Analogues of Antibacterial Natural Products

A thesis submitted in partial fulfillment of the requirement for the degree of

Doctor of Philosophy

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The work described in this thesis is entirely my own, except where I have either acknowledged help from a named source or given a reference to a published source or thesis. Text taken from another source will be enclosed in quotation marks and a reference given.

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ABSTRACT

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Elizabeth Anne Heaviside, St Catherine's College, University of Oxford
DPhil Thesis, Trinity Term 2012

This thesis is concerned with the synthesis and biological evaluation of structural mimics for the natural products 16-methyloxazolomycin and lemonomycin which display potent biological activity including antibacterial and antitumour activity.

Chapter 1 explores methods and approaches to the discovery of new antibacterial drugs and the challenges faced in this respect. It also gives an overview of the properties of the natural products investigated in the following chapters and summarises previous synthetic approaches to these molecules published in the scientific literature.

Chapter 2 describes the work carried out towards the synthesis of the diazabicyclo[3.2.1]octane unit of the tetrahydroisoquinoline antitumour antibiotic lemonomycin. The intended retrosynthesis of the natural product led to a 2,5-disubstituted pyrrolidine bearing a 1′-amino functional group; a series of routes were explored for the synthesis of this unit. Using (*S*)-pyroglutamic acid, strategies using Eschenmoser and thiolactim ether coupling reactions were investigated. A sequence based on the formation of a pyrrolidine ring from the cyclisation of an appropriately substituted oxime ether derived from L-phenylalanine was then implemented but a competing Beckmann rearrangement/Grob fragmentation prevented access to the desired heterocycle. Preliminary investigations were also carried out on the modification of cyclic imines derived from oxime ethers which did not undergo Beckmann rearrangement.

Chapter 3 describes the synthesis of a library of densely functionalised tetramic acid and pyroglutamate mimics for the right-hand fragment of 16-methyloxazolomycin, and their coupling with a *gem*-dimethylamide unit mimicking the middle fragment of the natural product. Tetramates were accessed through the Dieckmann cyclisation of *N*-acyloxazolidines and were derivatised with various alkyl halides. The pyroglutamates were accessed *via* the highly diastereoselective aldol cyclisation of *N*-acyloxazolidines formed by the amide coupling of a threonine derived oxazolidine and β-keto-acids. A series of β-keto-acids were synthesised through the acylation and subsequent ring-opening/decarboxylation reaction of Meldrum's acid. The formation of right-hand/middle fragment adducts was explored using cycloaddition, alkylation and Sonogashira chemistry before a Wittig protocol led to the formation of adducts (*E*)- and (*Z*)- **402** and **403**. Biological evaluation of the compounds synthesised in this chapter was carried out using both broth and hole-plate bioassays and active compounds were identified. Of particular note was that the Wittig adducts displayed a higher level of activity against Gram-negative *E. coli* than either the pyroglutamate or amide motifs alone.

ACKNOWLEDGEMENTS

Most importantly I would like to thank my supervisor, Prof. Mark Moloney. Thank you for sharing the ups and downs of this project with me; for encouraging and having confidence in me during the downs, and for being so animated on the occasions I got to deliver good news. Your enthusiasm is infectious and I hope I will be able apply to the same attitude to my future endeavours.

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Sam, thank you for endeavouring to remember chemistry and reading this thesis, the 'constructive criticism', however received, was appreciated. Now that this is out of the way, I can look forward to us becoming Mr and Dr! I love you.

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CONTENTS

ABBREVIATIONS

Ac	acetyl
AIBN	azobisisobutyronitrile
app.	apparent
aq.	aqueous
Ar	aryl
ATCC	American Type Culture Collection
ATP	adenosine triphosphate
BHT	2,6-di-*tert*-butyl-4-methylphenol
BOC	*tert*-butyloxycarbonyl
BOM	benzyloxymethyl
BOP	bis-(2-oxo-3-oxazolidinyl)phosphonic chloride
bp	boiling point
Bu	butyl
BuLi	*n*-butyllithium
c	concentration
CAN	ceric ammonium nitrate
CBz	carbobenzyloxy
clogD	calculated log of the distibution coefficient at pH 7.4
clogP	calculated log of the partition coefficient
CNS	central nervous system
Conc.	concentrated
COSY	2D 1H correlation spectroscopy
CSA	camphorsulphonic acid
d	doublet
DBAD	dibenzyl azodicarboxylate
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DCE	1,2-dichloroethane
DCM	dichloromethane
dd	doublet of doublets
de	diastereomeric excess
DEAD	diethyl azodicarboxylate

DEPT	distortionless enhancement by polarisation transfer
DIBAL	di-*iso*-butylaluminium hydride
DIPEA	*N*,*N*-di-*iso*-propylethylamine
DIPT	di-*iso*-propyltartrate
DMAP	4-(N,N-dimethylamino)pyridine
DMDO	dimethyldioxirane
DMF	dimethylformamide
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
dr	diastereomeric ratio
DTT	dithiothreitol
E. Coli	*Escherichia coli*
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
ee	enantiomeric excess
eq.	equivalents
ESI	electrospray ionisation
Et	ethyl
FAB	fast atom bombardment
FI	field ionisation
Fmoc	9-fluorenylmethoxycarbonyl
gem-	*geminal*
h	hour
HA	haemaglutinin
HATU	2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
Hek293	human embryonic kidney 293 cell
HMBC	heteronuclear multiple-bond correlation
HMDS	hexamethyldisilazide
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum Correlation
HTS	high throughput screening
IBX	*o*-iodoxybenzoic acid
IC50	inhibitory concentration of 50% of sample

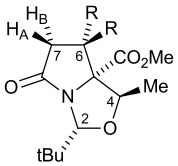
IR	Infra-red
LD50	lethal dose that kills 50% of the sample population
LDA	lithium diisopropylamide
Lit.	literature
M	molar
m	multiplet
Me	methyl
MIC	minimum inhibitory concentration
min	minutes
mol	moles
MOM	methoxymethyl ether
mp	melting point
MSA	molecular surface area
MTPA	α-methoxy-α-trifluoromethylphenylacetic acid
MW	molecular weight
NBS	*N*-bromosuccinimide
NMM	*N*-methylmorpholine
NMP	*N*-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
P	product
PBMC	peripheral blood mononuclear cell
PCC	pyridinium chlorochromate
ppm	parts per million
PSA	polar surface area
py	pyridine
q	quartet
qd	quartet of doublets
quant.	quantitative
RED-Al	sodium bis(2-methoxyethoxy)aluminiumhydride
Rf	retention factor
RNA	ribonucleic acid
ROESY	rotating-frame nuclear Overhauser effect spectroscopy

rt	room temperature
s	singlet
S. aureus	*Staphylococcus aureus*
Sat.	saturated
SM	starting material
t	*tertiary*
t	triplet
TAA	tetrahydroisoquinoline antitumour antibiotics
TBAF	tetra *n*-butylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TEA	triethylamine
TEBA	triethylbenzylammonium chloride
TEMPO	(2,2,6,6-tetramethyl-piperidin-1-yl)oxyl
TES	triethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	tri-*iso*-propylsilyl
TMEDA	tetramethylethylenediamine
TMS	trimethylsilyl
Ts	tosyl (*p*-touenesulphonyl)
UV	ultra violet
VT	variable temperature
w/v	weight per volume
δ	chemical shift

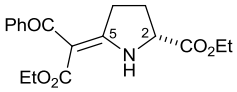
NOMENCLATURE

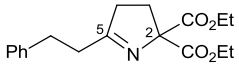
The following non-systematic numbering systems have been used throughout this thesis.

1. Bicyclic tetramates and pyroglutamates. In these templates *exo*- and *endo*- are also used to describe the position of substituents with respect to the bicyclic ring system. In the example below H_A is *endo*- and H_B in *exo*- with respect to the bicyclic ring.



2. Monocyclic lactams, enamines, imines, and pyroglutamates. Two examples are shown below.





STEREOCHEMISTRY

Absolute stereochemistry

Cahn-Ingold-Prelog (*R*,*S*) convention has been used throughout this thesis to describe chiral centres. For compounds containing double bonds, (*E*,*Z*) descriptors have been used to describe the geometry.

Relative stereochemistry

A dashed line is used to denote α-configuration, where the substituent lies behind the plane of the page, and a solid wedged line is used to denote β-configuration, where the substituent lies in front of the plane. Where a solid line is used it denotes unknown stereochemistry. A wavy line is used in the case that a mixture of isomers at that centre are present.

CHAPTER 1: INTRODUCTION

1.1 Overview

When the first antibacterial drugs were introduced in the first half of the 20th century they revolutionised healthcare and once-fatal illnesses could be treated to allow sufferers to make a full recovery. Before long, infections were found that did not respond to treatment using these antibiotics, and the first drug-resistant bacterial strains were identified. The 'Golden age' of antibiotics followed when most of the scaffolds seen in today's drug arsenal were discovered and characterised. However, after the clinical deployment of a new antibiotic drug, it can take as little as one year for resistant strains of bacteria to be detected.[1] In order to keep up with the continuing need for new drugs to treat resistant infections, incremental changes were made to these scaffolds and the rate of discovery of new scaffolds declined. According to Payne, the problem of resistance cannot be fully overcome by making small modifications to existing scaffolds and new scaffolds may have a longer period of utility than 2nd - current generation drugs based on old scaffolds.[2] It would be fair to say that there will never be an antibiotic drug to which resistance does not eventually develop; the challenge now is to develop new drugs, and methods for their use, that will maximise the time this takes. There is therefore a need not only for new drugs, but for new classes of drugs, with novel structural motifs, to slow the development of bacterial resistance.

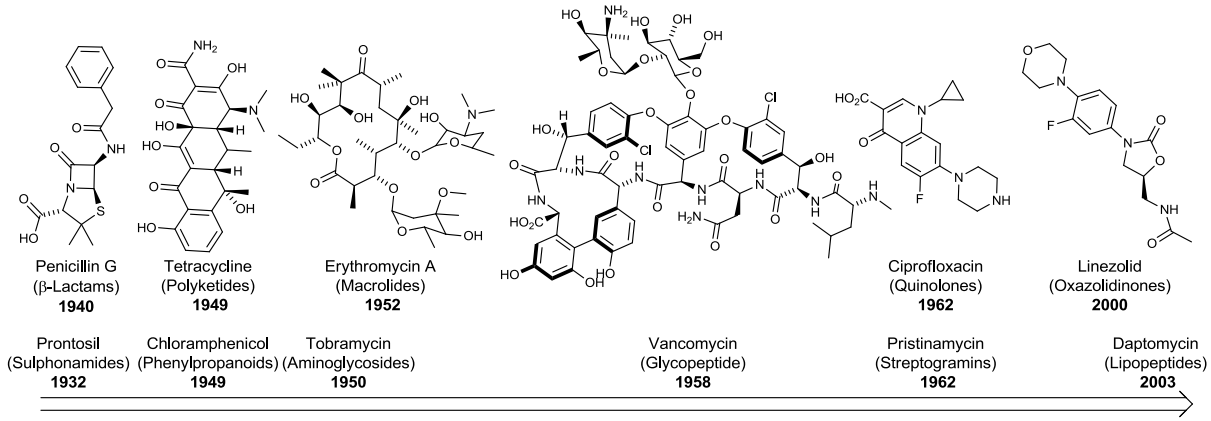


Figure 1.1 Timeline of the introduction of the main classes of antibiotic drugs.

1.2 Resistance

Resistance in bacterial infection arises from the evolution of strains of bacteria after exposure to antibiotics by a process of natural selection. This selects individuals with favourable mutations that confer resistance against an environmental pressure. An ability to limit the speed of evolution would help to slow the development of resistance to new antibiotics. There are many examples of how the actions of humans have caused accelerated evolution in other species and it is not limited to the evolution of resistance of bacteria and pests to their poisons. One example is the changes caused by heavy fishing pressure; fish have been shown to evolve slower growth rates and thinner bodies which can slip through nets.[1,3] Palumbi describes *'Humans as the world's greatest evolutionary force'* in his review of the topic which also details several strategies used for attempting to slow the evolution of resistance in bacteria, as well as other pest species.[1] An overkill strategy has been used in the treatment of HIV, where a cocktail of drugs is used to overwhelm the virus. The intention is that the strong dose of drugs leaves no virus able to reproduce, and therefore no way of introducing the variation needed for evolution to occur. This has also been used in the treatment of bacterial infections but this strategy is limited by the toxicity of the drugs involved and the possible side effects.[4] Another strategy employed in the treatment of bacterial infections is to withhold the most powerful drugs for the most severe cases where others have failed. The reduced use of a drug lengthens its life-span[4] because the selection pressure on the species it kills is reduced, and is therefore less likely to cause the evolution of resistant strains. Vancomycin is typically the antibiotic 'drug of last resort' but there are now strains that are resistant to this drug. Screening for resistance before treatment may also reduce the development of resistance by not exposing partially resistant populations to unsuitable drugs.

1.3 Drug Discovery

In order to identify active compounds, most industrial drug discovery programs screen large numbers of compounds using high throughput screening (HTS). Many compounds are tested simultaneously using an assay for the desired pathway, and any positive assays produce hit compounds. These hits are confirmed using more detailed assays along with other tests on the

compound's suitability as a potential drug. A number of analogues may be synthesised using combinatorial chemistry, high-throughput chemistry, or classical organic synthesis to begin analysis of the structure activity relationships. The most promising compounds at this stage are identified as leads and taken forward for further optimisation. Typically, lead structures will possess a high binding affinity for the protein target, moderate molecular weight and lipophilicity, not bind to human serum albumin, not be cytotoxic or readily metabolised, and be stable, soluble in water, permeable to cell membranes, and free of existing intellectual property.

1.4 Challenges to natural product orientated drug discovery

Between 1981 and 2006, two thirds of antibacterial new chemical entities were natural products or derived from natural products.[5] Despite this, the use of natural products for drug discovery has fallen out of favour in recent decades as it is less compatible with high-throughput screening (HTS) methods than compound libraries obtained by combinatorial chemistry.[6] Crude extracts of natural products can be used in HTS, but this often results in many false hits as these extracts may contain tannins, which are promiscuous protein binders. If any genuine hits are detected purification of the crude extract can take weeks to months, by which time the appropriate assay may no longer be running. There is also a risk that the active compounds that are isolated from the extracts are already known and therefore cannot be patented. The cost of collecting samples of natural products, along with the natural variability of the source material, is also problematic. If even these issues are overcome, and promising candidates are identified, the compounds will need to be obtained on multi-gram scales for the necessary development to take place and with so little being isolated from each extraction this is often a major problem. If the natural source is particularly low yielding, from an endangered species, or part of an endangered habitat then direct harvesting is unacceptable. One instance of this is the anticancer drug paclitaxel, isolated from the bark of the western yew (*T. brevifolia*) which grows predominantly in the forests of the Pacific Northwest, home to the endangered spotted owl. The isolation problem was solved by the identification of a semi-synthetic route using a related compound more readily available from the needles of another yew species (*T. baccata*).[7] The supply crisis also provided motivation

for the development of numerous total syntheses published by the academic community,[8] demonstrating the significant contribution that can be made by targeted synthetic studies. Many financial challenges to research also limit the development of new antibacterial therapies, including pipeline drugs that are insufficient to replace older drugs about to become generic and the limited profitability of single-use drugs that offer a cure.

From its inception, combinatorial chemistry was heralded as a breakthrough as it can create large compound libraries in a short space of time. Although a large amount of investment in the technique has been made, Newman and Cragg report that only one *de novo* new chemical entity (the antitumour compound sorafenib) resulting from this method of chemical discovery has been approved for drug use.[5] However, improved techniques for the identification, isolation, and structural elucidation of bioactive natural products are being developed to overcome the problems and the approach is now experiencing a renaissance as the advantages become clearer.[9]

1.5 Natural product inspired synthesis

1.5.1 Natural products as advanced starting points

Natural products as synthesised by living organisms arise from a relatively small selection of building blocks and biosynthetic pathways, yet they have extraordinary diversity in both structure and function. This makes them a vast source of inspiration for chemists and biologists. They also tend to exhibit more specific binding characteristics than artificially designed molecules. The reason for this lies in their evolutionary background. The secondary metabolites may have evolved to be beneficial to the host species (by keeping parasites and infections at bay) so that years of evolutionary optimisation have served as a screening process to develop the most active and specific compounds. They also tend to have better 'drug-like' qualities, such as bioactivity, pharmacokinetics, and solubility, than synthetic compound libraries. In their paper on the renaissance of natural products as drug candidates, Paterson and Anderson explain that "the structures of the [protein binding sites] of such natural products are often well conserved among proteins with markedly different genetic

sequences, such that secondary metabolites that have evolved for a certain purpose and mode of action by a producing organism may exert different, yet equally potent, effects in other settings."[10] They also often target proteins essential to life, which is consistent with them being defence substances for the producing organism. This is reflected in the observation that compound libraries based on natural product scaffolds have a higher hit rate than combinatorial libraries, which are often based on a large number of analogues of a small number of different scaffolds.[11]

1.5.2 Approaches to natural product orientated drug discovery

Many natural products have been shown to have antibacterial activity; in some cases they can be used unaltered for therapeutic purposes,[12] but more often structural analogues make better candidates. In order to investigate the activity of natural product analogues a library of compounds based on the natural product must be created. There are a variety of ways this may be achieved, for example by a semi-synthetic approach,[13] combinatorial biosynthesis[14] or diverted synthetic routes.[15] A semi-synthetic approach takes the natural product itself, isolated from the natural source, and makes chemical modifications through functional group transformation. In this scenario, analogues can be accessed rapidly but a limited supply of the natural product is still a potential problem and the scope of modifications is limited by the reactive groups present in the natural product. Combinatorial biosynthesis involves the use of genetic engineering to reconstruct biosynthetic pathways leading to structures related to the original natural product. This too has a limited diversity of analogues. Diverted synthesis, though more time consuming, offers the most flexibility in terms of the structure of analogues. A synthetic path to the desired natural product is designed that allows the introduction of structural variations at different stages on route to the target molecule.

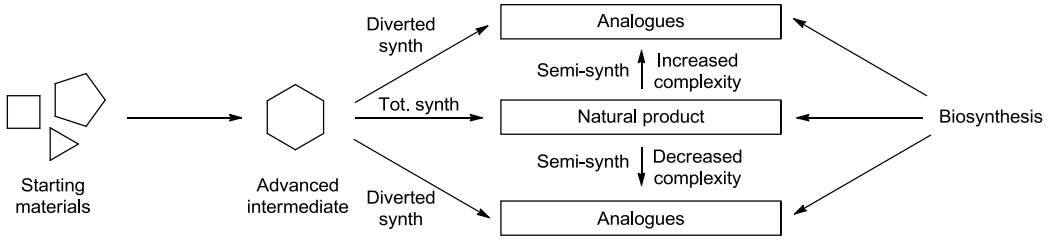


Figure 1.2 Approaches to NP analogues.[16]

One example of the success of diverted synthesis is E7389, a potent oncolytic agent currently in phase I clinical trials (Figure 1.3). It was discovered through the modification of an existing synthetic route[17] to the total synthesis of the natural product Halichondrin B, a marine natural product exhibiting high cytotoxicity.[18] The structure of E7389 is much simpler than that of Halichondrin B, but it retains much of the antimitotic properties and has the advantages of being more stable and easier to synthesise than the natural product itself.

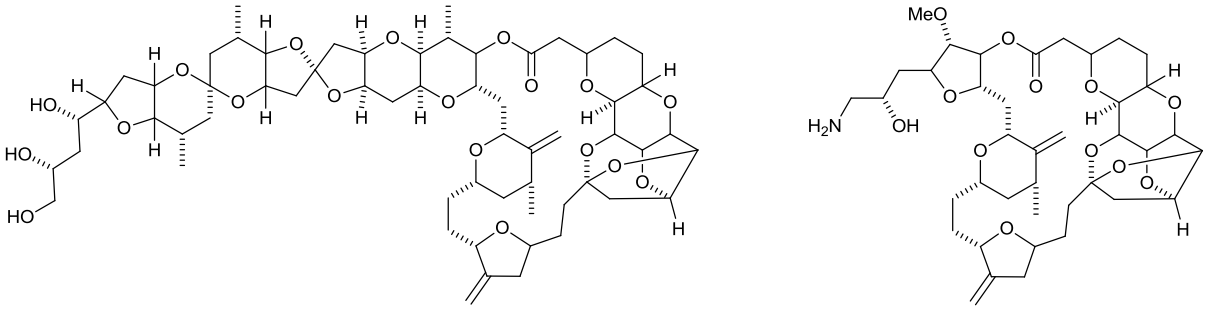


Figure 1.3 Structure of halichondrin B and synthetic analogue E7389.

This illustrates an important point about the activity of natural products. They usually have rigid scaffolds with defined three dimensional shapes, the purpose of which is to hold key atoms involved in the binding process in the correct orientation. If this arrangement is maintained in the synthetic analogues, structural complexity can be removed without fundamentally changing the activity of the compound. These simplifications can allow a practical, scalable synthesis to be developed much more quickly than would be possible for the parent compound.

The starting point for any natural product inspired synthetic study is the identification of a natural product of interest based on biological activity. Traditional medicines can be used as a source of leads and may give a higher probability of isolated compounds possessing activity and being non-toxic. However, this approach is only applicable for a small range of disorders that have traditional remedies. Random screening approaches are more common, and allow compounds to be tested against a wider range of diseases. Screening processes using almost pure extracts have been developed to help narrow the search for active compounds. Collections of natural products tend to be small and scattered around the world, due to the

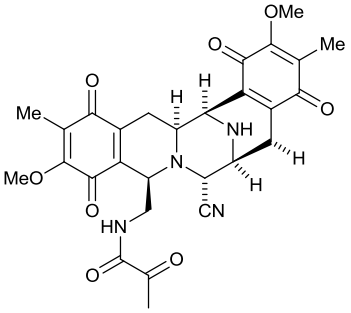
largely academic nature of this research. This means that the quantities of each natural product available for screening are small and that each compound may only be tested against one or two assays. One way of addressing this is to use natural product scaffolds such as alkaloids, terpenes, and peptides as a basis for creating a synthetic/semi-synthetic library. This brings together the chemical diversity of natural products and the synthetic feasibility to ensure that any leads identified can be optimised. As well as 'wet' bioassay analysis, it is also possible to identify hit compounds by *in silico* screening. New compounds may be compared to a series of tested, known compounds and allow the careful selection of which assays to replicate *in vitro* with the potentially very small amount of material available.

Some classes of natural products are known to have potent antibacterial bioactivity but insufficient work has been done to allow the development of drugs from these scaffolds. These include the tetrahydroisoquinoline antitumour antibiotics and the oxazolomycins, which form the focus of the work reported in this thesis.

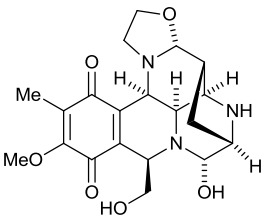
1.6 The tetrahydroisoquinoline antitumour antibiotics

The tetrahydroisoquinoline antitumour antibiotics (TAAs) are a class of alkaloids containing upwards of 55 members and are divided by Williams and Scott, in their comprehensive review of the TAAs,[19] into 3 groups: the saframycin, naphthyridinomycin, and quinocarcin families (Figure 1.4). The saframycin family (e.g. **1**) is the largest and includes the saframycins, safracins, renieramycins and ecteinbascidins. They are made up of five fused 6-membered rings, two of which are either quinones or hydroquinones. One of these aromatic rings is part of a tetrahydroisoquinoline unit attached to a piperazine ring. The naphthyridinomycins (e.g. **2**), cyanocyclines and bioxalomycins mostly possess six fused rings, four of which are 6-membered and two of which are 5-membered, including an oxazolidine motif. The aromatic part of these molecules is found in both the quinone and hydroquinone oxidation state. Quinocarcin (e.g. **3**), tetrazomine and lemonomycin all possess the tetrahydroisoquinoline framework, attached to a piperazine fused to a 5-membered ring. Quinocarcin and tetrazomine possess a phenol in place of the usual quinone/hydroquinone unit. All TAAs share a tetrahydroisoquinoline/piperazine tricyclic unit fused to either a

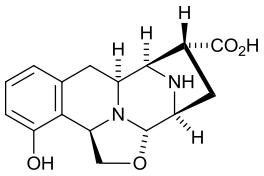
pyrrolidine or piperidine heterocycle. As there are so many members of this group of natural products, there are therefore many structural variations in the groups present around the core tetracyclic unit. Many of these alkaloids are potent, broad-spectrum antibiotics with strong antitumour activity exhibited across a wide range of cell lines and these properties, along with their structural complexity, have made them an attractive synthetic target for chemists.



Saframycin A **1**



Naphthyridinomycin **2**

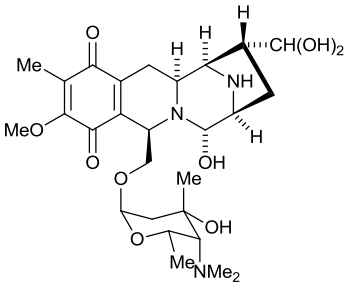


Quinocarcin **3**

Figure 1.4 Parent members of TAA families.

1.7 Lemonomycin

1.7.1 Structural elucidation



4

Figure 1.5 Lemonomycin **4.**

Lemonomycin **4** is a potent, broad-spectrum antibiotic which was first isolated in 1964 from a fermentation broth of the soil bacteria *Streptomyces candidus* (4 g of lemonomycin hydrochloride from a 2000 L culture broth).[20] Whaley *et al.* reported some physical characteristics of the compound though no molecular structure for the compound was proposed. It was described as a lemon yellow precipitate which decomposed without melting above 200 °C and had an optical rotation of $[\alpha]_D^{25}$ −116 (*c* 0.52 in $CHCl_3$). They also found that dimethylamine was a degradation product and identified that the molecule contained two basic sites. The full structural elucidation was only published in 2000 by He *et al.* after a

reinvestigation into the compound as part of an ongoing program into the discovery of new antibacterial agents.[21] A sample of the natural product (isolated by Whaley *et al.*) was purified by reverse-phase HPLC to give lemonomycin trifluoroacetate from which the molecular formula of the free base was established to be $C_{27}H_{41}N_3O_9$ by HMRS-ESI analysis.

The ^{1}H and ^{13}C NMR spectra for the trifluoroacetate salt were carefully studied in D_2O. A table of the chemical shifts and HMBC correlations as published by He *et al.* is included below (Table 1.1). The shift pattern indicated a quinone containing aglycon unit and an amino-sugar moiety. Correlations between carbons at 190.2 and 184.4 ppm and methyl protons at 1.96 and 3.91 indicated the methyl and methoxy groups in the quinone ring system. Careful study of the COSY, HMBC, and nOe (ROESY) spectra revealed the structure of the azabicyclic ring system. The signals C(1)H and C(3)H at 4.30 and 3.39 ppm were assigned due to the correlation of the C(1)*H* with *C*(8) and C(3)*H* with *C*(1) in the HMBC spectrum and an nOe enhancement places them in a *syn*-relationship on the fused ring system (Figure 1.6). COSY correlations linking C(11)*H* to C(15)*H*, C(14)H_2, C(13)*H* suggested a five membered ring fused to the piperazine and the C(16)*H* signal at 5.18 was attributed to the presence of an aldehyde hydrate. The structure of the amino-sugar was revealed by analysis of the ring proton coupling constants in addition to nOe and HMBC analysis. The broad doublet representing the anomeric proton C(1′)*H* had a small coupling constant, indicating an equatorial position. The remaining stereocentres in the ring were assigned by nOe enhancements observed between C(3′)*Me* and C(4′)*H* and C(5′)*H* along with small coupling constants between C(4)*H* and C(5)*H*. The 2,6-dideoxo-4-amino-pyranose unit is extremely rare in nature and this specific motif has only been observed in lemonomycin.

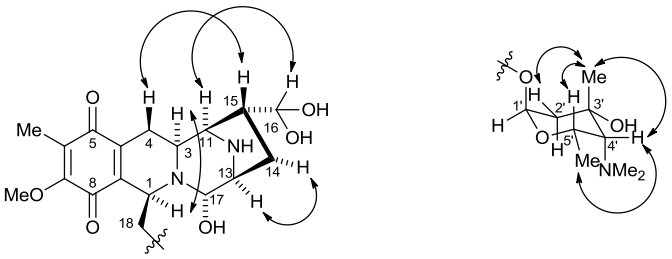


Figure 1.6 nOe enhancements of lemonomycin trifluoroacetate.

Atom number	^{1}H ppm (multiplicity, J Hz)	^{13}C ppm (substitution)	^{1}H-^{13}C HMBC correlation
1	4.30 (m)	54.4 (CH)	C-8,9,10,17
3	3.39 (br d, 9.0)	52.4 (CH)	C1,4,10,11,15
4	2.76 (br d, 17.5)	26.5 (CH_2)	C-5,9,10
	2.13 (m)		C-3,9,10,11
5	-	190.2 (C)	-
6	-	133.3 (C)	-
6-Me	1.96 (s)	11.0 (CH_3)	C-5,6,7
7	-	158.3 (C)	-
7-OMe	3.91 (s)	64.1 (CH_3)	C-7
8	-	184.6 (C)	-
9	-	140.7 (C)	-
10	-	144.5 (C)	-
11	4.03 (m)	62.9 (CH)	C-3,4,13,14,15,16
13	4.10 (br s)	63.4 (CH)	C-11,15,17
14	2.09 (m)	28.6 (CH_2)	C-11,15,16,17
	2.01 (m)		C-13,15,16,17
15	2.64 (ddd, 9.2, 4.5, 4.5)	43.3 (CH)	C-3,13,14,16
16	5.18 (d, 4.5)	92.9 (CH)	C-11,14,15
17	4.88 (d, 2.9)	81.4 (CH)	C-1,3
18	3.77 (br d, 10.0)	71.4 (CH_2)	C-9,1'
	3.64 (br d, 10.0)		C-1,9,1'
1'	5.08 (br d, 4.4)	99.9 (CH)	C-18,3',5'
2'	2.05 (m)	40.7 (CH_2)	C-1', 3', 4',3'-Me
	1.92 (m)	-	C-1', 3', 4',3'-Me
3'	-	69.7 (C)	-
3'-Me	1.33 (s)	31.4 (CH_3)	C-2',3',4'
4'	3.13 (br s)	72.7 (CH)	C-2',3',3'-Me,4'-NMe_2
4'-NMe_2	3.08 (s)	49.7 (CH_3)	C-4',4'-NMe
	3.07 (s)	44.4 (CH_3)	C-4',4'-NMe
5'	3.98 (br q, 7.2)	64.9 (CH)	C-1',4',6'
6'	1.46 (d,7.2)	20.0 (CH_3)	C-4',5'

Table 1.1 Chemical shift data and HMBC correlations of lemonomycin trifluoroacetate.

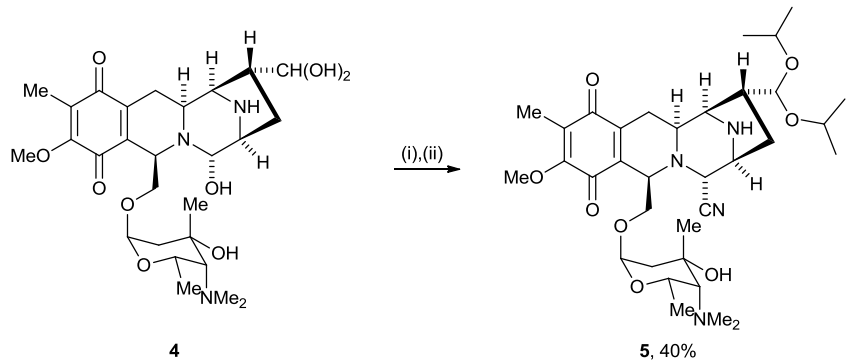
1.7.2 Biological activity

The activity of lemonomycin **4** against a range of Gram-negative and Gram-positive bacterial strains was reported by Whaley *et al.* who also compared its activity against a series of *Staphylococci* and *Streptococci* with that of the known antibiotics tetracycline, penicillin G and erythromycin. This data is summarised in Table 1.2.[20] The only tested bacterial strain that lemonomycin was not active against was *C. sporogenes*. In comparison to the known antibiotics, lemonomycin had similar or better activity in all cases, and was active against the strains of *S. aureus* and *Streptococcus* that were resistant to tetracycline and penicillin G. It was particularly potent against two species of *S. pyogenes* with MICs of less than 0.005 μg/mL. It was found that, when administered orally or subcutaneously, lemonomycin protected mice from staphylococcal and streptococcal infections, but was lethal at only slightly higher levels than the therapeutic dose.

Organism	MIC (μg/mL)			
	Lemono-mycin	**Tetra-cycline**	**Penicillin G**	**Erythro-mycin**
Mycobacterium smegmatis ATCC 607	6.2	-	-	-
Staphylococcus aureus ATCC 6548P	0.2	-	-	-
Streptococcus faecalis ATCC 8043	0.4	-	-	-
Bacillus subtilis ATCC 6633	0.05	-	-	-
Pseudomonas fluorescens ATCC 12633	1.6	-	-	-
Proteus vulgaris ATCC 9484	0.4	-	-	-
Escherichia coli ATCC9637	1.6	-	-	-
Salmonella gallinarum (Lederle 604)	0.8	-	-	-
Clostridium sporogenes ATCC7955	>100	-	-	-
Staphylococcus aureus (Lederle 4050B-122-7)	0.08	10	>100	1.5
S. aureus (Lederle 4050B-122-10)	0.15	>100	>100	3.0
S. aureus (Lederle 4050B-122-13)	0.15	2.5	0.08	3.0
S. aureus (Lederle 4050B-122-14)	0.15	>100	0.04	3.0
S. aureus Rose ATCC 14154	0.15	>100	>100	12.5
S. aureus Smith	0.04	2.5	0.8	1.5
Streptococcus faecalis ATCC 8043	0.4	2.5	2.5	0..4
S. pyogenes C203	<0.005	1.2	0.001	0.2
S. pyogenes (Lederle 8053B-40-2)	0.01	25	0.01	0.4
S. pyogenes (Lederle 8053B-40-3)	0.01	100	0.01	0.4
S. pyogenes NY5	<0.005	2.5	0.1	0.2
Streptococcus sp. λ-Strep. 11	5.0	>100	2.5	1.5
Streptococcus sp. β-Strep. 80	2.5	>100	2.5	1.5

Table 1.2 Biological activity of lemonomycin compared to known antibiotics.

Additional biological data was provided by He *et al.* and lemonomycin was also found to be active against methicillin resistant *Staphylococcus aureus* and vancomycin resistant *Enterococcus faecuem* with MIC values of 0.4 and 0.2 μg/mL respectively. Activity against human colon tumour cell line (HCT116) was also reported in lemonomycin **4** and the related semi-synthetic cyano-derivative **5** (Scheme 1.2) with IC_{50} values of 0.36 and 0.26 μg/mL.[21]



4 **5**, 40%

Scheme 1.2 *Reagents and conditions:* (i) *i*PrOH, 1% TFA, (ii) NaCN.

1.7.3 Biosynthesis

The biosynthesis of lemonomycin **4** itself has not been studied, but the origins of the structurally similar saframycin A **1** have been established. The tetrahydroisoquinoline

antitumour antibiotics derive from amino acid precursors; feeding studies carried out by Mikami *et al.* show that the central piperazine core of saframycin A derives from two molecules of tyrosine.[22] A culture of *Streptomyces lavendulae* 314 was fed with l-[U-^{14}C]-pyruvic acid, [2-^{14}C]-glycine, l-[U-^{14}C]-alanine, l-[*methyl*-^{14}C]-methionine, and l-[U-^{14}C]-tyrosine and the incorporation rate of these compounds was measured using radioactivity measurements. All except pyruvic acid were shown to be incorporated into the saframycin produced by the culture. The two *O*-Me, two *C*-Me and single *N*-Me substituents were all shown to derive from methionine and the C(1) side chain was shown to derive from alanine and glycine. These results were supported by ^{13}C labelled feeding studies using tyrosine, glycine and methionine, measuring the enrichment of the ^{13}C signals at the point in which the label in incorporated. A subsequent investigation established that the alanine and glycine residues were incorporated as the dipeptide unit, formed prior to its inclusion in the natural product (Figure 1.7).[23]

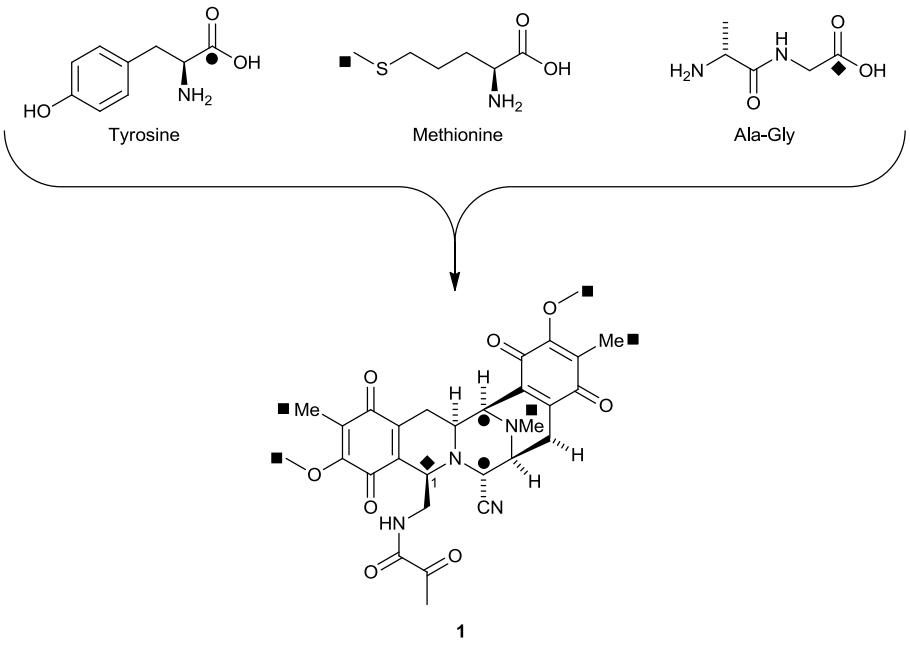


Figure 1.7 Biosynthesis of Saframycin A **1**.

Feeding studies have also been carried out on naphthyridinomycin **2**, which has a diazabicyclooctane core analogous to lemonomycin. After feeding *Streptomyces lusitanus* NRRL 8034 with labelled amino acid precursors, Zmijewsky *et al.* found that the piperazine core of this natural product was formed from one molecule of tyrosine and one of ornithine (Figure 1.8).[24] As in saframycin, the methyl substituents originate from methionine. It is

plausible that the lemonomycin **4** pyrrolidine ring may be derived from ornithine, as it is in naphthyridinomycin **2**, though it has also been postulated that it may originate from glutamic acid.[21]

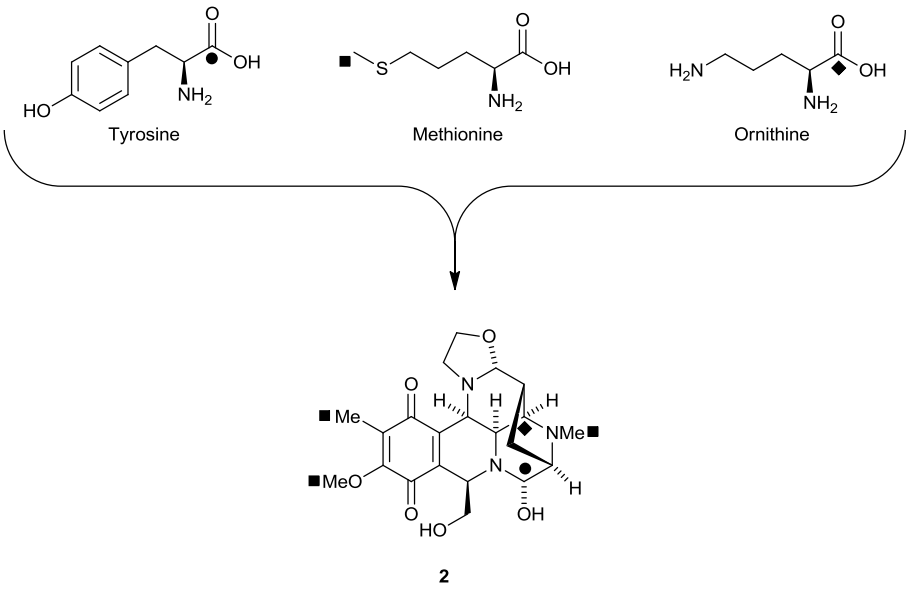


Figure 1.8 Biosynthesis of naphthyridinomycin **2**.

1.7.4 Biological mode of action

The biological modes of action of the TAA family have been studied extensively, though no studies relate specifically to lemonomycin **4**. The reported mode of action for all the TAAs which have been studied involves their interaction with DNA. The natural product saframycin A **1** has been reported to inhibit RNA synthesis at low concentrations and DNA synthesis at higher concentrations.[25] It was also noted that, when the natural product was reduced to the hydroquinone prior to testing, these effects were observable at lower concentrations. It was reported by Lown *et al.* that under mildly acidic conditions, the conjugate acid of saframycin can bind non-covalently to DNA.[26] A second, much slower mode of binding was also reported, in which the displacement of the cyano group by the guanine primary amino group in DNA was postulated, occurring *via* an intermediate iminium ion resulting from the loss of the cyano group. This mechanism of inhibition was studied by exposing ^{14}C labelled saframycin to DNA in the presence of the reducing agent dithiothreitol (DTT).[27] After the treatment of DNA with saframycin biosynthetically derived from ^{14}C labelled tyrosine, the DNA retained the ^{14}C label. A similar experiment in which the

saframycin was labelled with ^{14}C on the cyano group found that the label was not incorporated into the DNA. Based on the fact that DNA alkylation is increased if saframycin is reduced to the hydroquinone form, Hill and Remers proposed the following mechanism (Figure 1.9). Saframycin A **1** is reduced *in situ* to the hydroquinone form **6** which can facilitate the formation of the intermediate iminium ion. The phenol can donate electrons in order to push the cyano leaving group out, a process which leads to scission of the B-ring, giving the species **7**. The nitrogen then re-attacks the double bond formed from the ring scission to restore the phenol and give the iminium species **8**. This ion can then by attacked by the primary amine of a guanine residue to give adducts **9**. This mechanism explains the increased activity of reduced saframycins as the N lone pair could spontaneously expel the cyano group forming the iminium ion in one step, but the phenol increases the likelihood of ion formation. Similar mechanisms of action have also been proposed for the naphthyridinomycins[28] and ecteinascidins[29] which have a hydroxyl group in place of the cyano group of saframycin.

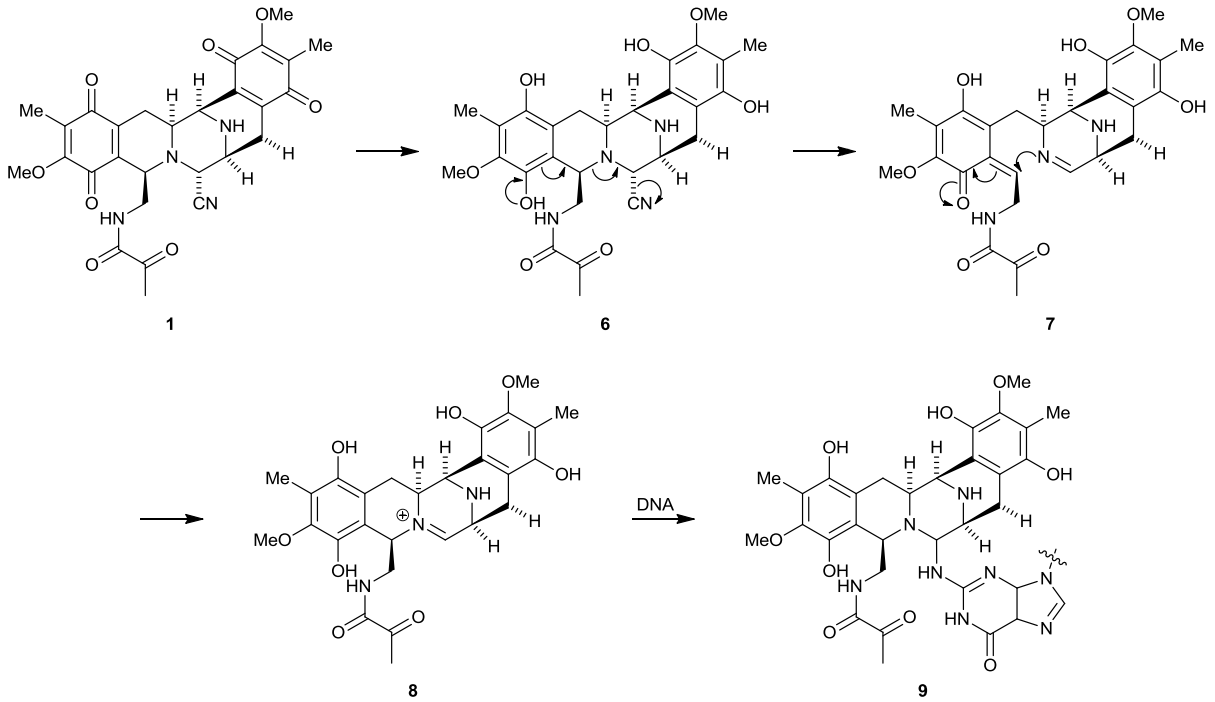


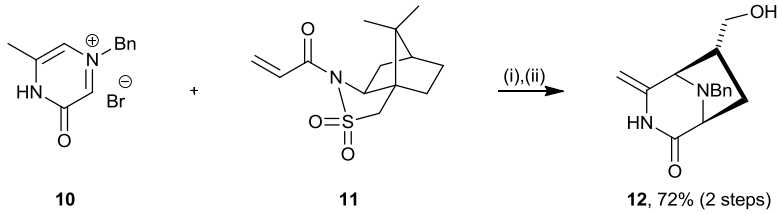
Figure 1.9 Proposed mechanism for DNA alkylation by saframycin A **1**.

It has also been demonstrated that the saframycins can cause cleavage of DNA strands under aerobic conditions.[26] This is consistent with the ability of quinones accept electrons and reduce molecular oxygen to superoxide, and mechanistic studies have shown that superoxide

and hydroxyl radicals can be formed in the presence of saframycin A hydroquinone **6**. This is suggested to be the major mode of action for the oxazolidine containing quinocarcins[30] and tetrazomines.[31]

1.8 Stoltz's total synthesis of lemonomycin

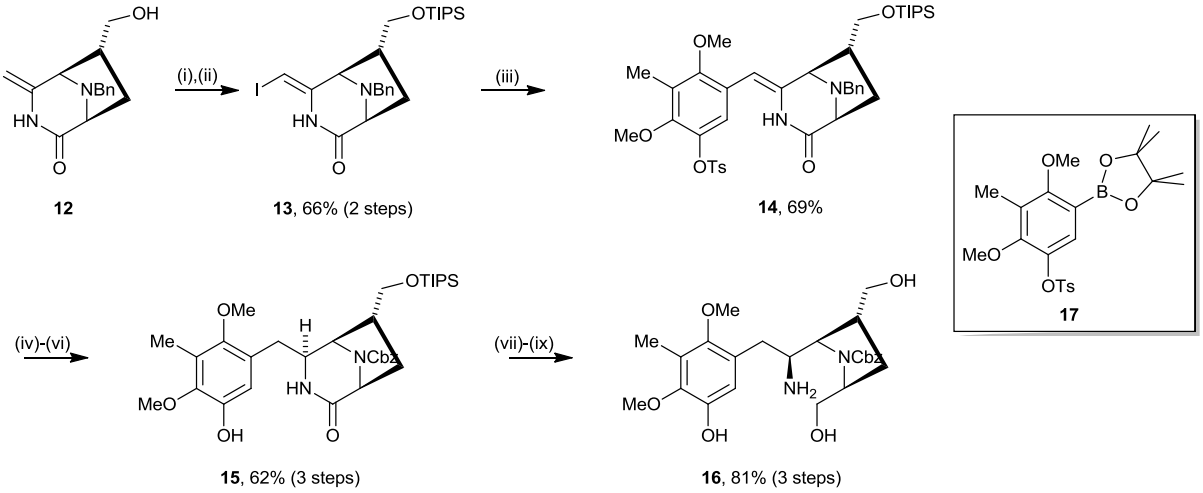
Despite being isolated in 1964, due to the long delay in its structural elucidation, lemonomycin is one of the newest members of the TAAs. To date, one total synthesis of lemonomycin has been published, by Stoltz *et al*. in 2003.[32] The synthesis utilised a stereoselective asymmetric Joule cycloaddition to give the fused piperazine/pyrrolidine unit and a Pictet-Spengler reaction to introduce the amino-sugar moiety and furnish the tetrahydroisoquinoline unit simultaneously, thus avoiding a late stage glycosylation reaction. The diazabicyclo[3.2.1]octane unit was established using a chiral auxiliary controlled dipolar cycloaddition of the type first described by Joule *et al.*.[33] The dipole was generated *in situ* by the deprotonation of bromide salt **10** with *N*-methylmorpholine and reacted with the dipolarophile, in this case the Oppolzer-sultam derived acrylamide **11**, to give a bicyclic compound which was treated with $NaBH_4$ to remove the auxiliary amide and furnish **12** in 72% yield (Scheme 1.3).



Scheme 1.3 *Reagents and conditions:* (i) NMM, MeCN, −20 °C, 72 h, (82%), (ii) $NaBH_4$, EtOH, 6 h (81%).

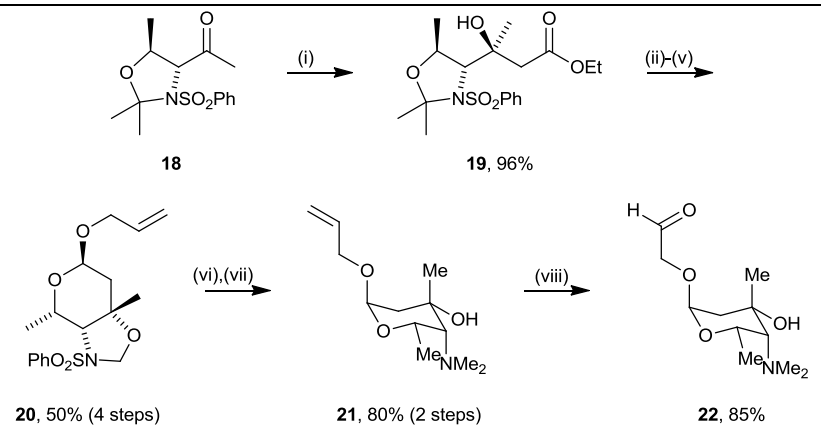
The diazabicycle **12** was *O*-protected with TIPSOTf and iodinated with ICl to give the vinyl iodide **13**. A Suzuki coupling reaction was used to join the vinyl iodide **13** with the boronic ester **17**, producing the aryl-enamide **14** in 69% yield. The trisubstituted double bond was then hydrogenated with concomitant cleavage of the *N*-Bn group. The resulting amine was then *N*-Cbz protected and the aromatic sulphonate ester group was cleaved to give compound **15** in 62% yield. After this species was found to be unreactive to Pictet-Spengler reactions, it was converted into the more reactive aminotriol **16**. This was achieved by activation of the

amide with Boc_2O, reduction with $NaBH_4$ and cleavage of the Boc groups and TIPS ether with methanolic HCl (Scheme 1.4).



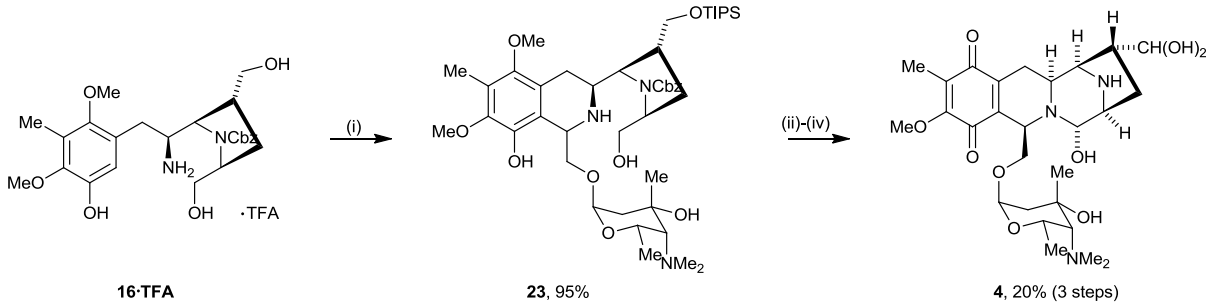
Scheme 1.4 *Reagents and conditions:* (i) TIPSOTf, DCM, 2,6-lutidine, (82%), (ii) ICl, DCM, 0 °C, (81%), (iii) **17**, $Pd(PPh_3)_4$, K_2CO_3, C_6D_6, MeOH, 70 °C, 4 h, (69%), (iv) Pd/C, H_2 (1000 psi), TFA, EtOH, 28 h, (72%) (v) CbzCl, DMAP, MeCN, (vi) KOTMS, MeCN, (87%, 2 steps), (vii) Boc_2O, DMAP, MeCN, (viii) $NaBH_4$, EtOH, (ix) HCl, MeOH (81%, 3 steps).

The synthesis of the aminopyranose unit began with compound **18** derived from D-threonine.[34,35] The Felkin-controlled addition of the lithium enolate of ethyl acetate provided the single diastereoisomer **19** in 96% yield. Subsequent acid-catalysed acetonide cleavage, lactonisation, conversion of the free OH and NH to the oxazolidine, diastereoselective reduction to the corresponding lactol, and conversion to the allyl alcohol gave the bicyclic compound **20** in 50% yield over four steps. Reductive cleavage of the oxazolidine and removal of the benzenesulphonyl group with RED-Al provided a secondary amine which was methylated by a reductive amination to give **21** in 80% yield. Oxidative cleavage of the allyl group using OsO_4 and $NaIO_4$ gave the α-glycosyloxy aldehyde **22** in 85% yield (Scheme 1.5).



Scheme 1.5 *Reagents and conditions:* (i) EtOAc, LDA, THF −78 °C, 2.5 h, (96%), (ii) HCl, H_2O, THF, 13 h, (86%), (iii) $(MeO)_2CH_2$, TMSOTf, (77%), (iv) DIBAL, THF, −78 °C, (96%), (v) MsOH, allyl alcohol, DCM, 13 h, (79%), (vi) RED-Al, toluene, 120 °C, 3 h, (86%), (vii) CH_2O (aq.), $NaBH_3CN$, (94%), (viii) OsO_4, $NaIO_4$, TFA, THF, H_2O, 0 °C, 16 h, (85%).

The conjugation of the α-glycosyloxy aldehyde **22** and aminotriol **16** was achieved using a novel Pictet-Spengler reaction. A mixture of **22** and the TFA salt of **16** was stirred in EtOH to furnish **23** in 95% yield. The tetrahydroisoquinoline **23** was converted to **4** in 20% yield by cleavage of the Cbz group, bis-Swern oxidation of the two primary alcohols and treatment with CAN to produce the quinone. The synthetic sample of **4**, prepared in a 3.1% yield over 15 linear steps, was identical to the sample isolated from natural sources (Scheme 1.6).



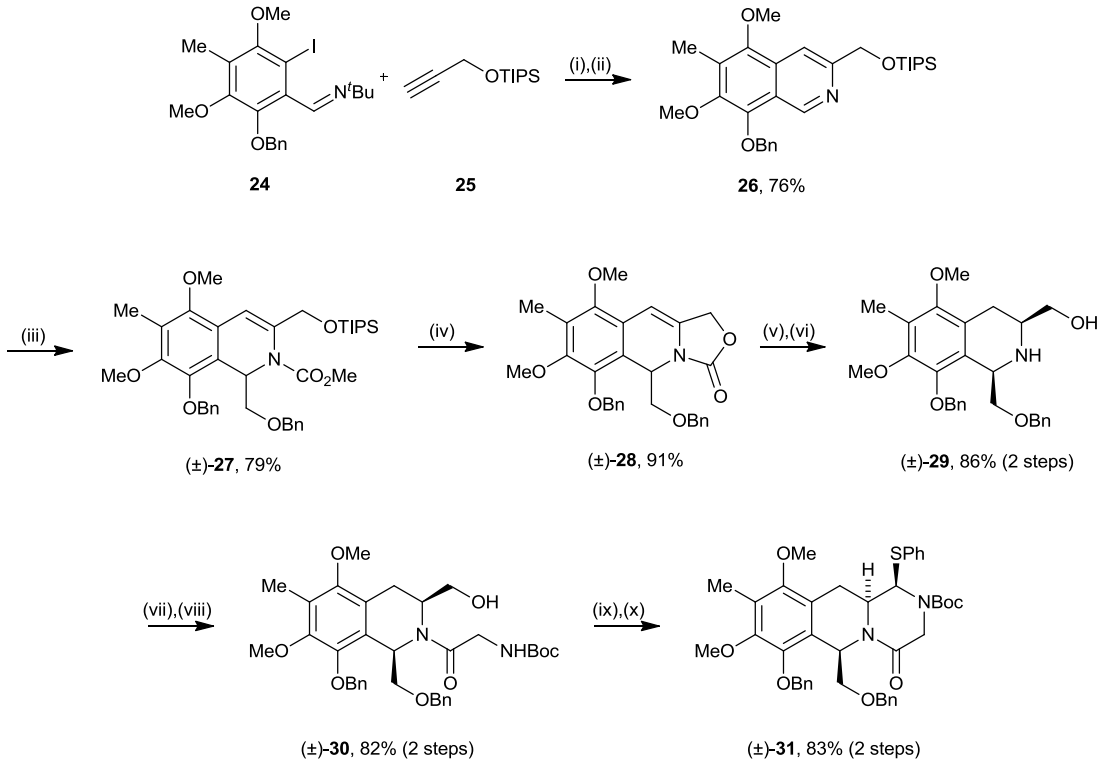
Scheme 1.6 *Reagents and conditions:* (i) **22**, EtOH, 23 °C, 63 h, (95%), (ii) H_2, Pd/C, EtOH, (74%), (iii) $(COCl)_2$, DMSO, TEA, −78 to 0 °C, then HCl (aq.), (52%), (iv) CAN (aq.), 0 °C (51%).

1.9 Synthetic studies of lemonomycin

The work by Stoltz was the first published work into the synthesis of lemonomycin, but since then four further research groups have made contributions to the literature in this area. Between 2005 and 2008, the groups of Magnus,[36,37] Fukuyama,[38] Zhu,[39,40] and Mulzer[41] have all published synthetic routes towards advanced intermediates of the lemonomycin aglycon unit. One synthesis of the glycosyl unit, lemonose, has also been published by Masson and Zhu in 2011.[42]

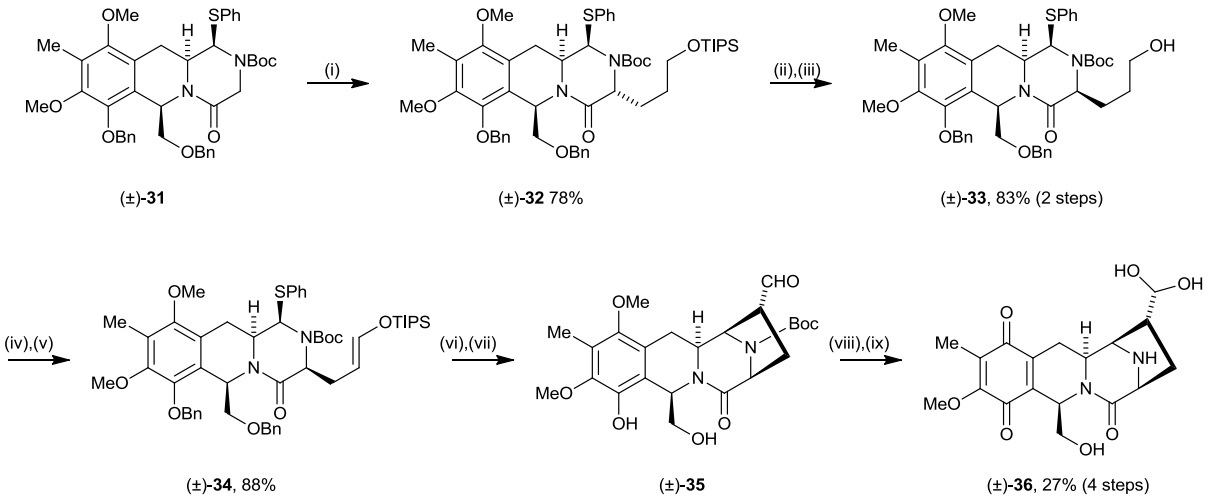
1.9.1 Magnus and Matthews' synthesis of (±)-lemonomycinone amide

The first synthesis of the lemonomycinone amide aglycon unit, in racemic form, was published by Magnus and Matthews in 2005.[36,37] Their synthesis proceeded *via* the intermediate (±)-**31**, which was also converted into the related natural product (±)-renieramycin. They began by synthesising the isoquinoline **26** in 76% yield from the iodoimine **24** and acetylene **25** under Castro[43] conditions using a modified Larock isoquinoline synthesis.[44] The species **26** was then treated with benzyloxymethyl lithium and quenched with methyl chloroformate to give (±)-**27** in 79% yield. The TIPS group was cleaved using TBAF and the resulting oxazolidinone (±)-**28**, produced in 91% yield, underwent ionic hydrogenation to reduce the olefin and then hydrazinolysis of the imine gave the 1,3-*cis*-disubstituted tetrahydroisoquinoline (±)-**29** in 86% yield. Silyl-activated amide coupling conditions were used to acylate the amine group with a mixed anhydride, leading to compound (±)-**30** in 82% yield. Swern oxidation gave the hemiaminal, which was converted to thioaminal (±)-**31** upon treatment with PhSH and TsOH (Scheme 1.7).



Scheme 1.7 *Reagents and conditions:* (i) CuI (1.2 eq.), TEA, 25 °C, 24 h, (ii) CuI (0.2 eq.), DMF, 100 °C, 1 h, (76%, 2 steps), (iii) $BnOCH_2Li$, THF, −78 °C, 2 h, then $ClCO_2Me$, (79%), (iv) TBAF, THF, 0 °C, 30 min, (91%), (v) TFA, Et_3SiH, 0 °C, 2 h, (vi) H_2NNH_2, KOH, $HOCH_2CH_2OH$, 150 °C, 3 h, (86%, 2 steps), (vii) TMSCl, TEA, THF, 0 °C, 2 h, (viii) *t*$BuOCOCCH_2NHBoc$, (82%, 2 steps), (ix) Swern, (88%), (x) HSPh, TsOH, DCM, −5-5 °C, 1.5 h, (95%).

Intermediate (±)-**31** was alkylated with the requisite alkyl iodide to form (±)-**32** as a single diastereoisomer in 78% yield. This compound possessed the undesired relative stereochemistry at the newly created chiral centre and was therefore epimerised by deprotonation with BuLi followed by diastereoselective reprotonation with BHT to give (±)-**33** in 83% yield after desilylation. Swern oxidation of the free alcohol followed by formation of the silyl enol ether gave the cyclisation precursor (±)-**34** in 88% yield. Formation of the final heterocycle was effected using the thiophile $AgBF_4$, giving the aldehyde (±)-**35**. Hydrogenolysis of the *O*-Bn groups gave the corresponding diol in 79% yield over two steps, and treatment with HCl to remove the Boc group formed the aldehyde hydrate, and finally the hydroquinone ring was oxidised to the quinone using CAN to furnish (±)-**36** in 35% yield (Scheme 1.8).

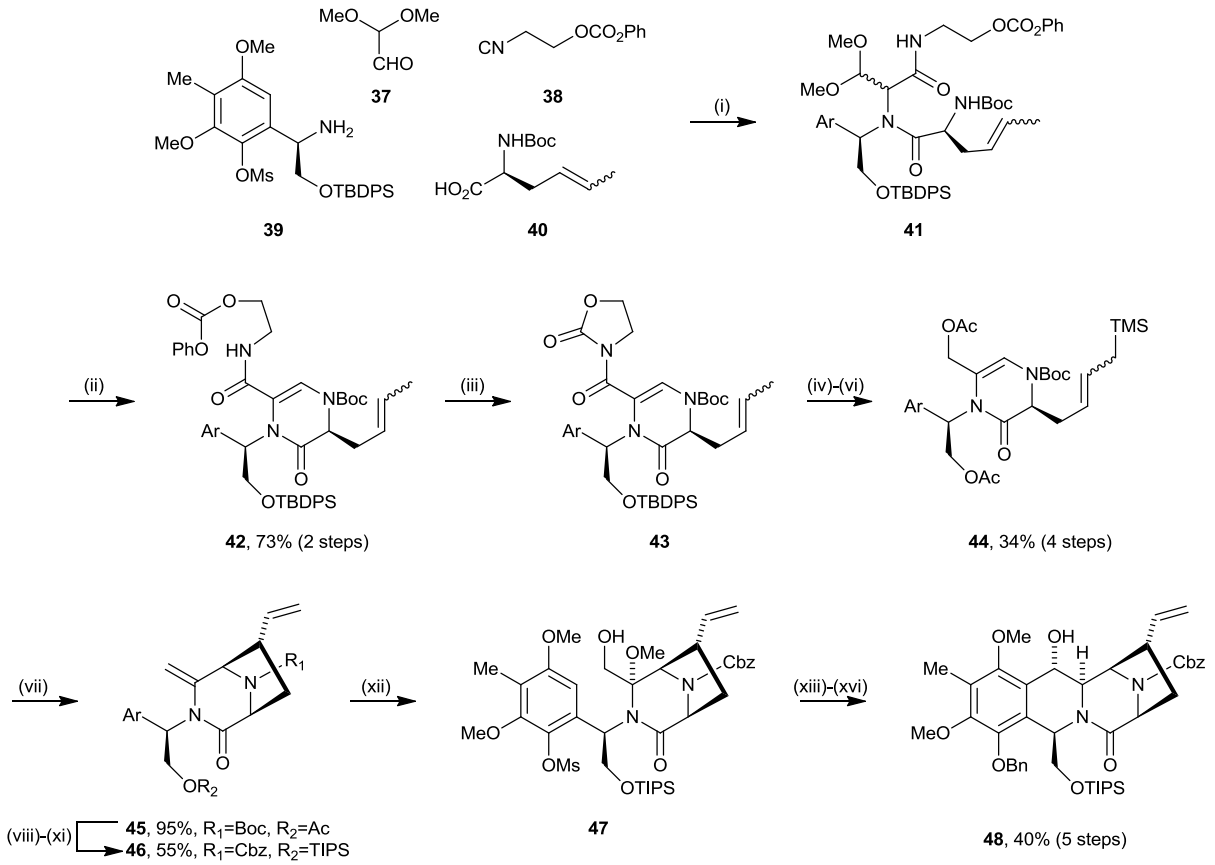


Scheme 1.8 *Reagents and conditions:* (i) KHMDS, THF, −78 °C, then RI, 30 min, (79%), (ii) BuLi (2.2 eq.), THF, −78 °C, 10 sec, then BHT in THF, (iii) 48% aq. HF, MeCN, 0 °C, (iv) Swern, (v) TIPSOTf, TEA, Et_2O, 25 °C, 25 °C, 16 h, (vi) $AgBF_4$, THF, 4 h, 25 °C, (vii) $Pd(OH)_2$, MeOH, 1 atm H_2, 25 °C, 12 h, (viii) 3 M aq. HCl, 1:1 MeOH:H_2O, 25 °C, 3 h, (ix) CAN, H_2O, 25 °C, 3 h.

1.9.2 Fukuyama's synthesis of the 3,8-diazabicyclo[3.2.1] skeleton

The synthesis of advanced intermediate **48** for lemonomycin was reported by Fukuyama *et al.* in 2005.[38] This was accomplished using an Ugi 4-CC reaction with the novel isocyanide **38**, a cross metathesis and an intramolecular Hosomi-Sakurai type reaction. The combination of the amino acid derivatives **39** and **40** with isocyanide **38** and **37** *via* a Ugi 4-CC reaction produced compound **41**. Treatment of **41** with CSA and quinoline resulted in the formation of cyclic enamide **42** in 73% yield over the two steps. The oxazolidinone **43** was formed by

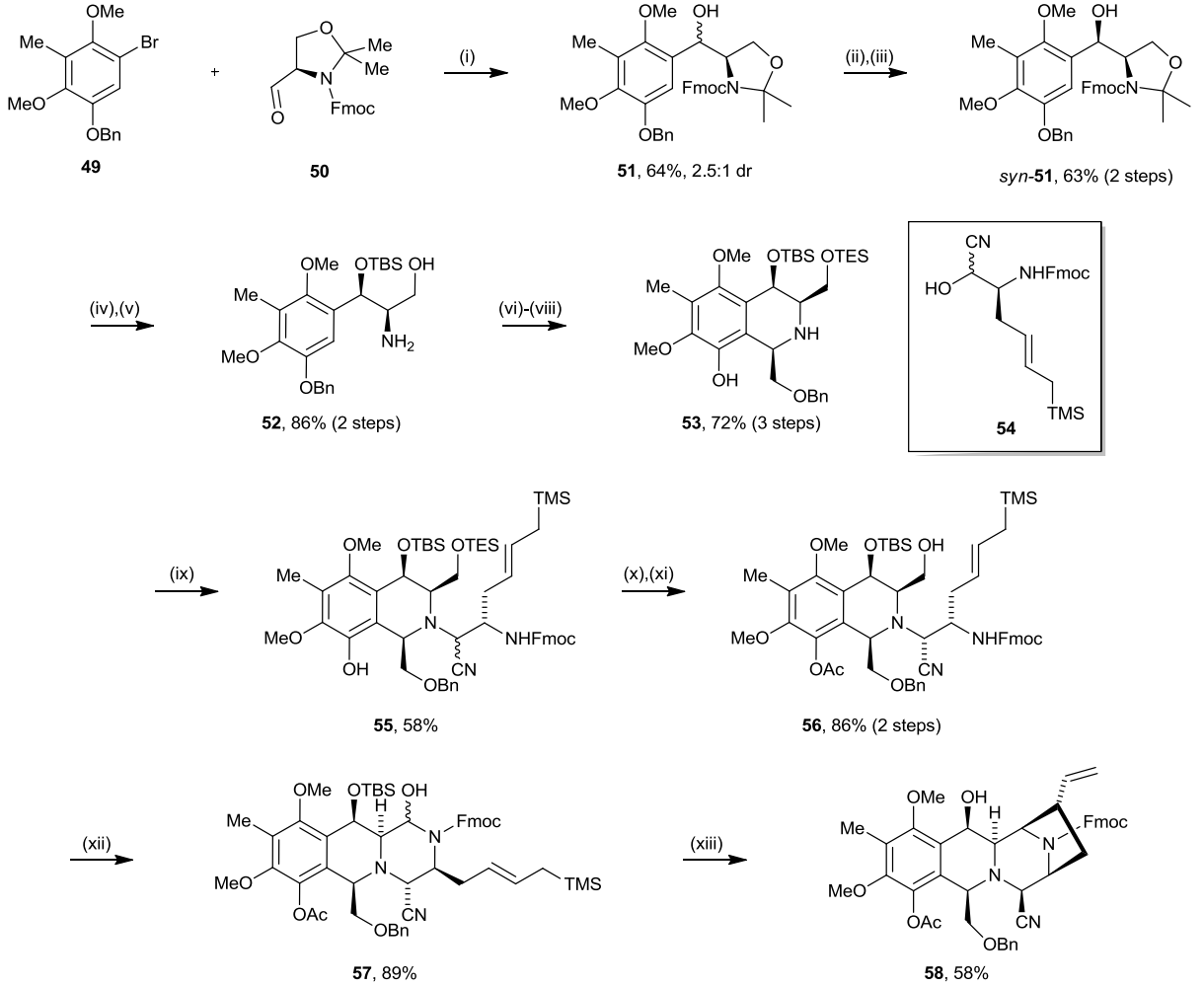
treating **42** with *t*BuOK. Reduction with $NaBH_4$ removed the heterocycle to leave a primary alcohol moiety. Removal of the TBDPS group from the remaining alcohol group and protection of both with Ac_2O was followed by cross metathesis with allyltrimethylsilane, giving **44** in 34% yield. Treatment with $BF_3.Et_2O$ promoted formation of the acylium cation and cyclisation of the allylsilane resulted in the formation of **45** as a single diastereoisomer in 95% yield. Protecting group manipulations gave the *N*-Cbz, *O*-TIPS **46** in 55% yield. DMDO oxidation of **46** provided **47**. Acylium ion mediated reduction removed the *O*-Me group and the *O*-Ms group on the hydroquinone was replaced with *O*-Bn to increase the electron donating ability of the aromatic ring. Finally the alcohol was oxidised with Dess-Martin periodinane and the resulting aldehyde was treated with TFA to effect the final cyclisation to give tetracyclic compound **48** (Scheme 1.9).



Scheme 1.9 *Reagents and conditions:* (i) 2,2,2-trifluoroethanol, rt to 50 °C, (ii) CSA, quinoline, PhMe, reflux, (73%, 2 steps), (iii) *t*BuOK, 4 Å mol. sieves, THF, 0 °C, (iv) $NaBH_4$, 3:1 THF:H_2O, (v) TBAF, THF, 50 °C then Ac_2O, Py, (68%, 3 steps), (vi) Grubbs II (2 mol%), allyltrimethylsilane, DCM, reflux (51%), (vii) $BF_3.Et_2O$, DCM, −78 °C, (95%), (viii) TMSOTf, DCM, 0 °C, (ix) CbzCl, DMAP, MeCN, (x) K_2CO_3, MeOH, (xi) TIPSOTf, 2,6-lutidine, DCM, 0 °C, (55%, 4 steps), (xii) dimethyldioxirane, Na_2SO_4, MeOH, −78 °C to rt then CSA, (xiii) $NaBH_3CN$, 9:1 TFA:THF, 0 °C, (xiv) $KOSiMe_3$, MeCN, 0 °C, BnBr, 50 °C, (53%, 3 steps), (xv) Dess-Martin periodinane, DCM, (xvi) 1:9 TFA:DCM, (76%, 2 steps).

1.9.3 Mulzer's synthesis of the tetracyclic core

Mulzer's approach to the tetracyclic core of lemonomycin utilises a Pictet-Spengler cyclisation to furnish the tetrahydroisoquinoline moiety, followed by a Strecker-type amino alkylation and an *N*-acylium cyclisation to furnish the remaining heterocycles. The aryl bromide **49** was converted, *via* the organolithium derivative, to the compound **51** in 64% yield mostly as the *syn*-isomer. The alcohol was subsequently oxidised and reduced in order to obtain exclusively the *syn*-isomer in 63% yield. The alcohol was then protected using TBSCl and the *N*-Fmoc and acetonide protecting groups were removed simultaneously in the presence of piperidine, giving amino-alcohol **52** in 86% yield. The primary hydroxyl group was TES protected and hydrogenolysis removed the benzyl group to give a phenol which underwent a Pictet-Spengler reaction with benzyloxyacetaldehyde to give the 1,3-*cis*-tetrahydroisoquinoline **53** as a single diastereoisomer in 72% yield over the three steps. Addition of the cyanohydrin **54** in trifluoroethanol gave **55** in 58% yield. Protection of the free phenol with Ac_2O and deprotection of the TES group from the primary alcohol gave **56** in 86% yield. Oxidation of the alcohol to the aldehyde using Dess-Martin periodinane resulted in *in situ* attack of the NH-Fmoc moiety to furnish the piperazine ring in compound **57** in 89% yield. Treatment with TFA promoted the formation of an acylium ion which is attacked by the silyl enol ether to form the final heterocyclic ring and give the tetracyclic lemonomycin intermediate **58** in 58% yield (Scheme 1.10).

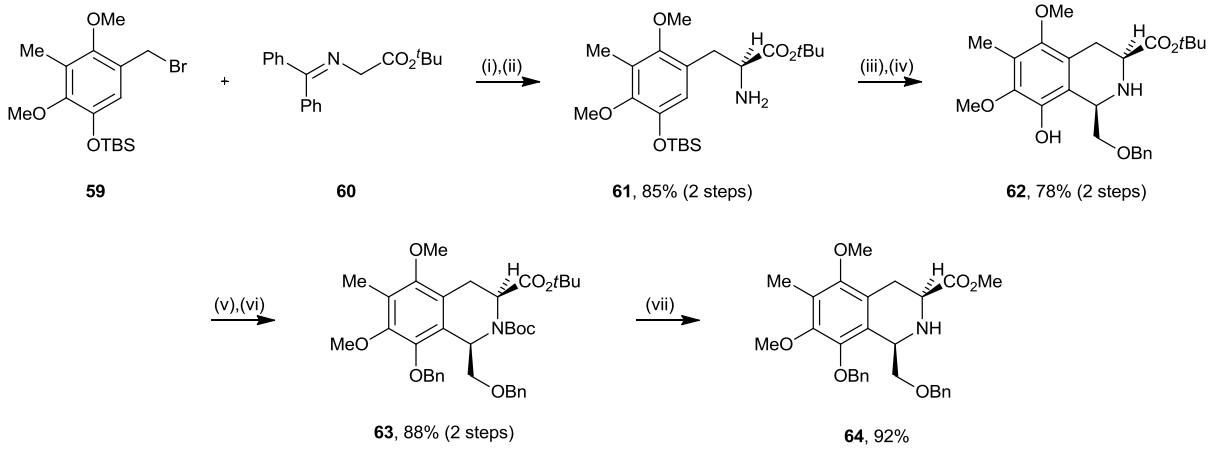


Scheme 1.10 *Reagents and conditions:* (i) *t*BuLi, then **50**, THF, −78 °C, (64%), (ii) PCC, DCM, rt, (75%), (iii) DIBAL-H, THF, 0 °C, (85%), (iv) TBSCl, imidizole, DMF, rt, (v) piperidine, THF, rt, (86%, 2 steps), (vi) TESCl, py, DCM, rt, (vii) Pd/C, H_2, MeOH, rt, (90%, 2 steps), (viii) benzyloxyacetaldehyde, AcOH, 4 Å mol. sieves, DCM, (81%), (ix) 2,2,2-trifluoroethanol, **54**, rt, (58%), (x) Ac_2O, py, DCM, rt, (98%), (xi) HF/py, py, THF, rt, (88%), (xii) Dess-Martin periodinane, DCM, rt, (89%), (xiii) TFA, THF, 0 °C, (58%).

1.9.4 Zhu's synthesis of (−)-lemonomycinone amide

Following on from an earlier publication on the synthesis of the tetracyclic core framework of lemonomycin,[39] Zhu *et al.* have since completed the synthesis of enantiopure (−)-lemonomycinone amide, an oxidised form of the lemonomycin aglycon unit.[40] This approach also uses a Pictet-Spengler reaction to furnish the tetrahydroisoquinoline unit, but unlike Stoltz's approach, this is carried out relatively early in the synthetic sequence. The tetrahydroisoquinoline **64** is made from the substituted benzyl bromide **59**, which is synthesised from 2,6-dimethoxytoluene in 7 steps and 61% overall yield. Enantioselective alkylation of **59** with **60** in the presence of Corey-Lygo's phase transfer catalyst, followed by hydrolysis of the imine functionality, gave **61** in 85% yield. This was followed by the Pictet-

Spengler cyclisation which used benzyloxyacetaldehyde added slowly to the aminophenol obtained from desilylation of the phenyl hydroxyl group of **61**, and gave the 1,3-*cis*-tetrahydroisoquinoline **62** in 78% yield over the two steps. The free amine was then protected with a Boc moiety before the phenol was benzylated to give **63** in 88% yield, which was transesterified with concomitant *N*-Boc cleavage to give **64** in 92% yield (Scheme 1.11).

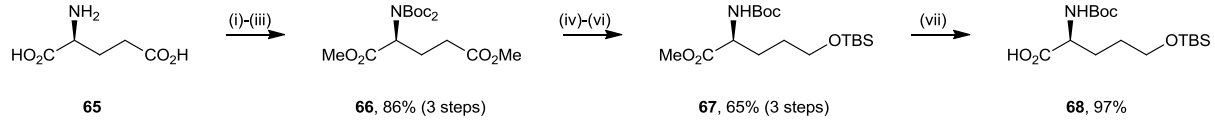


59 **60** **61**, 85% (2 steps) **62**, 78% (2 steps)

63, 88% (2 steps) **64**, 92%

Scheme 1.11 *Reagents and conditions:* (i) $CsOH.H_2O$, Corey-Lygo's phase transfer catalyst, DCM, −78 °C, 24 h, (ii) 1:1:1 THF:AcOH:H_2O, rt, 5 h, (85%, 2 steps), (iii) TBAF, THF, 0 °C, 4 h, (92%), (iv) $BnOCH_2CHO$, AcOH, DCM, 4Å mol. sieves, rt, 36 h, (85%), (v) Boc_2O, MeCN, DIPEA, rt, (90%), (vi) BnBr, Cs_2CO_3, DMF, NaI, rt, 6 h, (98%), (vii) $SOCl_2$, MeOH, reflux, 4 h, (92%).

The piperazine ring was then constructed by acylation on the tetrahydroisoquinoline nitrogen using the carboxylic acid **68**. This was prepared from L-glutamic acid **65** by first synthesising the di-Boc protected dimethyl ester **66** in 86% yield. Controlled reduction of the least hindered ester using DIBAL then $NaBH_4$ followed by silylation of the resulting alcohol and mono-deprotection of the amine gave **67** in 65% yield. LiOH hydrolysis of the remaining methyl ester gave the carboxylic acid **68** in 97% yield (Scheme 1.12).

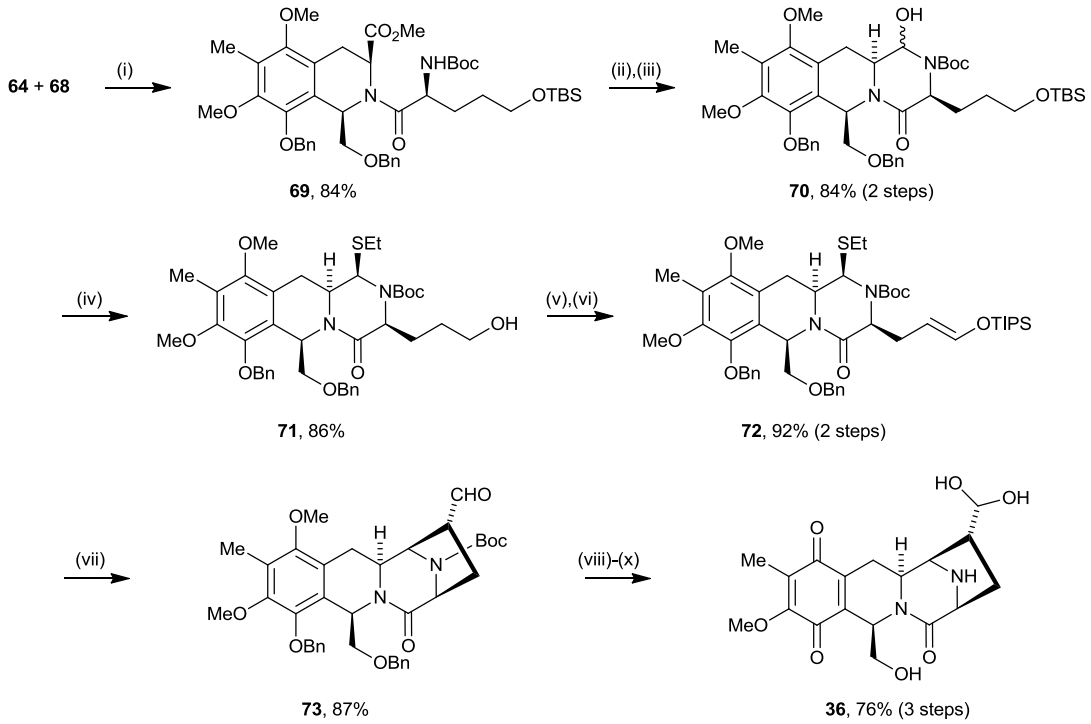


65 **66**, 86% (3 steps) **67**, 65% (3 steps) **68**, 97%

Scheme 1.12 *Reagents and conditions:* (i) TMSCl, MeOH, rt, 6 h, (ii) Boc_2O, TEA, MeOH, 48 h, (iii) Boc_2O, DMAP, NeCN, rt, overnight, (91%), (iv) DIBAL-H, Et_2O, 30 min, then $NaBH_4$, MeOH, rt, 1 h, (84%) (v) $CeCl_3.7H_2O$, NaI, MeCN, 45 °C, 1 h, (vi) TBSCl, DMF, imidizole, rt, overnight, (vii) LiOH, 3:1 THF:H_2O, 0 °C, 6 h (97%).

The carboxylic acid **68** was then used to acylate the tetrahydroisoquinoline **64**, giving the species **69** in 84% yield. The ester group was then reduced to the alcohol, and oxidised to the aldehyde which gave the hemiaminal **70** in 84% yield. Treatment of **70** with EtSH and $Hf(OTf)_4$ gave the amino thioether **71**, with concurrent desilylation, in 86% yield as a single

diastereoisomer. The free alcohol was then oxidised and the TIPS silyl enol ether **72** was formed in 92% yield. The intramolecular Mannich reaction of **72** with $AgBF_4$ furnished the final heterocycle of the lemonomycin framework and gave **73** in 87% yield. Hydrogenolysis removed the two *O*-Bn functionalities and following the protocols described by Magnus and Matthews,[36] HCl and CAN were used to convert the resulting diol to lemonomycinone amide **36** in 76% yield (0).

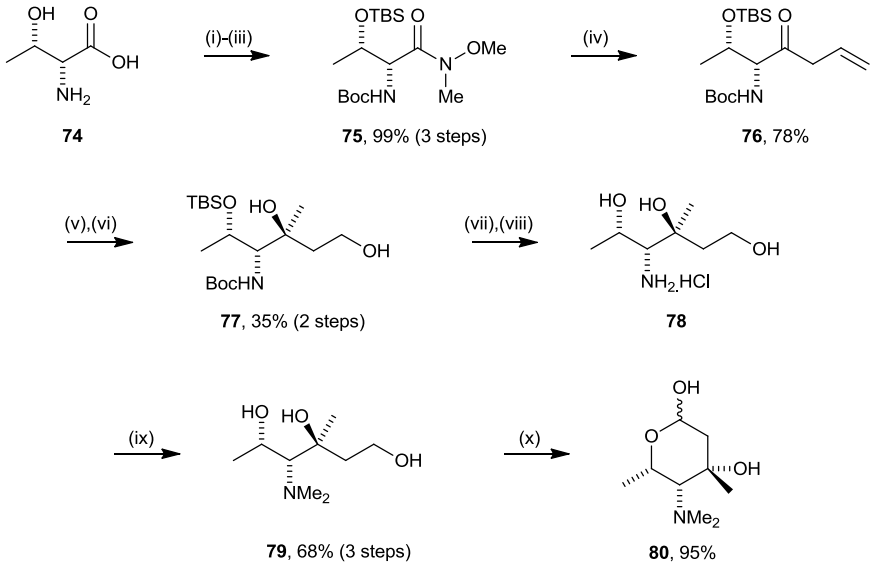


Scheme 1.13 *Reagents and conditions:* (i) HATU, HOAt, DIPEA, DMF, 0 °C, 8 h, then rt, 36 h, (84%), (ii) $LiBH_4$, MeOH, THF, rt, 8 h, (93%) (iii) Swern, (91%), (iv) $Hf(OTf)_4$ (0.1 eq.), EtSH, DCM, rt, 4 h, (86%) (v) Swern, (94%), (vi) TIPSOTf, TEA, Et_2O, rt, 12 h, (98%), (vii) $AgBF_4$, THF, rt, 8 h, (87%), (viii) $Pd(OH)_2$, MeOH, 0 °C, 6 h, (94%), (ix) 3 M aq. HCl, rt, 1:1 MeOH:H_2O, 3 h, (x) $(NH_4)_2Ce(NO_3)_6$, H_2O, rt, 3 h.

1.9.5 Lemonose sugar

Further to the synthesis of the aglycon unit of lemonomycin, Zhu, in collaboration with Masson, has more recently published a synthesis of the unique glycosyl unit, lemonose **80**.[42] Other than in Stoltz's total synthesis of lemonomycin, this is the only synthetic study of the pyranose system. The synthesis was carried out in ten steps from D-threonine with an 18% overall yield. The doubly protected Weinreb amide **75** was synthesised in 99% yield from D-threonine **74**. Addition of allylmagnesium bromide to **75** gave the ketone **76** in 78% yield. This was followed by the addition of methylmagnesium bromide to the ketone and ozonolysis of the double bond to give diol **77** in 35% yield over the two steps. Removal of both the *N*-

Boc and *O*-TBS groups gave the amino triol hydrochloride salt **78** in 27% yield. Reductive methylation of the free amino group with formaldehyde and $NaBH_4$ gave the dimethylamine **79** in 68% yield. Finally, the tertiary amino group of **79** was protonated with TFA before treatment with IBX effected the selective oxidation of the primary alcohol resulting in lactol **80** in 95% yield (Scheme 1.14).

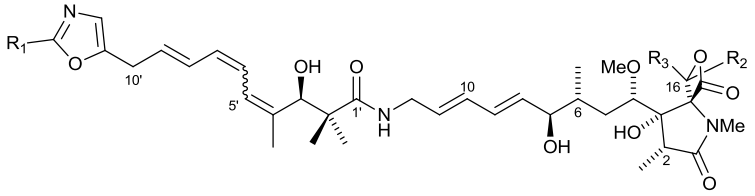


Scheme 1.14 *Reagents and conditions:* (i) Boc_2O, $NaHCO_3$, H_2O, dioxane, rt, 15 h, (ii) Me(OMe)NH.HCl, EDC, NMP, DCM, −15 °C, 1 h, (quant.), (iii) TBSCl, imidazole, DMF, rt, 15 h, (99%), (iv) allylMgBr, THF, −78 °C, 1 h, (78%), (v) MeMgBr, THF, 0 °C, 1 h, (44%), (vi) O_3, DCM, −78 °C then $NaBH_4$, EtOH, H_2O, 50 °C, 30 min, (81%), (vii) TBAF, THF, 0 °C, 3 h, (76%), (viii) anhydrous HCl, MeOH, rt, 15 h, (ix) formol, $NaBH_3CN$, AcOH, MeOH, rt, 8 h, (90%, 2 steps), (x) TFA, DMSO, rt then IBX, 2 h, (95%).

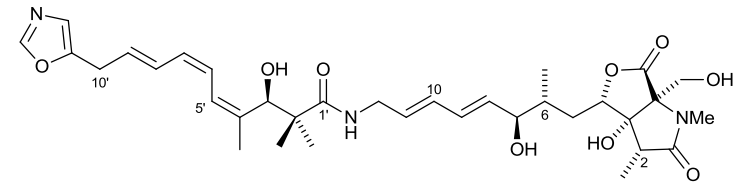
1.10 The oxazolomycins

The oxazolomycins are a family of natural products which exhibit a wide range of biological activity, including antiviral, antibacterial and cytotoxic activities.[45] There are currently 10 known members of the family, all isolated from various strains of *Streptomyces* bacteria. Oxazolomycin A **81a** is the parent member and was first isolated, and characterised, from a strain of *Streptomyces* spp. (typically 15-20 mg from a 10 L culture broth) during a search for antibiotics with inhibitory activity against Ehrlich ascites tumour, by Mori *et al.* in 1985.[46] The structure of the oxazolomycins can be broken down into three fragments; the right-hand spiro-fused β-lactone/γ-lactam moiety is attached by a diene spacer unit to an amide adjacent to a *gem*-dimethyl group leading to the left-hand triene with a terminal oxazole residue. With the exception of neooxazolomycin **82** and lajollamycin **83**, members of the oxazolomycin family typically differ either in the geometry of the left-hand triene, or in the substitution

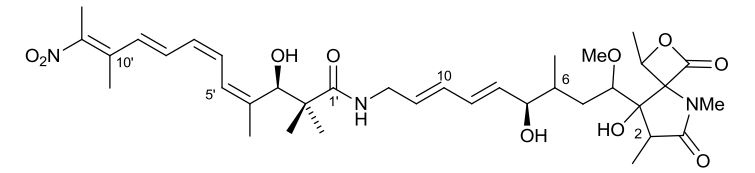
pattern on the right-hand β-lactone ring. Oxazolomycins B **81b** and C **81c** were isolated from a fermentation broth of *Streptomyces albus* JA3453 in 1998.[47] They are geometric isomers of the parent compound in the left-hand triene system with oxazolomycin A **81a** possessing 4′(*Z*),6′(*Z*),8′(*E*) geometry, and **81b** and **81c** possessing 4′(*E*),6′(*E*),8′(*E*) and 4′(*Z*),6′(*E*),8′(*E*) geometries respectively. 16-Methyloxazolomycin **81d**[48,49] has a methyl substituent on the β-lactone ring, with a 16-*S* configuration and the same triene geometry as oxazolomycin A. The curromycins,[50] A **81e** (possessing a 16-methoxymethyl substituent) and B **81f** (possessing a methyl group on the oxazole terminal residue as well as a 16-methyl substituent) both possess 4′(*Z*),6′(*Z*),8′(*E*) triene geometry and have been assumed to possess the same relative stereochemistry as 16-methyloxazolomycin on the β-lactone ring. Formed from the same strain of bacteria which also produces the oxazolomycins are KSM-2690 B **81g** and C **81h** which possess the 16-methyl substituent, and differ from each other in their triene geometry (*Z*,*Z*,*E* and *Z*,*E*,*E* respectively).[50] The stereochemistry of the β-lactone ring was determined by ROESY experiments, and showed that both products had a 16-*R* configuration, the opposite to that of **81d**, making **81g** the 16-epimer of 16-methyloxazolomycin. Neooxazolomycin **82**, produced by the strain *Streptomyces hygroscopicus*, has a different right-hand fragment to the other family members, and possesses a fused γ-lactone in place of the usual β-lactone, indicated by a peak at 1765 cm^{-1} in the IR spectrum of **82** rather than the usual 1825 cm^{-1}.[51] Finally, lajollamycin **83**, which was isolated from a strain of *Streptomyces nodosus* in 2005, differs in the left-hand fragment and does not contain an oxazole ring but instead possesses a tetraenyl-nitro terminus.[52] Additionally, lajollamycin **83** also bears a 16-methyl substituent on the β-lactone right-hand unit and key differences in the NOESY spectra of **83** and **81d** led to the prediction that the relative stereochemistry at C(15) or C(16) is different. The structures of the oxazolomycin family are outlined in Figure 1.10.



81a Oxazolomycin A: R_1 = H, R_2 = H, R_3 = H; 4'(Z),6'(Z),8'(E)
81b Oxazolomycin B: R_1 = H, R_2 = H, R_3 = H; 4'(E),6'(E),8'(E)
81c Oxazolomycin C: R_1 = H, R_2 = H, R_3 = H; 4'(Z),6'(E),8'(E)
81d 16-Methyloxazolomycin: R_1 = H, R_2 = Me, R_3 = H; 4'(Z),6'(Z),8'(E)
81e Curromycin A: R_1 = Me, R_2 = CH_2OCH_3, R_3 = H; 4'(Z),6'(Z),8'(E)
81f Curromycin B: R_1 = Me, R_2 = Me, R_3 = H; 4'(Z),6'(Z),8'(E)
81g KSM-2690 B: R_1 = H, R_2 = H, R_3 = Me; 4'(Z),6'(Z),8'(E)
81h KSM-2690 C: R_1 = H, R_2 = H, R_3 = Me; 4'(Z),6'(E),8'(E)



82 Neooxazolomycin



83 Lajollamycin

Figure 1.10 The oxazolomycin family.

1.11 Structural elucidation

1.11.1 Oxazolomycin A

The structure of oxazolomycin A **81a** was elucidated at the time of its discovery by Mori *et al.* and it was the first of the family to be fully characterised.[46] The molecular formula of $C_{35}H_{49}N_3O_9$ was indicated by elemental analysis and mass spectrometric methods (FAB-MS: *m/z* 656 $(M+H)^+$). The UV spectrum indicated the presence of a triene system, showing peaks at 265, 275 and 285 nm and an absorption at 230 nm corresponds to the conjugated diene chromophore. The IR spectrum revealed the presence of the β-lactone through absorption at 1825 cm^{-1}. The *Z* configuration of the trisubstituted double bond was confirmed by observation of an nOe enhancement between C(4′)*Me* and C(5′)*H*. A compound isolated in 1971 and named resistaphylin appears to be identical to oxazolomycin A in its physico-chemical properties.[53] The optical rotation of **81a** has been reported as $[\alpha]_D^{22}$ +30.3 (*c* 2.19 in MeOH) by Kanzaki *et al.*[47] ^{1}H-NMR details were provided for the diacetate, accessed by acetylation of oxazolomycin A, on the two free secondary hydroxyl groups, using Ac_2O and

pyridine.[46] The ^{1}H NMR spectrum of the diacetate was assigned with the aid of careful chemical degradation experiments to produce 8 fragments of simpler and more readily assignable structure.

1.11.2 16-Methyloxazolomycin

First reported in 1997 by Ryu *et al.*, 16-methyloxazolomycin **81d** was isolated from *Streptomyces* spp. (6 mg from a 10 L culture broth) as a pale yellow amorphous powder possessing an optical rotation of $[\alpha]_D^{23}$ +3.6 (*c* 0.52 in MeOH) and a molecular ion peak in the mass spectrum at *m/z* 692 ($[M+Na]^+$).[48] Its IR spectrum shows the characteristic stretches at 1825 and 1690 cm^{-1} that correspond to the β-lactone and amide functionalities. The UV spectrum shows maxima at 228 (23000), 266 (27200), 275 (32000) and 285 (27000) nm, consistent with the diene and triene subunits. The ^{1}H NMR spectrum of 16-methyloxazolomycin was understandably very similar to that reported for oxazolomycin A. Key differences between the two spectra are the presence of a doublet at 1.69 ppm corresponding to the 16-methyl group and a quartet at 4.82 ppm corresponding to the C(16)*H*. The oxymethylene signal in oxazolomycin A is also missing, indicating that the methyl group is attached here. The position of the methyl group was confirmed by the presence of HMBC cross peaks between C(16)*H* and *C*(3) and *C*(15), and between C(16)*Me* and *C*(16) and *C*(15). The stereochemistry of the β-lactone-γ-lactam spiro centre was deduced to be identical to oxazolomycin by NOESY experiments. The presence of NOESY cross peaks between C(16)*H* and C(3)O*H* and between C(16)*Me* and N*Me* lead to an *S* assignment for C(16) (Figure 1.11).

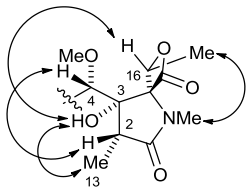
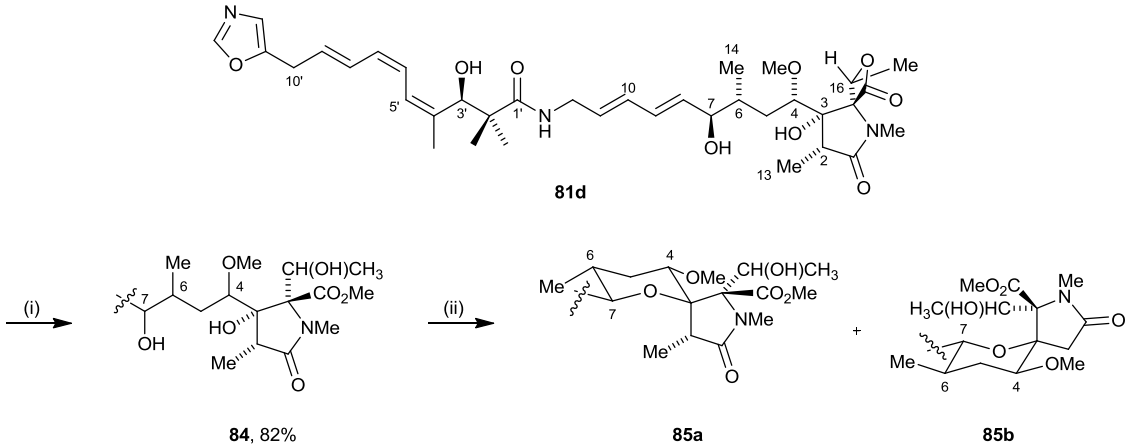


Figure 1.11 NOESY correlations used to assign the stereochemistry of the right-hand side of 16-methyloxazolomycin **81d**.

Another publication from the same group detailed degradation experiments that were used to confirm the assignment of the NMR data and determine the relative stereochemistry of C(4), C(6) and C(7).[49] In the first step of a series of chemical degradations, 16-

methyloxazolomycin **81d** was treated with 2N AcOH in MeOH at room temperature for two days to give methyl ester **84** in 82% yield. Ester **84** was treated with catalytic camphor sulphonic acid (CSA) in a 9:1 mixture of CH_3Cl and MeOH at room temperature to give tetrahydropyran/γ-lactam spiro compounds **85a** and **85b** only. The ratio of **85a** to **85b** was dependent on the reaction time with a 1.6:1 ratio of **a:b** observed after eight hours and a 4:1 ratio after 72 hours (Scheme 1.15).



Scheme 1.15 *Reagents and conditions:* (i) 2N AcOH/MeOH, 48 h, rt, (ii) CSA cat., $CHCl_3$:MeOH (9:1).

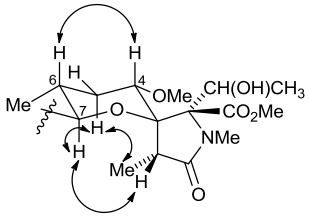
The ^{1}H NMR spectra of **81d**, **84**, **85a** and **85b** are detailed in Table 1.3 and were used to confirm the proposed structures. The signals at 4.26 and 4.08 ppm in the respective spectra of **85a** and **85b** were assigned to C(7)*H* and both appear as doublets, due to coupling with C(6)*H*, with coupling constants of 10.2 and 10.3 Hz. This large J value implies that C(7)*H* and C(6)*H* have a diaxial relationship. The signals corresponding to C(4)*H* at 3.52 and 3.48 ppm for **85a** and **85b** appear as doublets of doublets with first coupling constants of 10.8 and 10.6 Hz respectively. This suggests that C(4)*H* also occupies an axial position on the tetrahydropyran chair. These conclusions were supported by nOe enhancements between relevant protons in **85a** and **85b**, which are shown on Figure 1.12.

Position	δ_H - ppm (integral, multiplicity, J (Hz))			
	81d	**84**	**85a**	**85b**
C(2)*H*	2.34 (1H, q, 6.6)	2.33 (1H, q, 6.4)	2.36 (1H, q, 6.2)	2.34 (1H, q, 6.3)
C(4)*H*	3.38 (1H, t, 6.9)	3.40 (1H, t, 6.2)	3.52 (1H, dd, 10.8, 4.6)	3.48 (1H, dd, 10.6, 4.7)
C(5)H_2	1.95 (1H, m) 1.15 (1H, m)	1.97 (1H, m) 1.16 (1H, m)	1.98 (1H, m) 1.20 (1H, br t, 10.3)	2.17 (1H, m) 1.27 (1H, t, 10.3)
C(6)*H*	1.58 (1H, m)	1.58 (1H, m)	1.52 (1H, m)	1.64 (1H, m)
C(7)*H*	3.81 (1H, m)	3.82 (1H, m)	4.26 (1H, d, 10.2)	4.08 (1H, d, 10.3)
C(8)*H*	5.57 (1H, dd, 16.8, 11.2)	5.43 (1H, dd, 16.3, 10.8)	5.52 (1H)*	5.42 (1H, dd, 16.2, 10.2)

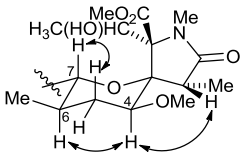
Position	δ_H - ppm (integral, multiplicity, J (Hz))			
	81d	**84**	**85a**	**85b**
C(9)*H*	6.14 (1H, dd, 11.2, 16.8)	8.98 (1H)*	6.39 (1H)*	6.32 (1H)
C(10)*H*	6.09 (1H, dd, 14.3, 10.8)	6.02 (1H, dd, 14.4, 10.2)	6.12 (1H, dd, 14.6, 9.9)	6.08 (1H, dd, 10.1, 14.6)
C(11)*H*	5.59 (1H, m)	5.62 (1H)*	5.55 (1H)*	5.60 (1H, m)
C(12)H_2	3.71 (2H, m)	3.73 (2H, m)	3.80 (2H, m)	3.84 (2H, m)
C(13)H_3	1.03 (3H, d. 6.6)	1.03 (3H, d. 6.8)	1.24 (3H, d. 6.2)	1.08 (3H, d. 6.3)
C(14)H_3	0.86 (3H, d, 6.2)	0.86 (3H, d, 6.2)	0.92 (3H, d, 6.2)	0.98 (3H, d, 6.3)
C(16)*H*	4.82 (1H, q, 6.3)	3.88 (1H, q, 6.5)	3.80 (1H, q, 6.4)	3.76 (1H, q, 6.6)
C(3')*H*	4.59 (1H, s)	4.56 (1H, s)	4.58 (1H, s)	4.56 (1H, s)
C(5')*H*	6.35 (1H, d, 11.3)	6.32 (1H, d, 11.4)	6.36 (1H)	6.82 (1H, d, 11.2)
C(6')*H*	6.27 (1H, dd, 10.9 11.3)	6.18 (1H, dd, 11.4 10.3)	6.24 (1H, dd, 10.1 11.3)	6.34 (1H)
C(7')*H*	5.90 (1H, dd, 11.5, 10.9)	5.95 (1H)	5.92 (1H, dd, 11.5, 10.1)	5.92 (1H, dd, 10.3, 11.6)
C(8')*H*	6.70 (1H, dd, 11.5, 14.5)	6.58 (1H, dt, 14.4, 14.8)	6.81 (1H, dd, 11.5, 14.3)	6.63 (1H, dd, 11.6, 15.1)
C(9')*H*	5.75 (1H, dt, 14.5, 4.5)	5.60 (1H)*	5.79 (1H, dt, 14.3, 4.4)	5.80 (1H, dt, 15.1, 4.6)
C(10')H_2	3.52 (2H, br d, 7.0)	3.55 (2H, br d, 7.1)	3.56 (2H, d, 7.1)	3.58 (2H, d, 7.2)
C(12')*H*	6.84 (1H, s)	6.84 (1H, s)	6.88 (1H, s)	6.91 (1H, s)
C(13')*H*	8.14 (1H, s)	8.14 (1H, s)	8.20 (1H, s)	8.18 (1H, s)
C(14')H_3	0.95 (3H, s)	0.95 (3H, s)	0.98 (3H, s)	0.99 (3H, s)
C(15')H_3	1.10 (3H, s)	1.10 (3H, s)	1.08 (3H, s)	1.14 (3H, s)
C(16')H_3	1.70 (3H, s)	1.70 (3H, s)	1.81 (3H, s)	1.86 (3H, s)
C(16)CH_3	1.69 (3H, d, 6.3)	1.69 (3H, d, 6.5)	1.76 (3H, d, 6.4)	1.74 (3H, d, 6.6)
NCH_3	2.77 (3H, s)	2.77 (3H, s)	2.86 (3H, s)	2.90 (3H, s)
OCH_3	3.15 (3H, s)	3.15 (3H, s)	3.40 (3H, s)	3.28 (3H, s)
C(3)O*H*	5.31 (1H, s)	5.31 (1H, s)	-	-
C(7)OH	4.82 (1H, s)	4.82 (1H, s)	-	-
C(3')OH	5.49 (1H, s)	5.31 (1H, s)	-	-
C(16)O*H*				
CO_2CH_3	-	3.60 (3H, s)	3.48 (3H, s)	3.51 (3H, s)
N*H*	7.63 (1H, br t, 5.9)	7.63 (1H, br t, 6.0)	7.54 (1H, br t, 5.6)	7.62 (1H, br t, 5.5)

*where multiplicities are not assigned it is due to overlap with other peaks.

Table 1.3 ^{1}H NMR data for compounds **81d**, **82**, **85a** and **85b**.



85a



85b

Figure 1.12 nOe enhancements for 16-methyloxazolomycin derivatives **85a** and **85b**.

The absolute stereochemistry of C(3′) and C(7) was determined by the modified Mosher method. The bis-(*R*)- and bis-(*S*)-MTPA esters were synthesised by treatment of **81d** with the respective Mosher's acid in dry pyridine. Analysis of the spectra of these compounds

suggested the configurations 3′*R*,7*R*. Due to the established relative configurations, the absolute assignment for **81d** was given as 2*R*,3*S*,4*S*,6*R*,15*S*,16*S*.

1.12 Biological activity of the oxazolomycins

The oxazolomycin family display a wide range of potent biological activity, including antibacterial, antiviral and *in vivo* antitumour activity, accompanied by relatively low toxicity (LD_{50} of 10.6 mg/kg for intraperitoneal injection in mice). These activities are summarised in Table 1.4.

Activity	**Natural product**									
	81a	**81b**	**81c**	**81d**	**81e**	**81f**	**81g**	**81h**	**82**	**83**
Viral activity:										
Influenza A MIC (μg/mL)	15.6									
Herpes simplex MIC (μg/mL)	15.6									
Vaccinia MIC (μg/mL)	15.6									
HIV replication					2.5	5				
Cytotoxic activity:										
P388 leukaemia IC_{50} (μg/mL)				0.23	0.06	0.19				
A-549 human lung adenocarcinoma IC_{50} (μg/mL)				4.6						
Human bladder carcinoma T24 IC_{50} (μg/mL)							10	10		
Mouse B16 melanoma					Active					
Friend leukaemia					Active					
Murine melanoma B16-F10 EC_{50} (μM)										9.6
Crown gall formation, MID (μg/disc)	0.8	0.8	0.8							
Alfalfa germination, MIC (μg/mL)	12.5	25.0	50.0							
Bacterial growth, MIC (μg/mL):										
Agrobacterium tumefaciens IFO13263	3.2	>100	>100							
Agrobacterium tumefaciens EHA101	6.3	100	>100							
Agrobacterium rhizogenes IFO13257	50	>100	>100							
Rhizobium loti IFO13336	>100	>100	>100							
Escherichia coli	>100	>100	>100							12
Bacillus subtilis	>100	>100	>100	5	Active		50			
Chlorella vulgaris IFO15941				10						
Micrococcus luteus					25	25	50			
Staphylococcus aureus (methicillin sensitive)										4
Staphylococcus aureus (methicillin resistant)										5
Staphylococcus aureus Smith							50			
Staphylococcus aureus FDA 209P					2.5	2.5				
Streptococcus pneumoniae (penicillin sensitive)										2
Streptococcus pneumoniae										1.5

Activity	Natural product									
	81a	**81b**	**81c**	**81d**	**81e**	**81f**	**81g**	**81h**	**82**	**83**
(penicillin resistant)										
Enterococcus faecalis (vancomycin sensitive)										14
Enterococcus faecium (vancomycin resistant)										20

Table 1.4 Biological activity of the oxazolomycin family.

1.12.2 Antiviral activity

This is mostly limited to **81a,** with a study by Tonew *et al.* revealing that the compound inhibits the replication of herpes simplex type 1, vaccinia and influenza A viruses in a dose-dependent manner. The tests were also carried out on Coxsackie A9 virus but this was not found to be inhibited by **81a**.[54] It is notable that whilst **81a** shows considerable antiviral activity, other members of the family are much less active (though **81b** and **81c** had not been isolated at the time of this publication). The curromycins A and B have been reported to inhibit the replication of human immunodeficiency virus in both acute and chronic infections and the authors suggest this may result from the curromycins affecting a late stage in the viral replication process.[55]

1.12.3 Cytotoxic activity

The parent members of the group have not been shown to have activity in this area though **81d**, curromycin A and B (**81e** and **f**), and KSM-2690 B and C (**81g** and **h**) have. Ryu *et al.* have shown that 16-methyloxazolomycin **81d** exhibits cytotoxicity against P388 leukaemia cells and A-549 human lung adenocarinoma cells with IC_{50} values of 0.23 and 4.6 μg/mL respectively.[48] Curromycin A and B also show cytotoxicity again P388 leukaemia cells with IC_{50}'s of 0.06 and 0.19 μg/mL[56] and **81b** is active against mouse B16 melanoma and Friend leukaemia cells as well.[57] Compounds KSM-2690 B and C **81g** and **81h** exhibit activity against human bladder carcinoma T24 cells with an IC_{50} of 10 μg/mL.[50] Related compound lajollamycin **83** has been reported to inhibit B16-F10 tumour cells *in vitro*.[52]

1.12.4 Antibacterial activity

The oxazolomycins A **81a**, B **81b** and C **81c** all inhibit crown gall formation (plant tumours). Oxazolomycin A also displays specific activity against two strains of *Agrobacterium*

tumefaciens and *Agrobacterium rhizgenes* (with MIC values of 3.2, 6.3 and 50 μg/mL respectively) that oxazolomycin B and C do not share. Other than these specific examples, no other antibacterial activity has been reported for the parent members of the group. Although very closely related in structure, the three parent members of the oxazolomycin family have significantly different biological activity in this area. This, along with the wide-ranging antibacterial activity of the remaining oxazolomycins, indicates that small structural variations can lead to marked consequences in the biological activity of these compounds.

All of the remaining family members except for KSM-2690 C **81h** and neooxazolomycin **82** have been shown to display antibacterial activity against a range of microbes. 16-Methyloxazolomycin **81d** displays activity against *Bacillus subtilis* 1069 and *Chlorella vulgaris* IFO15941 with MIC's of 5 and 10 μg/mL.[48] Both curromycin isomers **81e** and **f** (along with their diacetate and dipropionate ester analogues) inhibit crown gall formation on potato tubers.[58] Both **81e** and **f** also exhibit activity against *Staphylococcus aureus* FDA 209P with the same MIC of 2.5 μg/mL.[56] Curromycin A also shows activity against *Bacillus subtilis* IAM1026.[57] KSM-2690 C **81h** has not been shown to possess any antibacterial activity, but its isomer **81g** is moderately active against Gram-positive bacteria *Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus aureus* Smith each with an MIC of 50 μg/mL. The tetraenyl-nitro terminated lajollamycin **83** has the most wide-ranging reported antibacterial activity of all the family members and is active against both antibiotic sensitive and antibiotic resistant bacterial strains,[52] for example methicillin sensitive and resistant *S. aureus* with MIC values of 4 and 5 μg/mL, as well as *Streptococcus pneumonia*, *Enterococcus faecalis* and their respective penicillin and vancomycin resistant strains. Lajollamycin is also the only family member reported to display significant activity against Gram-negative *E. coli* (MIC 12 μg/ml).

1.13 Biosynthesis

The biosynthetic origins of most of the oxazolomycins have not been studied directly, but some details have been established for the parent member of the group, oxazolomycin A **81a**. The first study was carried out by Theiricke *et al.* who used a series of feeding experiments to

provide information about the building blocks of **81a**.[59] Feeding cultures of *Streptomyces albus* (strain JA3453) with each of [^{13}C]-labelled acetate, L-[*methyl*-^{13}C]methionine, L-[1-^{13}C]alanine and [1-^{13}C]glycine gave labelled samples of **81a** for which the relative abundances of ^{13}C were analysed, giving a picture of where carbons originating from each biological source were incorporated into the natural product. The diene and triene chains were both constructed using a polyketide pathway from acetate units. All five *C*-Me groups, the *O*-Me and the *N*-Me group were found to originate from methionine. The origin of the spiro ring system was not clear; it is formed from glycine, acetate, and a C_3 unit of unknown origin. The experiment using C_3 unit L-[1-^{13}C]-alanine resulted in no label incorporation into **81a**. The pattern for the C(1′)-C(16′) segment is in agreement with an earlier study of inthomycin,[60] a related compound possessing the oxazole and triene unit terminating in an amide. An experiment feeding labelled inthomycin to the oxazolomycin producing strain showed that inthomycin was not an intermediate in the synthesis of **81a** (Figure 1.13).

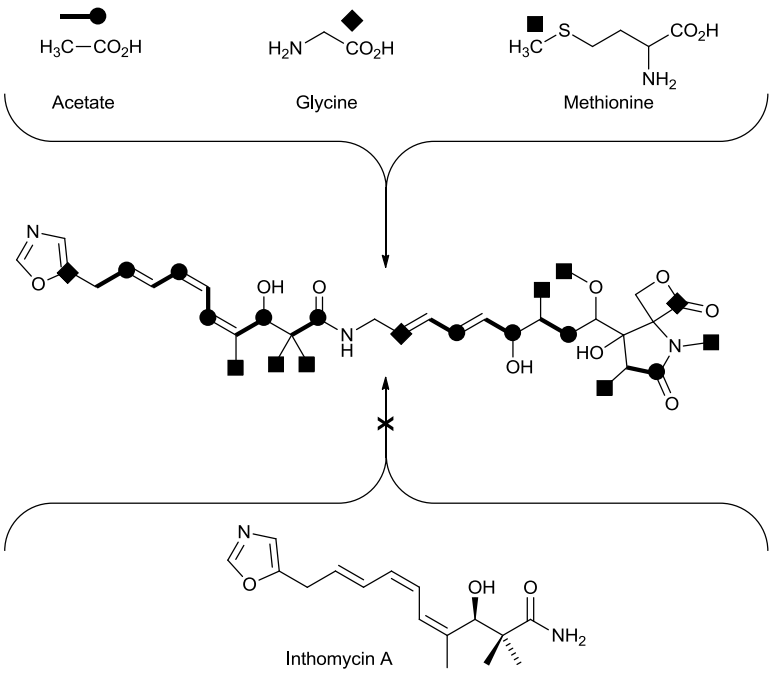


Figure 1.13 Biosynthesis of oxazolomycin.

In 2006, Zhao *et al.* identified the oxazolomycin biosynthetic gene cluster in *Streptomyces albus* JA3453 and deleted a 12kb DNA fragment containing six methoxymalonyl-ACP biosynthesis genes. This gave mutants that did not produce oxazolomycin, proving that the gene sequence removed is essential for its biosynthesis. This provided evidence that the

previously unaccounted for C(3)-C(4) moiety is derived from methoxymalonate.[61] A more recent publication used a series of *in vivo* and *in silico* experiments to propose a model for the biosynthesis of oxazolomycin.[62] They propose that the biosynthesis of oxazolomycin is controlled by a 79.5-kb DNA fragment that encodes for, amongst other things, polyketide synthases (PKSs), *trans*-acetyltransferases and enzymes for methoxymalonyl-acyl carrier protein (ACP) synthesis. Their model begins with the formation of a formylated glycine residues which becomes the oxazole ring terminus, and through the sequential incorporation of five malonyl-CoA, one glycine, three malonyl-CoA, one methoxymalonyl-ACP, one malonyl-CoA, and one serine unit as building blocks, the natural product is produced. The formation of spiro-fused γ-lactam/β-lactone unit occurs in the final stages of the synthesis. It also includes six *C*- and *N*-methylation events employing *S*-adenosyl-L-methionine.

1.14 Biological mode of action

The only detailed study of the mode of action of these compounds was carried out by Gräfe *et al.* in 1992 on the parent member **81a**. The group used an artificial lipid membrane model to show that oxazolomycin A is an effective protonophore at pH<7.0 and is able to transport both protons and monovalent cations, such as K^+, at pH>7.5 as a passive carrier. Several model system experiments led to the conclusion that **81a** is also a general uncoupler of oxidative phosphorylation.[54,63] The protonophoric properties observed below pH 7.0 could be attributed to the binding of protons to the nitrogen atom of the weakly basic oxazole ring, however this is surprising given the known pK_a values of the conjugate acids of oxazole (0.8) and isoxazole (−3.0).[64]

The life-cycle of the influenza A virus is known in some detail, and can be used to illustrate the importance of this mode of action. Membrane fusions which allow the ingestion of a virion by endocytosis into the host cell and expulsion of new infectious particles are vital to the success of the pathogen and have been extensively studied.[65] These processes are mediated by the membrane-bound glycoprotein haemaglutinin (HA). The membrane fusion between the host cell and the virion does not occur until the virion is inside the endocytotic vesicle, which is relatively acidic at pH ~5. Under these conditions, HA undergoes a

significant conformational reorganisation, thought to be triggered by protonation of six Asp side chain carboxylates, translating the 'fusion peptide' by ~100Å, and allowing it to bridge the viral and cellular membranes and therefore facilitate fusion.[66]

It has been shown that lipid soluble bases such as NH_3 and NMe_3 can inhibit viral infection by raising the pH of the normally acidic endosomes and preventing the requisite protonations.[45] This inhibits membrane fusion mediated by HA and the essential dispersal of viral RNA into the host cytoplasm is prevented. This is the basis of the modes of action of the antiviral drugs amantadine and rimantadine. These compounds act as protonophores to enhance the permeability of the endocytotic membrane to protons down the pH gradient and it is possible that the activity of oxazolomycin is due to a similar effect.

However, this mode of action could be expected to cause general metabolic disruption as the protonophoric activity provides a pathway additional to ATP synthetase for the transport of protons across the inner mitochondrial membrane, disrupting ATP synthesis with wide-ranging consequences. The low toxicity of oxazolomycin suggests that its protonophoric activity is selective for the endosomal membrane; the explanation for this selectivity is completely unknown. Unlike many other well-known antivirals, oxazolomycin may therefore target an early stage of the viral life-cycle and this unique mode of action makes it an antibiotic of significant interest.

1.15 Hatakeyama's total synthesis of oxazolomycin A

The oxazolomycin family pose a considerable synthetic challenge and whilst the volume of synthetic work published is slowly growing, the total synthesis of most members remains to be completed. There have been two reported routes to neooxazolomycin **82** and the most recent publication in this area is the first total synthesis of the parent member of the family, oxazolomycin A **81a**, reported in 2011. Hatakeyama *et al.* have successfully applied the traditional left-hand, middle and right-hand fragment retrosynthesis strategy to break down the structure into three component parts, **86**, **87** and **88**, which are united at a relatively late stage in the synthesis (Figure 1.14).[67] Whilst most approaches look to have these 3 fragments

completed before final tethering, their approach leaves the formation of the spiro-fused β-lactone to the very last step, instead installing the right-hand fragment as the dioxasilinane species **88** where both the acid and alcohol groups which make up the β-lactone are protected. This synthesis follows the publication in 2007 of a total synthesis of neooxazolomycin **82** by the same group, and uses much the same synthetic routes to make the various fragments of the natural product.[68] The other total synthesis of neooxazolomycin, published by Kende *et al.* in 1990, also successfully employs the 3 fragment approach.[69]

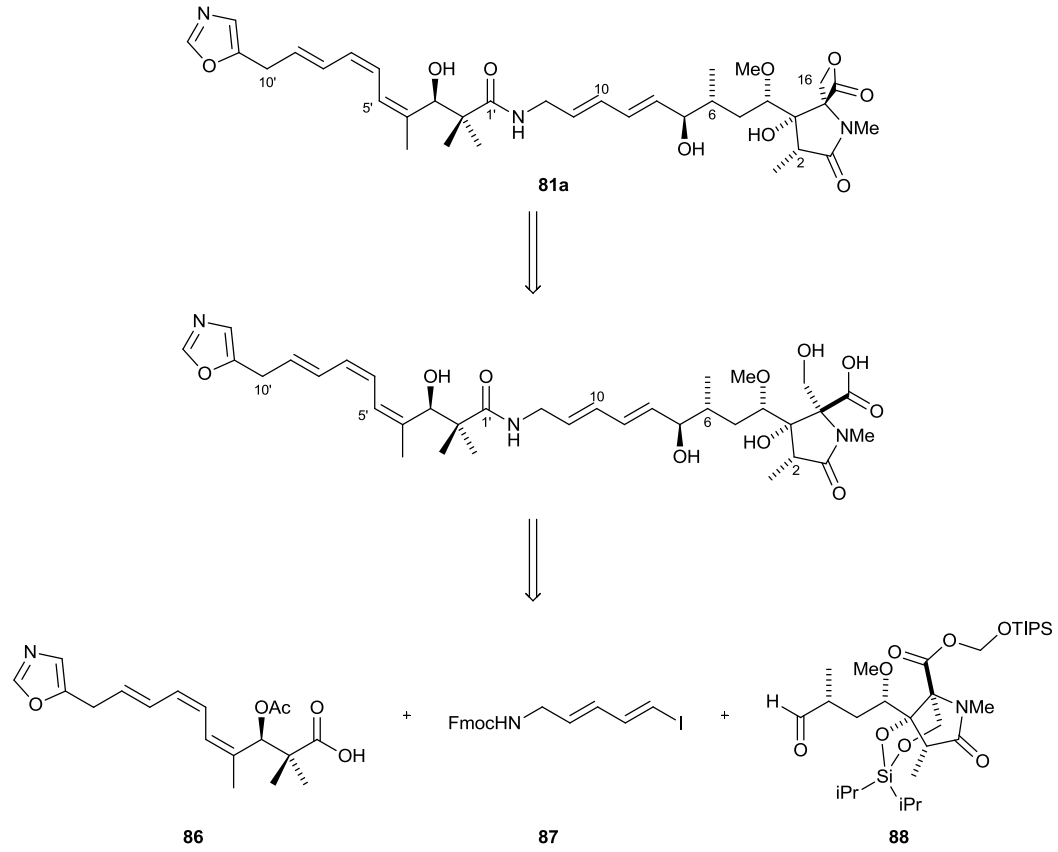
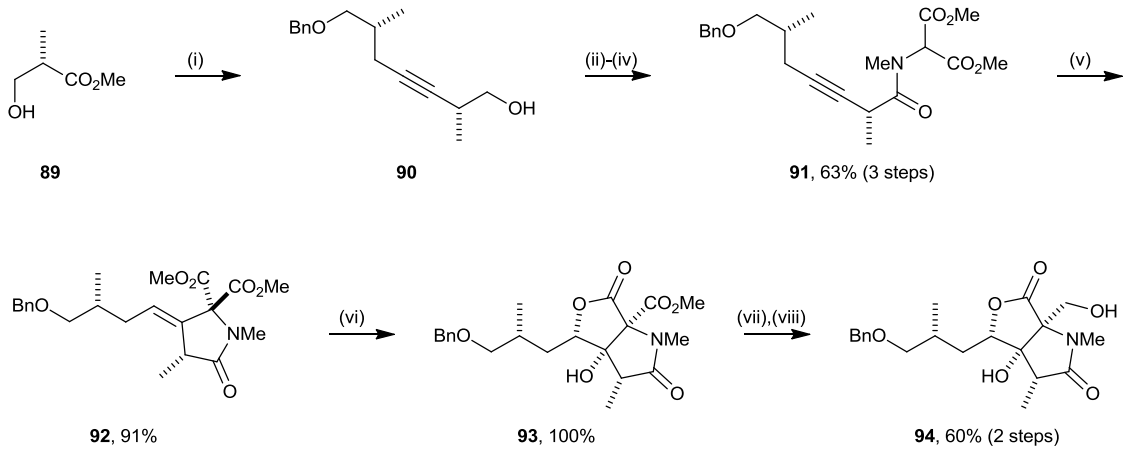


Figure 1.14 Hatakeyama's retrosynthesis of oxazolomycin A **81a**.

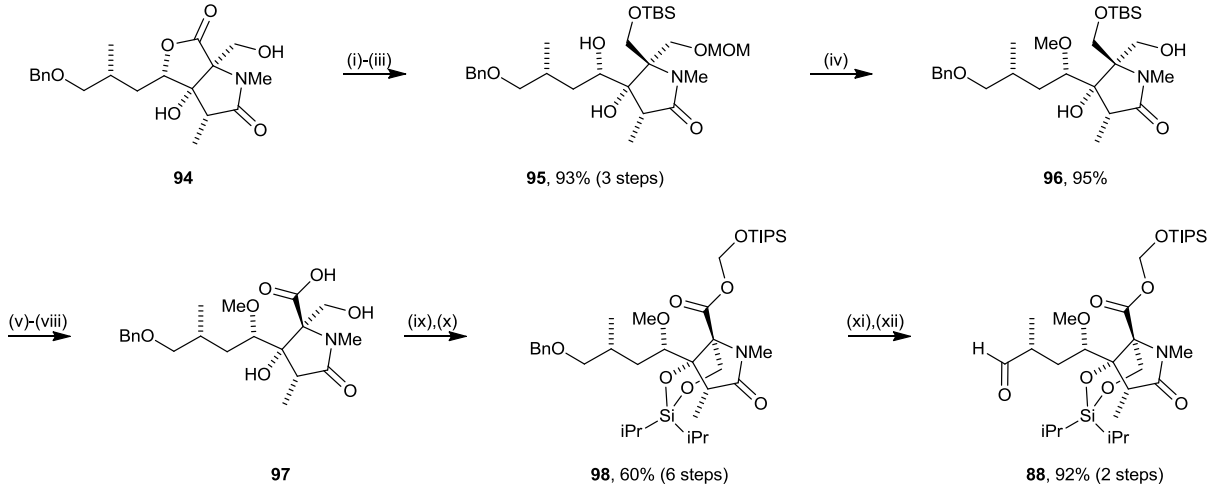
For the synthesis of the left-hand **86** and middle **87** fragments the group used improved versions of the methods described in their earlier paper and envisaged using the fragment **94**,[68] also used in the synthesis of neooxazolomycin, as an advanced intermediate in the synthesis of **88**. The hydroxyl group of compound **90**, synthesised in 3 steps from (*S*)-hydroxy-2-methylpropanoate **89** was oxidised with H_2CrO_4 to give the carboxylic acid, converted to the acid chloride, and reacted with diemethylaminomalonate to give amide **91** in 63% yield. Treatment with $In(OTf)_3$ and DBU resulted in a stereoselective cyclisation with the triple bond to give *E*-**92** in 91% yield which was then dihydroxylated with OsO_4 to

produce a diol which spontaneously cyclised to give the lactone **93** as the only isomer in quantitative yield. The stereoselectivity of this process is postulated to be caused by the conformation of **92** placing the terminal *O*-Bn group in a position that blocks one face of the double bond, supported by nOe enhancements measured in the reaction solvent system.[68] Finally, reduction of the remaining ester group gave **94**, the right-hand fragment of neooxazolomycin **82** (Scheme 1.16). Although similar, this sequence is an improvement on that reported in the synthesis of neooxazolomycin, with a yield here of 24% over 15 steps, versus 15% over 17 steps described in the previous publication.



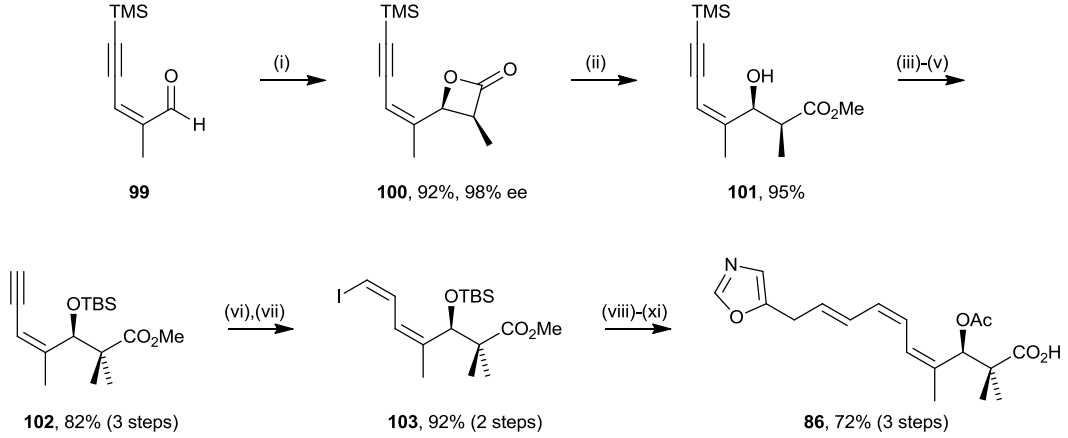
Scheme 1.16 *Reagents and conditions:* (i)[68] (a) TMSCl, (b) DIBAL, (c) Bestman-Ohira reagent, MeOH, (d) BuLi, ROH, TBAF, (ii) H_2CrO_4, aq. Acetone, 0 °C, (iii) $SOCl_2$, DCM, (iv) toluene, $(CO_2Me)_2CHNHMe$, (63%, 3 steps), (v) $In(OTf)_3$ (5mol%), DBU (4.4mol%), toluene, reflux, (91%) (vi) OsO_4 (0.4 eq.), NMO (4.4 eq.), 3:1 THF:H_2O, (100%), (vii) 4 M LiOH, THF, then 1 M HCl, (viii) $(COCl)_2$, DMF (cat.), DCM, then $NaBH_4$, 9:1 THF:MeOH, −78 °C (60%, 2 steps).

The elaboration of **94** to **88** began with MOM protection of the primary hydroxyl group, followed by reduction of the lactone ring with $NaBH_4$, and protection of the resulting primary hydroxyl with TBSOTf to give **95** in a 95% yield. Alkylation of the hindered secondary hydroxyl was achieved using trimethyloxonium tetrafluoroborate in 95% yield, producing **96**. The *O*-TBS group was removed and the alcohol oxidised in two steps to the carboxylic acid. Subsequent removal of the *O*-MOM group with $ZrCl_4$ gave the β-hydroxyl-acid **97**. A CH_2OTIPS ester was formed by treatment of **97** with triisopropylsilyloxymethyl(dodecyl)sulphane without affecting the primary hydroxyl group. The two remaining hydroxyl groups were protected as a dioxasilinane **98** in 60% yield over six steps. To furnish **88** in 92% yield, the *O*-Bn group was removed by hydrogenolysis and subsequent oxidation gave the aldehyde (Scheme 1.17).



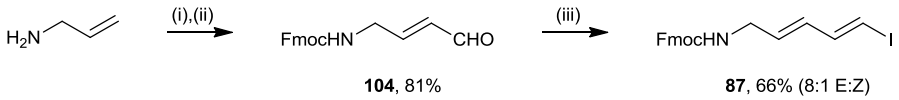
Scheme 1.17 *Reagents and conditions:* (i) MOMCl, *i*Pr_2NEt, DCM, (ii) $NaBH_4$, 1:1 THF:EtOH, (iii) TBSOTf, 2,6-lutidine, DCM, −78 °C, (93%, 3 steps), (iv) Me_3OBF_4, proton sponge, 4 Å mol. sieves, DCM, (95%), (v) TBAF, THF, (vi) H_2CrO_4, aq. acetone, 0 °C, (vii) $NaClO_2$, NaH_2PO_4, 2-methyl-4-butene, 5:1 *t*BuOH:H_2O, (viii) $ZrCl_4$ (0.3 eq.), *i*PrOH, reflux, (ix) *n*-$C_{12}H_{25}SCH_2OTIPS$, $CuBr_2$, *n*-Bu_4NBr, TEA, 4 Å mol. sieves, DCM, (67%, 5 steps), (x) *i*$Pr_2Si(OTf)_2$, 2,6-lutidine, DCE, reflux, (90%), (xi) H_2, $Pd(OH)_2$, EtOAc, (xii) Dess-Martin periodinane, $NaHCO_3$, DCM, (92%).

The left-hand fragment was also synthesised using a similar but much improved (by 17% and 3 steps) sequence to that used in the neooxazolomycin synthesis. The synthesis began with a *Cinchona* alkaloid-catalysed asymmetric cyclocondensation of aldehyde **99** with propionyl chloride using the Nelson protocol *via* a ketene to give **100** in 92% yield and 98% ee. Treatment with NaOMe gave the methyl ester **101** in 95% yield. Following the methylation of the lithium enolate, desilylation of the acetylene and TBS protection of the free hydroxyl, **102** was produced in 82% yield. Conversion to the vinyl iodide was done using the procedure described by Kende *et al.* in their total synthesis of neooxazolomycin, by first making the lithium salt of the acetylene, quenching with I_2 and carrying out a diimide reduction to furnish **103** in 92% yield.[69] Finally, the iodide was coupled to the requisite tributyl-stannane under Stille conditions, the *O*-TBS group was replaced with *O*-Ac and the methyl ester was hydrolysed to give the left-hand fragment **86** (Scheme 1.18).



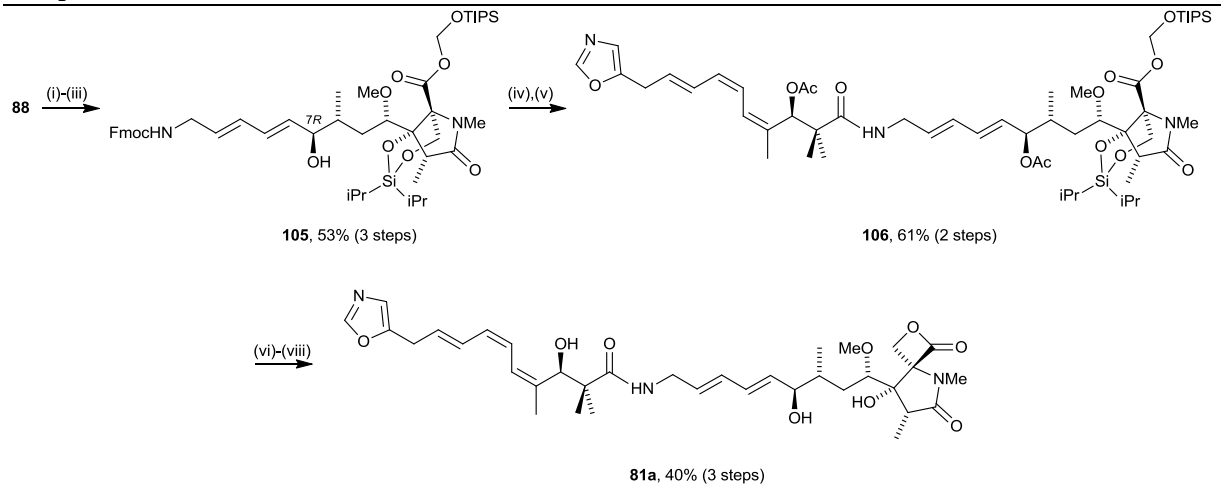
Scheme 1.18 *Reagents and conditions:* (i) catalyst (20 mol%), EtCOCl, $LiClO_4$ (4 eq.), *i*Pr_2NEt (2.5 eq.), 2:1 DCM:Et_2O, (92%), (ii) NaOMe, MeOH, (95%), (iii) LDA, MeI, THF, −20 °C, (84%), (iv) NaOMe, MeOH, (v) TBSOTf, 2,6-lutidine, DCM, 0 °C, (98%, 2 steps), (vi) BuLi, I_2, THF, −78 °C, (vii) *o*-$(NO_2)C_6H_4SO_2NHNH_2$, TEA, 1:1 *i*PrOH:THF, (92%, 2 steps), (viii) $Pd(PPh_3)_4$ (1 mol%), CuI (10 mol%), CsF (2 eq.), $RSnBu_3$, DMF, (83%), (ix) 47% HF, MeCN, (x) LiOH, 3:1 THF:MeOH, (xi) Ac_2O, py then $NaHCO_3$, MeOH (88%, 2 steps).

The middle fragment **87** was made in 3 steps from allylamine. First an Fmoc protecting group was added before a cross metathesis with acrolein was carried out using Hoveyda-Grubbs catalyst to give **104** in 81% yield. A Takai iodoalkenylation was then carried out with iodoform and $CrCl_2$ to give **87** in 66% yield (Scheme 1.19).



Scheme 1.19 *Reagents and conditions*: (i) FmocCl, $NaHCO_3$, dioxane, (96%), (ii) acrolein, Hoveyda-Grubbs II (5 mol%), DCM, (85%), (iii) $CrCl_2$, CHI_3, THF (66%).

Right-hand fragment aldehyde **88** was reacted with middle fragment vinyl iodide **87** using Nozaki-Hiyama-Kishi conditions (20 mol% $NiCl_2$, 6 eq. $CrCl_2$, 3:1 THF:DMSO) giving a 3:2 mixture of 7*R*-**105** with a 7*S*-epimer which was converted to the 7*R*- using an oxidation/reduction protocol eventually giving 7*R*-**105**:7*S*-**105** in a 4:1 ratio. Acylation of the hydroxyl, removal of the *N*-Fmoc group and condensation with a mixed anhydride of left-hand fragment carboxylic acid **86** gave amide **106** in 61% yield. Treatment with HF-pyridine and then LiOH removed the dioxasilinane and acetyl groups and finally, the β-lactone ring was formed in the presence of HATU and Hunig's base, giving oxazolomycin A **81a** in 40% yield (Scheme 1.20). The overall yield for this synthesis is 1.4% and it consists of 34 linear steps.



Scheme 1.20 *Reagents and conditions:* (i) **87**, $NiCl_2$ (20 mol%), $CrCl_2$ (6 eq.), 3:1 THF:DMSO, (ii) Dess-Martin periodinane, $NaHCO_3$, DCM, (iii) L-selectride, THF, −78 °C, (53%, 3 steps), (iv) Ac_2O, py, (91%), (v) DBU, DCM then mixed anhydride of **86**, BOPCl, TEA, DCM, (68%), (vi) HF.py, THF, (vii) LiOH, aq. THF then ion-exchange resin (H^+ form), (viii) HATU, DIPEA, THF, (40%, 3 steps).

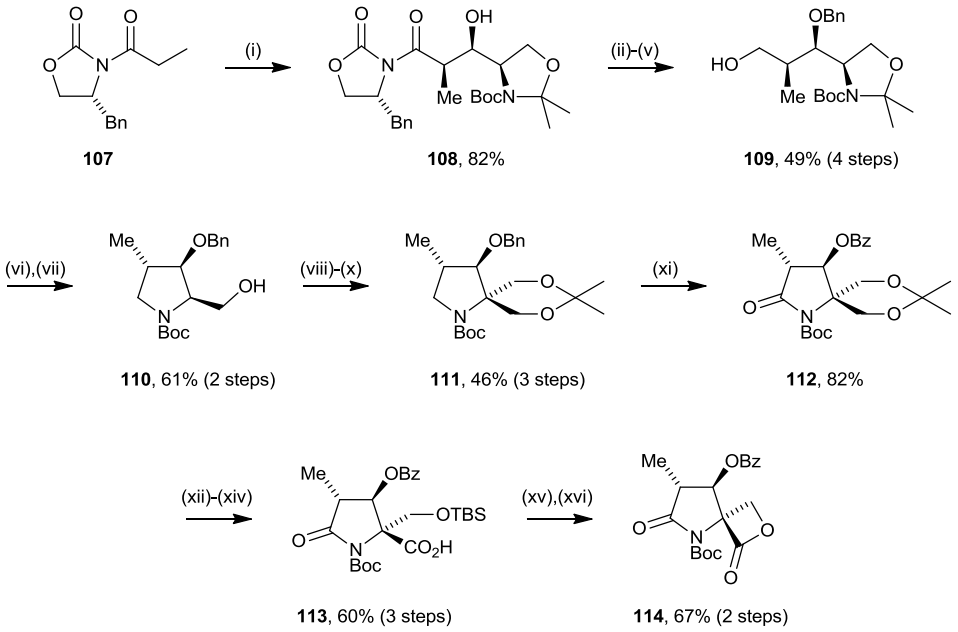
1.16 Synthetic studies of the oxazolomycins

Many groups have published work aimed at the synthesis of members of the oxazolomycin family, or fragments thereof. Most studies use the three fragment retrosynthesis to break down the molecules into simpler subunits and of particular interest to this thesis are those focussed on the right-hand unit consisting of the spiro fused β-lactone/γ-lactam ring system. As well as being a desirable target as part of an investigation into the chemistry of the oxazolomycin family, the β-lactone/γ-lactam unit has notable structural similarity to the pharmacophores of the omuralide and salinosporamide A, potent cancer cell cytotoxins that inhibit the 20S proteasome.[70]

1.16.1 Mohapatra's synthesis of the spiro-bicyclic γ-lactam/β-lactone 114

In 2006 Mohapartra *et al.* described the synthesis of the right-hand fragment γ-lactam/β-lactone unit found in the oxazolomycins.[71] The approach begins with an Evans' aldol reaction between (*R*)-oxazolidinone **107** and (*R*)-Garner's aldehyde, using Crimmins' modified method[72] with $TiCl_4$ catalysis to install the two stereocentres on the γ-lactam ring. The stereochemistry at the spiro centre is introduced by way of a selective mono-protection of a *gem*-dihydroxymethyl group. Thus, reaction of **107** and (*R*)-Garner's aldehyde gave **108** as the sole product in 82% yield, characterised by single crystal X-ray studies. Following treatment with lithium borohydride to give a diol, a series of protecting group manipulations

gave primary alcohol **109** in 49% yield. This was converted to the tosylate with TsCl and cyclisation was effected using TsOH in MeOH with concomitant removal of the acetonide group to give the pyrrolidine **110** in 61% yield over two steps. The alcohol of **110** was oxidised with IBX to give the corresponding aldehyde which was treated with formaldehyde and NaOH to install a *gem*-dihydroxymethyl group which was protected to give the isopropylidine derivative **111** in 46% yield. Compound **111** was then oxidised to pyrrolidinone **112** in 82% yield using $RuCl_3$ and $NaIO_4$. Removal of the isopropylidene group in the presence of TsOH regenerated the *gem*-dihydroxymethyl group which was selectively mono-protected with TBSCl. This selectivity was postulated to be a result of the *O*-Bz group blocking the approach of the sterically bulky electrophile. Oxidation of the remaining hydroxyl group gave compound **113** in 60% yield and desilylation followed by lactonisation under Mitsunobu conditions gave the spiro fused γ-lactam/β-lactone unit **114** in 67% yield (Scheme 1.21). The structure of **114** was confirmed using X-ray crystallography.

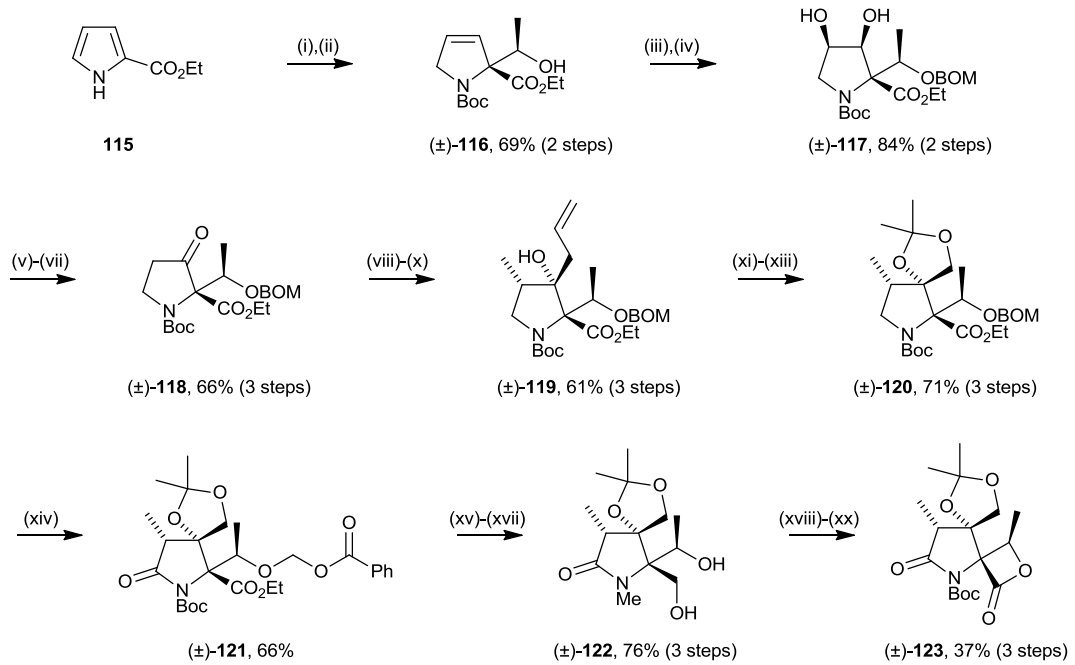


Scheme 1.21 *Reagents and conditions:* (i) (*R*)-Garner's aldehyde, $TiCl_4$, DIPEA, DCM, 0 °C, (82%), (ii) LiCl, $NaBH_4$, 2:1 EtOH:THF, rt, (75%), (iii) TBSCl, TEA, DMAP (cat.), DCM, rt, (82%), (iv) NaH, BnBr, DMF, 0 °C, (91%), (v) TBAF, THF, rt, (89%), (vi) TsCl, TEA, DCM, rt, (97%), (vii) TsOH, MeOH, rt, (63%), (viii) IBX, DMSO, THF, rt, (ix) H_2CO, 2 M NaOH, 7:1 THF:H_2O, rt, (55%, 2 steps), (x) DMP, TsOH, rt, (85%), (xi) $RuCl_3$, $NaIO_4$ (aq.), EtOAc, rt, (82%), (xii) TsOH, MeOH, rt, (86%), (xiii) TBSCl, imidazole, DCM, rt, (82%), (xiv) $RuCl_3$, $NaIO_4$ (aq.), EtOAc, rt, (86%), (xv) AcOH, THF, H_2O, rt, (77%), (xvi) PPh_3, DEAD, THF, −78 °C, rt, (88%).

1.16.2 Donohoe's synthesis of the right-hand fragment of KSM-2690 B

A 2007 publication by Donohoe *et al.* detailed a synthesis of the γ-lactam/β-lactone unit of KSM-2690 B **81g**, in racemic form.[73] **81g** possesses a C(16)-methyl substituent on the β-lactone ring, a feature also seen in the natural products 16-methyloxazolomycin **81d**, curromycin B **81f**, and KSM-2690 C **81h**, though **81d** and **f** have the reverse stereochemistry at C(16). Their approach began with an ammonia-free reductive aldol reaction of the commercially available pyrrole **115** and subsequent manipulation of the resulting functional groups produced (±)-**123**. The pyrrole **115** was protected as the *N*-Boc derivative and subjected to ammonia-free reductive aldol reaction conditions and, after transmetallation with magnesium bromide, was quenched with acetaldehyde. The resulting aldol adduct (±)-**116** was produced as a 4-5:1 mixture of chromatographically separable *anti*- and *syn*- isomers. Treatment of (±)-**116** with BOMCl protected the free hydroxyl, and dihydroxylation using modified Sharpless conditions gave the *syn*-diol (±)-**117** in 84% yield over two steps, resulting from selective attack of the face *anti* to the *O*-BOM group. A regioselective Mitsunobu reaction of the least hindered hydroxyl group using an iodide nucleophile followed by deiodination with indium hydride formed *in situ* gave a β-hydroxyester which was oxidised using Swern conditions to give the β-keto-ester (±)-**118** in 66% yield. The requisite methyl group was introduced by methylation of the enolate generated after treatment with NaHMDS and gave the isomer resulting from addition of the methyl group to the less hindered top face of the ring as the major one. This stereochemistry was reversed by deprotonation and reprotonation with the bulky proton source 2,6-di-*t*-butylphenol, selectively protonating the enolate from the top face. Allylmagnesium bromide was then used to carry out an addition to the ketone, resulting in homoallyl alcohol (±)-**119** in 61% yield over three steps. Ruthenium catalysed isomerisation to the allyl alcohol was followed by ozonolysis of the double bond and $NaBH_4$ reduction to the alcohol in one pot. The 1,2-diol was then converted to the acetonide (±)-**120** with dimethoxypropane in 71% yield. Oxidation with RuO_4 provided the lactam **121**, in 66% yield, in which the BOM group had also been oxidised at the benzylic position. Boc deprotection with $ZnBr_2$ and subsequent *N*-methylation followed removal of the oxidised BOM moiety and reduction of the ethyl ester with $LiBH_4$

gave diol (±)-**122** in 76% yield. Finally, formation of the β-lactone ring was achieved by selective oxidation of the primary alcohol to the corresponding acid by successive treatment with TEMPO/PhI(OAc)$_2$ and NaClO$_2$/NaH$_2$PO$_4$ buffer. Treatment with TsCl and pyridine gave (±)-**123** in 37% over the final three steps, the structure of which was confirmed by X-ray crystallography (Scheme 1.22).

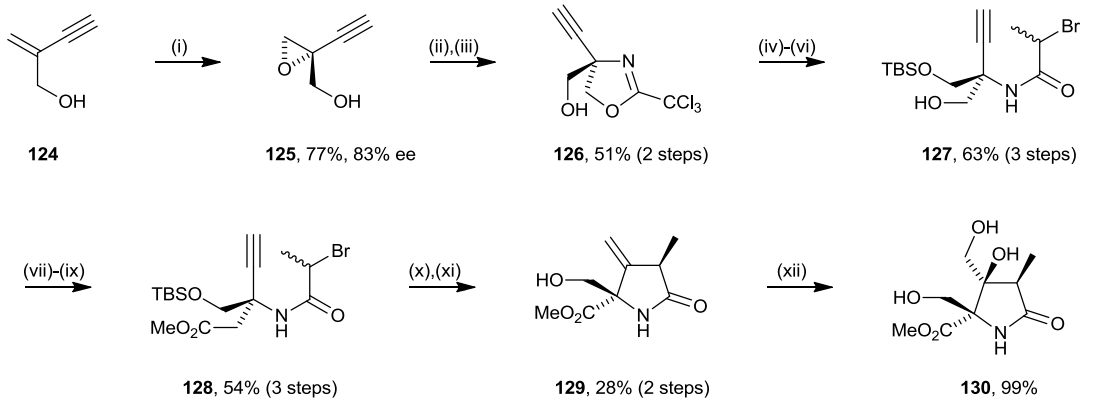


Scheme 1.22 *Reagents and conditions:* (i) Boc$_2$O, DMAP, DCM, (99%), (ii) LiDBB, THF, BMEA, −78 °C, MgBr$_2$.Et$_2$O/MeCHO, (70%, ~4:1 dr), (iii) BOMCl, *i*Pr$_2$NEt, DCM, (94%), (iv) cat. K$_2$OsO$_4$.2H$_2$O, K$_3$Fe(CN)$_6$, K$_2$CO$_3$, quinuclidine, *t*BuOH, H$_2$O, (90%), (v) PPh$_3$, DBAD, THF, rt then MeI, reflux, (vi) InCl$_3$, NaBH$_4$, MeCN, (70%, 2 steps), (vii) Swern, (95%), (viii) NaHMDS, 15-crown-5, THF, −78 °C then MeI (75%, 1:3.5 dr), (ix) NaHMDS, 15-crown-5, THF, −78 °C, then 2,6-di-*t*-butylphenol (96%, >10:1 dr), (x) allylmagnesium bromide, Et$_2$O, −78 °C, (86%), (xi) vinyloxytrimethylsilane, Grubbs II, toluene, reflux, (xii) O$_3$, DCM, MeOH, then NaBH$_4$, (xiii) CSA, 2,2-DMP, (71%, 3 steps), (xiv) cat. RuO$_2$.xH$_2$O, aq. NaIO$_4$, EtOAc, (66%), (xv) ZnBr$_2$, DCM, (xvi) Cs$_2$CO$_3$, MeI, (xvii) LiBH$_4$, MeOH, Et$_2$O, (76%, 3 steps), (xviii) TEMPO, PhI(OAc)$_2$, DCM, (93%), (xix) NaClO$_2$, NaH$_2$PO$_4$, *t*BuOH, H$_2$O, 2-methyl-2-butene, (xx) TsCl, py, DCM, −5 to 0 °C (40%, 2 steps).

1.16.3 Pattenden's synthesis of a common intermediate for the right-hand fragment of oxazolomycin A and neooxazolomycin

Pattenden *et al.* have described a synthetic approach to the pyrrolidinone triol **130** which serves as a common intermediate for the right-hand fragments of both oxazolomycin A **81a** and neooxazolomycin **82**, with conversion to both the spiro-fused β-lactone and fused γ-lactone plausible.[74] They use a Sharpless asymmetric epoxidation and a diastereoselective dihydroxylation to install the requisite stereocentres. Epoxidation of the enynol **124** under Sharpless conditions gave the epoxide **125** in 77% yield with an ee of 83% determined by

Mosher's ester analysis. Treatment with trichloroacetonitrile and DBU gave an acetimidate which was cyclised with $AlEt_2Cl$ to give the oxazoline **126** in 51% yield. TBS protection of the free hydroxyl followed by treatment with 1 M HCl revealed the ethynyl amine which was acylated with 2-bromopropionoyl chloride, giving **127** in 63% yield. The alcohol group of **127** was oxidised sequentially to the aldehyde and then carboxylic acid before being esterified using trimethylsilyldiazomethane giving the methyl ester **128** in 54% yield. A 2:1 mixture of α- and β-methylene-pyrrolidinone C(3) epimers resulted from the 5-*exo*-dig cyclisation of **128** when treated with Bu_3SnH and AIBN. These epimers were inseparable at this stage but after desilylation, the resulting primary alcohols were separated giving **129** in 28% yield over two steps as a single diastereoisomer. Finally, the triol **130** was furnished as a single diastereoisomer in 99% yield from the selective dihydroxylation of the top face of the 4-methylenepyrrolidinone using OsO_4-TMEDA (Scheme 1.23).

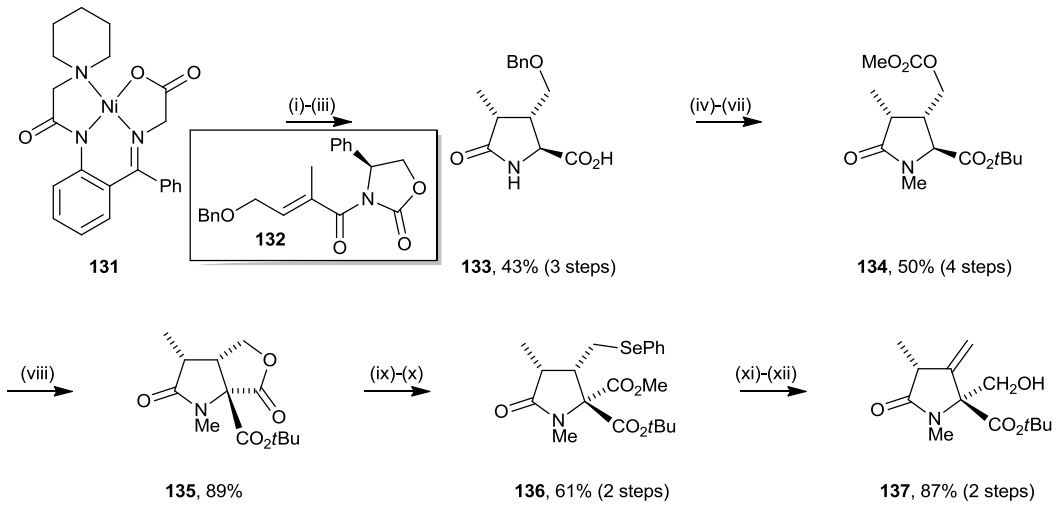


Scheme 1.23 *Reagents and conditions:* (i) 20% $Ti(OiPr)_4$, 26% D-(−)-DIPT, cumene hydroperoxide, DCM, −12 °C, 15 h, (77%, 83% ee), (ii) CCl_3CN, DBU, DCM, 0 °C, 1 h, (81%), (iii) $AlEt_2Cl$, DCM, 0 °C to rt, 15 h, (63%), (iv) TBSCl, imidazole, DCM, rt, 15 h, (83%), (v) 1M HCl, THF, rt, 3 h, (vi) 2-bromopropionoyl chloride, $NaHCO_3$, DCM, rt, 2 h, (76%, 2 steps) (vii) TPAP, NMO, DCM, rt, 2 h, (88%), (viii) $NaClO_2$, NaH_2PO_4, *t*BuOH, 2-methyl-2-butene, rt, 6 h, (83%), (ix) TMS-CHN_2, 1:2.5 MeOH:C_6H_6, rt, 1 h, (75%), (x) Bu_3SnH, AIBN, toluene, reflux, 2 h, (73%, 2:1 dr), (xi) TsOH, THF, H_2O, rt, 15 h, (39%, single diast.), (xii) OsO_4, TMEDA, DCM, −78 °C to rt, 3 h, (99%).

1.16.4 Ohfune's synthesis of methylene-pyrrolidine intermediate 137

An approach to a similar methylenepyrrolidinone intermediate has been described by Ohfune *et al.* in their paper published in 2008.[75] A key step in this synthesis is the reaction between the glycine Schiff base **131** and acrylate unit **132**. The stereoselective Michael addition in the presence of the organic base 1,5,7-triazabicyclo-[4,4,0]-dec-5-ene (TBD) gave the Michael adduct as a mixture of diastereoisomers in a 68:8:7:7:7:3 ratio. The pyrrolidinone **133** was

accessed in 43% yield by an acidic work up followed by treatment with base. The acid moiety was converted to the *t*-butyl ester and the nitrogen of the pyrrolidinone ring was methylated with MeI. Hydrogenolysis was used to cleave the *O*-Bn group and a methylcarbonate group was installed, giving compound **134** in 50% yield over four steps. Treatment with NaHMDS, followed by diazomethane, gave γ-butyrolactone **135** in 89% yield. The lactone ring opening was carried out by treatment with Ph_2Se_2 and $NaBH_4$ and esterification with diazomethane gave the diester **136** in 61% yield. Oxidative removal of the phenylselenyl group gave an exomethylene compound and chemoselective reduction of the methyl ester with $Zn(BH_4)_2$ gave the pyroglutamate **137** in 87% yield (Scheme 1.24).



Scheme 1.24 *Reagents and conditions:* (i) TBD (15 mol%), DMF, rt, (62%), (ii) 3 M HCl, (ii) 1 M NaOH, DCM, (70%, 2 steps), (iv) *t*BuOAc, 70% $HClO_4$, (v) MeI, NaH, THF, (73%, 2 steps), (vi) H_2, Pd/C, MeOH, (vii) $ClCO_2Me$, py, (69%, 2 steps), (viii) NaHMDS, THF then CH_2N_2, (89%), (ix)Ph_2Se_2, $NaBH_4$, EtOH, (x) CH_2N_2, (61%, 2 steps), (xi) 30% H_2O_2, THF, (97%), (xii) $Zn(BH_4)_2$, Et_2O, (90%).

1.17 Project aims

This thesis is divided into two parts, one focussing on mimics of the natural product 16-methyloxazolomycin **81d** and one concerned with lemonomycin **4** analogues. The work on 16-methyloxazolomycin aims to develop methodology to synthesise mimics for the γ-lactam/β-lactone fragment as densely functionalised tetramic acids and pyroglutamates and to assess their biological activity. The work on lemonomycin aims to begin the development of methodology for the synthesis of analogues based on the diazo-bicyclic fused piperazine/pyrrolidine core of the natural product using an unusual disconnection approach. This work is described in detail in the following chapters.

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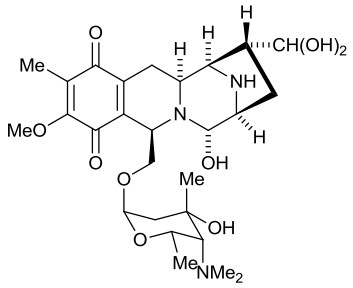
CHAPTER 2: RESULTS AND DISCUSSION

Synthesis of 2,5-disubstituted pyrrolidines as precursors to mimics for lemonomycin

This chapter describes studies aimed at the synthesis of mimics for the core 3,8-diazabicyclo[3.2.1]octane unit contained in the natural product lemonomycin **4**.



4

Figure 2.1 The structure of lemonomycin **4**.

A rapid synthesis of the core structure of lemonomycin is desired due to its potent, broad-spectrum antibiotic activity. A diverted total synthesis was envisaged alongside a program of investigation into the behaviour of mimics for this natural product which it was hoped may provide important and instructive information regarding structure-activity relationships. It was hoped that a library of analogues based on a simplified structural skeleton may be synthesised and biologically tested. It has been shown in the Moloney group's studies of the oxazolomycin natural products that simplified mimics can still possess antibacterial activity.[1,2,3,4]

It was envisaged that the following retrosynthesis could furnish lemonomycin (Figure 2.2). After attaining fragment **139**, a *cis*-2,5-disubstituted pyrrolidine, a ring closure between the amine and ester groups of the substituents, facilitated by their proximal *cis*-relationship, would give the 3,8-diazabicyclo[3.2.1]octane **138**. It is possible to imagine that this system would have a significant degree of structural rigidity; this would lend itself well to structure activity studies of various analogues with similar three dimensional arrangements. Subsequent introduction of the 2,6-dideoxyamino sugar moiety would lead towards the natural product **4**. As such, synthetic routes towards the synthesis of 2,5-disubstituted pyrrolidines would be investigated.

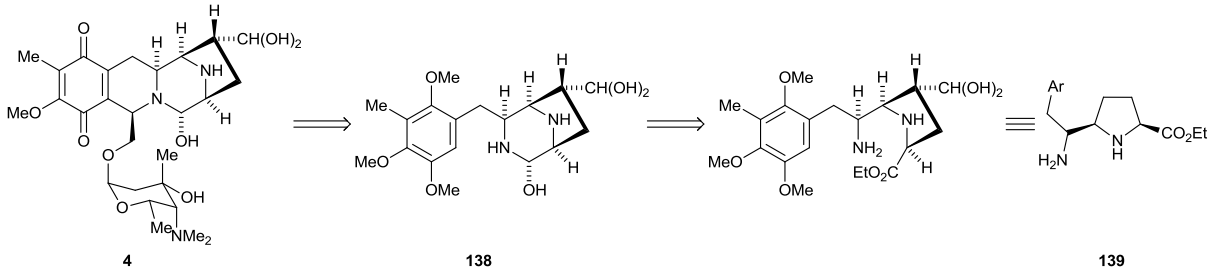


Figure 2.2 Retrosynthesis of lemonomycin **4**.

2.1 Previous work on the synthesis of 2,5-disubstituted pyrrolidines

Pyrrolidine scaffolds are found in many bioactive natural products, such as cocaine,[5] carzinophilin,[6] the novel glutamate antagonist kaitocephalin,[7] epibatidine,[8] the neuraminidase inhibitors A-315675 and A-192558,[9] and the protease inhibitor aeruginosin,[10] as well as lemonomycin.[11] Amongst the biological activity that has been reported, they have been shown to possess antifungal, antibacterial, insecticidal, haemolytic, anticholinergenic, and neuroexcitatory properties.[12] They are also of interest for their use as chiral auxiliaries[13] and their ability to induce well defined molecular architecture in peptidomimetic compounds. The lactam **141** (a selective antagonist for the NK-2 receptor) was designed as a mimetic for a β-turn such as that shown in **140** (Figure 2.3);[14] details of this and other peptidomimetic pyrrolidine scaffolds may be found in a review on the subject by Hanessian *et al.*.[12,15] Pyroglutamic acid **142** is an obvious candidate for the synthesis of functionalised pyrrolidines as the heterocyclic ring is present as well as a chiral centre at C(2); chemistry for the stereo-controlled manipulation of substituents at the C(3) and C(4) ring positions has been reported by Moloney *et al.* giving access to diversely substituted pyroglutamates.[16,17,18,19]

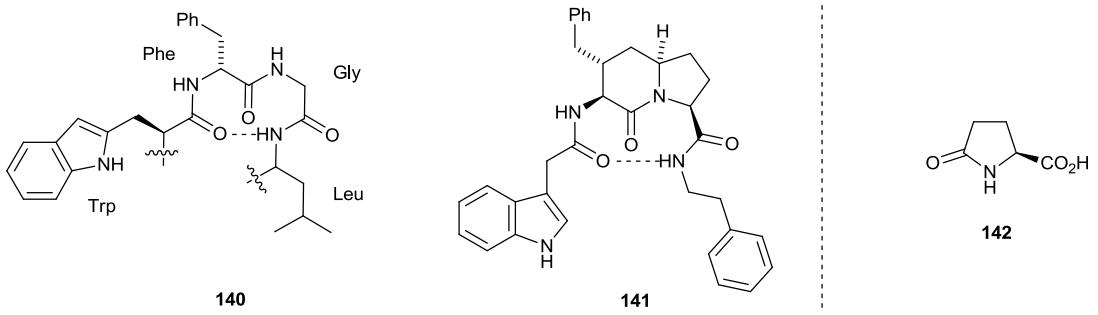
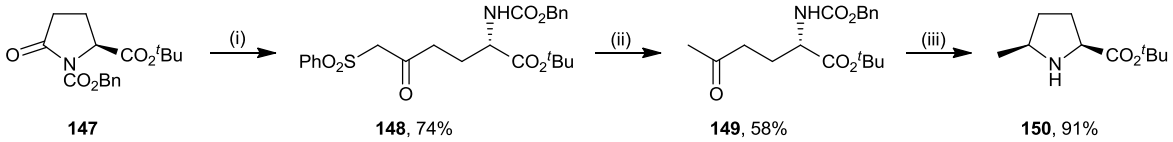


Figure 2.3 Partial structure of a hexapeptide including a Trp-Phe-Gly-Leu residue **140**, the β-turn mimetic **141** and pyroglutamic acid **142**.

2.1.2 Reduction of lactam carbonyl

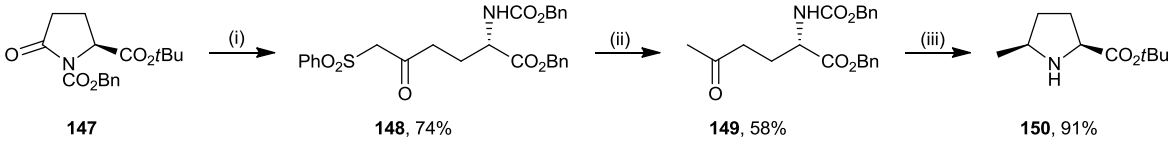
Approaches to the synthesis of 2,5-disubstituted pyrrolidines from pyroglutamic acid have focused on the modification of the lactam carbonyl group to introduce the C(5) substituent. Literature chemistry for this transformation relies on four main routes, as briefly outlined below. Reduction of the lactam carbonyl with DIBAL-H, $LiEt_3BH$, $NaBH_4$ or RedAl can produce a hemiaminal which will eliminate to form an *N*-acyliminium ion. This ion will react with a variety of nucleophiles such as organocuprates, allyl silanes, and stabilised phosphonates to give substituents at the C(5) position.[12] Langlois exploited this chemistry in a synthesis of the marine natural product **146**. *N*-Carboxymethyl-benzylpyroglutamate **143** was reduced with DIBAL-H and treated with acidified methanol to give the species **144** in 90% yield. This was then reacted with Me_3SiCN with the Lewis acid $SnCl_4$ to give the cyano product **145** in 86% yield as a 7:3 ratio of *cis*:*trans* pyrrolidines. Subsequent treatment with HCl produced the natural product in 86% yield (Scheme 2.1).[20]



Scheme 2.1 *Reagents and conditions:* (i) DIBAL-H, (ii) TsOH, MeOH, (iii) Me_3SiCN, $SnCl_4$, (iv) 6 N HCl, 24 h, propylene oxide, EtOH.

2.1.3 Ring opening/reclosure

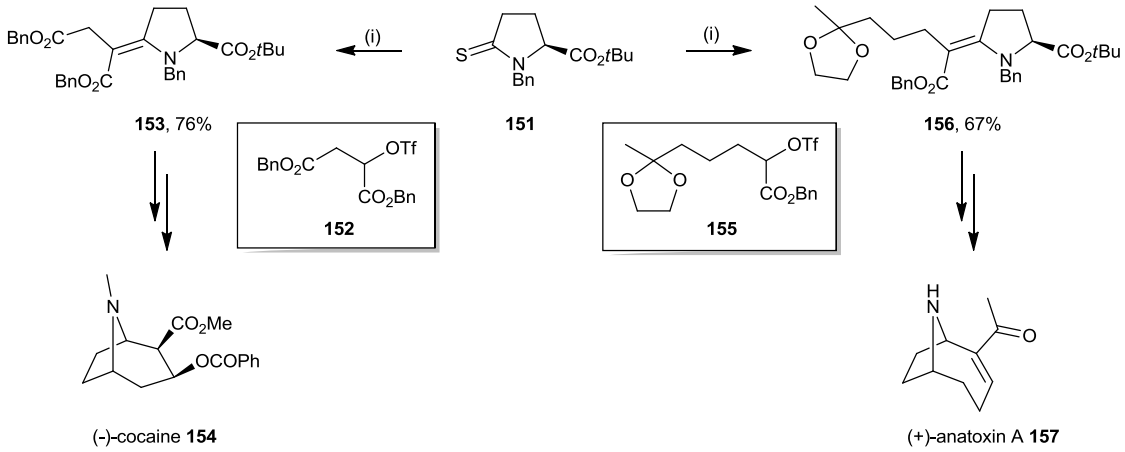
Another route involves the opening of the lactam ring followed by reclosure. Langlois also reported on the ring-opening of γ-lactams with lithium methylphenylsulphone to synthesise *cis*-2,5-disubstituted pyrrolidines.[21] Pyroglutamate **147** was ring-opened to form **148** in 74% yield after treatment with $PhSO_2CH_2Li$ in THF. This was then desulphonylated with 4-5% Na-Hg amalgam leading to **149** in 58% yield. The methylketone **149** underwent a cyclisation-reduction domino sequence in *N*-deprotection conditions (H_2, 1 atm, Pearlman's catalyst, rt) to give the pyrrolidine **150** in 91% yield (Scheme 2.2).



Scheme 2.2 *Reagents and conditions:* (i) $PhSO_2CH_2Li$, THF, −72 °C, (ii) 4-5% Na-Hg amalgam, (iii) H_2, 1 atm, Pearlman's catalyst, rt.

2.1.4 Eschenmoser coupling reaction

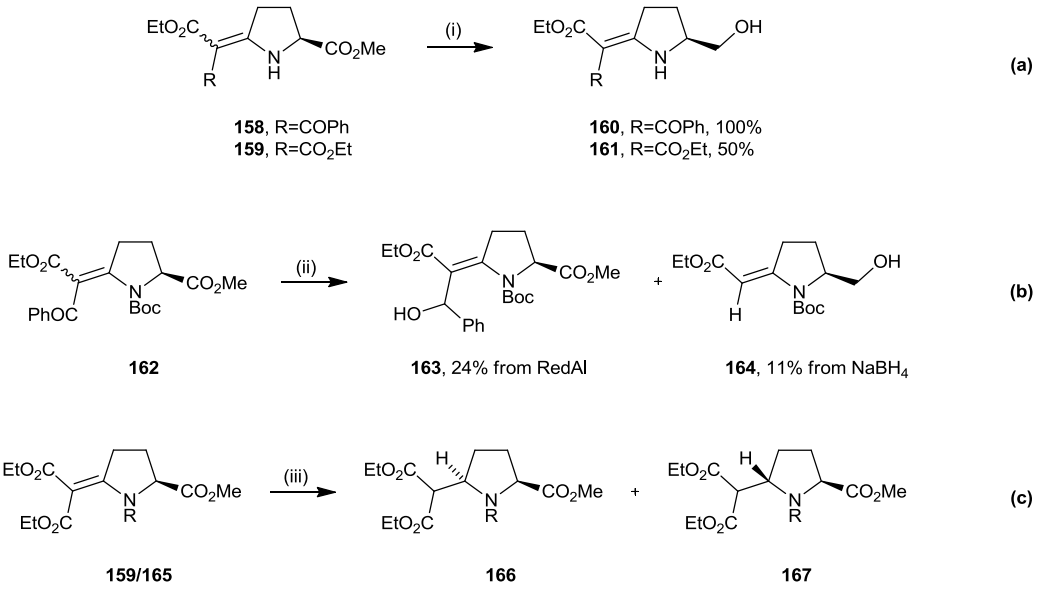
The Eschenmoser coupling reaction converts a thioamide into an enamine *via* an intermediate thioiminium salt from which elemental sulphur is extruded. Rapoport has extensively used Eschenmoser coupling reactions in the synthesis of natural products, and demonstrated that the reaction is most efficient for doubly activated ester systems, although conditions for less reactive systems have also been identified. Thus, the coupling between the thiolactam **151** derived from pyroglutamic acid and the triflate **152** is carried out in MeCN with the addition of PPh_3 and TEA or *N*-methylpiperidine to give the intermediate **153**, used in a synthesis of cocaine **154**, in 76% yield.[5] The same reaction conditions are used to couple the thiolactam and the carboxybenzyl triflate **155** to give the intermediate **156** in 67% yield; subsequent synthetic transformations gave anatoxin-A **157** (Scheme 2.3).[22]



Scheme 2.3 *Reagents and conditions:* (i) **152**/**155** MeCN, then PPh_3, DCM, TEA/*N*-methylpiperidine.

Following Rapoport's work, the use of Eschenmoser coupling reactions was investigated in the Moloney group for the synthesis of *cis* and *trans*-2,5-disubstituted pyrrolidines by the reduction of β-enamines.[23,24] The enamines **158** and **159** were synthesised according to the procedure described by Bachi *et al.*.[25] Attempted reduction of the enamines with $NaBH_4$ led to the production of alcohols **160** and **161** in 100 and 50% yields, resulting from the reduction of the C(2) ester group (Scheme 2.4, (a)). The non-reactivity was postulated to be due to the mesomeric donation of the nitrogen lone pair into the enamine system, and a Boc protected derivative was prepared in 73% yield by reaction of **158** with Boc_2O. This less electron-rich enamine system also demonstrated an apparent lack of reactivity in its reaction with Red-Al and $NaBH_4$. In the former, the alcohol **163** was produced in 24% yield from the reduction of

the ketone functionality. After the reaction with $NaBH_4$, the product was **164**, resulting from the reduction of the C(2) ester and debenzoylation, isolated in 11% yield (Scheme 2.4, (b)). Subsequently, hydrogenation protocols were tested, and it was discovered that the enamines may be reduced at high pressures of H_2, to give either the *cis*- or *trans*-2,5-disubstituted pyrrolidines. When **159** was treated with $PtO_2.H_2O$ in 3:1 AcOH:TFA with 4.5 atm of H_2, a 1:2 mixture of the *cis*- and *trans*- pyrrolidines **166** and **167** was formed. When the benzoyl derivative **165** was treated with 10% Pd/C in 10:1 EtOH:DCM with 5 atm of H_2, a 9:1 mixture of *cis*- and *trans*- pyrrolidines **166** and **167** resulted (Scheme 2.4, (c)). A protocol using $NaBH_3CN$ also reduced the enamine, but also gave an inseparable impurity.



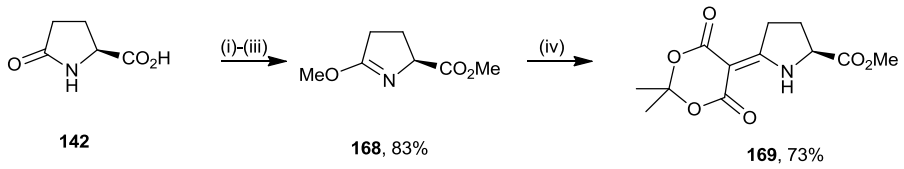
R	(iii) Reagents and conditions	Yield (%)	Ratio 166:167
H	10% Pd/C or Pt/C, H_2 (4.5 atm), 3:1 AcOH:TFA, rt 48 h	0	-
H	$PtO_2.H_2O$, H_2 (4.5 atm), 3:1 AcOH:TFA, rt, 48 h	100	1:2
COPh	10% Pd/C, H_2 (5 atm), 10:1 EtOH:DCM, rt 24 h	79	9:1
H	$NaBH_3CN$, EtOH, pH 3-5, 5 h	63 (impure)	-

Scheme 2.4 *Reagents and conditions:* (i) $NaBH_4$, EtOH, rt, (ii) Red-Al, 8:2 Et_2O:toluene, reflux, 24 h or $NaBH_4$, EtOH, reflux, 23 h, (iii) see table.

2.1.5 Lactim ether chemistry

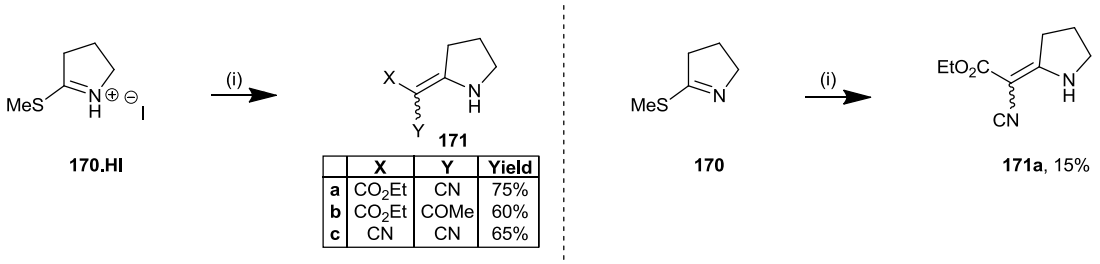
An alternative method for the synthesis of 2,5-disubstituted pyrrolidines involves the use of lactim ether chemistry. The reactions of lactim ethers with nucleophiles are relatively well documented in the literature.[26] For example, Nagasaka *et al.*[27] used the reaction of the lactim ether **168**, synthesised from pyroglutamic acid **142** in 83% yield, with the enolate of Meldrum's acid to furnish **169** in 73% yield (Scheme 2.5). Lactim ethers have, however, also

been reported to be problematic to form, with a large amount of the *N*-alkyl species being isolated, and that once formed, the *O*-alkyl species will readily rearrange to give the *N*-alkyl species on heating.[28]



Scheme 2.5 *Reagents and conditions:* (i) MeOH, H_2SO_4, (ii) Me_2SO_4, (iii) $KHCO_3$, (iv) Meldrum's acid, TEA.

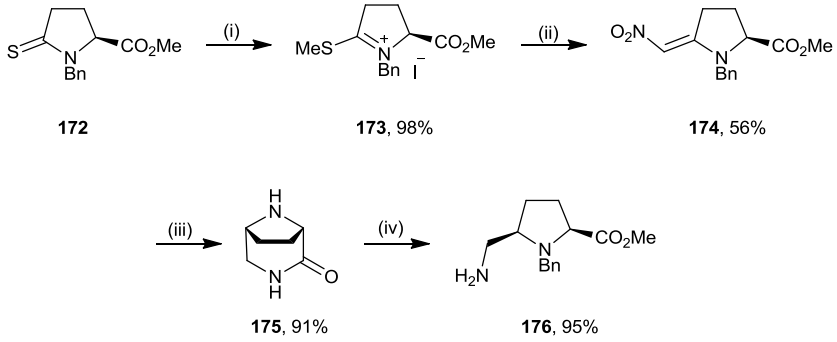
Fewer examples of the reactions of equivalent lactim thioethers have been documented, though the use of various nucleophiles has been reported. For example, Singh *et al.* reported that, when treated with activated methylene compounds, KF and TEBA in DCM, the hydroiodide salt **170.HI** formed enamines **171** in 60-75% yield. The reaction of the free lactim thioether **170** was reported to form **171a** in only 15% yield under the same conditions (Scheme 2.6).[29]



	X	Y	Yield
a	CO_2Et	CN	75%
b	CO_2Et	COMe	60%
c	CN	CN	65%

Scheme 2.6 *Reagents and conditions:* (i) XCH_2Y, KF, TEBA, DCM.

Anand *et al.* have also described the reaction of a lactim thioether derived from pyroglutamic acid reacting with a carbon nucleophile, this time in the form of nitromethane.[30] The lactim thioether salt **173** is synthesised from the analogous thiolactam **172** in 98% yield by treatment with MeI. The coupling reaction took place between nitromethane and **173** in DMF with an excess of TEA present to give the *E*-isomer **174** in 56% yield. This was then hydrogenated to reduce the nitro and enamine functionalities, and the product spontaneously cyclised to give the conformationally constrained piperazinone **175** in 91% yield. This was then ring opened to give the *cis*-2,5-disubstituted pyrrolidine **176** in 95% yield (Scheme 2.7).



Scheme 2.7 *Reagents and conditions:* (i) MeI, 2 h, (ii) nitromethane, TEA, DMF, rt, 2 h, (iii) H_2 (45 psi), 10% Pd-C, MeOH, 24 h, (iv) 2 N HCl in MeOH, rt, 1 h.

2.2 Synthesis of pyrrolidines from pyroglutamic acid

2.2.1 Eschenmoser coupling reaction

It was envisaged that an Eschenmoser protocol may be utilised in the preparation of 2,5-disubstitued pyrrolidines bearing the required 1′-amino substituent (Figure 2.4). Hussaini and Moloney reported the synthesis of 2,5-disubstituted pyrrolidines using an Eschenmoser coupling reaction between the thiolactam **177** and diethylbromomalonate and ethyl 2-bromo-3-oxo-3-phenylpropanoate **183**, followed by hydrogenation to give the *cis* and *trans* product.[23,24] More recently, the reduction of enamines to give 2,2,5-trisubstituted pyrrolidines under milder conditions using $NaBH_3CN$ has been reported.[31]

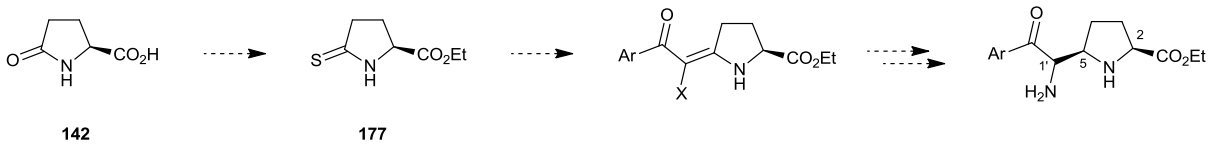
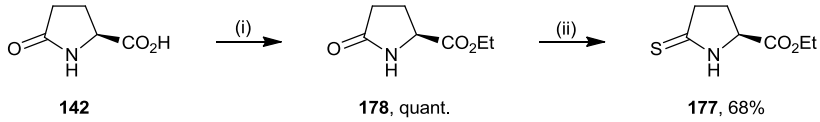


Figure 2.4 Envisaged synthesis of 2,5-disubstituted pyrrolidines by Eschenmoser sulphide contraction.

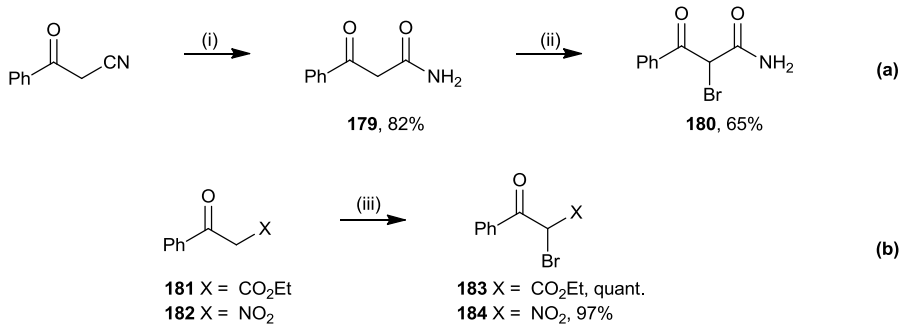
The thiolactam **177** was synthesised according to the published method[25] in two steps from (*S*)-pyroglutamic acid **142**; first it was converted to the ethyl ester **178** in quantitative yield, before being thionated with Lawesson's reagent in 68% yield (Scheme 2.8).



Scheme 2.8 *Reagents and conditions:* (i) H_2SO_4, EtOH, tol, 140 °C, 16 h, (ii) Lawesson's reagent, DCM, rt, 1.5 h.

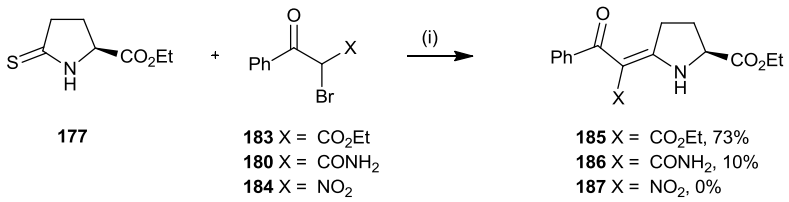
The electrophiles previously reported did not contain any nitrogen atoms, and so the suitable range of electrophiles needed to be expanded if it was going to be utilised in the synthesis of

lemonomycin mimics. Ethyl 2-bromo-3-oxo-3-phenylpropanoate **183** and nitrogen-containing electrophiles **180** and **184** were synthesised according to literature methods. The amido electrophile **180** was synthesised from benzoylacetonitrile by first undergoing acid-catalysed hydrolysis to give the amide **179** in 82% yield, and bromination of the central carbon using $CuBr_2$ in 65% yield (Scheme 2.9 (a)).[32] The electrophiles **183** and **184** were synthesised by a bromination of the enolic carbon in ethyl 2-benzoylethanoate **181** and benzoylnitromethane **182** using Br_2 in CCl_4 in very high yield (Scheme 2.9 (b)).[33]



Scheme 2.9 *Reagents and conditions:* (i) c.H_2SO_4, rt, 4 h, (ii) $CuBr_2$, EtOAc, rt, 6 h, (iii) Br_2, CCl_4, 5°C to rt, 3 h.

These electrophiles were treated with $NaHCO_3$ and added to a solution of thiolactam **177** (Scheme 2.10). The ester substituted electrophile **183** was successfully coupled to the thiolactam to give the enamine **185** in 73% yield after purification. The reaction of the amido electrophile **180** resulted in the isolation of the desired product **186** in 10% yield and a portion of **179** in 42% yield. None of the thiolactam was re-isolated. Following the reaction of the nitro-substituted electrophile **184**, no identifiable products were isolated, and neither in the crude ^{1}H NMR spectrum nor in that of the chromatography fractions were there any peaks consistent with the presence of the thiolactam starting material.

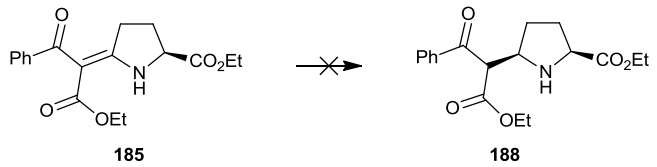


Scheme 2.10 *Reagents and conditions:* (i) $NaHCO_3$, DCM, 40-50 °C, 24 h.

As the nitro electrophile **184** did not give the enamine product, the only way to introduce the requisite nitrogen atom is through the use of the amido electrophile **180**. This route would require a rearrangement reaction to install the nitrogen at the correct position, as the

connectivity of the C and N atoms would need to be reversed. As the development of an optimised rearrangement protocol may require substantial quantities of material, the 10% yield of the enamine was unacceptable. As a result, an alternative approach to the synthesis of enamines of this type was sought.

The enamine **185** was subjected to the reported conditions for the reduction of trisubstituted enamines[31] and was stirred at rt with $NaBH_3CN$ in MeOH, acidified to pH 5 with HCl (Scheme 2.11). Analysis of the mixture by TLC indicated that no reaction had taken place after a period of 6 hours. The crude ^1H NMR was consistent with unreacted starting material indicating that forcing hydrogenation conditions would be required for the reduction of these systems.



Scheme 2.11 *Reagents and conditions:* (i) $NaBH_3CN$, MeOH, HCl (pH 5-6), 0 °C, rt, 6 h.

2.2.2 Lactim thioether coupling

It was envisaged that the substitution reactions of lactim thioethers such as **189** may provide an alternative synthetic route to disubstituted pyrrolidines possessing a 1′-amino functionality, and reported examples in the literature using nitro-substituted nucleophiles gave encouragement in this respect (Figure 2.5).[30] Preliminary work[34] within the group has begun to explore the scope of reactions using lactim thioethers, and it has been found that for an efficient reaction, activated methylene compounds were required, and a re-investigation was warranted.

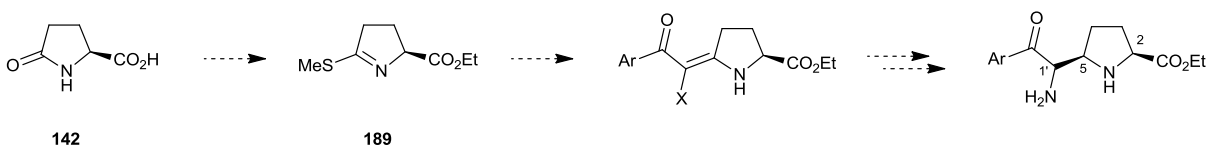
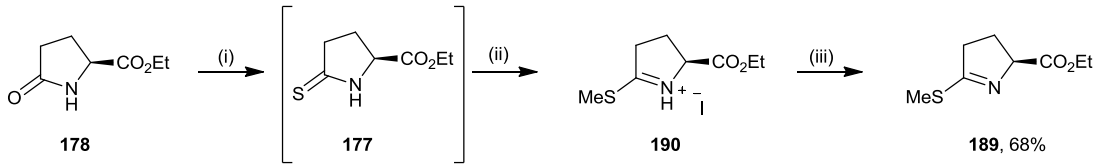


Figure 2.5 Envisaged synthesis of 2,5-disubstituted pyrrolidines using lactim thioether chemistry.

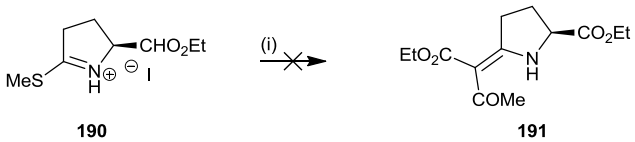
The lactim thioether **189** was first synthesised from the thiolactam **177** in 94% yield using MeI in THF stirred at rt for 6 h. Though this reaction proceeded almost quantitatively, the

purification of thiolactam **177** was problematic and time consuming on a large (>1 g) scale, generally giving low yields. An experiment using the crude thiolactam (passed through a plug of silica) immediately after formation was carried out. Following the reaction of the pyroglutamate **178** with Lawesson's reagent, the crude residue was immediately dissolved in acetone and MeI was added. The hydro-iodide salt **190** precipitated out of this solution and allowed for purification by a simple filtration at this stage. Subsequent basic aqueous washing of the salt furnished the free lactim thioether **189** in 68% yield from the lactam **178**, representing an increase in the overall yield (Scheme 2.12).



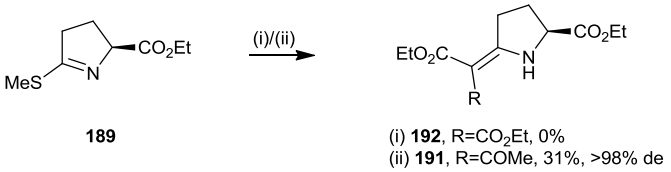
Scheme 2.12 *Reagents and conditions:* (i) Lawesson's reagent, DCM, rt, 1.5 h, (ii) MeI, acetone, rt, 15 min, (iii) K_2CO_3, H_2O, DCM.

The procedure reported by Singh *et al.*[29] was attempted on the hydroiodide salt **190**, by treating it with ethyl acetoacetate, KF and TEBA at room temperature. When TLC analysis showed that the starting materials were still present after 7 hours, the temperature was raised to 30 °C overnight, and then to 50 °C for a further 4 hours. However, unreacted starting materials were returned after work-up (Scheme 2.13).



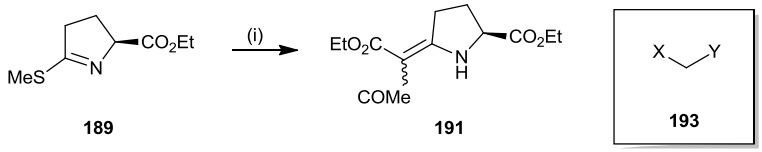
Scheme 2.13 *Reagents and conditions:* (i) EAA, KF, TEBA, DCM, rt-50 °C, 30 h.

A reaction between the free thioether and the enolate of diethyl malonate, formed by first treating the malonate with a base, was then attempted. Diethyl malonate was reacted with either NaH at 0 °C or BuLi at −78 °C. The lactim thioether **189** was then added to the mixtures and they were heated overnight (Scheme 2.14). Neither reaction showed any of the desired product **192** and only starting materials were obtained. A procedure using a higher temperature to try to force the reaction was then carried out, by heating **189** and ethyl acetoacetate to 120 °C in DMSO for 24 h. This procedure was successful and the resulting enamine **191** was isolated in 31% yield.



Scheme 2.14 *Reagents and conditions:* (i) Diethyl malonate, NaH, THF, 0-60 °C, 16 h, or Diethyl malonate, BuLi, toluene, −78 °C-65 °C, 16 h, (ii) EAA, DMSO, 120 °C, 24 h.

Following this success, the reactions of thiolactim ether **189** and ethyl acetoacetate were tested in a variety of solvents. In some cases, TEA was also added to see if this would accelerate the reaction. It was possible to measure the % conversion of the reaction by comparison of the integration of the peaks corresponding to C(2)*H* for the thioether **189** and the enamine **191** in the crude ^{1}H NMR spectra, which are seen at 4.7 and 4.5 ppm respectively. The results of these optimisation studies are outlined in the table below (Scheme 2.15). The results when using DMF and DMSO were similar, with slightly higher conversion being seen in the case of DMSO (Entries 1 and 2). In MeCN, DCM, and acetone, no product was identified. In the reactions carried out in methanol and $CHCl_3$, some changes to the starting material were observed, but unequivocal formation of products could not be established.

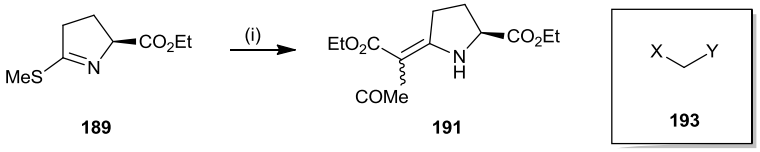


Entry	X	Y	Solvent	Base	Temperature	Time	Ratio of 189:191
1	CO_2Et	COMe	DMF	none	120 °C	24 h	74:26
				TEA	120 °C	~20 h	81:19
2	CO_2Et	COMe	DMSO	none	120 °C	~20 h	56:44
				TEA	rt	~20 h	100:0
					120 °C	24 h	59:41
						4 d	8:92
3	CO_2Et	COMe	MeCN	none	85 °C	20 h	100:0
				TEA	85 °C	20 h	100:0
4	CO_2Et	COMe	$CHCl_3$	none	65 °C	22 h	100:0
				TEA	65 °C	25 h	ester hydrolysis/decomposition
5	CO_2Et	COMe	DCM	TEA	40 °C	22 h	100:0
6	CO_2Et	COMe	Acetone	TEA	60 °C	40 h	100:0
7	CO_2Et	COMe	MeOH	TEA	65 °C	25 h	100:0
						44 h	partial decomposition

Scheme 2.15 *Reagents and conditions:* (i) **193**, see table.

As DMF and DMSO were the only solvents in which the reaction proceeded, DMSO was chosen for its lesser toxicity, and the reaction was carried out with other bases to see if the

conversion could be improved. Bases tested were TEA, DBU, K_2CO_3, and KO*t*Bu; only the reaction using TEA gave any of the desired product (Scheme 2.16). Optimal conditions used DMSO as the solvent and 1.1 eq. of TEA as a base.

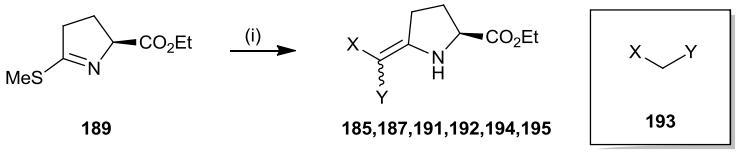


189 **191** **193**

Entry	X	Y	Solvent	Base	Temperature	Time	Ratio 189:191
1	CO_2Et	COMe	DMSO	TEA	120 °C	24 h	59:41
2	CO_2Et	COMe	DMSO	DBU	120 °C	24 h	partial decomposition
3	CO_2Et	COMe	DMSO	K_2CO_3	120 °C	24 h	100:0
						4 d	Partial decomposition
4	CO_2Et	COMe	DMSO	KO*t*Bu	120 °C	24 h	100:0
						4 d	100:0

Scheme 2.16 *Reagents and conditions:* (i) **193**, see table.

These conditions were then used with a range of activated methylene nucleophiles (Scheme 2.17). The % conversion varied between the different methylene nucleophiles. Notably, the CN substituted nucleophile was the only one which went to completion and **194** was isolated in 78% yield (Entry 2) as a 62:38 mixture of geometric isomers. This reaction also proceeded most quickly, requiring only 20 h to reach completion, whereas a number of the others were left for several days to achieve significant conversion. Of particular interest was the reaction of benzoylnitromethane (Entry 6) which would introduce a nitrogen atom at the desired location in the molecular skeleton. The first reaction gave a crude mixture with a complex ^{1}H NMR spectrum, indicating that decomposition had occurred. The experiment was repeated at a lower temperature of 60 °C and a 20% isolated yield of enamine **187** was achieved. Subsequent reduction of this species could give the desired 1′-amino substituent and allow for investigations into the second cyclisation to be carried out. Compound **187** was isolated as a single diastereoisomer, although it is not known whether it was the *E* or *Z* isomer. Compound **192** was also isolated in 12% yield following the reaction with diethyl malonate (Entry 5).

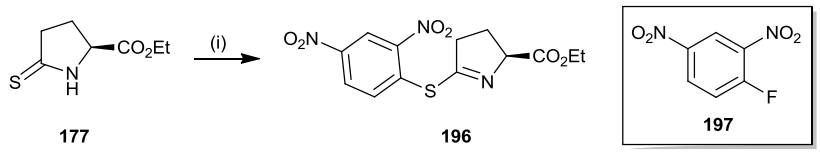


189 185,187,191,192,194,195 193

Entry	X	Y	Temperature	Time	Conversion	Product	Yield
1	CO_2Et	COMe	120 °C	4 d	92%	**191**	-
2	CO_2Et	CN	120 °C	20 h	100%	**194**	78%, 62:38 dr
3	CO_2Et	NCOMe	120 °C	20 h	0%	**195**	
				4 d	0%		-
4	CO_2Et	COPh	120 °C	20 h	15%	**185**	
				4 d	30%		-
5	CO_2Et	CO_2Et	120 °C	20 h	0%	**192**	
				4 d	58%		12%
6	COPh	NO_2	120 °C	18 h	decomposed	**187**	-
			60 °C	18 h	14%		
				3 d	30%		20%, >98% de

Scheme 2.17 *Reagents and conditions:* (i) MeI, K_2CO_3, THF, (ii) **193**, TEA, DMSO.

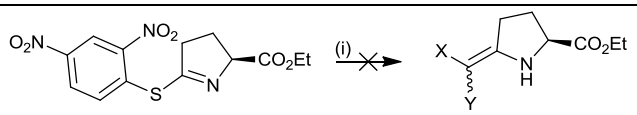
It was hoped that the introduction of a better leaving group, in the form of a 2,4-dinitrophenyl group, at the *S*-alkyl position would increase the reactivity of the 2*H*-pyrrole towards the desired nucleophiles. The aromatic thiolactim ether **196** was easily synthesised by treating the thiolactam **177** with 2,4-dinitrofluorobenzene **197** and K_2CO_3 in THF, but was found to be unstable to chromatography and could therefore not be separated from the excess **197** present in the reaction mixture (Scheme 2.18). A reaction using 1.0 equivalent of **197** did not proceed and gave unreacted thiolactam.



177 196 197

Scheme 2.18 *Reagents and conditions:* (i) 3.0 eq. **197**, K_2CO_3, THF, 50 °C, 7 h.

Attempts were made to carry out the coupling reaction using the crude thiolactim ether **196** under similar conditions to previous experiments, in DMSO with TEA heated to 60 °C, to avoid decomposition of the sensitive reagents. Only diethylmalonate was observed in the ^1H NMR spectrum of the crude material, and removal of the TEA did not change this outcome. Attempted reactions with benzoylnitromethane in $CHCl_3$ and MeCN were also unsuccessful (Scheme 2.19).

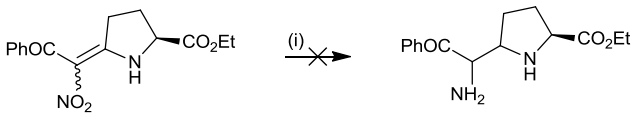


196 **191**, **187**

Entry	*Reagents and conditions*	Outcome
1	Diethyl malonate, DMSO, TEA, 60 °C, 18 h	DEM only in ^{1}H NMR spectrum
2	Diethyl malonate, DMSO, 100 °C, 18 h	DEM only in ^{1}H NMR spectrum
3	Benzoylnitromethane, $CHCl_3$, 60 °C, 18 h	Complex mixture
4	Benzoylnitromethane, MeCN, 60 °C, 18 h	Complex mixture

Scheme 2.19 *Reagents and conditions:* (i) see table.

An attempt was made to reduce the nitro-substituted enamine product **187** by hydrogenation using an H-cube apparatus. A 0.03 M solution of **187** in MeOH was passed three times through the H-cube, fitted with a Pd/C catalyst cartridge at a flow rate of 1 mL/min and a temperature of 40 °C. This process failed to reduce the enamine, and **187** was returned. This was not unexpected, as it has been reported that 4-5 atm of H_2 was required for the reduction of related enamine systems. A high pressure hydrogenation reaction was also attempted, using the conditions described by Hussaini,[23] with $PtO_2.H_2O$ catalyst and 5 atm of H_2. The crude material was analysed by mass spectrometry and peaks at *m/z* 275 and 329 which correspond to $[M+H^+]$ for the product in which the NO_2 group has been reduced and $[M+Na^+]$ for the product where enamine functionality has been reduced. No mass was found corresponding to the expected product **198** in which both groups are reduced. Column chromatography of the mixture isolated a portion of unreacted **187** in 10% yield and no other identifiable products (Scheme 2.20).



187 **198**

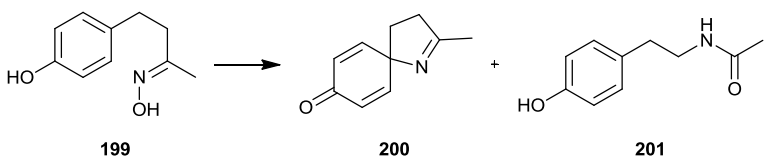
Scheme 2.20 *Reagents and conditions:* (i) Pd/C, H_2, MeOH, 40 °C (H-cube) or PtO_2, H_2 (5 atm), AcOH, TFA, rt, 48 h.

2.3 Pyrrolidine-forming cyclisation reactions

The previously discussed methods for the synthesis of 2,5-disubstituted pyrrolidines have focussed on the modification of pre-existing nitrogen heterocycles derived from pyroglutamic acid, but as indicated are hampered by a problematic final reduction step. Many strategies for the formation of new pyrrolidine motifs have been documented in the literature.[12] Amongst them are pericyclic reactions (1,3-dipolar[35] and [3+2] cycloadditions[36]), ring closing

metathesis[37] (of diallyl amines[38]), radical cyclisation,[39,40] Dieckmann cyclisation,[41] aldol cyclisation,[3] the electrophile (halogen,[42] chalcogen,[43] and metal cation[44]) mediated cyclisation of amines onto intramolecular C=C bonds, and catalytic hydroaminations[45] and chloroaminations.[46]

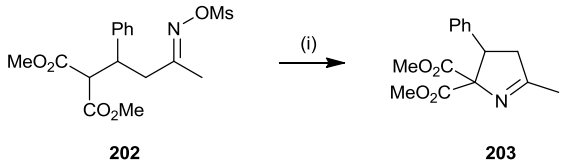
Cyclisations occurring from nucleophilic nitrogen onto internal electrophiles are very common, but cyclisations of internal nucleophiles onto an electrophilic nitrogen atom are much rarer. One reason for the lack of success is the competing Beckmann and Neber rearrangements. Narasaka *et al.* reported substitutions on the sp^2 nitrogen of the oxime **199**, once protonated, to give the spirocyclic system **200** in 4-91% yield under various conditions. The Beckmann rearranged product **201** was also isolated in 22-72% yield (Scheme 2.21).[47]



Reagent	Solvent	Products (%) 200	201
PPA	m-xylene	4	72
CF_3SO_3H	CH_3CN	47	37
CF_3SO_3H	CH_3NO_2	28	26
CF_3SO_3H	$ClCH_2CH_2Cl$	50	22
$(Bu_4N)ReO_4$ $+CF_3SO_3H$	$ClCH_2CH_2Cl$	91	0

Scheme 2.21 *Reagents and conditions:* (i) see table.

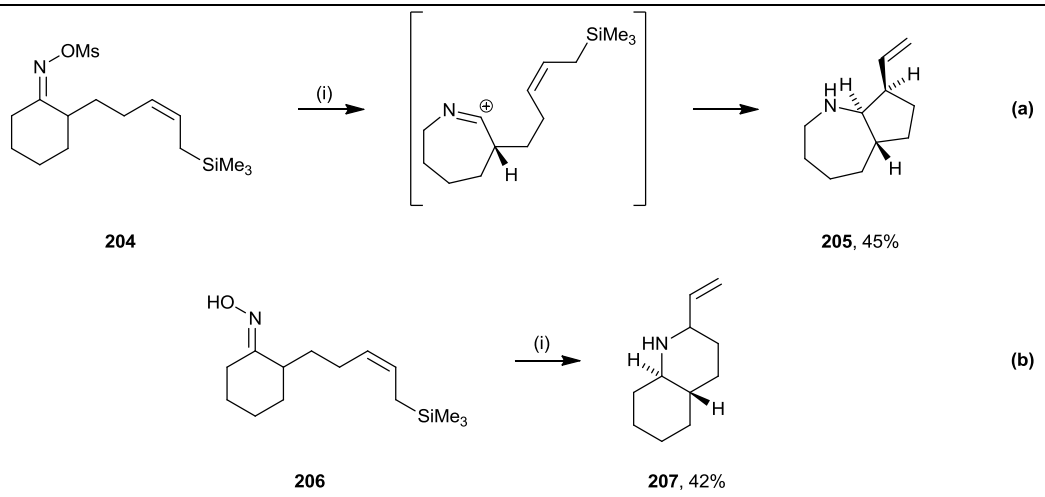
Narasaka *et al.* also reported that the cyclisation of the mesyloxime **202** was effected by a range of bases in DCM or DCE, to give **203** in 38-99% yield, with DBU in DCM giving the best result (Scheme 2.22).[48]



Base	Solvent	Yield 203
TEA	$ClCH_2CH_2Cl$	38%
DMAP	$ClCH_2CH_2Cl$	69%
DABCO	$ClCH_2CH_2Cl$	42%
DBU	DCM	99%

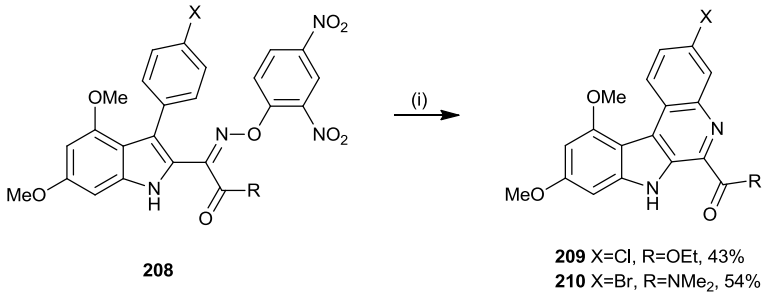
Scheme 2.22 *Reagents and conditions:* (i) see table.

Schinzer and Bo reported that when treated with DIBAL, the *Z*-mesyloxime **204** and the *E*-oxime **206**, gave different products. Mesyl oxime **204** underwent Beckmann rearrangement which the authors postulate to be encouraged by the weak Lewis acidity of DIBAL, followed by *in situ* trapping of the intermediate by the internal electron rich allyl silane to give **205** in 45% yield (Scheme 2.23, (a)). Reaction of the *E*-mesyloxime results in decomposition, but reaction of the species **206** gives the perhydroquinoline heterocycle **207** in 42% yield (Scheme 2.23, (b)).



Scheme 2.23 *Reagents and conditions:* (i) DIBAL, DCM, −78 to 0 °C.

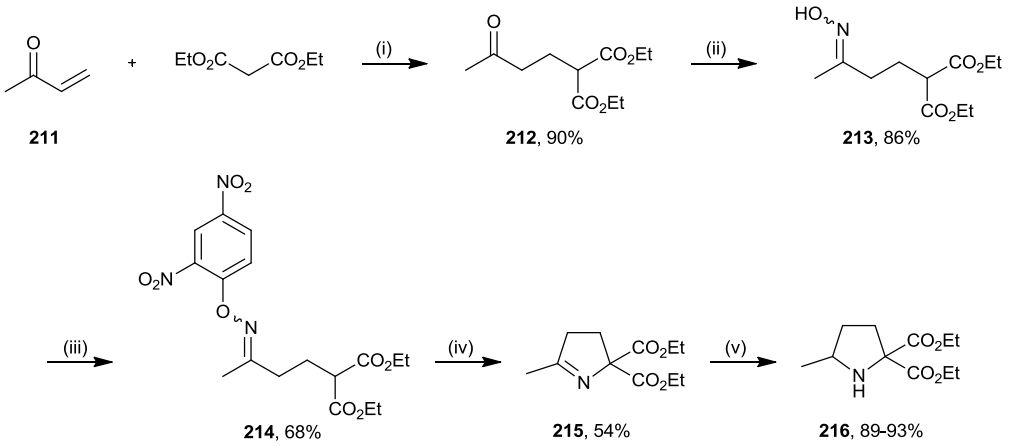
This approach has not been widely adopted, but Black *et al.* have reported the use of a similar reaction exploiting the leaving group ability of dinitrophenyl compounds **208** in the efficient formation of aromatic indolo[2,3,c]quinolines such as **209** and **210** in moderate yield (Scheme 2.24).[49]



Scheme 2.24 *Reagents and conditions:* (i) TEA, THF.

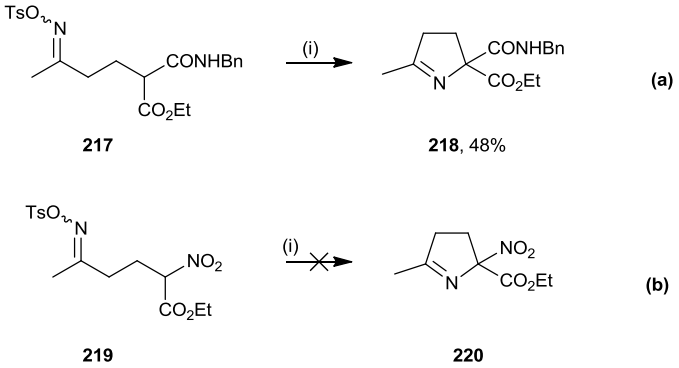
This cyclisation approach avoids the potentially problematic enamine reduction step, as the product is a cyclic imine which should be much more readily reduced. A preliminary study into the cyclisation of oxime ethers has been carried out in the Moloney group (unpublished work), and began with the simple oxime ether **214**, derived from methylvinylketone **211** and diethyl malonate. The Michael addition between **211** and diethyl malonate was carried out with K_2CO_3 in DCM giving ketone **212** in 90% yield, which was converted to the oxime **213** with $NH_2OH.HCl$ and TEA in 86% yield. The oxime was treated with NaOEt generated *in situ* and 2,4-dinitrofluorobenzene to give the oxime ether **214** in 68% yield. When treated with NaH a cyclisation between the methylene carbon and the sp^2 nitrogen occurred to give the 2*H*-pyrrole **215** in 54% yield. This cyclic imine was reduced with Pd/C and H_2 in an H-cube furnishing pyrrolidine **216** in 89% yield. The $NaBH_3CN$ reduction protocol was also

successfully used to produce **216** in 93% yield (Scheme 2.25).[50] The yields from this process were subsequently improved using a route *via* the activated tosyloxime, with the tosylation and cyclisation steps having a yield of 94 and 80% respectively. This route was also tested with a range of non-symmetrical malonates, with the yields for the pyrroline formation ranging from 52-65% using a modified cyclisation protocol *via* the tosyloxime route.



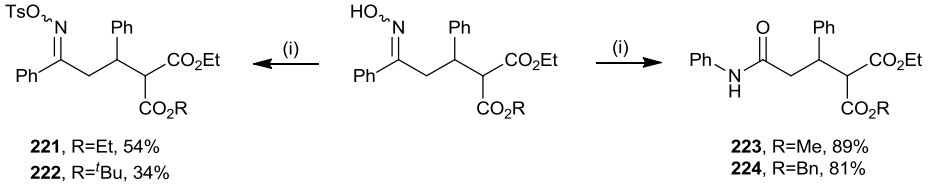
Scheme 2.25 *Reagents and conditions:* (i) K_2CO_3, DCM, rt, (ii) $NH_2OH.HCl$, TEA, EtOH, reflux, (iii) NaOEt, 1-fluoro-2,4-dinitrobenzene, EtOH, (iv) NaH, THF, reflux, (v) H_2, Pd/C, MeOH, or $NaBH_3CN$, HCl, MeOH.

The cyclisation was also shown to be feasible with malonamide-derived oxime ethers such as **217**, giving amido-pyrroline **218** in 48% yield (Scheme 2.26, (a)). Interestingly, although the synthesis of the nitro-derivative **219** proceeded as expected, the cyclisation did not produce any of the desired 2*H*-pyrrole **220** (Scheme 2.26, (b)).



Scheme 2.26 *Reagents and conditions:* (i) NaH, THF, reflux.

In the case of chalcone-derived oximes, Beckmann rearrangement products were observed, with the amides **223** and **224** being produced in 89 and 81% yield after treatment of the corresponding oximes with TsCl and TEA. Notably, the tosyloximes **221** and **222** appear to be stable to the rearrangement process and were isolated in 54 and 34% yields (Scheme 2.27).

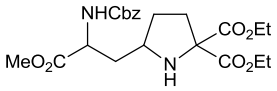


221, R=Et, 54%
222, R=^{t}Bu, 34%

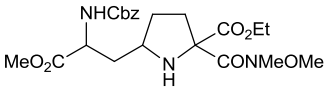
223, R=Me, 89%
224, R=Bn, 81%

Scheme 2.27 *Reagents and conditions:* (i) TsCl, TEA, DCM, rt.

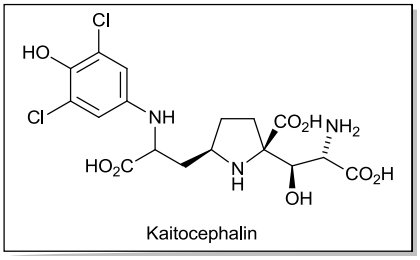
This methodology was subsequently applied to the synthesis of mimics **225** and **226** for the structure of the novel glutamate receptor antagonist kaitocephalin (Figure 2.6).



225



226



Kaitocephalin

Figure 2.6 Structures of mimics for the pyrrolidine core of kaitocephalin.

2.4 Synthesis of pyrrolidines by cyclisation of oxime-ethers

It was envisaged that the oxime ether cyclisation reaction outlined above may be used in the synthesis of 2,5-disubstituted pyrrolidines possessing a 1′-amino substituent, allowing for the formation of the bicyclic core of lemonomycin. The proposed route used *N*-protected L-phenylalanine **232** as a starting material, from which the α,β-unsaturated ketone **231** could be accessed by a Grignard addition to the Weinreb amide derivative of **232**. Michael addition of diethyl malonate and treatment with hydroxylamine would give the oxime **230**. Cyclisation of the nucleophilic carbon onto the sp^2 nitrogen would furnish the heterocycle **229**, which after reduction and deprotection, would give 1′-amino-pyrrolidine **228**. A second ring closure between the amine and one of the ester groups would give the bicyclic piperazinone **227** which forms the skeleton of lemonomycin **4** (Figure 2.7).

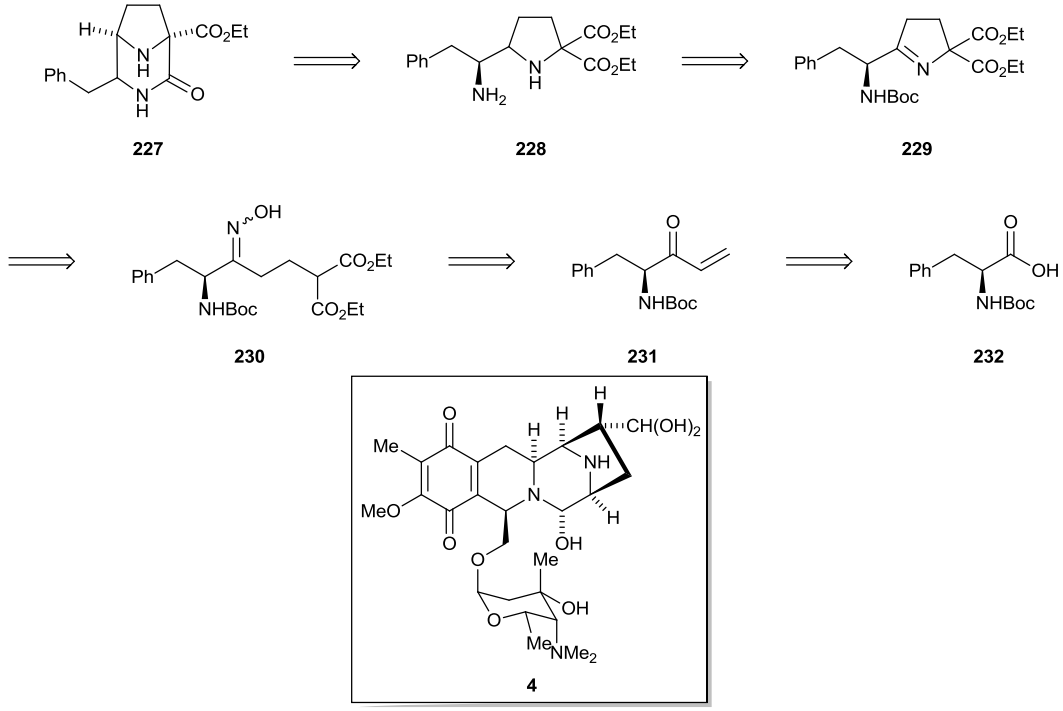
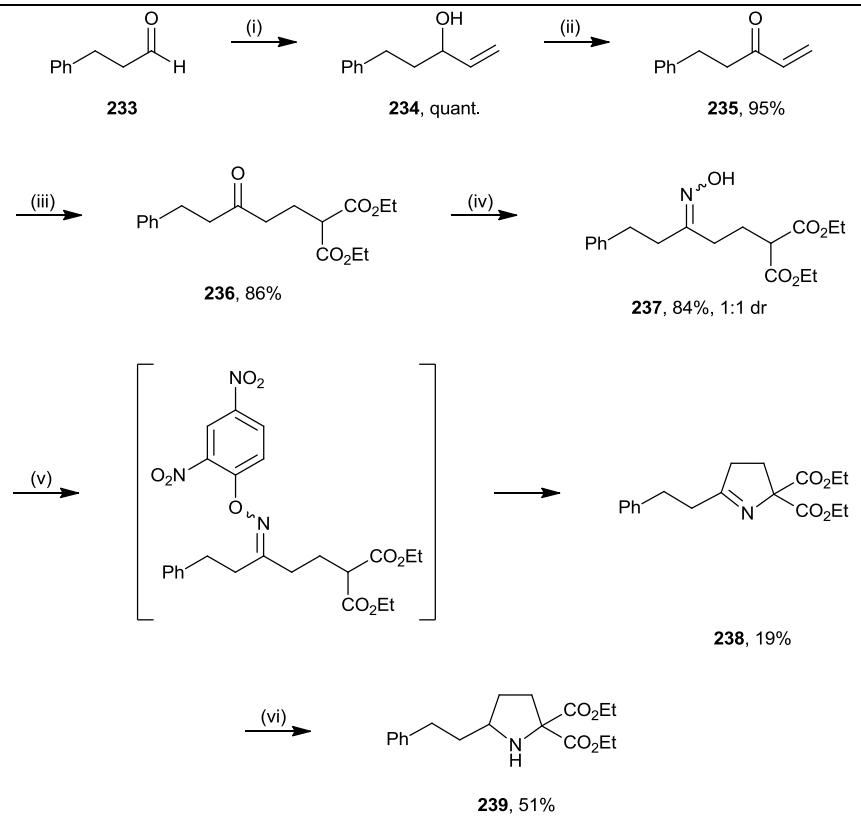


Figure 2.7 Retrosynthesis of the piperazine/pyrrolidine unit of lemonomycin **4**.

2.4.2 Model system

A model system containing an aromatic side chain was used to establish the viability of more substituted oximes in this scheme. 3-Phenylpropionaldehyde **233** was treated with vinylmagnesium bromide in Et_2O to give allylic alcohol **234** in quantitative yield. A Swern oxidation was carried out on the alcohol **234** to give the Michael acceptor **235** in 95% yield. Conjugate addition of diethyl malonate was carried out in DCM with K_2CO_3 to give the ketone **236** in a yield of 86%. Treatment of the ketone **236** with hydroxylamine hydrochloride and TEA in EtOH furnished the oxime **237** in 84% yield. The oxime **237** was then treated with NaOEt generated *in situ* by adding metallic Na to dry EtOH. A portion of 1-fluoro-2,4-dinitrobenzene **197** was added and the reaction was monitored by mass spectrometry until the starting material had been consumed. Purification of the crude reaction mixture gave the cyclised 2*H*-pyrrole **238** in 19% yield and no other identifiable products. This demonstrates that once formed, the oxime ether can undergo spontaneous cyclisation. It is likely that the oxime ether is highly reactive, due to its possessing such a good leaving group, and this could account for the low yield as any uncyclised material may be lost on the column. The heterocycle **238** was hydrogenated using an H-cube apparatus fitted with a Pd/C catalyst cartridge to give pyrrolidine **239** in 51% yield (Scheme 2.28).

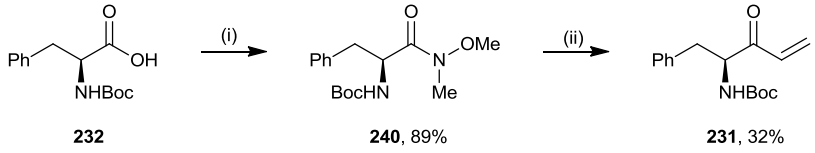


Scheme 2.28 *Reagents and conditions:* (i) Vinylmagnesium bromide, Et_2O, 0 °C, 10 min, (ii) DMSO, oxalyl chloride, DCM, −78 °C, 30 min, then TEA, 30 min, (iii) diethyl malonate, K_2CO_3, DCM, rt, 6 h, (iv) $NH_2OH.HCl$, TEA, EtOH, reflux, 7 h, (v) Na, EtOH, 1-fluoro-2,4-dinitrobenzene **197**, 0 °C to rt, 30 min, (vi) 0.01 M **238** in MeOH, Pd/C, H-cube, 0.5 ml/min, rt.

2.4.3 L-Phenylalanine route

With this success in hand, a sequence using *N*-Boc-phenylalanine was commenced. The requisite Michael acceptor **231** was constructed first by the conversion of *N*-Boc-Phe-OH **232** to the Weinreb amide **240** and subsequent Grignard addition to the amide giving the α,β-unsaturated ketone. The formation of Weinreb amide **240** was carried out using DCC and DMAP with NHMeOMe.HCl and TEA in a reaction that had a range of yields from 26-89%. This variation in yield was hypothesised to be a result of the amide product sticking to the silica gel of the column. A portion of crude material was therefore purified by rapid filtration through a short plug of silica to remove the urea by-product, and **240** was obtained in 89% yield and was pure by NMR spectroscopy. Of interest is that a portion of the same crude mixture purified by traditional chromatography resulted in a 33% yield of **240**. An alternative procedure utilising EDC with NHMeOMe.HCl, TEA was also used. This meant that the aqueous soluble urea could be removed by sequentially washing of the crude material with citric acid and $NaHCO_3$, and because this method negates the need for chromatography, yields

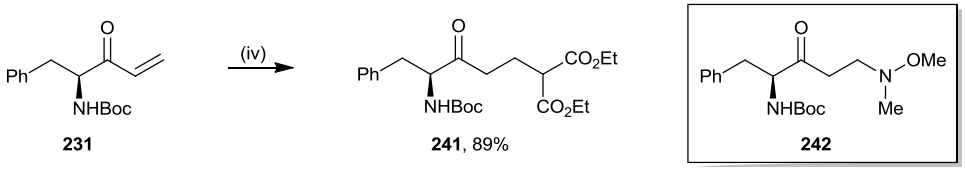
of around 57% were obtained. The Weinreb amide **240** was converted to the Michael acceptor **231** using vinylmagnesium bromide in THF at −78 °C warmed to rt over a few minutes to a few hours, however the yield of product for this reaction was found to be unreliable. This was attributed to an apparent acid sensitivity of the product. The original procedure included an aqueous work up in which the crude material was washed with sat. aq. NH_4Cl, but this process resulted in very low crude masses for the reaction; for example, in a reaction in which 500 mg of Weinreb amide **240** was used, only 270 mg of crude material was obtained. In a modification of this procedure, a reaction of the same scale was quenched with ice, warmed until melted and then extracted with Et_2O, furnishing 500 mg of crude material. The purification of this material also resulted in a large losses; chromatography resulted in the isolation of **231** in between 17 and 32% yield, even when passed through a small volume of silica (Scheme 2.29).



232 **240**, 89% **231**, 32%

Scheme 2.29 *Reagents and conditions:* (i) $NH_2OH.HCl$, TEA, DCC, DMAP, 0 °C to rt, 4 h, or $NH_2OH.HCl$, TEA, EDC, 0 °C to rt, 18 h, (ii) vinylmagnesium bromide, THF, −40/0 °C to rt.

As a result of these problems in obtaining large quantities of the material, a Michael addition was attempted with the crude sample of **231**. Treatment with diethyl malonate and K_2CO_3 in DCM gave a mixture that was then purified to give the ketones **241** and **242** in 32 and 30% yield respectively. The unwanted ketone **242** resulted from the Michael acceptor **231** reacting with the amine released in the previous reaction still present in the mixture. Reaction of pure acceptor **231** with diethyl malonate gave the ketone **241** in 89% yield (Scheme 2.30).

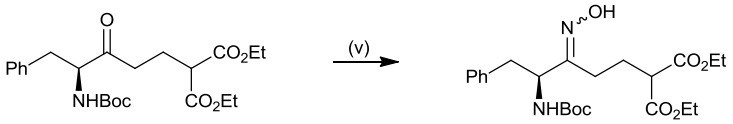


231 **241**, 89% **242**

Scheme 2.30 *Reagents and conditions:* (i) diethyl malonate, K_2CO_3, DCM, rt, 18 h.

The ketone **241** was converted to the corresponding oxime **230** by treating it with $NH_2OH.HCl$ and TEA in EtOH heated to reflux (Scheme 2.31). The oxime (*E*/*Z*)-**230** was isolated in 54% yield as an equimolar mixture of 3 species. The obvious source of isomerism

is the E/Z isomerism across the oxime double bond. The third species may originate from the restricted rotation around some of the bonds in the molecule as it bears a bulky Boc group close to a chiral centre. A variable temperature NMR experiment was conducted to establish whether rotamers may be the cause and the ^{1}H NMR spectrum in C_6D_6 was acquired at room temperature (Figure 2.8, (a)) and 343 K (Figure 2.8, (b)). In the region between 4.2 and 5.5 ppm, peaks corresponding to the N*H* and C(5)*H* protons are observed. In the room temperature spectrum, this is visible as 4-5 separate signals on the baseline. In the elevated temperature spectrum, there are 3 separate peaks. Additionally, the two peaks at 1.30 and 1.38 ppm for the *t*Bu groups at room temperature move much closer together at elevated temperature and appear at 1.35 and 1.37 ppm. The remaining signals between 2.00 and 3.50 ppm also appeared sharper. This shows that the NMR spectrum is simplified at elevated temperature. Additionally to the ^{1}H NMR spectrum being simplified at high temperature, studying of the ^{13}C, COSY, and HSQC spectra also acquired at 343 K suggested that the mixture now contained only 2 separate species, the *E*- and *Z*-oximes.



241 **230**, 54%

Scheme 2.31 *Reagents and conditions:* (i) $NH_2OH.HCl$, TEA, EtOH, reflux, 18 h.

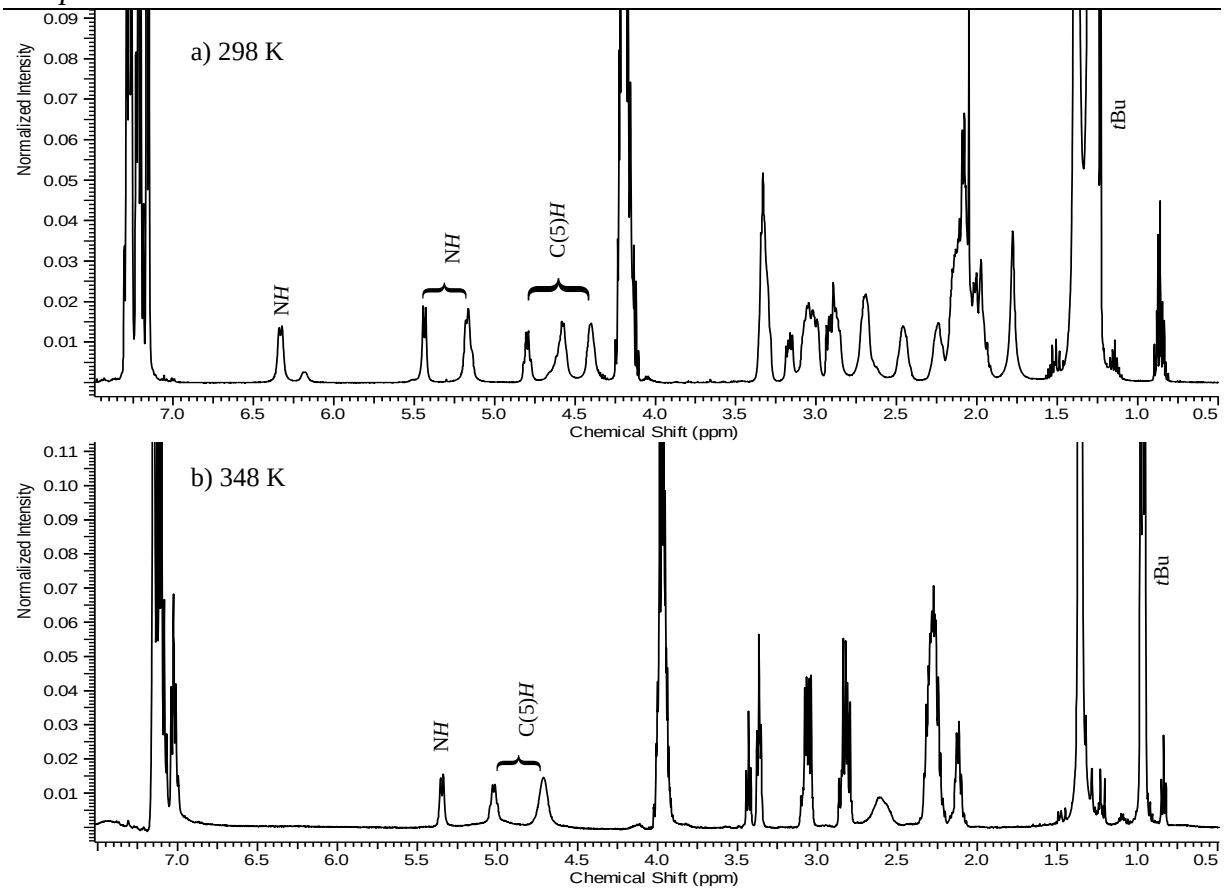
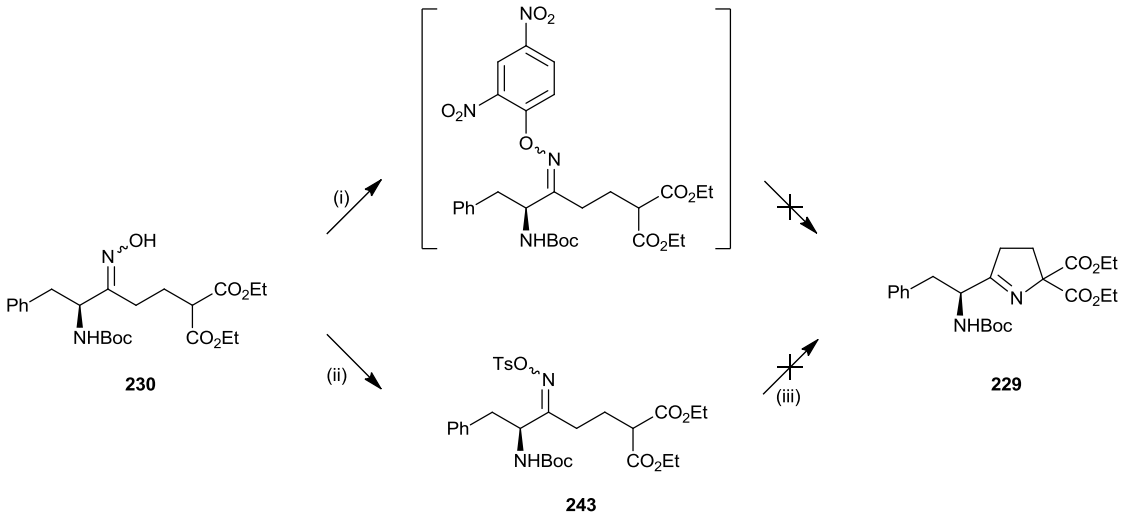


Figure 2.8 a) Room temperature and b) 348 K ^{1}H NMR spectra of oxime **230**.

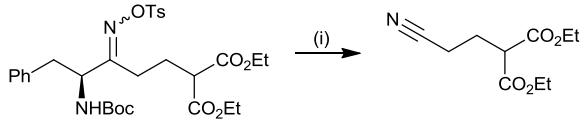
The oxime **230** was then treated with NaOEt and 1-fluoro-2,4-dinitrobenzene **197** in the hope of effecting a cyclisation, analogous to that seen in the simpler substrate, to give 2*H*-pyrrole **229** (Scheme 2.32). The reaction was monitored by mass spectrometry and a sample taken immediately after the addition of **197** contained a *m/z* peak at 455 (90% intensity), consistent with [M+Na^{+}] for the cyclised product. It also showed a peak at 639 (70% intensity), consistent with [M+Na^{+}] for the oxime ether mass, as well as a 100% intensity peak for the starting material. All three peaks continued to be observed in the samples taken after 15 and 50 min. However, after 1 h, the peaks relating to ether and cycle were no longer observed, and the starting material remained as the parent peak in subsequent samples spanning a 20 h time period. After work up, the crude ^{1}H NMR spectrum contained peaks consistent with the 1-fluoro-2,4-dinitrobenzene **197** but no other identifiable signals. An alternative route *via* the tosyl oxime was then trialled as recent work within the group had shown that these can be cyclised under basic conditions in a stepwise process.[50] The oxime **230** was treated with TsCl and TEA in DCM. The crude ^{1}H NMR spectrum showed some peaks consistent with the

presence of the tosyl oxime **243**, but column chromatography yielded no identifiable products. This was assumed to be as a result of the potentially reactive species having decomposed on the column, and a cyclisation reaction was attempted on the crude material. The crude tosyloxime **243** was treated with NaH in THF and left to stir at room temperature for 6 h. No *m/z* peak for either the starting material or product was observed in samples taken throughout the reaction period. TLC analysis of the crude material showed a complex mixture of products and chromatography resulted in several fractions of negligible mass (Scheme 2.32).



Scheme 2.32 *Reagents and conditions:* (i) Na, EtOH, then 1-fluoro-2,4-dinitrobenzene, 0-30 °C, 20h, (ii) TsCl, TEA, DCM, rt, 7 h, (iii) NaH, THF, rt, 6 h.

A cyclisation protocol using DBU to effect the cyclisation of the tosyloxime was also attempted. This used the crude tosyloxime **243**, which was dissolved in DCM and treated with DBU at 0 °C, warmed slowly to rt. TLC analysis showed consumption of the starting material and purification gave the nitrile compound **244**, resulting from a Beckmann rearrangement or fragmentation of the tosyloxime, in 73% yield. After migration of the side chain to displace the tosylate group, the resulting cation can fragment with the aid of the Boc protected nitrogen's lone pair to give the nitrile **244** rather than the usual amide product. Alternatively, the nitrile could be formed in one step *via* a Grob-type fragmentation in which the amino-alkyl and tosylate group are lost simultaneously to form the C-N triple bond (Scheme 2.33).

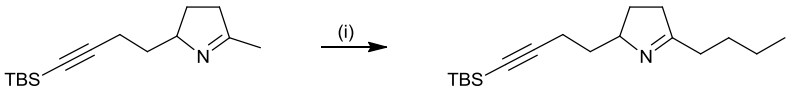


243 **244**, 73%

Scheme 2.33 *Reagents and conditions:* (i) DBU, DCM, 0 °C to rt, 7 h.

2.4.4 Modifications of 2*H*-pyrroles as cyclic imines

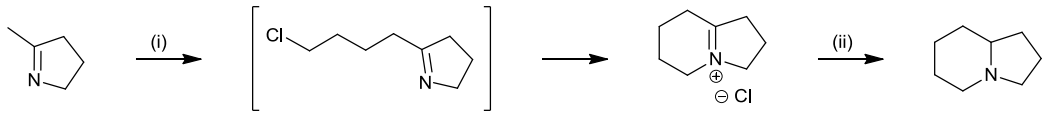
Following this, a literature review was conducted, and several papers were found which described the modification of 2*H*-pyrroles through deprotonation/alkylation chemistry of the cyclic imines. One example is the chain extension of the pyrroline **245**, converting the methyl substituent into a butyl chain by deprotonation with BuLi and alkylation with propyl bromide, furnishing **246** in 83% yield (Scheme 2.34).[51]



245 **246**, 83%

Scheme 2.34 *Reagents and conditions:* (i) BuLi, *n*-PrBr.

Another example utilises a dihalide to undergo a cascade double alkylation/cyclisation reaction to produce indolizidines and quinolizidines from cyclic imines in one step. The pyrroline **247** was treated with LDA and then 1-bromo-3-chloropropane to give the quaternary nitrogen salt **248** in 90% yield which was reduced with $LiAlH_4$ to give the indolizidine **249** in 59% yield (Scheme 2.35).[52]

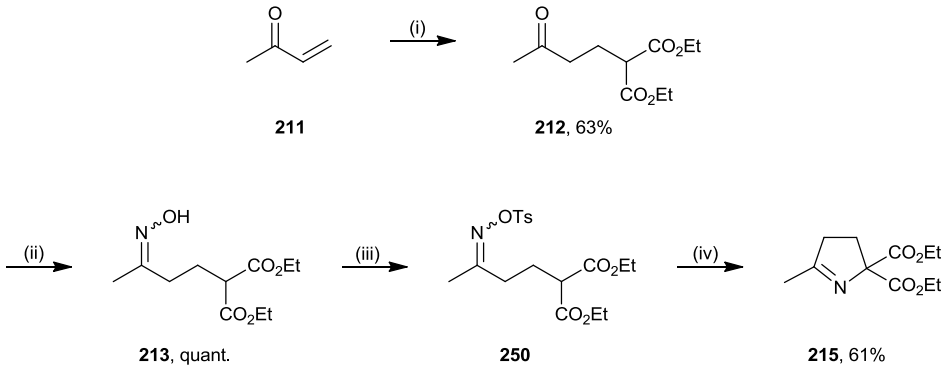


247 **248**, 90% **249**, 59%

Scheme 2.35 *Reagents and conditions:* (i) 1.1 eq. LDA, THF, −78 °C, then $Br(CH_2)_3Cl$ −78 °C to rt, (ii) $LiAlH_4$, Et_2O, rt.

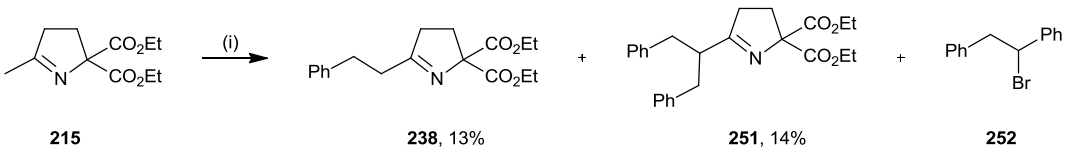
Some preliminary investigations into the modifications of cyclic imines formed by the oxime ether cyclisation were carried out. The methyl substituted 2*H*-pyrrole was synthesised from methyl vinyl ketone using the sequence described by Chandan and Moloney.[50] A Michael addition to methyl vinyl ketone **211** gave the diester **212** in 63% yield. The ketone **212** was then converted to the oxime **213** in quantitative yield. To effect the cyclisation to form 2*H*-pyrrole **215**, the oxime was first converted to the tosyloxime **250** by treatment with TsCl and TEA. The crude tosyloxime **250** was treated with DBU to give the 2*H*-pyrrole **215** in 61%

yield over the two steps (Scheme 2.36). The change to DBU as a base increased the yield of 2*H*-pyrrole isolated from the cyclisation reaction, when carried out using NaH, this reaction gave a yield of 13%.



Scheme 2.36 *Reagents and conditions:* (i) diethyl malonate, K_2CO_3, DCM, rt, 4 d, (ii) $NH_2OH.HCl$, TEA, EtOH, reflux, 7 h, (iii) TsCl, TEA, DCM, 0 °C to rt, 5 h, (iv) DBU, DCM, rt, 7 h.

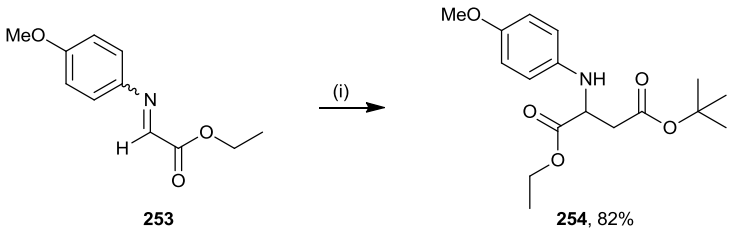
An alkylation experiment using a procedure adapted from that described by Helquist *et al.*[51] was conducted. The cyclic imine **215** was treated with 1.0 eq. BuLi and benzyl bromide at −78 °C and allowed to warm to 0 °C. The crude ^{1}H NMR spectrum for this reaction showed that no reaction had taken place. As there is a possibility that the BuLi could interact with the ester groups in the pyrroline, LDA was used in future reactions, and was formed *in situ* by stirring *i*Pr_2NH and BuLi in THF for 30 minutes at −78 °C. Using a procedure similar to that described by De Kimpe *et al.*,[52] the pyrroline was treated with LDA and BnBr, but once again no identifiable products were observed. A set of conditions were subsequently discovered in which a reaction did occur, and the product **238** was isolated in 13% yield. The reaction also produced the doubly-alkylated species **251** in 14% isolated yield and a portion of the by-product **252** formed from benzyl bromide reacting with itself (Scheme 2.37).



Scheme 2.37 *Reagents and conditions:* (i) 2.5 eq. *i*Pr_2NH, 5.0 eq. BuLi, 2.5 eq. BnBr, THF, −78 °C, 2 h.

Another method of modification of the cyclic imine is the nucleophilic addition to the imine bond, resulting in a 2,2,5,5-tetrasubstituted pyrrolidine. The addition of carbon nucleophiles to imines has been achieved with malonate derived nucleophiles[53] and organometallic reagents such as Grignards[54] and zinc enolates.[55,56] Luo *et al.* carried out a series of zinc

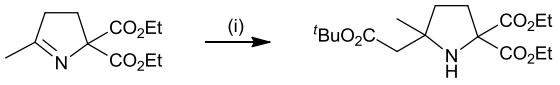
enolate additions to imines, for example the addition of *t*-butyl 2-bromozincacetate to imine **253**, giving **254** in 82% yield (Scheme 2.38).[56]



253 **254**, 82%

Scheme 2.38 *Reagents and conditions:* (i) *t*-Butyl 2-bromozinc-acetate, THF.

A suspension of activated zinc powder was treated with I_2 and *t*-butylbromoacetate at 40 °C in a sonic bath to form the Reformatsky enolate. The imine **215** was then added to this mixture, and further sonicated and stirred. The resulting mixture was purified to reveal a portion of the 2,2,5,5-tetrasubstituted pyrrolidine **255** in 20% yield (Scheme 2.39).



215 **255**, 20%

Scheme 2.39 *Reagents and conditions:* (i) Zn, I_2, tBuO$_2$CCH$_2$Br, THF, 40 °C, sonic bath.

2.5 Summary

This chapter has described the work towards the development of methodology for the synthesis of the diazabicyclo[3.2.1]octane unit of lemonomycin and the challenges faced in this respect. The retrosynthesis envisaged the formation of a *cis*-2,5-disubstituted pyrrolidine with subsequent formation of the fused piperazine ring, and as such aimed to build upon methodology developed in the group for the synthesis of 2,5-disubstituted pyrrolidines with the requirement of introducing a 1′-amino substituent. This differed from previous attempts to synthesise lemonomycin which used either a Joule cycloaddition or an *N*-acylium cyclisation of an existing piperazine to furnish this unit.

Initially, an Eschenmoser sulphide contraction between thiolactam **177**, derived from (*S*)-pyroglutamic acid and α-bromocarbonyl electrophiles was investigated for this purpose. It was found that whilst ethyl 2-bromo-3-oxo-3-phenylpropanoate reacted to give the product enamine in good yield, the nitrogen atom containing electrophiles gave little or none of the desired products.

A strategy employing the reaction of thiolactim ether **189**, also derived from (*S*)-pyroglutamic acid and activated methylene nucleophiles was then explored. Extensive optimisation studies were conducted using ethyl acetoacetate before the reaction was performed with a range of nucleophiles. With the exception of the cyano-substituted nucleophile, the yields for these reactions were disappointingly low and conversion percentages were moderate even after extended reaction times of up to 4 days. The nitro-substituted nucleophile did give a 20% yield of the enamine **187** and reduction of this compound to give the pyroglutamate was attempted. However, even under forcing conditions, using 5 atm of H_2, only the starting material was isolated. Future work could further investigate this reduction and the development of rearrangement protocols could allow for the utilisation of the cyano-substituted enamine.

A method involving the formation of a pyroglutamate from the cyclisation of an oxime ether was then pursued and was attractive as it avoided the problematic enamine reduction step. Once success had been achieved using a model system with an aromatic side chain, a synthesis based on L-phenylalanine was embarked upon. Numerous practical difficulties relating to the acid sensitivity of the compounds were overcome and the oxime **230** was synthesised. The cyclisation of **230** was attempted *via* the 2,4-dinitrophenyl oxime-ether and the tosyl-oxime. Whilst the *m/z* for the cyclic imine product was observed during the reaction, no product was obtained for the phenyloxime-ether mediated cyclisation. In the second case a 73% yield of the nitrile **244** resulting from the competing Beckmann rearrangement/Grob fragmentation of the tosyl oxime was obtained.

Finally, investigations into the modifications of the cyclic imines resulting from the cyclisations of oxime ethers were described. Methyl substituted 2*H*-pyrrole **215** was synthesised using the above methodology and electrophilic and nucleophilic modifications were carried out. Firstly, **215** was deprotonated using LDA and alkylated with benzyl bromide to give the mono- and di-alkylated 2*H*-pyrroles **238** and **251**. Secondly, **215** was treated with the Reformatsky zinc enolate under sonication to give the 2,2,5,5-tetrasubstituted

pyrrolidine **255**. Future work would aim to optimise and further investigate the scope of these promising preliminary investigations.

2.6 References

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CHAPTER 3: RESULTS AND DISCUSSION

Synthesis of mimics inspired by the structure of 16-methyloxazolomycin

This chapter describes the development of methodology based on the use of L-threonine as a chiral starting material for synthesising mimics of the right-hand fragment of 16-methyloxazolomycin **81d**. It describes the synthesis of a small library of alkylated tetramic acids and the use of an aldol approach to synthesise densely substituted pyroglutamates as mimics for the right-hand fragment of 16-methyloxazolomycin, as well as the generation of right-hand/middle fragment adducts containing a *gem*-dimethyl-amide unit as seen in the natural product.

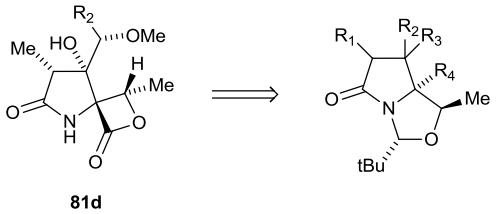


Figure 3.1 Target mimics for the right-hand fragment.

3.1 Background within the Moloney group

The Moloney group has an ongoing interest in the biological activity of analogues of the oxazolomycin natural products. The synthesis of oxazolomycin and its analogues has been approached using disconnections to form a left-hand **256**, middle **257** and right-hand fragment **258** (Figure 3.2). The chemistry of each of the three fragments has been examined separately and a small number of middle/right-hand hybrid structures have been synthesised.

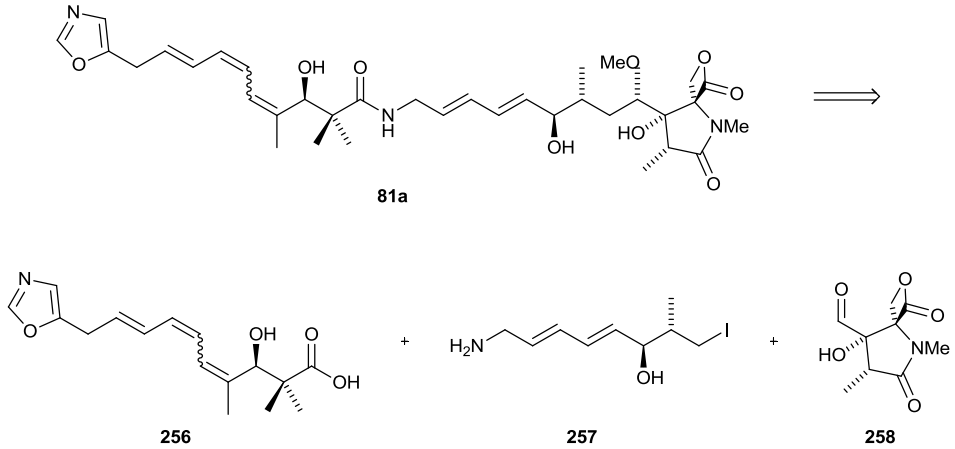
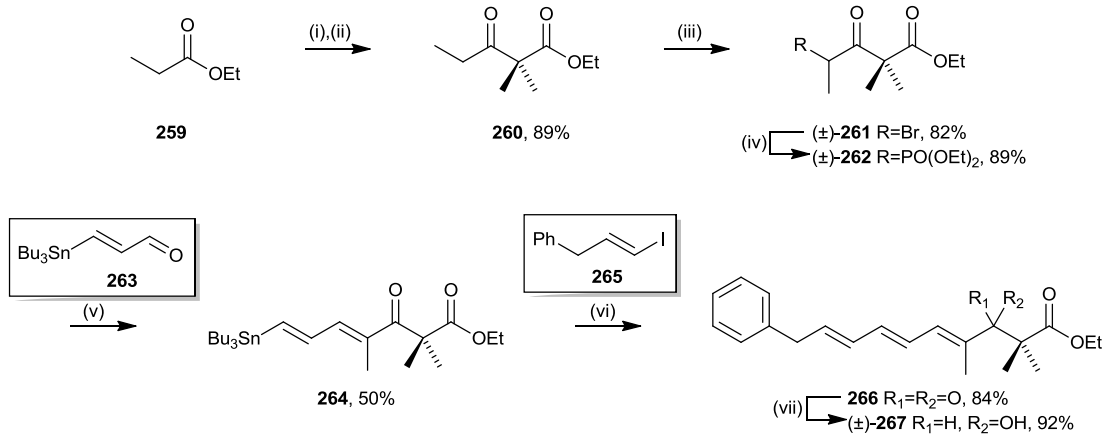


Figure 3.2 Left-hand, middle, and right-hand fragments of oxazolomycin.

3.1.2 Left-hand fragment

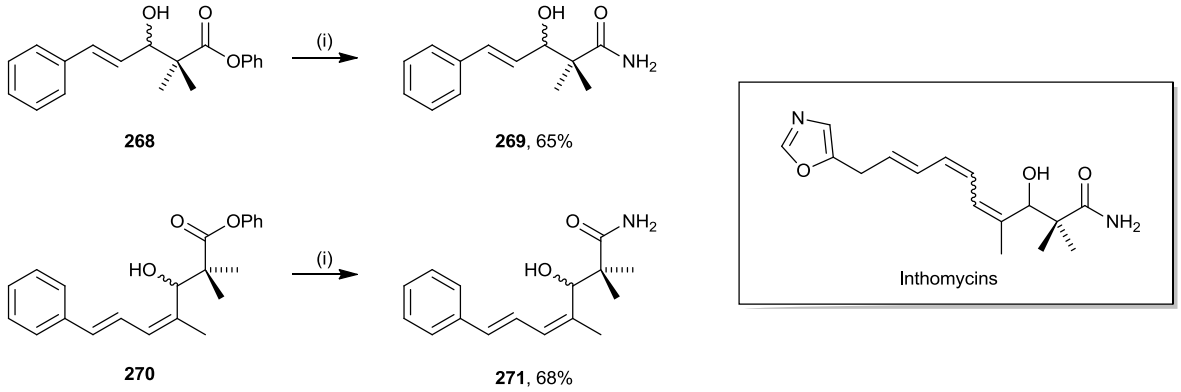
The synthesis of the left-hand triene-oxazole unit has been examined by first attempting the synthesis of the fragment **267**, where the sensitive oxazole residue was replaced with a phenyl group. The racemic ethyl ester (±)-(*E*),(*E*)-**267** has matching double bond geometries to oxazolomycin B.[1,2] The synthesis used a multi-component strategy and combined three main fragments, vinyl iodide **265**, α,β-unsaturated aldehyde **263**, and phosphonate (±)-**262**. The phosphonate (±)-**262** was synthesised from ethyl propanoate **259**; a Claisen condensation followed by methylation gave **260**, which was brominated to give (±)-**261** before substitution with $P(OEt)_3$. The allylic aldehyde **263** and phosphonate **262** were combined using a Horner-Wadsworth-Emmons reaction to give (*E*),(*E*)-**264** in 50% yield. Dienyl-stannane **264** was then coupled to the vinyl iodide **265** using a Stille reaction and the product (*E*),(*E*),(*E*)-**266** was produced in 84% yield, which was converted into the racemic alcohol (±)-**267** in 92% yield by reduction with $NaBH_4$ (Scheme 3.1). Attempts to use a similar approach for the synthesis of (*Z*),(*E*),(*E*)- and (*Z*),(*Z*),(*E*)- analogues resulted in isomeric mixtures which were difficult to separate and indicated the need for an efficient selective synthesis for each isomer.



Scheme 3.1 *Reagents and conditions:* (i) NaH, toluene, (ii) NaH, MeI, (iii) Br_2, AcOH, (iv) $P(OEt)_3$, toluene, (v) NaH, **263**, THF, (vi) $Pd(MeCN)_2Cl_2$, **265**, DMF, (vii) $NaBH_4$, EtOH.

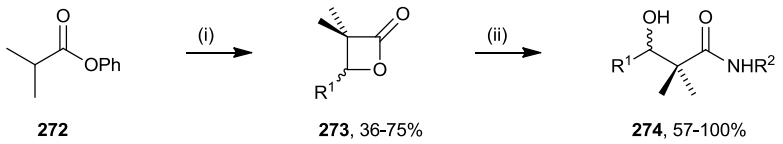
Displacement of the ester group with ammonia in simplified left-hand fragment analogues **268** and **270** (formed by a direct aldol reaction of the appropriate α,β-unsaturated aldehyde with phenyl isobutyrate) provided **269** and **271**, structural mimics of the inthomycin natural products (Scheme 3.2). The structure of inthomycin is identical to the left-hand fragment of oxazolomycin and terminates at the right-hand side with a primary amide functionality. Both

compounds were tested for biological activity against a range of organisms and the diene **271** was found to be active against *S. aureus* D267 and *E. coli* X580, and a potential lead structure against *S. pneumonie* ATCC49619 with an MIC of 64 μg/mL. It was also found that this potent activity was coupled with low toxicity, with **271** having an LD_{50} of >90.9 μM against Hek293 and PBMC cells.[3,4] Of note is that the inthomycins themselves are not known to possess any biological activity.



Scheme 3.2 *Reagents and conditions:* (i) Liq. NH_3, rt, 18 h.

A general method for the synthesis of a wide range of α,α-dimethyl-β-hydroxyamides bearing different substituents was also developed (Scheme 3.3) and the resulting library of amides were used to demonstrate the capacity of the *gem*-dimethyl group to induce a turn in the conformational shape of these molecules.[3,4] An aldol reaction between phenyl isobutyrate **272** and an aldehyde results in the *in situ* formation of the β-lactones **273**. Subsequent ring opening with a primary amine give the amides **274**.



Scheme 3.3 *Reagents and conditions:* (i) LDA, R^1CHO, THF, −78 to 0 °C, (ii) R^2NH_2, TEA, DCM, 40 °C, 1-4 d.

Molecular modelling was used to assess the minimum energy conformation of oxazolomycin B **81b** and the truncated fragment **276** (Figure 3.3). The fragment **276** was predicted to have a 'U' shaped conformation in which the *gem*-dimethyl group induces a turn, which is likely to be stabilised by an intramolecular H-bond between the NH and β-hydroxyl functionalities, which have a predicted proximity of 2.07 Å. The predicted minimum energy conformation of oxazolomycin B **81b** itself was also a 'U' shape, but this time with an NH-O distance of 4.07

Å, too far for effective H-bonding to occur. The synthetic analogues **275** and **277** were analysed by single crystal X-ray diffraction and whereas **277** showed an intramolecular H-bond stabilised hair-pin structure with and NH-O distance of 2.29 Å, **275** showed a linear structure stabilised by intermolecular H-bonds. It may be that these turns in the conformation of the oxazolomycins are important to their biological activity.

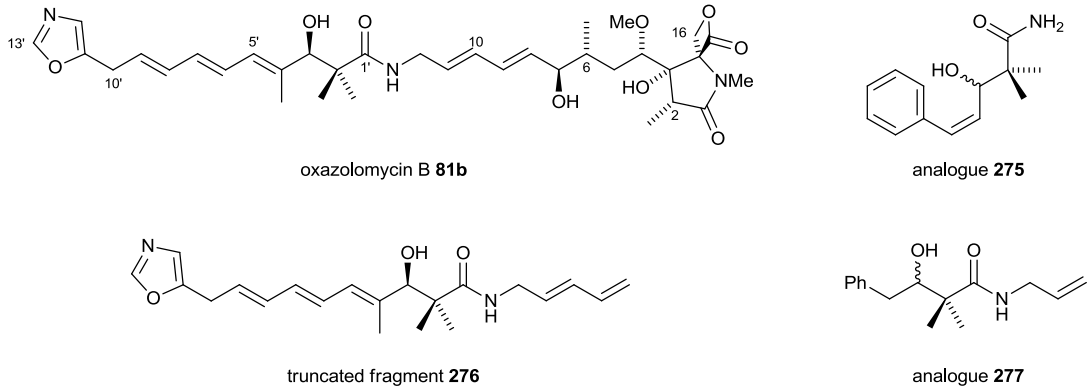
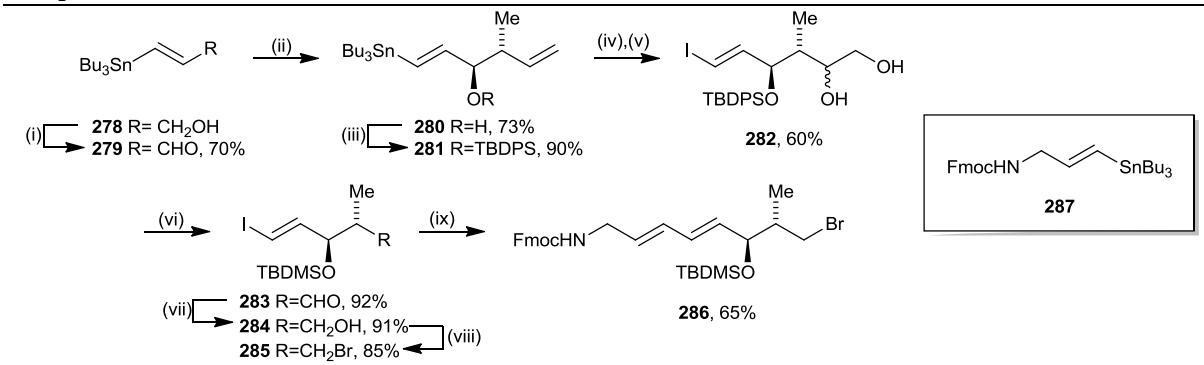


Figure 3.3 Structures subjected to molecular modelling and single crystal X-ray analysis.

3.1.3 Middle fragment

The middle fragment **286** has been synthesised in racemic form by Moloney and Wang (Scheme 3.4).[5] The strategy utilises a similar approach to Kende[6] and is based on a Stille coupling to join key components of the diene. Hiyama methodology[7] involving the Cr(II) mediated addition of crotyl bromide to an aldehyde was used to convert stannane **279** (accessible by oxidation of commercially available stannane **278**) to allylic alcohol **280** with the required *anti*-stereochemistry on reaction with crotyl bromide. Following silyl protection, **281** was converted to the iodide by treatment with I_2 and dihydroxylated with AD-mix-α to give **282**. Oxidative cleavage with $NaIO_4$ produced the aldehyde **283** and reduction with $NaBH_4$ gave the alcohol **284**, which was converted to the bromide **285** with CBr_4 and PPh_3. This was then used for Stille coupling with component **287**, derived from propargyl amine, to give the middle fragment **286** bearing *N*- and *O*-protection.



Scheme 3.4 *Reagents and conditions:* (i) PCC/DCM or swern, (ii) crotyl bromide, $CrCl_3/LiAlH_4$, (iii) TBDPSCl, imidazole, DCM, (iv) I_2, DCM, (v) AD-mix-α, *t*-BuOH, H_2O, (vi) $NaIO_4$, THF, H_2O, (vii) $NaBH_4$, EtOH, (viii) CBr_4, PPh_3, THF, (ix) **287**, $Pd(CH_3CN)_2Cl_2$/THF.

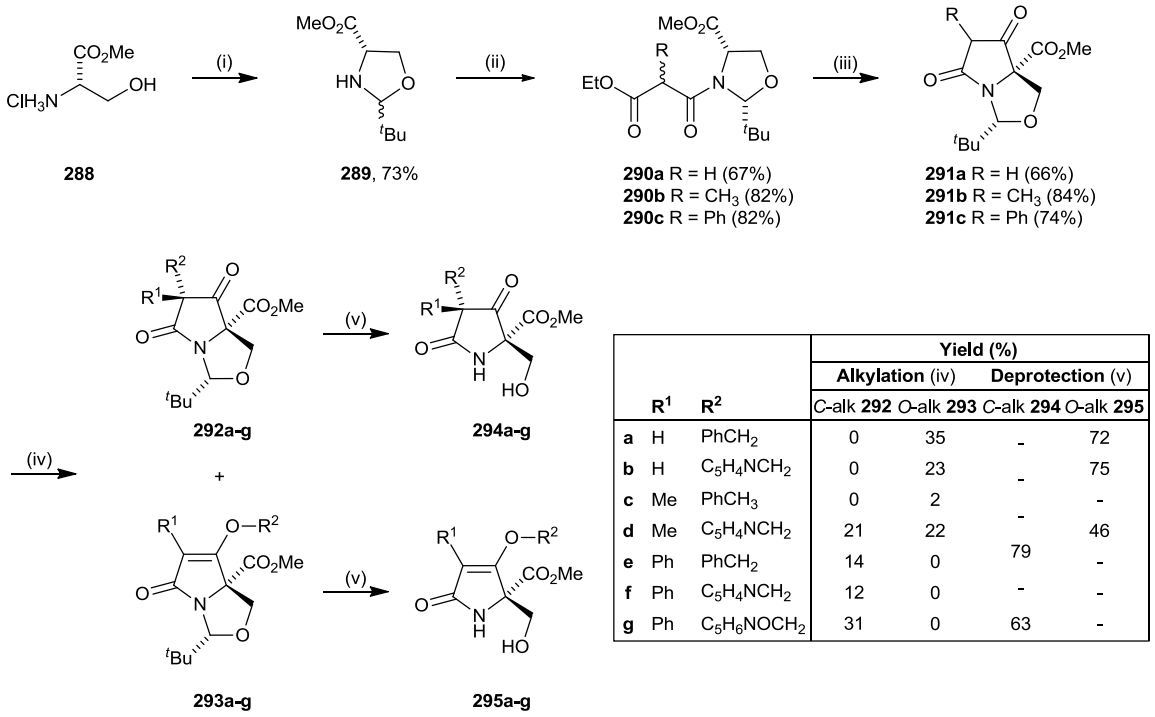
3.1.4 Right-hand fragment

The right-hand spiro-fused γ-lactam/β-lactone unit has been the focus of attention to develop rapid, stereocontrolled access to mimics based on the substituted tetramic acid/pyroglutamate motif. Effective synthetic methodology has been developed by Moloney and co-workers to access these enantiopure molecular skeletons using L-serine[8,9,10,11,12,13] and L-threonine[14,15] as chiral starting materials. The strategies employed have either been based around the Dieckmann or aldol cyclisation of *N*-acyloxazolidines, furnishing bicyclic tetramic acids and pyroglutamates. The approach utilises Seebach's principle of self-regeneration of stereochemistry,[16] and the propensity of oxazolidines to control the regio- and stereoselectivity of cyclisations.

3.1.4.1 Tetramic acids

A small library of analogues derived from serine has been generated and tested for antibacterial activity.[17] The tetramic acid core is accessed by the Dieckmann cyclisation of *N*-malonyl oxazolidines. Following the method described by Seebach,[18,19] L-serine was first converted to its methyl ester **288** and then condensed with pivalaldehyde under basic conditions to give the oxazolidine **289** in good yield (73%) as a 1:1 mixture of *cis*- and *trans*-isomers. The oxazolidine **289** was then coupled to substituted monoethyl malonates to give compounds **290** in which isomerism is only observed at C(2′). Subsequent Dieckmann cyclisation gave the tetramic acids **291**. The tetramate products **291a-c** were then alkylated, with *O*-alkylation to give **293a/b** occurring on the unsubstituted tetramate, *C*-alkylation to give **292e-g** occurring on the Ph-substituted tetramate and a combination of the two taking

place in the Me- substituted example. The yields of *O*- and *C*-alkylation are summarised in the table in Scheme 3.5, with the total isolated yield of alkylated products ranging from 2-42%. The resulting compounds were all deprotected using Corey-Reichard[20] conditions to give the corresponding alcohols **294a-g** and **295a-g** in 46-79% yield (Scheme 3.5).[17]



			Yield (%)			
			Alkylation (iv)		Deprotection (v)	
	R^1	R^2	*C*-alk **292**	*O*-alk **293**	*C*-alk **294**	*O*-alk **295**
a	H	$PhCH_2$	0	35	-	72
b	H	$C_5H_4NCH_2$	0	23	-	75
c	Me	$PhCH_3$	0	2	-	-
d	Me	$C_5H_4NCH_2$	21	22	79	46
e	Ph	$PhCH_2$	14	0	-	-
f	Ph	$C_5H_4NCH_2$	12	0	63	-
g	Ph	$C_5H_6NOCH_2$	31	0		-

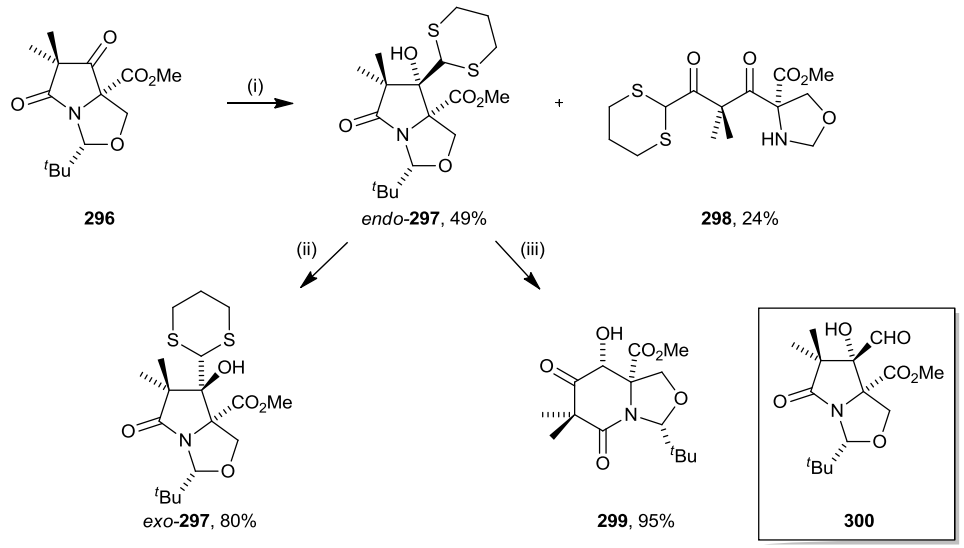
Scheme 3.5 *Reagents and conditions:* (i) *t*BuCHO, TEA, 40-60 petrol, reflux, (ii) DCC, DMAP, $EtO_2CCHRCO_2H$, DCM, rt, (iii) KO*t*Bu, *t*BuOH, reflux, (iv) NaH, R^2-Hal, THF, 0-40 °C, (v) $HS(CH_2)_3SH$, CF_3CH_2OH, HCl, rt.

Most of the simple tetramates displayed little or no biological activity when tested against *E. coli* and *S. aureus*, with the exceptions being **292d** and **292f** which were both active to *S. aureus*. The deprotected alcohols **294** and **295** had higher levels of activity, with **294e/f** and **295a** being active to both test organisms and the remaining **295b/d** being active towards *E. coli*.

3.1.4.2 Pyroglutamates from addition to the C(6) carbonyl

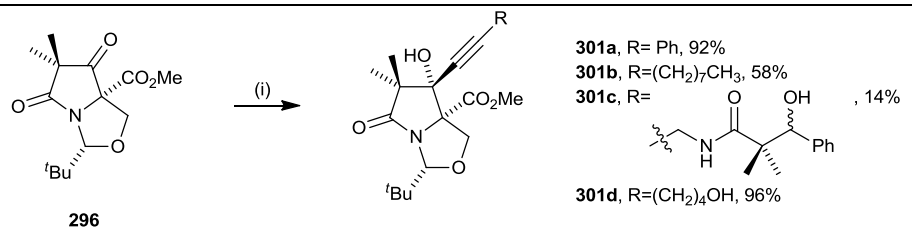
By way of probing the possibility that these Dieckmann products could be used in the synthesis of oxazolomycin, studies into the functionalisation of C(6) (where the oxazolomycin diene chain would be connected) have been conducted.[21,12] Addition of the lithium salt of 1,3-dithiane to tetramate **296** diastereoselectively produced *endo*-**297** in 49% yield along with a 24% yield of by-product **298**, resulting from a retro-aldol opening of the desired product

297.[12] The addition is kinetically controlled and the nucleophile approaches *anti*- to the *N*-lone pair and ends up on the more hindered *endo*- face of the bicyclic system (Scheme 3.6). The selectivity of this reaction is in keeping with the reported selectivity of hydride reduction also observed with these tetramates.[22,12] It is important to note that when exposed to mildly basic conditions (TEA in THF at 0 °C), the compound *endo*-**297** could be converted to the thermodynamically preferred isomer *exo*-**297** by a retro-aldol/aldol reclosure process in 80% yield. However, on attempted generation of aldehyde **300** by deprotection of the dithiane, the resulting product was ring expanded **299** which is generated by a related ring-opening/ring-closing pathway in very high yield.



Scheme 3.6 *Reagents and conditions:* (i) BuLi, 1,3-dithiane, THF, −78 °C, 2 h, (ii) TEA, THF, 0 °C, 1 h, (iii) MeI, $CaCO_3$, CH_3CN/H_2O, 70 °C, overnight.

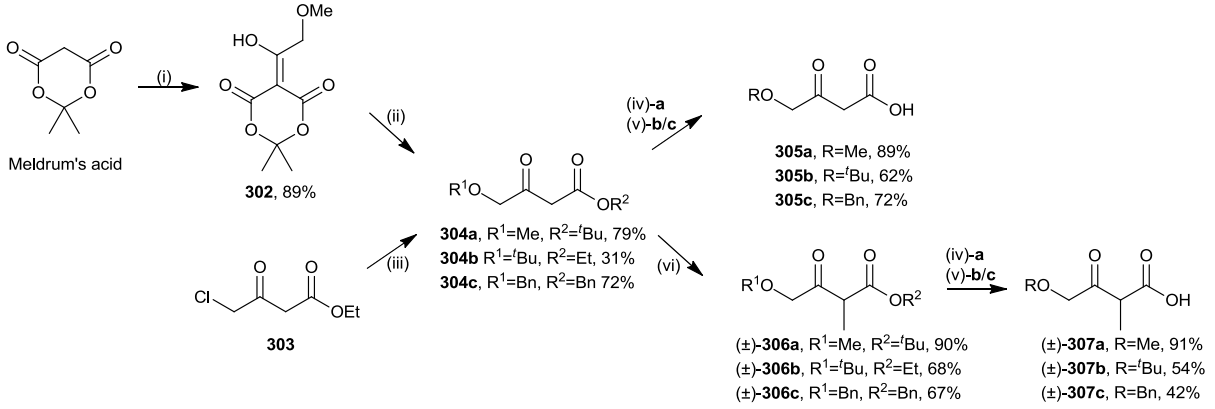
The tetramate **296** has also been successfully reacted with acetylide ions in reasonable yields (Scheme 3.7). The reaction still proceeds even with relatively complex acetylenes, such as that required for the synthesis of **301c**, although there is a marked decrease in yield for this reaction. The resulting derivatives have also been shown to be stable to further transformations and derivative **301d** has undergone extensive modifications to form the terminal bromide and then azide, before use in click chemistry reactions to form middle/right-hand fragment conjugates.[23] This approach to functionalisation of C(6) was limited by the need for geminal substitution to avoid synthetic complications arising from the highly acidic α-protons of the tetramic acid.[24]



Scheme 3.7 *Reagents and conditions:* (i) BuLi, RCCH, THF, −78 °C to rt, 6-20 h.

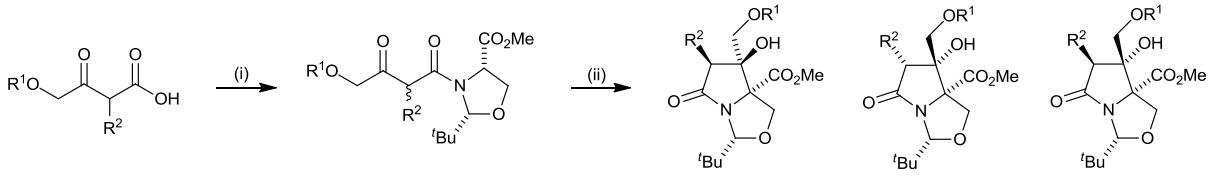
3.1.4.3 Pyroglutamates from aldol cyclisation approach

Following the apparently facile nature of the retro-aldol/aldol reclosure and related processes in the dithiane derivative above, an investigation into the use of aldol reaction to generate the bicyclic structure was undertaken.[25] In order to efficiently introduce the methoxy substituent present in oxazolomycin, two methods of synthesising δ-alkoxy-β-keto acids were developed. The first utilised the ability of Meldrum's acid to undergo ring opening decarboxylations in the presence of a nucleophile.[26] In this case, Meldrum's acid was acylated with methoxyacetyl chloride, and the resulting product **302** was produced in 89% yield. This Meldrum's acid derivative **302** was heated to reflux with *t*BuOH in toluene and a ring-opening/decarboxylation was effected to produce the β-keto-ester **304a** in 79% yield. This ester was alkylated in the α-position to install a methyl group in 90% yield to give (±)-**306a**, and both esters were hydrolysed with TFA in DCM to give the carboxylic acids **305a** and (±)-**307a** in 89% and 91% yields respectively. An alternative method used 4-chloroacetoacetate **303** reacted with alcohols *t*BuOH and BnOH to give the esters **304b** and **c** in 31% and 72% yield.[27] These were methylated to give (±)-**306b** and **c** each in around 67% yield. Each of the esters was hydrolysed with 1 M NaOH to give the four carboxylic acids, **305b** and **c** and (±)-**307b** and **c** in 42-72% yield (Scheme 3.8).



Scheme 3.8 *Reagents and conditions:* (i) methoxyacetyl chloride, py, DCM, rt, 3 h, (ii)1:1 *t*BuOH:toluene, reflux, 2 h, (iii) *t*BuOH/BnOH, NaH, THF, rt, 20 h, (iv) TFA, rt, 24 h, (v) NaOH, H_2O, rt, 30 h, (vi) *t*BuOK, MeI, THF, rt.

The β-keto-acids were coupled using EDC and DMAP to the oxazolidine **289** to give the aldol precursors **308a-f**, in low to moderate yields of 16-68% (Scheme 3.9). The *N*-acyloxazolidines were treated with NaOMe in MeOH and all underwent cyclisations to give bicyclic pyroglutamates **309-314**. Where the additional methyl substituent was lacking in **309-311**, both of the two possible diastereoisomers **a** and **b** were produced, in which **a** was the major isomer in each case. When the extra Me group was present, a third isomer was observed for R_1=Me and Bn (**312** and **314**), with only 2 observed for R^1=*t*Bu (**313**). The major isomer in this group varied as the R groups changed, probably as a result of steric interactions in the transition state.



305a R^1=Me, R^2=H
305b R^1=*t*Bu, R^2=H
305c R^1=Bn, R^2=H
307a R^1=Me, R^2=Me
307b R^1=*t*Bu, R^2=Me
307c R^1=Bn, R^2=Me

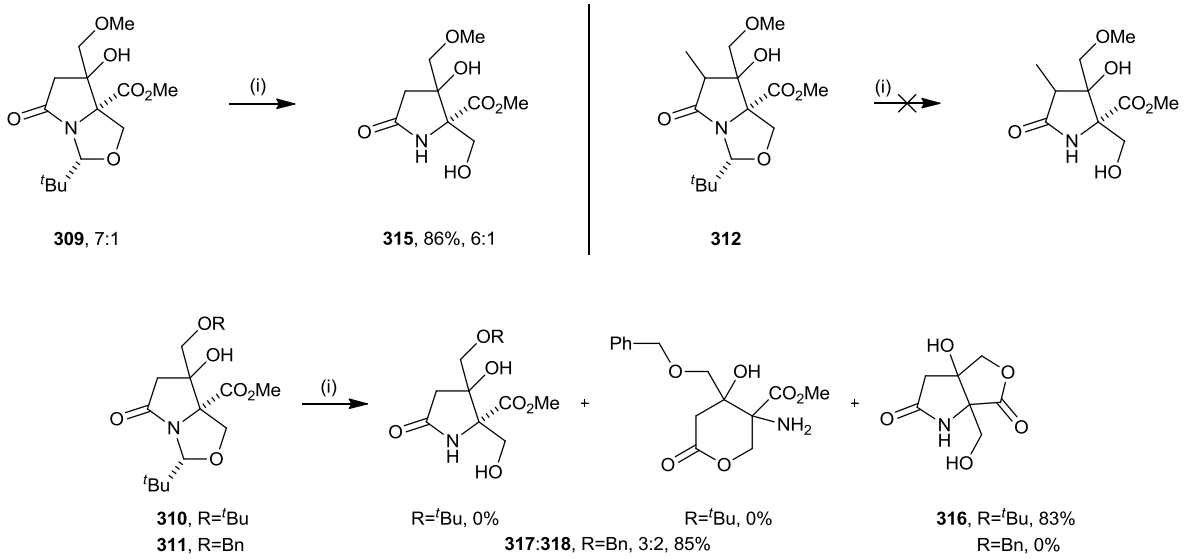
a R^1=Me, R^2=H, 32%
b R^1=*t*Bu, R^2=H, 41%
c R^1=Bn, R^2=H, 39%
d R^1=Me, R^2=Me, 68%
e R^1=*t*Bu, R^2=Me, 16%
f R^1=Bn, R^2=Me, 18%

309 R^1=Me, R^2=H, **a** 60%, **b** 8%
310 R^1=*t*Bu, R^2=H, **a** 55%, **b** 13%
311 R^1=Bn, R^2=H, **a** 53%, **b** 7%
312 R^1=Me, R^2=Me, **a** 6%, **b** 4%, **c** 8%
313 R^1=*t*Bu, R^2=Me, **a** 17%, **b** 38%, **c** 0%
314 R^1=Bn, R^2=Me, **a** 30%, **b** 13%, **c** 10%

Scheme 3.9 *Reagents and conditions:* (i) **289**, EDC, DMAP, DCM, rt, 5 h, (ii) NaOMe, MeOH, rt, 24 h.

The pyroglutamates were then subjected to the Corey-Reichard deprotection conditions[28] for which there were several outcomes (Scheme 3.10). The unsubstituted methoxy derivative **309** underwent the expected deprotection of the oxazolidine ring, to give the pyroglutaminol **315** in 86% yield. For the analogous methyl substituted **312** the reaction failed to proceed and only the starting material was isolated. In the cases of the *t*-butyl and benzyl derivatives **310**

and **311** rearrangements were a problem; the side product **316** was the sole product of the attempted deprotection of *t*-butyl derivative and a 3:2 ratio of the pyroglutaminol **317** and by-product **318** was formed in the benzyl case.



Scheme 3.10 *Reagents and conditions:* (i) 1,3-propanedithiol, 2% HCl in trifluoroethanol, rt, 12-20 h.

3.2 Tetramic acid mimics derived from L-threonine

Whilst the tetramic acid core is not strictly a component of the natural product oxazolomycin, it has been used as an intermediate in the synthesis of mimics for oxazolomycin which could therefore be reasonably described as a tetramic acid derivative. Tetramic acids are also of interest to synthetic chemists working in the area of antibacterials as there are numerous examples of natural products containing this template which exhibit biological activity.[29] As part of a wider investigation into the behaviour of pyroglutamates and tetramic acids derived from L-threonine (and therefore possessing the 16-methyl group present in the most antibacterially active members of the oxazolomycin family) it was felt that an investigation into the alkylations of L-threonine derived tetramic acids using this methodology was a useful starting point. It is postulated that the additional methyl group may have a significant effect on the biological activity of these templates and this work was expected to provide an insight into the characteristics and behaviour of L-threonine derived tetramic acids in order to help further develop chemistry aimed at the synthesis of the natural product 16-methyloxazolomycin **81d** (Figure 3.4).

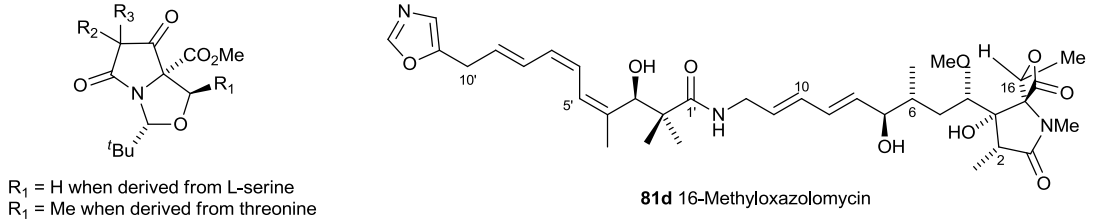
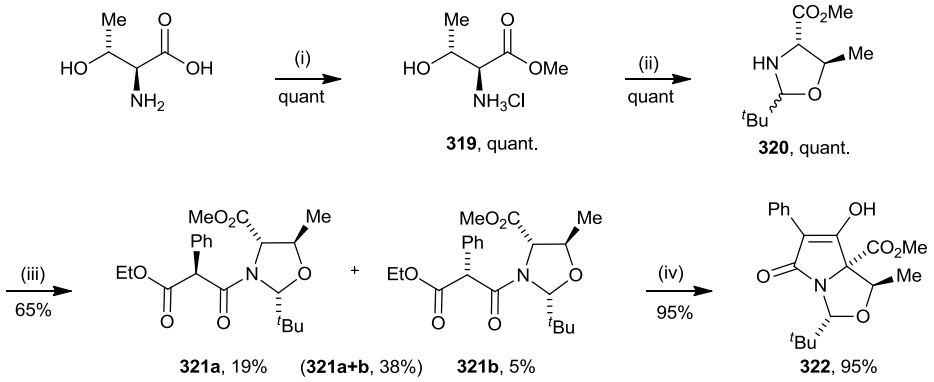


Figure 3.4 Tetramic acid template and 16-methyloxazolomycin **81d**.

3.2.2 Tetramic acid core

The tetramic acid intermediate **322** was first synthesised according to literature procedures set out by Moloney *et al.* from L-threonine in 4 steps.[15] Production of L-threonine methyl ester hydrochloride **319** proceeded in quantitative yield by treatment of L-threonine with $SOCl_2$ in MeOH and this was followed by a condensation reaction of the ester with pivalaldehyde to give the oxazolidine **320**, also in quantitative yield. Amide coupling between the oxazolidine and mono-ethyl phenylmalonate using DCC and DMAP gave diastereomeric amides **321a** and **321b** in 65% combined yield, which underwent a Dieckmann cyclisation upon treatment with KO*t*Bu to give the desired intermediate **322** in 95% yield (Scheme 3.11).

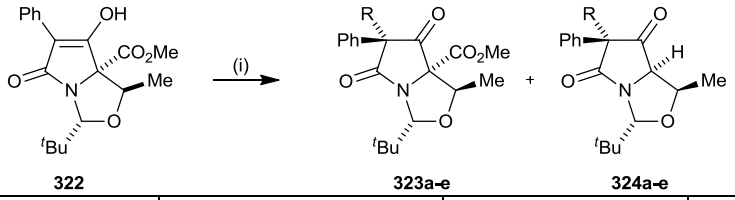


Scheme 3.11 *Reagents and conditions:* (i) $SOCl_2$, MeOH, 40 °C, (ii) *t*BuCHO, TEA, petrol, 60 °C, (iii) $EtO_2CCHPhCO_2H$, DCC, DMAP, DCM, rt, (iv) *t*BuOK, THF, 80 °C.

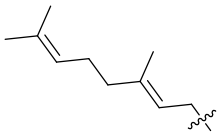
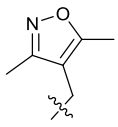
3.2.3 Alkylation of intermediate 322

With this in hand, attention turned to the alkylations of this intermediate with a variety of agents. Alkylating agents were chosen to synthesise analogues of the compounds identified in the study of L-serine derivatives[17] as well as some others it was thought would be of biological interest. Those included were benzyl, pyridylmethyl, dimethylisoxazolylmethyl, geranyl, and allyl halides. After treatment of **322** with NaH and benzyl bromide in THF, the compound **324a** was isolated as a single diastereoisomer in 50% yield, having undergone a

decarboxylation reaction in which the CO_2Me group had been lost. This species was the sole product isolated from this reaction. The decarboxylated **324b** was also the only species identified following the alkylation of **322** with allyl bromide, being isolated in 31% yield as a 76:23 mixture of diastereoisomers. For the reactions of **322** with 2-(bromomethyl)pyridine hydrobromide and geranyl bromide, both carboxylated species **323c** and **323d** were isolated in 8% yield along with portions of both decarboxylated species **324c** and **324d** in 13 and 5% yields respectively. Product **324c** was also a mixture of diastereoisomers, with a ratio of 65:35. The alkylation using 4-iodomethyl-3,5-dimethylisoxazole was the only one in which decarboxylation was not observed, with the compound **323e** being isolated in 59% yield. Following this observation, a reaction using benzyl iodide was carried out, in the hope that the more reactive alkylating agent may promote the production of **323a** over **324a**, but the decarboxylated **324a** was still the only product observed (isolated in 33% yield) (Scheme 3.12).



322 323a-e 324a-e

Alkylating agent	R	CO_2Me Product 323 (%)	Decarb Product 324 (%)
Benzyl bromide/Benzyl iodide	**a** CH_2Ph	0	50/33
Allyl bromide	**b** $CH_2CH{=}CH_2$	0	31 (73:26 dr at C(7))
2-(bromomethyl)pyridine hydrobromide	**c** CH_2Py	8	13 (65:35 dr at C(7))
Geranyl bromide	 **d**	8	5
4-Iodomethyl-3,5-dimethylisoxazole	 **e**	59	0

Scheme 3.12 *Reagents and conditions:* (i) NaH, alkylating agent, THF, 50 °C 3-24 h.

Stereochemical assignments of all single diastereoisomers were made by nOe analysis (Figure 3.5). For **324a** and **323e**, which were crystalline, this assignment was confirmed by acquisition of crystal structures from single crystal X-ray diffraction experiments (see Figure

3.6). In all cases the newly introduced substituent was found to occupy the *exo*- position with respect to the bicyclic ring system. This is in agreement with the results observed in the serine analogues.

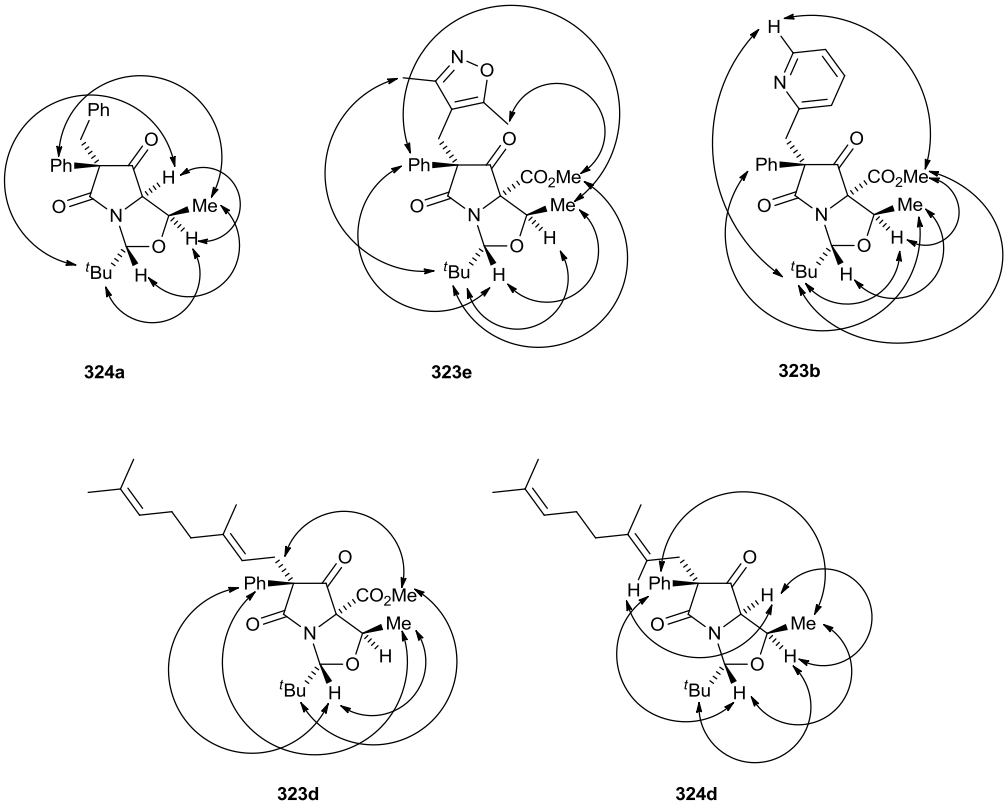


Figure 3.5 nOe enhancements for single diastereoisomers

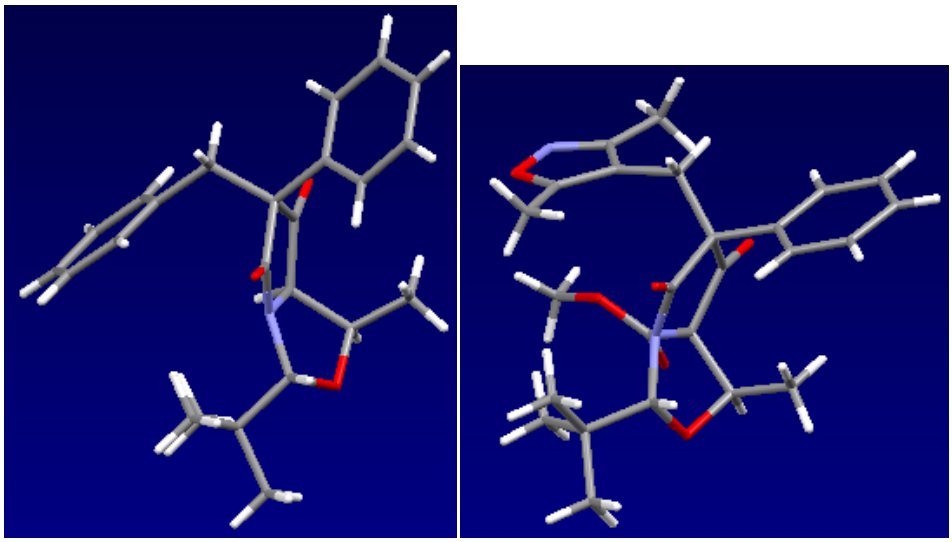
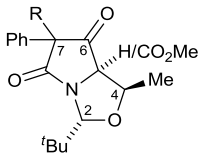


Figure 3.6 X-ray crystal structures for **324a** and **323e**.

The assignment of the major diastereoisomer of **324b** and **324c** as having the *exo*-C(7)R stereochemistry can be made by comparison of the chemical shift of the C(4)Me group in the ^{1}H NMR spectra of these compounds (Table 3.1). For the single diastereoisomers possessing

an *exo*-C(7)R group, the signal for the C(4)*Me* is a doublet between 0.58 and 0.79 ppm. For the major isomers of **324b** and **324c** the C(4)*Me* shifts are at 0.81 and 0.66 ppm, in close agreement with the range observed in the known isomers, indicating that they possess the same relative stereochemistry. For the minor isomers *epi*-**324b** and *epi*-**324c**, doublets corresponding to the C(4)*Me* group are observed at 1.40 and 1.37 ppm. This significant change in the chemical shift (around 0.6 ppm above the upper limit of the range for *exo*-C(7)R) indicates a change in the relative stereochemistry of the compound. They have been assigned as the *endo*-C(7)R isomers. These chemical shift differences provide a valuable individual method for assignment of stereochemistry in these compounds.



Number	R =	C(2)H	C(4)H	C(5)H	C(1')H$_2$	*t*Bu	C(4)Me	C(7)R stereochemistry
324a	Benzyl	5.12	4.33	3.50	3.41 3.52	0.70	0.73	*exo*
323c	CH_2Py	5.16	5.05	-	3.74	0.95	0.68	*exo*
323e	CH_2-isoxazole	5.12	5.08	-	3.12 3.21	0.93	0.58	*exo*
323d	Geranyl	5.24	4.51	4.19	2.85 2.98	1.00	0.79	*exo*
324d	Geranyl	5.15	5.13	-	2.98 3.11	0.96	0.79	*exo*
324c	CH_2Py major	5.13	4.45	4.40	3.53 3.89	0.76	0.66	*exo**
***epi*-324c**	CH_2Py minor	5.07	4.45	4.40	3.60 3.95	0.82	1.37	*endo**
324b	Allyl major	5.25	4.52	4.23	2.90	0.99	0.81	*exo**
***epi*-324b**	Allyl minor	5.20	3.71	3.63	2.93	0.99	1.40	*endo**

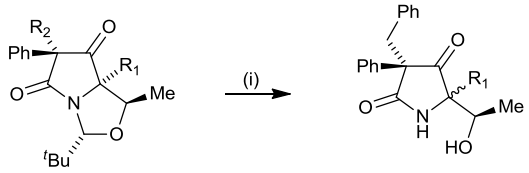
*assigned based on δ_H shift data

Table 3.1 Chemical shifts (ppm) of key protons in alkylated tetramic acids.

3.2.4 Deprotection of the oxazolidine ring

Subsequent deprotection of the oxazolidine to give the free alcohol and amide functionalities has been shown to lead to an increase in the biological activity of tetramic acids of this type derived from L-serine.[17] It was therefore desired to produce the deprotected forms of the above alkylated products. The production of a range of L-serine derived alcohols had been achieved with Corey and Reichard's[20] method using 1,3-dithiopropane in acidified trifluoroethanol in good yields.[17] Despite this precedent, the deprotection of these L-threonine

derivatives proved to be less straightforward. The deprotection of **324a** was carried out at 40 °C for 24 h using 1,3-dithiopropane in 1.5% HCl in 2,2,2-trifluoroethanol (Scheme 3.13). Following chromatography of the crude material, a sample of impure **325** was isolated, as a ~4:1 mixture of epimers at C(2). These isomers proved inseparable by chromatography, and the impurities co-eluted in the required polar solvent. An alternative procedure using 0.1 eq. of TFA was attempted but was unsuccessful, with TLC analysis indicating that no product had formed over a 3 day period and the ^{1}H NMR spectrum of the crude material appearing complex. The Corey-Reichard conditions were also tested with **323e**, in the hope that the presence of the CO_2Me group in this substrate would prevent the epimerisation and result in the production of only one product. After 24 h at 60 °C TLC analysis indicated no reaction had taken place so another 1.5 eq. of 1,3-dithiopropane and 2 drops of HCl were added. After a further 24 h of reaction time the starting material **323e** was the only observable spot on TLC analysis and was eventually isolated in 72% yield after chromatography. A harsher procedure where TFA was used as the solvent was also attempted on **323e**, and after 4 days TLC analysis still indicated the presence of starting materials. The ^{1}H NMR spectrum of the crude material was very complex and was attributed to the presence of TFA salts, but a basic aqueous wash did not improve the appearance. No further attempts to deprotect these substrates were made.



324a, R=Bn
323e, R=CH_2-isoxazole

325

Substrate	Reaction conditions	Outcome
324a	1.5 eq. 1,3-dithiopropane, 1.5% HCl in TFE, 40 °C, 24 h.	Very impure product **325**, 60% yield by mass in a 82:18 dr.
324a	0.1 eq. TFA, H_2O, TFE, 30 °C, 3 d.	TLC analysis showed only starting material **324a**. Complex crude ^{1}H NMR.
323e	1.0-2.5 eq. 1,3-dithiopropane, 1.5% HCl, TFE, 60 °C, 48 h.	**323e** isolated in 72% yield.
323e	Neat TFA, H_2O, rt, 4 d.	Broadened ^{1}H NMR. Basic aq. wash did not improve spectrum.

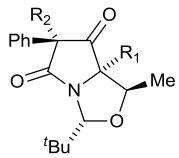
Scheme 3.13 *Reagents and conditions:* (i) see table.

Upon inspection of the crystal structure of **323e** it appears that both the protonation sites, the N and O, may be sterically hindered by the high level of substitution in the molecule. As

protonation is the first step in the acid-catalysed deprotection reaction this could reasonably be assumed to account for the lack of reactivity of compound **323e** and that varying the reaction conditions is unlikely to lead to any improvement. For the reaction of **324a**, it could be possible to improve the outcome using different conditions which may not promote epimerisation and lead to fewer impurities being introduced, though the X-ray crystal structure of **324a** indicates that the molecule is highly hindered making it likely that the reaction is operating on the boundary of feasibility.

3.2.5 Biological evaluation

Bioassays were performed to discover the antibacterial activity of the single diastereoisomers synthesised. The compounds were tested for activity against *S. aureus* D267 and *E. coli* X580 using the hole-plate method. None of the compounds tested exhibited any antibacterial activity in this assay, though it is notable that many of them did not fully dissolve in the requisite 70% DMSO in H_2O solvent (Table 3.2). In the case of **323c** a precipitate was found in the well following incubation and compound **323e** was used as a suspension. A broth assay against a wider panel of pathogens found that **323e** was active against *H. influenza* Hi4, a strain that does not possess efflux pumps, at 8 μg/mL, equal to the MIC of the reference compound linezolid (see appendix B). Efflux pumps are transport proteins located in the cytoplasmic membrane of cells which facilitate the removal of substances such as toxins and antibiotics from the cell by active transport; this is a common mechanism for antibacterial resistance. Pumps may be specific for one substrate or recognise many structurally dissimilar compounds and are associated with multiple drug resistance in bacteria.

Compound Number	**R_1**	**R_2**	***S. aureus* D267**		***E. coli* X580**	
			Zone size (mm)	**Rel. potency (Ceph C, %)**	**Zone size (mm)**	**Rel. potency (Ceph C, %)**
Tetramic acids 						
324a	H	Bn	0	-	0	-
323c	CO_2Me	CH_2Py	0	-	0	-
323d	CO_2Me	Geranyl	0	-	0	-

Compound Number	R_1	R_2	*S. aureus* D267		*E. coli* X580	
			Zone size (mm)	Rel. potency (Ceph C, %)	Zone size (mm)	Rel. potency (Ceph C, %)
324d	H	Geranyl	0	-	0	-
323e	CO_2Me	CH_2-isoxazolyl	0	-	0	-

Table 3.2 Bioassay results for alkylated tetramic acids.

3.3 Pyroglutamate mimics of the right-hand fragment of 16-methyloxazolomycin

Following the success of investigations into the aldol cyclisations of *N*-acyloxazolidines derived from L-serine, it was envisaged that this method may also be applied to the synthesis of mimics for the right-hand fragment of 16-methyloxazolomycin **81d** which possesses an additional methyl group on the β-lactone ring. The natural products Curromycin A **81e** and B **f**, KSM-2690 B **g** and C **h** and lajollamycin **83** also possess the 16-methyl substituent and are the most antibacterially active members of the oxazolomycin family. The introduction of this methyl group could be achieved by using L-threonine in place of L-serine in the formation of the oxazolidine. It has previously been shown that the threonine derived oxazolidines behave very similarly to serine oxazolidines in the amide coupling and Dieckmann cyclisation approaches.[30,31] The regio- and stereo-selective C(7) alkylations of threonine-derived tetramic acids have been described above and give credence to the hypothesis that the presence of the additional methyl group might affect the stereocontrol of reactions on the rigid bicyclic ring.

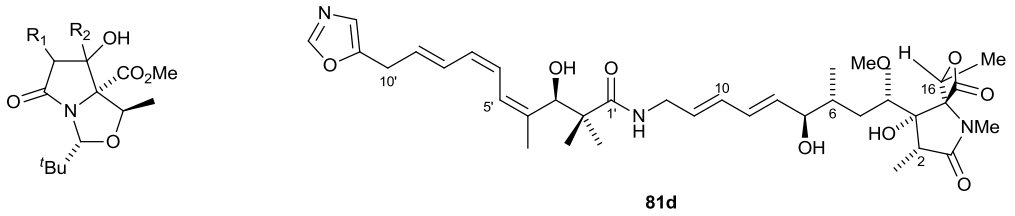


Figure 3.7 16-Methyloxazolomycin **81d** and the structure of proposed analogues.

The aim of this work was to construct a library of threonine-derived pyroglutamates and to investigate methods of varying the substituent on the C(6) side chain with a view to creating closer structural mimics of the natural product, ultimately incorporating the *gem*-dimethylamide fragment. This would be attempted using aldol methodology to effect cyclisations of *N*-acyloxazolidines obtained from the coupling of β-keto-acids and the threonine-derived oxazolidine **320** (Figure 3.8).

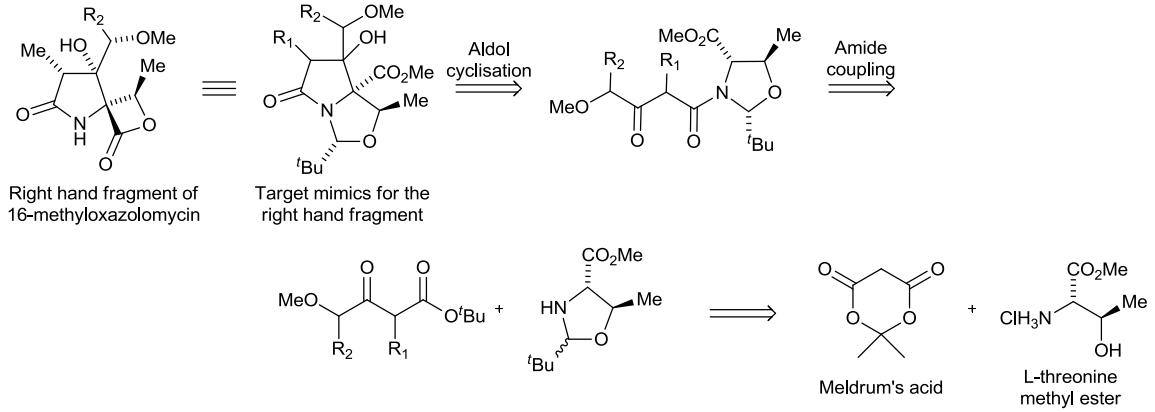
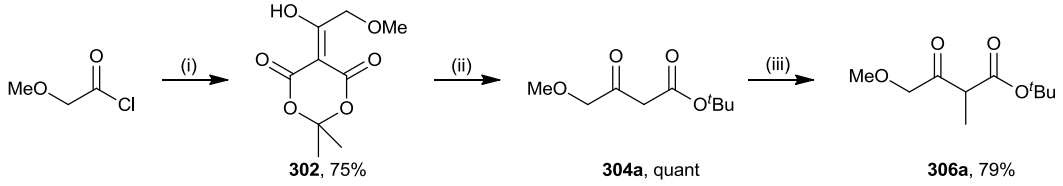


Figure 3.8 Retrosynthesis of proposed 16-methyloxazolomycin mimics.

3.3.2 β-Keto-ester synthesis

The sequence described by P. Fryer[26] was also replicated to generate the β-keto-ester **304a**. Meldrum's acid was acylated with methoxyacetyl chloride in DCM to give the enol tautomer product **302** in 75% yield and the subsequent ring opening/decarboxylation with *t*BuOH in toluene proceeded quantitatively to give **304a**. In order to introduce the methyl substituent present on the pyroglutamate core of the oxazolomycins, the β-keto-ester **304a** was then methylated in the α-position. Treatment with *t*BuOK and MeI in THF gave the α-methyl-β-keto-ester **306a** in 79% yield (Scheme 3.14).

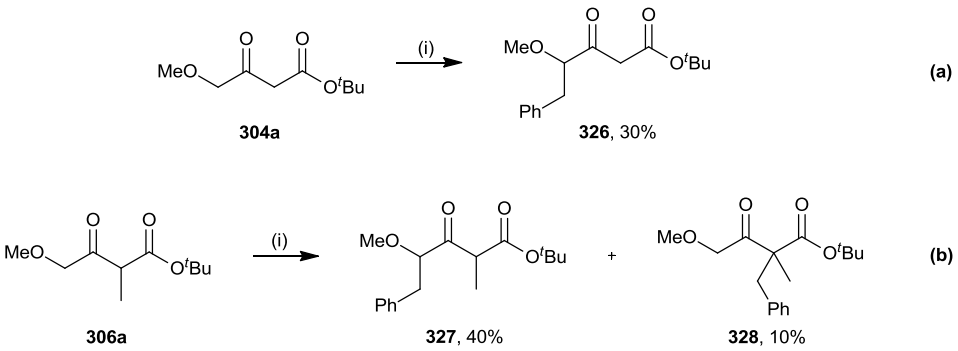


Scheme 3.14 *Reagents and conditions:* (i) Meldrum's acid, py, DCM, −10 °C to rt, 2 h, (ii) tol:*t*BuOH 1:1, reflux, 2 h, (iii) *t*BuOK, MeI, THF, rt, 5h.

3.3.3 γ-Alkylations of β-keto-esters

In order to develop a versatile synthetic route to enable the incorporation of different functional groups, and ultimately the rest of the oxazolomycin molecule into the pyroglutamate system, some time was taken to investigate the chemistry of the β-keto-esters to determine if alkylation in the γ-position as well as the α-position might be possible. Any substituent added to the γ-position at this stage would correspond to the position of the diene linker unit in the natural product. Following a literature procedure,[32] the β-keto-ester **304a** was treated with 1 eq. of NaH to deprotonate the α-C, followed by 1 eq. of BuLi to

deprotonate the γ-C and form the dianion. This species was bright yellow in colour and after 10 minutes stirring at 0 °C became slightly dulled. Addition of BnBr resulted in discolouration of the reaction mixture and, following work-up, the γ-benzyl-β-keto-ester **326** was isolated in 30% yield by column chromatography (Scheme 3.15 (a)). This yield is modest but nonetheless sufficient for purpose given the ease with which large quantities of **304** can be synthesised. The same procedure was applied to the ester **306a** and the resulting γ-benzyl ester **327** was isolated in 40% yield, along with the α-substituted **328** in 10% yield (Scheme 3.15 (b)).



304a **326**, 30%

306a **327**, 40% **328**, 10%

Scheme 3.15 *Reagents and conditions:* (i) NaH, BuLi, BnBr, THF, 0 °C to rt, 0.25 h.

With this success, other alkylating agents were used to investigate the scope of this reaction and to introduce a variety of substituents.

Whilst repeating these reactions in order to generate more material, it was noted that the procedure was unreliable and sometimes unexplainably produced no conversion to the desired product. As the colour changes that occurred during the reaction seemed to differ between repeat reactions, it was thought that the stability/formation of the dianion after the addition of BuLi may be the unreliable step. It was hoped that by adding DMPU to the reaction that the reactivity of the BuLi may be moderated and that the deprotonation may occur more effectively. With the addition of 0.1 eq. of DMPU, the reaction of **306a** did proceed to give some of the desired product **327**, in a reduced yield of 20%, along with 10% of the starting material, and 18% of the by-product **329** (Figure 3.9) resulting from a retro-aldol process. Increasing the stoichiometry of DMPU to 1.0 eq. only served to decrease the conversion to the product.

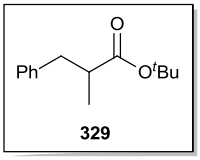
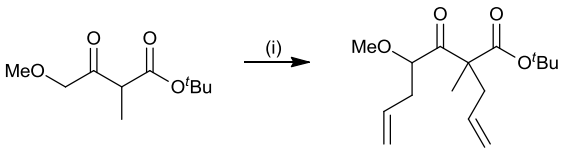


Figure 3.9 By-product **329**.

Allyl halides

The same conditions were applied to **306a** using allyl bromide in place of benzyl bromide and on examination of the crude ^{1}H NMR spectrum, only peaks corresponding to the starting β-keto-ester could be identified. It was suspected that perhaps allyl bromide was not a strong enough alkylating agent and so allyl iodide was used instead in a repeat reaction. On this occasion, the crude spectrum contained new peaks and column chromatography isolated a fraction of the doubly-alkylated compound **330** in 22%, along with a portion of the starting material (13%) (Scheme 3.16). Multiple spots were visible on the TLC plate but no other fractions could be unambiguously identified. Altering the procedure to add allyl iodide at −20 °C very slowly did not lead to the production of any of the desired monoalkylated product.

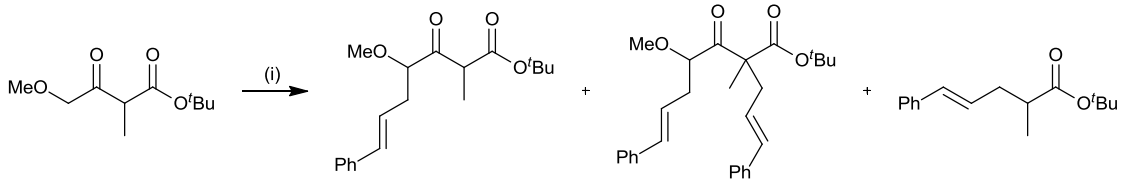


306a **330**, 22%

Scheme 3.16 *Reagents and conditions:* (i) NaH, BuLi, allyl iodide, THF, 0 °C to rt, 0.25 h.

Cinnamyl bromide

Cinnamyl bromide was also investigated as its reactivity is close to that of benzyl bromide, but under the original procedure no reaction was observed. When heated to 30 °C and left for 5 h instead of 15 min, there was a change observable in the crude ^{1}H NMR spectrum. After chromatography, the by-product **333** was identified in 18% yield, 7% of the starting material **306a** was recovered along with a fraction which might have contained the product **331**, but was not of sufficient purity to be unambiguously identified. The reaction was repeated with 0.1 eq. of DMPU and gave the by-product **333** in 28% yield, the doubly alkylated species **332** in 10% yield and returned both the starting materials cinnamyl bromide (30%) and ester **306a** (15%) (Scheme 3.17).



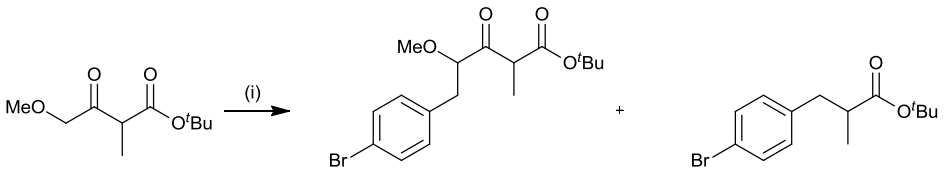
306a **331** **332** **333**

Entry	Reagents and conditions	Yield 331	Yield 332	Yield 333	% mass return of 306a
1	NaH, BuLi, cinnamyl bromide, THF, 0 °C to rt.	0	0	18	7
2	NaH, DMPU, BuLi, cinnamyl bromide, 0 °C to rt.	0	10	28	15

Scheme 3.17 *Reagents and conditions:* (i) See table.

p-Bromobenzyl bromide

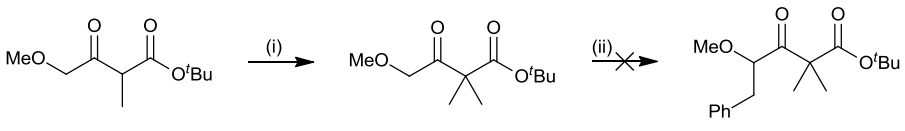
p-Bromobenzyl bromide was also tested since the bromo-aryl functionality had potential for manipulation through Pd-catalysed coupling reactions. Reaction under the DMPU conditions resulted in the desired product **334** in 18% yield, along with 8% of the by-product **335** and 18% of the starting ester **306a** (Scheme 3.18). Increase in the reaction time between the ester and both NaH and BuLi to allow for the complete formation of the mono-and di-anion did not increase the conversion to the desired product.



306a **334**, 18% **335**, 8%

Scheme 3.18 *Reagents and conditions:* (i) NaH, BuLi, DMPU, *p*-Br-BnBr, THF, 0 °C to rt, 0.25 h.

The dimethyl ester **336** was also synthesised in 34% yield by repeated methylation of the α-position in **306a** (Scheme 3.19). It was hoped that this would allow for the more straightforward alkylation of the mono-anion at the γ-position. Attempted alkylation of **306a** using LDA and BnBr was unsuccessful and only unreacted starting materials were visible in the crude ^{1}H NMR spectrum.



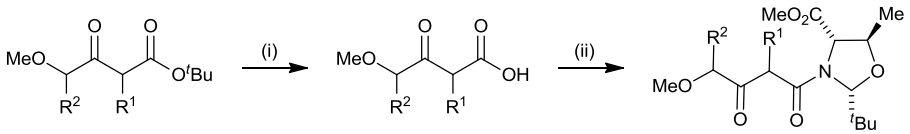
306a **336**, 34% **337**

Scheme 3.19 *Reagents and conditions:* (i) *t*BuOK, MeI, THF, rt, 5 h, (ii) LDA, BnBr, THF, 0 °C to rt.

Whilst this approach had produced sufficient material for the continuation of the synthetic sequence in the case of the benzyl derivatives, it was decided that the unreliable nature of these reactions made this approach unattractive for the generation of analogues and that alternative methods for modifying the substituents would be sought.

3.3.4 Elaboration to pyroglutamates

With β-keto-esters **304a**, **306a**, **326** and **327** in hand, attention turned to continuing the synthetic sequence to access functionalised pyroglutamates. The esters were all hydrolysed in a 1:1 mixture of TFA and DCM, to give the corresponding acids **305a**, **307a**, **338** and **339** in very high yield (Scheme 3.20). The carboxylic acids were then reacted with the oxazolidine **320** *via* an amide coupling reaction using DCC and DMAP in DCM to form the *N*-acyloxazolidines **340a-d**. It had been reported that the serine analogues of these oxazolidines were unstable to chromatography and that reaction using the crude product still furnished the desired pyroglutamates.[26] Purification of the *N*-acyloxazolidine **340a** was attempted and from a crude mixture weighing 330 mg, two fractions weighing 77 and 81 mg were isolated. Each of these fractions showed peaks in the ^{1}H NMR spectra that were consistent with the presence of the product, but both appeared more impure than the original crude mixture. It was decided that in following repetitions of this work the *N*-acyloxazolidines would be reacted on as crude mixtures.



304a R^1=H, R^2=H
306a R^1=Me, R^2=H
326 R^1=H, R^2=Bn
327 R^1=Me, R^2=Bn

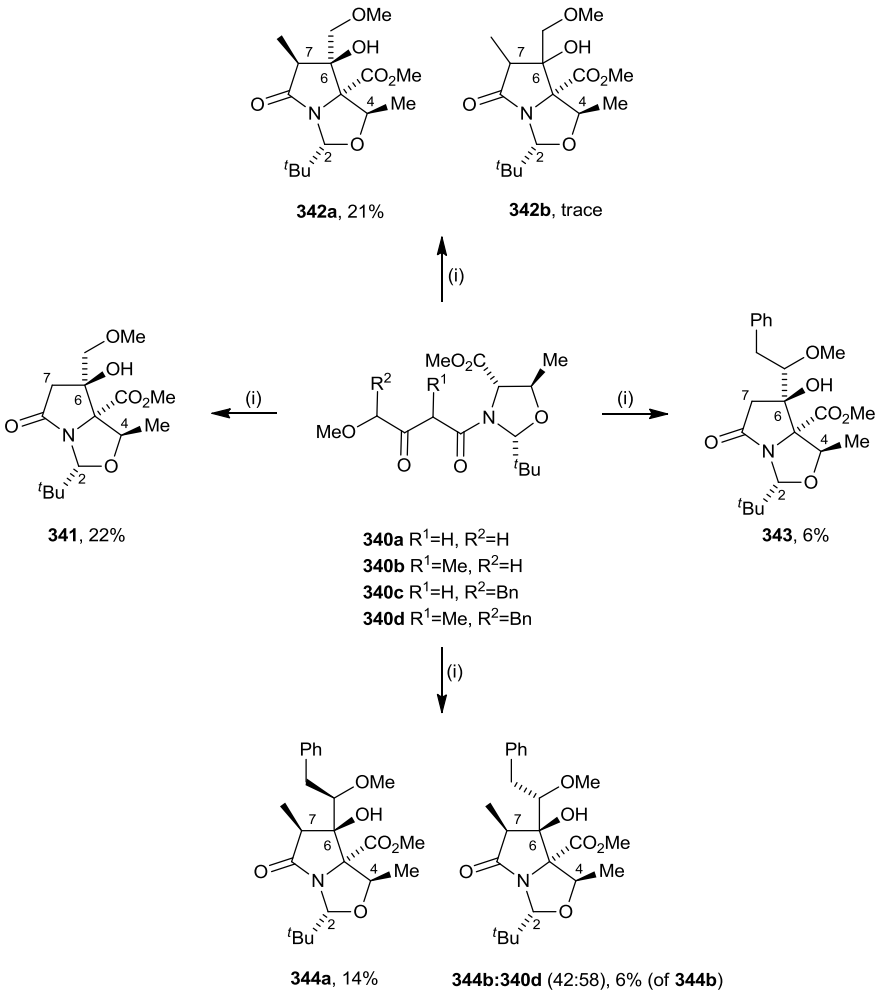
305a R^1=H, R^2=H, quant
307a R^1=Me, R^2=H, 93%
338 R^1=H, R^2=Bn, quant
339 R^1=Me, R^2=Bn, 98%

340a R^1=H, R^2=H
340b R^1=Me, R^2=H
340c R^1=H, R^2=Bn
340d R^1=Me, R^2=Bn

Scheme 3.20 *Reagents and conditions:* (i) DCM:TFA 1:1, rt, 2 h, (ii) **320**, DCC, DMAP, DCM, rt, 4 h.

The *N*-acyloxazolidines **340a-d** were all treated with NaOMe in MeOH for 24 h and then purified by column chromatography (Scheme 3.21). Purification of the crude mixture for the reaction of **340a** produced the single diastereoisomer **341** in 22% yield. No other pure products were isolated. Likewise, the reaction of **340c** produced only one diastereoisomer of the cyclised product **343** in 6% yield. Following the reaction of **340b** two isomers of the

bicyclic aldol product were isolated, the major isomer **342a** in 21% yield, along with another fraction containing **342a** and a trace amount of the minor isomer **342b** (visible only in the ^{1}H NMR spectra) in 10% yield. There was no other fraction containing the minor isomer. In the final case of the cyclisation of **340d**, there was a fraction of the major isomer **344a** isolated with a yield of 14%, along with a 48:52 mixture of a minor isomer **344b** with one isomer of the starting material **340d**; an effective 6% yield of the cyclised product. This indicates that although the attempted purification of **340a** was unsuccessful, these compounds could be stable to chromatography.



Scheme 3.21 *Reagents and conditions:* (i) NaOMe, MeOH, rt, 24 h.

3.3.5 Stereochemical assignment of the pyroglutamates

It was important to establish the stereochemistry of the major isomer in each case and to compare this to the stereochemistry of the previous library of serine derivatives. The first method employed nOe experiments conducted on the compounds **341-344,** for which the

results are shown in Figure 3.10. For the example case of **342a**, the stereochemistry of the oxazolidine ring was confirmed by the presence of enhancements between C(2)*H* and C(4)*Me*, both known to be on the *endo* face of the bicyclic ring system due to the self-regeneration of chirality[16] occurring upon *N*-acylation of the oxazolidine. The methyl ester was confirmed as being on the *exo* face through an enhancement to the C(2)*tBu*. An enhancement between C(2)*H* and C(7)*Me* indicated that the C(7)*Me* group occupied the *endo* face. The C(6)O*H* showed an enhancement to C(7)*Me*, indicating it had an *endo* position. However, the C(1′)H_2 showed enhancements to C(7)*Me*, C(7)*H* and C(4)*Me* (unexpected signals indicated in red in Figure 3.10). This pattern was also observed in the pyroglutamate **341** with enhancements seen between C(1′)H_2 and *endo*-C(7)*H*, *exo*-C(7)*H*, CO_2Me and C(4)*Me*. These signals did not appear to be consistent with either epimer at this carbon and so as well as acquiring X-ray crystal structures for the crystalline examples, molecular modelling calculations were carried out to predict the shape of each diastereoisomer to establish the likeliness of each fitting the nOe enhancements that had been observed.

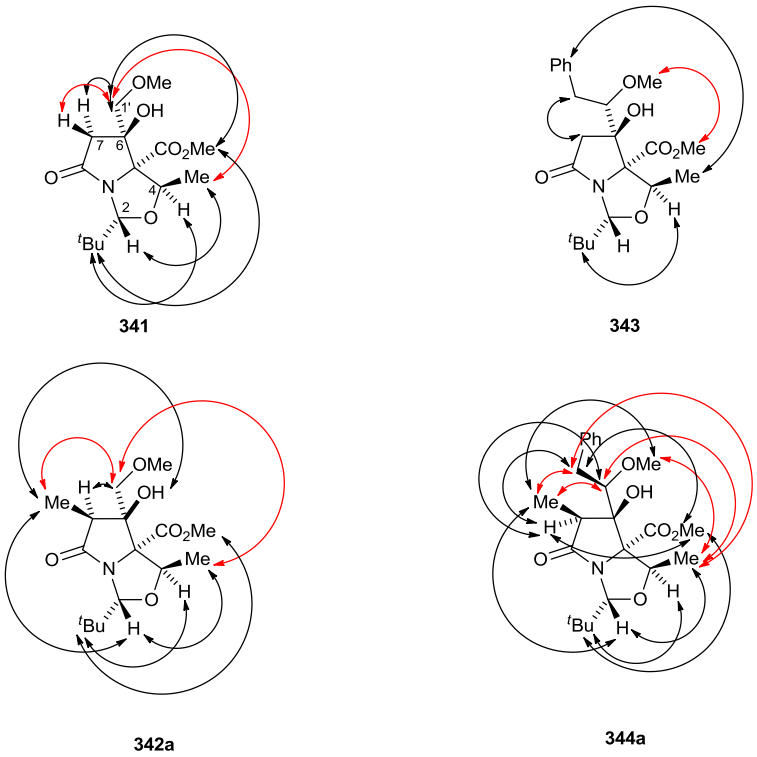


Figure 3.10 nOe enhancements for pyroglutamates **341-344**.

The molecular modelling was carried out using ChemBio3D Ultra, and after minimising the conformational strain on **341** the distance between C(4)Me and the closest C(1′)H was

predicted to be 3.25 Å. Single crystal X-ray analysis confirmed the stereochemistry of C(6) as having the OH in the *endo* position and the CH_2OMe in the *exo* position. It also showed the solid-state distance between the C(4)Me and C(1′)H proton to be 2.75 Å. At this internuclear distance, a strong nOe enhancement would be expected, and is consistent with the observations made. The crystal structure also allowed the distances between the C(7)H and C(1′)H and OH protons to be measured and they were between 2.6 and 3.7 Å in **341**. The stereochemistry of the **342a**, **344a** and **344b** were also confirmed by single crystal X-ray experiments (Figure 3.11 and Figure 3.12). All three compounds had the C(7)Me and C(6)OH in the *endo* position and all showed the same short internuclear distance between the C(4)Me and the C(1′)H protons, and between all the C(7)H and C(6) substituents.

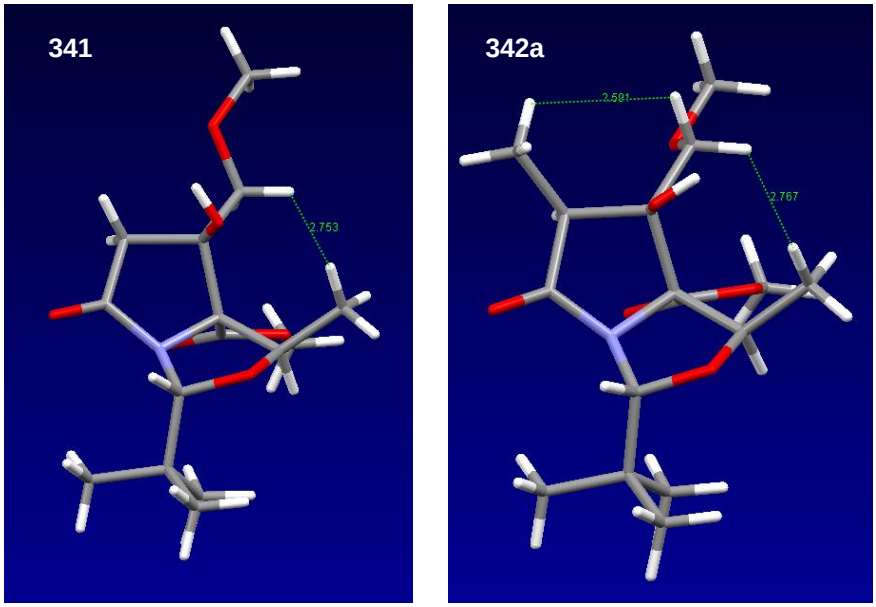


Figure 3.11 X-ray crystal structures of **341** and **342a**.

The minor isomer **344b** was crystallised from a mixture containing the *N*-acyloxazolidine **340d**. The major and minor isomers **344a** and **344b** were found to be epimers at the C(1′) stereocentre, the major isomer having a 1′-*S* configuration, with the minor being 1′-*R* (Figure 3.12). This means that the fundamental stereochemistry of the bicyclic ring system was the same for both isomers. The similarities between the pattern of nOe enhancements and predicted internuclear distances between all the pyroglutamates allowed the prediction that the remaining compound **343**, for which insufficient material was obtained to attempt the growth of a single crystal for diffraction experiments, would have the same relative stereochemistry

on the bicyclic ring system. The stereochemistry of the final C(1′) chiral centre in **343** remains unassigned.

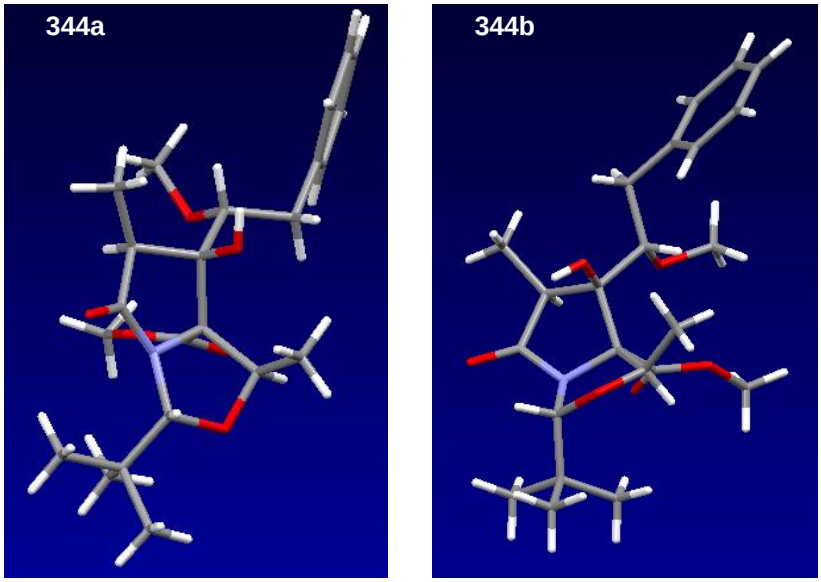


Figure 3.12 X-ray crystal structures of **344a** and **344b**.

It is of interest to note how this arrangement compares to that of the natural products. The pyroglutamate **344a,** which was the major product from the most highly substituted aldol reaction, possesses the same relative stereochemistry as the natural product **81d** at all 5 contiguous stereocentres (Figure 3.13). The correct absolute configuration could be accessed using the same sequence starting from the D-threonine instead of L-threonine. This means that the selectivity of the aldol reaction produces the correct stereochemistry for the natural product. This could be interpreted to indicate that this reaction sequence in some way mimics the biosynthetic pathway by which these compounds are produced in nature.

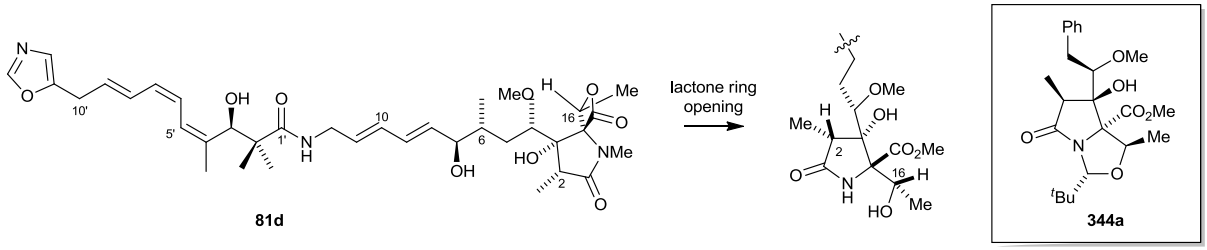


Figure 3.13 Depiction of the stereochemistry of the right-hand fragment of 16-methyloxazolomycin **81d**.

3.3.6 Varying the C(6)R group by using different acid chlorides

In light of the disappointing results obtained from the investigation into the γ-alkylations of β-keto-esters, a new way of introducing C(6) functionality to the pyroglutamates was desired. It

was decided that a more general investigation into the use of Meldrum's acid to synthesise β-keto-esters would be helpful and that it would, if successful, allow an investigation into the scope of the aldol cyclisation by using a series of different substituents. The use of a selection of acid chlorides would be tested, with the R groups of the acid chlorides resulting in different C(6) substituents on the pyroglutamate motif. Acid chlorides were chosen that contained groups that could allow further manipulation of the resulting pyroglutamates (exemplified in Figure 3.14). These were a double bond moiety, which could be modified in a variety of ways, a bromophenyl group which could be modified by Pd coupling reactions, and a thiol-ether, which it was envisaged may aid the γ-alkylation reaction through the stabilisation of adjacent anions.

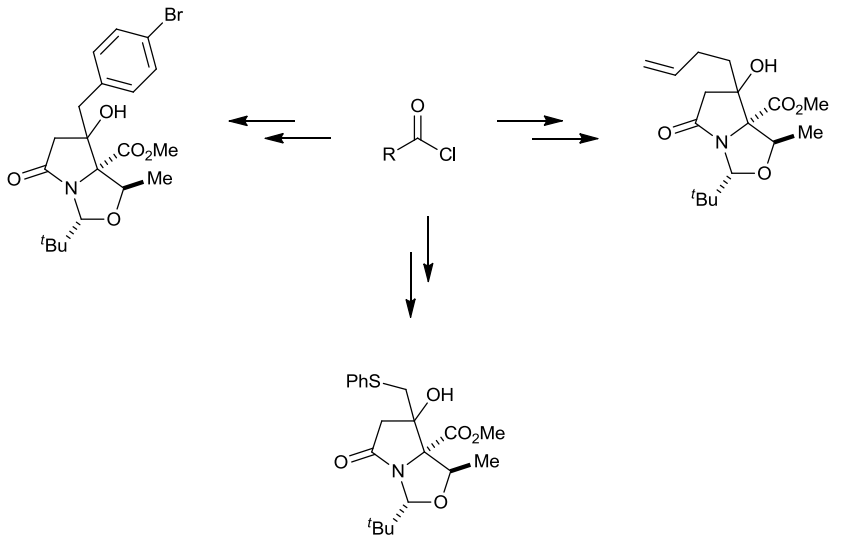
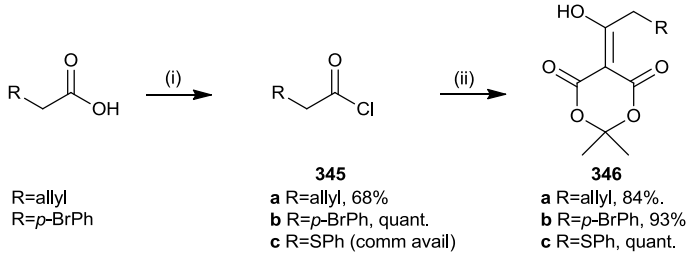


Figure 3.14 Chosen acid chlorides and their envisaged resulting pyroglutamates.

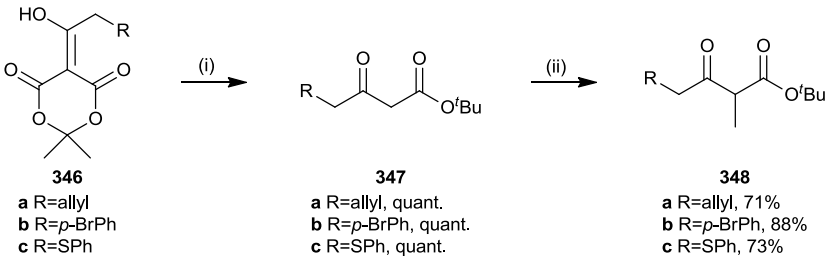
1.1.1.1 β-keto-ester synthesis

The acid chlorides 4-pentenoyl chloride **345a** and 4-bromophenylacetyl chloride **345b** were obtained in 68% and 100% yield by reaction of the requisite carboxylic acid with oxalyl chloride in DCM. The remaining 2-phenylthioacetyl chloride **345c** was commercially available. The acid chlorides were reacted with Meldrum's acid under the conditions described previously to give the enols **346a-c** in 84%-quantitative yield (Scheme 3.22).



Scheme 3.22 *Reagents and conditions:* (i) oxalyl chloride, DCM, 40 °C, 2 h, (ii) MA, py, DCM, rt, 5 h.

The β-keto-esters **347a**, **b**, and **c** were synthesised in quantitative yield by ring opening/decarboxylation of the enols **346a**, **b**, and **c**, carried out with *t*-BuOH in toluene. These β-keto-esters were methylated to install the requisite methyl substituent using *t*-BuOK and MeI in THF to give the α-methyl species **348 a**, **b**, and **c** in 71%, 88% and 73% yield respectively (Scheme 3.23).

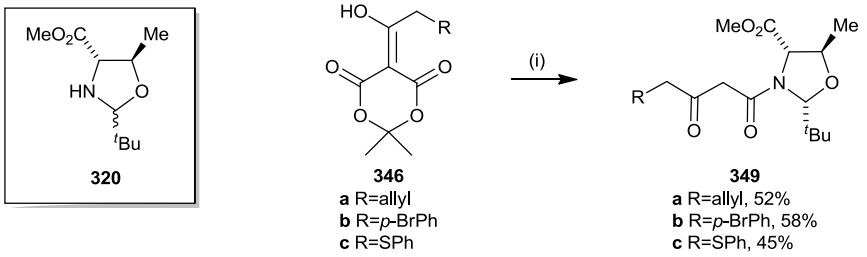


Scheme 3.23 *Reagents and conditions:* (i) 1:1 *t*BuOH:toluene, reflux, 2 h, (ii) *t*BuOK, MeI, THF, rt, 5 h.

3.3.6.2 Synthesis of *N*-acyloxazolidines

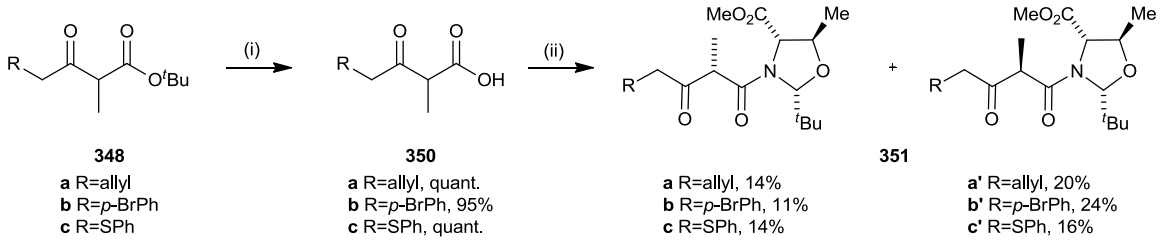
Concurrently to the synthesis of the previously described C(6)-methoxymethyl substituted pyroglutamates, an optimisation study of the original serine analogues was conducted and an alternative method for the synthesis of the unsubstituted *N*-acyloxazolidines by direct opening of the acylated Meldrum's acid species with the serine oxazolidine **289** was developed.[25] Thus, the Meldrum's acid derivative **346a** was treated with 0.9 eq. of the threonine oxazolidine **320** and heated to 60 °C in MeCN for 2 h. Following purification, the resulting oxazolidine **349a** was obtained in 52% yield. This method was also carried out with the bromophenyl **346b** and phenylthio **346c** derivatives to give the *N*-acyloxazolidines **349b** and **349c** in 58% and 45% yields respectively (Scheme 3.24). This method represented a time saving and increased the overall yield of these oxazolidines. The *N*-acyloxazolidine **349a** was also synthesised *via* the original method. Although the ring opening with *t*BuOH to form the

β-keto-ester and the subsequent ester hydrolysis reaction both proceed quantitatively, the amide coupling reaction to form **349a** had only a 28% yield as well as being two steps longer.



Scheme 3.24 *Reagents and conditions:* (i) **320**, MeCN, 60 °C, 2 h.

In order to synthesise the methyl analogues, the β-keto-esters **348a-c** were hydrolysed in greater than 95% yield with TFA to give the carboxylic acids **350a-c**. Amide coupling reactions using DCC and DMAP in DCM with the oxazolidine **320** resulted in the formation of 2 isomers (epimers at C(2′)) of each of the *N*-acyloxazolidines, **351a-c** in 34, 35 and 30% combined yield respectively (Scheme 3.25). The stereochemistry of the C(2′)Me group for **320b′** and **320c′** has been confirmed by X-ray crystallography (see Appendix C), and the configuration of **320a′** is assigned based on the relative polarities of the two epimers in each sample.



Scheme 3.25 *Reagents and conditions:* (i) 1:1 TFA:DCM, rt, 1.5 h, (ii) **320**, DCC, DMAP, DCM, rt, 4 h.

It is of interest to note that whilst the attempted purification of the methoxy-substituted *N*-acyloxazolidine **340a** indicated that these compounds were unstable to chromatography, all 6 variants in this library were purified successfully in this manner. It is however, notable that that the non-methylated *N*-acyloxazolidines **349c-a** have complex ^{1}H NMR spectra, and this may account for the previous report that use of the crude samples was better.[26]

3.3.6.3 Assignment of NMR spectra for *N*-acyloxazolidines

All the *N*-acyloxazolidine samples were been purified by column chromatography and appear as a single spot when studied by TLC analysis. Isomerism is possible from a variety of sources. Diastereoisomers can occur at C(2), as the *t*Bu can be *cis* or *trans* to the CO_2Me group, and in the methyl-substituted analogues it can also occur at C(2′). In previous experiments, if it is observed at all, the *trans* oxazolidine is minor compared to the *cis*.[14,33] It has also been observed that *N*-acyloxazolidines such as these can form rotamers,[15] resulting from hindered rotation of the acyl side chain caused by the bulky *t*Bu group on the oxazolidine ring. It is also possible that these compounds exist as a mixture of keto and enol tautomers, resulting from the acidic protons present between the two carbonyl groups of the acyl side chain. Any or all of these factors being present in a sample would make assignment of the NMR spectra challenging.

The interpretation of the ^{1}H NMR spectra of the unsubstituted *N*-acyloxazolidines **349** was problematic and did not reconcile easily with the proposed structures. In each case, some but not all of the signals appeared to be duplicated and the relative integrations of the peaks did not match. In the spectrum of **349a** (Figure 3.15) there are 2 singlets around 0.91 ppm corresponding to the *tBu* protons and 2 singlets at 3.75 ppm for CO_2*Me*, each with a slightly greater than 1:1 ratio. There are also 2 doublets almost overlapping at around 1.35 ppm corresponding to the C(5)*Me* and 2 broadened peaks corresponding to the C(5)*H* at around 4.70 ppm with the same ratios. Although this suggests that there are two species present, there is only one visible signal visible for C(2′)H_2, C(4)*H*, and C(2)*H* at 3.60, 4.30, and 5.40 ppm respectively, each of which integrate correctly compared to the major peak for the duplicated signals. The other signals are all multiplets and integrate to almost double their expected value, suggesting that they are overlapping peaks from 2 species. The same duplication pattern is observed in the ^{13}C spectra.

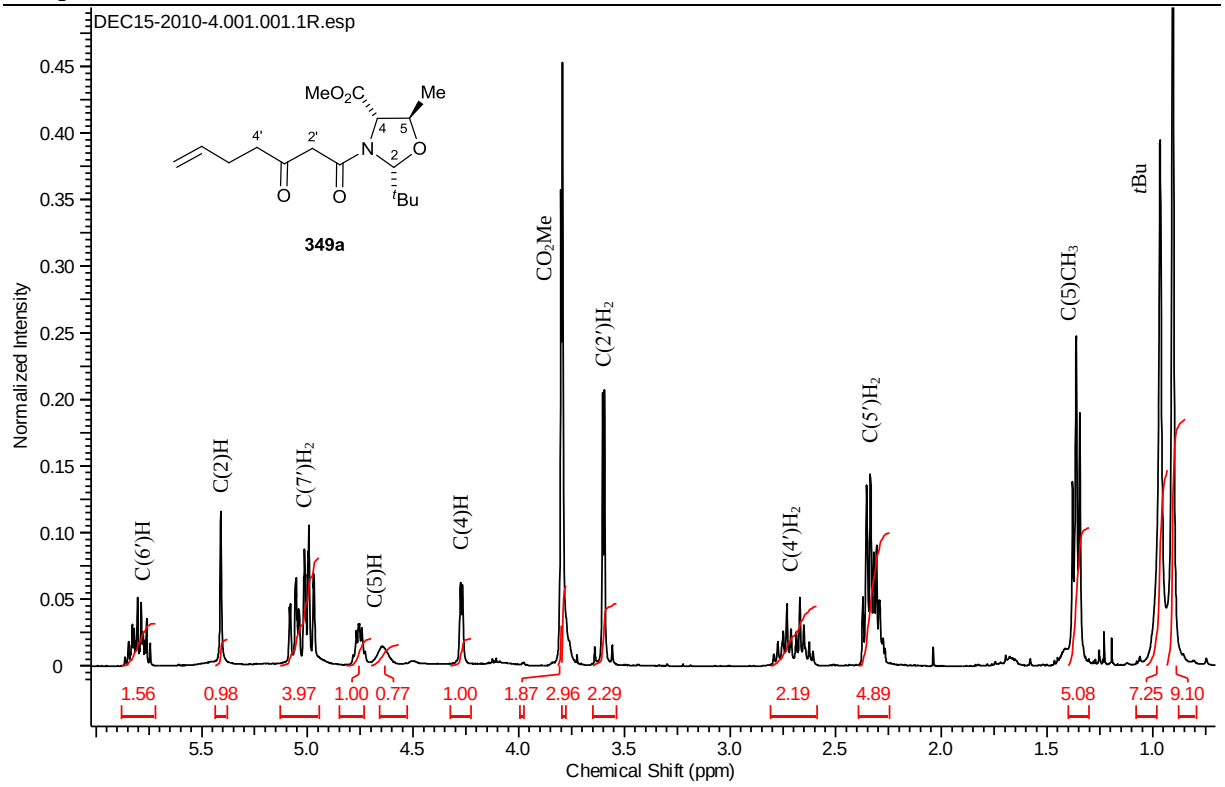


Figure 3.15 ^{1}H NMR for **349a** in $CDCl_3$.

For the bromoaryl *N*-acyloxazolidine **349b**, the $CDCl_3$ ^{1}H NMR spectrum (Figure 3.16, a) also appears to comprise of two species in roughly equal quantity, with the same peaks showing duplication and not as for **349a**. The two species in this mixture could reasonably be assumed to be the keto and enol tautomers, were it not for the lack of a vinylic CH for the enol form. In the hope of shifting the position of the keto-enol equilibrium, the ^{1}H NMR was repeated in MeOD (Figure 3.16, b). This spectrum appeared to be much simplified compared to the $CDCl_3$ spectrum. There is a second (lower integration) peak near to the *tBu* singlet at 0.90 ppm, and the aromatic C*H* at 7.20 and 7.50 ppm do appear to have smaller peaks next to them, but all other signals appear to be singular. There is an AB quartet that could be C(4′)H_2 at 3.85 ppm, but no signal that could be C(2′)H_2 for the keto-form, or a vinylic CH for the enol-form. One explanation for this could be that in MeOD, it is possible that the acidic protons on C(2′) could be exchanged for deuterium atoms, and therefore become invisible to NMR, however no ^{13}C signal for C(2′) could be found either.

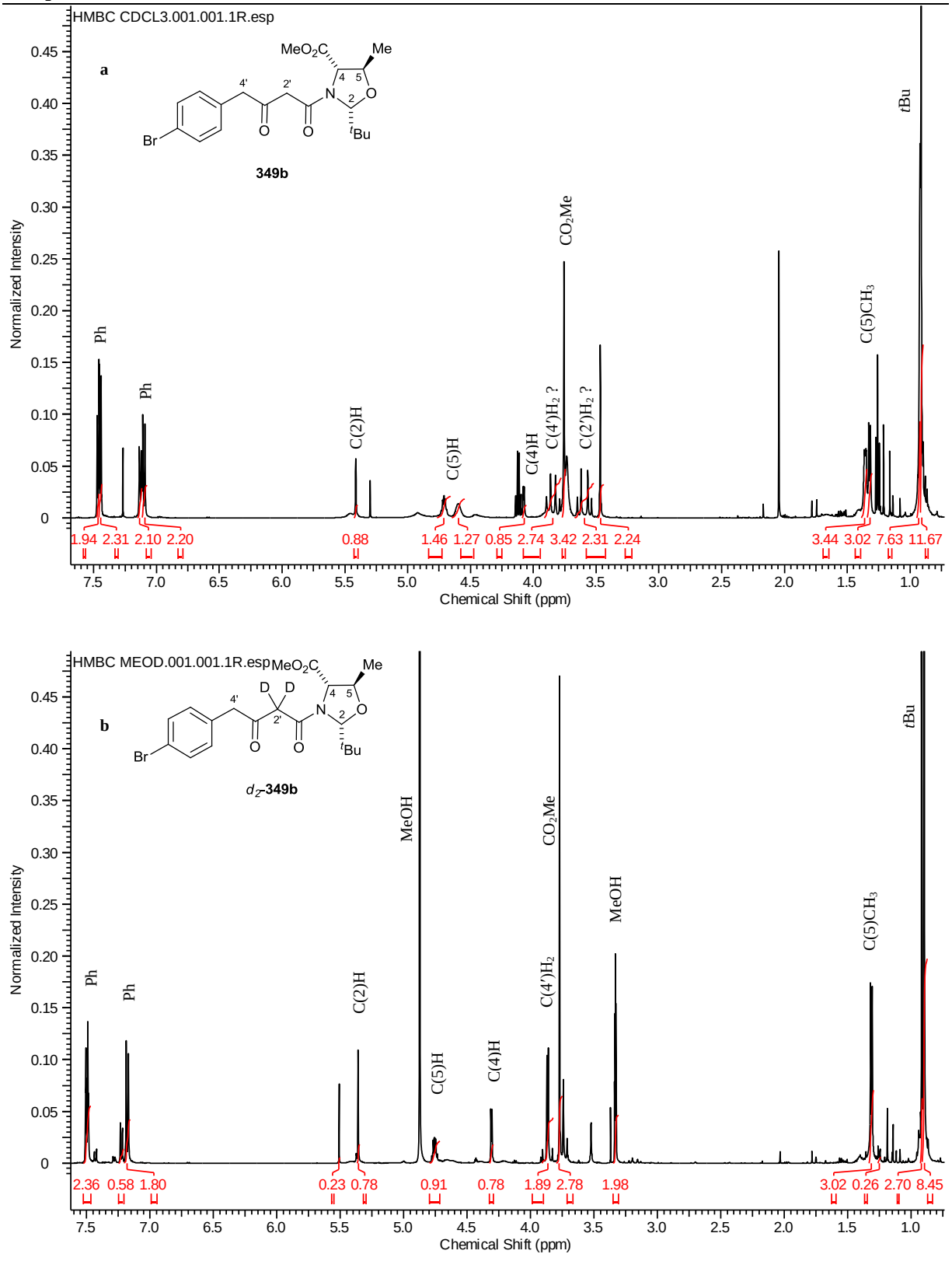


Figure 3.16 ^{1}H NMR for **349b** in (a) $CDCl_3$ and in (b) MeOD.

The same solvents were also used for **349c**. In $CDCl_3$ (Figure 3.17, a), the ^{1}H NMR spectrum shows 2 doublets for the C(5)*Me* around 1.25 ppm and 2 signals for the C(5)*H* around 4.6 ppm, and a number of overlapping signals around the 3.75 ppm CO_2*Me* peak that are in the

expected region for the C(2′)H_2 and C(4′)H_2. When repeated in MeOD (Figure 3.17, b), the spectrum appeared simplified and contained an AB quartet corresponding to a CH_2, assigned as C(4′)H_2, and no other CH_2 signals. This again could be due to deuterium exchange making the C(2′)H_2 invisible.

One explanation for the complexity of the spectra may be that the keto-enol equilibrium is in dynamic exchange at a speed that interferes with the acquisition of the NMR spectra. The relative speed at which the exchange occurs compared to the NMR timescale with have an effect on the spectra seen. If the exchange is very fast compared to the NMR time scale then the spectra will appear to be an "average" of the 2 structures. This can result in relaxation which can broaden peaks, so that they either appear as broad singlets rather than as multiplets, and sometimes so broadened that they are not distinguishable from the baseline and have effectively disappeared. These effects can be studied using variable temperature NMR (VT NMR). At low temperature, any exchange will slow down relative to the NMR time scale, and this should prevent any relaxation and allow the mixture to appear as 2 distinct components. Attempts to use this technique did not change the resulting ^{1}H NMR spectrum of **349c** in CD_2Cl_2, which appeared the same at 298 and 203 K.

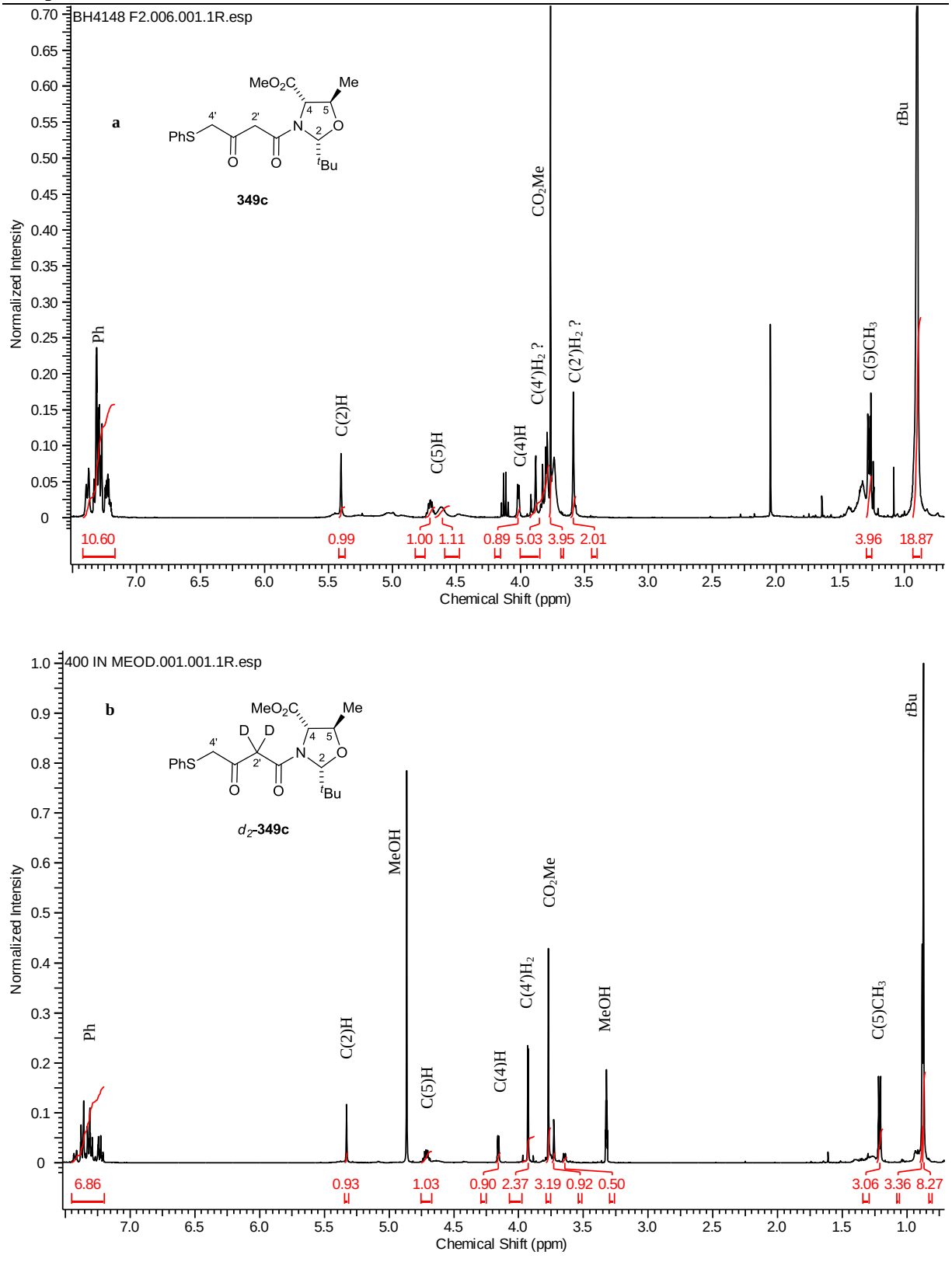


Figure 3.17 ^{1}H NMR for **349c** in (a) $CDCl_3$ and in (b) MeOD.

For each of the methyl-substituted analogues, 2 isomers were isolated and nOe experiments confirmed that the isomerism was a result of differing configurations at C(2′), rather than a different arrangement of the substituents on the oxazolidine ring (Figure 3.18). The ^{1}H NMR

spectra of these methyl substituted species were all unambiguously assigned and did not demonstrate any of the problems that were seen in the unsubstituted compounds described above. This difference may suggest that the inconsistencies observed stem from the dynamic exchange between keto and enol tautomers of the acyl side chain possessing acidic protons between the two carbonyl groups. The acidity of the protons at this position would be decreased with the addition of a methyl group, and so the enol tautomer will be comparatively disfavoured.

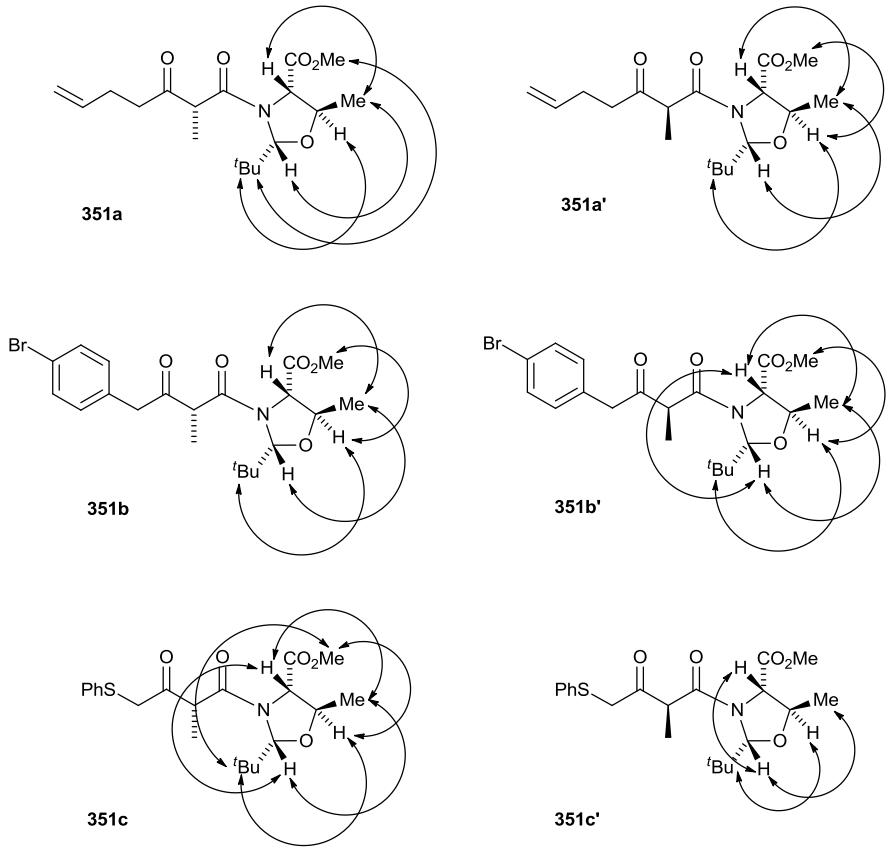
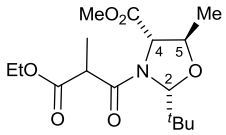


Figure 3.18 nOe enhancements for *N*-acyl-oxazolidines **351**.

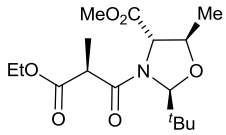
Because they appear to be mixtures, the unsubstituted *N*-acyloxazolidines **349** were not analysed by nOe. In order to rule out C(2) isomerism, the δ (ppm) of key ^{1}H and ^{13}C signals on the oxazolidine ring were compared to data obtained from known *cis* and *trans*-oxazolidines,[14] to see if there are any variations that could be attributed to a reversal in expected stereochemistry (Table 3.3). In the reference pair of oxazolidines *cis*-**352** and *trans*-**352**, there is very little difference between the ^{13}C chemical shifts. In the ^{1}H NMR spectra there is a significant difference between the shift of the C(2)*H* from 5.47 ppm in the *cis* to 5.10 ppm in the *trans*. In all of the compounds synthesised here the signal is much closer

(within 0.11 ppm) to 5.47, which is consistent with all the *N*-acyloxazolidines **349** and **351** having a *cis* relationship. The C(5)*H* shows a difference of 0.7 ppm between the *cis* and *trans* forms, and again, all the members of the current library show values in the region 0.15 ppm below the known *cis* isomer, consistent with a *cis* relationship. The C(4)*H* signal also shifts (from 4.52 to 3.85) between *cis* and *trans*, but the current compounds display a range of shifts, all between the two reference values, so this neither supports nor disagrees with the *cis* relationship. Though the structures had not been satisfactorily assigned, the subsequent aldol reactions gave 1-aza-3-oxabicyclo[3.3.0]octanes which have been fully characterised and assigned, and therefore confirm that the desired *N*-acyloxazolidine was present.

Compound	C(2) *H*	*C*(2) H	*t*Bu	*t*Bu	C(4) *H*	*C*(4) H	C(5)*H* (midpoint of m)	*C*(5) H	C(5) C*H*$_3$	C(5) *C*H$_3$
351a	5.42	96.2	0.93	25.9	4.11	65.8	4.78	75.9	1.35	20.3
351a	5.44	96.0	0.90	25.8	4.44	65.0	~4.78	76.0	1.32	20.2
351b'	5.42	96.1	0.90	25.9	3.92	65.0	4.65	76.2	1.19	20.0
351b	5.46	96.4	0.97	20.5	4.14	65.8	4.79	76.1	1.38	20.5
351c'	5.47	96.1	0.90	25.8	4.27	40.6	~4.69	76.4	1.27	20.1
351c	5.43	97.2	0.95	26.0	4.14	65.7	~4.81	76.0	1.37	20.4
349a	5.41	96.1	0.91	25.8	4.27	65.3	~4.75	76.1	1.35	20.1
349b	5.36	97.0	0.90	26.4	4.31	66.5	~4.76	77.5	1.32	20.4
349c	5.40	96.2	0.90	25.8	4.01	65.4	~4.70	76.1	~1.27	20.1
***cis*-352**	**5.47**	**95.7**	**0.88**	**25.7**	**4.52**	**65.2**	**~4.81**	**75.8**	**1.34**	**20.2**
***trans*-352**	**5.10**	**96.6**	**1.03**	**25.8**	**3.85**	**65.8**	**~4.14**	**75.8**	**1.34**	**20.2**



cis-**352**

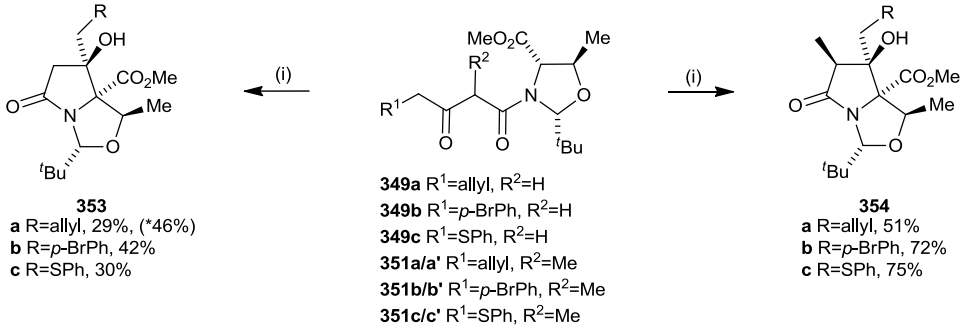


trans-**352**

Table 3.3 δ (ppm) for key signals in the ^{1}H and ^{13}C NMR spectra of selected *N*-acyloxazolidines.

3.3.6.4 Aldol cyclisations

The 6 *N*-acyloxazolidines were treated with NaOMe in MeOH for 24 h at rt and the pyroglutamates **353a-c** and **354a-c** were produced. The methyl substituted analogues **354a-c** were formed in significantly higher yields of 51-75% than the unsubstituted examples **353a-c** with yields of 30-46% (Scheme 3.26). This could be due to the Thorpe-Ingold[34] effect resulting from the extra substituent on the newly forming ring.



353
a R=allyl, 29%, (*46%)
b R=*p*-BrPh, 42%
c R=SPh, 30%

349a R^1=allyl, R^2=H
349b R^1=*p*-BrPh, R^2=H
349c R^1=SPh, R^2=H
351a/a' R^1=allyl, R^2=Me
351b/b' R^1=*p*-BrPh, R^2=Me
351c/c' R^1=SPh, R^2=Me

354
a R=allyl, 51%
b R=*p*-BrPh, 72%
c R=SPh, 75%

Scheme 3.26 *Reagents and conditions:* (i) NaOMe, MeOH, rt (*30 °C), 24 h.

Though the yields for the unsubstituted series are moderate, this represents the % conversion to the pyroglutamate, and most of the remaining *N*-acyloxazolidine is also recovered from the column and can be reused. It was hoped that a variation in the conditions might improve the conversion of the aldol cyclisation in these compounds. The reaction of **349a** was repeated with the addition of 5 h stirring at 30 °C (Table 3.4, entry 2), and also at 30 °C for 24 h (Table 3.4, entry 3), neither of which substantially changed the % conversion of the *N*-acyloxazolidine to pyroglutamate **353a** but a small increase in yield was achieved. Heating the reaction mixture to 60 °C overnight (Table 3.4, entry 4) resulted in a crude ^{1}H NMR spectrum which was complex and missing the C(4)Me peak associated with **353a**, the C(2)H peak associated with **349a** and any peak corresponding to a CO_2Me group. It was concluded that the elevated temperature had caused the decomposition of the materials. It was also noted, after repeat experiments, that the yields of pyroglutamates were sometimes much lower than expected from the % conversion. The % conversion remains relatively constant in each experiment, so variations in yield could be due to interactions between the components of the crude mixture and the acidic silica gel during column chromatography. Low isolated yields could be a result of retro-aldol processes occurring upon contact with silica, indicated by reactions in entries 5 and 9 where the isolated yield of the returned *N*-acyloxazolidine is higher than that predicted by the % conversion measured in the crude material (Table 3.4). The good return of starting material from the purification meant that material could be recycled in the next reaction and, although yields were not high, material was at least not being lost.

Entry	Reactant	R^1	R^2	Conditions	% conversion to 353/354	% mass return (max yield)	% mass return 349/351	% yield 353/354
1	**349a**	H	allyl	rt, 24 h	61	78 (47)	29	29
2	**349a**	H	allyl	rt 24 h, 30 °C 5 h	55	-	60	33
3	**349a**	H	allyl	30 °C, 24 h	67	92 (62)	51	46
4	**349a**	H	allyl	60 °C, 24 h	Decomposed	-	-	-
5	**349a**	H	allyl	30 °C, 24 h	67	83 (56)	41	35
6	**351a/a'**	Me	allyl	rt, 24 h	76	81 (62)	8	51
7	**349b**	H	CH_2-*p*-BrPh	rt, 24 h	~50	89 (45)	19	42
8	**351b/b'**	Me	CH_2-*p*-BrPh	rt, 24 h	80	92 (73)	12	72
9	**349c**	H	CH_2SPh	rt, 24 h	68	87 (59)	38	30
10	**351c/c'**	Me	CH_2SPh	rt, 24 h	>98	75 (73)	-	73*

*column chromatography was not carried out as impurities very minor.

Ratios calculated as average integration of product C(4)Me, C(7)H_2, C(2)H and C(4)H if non-overlapping, and starting material C(2)H and C(4)H protons.

Table 3.4 Conversion and yield values for aldol reactions.

3.3.6.5 Assigning the stereochemistry of the pyroglutamates

These reactions furnish bicyclic products containing up to 4 contiguous stereocentres and in each case the pyroglutamate was produced as a single diastereoisomer. In none of the examples were any other minor isomers observed, even in the crude spectra. The stereochemistry of the pyroglutamates **353-354** was analysed by nOe experiments (Figure 3.19). The enhancement patterns observed were similar to those seen in the methoxymethyl substituted series. Where overlapping of peaks did not prohibit observation, an enhancement between C(4)*Me* and C(1′)H_2 was seen. Enhancements between the C(1′)H_2 and the endo C(7)*H* or C(7)*Me* were also commonly observed. Both of these signals are indicated in red arrows on the diagrams below. As a result of the similarities to the previous library the stereochemistry was predicted to be analogous, with the CO_2Me and C(6)R being in the *exo* position and the C(6)OH and C(7)Me where present being in the *endo* position.

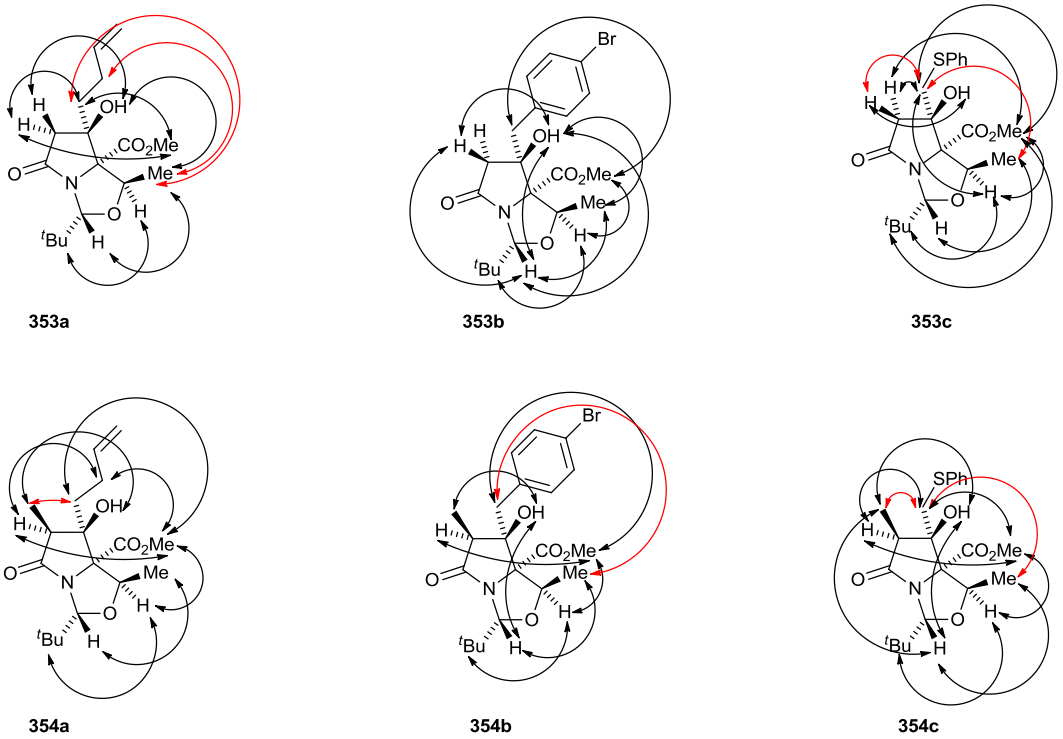


Figure 3.19 nOe enhancements of pyroglutamates **353-354**.

The compounds **353b**, **354b** and **354c** were crystalline and single crystal x-ray structures were obtained to unambiguously assign the stereochemistry (Figure 3.20). The C(4)Me-C(1′)H_2 internuclear distances were between 2.35 and 2.54 Å. The dihedral angles between C(6)CH_2 and C(7)H and C(7)Me in **354c** were 55° and 76° respectively in the solid state, meaning that the C(6)R groups effectively bisect the C(7) substituents, and explains why all the substituents are in close enough proximity to produce nOe enhancements.

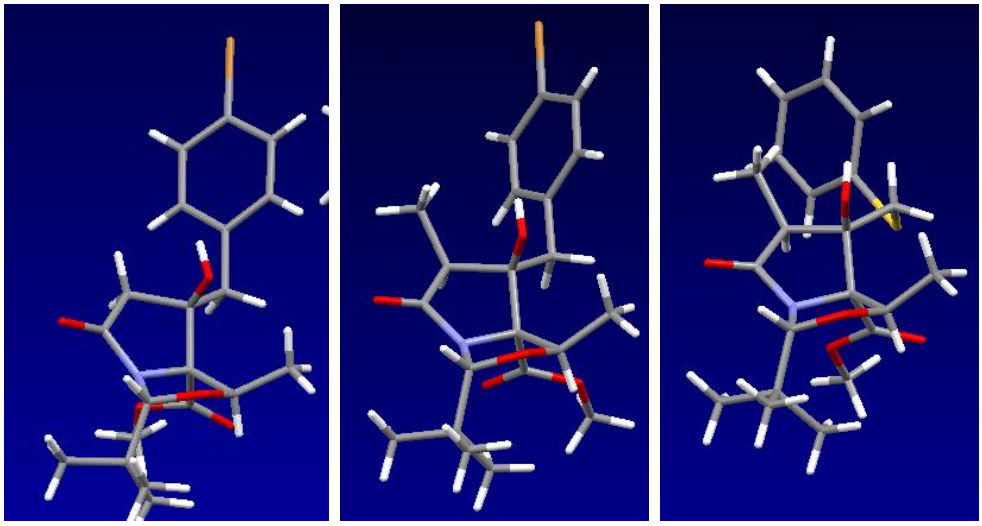
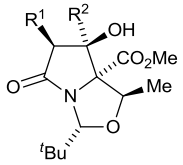


Figure 3.20 X-ray crystal structures for **353b**, **354b** and **354c**.

3.3.7 NMR analysis of the pyroglutamates

In order to compare the spectral data for the pyroglutamate library that had been generated, the key ^{1}H NMR chemical shifts have been tabulated below (Table 3.5). The chemical shifts of the protons bonded directly to the pyroglutamate backbone occupy a small range of chemical shifts. This suggests that the structures are rigid and that each proton is held in a similar environment in each example. The shift of C(2)*H* covers a range of only 0.07 ppm (between 4.99 and 5.06). The C(4)*H* appears with a range of 0.11 ppm between 4.72 and 4.83. The *endo*-C(7)*H*, whether on a methyl substituted analogue or not, generally appeared between 3.0 and 3.3 ppm. The C(4)*Me* group spans a range of 0.14 between 1.58 and 1.72 ppm, and the C(7)*Me* appeared between 1.02 and 1.21 except in the bromoaryl pyroglutamate **354b**. In this molecule, the C(7)*Me* showed a chemical shift of 0.65 ppm, 0.45 lower than the average of the other five. This is likely to be caused by the close proximity of the methyl group to one face of the aromatic ring, where it can be affected by ring currents.



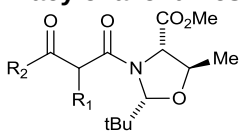
Compound	R^1	R^2	C(2) *H*	C(4) *H*	C(6) CH_2	C(7) *Me*	C(7) *H*	*tBu*	C(4) *Me*	C(6)R_2 stereo	C(7)R_1 Stereo
341	H	CH_2OMe	5.04	4.76	3.35 3.50	-	2.34 3.06	0.91	1.63	*exo**	-
342a	Me	CH_2OMe	5.03	4.72	3.42	1.05	3.19 -	0.92	1.68	*exo**	*endo**
343	H	CHBnOMe	5.00	4.75	3.71	-	1.78 3.29	0.92	1.68	*exo*	-
344a	Me	CHBnOMe	5.06	4.80	3.66 (CH)	1.15	3.20	0.94	1.72	*exo**	*endo**
344b	Me	CHBnOMe	4.99	4.73	3.79 (CH)	1.21	3.57	0.92	1.58	*exo**	*endo**
353a	H	CH_2allyl	5.06	4.79	1.38 1.94	-	2.37 3.04	0.91	1.70	*exo*	-
354a	Me	CH_2allyl	5.01	4.81	1.75	1.11	3.11	0.91	1.68	*exo*	*endo*
353b	H	CH_2-*p*-BrPh	4.99	4.79	1.85 3.06	-	2.45 3.06	0.89	1.76	*exo**	-
354b	Me	CH_2-*p*-BrPh	5.00	4.83	2.63 3.17	0.65	3.21	0.92	1.66	*exo**	*endo**
353c	H	CH_2SPh	5.05	4.75	3.03 3.37	-	2.33 2.89	0.90	1.69	*exo*	-
354c	Me	CH_2SPh	5.02	4.77	3.31	1.02	3.17	0.93	1.68	*Exo**	*endo**

*Confirmed through x-ray crystal structure

Table 3.5 Chemical shift (ppm) of key signals of the pyroglutamate library.

3.3.8 Biological evaluation

The *N*-acyloxazolidines and pyroglutamates synthesised were assayed against *S. aureus* D267 and *E. coli* X580 using the hole-plate method (see Appendix A). This phenotypic assay is not able to accurately measure MIC values for active compounds, but does allow a simple active/inactive result on antibacterial activity to be obtained quickly and easily. *N*-Acyloxazolidines **340c** and **349b** were the only compounds displaying activity against Gram-positive *S. aureus* with relative potencies of almost 10% relative to the cephalosporin C control. There were a number of active hits against Gram-negative *E. coli*. Four *N*-acyloxazolidines (**351a**, **351a′**, **349b**, **349c**) and four pyroglutamates (**353a**, **354a**, **353b**, **353c**) showed inhibition zones against *E. coli*, though it should be noted that for compounds **353a** and **353b** the zones were hazy, indicating only partial inhibition of bacterial growth. The relative potencies here are much smaller (0.03-0.04%) due to the higher sensitivity of *E. coli* to the reference compound cephalosporin C. Broth bioassays also identified three compounds with activity against *H. influenza* Hi4, **340c**, **353b**, and **354b** with MICs of 32, 64, and 16 μg/mL (see Appendix B). Though these active compounds have been identified, most of those tested exhibited no antibacterial activity. This was not unexpected, as previous work within the group has shown that simple tetramate systems rarely exhibit antibacterial activity.[35]

Compound number	R_1	R_2	*S. aureus* D267		*E. coli* X580	
			Zone size (mm)	Rel. potency (Ceph C, %)	Zone size (mm)	Rel. potency (Ceph C, %)
N-acyloxazolidines 						
340a	H	CH_2OMe	0	-	0	-
340b	Me	CH_2OMe	0	-	0	-
340c	H	CHBnOMe	13	9.73%	0	-
340d	Me	CHBnOMe	0	-	0	-
349a	H	Butenyl	0	-	0	-
351a'	Me	Butenyl	0	-	12.5	0.03%
351a	Me*	Butenyl	0	-	12	0.03%
349b	H	CH_2ArBr	12.5	9.99%	12	0.04%
351b'	Me	CH_2ArBr	0	-	0	-
351b	Me*	CH_2ArBr	0	-	0	-
349c	H	CH_2SPh	0	-	13	0.04%
351c'	Me	CH_2SPh	0	-	0	-
351c	Me*	CH_2SPh	0	-	0	-

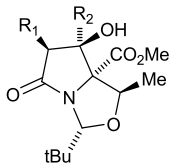
Compound number	R_1	R_2	*S. aureus* D267		*E. coli* X580	
			Zone size (mm)	Rel. potency (Ceph C, %)	Zone size (mm)	Rel. potency (Ceph C, %)
Pyroglutamates 						
341	H	CH_2OMe	0	-	0	-
342a	Me	CH_2OMe	0	-	0	-
343	H	CHBnOMe	0	-	0	-
344a	Me	CHBnOMe	0	-	0	-
344b	Me	CHBn*OMe	0	-	0	-
353a	H	Butenyl	0	-	12 (halo)	0.03%
354a	Me	Butenyl	0	-	14	0.04%
353b	H	CH_2ArBr	0	-	12 (halo)	0.04%
354b	Me	CH_2ArBr	0	-	0	-
353c	H	CH_2SPh	0	-	15	0.05%
354c	Me	CH_2SPh	0	-	0	-

Table 3.6 Bioassay results for *N*-acyloxazolidines and pyroglutamates.

3.4 Right-hand/middle fragment adducts

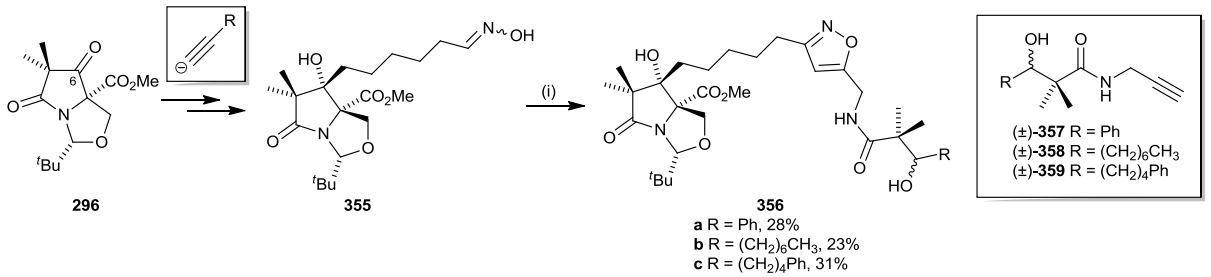
It has been shown that by tethering tetramic acid derived analogues for the right-hand fragment of oxazolomycin and a *gem*-dimethyl amide section mimicking the middle fragment, that the biological activity may be greater than that of the right-hand fragment alone.[23] It was envisaged that by addition of an amide side chain to these pyroglutamates the level of antibacterial activity observed may increase. Previously, when selecting acid chlorides for the pyroglutamate synthesis, the modification potential at this stage had been considered and as such a number of techniques were investigated.

3.4.1 Click chemistry

'Click'[36] chemistry has been used extensively in bioconjugation[37] to form heterocycle linkages between a variety of molecular entities and in the preparation of glycoconjugates.[38] Bagwell and Moloney used this 'click' chemistry strategy to form both triazole and isoxazole linked conjugates as mimics for oxazolomycin.[23] The linking groups were formed from the 1,3-dipolar cycloaddition of a terminal alkyne with either azide or nitrile oxide functionalities. Of these, the isoxazole-linked examples demonstrated more promising biological results, and were more active than the triazoles. This was postulated to be due to the highly polar triazole

linkage being a poor mimic for the middle fragment of oxazolomycin, which does not contain such polar subunits.

The isoxazole-linked oxazolomycin mimics were synthesised from the tetramate **296**; following addition of an alkyne to the carbonyl group at C(6), the oxime **355** was obtained in several steps. The oxime was treated with the propargylic amides (±)-**357-359**, NBS and TEA in DMF. The oxime is first converted to the bromo-oxime, which undergoes elimination to produce the nitrile oxide species *in situ*, followed by 1,3-dipolar cycloaddition with the triple bond of the amides (±)-**357-359** to give the isoxazole linked products **356** (Scheme 3.27).



Scheme 3.27 *Reagents and conditions:* (i) (±)-**357-359** NBS, TEA, DMF, rt, 18 h.

It was envisaged that a similar strategy could be employed to create similar adducts from the pyroglutamates synthesised in Section 3.3.6. The but-3-enyl substituted pyroglutamates **353a** and **354a** could be converted to the aldehyde by oxidative cleavage of the double bond, and then to the corresponding oxime by treatment with hydroxylamine to give a suitable substrate for the 'click' 1,3-dipolar cycloaddition reaction (Figure 3.21).

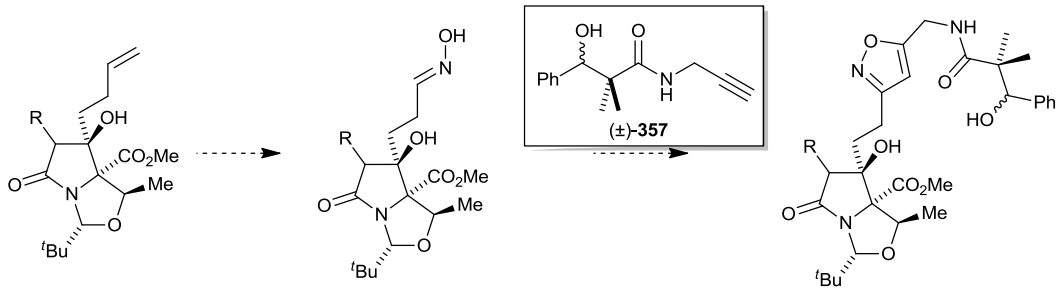
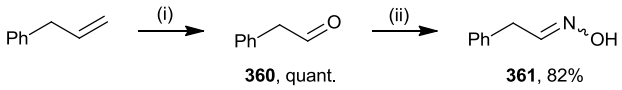


Figure 3.21 Envisaged synthesis of isoxazole linked conjugates.

Before committing material to the sequence, the steps were tested with allylbenzene. The oxidative cleavage of the double bond was carried out following a literature procedure using $RuCl_3$ and $NaIO_4$ in a 6:1 mixture of MeCN and H_2O.[39] The reaction proceeded in around 15

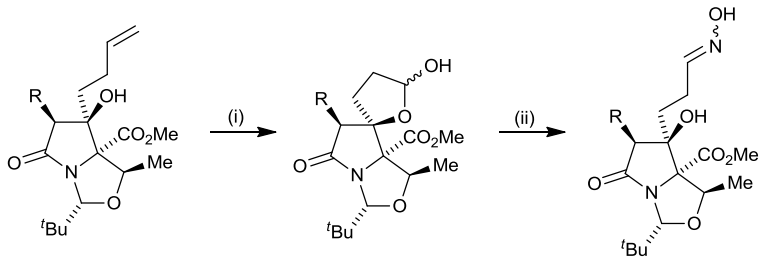
minutes to give the aldehyde **360** as the exclusive product. The aldehyde **360** was converted to the oxime **361** using hydroxylamine hydrochloride and Na_2CO_3 in THF and H_2O in 82% yield using a literature procedure (Scheme 3.28).[40]



360, quant. **361**, 82%

Scheme 3.28 *Reagents and conditions:* (i) $RuCl_3$, $NaIO_4$, 6:1 MeCN:H_2O, (ii) $NH_2OH.HCl$, H_2O, Na_2CO_3, THF, 0 °C to rt, 1 h.

The but-3-enyl-pyroglutamate **353a** was treated with $RuCl_3$ and $NaIO_4$ and the oxidative cleavage gave the species **362** in 74% yield. The structure of **362** results from the intramolecular cyclisation between the C(6) hydroxyl group and the aldehyde, forming a 5-membered lactol, and can be identified by the absence of an aldehyde proton signal at 9-11 ppm in the ^{1}H NMR spectrum and the presence of the two epimeric C(3′)*H* signals between 5.5 and 5.6 ppm and has the same *m/z* as the aldehyde. The lactol **362** was condensed with hydroxylamine to furnish the oxime **362** in 83% yield as a roughly 1:1 mixture of *E* and *Z* isomers. The methyl substituted pyroglutamate was also treated with the same reagents and gave the lactol **363** in 75% yield and the oxime **365** in 92% yield also with a 1:1 ratio of *E*:*Z* isomers (Scheme 3.29).



353a R=H
354a R=Me

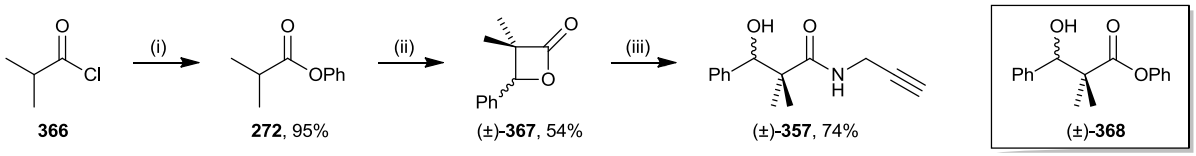
362 R=H, 74%
363 R=Me, 75%

364 R=H, 83%
365 R=Me, 92%

Scheme 3.29 *Reagents and conditions:* (i) $RuCl_3$, $NaIO_4$, 6:1 MeCN:H_2O, (ii) $NH_2OH.HCl$, H_2O, Na_2CO_3, THF, 0 °C to rt, 1 h.

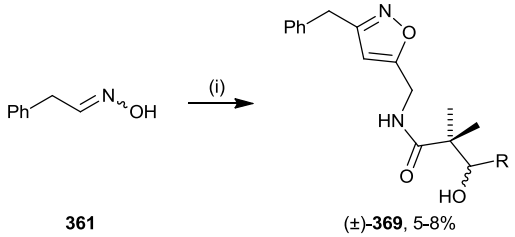
The phenyl substituted *gem*-dimethyl amide (±)-**357** was synthesised according to the protocol described by Moloney and Bagwell.[23] *iso*-Butyryl chloride **366** was reacted with phenol to give the phenyl ester **272** in 95% yield. This was then treated with LDA and benzaldehyde to give the β-lactone (±)-**367** in 54% yield, formed by intramolecular transesterification between the phenyl ester and the internal hydroxyl group. It is noteworthy that when performed on a large scale (>5 g) the ester (±)-**368** was the major product, isolated

in around 63%, with the β-lactone being isolated in around 13% yield. Finally, the amide (±)-**357** was furnished in 74% yield by opening the β-lactone ring with TEA and propargyl amine in DCM heated to reflux for 4 d (Scheme 3.30).



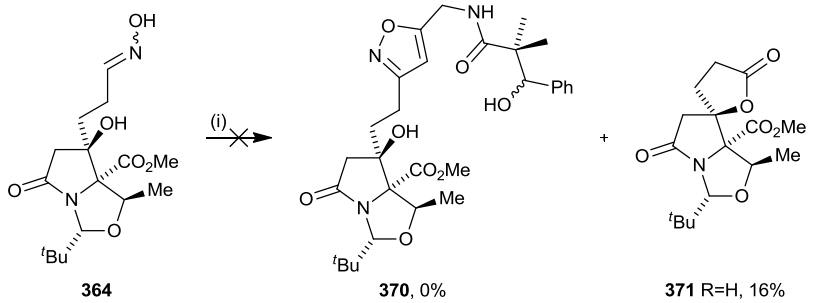
Scheme 3.30 *Reagents and conditions:* (i) py, phenol, DCM, 0 °C to rt, 30 min, (ii) LDA, PhCHO, THF, −78 to 0 °C, 2 h, (iii) TEA, propargyl amine, DCM, 40 °C, 4 d.

The cycloaddition reaction was then tested with the oxime **361** which was treated with NBS and TEA in the presence of the alkyne (±)-**357** using the procedure described by Dondoni *et al.*[38] Upon purification of the crude reaction mixture, the isoxazole (±)-**369** was isolated in 8% yield (Scheme 3.31). The unreacted oxime **361** and alkyne (±)-**357** were recovered in 40% and 60% yield respectively. A number of modifications to this procedure were tried to improve the yield of isoxazole. For example, the number of equivalents of NBS was increased from 1.1 to 2.0, and no production of (±)-**369** was seen. Increasing the number of equivalents of NBS to 5.0 resulted in decomposition and gave a complex mixture. Heating the reaction to 50 °C for 18 h did effect a reaction and an isoxazole CH was observed in the spectrum of the crude material, but the isolated yield was 5%, lower than the original 8%.



Scheme 3.31 *Reagents and conditions:* (i) NBS, TEA, alkyne (±)-**357**, DMF, rt, 18 h.

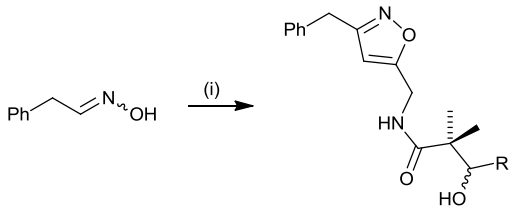
Nevertheless, an attempt was made at using this procedure on the pyroglutamate substrate, in the hope that enough material to perform a bioassay would be obtained. When the oxime **364** was subjected to the reaction conditions, none of the isoxazole was produced and no characteristic CH signal at 5.9 ppm in the ^{1}H NMR spectrum could be seen. Purification recovered 91% of the alkyne starting material (±)-**357** and 40% of the oxime **364**. A small amount (16%) of the lactone **371** was also identified (Scheme 3.32).



364 **370**, 0% **371** R=H, 16%

Scheme 3.32 *Reagents and conditions:* (i) NBS, TEA, alkyne (±)-**357**, DMF, rt, 18 h.

In light of these results, an alternative procedure for the cycloaddition was also investigated. Vaidya *et al.* used isoxazole forming 1,3-dipolar cycloadditions to conjugate a 1,4-benzodioxane unit, which is present in several bioactive molecules, with other biologically active fragments.[41] They utilise the aqueous bleach method described by Lee[42] to generate the nitrile oxide dipoles. Lee describes the use of NaOCl to form a variety of nitrile betaines from the oximes and hydrazones of aldehydes and their *in situ* trapping with a variety of dipolarophiles to form different heterocycles. This method uses the sodium hypochlorite to oxidise the oxime, *via* the chloro-oxime to the nitrile oxide. Benzyloxime **361** was stirred at rt with NaOCl, TEA and the alkyne (±)-**357** in DCM, and after an extended reaction time of 18 h from 5 h, gave the isoxazole (±)-**369** in 32% yield (Scheme 3.33). Whilst still modest, this yield is an improvement on that achieved using the NBS method.

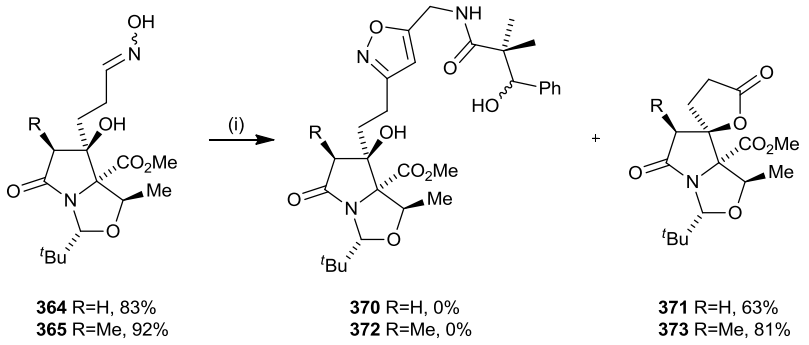


361 (±)-**369**, 32%

Scheme 3.33 *Reagents and conditions:* (i) 12% aq. NaOCl, TEA, alkyne (±)-**357**, DCM, rt 18 h.

This method was used on the pyroglutamate **364** and again, none of the isoxazole product **370** was observed. The lactone by-product **371** was isolated in 63% yield following chromatography, along with 8% of the alkyne (±)-**357**. This would indicate that following oxidation, the nitrile oxide species may cyclise with the intramolecular alcohol and then hydrolyse either in the aqueous conditions of the reaction or upon work-up. Previous reports have shown that the active nitrile oxide species will readily undergo reaction with an intramolecular group.[4] When the reaction was repeated with the methyl substituted

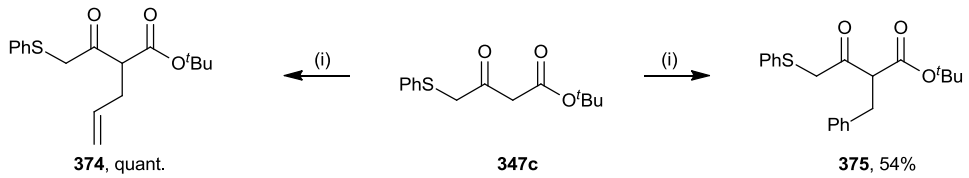
pyroglutamate **365** the by-product **373** was isolated in 81% yield along with 95% of the alkyne starting material (±)-**357** (Scheme 3.34). As there was a clear preference for this side reaction the 'click' chemistry approach was abandoned due to the unsuitability of this substrate. It is possible that this problem could be overcome by lengthening the carbon chain linking the pyroglutamate and alkene, so that the cyclisations of this type would be less favourable.



Scheme 3.34 *Reagents and conditions:* (i) 12% aq. NaOCl, TEA, alkyne (±)-**357**, DCM, rt 18 h.

3.4.2 Alkylations of PhS- substituted β-keto-esters

An investigation into the γ-alkylations of the γ-methoxy-β-keto-esters by reaction of their dianion with various alkylating agents has been described previously (Section 3.3.3). It was hoped that replacement of the methoxy group with a thiophenyl group may increase the success of this alkylation chemistry by stabilising the anion in the γ-position. The β-keto ester **347c** was treated NaH and BuLi, followed by various alkylating agents. With the alkylating agents allyl bromide and benzyl bromide, the sole products were in fact the α-alkylated esters **374** and **375** in quantitative and 54% yields respectively (Scheme 3.35).



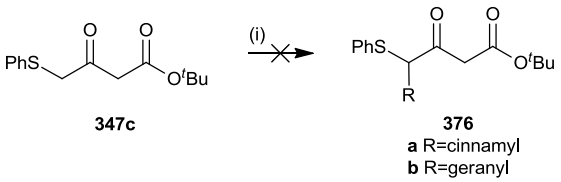
Scheme 3.35 *Reagents and conditions:* (i) NaH, BuLi, allyl bromide or benzyl bromide, THF, 0 °C to rt, 5 h.

The pK_a in DMSO of a proton between a carbonyl and a thiophenyl group is around 18.7, and the pK_a of a proton between a ketone and an ester is around 14.2 (Figure 3.22).[43] This means that the order of acidity in compound **347c** should be correct for the anion in the γ-position being the most reactive. This suggests that the dianion was not formed before addition of the

alkylating agent, although an obvious colour change was observed on addition of BuLi, indicating that the deprotonation had indeed occurred.

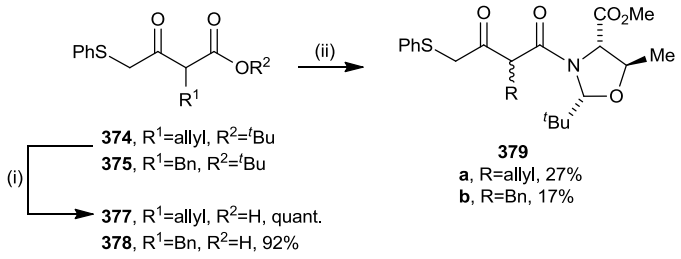
Figure 3.22 pK_a values for related compounds.

When the reaction was carried out with cinnamyl and geranyl bromides, no products were observed and the starting material **347c** was isolated in around 80% yield (Scheme 3.36). This may indicate that the nature of the alkylating agent has an important role in this reaction. Following this lack of success, it was decided to cease investigations into the γ-alkylation of β-keto-esters and look for an alternative approach to modifying the side chains of our pyroglutamates.



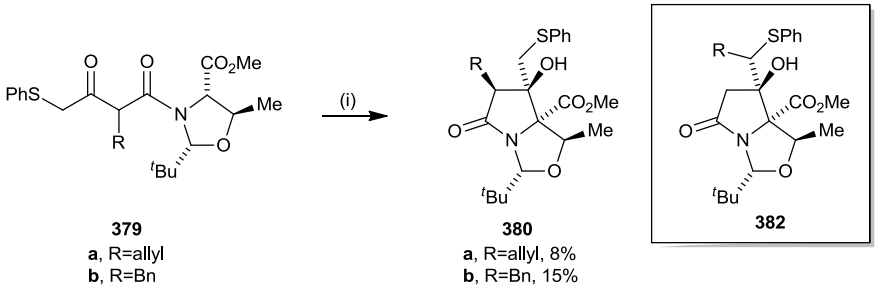
Scheme 3.36 *Reagents and conditions:* (i) NaH, BuLi, RBr, THF, 0 °C to rt, 5 h.

Having synthesised the substituted esters **374** and **375**, they were taken forward to see if the pyroglutamates could be synthesised in the presence of such bulkier groups at the α-position. The esters were hydrolysed with TFA in DCM to give the carboxylic acids **377** and **378** in high yield. The acids were then coupled to the oxazolidine **320** using DCC and DMAP to give the *N*-acyloxazolidines **379a** and **379b** in relatively low yields of 27 and 17%; as methyl substituted carboxylic acids gave lower yields for this reaction than the unsubstituted ones, it would be expected that higher steric bulk here would decrease the yield further (Scheme 3.37).



Scheme 3.37 *Reagents and conditions:* (i) 1:1 TFA:DCM, rt, 1.5 h, (ii) DCC, DMAP, **320**, DCM, rt, 5 h.

The aldol precursors **379a** and **b** were then cyclised with NaOMe and MeOH to give the pyroglutamates **380a** and **b** bearing substituents at C(6) (Scheme 3.38). These could be distinguished from the original target of **382** by the HMBC correlation between the benzyl/allyl CH_2 protons and *C*(6), and the absence of a correlation between with *C*SPh. The yields for these cyclisations were much lower than for previous examples, where a favourable Thorpe-Ingold effect resulted in increased yields for the cyclisation of the methyl substituted analogues. It may be that the increased bulk means the reaction is slower and had not been given sufficient time to reach completion.



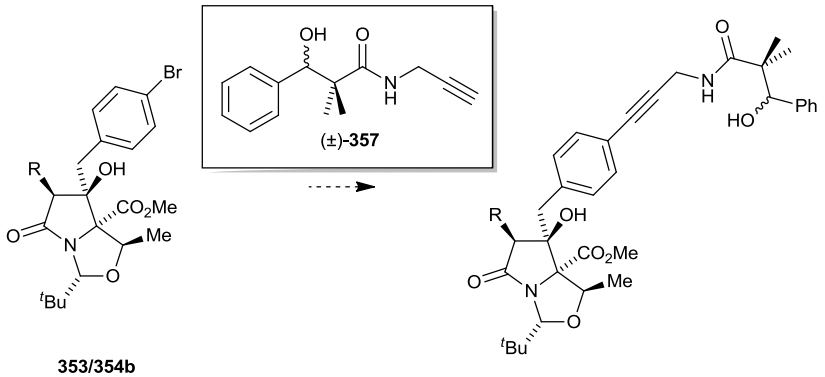
379
a, R=allyl
b, R=Bn

380
a, R=allyl, 8%
b, R=Bn, 15%

382

Scheme 3.38 *Reagents and conditions:* (i) NaOMe, MeOH, rt, 24 h.

3.4.3 Sonogashira strategy

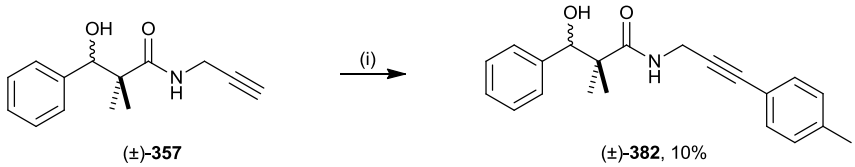


353/354b

Figure 3.23 Proposed strategy for synthesising phenyl-linked adducts.

Another strategy that was investigated is the Sonogashira coupling between aryl bromides and terminal alkynes. It was hoped that this would allow the coupling of the amide-alkyne fragment (±)-**357** directly with the pyroglutamates **343a** and **354a** bearing the *p*-bromobenzyl substituent (Figure 3.23). 4-Bromotoluene was used as a test reagent in place of the pyroglutamates to optimise the procedure. The first procedure used 5 mol% $Pd(PPh_3)_4$, 10 mol% CuI and TEA in THF, heated to 70 °C overnight. After purification, a trace amount of the product was isolated along with 20% of the 4-bromotoluene starting material. A similar

procedure using DMF, as well as the use of microwave rather than conventional heating, did not lead to an improvement in the extent of reaction. A procedure using $PdCl_2(PPh_3)_2$ under conventional heating resulted in little or no reaction occurring. However, in a slight modification of a literature method,[44] using 5 mol% $PdCl_2(PPh_3)_2$, 5 mol% CuI, PPh_3 and 13:1 *i*Pr_2NH:DMF as a solvent, heated in the microwave to 120 °C for 25 min, a 10% yield of the coupled product (±)-**382** was obtained (Scheme 3.39).



(±)-**357** (±)-**382**, 10%

Scheme 3.39 *Reagents and conditions:* (i) *p*-bromotoluene, $PdCl_2(PPh_3)_2$, CuI, PPh_3, 13:1 *i*Pr_2NH:DMF, microwave 120 °C, 25 min.

With the limitations recognised, this procedure was applied to the pyroglutamate system. The ^1H NMR spectrum of the crude material contained peaks that were consistent with the production of some of the desired product, but after chromatography, no identifiable fractions were isolated. Aryl bromides require higher temperatures to undergo Sonogashira reactions, and this means side reactions are more likely. Compounds possessing propargylic electron withdrawing groups (such as NH_2 or CO_2Me) are known to form allenes under elevated temperatures.[45,46] For most of the test reactions, column chromatography produced in excess of 5 fractions, each with very small mass, and often complex ^1H NMR spectra. It is also notable that although some fractions of the unreacted 4-bromotoluene were isolated for these reactions, the alkyne starting material (±)-**357** was never recovered. These observations are consistent with the occurrence of side reactions of a reactive intermediate such as an allene. The homodimer of the alkyne, a commonly observed by-product of Sonogashira reactions,[47,48] was also never observed.

3.4.4 Wittig chemistry

Following the unsuccessful investigation of many different strategies for the synthesis of right-hand/middle fragment adducts, a reassessment of potential synthetic routes was required. The most promising route investigated had been the 'click' chemistry route involving a 1,3-dipolar cycloaddition to form isoxazole linked adducts. Though successful in

moderate yield in the model system, competing intramolecular reactions of the oxidised intermediates had resulted in the production of a lactone by-product in high yield rather than the desired cycloaddition product. This sequence, however, began with the successful synthesis of the lactol protected aldehydes **362** and **363** in around 75% yield from the but-3-enyl pyroglutamates **353a** and **354b**, so with ready access to these substrates, it was decided that Wittig chemistry was an important route to investigate (Figure 3.24).

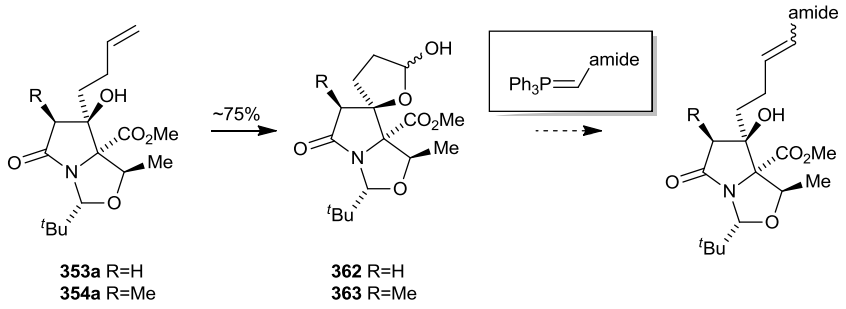
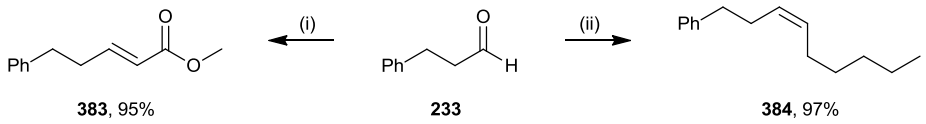


Figure 3.24 Proposed synthesis of Wittig coupled analogues.

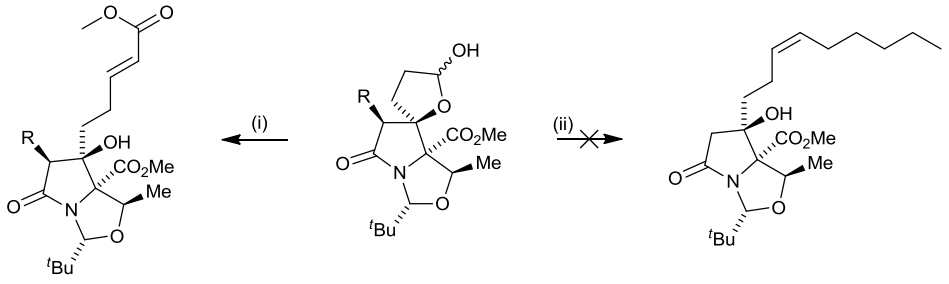
Successful protocols were established using a commercially available aldehyde and selection of ylids before attempts were made with the desired substrates. The reaction of 3-phenylpropionaldehyde **233** with the stabilised ylid methyl(triphenylphosphoranylidene)acetate gave the *E*-olefin **383** in 95% yield as a single isomer. The reaction of aldehyde **233** with an unstabilised ylid, formed *in situ* from the deprotonation of hexyltriphenylphosphonium bromide with BuLi, gave *Z*-olefin **384** in 97% yield (Scheme 3.40).



Scheme 3.40 *Reagents and conditions:* (i) Ph_3PCHCO_2Me, DCM, rt, 20 h, (ii) $H_{13}C_6PPh_3Br$, BuLi, −10 °C to rt, 5 h.

These protocols were then applied to the pyroglutamic lactols; thus, lactol **362** was reacted with the stabilised ylid and gave resulting *E*-alkene **385** in quantitative yield. The methyl analogue **363** gave the equivalent *E*-alkene **386** in 78% yield. Unfortunately, reaction of the lactol **362** with the unstabilised ylid resulted in the production of a complex mixture from which no identifiable products were isolated (Scheme 3.41). Nevertheless, the result of the

stabilised ylid Wittig reactions were promising and a synthesis for an ylid bearing the *gem*-dimethyl amide group was designed.



Scheme 3.41 *Reagents and conditions:* (i) Ph_3PCHCO_2Me, DCM, rt, 20 h, (ii) $H_{13}C_6PPh_3Br$, BuLi, −10 °C to rt, 5 h.

It was envisaged that an ylid containing the desired amide may be synthesised using aminoethanol as a linker between the phosphorylidene and amide groups. Aminoethanol could be used to open the β-lactone (±)-**367** to give the diol species (±)-**391**. This could be selectively acylated on the 1° alcohol to give the α-bromo-ester (±)-**390**, where the bromine could be displaced with PPh_3 to give the salt (±)-**389** with a final deprotonation furnishing the ylid (±)-**388** (Figure 3.25).

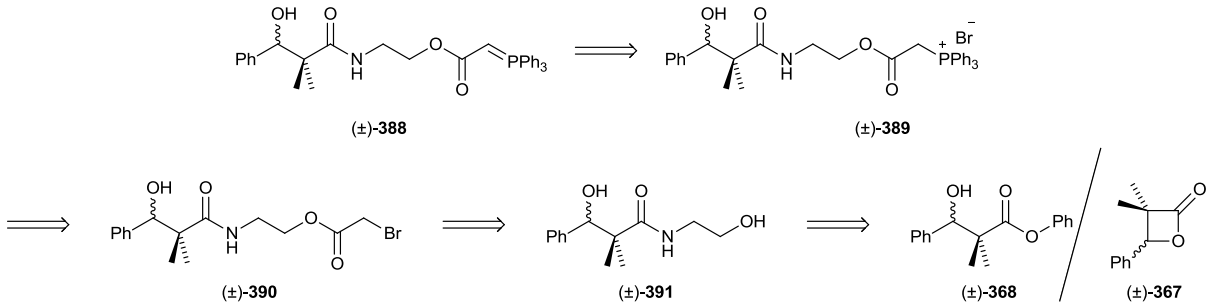
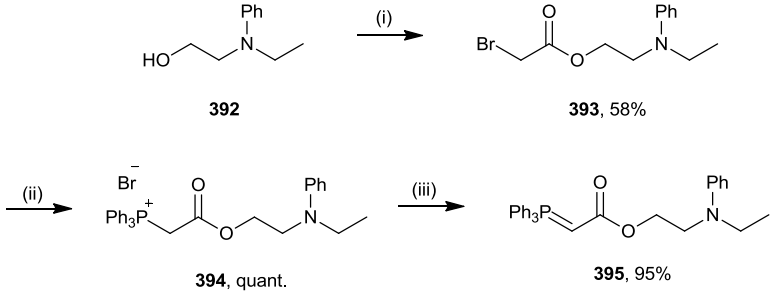


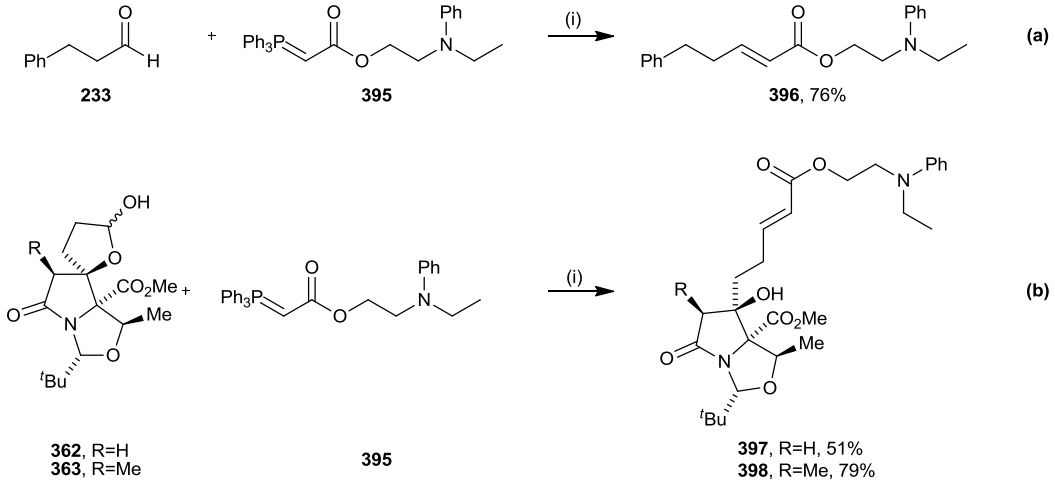
Figure 3.25 Retrosynthesis of ylid containing middle amide fragment mimic.

The final stages of this synthetic sequence were trialled using *N*-phenyl-*N*-ethylaminoethanol **392**, and if successful, the 3° amino group would provide an interesting comparison in bioactivity to the amide group present in the natural product. The alcohol **392** was reacted with bromoacetyl bromide giving α-bromo-ester **393** in 58% yield after purification. The bromide was quantitatively displaced with PPh_3 to give the salt **394**. The salt was converted to the ylid by simply shaking a DCM solution with 2 M NaOH in a separating funnel, followed by drying and concentration *in vacuo* to give the ylid **395** in 95% yield (Scheme 3.42).



Scheme 3.42 *Reagents and conditions:* (i) TEA, $BrCH_2COBr$, DCM, rt, 5 h, (ii) PPh_3, acetone, rt, 18 h, (iii) 2 M NaOH, DCM.

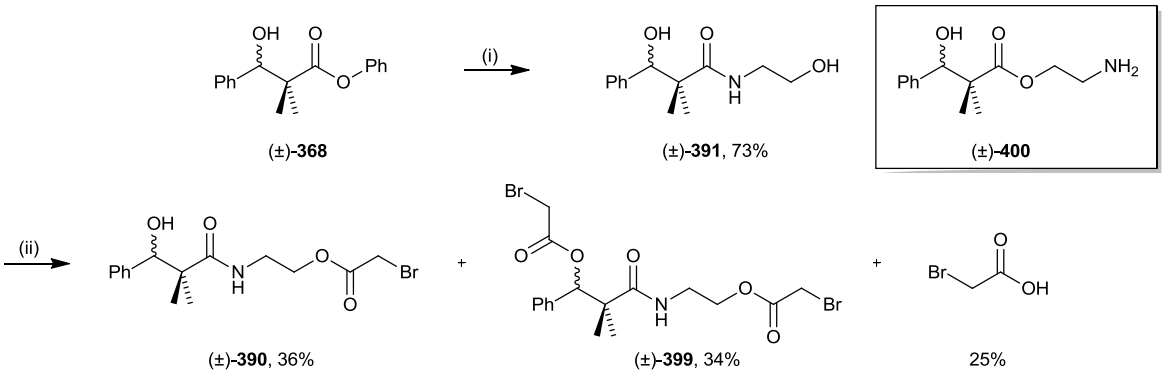
The reactivity of the ylid **395** was tested by treatment with 3-phenyl propionaldehyde **233** in DCM and the reaction gave exclusively the *E*-alkene **396** in 76% yield after removal of the Ph_3PO by filtering through a plug of silica with ice cold Et_2O (Scheme 3.43, (a)). Following this success, ylid **395** was also reacted with both lactols **362** and **363**, to give the alkenes **397** and **398** in 51% and 79% yields respectively (Scheme 3.43, (b)). In both cases only the *E*-alkene was observed.



Scheme 3.43 *Reagents and conditions:* (i) DCM, rt, 20 h.

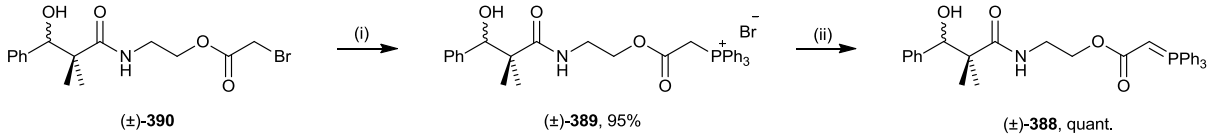
With this success in hand, efforts were turned to the synthesis of the amide conjugate. As envisaged, aminoethanol reacted selectively at the more nucleophilic nitrogen centre with the ester (±)-**368** to give the amide (±)-**391** in 73% yield. This species was differentiated from the ester (±)-**400** by inspection of the chemical shifts of the NCH_2 and OCH_2, and the characteristic appearance of the N*H* at 6.7ppm, as seen in all of the amide derivatives previously synthesised. The diol (±)-**391** was then reacted with bromoacetyl bromide. This reaction was cooled to 0 °C, and the acetyl bromide was added very slowly to minimise the production of the dibromide (±)-**399**, resulting from the acylation of both the 1° and 2°

alcohols. The desired product (±)-**390** in which the acylation has occurred only at the 1° alcohol was isolated in 36% yield. The doubly acylated product (±)-**399** was isolated in 34% yield (Scheme 3.44). A portion of the carboxylic acid was also isolated, which may have been present in the starting material or could be produced upon work-up from the residual acid bromide in the reaction mixture.



Scheme 3.44 *Reagents and conditions:* (i) aminoethanol, TEA, DCM, 40 °C, 2 d, (ii) TEA, $BrCH_2COBr$, DCM, 0 °C to rt, 5 h.

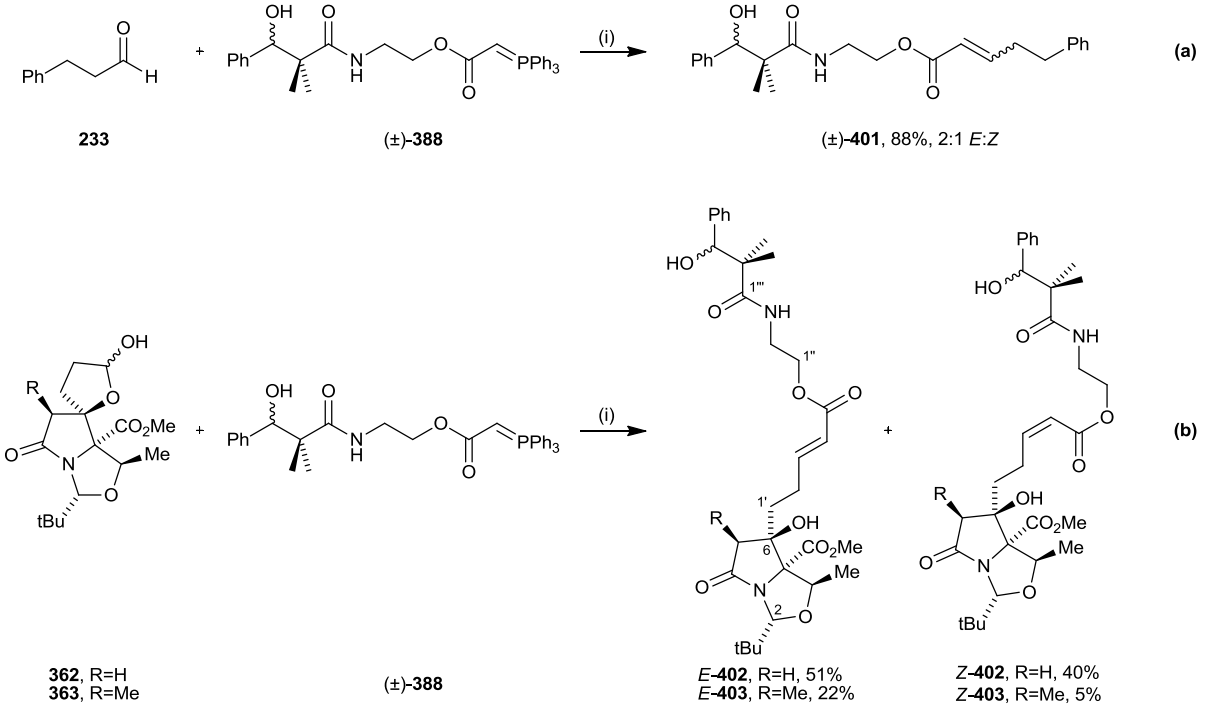
The monobromide (±)-**390** was reacted with PPh_3 in acetone to generate the phosphonium bromide salt (±)-**389** in 95% yield, and subsequent deprotonation with 2 M NaOH gave the ylid (±)-**388** quantitatively (Scheme 3.45).



Scheme 3.45 *Reagents and conditions:* (i) PPh_3, acetone, rt, 18 h, (ii) 2 M NaOH, DCM.

The ylid (±)-**388** was reacted with 3-phenylpropionaldehyde **233** to give *E*/*Z*-(±)-**401** in 88% yield as a 2:1 mixture of *E*:*Z* isomers (Scheme 3.46, (a)). With the reactivity of the ylid confirmed, the reaction was then carried out with the lactols **362** and **363**. Lactol **362** reacted to give *E*-**402** and *Z*-**402** in 51% and 40% yields respectively, both as a mixture of epimers at C(3‴). The crude ratio of E:Z had been measured as 73:30 by integration of the distinct carbon-carbon double bond protons. Lactol **363** reacted to give the C(3‴) epimeric products *E*-**403** and *Z*-**403** in 22% and 5% respectively (Scheme 3.46, (b)). The yields for these products are reduced because the method of purification that had been used for the previous Wittig products was not successful for these compounds. In previous cases, crude products had been filtered through a plug of silica gel using ice-cold Et_2O as the eluent to give the

olefinic products. Subsequent elution with EtOAc gave the Ph_3PO produced in the reaction. For the case of *E*/*Z*-**403**, the high polarity of the products resulted in the olefin eluting with the Ph_3PO, and the loss of some mass in the sample. Subsequently, the isomers were separated from Ph_3PO and each other by column chromatography using a graduated polarity eluent. For *E*/*Z*-**402**, this approach was employed immediately, and the loss of mass during the plug was avoided. In both cases, each of the geometric isomers are also a pair of epimers at C(3‴), and this leads to a surprisingly large effect on the chemical shift of the protons on the pyroglutamate core, which may have been thought to be too remote for an effect to be observed. For example, in the ^{1}H NMR spectrum of *E*-**402**, the C(2)*H* appears as 2 singlets of equal intensity at 5.02 and 5.03 ppm, and the *exo*-C(7)*H* signal at 3.00 ppm is also split into 2 overlapping doublets. All the other peaks in this spectrum appear to be a single signal. Most of the peaks correspond to 2 signals in the ^{13}C NMR spectrum.



Scheme 3.46 *Reagents and conditions:* (i) DCM, rt, 20 h.

3.4.5 Biological evaluation

The compounds synthesised in section 3.4 were subjected to biological evaluation using the hole-plate bioassay method. This included the lactones **362** and **364** from the 'click' chemistry reactions, various derivatives of the amide middle fragment mimics including the

ylid synthesis intermediates, and the adducts resulting from each of the Wittig reactions. The results of these bioassays are summarised in Table 3.7.

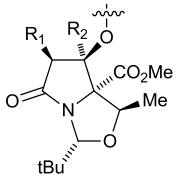
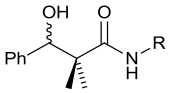
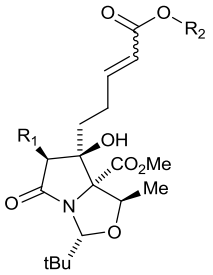
Compound Number	R_1	R_2	*S. Aureus* D267		*E. coli* X580	
			Zone size (mm)	Rel. potency (Ceph C, %)	Zone size (mm)	Rel. potency (Ceph C, %)
Pyroglutamate derivatives 						
362	H	Lactol	0	-	0	-
364	H	Oxime	0	-	0	-
371	H	Lactone	0	-	0	-
373	Me	Lactone	0	-	0	-
380a	Allyl	CH_2SPh	0	-	0	-
380b	Bn	CH_2SPh	0*dilute	-	0*dilute	-
Amides 						
(±)-357	Propargyl amide		0	-	15	0.03%
(±)-369	Amide-isoxazole test		Perhaps v. small halo	-	16	0.05%
(±)-382	Amide-sonog test		22	21.17%	15	0.04%
(±)-391	Ethanolamine Amide		0	-	0	-
(±)-390	Amide-bromide		25 (29 halo)	41.41%	40	1.72%
(±)-399	Amide-dibromide		30	105.96%	37	1.36%
(±)-401	Amide-test aldehyde		0, 14 (halo)	-	**15**, 17	0.05%
Wittig adducts 						
***E*-385**	H	Me	0	-	0	-
***E*-386**	Me	Me	0	-	0	-
***E*-397**	H	$(CH_2)_2NPhEt$	0	-	0	-
***E*-398**	Me	$(CH_2)_2NPhEt$	0	-	0	-
***E*-402**	H	Amide	0	-	**14**, 13	0.06%
***Z*-402**	H	Amide	0, 14 (halo)	-	15, 15	0.07%
***E*-403**	Me	Amide	0	-	14, 14	0.06%
***Z*-403**	Me	Amide	0	-	0, 11 (faint halo)	-

Table 3.7 Bioassay results for compounds in section 3.4.

The lactones **371** and **373** are both inactive to both test organisms, *S. aureus* and *E. coli*. Most of the amide derivatives displayed some biological activity, which is consistent with previously tested amide and ester libraries.[3] The propargylic amide (±)-**357** has been synthesised and biologically tested previously and gave a relative potency of 0.03% against *E. coli*, in agreement with the value obtained here. The compound (±)-**382** resulting from the model Sonogashira reaction gave a relative potency of 2.17% against *S. aureus* and 0.04% against *E. coli*. The model cycloaddition and Wittig reaction products (±)-**369** and (±)-**401** also displayed activity against *E. coli* both with a relative potency of 0.05%. The bromides (±)-**390** and (±)-**399** showed the highest activity, though this is most likely because of the general toxicity of alkyl halides.

The Wittig adducts **397** and **398** have linked the pyroglutamate core to a group that contains a 3° amine, and these compounds display no biological activity, along with the simple methyl substituted compounds **385** and **386**. Of interest is that the Wittig adducts **402** and **403** which have joined the pyroglutamate core to the middle fragment amide mimic all show some level of biological activity against one or both of the test organisms. In the case of *Z*-**403** the lack of significant activity can be rationalised by the low purity level of the sample meaning that the concentration of the actual hybrid structure was lower than 4 mg/mL. The other three conjugate structures, for which satisfactory purity was obtained, all show activity against *E. coli* with relative potencies between 0.06 and 0.07%. This can be compared to the activity of the butenyl-pyroglutamates **353a** and **354a**, with *E. coli* activity at 0.03 and 0.04%, and the amide (±)-**401** resulting from the Wittig reaction with 3-phenylpropionaldehyde, which has a 0.05% relative potency. Although modest, the activity of the Wittig adducts is greater than that of either of its component parts.

Broth assays of the above library identified three compounds with further activity. Compounds (±)-**401** and **380b** were active against *H. influenza* Hi4 with MIC values of 64 μg/mL. The compound (±)-**369** was also active against *S. pneumonia* Pn9 in the presence of 2.5% blood with an MIC of 64 μg/mL. Whilst this MIC value is quite high, any activity in

the presence of blood is important because binding to serum albumen often drastically reduces potency.

It is interesting to note that the parent oxazolomycin natural products display antibacterial activity against Gram-positive bacteria, and not Gram-negative. They have been recorded to inhibit *S. aureus*, but have been shown to be inactive against *E. coli* (except in the case of lajollamycin **83**), which is the opposite of the trend observed in the structural mimics synthesised in this thesis, in which more instances of *E. coli* activity have been noted.[49] However, Gram-negative activity is of considerable interest as such bacteria have significantly lower cell wall permeability as the result of a lipopolysaccharide outer membrane and thus the development of Gram-negative antibacterials is particularly challenging.[50]

3.5 Chemical informatics analysis of molecular properties

Comparison of the observed biological activity of compound libraries with their physiochemical properties has proved to be very useful in drug design and understanding structure-activity relationships.[50] Parameters that may be computationally calculated include polar surface area (PSA),[51] %PSA (PSA/MSA), and the partition coefficients *c*logP and *c*logD. Together these parameters can be used to assess the drug-like properties of molecules. The polarity of compounds affects their lipid and aqueous solubility, their ability to be transported into cells, and to bind to their intended target as well as other biological targets which may reduce efficacy or result in toxicity. The binding of blood serum can drastically reduce the bioactivity of some compounds, an effect which is a common difficulty in antibacterial discovery.[52]

Most well known in this area are ‘Lipinski’s rules’, which detail observations that most orally available drugs have a molecular weight of less than 500 and a logP of less than 5 as well as less than 5 H-bond donors and less than 10 H-bond acceptors. These rules relate only to the issues of cellular absorption of the molecules. More recent studies however, suggest that

more polar molecules may be required; one suggests that molecules with logP<3 and PSA>75 $Å^2$ will show greater safety in pre-clinical trials[53] and another reports that compounds with molecular weight <400 and logP<4 are more successful in assays of drug-like character.[54] Moreover, it has been noted that antibacterial drugs are, on average, more polar and larger than drugs for other disorders and do not in general comply with Lipinski's rules.[55]

Plotted in Figure 3.26 are the parameters of PSA and *c*logP against MSA for all the compounds from this chapter that were tested with the bacterial assay. Due to the relatively small sample size, and similar molecular properties of the compounds which have common structural motifs, conclusions about the range of 'chemical space' over which activity is most likely to be observed need to be made with care. The range over which biological activity is observed is largely the same as the total range of space occupied by the inactive compounds. It has been suggested that the optimum PSA of a non-CNS orally active drug is 70<PSA ($Å^2$)<120.[56] Although the majority of compounds tested fall into this range, there are active outliers at both extremities of PSA. Two active compounds (amide fragments (±)-**357** and (±)-**382**) have PSA of ~50 $Å^2$ and three (the Wittig adducts **402**-**403**) have a PSA of ~150 $Å^2$; the remaining actives are between 70 and 85 $Å^2$. The *c*logP of this compound library ranges from 0.72 to 6.81 and actives were identified between 1.71 and 4.65. Though these molecules are Lipinski compliant, the majority of active compounds have *c*logP>3 and so do not comply with the more recent analysis suggesting that more polar molecules are advantageous, especially in the field of antibacterials.

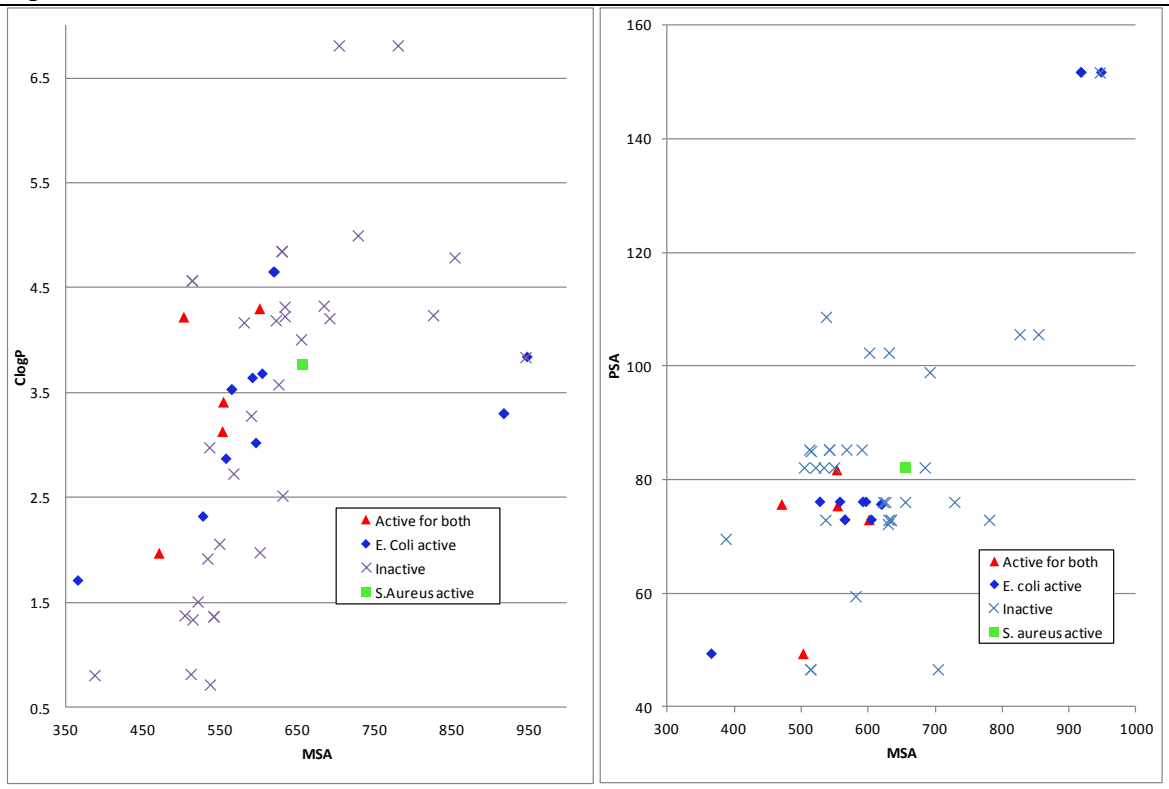


Figure 3.26 MSA versus *c*logP and PSA for active and inactive compounds assayed in the chapter.

3.6 Summary

This chapter has described work carried out with the aim of building sophisticated mimics for the right-hand fragment of 16-methyloxazolomycin and approaches to right hand-middle fragment adducts and their biological evaluation.

Firstly, a series of substituted tetramic acids (**323-324**) derived from L-threonine were synthesised using methodology established during a study of serine derived analogues. Mono-ethyl phenylmalonate was joined to the oxazolidine **320** derived from the condensation of L-threonine methyl ester with pivaalldehyde to give epimeric *N*-acyloxazolidines **321a** and **321b**, both with a *cis* relationship between the methyl ester and *t*Bu group as a result of self-regeneration of chirality. These species were cyclised using a Dieckmann reaction to give the tetramic acid **322**, which was alkylated using NaH and a series of alkyl halides to give substituted analogues **323c-e**, along with **324a-d** in which decarboxylation of the C(5)-CO_2Me had occurred. These compounds did not display antibacterial activity in hole-plate bioassays though one compound, **323e**, did exhibit activity at 8 μg/mL against *H. influenzae* Hi4 in a broth assay.

A related aldol approach was used to synthesise a series of methoxy-substituted pyroglutamates (**341**-**344**) from L-threonine. The synthetic route began with the acylation of Meldrum's acid with methoxyacetyl chloride and ring opening/decarboxylation with *t*BuOH to form β-keto-ester **304a**. This species was further functionalised using alkylations of its mono- and dianion, though it was found that the scope and reliability of this method were limited. The substituted β-keto-esters were hydrolysed to give the carboxylic acids and joined *via* amide coupling with the oxazolidine **320**. The resulting *N*-acyloxazolidines underwent a highly diastereoselective aldol cyclisation to give the pyroglutamates **341**-**344** as single diastereoisomers with the same relative stereochemistry as 16-methyloxazolomycin at all stereocentres. Disappointingly, none of these pyroglutamates displayed any biological activity when assayed either by the hole-plate method against *S. aureus* and *E. coli* or by broth assays against a wider range of bacterial strains.

A library of functionalised pyroglutamates was then synthesised by acylation of Meldrum's acid with 4-pentenoyl chloride, 4-bromophenylacetyl chloride, and 2-phenylthioacetyl chloride. Elaboration *via* the *N*-acyloxazolidines **349a-c** and **351a-c** gave the pyroglutamates **353** and **354 a-c** as single diastereoisomers with the same relative stereochemistry as the methoxy-substituted pyroglutamates. None of the pyroglutamates exhibited activity against *S. aureus* but four compounds, **353a**, **354a**, **353b**, and **353c**, showed zones of partial or full inhibition correlating to a potency of between 0.03 and 0.05% relative to cephalosporin C against *E. coli*. In the broth assay, both bromoaryl pyroglutamates **353b** and **354b** had activity against *H. influenza* Hi4 with MICs of 64 and 16 μg/mL.

The addition of a middle fragment mimic was investigated using several pathways. Alkylations of the dianion of the β-keto-ester derived from phenylthioacetyl chloride failed to produce the desired γ-alkylated species, instead giving the α-substituted analogues which were elaborated to the pyroglutamates **380a** and **b**. The Sonogashira coupling of *p*-bromotoluene and propargylic amide (±)-**357** was investigated under several literature procedures but no adduct was obtained from the reaction of pyroglutamate **353b**. A 'click'

chemistry strategy aiming to effect a 1,3-dipolar cycloaddition between the oxime derived from pyroglutamates **353a/b** and amide (±)-**357** was unsuccessful due to the preferential intramolecular reaction leading to lactones **371** and **373**. Finally, middle fragment adducts were synthesised using a Wittig reaction between the lactols **362** and **363** and the amino- and amido-ylids **395** and **388**, giving the Wittig adducts **397/8** and **402/3**. Many of the simple amide fragments resulting from model reactions displayed antibacterial activity against both *S. aureus* and *E. coli*, whilst the pyroglutamate-amide Wittig adducts displayed activity only against *E. coli* with relative potencies of 0.06-0.07%. Of interest is that only the amide adducts had activity while the amine analogues had none, suggesting that the *gem*-dimethyl amide unit of the oxazolomycins may play a pivotal role in their biological activity. It is also notable that the relative potency of the amide adducts is greater than either the pyroglutamate or amide unit alone.

In conclusion, a concise method for the diastereoselective synthesis of sophisticated mimics for the right-hand fragment of 16-methyloxazolomycin has been described. Further to this, a convenient and versatile method for the introduction of middle fragment mimics using a Wittig reaction has been developed, though some substitution was sacrificed. Future work would aim to reintroduce the methoxy substituent and to make adducts derived from ylids bearing more complex functionalisation, including possible mimics for the left-hand fragment of the natural product. Additionally, the deprotection of the oxazolidine ring and subsequent formation of the β-lactone ring would be an important future line of investigation.

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CHAPTER 4: EXPERIMENTAL SECTION

4.1 General Experimental

^{1}H NMR spectra were recorded on Bruker DPX200 (200 MHz), DPX400 (400 MHz), DQX400 (400 MHz), AVC500 (500 MHz), DRX500 (500 MHz) spectrometers. Chemical shifts (δ_H) are reported in parts per million (ppm) and are referenced to the residual protonated solvent peak. The abbreviations used to describe multiplicities are as follows: s (singlet), d (doublet), dd (double doublet), qd (quartet of doublets), t (triplet), dt (double triplet), q (quartet), m (multiplet), br (broad), and app. (apparent). Coupling constants (*J*) are given in Hertz (Hz). Two-dimensional COSY (correlation spectroscopy) spectra were obtained on a Bruker DXQ400 AVC500 or DRX500 spectrometer. Nuclear Overhauser experiments were performed using a Bruker AVC500, AVB500 or DRX500 spectrometer.

^{13}C NMR spectra were recorded on a Bruker DQX400 spectrometer at 100.6 MHz or a Bruker AVC500 spectrometer at 125.8 MHz with proton decoupling. Chemical shifts (δ_C) are reported in parts per million (ppm). Assignment was aided by the use of DEPT editing, HMQC, edited HSQC, and HMBC, performed on either DQX400 or AVC500 spectrometers.

Infrared (IR) spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer. Absorption maxima (υ_{max}) are reported in wavenumbers (cm^{-1}) and only selected peaks are reported.

Melting points were recorded using a Stuart Scientific SMP1 melting point apparatus in open capillaries and are uncorrected.

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter at the stated temperature, with concentrations given at g/100 mL; specific rotations are reported in 10^{-1} deg cm^2 g^{-1}.

Low resolution mass spectra (*m/z*) were recorded on a Fisons Platform spectrometer using electrospray ionisation (ESI) or a Fisons AutoSpec-oaTof spectrometer using electron impact

ionisation (EI) or field ionisation (FI). The *m/z* values of major peaks are reported in Daltons and their intensities given as percentages of the base peak. High resolution mass spectra (HRMS) were recorded on a Bruker microTOF (ESI).

Crystals for X-ray crystallography were grown from either of the following techniques (all at rt): slow evaporation from solutions of the requisite compound (EtOAc/Petrol/DCM/$CHCl_3$); slow vapour diffusion of petrol into a solution of the requisite compound in EtOAc.

Thin layer chromatography (TLC) was performed using Merck aluminium foil backed sheets precoated with 0.2 mm Kieselgel 60 F_{254}. Product spots were visualised by quenching of UV fluorescence (λ_{max} 254 nm), iodine vapour, or by staining with a 1% aqueous solution of $KMnO_4$ or a solution of 5% (w/v) phosphomolybdic acid in ethanol, followed by heating. Both dips were prepared according to J. Leonard, B. Lygo and G. Procter, "Advanced Practical Organic Chemistry", second edition, Blackie A & P, 1995. Retention factors (R_f) are quoted to the nearest 0.05. Flash column chromatography was carried out using Lancaster or Merck silica gel 60, 0.040-0.063 mm.

All reactions were carried out in oven-dried reaction flasks under an inert (N_2) atmosphere unless stated otherwise and where organometallic or other moisture sensitive reagents were involved, dry solvents were also used. Dry Et_3N, dry *i*Pr_2NH and dry pyridine were obtained by atmospheric pressure distillation over calcium hydride (5% w/v) under N_2 and stored over 4 Å molecular sieves under N_2. Dry DMSO was obtained by reduced pressure distillation over calcium hydride (5% w/v) and stored over 4 Å molecular sieves under N_2. Dry DMPU was obtained by storing over 4 Å molecular sieves for a minimum of 48 h. Dry DMF was purchased from Aldrich Chemicals Ltd and used as supplied. All other dry solvents were obtained by passage through a dry alumina column. 'Petrol' refers to the fraction of light petroleum ether boiling at 40-60 °C, unless otherwise stated, and was used as received. Water was purified by an Elix® UV-10 system.

Small scale reduced pressure distillations were performed using a horizontal Kugelrohr apparatus (Buchi GKR-51). Solvents were evaporated at 40 °C or below under reduced pressure on a Buchi RE111 rotavapor attached to a Vacuubrand CVC2 pump and pressure control system.

All reagents were obtained either from Aldrich Chemicals Ltd or Lancaster Chemicals Ltd and used as supplied unless otherwise stated. Alkyl halides and benzaldehyde were distilled prior to use. NBS was recrystallised prior to use.[1] *n*-BuLi was used as a solution in hexanes and was titrated against diphenylacetic acid before use.[2]

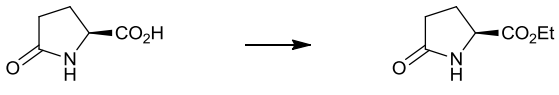
Reaction times are recorded in minutes (min), hours (h), and days (d). Temperatures below 25 °C were obtained using the following cold baths: 0 °C ice/water, −10 °C ice/water/salt, −20 °C to −78 °C acetone/CO_2(s). Organic layers were dried over $MgSO_4$ unless stated otherwise.

Microwave experiments were performed using a CEM Explorer.

4.2 Experimental for chapter 2

4.2.1 Experimental for Eschenmoser chemistry

(*S*)-Ethyl 5-oxypyrrolidine-2-carboxylate 178



142 **178**

EtOH (50 mL) and c.H_2SO_4 (1.25 mL, 2.5 mmol) were added to a suspension of (*S*)-pyroglutamic acid (25 g, 0.2 mol) in toluene (200 mL). The mixture was heated to 140 °C, using a condenser fitted with a Dean-Stark apparatus, and stirred for 16 h. The mixture was then cooled to rt before K_2CO_3 was added and stirring continued for a further 0.5 h. The solid was then removed by filtration of the resultant solution concentrated *in vacuo* to give **178** as a white solid (1.2 g, quant.); mp 40-42 ºC; {lit.[3] mp 48-50 °C}; $[\alpha]_D^{23}$ +25.7 (*c* 1.0 in $CHCl_3$)

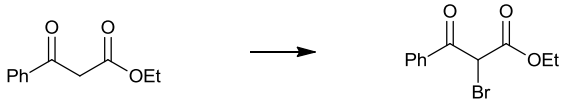
{lit.[3] $[\alpha]_D^{20}$ +2.4 (*c* 10.0 in EtOH)}; δ_H (200 MHz, $CDCl_3$) 1.23 (3H, t, *J* 7.0, OCH_2CH_3), 2.14-2.51 (4H, m, C(3)H_2, C(4)H_2), 4.14-4.29 (3H, m, OCH_2CH_3), C(2)*H*), 7.12-7.20 (1H, br s, N*H*).

(*S*)-Ethyl 5-thioxypyrrolidine-2-carboxylate 177

178 **177**

A solution of ester **178** (1.2 g, 7.8 mmol) in DCM (10 mL) was added to a suspension of Lawesson's reagent (1.71 g, 3.3 mmol) in DCM (10 mL) and the mixture was stirred for 1.5 h. The solvent was then removed *in vacuo* and purification *via* column chromatography (SiO_2, eluent 3:2 petrol:EtOAc) gave **177** as a yellow solid (97 mg, 68%); mp 37-38 ºC; {lit.[4] mp 35-36 °C}; $[\alpha]_D^{23}$ + 5.7 (*c* 1.0 in $CHCl_3$) {lit.[4] $[\alpha]_D^{20}$ +12.5 (*c* 4.0 in EtOH)}; δ_H (200 MHz, $CDCl_3$) 1.31 (3H, t, *J* 7.2, OCH_2CH_3), 2.25-2.43 (1H, m, C(3)H_AH_B), 2.46-2.66 (1H, m, C(3)H_AH_B), 2.90-3.03 (2H, m, C(4)H_2), 4.25 (2H, q, *J* 7.2, OCH_2CH_3), 4.53 (1H, dd, *J* 8.5, 6.1, C(2)*H*), 8.36 (1H, br s, N*H*).

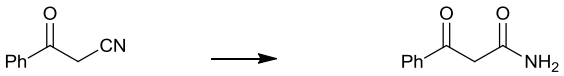
Ethyl 2-bromo-3-oxo-3-phenylpropanoate 183[5]



181 **183**

A solution of ethyl 3-oxo-3-phenylpropanoate **181** (12.5 mL, 72.2 mmol) in CCl_4 (35 mL) was cooled to −5 °C. A portion of Br_2 (4.24 mL, 82.8 mmol) was added dropwise over 15 min. The solution was then stirred at 0 °C for 1 h and then at rt for a further 3 h. The mixture was then heated to 60 °C, whereupon HBr gas was evolved. Once no further HBr bubbles evolved the mixture was concentrated *in vacuo* to give bromide **183** as a dark yellow oil (19.0 g, quant); δ_H (200 MHz, $CDCl_3$) 1.24 (3H, t, *J* 7.0, OCH_2CH_3), 4.28 (2H, q, *J* 7.0, OCH_2CH_3), 5.68 (1H, s, C*H*Br), 7.45-7.68 (3H, m, *o,p-Ph*), 7.96-8.11 (2H, m, *m-Ph*).

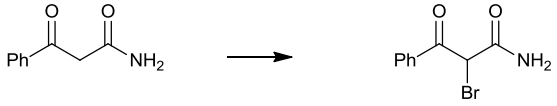
3-Oxo-3-phenylpropanamide 179[6]



179

A portion of benzoylacetonitrile (145 mg, 1 mmol) was dissolved in c.H_2SO_4 (5 mL) and stirred at rt for 4 h. The reaction mixture was then poured into iced water (30 mL) and sat. aq. K_2CO_3 was added until the solution was at pH 6.0 or below. The aqueous solution was then extracted with EtOAc (3 × 30 mL) and the combined organic layers were dried, filtered and concentrated *in vacuo* to give **179** as a pale yellow gum (134 mg, 82%); δ_H (200 MHz, $CDCl_3$) 4.00 (2H, s, C*H*$_2$), 7.44-7.67 (3H, m, *Ph*), 7.97-8.06 (2H, m, *Ph*).

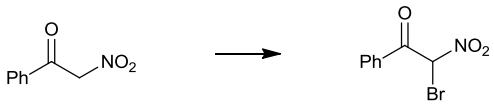
2-Bromo-3-oxo-3-phenylpropanamide 180[6]



179 **180**

A portion of 3-oxo-3-phenylpropanamide **179** (134 mg, 0.82 mmol) was dissolved in EtOAc (20 mL) and $CuBr_2$ (274 mg, 1.23 mmol) was added. The mixture was stirred at rt and monitored by TLC. When the starting material spot had faded after 6 h the mixture was filtered through a short plug of silica and concentrated *in vacuo* to give bromide **180** as a pale yellow solid (130 mg, 65%); mp 120-123 ºC; {lit.[6] mp 105-108 °C}; δ_H (200 MHz, $CDCl_3$) 5.62 (1H, s, C*H*Br), 6.63 (2H, br s, N*H*), 6.95 (1H, br s, N*H*), 7.45-7.55 (2H, m, *m-Ph*), 7.59-7.69 (1H, m, *p-Ph*), 7.98-8.05 (2H, m, *o-Ph*).

2-Bromo-2-nitro-1-phenylethanone 184

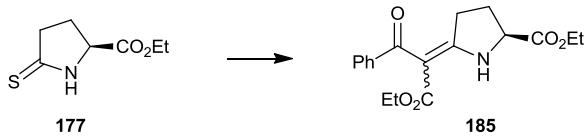


182 **184**

A solution of benzoylnitromethane **182** (300 mg, 1.8 mmol) in CCl_4 (5 mL) was cooled to −5 °C. A solution of Br_2 (0.11 mL, 2.2 mmol) in CCl_4 (2 mL) was added dropwise over 15 min. The resulting solution was stirred at 0 °C for 1 h and then at rt for a further 3 h. The mixture was heated to 60 °C whereupon HBr gas was evolved. Once no more HBr gas was evolved the mixture was cooled to rt and concentrated *in vacuo* to give **184** as a brown oil (433 mg,

97%); ν_{max} (film) 3019, 2919, 1691 (C=O); δ_H (400 MHz, $CDCl_3$) 7.33 (1H, s, C*H*Br), 7.59 (2H, s, *m-Ph*), 7.75 (1H, s, *p-Ph*), 8.01 (2H, s, *o-Ph*); δ_C (100 MHz, $CDCl_3$) 77.6 (*C*HBr), 129.8, 129.9 (*o,m-Ph*), 131.1 (*i-Ph*), 135.9 (*p-Ph*), 184.0 (*C*=O); *m/z* (ESI^-) 242 ($[M-H]^-$, 90%); HRMS (ESI^-) $C_8H_5BrNO_3^-$ ($[M-H]^-$) requires 241.9447, found 241.9450.

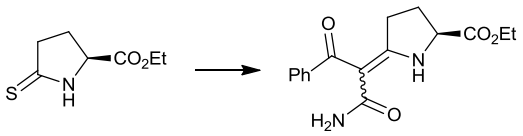
(*S*,*E*/*Z*)-Ethyl 5-(1′-ethoxycarbonyl-2′-phenyloxo-1′-ethylidene)pyrrolidine-2-carboxylate 185



177 **185**

Thiolactam **177** (200 mg, 0.1 mmol) and bromide **183** (560 mg, 2.10 mmol) were dissolved in DCM (20 mL) and $NaHCO_3$ (268 mg, 4.0 mmol) was added. The reaction mixture was heated to 50 °C and stirred for 24 h before being cooled to rt and filtered through a plug of celite. The filtrate was then concentrated *in vacuo* and purified *via* column chromatography (SiO_2, eluent 5:1 petrol:EtOAc) to give **185** in a 63:37 dr as a yellow oil (380 mg, 75%); R_f 0.3 (eluent 5:1 petrol:EtOAc); ν_{max} (film) 3309 (N−H), 1742, 1692 (C=O); δ_H (400 MHz, $CDCl_3$) 0.71-0.77 (3H, m, OCH_2CH_3'), 1.28-1.33 (3H, m, OCH_2CH_3), 2.14-2.30 (1H, m, C(3)H_AH_B), 2.36-2.48 (1H, m, C(3)H_AH_B), 3.08-3.35 (2H, m, C(4)H_2), 3.82 (0.63 × 2H, q, *J* 7.1, OCH_2CH_3', major), 3.90 (0.37 × 2H, q, *J* 7.2, OCH_2CH_3', minor), 4.20-4.29 (2H, m, OCH_2CH_3), 4.48-4.58 (1H, m, C(2)*H*), 7.28-7.68 (5H, m, *Ph*), 9.50 (br s, N*H*, min), 11.00 (br s, N*H*, maj); δ_C (100 MHz, $CDCl_3$) 13.4, 13.5, 14.1, 14.2 (OCH_2CH_3', OCH_2CH_3), 25.4, 26.1 (*C*(3)), 32.4, 33.1 (*C*(4)), 59.3, 59.6 (OCH_2CH_3) 60.9, 61.5 (*C*(2)), 61.8, 61.9 (OCH_2CH_3'), 96.7, 98.8 (*C*(5)), 126.7, 127.6, 127.7, 128.0, 129.7, 129.8, 130.7 (*o*, *m*, *p-Ph*), 142.5, 143.2 (*i-Ph*), 169.0, 169.6, 170.7, 171.0, 172.1, 173.3 (CO_2Et', CO_2Et, *C*(1′)), 195.1, 195.2 (C(2′)); *m/z* (ESI^+) 332 ($[M+H]^+$, 80%); HRMS (ESI^+) $C_{18}H_{22}NO_5^+$ ($[M+H]^+$) requires 332.1492, found 332.1495.

(*S*)-Ethyl 5-(1′-aminocarbonyl-2′-phenyloxo-1′-ethylidene)pyrrolidine-2-carboxylate 186

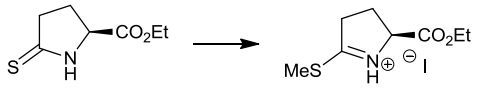


177 **186**

Thiolactam **177** (45 mg, 0.26 mmol) and bromide **180** (130 mg, 0.52 mmol) were dissolved in DCM (20 mL) and $NaHCO_3$ (87 mg, 1.0 mmol) was added. The reaction mixture was heated to 50 °C and stirred for 24 h before being cooled to rt and filtered through a plug of Celite. The filtrate was then concentrated *in vacuo* and purified *via* column chromatography (SiO_2, eluent 2:3 petrol:EtOAc) gave the single diastereoisomer **186** as a yellow oil (8 mg, 10%); R_f 0.35 (eluent 2:3 petrol:EtOAc); $[\alpha]_D^{23}$ +15.3 (*c* 0.4 in $CHCl_3$); ν_{max} (film) 3418 (N−H) 1739, 1621 (C=O); δ_H (500 MHz, $CDCl_3$) 1.30 (3H, t, *J* 7.1, OCH_2CH_3), 1.90-1.99 (1H, m, C(3)H_AH_B), 2.08-2.19 (2H, m, C(3)H_AH_B, C(4)H_AH_B), 2.22-2.31 (1H, m, C(4)H_AH_B), 4.23 (2H, q, *J* 7.1, OCH_2CH_3), 4.42 (1H, dd, *J* 7.6, 5.0, C(2)*H*), 7.38-7.55 (5H, m, *Ph*); δ_C (125 MHz, $CDCl_3$) 14.2 (OCH_2CH_3), 26.6 (C(3)), 34.9 (*C*(4)), 60.4 (*C*(2)), 61.9 (OCH_2CH_3), 99.4 (*C*(5)), 127.9, 128.5, 128.9, 130.9, 134.2 (*o*, *m*, *p*-*Ph*), 143.5 (*i*-*Ph*), 170.6, 171.6, 173.8 (*C*(1′), CO_2Et, $CONH_2$), 196.7 (*C*(2′)); *m/z* (ESI^-) 301 ($[M-H]^-$, 100%); HRMS (ESI^+) $C_{16}H_{19}N_2O_4^+$ ($[M+H]^+$) requires 303.1339, found 303.1338.

4.2.2 Experimental for thiolactim ether chemistry

(*S*)-Ethyl 5-(methylthio)-3,4-dihydro-2-pyrrole-2H-carboxylate hydroiodide 190[7]

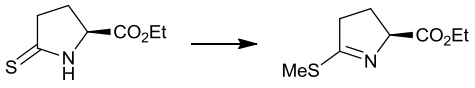


177 **190**

Thiolactam **177** (2.0 g, 17 mmol) was dissolved in acetone (10 mL) and MeI (3.14 mL, 51 mmol) was added dropwise. The resulting solution was stirred for 15 min during which a precipitate formed. The precipitate was filtered off and washed with Et_2O until it appeared white and dried in air to give **190** as a white powder (5.33 g, quant); mp 108-109 ºC; $[\alpha]_D^{23}$ +9.6 (*c* 1.0 in $CHCl_3$); ν_{max} (cell) 3684, 3020, 2400, 1746 (C=O); δ_H (400 MHz, $CDCl_3$) 1.33 (3H, t, *J* 7.1, $CO_2CH_2CH_3$), 2.36-2.46 (1H, m, C(3)H_AH_B), 2.80-2.92 (1H, m, C(3)H_AH_B), 4.28 (2H, q, *J* 7.1, $CO_2CH_2CH_3$), 5.19 (1H, m, C(2)*H*); δ_C (100 MHz, $CDCl_3$) 14.1

($CO_2CH_2CH_3$), 19.2 (SCH_3), 26.0 (*C*(3)), 38.4 (*C*(4)), 63.0 ($CO_2CH_2CH_3$), 65.9 (*C*(2)), 194.3 (*C*(5)), 168.1 (CO_2Et); *m/z* (ESI^+) 188 ($[M]^+$, 100%).[8]

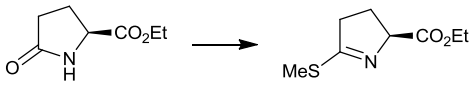
(*S*)-Ethyl 5-(methylthio)-3,4-dihydro-2-pyrrole-2H-carboxylate 189



177 **189**

Thiolactam **177** (100 mg, 0.57 mmol) was dissolved in dry THF (3 mL) and K_2CO_3 (237 mg, 1.73 mmol) and MeI (0.1 mL, 1.73 mmol) were added. The resulting mixture was stirred for 6 h at rt before being concentrated *in vacuo*. The residue was dissolved in $CHCl_3$ (20 mL), washed with sat. aq. $NaHCO_3$ (20 mL), dried, and concentrated *in vacuo* to give **189** as a pale yellow liquid (100 mg, 94%); R_f 0.4 (eluent 4:1 40-60 Petrol:EtOAc); $[\alpha]_D^{23}$ + 57.7 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 1738 (C=O); δ_H (400 MHz, $CDCl_3$) 1.25 (3H, t, *J* 7.1, OCH_2CH_3), 2.08-2.18 (1H, m, C(3)H_AH_B), 2.21-2.31 (1H, m, C(3)H_AH_B), 2.44 (3H, s, SCH_3), 2.57-2.66 (1H, m, C(4)H_AH_B), 2.70-2.80 (1H, m, C(4)H_AH_B), 4.16 (2H, q, *J* 7.1, OCH_2CH_3), 4.61-4.66 (1H, m, C(2)*H*); δ_C (100 MHz, $CDCl_3$) 13.8, 14.2 (OCH_2CH_3, SCH_3), 27.6 (*C*(3)), 38.6 (*C*(4)), 61.0 (OCH_2CH_3), 73.5 (*C*(2)), 172.8, 176.9 (*C*(5), CO_2Et); *m/z* (ESI^+) 188 ($[M+H]^+$, 55%); HRMS (ESI^+) $C_8H_{14}NO_2S^+$ ($[M+H]^+$) requires 188.0740, found 188.0738.

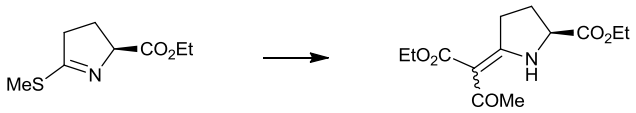
(*S*)-Ethyl 5-(methylthio)-3,4-dihydro-2-pyrrole-2H-carboxylate 189



178 **189**

A solution of ester **178** (2.7 g, 17 mmol) in DCM (20 mL) was added to a suspension of Lawesson's reagent (3.43 g, 8.5 mmol) in DCM (20 mL) and the mixture was stirred for 1.5 h. The solvent was then removed *in vacuo* and the residue was filtered through a short plug of SiO_2 (eluent EtOAc). Concentration *in vacuo* gave thiolactam **177** which was immediately dissolved in acetone (10 mL). MeI (3.14 mL, 50 mmol) was added and the solution stirred for 15 min during which a precipitate formed. The suspension was filtered and the precipitate washed with Et_2O until white in appearance. The solid was then dissolved in DCM (30 mL) and washed with dilute aq. K_2CO_3 (3 × 30 mL), dried and concentrated *in vacuo* to give **189** (2.2 g, 68%) as a colourless liquid.

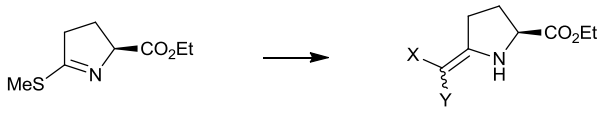
(2*S*)-Ethyl 5-(1′-ethoxycarbonyl-2′-methyloxo-1′-ethylidene)pyrrolidine-2-carboxylate 191



189 **191**

Ethyl acetoacetate (0.05 mL, 0.39 mmol) and **189** (50 mg, 0.27 mmol) were dissolved in DMSO (0.5 mL) and stirred at 120 °C for 16 h. The solution was then cooled to rt and H_2O (20 mL) was added. The mixture was extracted with DCM (3 × 15 mL) and the combined organic layers were washed with H_2O (4 × 20 mL), dried and concentrated *in vacuo* to give a crude mixture containing a 56:44 ratio of **189**:**191** Purification *via* column chromatography (SiO_2, eluent 20:3 petrol:EtOAc) gave **191** as a colourless oil (22 mg, 31%); R_f 0.3 (eluent 9:3 petrol:EtOAc); $[\alpha]_D^{23}$ −0.8 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3250 (N−H), 2982, 1743 (C=O), 1690 (C=O); δ_H (400 MHz, $CDCl_3$) 1.27-1.34 (6H, m, OCH_2CH_3), 2.13-2.21 (1H, m, C(3)H_AH_B), 2.31-2.38 (1H, m, C(3)H_AH_B), 2.43 (3H, s, $COCH_3$), 3.12-3.24 (2H, m, C(4)H_2), 4.18-4.25 (4H, m, OCH_2CH_3), 4.50 (1H, dd, *J* 9.0, 5.4, C(2)*H*); δ_C (100 MHz, $CDCl_3$) 14.1, 14.4 (OCH_2CH_3, OCH_2CH_3'), 25.3 (*C*(3)), 31.0 ($COCH_3$), 34.3 (*C*(4)), 59.7, 61.9 (OCH_2CH_3, OCH_2CH_3'), 61.4 (*C*(2)), 99.7 (*C*(1′)), 168.4, 170.8, 173.8 (*C*(5), CO_2Et, CO_2Et'), 198.3 (*C*(2′)); *m/z* (ESI^-) 268 ($[M-H]^-$, 100%); HRMS (ESI^+) $C_{13}H_{19}NNaO_5^+$ ($[M+Na]^+$) requires 292.1155, found 292.1160.

4.2.2.2 Optimisation reactions of lactim thioether



189

General Procedure A: Solvent variation

Ethyl acetoacetate (0.05 mL, 0.39 mmol) and lactim thioether **189** (50 mg, 0.27 mmol) were dissolved in the solvent and either the solution was stirred immediately or TEA (0.04 mL, 0.45 mmol) was added and the solution was stirred at the temperature stated. Aliquots were taken to assess the extent of reaction after various time periods. These were cooled to rt and H_2O (10 mL) was added. The mixture was extracted with DCM (3 × 15 mL) and the

combined organic layers were washed with H_2O (4 × 20 mL), dried and concentrated *in vacuo*.

A: The reagents were dissolved in DMF (0.5 mL) and stirred at 120 °C for 20 h to give a 63:37 mixture of **189**:**191**.

B: The reagents were dissolved in DMSO (0.5 mL) and stirred at 120 °C for 20 h to give a 56:44 mixture of **189**:**191**.

C: The reagents were dissolved in DMF (0.5 mL), TEA was added and the solution was stirred at 120 °C for 20 h to give a 81:19 mixture of **189**:**191**.

D: The reagents were dissolved in DMSO (0.5 mL), TEA was added and the solution was stirred at 120 °C for 20 h to give a 59:41 mixture of **189**:**191**, and for 4 d to give a 8:92 mixture.

E: The reagents were dissolved in MeCN (1 mL) and stirred at 85 °C for 20 h to give unreacted starting material.

F: The reagents were dissolved in MeCN (1 mL), TEA was added and the solution was stirred at 85 °C for 20 h to give unreacted starting material.

G: The reagents were dissolved in acetone (1 mL), TEA was added and the solution was stirred at 60 °C for 20 h to give unreacted starting material.

H: The reagents were dissolved in DCM (1 mL), TEA was added and the solution was stirred at 40 °C for 20 h to give unreacted starting material.

I: The reagents were dissolved in $CHCl_3$ (1 mL) and the solution was stirred at 65 °C for 20 h to give a complex inseparable mixture.

J: The reagents were dissolved in $CHCl_3$ (1 mL), TEA was added and the solution was stirred at 65 °C for 20 h to give unreacted starting material.

K: The reagents were dissolved in MeOH (1 mL) and the solution was stirred at 65 °C for 20 h to give a complex inseparable mixture.

L: The reagents were dissolved in MeOH (1 mL), TEA was added and the solution was stirred at 65 °C for 20 h to give unreacted starting material.

General Procedure B: Varying base

Ethyl acetoacetate (0.05 mL, 0.39 mmol) and lactim thioether **189** (50 mg, 0.27 mmol) were dissolved in DMSO (0.5 mL) and a portion of the base was added. The solution was stirred at 120 °C. Aliquots were taken to assess the extent of reaction after various time periods. These were cooled to rt and H_2O (10 mL) was added. The mixture was extracted with DCM (3 × 15 mL) and the combined organic layers were washed with H_2O (4 × 20 mL), dried and concentrated *in vacuo*.

A: The reaction using DBU (0.05 mL, 0.4 mmol) was stirred for 20 h to give a complex mixture of unidentifiable products.

B: The reaction using K_2CO_3 (41 mg, 0.4 mmol) was stirred for 4 d to give unreacted starting materials.

C: The reaction using KO*t*Bu (34 mg, 0.4 mmol) was stirred for 20 h to give unreacted starting materials and for 4 d to give a complex mixture of products.

General Procedure C: Varying nucleophile

The nucleophile (0.39 mmol) and lactim thioether **189** (50 mg, 0.27 mmol) were dissolved in DMSO (0.5 mL) and TEA (0.04 mL, 0.45 mmol) was added. The solution was stirred at 120 °C unless otherwise stated. Aliquots were taken to assess the extent of reaction after various time periods. These were cooled to rt and H_2O (10 mL) was added. The mixture was extracted with DCM (3 × 15 mL) and the combined organic layers were washed with H_2O (4 × 20 mL), dried and concentrated *in vacuo*.

A: The reaction using ethyl 2-cyanoacetate (0.04 mL, 0.39 mmol) was stirred for 20 h to give complete consumption of the starting material.

B: The reaction using ethyl 3-oxo-3-phenylpropanoate (0.07 mL, 0.39 mmol) was stirred for 20 h to give a 85:15 mixture of **189**:**185**, and for 4 d to give a 70:30 mixture.

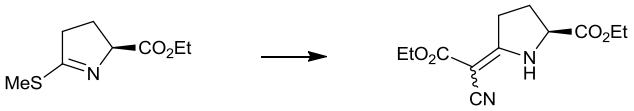
C: The reaction using diethyl malonate (0.06 mL, 0.39 mmol) was stirred for 20 h giving no observable production of **192**, and after 4 d gave a 58:42 mixture of **189**:**192**.

D: The reaction using ethyl acetamidoacetate (58 mg, 0.39 mmol) was stirred for 20 h and for 4 d, giving no observable production of **195**.

E: The reaction using benzoylnitromethane (66 mg, 0.39 mmol) was stirred for 20 h to give a complex mixture of unidentifiable products.

F: The reaction using benzoylnitromethane (66 mg, 0.39 mmol was stirred at 60 °C for 20 h to give a 86:14 mixture of **189**:**187**, and for 3 d to give a 70:30 mixture.

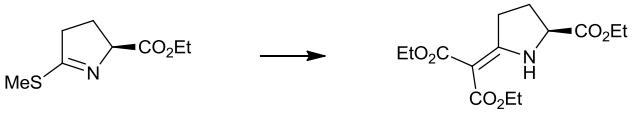
(2*S*)-Ethyl 5-(1′-cyano-2′-ethoxy-2′-oxoethylidene)pyrrolidine-2-carboxylate 194



189 **194**

Purification of the mixture described in **general procedure C:A** *via* column chromatography (SiO_2, eluent 9:3 petrol:EtOAc) gave **194** as a single diastereoisomer as a pale yellow oil (54 mg, 78%); R_f 0.4 (eluent 9:3 petrol:EtOAc); $[\alpha]_D^{23}$ −8.9 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3335 (N−H), 2983 (C−H), 2207 (C≡N), 1742(C=O), 1675 (C=O); δ_H (400 MHz, $CDCl_3$) 1.26 (6H, m, OCH_2CH_3, OCH_2CH_3'), 2.18-2.28 (1H, m, C(3)H_AH_B), 2.39-2.50 (1H, m, C(3)H_AH_B), 2.92-3.07 (2H, m, C(4)H_2), 4.17-4.25 (4H, m, OCH_2CH_3, OCH_2CH_3'), 4.54 (1H, dd, *J* 8.8, 5.6, C(2)*H*), 9.15 (1H, br s, N*H*); δ_C (100 MHz, $CDCl_3$) 14.1, 14.4 (OCH_2CH_3, OCH_2CH_3'), 25.2 (*C*(3)), 32.4 (*C*(4)), 60.6, 62.1 (OCH_2CH_3, OCH_2CH_3'), 62.2 (*C*(2)), 69.1 (*C*(1′)), 118.1 (*C*N), 167.4, 170.3, 173.3 (CO_2Et, *C*(2′), *C*(5)); *m/z* (ESI^-) 251 ($[M-H]^-$, 100%); HRMS (ESI^+) $C_{12}H_{16}N_2NaO_4^+$ ($[M+Na]^+$) requires 275.1002, found 275.1002.

(2*S*)-Ethyl 5-(1′-ethoxycarbonyl-2′-ethoxy-2′-oxoethylidene)pyrrolidine-2-carboxylate 192

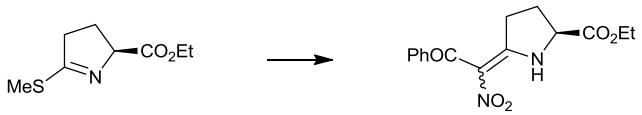


189 **192**

Purification of the mixture described in **general procedure C:C** *via* column chromatography (SiO_2, eluent 3:1 petrol:EtOAc) gave **192** as a pale yellow oil (10 mg, 12%); R_f 0.3 (eluent 3:1 petrol:EtOAc); $[\alpha]_D^{23}$ −6.7 (*c* 0.5 in $CHCl_3$) {lit.[9] $[\alpha]_D^{20}$ −28.9 (*c* 1.26 in $CHCl_3$)}; ν_{max} (film) 3317, 2981, 1743, 1650; δ_H (500 MHz, $CDCl_3$) 1.25-1.33 (9H, m, OCH_2CH_3), 2.14-2.21 (1H, m, C(3)H_AH_B), 2.32-2.41 (1H,m, C(3)H_AH_B), 3.09-3.23 (2H,m, C(4)H_2), 4.16-4.26 (6H, m, OCH_2CH_3), 4.44 (1H, dd, *J* 9.0, 5.7, C(2)*H*), 9.65 (1H, br s, N*H*); δ_C (125 MHz,

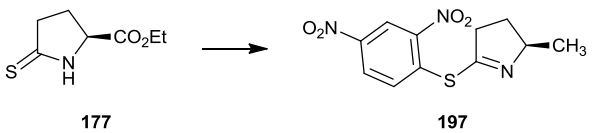
$CDCl_3$) 14.1, 14.3 ($CO_2CH_2\mathit{C}H_3$), 25.9 (*C*(3)), 33.1 (*C*(4)), 59.7, 59.8, 61.7 ($CO_2\mathit{C}H_2CH_3$), 60.8 (*C*(2)), 88.9 (*C*(1′)), 167.5, 169.4, 171.1, 172.1 ($\mathit{C}O_2Et$, *C*(5)); *m/z* (ESI^+) 322 ($[M+Na]^+$, 60%).

(2*S*)-Ethyl 5-(1′-nitro-2′-oxo-2′-phenylethylidene)pyrrolidine-2-carboxylate 187



Purification of the mixture described in **general procedure C:E** *via* column chromatography (SiO_2, eluent 9:3 petrol:EtOAc) gave **187** in a 38:62 dr as a pale yellow oil (23 mg, 20%); R_f 0.3 (eluent 9:3 petrol:EtOAc); ν_{max} (film) 3321 (N−H), 1740 (C=O); δ_H (400 MHz, $CDCl_3$) 1.30 (3H, m, $OCH_2C\mathit{H}_3$), 2.27-2.39 (1H, m, C(3)H_AH$_B$), 2.48-2.59 (1H, m, C(3)H$_A\mathit{H}_B$), 3.18 (0.62 × 2H, t, *J* 7.8, C(4)H_2, major), 3.38-3.57 (0.38 × 2H, m, C(4)H_2, minor), 4.23-4.34 (2H, m, $OC\mathit{H}_2CH_3$), 4.61-4.73 (1H, m, C(2)*H*), 7.37-7.55 (4H, m, *o,m-Ph*), 7.71-7.75 (0.62 × 1H, m, *p-Ph*, maj), 8.10-8.14 (0.38 × 1H, m, *p-Ph*, min); δ_C (125 MHz, $CDCl_3$) 14.3 ($OCH_2\mathit{C}H_3$), 25.2, 25.7 (*C*(3)), 34.2, 32.3 (*C*(4)), 62.0, 62.1, 62.3, 62.4 ($O\mathit{C}H_2CH_3$, *C*(2)), 126.5, 128.0, 128.2, 128.5, 129.7, 130.2, 131.1, 132.5, 133.7, 138.6, 139.4 (*Ph*), 165.6, 169.7, 169.7, 169.9 ($\mathit{C}O_2Et$, *C*(2′)), 189.3, 190.9 (*C*(5), *C*(1′)); *m/z* (ESI^+) 363 ($[M+NH_4+MeCN]^+$, 100%); HRMS (ESI^+) $C_{15}H_{16}N_2O_5^+$ ($[M+H]^+$) requires 305.1132, found 305.1132.

(*S*)-Ethyl 5-(2′,4′-dinitrophenylthio)-3,4-dihydro-2H-pyrrole-2-carboxylate 197



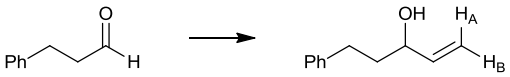
Method A: Thiolactam **177** (60 mg, 0.35 mmol) was dissolved in THF (3 mL) and K_2CO_3 (138 mg, 1.0 mmol) and 1-fluoro-2,4-dinitrobenzene (193 mg, 1.0 mmol) were added. The resulting mixture was stirred at 50 °C for 8 h before the solvent was removed *in vacuo*. The residue was dissolved in DCM (20 mL) and washed with sat.aq. $NaHCO_3$ (2 × 20 mL), dried and concentrated *in vacuo* to give a mixture of **197** and excess 1-fluoro-2,4-dinitrobenzene as a yellow oil which was used without further purification. Data for **197**: R_f 0.6 (eluent 3:1 petrol:EtOAc); δ_H (400 MHz, $CDCl_3$) 1.32 (3H, t, *J* 7.1, $CO_2CH_2C\mathit{H}_3$), 2.20-2.29 (1H, m,

C(3)H_AH_B), 2.32-2.42 (1H, m, C(3)H_AH_B), 2.80-2.90 (1H, m, C(4)H_AH_B), 2.93-3.03 (1H, m, C(4)H_AH_B), 4.23 (2H, q, *J* 7.1, $CO_2CH_2CH_3$), 4.74-4.80 (1H, m, C(2)*H*), 8.38-8.42 (1H, m, C(6′)*H*), 8.62-8.65 (C(5′)*H*), 8.88-8.90 (C(3′)*H*); δ_C (100 MHz, $CDCl_3$) 14.2 ($CO_2CH_2CH_3$), 26.9 (*C*(3)), 40.1 (*C*(4)), 61.4 ($CO_2CH_2CH_3$), 74.2 (*C*(2)), 119.9-135.9 (*Ph*), 157.4, 160.1 (*C*(5), CO_2Et); *m/z* (ESI$^-$) 338 ([M−H]$^-$, 100%).

Method B: Thiolactam **177** (60 mg, 0.35 mmol) was dissolved in THF (3 mL) and K_2CO_3 (138 mg, 1.0 mmol) and 1-fluoro-2,4-dinitrobenzene (67 mg, 0.35 mmol) were added. The resulting mixture was stirred at 50 °C for 8 h before the solvent was removed *in vacuo*. The residue was dissolved in DCM (20 mL) and washed with sat. aq. $NaHCO_3$ (2 × 20 mL), dried and concentrated *in vacuo* to give unreacted starting materials.

4.2.3 Experimental for oxime ether cyclisation chemistry

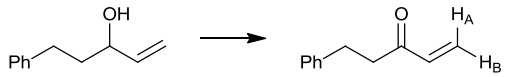
1-Phenylpent-4-ene-3-ol 234



233 **234**

A stirred solution of aldehyde **233** (0.5 mL, 3.72 mmol) in dry Et_2O (6 mL) was cooled to 0 °C, followed by the dropwise addition of vinylmagnesium bromide (3.91 mL, 1.0 M in THF, 3.91 mmol). The solution was stirred at 0 °C for 10 min before the addition of sat. aq. NH_4Cl (10 mL) and extraction with EtOAc (2 × 15 mL). The combined organic layers were washed with brine (20 mL), dried and concentrated *in vacuo* to give **234**[10] as a colourless oil (600 mg, quant); δ_H (200 MHz, $CDCl_3$) 1.82-1.97 (2H, m, C(1)H_2), 2,14 (1H, br s, O*H*), 2.71-2.82 (2H, m, C(2)H_2), 4.16 (1H, app. q, *J* 6.5, C(3)*H*), 5.18 (1H, d, *J* 10.2, C(5)H_B), 5.28 (1H, d, *J* 17.4, C(5)H_A), 5.85-6.04 (1H, m, C(4)*H*), 7.22-7.40 (5H, m, *Ph*).

1-Phenylpent-4-ene-3-one 235

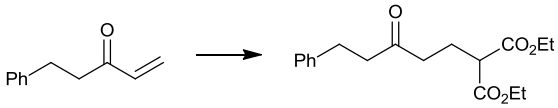


234 **235**

A solution of DMSO (0.9 mL, 12.0 mmol) in dry DCM (4 mL) at −78 °C was added *via* cannula to a stirred solution of oxalyl chloride (0.7 mL, 8.0 mmol) in DCM (4 mL) at −78 °C and the resulting mixture was stirred for 15 min. A solution of alcohol **234** (650 mg, 4.0

mmol) in DCM (2 mL) at −78 °C was added *via* cannula and the mixture stirred for a further 30 min. A portion of TEA (2.7 mL, 20 mmol) was added and the solution was allowed to warm to rt and stirred for 30 min. The mixture was then diluted with sat. aq. $NaHCO_3$ (20 mL) and extracted with EtOAc (2 × 20 mL) and the combined organic layers were washed with brine (30 mL), dried and concentrated *in vacuo* to give **235**[10] as a colourless oil (634 mg, 95%); δ_H (200 MHz, $CDCl_3$) 2.92-2.98 (4H, m, C(2)H_2, C(1)H_2), 5.84 (1H, dd, *J* 9.9, 1.7, C(5)H_B), 6.22 (1H, dd, *J* 17.8, 1.7, C(5)H_A), 6.39 (1H, dd, *J* 17.8, 9.9, C(4)*H*), 7.19-7.33 (5H, m, *Ph*).

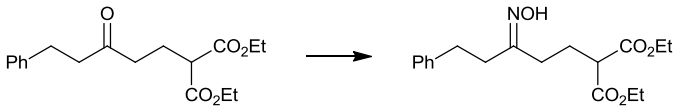
1-Phenyl-6-diethoxycarbonylhexan-3-one 236



235 **236**

Diethyl malonate (0.5 mL, 3.3 mmol) was dissolved in DCM (5 mL) and K_2CO_3 (911 mg, 6.6 mmol) was added. The mixture was stirred for 5 min before the addition of **235** (634 mg, 3.9 mmol) in DCM (2 mL). The mixture was stirred for a further 6 h before being diluted with DCM (20 mL) and washed with H_2O (2 × 20 mL), dried and concentrated *in vacuo* to give **236** as a colourless oil (1.04 g, 86%); ν_{max} (film) 2983, 1732 (C=O); δ_H (400 MHz, $CDCl_3$) 1.27 (6H, t, *J* 7.1, OCH_2CH_3), 2.14 (2H, app. q, *J* 7.3, C(5)H_2), 2.49 (2H, t, *J* 7.3, C(4)H_2), 2.68-2.76 (2H, m, C(2)H_2), 2.85-2.94 (2H, m, C(1)H_2), 3.36 (1H, t, *J* 7.3, C(6)H_2), 4.14-4.23 (4H, m, OCH_2CH_3), 7.14-7.30 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 14.0 (OCH_2CH_3), 22.4 (C(5)), 39.7 (*C*(4)), 41.1, 41.8 (*C*(6), *C*(2)), 44.2 (*C*(1)), 61.5 (OCH_2CH_3), 126.1, 128.2, 128.3, 128.4, 128.5 (*o*,*m*,*p*-*Ph*), 140.5 (*i*-*Ph*), 169.1 (CO_2Et), 208.4 (*C*(3)); *m/z* (ESI^+) 379 ($[M+NH_4+MeCN]^+$, 100%); HRMS (ESI^+) $C_{18}H_{24}O_5Na^+$ ($[M+Na]^+$) requires 343.1516, found 343.1513.

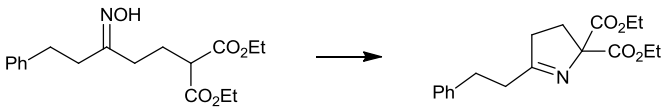
(*E*/*Z*)-1-Phenyl-6-diethyoxycarbonylhexan-3-hydroxyimine 237



236 **237**

Ketone **236** (150 mg, 0.47 mmol), hydroxylamine (65 mg, 0.94 mmol), and TEA (0.16 mL, 1.18 mmol) were dissolved in EtOH (10 mL). The mixture was heated to 85 °C for 7 h before the solvent was removed *in vacuo*. The residue was dissolved in DCM (25 mL) and washed with H_2O (2 × 20 mL), dried and concentrated *in vacuo* to give **237** in a 1:1 dr as a colourless oil (140 mg, 84%); R_f 0.2 (eluent 8:1 hexane:EtOAc); ν_{max} (film) 3251, 2982, 1730 (C=O), 1603; δ_H (400 MHz, $CDCl_3$) 1.25-1.30 (6H, m, OCH_2CH_3), 2.06-2.21 (3H, m, C(5)H_2, C(4)H_2-diastereoisomer A), 2.41-2.46 (0.5 × 2H, m, C(4)H_2-diastereoisomer B), 2.51-2.56 (0.5 × 2H, m, C(2)H_2-B), 2.62-2.67 (0.5 × 2H, m, C(2)H_2-A), 2.82-2.88 (2H, m, C(1)H_2), 3.37 (1H, app. t, *J* 7.3, C(6)*H*), 4.15-4.26 (4H, m, OCH_2CH_3), 7.18-7.32 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 14.1 (OCH_2CH_3), 24.6, 25.0 (*C*(5)), 25.7 (*C*(4)-B), 29.9 (*C*(2)-A), 31.5, 32.0 (*C*(1)), 32.4 (*C*(4)-A), 35.0 (*C*(2)-B), 51.2, 51.7 (*C*(6)), 61.5 (OCH_2CH_3), 126.7, 128.2, 128.3, 128.4, 128.5 (*o*,*m*,*p*-*Ph*), 141.1, 141.2 (*i*-*Ph*), 159.6 (*C*(3)), 169.1, 169.2 (CO_2Et); *m/z* (ESI^+) 394 ($[M+NH_4+MeCN]^+$, 100%); HRMS (ESI^+) $C_{18}H_{25}NNaO_5^+$ ($[M+Na]^+$) requires 358.1625, found 258.1624.

2,2-Diethoxycarbonyl-3,4-dihydro-5-phenylethyl-2H-pyrrole 238

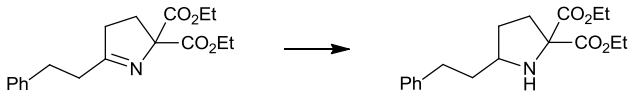


237 **238**

Oxime **237** (140 mg, 0.42 mmol) was dissolved in EtOH (10 mL) and Na (~30 mg) was added. The mixture was stirred for 15 min at rt, cooled to 0 °C and 1-fluoro-2,4-dinitrobenzene (86 mg, 0.46 mmol) was added. The mixture was then allowed to warm to rt and stirred for a further 30 min before being concentrated *in vacuo*. H_2O (15 mL) was added to the residue which was then extracted with EtOAc (2 × 20 mL) and the combined organic layers were dried and concetrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 10:1-4:1 petrol:EtOAc) gave **238** as a colourless oil (25 mg, 19%); R_f 0.4 (eluent 4:1 petrol:EtOAc); ν_{max} (film) 2982 (C−H), 1732 (C=O), 1637 (C=N); δ_H (500 MHz, $CDCl_3$) 1.30

(6H, t, *J* 7.2, OCH_2CH_3), 2.44 (2H, t, *J* 7.9, C(2′)H_2), 2.64 (2H, t, *J* 7.9, C(1′)H_2), 2.76 (2H, t, *J* 8.1, C(4)H_2), 2.99 (2H, t, *J* 8.1, C(3)H_2), 4.20-4.32 (4H, m, OCH_2CH_3), 7.19-7.32 (5H, m, *Ph*); δ_C (125 MHz, $CDCl_3$) 14.0 (OCH_2CH_3), 30.2 (*C*(3)), 32.6 (*C*(2′)), 35.3 (*C*(1′)), 38.4 (*C*(4)), 61.9 (OCH_2CH_3), 86.4 (*C*(2)), 126.1 (*p-Ph*), 128.3, 128.5 (*o,m-Ph*), 140.9 (*i-Ph*), 169.8 (*C*=O), 183.5 (*C*(5)); *m/z* (ESI^+) 318 ($[M+H]^+$, 95%); HRMS (ESI^+) $C_{18}H_{23}NNaO_4^+$ ($[M+Na]^+$) requires 340.1519, found 340.1518.

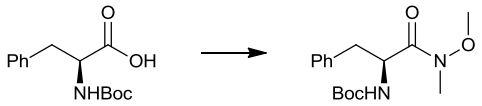
2,2-Diethoxycarbonyl-5-phenylethyl-pyrrolidine 239



238 **239**

Cyclisation product **238** (25 mg, 0.08 mmol) was dissolved in MeOH (8 mL) and passed through an H-Cube® using a Pd-C catalyst at a rate of 0.5 mL/min. The resulting solution was concentrated *in vacuo* to give **239** as a colourless oil (13 mg, 51%); ν_{max} (film) 3354 (N−H), 2936, 1732 (C=O); δ_H (500 MHz, $CDCl_3$) 1.28 (6H, t, *J* 7.1, OCH_2CH_3), 1.51-1.61 (1H, m, C(4)H_AH_B), 1.78-1.87 (1H, m, C(1′)H_AH_B), 1.97-2.09 (2H, m, C(4)H_AH_B, C(1′)H_AH_B), 2.27-2.35 (1H, m, C(3)H_AH_B), 2.51-2.58 (1H, m, C(3)H_AH_B), 2.63-2.76 (2H, m, C(2′)H_2), 3.39-3.48 (1H, m, C(5)*H*), 4.25 (4H, q, *J* 7.1, OCH_2CH_3), 7.17-7.22 (3H, m, *Ph*), 7.26-7.30 (2H, m, *Ph*); δ_C (125 MHz, $CDCl_3$) 14.0 (OCH_2CH_3), 30.8, 32.2, 33.2, 36.7 (*C*(3), *C*(4), *C*(1′), *C*(2′)), 59.7 (*C*(5)), 62.5 (OCH_2CH_3), 72.2 (*C*(2)), 125.9 (*p-Ph*), 128.3, 128.4 (*o,m-Ph*), 141.4 (*i-Ph*), 169.9 (*C*=O); *m/z* (ESI^+) 320 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{18}H_{26}NO_4^+$ ($[M+H]^+$) requires 320.1856, found 320.1857.

(*S*)-1-Phenyl-2-(*t*-butoxycarbonyl)amino-3-oxo-3-methoxy(methyl)aminopropane 240



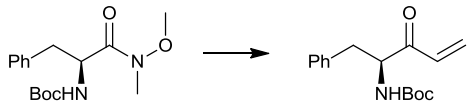
232 **240**

Method A: DCC (9.3 g, 45 mmol) and *N*,*O*-dimethylhydroxylamine hydrochloride **232** (4.4 g, 45 mmol) were added to a stirred solution of Boc-Phe-OH (10 g, 38 mmol) in DCM (70 mL). The solution was cooled to 0 °C and DMAP (220 mg, 1.8 mmol) and TEA (15.7 mL, 113 mmol) were added. The reaction mixture was allowed to warm to rt and stirred for 4 h.

The solid precipitate was filtered off and H_2O (50 mL) was added. The mixture was extracted with EtOAc (2 × 50 mL) and the combined organic layers were washed with brine (100 mL) before being dried and concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 5:1-1:1 petrol:EtOAc) gave amide **240** as a colourless viscous oil (780 mg, 89%, from purification of a 1 g portion of the crude material); R_f 0.2 (eluent 2:1 petrol:EtOAc); $[\alpha]_D^{23}$ + 23.1 (*c* 1.0 in $CHCl_3$) {lit.[11] $[\alpha]_D^{20}$ +29.0 (*c* 1.23 in $CHCl_3$)}; δ_H (400 MHz, $CDCl_3$) 1.37 (9H, s, C(C*H*$_3$)$_3$), 2.86 (1H, dd, *J* 13.4, 7.2, C(1)*H*$_A$H$_B$), 3.04 (1H, dd, *J* 13.4, 6.1, C(1)H$_A$*H*$_B$), 3.15 (3H, s, NC*H*$_3$), 3.63 (3H, s, NOC*H*$_3$), 4.87-5.01 (1H, m, C(2)*H*), 5.17-5.25 (1H, m, N*H*), 7.13-7.29 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 28.3 (C(*C*H$_3$)$_3$), 32.0 (N*C*H$_3$), 38.8 (*C*(1)), 51.5 (*C*(2)), 61.5 (NO*C*H$_3$), 79.5 (*C*Me$_3$), 126.7 (*p-Ph*), 128.3, 129.4 (*o*,*m-Ph*), 136.6 (*i-Ph*), 155.1 (*C*O$_2$*t*Bu), 172.3 (*C*ONMeOMe); *m/z* (ESI$^+$) 367 ([M+NH$_4$+MeCN]$^+$, 100%).

Method B: Boc-Phe-OH **232** (500 mg, 1.88 mmol) was dissolved in DCM (10 mL) and EDC (0.36 mL, 2.01 mmol) was added and the solution was cooled to 0 °C. A solution of $NH_2OH.HCl$ (195 mg, 2.01) and TEA (0.28 mL, 2.01 mmol) in DCM (10 mL) was added and the reaction mixture was allowed to warm to rt and stirred for a further 18 h. The DCM was removed *in vacuo* and the residue was redissolved in EtOAc (30 mL) then washed sequentially with 10% *w/v* aq. citric acid (10 mL, ~2 eq), 10% w/v aq. $NaHCO_3$ (30 mL) and brine (30 mL), dried and concenrated *in vacuo* to give **240** without further purification (262 mg, 57%).

(*S*)-1-Phenyl-2-(*t*-butoxylcabonyl)amino-3-oxo-pent-4-ene 231

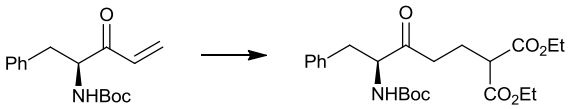


240 **231**

A stirred solution of amide **240** (1.2 g, 3.8 mmol) in dry Et_2O (20 mL) was cooled to −78 °C, followed by the dropwise addition of vinylmagnesium bromide (15.6 mL, 1.0 M in THF, 15.6 mmol). The solution was allowed to warm to −40 °C and stirred for 7 h before the addition of sat. aq. NH_4Cl (30 mL) and extraction with EtOAc (2 × 30 mL). The combined organic layers were washed with brine (40 mL), dried and concentrated *in vacuo* to give **231** as colourless oil (350 mg, 32%); R_f 0.5 (eluent 6:1 petrol:EtOAc); $[\alpha]_D^{23}$ + 80.1 (*c* 1.0 in $CHCl_3$);

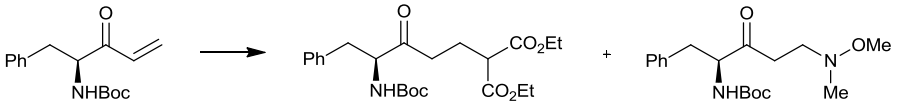
ν_{max} (film) 3352, 2978, 2934, 1705; δ_H (400 MHz, $CDCl_3$) 1.42 (9H, s, C(CH_3)$_3$), 2.99 (1H, dd, *J* 14.1, 5.5, C(1)H_AH_B), 3.15 (1H, dd, *J* 14.1, 6.4, C(1)H_AH_B), 4.84-4.90 (1H, m, C(2)*H*), 5.21-5.28 (1H, m, N*H*), 5.85 (1H, d, *J* 10.1, C(5)$H_{cis}H_{trans}$), 6.32-6.50 (2H, m, C(5)$H_{cis}H_{trans}$, C(4)*H*), 7.09-7.31 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 28.3 (C(*C*H_3)$_3$), 38.1 (*C*(1)), 58.2 (*C*(2)), 79.8 (*C*Me$_3$), 126.9, 128.5, 129.2, 129.4, 133.3 (*C*(4), *o,m,p-Ph*), 130.2 (*C*(5)), 135.9 (*i-Ph*), 155.1 (CO_2*t*Bu), 197.8 (C(3)); *m/z* (ESI^+) 334 ($[M+NH_4+MeCN]^+$, 85%); HRMS (ESI^+) $C_{16}H_{21}NNaO_3^+$ ($[M+H]^+$) requires 298.1414, found 298.1415.

(*S*)-1-Phenyl-2-(*t*-butoxycarbonyl)amino-6,6-diethoxycarbonyl-hex-3-one 241



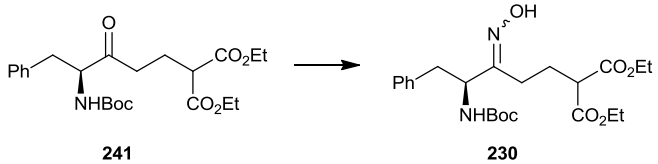
Diethylmalonate (0.19 mL, 1.27 mmol) was dissolved in DCM (7 mL) and K_2CO_3 (350 mg, 2.54 mmol) was added and the resulting mixture was stirred for 10 min at rt. After this time, a solution of ketone **231** (350 mg, 1.27 mmol) in DCM (5 mL) was added and the mixture was stirred for a further 18 h. The reaction mixture was filtered and then diluted with DCM (10 mL) and washed with H_2O (20 mL) before being dried and concetrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 10:1-3:1 40-60 petrol:EtOAc) gave **241** as a pale yellow oil (380 mg, 89%); R_f 0.2 (eluent 10:1 40-60 petrol:EtOAc); $[\alpha]_D^{23}$ + 20.2 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3379 (N−H), 2980, 1730 (C=O); δ_H (400 MHz, $CDCl_3$) 1.26 (6H, t, *J* 7.2, OCH_2CH_3), 1.40 (9H, s, C(CH_3)$_3$), 2.05-2.20 (2H, m, C(5)H_2), 2.43-2.60 (2H, m, C(4)H_2), 2.89-2.96 (1H, m, C(1)H_AH_B), 3.02-3.09 (1H, m, C(1)H_AH_B), 3.33 (1H, t, *J* 7.3, C(6)*H*), 4.14-4.23 (4H, m, OCH_2CH_3), 4.45-4.53 (1H, m, C(2)*H*), 5.09 (1H, br d, *J* 7.6, N*H*), 7.11-7.16 (2H, m, *Ph*), 7.21-7.32 (3H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 14.0 (OCH_2CH_3), 14.2 (C(*C*H_3)$_3$), 22.1 (*C*(5)), 37.6, 37.7 (*C*(1), *C*(4)), 50.6 (*C*(6)), 60.1 (*C*(2)), 61.5 (OCH_2CH_3), 79.9 (*C*Me$_3$), 127.0 (*p-Ph*), 128.6, 129.2 (*o,m-Ph*), 136.1 (*i-Ph*), 155.2 (*C*O_2*t*Bu), 169.0 (*C*O_2Et), 207.9 (*C*(3)); *m/z* (ESI^+) 458 ($[M+Na]^+$, 90%); HRMS (ESI^+) $C_{23}H_{33}NNaO_7^+$ ($[M+Na]^+$) requires 458.2149, found 458.2136.

(*S*)-1-Phenyl-2-(*t*-butoxycarbonyl)amino-5-(methoxy(methyl)amino)-pent-3-one 242



Diethylmalonate (0.66 mL, 8.8 mmol) was dissolved in DCM (10 mL) and K_2CO_3 (350 mg, 2.54 mmol) was added and the resulting mixture was stirred for 10 min at rt. After this time, a solution of crude ketone **231** (1.2 g, 4.4 mmol) in DCM (10 mL) was added and the mixture was stirred for a further 18 h. The reaction mixture was filtered and then diluted with DCM (10 mL) and washed with H_2O (20 mL) before being dried and concetrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 10:1-3:1 40-60 petrol:EtOAc) gave **241** as a pale yellow oil (344 mg, 30%); and **242** as a yellow oil (487, 32%); R_f 0.3 (eluent 3:1 40-60 petrol:EtOAc); $[\alpha]_D^{23}$ + 31.8 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3354 (N−H), 3063, 2979, 1741 (C=O); δ_H (400 MHz, $CDCl_3$) 1.38 (9H, s, C(C*H*$_3$)$_3$), 2.15 (3H, s, NC*H*$_3$), 2.58-2.74 (2H, m, C(4)*H*$_2$), 2.78-2.89 (2H, m, C(5)*H*$_2$), 2.90-2.99 (1H, m, (C(1)*H*$_A$H$_B$), 3.03-3.11 (1H, m, (C(1)H$_A$*H*$_B$), 3.39 (1H, s, OC*H*$_3$), 4.55 (1H, app. q, *J* 7.0, C(2)*H*), 5.20 (1H, d, *J* 7.6, N*H*), 7.12-7.30 (2H, m, *Ph*), 7.21-7.32 (3H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 28.3 (C(*C*H$_3$)$_3$), 37.4 (*C*(4)), 38.4 (*C*(1)), 44.9 (NC*H*$_3$), 54.7 (*C*(5)), 60.1 (*C*(2)), 60.3 (OC*H*$_3$), 79.7 (*C*Me$_3$), 126.9 (*p-Ph*), 128.6, 129.2 (*o,m-Ph*), 136.3 (*i-Ph*), 155.1 (*C*O$_2$*t*Bu), 207.9 (*C*(3)); *m/z* (ESI$^+$) 359 ([M+Na]$^+$, 60%); HRMS (ESI$^+$) $C_{18}H_{28}N_2NaO_4^+$ ([M+Na]$^+$) requires 359.1941, found 359.1939.

(*E/Z*)-(*S*)-1-Phenyl-2-(*t*-butoxycarbonyl)amino-6,6-diethoxycarbonyl-hex-3-hydroxyimine 230

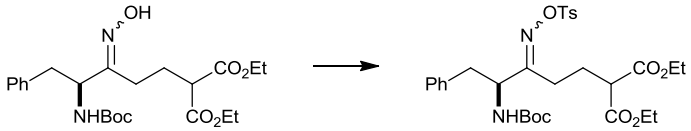


Ketone **241** (70 mg, 0.16 mmol), hydroxylamine hydrochloride (22 mg, 0.32 mmol) and TEA (0.06 mL, 0.4 mmol) were dissolved in EtOH and stirred at 85 °C for 18 h. The reaction mixture was cooled to rt and concentrated *in vacuo*. The residue was dissolved in DCM (10 mL) and washed with H_2O (10 mL) before being dried and concentrated *in vacuo*.

Purification *via* column chromatography (SiO_2, eluent 10:1-5:1 petrol:EtOAc) gave **230** as a white solid as a mixture of 3 isomers in roughly equal proportions (39 mg, 54%); R_f 0.2 (eluent 5:1 petrol:EtOAc); ν_{max} (film) 3374 (O−H), 2980, 1730 (C=O); δ_H (500 MHz, $CDCl_3$) 1.23-1.29 (6H, m, OCH_2CH_3), 1.30 (3H, s, $C(CH_3)_3$), 1.38 (5.5H, s, $C(CH_3)_3$), 1.91-2.18 (3H, m, C(5)H_2, C(4)H_2), 2.20-2.29 and 2.40-2.50 (1H, m, C(4)H_2), 2.66-2.74, 2.83-2.96, 2.98-3.10 and 3.14-3.20 (2H, m, 3 × C(1)H_2), 3.28-3.37 (1H, m, 3 × C(6)*H*), 4.10-4.25 (4H, m, OCH_2CH_3), 4.36-4.44, 4.53-4.62 and 4.75-4.84 (1H, m, 3 × C(2)*H*), 5.17 (0.35H, br d, *J* 8.51, N*H*), 5.44 (0.35H, br d, *J* 8.51, N*H*), 6.33 (0.3H, d, *J* 9.14, N*H*), 7.13-7.31 (5H, m, *Ph*), 8.16-8.39 (0.7H, m, 2 × NO*H*), 10.59 (0.3H, br s, NO*H*) ; δ_H (500 MHz, $CDCl_3$) 14.0, 14.1 (OCH_2CH_3), 24.5, 24.7 ($C(CH_3)_3$), 28.0, 28.3 (*C*(5), *C*(4)), 27.9, 39.4, 42.0 (*C*(1)), 51.1, 51.6, 51.7 (*C*(6)), 52.2, 54.3, 55.5 (*C*(2)), 61.4, 61.5 (OCH_2CH_3), 79.7, 79.8 (CMe_3), 126.6, 126.7, 128.3, 128.4, 128.5, 129.2, 129.5 (*o,m,p-Ph*), 136.9, 137.2, 137.4 (*i-Ph*), 155.0, 155.2 (CO_2tBu), 158.3 159.0, 159.1 (*C*(3)), 169.0, 169.1, 169.2 (CO_2Et) ; *m/z* (ESI^+) 473 ($[M+Na]^+$, 70%); HRMS (ESI^+) $C_{23}H_{34}N_2NaO_7^+$ ($[M+H]^+$) requires 473.2258, found 473.2257.

VT NMR at 343 K, a 68:32 dr: δ_H (500 MHz, C_6D_6) 0.95-0.99 (6H, m, $CO_2CH_2CH_3$), 1.35 (0.68 × 9H, s, $C(CH_3)_3$), 1.37 (0.32 × 9H, s, $C(CH_3)_3$), 2.06-2.17 (0.68 × 1H, m, C(3)H_AH_B-major), 2.20-2.36 (3H, m C(2)H_AH_B and C(3)H_AH_B-maj C(2)H_2 and C(3)H_2-minor), 2.50-2.71 (0.68 × 1H, m, C(2)H_AH_B-maj), 2.78-2.88 (1H, m, C(6)H_AH_B), 3.02-3.12 (C(6)H_AH_B), 3.37 (0.68 × 1H, t, *J* 6.9, C(1)*H*-maj), 3.43 (0.32 × 1H, t, *J* 7.1, C(1)*H*-min), 3.92-4.03 (4H, m, $CO_2CH_2CH_3$), 4.63-4.79 (0.68 × 1H, m, C(5)*H*-maj), 4.98-5,06 (0.32 × 1H, m, C(5)*H*-min), 5.35 (0.32 × 1H, d, *J* 8.5, N*H*-min); δ_H (500 MHz, C_6D_6) 13.7 ($CO_2CH_2CH_3$), 24.9, 25.2 (*C*(2)), 28.1, 28.1 ($C(CH_3)_3$), 30.5, 31.6 (*C*(3)), 38.2 (*C*(6)), 52.7, 54.1 (*C*(5)), 51.4, 51.9 (*C*(1)), 60.7, 60.8 ($CO_2CH_2CH_3$), 79.1 (CMe_3), 126.4, 126.5, 127.7, 127.8, 127.9, 128.0, 129.2, 129.6 (*o,m,p-Ph*), 137.7, 137.8 (*i-Ph*), 155.0 (CO_2tBu), 159.1 (*C*(4)), 168.7, 168.8 (CO_2Et).

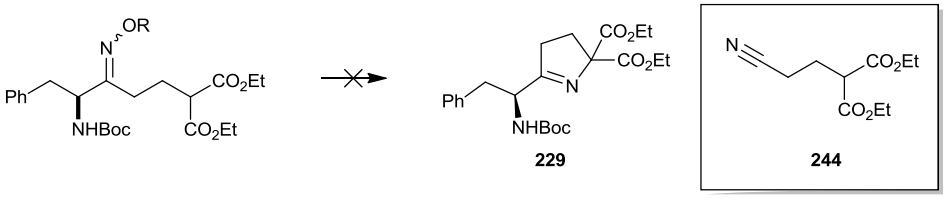
(*E*/*Z*)-(*S*)-1-Phenyl-2-(*t*-butoxycarbonyl)amino-6,6-diethoxycarbonyl-hex-3-hydroxyimine 243



230 **243**

Oxime **230** (110 mg, 0.26 mmol), and TEA (0.07 mL, 0.52 mmol) were dissolved in DCM (5 mL) and TsCl (55 mg, 0.28 mmol) was added. The mixture was stirred for 6 h before being diluted with DCM (15 mL) and washed with H_2O (15 mL) and sat. aq. $NaHCO_3$ (15 mL), dried and concentrated *in vacuo* to give the crude reaction mixture (160 mg) which was used without further purification.

Attempted synthesis of diethyl 5-(2′-phenyl-1′-(*t*-butoxycarbonyl)amino)ethyl-3,4-dihydro-2H-pyrrole-2,2-dicarboxylate 229



229 **244**

Method A: Oxime **230** (50 mg, 0.11 mmol) was dissolved in EtOH (5 mL) and Na (~10 mg) was added. The mixture was stirred for 15 min at rt, cooled to 0 °C and 1-fluoro-2,4-dinitrobenzene (25 mg, 0.13 mmol) was added. The mixture was then allowed to warm to rt and stirred for a further 30 min before being concentrated *in vacuo*. H_2O (10 mL) was added to the residue which was then extracted with EtOAc (2 × 10 mL) and the combined organic layers were dried and concetrated *in vacuo* to give a complex mixture of products.

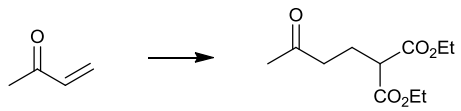
Method B: A solution of the crude **243** (160 mg, 0.26 mmol) in THF (5 mL) was added dropwise to a suspension of NaH (21 mg, 60% w.r.t. mineral oil, 0.53 mmol). The solution was warmed to rt and stirred for 6 h. The solution was concentrated *in vacuo* and the diluted with H_2O (10 mL) and extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with brine (30 mL), dried and concentrated *in vacuo*. Purification *via* column

chromoatography (SiO_2, eluent 2:1 petrol:EtOAc) gave a fraction with peaks which may be attributed to **229** (27 mg); R_f 0.3 (eluent 2:1 petrol:EtOAc).

Method C: A solution of the crude **243** (229 mg, 0.32 mmol) in DCM (5 mL) was cooled to 0 °C and DBU (0.12 mL, 0.8 mmol) was added. The reaction mixture was warmed to rt and stirred for 7 h. The mixture was diluted with sat. aq. NaHCO3 (20 mL) and extracted with DCM (3 × 15 mL). The combined organic layers were washed with brine (20 mL), dried and concentrated *in vacuo*. Purification *via* column chromatography gave 1,1-diethoxycarbonyl-3-cyanopropane **244**[12] as a colourless oil (50 mg, 73% from oxime); R_f 0.3 (eluent 6:1 petrol:EtOAc); δ_H (400 MHz, $CDCl_3$); 1.27 (6H, t, *J* 7.2, OCH_2CH_3), 2.23 (2H, app. q, *J* 7.2, C(2)H_2), 2.50 (2H, t, *J* 7.3, C(3)H_2), 3.49 (1H, t, *J* 7.2, C(1)*H*), 4.21 (4H, qd, *J* 7.2, 2.8, OCH_2); δ_C (100 MHz, $CDCl_3$) 14.0 (OCH_2CH_3), 15.1 (*C*(3)), 24.4 (*C*(2)), 50.1 (*C*(1)), 61.9 (OCH_2), 118.5 (*C*N), 168.1 (CO_2Et); *m/z* (ESI^+) 236 ($[M+Na]^+$, 100%).

4.2.4 Experimental for the modification of 2*H*-pyrroles

1,1-Diethoxycarbonyl-4-oxo-pentane 211[13]

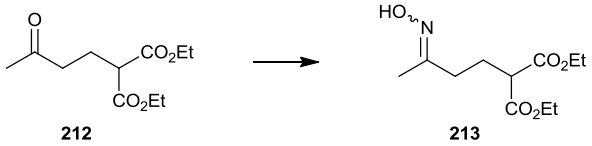


211 **212**

Diethyl malonate (16.2 mL, 107 mmol) was dissolved in DCM (70 mL) and K_2CO_3 (19.7 g, 142 mmol) was added and the resulting mixture was stirred for 10 min at rt. After this time, a solution of methylvinylketone **211** (5.95 mL, 71.3 mmol) in DCM (20 mL) was added and the reaction mixture was stirred for a further 4 d. The reaction mixture was filtered and then diluted with DCM (100 mL) and washed with H_2O (200 mL) before being dried and concetrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 10:1-3:1 petrol:EtOAc) gave **212**[13] as a colourless oil (13.3 g, 81%); R_f 0.15 (eluent 8:1 petrol:EtOAc); δ_H (400 MHz, $CDCl_3$) 1.26 (6H, t, *J* 7.2, $CO_2CH_2CH_3$), 2.11-2.17 (2H, m, C(2)H_2), 2.13 (3H, s, C(5)H_3), 2.54 (2H, t, *J* 7.3, C(3)H_2), 3.38 (1H, t, *J* 7.3, C(1)*H*), 4.19 (4H, q, *J* 7.2, $CO_2CH_2CH_3$); δ_C (100 MHz, $CDCl_3$) 14.0 ($CO_2CH_2CH_3$), 22.4 (*C*(2)), 29.9 (*C*(5)), 40.4

(*C*(3)), 50.7 (*C*(1)), 61.4 ($CO_2CH_2CH_3$), 169.1 (CO_2Et), 207.2 (*C*(4)); *m/z* (ESI^+) 253 ($[M+Na]^+$, 90%).

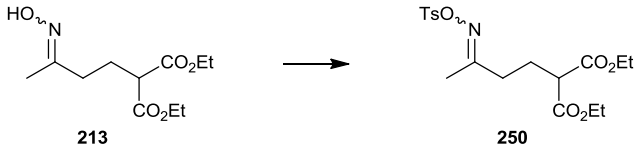
1,1-Diethoxycarbonyl-4-hydroxylimino-pentane 213[13]



212 **213**

Ketone **212** (6.97 g, 30.3 mmol), hydroxylamine hydrochloride (4.21 g, 60.6 mmol), and TEA (12.6 mL, 60.6 mmol) were dissolved in EtOH (40 mL). The mixture was heated to 85 °C and stirred for 7 h before the solvent was removed *in vacuo*. The residue was dissolved in DCM (100 mL) and washed with H_2O (2 × 80 mL), dried and concentrated *in vacuo* and after no further purification gave **213**[13] in a 3:1 dr as a colourless oil (7.42 g, quant); R_f 0.4 and 0.6 (eluent 2:1 petrol:EtOAc); ν_{max} (film) 3262 (O−H), 2983, 1734; δ_H (400 MHz, $CDCl_3$) 1.24-1.29 (6H, m, $CO_2CH_2CH_3$), 1.88 (3H, s, C(5)H_3), 2.07-2.17 (2H, m, C(2)H_2), 2.25 (0.75 × 2H, t, *J* 7.2, C(3)H_2 major), 2.42 (0.25 × 2H, t, *J* 7.9, C(3)H_2 minor), 3.34-3.48 (1H, m, C(1)*H*), 4.14-4.25 (4H, m, $CO_2CH_2CH_3$), 8.42 (1H, br s, O*H*); δ_C (100 MHz, $CDCl_3$) 13.5, 19.8 (C(5)), 14.0 ($CO_2CH_2CH_3$), 25.2 (*C*(2)), 26.3 (*C*(3) minor), 33.4 (*C*(3) major), 51.2 (*C*(1) major), 55.5 (*C*(1) minor), 61.4, 61.5 ($CO_2CH_2CH_3$), 157.0 (*C*(4)), 169.1 (CO_2Et); *m/z* (ESI^+) 268 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{11}H_{19}NNaO_5^+$ ($[M+Na]^+$) requires 268.1155, found 268.1154.

1,1-Diethoxycarbonyl-4-(tosyloxyimino)pentane 250

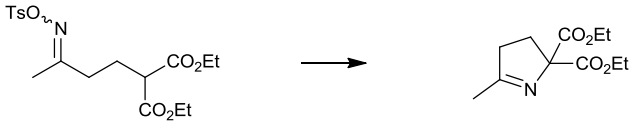


213 **250**

A solution of oxime **213** (3.0 g, 12.2 mmol) in DCM (20 mL) was cooled to 0 °C and TEA (4.26 mL, 30.4 mmol) was added. After stirring for 5 min tosyl chloride (4.65 g, 24.4 mmol) was added portionwise and the mixture was stirred at rt for 5 h. The mixture was then diluted with DCM (80 mL) and washed with H_2O (100 mL) and brine (50 mL), dried and concentrated in vacuo to give a crude mixture which was used without purification (6.3 g). Data for **250**; ν_{max} (film) 2983, 1731 (CO_2Et), 1370 (S=O), 1598; δ_H (400 MHz, $CDCl_3$)

1.29 (6H, t, *J* 7.1, $CO_2CH_2CH_3$), 1.93 (3H, s, C(5)H_2), 2.02-2.07 (2H, m, C(2)H_2), 2.41-2.47 (5H, m, C(3)H_2, Ph-CH_3), 3.31 (1H, t, *J* 7.3, C(1)*H*), 4.17-4.28 (4H, m, $CO_2CH_2CH_3$), 7.34 (2H, d, *J* 8.2, *m-Ph*), 7.85 (2H, d, *J* 8.2, *o-Ph*); δ_C (100 MHz, $CDCl_3$) 14.0 ($CO_2CH_2CH_3$), 19.7 (C(5)), 21.7 (*p*-CH_3), 24.4 (*C*(2)), 28.0 (*C*(3)), 51.2 (*C*(1)), 61.7 ($CO_2CH_2CH_3$), 128.8, 129.6 (*o,m-Ph*), 132.7 (*p-Ph*), 144.9 (*i-Ph*), 166.6, 168.6 (CO_2Et, *C*(4)); *m/z* (ESI^+) 422 ($[M+Ma]^+$, 70%); HRMS (ESI^+) $C_{18}H_{25}NNaO_7S^+$ ($[M+Na]^+$) requires 422.1244, found 422.1243.

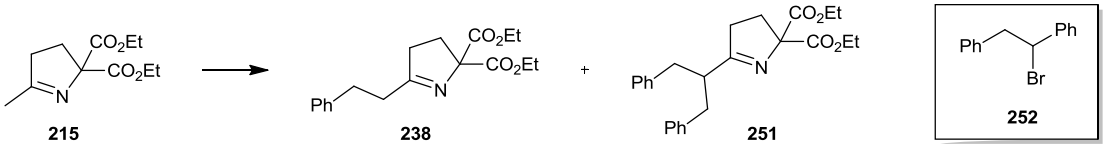
2,2-Diethoxycarbonyl-5-methyl-5-pyrroline 215



250 **215**

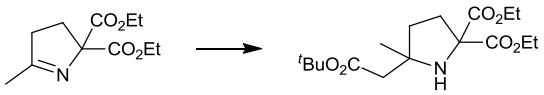
A solution of **250** (1.07 g portion of crude mixture, 1.94 mmol) in DCM (20 mL) was cooled to 0 °C and DBU (0.8 mL, 5.0 mmol) was added. The solution was warmed to rt and stirred for 7 h. The solution was then diluted with sat. aq. $NaHCO_3$ (30 mL) and extracted with DCM (2 × 20 mL). The combined organic layers were washed with brine (30 mL), dried and concentrated *in vacuo*. Purification *via* column chromoatography (SiO_2, eluent 2:1 petrol:EtOAc) gave **215**[13] as a bright yellow oil (269 g, 61% from ketone **212**); R_f 0.15 (eluent 2:1, petrol:EtOAc); ν_{max} (film) 3456, 2983, 1732, 1644; δ_H (400 MHz, $CDCl_3$) 1.28 (6H, t, *J* 7.1, $CO_2CH_2CH_3$), 2.15 (3H, s, C(5)CH_3), 2.46 (2H, t, *J* 7.8, C(3)H_2), 2.68 (2H, t, *J* 7.8, C(4)H_2), 4.20-4.30 (4H, m, $CO_2CH_2CH_3$); δ_C (100 MHz, $CDCl_3$) 14.0 ($CO_2CH_2CH_3$), 19.9 (C(5)CH_3), 30.4 (*C*(3)), 39.5 (*C*(4)), 61.9 ($CO_2CH_2CH_3$), 86.6 (*C*(2)), 169.9 (CO_2Et), 180.9 (*C*(4)); *m/z* (ESI^+) 228 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{11}H_{17}NNaO_4^+$ ($[M+Na]^+$) requires 250.1050, found 250.1052.

2,2-diethoxycarbonyl-3,4-dihydro-5-phenylethyl-2H-pyrrole 238 and 2,2-Diethoxycarbonyl-5-dibenzylmethyl-5-pyrroline 251



A solution of diisopropylamine (0.07 mL, 0.48 mmol) in THF (2 mL) was cooled to −78 °C and BuLi (0.69 mL, 1.45 M in hexane, 1.0 mmol) was added dropwise. The resulting solution was stirred at −78 °C for 50 min. After this time, a solution of **215** (100 mg, 0.44 mmol) in THF (1 mL) was added dropwise, and the mixture was stirred for a further 1 h at −78 °C. After this time, BnBr (0.1 mL, 0.88 mmol) was added and the mixture stirred for a further 2 h at −78 °C. The reaction was quenched with 2:1 sat. aq. NH_4Cl:MeOH (15 mL) at −78 °C, and the mixture extracted with Et_2O (3 × 10 mL). The combined organic layers were washed with brine (20 mL), dried and concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 8:1 to 2:1 petrol:EtOAc) gave **252** as a pale yellow oil (100 mg, 43% from BnBr); δ_H (400 MHz, $CDCl_3$) 3.51-3.65 (2H, m, PhCH_2), 5.20 (1H, t, *J* 7.6, C*H*Br), 7.23-7.61 (10H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 46.5 (PhCH_2), 55.5 (PhC*H*Br), 126.6, 126.9, 127.7, 128.4, 128.7, 128.8, 128.9, 129.1, 129.3 (*o,m,p-Ph*), 138.1, 141.6 (*i-Ph*); **251** as a pale yellow oil (25 mg, 14%); R_f 0.1 (eluent 4:1 petrol:EtOAc); ν_{max} (film) 3062, 2038, 2981, 1738, 1633; δ_H (100 MHz, $CDCl_3$) 1.29 (6H, t, *J* 7.1, $CO_2CH_2CH_3$), 2.15-2.22 (4H, m, C(3)H_2, C(4)H_2), 2.90-2.96 (2H, m, CH_2Ph), 2.98-3.04 (2H, m, CH_2Ph), 3.12-3.20 (1H, m, C*H*Bn_2), 4.23 (4H, q, *J* 7.1, $CO_2CH_2CH_3$), 7.14-7.26 (10H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 14.1 ($CO_2CH_2CH_3$), 29.6 (*C*(3)), 38.6 (*C*(4)), 39.8 (Ph*C*H_2), 46.5 (*C*HBn_2), 61.7 ($CO_2CH_2CH_3$), 86.3 (*C*(2)), 126.2, 126.4, 128.3, 128.4, 128.9, 129.0 (*o,m,p-Ph*), 139.5, 139.6 (*i-Ph*), 169.6 (CO_2Et), 186.7 (*C*(5)); *m/z* (ESI^+) 408 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{25}H_{29}NNaO_4^+$ ($[M+H]^+$) requires 430.1989, found 430.1988; and **238** as a pale oil (18 mg, 13%); data as previously.

2,2-Diethoxycarbonyl-5-methyl-5-(*tert*-butoxycarbonyl)methylpyrrolidine 255



215 **255**

Zinc powder was activated by stirring it with sat. aq. NH_4Cl for 15 min followed by decantation and successive washing with H_2O, EtOH and Et_2O, and drying *in vacuo* for 30 min. A suspension of activated zinc powder (360 mg, 5.51 mmol) and I_2 (28 mg, 0.22 mmol) in THF (20 mL) was placed in a sonic bath heated to 40 °C, and *t*-butyl bromoacetate (0.48 mL, 3.30 mmol) was added. The mixture was then sonicated for 20 min at 40 °C. The mixture was then stirred at rt for 30 min before a solution of imine **215** (250 mg, 1.1 mmol) was added and the mixture was sonicated for 30 min and 40 °C, then stirred for 30 min at rt. The reaction mixture was diluted with iced water (5 mL) and stirred for 5 min when sat. aq. NH_4Cl was added until all the precipitate had dissolved. The mixture was extracted with EtOAc (3 × 20 mL), dried and concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 5:1 petrol:EtOAc) gave **255** as a pale yellow oil (77 mg, 20 %); R_f 0.75 (eluent 3:1 petrol:EtOAc); ν_{max} (film) 3355, 2979, 1734; δ_H (400 MHz, $CDCl_3$) 1.20-1.30 (9H, m, $CO_2CH_2CH_3$, C(5)CH_3), 1.44 (9H, s, $C(CH_3)_3$), 1.62-1.74 (1H, m, C(4)H_AH_B), 1.86-1.96 (1H, m, C(4)H_AH_B), 2.27-2.49 (4H, m, C(3)H_2, CH_2CO_2tBu), 4.12-4.25 (4H, m, $CO_2CH_2CH_3$); δ_C (100 MHz, $CDCl_3$) 14.0 ($CO_2CH_2CH_3$), 27.0 (C(5)CH_3), 28.1 ($C(CH_3)_3$), 32.1 (*C*(3)), 37.5 (*C*(4)), 47.5 (CH_2CO_2tBu), 61.6 ($CO_2CH_2CH_3$), 72.2 (*C*(5)), 80.4 (CMe_3), 166.5 (CO_2tBu), 170.9, 171.8 (CO_2Et); *m/z* (ESI^+) 344 ($[M+H]^+$, 70%); HRMS (ESI^+) $C_{17}H_{29}NNaO_6^+$ ($[M+H]^+$) requires 366.1887, found 366.1887.

4.3 Experimental for chapter 3

4.3.1 General procedures

General procedure D: Synthesis of *N*-acyl-oxazolidines by amide coupling

A solution of (4*S*,5*R*)-2-*tert*-Butyl-4-methoxycarbonyl-5-methyloxazolidine **320** (1.0 eq.) in DCM (3 mL/mmol of **320**) was cooled to 0 °C before DCC (1.05 eq.) and DMAP (7 mol%) were added. A solution of the carboxylic acid (1.05 eq.) in DCM (1 mL/mmol of acid) was added and the mixture was stirred at 0 °C for 15 min and then at rt for 3-5 h. The mixture was then filtered, and the filtrate was concentrated *in vacuo* to give the crude product mixture.

General Procedure E: Acylation of Meldrum's acid

Pyridine (2.0 eq.) was added dropwise to a solution of Meldrum's acid (1.0 eq.) in DCM (10 mL/mmol of Meldrum's acid) at −10 °C and the resulting mixture was stirred for 20 min. A solution of acid chloride (1.0 eq.) in DCM (0.5 mL/mmol of acid chloride) was added dropwise and the resulting mixture was stirred for 1 h at −10 °C before being warmed to rt and stirred for a further 2 h. The mixture was quenched with 1M HCl (1 mL/mL DCM) and extracted with DCM (3×). The combined organic layers were washed with brine (50 mL), dried and concentrated *in vacuo* to give the crude product which was used without further purification.

General procedure F: Aldol cyclisation of *N*-acyl-oxazolidines

An amount of NaOMe (1.1 eq.) was added portionwise to a stirred solution of *N*-acyl-oxazolidine (1.0 eq.) in MeOH (10 mL/mmol of oxazolidine) and the resulting mixture was stirred at rt for 24 h. After this time, the mixture was partitioned between Et_2O (2 mL/mL MeOH) and sat. aq. NH_4Cl (2 mL/mL MeOH), and the aqueous layer was extracted with Et_2O (2×) before the combined organic layers were washed with brine (0.5 mL/mL Et_2O), dried and concentrated *in vacuo* to give the crude product mixture.

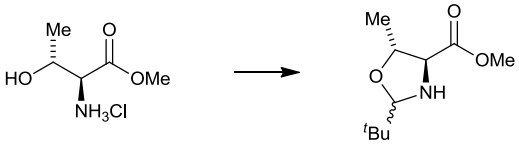
4.3.2 Experimental for tetramic acid mimics derived from L-threonine

L-Threonine methyl ester hydrochloride 319[14]

319

A flask of stirred MeOH (20 mL) was cooled to 0 °C and $SOCl_2$ (1.67 mL, 23 mmol) was added dropwise, followed by the portionwise addition of L-threonine (1.82 g, 15.3 mmol). The mixture was heated to 40 °C and stirred for 18 h. The mixture was then cooled and concentrated *in vacuo* to give **319** as a colourless gum (2.6 g, quant); $[\alpha]_D^{23}$ −2.9 (*c* 1.75 in EtOH) {lit.[15] $[\alpha]_D^{29}$ −14.9 (*c* 2.1 in H_2O)}; δ_H (400 MHz, MeOD) 1.34 (3H, d, *J* 6.6, C*H*Me), 3.86 (3H, s, OCH_3), 3.94 (1H, d, *J* 4.0, C*H*NH_3Cl), 4.26-4.33 (1H, m, C*H*OH); δ_C (100 MHz, MeOD) 19.5 (CH*C*H_3), 52.7 (O*C*H_3), 58.8 (*C*HNH_3Cl), 65.3 (*C*HOH), 168.6 (*C*O_2Me).

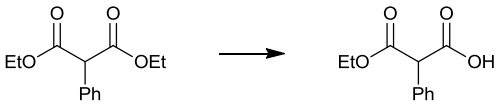
(4*S*,5*R*)-2-*tert*-Butyl-4-methoxycarbonyl-5-methyloxazolidine 320[14]



319 **320**

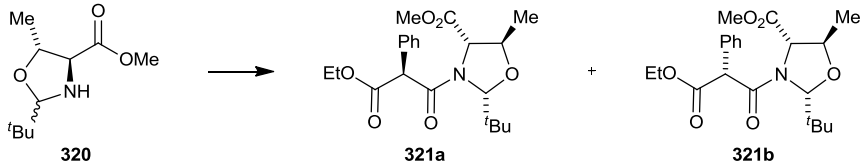
Pivalaldehyde (3.57 mL, 32.9 mmol) and TEA (3.6 mL, 26.3 mmol) were added to a stirred suspension of **319** (2.6g, 15.3 mmol) in 40-60 petrol (100 mL). The mixture was stirred at reflux for 18 h in a flask fitted with a Dean-Stark apparatus. The mixture was then cooled to rt and filtered. The residue was washed with petrol (3 × 30 mL) and the combined filtrate was concentrated *in vacuo* to give oxazolidine **320** as a colourless oil in a 1:1 dr (3.07 g, quant); δ_H (400 MHz, $CDCl_3$) 0.90 and 0.97 (9H, s, C(CH_3)$_3$), 1.33 and 1.37 (3H, d, *J* 6.1, C(5)CH_3), 2.80 (1H, br s, N*H*), 3.35 and 3.46 (1H, d, *J* 7.3 and 7.1, C(4)*H*), 3.75 and 3.77 (3H, s, CO_2CH_3), 3.78-3.88 (1H, m, C(5)*H*), 4.29 and 4.38 (1H, s, C(2)*H*); δ_C (100 MHz, $CDCl_3$) 18.9, 19.9 (C(5)*C*H_3), 24.8, 25.2 (C(*C*H_3)$_3$), 52.3, 52.4 (CO_2*C*H_3), 65.7, 67.2 (*C*(4)), 76.2, 77.3 (*C*(5)), 98.1, 98.8 (*C*(2)), 171.9, 172.6 (*C*O_2Me); *m/z* (ESI^+) 202 ($[M+H]^+$, 100%).

2-Ethyoxycarbonyl-2-phenylacetic acid[14]



A solution of diethyl phenylmalonate (9.13 mL, 42.3 mmol) in EtOH (50 mL) was cooled to 0 °C and KOH (2.37 g, 42.3 mmol) was added. The mixture was stirred at 10-20 °C for 5 h, and then stored in the fridge for 18 h. The mixture was concentrated *in vacuo* at 15-20 °C, the residue was dissolved in H_2O (30 mL) and extracted with Et_2O (3 × 20 mL). The aqueous layer was acidified with 1 M HCl and extracted with Et_2O (3 × 20 mL). The combined organic layers from the acidified extractions only were washed with brine (50 mL), dried and concentrated *in vacuo* to give the product as a white solid (5.3 g, 60%); mp 57-60 ºC; {lit.[16] mp 76-77 °C}; δ_H (400 MHz, $CDCl_3$) 1.27 (3H, t, *J* 7.2, $CO_2CH_2CH_3$), 4.19-4.29 (2H, m, $CO_2CH_2CH_3$), 4.66 (1H, s, C(2)*H*), 7.35-7.43 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 13.9 ($CO_2CH_2CH_3$), 57.5 (*C*(2)), 62.2 ($CO_2CH_2CH_3$), 128.5, 128.8, 129.2, 132.2 (*Ph*), 168.3 (CO_2Et), 173.3 (CO_2H); *m/z* (ESI^+) 231 ($[M+Na]^+$, 70%).

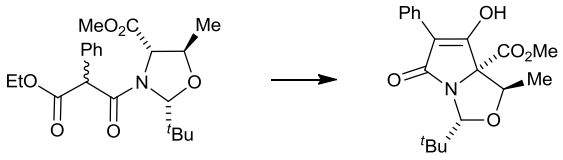
(2*R*,4*S*,5*R*,2′*S*)- and (2*R*,4*S*,5*R*,2′*R*)-2-*tert*-Butyl-3-((2′*S*)-2′-ethoxycarbonyl-2′-phenylethanoyl)-4-methoxycarbonyl-5-methyloxazolidine 321a and 321b[14]



Following **General procedure D** for the synthesis of *N*-acyloxazolidines, (4*S*,5*R*)-2-*t*-butyl-4-methoxycarbonyl-5-methyloxazolidine **320** (1.00 g, 4.97 mmol) was reacted with DCC (1.07 g, 5.22 mmol), DMAP (43 mg, 7 mol%) and the 2-ethyoxycarbonyl-2-phenylacetic acid (1.09 g, 5.22 mmol). Purification *via* column chromatography (SiO_2, eluent 6:1 40-60 petrol:EtOAc) gave **321a** as a white solid (377 mg, 19%); R_f 0.28 (eluent 6:1 petrol:EtOAc); mp 118-120 ºC; {lit.[14] mp 130-132 °C}; $[\alpha]_D^{23}$ +47.8 (*c* 1.0 in $CHCl_3$); {lit.[14] $[\alpha]_D^{22}$ −43.4 (*c* 2.0 in $CHCl_3$)}; δ_H (400 MHz, $CDCl_3$) 0.80 (3H, d, *J* 6.3, C(5)C*H*$_3$), 0.93 (9H, s, C(C*H*$_3$)$_3$), 1.28 (3H, t, *J* 7.1, $CO_2CH_2CH_3$), 3.87 (3H, s, CO_2CH_3), 4.05 (1H, d, *J* 2.8, C(4)*H*), 4.14-4.32 (4H, m, $CO_2CH_2CH_3$), 4.68 (1H, qd, *J* 6.3, 2.8, C(5)*H*), 4.90 (1H, s, C*H*Ph), 5.40 (1H, s, C(2)*H*), 7.33-7.40 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 14.0 ($CO_2CH_2CH_3$), 19.2 (C(5)CH_3), 25.7

($C(CH_3)_3$), 38.6 (*C*Me_3), 52.9 (OCH_3), 58.2 (*C*HPh), 61.7 ($CO_2CH_2CH_3$), 64.4 (*C*(4)), 75.7 (*C*(5)), 95.9 (*C*(2)), 128.6, 129.1, 129.3 (*o,m,p-Ph*), 168.2, 169.0, 170.0 (CO_2Et, CO_2Me, *C*(1′)); *m/z* (ESI^+) 805 ($[2M+Na]^+$, 100%); a mixture of **321a** and **321b** (730 mg, 38%), and **321b** as a colourless oil (96 mg, 5%); R_f 0.22 (eluent 6:1 40-60 petrol:EtOAc); $[\alpha]_D^{23}$ −6.03 (*c* 1.0 in $CHCl_3$); {lit.[14] $[\alpha]_D^{22}$ +87 (*c* 2.0 in $CHCl_3$)}; δ_H (400 MHz, $CDCl_3$) 0.82 (9H, s, $C(CH_3)_3$), 1.27 (3H, t, *J* 7.2, $CO_2CH_2CH_3$), 1.39 (3H, d, *J* 6.1, C(5)CH_3), 3.75 (3H, s, OCH_3), 4.23 (2H, q, *J* 7.2, $CO_2CH_2CH_3$), 4.49 (1H, d, *J* 2.8 C(4)*H*), 4.80-4.87 (1H, m, C(5)*H*), 4.92 (1H, s, C*H*Ph), 5.51 (1H, m, C(2)*H*), 7.31-7.42 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 14.1 ($CO_2CH_2CH_3$), 19.9 (C(5)CH_3), 25.7 ($C(CH_3)_3$), 37.7 (*C*Me_3), 52.7 (OCH_3), 57.3 (*C*HPh), 62.0 ($CO_2CH_2CH_3$), 65.6 (*C*(4)), 76.0 (*C*(5)), 96.0 (*C*(2)), 129.0, 128.3, 128.6, 129.7 (*o,m,p-Ph*), 133.0 (*i-Ph*), 168.4, 169.5, 170.0 (CO_2Et, CO_2Me, *C*(1′)); *m/z* (ESI^+) 805 ($[2M+Na]^+$, 100%).

(2*R*,4*R*,5*R*)-1-Aza-2-(*t*-butyl)-6-hydroxy-5-methoxycarbonyl-4-methyl-8-oxo-7-phenyl-3-oxabicyclo[3.3.0]oct-6-ene 322[14]

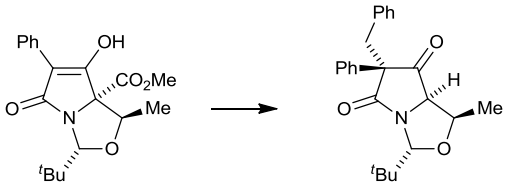


321a/b **322**

To a solution of *N*-acyloxazolidine **321** (4.00 g, 10.2 mmol) in THF (70 mL) was added KO*t*Bu (1.25 g, 11.2 mmol) and mixture was heated to reflux for 7 h. The mixture was then cooled to rt and partitioned between Et_2O (50 mL) and water (50 mL). The aqueous layer was acidified with 1 M HCl and extracted with Et_2O (2 x 50 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 4:1 EtOAc:MeOH) gave **322** as a white solid (3.75 g, 95%); R_f 0.25 (eluent 2:1 petrol:EtOAc); mp 95-95 ºC; $[\alpha]_D^{23}$ +130 (*c* 1.0 in $CHCl_3$); {lit.[14] $[\alpha]_D^{20}$ +87.2 (*c* 1.2 in MeOH)}; δ_H (400 MHz, $CDCl_3$) 0.94 (9H, s, $C(CH_3)_3$), 1.07 (3H, d, *J* 6.7, C(4)CH_3), 3.80 (3H, s, OCH_3), 4.83 (1H, s, C(2)*H*), 5.06 (1H, q, *J* 6.7, C(4)*H*), 7.26-7.42 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 14.5 (C(4)CH_3), 24.8 ($C(CH_3)_3$), 35.2 (*C*Me_3), 53.3

(OCH_3), 74.6 (*C*(4)), 76.6 (*C*(5)) 94.3 (*C*(2)), 110.0 (*C*(7)), 127.9, 128.0, 128.6, 129.0, 129.5 (*Ph*), 164.3 (CO_2Me), 169.9 (*C*(6)), 177.2 (*C*(8)); *m/z* (ESI^+) 344 ($[M-H]^-$, 100%).

(2*R*,4*R*,5*R*,7*S*)-1-Aza-2-(*t*-butyl)-4-methyl-6,8-dioxo-7-phenyl-7-benzyl-3-oxabicylco[3.3.0]octane 324a



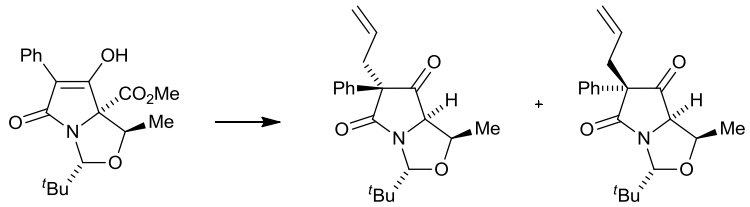
322 **324a**

Method A: A solution of the tetramic acid **322** (100 mg, 0.29 mmol) in THF (2 mL) was added dropwise to a stirred suspension of NaH (30 mg, 60% dispersion in paraffin, 0.73 mmol) in THF (10 mL) at 0 °C. The mixture was stirred at rt for 40 min, before the addition of BnBr (64 mg, 0.38 mmol). The mixture was then heated to 50 °C for 24 h, before being cooled to rt and partitioned between Et_2O (20 mL) and H_2O (10 mL). The organic layer was washed with brine (10 mL), dried and concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 50:1 to 30:1 40-60 petrol:EtOAc) gave **324a** as a colourless oil (55 mg, 50%); R_f 0.23 (eluent 40:1 40-60 petrol:EtOAc); $[\alpha]_D^{23}$ +205 (*c* 0.5 in $CHCl_3$); ν_{max} (film) 2971, 1763, 1707; δ_H (400 MHz, $CDCl_3$) 0.70 (9H, s, $C(CH_3)_3$), 0.73 (3H, d, *J* 6.6, C(4)CH_3), 3.41 (1H, d, *J* 12.6, CH_AH_BPh), 3.50 (1H, d, *J* 7.1, C(5)*H*), 3.52 (1H, d, *J* 12.6, CH_AH_BPh), 4.29-4.37 (1H, m, C(4)*H*), 5.12 (1H, s, C(2)*H*), 7.21-7.43 (8H, m, *Ph*), 7.72-7.77 (2H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 16.9 (C(4)CH_3), 24.4 ($C(CH_3)_3$), 36.5 (CMe_3), 47.0 (CH_2Ph), 65.5 (*C*(7)), 70.1 (*C*(5)), 73.4 (*C*(4)), 94.3 (*C*(2)), 127.2, 127.4, 127.6, 127.7, 127.8, 128.5, 128.6, 128.7, 128.9, 130.0, 130.5, 134.8 (*Ph*), 174.9 (*C*(6)), 208.8 (*C*(8)); *m/z* (ESI^+) 400 ($[M+Na]^+$, 80%); HRMS (ESI^+) $C_{24}H_{27}NNaO_3^+$ ($[M+Na]^+$) requires 400.1883, found 400.1882.

Method B: A solution of the tetramic acid **322** (200 mg, 0.60 mmol) in THF (2 mL) was added dropwise to a stirred suspension of NaH (60 mg, 60% dispersion in paraffin, 1.45 mmol) in THF (20 mL) at 0 °C. The mixture was stirred at rt for 40 min, before the addition of BnI (252 mg, 1.2 mmol). The mixture was then heated to 50 °C for 24 h, diluted with

NH_4Cl (5 mL) and the THF removed *in vacuo*. The mixture was then partitioned between Et_2O (30 mL) and NH_4Cl (15 mL). The organic layer was washed with brine (10 mL), dried and concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 50:1 to 30:1 40-60 petrol:EtOAc) gave **324a** as a colourless oil (74 mg, 33%).

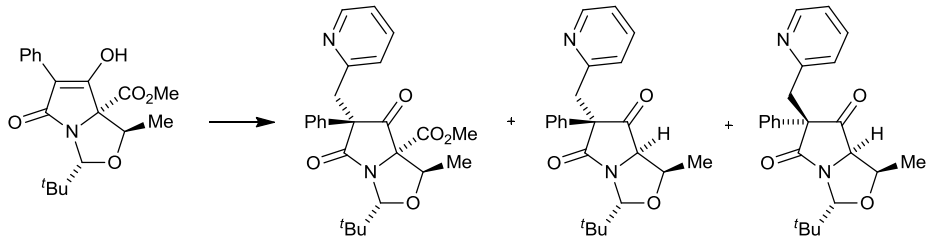
(2*R*,4*R*,5*R*,7*S*)- and (2*R*,4*R*,5*R*,7*R*)-1-Aza-2-(*t*-butyl)-4-methyl-6,8-dioxo-7-phenyl-7-allyl-3-oxabicyclo[3.3.0]octane 324b



A solution of the tetramic acid **322** (200 mg, 0.60 mmol) in THF (2 mL) was added dropwise to a stirred suspension of NaH (60 mg, 60% dispersion in paraffin, 1.44 mmol) in THF (10 mL) at 0 °C. The mixture was stirred at rt for 40 min, before the addition of allyl bromide (92 mg, 0.76 mmol). The mixture was then heated to 50 °C for 18 h, diluted with NH_4Cl (5 mL) and the THF removed *in vacuo*. The mixture was then partitioned between EtOAc (20 mL) and NH_4Cl (10 mL). The organic layer was washed with brine (10 mL), dried and concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 50:1 to 8:1 petrol:EtOAc) gave **324b** in a 73:27 dr as a colourless oil (60 mg, 31%); R_f 0.6 (eluent 8:1 40-60 petrol:EtOAc); ν_{max} (film) 2967, 1765, 1710; δ_H (400 MHz, $CDCl_3$) 0.81 (3H × 0.73, d, *J* 6.6, C(4)CH_3 major), 0.99 (9H, s, C(CH_3)$_3$), 1.40 (3H × 0.27, d, *J* 5.8, C(4)CH_3 minor), 2.90 (2H × 0.73, d, *J* 7.6, C(1′)H_2 maj), 2.93 (2H × 0.27, d, *J* 7.3, C(1′)H_2 min), 3.63 (1H × 0.27, d, *J* 9.4, C(5)*H* min), 3.67-3.75 (1H × 0.27, m, C(4)*H* min), 4.23 (1H × 0.73, d, *J* 6.8, C(5)*H* maj), 4.52 (1H × 0.73, app. quint., *J* 6.6, C(4)*H* maj), 5.15-5.24 (2H, m, C(3′)H_2), 5.15-5.24 (1H × 0.27, m, C(2)*H* min), 5.25 (1H × 0.73, s, C(2)*H* maj), 5.72-5.83 (1H, m, C(2′)*H*), 7.19-7.41 (3H, m, *Ph*), 7.57-7.60 (2H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 16.8 (C(4)*C*H$_3$ maj), 17.3 (C(4)*C*H$_3$ min), 24.9 (C(*C*H$_3$)$_3$), 36.0, 36.6 (*C*Me$_3$), 41.7 (*C*(1′) min), 44.4 (*C*(1′) maj), 63.6 (*C*(7′)), 70.2 (*C*(5) maj), 72.5 (*C*(5) min), 73.7 (*C*(4) maj), 74.7 (*C*(4) min), 94.5 (*C*(2) maj), 96.0 (*C*(2) min), 120.7 (*C*(2′)), 127.2, 127.4, 127.8, 128.0, 128.5, 128.8, 131.0 (*o,m,p-Ph*)

134.2, 135.2 (*i-Ph*), 175.5, 176.4 (*C*(8)), 206.7, 207.7 (*C*(6)); *m/z* (ESI^+) 350 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{20}H_{25}NNaO_3^+$ ($[M+Na]^+$) requires 350.1727, found 350.1721.

(2*R*,4*R*,5*R*,7*S*)-1-Aza-2-(*t*-butyl)-4-methyl-5-methoxycarbonyl-6,8-dioxo-7-phenyl-7-(pyridine-2-methyl-3-oxabicylco[3.3.0]octane 323b, (2*R*,4*R*,5*R*,7*S*)- and (2*R*,4*R*,5*R*,7*R*)-1-Aza-2-(*t*-butyl)-4-methyl-6,8-dioxo-7-phenyl-7-(pyridine-2-methyl-3-oxabicylco[3.3.0]octane 324b

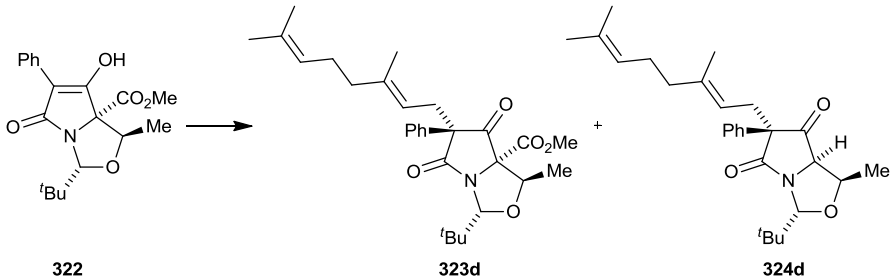


322 **323b** **324b** major **324b** minor

A solution of the tetramic acid **322** (100 mg, 0.29 mmol) in THF (2 mL) was added dropwise to a stirred suspension of NaH (30 mg, 60% dispersion in paraffin, 0.73 mmol) in THF (10 mL) at 0 °C. The mixture was stirred at rt for 40 min, before the addition of TEA (0.05 mL, 0.38 mmol) and 2-(bromomethyl)pyridine hydrobromide (95 mg, 0.38 mmol). The mixture was then heated to 50 °C for 24 h, before being cooled to rt and partitioned between EtOAc (20 mL) and H_2O (10 mL). The organic layer was washed with brine (10 mL), dried and concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 4:1 to 40-60 petrol:EtOAc) gave **323b** as a yellow oil (9 mg, 7.5%); R_f 0.2 (eluent 4:1 40-60 petrol:EtOAc); $[\alpha]_D^{23}$ +108 (*c* 0.27 in $CHCl_3$); ν_{max} (film) 2961, 1717, 1591, 1574; δ_H (500 MHz, $CDCl_3$) 0.68 (3H, d, *J* 6.9, C(4)CH_3), 0.95 (9H, s, C(CH_3)$_3$), 3.69 (3H, s, CO_2CH_3), 3.74 (2H, AB-q, *J* 14.2, CH_2Py), 5.05 (1H, q, *J* 6.6, C(4)*H*), 5.16 (1H, s, C(2)*H*), 6.89-6.92 (1H, m, *o-Py*), 7.09-7.13 (1H, m, *p-Py*), 7.28-7.36 (3H, m, *m,p-Ph*), 7.46-7.50 (1H, m, *m-Py*), 7.69-7.72 (2H, m, *o-Ph*), 8.44-8.46 (1H, m, α-*Py*); δ_C (125 MHz, $CDCl_3$) 15.9 (C(4)CH_3), 25.0 (C(CH_3)$_3$), 35.5 (CMe_3), 48.0 (CH_2Py), 53.6 (CO_2CH_3), 61.5 (*C*(7)), 76.7 (*C*(4)), 81.7 (*C*(5)), 97.6 (*C*(2)), 122.1 (*p-Py*), 124.3 (*o-Py*), 127.7 (*o-Ph*), 127.9, 128.5 (*m,p-Ph*), 133.5 (*i-Ph*), 135.8 (*m-Py*), 149.0 (α-*Py*), 154.9 (*i-Py*), 167.5 (CO_2Me), 177.3 (*C*(8)), 202.1 (*C*(6)); *m/z* (ESI^+) 437 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{25}H_{28}N_2NaO_5^+$ ($[M+Na]^+$) requires 459.1890, found 459.1890; and **324b** in 65:35 dr as a pale yellow oil (14 mg, 13 %); R_f 0.25 (eluent 4:1

40-60 petrol:EtOAc); ν_{max} (film) 3060, 2969, 1765, 1705, 1592, 1570; δ_H (500 MHz, $CDCl_3$) 0.66 (3H × 0.65, d, *J* 6.6, C(4)CH_3 major), 0.76 (9H × 0.65, s, C(CH_3)$_3$ major), 0.82 (9H × 0.35, s, C(CH_3)$_3$ minor), 1.37 (3H × 0.35, d, *J* 5.7, C(4)CH_3 min), 3.53 (1H × 0.65, d, *J* 15.0, CH_AH$_B$Py maj), 3.60 (1H × 0.35, d, *J* 15.0, CH_AH$_B$Py min), 3.89 (1H × 0.65, d, *J* 15.0, CH$_A$$H_B$Py maj), 3.95 (1H × 0.35, d, *J* 15.0, CH$_A$$H_B$Py min), 4.39-4.42 (1H, m, C(5)*H*), 4.42-4.48 (1H, m, C(4)*H*), 5.07 (1H × 0.35, s, C(2)*H* min), 5.13 (1H × 0.65, s, C(2)*H* maj), 7.13-7.20 (2H, m, *o-Py*, *p-Py*), 7.28-7.41 (3H, m, *m*,*p-Ph*), 7.51-7.55 (2H × 0.35, m, *o-Ph* min), 7.59-7.65 (1H, m, *m-Py*), 7.73-7.78 (2H × 0.65, m, *o-Ph* maj), 8.43-8.45 (1H × 0.35, m, α-*Py* min), 8.47-8.49 (1H × 0.65, m, α-*Py* maj); δ_C (125 MHz, $CDCl_3$) 17.0 (C(4)*C*H$_3$ maj), 17.4 (C(4)*C*H$_3$ min), 24.6, 24.7 (C(*C*H$_3$)$_3$), 36.1 (*C*Me$_3$ min), 36.6 (*C*Me$_3$ maj), 45.9 (*C*H$_2$Py min), 48.7 (*C*H$_2$Py maj), 63.2, 62.3 (*C*(7)), 70.6 (*C*(5) maj), 72.9 (*C*(5) min), 73.5 (*C*(4) maj), 74.8 (*C*(4) min), 94.1 (*C*(2) maj), 95.5 (*C*(2) min), 122.0, 122.2, 123.0, 123.4, 123.5, 123.9, 125.2, 127.2, 127.4, 127.8, 128.0, 128.5, 128.6, 128.9, 129.3, 129.5, 135.1, 136.0, 136.7, 136.8, 137.0 (*o,m,p-Ph*, *o,m,p-Py*), 148.0, 149.6 (α-*Py*), 156.0, 156.1 (*i-Py*), 174.9, 176.4 (*C*(8)), 205.3, 206.8 (*C*(6)); *m/z* (ESI$^+$) 379 ([M+H]$^+$, 80%); HRMS (ESI$^+$) $C_{23}H_{27}N_2O_3^+$ ([M+H]$^+$) requires 379.2016, found 379.2015.

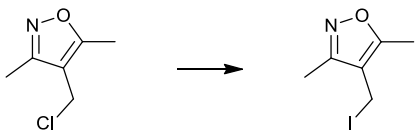
(2*R*,4*R*,5*R*,7*S*)-1-Aza-2-(*t*-butyl)-4-methyl-5-methoxycarbonyl-6,8-dioxo-7-phenyl-7-((*E*)-3′,7′-dimethylocta-2′,6′-dienyl)-3-oxabicyclo[3.3.0]octane 323d and (2*R*,4*R*,5*R*,7*S*)-1-Aza-2-(*t*-butyl)-4-methyl-6,8-dioxo-7-phenyl-7-((*E*)-3′,7′-dimethylocta-2′,6′-dienyl)-3-oxabicyclo[3.3.0]octane 324d



A solution of the tetramic acid **322** (257 mg, 0.74 mmol) in THF (2mL) was added dropwise to a stirred suspension of NaH (75 mg, 60% dispersion in paraffin, 1.84 mmol) in THF (20 mL) at 0 °C. The mixture was stirred at rt for 40 min, before the addition of geranyl bromide (0.18 mL, 0.97 mmol). The mixture was then heated to 50 °C for 24 h, before being cooled to

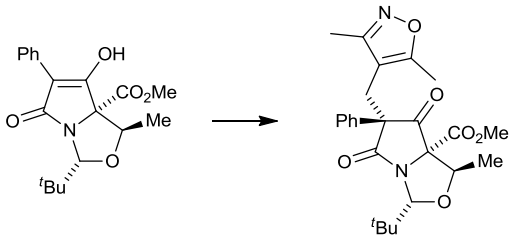
rt and partitioned between Et_2O (30 mL) and H_2O (15 mL). The organic layer was washed with brine (15 mL), dried and concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 20:1 petrol:EtOAc) gave a 1:1 **323d**:**324d** mixture (113 mg, 36%) which was partially separated by further column chromatography (SiO_2, eluent 1:1 DCM:petrol) to give **323d** as a colourless oil (29 mg, 8.5%); R_f 0.45 (eluent 20:1 petrol:EtOAc), 0.42 (eluent 1:1 DCM:petrol); $[\alpha]_D^{23}$ +83.2 (*c* 1.45 in $CHCl_3$); ν_{max} (film) 3478, 2964, 2731, 1741, 1600; δ_H (500 MHz, $CDCl_3$) 0.79 (3H, d, *J* 6.6, C(4)CH_3), 0.96 (9H, s, C(CH_3)$_3$), 1.51 (3H, s, C(3′)CH_3), 1.55 and 1.64 (3H, s, C(7′)CH_3 and C(8′)H_3), 1.87-2.00 (4H, m, C(4′)H_2, C(5′)H_2), 2.98 (1H, dd, *J* 14.5, 7.3, C(1′)H_AH$_B$), 3.12 (1H, dd, *J* 14.5, 7.3, C(1′)H$_A$$H_B$), 3.84 (3H, s, CO_2CH_3), 4.97-5.04 (2H, m, C(2′)*H*, C(6′)*H*), 5.13 (1H, q, *J* 6.6, C(4)*H*), 5.15 (1H, s, C(2)*H*), 7.25-7.35 (3H, m, *m,p-Ph*), 7.62-7.66 (2H, m, *o-Ph*); δ_C (125 MHz, $CDCl_3$) 15.6 (C(4)*C*H$_3$), 16.1 (C(3′)*C*H$_3$), 17.6, 25.6 (C(7′)*C*H$_3$, C(8′)), 24.8 (C(*C*H$_3$)$_3$), 26.4, 39.7 (*C*(4′), *C*(5′)), 35.4 (*C*Me$_3$), 38.5 (*C*(1′)), 53.5 (CO_2CH_3), 61.4 (*C*(7)), 76.1 (*C*(4)), 81.6 (*C*(5)), 97.3 (*C*(2)), 116.2 (*C*(2′)), 124.0 (*C*(6′)), 127.6, 127.7 (*o,p-Ph*), 128.2 (*m-Ph*), 131.3 (*C*(7′)), 133.2 (*i-Ph*), 140.4 (*C*(3′)), 167.8 (*C*O$_2$Me), 177.9 (*C*(8)), 203.4 (*C*(6)); *m/z* (ESI$^+$) 504 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{29}H_{39}NNaO_5^+$ ([M+Na]$^+$) requires 504.2720, found 504.2721; a mixture of **323d** and **324d** (24 mg, 6.8%); and **324d** as a colourless oil (14 mg, 5%); R_f 0.45 (eluent 20:1 40-60 petrol:EtOAc), 0.25 (eluent 1:1 DCM:petrol); $[\alpha]_D^{23}$ +308 (*c* 0.7 in $CHCl_3$); ν_{max} (film) 3406, 2966, 1767, 1709; δ_H (500 MHz, $CDCl_3$) 0.80 (3H, d, *J* 6.6, C(4)CH_3), 1.00 (9H, s, C(CH_3)$_3$), 1.59 and 1.68 (3H, s, C(7′)CH_3 and C(8′)H_3), 1.64 (3H, s, C(3′)CH_3), 1.99-2.10 (4H, m, C(4′)H_2, C(5′)H_2), 2.85 (1H, dd, *J* 13.6, 7.1 (C(1′)H_AH$_B$), 2.97 (1H, dd, *J* 13.6, 8.5, C(1′)H$_A$$H_B$), 4.19 (1H, app. quint., *J* 6.6, C(4)*H*), 5.02-5.07 (1H, m, C(6′)*H*), 5.18-5.23 (1H, m, C(2′)*H*), 5.25 (1H, s, C(2)*H*), 7.25-7.37 (3H, m, *m,p-Ph*), 7.61-7.66 (2H, m, *o-Ph*); δ_C (125 MHz, $CDCl_3$) 16.3 (C(3′)*C*H$_3$), 16.8 (C(4)*C*H$_3$), 17.6, 25.6(C(7′)*C*H$_3$, *C*(8′)), 24.8 (C(*C*H$_3$)$_3$), 26.3 (*C*(5′)), 36.6 (*C*Me$_3$), 39.4 (*C*(1′)), 39.8 (*C*(4′)), 63.4 (*C*(7)), 70.2 (*C*(5)), 73.8 (*C*(4)), 94.5 (*C*(2)), 116.6 *C*(2′)), 123.7 (*C*(6′)), 127.3 (*o-Ph*), 127.6 (*m-Ph*), 128.4 (*p-Ph*), 132.0 (*C*(7′)), 134.7 (*i-Ph*), 141.4 (*C*(3′)), 176.1 (*C*(8)), 208.4 (*C*(6)); *m/z* (ESI$^+$) 446 ([M+Na]$^+$, 80%), 869 ([2M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{27}H_{37}NNaO_3^+$ ([M+Na]$^+$) requires 446.2666, found 446.2662.

4-Iodomethyl-3,5-dimethylisoxazole



A mixture of 4-chloromethyl-3,5-dimethylisoxazole (500 mg, 3.4 mmol) and NaI (611 mg, 4.1 mmol) was heated to 56 °C for 18 h. The mixture was cooled, filtered and the filtrate concentrated *in vacuo* to give the product as a brown oil (805 mg, quant); δ_H (200 MHz, $CDCl_3$) 2.27 (3H, s, C(5)CH_3), 2.32 (3H, s, C(3)CH_3), 4.11 (2H, s, CH_2I); *m/z* (ESI$^+$) 260 ([M+Na]$^+$, 100%).

(2*R*,4*R*,5*R*,7*S*)-1-Aza-2-(*t*-butyl)-4-methyl-5-methoxycarbonyl-6,8-dioxo-7-phenyl-7-(3′,5′-dimethylioxazol-4-ylmethyl)-3-oxabicylo[3.3.0]octane 323e

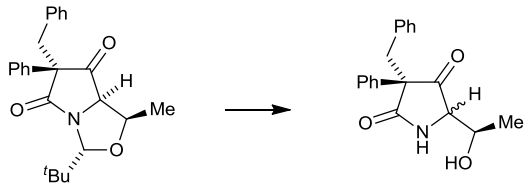


322 **323e**

A solution of the tetramic acid **322** (100 mg, 0.29 mmol) in THF (2 mL) was added dropwise to a stirred suspension of NaH (30 mg, 60% dispersion in paraffin, 0.75 mmol) in THF (10 mL) at 0 °C. The mixture was stirred at rt for 40 min, before the addition of 4-iodomethyl-3,5-dimethylisoxazole (137 mg, 0.58 mmol) in THF (2 mL). The mixture was then heated to 50 °C for 3 h, before being cooled to rt and partitioned between EtOAc (20 mL) and H_2O (10 mL). The organic layer was washed with brine (10 mL), dried and concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 20:1 petrol:EtOAc) gave **323e** as a sticky white solid (75 mg, 59%); R_f 0.25 (eluent 8:1 petrol:EtOAc); $[\alpha]_D^{23}$ +111 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 2962, 1714, 1629; δ_H (400 MHz, $CDCl_3$) 0.58 (3H, d, *J* 6.8, C(4)CH_3), 0.93 (9H, s, C(CH_3)$_3$), 1.91 (3H, s, C(5′)CH_3), 2.03 (3H, s, C(3′)CH_3), 3.12 (1H, d, *J* 14.8, CH_AH$_B$Ar), 3.21 (1H, d, *J* 14.8, CH$_A$$H_B$Ar), 3.75 (3H, s, CO_2CH_3), 5.08 (1H, q, *J* 6.8, C(4)*H*), 5.12 (1H, s, C(2)*H*), 7.29-7.36 (3H, m, *m*,*p*-*Ph*), 7.76-7.81 (2H, m, *o*-*Ph*); δ_C (100 MHz, $CDCl_3$) 9.7 (*C*(5′)), 11.0 (*C*(3′)), 15.8 (*C*(4)), 24.9 (C(*C*H$_3$)$_3$), 34.5 (*C*H$_2$Ar), 35.5 (*C*Me$_3$), 53.8 (CO_2CH_3), 60.0 (*C*(7)), 76.0 (*C*(4)), 81.5 (*C*(5)), 98.2 (*C*(2)), 107.6 (*C*(4′)), 127.3, 128.3,

128.7, 128.8 (*o,m,p-Ph*), 133.6 (*i-Ph*), 160.2 (*C*(5′)), 167.7, 168.1 (*C*(3′), *C*O_2Me), 177.2 (*C*(8)), 203.5 (*C*(6)); *m/z* (ESI$^+$) 477 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{25}H_{30}N_2NaO_6^+$ ([M+Na]$^+$) requires 477.1996, found 477.1996.

(4*S*,2*R*,1′*R*)- and (4*S*,2*R*,1′*S*)-4-Benzyl-4-phenyl-2-(1′-hydroxyethyl)-pyrrolidine-2,4-dione 325



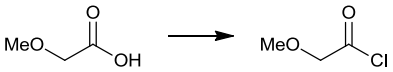
324a **325**

Method A: A solution of **324a** (40 mg, 0.11 mmol) in 1.5% (w/v) HCl in 2,2,2-trifluoroethanol (5 mL) and propan-1,3-dithiol (10 µL, 0.11 mmol) was added. The mixture was stirred for 7 h at rt and then partitioned between EtOAc (10 mL) and H_2O (10 mL). The aqueous layer was concentrated *in vacuo* to give a crude mixture which was purified by column chromatography (SiO_2, eluent 40:1 petrol:EtOAc to EtOAc) to give an impure sample of **325** in an 82:18 dr as a yellow oil (20 mg, 61%); ν_{max} (film) 3251 (br, N−H), 2926, 1694, 1602; δ_H (500 MHz, $CDCl_3$) 0.96 (3H × 0.82, d, *J* 6.31, C*H*$_3$ major), 1.14 (3H × 0.18, d, *J* 6.62, C*H*$_3$ minor), 3.02 (1H × 0.18, d, *J* 3.2, C(2)*H* min), 3.15 (1H × 0.82, d, *J* 3.2, C(2)*H* maj), 3.39 (1H × 0.82, d, *J* 12.9 CH_AH_BPh maj), 3.41 (1H × 0.18, d, *J* 12.9, CH_AH_BPh min), 3.49 (1H × 0.82, d, *J* 12.9, CH_AH_BPh maj), 3.50 (1H × 0.18, d, *J* 12.9, CH_AH_BPh min), 3.77-3.83 (1H, m, C*H*OH), 6.90 (1H × 0.82, br s, N*H* maj), 7.22-7.40 (8H, *o,m,p*-CH_2*Ph*, *m,p-Ph*), 7.60-7.64 (2H, m, *o-Ph*); δ_C (125 MHz, $CDCl_3$) 18.3 (*C*H$_3$ maj), 21.0 (*C*H$_3$ min), 43.2, 43.9 (*C*H_2Ph), 60.4 (*C*(4)), 67.2, 67.3, 67.7 (*C*(2) maj, *C*(2)min, *C*HOH), 126.7, 126.9, 127.0, 127.0, 127.4, 127.5, 128.0, 128.0, 128.4, 128.5, 128.6, 128.6, 128.7, 128.8, 128.8, 129.0, 129.1, 129.2, 130.2, 130.6 (*o,m,p-Ph*, *o,m,p*-CH_2*Ph*), 134.8, 134.9, 135.4, 135.5 (*i-Ph*, *i*-CH_2*Ph*), 174.9, 175.1 (*C*(5)), 209.8, 210.5 (*C*(3)); *m/z* (ESI$^+$) 332 ([M+Na]$^+$, 100%).[8]

Method B: Two drops of TFA were added to solution of **324a** (25 mg, 0.06 mmol) in 2,2,2-trifluoroethanol (5 mL). The mixture was stirred at rt for 24 h, and then for 3 d at 30 °C. After this time, the mixture was concentrated *in vacuo* to give a complex mixture.

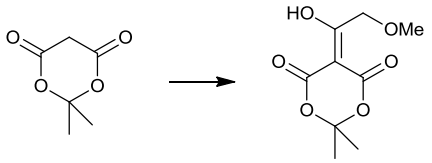
4.3.3 Experimental for the synthesis of functionalised pyroglutamates

Methoxyacetyl chloride[17]



Methoxyacetic acid (8.5 ml, 110 mmol) was added dropwise to a flask of stirring thionyl chloride (24.2 mL, 332 mmol) and stirred at rt for 15 min. The mixture was then heated to reflux at 110 °C for 2 h. The mixture was allowed to cool and then purified by distillation at atmospheric pressure to give the product as a colourless oil (7.67 g, 64%); bp 98 ºC; {lit.[17] bp 98 °C}; δ_H (400 MHz, $CDCl_3$) 3.49 (3H, s, CH_3), 4.36 (2H, s, CH_2); δ_C (100 MHz, $CDCl_3$) 59.7 (*C*H_3), 77.5 (*C*H_2), 171.8 (*C*=O).

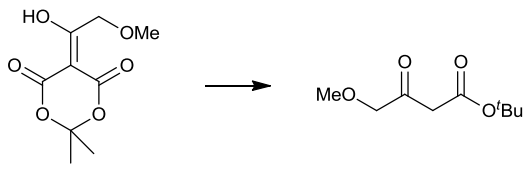
5-(2′-Methoxyacetyl)-2,2-dimethyl-1,3-dioxane-4,6-dione 302[18]



302

Following **general procedure E**, Pyridine (3.46 mL, 43.0 mmol), Meldrum's acid (3.10 g, 21.5 mmol) and methoxyacetyl chloride (2.56 g, 23.7 mmol) were reacted to give **302** as a dark brown oil (3.5 g, 75%) which was used without further purification; δ_H (400 MHz, $CDCl_3$) 1.75 (6H, s, C(CH_3)$_2$), 3.52 (3H, s, OCH_3), 4.86 (CH_2); δ_C (100 MHz, $CDCl_3$) 26.9 (C(2)(*C*H_3)$_2$), 59.9 (O*C*H_3), 72.1 (*C*H_2), 90.1 (*C*(5)), 105.9 (*C*(2)), 162.9 (*C*=O), 194.1 (*C*OCH_2OMe); *m/z* (ESI$^+$) 215 ([M−H]$^-$, 40%).

***t*-Butyl 4-methoxy-3-oxo-butanoate 304a**[18]

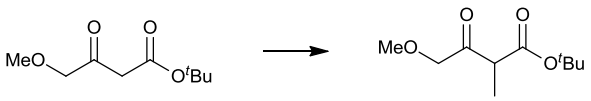


302 **304a**

A solution of **302** (3.5 g, 16.2 mmol) in 1:1 toluene:*t*BuOH (36 mL) was stirred at reflux for 2 h. The mixture was cooled to rt and concentrated *in vacuo* to give **304a** as a dark brown liquid (2.2 g, 73%); R_f 0.3 (eluent 9:1 petrol:EtOAc); δ_H (400 MHz, $CDCl_3$) 1.47 (9H, s,

$C(CH_3)_3$), 3.41 (2H, s, C(2)H_2), 3.42 (3H, s, OCH_3), 4.08 (2H, s, C(4)H_2); δ_C (100 MHz, $CDCl_3$) 27.9 ($C(CH_3)_3$), 47.2 (*C*(2)), 59.3 (OCH_3), 77.3 (*C*(4)), 83.4 (CMe_3), 166.2 (*C*(1)), 202.0 (*C*(3)); *m/z* (ESI^+) 211 ($[M+Na]^+$, 50%), 399 ($[2M+Na]^+$, 75%).

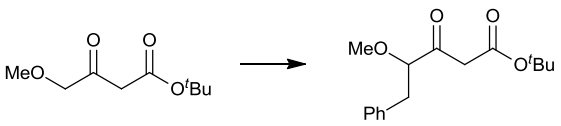
_t_-Butyl 4-methoxy-2-methyl-3-oxobutanoate 306a[18]



304a **306a**

A solution of **304a** (2.0 g, 10.6 mmol) in THF (30 mL) was stirred at 0 °C and *t*BuOK (1.30 g, 11.7 mmol) was added. The mixture was then warmed to rt and stirred for 40 min before the addition of MeI (0.73 mL, 11.7 mmol). The mixture was left to stir for a further 5 h at rt. After this time the mixture was partitioned between Et_2O (30 mL) and brine (30 mL). The aqueous layer was extracted with Et_2O (2 × 20 mL) and the combined organic layers were dried and concentrated *in vacuo* to give **306a** as a yellow oil (1.60 g, 74%); R_f 0.4 (eluent 9:1 petrol:EtOAc); δ_H (400 MHz, $CDCl_3$) 1.30 (3H, d, *J* 7.1, C(2)CH_3), 1.45 (9H, s, $C(CH_3)_3$), 3.41 (3H, s, OCH_3), 3.55 (1H, q, *J* 7.1, C(2)*H*), 4.07-4.15 (2H, m, C(4)H_2); δ_C (100 MHz, $CDCl_3$) 12.2 (C(2)CH_3), 27.9 ($C(CH_3)_3$), 50.1 (*C*(2)), 59.2 (OCH_3), 76.8 (*C*(4)), 81.8 (CMe_3), 169.4 (*C*(1)), 204.5 (*C*(3)); *m/z* (ESI^+) 225 ($[M+Na]^+$, 80%), 427 ($[2M+Na]^+$, 100%).

_t_-Butyl 4-methoxy-5-phenyl-3-oxo-pentanoate 326

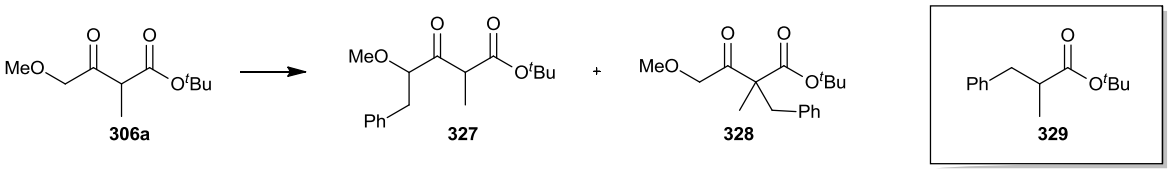


304a **326**

A stirred suspension of NaH (47 mg, 1.17 mmol) in THF (20 mL) was cooled to 0 °C and a solution of **304a** (200 mg, 1.06 mmol) in THF (2 mL) was added dropwise. Stirring was continued for 10 min at 0 °C before the dropwise addition of BuLi (0.7 mL, 1.6 M in hexanes, 1.12 mmol). After stirring for a further 10 min, BnBr (0.14 mL, 1.17 mmol) was added in one portion and the mixture was allowed to warm to rt over 15 min. The mixture was quenched with 2M HCl (7 mL) and extracted with Et_2O (2 × 15 mL). The combined organic layers were washed with H_2O until the aqueous layer remained neutral, dried and concentrated *in vacuo*. Purification by column chromatography (SiO_2, eluent 10:1 40-60 petrol:EtOAc) gave

326 as a colourless oil (90 mg, 30%) as a 3:1 keto:enol mixture; R_f 0.5 (eluent 10:1 40-60 petrol:EtOAc); ν_{max} (film) 3064, 3030, 2980, 2829, 1717, 1650; δ_H (400 MHz, $CDCl_3$) keto: 1.47 (9H, s, C(CH_3)$_3$), 2.93 (1H, d, *J* 7.8, C(5)H_AH$_B$), 3.02 (1H, d, *J* 4.6, C(5)H$_A$$H_B$), 3.28 (1H, d, *J* 5.1, C(2)H_AH$_B$), 3.32 (3H, s, OCH_3), 3.46 (1H, d, *J* 15.9, C(2)H$_A$$H_B$), 3.92 (1H, dd, *J* 7.8, 4.6, C(4)*H*), 7.20-7.33 (5H, m, *Ph*), enol: 1.51 (9H, s, C(CH_3)$_3$), 2.90 (1H, d, *J* 7.8 C(5)H_AH$_B$), 3.06 (1H, d, *J* 4.3, C(5)H$_A$$H_B$), 3.32 (3H, s, OC$H_3$), 3.76 (1H, dd, *J* 8.6, 4.3, C(4)*H*), 5.11 (1H, s, C(2)*H*), 7.20-7.33 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$)[19] 28.0, 28.3 (C(*C*H$_3$)$_3$), 37.8 (*C*(5)), 46.7 (*C*(2) keto), 57.9, 58.7 (O*C*H$_3$), 81.8 (*C*Me$_3$), 87.6 (*C*(4) keto), 90.7 (*C*(2) enol), 126.7, 128.2, 128.4, 129.3, 129.4 (*o,m,p-Ph*), 136.8 (*i-Ph*), 166.4 (*C*(1)), 205.5 (*C*(3)); *m/z* (ESI$^+$) 301 ([M+Na]$^+$, 80%), 579 ([2M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{16}H_{22}NaO_4^+$ ([M+Na]$^+$) requires 301.1410, found 301.1410; and a fraction of unreacted **304a** (70 mg, 35%).

_t_-Butyl 4-methoxy-2-methyl-3-oxo-5-phenylpentanoate 327 and _t_-butyl 2-benzyl-2-methyl-4-methoxy-3-oxobutanoate 328



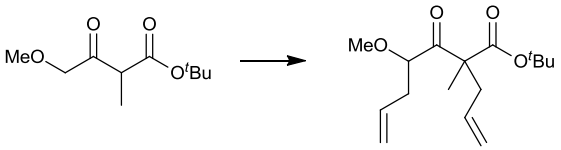
Method A: A stirred suspension of NaH (43 mg, 1.00 mmol) in THF (20 mL) was cooled to 0 °C and a solution of **306a** (200 mg, 1.0 mmol) in THF (2 mL) was added dropwise. Stirring was continued for 10 min at 0 °C before the dropwise addition of BuLi (0.73 mL, 1.6 M in hexanes, 1.09 mmol). After stirring for a further 10 min, BnBr (0.13 mL, 1.09 mmol) was added in one portion and the mixture was allowed to warm to rt over 15 min. The mixture was quenched with 2M HCl (7 mL) and extracted with Et_2O (2 × 15 mL). The combined organic layers were washed with H_2O until the aqueous layer remained neutral, dried and concentrated *in vacuo*. Purification by column chromatography (SiO_2, eluent 40:1 to 20:1 40-60 petrol:EtOAc) gave **327** in a 47:43 dr as a colourless oil (117 mg, 40%); R_f 0.5 (eluent 50:1 40-60 petrol:EtOAc); ν_{max} (film) 3437, 3030, 2980, 2830, 1716, 1604; δ_H (100 MHz, $CDCl_3$) major diastereomer: 1.18 (3H, d, *J* 7.3, C(2)CH_3), 1.44 (9H, s, C(CH_3)$_3$), 2.88-2.93 (1H, m, C(5)H_AH$_B$), 3.11 (1H, dd, *J* 14.0, 4.2, C(5)H$_A$$H_B$), 3.32 (3H, s, OC$H_3$), 3.56-3.63 (1H, m,

C(4)*H*), 7.19-7.33 (5H, m, *Ph*), minor diastereoisomer: 1.25 (3H, d, *J* 7.1, C(2)CH_3), 1.46 (9H, s, C(CH_3)$_3$), 2.88-2.93 (1H, m, C(5)H_AH$_B$), 3.04 (1H, dd, *J* 14.2, 4.0, C(5)H$_A$$H_B$), 3.27 (3H, s, OC$H_3$), 3.56-3.63 (1H, m, C(2)*H*), 2.97-4.03 (1H, m, C(4)*H*), 7.19-7.33 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 12.5 (C(2)*C*H$_3$), 27.9 (C(*C*H$_3$)$_3$), 37.2 (*C*(5) major), 38.5 (*C*(5) minor), 49.4, 50.1 (*C*(2)), 58.6, 58.9 (O*C*H$_3$), 81.4, 81.8 (*C*Me$_3$), 86.9, 87.7 (*C*(4)), 126.6, 128.3, 128.4, 129.3, 129.6 (*o,m,p-Ph*), 137.2 (*i-Ph*), 169.3, 169.8 (*C*(1)), 207.2, 207.8 (*C*(3)); *m/z* (ESI$^+$) 315 ([M+Na]$^+$, 80%), 291 ([M−H]$^-$, 100%); HRMS (ESI$^+$) $C_{17}H_{24}NaO_4^+$ ([M+Na]$^+$) requires 315.1567, found 315.1565; and **328** as a colourless oil (28 mg, 10%); R_f 0.25 (eluent 50:1 40-60 petrol:EtOAc), ν_{max} (film) 2980, 2935, 2825, 1714, 1605; δ_H (400 MHz, $CDCl_3$) 1.28 (3H, s, C(2)CH_3), 1.43 (9H, s, C(CH_3)$_3$), 3.16 (1H, d, *J* 13.9, CH_AH$_B$Ph), 3.22 (1H, d, *J* 13.9, CH$_A$$H_B$Ph), 3.39 (3H, s, OC$H_3$), 4.11 (1H, d, *J* 17.1, C(4)H_AH$_B$), 4.17 (1H, d, *J* 17.1, C(4)H$_A$$H_B$), 7.10-7.14 (2H, m, *Ph*), 7.20-7.28 (3H, m, *Ph*); δ_C (100 MHz, $CDCl_3$)[20] 19.2 (C(2)*C*H$_3$), 27.8 (C(*C*H$_3$)$_3$), 40.4 (*C*H$_2$Ph), 59.1 (O*C*H$_3$), 75.7 (*C*(4)), 82.0 (*C*Me$_3$), 126.8, 128.1, 130.4 (*o,m,p-Ph*), 136.4 (*i-Ph*), 171.1 (*C*(1)), 205.1 (*C*(3)); *m/z* (ESI$^+$) 315 ([M+Na]$^+$, 80%), 607 ([2M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{17}H_{24}NaO_4^+$ ([M+Na]$^+$) requires 315.1567, found 315.1567.

Method B: A stirred suspension of NaH (22 mg, 0.54 mmol) in THF (60 mL) was cooled to 0 °C and DMPU (6μL, 0.05 mmol) was added. A solution of **306a** (100 mg, 0.5 mmol) in THF (2 mL) was added dropwise. Stirring was continued for 10 min at 0 °C before the dropwise addition of BuLi (0.0.33 mL, 1.57 M in hexanes, 0.52 mmol). After stirring for a further 10 min, BnBr (0.07 mL, 0.54 mmol) was added in one portion and the mixture was allowed to warm to rt over 15 min. The mixture was quenched with 2M HCl (7 mL) and extracted with Et_2O (2 × 15 mL). The combined organic layers were washed with H_2O until the aqueous layer remained neutral, dried and concentrated *in vacuo*. Purification by column chromatography (SiO_2, eluent 40:1 to 20:1 40-60 petrol:EtOAc) gave by-product **329**[21] as a colourless oil (40 mg, 18%); R_f 0.28 (eluent 40:1 petrol:EtOAc); δ_H (400 MHz, $CDCl_3$) 1.13 (3H, d, *J* 6.6, C(2)CH_3), 1.39 (9H, s, C(CH_3)$_3$), 2.60-2.70 (2H, m, C(2)*H*, C(3)H_AH$_B$), 2.98 (1H, dd, *J* 16.3, 10.2, C(3)H$_A$$H_B$), 7.17-7.32 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 17.0

(C(2)*C*H_3), 28.0 (C(*C*H_3)$_3$), 39.8 (*C*(3)), 42.3 (*C*(2)), 80.0 (*C*Me_3), 126.1, 128.2, 129.0 (*o,m,p-Ph*), 139.7 (*i-Ph*), 175.5 (*C*(1)); *m/z* (ESI$^+$) 243 ([M+Na]$^+$, 100%); product **327** (60 mg, 20%); data as above; and unreacted starting material **306a** (20 mg, 20%).

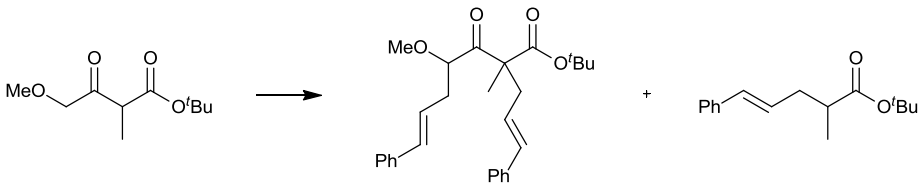
_t_-Butyl 2,4-diallyl-4-methoxy-2-methyl-3-oxohept-6-enoate 330



306a **330**

A stirred suspension of NaH (52 mg, 1.30 mmol) in THF (20 mL) was cooled to 0 °C and a solution of **306a** (238 mg, 1.00 mmol) in THF (2 mL) was added dropwise. Stirring was continued for 10 min at 0 °C before the dropwise addition of BuLi (0.86 mL, 1.6 M in hexanes, 1.24 mmol). After stirring for a further 10 min, allyl iodide (0.12 mL, 1.30 mmol) was added in one portion and the mixture was allowed to warm to rt over 15 min. The mixture was quenched with 2M HCl (7 mL) and extracted with Et_2O (2 × 15 mL). The combined organic layers were washed with H_2O until the aqueous layer remained neutral, dried and concentrated *in vacuo*. Purification by column chromatography (SiO_2, eluent 40:1 to 20:1 40-60 petrol:EtOAc) gave **330** in an 8:1 dr as a colourless oil (41 mg, 22%); R_f 0.5 (eluent 20: 40-60 petrol:EtOAc); ν_{max} (film) 3078, 2980, 1714, 1641; δ_H (400 MHz, $CDCl_3$) 1.27 (3H, s, C(2)CH_3), 1.44 (9H, s, C(CH_3)$_3$), 2.46-2.63 (4H, m, C(5)H_2, C(1′)H_2), 3.37 (3H, s, OCH_3), 3.87 (1H, t, *J* 5.1, C(4)*H*), 5.00-5.15 (4H, m, C(7)H_2, C(3′)H_2), 5.60-5.82 (2H, m, C(6)*H*, C(2′)*H*); δ_C (100 MHz, $CDCl_3$) 18.9 (C(2)*C*H_3), 28.0 (C(*C*H_3)$_3$), 35.0, 39.7 (*C*(5), *C*(2′)), 57.7 (O*C*H_3), 81.3 (*C*Me_3), 84.8 (*C*(4)), 117.7, 118.5 (*C*(7), *C*(3′)), 133.1 , 133.7 (*C*(6), *C*(2′)), 171.0 (*C*(1)), 207.3 (*C*(3)); *m/z* (ESI$^+$) 305 ([M+Na]$^+$, 80%), 281 ([M−H]$^-$, 40%); HRMS (ESI$^+$) $C_{16}H_{26}NaO_4^+$ ([M+Na]$^+$) requires 305.1723, found 305.1723.

_t_-Butyl 2,4-dicinnamyl-4-methoxy-2-methyl-3-oxohept-6-enoate 332 and _t_-butyl 2-cinnamyl-propanoate 333



306a **332** **333**

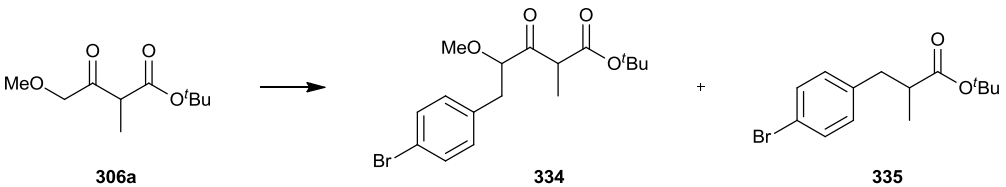
Method A: A stirred suspension of NaH (22 mg, 0.54 mmol) in THF (5 mL) was cooled to 0 °C and a solution of **306a** (100 mg, 0.50 mmol) in THF (2 mL) was added dropwise. Stirring was continued for 10 min at 0 °C before the dropwise addition of BuLi (0.36 mL, 1.44 M in hexanes, 0.52 mmol). After stirring for a further 10 min, cinnamyl bromide (107 mg, 0.54 mmol) was added in one portion and the mixture was allowed to warm to rt over 15 min. The mixture was quenched with 2M HCl (7 mL) and extracted with Et_2O (2 × 15 mL). The combined organic layers were washed with H_2O until the aqueous layer remained neutral, dried and concentrated *in vacuo* to give unreacted starting materials.

Method B: A stirred suspension of NaH (22 mg, 0.54 mmol) in THF (5 mL) was cooled to 0 °C and a solution of **306a** (100 mg, 0.50 mmol) in THF (2 mL) was added dropwise. Stirring was continued for 10 min at 0 °C before the dropwise addition of BuLi (0.36 mL, 1.44 M in hexanes, 0.52 mmol). After stirring for a further 10 min, cinnamyl bromide (107 mg, 0.54 mmol) was added in one portion and the mixture was allowed to warm to rt for 5 h. The mixture was quenched with 2M HCl (7 mL) and extracted with Et_2O (2 × 15 mL). The combined organic layers were washed with H_2O until the aqueous layer remained neutral, dried and concentrated *in vacuo*. Column chromatography (SiO_2, eluent 80:1 to 40:1 petrol:EtOAc) gave by-product **333** as a colourless oil (35 mg, 28%); R_f 0.3 (eluent 40:1 petrol:EtOAc); ν_{max} (film) 3060, 3026, 2976, 2934, 1727; δ_H (400 MHz, $CDCl_3$) 1.18 (3H, d, *J* 6.8, C(2)CH_3), 1.45 (9H, s, C(CH_3)$_3$), 2.27-2.38 (1H, m, C(3)H_AH$_B$), 2.46-2.58 (2H, m, C(3)H$_A$$H_B$, C(2)*H*), 6.13-6.23 (1H, m, C(4)*H*), 6.43 (1H, d, *J* 15.9, C(5)*H*), 7.19-7.36 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 16.8 (C(2)*C*H$_3$), 28.1 (C(*C*H$_3$)$_3$), 37.2 (*C*(3)), 40.5 (*C*(2)), 80.1 (*C*Me$_3$), 126.0, 127.0, 128.5 (*o,m,p-Ph*), 127.6 (*C*(4)), 131.8 (*C*(5)), 137.5 (*i-Ph*), 175.3

(*C*(1)); *m/z* (ESI$^+$) 269 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{16}H_{22}NaO_2^+$ ([M+Na]$^+$) requires 269.1512, found 269.1513; and unreacted **306a** (7 mg, 7%).

Method C: A stirred suspension of NaH (22 mg, 0.54 mmol) in THF (5 mL) was cooled to 0 °C and DMPU (6 µL, 0.05 mmol) was added. A solution of **306a** (100 mg, 0.50 mmol) in THF (2 mL) was then added dropwise. Stirring was continued for 10 min at 0 °C before the dropwise addition of BuLi (0.36 mL, 1.44 M in hexanes, 0.52 mmol). After stirring for a further 10 min, benzyl bromide (116 mg, 0.54 mmol) was added in one portion and the mixture was allowed to warm to rt for 2 h. The mixture was quenched with 2M HCl (7 mL) and extracted with Et_2O (2 × 15 mL). The combined organic layers were washed with H_2O until the aqueous layer remained neutral, dried and concentrated *in vacuo*. Column chromatography (SiO_2, eluent 80:1 to 40:1 petrol:EtOAc) gave unreacted cinnamyl bromide (30 mg, 29%); by-product **333** (35 mg, 28%); double-alkylated **332** as a colourless oil (11 mg, 7%); R_f 0.2 (eluent 40:1 petrol:EtOAc); ν_{max} (film) 3469, 2978, 29.3, 1715; δ_H (400 MHz, $CDCl_3$) 1.32-1.34 (3H, m, C(2)CH_3), 1.42-1.44 (9H, s, C(CH_3)$_3$), 2.57-2.84 (4H, m, C(5)H_2, C(1′)H_2), 3.41-3.44 (3H, s, OCH_3), 3.98-4.02 (1H, m, C(4)*H*), 6.01-6.26 (2H, m, C(7)*H*, C(3′)*H*), 6.37-6.52 (2H, m, C(6)*H*, C(2′)*H*), 7.17-7.36 (10H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 19.1, 19.3 (C(2)*C*H$_3$), 28.0, 28.1 (C(*C*H$_3$)$_3$), 34.3, 34.8 (*C*(5)), 38.4, 39.3 (*C*(1′)), 57.6, 57.9 (O*C*H$_3$), 85.2, 85.6 (*C*(4)), 124.9, 124.9, 125.2, 125.4 (*C*(7), *C*(3′)), 126.1, 126.1, 127.1, 127.2, 128.4, 128.5 (*o,m,p-Ph*), 132.8, 132.9, 133.3, 133.5 (*C*(6), *C*(2′)), 137.2, 137.3 (*i-Ph*), 170.9, 171.0 (*C*(1)), 207.6, 207.7 (*C*(3)); *m/z* (ESI$^+$) 457 ([M+Na]$^+$, 40%); HRMS (ESI$^+$) $C_{28}H_{34}NaO_4^+$ ([M+Na]$^+$) requires 457.2349, found 457.2335; and unreacted **306a** (15 mg, 15%).

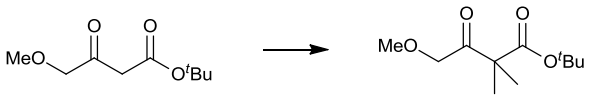
***t*-butyl 4-methoxy-5-(4′-bromophenyl)-2-methyl-3-oxohept-6-enoate 334**



A stirred suspension of NaH (22 mg, 0.54 mmol) in THF (5 mL) was cooled to 0 °C and DMPU (6 µL, 0.05 mmol) was added. A solution of **306a** (100 mg, 0.50 mmol) in THF (2

mL) was then added dropwise. Stirring was continued for 10 min at 0 °C before the dropwise addition of BuLi (0.33 mL, 1.58 M in hexanes, 0.52 mmol). After stirring for a further 10 min, 4-bromobenzyl bromide (135 mg, 0.54 mmol) was added in one portion and the mixture was allowed to warm to rt for 2 h. The mixture was quenched with 2M HCl (7 mL) and extracted with Et_2O (2 × 15 mL). The combined organic layers were washed with H_2O until the aqueous layer remained neutral, dried and concentrated *in vacuo*. Column chromatography (SiO_2, eluent 50:1 to 20:1 petrol:EtOAc) gave **334** in a 59:41 dr as a colourless oil (33 mg, 18%); R_f 0.15 (eluent 20:1 petrol:EtOAc); ν_{max} (film) 2980, 2936, 1741, 1716; δ_H (400 MHz, $CDCl_3$) major isomer- 1.19 (3H, d, *J* 7.3, C(2)CH_3), 1.43 (9H, s, C(CH_3)$_3$), 2.87-2.91 (1H, m, C(5)H_AH$_B$), 2.96-3.08 (1H, m, C(5)H$_A$$H_B$), 3.33 (3H, s, OC$H_3$), 3.57 (1H, q, *J* 7.3, C(2)*H*), 3.96 (1H, dd, *J* 6.8, 4.2, C(4)*H*), 7.08 (2H, d, *J* 8.3, C(2′)H, C(6′)*H*), 7.39 (2H, d, *J* 8.3, C(3′)*H*, C(5′)*H*), minor isomer- 1.26 (3H, d, *J* 7.1, C(2)CH_3), 1.46 (9H, s, C(CH_3)$_3$), 2.83-2.87 (1H, m, C(5)H_AH$_B$), 2.96-3.08 (1H, m, C(5)H$_A$$H_B$), 3.27 (3H, s, OC$H_3$), 3.62 (1H, q, *J* 7.1, C(2)*H)*, 3.92 (1H, dd, *J* 8.8, 3.8, C(4)*H*), 7.13 (2H, d, *J* 8.3, C(2′)*H*, C(6′)*H*), 7.42 (2H, d, *J* 8.3, C(3′)*H*, C(5′)*H*); δ_C (100 MHz, $CDCl_3$) 12.5, 12.6 (C(2)*C*H$_3$), 27.9, 28.0 (C(*C*H$_3$)$_3$), 36.3 (*C*(5) maj), 37.0 (*C*(5) min), 49.4 (*C*(2) maj), 50.0 (*C*(2) min), 58.6 (O*C*H$_3$ min), 59.0 (O*C*H$_3$ maj), 81.5, 818 (*C*Me$_3$), 86.5 (*C*(4) maj), 87.0 (*C*(4) min), 120.6 (*C*(4′)), 131.1, 131.2, 131.4, 131.5 (*C*(2′), *C*(3′), *C*(5′), *C*(6′)), 136.1, 136.6 (*C*(1′)), 169.3, 169.8 (*C*(1)), 207.0, 207.5 (*C*(3)); *m/z* (ESI$^+$) 393 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{17}H_{23}BrNaO_4^+$ ([M+Na]$^+$) requires 393.0672, found 393.0666; and by-product **335** as a colourless oil (12 mg, 8%); R_f 0.33 (eluent 20:1 petrol:EtOAc); ν_{max} (film) 2976, 2933, 1721; δ_H (400 MHz, $CDCl_3$) 1.12 (3H, d, *J* 6.6, C(2)CH_3), 1.38 (9H, s, C(CH_3)$_3$), 2.56-2.63 (2H, m, C(2)*H*, C(3)H_AH$_B$), 2.91 (1H, dd, *J* 16.4, 10.1, C(3)H$_A$$H_B$), 7.06 (2H, d, *J* 8.5, C(2′)*H*, C(6′)*H*), 7.40 (2H, d, *J* 8.5, C(3′)*H*, C(5′)*H*); δ_C (100 MHz, $CDCl_3$) 17.0 (C(2)*C*H$_3$), 28.1 (C(*C*H$_3$)$_3$), 39.1 (*C*(3)), 42.1 (*C*(2)), 80.2 (*C*Me$_3$), 120.0 (*C*(4′)), 130.8 (*C*(2′), *C*(6′)), 131.3 (*C*(3′), *C*(5′)), 138.7 (*C*(1′)), 175.1 (*C*(1)); *m/z* (ESI$^+$) 321 ([M+Na]$^+$, 40%); HRMS (ESI$^+$) $C_{14}H_{19}BrNaO_2^+$ ([M+Na]$^+$) requires 321.0461, 323.0441 found 321.0460, 323.0443.

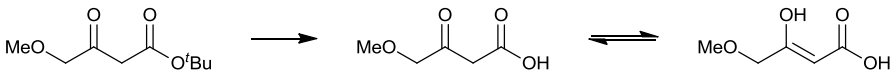
_t_-Butyl 4-methoxy-2,2-dimethyl-3-oxo-butanoate 336



304a **336**

A solution of **304a** (220 mg, 1.17 mmol) in THF (10 mL) was stirred at 0 °C and *t*BuOK (317 mg, 2.60 mmol) was added. The mixture was then warmed to rt and stirred for 40 min before the addition of MeI (0.16 mL, 2.60 mmol). The mixture was left to stir for a further 5 h at rt. After this time the mixture was partitioned between Et_2O (15 mL) and brine (15 mL). The aqueous layer was extracted with Et_2O (2 × 10 mL) and the combined organic layers were dried and concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 50:1 40-60 petrol:EtOAc) gave **336** as a colourless oil (85 mg, 34%); R_f 0.5 (eluent 9:1 40-60 petrol:EtOAc); ν_{max} (film) 3446, 2981, 2936, 2826, 1715, 1634; δ_H (400 MHz, $CDCl_3$) 1.28 (6H, s, C(2)(CH_3)$_2$), 1.40 (9H, s, C(CH_3)$_3$), 3.33 (3H, s, OCH_3), 4.10 (2H, s, C(4)H_2); δ_C (100 MHz, $CDCl_3$) 21.6 (C(2)(*C*H$_3$)$_2$), 27.7 (C(*C*H$_3$)$_3$), 54.1 (*C*(2)), 59.0 (O*C*H$_3$), 75.1 (*C*(4)), 81.4 (*C*Me$_3$), 172.2 (*C*(1)), 205.7 (*C*(3)); *m/z* (ESI$^+$) 239 ([M+Na]$^+$, 90%), 239 ([2M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{11}H_{20}NaO_4^+$ ([M+Na]$^+$) requires 239.1254, found 239.1251.

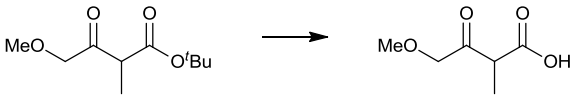
4-Methoxy-3-oxobutanoic acid 305a[18]



304a **305a**

A solution of **304a** (200 mg, 1.06 mmol) in DCM (1 mL) was cooled to 0 °C and TFA (1 mL) was added dropwise. The resulting solution was stirred for 24 h at rt before the solvent was removed *in vacuo* to give **305a** as a yellow oil (140 mg, quant) in a 5:1 keto:enol ratio; δ_H (400 MHz, $CDCl_3$) keto-tautomer - 3.44 (3H, s, OCH_3), 3.57 (2H, s, C(2)H_2), 4.10 (2H, s, C(4)H_2), 9.73 (1H, br s, O*H*), enol-tautomer - 3.44 (3H, s, OCH_3), 4.02 (2H, s, C(4)H_2), 5.32 (1H, s, C(2)*H*), 9.73 (1H, br s, O*H*); δ_C (100 MHz, $CDCl_3$) 45.1 (keto-*C*(2)) 59.2, 59.3 (2 × O*C*H$_3$), 71.1 (enol-*C*(4)), 77.2 (keto-*C*(4)), 87.8 (enol-*C*(2)), 171.9 (keto-*C*(1)), 201.8 (keto-*C*(8)).

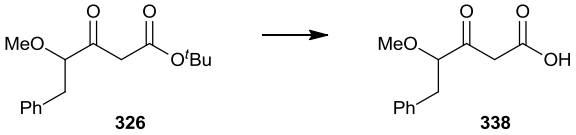
4-Methoxy-2-methyl-3-oxobutanioic acid 307a[18]



306a **307a**

A solution of **306a** (282 mg, 1.39 mmol) in DCM (1.5 mL) was cooled to 0 °C and TFA (1.5 mL) was added dropwise. The resulting solution was stirred for 3 h at rt before the solvent was removed *in vacuo* to give **307a** as an orange oil (190 mg, 93%); δ_H (400 MHz, $CDCl_3$) 1.39 (3H, d, *J* 7.3, C(2)CH_3), 3.44 (3H, s, OCH_3), 3.76 (1H, q, *J* 7.3, C(2)*H*), 4.13-4.17 (2H, m, C(4)H_2), 6.50 (1H, br s, O*H*); δ_C (100 MHz, $CDCl_3$) 12.2 (C(2)*C*H_3), 48.5 (*C*(2)), 59.4 (O*C*H_3), 76.7 (*C*(4)), 175.2 (*C*(1)), 204.1 (*C*(3)).

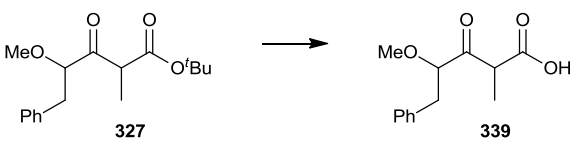
4-Methoxy-3-oxo-5-phenylpentanoic acid 338



326 **338**

A solution of **326** (90 mg, 0.32 mmol) in DCM (0.5 mL) was cooled to 0 °C and TFA (0.5 mL) was added dropwise. The resulting solution was stirred for 1.5 h at rt before the solvent was removed *in vacuo* to give **338** as an yellow oil (78 mg, quant); R_f 0.56 (eluent 3:1 40-60 petorl:EtOAc); ν_{max} (film) 2936, 1714, 1496, 1455; δ_H (400 MHz, $CDCl_3$) 2.91-2.91 (1H, m, C(5)H_AH_B), 3.03-3.09 (1H, m, C(5)H_AH_B), 3.35 (3H, s, OCH_3), 3.40 (1H, d, *J* 16.9, C(2)H_AH_B), 3.59 (1H, d, *J* 16.9, C(2)H_AH_B), 3.95 (1H, dd, *J* 7.3, 4.6, C(4)*H*), 7.16-7.34 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 37.7 (*C*(5)), 44.5 (*C*(2)), 58.8 (O*C*H_3), 87.5 (*C*(4)), 126.9, 128.5, 129.4 (*o,m,p-Ph*), 136.3 (*i-Ph*), 171.5 (*C*(1)), 205.8 (*C*(3)); *m/z* (ESI$^+$) 245 ([M+Na]$^+$, 80%), 221 ([M−H]−, 100%); HRMS (ESI$^+$) $C_{12}H_{14}NaO_4^+$ ([M+H]$^+$) requires 245.0784, found 245.788.

4-Methoxy-2-methyl-3-oxo-5-phenylpentanoic acid 339



327 **339**

A solution of **327** (357 mg, 1.22 mmol) in DCM (1.7 mL) was cooled to 0 °C and TFA (1.7 mL) was added dropwise. The resulting solution was stirred for 1.5 h at rt before the solvent

was removed *in vacuo* to give **339** in a 53:47 dr as an orange oil (283 mg, 98%); ν_{max} (film) 2939, 1713, 1496, 1455; δ_H (400 MHz, $CDCl_3$) 1.24 (3H × 0.53, d, *J* 7.3, C(2)CH_3 major), 1.28 (3H × 0.47, d, *J* 7.1, C(2)CH_3 minor), 2.89-3.00 (1H, m, C(5)H_AH_B), 3.06-3.15 (1H, m, C(5)H_AH_B), 3.28 (3H × 0.47, s, OCH_3 min), 3.30 (3H × 0.53, s, OCH_3 maj), 3.60-3.72 (1H, m, C(2)*H*), 3.99-4.05 (1H, m, C(4)*H*), 7.18-7.32 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 12.4 (C(2)*C*H_3), 37.4, 38.2 (C(5)), 48.2, 48.9 (*C*(2)), 58.6, 58.9 (O*C*H_3), 87.0, 87.7 (*C*(4)), 126.6, 126.7, 128.4, 128.5, 129.3, 129.4, 129.6 (*o,m,p-Ph*), 136.8, 137.1 (*i-Ph*), 175.5, 176.2 (*C*(1)), 206.8, 207.1 (*C*(3)); *m/z* (ESI^+) 259 ($[M+Na]^+$, 80%), 235 ($[M-H]^-$, 80%); HRMS (ESI^+) $C_{13}H_{16}NaO_4^+$ ($[M+Na]^+$) requires 259.0941, found 259.0947.

(2*R*,4*S*,5*R*)-2-*t*-Butyl-3-(4′-methoxy-3′-oxobutanoyl)-4-methoxycarbonyl-5-methyloxazolidine 340a

305a **340a**

Following **general procedure D** for the synthesis of *N*-acyloxazolidines, (4*S*,5*R*)-2-*tert*-butyl-4-methoxycarbonyl-5-methyloxazolidine **320** (211 mg, 1.05 mmol) was reacted with DCC (227 mg, 1.01 mmol), DMAP (9 mg, 7 mol%) and the acid **305a** (144 mg, 1.10 mmol). The crude mixture of **340a** (350 mg) was used without purification; *m/z* (ESI^+) 338 ($[M+Na]^+$, 50%).

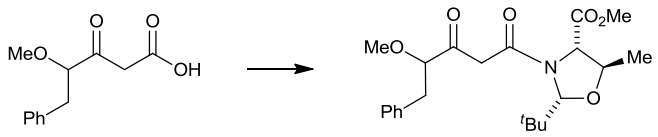
(2*R*,4*S*,5*R*)-2-*t*-Butyl-3-(4′-methoxy-2′-methyl-3′-oxobutanoyl)-4-methoxycarbonyl-5-methyloxazolidine 340b

307a **340b**

Following **general procedure D** for the synthesis of *N*-acyloxazolidines, (4*S*,5*R*)-2-*tert*-butyl-4-methoxycarbonyl-5-methyloxazolidine **320** (248 mg, 1.23 mmol) was reacted with DCC (268 mg, 1.30 mmol), DMAP (10 mg, 7 mol%) and the acid **307a** (190 mg, 1.30 mmol).

The crude mixture of **340b** (340 mg) was used without purification; *m/z* (ESI^+) 352 ($[M+Na]^+$, 20%), 681 ($[2M+Na]^+$, 100%).

(2*R*,4*S*,5*R*)-2-*t*-Butyl-3-(4′-methoxy-5′-phenyl--3′-oxopentanoyl)-4-methoxycarbonyl-5-methyloxazolidine 340c



338 **340c**

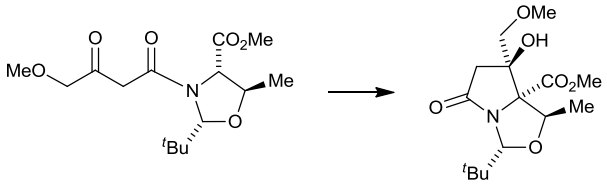
Following **general procedure D** for the synthesis of *N*-acyloxazolidines, (4*S*,5*R*)-2-*tert*-butyl-4-methoxycarbonyl-5-methyloxazolidine **320** (67 mg, 0.33 mmol) was reacted with DCC (72 mg, 0.35 mmol), DMAP (3 mg, 7 mol%) and the acid **338** (78 mg, 0.35 mmol). The crude mixture of **340c** (115 mg) was used without purification; ν_{max} (film) 3322, 2932, 2118, 1745, 1633; *m/z* (ESI^+) 428 ($[M+Na]^+$, 70%), 833 ($[2M+Na]^+$, 100%); HRMS (ESI^+) $C_{22}H_{31}NNaO_6^+$ ($[M+Na]^+$) requires 428.2044, found 428.2041.

(2*R*,4*S*,5*R*)-2-*t*-Butyl-3-(4′-methoxy-5′-phenyl-2′-methyl-3′-oxopentanoyl)-4-methoxycarbonyl-5-methyloxazolidine 340d

339 **340d**

Following **general procedure D** for the synthesis of *N*-acyloxazolidines, (4*S*,5*R*)-2-*tert*-butyl-4-methoxycarbonyl-5-methyloxazolidine **320** (229 mg, 1.14 mmol) was reacted with DCC (247 mg, 1.20 mmol), DMAP (10 mg, 7 mol%) and the acid **339** (236 mg, 1.20 mmol). The crude mixture of **340d** (438 mg) was used without purification; ν_{max} (film) 3339, 3030, 2934, 2118, 1822, 1741, 1651; *m/z* (ESI^+) 442 ($[M+Na]^+$, 60%), 861 ($[2M+Na]^+$, 90%), 418 ($[M-H]^-$, 30%); HRMS (ESI^+) $C_{23}H_{33}NNaO_6^+$ ($[M+Na]^+$) requires 422.2200, found 422.2198.

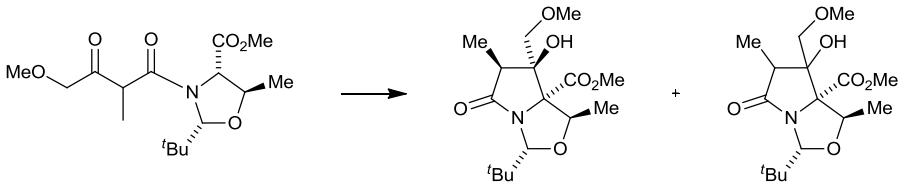
(2*R*,4*R*,5*R*,6*R*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-methoxymethyl-8-oxo-3-oxabicylco[3.3.0]octane 341



340a **341**

Following **general procedure F** for the aldol cyclisation of *N*-acyloxazolidines, **340a** (440 mg, 1.40 mmol) and NaOMe (83 mg, 1.53 mmol) were reacted to give the crude reaction mixture. Purification *via* column chromatography (SiO_2, eluent 3:1 40-60 petrol:EtOAc) gave **341** as a white solid (99 mg, 22%); R_f 0.1 (eluent 3:1 40-60 petrol:EtOAc); mp 144-147 ºC; $[\alpha]_D^{23}$ +26.7 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3404 (br), 2956, 1704; δ_H (400 MHz, $CDCl_3$) 0.91 (9H, s, C(CH_3)$_3$), 1.63 (3, d, *J* 6.6, C(4)CH_3), 2.34 (1H, d, *J* 16.2, C(7)H_AH$_B$), 3.06 (1H, d, *J* 16.2, C(7)H$_A$$H_B$), 3.09 (1H, s, O*H*), 3.35 (1H, d, *J* 9.7, CH_AH$_B$OMe), 3.36 (3H, s, OCH_3), 3.50 (1H, d, *J* 9.7, CH$_A$$H_B$OMe), 3.77 (3H, s, CO_2CH_3), 4.76 (1H, q, *J* 6.6, C(4)*H*), 5.04 (1H, s, C(2)*H*); δ_C (100 MHz, $CDCl_3$) 15.5 (C(4)*C*H$_3$), 25.6 (C(*C*H$_3$)$_3$), 37.0 (*C*Me$_3$), 45.8 (*C*(7)), 52.6 (CO_2CH_3), 59.3 (OCH_3), 74.3 (*C*H$_2$OMe), 78.6 (*C*(4)), 79.0, 82.2 (*C*(5), *C*(6)), 171.5 (*C*O$_2$Me), 178.2 (*C*(8)); *m/z* (ESI$^-$) 314 ([M−H]$^-$, 100%); HRMS (ESI$^+$) $C_{15}H_{26}NO_6^+$ ([M+H]$^+$) requires 316.1755, found 316.1754.

(2*R*,4*R*,5*R*,6*R*,7*S*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-methoxymethyl-7-methyl-8-oxo-3-oxabicyclo[3.3.0]octane 342a

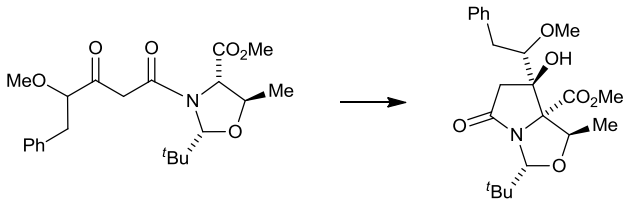


340b **342a** **342b**

Following **general procedure F** for the aldol cyclisation of *N*-acyloxazolidines, **340b** (280 mg, 0.85 mmol) and NaOMe (50 mg, 0.94 mmol) were reacted to give the crude reaction mixture. Purification *via* column chromatography (SiO_2, eluent 3:1 40-60 petrol:EtOAc) gave **342a** as a colourless solid (30 mg, 11%); R_f 0.1 (eluent 3:1 40-60 petrol:EtOAc); mp 120-130 ºC; $[\alpha]_D^{23}$ −31.7 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3457 (br), 2956, 1725; δ_H (400 MHz, $CDCl_3$)

0.92 (9H, s, C(CH_3)$_3$), 1.05 (3H, d, *J* 7.1, C(7)CH_3), 1.68 (3H, d, *J* 6.6, C(4)CH_3), 2.53 (1H, s, O*H*), 3.19 (1H, q, *J* 7.1, C(7)*H*), 3.33 (3H, s, OCH_3), 3.40 (1H, d, *J* 9.9, CH_AH$_B$OMe), 3.47 (1H, d, *J* 9.9, CH$_A$$H_B$OMe), 3.76 (3H, s, CO$_2CH_3$), 4.72 (1H, q, *J* 6.6, C(4)*H*), 5.03 (1H, s, C(2)*H*); δ_C (100 MHz, CDCl$_3$) 6.6 (C(7)*C*H$_3$), 15.5 (C(4)*C*H$_3$), 25.7 (C(*C*H$_3$)$_3$), 37.3 (*C*Me$_3$), 45.4 (*C*(7)), 52.6 (CO$_2$*C*H$_3$), 59.2 (O*C*H$_3$), 72.4 (*C*H$_2$OMe), 77.2 (*C*(5)), 78.9 (*C*(4)), 85.2 (*C*(6)), 96.6 (*C*(2)), 171.5 (*C*O$_2$Me), 180.5 (*C*(8)); *m/z* (ESI$^+$) 352 ([M+Na]$^+$, 90%), (ESI$^-$) 328 ([M−H]$^-$, 100%); HRMS (ESI$^+$) $C_{16}H_{27}NNaO_6^+$ ([M+Na]$^+$) requires 352.1731, found 352.1725; and another fraction of **342a** containing a trace amount of an unassigned diastereoisomer **342b** as a colourless oil (30 mg, 10%).

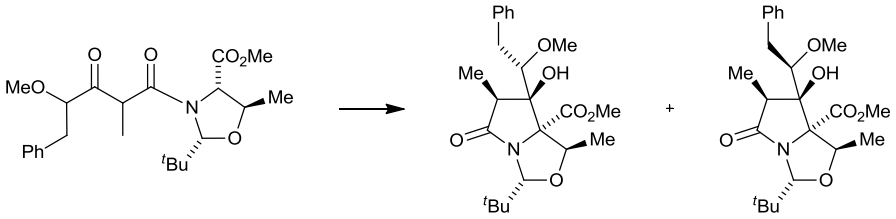
(2*R*,4*R*,5*R*,6*R*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(1′-methoxy-2′-phenylethyl)-8-oxo-3-oxabicyclo[3.3.0]octane 343



340c **343**

Following **general procedure F** for the aldol cyclisation of *N*-acyloxazolidines, *N*-acyl-oxazolidine **320** (107 mg, 0.26 mmol) and NaOMe (16 mg, 0.29 mmol) were reacted to give the crude reaction mixture. Purification *via* column chromatography (SiO$_2$, eluent 20:1 40-60 petrol:EtOAc) gave **343** as a colourless oil (6.5 mg, 6 %); R_f 0.05 (eluent 8:1 40-60 petrol:EtOAc); $[\alpha]_D^{23}$ +11.6 (*c* 0.32 in CHCl$_3$); ν_{max} (film) 2956, 1698; δ_H (500 MHz, CDCl$_3$) 0.92 (9H, s, C(CH_3)$_3$), 1.68 (3H, d, *J* 6.6, C(4)CH_3), 1.78 (1H, d, *J* 16.1, C(7)H_AH$_B$), 2.77 (1H, dd, *J* 14.7, 6.0, C(2′)H_AH$_B$), 3.13 (3H, s, OCH_3), 3.15 (1H, dd, *J* 14.7, 4.1, C(2′)H$_A$$H_B$), 3.27-3.32 (1H, m, C(7)H$_A$$H_B$), 3.71 (1H, dd, *J* 6.0, 4.1, C(1′)*H*), 3.76 (3H, s, CO$_2$CH_3), 4.75 (1H, q, *J* 6.6, C(4)*H*), 5.00 (1H, s, C(2)*H*), 7.22-7.31 (5H, m, *Ph*); δ_C (125 MHz, CDCl$_3$) 15.4 (C(4)*C*H$_3$), 25.8 (C(*C*H$_3$)$_3$), 35.5 (*C*(2′)), 37.2 (*C*Me$_3$), 44.1 (*C*(7)), 52.4 (CO$_2$*C*H$_3$), 57.8 (O*C*H$_3$), 78.1 (*C*(5)), 78.9 (*C*(4)), 81.9 (*C*(1′)), 87.4 (*C*(6)), 96.4 (*C*(2)), 126.6 (*p-Ph*), 128.2, 128.6, 129.3, 129.6 (*o*,*m-Ph*), 138.3 (*i-Ph*), 171.5 (*C*O$_2$Me), 178.5 (*C*(8)); *m/z* (ESI$^+$) 406 ([M+H]$^+$, 30%), 428 ([M+Na]$^+$, 80%), 404 ([M−H]$^-$, 100%); HRMS (ESI$^+$) $C_{22}H_{31}NNaO_6^+$ ([M+H]$^+$) requires 428.2044, found 428.2026.

(2*R*,4*R*,5*R*,6*R*,7*S*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(1′-methoxy-2′-phenylethyl)-7-methyl-8-oxo-3-oxabicyclo[3.3.0]octane 344a and (2*R*,4*R*,5*R*,6*R*,7*R*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(1′-methoxy-2′-phenylethyl)-7-methyl-8-oxo-3-oxabicyclo[3.3.0]octane 344b



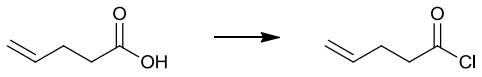
340d **344a** **344b**

Following **general procedure F** for the aldol cyclisation of *N*-acyloxazolidines, *N*-acyl-oxazolidine **340d** (400 mg, 0.95 mmol) and NaOMe (57 mg, 1.05 mmol) were reacted to give the crude reaction mixture. Purification *via* column chromatography (SiO_2, eluent 20:1 40-60 petrol:EtOAc) gave **344a** as a white solid (54 mg, 14%); R_f 0.1 (eluent 8:1, 40-60 petrol:EtOAc); mp 198-201 ºC; $[\alpha]_D^{23}$ +4.9 (*c* 0.63 in $CHCl_3$); ν_{max} (film) 3315, 2937, 1736, 1682; δ_H (400 MHz, $CDCl_3$) 0.94 (9H, s, C(C*H*$_3$)$_3$), 1.15 (3H, d, *J* 7.0, C(7)C*H*$_3$), 1.72 (3H, d, *J* 6.6, C(4)C*H*$_3$), 2.63-2.77 (2H, m, C(2′)*H*$_2$), 2.96 (3H, s, OC*H*$_3$), 3.20 (1H, q, *J* 7.0, C(7)*H*), 3.23 (1H, s, O*H*), 3.66 (1H, dd, *J* 10.0, 2.4, C(1′)*H*), 3.83 (3H, s, CO_2CH_3), 4.80 (1H, q, *J* 6.6, C(4)*H*), 5.06 (1H, s, C(2)*H*), 7.21-7.35 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 7.6 (C(7)*C*H$_3$), 16.2 (C(4)*C*H$_3$), 25.7 (C(*C*H$_3$)$_3$), 37.3 (*C*Me$_3$), 38.4 (*C*(2′)), 45.2 (*C*(7)), 52.8 (CO_2CH_3), 61.7 (O*C*H$_3$), 76.7 (*C*(5)), 79.3 (*C*(4)), 83.3 (*C*(1′)), 87.3 (*C*(6)), 96.1 (*C*(2)), 126.7 (*p-Ph*), 128.6, 129.3 (*o,m-Ph*), 138.4 (*i-Ph*), 172.4 (*C*O_2Me), 179.6 (*C*(8)); *m/z* (ESI^+) 418 ($[M-H]^-$, 95%); HRMS (ESI^+) $C_{23}H_{34}NO_6^+$ ($[M+H]^+$) requires 420.2381, found 420.2375; and a 58:42 mixture **(F3)** of the starting material **340d** and **344b** as a colourless gum; R_f 0.25 (eluent 8:1 40-60 petrol:EtOAc); ν_{max} (film) 3447, 2936, 1728; **340d** - δ_H (400 MHz, $CDCl_3$) 0.90 (9H, s, C(C*H*$_3$)$_3$), 1.19 (3H, d, *J* 6.8, C(2′)C*H*$_3$), 1.50 (3H, d, *J* 6.1, C(5)C*H*$_3$), 2.63 (1H, q, *J* 6.8, C(2′)*H*), 2.88 (1H, dd, *J* 14.0, 5.2, C(5′)*H*$_A$H$_B$), 3.12 (1H, dd, *J* 14.0, 5.2, C(5′)H$_A$*H*$_B$), 3.30 (3H, s, OC*H*$_3$), 3.72 (3H, s, CO_2CH_3), 3.93 (1H, dd, *J* 5.2, 4.4, C(4′)*H*), 4.27 (1H, d, *J* 7.1, C(4)*H*), 4.51-4.58 (1H, m, C(5)*H*), 5.50 (1H, s, C(2)*H*), 7.12-7.32 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 8.2 (C(2′)*C*H$_3$), 21.0 (*C*(5)CH$_3$), 25.8/26.0 (C(*C*H$_3$)$_3$), 37.2/38.6 (*C*Me$_3$), 37.9 (*C*(5′)), 50.1 (*C*(2′)), 58.0/58.2 (O*C*H$_3$), 66.2 (*C*(4)), 77.6 (*C*(5)), 86.3 (*C*(4′)), 96.6 (*C*(2)), 126.3,

126.6, 128.3, 128.6, 129.1, 129.3, 129.4, 129.8 (*o,m,p-Ph*), 136.5 (*i-Ph*), 170.4 (*C*O_2Me), 172.8 (*C*(1′)), 206.8 (*C*(3′)); **344b** - δ_H (400 MHz, $CDCl_3$) 0.92 (9H, s, C(CH_3)$_3$), 1.21 (3H, d, *J* 7.3, C(7)CH_3), 1.58 (3H, d, *J* 6.8, C(4)CH_3), 2.75 (1H, dd, *J* 14.4, 6.6, C(2′)H_AH_B), 3.06 (3H, s, OCH_3), 3.24 (1H, dd, *J* 14.4, 4.6, C(2′)H_AH_B), 3.57 (1H, q, *J* 7.1, C(7)*H*), 3.76 (3H, s, CO_2CH_3), 3.77-3.81 (1H, m, C(1′)*H*), 4.73 (1H, q, *J* 6.8 C(4)*H*), 4.99 (1H, s, C(2)*H*), 7.12-7.32 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 12.9 (C(7)*C*H_3), 15.4 (C(4)*C*H_3), 25.8/26.0 (C(*C*H_3)$_3$), 36.9 (*C*(2′)), 37.2/38.6 (*C*Me$_3$), 44.9 (*C*(7)), 52.4 (CO_2CH_3), 58.0/58.2 (OCH_3), 76.54 (*C*(5)), 79.0 (*C*(4)), 82.7 (*C*(1′)), 88.7 (*C*(6)), 96.4 (*C*(2)), 140.1 (*i-Ph*), 171.6 (*C*O_2Me), 180.7 (*C*(8)); *m/z* (ESI$^+$) 420 ($[M+H]^+$, 60%), 442 ($[M+Na]^+$, 90%), 418 ($[M-H]^-$, 100%); HRMS (ESI$^+$) $C_{23}H_{34}NO_6^+$ ($[M+H]^+$) requires 420.2381, found 420.2374.

4.3.4 Experimental for varying C(6) group by using different acid chlorides

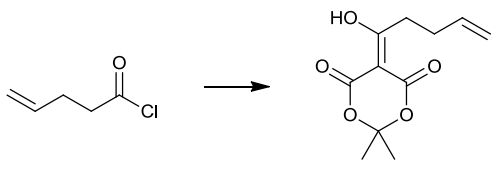
4-Pentenoyl chloride 345a[22]



345a

A solution of 4-pentenoic acid (500 mg, 5 mmol) in DCM (3 mL) was stirred at rt, and oxalyl chloride (0.44 mL, 5.0 mmol) was added dropwise over 5 min. The resulting solution was heated to 40 °C for 2 h. The mixture was then concentrated *in vacuo* before a portion of DCM (2 mL) was added and the solution was reconcentrated to give **345a** as a pale pink oil (405 mg, 68%); δ_H (400 MHz, $CDCl_3$) 2.43-2.50 (2H, m, C(3)H_2), 3.00 (2H, t, *J* 7.2, C(2)H_2), 5.07-5.16 (2H, m, C(5)H_2), 5.74-5.86 (1H, m, C(4)*H*); δ_C (100 MHz, $CDCl_3$) 28.9 (*C*(3)), 46.2 (*C*(2)), 116.9 (*C*(5)), 134.6 (*C*(6)), 173.2 (*C*(1)).

5-(4′-Pentenoyl)-2,2-dimethyl-1,3-dioxane-4,6-dione 346a

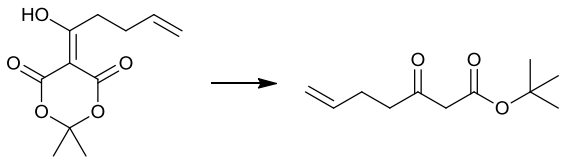


345a **346a**

Pyridine (0.49 mL, 6.13 mmol) was added to a solution of Meldrum's acid (447 mg, 3.1 mmol) in DCM (5 mL) at −10 °C and stirred for 20 min. A solution of acid chloride **345a**

(405 mg, 3.41 mmol) in DCM (2 mL) was added dropwise and the resulting mixture was stirred for 1 h at −10 °C before being warmed to rt and stirred for a further 2 h. The mixture was quenched with 1M HCl (5 mL) and extracted with DCM (3 × 5 mL). The combined organic layers were washed with brine (10 mL), dried and concentrated *in vacuo* to give **346a** as a yellow oil (644 mg, 84%) which was used without further purification; ν_{max} (film) 3080, 3002, 2922, 1739, 1667, 1574; δ_H (400 MHz, $CDCl_3$) 1.74 (3H, s, CH_3), 1.79 (3H, s, CH_3), 2.44-2.51 (2H, m, C(3′)H_2), 3.21 (2H, t, *J* 7.5, C(2′)H_2), 5.01-5.13 (2H, m, C(5′)H_2), 5.78-5.91 (1H, m, C(4′)*H*); δ_C (100 MHz, $CDCl_3$) 26.8, 27.6 (*C*H_3), 29.8 (*C*(3′)), 34.9 (*C*(2′)), 91.6 (*C*(5′)), 104.9 (C(2)), 116.2 (C(5′)), 136.0 (*C*(4′)), 170.5 (*C*(1), *C*(3)), 197.0 (*C*(1′)); *m/z* (ESI^-) 255 ($[M-H]^-$, 25%); HRMS (ESI^+) $C_{11}H_{14}NaO_5^+$ ($[M+Na]^+$) requires 249.0733, found 249.0739.

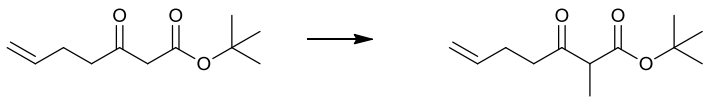
_t_-Butyl 3-oxohept-6-enoate 347a



346a **347a**

A solution of **346a** (312 mg, 1.4 mmol) in 1:1 toluene:*t*-BuOH (4 mL) was stirred at reflux for 2 h. The mixture was cooled to rt and concentrated *in vacuo* to give **347a** as a yellow liquid (290 mg, quant.); R_f 0.55 (eluent 50:1 petrol:EtOAc); ν_{max} (film) 3080, 2981, 1734, 1643; δ_H (400 MHz, $CDCl_3$) 1.47 (9H, s, C(CH_3)$_3$), 2.32-2.39 (2H, m, C(5)H_2), 2.64 (2H, t, *J* 7.3, C(4)H_2), 3.36 (2H, s, C(2)*H*), 4.97-5.11 (2H, m, C(7)H_2), 5.75-5.89 (1H, m, C(6)*H*); δ_C (100 MHz, $CDCl_3$) 27.4 (*C*(5)), 27.9 (C(*C*H_3)$_3$), 41.9 (*C*(4)), 50.7 (*C*(2)), 82.0 (*C*Me$_3$), 115.4 (*C*(7)), 136.7 (*C*(6)), 166.5 (*C*(1)), 202.3 (*C*(3)); *m/z* (ESI^+) 221 ($[M+Na]^+$, 75%), 419 ($[2M+Na]^+$, 100%); HRMS (ESI^+) $C_{11}H_{18}NaO_3^+$ ($[M+Na]^+$) requires 221.1148, found 221.1144.

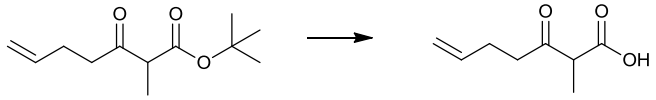
_t_-Butyl 2-methyl-3-oxohept-6-enoate 348a[23]



347a 348a

A solution of **347a** (277 mg, 1.4 mmol) in THF (7 mL) was stirred at 0 °C and *t*BuOK (180 mg, 1.46 mmol) was added. The mixture was then warmed to rt and stirred for 40 min before the addition of MeI (0.09 mL, 1.46 mmol). The mixture was left to stir for a further 5 h at rt. After this time the mixture was partitioned between Et_2O (15 mL) and brine (15 mL). The aqueous layer was extracted with Et_2O (2 × 10 mL) and the combined organic layers were dried and concentrated *in vacuo*. Purification by column chromatography (SiO_2, eluent 100:1 to 50:1 petrol:EtOAc) gave **348a** as a yellow oil (128 mg, 43%); R_f 0.45 (eluent 50:1 petrol:EtOAc); ν_{max} (film) 2980, 1715, 1642; δ_H (400 MHz, $CDCl_3$) 1.28 (3H, d, *J* 7.1, C(2)CH_3), 1.45 (9H, s, C(CH_3)$_3$), 2.30-2.37 (2H, m, C(5)H_2), 2.50-2.73 (2H, m, C(4)H_2), 3.42 (1H, q, *J* 7.1, C(2)*H*), 4.96-5.06 (2H, m, C(7)H_2), 5.74-5.85 (1H, m, C(6)*H*); δ_C (100 MHz, $CDCl_3$) 12.6 (C(2)*C*H$_3$), 27.6 (*C*(5)), 27.9 (C(*C*H$_3$)$_3$), 40.4 (*C*(4)), 53.9 (*C*(2)), 81.7 (*C*Me$_3$), 115.3 (*C*(7)), 136.9 (*C*(6)), 169.7 (*C*(1)), 205.4 (*C*(3)); *m/z* (ESI$^+$) 235 ([M+Na]$^+$, 100%).

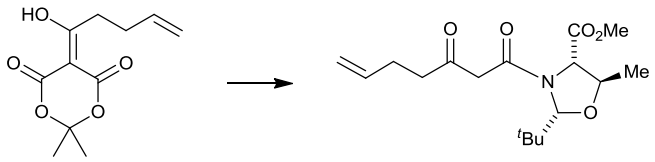
2-methyl-3-oxohept-6-enoic acid 350a



348a 350a

A solution of **348a** (128 mg, 0.60 mmol) in DCM (0.7 mL) was cooled to 0 °C and TFA (0.7 mL) was added dropwise. The resulting solution was stirred for 1.5 h at rt before the solvent was removed *in vacuo* to give **350a** as a colourless oil (103 mg, quant); ν_{max} (film) 2984, 1712, 1642; δ_H (400 MHz, $CDCl_3$) 1.39 (3H×0.5, d, *J* 7.3, C(2)CH_3-keto), 1.42 (3H×0.5, s, C(2)CH_3-enol), 2.30-2.40 (2H, m, C(5)H_2), 2.50-2.81 (2H, m, C(4)H_2), 3.59 (1H×0.5, q, *J* 7.3, C(2)*H*-keto), 4.96-5.09 (2H, m, C(7)H_2), 5.74-5.87 (1H, m, C(6)H_2); δ_C (125 MHz, $CDCl_3$) 12.9 (C(2)*C*H$_3$-keto), 22.0 (C(2)*C*H$_3$-enol), 27.4, 27.8 (*C*(5)), 37.2, 41.1 (*C*(4)), 52.2 (*C*(2)), 115.2, 115.6 (*C*(7)), 136.5, 137.3 (*C*(6)), 178.9 (*C*(1)), 205.1 (*C*(3)); *m/z* (FI$^+$) 156 ([M]$^+$, 100%); HRMS (FI$^+$) $C_8H_{12}O_3^+$ ([M]$^+$) requires 156.0786, found 156.0784.

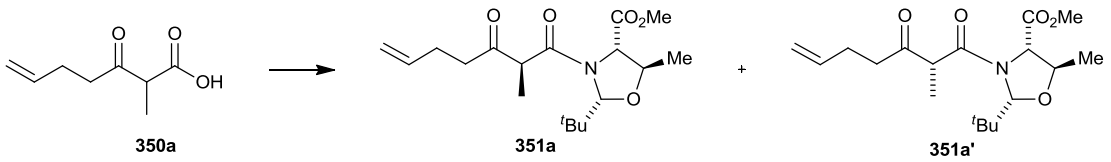
(2*R*,4*S*,5*R*)-2-*t*-Butyl-3-(3′-oxohept-6′-enoyl)-4-methoxycarbonyl-5-methyloxazolidine 349a



346a 349a

Acylated Meldrum's acid **346a** (2.5 g, 11.0 mmol) was dissolved in MeCN (25 mL) and oxazolidine **320** (2.11 mg, 10.5 mmol) in MeCN (25 mL) was added. The resulting mixture was stirred at 60 °C for 2 h and then concentrated *in vacuo* and purified by column chromatography (SiO_2, eluent 20:2 to 5:1 petrol:EtOAc) to give **349a** as a yellow oil (1.86 g, 52%); R_f 0.2 (eluent 20:1 petrol:EtOAc); ν_{max} (film) 2958, 1753, 1667, 1632; δ_H (400 MHz, $CDCl_3$) Of the major component; 0.91 (9H, s, C(CH_3)$_3$), 1.35 (3H, d, *J* 6.3, C(5)CH_3), 2.28-2.38 (2H, m, C(5′)H_2), 2.59-2.81 (2H, m, C(4′)H_2), 3.60 (2H, AB q, *J* 14.8, C(2′)H_2), 3.79 (3H, s, CO_2CH_3), 4.27 (1H, d, *J* 3.8, C(4)*H*), 4.72-4.49 (1H, m, C(5)*H*), 4.96-5.09 (2H, m, C(7′)H_2), 5.41 (1H, s, C(2)*H*), 5.74-5.86 (1H, m, C(6′)*H*); δ_C (100 MHz, $CDCl_3$) 20.1 (C(5)*C*H$_3$), 25.8 (C(*C*H$_3$)$_3$), 26.7 (*C*(5′)), 37.8 (*C*Me$_3$), 42.3 (*C*(4′)), 50.7 (*C*(2′)), 52.5 (CO_2CH_3), 65.3 (*C*(4)), 76.1 (*C*(5)), 96.1 (*C*(2)), 115.5 (*C*(7′)), 136.6 (*C*(6′)), 168.0 (*C*(1′)), 170.3 (*C*O$_2$Me), 203.8 (*C*(3′)); *m/z* (ESI$^+$) 326 ([M+H]$^+$, 55%), 348 ([M+Na]$^+$, 70%), 673 ([2M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{17}H_{27}NNaO_5^+$ ([M+Na]$^+$) requires 348.1777, found 348.1781.

(2*R*,4*S*,5*R*,2′*R*)- and (2*R*,4*S*,5*R*,2′*S*)-2-*t*-Butyl-3-(3′-oxo-2′-methylhept-6′-enoyl)-4-methoxycarbonyl-5-methyloxazolidine 351a and 351a′

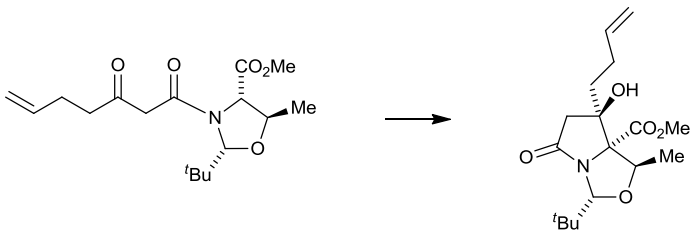


350a 351a 351a'

Follwing **General Procedure D** for the synthesis of *N*-acyloxazolidines, oxazolidine **320** (522 mg, 2.6 mmol) was reacted with DCC (562 mg, 2.78 mmol), DMAP (23 mg, 7 mol%), and acid **350a** (434 mg, 2.78 mmol) in DCM (13 mL). Purification *via* column chromatography (SiO_2, eluent 10:1 petrol:EtOAc) gave **351a** as a colourless oil (130 mg, 14%); R_f 0.74 (eluent 3:1 petrol:EtOAc); $[\alpha]_D^{23}$ +2.42 (*c* 0.44 in $CHCl_3$); ν_{max} (film) 2977,

2958, 1747, 1729, 1664; δ_H (400 MHz, $CDCl_3$) 0.90 (9H, s, C(C*H*$_3$)$_3$), 1.32 (3H, d, *J* 6.1, C(5)C*H*$_3$), 1.50 (3H, d, *J* 6.8, C(2′)C*H*$_3$), 2.30 (2H, app. q, *J* 6.8, C(5′)*H*$_2$), 2.57-2.74 (2H, m, C(4′)*H*$_2$), 3.66 (1H, q, *J* 6.8, C(2′)*H*), 3.81 (3H, s, CO_2CH_3), 4.44 (1H, d, *J* 3.0, C(4)*H*), 4.73-4.82 (1H, m, C(5)*H*), 4.95-5.06 (2H, m, C(7′)*H*$_2$), 5.44 (1H, s, C(2)*H*), 5.72-5.84 (1H, m, C(6′)*H*); δ_C (125 MHz, $CDCl_3$) 15.0 (C(2′)*C*H$_3$), 20.2 (C(5)*C*H$_3$), 25.8 (C(*C*H$_3$)$_3$), 27.2 (*C*(5′)), 37.8, 38.3 (*C*(4′), *C*Me$_3$), 52.8 (CO_2CH_3), 54.1 (*C*(2′)), 65.0 (*C*(4)), 76.0 (*C*(5)), 96.0 (*C*(2)), 115.3 (*C*(7′)), 136.7 (*C*(6′)), 170.2 (*C*O_2Me), 171.5 (*C*(1′)), 207.6 (*C*(3′)); *m/z* (ESI$^+$) 362 ([M+Na]$^+$, 40%), 701 ([2M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{18}H_{29}NNaO_5^+$ ([M+Na]$^+$) requires 362.1938, found 362.1931; and **351a′** as a yellow oil (180 mg, 20%); R_f 0.1 (eluent 10:1 petrol:EtOAc); $[\alpha]_D^{23}$ −4.2 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 2977, 2958, 2936, 1747, 1728, 1663; δ_H (400 MHz, $CDCl_3$) 0.93 (9H, s, C(C*H*$_3$)$_3$), 1.29 (3H, d, *J* 7.1, C(2′)C*H*$_3$), 1.35 (3H, d, *J* 6.3, C(5)C*H*$_3$), 2.29-2.38 (2H, m, C(5′)*H*$_2$), 2.58-2.77 (2H, m, C(4′)*H*$_2$), 3.49 (1H, q, *J* 7.1, C(2′)*H*), 3.81 (3H, s, CO_2CH_3), 4.11 (1H, d, *J* 4.3, C(4)*H*), 4.74-4.81 (1H, m, C(5)*H*), 4.93-5.05 (2H, m, C(7′)*H*$_2$), 5.42 (1H, s, C(2)*H*), 5.71-5.86 (1H, m, C(6′)*H*); δ_C (100 MHz, $CDCl_3$) 12.9 (C(2′)*C*H$_3$), 20.3 (C(5)*C*H$_3$), 25.9 (C(*C*H$_3$)$_3$), 27.5 (*C*(5′)), 38.0 (*C*Me$_3$), 39.1 (*C*(4′)), 52.4, 52.9 (*C*(2′), CO_2CH_3), 65.8 (*C*(4)), 75.9 (*C*(5)), 96.2 (*C*(2)), 115.1 (*C*(7′)), 137.1 (*C*(6′)), 169.9 (*C*O_2Me), 172.4 (*C*(1′)), 204.2 (*C*(3′)); *m/z* (ESI$^+$) 362 ([M+Na]$^+$, 30%), 701, ([2M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{18}H_{29}NNaO_5^+$ ([M+Na]$^+$) requires 362.1938, found 362.1925.

(2*R*,5*R*,4*R*,6*R*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(but-3′-enyl)-8-oxo-oxabicyclo[3.3.0]octane 353a

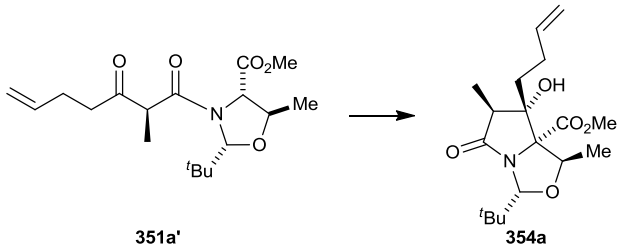


349a **353a**

Following **General Procedure F** for the aldol cyclisation of *N*-acyloxazolidines, *N*-acyloxazolidine **349a** (143 mg, 0.44 mmol) and NaOMe (26 mg, 0.48 mmol) were reacted to give a crude mixture which was purified *via* column chromatography (SiO_2, eluent 20:1-6:1)

to give **349a** (74 mg, 51%) and **353a** as a colourless oil (67 g, 46%); R_f 0.6 (eluent 2:1 petrol:EtOAc); $[\alpha]_D^{23}$ +18.0 (*c* 0.95 in $CHCl_3$); ν_{max} (film) 3418 (OH), 3078, 2955, 1702 (ester C=O), 1642 (amide C=O); δ_H (400 MHz, $CDCl_3$) 0.91 (9H, s, C(CH_3)$_3$), 1.33-1.43 (1H, m, C(1′)H_AH$_B$), 1.70 (3H, d, *J* 6.6, C(4)CH_3), 1.90-1.98 (1H, m, C(1′)H$_A$$H_B$), 1.99-2.10 (1H, m, C(2′)H_AH$_B$), 2.30-2.37 (1H, m, C(2′)H$_A$$H_B$), 2.37 (1H, d, *J* 15.8, C(7)H_AH$_B$), 3.04 (1H, d, *J* 15.8, C(7)H$_A$$H_B$), 3.14 (1H, br s, O*H*), 3H, s, CO_2CH_3), 4.79 (1H, q, *J* 6.6, C(4)*H*), 4.98-5.10 (2H, m, C(4′)H_2), 5.06 (1H, s, C(2)*H*), 5.76-5.87 (1H, m, C(3′)*H*); δ_C (100 MHz, $CDCl_3$) 15.3 (C(4)*C*H$_3$), 25.7 (C(*C*H$_3$)$_3$), 28.6 (*C*(2′)), 35.0 (*C*(1′)), 37.4 (*C*Me$_3$), 47.4 (*C*(7)), 52.7 (CO_2CH_3), 78.5 (*C*(4)), 80.9, 84.8 (*C*(6), *C*(5)), 96.2 (*C*(2)), 115.5 (*C*(4′)), 137.8 (*C*(3′)), 172.0, 178.5 (*C*O$_2$Me, *C*(8)); *m/z* (ESI$^+$) 348 ([M+Na]$^+$, 90%), 673 ([2M+Na]$^+$, 100%), 324 ([M−H]$^-$, 100%); HRMS (ESI$^+$) $C_{17}H_{27}NNaO_5^+$ ([M+H]$^+$) requires 348.1781, found 348.1771.

(2*R*,5*R*,4*R*,6*R*,7*S*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(but-3′-enyl)-7-methyl-8-oxo-oxabicyclo[3.3.0]octane 354a and (2*R*,4*S*,5*R*,2′*R*)-2-*t*-Butyl-3-(3′-oxo-2′-methylhept-6′-enoyl)-4-methoxycarbonyl-5-methyloxazolidine 351a

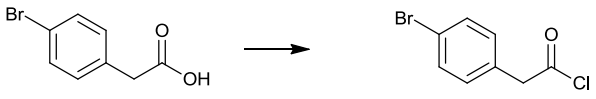


351a' **354a**

Following **General Procedure F**, *N*-acyl-oxazolidine **351a′** (258 mg, 0.76 mmol) and NaOMe (45 mg, 0.84 mmol) were reacted to give a crude mixture which was purified *via* column chromatography (SiO_2, eluent 10:1 petrol:EtOAc) to give **354a** as a colourless oil (132 mg, 51%); R_f 0.56 (eluent 3:1 40-60 petrol:EtOAc); $[\alpha]_D^{23}$ +1.88 (*c* 0.65 in $CHCl_3$); ν_{max} (film) 3454 (OH), 3071, 2957, 2251, 1725; δ_H (400 MHz, $CDCl_3$) Major – 0.91 (9H, s, C(CH_3)$_3$), 1.11 (3H, d, *J* 7.2, C(7)*C*H$_3$), 1.68 (3H, d, *J* 6.6, C(4)CH_3), 1.70-1.80 (2H, m, C(1′)H_2), 2.08-2.20 (1H, m, C(2′)H_AH$_B$), 2.21-2.31 (1H, m, C(2′)H$_A$$H_B$), 3.11 (1H, q, *J* 7.2, C(7)*H*), 3.79 (3H, s, CO_2CH_3), 4.81 (1H, q, *J* 6.6, C(4)*H*), 4.94-5.07 (2H, m, C(4′)H_2), 5.01 (1H, s, C(2)*H*), 5.73-5.85 (1H, m, C(3′)*H*); δ_C (125 MHz, $CDCl_3$) 7.4 (C(7)*C*H$_3$), 15.9 (C(4)*C*H$_3$), 28.3 (*C*(2′)), 35.0 (*C*(1′)), 37.3 (*C*(CH$_3$)$_3$), 48.3 (*C*(7)), 52.7 (CO_2CH_3), 78.8

(*C*(4)), 79.1 (*C*(5)), 86.3 (*C*(6)), 96.1, (*C*(2)), 115.3 (*C*(4′)), 137.8 (*C*(3′)), 172.0 (*C*O_2Me), 180.0 (*C*(8)); *m/z* (ESI$^+$) 362 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{18}H_{29}NNaO_5^+$ ([M+Na]$^+$) requires 362.1938, found 362.1923; and **351a** (50 mg, 38%).

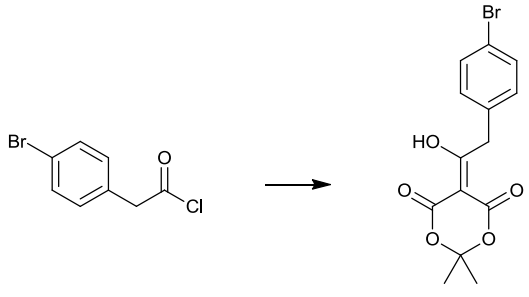
4-Bromophenylacetyl chloride 345b[24]



345b

A solution of 4-bromophenylacetic acid (1.0 g, 4.65 mmol) in DCM (6 mL) was stirred at rt, and oxalyl chloride (0.6 mL, 6.9 mmol) was added dropwise over 5 min. The resulting solution was heated to 40 °C for 4 h. The mixture was then concentrated *in vacuo* before a portion of DCM (3 mL) was added and the solution reconcentrated to give **345b** as a colourless oil (1.13 g, quant); δ_H (400 MHz, $CDCl_3$) 4.11 (2H, s, CH_2), 7.16 (2H, d, *J* 8.2, C(2)*H*, C(6)*H*), 7.52 (2H, d, *J* 8.2, C(3)*H*, C(5)*H*); δ_C (100 MHz, $CDCl_3$) 52.3 (*C*H_2), 122.4 (*C*(1)), 130.2 (*C*(4)), 131.2, 132.1 (*C*(2), *C*(3), *C*(5), *C*(6)), 171.5 (*C*=O); *m/z* (TOF FI$^+$) 233 (M$^+$, 100%).

5-(4′-Bromophenylacetoyl)-2,2-dimethyl-1,3-dioxane-4,6-dione 346b

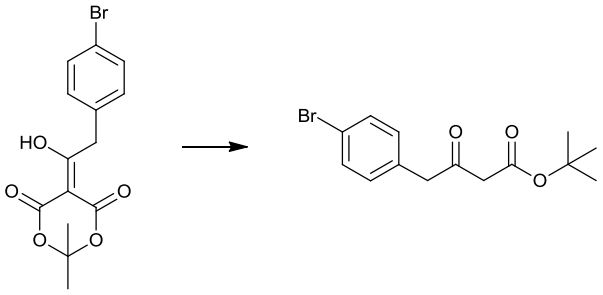


345b **346b**

Pyridine (0.75 mL, 9.3 mmol) was added to a solution of Meldrum's acid (670 mg, 4.65 mmol) in DCM (20 mL) at −10 °C and stirred for 20 min. A solution of acid chloride **345b** (1.08 g, 4.65 mmol) in DCM (5 mL) was added dropwise and the resulting mixture was stirred for 1 h at −10 °C before being warmed to rt and stirred for a further 2 h. The mixture was quenched with 1M HCl (10 mL) and extracted with DCM (3 × 15 mL). The combined organic layers were washed with brine (20 mL), dried and concentrated *in vacuo* to give **346b** as a red solid (1.49 g, 93%); mp 112-115 ºC; ν_{max} (film) 3000, 1741, 1648; δ_H (400 MHz,

$CDCl_3$) 1.73 (6H, s, CH_3), 4.37 (2H, s, CH_2), 7.27 (2H, d, *J* 8.3, C(2′)*H*, C(6′)*H*), 7.46 (2H, d, *J* 8.3, C(3′)*H*, C(5′)*H*); δ_C (100 MHz, $CDCl_3$) 26.8 (*C*H_3), 40.2 (*C*H_2), 91.5 (*C*(5)), 105.1 (*C*(2)), 121.7 (*C*(4′)), 131.3 (*C*(2′), *C*(6′)), 131.8 (*C*(3′), *C*(5′)), 133.0 (*C*(1′)), 170.5 (*C*(4), *C*(6)), 193.8 (*C*=O); *m/z* (ESI^-) 388 ($[M-H]^-$, 100%); HRMS (ESI^+) $C_{14}H_{13}BrO_5^+$ ($[M+Na]^+$) requires 362.9839, 364.9829, found 362.9826, 364.9807.

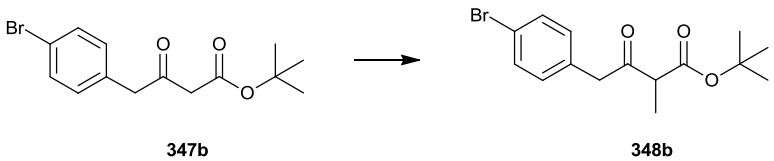
_t_-Butyl 4-(4′-bromophenyl)3-oxobutanoate 347b



346b **347b**

A solution of **346b** (750 mg, 2.19 mmol) in 1:1 toluene:*t*-BuOH (10 mL) was stirred at reflux for 2 h. The mixture was cooled to rt and concentrated *in vacuo* to give **347b** as a pale yellow oil (680 mg, quant); R_f 0.6 (eluent, 5:1 petrol:EtOAc) ν_{max} (film) 2979, 1731, 1648; δ_H (400 MHz, $CDCl_3$) 1.49 (9H, s, C(CH_3)$_3$), 3.41 and 3.79 (2×2H, s, C(2)H_2, C(4)H_2), 7.10 (2H, d, *J* 7.3, C(2′)*H*, C(6′)*H*), 7.49 (2H, d, *J* 7.3, C(3′)*H*, C(5′)*H*); δ_C (100 MHz, $CDCl_3$) 28.0 (C(*C*H_3)$_3$), 49.1, 49.8 (*C*(2), *C*(4)), 82.3 (*C*Me$_3$), 121.4 (*C*(4′)), 131.3 (*C*(2′), *C*(6′)), 131.9 (*C*(3′), *C*(5′)), 132.3 (*C*(1′)), 166.2 (*C*(1)), 200.2 (*C*(3)); *m/z* (ESI^+) 335, 337 ($[M+Na]^+$, 95%); HRMS (ESI^+) $C_{14}H_{17}BrNaO_3^+$ ($[M+Na]^+$) requires 335.0253, 337.0233, found 335.0243, 337.0225.

***t*Butyl 4-(4′-bromophenyl)-2-methyl-3-oxobutanaoate 348b**

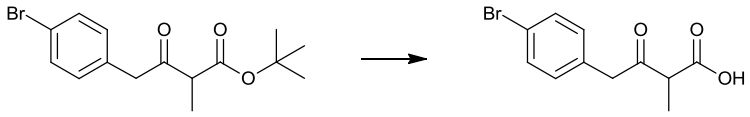


347b **348b**

A solution of **347b** (638 mg, 2.19 mmol) in THF (15 mL) was stirred at 0 °C and *t*BuOK (280 mg, 2.29 mmol) was added. The mixture was then warmed to rt and stirred for 40 min before the addition of MeI (0.14 mL, 2.29 mmol). The mixture was left to stir for a further 5 h at rt. After this time the mixture was partitioned between Et_2O (25 mL) and brine (20 mL). The

aqueous layer was extracted with Et_2O (2 × 10 mL) and the combined organic layers were dried and concentrated *in vacuo* to give **348b** as a yellow oil (631 mg, 88%); R_f 0.75 (eluent 5:1 40-60 petrol:EtOAc); ν_{max} (film) 2983, 2937, 2360, 1745, 1715; δ_H (400 MHz, $CDCl_3$) 1.29 (3H, d, *J* 7.1, C(2)CH_3), 1.46 (9H, s, C(CH_3)$_3$), 3.53 (1H, q, *J* 7.1, C(2)*H*), 3.76 (1H, d, *J* 16.4, C(4)H_AH$_B$), 3.84 (1H, d, *J* 16.4, C(4)H$_A$$H_B$), 7.08 (2H, d, *J* 8.5, C(2′)*H*, C(6′)*H*), 7.45 (2H, d, *J* 8.5, C(3′)*H*, C(5′)*H*); δ_C (100 MHz, $CDCl_3$) 12.7 (C(2)*C*H$_3$), 27.9 (C(*C*H$_3$)$_3$), 47.7 (*C*(4)), 53.1 (*C*(2)), 82.1 (*C*Me$_3$), 121.2 (*C*(4′)), 131.1 (*C*(2′), *C*(6′)), 131.5 (*C*(3′), *C*(5′)), 132.6 (*C*(1′)), 169.4 (*C*(1)), 203.0 (*C*(3)); *m/z* (ESI$^+$) 349, 351 ([M+Na]$^+$, 80%), *m/z* (ESI$^+$) 675, 677, 679 ([2M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{15}H_{19}BrO_3^+$ ([M+Na]$^+$) requires 349.0410, 351.0390, found 349.0396, 351.0378.

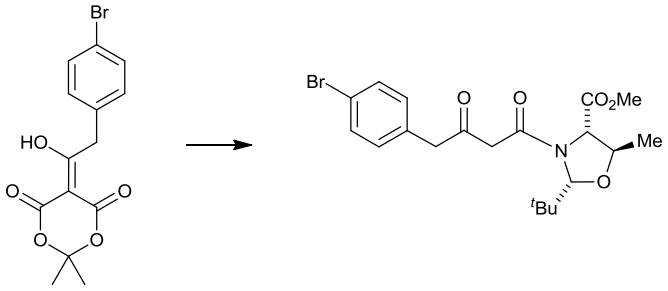
4-(4′-bromophenyl)-2-methyl-3-oxobutaoic acid 350b



348b **350b**

A solution of **348b** (631 mg, 1.92 mmol) in DCM (4 mL) was cooled to 0 °C and TFA (4 mL) was added dropwise. The resulting solution was stirred for 1.5 h at rt before the solvent was removed *in vacuo* to give **350b** as a yellow oil (499 mg, 95%) in a 3:1 mixture of keto and enol forms; ν_{max} (film) 2980, 1712; δ_H (400 MHz, $CDCl_3$) keto – 1.37 (3H, d, *J* 7.1, C(2)CH_3), 3.69 (1H, q, *J* 7.1, C(2)*H*), 3.84 (1H, d, *J* 16.0, C(4)H_AH$_B$), 3.90 (1H, d, *J* 16.0, C(4)H$_A$$H_B$), 7.06-7.12 (2H, m, *Ph*), 7.42-7.50 (2H, m, *Ph*), 12.40 (1H, br s, O*H*), enol – 1.47 (3H, s, C(2)CH_3), 3.66 (2H, s, C(4)H_2), 7.06-7.12 (2H, m, *Ph*), 7.42-7.50 (2H, m, *Ph*), 12.40 (1H, br s, O*H*); δ_C (100 MHz, $CDCl_3$) 12.8 (C(2)CH3 keto), 22.0 (C(2)*C*H$_3$ enol), 48.0 (*C*(4) keto), 48.9 (*C*(4) enol), 51.5 (*C*(2) keto), 121.0, 121.4 (*C*(4′)), 131.1, 131.3, 131.6, 131.8, 131.9, 132.0, 133.3 (*Ph*, *C*(2) enol), 154.1, 154.2 (*C*(1′)), 175.4 (*C*(1)), 202.4 (*C*(3) keto), 202.4 (*C*(3) enol); *m/z* (ESI$^+$) 292, 294 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{11}H_{11}BrNaO_3^+$ ([M+Na]$^+$) requires 292.9784, 294.9764, found 292.9780, 294.9763.

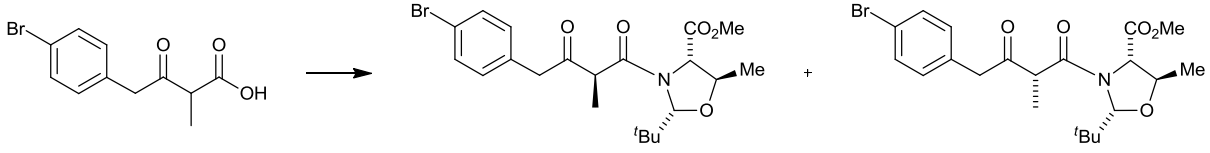
(2*R*,4*S*,5*R*)-2-*t*-Butyl-3-(3′-oxo-4′-(4′′-*p*-bromophenyl)butanoyl)-4-methoxycarbonyl-5-methyloxazolidine 349b



346b **349b**

Acylated Meldrum's acid **346b** (700 mg, 2.05 mmol) was dissolved in MeCN (10 mL) and oxazolidine **320** (392 mg, 1.95 mmol) in MeCN (5 mL) was added. The resulting mixture was stirred at 60 °C for 2 h and then concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 10:1 petrol:EtOAc) gave **349b** as a colourless oil (524 mg, 58%); R_f 0.45 (eluent 3:1 petrol:EtOAc); ν_{max} (film) 2957, 1747, 1685; δ_H (500 MHz, MeOD) 0.90 (9H, s, C(C*H*$_3$)$_3$), 1.32 (3H, d, *J* 6.0, C(5)C*H*$_3$), 3.77 (3H, s, CO_2CH_3), 3.87 (2H, AB q, *J* 16.4, C(4′)*H*$_2$), 4.31 (1H, d, *J* 3.4, C(4)*H*), 4.73-4.79 (1H, m, C(5)*H*), 5.36 (1H, s, C(2)*H*), 7.18 (2H, d, *J* 8.2, *o-Ph*), 7.50 (2H, d, *J* 8.2, *m-Ph*); δ_C (125 MHz, MeOD) 20.4 (C(5)*C*H$_3$), 26.4 (C(*C*H$_3$)$_3$), 38.8 (*C*Me$_3$), 53.3 (*C*(4′)), 66.5 (*C*(4)), 77.5 (*C*(5)), 97.2 (*C*(2)), 122.2 (*C*Br), 132.7, `132.9 (*o,m-Ph*), 134.5 (*i-Ph*), 171.0, 171.5 (CO_2CH_3, *C*(1′)), 203.4 (*C*(3′)); *m/z* (ESI$^+$) 462/464 ([M+Na]$^+$, 75%), 903 ([2M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{20}H_{26}BrNNaO_6^+$ ([M+Na]$^+$) requires 462.0887, 464.0867, found 462.0869, 464.0850.

(2*R*,4*S*,5*R*,2′*R*)- and (2*R*,4*S*,5*R*,2′*S*)-2-*t*-Butyl-3-(3′-oxo-4′-(4′′-bromophenyl)-2′-methylbutanoyl)-4-methoxycarbonyl-5-methyloxazolidine 351b and 351b′

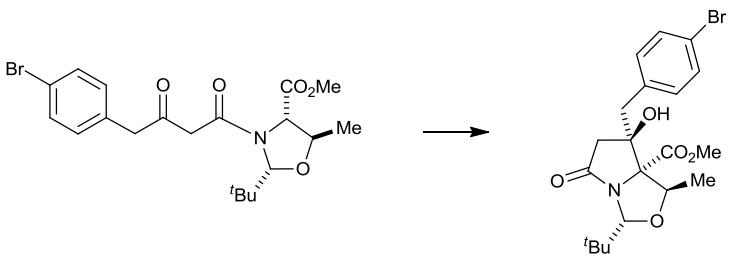


350b **351b** **351b'**

Following **General procedure D** for the synthesis of *N*-acyloxazolidines, oxazolidine **320** (336 mg, 1.67 mmol) was reacted with DCC (344 mg, 1.67 mmol), DMAP (14 mg, 7 mol%), and the acid **350b** (477 mg, 1.76 mmol). Purification *via* column chromatography (SiO_2, eluent 10:1 40-60 petrol:EtOAc) gave **351b** as a pale yellow oil (86 mg, 11%); R_f 0.4 (eluent

5:1 40-60 petrol:EtOAc); $[\alpha]_D^{23}$ +19.2 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 2957, 1748, 1713, 1662; δ_H (400 MHz, $CDCl_3$) 0.90 (9H, s, $C(CH_3)_3$), 1.19 (3H, d, *J* 6.1, C(5)CH_3), 1.53 (3H, d, *J* 6.9, C(2′)CH_3), 3.66 (1h, q, *J* 6.9, C(2′)*H*), 3.67 (3H, s, CO_2CH_3), 3.77-3.84 (2H, m, C(4′)H_2), 3.92 (1H, d, *J* 4.3, C(4)*H*), 4.65 (1H, qd, *J* 6.1, 4.3, C(5)*H*), 5.42 (1H, s, C(2)*H*), 7.05 (2H, d, *J* 8.3, C(2″)*H*, C(6″)*H*), 7.45 (2H, d, *J* 8.3, C(3″)*H*, C(5″)*H*); δ_C (100 MHz, $CDCl_3$) 15.0 (C(2′)CH_3), 20.0 (C(5)CH_3), 25.9 ($C(CH_3)_3$), 38.0 (CMe_3), 45.7 (*C*(4′)), 52.8, 53.0 (CO_2CH_3, *C*(2′)), 65.0 (*C*(4)), 76.2 (*C*(5)), 96.1 (*C*(2)), 121.4 (*C*(4″)), 131.3, 131.5, 131.7, 131.9 (*C*(2″), *C*(6″), *C*(3″), *C*(5″)), 132.3 (*C*(1″), 170.0, 171.4 (CO_2Me, *C*(1′)), 205.0 (*C*(3′)); *m/z* (FI^+) 453 (M^+, 100%), 455 (M^+, 100%); HRMS (FI^+) $C_{21}H_{28}BrNO_5^+$ (M^+) requires 453.1151, 455.1133, found 453.1380, 455.1338; and **351b′** as a yellow solid (185 mg, 24%); R_f 0.2 (eluent 5:1 40-60 petrol:EtOAc); mp 148-150 ºC; $[\alpha]_D^{23}$ +1.7 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 2958, 2359, 1747, 1733, 1662; δ_H (400 MHz, $CDCl_3$) 0.97 (9H, s, $C(CH_3)_3$), 1.32 (3H, d, *J* 6.9, C(2′)CH_3), 1.38 (3H, d, *J* 6.1, C(5)CH_3), 3.60 (1H, q, *J* 6.9, C(2′)*H*), 3.82 (3H, s, CO_2CH_3), 3.82-3.93 (2H, m, C(4′)H_2), 4.14 (1H, d, *J* 4.3, C(4)*H*), 4.75-4.83 (1H, m, C(5)*H*), 5.46 (1H, s, C(2)*H*), 7.10 (2H, d, *J* 8.3, C(2″)*H*, C(6″)*H*), 7.42 (2H, d, *J* 8.3, (C(3″)*H*, C(5″)*H*); δ_C (100 MHz, $CDCl_3$) 13.0 (C(2′)CH_3), 20.5 (C(5)CH_3), 26.0 ($C(CH_3)_3$), 38.2 (CMe_3), 45.9 (*C*(4′)), 52.6, 53.0 (CO_2CH_3, *C*(2′)), 65.8 (*C*(4)), 76.1 (*C*(5)), 96.4 (*C*(2)), 120.9 (*C*(4″)), 131.2, 131.4, 131.5, 131.9 (*C*(2″), *C*(6″), *C*(3″), *C*(5″)), 132.9 (*C*(1″)), 170.0, 172.1 (CO_2Me, *C*(1″)), 201.7 (*C*(3″)); *m/z* (FI^+) 453 (M^+, 100%), 455 (M^+, 100%); HRMS (FI^+) $C_{21}H_{28}BrNO_5^+$ (M^+) requires 453.1151, 455.1133, found 453.1393, 455.1442.

(2*R*,4*R*,5*R*,6*R*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(*p*-bromophenyl)-8-oxo-3-oxabicyclo[3.3.0]octane 353b

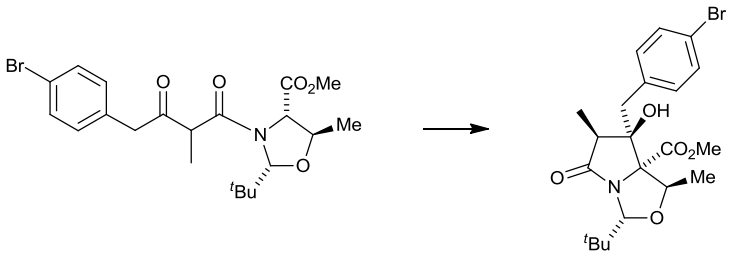


349b **353b**

Following **General Procedure F**, *N*-acyl-oxazolidine **349b** (460 mg, 1.05 mmol) and NaOMe (62 mg, 1.15 mmol) were reacted to give a 1:1 mixture of SM:P which was purified *via*

column chromatography (SiO_2, eluent DCM) to give **349b** (115 mg) and **353b** as a yellow solid (198 mg, 42%); R_f 0.1 (eluent DCM); mp 122-125 ºC; $[\alpha]_D^{23}$ −4.6 (*c* 0.63 in $CHCl_3$); ν_{max} (film) 3414, 2956, 2874, 1703, 1488; δ_H (400 MHz, $CDCl_3$) 0.89 (9H, s, C(CH_3)$_3$), 1.76 (3H, d, *J* 6.6, C(4)CH_3), 1.85 (1H, d, *J* 15.9, C(1′)H_AH$_B$), 2.45 (1H, d, *J* 13.4, C(7)H_AH$_B$), 3.04-3.09 (2H, m, C(1′)H$_A$$H_B$, C(7)H$_A$$H_B$), 3.19 (1H, br s, O*H*), 3.83 (3H, s, CO_2CH_3), 4.79 (1H, q, *J* 6.6, C(4)*H*), 4.99 (1H, s, C(2)*H*), 7.04 (2H, d, *J* 8.3, *o-Ph*), 7.39 (2H, d, *J* 8.3, *m-Ph*); δ_C (100 MHz, $CDCl_3$) 15.3 (C(4)*C*H$_3$), 25.7 (C(*C*H$_3$)$_3$), 37.4 (*C*Me$_3$), 41.3 (*C*(7)), 47.2 (*C*(1′)), 52.9 (CO_2CH_3), 78.6 (*C*(4)), 80.6 (*C*(5)), 83.9 (*C*(6)), 96.2 (*C*(2)), 121.2 (*i-Ph*), 131.7, 131.9 (*o,m-Ph*), 143.4 (*C*Br), 172.0 (*C*O$_2$Me), 178.0 (*C*(8)); *m/z* (ESI$^-$) 438/440 ([M−H]$^-$, 100%); HRMS (ESI$^+$) $C_{20}H_{26}NNaO_5^+$ ([M+Na]$^+$) requires 462.0887, 464.0867, found 462.0879, 464.0855.

(2*R*,4*R*,5*R*,6*R*,7*S*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(*p*-bromophenyl)-7-methyl-8-oxo-3-oxabicyclo[3.3.0]octane 354b



351b/b' **354b**

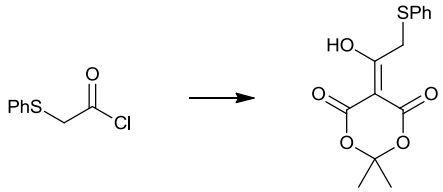
Following **General Procedure F**, *N*-acyl-oxazolidine **351b′** (171 mg, 0.38 mmol) and NaOMe (22 mg, 0.41 mmol) were reacted to give a 21:79 mixture of SM:P.

Following **General Procedure F**, *N*-acyl-oxazolidine **351b** (71 mg, 0.15 mmol) and NaOMe (10 mg, 0.17 mmol) were reacted to give a 22:78 mixture of SM:P.

The combined crude products from both reaction were purified by column chromatography (SiO_2, eluent 5:1 petrol:EtOAc) to give **345b** as a colourless solid (124 mg, 72%); R_f 0.35 (eluent 5:1 petrol:EtOAc); mp 150-153 ºC; $[\alpha]_D^{23}$ +1.82 (*c* 0.37 in $CHCl_3$); ν_{max} (film) 3458, 2955, 2873, 1708, 1659; δ_H (400 MHz, $CDCl_3$) 0.65 (3H, d, *J* 7.1, C(7)CH_3), 0.92 (9H, s, C(CH_3)$_3$), 1.66 (3H, d, *J* 6.6, C(4)CH_3), 2.09 (1H, br s, OH), 2.63 (1H, d, *J* 14.2, C(1′)H_AH$_B$), 3.17 (1H, d, *J* 14.2, C(1′)H$_A$$H_B$), 3.21 (1H, q, *J* 7.1, C(7)*H*), 3.85 (3H, s, CO_2CH_3), 4.83 (1H, q, *J* 6.6, C(4)*H*), 5.00 (1H, s, C(2)*H*), 7.14 (2H, d, *J* 8.3, *o-Ph*), 7.43 (2H, d, *J* 8.3, *m-Ph*); δ_C

(100 MHz, $CDCl_3$) 7.5 (C(7)*C*H_3), 15.5 (C(4)*C*H_3), 25.8 (C(*C*H_3)$_3$), 37.4 (*C*Me_3), 41.1 (*C*(1′)), 48.2 (*C*(7)), 52.9 (CO_2*C*H_3), 78.8 (*C*(4)), 79.1 (*C*(5)), 85.4 (*C*(6)), 96.6 (*C*(2)), 121.4 (*i-Ph*), 131.5 (*o-Ph*), 132.5 (*m-Ph*), 134.0 (*C*Br), 172.1 (*C*O_2Me), 180.3 (*C*(8)); *m/z* (ESI^+) 476/478 ($[M+Na]^+$, 35%), 929, 931, 933 ($[2M+Na]^+$, 100%); HRMS (ESI^+) $C_{21}H_{28}BrNNaO_5^+$ ($[M+Na]^+$) requires 476.1043, 478.1024, found 476.1047, 478.1032.

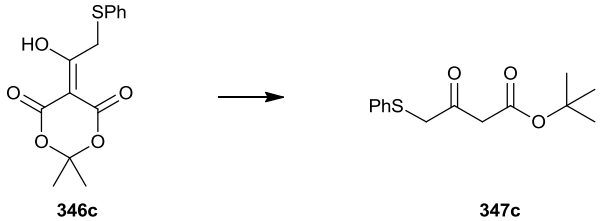
5-(1′-hydroxy-2′-(phenylthio)ethylidene))-2,2-dimethyl-1,3-dioxane-4,6-dione 346c



345c **346c**

Following **General Procedure E**, pyridine (0.86 mL, 10.7 mmol) and Meldrum's acid (771 mg, 5.35 mmol) in DCM (25 mL) were combined with a solution of acid chloride **345c** (1.00 g, 5.35 mmol) in DCM (5 mL) to give **346c** as an orange oil (1.62 g, quant); ν_{max} (film) 3000, 1737, 1666; δ_H (400 MHz, $CDCl_3$) 1.67 (6H, s, C*H*$_3$), 4.38 (2H, s, C(2′)*H*$_2$), 7.28-7.32 (3H, m, *Ph*), 7.47-7.51 (2H, m, *Ph*), 14.57 (1H, br s, O*H*); δ_C (100 MHz, $CDCl_3$) 26.8 (*C*H_3), 36.4 (*C*(2′)), 91.1 (*C*(5)), 105.2 (*C*(2)), 127.0, 128.0, 132.1 (*o,m,p-Ph*), 133.5 (*i-Ph*), 170.4 (*C*(4), *C*(6)), 192.1 (*C*(1′)); *m/z* (ESI^-) 293 ($[M-H]^-$, 40%); HRMS (ESI^-) $C_{14}H_{13}O_5S^-$ ($[M-H]^-$) requires 293.0489, found 293.0489.

***t*-Butyl (4-phenylthio)-3-oxo-butanoate 347c**

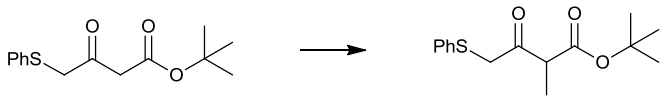


346c **347c**

A solution of **346c** (714 mg, 2.42 mmol) in 1:1 toluene:*t*-BuOH (10 mL) was stirred at reflux for 2 h. The mixture was cooled to rt and concentrated *in vacuo* to give **347c** as a brown liquid (643 mg, quant); R_f 0.75 (eluent 5:1 40-60 petrol:EtOAc); ν_{max} (film) 3060, 2979, 1715, 1649; δ_H (400 MHz, $CDCl_3$) 1.47 (9H, s, C(C*H*$_3$)$_3$), 3.56 (2H, s, C(2)*H*$_2$), 3.82 (2H, s, C(4)*H*$_2$), 7.22-7.37 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 27.9 (C(*C*H_3)$_3$), 43.9 (*C*(4)), 47.8 (*C*(2)), 82.3 (*C*Me_3), 127.1, 129.2, 129.7 (*o,m,p-Ph*), 134.3 (*i-Ph*), 166.2 (*C*(1)), 198.4 (*C*(3));

m/z (ESI$^+$) 289 ([M+Na]$^+$, 80%); HRMS (ESI$^+$) $C_{14}H_{18}NaO_3S^+$ ([M+Na]$^+$) requires 289.0867, found 289.0869.

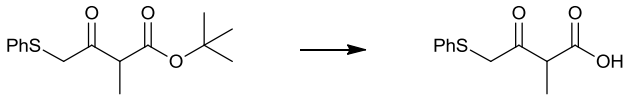
_t_-Butyl (4-phenylthio)-3-oxo-2-methylbutanoate 348c



347c **348c**

A solution of **347c** (717 mg, 2.56 mmol) in THF (20 mL) was stirred at 0 °C and *t*BuOK (328 mg, 2.68 mmol) was added. The mixture was then warmed to rt and stirred for 40 min before the addition of MeI (0.17 mL, 2.68 mmol). The mixture was left to stir for a further 5 h at rt. After this time the mixture was partitioned between Et_2O (20 mL) and brine (20 mL). The aqueous layer was extracted with Et_2O (2 × 10mL) and the combined organic layers were dried and concentrated *in vacuo* to give **348c** as a brown oil (620 mg, 86%); R_f 0.85 (eluent 5:1 petrol:EtOAc); ν_{max} (film) 3059, 1737, 1713; δ_H (400 MHz, $CDCl_3$) 1.27 (3H, d, *J* 7.1, C(2)CH_3), 3.84 (1H, q, *J* 7.1, C(2)*H*), 3.81-3.92 (2H, m, C(4)H_2), 7.26-7.36 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 12.8 (C(2)*C*H$_3$), 27.9 (C(*C*H$_3$)$_3$), 43.9 (*C*(4)), 50.9 (*C*(2)), 82.1 (*C*Me$_3$), 127.0 (*p-Ph*), 129.0, 129.1, 129.2, 129.7 (*o,m,p-Ph*), 134.6 (*i-Ph*), 169.2 (*C*(1)), 201.1 (*C*(3)); *m/z* (ESI$^+$) 303 ([M+Na]$^+$, 75%); HRMS (ESI$^+$) $C_{15}H_{20}NaO_3S^+$ ([M+Na]$^+$) requires 303.1025, found 303.1023.

2-Methyl-3-oxo-4-(phenylthio)butanoic acid 350c

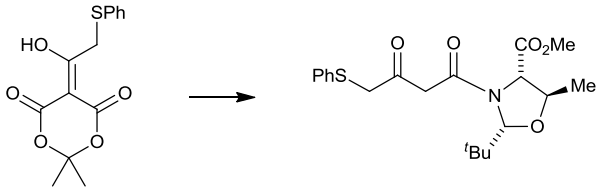


348c **350c**

A solution of **348c** (620 mg, 2.21 mmol) in DCM (4 mL) was cooled to 0 °C and TFA (4 mL) was added dropwise. The resulting solution was stirred for 2 h at rt before the solvent was removed *in vacuo* by co-evaporation with toluene to give **350c** as a brown oil (490 mg, quant) which was used without further purification; ν_{max} (film) 3059, 2985, 2940, 1707, 1583; δ_H (400 MHz, $CDCl_3$) 1.35 (3H, d, *J* 7.1, C(2)CH_3), 3.86, 3.91 (2H, AB q, *J* 15.3, C(4)H_2), 4.03 (1H, q, *J* 7.1, C(2)*H*), 7.15-7.38 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 12.9 (*C*H$_3$), 43.5 (*C*(4)),

49.1 (*C*(2)), 125.3, 127.3, 128.2, 129.2, 130.0 (*o,m,p-Ph*), 133.9 (*i-Ph*), 175.1 (*C*O_2H), 199.5 (*C*(3)); *m/z* (ESI$^+$) 223 ([M−H]$^-$, 40%).[8]

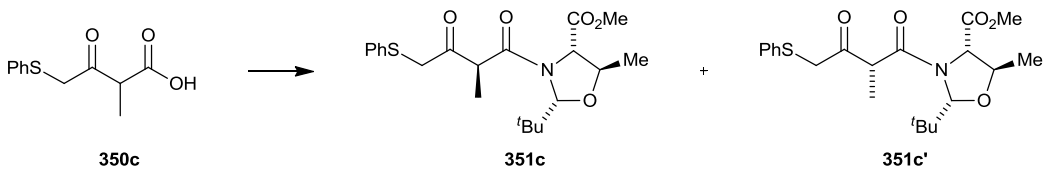
(2*R*,4*S*,5*R*) 2-*t*Butyl-3-(3′-oxo-4′-phenylthiobutanoyl)-4-methoxycarbonyl-5-methyloxazolidine 349c



346c **349c**

Acylated Meldrum's acid **346c** (907 mg, 3.1 mmol) was dissolved in MeCN (15 mL) and oxazolidine **320** (563 mg, 2.8 mmol) in MeCN (8 mL) was added. The resulting mixture was stirred at 60 °C for 2 h and then concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 20:1-5:1 petrol:EtOAc) gave **349c** as a yellow oil (558 mg, 45 %) as a mixture of keto and enol forms; R_f 0.37(eluent 5:1 pterol:EtOAc); ν_{max} (film) 2975, 2957, 1745, 1716, 1662; δ_H (400 MHz, MeOD) 0.87 (s, C(CH_3)$_3$), 1.21 (3H, d, *J* 6.3, C(5)CH_3), 3.77 (3H, s, CO_2CH_3), 3.93 (2H, AB q, *J* 15.4, C(4′)H_2), 4.16 (1H, d, *J* 3.8, C(4)*H*), 4.68-4.74 (1H, m, C(5)*H*), 5.33 (1H, s, C(2)*H*), 7.21-7.45 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 19.2 (C(5)*C*H$_3$), 25.3 (C(*C*H$_3$)$_3$), 37.8 (*C*Me$_3$), 43.1 (*C*(4′)) 52.3 (CO_2CH_3), 65.4 (*C*(4)), 76.3 (*C*(5)), 96.1 (*C*(2)), 126.9 (*p-Ph*), 129.2, 129.3 (*o,m-Ph*), 170.4, 170.5 (*C*O_2Me, C(1′)), 200.0 (*C*(3′)); *m/z* (ESI$^+$) 809 ([2M+Na]$^+$, 100%), 394 ([M+H]$^+$, 80%); HRMS (ESI$^+$) $C_{20}H_{28}NO_5S^+$ ([M+H]$^+$) requires 394.1680, found 394.1683.

(2*R*,4*S*,5*R* ,2′*R*)- and (2*R*,4*S*,5*R* ,2′*S*)-2-*t*Butyl-3-(3′-oxo-2′-methyl-4′-phenylthiobutanoyl)4-methoxycarbonyl-5-methyloxazolidine 351c and 351c′

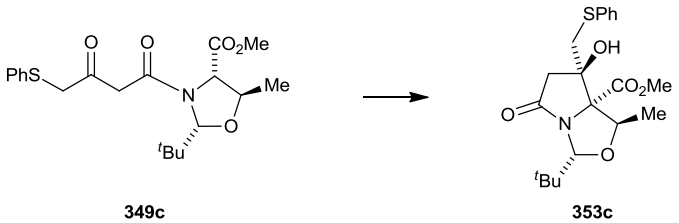


350c **351c** **351c'**

Follwing **General Procedure D** for the synthesis of *N*-acyloxazolidines, oxazolidine **320** (422 mg, 2.1 mmol) was reacted with DCC (457 mg, 2.2 mmol), DMAP (18 mg, 7 mol%), and the acid **350c** (495 mg, 2.2 mmol). Purification *via* column chromatography (SiO_2, eluent 10:1 petrol:EtOAc) gave **350c** as a yellow oil (117 mg, 14%); R_f 0.4 (eluent 20:1

petrol:EtOAc); $[\alpha]_D^{23}$ −12.2 (*c* 0.72 in $CHCl_3$); ν_{max} (film) 2975, 2957, 1746, 1717, 1661; δ_H (400 MHz, $CDCl_3$) 0.90 (9H, s, C(C*H*$_3$)$_3$), 1.27 (3H, d, *J* 6.3, C(5)C*H*$_3$), 1.48 (3H, d, *J* 7.1, C(2′)C*H*$_3$), 3.78 (3H, s, CO_2CH_3), 3.80-3.93 (3H, m, C(2′)*H*, C(4′)*H*$_2$), 4.27 (1H, d, *J* 4.3, C(4)*H*), 4.65-4.72 (1H, m, C(5)*H*), 5.47 (1H, s, C(2)*H*), 7.19-7.24 (1H, m, *Ph*), 7.27-7.30 (4H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 15.0 (C(2′)*C*H$_3$), 20.1 (C(5)*C*H$_3$), 25.8 (C(*C*H$_3$)$_3$), 38.0 (*C*Me$_3$), 40.6 (*C*(4′)), 50.5 (*C*(2′)), 52.7 (CO_2CH_3), 65.3 (*C*(4)), 76.4 (*C*(5)), 96.1 (*C*(2)), 126.9 (*p-Ph*), 128.5, 129.3 (*o,m-Ph*), 134.0 (*i-Ph*), 170.1 (*C*O$_2$Me), 172.4 (*C*(1′)), 202.2 (*C*(3′)); *m/z* (ESI^+) 408 ($[M+H]^+$, 5%), 430 ($[M+Na]^+$, 45%), 837 ($[2M+Na]^+$, 100%); HRMS (ESI^+) $C_{21}H_{29}NNaO_5S^+$ ($[M+Na]^+$) requires 430.1659, found 430.1647; and **350c′** as a yellow solid (140 mg, 16%); R_f 0.2 (eluent 20:1 petrol:EtOAc); mp 102-106 ºC; $[\alpha]_D^{23}$ −22.5 (*c* 0.39 in $CHCl_3$); ν_{max} (film) 2976, 2957, 1742, 1660; δ_H (400 MHz, $CDCl_3$) 0.95 (9H, s, C(C*H*$_3$)$_3$), 1.34 (3H, d, *J* 6.8, C(2′)C*H*$_3$), 1.37 (3H, d, *J* 6.1, C(5)C*H*$_3$), 3.81-3.88 (4H, m, CO_2CH_3, C(2′)*H*), 3.91, 3.98 (2H, AB q, *J* 15.8, C(4′)*H*$_2$), 4.14 (1H, d, *J* 4.0, C(4)*H*), 4.78-4.85 (1H, m, C(5)*H*), 5.43 (1H, s, C(2)*H*), 7.20 (1H, t, *J* 7.3, *p-Ph*), 7.28 (2H, app. t, *J* 7.6, *m-Ph*), 7.35 (2H, d, *J* 7.8, *o-Ph*); δ_C (100 MHz, $CDCl_3$) 13.2 (C(2′)*C*H$_3$), 20.4 (C(5)*C*H$_3$), 26.0 (C(*C*H$_3$)$_3$), 38.1 (*C*Me$_3$), 42.0 (*C*(4′)), 50.8 (*C*(2′)), 53.1 (CO_2CH_3), 65.7 (*C*(4)), 76.0 (*C*(5)), 97.2 (*C*(2)), 126.7 (*p-Ph*), 129.0, 129.5 (*o,m-Ph*), 134.8 (*i-Ph*); *m/z* (ESI^+) 408 ($[M+H]^+$, 5%), 430 ($[M+Na]^+$, 55%), 837 ($[2M+Na]^+$, 100%); HRMS (ESI^+) $C_{21}H_{29}NNaO_5S^+$ ($[M+Na]^+$) requires 430.1659, found 430.1646.

(2*R*,4*R*,5*R*,6*R*)-1-Aza-2-*t*-butyl-4methyl-5-methoxycarbonyl-6-hydroxy-6-(phenylthio-methyl)-8-oxo-3-oxabicyclo[3.3.0]octane 353c

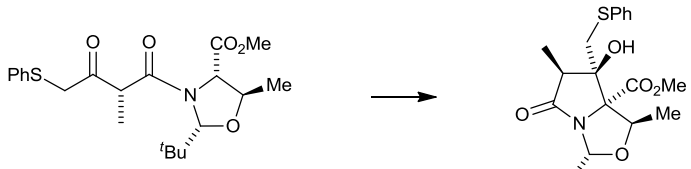


349c **353c**

Following **General Procedure F** for the aldol cyclisation of *N*-acyloxazolidines, *N*-acyl-oxazolidine **320** (550 mg, 1.39 mmol) and NaOMe (84 mg, 1.55 mmol) were reacted to give a crude mixture which was purified *via* column chromatography (SiO_2, eluent 5:1 petrol:EtOAc) to give **353c** as a pale yellow oil (167 mg, 30%); R_f 0.25 (eluent 5:1

petrol:EtOAc); $[\alpha]_D^{23}$ +8.9 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3400, 2956, 2874, 1743, 1703, 1584, 1481; δ_H (400 MHz, $CDCl_3$) 0.90 (9H, s, C(C*H*$_3$)$_3$), 1.69 (3H, d, *J* 6.6, C(4)C*H*$_3$), 2.33 (1H, d, *J* 15.8, C(7)*H*$_A$H$_B$), 2.89 (1H, d, *J* 15.8, C(7)H$_A$*H*$_B$), 3.03 (1H, d, *J* 13.9, C(1′)*H*$_A$H$_B$), 3.34 (1H, s, O*H*), 3.37 (1H, d, *J* 13.9, C(1′)H$_A$*H*$_B$), 3.82 (3H, s, CO_2CH_3), 4.75 (1H, q, *J* 6.6, C(4)*H*), 5.05 (1H, s, C(2)*H*), 7.22-7.33 (3H, m, *Ph*), 7.38-7.41 (2H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 15.4 (C(4)*C*H$_3$), 25.7 (C(*C*H$_3$)$_3$), 37.4 (*C*Me$_3$), 41.8 (*C*(1′)), 47.8 (*C*(7)), 53.0 (CO_2CH_3), 78.5 (*C*(4)), 80.1 (*C*(5)), 83.6 (*C*(6)), 96.6 (*C*(2)), 127.4 (*p-Ph*), 129.4, 130.5 (*o,m-Ph*), 135.0 (*i-Ph*), 171.6 (CO_2Me), 177.7 (*C*(8)); *m/z* (ESI^+) 392 ($[M-H]^-$, 100%); HRMS (ESI^+) $C_{20}H_{26}NO_5S^+$ ($[M-H]^-$) requires 392.01537, found 392.1533.

(2*R*,4*R*,5*R*,6*R*,7*S*)-1-Aza-2-*t*-butyl-4methyl-5-methoxycarbonyl-6-hydroxy-6-(phenylthio-methyl)-7-methyl-8-oxo-3-oxabicyclo[3.3.0]octane 354c

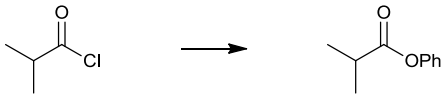


351c' **354c**

Following **General Procedure F** for the aldol cyclisation of *N*-acyloxazolidines, *N*-acyl-oxazolidine **351c′** (122 mg, 0.3 mmol) and NaOMe (18 mg, 0.33 mmol) were reacted to give **354c** as a yellow solid (92 mg, 75%); R_f 0.25 (eluent 5:1 petrol:EtOAc); mp 150-152 ºC; $[\alpha]_D^{23}$ +6.7 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3367 (br), 2956, 2938, 2873, 1722, 1695; δ_H (400 MHz, $CDCl_3$) 0.93 (9H, s, C(C*H*$_3$)$_3$), 1.02 (3H, d, *J* 7.1, C(7)C*H*$_3$), 1.68 (3H, d, *J* 6.6, C(4)C*H*$_3$), 2.82 (1H, br s, O*H*), 3.17 (1H, q, *J* 7.1, C(7)*H*), 3.31 (2H, s, C*H*$_2$SPh), 3.83 (3H, s, CO_2CH_3), 4.77 (1H, q, *J* 6.6, C(4)*H*), 5.02 (1H, s, C(2)*H*), 7.25-7.34 (3H, m, *m,p-Ph*), 7.41 (2H, d, *J* 8.3, *o-Ph*); δ_C (125 MHz, $CDCl_3$) 7.2 (C(7)*C*H$_3$), 15.7 (C(4)*C*H$_3$), 25.7 (C(*C*H$_3$)$_3$), 37.3 (*C*Me$_3$), 41.4 (*C*H$_2$Ph), 47.9 (*C*(7)), 52.9 (CO_2CH_3), 78.3 (*C*(4)), 78.8 (*C*(5)), 85.1 (*C*(6)), 96.4 (*C*(2)), 127.4, 129.3 (*m,p-Ph*), 130.6 (*o-Ph*), 135.6 (*i-Ph*), 171.6 (CO_2Me), 179.6 (*C*(8)); *m/z* (ESI^-) 406 ($[M-H]^-$, 40%); HRMS (ESI^-) $C_{21}H_{28}NO_5S^-$ ($[M-H]^-$) requires 406.1694, found 406.1707.

4.3.5 Experimental for synthesis of right hand-middle fragment adducts

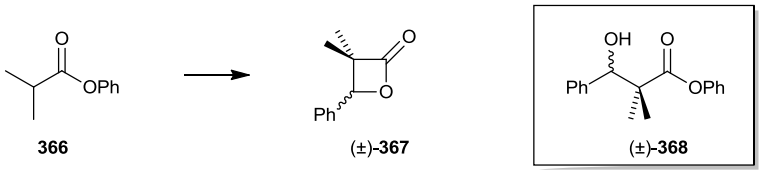
Phenyl isobutyrate 366[25]



366

A solution of phenol (1.76 g, 18.7 mmol) and pyridine (1.66 mL, 20.6 mmol) in DCM (80 mL) was cooled to 0 °C and *iso*-butyryl chloride (1.98 mL, 18.7 mmol) was added dropwise. The resulting solution was stirred at 0 °C for 10 min before being warmed to rt and stirred for a further 30 min. A portion of H_2O (80 mL) was added and the layers were separated. The aqueous layer was extracted with DCM (3 × 20 mL), and the combined organic layers were washed sequentially with 0.5 M HCl (2 × 100 mL), H_2O (100 mL), cold 4 M NaOH (2 × 100 mL) and brine (100 mL). The organic layer was then dried and concentrated *in vacuo* to give **366** as a colourless oil (2.92 g, 95%); ν_{max} (film) 2976, 2940, 2880, 1754, 1594; δ_H (400 MHz, $CDCl_3$) 1.34 (6H, d, *J* 7.1, C*H*$_3$), 2.82 (1H, septet, *J* 7.1, C*H*), 7.07-7.11 (2H, m, *o-Ph*), 7.21-7.27 (1H, m, *p-Ph*), 7.36-7.42 (2H, m, *m-Ph*); δ_C (100 MHz, $CDCl_3$) 18.9 (*C*H$_3$), 34.2 (*C*H), 121.5 (*o-Ph*), 125.6 (*p-Ph*), 129.3 (*m-Ph*), 150.9 (*i-Ph*), 175.6 (*C*=O); *m/z* (ESI$^+$) 187 ([M+Na]$^+$, 100%).

(±)-3,3-dimethyl-4-phenyl-2-oxetanone (±)-367[26]



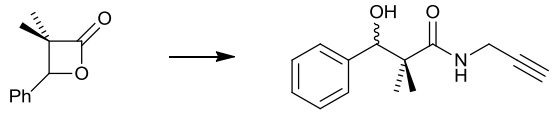
366 **(±)-367** **(±)-368**

A solution of *i*Pr$_2$NH (2.74 mL, 19.7 mmol) in THF (17 mL) was cooled to 0 °C. A solution of BuLi (11.6 mL, 1.53 M in hexanes, 17.7 mmol) was added dropwise, and the resulting solution was stirred for 30 min at 0 °C. After this time, the solution was cooled to −78 °C and a solution of **366** (2.90 g, 17.7 mmol) in THF (15 mL) was added dropwise *via* cannula. The solution was stirred for a further 30 min at −78 °C. This was followed by the dropwise addition of benzaldehyde (1.49 mL, 14.7 mmol) and a further 30 min stirring at −78 °C. The solution was warmed to 0 °C and 1 M NaOH (25 mL) was added. The cold bath was removed and the mixture left to stir for 5 min before being partitioned between Et_2O (50 mL)

and H_2O (50 mL). The aqueous layer was extracted with Et_2O (3 × 15 mL) and the combined organic layers were washed with 1 M NaOH (2 × 20 mL) and brine (30 mL) before being dried and concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 20:1 petrol:EtOAc) gave (±)-**367** as a colourless oil (1.42 g, 54%); R_f 0.3 (eluent 20:1 petrol:EtOAc); ν_{max} (film) 2972, 2933, 2873, 1820, 1463; δ_H (400 MHz, $CDCl_3$) 0.90 (3H, s, C(3)CH_3), 1.58 (3H, s, C(3)CH_3), 5.33 (1H, s, C(4)*H*), 7.27 (2H, d, *J* 7.3, *o-Ph*), 7.35 (1H, t, *J* 7.3, *p-Ph*), 7.42 (2H, att t, *J* 7.3, *m-Ph*); δ_C (100 MHz, $CDCl_3$) 17.8, 22.5 (C(3)(*C*H_3)$_2$), 56.7 (*C*(3)), 82.4 (*C*(4)), 125.2, 128.5, 128.7 (*o,m,p-Ph*), 135.3 (*i-Ph*), 175.0 (*C*(2)); *m/z* (ESI^+) 375 ($[2M+Na]^+$, 35%), 199 ($[M+Na]^+$, 20%).

When conducted on a larger scale, this reaction produced majoratively the ester phenyl 3-hydroxy-2,2-dimethyl-3-phenylpropanoate (±)-**368**,[27] an intermediate in the production of the β-lactone, as a yellow oil; δ_H (400 MHz, $CDCl_3$) 1.29 (3H, s, CH_3), 1.32 (3H, s, CH_3), 2.92 (1H, br s, O*H*), 5.09 (1H, s, C*H*OH), 7.05-7.09 (2H, m, *o*-O*Ph*), 7.23-7.27 (1H, m, *p*-O*Ph*), 7.31-7.43 (7H, m, *m*-O*Ph*, *o,m,p-Ph*); δ_C (125 MHz, $CDCl_3$) 19.1, 22.9 (*C*H_3), 48.2 (*C*Me_2), 78.6 (*C*HOH), 121.5 (*o*-O*Ph*), 125.9 (*p*-O*Ph*), 12.8, 127.9, 128.0, 129.4 (*m*-O*Ph*, *o,m,p-Ph*), 139.9 (*i-Ph*), 150.8 (*i*-O*Ph*), 176.2 (*C*=O); *m/z* (ESI^+) 293 ($[M+Na]^+$, 20%), 309 ($[M+K]^+$, 100%).

(±)-*N*-(prop-2-ynyl)-3-hydroxy-2,2-dimethyl-3-phenylpropanamide (±)-357[28]



(±)-**367** (±)-**357**

A mixture of (±)-**367** (1.34 g, 7.63 mmol), propargylamine (2.44 mL, 38.2 mmol) and TEA (1.16 mL, 18.4 mmol) in DCM (40 mL) was stirred at reflux for 3 days. After this time the solvent was removed *in vacuo* and the resulting residue was purified *via* column chromatography (SiO_2, eluent 7:3 petrol:EtOAc) to give (±)-**357** as a pale yellow oil (1.30 g, 74%); R_f 0.3 (eluent 7:3 petrol:EtOAc); ν_{max} (film) 3297, 2975, 2933, 2873, 1639, 1522, 1453; δ_H (400 MHz, $CDCl_3$) 1.08 (3H, s, CH_3), 1.21 (3H, s, CH_3), 2.24 (1H, t, *J* 2.7, C≡C*H*), 3.96-4.06 (3H, m, NHCH_2, O*H*), 4.67 (1H, d, *J* 4.6, C*H*OH), 6.41 (1H, br s, N*H*), 7.25-7.33 (5H,

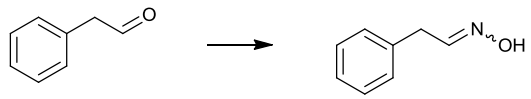
m, *Ph*); δ_C (125 MHz, $CDCl_3$) 20.4 (*C*H_3), 24.3 (*C*H_3), 29.2 (*C*(1′)), 46.3 (*C*(2)), 71.6 (*C*(3′)), 79.4 (*C*(2′)), 79.8 (*C*(3)), 127.5, 127.6, 127.8 (*o,m,p-Ph*), 140.5 (*i-Ph*), 177.2 (*C*(1)); *m/z* (ESI^-) 230 ($[M-H]^-$, 100%).

2-Phenylacetaldehyde 360[29]

360

Allyl benzene (500 mg, 4.23 mmol) was dissolved in 6:1 MeCN:H_2O (29 mL) and $RuCl_3$ (4.2 mL, 0.035 M solution in H_2O, 0.17 mmol) was added. Portions of $NaIO_4$ (2.7 g, 12.7 mmol) were added over 5 min and the solution lightened in colour. After 2 h, the reaction as quenched with sat. aq. $Na_2S_2O_3$ (15 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with H_2O (30 mL) and brine (30 mL), before being dried and concentrated *in vacuo* to give **360** as a colourless oil (642 mg) which was used without further purification; δ_H (400 MHz, $CDCl_3$) 3.70 (2H, d, *J* 2.0, C*H*$_2$), 7.21-7.42 (5H, m, *Ph*), 9.75-9.77 (1H, m, C*H*O); δ_C (100 MHz, $CDCl_3$) 50.6 (*C*H_2), 127.4, 129.0, 129.8 (*o,m,p-Ph*), 131.8 (*i-Ph*), 199.5 (*C*=O); *m/z* (FI^+) 120 (M^+, 100%).

(*E*/*Z*)-2-Phenylacetaldehyde oxime 361

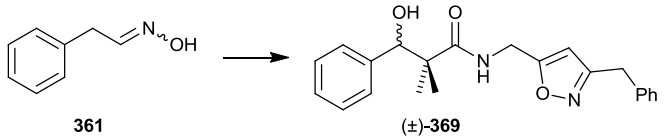


360 **361**

A solution of $NH_2OH.HCl$ (420 mg, 6.05 mmol) in H_2O (11 mL) was added dropwise to a stirred solution of aldehyde **360** (242 mg, 2.02 mmol) in THF (13 mL). The mixture was then cooled to 0 °C and Na_2CO_3 (2.02 mL, 1.0 M in H_2O, 2.02 mmol) was added dropwise. The cold bath was removed and the mixture stirred at rt for 1.5 h. After this time, H_2O (10 mL) was added and the mixture extracted with EtOAc (3 × 20 mL). The combined organic layers were dried and concentrated *in vacuo* to give **361** as a white solid (224 mg, 82%); R_f 0.7 (eluent 7:3 petrol:EtOAc); δ_H (400 MHz, $CDCl_3$) 3.57 (2H, d, *J* 6.3, C*H*$_2$-A), 3.78 (2H, d, *J* 5.3, C*H*$_2$-B), 6.94 (1H, t, *J* 5.3, C*H*NOH-B), 7.22-7.38 (10H, m, *Ph*-A+B), 7.58 (1H, t, *J* 6.3, C*H*NOH-A), 8.81 and 9.27 (1H, br s, O*H*-A+B); δ_C (125 MHz, $CDCl_3$) 31.7 (*C*H_2-B), 35.9

(*C*H_2-A), 126.7, 126.9, 128.8, 128.9 (*o,m,p-Ph*-A+B), 136.1, 136.6 (*i-Ph*-A+B), 150.7, 150.9 (C=NO*H*-A+B); *m/z* (FI^+) 135 (M^+, 100%).

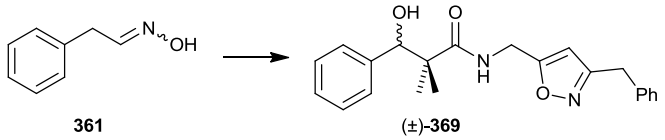
(±)-(*R*/*S*)-*N*-((3′-benzylisoxazol-5′-yl)methyl)-3-hydroxy-2,2-dimethyl-3-phenylpropanamide (±)-369



361 (±)-**369**

A portion of NBS (295 mg, 1.66 mmol) was added to a stirred solution of oxime **361** (203 mg, 1.50 mmol) and alkyne (±)-**357** (348 mg, 1.50 mmol) in anhydrous DMF (30 mL) at rt. The resultant mixture was stirred at rt for 30 min. The mixture was then cooled to 0 °C and TEA (1.65 mL, 1.0 M in DMF, 1.65 mmol) was added dropwise. The cold bath was removed and the mixture allowed to stir at rt for 18 h. After this time, H_2O (60 mL) was added and the mixture was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with H_2O (2 × 30 mL) and brine (30 mL), dried and concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 10:1-2:1 petrol:EtOAc) gave oxime **361** (83 mg, 40%), alkyne (±)-**357** (210 mg, 60%), and (±)-**369** as a yellow oil (42 mg, 8%); R_f 0.14 (eluent 7:3 40-60 petrol:EtOAc); ν_{max} (film) 3344, 3063, 3030, 2975, 2933, 1719, 1645; δ_H (500 MHz, $CDCl_3$) 1.08 (3H, s, C(2)CH_3), 1.20 (3H, s, C(2)CH_3), 3.97 (2H, s, CH_2Ph), 4.48 (2H, d, *J* 5.7, CH_2NH), 4.67 (1H, s, C(3)*H*), 5.92 (1H, s, C(4′)*H*), 6.76 (1H, br s, N*H*), 7.19-7.35 (10H, m, *Ph*); δ_C (125 MHz, $CDCl_3$) 20.3 (C(2)*C*H_3), 24.4 (C(2)*C*H_3), 32.5, *C*H_2Ph), 53.3 (*C*H_2NH), 46.6 (*C*(2)), 79.8 (*C*(3)), 102.3 (*C*(4′)), 127.0, 127.5, 127.9, 128.0, 128.8, 128.9 (*o,m,p-Ph*), 137.1, 140.5 (*i-Ph*), 163.3 (*C*(3′)), 168.8 (*C*(5′)), 177.6 (*C*(1)); *m/z* (ESI^+) 363 ($[M-H]^-$, 100%); HRMS (ESI^+) $C_{22}H_{24}N_2NaO_3^+$ ($[M+Na]^+$) requires 387.1679, found 387.1660.

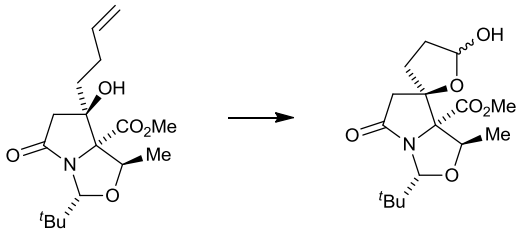
Alternative procedure for the synthesis of (±)-(*R*/*S*)-*N*-((3′-benzylisoxazol-5′-yl)methyl)-3-hydroxy-2,2-dimethyl-3-phenylpropanamide (±)-369



361 (±)-**369**

Alkyne (±)-**357** (66 mg, 0.28 mmol), NaOCl (0.31 mL, 0.46 mmol) and TEA (0.01 mL, 0.06 mmol) were stirred in DCM (5 mL) at 0 °C, and oxime **361** (39 mg, 0.28 mmol) was added dropwise over 15 min. The resulting mixture was allowed to warm to rt and stirred for 18 h. The mixture was diluted with H_2O (10 mL) and the phases were separated. The aqueous phase was extracted with DCM (2 × 10 mL) before the combined organic layers were dried and concentrated *in vacuo*. Purification by column chromatography (SiO_2, eluent 7:3 petrol:EtOAc) gave unreacted (±)-**357** (39 mg, 59%) and (±)-**369** (34 mg, 32%); data as above.

(2*R*,5*R*,4*R*,6*R*)-Spiro-[1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(but-3′-enyl)-8-oxo-3-oxabicyclo[3.3.0]octane]-6,5′-[3′(*R*/*S*)-hydroxytetrahydrofuran] 362

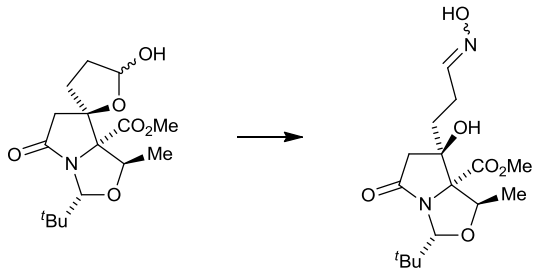


353a **362**

Alkene **353a** (87 mg, 0.27 mmol) was dissolved in 6:1 MeCN:H_2O (2 mL) and $RuCl_3$ (0.27 mL, 0.035 M solution in H_2O, 9.0 μmol) was added. Portions of $NaIO_4$ (171 mg, 0.80 mmol) were added over 5 min and the solution lightened in colour. After 10 min, the reaction was quenched with sat. aq. $Na_2S_2O_3$ (5 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with H_2O (15 mL) and brine (15 mL), before being dried and concentrated *in vacuo* to give **362** in a 76:24 dr as a colourless oil (65 mg, 74%): ν_{max} (film) 2959, 2906, 2872, 1790, 1726; δ_H (400 MHz, $CDCl_3$) 0.88-0.91 (9H, m, C(C*H*$_3$)$_3$), 1.49 (3H × 0.76, d, *J* 6.3, C(4)C*H*$_3$-A), 1.63 (3H × 0.24, d, *J* 6.7, C(4)C*H*$_3$-B), 1.90-2.34 (4H,

m, C(1′)H_2, C(2′)H_2), 2.62 (1H × 0.24, d, *J* 16.2, C(7)H_AH_B-B), 2.81 (1H × 0.76, d, *J* 16.0, C(7)H_AH_B-A), 3.04 (1H × 0.76, d, *J* 16.0, C(7)CH_AH_B-A), 3.13 (1H × 0.24, d, *J* 16.2, C(7)H_AH_B-B), 3.79 (3H × 0.76, s, CO_2CH_3-A), 3.83 (3H × 0.24, s, CO_2CH_3-B), 4.00 (1H × 0.24, br s, O*H*-B), 4.18 (1H × 0.76, br s O*H*-A), 4.74 (1H × 0.76, q, *J* 6.3, C(4)*H*-A), 4.84 (1H × 0.24, q, *J* 6.7, C(4)*H*-B), 4.98 (1H × 0.76, s, C(2)*H*-B), 5.01 (1H × 0.24, C(2)*H*-A), 5.54 (1H × 0.24, br s, C(3′)*H*-B), 5.59 (1H × 0.76, br s, C(3′)*H*-A); δ_C (100 MHz, $CDCl_3$) 15.1, 15.6 (C(4)*C*H_3), 25.6, 25.9 (C(*C*H_3)$_3$), 30.2, 30.7, 32.6, 33.1 (*C*(1′), *C*(2′)), 36.8, 37.2 (*C*Me_3), 47.4, 48.2 (*C*(7)), 52.8, 53.2 (CO_2CH_3), 77.9, 78.1 (*C*(4)), 79.3, 79.5 (*C*(5)), 91.1, 91.8 (*C*(6))), 95.6, 95.9 (*C*(2)), 98.5, 99.4 (*C*(3′)), 171.8, 173.5 (*C*O_2Me), 177.6, 178.4 (*C*(8)); *m/z* (ESI^+) 677 ($[2M+Na]^+$, 100%), (ESI^-) 326 ($[M-H]^-$, 100%); HRMS (ESI^+) $C_{16}H_{26}NO_6^+$ ($[M+H]^+$) requires 328.1755, found 328.1753.

(2*R*,4*R*,5*R*,6*R*)-1-Aza-2-*t*-butyl-4methyl-5-methoxycarbonyl-6-hydroxy-6-(3′-(hydroxyimino)propyl)-8-oxabicyclo[3.3.0]octane 364

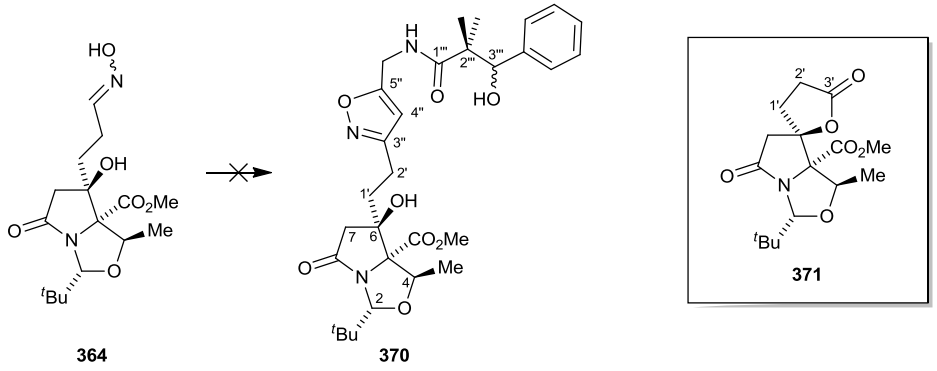


362 **364**

A solution of $NH_2OH.HCl$ (41 mg, 0.6 mmol) in H_2O (1.2 mL) was added dropwise to a stirred solution of aldehyde **362** (65 mg, 0.2 mmol) in THF (1.5 mL). The mixture was then cooled to 0 °C and Na_2CO_3 (0.2 mL, 1.0 M in H_2O, 0.2 mmol) was added dropwise. The cold bath was removed and the mixture stirred at rt for 1.5 h. After this time, H_2O (5 mL) was added and the mixture extracted with EtOAc (3 × 10 mL). The combined organic layers were dried and concentrated *in vacuo* to give **364** in a 1.5:1 dr as a colourless oil (57 mg, 83%); ν_{max} (film) 3396 (br), 2958, 2875, 1792, 1702; δ_H (400 MHz, $CDCl_3$) 0.90 and 0.92 (9H, s, C(CH_3)$_3$), 1.45-1.54 (1H, m, C(1′)H_AH_B), 1.68 and 1.69 (3H, d, *J* 6.5 and 6.6, C(4)CH_3), 2.02-2.14 (1H, m, C(1′)H_AH_B), 2.37 and 2.62 (1H, d, *J* 15.4 and 15.8, C(7)H_AH_B), 3.01 and 3.14 (1H, d, *J* 15.4, 15.8, C(7)H_AH_B), 3.80 and 3.84 (3H, s, CO_2CH_3), 4.78 and 4.85 (1H, q, *J* 6.5 and 6.6, C(4)*H*), 5.06 and 5.07 (1H, s, C(2)*H*), 6.79 and 7.40 (1H, t, *J* 5.3 and 5.9, C(3′)*H*),

8.73 (1H, br s, O*H*); δ_C (100 MHz, $CDCl_3$) 15.2 (C(4)*C*H_3), 21.0 (*C*(2′)), 25.5, 25.7 (C(*C*H_3)$_3$), 31.8, 32.1 (*C*(1′)), 37.1, 37.4 (*C*Me$_3$), 47.0, 47.3 (*C*(7)), 52.9, 53.2 (CO_2C*H*$_3$), 77.9, 78.6 (*C*(4)), 80.9, 81.1 (*C*(5)), 84.3, 84.5 (*C*(6)), 96.3 (*C*(2)), 151.6 (*C*(3′)), 172.0 (*C*O_2Me), 179.1 (*C*(8)); *m/z* (ESI$^+$) 707 ([2M+Na]$^+$, 100%), (ESI$^-$) 341 ([M−Na]$^-$, 100%); HRMS (ESI$^+$) $C_{16}H_{27}N_2O_6^+$ ([M+H]$^+$) requires 343.1864, found 343.1860.

Attempted synthesis of (2*R*,4*R*,5*R*,6*R*,3‴*R*)- and (2*R*,4*R*,5*R*,6*R*,3‴*S*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(2′-(5″-((3‴-hydroxy-2‴,2‴-dimethyl-3‴-phenylpropanamido)methyl)isoxazol-3″-yl)ethyl)-8-oxabicyclo[3.3.0]octane 370

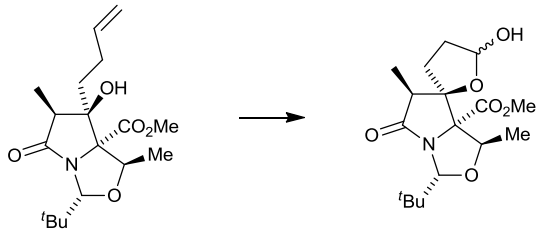


Method A: A portion of NBS (29 mg, 0.17 mmol) was added to a stirred solution of oxime **364** (52 mg, 0.15 mmol) and alkyne (±)-**357** (35 mg, 0.15 mmol) in anhydrous DMF (3 mL) at rt. The resultant mixture was stirred at rt for 30 min. The mixture was then cooled to 0 °C and TEA (0.17 mL, 1.0 M in DMF, 0.17 mmol) was added dropwise. The cold bath was removed and the mixture allowed to stir at rt for 18 h. After this time, H_2O (7 mL) was added and the mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with H_2O (2 × 10 mL) and brine (10 mL), dried and concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 3:1-1:1 petrol:EtOAc) gave alkyne (32 mg, 91%), oxime (21 mg, 52%), and (2*R*,5*R*,4*R*,6*R*)-Spiro-[1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(but-3′-enyl)-8-oxo-3-oxabicyclo[3.3.0]octane]-6,5′-[3′-oxotetrahydrofuran] **371** as a colourless oil (8 mg, 16%); R_f 0.05 (eluent 2:1 petrol:EtOAc); $[\alpha]_D^{23}$ +13.8 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3438 (br), 2958, 2875, 1790, 1726; δ_H (400 MHz, $CDCl_3$) 0.92 (9H, s, C(C*H*$_3$)$_3$), 1.50 (2H, d, *J* 6.6, C(4)C*H*$_3$), 2.09-2.15 (1H, m, C(1′)*H*$_A$H$_B$), 2.44-2.52 (1H, m, C(1′)H$_A$*H*$_B$), 2.58-2.64 (3H, m, C(2′)*H*$_2$, C(7)*H*$_A$H$_B$), 3.14 (1H, d, *J* 15.8 C(7)H$_A$*H*$_B$), 3.85 (3H, s, CO_2C*H*$_3$), 4.86 (1H, q, *J* 6.6, C(4)*H*), 5.07 (1H, s, C(2)*H*); δ_C (100

MHz, $CDCl_3$) 15.6 (C(4)*C*H_3), 25.5 (C(*C*H_3)$_3$), 27.8 (*C*(1′)), 28.3 (*C*(2′)), 37.1 (*C*Me_3), 46.7 (*C*(7)), 53.2 (CO_2*C*H_3), 77.9 (*C*(4)), 79.3 (*C*(5)), 91.2 (*C*(6)), 96.1 (*C*(2)), 170.6 (*C*O_2Me), 173.3 (*C*(3′)), 175.1 (*C*(8)); *m/z* (ESI^-) 324 ($[M-H]^-$, 45%); HRMS (FI^+) $C_{16}H_{23}NO_6^+$ (M^+) requires 325.1520, found 325.1382.

Method B: Alkyne (±)-**357** (39 mg, 0.17 mmol), NaOCl (0.18 mL, 0.27 mmol) and TEA (5 µL, 0.034 mmol) were stirred in DCM (5 mL) at 0 °C, and oxime **364** (75 mg, 0.17 mmol) was added dropwise over 15 min. The resulting mixture was allowed to warm to rt and stirred for 18 h. The mixture was diluted with H_2O (10 mL) and the phases were separated. The aqueous phase was extracted with DCM (2 × 10 mL) before the combined organic layers were dried and concentrated *in vacuo*. Purification by column chromatography (SiO_2, eluent 7:3 petrol:EtOAc) gave alkyne (±)-**357** (33 mg, 84%) and **371** (24 mg, 63%).

(2*R*,5*R*,4*R*,6*R*,7S)-Spiro-[1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(but-3′-enyl)-7-methyl-8-oxo-3-oxabicyclo[3.3.0]octane]-6,5′-[3′(*R*/*S*)-hydroxytetrahydrofuran] 363

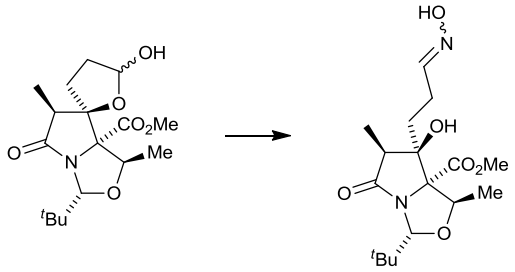


354a **363**

Alkene **354a** (49 mg, 0.14 mmol) was dissolved in 6:1 MeCN:H_2O (1 mL) and $RuCl_3$ (0.14 mL, 0.035 M solution in H_2O, 5.0 µmol) was added. Portions of $NaIO_4$ (92 mg, 0.43 mmol) were added over 5 min and the solution lightened in colour. After 10 min, the reaction was quenched with sat. aq. $Na_2S_2O_3$ (5 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with H_2O (15 mL) and brine (15 mL), before being dried and concentrated *in vacuo* to gave **363** in a 66:34 dr as a colourless oil (36 mg, 75%); ν_{max} (film) 3437, 2957, 2875, 1789, 1722; δ_H (400 MHz, $CDCl_3$) 0.89 and 0.90 (9H, s, C(C*H*$_3$)$_3$), 1.03 and 1.14 (3H, d, *J* 7.1, C(7)C*H*$_3$), 1.44 and 1.66 (3H, d, *J* 6.8, 6.6, C(4)C*H*$_3$), 1.72-1.97 (1H, m, C(1′)*H*$_A$H$_B$), 1.98-2.21 (2H, m, C(1′)H$_A$*H*$_B$, C(2′)*H*$_A$H$_B$), 2.39-2.69 (1H, m,

C(2′)H_AH_B), 2.99 and 3.14 (1H, q, *J* 7.1, C(7)*H*), 3.77 and 3.78 (3H, s, CO_2CH_3), 4.74-4.84 (1H, m, C(4)*H*), 4.96 and 4.98 (1H, s, C(2)*H*), 5.54-5.63 (1H, m, C(3′)*H*); δ_C (100 MHz, $CDCl_3$) 7.8 and 8.4 (C(7)*C*H_3), 15.2 and 15.8 (C(4)*C*H_3), 25.4 and 25.6 (C(*C*H_3)$_3$), 29.4 and 29.8 (*C*(2′)), 32.9 and 33.0 (*C*(1′)), 37.0 and 37.0 (*C*Me_3), 47.9 and 49.7 (*C*(7)), 52.6 and 52.6 (CO_2CH_3), 77.8 and 78.2 (*C*(4)), 78.1 and 79.5 (*C*(5)), 94.1, 94.9 (*C*(6)) 95.0 and 95.3 (*C*(2)), 99.6 and 100.3 (*C*(3′)), 172.1 and 172.3 (*C*O_2Me), 179.1 and 179.3 (*C*(8)); *m/z* (ESI^+) 364 ($[M+Na]^+$, 95%), 340 ($[M-H]^-$, 50%); HRMS (ESI^+) $C_{17}H_{27}NNaO_6^+$ ($[M+Na]^+$) requires 364.1731, found 364.1723.

(2*R*,4*R*,5*R*,6*R*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(3′-(hydroxyimino)propyl)-7-methyl-8-oxabicyclo[3.3.0]octane 365

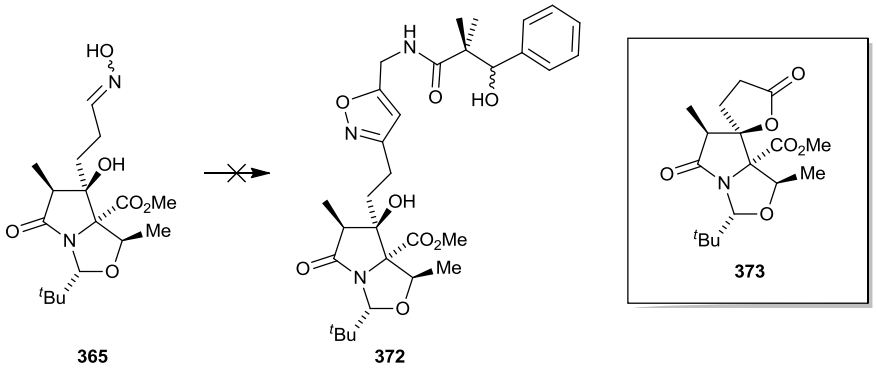


363 **365**

A solution of $NH_2OH.HCl$ (22 mg, 0.3 mmol) in H_2O (0.6 mL) was added dropwise to a stirred solution of aldehyde **363** (36 mg, 0.1 mmol) in THF (0.6 mL). The mixture was then cooled to 0 °C and Na_2CO_3 (0.1 mL, 1.0 M in H_2O, 0.1 mmol) was added dropwise. The cold bath was removed and the mixture stirred at rt for 1.5 h. After this time, H_2O (5 mL) was added and the mixture extracted with EtOAc (3 × 10 mL). The combined organic layers were dried and concentrated *in vacuo* to gave **365** in a 1.5:1 dr as a colourless oil (33 mg, 92%); ν_{max} (film) 3400 (br), 2957, 2876, 1788, 1722; δ_H (400 MHz, $CDCl_3$) 0.91, 0.92 (9H, s, C(C*H*$_3$)$_3$), 1.09-1.15 (3H, m, C(7)C*H*$_3$), 1.49, 1.67 (3H, d, *J* 6.6, C(4)C*H*$_3$), 1.73-1.3, 2.09-2.20 (2H, m, C(1′)*H*$_2$), 2.37-2.53, 2.54-2.69 (2H, m, C(2′)*H*$_2$), 3.06-3.22 (1H, m, C(7)*H*), 3.79, 3.84 (3H, s, CO_2CH_3), 4.82 (1H, q, *J* 6.6, C(4)*H*), 5.02, 5.06 (1H, s, C(2)*H*), 6.70-6.78, 7.44-7.48 (C(3′)*H*); δ_C (100 MHz, $CDCl_3$) 6.6, 7.3 (C(7)*C*H_3), 15.5, 16.0 (C(4)*C*H_3), 24.7, 28.4 (*C*(2′)), 25.5, 32.3 (*C*(1′)), 37.3, 37.3 (*C*Me_3), 48.8, 48.9 (*C*(7)), 52.8, 53.2 (CO_2CH_3), 78.2, 79.0 (*C*(4)), 79.2, 79.5 (*C*(5)), 85.8, 86.0 (*C*(6)), 95.9, 96.4 (*C*(2)), 151.4, 152.0 (*C*(3′)), 172.0, 172.3 (*C*O_2Me), 180.0, 180.1 (*C*(8)); *m/z* (ESI^+) 735 ($[2M+Na]^+$, 100%), (ESI^-) 355

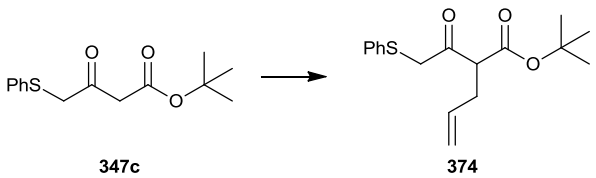
([M−Na]$^-$, 20%); HRMS (ESI$^+$) $C_{17}H_{28}N_2NaO_6^+$ ([M+Na]$^+$) requires 379.1840, found 379.1835.

Attempted synthesis of (2*R*,4*R*,5*R*,6*R*,3′′′*R*)- and (2*R*,4*R*,5*R*,6*R*,3′′′*S*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(2′-(5′′-((3′′′-hydroxy-2′′′,2′′′-dimethyl-3′′′-phenylpropanamido)methyl)isoxazol-3′′-yl)ethyl)-7-methyl-8-oxabicyclo[3.3.0]octane 372



Alkyne (±)-**357** (21 mg, 0.09 mmol), NaOCl (0.11 mL, 0.15 mmol) and TEA (2 μL, 0.018 mmol) were stirred in DCM (5 mL) at 0 °C, and oxime (33 mg, 0.09 mmol) in DCM (2 mL) was added dropwise over 15 min. The resulting mixture was allowed to warm to rt and stirred for 18 h. The mixture was diluted with H_2O (10 mL) and the phases were separated. The aqueous phase was extracted with DCM (2 × 10 mL) before the combined organic layers were dried and concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 2:1 petrol:EtOAc) gave propargylic amide (±)-**357** (19 mg, 95%) and (2*R*,5*R*,4*R*,6*R*,7*S*)-spiro-[1-aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(but-3′-enyl)-7-methyl-8-oxo-3-oxabicyclo[3.3.0]octane]-6,5′-[2′-oxo-tetrahydrofuran] **373** as a colourless oil (25 mg, 81%); R_f 0.3 (eluent 2:1 petrol:EtOAc); $[\alpha]_D^{23}$ −7.53 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3435, 2957, 2924, 2873, 1788, 1726; δ_H (400 MHz, $CDCl_3$) 0.92 (9H, s, C(CH_3)$_3$), 1.11 (3H, d, *J* 7.0, C(7)CH_3), 1.49 (3H, d, *J* 6.6, C(4)CH_3), 2.10-2.19 (1H, m, C(1′)H_AH$_B$), 2.54-2.71 (3H, m, C(1′)H$_A$$H_B$, C(2′)$H_2$), 3.19 (1H, q, *J* 7.0, C(7)*H*), 3.84 (3H, s, CO_2CH_3), 4.82 (1H, q, *J* 6.6, C(4)*H*), 5.06 (1H, s, C(2)*H*); δ_C (100 MHz, $CDCl_3$) 6.6 (C(7)*C*H$_3$), 15.5 (C(4)*C*H$_3$), 25.5 (C(*C*H$_3$)$_3$), 25.5 (*C*(1′)), 28.4 (*C*(2′)), 37.3 (*C*Me$_3$), 48.9 (*C*(7)), 53.2 (CO_2CH_3), 78.0 (*C*(4)), 78.2 (*C*(5)), 94.0 (*C*(6)), 96.4 (*C*(2)), 170.9 (*C*O$_2$Me), 174.1 (*C*(3′)), 177.4 (*C*(8)); *m/z* (ESI$^+$) 362 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{17}H_{25}NNaO_6^+$ ([M+Na]$^+$) requires 362.1574, found 362.1581.

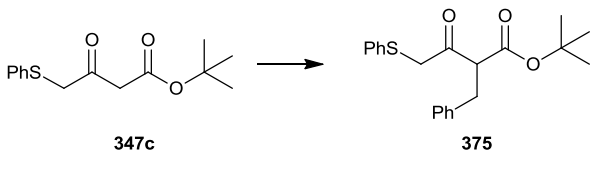
_t_-Butyl 3-oxo-2-allyl-4-(phenylthio)-butanoate 374



347c **374**

A stirred suspension of NaH (33 mg, 0.83 mmol) in THF (6 mL) was cooled to 0 °C and a solution of **347c** (200 mg, 0.75 mmol) in THF (2 mL) was added dropwise. Stirring was continued for 10 min at 0 °C before the dropwise addition of BuLi (0.53 mL, 1.48 M in hexanes, 0.79 mmol). After stirring for a further 10 min, allyl bromide (0.07 mL, 0.83 mmol) was added in one portion and the mixture was allowed to warm to rt over 15 min. The mixture was quenched with 2 M HCl (7 mL) and extracted with Et_2O (2 × 15 mL). The combined organic layers were washed with H_2O until the aqueous layer remained neutral, dried and concentrated *in vacuo* to give *t*-butyl 3-oxo-2-allyl-4-(phenylthio)-butanoate **374** as a yellow oil (232 mg, quant.): R_f 0.2 (eluent 50:1 petrol:EtOAc); ν_{max} (film) 2978, 2927, 1738, 1710; δ_H (400 MHz, $CDCl_3$) 1.46 (9H, s, (C(CH_3)$_3$), 2.37-2.47 (1H, m, C(1′)H_AH$_B$), 2.51-2.62 (1H, m, C(1′)H$_A$$H_B$), 3.55 (1H, d, *J* 15.4, C(4)H_AH$_B$), 3.69 (1H, d, *J* 15.4, C(4)H$_A$$H_B$), 3.79 (1H, app. t, *J* 7.5, C(2)*H*), 5.07-5.17 (2H, m, C(3′)H_2), 5.76-5.89 (1H, m, C(2′)*H*), 7.26-7.42 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 28.0 (C(*C*H$_3$)$_3$), 33.8 (*C*(1′)), 47.6 (*C*(4)), 56.0 (*C*(2)), 82.0 (*C*Me$_3$), 118.0 (*C*(3′)), 128.7, 128.9, 129.1 (*o,m,p-Ph*), 133.9 (*C*(2′)), 134.6 (*i-Ph*), 166.3 (*C*(1)), 198.2 (*C*(3)); *m/z* (ESI$^+$) 329 ([M+Na]$^+$, 15%), 635 ([2M+Na]$^+$, 20%); HRMS (ESI$^+$) $C_{17}H_{22}NaO_3S^+$ ([M+Na]$^+$) requires 329.1182, found 329.1182.

_t_-Butyl 3-oxo-2-benzyl-4-(phenylthio)-butanoate 375

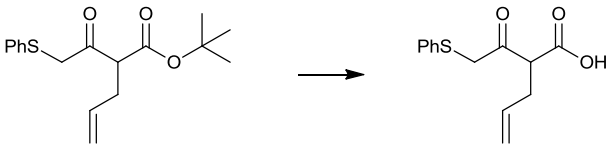


347c **375**

A stirred suspension of NaH (17 mg, 0.41 mmol) in THF (4 mL) was cooled to 0 °C and a solution of **347c** (100 mg, 0.38 mmol) in THF (1 mL) was added dropwise. Stirring was continued for 10 min at 0 °C before the dropwise addition of BuLi (0.27 mL, 1.48 M in hexanes, 0.39 mmol). After stirring for a further 10 min, BnBr (0.05 mL, 0.41 mmol) was added in one portion and the mixture was allowed to warm to rt over 15 min. The mixture

was quenched with 2 M HCl (7 mL) and extracted with Et_2O (2 × 15 mL). The combined organic layers were washed with H_2O until the aqueous layer remained neutral, dried and concentrated *in vacuo*. Purification by column chromatography (SiO_2, eluent 50:1 40-60 petrol:EtOAc) gave *t*-butyl 3-oxo-2-benzyl-4-(phenylthio)-butanoate **375** as a yellow oil (91 mg, 54%) in a 4:1 mixture with **347c**; R_f 0.2 (eluent 50:1 petrol:EtOAc); ν_{max} (film) 2978, 2931, 1737, 1709; δ_H (400 MHz, $CDCl_3$) 1.41 (9H, s, C(CH_3)$_3$), 2.96 (1H, dd, *J* 14.4, 7.2, C(1′)H_AH$_B$), 3.22 (1H, dd, *J* 14.4, 7.6, C(1′)H$_A$$H_B$), 3.52 (1H, d, *J* 15.3, C(4)H_AH$_B$), 3.63 (1H, d, *J* 15.3, C(4)H$_A$$H_B$), 4.04 (1H, app. t, *J* 7.4, C(2)*H*), 7.14-7.37 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 27.9 (C(*C*H$_3$)$_3$), 35.9 (*C*(5)), 48.2 (*C*(2)), 57.9 (*C*(4)), 82.0 (*C*Me$_3$), 126.7-129.6 (*o,m,p-Ph*, -S*Ph*), 133.8 (*i*-S*Ph*), 138.0 (*i-Ph*), 166.1 (*C*(1)), 198.1 (*C*(3)); *m/z* (ESI$^-$) 355 ([M−H]$^-$, 100%); HRMS (ESI$^+$) $C_{21}H_{24}NaO_3S^+$ ([M+Na]$^+$) requires 379.1228, found 379.1338.

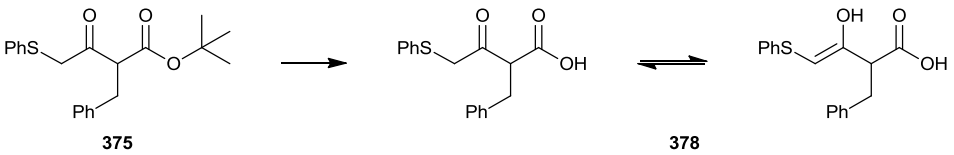
2-Allyl-3-oxo-4-(phenylthio)-butanoic acid 377



374 **377**

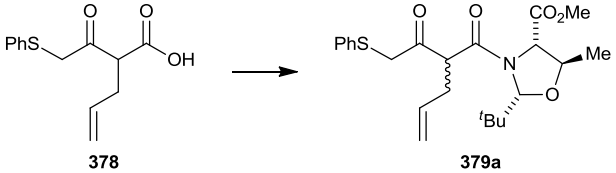
A solution of **374** (232 mg, 0.75 mmol) in DCM (1.0 mL) was cooled to 0 °C and TFA (1.0 mL) was added dropwise. The resulting solution was stirred for 1.5 h at rt before the solvent was removed *in vacuo* to give **377** as a pale yellow oil (190 mg, quant.); ν_{max} (film) 3077, 2923, 1704; δ_H (400 MHz, $CDCl_3$) 2.43-2.64 (2H, m, C(1′)H_2), 3.75-3.79 (3H, m, C(4)H_2, C(2)*H*), 5.09-5.20 (2H, m, C(3′)H_2), 5.76-5.89 (1H, m, C(2′)*H*), 7.28-7.42 (5H, m, *Ph*), 8.57 (1H, br s, O*H*); δ_C (100 MHz, $CDCl_3$)[30] 33.7 (*C*(1′)), 45.0 (*C*(4)), 56.5 (*C*(2)), 118.5 (*C*(3′)), 129.0, 129.2, 129.3, 130.0, 130.7 (*o,m,p-Ph*), 133.3, 134.0 (*C*(2′), *i-Ph*), 198.8 (*C*(3)); *m/z* (ESI$^+$) 273 ([M+Na]$^+$, 100%), (ESI$^-$) 249 ([M−H]$^-$, 60%); HRMS (ESI$^+$) $C_{13}H_{24}NaO_3S^+$ ([M+Na]$^+$) requires 273.0556, found 273.0561.

2-Benzyl-3-oxo-4-(phenylthio)-butanoic acid 378



A solution of **375** (90 mg, 0.26 mmol) in DCM (0.5 mL) was cooled to 0 °C and TFA (0.5 mL) was added dropwise. The resulting solution was stirred for 1.5 h at rt before the solvent was removed *in vacuo* to give **378** as a yellow oil (71 mg, 92%) in a 3:1 mixture of keto:enol tautomers; ν_{max} (film) 3061, 3028, 2928, 1705; δ_H (400 MHz, $CDCl_3$) keto form - 2.89-3.28 (2H, m, C*H*$_2$Ph), 3.66-3.72 (2H, m, C(4)*H*$_2$), 4.03 (1H, app. t, *J* 7.6, C(2)*H*), 7.15-7.45 (10H, m, *Ph*, S*Ph*); enol form - 2.89-3.28 (2H, m, C*H*$_2$Ph), 4.30 (1H, app. t, *J* 7.5, C(2)*H*), 4.83 (1H, s, C(4)*H*), 7.15-7.45 (10H, m, *Ph*, S*Ph*); δ_C (100 MHz, $CDCl_3$) 34.0, 35.9 (*C*H$_2$Ph), 45.9 (*C*(4) keto), 57.1 (*C*(2) enol), 58.3 (*C*(2) keto), 90.1 (*C*(4) enol), 126.8, 126.9, 127.2, 127.5, 128.3, 128.4, 128.5, 128.6, 129.0, 129.1, 129.7, 130.0 (*o,m,p-Ph*, -S*Ph*), 133.4, 134.0 (*i-Ph*), 137.7, 140.0 (*i*-S*Ph*), 171.8, 173.8 (*C*(1)), 198. 3, 198.8 (*C*(3)); *m/z* (ESI$^+$) 323 ([M+Na]$^+$, 100%), (ESI$^-$) 299 ([M−H]$^-$, 100%); HRMS (ESI$^+$) $C_{17}H_{16}NaO_3S^+$ ([M+Na]$^+$) requires 323.0712, found 323.0716.

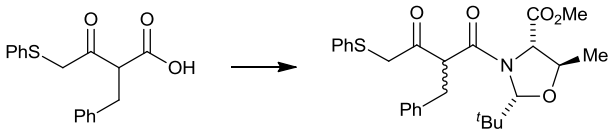
(2*R*,4*S*,5*R*)-2-*t*-Butyl-3-(2′-allyl-3′-oxo-4′-phenylthio-butanoyl)-4-methoxycarbonyl-5-methyloxazolidine 379a



Follwing **General Procedure D** for the synthesis of *N*-acyloxazolidines, oxazolidine **320** (169 mg, 0.84 mmol) was reacted with DCC (183 mg, 0.88 mmol), DMAP (7 mg, 7 mol%) and acid **378** (211 mg, 0.84 mmol). Purification *via* column chromatography gave **379a** as a mixture of indeterminate dr as a pale yellow oil (100 mg, 27%); R_f 0.2 (eluent 20:1 petrol:EtOAc); ν_{max} (film) 2956, 2926, 2871, 2855, 1746, 1714, 1667, 1625; δ_H (400 MHz, $CDCl_3$) 0.89 (9H, s, C(C*H*$_3$)$_3$), 1.32-1.40 (3H, m, C(5)C*H*$_3$) 2.41-2.73 (2H, m, C(1″)*H*$_2$), 3.72-3.82 (4H, m, CO$_2$C*H*$_3$), C(4′)*H*$_A$H$_B$), 3.90-3.95 (1H, m, C(2′)*H*), 4.00 (1H, d, *J* 15.2, C(4′)H$_A$*H*$_B$), 3.81 and 4.26 (1H, d, *J* 2.8 and 3.5, C(4)*H*), 4.66-4.72 and 4.73-4.79 (1H, m,

C(5)*H*), 5.07-5.20 (2H, m, C(3′′)H_2), 5.38-5.43 (1H, m, C(2)*H*), 5.75-5.91 (1H, m, C(2′′)*H*), 7.25-7.34 (3H, m, *o,p-Ph*), 7.34-7.46 (2H, m, *m-Ph*); δ_C (100 MHz, $CDCl_3$) 19.9, 20.0 (C(5)*C*H_3), 25.8 (C(*C*H_3)$_3$), 33.9 (*C*(1′′)), 37.7 (*C*Me$_3$), 46.9, 48.4 (*C*(4′)), 52.6, 52.7 (CO_2*C*H_3), 55.9, 46.0 (*C*(2′)), 65.1 (*C*(4)), 75.9 (*C*(5)), 95.9 (*C*(2)), 117.9, 118.0 (*C*(3′′)), 127.8, 127.9 (*p-Ph*), 128.7, 128.9, 129.0, 129.2 (*o,m-Ph*), 132.4, 133.7 (*i-Ph*), 134.0, 134.2 (*C*(2′′)), 167.9 (*C*(1′)), 170.1 (*C*O_2Me), 185.8 (*C*(3′)); *m/z* (ESI$^+$) 456([M+Na]$^+$, 100%), (ESI$^-$) 432 ([M−H]$^-$, 100%); HRMS (ESI$^+$) $C_{23}H_{31}NNaO_5S^+$ ([M+Na]$^+$) requires 456.1815, found 456.1809.

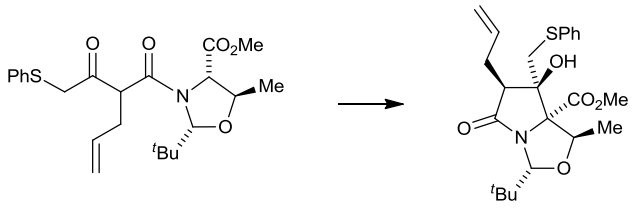
(2*R*,4*S*,5*R*)-2-*t*-Butyl-3-(2′-benzyl-3′-oxo-4′-phenylthio-butanoyl)-4-methoxycarbonyl-5-methyloxazolidine 379b



378 **379b**

Follwing **General Procedure D** for the synthesis of *N*-acyloxazolidines, oxazolidine **320** (47 mg, 0.24 mmol) was reacted with DCC (51 mg, 0.25 mmol), DMAP (2 mg, 7 mol%) and acid **378** (71 mg, 0.24 mmol). Purification *via* flash column chromatography (SiO_2, eluent 20:1 petrol:EtOAc) gave **379b** in a 1:1 dr as a colourless oil (20 mg, 17%); R_f 0.2 (eluent 20:1 petrol:EtOAc); ν_{max} (film) 3029, 2975, 2956, 2933, 2873, 1745, 1715, 1667, 1625; δ_H (500 MHz, $CDCl_3$) 0.87 (9H, s, C(CH_3)$_3$), 1.16 and 1.22 (3H, d, *J* 6.3, C(5)CH_3), 2.98-3.11 (1H, m, CH_AH$_B$Ph), 3.16-3.30 (1H, m, CH$_A$$H_B$Ph), 3.61-3.82 (5H, m, CO_2CH_3, C(4′)H_2-epimer A, C(4′)H_AH$_B$-epimer B, C(4)*H*-A), 3.95 (0.5 × 1H, d, *J* 15.1, C(4′)H$_A$$H_B$-B), 4.07-4.12 (0.5 × 2H, m, C(4′)*H*-B, C(2′)*H*-A), 4.17 (0.5 × 1H, app. t, *J* 7.6, C(2′)*H*-B), 4.63-4.68 and 4.69-4.73 (1H, m, C(5)*H*), 5.20 and 5.39 (1H, s, C(2)*H*), 7.14-7.32 (10H, m, *Ph*, S*Ph*); δ_C (125 MHz, $CDCl_3$) 19.8, 19.9 (C(5)*C*H_3), 25.7, 25.7 (C(*C*H_3)$_3$), 36.0, 36.3, 37.6, 37.7 (*C*H_2Ph, *C*Me$_3$), 48.2, 48.6 (*C*(4′)), 52.5, 52.7 (CO_2*C*H_3), 57.5, 57.8 (*C*(2′)), 64.9, 64.9 (*C*(4)), 75.7, 75.8 (*C*(5)), 95.8, 95.9 (*C*(2)), 126.8-129.2 (*o,m,p-Ph*, -S*Ph*), 133.0, 133.5 (*i*-S*Ph*), 137.5, 137.6 (*i-Ph*), 167.5, 168.1 (*C*(1′)), 170.0, 170.1 (*C*O_2Me), 199.6, 200.1 (*C*(3′)); *m/z* (ESI$^-$) 482 ([M−H]$^-$, 100%), (ESI$^+$) 506 ([M+Na]$^+$, 60%); HRMS (ESI$^+$) $C_{27}H_{33}NNaO_5S^+$ ([M+Na]$^+$) requires 506.1972, found 506.1980.

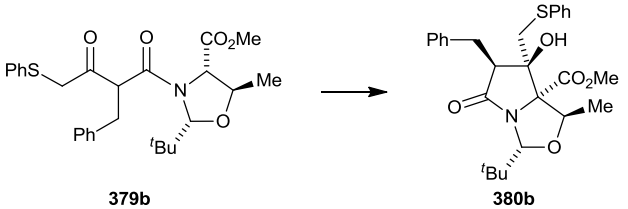
(2*R*,4*R*,5*R*,6*R*,7*S*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(phenylthio-methyl)-7-allyl-8-oxo-3-oxabicyclo[3.3.0]octane 380a



379a **380a**

Following **General Procedure F** for the aldol cyclisation of *N*-acyloxazolidines, *N*-acyloxazolidine **379a** (100 mg, 0.23 mmol) and NaOMe (14 mg, 0.25 mmol) were reacted to give a crude mixture which was purified *via* column chromatography (SiO_2, eluent 10:1 petrol:EtOAc) to give **380a** as an off-white solid (8 mg, 8%); R_f 0.1 (eluent 10:1 petrol:EtOAc); mp 95-97 ºC; $[\alpha]_D^{23}$ −5.92 (*c* 0.4 in $CHCl_3$); ν_{max} (film) 3430, 2955, 2923, 2854, 1725; δ_H (500 MHz, $CDCl_3$) 0.93 (9H, s, C(C*H*$_3$)$_3$), 1.65 (3H, d, *J* 6.6, C(4)C*H*$_3$), 2.29-2.38 (1H, m, C(1′′)*H*$_A$H$_B$), 2.46-2.57 (1H, m, C(1′′)H$_A$*H*$_B$), 2.89 (1H, s, O*H*), 3.18 (1H, app. t, *J* 6.2, C(7)*H*), 3.30 (1H, d, *J* 14.2, C(1′)*H*$_A$H$_B$), 3.40 (1H, d, *J* 14.2, C(1′)H$_A$*H*$_B$), 3.83 (3H, s, CO_2CH_3), 4.81 (1H, q, *J* 6.6, C(4)*H*), 5.00-5.13 (2H, m, C(3′′)*H*$_2$), 5.05 (1H, s, C(2)*H*), 5.93-6.03 (1H, m, C(2′′)*H*), 7.24-7.41 (5H, m, *Ph*); δ_C (125 MHz, $CDCl_3$) 14.1 (C(4)*C*H$_3$), 25.6 (C(*C*H$_3$)$_3$), 28.1 (*C*(1′′)), 37.1 (*C*Me$_3$), 41.3 (*C*(1′)), 52.3 (*C*(7)), 53.0 (CO_2CH_3), 78.6, 78.7 (*C*(4), *C*(5)), 85.7 (*C*(6)), 96.0 (*C*(2)), 117.1 (*C*(3′′)), 127.3, 129.3, 130.3 (*o*,*m*,*p*-*Ph*), 135.6, 136.5 (*i*-*Ph*, *C*(2′′)), 171.7 (*C*O$_2$Me), 178.1 (*C*(8)); *m/z* (ESI$^+$) 456 ([M+Na]$^+$, 75%), (ESI$^-$) 432 ([M−H]$^-$, 100%); HRMS (ESI$^+$) $C_{23}H_{31}NNaO_5S^+$ ([M+Na]$^+$) requires 456.1815, found 456.1811.

(2*R*,4*R*,5*R*,6*R*,7*S*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(phenylthio-methyl)-7-benzyl-8-oxo-3-oxabicyclo[3.3.0]octane 380b

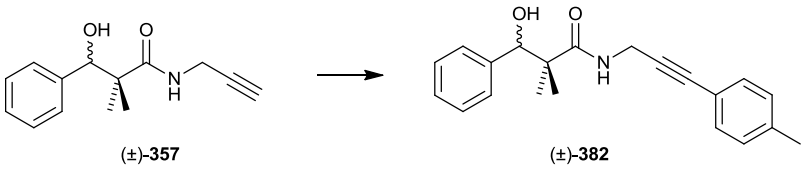


379b **380b**

Following **General Procedure F** for the aldol cyclisation of *N*-acyloxazolidines, *N*-acyloxazolidine **379b** (20 mg, 4.0 μmol) and NaOMe (3 mg, 5.0 μmol) were reacted to give a

crude mixture which was purified *via* column chromatography (SiO_2, eluent 10:1 petrol:EtOAc) to give **380b** as a colourless oil (3 mg, 15%); R_f 0.3 (eluent 6:1 petrol:EtOAc); $[\alpha]_D^{23}$ +7.56 (*c* 0.15 in $CHCl_3$); ν_{max} (film) 3437, 2956, 2924, 2854, 1722; δ_H (500 MHz, $CDCl_3$) 0.94 (9H, s, C(C*H*$_3$)$_3$), 1.65 (3H, d, *J* 6.6, C(4)C*H*$_3$), 2.89 (1H, dd, *J* 14.8, 7.4, C*H*$_A$H$_B$Ph), 2.98 (1H, d, *J* 14.3, C(1′)*H*$_A$H$_B$), 3.08 (1H, d, *J* 14.3, C(1′)H$_A$*H*$_B$), 3.16 (1H, dd, *J* 14.8, 6.0, CH$_A$*H*$_B$Ph), 3.42 (1H, app. t, *J* 6.6, C(7)*H*), 3.79 (3H, s, CO_2CH_3), 4.91 (1H, q, *J* 6.6, C(4)*H*), 5.05 (1H, s, C(2)*H*), 7.16-7.30 (10H, m, *Ph*, S*Ph*); δ_C (125 MHz, $CDCl_3$) 16.9 (C(4)*C*H$_3$), 25.6 (C(*C*H$_3$)$_3$), 29.6 (*C*H$_2$Ph), 37.1 (*C*Me$_3$), 41.6 (*C*(1′)), 52.9 (CO_2CH_3), 54.8 (*C*(7)), 78.4, 79.2 (*C*(4), *C*(5)), 85.0 (*C*(6)), 95.1 (*C*(2)), 126.3, 127.2, 128.5, 129.2, 129.3, 130.0 (*o,m,p-Ph*, -S*Ph*), 135.4 (*i*-S*Ph*), 139.7 (*i-Ph*), 171.9 (*C*O$_2$Me), 177.2 (*C*(8)); *m/z* (ESI^+) 506 ($[M+Na]^+$, 95%), (ESI^-) 506 ($[M-Na]^-$, 100%); HRMS (ESI^+) $C_{27}H_{33}NNaO_5S^+$ ($[M+Na]^+$) requires 506.1972, found 506.1976.

(±)-3-Hydroxy-2,2-dimethyl-3-phenyl-*N*-(3-(*p*-tolyl)prop-2-yn-1-yl)propanamide (±)-382



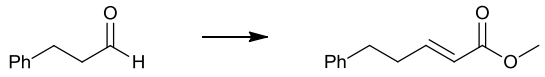
(±)-**357** (±)-**382**

Method A: A solution of *p*-tolyl bromide (0.05 mL, 0.39 mmol), alkyne (±)-**357** (100 mg, 0.43 mmol), CuI (8 mg, 0.04 mmol), and tetrakis(triphenylphophine)Palladium (0) (23 mg, 0.02 mmol) in TEA (4 mL and THF (1.3 mL) was stirred under N_2 at 70 °C for 18 h. The mixture was cooled to rt and H_2O (10 mL) was added. The mixture was extracted with EtOAc (3 × 10 mL) and the combined organic layers were dried and concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 7:3 petrol:EtOAc) gave (±)-**382** as an orange oil (7 mg, 5%); R_f 0.3 (eluent 7:3); ν_{max} (film) 3338, 2975, 2923, 1642, 1511; δ_H (500 MHz, $CDCl_3$) 1.12 (3H, s, C(2)C*H*$_3$), 1.27 (3H, s, C(2)C*H*$_3$), 2.36 (3H, s, *p*-C*H*$_3$), 4.07 (1H, br s, O*H*), 4.24 (2H, dd, *J* 17.7, 5.0, C(1′)*H*$_A$H$_B$), 4.31 (2H, dd, *J* 17.7, 5.4, C(1′)H$_A$*H*$_B$), 4.70 (1H, d, *J* 3.8, C*H*OH), 6.28 (1H, br s, N*H*), 7.14 (2H, d, *J* 7.9, *m-H*), 7.21-7.36 (2H, m, *o-H*); δ_C (125 MHz, $CDCl_3$) 21.5 (C(2)CH3), 24.4 (C(2)CH3), 30.2 (C(1′)), 46.3 (C(2)), 79.8 (C(3)), 80.1 (C(2′)), 83.7 (C(3′)), 119.4 (i-Ph tolyl ring), 127-132 (o,m,p-Ph), 138.6 (p-Ph

tolyl ring), 140.5 (i-Ph unsubs ring), 177.2 (C(1)); *m/z* (ESI^+) 320 ($[M-H]^-$, 50%); HRMS (ESI^+) $C_{21}H_{23}NNaO_2^+$ ($[M+Na]^+$) requires 344.1621, found 344.1624.

Method B: Aryl bromide (37 mg, 0.22 mmol), $PdCl_2(PPh_3)_2$ (8 mg, 0.01 mmol), CuI (2 mg, 0.01 mmol), PPh_3 (10 mg, 0.04 mmol), and alkyne (±)-**357** (50 mg, 0.22 mmol), $NHiPr_2$ (0.4 mL) and DMF (0.12 mL) were added to a microwave tube and stirred at 120 °C for 25 min in a microwave. The mixture was then diluted with Et_2O (5 mL), filtered and poured into 0.1 M HCl (5 mL). The mixture was separated and the aqueous layer was extracted with Et_2O (3 × 10 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (20 mL) and H_2O (20 mL), before being dried and concentrated *in vacuo* to give the crude mixture. Purification *via* column chromatography gave (±)-**382** (7 mg, 10%).

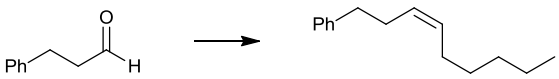
(*E*)-Methyl 5-phenylpent-2-enoate 383[31]



233 **383**

A portion of the aldehyde **233** (90 mg, 0.667 mmol) and phosphorous ylid methyl(triphenylphosphoranylidene)acetate (348 mg, 1.0 mmol) were dissolved in DCM (2 mL) and stirred for 20 h at rt. The solvent was removed *in vacuo* and the residue was filtered through a plug of silica (eluent ice cold Et_2O) to give **383** as a colourless oil (120 mg, 95%); δ_H (400 MHz, $CDCl_3$) 2.51-2.58 (2H, m, C(4)H_2), 2.79 (2H, t, *J* 7.7, C(5)H_2), 3.74 (3H, s, CO_2CH_3), 5.68 (1H, d, *J* 15.7, C(2)*H*), 7.02 (1H, dt, *J* 15.7, 6.8, C(3)*H*), 7.17-7.33 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 33.9 (*C*(4)), 34.3 (*C*(5)), 51.4 (*C*O_2Me), 121.4 (*C*(2)), 126.2, 128.3, 128.5 (*o,m,p-Ph*), 140.7 (*i-Ph*), 148.4 (*C*(3)), 167.6 (*C*(1)).

(*Z*)-1-Phenyl-non-3-ene 384[32]

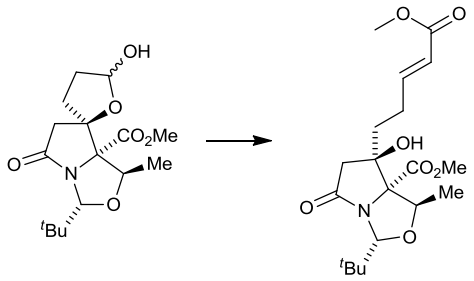


233 **384**

A suspension of hexyltriphenylphosphonium bromide (429 mg, 1.0 mmol) in dry THF (8 mL) was cooled to −10 °C and BuLi (0.78 mL, 1.4 M in hexanes, 1.1 mmol) was added dropwise over 10 min. The mixture was stirred for 1 h at between −5 and −10 °C. The aldehyde **233**

(0.11 mL, 0.86 mmol) in dry THF (2 mL) was added dropwise over 10 min and the mixture was allowed to warm to rt over 2 h, before being stirred at rt for a further 3 h. After this time H_2O (1 mL) was added and the THF was removed *in vacuo* before the mixture was further diluted with H_2O (9 mL) and extracted with EtOAc (2 × 10 mL). The combined organic layers were dried and concentrated *in vacuo* to give the crude mixture which was filtered through a plug of silica (eluent ice cold Et_2O) to give **384** as a colourless oil (170 mg, 97%); δ_H (400 MHz, $CDCl_3$) 0.91 (3H, t, *J* 6.9 C(9)H_3), 1.23-1.36 (6H, m, C(6)H_2, C(7)H_2, C(8)H_2), 1.97-2.05 (2H, m, C(5)H_2), 2.35-2.42 (2H, m, C(2)H_2), 2.69 (2H, t, *J* 7.7, C(1)H_2), 5.38-5.48 (2H, m, C(3)*H*, C(4)*H*), 7.18-7.34 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 14.1 (*C*(9)), 22.6, 29.2, 31.6 (*C*(6), *C*(7), *C*(8)), 27.2 (*C*(5)), 29.3 (*C*(1)), 125.8, 128.3, 128.5, 128.6, 130.8 (*o,m,p-Ph*, *C*(3)), 131.2 (*C*(4)), 142.2 (*i-Ph*).

(2*R*,4*R*,5*R*,6*R*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-((*E*)-4′-methoxycarbonyl-but-3′-enyl)-8-oxo-oxabicyclo[3.3.0]octane 385

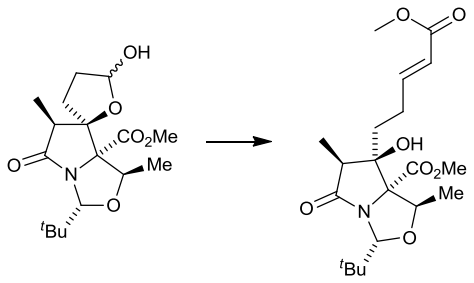


362 **385**

A portion of the aldehyde **362** (61 mg, 0.19 mmol) and phosphorous ylid methyl(triphenylphosphoranylidene)acetate (97 mg, 0.28 mmol) were dissolved in DCM (1 mL) and stirred for 20 h at rt. The solvent was removed *in vacuo* and the crude residue was filtered through a plug of silica (eluent ice cold Et_2O) to give **385** as a colourless oil (75 mg, quant); R_f 0.4 (eluent 2:1 petrol:EtOAc); $[\alpha]_D^{23}$ +21.0 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3413 (br), 2955, 2927, 2873, 1721, 1701, 1657; δ_H (400 MHz, $CDCl_3$) 0.90 (9H, s, C(CH_3)$_3$), 1.36-1.45 (1H, m, C(1′)H_AH$_B$), 1.69 (3H, d, *J* 6.6, C(4)CH_3), 1.93-2.01 (1H, m, C(1′)H$_A$$H_B$), 2.10-2.22 (1H, m, C(2′)H_AH$_B$), 2.39 (1H, d, *J* 15.7, C(7)H_AH$_B$), 2.48-2.59 (1H, m, C(2′)H$_A$$H_B$), 3.23 (1H, d, *J* 15.7, C(7)H$_A$$H_B$), 3.72 (3H, s, C(5′)O$_2CH_3$), 3.79 (1H, s, CO$_2CH_3$), 4.78 (1H, q, *J* 6.6, C(4)*H*), 5.05 (1H, s, C(2)*H*), 5.84 (1H, dt, *J* 15.7, 1.5. C(4′)*H*), 6.94 (1H, dt, *J* 15.7, 6.8, C(3′)*H*); δ_C (100 MHz, $CDCl_3$) 15.3 (C(4)*C*H$_3$), 25.7 (C(*C*H$_3$)$_3$), 27.0 (*C*(2′)), 34.4 (*C*(1′)),

37.4 (*C*Me$_3$), 47.2 (*C*(7)), 51.5 (C(5′)O$_2$*C*H$_3$), 52.8 (CO$_2$*C*H$_3$), 78.5 (*C*(4)), 80.9 (*C*(5)), 84.3 (*C*(6)), 96.0 (*C*(2)), 121.5 (*C*(4′)), 148.2 (*C*(3′)), 166.9 (*C*(5′)), 171.9 (*C*O$_2$Me), 178.3 (*C*(8)); *m/z* (ESI$^-$) 382 ([M−H]$^-$, 80%); HRMS (ESI$^+$) $C_{19}H_{29}NaNO_7^+$ ([M+Na]$^+$) requires 406.1836, found 406.1838.

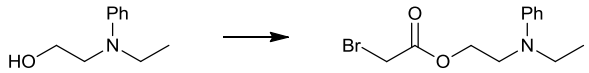
(2*R*,4*R*,5*R*,6*R*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-((*E*)-4′-methoxycarbonyl-but-3′-enyl)-7-methyl-8-oxo-oxabicyclo[3.3.0]octane 386



363 **386**

A portion of the aldehyde **363** (39 mg, 0.11 mmol) and phosphorous ylid methyl(triphenylphosphoranylidene)acetate (60 mg, 0.17 mmol) were dissolved in DCM (0.5 mL) and stirred for 20 h at rt. The solvent was removed *in vacuo* and the crude residue filtered through a plug of silica (eluent ice cold Et$_2$O) to give **386** as a colourless oil (34 mg, 78%); R_f 0.5 (eluent 2:1 petrol:EtOAc); $[\alpha]_D^{23}$ +4.1 (*c* 1.0 in CHCl$_3$); ν_{max} (film) 3436 (br), 2955, 1721, 1658; δ_H (400 MHz, CDCl$_3$) 0.91 (9H, s, C(C*H*$_3$)$_3$), 1.11 (3H, d, *J* 7.1 C(7)C*H*$_3$), 1.68 (3H, d, *J* 6.6, C(4)C*H*$_3$), 1.70-1.84 (2H, m, C(1′)*H*$_2$), 2.25-2.38 (1H, m, C(2′)*H*$_A$H$_B$), 2.41-2.53 (1H, m, C(2′)H$_A$*H*$_B$), 3.11 (1H, q, *J* 7.1, C(7)*H*), 3.72 (3H, s, C(5′)O$_2$C*H*$_3$), 3.79 (3H, s, CO$_2$C*H*$_3$), 4.80 (1H, q, *J* 6.6, C(4)*H*), 5.01 (1H, s, C(2)*H*), 5.83 (1H, dt, *J* 15.7, 1.5, C(4′)*H*), 6.91 (1H, dt, *J* 15.7, 6.8, C(3′)*H*); δ_C (100 MHz, CDCl$_3$) 7.3 (C(7)*C*H$_3$), 15.9 (C(4)*C*H$_3$), 25.7 (C(*C*H$_3$)$_3$), 26.8 (*C*(2′)), 34.2 (*C*(1′)), 37.3 (*C*Me$_3$), 48.5 (*C*(7)), 51.5 (*C*(5′)O$_2$Me), 52.8 (*C*O$_2$Me), 78.8 (*C*(4)), 79.2 (*C*(5)), 85.8 (*C*(6)), 96.0 (*C*(2)), 121.4 (*C*(4′)), 148.2 (*C*(3′)), 166.9 (*C*(5′)), 172.0 (*C*O$_2$Me), 179.7 (*C*(8)); *m/z* (ESI$^+$) 398 ([M+H]$^+$, 80%), 817 ([2M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{20}H_{31}NNaO_7^+$ ([M+Na]$^+$) requires 420.1983, found 420.1984.

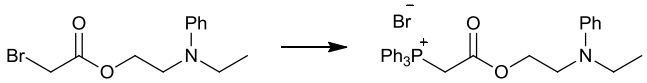
2-(Ethyl(phenyl)amino)ethyl 2-bromoacetate 393



392 **393**

Aminoethanol **392** (1.0 g, 6.1 mmol) was dissolved in dry DCM (7 mL) and TEA (0.84 mL, 6.1 mmol) was added. The mixture was stirred for 10 min before the dropwise addition of bromoacetyl bromide (0.58 mL, 6.7 mmol) over 5 min. The mixture was stirred for 5 h at rt and then concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 20:1 petrol:EtOAc) gave **393** as a pale yellow oil (1.02 g, 58%); R_f 0.9 (eluent 3:1 petrol:EtOAc); ν_{max} (film) 3025, 2969, 2895, 1736, 1597, 1504; δ_H (400 MHz, $CDCl_3$) 1.19 (3H, t, *J* 7.1, C*H*$_3$), 3.43 (2H, q, *J* 7.1, C*H*$_2$CH$_3$), 3.61 (2H, t, *J* 6.3, C*H*$_2$NEtPh), 3.82 (2H, s, C*H*$_2$Br), 4.35 (2H, t, *J* 6.3, C*H*$_2$O), 6.68-6.77 (3H, m, *Ph*), 7.22-7.27 (2H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 12.2 (*C*H$_3$), 25.7 (*C*Br), 45.3 (*C*H$_2$CH$_3$), 48.5 (*C*H$_2$NEtPh), 112.1, 116.5, 129.4 (*o*,*m*,*p*-*Ph*), 147.4 (*i*-*Ph*), 167.2 (*C*=O); *m/z* (ESI$^+$) 286/288 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{12}H_{17}BrNO_2^+$ ([M+H]$^+$) requires 286.0437/288.0417, found 286.0439/288.0420.

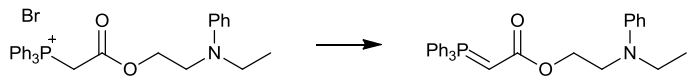
(2-(2′-(ethyl(phenyl)emino)ethoxy)-2-oxoethyl)triphenylphosphonium bromide 394



393 **394**

Bromide **393** (100 mg, 0.35 mmol) was added to a stirred solution of PPh_3 (92 mg, 0.35 mmol) in acetone (2 mL) and the resulting mixture was stirred for 18 h at rt. The solvent was removed *in vacuo* to give **394** as a pale brown sticky foam (200 mg, quant); δ_H (400 MHz, $CDCl_3$) 1.07 (3H, t, *J* 7.1, C*H*$_3$), 3.33 (2H, q, *J* 7.1, C*H*$_2$CH$_3$), 3.51 (2H, t, *J* 6.1, C*H*$_2$NEtPh), 4.19 (2H, t, *J* 6.1, C*H*$_2$O), 5.56 (2H, d, J_{HP} 13.4, C*H*$_2$P), 6.73-6.81 (3H, m, *o*,*p*-N*Ph*), 7.20-7.25 (2H, m, *m*-N*Ph*), 7.61-7.67 (2H, m, *m*-P*Ph*$_3$), 7.74-7.80 (1H, m, *p*-P*Ph*$_3$), 7.81-7.88 (2H, m, *o*-P*Ph*$_3$); δ_C (100 MHz, $CDCl_3$) 11.7 (*C*H$_3$), 32.7 (d, J_{CP} 57.5, *C*H$_2$PPh$_3$), 46.1 (*C*H$_2$CH$_3$), 49.0 (*C*H$_2$NEtPh), 63.3 (*C*H$_2$O), 113.4 (*o*-N*Ph*), 117.8, 118.1 (*p*-N*Ph*, *i*-P*Ph*$_3$), 129.5 (*m*-N*Ph*), 128.6, 130.3, 130.4, 133.5, 133.8, 133.9, 135.2, 135.3 (*o*,*m*,*p*-P*Ph*$_3$) 146.0 (*i*-N*Ph*), 164.6 (d, J_{CP} 3.2, *C*=O); *m/z* (ESI$^+$) 468 (M$^+$, 100%); HRMS (ESI$^+$) $C_{30}H_{31}NO_2P^+$ (M$^+$) requires 468.2087, found 468.2089.

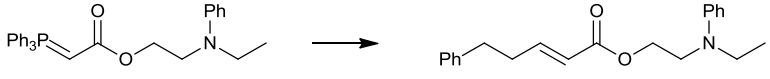
2′-(ethyl(phenyl)amino)ethyl 2-(triphenylphosphoranylidene)acetate 395



394 395

The PBr salt **394** was dissolved in DCM (10 mL) and shaken in a separating funnel with aq. 2 M NaOH (5mL). The layers were separated and the aqueous layer was extracted with DCM (2 × 5 mL). The combined organic layers were dried and concentrated *in vacuo* to give **395** as a pale yellow gum (40 mg, 95%); ν_{max} (film) 3057, 2968, 2892, 1614, 1598; δ_H (500 MHz, $CDCl_3$) 0.99-1.15 (3H, m, C*H*$_3$), 2.90 (1H, br s, C(2)*H*), 3.11-3.52 (4H, m, C(2′)*H*$_2$, NC*H*$_2$CH$_3$), 3.98-4.15 (2H, m, C(1′)*H*$_2$), 6.59-6.74 (3H, m, *o*,*p*-N*Ph*), 7.17 (2H, app. t, *J* 7.7, *m*-N*Ph*), 7.42-7.50 (2H, m, *m*-P*Ph*$_3$), 7.53-7.59 (1H, m, *p*-P*Ph*$_3$), 7.62-7.71 (2H, m, *o*-P*Ph*$_3$); δ_C (125 MHz, $CDCl_3$) 12.1 (*C*H$_3$), 44.8, 45.2 (NCH$_2$CH$_3$, *C*(2′)), 59.3 (*C*(1′)), 111.7 (*o*-N*Ph*), 115.4 (*p*-N*Ph*), 129.2 (*m*-N*Ph*), 128.4, 128.5, 128.7, 128.8, 132.1, 133.2, 133.6 (*o*,*m*,*p*-P*Ph*$_3$), 147.8 (*i*-N*Ph*), 171.0 (*C*=O); *m/z* (ESI$^+$) 468 ([M+H]$^+$, 90%); HRMS (ESI$^+$) $C_{30}H_{31}NO_2P^+$ ([M+H]$^+$) requires 468.2087, found 468.2086.

(*E*)-2′-(ethyl(phenyl)amino)ethyl 5-phenylpent-2-enoate 396

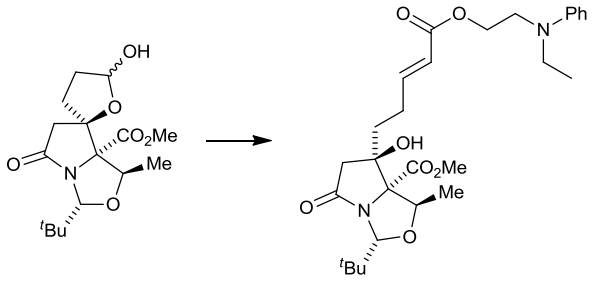


395 396

A portion of the aldehyde **233** (7 µL, 0.06 mmol) and phosphorous ylid **395** (40 mg, 0.09 mmol) were dissolved in DCM (0.5 mL) and stirred for 20 h at rt. The solvent was removed *in vacuo* and the residue was filtered through a plug of silica (eluent ice cold Et_2O) to remove the Ph_3PO and then separated from the starting aldehyde by column chromatography (SiO_2, eluent 50:1 petrol:EtOAc), giving **396** as a pale yellow oil (14 mg, 76%); R_f 0.2 (eluent 50:1 petrol:EtOAc); ν_{max} (film) 2970, 2931, 2897, 1718, 1653, 1598, 1505; δ_H (500 MHz, $CDCl_3$) 1.18 (3H, t, *J* 7.1, C*H*$_3$), 2.51-2.58 (2H, m, C(4)*H*$_2$), 2.79 (2H, t, *J* 6.7, C(5)*H*$_2$), 3.42 (2H, q, *J* 7.1, C*H*$_2$CH$_3$), 3.59 (2H, t, *J* 6.5, C(2′)*H*$_2$), 4.30 (2H, t, *J* 6.5, C(1′)*H*$_2$), 5.85 (1H, dt, *J* 15.6, 1.5, C(2)*H*), 6.70 (1H, t, *J* 7.3, *p*-N*Ph*), 6.75 (2H, d, *J* 8.2, *o*-N*Ph*), 7.01 (1H, dt, *J* 15.6, 6.9, C(3)*H*), 7.18-7.26 (5H, m, *m*-N*Ph*, *o*,*p*-*Ph*), 7.31 (2H, app. t, *J* 7.4, *m*-*Ph*); δ_C (125 MHz, $CDCl_3$) 12.2 (*C*H$_3$), 33.9 (*C*(4)), 34.3 (*C*(5)), 45.3 (*C*H$_2$CH$_3$), 48.8 (*C*(2′)), 61.6 (*C*(1′)), 111.9

(*o*-N*Ph*), 116.1 (*p*-N*Ph*), 121.5 (*C*(2)), 126.3, 128.3, 128.6, 129.3 (*m*-N*Ph*, *o,m,p-Ph*), 140.7 (*i-Ph*), 147.6 (*i*-N*Ph*), 148.7 (*C*(3)), 166.4 (*C*=O); *m/z* (ESI^+) 324 ($[M+H]^+$, 100%), 346 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{21}H_{26}NO_2^+$ ($[M+H]^+$) requires 324.1958, found 324.1955.

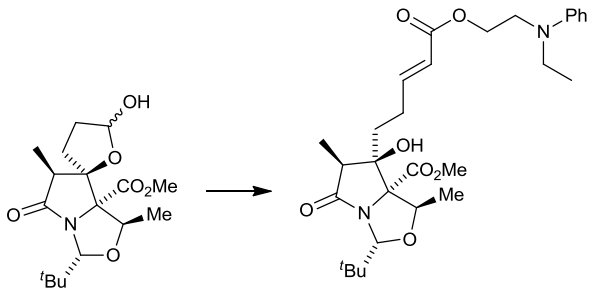
(2*R*,4*R*,6*R*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(*E*-5′-(2″-ethyl(phenyl)amino)-ethoxy)-5′-oxopent-3′-en-1′-yl)-8-oxo-3-oxabicyclo[3.3.0]octane 397



362 **397**

A portion of the aldehyde **362** (26 mg, 0.08 mmol) and phosphorous ylid **395** (56 mg, 0.12 mmol) were dissolved in DCM (1 mL) and stirred for 20 h at rt. The solvent was removed *in vacuo* and the crude residue was filtered through a plug of silica (eluent ice cold Et_2O) to give **397** as a yellow oil (21 mg, 51%); R_f 0.72 (eluent 1:1 petrol:EtOAc); $[\alpha]_D^{22}$ +12.4 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3410, 2958, 2873, 1718, 1699, 1654, 1598; δ_H (500 MHz, $CDCl_3$) 0.92 (9H, s, C(C*H*$_3$)$_3$), 1.18 (3H, t, *J* 7.1, NCH$_2$C*H*$_3$), 1.37-1.44 (1H, m, C(1′)*H*$_A$H$_B$), 1.70 (3H, d, *J* 6.6, C(4)C*H*$_3$), 1.94-2.01 (1H, m, C(1′)H$_A$*H*$_B$), 2.12-2.21 (1H, m, C(2′)*H*$_A$H$_B$), 2.38 (1H, d, *J* 15.8, C(7)*H*$_A$H$_B$), 2.49-2.58 (1H, m, C(2′)H$_A$*H*$_B$), 3.05 (1H, d, *J* 15.8, C(7)H$_A$*H*$_B$), 3.42 (2H, q, *J* 7.1, NC*H*$_2$CH$_3$), 3.56 (1H, s, O*H*), 3.59 (2H, t, *J* 6.4, C(2″)*H*$_2$), 3.81 (3H, s, CO$_2$C*H*$_3$), 4.30 (2H, t, *J* 6.4, C(1″)*H*$_2$), 4.80 (1H, q, *J* 6.6, C(4)*H*), 5.07 (1H, s, C(2)*H*), 5.85 (1H, d, *J* 15.6, C(4′)*H*), 6.69 (1H, t, *J* 7.3, *p-Ph*), 6.75 (2H, d, *J* 7.9, *o-Ph*), 6.93 (1H, dt, *J* 15.6, 6.9, C(3′)*H*), 7.20-7.25 (2H, m, *m-Ph*); δ_C (125 MHz, $CDCl_3$) 12.2 (NCH$_2$*C*H$_3$), 15.3 (C(4)*C*H$_3$), 25.7 (C(*C*H$_3$)$_3$), 27.1 (*C*(2′)), 34.3 (*C*(1′)), 37.4 (*C*Me$_3$), 45.2 (N*C*H$_2$CH$_3$), 47.2 (*C*(7)), 48.8 (*C*(2″)), 52.7 (CO$_2$*C*H$_3$), 61.6 (*C*(1″)), 78.5 (*C*(4)), 80.8 (*C*(5)), 84.4 (*C*(6)), 96.1 (*C*(2)), 111.9 (*o-Ph*), 116.2 (*p-Ph*), 121.6 (*C*(4′)), 129.3 (*m-Ph*), 147.6 (*i-Ph*), 148.4 (*C*(3)), 166.3 (*C*(5′)), 171.9 (*C*O$_2$Me), 178.2 (*C*(8)); *m/z* (ESI^+) 539 ($[M+Na]^+$, 100%), 517 ($[M+H]^+$, 80%); HRMS (ESI^+) $C_{28}H_{40}N_2NaO_7^+$ ($[M+H]^+$) requires 539.2728, found 539.2728.

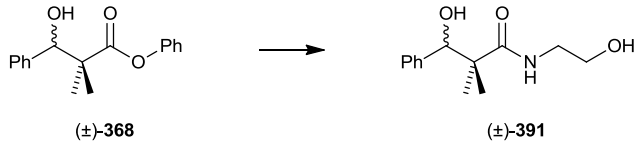
(2*R*,4*R*,6*R*,7*S*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-(*E*-5′-(2′′-ethyl(phenyl)amino)-ethoxy)-5′-oxopent-3′-en-1′-yl)-7-methyl-8-oxo-3-oxabicyclo[3.3.0]octane 398



363 **398**

A portion of the aldehyde **363** (34 mg, 0.10 mmol) and phosphorous ylid **395** (66 mg, 0.15 mmol) were dissolved in DCM (1 mL) and stirred for 20 h at rt. The solvent was removed *in vacuo* and the crude residue was filtered through a plug of silica (eluent ice cold Et_2O) to give **398** as a pale yellow oil (42 mg, 79%); R_f 0.83 (eluent 1:1 petrol:EtOAc); $[\alpha]_D^{22}$ −2.9 (*c* 0.7 in $CHCl_3$); ν_{max} (film) 3444, 2958, 2873, 1717, 1655, 1598; δ_H (400 MHz, $CDCl_3$) 0.93 (9H, s, C(C*H*$_3$)$_3$), 1.13 (3H, d, *J* 7.1, C(7)C*H*$_3$), 1.18 (3H, t, *J* 7.1, NCH$_2$C*H*$_3$), 1.70 (3H, d, *J* 6.6, C(4)C*H*$_3$), 1.70-1.85 (2H, m, C(1′)*H*$_2$), 2.27-2.38 (1H, m, C(2′)*H*$_A$H$_B$), 2.41-2.55 (1H, m, C(2′)H$_A$*H*$_B$), 3.13 (1H, q, *J* 7.1, C(7)*H*), 3.42 (2H, q, *J* 7.1, NC*H*$_2$CH$_3$), 3.59 (2H, t, *J* 6.3, C(2′′)*H*$_2$), 3.80 (3H, s, CO$_2$C*H*$_3$), 4.30 (2H, t, *J* 6.3, C(1′′)*H*$_2$), 4.82 (1H, q, *J* 6.6, C(4)*H*), 5.03 (1H, s, C(2)*H*), 5.83 (1H, dt, *J* 15.7, 1.4, C(4′)*H*), 6.69 (1H, t, *J* 7.2, *p*-N*Ph*), 6.74 (2H, d, *J* 8.1, *o*-N*Ph*), 6.91 (2H, dt, *J* 15.7, 6.8, C(3′)*H*), 7.19-7.25 (2H, m, *m*-N*Ph*); δ_C (100 MHz, $CDCl_3$) 7.4 (C(7)CH$_3$), 12.2 (NCH$_2$*C*H$_3$), 15.9 (C(4)*C*H$_3$), 25.8 (C(*C*H$_3$)$_3$), 26.8 (*C*(2′)), 34.2 (*C*(1′)), 37.3 (*C*Me$_3$), 45.2 (N*C*H$_2$CH$_3$), 48.5 (*C*(7)), 48.8 (*C*(2′′)), 52.8 (CO$_2$*C*H$_3$), 61.7 (*C*(1′′)), 78.8 (*C*(4)), 79.1 (*C*(5)), 85.9 (*C*(6)), 96.1 (*C*(2)), 112.0 (*o*-N*Ph*), 116.2 (*p*-N*Ph*), 121.5 (*C*(4′)), 129.3 (*m*-N*Ph*), 147.6 (*i*-N*Ph*), 148.5 (*C*(3′)), 166.3 (*C*(5′)), 171.9 (*C*O$_2$Me), 179.7 (*C*(8)); *m/z* (ESI$^+$) 531 ([M+H]$^+$, 100%), 553 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{29}H_{43}N_2O_7^+$ ([M+H]$^+$) requires 531.3065, found 531.3066.

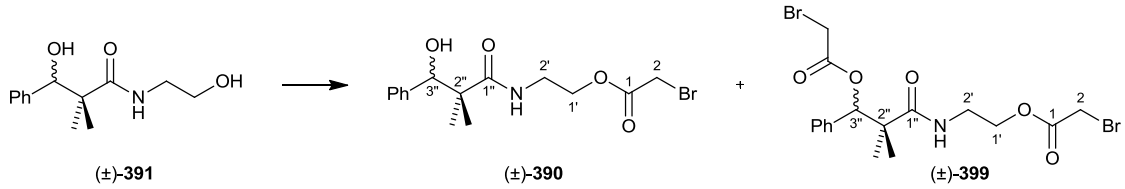
(±)-3-Hydroxy-*N*-(2′hydroxyethyl)-2,2-dimethyl-3-phenylpropanamide (±)-391



(±)-**368** (±)-**391**

The ester (±)-**368** (200 mg, 0.74 mmol), TEA (0.11 mL, 0.81 mmol) and aminoethanol (0.22 mL, 3.7 mmol) were dissolved in DCM (4 mL) and stirred at 40 °C for 2 days. The solvent was then removed *in vacuo* and the residue was purified *via* column chromatography (SiO_2, eluent 4:1 petrol:EtOAc then EtOAc) to give (±)-**391** as a yellow oil (268 mg, 73%); R_f 0.08 (eluent 4:1 petrol:EtOAc); ν_{max} (film) 3344, 2971, 2937, 2877, 1736, 1629, 1532; δ_H (400 MHz, $CDCl_3$) 1.03 (3H, s, CH_3), 1.10 (3H, s, CH_3), 3.19-3.28 (1H, m, C(1′)H_AH_B), 3.32-3.41 (1H, m, C(1′)H_AH_B), 3.52-3.62 (2H, m, C(2′)H_2), 3.81 (br s, O*H*), 4.65 (1H, s, C(3)*H*), 4.79 (br s, O*H*), 6.73 (1H, t, *J* 5.4, N*H*), 7.20-7.31 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 19.7 (*C*H_3), 24.1 (*C*H_3), 42.2 (*C*(1′)), 46.5 (*C*(2)), 61.5 (*C*(2′)), 79.4 (*C*(3)), 127.5, 127.8, 127.9 (*o,m,p-Ph*), 140.5 (*i-Ph*), 178.7 (*C*(1)); *m/z* (ESI^+) 260 ($[M+Na]^+$, 60%), 497 ($[2M+Na]^+$, 100%); HRMS (ESI^+) $C_{13}H_{19}NO_3^+$ ($[M+H]^+$) requires 260.1257, found 260.1258.

(±)-2′-(3′′-hydroxy-2′′,2′′-diemethyl-3′′-phenylpropanamido)ethyl 2-bromoacetate (±)-390 and (±)-2′-(3′′-(bromoacetoxy)-2′′,2′′-diemethyl-3′′-phenylpropanamido)ethyl 2-bromoacetate (±)-399

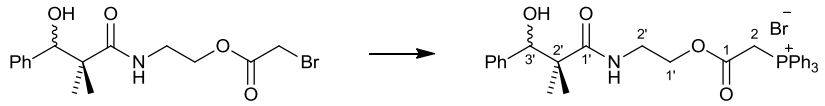


(±)-**391** (±)-**390** (±)-**399**

Diol (±)-**391** (158 mg, 0.67 mmol) was dissolved in dry DCM (3 mL) and TEA (0.07 mL, 0.09 mmol) was added. The mixture was stirred for 10 min before it was cooled to 0 °C and bromoacetyl bromide (0.06 mL, 0.73 mmol) was added dropwise over 5 min. The mixture was stirred for 5 h at rt and then concentrated *in vacuo*. Purification *via* column chromatography (SiO_2, eluent 5:1 to 1:1) gave (±)-**399** as a orange oil (40 mg, 34%); R_f 0.4 (eluent 1:1 petrol:EtOAc); ν_{max} (film) 3422 (NH), 2975, 1735, 1650, 1528; δ_H (400 MHz, $CDCl_3$) 1.16 (3H, s, CH_3), 1.24 (3H, s, CH_3), 3.55 (2H, app. q, *J* 5.4, C(2′)H_2), 3.88 (2H, s, CH_2Br), 4.17-4.31 (2H, m, C(1′)H_2), 6.01 (1H, s, C(3′′)*H*), 6.28 (1H, t, *J* 5.2, N*H*), 7.26-7.35

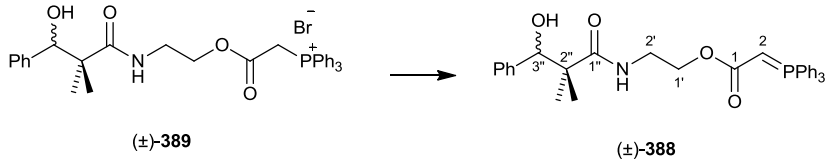
(5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 20.6 (*C*H_3), 22.3 (*C*H_3), 25.6 (*C*(2)), 25.9 (*C*H_2Br), 38.9 (*C*(2′)), 46.9 (*C*(2″)), 64.9 (*C*(1′)), 81.5 (*C*(3″)), 127.5,128.1, 128.4 (*o,m,p-Ph*), 135.9 (*i-Ph*), 165.5, 167.4 (*C*(1), *C*(1″)), 175.3 (*C*=O, amide); *m/z* (ESI^+) 499/501/503 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{17}H_{21}Br_2NNaO_5^+$ ($[M+Na]^+$) requires 501.9659, found 501.9654; and (±)-**390** as a pale orange oil (59 mg, 36%); R_f 0.3 (eluent 1:1 petrol:EtOAc); ν_{max} (film) 3359, 2973, 1737, 1637, 1533; δ_H (400 MHz, $CDCl_3$) 1.09 (3H, s, C*H*$_3$), 1.20 (3H, s, C*H*$_3$), 3.54 (2H, app. q, *J* 5.4, C(2′)*H*$_2$), 3.80 (2H, s, C(2)*H*$_2$), 4.22-4.26 (2H, m, C(1′)*H*$_2$), 4.68 (1H, s, C(3″)*H*), 6.48 (1H, br s, N*H*), 7.24-7.32 (5H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 20.4 (*C*H_3), 24.2 (*C*H_3), 25.5 (*C*(2)), 38.5 (*C*(2′)), 46.4 (*C*(2″)), 64.6 (*C*(1′)), 79.7 (*C*(3″)), 127.5, 127.8, 127.9 (*o,m,p-Ph*), 140.5 (*i-Ph*), 167.4 (*C*(1)), 178.1 (*C*(1″)); *m/z* (ESI^+) 380/382 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{15}H_{20}BrNNaO_4^+$ ($[M+H]^+$) requires 382.0448, found 382.0444.

(±)-2′-(3″-hydroxy-2″,2″-diemethyl-3″-phenylpropanamido)ethyltriphenylphosphonium bromide (±)-389



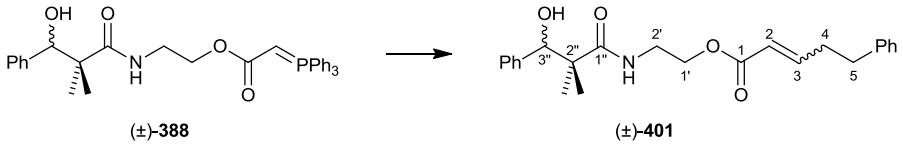
Bromide (±)-**390** (37 mg, 0.1 mmol) was added to a stirred solution of PPh_3 (29 mg, 0.1 mmol) in acetone (1 mL) and the resulting mixture was stirred for 18 h at rt. The solvent was removed *in vacuo* to give (±)-**389** as a white powder (59 mg, 95%); mp 108-111 ºC; ν_{max} (film) 3294 (br), 3057, 2971, 1731, 1643; δ_H (400 MHz, $CDCl_3$) 1.14 (3H, s, CH_3), 1.19 (3H, s, C*H*$_3$), 3.50 (2H, app. q, *J* 5.0, C(2′)*H*$_2$), 4.15-4.21 (1H, m, C(1′)*H*$_A$H$_B$), 4.25-4.32 (1H, m, C(1′)H$_A$*H*$_B$), 4.80 (1H, s, C(3″)*H*), 5.47 (1H, dd, *J* 17.2, 12.5, C(2)*H*$_A$H$_B$), 5.62 (1H, dd, *J* 17.2, 12.5, C(2)H$_A$*H*$_B$); δ_C (100 MHz, $CDCl_3$) 20.0 (*C*H_3), 24.6 (*C*H_3), 32.6 (*C*(2)), 38.6 (*C*(2′)), 65.9 (*C*(1′)), 79.1 (*C*(3″)), 127.1, 127.4, 128.0, 128.4, 128.7, 130.3, 130.4, 130.5, 133.6, 133.8, 133.8, 133.9, 135.2 (*o,m,p,i*-P*Ph*$_3$, *o,m,p-Ph*), 141.0 (*i-Ph*), 164.8 (*C*(1)), 179.2 (*C*(1″)); *m/z* (ESI^+) 540 (M^+, 90%); HRMS (ESI^+) $C_{33}H_{35}NO_4P^+$ (M^+) requires 540.2298, found 540.2293.

(±)-2′-(3′′-Hydroxy-2′′,2′′-diemthyl-3′′-phenylpropanamido)ethyl 2-(triphenylphosphoranylidene)acetate (±)-388



The salt (±)-**389** (50 mg, 0.08 mmol) was dissolved in DCM (10 mL) and shaken in a separating funnel with aq. 2M NaOH (5mL). The layers were separated and the aqueous layer was extracted with DCM (2 × 5 mL). The combined organic layers were dried and concentrated *in vacuo* to give (±)-**388** as a yellow oil (43 mg, quant.); ν_{max} (film) 3324 (br), 3059, 2971, 1732, 1612, 1527, 1437; δ_H (400 MHz, $CDCl_3$) 0.91 (3H, s, CH_3), 1.07 (3H, s, CH_3), ~2.80 (very br s, C(2)*H*), 3.20-3.32 (2H, m, C(2′)H_2), 4.00-4.10 (2H, m, C(1′)H_2), 4.58 (1H, s, C(3′′)*H*), 5.24 (1H, br s, O*H*), 7.38-7.79 (20H, m, *Ph*, P*Ph*$_3$); δ_C (100 MHz, $CDCl_3$) 20.0 (*C*H$_3$), 24.2 (*C*H$_3$), 39.0 (*C*(2′)), 45.4 (*C*(2′′)), 60.3 (*C*(1′)), 80.0 (*C*(3′′)), 127.5-133.8 (*Ph*, P*Ph*$_3$), 140.9 (*i-Ph*), 177.9, 178.6 (*C*(1), *C*(1′′)); *m/z* (ESI$^+$) 540 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{33}H_{35}NO_4P^+$ ([M+H]$^+$) requires 540.2298, found 540.2314.

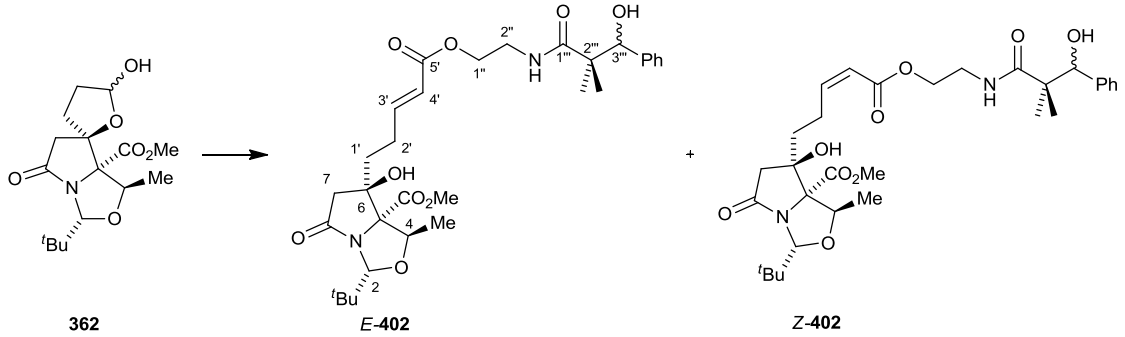
(±)- (*E*/*Z*)-2′-(3′′-Hydroxy-2′′,2′′-dimethyl-3′′-pheynlpropanamido)ethyl 5-phenylpent-2-enoate (±)-401



A portion of the aldehyde **233** (6 µL, 0.04 mmol) and phosphorous ylid (±)-**388** (35 mg, 0.06 mmol) were dissolved in DCM (0.5 mL) and stirred for 20 h at rt. The solvent was removed *in vacuo* and the crude residue was purified by column chromatography (SiO_2, eluent 20:1 to 5:1) to give (±)-**401** as a 2:1 mixture of *E*:*Z* isomers as a colourless oil (14 mg, 88%); R_f 0.5 (eluent 1:1 petrol:EtOAc); ν_{max} (film) 3348, 3062, 6029, 2928, 1718, 1657, 1533; δ_H (500 MHz, $CDCl_3$) 1.07 and 1.21 (3H, s, C(2′′)CH_3-*Z*), 1.08 and 1.22 (3H, s, C(2′′)CH_3-*E*), 2.51-2.57 (2H, m, C(4)H_2-*E*), 2.74-2.80 (2H, m, C(5)H_2), 2.90-3.00 (2H, m, C(4)H$_2$-*Z*), 3.50-3.56 (2H, m, C(1′)H_2), 4.19-4.25 (2H, m, C(2′)H_2), 4.66 (1H, br s, C(3′′)*H*), 5.76 (1H, dt, *J* 11.5, 1.6, C(3)*H-Z*), 5.82 (1H, dt, *J* 15.8, 1.6, C(3)*H-E*), 6.29 (1H, dt, *J* 11.5, 7.4, C(2)*H-Z*), 6.38-

6.46 (1H, m, N*H*), 7.01 (1H, dt, *J* 15.8, 6.9, C(2)*H*-*E*), 7.15-7.32 (10H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 20.4, 20.5 (C(2′′)*C*H_3), 24.3, 24.3 (C(2′′) *C*H_3), 30.5 (*C*(4)-*Z*), 33.5 (*C*(4) – *E*), 34.3, 34.9 (*C*(5)), 39.0, 39.1 (*C*(2′)), 46.2 (*C*(2′′)), 62.4, 62.7 (*C*(1′)), 79.8, 79.9 (*C*(3′′)), 119.5 (*C*(3)-*Z*), 121.1 (*C*(3)-*E*), 126.1, 126.2, 127.4, 127.5, 127.7, 127.8, 128.3, 128.4, 128.4, 128.5, 128.5 (*o,m,p-Ph*), 140.6, 140.6, 140.6, 140.9 (*i-Ph*), 149.9 (*C*(2)-*E*), 150.4 (*C*(2)-*Z*), 166.3, 166.7 (*C*(1)), 177.8, 177.9 (*C*(1′′)); *m/z* (ESI^+) 396 ($[M+H]^+$, 100%), 418 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{24}H_{29}NNaO_4^+$ ($[M+Na]^+$) requires 418.1989, found 418.1989.

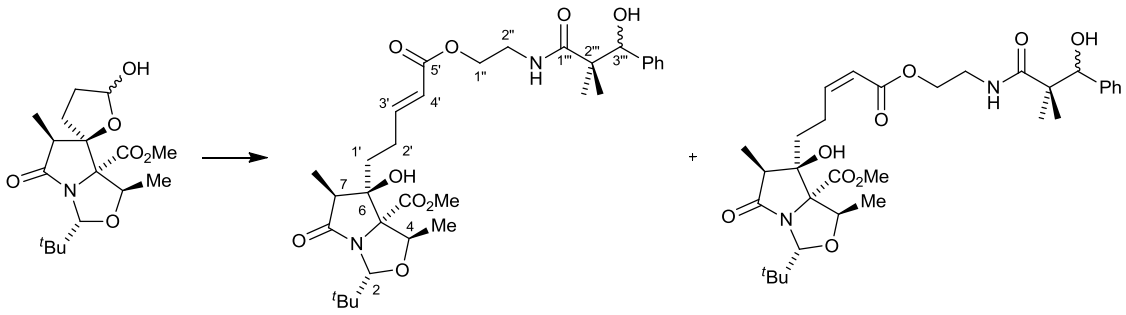
(2*R*,4*R*,5*R*,6*R*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-((*E*)-5′-(2′′-(3′′′-hydroxy-2′′′,2′′′-diemethyl-3′′′-phenylpropanamido)ethoxy)-5′-oxopent-3′-en-1′-yl)-8-oxo-3-pxabicyclo[3.3.0]octane *E*-402 and (2*R*,4*R*,5*R*,6*R*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-((*Z*)-5′-(2′′-(3′′′-hydroxy-2′′′,2′′′-diemethyl-3′′′-phenylpropanamido)ethoxy)-5′-oxopent-3′-en-1′-yl)-8-oxo-3-pxabicyclo[3.3.0]octane *Z*-402



A portion of the aldehyde **362** (18.2 mg, 0.06 mmol) and phosphorous ylid (±)-**388** (36 mg, 0.07 mmol) were dissolved in DCM (0.5 mL) and stirred for 20 h at rt. The solvent was removed *in vacuo* to give a crude product mixture containing a 3.5:1 mixture of the *E*:*Z* isomers. Purification *via* column chromatography (SiO_2, eluent 5:1 to 1:1 petrol:EtOAc) gave *Z*-**402** as 1:1 a mixture of epimers at C(3′′′) as a colourless oil (13 mg, 40%); R_f 0.7 (eluent EtOAc); ν_{max} (film) 3368, 2956, 2874, 1714, 1643, 1534; δ_H (500 MHz, $CDCl_3$) 0.88 and 0.90 (9H, s, C(CH_3)$_3$), 1.08 and 1.09 (3H, s, C(2′′′)CH_3), 1.17 and 1.19 (3H, s, C(2′′′)CH_3), 1.46-1.54 (1H, m, C(1′)H_AH$_B$), 1.67-1.70 (1H, m, C(4)CH_3), 2.00-2.08 (1H, m, C(1′)H$_A$$H_B$), 2.36 and 2.41 (1H, d, *J* 15.5, and 15.8, C(7)H_AH$_B$), 2.68-2.81 (2H, m, C(2′)H_2), 2.90-2.98 (1H, m, C(7)H$_A$$H_B$), 3.47-3.52 (1H, m, C(2′′)H$_A$$H_B$-epimer A), 3.58 (2H, app. q, *J* 5.4, C(2′′)H_2-

epimer B), 3.63-3.70 (1H, m, C(2″)H_AH_B-A) altogether (2H), 3.72 and 3.98 (1H, br s, O*H*), 3.79 (3H, s, CO_2CH_3), 4.20-4.30 (2H, m, C(1‴)H_2), 4.70 (1H, br s, C(3‴)*H*), 4.73-4.78 (1H, m, C(4)*H*), 4.96 and 4.99 (1H, s, C(2)*H*), 5.80-5.84 (1H, m, C(4′)*H*), 6.26-6.30 (1H, m, C(3′)*H*), 6.55-6.62 (1H, m, N*H*), 7.23-7.34 (5H, m, *Ph*); δ_C (125 MHz, $CDCl_3$) 15.3, 15.4 (C(4)*C*H_3), 20.1, 20.3 (C(2‴)*C*H_3), 24.3, 24.3, 24.4, 24.5 (C(2‴)*C*H_3, *C*(2′)), 35.7, 35.7 (C(*C*H_3)$_3$), 34.4 (*C*(1′)), 37.4 (*C*Me_3), 38.8, 38.8 (*C*(2″)), 46.4, 46.5 (*C*(2‴)), 47.4, 47.5 (*C*(7)), 52.7, 52.7 (CO_2CH$_3$), 63.2, 63.3 (*C*(1″)), 78.5 (*C*(4)), 79.4, 79.7 (*C*(3‴)), 80.8 (*C*(5)), 84.2, 84.3 (*C*(6)), 96.4, 96.5 (*C*(2)), 120.3, 120.4 (*C*(4′)), 127.5, 127.6, 127.7, 127.7, 127.8, 127.9 (*o,m,p-Ph*), 140.5, 140.6 (*i-Ph*), 149.7, 149.9 (*C*(3′)), 166.9, 167.0 (*C*(5′)), 172.1 (*C*O_2Me), 177.8, 178.0 (*C*(1‴)), 178.5, 178.9 (*C*(8)); *m/z* (ESI^+) 611 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{31}H_{44}N_2NaO_9^+$ ($[M+Na]^+$) requires 611.2939, found 611.2930; and *E*-**402** as a 1:1 mixture of epimers at C(3‴) as a colourless oil (17 mg, 51%); R_f 0.65 (eluent EtOAc); ν_{max} (film) 3369, 2956, 2874, 1704, 1649, 1534; δ_H (500 MHz, $CDCl_3$) 0.90 (9H, s, C(CH_3)$_3$), 1.08 and 1.21 (3H, s, C(2‴)CH_3), 1.38-1.46 (1H, m, C(1′)H_AH_B), 1.68 (3H, d, *J* 6.6, C(4)CH_3), 1.95-2.02 (1H, m, C(1′)H_AH_B), 2.16-2.25 (1H, m, C(2′)H_AH_B), 2.34 (1H, d, *J* 15.8, C(7)H_AH_B), 2.47-2.56 (1H, m, C(2′)H_AH_B), 2.96-3.03 (1H, m, C(7)H_AH_B), 3.35 and 3.40 (1H, s, O*H*), 3.49-3.60 (2H, m, C(2″)H_2), 3.78 (3H, s, CO_2CH_3), 4.21-4.26 (2H, m, C(1″)H_2), 4.34 (1H, br s, O*H*), 4.67 (1H, d, *J* 2.5, C(3‴)*H*), 4.77 (1H, q, *J* 6.6, C(4)*H*), 5.02 and 5.03 (1H, s, C(2)*H*), 5.85 (1H, d, *J* 15.8, C(4′)*H*), 6.91-6.98 (1H, m, C(3′)*H*), 7.23-7.31 (5H, m, *Ph*); δ_C (125 MHz, $CDCl_3$) 15.2, 15.3 (C(4)*C*H_3), 20.3, 20.4, 24.4, 24.4 (C(2‴)*C*H_3), 25.6, 25.7 (C(*C*H_3)$_3$), 27.1, 27.1 (*C*(2′)), 34.1, 34.5 (*C*(1′)), 37.4 (*C*Me_3), 38.9, 39.0 (*C*(2″)), 46.3, 46.3 (*C*(7)), 47.3, 47.4 (*C*(2‴)), 52.7, 52.9 (CO_2CH$_3$), 62.9 (*C*(1″)), 78.4, 78.5 (*C*(4)), 79.7, 79.8 (*C*(3‴)), 80.8, 80.8 (*C*(5)), 84.2, 84.2 (*C*(6)), 96.2, 96.3 (*C*(2)), 121.4, 121.4 ((*C*(4′)), 127.5, 127.6, 127.7, 127.8, 127.8, 128.5 (*o,m,p-Ph*), 140.7 (*i-Ph*), 149.0, 149.8 (*C*(3′)), 166.5 (*C*(5′)), 171.8, 172.1 (*C*O_2Me), 177.9, 178.2 (*C*(8), *C*(1‴)); *m/z* (ESI^+) 611 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{31}H_{44}N_2NaO_9^+$ ($[M+Na]^+$) requires 611.2939, found 611.2947.

(2*R*,4*R*,5*R*,6*R*,7*S*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-((*E*)-5′-(2″-(3‴-hydroxy-2‴,2‴-diemethyl-3‴-phenylpropanamido)ethoxy)-5′-oxopent-3′-en-1′-yl)-7-methyl-8-oxo-3-pxabicyclo[3.3.0]octane *E*-403 and (2*R*,4*R*,5*R*,6*R*,7*S*)-1-Aza-2-*t*-butyl-4-methyl-5-methoxycarbonyl-6-hydroxy-6-((*Z*)-5′-(2″-(3‴-hydroxy-2‴,2‴-diemethyl-3‴-phenylpropanamido)ethoxy)-5′-oxopent-3′-en-1′-yl)-7-methyl-8-oxo-3-oxabicyclo[3.3.0]octane *Z*-403



A portion of the aldehyde **363** (23 mg, 0.07 mmol) and phosphorous ylid (±)-**388** (44 mg, 0.08 mmol) were dissolved in DCM (1 mL) and stirred for 20 h at rt. The solvent was removed *in vacuo* to give the crude residue. Purification *via* column chromatography (SiO_2, eluent 5:1 to 1:1 petrol:EtOAc) gave *Z*-**403** as a colourless oil (2 mg, 5%) as a 1:1 mixture of epimers at C(3‴); R_f 0.82 (eluent EtOAc); ν_{max} (film) 3351, 2956, 2875, 1718, 1645, 1534; δ_H (500 MHz, $CDCl_3$) 0.92 and 0.93 (9H, s C(CH_3)$_3$), 1.09 and 1.09 (3H, s, C(2‴)CH_3), 1.14 and 1.15 (3H, d, *J* 2.5 and 2.8, C(7)CH_3), 1.20 and 1.22 (3H, s, C(2‴)CH_3), 1.66-1.69 (3H, m, C(4)CH_3), 1.71-1.79 (1H, m, C(1′)H_AH$_B$), 1.80-1.88 (1H, m, C(1′)H$_A$$H_B$), 2.66-2.77 (1H, m, C(2′)H_AH$_B$), 2.78-2.91 (1H, m, C(2′)H$_A$$H_B$), 3.06-3.14 (1H, m, C(7)*H*), 3.52-3.61 (2H, m, C(2″)H_2), 3.79 (3H, s, CO_2CH_3), 4.21-4.27 (C(1″)H_2), 4.66-4.70 (1H, m, C(3‴)*H*), 4.76-4.83 (1H, m, C(4)*H*), 5.02 and 5.03 (1H, s, C(2)*H*), 5.74-5.85 (1H, m, C(4′)*H*), 6.27-6.33 (1H, m, C(3′)*H*), 6.41 (1H, br s, N*H*), 6.93 (1H, dt, *J* 15.5, 6.9, C(3′)*H*), 7.27-7.31 (5H, m, *Ph*); δ_C (125 MHz, $CDCl_3$) 7.4, 7.5 (C(7)*C*H$_3$), 15.9, 15.9 (C(4)*C*H$_3$), 20.2, 20.3 (C(2‴)*C*H$_3$), 23.7, 23.7 (*C*(2′)), 24.3, 24.3 (C(2‴)*C*H$_3$), 25.7, 25.8 (C(*C*H$_3$)$_3$), 34.0, 34.9 (*C*(1′)), 37.3 (*C*Me$_3$), 38.9, 39.0 (*C*(2″)), 46.2, 46.3 (*C*(2‴)), 48.5, 48.6 (*C*(7)), 52.7, 52.9 (CO_2CH_3), 62.9 (*C*(1″)), 78.8, 78.8 (*C*(4)), 79.2, .79.2 (*C*(3‴)), 79.8, 79.9 (*C*(5)), 85.7 (*C*(6)), 96.1, 96.2 (*C*(2)), 119.8, 119.8 (*C*(4′)), 127.5, 127.7, 127.8, 127.8, 128.4, 128.5 (*o,m,p-Ph*), 140.5 (*i-Ph*), 150.2, 150.3 (*C*(3′)), 166.5 (*C*(5′)), 172.3 (*C*O$_2$Me), 177.8 (*C*(1‴)), 179.6, 180.0 (*C*(8)); *m/z* (ESI$^+$) 625

([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{32}H_{46}N_2NaO_9^+$ ([M+Na]$^+$) requires 625.3096, found 625.3103; and *E*-**403** as a colourless oil (9 mg, 22%) as a 1:1 mixture of epimers at C(3‴); R_f 0.7 (eluent EtOAc); ν_{max} (film) 3376, 2956, 2875, 1711, 1649, 1534; δ_H (500 MHz, $CDCl_3$) 0.92 (9H, s, C(C*H*$_3$)$_3$), 1.09 (3H, s, C(2‴)C*H*$_3$), 1.12 (3H, d, *J* 6.9, C(7)C*H*$_3$), 1.21 (3H, br s, C(2‴) C*H*$_3$), 1.67 and 1.68 (3H, d, *J* 6.6, C(4)C*H*$_3$), 1.70-1.75 (1H, m, C(1′)*H*$_A$H$_B$), 1.77-1.85 (1H, m, C(1′)H$_A$*H*$_B$), 2.27-2.38 (1H, m, C(2′)*H*$_A$H$_B$), 2.43-2.54 (1H, m, C(2′)H$_A$*H*$_B$), 3.11 (1H, q, *J* 6.9, C(7)*H*), 3.52-3.58 (2H, m, C(2″)*H*$_2$), 3.78 and 3.79 (3H, s, CO_2CH_3), 4.18 (1H, br s, OH), 4.24 (2H, t, *J* 5.3, C(1″)*H*$_2$), 4.67 and 4.68 (1H, s, C(3‴)*H*), 4.80 (1H, q, *J* 6.6, C(4)*H*), 5.01 (1H, s, C(2)*H*), 5.82 (1H, br d, *J* 15.5, C(4′)*H*), 6.43 (1H, br, N*H*), 6.93 (1H, dt, *J* 15.5, 6.9, C(3′)*H*), 7.23-7.32 (5H, m, Ph); δ_C (125 MHz, $CDCl_3$) 7.4 (C(7)*C*H$_3$), 15.9 (C(4)*C*H$_3$), 20.3, 24.3 (C(2‴)*C*H$_3$), 25.7 (C(*C*H$_3$)$_3$), 26.8 (*C*(2′)), 34.1 (*C*(1′)), 37.3 (*C*Me$_3$), 39.1 (*C*(2″)), 46.2 (*C*(2‴)), 48.5 (*C*(7)), 52.8 (CO_2CH_3), 62.9 (*C*(1″)), 78.8, 79.1, 79.9 (*C*(4), *C*(5), *C*(3‴)), 85.8 (*C*(6)), 96.2 (*C*(2)), 121.1 (*C*(4′)), 127.5, 127.7, 127.9 (*o,m,p-Ph*), 140.6 (*i-Ph*), 149.0 (*C*(3′)), 166.5 (*C*(5′)), 171.9 (*C*O$_2$Me), 177.9 (*C*(1‴)), 179.6 (*C*(8)); *m/z* (ESI$^+$) 625 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{32}H_{46}N_2NaO_9^+$ ([M+Na]$^+$) requires 625.3096, found 625.3108.

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APPENDIX A: Hole-plate bioassay information

Bioassay plates were kindly prepared by Wendy Sobey. Samples were tested on agar plates inoculated with *E. coli* X580 and *S. aureus* D267. A 4 mg/mL solution in 7:3 DMSO:H_2O of each compound to be tested was prepared. Up to 5 wells of 100 μL in size were cut into the each of the agar plates. A 100 μL portion of each solution was loaded into a well, and the plates were covered and incubated at 37 °C for between 16 and 20 h. The plates were then inspected and the sizes of any zones of inhibition were noted. A full set of calibration data was also collected simultaneously with each run of bioassays.

A.1 Calibration data for hole-plate bioassays

An example of a calibration data set is included below with accompanying graphs from which relative potencies were calculated. Zone sizes for a number of moles of compounds were used to obtain an equivalent number of moles of ceph C a ratio of these gives the relative potency.

Conc. stock soln. (μg/mL)	Vol. Of Ceph C (μL/well)	Vol. Of water (mL/well)	Mass Ceph C (μg/ well)	Moles Ceph C (nmol/ well)	Zone size (mm)	Mass Ceph C (g)	Log (mol ceph C)
E. Coli							
10	20	80	0.2	0.481464	15	2E-07	-9.31744
	40	60	0.4	0.962927	20	4E-07	-9.01641
	60	40	0.6	1.444391	22	6E-07	-8.84032
	80	20	0.8	1.925855	24	8E-07	-8.71538
	100	0	1	2.407318	28	0.000001	-8.61847
100	20	80	2	4.814636	31	0.000002	-8.31744
	40	60	4	9.629273	36	0.000004	-8.01641
	60	40	6	14.44391	39	0.000006	-7.84032
	80	20	8	19.25855	40	0.000008	-7.71538
	100	0	10	24.07318	42	0.00001	-7.61847
S. Aureus							
1000	20	80	20	48.14636	0	N/A	N/A
	40	60	40	96.29273	15	0.00004	-7.01641
	60	40	60	144.4391	18	0.00006	-6.84032
	80	20	80	192.5855	20	0.00008	-6.71538
	100	0	100	240.7318	21	0.0001	-6.61847

Table A.1 Calibration data collected in January 2012.

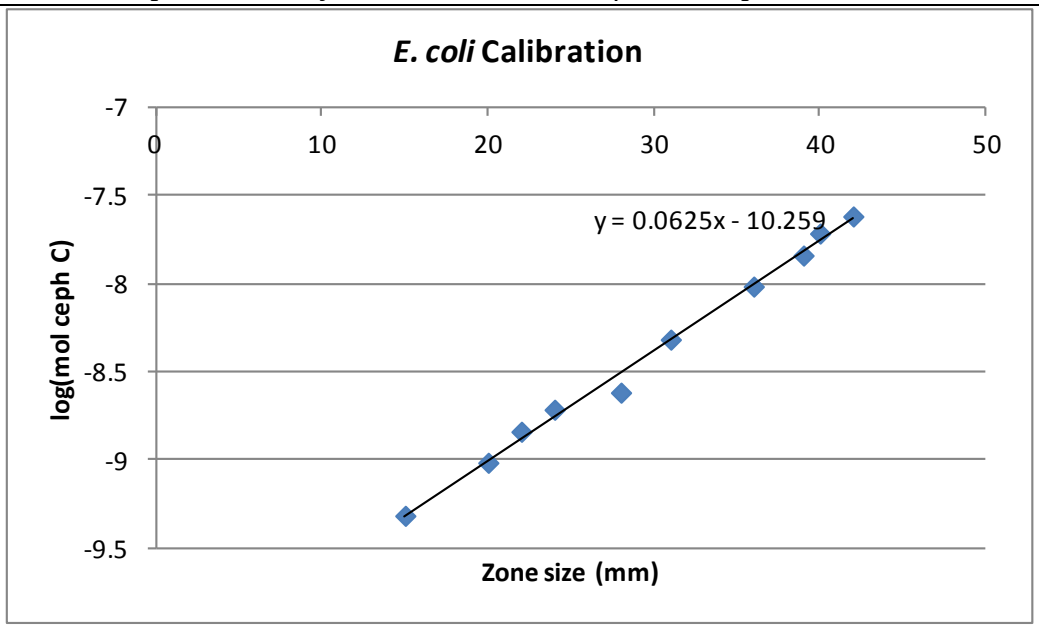


Figure A.1 Calibration graph for *E. coli*.

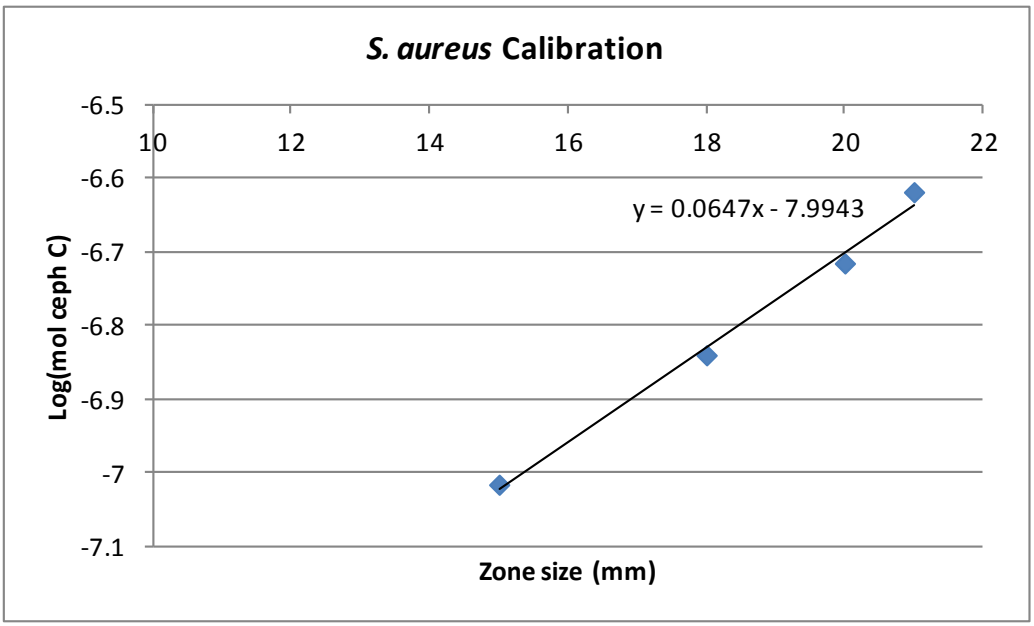
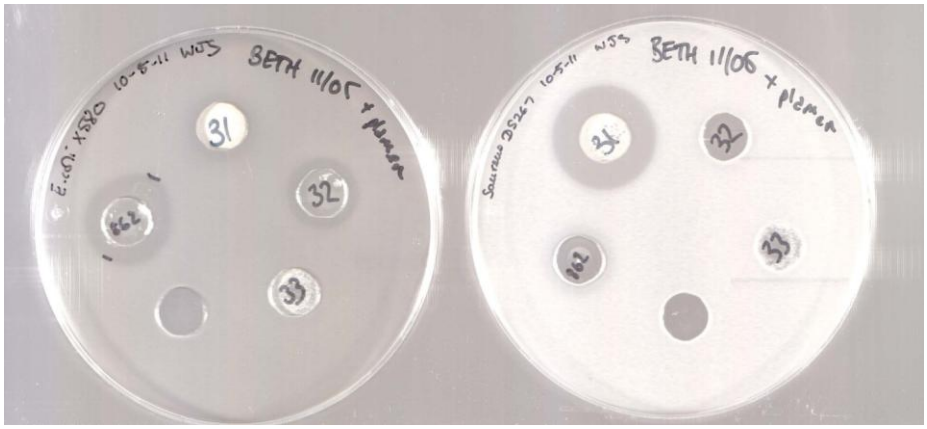


Figure A.2 Calibration graph for *S. aureus*.



Scheme 4.1 Figure A.3 Example of the appearance of zones of inhibition.

A.2 Relative potency calculations

							S. aureus D267		*E. coli* X580	
Compound number	**Zone size S. Aureus**	**Rel potency (%)**	**Zone size E. Coli**	**Rel potency (%)**	**FW compound**	**Conc compound (mol/well)**	**Log conc ceph C (mol/well)**	**Eq conc. Ceph C (mol/well)**	**Log conc (mol/well)**	**Eq conc. Ceph C (mol/well)**
	Measured from plate	(Conc compound /conc ceph C) ×100	Measured from plate	(Conc compound /conc ceph C) ×100		(0.004g/FW)×0.1	From graph	10^x	From graph	10^x
323c	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
323d	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
323e	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
324a	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
324b	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
324d	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
340a	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
340b	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
340c	13	9.735	0	0.0000	405.5	9.864E-07	-7.018	9.603E-08	N/A	N/A
340d	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
341	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
342a	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
343	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
344a	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
344b	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
349a	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
349b	12.5	9.994	12	0.0379	440.3	9.085E-07	-7.042	9.079E-08	-9.463	3.443E-10
349c	0	0.000	13	0.0388	393.5	1.017E-06	N/A	N/A	-9.405	3.940E-10

							S. aureus D267		*E. coli* X580	
Compound number	**Zone size S. Aureus**	**Rel potency (%)**	**Zone size E. Coli**	**Rel potency (%)**	**FW compound**	**Conc compound (mol/well)**	**Log conc ceph C (mol/well)**	**Eq conc. Ceph C (mol/well)**	**Log conc (mol/well)**	**Eq conc. Ceph C (mol/well)**
351a	0	0.000	12	0.0292	339.4	1.179E-06	N/A	N/A	-9.463	3.443E-10
351a'	0	0.000	12.5	0.0313	339.4	1.179E-06	N/A	N/A	-9.434	3.683E-10
351b	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
351b'	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
351c	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
351c'	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
353a	0	0.000	12	0.0280	325.4	1.229E-06	N/A	N/A	-9.463	3.443E-10
353b	0	0.000	12	0.0379	440.3	9.085E-07	N/A	N/A	-9.463	3.443E-10
353c	0	0.000	15	0.0507	393.5	1.017E-06	N/A	N/A	-9.288	5.158E-10
354a	0	0.000	14	0.0383	339.4	1.179E-06	N/A	N/A	-9.346	4.508E-10
354b	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
354c	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
(±)-357	0	0.000	15	0.0298	231	1.732E-06	N/A	N/A	-9.288	5.158E-10
362	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
364	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
(±)-369	0	0.000	16	0.0538	364.4	1.098E-06	N/A	N/A	-9.229	5.902E-10
371	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
373	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
(±)-382	22	21.168	15	0.0414	321.4	1.245E-06	-6.579	2.635E-07	-9.288	5.158E-10
(±)-390	25	41.418	40	1.7179	394.5	1.014E-06	-6.377	4.200E-07	-7.759	1.742E-08
(±)-391	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
(±)-399	30	105.964	37	1.3551	479.2	8.347E-07	-6.053	8.845E-07	-7.947	1.131E-08
(±)-401	0	0.000	15	0.0472	395.5	1.011E-06	N/A	N/A	-9.322	4.770E-10

							***S. aureus* D267**		***E. coli* X580**	
Compound number	**Zone size S. Aureus**	**Rel potency (%)**	**Zone size E. Coli**	**Rel potency (%)**	**FW compound**	**Conc compound (mol/well)**	**Log conc ceph C (mol/well)**	**Eq conc. Ceph C (mol/well)**	**Log conc (mol/well)**	**Eq conc. Ceph C (mol/well)**
380a	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
380b	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
E-385	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
E-386	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
E-397	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
E-398	0	0.000	0	0.0000	N/A	N/A	N/A	N/A	N/A	N/A
E-402	0	0.000	14	0.0608	588.7	6.795E-07	N/A	N/A	-9.384	4.130E-10
E-403	0	0.000	14	0.0622	602.7	6.637E-07	N/A	N/A	-9.384	4.130E-10
Z-402	0	0.000	15	0.0702	588.7	6.795E-07	N/A	N/A	-9.322	4.770E-10
Z-403	0	0.000	0	0.0000	602.7	6.637E-07	N/A	N/A	N/A	N/A

Table A.2 Values used in the calculation of relative potencies.

A.3 Hole-plate bioassay data and chemical informatics parameters

Chemical informatics parameters are presented alongside bioassay zone sizes and relative potencies for all the compounds tested in chapter 3.

Compound	Log P	PSA	MSA	%PSA	Log D	S. aureus D267 (mm)	Rel. pot. S. Aureus	E. coli X580 (mm)	Rel. pot. E. Coli	Notes
323c	4.17	59.50	580.80	10.24	4.17	-	-	-	-	Poor solubility
323d	6.81	72.91	780.35	9.34	6.81	-	-	-	-	Poor solubility
323e	4.21	98.94	691.60	14.31	4.21	-	-	-	-	Poor solubility
324a	1.37	85.30	541.36	15.76	1.20	-	-	-	-	
324b	4.57	46.61	513.34	9.08	4.52	-	-	-	-	Poor solubility
324d	6.81	46.61	703.93	6.62	6.81	-	-	-	-	Poor solubility
340a	1.51	82.14	520.81	15.77	1.45	-	-	-	-	
340b	2.06	82.14	548.80	14.97	1.99	-	-	-	-	
340c	3.77	82.14	656.27	12.52	3.67	13	9.74	-	-	
340d	4.33	82.15	684.29	12.01	4.21	-	-	-	-	
341	0.82	85.30	511.80	16.67	0.66	-	-	-	-	
342a	5.51	46.61	587.26	7.94	5.51	-	-	-	-	Poor solubility
343	2.73	85.30	567.12	15.04	2.73	-	-	-	-	
344a	3.28	85.30	590.00	14.46	3.28	-	-	-	-	Poor solubility
344b	3.28	85.30	675.00	12.64	3.28	-	-	-	-	Poor solubility
349a	2.98	72.91	535.77	13.61	2.91	-	-	-	-	
349b	4.30	72.91	601.57	12.12	4.22	13	9.99	12	0.04	
349c	3.68	72.91	604.88	12.05	3.61	-	-	13	0.04	
351a	3.53	72.91	565.49	12.89	3.45	-	-	12	0.03	
351a'	3.53	72.91	565.19	12.90	3.45	-	-	13	0.03	
351b	4.85	72.91	630.02	11.57	4.77	-	-	-	-	
351b'	4.85	72.19	629.32	11.47	4.77	-	-	-	-	
351c	4.23	72.91	633.66	11.51	4.16	-	-	-	-	Poor solubility
351c'	4.32	72.91	633.52	11.51	4.16	-	-	-	-	
353a	2.32	76.07	528.00	14.41	2.17	-	-	12	0.03	
353b	3.64	76.07	592.16	12.85	3.49	-	-	12	0.04	
353c	3.02	76.07	596.68	12.75	2.88	-	-	15	0.05	
354a	2.87	76.07	557.87	13.64	2.71	-	-	14	0.04	
354b	4.19	76.07	622.12	12.23	4.03	-	-	-	-	
354c	3.58	76.07	625.52	12.16	3.42	-	-	-	-	
(±)-357	1.71	49.33	365.79	13.49	1.72	-	-	15	0.03	
362	1.34	85.03	514.23	16.54	1.16	-	-	-	-	
364	0.72	108.66	536.79	20.24	0.56	-	-	-	-	
(±)-369	3.41	75.36	554.50	13.59	3.47	-	-	16	0.05	

Compound	Log P	PSA	MSA	%PSA	Log D	S. aureus D267 (mm)	Rel. pot. S. Aureus	E. coli X580 (mm)	Rel. pot. E. Coli	Notes
371	1.38	82.14	503.87	16.30	1.38	-	-	-	-	
373	1.92	82.14	533.31	15.40	1.92	-	-	-	-	
(±)-382	4.22	49.33	503.07	9.81	4.21	22	21.17	15	0.04	Poor solubility
(±)-390	1.97	75.63	471.08	16.05	1.97	25, 29 (halo)	41.42	40	1.72	
(±)-391	0.81	69.56	386.98	17.98	0.81	-	-	-	-	
(±)-399	3.13	81.70	555.90	14.70	3.13	30	105.96	37	1.36	
(±)-401	4.65	75.63	619.34	12.21	4.65	-	-	15	0.05	
380a	4.01	76.07	654.85	11.62	4.01	-	-	-	-	
380b	5.00	76.07	728.49	10.44	5.00	-	-	-	-	
E-385	1.98	102.37	601.26	17.03	1.98	-	-	-	-	
E-386	2.52	102.37	630.71	16.23	2.52	-	-	-	-	
E-397	4.24	105.61	826.02	12.79	4.24	-	-	-	-	
E-398	4.79	105.61	853.75	12.37	4.76	-	-	-	-	
E-402	3.30	151.70	917.90	16.53	3.30	-	-	14	0.06	
E-403	3.84	151.70	948.28	16.00	3.84	-	-	14	0.06	
Z-402	3.30	151.70	918.20	16.52	3.30	-	-	15	0.07	
Z-403	3.84	151.70	945.81	16.04	3.84	-	-	-	-	

Table A.3 Chemical informatics parameters and bioassay results.

APPENDIX B: Broth bioassay information

Broth assays were conducted at Galapagos SASU, 102 Avenue Gaston Roussel, 93230 Romainville, Paris, France.

Evaluation of antibiotic activity was performed using standard methodology (Clinical and Laboratory Standards Institute, 2006, M7-A7, Methods for antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 7^{th} ed., CLSI, Wayne, PA). Compounds were diluted in DMSO to obtain 2.56 mg/mL, then 100 µL aliquots were diluted in Mueller Hinton broth to 0.256 mg/mL and assayed against the bacterial panel by incubation in 96 well microplates at 37 °C for 24 h. The MIC was determined by visually reading the first concentration where no growth (no turbidity) appears. Reference compounds were ciprofloxacine (1.28 mg/mL frozen aliquot) and linezolid (2 mg/mL).

B.1 Broth bioassay data

		Strains	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
			S. aureus						*E. faecalis*	*E. faecium*	*S. pneumoniae*			*H. influenzae*		*E. coli*		*P. aeruginosa*
			Sa1	Sa26	Sa26 + 10% Serum Human	Sa26 + 50% Serum Human	Sa4	Sa2	Ecalis1	Ecium1	Pn1	Pn9	Pn9+2.5%blood	Hi3	Hi4	Ec1	Ec50	Pa1
		Pheno-type	ATCC13709 *in vivo*	ATCC25923	Serum effect	Serum effect	Oxford	MRSA, *in vivo*	ATCC29212 VanS	VanA	ATCC49619	PenR	Blood effect	ATCC 31517 MMSA	LS2 Efflux ko	ATCC25922	Ec49 No Efflux	ATCC 27853
		Cipro	0.06	0.5	1	0.5	0.12	16	1	16	1	1	1	<=0.03	<=0.03	<=0.03	<=0.03	0.5
		Liné	2	2	2	2	2	2	2	2	1	0.5	0.5	16	8	>32	>32	>32
G321623	**323d**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G321622	**323d**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G321613	**323e**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	**8**	>64	>64	>64
G321612	**324a**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G321616	**324b**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	32	>64	>64	>64
G321617	**324c**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G321611	**340a**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64

		Strains	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
			S. aureus						*E. faecalis*	*E. faecium*	*S. pneumoniae*			*H. influenzae*		*E. coli*		*P. aeruginosa*
			Sa1	Sa26	Sa26 + 10% Serum Human	Sa26 + 50% Serum Human	Sa4	Sa2	Ecalis1	Ecium1	Pn1	Pn9	Pn9+2.5%blood	Hi3	Hi4	Ec1	Ec50	Pa1
		Pheno-type	ATCC13709 *in vivo*	ATCC25923	Serum effect	Serum effect	Oxford	MRSA, *in vivo*	ATCC29212 VanS	VanA	ATCC49619	PenR	Blood effect	ATCC 31517 MMSA	LS2 Efflux ko	ATCC25922	Ec49 No Efflux	ATCC 27853
		Cipro	0.06	0.5	1	0.5	0.12	16	1	16	1	1	1	<=0.03	<=0.03	<=0.03	<=0.03	0.5
		Liné	2	2	2	2	2	2	2	2	1	0.5	0.5	16	8	>32	>32	>32
G321610	**340b**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G321619	**340c**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	**32**	>64	>64	>64
G321618	**340d**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G321615	**341**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G321614	**342a**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G321620	**344a**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G321621	**344b**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	**64**	>64	>64	>64

			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
			S. aureus						*E. faecalis*	*E. faecium*	*S. pneumoniae*			*H. influenzae*		*E. coli*		*P. aeruginosa*
		Strains	**Sa1**	**Sa26**	**Sa26 + 10% Serum Human**	**Sa26 + 50% Serum Human**	**Sa4**	**Sa2**	**Ecalis1**	**Ecium1**	**Pn1**	**Pn9**	**Pn9+2.5%blood**	**Hi3**	**Hi4**	**Ec1**	**Ec50**	**Pa1**
		Pheno-type	**ATCC13709 *in vivo***	**ATCC25923**	**Serum effect**	**Serum effect**	**Oxford**	**MRSA, *in vivo***	**ATCC29212 VanS**	**VanA**	**ATCC49619**	**PenR**	**Blood effect**	**ATCC 31517 MMSA**	**LS2 Efflux ko**	**ATCC25922**	**Ec49 No Efflux**	**ATCC 27853**
		Cipro	0.06	0.25	0.5	0.25	<=0.03	16	0.5	16	1	1	1	<=0.03	<=0.03	<=0.03	<=0.03	0.5
		Liné	2	2	2	2	2	2	1	1	1	0.5	1	16	8	>32	8	>32
G445452	**343**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G445460	**353a**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G445461	**353b**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	**64**	>64	>64	>64
G445463	**353c**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G445465	**354a**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G445462	**354b**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	**16**	>64	>64	>64
G445464	**354c**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G445468	**362**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G445469	**364**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64

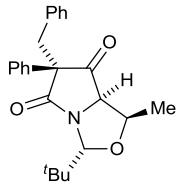
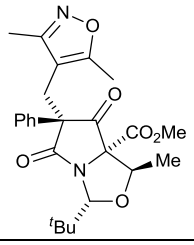
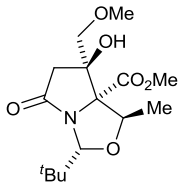
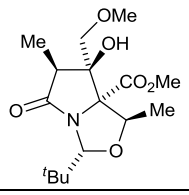
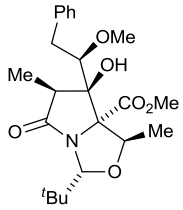
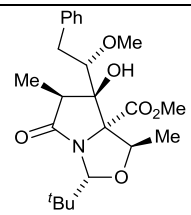
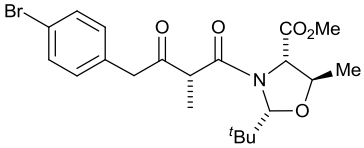
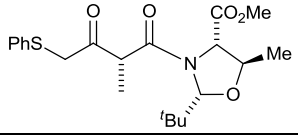
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
			S. aureus						*E. faecalis*	*E. faecium*	*S. pneumoniae*			*H. influenzae*		*E. coli*		*P. aeruginosa*
		Strains	**Sa1**	**Sa26**	**Sa26 + 10% Serum Human**	**Sa26 + 50% Serum Human**	**Sa4**	**Sa2**	**Ecalis1**	**Ecium1**	**Pn1**	**Pn9**	**Pn9+2.5%blood**	**Hi3**	**Hi4**	**Ec1**	**Ec50**	**Pa1**
		Pheno-type	**ATCC13709 *in vivo***	**ATCC25923**	**Serum effect**	**Serum effect**	**Oxford**	**MRSA, *in vivo***	**ATCC29212 VanS**	**VanA**	**ATCC49619**	**PenR**	**Blood effect**	**ATCC 31517 MMSA**	**LS2 Efflux ko**	**ATCC25922**	**Ec49 No Efflux**	**ATCC 27853**
		Ciprο	0.06	0.25	0.5	0.25	<=0.03	16	0.5	16	1	1	1	<=0.03	<=0.03	<=0.03	<=0.03	0.5
		Liné	2	2	2	2	2	2	1	1	1	0.5	1	16	8	>32	8	>32
G445467	**(±)-369**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	**64**	>64	>64	>64	>64	>64
G445453	**371**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G445470	**373**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G445466	**(±)-382**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G445475	**(±)-401**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	**64**	>64	>64	>64
G445471	**380a**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G445472	**380b**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	**64**	>64	>64	>64
G445473	**E-385**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G445474	**E-386**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64

			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
			S. aureus						*E. faecalis*	*E. faecium*	*S. pneumoniae*			*H. influenzae*		*E. coli*		*P. aeruginosa*
		Strains	**Sa1**	**Sa26**	**Sa26 + 10% Serum Human**	**Sa26 + 50% Serum Human**	**Sa4**	**Sa2**	**Ecalis1**	**Ecium1**	**Pn1**	**Pn9**	**Pn9+2.5%blood**	**Hi3**	**Hi4**	**Ec1**	**Ec50**	**Pa1**
		Pheno-type	**ATCC13709 *in vivo***	**ATCC25923**	**Serum effect**	**Serum effect**	**Oxford**	**MRSA, *in vivo***	**ATCC29212 VanS**	**VanA**	**ATCC49619**	**PenR**	**Blood effect**	**ATCC 31517 MMSA**	**LS2 Efflux ko**	**ATCC25922**	**Ec49 No Efflux**	**ATCC 27853**
		Cipro	0.06	0.25	0.5	0.25	<=0.03	16	0.5	16	1	1	1	<=0.03	<=0.03	<=0.03	<=0.03	0.5
		Liné	2	2	2	2	2	2	1	1	1	0.5	1	16	8	>32	8	>32
G445454	**E-397**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G445455	**E-398**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G445458	**E-402**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G445457	**E-403**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G445459	**Z-402**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
G445456	**Z-403**	**1**	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64

Table B.1 Broth bioassay results.

APPENDIX C: X-ray crystal structures

Crystalographic data for relevant compounds is provided on a CD attached to the inside of the back cover. The contents of the CD are detailed in the table below.

Compound Number	Structure	X-ray file name
324a		6276
323e		6274
341		6298
342a		6297 and 6327 (allotropes)
344a		6326
344b		6325
351b'		6333
351c'		6343

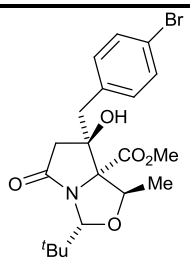
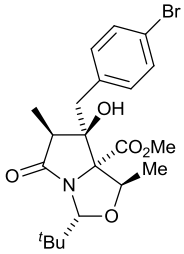
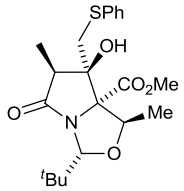
353b		6348
354b		6349
354c		6345

Table C.1 Contents of CD appendix.