was added, the mixture was stirred overnight under a static pressure of hydrogen at 35 °C. The solution was then filtered through celite, and the solvents were removed under reduced pressure. The resulting oil was dissolved in anhydrous dichloromethane (3.1 mL), and S -phenyl 4-methylbenzene-1-sulfonothioate **189** (0.481 g, 2 mmol, 2.9 equiv) and triethylamine (0.15 mL, 1.1 mmol, 1.6 equiv) were added. The mixture was stirred under an atmosphere of nitrogen for 5 h, the solvents were removed under reduced pressure, and the resulting pale yellow residue was purified by column chromatography using petroleum ether (40−60 °C)/ethyl acetate (9:1) as the eluent, yielding diastereoisomers **187ab** as an inseparable mixture in a 0.9:1 ratio as a pale yellow oil (0.281 g, 67% over the two steps). **Data for the minor diastereoisomer 187a**: ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.50 (dd, *J* = 8.2, 1.3 Hz, 2H, H19), 7.48−7.31 (m, 3H, H20, H18), 7.28 (d, *J* = 8.7 Hz, 2H, H13), 6.84 (d, *J* = 8.8 Hz, 2H, H12), 4.85 (d, *J* = 15.2 Hz, 1H, H10), 4.41 (d, *J* = 15.2 Hz, 1H, H10), 3.79 (s, 3H, H15), 3.44 (s, 3H, H6), 2.26 (dd, *J* = 15.2, 6.8 Hz, 1H, H7), 1.95 (dd, *J* = 15.2, 5.6 Hz, 1H, H7), 1.45 (s, 3H, H16), 1.29 (d, *J* = 6.7 Hz, 1H H8), 0.73 (d, *J* = 6.7 Hz, 3H, H9), 0.70 (d, *J* = 6.6 Hz, 3H, H9); ^{13}C NMR (126 MHz, $CDCl_3$) δC 200.4, 171.8, 168.0, 159.2, 137.9, 130.6, 130.1, 129.0, 128.6, 128.2, 113.9, 75.7, 55.4, 53.1, 53.0, 45.0, 40.1, 24.0, 23.7, 18.3; one peak unobserved in ^{13}C NMR

spectrum. **Data for the major diastereoisomer 187b**: ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.61 (d, *J* = 7.0 Hz, 2H, H8), 7.47−7.31 (m, 3H, H20, 18), 7.22 (d, *J* = 8.7 Hz, 2H, H13), 6.81 (d, *J* = 8.8 Hz, 2H, H12), 4.96 (d, J = 15.0 Hz, 1H, H10), 4.04 (d, *J* = 15.0 Hz, 1H, H10), 3.77 (s, 3H, H15), 3.15 (s, 3H, H6), 2.14 (dd, *J* = 15.0, 5.7 Hz, 1H, H7), 1.74 (d, *J* = 6.5 Hz, 1H, H8), 1.60 (dd, *J* = 15.0, 6.5 Hz, 1H, H7), 1.54 (s, 3H, H16), 0.95 (d, *J* = 6.6 Hz, 3H, H9), 0.85 (d, *J* = 6.6 Hz, 3H, H9); ^{13}C NMR (126 MHz, $CDCl_3$) δC 204.2, 172.6, 168.0, 159.4, 137.8, 130.8, 130.4, 129.0, 128.3, 127.8, 113.9, 74.9, 55.4, 53.2, 52.9, 43.0, 38.4, 24.6, 23.9, 20.8; one peak unobserved in ^{13}C NMR spectrum. **Data for the mixture of diastereoisomers 187ab**: ν_{max}(thin film)/cm^{-1} 2957 C-H (s), 1771 C=O (s), 1745 C=O (s), 1700 C=O (s); HRMS (ASAP-TOF) m/z [M + H]$^{+}$ calcd for $[C_{25}H_{30}NO_5S]^{+}$ 456.1845, found 456.1854.

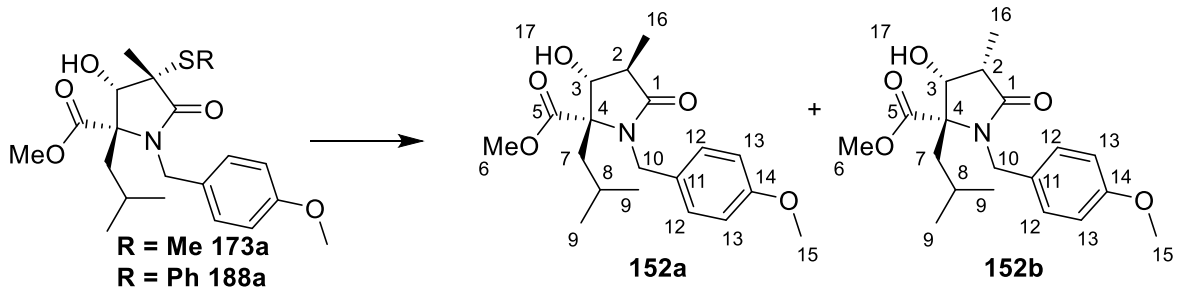
(2S,3S,4S)-Methyl 3-Hydroxy-2-isobutyl-1-(4-methoxybenzyl)-4-methyl-5-oxo-4-(phenylthio)pyrrolidine-2-carboxylate 188a and (2S,3R,4R)-Methyl 3-Hydroxy-2-isobutyl-1-(4-methoxybenzyl)-4-methyl-5-oxo-4-(phenylthio)-pyrrolidine-2-carboxylate 188b



The mixture of diastereoisomers **187ab** (0.2059 g, 0.45 mmol) was dissolved in ethanol (9 mL) and the solution cooled to −10 °C. Sodium borohydride (0.012 g, 0.3 mmol, 0.7 equiv) was added and the mixture stirred for 30 m before being quenched by addition of water (1 mL). The solvents were removed under reduced pressure, and the residue was dissolved in dichloromethane. The organic layer was washed twice each with water (20 mL) and brine (20 mL). The organic layer was dried (sodium sulphate) and filtered, and the solvents were removed under reduced pressure. The resulting residue was purified by column chromatography using petroleum ether (40−60 °C)/ethyl acetate (6:1) as the eluent to give **188a** and **188b** as colourless oils. **Data for the minor, first-eluting diastereoisomer 188a**: (yield 0.063 g, 30%) ν_{max} (thin film)/cm^{-1} 3423 O-H (b), 2957 C-H (s), 1740 C=O (s), 1701 C=O

(s); $[\alpha]^{D\ 22}$ = −60.0 (*c* 0.34, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.55 (d, *J* = 6.9 Hz, 2H, H19), 7.40−7.28 (m, 3H, H20, 18), 7.18 (d, *J* = 8.7 Hz, 2H, H13), 6.81 (d, *J* = 8.7 Hz, 2H, H12), 4.71 (d, *J* = 15.7 Hz, 1H, H3), 4.52−4.45 (m, 2H, H21, 10), 4.09 (d, *J* = 10.3 Hz, 1H, H10), 3.78 (s, 3H, H15), 3.66 (s, 3H, H6), 1.95 (dd, *J* = 14.6, 6.1 Hz, 1H, H7), 1.78 (d, *J* = 6.4 Hz, 1H, H8), 1.66 (dd, *J* = 14.6, 5.0 Hz, 1H, H7), 1.44 (s, 3H, H16), 0.87 (d, *J* = 6.6 Hz, 3H, H9), 0.76 (d, *J* = 6.6 Hz, 3H, H9); ^{13}C NMR (126 MHz, $CDCl_3$) δC 174.1, 173.6, 158.8 Ar, 137.5 Ar, 130.0 Ar, 129.5 Ar, 129.2 Ar, 129.1 Ar, 128.8 Ar, 113.8 Ar, 80.3 C3, 68.0 C4 or 2, 56.0 C4 or 2, 55.4 C15, 52.7 C6, 44.8 C10, 43.7 C7, 24.5 C9, 24.1 C9, 23.6 C8, 23.2 C16; HRMS (ASAP-TOF) m/z $[M + H]^+$ calcd for $[C_{25}H_{32}NO_5S]$+ 458.2001, found 458.2003. **Data for the major second-eluting diastereoisomer 188b**: (yield 0.073 g, 35%); ν_{max} (thin film)/cm^{-1} 3405, 2957, 2930, 1736, 1683; $[\alpha]^{D\ 19}$ = −5.8 (*c* 13.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.63 (d, *J* = 6.7 Hz, 2H, H19), 7.42−7.33 (m, 3H H20, 18), 7.15 (d, *J* = 8.7 Hz, 2H, H13), 6.83 (d, *J* = 8.7 Hz, 2H, H12), 4.92 (d, *J* = 16.1 Hz, 1H, H10), 4.44 (d, *J* = 16.1 Hz, 1H, H10), 4.41 (d, *J* = 3.8 Hz, 1H, H3), 3.79 (s, 3H, H15), 3.67 (s, 3H, H6), 3.65 (d, *J* = 3.9 Hz, 1H, H21), 1.93-1.86 (m, 1H, H7), 1.81−1.72 (m, 2H, H7,8), 1.29 (s, 3H, H16), 0.93 (d, *J* = 6.5 Hz, 3H, H9), 0.76 (d, *J* = 6.3 Hz, 3H, H9); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.4, 173.2, 158.6 Ar, 136.4 Ar, 130.5 Ar, 129.5 Ar, 129.3 Ar, 128.7 Ar, 128.3 Ar, 113.9 Ar, 75.4 C3, 71.8 C4 or 2, 62.3 C4 or 2, 55.4 C15, 52.5 C6, 45.4 C10, 41.3 C7, 24.3 C9, 24.2 C9, 23.7 C8, 22.4 C16; HRMS (FTMS-NSI) m/z $[M + H]^+$ calcd for $[C_{25}H_{32}NO_5S]^+$ 458.1996, found 458.1987.

(2S,3R,4S)-Methyl-3-Hydroxy-2-isobutyl-1-(4-methoxybenzyl)-4-methyl-5-oxopyrrolidine-2 carboxylate 152a and (2S,3R,4R)-Methyl 3-Hydroxy-2-isobutyl-1-(4-methoxybenzyl)-4-methyl-5-oxopyrrolidine-2-carboxylate 152b



Desulphurization by treatment with Raney® nickel where R = Me

Raney® nickel 2800 grade was washed with ethanol and dried under an atmosphere of argon. A micro spatula portion was added to a solution of **173a** (0.02 g, 0.05 mmol) in ethanol (2.3 mL). The mixture was heated to reflux for 4 h. The Raney® nickel was removed by filtration, and solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using petroleum ether (40−60 °C)/ethyl acetate (3:1) as the eluent, to give **152a** and **152b** as a 3:1 mixture of diastereoisomers (5.7 mg, 7%).

Desulphurization by treatment with Raney® nickel where R = Ph

Raney® nickel 2800 grade was washed with ethanol and dried under an atmosphere of argon. A portion was added to a solution of **188a** (0.0237 g, 0.05 mmol) in ethanol (1 mL). The mixture was heated to reflux for 4 h. The Raney® nickel was removed by filtration, and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using ethyl acetate/petroleum ether (40−60 °C) (1:1) as the eluent, affording **152a** and **152b** as a 3:1 mixture of diastereoisomers (7.4 mg, 42%).

Desulphurization by treatment with AIBN/organotin where R = Ph

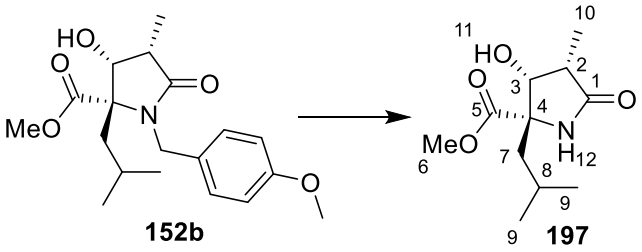
Lactam **188a** (0.057 g, 0.12 mmol) and AIBN (0.0042 g, 0.026 mmol, 0.2 equiv) were dissolved in anhydrous acetone (0.9 mL). Tributyl-tinhydride (0.11 mL, 0.41 mmol, 3.4 equiv) was added, and the mixture was heated to reflux under a nitrogen atmosphere overnight. The solvents were removed under reduced pressure, and the residue was purified by column chromatography using ethyl acetate/petroleum ether (40−60 °C) (1:3) as the eluent, and a

90:10 silica/potassium carbonate mix as the stationary phase, to afford **152ab** as a colourless oil in a 1:2 mixture of diastereoisomers (0.0405 g, 93%).

Desulphurization by treatment with AIBN/TTMS where R = Ph

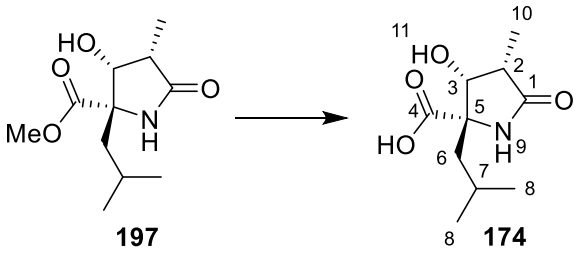
Lactam **188a** (0.064 g, 0.14 mmol) and AIBN (0.007 g, 0.043 mmol) were dissolved in anhydrous acetone (1 mL). Tris(trimethylsilyl)silane (0.14 mL, 0.45 mmol, 3.2 equiv) was added, and the mixture was heated to reflux under a nitrogen atmosphere overnight. The solvents were removed under reduced pressure, and the residue was purified by column chromatography using ethyl acetate/petroleum ether (40−60 °C) (1:1) as the eluent, to afford **152a** as a colourless oil (0.007 g, 14%) and **152b** as a colourless oil (0.036 g, 74%). **Data for the first-eluting diastereoisomer 152a**: $[\alpha]^{D\ 19}$ = +7.4 (*c* 0.42, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3356, 2957, 2918, 1741, 1673 δH 7.16 (d, *J* = 8.7 Hz, 2H, H13), 6.81 (d, *J* = 8.7 Hz, 2H, H12), 4.69 (d, *J* = 15.3 Hz, 1H, H10), 4.17 (d, *J* = 15.3 Hz, 1H, H10), 3.90 (t, *J* = 9.8 Hz, 1H, H3), 3.78 (s, 3H, H15), 3.34 (s, 3H, H6), 2.84 (d, *J* = 9.8 Hz, 1H, H17), 2.71 (dq, *J* = 9.6, 7.0 Hz, 1H, H2), 2.15 (dd, *J* = 14.4, 7.5 Hz, 1H, H7), 1.92−1.81 (m, 1H, H8), 1.55 (dd, *J* = 14.5, 4.5 Hz, 1H, H7), 1.32 (d, *J* = 7.0 Hz, 3H, H16), 0.97−0.88 (m, 6H, H9); ^{13}C NMR (126 MHz, $CDCl_3$) δC 175.5, 172.4, 159.0 Ar, 129.7 Ar, 129.4 Ar, 113.9 Ar, 79.4 C3, 69.1 C4, 55.4 C2, 52.1 C15, 43.4 C6, 43.3 C10, 41.9 C7, 24.7 C8, 24.0 C9, 23.5 C9, 13.7 C16; HRMS (NSI-FTMS) m/z $[M + H]^+$ calcd for $[C_{19}H_{28}NO_5]^+$ 350.1962, found 350.1964. **Data for the second-eluting diastereoisomer 152b**: $[\alpha]^{D\ 22}$ = −27 (*c* 0.67, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3374 O-H (b), 2956 C-H (s), 1742 C=O (s), 1672 C=O (s); ^1H NMR (500 MHz, $CDCl_3$) δH 7.17 (d, *J* = 8.8 Hz, 2H, H13), 6.81 (d, *J* = 8.8 Hz, 2H, H12), 4.67 (d, *J* = 15.4 Hz, 1H, H10), 4.47−4.36 (m, 2H, H10, 3), 3.77 (s, 3H, H15), 3.46 (s, 3H, H6), 3.03 (d, *J* = 7.6 Hz, 1H H17), 2.76 (d, *J* = 7.5 Hz, 1H, H2), 1.99 (dd, *J* = 14.4, 6.1 Hz, 1H, H7), 1.77−1.67 (m, *J* = 12.9, 6.5 Hz, 1H, H16), 1.61 (dd, *J* = 14.4, 6.0 Hz, 1H, H7), 1.27 (d, *J* = 7.6 Hz, 3H, H16), 0.88 (d, *J* = 6.6 Hz, 3H, H9), 0.84 (d, *J* = 6.6 Hz, 3H, H9); ^{13}C NMR (126 MHz, $CDCl_3$) δC 176.6, 172.3, 158.7 Ar, 130.1 Ar, 128.9 Ar, 113.8 Ar, 73.4 C3, 72.5 C4, 55.4 C2, 52.2 C15, 44.7 C6, 43.3 C10, 39.9 C7, 24.3 C8, 24.1 C9, 24.0 C9, 9.5 C16; HRMS (NSI-FTMS) m/z $[M + H]^+$ calcd for $[C_{19}H_{28}NO_5]^+$ 350.1962, found 350.1964.

(2S,3R,4S)-Methyl 3-Hydroxy-2-isobutyl-4-methyl-5-oxopyrrolidine-2-carboxylate 197



Lactam **152b** (0.036 g, 0.10 mmol) was dissolved in a 3:1 mixture of acetonitrile/water (1 mL). CAN (0.287 g, 0.52 mmol, 5.2 equiv) was added and the mixture stirred at room temperature until TLC showed the reaction was complete. The mixture was extracted with ethyl acetate (3 × 20 mL), the combined organic layers were washed with brine (20 mL), dried (sodium sulphate), filtered, and the solvents were removed under reduced pressure. The residue was purified by column chromatography using ethyl acetate/petroleum ether (40−60 °C) (9:1) as the eluent, affording **197** as a colourless solid (0.023 g, 62%). Mp 147-156 °C ν_{max} (thin film)/cm^{-1} 3434 O-H (b), 2959 C-H (s), 2079, 1725 C=O (s), 1641 C=O (s); $[\alpha]^{D\ 23}$ = −2.85 (*c* 0.42, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δH 8.20 (s, 1H, H12), 5.09 (d, *J* = 11.6 Hz, 1H, H3), 4.06 (dd, *J* = 11.4, 5.1 Hz, 1H, H2), 3.83 (s, 3H, H6), 2.72−2.65 (m, 1H, H11), 2.00 (dd, *J* = 13.8, 8.7 Hz, 1H, H7), 1.78−1.68 (m, 1H, H8), 1.51 (dd, *J* = 13.8, 5.1 Hz, 1H, H7), 1.18 (d, *J* = 7.3 Hz, 3H, H10), 0.96 (d, *J* = 6.7 Hz, 3H, H9), 0.87 (d, *J* = 6.6 Hz, 3H, H9); ^{13}C NMR (126 MHz, $CDCl_3$) δC 178.1, 174.2, 78.9 C3, 72.0 C4, 52.8 C2, 43.5 C6, 40.1 C7, 25.0 C8, 24.0 C9, 21.9 C9, 7.7 C10; HRMS (NSI-FTMS) m/z $[M + H]^+$ calcd for $[C_{11}H_{20}NO_4]^+$ 230.1387, found 230.1388.

(2S,3R,4S)-3-Hydroxy-2-isobutyl-4-methyl-5-oxopyrrolidine-2-carboxylic Acid 174[77]

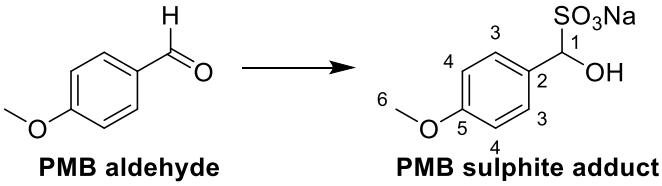


Lactam **197** (0.01 g 0.04 mmol) was dissolved in aqueous sodium hydroxide (0.5 M, 0.86 mL) and kept at 4 °C. The progress of the reaction was monitored by TLC. When all the starting material was consumed (approximately 24 h), the solution was acidified to pH 3 with HCl (1 M), and the solvents were removed under reduced pressure. The residue was dissolved in hot THF and filtered through cotton wool. The solvents were removed under reduced pressure. The residue was purified by column chromatography using a dichloromethane/methanol/acetic acid (90:9:1) mixture as the eluent affording **174** as a pale brown residue (0.078 g, 86%): ν_{max} (thin film)/cm^{-1} 3405 O-H (b), 2923 C-H (s), 1725 C=O (s), 1686 C=O (s); $[\alpha]^{D\ 20}$ = −10 (*c* 0.28, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δH 4.10 (d, *J* = 5.2 Hz, 1H, H3), 2.79−2.71 (m, 1H, H2), 1.94 (dd, *J* = 13.8, 8.3 Hz, 1H, H7), 1.76−1.65 (m, 1H, H8), 1.57 (dd, *J* = 13.8, 4.7 Hz, 1H, H7), 1.11 (d, *J* = 7.2 Hz, 3H, H10), 0.95 (d, *J* = 6.7 Hz, 3H, H9), 0.92 (d, *J* = 6.7 Hz, 3H, H9); ^{13}C NMR (126 MHz, CD_3OD) δC 180.0 C4, 175.4 C1, 78.1 C3, 45.2 C5, 41.4 C2, 25.9 C6, 24.8 C7, 23.0 C8, 20.8 C8, 8.4 C10; HRMS (NSI-FTMS) m/z $[M + H]^+$ calcd for $[C_{10}H_{18}NO_4]^+$ 214.1084, found 214.1085.

3.2.2 Experimental for procedures starting from L-serine

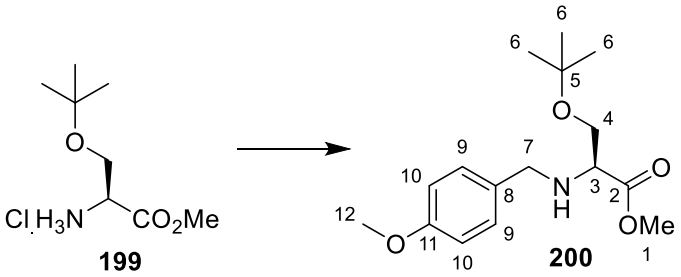
3.2.2.1 Experimental toward the synthesis of omuralide

Sodium hydroxy(4-methoxyphenyl)methanesulfonate[107]



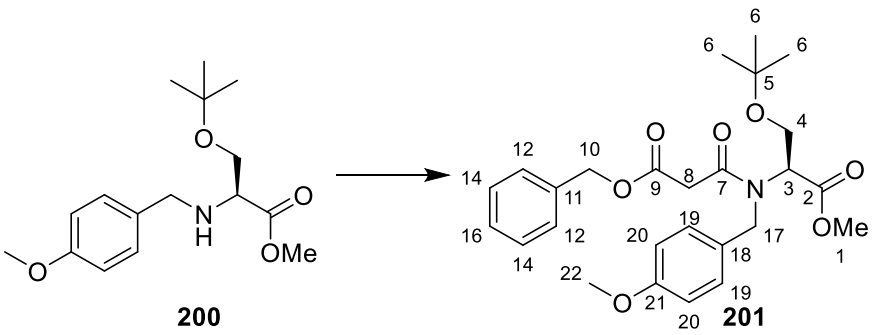
p-Methoxybenzaldehyde (20 mL, 0.164 mol, 1.2 equiv) was stirred in ethanol (330 mL). An aqueous solution of sodium sulphite (17.56 g, 0.139 mol in 34 mL) was slowly added forming a cloudy precipitate. This solution was stirred for 16 h at 30 °C then stirred in an ice bath for 2 hours allowing further precipitation. The resulting suspension was filtered, washed with hexane and dried in a vacuum oven to yield the PMB sulphite adduct as a fluffy white solid (33.72 g, 81%). Mp 166-168 °C (decomp.) (lit[203] 155-157 °C (decomp.); ν_{max} (solid)/cm^{-1} 3226 O-H (b), 1516, 1249; ^{1}H NMR (500 MHz, DMSO) δH 7.34 (d, *J* = 8.3 Hz, 2H, Ar), 6.79 (d, *J* = 8.8 Hz, 2H, Ar), 5.56 (d, *J* = 5.0 Hz, 1H, H1), 4.87 (d, *J* = 5.0 Hz, 1H, H1), 3.72 (s, 3H, H6), hydroxyl proton unobserved in ^{1}H NMR spectrum; ^{13}C NMR (126 MHz, DMSO) δC 158.6 Ar, 131.6 Ar, 129.1 Ar, 112.6 Ar, 84.7 C1, 55.2 C6.

(S)-methyl 3-(tert-butoxy)-2-((4-methoxybenzyl)amino)propanoate 200



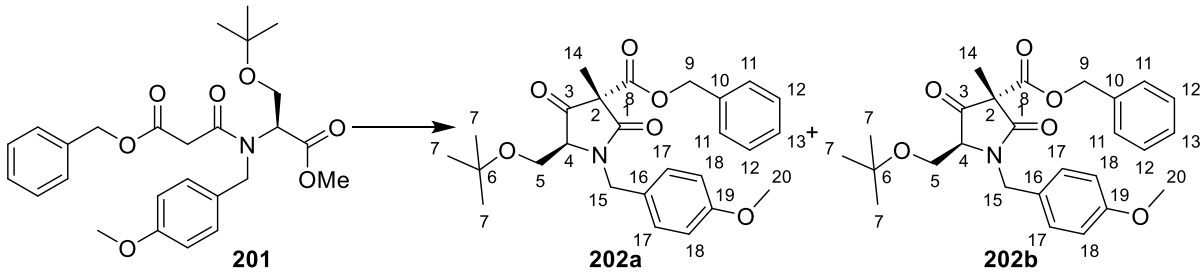
t-Bu-O-Serine methyl ester hydrochloride **199** (5.00 g, 23.61 mmol) was dissolved in methanol (250 mL). The PMB sulphite salt (9 g, 37.46 mmol, 1.6 equiv) was added along with triethylamine (3 mL, 21.52 mmol 0.9 equiv). The stirred solution was cooled with an ice bath and sodium cyanoborohydride (9.09 g, 144 mmol, 6.1 equiv) was added in small portions. The suspension was left overnight and allowed to reach room temperature. The solution was evaporated and dissolved in ethyl acetate (200 mL). The organic solution was washed with equal amounts of brine, saturated sodium sulphite solution and water, then dried (magnesium sulphate), filtered, and evaporated under reduced pressure to produce 5.92 g of crude **200**, which was used in the next step with no further purification. For analytical purposes a portion was purified by column chromatography on silica gel using petroleum ether (40-60 °C)/ethyl acetate (9:1) to yield **200** as a colourless oil. $[\alpha]^{D\ 24}$ = –23.61 (*c* 1.05, $CHCl_3$) ν_{max} (thin film)/cm^{-1}: 3434 N-H (s), 2974 C-H (s), 1743 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.25 (d, *J* = 7.5 Hz, 2H, H10), 6.85 (d, *J* = 8.7 Hz, 2H, H9), 3.83 (d, *J* = 12.8 Hz, 1H, H7), 3.79 (s, 3H, H12), 3.72 (s, 3H, H1), 3.65 (d, *J* = 12.8 Hz, 1H, H7), 3.60 (dd, *J* = 8.7, 5.3 Hz, 1H, H4), 3.55 (dd, *J* = 8.7, 4.9 Hz, 1H, H4), 3.43 (t, *J* = 5.1 Hz, 1H, H3), 2.14 (s, 1H), 1.14 (s, 9H, H6); ^{13}C NMR (126 MHz, $CDCl_3$) δC 174.1 C2, 158.8 Ar, 132.1 Ar, 129.7 Ar, 113.9 Ar, 73.3 C5, 63.3 C4, 61.1 C3, 55.4 C2, 51.8 C3, 51.5 C7, 27.5 C6; HRMS (NSI-FTMS) *m/z* $[M+H]^+$ Calcd for $[C_{16}H_{26}NO_4]^+$ 296.1856 found 296.1859.

(S)-benzyl 3-((3-(tert-butoxy)-1-methoxy-1-oxopropan-2-yl)(4-methoxybenzyl)amino)-3-oxopropanoate 201



Crude amine **200** (5.92 g, 20.05 mmol) was dissolved in anhydrous DCM (182 mL) in a flame dried flask. The benzyl malonic half ester **146** (11.59 g, 49.89 mmol, 2.5 equiv) was added along with EDAC.HCl (10.30 g, 53.72 mmol 2.7 equiv), DMAP (0.5 g, 4.1 mmol, 0.2 equiv) and N-methyl morpholine (5.5 mL, 50 mmol, 2.5 equiv). The solution was stirred for 16 h under an atmosphere of nitrogen. HCl (1 M aq solution) (6 mL) was added, the solution transferred into a separating funnel, and washed with equal amounts of water and brine. The organic layer was dried (magnesium sulphate), filtered and evaporated under reduced pressure to form **201** as a yellow oil, which was purified by column chromatography on silica gel using petroleum ether (40-60 °C)/ethyl acetate (8:2) as the eluent, to produce a pale-yellow oil. (5.49 g, 49% over the 2 steps) $[\alpha]^{D\ 24}$ = –23.61 (*c* 1.05, $CHCl_3$) (97% ee); ν_{max} (neat)/cm^{-1}: 3434, 2974 C-H (s), 1743 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) (major conformation) δH 7.40 – 7.29 (m, 5H H12, 14, 16), 7.24 (d, *J* = 8.8 Hz, 2H, H20), 6.86 (d, *J* = 8.7 Hz, 2H, H19), 5.15 (s, 2H, H8 or H10), 4.69 (s, 2H, H8 or 10), 4.58 (dd, *J* = 7.5, 3.4 Hz, 1H, H4), 3.90 (dd, *J* = 9.7, 7.6 Hz, 1H, H4), 3.80 – 3.74 (m, 4H, H22 and 3), 3.67 (s, 3H, H1), 3.51 (d, *J* = 15.4 Hz, 1H, H17), 3.43 (d, *J* = 15.4 Hz, 1H, H17), 1.06 (s, 9H, H6); ^{13}C NMR (126 MHz, $CDCl_3$) δC 169.7, 167.3, 167.2, 159.1, 128.8, 128.6, 128.4, 128.1, 114.2, 73.4, 67.1, 60.7, 59.7, 55.4, 52.3, 52.1, 41.5, 27.3, two peaks unobserved in ^{13}C NMR spectrum; HRMS (NSI-FTMS) *m/z* $[M+H]^+$ Calcd for $[C_{26}H_{34}NO_7]^+$ 472.2330 found 472.2325. Determined by HPLC to be 97% ee. Determination of ee was found by HPLC using an AD-H Chiralpak column 90:10 hexane/IPA, 230 nm, 0.8 mL/min, 15 °C.

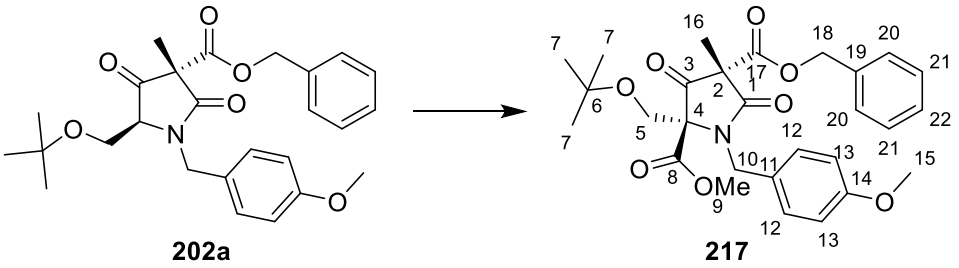
(3S,5S)-benzyl 5-(tert-butoxymethyl)-1-(4-methoxybenzyl)-3-methyl-2,4-dioxopyrrolidine-3-carboxylate 202a and (3R,5S)-benzyl 5-(tert-butoxymethyl)-1-(4-methoxybenzyl)-3-methyl-2,4-dioxopyrrolidine-3-carboxylate 202b



The cyclization precursor **201** (1.605 g, 3.4 mmol) was dissolved in ether (9.8 mL), TBAF (1 M in THF) (7 mL, 7 mmol, 2.1 equiv) was added and the solution was stirred for 10 m, evaporated under reduced pressure, and placed under high vacuum for approximately 2 h. To the resulting white solid was added THF (9.8 mL) and the solution was cooled to -12 °C. MeI (2.1 mL, 34 mmol, 10 equiv) was added and the solution stirred for 72 h. Water (approximately 3 mL) was added and the solution was allowed to warm to room temperature where the solution was dissolved in ethyl acetate (100 mL) and washed with equal amounts of water and brine. The organic layer was dried (magnesium sulphate), filtered, and evaporated. The resulting brown oil was purified by column chromatography on silica gel using petroleum ether (40-60 °C)/ethyl acetate (9:1) as the eluent, to produce **202ab** as a 3:1 ratio of diastereoisomers (1.0273 g, 67%) as a clear oil which could be partially separated **Data for the major, first eluting diastereoisomer 202a:** (Yield 0.491 g, 32%), $[\alpha]^{D\ 19.5}$ = –91.3 (*c* 1.06, $CHCl_3$) (98% ee) ν_{max} (neat)/cm^{-1}: 2934 C-H (s), 1781 C=O (s), 1749 C=O (s), 1698 C=O (s); ^{1}H NMR (400 MHz, $CDCl_3$) δH 7.39 – 7.33 (m, 3H, H13, 11), 7.28 – 7.23 (m, 2H, H12), 7.01 (d, J = 8.4 Hz, 2H, H18), 6.60 (d, J = 8.7 Hz, 2H, H17), 5.38 (d, J = 15.1 Hz, 1H, H9), 5.21 (d, J = 12.3 Hz, 1H, H15), 5.10 (d, J = 12.3 Hz, 1H, H15), 3.86 (t, J = 2.3 Hz, 1H, H4), 3.83 (d, J = 15.1 Hz, 1H, H9), 3.74 (s, 3H, H20), 3.58 (dd, J = 9.9, 2.1 Hz, 1H, H5), 3.53 (dd, J = 9.9, 2.4 Hz, 1H, H5), 1.56 (s, 3H, H14), 1.12 (s, 9H, H7); ^{13}C NMR (126 MHz, $CDCl_3$) δC 205.0, 170.3, 166.0, 159.3 Ar, 135.0 Ar, 129.3 Ar, 128.8 Ar, 128.6 Ar, 128.4 Ar, 126.7 Ar, 114.3 Ar, 73.9 C5, 68.2 C9, 65.2 C6, 58.8 C2, 57.2 C4, 55.4 C20, 43.1 C15, 27.3 C7, 15.3 C14; HRMS (NSI-FTMS) *m/z* $[M+H]^+$ Calcd for $[C_{26}H_{32}NO_6]^+$ 254.2224 found 454.2218. Determined by HPLC to be 79% ee recrystalization in IPA was found to have an enriched supernatent of 98% ee. Determination of ee was found by HPLC using an AD-H Chiralpak column 80:20 hexane/IPA, 230 nm, 0.8 mL/min, 25 °C. **Data**

for the partially purified, minor, second eluting diastereomer 202b: (Yield 0.416 g, 35%), ^{1}H NMR (400 MHz, $CDCl_3$) δH 7.38 – 7.28 (m, 5H, H16, 14, 12), 7.22 (d, *J* = 8.6 Hz, 2H, H20), 6.82 (d, *J* = 8.7 Hz, 2H, H19), 5.25 – 5.16 (m, 2H H9-15), 5.11 (d, *J* = 12.2 Hz, 1H, H9-15), 4.31 (d, *J* = 14.3 Hz, 1H, H9 or 15), 3.87 (dd, *J* = 7.2, 2.6 Hz, 1H, H5 or 4), 3.78 (s, 3H, H20), 3.59 (dd, *J* = 9.7, 2.5 Hz, 1H, H5 or 4), 3.48 (dd, *J* = 9.7, 7.1 Hz, 1H, H5 or 4), 1.52 (s, 3H, H14), 1.02 (s, 9H, H7); ^{13}C NMR (101 MHz, $CDCl_3$) δ 204.0, 169.6, 165.6, 159.4 Ar, 135.0 Ar, 130.0 Ar, 128.8 Ar, 128.7 Ar, 128.3 Ar, 128.0 Ar, 114.2 Ar, 74.0 C6, 68.2 C5, 65.5 C9, 62.8 C4, 58.5 C2, 55.4 C20, 44.5 C15, 27.2 C7, 16.7 C14.

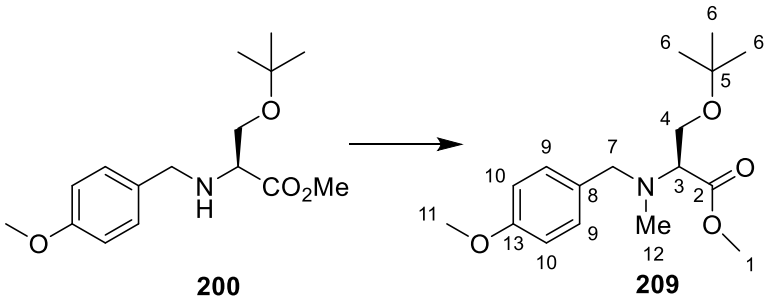
(2R,4S)-4-benzyl 2-methyl 2-(tert-butoxymethyl)-1-(4-methoxybenzyl)-4-methyl-3,5-dioxopyrrolidine-2,4-dicarboxylate 217



Lactam **202a** (0.1125 g, 0.25 mmol), in a flame dried flask, was dissolved in anhydrous THF (4.5 mL) and cooled to -40 °C and placed under an atmosphere of nitrogen. LiHMDS (1 M in THF/ethylbenzene) (0.56 mL, 0.56 mmol, 2.2 equiv) was added with DMPU (0.9 mL, 0.75 mmol, 3 equiv) and the solution left for 0.5 h. Methyl cyanoformate was added (0.09 mL, 1.13 mmol, 4.5 equiv) and stirring was continued for 3 h. Saturated NH_4Cl solution (0.2 mL) was used to quench the solution, which was allowed to warm to room temperature. The crude solution had water added (20 mL) which was extracted with equal amounts of ethyl acetate twice. The combined organic extracts were washed with water (20 mL), and with brine (2 x 20 mL) before being dried (sodium sulphate), filtered, and evaporated under reduced pressure. The resulting residue was purified by column chromatography using a petroleum ether (40-60 °C)/ethyl acetate (9:1) as the eluent to yield **217** as a white crystalline solid (0.0881 g, 69%). Mp 90-94 °C $[\alpha]^{D\ 25}$ = +2.85 (*c* 1.12, $CHCl_3$) (supernatant of up to 93 % ee could be obtained after recrystallization from IPA) ν_{max} (neat)/cm^{-1}: 3434, 2974 C-H (s), 1743 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.39 – 7.30 (m, 5H, H20, 21, 22), 7.18 (d, *J* = 8.7 Hz, 2H, H13), 6.70 (d, *J* = 8.7 Hz, 2H, H12), 5.24 (d, *J* = 12.5 Hz, 1H, H18 or 10), 5.16 (d, *J* = 12.5 Hz, 1H, H18 or 10), 4.72 (d, *J* = 15.3 Hz, 1H, H18 or 10), 4.57 (d, *J* = 15.3 Hz, 1H, H5), 3.83 (d, *J* = 9.7 Hz, 1H,

H15), 3.75 (s, 3H, H5), 3.73 (d, *J* = 9.7 Hz, 1H, H9), 3.53 (s, 3H, H16), 1.66 (s, 3H, H16), 0.87 (s, 9H, H7); ^{13}C NMR (126 MHz, $CDCl_3$) δC 199.7, 170.7, 166.3, 165.1 Ar, 159.0 Ar, 135.1 Ar, 129.5 Ar, 128.7 Ar, 128.6 Ar, 128.5 Ar, 128.0 Ar, 113.7 Ar, 74.1 C5, 68.1 C18, 61.0 C2, 57.7 C4, 55.5 C15, 53.4 C9, 44.7 C10, 26.8 C7, 18.9 C16, one carbon peak unobserved in ^{13}C spectrum ; (NSI-FTMS) *m/z* $[M+H]^+$ Calcd for $[C_{28}H_{34}NO_8]^+$ 512.2279 found 512.2271. Determination of ee was found by HPLC using an AD-H Chiralpak column 90:10 hexane/IPA, 230 nm, 0.8 mL/min, 25 °C.

(S)-methyl 3-(tert-butoxy)-2-((4-methoxybenzyl)(methyl)amino)propanoate 209



Alkylation method:

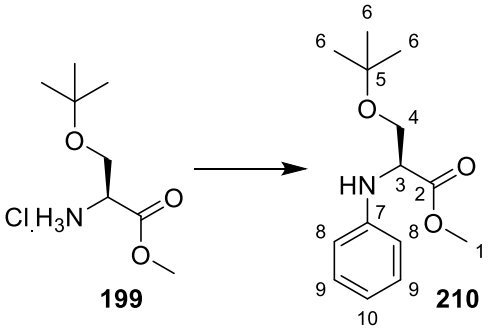
Amine **200** (0.1289 g, 0.436 mmol) was dissolved in DMF (6 mL). K_2CO_3 (0.16 g, 1.16 mmol, 2.7 equiv) and MeI (0.06 mL, 0.96 mmol, 2.2 equiv) were added and the solution stirred at room temperature until TLC showed consumption of the starting material (about 1 h). Water (5 mL) was added and all solvents were removed under reduced pressure. The resulting residue was dissolved in ethyl acetate (75 mL) and water (75 mL). The aqueous layer was removed and the organic washed with a further 75 mL of water and brine (2 x 75 mL). The organic solution was dried (magnesium sulphate), filtered, and evaporated under reduced pressure to provide **209** as a colourless oil. The oil was purified with column chromatography using petroleum ether (40-60 °C)/ethyl acetate (9:1) as the eluent to give **209** (0.0834 g, 62%).

Reductive amination method:

Amine **200** (0.1415 g, 0.48 mmol) and *para*-formaldehyde (0.072 g, 2.38 mmol, 5 equiv) were stirred in MeOH (6.6 mL) for 5 h. Sodium cyanoborohydride (0.072 g, 1.14 mmol, 2.4 equiv) was added and the solution stirred for 16 h. The solvents were evaporated under vacuum and the resulting crude material dissolved in ethyl acetate (75 mL) and water (75 mL). The aqueous layer was removed and the organic washed with a further 75 mL of water and brine (2 x 75

mL). The organic was dried (magnesium sulphate), filtered, and evaporated under reduced pressure, to provide **209** as a colourless oil which could be used without further purification (0.1094 g, 74%). For characterization purposes however, a small amount was purified using column chromatography. $[\alpha]^{D\,23}$ = –46.5 (*c* 1.6, $CHCl_3$); ν_{max} (neat)/cm^{-1}: 2974 C-H (s), 2951 C-H (s), 1736 C=O (s), 1612; ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.25 (d, *J* = 9.6 Hz, 2H, H10), 6.84 (d, *J* = 8.6 Hz, 2H, H9), 3.80 (s, 3H, H10), 3.76 (d, *J* = 7.6 Hz, 1H, H3), 3.74 (s, 3H, H1), 3.70 (d, *J* = 13.3 Hz, 1H, H7), 3.62 (m, 2H, H7 and 4), 3.51 (dd, *J* = 7.4, 5.7 Hz, 1H, H4), 2.29 (s, 3H, H12), 1.17 (s, 9H, H8); ^{13}C NMR (126 MHz, $CDCl_3$) δC 172.0, 158.8 Ar, 131.5 Ar, 130.1 Ar, 113.8 Ar, 73.3 C5, 66.1 C4, 61.3 C3, 58.8 C12, 55.4 C11, 51.2 C1, 39.0 C12, 27.5 C6; HRMS (NSI-FTMS) m/z $[M + H]^+$ calcd for $[C_{17}H_{28}NO_4]^+$ 310.2013, found 310.2016.

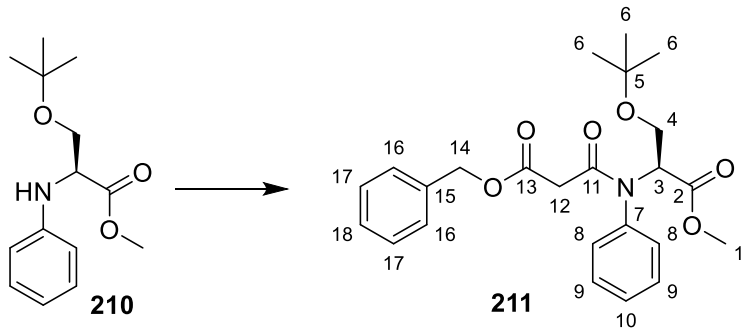
Methyl (2*S*)-2-anilino-3-*tert*-butoxypropanoate 210[204]



Amine **210** (1.249 g, 5.9 mmol) was dissolved in in anhydrous DCM (40 mL). Phenyl boronic acid (1.447 g, 11.94 mmol, 2 equiv) was added, along with $Cu(OAc)_2$ (1.2 g, 6.6 mmol, 1.1 equiv). Triethylamine (1.65 mL, 11.8 mmol, 2 equiv) and molecular sieve (4 Å, 4.4 g) were also added and the reaction was placed under a static atmosphere of oxygen and stirred for 3 days. Ammonium hydroxide (1 M aqueous solution, 40 mL) was added, the suspension stirred for 40 m, filtered through diatomaceous earth and transferred into a separating funnel. The organic layer was removed and the aqueous extracted again with an equal portion of DCM. The organic layers were combined, washed with water (80 mL) and brine (80 mL), dried (magnesium sulphate), filtered and evaporated under reduced pressure. The resulting residue was purified by column chromatography using petroleum ether (40-60 °C)/ether 18:1 as the eluent to yield **210** as a cream coloured solid (0.319 g, 22%). Recrystallization from petroleum ether yielded crystals of 95% ee and a racemic supernatant from 72% ee. Mp 52-59 °C (lit[205] 47-50 °C); $[\alpha]^{D\,22}$ = -13.69 (*c* 1.11, $CHCl_3$) (95% ee) (lit[205] $[\alpha]^{D\,24}$ = -10.7 (*c* 1.1, $CHCl_3$) (71% ee)); ν_{max} (neat)/cm^{-1}: 3398 N-H (b), 2975 C-H (s), 1751 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.20

– 7.14 (m, 2H, H8), 7.75 (td, *J* = 7.4, 0.8 Hz, 1H, H10), 6.64 (d, *J* = 8.2 Hz, 2H, H9), 4.62 (s, 1H, NH), 4.20 (t, *J* = 4.1 Hz, 1H, H3), 3.78 (dd, *J* = 8.8, 4.0 Hz, 1H, H4), 3.73 (s, 3H, H1), 3.69 (dd, *J* = 8.8, 4.2 Hz, 1H, H4), 1.17 (s, 9H, H6); ^{13}C NMR (126 MHz, $CDCl_3$) δC 172.0 C2, 146.9 Ar, 129.40 Ar, 118.5 Ar, 113.8 Ar, 73.70 C5, 62.60 C4, 57.4 C3, 52.3 C1, 27.5 C6. Determination of ee was found by HPLC using an Eurocel 01 Knauer column 90:10 hexane/IPA, 230 nm, 0.8 mL/min, 25 °C.

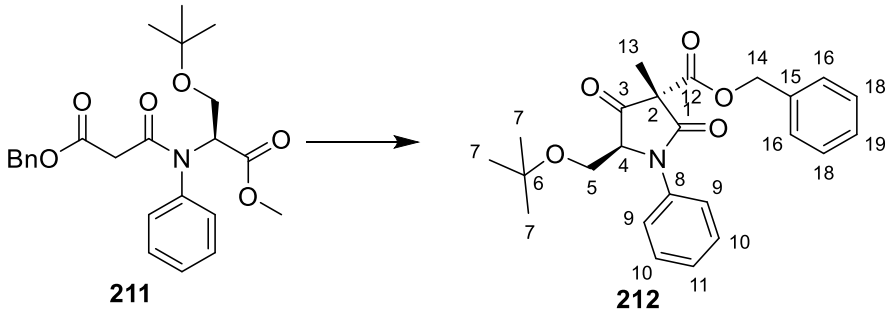
(S)-benzyl 3-((3-(tert-butoxy)-1-methoxy-1-oxopropan-2-yl)(phenyl)amino)-3-oxopropanoate 211



Amine **210** (0.2308 g, 0.91 mmol) was dissolved in anhydrous DCM (7 mL) in a flame dried flask. The benzyl malonic half ester **146** (0.47 g, 2.02 mmol, 2.2 equiv) was added along with EDAC.HCl (0.467 g, 2.4 mmol 2.7 equiv), DMAP (0.021 g, 0.17 mmol, 0.19 equiv) and N-methylmorpholine (0.23 mL, 2.09 mmol, 2.3 equiv). The solution was stirred for 16 h under a nitrogen atmosphere. HCl (0.3 mL, 1 M solution) was added, and the solution diluted with DCM (50 mL). The solution was transferred into a separating funnel and washed with equal amounts of water and brine. The organic layer was dried (magnesium sulphate), filtered and evaporated under reduced pressure, to form **211** as a yellow oil which was purified by column chromatography using petroleum ether (40-60 °C)/ethyl acetate (9:1 to 7:3) as the eluent to produce clear oil (0.1136 g, 71%). $[\alpha]^{D\ 23}$ = +1.48 (*c* 0.54, $CHCl_3$) (95 % ee); ν_{max} (neat)/cm^{-1}: 3022 C-H (unsaturated, s), 2974 C-H (s), 1743 C=O (s), 1663 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.45 (s, 2H, Ar), 7.38 – 7.28 (m, 8H, Ar), 5.10 (s, 2H, H12), 4.69 – 4.64 (m, 1H H3 or 4), 3.81 – 3.78 (m, 2H, H3 or 4), 3.75 (s, 3H, H1), 3.24 (d, *J* = 15.8 Hz, 1H, H14), 3.20 (d, *J* = 15.8 Hz, 1H, H14), 1.08 (s, 9H, H6); ^{13}C NMR (126 MHz, $CDCl_3$) δC 169.8, 167.3, 166.4, 141.5 Ar, 135.6 Ar, 129.6 Ar, 129.5 Ar, 128.8 Ar, 128.6 Ar, 128.5 Ar, 128.4 Ar, 73.5 C14, 67.1 C5, 62.0 C4, 59.2 C12, 52.4 C3, 42.2 C1, 27.4 C6; (NSI-FTMS) *m/z* $[M+H]^+$ Calcd for $[C_{24}H_{30}NO_6]^+$ 428.2068 found

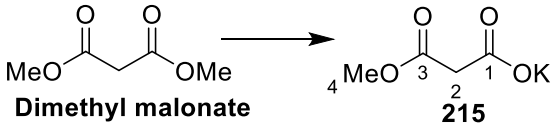
428.2065. Determined by HPLC to be 95% ee. Determination of ee was found by HPLC using an AD-H Chiralpak column 80:20 hexane/IPA, 230 nm, 0.8 mL/min, 25 °C.

(3S,5S)-benzyl 5-(tert-butoxymethyl)-3-methyl-2,4-dioxo-1-phenylpyrrolidine-3-carboxylate 212



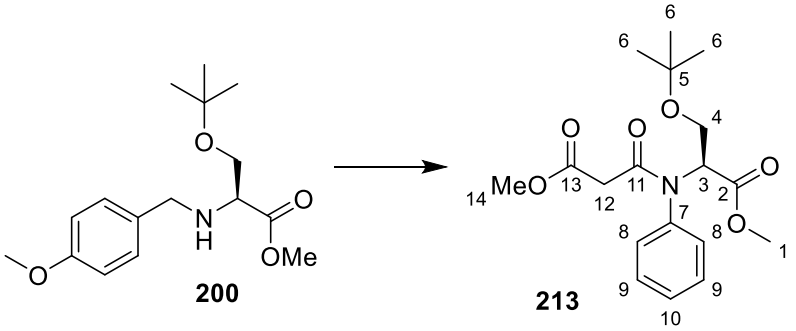
The Dieckmann cyclization precursor **211** (0.104 g, 0.24 mmol) was dissolved in ether (0.67 mL), TBAF (1 M in THF), (0.5 mL, 0.5 mmol 2.1 equiv) was added and the solution was stirred for 10 m, evaporated under reduced pressure, and placed under high vacuum for approximately 2 h. To the resulting white solid was added THF (0.67 mL), and the reaction was cooled to -12 °C. MeI (0.07 mL, 1.12 mmol, 4.7 equiv) was added and the suspension stirred for 72 h. Water (0.1 mL) was added and the solution was allowed to warm to room temperature where the solution was dissolved in ethyl acetate (30 mL) and washed with an equal amount of water and brine. The organic layer was dried (magnesium sulphate), filtered, and evaporated under reduced pressure. The resulting brown oil was purified by column chromatography with petroleum ether (40-60 °C)/ethyl acetate (9:1) as the eluent. Only the major diastereoisomer **212** could be isolated (0.008 g, 8%) Mp 121-124 °C $[\alpha]^{D\ 22}$ = +14.2 (*c* 0.81, $CHCl_3$) (87 % ee) ν_{max} (neat)/cm^{-1}: 2977 C-H (s), 2253, 1782 C=O (s), 1752 C=O (s), 1702 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.44 – 7.23 (m, 10H, Ar), 5.18 (s, 2H, H14), 4.57 (t, *J* = 2.1 Hz, 1H, H4), 3.68 (dd, *J* = 9.6, 1.8 Hz, 1H, H5), 3.39 (dd, *J* = 9.6, 2.4 Hz, 1H, H5), 1.65 (s, 3H, H13), 1.02 (s, 9H, H7); ^{13}C NMR (126 MHz, $CDCl_3$) δC 204.5, 169.5, 165.8, 135.8 Ar, 135.2 Ar, 129.4 Ar, 128.8 Ar, 128.5 Ar, 127.8 Ar, 127.5 Ar, 125.6 Ar, 73.9 C6, 68.8 C5, 68.0 C14, 59.7 C2, 57.9 C4, 27.2 C7, 15.2 C13; (NSI-FTMS) *m/z* $[M+H]^+$ Calcd for $[C_{24}H_{28}NO_5S]^+$ 410.1962 found 410.1956. Determined by HPLC to be 87% ee. Determination of ee was found by HPLC using an AD-H Chiralpak column 80:20 hexane/IPA, 230 nm, 0.8 mL/min, 25 °C.

Potassium 3-methoxy-3-oxopropanoate 215[206]



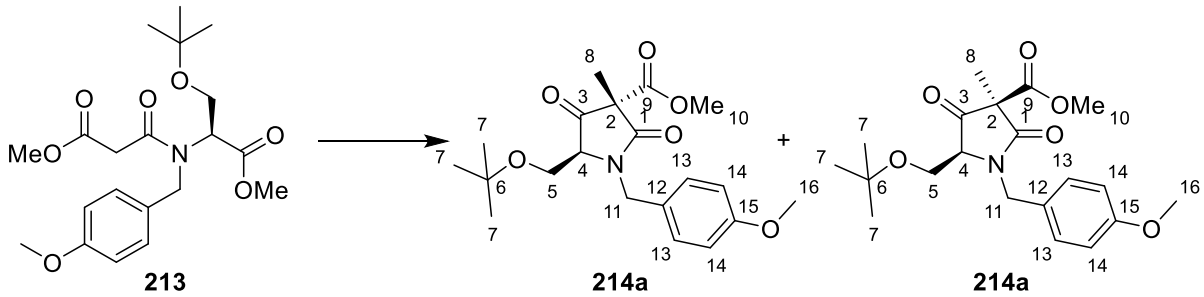
KOH (22.06 g, 393.18 mmol, 1.2 equiv) was dissolved in methanol (75 mL). The resulting solution was added to a beaker of dimethyl malonate (38 mL, 332.5 mmol) dissolved in methanol (85 mL). A white precipitate was formed which was collected by vacuum filtration and dried in a vacuum oven to provide (26.35 g 51%) of **215**, collected as a white solid. Mp: 204-209 °C (lit[207] 204-207 °C); ν_{max} (solid)/cm^{-1}: 1726 C=O (s), 1595, 1368; ^{1}H NMR (500 MHz, D_2O) δH 3.75 (s, 3H, H4), 3.34 (s, 2H, H3); ^{13}C NMR (101 MHz, D_2O) δC 174.8, 172.6, 53.2 C3, 45.0 C4.

(S)-methyl 3-(tert-butoxy)-2-(3-methoxy-N-(4-methoxybenzyl)-3-oxopropanamido)propanoate 213



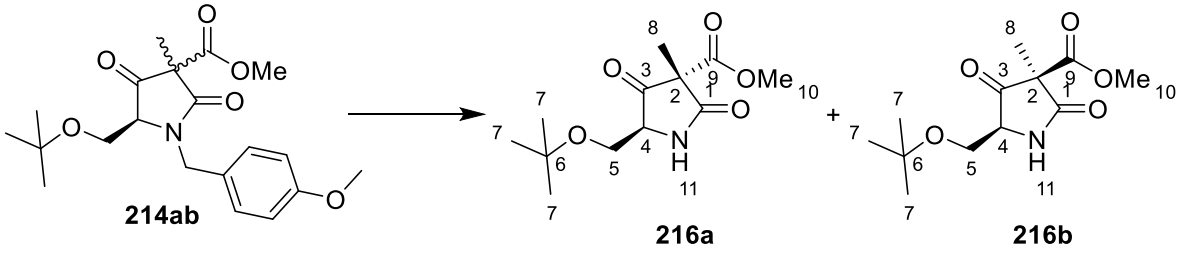
Amine **200** (0.5558 g, 1.88 mmol) was dissolved in anhydrous DCM (17.5 mL) in a flame dried flask. The methyl malonic half ester **215** (0.692 g, 4.43 mmol, 2.4 equiv) was added along with EDAC.HCl (1.06 g, 5.53 mmol, 2.9 equiv), DMAP (0.0405 g, 0.33 mmol, 0.18 equiv) and N-methylmorpholine (0.5 mL, 4.54 mmol, 2.4 equiv). The solution was stirred for 24 h under an atmosphere of nitrogen. HCl (1 M, 0.5 mL) was added, the solution transferred into a separating funnel, and washed with equal amounts of water and brine. The organic layer was dried (magnesium sulphate), filtered, and evaporated under reduced pressure to form a yellow oil, which was purified by column chromatography using petroleum ether (40-60 °C)/ethyl acetate (8:2) as the eluent to produce the desired compound **213** as a pale-yellow oil (0.54 g, 73%). $[\alpha]^{D\ 26}$ = -42.0 (*c* 1.13, $CHCl_3$); ν_{max} (neat)/cm^{-1}: 2973 C-H (s), 1744 C=O (s), 1656 C=O (s), 1514; ^{1}H NMR (400 MHz, $CDCl_3$) δH 7.29 (d, *J* = 7.5 Hz, 2H, H17), 6.91 (d, *J* = 8.7 Hz, 2H, H16), 4.73 (s, 2H, H12), 4.60 (dd, *J* = 7.6, 3.4 Hz, 1H, H4 or 3), 3.93 (dd, *J* = 9.7, 7.6 Hz, 1H, H4 or 3), 3.86 – 3.81 (m, 4H, H19, 3 or 4), 3.74 (s, 3H, H1 or 14), 3.72 (s, 3H, H1 or 14), 3.50 (d, *J* = 15.3 Hz, 1H, H7), 3.40 (d, *J* = 15.3 Hz, 1H, H7), 1.10 (s, 9H, H6); ^{13}C NMR (101 MHz, $CDCl_3$) δC 169.7, 167.8, 167.4, 159.2 Ar, 128.8 Ar, 128.1 Ar, 114.2 Ar, 73.5 C5, 60.7 C4, 59.8 C12, 55.4 C3, 52.5 C19, 52.3 C14 or 1, 52.2 C1 or 14, 41.3 C7, 27.3 C6; (NSI-FTMS) *m/z* $[M+H]^+$ Calcd for $[C_{20}H_{30}NO_7]^+$ 396.2017 found 396.2016.

(3S,5S)-methyl 5-(tert-butoxymethyl)-1-(4-methoxybenzyl)-3-methyl-2,4-dioxopyrrolidine-3-carboxylate 214a and (3R,5S)-methyl 5-(tert-butoxymethyl)-1-(4-methoxybenzyl)-3-methyl-2,4-dioxopyrrolidine-3-carboxylate 214b



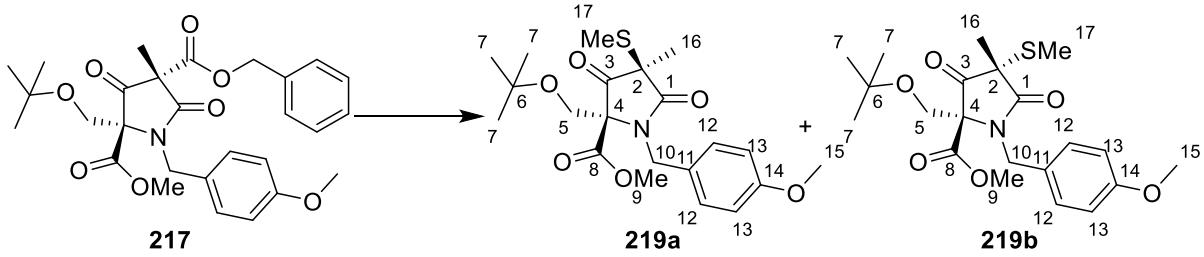
The Dieckmann cyclization precursor **213** (0.2219 g, 0.56 mmol) was dissolved in ether (1.6 mL). TBAF (1 M in THF), (1.6 mL, 1.6 mmol, 3 equiv) was added and the solution stirred for 5 m. Solvents were removed under reduced pressure to form a brown oil. The oil was dissolved in THF (1.6 mL) and cooled to -12 °C, MeI (0.15 mL, 2.4 mmol, 4.3 equiv) was added and the suspension stirred for 58 h. The solution was quenched with water (1 mL) and the reaction mixture was pipetted crude onto a short silica gel column. The column was washed with ethyl acetate until full elution of the product had occurred. The partially purified 3:1 mixture of diastereoisomers **214ab** was collected as a yellow oil and used onto the next step without further purification (0.1832 g, 87%). **Data for the major diastereoisomer 214a:** ^{1}H NMR (400 MHz, $CDCl_3$) δH 7.21 (d, *J* = 8.5 Hz, 2H, H14), 6.87 (d, *J* = 8.6 Hz, 2H, H13), 5.35 (d, *J* = 15.0 Hz, 1H, H11), 3.93 (d, *J* = 15.0 Hz, 1H, H11), 3.93 (t, *J* = 2.2 Hz, 1H, H4), 3.80 (s, 3H, H16), 3.73 (s, 3H, H10), 3.60 (dd, *J* = 9.8, 2.2 Hz, 1H, H5), 3.56 (dd, *J* = 9.9, 2.4 Hz, 1H, H5), 1.53 (s, 3H, H8), 1.11 (s, 9H, H7); ^{13}C NMR (101 MHz, $CDCl_3$) δC 205.0, 170.5, 166.7, 159.5, 129.5, 127.2, 114.4, 73.9 C6, 65.4 C5, 58.7 C2, 57.4 C4, 55.5 C16, 53.4 C10, 43.4 C11, 27.3 C7, 15.2 C8. **Data for the minor diastereoisomer 214b:** ^{1}H NMR (400 MHz, $CDCl_3$) δH 7.23 (d, *J* = 6.6 Hz, 2H, H14), 6.85 (d, *J* = 7.2 Hz, 2H, H13), 5.27 (d, *J* = 14.6 Hz, 1H, H11), 4.23 (d, *J* = 14.5 Hz, 1H, H11), 3.86 (dd, *J* = 5.8, 2.7 Hz, 1H, H5), 3.79 (s, 3H, H16), 3.75 (s, 3H, H10), 3.69 (m, 2H, H5, 4), 1.52 (s, 3H, H8), 1.17 (s, 9H, H7); ^{13}C NMR (101 MHz, $CDCl_3$) δC 204.0, 169.0, 166.3, 159.5, 129.0, 127.8, 114.3, 74.0 C6, 65.1 C5, 61.3 C2, 58.3 C4, 55.4 C16, 53.4 C10, 44.2 C11, 27.4 C7, 17.4 C8. **Data for both diastereoisomers:** ν_{max} (neat)/cm^{-1}: 2974 C-H (s), 1782 C=O (s), 1750 C=O (s), 1698 C=O (s), 1514; (NSI-FTMS) *m/z* $[M+H]^+$ Calcd for $[C_{20}H_{28}NO_6]^+$ 378.1911 found 378.1913.

(3S,5S)-methyl 5-(tert-butoxymethyl)-3-methyl-2,4-dioxopyrrolidine-3-carboxylate 216a and (3R,5S)-methyl 5-(tert-butoxymethyl)-3-methyl-2,4-dioxopyrrolidine-3-carboxylate 216b



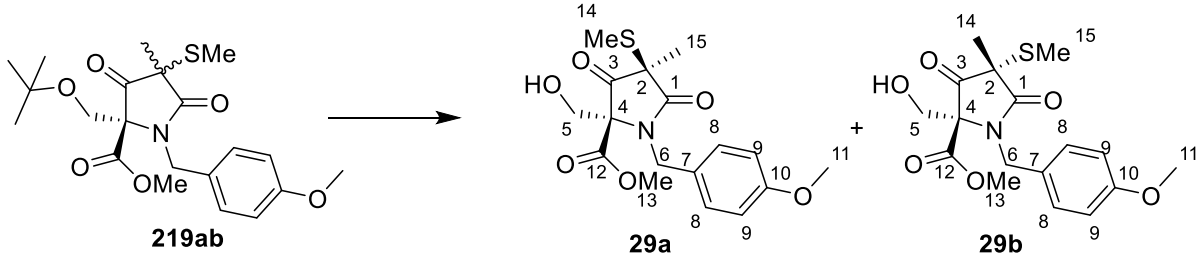
The mixture of diastereoisomers **214ab** (0.1251 g, 0.331 mmol) was dissolved in a MeCN/H_2O mixture (3:1) (3.5 mL). CAN (0.9 g, 1.64 mmol, 5 equiv) was added and the solution stirred vigorously until consumption of the starting material had occurred (approx 1.5 h). Ethyl acetate (25 mL) was added and the solution washed with water (25 mL). The aqueous was re-extracted with ethyl acetate (25 mL) and both organic layers were combined. The organic solution was further washed with water (25 mL) and brine (25 mL). The organic layer was dried (magnesium sulphate), filtered, and the solvents removed under reduced pressure. The crude material was purified by column chromatography with petroleum ether (40-60 °C)/ethyl acetate (8:2 – 7:3) as the eluent to provide a mixture of partially separable diastereoisomers (0.0483 g, 57% total) from which only **216a** could be obtained uncontaminated (0.01 g, 12%). **Data for the major, first eluting diastereoisomer 216a:** Mp 111-116 °C; $[\alpha]^{D\ 24}$ = -46.2 (*c* 0.91, $CHCl_3$); ν_{max} (neat)/cm^{-1}: 3234, 2976 C-H (s), 1785 C=O (s), 1750 C=O (s), 1706 C=O (s); ^{1}H NMR (400 MHz, $CDCl_3$) δH 6.72 (d, *J* = 39.7 Hz, 1H, H11), 4.18 (dd, *J* = 9.2, 3.6 Hz, 1H, H5), 3.77 – 3.69 (m, 4H, H5, 10), 3.53 (t, *J* = 9.1 Hz, 1H, H4), 1.55 (s, 3H, H8), 1.19 (s, 9H, H7); ^{13}C NMR (101 MHz, $CDCl_3$) δC 204.4, 171.4, 166.2 C1, 74.3 C6, 63.4 C5, 62.4 C2, 58.5 C4, 53.6 C10, 27.5 C7, 16.7 C8; (NSI-FTMS) *m/z* $[M+H]^+$ Calcd for $[C_{12}H_{20}NO_5]^+$ 258.1336 found 258.1339. **Data for the minor, second eluting diastereoisomer 216b:** ^{1}H NMR (400 MHz, $CDCl_3$) δH 6.73 – 6.31 (m, 1H, H11), 4.31 (t, J = 3.6 Hz, 1H, H4), 3.74 (s, 3H, H10), 3.61 (d, J = 3.6 Hz, 2H, H4), 1.51 (s, 3H, H8), 1.14 (s, 9H, H7); ^{13}C NMR (101 MHz, $CDCl_3$) δC 205.2, 172.2, 166.3, 74.1 C6, 63.5 C5, 61.0 C2, 58.6 C4, 53.6 C10, 27.4 C7, 15.1 C8.

(2R,4R)-methyl 2-(tert-butoxymethyl)-1-(4-methoxybenzyl)-4-methyl-4-(methylthio)-3,5-dioxopyrrolidine-2-carboxylate 219a and (2R,4S)-methyl 2-(tert-butoxymethyl)-1-(4-methoxybenzyl)-4-methyl-4-(methylthio)-3,5-dioxopyrrolidine-2-carboxylate 219b



Methyl ester **217** (0.1426 g, 0.31 mmol) was dissolved in THF (0.7 mL) and heated to 35 °C. A micro-spatula full of $Pd(OH)_2/C$ was added and the stirred solution was placed under a static atmosphere of hydrogen. After 16 h this solution was filtered through diatomaceous earth and washed through with DCM. The solution was evaporated to produce a crude foam which was dissolved in anhydrous DCM (0.5 mL). Sulphonothioate **186** (0.1090 g, 0.53 mmol, 1.7 equiv) was added to this along with triethylamine (0.05 mL, 0.35 mmol, 1.1 equiv) in a flame dried flask. The solution was stirred at 20 °C for 5 h under an atmosphere of nitrogen then evaporated, to a crude yellow oil. Purification was achieved using column chromatography with petroleum ether (40-60 °C)/ethyl acetate (9:1) as the eluent to produce a 4:1 (by NMR) mixture of inseparable diastereoisomers **219ab** as a pale-yellow oil (0.127 g, 70%). **Data for the major diastereoisomer 219a:** ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.26 (d, *J* = 8.7 Hz, 2H, H13), 6.82 (d, *J* = 8.7 Hz, 2H, H12), 4.74 (d, *J* = 15.2 Hz, 1H, H10), 4.44 (d, *J* = 15.2 Hz, 1H, H10), 3.89 (d, *J* = 9.6 Hz, 1H, H5), 3.78 (s, 3H, H15), 3.72 (d, *J* = 9.6 Hz, 1H, H5), 3.47 (s, 3H, H9), 2.16 (s, 3H, H17), 1.57 (s, 3H, H16), 1.01 (s, 9H, H7); ^{13}C NMR (101 MHz, $CDCl_3$) δ 201.6, 172.5, 166.2, 159.3 Ar, 130.3 Ar, 128.4 Ar, 113.8 Ar, 76.2 C6, 74.3 C5, 60.3 C2 or 4, 55.4 C4 or 2, 52.0 C15, 49.7 C9, 44.1 C10, 27.1 C7, 18.0 C16, 12.6 C17. **Data for the minor diastereoisomer 219b:** ^{1}H NMR (500 MHz, $CDCl_3$) δC 7.26 (d, *J* = 8.7 Hz, 2H, H13), 6.82 (d, *J* = 8.7 Hz, 2H, H12), 4.84 (d, *J* = 15.1 Hz, 1H, H10), 4.36 (d, *J* = 15.1 Hz, 1H, H10), 4.00 (d, *J* = 10.2 Hz, 1H, H5), 3.86 (d, *J* = 10.2 Hz, 1H, H5), 3.78 (s, 3H, H15), 3.37 (s, 3H, H9), 2.20 (s, 3H, H17), 1.61 (s, 3H, H16), 1.08 (s, 9H, H7); ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.4, 172.7, 166.7, 159.3 Ar, 130.4 Ar, 128.0 Ar, 113.8 Ar, 75.8 C6, 74.4 C5, 59.7 C2 or 4, 55.4 C4 or 2, 53.1 C15, 50.7 C9, 44.2 C10, 27.1 C7, 20.7 C16, 12.9 C17. **Data for both diastereoisomers:** ν_{max} (neat)/cm^{-1}: 3020, 1741 C=O (s), 1699 C=O (s); (NSI-FTMS) *m/z* $[M+H]^+$ Calcd for $[C_{21}H_{30}NO_6S]^+$ 424.1788 found 424.1790.

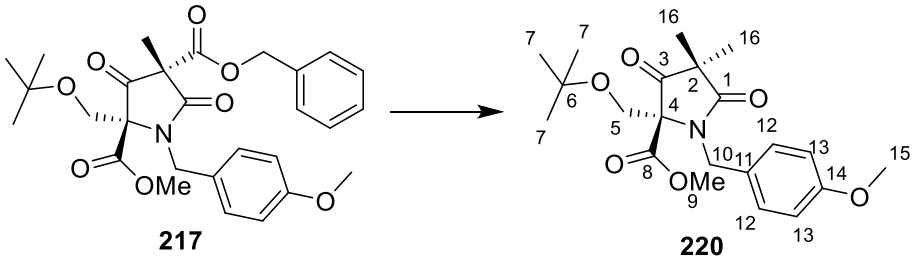
(2R,4R)-methyl 2-(hydroxymethyl)-1-(4-methoxybenzyl)-4-methyl-4-(methylthio)-3,5-dioxopyrrolidine-2-carboxylate 29a[76] **and (2R,4S)-methyl 2-(hydroxymethyl)-1-(4-methoxybenzyl)-4-methyl-4-(methylthio)-3,5-dioxopyrrolidine-2-carboxylate 29b**



The 4:1 mixture of inseparable diastereoisomers **219ab** (0.0297 g, 0.07 mmol) in a flame dried flask was dissolved in anhydrous DCM (0.15 mL) and TFA (0.15 mL). The solution was stirred under nitrogen for 1.5 h or until TLC showed complete consumption of starting material. The solution was diluted with DCM (50 mL), and an equal amount of water was carefully added and used to wash the organic layer. The organic solution was further washed with an equal amount of a $NaHCO_3$ solution. The organic layer was dried (sodium sulphate), filtered, and evaporated to a brown residue. The residue was purified by column chromatography using petroleum ether (40-60 °C)/ethyl acetate (2:1) as the eluent to produce **29ab** as a 2:1 mixture of inseparable isomers (0.0195 g, 76%). **Data for the major diastereomer 29a:** ^{1}H NMR (400 MHz, $CDCl_3$) δH 7.33 (d, *J* = 8.6 Hz, 2H, H9), 6.86 (d, *J* = 8.6 Hz, 2H, H8), 5.08 (d, *J* = 15.2 Hz, 1H, H6), 4.35 (d, *J* = 15.2 Hz, 1H, H6), 4.17 (d, *J* = 12.0 Hz, 1H, H5), 3.79 (d, *J* = 12.8 Hz, 4H, H5 and 11), 3.67 (s, 3H, H13), 2.12 (s, 3H, H4), 1.54 (s, 3H, H5), hydroxyl proton unobserved in ^{1}H NMR spectrum; ^{13}C NMR (101 MHz, $CDCl_3$) δC 199.1, 172.2, 165.7, 159.7 Ar, 129.9 Ar, 128.8 Ar, 114.6 Ar, 77.6 C5, 61.8 C2 or 4, 55.4 C2 or 4, 53.4 C11, 49.7 C13, 44.3 C6, 16.9 C15, 12.4 C14. Analysis on HPLC determined ee to be 58%. **Data for the minor diastereomer 29b:** ^{1}H NMR (400 MHz, $CDCl_3$) δH 7.27 (d, *J* = 9.7 Hz, 2H, H9), 6.84 (d, *J* = 7.2 Hz, 2H, H8), 4.68 (d, *J* = 15.0 Hz, 1H, H6), 4.60 (d, *J* = 15.0 Hz, 1H, H6), 4.20 (d, *J* = 11.8 Hz, 1H, H5), 4.07 (d, *J* = 12.6 Hz, 1H, H5), 3.77 (s, 3H, H11), 3.42 (s, 3H, H13), 2.17 (s, 3H, C15), 1.61 (s, 3H, C14), hydroxyl proton unobserved in ^{1}H NMR spectrum; ^{13}C NMR (101 MHz, $CDCl_3$) δC 200.3, 172.2, 166.7, 159.6 Ar, 130.3 Ar, 127.9 Ar, 114.3 Ar, 76.6 C5, 62.01 C2 or 4, 55.4 C2 or 4, 53.4 C11, 49.5 C13, 44.4 C6, 18.1 C15, 12.2 C14. Analysis on HPLC determined ee to be 41% ee. **Data for both diastereoisomers:** ν_{max} (neat)/cm^{-1}: 3419, 3000, 2932 C-H (s), 1776 C=O (s), 1742 C=O (s), 1699 C=O (s); (NSI-FTMS) *m/z* $[M+H]^+$ Calcd for $[C_{17}H_{22}NO_6S]^+$ 368.1162 found 368.1165.

Determination of ee was found by HPLC using an AD-H Chiralpak column 80:20 hexane/IPA, 230 nm, 0.8 mL/min, 25 °C.

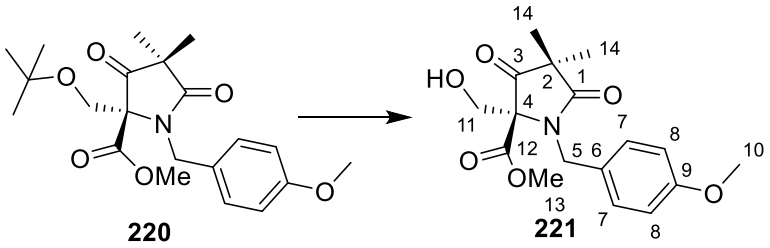
(R)-methyl 2-(tert-butoxymethyl)-1-(4-methoxybenzyl)-4,4-dimethyl-3,5-dioxopyrrolidine-2-carboxylate 220



Methyl ester **217** (0.0832 g, 0.16 mmol) was dissolved in THF (0.5 mL) and the solution heated to 35 °C. $Pd(OH)_2/C$ (20% nominally on water) (0.604 g) was added and the solution put under a static atmosphere of hydrogen. After 16 h this solution was filtered through diatomaceous earth and washed through with DCM. The solution was evaporated to produce a crude foam which was dissolved in DCM (0.6 mL). The crude compound was stirred under an atmosphere of nitrogen and triethylamine was added (0.04 mL, 0.29 mmol, 1.8 equiv). After 15 m MeI was added (0.04 mL 0.64 mmol, 4 equiv). The solution was stirred for a further 5 h, diluted with DCM (50 mL), and washed with water (50 mL) and brine (50 mL). The organic layer was dried (sodium sulphate), filtered, evaporated under reduced pressure and purified with column chromatography using petroleum ether (40-60 °C)/ethyl acetate (8:2) as the eluent to produce a pink oil. The oil was dissolved in DCM and washed with a saturated sodium thiosuphate solution until the organic layer became clear, then, dried with sodium sulphate, filtered, and evaporated under reduced pressure to produce **220** as a colourless oil. (0.0286 g, 46% over the 2 steps). $[\alpha]^{D\ 22}$ = +50 (*c* 0.31, $CHCl_3$) (86% ee); ν_{max} (neat)/cm^{-1}: 3019, 2978 C-H (s), 1779 C=O (s), 1742 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.22 (d, *J* = 8.7 Hz, 2H, H13), 6.81 (d, *J* = 8.7 Hz, 2H, H12), 4.87 (d, *J* = 15.1 Hz, 1H, H10), 4.19 (d, *J* = 15.1 Hz, 1H, H10), 3.89 (d, *J* = 9.8 Hz, 1H, H5), 3.77 (s, 3H, H15), 3.75 (d, *J* = 9.8 Hz, 1H, H5), 3.31 (s, 3H, H9), 1.34 (s, 3H, H16), 1.28 (s, 3H, H16), 1.05 (s, 9H, H7); ^{13}C NMR (126 MHz, $CDCl_3$) δC 209.1, 177.3, 166.9, 159.3, 130.5 Ar, 128.2 Ar, 113.8 Ar, 75.8 C6, 74.1 C5, 58.9 C2 or 4, 55.4 C4 or 2, 52.8 C15, 46.1 C9, 43.3 C10, 27.2 C7, 22.2 C16, 20.6 C16; HRMS (NSI-FTMS) *m/z* $[M+H]^+$ Calcd for $[C_{21}H_{30}NO_6]^+$ 392.2068 found 392.2068. Analysis on HPLC determined ee to be 86% ee. Determination of

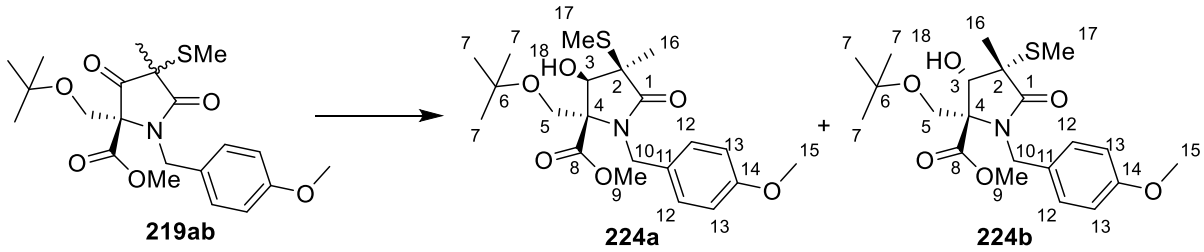
ee was found by HPLC using an Eurocel 01 Knauer column 95:5 hexane/IPA, 230 nm, 0.8 mL/min, 25 °C.

(R)-methyl 2-(hydroxymethyl)-1-(4-methoxybenzyl)-4,4-dimethyl-3,5-dioxopyrrolidine-2-carboxylate 221



Lactam **220** (0.0109 g, 0.027 mmol) was dissolved in dry DCM (0.1 mL) and TFA (0.1 mL). The solution was stirred under nitrogen for 1.5 h or until TLC showed complete consumption of starting material. The solution was diluted up to 50 mL with DCM and an equal amount of water was carefully added and used to wash the organic layer. The organic layer was further washed with a saturated $NaHCO_3$ solution (50 mL). The organic layer was then dried (sodium sulphate), filtered, and evaporated to a white solid. This was purified by column chromatography using petroleum ether (40-60 °C)/ethyl acetate (2:1) as the eluent to produce the deprotected alcohol **221** as a gummy residue (0.0062 g, 67%). $[\alpha]^{D\ 24}$ = -7.74 (*c* 0.62, $CHCl_3$) (86% ee); ν_{max} (neat)/cm^{-1}: 3396 O-H (b), 2919 C-H (s), 1778 C=O (s), 1742 C=O (s), 1678 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.30 (d, *J* = 8.7 Hz, 2H, H8), 6.86 (d, *J* = 8.7 Hz, 2H, H7), 4.89 (d, *J* = 15.1 Hz, 1H, H5), 4.31 (d, *J* = 15.1 Hz, 1H, H5), 4.14 (dd, *J* = 12.3, 8.6 Hz, 1H, H11), 3.85 (dd, *J* = 12.3, 4.4 Hz, 1H, H11), 3.79 (s, 3H, H10), 3.55 (s, 3H, H13), 1.35 (s, 3H, H14), 1.28 (s, 3H, H14), 1.17 (dd, *J* = 8.6, 4.5 Hz, 1H, OH); ^{13}C NMR (126 MHz, $CDCl_3$) δC 208.3, 177.3, 166.4, 159.6 , 130.1 Ar, 128.7 Ar, 114.5 Ar, 77.3 C11, 60.8 C4 or 2, 55.4 C 2 or 4, 53.2 C10, 46.1 C13, 43.7 C5, 22.0 C14, 20.5 C14; HRMS (NSI-FTMS) *m/z* $[M+H]^+$ Calcd for $[C_{17}H_{22}NO_6]^+$ 336.1442 found 336.1443. Determined by HPLC to be 86% ee. Determination of ee was found by HPLC using an AD-H Chiralpak column 80:20 hexane/IPA, 230 nm, 0.8 mL/min, 25 °C.

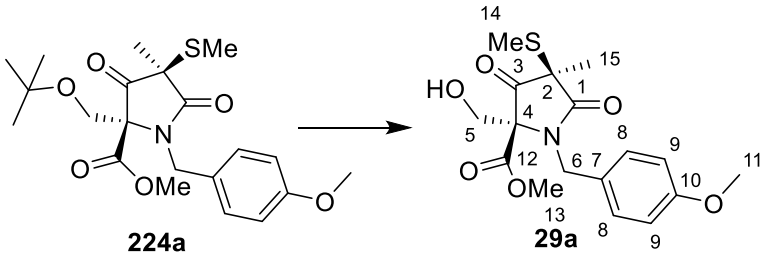
(2R,3R,4R)-methyl 2-(tert-butoxymethyl)-3-hydroxy-1-(4-methoxybenzyl)-4-methyl-4-(methylthio)-5-oxopyrrolidine-2-carboxylate 224a and (2R,3S,4S)-methyl 2-(tert-butoxymethyl)-3-hydroxy-1-(4-methoxybenzyl)-4-methyl-4-(methylthio)-5-oxopyrrolidine-2-carboxylate 224b



The mixture of diastereoisomers **219ab** (0.1407 g, 0.33 mmol) was dissolved in ethanol (9 mL) and the solution was cooled with an ice bath. Sodium borohydride (0.0073 g, 0.19 mmol, 0.6 equiv) was added and the reaction was stirred for 20 m. Water (40 mL) was added to quench the reaction and the resulting solution was extracted with equal amounts of ethyl acetate (3 x 40 mL). The combined organic fractions were washed with and equal amount of brine, dried (sodium sulphate), filtered and evaporated. The resulting residue was purified using column chromatography with petroleum ether (40-60 °C)/ethyl acetate (8:2 - 2:1) as the eluent to provide 2 separable diastereoisomers **224a** and **224b** as gummy oils. The first eluting diastereoisomer (0.0764 g, 54%) and the second (0.0181 g, 13%). Analysis on chiral HPLC showed the of the diastereoisomers were 67% and 66% ee respectively. Diastereomer **224a** however upon re-crystalization from IPA gave 0.0511 g of material from the supernatant at 99% ee. **Data for major, first eluting diastereoisomer 224a:** $[\alpha]^{D\ 23}$ = +6.2 (*c* 0.71, $CHCl_3$) (99% ee); ν_{max} (neat)/cm^{-1}: 3418, 2973, 2926, 1743, 1697; ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.24 (d, *J* = 8.7 Hz, 2H, H13), 6.81 (d, *J* = 8.7 Hz, 2H, H12), 4.71 (d, *J* = 15.2 Hz, 1H, H10), 4.46 (d, *J* = 15.2 Hz, 1H, H10), 3.99 – 3.87 (m, 2H, H3 and 18), 3.82 (d, *J* = 9.7 Hz, 1H, H5), 3.78 (s, 3H, H15), 3.65 (s, 3H, H9), 3.44 (d, *J* = 9.7 Hz, 1H, H5), 2.12 (s, 3H, H17), 1.60 (s, 3H, H16), 1.04 (s, 9H, H7); ^{13}C NMR (126 MHz, $CDCl_3$) δC 173.0, 172.1, 158.9, 130.4 Ar, 129.6 Ar, 113.7 Ar, 77.9 C3, 74.1 C6, 70.7 C5, 62.8 C4 or 2, 55.4 C2 or 4, 53.4 C15, 52.5 C9, 45.2 C10, 27.2 C7, 22.9 C18, 12.3 C16; HRMS (NSI-FTMS) *m/z* $[M+H]^+$ Calcd for $[C_{21}H_{32}NO_6S]^+$ 426.1945 found 426.1942. **Data for the minor, second eluting diastereomer 224b:** $[\alpha]^{D\ 22}$ = +5.26 (*c* 0.38, $CHCl_3$) (66% ee); ν_{max} (neat)/cm^{-1}: 3385 O-H (b), 3016, 2975 C-H (s), 1743 C=O (s), 1686 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18 (d, *J* = 8.6 Hz, 2H, H13), 6.81 (d, *J* = 8.7 Hz, 2H, H12), 4.77 (d, *J* = 15.5

Hz, 1H, H10), 4.43 (d, *J* = 15.5 Hz, 1H, H3), 4.32 (s, 1H, H3), 3.96 (d, *J* = 9.7 Hz, 1H, H5), 3.77 (s, 3H, H15), 3.75 (s, 1H, H10), 3.67 (d, *J* = 9.7 Hz, 1H, H5), 3.64 (s, 3H, H15), 2.19 (s, 3H, H17), 1.53 (s, 3H, H16), 1.02 (s, 9H, H7);^{13}C NMR (126 MHz, $CDCl_3$) δ 173.8, 171.7, 158.8 Ar, 130.3 Ar, 128.9 Ar, 113.7 Ar, 78.0 C3, 74.4 C6, 71.6 C5, 62.1 C2 or 4, 55.7 C4 or 2, 55.4 C15, 52.7 C9, 45.3 C10, 27.1 C7, 22.6 C17, 12.7 C16; HRMS (NSI-FTMS) *m/z* $[M+H]^+$ Calcd for $[C_{21}H_{32}NO_6S]^+$ 426.1945 found 426.1945. Determination of ee was found by HPLC using an AD-H Chiralpak column 90:10 hexane/IPA, 230 nm, 0.8 mL/min, 25 °C.

(2R,3R,4R)-methyl 3-hydroxy-2-(hydroxymethyl)-1-(4-methoxybenzyl)-4-methyl-4-(methylthio)-5-oxopyrrolidine-2-carboxylate 29a[76]

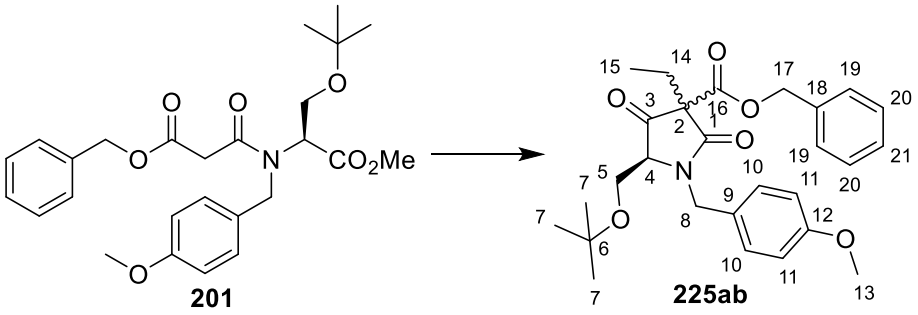


Alcohol **224a** (0.0427 g, 0.1 mmol) was dissolved in anhydrous DCM (0.21 mL) in a flame dried flask. TFA (0.21 mL) was added and the reaction was stirred under an atmosphere of nitrogen until TLC showed complete consumption of starting material (about 1.5h). The reaction mixture was diluted with DCM (20 mL) and quenched with water (20 mL). The organic layer was removed and the aqueous extracted again with DCM (20 mL). The organic layers were combined and washed with saturated $NaHCO_3$ and brine (20 mL of each), dried (sodium sulphate), filtered, and evaporated under reduced pressure. The resulting residue was purified by column chromatography using petroleum ether (40-60 °C)/ethyl acetate 1:1 as the eluent to yield **29a** as a white solid (0.0279 g, 75%). Mp 128 – 130 °C (lit[29] 129 °C); $[\alpha]^{D\ 23}$ = –33.84 (*c* 0.13, $CHCl_3$) (99% ee) (lit[29] $[\alpha]^{D\ 23}$ = –41.8 (*c* 0.1, $CHCl_3$)); ν_{max} (neat)/cm^{-1}: 3416 O-H (b), 2925 C-H (s), 2852, 1737 C=O (s), 1675 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.29 (d, *J* = 8.5 Hz, 2H, H9), 6.85 (d, *J* = 8.7 Hz, 2H, H8), 5.11 (d, *J* = 15.3 Hz, 1H, H6), 4.13 (d, *J* = 7.7 Hz, 1H, H3 or 5), 4.05 (d, *J* = 15.3 Hz, 1H, H6), 3.85 – 3.77 (m, 5H, H11 and 3 or 5 or OH), 3.76 (s, 3H, H13), 3.67 (d, *J* = 8.1 Hz, 1H, H3 or 5 or OH), 2.14 (s, 3H, H14), 1.61 (s, 3H, H15); ^{13}C NMR (126 MHz, $CDCl_3$) δC 173.5, 171.6, 159.5 Ar, 129.8 Ar, 129.6 Ar, 114.6 Ar, 76.8 C3, 72.4 C5, 62.5 C2

or 4, 55.4 C4 or 2, 53.4 C11, 52.9 C13, 44.8, 22.9 C14 12.4 C15; HRMS (NSI-FTMS) *m/z* $[M+H]^+$ Calcd for $[C_{17}H_{24}NO_6S]^+$ 370.1319 found 370.1320. Analysis on HPLC determined ee to be 99%. Determination of ee was found by HPLC using an AD-H Chiralpak column 80:20 hexane/IPA, 230 nm, 0.8 mL/min, 25 °C.

3.2.2.2 Experimental toward the synthesis of salinosporamide B

(3S,5S)-benzyl 5-(tert-butoxymethyl)-3-ethyl-1-(4-methoxybenzyl)-2,4-dioxopyrrolidine-3-carboxylate and (3R,5S)-benzyl 5-(tert-butoxymethyl)-3-ethyl-1-(4-methoxybenzyl)-2,4-dioxopyrrolidine-3-carboxylate 225ab



Cyclization ran at room temperature:

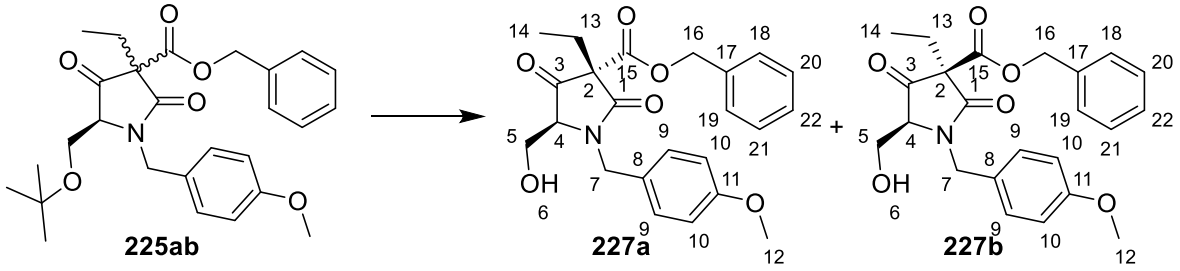
The cyclization precursor **201** (1.5562 g, 3.3 mmol) was dissolved in ether (9.5 mL), TBAF (1 M in THF, 6.6 mL, 6.6 mmol, 2 equiv) was added until a white precipitate formed (<5 m). Solvents were removed under reduced pressure and the resulting white solid suspended in THF (9.5 mL) under a nitrogen atmosphere. EtI was added (0.58 mL, 7.21 mmol, 2.2 equiv) and the solution stirred for 15 h. A second aliquot of EtI was added (0.58 mL, 7.21 mmol, 2.2 equiv) and the solution stirred for a further 5 h. Water (20 mL) was added and the resulting mixture was extracted with DCM (3 x 50 mL). The organic layers were combined and washed with water (50 mL), and brine (50 mL). The organic extractions were dried (magnesium sulphate) filtered, and evaporated under reduced pressure to provide the crude material as a yellow oil. The oil was purified by column chromatography using petroleum ether (40-60 °C)/ethyl acetate (9:1) as the eluent to provide **225ab** as a 1:0.4 mixture of diastereoisomers through NMR as a pale-yellow oil (0.669 g, 43%).

Cyclization ran at 0 °C to rt:

The cyclization precursor **201** (0.6304 g, 1.34 mmol) was dissolved in ether (3.8 mL). TBAF (1 M in THF, 2.7 mL, 2.7 mmol, 2 equiv) was added until a white precipitate formed (<5 m). Solvents were removed under reduced pressure and the resulting white solid suspended in THF (3.8 mL) under a nitrogen atmosphere. EtI was added at 0 °C (0.95 mL, 11.8 mmol, 8.8 equiv) and the solution was allowed to warm slowly to room temperature over 20 h. Water (20 mL) was added and the resulting mixture was extracted with DCM (3 x 20 mL). The organic

layers were combined and washed with water (50 mL) and brine (50 mL). The organic extractions were dried (magnesium sulphate), filtered, and evaporated under reduced pressure to provide the crude material as a yellow oil. The oil was purified by column chromatography using petroleum ether (40-60 °C)/ethyl acetate (9:1) as the eluent to provide **225ab** as a 1:0.65 mixture of diastereoisomers from NMR as a pale yellow oil (0.2236 g, 36%). **Data for the major diastereoisomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δH 7.39 – 7.19 (m, 5H, H21, 20, 19), 7.03 (d, *J* = 8.5 Hz, 2H, H11), 6.62 (d, *J* = 8.7 Hz, 2H, H12), 5.41 (d, *J* = 15.0 Hz, 1H, H7), 5.17 (d, *J* = 10.5 Hz, 1H, H8 or 17), 5.09 (d, *J* = 12.3 Hz, 1H, H8 or 17), 3.90 (d, *J* = 15.0 Hz, 1H, H8 or 17), 3.83 (t, *J* = 2.6 Hz, 1H, H4), 3.74 (s, 3H, H13), 3.66 (dd, *J* = 10.0, 2.5 Hz, 1H, H5), 3.54 (dd, *J* = 10.0, 2.8 Hz, 1H, H5), 2.23 (m, 2H, H14), 1.12 (s, 9H, H7), 0.95 (t, *J* = 7.5 Hz, 3H, H15); ^{13}C NMR (101 MHz, $CDCl_3$) δC 204.4, 169.2, 165.5, 159.2, 135.0, 129.4, 128.8, 128.3, 126.9, 114.2, 73.8, 68.0, 64.9, 63.4, 55.4, 43.3, 27.2, 23.8, 8.8, two carbon peaks unobserved in ^{13}C spectrum. **Data for the minor diastereoisomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δH 7.42 – 7.18 (m, 7H, C21, 20, 19), 6.81 (d, *J* = 8.7 Hz, 2H, H10), 5.30 – 5.16 (m, 3H, H8 or 17), 4.28 (d, *J* = 14.3 Hz, 1H, H8 or 17), 3.79 – 3.74 (m, 4H, H13 and 18 or 17), 3.60 (dd, *J* = 9.7, 2.5 Hz, 1H, H5), 3.46 (dd, *J* = 9.7, 7.1 Hz, 1H, H5), 2.30 – 2.14 (m, 2H, H14), 1.03 (s, 9H, H7), 0.72 (t, *J* = 7.5 Hz, 3H, H15). ^{13}C NMR (101 MHz, $CDCl_3$) δC 204.3, 168.6, 165.1, 159.2, 134.9, 130.2, 128.7, 128.5, 127.9, 114.0, 73.9, 67.9, 66.2, 63.4, 62.6, 55.3, 44.4, 27.1, 25.5, 8.5, one carbon peak unobserved in ^{13}C spectrum. **Data for the mix of diastereoisomers:** ν_{max} (neat)/cm^{-1}: 3450, 2974 C-H (s), 1778 C=O (s), 1747 C=O (s), 1696 C=O (s); HRMS (NSI-FTMS) m/z $[M + H]^+$ calcd for $[C_{27}H_{34}NO_6]^+$ 468.2381, found 468.2374.

(3S,5S)-benzyl 3-ethyl-5-(hydroxymethyl)-1-(4-methoxybenzyl)-2,4-dioxopyrrolidine-3-carboxylate and (3R,5S)-benzyl 3-ethyl-5-(hydroxymethyl)-1-(4-methoxybenzyl)-2,4-dioxopyrrolidine-3-carboxylate

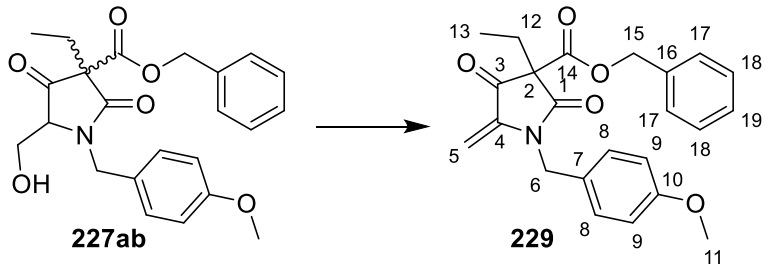


The inseparable mixture of diastereoisomers **225ab** (0.066 g, 0.14 mmol) of the 1:0.4 mixture of diastereoisomers from the room temperature cyclization was dissolved in DCM (0.3 mL) with TFA (0.3 mL). The solution was stirred at room temperature under argon until complete consumption of the starting material (about 1h) had occurred. DCM (40 mL) was added and the organic solution was carefully washed with water (20 mL x 2) and saturated sodium hydrogen carbonate solution (20 mL x 2). The organic layer was dried (magnesium sulphate), filtered, and evaporated under reduced pressure to produce a colourless residue. The diastereoisomers could be separated by column chromatography using petroleum ether (40-60 °C)/ethyl acetate (8:2) as the eluent, the first eluting diastereoisomer as a colourless oil (0.010 g, 18%) and the second as a colourless oil (0.032 g, 55%). The first eluting diastereomer was found to have an ee of 66%, the second was found to have an ee of 45%.

The inseparable mixture of diastereoisomers **225ab** (0.2236 g, 0.47 mmol) of the 1:0.65 mixture of diastereoisomers from the 0 °C cyclization was dissolved in DCM (1 mL) with TFA (1 mL). The solution was stirred at room temperature under argon until complete consumption of the starting material (about 1h) had occurred. DCM (50 mL) was added and the organic solution was carefully washed with water (30 mL x 2) and saturated sodium hydrogen carbonate solution (30 mL x 2). The organic layer was dried (magnesium sulphate), filtered, and evaporated under reduced pressure to produce a colourless residue. The diastereoisomers could be separated using column chromatography with petroleum ether (40-60 °C)/ethyl acetate as the eluent. The first eluting diastereoisomer as a colourless oil (0.058 g, 29%) and the second as a colourless oil (0.0902 g, 46%). The first eluting diastereomer was found to have an ee of 78%, the second was found to have an ee of 60%.

Data for the first eluting diatereoisomer: $[\alpha]^{D\ 24}$ = -34 (*c* 0.73, $CHCl_3$) (66% ee); ν_{max} (neat)/cm^{-1}: 3448 O-H (s), 2939 C-H (s), 1776 C=O (s), 1744 C=O (s), 1682 C=O (s); ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.40 – 7.33 (m, 3H, H22, 18, 19), 7.30 (dd, *J* = 7.6, 1.8 Hz, 2H, H20, 21), 7.20 (d, *J* = 8.6 Hz, 2H, H10), 6.81 (d, *J* = 8.7 Hz, 2H, H9), 5.19 (d, *J* = 12.2 Hz, 1H, H7 or 16), 5.14 (d, *J* = 12.2 Hz, 1H, H7 or 16), 5.13 (d, *J* = 14.18 Hz, 1H, H7 or 16), 4.30 (d, *J* = 14.8 Hz, 1H, H7 or 16), 3.90 (dd, *J* = 12.2, 3.3 Hz, 1H, H5), 3.78 (s, 3H, H12), 3.75 (dd, *J* = 7.7, 4.5 Hz, 1H, H5), 3.67 – 3.64 (m, 1H, H4), 2.36 – 2.18 (m, 2H, H13), 0.81 (t, *J* = 7.5 Hz, 3H, H14); ^{13}C NMR (101 MHz, $CDCl_3$) δC 205.7, 169.0, 165.8, 159.6 Ar, 134.7 Ar, 129.7 Ar, 128.9 Ar, 128.9 Ar, 128.4 Ar, 127.4 Ar, 114.6 Ar, 68.5 C5, 67.5 C2 or 16, 63.9 C2 or 16, 60.3 C4, 55.4 C12, 44.2 C7, 25.4 C13, 8.7 C14; HRMS (FTMS-NSI) m/z $[M + H]^+$ calcd for $[C_{23}H_{26}NO_6]^+$ 412.1755, found 412.1753. **Data for the second eluting diastereoisomer:** $[\alpha]^{D\ 24}$ = -72.4 (*c* 1.1, $CHCl_3$) (60% ee); ν_{max} (neat)/cm^{-1}: 3440 O-H (s), 2940 C-H (s), 2253, 1780 C=O (s), 1747 C=O (s), 1688 C=O (s); ^{1}H NMR (400 MHz, $CDCl_3$) δH 7.29 – 7.25 (m, 3H, H22, 18, 19), 7.15 – 7.12 (m, 2H, H20, 21), 7.02 (d, *J* = 8.6 Hz, 2H, H10), 6.59 (d, *J* = 8.7 Hz, 2H, H9), 5.14 (d, *J* = 15 Hz, 1H, H7 or 16), 5.09 (d, *J* = 12.3 Hz, 1H, H7 or 16), 5.04 (d, *J* = 12.3 Hz, 1H, H7 or 16), 4.10 (d, *J* = 15.0 Hz, 1H, H7 or 16), 3.91 (dd, *J* = 12.0, 2.7 Hz, 1H, H5) 3.81 – 3.78 (m, 1H, H4), 3.72 (dd, *J* = 12.0, 3.6 Hz, 1H, H5), 3.68 (s, 3H, H12), 2.24 – 2.10 (m, 2H, H13), 0.85 (t, *J* = 7.5 Hz, 3H, H14). ^{13}C NMR (101 MHz, $CDCl_3$) δC 204.9, 169.2, 165.1, 159.5 Ar, 134.9 Ar, 129.5 Ar, 128.9 Ar, 128.7 Ar, 128.3 Ar, 126.9 Ar, 114.5 Ar, 68.2 C5, 66.3 C2 or 16, 63.7 C2 or 16, 58.7 C4, 55.4 C12, 43.9 C7, 23.8 C13, 8.8 C14; HRMS (FTMS-NSI) m/z $[M + H]^+$ calcd for $[C_{23}H_{26}NO_6]^+$ 412.1755, found 412.1752. Determination of ee was found by HPLC using an AD-H Chiralpak column 80:20 hexane/IPA, 230 nm, 0.8 mL/min, 25 °C.

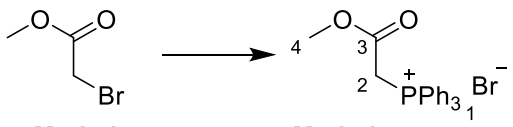
Benzyl 3-ethyl-1-(4-methoxybenzyl)-5-methylene-2,4-dioxopyrrolidine-3-carboxylate 229



The racemic mixture of diastereoisomers **227ab** (0.0336 g, 0.082 mmol) was dissolved in anhydrous THF (1.7 mL) under an atmosphere of nitrogen and cooled to -78 °C. LiHMDS (1 M in THF, 0.25 mL, 0.25 mmol, 3 equiv) was added along with DMPU (0.3 mL, 2.48 mmol, 30 equiv). The solution was stirred for 30 m before methyl cyanoformate (0.03 mL, 0.377 mmol, 4.6 equiv) was added. The solution was further stirred at -78 °C for 3 h then, saturated ammonium chloride solution (0.3 mL) was added and the mixture was allowed to warm to room temperature. Ethyl acetate was added (30 mL) and the solution was washed with water (30 mL). The aqueous layer was re-extracted with ethyl acetate (30 mL) and the organic extractions were combined. The combined organic layers were washed with water (30 mL) and brine (30 mL). The organic layer was dried (magnesium sulphate), filtered, and evaporated under reduced pressure to provide a pale brown oil. The oil was purified by column chromatography using petroleum ether (40-60 °C)/ethyl acetate (9:1) as the eluent to provide **229** as a colourless residue, which appeared green in solution (0.0085 g, 27%). ν_{max} (neat)/cm^{-1}: 2936 C-H (s), 1769 C=O (s), 1720 C=O (s), 1631; ^{1}H NMR (500 MHz, $CDCl_3$) δH 7.38 – 7.30 (m, 3H H19, 17), 7.25 – 7.21 (m, 2H, H18), 7.08 (d, *J* = 8.8 Hz, 2H, H9), 6.67 (d, *J* = 8.7 Hz, 2H, H8), 5.23 – 5.19 (m, 2H, H5, 15 or 6), 5.12 (d, *J* = 12.4 Hz, 1H, H15 or 6), 5.06 (d, *J* = 15.2 Hz, 1H, H15 or 6), 4.61 (d, *J* = 15.3 Hz, 1H, H15 or 6), 4.53 (d, *J* = 2.3 Hz, 1H, H5), 3.74 (s, 3H, H11), 2.30 (q, *J* = 7.5 Hz, 2H, H12), 0.85 (t, *J* = 7.5 Hz, 3H, H13); ^{13}C NMR (126 MHz, $CDCl_3$) δC 192.7, 169.0, 164.9, 159.3, 141.0, 134.9, 128.8, 128.6, 128.6, 128.1, 126.1, 114.3, 94.2, 68.2, 62.3, 55.4 C11, 43.6 C6, 24.8 C12, 8.4 C13; HRMS (NSI-FTMS) m/z [M + H]+ calcd for $[C_{23}H_{24}NO_5]$+ 394.1649, found 394.1651.

3.2.3 Experimental procedures toward the synthesis of hydroxy leucine

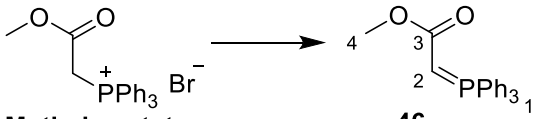
(2-methoxy-2-oxoethyl)triphenylphosphonium bromide[208]



Methyl Bromo acetate **Methyl acetate Phosphonium salt**

To triphenylphosphine (5.11 g, 19.48 mmol) was added ethyl acetate (44 mL). Methyl bromoacetate (1.85 mL, 19.54 mmol, 1 equiv) was added dropwise and the solution stirred for 24 h. The resulting white precipitate was collected through suction filtration, washed with ether, and dried in a vacuum oven to provide the phosphonium salt (6.5 g, 80%). Mp 156-158 °C (lit[209] 162 °C); ν_{max} (neat)/cm^{-1}: 3055, 3009, 2954 C-H (s), 2193, 1727 C=O (s); ^{1}H NMR ($CDCl_3$, 500 MHz) δH: 7.84 – 7.77 (m, 6H, Ar), 7.74 – 7.69 (m, 3H, Ar), 7.64 – 7.57 (m, 6H, Ar), 5.45 (d, *J* = 13.6 Hz, 2H, H2) 3.49 (s, 3H, H4); ^{13}C NMR ($CDCl_3$, 100 MHz) δC 165.0 (d) C3, 135.2 (d), 133.9 (d), 130.3 (d), 117.7 (d), 53.4 (d) C4, 32.9 (d) C4.

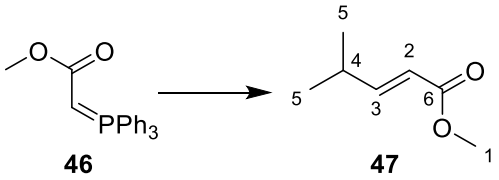
Methyl 2-(triphenylphosphoranylidene)acetate 46[208]



Methyl acetate Phosphonium salt **46**

Methyl acetate phosphonium salt (6.394 g) was dissolved in DCM (40 mL). The solution was washed with an NaOH solution (1 M, 30 mL). The organic layer was removed and the aqueous re-extracted with DCM (40 mL). The organic layers were combined and washed with an equal amount of brine solution. The organic layer was dried (magnesium sulphate), filtered, and evaporated to provide **46** as an off-white solid (5.096 g, 99%). Mp 166.5-168 °C (lit[209] 165 °C); ν_{max}(neat)/cm^{-1}: 3058, 2943 C-H (s), 1619; ^{1}H NMR ($CDCl_3$, 400 MHz) δH: 7.70 – 7.60 (m, 6H, H1, Ar), 7.59 – 7.50 (m, 3H, H1, Ar), 7.50 – 7.40 (m, 6H, H1, Ar), 3.53 (bs, 3H, H4) 2.90 (bs, 1H, H2); ^{13}C NMR ($CDCl_3$, 100 MHz) δC: 133.1 (d) Ar, 132.1 (d) Ar, 128.9 (d) Ar.

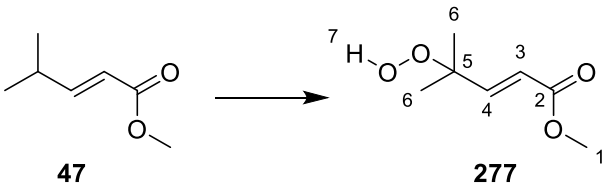
(E)-methyl 4-methylpent-2-enoate 47[33]



46 **47**

Ylid **46** (11.56 g, 34.57 mmol) was dissolved in anhydrous DCM (36 mL) and the solution was cooled to 0 °C using an ice bath. *iso*-Butyraldehyde (2.8 mL, 30.68 mmol, 1.1 equiv) was added and the solution stirred for 20 h being allowed to gradually warm to room temperature. The DCM was removed under reduced pressure, and the crude olefin was extracted from the resulting solid by washing with pentane. The suspension was filtered, and the pentane removed under reduced pressure. The olefin was further purified using Kugelrohr distillation apparatus to afford **47** as a colourless liquid (2.81 g, 63%). ν_{max}(neat)/cm^{-1}: 2964 C-H (s), 2873, 1727 C=O (s); ^{1}H NMR ($CDCl_3$, 500 MHz) δH: 6.95 (dd, *J* = 15.7, 6.7 Hz, 1H, H3) 5.77 (dd, *J* = 15.7, 1.5 Hz, 1H, H3), 3.72 (s, 3H, H3), 2.50 – 2.41 (m, 1H, H1) 1.06 (d, *J* = 10 Hz, 6H, H6); ^{13}C NMR ($CDCl_3$, 100 MHz) δC: 167.6 C1, 155.9 C3, 118.4 C4, 51.5 C5, 31.1 C1, 21.4 C6 and 7.

Peroxide (E)-methyl 4-hydroperoxy-4-methylpent-2-enoate 277

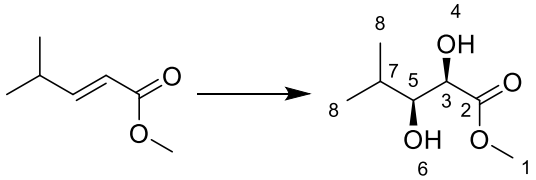


47 **277**

Conversion of enone **47** to peroxide **277** occurred spontaneously in air and light.

ν_{max}(neat)/cm^{-1}: 3386 O-H (b), 2985 C-H (s), 2954, 1706, C=O (s), 1660; ^{1}H NMR (400 MHz, $CDCl_3$) δH 7.03 (d, *J* = 16.1 Hz, 1H, H4), 5.98 (d, *J* = 16.1 Hz, 1H, H3), 3.75 (s, 3H, H1), 1.38 (s, 6H, H6); ^{13}C NMR (101 MHz, $CDCl_3$) δC 167.3 C1, 151.5 C3, 120.5 C4, 81.9 C5, 51.9 C1, 24.1 C6 and 7. *m/z* (NSI-FTMS) $[M+H]^+$: calcd for $[C_7H_{13}O_4]^+$ 161.0808, found 161.0806.

(2R,3S)-methyl 2,3-dihydroxy-4-methylpentanoate 48[33]



47 **48**

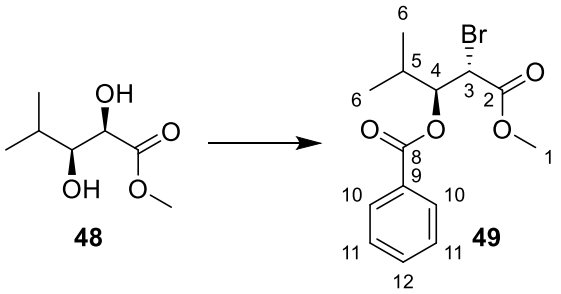
Racemic synthesis:

$K_2OsO_4.2H_2O$ (21.6 mg, 0.058 mmol, 0.008 equiv) was added to a flask with *t*-butanol (9 mL), H_2O (5 mL) and N-methylmorpholine N-oxide (50% wt in H_2O) (5 mL). Enone **47** (0.8955 g, 6.98 mmol) was added over 24 h using a syringe pump. The solution was further stirred for 2 days, then, sodium sulphite (4 g) was carefully added and the solution was stirred for another hour. Water (20 mL) was added and the aqueous layer was extracted with ethyl acetate (20 mL x 3). The organic layers were combined and washed with a saturated sodium sulphite solution (20 mL). The organic layers were dried (sodium sulphate), filtered, and evaporated to yield diol **48** as a clear oil which cooled to a waxy white solid (0.872 g, 77%).

Enantioselective synthesis:

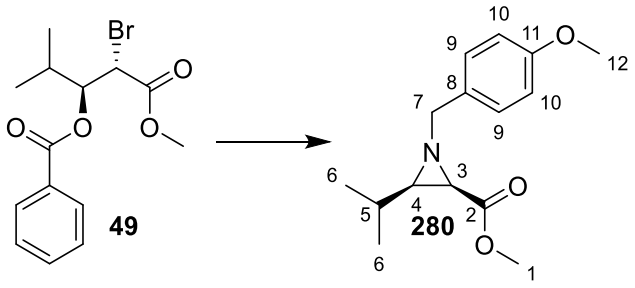
To a flask containing AD-mix α (31.167 g), was added a *t*-butanol/water solution (1:1) (220 mL). The suspension was stirred for 30 m then enone **47** (2.707 g, 21.12 mmol) was slowly added and the solution was stirred for 24 h. The solution was carefully quenched with saturated sodium sulphite solution and stirred for a further 30 m, then, extracted with ethyl acetate (100 mL x 3). The organic layers were combined and washed with a saturated sodium sulphite solution (100 mL). The organic layers were dried (magnesium sulphate), filtered and evaporated under reduced pressure to yield diol **48** (2.326 g, 68%) at 90% ee. The enantiomeric excess was enriched by dissolving the solid in 50 mL of a 9:1 petroleum ether/ethyl acetate solution and cooling to -20 °C to yield white waxy crystals (1.717 g, 50%) 96% ee. ν_{max}(neat)/cm^{-1}: 3368 O-H (b), 2960 C-H (s), 2874, 1741 C=O (s); $[\alpha]^{D\ 23}$ = +22.92 (*c* 0.72, $CHCl_3$) (96% ee), (lit[33] $[\alpha]^{D\ 23}$ = -10.7 (*c* 1, $CHCl_3$)); ^{1}H NMR (500 MHz, $CDCl_3$) δH 4.30 (s, 1H, H4 or 6), 3.83 (s, 3H, H1), 3.51 (t, *J* = 7.7 Hz, 1H, H5), 3.01 (s, 1H, H4 or 6), 1.95 – 1.81 (m, 2H, H7 and 3), 1.05 (d, *J* = 6.7 Hz, 3H, H8), 0.98 (d, *J* = 6.7 Hz, 3H, H8); ^{13}C NMR (101 MHz, $CDCl_3$) δC 174.8 C2, 78.0 C3, 71.5 C5, 52.9 C1, 31.2 C7, 19.2 C8, 19.1 C8.

(2S,3S)-2-bromo-1-methoxy-4-methyl-1-oxopentan-3-yl benzoate 49[33]



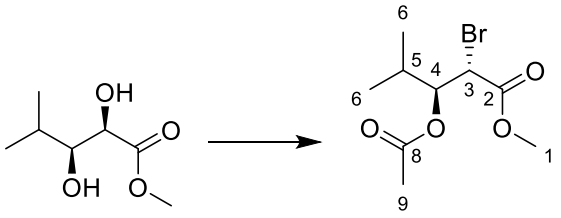
Diol **48** (0.5335 g, 3.29 mmol) was dissolved in anhydrous DCM (7 mL). Trimethyl-orthobenzoate (0.73 mL, 4.25 mmol, 1.3 equiv) was added with $BF_3.OEt_2$ (0.02 mL, 0.16 mmol, 0.05 equiv) and the solution stirred at room temperature for 2 h. Triethyl amine (0.02 mL) was added and the solution evaporated to dryness. The residue was re-dissolved in DCM (7 mL) and acetyl bromine (0.25 mL, 3.4 mmol, 1 equiv.) was added. After 3 h water was added (100 mL) and the solution extracted with DCM (100 mL x 2). The organic layers were combined and dried (magnesium sulphate), filtered, and evaporated. The resulting brown oil **49** (0.908 g, 84%) was pure enough to be used without further purification, but a small portion was purified by column chromatography for characterization purposes using petroleum ether (40-60 °C)/ethyl acetate as the eluent. ν_{max}(neat)/cm^{-1}: 2967 C-H (s), 1750 C=O (s), 1729 C=O (s), $[\alpha]^{D\ 26}$ = +34.43 (c 0.73, $CHCl_3$) (94% ee) (lit[33] $[\alpha]^{D\ 25}$ = +31.1 (*c* 1.01, $CHCl_3$)); ^{1}H NMR ($CDCl_3$, 400 MHz) δH: 8.06 – 8.00 (m, 2H, H11), 7.61 – 7.55 (m, 1H, H12), 7.48 – 7.41 (m, 2H, H10), 5.57 (dd, *J* = 8.8, 3.9 Hz, 1H, H4) 4.48 (d, *J* = 8.8 Hz, 1H, H3), 3.68 (s, 3H, H1), 2.51 – 2.39 (m, 1H, H5), 1.02 (d, *J* = 6.9 Hz, 6H, H6); ^{13}C NMR ($CDCl_3$, 100 MHz) δC 168.4, 165.4, 133.4, 129.9, 129.6, 128.6, 77.2 C4, 53.3 C3, 44.5 C1, 29.5 C5, 19.7 C6, 15.9 C6.

(2R,3R)-methyl 3-isopropyl-1-(4-methoxybenzyl)aziridine-2-carboxylate 280



Ester **49** 0.0566 g (0.17 mmol) was dissolved in dry THF (1.6 mL). *p*-Methoxybenzylamine (0.03 mL, 0.229 mmol, 1.35 equiv) was added at -78 °C and allowed to warm to room temperature over 3 days. The solution was evaporated and purified by column chromatography using petroleum ether (40-60 °C)/ethyl acetate (8:2) as the eluent, to produce (0.0113 g, 25%) of aziridine **280** as a clear oil. ν_{max}(neat)/cm^{-1}: 3432, 2959 C-H (s), 1747 C=O (s); ^{1}H NMR ($CDCl_3$, 400 MHz) δH: 7.24 (d, *J* = 8.7 Hz, 2H, H10), 6.85 (d, *J* = 8.7 Hz, 2H, H9), 3.79 (s, 3H, H12), 3.71 (s, 3H, H1), 3.54 (d, *J* = 12.9 Hz, 1H, H7), 3.43 (d, *J* = 12.9 Hz, 1H, H7) 2.25 (d, *J* = 6.4 Hz, 1H, H3), 1.63 – 1.52 (m, 2H, H4 and 5), 0.89 (d, *J* = 6.3 Hz, 3H, H6), 0.82 (d, *J* = 6.4 Hz, 3H, H6); ^{13}C NMR ($CDCl_3$, 100 MHz) δC: 170.6, 159.1 Ar, 129.0 Ar, 113.8 Ar, 63.8, 55.4, 53.6, 52.1, 42.7 C7, 27.5 C5, 21.0 C6, 19.7 C6; one quaternary Ar peak unobserved in ^{13}C NMR spectrum; m/z (NSI-FTMS) $[M+H]^+$: calcd for $[C_{15}H_{22}O_3N]^+$ 264.1594, found 264.1598.

(2S,3S)-methyl 3-acetoxy-2-bromo-4-methylpentanoate 285

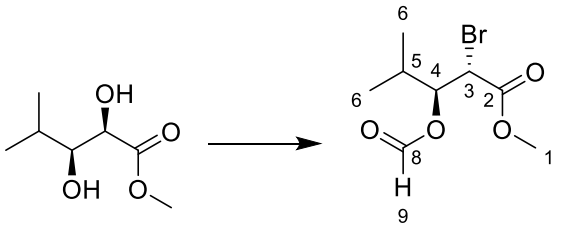


48 **285**

Ester **48** (0.0367 g, 0.226 mmol) was dissolved in anhydrous DCM (0.5 mL). Trimethyl-orthoacetate (0.05 mL, 0.39 mmol, 1.7 equiv) was added with 1 drop of $BF_3.OEt_2$ and the solution stirred at room temperature for 2 h. Triethyl amine (1 drop) was added and the solution evaporated to dryness. The residue was then re-dissolved in DCM (0.5 mL) and acetyl bromine (0.05 mL, 0.67 mmol, 3 equiv.) was added. After 3 h the reaction was quenched with water (20 mL) and the solution extracted with DCM (50 mL x 2). The organic layers were combined and dried (magnesium sulphate), filtered, and evaporated. The resulting brown oil **285** (0.0547 g, 91%) was characterized and used without further purification. ν_{max}(neat)/cm^{-1}: 3021, 2970 C-H (s), 1751 C=O (s); $[\alpha]^{D\ 21}$ = +10.41 (*c* 0.48, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δH 5.31 (dd, *J* = 9.0, 3.8 Hz, 1H, H4), 4.29 (d, *J* = 9.0 Hz, 1H, H3), 3.75 (s, 3H, H1), 2.40 – 2.27 (m, 1H, H5 and 3), 2.04 (s, 3H, H9), 0.93 (d, *J* = 6.9 Hz, 3H, H6), 0.90 (d, *J* = 6.9 Hz, 3H, H6); ^{13}C NMR (126 MHz, $CDCl_3$) δC 169.7, 168.4, 76.5 C4, 53.2 C1, 44.3 C3, 29.0 C9, 20.7 C5, 19.5 C6, 15.5 C6. Unfortunately, although attempted, accurate mass data was unable to be obtained for this compound.

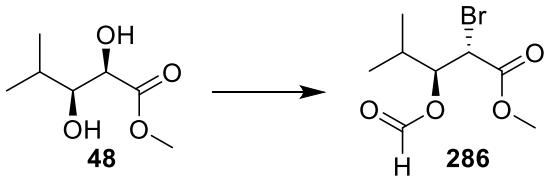
Two step procedure to 286 from 48:

(2S,3S)-methyl 2-bromo-3-(formyloxy)-4-methylpentanoate 286



Ester **48** (0.0731, 0.45 mmol), was dissolved in anhydrous DCM (0.9 mL). Trimethyl-orthoformate (0.07 mL, 0.63 mmol, 1.4 equiv) was added with 1 drop of $BF_3.OEt_2$ and the solution stirred at room temperature for 2 h. 1 drop of triethyl amine was added and the solution evaporated to dryness. The residue was re-dissolved in DCM (0.9 mL) and acetyl bromine (0.04 mL, 0.54 mmol, 1.2 equiv.) was added. After 2 hours water was used to quench the reaction (20 mL) and the solution extracted with DCM (50 mL x 2). The organic layers were combined and dried (magnesium sulphate), filtered, and evaporated. The resulting brown oil **286** was purified using column chromatography with petroleum ether (40-60 °C)/ethyl acetate (9:1) as the eluent to provide **286** (0.0568 g, 91%). ν_{max}(neat)/cm^{-1}: 3446, 2969 C-H (s), 2881, 1751 C=O (s), 1732 C=O (s); $[\alpha]^{D\ 25}$ = −13.36 (94% ee) (*c* 1.01, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δH 8.08 (s, 1H, H9), 5.40 (dd, *J* = 9.2, 3.3 Hz, 1H, H4), 4.32 (d, *J* = 9.2 Hz, 1H, H3), 3.77 (s, 3H, H1), 2.40 (dtd, *J* = 13.7, 6.9, 3.6 Hz, 1H, H3), 0.97 (d, *J* = 6.9 Hz, 3H, H6), 0.93 (d, *J* = 6.8 Hz, 3H, H6); ^{13}C NMR (101 MHz, $CDCl_3$) δC 168.3, 159.8, 76.6 C4, 53.4 C1, 43.5 C3, 28.9 C5, 19.6 C6, 15.3 C6. Unfortunately, although attempted, accurate mass data was unable to be obtained for this compound.

(2S,3S)-methyl 2-bromo-3-hydroxy-4-methylpentanoate 286

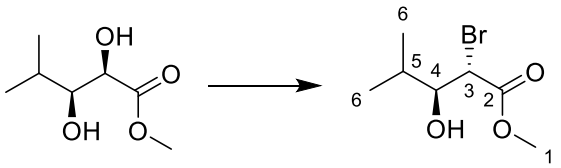


The bromo-formate ester **48** (0.2751 g, 1.09 mmol) was dissolved in MeOH (13.5 mL). (±)-CSA (0.338 g, 1.46 mmol, 1.3 equiv) was added, and the solution stirred for 5 h. A further portion

of (±)-CSA (0.1 g, 0.43 mmol, 0.4 equiv) was added and the reaction left until TLC showed complete consumption of the starting material had occurred. The solvents were removed under reduced pressure and the residue dissolved in ether (75 mL). The organic layer was washed with water (50 mL) and saturated $NaHCO_3$ solution (50 mL). The organic layer was dried (magnesium sulphate), filtered, and evaporated under reduced pressure. To provide bromo-hydrin **286** (0.217 g, 89%) which could be used without further purification.

Two step procedure to 286 from 48

(2S,3S)-methyl 2-bromo-3-hydroxy-4-methylpentanoate 286

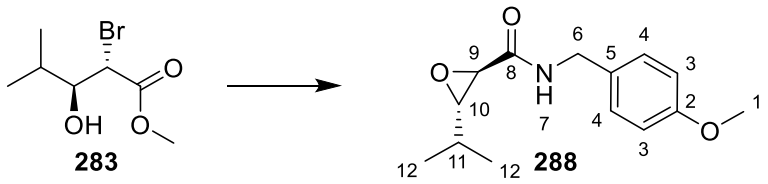


48 **286**

Diol **48** (0.537 g, 3.31 mmol), was dissolved in anhydrous DCM (6.5 mL). Trimethyl-orthoformate (0.5 mL, 4.57 mmol, 1.4 equiv) was added with $BF_3.OEt_2$ (0.02 mL, 0.16 mmol, 0.05 equiv) and the solution stirred at room temperature for 2 h. Triethyl amine (0.02 mL) was added and the solution evaporated to dryness. The residue was re-dissolved in DCM (6.5 mL) and acetyl bromine (0.28 mL, 3.78 mmol, 1.1 equiv) was added. After 2 h methanol (3.9 mL) and (±)-CSA (2.00 g, 8.6 mmol, 2.6 equiv) was added and the solution was left to stir for a further 2 hours. The solution was diluted with DCM (50 mL). Water (50 mL) was added and the organic layer was separated. The aqueous later was extracted again with DCM (50 mL). The organic layers were combined, washed with saturated $NaHCO_3$ solution (50 mL) and dried (magnesium sulphate), filtered, and evaporated. The resulting brown oil was purified using column chromatography with petroleum ether (40-60 °C)/ethyl acetate (9:1) as the eluent to provide **286** (0.4952 g, 67%). ν_{max}(neat)/cm^{-1}: 3455 O-H (b), 2962 C-H (s), 2877, 1736 C=O (s); $[\alpha]^{D\ 24}$ = −34.6 (96% ee) (*c* 0.75, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δH 4.22 (d, *J* = 8.2 Hz, 1H, H4), 3.87 (ddd, *J* = 8.3, 6.2, 3.9 Hz, 1H, H3), 3.81 (s, 3H, H1), 2.50 (d, *J* = 6.3 Hz, 1H, OH), 2.14 (dtd, *J* = 13.7, 6.9, 3.9 Hz, 1H, H5), 1.02 (d, *J* = 6.9 Hz, 3H, H6), 0.92 (d, *J* = 6.8 Hz, 3H, H6); ^{13}C NMR (126 MHz, $CDCl_3$) δC 170.4 C2, 76.4 C4, 53.2 C1, 45.4 C3, 29.5 C5, 19.0 C6, 15.0 C6.

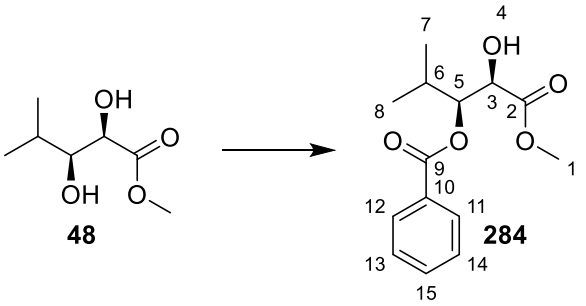
Unfortunately, although attempted, accurate mass data was unable to be obtained for this compound.

(2R,3S)-3-isopropyl-N-(4-methoxybenzyl)oxirane-2-carboxamide 288



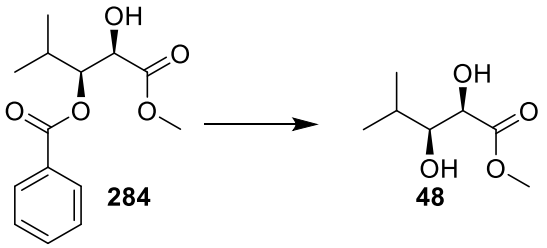
Bromohydrin **283** (0.1053 g, 0.47 mmol) was dissolved in THF (4.4 mL). *p*-Methoxybenzylamine (0.12 mL, 0.91 mmol, 2 equiv) was added with triethylamine (0.06 mL, 0.43 mmol, 0.9 equiv) and the solution was stirred overnight. The solution was evaporated, dissolved in ethyl acetate and washed with equal amounts of water (x2) and brine (x2). The organic layer was dried (magnesium sulphate), filtered, and evaporated. The resulting residue was purified by column chromatography using petroleum ether (40-60 °C)/ethyl acetate (8:2) as the eluent to provide epoxy amide **288** as a white crystalline solid (0.078 g, 67%). Mp 75-77 °C ν_{max} (neat)/cm^{-1}: 3321, 2964 C-H (s), 2934, 1655 C=O (s), 1534, 1514; $[\alpha]^{D\ 22}$ = −3.5 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δH 7.17 (d, *J* = 8.8 Hz, 2H, H3), 6.86 (d, *J* = 8.7 Hz, 2H, H4), 6.34 (s, 1H, H7), 4.35 (d, *J* = 5.9 Hz, 2H, H6), 3.80 (s, 3H, H1), 3.31 (d, *J* = 2.2 Hz, 1H, H9), 2.75 (dd, *J* = 6.4, 2.2 Hz, 1H, H10), 1.72 – 1.60 (m, 1H, H11), 1.01 (d, *J* = 4.2 Hz, 3H, H12), 1.00 (d, *J* = 4.3 Hz, 3H, H12); ^{13}C NMR (101 MHz, $CDCl_3$) δC 168.6 C8, 159.3, 129.8, 129.3, 114.3, 64.8 C9, 55.5 C10, 54.7 C1, 42.5 C6, 30.3 C11, 18.6 C12, 18.2 C12; m/z (NSI-FTMS) $[M+H]^+$ calcd for 250.1438 $[C_{14}H_{20}NO_3]^+$ found 250.1440. Determination of ee was found by HPLC using an AD-H Chiralpak column 80:20 hexane/IPA, 230 nm, 0.8 mL/min, 25 °C.

(2R,3S)-2-hydroxy-1-methoxy-4-methyl-1-oxopentan-3-yl benzoate 284



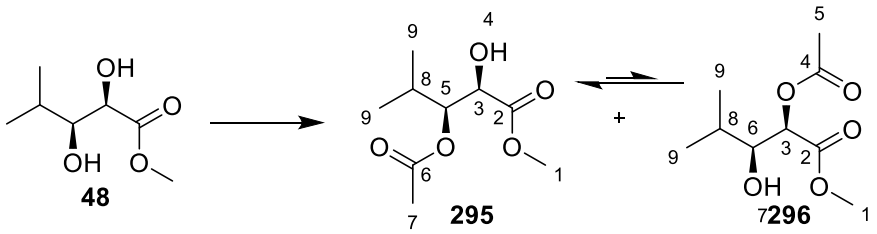
Diol **48** (0.274 g, 1.69 mmol) was dissolved in dry dichloromethane (3.4 mL). Trimethoxy orthobenzoate (0.4 mL, 2.3 mmol, 1.4 equiv) was added along with 1 drop of $BF_3.OEt_2$. The solution was stirred for 2 h. Water (1 mL) was added and the solution vigorously stirred for a further 1 h. The solution was diluted with water (approximately 20 mL) and extracted twice with equal amounts of dichloromethane. The organic was dried (magnesium sulphate), filtered, and evaporated. Purification using column chromatography with petroleum ether (40-60 °C)/ethyl acetate as the eluent provided **284** as a clear oil (0.317 g, 70%). ν_{max} (neat)/cm^{-1}: 3490 O-H (b), 2968 C-H (s), 1744 C=O (s), 1721 C=O (s), 1272; $[\alpha]^{D\ 19}$ = −45.1 (*c* 0.51, $CHCl_3$), (94 % ee); ^{1}H NMR ($CDCl_3$, 400 MHz) ^{1}H NMR (500 MHz, $CDCl_3$) δH 8.01 (dd, *J* = 8.4, 1.3 Hz, 2H, H13 and 14), 7.61 – 7.52 (m, 1H, H15), 7.43 (t, *J* = 7.8 Hz, 2H, H12 and 11), 5.14 (dd, *J* = 9.1, 2.0 Hz, 1H, H5), 4.46 (dd, *J* = 7.9, 1.9 Hz, 1H, H3), 3.71 (s, 3H, H1), 3.02 (d, *J* = 8.0 Hz, 1H, H4), 2.40 – 2.25 (m, 1H, H6), 1.09 (d, *J* = 6.8 Hz, 3H, H7 or 8), 0.99 (d, *J* = 6.8 Hz, 3H, H8 or 7); ^{13}C NMR ($CDCl_3$, 100 MHz) δC: 173.6, 166.0, 133.3, 129.9, 129.8, 128.6, 79.6 C3, 70.6 C5, 52.9 C1, 29.3 C6, 19.0 C7 or 8, 19.0 C7 or 8; m/z (TOF-ASAP) $[M+H]^+$ calcd for 267.1233 $[C_{14}H_{19}O_5]^+$ found 267.1227.

(2R,3S)-methyl 2,3-dihydroxy-4-methylpentanoate 48



Benzoyl ester **284** (0.036 g, 0.135 mmol) was dissolved in methanol. The solution was cooled with an ice bath and potassium *tert*-butoxide (0.019 g, 0.17 mmol, 1.3 equiv) was added. After 1 h, the solution was neutralised with amberlite resin H^+ 120. After neutralisation the solution was filtered, evaporated, and purified by column chromatography, using petroleum ether (40-60 °C)/ethyl acetate as the eluent to provide diol **48** as a waxy white solid (0.018 g, 84%).

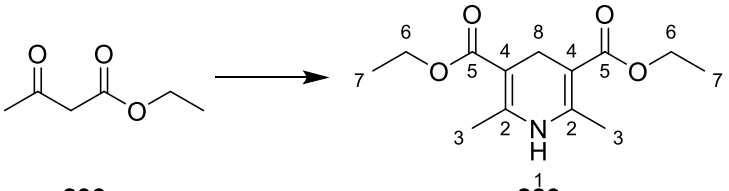
(2R,3S)-methyl 3-acetoxy-2-hydroxy-4-methylpentanoate 295 and (2R,3S)-methyl 2-acetoxy-3-hydroxy-4-methylpentanoate 296



Diol **48** (0.0881 g, 0.54 mmol) was dissolved in dry dichloromethane (1.2 mL). Trimethoxy orthoacetate (0.09 mL, 0.7 mmol, 1.3 equiv) was added along with 1 drop of $BF_3.OEt_2$. The solution was stirred for 2 h. Water (0.3 mL) was added and the solution vigorously stirred for a further 1 h. The solution was diluted with water (approximately 20 mL) and extracted twice with equal amounts of dichloromethane. The organic solution was dried (magnesium sulphate), filtered, and evaporated. Purification by column chromatography using petroleum ether (40-60 °C)/ethyl acetate (9:1) as the eluent provided **295** as a clear oil (0.0344 g, 31%) and **296** as a clear oil (0.0125 g, 11%), which rapidly converted to **295** upon standing in chloroform. **First eluting regioisomer 295:** ν_{max}(neat)/cm^{-1}: 3491 O-H (b), 2966 C-H (s), 1748 C=O (s), 1272; $[\alpha]^{D\ 24}$ = −115.1 (*c* 0.76, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δH: 4.85 (dd, *J* = 9.3, 1.8 Hz, 1H, H6), 4.33 (d, *J* = 1.6 Hz, 1H, H3), 3.73 (s, 3H, H1), 2.86 (bs, 1H, H7), 2.21 – 2.08 (m, 1H, H8), 2.03 (s, 3H, H5), 1.01 (d, *J*= 6.8 Hz, 3H, H9), 0.91 (d, *J*= 6.7 Hz, 3H, H9); ^{13}C NMR ($CDCl_3$,

100 MHz) δC 173.7, 170.4, 79.1 C3, 70.2 C6, 52.8 C1, 28.9 C5, 20.7 C8, 19.0 C9, 18.8 C9; m/z (FTMS-NSI) $[M+Na]^+$ calcd for 227.0895 $[C_{14}H_{19}O_5]^+$ found 227.0891. **Second eluting regioisomer 296:** ^{1}H NMR ($CDCl_3$, 400 MHz) δH: 5.21 (d, *J* = 2.6, 1H, H3), 3.77 (s, 3H, H1), (dd, *J* = 8.5, 2.6 Hz, 1H, H6), 2.18 (s, 3H, H5), 1.96 (bs, 1H, OH), 1.85 – 1.70 (m, 1H, H8), 1.05 (d, J= 6.7 Hz, 3H, H9), 0.90 (d, *J* = 6.8 Hz, 3H, H9); ^{13}C NMR ($CDCl_3$, 100 MHz) δC 170.3, 169.5, 76.8 C6 or 3, 73.3 C3 or 6, 52.7 C1, 31.2 C5, 20.7 C8, 19.1 C9, 18.9 C9.

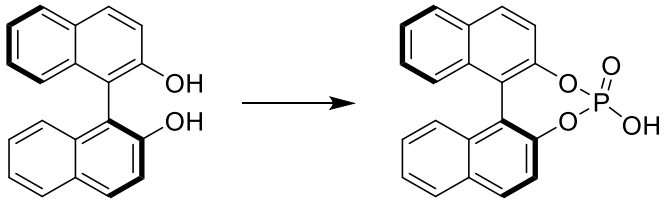
(S)-1-((4-methoxybenzyl)amino)-4-methyl-1,2-dioxopentan-3-yl benzoate 289[196]



293 **289**

Formaldehyde (aqueous 37%), (0.81 mL, 10.8 mmol, 1.1 equiv), methanol (15 mL), ethyl acetoacetate (2.7 mL, 21.16 mmol, 2.1 equiv), ammonium acetate (0.77 g, 9.98 mmol), *p*-toluenesulphonic acid monohydrate (0.049 g, 0.28 mmol, 0.028 equiv) were stirred at room temperature for 16 h. The resulting suspension was evaporated to a crude yellow solid which was recrystalized twice with methanol to provide Hantzsch's ester **289** as a yellow solid (0.57 g, 23%). Mp 160-167 °C (lit[210] 164-166 °C); ν_{max}(neat)/cm^{-1}: 3349 N-H (b), 2987 C-H (s), 1694 C=O (s), 1657 C=O (s); ^{1}H NMR (400 MHz, $CDCl_3$) δH 5.11 (s, 1H, H1), 4.17 (q, *J* = 7.1 Hz, 4H, H6), 3.26 (s, 2H, H8), 2.19 (s, 6H, H3), 1.28 (t, *J* = 7.1 Hz, 6H, H7); ^{13}C NMR (101 MHz, $CDCl_3$) δC 168.2 C5, 144.9 C4, 99.7 C2, 59.8 C6, 24.9 C8, 19.4 C3, 14.6 C7.

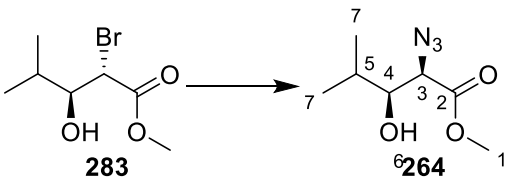
(11bS)-4-hydroxydinaphtho[2,1-d:1',2'-f][1,3,2]dioxaphosphepine 4-oxide 290[195]



292 **290**

S-Binol **292** (1.021 g, 3.57 mmol) was dissolved in dry pyridine (7.1 mL). $POCl_3$ (0.67 mL, 6.43 mmol, 1.8 equiv) was added and the mixture was heated to 75 °C. After 5 h the mixture was cooled and water (7.2 mL) was added and the solution was stirred for a further 2 h. DCM (150 mL) was added and the pyridine was removed with HCl (4 M, 150 mL). The organic layer was dried (sodium sulphate), filtered, and evaporated under reduced pressure to afford a crude which was purified by column chromatography using DCM/MeOH (9:1) as the eluent to afford **290** as a beige solid (0.576 g, 46%). Mp >300 °C (lit[211] 236-239 °C) $[\alpha]^{D\ 23}$ = +583 (*c* 0.18, $CHCl_3$) (lit[212] $[\alpha]^{D\ 20}$ = +607 (*c* 1, MeOH)); ν_{max} (neat)/cm^{-1}: 1237, 1096; ^{1}H NMR (500 MHz, DMSO) δH 8.05 (dd, *J* = 19.7, 8.1 Hz, 4H), 7.46 (d, *J* = 6.6 Hz, 4H), 7.38 – 7.27 (m, 2H), 7.22 (d, J = 8.1 Hz, 2H); ^{13}C NMR (126 MHz, DMSO) δC 146.1, 131.9, 130.5, 130.1, 128.5, 126.3, 126.1, 124.7, 122.3, 121.6.

(2R,3S)-methyl 2-azido-3-hydroxy-4-methylpentanoate 264

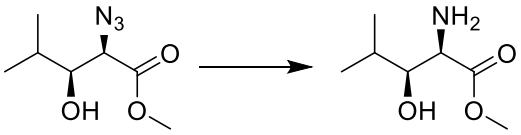


283 ^{6}**264**

Bromohydrin **283** (0.1469 g, 0.65 mmol) was dissolved in DMF (5.8 mL). Sodium azide (0.144 g, 2.21 mmol, 3.4 equiv) was added and the solution was stirred for 20 h. The DMF was evaporated under reduced pressure and the resulting residue dissolved in ethyl acetate (50 mL) and water (25 mL). The aqueous layer was removed and the organic layer washed again with brine (25 mL). Both aqueous layers were removed and extracted with ethyl acetate (50 mL). The organic layers were dried (sodium sulphate), filtered, and evaporated under reduced pressure. The azide **264** was produced as a clear oil (0.098 g, 80%). Although the product was used without purification, a small portion was purified by column chromatography for characterization purposes using petroleum ether (40-60 °C)/ethyl acetate (8:2) as the eluent.

ν_{max}(neat)/cm^{-1}: 3483 O-H (b), 2963 C-H (s), 2876, 2117, 1747 C=O (s), 1438; $[\alpha]^{D\ 25}$ = +36.7 (*c* 0.64, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δH 4.03 (d, *J* = 2.9 Hz, 1H, H3), 3.84 (s, 3H, H1), 3.70 (dd, *J* = 8.4, 2.9 Hz, 1H, H4), 1.96 (s, 1H, H6), 1.91 – 1.81 (m, 1H, H7), 1.05 (d, *J* = 6.7 Hz, 3H, H7), 0.95 (d, *J* = 6.7 Hz, 3H, H7). ^{13}C NMR (101 MHz, $CDCl_3$) δC 170.2 C2, 78.0 C4, 64.2 C3, 53.0 C1, 31.4 C5, 19.1 C7, 18.7 C7; m/z (ASAP-TOF) $[M+H-N_2]^+$ calcd for 160.0974 $[C_7H_{14}NO_3]^+$ found 160.0975.

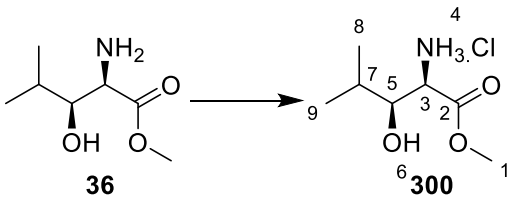
(2R,3S)-methyl 2-amino-3-hydroxy-4-methylpentanoate 36



264 **36**

The azido alcohol **264** (0.0853 g, 0.45 mmol) was dissolved in MeOH (0.5 mL) and the solution gassed with nitrogen. Pd/C (0.0210 g, 10% by weight) was added and the suspension placed under a static atmosphere of hydrogen. The suspension was stirred overnight, filtered through celite, and washed with methanol. The resulting solution was evaporated under reduced pressure to produce the amino ester **36**, which was used onto the next step without further purification (0.0734 g, quant).

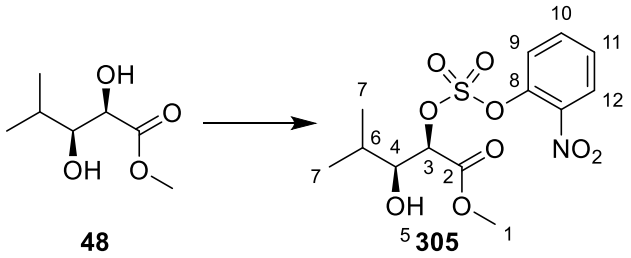
(2R,3S)-3-hydroxy-1-methoxy-4-methyl-1-oxopentan-2-aminium chloride 300



36 **300**

The amino acid ester **36** (0.0128 g, 0.079 mmol) was dissolved in MeOH (0.54 mL) a solution of methanolic HCl (3 M, 0.54 mL) was added and stirred for 3 h. The solution was evaporated to produce **300** as a waxy yellow residue (0.0155 g, quant). ν_{max}(neat)/cm^{-1}: 3423 O-H (b), 2965 C-H (s), 2929, 2027, 1743 C=O (s); $[\alpha]^{D\ 24}$ = -4.8 (*c* 1.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δH 8.35 (s, 3H, H4), 4.26 (s, 1H, H5), 4.39 – 3.64 (m, 5H, H3, 1, 6), 1.99 (s, 1H, H7), 1.11 – 0.91 (m, 6H, H8 and 9); ^{13}C NMR (101 MHz, $CDCl_3$) δC 169.4 C2, 75.1 C5, 56.6 C3, 53.9 C1, 30.4 C7, 19.8 C9 or 8, 17.5 C8 or 9.

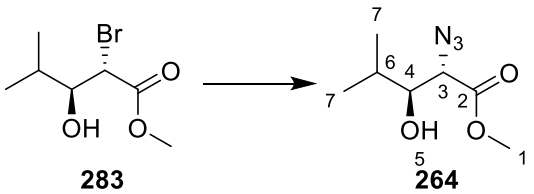
(2R,3S)-methyl 3-hydroxy-4-methyl-2-(((2-nitrophenoxy)sulfonyl)oxy)pentanoate 305



Diol **48** (0.4589 g, 2.83 mmol) was dissolved in anhydrous DCM (22 mL). 2-Nosyl chloride (0.728 g, 3.82 mmol, 1.3 equiv) was added in one portion, triethyl amine (0.4 mL, 2.86 mmol, 1 equiv) was slowly added over 0.5 h. The solution was stirred for 3 h. HCl (1 M, 1 mL), was added with DCM (75 mL) and the solution was washed with an equal portion of brine. The organic layer was dried (magnesium sulphate), filtered, and evaporated to dryness. The resulting yellow oil was purified by column chromatography using petroleum ether (40-60 °C)/ethyl acetate (7:3) as the eluent to provide **305** as a pale-yellow oil. (0.605 g, 62 %). ν_{max}(neat)/cm^{-1}: 3544 O-H (b), 3101, 2964 C-H (s), 1766 C=O (s), 1547; $[\alpha]^{D\,26}$ = −23.3 (*c* 0.73, $CHCl_3$) (96% ee); ^{1}H NMR (500 MHz, $CDCl_3$) δH 8.19 (dd, *J* = 7.3, 1.2 Hz, 1H, Ar), 7.85 – 7.75 (m, 3H, Ar), 5.24 (d, *J* = 3.0, 1H, H3), 3.75 – 3.68 (m, 4H, H4 and 1), 2.00 – 1.80 (m, 2H, H6 and 5), 1.05 (d, *J* = 6.7 Hz, 3H, H7), 0.98 (d, *J* = 6.7 Hz, 3H, H7); ^{13}C NMR (126 MHz, $CDCl_3$) δC 167.7 C2, 148.4 Ar, 135.1 Ar, 132.5 Ar, 131.5 Ar, 130.3 Ar, 125.0 Ar, 80.9 C3, 77.3 C4, 53.0 C1, 30.7 C6, 18.0 C7, 18.4 C7; m/z (ASAP-TOF) $[M+H]^+$ calcd for 348.0753 $[C_{13}H_{18}NO_8S]^+$ found 348.0752. Determination of ee was found by HPLC using an AD-H Chiralpak column 25:75 hexane/IPA, 254 nm, 0.8 mL/min, 25 °C.

(2S,3S)-methyl 2-azido-3-hydroxy-4-methylpentanoate 264[39]

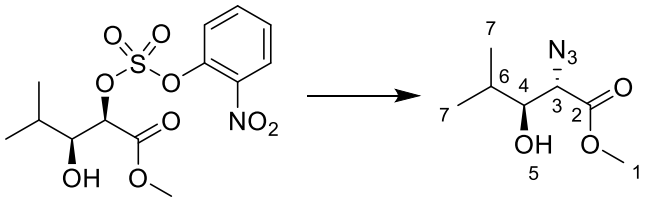
Synthesis of 264 from 283 *via* the epoxide



283 **264**

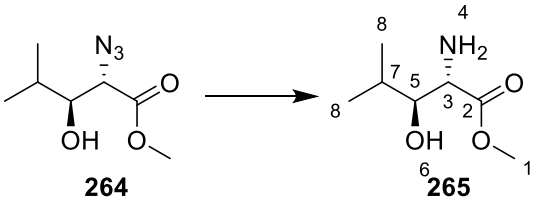
Bromo-hydrin **283** (0.0285 g, 0.13 mmol) was dissolved in PEG-400 (0.23 mL). Triethylamine (0.025 mL, 0.18 mmol, 1.4 equiv) was added and the solution and stirred for 1 hour. Sodium azide (0.034 g, 0.52 mmol, 4 equiv) was added and the suspension stirred for 36 h. Water (20 mL) was added and the solution extracted with ethyl acetate (20 mL x 2). The solution was dried with sodium sulphate, filtered and evaporated to a colourless oil. The crude material was purified by column chromatography using petroleum ether (40-60 °C)/ethyl acetate (9:1) as the eluent to produce azide **264** as a clear residue (2.1 mg, 9%).

Synthesis of 264 from the nosylated precursor 305



Nosylated diol **305** (0.5933 g, 1.63 mmol) was dissolved in DMF (18 mL). Sodium azide (0.304 g, 4.68 mmol, 2.9 equiv) was added and the mixture stirred at room temperature overnight. The solution was evaporated to dryness and water (50 mL) was added along with ethyl acetate (50 mL). The organic layer was removed, and the aqueous solution re-extracted with ethyl acetate (50 mL). The organic layers were combined and washed with brine (50 mL). The organic layer was dried (magnesium sulphate), filtered, and evaporated under reduced pressure. The resulting pale-yellow oil **264** (0.3051 g, 95%) could be used without further purification, however a small portion was purified for characterization purposes by column chromatography using petroleum ether (40-60 °C)/ethyl acetate (9:1) as the eluent. ν_{max}(neat)/cm^{-1}: 3435 O-H (b), 3020, 2966 C-H (s), 2113, 1741 C=O (s); $[\alpha]^{D\ 26}$ = −66 (*c* 1, $CHCl_3$); (Lit[39] $[\alpha]^{D\ 23}$ = -47.6 (*c* 1.1, $CHCl_3$)); ^{1}H NMR (400 MHz, $CDCl_3$) δH 3.93 (d, *J* = 6.5 Hz, 1H, H3), 3.84 (s, 3H, H1), 3.68 (dd, *J* = 6.5, 5.5 Hz, 1H, H4), 2.00 – 1.87 (m, 1H, H6), 1.61 (bs, 1H, H5), 1.00 (d, *J* = 6.8 Hz, 3H, H7), 0.97 (d, *J* = 6.8 Hz, 3H, H7); ^{13}C NMR (126 MHz, $CDCl_3$) δC 170.2 C1, 76.6 C4, 64.0 C3, 52.9 C1, 30.30 C6, 19.4 C7, 16.6 C7.

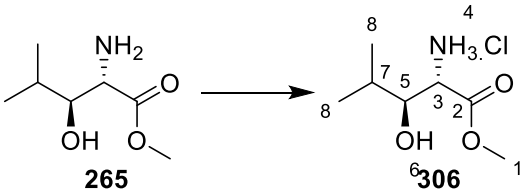
(2S,3S)-methyl 2-amino-3-hydroxy-4-methylpentanoate 265



The azido alcohol **264** (0.2160 g, 1.15 mmol) was dissolved in MeOH (1.2 mL) and the solution gassed with nitrogen. Pd/C (0.042 g, 10% by weight) was added, and the suspension placed under a static atmosphere of hydrogen. The suspension was stirred overnight, filtered through celite, and washed with methanol. The resulting solution was evaporated under

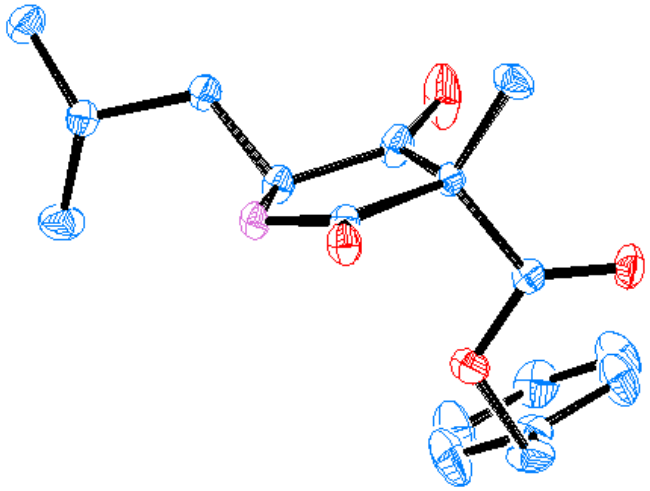
reduced pressure, and purified by column chromatography using 99% Ethyl acetate 1% TEA to 10% MeOH/DCM as the eluent to produce amine **265** as a clear oil. (0.0737 g, 41%). ν_{max} (neat)/cm^{-1}: 3366 O-H (b), 2959 C-H (s), 2874, 1735 C=O (s); $[\alpha]^{D\ 25}$ = +31.6 (*c* 0.6, $CHCl_3$); (Lit[39] $[\alpha]^{D\ 23}$ = +8.6 (*c* 0.7, $CHCl_3$)) ^{1}H NMR (400 MHz, $CDCl_3$) δH 3.75 (s, 3H, H1), 3.63 (d, *J* = 4.9 Hz, 1H, H5), 3.47 – 3.41 (m, 1H, H3), 2.19 (s, 4H, H6 and 4), 1.78 (p, *J* = 6.8 Hz, 1H, H7), 0.96 (t, *J* = 7.3 Hz, 6H, H8); ^{13}C NMR (101 MHz, $CDCl_3$) δC 174.9 C2, 78.6 C5, 56.8 C1, 52.2 C3, 30.7 C7, 19.6 C8, 18.0 C8. HRMS (NSI-FTMS) m/z $[M + H]^+$ calcd for $[C_7H_{16}NO_3]^+$ 162.1125, found 162.1122.

(2S,3S)-3-hydroxy-1-methoxy-4-methyl-1-oxopentan-2-aminium chloride 306

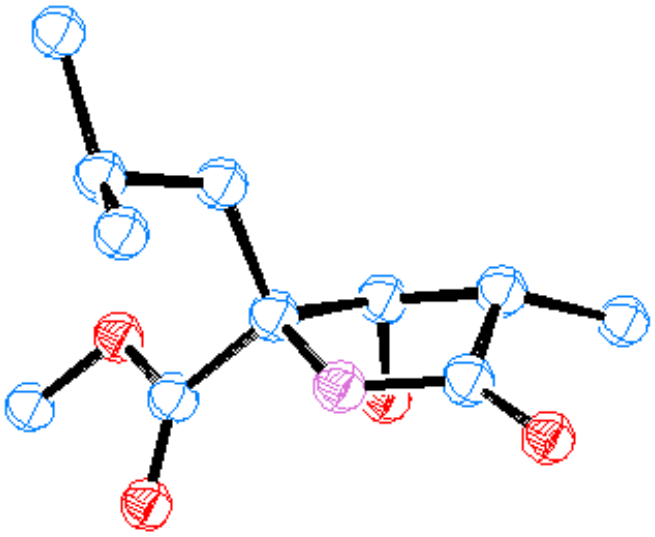


The amino acid ester **265** (0.0555 g, 0.344 mmol) was dissolved in MeOH (2.4 mL) a solution of methanolic HCl was added (3 M, 2.4 mL) and the solution stirred for 3 h. The solution was evaporated to a waxy yellow residue providing the hydrochloride salt **306** (0.068 g, quant.). ν_{max} (neat)/cm^{-1}: 3391 O-H (b), 2965 C-H (s), 1745 C=O (s), 1618; $[\alpha]^{D\ 25}$ = +28.1 (*c* 0.48, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δH 8.35 (s, 3H, H4), 4.88 (s, 1H, H5), 4.39 (s, 1H, H3), 3.84 (s, 4H, H1 and 6), 1.90 (s, 1H, H7), 1.12 – 0.79 (m, 6H, H8); ^{13}C NMR (126 MHz, $CDCl_3$) δC 168.5 C2, 76.2 C5, 56.4 C3, 53.6 C1, 31.1 C7, 20.1 C8, 19.1 C8. HRMS (NSI-FTMS) m/z $[M - Cl]^+$ calcd for $[C_7H_{16}NO_3]^+$ 162.1125, found 162.1121.

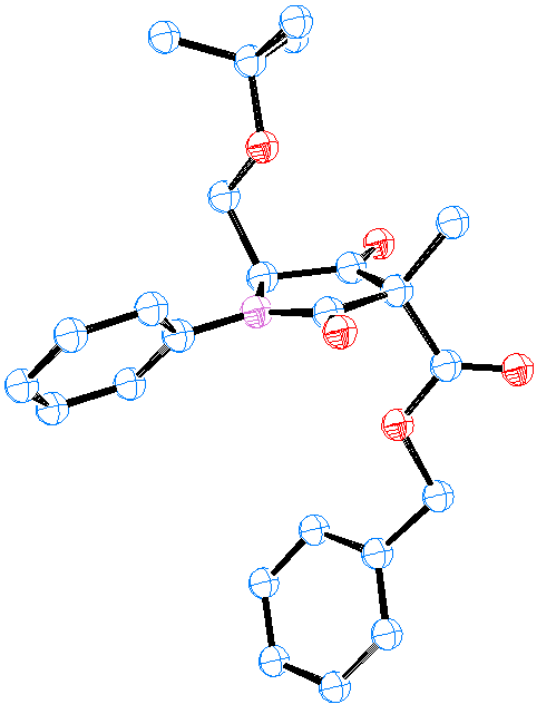
3.3 X-ray crystallography data



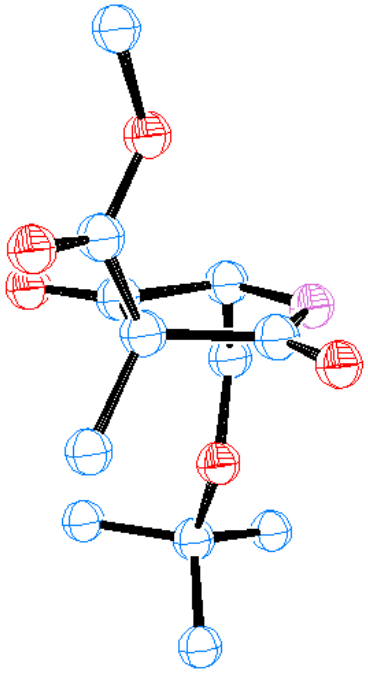
Compound number	**179**
Space group	P 2_1/n (14)
Cell lengths	**a** 15.59060(16) **b** 5.83233(5) **c** 19.3142(2)
Cell angles	α 90.0000 β 112.9800(13) γ 90.0000
Cell volume	1616.86
Z, Z’	**Z**: 4 **Z'**: 0
R-factor %	4.11



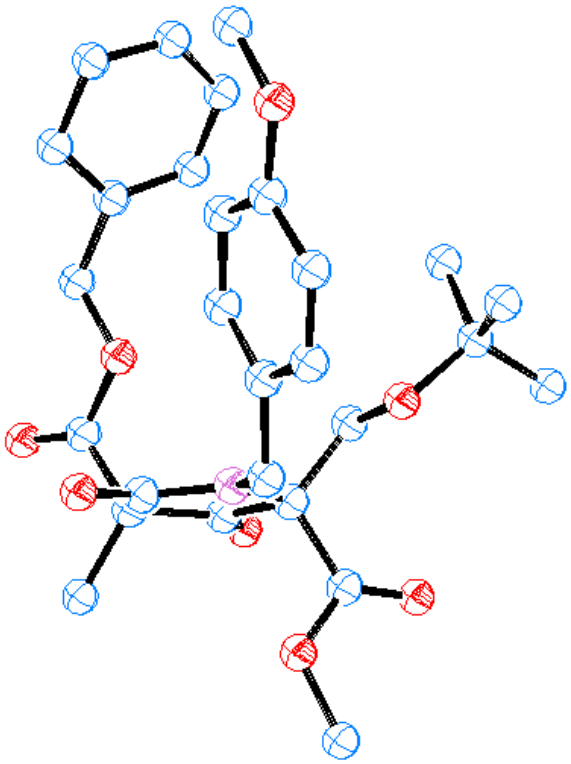
Compound number	**197**
Space group	P 1 (2)
Cell lengths	**a** 10.3698(15) **b** 11.4667(12) **c** 12.0264(11)
Cell angles	α 65.754(8) β 80.975(12) γ 85.855(12)
Cell volume	1287.69
Z, Z’	**Z**: 0 **Z'**: 0



Compound number	**212a**
Space group	P 2_1 2_1 2_1 (19)
Cell lengths	**a** 6.24701(5) **b** 17.78990(14) **c** 20.26860(16)
Cell angles	**a** 90.0000 **b** 90.0000 **g** 90.0000
Cell volume	2252.52
Z, Z’	**Z**: 0 **Z'**: 0

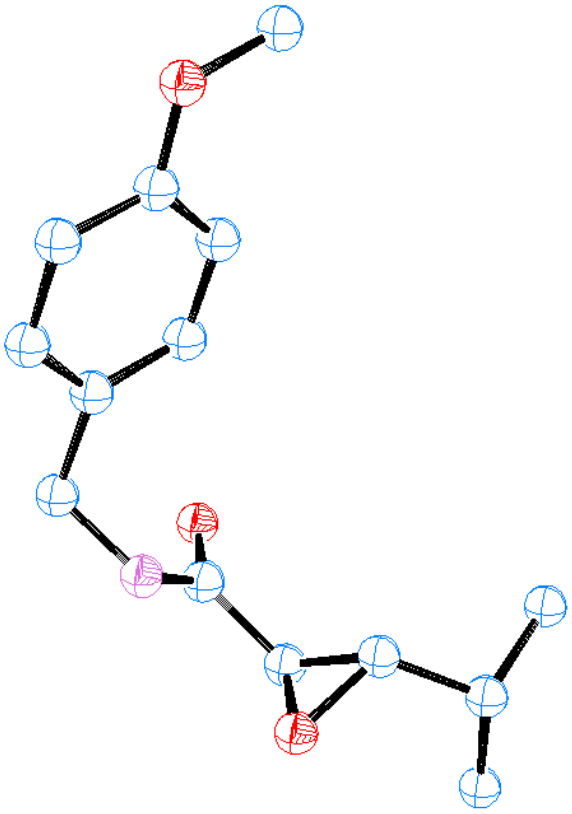


Compound number	**216a**
Space group	P 2_1 (4)
Cell lengths	**a** 8.41466(6) **b** 22.42870(13) **c** 11.38440(9)
Cell angles	α 90.0000 β 107.7070(8) γ 90.0000
Cell volume	2046.79
Z, Z’	**Z**: 0 **Z'**: 0

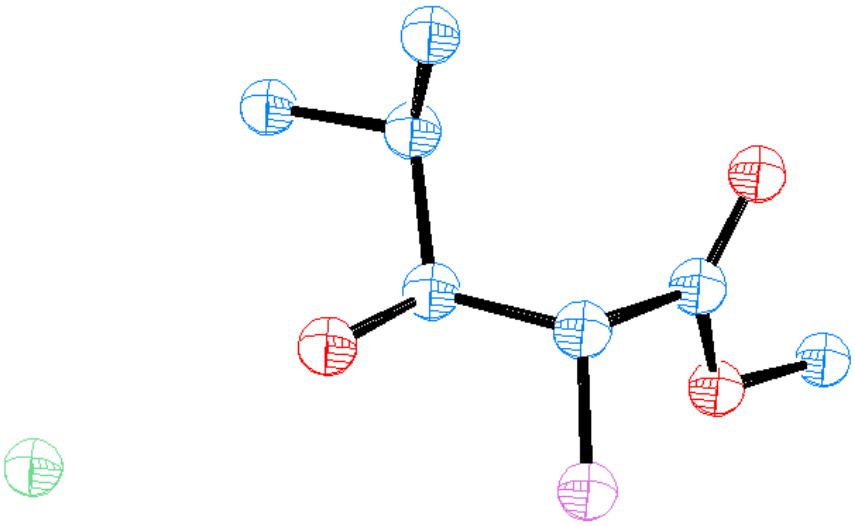


Compound number	**217**
Space group	P 2_1 (4)
Cell lengths	**a** 10.3313(2) **b** 10.2519(2) **c** 26.4465(6)
Cell angles	α 90.0000 β 98.891(2) γ 90.0000
Cell volume	2767.44
Z, Z’	**Z**: 0 **Z'**: 0

Compound number	(±)-**217**
Space group	P 2_1/n (14)
Cell lengths	**a** 14.29830(9) **b** 10.41200(6) **c** 18.66340(11)
Cell angles	**a** 90.0000 **b** 105.0690(6) **g** 90.0000
Cell volume	2682.95
Z, Z’	**Z**: 0 **Z'**: 0



Compound number	**288**
Space group	P 2_1 (4)
Cell lengths	**a** 8.19162(12) **b** 5.13934(7) **c** 15.5952(2)
Cell angles	α 90.0000 β 92.5974(13) γ 90.0000
Cell volume	655.876
Z, Z’	**Z**: 0 **Z'**: 0



Compound number	**156**
Space group	P 2_1/n (14)
Cell lengths	**a** 5.61665(15) **b** 22.0705(7) **c** 8.3267(3)
Cell angles	α 90.0000 β 98.395(3) γ 90.0000
Cell volume	1021.14
Z, Z’	**Z**: 0 **Z'**: 0

4.0 References

1 D. J. Newman and G. M. Cragg, *J. Nat. Prod.*, 2016, **79**, 629–661.

2 D. A. Dias, S. Urban and U. Roessner, *Metabolites*, 2012, **2**, 303–336.

3 S. Omura, T. Fujimoto, K. Otoguro, K. Matsuzaki, R. Moriguchi, H. Tanaka and Y. Sasaki, *J. Antibiot. (Tokyo).*, 1991, **44**, 113–116.

4 S. Ōmura, K. Matsuzaki, T. Fujimoto, K. Kosuge, T. Furuya, S. Fujita and A. Nakagawa, *J. Antibiot. (Tokyo).*, 1991, **44**, 117–118.

5 E. J. Corey and G. A. Reichard, *J. Am. Chem. Soc.*, 1992, **114**, 10677–10678.

6 T. Sunazuka, T. Nagamitsu, K. Matsuzaki, H. Tanaka and S. Ōmura, *J. Am. Chem. Soc.*, 1993, **115**, 5302.

7 G. Fenteany, R. F. Standaert, G. A. Reichard, E. J. Corey and S. L. Schreiber, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**, 3358–3362.

8 G. Fenteany, R. F. Standaert, W. S. Lane, S. Choi, E. J. Corey and S. L. Schreiber, *Science*, 1995, **268**, 726–731.

9 L. R. Dick, A. A. Cruikshank, F. D. Melandri, L. Sandra and R. L. Stein, 1996, **272**, 182–188.

10 L. R. Dick, A. A. Cruikshank, A. T. Destree, L. Grenier, T. A. McCormack, F. D. Melandri, S. L. Nunes, V. J. Palombella, L. A. Parent, L. Plamondon and R. L. Stein, *J. Bol. Chem.*, 1997, **272**, 182–188.

11 M. Groll, L. Ditzel, J. Löwe, D. Stock, M. Bochtler, H. D. Bartunik and R. Huber, *Nature*, 1997, **386**, 463–471. Image from the RCSB PDB (rcsb.org) of PDB ID IRYP

12 A. K. Nussbaum, T. P. Dick, W. Keilholz, M. Schirle, S. Stevanovic, K. Dietz, W. Heinmeyer, M. Groll, D. H. Wolf, R. Huber, H. Rammensee and H. Schild, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 12504–12509.

13 J. ADAMS, *Cancer Treat. Rev.*, 2003, **29**, 3–9.

14 M. Groll, E. P. Balskus and E. N. Jacobsen, *J. Am. Chem. Soc.*, 2008, **130**, 14981–14983.

15 M. A. Gräwert and M. Groll, *Chem. Commun.*, 2012, **48**, 1364–1378.

16 E. P. Balskus and E. N. Jacobsen, *J. Am. Chem. Soc.*, 2006, **128**, 6810–6812.

17 J. Watt, G. Hughes, S. Walpole, S. Monaco, G. R. Stephenson, P. Bulman Page, A. Hemmings, J. Angulo and A. Chantry, *Chem. - A Eur. J.*, 2018, **24**, 17677–17680.

18 S. Tsukamoto and H. Yokosawa, *Planta Med.*, 2010, **76**, 1064–1074.

19 J. Adams, *Nat. Rev. Cancer*, 2004, **4**, 349–360.

20 L. J. Crawford, B. Walker and A. E. Irvine, *J. Cell Commun. Signal.*, 2011, **5**, 101–110.

21 J. Adams, V. J. Palombella, E. A. Sausville, J. Johnson, A. Destree, D. D. Lazarus, J. Maas, C. S. Pien, S. Prakash and P. J. Elliott, *Cancer Res.*, 1999, **300**, 2615–2622.

22 G. Fenteany and S. L. Schreiber, *J. Bol. Chem.*, 1998, **273**, 8545–8548.

23 R. LeBlanc, L. P. Catley, T. Hideshima, S. Lentzsch, C. S. Mitsiades, N. Mitsiades, D. Neuberg, O. Goloubeva, C. S. Pien, J. Adams, D. Gupta, P. G. Richardson, N. C. Munshi and K. C. Anderson, *Cancer Res.*, 2002, **62**, 4996–5000.

24 S. Frantz and A. Paramore, *Nat. Rev. drug Discov.*, 2003, **2**, 611–612.

25 M. Pirrung and C. Heathcock, *J. Org. Chem.*, 1980, **45**, 1727–1728.

26 E. J. Corey, G. A. Reichard and R. Kania, *Tetrahedron Lett.*, 1993, **34**, 6977–6980.

27 M. Braun and H. Sacha, *Angew. Chemie (International Ed. English)*, 1991, **85**, 1318–1320.

28 E. J. Corey, W. Li and G. A. Reichard, *J. Am. Chem. Soc.*, 1998, **120**, 2330–2336.

29 E. J. Corey, L. Weidong and T. Nagamitsu, *Angew. Chemie - Int. Ed.*, 1998, **37**, 1676–1679.

30 D. B. Dess and J. C. Martin, *J. Org. Chem.*, 1983, **48**, 4155–4156.

31 H. C. Brown and K. S. Bhat, *J. Am. Chem. Soc.*, 1986, **108**, 293–294.

32 J. S. Panek and C. E. Masse, *Angew. Chemie - Int. Ed.*, 1999, **38**, 1093–1095.

33 F. Soucy, L. Grenier, M. L. Behnke, A. T. Destree, T. A. McCormack, J. Adams and L. Plamondon, *J. Am. Chem. Soc.*, 1999, **121**, 9967–9976.

34 P. R. Fleming and K. B. Sharpless, *J. Org. Chem.*, 1991, **56**, 2869–2875.

35 P. Rullière, J. Grisel, C. Poittevin, P. Cividino, S. Carret and J. F. Poisson, *Org. Lett.*, 2016, **18**, 2824–2827.

36 P. Rullière, A. Cannillo, J. Grisel, P. Cividino, S. Carret and J. F. Poisson, *Org. Lett.*, 2018, **20**, 4558–4561.

37 E. J. Corey, W. D. Z. Li, T. Nagamitsu and G. Fenteany, *Tetrahedron*, 1999, **55**, 3305–3316.

38 E. J. Corey and W. Z. Li, *Tetrahedron Lett.*, 1998, **39**, 7475–7478.

39 P. Saravanan and E. J. Corey, *J. Org. Chem.*, 2003, **68**, 2760–2764.

40 R. A. Shenvi and E. J. Corey, *J. Am. Chem. Soc.*, 2009, **131**, 5746–5747.

41 R. H. Feling, G. O. Buchanan, T. J. Mincer, C. A. Kauffman, P. R. Jensen and W. Fenical, *Angew. Chemie - Int. Ed.*, 2003, **42**, 355–357.

42 P. G. Williams, G. O. Buchanan, R. H. Feling, C. A. Kauffman, P. R. Jensen and W. Fenical, *J. Org. Chem.*, 2005, **70**, 6196–6203.

43 K. A. Reed, R. R. Manam, S. S. Mitchell, J. Xu, S. Teisan, T. H. Chao, G. Deyanat-Yazdi, S. T. C. Neuteboom, K. S. Lam and B. C. M. Potts, *J. Am. Chem. Soc.*, 2006, **128**, 5136-5141.

44 M. Stadler, J. Bitzer, A. Mayer-Bartschmid, H. Müller, J. Benet-Buchholz, F. Gantner,

H. V. Tichy, P. Reinemer and K. B. Bacon, *J. Nat. Prod.*, 2007, **70**, 246–252.

45 M. Groll, R. Huber and B. C. M. Potts, *J. Am. Chem. Soc.*, 2006, **128**, 5136–5141.

46 B. C. Potts and K. S. Lam, *Mar. Drugs*, 2010, **8**, 835–880.

47 V. R. Macherla, S. S. Mitchell, R. R. Manam, K. A. Reed, T. H. Chao, B. Nicholson, G. Deyanat-Yazdi, B. Mai, P. R. Jensen, W. F. Fenical, S. T. C. Neuteboom, K. S. Lam, M. A. Palladino and B. C. M. Potts, *J. Med. Chem.*, 2005, **48**, 3684–3687.

48 L. R. Reddy, J. F. Fournier, B. V. S. Reddy and E. J. Corey, *J. Am. Chem. Soc.*, 2005, **127**, 8974–8976.

49 R. R. Manam, V. R. Macherla, G. Tsueng, C. W. Dring, J. Weiss, S. T. C. Neuteboom, K. S. Lam and B. C. Potts, *J. Nat. Prod.*, 2009, **72**, 295–297.

50 L. R. Reddy, P. Saravanan and E. J. Corey, *J. Am. Chem. Soc.*, 2004, **126**, 6230–6231.

51 G. S. Cortez, R. L. Tennyson and D. Romo, *J. Am. Chem. Soc.,* 2001, **123**, 7945–7946.

52 M. Gil, N. Henry and D. Romo, *Org. Lett.*, 2007, **9**, 2143–2146.

53 H. Nguyen, G. Ma, T. Gladysheva, T. Fremgen and D. Romo, *J. Org. Chem.*, 2011, **76**, 2–12.

54 W. Wei, S. Lin, M. Chen, T. Liu, A. Wang, J. Li, Q. Guo and X. Shang, *J. Nat. Prod.*, 2017, **80**, 201–204.

55 H. J. Shin, T. S. Kim, H. S. Lee, J. Y. Park, I. K. Choi and H. J. Kwon, *Phytochemistry*, 2008, **69**, 2363–2366.

56 T. Mori, K. Takahashi, M. Kashiwabara and D. Uemura, *Tetrahedron Lett.*, 1985, **26**, 1073–1076.

57 C. L. Bagwell, M. G. Moloney and A. L. Thompson, *Bioorganic Med. Chem. Lett.*, 2008, **18**, 4081–4086.

58 K. Eto, M. Yoshino, K. Takahashi, J. Ishihara and S. Hatakeyama, *Org. Lett.*, 2011, **13**, 5398–5401.

59 K. Ko, S. H. Lee, S. H. Kim, E. H. Kim, K. B. Oh, J. Shin and D. C. Oh, *J. Nat. Prod.*, 2014, **77**, 2099–2104.

60 H. Ogawa, A. Iwasaki, S. Sumimoto, M. Iwatsuki, A. Ishiyama, R. Hokari, K. Otoguro, S. Omura and K. Suenaga, *Org. Lett.*, 2017, **19**, 890–893.

61 Y. K. T. Lam, O. D. Hensens, R. Ransom, R. A. Giacobbe, J. Polishook and D. Zink, *Tetrahedron*, 1996, **52**, 1481–1486.

62 K. Tanaka, K. Kobayashi and H. Kogen, *Org. Lett.*, 2016, **18**, 1920–1923.

63 P. Sauleau, P. Retailleau, J. Vacelet and M. L. Bourguet-Kondracki, *Tetrahedron*, 2005, **61**, 955–963.

64 R. a Meronuck, J. a Steele, C. J. Mirocha and C. M. Christensen, *Appl. Microbiol.*, 1972, **23**, 613–617.

65 B. J. L. Royles, *Chem. Rev.*, 1995, **95**, 1981–2001.

66 X. Mo, Q. Li and J. Ju, *RSC Adv.*, 2014, **4**, 50566–50593.

67 P. C. B. Page, A. S. Hamzah, D. C. Leach, S. M. Allin, D. M. Andrews and G. A. Rassias, *Org. Lett.*, 2003, **5**, 353–355.

68 L. N. Mander and S. P. Sethi, *Tetrahedron Lett.*, 1983, **24**, 5425–5428.

69 P. C. Page, D. C. Leach, C. M. Hayman, A. S. Hamzah, S. M. Allin and V. McKee, *Synlett*, 2003, **7**, 1025–1027.

70 A. E. Horton, PhD thesis, *University of East Anglia*, **2015**.

71 S. M. Allin, C. I. Thomas, K. Doyle and M. R. J. Elsegood, *J. Org. Chem.*, 2005, **70**, 357–359.

72 A. P. Krapcho, G. A. Glynn and B. J. Grenon, *Tetrahedron Lett.*, 1967, **8**, 215–217.

73 P. Garner, J. T. Anderson and S. Dey, *J. Org. Chem.*, 1998, **63**, 5732–5733.

74 C. J. Brennan, G. Pattenden and G. Rescourio, *Tetrahedron Lett.*, 2003, **44**, 8757–8760.

75 P. C. B. Page, R. L. Goodyear, A. E. Horton, Y. Chan, R. Karim, M. A. O. Connell, C. Hamilton, A. M. Z. Slawin, B. R. Buckley and S. M. Allin, *J. Org. Chem.*, 2017, **82**, 12209–12223.

76 G. Pattenden and G. Rescourio, *Org. Biomol. Chem.*, 2008, **6**, 3428–3438.

77 S. Tekkam, M. A. Alam, C. Jonnalagadda and V. R. Mereddy, *Chem. Commun.*, 2011, **47**, 3219–3221.

78 D. Bonnet, J. F. Margathe, S. Radford, E. Pflimlin, S. Riché, P. Doman, M. Hibert and A. Ganesan, *ACS Comb. Sci.*, 2012, **14**, 323–334.

79 S. Yamada, C. Hongo, R. Yoshioka and I. Chibata, *J. Org. Chem.*, 1983, **48**, 843–846.

80 M. D. Toney, *Arch. Biochem. Biophys.*, 2005, **433**, 279–287.

81 M. A. Smith, V. Mack, A. Ebneth, I. Moraes, B. Felicetti, M. Wood, D. Schonfeld, O. Mather, A. Cesura and J. Barker, *J. Biol. Chem.*, 2010, **285**, 12873–12881.

82 M. Pugniere, C. San Juan and A. Previero, *Biotechnol. Lett.*, 1985, **7**, 31–36.

83 J. Poncet, P. Jouin and B. Castro, *J. Chem. Soc., Perkin Trans. 1*, 1990, **0**, 611–616.

84 W. Pearlman, *Tetrahedron Lett.*, 1967, 1663–1664.

85 K. Fujiki, N. Tanifuji, Y. Sasaki and T. Yokoyama, *Synthesis (Stuttg).*, 2002, **3**, 343–348.

86 United states Pat. Off., 1,628,190, 1927.

87 J. Bougault, E. Cattelain and P. Chabrierm, *Compt. rend.*, 1939, **208**, 657.

88 R. Mozingo, D. E. Wolf, S. A. Harris and K. Folkers, *J. Am. Chem. Soc.*, 1943, **65**, 1013–1016.

89 H. Hauptmann and B. Wladislaw, *J. Am. Chem. Soc.*, 1950, **72**, 710.

90 S. Imaizumi, *Chem. Abstr.*, 1960, **54**, 1403h.

91 Kharasch and Meyers, in *The Chemistry of Organic sulfur compounds*, Volume 2., 1966, pp. 35–71.

92 T. D. Stewart and D. Lipkin, *J. Am. Chem. Soc.*, 1939, **61**, 3297–3300.

93 Y. Izumi, M. Imaida, H. Fukawa and S. Akabori, *Bull. Chem. Soc. Jpn.*, 1963, **36**, 155–160.

94 Y. Ueno, T. Miyano and M. Okawara, *Tetrahedron Lett.*, 1982, **23**, 443–446.

95 H. Natsugari, N. Tamura, K. Yoshioka and M. Ochiai, *J. Chem. Soc., Perkin Trans. 1*, 1983, **0**, 403–411.

96 F. Marr, R. Fröhlich and D. Hoppe, *Tetrahedron: Asymmetry*, 2002, **13**, 2587–2592.

97 F. Dénès, C. H. Schiesser and P. Renaud, *Chem. Soc. Rev.*, 2013, **42**, 7900–7942.

98 I. Kadota, H. Takamura, H. Nishii and Y. Yamamoto, *J. Am. Chem. Soc.*, 1995, **117**, 1173–1174.

99 J. Wei and J. T. Shaw, *Org. Lett.*, 2007, **9**, 4077–4080.

100 S. S. Bari, P. Venugopalan and R. Arora, *Tetrahedron Lett.*, 2003, **44**, 895–897.

101 D. C. Harrowven, I. L. Guy and M. I. T. Nunn, *Chem. Commun.*, 2004, **0**, 1966–1969.

102 D. C. Harrowven, D. P. Curran, S. L. Kostiuk, I. L. Wallis-Guy, S. Whiting, K. J. Stenning, B. Tang, E. Packard and L. Nanson, *Chem. Commun.*, 2010, **46**, 6335–6337.

103 C. Chatgilialoglu, C. Ferreri, Y. Landais and V. I. Timokhin, *Chem. Rev.*, 2018, **118**, 6516–6572.

104 P. Šafář, J. Žúžiová, Š. Marchalín, E. Tóthová, N. Prónayová, Ľ. Švorc, V. Vrábel and A. Daïch, *Tetrahedron Asymmetry*, 2009, **20**, 626–634.

105 E. J. Corey and W. D. Li, *Chem. Pharm. Bull. (Tokyo).*, 1999, **47**, 1–10.

106 T. C. Byung and K. K. Sang, *Tetrahedron*, 2005, **61**, 5725–5734.

107 M. Barniol-Xicota, A. L. Turcu, S. Codony, C. Escolano and S. Vázquez, *Tetrahedron Lett.*, 2014, **55**, 2548–2550.

108 S. D. Bull, S. G. Davies, S. W. Epstein and J. V. A. Ouzman, *Chem. Commun.*, 1998, **0**, 659- 660.

109 K. N. White and J. P. Konopelski, *Org. Lett.*, 2005, **7**, 4111–4112.

110 A. S. Guram, R. A. Rennels and S. L. Buchwald, *Angew. Chemie (International Ed. English)*, 1995, **34**, 1348–1350.

111 J. Louie and J. F. Hartwig, *Tetrahedron Lett.*, 1995, **36**, 3609–3612.

112 P. Y. S. Lam, C. G. Clark, S. Saubern, J. Adams, M. P. Winters, D. M. T. Chan and A. Combs, *Tetrahedron Lett.*, 1998, **39**, 2941–2944.

113 D. Seebach, T. Maetzke, W. Petter, B. Kloetzer and D. A. Plattner, *J. Am. Chem. Soc.*, 1991, **113**, 1781–1786.

114 A. I. Meyers, M. A. Seefeld, B. A. Lefker and J. F. Blake, *J. Am. Chem. Soc.*, 1997, **119**, 4565–4566.

115 K. Ando, N. S. Green, Y. Li and K. N. Houk, *J. Am. Chem. Soc.*, 1999, **121**, 5334–5335.

116 A. Furst and P. A. Plattner, *Helv. Chim. Acta*, 1949, **32**, 275–283.

117 O. Wallach, *Liebigs ann. Chem.*, 1895, **286**, 90–143.

118 C. P. Brock, W. B. Schweizer and J. D. Dunitz, *J. Am. Chem. Soc.*, 1991, **113**, 9811–9820.

119 Y. Wu, D. C. Limburg, D. E. Wilkinson, M. J. Vaal and G. S. Hamilton, *Tetrahedron*, 2000, **41**, 2847–2849.

120 B. Li, M. Berliner, R. Buzon, C. K. F. Chiu, S. T. Colgan, T. Kaneko, N. Keene, W. Kissel, T. Le, K. R. Leeman, B. Marquez, R. Morris, L. Newell, S. Wunderwald, M. Witt, J. Weaver, Z. Zhang and Z. Zhang, *J. Org. Chem.*, 2006, **71**, 9045–9050.

121 B. J. E. Baldwin, *J. Chem. Soc., Chem. Commun.*, 1976, **0**, 734–736.

122 D. J. Buchanan, D. J. Dixon and F. A. Hernandez-Juan, *Org. Lett.*, 2004, **6**, 1357–1360.

123 A. Massa, A. Scettri, R. Filosa and L. Capozzolo, *Tetrahedron Lett.*, 2009, **50**, 7318–7321.

124 A. Endo and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2005, **127**, 8298–8299.

125 K. Takahashi, M. Midori, K. Kawano, J. Ishihara and S. Hatakeyama, *Angew. Chemie - Int. Ed.*, 2008, **47**, 6244–6246.

126 N. P. Mulholland, G. Pattenden and I. A. S. Walters, *Org. Biomol. Chem.*, 2006, **4**, 2845–2846.

127 N. P. Mulholland, G. Pattenden and I. A. S. Walters, *Org. Biomol. Chem.*, 2008, **6**, 2782–2789.

128 M. Gil, N. Henry and D. Romo, *Org. Lett.*, 2007, **9**, 2143–2146.

129 M. Frigerio, M. Santagostino and S. Sputore, *J. Org. Chem.*, 1999, **64**, 4537–4538.

130 D. J. Atkinson, B. J. Naysmith, D. P. Furkert and M. A. Brimble, *Beilstein J. Org. Chem.*, 2016, **12**, 2325–2342.

131 H. He, R. T. Williamson, B. Shen, E. I. Graziani, H. Y. Yang, S. M. Sakya, P. J. Petersen and G. T. Carter, *J. Am. Chem. Soc.*, 2002, **124**, 9729–9736.

132 Y. Koiso, M. Natori and S. Iwasaki, *Tetrahedron Lett.*, 1992, **33**, 4157–4160.

133 Y. Koiso, Y. Li and S. Iwasaki, *J. Antibiot. (Tokyo).*, 1994, **47**, 765–773.

134 D. P. Bonner, J. O. Sullivan, S. K. Tanaka, J. M. Clark and R. R. Whitney, *J. Antibiot. (Tokyo).*, 1988, 1745–1751.

135 A. A. Tymiak, T. J. Mccormick and S. E. Unger, *J. Org. Chem.*, 1989, **54**, 1149–1157.

136 H. Umezawa, *Lloydia*, 1977, **40**, 67.

137 T. Takita, Y. Muraoka, T. Nakatani, A. Fujii, Y. Umezawa, H. Naganawa and H. Umezawa, *J. Antibiot. (Tokyo).*, 1978, **XXXI**, 801–804.

138 J. Watanabe, N. Nakada, S. Sawairi, H. Shimada, S. Ohshima, T. Kamiyama and M. Arisawa, *J. Antibiot. (Tokyo).*, 1994, **47**, 32–36.

139 T. Kamiyama, N. Shimma, T. Ohtsuka, N. Nakayama, Y. Itezono, N. Nakada, J. Watanabe, K. Yokose and N. Roche, *J. Antibiot. (Tokyo).*, 1994, **47**, 37.

140 M. K. Renner, Y. Shen, X. Cheng, P. R. Jensen, W. Frankmoelle, C. A. Kauffman, W. Fenical, E. Lobkovsky and J. Clardy, *J. Am. Chem. Soc.*, 1999, **121**, 11273–11276.

141 M. H. McCormick, W. M. Stark, G. E. Pittenger, R. C. Pittenger and J. M. McGuire, *Antibiot. Annu.*, 606–611.

142 D. A. Evans, M. R. Wood, B. W. Trotter, T. I. Richardson, J. C. Barrow and J. L. Katz, *Angew. Chemie - Int. Ed.*, 1998, **37**, 2700–2704.

143 G. M. Sheldrick, P. G. Jones, O. Kennard, D. H. Williams and G. A. Smith, *Nature*, 1978, **271**, 223–225.

144 C. M. Harris, H. Kopecka and T. M. Harris, 1983, **5**, 6915–6922.

145 Y. Gao, *Nat. Prod. Rep.*, 2002, **19**, 100–107.

146 K. C. Nicolaou, C. N. C. Boddy, S. Bräse and N. Winssinger, *Angew. Chemie Int. Ed.*, 1999, **38**, 2096–2152.

147 WHO, *The selection and use of essential medicines*, 2017.

148 D. L. Boger, *J. Org. Chem.*, 2017, **82**, 11961–11980.

149 H. Nakata, K. Maeda, T. Miyakawa, S. Shibayama, M. Matsuo, Y. Takaoka, M. Ito, Y. Koyanagi and H. Mitsuya, *J. Virol.*, 2005, **79**, 2087–2096.

150 J. Ehrlich, Q. R. Bartz, R. M. Smith, D. A. Joslyn and P. R. Burkholder, *Science.*, 1947, **106**, 417–417.

151 M. C. Rebstock, H. M. Crooks, J. Controulis and Q. R. Bartz, *J. Am. Chem. Soc.*, 1949, **71**, 2458–2462.

152 J. Controulis, M. C. Rebstock and H. M. Crooks, *J. Am. Chem. Soc.*, 1949, **71**, 2463–2468.

153 H. Maehr, C. M. Liu, N. J. Palleroni, J. Smallheer, L. Todaro, T. H. Williams and J. F. Blount, *J Antibiot*, 1986, **39**, 17–25.

154 T. Smitka, J. Deeter and A. Hunt, *J. Antibiot. (Tokyo).*, 1988, 726–733.

155 Y. Hayakawa, M. Nakagawa, Y. Toda and H. Seto, *Agric. Biol. Chem.*, 1990, **54**, 1007–1011.

156 P. W. Ford, K. R. Gustafson, T. C. McKee, N. Shigematsu, L. K. Maurizi, L. K. Pannell, D.

E. Williams, E. D. De Silva, P. Lassota, T. M. Allen, R. Van Soest, R. J. Andersen and M. R. Boyd, *J. Am. Chem. Soc.*, 1999, **121**, 5899–5909.

157 Nishiyama, K. Sugawara, K. Tomita, H. Yamamoto, H. Kamei and T. Oki, *J. Antibiot. (Tokyo).*, 1993, **46**, 921–927.

158 R. P. Maskey, S. Fotso, M. Sevvana, I. Usón, I. Grün-Wollny and H. Laatsch, *J. Antibiot. (Tokyo).*, 2006, **59**, 309–314.

159 K. Umezawa, K. Nakazawa, Y. Ikeda, H. Naganawa and S. Kondo, *J. Org. Chem.*, 1999, **64**, 3034–3038.

160 Y. Uchihata, N. Ando, Y. Ikeda, S. Kondo, M. Hamada and K. Umezawa, *J. Antibiot. (Tokyo).*, 2002, **55**, 1–5.

161 Y. Sakai, T. Yoshida, T. Tsujita, K. Ochiai, T. Agatsuma, Y. Saitoh, F. Tanaka, T. Akiyama, S. Akinaga and T. Mizukami, *J Antibiot*, 1997, **50**, 659–664.

162 M. Izumikawa, M. Takagi and K. Shin-Ya, *J. Nat. Prod.*, 2012, **75**, 280–284.

163 J. C. Sheehan, D. Mania, S. Nakamura, J. A. Stock and K. Maeda, *J. Am. Chem. Soc.*, 1968, **90**, 462–470.

164 T. Kinoshita, Y. Kono, S. Takeuchi and J. M. Daly, *Agric. Biol. Chem.*, 1989, **53**, 1283–1290.

165 K. Fukushima, T. Arai, Y. Mori, M. Tsuboi and M. Suzuki, *J. Antibiot.*, 1983, **36**, 1606–1612.

166 G. L. Helms, R. E. Moore, W. P. Niemczura, G. M. L. Patterson, K. B. Tomer and M. L. Gross, *J. Org. Chem.*, 1988, **53**, 1298–1307.

167 D. A. Evans and A. E. Weber, *J. Am. Chem. Soc.*, 1986, **108**, 6757–6761.

168 D. A. Evans, E. B. Sjogren, A. E. Weber and R. E. Conn, *Tetrahedron Lett.*, 1987, **28**, 39–42.

169 R. Thayumanavan, F. Tanaka and C. F. Barbas, *Org. Lett.*, 2004, **6**, 3541–3544.

170 E. J. Corey, D. H. Lee and S. Choi, *Tetrahedron Lett.*, 1992, **33**, 6735–6738.

171 J. P. Genet, C. Pinel, S. Mallart, S. Juge, S. Thorimbert and J. A. Laffitte, *Tetrahedron: Asymmetry*, 1991, **2**, 555–567.

172 R. Noyori, T. Ikeda, T. Ohkuma, M. Widhalm, M. Kitamura, H. Takaya, S. Akutagawa, N. Sayo, T. Saito, T. Taketomi and H. Kumobayashi, *J. Am. Chem. Soc.*, 1989, **111**, 9134–9135.

173 K. Makino, N. Okamoto, O. Hara and Y. Hamada, *Tetrahedron Asymmetry*, 2001, **12**, 1757–1762.

174 C. Mordant, P. Dünkelmann, V. Ratovelomanana-Vidal and J. P. Genet, *European J. Org. Chem.*, 2004, 3017–3026.

175 C. Mordant, P. Dünkelmann, V. Ratovelomanana-Vidal and J. P. Genet, *Chem. Commun.*, 2004, **4**, 1296–1297.

176 K. Makino, T. Goto, Y. Hiroki and Y. Hamada, *Angew. Chemie - Int. Ed.*, 2004, **43**, 882–884.

177 Y. Gao and K. B. Sharpless, *J. Am. Chem. Soc.*, 1988, **110**, 7538–7539.

178 T. Nagamitsu, T. Sunazuka, H. Tanaka, O. Satoshi, P. A. Sprengeler and A. B. Smith, 1996, **118**, 3584–3590.

179 T. Katsuki and K. B. Sharpless, *J. Am. Chem. Soc.*, 1980, **102**, 5974–5976.

180 O. Makowka, *Berichte der Dtsch. Chem. Gesellschaft*, 1908, **41**, 943–944.

181 R. Criegee, B. Marchand and H. Wannowius, *Justus Liebig's Ann. der Chemie*, 1942, **550**, 99–133.

182 S. G. Hentges and K. B. Sharpless, *J. Am. Chem. Soc.*, 1980, **102**, 4263–4265.

183 E. N. Jacobsen, I. Markó, W. S. Mungall, G. Schröder and K. B. Sharpless, *J. Am. Chem. Soc.*, 1988, **110**, 1968–1970.

184 V. VanRheenen, R. C. Kelly and D. Y. Cha, *Tetrahedron Lett.*, 1976, **17**, 1973–1976.

185 K. B. Sharpless, W. Amberg, Y. L. Bennani, G. A. Crispino, J. Hartung, K. S. Jeong, H. L. Kwong, K. Morikawa, Z. M. Wang, D. Xu and X. L. Zhang, *J. Org. Chem.*, 1992, **57**, 2768–2771.

186 N. H. Cromwell, R. D. Babson and C. E. Harris, *J. Am. Chem. Soc.*, 1943, **65**, 312–315.

187 N. H. Cromwell, D. L. Nagel and P. B. Woller, *J. Org. Chem.*, 1971, **36**, 3911–3917.

188 T. W. Greene and P. G. M. Wuts, *Protective groups in organic synthesis*, John Wiley and Sons, 2nd edition., 1991.

189 I. A. Khan and A. K. Saxena, *J. Org. Chem.*, 2013, **78**, 11656–11669.

190 A. De Mico, R. Margarita, L. Parlanti, A. Vescovi and G. Piancatelli, *J. Org. Chem.*, 1997, **62**, 6974–6977.

191 A. Nooy, A. Besemer and H. Bekkum, *Synthesis (Stuttg).*, 1996, 1153–1174.

192 T. Akiyama, J. Itoh, K. Yokota and K. Fuchibe, *Angew. Chemie - Int. Ed.*, 2004, **43**, 1566–1568.

193 D. Uraguchi and M. Terada, *J. Am. Chem. Soc.*, 2004, **126**, 5356–5357.

194 R. I. Storer, D. E. Carrera, Y. Ni and D. W. C. MacMillan, *J. Am. Chem. Soc.*, 2006, **128**, 84–86.

195 X.-L. Liu, Z.-B. Yu, B.-W. Pan, L. Chen, T.-T. Feng and Y. Zhou, *J. Heterocycl. Chem.*, 2012, **52**, 628.

196 A. Kumar, S. Sharma and R. A. Maurya, *Adv. Synth. Catal.*, 2010, **352**, 2227–2232.

197 B. Das, V. S. Reddy, F. Tehseen and M. Krishnaiah, *Synthesis (Stuttg).*, 2007, 666–668.

198 K. S. Olivier and M. S. Van Nieuwenhze, 2010, **12**, 1680–1683.

199 G. Sabitha, R. S. Babu, M. S. K. Reddy and J. S. Yadav, *Synthesis (Stuttg).*, 2002, 2254–

2258.

200 G. Sabitha, R. S. Babu, M. Rajkumar and J. S. Yadav, *Org. Lett.*, 2002, **4**, 343–345.

201 M. J. Martin, R. Rodriguez-Acebes, Y. Garcia-Ramos, V. Martinez, C. Murcia, I. Digon, I. Marco, M. Pelay-Gimeno, R. Fernández, F. Reyes, A. M. Francesch, S. Munt, J. Tulla-Puche, F. Albericio and C. Cuevas, *J. Am. Chem. Soc.*, 2014, **136**, 6754–6762.

202 G. Y. Zhang, S. S. Lv, A. Shoberu and J. P. Zou, *J. Org. Chem.*, 2017, **82**, 9801–9807.

203 C. R. Pandit and N. S. Mani, *Synthesis (Stuttg).*, 2009, **23**, 4032–4036.

204 World intellectual property organization WO 2007/026920 A2, 2007.

205 S. M. King and S. L. Buchwald, *Org. Lett.*, 2016, **18**, 4128–4131.

206 Z. Noszticzius, W. D. McCormick and H. L. Swinney, *J. Phys. Chem.*, 1987, **91**, 5129–5134.

207 M. Nakane, H. Gollman, C. R. Hutchinson and P. L. Knutson, *J. Org. Chem.*, 1980, **45**, 2536–2538.

208 R. B. Boers, Y. P. Randulfe, H. N. S. Van Der Haas, M. Van Rossum-Baan and J. Lugtenburg, *European J. Org. Chem.*, 2002, 2094–2108.

209 T. Müller, M. Göhl, I. Lusebrink, K. Dettner and K. Seifert, *European J. Org. Chem.*, 2012, 2323–2330.

210 D. S. Rekunge, C. K. Khatri and G. U. Chaturbhuj, *Tetrahedron Lett.*, 2017, **58**, 1240–1244.

211 L. Qin, P. Wang, Y. Zhang, Z. Ren, X. Zhang and C. S. Da, *Synlett*, 2016, **27**, 571–574.

212 W. Arnold, J. J. Daly and R. Imhof, *Tetrahedron Lett.*, 1983, **24**, 343–346.