# Customising Lactacystins: Studies Towards the Total Synthesis of Lactacystin and its Analogues

by

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A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of

Doctor of Philosophy

at

The University of East Anglia

September 2015

# 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 this or any other institution for a degree.

Alexandra Horton

September 2015

# Abstract

This thesis consists of four chapters. The first contains a literature review of the isolation, previous total syntheses, biological activity and structure activity relationships of lactacystin and its analogues. Chapter two discusses our work towards the total synthesis of lactacystin and its analogues starting from three different amino acids. Chapter three contains the experimental details of our work, and the final chapter contains the details of our work on the biological testing of some of our advanced intermediates towards deoxylactacystin.

Our synthetic approach towards lactacystin and its analogues starts from a simple amino acid derivative; using different amino acid derivatives as starting material, the C5 position is easily altered. The starting material is then advanced to a suitable diester for Dieckmann cyclization to form the lactam core found in the natural product.

The next key step in our approach follows Mander's acylation protocol to form the C5 quaternary centre using methyl cyanoformate to install a methyl ester group in a selective manner. This step results in the fully functionalized core of lactacystin.

At this stage we had two possible routes. First, we investigated the reduction of the ketone at C6 followed by attempted decarboxylation at C7; this route ultimately proved unsuccessful. The second route inverted the reaction order; performing the decarboxylation at C7 first followed by attempts to reduce the ketone at C6. The reduction was unsuccessful and so a thiomethyl derivative was employed to allow the ketone to be successfully reduced followed by removal of the thiomethyl group using Raney nickel.

Chapter four has been written as a stand-alone chapter. Four advanced intermediates towards deoxylactacystin were chosen to undergo biological testing. Compounds were tested for their anti-proliferative effects against the HL-60 cell line using an MTS assay and their ability to inhibit the chymotrypsin-like activity in the 20S proteasome.

## Acknowledgements

I would like to thank my supervisor Prof. Phil Page for the opportunity to work as part of his group and for his guidance, advice and support throughout my research. I would like to thank Dr. Chris Hamiliton and Dr. Maria O'Connell for their time, patience and help in the biological testing part of my project. I would also like to thank the BBSRC and Charnwood Molecular Ltd. for funding this project.

I am very grateful to Dr. Yohan Chan and would like to thank him for putting up with me over the last four years! His advice and guidance throughout my time here at the UEA has been invaluable.

To James and Ryan, who have been there for me and helped me from day one, thank you. I couldn't have done it without you both and our Friday lunches! To the rest of the Page group, and all my other friends on the 3rd floor, thank you. My time here at the UEA has been a lot of fun.

Last, but by no means least, I would like to thank my parents. I am forever grateful to them for every opportunity they have given me throughout my life. I wouldn't be where I am today without their continued, and unconditional, love and support. Thank you!

# List of Abbreviations

| | |
|---|---|
| Å | ångström |
| ABCN | 1,1′-azobis(cyclohexanecarbonitrile) |
| Ac | acetyl |
| $[\alpha]_D$ | specific optical rotation at the sodium D line |
| AIBN | azobisisobutyronitrile |
| aq. | aqueous |
| $B_{AC}2$ | base-catalysed acyl cleavage |
| $B_{AL}2$ | base-catalysed alkyl cleavage |
| Bn | benzyl |
| BOPCl | bis (2-oxo-3-oxazolidinyl) phosphinic chloride |
| b.p. | boiling point |
| Bu | butyl |
| *i*-Bu | *iso*-butyl |
| *n*BuLi | *n*-butyllithium |
| CAN | ceric ammonium nitrate |
| CDI | 1,1'-carbonyldiimidazole |
| $cm^{-1}$ | wavenumber |
| Conc. | concentrated |
| Δ | heat |
| δ | chemical shift |
| °C | degrees Celsius |
| d | day(s) |
| DCC | *N*,*N*'-dicyclohexylcarbodiimide |
| DEAD | diethyl azodicarboxylate |
| DHTD | 1,6-dimethyl-1,5,7-hexahydro- 1,4,6,7-tetrazocin-2,5-dione |
| DIBAL-H | diisobutyl aluminium hydride |
| DIPEA | *N*,*N*-diisopropylethylamine |
| DMAP | 4-*N*,*N* –dimethylaminopyridine |
| DMF | dimethylformamide |
| DMPU | 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone |
| DMSO | dimethylsulfoxide |

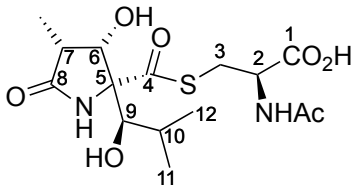
| | |
|---|---|
| EDAC·HCl | *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride |
| Equiv. | equivalents |
| Et | ethyl |
| g | gram(s) |
| h | hour(s) |
| HATU | 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*] pyridinium 3-oxid hexafluorophosphate |
| HMPA | hexamethylphosphoramide |
| IPA | *iso*-propanol |
| IR | infrared |
| *J* | coupling constant |
| kD | kilodalton(s) |
| LAH | lithium aluminium hydride |
| LDA | lithium diisopropylamine |
| LiHMDS | lithium hexamethyldisilazide |
| M | molar |
| Me | methyl |
| MHz | megahertz |
| min. | minute |
| ml | millilitre |
| mmol | millimole |
| mol | mole |
| MPV | Meerwein-Ponndorf-Verley |
| MS | mass spectrometry |
| NAC | *N*-acetylcysteine |
| NMR | nuclear magnetic resonance |
| NMM | *N*-methylmorpholine |
| $Pd(OH)_2/C$ | palladium hydroxide on carbon |
| Ph | phenyl |
| PMB | 4-methoxybenzyl |
| ppm | parts per million |
| *i*-Pr | *iso*-propyl |
| Red-Al® | sodium bis(2-methoxyethoxy)aluminium hydride |
| RT | room temperature |

| | |
|---|---|
| TBAF | tetrabutylammonium fluoride |
| TBDMS-Cl | *tert*-butyldimethylsilyl chloride |
| TBP | tributylphosphine |
| Tf | trifluoromethanesulfonate (triflate) |
| TFA | trifluoroacetate |
| TFAA | trifluoroacetic anhydride |
| THF | tetrahydrofuran |
| TIPS-Cl | triisopropylsilyl chloride |
| TLC | thin layer chromatography |
| TMAD | tetramethylazodicarboxamide |
| TPP | triphenylphosphine |
| *p*-TSA | *para*-toluenesulfonic acid |
| TTMSS | tris(trimethylsilyl)silane |
| UV | ultraviolet |
| VT | variable-temperature |

# Contents

# 1.0 Introduction

Lactacystin **1** is a microbial metabolite first isolated by Ōmura *et al*. in 1991 from *Streptomyces sp*. OM-6519.[1, 2] The microbial metabolites were screened to determine if they could induce differentiation of the Neuro 2A (mouse neuroblastoma) cell line. Lactacystin was found to induce neurite outgrowth and inhibit cell proliferation in the Neuro 2A cell line as well as inhibiting growth in the osteosarcoma cell line in humans.[3] Its effect on Neuro 2A cell lines was found to mimic the action of neurotrophic factors (NTF). NTFs are responsible for the maintenance and survival of nerve cells, without which the nervous system cannot function correctly, and nerve-related diseases such as Alzheimer's and Parkinson's can occur as a result.




**1**

**Figure 1.** Lactacystin

In 1994 Fenteany *et al*. studied the activity of lactacystin analogues to determine which structural features were essential for biological activity.[3] They found that the activity could be greatly affected by the groups on the γ-lactam ring. The groups and stereochemistry at the C5, C6 and C7 positions including the configuration of the C9 carbon are important. Modifications can result in partial or complete loss of activity. In contrast the *N*-acetyl-L-cysteine (NAC) moiety was found to play no part in the activity and this group could be changed with no effect on activity. It was also found that the inactive compounds do not compete with the action of lactacystin. They observed that the analogues that are most active in cell cycle progression and neurite outgrowth all have the potential to form β-lactones.

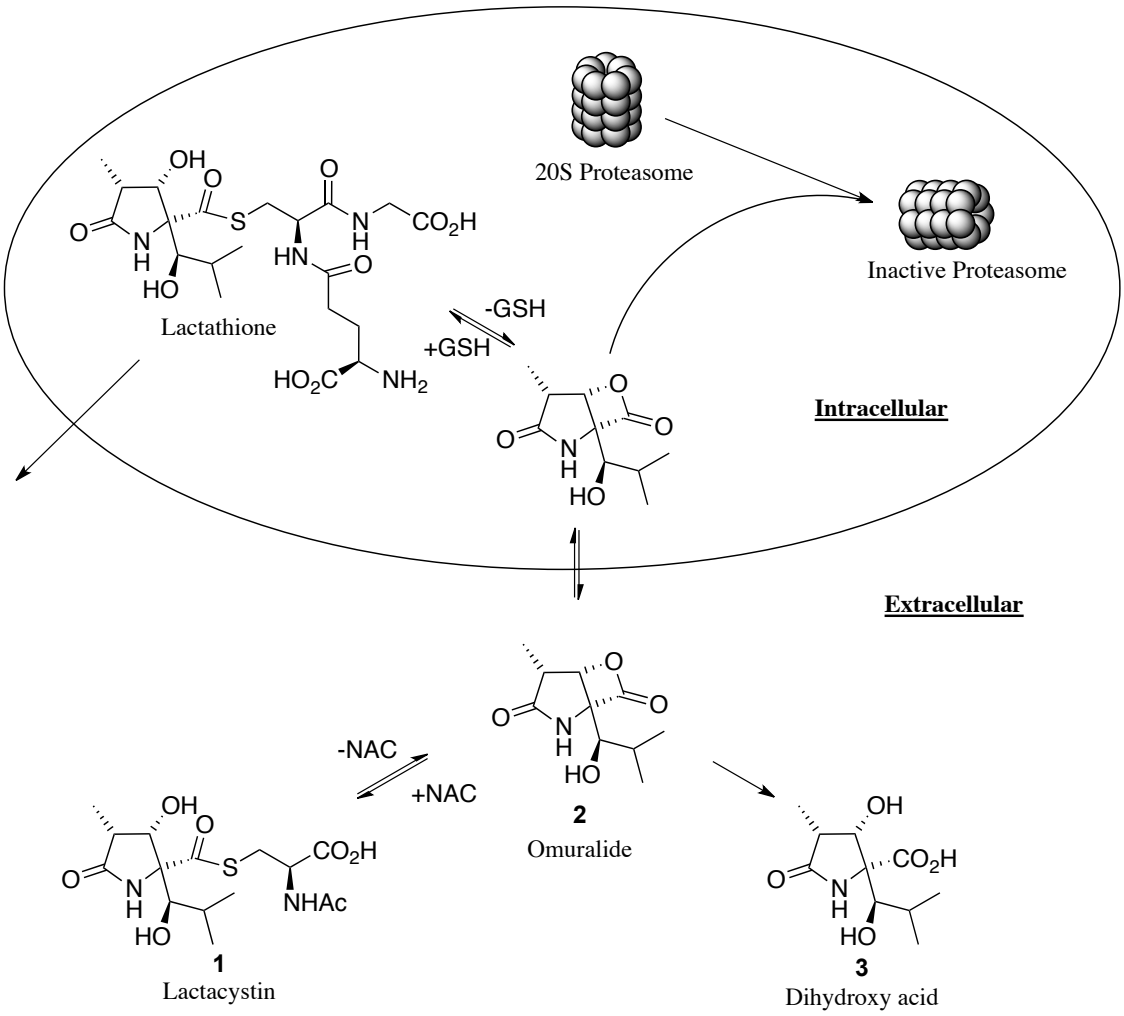
Not long after, Schreiber[4] carried out labeling studies to identify the specific cellular target of lactacystin. Tritium-labeled lactacystin was used and the 20S proteasome was identified as the target. The study tested a series of lactacystin analogues and found that

the key to activity was the electrophilic carbonyl group at the C4 position. The C4 carbonyl group of the thioester in lactacystin and the β-lactone analogue were found to be the reactive electrophiles. Hydrolysis of the lactacystin thioester resulted in the corresponding acid, a compound which showed no biological activity.

## 1.1 Mechanistic Studies into the Action of Lactacystin

Dick *et al*. described the mechanism of proteasome inhibition by lactacystin.[5] Following the discoveries by Fenteany and Schreiber,[3, 4] they were able to show that lactacystin underwent hydrolysis in aqueous solution, at pH 8, to form NAC and the dihydroxy acid **3** through the β-lactone intermediate **2**. They discovered that proteasome inhibition is not caused by lactacystin but the β-lactone, also known as omuralide, exclusively. At pH 6.3, lactacystin is stable but also inactive as a proteasome inhibitor.

Further *in vitro* studies showed that lactacystin itself cannot permeate through cell walls, but the β-lactone derivative can. The efficiency of lactacystin as a proteasome inhibitor is thus dependent on its ability to form the β-lactone **2**. Once inside the cell the β-lactone can undergo several reactions, shown in **Scheme 1**.[6] Hydrolysis can occur to form the inactive dihydroxy acid **3**, it can react with glutathione (GSH) to form lactathione, which is analogous to lactacystin and can reform the β-lactone, and finally it can acylate the threonine residue in the proteasome, resulting in inhibition.




**Scheme 1.** Mechanism of action of lactacystin in cells.[6]

### 1.1.1 The 20S Proteasome

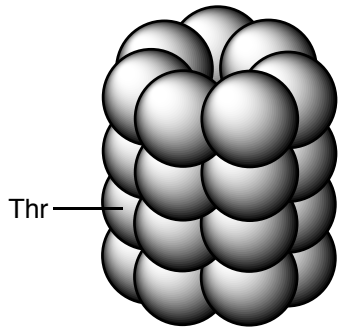



**Figure 2.** The 20S proteasome.

The 20S proteasome (**Figure 2**) was found to be the specific cellular target of lactacystin and its derivatives.[4] Lactacystin was found to inhibit the trypsin-like (proteases that cleave peptide bonds in the position following a positively charged amino acid such as lysine), chymotrypsin-like (the hydrophobic nature of the S1 pocket makes it specific for medium to large hydrophobic residues) and peptidylglutamyl-peptide hydrolysing (cleavage of peptide bonds in the position following acidic or branched-chain amino acids) activity in the enzyme complex.[4] The trypsin- and chymotrypsin-like activity are both irreversibly inhibited by lactacystin.

The 20S proteasome is a large (~700 kD) protein complex.[7] It is cylindrical in shape with a hollow center. It is made up of a stack of 4 doughnut-shaped rings, each consisting of 7 protein subunits, stacked on top of each other. The two outer rings consist of α-type subunits while the inner rings contain β-type subunits. The 20S proteasome is capped by 19S proteasomes at either end to make up the whole 26S proteasome unit. A crystal structure of the 20S proteasome can be seen below (**Figure 3**).

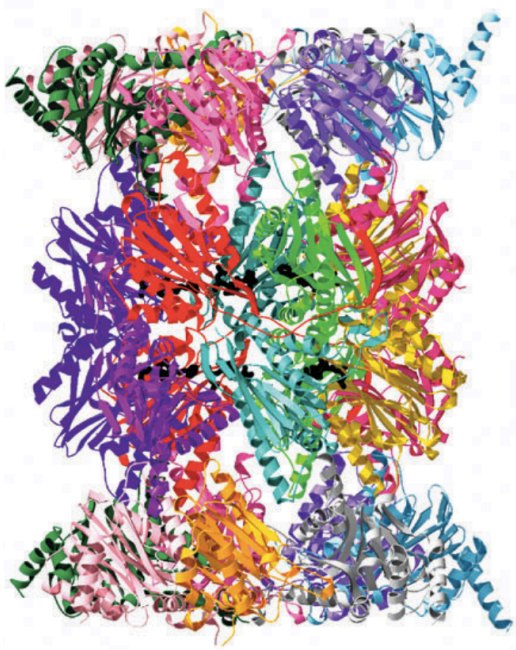


**Figure 3.** A crystal structure of the 20S proteasome.[8]

The proteasome is essential in regulating many processes within the cell, including cellular function and homeostasis.[9] The ubiquitin proteasome pathway (UPP, **Figure 4**) is responsible for the majority of intracellular protein degradation. This is important for cell growth and survival, for both healthy and tumour cells.

There are two stages to the UPP. The first stage, ubiquitin tagging, occurs when the ubiquitin-activating enzyme E1 covalently binds ubiquitin; the ubiquitin is then transferred to the ubiquitin-conjugating enzyme E2. Finally, the E3 ubiquitin ligase transfers the ubiquitin to the target protein. The second stage, proteolytic degradation, occurs when the ubiquitin-tagged proteins are transported to the proteasome. Polyubiquitin chains are produced by conjugation of ubiquitin moieties and act as a signal to target the protein for degradation. Different polyubiquitin chain lengths result in different functions, one of which is protein degradation. The ubiquitin molecules are removed and the protein is fed into the proteasome where it is broken down into small peptide units.

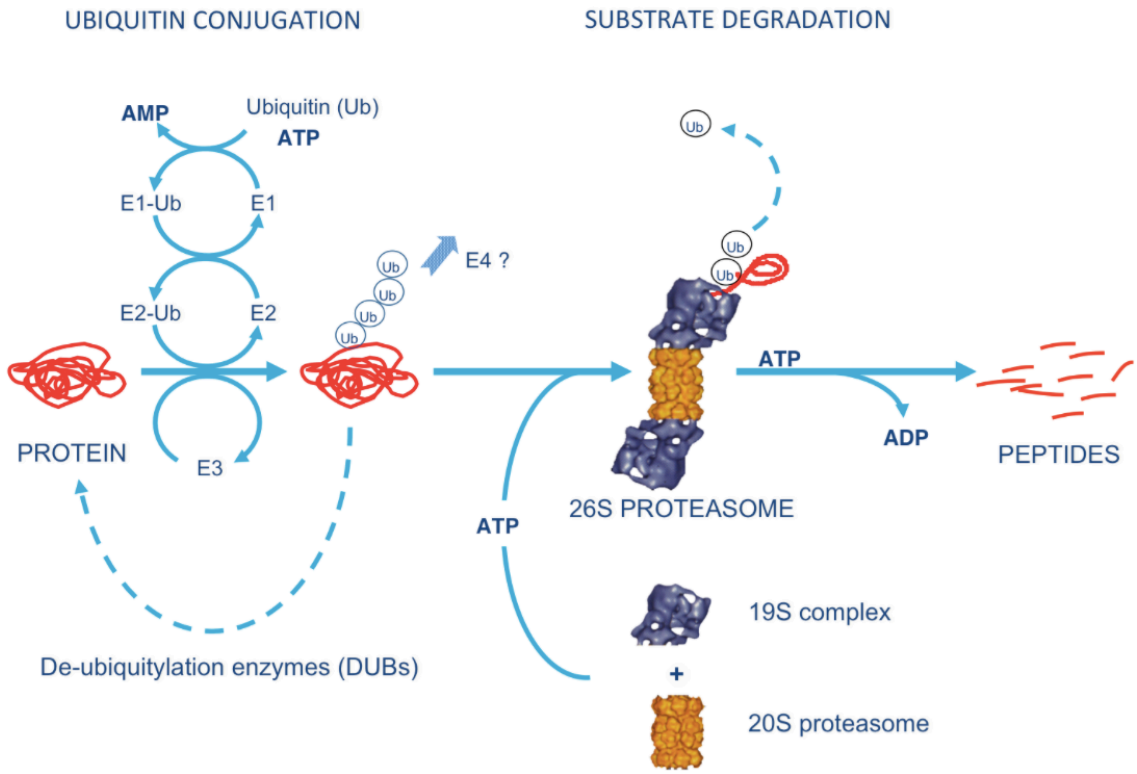
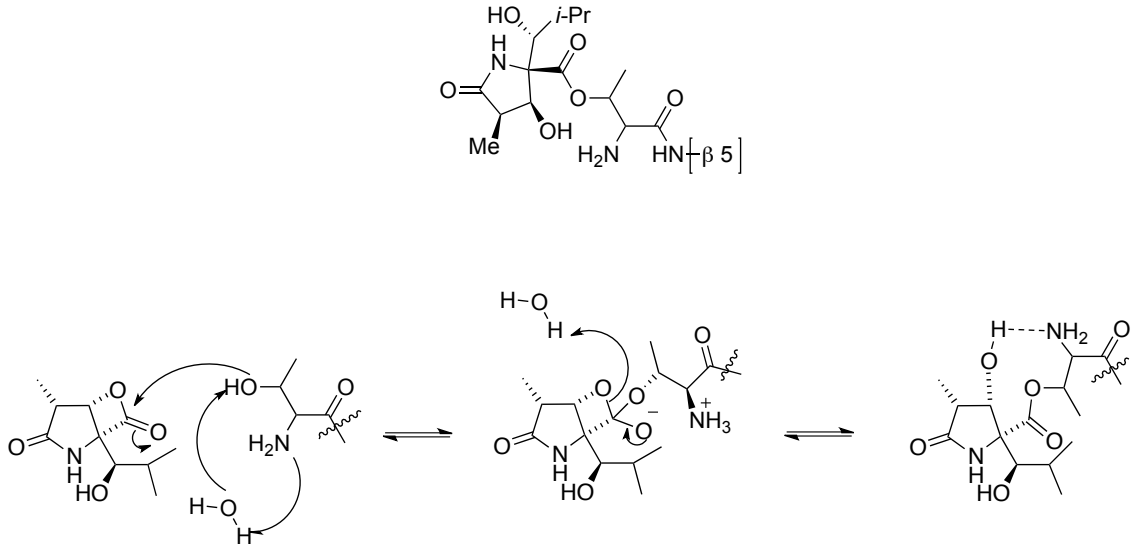



**Figure 4.** The ubiquitin proteasome pathway.[10]

Inhibition of the proteasome stops the process of protein degradation, thereby inducing apoptosis.[9] If cancer cells can be specifically targeted for proteasome inhibition, these cells will undergo apoptosis leaving behind only healthy cells. Proteasome inhibition is a key strategy for anti-cancer therapy and is of great interest in current research.

Lactacystin, or rather the β-lactone, acts by acylating the amino terminal threonine residue of one of the β-type protein subunits of the 20S proteasome.[8] The hydroxyl group on the threonine attacks the carbonyl moiety of the lactone, resulting in ring opening and the formation of an ester linkage between the proteasome and lactacystin. Hydrogen bonding can also be observed between the hydroxyl group formed from the lactone ring opening and the amine of the threonine residue, **Scheme 2**.



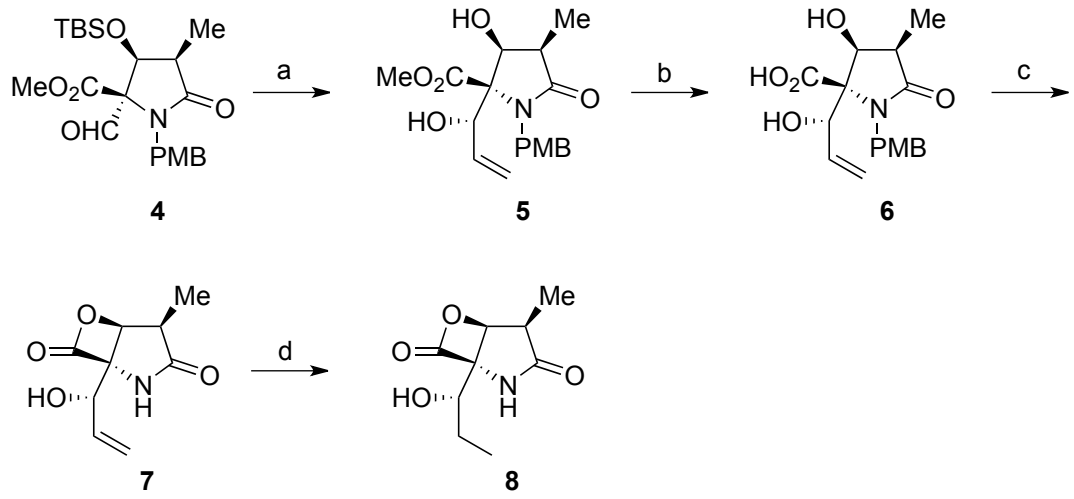
**Scheme 2.** Deactivation of the 20S proteasome by acylation of a terminal threonine residue.[8]

## 1.1.2 Structure Activity Relationship (SAR) Studies

Although early work had touched on the importance of structure and stereochemistry in the biological activity of lactacystin, it was Corey who in 1999 completed the most comprehensive SAR study to date.[11]

The relative stereochemistry at the C5 and C6 positions are important for the formation of the β-lactone. The C5 hydroxyisobutyl group and the stereochemistry of the hydroxyl group at the C9 position are important for binding in the hydrophobic pocket of the lactacystin-proteasome complex. This was shown using X-ray crystallography by Groll *et al*.[7] As stated above, the NAC moiety is not important to the activity of lactacystin.

In 1998 the Corey group designed a new enantioselective synthesis that would allow functionalization of an advanced intermediate enabling the generation of a variety of analogues.[12] They first studied the effect of replacing the isopropyl moiety at C9 with other lipophilic groups, such as ethyl (**Scheme 3**) and propyl (**Scheme 4**), while leaving the hydroxyl group also at C9 in place.

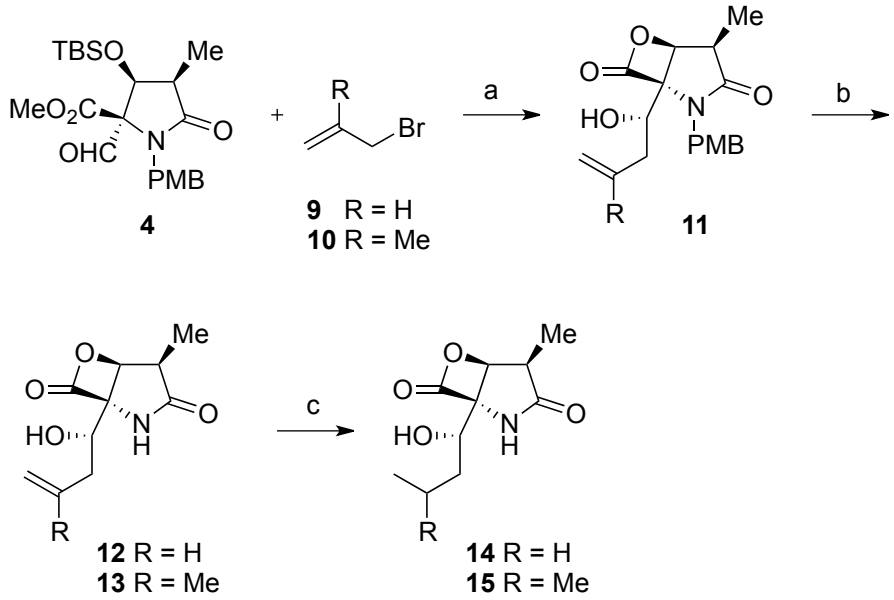



**Reagents and Conditions**: a) 1. Vinylmagnesium bromide, TMSCl, THF, –40 °C; 2. TFA/$H_2O$ (4:1), 50 °C, 4 h; b) LiOH, THF/$H_2O$ (1:1). 23 °C, 0.5 h; c) 1. BOPCl, $Et_3N$, $CH_2Cl_2$, 23 °C, 0.5 h; 2. CAN, $CH_3CN/H_2O$ (3:1), 23 °C, 1 h, 35% (5 steps); d) $H_2$, Pd/C (10%), EtOH, 2 h, >99%.

**Scheme 3.**

The advanced intermediate **4** was prepared in 6 steps from dimethyl methylmalonate.[12] A Grignard addition onto **4** in tetrahydrofuran in the presence of trimethylsilyl chloride followed by desilylation afforded the allylic alcohol **5**. Saponification of the methyl ester to form the dihydroxy acid followed by β-lactonization using bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl) and triethylamine and removal of the *p*-methoxybenzyl group using ceric ammonium nitrate (CAN) led to the formation of β-lactone **7** in 35% yield over 5 steps. The hydrogenation of **7** afforded the corresponding C9-ethyl analogue **8** in >99% yield.

The synthesis of analogues **14** and **15** follows a similar route (**Scheme 4**): a chromium-catalysed addition of allyl and methylallyl bromide to **4**, followed again by a saponification reaction, β-lactonization and *p*-methoxybenzyl group removal, resulted in compounds **12** and **13**, respectively. The catalytic hydrogenation of **12** and **13** afforded the corresponding omuralide analogues, **14** and **15**.



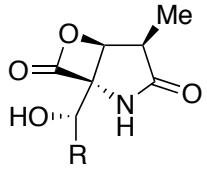

**Reagents and Conditions**: a) 1. $CrCl_3$(cat.), Mn(0), TMSCl, THF, 23 °C, 12 h, 50%; 2. 5% HF/$CH_3CN$, 23 °C, 4 h, 96%; 3. LiOH, THF/$H_2O$ (1:1). 23 °C, 2 h, 93%; 4. BOPCl, $Et_3N$, $CH_2Cl_2$, 23 °C, 1 h, 95%; b) CAN, $CH_3CN/H_2O$ (3:1), 23 °C, 1 h, 65%; c) $H_2$, Pd/C (10%), EtOH, 23 °C, 2 h, 99%.

**Scheme 4.**

The synthesis of analogue **16** (R=H, **Table 1**) was completed by reduction of the aldehyde group of **4** to the corresponding alcohol followed by saponification, β-lactonization and *p*-methoxybenzyl group removal as described above. The C9-phenyl analogue **17** was prepared in a route analogous to the one used to prepare **7** and **8** using a Grignard addition to introduce the desired functionality.

In the studies below (**Tables 1-3**),[11] the different analogues' ability to inhibit the proteasome was tested by measuring the rates of inhibition of chymotrypsin-like peptidase activity using purified 20S proteasome from a bovine brain. Investigations into the effect of functionalization at C9 (**Table 1**) supported Schrieber's early work. Even subtle changes to the group, using a slightly larger or smaller group than the original isopropyl, resulted in great loss of activity. When replaced with a much smaller group, for example just a proton (**16**), the activity was dramatically reduced. When replaced with a much larger group, as in the case of the C9-phenyl analogue **17**, a complete lack of inhibition was observed.

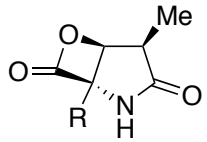
**Table 1.** Kinetics of inhibition by C9 functionalized β-lactone analogues of lactacystin.



| Compound | Analogue Structure | $k_{assoc}$ ($M^{-1}s^{-1}$) |
|---|---|---|
| **2** | R = $CH(CH_3)_2$ | 3059 ± 478 |
| **7** | R = $CH{=}CH_2$ | 188 ± 11 |
| **8** | R = $C_2H_5$ | 290 ± 12 |
| **16** | R = H | 9.7 ± 6.2 |
| **17** | R = $C_6H_5$ | No inhibition |
| **12** | R = $CH_2CH{=}CH_2$ | 255 ± 40 |
| **13** | R = $CH_2C(CH_3){=}CH_2$ | 64.7 ± 2.2 |
| **14** | R = $CH_2CH_2CH_3$ | 192 ± 35 |
| **15** | R = $CH_2CH(CH_3)_2$ | 17.4 ± 2.4 |

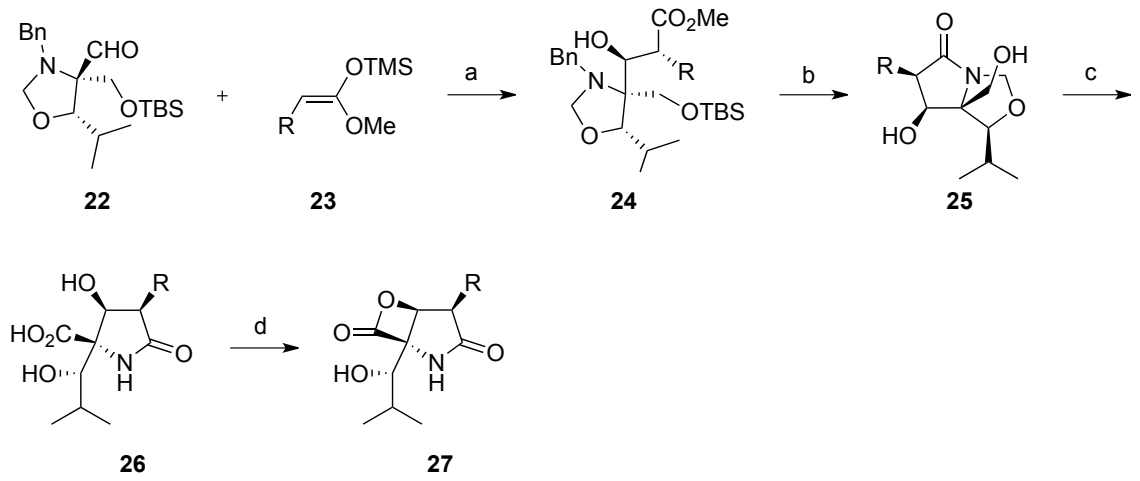
The effect of other substituents at the C5 position was also studied (**Table 2**). With a ketone in place of the C9 hydroxyl moiety in lactacystin, a complete lack of inhibition was observed. The importance of the stereochemistry at the C9 position was also confirmed: the activity is dramatically reduced when the stereochemistry is inverted. Interestingly, in the case of both C9 and C5 functionalization, the inhibition was at its highest when lactacystin was used, and no modification led to any improvement.

**Table 2.** Kinetics of inhibition by C5 functionalized β-lactone analogues of lactacystin.



| Compound | Analogue Structure | $k_{assoc}$ ($M^{-1}s^{-1}$) |
|---|---|---|
| **2** | R =  | 3059 ± 478 |
| **18** | R = $C(O)CH(CH_3)_2$ | No inhibition |
| **19** | R =  | 65 ± 9.6 |
| **20** | R = $CH_2CH(CH_3)_2$ | 235 ± 16 |
| **21** | R = $CH{=}C(CH_3)_2$ | 98 ± 5 |

The importance of the C7 substituent was also investigated in relation to proteasome inhibition (**Table 3**). **Scheme 5** shows the synthetic route to a variety of analogues where functionalization was achieved by the early addition of different alkenes. Reaction of the alkene with the aldehyde of **22** using a magnesium-catalysed *anti*-aldol approach gave **24**.[13] Hydrogenation of **24** followed by cyclization and deprotection of the primary alcohol gave the bicyclic compound **25**. The dihydroxy acid **26** was prepared over three steps and then converted into the corresponding β-lactone **27** using BOPCl. Again, the ability to inhibit the proteasome was tested by measuring the rates of inhibition of chymotrypsin-like peptidase activity.

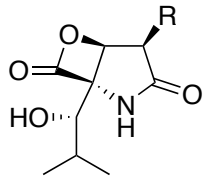



**Reagents and Conditions**: a) 1. $MgI_2$, $CH_2Cl_2$, –20 °C – 0 °C; 2. $K_2CO_3$, MeOH, 23 °C, 75-80% (2 steps); b) 1. $H_2$, Pd/C (10%), EtOH, $(i\text{-Pr})_2NEt$ (cat.), 23 °C, 30 h; 2. MeOH, 55 °C, 20 h; 3. 5% HF/$CH_3CN$, 23 °C, 24 h, 76-84% (3 steps); c) 1. DMSO, $(COCl)_2$, $Et_3N$, $CH_2Cl_2$, –78 °C; 2. $NaClO_2$, $NaH_2PO_4$, *t*-BuOH/2-Me-2-butene, 23 °C, 0.5 h; 3. $HS(CH_2)_3SH$, 2% HCl (g), $CF_3CH_2OH$, 50 °C, 7 h, 65-75% (3 steps); d) BOPCl, $Et_3N$, $CH_2Cl_2$, 23 °C, >90%.

**Scheme 5.**

In contrast with the already optimized C5 and C9 substituents, variations at the C7 group have yielded compounds with increased activity. When the methyl group was replaced with a proton (compound **29**), the activity dropped dramatically. However, when three of the analogues where the substituent was larger than a methyl group were tested, the inhibitory activity more than doubled. Interestingly, the C7-ethyl substituent (compound **28**) is found in salinosporamide B, an analogue of another naturally occurring proteasome inhibitor that shares the same core structure and mode of action as lactacystin.

**Table 3.** Kinetics of inhibition by C7 functionalized β-lactone analogues of lactacystin.



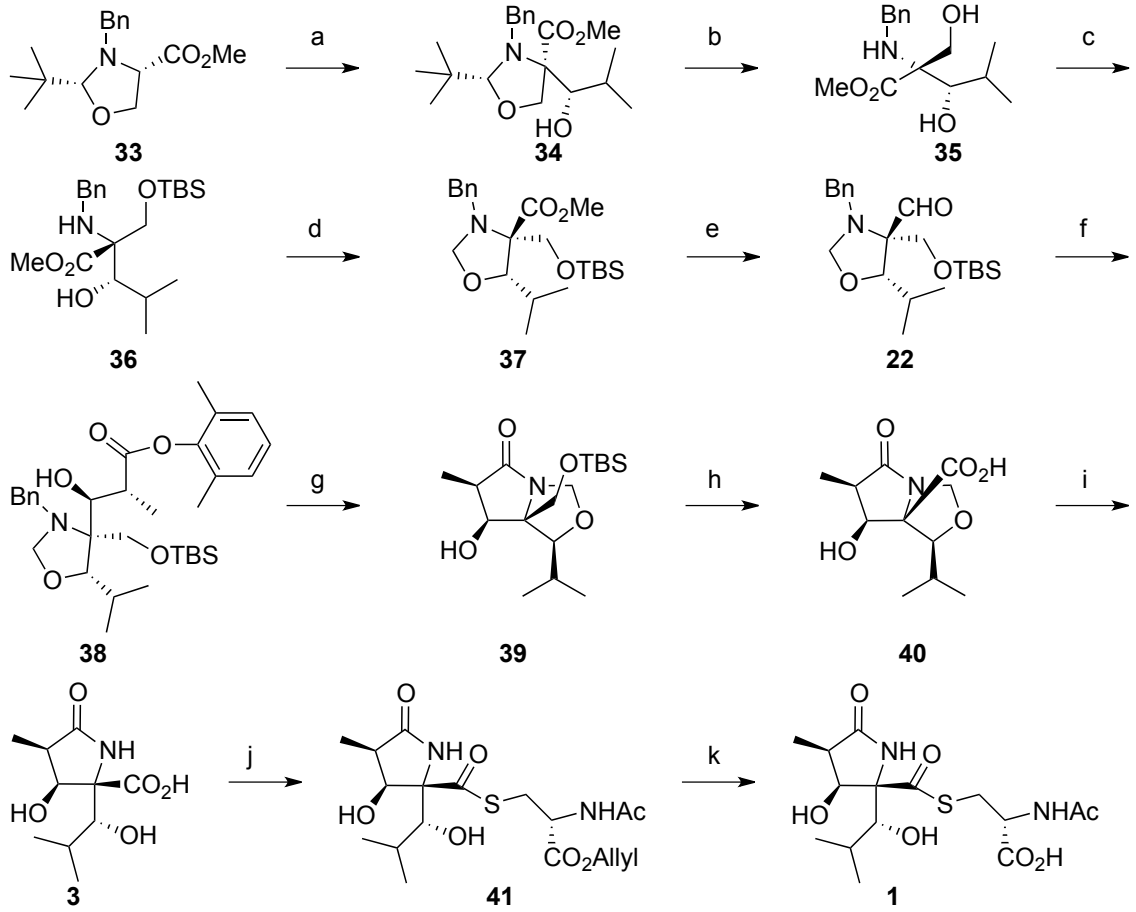

| Compound | Analogue Structure | $k_{assoc}$ ($M^{-1}s^{-1}$) |
|---|---|---|
| **2** | R = $CH_3$ | 3059 ± 478 |
| **28** | R = $CH_2CH_3$ | 6679 ± 553 |
| **29** | R = H | 450 ± 77 |
| **30** | R = $CH_2C_6H_5$ | 2227 ± 180 |
| **31** | R = $(CH_2)_3CH_3$ | 7275 ± 466 |
| **32** | R = $CH(CH_3)_2$ | 8465 ± 1572 |

In conclusion, it is clear to see that Nature has nearly optimized lactacystin's structure for proteasome inhibition. The combined studies into the functionalization of the C5 and C9 positions clearly demonstrate this. There is however scope for improvement at the C7 position for a novel proteasome inhibitor to be designed to achieve increased activity.

## 1.1.3 Previous Syntheses of Lactacystin

### 1.1.3.1 The Corey Syntheses

Corey and Reichard reported the first total synthesis of lactacystin in 1992.[14] Lactacystin was successfully synthesized in 6% yield over 13 steps from **33** (**Scheme 6**).




**Reagents and Conditions**: a) LDA, LiBr, *iso*-butyraldehyde, THF, –78 °C, 51%; b) MeOH, TfOH, 80 °C, 91%; c) TBSCl, imidazole, DMF, 23 °C, 97%; d) TsOH, $(CH_2O)_n$, benzene, 96%; e) 1. $LiBH_4$/THF, MeOH, 23 °C; 2. DMSO, $(COCl)_2$, $Et_3N$, $CH_2Cl_2$, –78 °C, 85%; f) LDA, 2,6-dimethylphenylpropionate, THF, –78 °C, 48%; g) $H_2$, Pd/C, EtOH, 23 °C, 87%; h) 1. 5% HF/$CH_3CN$, 23 °C, 90%; 2. DMSO, $(COCl)_2$, $Et_3N$, $CH_2Cl_2$, –78 °C, 73%; 3. $NaClO_2$ $NaH_2PO_4$, *t*-BuOH, 2-methyl-2-butene, 23 °C, >95%; i) 1,3-propanedithiol, 2% HCl/$CF_3CH_2OH$, 50 °C, >95%; j) *N*-acetylcysteine allyl ester, BOPCl, $Et_3N$, $CH_2Cl_2$, 23 °C, 79%; k) $Pd(Ph_3P)_4$, $HCO_2H$, $Et_3N$, THF, 23 °C, 84%.

**Scheme 6.**

To begin, *N*-benzylserine methyl ester was converted into the *cis*-oxazolidine derivative **33**, which was isolated as a mixture of diastereoisomers in a 9:1 ratio. An aldol condensation of **33** with *iso*-butyraldehyde gave the oxazolidine **34** in excellent diastereoisomeric purity (>98%) after recrystallization from pentane. Lithium bromide was found to be essential as low yields and poor selectivity were observed in its absence. The oxazolidine ring-opening, followed by the silyl protection of the primary alcohol, gave compound **36**. The formation of a new oxazolidine **37** proceeded using formaldehyde in 96% yield. Reduction of the ester moiety followed by a re-oxidation gave aldehyde **22**. The aldol condensation with 2,6-dimethylphenylpropionate under Pirrung-Heathcock *anti*-aldol conditions[15] resulted in the formation of oxazolidine **38** as the major diastereoisomer. The bicyclic lactam **39** was obtained by catalytic hydrogenation of **38**. Desilylation followed by the oxidation of the primary alcohol gave the acid **40**. The acid-catalysed transfer of methylene to 1,3-propanedithiol was used to ring-open the oxazolidine resulting in the dihydroxy acid **3** in 95% yield. The final steps included a reaction with *N*-acetylcysteine to introduce the NAC side chain; deallylation of **41** afforded lactacystin.

The attractive feature in this total synthesis, apart from a good overall yield, is how few purification steps requiring chromatography are needed. However, as mentioned above, the main drawback of this synthesis was the poor selectivity of the aldol condensation of **22**. During the transition state of the reaction of aldehyde **22** with 2,6-dimethylphenylpropionate under Pirrung-Heathcock *anti*-aldol conditions, the benzyl and isopropyl groups of the oxazoline ring are *cis* to each other. The *re* and *si* faces are not sterically different enough to induce a stereoselective attack of the aldehyde carbonyl moiety (**Figure 5**).[16]

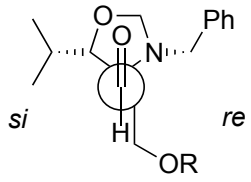
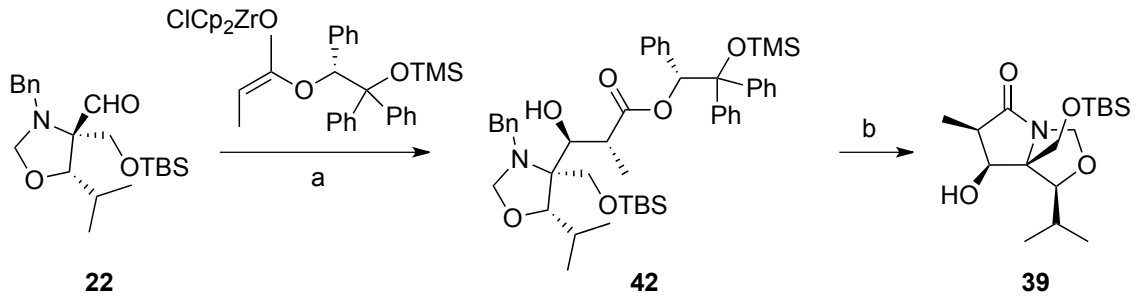



**Figure 5.**

Investigations were then carried out to improve the selectivity. Corey used the methodology developed by Braun that utilised a chiral zirconium enolate of 2-siloxy-1,2,2-triphenylpropionate.[17] When the (*R*)-zirconium enolate was used, aldehyde **22** was converted into four diastereoisomeric products in a 32:2:2:1 ratio. The required diastereoisomer for the natural product, with a (6*S*,7*R*) configuration, was isolated as the major diastereoisomer in 80% yield. Using the (*S*)-enantiomer of the zirconium enolate, attack occurred onto the *re* face giving the (6*R*,7*S*)-diastereoisomer as the major product. The hydrogenation of **42** followed by diazomethane treatment and, finally, cyclization in methanol gave the reported bicyclic lactam **39** (**Scheme 7**).[16]



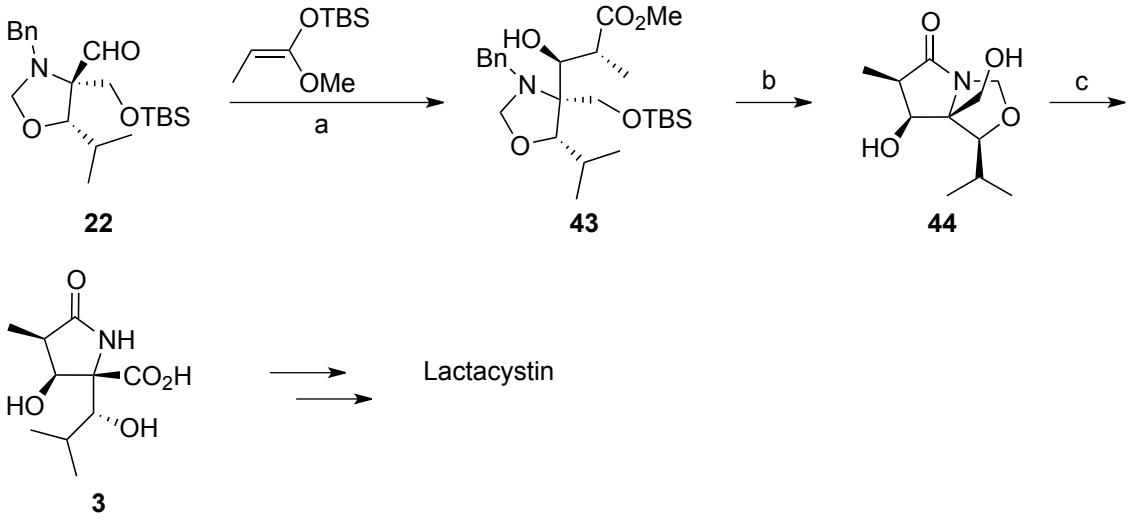

**Reagents and Conditions**: a) THF, –20 °C, 80%; b) 1. $H_2$, Pd/C; 2. $CH_2N_2$; 3. MeOH, 64%.

**Scheme 7.**

Although the selectivity was improved, long reaction times and the large excess of the zirconium enolate required were not desirable. Unhappy with these limitations, the Corey group made further improvement to the reaction, this time employing a magnesium-catalysed *anti*-aldol reaction.[13] The chelation of a metal to the nitrogen of the *N*-benzyl and the oxygen of the aldehyde enabled a Mukaiyama-type aldol process to occur.

The test reactions using the *t*-butyldimethylsilyl enol ether of ethyl acetate and various Lewis acids, such as $TiCl_4$, resulted in no aldol product and decomposition of the aldehyde. Reactions with bicoordinate Lewis acids and the *t*-butyldimethylsilyl enol ether of ethyl acetate afforded neither the aldol nor decomposition products. These results led the group to believe that the nitrogen of the *N*-benzyl was too sterically hindered to coordinate to the metal. However, $MgI_2$ was then tested and complete diastereoselectivity was observed. With optimized conditions in hand, the reaction was

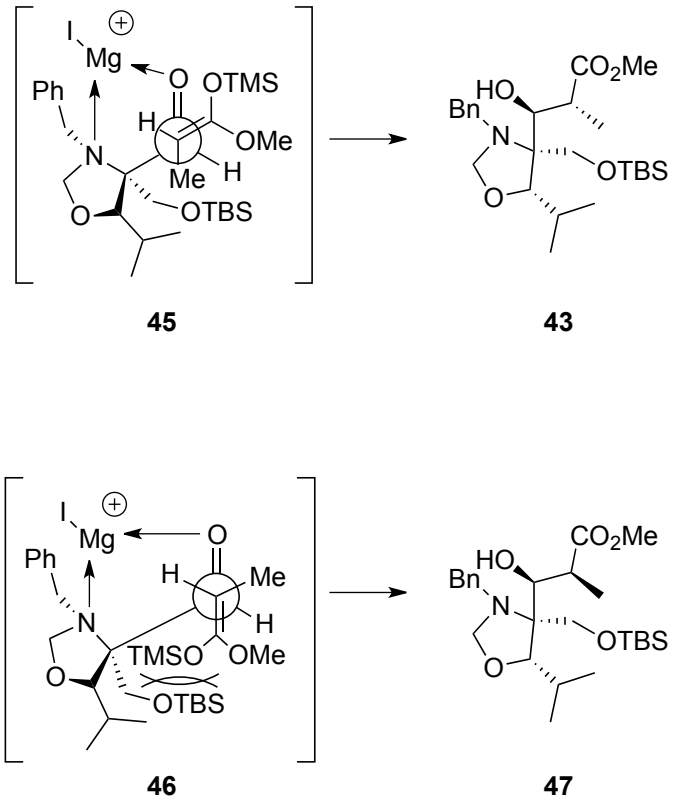
repeated using the (*E*)-trimethylsilyl enol ether of methyl propionate (**Scheme 8**). High *si* selectivity was observed, and the desired *anti* aldol product **43** was isolated from a 9:1 mixture of *anti*/*syn* diastereoisomers using silica gel chromatography. The dihydroxy acid **3** was obtained after oxidation of the primary alcohol followed by acid-catalysed transfer of methylene to 1,3-propanedithiol to ring-open the oxazolidine.




**Reagents and Conditions**: a) $MgI_2$, $CH_2Cl_2$, –20 °C; b) 1. $H_2$, Pd/C, EtOH, DIPEA, 23 °C, 30 h; 2. MeOH, 55 °C, 20 h; 3. 5% HF/$CH_3CN$, 23 °C, 24 h, 76%; c) 1. $Et_3N$, DMSO, $(COCl)_2$, $CH_2Cl_2$, –78 °C; 2. $NaClO_2$, $NaH_2PO_4$, *t*-BuOH, 23 °C; 3. 1,3-propanedithiol, HCl, $CF_3CH_2OH$, 55 °C, 8 h, 77%.

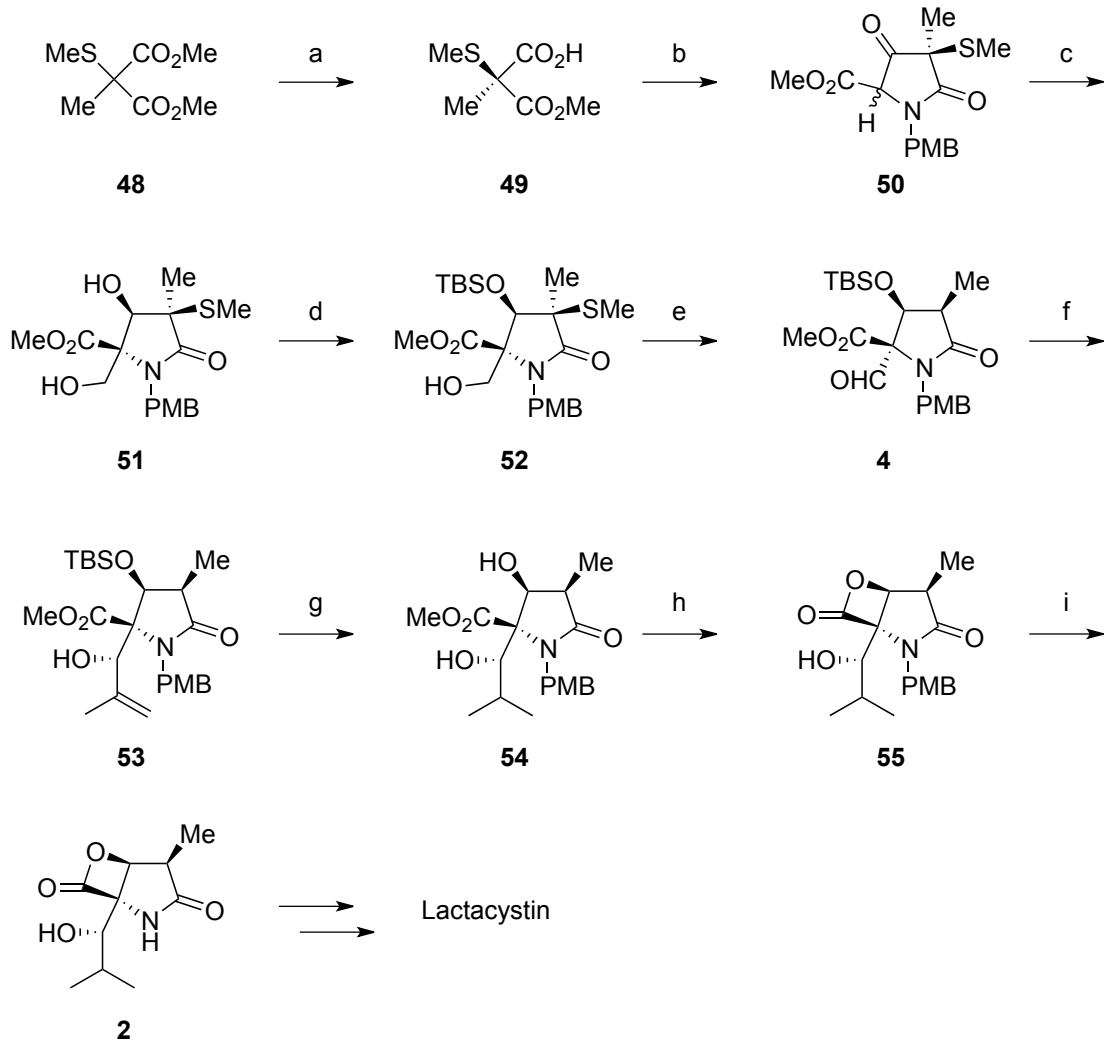
**Scheme 8.**

The selectivity observed could be explained by the low energy transition state that resulted from the chelation of $MgI^+$. There is much less steric repulsion in **45** compared to other geometries. For example, the antiperiplanar arrangement would show much higher steric repulsion (**Scheme 9**).[13]




**Scheme 9.**

In 1998, Corey and Nagamitsu reported a different approach to the total synthesis of lactacystin.[12] The new route was not only shorter but was most impressive due to the formation of an advanced intermediate that allowed ready access to various lactacystin analogues. The introduction of the isopropyl, or other lipophilic groups, could be carried out late in the synthesis. This route employs a blocking group at the C7 position; the group chosen was a thiomethyl group: importantly, it is not labile but it is also bulky enough to induce stereoselectivity in the hydroxymethylation of **50**.




**Reagents and Conditions**: a) 1. PLE, $H_2O$, pH 7.3, 23 °C, 24 h, 97%; 2. Recrystallization of the quinine salt, 62%, 95% *ee*; b) 1. $(COCl)_2$, DMF (cat.), 23 °C, 1 h; 2. PMB-$NHCH_2CO_2Me$, $Et_3N$, $CH_2Cl_2$, 23 °C, 1 h, 99%; 3. LDA, THF, –78 °C, 2 h, 93%; c) 1.DBU, THF, –78 °C, then aq. $CH_2O$, –78 °C, 0.5 h, 90%; 2. $NaBH(OAc)_3$, HOAc, 23 °C, 1 h, recrystallization, 95%, 99% *ee*; d) 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%; e) 1. Raney Ni, EtOH, 0 °C, 1 h, 82%; 2. Dess-Martin reagent, $CH_2Cl_2$, 23 °C, 1 h, 95%; f) $H_2C$=C(Me)MgBr, TMSCl, THF, –40 °C, 0.5 h, 97%; g) 1. $H_2$, Pd/C, EtOH, 23 °C, 12 h, 99%; 2. $CF_3CO_2H/H_2O$ (4:1), 50 °C, 4 h, 87%; h) 1. LiOH, THF/$H_2O$ (1:1), 23 °C, 0.5 h; 2. BOPCl, $Et_3N$, $CH_2Cl_2$, 23 °C, 0.5 h, 90%; i) $Ce^{IV}$, $CH_3CN/H_2O$ (3:1), 23 °C, 1 h, 62%.

**Scheme 10.**

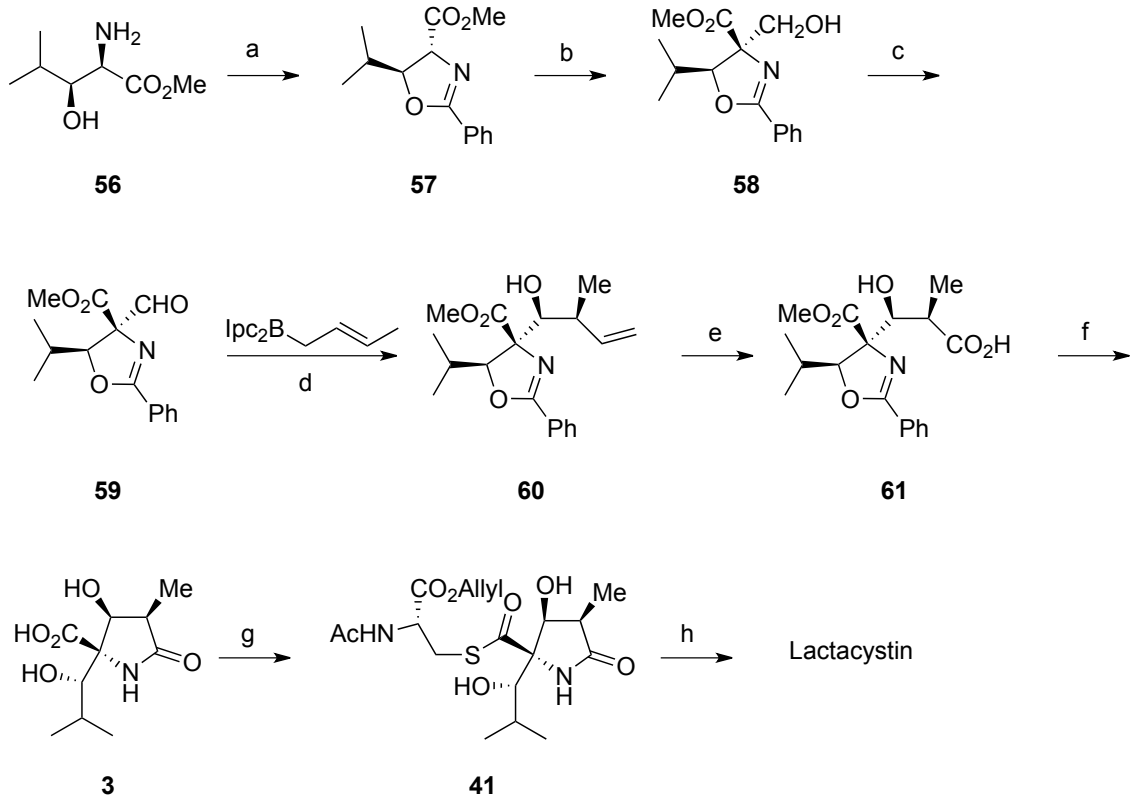
The achiral thiomethyl derivative **48** was synthesized using dimethyl methylmalonate with sodium hydride and MsCl in tetrahydrofuran. The compound **48** was then converted to the chiral acid **49** by enantioselective hydrolysis using porcine liver

esterase, and the acid **49** was obtained in 95% *ee* after recrystallization. The Dieckmann cyclization precursor was prepared after conversion of acid **49** to the corresponding acyl chloride using oxalyl chloride followed by coupling to *N*-*p*-methoxybenzylglycinate; treatment of the newly formed compound with LDA led to the formation of the γ-lactam product **50** as a 1:1 mixture of diastereoisomers at the C5 position. The hydroxymethylation of lactam **50** proceeded with high stereoselectivity (9:1) as hoped. A stereospecific ketone reduction gave the dihydroxy lactam **51** in 86% yield over the two steps. TBS protection of the secondary hydroxyl moiety was achieved by selective esterification of the primary hydroxyl group followed by silylation of the secondary hydroxyl moiety; the cleavage of the ester group was then performed to access the primary alcohol. The desulfurization of **52** was achieved in high stereoselectivity (10:1) by treatment with Raney nickel. A Dess-Martin periodinane oxidation gave aldehyde **4** in 78% yield over two steps from **52**.[18] A Grignard addition onto **4** in the presence of TMSCl afforded the desired product **53**. Use of TMSCl was vital in this reaction; its ability to trap the alkoxide ion prevented a rapid retro-aldol cleavage. The hydrogenation and desilylation of **53** followed by the saponification of the methyl ester resulted in the intermediate needed for the β-lactonization using BOPCl and triethylamine. Finally, the removal of the PMB protecting group using CAN gave omuralide **2**, which can easily be converted to lactacystin upon treatment with *N*-acetylcysteine and triethylamine.

With the advanced intermediate **4** in hand, various Grignard additions could be carried out to achieve a range of analogues. This is an efficient, stereocontrolled synthesis that is both relatively simple and economic in terms of reagents used.

### 1.1.3.2 The Ōmura Syntheses

Shortly after the first synthesis by Corey, the Ōmura group reported their route to lactacystin.[19] Using (2*R*,3*S*)-β-hydroxyleucine methyl ester **56** as the starting material, lactacystin was successfully synthesized in 13% yield over 10 steps (**Scheme 11**). The treatment of the methyl ester **56** with methyl benzimidate gave the *trans*-oxazoline **57**. Following Seebach's protocol,[20] an aldol condensation of **57** and formaldehyde was performed giving **58** in 98% de and 85% yield.




**Reagents and Conditions**: a) Ph(MeO)C=NH, 72%; b) LiHMDS, formaldehyde, 85%; c) Moffat oxidation; d) 1. (*E*)-crotyldiisopinocampheylborane, THF, –78 °C; 2. $OH^-$, $H_2O_2$, 70% (2 steps); e) 1. $O_3$, DMS; 2. $NaClO_2$, $NaH_2PO_4$, 56% (2 steps); f) 1. Pd, $HCO_2NH_4$; 2. 0.1 M NaOH, 82% (2 steps); g) BOPCl, $Et_3N$, *N*-acetyl-L-cysteine allyl ester, 79%; h) $(Ph_3P)_4Pd$, 81%.

**Scheme 11.**

The next step, oxidation of the primary alcohol, proved challenging. After unsuccessful attempts to obtain the aldehyde **59** using the Swern[21] and Parikh-Doering[22] oxidations, pyridinium chlorochromate (PCC),[23] and Dess-Martin periodinane,[18] the Moffat oxidation provided the desired derivative **59**.[24] A drawback to this methodology was a deformylation reaction occurring during aqueous work-up and silica gel chromatography; giving the *trans*-oxazoline **57**. To prevent the deformylation, the aldehyde was isolated with a non-aqueous work-up and no further purification.

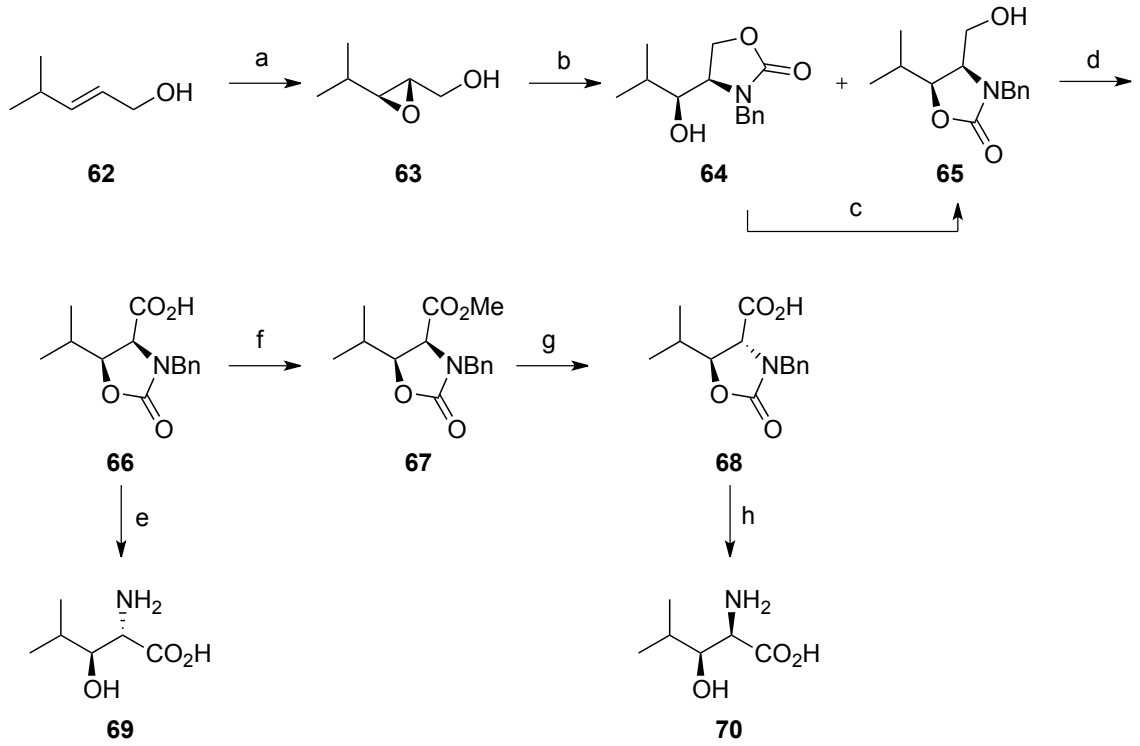
In 1986, Brown and co-workers described an allylboration methodology using (*E*)-crotyldiisopincampheylborane.[25] The high reactivity of organometallic reagents used in the allylation of aldehydes often results in poor levels of both regio- and stereoselectivity. Brown developed a new regio- and stereoselective preparation of optically active (*Z*)- and (*E*)-crotylboranes that was used in the allylation of aldehydes.

When the aldehyde **59** was subjected to the Brown conditions, the desired β-methyl homoallylic alcohol **60** was obtained as the major product alongside another diastereoisomer in 70% yield in a 4:1 ratio. Other methodologies were found to give lower selectivity. The use of the (*E*)-crotylchromium(II) reagent as described by Hiyama[26] and (*E*)-crotylpinacol borane as described by Roush[27] both gave the alcohol as a mixture of diastereoisomers in a 2:1 ratio. Roush was able to increase the ratio to 3:1 by using (*R,R*)-(*E*)-crotyl tartrate borane.[28] Conversion of the alkene to the carboxylic acid derivative **61** was achieved in one step using ruthenium(III) chloride and sodium periodate; however, low yields (11%) were obtained. The yield was improved using a two-step procedure consisting of the ozonolysis of **60**, with a reductive work-up using dimethylsulfide, followed by a selective oxidation, giving **61** in 56% yield.

Formation of the dihydroxy acid **3** proved difficult: both hydrogenolysis (using Pd/C, $Pd(OH)_2$, or palladium black with $H_2$ in methanol) and hydrolysis under acidic conditions (heating under reflux in 6 M HCl) were unsuccessful. Fortunately, catalytic transfer hydrogenation conditions using palladium black, ammonium formate and acetic acid under reflux followed, without purification, by ester hydrolysis using sodium hydroxide gave the dihydroxy acid **3** in 79% yield. The transformation of **3** to (+)-lactacystin was carried out as described by Corey.[14]

Continuing their investigations, the Ōmura group reported improvements to their original synthesis.[29] This work focused not only on the total synthesis but also on the analysis of the biological activity of lactacystin and analogues. Although improvements were made to the synthesis of the 3-hydroxyleucine starting material, the majority of the synthesis was unchanged.

Several approaches to making 3-hydroxyleucine have been reported, but the methods were not considered suitable as they either lacked generality, producing only one of the four possible diastereoisomers, or required a chiral non-racemic catalyst.[30] These limitations were undesirable as multigram quantities were needed for biological analysis.

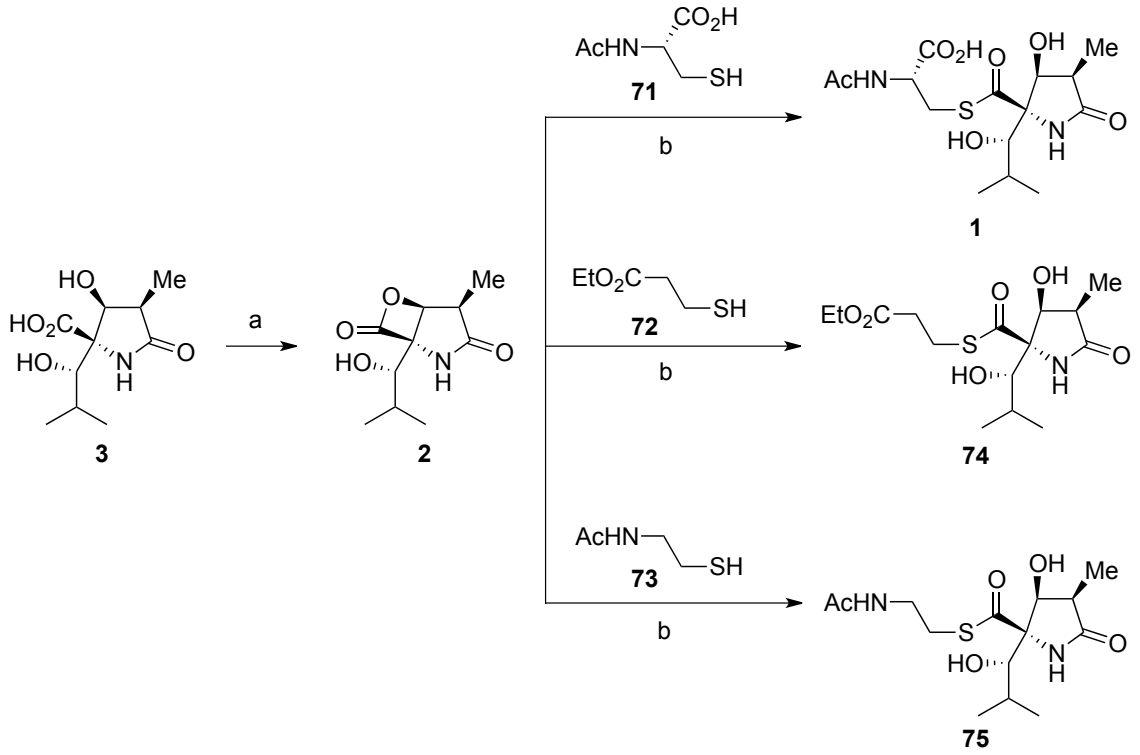



**Reagents and Conditions:** a) $PhC(Me)_2O_2H$, $Ti(O\text{-}i\text{-}Pr)_4$, (+)-DIPT, 82%; b) NaH, BnNCO, THF, reflux, 2 h; c) NaH, THF, reflux; d) Jones oxidation, 100%; e) 1. 2 M KOH; 2. $H_2$, $Pd(OH)_2$, 98% (2 steps); f) $CH_2N_2$, 87%; g) KOH, EtOH, 96%; h) 1. 2 M KOH; 2. $H_2$, $Pd(OH)_2$, 98% (2 steps).

**Scheme 12.**

The method developed by Ōmura was both simple and concise, using commercially available starting materials. This method also required little chromatography, as only **63** and **65** required purification. They employed a catalytic Sharpless epoxidation[31] to give **63**, a benzyl isocyanate-induced epoxide ring-opening to give **64**, and the epimerization of the oxazolidinone ester **67** as the key steps to (2*R*, 3*S*) 3-hydroxyleucine **70**.

With an improved synthesis of 3-hydroxyleucine available, the dihydroxy acid **3** was synthesized following the route described above. Lactacystin and two analogues were then prepared (**Scheme 13**).



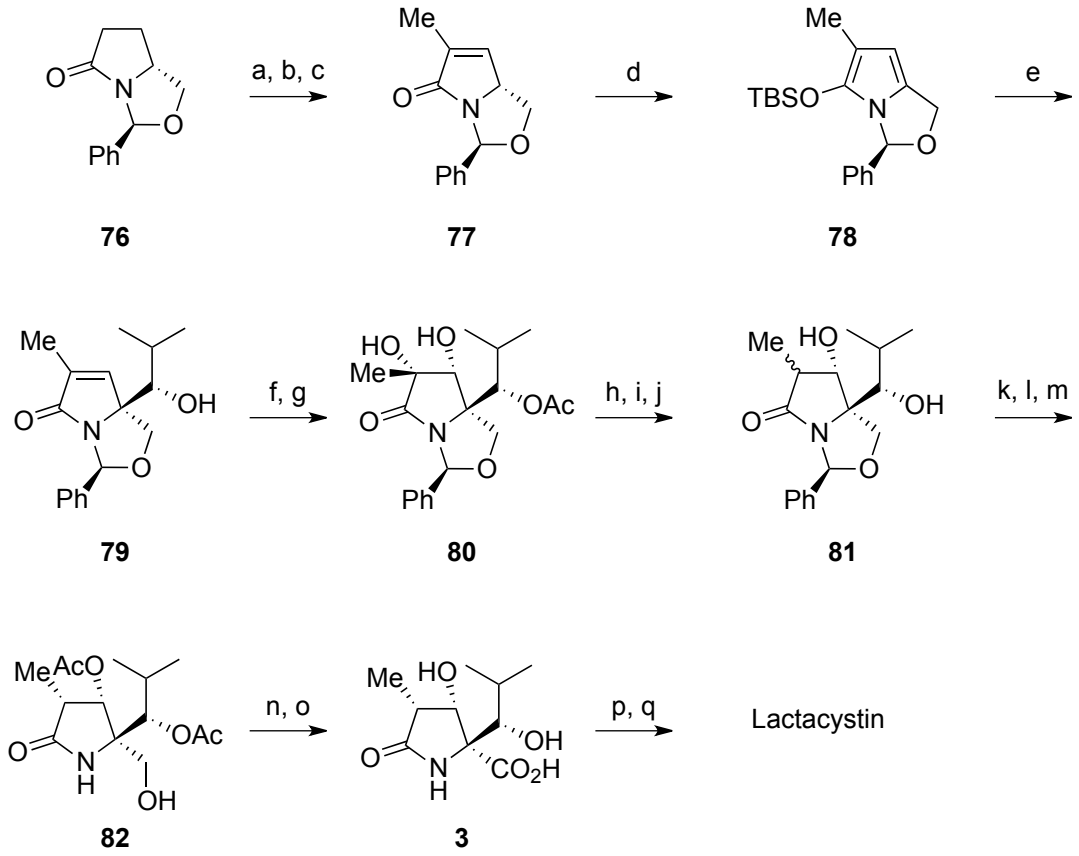

**Reagents and Conditions:** a) BOPCl, $Et_3N$, $CH_2Cl_2$, 85%; b) $Et_3N$, $CH_2Cl_2$, **1**: 64%, **74**: 89%, **75**: 77%.

**Scheme 13.**

Departing from Corey's route, an intramolecular coupling using BOPCl was performed on dihydroxy acid **3** in the presence of $Et_3N$ to form the β-lactone **2** in 85% yield. Interestingly, lactone **2** was converted into (+)-lactacystin using *N*-acetylcysteine, instead of *N*-acetyl-L-cysteine allyl ester, eliminating the need for a deprotection step. Using the same procedure, the novel lactacystin analogues **74** and **75** were prepared from the condensation of lactone **2** with thiols **72** and **73**, respectively.

### 1.1.3.3 The Baldwin Synthesis

While the Corey and Ōmura groups were developing their synthetic routes, Baldwin and co-workers were also investigating a different approach, **Scheme 14**.[32]




**Reagents and Conditions:** a) LDA, MeI, THF, –78 °C, 95%; b) LDA, PhSeBr, THF, –78 °C, 79%; c) $O_3$, $CH_2Cl_2$, –78 °C, pyridine, RT, 87%; d) TBSOTf, 2,6-lutidine, $CH_2Cl_2$, RT, 89%; e) *i*-PrCHO, $SnCl_4$, ether, –78 °C, 55%; f) $Ac_2O$, pyridine, RT, 99%; g) $OsO_4$, *N*-methylmorpholine *N*-oxide, aq. acetone, RT, 87%; h) *N,N'*-thiocarbonyldiimidazole, THF, RT, 91%; i) $Bu_3SnH$, AIBN, toluene, reflux, 94%; j) 2 M NaOH/MeOH (1:3), 0-3 °C, 94%; k) $H_2$, Pd/C, HCl, MeOH, RT, 87%; l) $Et_3SiCl$, pyridine, $Ac_2O$, RT, 89%; m) 40% HF, $CH_3CN$, RT, 91%; n) Jones' reagent, acetone, 0 °C to RT, 91%; o) 0.2 M NaOH, RT, quant.; p) (*R*)-*N*-acetylcysteine allyl ester, BOPCl, $Et_3N$, DCM, RT, 60%; q) $Pd(PPh_3)_4$, $HCO_2HNEt_3$, THF, RT, 88%.

**Scheme 14.**

Using (*R*)-glutamic acid as the starting material, the bicyclic oxazolidine **76** was prepared over three steps. A sequence of methylation followed by selenenylation and ozonolysis led to the unsaturated bicyclic oxazolidine derivative **77**. Treatment of **77** with TBSOTf and 2,6-lutidine gave the siloxypyrrole intermediate **78** as a crystalline solid in 89% yield. Aldol reaction of **78** and isobutyraldehyde resulted in the formation of the major isomer **79** and the corresponding secondary alcohol epimer in a 9:1 ratio. Acylation of the free hydroxyl group followed by dihydroxylation, using osmium tetroxide and *N*-methylmorpholine *N*-oxide, gave the diol **80** as a single diastereoisomer

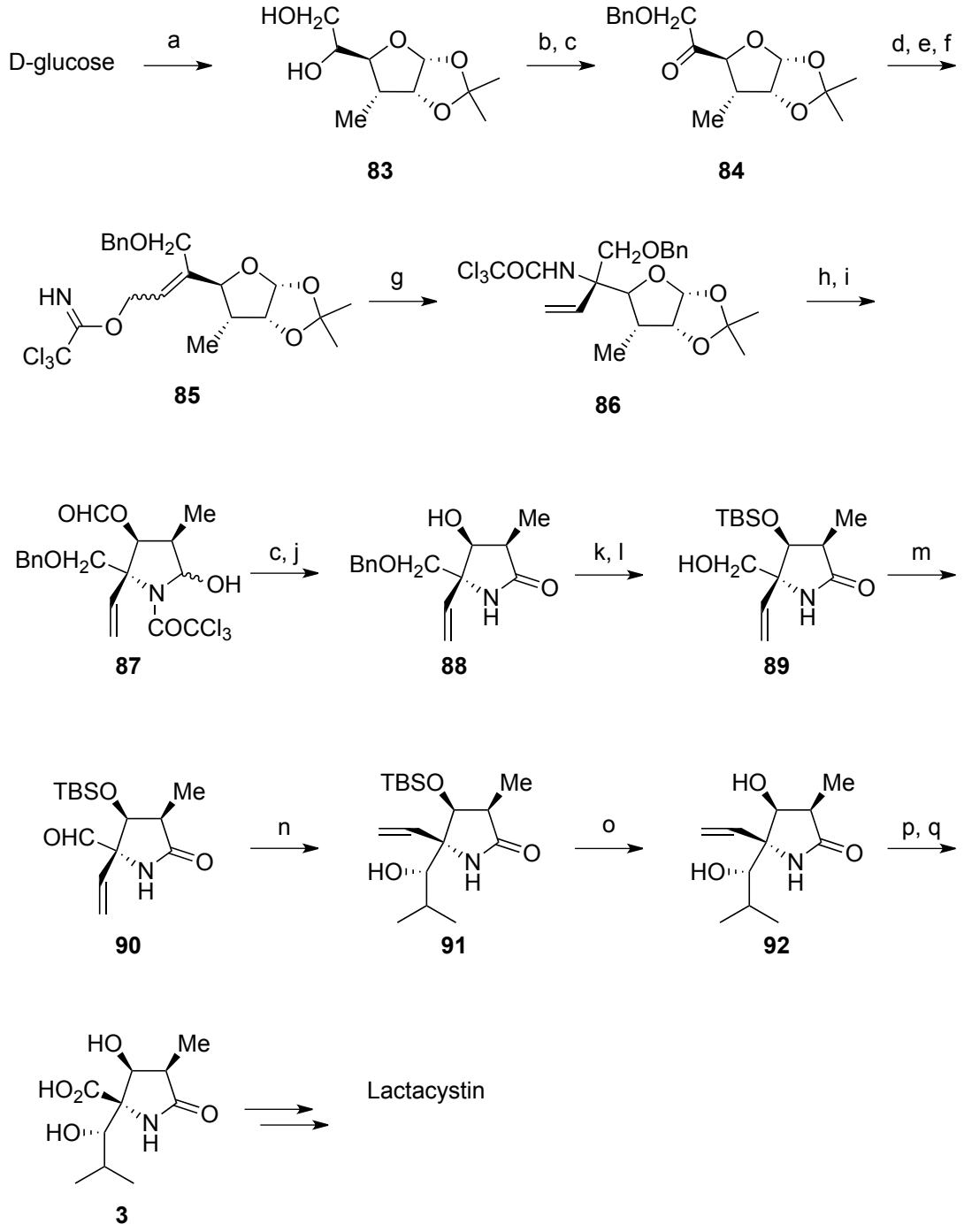
in 87% yield. Removal of the tertiary hydroxyl was achieved using the Barton-McCombie deoxygenation reaction through the formation of a thiocarbamate followed by radical decarboxylation using $Bu_3SnH$ and AIBN as the radical initiator. The method was not diastereoselective and resulted in a 1:1 mixture of epimers. Treatment of the diastereoisomeric mixture with 0.5 M NaOH in MeOH resulted in removal of the acetyl group and epimerization at the C6 position to give the desired *syn*-isomer **81** (*syn*:*anti*, 73:10).

Hydrogenolysis of **81** resulted in the cleavage of the oxazolidine moiety, giving the key lactam core of lactacystin. A sequence of selective protections and deprotections of the primary and secondary alcohol groups afforded primary alcohol **82**. Using an excess of the Jones' reagent, the primary alcohol was converted to the corresponding carboxylic acid. Finally, deprotection of the secondary alcohol groups gave the dihydroxy acid **3** in 91% yield. In conclusion, the Baldwin group successfully completed the synthesis of lactacystin from **76** in 17 steps and 8% overall yield.

### 1.1.3.4 The Chida Synthesis

In 1995, Chida *et al*. reported work on the total synthesis of (+)-lactacystin from D-glucose (**Scheme 15**).[33] 3-Deoxy-1,2-*O*-isopropylidene-3-*C*-methyl-α-D-allofuranose **83** was chosen as the starting material. It is a known compound prepared over four steps from diacetone-D-glucose.[34]

Treatment of **83** with dibutyltin oxide and benzyl bromide followed by oxidation of the secondary alcohol using Jones' reagent led to the formation of **84**. A Wittig reaction was used on **84** to give the corresponding alkene as a 1:1 mixture of diastereoisomers. The diastereoisomeric mixture was subjected to a DIBAL reduction to form the allylic alcohol in 90% yield. Treatment of the allylic alcohol with trichloroacetonitrile and sodium hydride in diethyl ether afforded the trichloroacetimidate **85**, which was, without purification, heated in toluene at 150 °C for 89 h. Overman rearrangement[35] led to the formation of product **86** as an inseparable mixture of diastereoisomers in a 4.8:1 ratio.



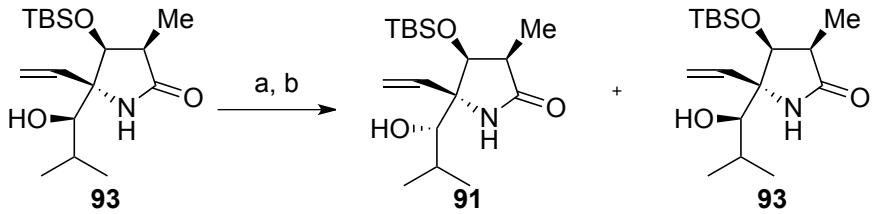

**Reagents and Conditions:** a) See ref. 34; b) $Bu_2SNO$, toluene, reflux, then BnBr, CsF, DMF, RT; c) Jones' reagent ($CrO_3$ in dil. $H_2SO_4$) acetone, 0 °C; d) $Ph_3P{=}CHCO_2Et$, toluene, 60 °C; e) DIBAL-H, $CH_2Cl_2$, –15 °C; f) trichloroacetonitrile, NaH (60 mol%), $Et_2O$, RT; g) toluene, heat at 150 °C (in a sealed tube), 89 h; h) TFA/$H_2O$ (3:2), 0 °C; i) $NaIO_4$, MeOH/$H_2O$ (1:1), RT; j) $NaBH_4$, MeOH, 0 °C; k) *tert*-butyldimethylsilyl trifluoromethanesulfonate, 2,6-lutidine, $CH_2Cl_2$, RT; l) Na, Liq. $NH_3$-THF, –78 °C; m) $Me_2SO$, DCC, TFA, pyridine, benzene, RT; n) *i*PrMgBr, THF –20 °C to RT; o) TFA/$H_2O$ (4:1), 50 °C; p) $O_3$, $CH_2Cl_2$, –78 °C, then $Me_2S$; q) $NaClO_2$, $NaH_2PO_4$, $HOSO_2NH_2$, *tert*-butanol/ $H_2O$, RT.

**Scheme 15.**

Hydrolysis under acidic conditions of the diastereoisomeric mixture resulted in the corresponding lactol in 72% yield after silica gel chromatography. A periodate oxidation led to oxidative cleavage followed by cyclization to form the pyrrolidine **87**. A Jones oxidation to form the corresponding lactam followed by a sodium borohydride reduction to remove both the *N*-trichloroacetyl and *O*-formyl protecting groups gave **88**.

Silyl protection of the secondary alcohol followed by debenzylation gave **89**. A Moffat oxidation afforded the aldehyde **90**, which was then used in the next step without purification. Grignard addition using isopropylmagnesium bromide in THF resulted in a mixture of diastereoisomers at the C9 position. The desired diastereoisomer **91** was isolated in 35% yield. Deprotection of the secondary alcohol of **91** with aqueous trifluoroacetic acid gave **92**. Formation of the dihydroxy acid **3** was achieved by ozonolysis to form an aldehyde moiety followed by selective oxidation to give the corresponding carboxylic acid. The synthesis of (+)-lactacystin was then completed following Corey's methodology.[14]

The main drawback of this route was the poor selectivity of the Grignard addition onto aldehyde **90** leading to the formation of alcohol **91** in 35% yield. Indeed, the presence of unwanted diastereoisomers is not desirable in total synthesis. However, the oxidation of the unwanted diastereoisomer **93** followed by a reduction using triisobutylaluminium in $CH_2Cl_2$ at 0 °C gave the desired isomer **91** as the major product (70% yield) alongside **93** in 7% yield, **Scheme 16.**



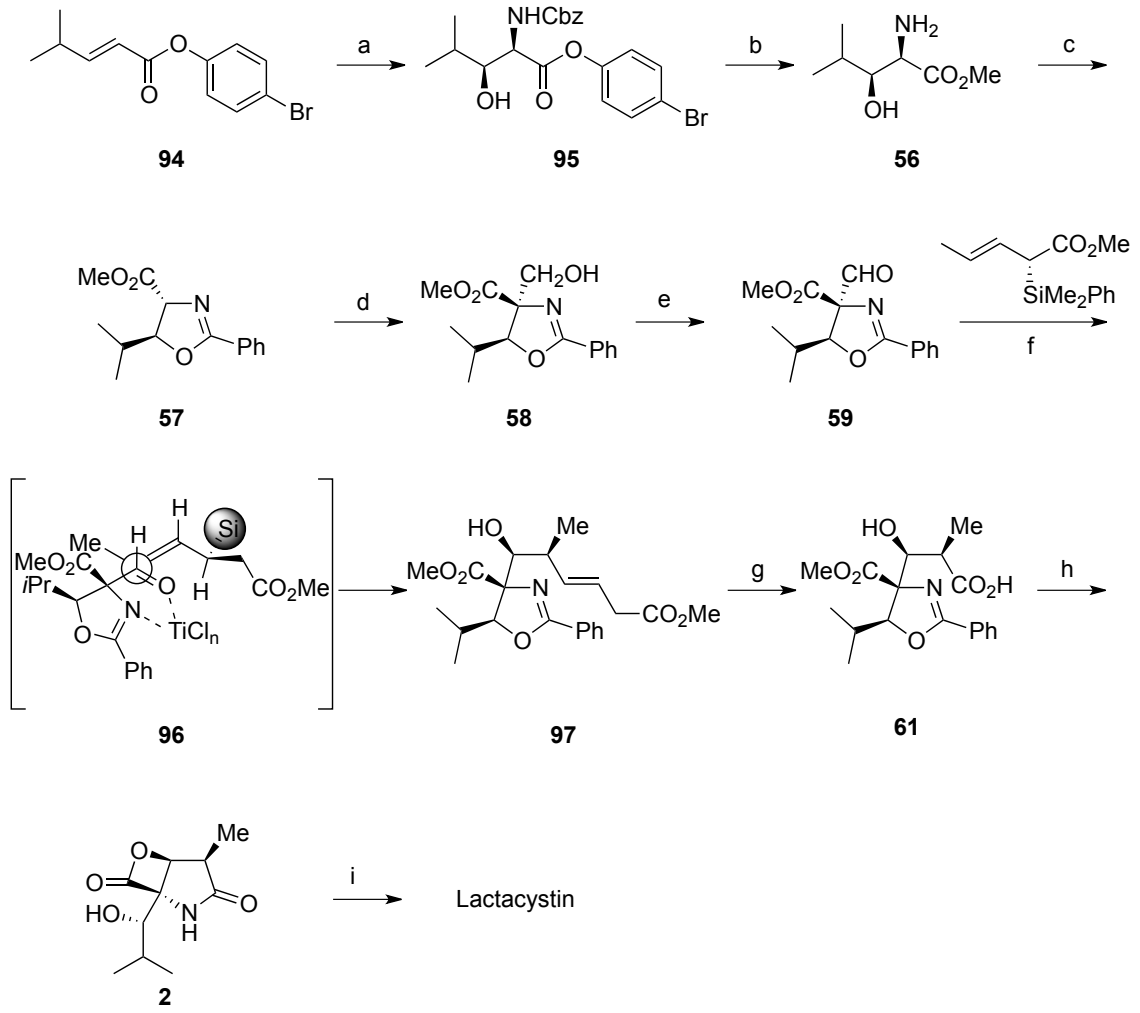

**Reagents and Conditions:** a)$Me_2SO$, DCC, TFA, pyridine, benzene, RT; b) triisobutylaluminium, $CH_2Cl_2$-hexane, 0 °C, **91**: 70%, **93**: 7%.

**Scheme 16.**

### 1.1.3.5 The Panek Synthesis

The Panek synthesis is similar to that of the Ōmura group.[36] Although both syntheses share the oxazoline **57** as a common intermediate, their approaches were slightly different. Panek and co-workers performed an asymmetric aminohydroxylation of (*p*-bromophenyl)-4-methyl-2-pentenoate, **94**, under Sharpless conditions followed by reaction with CbzNNaCl to give **95** in excellent *ee* (above 99%) after recrystallization. Transesterification to form the methyl ester followed by removal of the Cbz protecting group under catalytic hydrogenation conditions afforded **56**. The *trans*-oxazoline intermediate **57** was then formed upon treatment of **56** with trimethylorthobenzoate and *p*-toluenesulfonic acid. This is a much shorter and more efficient route to the *trans*-oxazoline **57** than that reported by Ōmura.[29] The next two steps to intermediate **59** were achieved as described above in **Scheme 11**.

Another key step of the Panek route is the *anti*-selective crotylation reaction of **59** in the presence of $TiCl_4$ to give **97** in high selectivity and yields of 50-60%. Ozonolysis of **97** in the presence of sodium chlorite led to the formation of known intermediate **61**, which allowed Panek and co-workers to complete the synthesis of lactacystin employing literature procedures.[12, 29]




**Reagents and Conditions:** a) $K_2[OsO_2(OH)_4]$ (5 mol%), $(DHQ)_2AQN$ (5 mol%), CbzNNaCl, *n*PrOH, $H_2O$, RT, 4 h, 60%; b) 1. $Ti(OiPr)_4$, MeOH, RT; 2. $H_2$, 10% Pd/C, MeOH, 100% (2 steps); c) $PhC(OMe)_3$, *p*-TsOH, DME, reflux, 4 h, 85%; d) LiHMDS, formaldehyde, 85%; e) Moffat [O]; f) $TiCl_4$, –78 °C to –35 °C, 60%; g) 1. $O_3$, DMS; 2. $NaClO_2$, $NaH_2PO_4$, 90% (2 steps); h)1. Pd, $HCO_2NH_4$; 2. 0.1 M NaOH, 82% (2 steps); 3. BOPCl, $Et_3N$, 80% (3 steps); i) *N*-acetyl-L-cysteine allyl ester, $Et_3N$, $CH_2Cl_2$, RT, 70%.

**Scheme 17.**

### 1.1.3.6 The Pattenden Synthesis

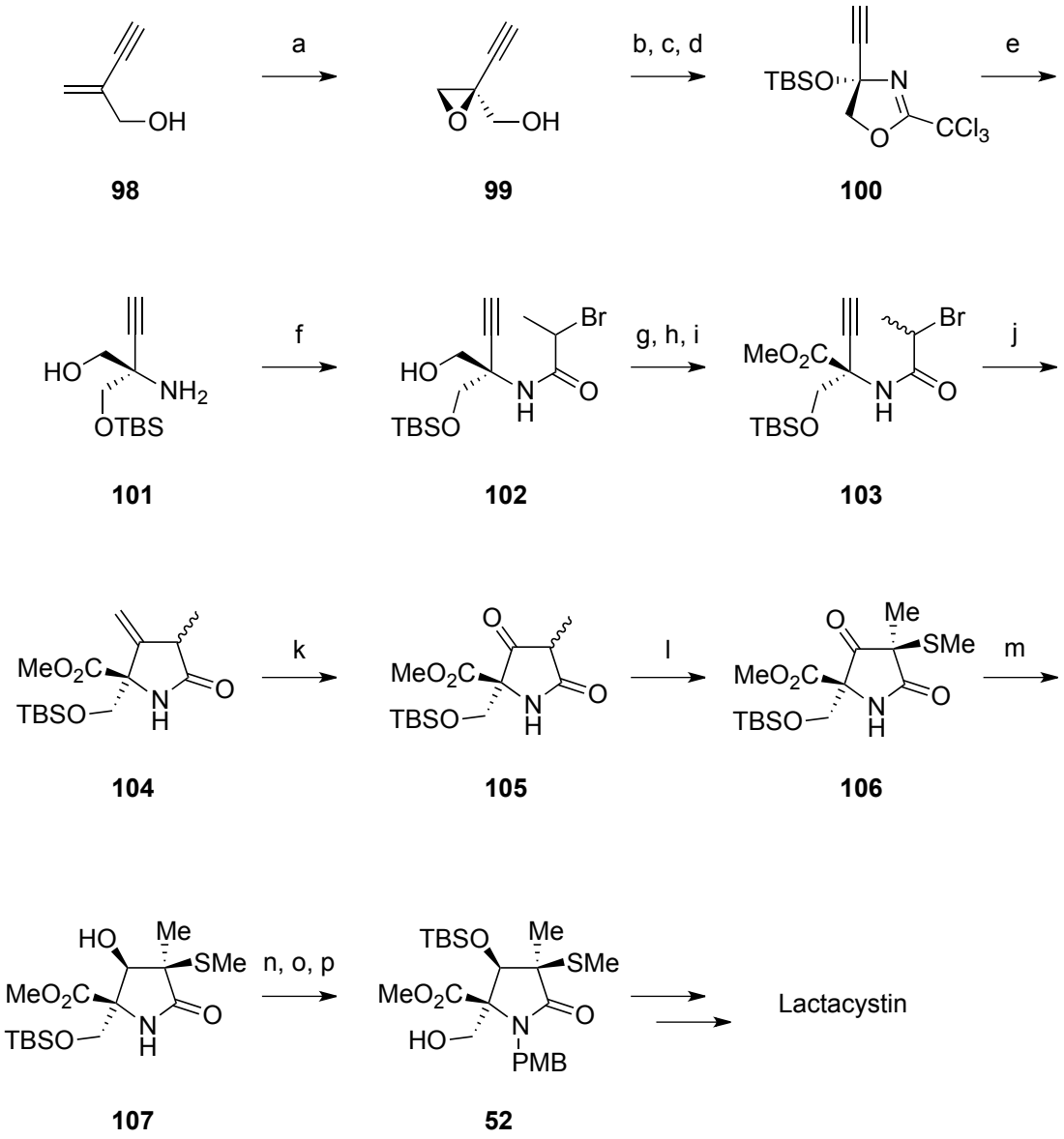
Pattenden *et al*. reported a different route to the total synthesis of lactacystin employing a radical-mediated approach to give the lactam core, **Scheme 18**.[37] 2-Ethynylpropenol was submitted to Sharpless epoxidation conditions,[31] and the corresponding chiral epoxide **99** was obtained in 66% yield and 86% ee. Conversion of the epoxide to the oxazoline followed by the protection of the primary alcohol using TBSOTf gave **100**, and its absolute stereochemistry was confirmed using X-ray crystallography. Treatment of **100** with 1 M hydrochloric acid resulted in the ring-opening of the oxazoline to give

the amino alcohol **101** as the hydrochloride salt. Without purification, **101** was reacted with 2-bromopropionyl chloride to give the amide **102** as a mixture of diastereoisomers in a 1:1 ratio. The primary alcohol moiety was then converted into the corresponding aldehyde using Dess-Martin periodinane followed by further oxidation to the carboxylic acid; finally, an esterification reaction using trimethylsilyldiazomethane gave methyl ester **103** in 62% yield over three steps.

The radical-mediated 5-*exo*-dig cyclization was achieved when the bromoamide **103** was treated with $Bu_3SnH$-AIBN in toluene under reflux.[38] The lactam **104** was isolated using silica gel chromatography in 60% yield as a mixture of epimers at the C7 position in a 2:1 ratio.

Ozonolysis of **104** with a reductive work-up using dimethyl sulfide gave **105** as a mixture of diastereoisomers in a 2:1 ratio. Unfortunately, attempts to epimerize the C7 centre were unsuccessful. The group then decided to employ a strategy reported by Corey that made use of a thiomethyl derivative.[12] Corey had already shown that Raney nickel could be used in desulfurization in a selective manner. The mixture of epimers was treated with *S*-methyl-*p*-toluenethiosulfonate to form the thiomethyl derivative **106** in high diastereoisomeric excess (87:13). The addition was determined to be *anti* to the bulky $CH_2OTBS$ group at the C5 position.

Diastereoselective ketone reduction of **106** was achieved using zinc borohydride, giving **107** in 79% yield. The TBS protection of the resulting secondary alcohol followed by *p*-methoxybenzyl protection and selective deprotection of the primary alcohol gave the advanced intermediate **52** reported by Corey.

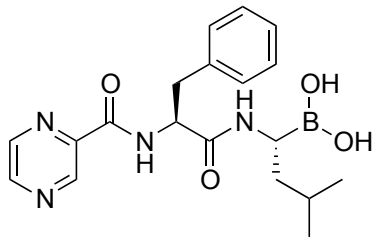



**Reagents and Conditions:** a) L-(+)-DIPT, $Ti(OiPr)_4$, cumene peroxide, $CH_2Cl_2$, –10 °C, 66%; b) $Cl_3CCN$, DBU, 0 °C, 66%; c) $Et_2AlCl$, $CH_2Cl_2$, 0 ° to RT, 78%; d) TBSOTf, 2,6-lutidine, $CH_2Cl_2$, 0 °C to RT, 97%; e) 1 M HCl, THF, RT; f) $CH_3CH(Br)COCl$, $NaHCO_3$, $CH_2Cl_2$, RT, 76% (2 steps); g) Dess-Martin periodinane, $CH_2Cl_2$, 0 °C; h) $NaClO_4$, $NaH_2PO_4$, *t*-BuOH, 2-methyl-2-butene, RT; i) $Me_3SiCHN_2$, MeOH-benzene, RT, 62%; j) $Bu_3SnH$, AIBN, toluene, reflux, 60%; k) $O_3$, MeOH, –78 °C, 15 min then $Me_2S$, –78 °C to RT, 75%; l) *p*-$MeC_6H_4SO_2Me$, $Et_3N$, $CH_2Cl_2$, RT, 78%; m) $Zn(BH_4)_2$, (4.4 M in THF), 0 °C, 79%; n) TBSOTf, 2,6-lutidine, $CH_2Cl_2$, 0 °C to RT, 80%; o) PMBBr, NaH, DMF, 0 °C to RT, 73%; p) HF-pyridine, THF, RT to 40 °C, 89%.

**Scheme 18.**

## 1.2 Other Proteasome Inhibitors

There are many classes of proteasome inhibitors; these include, but are not limited to, flavonoids, triterpenoids, β-lactones, peptides boronates and syrbactins.[39] Bortezomib, a peptide boronate, was the first proteasome inhibitor to be used in clinical trials for the treatment of multiple myeloma and is now fully approved by the Food and Drug Administration (FDA) in the USA and the European Medicines Agency (EMA). Bortezomib inhibits the chymotrypsin-like activity of the 26S proteasome and binds covalently and reversibly to the β5 residue in the core 20S proteasome.[40]




**108**

**Figure 6.** Bortezomib

Lactacystin is one of the β-lactone class of inhibitors (**Figure 7**). The inhibitors of this class also share other structural features similar to those of lactacystin that have shown to be important in SAR studies of lactacystin's ability to inhibit the 20S proteasome.

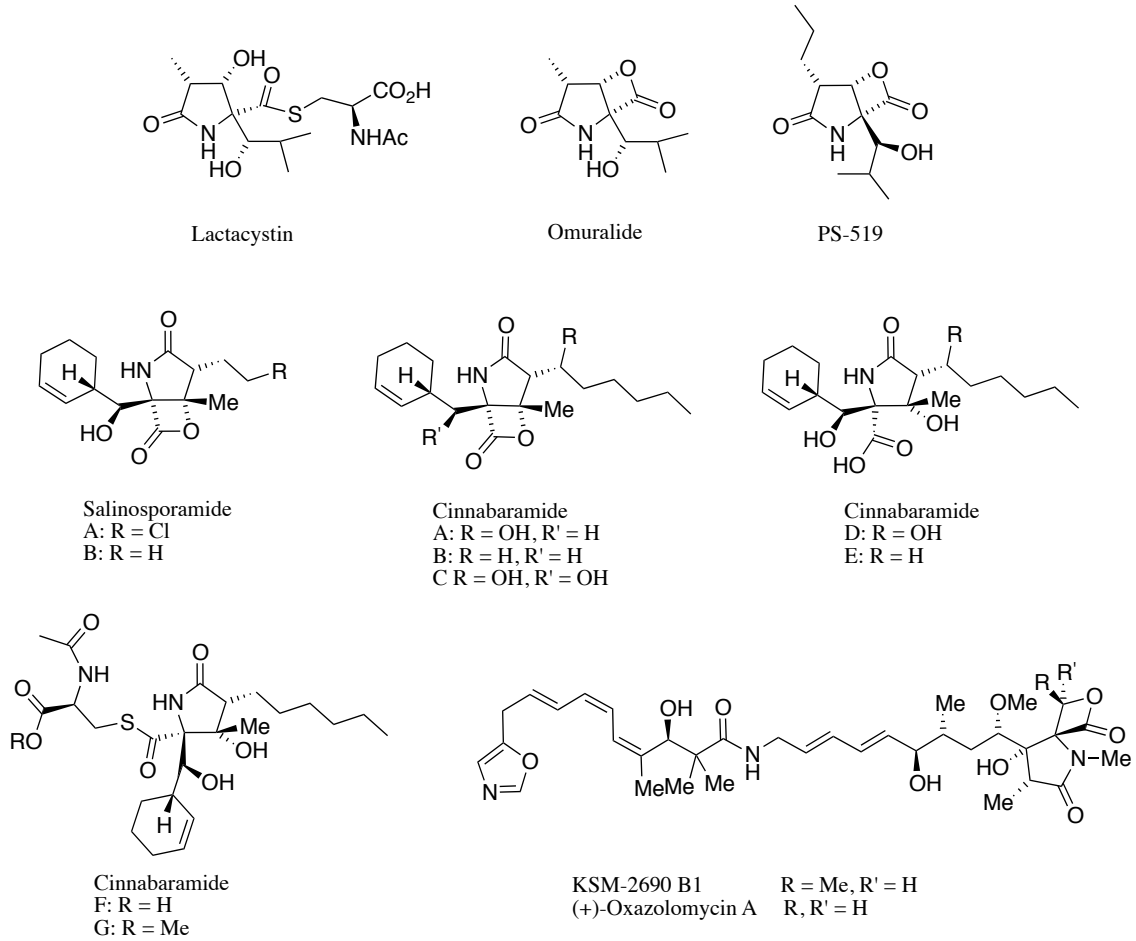
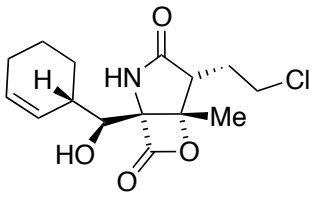



**Figure 7.** The structures of other β-lactone proteasome inhibitors.

Salinosporamide A **109** has proved a popular synthetic target due to its high potency, and is discussed in detail below. PS-519 is a novel proteasome inhibitor with structural similarities to both omuralide and salinosporamide. The cinnabaramides A-G were also isolated from a strain of *Streptomyces* and share the same cyclohexenyl moiety found in salinosporamide A.[41] Oxazolomycin A, as well as having structural similarities to lactacystin, is the parent member of a family of compounds (including KSM-2690 B1) showing antibacterial, antiviral and antitumour activity. The first total synthesis of oxazolomycin A was reported by the Hatakeyama group in 2011.[42]

### 1.2.1 The Salinosporamides

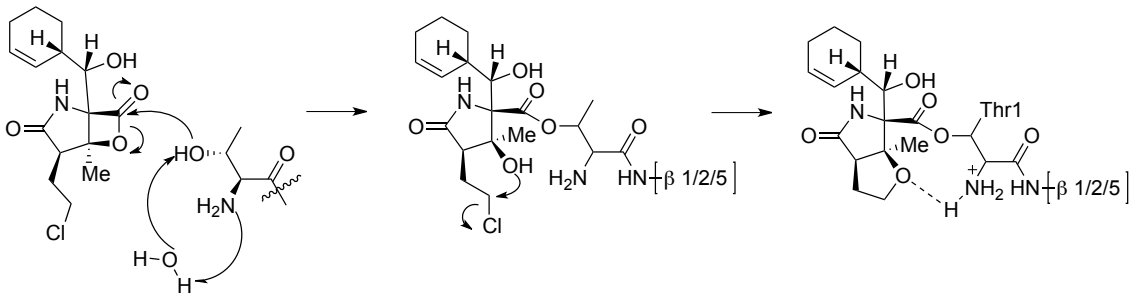
After lactacystin, the most commonly studied member of this lactam-based proteasome inhibitor family is salinosporamide A. It is a particularly interesting compound as its high potency as an anti-cancer agent meant it reached Phase I human clinical trials for the treatment of multiple myeloma only three years after its discovery in 2003.




**109**

**Figure 8.** Salinosporamide A

In 2003, Feling and co-workers reported the isolation of salinosporamide A **109** from the CNB-392 strain of the bacterium *Salinospora tropica*.[43] Unlike lactacystin, salinosporamide A is naturally occurring in its β-lactone form. The key structural differences are the cyclohexenyl group that replaces the isopropyl group at C9, the replacement of the proton with a methyl group at C6 and the chloroethyl chain replacing the methyl group at C7. Salinosporamides A-K have been isolated and all have slightly different functional groups around the same core bicyclic ring structure. Salinosporamide A binds to the 20S proteasome in a similar way to omuralide but has been found to be more effective as a proteasome inhibitor than omuralide.[44]

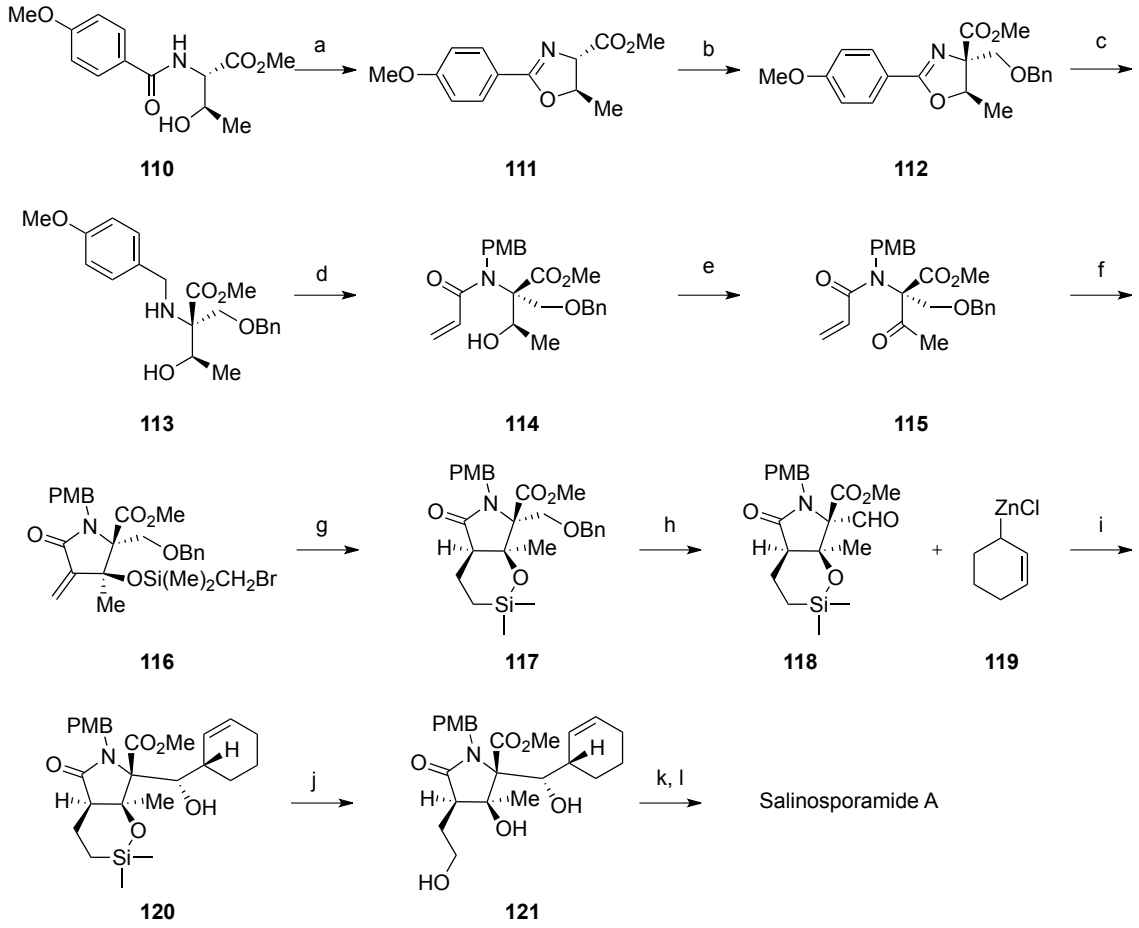



**Scheme 19.** The binding of salinosporamide A to the 20S proteasome.

## 1.2.2 Previous Syntheses of Salinosporamide A

### 1.2.2.1 The Corey Synthesis

There have been many reported syntheses of salinosporamide A. As in the case of lactacystin, the first of these was by the Corey group in 2004, **Scheme 20**.[45]




**Reagents and Conditions:** a) *p*-TsOH, toluene, reflux, 12 h, 80%; b) LDA, THF, HMPA, –78 °C, $ClCH_2OBn$, 4 h, 69%; c) $NaCNBH_3$, AcOH, 40 °C, 12 h, 90%; d) 1. TMSCl, $Et_2O$, 23 °C, 12 h; 2. Acryloyl chloride, *i*-$Pr_2Net$, $CH_2Cl_2$, 1 h, 0 °C, then $H^+$, $Et_2O$, 23 °C, 1 h, 96%; e) Dess-Martin periodinane, 23 °C, 1 h, 96%; f) 1. Quinuclidine, DME, 0 °C, 7 d, 90%; 2. $BrCH_2Si(CH_3)_2Cl$, $Et_3N$, DMAP, $CH_2Cl_2$, 0 °C, 30 min, 95%; g) $Bu_3SnH$, AIBN, benzene, reflux, 8 h, 89%; h) 1. Pd/C, EtOH, $H_2$ (1 atm), 18 h, 95%; 2. Dess-Martin periodinane, 23 °C, 1 h, 95%; i) THF, –78 °C, 5 h, 88%; j) KF, $KHCO_3$, $H_2O_2$, THF/MeOH (1:1), 23 °C, 18 h, 92%; k) CAN, MeCN/$H_2O$ (3:1), 0 °C, 1 h, 83%; l) 1. 3 M LiOH/THF (3:1), 5 °C, 4 d; 2. BOPCl, pyridine, $CH_2Cl_2$, 23 °C, 1 h; 3. $Ph_3PCl_2MeCN$, pyridine, 23 °C, 1 h, 65% (3 steps).

**Scheme 20.**

Acylation of (*S*)-threonine methyl ester using 4-methoxybenzoyl chloride afforded the amide **110**. Cyclization of **110** to form the oxazoline **111** was achieved upon reflux in toluene with *p*-toluenesulfonic acid. Deprotonation of **111** with LDA followed by alkylation of the resulting enolate with chloromethyl benzyl ether allowed the formation of **112** in 69% yield. The tertiary stereocentre formed in this reaction has the same absolute configuration as the natural product. Reduction of **112** with sodium cyanoborohydride cleaves the oxazoline, giving **113** in 90% yield. Formation of **114** was achieved over two steps: alcohol **113** was first converted to the corresponding TMS ether using trimethylsilyl chloride and triethylamine; then, *N*-acylation using acryloyl chloride and acidic work-up using aqueous hydrochloric acid to remove the TMS group led to the formation of **114**.

The formation of the lactam was achieved through a well-designed sequence that also established the desired stereochemistry at the C5 and C6 positions. A Dess-Martin periodinane oxidation gave the keto amide ester **115**, which was then cyclized under Baylis-Hillman aldol conditions. Cyclization resulted in a mixture of diastereoisomers at the C6 position in a 9:1 ratio. Silylation of the desired diastereoisomer gave **116**, which was then transformed into the bicyclic compound **117** using a radical-mediated cyclization using tributyltin hydride and AIBN. Cleavage of the benzyl ether was achieved under standard hydrogenation conditions using Pd/C. A Dess-Martin periodinane oxidation was used again, to form the aldehyde **118** in 95% yield.

The introduction of the 2-cyclohexenyl group was achieved by addition of 2-cyclohexenylzinc chloride **119**. Synthesis of 2-cyclohexenylzinc chloride was achieved using a palladium-catalysed 1,4-addition of tributyltin hydride to 1,3-cyclohexadiene to give 2-cyclohexenyl-tri-*n*-butyltin which underwent transmetallation using *n*-butyllithium and zinc chloride to give the desired 2-cyclohexenylzinc chloride. The reaction of 2-cyclohexenylzinc chloride with **118** in THF at –78 °C gave **120** in 88% yield. Oxidation of **120** under Tamao-Fleming conditions gave the triol **121** in 92% yield.

The next three steps of the synthesis follow literature reported by the Corey group in the synthesis of lactacystin. CAN was used to remove the *p*-methoxybenzyl group, the

methyl ester was saponified using lithium hydroxide, and β-lactonization was achieved using BOPCl. Finally, the reaction with triphenylphosphine dichloride in acetonitrile-pyridine gave the natural product salinosporamide A in 14% yield over 17 steps.

#### 1.2.2.2 The Ōmura Synthesis

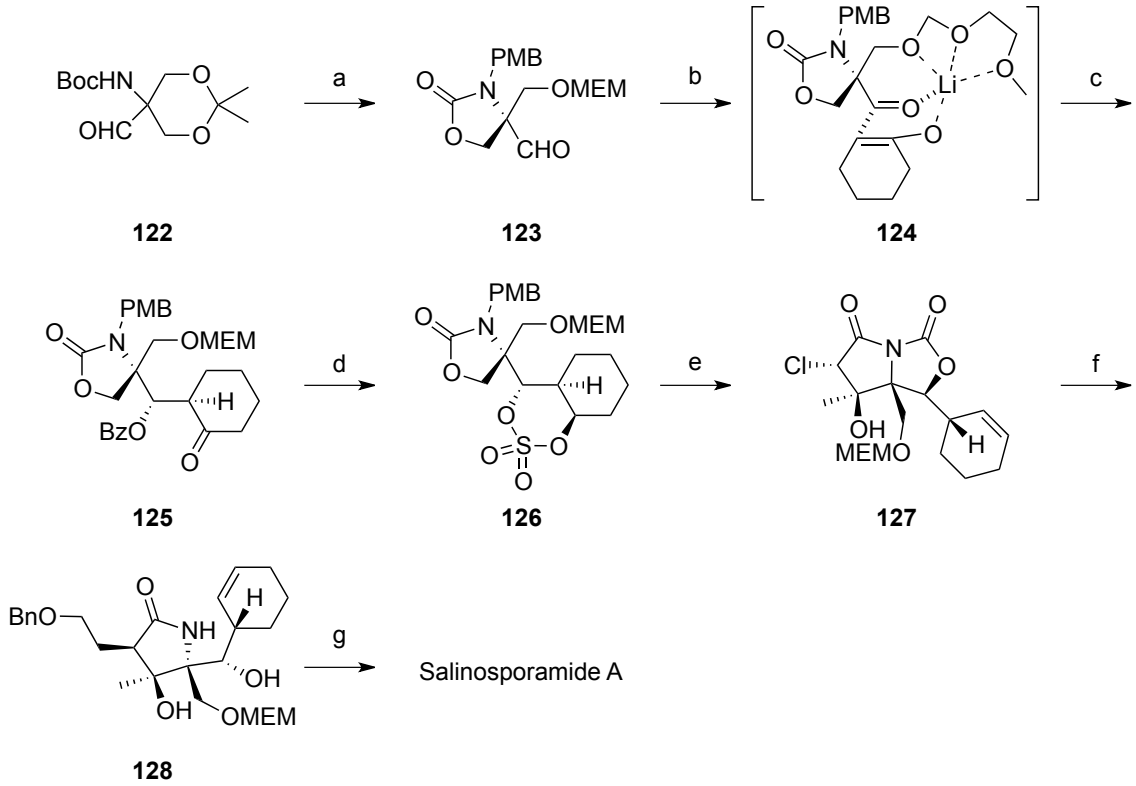
After Corey, other groups reported the total synthesis of salinosporamide A, including the Ōmura group in 2008.[46] Unlike Corey and other subsequent researchers, such as Romo and Danishefsky, who all install the cyclohexene ring in the same way at a late stage in the synthesis,[47, 48] Ōmura and co-workers reported a new approach to construct the cyclohexene ring in the early stages of their synthesis, **Scheme 21**.

The aldehyde starting material **122** was converted over multiple steps into the carbamate **123**. The precursor to the cyclohexene moiety was then introduced at this early stage. Simultaneous installation of the C5 and C6 stereogenic centres was achieved by an aldol reaction between the aldehyde **123** and cyclohexanone, and the reaction quenched with benzoyl chloride. The large diastereoisomeric ratio (20:1) observed in **125** was achieved due to formation of the highly chelated intermediate **124**. This reaction was also attempted using Corey's methodology: 2-cyclohexenylzinc chloride was added to the aldehyde **123**; however, low diastereoselectivity was observed.[45]

Conversion of the cyclohexanone moiety to the corresponding cyclohexene proved more difficult than the group envisaged. Reduction of an enol triflate using palladium chemistry, the Shapiro reaction, and a reduction-dehydration procedure were attempted but unsuccessful. Finally, they settled on a method that utilized a cyclic sulfate intermediate **126**, which, after elimination, gave the required cyclohexene moiety.

The construction of the γ-lactam core was achieved over multiple steps via the bicyclic compound **127**. Ring-opening of the lactone gave the core lactam ring which was then subjected to multiple protection and deprotection reactions, β-lactonization and chlorination reactions to give salinosporamide A. Although a relatively long route, the

ability to stereoselectively construct the cyclohexene ring at an early stage is the most interesting feature of this synthesis.

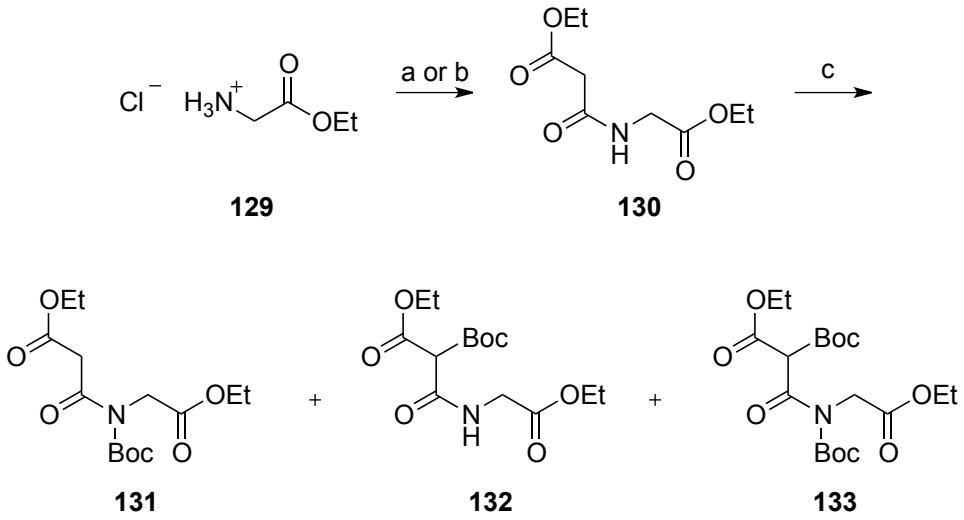



**Reagents and Conditions:** a) 1. $Ph_3P^+CH_3Br^-$, NaHMDS, THF, RT, 97%; 2. *i*-TsOH·$H_2O$, MeOH, RT, 82%; 3. Lipase vinyl acetate, *i*-$Pr_2O$, RT; 4. TBDPSCl, imidazole, DMF, RT, 94%, 97% ee (2 steps); 5. $K_2CO_3$, MeOH, RT; 6. MEMCl, *i*-$Pr_2$Net, $CH_2Cl_2$, RT, 94% (2 steps); 7. TBAF, THF, RT; 8. NaH, THF/DMF, then PMBCl, RT, 97% (2 steps); 9. $OsO_4$, NMO, acetone/$H_2O$, RT; 10. $NaIO_4$, MeOH/$H_2O$, RT, 92% (2 steps); b) LDA, cyclohexanone, THF, –78 °C; c) BzCl, 79%; d) 1. $NaBH_4$, $CeCl_3$·$7H_2O$, MeOH, RT; 2. $K_2CO_3$, MeOH, RT, 95% (2 steps); 3. $SOCl_2$, pyridine, $CH_2Cl_2$, RT; 4. $RuCl_3$, $NaIO_4$, MeCN/$CCl_4$/$H_2O$, RT, 94% (2 steps); e) 1. DBU, toluene, 100 °C; 2. TsOH·$H_2O$, dioxane, RT, 97% (2 steps); 2. NaH, THF/EtOH, RT; 3. $(COCl)_2$, DMSO, $Et_3N$, $CH_2Cl_2$, –78 °C, 92% (2 steps); 4. MeMgBr, THF, –78 °C; 2. Dess-Martin periodinane, $CH_2Cl_2$, RT, 100% (2 steps); 5. CAN, MeCN/$H_2O$, 0 °C, 92%; 6. LiHMDS, THF, –78 °C, then chloroacetyl chloride, 63%; f) 1. $SmI_2$, LiCl, benzyloxyacetaldehyde, THF, –78 °C, 83%; 2. MsCl, $Et_3N$, $Me_3N$·HCl, toluene, RT; 3. NaOH, MeOH/$H_2O$, 0 °C, 81% (2 steps); 4. $LiEt_3BH$, THF, 0 °C, 77%; g) 1. TESCl, NaI, MeCN, 0 °C, 100%; 2. TESOTf, 2,6-lutidine, $CH_2Cl_2$, –78 °C, 48%; 3. HF·pyridine, THF, 0 °C, 90%; 4. Dess-Martin periodinane, $CH_2Cl_2$, RT; 2. $NaClO_2$, 2-methyl-2-butene, $Na_2HPO_4$, *t*-BuOH/$H_2O$, RT; 5. Li, $NH_3$, *t*-BuOH/THF, –78 °C; 6. BOPCl, pyridine/$CH_2Cl_2$, RT; 7. $Ph_3PCl_2$, pyridine/MeCN, RT, 48% (5 steps); 8. HF·pyridine, THF, RT, 84%.

**Scheme 21.**

## 1.3 Previous Work in the Page Group

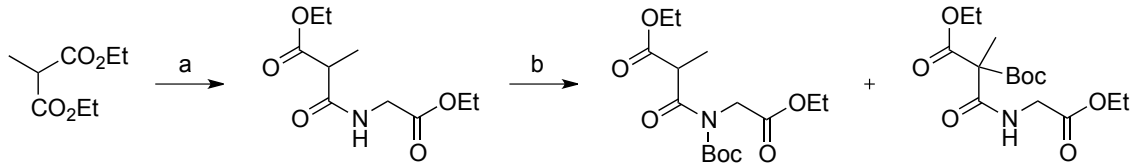
Investigations within the group began focusing on the construction of the lactam core of lactacystin. One of the key steps was the use of a Dieckmann cyclization to obtain the core. The synthesis of the unprotected cyclization precursor **130** proceeded without difficulty; however, the protection of the amide moiety proved challenging, **Scheme 22**.[49]




**Reagents and Conditions:** a) 1. NaOH; 2. Ethyl malonyl chloride, $CH_2Cl_2$, 57%; b) potassium ethyl malonate, DCC, $Et_3N$, aq. MeCN, 81%; c) $Boc_2O$, $Et_3N$, DMAP, $CH_2Cl_2$, **131**: 15%, **132**: 13%, **133**: 11%.

**Scheme 22.**

The use of di-*tert*-butyl dicarbonate resulted in a mixture of both *N*- and *C*-Boc products **131**, **132** and **133**, in low yields. Compounds **132** and **133** were both subjected to Dieckmann cyclization conditions; unfortunately, the desired products were not isolated. A possible solution to avoid the formation of the *C*-Boc product would be the introduction of a methyl group at the C7 position prior to the Boc protection, **Scheme 23**.



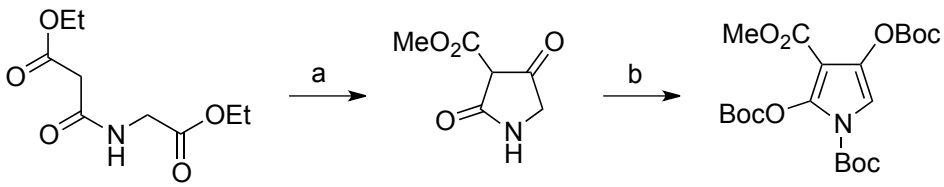

**134** **135** **136** **137**

**Reagents and Conditions:** a) 1. KOH, EtOH, 24 h, RT, 87%; 2. glycine ethyl ester, DCC, $Et_3N$, $CH_2Cl_2$, 82%; b) $Boc_2O$, DMAP, MeCN, RT, **136**: 60%, **137**: 11%.

**Scheme 23.**

Compound **135** was prepared over two steps from methylmalonate diethyl ester in 71% yield. The use of di-*tert*-butyl dicarbonate with triethylamine and DMAP as a catalyst in dichloromethane gave compounds **136** and **137** in 30% and 18% yields, respectively. Changing the solvent to acetonitrile and removing the triethylamine led to the formation of the desired *N*-Boc product **136** as the major compound in 60% yield alongside a small amount of **137** (11%). Compounds **135**, **136** and **137** were all subjected to a number of cyclization conditions: the desired products were not observed and, instead, transesterification occurred, complex mixtures were formed and in some cases the starting material was recovered.

Cyclization of the unprotected Dieckmann cyclization precursor **130** gave the lactam **138** in 70% yield, **Scheme 24**. Compound **138** was insoluble in common organic solvents, and attempts were made to further functionalize the amide nitrogen to increase solubility. Again, the Boc protection did not proceed as desired and the trisubstituted pyrrole **139** was isolated in 14% yield

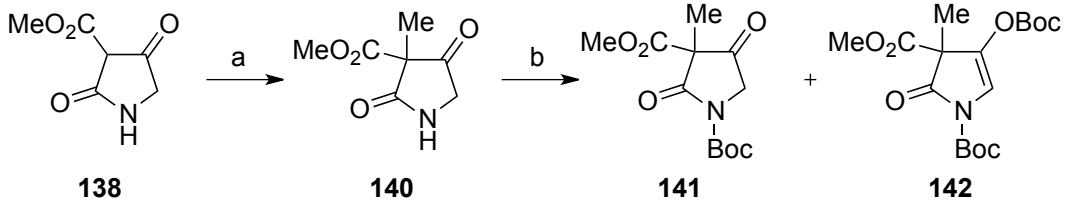



**130** **138** **139**

**Reagents and Conditions:** a) Na/MeOH, toluene, reflux, 70%; b) $Boc_2O$, $Et_3N$, DMAP, MeCN, RT, 14%.

**Scheme 24.**

Attempts were then made to alkylate lactam **138** at the C3 position using methyl iodide, **Scheme 25**. The use of tetrabutylammonium fluoride (TBAF) both as a base and phase transfer catalyst solved the problem of insolubility mentioned above, and the methylated product **140** was obtained in 82% yield. With the presence of the quaternary centre at the C3 position preventing aromatization to the pyrrole, the reaction with di-*tert*-butyl dicarbonate and DMAP in acetonitrile gave the *N*-Boc protected lactam **141** in 76% yield alongside trace amounts of **142**.



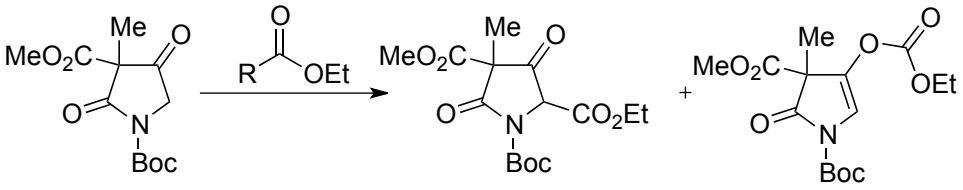

**138** **140** **141** **142**

**Reagents and Conditions:** a) TBAF, THF, MeI, RT, 82%; b) $Boc_2O$, DMAP, MeCN, RT, **141**: 76%.

**Scheme 25.**

Introduction of the functionality at the C5 position was the next step. Acylation was investigated using a variety of bases under different conditions (**Table 4**).[49] Although most methods proved unsuccessful, the desired product **143** was obtained in 10% yield when ethylcyanoformate was used. A possible reason for the low yields observed was thought to be the steric hindrance caused by the Boc group.

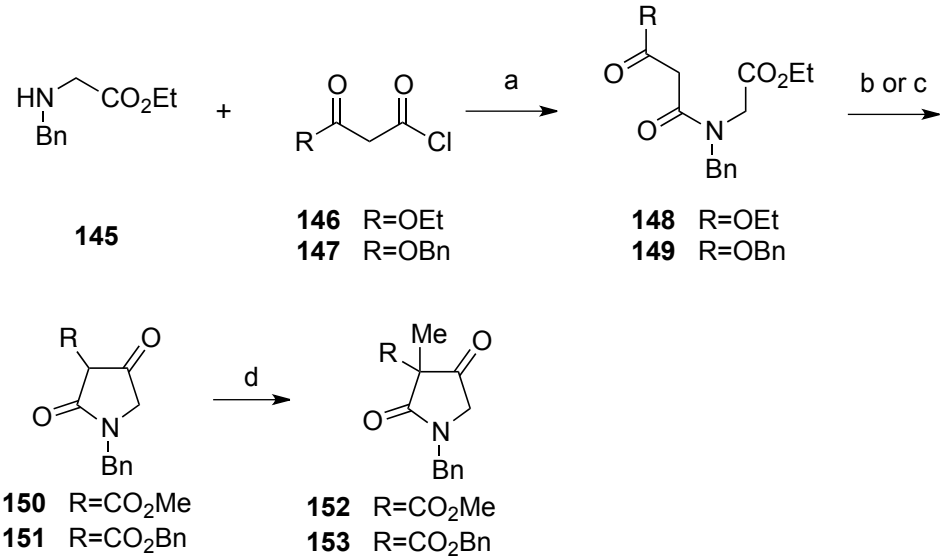
**Table 4.** Investigations into the acylation of **141**.




**141** **143** **144**

| Conditions | R | 143 | 144 |
|---|---|---|---|
| LDA, THF, –78 °C | Cl | 10% | 12% |
| LiHMDS, THF, –78 °C | Cl | - | - |
| *t*-BuOK, THF, –78 °C | Cl | - | 35% |
| t-BuOK, THF, –78 °C | OEt | - | - |
| KH, benzene, reflux | OEt | - | - |
| LDA, THF, –78 °C | CN | 10% | - |

With multiple problems arising from the use of Boc as a protecting group, investigations were then focused on groups which would be both smaller and less likely to lead to *C*-acylation. *N*-Benzyl glycine ethyl ester **145** was chosen as the starting material: it can be easily prepared from the condensation of ethyl bromoacetate and benzylamine.




**Reagents and Conditions:** a) pyridine, DMAP, $CH_2Cl_2$, RT, 24 h, **148**: 91%, **149**: 95%; b) For **148**; Na/MeOH, reflux, 88%; c) For **149**; NaH, benzene, 6 h, 63%; d) TBAF, MeI, 24 h, **152**: 77%, **153**: 70%.

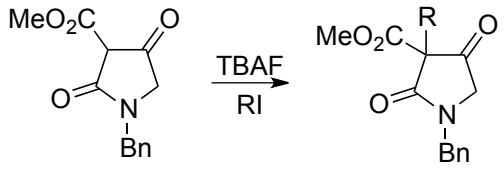
**Scheme 26.**

Condensation of ethyl malonyl chloride **146** or benzyl malonyl chloride **147** and *N*-benzyl glycine ethyl ester **145** led to the formation of the Dieckmann cyclization precursors **148** and **149** in 91% and 95% yield, respectively. The Dieckmann cyclization of **148** using sodium/methanol under reflux gave **150** in 88% yield. Treatment of **149** with sodium/benzyl alcohol gave **151** in a low yield of 14%. This methodology was improved by using sodium hydride in benzene, which gave **151** in 63% yield.

The next step was to introduce the methyl group at the C7 position. Unfortunately, both **150** and **151** were poorly soluble in common organic solvents. Attempts at methylation using various methods including NaH/MeI, $^t$BuOK/BuOH/MeI and $K_2CO_3$/MeI all failed to give the desired products. After further investigation, the use of TBAF with methyl iodide in THF was found to be successful and gave the methylated products **152** and **153** in high yields (77% and 70%, respectively).

Alkylation of **150** using various electrophiles was then carried out to investigate the efficiency of this reaction, **Table 5**.[50] This is also interesting as it suggests this methodology could allow access to a variety of lactacystin analogues.

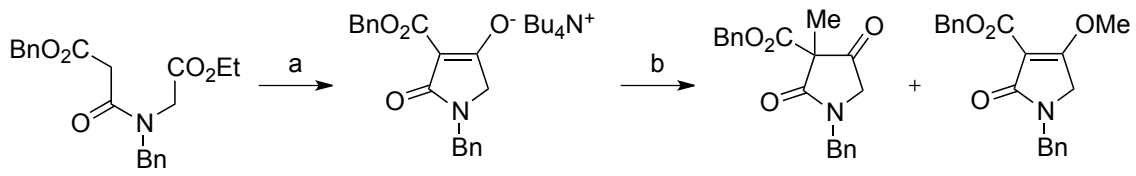
**Table 5.** Alkylation of **150** using TBAF (1.2 equiv.) and RI (2 equiv.) in THF.




**150** **154**

| R | Yield (%) |
|---|---|
| Me | 77 |
| Et | 63 |
| $PhCH_2$ | 89 |
| $CH_2$=$CHCH_2$ | 60 |
| PhCH= $CHCH_2$ | 64 |
| $EtO_2$ $CCH_2$ | 46 |

Later work details improvements made to the Dieckmann cyclization step; instead of using sodium hydride in benzene as described above, it was found that treatment of **149** with TBAF in diethyl ether at room temperature gave the tetrabutylammonium salt **155**. Without purification, treatment of the salt with methyl iodide in THF gave **153** in 73% yield from **149**, **Scheme 27**. As well as the desired product, the enol ether regioisomer **156** was also isolated (**153**:**156**, 3.5:1). These milder conditions are much more attractive for a synthesis.

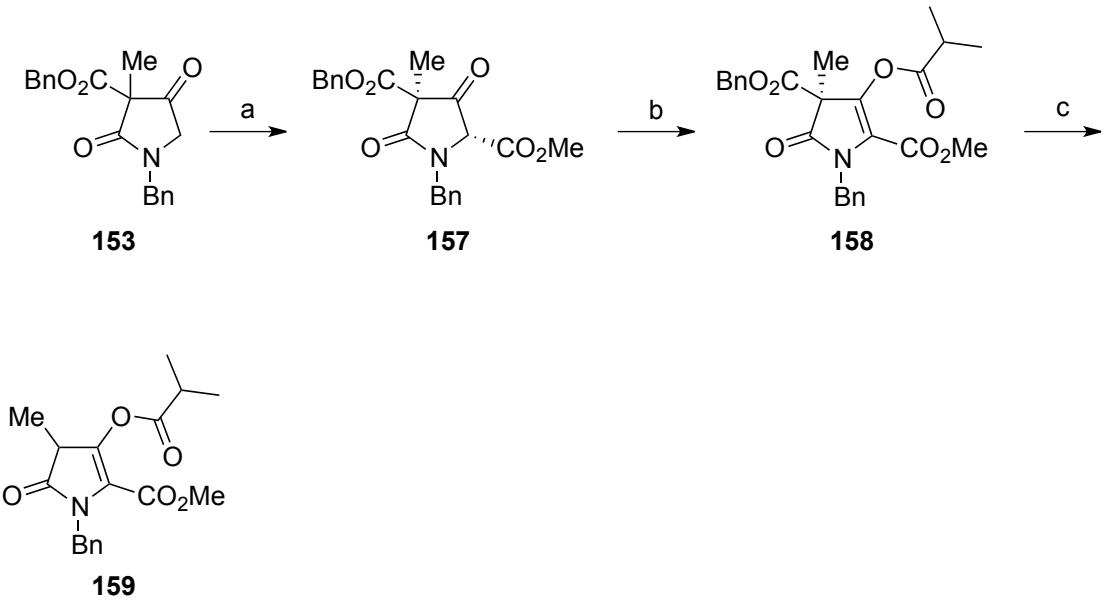



**149** **155** **153** **156**

**Reagents and Conditions:** a) TBAF, $Et_2O$, RT, 24 h; b) MeI, THF, RT, 24 h, 73%.

**Scheme 27.**

Another key step in the synthesis is the introduction of the functionality at the C5 position following Mander's protocol, **Scheme 28**. Deprotonation of **153** using lithium bis(trimethylsilyl)amide (LiHMDS) in the presence of 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU) generated the corresponding enolate; methyl cyanoformate was then added to the solution to give **157** in 75% yield as a 5:1 mixture of diastereoisomers. As observed above (**Table 4**), the use of Mander's reagent did not lead to any competing *O*-acylation.

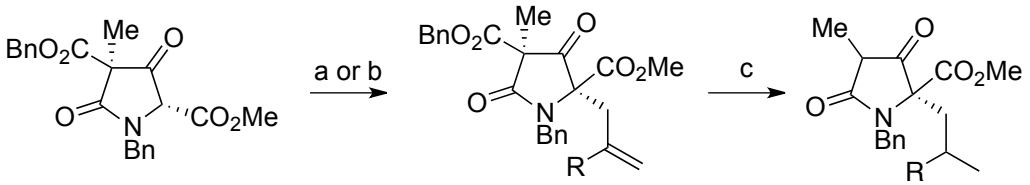



**Reagents and Conditions:** a) LiHMDS, DMPU, THF, $MeO_2CCN$, 75%; b) $CH_3CHCOCl$, pyridine, $CH_2Cl_2$, RT, 24 h, 68%; c) $H_2$, Pd/C, THF, 98%.

**Scheme 28.**

Formation of the quaternary centre at the C5 position using isobutyraldehyde under various conditions including Mukaiyama's protocol proved unsuccessful. After the use of methyl cyanoformate gave exclusively the *C*-alkylated product in the previous step, attempts were made to alkylate compound **157** using isobutyryl cyanide. Unfortunately, the desired alkylated product was not isolated. When isobutyryl chloride was used, only the *O*-acylation product was observed; **158** was isolated in 68% yield. When **158** was subjected to hydrogenolysis conditions, the decarboxylated product **159** was obtained in 98% yield.

To prevent the *O*-acylation, the reaction conditions were modified.[51] Treatment of **157** with sodium hydride in DMF followed by the addition of allyl bromide gave **160** in 44% yield; no *O*-allylation was observed. When the reaction was repeated using methallyl bromide, the analogous compound **161** was prepared in much higher yield (75%).




**157**

**160** R=H
**161** R=Me

**162** R=H
**163** R=Me

**Reagents and Conditions:** a) NaH, DMF, allyl bromide, RT, **160**: 44%; b) NaH, DMF, methallyl bromide, RT, **161**: 75%; c) $H_2$, Pd/C, THF, **162**: 90%.

**Scheme 29.**

When **160** was subjected to standard hydrogenation conditions, the decarboxylation of the benzyl ester with concomitant reduction of the double bond occurred to give **162** in 90% yield. This advanced intermediate contains the complete carbon skeleton of *clasto*-lactacystin dihydroxyacid and was achieved after only five steps.[51]

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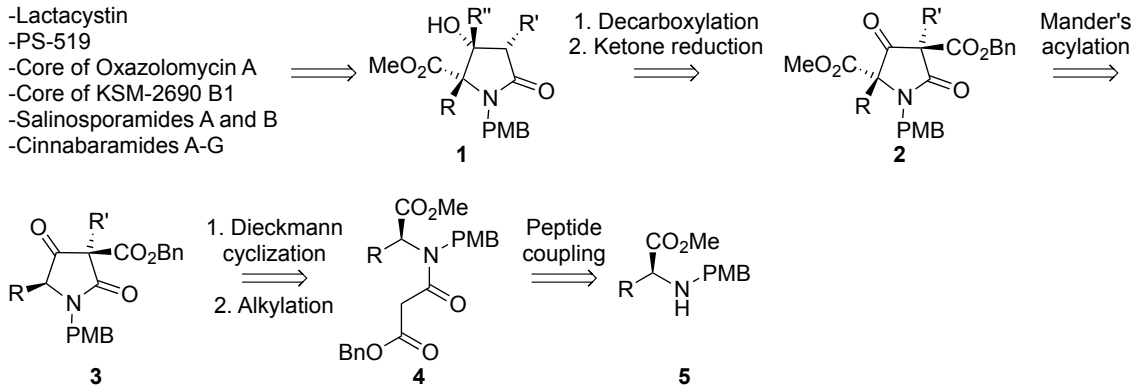
# 2.0 Results and Discussion

## 2.1 Retrosynthetic Analysis of Lactacystin and Analogues

Lactacystin and its analogues share the same substituted lactam core, but differ at the C5 and C7 positions, allowing us to consider a common retrosynthetic approach. The retrosynthetic analysis below (**Scheme 1**) shows the key steps required for a synthesis of lactacystin and its analogues. Using a commercially available amino acid derivative as the starting material, the C5 functionality is present from the start, and the use of different amino acids allows variation at this position. Key steps include a Dieckmann cyclization and a Mander's acylation to form the lactam ring and the C5 quaternary centre, respectively. The use of a range of alkylating agents during the tandem Dieckmann cyclization/alkylation should allow the installation of a C7 group that differs between the natural products as well as allowing access to novel structures. A decarboxylation at the C7 position and the reduction of the ketone moiety at the C6 position would then be required to give the fully functionalized core found in this class of proteasome inhibitors.




**Scheme 1.**

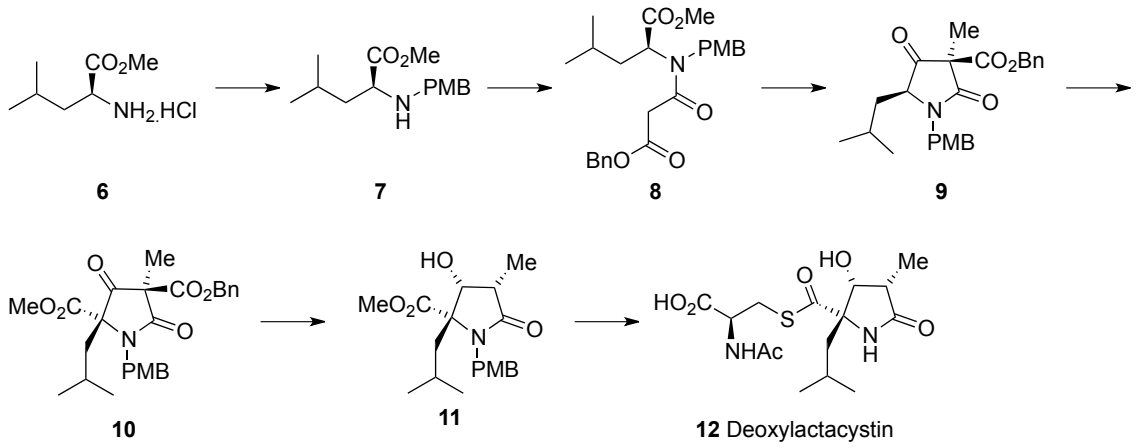
The work presented below details the use of L-leucine, L-serine and L-valine derivatives as the starting materials. Work has mainly been focused on the use of L-leucine (which will lead to the formation of deoxylactacystin) as it has the *iso*-butyl group present from the start and shares the closet structural similarity to lactacystin. We hope that by using a chiral starting material, in this case L-leucine, we can induce the desired

stereochemistry at the C5, C6 and C7 positions throughout the synthesis. Indeed, this β-lactone class of inhibitors is particularly challenging due to the number of stereocentres that need to be generated and controlled during the synthesis.

## 2.2 Synthesis of the Leucine Analogue

### 2.2.1 Proposed Synthetic Route Towards Deoxylactacystin

Using L-leucine methyl ester hydrochloride **6** as the starting material, we hoped to synthesize the advanced intermediate **11** that was reported by Corey in 1999[1] using the route developed by the Page group as described above (**Chapter 1**).

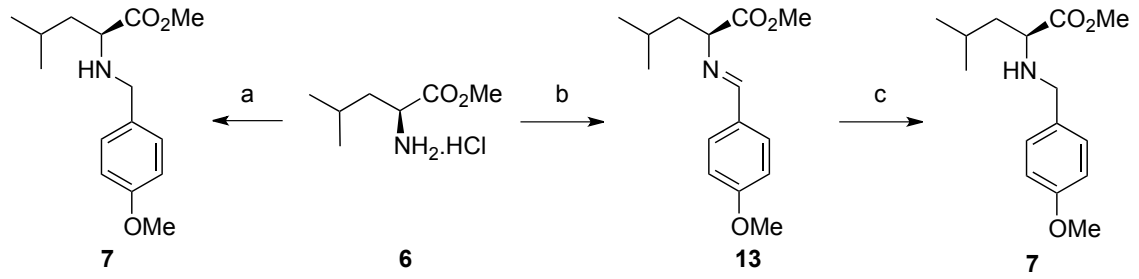



**Scheme 2.**

### 2.2.2 Synthesis of the Dieckmann Cyclization Precursor 8

Due to the problems encountered when using the Boc and benzyl protecting groups described above, we turned our attention towards the 4-methoxybenzyl moiety as the protecting group. Corey reported the use of this group and its easy removal using ceric ammonium nitrate in his early approach to the synthesis of lactacystin.[2] Compound **7** can be synthesized in one-pot from commercially available L-leucine methyl ester hydrochloride **6** using triethylamine, 4-methoxybenzaldehyde and sodium borohydride in methanol; however, low yields (53%) were obtained. The reaction did not afford reproducible yields and, at times, resulted in an inseparable mixture of the desired

product **7** and *p*-methoxybenzylalcohol. This result indicated that full conversion to the imine **13** was vital before introduction of the reducing reagent. Hence, a two-step procedure was investigated.

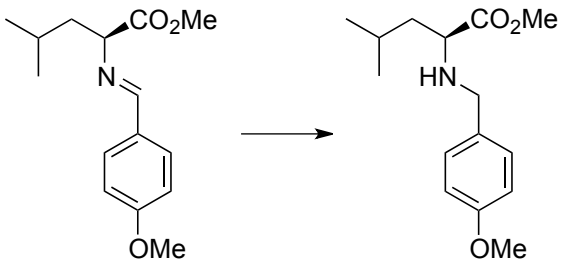



**Reagents and Conditions:** a) 4-methoxybenzaldehyde, $Et_3N$, $NaBH_4$, MeOH, 53%; b) 4-methoxybenzaldehyde, acetic acid, toluene, reflux, 100%; c) $NaBH_3CN$, acetic acid, MeOH, 93%.

**Scheme 3.**

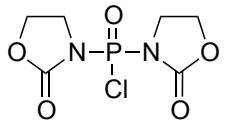
In order to improve the conversion to the imine, 4-methoxybenzaldehyde and acetic acid were heated under reflux in toluene using a Dean-Stark apparatus to give **13**, which was used without further purification. The use of the Dean-Stark apparatus drove the reaction equilibrium to the required imine by removal of water. It is easy to determine whether full conversion to the imine has occurred by $^1$H NMR spectrum analysis of the crude product. If the aldehyde starting material is still present a characteristic singlet peak can be seen just below 10 ppm. The reduction of **13** was achieved using sodium borohydride and acetic acid in methanol; however, compound **7** was isolated in 58% yield, a marginal improvement on the method described above. When sodium cyanoborohydride, a reducing reagent known to be selective towards imine reduction, was used, amine **7** was obtained in 93% yield. Because acetic acid is required to control the pH of this reaction, the use of sodium cyanoborohydride can be dangerous as toxic hydrogen cyanide gas can be released. Sodium triacetoxyborohydride is often used as a safer alternative to sodium cyanoborohydride for imine reductions. It is known that sodium borohydride can react with acetic acid to form sodium triacetoxyborohydride; this could have occurred in the two-step reductive amination described above. However, when the reaction was carried out with commercially available sodium triacetoxyborohydride, no reaction occurred and the starting material was recovered.

**Table 1.** Investigation into the reduction of compound **13**.



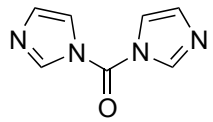

| Reaction Conditions | Reducing Reagent | Yield (%) |
|---|---|---|
| AcOH, MeOH, 0 °C to RT | $NaBH_4$ | 53 |
| AcOH, MeOH, 0 °C to RT | $NaBH_3CN$ | 93 |
| MeOH, 0 °C to RT | $NaBH(OAc)_3$ | S.M. |

The Dieckmann cyclization precursor **8** was then synthesized using a peptide coupling reaction. There are many peptide coupling reagents reported in the literature; these include (but are not limited to) phosphonium reagents (for example: BOPCl, first used by Corey to form the lactone moiety in omuralide), uronium reagents (for example: HATU, a reagent commonly used in the synthesis of macrocycles), carbodiimide reagents (for example, EDAC·HCl) and imidazolium reagents (for example, CDI).[3]



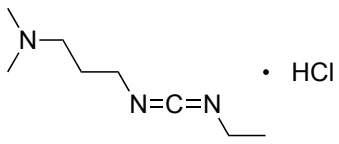
BOPCl

Bis(2-oxo-3-oxazolidinyl)phosphinic chloride



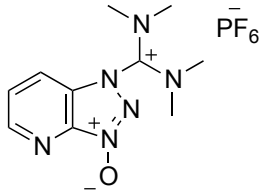
CDI

1,1'-Carbonyldiimidazole



EDAC·HCl

*N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride

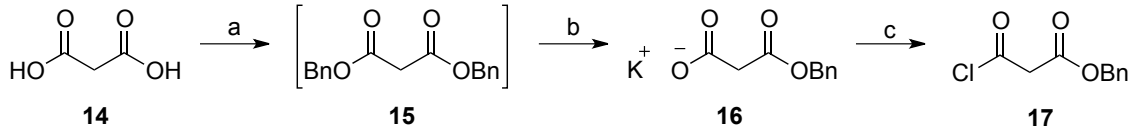


HATU

1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*] pyridinium-3-oxid hexafluorophosphate

**Figure 1.** Coupling reagents.

The synthesis of potassium benzyloxycarbonyl acetate **16** required for the peptide coupling was achieved over two steps from malonic acid in 72% yield (**Scheme 4**).[4]

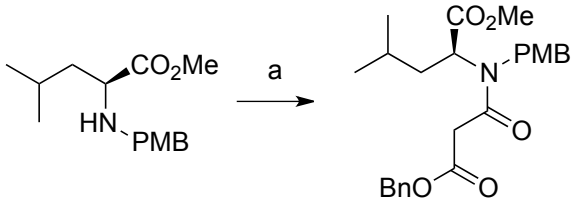



**14** **15** **16** **17**

**Reagents and Conditions:** a) benzyl alcohol, *p*-TsOH, toluene, reflux, overnight; b) KOH in BnOH (1 M), 72% (2 steps); c) oxalyl chloride, toluene, 34 °C, 24 h.

**Scheme 4.**

We chose to use a commercially available carbodiimide coupling reagent, *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDAC·HCl), due to its ease of handling and because the by-product formed is easily removed during work-up as it is water-soluble under acidic conditions. The treatment of **7** with potassium benzyloxycarbonyl acetate **16**, EDAC·HCl, 4-(dimethylamino)pyridine (DMAP) and *N*-methylmorpholine (NMM) in dichloromethane gave **8** in 72% yield after silica gel chromatography purification.




**7** **8**

**Reagents and Conditions:** a) potassium benzyloxycarbonyl acetate **16**, EDAC·HCl, DMAP, NMM, $CH_2Cl_2$, 72%.

**Scheme 5.**

Room temperature $^1$H NMR spectrum analysis shows the presence of two rotamers, and so a variable-temperature (VT) $^1$H NMR experiment was carried out in deuteriated dimethylsulfoxide (*d*6-DMSO) at 25, 75 and 100 °C, **Figure 2**. Analysis of the resulting spectra clearly shows the peaks beginning to coalesce as the temperature is increased.

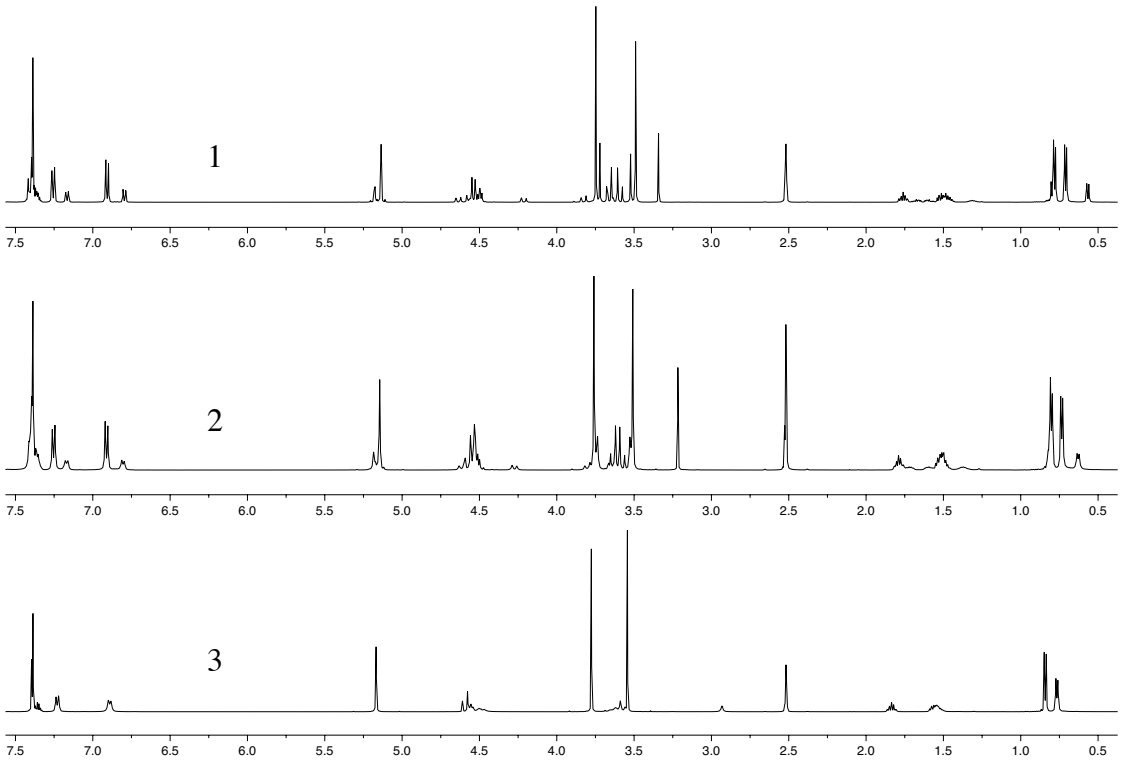
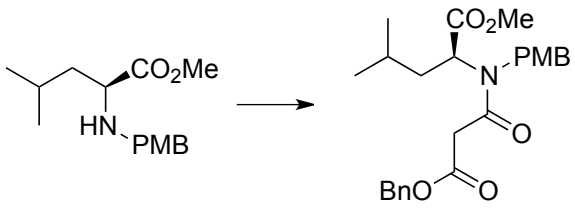



**Figure 2.** Variable temperature $^{1}$H NMR spectra of compound **8** at **1**: 25 °C, **2**: 75 °C and **3**: 100 °C.

The optimization of this reaction was investigated using methods other than carbodiimide coupling. The treatment of potassium benzyloxycarbonyl acetate **16** with oxalyl chloride gave the corresponding acid chloride **17** without purification in quantitative yield. The addition of the acyl chloride to **7** in the presence of pyridine and DMAP gave the Dieckmann precursor **8** in only 58% yield. The coupling reagent propane phosphonic acid anhydride, known commercially as T3P®, was also used for this reaction.[5] The by-products formed from using T3P® are all water-soluble and can be easily removed during aqueous work-up, which aids the purification of the desired compound. T3P® is also a greener reagent as it is less toxic than the carbodiimide reagents. The treatment of **7** with potassium benzyloxycarbonyl acetate **16**, *N*,*N*-diisopropylethylamine (DIPEA or Hünig's base) and T3P® in tetrahydrofuran gave **8** in 62% yield.

**Table 2.** Synthesis of the Dieckmann cyclization precursor **8** from **7**.

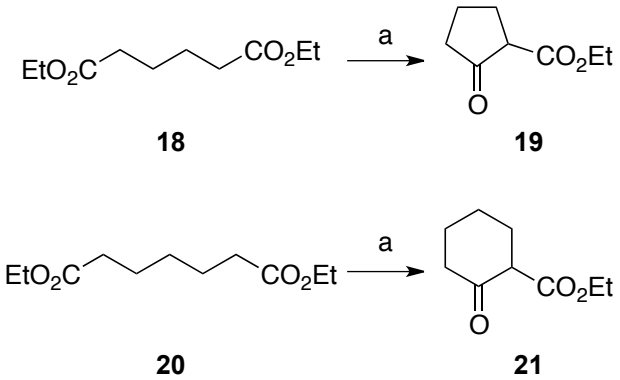



**7** **8**

| Reaction Conditions | Coupling Reagent | Yield (%) |
|---|---|---|
| **16**, NMM, DMAP, $CH_2Cl_2$ | EDAC·HCl | 72 |
| **17**, pyridine, DMAP, $CH_2Cl_2$ | None | 58 |
| **16**, DIPEA, THF | T3P® | 62 |

## 2.2.3 The Dieckmann Cyclization

The Dieckmann cyclization, also known as the Dieckmann condensation, is a form of intramolecular Claisen condensation used to synthesize cyclic β-ketoesters of varying ring size. In 1894, Dieckmann reported the reaction of the diesters **18** and **20** with sodium in ethanol to give the corresponding cyclic β-ketoesters **19** and **21**, respectively.[6]



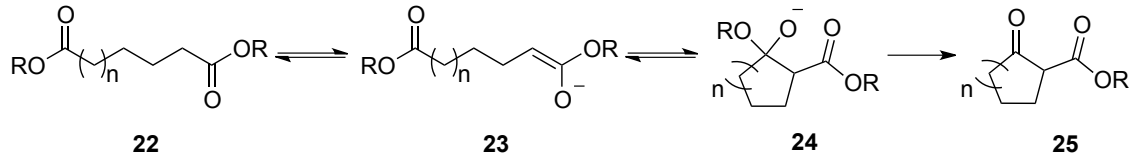

**18** **19**

**20** **21**

**Reagents and Conditions:** a) Na, EtOH.

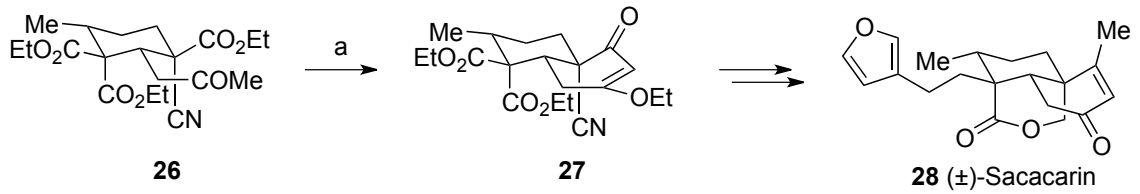
**Scheme 6.**

When diester **22** is treated with a base, the ester enolate intermediate **23** is formed followed by attack of the enolate onto the other ester moiety to give the cyclic β-ketoester **25**. The rate-determining step of the reaction is the ring formation step, the attack of the ester enolate onto the carbonyl of the second ester moiety.




**Scheme 7.** The Dieckmann condensation mechanism.

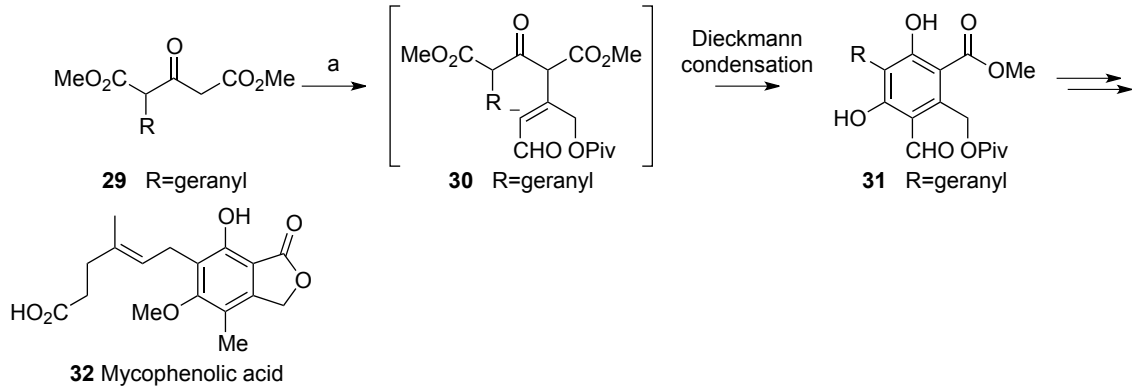
There are many natural product syntheses in the literature that employ a Dieckmann condensation. Grossman and co-workers reported the synthesis of (±)-sacacarin **28** in 1996 in which a Dieckmann condensation was used to synthesize the second ring moiety of the natural product in high yield (90%).[7]




**Reagents and Conditions:** a) 1. NaOEt, EtOH, reflux; 2. TsOH (cat), EtOH, $C_6H_6$-$H_2O$, reflux, 90% (2 steps).

**Scheme 8.** The synthesis of (±)-sacacarin.

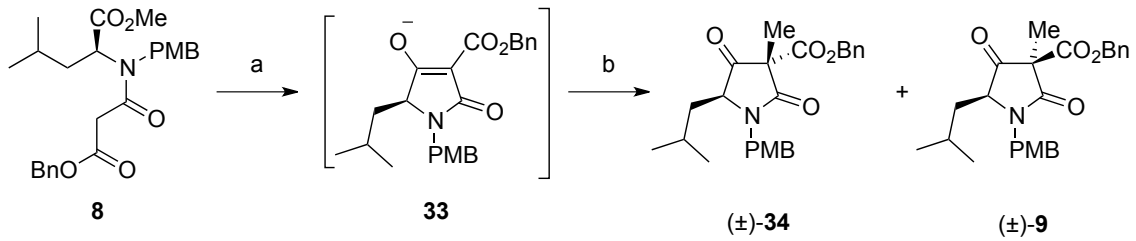
In 2003, Covarrubias-Zúñiga reported the synthesis of mycophenolic acid.[8] 2-Geranyl-1,3-acetonedicarboxylate **29** was treated with sodium hydride followed by the addition of a protected alkynal to form the enolate which then underwent Dieckmann condensation to give the substituted aromatic ring of mycophenolic acid **31** in 33% yield (**Scheme 9**).




**Reagents and Conditions:** a) 1. NaH, THF, RT; 2. Protected alkynal, 2 h; 3. HCl 33% (3 steps).

**Scheme 9.** The synthesis of mycophenolic acid.

As described above (**Chapter 1**, **Scheme 27**), the Page group reported a two-step process wherein cyclization occurs first using TBAF in diethyl ether to form the tetrabutylammonium salt, followed by the methylation step in tetrahydrofuran to install the C7 functionality. Methylation occurs through the enolate intermediate **33** resulting in the formation of the two diastereoisomers (±)-**34** and (±)-**9** in 30 and 29% yield, respectively (**Scheme 10**). Unfortunately, diastereoisomers (±)-**34** and (±)-**9** are difficult to separate using silica gel chromatography because of their similar polarity, leading to the isolation of a mixture of (±)-**34** and (±)-**9** (as well as the pure diastereoisomers).




**8** **33** (±)-**34** (±)-**9**

**Reagents and Conditions:** a) TBAF, $Et_2O$, RT, overnight; b) MeI, THF, RT, overnight, (±)-**34**: 30%, (±)-**9**: 29% (2 steps).

**Scheme 10.**

Disappointingly, the reaction did not appear to be diastereoselective. Due to the planarity of enolate intermediate **33**, the small size of the methyl group and the bulky group at C5 being too far away, the addition does not favour either face, resulting in the formation of two diastereoisomers in a 1:1 mixture. Furthermore, specific rotation measurements show racemization had occurred at this point.

Unfortunately, we were unable to obtain crystals suitable for X-ray crystallography of (±)-**34** and (±)-**9**; however, work within the group yielded crystals of the *p*-nitro benzyl analogue of (±)-**34** (**Figure 3**) allowing us to deduce the relative configuration of (±)-**34** and (±)-**9**.

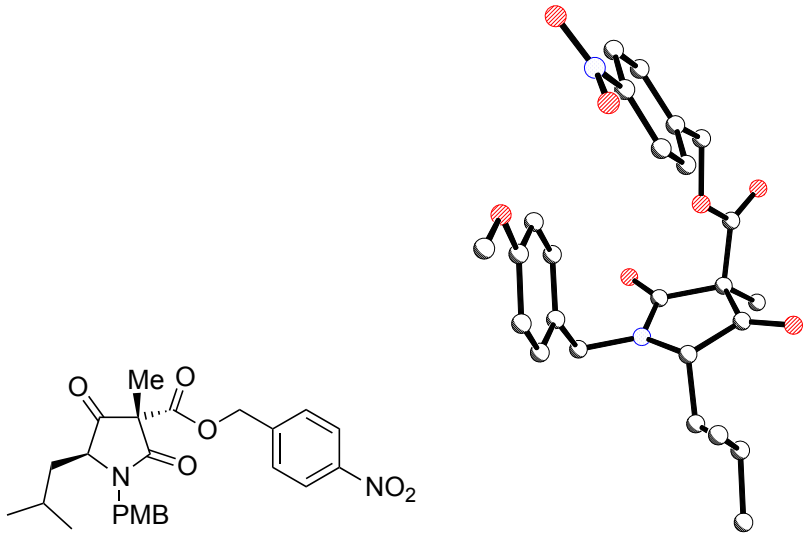
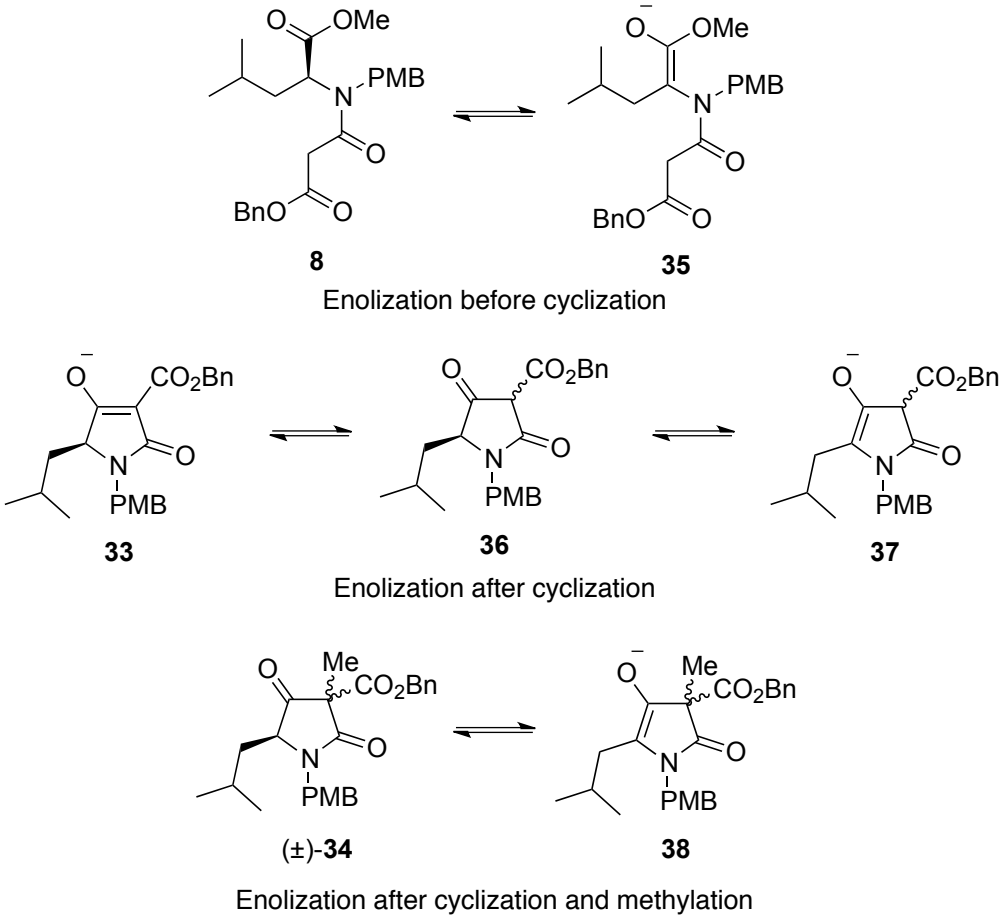


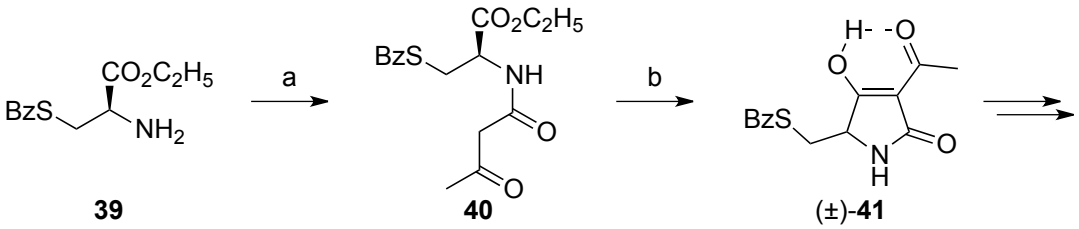

**Figure 3.** X-ray crystallographic structure of the *p*-nitro benzyl analogue of (±)-**34**.

The cyclization results in the formation of two stereocentres and if the only enolate generated was enolate **33**, two non-racemic diastereoisomers would be the only products formed. There are three possible explanations for the racemization: either the racemization occurs before the cyclization (**35**), or after the cyclization but before the methylation (**37**), or after the methylation (**38**); it could also possibly occur during more than one step. We do not believe racemization occurs before cyclization; indeed, the difference in pKa between the enolisable proton of the amino ester (pKa≈25) and the malonyl protons (pKa≈13) leads us to believe racemization should not occur at this point. Enolization through the C5 position could occur before or after methylation, as methoxide is generated during the cyclization step, so deprotonation at C5 would be possible. Interestingly, we were unable to observe any products resulting from the methylation at C5. Due to the large excess of methyl iodide used, if enolization occurred after methylation at C7 to form **38**, we would also expect to see methylation at C5. However, if enolization occurs first, selective methylation at C7 may be observed because the proton at C7 (pKa≈11) is much more acidic than that at C5 (pKa≈25) making **33** the thermodynamically more stable enolate form; hence, the equilibrium between **33** and **37** is shifted towards **33**.




**Scheme 11.**

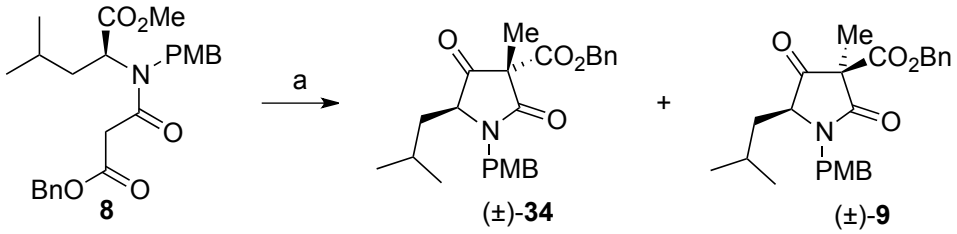
This result is not unprecedented; racemization during Dieckmann cyclization has been reported previously. In 1990, Poncet and co-workers reported the '*racemisation during the synthesis of tetramic acids via Dieckmann cyclisation*'.[9] In 1964, Lukas reported the total synthesis of holomycin in which a key step was the cyclization of **40** with sodium ethoxide to form the racemic lactam (±)-**41**.[10]




**Reagents and Conditions:** a) diketene, EtOH, RT; b) NaOEt, EtOH, benzene, reflux.

**Scheme 12.**

A one-pot tandem Dieckmann cyclization/methylation synthesis was also attempted with no notable loss in yield. Treatment of **8** with TBAF and methyl iodide in tetrahydrofuran gave the diastereoisomers (±)-**34** and (±)-**9** in 22% and 39% yield, respectively (**Scheme 13**). When diethyl ether was used instead of tetrahydrofuran, no reaction was observed and the starting material was recovered.




**Reagents and Conditions:** a) TBAF, MeI, THF, (±)-**34**: 22%, (±)-**9**: 39%.

**Scheme 13.**

Although (±)-**34** and (±)-**9** were isolated in different yields, the $^{1}$H NMR spectrum analysis of the reaction mixture shows the formation of the diastereoisomers in a 1:1 ratio, as observed in the two-step procedure.

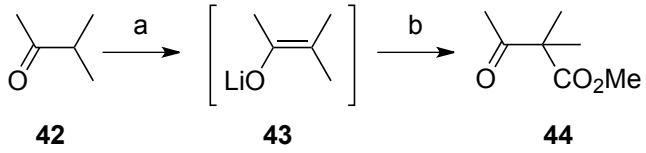
The formation of unwanted diastereoisomers is never an attractive feature in total synthesis as only one has the correct relative stereochemistry required thereby reducing the yield of usable material.

## 2.2.4 The Mander's Acylation

The next step in our synthesis was the introduction of the C5 functionality that will eventually be used to form the β-lactone moiety, found to be essential to the inhibitors' ability to bind to the 20S proteasome.

In 1983, Lewis Mander reported the '*Regioselective synthesis of β-ketoesters from lithium enolates and methyl cyanoformate*' in which he described the treatment of a range of ketones with lithium diisopropylamine (LDA), hexamethylphosphoramide (HMPA) and methyl cyanoformate in tetrahydrofuran at −78 °C to give the corresponding β-ketoesters.[11] This method was the first of its kind to allow access to

these types of compounds in consistently high yields with complete chemoselectivity. This reaction can also be carried out using methyl chloroformate instead of methyl cyanoformate.[12, 13]

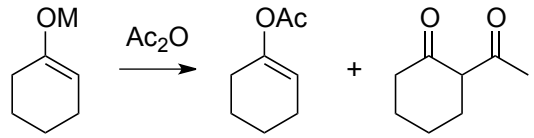



**Reagents and Conditions:** a) LDA, THF b) HMPA, methyl cyanoformate.

**Scheme 14.**

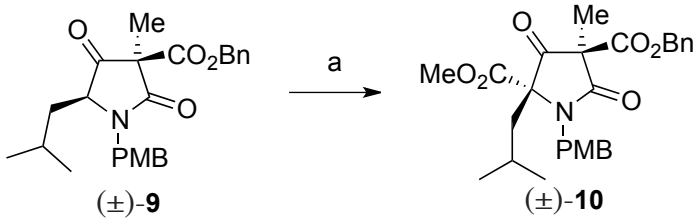
Older methods such as the reaction with acyl halides or anhydrides often lacked selectivity and resulted in a mixture of both the *C*- and *O*-alkylated products. Acylation of the cyclohexanone enolate **45** gives different ratios of *C*- and *O*-alkylated products depending on the metal cation and solvent used (**Table 3**).[14]

**Table 3.** A comparison between the *C*- and *O*-alkylation when reacting with acetic anhydride.




| Metal Cation | Solvent | Yield of 46 | Yield of 47 |
|---|---|---|---|
| $Li^+$ | Dimethoxyethane (DME) | 49% | 16% |
| $Mg^{2+}$ | Diethyl ether | 25% | 43% |

With crystallographic data analysis showing that compound (±)-**9** has the desired relative stereochemistry, the acylation reaction was carried out following a procedure similar to Mander's (**Scheme 15**).




**Reagents and Conditions:** a) LiHMDS, DMPU, methyl cyanoformate, THF, –78 °C, 86%.

**Scheme 15.**

Compound (±)-**9** was treated with LiHMDS (freshly prepared from *n*-butyl lithium and hexamethyldisilazane) in tetrahydrofuran at –78 °C and the reaction mixture stirred for one hour. 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU) and methyl cyanoformate were then added. The reaction was stirred at –78 °C for four hours after which it was quenched using an aqueous solution of ammonium chloride. The desired compound (±)-**10** was isolated in 79% yield. Due to the reaction going through an enolate intermediate, addition of the methyl ester group could occur from the top or bottom face resulting in the formation of diastereoisomers. However, this was not observed; analysis of the $^{1}$H NMR spectrum confirms that the acylation is stereoselective as a single product was observed, and crystallographic data of the product obtained from the reduction of the ketone moiety of (±)-**10** (**Figure 5**, compound (±)-**51**) shows that the C5 methyl ester moiety adds to the opposite side of the C7 benzyl ester. When we compare the stereochemistry of lactacystin and (±)-**10** (**Figure 4**) we can see that the correct relative stereochemistry has been achieved.

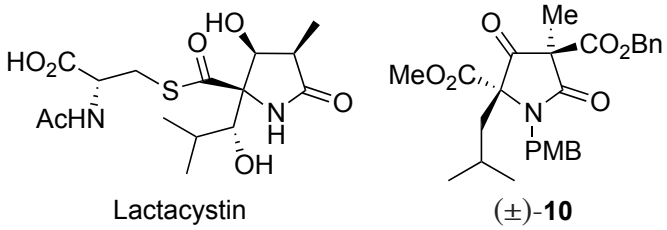
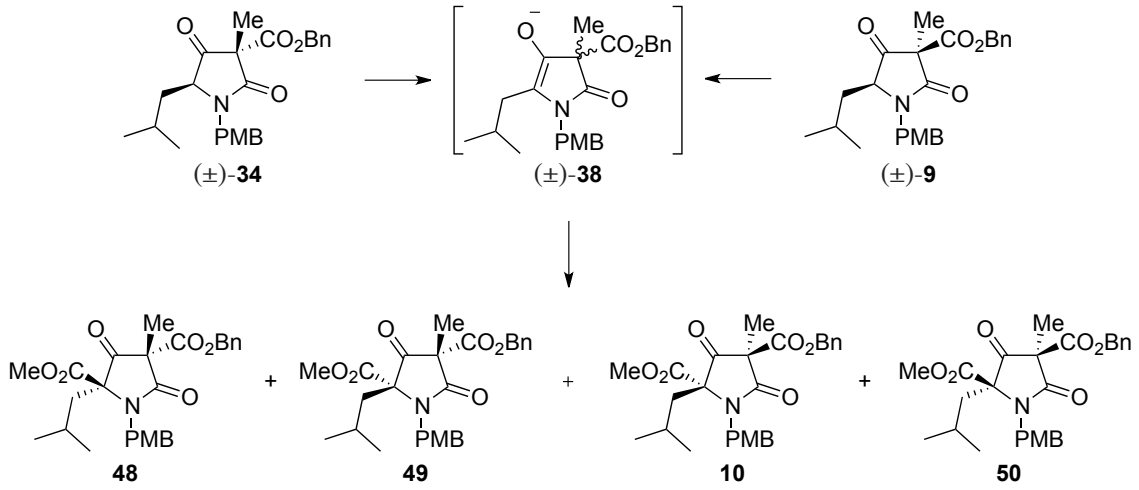



**Figure 4.** Relative configuration of (±)-**10**.

Due to the racemization occurring during the Dieckmann cyclization/methylation step and the stereoselective nature of the acylation, we thought that the separation of the diastereoisomers (±)-**34** and (±)-**9** might not be necessary. Indeed, (±)-**34** and (±)-**9** should lead to the same racemic enolate (±)-**38**. Then, in theory, if the acylation reaction

were not selective, racemic enolate (±)-**38** would lead to four possible stereoisomers: **48**, **49**, **10** and **50**. It is important to note here that compounds **48** and **10**, as well as **49** and **50**, are enantiomers, respectively.



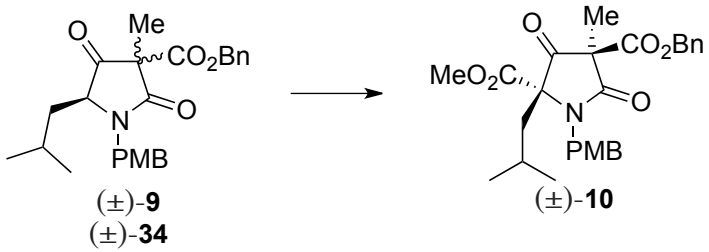

**Scheme 16.** Investigation into the Mander's acylation reaction.

As the acylation is selective, we predicted that compound (±)-**34** should behave in the same way as (±)-**9**, and lead to the formation of (±)-**10**. Indeed, when compound (±)-**34** was subjected to the acylation conditions described above, only compound (±)-**10** was isolated in 73% yield. NMR and IR spectroscopic and mass spectrometric analysis of the product obtained showed the compound isolated from this reaction was the same as that isolated from the reaction of (±)-**9**. To further support this conclusion, the reaction was attempted using a mixture of (±)-**34** and (±)-**9**. Unsurprisingly, only one compound was isolated from this reaction, compound (±)-**10**.

When we first started investigating this reaction, the LiHMDS was prepared *in situ* from the reaction of *n*-butyl lithium and hexamethyldisilazane in tetrahydrofuran at –78 °C giving (±)-**10** in 79% yield. The yield was increased to 86% when commercially available LiHMDS was used. This difference in yield may be due in part to the accumulative internal error associated with the preparation of LiHMDS and subsequent cannulation of the base into the reaction mixture. In 2013, Pfizer reported the synthesis of filibuvir in which they use LiHMDS to perform a Dieckmann cyclization.[15] They describe how both the source of LiHMDS and the mode of addition of LiHMDS can affect the yield of product obtained. They report a difference in yield when using *n*-

BuLi or Li metal as the lithium source and a difference in yield when using LiHMDS from different suppliers. They stated that they did not know why there was a difference and concluded by describing the results as an '*unexplained LiHMDS source variation*'.

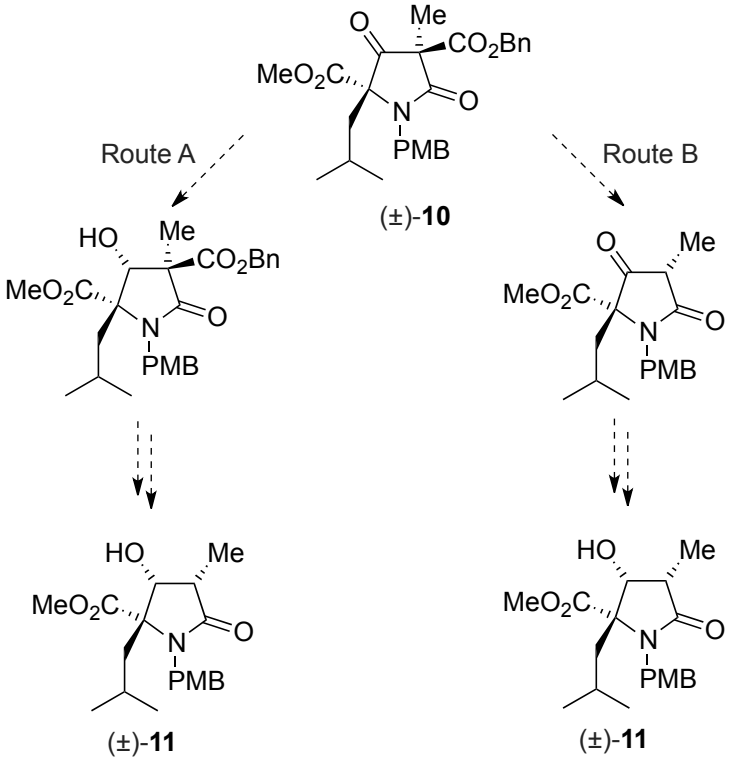
**Table 4.** Investigation into the Mander's acylation reaction.




| Starting Material | Base | Yield of (±)-10 (%) |
|---|---|---|
| (±)-**9** | LiHMDS (made *in situ*) | 79 |
| (±)-**34** | LiHMDS (made *in situ*) | 73 |
| Mixture of (±)-**9** and (±)-**34** | LiHMDS (made *in situ*) | 78 |
| Mixture of (±)-**9** and (±)-**34** | LiHMDS (commercial) | 86 |

## 2.2.5 Steps Towards the Synthesis of (±)-11

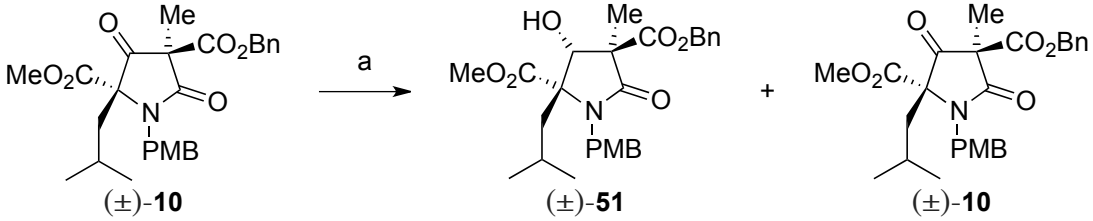
At this stage in the synthesis, two routes are possible to obtain the desired intermediate; the first possibility involves the reduction of the ketone at C6 followed by decarboxylation of the benzyl ester moiety at the C7 position (**Scheme 17**, Route A), while the second route starts with the decarboxylation of the benzyl ester moiety at the C7 position followed by reduction of the ketone at C6 (**Scheme 17**, Route B).




**Scheme 17.**

## 2.2.5.1 Route A Towards (±)-11

### 2.2.5.1.1 Reduction of the Mander's Reaction Product, (±)-10




**Reagents and Conditions:** a) $NaBH_4$, EtOH, 0 °C to RT, 30 min, 43-57%.

**Scheme 18.**

Under standard conditions using sodium borohydride (**Scheme 18**), compound (±)-**51** was isolated as a single diastereoisomer in yields of 43-57%. Crystallographic studies show that the correct relative stereochemistry was achieved when compared to that of lactacystin; the hydroxyl moiety, the methyl group and the methyl ester, at the C6, C7 and C5 positions, respectively, are all on the same side relative to each other.

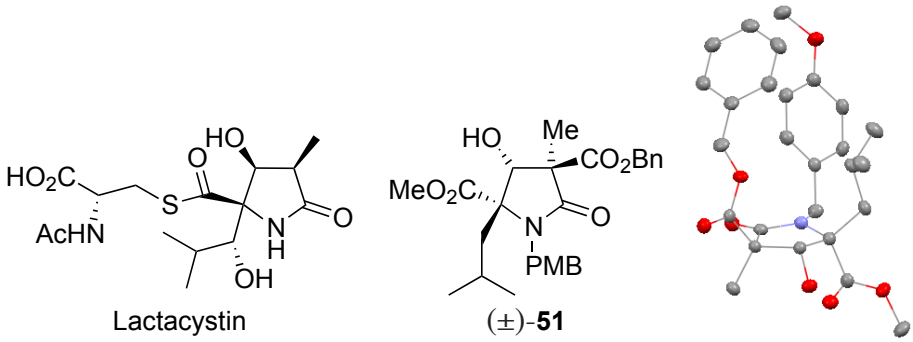
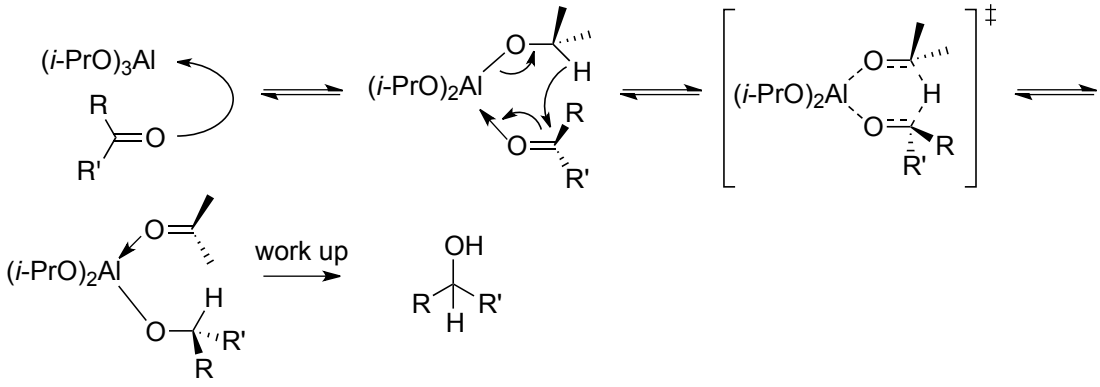



**Figure 5.** X-ray crystallographic structure of (±)-**51**.

In an attempt to optimize this reaction, other reducing reagents were screened under varying conditions (**Table 5**). The use of sodium cyanoborohydride or sodium triacetoxyborohyride resulted in full recovery of the starting material. Attempts using DIBAL also led to the recovery of the starting material probably due to the bulky nature of DIBAL.

The Meerwein-Ponndorf-Verley (MPV) reduction (the reverse reaction, the oxidation of the alcohol, is known as the Oppenauer oxidation) is a well-documented reaction that uses an excess of aluminium isopropoxide and *iso*-propyl alcohol to reduce ketones to the corresponding alcohols.[16] We had hoped to use this reaction as one of its key features is its highly diastereoselective nature when applied to rigid cyclic compounds.

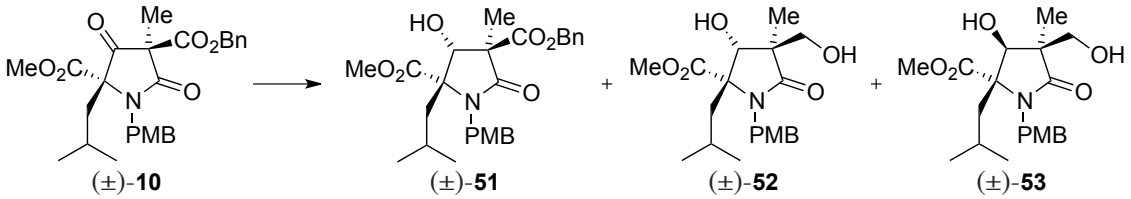



**Scheme 19.** The Meerwein-Ponndorf-Verley reduction.

Formation of the aluminium complex occurs at the less hindered face; this then directs the subsequent formation of the six-membered transition state and therefore the hydride transfer. Unfortunately, this approach was not successful and the starting material was

recovered (**Table 5**, Entries **12** and **13**). One of the drawbacks of the MPV reduction is that it is greatly affected by steric hindrance; hence, we believe, with a relatively small 5-membered ring and large groups at the C5 and C7 positions, the steric hindrance was too great.

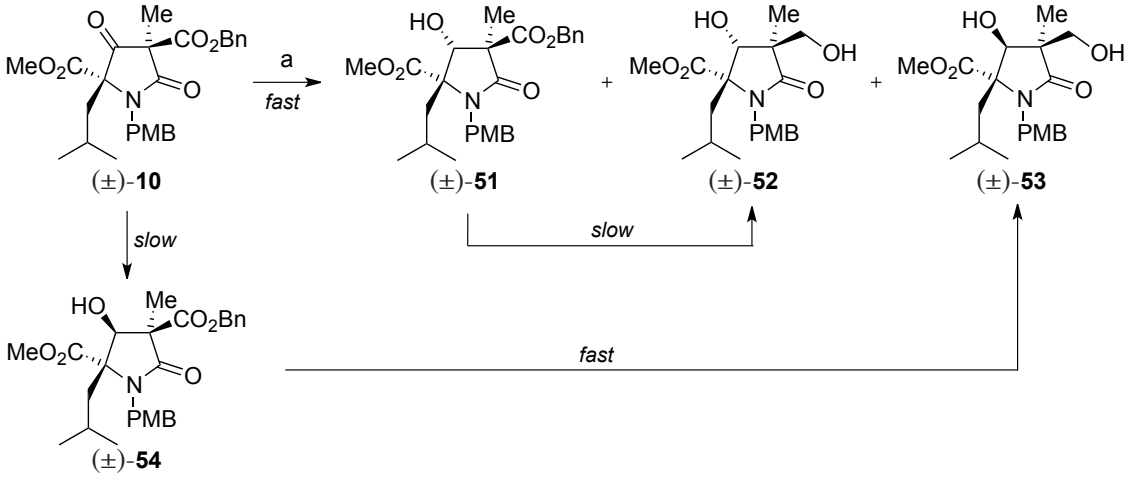
**Table 5.** Investigation into the reduction of (±)-**10**.




(±)-**10** (±)-**51** (±)-**52** (±)-**53**

| Entry | Reducing Reagent | Reactions Conditions | Product | Yield (%) |
|---|---|---|---|---|
| **1** | $NaBH_3CN$ (2 equiv.) | Acetic acid, MeOH, 0 °C – RT, 4 h | S.M. | 100 |
| **2** | $NaBH_3CN$ (2 equiv.) | Acetic acid, MeOH, 0 °C – RT, 48 h | S.M. | 100 |
| **3** | $Na(AcO)_3BH$ (10 equiv.) | $CH_2Cl_2$, 0 °C – RT, 24 h | S.M. | 100 |
| **4** | $NaBH_4$ (2 equiv.) | Acetic acid, MeOH, 0 °C – RT, 4 h | S.M. | 100 |
| **5** | $NaBH_4$ (0.7 equiv.) | EtOH, –10 °C, 2 h | (±)-**51**<br>(±)-**52** or (±)-**53**<br>(±)-**52** or (±)-**53** | 42<br>29<br>10 |
| **6** | $NaBH_4$ (2 equiv.) | EtOH, 0 °C – RT, 30 min | (±)-**51** | 43-57 |
| **7** | $NaBH_4$ (0.7 equiv.) | EtOH, –20 °C, 1 h | (±)-**51** | 51 |
| **8** | $NaBH_4$ (0.7 equiv.) | EtOH, –10 °C, 1 h | (±)-**51** | 54 |
| **9** | $NaBH_4$ (0.7 equiv.) | EtOH, –10 °C, 30 min | S.M.<br>(±)-**51** | 31<br>52 |
| **10** | DIBAL (1 equiv.) | THF, 60 °C, 4 h | S.M. | 100 |
| **11** | DIBAL (2 equiv.) | THF, 60 °C, 4 h | S.M. | 100 |
| **12** | $(i\text{PrO})_3Al$ (10 equiv.) | *i*-PrOH, reflux, 24 h | S.M. | 100 |
| **13** | $(i\text{PrO})_3Al$ (10 equiv.) | *i*-PrOH, Dean-Stark reflux, 24 h | S.M. | 100 |

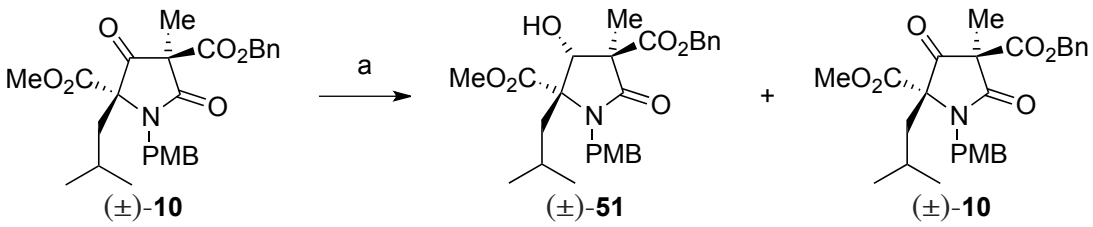
With all attempts failing when changing the reducing reagent, optimization of the reaction conditions using sodium borohydride was carried out. It became clear that both reaction time and temperature were significant factors in the success of this reaction.




**Reagents and Conditions:** a) $NaBH_4$, EtOH, –10 °C, 2 h, (±)-**51**: 42%, (±)-**52** or (±)-**53**: 29%, (±)-**52** or (±)-**53**: 10%.

**Scheme 20.**

When using sodium borohydride as the reducing reagent at –10 °C for two hours (**Table 5**, Entry **5**), the desired compound (±)-**51** was isolated as the first eluting compound after silica gel column chromatography in 42% yield. Two over-reduced products, diastereoisomers (±)-**52** and (±)-**53**, were also isolated from the reaction. Although we know the structures of (±)-**52** and (±)-**53**, we were unable to determine their relative configurations. The second eluting compound was isolated in 29% yield and the third in 10% yield. As we do not isolate compound (±)-**54**, we can predict the relative rates of the reaction. The formation of (±)-**51** must be much faster compared to that of (±)-**54**; however, (±)-**54** must then be converted to (±)-**53** very quickly (**Scheme 20**).




**Reagents and Conditions:** a) $NaBH_4$, EtOH, –10 °C, 30 min, (±)-**51**: 52%, (±)-**10**: 31%.

**Scheme 21.**

Optimization of the conditions using sodium borohydride led to the isolation of the product (±)-**51** in 52% yield as well as the recovery of starting material (±)-**10** in 31% yield (**Table 5**, Entry **9**).

Fortunately, this reaction was completely stereoselective. The formation of (±)-**54** under these conditions is so slow that, when left for only 30 minutes, only one diastereoisomer was ever isolated and, as described above, no trace of (±)-**54** was ever observed. Although this is a positive result, as the correct relative stereochemistry in relation to lactacystin (**Figure 5**) has been achieved, this is an interesting result because hydride addition occurs from the opposite face to that which one would predict. The crystal structure shows the benzyl ester, *iso*-butyl and PMB groups all occupying space above the ring; we can assume this would be the same for the starting material (±)-**10**. It would, therefore, not be unprecedented to expect the hydride to approach from underneath, which is the least sterically hindered face, resulting in the hydroxyl group being on the same side as the benzyl ester, *iso*-butyl and PMB groups. When using Newman projections to predict the stereochemistry of the product obtained from reduction the hydroxyl group would, again, be expected to form on the same side as the benzyl ester, *iso*-butyl and PMB groups.

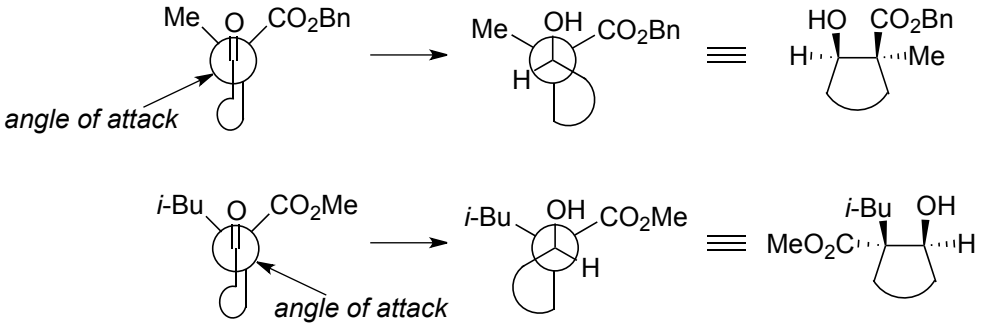
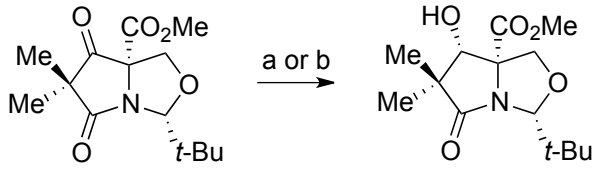



**Figure 6.** The Newman projection for the prediction of the stereoselectivity of the ketone reduction.

In 2008, Moloney and co-workers reported the '*equilibration in bicyclic pyroglutamates by ring opening-reclosure*' in which the stereoselective ketone reduction of **55** is described using $NaBH_4$ in ethanol or LAH in THF to give the corresponding alcohol **56** (**Scheme 22**).[17]



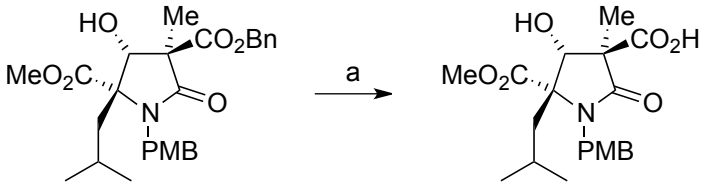

**55** **56**

**Reagents and Conditions:** a) $NaBH_4$, EtOH, 0 °C, 1 h; b) $LiAlH_4$, THF, –78 °C, 1 h.

**Scheme 22.**

They state that the selectivity is achieved by *endo*-addition of the small hydride nucleophile *anti*- to the lone pair of the nitrogen. In our case the lone pair on the nitrogen is on the bottom face and so the hydride will attack from the top face (the same face as the benzyl ester, *iso*-butyl and PMB groups) resulting in the hydroxyl group being on the same side as the methyl ester group at C5 and the methyl group at C7.

### 2.2.5.1.2 Decarboxylation of Compound (±)-51




(±)-**51** (±)-**57**

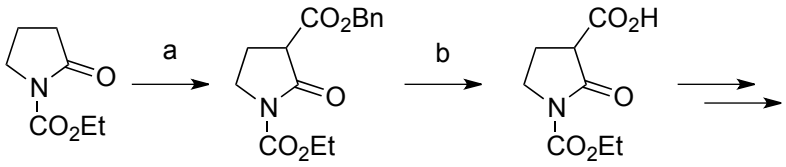
**Reagents and Conditions:** a) $H_2$, $Pd(OH)_2/C$, THF, quant.

**Scheme 23.**

When (±)-**51** was treated under hydrogenolysis conditions using palladium hydroxide on carbon and a hydrogen balloon the corresponding carboxylic acid (±)-**57** was isolated in quantitative yield without purification. We had hoped that the treatment of (±)-**51** under the hydrogenolysis conditions would proceed with concomitant decarboxylation due to the presence of the carbonyl moiety in the β position enabling formation of the enolate. This was not the case however, and the corresponding carboxylic acid (±)-**57** was the only product isolated from this reaction. For decarboxylation to occur the intermediate must be stabilized through conjugation, usually through the enolate. When the ketone of (±)-**10** is reduced to a hydroxyl moiety, the enolate can no longer be formed. The carbonyl group at C8 is part of an amide

moiety and conjugation between the nitrogen atom lone pair and the carbonyl group is possibly too strong to allow enolization to proceed through this position.

A literature search shows that this result is not unprecedented. There are many reports of the debenzylation of substituted γ-lactams to form the corresponding carboxylic acid.[18] In 1994, Leonard and co-workers reported *'a sulfolene-based intramolecular Diels-Alder approach to the synthesis of manzamine A*' in which they subject the benzyl ester **59** to hydrogenolysis conditions using $H_2$ and 5% Pd/C in diethyl ether, and the product obtained is the corresponding carboxylic acid **60**.[19]

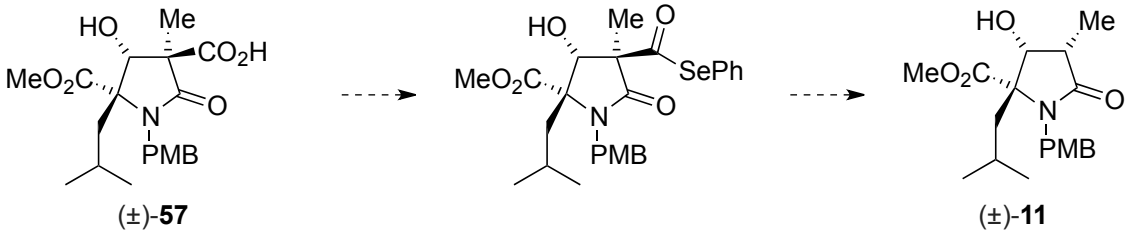



**Reagents and Conditions:** a) 1. $(Me_3Si)_2NLi$, THF; 2. $PhCH_2COCN$; b) $H_2$, 5% Pd/C, $Et_2O$.

**Scheme 24.**

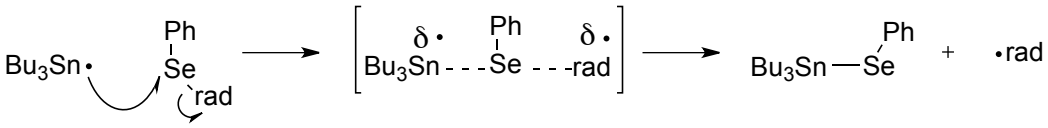
### 2.2.5.1.2.1 Radical-Mediated Decarbonylation of the Acyl Selenide

As the hydrogenolysis of (±)-**51** yielded the carboxylic acid (±)-**57** instead of the desired (±)-**11**, methods to achieve the decarboxylation of (±)-**57** were investigated. First, we decided to carry out the synthesis of an acyl selenide from the carboxylic acid (±)-**57** followed by a radical-mediated decarbonylation to give the desired advanced intermediate (±)-**11.**




**Scheme 25.**

Selenium compounds have many uses in organic synthesis including in radical reactions as precursors. The C-Se and Se-H bonds are considered relatively weak, this along with the polarizability of the selenium atom makes organoselenides useful compounds for radical chemistry.[20]




**Scheme 26.**

Tributyltin hydride ($Bu_3SnH$), tributylgermanium hydride ($Bu_3GeH$) and tris(trimethylsilyl)silane (TTMSS) are commonly used as radical initiators, where the precursor is a halide, for example bromides and iodides. Selenide groups, specifically phenylselenenyl (PhSe) groups, have been used to replace the halides, and rate studies show that abstraction rates with $Bu_3Sn$•, $Bu_3Ge$•, and primary alkyl ($RCH_2$•) radicals are comparable to that of the corresponding bromide, but smaller than the corresponding iodide by several orders of magnitude.[21]

**Table 6.** Reaction constants for the abstraction of PhSe, Br and I.

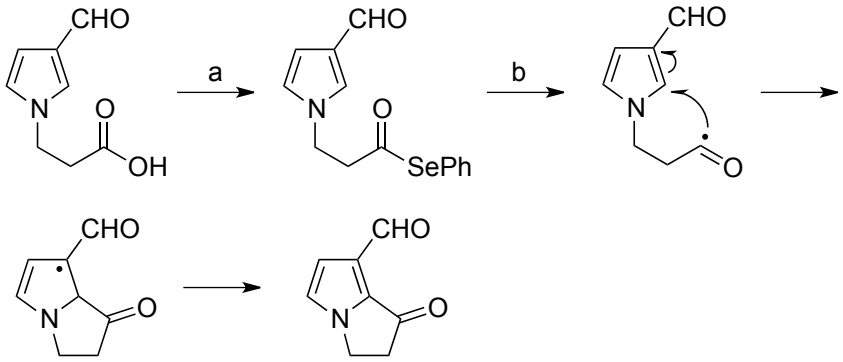
| Radical | Compound | Temperature (°C) | Rate Constant ($M^{-1}s^{-1}$) |
|---|---|---|---|
| $RCH_2$• | PhSe-$CH_2CO_2Et$ | 50 | 1.0 x $10^5$ |
| $RCH_2$• | Br-$CH_2CO_2Et$ | 50 | 0.7 x $10^5$ |
| $RCH_2$• | I-$CH_2CO_2Et$ | 50 | 2.6 x $10^7$ |
| $Bu_3Sn$• | PhSe-$CH_2CO_2Et$ | 25 | 1.2 x $10^8$ |
| $Bu_3Ge$• | PhSe-$CH_2CO_2Et$ | 25 | 9.2 x $10^8$ |

Interestingly, when acyl selenides are used, the abstraction of the phenylselenenyl group is faster than when using alkylphenylselenides. The most common reagents for the abstraction of the PhSe group from an acyl selenide are $Bu_3SnH$ and TTMSS; however, $Bu_3GeH$ has also been shown to work in the same way. Acyl selenides are easily synthesized from the corresponding carboxylic acid.

As well as the abstraction rates, selenenyl groups are a popular choice as they are often referred to as '*radical protective groups*'. Indeed, when compared to the halides, they

can survive many more synthetic transformations, allowing them to be introduced at a much earlier stage of the synthesis if necessary. Another advantage of acyl selenides compared to acyl halides is their stability: it is often possible to purify acyl selenides using silica gel column chromatography.

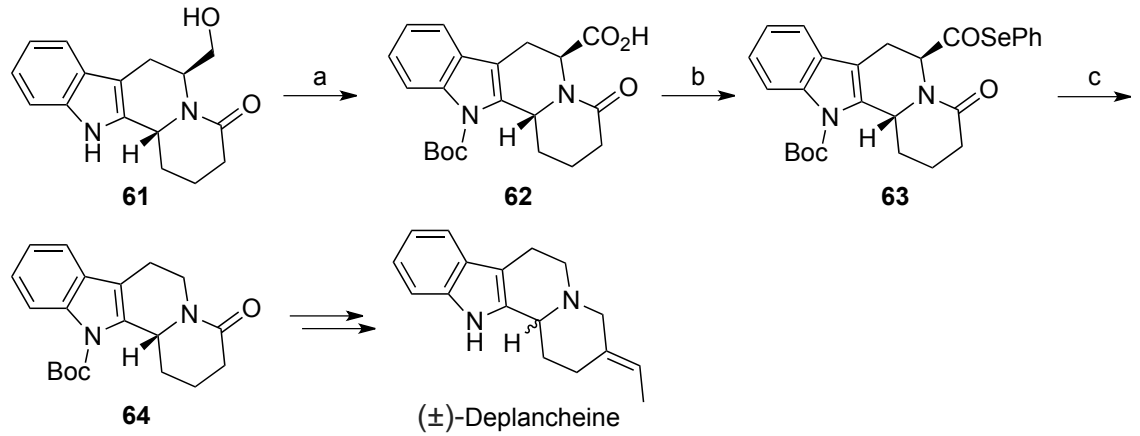
Acyl selenides are often used to generate acyl radicals that are then cyclized (**Scheme 27**).[22] The main drawback of this reaction is the unwanted loss of CO; performing the reaction under an atmosphere of CO can easily prevent this. In our proposed route this is not a problem, as decarbonylation is required to give the desired product.




**Reagents and Conditions:** a) $Bu_3P$, $(PhSe)_2$, $CH_2Cl_2$, 24 h; b) $Bu_3SnH$, AIBN, CO, $CH_3CN$.

**Scheme 27.**

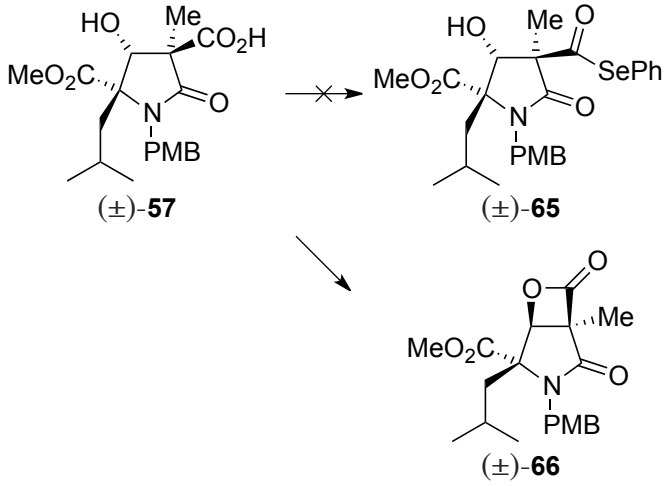
In 2005, the Allin group reported the synthesis of the indole alkaloid deplancheine by employing the radical-mediated decarbonylation of an acyl selenide (**Scheme 28**).[23] The alcohol **61** was oxidized to the carboxylic acid **62**, which was then treated with diphenyldiselenide and tributylphosphine (TBP) in dichloromethane to give the corresponding acyl selenide **63**. The radical-mediated decarbonylation of **63** was achieved upon treatment with *n*-$Bu_3SnH$ and AIBN as the radical initiator in toluene to give **64**.




**Reagents and Conditions:** a) 1. IBX, DMSO, RT, 24 h, 70%; 2. $Et_3N$, $(Boc)_2O$, DMAP, THF, RT, 4 h, 98%; 3. $NaClO_2$, $NaH_2PO_4$, 1-methyl-1-cyclohexene, $CH_3CN$, *t*-BuOH, $H_2O$, 0 °C to RT, 18 h, 83%; b) $(PhSe)_2$, $PBu_3$, $CH_2Cl_2$, 0 °C to RT, 18 h, 83%; c) *n*-$Bu_3SnH$, AIBN, toluene, 80 °C, 2 h, 73%.

**Scheme 28.**

Following the reported procedure, the carboxylic acid (±)-**57** was treated with diphenyldiselenide and tributylphosphine in anhydrous dichloromethane at room temperature overnight.[23] Unfortunately, the desired product was not observed and the β-lactone (±)-**66** was the only product isolated from the complex mixture in a 43% yield. IR spectrum analysis shows a stretch at 1842 $cm^{-1}$ which is a characteristic stretch for the C=O functionality of the β-lactone.




**Reagents and Conditions:** a) $Bu_3P$, $(PhSe)_2$, $CH_2Cl_2$, 24 h, (±)-**66**: 43%.

**Scheme 29.**

At first, this result was surprising as the formation of a *trans* β-lactone is not possible. However, with tributylphosphine present in the reaction mixture, a Mitsunobu-like reaction could have occurred. The most common conditions for the Mitsunobu reaction use triphenylphosphine (TPP) and diethyl azodicarboxylate; however, in 1999 Tsunoda and co-workers reported the use of '*new Mitsunobu reagents in the C-C bond formation*' in which new systems using TMAD-TBP and DHTD-TBP were found to be more efficient in the *N*-alkylation of primary alcohols than the original DEAD-TPP system (**Figure 7**).[24]

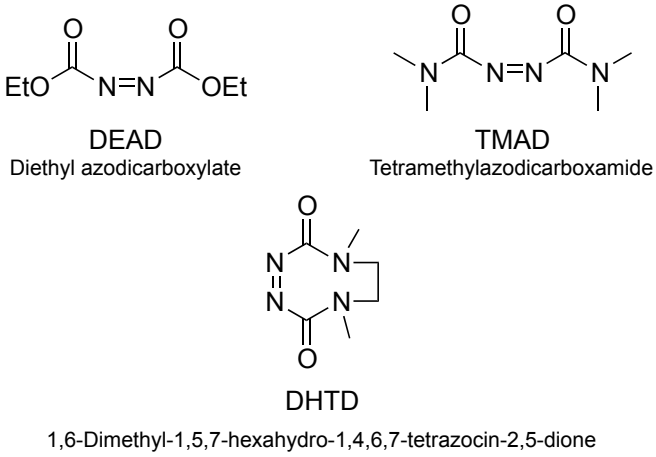
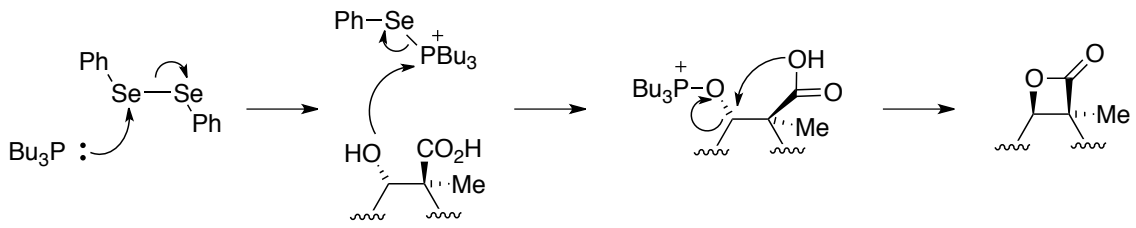



**Figure 7.** Azo-type Mitsunobu reagents.

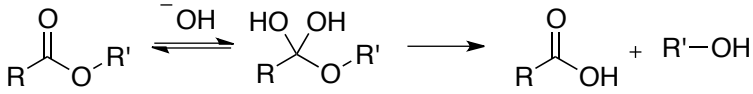
**Scheme 30** describes our proposed mechanism for the formation of the β-lactone (±)-**66**. The phosphine is activated upon reaction with diphenyldiselenide, and the phosphonium selenide is then attacked by the hydroxyl group. An intramolecular lactonization occurs resulting in formation of the β-lactone.



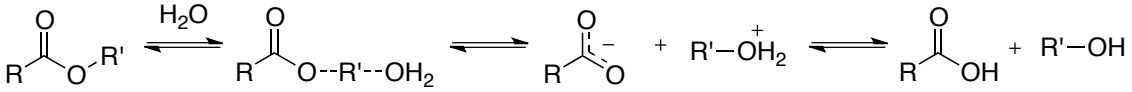
**Scheme 30.**

Consideration of the reverse reaction, the hydrolysis of the β-lactone, and the principle of microscopic reversibility can help support the theory described above. Ester

hydrolysis most commonly occurs through the base-catalysed acyl cleavage ($B_{AC}2$) mechanism; however, there are some reports of the very rare base-catalysed alkyl cleavage ($B_{AL}2$) mechanism (**Scheme 31**).[25]



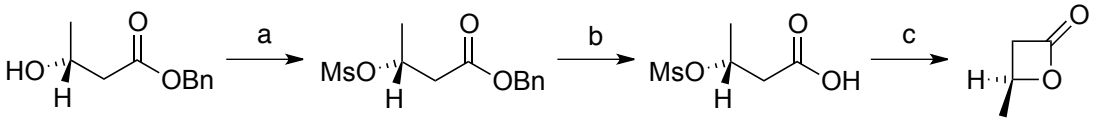
Base-catalysed acyl cleavage ($B_{AC}2$) mechanism.



Base-catalysed alkyl cleavage ($B_{AL}2$) mechanism.

**Scheme 31.**

In 1993, Douglas and co-workers reported their study of the hydrolysis of $^{18}$O-methyl triphenylacetate: even when conditions designed to halt the $B_{AC}2$ mechanism were used, it was still found to be the predominant mechanism and only about 5% of the reaction proceeded through the $B_{AL}2$ mechanism, indicating that the $B_{AL}2$ mechanism is intrinsically unfavourable.[25] In 2013, Casado and co-workers reported the calculated energy barriers for the neutral hydrolysis of a series of lactones.[26] These energies suggested that β-lactones are susceptible to neutral hydrolysis and that some favour the $B_{AL}2$ mechanism.

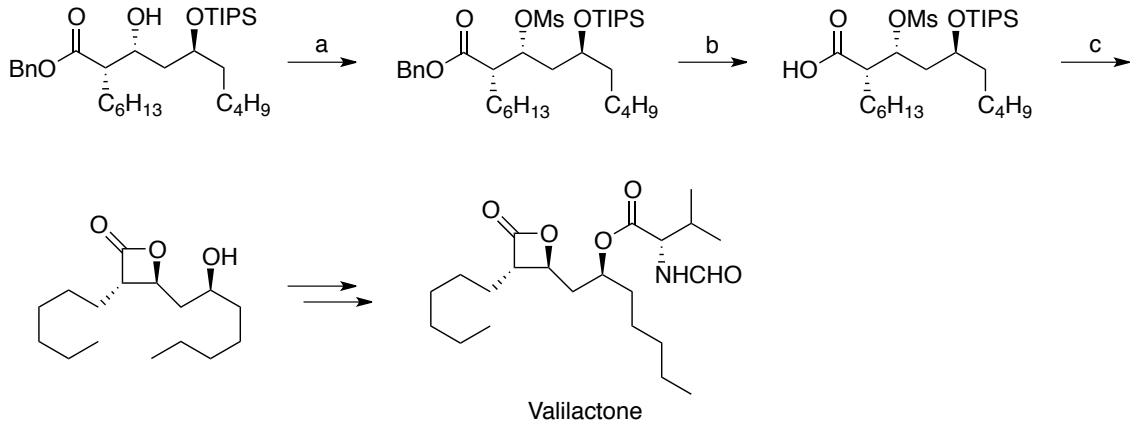


**Reagents and Conditions:** a) Pyridine, mesyl chloride, $CH_2Cl_2$, 5 °C for 40 h then 25 °C for 5 h, 98%; b) HBr in acetic acid, $CH_2Cl_2$, 25 °C for 6 h then –15 °C for 16 h, 71%; c) $H_2O$, $NaHCO_3$, pH 7.5, 45%.

**Scheme 32.**

In 1990 Lenz reported a three-step sequence to form a β-lactone in which the configuration of the β-carbon was inverted (**Scheme 32**).[27] The β-lactone was formed by conversion of the hydroxyl group into a good leaving group using mesyl chloride (MsCl) and triethylamine, followed by cleavage of the benzyl group under

hydrogenolysis conditions to give the corresponding carboxylic acid, and finally, an intramolecular $S_N2$ reaction initiated by the treatment of the carboxylic acid with base. Other groups have since applied this strategic approach to β-lactone formation. In 2006, Wu and co-workers employed this methodology in the total synthesis of valilactone (**Scheme 33**).[28]




**Reagents and Conditions:** a) mesyl chloride, $Et_3N$, $CH_2Cl_2$, 0 °C 3 h, 87%; b) $Pd(OH)_2$, $H_2$, EtOAc/MeOH, RT, 7 h, 100%; c) $K_2CO_3$, THF, RT, 12 h, 71%.

**Scheme 33.**

NOESY NMR analysis of compound (±)-**66** was performed to support our findings and help confirm relative stereochemistry; the NOESY spectrum shows through space interactions as opposed to through bond interactions (*J*-coupling). Through space interactions were observed between the methyl group at C7 and the ring proton at C6; they are on the same side of the ring, confirming the *cis*-lactone structure.

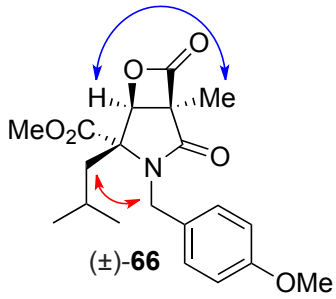
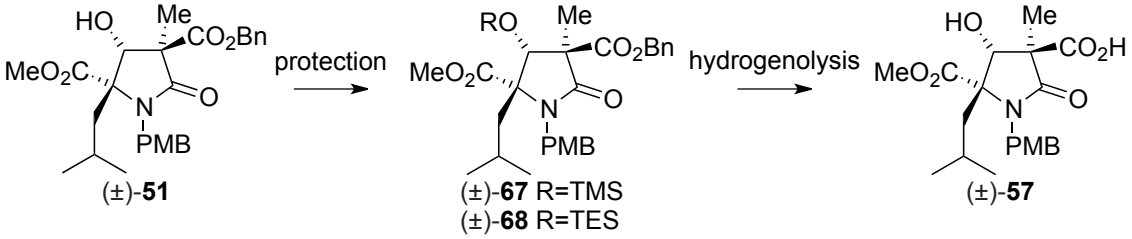



**Figure 8.** NOESY spectrum analysis interpretation of (±)-**66**.

### 2.2.5.1.2.1.1 Silyl Protection

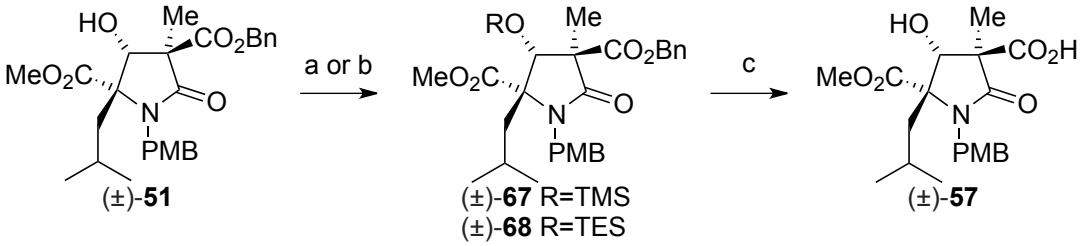
In order to prevent lactonization, protection strategies were investigated. The most common reagents for protecting hydroxyl groups are silylating reagents, for example triisopropylsilyl chloride (TIPS-Cl) and *tert*-butyldimethylsilyl chloride (TBDMS-Cl), as they are often easily added and removed using fluoride. Under standard conditions, treatment of (±)-**51** with TIPS-Cl or TBDMS-Cl proved unsuccessful and the starting material was recovered. Attempts were made using triisopropylsilyl triflate as it is more reactive than its chloride counterpart but the same result was observed. We believe the failure of this reaction is due to steric hindrance, the large benzyl ester at C7 and the two substituents at C5 prevented the addition of the bulky TIPS and TBDMS groups.

**Table 7.** Investigation into the protection of the hydroxyl group of (±)-**51** and subsequent hydrogenolysis.




| Protecting Reagent | Step 1: Protection | Step 2: Hydrogenolysis |
|---|---|---|
| TIPS-Cl | Starting material | N/A |
| TIPS-OTf | Starting material | N/A |
| TBDMS-Cl | Starting material | N/A |
| TMS-Cl | (±)-**67** | (±)-**57** |
| TES-Cl | (±)-**68** | (±)-**57** |

Trimethylsilyl chloride (TMS-Cl) and triethylsilyl chloride (TES-Cl) are not commonly used as protecting reagents as the TMS and TES groups are often poorly stable and cannot survive other synthetic transformations. As we believed the reason the other silyl groups were unsuccessful was due to the groups being too bulky, we decided to test both TMS- and TES-Cl.

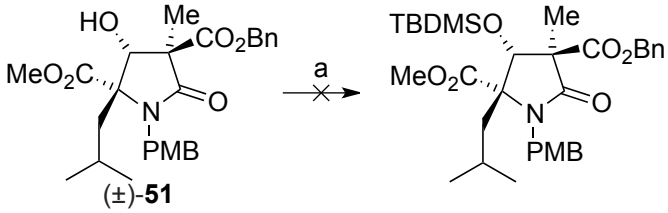



**Reagents and Conditions:** a) TMSCl, imidazole, DMF, 35 °C, 24 h, quant.; b) TESCl, imidazole, DMF, 35 °C, 24 h, quant.; c) $H_2$, $Pd(OH)_2/C$, THF, 30 °C.

**Scheme 34.**

The use of both the TMS and TES groups was successful and compounds (±)-**67** and (±)-**68** were obtained in quantitative yields without purification. Unfortunately, when subjected to the hydrogenolysis conditions, both groups were removed resulting in the isolation of compound (±)-**57**.

In 2008, Stawinski reported work describing hydroxyl protection using *N*-methylimidazole and iodine with various silyl chloride protecting groups.[29] It was found that these conditions not only decreased reaction times but also enabled protection where, before, under standard conditions (i.e. not using iodine) protection was unsuccessful. This promising work led us to try the Stawinski conditions; however, we were only able to recover the starting material.



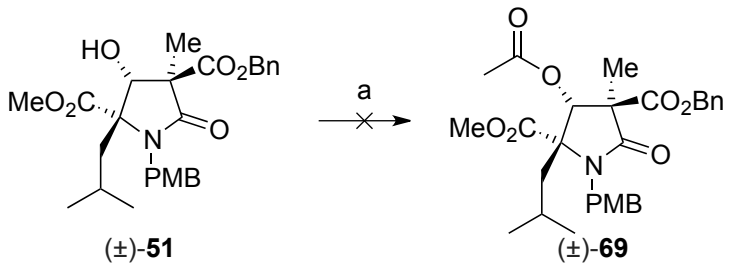

**Reagents and Conditions:** a) TBDMSCl, imidazole, $I_2$, THF, RT, 24 h.

**Scheme 35.**

## 2.2.5.1.2.1.2 Acetate Protection

The synthesis required a protecting group reagent that was reactive enough and small enough while at the same time forming a stable product. The reaction of (±)-**51** with acetyl chloride in an attempt to form the acetate was unsuccessful. Although seemingly

small enough, it did not appear reactive enough under the conditions used and full recovery of the starting material was obtained.



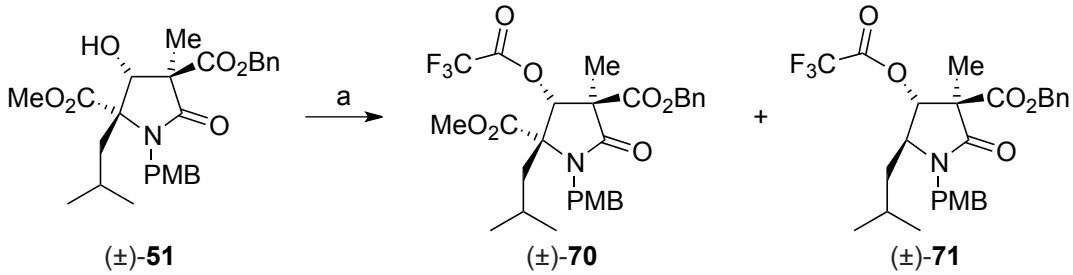

(±)-**51** (±)-**69**

**Reagents and Conditions:** a) acetyl chloride, $Et_3N$, DMAP, $CH_2Cl_2$, RT, 24 h.

**Scheme 36.**

## 2.2.5.1.2.1.3 Trifluoroacetate Protection

Trifluoroacetic anhydride is a well-documented reagent used in the protection of hydroxyl groups.[30] Treatment of (±)-**51** with trifluoroacetic anhydride and pyridine in anhydrous diethyl ether gave the desired compound (±)-**70**, isolated in 52% yield after silica gel column chromatography.



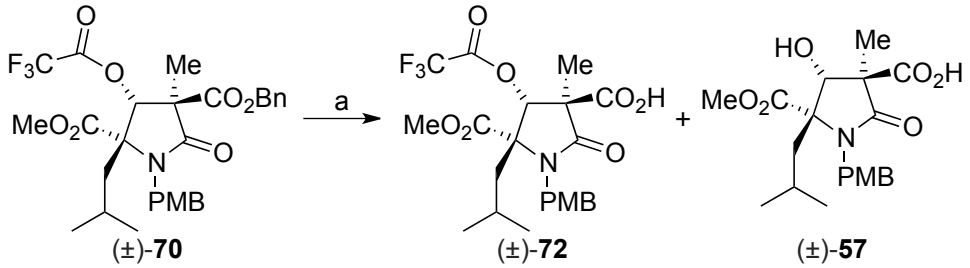

(±)-**51** (±)-**70** (±)-**71**

**Reagents and Conditions:** a) $(CF_3CO)_2O$, pyridine, $Et_2O$, (±)-**70**:52%, (±)-**71**:21%.

**Scheme 37.**

Upon purification, a second compound similar in structure to the desired product (±)-**70** was isolated. Compound (±)-**71** is the suggested structure for the product isolated as supported by $^1H$ NMR, $^{13}C$ NMR, $^{19}F$ NMR, IR and MS data. The $^1H$ NMR spectrum of the crude product mixture before purification did not show the presence of (±)-**71** and so this was believed to be a decomposition product caused by the chromatography. To prevent this, all subsequent reactions were carried out without purification of (±)-**70**.

Treatment of (±)-**70** under hydrogenolysis conditions resulted in the formation of two products in a 1.5:1 ratio according to analysis of the $^{1}$H NMR spectrum of the crude mixture. The first eluting compound was the desired product (±)-**72** in 20% yield. (±)-**57** was also isolated from the reaction mixture in 16% yield and was formed by the trifluoroacetate protecting group being removed during the reaction.

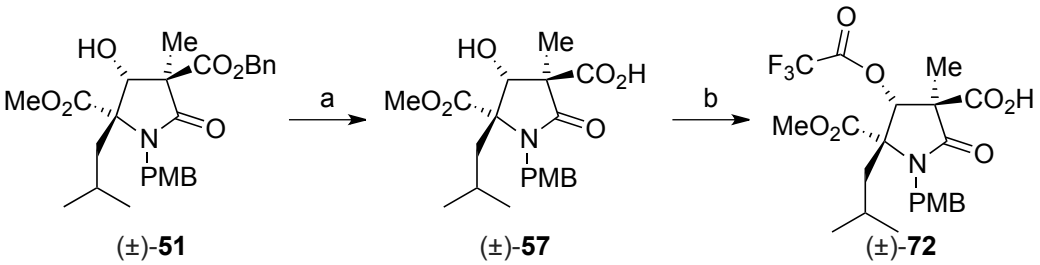



(±)-**70** (±)-**72** (±)-**57**

**Reagents and Conditions:** a) $H_2$, $Pd(OH)_2/C$, THF, 30 °C, (±)-**71**: 20%, (±)-**57**: 16%.

**Scheme 38.**

Although this route was successful, the yield was unsatisfactory. To improve the route, the order of the reactions was inverted to make the carboxylic acid (±)-**57** first, and then protect the hydroxyl group. Treatment of (±)-**57** with trifluoroacetic anhydride gave (±)-**72** in 81% yield (**Scheme 39**).



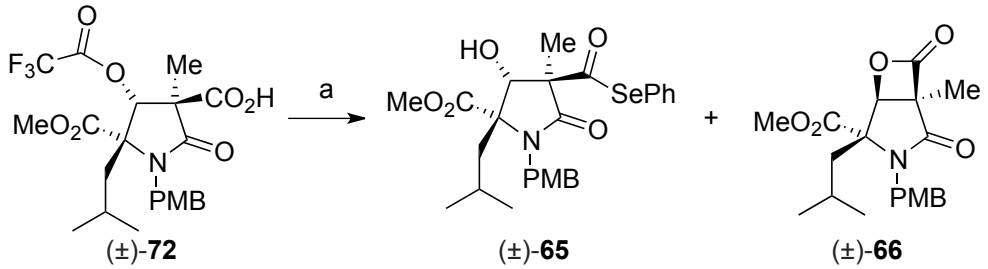

(±)-**51** (±)-**57** (±)-**72**

**Reagents and Conditions:** a) $H_2$, $Pd(OH)_2/C$, THF, 30 °C, quant.; b) $(CF_3CO)_2O$, pyridine, $Et_2O$, 81%.

**Scheme 39.**

### 2.2.5.1.2.2 Synthesis of the Acyl Selenide from the Protected Carboxylic Acid (±)-72 and Subsequent Radical-Mediated Decarbonylation

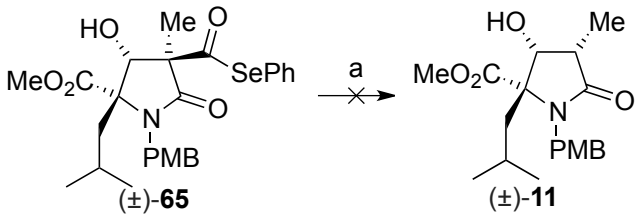
With (±)-**72** in hand, the acyl selenide synthesis was attempted under the conditions described above (**2.2.5.1.2.1**, **Scheme 29**) in the hope that the protecting group would prevent the lactonization reaction.[23] This reaction resulted in a complex mixture and, although successful, the acyl selenide (±)-**65** was isolated in a low yield (31%). The β-lactone (±)-**66** was isolated alongside the desired product in 10% yield. Unfortunately, no other products were isolated in significant yield from this reaction and starting material was not recovered.




**Reagents and Conditions:** a) $Bu_3P$, $(PhSe)_2$, $CH_2Cl_2$, 24 h, (±)-**65**: 31%, (±)-**66**: 10%.

**Scheme 40.**

Following the procedure reported by Allin in 2005, the acyl selenide (±)-**65** was treated with tri-*n*-butyltin hydride and 1,1′-azobis(cyclohexanecarbonitrile) (ABCN) as the radical initiator in anhydrous toluene.[23] Purification of this product proved extremely challenging; $^1$H NMR analysis of the main fractions isolated after silica gel column chromatography showed the extensive presence of tin residues in the samples (characteristic peaks due to tin residues were observed from around 0.8-1.7 ppm).




**Reagents and Conditions:** a) *n*-$Bu_3SnH$, ABCN, toluene, 80 °C, 2 h.

**Scheme 41.**

Mixtures containing tin reagents are notoriously hard to purify. The most common methodology, partitioning between acetonitrile and hexane, proved unsuccessful in our hands. In 2004, Harrowven reported the use of a '*KF-silica stationary phase for the chromatographic removal of tin residues from organic compounds*'.[31] Although the amount of tin residues was significantly reduced using this method, we were unable to remove them completely. Other purification methodologies, including stirring the mixture with KF overnight before purification, also proved unsuccessful.

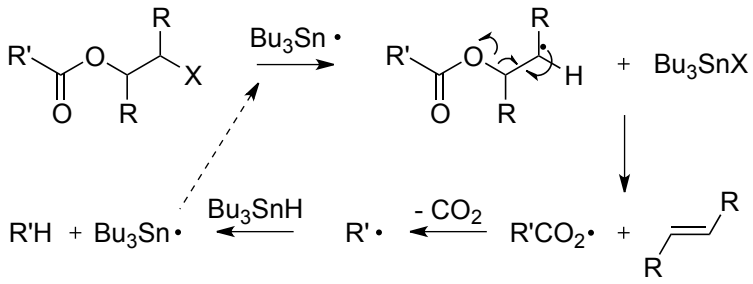
With the tin residues causing such problems in purification, tributylgermanium hydride and tris(trimethylsilyl)silane were used in the reaction instead of the tributyltin hydride. Unfortunately, both methods proved unsuccessful and resulted in complex mixtures, neither the desired compound nor starting material being isolated.

The lack of positive results and various problems arising when using the radical-mediated decarboxylation route led us to investigate alternative decarboxylation strategies.

### 2.2.5.1.2.3 The Barton Decarboxylation

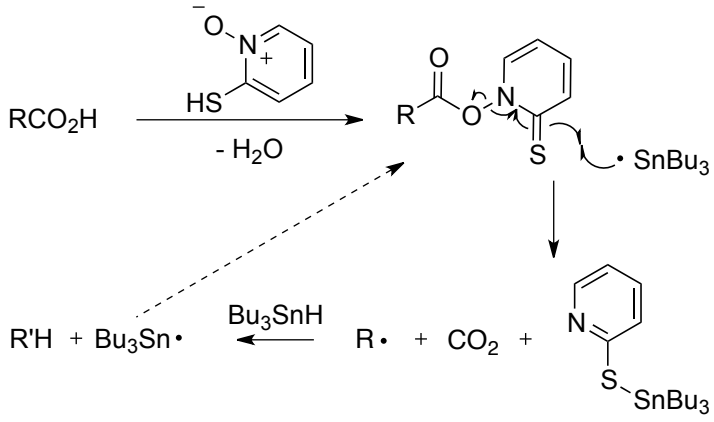
The Barton decarboxylation is another example of a radical-mediated reaction. In 1980, Barton reported '*a new radical decarboxylation reaction for the conversion of carboxylic acids into hydrocarbons*' using tributyltin hydride.[32] Barton suggested that carboxyl radicals could be generated if an efficient alkene-forming radical-mediated fragmentation reaction could be achieved (**Scheme 42**).[32]

Earlier work had shown that, in the steroidal series, the reduction of vicinal chlorohydrin esters using tributyltin hydride resulted only in dehalogenation without any fragmentation of the intermediate radical. Barton believed that the driving force for successful carboxyl radical generation was the formation of a conjugated alkene. Decarboxylation would then occur, resulting in another radical species, followed by hydrogen atom transfer from tributyltin hydride to give the desired hydrocarbon product.



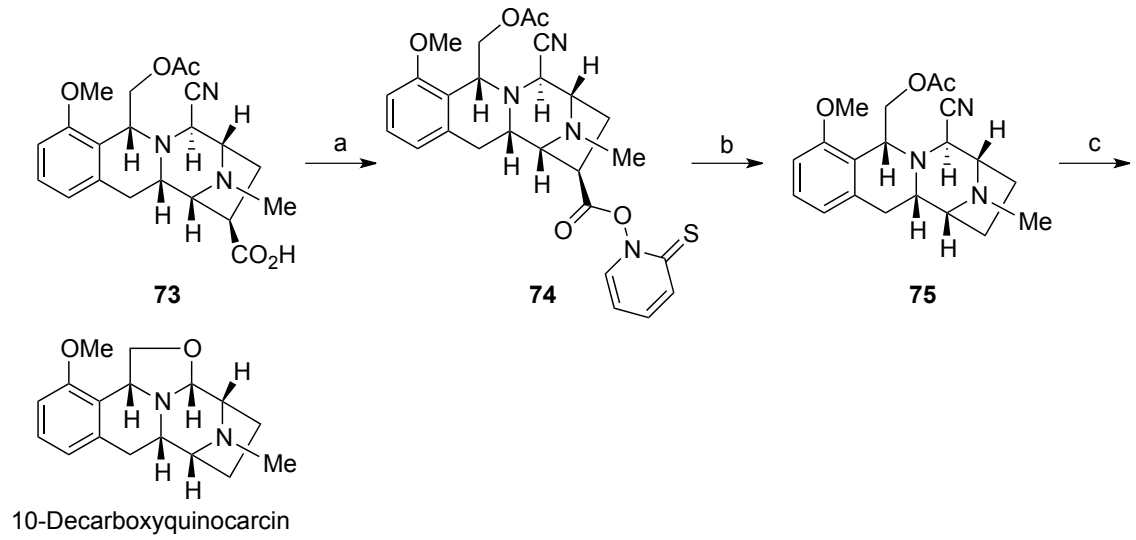

**Scheme 42.**

Later, in 1983, Barton reported '*new and improved methods for the radical decarboxylation of acids*'.[33] The reaction of *N*-hydroxypyridine-2-thione with a carboxylic acid gives the corresponding ester, which can then undergo a radical decarboxylation to form the corresponding alkane upon treatment with tributyltin hydride or *t*-butylmercaptan (**Scheme 43**). These *N*-hydroxypyridine-2-thione esters are now more commonly referred to as '*Barton esters*'.




**Scheme 43.**

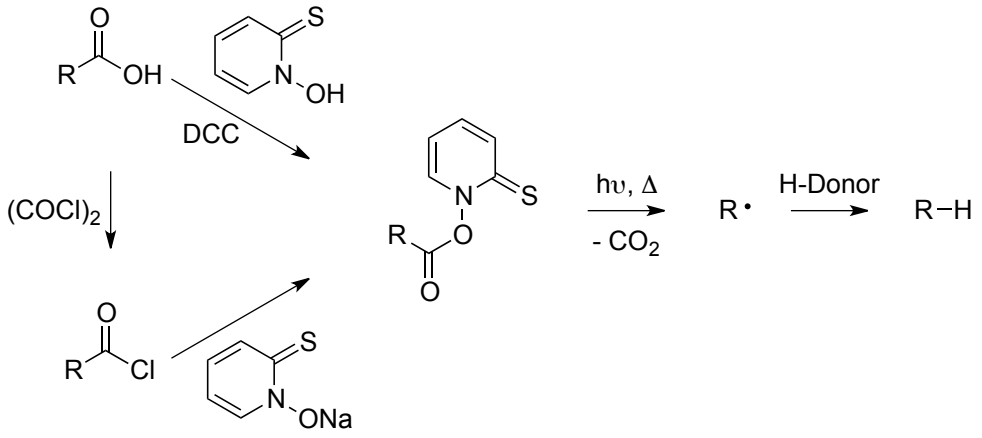
There are many natural product syntheses in the literature that employ a Barton decarboxylation. (–)-Quinocarcin is an antitumour antibiotic, and, when the Terashima group were carrying out structure activity relationship studies, they prepared the 10-decarboxyquinocarcin analogue employing the Barton decarboxylation methodology (**Scheme 44**).[34] The esterification of carboxylic acid **73** was achieved using *N*-hydroxypyridine-2-thione with *N*,*N*'-dicyclohexylcarbodiimide (DCC) and DMAP in benzene. Subjecting the thiohydroxamate ester to Barton radical decarboxylation conditions using AIBN and tributyltin hydride resulted in full decarboxylation and **75** was isolated in 65% yield over the two steps.




**Reagents and Conditions:** a) 2-mercaptopyridine-*N*-oxide, DCC, DMAP, benzene, reflux; b) *n*-$Bu_3SnH$, AIBN, benzene, reflux, 65% (2 steps); c) 1. 1 M NaOH, MeOH, RT, 98%; 2. $AgNO_3$, MeOH, RT, 81%.

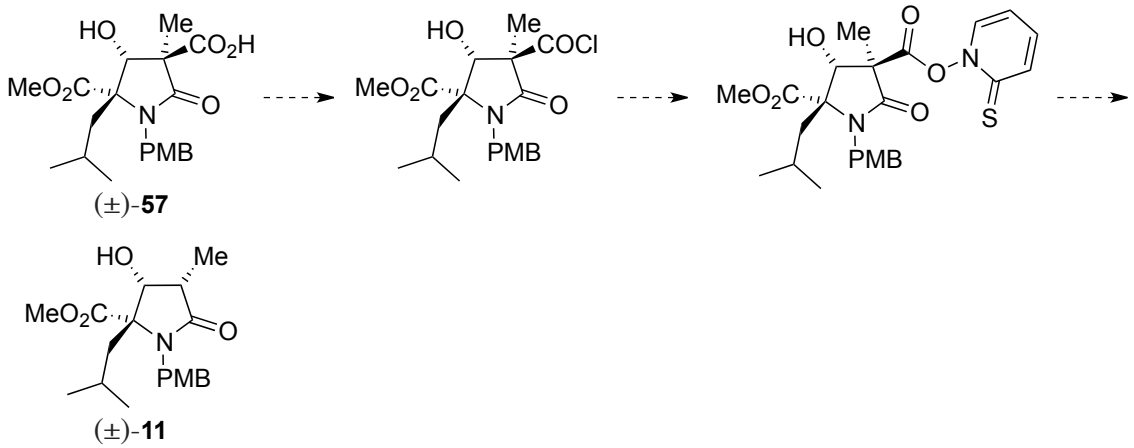
**Scheme 44.**

The original method for the Barton decarboxylation employs tributyltin hydride as the hydrogen donor and to propagate the radical reaction. Tributylgermanium hydride and TTMSS can also be used in the same way. Furthermore, recent methods report the use of a photochemical approach. Because of the issues encountered by us when tributyltin- and tributylgermanium hydride and TTMSS were used, we decided to employ the photochemical approach reported by Ko in 2011 (**Scheme 45**).[35] Ko also reported that chloroform could successfully be used as a H-donor instead of tributyltin hydride, reducing both the cost and toxicity of such reactions.



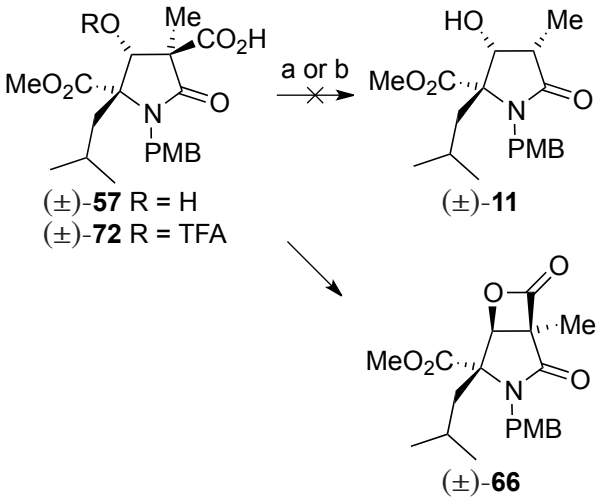

**Scheme 45.**

Our approach would involve the conversion of the carboxylic acid into the corresponding acyl chloride, followed by addition of 2-mercaptopyridine *N*-oxide sodium salt to form the Barton ester, and finally decarboxylation to give the desired intermediate (±)-**11 (Scheme 46**).




**Scheme 46.**

The carboxylic acid (±)-**57** was treated with oxalyl chloride in chloroform and dimethylformamide (DMF) to produce the corresponding acyl chloride. The acyl chloride solution was then added to a solution of 2-mercaptopyridine *N*-oxide sodium salt in chloroform using a cannula under the irradiation of a UV lamp.




**Reagents and Conditions:** a) (±)-**57**, oxalyl chloride, 2-mercaptopyridine *N*-oxide sodium salt, DMF, $CHCl_3$; b) (±)-**57** or (±)-**72**, EDAC·HCl, NMM, DMAP, 2-mercaptopyridine *N*-oxide sodium salt, THF.

**Scheme 47.**

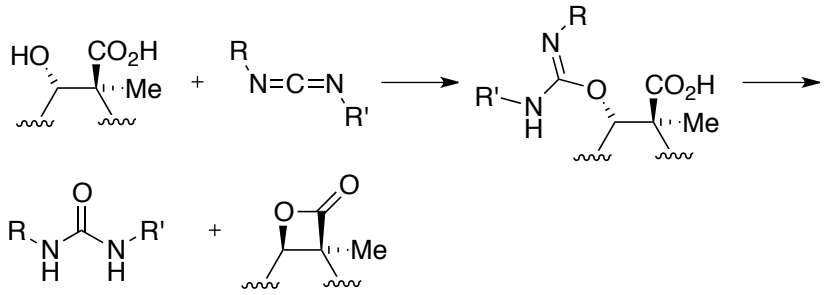
Unfortunately, $^1$H NMR spectrum analysis of the reaction mixture showed the formation of the β-lactone (±)-**66** as the major product (described above **2.2.5.1.2.1**, **Scheme 29**), and the expected signals corresponding to the thiohydroxamate ester were not observed, leading us to the believe that the desired product was not formed in the reaction.

Attempts were also made to couple the 2-mercaptopyridine *N*-oxide sodium salt to the carboxylic acid using EDAC·HCl as described in section **2.2.2**. This was carried out on both the unprotected alcohol (±)-**57** and the protected compound (±)-**72**; however, both gave the same result: the major product observed was β-lactone (±)-**66**.

In the reaction to form the acyl selenide (described above **2.2.5.1.2.1**, **Scheme 29**), we suggested that the formation of the β-lactone was due to a Mitsunobu-like reaction occurring in the presence of tributylphosphine; however, this is not the case with respect to the Barton decarboxylation as there is no phosphine present. In order to form the β-lactone, the hydroxyl moiety must be converted into a leaving group followed by the attack of the carboxylate onto the C6 position to form the lactone moiety (this is still a Mitsunobu-like reaction in terms of transformation but not in terms of reagents used).

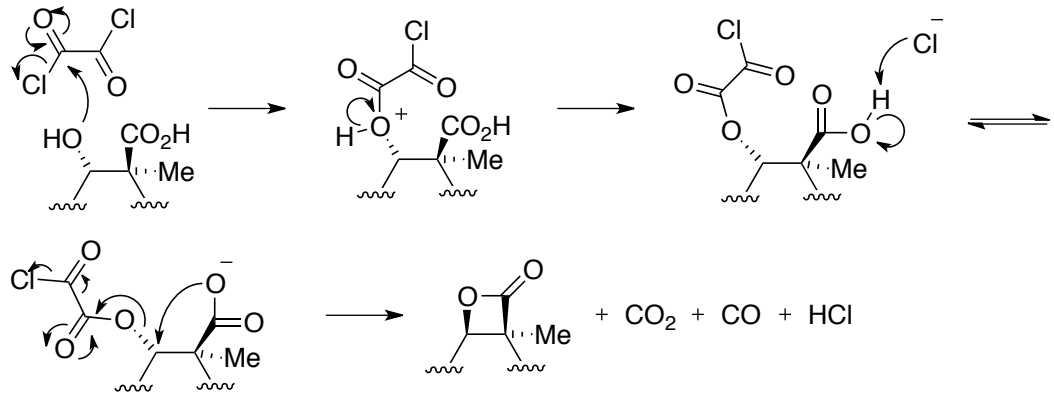
In the first coupling reaction, with the unprotected (±)-**57**, the EDAC·HCl may have reacted with the hydroxyl moiety instead of the acid transforming it into a leaving

group, the NMM could then act as a base to deprotonate the acid resulting in an intramolecular lactonization to form the β-lactone (**Scheme 48**). In the second reaction, when the hydroxyl was protected with a trifluoroacetate group (compound (±)-**72**), the C6 moiety is already a suitable leaving group and so, again, deprotonation of the acid could have resulted in an intramolecular lactonization to form the β-lactone.



**Scheme 48.**

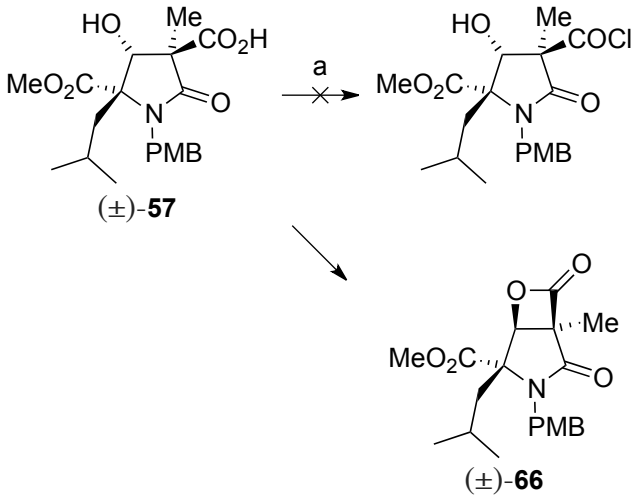
The outcome of the reaction of the β-hydroxy acid (±)-**57** with oxalyl chloride was at first more confusing. However, if we consider that the acyl chloride did not form and instead the oxalyl chloride reacted with the hydroxyl group we can apply the same reasoning described above. Oxalyl chloride would react with the hydroxyl moiety, creating a suitable leaving group at the C6 position (**Scheme 49**).



**Scheme 49.**

To test our theory the carboxylic acid (±)-**57** was treated with oxalyl chloride in chloroform and DMF as described above. After stirring for 4 h the solvent was removed under reduced pressure, and $^{1}$H NMR spectrum analysis of the crude product mixture

showed that the β-lactone (±)-**66** had been formed. After silica gel column chromatography the β-lactone (±)-**66** was isolated in quantitative yield.



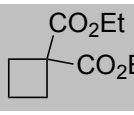
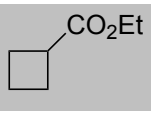
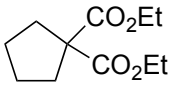
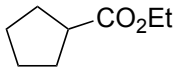

**Reagents and Conditions:** a) oxalyl chloride, DMF, $CHCl_3$, RT, 4 h, (±)-**66**: quant.

**Scheme 50.**

## 2.2.5.1.2.4 The Krapcho Decarboxylation

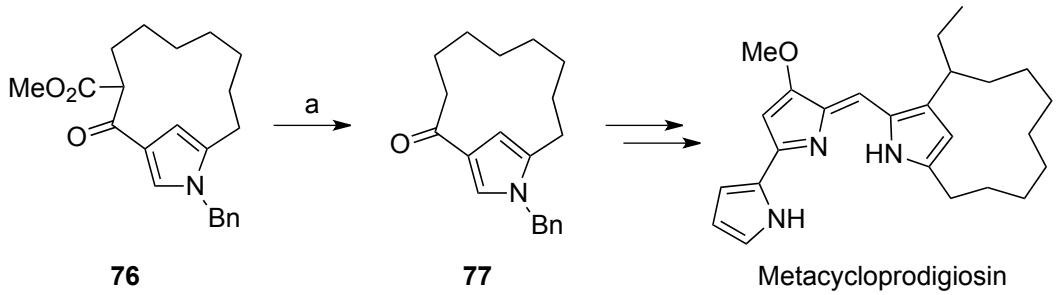
In 1967, Krapcho reported the use of sodium cyanide and DMSO to achieve the decarboxylation of geminal diesters in one step (**Table 8**).[36] Previously, this type of transformation could only be carried out in three steps: saponification to the diacid, decarboxylation of the diacid, then finally esterification to give the desired ester. Not only did Krapcho's method reduce the number of synthetic steps, but in so doing yields were also increased.

**Table 8.** Decarboxylation of 1,1-diesters with sodium cyanide in DMSO.

| Reactant | Product | Yield (%) |
|---|---|---|
| $CH_3CH(CO_2Et)_2$ | $CH_3CH_2CO_2Et$ | 75 |
| $CH_3CH_2CH(CO_2Et)_2$ | $CH_3CH_2CH_2CO_2Et$ | 80 |
|  |  | 75 |
|  |  | 75 |

Since 1967, the scope of this reaction has been thoroughly investigated and the toxic sodium cyanide has been replaced by lithium or sodium chloride, now the most commonly used reagents for this transformation. An important structural requirement for this reaction is the presence of an electron-withdrawing group in the beta position; β-ketoesters and malonic esters can both undergo decarboxylation using this method.

The Krapcho decarboxylation has many synthetic applications. Metacycloprodigiosin is an immunosuppressive alkaloid, and in 1999, the Fürstner group reported the use of Krapcho conditions in their synthesis.[37] Treatment of the methyl ester **76** with sodium chloride in wet DMSO resulted in the desired decarboxylated product **77** in 91% yield, which was then advanced to the desired product, metacycloprodigiosin.

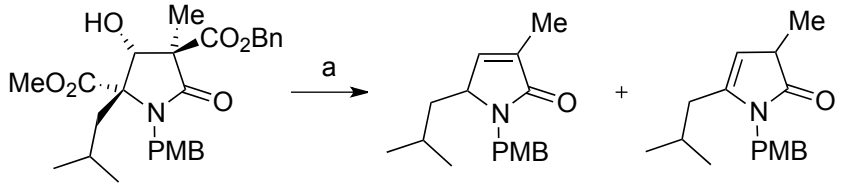



**76** **77** Metacycloprodigiosin

**Reagents and Conditions:** a) NaCl, $H_2O$, DMSO, 180-190 °C, 1.5 h, 91%.

**Scheme 51.**

We hoped to use this method to decarboxylate the benzyl ester of (±)-**51** at the C7 position, using the carbonyl group of the amide to form the enolate, and in doing so forming benzyl chloride and carbon dioxide as the by-products. One possible problem was the presence of the methyl ester group at the C5 position; however, without the presence of the β-ketone at C6 we hoped that this group would be left intact.




(±)-**51** (±)-**78** (±)-**79**

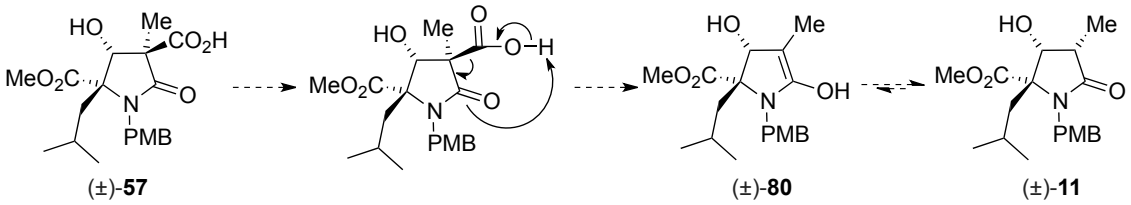
**Reagents and Conditions:** a) LiCl, DMF, 135 °C, 4 h, (±)-**78**: 49% and (±)-**79**: 38%.

**Scheme 52.**

The β-ketoester (±)-**51** was treated with lithium chloride in DMF and heated at 135 °C for 4 h.[38] After silica gel column chromatography, compounds (±)-**78** and (±)-**79** were isolated in 49% and 38% yield, respectively. $^1$H NMR spectrum analysis of the crude reaction mixture showed that these compounds were formed in a 1:1 ratio. Unfortunately, the benzyl and methyl ester and the hydroxyl moiety were removed.

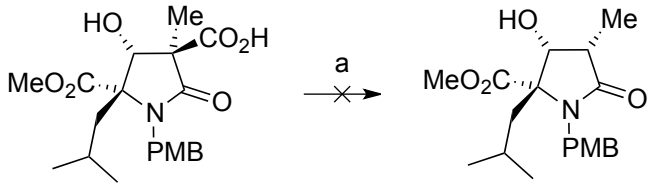
### 2.2.5.1.2.5 Acid-Catalysed Decarboxylation

As the methods described above were unsuccessful, an acid-catalysed decarboxylation was attempted. The mechanism for this reaction can be seen in **Scheme 53**; the intramolecular deprotonation of the carboxylic acid and subsequent loss of $CO_2$ give the alkene (±)-**80**; however, the equilibrium will be shifted towards (±)-**11** due to the increased stability of the amide, and the acid present in the reaction.




**Scheme 53.**

Treatment of the carboxylic acid (±)-**57** with 6 M hydrochloric acid (HCl) and heating to reflux resulted in a complex mixture. At first it appeared that the reaction may have been successful; IR analysis showed stretches at 1733, 1674 and 3387 cm$^{-1}$ corresponding to the carbonyl of the ester and amide, and the hydroxyl moieties (no characteristic carboxylic acid stretches were observed), respectively. Mass spectrometry data correlates to the desired product (±)-**11** with the formula for $[M+H]^+$ as $[C_{19}H_{25}NO_5+H]^+$ with mass 350.20. Unfortunately, despite multiple purification attempts using silica gel column chromatography, we were unable to isolate a single product cleanly. $^1$H NMR spectrum analysis showed at least three compounds had co-eluted. Without the ability to separate the products, we cannot confirm whether the desired product is present.




(±)-**57** (±)-**11**

**Reagents and Conditions:** a) 6 M HCl, $H_2O$, reflux, 4 h.

**Scheme 54.**

As the initial results were promising, the reaction was performed with a weaker acid, *p*-toluenesulfonic acid (*p*-TSA), in the hope of preventing any possible decomposition caused by using a strong acid such as 6 M HCl. Unfortunately, when this reaction was performed under reflux a complex mixture was again formed and no products could be isolated; the product described above was not observed in the $^1$H NMR spectrum of the crude mixture. To prevent decomposition, the reaction was repeated under milder conditions, at both 80 °C and room temperature; however, in both cases, no reaction occurred and the starting material was recovered.

**Table 9.** Investigation into the acid-catalysed decarboxylation of (±)-**57**.

| Acid | Conditions | Result |
|---|---|---|
| 6 M HCl | Reflux | Complex mixture |
| *p*-TSA | Reflux | Complex mixture |
| *p*-TSA | 80 °C | Starting material |
| *p*-TSA | RT | Starting material |

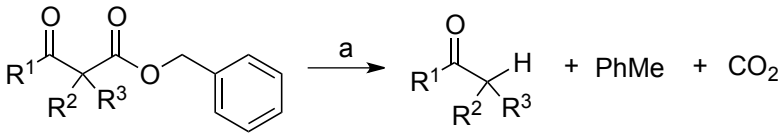
## 2.2.5.2 Route B Towards (±)-11

### 2.2.5.2.1 Decarboxylation of the Mander's Reaction Product, (±)-10

With the Route A (**Scheme 17**) proving so challenging, we decided instead to perform the decarboxylation of the benzyl ester moiety at the C7 position followed by reduction of the ketone at C6 (**Scheme 17**, Route B).

The hydrogenolysis of benzyl β-ketoesters using palladium and a hydride source is a well-known reaction that results in the corresponding ketone (**Scheme 55**). There are

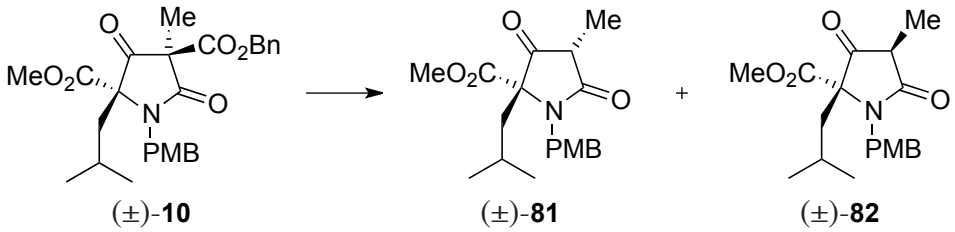
many examples of this type of reaction in the literature; the most common reaction conditions involve using hydrogen gas as the hydride source and catalytic amounts of Pd/C.[39]




**Reagents and Conditions:** a) $Pd^0$, hydride source.

**Scheme 55.**

Following route B, when (±)-**10** was treated under hydrogenolysis conditions using palladium hydroxide on carbon and a hydrogen balloon, debenzylation and decarboxylation occurred, but led to a 1:1 mixture of diastereoisomers at the C7 position, compounds (±)-**81** and (±)-**82** (**Scheme 56**).




**Reagents and Conditions:** a) $H_2$, $Pd(OH)_2/C$, THF, 30 °C, >90%.

**Scheme 56.**

As described above, this is, again, due to the planarity of the enolate intermediate formed during the reaction. The hydrogenolysis results in the removal of the benzyl group, and concomitant loss of $CO_2$ occurs via the enolate. Unfortunately, these diastereoisomers were inseparable on silica gel column chromatography. It was also found that the product was relatively unstable on both silica gel and alumina, and for this reason was used without purification in the following step.

The reaction did not afford reproducible yields; hence, work was undertaken to find optimal conditions. The reaction outcome can be influenced by both the reaction time and temperature. When the reaction time was shorter than 12 hours, $^1$H NMR spectrum analysis of the mixture showed the reaction had not gone to completion. However, we

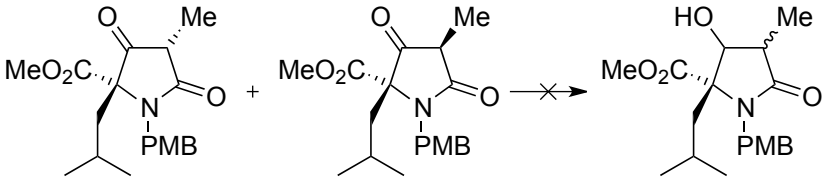
were unable to isolate and characterize any product due to the compounds' instability on both silica gel and alumina. When the reaction time was longer than 72 hours, complete decomposition occurred. Temperature also played an important role in this reaction; at first, the reaction was carried out at room temperature, which led to irreproducible results. As room temperature can vary we decided to remove this variable by keeping the reaction at 30 °C. Finally, we settled on the optimized conditions described in **Scheme 56**; using $Pd(OH)_2/C$ and under a hydrogen atmosphere using a balloon in anhydrous THF at 30 °C.

When the benzyl ester group is removed, the resulting proton at the C7 position is relatively acidic as the 1,3-keto-amide moiety in the lactam ring has a pKa similar to that of a diester (pKa≈11-13). The compounds (±)-**81** and (±)-**82** can undergo enolization as observed in earlier steps of the synthesis, and this may account for the unstable nature of this compound. While the outcome of the decarboxylation step was not the desired one, the reduction of the ketone moiety was attempted on the diastereoisomeric mixture in the hope that the corresponding products could be separated at a later stage.

#### 2.2.5.2.2 Reduction of the Diastereoisomeric Mixture, (±)-81 and (±)-82

The reduction of the ketone at the C6 position proved much more challenging than expected. Reduction using sodium borohydride, sodium triacetoxyborohydride and sodium cyanoborohydride all proved unsuccessful under a variety of reaction conditions. These reactions either resulted in the recovery of the starting material, decomposition, complex mixtures or isolation of compounds closely related to the desired product.

**Table 10.** Investigation into the reduction of (±)-**81** and (±)-**82**.




(±)-**81** (±)-**82**

| Reducing Reagent | Conditions | Result |
|---|---|---|
| $NaBH_4$ | EtOH, 0 °C, 30 min | Complex mixture |
| $NaBH_3CN$ | MeOH, 0 °C – RT, 2 h | S.M. |
| $NaBH(OAc)_3$ | Acetic acid, RT, 1 h | Unidentified product |
| 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® | $CH_2Cl_2$, –78 °C, 1-2 h | Complex mixture |

$^1$H NMR spectrum analysis of the starting material (±)-**81** and (±)-**82** showed the required doublet corresponding to the methyl group at C7, and the lack of this doublet peak was immediately clear from the $^1$H NMR spectrum analysis of the reaction mixtures that gave related compounds. The presence of a singlet peak corresponding to the methyl group indicated that the C7 position was now a quaternary centre again.

Following the reported procedure, the diastereoisomeric mixture of (±)-**81** and (±)-**82** was treated with sodium triacetoxyborohydride in acetic acid at room temperature for one hour.[40] The desired compound was not isolated but a product closely related to the staring material was isolated. In an attempt to determine the structure of the product isolated, a combination of $^1$H NMR, $^{13}$C NMR, COSY, HSQC, DEPT and IR spectroscopic analysis was used. Unfortunately, the mass spectrometry data did not correlate to any predicted structure. **Figure 9** below shows the $^1$H NMR spectrum of the compound isolated after reduction of (±)-**81** and (±)-**82** with sodium triacetoxyborohydride.

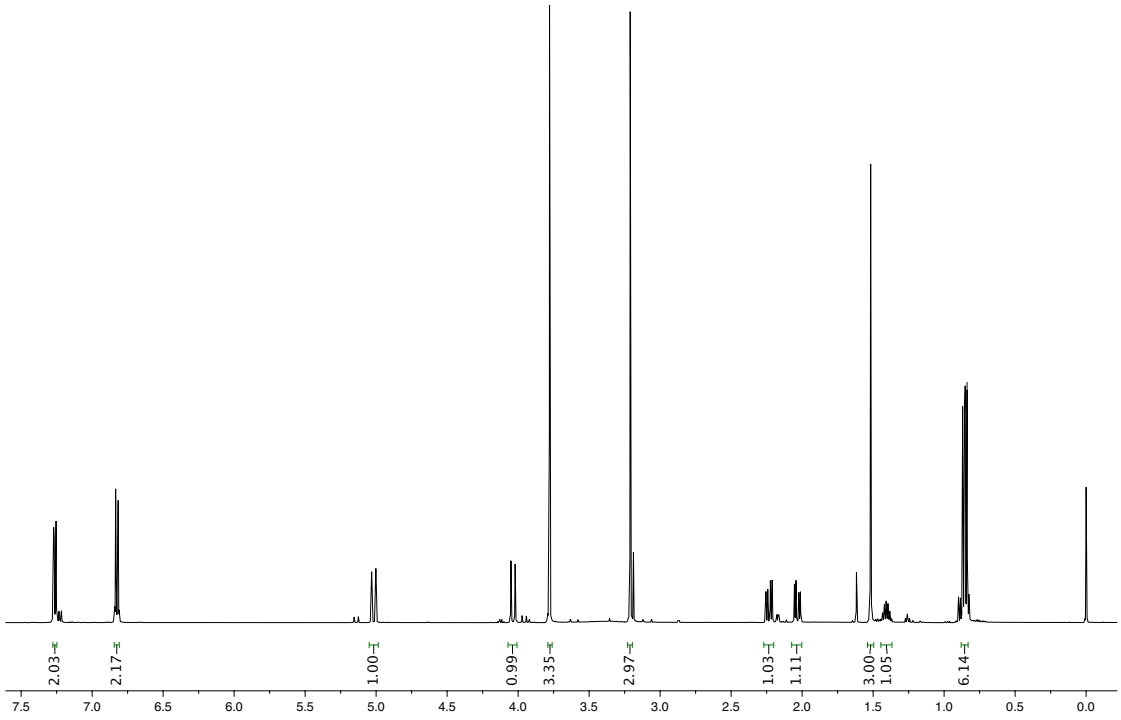
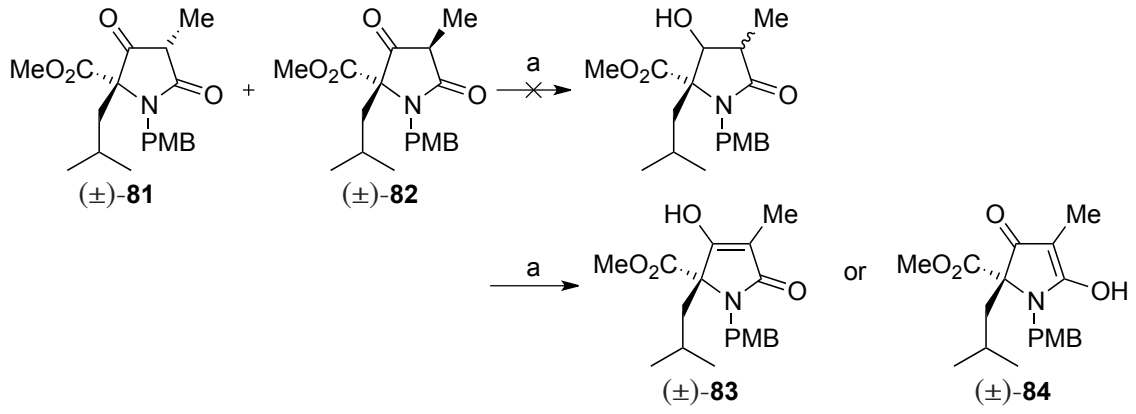



**Figure 9.** $^{1}$H NMR spectrum of the compound isolated after reduction of (±)-**81** and (±)-**82** with sodium triacetoxyborohydride.

Analysis of the $^{1}$H NMR spectrum shows that the signal corresponding to the methyl group at the C7 position is no longer a doublet and is instead a singlet, indicating that the C7 carbon is now quaternary. The signals corresponding to the two methoxy groups, the *iso*-butyl group and the PMB group are all present.




**Reagents and Conditions:** a) $NaBH(OAc)_3$, acetic acid, RT, 1 h.

**Scheme 57.**

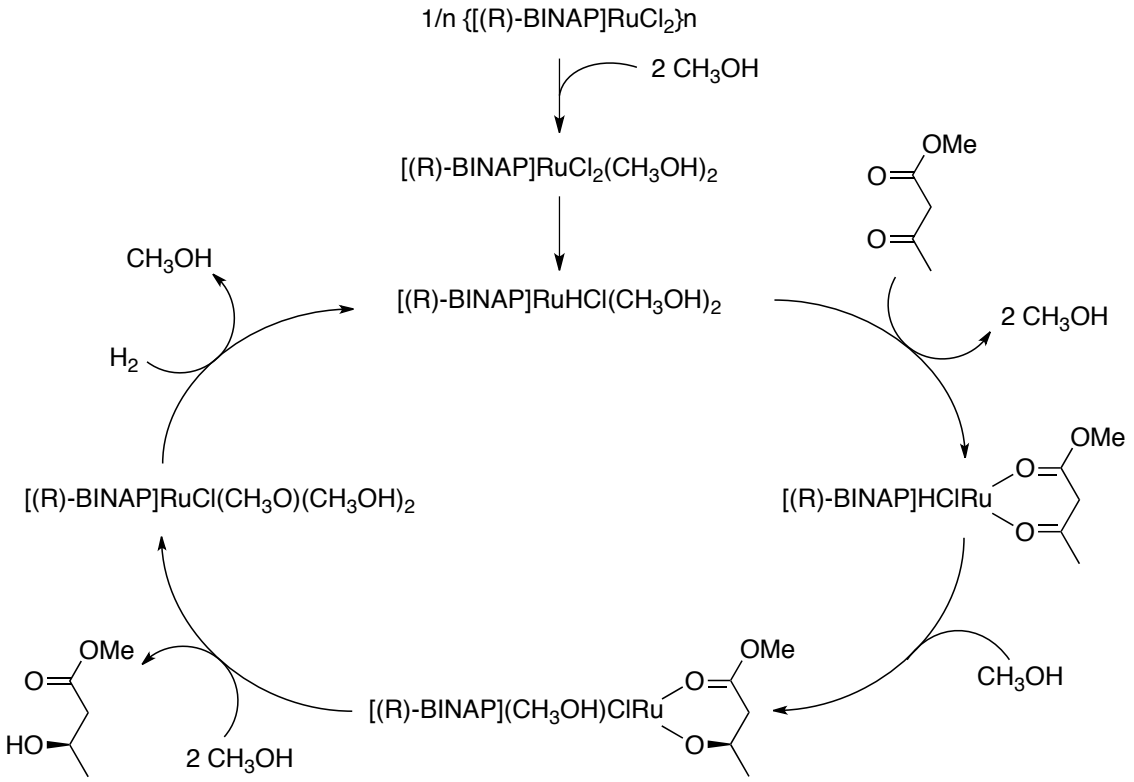
At first, we proposed the structure to be (±)-**83**; all the signals in the $^{1}$H NMR spectrum appeared to correlate to this structure (**Scheme 57**). However, upon analysis of the IR spectrum, three carbonyl stretches similar to those in the starting material can be seen. A stretch at 3363 $cm^{-1}$ also indicates the presence of a hydroxyl group. We know addition of the hydroxyl to the C7 position is not possible but also that if the ketone at C6 had been reduced, we would not see the carbonyl stretch in the IR spectrum or the ketone signal in the $^{13}$C NMR spectrum at 205.2 ppm. The same logic can be applied to the amide moiety; the enol (±)-**84** cannot be the product as both the stretch in the IR spectrum and the peak in the $^{13}$C NMR spectrum are present. Unfortunately, the accurate mass spectrometry data did not correlate with any of the theorized structures and so, without X-ray crystallographic data, we cannot be certain of the structure of this compound.

The use of lithium aluminium hydride (LAH) was also investigated. Although LAH is a stronger reducing reagent than the borohydrides mentioned above and can be used to reduce esters and amides as well as ketones, we hoped to control this by using low temperatures and monitoring the reaction by thin layer chromatography. Both LAH powder and LAH in solution (1 M in tetrahydrofuran) were used at temperatures of 0 °C and −78 °C. Unfortunately, neither resulted in successful isolation of the desired compound. Sodium bis(2-methoxyethoxy)aluminium hydride (Red-Al®) is comparable in reactivity to LAH and was also used in an attempt to reduce the ketone but proved equally unsuccessful. Di-isobutyl aluminium hydride (DIBAL) is most commonly used in the reduction of esters to aldehydes; however, as other reagents had failed, the reaction was repeated using a DIBAL solution (1 M in tetrahydrofuran). Unfortunately, this was again unsuccessful. In both the Red-Al® and DIBAL reactions, analysis of the $^{1}$H NMR spectra of the reaction mixtures showed similarities to the starting material; however, we were unable to purify the compound to a sufficient standard to allow structure identification. No starting material was recovered from either reaction.

### 2.2.5.2.3 The Noyori Asymmetric Hydrogenation Reaction

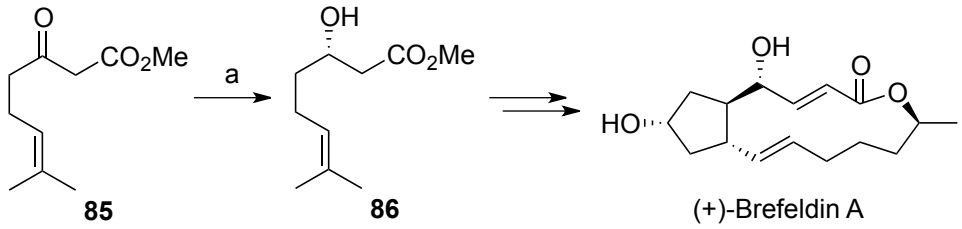
As a final attempt to reduce the ketone, we employed a strategy using the Noyori catalyst for asymmetric hydrogenation. Noyori was joint winner of the Nobel Prize in

2001 for his work on asymmetric hydrogenation reactions. In 1987, Noyori reported the use of a ruthenium catalyst, $RuCl_2$[(*R*)-BINAP], in the catalytic asymmetric reduction of methyl acetoacetate.[41]




**Scheme 58.** The catalytic cycle for the Noyori asymmetric hydrogenation.

There are many reported uses of the Noyori asymmetric hydrogenation reaction in total synthesis. In 1991, Robinson and co-workers reported the synthesis of the antibiotic (+)-brefeldin A, wherein the β-ketoester **85** was converted to **86** in 96% yield and high enantiomeric ratio (99:1) using Noyori's approach.[42]




**Reagents and Conditions:** a) $RuCl_2$·cyclooctadiene, BINAP, $H_2$, MeOH, 50 psi, 80 °C, 6 h, 96%.

**Scheme 59.** The synthesis of (+)-brefeldin A.

Since Noyori's original report in 1987, the catalyst has been improved.[43] We used the commercially available $RuCl_2$[(*R*)–DM–BINAP][(*R*)–DAIPEN] catalyst (**Figure 10**). Treatment of (±)-**81** and (±)-**82** with potassium carbonate and 5 mol% of the ruthenium catalyst in a 5:1 mixture of *iso*-propanol and tetrahydrofuran did not yield the desired compound. This reaction was carried out under a hydrogen atmosphere using a balloon and also under a pressure of 3 bar, but both sets of reaction conditions led to the same products.

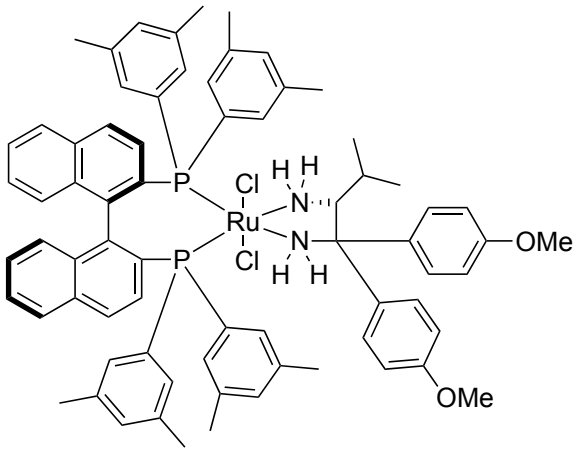
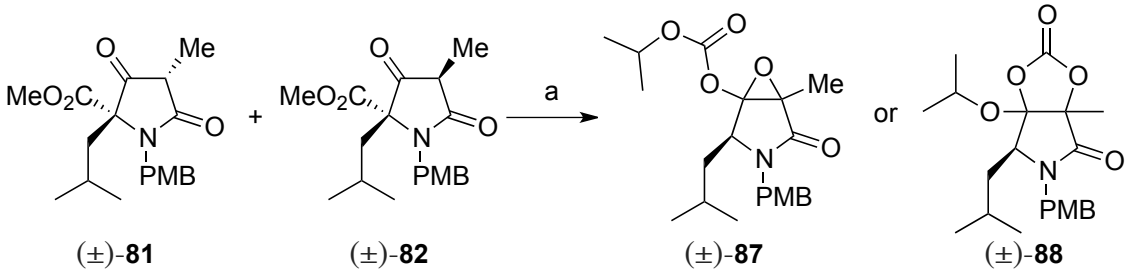



**Figure 10.** The $RuCl_2$[(*R*)–DM–BINAP][(*R*)–DAIPEN] catalyst.

After purification using silica gel column chromatography two compounds were isolated. Our proposed structures for the first eluting compound are either (±)-**87** or (±)-**88**. The proposed structures have been deduced using NMR, IR and MS data analysis as we were unable to obtain any crystals suitable for X-ray crystallographic analysis. IR spectrum analysis showed two absorptions corresponding to carbonyl stretches; one at 1758 $cm^{-1}$, slightly higher than the characteristic ester or ketone stretches, and one at 1670 $cm^{-1}$ representing the amide; there was no signal in the hydroxyl region. The mass spectrometry data suggest a possible formula for $[M+H]^+$ as $[C_{21}H_{29}NO_6+H]^+$ with mass 392.21. Both the proposed structures (±)-**87** and (±)-**88** match this data.




(±)-**81** (±)-**82** (±)-**87** (±)-**88**

**Reagents and Conditions:** a) $RuCl_2$[(*R*)–DM–BINAP][(*R*)–DAIPEN] (5 mol%), IPA:THF (5:1), 3-6 days.

**Scheme 60.**

Unfortunately, we are unable to give a definitive mechanism as to how this reaction might proceed to give either of the proposed compounds (±)-**87** and (±)-**88**. In both suggested structures, it appears first that the ester group at the C5 position has migrated to the C6 position, and secondly that the *iso*-propanol has acted as a nucleophile in some way. $^1$H NMR spectrum analysis (**Figure 11**) shows that the splitting pattern corresponding to the $CH_2$ of the *iso*-butyl is now two sets of multiplets, this presumably arising from coupling with each other, the CH of the *iso*-butyl and one other proton (the new ring proton at the C5 position). Analysis of the COSY H-H spectrum shows a correlation between the multiplets corresponding to the methylene proton and a single proton at around 4 ppm; the splitting pattern for this proton is a doublet of doublets and represents the ring proton at the C5 position. The signal corresponding to the methyl ester is not present, and signals corresponding to the two methyl groups (two doublets at around 1.25 ppm) and the methine unit (a multiplet at around 5 ppm) of an *iso*-propoxy moiety are now present.

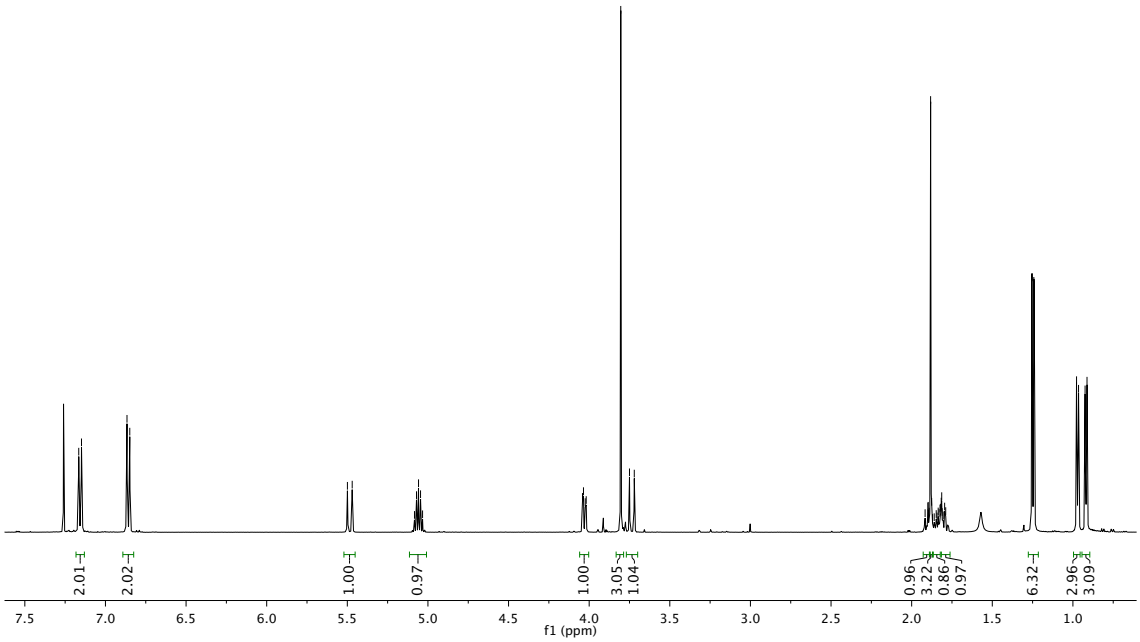



**Figure 11.** $^{1}$H NMR spectrum of the first eluting compound isolated after reduction of (±)-**81** and (±)-**82** with the Noyori catalyst.

The second compound isolated from this reaction was an interesting discovery. The data greatly resembled those of the compound isolated from the reduction of (±)-**81** and (±)-**82** with sodium triacetoxyborohydride, suggesting that the products from these two separate reactions could be diastereoisomers. The IR spectrum showed the same three carbonyl and hydroxyl signals at similar frequencies, the mass spectrometry data for both reactions showed $[M+H]^+$ peaks at 364.17, and both the $^{13}$C NMR spectra showed a characteristic ketone signal. A comparison of the $^{1}$H NMR spectra for both compounds (**Figure 12**) shows just how similar they are, the only significant difference is the peak for the methylene of the *iso*-butyl group (in the region of 1.9 – 2.3 ppm): instead of being two clearly separated doublet of doublets peaks, as the product obtained from the sodium triacetoxyborohydride reaction, in the compound isolated from the Noyori reaction, these peaks have coalesced (highlighted in **Figure 12**). The data do not give us enough information to assign a structure to this compound.

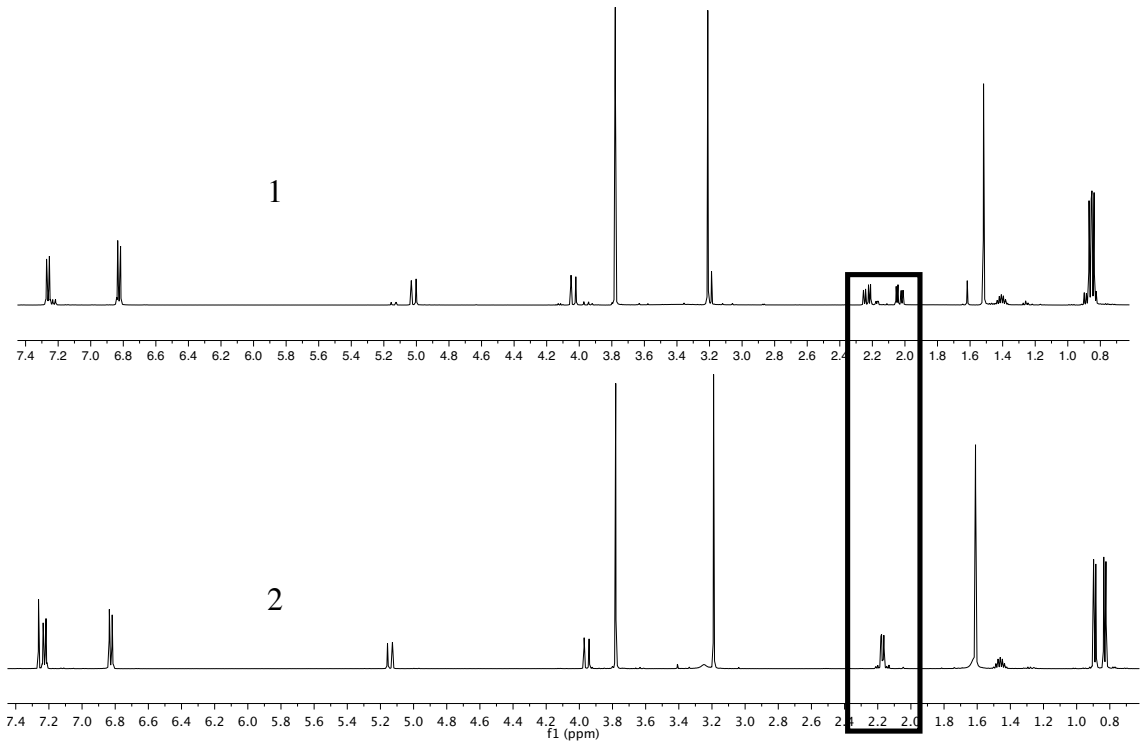
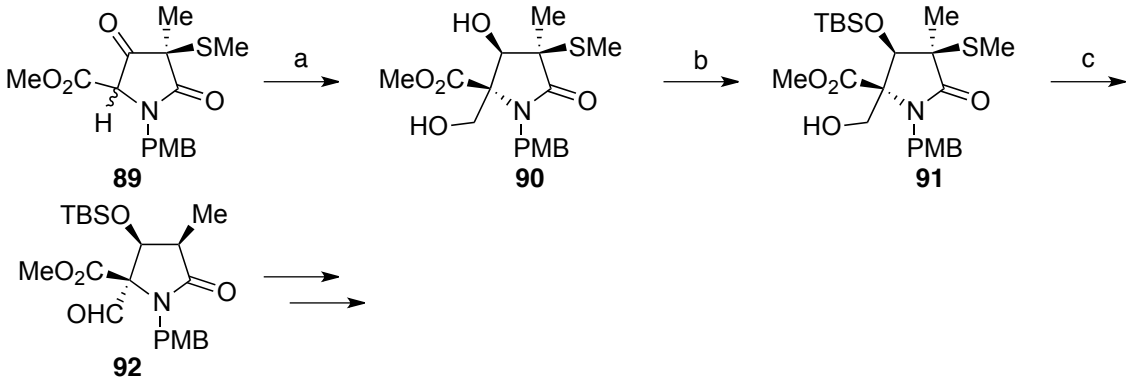



**Figure 12.** A comparison between the $^{1}$H NMR spectrum after reduction of (±)-**81** and (±)-**82** with **1**: sodium triacetoxyborohydride and **2**: the Noyori catalyst.

### 2.2.5.2.4 Synthesis of the Thiomethyl Derivative

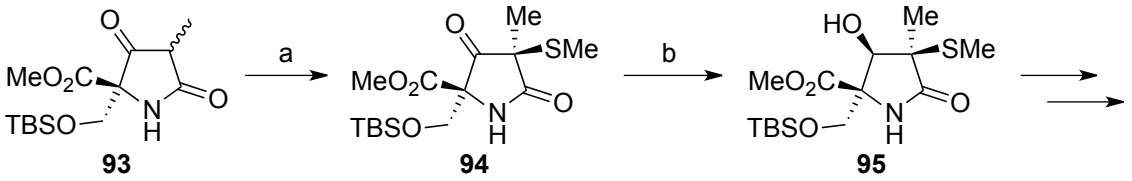
With the reduction of (±)-**81** and (±)-**82** proving unsuccessful under a range of conditions, a new approach was investigated. Both Corey's and Pattenden's syntheses of lactacystin are reviewed in detail above (**Chapter 1**): a thiomethyl derivative is employed in their synthetic approaches.[2, 40] Corey describes the use of a thiomethyl group as both a blocking group and a group to induce stereoselectivity in the hydroxymethylation of **89** (**Scheme 61**). Desulfurization of **91** was achieved in high stereoselectivity (10:1) by treatment with Raney nickel.




**Reagents and Conditions:** a) 1. DBU, THF, –78 °C, then aq. $CH_2O$, –78 °C, 0.5 h, 90%; 2. $NaBH(OAc)_3$, HOAc, 23 °C, 1 h, recrystallization, 95%, 99% *ee*; b) 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%; c) 1. Raney Ni, EtOH, 0 °C, 1 h, 82%; 2. Dess-Martin reagent, $CH_2Cl_2$, 23 °C, 1 h, 95%.

**Scheme 61.**

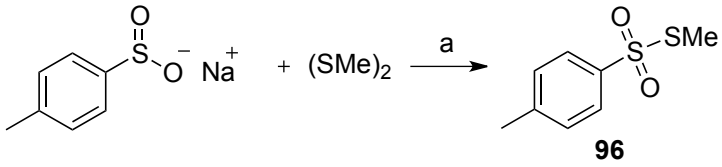
Pattenden reported the isolation of compound **93** as an inseparable mixture of diastereoisomers in a 2:1 ratio (**Scheme 62**). Interestingly, Pattenden does not mention if attempts were made to reduce the ketone **93**, and instead uses the findings described by Corey to improve the diastereoisomeric ratio.




**Reagents and Conditions:** a) *p*-$MeC_6H_4SO_2Me$, $Et_3N$, $CH_2Cl_2$, RT, 78%; b) $Zn(BH_4)_2$, (4.4 M in THF), 0 °C, 79%.

**Scheme 62.**

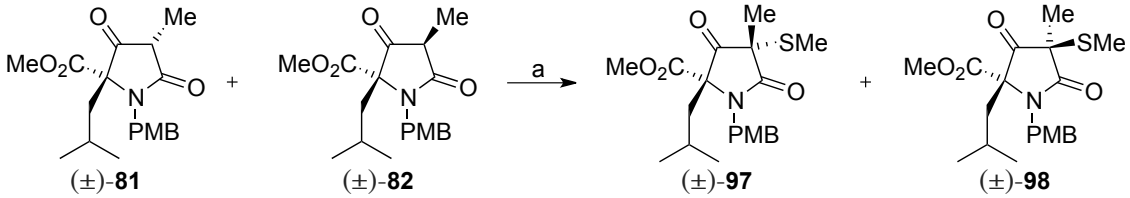
In Pattenden's procedure compound **93** was treated with *S*-methyl-*p*-toluenethiosulfonate **96** and triethylamine resulting in the formation of a mixture of diastereoisomers at the C7 position in a 7:1 ratio; compound **94** was isolated as the major diastereoisomer after silica gel column chromatography. Compound **94** was reduced using zinc borohydride to give **95** as a single diastereoisomer. Then, following the procedure reported by Corey, the thiomethyl group was removed in a stereoselective manner using Raney nickel. We hoped to employ the same strategy in our synthetic route.




**Reagents and Conditions:** a) $CH_2Cl_2$, $I_2$, RT, quant.

**Scheme 63.**

Thus, *S*-methyl-*p*-toluenethiosulfonate **96** was synthesized following Fujiki's procedure (**Scheme 63**).[44] Treatment of the diastereoisomeric mixture of (±)-**81** and (±)-**82** with (*S*)-methyl-*p*-toluenethiosulfonate and triethylamine in dichloromethane resulted in the isolation of compounds (±)-**97** and (±)-**98** as an inseparable mixture of diastereoisomers in a 2:1 ratio and 47% yield after silica gel column chromatography. Using a NOESY NMR spectrum analysis, compound (±)-**98** was found to be the major isomer. While Pattenden was able to improve the diastereoisomeric ratio from 2:1 to 87:13, we were not as fortunate.




**Reagents and Conditions:** a) *p*-$MeC_6H_4SO_2Me$, $Et_3N$, $CH_2Cl_2$, RT, (±)-**97** and (±)-**98**: 47%.

**Scheme 64.**

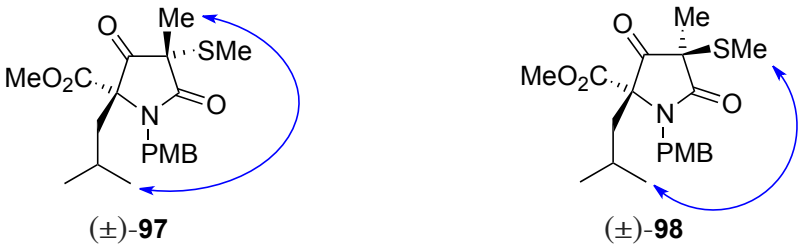



**Figure 13.** NOESY spectrum analysis interpretation to assign relative stereochemistry to the inseparable mixture of (±)-**97** and (±)-**98**.

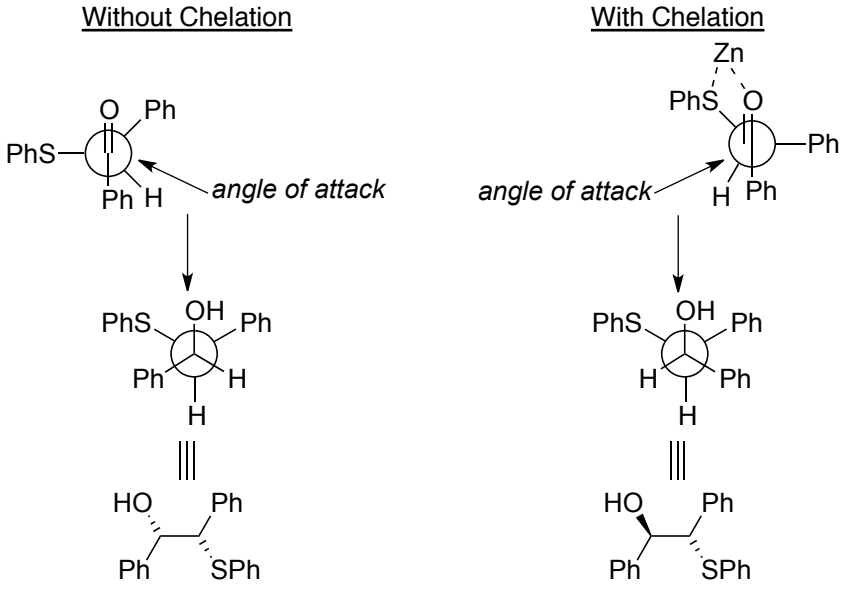
Trace amounts of another inseparable mixture of isomers in a 2:1 ratio were also isolated from the reaction. In an attempt to assign a structure to the compounds isolated a combination of $^{1}H$ NMR, $^{13}C$ NMR, COSY, HSQC, DEPT and IR spectra analyses

was used. $^{1}$H NMR spectrum analysis of the mixture shows a singlet peak at 1.61 ppm corresponding to the methyl group at the C7 position, indicating that the C7 carbon is a quaternary centre. IR analysis shows absorptions at 3355, 1783, 1747 and 1684 cm$^{-1}$ indicating the presences of a hydroxyl group and three carbonyl groups. Unfortunately we were unable to assign structures using the data obtain.

### 2.2.5.2.4.1 Reduction of the Thiomethyl Derivative

Pattenden reported the use of zinc borohydride to reduce the ketone moiety in **94** to give the corresponding alcohol **95** in 79% yield (**Scheme 62**).[40] In this case, the stereoselectivity is achieved by chelation of the zinc to the oxygen atom of the ketone and the sulfur atom of the thiomethyl group resulting in hydride attack from the opposite side to the thiomethyl group, i.e. the least hindered face.

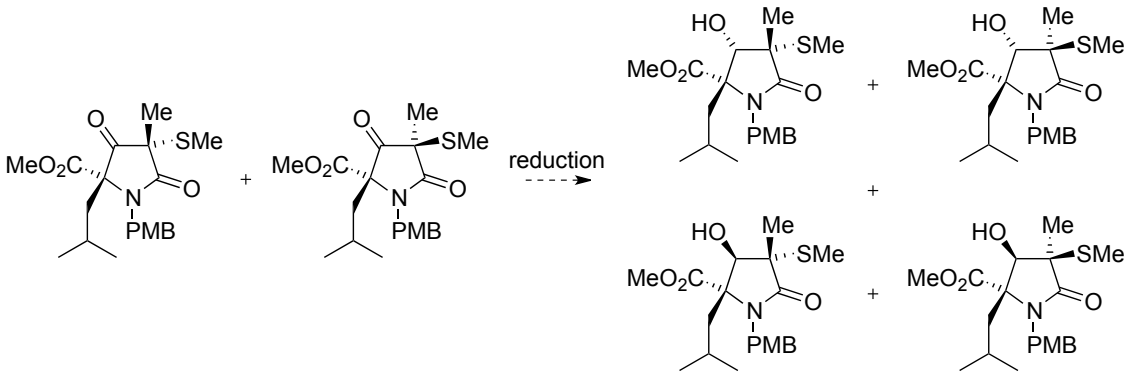
**Scheme 65** shows how chelation can alter the stereochemistry of the reduction product. The chelation of the zinc centre to the oxygen and sulfur atoms causes rotation, and, therefore, changes the conformation of the starting material. This conformational change means the least hindered face, and so the angle of attack, is now on the opposite side with respect to the non-chelated model.




**Scheme 65.**

Although there are many reports of the use of zinc borohydride for stereoselective reduction, it is not a common, commercially available reagent.[45] Zinc borohydride is prepared from commercially available zinc chloride and sodium borohydride in diethyl ether.

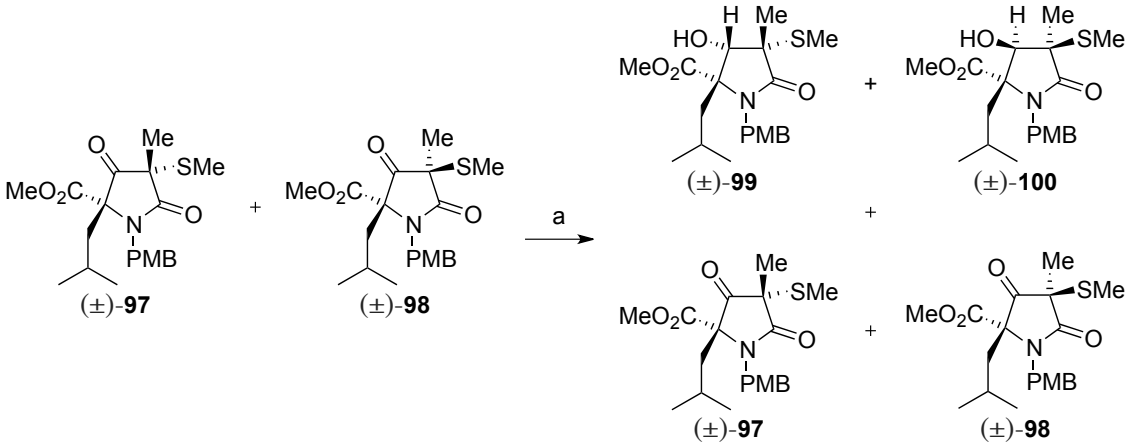
In contrast, Corey reported the reduction of ketone **89** (after hydroxymethylation) using sodium triacetoxyborohydride in acetic acid to give **90** as a single diastereoisomer in 95% yield.[2] The reduction was selective without the need for zinc borohydride. Indeed, chelation control is an important factor in selective reductions for acyclic substrates and cyclic substrates with similar sized substituents. In Corey's case, the substituents' size difference is sufficient to induce the desired outcome. As Corey had shown the reduction was selective using sodium triacetoxyborohydride, and zinc borohydride is not commercially available, we attempted the ketone reduction of the mixture of (±)-**97** and (±)-**98** under optimized reduction conditions described above using sodium borohydride in ethanol (section **2.2.5.1.1**).




**Scheme 66.**

As the starting material is a mixture of two racemic diastereoisomers, if the reduction is not selective, a possible four racemic products could be formed (**Scheme 66**). $^{1}$H NMR spectrum analysis of the reaction mixture showed the presence of four compounds, in a 6:6:4:1 ratio approximately. Fortunately, the reduction was successful, and selective, and after silica gel column chromatography, only two reduction products, compounds (±)-**99** and (±)-**100**, were isolated in 17% and 25% yield, respectively. The other two compounds observed in the $^{1}$H NMR spectrum of the reaction mixture were the two

starting material diastereoisomers, isolated as an inseparable diastereoisomeric mixture of (±)-**97** and (±)-**98** in 23% yield.




**Reagents and Conditions:** a) $NaBH_4$, EtOH, –10 °C, 30 min, (±)-**99**: 17%, (±)-**100**: 25% and (±)-**97** and (±)-**98**: 23%.

**Scheme 67.**

The ratio of diastereoisomers in the starting material (2:1) compared to the ratio of recovered starting material diastereoisomers after the reaction (6:1) gives us information on the rate of reduction: the minor diastereoisomer is reacting faster than the major diastereoisomer.

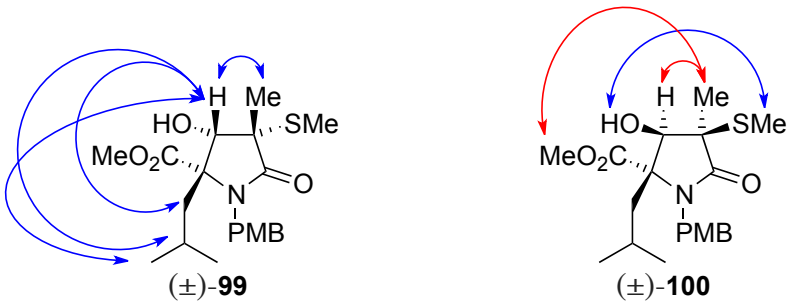
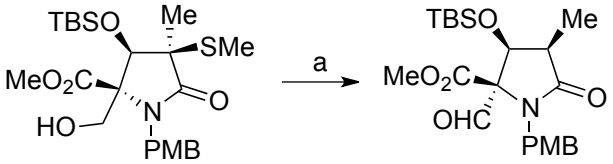



**Figure 14.** NOESY spectrum analysis interpretation of (±)-**99** and (±)-**100**.

The relative stereochemistry shown in compounds (±)-**99** and (±)-**100** was assigned from NOESY NMR spectrum analysis **(Figure 14)**. Unfortunately, we were unable to obtain crystals of these compounds suitable for X-ray crystallography.

### 2.2.5.2.4.2 Desulfurization of the Thiomethyl Derivative

Corey reported the selective desulfurization of **91** using Raney nickel in ethanol at 0 °C to give **92** (**Scheme 68**).[2] The product **92** displays opposite configuration of the methyl group at the C7 position to that observed in the starting material. It has been suggested that the mechanism for desulfurization using Raney nickel may proceed through a radical intermediate. This difference in configuration at the C7 position may be due to equilibration during the radical process. Due to the structural and configurational similarities between compound (±)-**99** and Corey's compound **91**, we hoped that a similar equilibration process may occur.

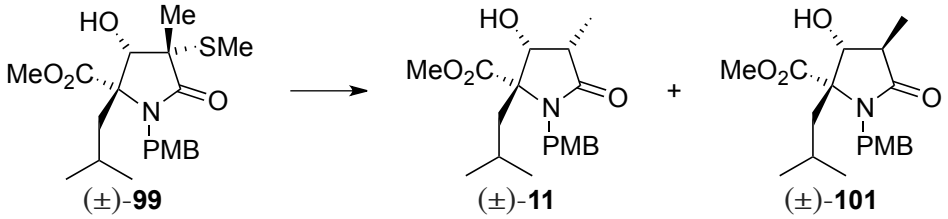



**91** **92**

**Reagents and Conditions:** a) 1. Raney Ni, EtOH, 0 °C, 1 h, 82%; 2. Dess-Martin reagent, $CH_2Cl_2$, 23 °C, 1 h, 95%.

**Scheme 68.**

Unfortunately, treatment of (±)-**99** with Raney nickel in ethanol at 0 °C did not give the desired product (±)-**11**, and the starting material was not recovered either. The $^1$H NMR spectrum showed the peak corresponding to the thiomethyl group at 2.16 ppm still present.




(±)-**99** (±)-**11** (±)-**101**

**Reagents and Conditions:** a) Raney Ni, EtOH, reflux, 4 h.

**Scheme 69.**

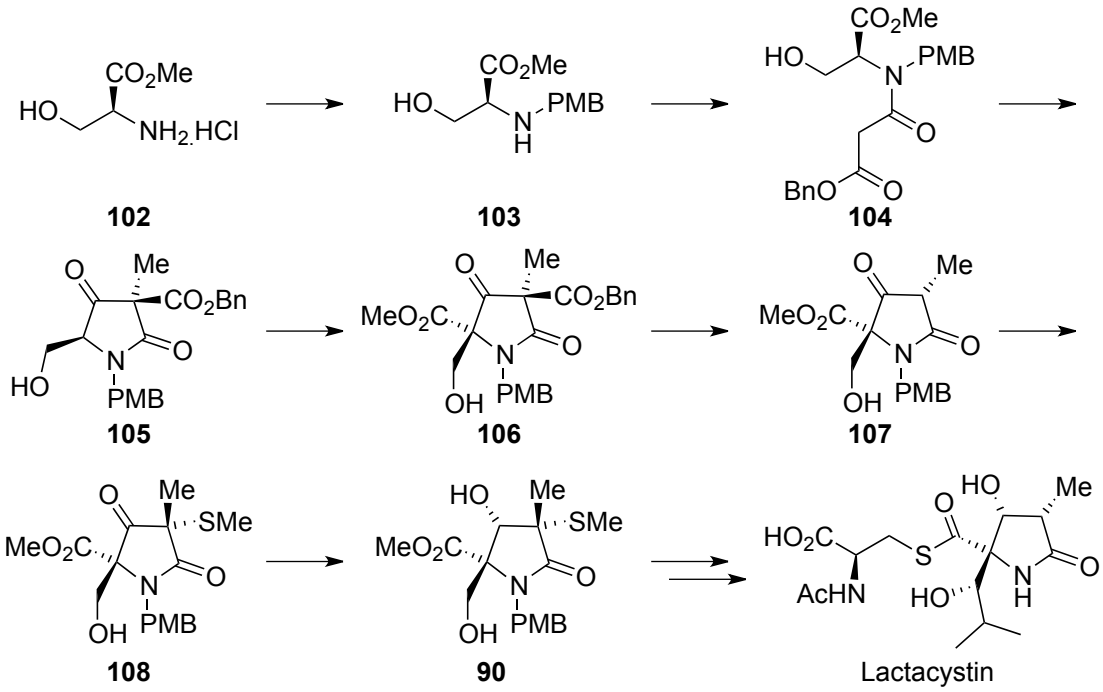
When (±)-**99** was treated with Raney nickel in ethanol and heated under reflux for 4 h, $^{1}$H NMR spectrum analysis of the product isolated after silica gel chromatography appeared to show the desired compound (±)-**11** alongside diastereoisomer (±)-**101** as an inseparable mixture. These compounds were observed in a 3:1 ratio; however, we were unable to determine which diastereoisomer is the major product. A NOESY NMR experiment was attempted to assign relative stereochemistry; however, no correlations were observed. The diastereoisomeric ratio is, however, large enough to enable us to distinguish between the diastereoisomers and assign the $^{1}$H NMR spectrum of the major product. We were confident that desulfurization had been successful as the $^{1}$H NMR spectrum clearly showed the signal corresponding to the methyl group at the C7 position as a doublet, coupling with the new ring proton at the C7 position. The mass spectrometry data correlates to the desired product (±)-**11** with the formula for $[M+H]^{+}$ as $[C_{19}H_{25}NO_5+H]^{+}$ with mass 350.20. Using a combination of $^{1}$H, COSY, $^{13}$C, DEPT and HSQC NMR, IR and MS data analyses, we believe the desired compound, Corey's intermediate (±)-**11**, was successfully synthesized. Unfortunately, we were unable to separate the diastereoisomers. Although (±)-**11** is an advanced intermediate synthesized by Corey, unfortunately, no data for the compound was reported.

## 2.3 Synthesis of the Serine Analogue

### 2.3.1 Proposed Synthetic Route Towards Lactacystin

At the same time as work was being carried out on the leucine analogue, the synthesis using a different amino acid starting material, L-serine, was also being investigated. In comparison to the leucine derivative, when using serine, the hydroxyl functionality at C9 found in lactacystin is present from the beginning. The hydroxyl functionality was proven to be very important in structure activity relationship (SAR) studies, and is found in many of the lactacystin analogues, for example the salinosporamides and the cinnabaramides (**Chapter 1**). We hoped that, by using serine, our synthetic route would allow ready access to these natural products and possible novel analogues with groups tailored to give high biological activity.

Using L-serine methyl ester hydrochloride **102** as the starting material, we hoped to synthesize the advanced intermediate **90**, reported by Corey in 1998, using our route developed for the leucine analogue (**Scheme 70**).[2]

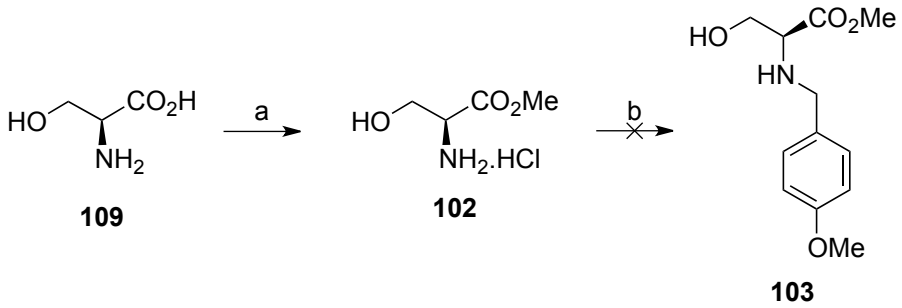



**Scheme 70.**

## 2.3.2 Synthesis from L-Serine

### 2.3.2.1 Protection of the Amine with 4-Methoxybenzaldehyde

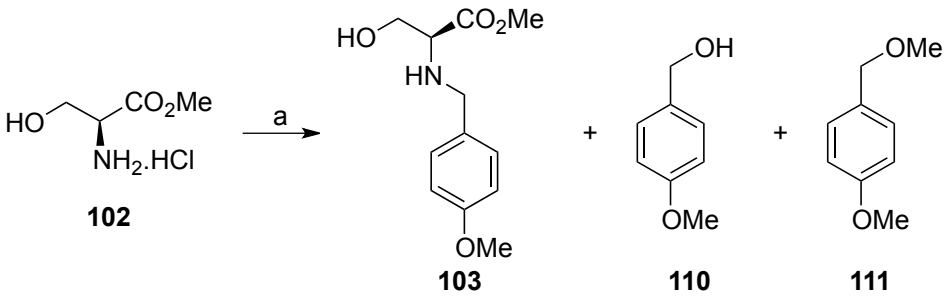
Esterification of commercially available L-serine with acetyl chloride in methanol gave L-serine methyl ester hydrochloride in quantitative yield, without purification.




**Reagents and Conditions:** a) Acetyl chloride, MeOH, reflux, quant.; b) 1. 4-Methoxybenzaldehyde, acetic acid, toluene, reflux; 2. $NaBH_3CN$, acetic acid, MeOH.

**Scheme 71.**

Using the optimized conditions described above (section **2.2.2**), L-serine methyl ester hydrochloride was treated with 4-methoxybenzaldehyde and acetic acid in toluene under reflux using a Dean-Stark apparatus. Unfortunately, the reaction was not successful, decomposition occurred and the imine was not observed when the $^1H$ NMR spectrum of the reaction mixture was analysed. L-Serine methyl ester hydrochloride is not soluble in toluene and decomposes when heated under reflux in toluene.

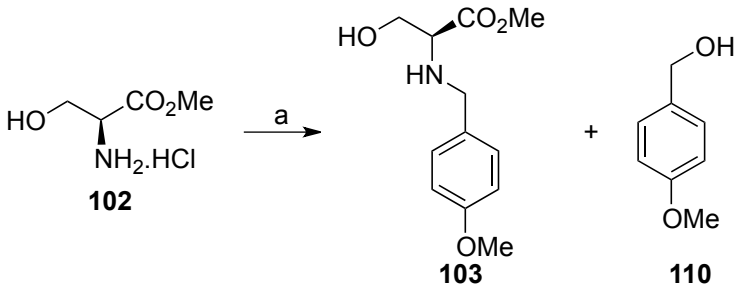



**Reagents and Conditions:** a) 4-methoxybenzaldehyde, $NaBH_3CN$, acetic acid, MeOH, **103**: 4%.

**Scheme 72.**

A one-pot procedure was attempted whereby treatment of L-serine methyl ester hydrochloride with 4-methoxybenzaldehyde, sodium cyanoborohydride and acetic acid in methanol resulted in the formation of three products. The major products observed were 4-methoxybenzylalcohol **110** and 1-methoxy-4-(methoxymethyl)benzene **111**. The desired compound **103** was the minor product, isolated in 4% yield. Unlike the leucine derivative, the by-products could be separated from the desired compound **103** using silica gel column chromatography.

In 1993, Yoo and co-workers reported the synthesis of PMB protected L-serine methyl ester through a one-pot procedure using triethylamine, 4-methoxybenzaldehyde, hydrogen and palladium on carbon in methanol.[46] Following their procedure, compound **103** was isolated in 64% yield. As well as increasing the yield of the desired product, the only by-product observed in this reaction was 4-methoxybenzylalcohol **110**.




**Reagents and Conditions:** a) 4-methoxybenzaldehyde, triethylamine, $H_2$, Pd/C, MeOH, **103**: 64%.

**Scheme 73.**

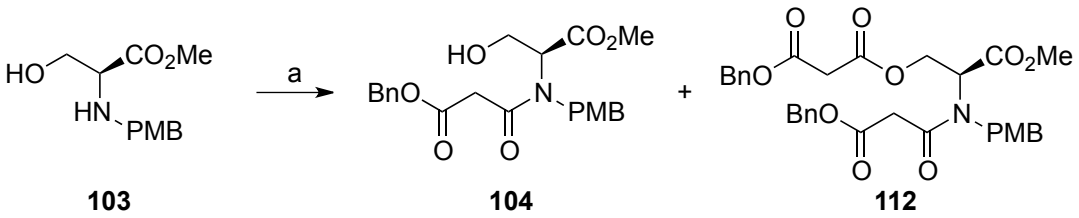
Attempts were made to optimize the reaction. The by-product (4-methoxybenzylalcohol) is formed when full conversion to the imine intermediate is not achieved; if full conversion can be achieved (as observed in the case of the leucine analogue), the yield of **103** should increase. L-serine methyl ester hydrochloride was treated with triethylamine in methanol in the presence of sodium sulfate. After stirring, the sodium sulfate was filtered off and the solvent removed under reduced pressure before analysis of the resulting material. This reaction was investigated under a range of conditions: increasing the reaction time, the use of 4 Å molecular sieves instead of sodium sulfate, and changing the solvent to dichloromethane. Under every set of

reaction conditions attempted, conversion to the imine was not complete according to $^1$H NMR spectrum analysis of the crude reaction mixture.

### 2.3.2.2 Synthesis of the Dieckmann Cyclization Precursor

#### 2.3.2.2.1 Peptide Coupling to the PMB Protected Amine

Applying the optimized coupling conditions described above (section **2.2.2**), compound **103** was treated with potassium benzyloxycarbonyl acetate **16**, EDAC·HCl, DMAP and NMM in dichloromethane. Two compounds were isolated from the reaction after silica gel column chromatography. The desired compound **104** was isolated in a low yield (14%). Compound **112**, the result of coupling occurring at both the amine and hydroxyl moieties, was isolated as the major product in 31% yield. This result is neither unprecedented nor unexpected; there are many reports in the literature of the coupling of a carboxylic acid to a hydroxyl moiety in the presence of carbodiimide coupling reagents.[47]

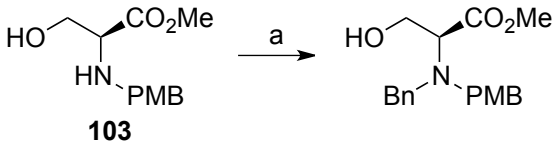



**103** **104** **112**

**Reagents and Conditions:** a) potassium benzyloxycarbonyl acetate **16**, EDAC·HCl, DMAP, NMM, $CH_2Cl_2$, **104**: 14%, **112**: 31%.

**Scheme 74.**

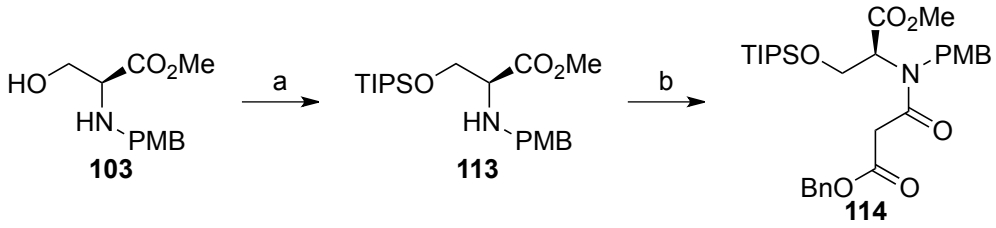
To prevent coupling to the hydroxyl moiety, a protection strategy was needed. Previous work in the group described the attempt at benzyl protection, however, this was found to be unsuccessful, and treatment of **103** with benzylbromide and potassium carbonate in DMF resulted in the formation of the tertiary amine as the only product.




**Reagents and Conditions:** a) benzylbromide, potassium carbonate, DMF.

**Scheme 75.**

As described above in our work on the leucine analogue, the most common reagents used to protect hydroxyl moieties are silyl reagents. A possible problem with this route is the use of TBAF during the Dieckmann cyclization step (competing reactions between removal of the protecting group and cyclization may occur), but we still felt it was a worthwhile route to investigate.

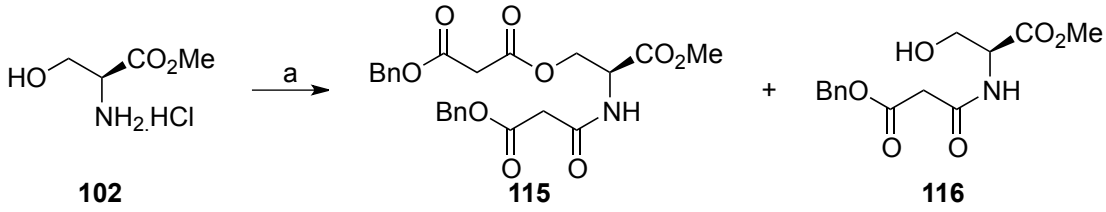



**Reagents and Conditions:** a) TIPS-Cl, imidazole, DMF, reflux, 90%; b) potassium benzyloxycarbonyl acetate **16**, EDAC·HCl, DMAP, NMM, $CH_2Cl_2$, 91%.

**Scheme 76.**

Treatment of **103** with TIPS-Cl and imidazole in DMF under reflux gave compound **113** in 90% yield. Subsequent peptide coupling, under optimized conditions, gave the tertiary amide **114** in 91% yield. None of the double-coupled product **112** was observed.

### 2.3.2.2.2 Peptide Coupling to L-Serine Methyl Ester Hydrochloride

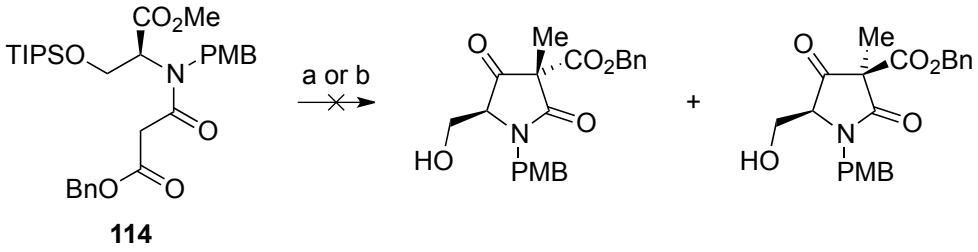



**Reagents and Conditions:** a) potassium benzyloxycarbonyl acetate **16**, EDAC·HCl, DMAP, NMM, $CH_2Cl_2$, **115**: 8%, **116**: 52%.

**Scheme 77.**

The coupling of **16** to the unprotected L-serine methyl ester hydrochloride was also investigated. When L-serine methyl ester hydrochloride was subjected to optimized peptide coupling conditions, compounds **115** and **116** were isolated in 8% and 52% yields, respectively. Interestingly, the major product of this reaction is the opposite to that observed above. Coupling occurs preferentially to the nitrogen with only small amounts of the *bis*-coupled compound observed; this may be due to the absence of the PMB group making the nitrogen atom much more reactive.

### 2.3.2.3 The Dieckmann Cyclization



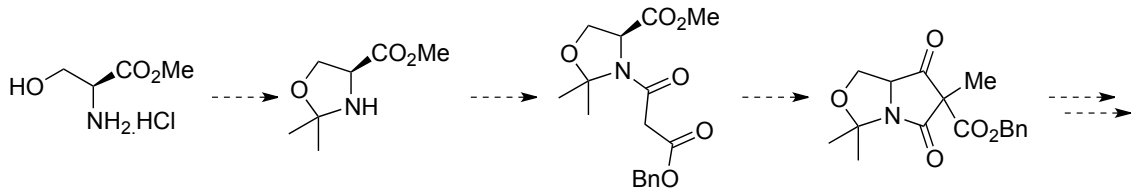

**Reagents and Conditions:** a) TBAF, MeI, THF; b) 1. TBAF, $Et_2O$, RT, overnight; 2. MeI, THF, RT, overnight.

**Scheme 78.**

Subjecting compound **114** to the optimized one-pot conditions for the Dieckmann cyclization proved unsuccessful, resulting in a complex mixture from which nothing was isolated. As predicted the reaction did not proceed cleanly and this was perhaps due to the competing reactions of TBAF; the cyclization and deprotection. This reaction was

also attempted under the original two-step conditions reported by Page, however, it was again unsuccessful, and no starting material or desired product was isolated from the reaction.

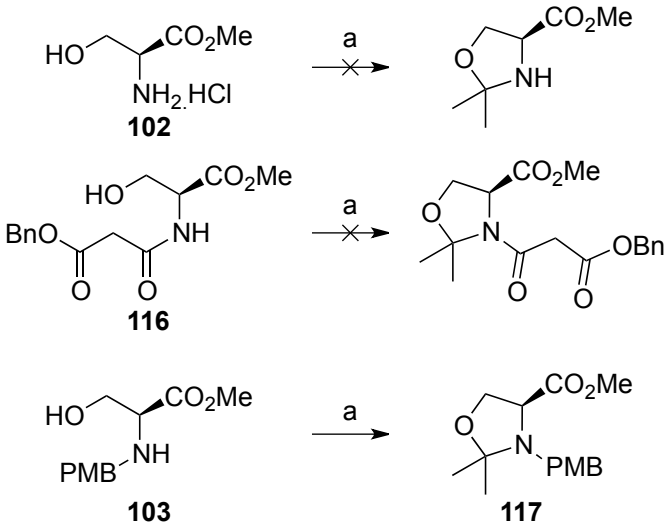
### 2.3.2.4 The Oxazolidine Approach




**Scheme 79.**

In conjunction with the work described above investigation into a route to simultaneously protect the amine and hydroxyl moieties was carried out. We hoped that forming the oxazolidine intermediate would not only reduce the number of steps in our synthetic route but that it might also have an effect on the ratio of diastereoisomers formed in the Dieckmann cyclization.

Following the method reported by Siciliano in 2014, compound **102** was treated with camphorsulfonic acid (CSA) and 2,2-dimethoxypropane (DMP) in toluene under reflux.[48] Unfortunately, this reaction proved unsuccessful and the starting material was recovered. Compound **116** was subjected to the same conditions and, again, only starting material was recovered. It was unclear as to why this reaction was not successful- but it may be due to the unstable nature of the oxazolidine.




**Reagents and Conditions:** a) CSA, DMP, toluene, reflux, **117**: 97%.

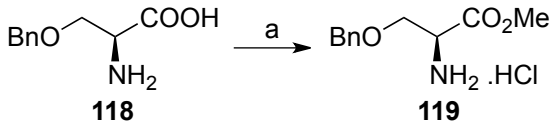
**Scheme 80.**

Compound **103** was subjected to the same conditions and, surprisingly, gave **117** in 97% yield. This compound was not purified due to its instability on silica gel. The $^{1}H$ NMR spectrum is relatively clean for this compound, however, decomposition occurs quickly as IR spectrum analysis show stretches corresponding to the starting material as well as the product, and mass spectrometry data analysis shows not only the product peak for $[M+H]^{+}$ at 280.15 but also the starting material peak for $[M+H]^{+}$ at 240.12. Although it is interesting that this reaction worked, the product was not of any use in our synthetic approach so we did not continue investigations any further.

## 2.3.3 Synthesis from *O*-Benzyl-L-Serine

### 2.3.3.1 Protection of the Amine

As we had predicted the possibility of competing reactions during the cyclization of the TIPS protected compound **114**, we obtained commercially available *O*-benzyl-L-serine to use as the starting material. Esterification of *O*-benzyl-L-serine **118** with acetyl chloride in methanol gave the *O*-benzyl-L-serine methyl ester hydrochloride salt **119** in quantitative yield without purification.

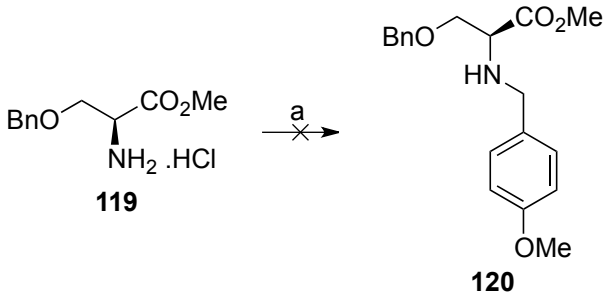



**118** **119**

**Reagents and Conditions:** a) Acetyl chloride, MeOH, reflux, quant.

**Scheme 81.**

With **119** in hand, protection of the amine moiety was the next step in the synthesis. As described above, the *O*-benzyl-L-serine derivative was also unstable under reflux, and, due to the presence of the benzyl group, the protection method reported by Yoon using hydrogenation conditions could not be used.

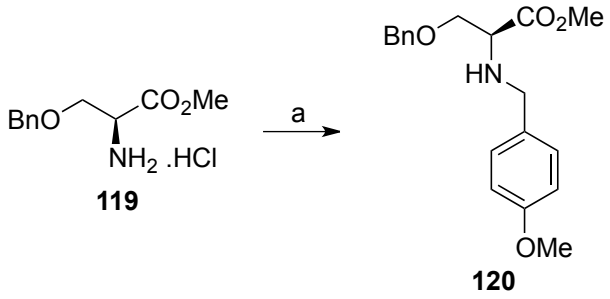



**119** **120**

**Reagents and Conditions:** a) 4-Methoxybenzaldehyde, triethylamine, $NaBH_3CN$, MeOH.

**Scheme 82.**

Treatment of **119** with triethylamine, 4-methoxybenzaldehyde and sodium cyanoborohydride in methanol did not afford the desired product. The major product isolated from the reaction mixture was *p*-methoxybenzylalcohol, a by-product formed as a result of the reduction of the 4-methoxybenzaldehyde starting material.

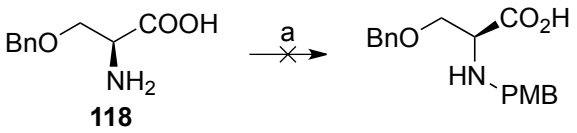



**119** **120**

**Reagents and Conditions:** a) 4-Methoxybenzyl chloride, potassium carbonate, $CH_3CN$, 45%.

**Scheme 83.**

With the protection of the amine group proving much more challenging than expected, we decided to investigate a different route. Treatment of **119** with 4-methoxybenzyl chloride and potassium carbonate in acetonitrile gave compound **120** in 45% yield. Unfortunately, as well as being low yielding, this reaction was not easily reproducible.[49]



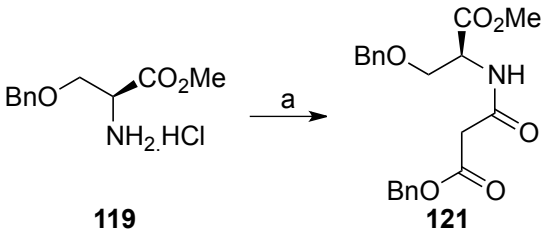

**118**

**Reagents and Conditions:** a) 4-Methoxybenzaldehyde, triethylamine, $NaBH_4$, MeOH.

**Scheme 84.**

As the PMB protection of **119** was proving difficult, we considered inverting the order of the reactions; i.e. attempting the protection before the esterification. Treatment of *O*-benzyl-L-serine **118** with 4-methoxybenzaldehyde, triethylamine and sodium borohydride in methanol resulted in the formation of a colourless solid. Interestingly, this solid was insoluble in all common organic solvents.

### 2.3.3.2 Synthesis of the Dieckmann Cyclization Precursor




**119** **121**

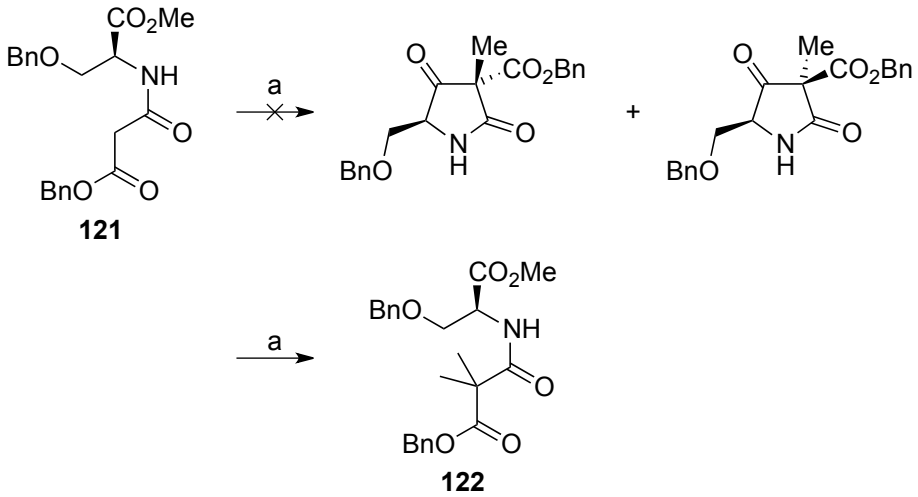
**Reagents and Conditions:** a) potassium benzyloxycarbonyl acetate **16**, EDAC·HCl, DMAP, NMM, $CH_2Cl_2$, 69%.

**Scheme 85.**

With PMB protection proving so difficult we decided to attempt the peptide coupling using *O*-benzyl-L-serine methyl ester hydrochloride as the starting material. Subjecting **119** to our optimized conditions gave **121** in 69% yield.

In comparison to the Dieckmann cyclization precursor of the leucine derivative **8**, rotamers were not observed in compound **121**; this is probably due to the lack of the PMB group, allowing free rotation around the amide C-N bond.

### 2.3.3.3 The Dieckmann Cyclization




**Reagents and Conditions:** a) TBAF, MeI, THF, 30%.

**Scheme 86.**

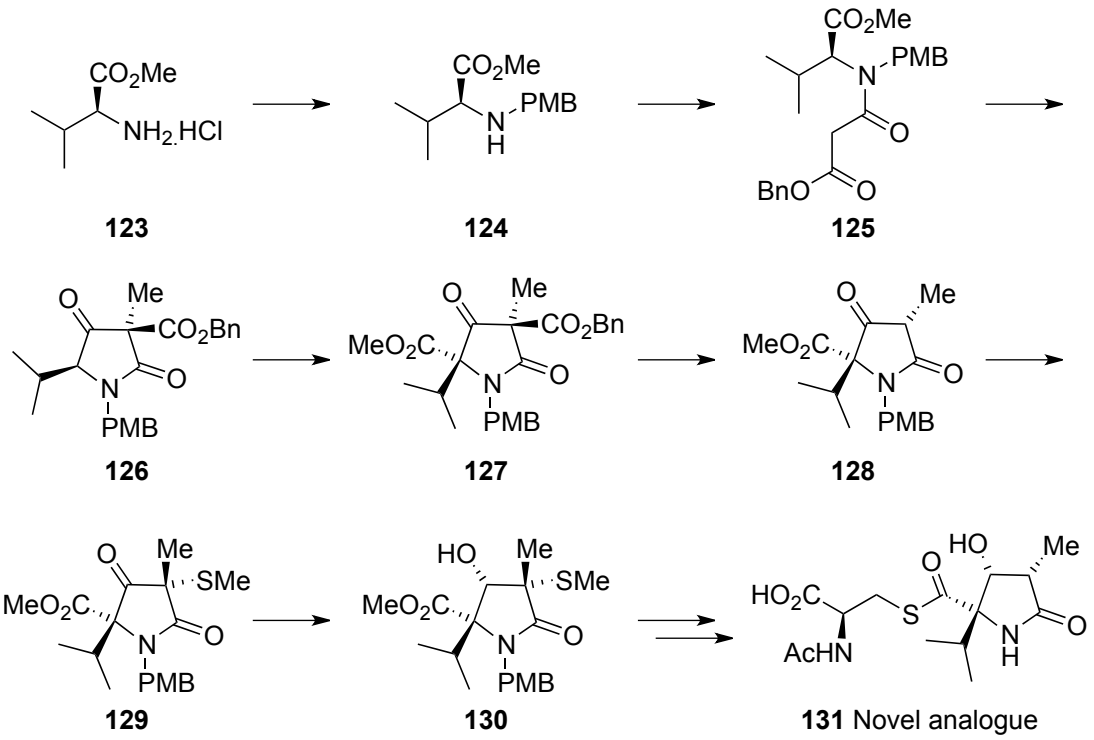
When the *O*-benzyl-L-serine derivative **121**, without the PMB protecting group, was subjected to optimized cyclization conditions, the desired compound was not observed and compound **122** was isolated in 30% yield. Deprotonation of the acidic malonyl-type protons (pKa≈13) occurs as expected and, instead of the desired intramolecular cyclization and alkylation reaction, alkylation at this position occurred twice to give the quaternary centre due to the excess of methyl iodide present in the reaction.

This result may be due to reduced reactivity, or it is possible that the sizes of the functional groups present are preventing cyclization in that the groups are too big and cyclization is not favourable.

## 2.4 Synthesis of the Valine Analogue

### 2.4.1 Proposed Synthetic Route Towards a Novel Analogue of Lactacystin

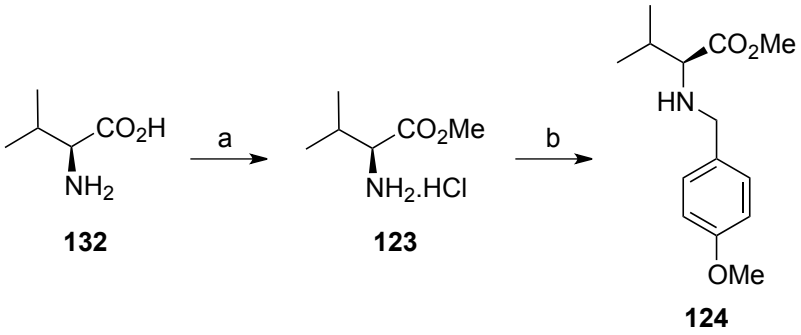
As one of the aims of this project is to design a synthetic route that allows ready access to both natural and novel analogues of lactacystin, L-valine methyl ester hydrochloride was also used as a starting material. Valine is similar in structure to leucine; it does not have the added hydroxyl functionality that played a significant role in the complications in the synthesis of the serine analogue.




**Scheme 87.**

## 2.4.2 Synthesis of the Dieckmann Cyclization Precursor

Esterification of commercially available L-valine with acetyl chloride in methanol gave L-valine methyl ester hydrochloride **123** in quantitative yield, without purification. As described above, L-valine has a similar structure to L-leucine, and it behaves in a chemically similar manner to L-leucine, unlike the serine derivatives.

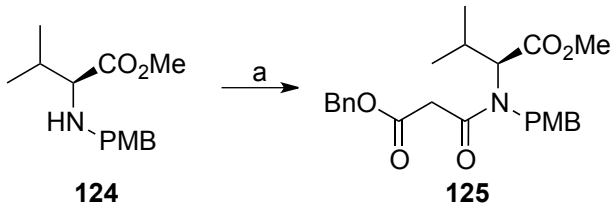



**132** **123** **124**

**Reagents and Conditions:** a) Acetyl chloride, MeOH, reflux, quant.; b) 1. 4-Methoxybenzaldehyde, acetic acid, toluene, reflux, quant.; 2. $NaBH_3CN$, acetic acid, MeOH, 74%.

**Scheme 88.**

Using the optimized conditions described above in our investigations of the leucine analogue (section **2.2.2**), L-valine methyl ester hydrochloride **123** was treated with 4-methoxybenzaldehyde and acetic acid then heated under reflux in toluene using a Dean-Stark apparatus. Full conversion to the imine was achieved, and reduction was performed using sodium cyanoborohydride. The desired compound **124** was isolated in 74% yield.




**124** **125**

**Reagents and Conditions:** a) Potassium benzyloxycarbonyl acetate **16**, EDAC·HCl, DMAP, NMM, $CH_2Cl_2$, 66%.

**Scheme 89.**

Treatment of **124** with potassium benzyloxycarbonyl acetate **16**, EDAC·HCl, DMAP and NMM in dichloromethane under optimized conditions gave compound **125** after silica gel column chromatography in 66% yield, a comparable yield to that achieved when using the leucine derivative (72%).

As was the case with the leucine derivative, room temperature $^{1}$H NMR spectrum analysis shows the presence of two rotamers. A variable-temperature (VT) $^{1}$H NMR experiment was carried out in deuteriated dimethylsulfoxide (*d*6-DMSO) at 25, 75 and 100 °C (**Figure 15**). Analysis of the resulting spectra does not show a significant difference between the spectra at 25 °C and 75 °C, but at 100 °C the peaks are clearly beginning to coalesce.

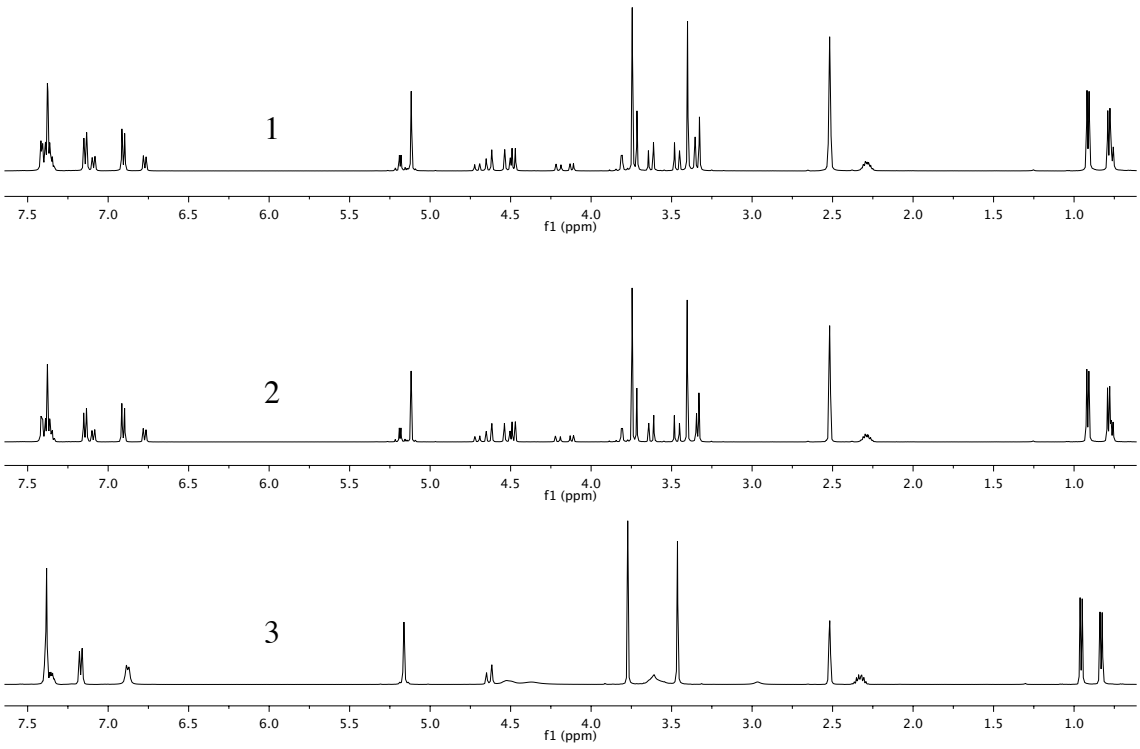
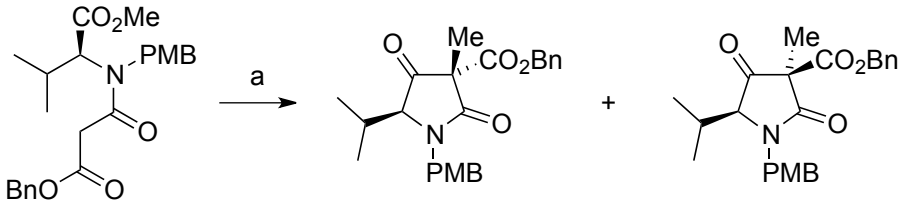



**Figure 15.** Variable temperature $^{1}$H NMR spectra of compound **125** at **1**: 25 °C, **2**: 75 °C and **3**: 100 °C.

## 2.4.3 The Dieckmann Cyclization




**125** (±)-**132** (±)-**126**

**Reagents and Conditions:** a) TBAF, MeI, THF, (±)-**132**: 25%, (±)-**126**: 41%.

**Scheme 90.**

Subjecting **125** to optimized one-pot tandem Dieckmann cyclization/methylation (section **2.2.3**) resulted in the formation of two diastereoisomers. Though we fully expected racemization to occur at this point and know (due to this racemization) it is not necessary to separate the diastereoisomers for the next step in our synthesis, the diastereoisomers were separated using silica gel column chromatography for analytical purposes. Compounds (±)-**132** and (±)-**126** were obtained in 25% and 41% yields, respectively. $^{1}$H NMR spectrum analysis of the reaction mixture before separation shows a 2:1 diastereoisomeric ratio; this reaction thus appears to be more diastereoselective than that of the leucine derivative. This increased selectivity is probably a result of the *iso*-propyl moiety at C5 in the product; the steric bulk of the *iso*-propyl group is closer to the ring than that of the *iso*-butyl group, therefore increasing steric hindrance on the top face. As expected, specific rotation measurements of each diastereoisomer confirmed that racemization had occurred at this point.

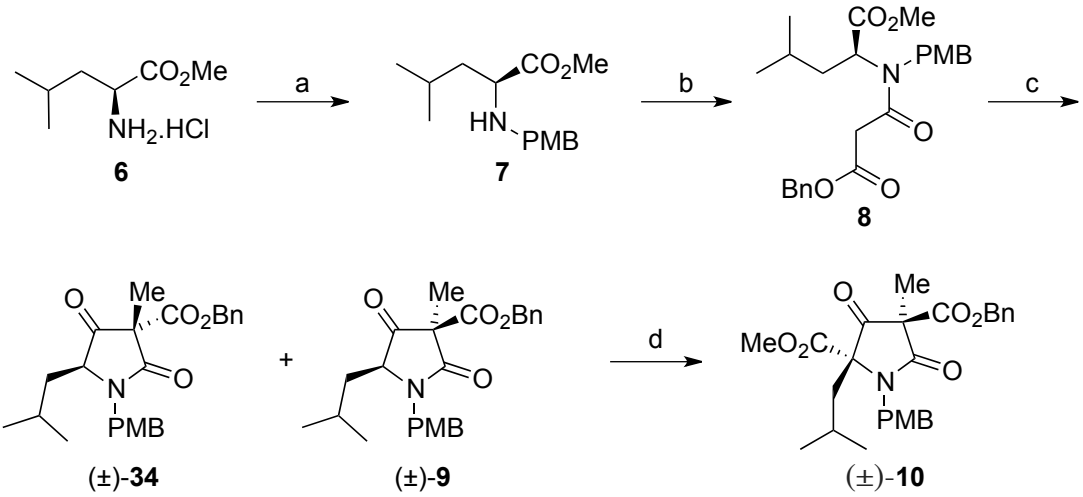
## 2.5 Conclusion

One of the aims of our work was to design a synthetic approach that can be applied to the synthesis of lactacystin and its analogues. We began our synthesis from a commercially available amino acid derivative (either from L-leucine, L-serine or L-valine); key steps in our approach included a tandem Dieckmann cyclization/alkylation to form the lactam ring and install the C7 functionality, and a Mander's acylation to form the C5 quaternary centre.

Our work was mainly focused on the use of the L-leucine derivative (L-leucine methyl ester hydrochloride) as the starting material, which would lead to the formation of deoxylactacystin. The Dieckmann cyclization precursor was synthesized following protection of the amine using 4-methoxybenzaldehyde followed by coupling to potassium benzyloxycarbonyl acetate using the carbodiimide reagent, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDAC·HCl).

The lactam core (common throughout the lactacystin analogues) was formed by a tandem Dieckmann cyclization/alkylation reaction. Previous work in the Page group described a two-step procedure, wherein cyclization occurs first using TBAF in diethyl ether to form the tetrabutylammonium salt, followed by the methylation step in tetrahydrofuran to install the C7 functionality. We were able to improve on this and complete the step as a one-pot procedure with no notable loss in yield. Disappointingly, the reaction did not appear to be diastereoselective and it appeared that racemization had also occurred during this step.

Formation of the C5 quaternary centre was achieved using a Mander's acylation by treatment of (±)-**9** and/or (±)-**34** with methylcyanoformate and LiHMDS. This reaction is completely stereoselective and addition occurs at the C5 position to the opposite side of the C7 benzyl ester.

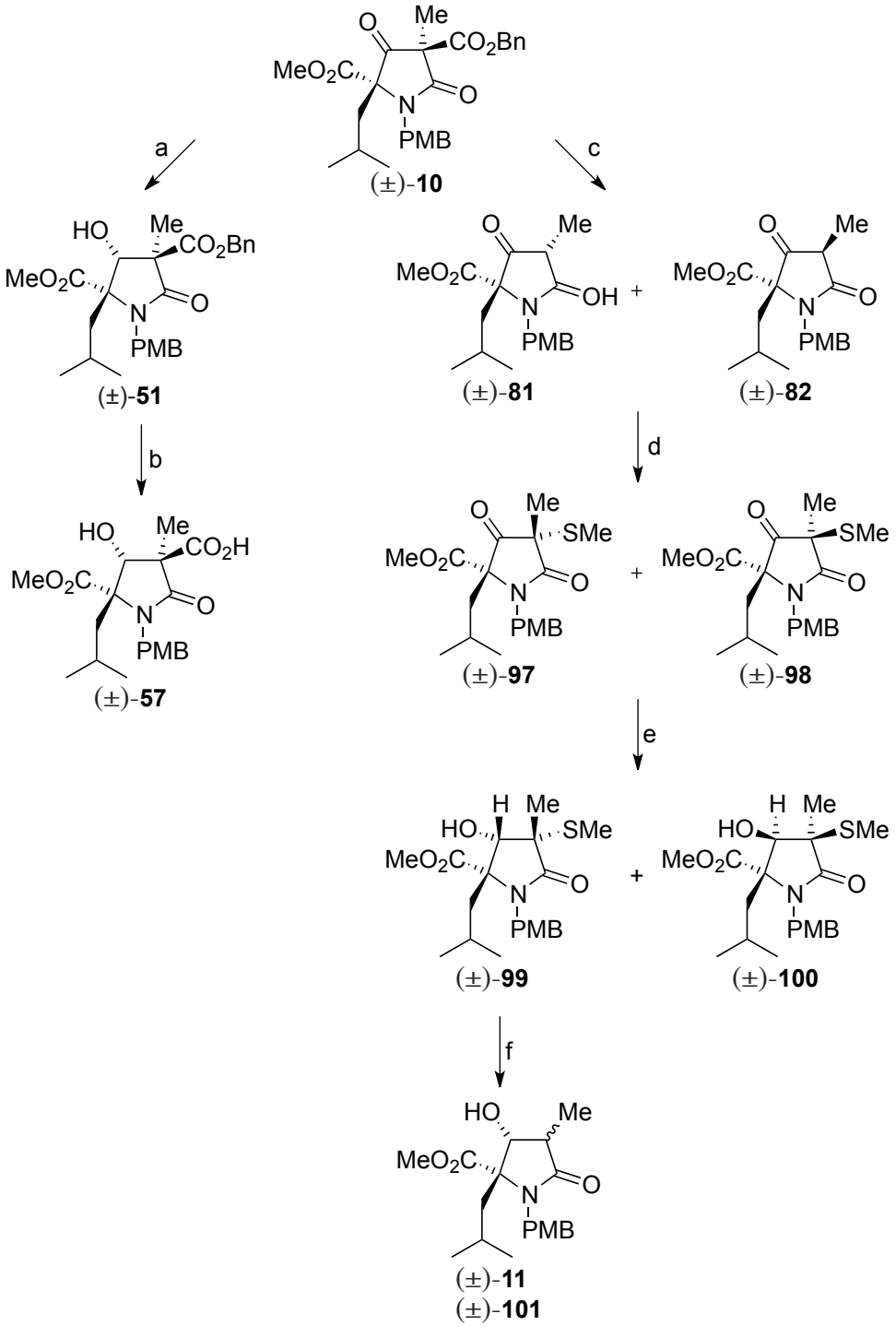



**Reagents and Conditions:** a) 1. 4-Methoxybenzaldehyde, acetic acid, toluene, reflux, 100%; 2. $NaBH_3CN$, acetic acid, MeOH, 93%; b) Potassium benzyloxycarbonyl acetate **16**, EDAC·HCl, DMAP, NMM, $CH_2Cl_2$, 72%; c) TBAF, MeI, THF, (±)-**34**: 22%, (±)-**9**: 39%; d) LiHMDS, DMPU, methyl cyanoformate, THF, –78 °C, 86%.

**Scheme 91.**

At this point, the synthesis could be completed using two possible routes. Route A consists of the reduction of the ketone at the C6 position of (±)-**10** to give (±)-**51**, followed by decarboxylation of the benzyl ester moiety at the C7 position. When (±)-**51** was treated under hydrogenolysis conditions, debenzylation occurred resulting in the carboxylic acid (±)-**57**. Many attempts were made to remove the carboxylic acid moiety, including a radical-mediated approach involving the synthesis of an acyl selenide, the Barton decarboxylation, the Krapcho decarboxylation and an acid-catalysed decarboxylation; unfortunately, none proved successful.

Route B inverted the order of the reactions; decarboxylation under hydrogenolysis conditions followed by the reduction of the ketone moiety at the C6 position. Treatment of (±)-**10** under hydrogenolysis conditions resulted in debenzylation with concomitant decarboxylation to give (±)-**81** and (±)-**82** as a mixture of inseparable diastereoisomers in a 1:1 ratio. All the attempts to reduce the ketone moiety at the C6 position proved unsuccessful. A strategy using a thiomethyl derivative reported by Corey in 1998 was then employed. Following Corey's method, we successfully synthesized the desired advanced intermediate (±)-**11**, however, it was isolated alongside diastereoisomer (±)-**101** as an inseparable mixture in a 3:1 ratio.




**Reagents and Conditions:** a) $NaBH_4$, EtOH, –10 °C, 30 min, 52%; b) $H_2$, $Pd(OH)_2/C$, THF, quant.; c) $H_2$, $Pd(OH)_2/C$, THF, 30 °C, >90%; d) *p*-$MeC_6H_4SO_2Me$, $Et_3N$, $CH_2Cl_2$, RT, (±)-**97** and (±)-**98**: 47%; e) $NaBH_4$, EtOH, –10 °C, 30 min, (±)-**99**: 17%, (±)-**100**: 25%; f) Raney Ni, EtOH, reflux, 4 h.

**Scheme 92.**

Using L-serine methyl ester hydrochloride as the starting material proved much more challenging. The extra hydroxyl functionality present in serine appears to affect the chemical behaviour of this compound. It does not have the same properties as the L-leucine derivative; for example, it is not soluble in the same solvents and so, from the start we had to alter our reaction conditions to compensate for this. We used L-serine methyl ester hydrochloride and, because the free hydroxyl group was causing problems, *O*-benzyl-L-serine methyl ester hydrochloride as starting materials. We have not yet been able to successfully synthesize the lactam core from the L-serine derivatives.

Fortunately, our preliminary work using L-valine methyl ester hydrochloride as the starting material to synthesize a novel lactacystin analogue is proving promising. The L-valine derivative appears, at present, to behave in a similar way to the L-leucine derivative. Following the same protection, coupling and cyclization steps, the substituted lactam core has been successfully synthesized.

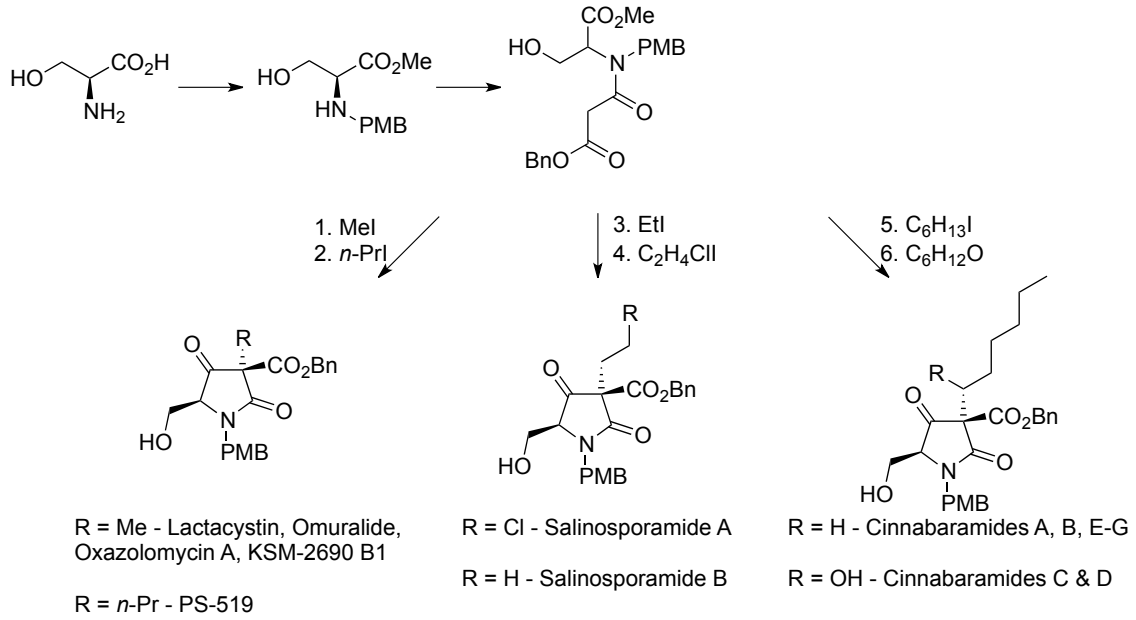
## 2.6 Recommended Future Work

Work in the immediate future should focus on the stereoselective desulfurization of compound (±)-**99**. As the reaction was carried out under reflux, we would hope that by reducing the temperature, the stereoselectivity could be controlled. Once desulfurization is successful the synthetic route should be applied to the L-valine derivative to synthesize a novel lactacystin analogue.

As the use of serine as the starting material means the hydroxyl functionality found in lactacystin is present from the start; in theory this should reduce the overall number of steps require in the total synthesis when compared to using the leucine derivative. Cyclization of the serine derivative to form the lactam core should be investigated further.

The tandem Dieckmann cyclization/alkylation step could be investigated further still by changing the alkylating agent. This would allow ready access to a variety of analogues. If cyclization using different alkylating agents can be achieved using the L-leucine derivative, and successful cyclization of the serine derivative is achieved, this

methodology can then be applied to the L-serine derivative to allow ready access to a range of analogues. **Scheme 93** details how the use of different alkylating agents results in the formation of the lactam core where the functionality at C7 corresponds to different analogues, for example by using ethyl iodide, the C7 group is now that found in salinosporamide B.




**Scheme 93.**

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# 3.0 Experimental

## 3.1 General Experimental

### 3.1.1 Preparation of Reagents, Solvents and Glassware

Commercially obtained reagents were used as supplied, without further purification, and stored in accordance to the supplier's recommendations, unless otherwise stated.

Solvents used for reactions and column chromatography were purified as required. Petroleum ether 40/60 (the fraction which boils between 40 °C and 60 °C) was distilled before use. Tetrahydrofuran and diethyl ether were dried over the sodium/benzophenone ketyl radical, then distilled. Dichloromethane was dried over calcium chloride and then distilled. Toluene was dried over sodium and then distilled. All other solvents were used as supplied from the manufacturer.

For reactions performed under anhydrous conditions, glassware was dried in the oven at 150 °C before being cooled in a desiccator over silica gel. Glassware was sealed with a septum cap before being flushed with nitrogen. Solvents used in anhydrous reactions were freshly prepared each time and added using a syringe.

### 3.1.2 Analysis of Compounds: Spectroscopic Techniques

Proton, carbon, fluorine and selenium NMR experiments (including 2D experiments) were carried out on a Bruker Advance III 500 MHz NMR spectrometer at 500, 126, 471 and 95 MHz, respectively. Samples were dissolved in the specified deuteriated solvents. All spectra were processed using MestReNova software, and chemical shifts were reported in ppm relative to either tetramethylsilane (TMS) or the residual solvent peak. Multiplicities are reported as either singlets (s), doublets (d), apparent doublets (app d) doublet of doublets (dd), triplets (t), quartets (q) or multiplets (m). Coupling constants (*J* values) are reported in Hertz (Hz).

Melting points were carried out on a Büchi B-545 instrument. All melting points were observed manually and are quoted as a range. Literature values are reported where available.

Mass spectra were obtained from the EPSRC UK National Mass Spectrometry Facility (NMSF) at Swansea University (previously known as the National Mass Spectrometry Service Centre, NMSSC).

Infra-Red (IR) spectra were recorded using a Perkin-Elmer Spectrum 100 FT-IR spectrometer and processed using Spectrum Express Application software, Version: 1.02.00.0014.

Specific rotation measurements were recorded using a Bellingham and Stanley ADP-440 polarimeter operating at the sodium (D) line emission of $\lambda$ = 589 nm at the specified temperature. Solutions used were prepared in a volumetric flask using spectrophotometric grade solvents. Literature values are quoted where available.

### 3.1.3 Chromatographic Techniques

Thin layer chromatography (TLC) was carried out on Merck aluminium backed plates coated with 0.2 mm Kiesegel 60 $GF_{254}$. Individual solvent systems are reported in the experimental procedures below. TLC plates were visualised under UV irradiation and/or stained using phosphomolybdic acid solution or potassium permanganate solution.

Flash column chromatography was carried out using glass columns packed with Kieselgel 40-63 μm silica gel. Individual solvent systems are reported in the experimental procedures below.

### 3.1.4 Numbering System

The numbering system used to assign $^{1}H$ and $^{13}C$ chemical shifts is designed by the author to simplify characterization.

## 3.2 Individual Experimental Procedures and Characterization

### 3.2.1 General Procedures

#### 3.2.1.1 General Procedure for the Esterification of an Amino Acid

Acetyl chloride (3 equiv.) was added slowly to methanol (30 mL/g of amino acid) while maintaining the temperature below 0 °C. After 30 min, the amino acid was added as a solid at 0 °C and the reaction mixture stirred for another 30 min. The reaction mixture was heated under reflux overnight. The solvent was removed under reduced pressure to yield the desired product as a solid without further purification.

#### 3.2.1.2 General Procedure for the Peptide Coupling using EDAC·HCl

The amine was dissolved in anhydrous dichloromethane (30 mL/g of amine). NMM (1.1-2 equiv.), benzyl malonic half ester (1.1-2 equiv.), EDAC·HCl (1.5-2.5 equiv.) and DMAP (0.2 equiv.) were added to the reaction mixture. The reaction mixture was stirred under an atmosphere of nitrogen for 20 h. An aqueous solution of HCl (1 M, 1 mL/g of amine) was added to the reaction mixture and stirring was continued for a further 30 min. The organic layer was separated, washed with water (2 x 30 mL/g of amine), dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness under reduced pressure. The residue was purified using column chromatography on silica gel.

#### 3.2.1.3 General Procedure for the One-pot Dieckmann Cyclization

The diester was dissolved in THF (50 mL/g of diester) under an atmosphere of nitrogen, and TBAF (1 M in THF, 3.5 equiv.) added. The reaction mixture was allowed to stir for 30 min at room temperature. The solution was cooled to 0 °C, iodomethane (4 equiv.)

added and stirred overnight. Water was added, and the solvents were removed under reduced pressure. The residue was dissolved in dichloromethane (50 mL/g of diester) and the resulting solution was washed with water (2 x 100 mL/g of diester), brine (2 x 100 mL/g of diester), dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness under reduced pressure. The residue was purified using column chromatography on silica gel.

#### 3.2.1.4 General Procedure for the Mander's Acylation Reaction

**Procedure A:** Hexamethyldisilazane (3 equiv.) was dissolved in anhydrous THF (10 mL/g of lactam) and the solution was cooled to –78 °C. *n*-Butyl lithium (2.5 M solution in hexanes, 3 equiv.) was added. The solution was allowed to stir for 30 min and DMPU (1.5 equiv.) added. The lactam starting material was dissolved in anhydrous THF (10 mL/mg of lactam), the resulting solution cooled to –78 °C, and DMPU (1.5 equiv.) added. The solution of LiHMDS was added dropwise to the solution of the lactam using a cannula. The mixture was stirred at –78 °C for 30 min. Methyl cyanoformate (5 equiv.) was added and the mixture stirred for a further 4 h at –78 °C. Saturated aqueous $NH_4Cl$ (1 mL/g of lactam) was added at –78 °C. The mixture was allowed to reach room temperature and the solvent removed under reduced pressure. The residue was dissolved in ethyl acetate and the resulting solution washed with water (2 x 10 mL/mg of lactam) and brine (2 x 10 mL/mg of lactam). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under reduced pressure. The residue was purified using column chromatography on silica gel.

**Procedure B:** The lactam starting material was dissolved in anhydrous THF (40 mL/g of lactam) and the solution cooled to –78 °C. DPMU (3 equiv.) and LiHMDS (1 M in THF, 2 equiv.) were added and the mixture stirred at –78 °C for 30 min. Methyl cyanoformate (3 equiv.) was added and the mixture stirred for a further 4 h at –78 °C. Saturated aqueous $NH_4Cl$ (1 mL/g of lactam) was added at –78 °C. The mixture was allowed to reach room temperature and the solvent removed under reduced pressure. The residue was dissolved in ethyl acetate and the resulting solution washed with water (2 x 40 mL/g of lactam) and brine (2 x 40 mL/g of lactam). The organic layer was dried

over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure. The residue was purified using column chromatography on silica gel.

### 3.2.1.5 General Procedure for the Ketone Reduction using $NaBH_4$

The ketone was dissolved in ethanol (50 mL/g of ketone). The mixture was cooled to −10 °C followed by the addition of $NaBH_4$ (0.5 equiv.). The reaction mixture was stirred at −10 °C for 30-120 min after which it was quenched with water (10 mL/g of ketone). The solvent was removed under reduced pressure and the resulting residue re-dissolved in ethyl acetate (50 mL/g of ketone). The mixture was washed with water (2 x 50 mL/g of ketone) and then brine (2 x 50 mL/g of ketone). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure. The residue was purified using column chromatography on silica gel (light petroleum ether/ethyl acetate).

### 3.2.1.6 General Procedure for the Treatment of the Benzyl Ester Under Hydrogenolysis Conditions

The benzyl ester was dissolved in anhydrous THF (5 mL/g of ester), to this was added $Pd(OH)_2$ / C (50% by weight). The reaction mixture was purged with nitrogen and treated with a balloon of hydrogen overnight at room temperature. The reaction mixture was filtered through celite and the solvent removed under reduced pressure.

### 3.2.1.7 General Procedure for the Formation of an Acyl Selenide

The carboxylic acid was dissolved in anhydrous dichloromethane (5 mL/g of carboxylic acid) under a nitrogen atmosphere and diphenyldiselenide (1.5 equiv.) added. The mixture was cooled to 0 °C and tributylphosphine (2 equiv.) added. The solution was warmed to room temperature and stirred overnight. The solution was diluted with dichloromethane (10 mL/g of carboxylic acid) and water (10 mL/g of carboxylic acid). The aqueous layer was extracted again with dichloromethane (10 mL/g of carboxylic acid). The organic fractions were combined, washed with brine (5 mL/g of carboxylic

acid), dried over anhydrous magnesium sulfate and evaporated to dryness under reduced pressure. The residue was purified using column chromatography on silica gel (light petroleum ether/ethyl acetate).

### 3.2.1.8 General Procedure for the Silyl Protection of the Hydroxyl Moiety

The silyl protection reagent (1.2 equiv.) and imidazole (2.5 equiv.) were added to a solution of the reduced Mander's reaction product in DMF (5 mL/g of reduced Mander's reaction product). The reaction mixture was stirred at 35 °C under an atmosphere of nitrogen overnight. The reaction was quenched with water (1 mL/g of reduced Mander's reaction product) and the solvent removed under reduced pressure. The resulting product was re-dissolved in diethyl ether (5 mL/g of reduced Mander's reaction product) and washed with water (2 x 10 mL/g of reduced Mander's reaction product) and brine (2 x 10 mL/g of reduced Mander's reaction product). The organic layer was dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness with no further purification.

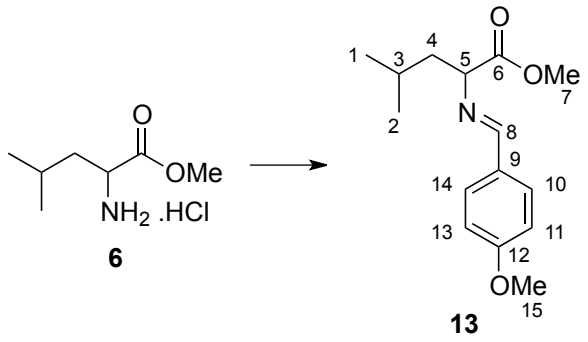
### 3.2.1.9 General Procedure for the Protection of the Hydroxyl Moiety at C6 using Trifluoroacetic Anhydride

The hydroxyl compound was dissolved in dry diethyl ether (10 mL/g of hydroxyl). The mixture was cooled to *ca*. 0 °C. Pyridine (2.5 equiv.) and trifluoroacetic anhydride (2.5 equiv.) were added to the reaction mixture. The reaction was monitored using TLC (light petroleum ether/ethyl acetate). On completion, pentane (10 mL/g of hydroxyl) was added and the mixture filtered through celite to remove the pyridinium trifluoroacetate by-product. The solvent was removed under reduced pressure and the resulting residue re-dissolved in dichloromethane (10 mL/g of hydroxyl), washed with water (2 x 10 mL/g of hydroxyl) and brine (2 x 10 mL/g of hydroxyl). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under reduced pressure.

## 3.2.2 Individual Experimental Procedures

### 3.2.2.1 Synthesis from L-Leucine

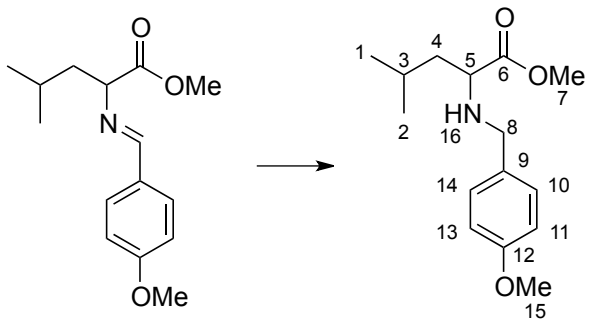
**R2-{[1-(4-Methoxy-phenyl)-meth-(E)-ylidene]-amino}-4-methyl-pentanoic acid methyl ester 13**




L-Leucine methyl ester hydrochloride (10.01 g, 0.06 mol) and 4-methoxybenzaldehyde (7.30 mL, 0.06 mmol, 1.1 equiv.) were dissolved in toluene (100 mL). Acetic acid (2 mL) was added and the reaction mixture heated under reflux using a Dean-Stark apparatus overnight. The solvent was evaporated to dryness under reduced pressure. The resulting product was obtained as a brown oil (15.82 g, quant.).

$\nu_{max}$ (thin film)/cm$^{-1}$ 2956 and 1739. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H, H8), 7.73 (app d, 2H, *J* = 8.9 Hz, H11 and H13), 6.93 (app d, 2H, *J* = 8.9 Hz, H10 and H14), 4.05 (dd, 1H, *J* = 8.8, 5.6 Hz, H5), 3.85 (s, 3H, H15), 3.74 (s, 3H, H7), 1.87–1.79 (m, 2H, H4), 1.61–1.54 (m, 1H, H3), 0.94 (d, 3H, *J* = 6.6 Hz, H1 or H2), 0.89 (d, 3H, *J* = 6.6 Hz, H1 or H2).

**2-(4'-Methoxy-benzylamino)-4-methyl-pentanoic acid methyl ester 7**[1]




**Direct synthesis of compound 7**: L-Leucine methyl ester hydrochloride (0.99 g 5.45 mmol) was dissolved in methanol (50 mL). Triethylamine (0.76 mL, 5.45 mmol) and 4-methoxybenzaldehyde (0.75 mL, 6.81 mmol, 1.25 equiv.) were added and the reaction mixture was stirred at room temperature for 90 min. The reaction mixture was cooled to 0 °C and sodium borohydride (0.38 g, 13.62 mmol, 2 equiv.) added. Stirring was continued for a further 30 min. The solvent was removed under reduced pressure and the resulting residue re-dissolved in ethyl acetate (75 mL). The organic layer was washed with water (2 x 30 mL), brine (2 x 30 mL) and saturated aqueous $Na_2CO_3$ (2 x 30 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness under reduced pressure. The title compound was obtained as a yellow/brown oil (0.77 g, 53%).

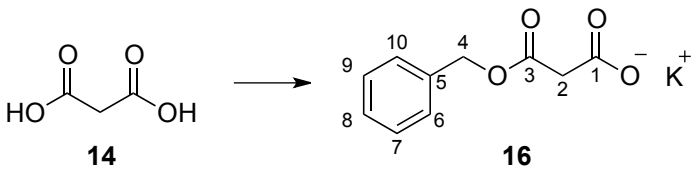
**Synthesis of compound 7 using the Dean-Stark protocol**

**Procedure A:** Compound **13** (15.82 g, 0.06 mol) was dissolved in ethanol (100 mL) and sodium borohydride (1.61 g, 0.04 mol, 0.7 equiv.) added slowly in small portions. The reaction was monitored by TLC then quenched with water (30 mL). The solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (50 mL) and the resulting solution was washed with water (2 x 50 mL), brine (2 x 50 mL) and saturated aqueous $Na_2CO_3$ (2 x 50 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness under reduced pressure. The title compound was obtained as a yellow/brown oil (8.42 g, 58%).

**Procedure B:** Compound **13** (22.32 g, 0.85 mol) and acetic acid (4.5 mL) were dissolved in methanol (200 mL). The reaction mixture was cooled to 0 °C and sodium cyanoborohydride (10.66 g, 0.17 mol, 2 equiv.) added slowly in small portions. The reaction was stirred for 30 min at 0 °C then allowed to reach room temperature and stirred for a further 5 h. Water (20 mL) was added to quench the reaction mixture and the solvents were removed under reduced pressure. The residue was dissolved in dichloromethane (100 mL), washed with water (2 x 100 mL), brine (2 x 100 mL) and a saturated solution of $Na_2CO_3$ (2 x 100 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness under reduced pressure. The title compound was obtained as a yellow/brown oil (20.89 g, 93%).

Found (NSI): $[M+H]^+$ 266.1755; $[C_{15}H_{23}NO_3+H]^+$ requires 266.1751. $\nu_{max}$ (thin film)/$cm^{-1}$ 2955 and 1733. $[\alpha]_D$ = +7 (*c* 1.5, $CDCl_3$, 25 °C, lit –31.17, *c* 1.5, $CDCl_3$, 27 °C).[1] $^1$H NMR (500 MHz, $CDCl_3$) δ 7.23 (app d, 2H, *J* = 8.6 Hz, H11 and H13), 6.84 (app d, 2H, *J* = 8.6 Hz, H10 and H14), 3.77 (d, 3H, *J* = 0.8 Hz, H15), 3.73 (d, 1H, *J* = 12.7 Hz, H8), 3.70 (s, 3H, H7), 3.54 (d, 1H, *J* = 12.7 Hz, H8), 3.29 (dd, 1H, *J* = 7.7, 6.8 Hz, H5), 1.82 – 1.72 (m, 2H, H3 and H16), 1.46 (ddd, 2H, *J* = 7.4, 6.7, 2.1 Hz, H4), 0.91 (d, 3H, *J* = 6.7 Hz, H1 or H2), 0.84 (d, 3H, *J* = 6.7 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 176.5 (C6), 158.7 (C12), 132.0 (C9), 129.4 (C11 and C13), 113.7 (C10 and C14), 59.1 (C5), 55.2 (C15), 51.6 (C8), 51.5 (C7), 42.8 (C4), 24.9 (C3), 22.8 (C1 or C2), 22.2 (C1 or C2).

**Potassium benzyloxycarbonyl acetate 16**[2]

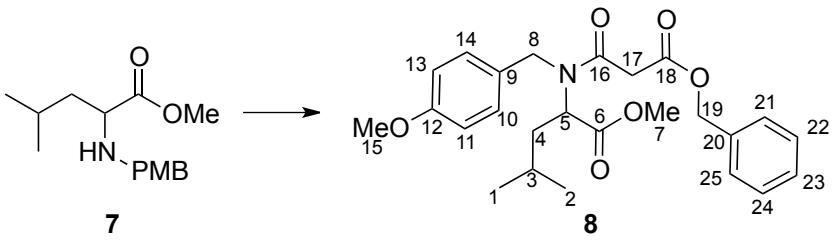



Malonic acid (50.10 g, 0.48 mol), benzyl alcohol (105 mL, 1.01 mol, 2.1 equiv.) and *p*-TsOH (0.92 g, 4.81 mmol, 0.01 equiv.) were dissolved in toluene (500 mL) and heated under reflux using a Dean-Stark apparatus overnight. The solvents were removed under reduced pressure and the residue was dissolved in a solution of KOH in BnOH (1 M,

26.00 g in 480 mL). A yellow solid precipitated which was then filtered and washed with diethyl ether (300 mL). The solid was dried in the vacuum oven overnight yielding the title compound as a colourless solid (80.10 g, 72%).

$\nu_{max}$ (solid) $cm^{-1}$ 1722 and 1599. mp 199-202 °C, lit mp 201 °C.[2] $^1$H NMR (500 MHz, $D_2O$) δ 7.40 – 1.35 (m, 5H, H6-10), 5.15 (s, 2H, H4), 3.29 (s, 2H, H2). $^{13}$C NMR (126 MHz, $D_2O$) δ 173.9 (C1 or C3), 171.1 (C1 or C3), 135.6 (C5), 128.6 (C6-10), 67.2 (C4), 44.6 (C2).

**2-{(4-Methoxy-benzyl)-[2-(benzyloxycarbonyl)-acetyl]-amino}-4-methyl-pentanoic acid methyl ester 8**




**Coupling using EDAC·HCl:** Compound **7** (9.84 g, 0.37 mol) was subjected to the general procedure for peptide coupling using EDAC·HCl 17.73 g, 0.93 mol, 2.5 equiv.), NMM (8.2 mL, 0.75 mol, 2 equiv.), benzyl malonic half ester (17.19 g, 0.75 mol, 2 equiv.) and DMAP (0.90 g, 0.07 mol, 0.2 equiv.). The residue was purified using column chromatography on silica gel (30% ethyl acetate in light petroleum ether). Compound **8** was isolated as a yellow oil (11.73 g, 72%).

**Coupling using T3P®:** Compound **7** (0.22 g, 0.80 mmol) was dissolved in THF (5 mL) and benzyl malonic half ester (0.23 g, 0.10 mmol, 1.2 equiv.) was added. The mixture was cooled to 0 °C and DIPEA (0.26 mL, 1.49 mmol, 1.8 equiv.), followed by T3P® (50% solution in THF, 0.79 g, 1.29 mmol, 1.5 equiv.) were added. The reaction was allowed to reach room temperature and then stirred for 20 h. Water (10 mL) was then added to the reaction mixture and extracted with ethyl acetate (2 x 10 mL). The organic layers were combined, washed with water (2 x 20 mL) and brine (2 x 20 mL), dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness under reduced

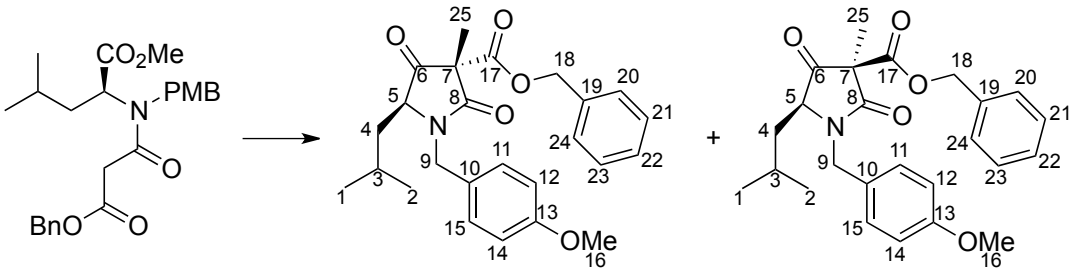
pressure. The residue was purified using column chromatography on silica gel (30% ethyl acetate in light petroleum ether) to yield the title compound as a yellow oil (0.23 g, 62%).

**Coupling from the acid chloride of the malonic half ester:** Potassium benzyloxycarbonyl acetate (0.72 g, 3.08 mmol) was suspended in toluene (5 mL) and oxalyl chloride (0.83 mL, 9.87 mmol, 3.2 equiv.) added. The reaction mixture was stirred at 34 °C for 24 h. The solvent and excess oxalyl chloride were removed under reduced pressure to give the corresponding acyl chloride which was used without purification. Compound **7** (1.01 g, 3.70 mmol, 1.2 equiv.) was dissolved in anhydrous dichloromethane (20 mL) and the solution cooled to 0 °C. The acyl chloride was dissolved in anhydrous dichloromethane (10 mL) and added to the amine solution. While maintaining the temperature at 0 °C, pyridine (0.01 mL, 0.03 mmol, 0.01 equiv.) and DMAP (0.02 g, 0.15 mmol, 0.05 equiv.) were added. After addition the reaction mixture was allowed to reach room temperature and stirred for 4 h. The reaction mixture was washed with an aqueous solution of HCl (5%, 20 mL), dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness under reduced pressure. The residue was purified using column chromatography on silica gel (30% ethyl acetate in light petroleum ether) to yield the title compound as a yellow oil (0. 97 g, 58%).

Found (NSI): $[M+H]^+$ 442.2226; $[C_{25}H_{31}NO_6+H]^+$ requires 442.2224. $\nu_{max}$ (thin film)/$cm^{-1}$ 2955, 1740 and 1655. $[\alpha]_D$ = +12 (*c* 1, $CHCl_3$, 25 °C).

**Major Rotamer**: $^1$H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.33 (m, 5H, H21-25), 7.15 (app d, 2H, *J* = 8.7 Hz, H11 and H13), 6.85 (app d, 2H, *J* = 8.7 Hz, H10 and H14), 5.16 (d, 2H, *J* = 2.4 Hz, H17), 4.82 (dd, 1H, *J* = 7.4, 6.3 Hz, H5), 4.55 (d, 1H, *J* = 17.1 Hz, H8), 4.42 (d, 1H, *J* = 17.1 Hz, H8), 3.79 (s, 3H, H15), 3.57 (s, 3H, H7), 3.48 (d, 2H, *J* = 3.7 Hz, H19), 1.89 – 1.81 (m, 1H, H4), 1.58 – 1.53 (m, 1H, H4 and H3) 0.88 (d, 3H, *J* = 6.3 Hz, H1 or H2), 0.80 (d, 3H, *J* = 6.3 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.7, 167.2, 159.2, 135.4, 128.6 (Ar), 128.4 (Ar), 128.4 (Ar), 128.1, 127.96 (C11 and C13), 114.2 (C10 and C14), 67.2 (C17), 56.1 (C5), 55.3 (C15), 52.0 (C7), 50.0 (C8), 41.7 (C19), 38.3 (C4), 25.1 (C3), 22.6 (C1 or C2), 22.3(C1 or C2).

**The Dieckmann Cyclization**




**Two-step synthesis:** Diester **8** (8.86 g, 0.02 mol) was dissolved in diethyl ether (50 mL) under an atmosphere of nitrogen. TBAF (1 M solution in THF, 50 mL, 0.05 mol, 2.5 equiv.) was added and the reaction mixture was stirred overnight. The solvents were removed under reduced pressure and the resulting residue was dissolved in THF (50 mL) under an atmosphere of nitrogen and the solution was cooled to 0 °C. Iodomethane (5.00 mL, 0.08 mol, 4 equiv.) was added and the reaction was stirred overnight. The solvent was removed under reduced pressure and the resulting residue was dissolved in dichloromethane (50 mL). The solution was washed with water (2 x 50 mL), dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness under reduced pressure. The resulting residue was purified using column chromatography on silica gel (20% ethyl acetate in light petroleum ether). The first eluting diastereoisomer (±)-**34** was obtained as a pale yellow oil (2.59 g, 30%), the second eluting diastereoisomer (±)-**9** was obtained as a darker yellow oil (2.47 g, 29%).

**One-pot synthesis:** Diester **8** (0.21 g, 0.5 mmol) was subjected to the general procedure for the one-pot Dieckmann cyclization using TBAF (1 M in THF, 1.60 mL, 1.6 mmol, 3.5 equiv.) and iodomethane (0.12 mL, 1.8 mmol, 4 equiv.). The residue was purified using column chromatography on silica gel (20% ethyl acetate in light petroleum ether as the eluent). The first eluting diastereoisomer (±)-**34** was obtained as a pale yellow oil (0.044 g, 22%), the second eluting diastereoisomer (±)-**9** was obtained as a dark yellow oil (0.08 g, 39%).

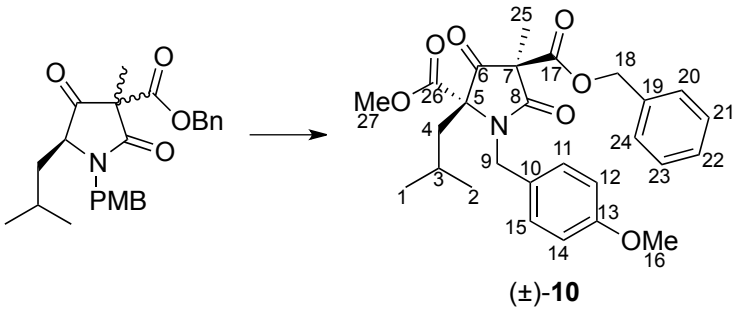
**(±)-(3*S*,5*S*)-*N*-(4'-Methoxybenzyl)-3-methyl-5-(2'-methylpropyl)-pyrrolidin-2,4-dione-3-carboxylic acid benzyl ester (±)-34**

Found (NSI): $[M+Na]^+$ 424.2110; $[C_{27}H_{31}NO_7+Na]^+$ requires 424.2118. $\nu_{max}$ (thin film)/$cm^{-1}$ 2958, 1778, 1747 and 1697. $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.35 (dd, 3H, *J* = 5.0, 1.9 Hz, Ar), 7.24 – 7.21 (m, 2H, Ar), 7.02 (app d, 2H, *J* = 8.6 Hz, H12 and H14), 6.59 (app d, 2H, *J* = 8.6 Hz, H11 and H15), 5.42 (d, 1H, *J* = 15.0 Hz, H9), 5.19 (d, 1H, *J* = 12.3 Hz, H18), 5.09 (d, 1H, *J* = 12.3 Hz, H18), 3.85 (dd, 1H, *J* = 7.8, 3.9 Hz, H5), 3.80 (d, 1H, *J* = 15.0 Hz, H9), 3.73 (s, 3H, H16), 1.82 – 1.73 (m, 1H, H3), 1.67 – 1.61 (m, 1H, H4), 1.58 (s, 3H, H25), 1.56 – 1.48 (m, 1H, H4), 0.87 (d, 3H, *J* = 6.6 Hz, H1 or H2), 0.76 (d, 3H, *J* = 6.6 Hz, H1 or H2). $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 205.9 (C6), 169.6 (C8), 165.6 (C17), 159.2 (C13), 134.8 (C10 and C19), 129.4 (C12 or C14), 128.7 (Ar), 128.5 (Ar), 128.2 (Ar), 126.2 (C7), 114.1 (C11 or C15), 68.1 (C18), 62.4 (C5), 58.6, 55.2 (C16), 43.2 (C9), 37.9 (C4), 24.6 (C3), 23.2 (C1 or C2), 22.4 (C1 or C2), 16.1 (C25).

**(±)-(3*R*,5*S*)-*N*-(4'-Methoxybenzyl)-3-methyl-5-(2'-methylpropyl)-pyrrolidin-2,4-dione-3-carboxylic acid benzyl ester (±)-9**

Found (NSI): $[M+Na]^+$ 424.2108; $[C_{27}H_{31}NO_7+Na]^+$ requires 424.2118. $\nu_{max}$ (thin film)/$cm^{-1}$ 2927, 1775, 1746 and 1696. $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.26 (m, 5H, H20-24), 7.13 (app d, 2H, *J* = 8.6 Hz, H12 and H14), 6.83 (app d, 2H, *J* = 8.6 Hz, H11 and H15), 5.26 (d, 1H, *J* = 12.1 Hz, H18), 5.19 (d, 1H, *J* = 14.9 Hz, H9), 5.08 (d, 1H, *J* = 12.1 Hz, H18), 4.01 (d, 1H, *J* = 14.9 Hz, H9), 3.79 (s, 3H, H16), 3.67 (t, 1H, *J* = 6.8 Hz, H5), 1.77 – 1.72 (m, 1H, H3), 1.54 (s, 3H, H25), 1.50 (dd, 2H, *J* = 7.4, 6.5 Hz, H4), 0.73 (d, 3H, *J* = 6.5 Hz, H1 or H2), 0.70 (d, 3H, *J* = 6.5 Hz, H1 or H2). $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 205.6 (C6), 169.3 (C8), 165.6 (C17), 159.4 (C13), 134.7 (C10 and C19), 129.4 (C12 and C14), 128.7, 128.6, 128.4, 127.1, 114.3 (C11 and C15), 68.2 (C18), 62.4 (C5), 58.6, 55.30 (C16), 43.5 (C9), 38.9 (C4), 24.4 (C3), 23.0 (C1 or C2), 21.9 (C1 or C2), 16.6 (C25).

**(±)-(3*R*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-5-(2'-methylpropyl)-pyrrolidin-2,4-dione-3,5-dicarboxylic acid-3-benzyl ester-5-methyl ester (±)-10**




(±)-**10**

Synthesized according to the general procedure for the Mander's acylation reaction (Procedure A) from the first eluting diastereoisomer (±)-**34**. Compound (±)-**34** (0.16 g, 0.38 mmol), hexamethyldisilazane (0.24 mL, 1.13 mmol, 3 equiv.), n-BuLi (2.5 M in THF, 0.46 mL, 1.13 mmol, 3 equiv.), DMPU (0.07 mL, 0.57 mmol, 1.5 equiv.) and methyl cyanoformate (0.15 mL, 1.88 mmol, 5 eqiuv.). Compound (±)-**10** was isolated as an off-white solid (0. 13 g, 73%).

Synthesized according to the general procedure for the Mander's acylation reaction (Procedure A) from the second eluting diastereoisomer (±)-**9**. Compound (±)-**9** (0.22 g, 0.52 mmol), hexamethyldisilazane (0.32 mL, 1.56 mmol, 3 equiv.), n-BuLi (2.5 M in THF, 0.62 mL, 1.56 mmol, 3 equiv.), DMPU (0.06 mL, 0.78 mmol, 1.5 equiv.) and methyl cyanoformate (0.20 mL, 2.60 mmol, 5 equiv.). Compound (±)-**10** was isolated as an off-white solid (0.19 g, 79%).

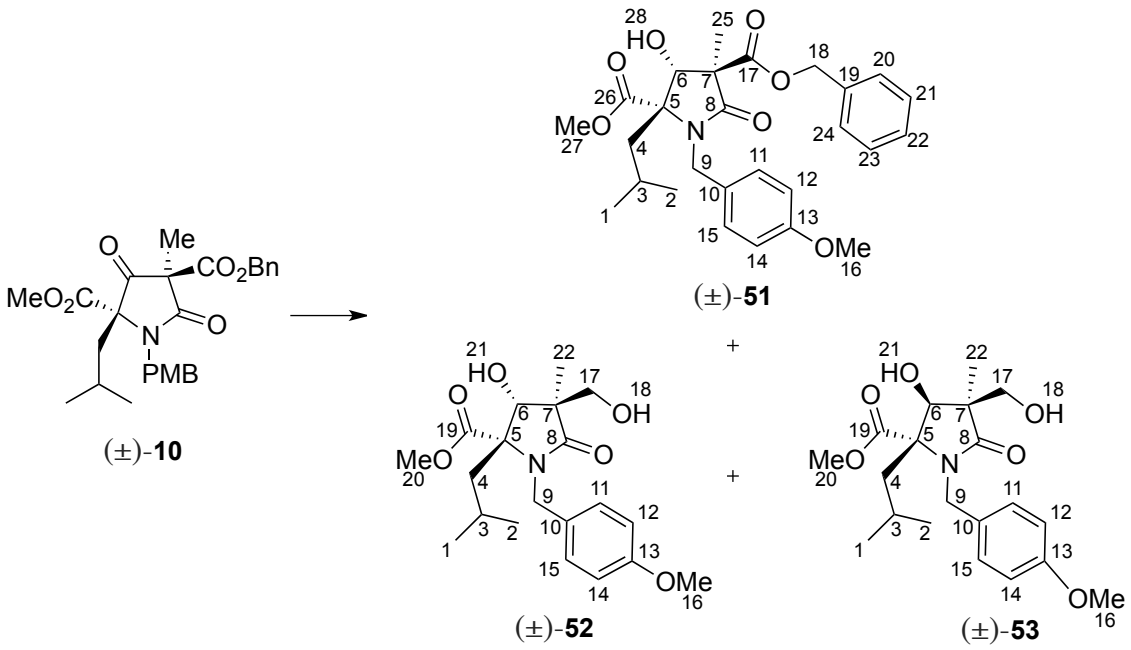
Synthesized according to the general procedure for the Mander's acylation reaction (Procedure A) from the mixture of diastereoisomers (±)-**34** and (±)-**9**. A mixture of compounds (±)-34 and (±)-**9** (0.34 g, 0.80 mmol, 3 equiv.), hexamethyldisilazane (0.50 mL, 2.41 mmol, 3 equiv.), n-BuLi (2.5 M in THF, 0.96 mL, 2.41 mmol, 3 equiv.), DMPU (0.15 mL, 1.20 mmol, 1.5 equiv.) and methyl cyanoformate (0.32 mL, 4.01 mmol, 5 equiv.). Compound (±)-**10** was isolated as an off-white solid (0.34 g, 78%).

Synthesized according to the general procedure for the Mander's acylation reaction (Procedure B) from a mixture of diastereoisomers (±)-**34** and (±)-**9**. A mixture of

compounds (±)-**34** and (±)-**9** (0.34 g, 0.8 mmol), DPMU (1.10 mL, 9.19 mmol, 3 equiv.), LiHMDS (1 M in THF, 6.1 mL, 6.13 mmol, 2 equiv.) and methyl cyanoformate (0.78 mL, 9.19 mmol, 3 equiv.). Compound (±)-**10** was isolated as an off-white solid (1.26 g, 86%).

Found (NSI): $[M+Na]^+$ 504.1979; $[C_{27}H_{31}NO_7+Na]^+$ requires 504.1993. $\nu_{max}$ (thin film)/$cm^{-1}$ 2958, 1782, 1751 and 1699. mp 79-82 °C. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.28 (m, 5H, H20-24), 7.19 (app d, 2H, *J* = 8.7 Hz, H12 and H14), 6.77 (app d, 2H, *J* = 8.7 Hz, H11 and H15), 5.20 (d, 1H, *J* = 12.1 Hz, H18), 5.14 (d, 1H, *J* = 12.1 Hz, H18), 4.89 (d, 1H, *J* = 15.0 Hz, H9), 4.15 (d, 1H, *J* = 15.0 Hz, H9), 3.77 (s, 3H, H16), 3.23 (s, 3H, H27), 2.15 (dd, 1H, *J* = 15.2, 5.4 Hz, H4), 1.86 (dd, 1H, *J* = 15.2, 6.3 Hz, H4), 1.72 (s, 3H, H25), 1.47 – 1.41 (m, 1H, H3), 0.65 (d, 3H, *J* = 6.6 Hz, H1 or H2), 0.52 (d, 3H, *J* = 6.6 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 201.8 (C6), 170.8 (C8), 167.6 (C17 or C26), 165.2 (C17 or C26), 159.23 (C13), 134.5 (C10 and C19), 130.3 (C12 and C14), 128.7 (Ar), 128.6 (Ar), 128.6 (Ar), 127.6 (C5 or C7), 113.8 (C11 and C15), 76.1 (C5 or C7), 68.5 (C18), 58.4, 55.3 (C16), 52.9 (C27), 43.9 (C9), 38.7 (C4), 24.2 (C1 or C2), 23.4 (C1 or C2), 23.1 (C3), 18.9 (C25).

**Reduction of the Mander's Acylation Reaction Product (±)-10**




**Procedure A**: Compound (±)-**10** (0.15 g, 0.32 mmol) was subjected to the general procedure for ketone reduction using $NaBH_4$ (0.01 g, 0.22 mmol, 0.5 equiv.). The reaction was stirred at −10 °C for 2 h. The residue was purified using column chromatography on silica gel (light petroleum ether/ethyl acetate, 2:1). Compound (±)-**51** was isolated as a white foam (0.07 g, 42%). Compound (±)-**52** or (±)-**53** (0.04 g, 29%). Compound (±)-**52** or (±)-**53** (0.01 g, 10%).

**Procedure B**: Compound (±)-**10** (0.21 g, 0.44 mmol, 1 equiv.) was subjected to the general procedure for ketone reduction using $NaBH_4$ (0.01 g, 0.22 mmol, 0.5 equiv.). The reaction was stirred at −10 °C for 30 min. The residue was purified using column chromatography on silica gel (light petroleum ether/ethyl acetate, 2:1). Compound (±)-**51** was isolated as a white foam (0.11 g, 52%). Starting material (0.07 g, 31%).

**(±)-(3*R*,4*R*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-4-hydroxy-5-(2'-methylpropyl)-pyrrolidin-2-one-3,5-dicarboxylic acid-3-benzyl ester-5-methyl ester (±)-51**

Found (NSI): $[M+Na]^+$ 506.2134; $[C_{27}H_{33}NO_7+Na]^+$ requires 506.2149. $\nu_{max}$ (thin film)/$cm^{-1}$ 3373, 1735 and 1676. $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.33 (m, 5H, H20-24), 7.11 (app d, 2H, *J* = 8.7 Hz, H12 and H14), 6.68 (app d, 2H, *J* = 8.7 Hz, H11

and H15), 5.27 (d, 1H, *J* = 12.4 Hz, H18), 5.24 (d, 1H, *J* = 12.4 Hz, H18), 4.94 (d, 1H, *J* = 16.1 Hz, H9), 4.69 (d, 1H, *J* = 8.4 Hz, H6), 4.39 (d, 1H, *J* = 16.1 Hz, H9), 3.75 (s, 3H, H16), 3.70 (s, 3H, H27), 3.14 (d, 1H *J* = 8.4 Hz, H28), 1.71 – 1.67 (m, 2H, H4), 1.67 – 1.62 (m, 1H, H3), 1.48 (s, 3H, H25), 0.83 (d, 3H, *J* = 6.2 Hz, H1 or H2), 0.70 (d, 3H, *J* = 6.2 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.8, 172.18, 170.8, 158.5, 135.1, 130.3, 128.7 (Ar), 128.4 (Ar), 128.3 (Ar), 127.9 (C12 and C14), 113.8 (C11 and C15), 77.5 (C6), 72.1, 67.7 (C18), 56.2, 55.3 (C16), 52.5 (C27), 45.1 (C9), 40.3 (C4), 24.1 (C1 or C2), 24.1 (C3), 23.4 (C1 or C2), 19.2 (C25).

**(±)-(3*R*,4*R*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-3-hydroxymethyl-4-hydroxy-5-(2'-methylpropyl)-pyrrolidin-2-one-5-carboxylic acid methyl ester (±)-52**

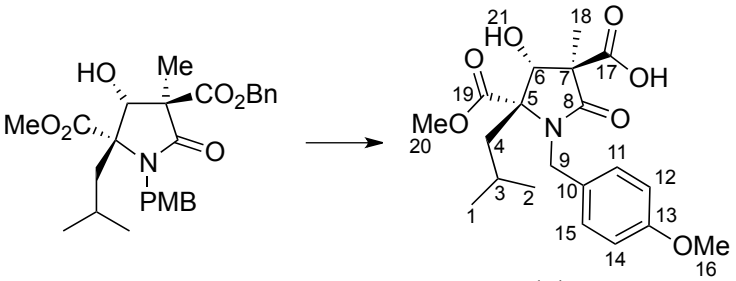
**(±)-(3*R*,4*S*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-3-hydroxymethyl-4-hydroxy-5-(2'-methylpropyl)-pyrrolidin-2-one-5-carboxylic acid methyl ester (±)-53**

Compound (±)-**52** or (±)-**53**: Found (NSI): $[M+H]^+$ 380.2065; $[C_{20}H_{29}NO_6+H]^+$ requires 380.2068. $\nu_{max}$ (thin film)/$cm^{-1}$ 3356, 2961, 1735 and 1671. $^1$H NMR (500 MHz, $CDCl_3$) δ 7.15 (app d, 2H, *J* = 8.6 Hz, H12 and H 14), 6.83 (app d, 2H *J* = 8.6 Hz, H11 and H15), 4.99 (d, 1H, *J* = 16.2 Hz, H9), 4.70 (d, 1H, *J* = 7.3 Hz, H6), 4.40 (d, 1H, *J* = 16.2 Hz, H9), 4.27 (s, 1H, H21), 3.96 – 3.88 (m, 2H, H17), 3.78 (s, 3H, H16), 3.73 (s, 3H, H20), 2.95 (s, 1H, H18), 1.77 (dd, 1H, *J* = 12.8, 5.7 Hz, H4), 1.74 – 1.69 (m, 1H, H3), 1.66 (dd, 1H, *J* = 12.8, 4.9 Hz, H4), 0.99 (s, 3H, H22), 0.91 (d, 3H, *J* = 6.3 Hz, H1 or H2), 0.74 (d, 3H, *J* = 6.3 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 176.69 (C8), 173.58 (C19), 158.51 (C13), 130.64 (C10), 127.85 (C12 and C14), 113.91 (C11 and C15), 79.03 (C6), 72.87 (C5 or C7), 65.99 (C17), 55.26 (C16), 52.28 (C20), 49.26 (C5 or C7), 44.96 (C9), 40.38 (C4), 24.14 (C1 or C2), 24.12 (C3), 23.52 (C1 or C2), 18.17 (C22).

Compound (±)-**52** or (±)-**53**: Found (NSI): $[M+H]^+$ 380.2065; $[C_{20}H_{29}NO_6+H]^+$ requires 380.2068. $\nu_{max}$ (thin film)/$cm^{-1}$ 3393, 2955, 1738 and 1669. $^1$H NMR (500 MHz, $CDCl_3$) δ 7.11 (app d, 2H, *J* = 8.7 Hz, H12 and H14), 6.82 (app d, 2H, *J* = 8.7 Hz, H11 and H15), 4.59 (d, 1H, *J* = 15.6 Hz, H9), 4.45 (d, 1H *J* = 15.6 Hz, H9), 4.43 (d, 1H *J* = 10.8 Hz, H6), 3.82 (dd, 1H *J* = 10.8, 5.3 Hz, H17), 3.78 (s, 3H, H16), 3.56 (dd, 1H, *J* = 10.8,

5.4 Hz, H17), 3.44 (s, 3H, H20), 2.10 (dd, 1H, *J* = 14.2, 7.3 Hz, H4), 1.98 – 1.92 (m, 1H, H3), 1.57 (dd, 1H, *J* = 14.2, 4.8 Hz, H4), 1.14 (s, 3H, H22), 0.87 (d, 3H *J* = 2.2 Hz, H1 or H2), 0.86 (d, 3H, *J* = 2.2 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 177.56 (C8 or C19), 158.73 (C8 or C19), 128.60 (C12 and C14), 113.86 (C11 and C15), 74.86 (C6), 68.14 (C5 or C7), 66.51 (C17), 55.30 (C16), 52.20 (C20), 48.57 (C5 or C7), 44.16 (C4), 43.67 (C9), 24.46 (C1 or C2), 23.57 (C1 or C2), 23.49 (C3), 13.51 (C22).

**(±)-(3*R*,4*R*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-4-hydroxy-5-(2'-methylpropyl)-pyrrolidin-2-one-3,5-dicarboxylic acid-5-methyl ester (±)-57**

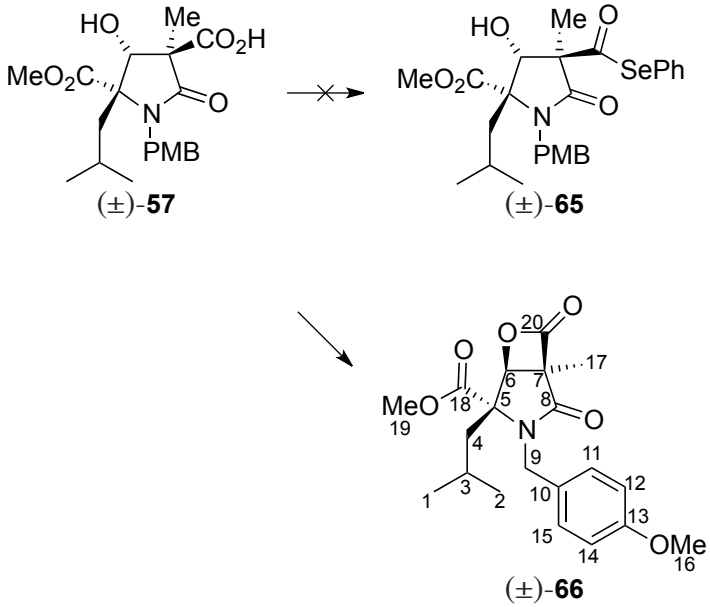



(±)-**51** (±)-**57**

Compound (±)-**51** (1.29 g, 2.7 mmol) was subjected to the general procedure for the treatment of the benzyl ester under hydrogenolysis conditions using $Pd(OH)_2$/ C (0.65 g). The product (±)-**57** was obtained as a colourless solid (0.89 g, 86%). No further purification was carried out.

Found (NSI): $[M-H]^-$ 392.1704; $[C_{20}H_{27}NO_7-H]^-$ requires 392.1715. $\nu_{max}$ (thin film)/cm$^{-1}$ 3343, 1741 and 1673. mp 108-111 °C. $^1$H NMR (500 MHz, $CDCl_3$) δ 7.14 (app d, 2H, *J* = 8.6 Hz, H12 and H14), 6.85 (app d, 2H, *J* = 8.6 Hz, H11 and H15), 4.98 (d, 1H, *J* = 16.2 Hz, H9), 4.91 (s, 1H, H6), 4.54 (d, 1H, *J* = 16.2 Hz, H9), 3.79 (s, 3H, H16), 3.77 (s, 3H, H20), 1.89 – 1.84 (m, 1H, H4), 1.70 – 1.64 (m, 2H, H3 and H4), 1.44 (s, 3H, H18), 0.90 (d, 3H, *J* = 6.3 Hz, H1 or H2), 0.75 (d, 3H, *J* = 6.3 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.2, 158.8, 127.9 (C12 and C14), 114.1 (C11 and C15), 77.2 (C5 or C7), 74.4 (C6), 72.3 (C5 or C7), 55.3 (C16), 52.7 (C20), 45.4 (C9), 39.5 (C4), 24.2 (C3), 24.0 (C1 or C2), 23.3 (C1 or C2), 20.9 (C18).

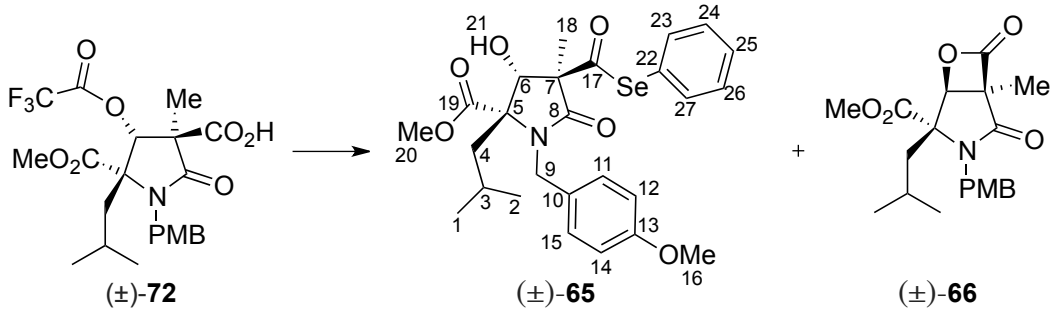
**(±)-(3*R*,4*S*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-5-(2'-methylpropyl)-3-azabicyclo[3.2.0] heptane-2,7-dione-5-carboxylic acid methyl ester (±)-66**




Compound (±)-**57** (0.13 g, 0.3 mmol) was subjected to the general procedure for the formation of an acyl selenide using diphenyldiselenide (0.16 g, 0.5 mmol, 1.5 equiv.) and tributylphosphine (0.16 mL, 0.7 mmol, 2 equiv.). Compound (±)-**66** was isolated as an off-white solid (0.05 g, 43%).

Found (NSI): $[M+H]^+$ 376.1756; $[C_{20}H_{25}NO_6+H]^+$ requires 376.1755. $\nu_{max}$ (thin film)/$cm^{-1}$ 1842, 1731 and 1702. mp 104-109 °C $^1$H NMR (500 MHz, $CDCl_3$) δ 7.12 (app d, 2H, *J* = 8.7 Hz, H12 and H13), 6.83 (app d, 2H, *J* = 8.7 Hz, H11 and H15), 5.15 (s, 1H, H6), 4.51 (d, 2H, *J* = 1.8 Hz, H9), 3.78 (s, 3H, H16), 3.52 (s, 3H, H19), 2.11 (dd, 1H, *J* = 13.4, 7.7 Hz, H4), 1.77 – 1.71 (m, 1H, H3), 1.67 (s, 3H, H17), 1.62 (dd, 1H, *J* = 13.4, 5.5 Hz, H4), 0.93 (d, 3H, *J* = 6.6 Hz, H1 or H2), 0.86 (d, 3H, *J* = 6.6 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 169.7, 168.4, 165.8, 159.0, 128.8, 128.8 (C12 and C14), 114.0 (C11 and C15), 76.0 (C6), 68.6 (C5 or C7), 64.9 (C5 or C7), 55.3 (C16), 52.7 (C19), 43.9 (H9), 37.3 (C4), 24.6 (C3), 23.8 (C1 or C2), 22.8 (C1 or C2), 11.7 (C17).

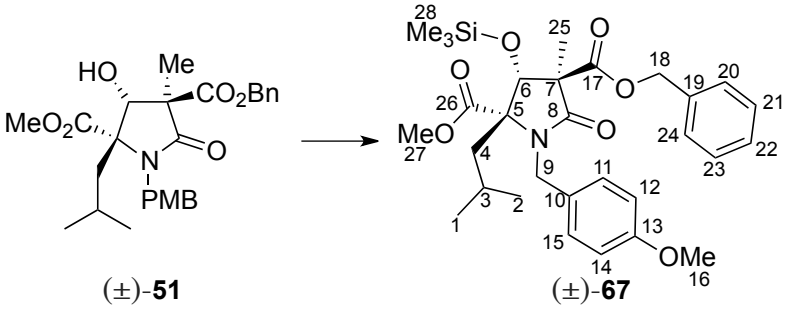
**(±)-(3*R*,4*R*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-4-hydroxy-5-(2'-methylpropyl)-pyrrolidin-2-one-3-phenylseleno ester-5-carboxylic acid methyl ester (±)-65**




Compound (±)-**72** (0.36 g, 0.7 mmol) was subjected to the general procedure for the formation of an acyl selenide using diphenyldiselenide (0.35 g, 1.0 mmol, 1.5 equiv.) and tributylphosphine (0.36 mL, 0.7 mmol, 2 equiv.). The desired compound (±)-**65** was isolated as a yellow oil (0.12 g, 31%). Compound (±)-**66** was also isolated (0.03 g, 10%).

$\nu_{max}$ (thin film)/cm$^{-1}$ 3423, 1736 and 1690. $^{1}$H NMR (500 MHz, $CDCl_3$) δ 7.55 – 7.51 (m, 2H, H23 and H27), 7.44 – 7.37 (m, 3H, H24-26), 7.22 (app d, 2H, *J* = 8.6 Hz, H12 and H14), 6.86 (app d, 2H, *J* = 8.6 Hz, H11 and H15), 4.98 (d, 1H, *J* = 16.1 Hz, H9), 4.84 (d, 1H, *J* = 5.2 Hz, H6), 4.49 (d, 1H, *J* = 16.1 Hz, H9), 3.79 (s, 3H, H16), 3.73 (s, 3H, H20), 2.94 (d, 1H, *J* = 5.2 Hz, H21), 1.85 – 1.77 (m, 1H, H4), 1.72 – 1.64 (m, 2H, H4 and H3), 1.51 (s, 3H, H18), 0.87 (d, 3H, *J* = 6.4 Hz, H1 or H2), 0.75 (d, 3H, *J* = 6.4 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 204.9, 172.6, 172.5, 158.6, 136.2, 130.2, 129.3 (Ar), 129.1 (Ar), 128.1 (C12 and C14), 126.6 (Ar), 114.0 (C11 and C12), 77.0 (C6), 71.5 (C5 or C7), 64.3 (C5 or C7), 55.3 (C16), 52.5 (C20), 45.0 (C9), 40.3 (C4), 24.1 (C3), 24.1 (C1 or C2), 23.5 (C1 or C2), 21.4 (C18). $^{77}$Se NMR (95 MHz, $CDCl_3$) δ 700.24.

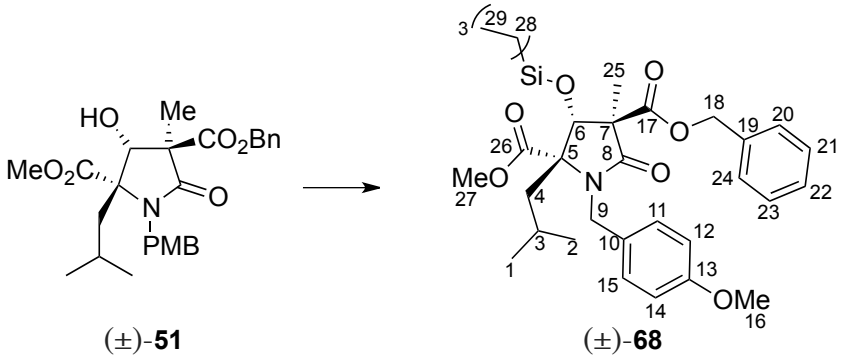
**(±)-(3*R*,4*R*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-4-(*tri*-methylsilanyloxy)-5-(2'-methylpropyl)-pyrrolidin-2-one-3,5-dicarboxylic acid-3-benzyl ester-5-methyl ester (±)-67**




Compound (±)-**51** (0.41 g, 0.8 mmol) was subjected to the general procedure for the silyl protection of the hydroxyl moiety using trimethylsilyl chloride (0.13 mL, 1.0 mmol, 1.2 equiv.) and imidazole (0.15 g, 2.1 mmol, 2.5 equiv.). No further purification was carried out. The product (±)-**67** was obtained as a yellow oil (0.48 g, quant.).

Found (NSI): $[M+H]^+$ 556.2728; $[C_{30}H_{41}NO_7Si+H]^+$ requires 556.2725. $\nu_{max}$ (thin film)/$cm^{-1}$ 2957, 1740 and 1697. $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.47 – 7.43 (m, 5H, H20-24), 7.19 (app d, 2H, *J* = 8.7 Hz, H11 and H15), 6.75 (app d, 2H, *J* = 8.7 Hz, H12 and H14), 5.35 (d, 1H, *J* = 12.2 Hz, H18), 5.18 (d, 1H, *J* = 12.2 Hz, H18), 4.74 (d, 1H, *J* = 16.0 Hz, H9), 4.65 (d, 1H, *J* = 16.0 Hz, H9), 4.50 (s, 1H, H6), 3.83 (s, 3H, H16), 3.57 (s, 3H, H27), 1.97 (dd, 1H, *J* = 15.1, 8.4 Hz, H4), 1.90 (dd, 1H, *J* = 15.1, 3.8 Hz, H4), 1.84 – 1.78 (m, 1H, H3), 1.65 (s, 3H, H25), 0.81 (d, 3H, *J* = 6.6 Hz, H1 or H2), 0.70 (d, 3H, *J* = 6.6 Hz, H1 or H2), 0.22 (s, 9H, H28). $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 173.3, 172.8, 169.4, 158.4, 135.4, 129.6, 128.7 (Ar), 128.6 (Ar), 128.3 (Ar), 128.2 (C12 and C15), 113.6 (C11 and C15), 81.1 (C6), 71.5 (C5 or C7), 67.4 (C18), 56.6 (C5 or C7), 55.3 (C16), 52.1 (C27), 46.2 (C9), 40.1 (C4), 24.8 (C1 or C2), 23.63 (C3), 23. (C1 or C2), 21.2 (C25), 0.1 (C28).

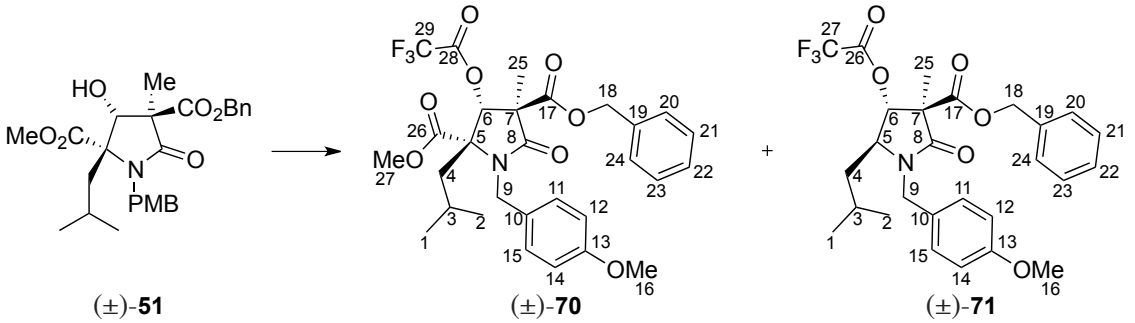
**(±)-(3*R*,4*R*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-4-(*tri*-ethylsilanyloxy)-5-(2'-methylpropyl)-pyrrolidin-2-one-3,5-dicarboxylic acid-3-benzyl ester-5-methyl ester (±)-68**




Compound (±)-**51** (0.11 g, 0.2 mmol) was subjected to the general procedure for the silyl protection of the hydroxyl moiety using triethylsilyl chloride (0.05 ml, 0.3 mmol, 1.2 equiv.) and imidazole (0.04 g, 0.6 mmol, 2.5 equiv.). No further purification was carried out. The product (±)-**68** was obtained as a yellow oil (0.13 g, quant.).

Found (NSI): $[M+H]^+$ 598.3178; $[C_{33}H_{47}NO_7Si+H]^+$ requires 598.3195. $\nu_{max}$ (thin film)/$cm^{-1}$ 2957, 1741 and 1697. $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.33 (m, 5H, H20-24), 7.08 (app d, 2H, *J* = 8.6 Hz, H 12 and H14), 6.64 (app d, 2H, *J* = 8.6 Hz, H11 and H15), 5.21 (d, 1H, *J* = 12.2 Hz, H18), 5.12 (d, 1H *J* = 12.2 Hz, H18), 4.61 (d, 1H *J* = 16.0 Hz, H9), 4.52 (d, 1H, *J* = 16.0 Hz, H9), 4.38 (s, 1H, H6), 3.73 (s, 3H, H16), 3.46 (s, 3H, H27), 1.88 (d, 2H, *J* = 7.3 Hz, H4), 1.78 – 1.70 (m, 1H, H2), 1.56 (s, 3H, H25), 0.96 (t, 9H, *J* = 7.9 Hz, H29), 0.69 (d, 3H, *J* = 6.6 Hz, H1 or H2), 0.63 (q, 6H, *J* = 8.1 Hz, H28), 0.59 (d, 3H, *J* = 6.6 Hz, H1 or H2). $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 173.5, 172.8, 169.3, 158.4, 135.23, 129.5, 128.7 (Ar), 128.5 (Ar), 128.3 (Ar), 128.2 (C12 and C14), 113.6 (C11 and C15), 81.4 (C6), 71.7 (C5 or C7), 67.5 (C18), 56.7 (C5 or C7), 55.2 (C16), 52.1 (C27), 46.5 (C9), 39.8 (C4), 25.0 (C1 or C2), 23.7 (C3), 22.7 (C1 or C2), 21.5 (C25), 6.7 (C29), 4.8 (C28).

**Protection of the Hydroxyl Moiety at C6 Using Trifluoroacetic Anhydride**




**Procedure A:** Compound (±)-**51** (0.35 g, 0.7 mmol) was subjected to the general procedure for the protection of the hydroxyl moiety at C6 using trifluoroacetic anhydride (0.25 mL, 1.8 mmol, 2.5 equiv.) and pyridine (0.15 mL, 1.8 mmol, 2.5 equiv.). Purification using column chromatography on silica gel (light petroleum ether/ethyl acetate 1:1) gave compounds (±)-**70** (0.22 g, 52%) and (±)-**71** (0.08 g, 21%).

**Procedure B:** Compound (±)-**51** (1.21 g, 2.5 mmol) was subjected to the general procedure for the protection of the hydroxyl moiety at C6 using trifluoroacetic anhydride (0.87 mL, 6.2 mmol, 2.5 equiv.) and pyridine (0.50 mL, 6.2 mmol, 2.5 equiv.). No further purification was carried out. Compound (±)-**70** was isolated as a colourless oil (1.45 g, quant.).

**(±)-(3*R*,4*R*,5*S*)-*N*-(4'-Methoxybenzyl)-3-methyl-5-(2'-methylpropyl)-4-(trifluoroacetoxy)-pyrrolidin-2-one-3,5-dicarboxylic acid-3-benzyl ester-5-methyl ester (±)-70**

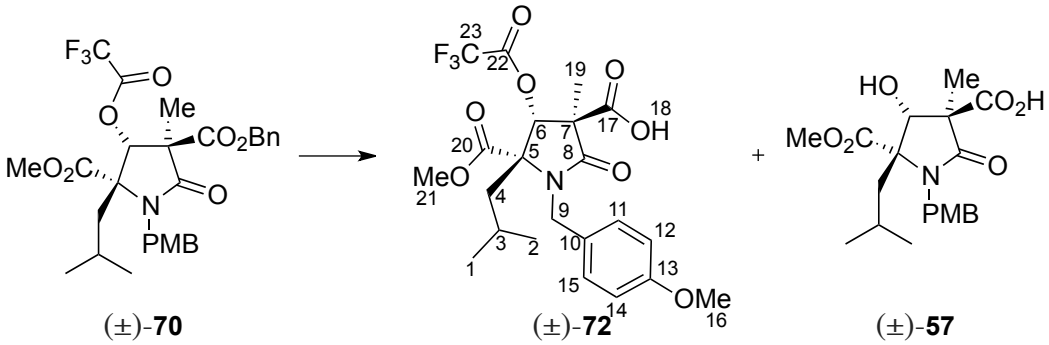
$\nu_{max}$ (thin film)/cm$^{-1}$ 1796, 1742 and 1708. $^{1}$H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.33 (m, 5H, H20-24), 7.09 (app d, 2H, *J* = 8.7 Hz, H12 and H14), 6.68 (app d, 2H, *J* = 8.7 Hz, H11 and H15), 6.05 (s, 1H, H6), 5.15 (s, 2H, H18), 4.85 (d, 1H, *J* = 16.1 Hz, H9), 4.57 (d, 1H, *J* = 16.1 Hz, H9), 3.75 (s, 3H, H16), 3.65 (s, 3H, H27), 1.72 (qd, 2H, *J* = 14.7, 6.0 Hz, H4), 1.62 (s, 3H, H25), 1.55 – 1.47 (m, 1H, H3), 0.70 (d, *J* = 6.6 Hz, 3H, H1 or H2), 0.67 (d, *J* = 6.6 Hz, 3H, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.0, 171.0, 168.0, 158.7, 134.6, 129.3, 128.7 (Ar), 128.5 (Ar), 128.5 (Ar), 128.0 (C12 and C14), 113.9 (C11 and C15), 79.8 (C6), 70.0, 68.2 (C18), 55.5, 55.2 (C16), 53.0 (C27),

45.0 (C9), 39.9 (C4), 23.7 (C1 or C2), 23.6 (C3), 23.4 (C1 or C2), 19.9 (C25). $^{19}$F NMR (471 MHz, $CDCl_3$) δ -74.45.

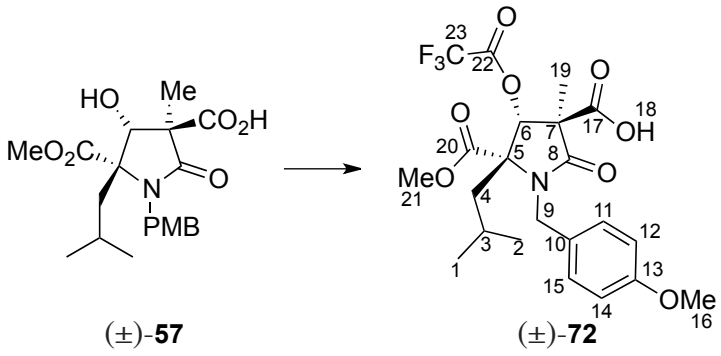
**(±)-(3*R*,4*R*,5*S*)-*N*-(4'-Methoxybenzyl)-3-methyl-5-(2'-methylpropyl)-4-(trifluoroacetoxy)-pyrrolidin-2-one-3-carboxylic acid benzyl ester (±)-71**

$\nu_{max}$ (thin film)/cm$^{-1}$ 1794, 1746 and 1705. $^1$H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.33 (m, 4H, H20, H21, H23 and H24), 7.24 (dd, 1H, *J* = 4.0, 2.1 Hz, H22), 7.08 (app d, 2H, *J* = 8.5 Hz, H12 and H14), 6.62 (app d, 2H, *J* = 8.5 Hz, H11 and H15), 5.26 (d, 1H, *J* = 12.2 Hz, H18), 5.18 (d, 1H, *J* = 15.1 Hz, H9), 5.12 (d, 1H, *J* = 6.5 Hz, H6), 5.03 (d, 1H, *J* = 12.2 Hz, H18), 3.79 (d, 1H, *J* = 15.1 Hz, H9), 3.75 (s, 3H, H16), 3.63 (ddd, 1H, *J* = 10.4, 6.5, 3.7 Hz, H5), 1.74 – 1.67 (m, 1H, H4), 1.65 (s, 3H, H25), 1.42 – 1.37 (m, 1H, H3) 1.30 – 1.28 (m, 1H, H4), 0.88 (d, 3H, *J* = 6.5 Hz, H1 or H2), 0.65 (d, 3H, *J* = 6.5 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 169.9, 167.6, 159.1, 129.4 (C12 and C14), 128.7 (Ar), 128.5 (Ar), 128.4 (Ar), 126.4, 114.0 (C11 and C15), 100.0, 83.3 (C6), 77.2, 67.9 (C18), 56.1, 56.0 (C5), 55.2 (C16), 43.5 (C9), 41.2 (C4), 29.7, 24.3 (C1 or C2), 24.1 (C3), 20.8 (C1 or C2), 19.5 (C25). $^{19}$F NMR (471 MHz, $CDCl_3$) δ -75.07.

**(±)-(3*R*,4*R*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-4-(trifluoroacetoxy)-5-(2'-methylpropyl)-pyrrolidin-2-one-3,5-dicarboxylic acid-5-methyl ester (±)-72**



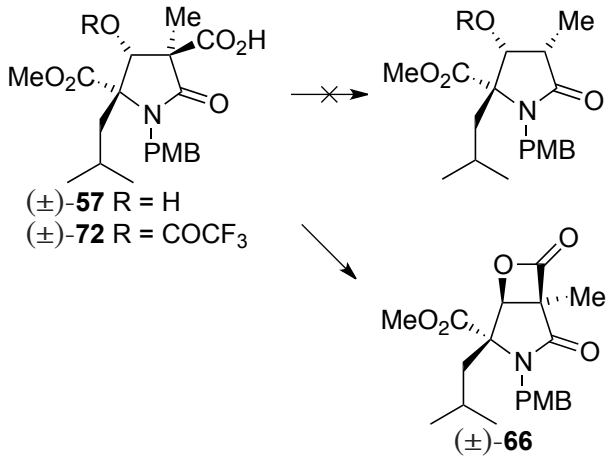

Compound (±)-**70** (1.30 g, 2.6 mmol) was subjected to the general procedure for the treatment of the benzyl ester under hydrogenolysis conditions using $Pd(OH)_2$/ C (0.65 g). Purification using column chromatography on silica gel (light petroleum ether/ethyl acetate 2:1, then 10% methanol in dichloromethane) gave the desired product (±)-**72** as a colourless oil (0.22 g, 20%) and compound (±)-**57** (0.14 g, 16%).




Compound (±)-**57** (0.30 g, 0.80 mmol) was subjected to the general procedure for the protection of the hydroxyl moiety at C6 using trifluoroacetic anhydride (0.26 mL, 1.90 mmol, 2.5 equiv.) and pyridine (0.15 mL, 1.90 mmol, 2.5 equiv.). No further purification was carried out. The desired compound (±)-**72** was isolated as a yellow oil (0.30 g, 81%).

$\nu_{max}$ (thin film)/cm$^{-1}$ 1799, 1742, 1707 and 1676. $^{1}$H NMR (500 MHz, $CDCl_3$) δ 7.11 (app d, 2H, *J* = 8.7 Hz, H12 and H14), 6.87 (app d, 2H, *J* = 8.7 Hz, H11 and H15), 6.35 (s, 1H, H6), 5.08 (d, 1H, *J* = 16.2 Hz, H9), 4.57 (d, 1H, *J* = 16.2 Hz, H9), 3.82 (s, 3H, H16), 3.80 (s, 3H, H21), 1.74 (dd, 1H, *J* = 14.0, 6.6 Hz, H4), 1.56 (s, 3H, H19), 1.55 – 1.53 (m, 1H, H3), 1.50 (dd, 1H, *J* = 14.0, 4.2 Hz, H4), 0.72 (d, 3H, *J* = 6.4 Hz, H1 or H2), 0.68 (d, 3H, *J* = 6.4 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 175.1, 170.7, 168.6, 159.1, 128.4, 127.7 (C12 and C14), 114.2 (C11 and C15), 77.9 (C6), 70.9, 55.3 (C16), 53.8, 53.3 (C21), 45.5 (C9), 40.3 (C4), 23.9 (C1 or C2), 23.3 (C1 or C2), 23.2 (C3), 21.1 (C19). $^{19}$F NMR (471 MHz, $CDCl_3$) δ -74.76.

**The Barton Decarboxylation**




**Procedure A**: Compound (±)-**57** (0.10 g, 0.24 mmol) was dissolved in chloroform (5 mL) with oxalyl chloride (0.03 mL, 0.32 mmol, 1.2 equiv.) and 1 drop of DMF. The reaction was stirred for 2 h. In a separate flask, 2-mercaptopyridine *N*-oxide sodium salt (0.06 g, 0.39 mmol, 1.5 equiv.) was dissolved in chloroform (5 mL). The acid chloride solution was added to the 2-mercaptopyridine *N*-oxide sodium salt solution dropwise using a cannula whilst irradiating with a UV lamp. The reaction was left under UV irradiation for 3 h after which the solvent was removed and the residue was re-dissolved in diethyl ether. The organic phase was washed with a solution of HCl (1 M, 3 x 20 mL), brine (3 x 20 mL), dried over anhydrous magnesium sulfate and evaporated to dryness under reduced pressure.

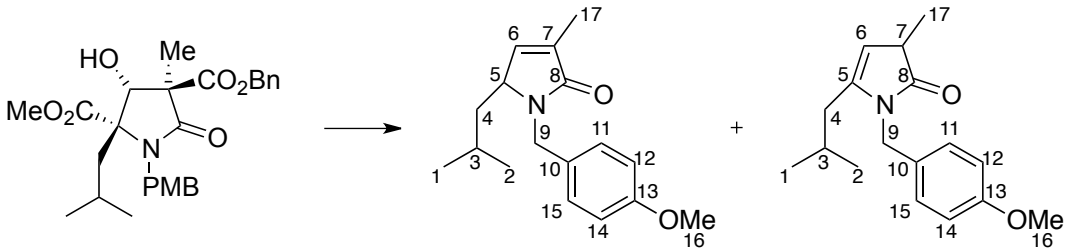
**Procedure B**: The carboxylic acid starting material was dissolved in anhydrous dichloromethane (50 mL/g of carboxylic acid). NMM (2 equiv.), 2-mercaptopyridine *N*-oxide sodium salt (2 equiv.), EDAC·HCl (2.5 equiv.) and DMAP (0.2 equiv.) were added to the reaction mixture. The reaction mixture was stirred under an atmosphere of nitrogen for 20 h. An aqueous solution of HCl (1 M, 3 mL) was added to the reaction mixture and stirring was continued for a further 30 min. The organic layer was separated, washed with water (3 x 20 mL), dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness under reduced pressure.

Compound (±)-**57** (0.10 g, 0.24 mmol) was subjected to the conditions described above in procedure B using NMM (0.05 mL, 0.49 mmol, 2 equiv.), 2-mercaptopyridine *N*-

oxide sodium salt (0.07 g, 0.49 mmol, 2 equiv.), EDAC·HCl (0.12 g, 0.62 mmol, 2.5 equiv.) and DMAP (0.01 g, 0.05 mmol, 0.2 equiv.). Compound (±)-**66** was isolated.

Compound (±)-**72** (0.12 g, 0.25 mmol) was subjected to the conditions described above in procedure B using NMM (0.06 mL, 0.50 mmol, 2 equiv.), 2-mercaptopyridine *N*-oxide sodium salt (0.08 g, 0.50 mmol, 2 equiv.), EDAC·HCl (0.12 g, 0.62 mmol, 2.5 equiv.) and DMAP (0.01 g, 0.05 mmol, 0.2 equiv.). Compound (±)-**66** was isolated.

**The Krapcho Decarboxylation**




(±)-**51** (±)-**78** (±)-**79**

Compound (±)-**51** (0.18 g, 0.4 mmol) was dissolved in wet DMF (5 mL) and LiCl (0.05 g, 1.1 mmol, 3 equiv.) added to the mixture. The reaction was heated at 135 °C for 4 h. The solution was cooled to room temperature. An aqueous solution of $NH_4OH$ (5%) was added to the reaction mixture. The aqueous phase was extracted with diethyl ether (3 x 10 mL). The organic layers were combined, washed with brine (2 x 10 mL), dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness. The crude product was purified by silica gel column chromatography using 20% ethyl acetate in light petroleum ether to give the first eluting compound (±)-**78** as an oil (0.05 g, 49%) and the second eluting compound (±)-**79** as an oil (0.04 g, 38%).

**(±)-*N*-(4'-Methoxybenzyl)-3-methyl-5-(2'-methylpropyl)-3,4-dehydropyrrolidin-2-one (±)-78**

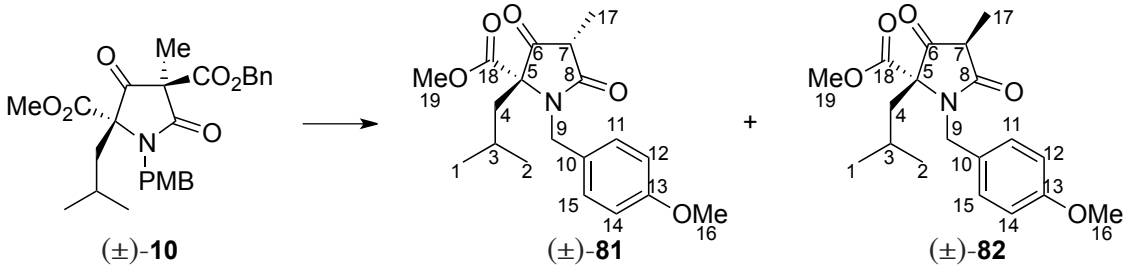
$\nu_{max}$ (thin film)/$cm^{-1}$ 1713 and 1671. $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.15 (app d, 2H, *J* = 8.6 Hz, H12 and H14), 6.82 (app d, 2H, *J* = 8.6 Hz, H11 and H15), 4.57 (d, 2H, *J* = 2.0 Hz, H9), 4.51 (dt, 1H, *J* = 9.5, 2.2 Hz, H5), 3.78 (s, 3H, H16), 2.91 (ddd, 1H, *J* = 16.1, 9.7, 2.0 Hz, H4), 2.72 – 2.61 (m, 1H, H6), 2.39 – 2.31 (m, 1H, H3), 2.22 (ddd, 1H, *J* =

16.1, 6.3, 2.4 Hz, H4), 1.29 (d, 3H, *J* = 7.2 Hz, H17), 0.92 (d, 3H, *J* = 4.9 Hz, H1 or H2), 0.91 (d, 3H, *J* = 4.9 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 178.3, 158.7, 135.8, 128.70, 128.5 (C12 and C14), 113.8 (C11 and C15), 109.7 (C5), 55.2 (C16), 43.1 (C9), 35.1 (C6), 30.3 (C4), 26.8 (C3), 23.6 (C1 or C2), 23.5 (C1 or C2), 17.3 (C17).

**(±)-*N*-(4'-Methoxybenzyl)-3-methyl-5-(2'-methylpropyl)-4,5-dehydropyrrolidin-2-one (±)-79**

$\nu_{max}$ (thin film)/cm$^{-1}$ 1675. $^1$H NMR (500 MHz, $CDCl_3$) δ 7.15 (app d, 2H, *J* = 8.6 Hz, H12 and H14), 6.83 (app d, 2H, *J* = 8.6 Hz, H11 and H15), 6.70 – 6.66 (m, 1H, H7), 5.09 (d, 1H, *J* = 15.0 Hz, H9), 4.01 (d, 1H, *J* = 15.0 Hz, H9), 3.78 (s, 4H, H16 and H6), 1.92 (t, 3H, *J* = 1.7 Hz, H17), 1.68 – 1.60 (m, 1H, H3), 1.65 (d, 1H, *J* = 9.7 Hz, H4), 1.25 (d, 1H, *J* = 9.7 Hz, H4), 0.89 (d, 3H, *J* = 6.1 Hz, H1 or H2), 0.85 (d, 3H, *J* = 6.1 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.7, 158.9, 140.6 (C7), 134.5, 129.9, 129.3 (C12 and C14), 114.0 (C11 and C15), 58.1 (C6 or C16), 55.3 (C7 or C16), 43.3 (C9), 39.8 (C4), 25.1 (C3), 23.9 (C1 or C2), 22.3 (C1 or C2), 11.3 (C17).

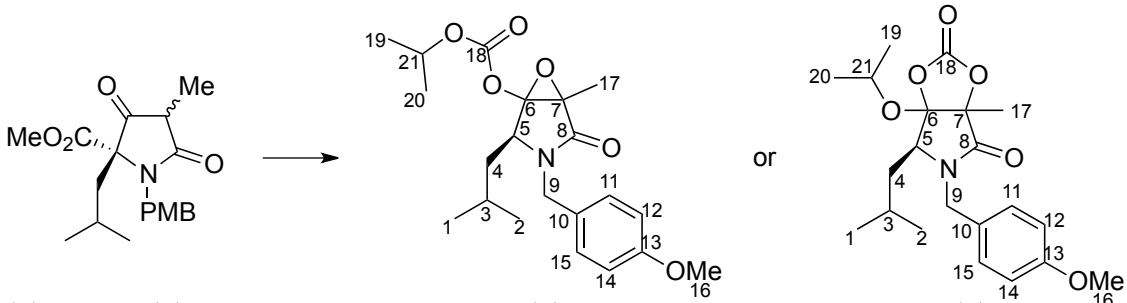
**(±)-(3*R*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-5-(2'-methylpropyl)-pyrrolidin-2-one-5-carboxylic acid methyl ester (±)-81 and (±)-(3*S*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-5-(2'-methylpropyl)-pyrrolidin-2-one-5-carboxylic acid methyl ester (±)-82**




Compound (±)-**10** (0.33 g, 0.70 mmol) was subjected to the general procedure for the treatment of the benzyl ester under hydrogenolysis conditions using $Pd(OH)_2$/ C (0.17 g). The product was obtained as an inseparable mixture of diastereoisomers (±)-**81** and (±)-**82** in a 1:1 ratio as a colourless oil (0.26 g, quant.). No further purification was carried out.

Found (NSI): $[M+H]^+$ 348.1806; $[C_{19}H_{25}NO_5+H]^+$ requires 348.1085. $\nu_{max}$ (thin film)/$cm^{-1}$ 2955, 1743 and 1638. $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.25 (app d, 4H, *J* = 8.7 Hz, H12 and H14), 6.82 (app d, 4H, *J* = 8.7 Hz, H11 and H15), 5.22 (d, 1H, *J* = 14.8 Hz, H9), 4.97 (d, 1H, *J* = 14.8 Hz, H9), 4.02 (d, 1H, *J* = 14.8 Hz, H9), 3.84 (d, 1H, *J* = 14.8 Hz, H9), 3.78 (s, 6H, H16), 3.20 – 3.18 (m, 1H, H7), 3.17 (s, 3H, H19), 3.14 (s, 3H, H19), 2.89 (q, 1H, *J* = 7.4 Hz, H7), 2.17 – 2.08 (m, 4H, H4), 1.45 (d, 3H, *J* = 7.6 Hz, H17), 1.45 – 1.37 (m, 2H, H3) 1.37 (d, 3H, *J* = 7.6 Hz, H17), 0.92 (d, 3H, *J* = 6.8 Hz, H1 or H2), 0.86 (d, 3H, *J* = 6.8 Hz, H1 or H2), 0.78 (d, 3H, *J* = 6.6 Hz, H1 or H2), 0.76 (d, 3H, *J* = 6.6 Hz, H1 or H2). $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 207.4, 206.0, 173.6, 173.2, 167.9, 159.3, 130.8 (C12 and C14), 130.8 (C12 and C14), 127.7, 113.8 (C11 and C15), 113.7 (C11 and C15), 75.7, 75.2, 55.3 (C16), 52.8 (C19), 44.5 (C7), 44.2 (C7), 43.7 (C9), 38.1 (C4), 36.9 (C4), 25.61, 23.9 (C1, C2 or C3), 23.8 (C1, C2 or C3), 23.7 (C1, C2 or C3), 23.6 (C1, C2 or C3), 23.6 (C1, C2 or C3), 23.5 (C1, C2 or C3), 12.5 (C17), 10.0 (C17).

**The Noyori Asymmetric Hydrogenation Reaction**




(±)-**81** and (±)-**82** (±)-**87** (±)-**88**

**Procedure A**: The mixture of compounds (±)-**81** and (±)-**82** (0.15 g, 0.43 mmol) and potassium carbonate (0.01 g, 0.25 equiv.) were dissolved in a mixture of 2-propanol and THF (5:1, 3 mL). The reaction was flushed with argon for 5 min after which the catalyst, $RuCl_2$[(*R*)–DM–BINAP][(*R*)–DAIPEN] (0.03 g, 5 mol%), was added and flushed with argon for a further 5 min. The argon was replaced with a balloon of hydrogen and the reaction mixture left to stir for 6 d. The solvent was removed under reduced pressure and the resulting residue re-dissolved in toluene (5 mL) and washed with water (2 x 5 mL). The organic layer was dried over anhydrous magnesium sulfate,

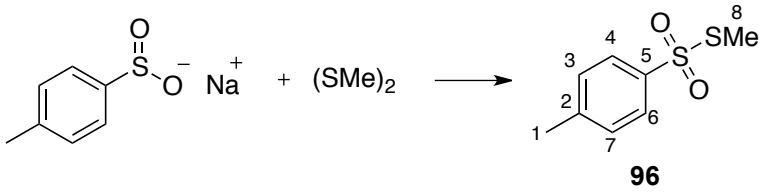
filtered, and evaporated to dryness under reduced pressure. The residue was purified using column chromatography on silica gel (light petroleum ether/ethyl acetate, 3:1). Two products were isolated from the reaction mixture, the first with the possible structure (±)-**87** or (±)-**88**, the second is unknown.

**Procedure B**: The mixture of compounds (±)-**81** and (±)-**82** (0.12 g, 0.38 mmol) and potassium carbonate (0.01 g, 0.25 equiv.) were dissolved in a mixture of 2-propanol and THF (5:1, 3 mL). The reaction was degassed via nitrogen/vacuum for 5 min after which the catalyst, $RuCl_2$[(*R*)–DM–BINAP][(*R*)–DAIPEN] (0.02 g, 5 mol%), was added. The reaction was hydrogenated at 40 psi for 3 d. The solvent was removed under reduced pressure and the resulting residue re-dissolved in toluene (5 mL) and washed with water (2 x 5 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness under reduced pressure. The residue was purified using column chromatography on silica gel (light petroleum ether/ethyl acetate, 3:1). Two products were isolated from the reaction mixture, the first with the possible structure (±)-**87** or (±)-**88**, the second is unknown.

**(±)-(5*R*)-3,4-Dehydro-*N*-(4'-methoxybenzyl)-3-methyl-4-(isopropoxy carbonate)-5-(2'-methylpropyl)-pyrrolidin-2-one-3,4-oxide (±)-87 or (±)-(5*R*)-3,4-Dehydro-*N*-(4'-methoxybenzyl)-3-methyl-4-isopropoxy-5-(2'-methylpropyl)-pyrrolidin-2-one-3,4-carbonate (±)-88**

Found (NSI): $[M+H]^+$ 392.2063; $[C_{21}H_{29}NO_6+H]^+$ requires 392.2068. $\nu_{max}$ (thin film)/$cm^{-1}$ 1758 and 1669. $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.16 (app d, 2H, *J* = 8.6 Hz, H12 and H14), 6.86 (app d, 2H, *J* = 8.6 Hz, H11 and H15), 5.48 (d, 1H, *J* = 14.8 Hz, H9), 5.06 (p, 1H, *J* = 6.3 Hz, H21), 4.03 (dd, 1H, *J* = 8.5, 3.1 Hz, H5), 3.80 (s, 3H, H16), 3.74 (d, 1H, *J* = 14.8 Hz, H9), 1.93 – 1.88 (m, 1H, H4), 1.88 (s, 3H, H17), 1.87 – 1.82 (m, 1H, H4), 1.82 – 1.76 (m, 1H, H3), 1.25 (d, 3H, *J* = 1.0 Hz, H19 or H20), 1.24 (d, 3H, *J* = 1.0 Hz, H19 or H20), 0.97 (d, 3H, *J* = 6.1 Hz, H1 or H2), 0.92 (d, 3H, *J* = 6.1 Hz, H1 or H2). $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 166.2, 165.6, 163.8, 159.6, 129.5 (C12 and C14), 126.6, 114.4 (C11 and C15), 83.6, 71.3 (C21), 55.4 (C16), 55.0 (C5), 45.9 (C9), 41.0 (C4), 24.5 (C3), 23.4 (C1 or C2), 21.7 (C7), 21.6 (C1 or C2), 21.5 (C19 and C20).

**Preparation of *S*-Methyl-*p*-toluenethiosulfonate 96**

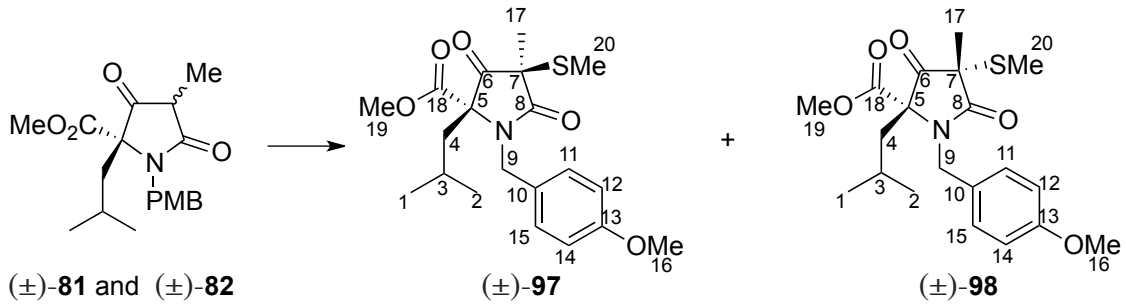



**96**

Sodium *p*-toluenesulfinate (3.23 g, 18.13 mmol, 3.2 equiv.) was dissolved in dichloromethane (50 mL) and dimethyl disulfide (0.50 mL, 5.66 mmol) added to the solution. Iodine (2.81 g, 11.33 mmol, 2 equiv.) was added to the mixture with vigorous stirring. The reaction mixture was stirred and monitored by TLC until the dimethyl disulfide was consumed. The mixture was diluted with dichloromethane (30 mL) followed by the addition of aqueous $Na_2S_2O_3$ (1 M) with stirring until the iodine colour disappeared. The organic layer was washed with water (2 x 50 mL), dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under reduced pressure. The product precipitated as an off-white solid (1.15 g, quant.).

$\nu_{max}$ (thin film)/cm$^{-1}$ 2926 and 1594. $^1$H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, 2H, $J$ = 8.3 Hz), 7.36 (d, 2H, $J$ = 8.3 Hz), 2.50 (s, 3H), 2.46 (s, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 144.9, 140.9, 129.9, 127.2, 21.7, 18.1.

**Synthesis of the Thiomethyl Derivative from (±)-81 and (±)-82**




(±)-**81** and (±)-**82** (±)-**97** (±)-**98**

Triethylamine (0.2 mL, 1 mmol, 1.2 equiv.) and *S*-methyl-*p*-toluenethiosulfonate (0.18 g, 0.8 mmol) were added to a solution of the mixture of (±)-**81** and (±)-**82** (0.30 g, 0.86 mmol) in dichloromethane (5 mL) at room temperature under a nitrogen atmosphere.

The mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue purified using column chromatography on silica gel (10% ethyl acetate in light petroleum ether) to yield compounds (±)-**97** and (±)-**98** as an inseparable mixture (0.16 g, 47%).

Compounds (±)-**97** and (±)-**98**: Found (NSI): $[M+H]^+$ 394.1682; $[C_{20}H_{27}NO_5S+H]^+$ requires 394.1683. $\nu_{max}$ (thin film)/$cm^{-1}$ 2960, 1769, 1745 and 1698.

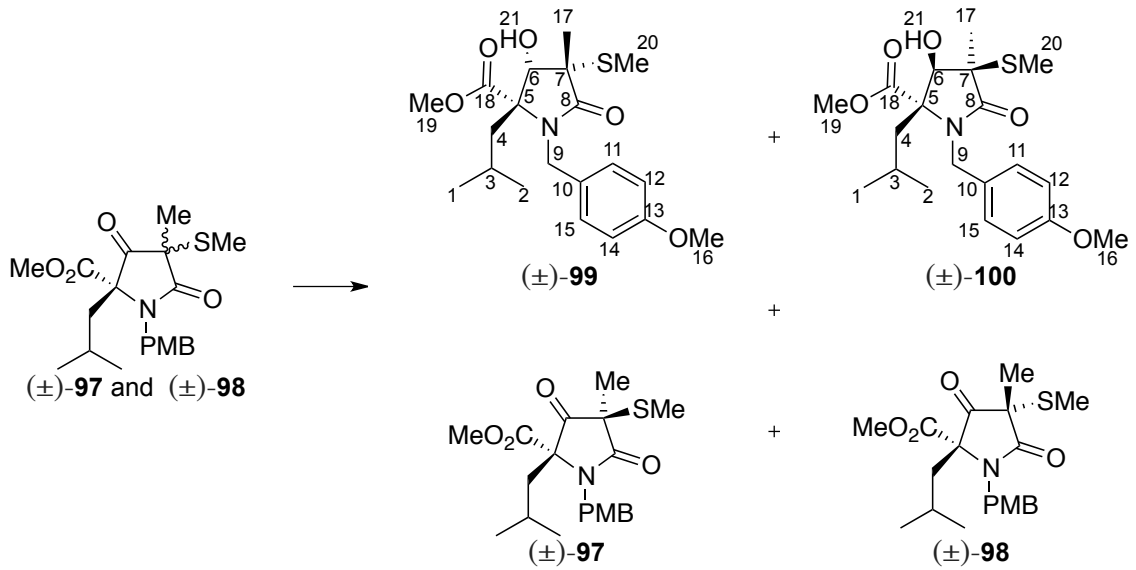
**(±)-(3*R*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-3-methylsulfanyl-5-(2'-methylpropyl)-pyrrolidin-2,4-dione-5-carboxylic acid methyl ester (±)-97**

$^1$H NMR (500 MHz, $CDCl_3$) δ 7.27 (app d, 2H, *J* = 8.7 Hz, H12 and H14), 6.83 (app d, 2H, *J* = 8.7 Hz, H11 and H15), 4.80 (d, 1H, *J* = 15.2 Hz, H9), 4.43 (d, 1H, *J* = 15.2 Hz, H9), 3.78 (s, 3H, H16), 3.45 (s, 3H, H19), 2.25 (dd, 1H, *J* = 15.2, 6.8 Hz, H4), 2.15 (s, 3H, H20), 1.96 (dd, 1H, *J* = 15.2, 5.7 Hz, H4), 1.58 (s, 3H, H17), 1.38 – 1.29 (m, 1H, H3), 0.75 (d, 3H, *J* = 4.5 Hz, H1 or H2), 0.74 (d, 3H, *J* = 4.5 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 200.2, 171.7, 167.9, 159.0, 129.8 (C12 and C14), 128.5, 113.8 (C11 and C15), 75.4, 55.3 (C16), 52.9 (C19), 49.3, 44.9 (C9), 40.0 (C4), 23.9 (C3), 23.5 (C1 and C2), 16.9 (C17), 12.3 (C20).

**(±)-(3*S*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-3-methylsulfanyl-5-(2'-methylpropyl)-pyrrolidin-2,4-dione-5-carboxylic acid methyl ester (±)-98**

$^1$H NMR (500 MHz, $CDCl_3$) δ 7.24 (app d, 2H, *J* = 8.6 Hz, H12 and H14), 6.82 (app d, 2H, *J* = 8.6 Hz, H11 and H15), 4.96 (d, 1H, *J* = 14.9 Hz, H9), 4.03 (d, 1H, *J* = 14.9 Hz, H9), 3.77 (s, 3H, H16), 3.16 (s, 3H, H19), 2.28 (s, 3H, H20), 2.24 (dd, 1H, *J* = 15.3, 5.4 Hz, H4), 1.98 (dd, 1H, *J* = 15.3, 6.2 Hz, H4), 1.74 (dtd, 1H, *J* = 13.1, 6.6, 1.2 Hz, H3), 1.67 (s, 3H, H17), 0.93 (d, 3H, *J* = 6.6 Hz, H1 or H2), 0.87 (d, 3H, *J* = 6.6 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 202.6, 172.4, 168.0, 159.3, 130.7 (C12 and C14), 127.5, 113.8 (C11 and C15), 75.2, 55.3 (C16), 52.8 (C19), 48.2, 43.7 (C9), 38.3 (C4), 24.6 (C1 or C2), 23.9 (C1 or C2), 23.7 (C3), 18.5 (C17), 11.6 (C20).

**Reduction of the Thiomethyl Derivative (±)-97 and (±)-98**




The inseparable mixture of compounds (±)-**97** and (±)-**98** (0.088 g, 0.22 mmol) was subjected to the general procedure for ketone reduction using $NaBH_4$ (0.004 g, 0.11 mmol, 0.5 equiv.). The mixture was stirred at –10 °C for 30 minutes. After quenching and subsequent washing the resulting residue was purified using column chromatography on silica gel (light petroleum ether/ethyl acetate, 3:1). The first eluting diastereoisomer (±)-**99** was obtained as a yellow oil (0.015 g, 17%), the second eluting diastereoisomer (±)-**100** was obtained as a dark yellow oil (0.023 g, 25%). The starting material, an inseparable mixture of compounds (±)-**97** and (±)-**98**, was recovered (0.020 g, 23%).

**(±)-(3*S*,4*S*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-3-methylsulfanyl-4-hydroxy-5-(2'-methylpropyl)-pyrrolidin-2-one-5-carboxylic acid methyl ester (±)-99**

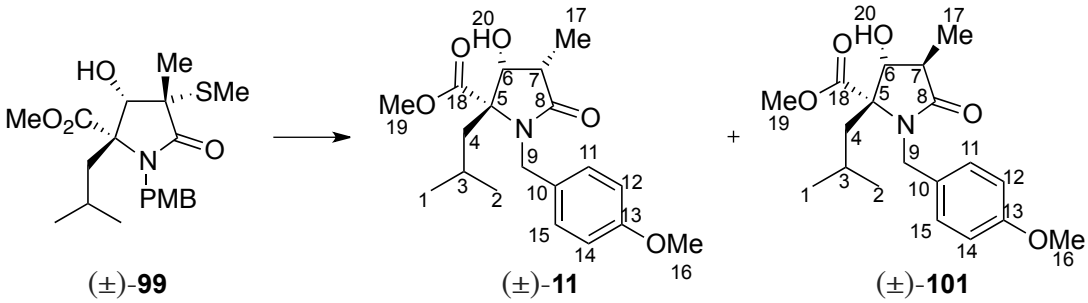
Found (NSI): $[M+H]^+$ 396.1830; $[C_{20}H_{29}NO_5S+H]^+$ requires 396.1839. $\nu_{max}$ (thin film)/$cm^{-1}$ 3432, 2957, 2927, 1741 and 1698. $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.15 (app d, 2H, *J* = 8.7 Hz, H12 and H14), 6.82 (app d, 2H, *J* = 8.7 Hz, H11 and H15), 4.84 (d, 1H, *J* = 15.9 Hz, H9), 4.47 (d, 1H, *J* = 10.0 Hz, H21), 4.36 (d, 1H, *J* = 15.9 Hz, H9), 4.03 (d, 1H, *J* = 10.0 Hz, H6), 3.78 (s, 3H, H16), 3.66 (s, 3H, H19), 2.14 (s, 3H, H20), 1.85 (dd, 1H, *J* = 14.5, 6.0 Hz, H4), 1.77 – 1.72 (m, 1H, H3), 1.65 (dd, 1H, *J* = 14.5, 4.5 Hz, H4), 1.63 (s, 3H, H17), 0.82 (d, 3H, *J* = 6.6 Hz, H1 or H2), 0.71 (d, 3H, *J* = 6.6 Hz,

H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 174.6, 173.1, 158.7, 130.2, 128.7 (C12 and C14), 113.8 (C11 and C15), 81.3 (C6), 67.4, 55.3 (C16), 52.7 (C19), 51.5, 44.8 (C9), 44.0 (C4), 24.4 (C1 or C2), 23.9 (C1 or C2), 23.5 (C3), 21.8 (C17), 11.5 (C20).

**(±)-(3*R*,4*R*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-3-methylsulfanyl-4-hydroxy-5-(2'-methylpropyl)-pyrrolidin-2-one-5-carboxylic acid methyl ester (±)-100**

Found (NSI): $[M+H]^+$ 396.1832; $[C_{20}H_{29}NO_5S+H]^+$ requires 396.1839. $\nu_{max}$ (thin film)/cm$^{-1}$ 3484, 2957, 2928, 1738 and 1682. $^1$H NMR (500 MHz, $CDCl_3$) δ 7.15 (app d, 2H, *J* = 8.7 Hz, H12 and H14), 6.82 (app d, 2H, *J* = 8.7 Hz, H11 and H15), 4.89 (d, 1H, *J* = 16.0 Hz, H9), 4.47 (d, 1H, *J* = 3.3 Hz, H6), 4.43 (d, 1H, *J* = 16.0 Hz, H9), 3.78 (s, 3H, H16), 3.70 (s, 3H, H19), 3.20 (d, 1H, *J* = 3.3 Hz, H21), 2.15 (s, 3H, H20), 1.88 (dd, 1H, *J* = 13.7, 6.3 Hz, H4), 1.80 (dd, 1H, *J* = 13.7, 5.3 Hz, H4), 1.76 – 1.71 (m, 1H, H3), 1.50 (s, 3H, H17), 0.91 (d, 3H, *J* = 6.6 Hz, H1 or H2), 0.77 (d, 3H, *J* = 6.6 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 173.2, 173.1, 158.5, 130.5, 128.2 (C12 and C14), 113.8 (C11 and C15), 75.9 (C6), 71.6, 57.5, 55.3 (C16), 52.4 (C19), 45.2 (C9), 40.7 (C4), 24.2 (C3) 24.2 (C1 or C2), 23.5 (C1 or C2), 21.9 (C17), 12.6 (C20).

**(±)-(3*S*,4*R*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-4-hydroxy-5-(2'-methylpropyl)-pyrrolidin-2-one-5-carboxylic acid methyl ester (±)-11 and (±)-(3*R*,4*R*,5*R*)-*N*-(4'-Methoxybenzyl)-3-methyl-4-hydroxy-5-(2'-methylpropyl)-pyrrolidin-2-one-5-carboxylic acid methyl ester (±)-101**



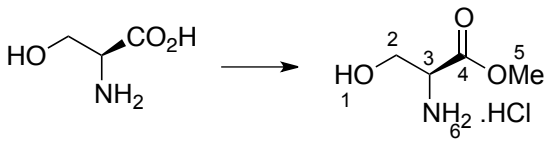

Raney nickel was washed using ethanol and dried under an atmosphere of nitrogen before being added to a solution of (±)-**99** (0.02 g, 0.05 mmol) in ethanol. The reaction mixture was heated under reflux for 4 h. After removal of the Raney nickel by filtration the solvent was removed under reduced pressure. The resulting residue was purified using column chromatography on silica gel (light petroleum ether/ethyl acetate, 3:1). Compounds (±)-**11** and (±)-**101** were isolated as an inseparable mixture of diastereoisomers in a 3:1 ratio.

Found (NSI): $[M+H]^+$ 350.1963; $[C_{19}H_{27}NO_5+H]^+$ requires 350.1962.

**Major diastereoisomer**: $^1$H NMR (500 MHz, $CDCl_3$) δ 7.11 (app d, 2H, *J* = 8.7 Hz, H12 and H14), 6.75 (app d, 2H, *J* = 8.7 Hz, H11 and H15), 4.75 (d, 1H, *J* = 15.9 Hz, H9), 4.65 (dd, 1H, *J* = 10.8, 5.2 Hz, H6), 4.25 (d, 1H, *J* = 15.9 Hz, H9), 3.71 (s, 3H, H16), 3.58 (s, 3H, H19), 2.71 (qd, 1H, *J* = 7.3, 4.9 Hz, H7), 1.75 (dd, 1H, *J* = 13.3, 6.4 Hz, H4), 1.67 – 1.60 (m, 1H, H3), 1.56 (dd, 1H, *J* = 13.3, 5.7 Hz, H4), 1.18 (d, 3H, *J* = 7.2 Hz, H17), 0.85 (d, 3H, *J* = 6.6 Hz, H1 or H2), 0.73 (d, 3H, *J* = 6.6 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 175.9, 172.0, 158.5, 131.1, 128.3 (C12 and C14), 113.8 (C11 and C15), 72.3 (C6), 72.0, 55.3 (C16), 52.1 (C19), 43.8 (C9), 41.1 (C7), 39.2 (C4), 24.1 (C1 or C2), 24.0 (C3), 23.4 (C1 or C2), 8.6 (C17).

### 3.2.2.2 Synthesis from L-Serine

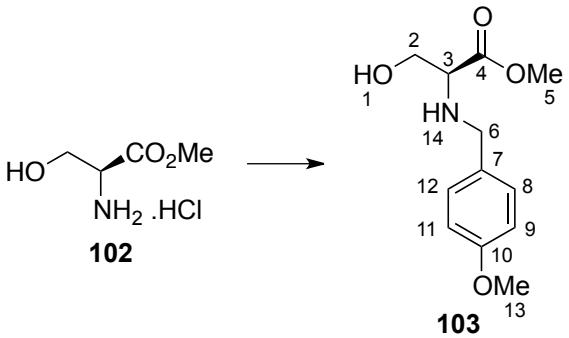
**L-Serine methyl ester hydrochloride 102**[3]




L-Serine (10.06 g, 0.10 mol) was subjected to the general procedure for the esterification of an amino acid using acetyl chloride (20 mL, 0.29 mol, 3 equiv.). The product L-serine methyl ester hydrochloride **102** was obtained as off-white solid (14.90 g, quant.). No further purification was carried out.

Found (APCI): $[M+H]^+$ 120.0653; $[C_4H_9NO_3+H]^+$ requires 120.0655. mp 159-164 °C, lit 155-163 °C.[3] $[\alpha]_D$ = +4 (*c* 2, MeOH, 25 °C, lit +5, *c* 2, MeOH, 20 °C).[3] $^1$H NMR (500 MHz, $D_2O$) δ 4.13 (t, 1H, *J* = 3.5 Hz, H3), 3.96 (dd, 1H, *J* = 12.9, 4.3 Hz, H2), 3.85 (dd, 1H, *J* = 12.9, 3.7 Hz, H2), 3.71 (s, 3H, H5). $^{13}$C NMR (126 MHz, $D_2O$) δ 168.9 (C4), 59.2 (C2), 54.7 (C3), 53.7 (C5).

**2-(4'-Methoxy-benzylamino)-3-hydroxy-propanoic acid methyl ester 103**[4]

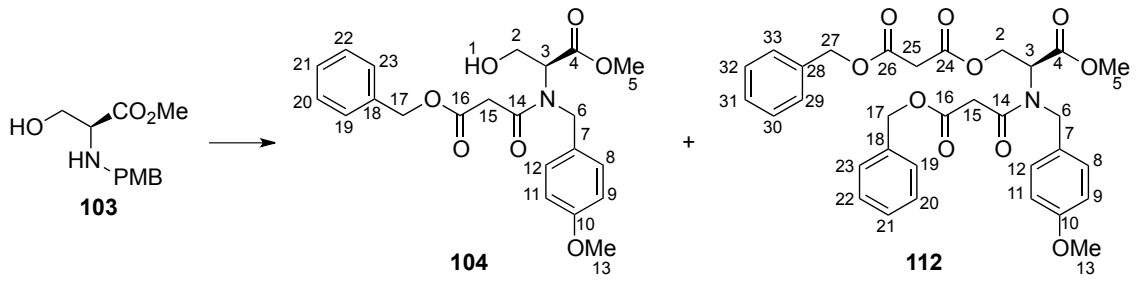



L-Serine methyl ester hydrochloride (0.36 g, 2 mmol) was dissolved in methanol (10 mL) and triethylamine (0.48 mL, 3.4 mmol, 1.5 equiv.) added. The reaction mixture was stirred for 1 h. 4-Methoxybenzaldehyde (0.31 mL, 2.5 mmol, 1.1 equiv.) was added

and the reaction mixture stirred for a further hour. The reaction mixture was purged with nitrogen and Pd/C (10% by weight, 0.04 g) added, the mixture was again purged with nitrogen and then treated with hydrogen under balloon pressure overnight at room temperature. The reaction mixture was filtered through celite and the solvents removed under reduced pressure. The residue was purified using column chromatography on silica gel (light petroleum ether/ethyl acetate 1:1) to yield the title compound as a pale yellow oil (0.35 g, 64%).

Found (NSI): $[M+H]^+$ 240.1228; $[C_{12}H_{17}NO_4+H]^+$ requires 240.1230. $\nu_{max}$ (thin film)/$cm^{-1}$ 3321, 2953, and 1736. $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.24 (app d, 2H, *J* = 8.7 Hz, H9 and H11), 6.86 (app d, 2H, *J* = 8.7 Hz, H8 and H12), 3.83 (d, 1H, *J* = 13.1 Hz, H6), 3.80 (s, 3H, H13), 3.77 (dd, 1H, *J* = 10.8, 4.6 Hz, H2), 3.75 (s, 3H, H5), 3.67 (d, 1H, *J* = 13.1 Hz, H6), 3.60 (dd, 1H, *J* = 10.8, 6.5 Hz, H2), 3.43 (dd, 1H, *J* = 6.5, 4.5 Hz, H3). $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 173.5 (C4), 158.9 (C10), 131.4 (C7), 129.5 (C9 and C11), 113.9 (C8 and C12), 62.4 (C2), 61.6 (C3), 55.3 (C13), 52.2 (C5), 51.5 (C6).

**Peptide Coupling**




Compound **103** (0.93 g, 3.9 mmol) was subjected to the general procedure for peptide coupling using EDAC·HCl (0.64 g, 9.7 mmol, 2.5 equiv.), NMM (0.85 mL, 7.7 mmol, 2 equiv.), benzyl malonic half ester (1.80 g, 7.7 mmol, 2 equiv.) and DMAP (0.10 g, 0.8 mmol, 0.2 equiv.). The residue was purified using column chromatography on silica gel (light petroleum ether/ethyl acetate, 2:1). Compound **104** was isolated as a yellow oil (0.23 g, 14%). Compound **112** was isolated as a colourless oil (0.42 g, 31%).

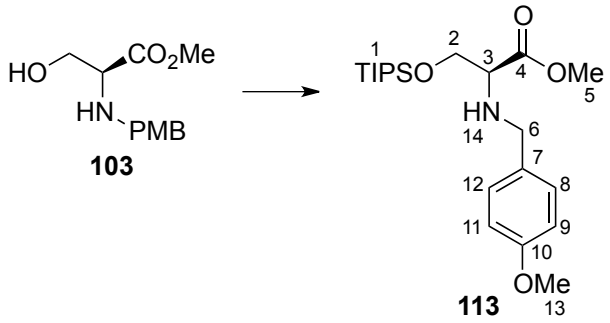
**2-{(4'-Methoxy-benzyl)-[2'-(benzyloxycarbonyl)-acetyl]-amino}-3-hydroxy-propanoic acid methyl ester 104**

Found (NSI): $[M+H]^+$ 416.1698; $[C_{22}H_{25}NO_7+H]^+$ requires 416.1704. $\nu_{max}$ (thin film)/$cm^{-1}$ 3456, 2953, 1740 and 1651. $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.29 – 7.25 (m, 5H, H18-23), 7.14 (app d, 2H, *J* = 8.5 Hz, H9 and H11), 6.78 (app d, 2H, *J* = 8.5 Hz, H8 and H12), 5.13 – 5.04 (m, 2H, H17), 4.51 – 4.38 (m, 2H, H6), 4.04 – 3.97 (m, 2H, H2 and H3), 3.81 – 3.74 (m, 1H, H2), 3.70 (s, 3H, H13), 3.58 (s, 3H, H5), 3.51 (d, 1H, *J* = 15.8 Hz, H15), 3.41 (d, 1H, *J* = 15.8 Hz, H15). $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 170.0, 167.3, 159.5, 135.2 (C10), 128.7 (Ar), 128.5 (Ar), 128.5 (C9 and C11), 127.3 (C7 and C18), 114.4 (C8 and C12), 67.5 (C17), 61.5 (C3), 60.7 (C2), 55.3 (C13), 53.2 (C6), 52.3 (C5), 41.4 (C15).

**2-{(4'-Methoxy-benzyl)-[2'-(benzyloxycarbonyl)-acetyl]-amino}-3-(2'-(benzyloxycarbonyl)-acetyl]-oxy)-propanoic acid methyl ester 112**

Found (NSI): $[M+H]^+$ 592.2171; $[C_{32}H_{33}NO_{10}+H]^+$ requires 592.2177. $\nu_{max}$ (thin film)/$cm^{-1}$ 2954, 1741 and 1655. $[\alpha]_D$ = –33.6 (*c* 1, $CHCl_3$, 25 °C). $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.39 – 7.29 (m, 10H, H19-23 and H29-33), 7.20 (app d, 2H, *J* = 8.7 Hz, H9 and H11), 6.85 (app d, 2H, *J* = 8.7 Hz, H8 and H12), 5.16 (s, 4H, H17 and H27), 4.71 (dd, 1H, *J* = 11.7, 4.5 Hz, H2), 4.59 (d, 1H, *J* = 16.6 Hz, H6), 4.57 (dd, 1H *J* = 11.7, 8.1 Hz, H2), 4.43 (d, 1H, *J* = 16.6 Hz, H6), 4.39 (dd, 1H, *J* = 8.1, 4.5 Hz, H3), 3.78 (s, 3H, H13), 3.65 (s, 3H, H5), 3.56 – 3.45 (m, 2H, H15 or H25), 3.32 (d, 2H *J* = 2.1 Hz, H15 or H25). $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 168.2, 167.1, 166.9, 166.1, 165.9, 159.4, 135.3, 135.2, 128.6 (Ar), 128.6 (Ar), 128.6 (C9 and C11), 128.5 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 127.3, 114.2 (C8 and C12), 67.3 (C17 and C27), 63.2 (C2), 58.3 (C3), 55.3 (C13), 52.7 (C6), 52.5 (C5), 41.3 (C15 or C25), 41.3 (C15 or C25).

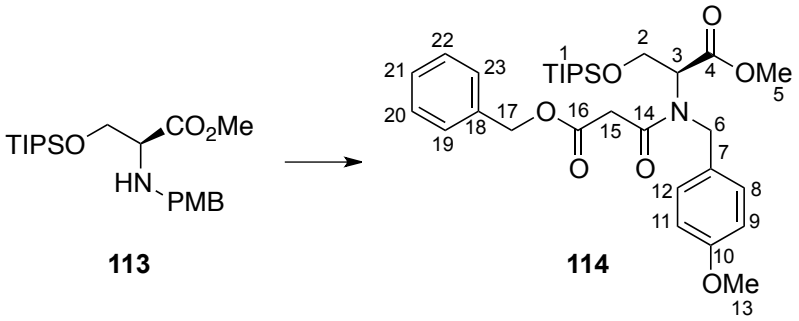
**2-(4'-Methoxy-benzylamino)-3-(*tri-iso*-propylsilanyloxy)-propanoic acid methyl ester 113**




Compound **103** (2.07 g, 8.7 mmol) was subjected to the general procedure for the silyl protection of the hydroxyl moiety using triisopropyl silyl chloride (2.23 mL, 10.4 mmol, 1.2 equiv.) and imidazole (1.48 g, 21.7 mmol, 2.5 equiv.). The product **113** was obtained as a yellow oil (3.10 g, 90%). No further purification was carried out.

Found (NSI): $[M+H]^+$ 396.2558; $[C_{21}H_{37}NO_4Si+H]^+$ requires 396.2565. $\nu_{max}$ (thin film)/$cm^{-1}$ 2943, 2866 and 1743. $[\alpha]_D = -19.2$ (*c* 1, $CHCl_3$, 25 °C). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.25 (app d, 2H, *J* = 8.6 Hz, H8 and H12), 6.85 (app d, 2H, *J* = 8.6 Hz, H9 and H11), 3.97 (dd, 1H, *J* = 9.5, 4.8 Hz, H2), 3.90 (dd, 1H, *J* = 9.5, 5.0 Hz, H2), 3.84 (d, 1H, *J* = 12.9 Hz, H6), 3.79 (s, 3H, H13), 3.72 (s, 3H, H5), 3.66 (d, 1H, *J* = 12.9 Hz, H6), 3.41 (t, 1H, *J* = 4.9 Hz, H3), 1.09 – 0.99 (m, 21H, H1). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 173.9 (C4), 158.7 (C10), 132.0 (C7), 129.4 (C9 and C11), 113.8 (C8 and C12), 65.0 (C2), 62.3 (C3), 55.3 (C13), 51.7 (C5), 51.3 (C6), 17.9 (C1), 11.9 (C1).

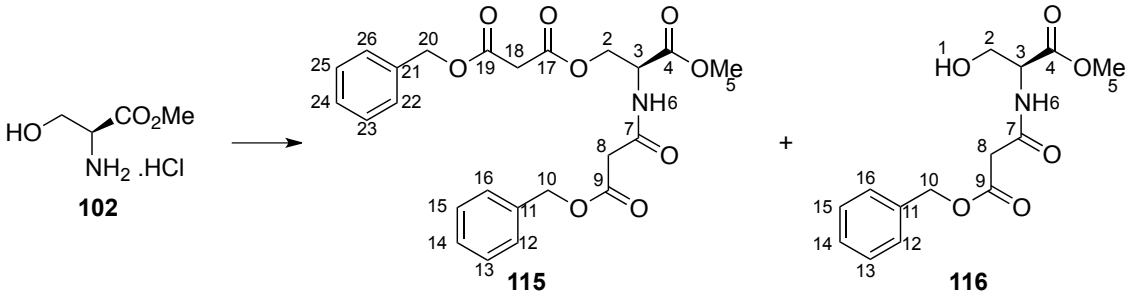
**2-{(4'-Methoxy-benzyl)-[2'-(benzyloxycarbonyl)-acetyl]-amino}-3-(*tri-iso*-propylsilanyloxy)-propanoic acid methyl ester 114**




Compound **113** (2.00 g, 5.0 mmol) was subjected to the general procedure for peptide coupling using EDAC·HCl (2.42 g, 12.6 mmol, 2.5 equiv.), NMM (1.10 mL, 10.1 mmol, 2 equiv.), benzyl malonic half ester (2.35 g, 10.1 mmol, 2 equiv.) and DMAP (0.12 g, 1.0 mmol, 0.2 equiv.). The residue was purified using column chromatography on silica gel (light petroleum ether/ethyl acetate, 2:1). Compound **114** was isolated as a yellow oil (2.65 g, 91%).

Found (NSI): $[M+H]^+$ 572.3030; $[C_{31}H_{45}NO_7Si+H]^+$ requires 572.3038. $\nu_{max}$ (thin film)/$cm^{-1}$ 2944, 2866, 1743 and 1659. $[\alpha]_D$ = +4.4 (*c* 1, $CHCl_3$, 25 °C). $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.36 – 7.32 (m, 5H, H19-23), 7.25 (app d, 2H, *J* = 9.1 Hz, H8 and H12), 6.86 (app d, 2H, *J* = 9.1 Hz, H9 and H11), 5.14 (s, 2H, H17), 4.74 (s, 2H, H6), 4.59 (dd, 1H, *J* = 7.4, 3.7 Hz, H3), 4.26 (dd, 1H, *J* = 10.7, 7.5 Hz, H2), 4.17 (dd, 1H, *J* = 10.7, 3.7 Hz, H2), 3.79 (s, 3H, H13), 3.66 (s, 3H, H5), 3.52 – 3.41 (m, 2H, H15), 1.02 – 0.98 (m, 21H, H1). $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 169.4 (C16), 167.2 (C4), 167.0 (C14), 159.1 (C10), 135.5 (C7 and C18), 128.5 (Ar), 128.3 (Ar), 128.0 (C9 and C11), 114.2 (C8 and C12), 67.1 (C17), 62.4 (C2), 60.9, (C3) 55.3 (C13), 52.1 (C6), 52.0 (C5), 41.4 (C15), 17.9, 17.9 (C1), 11.8 (C1).

**Peptide Coupling**




Compound **102** (0.35 g, 2.2 mmol) was subjected to the general procedure for peptide coupling using EDAC·HCl (0.64 g, 3.4 mmol, 1.5 equiv.), NMM (0.27 mL, 2.5 mmol, 1.1 equiv.), benzyl malonic half ester (0.57 g, 2.5 mmol, 1.1 equiv.) and DMAP (0.05 g, 0.4 mmol, 0.2 equiv.). The residue was purified using column chromatography on silica gel (30% ethyl acetate in light petroleum ether). Two compounds were isolated in a 1:6 ratio, the first eluting compound **115** (0.08 g, 8%) and the second eluting compound **116** (0.34 g, 52%).

**2-(2'-(benzyloxycarbonyl)-acetyl-amino)-3-(2'-(benzyloxycarbonyl)-acetyl]-oxy)-propanoic acid methyl ester 115**

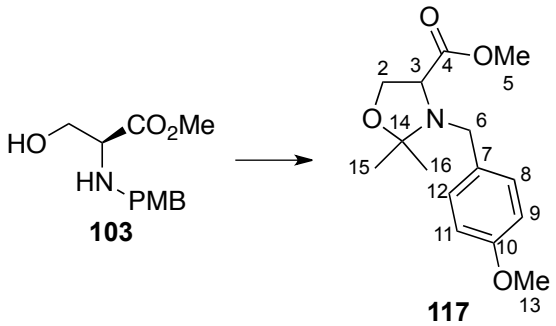
Found (NSI): $[M+H]^+$ 472.1593; $[C_{24}H_{25}NO_9+H]^+$ requires 472.1602. $\nu_{max}$ (thin film)/$cm^{-1}$ 3354, 2955, 1739 and 1683. $[\alpha]_D$ = +35 (*c* 1, $CHCl_3$, 25 °C). $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.75 (d, 1H, *J* = 7.8 Hz, H6), 7.41 – 7.29 (m, 10H, H12-16 and H22-26), 5.17 (d, 4H, *J* = 1.9 Hz, H10 and H20), 4.89 (dt, 1H, *J* = 7.6, 3.6 Hz, H3), 4.55 (dd, 1H, *J* = 11.4, 3.5 Hz, H2), 4.50 (dd, 1H, *J* = 11.4, 3.7 Hz, H2), 3.75 (s, 3H, H5), 3.42 (s, 2H, H18), 3.37 (s, 2H, H8). $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 169.3, 168.5, 166.2, 165.9, 165.0, 135.1 (C11 or C21), 135.0 (C11 or C21), 128.7 (Ar), 128.6 (Ar), 128.6 (Ar), 128.4 (Ar), 128.3 (Ar), 67.5 (C10 or C20), 67.4 (C10 or C20), 64.4 (C2), 53.0 (C5), 51.6 (C3), 41.2 (C8 or C18), 41.1 (C8 or C18).

**2-(2'-(benzyloxycarbonyl)-acetyl-amino)-3-hydoxy-propanoic acid methyl ester 116**

Found (NSI): $[M+H]^+$ 296.1127; $[C_{14}H_{17}NO_6+H]^+$ requires 296.1129. $\nu_{max}$ (thin film)/$cm^{-1}$ 3355, 2955, 1740 and 1661. $[\alpha]_D$ = +26 (*c* 1, $CHCl_3$, 25 °C). $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.80 (d, 1H, *J* = 7.6 Hz, H6), 7.38 – 7.29 (m, 5H, H12-16), 5.16 (s, 2H,

H10), 4.65 (dt, 1H, *J* = 7.4, 3.6 Hz, H3), 3.95 (dd, 1H, *J* = 11.4, 3.7 Hz, H2), 3.88 (dd, 1H, *J* = 11.4, 3.5 Hz, H2), 3.74 (s, 3H, H5), 3.42 (s, 2H, H8), 3.13 (s, 1H, H1). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 170.8 (C9), 168.7 (C4), 165.7 (C7), 135.0 (C11), 128.7 (Ar), 128.6 (Ar), 128.4 (Ar), 67.4 (C10), 62.7 (C2) 54.9 (C3), 52.7 (C5), 41.5 (C8).

**_N_-(4'-Methoxybenzyl)-2,2-dimethyl-1,3-oxazolidine-4-carboxylic acid methyl ester 117**



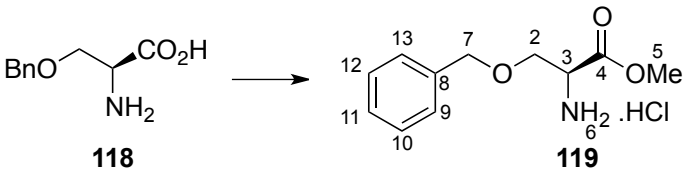

PMB protected L-serine methyl ester **103** (0.12 g, 0.5 mmol) and camphorsulfonic acid (0.02 g, 0.1 mmol, 0.2 equiv.) were dissolved in toluene (5 mL). 2,2-Dimethoxypropane (0.3 g, 0.3 mmol, 0.5 equiv.) was added to the reaction mixture. The reaction mixture was heated under reflux for 45 min at which point the reaction was cooled to room temperature and the solvent removed under reduced pressure. The residue was re-dissolved in toluene (5 mL) and another portion of DMP (0.3 g, 0.3 mmol, 0.5 equiv.) added. This process was repeated 3 times then the solvent removed and residue re-dissolved in diethyl ether (5 mL). The solution was then partitioned with aqueous $NaHCO_3$ (5%, 15 mL). The aqueous phase was then extracted with diethyl ether (2 x 10 mL). The organic layers were combined, dried over magnesium sulfate and the solvent removed under reduced pressure. The resulting product **117** was isolated as an oil (0.13 g, 97%). No further purification was carried out.

Found (NSI): $[M+H]^+$ 280.1538; $[C_{15}H_{21}NO_4+H]^+$ requires 280.1543. $\nu_{max}$ (thin film)/cm$^{-1}$ 2953 and1737. $^1$H NMR (500 MHz, $CDCl_3$) δ 7.26 (app d, 2H, *J* = 8.7 Hz, H8 and H12), 6.83 (app d, 2H, *J* = 8.7 Hz, H9 and H11), 4.11 (dd, 1H, *J* = 8.4, 7.8 Hz, H2), 3.96 (dd, 1H, *J* = 8.4, 5.4 Hz, H2), 3.89 (d, 1H, *J* = 13.3 Hz, H6), 3.79 (s, 3H,

H13), 3.64 (d, 1H, *J* = 13.3 Hz, H6), 3.61 (dd, 1H, *J* = 7.8, 5.5 Hz, H3), 3.45 (s, 3H, H5), 1.37 (s, 3H, H15 or H16), 1.29 (s, 3H, H15 or H16). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 173.1 (C4), 159.1 (C10), 130.9 (C7), 129.7 (C9 and C11), 114.0 (C8 and C12), 66.7 (C2), 55.3 (C13), 51.7 (C5), 51.4 (C6), 26.7 (C15 or C16), 22.1 (C15 or C16).

### 3.2.2.3 Synthesis from *O*-Benzyl-L-Serine

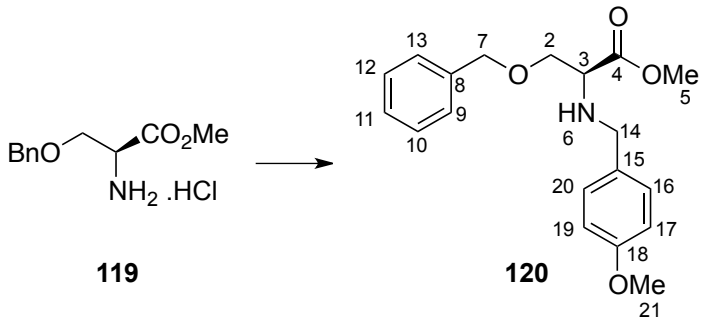
**_O_-Benzyl-L-serine methyl ester hydrochloride**[5]




*O*-Benzyl-L-serine (0.21 g, 1.08 mmol) was subjected to the general procedure for the esterification of an amino acid using acetyl chloride (0.22 mL, 3.26 mmol, 3 equiv.). The product **119** was obtained as off-white solid (0.27 g, quant.). No further purification was carried out.

Found (NSI): $[M+H]^+$ 210.1122; $[C_{11}H_{15}NO_3+H]^+$ requires 210.1125. mp 141-145 °C, lit 165-166 °C.[5] $[\alpha]_D$ = +9 (*c* 1, $CH_3OH$, 25 °C, lit +6.9, *c* 1, $CH_3OH$, 20 °C).[5] $^1$H NMR (500 MHz, $D_2O$) δ 7.42 – 7.32 (m, 5H, H8-13), 4.60 (d, 1H, *J* = 12.0 Hz, H7), 4.52 (d, 1H, *J* = 12.0 Hz, H7), 4.32 (dd, 1H, *J* = 4.3, 3.2 Hz, H3), 3.95 (dd, 1H, *J* = 11.1, 4.3 Hz, H2), 3.86 (dd, 1H, *J* = 11.1, 3.2 Hz, H2), 3.76 (S, 3H, H5). $^{13}$C NMR (126 MHz, $D_2O$) δ 168.68 (C4), 136.73 (C8), 128.75 (Ar), 128.48 (Ar), 128.38 (Ar), 73.11 (C7), 66.35 (C2), 53.71 (C5), 53.15 (C3).

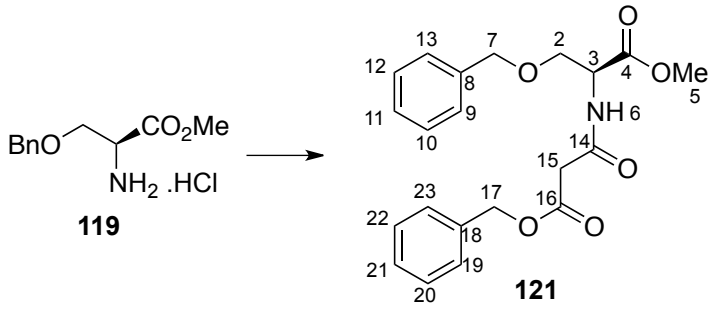
**2-(4'-Methoxy-benzylamino)-3-benzyloxy-propanoic acid methyl ester 120**




*O*-Benzyl-L-serine methyl ester hydrochloride (0.20 g, 0.81 mmol, 1.1 equiv.) was dissolved in acetonitrile (5 mL) and 4-methoxybenzyl chloride (0.10 mL, 0.74 mmol) and potassium carbonate (0.11 g, 0.81 mmol, 1.1 equiv.) were added. The reaction mixture was heated under reflux overnight. The solvent was removed under reduced pressure and the residue purified using column chromatography on silica gel (light petroleum ether : ethyl acetate, 2:1) to yield the title compound as an oil (0.12 g, 45%).

Found (NSI): $[M+H]^+$ 330.1697; $[C_{19}H_{23}NO_4+H]^+$ requires 330.1700. $\nu_{max}$ (thin film)/$cm^{-1}$ 1739. $[\alpha]_D$ = +14 (*c* 1, $CHCl_3$, 25 °C). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.28 – 7.18 (m, 5H, H9-13), 7.17 (app d, 2H, *J* = 8.7 Hz, H17 and H19), 6.77 (app d, 2H, *J* = 8.7 Hz, H16 and H20), 4.45 (d, 1H, *J* = 12.2 Hz, H7), 4.41 (d, 1H, *J* = 12.2 Hz, H7), 3.75 (d, 1H, *J* = 12.7 Hz, H14), 3.71 (s, 3H, H21), 3.65 (s, 3H, H5), 3.61 (dd, 2H, *J* = 8.7, 4.9 Hz, H2), 3.58 (d, 1H, *J* = 12.7 Hz, H14), 3.43 (t, 1H, *J* = 4.9 Hz, H3), 2.10 (s, 1H, H6). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 173.6 (C4), 158.8 (C18), 137.9 (C8), 131.6 (C15), 129.6 (C17 and C19), 128.4 (Ar), 127.7 (Ar), 127.6 (Ar), 113.8 (C16 and C20), 73.2 (C7), 71.0 (C2), 60.4 (C3), 55.3 (C21), 52.0 (C5), 51.4 (C14).

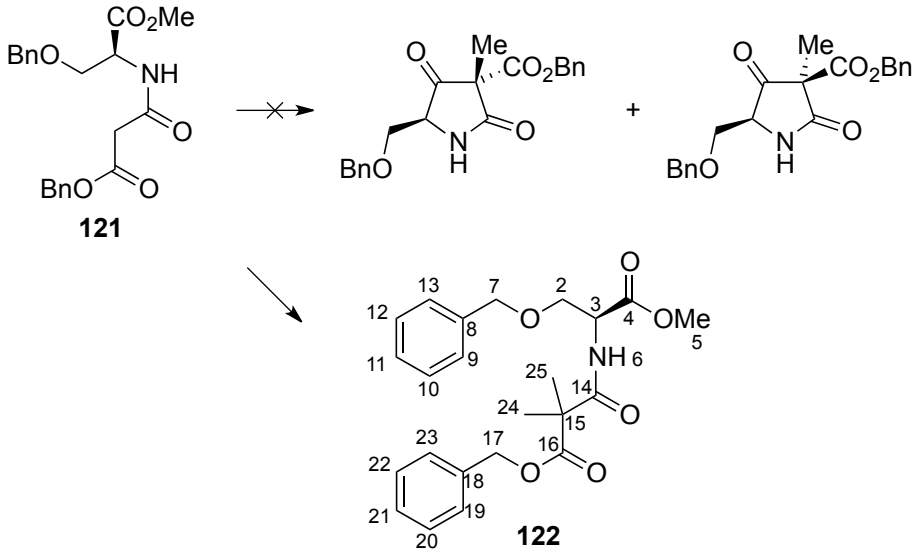
**2-(2'-(Benzyloxycarbonyl)-acetyl-amino)-3-benzyloxy-propanoic acid methyl ester 121**




*O*-Benzyl-L-serine methyl ester hydrochloride **119** (0.20 g, 0.82 mmol) was subjected to the general procedure for peptide coupling using EDAC·HCl (0.39 g, 2.04 mmol, 2.5 equiv.), NMM (0.18 mL, 1.63 mmol, 2 equiv.), benzyl malonic half ester (0.21 g, 0.90 mmol, 2 equiv.), and DMAP (0.02g, 0.16 mmol, 0.2 equiv.). The residue was purified using column chromatography on silica gel (light petroleum ether/ethyl acetate, 2:1). Compound **121** was isolated as an off-white solid (0.22 g, 69%).

Found (NSI): $[M+H]^+$ 386.1588; $[C_{19}H_{23}NO_4+H]^+$ requires 386.1598. $\nu_{max}$ (thin film)/$cm^{-1}$ 3353, 1744 and 1679. mp 104-109 °C. $[\alpha]_D$ = +24 (*c* 1, $CHCl_3$, 25 °C). $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.74 (d, 1H, *J* = 7.7 Hz, H6), 7.38 – 7.27 (m, 10H, H9-13 and H19-23), 5.19 (s, 2H, H17), 4.76 (dt, 1H, *J* = 7.9, 3.3 Hz, H3), 4.53 (d, 1H, *J* = 12.2 Hz, H7), 4.48 (d, 1H, *J* = 12.2 Hz, H7), 3.89 (dd, 1H, *J* = 9.6, 3.3 Hz, H2), 3.73 (s, 3H, H5), 3.69 (dd, 1H, *J* = 9.6, 3.3 Hz, H2), 3.39 (d, 2H, *J* = 2.8 Hz, H15). $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 170.4, 168.6, 164.9, 137.5, 135.1, 128.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.5 (Ar), 127.9 (Ar), 127.7 (Ar), 73.3 (C7), 69.4 (C2), 67.3 (C17), 52.9 (C3), 52.6 (C5), 41.3 (C15).

**The Dieckmann Cyclization**



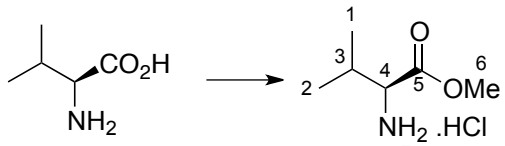

The diester **121** (0.17 g, 0.43 mmol) was subjected to the general procedure for the one-pot Dieckmann cyclization using TBAF (1 M in THF, 1.5 mL, 1.5 mmol, 3.5 equiv.) and iodomethane (0.1 mL, 1.7 mmol, 4 equiv.). The residue was purified using column chromatography on silica gel (light petroleum ether/ethyl acetate, 4:1). Compound **122** was isolated as a colourless oil (0.05 g, 30%).

**2-(2'-(Benzyloxycarbonyl)-2'-methyl-propionyl-amino)-3-benzyloxy-propanoic acid methyl ester**

Found (NSI): $[M+H]^+$ 386.1588; $[C_{19}H_{23}NO_4+H]^+$ requires 386.1598. $\nu_{max}$ (thin film)/$cm^{-1}$ 3353, 1744 and 1679. $[\alpha]_D$ = +22 (*c* 1, $CHCl_3$, 25 °C). $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.28 – 7.14 (m, 10H, H9-13 and H19-23), 7.03 (d, 1H, *J* = 7.6 Hz, H6), 5.10 (s, 2H, H17), 4.62 (dt, 1H, *J* = 7.8, 3.2 Hz, H3), 4.43 – 4.34 (m, 2H, H7), 3.78 (dd, 1H, *J* = 9.5, 3.2 Hz, H2), 3.64 (s, 3H, H5), 3.54 (dd, 1H, *J* = 9.5, 3.3 Hz, H2), 1.43 (s, 3H, H24 or H25), 1.41 (s, 3H, H24 or H25). $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 174.1 (C4), 171.8 (C16), 170.6 (C14), 137.7 (C18), 135.7 (C8), 128.7 (Ar), 128.6 (Ar), 128.4 (Ar), 128.1 (Ar), 128.0 (Ar), 127.7 (Ar), 73.3 (C7), 69.4 (C2), 67.3 (C17), 53.0 (C3), 52.6 (C5), 50.2, 23.6 (C24 or C25), 23.5 (C24 or C25).

### 3.2.2.4 Synthesis from L-Valine

**L-Valine methyl ester hydrochloride 123**[6]

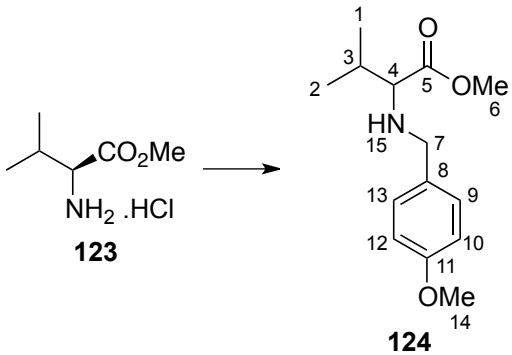



**132** **123**

L-valine (10.11 g, 0.09 mol) was subjected to the general procedure for the esterification of an amino acid using acetyl chloride (18 mL, 0.26 mol, 3 equiv.). The product **123** was obtained as off-white solid (14.46 g, quant.). No further purification was carried out.

$[\alpha]_D$ = +12 (*c* 2, $H_2O$, 25 °C, lit +15°, *c* 2, $H_2O$).[6] mp 170-173 °C, lit 171-173 °C.[6] $^1$H NMR (500 MHz, $D_2O$) δ 3.98 (d, 1H, *J* = 4.7 Hz, H4), 3.80 (s, 3H, H6), 2.30 (m, 1H, H3), 0.98 (d, 3H, *J* = 6.8 Hz, H 1 or H2), 0.96 (d, 3H, *J* = 6.8 Hz, H 1 or H2). $^{13}$C NMR (126 MHz, $D_2O$) δ 170.4 (C5), 58.4 (C6), 53.4 (C4), 29.3 (C3), 17.3 (C1 or C2), 17.0 (C1 or C2).

**2-(4'-Methoxy-benzylamino)-3-methyl-butanoic acid methyl ester 124**[7]



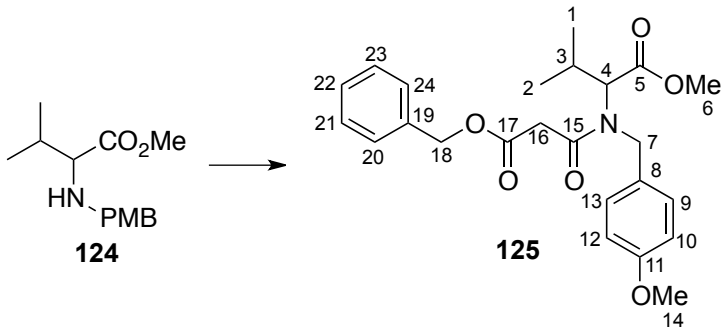

**123** **124**

L-Valine methyl ester hydrochloride (1.50 g, 8.89 mmol) and 4-methoxybenzaldehyde (1.12 mL, 9.77 mmol, 1.1 equiv.) were dissolved in toluene (50 mL) and acetic acid (0.25 mL, 4.44 mmol 0.5 equiv.) added. The reaction mixture was heated under reflux

using a Dean-Stark apparatus overnight. The solvent was removed under reduced pressure and the residue re-dissolved in methanol (50 mL) and acetic acid (0.25 mL, 4.44 mmol, 0.5 equiv.) added. The reaction mixture was cooled to 0 °C and sodium cyanoborohydride (1.12 g, 17.77 mmol, 2 equiv.) was added slowly in small portions. The reaction mixture was stirred for 30 min at 0 °C then allowed to warm to room temperature and stirred overnight. The reaction was quenched with water (10 mL). The solvents were removed under reduced pressure and the residue was dissolved in dichloromethane (50 mL) and washed with water (2 x 50 mL), brine (2 x 50 mL) and $Na_2CO_3$ (2 x 50 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness under reduced pressure. The product **124** was obtained as a yellow oil (2.09 g, 94%). No further purification was carried out.

Found (NSI): $[M+H]^+$ 252.1595; $[C_{14}H_{22}NO_3+H]^+$ requires 252.1594. $\nu_{max}$ (thin film)/$cm^{-1}$ 1733. $[\alpha]_D$ = +7 (*c* 1, $CHCl_3$, 25 °C). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.25 (app d, 2H, *J* = 8.7 Hz, H10 and H12), 6.85 (app d, 2H, *J* = 8.7 Hz, H9 and H13), 3.79 (s, 3H, H14), 3.75 (d, 1H, *J* = 12.9 Hz, H7), 3.71 (s, 3H, H6), 3.52 (d, 1H, *J* = 12.9 Hz, H7), 3.00 (d, 1H, *J* = 6.1 Hz, H4), 1.90 (m, 1H, H3), 0.94 (d, 3H, *J* = 6.8 Hz, H1 or H2), 0.92 (d, 3H, *J* = 6.8 Hz, H1 or H2). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 175.8 (C5), 158.7 (C11), 132.2 (C8), 129.4 (C10 and C12), 113.7 (C9 and C13), 66.5 (C4), 55.3 (C14), 52.0 (C7), 51.4 (C6), 32.0 (C3), 19.3 (C1 or C2), 18.7 (C1 or C2).

**2-{(4'-Methoxy-benzyl)-[2'-(benzyloxycarbonyl)-acetyl]-amino}-3-methyl-butanoic acid methyl ester 125**




Compound **124** (1.16 g, 6.20 mmol) was subjected to the general procedure for peptide coupling using EDAC·HCl (2.99 g, 15.49 mmol, 2.5 equiv.), NMM (1.40 mL, 12.39 mmol, 2 equiv.), benzyl malonic half ester (3.02 g, 9.30 mmol, 1.5 equiv.) and DMAP (0.16 g, 1.24 mmol, 0.2 equiv.). The residue was purified using column chromatography on silica gel (light petroleum ether/ethyl acetate, 4:1). Compound **125** was isolated as a yellow oil (1.75 g, 66%).

Found (NSI): $[M+H]^+$ 428.2066; $[C_{24}H_{29}NO_6+H]^+$ requires 428.2068. $\nu_{max}$ (thin film)/$cm^{-1}$1741 and 1655. $[\alpha]_D$ = +6 (*c* 1, $CHCl_3$, 25 °C).

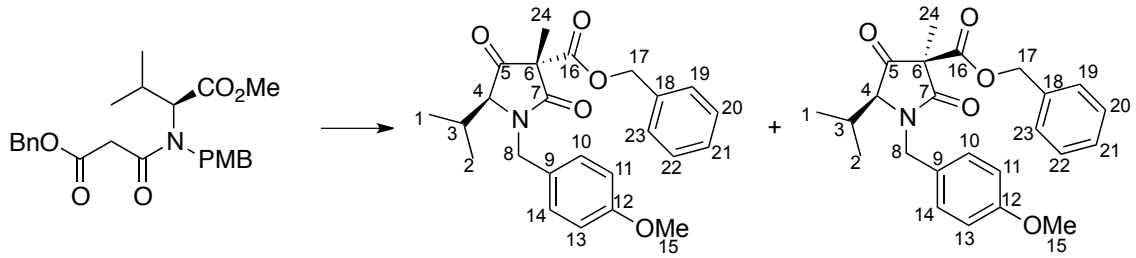
**Major rotamer:** $^1$H NMR (500 MHz, $CDCl_3$) δ 7.42 – 7.28 (m, 5H, H20-24), 7.04 (app d, 2H, *J* = 8.6 Hz, H10 and H12), 6.83 (app d, 2H, *J* = 8.6 Hz, H9 and H13), 5.14 (d, 2H, *J* = 2.3 Hz, H18), 4.85 (d, 1H, *J* = 10.4 Hz, H4), 4.59 (d, 1H, *J* = 17.5 Hz, H7), 4.53 (d, 1H, *J* = 17.5 Hz, H7), 3.77 (s, 3H, H14), 3.48 (d, 1H, *J* = 15.5 Hz, H16), 3.45 (s, 3H, H6), 3.39 (d, 1H, *J* = 15.5 Hz, H16), 2.32 – 2.26 (m, 1H, H3), 0.96 (d, 3H, *J* = 6.7 Hz, H1 or H2), 0.89 (d, 3H, *J* = 6.7 Hz, H1 or H2).

**Minor rotamer:** $^1$H NMR (500 MHz, $CDCl_3$) δ 7.42 – 7.28 (m, 5H, H20-24), 7.17 (app d, 2H, *J* = 8.6 Hz, H10 and H12), 6.73 (app d, 2H, *J* = 8.6 Hz, H9 and H13), 5.21 (d, 2H, *J* = 2.1 Hz, H18), 4.87 (d, 1H, *J* = 15.2 Hz, H7), 4.28 (d, 1H, *J* = 15.2 Hz, H7), 3.82 (d, 1H, *J* = 10.9 Hz, H4), 3.75 (s, 3H, H14), 3.69 (d, 2H, *J* = 2.4 Hz, H16), 3.35 (s, 3H,

H6), 2.40 – 2.32 (m, 1H, H3), 0.93 (d, 3H, *J* = 6.6 Hz, H1 or H2), 0.77 (d, 3H, *J* = 6.6 Hz, H1 or H2).

$^{13}$C NMR (126 MHz, $CDCl_3$) δ 170.7, 169.8, 167.5, 167.3, 167.2, 167.2, 159.0, 158.60, 135.4, 135.3, 129.4, 128.6, 128.6, 128.4, 128.4, 128.4, 128.2, 127.2, 114.2, 113.5, 67.2, 66.7, 62.1, 60.4, 55.3, 52.0, 51.7, 48.4, 45.6, 42.1, 41.6, 28.1, 27.6, 19.9, 18.6.

**The Dieckmann Cyclization**




**125** (±)-**132** (±)-**126**

The diester **125** (1.74 g, 4.08 mmol) was subjected to the general procedure for the one-pot Dieckmann cyclization using TBAF (1 M in THF, 14.30 mL, 14.28 mmol, 3.5 equiv.) and iodomethane (1 mL, 16.32 mmol, 4 equiv.). The residue was purified using column chromatography on silica gel (20% ethyl acetate in light petroleum ether). The first eluting diastereoisomer (±)-**132** was obtained as a yellow oil (0.43 g, 25%) and the second eluting diastereoisomer (±)-**126** was obtained as an off-white solid (0.68 g, 41%).

**(±)-(3*S*,5*S*)-*N*-(4'-Methoxybenzyl)-3-methyl-5-(1'-methylethyl)-pyrrolidin-2,4-dione-3-carboxylic acid benzyl ester (±)-132**

Found (NSI): $[M+H]^+$ 427.2225; $[C_{24}H_{27}NO_5+NH_4]^+$ requires 427.2227. $\nu_{max}$ (thin film)/cm$^{-1}$ 2964, 1776, 1748 and 1697. $^1$H NMR (500 MHz, $CDCl_3$) δ 7.39 – 7.33 (m, 3H, Ar), 7.25 – 7.22 (m, 2H, Ar), 6.99 (app d, 2H, *J* = 8.6 Hz, H10 and H14), 6.57 (app d, 2H, *J* = 8.6 Hz, H11 and H13), 5.43 (d, 1H, *J* = 15.0 Hz, H8), 5.21 (d, 1H, *J* = 12.3 Hz, H17), 5.06 (d, 1H, *J* = 12.3 Hz, H17), 3.77 (d, 1H, *J* = 15.0 Hz, H8), 3.75 (d, 1H, *J* = 3.4 Hz, H4), 3.72 (s, 3H, H15), 2.18 – 2.14 (m, 1H, H3), 1.53 (s, 3H, H24), 1.05 (d, 3H, *J* = 7.1 Hz, H1 or H2), 0.82 (d, 3H, *J* = 7.1 Hz, H1 or H2). $^{13}$C NMR (126 MHz,

$CDCl_3$) δ 205.5, 170.0, 159.2, 134.8, 129.4 (C11 and C13), 128.8 (Ar), 128.6 (Ar), 128.3 (Ar), 126.0, 114.2 (C10 and C14), 68.3 (C4), 68.2 (C17), 59.0 (C6), 55.2 (C15), 42.9 (C8), 27.7 (C3), 18.2 (C1 or C2), 16.4 (C1 or C2), 15.0 (C 24).

**(±)-(3*R*,5*S*)-*N*-(4'-Methoxybenzyl)-3-methyl-5-(1'-methylethyl)-)-pyrrolidin-2,4-dione-3-carboxylic acid benzyl ester (±)-126**

Found (NSI): $[M+H]^+$ 410.1963; $[C_{24}H_{27}NO_5+H]^+$ requires 410.1962. $\nu_{max}$ (thin film)/$cm^{-1}$ 2966, 1773, 1743 and 1695. mp 96-99 °C. $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.36 – 7.28 (m, 5H, H19-23), 7.13 (app d, 2H, *J* = 8.6 Hz, H11 and H13), 6.83 (app d, 2H, *J* = 8.6 Hz, H10 and H14), 5.25 (d, 1H, *J* = 14.9 Hz, H8), 5.19 (d, 1H, *J* = 12.2 Hz, H17), 5.14 (d, 1H, *J* = 12.2 Hz, H17), 3.96 (d, 1H, *J* = 14.9 Hz, H8), 3.78 (s, 3H, H15), 3.63 (d, 1H, *J* = 3.5 Hz, H4), 2.19 – 2.14 (m, 1H, H3), 1.54 (s, 3H, H24), 1.03 (d, 3H, *J* = 7.1 Hz, H1 or H2), 0.70 (d, 3H, *J* = 7.1 Hz, H1 or H2). $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 205.0, 170.3, 165.6, 159.4, 134.8, 129.4 (C11 and C13), 128.5 (Ar), 128.5 (Ar), 128.4 (Ar), 127.1, 114.4 (C10 and C14), 68.0 (C4), 68.0 (C17), 59.0 (C6), 55.3 (C15), 43.4 (C8), 28.3 (C3), 18.6 (C1 or C2), 18.3 (C24), 16.4 (C1 or C2).

## 3.3 References

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# 4.0 Biological Activity Studies

## 4.1 Introduction

Proteasome inhibition is a key strategy for anti-cancer therapy and is of great interest in current research. The proteasome is essential in regulating many processes within the cell, including cellular function and homeostasis.[1] The ubiquitin proteasome pathway regulates the processes that are important for cell growth and survival, for both healthy and tumour cells. Inhibition of the proteasome stops the process of protein degradation, thereby inducing apoptosis. If cancer cells can be specifically targeted for proteasome inhibition, these cells will undergo apoptosis leaving behind only healthy cells.

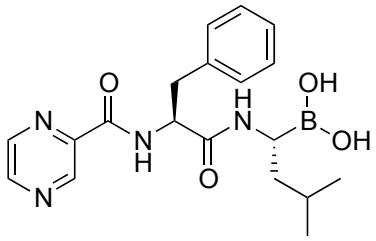



**Figure 1.** Bortezomib

Bortezomib was the first proteasome inhibitor to be used in clinical trials for the treatment of multiple myeloma and is now fully approved by the Food and Drug Administration (FDA) in the USA.[2] It was found to be successful at inducing apoptosis in cancer cell lines whilst having little cytotoxic effects on healthy cells.[3] Unfortunately, in many cases, patients either failed to respond to treatment or relapse occurred (when using Bortezomib alone or as part of combination therapies).[4] This resistance to Bortezomib has led to the need for second generation inhibitors.

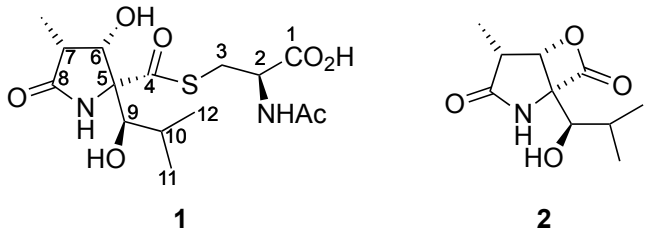



**Figure 2.** Structures of lactacystin **1** and its active form, omuralide **2**.

Lactacystin **1** is a microbial metabolite isolated from *Streptomyces sp*. OM-6519.[5,6] It has anti-tumor effects *in vitro* and *in vivo*. It has been shown to inhibit tumour growth in an animal model of malignant glioma,[7] and prevent cell proliferation in the human Neuro 2A neuroblastoma cell line and the human osteosarcoma cell line.[8] Lactacystin itself cannot permeate through cell walls, but the β-lactone derivative, also known as omuralide **2**, can. The efficiency of lactacystin as a proteasome inhibitor is thus dependent on its ability to form the β-lactone **2**. The 20S proteasome (**Figure 3**) was found to be the specific cellular target of lactacystin and its derivatives.[9]

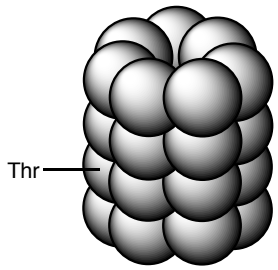



**Figure 3.** The 20S proteasome.

In 1994, Fenteany *et al*. studied the activity of lactacystin analogues to determine which structural features were essential for biological activity.[8] They found that the activity could be greatly affected by the groups on the γ-lactam ring and modifications (including changing the groups completely or altering the stereochemistry) at the C5, C6, C7 and C9 positions can result in partial or complete loss of activity. The case where this does not apply is in the *N*-acetyl-L-cysteine (NAC) moiety at C5; it was found to play no part in the activity and this group could be changed with no effect on activity.

Lactacystin was found to inhibit the trypsin-like (proteases that cleave peptide bonds in the position following a positively charged amino acid such as lysine), chymotrypsin-like (the hydrophobic nature of the S1 pocket makes it specific for medium to large hydrophobic residues) and peptidylglutamyl-peptide hydrolysing (cleavage of peptide bonds in the position following acidic or branched-chain amino acids) activities in the enzyme complex.[9] The trypsin- and chymotrypsin-like activities are both irreversibly inhibited by lactacystin.

In this study, four compounds were selected to be tested for biological activity (**Figure 4**); including proteasome inhibition and anti proliferative effects. These compounds were chosen due to their structural similarities to lactacystin and omuralide in the hope that they would exhibit similar biological activity. Each compound was tested to determine its $IC_{50}$ value against the human leukemia HL-60 cell line (using an MTS cell viability assay) and its ability to inhibit the 20S proteasome (using an enzyme inhibition assay).

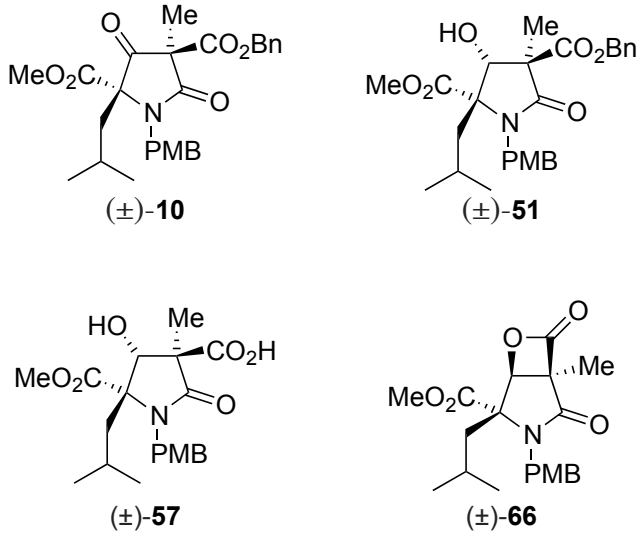
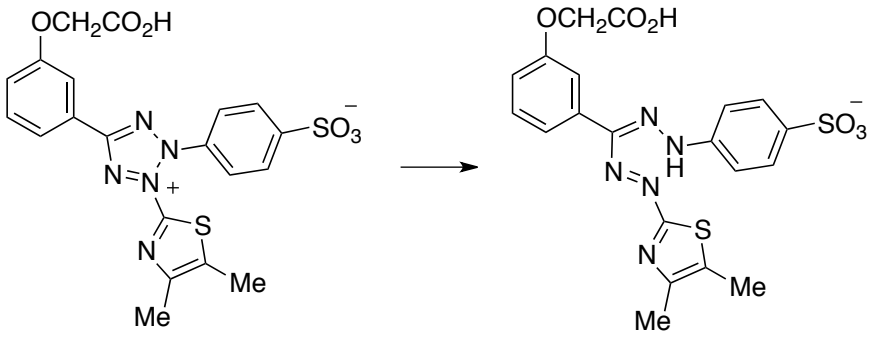



**Figure 4.** Structures of the compounds chosen for biological activity studies.

## 4.2 Anti-Proliferative Studies

### 4.2.1 Introduction

Cell viability assays are used to screen compounds against a specific type of cell to determine if the compound shows direct cytotoxic effects, which can lead to cell death, or has an effect on cell proliferation. An MTS assay is a colourimetric method used to determine cell viability in proliferation or cytotoxicity assays. In living cells, MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) is bioreduced to form a formazan product that is red in colour. The reduction is believed to require NADH or NADPH. If the cells are killed, reduction of the MTS is no longer possible and formazan is not formed (i.e. the red colour of formazan is not seen). Cell viability is measured in terms of absorbance; formazan dye absorbs at 492 nm. The formation of formazan product is directly proportional to the number of viable cells present.




**Scheme 1.** Conversion of MTS to formazan.

An MTS assay is used to determine the $IC_{50}$ value of a compound against a specific cell line. $IC_{50}$ is defined as the '*half maximal inhibitory concentration*'. It is a measure of how effective a compound is at inhibiting a specific biological process. The $IC_{50}$ value can be calculated using the absorbance values of a compound at varying inhibitor concentrations. The $IC_{50}$ value will vary between cell lines; inhibitors show different specific effects in different cell types.

The HL-60 cell line is often used in cell viability assays. HL-60 cells are human promyelocytic leukemia cells established in 1977 from a single patient with acute myeloid leukemia. Proliferation of HL-60 cells occurs in suspension culture with a doubling time of 20 – 45 h.[10]

## 4.2.2 Results and Discussion

In order to confirm that our assay was working and to produce a positive control for our studies, we investigated the anti-proliferative activity of omuralide in HL-60 cells. 3 x $10^4$ cells were seeded in wells of a 96 well plate. The positive control (containing only media), negative control (containing only HL-60's) and the vehicle control (containing HL-60's and DMSO) were set up and the remaining wells treated with omuralide at varying concentrations from 0 – 20 μM. After incubation for 72 h at 37 °C, followed by addition of MTS and further incubation for 4 h at 37 °C, the absorbance was measured at 492 nm. **Figure 5** shows the 96 well plate after MTS addition and incubation. An obvious colour difference is observed as the concentration of omuralide present decreases.

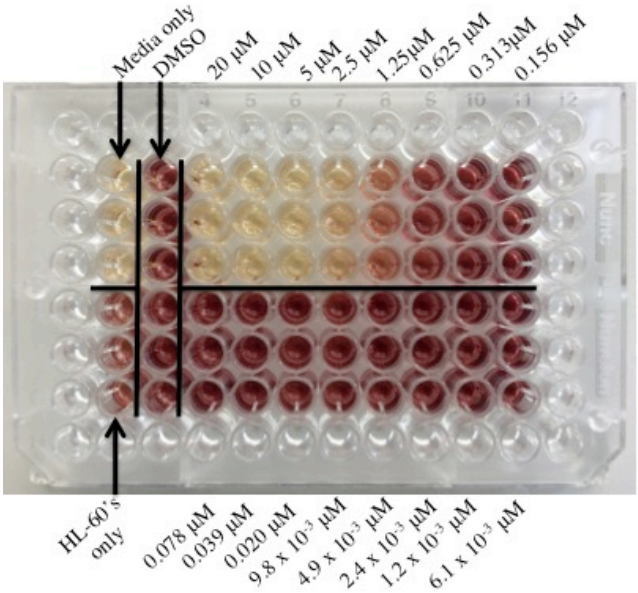


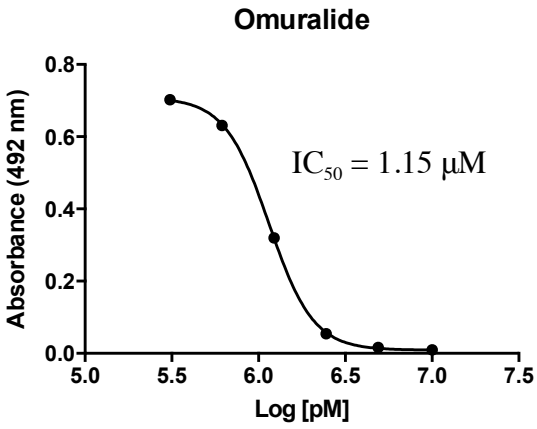

**Figure 5**. Cell viability assay for omuralide after incubation with MTS

The red colour, due to the presence of formazan, cannot be seen at concentrations 20, 10, 5, 2.5 and 1.25 μM. At these concentrations the compounds have decreased cell viability and the bioreduction of MTS to formazan does not occur. The change from yellow to red gives us an indication of where the $IC_{50}$ value will be; between 1.25 and 0.625 μM. **Table 1** shows the absorbance readings obtained and from this a graph can be plotted. Using this graph the $IC_{50}$ value for omuralide against the HL-60 cell line was calculated to be 1.15 μM.

**Table 1.** MTS assay results for omuralide

| Concentration (μM) | Log[pM] | Absorbance (492 nm) |
|---|---|---|
| 0.31 | 5.49 | 0.702 |
| 0.63 | 5.80 | 0.631 |
| 1.25 | 6.10 | 0.320 |
| 2.50 | 6.40 | 0.054 |
| 5.00 | 6.70 | 0.016 |
| 10.00 | 7.00 | 0.010 |

**Figure 6.** Determination the $IC_{50}$ value of omuralide using GraphPad Prism




The next step was to test the compounds made in our laboratories to see how they compare to omuralide. 3 x $10^4$ cells were seeded in wells of a 96 well plate. The positive control (containing only media), negative control (containing only HL-60's) and the vehicle control (containing HL-60's and DMSO) were set up and the remaining wells treated with compounds (±)-**10**, (±)-**51**, (±)-**57** and (±)-**66** at varying concentrations from 0 – 1000 μM. After incubation for 72 h at 37 °C, followed by addition of MTS and further incubation for 4 h at 37 °C, the absorbance was measured at 492 nm.

**Figure 7** shows the 96 well plates after MTS addition and incubation. An obvious colour difference is observed as the concentration decreases. In compounds (±)-**51** and (±)-**66** the red colour, due to the presence of formazan, cannot be seen at concentrations of 1000, 500 and 100 μM. At these concentrations the compounds have decreased cell viability and the bioreduction of MTS to formazan does not occur. Again, the change

from yellow to red gives us an indication of where the $IC_{50}$ value will be; between 100 and 50 μM. Compound (±)-**57** appears to decrease cell viability at concentrations of 1000 and 500 μM and compound (±)-**10** does not appear to decrease cell viability even at a concentration of 1000 μM (i.e. the $IC_{50}$ value is greater than 1000 μM). The $IC_{50}$ values are calculated using a graph of absorbance (at 492 nm) vs. log concentration (pM).

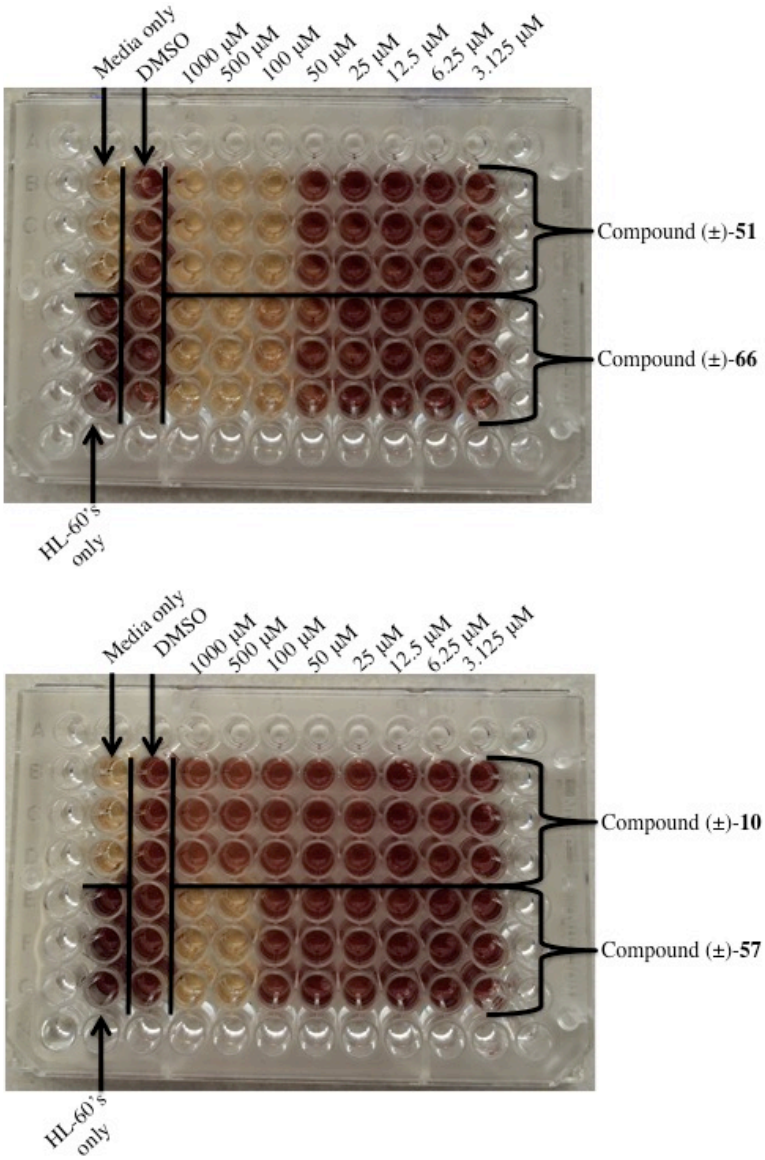



**Figure 7**. Cell viability assays for compounds (±)-**10**, (±)-**51**, (±)-**57** and (±)-**66** after incubation with MTS

**Table 2.** MTS assay results for compound (±)-**51**

| Concentration (μM) | Log[pM] | Absorbance (492 nm) |
|---|---|---|
| 6.25 | 6.79 | 0.865 |
| 12.5 | 7.09 | 0.847 |
| 25 | 7.39 | 0.831 |
| 50 | 7.69 | 0.621 |
| 100 | 8.00 | 0.024 |
| 500 | 8.69 | 0.024 |
| 1000 | 9.00 | 0.024 |

**Figure 8.** Determination the $IC_{50}$ value of compound (±)-**51** using GraphPad Prism

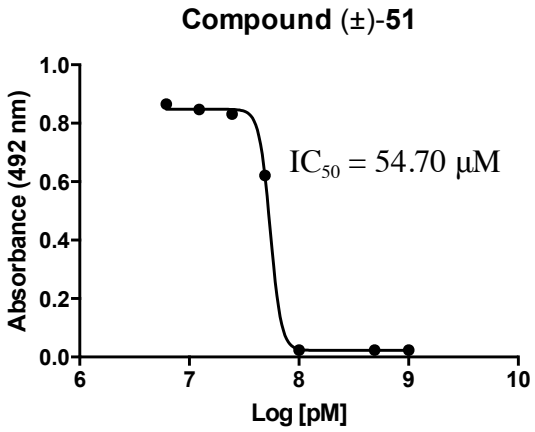



**Table 3.** MTS assay results for compound (±)-**57**

| Concentration (μM) | Log[pM] | Absorbance (492 nm) |
|---|---|---|
| 6.25 | 6.79 | 0.986 |
| 12.5 | 7.09 | 0.878 |
| 25 | 7.39 | 0.660 |
| 50 | 7.69 | 0.678 |
| 100 | 8.00 | 0.571 |
| 500 | 8.69 | 0.005 |
| 1000 | 9.00 | 0.005 |

**Figure 9.** Determination the $IC_{50}$ value of compound (±)-**57** using GraphPad Prism

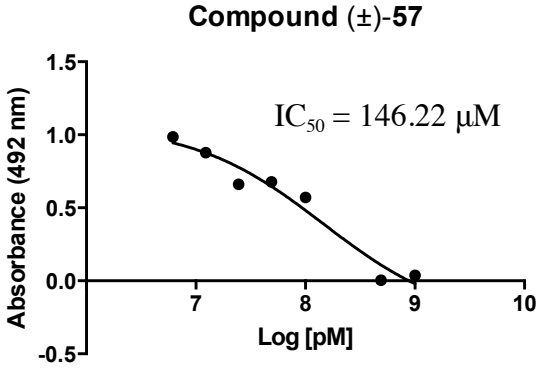
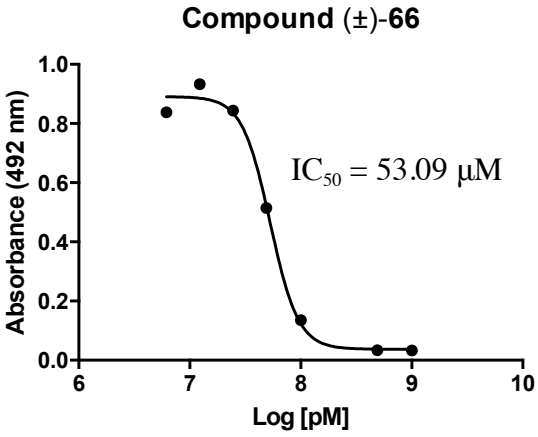



**Table 4.** MTS assay results for compound (±)-**66**

| Concentration (μM) | Log[pM] | Absorbance (492 nm) |
|---|---|---|
| 6.25 | 6.79 | 0.838 |
| 12.5 | 7.09 | 0.933 |
| 25 | 7.39 | 0.844 |
| 50 | 7.69 | 0.515 |
| 100 | 8.00 | 0.135 |
| 500 | 8.69 | 0.034 |
| 1000 | 9.00 | 0.033 |

**Figure 10.** Determination the $IC_{50}$ value of compound (±)-**66** using GraphPad Prism



**Tables 2-4** show the absorbance readings obtained for compounds (±)-**51**, (±)-**57** and (±)-**66**: the absorbance decreases as the compound concentration increases. Looking at the absorbance data, and the assay, the $IC_{50}$ value can be estimated to be between 50 and 100 μM, 100 and 500 μM and 50 and 100 μM respectively. The data obtained was used to plot a graph for each compound from which the $IC_{50}$ value was calculated to be. The calculated $IC_{50}$ values for compounds (±)-**51**, (±)-**57** and (±)-**66** are 54.70 μM, 146.22 μM and 53.09 μM, respectively.

The results obtained for compound (±)-**57** should be viewed with caution; they do not give the standard dose-response curve. More data points over a larger range may result in data more likely to give the standard dose-response curve.

When we compare the data obtained for our compounds to that obtained for omuralide (**Table 5**) we can see that the $IC_{50}$ values are considerably higher for our compounds; they do not decrease cell viability at the same level. A low $IC_{50}$ value is an essential feature for a compound if it were to be used as a therapeutic drug; this is because the dosage of the drug required would also be low.

**Table 5**. $IC_{50}$ concentrations for compounds (±)-**10** (±)-**51**, (±)-**57** and (±)-**66** and omuralide.

| Compound | $LogIC_{50}$ [pM] | $IC_{50}$ [μM] |
|---|---|---|
| (±)-**51** | 7.74 | 54.70 |
| (±)-**57** | 8.17 | 146.22 |
| (±)-**66** | 7.73 | 53.09 |
| (±)-**10** | - | > 1000 |
| **Omuralide** | 6.06 | 1.15 |

Compound (±)-**66** has a β-lactone moiety (albeit at a different position on the ring) also found in the natural product and so we could expect it to have a similar anti-proliferative effect on HL-60's as omuralide. Although not as low as omuralide compound (±)-**66** has an $IC_{50}$ value closest to that of omuralide.

Compound (±)-**10** is the least similar in structure to omuralide and the other compounds tested; it contains a ketone moiety at the C6 position not found in any of the other compounds or the natural product. Structural differences will affect levels of activity.

### 4.2.3 Conclusion and Future Work

$IC_{50}$ values were successfully calculated for compounds (±)-**51**, (±)-**57** and (±)-**66** against the HL-60 cell line. Unfortunately, none of these were in the same order of magnitude as that calculated for omuralide but all showed anti-proliferative effects in the HL-60 cell line. Compound (±)-**10** had little effect on cell viability at the concentrations tested.

We were unable to find any literature reporting the study of the anti-proliferative effects of omuralide on HL-60 cells. This makes the work reported here novel and potentially very interesting and worthy of further investigation. Further investigation is also needed into structure activity relationships. It is clear from the results that structure plays a key role in the observed level of activity; compounds (±)-**10** and (±)-**66** show vastly different anti-proliferative effects.

## 4.2.4 Experimental

### 4.2.4.1 Chemicals

The human promyelocytic leukemia cells (HL-60's) were purchased from the European Collection of Cell Cultures (ECCC, Porton Down, UK). Foetal calf serum (FCS) was purchased from Biosera. RPMI-1640 media and L-glutamine were purchased from Invitrogen. MTS was purchased from Promega.

Compounds (±)-**10**, (±)-**51**, (±)-**57** and (±)-**66** were synthesized in our laboratories and dissolved in the required volume of DMSO to give a 100 mM stock solution. From the stock solution serial dilutions were made (in DMSO) to give 50, 10, 5, 2.5, 1.25, 0.625 and 3.125 mM stocks to be used in the MTS assay.

### 4.2.4.2 Cell Culture

The HL-60 cells were cultured in RPMI-1640 media that contained 2mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin and 10% FCS. Cells were maintained at 37 °C in a 5% $CO_2$ atmosphere. HL-60 density in culture was maintained between $1 \times 10^5$ and $9 \times 10^5$ cells / mL in 75 $cm^2$ flasks. Every 3.5 days the cultures were split with fresh media and used for experimentation until passage 30. HL-60's were diluted to 1 in 2 using trypan blue and counted using a Malassez haemocytometer with light microscopy.

### 4.2.4.3 MTS assay

The MTS assays were carried out, following the manufacturer's instructions, using the CellTilter 96® $Aqu_{eous}$ One Solution Cell Proliferation Assay kit. The outer wells of a 96 well plate were filled with 250 μl of water to aid in the prevention of evaporation of other wells on the plate. 100 μl of 3 x $10^4$ cells were seeded in each well. Three sets of control wells made up; one containing only media (positive control), one containing only HL-60 cells (negative control) and one containing HL-60 cells and DMSO (vehicle

control). The remaining wells were seeded with HL-60 cells and either compounds (±)-**10**, (±)-**51**, (±)-**57** or (±)-**66** (at varying concentrations). Each experiment was carried out in triplicate. The plate was incubated for 72 h at 37 °C with 5% $CO_2$. After incubation 10 μl of MTS assay reagent was added to each well and the plate incubated for a further 4 h at 37 °C with 5% $CO_2$.

Absorbance was measured at 492 nm using a BMG Labtech POLARstarOPTIMA microplate reader. $IC_{50}$ values were calculated from a graph of log[pM] vs. absorbance (492 nm) using GraphPad Prism Version 6.0.

## 4.3 Enzyme Inhibitor Studies

### 4.3.1 Basic Enzyme Kinetics

It is important to understand the 'normal' action of an enzyme before discussing the effect of an inhibitor on enzyme activity. When a substrate binds and is turned over by the enzyme the product is produced. This can be represented by the scheme below and also illustrated graphically (**Figure 11**).

$$E + S \underset{\text{FAST}}{\overset{K_M}{\rightleftharpoons}} ES \xrightarrow[\text{SLOW}]{k_{cat}} E + P$$

**Scheme 2**. General scheme for enzyme catalysed substrate turnover.

The term $K_M$ is known as the Michaelis constant and is defined as the '*concentration of substrate leading to half saturation of the enzyme active sites under steady state conditions*'.[10] Upon mixing E + S the initial equilibrium to form the enzyme-substrate (ES) complex is rapidly established, usually within μ seconds. The term $k_{cat}$ is defined as the rate constant for the slowest 'rate determining' step of the steps that then lead to product formation.[11]

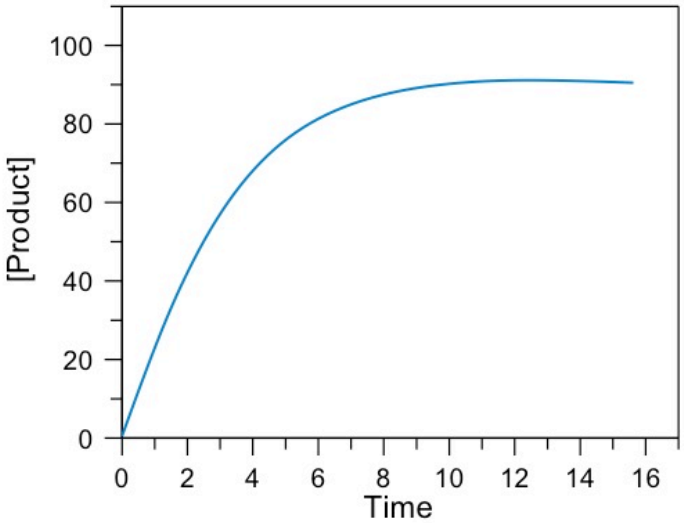



**Figure 11**. General graphical illustration for enzyme activity without the presence of an inhibitor.

The deviation from linearity in the graph illustrates the substrate depletion after time; when all the substrate has been turned over by the enzyme, no more product can be formed. The rate of product formation is defined as $[ES]k_{cat}$. As product is formed the concentration of substrate decreases so the E + S $\rightleftharpoons$ ES equilibrium shifts to the left; the concentration of ES drops so the rate of product formation gradually decreases until all substrate is consumed.

If the initial rate of product formation is measured across a range of different substrate concentrations, a plot of the initial rate *vs*. [S] gives a curve which is described by the Michaelis-Menten equation:

$$v = \frac{V_{\max}[S]}{K_M + [S]}$$

From this, it is possible to obtain a value for the term $V_{max}$. When the enzyme is saturated with substrate, this is the $V_{max}$ and is defined as the maximum velocity (rate) of product formation at infinite substrate concentration.[11]

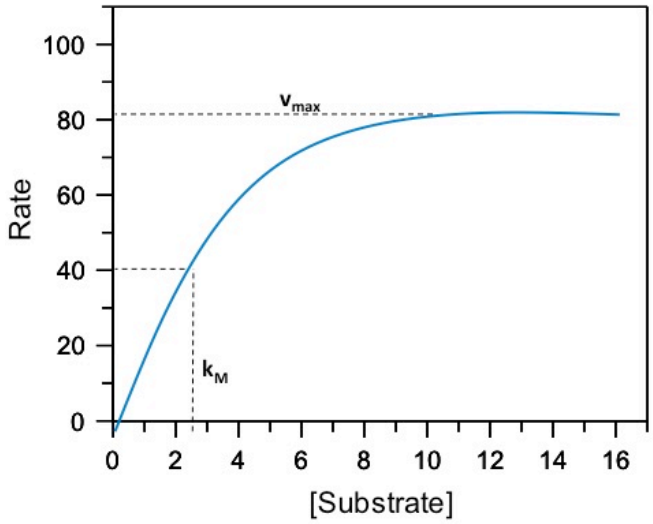



**Figure 12**. General graphical illustration to determine the rate of product formation.

#### 4.3.1.1 The Difference Between Reversible and Time-Dependent Covalent Inhibitors

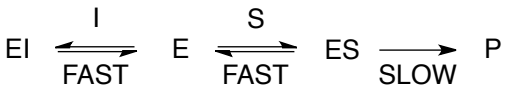
Reversible inhibitors bind to the enzyme through non-covalent forces including, hydrophobic and electrostatics forces, hydrogen bonding and van der Waals interactions. There are three types of reversible inhibition derived from the equilibrium reactions described above; competitive inhibition, non-competitive inhibition and uncompetitive inhibition.

Competitive inhibitors are those that bind to the enzyme in place of the substrate, i.e. they are mutually exclusive, either the substrate binds or the inhibitor binds. They cannot bind at the same time and so formation of the enzyme-substrate (ES) complex is affected.

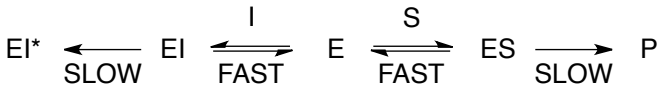
Non-competitive inhibitors are those that show binding affinity for both the free enzyme and the ES complex (or subsequent species). They can result in the formation of an enzyme-inhibitor (EI) complex and/or an enzyme-substrate-inhibitor (ESI) complex.

Uncompetitive inhibitors are those that show no binding affinity for the free enzyme but bind exclusively to the ES complex (or subsequent species). Inhibition can only occur after the formation of the enzyme-substrate complex, i.e. the E + S $\rightleftharpoons$ ES reaction is not affected but the ES → ES* reaction is.

Competitive covalent inhibitors bond irreversibly to the enzymes active site and as a result, modify the enzyme and activity is permanently reduced. Many irreversible inhibitors first bind reversibly to the enzyme forming the EI complex, before covalent bonding occurs (EI*). The rate of EI formation is very fast (μ seconds) compared to that of EI* formation (seconds/minutes) and so these inhibitors are often referred to as slow binding.[9]




Competitive inhibition




Competitive covalent inhibition

**Scheme 3.**

The initial curve in the presence of a time-dependent covalent inhibitor is similar to that of a classical reversible inhibitor (**Figure 13**). This is because the first (reversible) step is very fast and the second (irreversible) step is much slower. When the inhibitor bonds irreversibly the substrate is no longer turned over by the enzyme, no product is formed and the curve begins to level off. The formation of the EI* complex (the slow step) shifts the equilibrium towards EI* and so the concentration of ES drops which in turn results in reduced product formation.

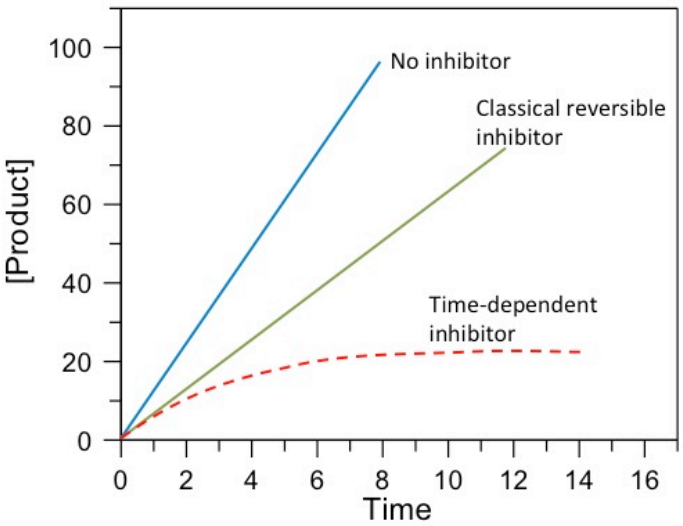
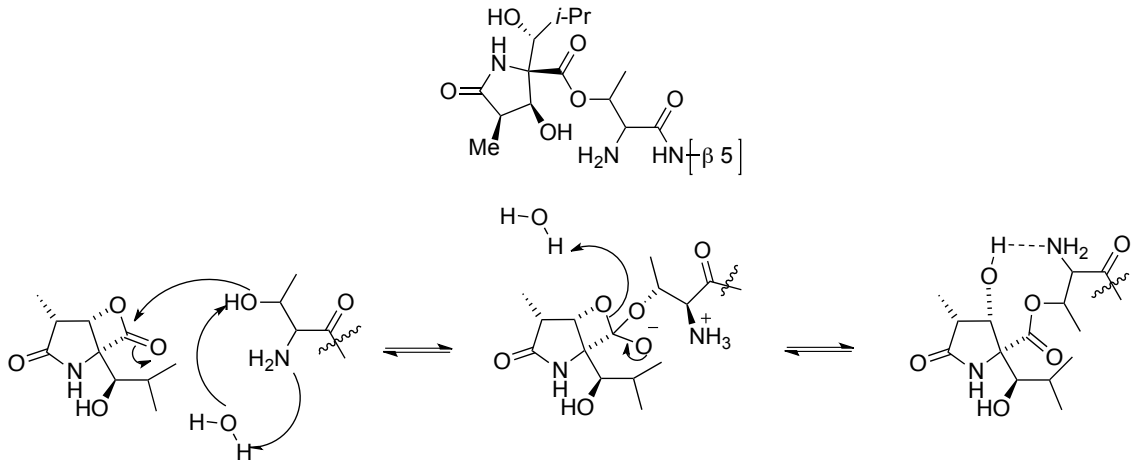



**Figure 13**. General graphical illustration to compare product formation over time in the presence of no inhibitor, a classical reversible inhibitor and a time-dependent covalent inhibitor.

### 4.3.1.2 Proteasome Inhibition by Omuralide

Omuralide acts as a competitive irreversible inhibitor of trypsin- and chymotrypsin-like activity in the 20S proteasome.[9] Cell apoptosis is induced as a result of this inhibition.[12] Omuralide acts by acylating the amino terminal threonine residue of one of the β-type protein subunits of the 20S proteasome, this blocks the active site and stops normal working of the proteasome.[13]




**Scheme 4.** Deactivation of the 20S proteasome by acylation of a terminal threonine residue. [8]

### 4.3.2 Results and Discussion

As described above, omuralide inhibits the trypsin-like and chymotrypsin-like activity in the 20S proteasome. We decided to test our compounds against the chymotrypsin-like activity using a commercially available assay kit containing the 20S proteasome and the chymotrypsin-like substrate (Suc-LLVY-AMC). The AMC (7-amino-4-methylcoumarin) part of the substrate is a fluorescent tag, when the substrate binds and is turned over by the enzyme the AMC tag is released and fluorescence is observed and measured. Continuous kinetic readings were performed over 1 h using a microplate reader.

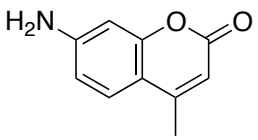



**Figure 14.** AMC (7-amino-4-methylcoumarin).

As we know the mechanism of action of omuralide, we can deduce which compounds may behave in a similar manner. Compound (±)-**66** also has a β-lactone moiety (albeit at a different position on the ring) also found in the natural product and so we would expect a similar mode of action to omuralide. Esters are also reactive towards nucleophiles, although less so than lactones; the ester moiety at C5 present in all compounds could also be used in the reaction with threonine.

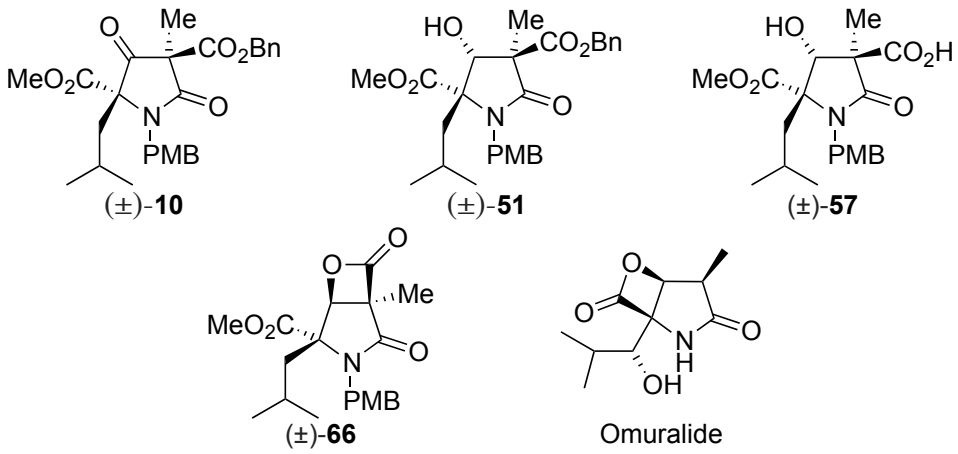



**Figure 15.** The structures of the compounds being screened.

Two standard experiments were run to obtain data that could be used to compare the results for compounds (±)-**10**, (±)-**51**, (±)-**57** and (±)-**66**. The first standard experiment contained only substrate and 20S proteasome, this gives us data to represent the normal enzyme activity without the presence of an inhibitor. The second standard was run with only the substrate to give a baseline reading. Compounds (±)-**10**, (±)-**51**, (±)-**57** and (±)-**66** (500 μM) were then screened for inhibition of the 20S proteasome chymotrypsin activity, compared to 1 μM of omuralide.

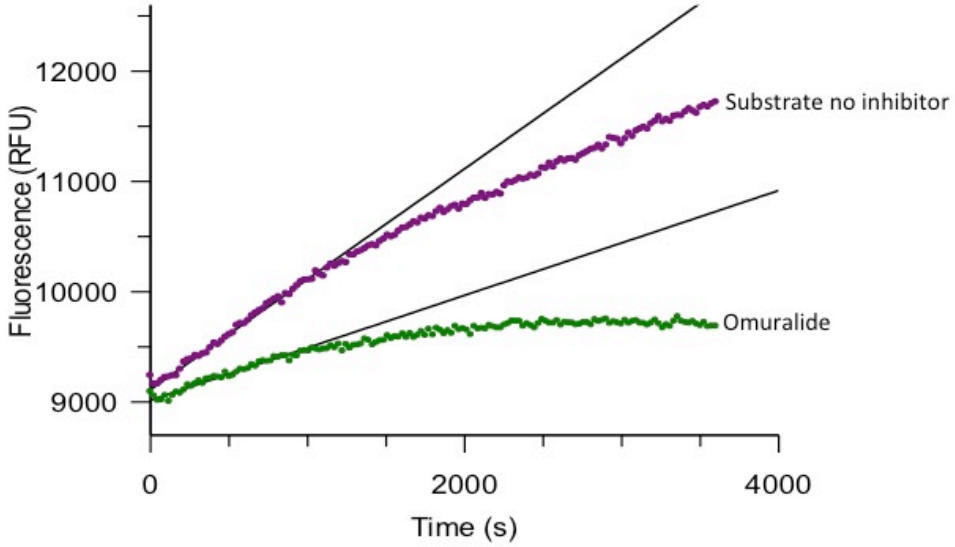



**Figure 16.** A graph of proteasome activity over time with no inhibitor and with omuralide.

**Figure 16** shows the standard curve obtained from the substrate with no inhibitor present (the purple line). Fluorescence increases over time; the substrate binds and is being turned over by the enzyme and the fluorescent tag is being released. Deviation from linearity occurs after time due to substrate depletion.

In the case of omuralide (**Figure 16**, the green line), we can see that after about 1000 s the line starts to level off; the inhibitor is binding to the enzyme in the place of the substrate and so the fluorescent tag is not being released and no further increase in fluorescence is observed. Under these assay conditions the initial percentage inhibition can be calculated from the line gradients; the initial percentage inhibition for omuralide against the chymotrypsin-like activity of the 20S proteasome was calculated to be 47%.

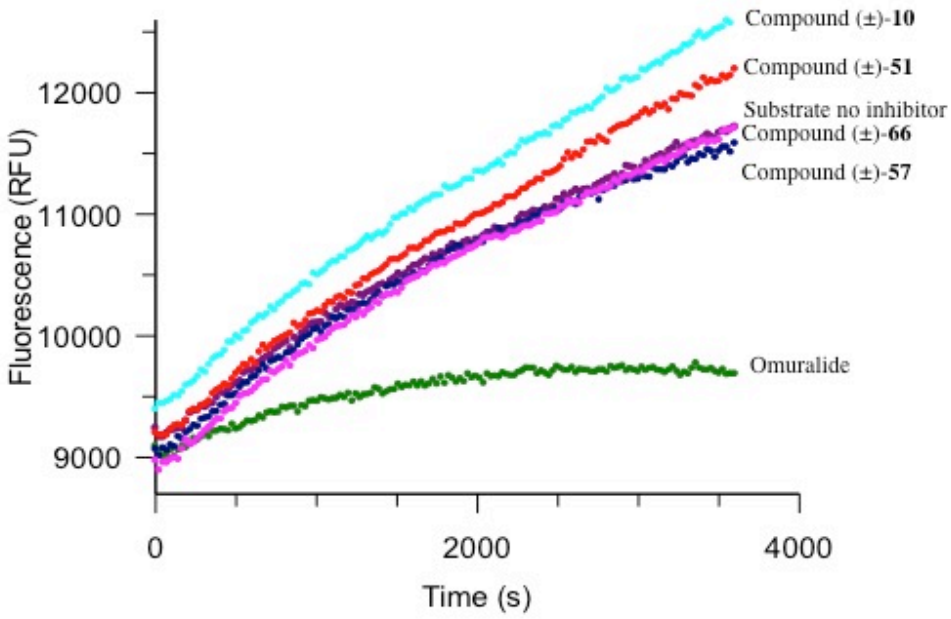



**Figure 17.** A graph of proteasome activity over time in the presence of different inhibitors, compounds (±)-**10**, (±)-**51**, (±)-**57**, (±)-**66** and omuralide.

**Figure 17** shows the results of screening for compounds (±)-**10**, (±)-**51**, (±)-**57** and (±)-**66** against that of the standard substrate curve and that obtained for the known inhibitor omuralide. The curves obtained show similar linearity to that of the substrate standard (again with deviation from linearity due to substrate depletion) thereby indicating that they are not inhibiting the chymotrypsin-like activity in the 20S proteasome. The curve obtained for omuralide is exactly as expected from a time-dependent covalent inhibitor; the initial rate is similar to that where no inhibitor is present as the first step is reversible however after time it covalently binds to the proteasome and the curve levels off. The percentage activity was calculated for each compound (**Table 6**).

Also evident from this graph is that the initial RFU values for some of the compounds being tested is higher than that of the substrate only standard; this is due to the compounds themselves fluorescing, this will have no effect on the gradient and so does not affect the results obtained.

**Table 6**. Calcualted activites for compounds tested against the chymotrypsin activity of the 20S proteasome.

| Inhibitor | % Activity | Time-Dependent |
|---|---|---|
| None | 100 | - |
| Omuralide | 47 ± 2 | Yes |
| (±)-**10** | 113 ± 2 | - |
| (±)-**51** | 109 ± 1 | - |
| (±)-**57** | 106 ± 1 | - |
| (±)-**66** | 105 ± 2 | - |

### 4.3.3 Conclusion and Future Work

Compounds (±)-**10**, (±)-**51**, (±)-**57** and (±)-**66** do not appear to inhibit the chymotrypsin-like activity of the 20S proteasome as in the case of omuralide. There are many factors to consider in what makes a compound a successful inhibitor; the functional groups present and the overall size of the compound are both important. The protecting group (PMB) present on all the compounds tested is likely to cause the lack of activity, this group makes the compounds much larger than the natural product and so they may not fit into the active site of the enzyme. It is also possible that the functional groups present in compounds (±)-**10**, (±)-**51**, (±)-**57** and (±)-**66** are not optimal for inhibition.

Compound (±)-**66** is the most interesting of those tested as it has the lactone motif, albeit at the C6-7 position rather than the C5-6 position, found in omuralide. It is expected that this would act chemically in the most similar way to the natural product.

Future work should include the deprotection and re-screening of the compounds against the chymotrypsin-like activity. To achieve a full set of data the deprotected compounds should be screened against the trypsin- and caspase-like activity in the 20S proteasome. It is possible for a compound to inhibit one type of activity and not another.

### 4.3.4 Experimental

The proteasome assays were carried out, following the manufacturer's instructions, using the VIVAdetect™ 20S Proteasome Assay Kit PLUS. The kit contained 10x VIVAdetect™ proteasome assay buffer, 20S proteasome, Suc-LLVY-AMC (chymotrypsin-like substrate), VIVAdetect™ 96 well assay plate and VIVAdetect™ AMC standard. Each experiment was performed in duplicate at 25 °C.

AMC standard experiment: AMC standard was diluted with the provided VIVAdetect™ proteasome assay buffer to give a final assay concentration of 1.6 μM.

Substrate control experiment: The substrate Suc-LLVY-AMC (chymotrypsin-like substrate) was diluted with the provided VIVAdetect™ proteasome assay buffer and DMSO to give a final assay concentration of 100 μM.

Substrate plus 20S proteasome control experiment: The substrate Suc-LLVY-AMC (chymotrypsin-like substrate) and the 20S proteasome were diluted with the provided VIVAdetect™ proteasome assay buffer and DMSO to give a final assay concentration of substrate of 100 μM and 20S proteasome of 2.5 nM.

Inhibitor testing experiments: Omuralide and compounds (±)-**10**, (±)-**51**, (±)-**57** and (±)-**66** were individually tested for their ability to inhibit the 20S proteasome. For each experiment the substrate Suc-LLVY-AMC (chymotrypsin-like substrate) and the 20S proteasome were diluted with the provided VIVAdetect™ proteasome assay buffer and DMSO to give a final assay concentration of substrate of 100 μM and 20S proteasome of 2.5 nM. Omuralide was tested at a final assay concentration of 1 μM, and compounds (±)-**10**, (±)-**51**, (±)-**57** and (±)-**66** were tested at a final assay concentration of 500 μM. The buffer solution, the substrate and the compound being tested were all added together then incubated at 25 °C for 15 min before the addition of the 20S proteasome.

The fluorescence (measured in relative fluorescence units) was measured using a BMG Labtech POLARstarOPTIMA microplate reader with filters for 340 nm excitation and 480 nm emission. A continuous kinetic analysis was performed for 1 h. Analysis of the

results obtained was performed by plotting graphs of fluorescence (RFU) vs. time (s) using GraFit Version 5.0.10.

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