Tetramates as antibacterial and anticancer core scaffolds

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 work, except where I have acknowledged data generated from, or in collaboration with, a named source.

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ABSTRACT

Tetramates as antibacterial and anticancer core scaffolds

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This thesis is concerned with the synthesis of small molecules that mimic tetramate- and pyrrolidinone-containing natural products, and the evaluation of their antibacterial and anticancer properties.

Chapter 1 exposes the need for new antibiotics due to the emergence of resistance to current drugs, and it discusses the value of the natural product inspired approach for drug discovery. It also gives an overview of the structure, synthesis and biological properties of the natural products pramanicin and oxazolomycin, both of which contain a pyrrolidinone core.

Chapter 2 describes an efficient synthetic route which allowed the preparation of a library of acyltetramates via Grignard addition to a Weinreb amide. This route was also used to prepare mimics of the acyl chain of pramanicin, comprising dienones and epoxy-enones of different lengths, and of the left-hand segment of oxazolomycin.

In Chapter 3, reduction of the tetramate core of different closely-related systems was performed, and it was found that the diastereochemical outcome could be controlled by appropriate choice of substituents in the bicyclic system. Preparation of reduced acyltetramates was only successful via hydrogenation of the Weinreb amide and subsequent Grignard substitution.

Chapter 4 describes the introduction of β-lactones to pyrrolidinones. Conditions for the selective formation of either spiro- or fused-β-lactones were found, which relied on the activation of the precursor β-hydroxyacid, accessed via *N*,*O*-acetal deprotection and ester saponification of the bicyclic systems. Further functionalisation of these analogues to mimic oxazolomycin was thwarted by the instability of some of the intermediates.

Finally, the biological properties of the analogues are discussed in Chapter 5. The antibacterial activity against Gram-negative *E. coli* and Gram-positive *S. aureus* was examined using the hole-plate method, which showed that the aliphatic lipophilic tetramates were the most potent inhibitors, with selective Gram-positive activity. An anticancer high-content screen was also designed, but only weak activities of the synthesised compounds were observed against four cancer cell lines.

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ABBREVIATIONS

A. fumigatus	*Aspergillus fumigatus*
A. tumefaciens	*Agrobacterium tumefaciens*
Ac	acetyl
AG	aminoglycoside
anh.	anhydrous
ATCC	American Type Culture Collection
ATR	attenuated total reflectance
B. subtilis	*Bacillus subtilis*
BCNP	biochemical natural product
BIOS	biology-oriented synthesis
Boc	*tert*-butyloxycarbonyl
BOPCl	bis(2-oxo-3-oxazolidinyl)phosphinic chloride
BL	β-lactam
Bn	benzyl
Bu	butyl
Bz	benzoyl
C. albicans	*Candida albicans*
C. krusei	*Candida krusei*
C. vulgaris	*Chlorella vulgaris*
CA-L	caspase-like
cat.	catalytic
CephC	Cephalosporin C
CFU	colony-forming unit
CI	chemical ionisation
clogD	calculated distribution coefficient between octanol and water of ionised and non-ionised forms of a compound at a certain pH
clogP	calculated partition coefficient between octanol and water
CMC	comprehensive medicinal chemistry
CNS	central nervous system
COSY	correlation spectroscopy
CRUK	cancer research United Kingdom
CT-L	chymotrypsin-like

d	day
DBU	1,8-diazabicycloundec-7-ene
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DCT	docetaxel
de	diastereomeric excess
DEAD	diethyl azodicarboxylate
DEPT	distortionless enhancement by polarisation transfer
DIAD	diisopropyl azodicarboxylate
DIBAL-H	diisobutylaluminum hydride
DIPEA	*N*,*N*-diisopropylethylamine
DMAD	dimethyl acetylenedicarboxylate
DMAP	4-dimethylaminopyridine
DMBQ	2,6-dimethyl-1,4-benzoquinone
DMEM	Dulbecco's modified Eagle's medium
DMF	*N*,*N*-dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethyl sulfoxide
DOS	diversity-oriented synthesis
dr	diastereomeric ratio
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
E	*trans*
E. coli	*Escherichia coli*
E. faecalis	*Enterococcus faecalis*
EC_{50}	concentration of a compound required to achieve 50% of the maximum response
ED_{50}	concentration of a compound required to achieve the desired effect on 50% of a population in a cellular or biochemical assay
EDTA	ethylenediaminetetraacetic acid
ee	enantiomeric excess
EI	electron ionisation
EMA	European Medicines Agency
epi	epimer
eq	equivalents

ESI	electrospray ionisation
Et	ethyl
FBS	fetal bovine serum
FDA	Food and Drug Administration
FI	field ionisation
FQ	fluoroquinolone
FT	Fourier transform
GDH	glucose dehydrogenase
GSK	GlaxoSmithKline
h	hour
H. influenziae	*Haemophilus influenziae*
HA	haemagglutinin
HCS	high-content screen
HESI-II	heated electrospray ionisation
HIV	human immunodeficiency virus
HMBC	heteronuclear multiple bond correlation
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum correlation
HTS	high-throughput screen
HUVEC	human umbilical vein endothelial cell
HWE	Horner-Wadsworth-Emmons
IC_{50}	concentration of a compound required to achieve 50% inhibition in a cellular or biochemical assay
IR	infrared
J	coupling constant
JNK	c-jun-*N*-terminal kinase
K. pneumoniae	*Klebsiella pneumoniae*
LC	liquid chromatography
LC_{80}	concentration of a compound required to achieve 80% inhibition of the growth of a tested population
LRMS	low resolution mass spectrometry
M. luteus	*Micrococcus luteus*
m.p.	melting point

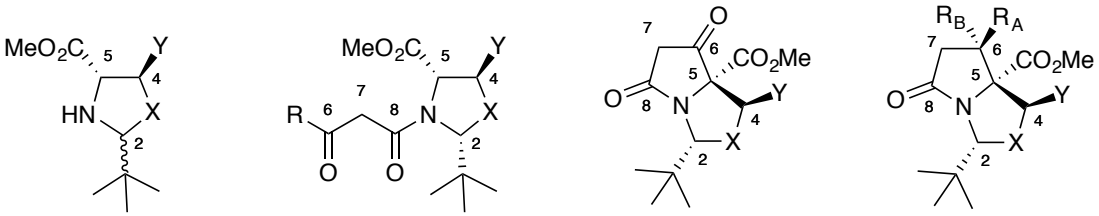
m/z	mass/charge ratio
MAPK	mitogen-activated protein kinase
*m*CPBA	*meta*-chloroperoxybenzoic acid
MDR	multidrug-resistant
Me	methyl
MHB	Mueller Hinton broth
MIC	minimum inhibitory concentration
MID	minimum inhibitory dilution
min	minute
MM	multiple myeloma
MOA	mechanism of action
MRSA	methicillin-resistant *Staphylococcus aureus*
Ms	methanesulfonyl
MS	mass spectrometry
MSA	molecular surface area
MW	molecular weight
NAD	nicotinamide adenine dinucleotide
NME	new molecular entity
NMNP	non-mammalian natural product
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
P	protecting group
P. aeruginosa	*Pseudomonas aeruginosa*
p-Ts	*para*-toluenesulfonyl
PBS	phosphate buffered saline
PC	principal component
PCC	pyridinium chlorochromate
Ph	phenyl
PMB	*para*-methoxybenzyl
ppm	parts per million
Pr	propyl
PSA	polar surface area
pyr.	pyridine

quant.	quantitative
R_f	retention factor
RNA	ribonucleic acid
RNAP	RNA polymerase
ROESY	rotating frame nuclear Overhauser effect
RPMI	Roswell Park Memorial Institute
rt	room temperature
S. aureus	*Staphylococcus aureus*
S. pneumoniae	*Streptococcus pneumoniae*
SAR	structure-activity relationship
sp.	species (singular)
SPCA	sparse principal component analysis
spp.	species (plural)
spt	selective population transfer
SRS	self-regeneration of stereocentres
t	tertiary
T-L	trypsin-like
TBAF	tetrabutylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TDI	Target Discovery Institute
TES	triethylsilyl
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMEDA	tetramethylethylenediamine
TMS	trimethylsilyl / trimethylsilane
topoIV	topoisomerase IV
TosMIC	toluenesulfonylmethyl isocyanide
UPPS	undecaprenyl pyrophosphate synthase
UV	ultraviolet
v/v	volume to volume
w/w	weight to weight

XPS	X-ray photoelectronic spectroscopy
Z	*cis*

NOMENCLATURE

The following non-systematic numbering systems have been used throughout this thesis for oxazolidines/thiazolidines, *N*-acyloxazolidines/thiazolidines and bicyclic tetramates and pyroglutamates.



a: X = O, Y = H (serine system)

b: X = S, Y = H (cysteine system)

c: X = O, Y = Me (threonine system)

Assignment of the ^{1}H and ^{13}C NMR signals in the Experimental Section follows these numbering systems, unless otherwise specified in the compound structures.

In bicyclic tetramates and pyroglutamates, *exo*- and *endo*- are used to describe the position of the substituents with respect to the bicyclic ring system. In the example above, R_A is *endo*- and R_B is *exo*-.

In tricarbonyl systems, *exo*- and *endo*- descriptives refer to the carbonyl groups outside and inside the ring respectively.

STEREOCHEMISTRY

The Cahn-Ingold-Prelog rules have been used throughout this thesis to describe chiral centres as *R* and *S* and double bonds as *E* and *Z*.

A dashed line is used to denote a bond that lies behind the plane of the page, and a wedged line denotes a bond that lies in front of the plane of the page. A wavy bond represents the presence of a mixture of isomers at that chiral centre, and a solid line signifies that the stereochemistry at that chiral centre is unknown.

CHAPTER 1: INTRODUCTION

1.1 Antibacterials

The introduction of sulfonamides and penicillin in the first half of the 20th Century, and the discovery of further drug classes against bacterial infections during the 'golden era' of antibacterial drug discovery contributed significantly to improved health in society and increased life expectancy.[1] However, in parallel with their discovery, resistance to antibiotics by their target microorganisms also emerged, and resistance has now evolved to every antibiotic ever placed into clinical practice (Figure 1.1).[2]

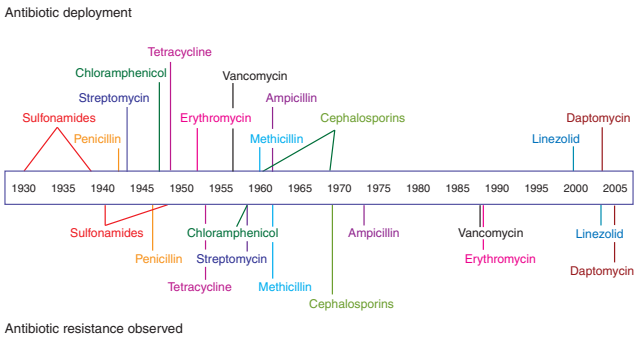


Figure 1.1. Timeline of antibiotic deployment and evolution of resistance. Reprinted by permission from Macmillan Publishers Ltd: Nature Chemical Biology (Clatworthy, A. E.; Pierson, E.; Hung, D. T. *Nat. Chem. Biol.* **2007**, *3* (9), 541), copyright (2007).

Acquired resistance can arise from mutations that occur during replication or by gaining genes that encode a mechanism of resistance by horizontal transfer from other bacteria.[3] Each acquired resistance mechanism may render the bacterium resistant to many or all the antibacterial agents of the same class.[4] There is therefore a need to find new antibiotic candidates that act at new targets and/or via distinct mechanisms.

Paradoxically, despite the rapidly growing number of multidrug-resistant (MDR) strains and the urgent clinical demand for new drugs, many pharmaceutical companies have made strategic decisions to leave the field of antibacterial agents. Antibiotic treatment is generally low-cost and

short-term, which leads to lower revenue compared to drugs in other fields such as oncology or autoimmune conditions, and ultimately yields poorer return on investment.[5]

Other than economics, there are additional challenges associated with antibiotic drug discovery.[5] The outer membrane of Gram-negative bacteria is a barrier for amphiphatic compounds and the inner membrane restricts the penetration of hydrophilic substances, resulting in a perfect barrier. Moreover, it also contains MDR pumps which extrude any compounds that leak in through the membrane.[6] As a result of poor penetration, antibiotics are usually only effective at much higher concentrations than typical drugs, which increases the risk of toxicity.[5]

High-throughput screening (HTS) campaigns to find new antibacterial drugs with novel modes of action have had very low success rates.[2] Marketed antibacterials are on average more hydrophilic and slightly larger than other drugs (Figure 1.2) and tend not to follow Lipinski's 'rule of five',[7] while most commercial compound collections are biased towards compounds that do.

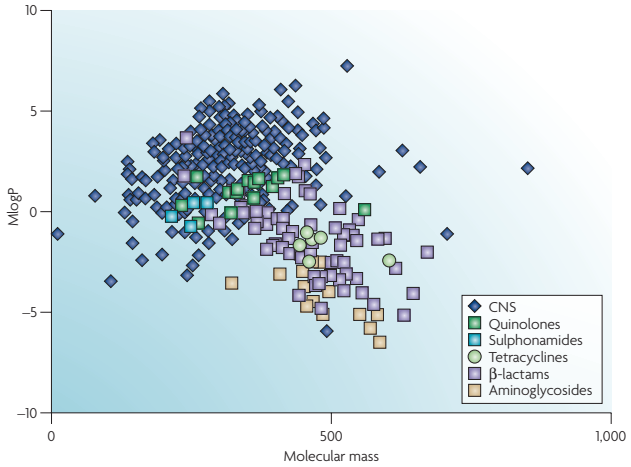


Figure 1.2. Calculated logP and molecular mass of marketed drugs for central nervous system (CNS) disorders compared with marketed antibacterial drugs. Similar observations for other mammalian target classes (data not shown). Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Drug Discovery (Payne, D. J.; Gwynn, M. N.; Holmes, D. J.; Pompliano, D. L. *Nat. Rev. Drug Discov.* **2007**, *6* (1), 29), copyright 2007.

In addition to the low hit rate of antibacterial HTSs, such hits are frequently later found to lack whole-cell activity. One solution is to perform non-target based, whole-cell (phenotypic),

screening. This strategy, although less sensitive than molecular screens, evaluates all targets in their physiological context simultaneously. A secondary assay to rule out non-specific nuisance compounds is also required.[8] Once hits have been selected, further studies are needed to identify the molecular target before starting lead optimisation.

1.2 Different approaches towards drug discovery

1.2.1 Natural products for drug discovery

Natural products have long been valuable sources of inspiration for drug design, and they have been particularly effective in the areas of cancer and infectious diseases. By the end of 2013, the Food and Drug Administration (FDA) had approved 547 natural products and derivatives, which represents 38% of all FDA-approved new molecular entities (NMEs).[9] 9.8% of all FDA-approved NMEs are unmodified natural products, and each of these has led to three synthetic derivatives on average.

Natural products are often referred to as privileged structures – they have been selected by evolutionary pressures to interact with biological targets for specific purposes, and they tend to possess well-defined three-dimensional structures. The structural features of natural products are known to differ from those of synthetic drug-like molecules in many regards.[10] Several studies have attempted to measure those features,[11–14] and some have revealed that natural products typically have a higher number of chiral centres and of sp^3-hybridised bridgehead atoms, increased steric complexity, higher number of hydrogen-bond donors and acceptors and greater molecular rigidity. Although they have a lower ratio of aromatic rings to total heavy atoms, the diversity of ring systems is bigger.

An analysis of the 126,140 compounds in The Dictionary of Natural Products revealed that only 60% comply with Lipinski's 'rule of five', with 18% violating the rules in at least two criteria.[15,16] Another study by T. Rodrigues *et al.* has shown that, while natural products contain a greater fraction of undesired sub-structural elements than synthetic drugs, particularly Michael

acceptors, they have a lower target promiscuity.[16] Similar findings were reported by P. A. Clemons and co-workers, who measured protein-binding of a collection of molecules from different sources against 100 different proteins and found natural products to have a bigger binding selectivity.[17] These data support the view of natural products as privileged structures, and also indicate that the rigorous application of decision guidelines has the dangerous potential to exclude compounds that could be good drug candidates.[18]

1.2.2 Structural analogues of natural products

In some cases, in spite of the high potency of natural products, the preparation of analogues could enhance their drug-like properties such as bioactivity, pharmacokinetics, solubility and/or safety. There are different ways to prepare natural product analogues (Figure 1.3). Firstly, in the semi-synthetic approach, the isolated natural product is modified by simple functional group transformations. This approach has been employed to prepare analogues of glycopeptide antibiotics with improved water solubility.[19] A second approach is combinatorial biosynthesis,[20,21] where genetic engineering is used to modify biosynthetic pathways to natural products in order to produce altered structures. While these approaches allow for the rapid access to analogues, the scope of modification is intrinsically limited by the reactive groups present in the natural product.

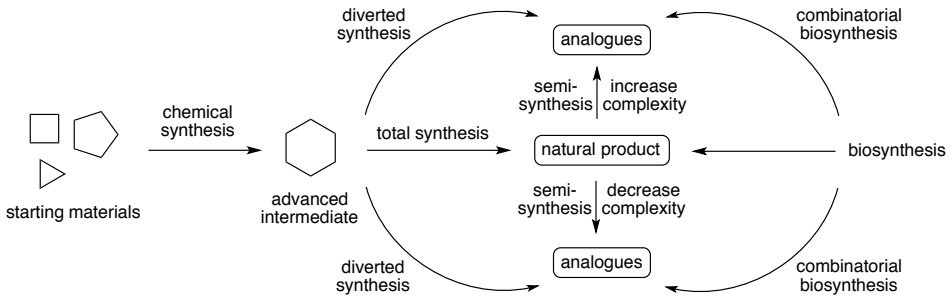


Figure 1.3. Approaches to natural product analogues. Adapted with permission from Njardarson, J. T.; Gaul, C.; Shan, D.; Huang, X.-Y.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126* (4), 1038. Copyright 2004 American Chemical Society.

A third approach is diverted total synthesis,[22] where a synthetic path to the natural product is designed that allows the introduction of structural modifications *en route* to the final compound.

This approach, which offers the highest flexibility, has been used to prepare several simplified analogues of natural products. One example comes from the work of S. J. Danishefsky and co-workers[22] on the development of analogues of (+)-migrastatin (Figure 1.4), an inhibitor of tumour cell migration.[23] They prepared several compounds with the core structure of the natural product and found that the IC_{50} in the migration inhibition assay of simplified analogue **1** was reduced by 3 orders of magnitude. Ketone **2**, which was also considerably more active than the natural product, was expected to have higher *in vivo* stability.

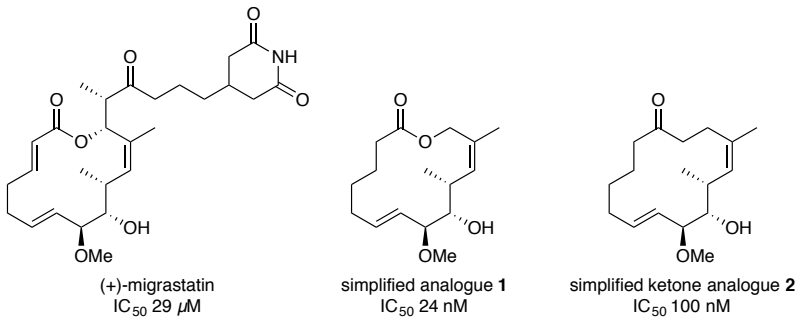


Figure 1.4. Structure of (+)-migrastatin and synthetic analogues prepared by J. T. Njardarson *et al.*[22] with IC_{50} values in the migration assay with 4T1 tumour cells.

Therefore, the diverted total synthesis approach enables the identification of natural product analogues, with preserved or enhanced biological activities over the parent compound, that can be synthesised more effectively. This is particularly attractive for scale-up purposes, which is key for biological assays, clinical studies and commercialisation. Moreover, it also allows for the preparation of compounds with improved pharmacological properties.

Other than structural analogues, another method to exploit the natural product structures is the preparation of synthetic mimetics, which has been particularly effective for the preparation of non-peptide peptidomimetics. An example of this approach is the preparation of azepine mimetics of cryptophycins, depsipeptides isolated from terrestrial algae.[24] (+)-Cryptophycin-1 (Figure 1.5), the most abundant member of the family, shows potent activity against a variety of tumour cell lines, which derives from its ability to inhibit tubulin polymerisation.[25] However, (+)-cryptophycin-1 possesses poor hydrolytic stability due to the ester functionality. To improve

its pharmacokinetic properties, A. B. Smith and co-workers used an azepine scaffold as a non-peptide synthetic mimetic to which they attached residues that resembled the overall geometry of the natural product.[26] They studied the stereochemistry and composition of the side-chains to both optimise the biological response and improve hydrolytic stability and bioavailability. They observed modest activities of the synthesised mimetics against several human cell lines, but these could be improved by further modification of the azepine systems.

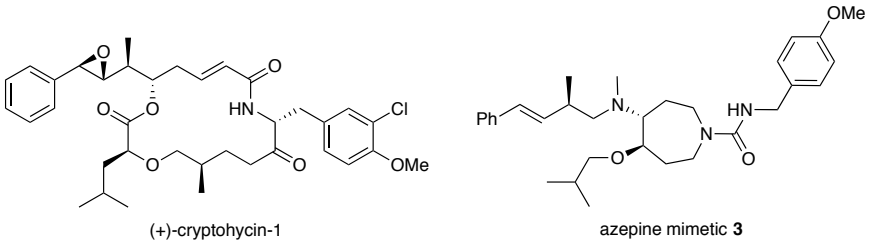


Figure 1.5. Structure of (+)-cryptophycin-1 and azepine mimetic **3** prepared by A. B. Smith and co-workers.[26]

1.2.3 High-throughput screening and combinatorial chemistry

Despite the value of natural products for drug design, since the 1970s the number of natural product-based NMEs started to decrease and in 2013 they represented only a 24% of the NMEs per year (Figure 1.6).[9] The effort required to isolate and identify natural products and the difficulty of their total syntheses has led to a move toward synthetic drugs in the pharmaceutical industry.[27] Natural products are often only available in extremely small quantities, representing much less than 1% by weight of the crude extract. In some cases, organic synthesis can be used to overcome supply problems,[28] but the often highly complex structures of natural products result in long and low-yielding synthetic routes, which hampers the scale-up and provision of these compounds.[29]

Another limitation of natural products is that they "may not display enough versatility to be suitable for treatment of all heritable human diseases".[30] They tend to target proteins that are essential to an organism, and additional sources of small molecules are required to cover the treatment of every human disease.

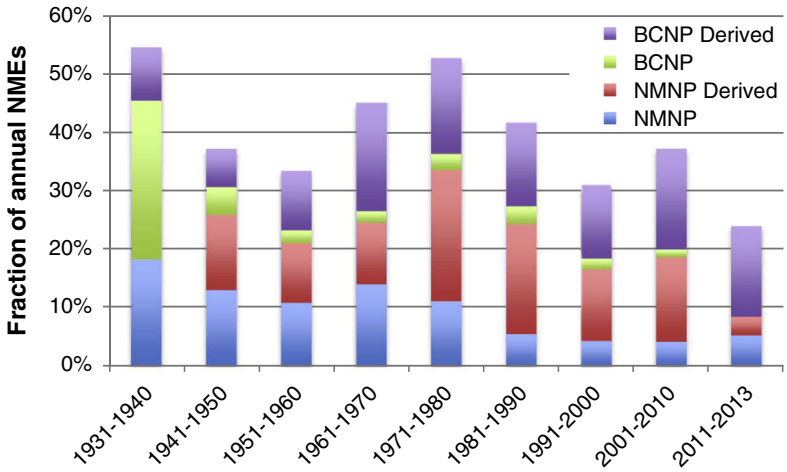


Figure 1.6. Fraction of annually approved NMEs separated into biochemical natural products (BCNPs) and non-mammalian natural products (NMNPs), as well as their semisynthetic or synthetic derivatives. Reprinted from Drug Discovery Today, 21 (2), Patridge, E.; Gareiss, P.; Kinch, M. S.; Hoyer, D., An analysis of FDA-approved drugs: natural products and their derivatives, 204-207, Copyright (2016), with permission from Elsevier.

A popular approach to drug discovery in the pharmaceutical industry is the HTS of hundreds or thousands of compounds. Combinatorial chemistry, with its ability to generate big libraries of molecules, seems to provide a perfect fit. However, this strategy has proven not to be as successful as expected, and between 1981 and 2010 only one NME designed with this method was reported: sorafenib (Nexavar), approved for the treatment of renal cell carcinoma and hepatocellular carcinoma.[31]

Although the use of combinatorial chemistry coupled with HTS is fast, it frequently results in low hit rates. These libraries are often designed based on chemical accessibility and achievable size rather than biologically relevant structures.[32] They also tend to be biased towards Lipinski's 'rule of five' and other rigorous guidelines, which might hinder the discovery of active compounds, as previously discussed.

1.2.4 Diversity-oriented synthesis and biology-oriented synthesis

To address the lack of diversity of combinatorial chemistry, another approach is diversity-oriented synthesis (DOS), where structurally and functionally different compound libraries are generated from a small number of precursors. These libraries, although smaller, cover a wider

chemical space and enable the exploration of regions that are often underrepresented in combinatorial libraries. The complexity and diversity of these compounds make them more likely to be successful in a phenotypic screen.[33,34]

D. R. Spring and co-workers have proven DOS to have some potential in the field of antibiotics, where combinatorial libraries have had little success because of the narrow focus of bioactive space and the bias towards known targets.[2] In one of their reports in the field, they generated a library of 223 compounds with 30 discrete molecular frameworks from a simple diazoacetate starting material,[35] and 64 of these displayed activity against two epidemic strains of methicillin-resistant *S. aureus* (MRSA) in cellular assays.[36] They performed further studies with the most potent compound and found that it had low toxicity in mammalian cells and that it operated as an inhibitor of dihydrofolate reductase.

Bringing together the significance of natural products for inspiration in drug discovery, and the power of DOS to generate libraries of diverse and complex compounds, H. Waldmann and co-workers introduced the concept of biology-oriented synthesis (BIOS).[37] This approach classifies core scaffolds with known biological activity against a certain protein target, and creates small compound libraries based on one of these scaffolds. The synthesis of these molecules usually requires elaborate chemistry and multi-step sequences, thus the library sizes are usually smaller but more focused, and usually with higher hit rates.[38] The Waldmann group has had much success with this concept, and they have found potent and selective inhibitors of various protein phosphatases[37] and a small-molecule activator of the Wnt-pathway[39] amongst others. For the latter, they designed a one-pot 4-7 step synthesis of multicyclic oxepanes that resemble the structure of biologically active natural products (Figure 1.7). They prepared a library of 125 oxepane analogues and screened them in a reporter gene assay monitoring the Wnt-signalling modulation. They found some active compounds, which could be used to perform structure-activity relationship (SAR) studies.

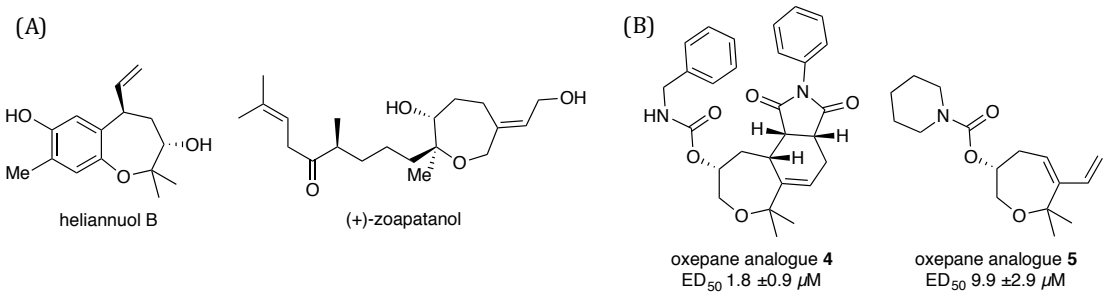


Figure 1.7. (A) Examples of bioactive natural products with an oxepane scaffold; (B) oxepane analogues prepared by H. Waldmann and co-workers,[39] with ED_{50} values based on their activity in the reporter gene assay monitoring the Wnt-signalling modulation.

1.3 β-Lactones

1.3.1 The 26S proteasome: structure and function

The 26S proteasome, also known as 'the proteasome', is a 2.5 MDa multisubunit enzyme complex located in the nucleus and cytoplasm of eukaryotic cells. It is responsible for the degradation of unneeded or damaged proteins in cells, controlling key cellular processes such as the cell cycle, DNA repair, apoptosis and immune and inflammatory responses.[40]

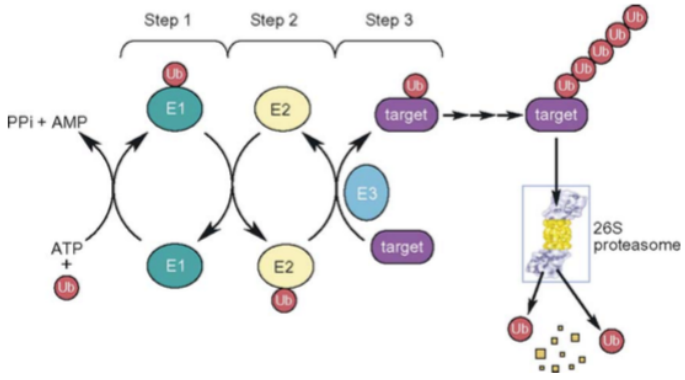


Figure 1.8. Ubiquitination of proteins targeted to the proteasome. Reprinted from Cancer Treatment Reviews, 29 (3), Adams, J., The proteasome: structure, function, and role in the cell, 3, Copyright (2003), with permission from Elsevier.

The degradation process, known as the ubiquitin-proteasome pathway, can be divided into two main steps: marking of a protein with a polyubiquitin chain followed by degradation by the 26S proteasome (Figure 1.8). For a protein to be recognised by the proteasome, ubiquitin, a 76-amino acid protein, is first attached to the ε-NH_2 group of a lysine residue. This first step is carried out

by a cascade of enzymes: E1, E2 and E3.[41,42] Firstly, E1 uses ATP to activate ubiquitin, which is transferred to the carrier protein E2. E3, a protein ligase, catalyses the transfer of the activated ubiquitin from E2 to the target protein. This process takes place several times, forming a chain of ubiquitins attached to the substrate which tags it for destruction by the proteasome. With 500 to 1000 different ubiquitin ligases, most of the substrate selectivity occurs in E3, which is essential for a regulated and coordinated degradation of cellular proteins.

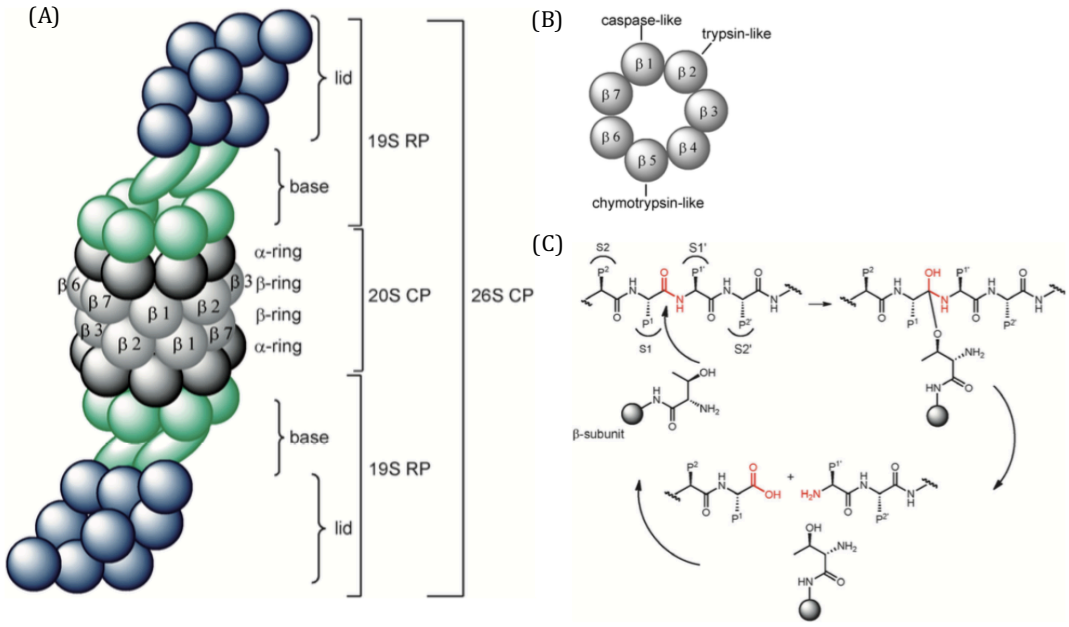


Figure 1.9. (A) 19S and 20S units of the 26S proteasome; (B) positioning of catalytic subunits in the β-ring; (C) catalytic mechanism of the 26S proteasome. Reprinted by permission of Rentsch, A.; Landsberg, D.; Brodmann, T.; Bülow, L.; Girbig, A.-K.; Kalesse, M. *Angew. Chem., Int. Ed.* **2013**, *52* (21), 5450. Copyright 2013 by John Wiley and Sons, Inc.

The 26S proteasome consists of a core cylindrical 20S particle composed of four stacked rings (two α and two β), each with seven subunits that host the catalytic centres (Figure 1.9 A). The 20S proteasome is associated with two 19S regulatory particles, which contain the polyubiquitin binding sites. During the degradation process, six ATPases unfold the protein and open a channel in the α ring of the 20S particle, giving ubiquitinated proteins access to the catalytic pocket. The polyubiquitin chain is first removed at the 19S unit and the proteins are then degraded at one of the three catalytic subunits (Figure 1.9 B): chymotrypsin-like (CT-L), trypsin-like (T-L) or caspase-like (CA-L). They mainly target hydrophobic residues, basic aminoacids and acidic

aminoacids respectively.[43,44] In all three cases, the hydroxy group of an *N*-terminal threonine acts as a nucleophile (Figure 1.9 C) and cleaves a peptide bond. Unlike most proteases, which cleave the target substrate once and release the two fragments, the proteasome cuts the target peptides at multiple sites, generating fragments ranging from 3 to 22 residues in length.[45]

1.3.2 Proteasome inhibition as a target for cancer therapy

Several proteasome inhibitors have been reported,[46–50] which bind to active sites in the 20S core particle and interfere with its activity. They are usually short peptides with a pharmacophore that interacts with a catalytic residue, either reversibly or irreversibly, while the peptide portion is specific for the substrate binding pocket. In spite of the multiple active sites found in the proteasome, inhibition of all of them is not required for a significant reduction of its activity. Inactivation of the chymotrypsin-like active site is sufficient for a significant reduction of protein degradation, whereas the effect of inhibiting the trypsin-like or caspase-like sites is lower.[45,51–54] Furthermore, inhibitors of the chymotrypsin-like site are usually more hydrophobic and cell-permeable than inhibitors of trypsin-like or caspase-like sites, which have charged residues. In consequence, most proteasome inhibitors act on the chymotrypsin-like binding site, with weaker effects on the two other sites.

Based on their pharmacophore, proteasome inhibitors can be classified into five classes: peptide aldehydes, peptide boronates, peptide vinyl sulfones, peptide epoxyketones and β-lactones (Table 1.1).[55] Peptide boronates are amongst the most promising inhibitors;[48,56,57] they are up to 100-fold more active than the aldehyde analogues and are highly selective. Most have a low molecular weight, high stability and simple synthetic routes. A representative example is bortezomib (Figure 1.10), which binds reversibly to the chymotrypsin-like active site of the proteasome and inhibits proteolysis.

Table 1.1. Classes of proteasome inhibitors, representative compounds and inhibition of different active sites. Adapted from Cancer & Biology, 8 (8), Kisselev, A. F.; Goldberg, A. L., Proteasome inhibitors: from research tools to drug candidates, 739-758, Copyright (2001), with permission from Elsevier.

Class		Compound	K_i [nM]			LC_{80} [μM]
			CT-L	T-L	C-L	
Reversible inhibitors	Peptide aldehydes	MG132	2	27600	900	0.4
		PSI[58]	IC_{50} 250 nM	-	-	-
		CEP1612[59]	<2	>1000	-	-
	Peptide boronates	MG262[56]	0.03	-	-	0.04
		bortezomib[56]	0.62	-	-	0.02
		PS273[60]	k_{ass} 0.15 $M^{-1}s^{-1}$	-	-	-
Irreversible inhibitors	Peptide vinyl sulfones	NLVS[47]	13400	422	100	8
		YLVS[47]	1500	560	77	-
	Peptide epoxyketones	Dihydroeponemycin[49]	65	4.4	61	-
		Epoxomicin[61]	37000	79	37	0.03
		YU101[61]	166000	7.1	21	0.25
	β-Lactones	lactacystin[49]	675	30	4	4
		omuralide[50]	8500	253	37	-

Proteasome inhibition can lead to significant changes in certain protein levels, resulting in a specific cellular response. Cancer cells generally have an increased metabolism and higher levels of oxidative stress, cytokines and growth factors than normal cells, which leads to higher proteasome activity in tumoural tissue. Therefore, they are more sensitive to proteasome inhibition than healthy cells, making the proteasome a potential target for cancer therapy.[62] For example, I. Nickeleit *et al*. have shown that argyrin A (Figure 1.10), a cyclic peptide that inhibits the proteasome, prevents destruction of the cyclin kinase inhibitor $p27^{kip1}$ and induces apoptosis selectively in cancer cells *in vitro*.[63]

The FDA approval of bortezomib for the treatment of multiple myeloma (MM) in 2003 confirmed the effectiveness of proteasome inhibition as a therapeutic strategy. One year later, it was also introduced into the European market. Despite its potent activity, both intrinsic and acquired

resistance have been observed in MM patients treated with bortezomib,[64] which can be overcome if used in combination with other anticancer agents.[65,66]

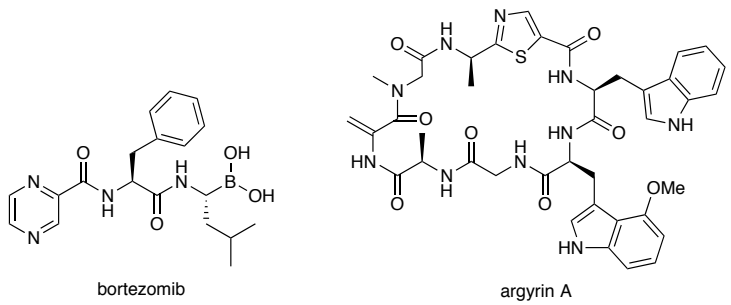


Figure 1.10. Structure of proteasome inhibitors bortezomib and argyrin A.

1.3.3 β-Lactones as proteasome inhibitors

β-Lactones are amongst the earliest known proteasome inhibitors. Their mechanism of action (MOA) is based on acylation of the *N*-terminal threonine group of the proteasome through opening of the β-lactone.

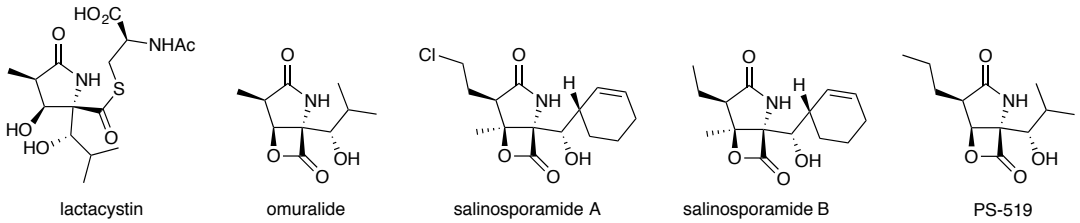


Figure 1.11. β-Lactone natural and synthetic proteasome inhibitors.

Lactacystin (Figure 1.11), a *Streptomyces* metabolite, was the first non-peptidic natural proteasome inhibitor to be isolated.[67] It was found to irreversibly block the activity of the β5 (chymotrypsin-like) subunit of mammalian proteasome.[68] L. R. Dick *et al*. found that the active form is not the thioester lactacystin, but *in vitro* it undergoes cyclisation to the β-lactone,[46] known as omuralide. In contrast to the inactive form, omuralide is permeable to plasma membranes of mammalian cells and can acylate the threonines in the proteasome's active site.

β-Lactones are the least stable of all proteasome inhibitors: they easily hydrolyse at neutral pH in water and exist in mammalian cells in equilibrium with lactathione through reaction with glutathione. Additionally, despite being considered as irreversible inhibitors, their adduct with the proteasome slowly hydrolyses in water ($t_{1/2} \approx 20$ h).[46]

Salinosporamides A and B, which are structurally related to omuralide, contain some unique structural features that enhance their potency *in vitro* and *in vivo*.[69] Having the cyclohexene ring instead of the isopropyl group results in favourable hydrophobic interactions in the S1 binding pocket of the β5 subunit, which is responsible for key enzyme-substrate interactions during peptide hydrolysis. This probably explains why salinosporamide A and B are more potent inhibitors of the CT-L active site than omuralide (Table 1.2).[70] Moreover, the chloroethyl group found in salinosporamide A accounts for the lower IC_{50} values than salinosporamide B.[70,71] After attachment of the β-lactone of salinosporamide A to the proteasome, the new alcohol group undergoes an intramolecular nucleophilic attack to form a cyclic tetrahydrofuran ring, which prevents hydrolysis of the ester linkage to the proteasome. The effectiveness of this irreversible binding is evidenced when comparing the recovery of blood 20S proteasome activity of salinosporamide A and PS-519.[69] While the latter has shown full recovery to basal levels within 24 h after administration to healthy volunteers,[72] in mice treated with salinosporamide A it was only partially recovered after 7 days.[65] This recovery could be attributed to red blood cell turnover.

Table 1.2. Rabbit 20S proteasome inhibition of β-lactone natural products. Adapted with permission from Macherla, V. R.; Mitchell, S. S.; Manam, R. R.; Reed, K. A.; Chao, T.-H.; Nicholson, B.; Deyanat-Yazdi, G.; Mai, B.; Jensen, P. R.; Fenical, W. F.; Neuteboom, S. T. C.; Lam, K. S.; Palladino, M. A.; Potts, B. C. M. *J. Med. Chem.* **2005**, *48* (11), 3684. Copyright 2016 American Chemical Society.

Compound	**IC_{50} [nM]**		
	CT-L	**T-L**	**CA-L**
omuralide	57 ±6	540	>10000
salinosporamide A	2.6 ±0.2	21 ±0.3	430 ±60
salinosporamide B	27 ±4	640 ±60	1200 ±100

Salinosporamide A inhibits the three proteolytic sites of the 20S proteasome without affecting other proteases and blocks cell proliferation of various tumour cell lines, including some bortezomib-resistant ones.[73] It was acquired by Nereus Pharmaceuticals under the name NPI-0052 and is being developed as a new agent against human cancer. It has been termed as an orphan drug against multiple myeloma by the FDA (2013) and the EMA (2014) and it is currently in Phase I human clinical trials for multiple myeloma.

1.4 Pramanicin

1.4.1 Isolation and structure determination

Pramanicin (Figure 1.12) was first isolated in 1994 by R. E. Schwartz and co-workers[74] from a sterile fungus (MF5868=BW283, Merck Microbial Resources Culture Collection) which was found in unidentified grass in New Jersey. The fungus was later identified as a species of *Stagonospora*.[75] Its isolation consisted of extraction, partition and silica gel and preparative HPLC chromatography to give pramanicin as a white crystalline solid.

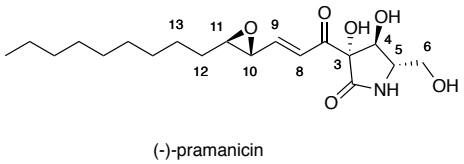


Figure 1.12. Structure of (-)-pramanicin.

Its structure determination was achieved with ^{1}H, ^{13}C, DEPT, HMBC and COSY NMR experiments in CD_3OD as well as MS fragmentation and IR absorption. The relative stereochemistry of the lactam ring was determined using ROESY correlations, which showed strong signals between the *C*(3)-hydroxyl proton, *H*(4) and *H*(6)s but not with *H*(5). The small coupling constants between *H*(10) and *H*(11) (2 Hz) suggested a *trans* orientation of the epoxide. This was confirmed with ROESY correlations of *H*(9) with *H*(11) and of *H*(10) with *H*(12) and *H*(13), but not between *H*(10) and *H*(11). The absolute configuration of pramanicin and the stereochemistry of the side-chain relative to the lactam entity were determined in 1999 by A. G. M. Barrett *et al.*[76,77] They achieved

its first total synthesis and assigned all the stereocentres by comparing the NMR spectra and the optical rotation of (+)-pramanicin and its diastereomer at the epoxide with the ones reported for the isolated natural product.

1.4.2 Biological activity

The antifungal and antibacterial activities of (-)-pramanicin were studied by Schwartz *et al*. and are summarised in Table 1.3.[74] They used microtitre dish broth dilution assays where the minimum inhibitory concentration (MIC) was defined as the lowest concentration with visible growth inhibition.

Table 1.3. Antimicrobial activity of (-)-pramanicin.[74]

Organism	Merck Culture Number	MIC [μM]
Candida albicans	MY 1055	20.0
Candida parapsilosis	MY 1010	4.0
Candida tropicalis	MY 2167	20.0
Candida krusei	MY 2168	>100.0
Cryptococcus neoformans	MY 2061	20.0
Cryptococcus neoformans	MY 2062	0.062
Aspergillus fumigatus	MF 5668	100.0
Bacillus subtilis	MB 964	4.0

Pramanicin has also been reported to possess physiological activities in mammalian blood vessels. Endothelial cells form the linings of blood vessels and control the blood supply in almost every region of the body; they play a key role in tissue growth and repair.[78] Drugs that act on endothelial cells could have the potential to block the growth of tumours, hence the interest in endothelial cell biology for cancer treatment. C.-Y. Kwan and co-workers found that pramanicin can make the cell membrane permeable to Ca^{2+}, which raises the cytosolic Ca^{2+}, leads to NO release and eventually causes endothelial cell death.[79,80] This might result from partition of pramanicin into the lipid bilayers of the cell membrane owing to its hydrophobic nature, but the exact mechanism remains unclear.

C.-Y. Kwan and co-workers also observed that pramanicin can selectively cause endothelium-dependent vascular relaxation mediated by the NO pathway at 4-100 μM. This was shown to have minimal direct effect on the contractility of smooth muscle cells, but it caused an initial slow vascular relaxation which was followed by a cytotoxic effect that eventually resulted in the loss of vasorelaxant function.[79] They also investigated the role of pramanicin's epoxy group on its vasorelaxation properties. Diene **6**, which was isolated as a by-product during fermentation,[81] and saturated analogue **7**, prepared from the hydrogenation of **6** (Figure 1.13), both retained partial activity, indicating that the epoxide of the natural product is optimal but not essential for its vascular activity.[82]

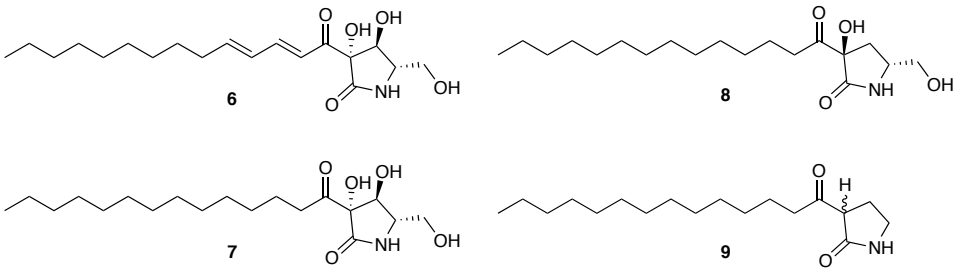


Figure 1.13. Analogues of pramanicin studied by C.-Y. Kwan and co-workers (**6**,**7**)[82] and C. Bodur *et al.* (**6**,**8**,**9**).[83]

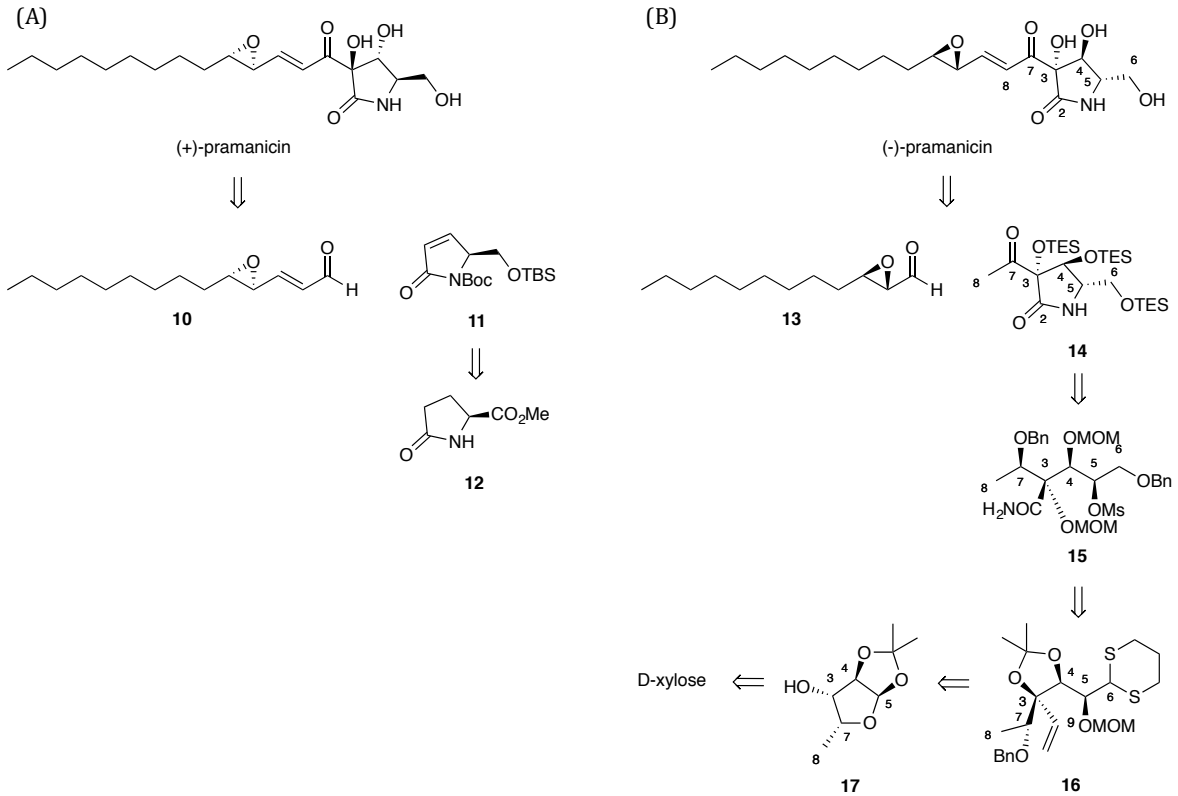
In addition to Kwan's work, O. Kutuk *et al*. have found that pramanicin induces an apoptotic effect on Jurkat T leukemia cells in a dose-dependent manner[84]. c-Jun-*N*-terminal kinases (JNK) and p38 are two groups of mitogen-activated protein kinases (MAPK) which are known to be involved in pro-apoptotic and anti-apoptotic signalling pathways.[85,86] In their study, O. Kutuk *et al*. reported that pramanicin activates the JNK and p38 kinase cascades, and it also regulates the release of cytochrome *c* and activates caspase-9/-3. These results proved pramanicin to be a potential apoptosis-inducing agent in Jurkat cells.

More recently, C. Bodur *et al.* showed that pramanicin analogue **6** induces apoptosis in human colon cancer cells through Bcl-2 downregulation and p38 activation.[83] At 100 μM, **6** decreased the cell viability by 70% compared to the control, while the natural product only caused an 8%

decrease. Analogues **8**,**9** (Figure 1.13), although less active, also retained significant activity. These results indicate that the key pharmacophore is, at most, an acylated pyrrolidinone, and that the epoxide, diene, hydroxymethyl and alcohol groups enhance anticancer activity but are not essential.

1.4.3 Total synthesis

To date, two total syntheses of pramanicin have been described (Scheme 1.1). The first one, reported by A. G. M. Barrett and co-workers in 1999,[76] gave in 28 steps (18 steps longest linear sequence) the enantiomer of the natural product. In 2006, K. Tadano and co-workers reported the total synthesis of (-)-pramanicin in 38 steps (31 steps longest linear sequence).



Scheme 1.1. Retrosynthetic analysis of the total synthesis of (A) (+)-pramanicin by Barrett[76] and (B) (-)-pramanicin by Tadano.[87]

Barrett's synthesis used aldehyde **10** to introduce the fatty chain of pramanicin, which was accessed in seven steps starting from decanal, using a double Horner-Wadsmorth-Emmons (HWE) strategy and an enantioselective Sharpless epoxidadion. The aldehyde was coupled to α,β-

unsaturated lactam **11**, accessible from methyl L-pyroglutamate **12** in five steps. Coupling was achieved via a one-pot Michael addition of an aminosilyl zincate species to the lactam, followed by quenching of the resultant enolate with aldehyde **10** and ethanolysis of the resulting silylamine. A subsequent oxidation sequence gave (+)-pramanicin.

Tadano employed a late-stage aldol condensation of aldehyde **13**, an intermediate of Barrett's synthesis of **10**, and the lactam head group **14**. In this case the lactam was already poly-hydroxylated, and it was accessed from D-xylose as summarised in Scheme 1.1 B. Key steps of the route were the addition of vinyl Grignard and 1,3-dithiane to **17**, which proceeded with high levels of diastereoselectivity through steric control. The least efficient transformation of the sequence was the cyclisation of amide **15**, which gave the desired γ-lactam in only 52% yield from attack of the amide anion. The low yield was due to concomitant attack of the imidate anion to give the γ-lactone.

1.5 Oxazolomycin

1.5.1 Isolation and structure determination

Oxazolomycin A (Figure 1.14) belongs to a family of natural products isolated from different species of *Streptomyces*.[88] These compounds possess an unusual spiro-β-lactone/γ-lactam and a terminal oxazole linked by triene and (*E*,*E*)-diene spacers. The different members of the oxazolomycin family differ in the triene geometry and methyl substitution in the oxazole and lactone rings.

Oxazolomycin was first isolated by Mori *et al*., who were searching for antibiotics with inhibitory activity against Ehrlich ascites tumour from a strain of *Streptomyces* spp.[89] Its isolation consisted of extraction, partition and column chromatography and HPLC purification to give oxazolomycin A and neooxazolomycin.

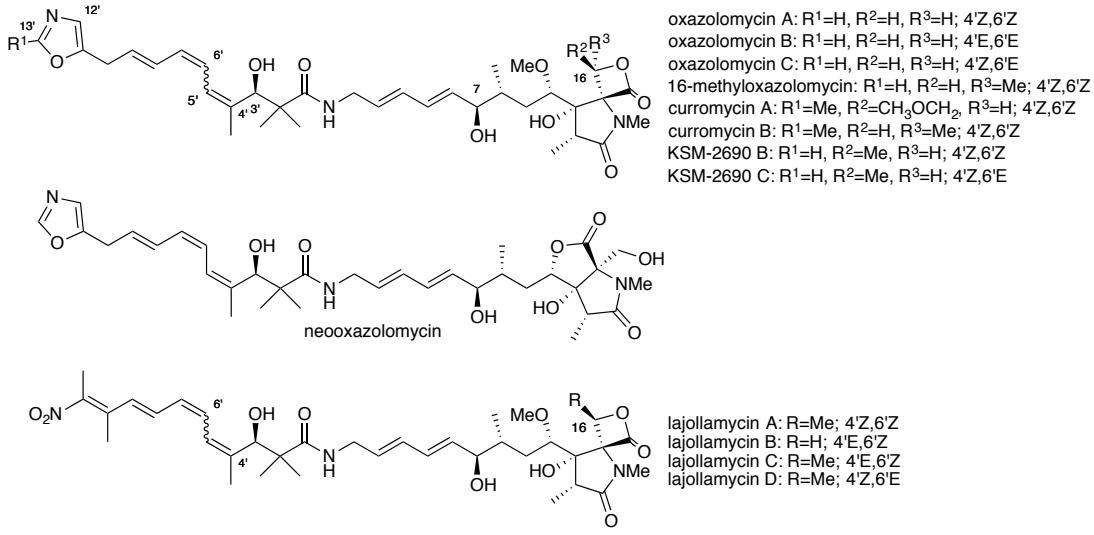


Figure 1.14. Structure of oxazolomycins.

The UV spectra of the isolated oxazolomycin A suggested its triene and diene chromophores,[90] and the *Z* configuration of the *C*(4')-*C*(5') double bond was confirmed from a 5.9% NOE between C(4')*Me* and *H*(5'). The IR absorption at 1825 cm^{-1} revealed the presence of the β-lactone moiety. Hydrogenation and consequent ^{1}H and ^{13}C NMR analysis of the decahydrooxazolomycin indicated the presence of a 5-substituted oxazole ring, with characteristic *H*(12') and *H*(13') singlets. Final structure determination was achieved through careful characterisation of the fragments derived from its ozonolysis and subsequent derivatisation.

Oxazolomycins B and C were first isolated in 1998 by H. Kanzaki *et al.* from *Streptomyces albus* JA3453.[91] They are geometrical isomers of oxazolomycin A, with *EEE* and *ZEE* triene geometries respectively, as indicated by the COSY and *J* values of their ^{1}H NMR olefinic protons.

Other members of the oxazolomycin family, also isolated from strains of *Streptomyces*, are 16-methyloxazolomycin,[92,93] curromycins A,B[94] and KSM-2690 B,C,[95] which have different oxazole and lactone substituents. Neooxazolomycin bears a γ-lactone instead of the β-lactone.[96] The last members of the family to be isolated were lajollomycins A-D,[97,98] which possess an unusual nitro-tetraene moiety instead of the oxazole ring and differ in the *C*(16)-substituent and the tetraene geometry.

1.5.2 Biological activity

The oxazolomycins exhibit a wide range of biological activities, summarised in Table 1.4.

Agrobacterium tumefaciens[99] is a Gram-negative soil bacterium which causes crown gall tumours in eudicot plants. S. Kawai *et al*. were interested in understanding the mechanism of tumour induction, and in the search for chemical probes they found that oxazolomycin A inhibited crown gall formation at 0.8 μg/disk. They also tested its activity against several bacteria and found it be selective against *A. tumefaciens*.[100] Oxazolomycins B,C and curromycins A,B were later studied by H. Kanzaki and co-workers[91] and K. Kawazu and co-workers[101] respectively. They all showed inhibition of crown gall formation, and the latter also displayed selective antibacterial activity against *A. tumefaciens*. Preparation of diacetate and dipropionate analogues (at *C*(7) and *C*(3')) of curromycin resulted in loss of its antibacterial activity, which showed the potential value of these compounds to study the mechanism of plant transformation by *A. tumefaciens*.

Regarding the antibacterial activity of other members of the family, 16-methyloxazolomycin showed inhibition of *B. subtilis* and *C. vulgaris*[92] and KSM-2690B of *B. subtilis*, *S. aureus* and *M. luteus*.[95]

With respect to the anticancer activity of these compounds, little is known about the cytotoxic activities of oxazolomycins A-C and only oxazolomycin A has been reported to display activity against P388 leukemia[89] and human bladder carcinoma T24.[95] Amongst the other members, noteworthy 16-methyloxazolomycin and curromycin A have been reported to exhibit cytotoxicity against P388 leukemia.[92,94] Moreover, the former exhibits activity against A-549 human lung adenocarcinoma and human bladder carcinoma T24,[95] and the latter against mouse B16 melanoma and friend leukemia.

Table 1.4. Biological activity of oxazolomycins. Adapted from M. G. Moloney *et al*.[88]

Activity		Ox[91]			16-MeOx	Curro[101]		KSM-2690B[95]
		A	B	C		A	B	
Crown Gall formation, MID [μg/disc]		0.8	0.8	0.8		3.2	3.2	
Alfaalfa germination, MID [μg/disc]		12.5	25. 0	50.0		12.5[91]	12.5[91]	
Bacterial growth MIC [μg/mL]	*Agrobacterium tumefaciens*	3.1[100]	>100	>100		6.3	6.3	
	Agrobacterium rhizogenes	50	>100	>100				
	Rhizobium loti	>100	>100	>100				
	Escherichia coli	>100	>100	>100		100	100	
	Bacillus subtilis	>100	>100	>100	5.0[92]	>100	>100	23 mm[b]
	Staphylococcus aureus	>100			n.a.[a,95]			25 mm[b]
	Micrococcus luteus							15 mm[b]
	Chlorella vulgaris				10[92]			
Cytotoxic activity IC_{50} [μg/mL]	P388 leukemia	active[89]			0.23[92]	active[94]		
	Human bladder carcinoma T24	10[95]			10[95]			10
	A-549 human lung adenocarcinoma				4.6[92]			
	B16 melanoma					active[94]		
	Friend leukemia					active[94]		
	Ehrlich ascites carcinoma	active[89]						
Viral activity [μg/mL]	Infleunza A, MIC	15.6[102]						
	Herpes simplex, MIC	15.6[102]						
	Vaccinia, MIC	15.6[102]						
	HIV, IC_{50}					6.5[103]	20[103]	

[a]n.a. = not active.

[b]inhibition zone (diameter) using paper-disk diffusion method at 50 μg/mL.

The antiviral activity of oxazolomycin A has been studied by E. Tonew and co-workers, who showed that it reduced plaque formation of enveloped DNA and RNA viruses by more than 90% in the range of the maximum tolerated dose.[102] They found that it prevented replication of

influenza A, herpes simplex and vaccinia in a dose-dependent manner. In addition to E. Tonew's work, M. Nakamura *et al*. reported that curromycins A and B inhibit the replication of human immunodeficiency virus (HIV) in both acute and chronic infections, by affecting a late step in the virus replication cycle.[103]

Truncated versions of oxazolomcins A-C, which are essentially the left-hand fragment until the amide group, have also been isolated from a *Streptomyces sp.* and named inthomycins A-C.[104] They have been identified as potential new types of herbicides as they inhibit cellulose biosynthesis selectively.[105]

The antibacterial, anticancer and antiviral activities of different members of the oxazolomycin family show that small structural differences can lead to big changes in bioactivity. Therefore, detailed SAR studies of these compounds could identify novel lead structures for drug discovery.

1.5.3 Mode of action

The mode of antiviral action of oxazolomycin A has been studied by Gräfe *et al.*[102,106] They used an artificial lipid membrane model to show that oxazolomycin A is an effective protonophore at pH <7.0, and it conveys protons and monovalent cations at pH >7.5. Membrane fusions, essential for the endocytosis of a virion into the host cell and the expulsion of new infectious particles, are mediated by haemagglutinin (HA), a membrane-bound glycoprotein.[107–109] These fusions are triggered by protonation of Asp residues in the protein. It has been shown that protonophores can inhibit viral infection by preventing these protonations. In fact, the two anti-influenza drugs amantadine and rimantidine act through this mechanism.[110,111]

The observed antibacterial and anticancer activity of oxazolomycin could also derive from a similar membranophilic behaviour, similar to lipopeptaibols, a family of membrane active antimicrobial peptides.[112]

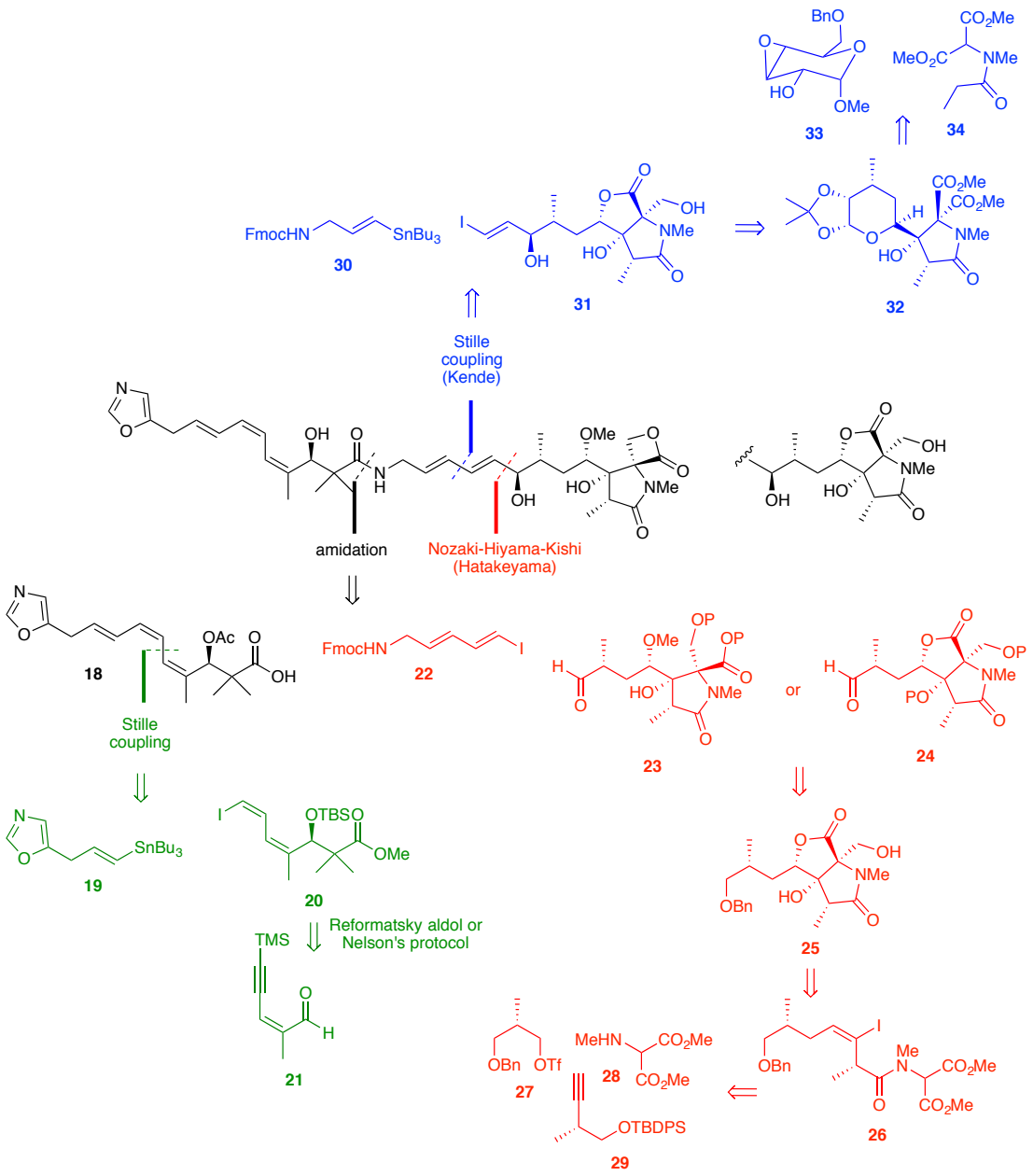
Molecular modelling, NMR and X-ray crystallography analysis of a truncated version of oxazolomycin showed that it might exist as a U-shaped structure induced by the geminal dimethylamide.[113] This arrangement would give a polar lactam-lactone head group and a non-polar tail, and this could be an important element for its biological activity. It is hence expected that the preparation of analogues with different substituents around the *gem*-dimethyl amide might help to better understand the mechanism of action of this family of natural products.

1.5.4 Total synthesis

Given the wide range of biological activities of oxazolomycins, and the need to fully understand their biological mechanism of action and therapeutic potential, the design of effective synthetic routes towards both the natural product itself and analogues is of interest.

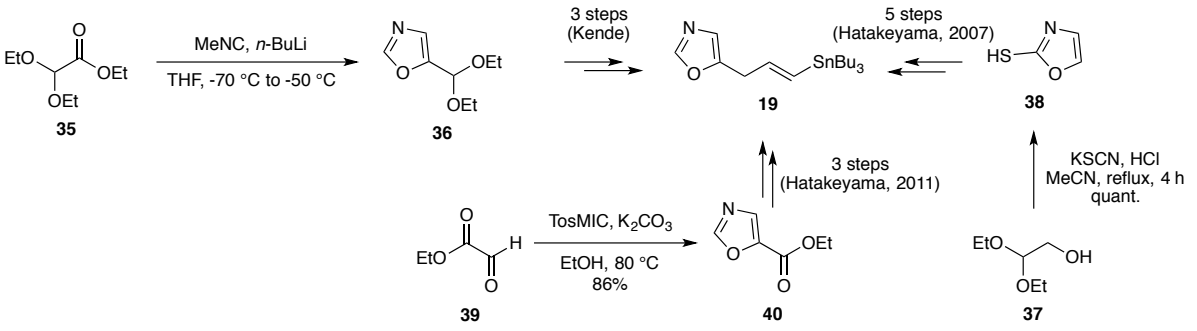
The high complexity of these compounds, with the highly functionalised β-lactone/γ-lactam unit, polyene and oxazole moieties, has attracted the attention of several research groups yet only three total syntheses have been reported: neooxazolomycin by A. S. Kende in 1990[114] and S. Hatakeyama in 2007,[115] and oxazolomycin A by S. Hatakeyama in 2011.[116]

Scheme 1.2 shows the key disconnections used for the total syntheses of oxazolomycin A and neooxazolomycin. The three syntheses accessed the left hand fragment **18** via Stille coupling of stannane **19** and vinyl iodide **20**. The oxazole heterocycle was accessed through different procedures for each reported synthesis, as outlined in Scheme 1.3. Vinyl iodide **20** was prepared by Kende via a stereoselective Reformatsky-type aldol with aldehyde **21** to introduce the new stereocentre with the desired configuration. For oxazolomycin A, Hatakeyama instead performed a *Cinchona* alkaloid-catalysed asymmetric cyclocondensation, a protocol reported by S. G. Nelson and co-workers,[117] between aldehyde **21** and propionyl chloride, which after methanolysis afforded the desired alcohol in 98% ee.



Scheme 1.2. Retrosynthetic analysis of the reported total syntheses of oxazolomycin A[116] and neooxazolomycin.[114,115]

Kende's approach to the diene moiety involved another Stille coupling, between stannane **30**, readily available from propargylamine, and vinyl iodide **31**. Alternatively, Hatakeyama initially prepared the middle fragment **22**, in 3 steps from allylamine, utilising a second generation Hoveyda-Grubbs catalyst followed by Takai's iodoalkenylation.[118] Fragment **22** was then coupled to the right-hand segment through a $CrCl_2$/$NiCl_2$-mediated Nozaki-Hiyama-Kishi reaction.



Scheme 1.3. Reported syntheses of oxazole **19**.[115,119,120]

Regarding the right-hand fragment, Kende's route to **31** employed anhydrosugar **33**[121] as a chiral source, which after manipulation was coupled to α-amidomalonate **34** to give lactam **32** as a separable 1:1.4 mixture of diastereomers. Lactam **32** was then rearranged to the lactone-lactam core of **31**. In Hatakeyama's syntheses, compound **25** was used to access the lactam-lactone core of both oxazolomycin and neooxazolomycin. It was synthesised by a palladium-catalysed cyclisation of amide **26** followed by stereoselective dihydroxylation and concomitant lactonisation. For oxazolomycin, the γ-lactone was cleaved, the generated secondary alcohol methylated and subjected to a reduction-oxidation sequence to **23**. After coupling with the middle and left fragments, the last step of the synthesis was the generation of the β-lactone with HATU[122] and DIPEA.

1.6 Previous work in the Moloney group

1.6.1 Biological relevance of tetramic acids

Tetramic acids (pyrrolidine-2,4-diones) are found in a wide range of natural products with diverse biological activities (Figure 1.15).[123–125] Some examples are streptolydigin, which inhibits bacterial RNA polymerase (RNAP)[126] and equisetin, which is active against several strains of Gram-positive bacteria and inhibits HIV-1 integrase.[127] JBIR-22 and harzianic acid belong to the same subfamily of closely-related tetramic acids as equisetin. The former is the first example of a tetramic acid that acts as a protein-protein interaction inhibitor and it can inhibit the proteasome assembly factor,[128] and the latter has been reported to possess antifungal and plant growth

promoting activity.[129] Reutericyclin is bactericidal to Gram-positive bacteria, based on its activity as a proton-ionophore, but Gram-negative bacteria have shown resistance.[130,131] Cyclopiazonic acid has been reported to cause endothelium relaxation via raising cytosolic Ca^{2+} levels,[79,132,133] similar to pramanicin, and magnesidin A inhibits various Gram-positive bacteria and prevents the decay of foodstuffs caused by spore-generating organisms,[134] giving it a potential application as a food preservative.

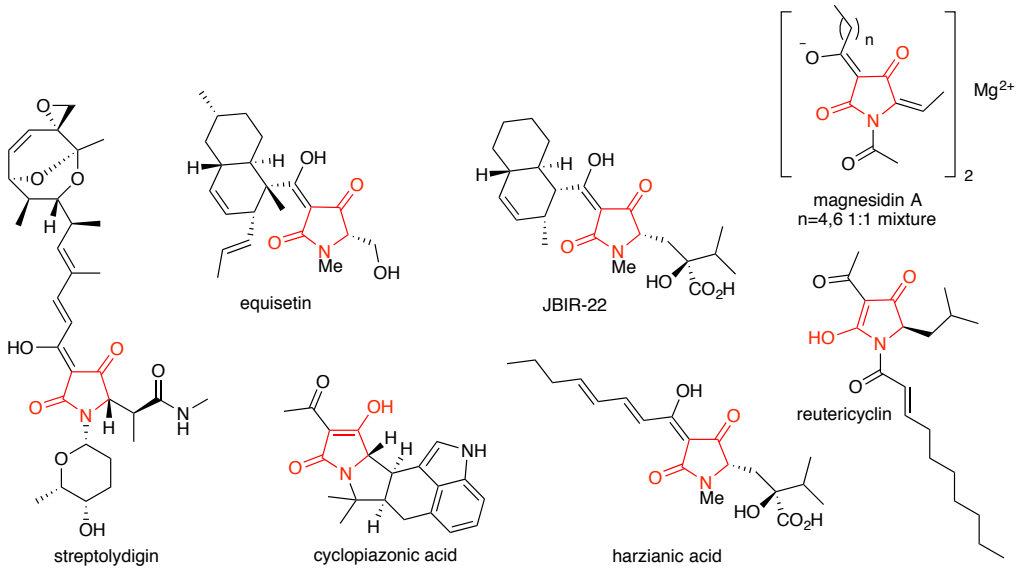


Figure 1.15. Example of biologically active natural products containing a tetramate core.

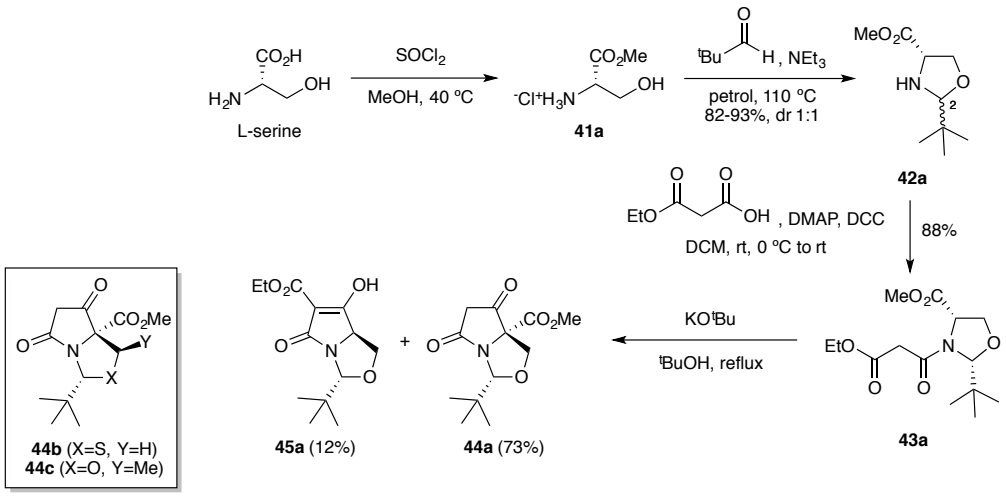
Derivatives of tetramates, such as hydroxylated pyrrolidinones, are also of interest; two examples are pramanicin and oxazolomycin (*vide infra*).

1.6.2 Preparation of a tetramic acid core

The Moloney group has developed highly effective methodology to construct the chiral tetramic acid **44a**. While the tetramate itself has shown to possess none or little intrinsic antibacterial activity, it can be used as a template for further functionalisation to generate libraries of mimics of natural products with potent biological activities.[135]

The synthesis of tetramate **44a**[136] starts with L-serine methyl ester **41a**, which is condensed with pivaldehyde with removal of water and *N*-acylated with ethyl hydrogen malonate under standard

coupling conditions (Scheme 1.4). Subsequent Dieckmann cyclisation gives a 3:1 mixture of methyl ester **44a** and ethyl ester **45a**, which can be easily separated with 1 M aqueous NaH_2PO_4, where **45a** is deprotonated while **44a** stays in the organic phase. The chiral bicyclic tetramate can also be prepared starting from L-cysteine or L-threonine to give **44b** and **44c** respectively. The synthesis relies on Seebach's 'self-regeneration of stereocentres' (SRS),[137] where a temporary stereocentre (in this case *C*(2)) is introduced to avoid racemisation. More details on the regio- and diastereoselectivities observed in this sequence are discussed in Chapter 2.



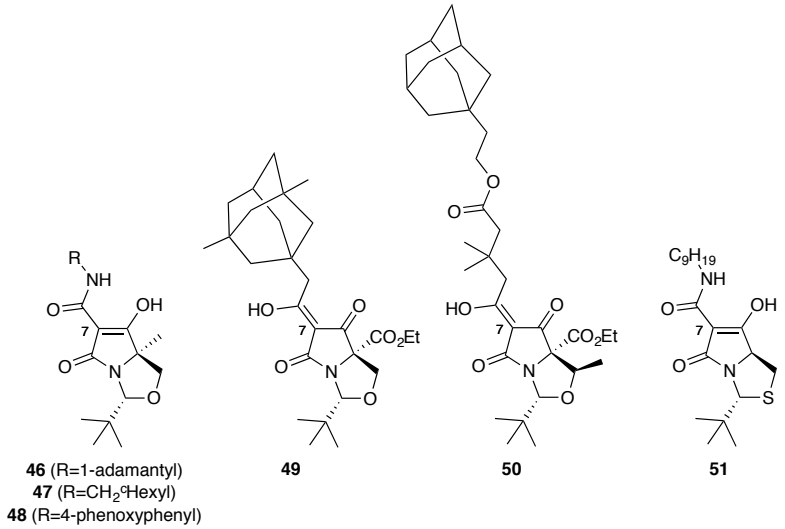
Scheme 1.4. Synthesis of tetramic acid **44**.[136]

1.6.3 3-Acyl tetramic acids

As observed in Figure 1.15, 3-acyl tetramic acids (*C*(7)-acyl with the numbering used throughout this thesis for tetramate core **44**) are common structural skeletons in natural products. M. G. Moloney and co-workers recently prepared a library of 162 analogues, including *C*(7)-acyl, -carboxamide and -enamine analogues, and found that some of these compounds, especially those with lipophilic aliphatic and bulky aromatic groups, had low MIC values against *S. aureus* (Table 1.5).[135] This phenotypic activity correlated with inhibition of two enzymes: bacterial RNAP and undecaprenyl pyrophosphate synthase (UPPS), which operate in independent metabolic pathways. SAR studies revealed that the *C*(7)-acyl/carboxamide oxygen and the *tert*-

butyl group were essential for this inhibition, possibly by forming key hydrogen bonds and hydrophobic interactions in the binding pocket respectively.

Table 1.5. Antibacterial activity of selected compounds from Y.-C. Jeong *et al.*[135]



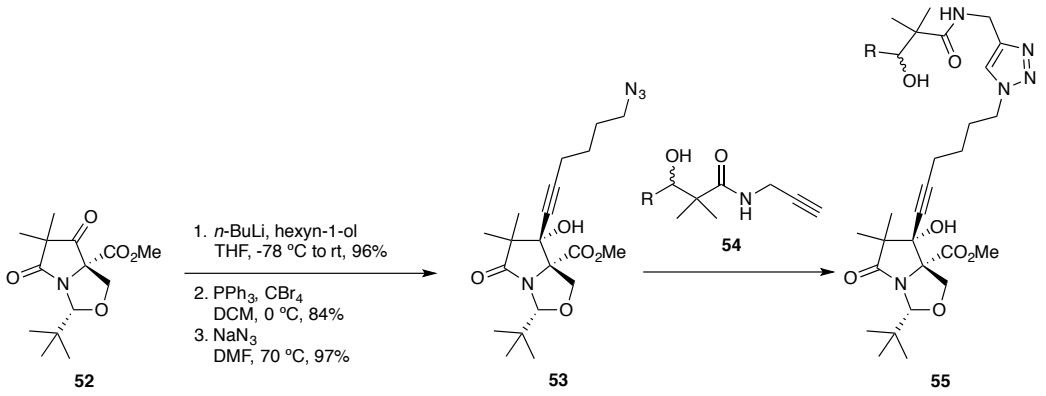
Compound	MIC [μg/mL]				IC_{50} [μM]	
	S. aureus	*E. coli*	*S. pneumonia*	*H. influenzae*	RNAP	UPPS
46	1	>64	1	2	17	0.38
47	4	>64	1	4	31	0.68
48	1	>64	0.5	1	7.3	0.78
49	2	>64	0.5	64	4.0	3.2
50	1	>64	1	>64	2.4	1.9
51	1	>64	0.5	>64	16	0.17

In spite of their significant dual enzyme inhibition, these analogues showed high plasma protein binding, which lowered their bioavailability and might explain why they were found to be ineffective *in vivo*. However, this study demonstrates the feasibility of natural product inspired tetramates, and further optimisation of their structures might lead to novel antibiotics.

1.6.4 Functionalisation of the *C*(6)-position

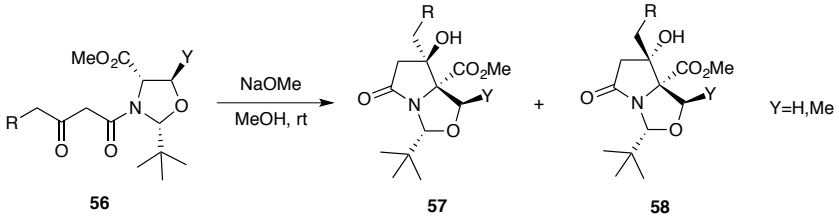
In addition to introducing acyl groups to the *C*(7)-position of the tetramate core, the Moloney group has also established methodology to functionalise the *C*(6)-position.

One approach is to treat the tetramic acid with a nucleophile, such as an alkyne, which proceeds diastereoselectively through a kinetically controlled attack to the *endo*-face of the bicyclic system. An example is the work of C. L. Bagwell *et al*. on mimics of oxazolomycin,[138] where tetramate **53**[139] was linked with **54**, a derivative of the mid-segment of oxazolomycin, through a [3+2] cycloaddition reaction (Scheme 1.5).



Scheme 1.5. *C*(6)-functionalisation of the tetramate core to prepare analogues of oxazolomycin.[138]

Another approach is through aldol cyclisation of malonamides **56**,[140,141] giving a mixture of diastereomers **57** and **58** (Scheme 1.6). In this case alcohol **57**, which has the opposite *C*(6)-stereochemistry to alcohol **53**, is the major isomer, placing the bulkier CH_2R group in the *exo*-face.

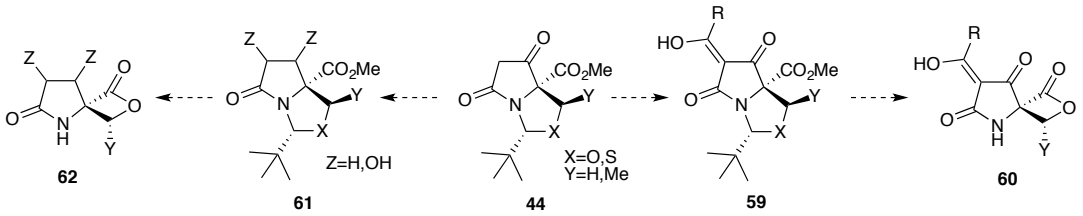


Scheme 1.6. Aldol cyclisation of malonamide **56**.[140,141]

1.7 Aims of the project

This project focuses on pramanicin and oxazolomycin. The main objective is to develop chemical transformations of the common tetramate core **44** to rapidly synthesise analogues that mimic the natural product structures. Diversity will be introduced in the positions indicated in Scheme 1.7,

and the three systems serine (X = O, Y = H), cysteine (X = S, Y = H) and threonine (X = O, Y = Me) will be compared in terms of structure, reactivity and biological activity.



Scheme 1.7. Synthetic aims.

Firstly, methodology will be developed to access analogues **59** with a range of R groups, and elaborate them to introduce a 'pramanicin-like' acyl chain. Secondly, reduction/oxidation of the tetramate core to **61** would give access to pyroglutamate systems analogous to the natural products. Finally, preparation of spiro-β-lactones **60** and **62** could increase the biological activity of these compounds through proteasome inhibition.

The synthesised compounds will be assessed to examine their antibacterial and anticancer activities. The overall aim of the project is to demonstrate that tetramates provide valuable 3D-templates suitable for the natural product inspired approach to drug discovery.

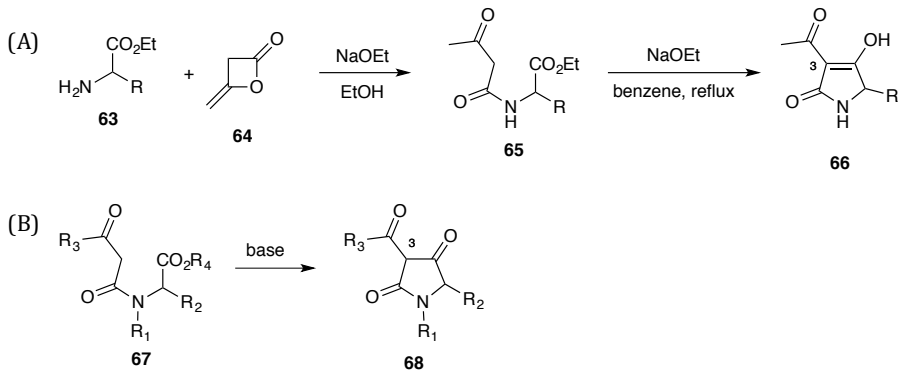
CHAPTER 2: ACYLATION OF THE TETRAMATE CORE

This chapter describes the functionalisation of the tetramate core via acylation of the $C(7)$-position. Firstly, a Weinreb amide precursor was prepared, which allowed the preparation of a range of acylated tetramic acids with different functionalities. These systems were then further elaborated to mimic the side-chains of the natural products pramanicin and oxazolomycin.

2.1 Introduction

2.1.1 Reported syntheses of 3-acyltetramic acids

Given the biological relevance of 3-acyltetramic acids (Section 1.6.1), several research groups have developed synthetic methodologies to construct these challenging systems. There are two main routes for their synthesis, depending on whether the acyl group is incorporated before or after construction of the tetramic acid core.

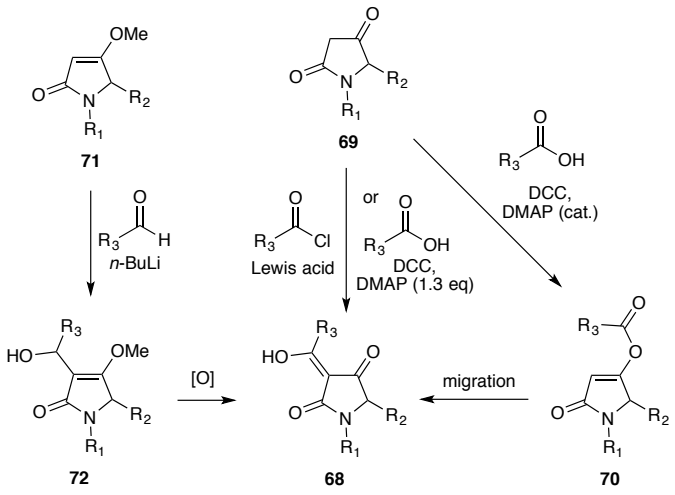


Scheme 2.1. (A) Two-step synthesis of 3-acyltetramic acids by R. N. Lacey;[142] (B) general Lacey-Dieckmann cyclisation.

The first synthetic approach to 3-acyltetramic acids was reported by R. N. Lacey[142] and it involved a two-step synthesis from readily available esters of α-amino acids (Scheme 2.1 A). The α-amino esters were converted into the corresponding acetoacetyl derivatives via condensation with diketene, which then cyclised upon treatment with sodium ethoxide. This Dieckmann-type cyclisation has been widely applied to the preparation of tetramate-containing natural products,

including equisetin[143] and cyclopiazonic acid.[144] However, this method is limited to the availability of the *N*-acetoacetyl-α-amino ester. For example, *N*-acyl tetramic acids such as that in reutericyclin and magnesidin A (Figure 1.15) cannot be accessed due to the difficulty of preparing the *N*-diacyl precursor.[145] Another limitation of this method is the potential racemisation under basic conditions when R_2 is not H (Scheme 2.1 B).

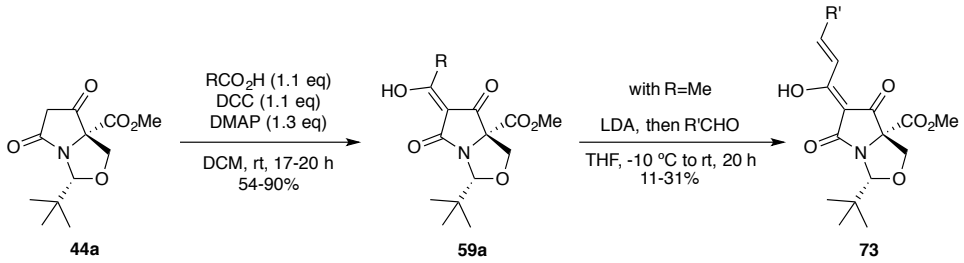
The second option is to introduce the acyl group after preparation of the tetramic acid core (Scheme 2.2). This can be achieved with the appropriate acid chloride in the presence of Lewis acids such as $BF_3{\cdot}OEt_2$ or $TiCl_4$,[146–148] or through lithiation of methyl tetramate **71** and reaction with aldehydes, followed by oxidation and demethylation.[149,150] Another option is a two-step sequence where tetramic acid **69** is first *O*-acylated with the corresponding carboxylic acid under coupling conditions (DCC and a catalytic amount of DMAP) and then migrated, *in situ* or stepwise, with triethylamine,[151] triethylamine and hydroxybenzotriazole,[152] acetone cyanohydrin[153] or DMAP.[145]



Scheme 2.2. Synthetic approaches to 3-acyltetramic acids via incorporation of the acyl chain after cyclisation.

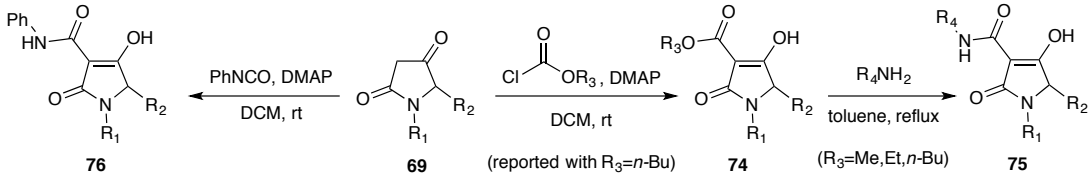
The Moloney group has used the *in situ* migration of *O*-acyl to *C*-acyltetramic acids to introduce acyl groups into the tetramate core **44a** in one step (Scheme 2.3).[145] Further manipulation of the

side-chain to alkenes **73** provided simple hybrid mimics of equisetin and pramanicin, which exhibited significant antibacterial activity against Gram-positive *S. aureus*.[154]



Scheme 2.3. Introduction and manipulation of *C*(7)-acyl side-chains to tetramic acid **44a**.[154]

Despite the numerous examples on the acylation of tetramic acids, there are only a handful of examples on the incorporation of amides and esters to the tetramate core. In most of the reported alkoxycarbonyl-tetramates, the ester functionality is introduced prior to Dieckmann cyclisation (Scheme 2.1 B, R_3 = OR), and only Y.-C. Jeong and co-workers have reported the direct *C*-acyloxylation of tetramates with butyl chloroformate (1.2 eq) and DMAP (2.2 eq), as shown in Scheme 2.4.[135,155] Regarding carboxamide-tetramates, in most cases the synthesis relies on ester-amide exchange, refluxing the ester tetramic acid and a primary amine,[135,155,156] in some cases with triethylamine[157] or acetic acid[158] and/or microwave irradiation.[158,159] Direct incorporation of the amide group has only been accomplished for *N*-phenylamide **76**, treating tetramate **69** with phenyl isocyanate (1.1 eq) and DMAP (1.3 eq).[135]



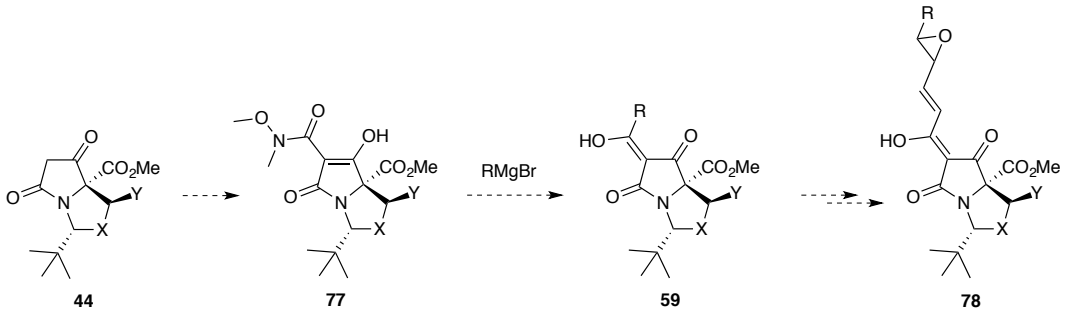
Scheme 2.4. Reported syntheses of alkoxycarbonyl- and carboxamide-tetramates.

2.1.2 Synthetic objectives

In spite of the range of reported methodologies to introduce acyl groups into tetramic acids, they all rely on the availability of the acyl chain as acid chlorides, aldehydes or carboxylic acids, which

limits the range of functional groups that can be introduced. For instance, enoyls and polyenoyls are common motifs in bioactive 3-acyltetramic acids. Although they can be accessed by manipulation of the acyl chain after its introduction, these transformations can be challenging as evidenced by the low yields of analogues **73**.

The first objective of the work reported in this Chapter was to develop a more general method to prepare these analogues which allowed the introduction of a wider range of acyl functionalities. It was envisaged that Weinreb amide **77** would be an effective precursor, which could be substituted with Grignard reagents (Scheme 2.5). These systems would be further elaborated to prepare the acyl chain of pramanicin **78**. Given the promising biological data of compounds **73**,[154] these systems are expected to possess potent antibacterial activities.

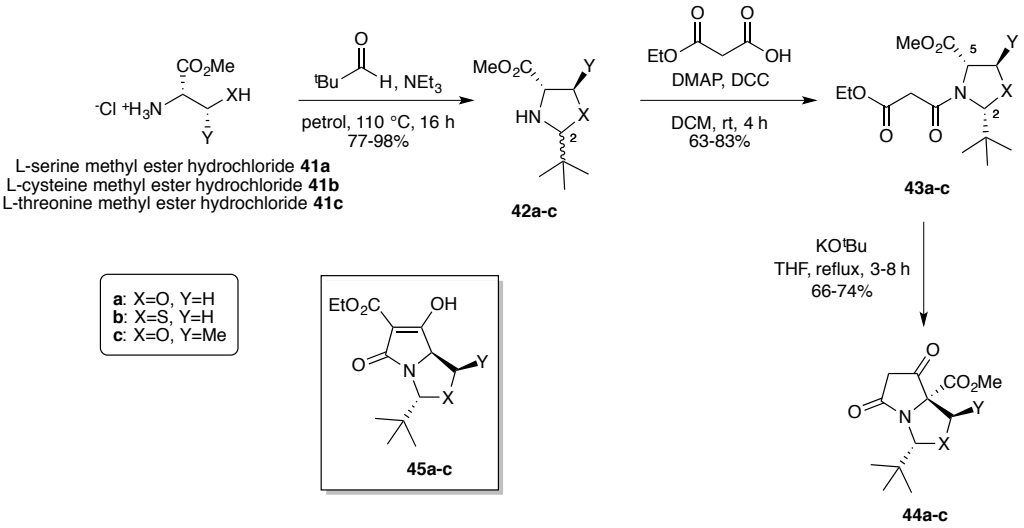


Scheme 2.5. Synthetic objectives for Chapter 2.

2.2 Synthesis of tetramate cores derived from serine, cysteine and threonine

Tetramates **44a-c** were prepared starting from L-serine,[136] L-cysteine[135] and L-threonine[160] methyl esters using reported methods, as shown in Scheme 2.6. Firstly, condensation with pivaldehyde according to Seebach's protocol[137] gave oxazolidines **42a-c** as mixtures of *C*(2)-epimers. *N*-Acylation with ethyl hydrogen malonate, DCC and DMAP provided malonamides **43a-c**, in this case mainly as the *cis* diastereomers (*cis*/*trans* refers to the relative stereochemistry between *H*(2) and *H*(5)), placing the three contiguous substituents of the five membered ring in pseudo-equatorial positions. This change on the *cis*-*trans* ratio derives from the fact that **42a-c**

can freely equilibrate through ring-chain tautomerism.[161] As observed in Table 2.1, the formation of **43a** proceeded in higher yields than for **43b,c**. For **43b**, this could be due to distortion of the five membered ring by the bigger sulfur atom, which could increase the stability of the *trans* diastereomer. For **43c**, the additional methyl group, which is placed in pseudo-axial position, could destabilise the *cis* isomer; in fact, this could also account for the bigger ratio of *trans*-oxazolidine **42c** when compared to the other two systems.



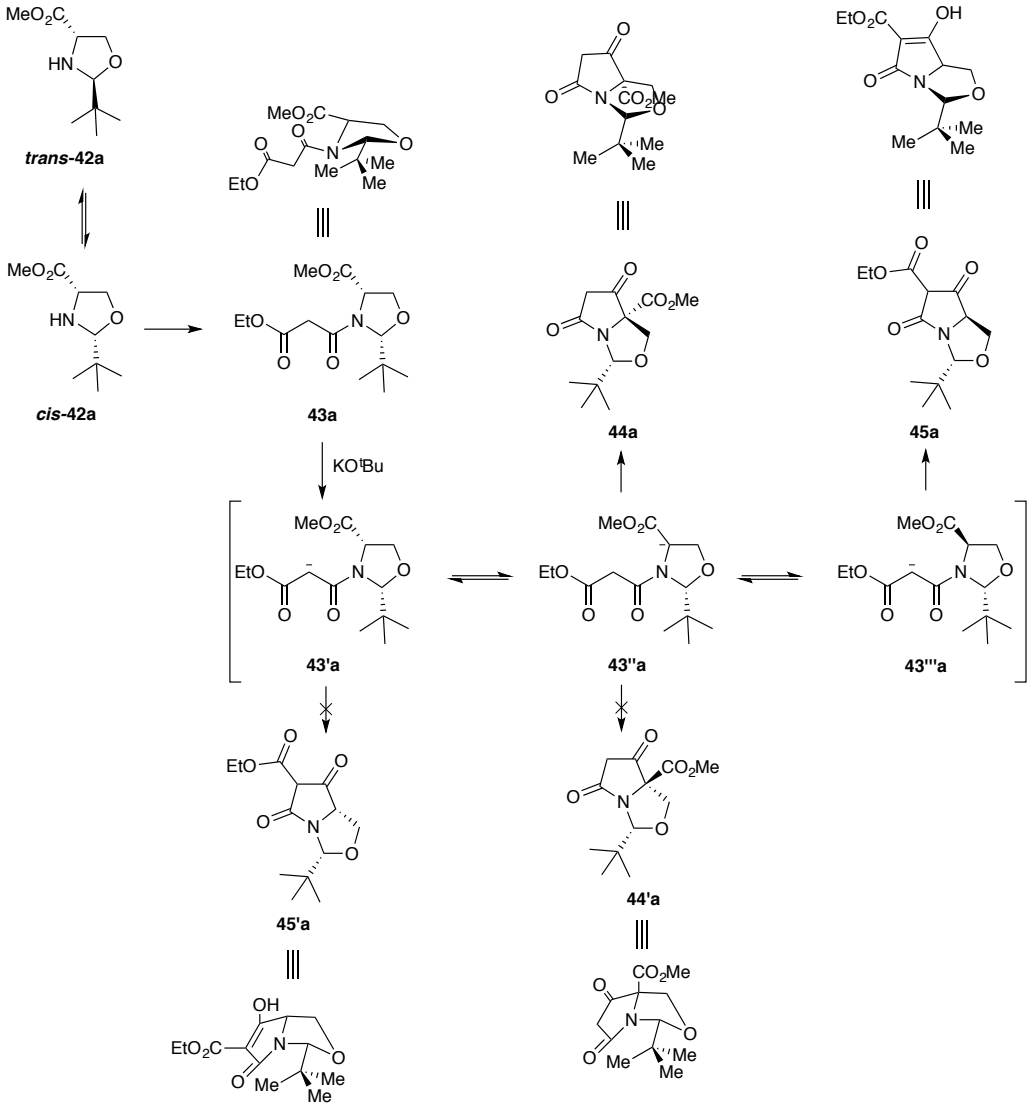
Scheme 2.6. Preparation of tetramate cores **44a-c**.

Table 2.1. Yields of the three steps for the preparation of tetramates derived from serine, cysteine and threonine.

	Condensation		***N*-Acylation**	**Cyclisation**	
	42 *cis*:*trans*	**Yield**	**Yield**	**Time**	**Yield**
a (X=O, Y=H)	1.4:1	97%	83%	3 h	74%
b (X=S, Y=H)	1.5:1	98%	75%	3 h	66%
c (X=O, Y=Me)	1.0:1	77%	63%	8 h	69%

Finally, ring closure with KO^{t}Bu gave bicyclic methyl esters **44a-c** in 66-74% yields, together with smaller amounts of ethyl esters **45a-c**. As illustrated in Scheme 2.7 for the serine series, the *tert*-butyl group plays a key role in the regio- and diastereoselectivity observed during the cyclisation of **43a**. Out of the four possible products derived from its deprotonation, ethyl ester **45'a** and methyl ester **44'a** cannot be formed as the bulky *tert*-butyl group would be placed in the *endo*-

face of the bicyclic system.[162,163] Additionally, ethyl ester **45a** is formed as the minor product due to the lower stability of *trans*-oxazolidine **43'''a**.



Scheme 2.7. Dieckmann cyclisation of *N*-acyloxazolidine **43a**, adapted from M. D. Andrews *et al*.[136] and Y.-C. Jeong *et al*.[161]

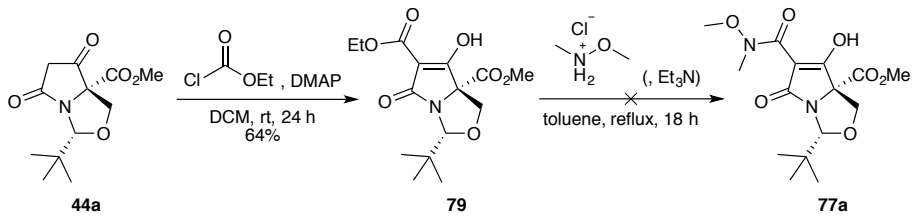
M. D. Andrews and co-workers have also reported that the cyclisation of **43a** proceeds with high levels of enantioselectivity, giving methyl ester **44a** in >90% enantiomeric excess (ee). The minor enantiomer probably derives from the presence of small amounts of the *trans*-oxazolidine in the starting material, as Y.-C. Jeong and co-workers showed that epimerisation at *C*(2) does not occur during cyclisation.[161] However, the optical purity of the corresponding cysteine and threonine systems **44b** and **44c** has yet not been fully evaluated. For this purpose, racemic samples of

tetramates **44a-c** were prepared starting from DL-serine, cysteine, and threonine, and compared with the enantiopure ones by chiral HPLC (Appendix E). The ee value of **44a** was confirmed to be high (96%), and both **44b** and **44c** showed no detectable presence of the other enantiomer. These results confirm the effectiveness of Seebach's protocol for the control of such enolate-mediated processes,[164] and further emphasise the value of *tert*-butyl oxazolidines/thiazolidines in chemoselective Dieckmann ring closures.

2.3 Preparation of Weinreb amide 77

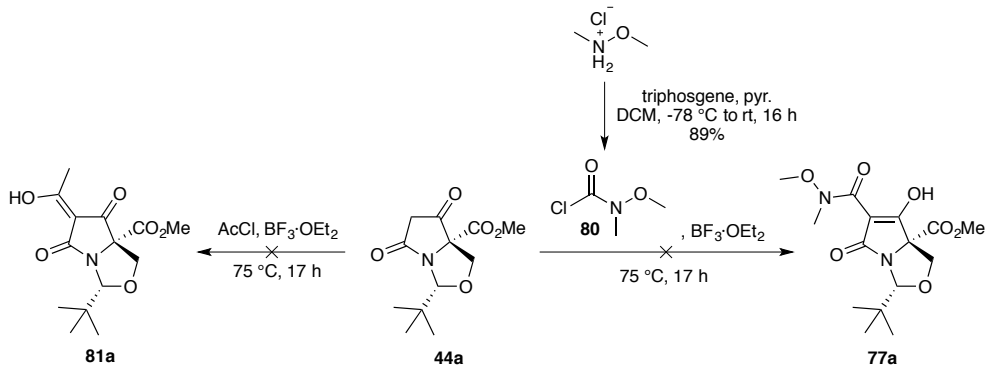
2.3.1 Attempted introduction of the Weinreb amide to the tetramate core

With tetramic acid **44a** in hand, the initial approach to the synthesis of Weinreb amide **77a** was via ester-amide exchange with ester **79**. Treatment of tetramate **44a** with ethyl chloroformate and DMAP, adapted from Y.-C. Jeong *et al.*'s[135] procedure, yielded the ethyl ester (Scheme 2.8). Ester **79** was found to be relatively unstable and it easily hydrolysed when stored at room temperature or purified by column chromatography, thus it was only purified via work-up as described for ester **45a** (Section 1.6.2). Unfortunately, none of the desired amide was formed when the ethyl ester was refluxed with *N*,*O*-dimethylhydroxylamine hydrochloride, and only a complex mixture of hydrolysed and decarboxylated products was observed in the LRMS and NMR spectra of the crude product. Similar results were obtained when adding 1 equivalent of Et_3N, but in this case one major product was observed in the crude NMR, with high molecular weight values in the LRMS spectrum which seemed to correspond to a dimeric structure. ^{1}H NMR analysis showed the presence of *H*(2), *H*(4)s, *tert*-butyl and CO_2CH_3, but no *H*(7)s and no peaks for the ethyl ester or the amide, which might indicate dimerisation at the *C*(7)-position, potentially via initial hydrolysis of the ethyl ester (or of the Weinreb amide if formed).



Scheme 2.8. Attempted synthesis of Weinreb amide **77a** via ester-amide exchange.

Alternatively, the use of R. C. F. Jones and co-workers conditions[148] with carbamic chloride **80** and a strong Lewis acid ($BF_3{\cdot}OEt_2$) resulted in degradation of the tetramate core, which could be due to deprotection of the *N*,*O*-acetal, and no product was observed by NMR or LRMS analysis (Scheme 2.9).

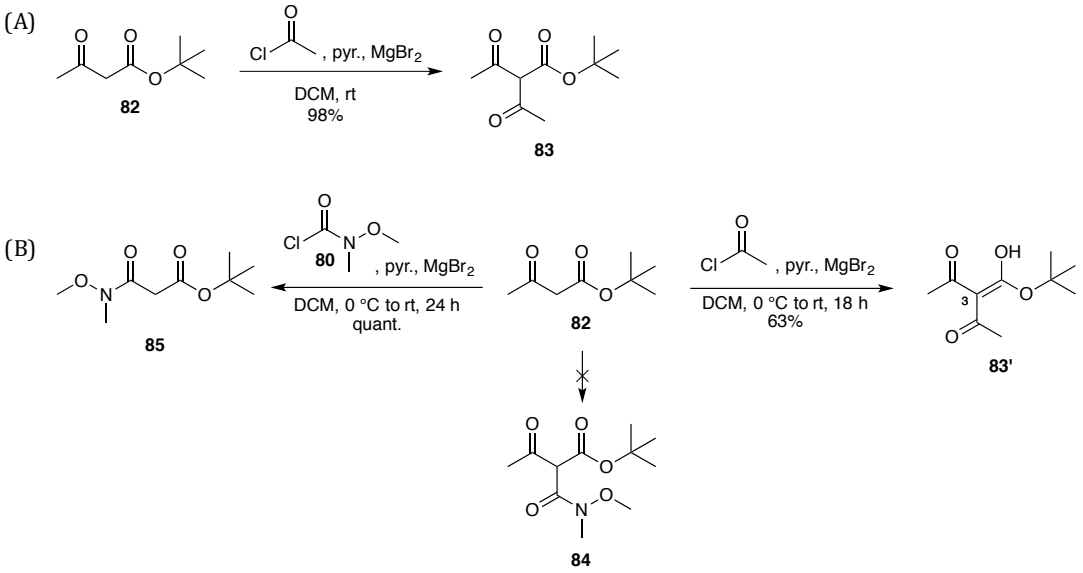


Scheme 2.9. Attempted acylation of tetramic acid **44a** with acyl chloride and a strong Lewis acid.

D. Sucunza *et al.* have described the acetylation of dicarbonyl **82** with acetyl chloride in the presence of pyridine and magnesium bromide in high yield (Scheme 2.10 A).[165] It was of interest to investigate whether these conditions would be also applicable to the introduction of amides, and if they could also be used for the acylation of tetramates. Firstly, the reported conditions were reproduced and tricarbonyl **83'** was obtained in 63% yield. This compound was found to be in the enol form, contrary to what had been reported, as evidenced by the lack of *H*(3) and the low field value of *C*(3) (110.6 ppm).

When acetoacetate **82** was treated with pyridine, magnesium bromide and carbamic chloride **80** instead of acetyl chloride, Weinreb amide **85** was isolated in quantitative yields (Scheme 2.10 B).

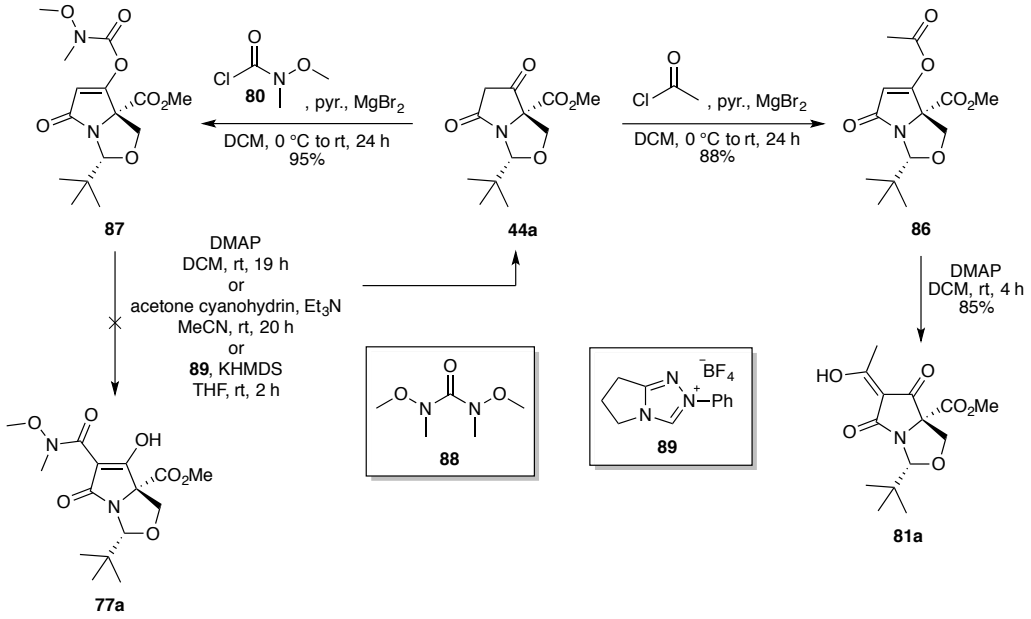
This result shows that the desired tricarbonyl amide **84** was initially formed, but it underwent deacetylation, and it proves that acylamidation of dicarbonyls is possible under these conditions.



Scheme 2.10. (A) Acetylation of *tert*-butyl acetoacetate reported by D. Sucunza *et al.*;[165] (B) acetylation and acylamidation of *tert*-butyl acetoacetate.

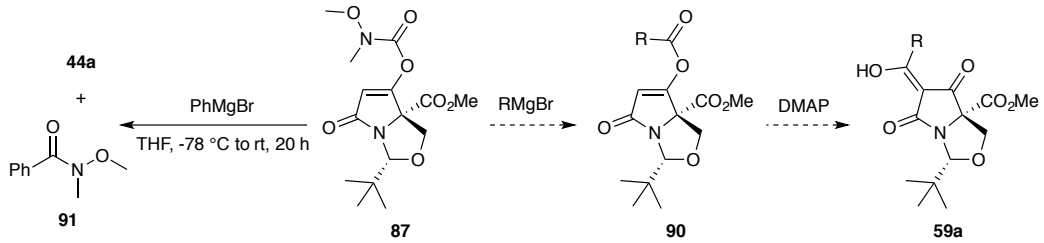
Application of Sucunza's acetylation conditions to tetramate **44a** resulted in exclusive *O*-acylation, but *O*-acetal **86** could be converted to *C*-acetyl **81a** via acyl migration with 1.5 equivalents of DMAP, as shown in Scheme 2.11. The same transformation was attempted in one step, adding DMAP to the mixture of pyridine, magnesium bromide and acetyl chloride, but only *O*-acetyl **86** was isolated.

Likewise, attempted acylamidation of **44a** furnished only carbamate **87**, which could only be separated from urea **88**, a by-product of the synthesis of **80**, after drying it under reduced pressure for several hours. In this case, migration with DMAP failed to give Weinreb amide **77a**, and instead **44a** was recovered; the same was observed with acetone cyanohydrin.[153] With triazolium salt **89** and KHMDS, which form a carbene *in situ* that has been shown to promote *O*- to *C*-acyl transfers,[166] starting material **87** was recovered.



Scheme 2.11. *O*-Acylation of tetramate **44a** and attempted migration.

In view of the possibility to perform *O*- to *C*-acyl migrations, but not with acylamides, the initial Grignard reaction on Weinreb amide **87**, followed by acyl migration, seemed to be a plausible approach to analogues **59a** (Scheme 2.12). However, reaction of **87** with phenylmagnesium bromide returned tetramic acid **44a** and benzamide **91**, as a result of the tetramate being a better leaving group than the amine.

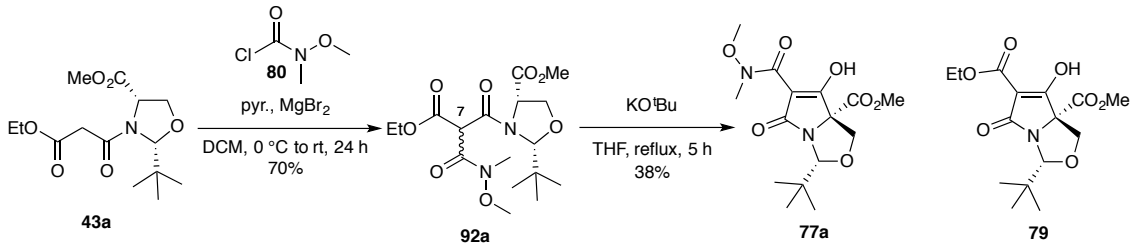


Scheme 2.12. Grignard addition to carbamate **87**.

2.3.2 Introduction of the Weinreb amide prior to cyclisation

Given the difficulties encountered in the preparation of Weinreb amide **77a** via acylation of the tetramate core, another strategy was devised where the amide would be introduced before cyclisation. For this purpose, *N*-acyloxazolidine **43a** was subjected to Sucunza's acylation conditions, and in this case the desired *C*-acylated product **92a** was formed (Scheme 2.13). This

tricarbonyl system existed in the keto form, as indicated by the presence of an *H*(7) singlet on the ^{1}H NMR spectrum, and it was formed as a 1.1:1 mixture of diastereomers, which were not separated.

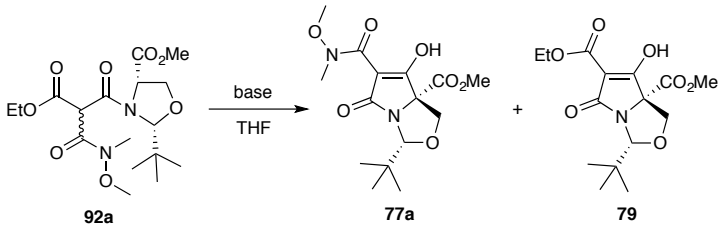


Scheme 2.13. Preparation of Weinreb amide **77a** via acylamidation prior to cyclisation.

It was expected that Dieckmann cyclisation of **92a** would give Weinreb amide **77a** preferentially over ethyl ester **79**, favoured by the better leaving group ability of the ethoxide than the amine. However, treatment of **92a** with KO^{t}Bu under reflux gave an inseparable 1:1 mixture of **77a** and **79**. After screening different bases (Table 2.2), it was gratifying to find that treatment of **92a** with DBU at room temperature for 24 h gave Weinreb amide **77a** almost exclusively, with no ethyl ester observed by NMR. LC/MS analysis of the crude product indicated the presence of the ethyl ester in <1%. This synthesis could be easily scaled up and up to 4.6 g of **77a** have been prepared.

The same conditions were employed for the preparation of Weinreb amide **77b** (starting from cysteine), and for **77c** (starting from threonine) 5 equivalents of DBU had to be added to reach full conversion, probably due to a slower reaction resulting from hindrance from the methyl group in *C*(4). Noteworthy, it would seem that this sequence could also be applied to the synthesis of different *C*(7)-carboxamide-tetramates.

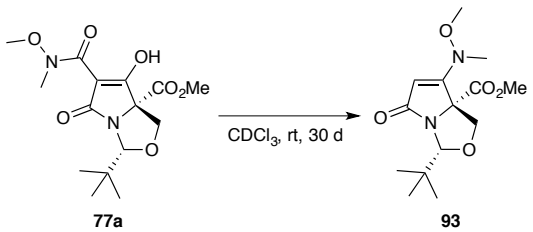
Table 2.2. Base screen for the cyclisation of tricarbonyl **92a**.



Entry	Base	pK_a	Temperature	Time [h]	Yield [%]	Ratio 77a:79
1	Pyridine	5.2	rt	28	0	-
2	Et_3N	10.8	rt	24	0	-
3	Piperidine	11.0	rt	24	quant.	1:1[a]
4	DBU	12	rt	24	84	110:1[b]
5	KO^tBu	17	reflux	5	76	1:1[a]

[a]Determined by NMR
[b]Determined by LC/MS

During the characterisation of the Weinreb amides, it was observed that when storing them in $CDCl_3$ for several days a new set of peaks appeared in the NMR spectrum. So as to understand the degradation path, a sample of **77a** was stirred in $CDCl_3$ at room temperature for 30 days, whereupon all the Weinreb amide had reacted. Column purification and full characterisation of the degradation product indicated that it corresponded to enamine **93** (Scheme 2.14). This probably arose from hydrolysis and decarboxylation, and reaction of the tetramate with released amine.



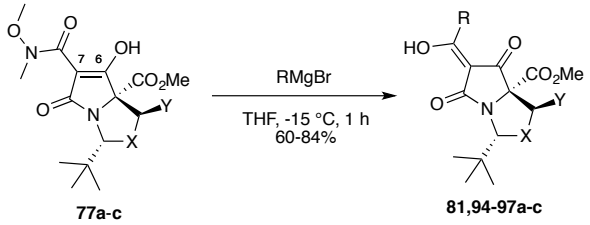
Scheme 2.14. Degradation of Weinreb amide **77a** in $CDCl_3$.

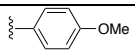
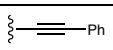
2.4 Preparation of acylated analogues

2.4.1 Grignard addition to the Weinreb amide

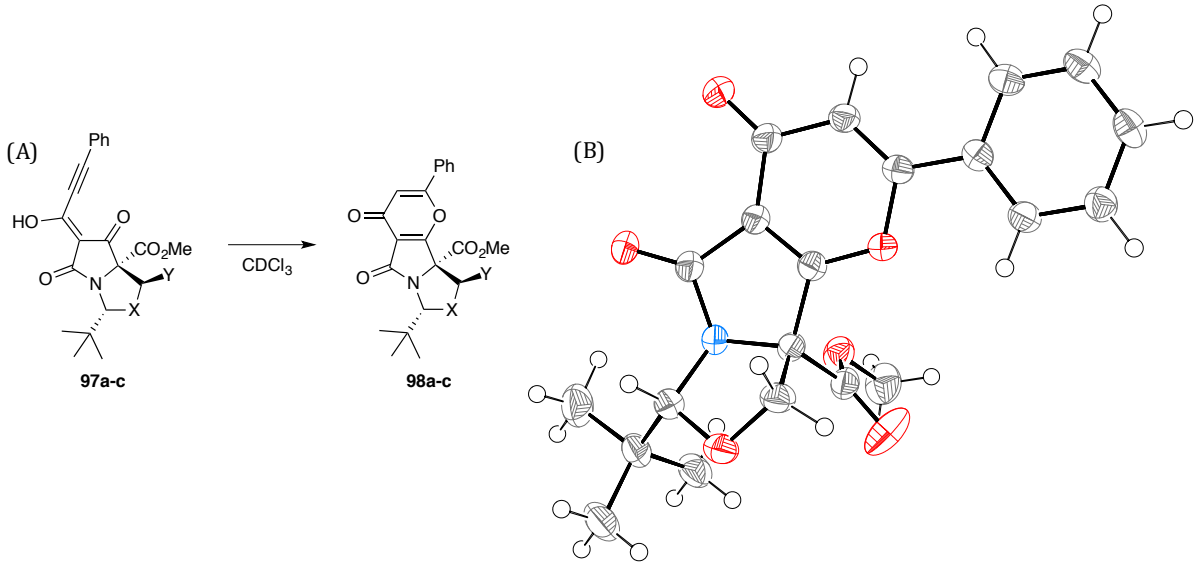
With Weinreb amides **77a-c** in hand, treatment with 2 equivalents of the desired magnesium bromide gave *C*(7)-acylated compounds **81,94-97a-c** in 60-84% yield (Table 2.3), allowing the introduction of alkyl, aryl, alkenyl and alkynyl functionalities. Remarkably, high regioselectivity was observed for this transformation, with no significant amounts of over-addition products or attack to the *C*(6)-position or the methyl ester observed by NMR or LRMS analysis. This high efficiency probably derives from the stability of the magnesium-chelated tetrahedral intermediate,[167] avoiding over-addition, as well as the deprotonated enolate in *C*(6), which might prevent addition onto this position, and the hindered nature of the methyl ester.

Table 2.3. Grignard reaction of Weinreb amides **77a-c**.



Compound	R	Yield [%]		
		a (X=O, Y=H)	b (X=S, Y=H)	c (X=O, Y=Me)
81	Me	82	63	71
94	Ph	84	76	74
95		83	75	60
96		79	72	71
97		81	66	81

Interestingly, it was found that alkynes **97a-c** slowly degraded to a single product when stored in $CDCl_3$ for several days. NMR analysis of the product indicated that it corresponded to tricyclic compounds **98a-c**, which was later confirmed by crystal structure of **98a** (Scheme 2.15).



Scheme 2.15. (A) Isomerisation of alkynes **97a-c**; (B) crystal structure of **98a**.

2.4.2 Tautomeric forms of acylated analogues

Acyltetramates **81,94-97a-c** could exist as the tautomeric forms shown in Figure 2.1. Analysis of related 3-acyl tetramic acids has shown that the interconversion between internal tautomers A-B and C-D is a fast process, giving NMR signal peaks that are an average of both forms. Conversely, the equilibrium between external tautomers AB-CD is slow and they often give separate NMR signals.[168,169] The tautomeric forms can be distinguished by ^{13}C NMR spectroscopy, due to the differing inductive effects arising from keto-enol forms and hydrogen bonding.[145] For instance, the enolic carbonyl on $C(6)$ in form A should resonate at higher field than the corresponding free carbonyl in forms C and D, while in form B it should occur at lower field due to hydrogen bonding from the $C(9)$-enol group. For the same reason, the hydrogen bonded $C(8)$ in form D should resonate more downfield than in forms A and B, and at higher field in form C.

^{1}H and ^{13}C NMR analysis of acylated compounds **81,94-97a-c** in $CDCl_3$ revealed that they exist as one major tautomer. For analogues **81a-c** and **96a-c** (alkyl and alkenyl analogues) a minor tautomeric form was observed in ratios ranging from 6.5:1 to 3.2:1, which was not observed for analogues having aryl or alkynyl acyl groups.

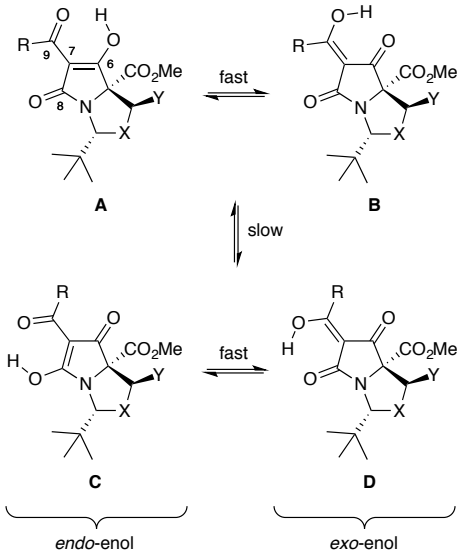


Figure 2.1. Tautomeric forms of *C*(7)-acyltetramates.

Table 2.4. Representative ^{13}C NMR signals of acylated analogues in $CDCl_3$.

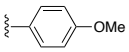
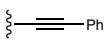
Compound	R	X	Y	AB:CD	*C*(6)		*C*(8)		*C*(9)	
					AB	CD	AB	CD	AB	CD
81a	Me	O	H	1:4.2	195.7	188.1	172.5	180.0	190.2	188.6
81b		S	H	1:6.5	196.5	188.9	170.3	177.4	189.1	187.6
81c		O	Me	1:3.2	196.0	188.6	172.2	179.5	189.2	187.3
94a	Ph	O	H	0:1	-	186.4	-	182.6	-	183.8
94b		S	H	0:1	-	187.2	-	179.9	-	183.0
94c		O	Me	0:1	-	186.8	-	182.0	-	182.6
95a		O	H	0:1	-	186.6	-	183.3	-	182.8
95b		S	H	0:1	-	187.3	-	180.6	-	182.2
95c		O	Me	0:1	-	186.9	-	182.6	-	181.7
96a		O	H	1:3.6	196.9	188.1	172.9	180.7	177.4	176.9
96b		S	H	1:3.8	197.6	188.9	170.7	178.1	176.7	176.3
96c		O	Me	1:3.5	197.2	188.4	172.5	180.1	176.3	175.8
97a		O	H	0:1	-	186.1	-	179.4	-	160.9
97b		S	H	0:1	-	186.9	-	176.9	-	160.1
97c		O	Me	0:1	-	186.4	-	178.8	-	159.7
77a	Weinreb amide	O	H	1:0	185.7	-	180.7	-	166.5	
77b		S	H	1:0	185.6	-	179.9	-	166.5	
77c		O	Me	1:0	185.7	-	179.0	-	166.3	
79	Ester	O	H	1:0	184.4	-	172.5	-	167.2	

Table 2.4 shows the carbonyl ^{13}C NMR signals for the major and minor tautomeric forms of these compounds, which were assigned using HMBC correlations. The smallest differences in chemical shifts between the two external tautomeric forms are on *C*(9), which would correspond to forms A and C or B and D. The major tautomeric forms show a downfield *C*(8) and a higher field *C*(6) when compared to the minor tautomers, which would match *Z-exo*-enol form D. The lower field *C*(6) and higher field *C*(8) of the minor tautomer are in accordance with *E-exo*-enol form B. The small differences observed on the *C*(9) (and on the *C*(7), see Experimental section) between both forms could arise from different contributions of the internal tautomers.[168]

In order to gain further evidence to this assignment, a ^{1}H-^{13}C NOE experiment of compound **81a** in C_6D_6 was performed. Irradiation of the R = Me of the major tautomeric form showed a 4.5% enhancement on carbonyl *C*(6) and only 1.3% on *C*(8) (Figure 2.2). This is consistent with the structure of forms CD, while the opposite should be observed for AB.

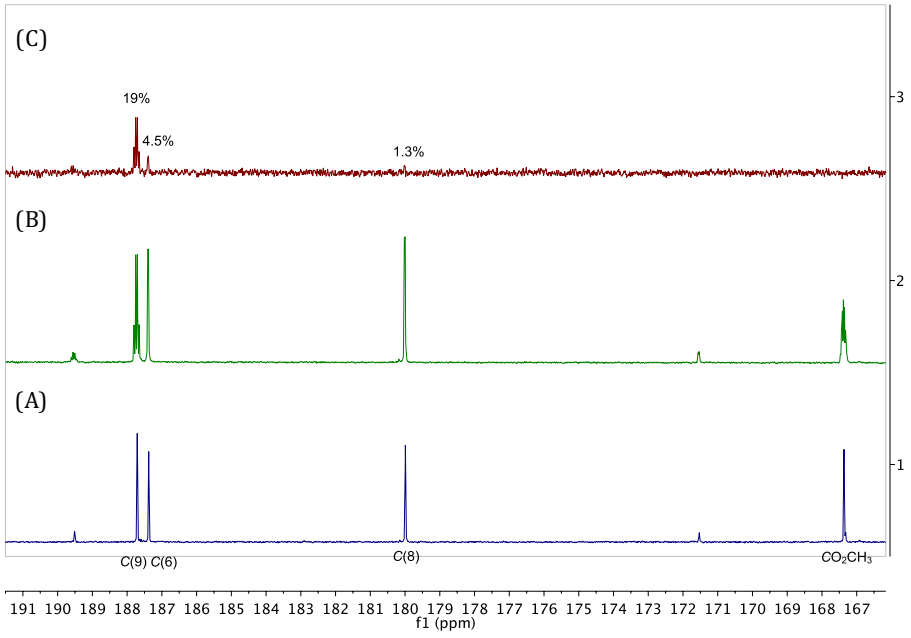


Figure 2.2. ^{1}H-^{13}C NOE of compound **81a**. Carbonyl region of the (A) standard ^{13}C NMR; (B) off-resonance decoupled ^{13}C NMR; (C) difference between ^{13}C NMR with and without irradiation of C(9)*Me*.

This assignment is also consistent with a study of 3-acyltetramic acids by Y.-C. Jeong[145] which indicated that *N*-H and *N*-alkyl derivatives favour form D while the *N*-acyl ones exist as a mixture of external tautomers AB and D in approximately equal ratio.

The ratio of tautomeric forms observed by NMR analysis depended on the solvent used. With non-polar solvents (C_6D_6, CD_2Cl_2 and $CDCl_3$), a mixture of AB and CD forms ranging from 1:3.8 to 1:4.5 for **81a** was observed. Conversely, polar solvents (CD_3OD and CD_3CN) displayed only one set of peaks with broader carbonyl and *C*(7) signals (Figure 2.3), which could result from solvent-assisted proton transfer reactions[145] which could accelerate the equilibrium between the tautomeric forms.

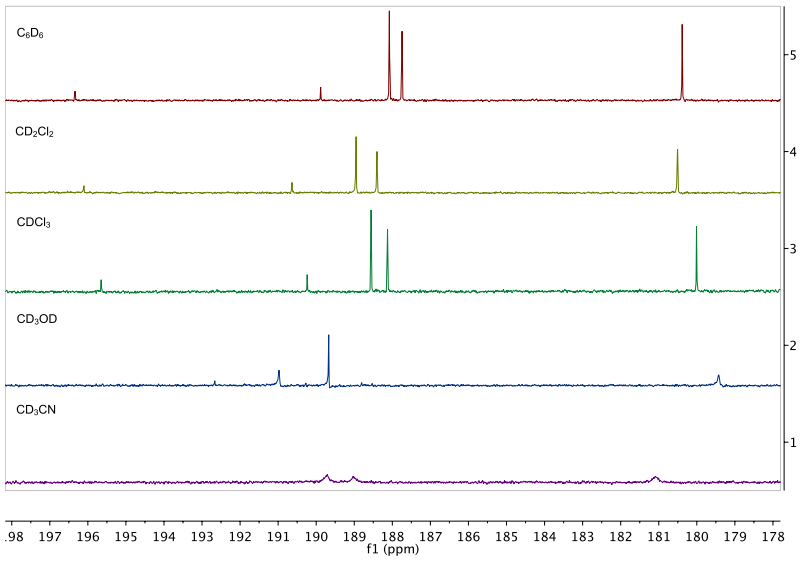


Figure 2.3. Carbonyl ^{13}C NMR (125 MHz) region of acetylated analogue **81a** in different solvents.

In contrast to 3-acyltetramates, energy calculations have shown that the major tautomeric form of 3-carboxamide and 3-alkoxycarbonyl tetramic acids is A.[155] The high field *C*(6)-enol of both Weinreb amide **77a** and ethyl ester **79** (Table 2.4) are in accordance with this assignment. For the ethyl ester, the chemical shift of *C*(8) being similar to the minor form of the acylated analogues is also consistent. Conversely, for the Weinreb amides the lactam carbonyl is observed at unexpected low field. It is worth mentioning that these amides displayed some broad ^{13}C NMR signals (Figure 2.4 A) which thwarted their assignment. A large number of scans were required to

observe, in ^{13}C NMR spectra, the signals of N*C*H$_3$, NO*C*H$_3$, *C*(6), *C*(7) and *C*(8), which were weaker than some minor impurities present in the product but could be identified with HSQC and HMBC correlations (Figure 2.4 B). This phenomenon could result from a combination of the tautomeric and rotameric forms found in these compounds. In fact, in some of the reported carboxamide-tetramic acids, these signals are not mentioned in their ^{13}C NMR spectroscopic characterisation.[135]

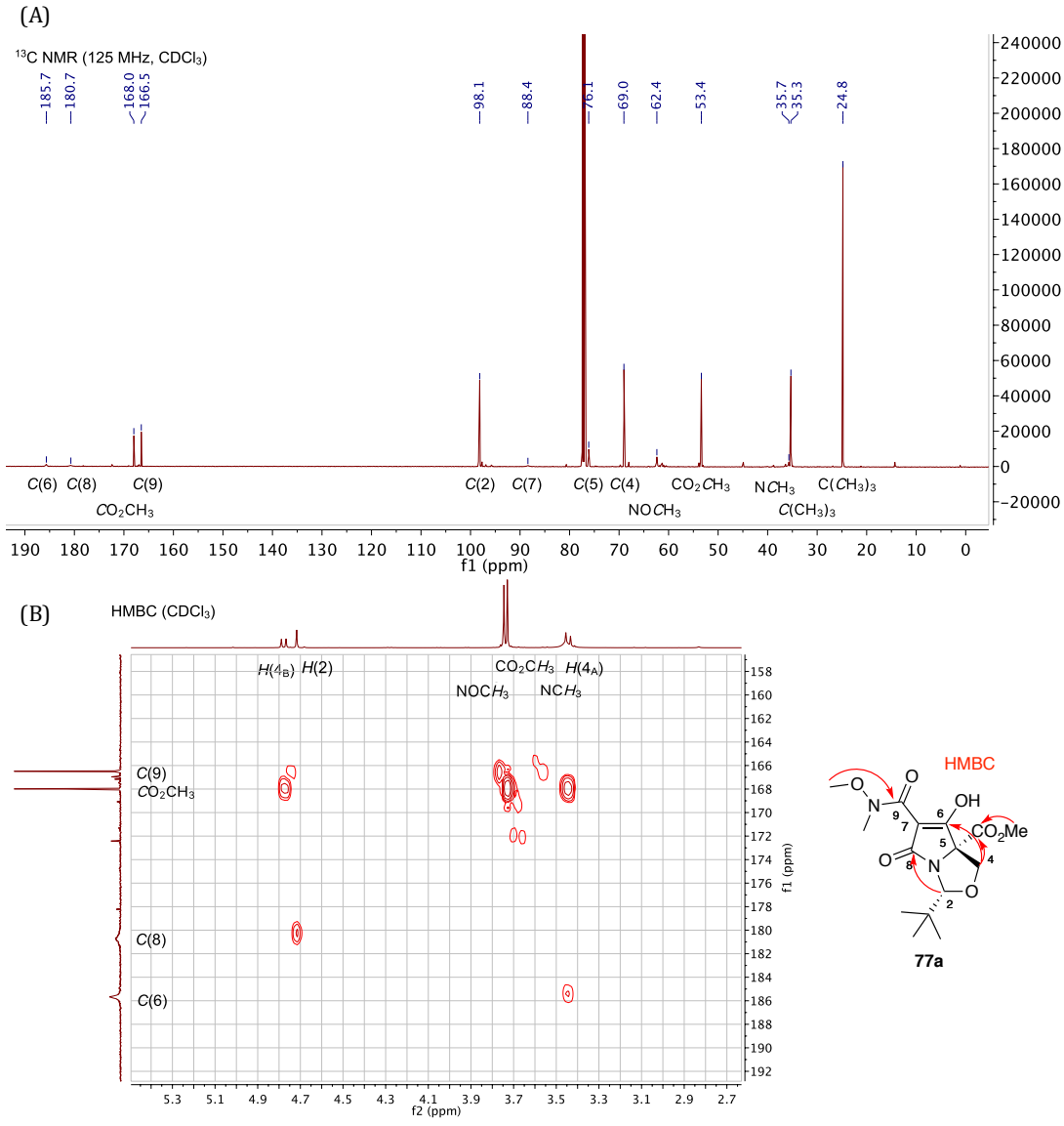


Figure 2.4. (A) ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of Weinreb amide **77a** showing some broad signals; (B) carbonyl region of the HMBC spectrum and HMBC correlations of **77a**.

2.4.3 Metal chelation of acylated analogues

The tricarbonyl moiety of acyltetramic acids provides a suitable site for bidentate metal complexation. It has been reported that these compounds can chelate Cu^{2+}, Mg^{2+}, Ca^{2+}, Fe^{3+} and Ni^{2+} metal cations amongst others,[170–172] and in some cases the natural tetramic acids have been isolated as their chelated salts (magnesidin A, Figure 1.15).[146,173,174] These metal complexing properties are key for the mode of action of many biologically active tetramic acids. For example, oleficin, active against Gram-positive bacteria, depletes the Mg^{2+} and Ca^{2+} contents of mitochondria and inhibits yeast respiration,[175] and tetronasin, active against several ruminal bacteria, depolarises the bacterial membrane by facilitating Ca^{2+}/H^{+} exchange.[176]

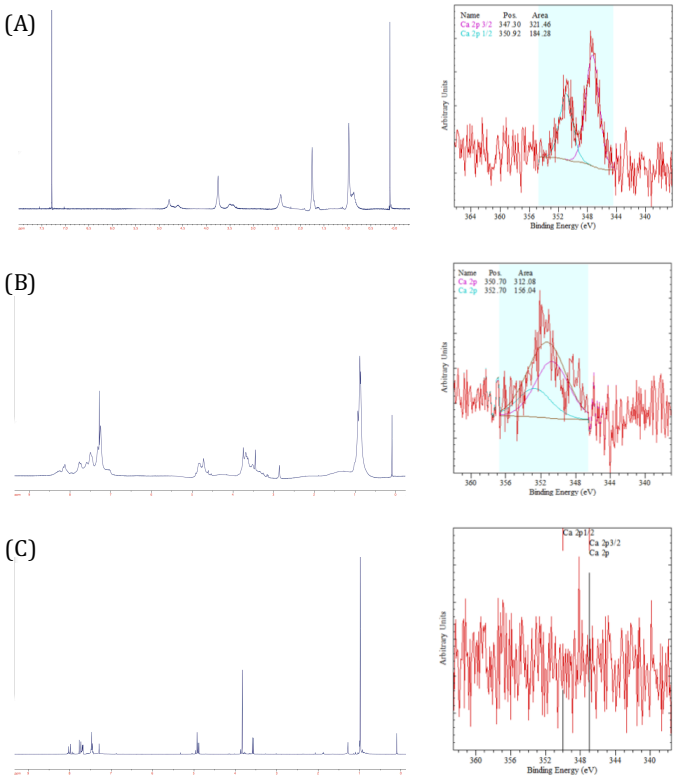


Figure 2.5. ^{1}H NMR (400 MHz, $CDCl_3$) and XPS calcium region of (A) **81a** after silica purification; (B) **96a** after silica purification; (C) **96a** after washing with 2 M HCl.

The chelating ability of acylated tetramic acids has also been reported to hamper purification, given their high affinity for silica and big tendency to chelate trace amounts of metals, such as Ca^{2+}, Mg^{2+} and Fe^{2+}, found in commercial silica gel.[174,177,178] Indeed, all *C*(7)-acyl analogues gave broad

NMR signals after silica gel purification, and they had to be washed with 2 M HCl to obtain metal-free samples with well-resolved NMR spectra (Figure 2.5). In some cases, the chelated metals have been identified using MS, but for the compounds synthesised herein no peaks corresponding to complexed compounds were observed.

Table 2.5. XPS results.

Sample	Element [%]			
	Carbon	Oxygen	Nitrogen	Calcium
81a after silica purification	46.0	47.6	3.8	2.5
96a after silica purification	49.2	44.3	3.7	2.8
96a after 2 M HCl wash	49.5	48.7	1.4	0

The solid nature of all the complexed samples rendered the opportunity to use X-ray photoelectronic spectroscopy (XPS) to identify the chelated metals. XPS analysis of the chelated **81a** and **96a** revealed the presence of calcium in both cases, but no other possible metals (Mg, Fe, Co…) were detected (Figure 2.5 A,B, Table 2.5). After washing **96a** with 2 M HCl and analysing the solid metal-free sample again by XPS, no detectable amounts of calcium were found (Figure 2.5 C, Table 2.5). Furthermore, when the metal-free sample, showing sharp NMR peaks, was washed with 2 M aqueous $Ca(NO_3)_2$, broadening of the NMR signals was observed. These results indicate that the difficult purification of *C*(7)-acyltetramic acids by column chromatography might be due to chelation to trace amounts of calcium found in the silica gel.

2.5 Manipulation of the tetramate core of acylated analogues

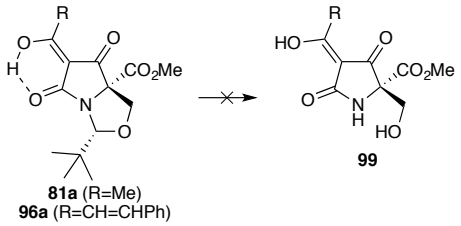
In order to alter the physicochemical properties of these analogues, and to evaluate the influence on biological activity, *N*,*O*-acetal deprotection and decarboxylation were of interest.

2.5.1 *N*,*O*-Acetal deprotection

As observed by S. W. B. Tan *et al.* for analogous compounds,[154] the acylated analogues were unreactive towards *N*,*O*-acetal deprotection. All the tested conditions, including Corey-Richard

protocol with 1,3-propanedithiol,[179] used for the deprotection of similar systems,[136,160,180–182] and other acidic conditions (Table 2.6) resulted in quantitative recovery of starting material.

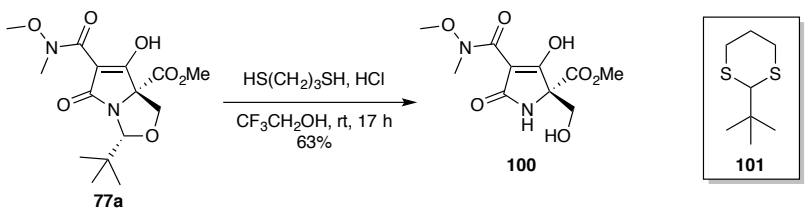
Table 2.6. Attempted *N*,*O*-acetal deprotection of acylated analogues.



81a (R=Me)
96a (R=CH=CHPh)

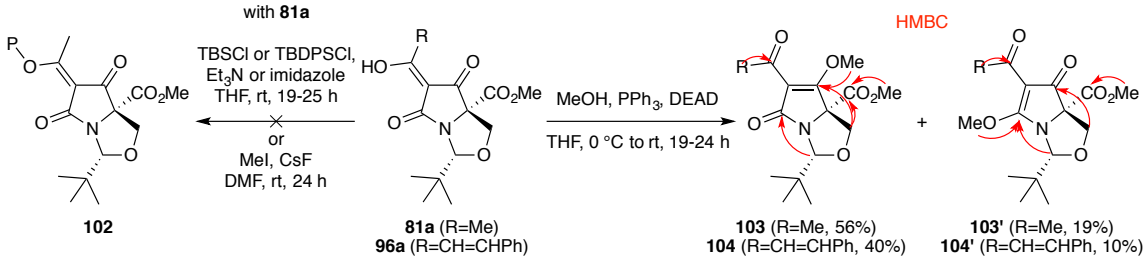
Entry	Conditions	Outcome
1	$HS(CH_2)_3SH$ (1.1 eq) HCl (2%) in CF_3CH_2OH, rt, 15 h[179]	Recovered starting material.
2	1:1:3 HCO_2H/H_2O/THF, rt, 17 h[183]	
3	TFA (2 eq) 1:1 THF/H_2O, rt, 20 h[184]	
4	TFA (20 eq) DCM, rt, 18 h	
5	$BiBr_3$ (0.1 eq) MeCN, rt, 20 h[185]	
6	BBr_3 (1 eq) DCM, rt, 24 h	

It was suspected that the lack of reactivity of the acylated products towards hydrolysis was due to the *exo*-enol moiety, which stabilised the lactam through hydrogen bonding. The successful deprotection of Weinreb amide **77a** (Scheme 2.16), which has a free lactam carbonyl, would support this supposition. This outcome also provided further evidence to the tautomeric assignment of the carboxamide compounds. Given the high polarity of alcohol **100**, column purification was unsuccessful, but dithiane **101** could be removed by dissolving the crude product in MeOH and washing it with petrol.



Scheme 2.16. Successful *N*,*O*-acetal deprotection of Weinreb amide **77a**.

These results suggested that protection of the enolic moiety should allow subsequent *N*,*O*-acetal hydrolysis. However, the enol group also proved to be highly unreactive, probably due to its extensive delocalisation in the tricarbonyl system. Attempted silylation with *tert*-butyldimethylsilyl (TBS) chloride or *tert*-butyldiphenylsilyl (TBDPS) chloride in the presence of imidazole or triethylamine gave unreacted starting material. Similarly, upon treatment with MeI in the presence of CsF, conditions reported for the *O*-alkylation of tetronic acids,[186] no reaction occurred. Finally, treatment of **81a** with methanol under Mitsunobu reaction conditions[187] gave the methylated product, which was found to be a separable 3:1 mixture of enol ethers (Scheme 2.17). HMBC correlations of both products indicated that the major one corresponded to methylation of the *C*(6)-ketone, and the *C*(8)-amide in the minor one. This result was unexpected, and it indicated that despite the higher stability of the *exo*-enol forms, the *endo*-enol ethers are formed preferentially. The same was observed when methylating alkene **96a**, which in this case was isolated as a 4:1 inseparable mixture of **104** and **104'**.



Scheme 2.17. Enol protection of acylated analogues.

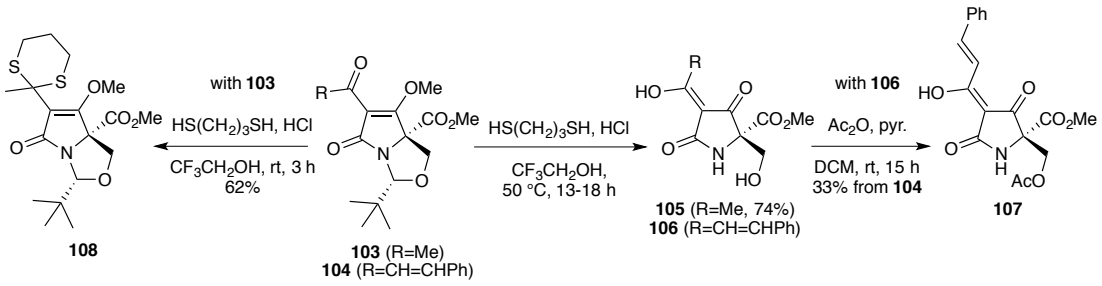
Comparison of the ^{13}C NMR spectra of the enol starting materials and the enol ether products (Table 2.7) supports their structures: *C*(9) is found at lower field in the enol ethers, which have a free carbonyl, the major product has a higher field *C*(8) compared to the enol starting material, where it is hydrogen bonded, and the chemical shift of *C*(6) is similar for the enol starting material and the minor product.

Table 2.7. Representative ^{13}C NMR signals for enol and enol ether analogues in $CDCl_3$.

	R=Me			R=CH=CHPh		
	81a*	**103**	**103'**	**96a***	**104**	**104'**
***C*(6)**	188.1	175.3	189.4	188.1	175.4	189.5
***C*(8)**	180.0	175.6	184.3	180.7	175.8	185.9
***C*(9)**	188.6	195.3	193.1	176.9	186.5	183.9

*Major tautomer.

With the protected enol, *N,O*-acetal deprotection of **103** was successful and alcohol **105** was obtained in 74% yield when using Corey-Richard's protocol with propanedithiol at 50 °C (Scheme 2.18). When the reaction was performed at room temperature, dithiane **108** was obtained instead. However, hydrolysis of alkene **104** proved to be more challenging. While the formation of **106** was indicated by LRMS and by the presence of dithiane **101** in the crude NMR spectrum, no product was recovered after attempted purification by *flash* column chromatography. This could be explained by a combination of factors: firstly, its high polarity and metal chelation, and secondly, its possible instability towards a retro-aldol reaction, which would be more favourable in **106** than in **105** due to further delocalisation into the alkenone-aromatic system. Indeed, purification of protected alcohol **107**, prepared by acetylation of the crude **106**, was successful.

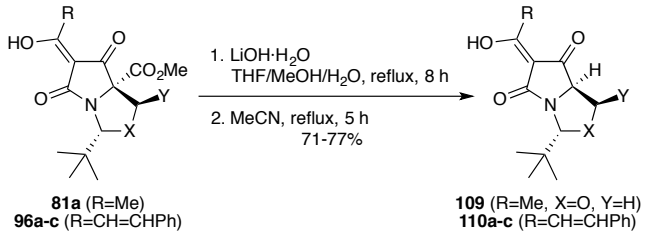


Scheme 2.18. *N,O*-Acetal deprotection of enol ethers **103** and **104**.

2.5.2 Decarboxylation

Acylated analogues **81a** and **96a-c** were hydrolysed with an excess of LiOH, and reflux of the crude acids in MeCN gave the decarboxylated products as single diastereomers. The *tert*-butyl group

probably again played an important role in the selectivity observed, as the alternative diastereomer would place this bulky group in the *endo*-side of the bicyclic system.

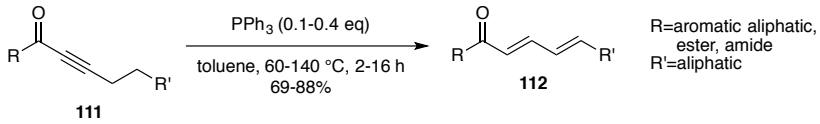


Scheme 2.19. Decarboxylation of acylated analogues **81a** and **96a-c**.

2.6 Side-chain elaboration: preparation of mimics of pramanicin

2.6.1 Grignard addition of aliphatic alkynes

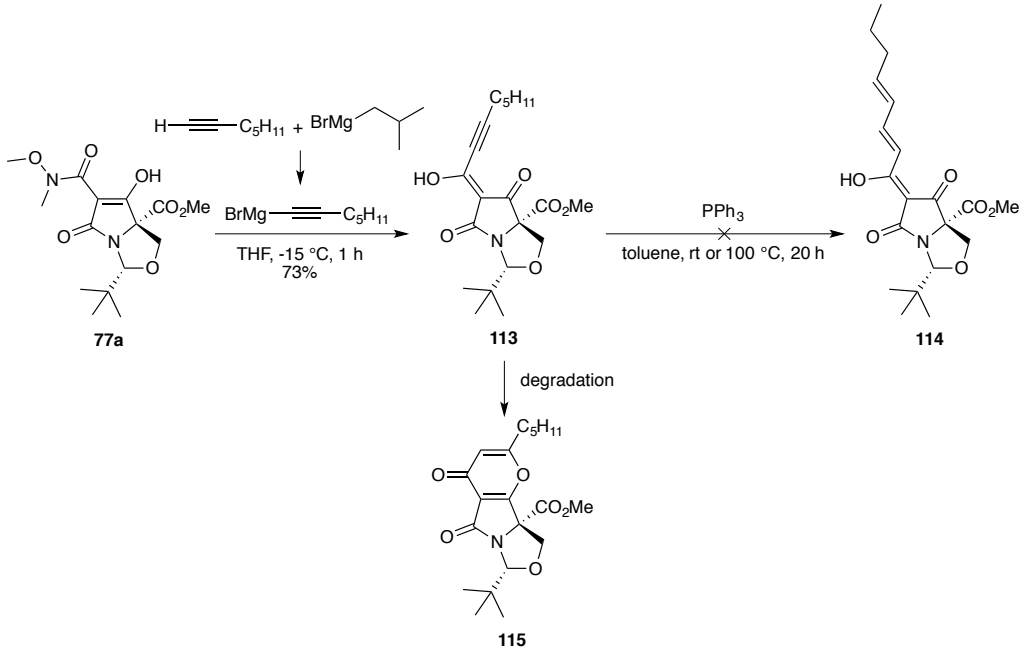
B. M. Trost and co-workers have reported that ynones **111** can be isomerised to the corresponding conjugated dienones **112** when heated with catalytic amounts of triphenylphosphine (Scheme 2.20).[188] This transformation is particularly efficient with aromatic ketones; with aliphatic ones slightly higher temperatures are needed, and esters and amides require the addition of acetic acid (0.5 eq).



Scheme 2.20. Isomerisation of ynones reported by B. M. Trost and co-workers.[188]

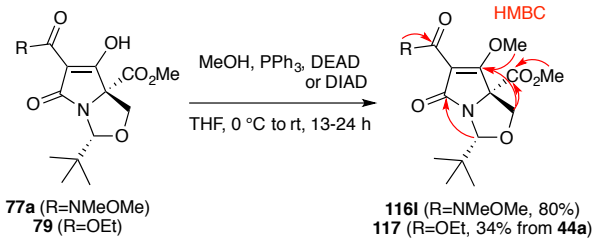
It was envisaged that Trost's procedure could be used to prepare dienone **114** (Scheme 2.21), and subsequent epoxidation would give simple mimics of pramanicin, bearing its acyl chain. Weinreb amide **77a** was reacted with heptynylmagnesium bromide, prepared *in situ* from 1-heptyne, to give **113** in 73% yield. Unfortunately, this alkyne was found to be highly unstable and it degraded to tricycle **115** within a few hours. This degradation, which had already been observed for analogues **97a-c**, probably proceeded at lower rates in the aromatic analogues due to conjugation of the alkyne with the phenyl group, which decreased its reactivity. When isomerisation of **113**

was attempted immediately after column purification or with the crude product, no desired dienone was formed, and only degradation product **115** was obtained.



Scheme 2.21. Unsuccessful preparation of dienone **114**.

In view of the low stability of **113**, probably involving an intramolecular attack of the *endo*-enol to the alkyne, enol protection was investigated. Attempted methylation of the crude alkyne under the previously described Mistunobu conditions failed to give the desired product, and a complex crude mixture was observed by NMR spectroscopy. Thus, protection of the Weinreb amide prior to Grignard addition seemed to be the way forward. The aforementioned protecting conditions yielded methylated Weinreb amide **116I** as a single isomer, with the enolate on $C(6)$ as indicated by HMBC correlations (Scheme 2.22). For the purpose of comparison, methylated ethyl ester **117** was also prepared, and it was also obtained as a single $C(6)$-enol ether. As shown in Table 2.8, the $C(6)$ ^{13}C NMR signal shifts to higher fields in the methylated products as expected, and $C(9)$ is observed slightly lower field in the enol derivatives, probably due to hydrogen bonding.



Scheme 2.22. Enol protection of Weinreb amide **77a** and ethyl ester **79**.

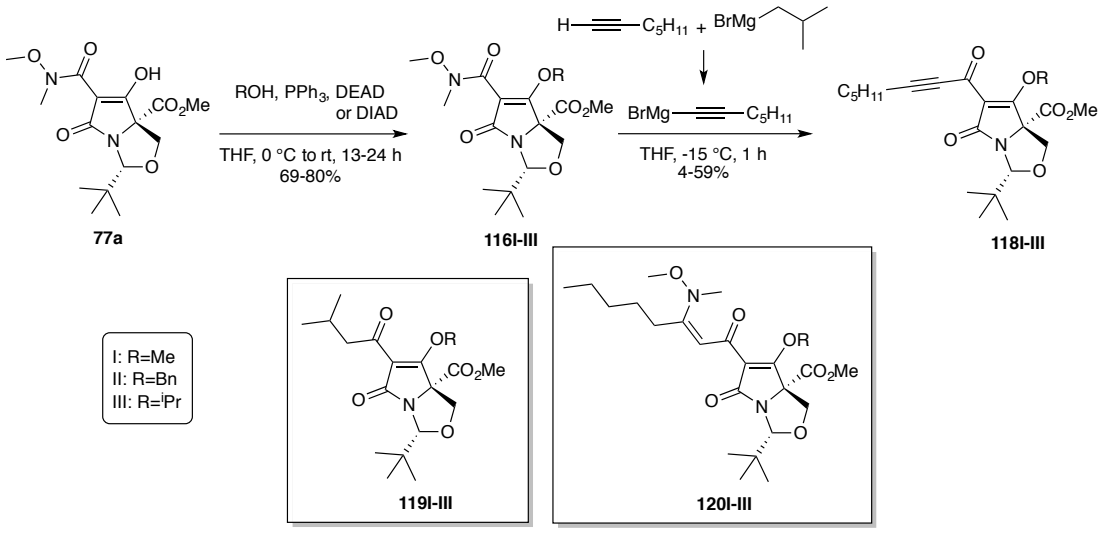
Table 2.8. Representative ^{13}C NMR (500 MHz, $CDCl_3$) signals for the enol and enol ether forms of Weinreb amide and ethyl ester tetramates.

	Weinreb amide		**Ethyl ester**	
	77a	**116I**	**79**	**117**
***C*(6)**	185.7	169.7	184.4	174.0
***C*(8)**	180.7	174.9	172.5	174.1
***C*(9)**	166.5	163.0	167.2	162.1

When **116I** was subjected to Grignard addition, alkyne **118I** was formed in extremely low yields owing to the formation of numerous by-products (Scheme 2.23): firstly, deprotection of the enol group to Weinreb amide **77a** and alkyne **113** (which could derive from either deprotection of **118I** or alkyne addition to **77a**); secondly, reaction of isobutylmagnesium bromide with Weinreb amide **116I** and thirdly, the formation of enamine **120I**. Even though the isolation of **120I** was unexpected, its formation during the reaction of Weinreb amides and alkynes had been observed by J. Nielsen[189] and A. Choudhury,[190] who actually failed to obtain any of the desired alkyne.

The formation of isobutyl ketone **119I** could be reduced, although not fully avoided, by reacting 1-heptyne with isobutylmagnesium bromide for a longer time. However, this change only increased the yield of the formation of **118I** to 27%, with the other side-products still being formed in significant amounts. Deprotection of the enol ether was likely to originate from attack of the alkynyl Grignard onto the methyl group, since the highly delocalised tricarbonyl system makes for a very good leaving group. It was envisaged that this deprotection could be impeded by employing a bulkier protecting group. Indeed, an improvement was observed with benzyl and isopropyl groups, but it was accompanied by a decrease in the yield of the initial protection step

(Table 2.9). With bulkier groups such as benzhydryl or *tert*-butyl, there was low or no conversion. The use of the isopropoxy enolate seemed like a good balance, where the protection could be achieved in acceptable yield and the Grignard addition proceeded with no detectable deprotection. The still relatively low yield was due to the formation of enamine **120III** as a major side-product, together with minor amounts of isobutyl **119III**.



Scheme 2.23. Preparation of alkynes **118I-III** with protected Weinreb amides **116I-III**.

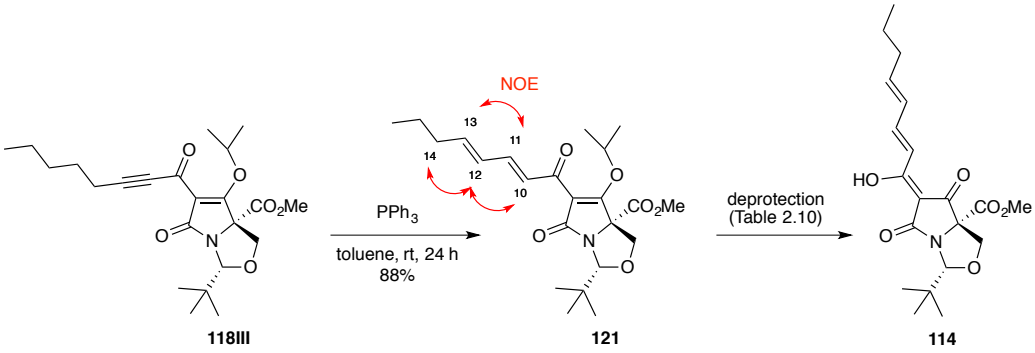
Table 2.9. Screen of enol protecting groups to optimise the formation of alkynes **118I-III**.

R	Protection		Grignard addition	
	Time	Yield	Yield	Side-products
Me	13 h	80%	27%	**77a**, **113**, **119**, **120**
Bn	18 h	71%	35%	**77a**, **113**, **119**, **120**
^{i}Pr	24 h	69%	59%	**119**, **120**
$CHPh_2$	24 h	21%	-	-
^{t}Bu	24 h	Recovered starting material	-	-

2.6.2 Isomerisation and deprotection

With alkyne **118III** in hand, isomerisation to the dienone occurred in high yields when using stoichiometric amounts of triphenylphosphine at room temperature for 24 h. Diene **121** was obtained as a single (*E*,*E*)-regioisomer, evidenced by the large coupling constants ($J_{10,11}$ = $J_{12,13}$ =

15.0 Hz) and further confirmed by an NOE experiment, which showed strong correlations between *H*(10)-*H*(12), *H*(12)-*H*(14) and *H*(11)-*H*(13), as indicated in Scheme 2.24.



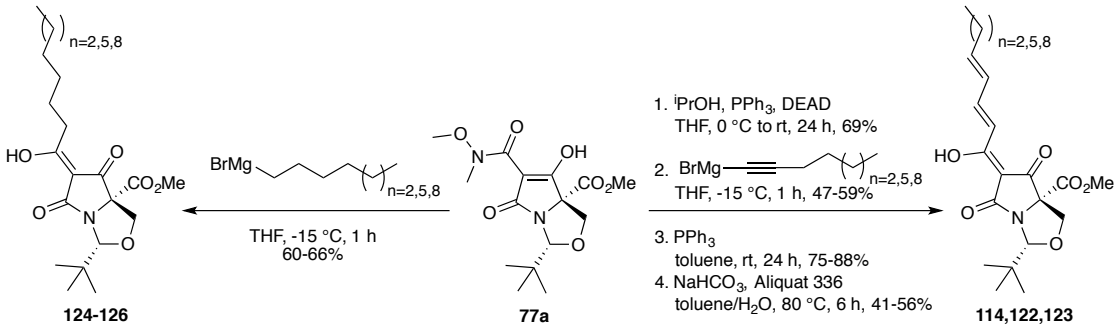
Scheme 2.24. Isomerisation and deprotection to dienone **114**.

Table 2.10. Attempted conditions for the enol deprotection of **121**.

Entry	Conditions	Temperature	Yield [%]	Outcome
1	BBr_3 (1.0 eq) DCM, 1 h	rt	37	Crude NMR shows aldehyde peak.
2	BBr_3 (0.33 eq) DCM, 30 min	rt	44	Crude NMR shows small aldehyde peak.
3	BBr_3 (1.0 eq) DCM, 30 min	-78 °C	15	Some starting material left.
4	BCl_3 (1.0 eq) DCM, 30 min	rt	20	Crude NMR shows aldehyde peak.
5	$NaHCO_3$ (1.2 eq), Aliquat 336 toluene/H_2O, 18 h	rt	0	Recovered starting material.
6	$NaHCO_3$ (10 eq), Aliquat 336 toluene/H_2O, 19 h	rt	0	Recovered starting material.
7	$NaHCO_3$ (1.2 eq), Aliquat 336 toluene/H_2O, 18 h	80 °C	37	50% recovered starting material.
8	$NaHCO_3$ (1.2 eq), $^tBu_3BnNCl$ toluene/H_2O, 8 h	80 °C	0	Recovered starting material.
9	$NaHCO_3$ (2.5 eq), Aliquat 336 toluene/H_2O, 18 h	80 °C	41	30% recovered starting material.
10	$NaHCO_3$ (20 eq), Aliquat 336 toluene/H_2O, 6 h	80 °C	56	Full conversion.

Enol deprotection of **121** with BBr_3 furnished tetramate **114** in low yields. The crude NMR spectrum showed the presence of a low field aldehyde peak, which probably corresponded to pivaldehyde and indicated that *N*,*O*-acetal deprotection occurred under these conditions. This acetal hydrolysis took place only before removal of the isopropoxy group, as it had been observed (Section 2.5.1) that free acyltetramic acids are stable towards hydrolysis. Thus, the reaction was trialled at a lower temperature and with the weaker Lewis acid BCl_3, but no improvement was observed (Table 2.10).

In an attempt to optimise the reaction by preventing acetal hydrolysis, enol deprotection was examined with $NaHCO_3$ under phase-transfer catalysis conditions. Gratifyingly, when using an excess of the base with stoichiometric amounts of Aliquat 336 at 80 °C, the reaction was completed after 6 h and NMR analysis of the crude product showed the desired product with no signs of acetal hydrolysis. Unfortunately, column chromatography purification was necessary to remove the remaining Aliquat 336, which notably decreased the yield of the reaction due to the high affinity of the tetramate for silica.



n	Yield [%]
2	60
5	61
8	66

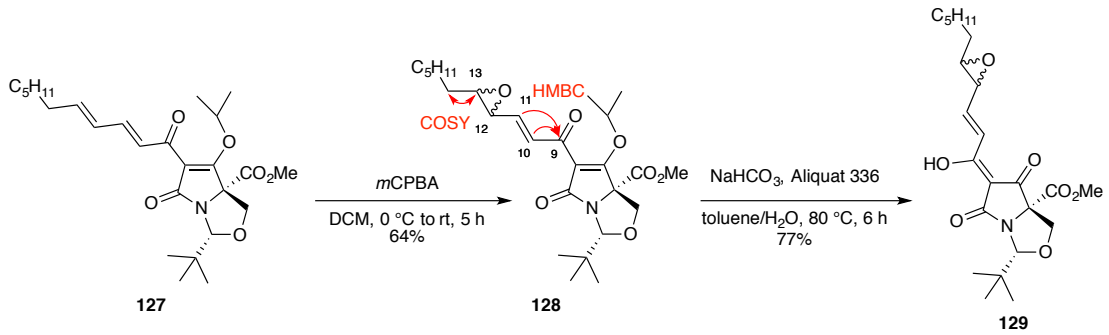
n	Yield [%]		
	Grignard	Isomerisation	Deprotection
2	59	88	56
5	54	81	48
8	47	75	41

Scheme 2.25. Preparation of dienone-tetramic acids **114**,**122**,**123** and corresponding saturated analogues **124**-**126**.

This sequence provided a reliable route to dienone-tetramic acids, and it was used to prepare analogues with different chain lengths (Scheme 2.25), which will be compared in terms of biological activity. Saturated derivatives **124-126**, readily available from Grignard reaction with Weinreb amide **77a**, will also be assayed to examine the role of the diene functionality.

2.6.3 Epoxidation of dienoyl analogue

Epoxidation of dienone **127** with *m*CPBA afforded epoxy-enone **128** regioselectively, via reaction of the more electron rich remote double bond, as a 1:1 mixture of diastereomers (Scheme 2.26). The position of the epoxide was indicated by COSY correlations between one of the epoxide protons and the aliphatic region, and HMBC correlations between the carbonyl and the olefinic protons, as indicated in Scheme 2.26. The resemblance between the spectroscopic data of epoxide **128** and the one reported for pramanicin further supports the assignment (Figure 2.6).



Scheme 2.26. Epoxidation of dienoyl **127** and enol deprotection.

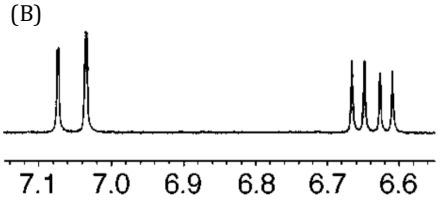
Direct epoxidation of tetramate **122** was slower, probably due to bigger delocalisation of the dienone moiety. Moreover, purification of epoxide **129** was unsuccessful given that, firstly, when the crude product was washed with a pH 7.5 buffer to remove *m*-chlorobenzoic acid, the tetramate also dissolved in the aqueous layer via deprotonation of the enol group. Secondly, both the product and the acid had the same retention factor when columned in silica.

Enol deprotection of **128** provided tetramate **129**, which represents a simple mimic of pramanicin bearing a similar epoxy-enone acyl chain linked to a tetramic acid core.

(A)

Atom	Pramanicin (400 MHz, CD_3OD)	128 (500 MHz, $CDCl_3$)
10	7.05 (dd, *J* 15.6, 0.7)	7.16 (dd, *J* 15.6, 0.5), 7.23 (dd, *J* 15.6, 0.5)
11	6.64 (dd, *J* 15.6, 7.0)	6.59 (dd, *J* 15.6, 8.0), 6.65 (dd, *J* 15.6, 7.3)
12	3.31 (ddd, *J* 7, 1.9, 0.7)	3.28 (dd, *J* 7.7, 1.8)
13	2.93 (ddd, *J* 6.2, 4.9, 1.9)	2.95 (m)

(B)



(C)

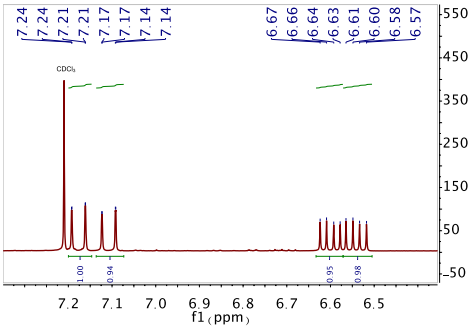
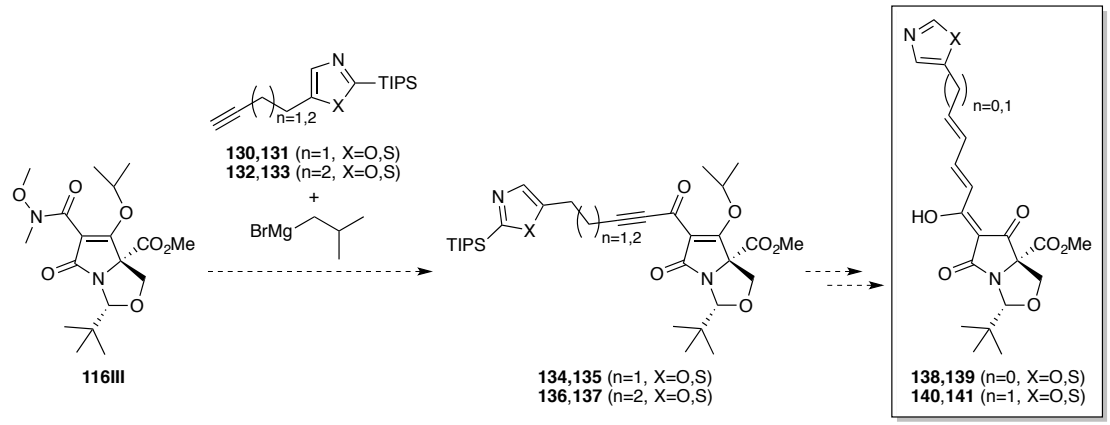


Figure 2.6. Comparison of spectroscopic data of the natural product pramanicin[74] and synthetic compound **128**. (A) ^{1}H NMR olefin and epoxide signals; ^{1}H NMR olefinic region for (B) pramanicin (400 MHz, $CDCl_3$)[76,77] and (C) **128** (500 MHz, $CDCl_3$).

2.7 Side-chain elaboration: preparation of mimics of oxazolomycin

It was of interest to extend the scope of the method designed for the preparation of dienone-tetramates, to further develop the acyl chain of the analogues. Pramanicin mimics **114**,**122**-**126**,**129** bear highly hydrophobic acyl chains, which could increase plasma protein binding and decrease their activity *in vivo*.[135] Hence, the introduction of more polar side-chains was of interest, and it could be achieved through the addition of heterocycles. In particular, oxazole **140** (Scheme 2.27) would constitute a simple mimic of the left-hand segment of oxazolomycin, and the synthesis could be extended to derivatives with a shorter side-chain and with a thiazole ring (**138**,**139**,**141**). These analogues could be accessed using the described protection-Grignard-isomerisarion-deprotection sequence, but in this case alkynes **130-133** were not commercially available.

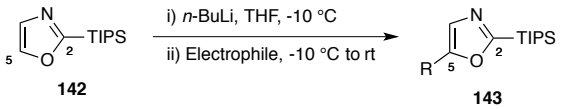


Scheme 2.27. Proposed synthesis of oxazolomycin mimics **138**-**141**.

2.7.1 Synthesis of oxazole/thiazole-alkynes

R. A. Miller *et al.* have reported that *C*(5)-substituted oxazoles **143** can be accessed from *C*(2)-protected oxazole **142**, which is first metalated with *n*-BuLi and then reacted with a variety of electrophiles as indicated in Table 2.11. They have also reported that oxazole can be *C*(2)-silylated with TBS, trimethylsilyl (TMS) and triisopropylsilyl (TIPS) triflate, but only the TIPS-protected oxazole **142** is stable to water and column chromatography.[191] Interestingly, when using TIPSCl instead of TIPSOTf, only *O*-silylated ring-open oxazole is formed.

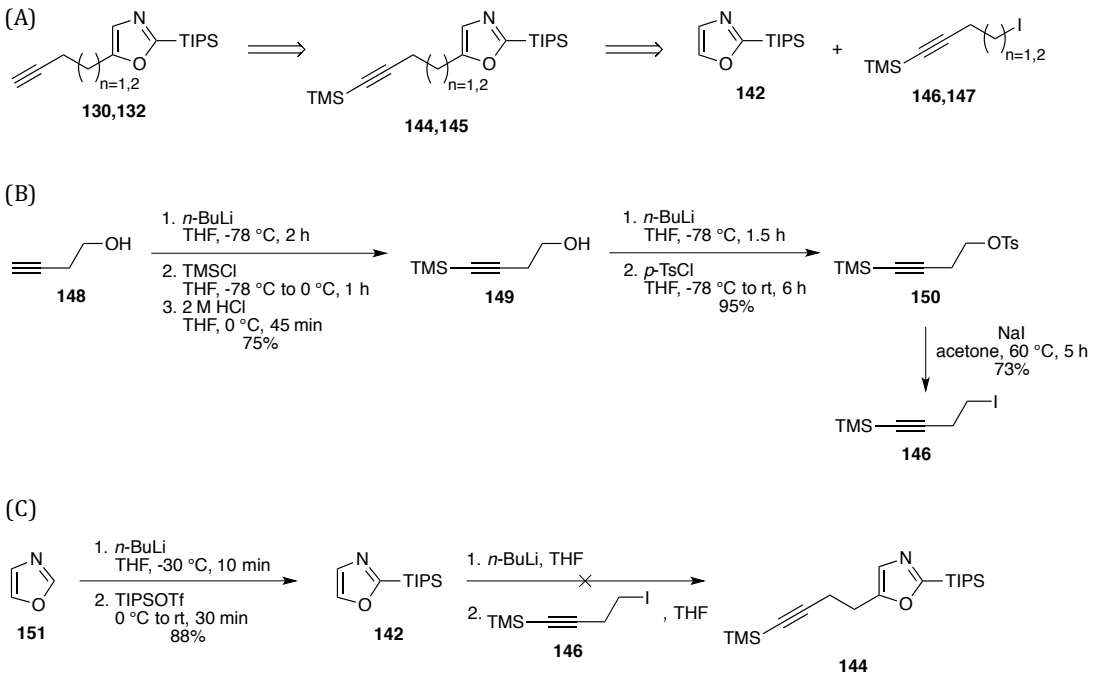
Table 2.11. Lithiation and reaction of TIPS-protected oxazoles reported by R. A. Miller *et al.*[192]



Entry	Electrophile	R	Yield [%]
1	MeI	Me	86
2	EtI	Et	94
3	benzaldehyde	CHOHPh	84
4	TMSCl	TMS	89
5	TBSCl	TBS	93
6	benzylbromide	CH_2Ph	79
7	tributyltinchloride	$Sn(Bu)_3$	75
8	allyl bromide	allyl	75
9	benzonitrile	COPh	86
10	DMF	CHO	80

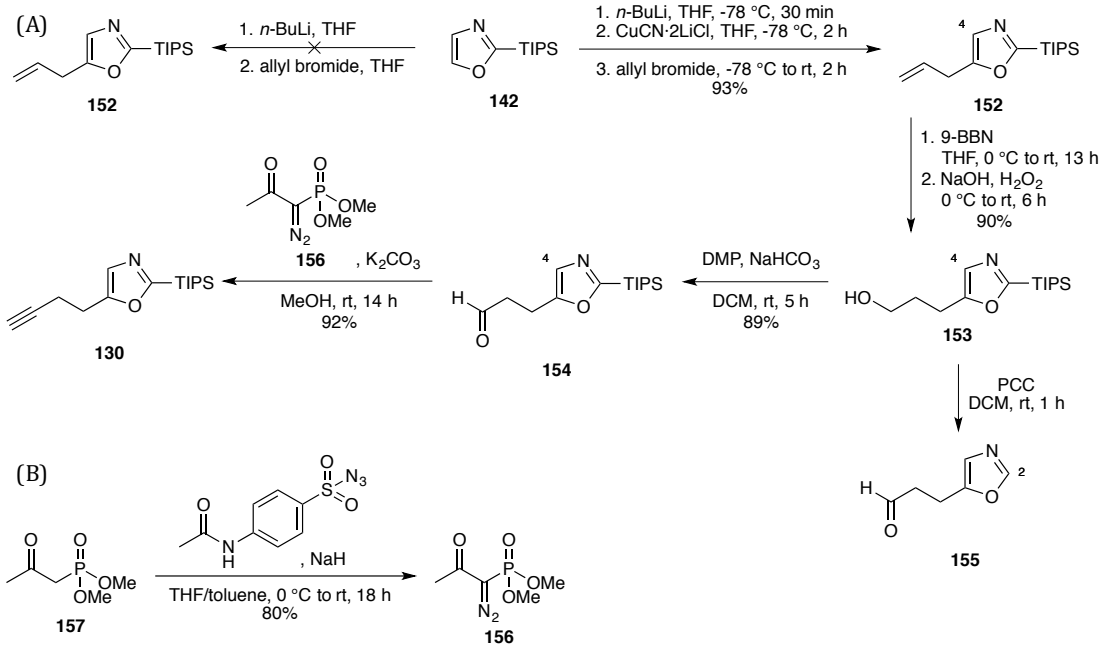
Therefore, it was initially thought that alkynes **130,132** could be prepared by alkylation of the *C*(2)-protected oxazole **142** with iodides **146,147** (Scheme 2.28 A). The TMS group in the terminal alkyne, orthogonal to the TIPS group in oxazole, could then be removed to give alkynes **130**,**132**.

Starting from 3-butyn-1-ol, alkyne protection, alcohol activation and displacement with NaI gave iodide **146**, but all attempts to alkylate the *C*(5)-lithiated oxazole, using different temperatures and reaction times, were unsuccessful and only starting material was recovered (Scheme 2.28 B,C). In an alternative synthetic route towards the synthesis of alkynes **130,132**, it was decided to react protected oxazole **142** with an electrophile that had already been employed by Miller *et al.* If allyl bromide was used (Table 2.11, Entry 8), succeeding manipulation of the alkene could give access to alkynes. Surprisingly, after treatment of **142** with *n*-BuLi at -10 °C followed by allyl bromide as reported, starting material was recovered. Again, the use of different temperatures and reaction times did not change the outcome.



Scheme 2.28. (A) Proposed synthesis of alkynes **130**,**132**; (B) preparation of iodide **146**; (C) unsuccessful alkylation of TIPS-protected oxazole.

Following the apparent lack of reactivity of the *C*(5)-lithiated oxazole towards S_N2 reactions with alkyl and allyl halides, the alternative S_N2' reaction was tested through transmetallation to the cuprate. Satisfyingly, treatment of oxazole **142** with *n*-BuLi followed by addition of a freshly prepared solution of CuCN·2LiCl in THF and allyl bromide gave alkene **152** in 93% yield, together with 7% remaining starting material which could not be separated by column chromatography (Scheme 2.29 A).

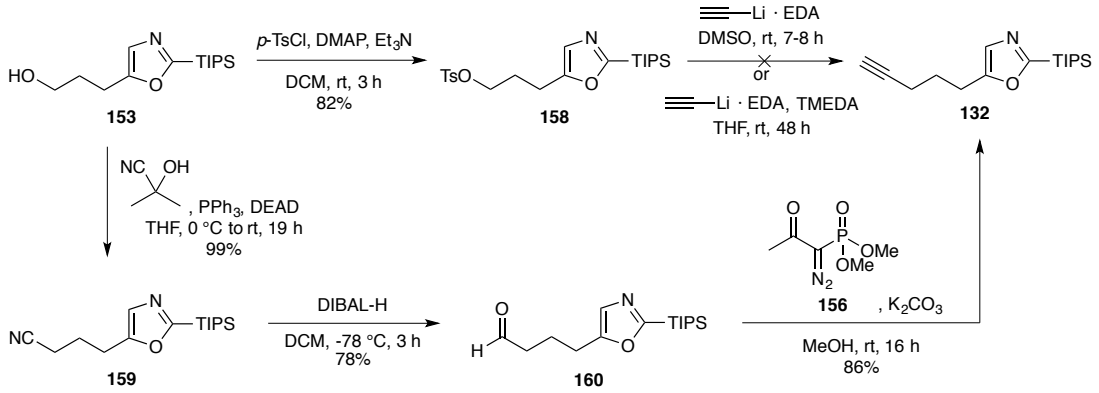


Scheme 2.29. (A) Successful *C*(5)-substitution of oxazole **142** and preparation of alkyne **130**; (B) preparation of the Ohira-Bestmann reagent **156**.

Hydroboration of alkene **152** provided alcohol **153** in high yield. Oxidation with PCC gave the volatile TIPS-deprotected aldehyde **155**, as indicated by the characteristic *H*(2) at 7.78 ppm in the crude ^{1}H NMR spectrum, which was found at a lower field than *H*(4) (6.81-6.83 ppm for compounds **152-154**). Alternatively, with Dess-Martin periodinane (DMP) the desired aldehyde **154** was formed in 69% yield, together with 10% of deprotected aldehyde **155**. TIPS-deprotection was probably catalysed by the acetic acid formed during the reaction; hence the reaction was performed using 1 equivalent of sodium bicarbonate, which increased the yield to 89%. Finally, aldehyde **154** was converted into alkyne **130** using the Bestmann reagent **156** in good yield. The Bestmann reagent was prepared following a reported one-step procedure[193] which utilises

4-acetamidobenzenesulfonyl azide as the diazo transfer reagent instead of the more hazardous tosyl azide (Scheme 2.29 B).

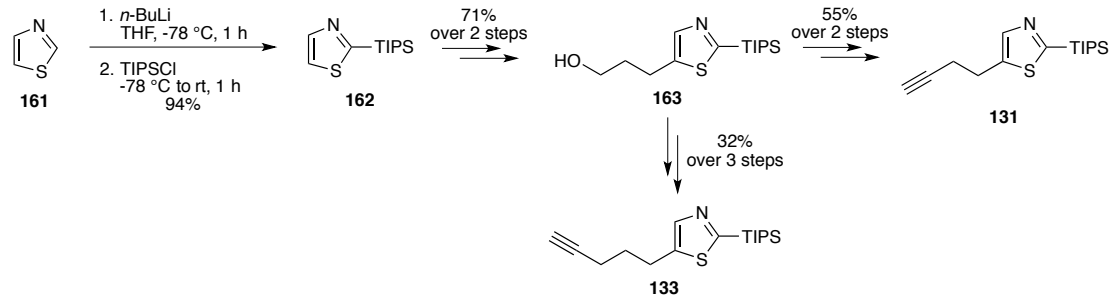
For the preparation of the longer alkyne **132**, alcohol **153** was activated as the tosylate in an attempt to displace it with lithium acetylide (Scheme 2.30). However, with 1.5 equivalents of the acetylide in DMSO only traces of product were isolated, and with an excess no product was observed. The reaction was also trialled adding TMEDA in THF, but in this case mainly starting material was recovered.



Scheme 2.30. Preparation of alkyne **132**.

Since the use of the Bestmann reagent had proven to be valuable for the synthesis of alkyne **130**, it was expected that a similar synthesis could be adapted for **132**. For the preparation of the longer chain aldehyde **160**, the additional carbon could be added through incorporation of a nitrile group, which would then be reduced to the aldehyde. The Mitsunobu-Wilk procedure,[194] which employs Mitsunobu conditions with acetone cyanohydrin as the acidic component and the source of cyanide ion, proved to be very successful for the conversion of alcohol **153** into nitrile **159**. Subsequent reduction with DIBAL-H and treatment with Bestmann reagent yielded alkyne **132**.

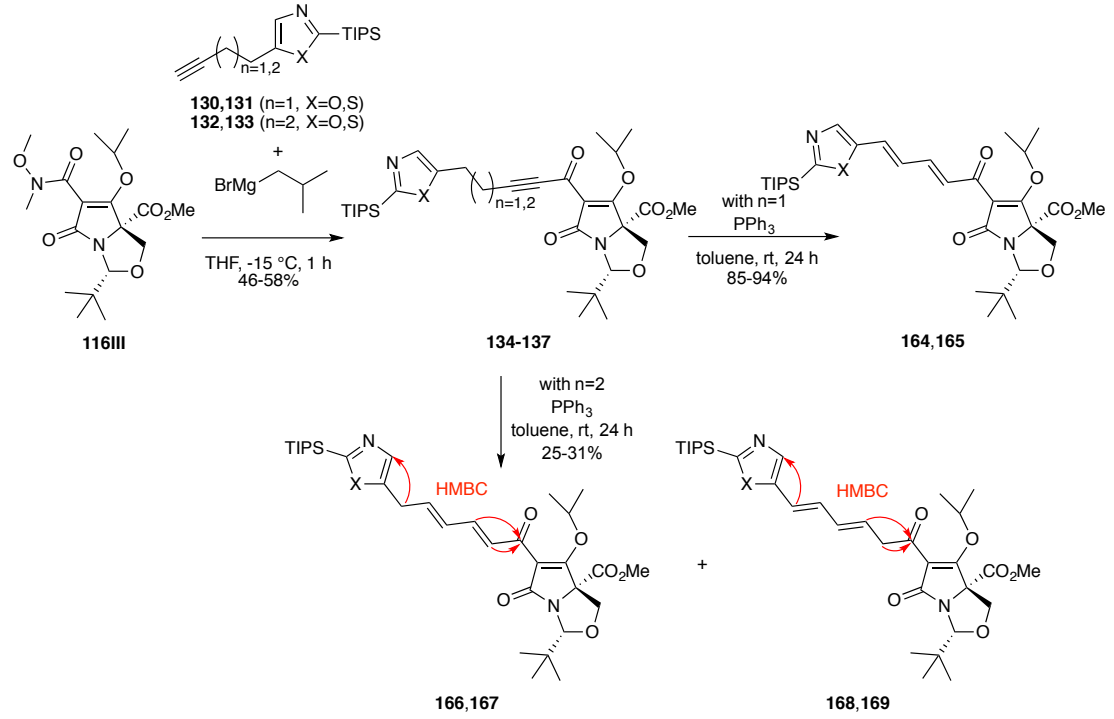
The same synthetic sequence could be used to access alkynes **131** and **133** from TIPS-protected thiazole **162**, which was prepared in this case reacting the *C*(2)-lithiated thiazole with TIPS chloride (Scheme 2.31).



Scheme 2.31. Preparation of alkyne-thiazoles **131** and **133**.

2.7.2 Introduction of heterocyclic alkynes to the tetramate core

With alkynes **130-133** in hand, reaction with Weinreb amide **116III** gave the alkynoyl analogues **134-137**, with yields of 46-58%. As observed before, the enamine side-product, which was formed in up to 1:1 ratio with the desired product, accounts for the low yields.



Scheme 2.32. Coupling of alkynes **130-133** with Weinreb amide **116III** and isomerisation to dienes.

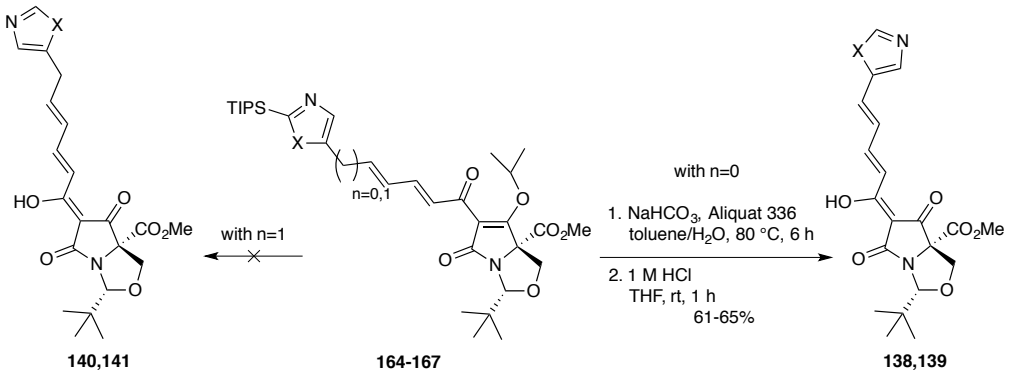
Interestingly, isomerisation of the alkynes was achieved in high yields where n=1 (Table 2.12), but where n=2 a 1:1 mixture of isomers was obtained. HMBC correlations indicated that the undesired isomer **168,169** was the by-product of the reaction, as indicated in Scheme 2.32. The

chemical shift of the ketone carbonyl was also descriptive, which was found at higher field for the desired isomer as expected for an alkenyl ketone.

Table 2.12. Yields of the Grignard addition of alkynes **130**-**133** to Weinreb amide **116III** and isomerisation to dienes **164**-**167**.

n	X	Yield [%]	
		Grignard	Isomerisation
1	O	58	94
1	S	58	85
2	O	54	31
2	S	46	25

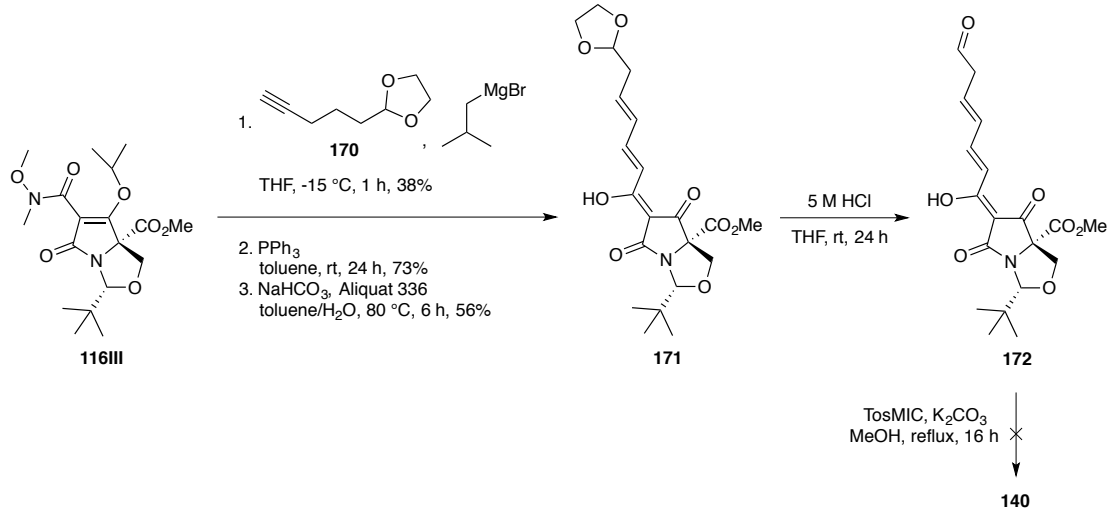
Deprotection of the isopropoxy group with $NaHCO_3$ and Aliquat 336, and subsequent TIPS oxazole/thiazole hydrolysis with 1 M HCl in THF for 1 h yielded the target compounds **138**,**139**, which were isolated after column chromatography and subsequent acid wash in 61-65% yield (Scheme 2.33). Disappointingly, the compounds bearing the additional -CH_2- group, analogous to oxazolomycin, experienced degradation when the enol was deprotected. The crude ^{1}H NMR spectrum showed complex broad peaks, with no desired mass observed by LRMS analysis, and attempted column chromatography did not provide any of the desired product.



Scheme 2.33. Enol deprotection of dienes **164**-**167**.

An alternative approach to the synthesis of **140** would be to deprotect the enol group before addition of the oxazole ring. A. M. van Leusen *et al.* reported that oxazoles can be prepared through condensation of tosylmethyl isocyanide (TosMIC) with an aldehyde under basic conditions.[195]

Thus, it was hoped that **140** could be accessed via a late-stage preparation of the heterocycle from condensation of aldehyde **172** with TosMIC (Scheme 2.34).



Scheme 2.34. Attempted synthesis of oxazole **140** via late-stage introduction of the heterocycle.

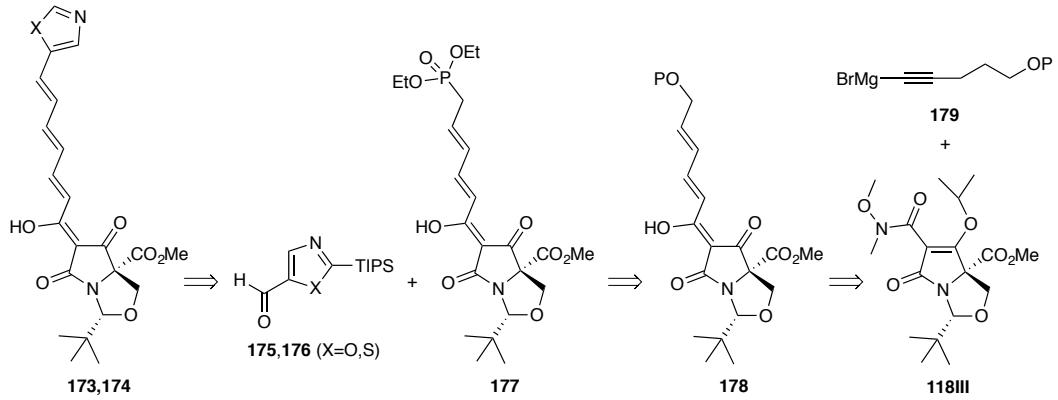
Coupling of alkyne **170**, available from 5-hexyn-1-ol in two steps,[196] to Weinreb amide **116III** followed by isomerisation and enol deprotection gave dioxolane **171**. Upon treatment with 5 M HCl for 24 h, aldehyde **172** was obtained as indicated by the characteristic low field aldehyde peak in the ^{1}H NMR spectrum and the desired mass by LRMS, but it was not further purified. Unfortunately, when the aldehyde was treated with TosMIC and K_2CO_3 overnight only broad peaks were observed in the crude NMR spectrum with no characteristic oxazole signals, and again no desired product was detected by LRMS. It would appear that either aldehyde **172** degraded under these conditions, or **140** was formed but it was unstable.

In fact, the instability of inthomycins (natural products containing the left-hand segment of oxazolomycin, see Section 1.5.2) has been documented,[197,198] and this could explain the difficulty, or impossibility, to prepare analogues **140**,**141**. Additionally, it could be that conjugation with the tetramate core further destabilises these structures. Therefore, it could be reasoned that similar systems, but where the tetramic acid has been reduced at $C(6)$ or the $C(7)$-position oxidised, analogous to the pyrrolidinone core of pramanicin, might present enhanced stabilities. The

synthetic efforts towards reduced tetramic acids, including the reduction of *C*(7)-acylated systems, is described in Chapter 3.

2.7.3 Attempted preparation of trienes

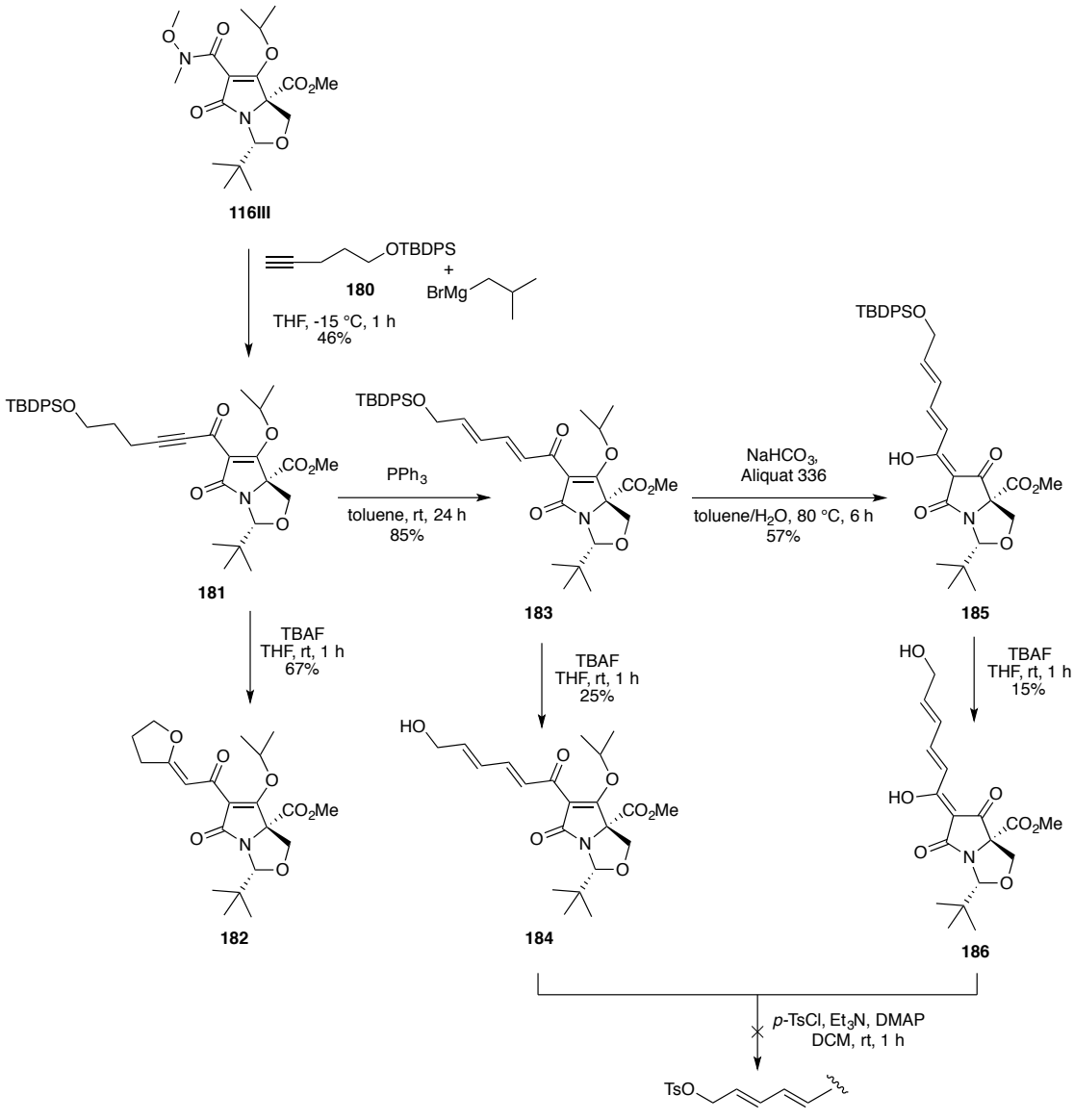
With a view to expanding the library of heterocyclic analogues, and having closer mimics to the left-hand fragment of oxazolomycin, the synthesis of trienes **173**,**174** was examined. Scheme 2.35 shows the retrosynthetic plan, where the third double bond would be introduced via HWE olefination of phosphonate **177** with aldehydes **175**,**176**. Oxazole **175** can be accessed from TIPS-protected oxazole **142** and *N*,*N*-dimethylformamide (DMF) (Table 2.11, Entry 10),[192] and a similar method should also be applicable to thiazole **176**. Preparation of protected alcohol **178** using the developed methodology, and further deprotection, activation and displacement to **177** seemed to be amenable.



Scheme 2.35. Retrosynthetic approach to heterocyclic trienes.

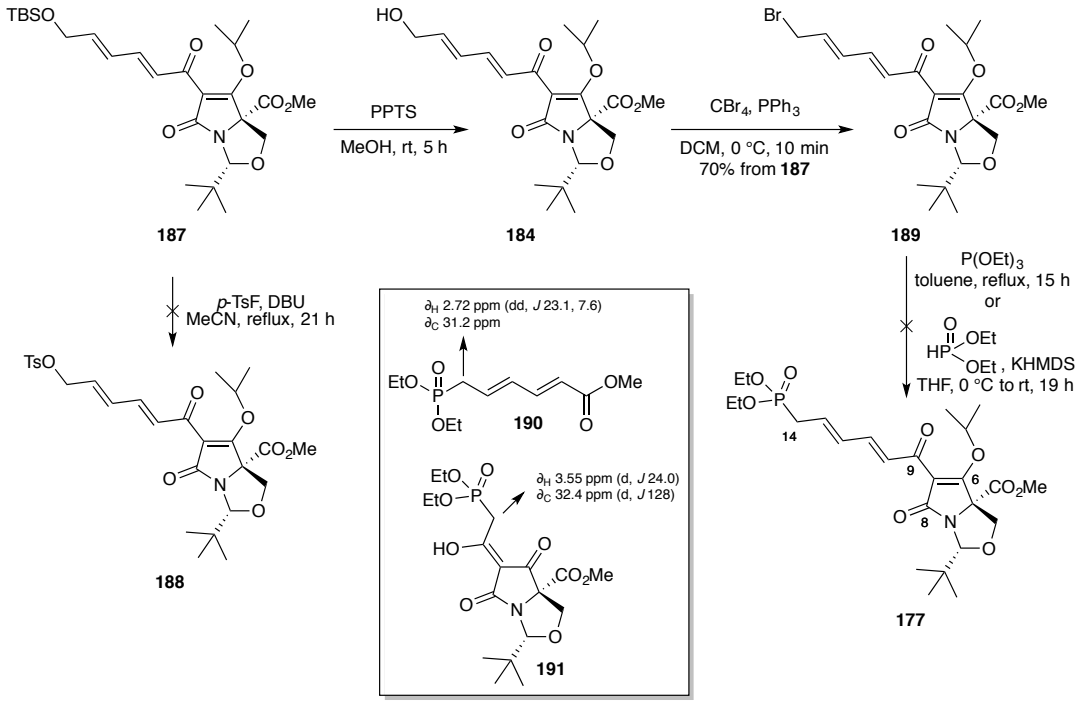
Silyl ether **180**, prepared from pent-4-yn-1-ol, was coupled with Weinreb amide **116III** (Scheme 2.36). Interestingly, silyl deprotection of **181** with tetrabutylammonium fluoride (TBAF) resulted in instant cyclisation to dihydrofuran **182**. Instead, isomerisation of the alkyne followed by silyl deprotection gave full conversion to the desired alcohol. However, after column purification to remove the TBAF impurities and TBDPSOH, the isolated yield of **184** was only 25%. Low and not reproducible yields were also observed for the preparation of alcohol **186**, where the enol group had been previously hydrolysed. The low efficiency of this transformation

probably arises from the low stability of these alcohols, which could undergo an intramolecular cyclisation similarly to the formation of **182**. More evidence to this assumption is that, when trying to tosylate these alcohols with triethylamine, tosyl chloride and DMAP, upon addition of the base there was a pronounced colour change from yellow to dark orange-brown. After 1 h, thin layer chromatography (TLC) indicated complete consumption of the starting material, but no desired product was observed by LRMS. The crude NMR spectrum exhibited the characteristic tetramate *H*(2) singlet and *H*(4) doublets, but the alkene signals seemed to have shifted and broadened; the products obtained after column chromatography could not be identified.



Scheme 2.36. Preparation of alcohols **184** and **186** and unsuccessful tosylation.

V. Gembus and co-workers have reported that silyl ethers can be interconverted into tosylates using *p*-toluenesulfonyl fluoride and catalytic amounts of DBU, and this method can be applied to TMS, triethylsilyl (TES) and TBS ethers.[199] Given the instability of alcohols **184** and **186**, direct conversion of the silyl ether into a leaving group was a promising alternative. For this reason, TBS ether **187** (Scheme 2.37) was prepared and treated with *p*-TsF and DBU as reported, but only starting material was recovered. However, deprotection of **187** to the alcohol and removal of the acid via work-up gave crude alcohol **184** which was pure enough to be used in the following step. This gave access to more material and conversion to bromide **189** could be investigated. With PBr_3, bromide **189** was formed together with other unidentified products, but with the Appel reagent[200] (triphenylphosphine/tetrabromomethane) the bromide was isolated in 70% yield over the two steps, with no significant amounts of side-products.



Scheme 2.37. Attempted preparation of phosphonate **177** and reported examples of related phosphonate systems.[154,201]

For the conversion of bromide **189** into phosphonate **177**, the use of Arbuzov reaction conditions[202,203] (excess of triethyl phosphite under refluxing toluene) failed to give any of the desired product, with only degradation observed by NMR analysis. Instead, displacement of the

bromide with diethyl phosphite and KHMDS (with NaH, a big excess of base was needed to reach full conversion) gave, after column purification, a 2:1 mixture of products which showed the desired mass by HRMS analysis. However, careful NMR analysis revealed that none of the products belonged to phosphonate **177**. The major product showed chemical shifts of *H*(14)s (5.17, 5.28 ppm) and *C*(14) (117.9 ppm) which were significantly lower field than expected (Scheme 2.37 shows the chemical shifts of related systems[154,201]). A ^{1}H-^{31}P HMBC experiment indicated that the phosphorous had stronger correlations to olefinic protons than to *H*(14)s. Additionally, only three low field carbon signals were observed, corresponding to *C*(6), *C*(8) and CO_2CH_3, and when enol deprotection of the synthesised phosphonate was attempted with $NaHCO_3$/Aliquat 336, only starting material was recovered. It would seem that an intramolecular reaction involving the *C*(9)-carbonyl could have occurred, and the *H*(14)s could be attached to the oxygen atom. Unfortunately, it was not possible to determine the exact structure. When HWE olefination was attempted with the unidentified product, treating it with valeraldehyde and any of the bases potassium *tert*-butoxide,[154] KHMDS[204] or LDA,[205] the crude NMR only showed broad peaks, probably arising from degradation.

2.8 Summary of Chapter 2 and future work

In this chapter, it was shown that the *C*(7)-position of tetramic acids can be functionalised with esters, amides and ketones to prepare interesting, and synthetically challenging, tricarbonyl structures. The former could be introduced directly into the tetramate core, using the required chloroformate together with stoichiometric amounts of DMAP. Amides had to be introduced before Dieckmann cyclisation, treating malonamide **43** with the corresponding carbamic chloride, followed by cyclisation with DBU. Finally, the synthesis of Weinreb amide **77** and subsequent reaction with Grignards allowed the preparation of a small library of alkyl, aryl, alkenyl and alkynyl keto-derivatives.

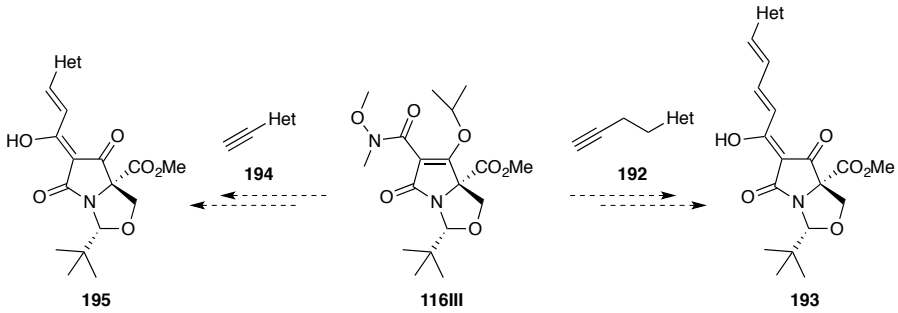
^{13}C NMR analysis of these tricarbonyl scaffolds revealed that the main tautomeric form of the ketone analogues is *Z*-*exo*-enol, in some cases with small contributions of the *E*-*exo*-enol, while esters and amides exist mainly as the *C*(6)-*endo*-enol. Also, XPS analysis indicated that these systems can chelate traces of calcium found in commercial silica gel.

The tetramate core of the acylated analogues was modified via hydrolysis of the *N*,*O*-acetal and decarboxylation of the methyl ester. Moreover, the acyl chain was elaborated to mimic key features of the natural products pramanicin and oxazolomycin. Dienoyl structures were prepared, accessible from isomerisation of the alkynone analogues. Due to the instability of these, the enol group in the tetramate core was initially protected, which was achieved under modified Mitsunobu conditions. Regioselective epoxidation furnished a mimic bearing the side-chain of pramanicin.

Furthermore, the lipohilicity of these compounds was decreased by preparing analogues with heterocycles in the acyl chain. Oxazole and thiazole alkynes were synthesised and introduced into the tetramate core to prepare simple mimics of the left-hand segment of oxazolomycin. However, preparation of some of these compounds was unsuccessful due to degradation.

In short, a range of *C*(7)-acylated analogues with a wide variety of functionalities, some with close features to biologically-active natural products, were synthesised. The antibacterial and anticancer properties of these compounds were evaluated and they are described in Chapter 5.

Further elaboration of these systems was thwarted by the instability of some of the intermediates. Future efforts could focus on the successful oxazole and thiazole compounds, to prepare analogues **193** with a wider range of heterocycles (Scheme 2.38). Also, the simpler alkynes **194** could be prepared and introduced to the tetramate core, followed by partial reduction to alkenones **195**.



Scheme 2.38. Proposed future work for Chapter 2.

CHAPTER 3: REDUCTION OF THE TETRAMATE CORE

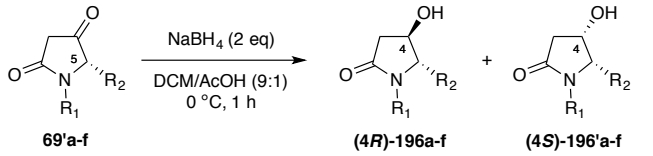
Reduced tetramic acids, or 3-hydroxypyrrolidinones, are found in several natural products possessing interesting biological activities. The stereoselective reduction of the tetramate core is key to the preparation of mimics of such compounds. This chapter compares the diastereoselective reduction of tetramates derived from the three systems serine, cysteine and threonine. It also describes the challenges encountered during the attempted reduction of *C*(7)-acyltetramic acids, which was achieved under hydrogenation conditions. Some content in this chapter has been reproduced from Josa-Culleré, L.; Moloney, M. G.; Thompson, A. L. *Synlett* **2016**, *27* (11), 1677, copyright (2016) Georg Thieme Verlag KG.

3.1 Introduction

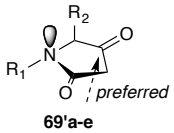
3.1.1 Stereoselective reduction of tetramic acids

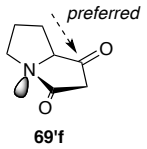
Poncet and co-workers[206] described the reduction of tetramic acids **69'a-f** with sodium acetoxyborohydride generated *in situ* (Scheme 3.1). Studying several substrates, with different *N*- and *C*(5)-substituents, they found that hydride attack to the carbonyl group took place *anti*- to the nitrogen lone pair, presumably due to repulsive electrostatic interactions. In the case of analogues **69'a-e**, calculation of the lowest energy conformation showed that the nitrogen atom is non-planar, placing the *N*-R_1 on the opposite site to R_2. Bulkier R_1 substituents increase the pyramidalisation of the nitrogen, accounting for the bigger diastereoselectivity towards the 4*S* alcohol.

In the case of bicyclic derivative **69'f**, the reduction proceeded with a reversal of stereoselectivity, giving the 4*R* alcohol mainly. Hydride attack took place from the more hindered *endo*-face, led again by stereoelectronic effects; however, in this case the bicyclic structure places the nitrogen lone pair on the opposite site of the ring.



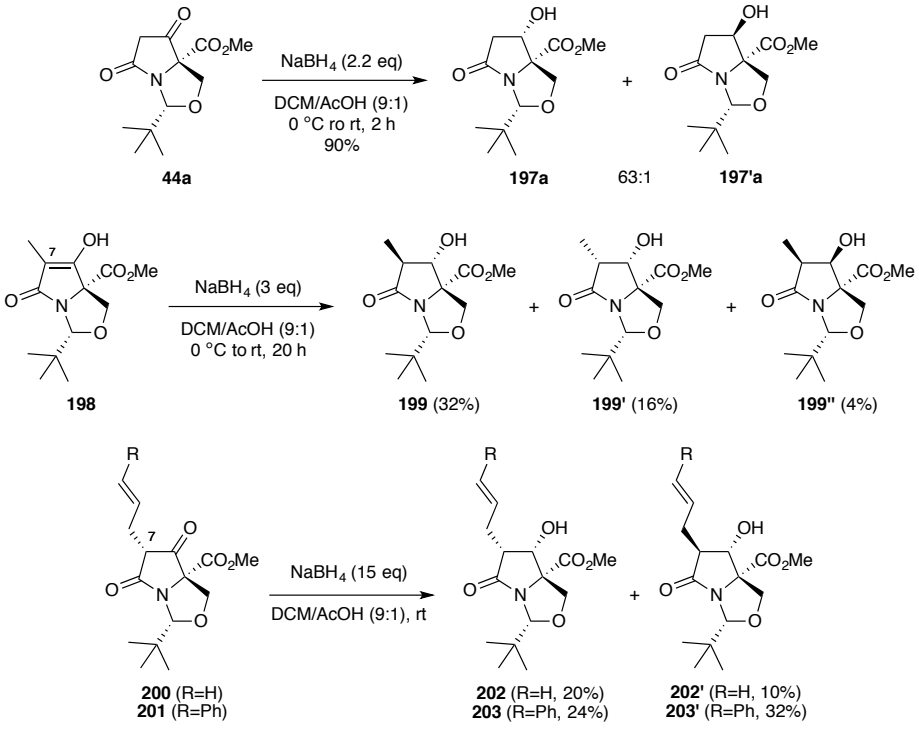
Compound	R_1	R_2	(*R*)/(*S*) ratio
69'a	H	^{i}Bu	32:68
69'b	Me	^{i}Bu	15:85
69'c	^{i}Pr	^{i}Bu	0:100
69'd	Me	^{i}Pr	34:66
69'e	^{i}Pr	Me	4:96
69'f	-$(CH_3)_2$-		95:5





Scheme 3.1. Stereoselective reduction of tetramic acids **69'a-f** reported by Poncet and co-workers.[206]

The Moloney group has employed the stereochemical outcome observed by Poncet and co-workers for the reduction of bicyclic tetramic acids. In the case of tetramate **44a**, the reduction proceeded with excellent levels of diastereoselectivity and good yield to give almost exclusively alcohol **197a** (Scheme 3.2).[182] As previously observed for **69'f**, hydride attack took place from the *endo*-face of the bicyclic system.



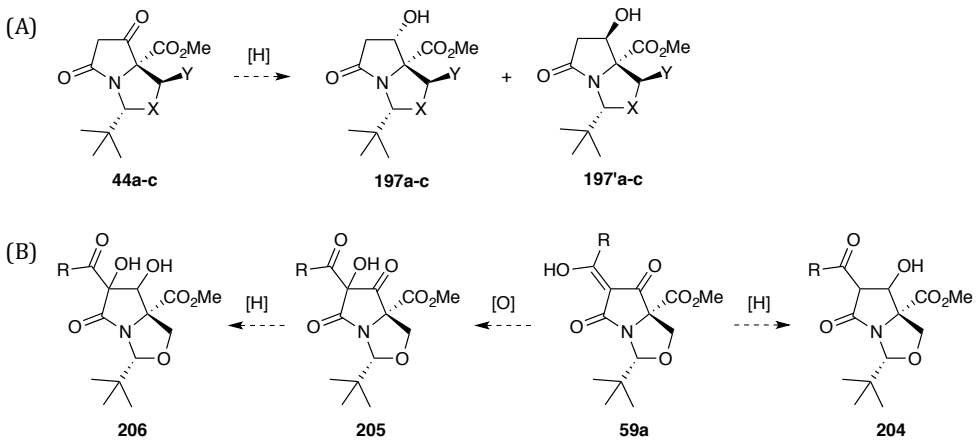
Scheme 3.2. Reported examples of the reduction of $C(7)$-unsubstituted and $C(7)$-alkyltetramic acids.[182,207]

For *C*(7)-alkyl tetramates **198**,**200**,**201**, the reduction was less efficient and lower yields were observed due to the increased steric hindrance from the *C*(7)-substituent.[182,207] Additionally, these were formed as epimeric mixtures at *C*(7).

3.1.2 Synthetic objectives

Following Matthews and co-workers' work on the stereoselective reduction of tetramate **44a**, it was of interest to reduce the alternative tetramates **44b** and **44c** (Scheme 3.3 A). These bicyclic systems have a bulkier sulfur atom and a methyl group respectively that could hinder the attack of the hydride and might lead to an alternative diastereomeric preference.

Moreover, the tetramate core of acylated analogues **59a**, prepared as described in Chapter 2, would be further elaborated by reduction of the *C*(6)-ketone and oxidation of the *C*(7)-position (Scheme 3.3 B). This could lead to a new library of compounds, allowing for an interesting comparison with the previous acylated analogues in terms of structure and biological activities.



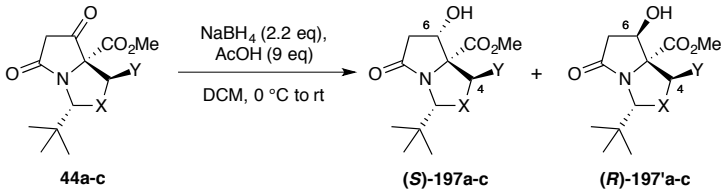
Scheme 3.3. Synthetic objectives for Chapter 3.

3.2 Reduction of tetramates derived from serine, cysteine and threonine

With tetramates **44a-c** prepared as described in Section 2.2, the reported results for the reduction of **44a** were initially reproduced and it was found that the 6*S* alcohol **197a** was formed in 96% diastereomeric excess (de) over **197'a**, as determined by LC/MS analysis (Appendix D), and in

quantitative yield (Table 3.1). Tetramate **44b** gave alcohol **197b** in quantitative yield, with identical stereoselectivity, and the alternative diastereomer **197'b** was not observed. With **44c**, diastereomer **197c** was still the major one, but with a decreased de of 68%. However, while the reduction of tetramates **44a** and **44b** was completed in good yield in 2 hours, for **44c**, even after 2 days there was still starting material left, giving the alcohol in only 51% yield, indicative of the steric hindrance resulting from the methyl group in the *C*(4)-position. The stereochemistry of these compounds was assigned by NOE analysis (Appendix C), and it was further confirmed by crystal structure of alcohol **197b** (Figure 3.1).

Table 3.1. Reduction of tetramic acids **44a-c**.



Product	time	Yield	de of (*S*)
197a	2 h	quant.	96
197b	2 h	quant.	100
197c	2 d	51%	68

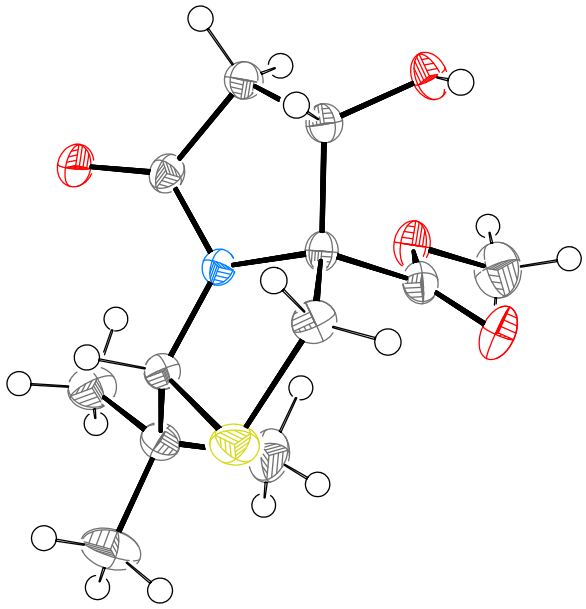
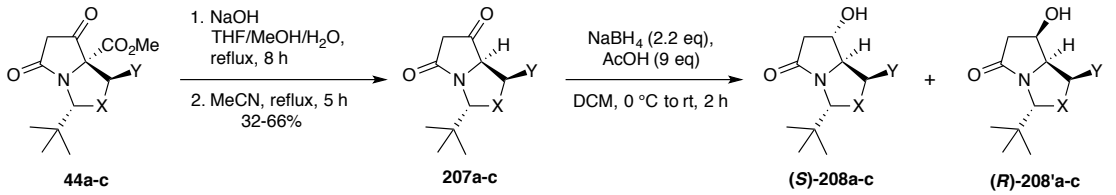
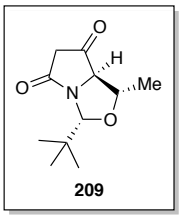


Figure 3.1. X-ray crystal structure of alcohol **197b**.

The high diastereoselectivity observed in all cases for the reduction of **44a-c** is probably due to hindering of the hydride attack by either the nitrogen lone pair or the methyl ester in the bicyclic system, or a combination of both. To confirm this, and to determine whether the selectivity could be changed, decarboxylation of tetramic acids **44a-c** was examined (Scheme 3.4). The methyl ester was treated with sodium hydroxide under reflux, and the generated carboxylic acid refluxed in acetonitrile[161] to give tetramates **207a-c**. Decarboxylated products **207a** and **207b** were formed as single diastereomers, with the bulky *tert*-butyl group in the *exo*-face of the bicyclic system, as demonstrated by NOE correlations. Interestingly, after careful NOE analysis, it was found that decarboxylation of **44c** furnished a 2.8:1 mixture of diastereomers **207c** and **209**. It seems that this would involve an elimination-re-addition of the intermediate enolate generated during decarboxylation.



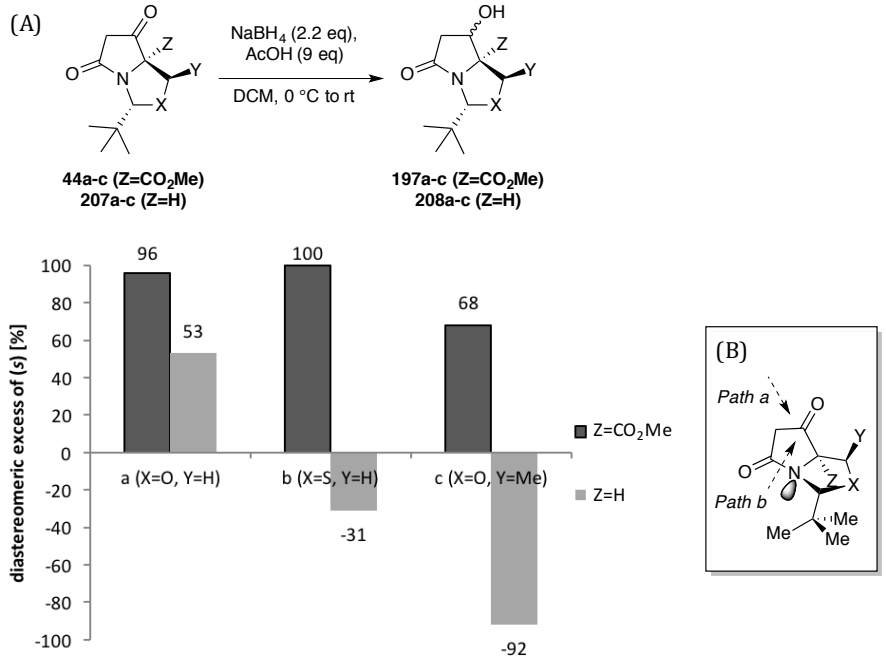


Product	Yield	de of (*S*)
208a	99%	53
208b	86%	-31
208c	92%	-92

Scheme 3.4. Preparation and reduction of decarboxylated tetramates.

Decarboxylated compounds **207a-c** were subjected to reduction with sodium acetoxyborohydride to give alcohols **208a-c** and **208'a-c** (Scheme 3.4), and the structures were again assigned by NOE correlations. Using LC/MS analysis, it was clear that a significant decrease in stereoselectivity occurred when compared to the methoxycarbonyl substituted systems **44a-c** (Scheme 3.5 A). For serine, **208a** was still the major product, but with a reduced 53% diastereomeric excess over the other possibility **208'a**. For cysteine and threonine, the selectivity

was inverted and isomers **208'b** and **208'c** were obtained in preference to **208b** and **208c**, with de values of 31% and 92% respectively.



Scheme 3.5. (A) Diastereomeric excess of the 4*S* diastereomer for the reduction of tetramates **44a-c** and **207a-c**; (B) hydride attack to the *C*(6)-carbonyl. Reproduced from Josa-Culleré, L.; Moloney, M. G.; Thompson, A. L. *Synlett* **2016**, *27* (11), 1677, copyright (2016) Georg Thieme Verlag KG.

In the addition of hydride to these systems, it would appear then that for tetramates **44a-c**, which possess an immediately adjacent bridgehead carboxymethyl group, the dominant route of entry is by Path a (Scheme 3.5 B), which also avoids the *N*-lone pair and the *exo*-*tert*-butyl group. However, for tetramates **207a-c**, the absence of the bridgehead carboxymethyl group but also the presence of the large ring sulfur atom of cysteine or the *C*(4)-methyl of threonine is enough to force hydride attack to the *exo*-face of the ring, and for **207c**, this is now the dominant mode of entry.

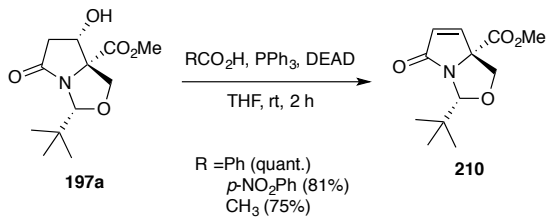
With these results, it has been shown that the diastereomeric outcome for the reduction of the tetramate core can be controlled by appropriate choice of the bridgehead group, the *C*(4)-substituent and the ring heteroatom.

Of interest was that the ^{1}H and ^{13}C NMR spectra of alcohols **197a-c** and **208a-c** showed some consistent differences: the 6*S* alcohols showed *H*(6) at higher field and *C*(6) at lower field than the alternative 6*R* diastereomers (Table 3.2). Also, as observed by Andrews *et al*. for similar compounds,[182] the difference in chemical shift between $H(4_{endo})$ and $H(4_{exo})$ was significantly bigger for the 6*S* alcohols than for 6*R* alcohols, and the opposite was true for *H*(7). Therefore, while NOE analysis was performed on all the synthesised alcohols, diastereomeric assignment of new alcohol analogues would be directly possible from ^{1}H and ^{13}C NMR analysis.

Table 3.2. Representative chemical shifts of 6*S* and 6*R* alcohols **197a-c** and **208a-c**. Adapted from Josa-Culleré, L.; Moloney, M. G.; Thompson, A. L. *Synlett* **2016**, *27* (11), 1677, copyright (2016) Georg Thieme Verlag KG.

Compound	*H*(6) [ppm]		*C*(6) [ppm]		$H(4_A)$-$H(4_B)$ [ppm]		$H(7_A)$-$H(7_B)$ [ppm]	
	(6*S*)	(6*R*)	(6*S*)	(6*R*)	(6*S*)	(6*R*)	(6*S*)	(6*R*)
197a	4.56	-	73.8	-	1.32	-	0.34	-
197b	4.43	-	76.6	-	0.87	-	0.24	-
197c	4.61	-	69.7	-	-	-	0.31	-
208a	4.36	4.55	69.1	66.5	1.15	0.0	0.06	0.63
208b	4.29	4.58	68.8	66.7	0.60	0.24	0.22	0.47
208c	-	4.95	-	65.6	-	-	-	0.42

Attempted inversion of configuration of alcohol **197a** was unsuccessful. The use of Mitsunobu conditons[208] with DEAD, triphenylphosphine and benzoic acid gave the elimination product **210** exclusively (Scheme 3.6). The same was observed when using the more acidic 4-nitrobenzoic acid or the less bulky acetic acid. These results confirm the big steric hindrance of the *C*(6)-position.



Scheme 3.6. Unsuccessful inversion of configuration of alcohol **197a** under Mitsunobu conditions.

3.3 Synthesis of reduced acylated analogues

Having been able to reduce the tetramate core of **44a** with high diastereoselectivities, it was of interest to study the reduction of the acylated compounds. If the stereochemical control was maintained, this would give access to analogues of pramanicin bearing the *C*(6)-hydroxyl group with the same relative configuration as the natural product, as shown in Figure 3.2.

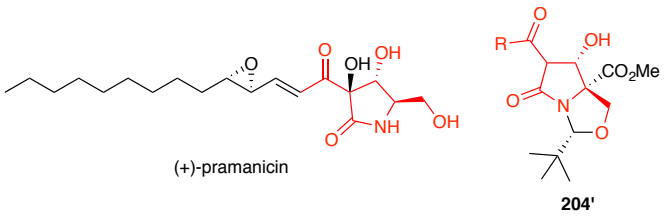
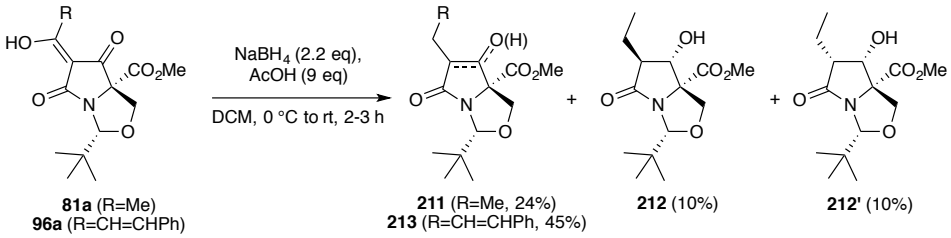


Figure 3.2. Relationship of (+)-pramanicin with the target reduced analogues **204'**.

3.3.1 Reduction of acylated analogues

For the reduction of acetyl **81a**, the same reduction conditions with $NaBH(OAc)_3$ were initially attempted. Unfortunately, attack of the hydride took place on the more accessible *exo*-carbonyl group instead of the desired *C*(6)-ketone (Scheme 3.7). The generated alcohol underwent elimination, followed by conjugated reduction of the enone with a second equivalent of hydride. As a result, *C*(7)-alkyl tetramate **211** was isolated in 24% yield together with alcohols **212** and **212'** (10% of each), which could be separated by *flash* column chromatography.



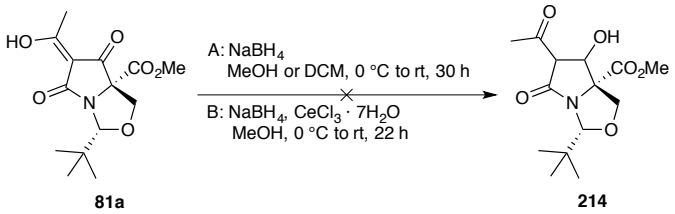
Scheme 3.7. Reduction of *C*(7)-acylated analogues.

Tetramate **211** existed as a keto-enol mixture, as evidenced by the presence of positive cross peaks in the NOE spectrum, which hampered the stereochemical assignment of the keto form. Both alcohols **212** and **212'** were generated through attack of the hydride from the *endo*-face of

the bicyclic system, consistent with the selectivity observed for the reduction of tetramate **44a** and with the reported reduction of similar *C*(7)-alkyl systems (Scheme 3.2).[207]

Similar results were observed for the more conjugated system **96a**, although a more complex mixture of products was obtained from which only tetramate **213** was isolated.

Reduction of **81a** was also attempted without acetic acid, with the hope that the less bulky $NaBH_4$, instead of $NaBH(OAc)_3$, would have more access to *C*(6). However, after stirring for 30 h, the starting material was recovered, indicative of the need of the acid to activate the carbonyl group for hydride attack. Luche reduction with $CeCl_3$[209] also resulted in recovered starting material and none of the desired **214** was obtained (Scheme 3.8).

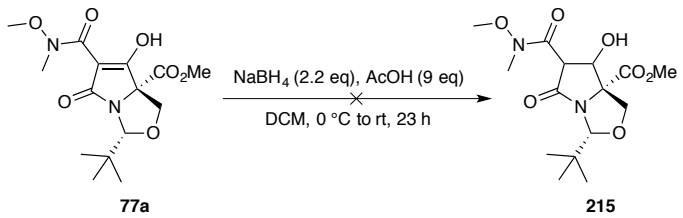


Scheme 3.8. Unsuccessful reduction of acetyltetramate **81a**.

As observed in Chapter 2, methylation of the acylated analogues generates the enol ether in the *C*(6)-position. Therefore, protection of the *exo*-enol is not a possibility for the preparation of acylated alcohols **204**.

3.3.2 Attempted reduction of the Weinreb amide

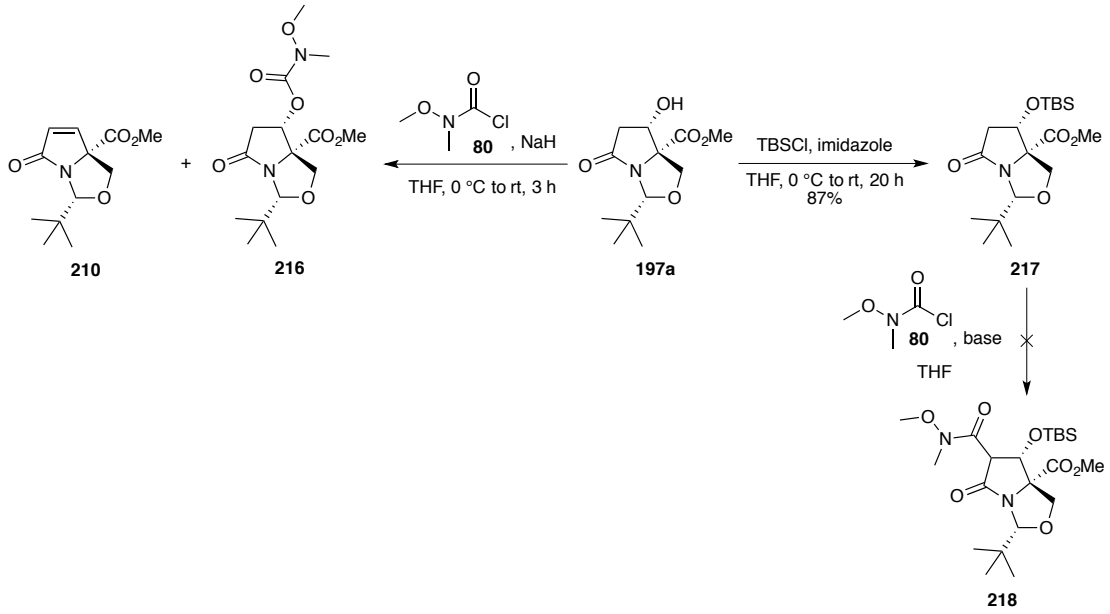
An alternative route for the synthesis of alcohols **204** would be to reduce the Weinreb amide, which could be later subjected to Grignard addition. In this case, the *C*(6)-enol should reduce preferentially to the less reactive amide. However, when Weinreb amide **77a** was stirred with $NaBH(OAc)_3$ overnight, only starting material together with minor unidentified products were obtained, with no alcohol **215** observed by NMR or MS analysis (Scheme 3.9).



Scheme 3.9. Attempted reduction of the Weinreb amide.

3.3.3 Attempted acylation of the reduced tetramic acid

Following the unsuccessful reduction of both the acylated analogues and the Weinreb amide, the possibility to acylate the reduced tetramate core was investigated. Unsurprisingly, direct treatment of alcohol **197a** with methoxy(methyl)carbamic chloride **80** gave the *O*-acylated product **216**, which was not isolated, together with alkene **210**. Alternatively, with the TBS-protected analogue **217**, introduction of the Weinreb amide was unsuccessful, and mainly starting material was recovered (Scheme 3.10, Table 3.3).



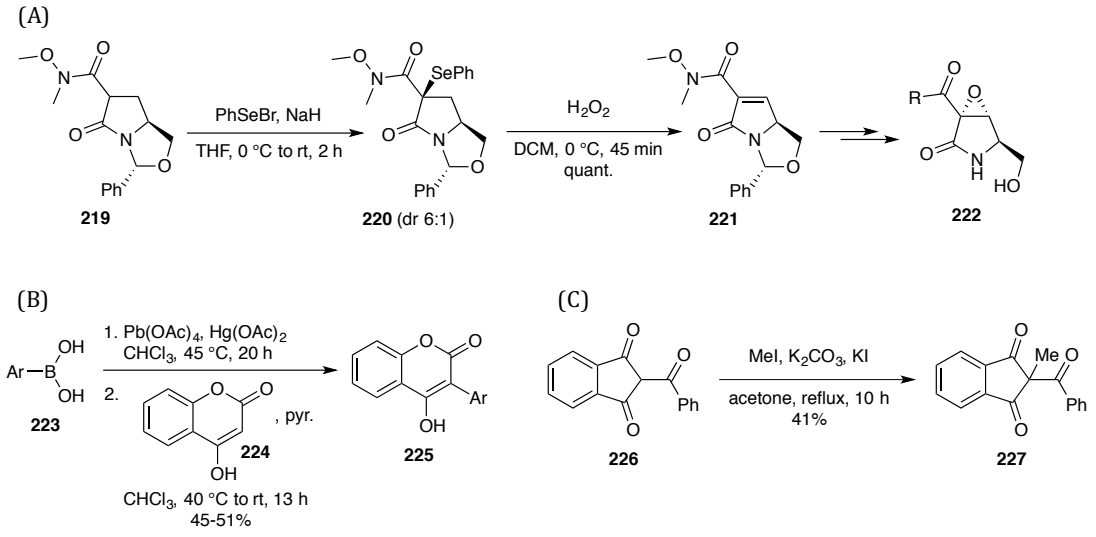
Scheme 3.10. Attempted introduction of the Weinreb amide to the reduced tetramate.

Table 3.3. Conditions used for the attempted acylation of silyl ether **217**.

Entry	Conditions	Outcome
1	NaH (3 eq), **80** (2 eq) THF, 0 °C to rt, 20 h	Recovered starting material.
2	NaH (3 eq), **80** (2 eq) THF, 0 °C to reflux, 18 h	Recovered starting material.
3	LDA (1.1 eq), **80** (1.1 eq) THF, -78 °C to 0 °C, 1 h	1.5:1 mixture of starting material **217** and elimination product **210**.
4	LDA (1.1 eq), **80** (10 eq) THF, -78 °C to rt, 17 h	Recovered starting material.

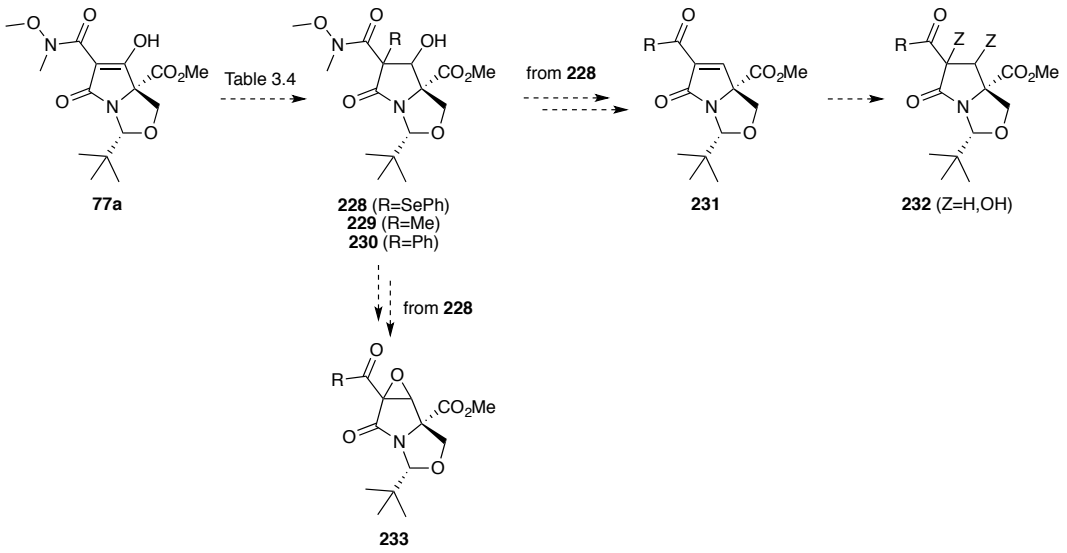
3.3.4 Attempted selenation/alkylation of the Weinreb amide

Given the inertness of Weinreb amide **77a** towards reduction, it was decided to instead study its *C*(7)-selenation. A similar analogue **220** (Scheme 3.11 A) had been previously prepared by the Moloney group, which was subjected to oxidative elimination to alkene **221** and further elaborated to give a library of biologically active compounds **222**.[210]



Scheme 3.11. Reported examples of the selenation[210] and alkylation[211,212] of dicarbonyl and tricarbonyl systems.

In the case of Weinreb amide **77a**, the resulting β-phenylselenoalcohol **228** could be subjected to epoxidation[213] or elimination (Scheme 3.12).[214,215] The resulting alkene **231** could be hydroxylated, dihydroxylated or epoxidised and reacted with Grignards to give a library of analogues **232**,**233** with a reduced/oxidised tetramate core.



Scheme 3.12. Proposed selenation of Weinreb amide **77a** and further functionalisation.

Table 3.4. Attempted conditions for the selenation/alkylation of Weinreb amide **77a**.

Entry	R	Conditions	Literature example*	Outcome
1	SePh	PhSeBr (1.5 eq), NaH (1.5 eq) THF, rt, 18 h	A[210]	Recovered starting material.
2	SePh	PhSeCl (1.5 eq), NaH (1.5 eq) THF, rt, 18 h	Adapted from A[210]	Recovered starting material.
3	SePh	PhSeBr (1.5 eq), NaH (1.5 eq) THF, reflux, 18 h		Starting material and other unidentified minor products.
4	SePh	PhSeBr (4 eq), K_2CO_3 (4 eq), KI (0.15 eq) Acetone, reflux, 14 h		Degradation products.
5	SePh	PhSeBr (1.5 eq), LiHMDS (1.5 eq) THF, -78 °C to rt, 21 h		Recovered starting material.
6	SePh	PhSeBr (1.5 eq), DBU (1.5 eq) THF, rt to reflux, 24 h		Crude NMR shows tetramate **44a**.
7	Ph	1. $PhB(OH)_2$ (1.1 eq), $Pb(OAc)_4$ (1.1 eq), $Hg(OAc)_2$ (0.1 eq) $CHCl_3$, 45 °C, 20 h 2. **77a** (1.0 eq), pyr. (3 eq) $CHCl_3$, 40 °C to rt, 17 h	B[211]	Recovered starting material.
8	Me	MeI (4 eq), K_2CO_3 (4 eq), KI (0.15 eq) Acetone, reflux, 23 h	C[212]	Starting material and traces of *O*-methylated product.

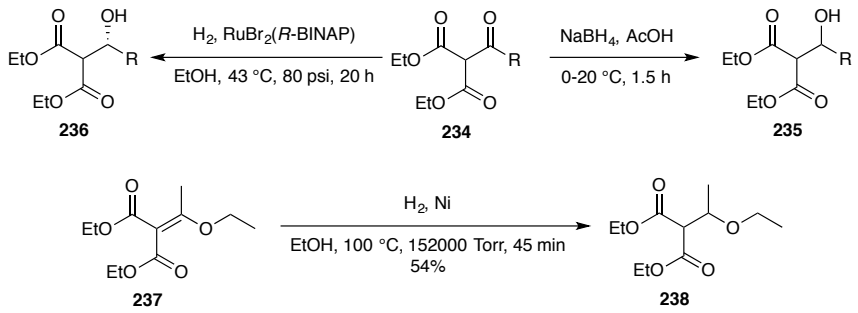
*Refers to Scheme 3.11.

Unfortunately, selenation of **77a** was unsuccessful (Table 3.4, Entries 1-6). Treatment with PhSeBr or PhSeCl with a range of bases (NaH, K_2CO_3, LiHMDS and DBU) and temperatures (-78 °C

to reflux in acetone and THF) only resulted in either recovery of starting material or formation of unidentified products, with no selenated product observed in the crude NMR and MS spectra.

Alkylation of the $C(7)$-position under conditions used for similar systems (Scheme 3.11 B,C) was also unsuccessful (Table 3.4, Entries 7-8).

The lack of reactivity of the Weinreb amide towards $C(6)$-reduction and $C(7)$-substitution could be due to steric hindrance, but also to the big stability of this highly conjugated tricarbonyl system. In fact, there are only a handful of literature examples on the reduction of tricarbonyls (Scheme 3.13). They concern the reduction of β-keto diesters **234** either with $NaBH_4$ and AcOH[216] or through an asymmetric hydrogenation with a BINAP ruthenium(II) catalyst (used for the total synthesis of scymnol),[217] but in these cases the ketone is in the keto form. An interesting example is enol ether **237**, which was converted to ether **238** by H. M. Barnes *et al.*, but hydrogenation at high temperature (100 °C) and pressure (152000 Torr) was needed.[218] Inspired by the successful reduction of this system, it was decided to study the $NaBH_4$ reduction and the hydrogenation of the protected Weinreb amide, as described in the next section.

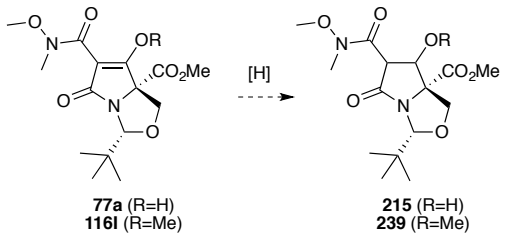


Scheme 3.13. Reported examples of the reduction of (A) β-keto diesters[216,217] and (B) diethyl 2-(1-ethoxyethylidene)-malonate.[218]

3.3.5 Reduction of the protected Weinreb amide

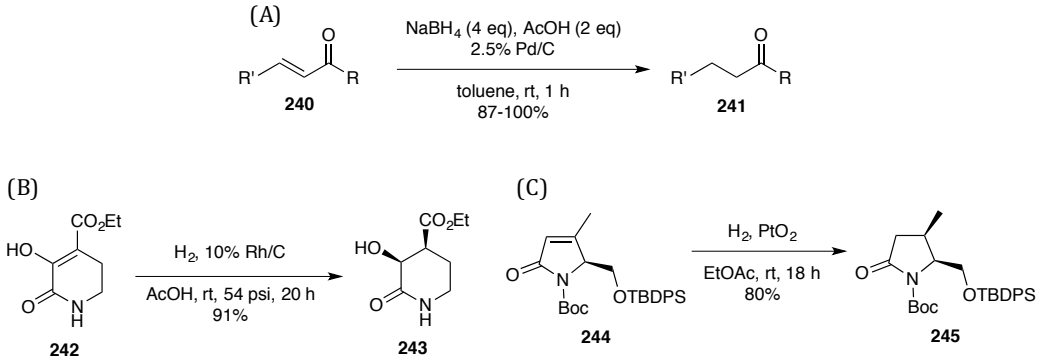
Attempted reduction of methylated Weinreb amide **116I** with $NaBH_4$ or $NaBH(OAc)_3$ resulted in quantitative recovery of starting material (Table 3.5, Entries 1-2).

Table 3.5. Attempted conditions for the reduction of protected Weinreb amide **116I**.



Entry	R	Conditions	Literature example*	Outcome
1	Me	$NaBH_4$ (2.2 eq), AcOH (9 eq) DCM, rt, 23 h		Recovered starting material.
2	Me	$NaBH_4$ (2.2 eq) MeOH, rt, 22 h		Recovered starting material.
3	Me	$NaBH_4$ (4 eq), AcOH (2 eq), 2.5% Pd/C toluene, rt, 22 h	A[219]	Recovered starting material.
4	H	H_2, 10% Rh/C, AcOH, rt, 3.5 bar, 21 h	B[220]	Recovered starting material.
5	Me	H_2, 10% Rh/C AcOH, rt, 3.5 bar, 21 h	B[220]	Recovered starting material.
6	Me	H_2, 10% Pd/C EtOAc, rt, 2 bar, 15 h		Crude NMR shows 5:1 mixture of **116I** and **239** (product not isolated).
7	Me	H_2, 20% PtO_2 EtOAc, rt, 3 h	C[221]	Crude NMR shows full conversion to **239** as a single diastereomer in quant. yield.

*Refers to Scheme 3.14.



Scheme 3.14. (A) Selective 1,4-conjugate reduction of α,β-unsaturated carbonyl compounds reported by A. Russo et al.;[219] hydrogenation of (B) conjugated enol **242**[220] and (C) unsaturated lactam **244**.[221]

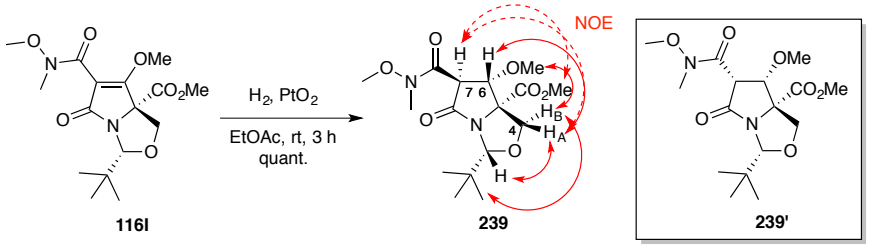
A. Russo *et al*. reported the selective 1,4-conjugate reduction of a range of α,β-unsaturated carbonyl compounds **240**, including ketones, esters and amides, using $NaBH_4$ in combination with

palladium catalysis (Scheme 3.14 A).[219] Thus, it was expected that the application of these conditions to methylated analogue **116I** could provide ether **239**. However, only starting material was recovered.

A further attempt to prepare alcohol **215** or ether **239** was through hydrogenation (Table 3.5, Entries 4-7). When **77a** was treated with hydrogen gas at 3.5 bar in the presence of Rh/C in AcOH, conditions used for the reduction of the conjugated enol **242** (Scheme 3.14 B),[220] only starting material was recovered. Again, it was thought that the stabilising delocalisation of the tetramate could be decreasing its reactivity, but enol ether **116I** also returned starting material. Alternatively, with Pd/C as the catalyst and at 2 bar, the crude NMR spectrum showed for the first time a new set of peaks matching the desired product **239**, but with only a 20% conversion.

Hydrogenation of unsaturated lactam **244**, at atmospheric pressure with PtO_2, has been reported to give **245** and proceed with high levels of diastereoselectivity (Scheme 3.14 C).[221] When the same conditions were applied to enol ether **116I**, after 3 h both TLC and MS analysis indicated full conversion, and the crude NMR spectrum showed ether **239** as a single diastereomer, with no need for further purification after filtration through Celite. Interestingly, both **116I** and **239** had the same R_f on TLC but only the starting material was visible under UV.

Initially, it was hypothesised that the ether would have the stereochemistry of isomer **239'** (Scheme 3.15), with addition of hydrogen from the *endo*-face, analogously to the hydride attack to tetramate **44a**, and via *syn*- addition as expected for a hydrogenation. NOE analysis indicated the stereochemistry of *C*(6) to be as predicted: irradiation of *H*(6) gave enhancement of $H(4_A)$ but not $H(4_B)$. Analysis of *C*(7) was less indicative, as only weak enhancement of $H(4_A)$ was observed, as well as a small one to CO_2Me, and correlations between *H*(6) and *H*(7) were obscured by the spt (selective population transfer) effect, showing both positive and negative cross-peaks. Consequently, crystals of ether **239** were grown and analysed, and it was of surprise to find that **239** was a *C*(7)-epimer of the expected structure (Figure 3.3).



Scheme 3.15. Successful preparation of ether **239** via hydrogenation with PtO_2.

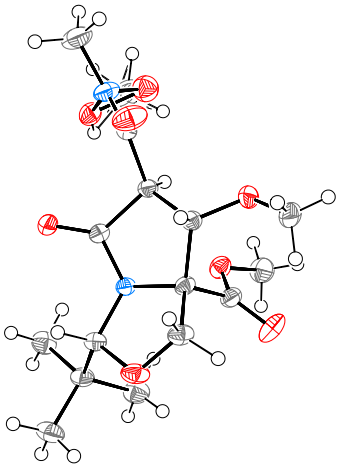
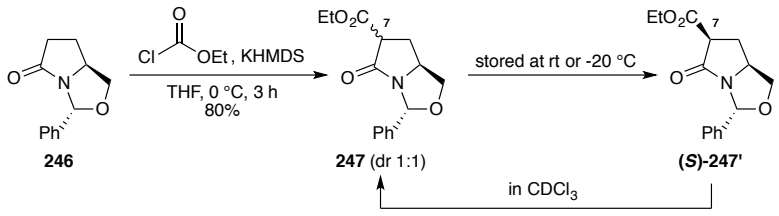


Figure 3.3. X-ray crystal structure of **239**. The average structure is disordered.

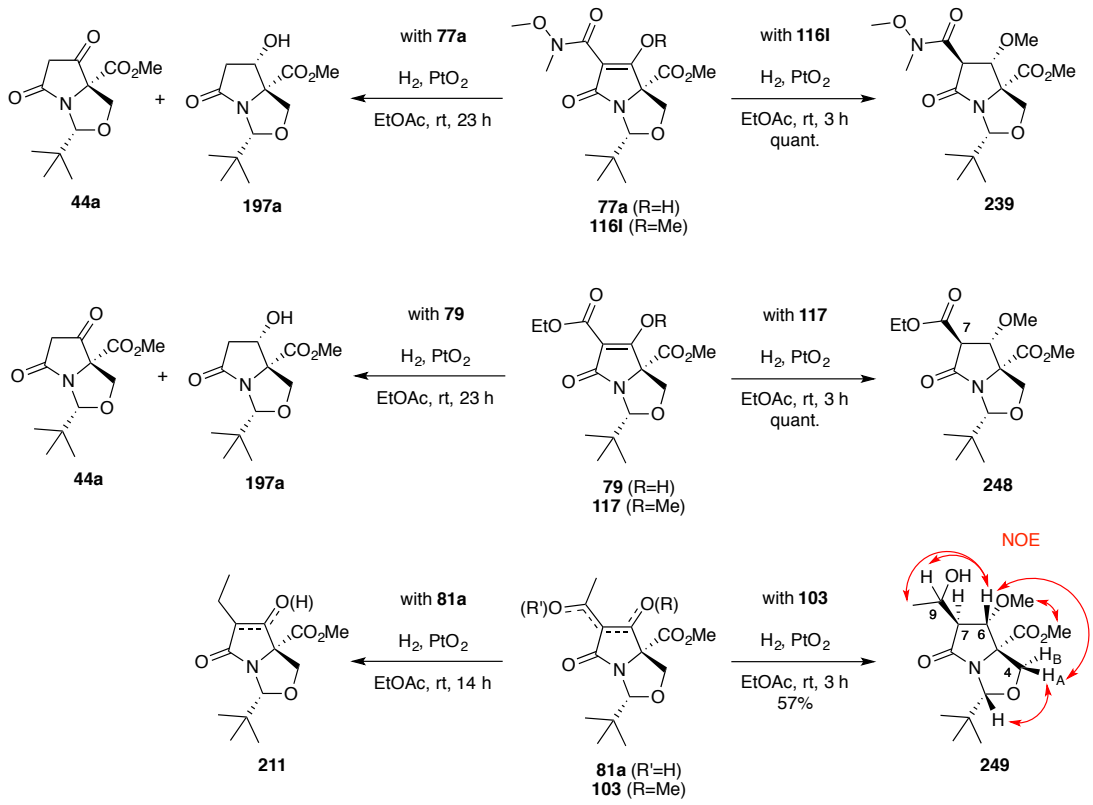
S. W. B. Tan had actually observed that ester **247** (Scheme 3.16), which was initially formed as a 1:1 mixture of diastereomers when acylating pyrrolidinone **246**, slowly isomerised to give the 7*S* *endo*-diastereomer when stored in the pure form at room temperature or at -20 °C.[222] When dissolving it in $CDCl_3$, it isomerised back to a mixture of *C*(7)-epimers. It would seem that **239'** was initially formed when hydrogenating **116l**, but isomerisation occurred in the reaction mixture and in this case the additional steric interactions between the Weinreb amide and the methoxy group favoured the *endo*-diastereomer. No isomerisation to the *exo*-diastereomer was observed when storing **239** in $CDCl_3$.



Scheme 3.16. Acylation of **246** and isomerisation to the 7*S* isomer reported by S. W. B. Tan.[222]

3.3.6 Hydrogenation of acylated compounds

Hydrogenation with PtO_2 of methylated ethyl ester **117** proceeded diastereoselectively, analogously to **116l**, to give ether **248** as a single isomer (Scheme 3.17). NOE analysis was again not determining, as no correlations were observed for *H*(7), but the same diastereomerical outcome as for **239** would be expected. Interestingly, when the free enol forms, **77a** and **79**, were subjected to the same conditions, tetramate **44a** and alcohol **197a** were formed, probably arising from hydrolysis of the amide and the ester, followed by decarboxylation. Tetramate **44a** then underwent diastereoselective hydrogenation of the ketone to the alcohol.



Scheme 3.17. Hydrogenation of enol and enol ether forms of *C*(7)-carboxamide, -alkoxy and -acyltetramic acids.

With acetyl **81a**, after 14 h the crude NMR spectrum showed mainly *C*(7)-ethyl **211**, together with trace amounts of the alcohols, analogous to what had been observed for its reduction with $NaBH(OAc)_3$ (Scheme 3.7). Instead, with methylated **103**, alcohol **249** was formed as a single

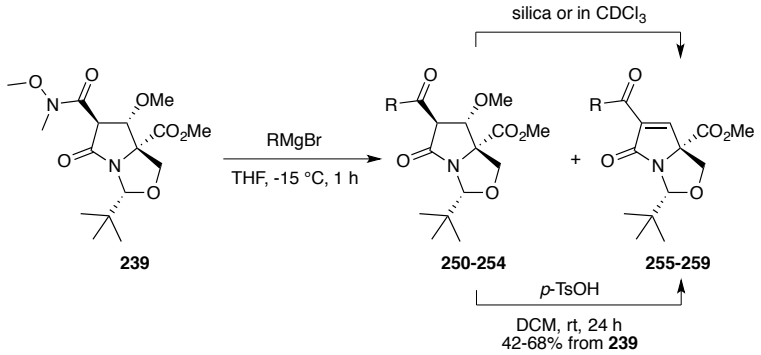
diastereomer (unassigned at *C*(9)). Irradiation of *H*(6) showed strong enhancement of $H(4_A)$ and *H*(9), which supported the configuration at *C*(6) and *C*(7).

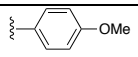
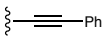
3.3.7 Grignard addition to Weinreb amide 239

As shown in Scheme 3.17, preparation of acylated alcohols **204** cannot be achieved via direct hydrogenation of the acylated tetramates, as the *exo*-enol group undergoes reduction. Moreover, this would be restricted to the alkyl and aryl analogues since the alkenyl and alkynyl ones would also undergo side-chain hydrogenation. Consequently, Grignard addition to Weinreb amide **239** seemed to be a plausible alternative.

Treatment of Weinreb amide **239** with 1 equivalent of methylmagnesium bromide gave acetyl **250** together with small amounts of the elimination product **255** (Table 3.6). Unfortunately, purification of the desired product by *flash* column chromatography was not possible. The acidic silica gel seemed to catalyse the formation of **255**, and mixtures of both products were collected. Elimination also occurred when storing ether **250** in $CDCl_3$ for several days, but no isomerisation at *C*(7) was detected.

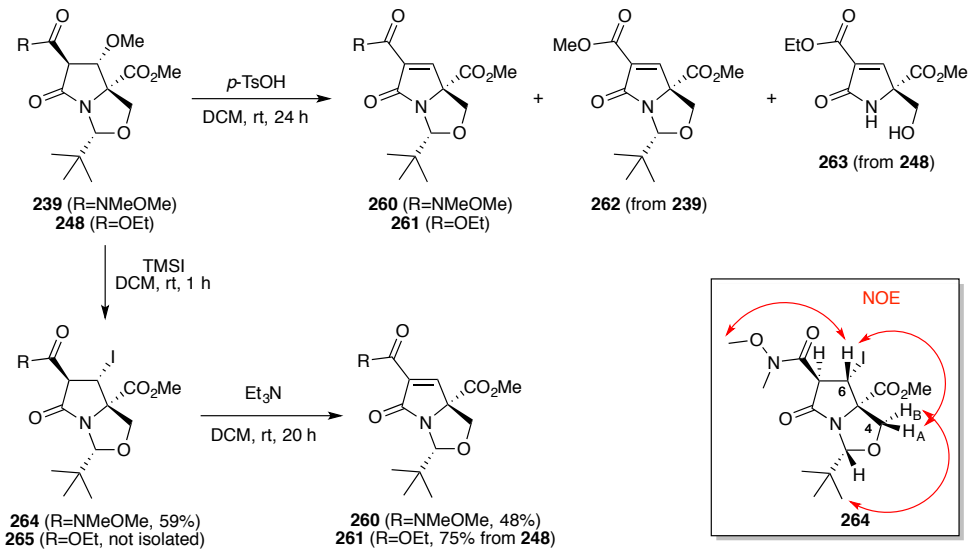
Table 3.6. Grignard addition to Weinreb amide **239** and elimination.



Compound	R	Yield from 239 [%]
255	Me	48
256	Ph	68
257		50
258		49
259		42

Weinreb amide **239** was substituted with different Grignard reagents, which also yielded the acylated analogues **251**-**254** as mixtures with the elimination products **256**-**259**. Formation of the enones could also be accelerated by treatment with 0.5 equivalents of *p*-toluenesulfonic acid for 24 h, and they were isolated in 42-68% yield over the two steps.

Elimination of Weinreb amide **239** was less efficient, which had already been evidenced by its stability in $CDCl_3$ contrary to ketones **250**-**254**, probably owing to the weaker electron withdrawing nature of the amide. With 0.5 equivalents of *p*-toluenesulfonic acid, mainly starting material was recovered together with small amounts of enone **260** and other unidentified by-products (Scheme 3.18). With 2 equivalents of the acid, the ether starting material was consumed but the crude NMR spectrum showed a mixture of products, from which methyl ester **262** was isolated. Alternatively, it was envisaged that the alkene could be accessed through demethylation of **239** to the alcohol, followed by elimination via the mesylate with Et_3N and MsCl. However, upon treatment of methyl ether **239** with TMSI, iodide **264** was formed instead of the alcohol, which could arise from initial elimination followed by conjugate addition of the iodide. NOE correlations between $H(4_A)$ and $H(6)$ suggested the stereochemistry as drawn in Scheme 3.18, and the assignment was confirmed by crystal structure (Figure 3.4). Treatment of the iodide with Et_3N overnight furnished enone **260** in 48% yield along with 26% recovered starting material **264**.



Scheme 3.18. Preparation of enones **260** and **261**.

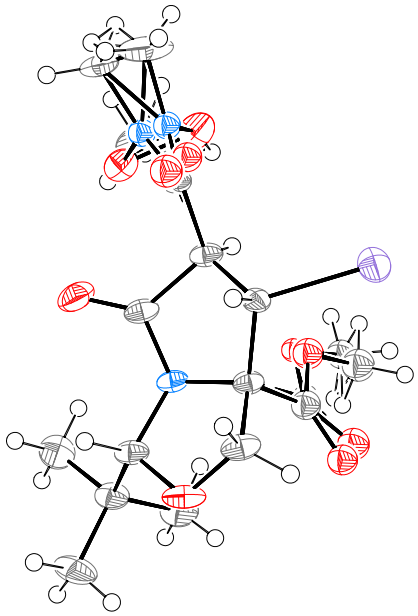


Figure 3.4. X-ray crystal structure of iodide **264**. The average structure is disordered, but additional satellite peaks have been identified and the modulated structure gives an ordered structure.

With regards to ethyl ester **248**, stirring with 2 equivalents of *p*-TsOH for 24 h resulted in less than 50% conversion to enone **261**, and either with a bigger excess of acid (10 equivalents) or under reflux, the crude NMR spectrum seemed to show a mixture of desired product and the *N*,*O*-acetal deprotected alcohol **263**, evidenced by the corresponding mass observed by LRMS analysis and the characteristic aldehyde peak in the crude NMR spectrum (after simple evaporation of the reaction mixture). This result would again be consistent with the lower electron withdrawing ability of the ester functionality compared to the ketone group. Formation of enone **261** via the iodide, which could not be isolated as it eliminated during silica column chromatography, was achieved in 75% yield over the two steps.

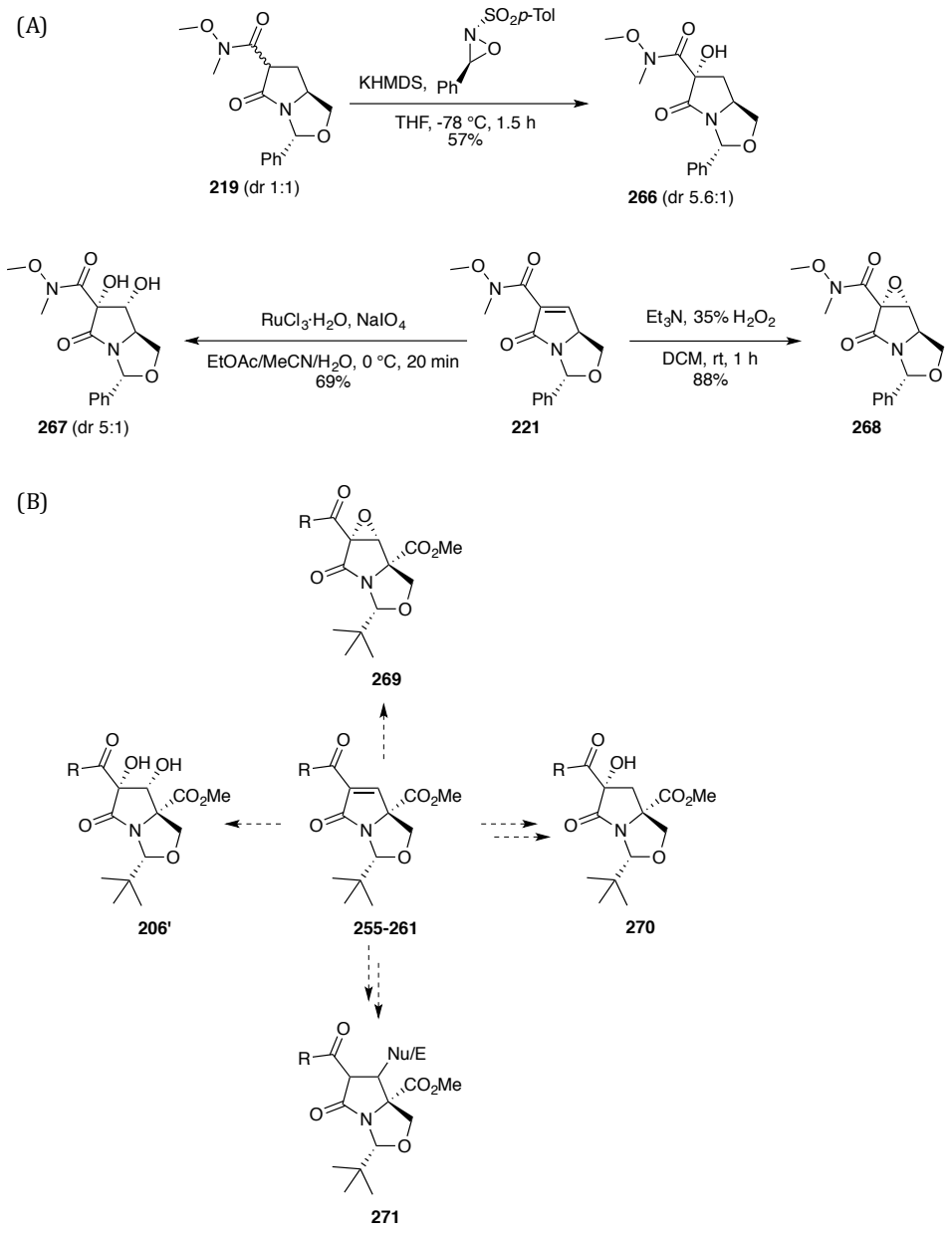
3.4 Summary of Chapter 3 and future work

In this chapter, it has been shown that reduction of the tetramate core can be achieved with high levels of diastereoselectivity, and small changes in the bicyclic lactam can alter the selectivity of the transformation. These changes include substitution of the ring heteroatom to sulfur, addition of a methyl group in $C(4)$, or elimination of the bridgehead carboxymethyl group.

For the preparation of reduced *C*(7)-acyltetramates, reduction of the *exo*-carbonyl group took place instead of the more hindered *endo*-*C*(6)-ketone. Reduction of the Weinreb amide was also unsuccessful, given its inertness probably owing to the highly delocalised tricarbonyl system, and so was its *C*(7)-selenation/alkylation.

Finally, it was found that the reduced Weinreb amide could be accessed via hydrogenation of the enol ether with PtO_2, which upon attempted deprotection with TMSI gave the iodide instead of the alcohol. The methyl ether form of the Weinreb amide could be substituted with alkyl, aryl, alkenyl and alkynyl Grignard reagents, but purification of these analogues was thwarted by their instability towards elimination to the enones. These transformations have enabled the preparation of several analogues with different oxidation levels and different *C*(7)-acyl side-chains, which are likely to render an interesting comparison in terms of biological activities which are discussed in Chapter 5.

Enones **255-261** open the door to the functionalisation of the tetramate core to prepare reduced/oxidised analogues with different acyl substituents, following reported methods for similar systems as shown in Scheme 3.19.[210,222] The enones could also be manipulated via nucleophilic conjugate addition, and the formation of iodide **264** supports this possibility. Moreover, the iodide could be used for the substitution with electrophiles, for instance via the organozinc derivative.[223,224]



Scheme 3.19. (A) Reported functionalisation of Weinreb amides **219** and **221**;[210,222] (B) proposed future work for Chapter 3.

CHAPTER 4: INTRODUCTION OF β-LACTONES TO THE TETRAMATE CORE

This chapter describes the functionalisation of the tetramate core via introduction of β-lactones. Firstly, conditions for the introduction of both fused- and spiro-β-lactones into a simple reduced tetramate were found. Secondly, a more elaborated pyrrolidinone system, with the same relative stereochemistry as oxazolomycin, was prepared, lactonised and further functionalised.

4.1 Introduction

With a complex bicyclic framework and several contiguous stereocentres, the challenging lactam-lactone core of β-lactone-containing natural products has attracted tremendous attention amongst synthetic organic chemists. Moreover, the ability of these compounds to inhibit the 20S proteasome, leading to potential roles in diverse disease therapies,[225] results in their total syntheses, as well as the preparation of analogues, being of importance.

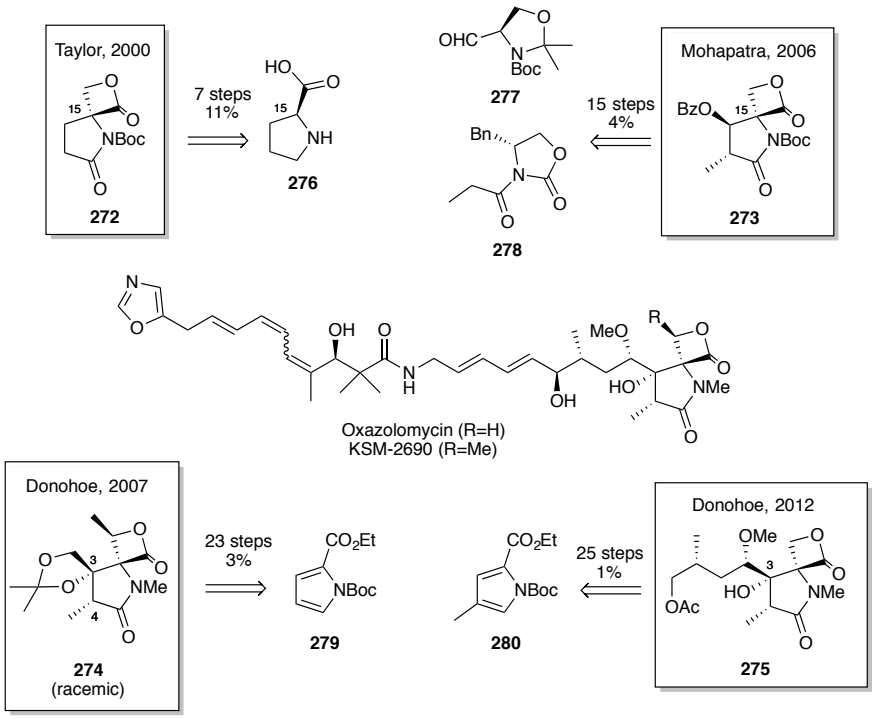
4.1.1 Spiro-β-lactones

Even though there is only one reported total synthesis of oxazolomycin A[116] (Section 1.5.4), several groups have reported synthetic efforts towards its pyrrolidinone core, which contains a spiro-β-lactone. Scheme 4.1 illustrates four relevant examples, and Table 4.1 summarises key features of these syntheses.

The first two syntheses commenced with chiral starting materials. Taylor's approach[226] took advantage of Seebach's SRS to prepare enantiopure material, while Mohapatra[227] relied on an aldol reaction between **277** and **278** which introduced most of the stereocentres in the system. Instead, Donohoe started with an achiral pyrrole, which in his first synthesis[228] led to racemic material, but in the second one[229] the enantiopure compound was obtained via enzymatic

desymmetrisation.[230] The quaternary centres were then introduced stepwise. In the four syntheses the pyrrolidine was oxidised at a late-stage with RuO_4.

Formation of the spiro-β-lactone was the last or second last step of the sequence in all cases, given the lability of this functional group. It was accessed from the β-hydroxyacid, where lactonisation was achieved under Mitsunobu conditions, with tosyl chloride and triethylamine or with HBTU and triethylamine (HATU and DIPEA were used for the total synthesis[116]).



Scheme 4.1. Reported syntheses of the pyrrolidinone core of oxazolomycin and KSM-2690.[226–229]

In spite of the success of these syntheses to prepare the core of oxazolomycin with the appropriate stereochemistry, they are not designed for the synthesis of analogues. They involve a large number of steps with low overall yields, in some cases with hazardous and/or expensive reagents which would hamper the scale-up. Additionally, they do not allow for flexible late-stage introduction of structural diversity.

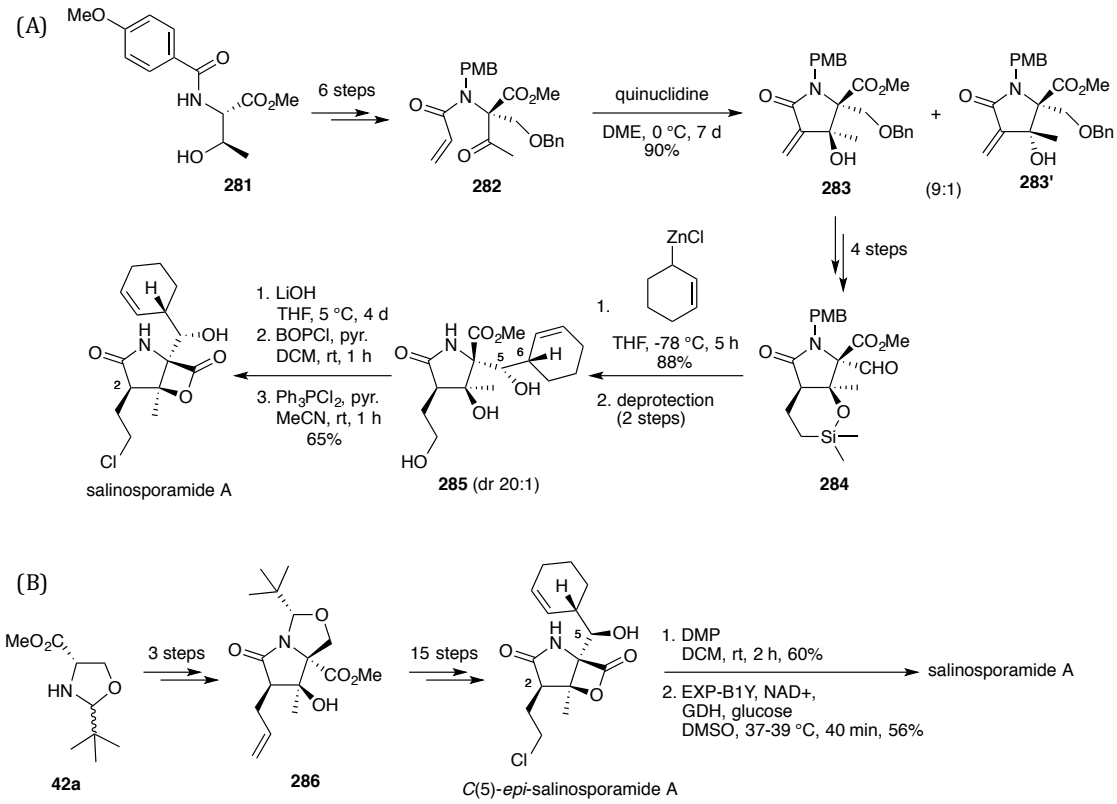
Table 4.1. Key features of reported syntheses of the pyrrolidinone core of oxazolomycin.

Group (year)	Key features	Lactonisation
R. J. K. Taylor (2000)[226]	- Enantioselective synthesis utilising Seebach's SRS.[137] - *C*(15)-hydroxymethylation with BOMCl.	DMAD, PPh_3 THF, -78 °C to rt, 10 min 86%
D. K. Mohapatra (2006)[227]	- Evans' aldol[231] between **277** and **278**. - Introduction of a *gem*-hydroxymethyl at *C*(15) via a crossed Cannizzaro reaction.	DEAD, PPh_3 THF, -78 °C to rt 88%
T. J. Donohoe (2007)[228]	- Ammonia-free Birch reductive aldol of protected pyrrole. - Diastereoselective introduction of C(4)*Me* via methylation of the β-ketoester followed by reprotonation with a bulky proton source. - *C*(3)-hydroxymethylation via allyl Grignard addition, alkene isomerisation and ozonolysis.	*p*-TsCl, pyr. DCM, -5 °C to 0 °C, 20 h 40% (2 steps)
T. J. Donohoe (2012)[229]	- Enantiopure product using enzymatic desymmetrisation. - Diastereoselective organocerium addition to a *C*(3)-aldehyde.	HBTU, Et_3N DCM, 0 °C to rt, 16 h 64% (2 steps)

4.1.2 Fused-β-lactones

The less complex structure of salinosporamide, which contains a fused-β-lactone, has allowed not only the design of several total syntheses,[232] but also the provision of closely-related analogues.

E. J. Corey published the first total synthesis of salinosporamide A in 2004 (Scheme 4.2 A),[233] which is also the shortest and highest yielding stereoselective route amongst the reported syntheses to date. His approach started with the L-threonine derivative **281** and had as key steps the ring closure of **282** by means of an internal Baylis-Hillman-aldol reaction with quinuclidine to give lactam **283** with high levels of diastereoselectivity, and the simultaneous construction of the *C*(5)- and *C*(6)-stereocentres via allylation of aldehyde **284** with 2-cyclohexenylzinc chloride.



Scheme 4.2. Total synthesis of salinosporamide A by (A) E. J. Corey in 2004[233] and (B) Nereus Pharmaceuticals in 2007.[234]

Analogously to the spiro-β-lactone of oxazolomycin, in most of the reported syntheses of salinosporamide A[233,235–239] the fused-β-lactone was introduced towards the end of the synthesis, via activation of the β-hydroxyacid with BOPCl and pyridine. The last step was an OH-Cl exchange with triphenylphosphine dichloride. In the synthesis reported by Nereus Pharmaceuticals (Scheme 4.2 B),[234] after the chlorination step they had the *C*(5)-epimer of the natural product, and the correct stereocentre was established through oxidation with DMP followed by reduction with an enantioselective ketoreductase enzyme. It is worth noting that this synthesis relied on Seebach's SRS and made use of intermediates such as **286**, closely related to some analogues prepared by the Moloney group.

With the OH-Cl exchange being the last step of the synthesis of salinosporamide A, analogues with different *C*(2)-substituents are easily accessible. Nereus Pharmaceuticals have prepared a library of derivatives with different R_1 groups (Figure 4.1) including halogens, non-halogen leaving

groups such as tosylate and mesylate, and non-leaving groups like methyl or alcohol. Other chemical alterations of the natural product included modification of the $C(5)$-cyclohexenyl ring through hydrogenation, epoxidation and subsequent epoxide opening.[70] More chemical diversity on R_2 was introduced through gene inactivation in combination with mutasynthesis.[240] Overall, it was found that the ability of the natural product to inhibit the proteasome could not be substantially improved with these structural modifications.

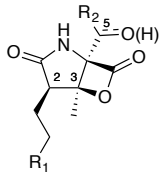
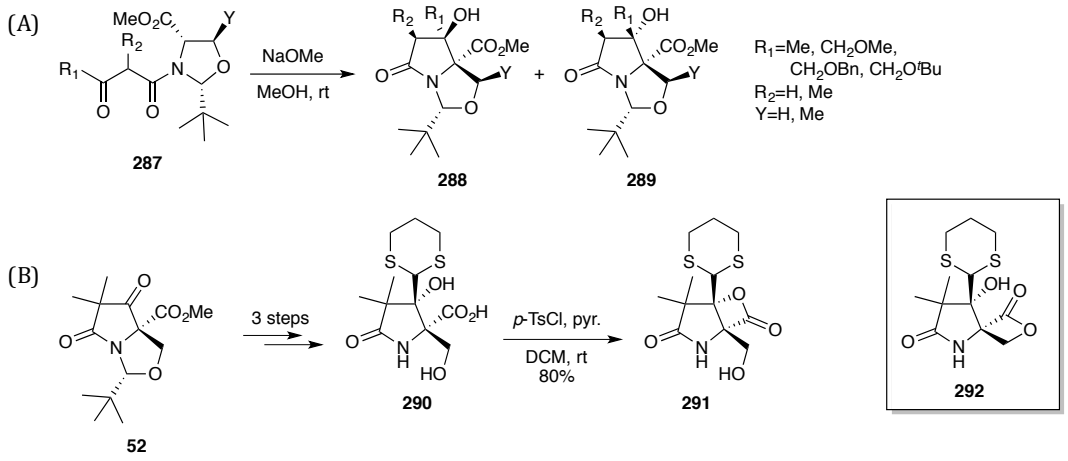


Figure 4.1. Structural diversity of salinosporamide for SAR studies.

4.1.3 Previous work in the Moloney group

Taylor's approach to oxazolomycin[226] and Ling's approach to salinosporamide[234] demonstrate the value of Seebach's protocol for the introduction of stereocentres in a diastereocontrolled fashion, to prepare the pyrrolidinone core of natural products. The Moloney group has also developed methodology to access mimics of such compounds (Scheme 4.3 A). They have shown that aldol ring closure of malonamides **287** gives elaborated pyroglutamates **288** and **289**.[140,141,160,241]

It has also been shown that the tetramate core can be used to prepare pyroglutamic acid **290**, accessed via a kinetically controlled addition of the lithium salt of 1,3-dithiane to **52**,[139] and that it can be lactonised regioselectively to fused-β-lactone **291** instead of spiro-β-lactone **292** (Scheme 4.3 B).[180]



Scheme 4.3. Previous work in the Moloney group involving (A) preparation of an elaborated pyrroldinone core;[140,141,160,241] (B) introduction of a fused-β-lactone.[139,180]

4.1.4 Synthetic objectives

The objective of this chapter was to prove that the reduced tetramate core derived from serine can be used to prepare both fused- and spiro-β-lactones, and to introduce chemical diversity which could be used for SAR studies. While *C*(2)- and *C*(5)-substitutions of salinosporamide have been studied in detail, modification of *C*(3) has not, thus the identification of a synthetic route which allows for late-stage functionalisation of this position would be beneficial. Moreover, this would also permit the preparation of mimics of the pyrrolidinone core of oxazolomycin.

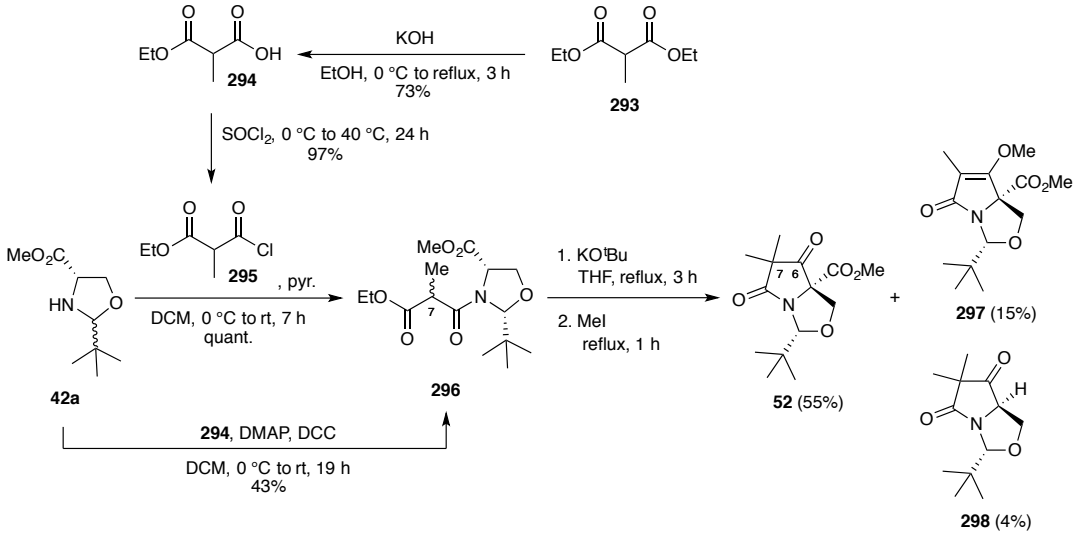
4.2 Introduction of fused- and spiro-β-lactones to the tetramate core

4.2.1 Preparation of a fused-β-lactone

Tetramate **52** was chosen as a simple system for the initial studies towards the formation of spiro-β-lactones. The lack of alcohol group in *C*(6) should avoid the formation of the fused-β-lactone, and the dimethyl substitution at *C*(7) was chosen to avoid the formation of the enol form of the ketone and to decrease the polarity of the intermediates of the proposed synthesis, which was expected to facilitate purification.

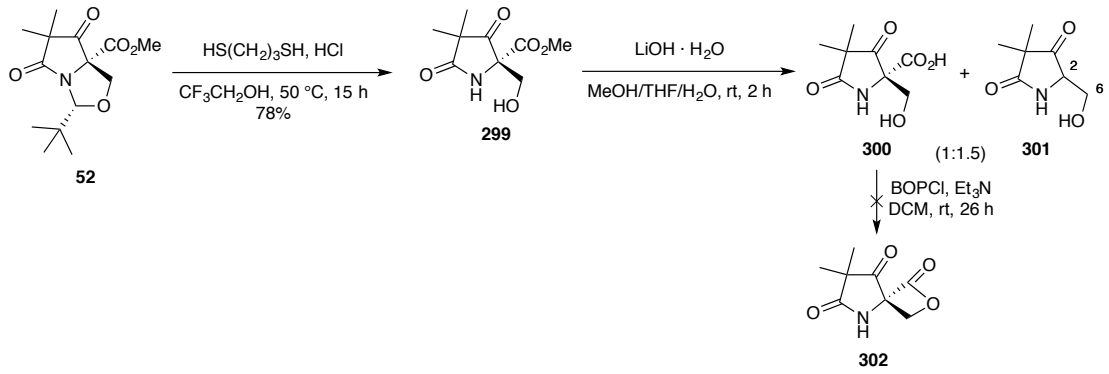
The formation of *N*-acyl oxazolidine **296** (Scheme 4.4) under the previously described coupling conditions with ethyl α-methylmalonic acid **294**, DCC and DMAP proceeded at slower rates than

for **43a**, and in only 43% yield, probably as a result of the increased steric hindrance from the *C*(7)-methyl group. Alternatively, when oxazolidine **42a** was treated with α-methylmalonyl chloride **295**, generated in 2 steps from diethylmethylmalonate,[242] in the presence of pyridine, malonamide **296** was formed in quantitative yields as a 1.5:1 mixture of diastereomers which were only separated for characterisation purposes. Dieckmann cyclisation with potassium *tert*-butoxide followed by methylation in one-pot gave *gem*-dimethyl tetramate **52**, along with a small amount of enol ether **297**. When the Dieckmann cyclisation was performed at large scale (5.6 g), small amounts (4%) of decarboxylated product **298** were also collected.



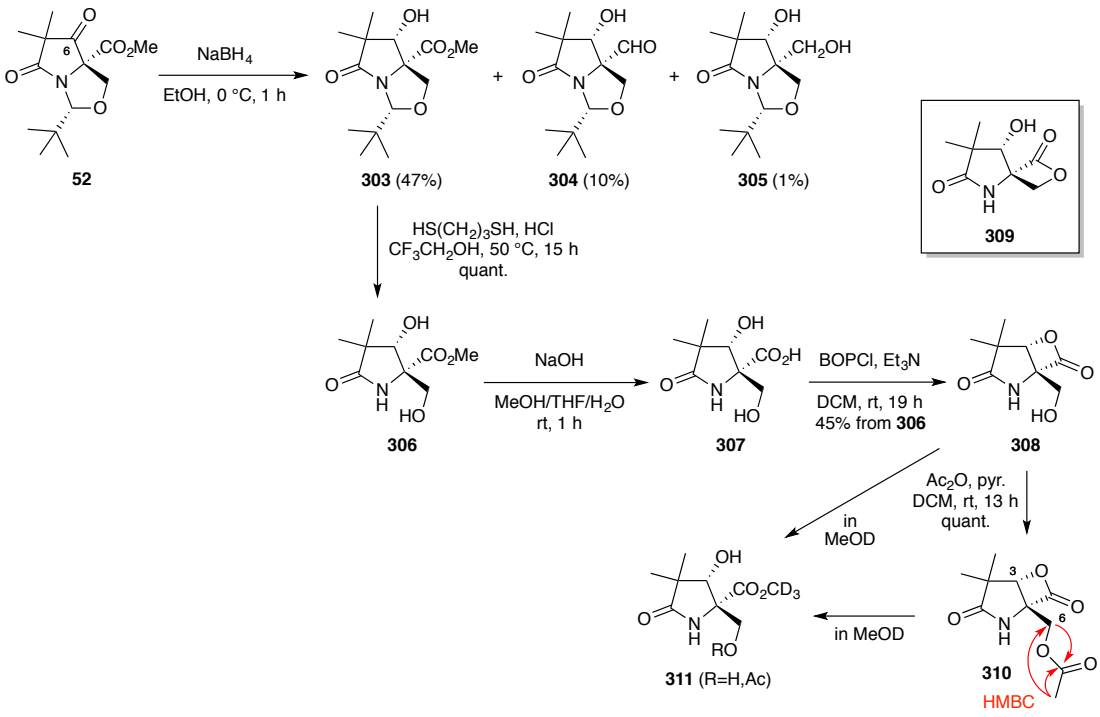
Scheme 4.4. Preparation of tetramate **52**.

N,O-Acetal deprotection of **52** with 1,3-propanedithiol furnished alcohol **299** (Scheme 4.5), which upon saponification with LiOH gave carboxylic acid **300** as a 1:1.5 mixture with the decarboxylated product, evidenced by a dominant 156.1 *m/z* peak on the LRMS spectrum (corresponding to $[M-H]^-$ of **301**) and triplet and doublet of doublet signals from *H*(2) and *H*(6)s in the crude NMR spectrum. Attempted lactonisation of the crude mixture with BOPCl and Et_3N gave a complex crude mixture from which no product was obtained after *flash* column chromatography. Therefore, it was evident that a different substrate was required to study the preparation of spiro-β-lactones, which lacked a carbonyl group at *C*(6) to avoid decarboxylation of the β-hydroxyacid.



Scheme 4.5. Attempted synthesis of spiro-β-lactone **302**.

Reduction of tetramate **52** with $NaBH(OAc)_3$ in DCM gave alcohol **303** as a single diastereomer, as indicated by NOE correlations, but the reaction was much slower than with tetramate **44a** and gave only 75% conversion after 18 h. This result shows the increased steric hindrance of the *C*(6)-carbonyl from the *gem*-methyl groups. Reduction with $NaBH_4$ in EtOH[139] was completed in 1 h and also proceeded diastereoselectively (Scheme 4.6). The low yield of the transformation was due to the formation of small amounts of aldehyde **304** and alcohol **305**, which hampered the purification of the desired product. *N*,*O*-acetal deprotection of **303** yielded alcohol **306**, which upon treatment with LiOH gave acid **307** in low yields (37%) owing to its high polarity. Some acid remained in the aqueous phase (previously acidified with 1 M HCl) even after extracting with EtOAc 20 times. Instead, the crude mixture was concentrated under reduced pressure after acidification, which would be used directly in the following step. For this purpose, NaOH was chosen instead of LiOH as it facilitated the removal of water (because of the higher hygroscopic properties of LiCl than NaCl).



Scheme 4.6. Synthesis of fused-β-lactone **308**.

When the crude acid **307** was reacted with BOPCl and Et_3N, one single product was obtained, which after *flash* column chromatography showed the molecular weight of the β-lactone by LRMS analysis. Following P. Angelov *et al.*'s results (Scheme 4.3 B),[180] it was expected that the fused-β-lactone **308**, and not spiro-**309**, would have been formed, given that the energy difference between both is of the same order of magnitude than that of their reported substrates **291** and **292**, as indicated in Table 4.2. To further support this assignment, the free alcohol of the β-lactone was acetylated and analysed by HMBC spectroscopy, which showed correlations of the acetyl carbonyl and methyl groups with *H*(6)s but not with *H*(3).

Table 4.2. Minimum energy calculations of fused- and spiro-β-lactones. Calculated using Chem3D MM2 energy minimisation.

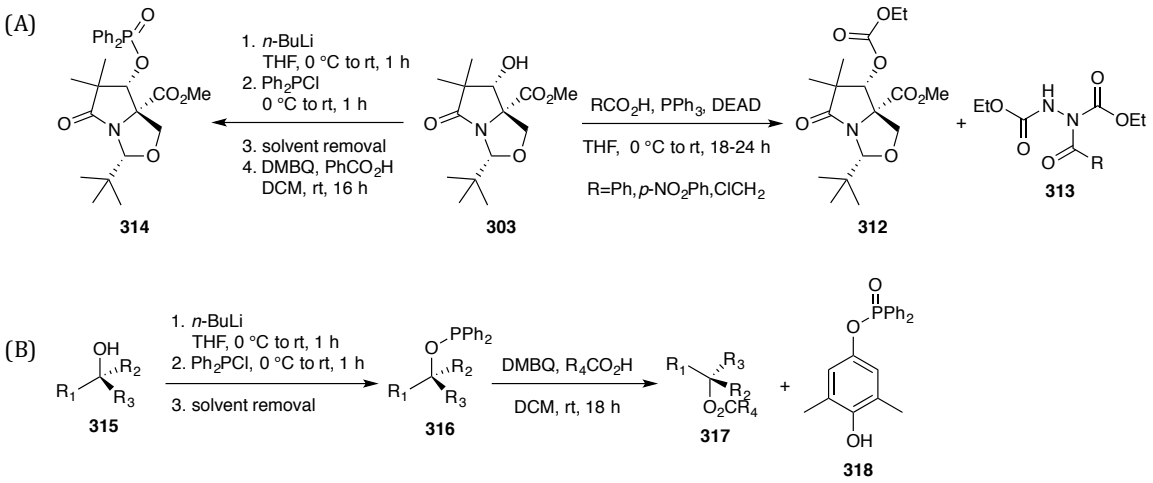
Compound	Minimum energy [kcal/mol]
291 (fused)	52.49
292 (spiro)	56.80
308 (fused)	38.13
309 (spiro)	44.22

Of interest is that, while **308** and **310** were stable in $CDCl_3$, when kept in CD_3OD for 3 days they showed 50% conversion to ester **311**, resulting from attack of the deuterated methanol to the labile β-lactone.

4.2.2 Preparation of a spiro-β-lactone

For the generation of the alternative spiro-β-lactone derived from pyroglutamate **303**, it would be necessary to either protect the *C*(6)-alcohol or to invert its stereochemistry, which would leave the β-hydroxyacid precursor with only one possible mode of cylisation.

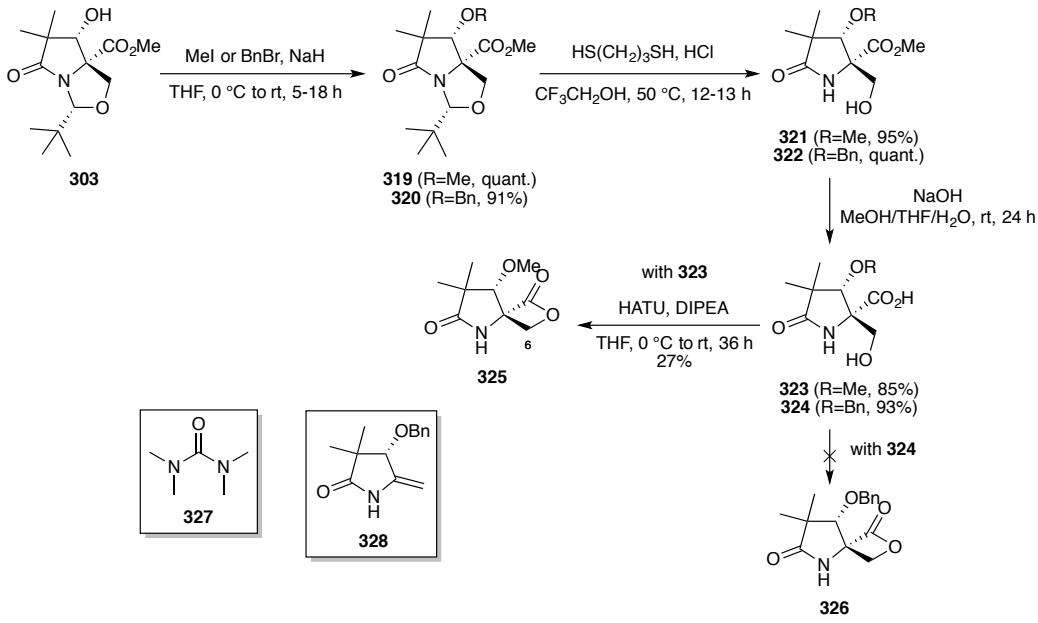
The previous attempt to invert the configuration of alcohol **197a** had been thwarted by its preference to undergo elimination. This side-reaction would not be possible for *gem*-dimethyl alcohol **303**, hence its inversion of configuration under Mitsunobu conditions[208] was examined. However, carbonate **312** and not the desired inversion product was formed when the alcohol was treated with DEAD, triphenylphosphine and any of benzoic, *para*-nitrobenzoic or chloroacetic acid, along with hydrazine **313** (Scheme 4.7); with acetic acid, only starting material and the acetylhydrazine were recovered. These results support the highly hindered nature of the *C*(6)-position.



Scheme 4.7 (A) Unsuccessful inversion of configuration of alcohol **303**; (B) reported synthesis of inverted esters from bulky secondary and tertiary alcohols.[243,244]

Mukaiyama and co-workers have developed a protocol to prepare inverted esters which can be applied to bulky secondary and tertiary alcohols.[243,244] The method, which is outlined in Scheme 4.7 B, involves initial conversion of the alcohol into its alkoxyphosphine, followed by treatment with a carboxylic acid and 2,6-dimethyl-1,4-benzoquinone (DMBQ). However, when alcohol **303** was subjected to these conditions, a non-separable mixture of starting material and phosphine oxide **314** was obtained after column chromatography, and no inversion product.

Since it was established that it was not possible to invert the configuration of alcohol **303**, the alternative alcohol protection was investigated (Scheme 4.8). Displacement with MeI, followed by *N*,*O*-acetal deprotection and saponification gave carboxylic acid **323** in high yields, and in this case it could be isolated after work-up. Attempted lactonisation with BOPCl and Et_3N resulted in a complex crude mixture, but instead with HATU and DIPEA, conditions used for the total synthesis of oxazolomycin A,[116] the spiro-β-lactone was isolated in 27% yield, as a non-separable mixture with urea **327** (derived from HATU) after column chromatography. The crude NMR spectrum showed another set of distinct *H*(6) doublets, in a 2:1 ratio with those of **325**. This by-product, which could not be isolated after column chromatography, might account for the low yield of the lactonisation reaction.



Scheme 4.8. Successful synthesis of spiro-β-lactone **325**.

Preparation of the benzyl ether β-lactone would be of interest as it could later be deprotected under hydrogenation conditions, and it would make an interesting comparison with the fused-β-lactone **308**. Additionally, its reduced polarity would be likely to enable the separation from urea **327**. However, attempted lactonisation of acid **324** with BOPCl and Et_3N or HATU and DIPEA was unsuccessful. Other methods used for the lactonisation of β-hydroxyacids such as the Mitsunobu conditions[245] or via the Yamaguchi anhydride[246,247] also failed to give any of the desired product (Table 4.3). It could be that the bulkier benzyl group hindered activation of the acid, or that it destabilised the β-lactone. In all cases, the crude NMR spectrum showed a complex mixture with unidentified by-products, which could derive from degradation of the acid or of the β-lactone after being formed. The crude NMR spectrum from the reaction with HATU and DIPEA showed doublet signals in the olefinic region, which could correspond to enamide **328** (Scheme 4.8).[180]

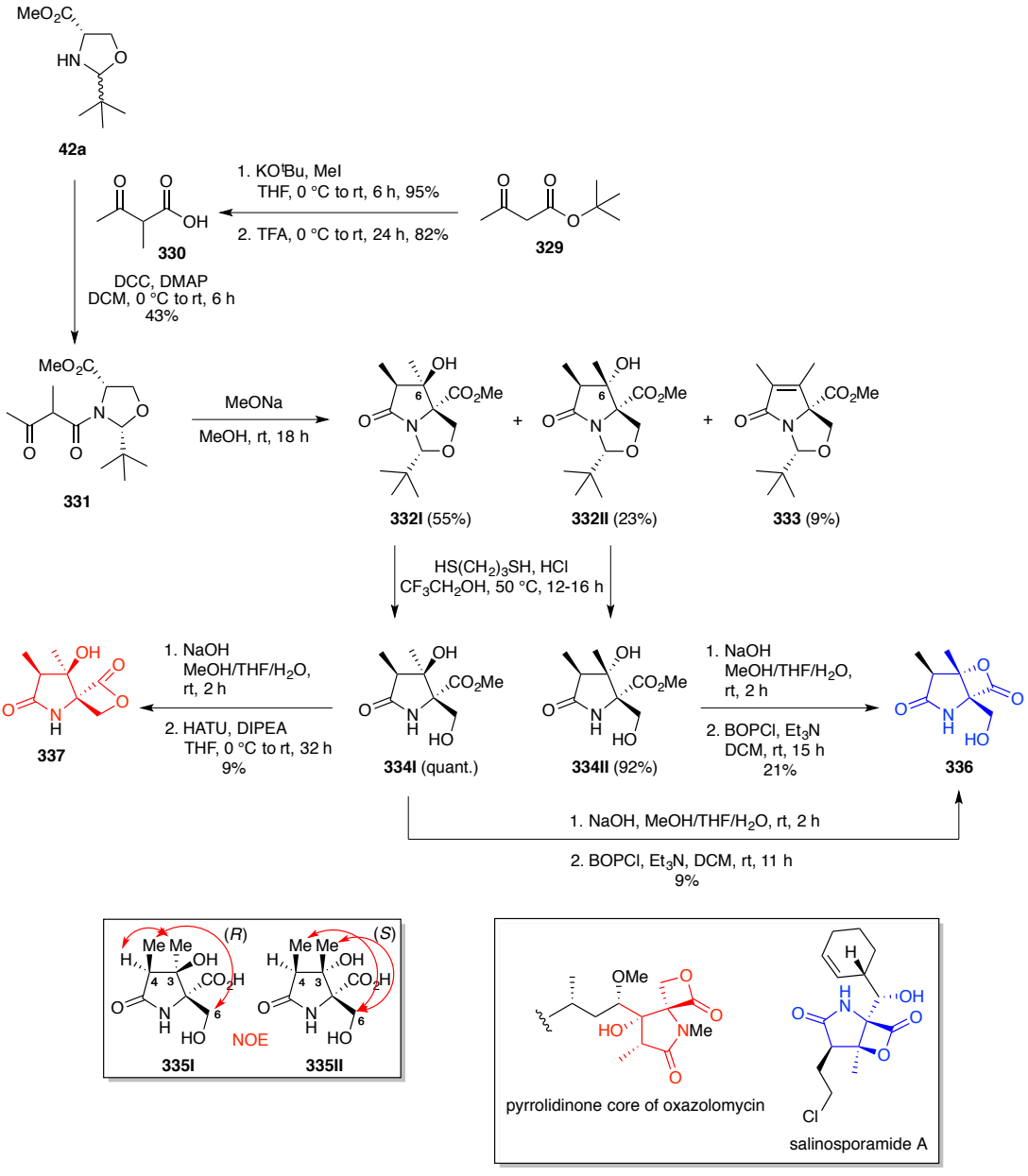
Table 4.3. Attempted conditions for the lactonisation of carboxylic acids **323** and **324**.

Entry	R	Conditions	Outcome
1	Me	BOPCl, Et_3N THF, rt, 17 h	Complex crude mixture, no product isolated after column chromatography.
2	Me	HATU, DIPEA THF, 0 °C to rt, 36 h	Spiro-β-lactone **325** formed in 27% yield as an inseparable mixture with urea **327**.
3	Bn	BOPCl, Et_3N THF, rt, 18 h	No product isolated after column chromatography, unidentified by-products.
4	Bn	HATU, DIPEA THF, 0 °C to rt, 33 h	No product isolated after column chromatography. Crude NMR shows doublets at 5-5.5 ppm which could correspond to enamide **328**.
5	Bn	Ph_3P, DEAD THF, 0 °C to rt, 20 h	Complex crude mixture with some starting material, no product isolated after column chromatography.
6	Bn	2,4,6-$Cl_3C_6H_2COCl$, Et_3N, DMAP DCM, rt, 1 h	Full conversion of the starting material after 1 h, complex crude mixture.

4.3 Preparation of a pyrrolidinone core with *C*(6)Me

Aldol cyclisation of malonamide **331** gave pyrrolidinone scaffolds **332I** and **332II** as a 2.4:1 separable mixture (Scheme 4.9), assigned by NOE correlations, together with small amounts of the elimination product **333**. Remarkably, this ring closure gave only two out of four possible

diastereomers, with the major one **332I** possessing the same relative stereochemistry as oxazolomycin. The absolute stereochemistry of the natural product could be accessed starting the synthesis with D-serine instead of L-serine. Moreover, another interesting factor about diastereomers **332I** and **332II** is that, in view of the results from the previous section, each of them should cyclise selectively to give spiro- and fused-β-lactones respectively.



Scheme 4.9. Preparation of the β-lactone/γ-lactam core of oxazolomycin and salinosporamide.

The *N,O*-acetal unit of the minor diastereomer **332II** was hydrolysed in high yields and the ester saponified with NaOH. The β-hydroxyacid was again too polar to be extracted from the aqueous

layer, thus the crude mixture was acidified, concentrated *in vacuo* and directly used in the following step. Lactonisation with BOPCl and Et_3N gave fused-β-lactone **336** selectively, closely related to the β-lactone/γ-lactam of salinosporamide.

With regards to the major diastereomer **332I**, it was a surprise to find that after being subjected to the same three-step sequence, the same fused-β-lactone **336** was obtained. NMR analysis of the crude acid revealed a 1:1 mixture of isomers, which seemed to derive from a retro-aldol reaction during base hydrolysis, which equilibrated disatereomers **335I** and **335II**. NMR analysis of the crude acid derived from the minor isomer also showed some equilibration, but with a 1:5 diastereomeric ratio of **335I** and **335II**. NOE correlations supported these assignments: acid **335I** showed stronger correlations between C(3)*Me* and *H*(4) than acid **335II**, which showed stronger correlations between *H*(6) and C(3)*Me*. Thus, it would appear that while the 6*R* isomer **332I**, with the C(6)*Me* in the *exo*-face of the bicyclic system, is more favourable than the 6*S* one, after *N*,*O*-acetal deprotection, the steric hindrance of the methyl group is reduced, and it prefers the opposite stereochemistry, which can be achieved by the retro-aldol process.

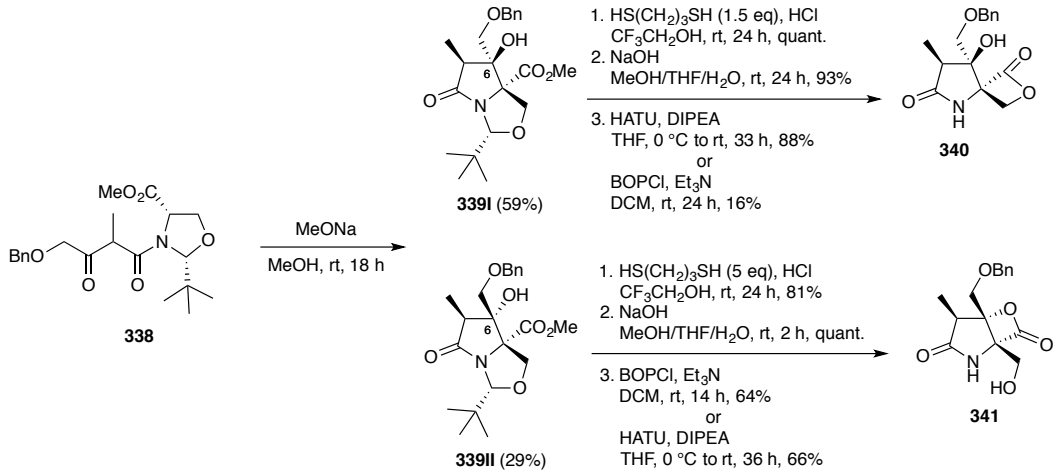
Lactonisation of the crude acid mixture derived from **334I** with HATU and DIPEA furnished a separable 2.5:1 mixture of fused-β-lactone **336** and spiro-β-lactone **337**, with the latter isolated in 9% yield over the two steps. The change in ratio (1:1 diastereomeric acid mixture to 2.5:1 regiomeric β-lactone mixture) could be due to either equilibration of the acids during the reaction or degradation of the spiro-β-lactone.

Formation of the β-lactones was evidenced by their mass in HRMS spectra and by the characteristic IR absorption at 1825-1832 cm^{-1} from the lactone carbonyl.

4.4 Preparation of a pyrrolidinone core with *C*(6)CH_2Bn

4.4.1 Analogues derived from serine

In the interest of further functionalising these systems, to prepare closely related pyrrolidinones suitable for SAR studies, the benzyloxy system **339** would be valuable. Hydrogenation to the free alcohol should allow for substitution of the primary alcohol, more available than the hindered tertiary one.



Scheme 4.10. Preparation of spiro-β-lactone **340** and fused-β-lactone **341**.

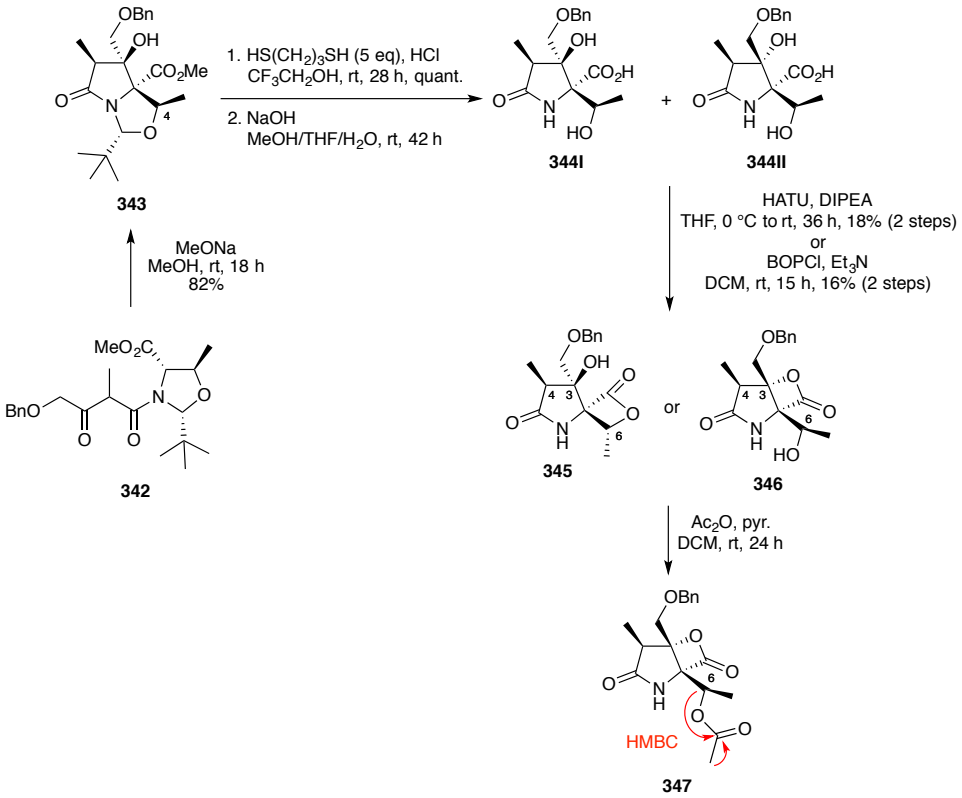
Aldol cyclisation of malonamide **338**, prepared analogously to **331**, gave a 2:1 separable mixture of diastereomers **339I** and **339II** (Scheme 4.10). *N*,*O*-acetal deprotection had to be performed at room temperature, since at 50 °C the benzyl ether was also partially deprotected. Interestingly, while acetal hydrolysis of the major diastereomer was completed after 24 h, for the minor one only 80% conversion was achieved even with 5 equivalents of 1,3-propanedithiol, presumably owing to the presence of the benzyloxymethyl group in the *endo*-face of the bicyclic system, which might hinder access to the acetal. Conversely, for ester hydrolysis, with the minor isomer the reaction was completed in 2 h, while for the major one after the same time only 35% conversion was observed in the crude NMR spectrum, and it required stirring overnight to achieve full conversion. In this case, the benzyloxymethyl group of the major isomer seemed to hinder access to the methyl ester. Another interesting comparison is that the acid equilibration which had been

observed for the *C*(6)Me analogues did not occur in the *C*(6)OBn derivatives, which were isolated after simple work-up as single diastereomers.

Lactonisation of the acids furnished β-lactones **340** and **341** in good yields, showing the expected MH^+ on their HRMS spectra and high wavelength carbonyl absorptions at 1813-1827 cm^{-1}.

4.4.2 Analogues derived from threonine

It was envisaged that it would also be valuable to prepare these analogues starting from threonine, since the additional methyl group should give, for the major isomer, the β-lactone/γ-lactam core of 16-methyloxazolomycin, which has been shown to have potent bioactivities (Section 1.5.2). For the minor isomer, the increased hydrophobicity from the additional methyl group could also be beneficial for its potential proteasome inhibition properties.[232]



Scheme 4.11. Preparation of a β-lactone derived from threonine.

Ring closure of malonamide **342** gave pyroglutamate **343** a single diastereomer (Scheme 4.11). The increased stereoselectivity of this aldol cyclisation probably arose from additional steric

interactions between the *C*(4)-methyl and the bulky benzyloxymethyl groups in the alternative diastereomer, which was not formed. On the contrary, after *N*,*O*-acetal deprotection and ester hydrolysis, where the second required long reaction times, a 2:1 diastereomeric mixture of acids was obtained, together with other minor unidentified by-products. NOE analysis of the complex crude mixture was not sufficient to identify which diastereomer was the dominant one. While NOE interactions in bicyclic tetramates are usually invaluable to determine the relative stereochemistry, for the five-membered rings obtained after *N*,*O*-acetal hydrolysis, the correlations are often not reliable.

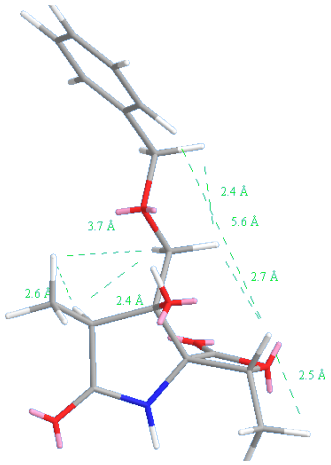
When the crude acid mixture was subjected to β-lactonisation and subsequent column chromatography purification, only one product was isolated, which displayed the correct MH^+ by HRMS analysis and the β-lactone C=O stretch at 1821 cm^{-1}. However, assignment of which one of the spiro- or fused- structures had been formed proved to be challenging. Cyclisation of the acids proceeded in similar yields when using either BOPCl and Et_3N or HATU and DIPEA; by comparison to the results from previous sections this would seem to indicate that the fused-β-lactone had been formed. NOE analysis gave some contradictory data: strong correlations were observed between *H*(4) and C(3)CH_2, which would be consistent with spiro-**345**, but also between C(4)CH_3 and C(3)CH_2, indicative of fused-**346**. *H*(6) and C(3)CH_2 also showed some NOE interactions, which initially seemed to agree with the structure of **346**, but the minimum energy conformations of both compounds (generated using MM2-minimised molecular model with ChemBio3D Ultra version 15) indicated that the distance between *H*(6) and C(3)CH_2 is in fact smaller for spiro-**345** than for fused-**346** (Figure 4.2). Another notable difference between both is the close distance between *H*(6) and one of the aromatic protons for the fused-β-lactone; a weak enhancement of the aromatic protons was observed when irradiating *H*(6).

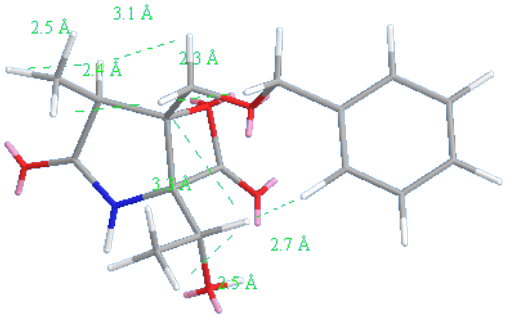
In order to gain further evidence for the assignment of the β-lactone, acetylation of the free alcohol was attempted. Even after stirring with an excess of acetic anhydride and pyridine overnight, the conversion was only 25%. This lack of reactivity would seem to be consistent with the hindered

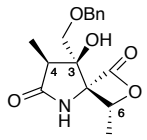
tertiary alcohol of the spiro- structure, however when the crude mixture was analysed by HMBC spectroscopy, correlations were found between the acetyl carbonyl and *H*(6), which would indicate fused-**347**.

Despite having more evidence to support the formation of the fused-β-lactone over the spiro- one, its crystal structure would be needed to unequivocally assign the structure.

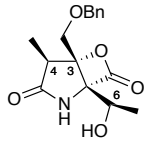
(A)







345



346

(B)

Atoms	Distance [Å]	
	345 (spiro)	**346** (fused)
H(4)-C(4)C*H*$_3$	2.6	2.5
H(4)-C(3)C*H*$_2$	2.4	3.1
C(3)C*H*$_2$-C(4)C*H*$_3$	3.7	2.4
H(6)-C(6)C*H*$_3$	2.5	2.5
C(3)C*H*$_2$-OC*H*$_2$Ph	2.4	2.3
H(6)-C(3)C*H*$_2$	2.7	3.3
H(6)-Ph	5.6	2.7

(C)

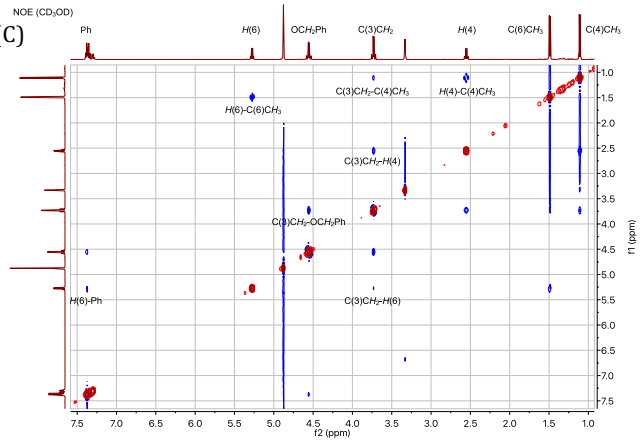


Figure 4.2. (A) Minimum energy conformations of β-lactones **345** and **346** (generated using MM2-minimised molecular model with ChemBio3D Ultra version 15); (B) distances between relevant atoms; (C) NOE spectrum of the synthesised β-lactone.

It is remarkable how the addition of a single methyl group is capable of altering the stereo- and regiochemical outcome of these processes: the diastereomeric excess of the aldol cyclisation of **342**, the equilibration of the carboxylic acids and the β-lactone formation.

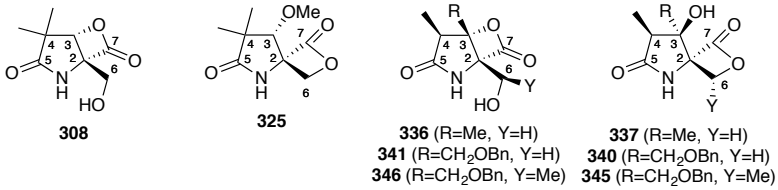
4.5 NMR spectroscopic characteristics of β-lactones

With three fused- and three spiro-β-lactones prepared, it was of value to determine whether their spectroscopic data showed any consistent differences, which could assist with the assignment of newly synthesised β-lactones. Both the comparison between fused- and spiro- structures, but also with their methyl ester precursors, was of interest.

The chart in Figure 4.3 A illustrates the difference (in absolute values) in ^{13}C NMR chemical shifts between fused- and spiro-β-lactones derived from the same 'system' (between **308**-**325**, **336**-**337** and **340**-**341**). It shows that the most distinct chemical shift is for *C*(6), with differences between both structures of 11-12 ppm, followed by *C*(3) with 7-10 ppm. On the other hand, the ^{13}C NMR signals of *C*(2), *C*(4), *C*(5) and *C*(7) are comparable. Similar observations can be made when comparing, for both fused- and spiro-β-lactones, the ^{13}C NMR chemical shifts of the β-lactone and its methyl ester precursor (Figure 4.3 B), with the biggest and most consistent discrepancies found in *C*(6).

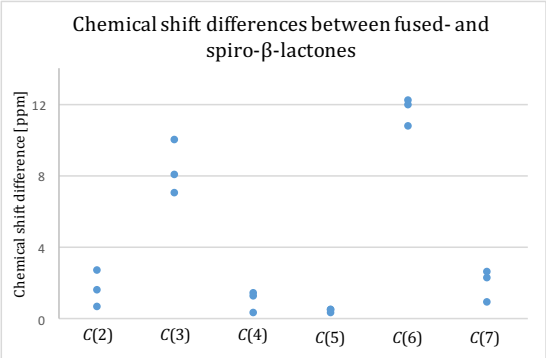
Having a closer look at these values, the table in Figure 4.3 C shows that spiro-β-lactones have *C*(6) chemical shifts 3.6-4.7 ppm higher than the parent methyl esters, while for the fused- ones these are 6.7-8.1 ppm lower. For *C*(3), the opposite trend is observed but the differences are less pronounced, and for **325** this is not in agreement. It could be that the differences between the *C*(3) of fused- and spiro- structures are also a result of their different stereochemistries (3*S* and 3*R* respectively), rather than the nature of the β-lactone.

The ^{1}H NMR signals of *H*(6) are larger for spiro-β-lactones when compared to both the corresponding fused-β-lactone and their methyl ester precursor, while the *H*(6) values of fused-β-lactones are similar to the methyl ester. Another distinct feature is the coupling constant of the *H*(6)s: this value is 11-13 Hz for fused-β-lactones and for the methyl esters, while there is a notable decrease for spiro-β-lactones, for which it halves to 6 Hz.

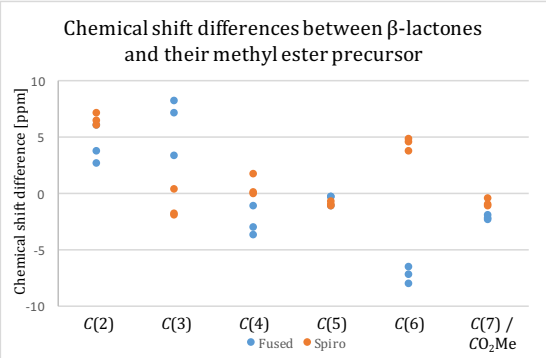


308 | **325** | **336** (R=Me, Y=H), **341** (R=CH_2OBn, Y=H), **346** (R=CH_2OBn, Y=Me) | **337** (R=Me, Y=H), **340** (R=CH_2OBn, Y=H), **345** (R=CH_2OBn, Y=Me)

(A)



(B)



(C)

Compound	Chemical shift [ppm]						J coupling [Hz]	
	C(3)		*C*(6)		*H*(6)[a]		*H*(6)	
	β-L	M. E.	β-L	M. E.	β-L	M. E.	β-L	M. E.
308 (fused)	81.8	78.5	58.7	66.0	3.87	3.86	12.2	11.2
325 (spiro)	88.8	88.5	70.6	66.2	4.47	3.90	6.0	11.4
336 (fused)	87.5	79.4	57.0	65.1	3.88	3.84	12.6	10.8
337 (spiro)	77.5	79.4	67.7	64.1	5.03	4.01	6.3	11.6
341 (fused)	87.6	80.6	57.2	63.9	3.94	3.92	12.5	10.8
340 (spiro)	79.6	81.6	69.4	64.7	4.57	4.02	6.0	11.2
345/346	87.5	79.4	77.5	70.8	4.25	4.18	6.5[b]	6.5[b]

[a]Average chemical shift between $H(6_A)$ and $H(6_B)$.
[b]Coupling between *H*(6) and C(6)CH_3.

Figure 4.3. ^{13}C NMR chemical shift differences between (A) fused- and spiro-β-lactone regioisomers and (B) β-lactones and their methyl ester precursors; (C) comparison of relevant ^{13}C NMR and ^{1}H chemical shifts and *J* couplings for fused- and spiro-β-lactones (β-L) and their methyl ester (M. E.) precursors.

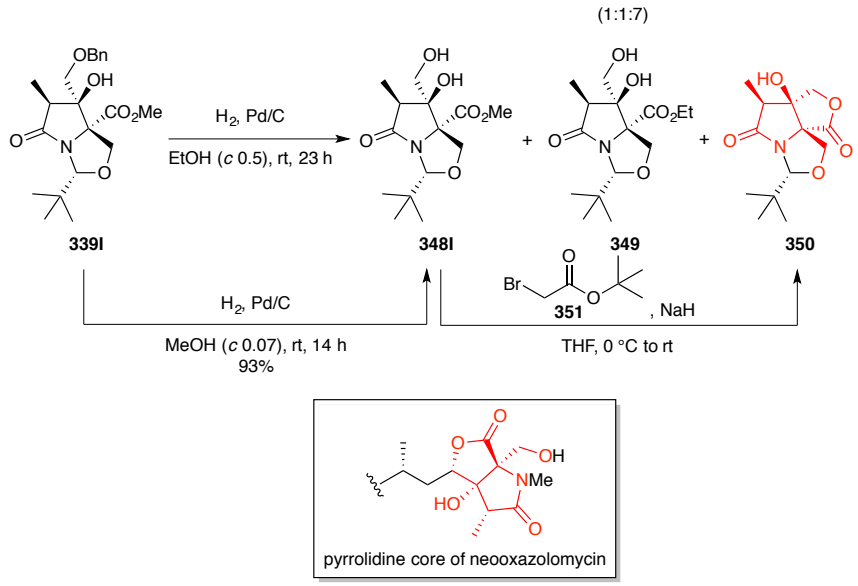
Unfortunately, even with distinctive trends observed for fused- and spiro- structures, spectroscopic analysis of the β-lactone derived from threonine was again contradictory: *C*(3) has a larger chemical shift than the methyl ester, and *H*(6) is similar for both, consistent with a fused-structure, but *C*(6) also has a bigger chemical shift, which would indicate the spiro- structure.

4.6 Further functionalisation of the pyrrolidinone core

4.6.1 Substitution of a short chain alcohol

The first attempt to deprotect the benzyloxy group of **339I** under hydrogenation with Pd/C in EtOH delivered a mixture of products, with the desired alcohol **348I** and ethyl ester **349** as the

minor ones. The major product was γ-lactone **350**, where the deprotected alcohol underwent intramolecular cyclisation with the ester. Despite it not being the desired product, isolation of this side-product is worthy as it actually possesses the same core as neooxazolomycin (Scheme 4.12). This structure was confirmed by X-ray crystallography (Figure 4.4). The yield of the desired alcohol **348I** could be increased to 93% by performing the reaction in MeOH instead of EtOH, at shorter reaction times and under higher dilution.



Scheme 4.12. Hydrogenation of benzyloxy **339I** and attempted alcohol substitution.

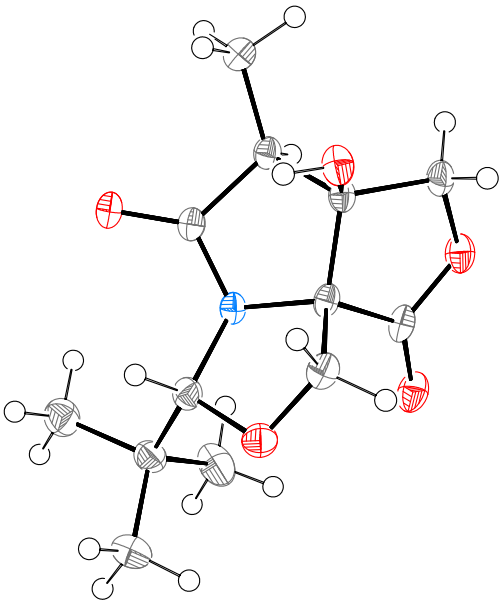
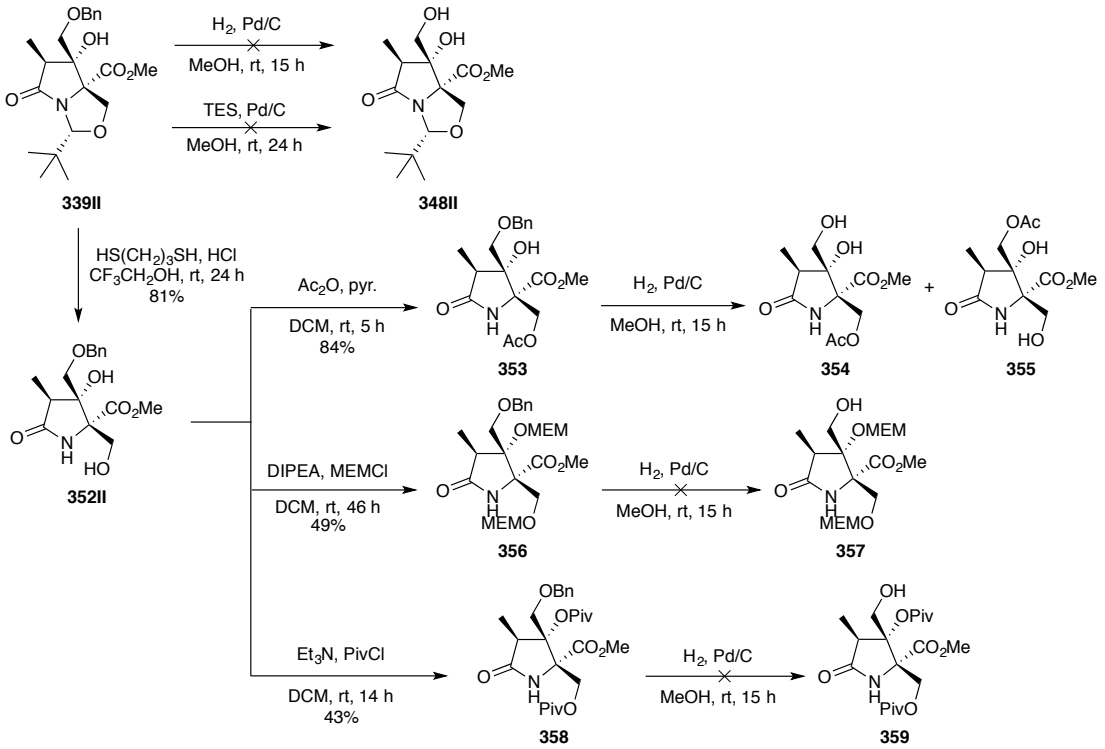


Figure 4.4. X-ray crystal structure of **350**.

That alcohol **348I** could be used to functionalise the pyrrolidinone core via nucleophilic substitution with electrophiles was first tested by reacting it with commercial bromide **351**. Unfortunately, the alcohol preferred to undergo intramolecular substitution and only **350** was obtained even when using a large excess of the bromide.

It was expected that alcohol substitution with the minor isomer would be possible, given that in this case the primary alcohol is on the opposite side from the methyl ester, hence intramolecular cyclisation would not be a possibility. However, hydrogenation of **339II** resulted in quantitative recovery of the benzyloxy starting material, and so did treatment with TES and Pd/C.[248] This lack of reactivity may originate from the hindered nature of the benzyl ether, which is in the *endo*-face of the bicyclic system.



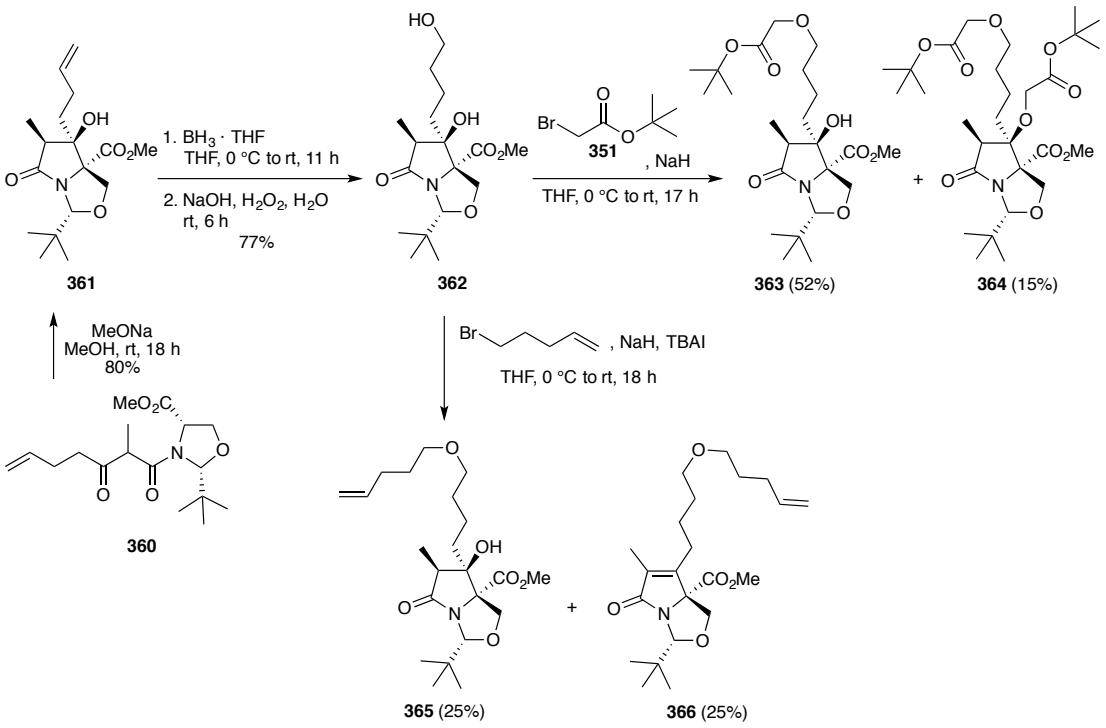
Scheme 4.13. Attempted deprotection of benzyl ethers **339II** and **352II**.

Thus, *N*,*O*-acetal deprotection prior to hydrogenation should enhance access to the benzyloxy group, although previous orthogonal protection of the new primary alcohol would be required. As shown in Scheme 4.13, a number of problems were encountered in this route. Firstly, acetylation

of alcohol **352II** furnished mono-acetyl **353**, but subsequent hydrogenation gave a 1.3:1 mixture of **354** and **355**, probably via intramolecular acetyl migration. Secondly, for the protection as either MEM ether or pivalate ester an excess of reagents was required to reach full conversion, which resulted in a mixture of products, with double protected **356** and **358** as the main isolated products. Upon attempted hydrogenation, only starting material was recovered in both cases, probably owing to steric hindrance. Thus, it was clear that this strategy was not worth pursuing.

4.6.2 Substitution of a long chain alcohol

Another means of avoiding the intramolecular cyclisation of **348I** would be to prepare a longer chain alcohol, such as **362** (Scheme 4.14). For this purpose, malonamide **360** was prepared and subjected to aldol ring closure, which gave alkene **361** as a single diastereomer. Hydroboration-oxidation yielded alcohol **362**, which in this case could undergo substitution with bromide **351** to give ether **363** in moderate yield, along with some double addition product.

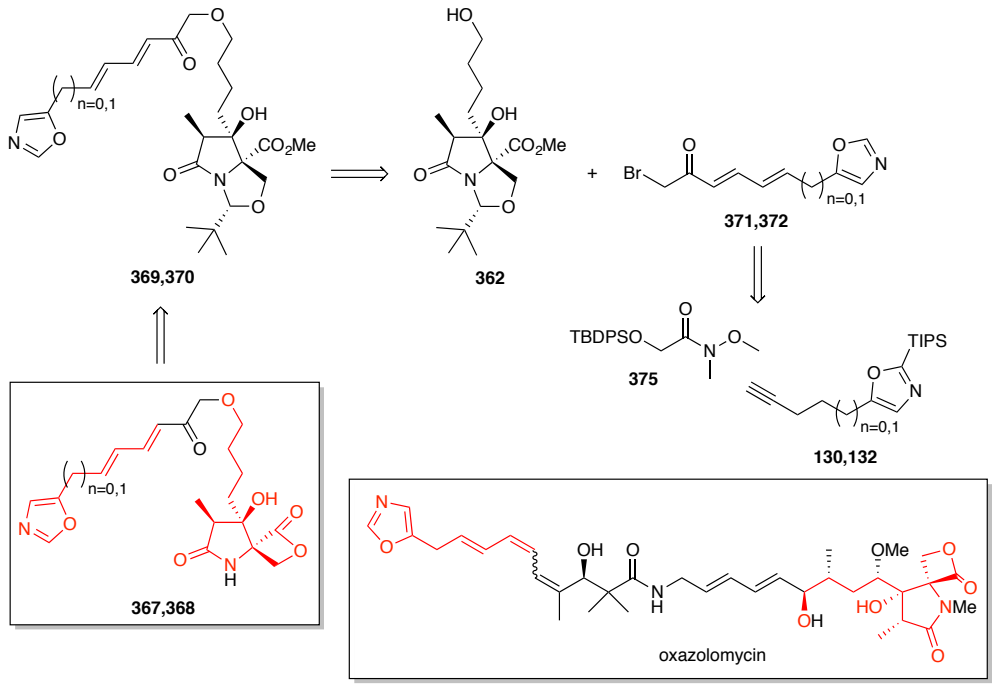


Scheme 4.14. Preparation of alkene **361**, followed by hydroboration-oxidation and alcohol substitution.

Substitution of the alcohol with less reactive bromides, although possible, was less efficient. The formation of ether **365** required the addition of TBAI and an excess of base, which led to concomitant elimination to alkene **366**.

4.6.3 Attempted synthesis of simple mimics of oxazolomycin

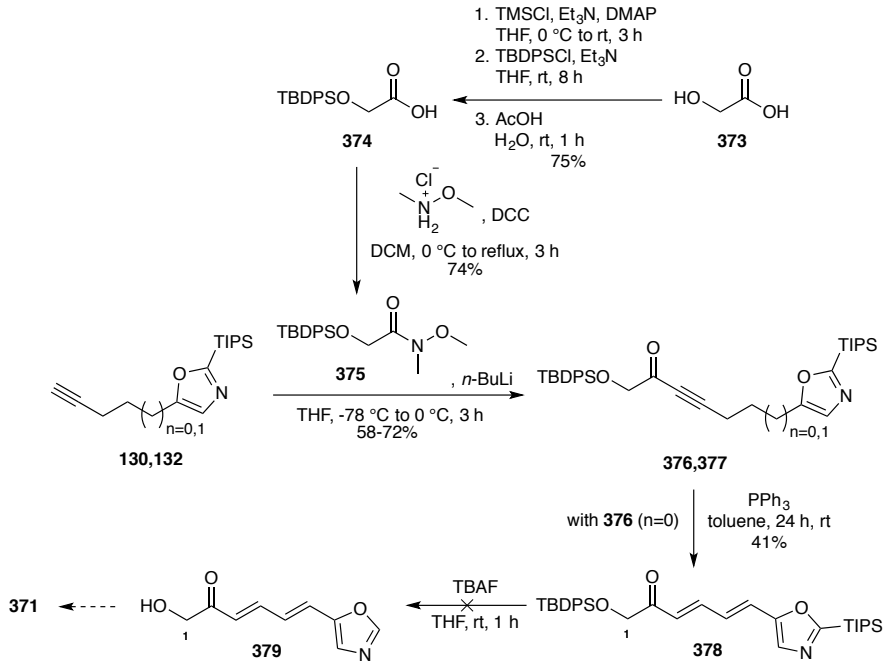
Analogues **367,368** could be considered simple mimics of oxazolomycin, bearing the β-lactone/γ-lactam moiety linked to the left-hand segment, and they could be synthesised from alcohol **362** and bromides **371,372** (Scheme 4.15). The bromides should be accessible from Weinreb amide **375** and alkynes **130,132**, employing the protocols developed in Chapter 2.



Scheme 4.15. Retrosynthetic approach to the simple mimics of oxazolomycin **367**,**368**.

Alkynes **130**,**132** were coupled to Weinreb amide **375**, prepared from glycolic acid using reported methods (Scheme 4.16).[249,250] Isomerisation of the shorter alkyne **376** (n=0) gave (*E*,*E*)-diene **378**, which required 2 equivalents of triphenylphosphine to achieve completion. The low isolated yield was unexpected, as the crude NMR spectrum showed essentially desired product and aromatic signals from PPh_3, and it could be that the dienone was not particularly stable to silica gel. Isomerisation of the longer alkyne **377** was expected to be less efficient, in view of the results

from the analogous alkyne **136** (Scheme 2.32, Section 2.7.2). Indeed, the crude NMR showed a mixture of products, and after column chromatography only small amounts of desired dienone product were collected, which could result again from low stability.



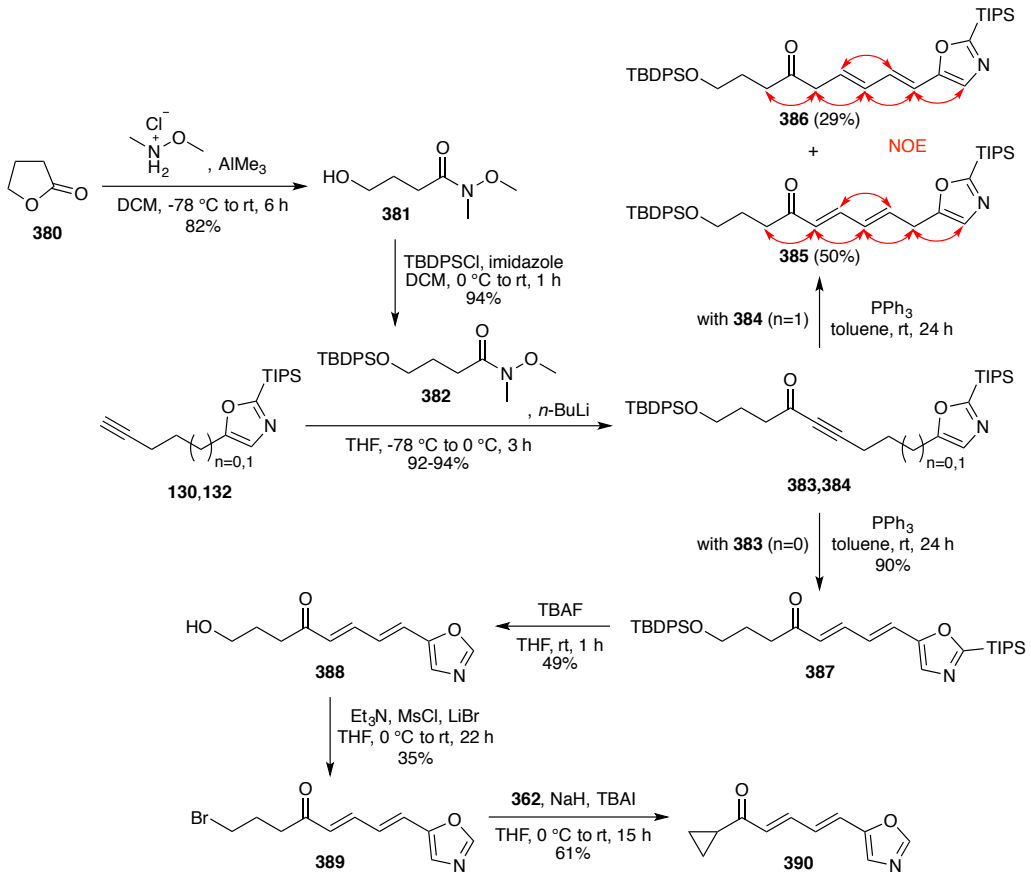
Scheme 4.16. Attempted synthesis of bromides **371**,**372**.

Transformation of **378** into alcohol **379** was unsuccessful. When the silyl ether was treated with TBAF for 1 h, the crude NMR spectrum showed only signals from the TBDPS and TIPS groups, but no *H*(1) singlet or alkene signals. When the reaction mixture was evaporated *in vacuo* instead of being subjected to aqueous work-up, the crude NMR spectrum showed a low field singlet at 9 ppm and broad signals in the olefinic region. It could be that the alcohol spontaneously oxidised to the aldehyde upon hydrolysis of the silyl ether.

It was plausible that degradation of α-hydroxyketone **379** could be avoided by increasing the distance between the alcohol and the carbonyl functionalities, such as in **388** (Scheme 4.17).

Weinreb amide **382** was prepared following reported methods[251,252] and coupled to alkynes **130**,**132** in high yields. Isomerisation of the shorter alkyne **383** yielded diene **387**, while for the longer one the desired diene **385** (assigned with HMBC correlations) was isolated in 50% yield,

along with its regioisomer **386**. The silyl ether of **387** was deprotected with 2 equivalents of TBAF to furnish alcohol **388**, and subsequently brominated to **389**. In both of these reactions, the crude NMR spectrum showed almost exclusively the desired product (with 20% remaining starting material for the second one), and the low yields could again be due to the low stability of these intermediates during column chromatography purification.

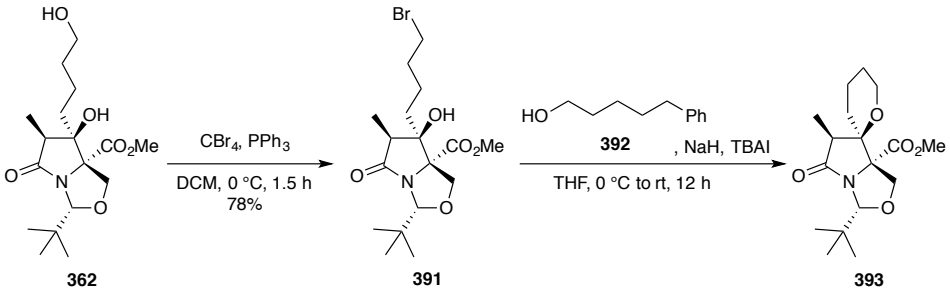


Scheme 4.17. Synthesis of bromide **389** and attempted substitution with alcohol **362**.

With bromide **389** in hand, displacement of alcohol **362** was attempted. Unfortunately, it was highly disappointing to recover quantitative unreacted alcohol, while the bromide was converted into cyclopropyl **390**, presumably via an intramolecular cyclisation of the enolate.

Due to the apparent trend of bromide **389** to cyclise intramolecularly, an alternative approach was devised where the leaving group would be introduced into the pyrrolidinone core, and reacted with alcohol **388**. However, when this was tested with bromide **391**, prepared via Appel

reaction[200] of alcohol **362**, and the commercial alcohol **392**, again an intramolecular cyclisation occurred and spirocyclic compound **393** was formed instead of the desired ether, as shown in Scheme 4.18.



Scheme 4.18. Attempted substitution of bromide **391**.

4.7 Summary of Chapter 4 and future work

This chapter demonstrates the feasibility of using reduced versions of the tetramate core to introduce both fused- and spiro-β-lactones.

With the first tetramate system used, bearing a *gem*-dimethyl in *C*(7), *C*(6)-ketone reduction was required to avoid decarboxylation of the β-hydroxyacid, precursor of the β-lactones. The reduction proceeded diastereoselectively, and after *N*,*O*-acetal deprotection and ester saponification, ring closure with BOPCl and Et_3N furnished the fused-β-lactone regioselectively, via reaction of the carboxylic acid with the *C*(6)-alcohol. Preparation of the alternative spiro-β-lactone was also possible, with previous methylation of the *C*(6)-hydroxyl and using HATU and DIPEA.

Generation of more elaborated pyrrolidinone systems relied on the intramolecular aldol cyclisation of malonamides, which generally furnished mixtures of two diastereomers. The major one had the same relative stereochemistry as oxazolomycin, while the minor one was similar to salinosporamide. Both were subjected to lactonisation to give spiro- and fused- structures respectively, similar to the natural products. A pyrrolidinone analogue derived from threonine

was also synthesised, but identification of the product obtained after lactonisation was challenging.

The ^{1}H and ^{13}C NMR spectra of the synthesised fused- and spiro-β-lactones showed some consistent trends. The biggest differences were observed for $C(6)$, where the former had lower shift values than the latter, with differences of up to 12 ppm. The coupling constants of $H(6)$s were also distinctive: the former had similar values (12.2-12.6 ppm) than their methyl ester precursors, while for the latter a large decrease was observed, giving values of 6.0-6.3 ppm.

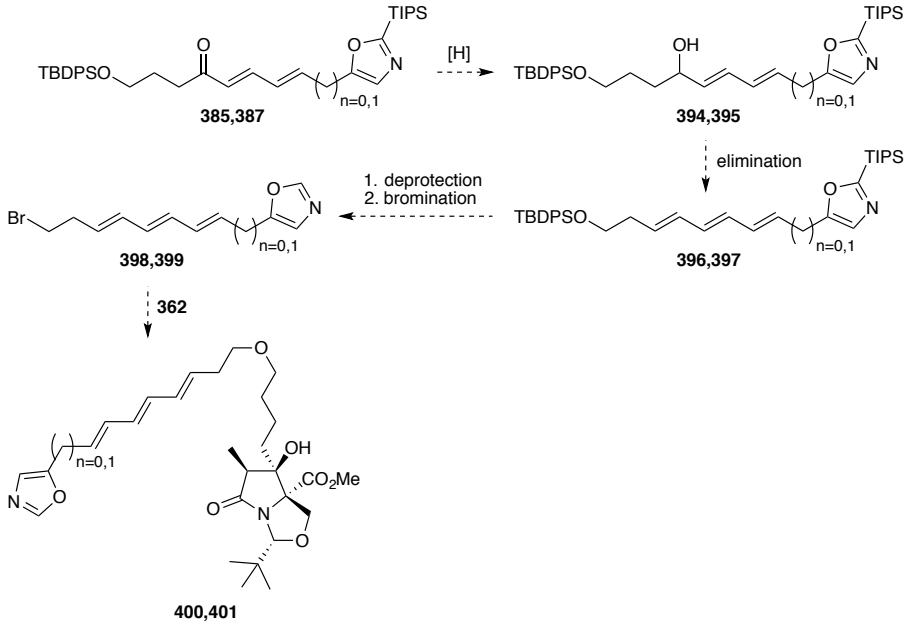
It was envisaged that further functionalised analogues of these systems would be accessible by means of nucleophilic substitution of a $C(6)$-hydroxymethyl derivative. Initially, this approach was thwarted by the intramolecular cyclisation of the alcohol, which interestingly provided the core of neooxazolomycin. This side-reaction could be avoided through preparation of a longer chain alcohol, a $C(6)$-hydroxybutyl, which was shown to undergo substitution with bromides.

For the preparation of simple mimics of oxazolomycin, possessing both its pyrrolidinone core and the left-hand segment, the preparation of oxazole-diene bromides was investigated. Unfortunately, the route was hampered by the instability of some of the intermediates and the side-reaction of the synthesised bromide, which underwent intramolecular cyclisation via the enolate instead of substitution of alcohol **362**.

In brief, elaborated pyrrolidinones derived from serine and threonine have been prepared, which have been manipulated by introduction of fused- and spiro-β-lactones and by functionalisation of the $C(6)$-position.

Future efforts to prepare mimics of oxazolomycin could involve the synthesis of bromides **398,399** (Scheme 4.19), to give analogues **400,401**. Conversion of the carbonyl group into the alkene should not only avoid the intramolecular side-reaction, but also give mimics with even closer structures to the natural product. Future work could also focus on the substitution of

alcohol **362** with simple commercial alkene bromides, to prepare derivatives such as alkene **365**. The double bonds could then be elaborated, for example by cross-coupling reactions, epoxidation, hydroxylation and dihydroxylation. Furthermore, alcohol **362** could be oxidised to the aldehyde and subjected to Wittig or Julia olefinations.



Scheme 4.19. Proposed future work for Chapter 4.

CHAPTER 5: EVALUATION OF BIOLOGICAL PROPERTIES

This final chapter reports the evaluation of the antibacterial and anticancer properties of analogues synthesised in Chapters 2-4. The antibacterial activity was evaluated using the hole-plate method, which revealed that aliphatic lipophilic *C*(7)-acyltetramates inhibit Gram-positive *S. aureus* selectively. This activity was confirmed with a broth dilution assay, which showed that some of the analogues possess MIC values <2 μg/mL. For the anticancer activity, a high-content screen was designed and performed, which identified some compounds active against several cell lines. The IC_{50} values of these were later found to be 10-20 μM.

5.1 Antibacterial properties

5.1.1 Introduction

5.1.1.1 The challenges of antibacterial drug discovery

Following the determination of the complete DNA sequence of a bacterial genome from *Haemophilus influenzae* in 1995, GlaxoSmithKline (GSK) spent six years (1995-2001) evaluating more than 300 genes as potential targets for novel antibiotics.[2] 160 of these were identified as essential bacterial targets and 70 high-throughput screens, mostly target-based, were run with the company's synthetic chemical collection with the objective of finding a novel antibacterial compound with either Gram-positive or broad-spectrum activity. To their disappointment, only 16 HTSs provided hits, from which only 5 gave rise to leads. This represents a four- to five-fold lower success rate than for targets from other therapeutic areas.

With this lack of success, Payne and co-workers[2,253] recognised that "a different paradigm for antibacterials is needed". Firstly, compounds with single enzyme targets have a larger chance of evolving resistance by single point mutation; thus, small molecules that target multiple enzymes from more than one family would be advantageous. Secondly, chemical diversity of the HTS is essential. As discussed in Chapter 1, known antibacterials often do not follow Lipinski's 'rule of

five',[7] while most pharmaceutical chemical collections have compounds that do. It is necessary to generate libraries with compounds that have the required physicochemical properties for antibacterials.

Payne also identified the challenges of converting good enzyme inhibitors into permeative molecules. For this purpose, whole-cell assays are attractive, where the required characteristics of an antibacterial, such as cell penetration, are already a prerequisite for obtaining hits. However, subsequent identification of the MOA, required for a rational SAR, is often difficult. A possible approach is that of J. A. DeVito *et al.*, who engineered strains of *E. coli* to under-express a single gene product, making each of them highly susceptible to specific inhibitors of that gene target.[254]

5.1.1.2 Known classes of antibacterial drugs

Antibiotics can either kill bacteria (bactericidal) or inhibit growth (bacteriostatic). Common targets for the bactericidal activity of antibiotics are bacterial cell wall biosynthesis, protein biosynthesis and nucleic acid synthesis.[255] Figure 5.1 shows examples of known antibiotics from different classes.

Examples of antibiotics inhibiting bacterial cell wall biosynthesis are β-lactams and glycopeptides. The peptidoglycan layer of bacterial cell wall, essential for its structural integrity, consists of strands of covalently crosslinked peptides and glycans. β-Lactams act by inhibiting the transpeptidase enzymes, which are responsible for the late stages of peptidoglycan biosynthesis.[256] This results in osmotic instability or autolysis and leads to bacterial death. On the other hand, glycopeptides such as vancomycin form stable complexes with the D-Ala-D-Ala subunit of the peptidoglycan layer, which increases steric hindrance and prevents the transpeptidases from adding new subunits, again leading to a weaker cell wall.[257]

Protein synthesis is a multi-step process than implicates several enzymes. However, the majority of the antibacterial compounds that inhibit protein biosynthesis act at the ribosome, which is

constituted by two subunits: 30S and 50S. Initiation, elongation and termination of protein assembly by the ribosome involve a large number of steps, which has led to several classes of protein-synthesis inhibitors. Examples of antibiotics that act at the 30S subunit are aminoglycosides, which perturb the elongation of nascent chains and cause premature chain termination,[258] and tetracyclines, which prevent association of aminoacyl-tRNA with the bacterial ribosome.[259] Chloramphenicol, macrolides, streptogramins and oxazolidinones are other classes of antibiotics, which act on the 50S subunit.[260]

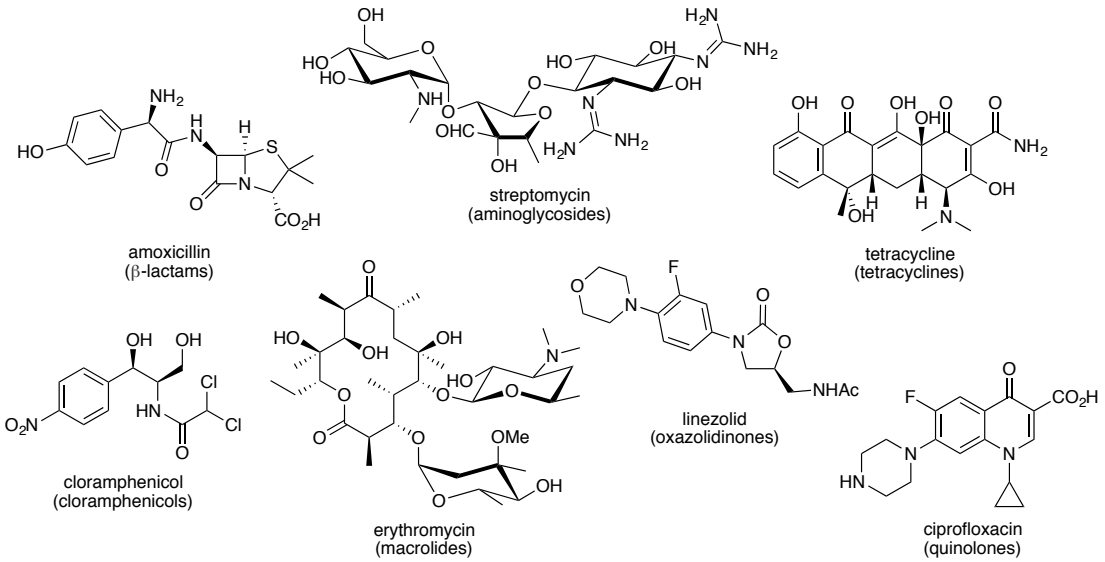


Figure 5.1. Examples of known antibiotics from different classes.

Inhibitors of nucleic acid synthesis act on enzymes responsible for DNA replication and repair. DNA gyrase and topoisomerase IV (topoIV) are enzymes responsible for unlinking DNA after each round of replication, and for catalysing negative supercoiling. Quinolones and fluoroquinolones are synthetic classes of antibiotics that inhibit these enzymes, promoting double-stranded DNA breakage.[261]

5.1.2 Evaluation of the antibacterial activity of synthesised analogues

The antibacterial activity of the synthesised analogues was initially evaluated using the hole-plate method. This phenotypic assay was chosen as it provided quick results for preliminary evaluation of the antibacterial properties, giving also a direct method of assessing bacterial cell wall

permeability. However, unlike broth assays, this assay does not provide MIC values of active compounds.

Some of the compounds were also sent to SeaLife Pharma for the determination of MIC values and for preliminary toxicity evaluation.

5.1.2.1 Hole-plate method

Whole-cell antibacterial assays were performed using the hole-plate method[262] with Gram-positive *Staphylococcus aureus* DS267 and Gram-negative *Escherichia coli* X580. Samples were prepared as 4 mg/mL solutions in 1:1 MeOH/DMSO, and diluted to lower concentrations where necessary. A 100 µL aliquot of each solution was loaded into wells of 10 mm of diameter in agar plates and incubated at 37 °C for 16-20 h. The diameters of the resultant inhibition zones were measured along two perpendicular axes and averaged to obtain the zone of inhibition. The assays were repeated in duplicate. A negative control was run with solvent alone.



Figure 5.2. Example of an agar plate after incubation with samples overnight, showing clear inhibition zones.

Cephalosporin C (CephC), a known antibiotic of the β-lactam class, was used as a standard. Calibration curves were performed at 2-100 µg/mL for *E. coli* and 200-1000 µg/mL for *S. aureus*. The relative potency of the analogues was estimated by reference to CephC, calculated as the moles of CephC required to give the same zone size as the compound of study, with respect to its number of moles.

Results for the antibacterial activities of selected compounds are represented in bar charts (full data in Appendix F), indicating the zone diameter at the specified concentration. A larger zone size signifies a stronger antibacterial activity. A 'H' represents a 'halo' zone of inhibition, where a zone of reduced bacterial density was observed instead of a clear inhibition zone (Figure 5.2). A 'P' signifies that a solid was observed in the well after incubation, indicating that some of the compound precipitated before diffusing into the agar plate.

5.1.2.2 Compounds from Chapter 2: hole-plate assay

Figure 5.3 presents the antibacterial activity of tetramate cores **44a-c** and the first small library of *C*(7)-acyltetramates, including the Weinreb amide and the acetyl, aryl and alkenyl analogues. The alkynyl analogues were not tested due to their low stability. Most of the compounds showed no activity against *E. coli*, with only the aryl derived from cysteine showing a small halo zone.

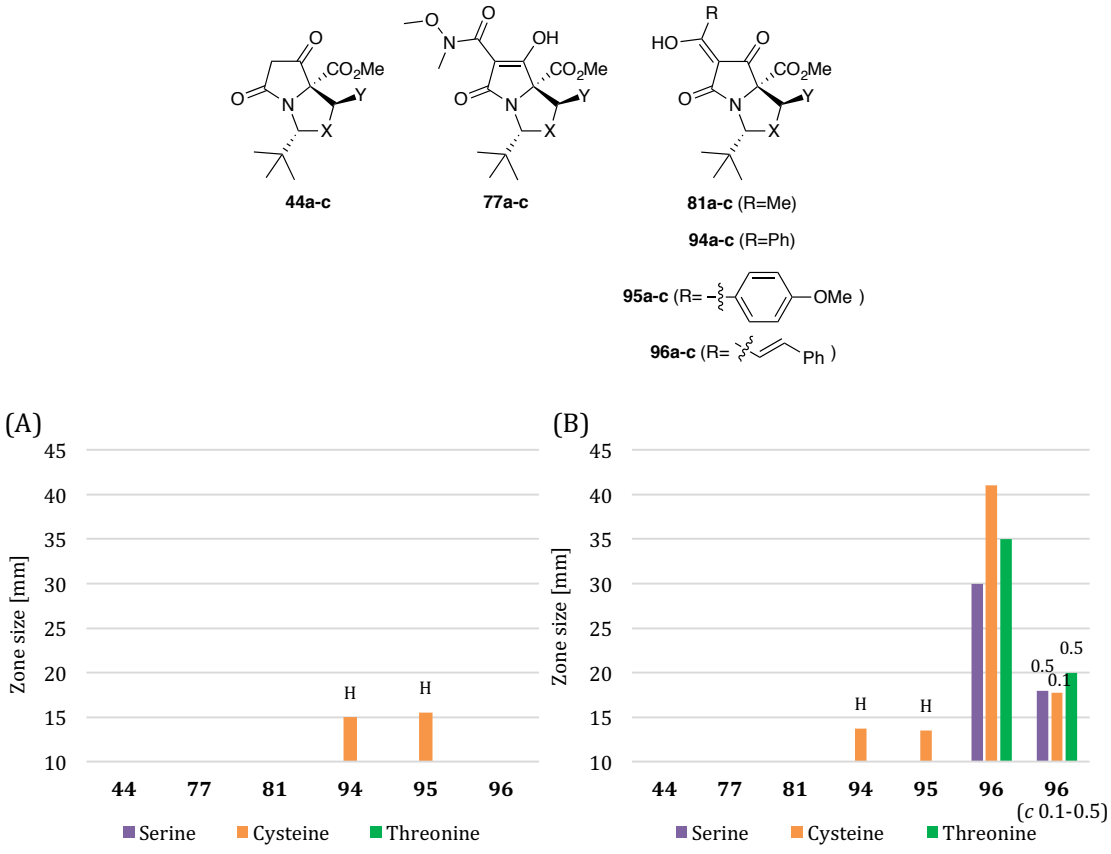


Figure 5.3. Antibacterial activity of *C*(7)-acylated analogues at 4 mg/mL (except **96a-c**, also tested at 0.1-0.5 mg/mL) against (A) *E. coli* and (B) *S. aureus*.

The activity against *S. aureus* was highly dependent on the nature of the acyl group. The non-acylated tetramate cores showed no activity, and neither did the Weinreb amide or the acetylated compounds. The aryl ones showed only halo zones for the cysteine systems. Conversely, the alkenyl analogues exhibited high potencies, with zone sizes of 18-20 mm at 0.1-0.5 mg/mL. This would represent a relative potency with respect to CephC of 1.6, 7.9 and 2.2 for the serine, cysteine and threonine systems respectively.

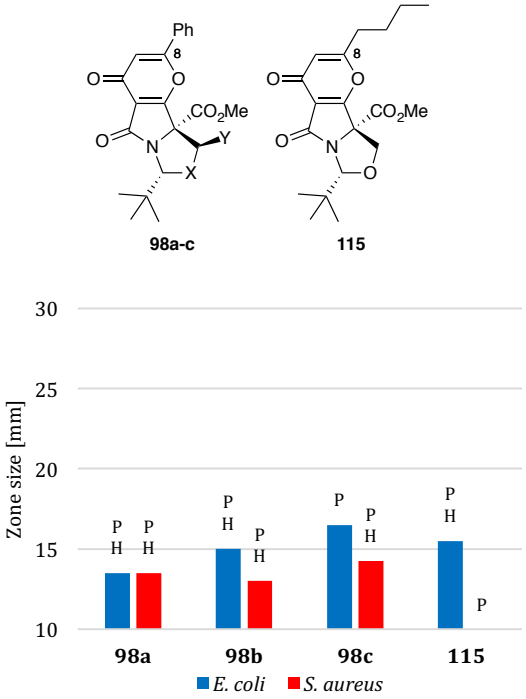


Figure 5.4. Antibacterial activity of tricyclic analogues at 4 mg/mL against *E. coli* and *S. aureus*.

Tricyclic compounds **98a-c** and **115**, derived from the degradation of alkynone tetramates, were also examined. The four compounds precipitated in the wells after overnight incubation, probably as a result of their low polarities, which decreased their solubility. Nevertheless, they still showed weak activities against *E. coli* and *S. aureus*, with bigger inhibition zones for the former. This would suggest that optimisation of these structures to improve solubility, for example via introduction of polar heterocycles at *C*(8) or deprotection of the *N*,*O*-acetal, could result in a new series of tetramate-derived analogues with interesting bioactivities. In fact, a recent report from the Moloney group indicated that the related polycyclic derivative **402** (Figure 5.5 A) exhibited some

Gram-positive activity,[263] but in their case the two amino groups significantly increased polarity, and consequently solubility, of the compound.

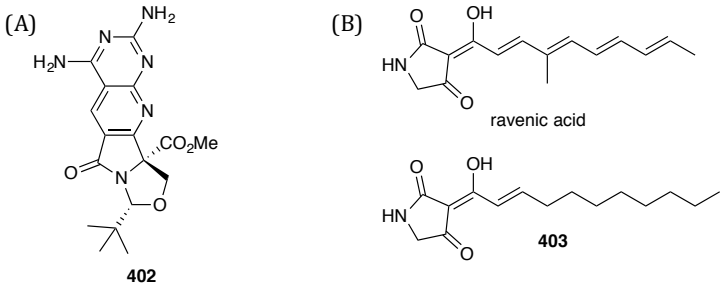


Figure 5.5. (A) Polycyclic derivative prepared by the Moloney group;[263] (B) enoyltetramates studied by R. Schobert and co-workers.[264]

Interestingly, acetoxy **86** showed some inhibition of *S. aureus*, but not carbamate **87** (Figure 5.6). Given the leaving group nature of the tetramate core (Section 2.3.1), the activity observed for **86** could be ascribed to its ability to acetylate its target(s), and the stronger carbamate bond in **87** could account for its lack of activity.

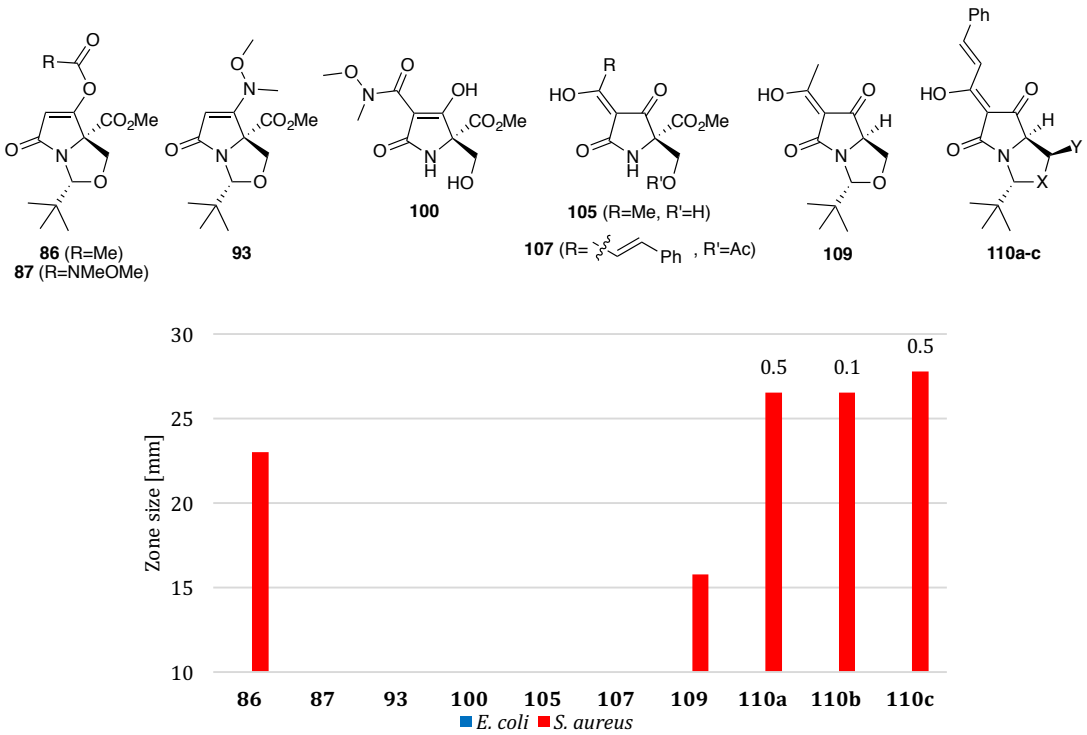
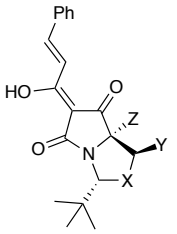


Figure 5.6. Antibacterial activity of modified *C*(7)-acylated analogues and derivatives at 4 mg/mL, except **110a-c** at 0.1-0.5 mg/mL, against *E. coli* and *S. aureus*.

The *N*,*O*-acetal deprotected products **100**, **105** and **107** displayed no inhibition, which would be consistent with results from Y.-C. Jeong *et al.* which showed that the *tert*-butyl group of related bicyclic *C*(7)-acyltetramates was essential for antibacterial activity.[135] Conversely, decarboxylation led to an improvement of Gram-positive activity: acetyl **109** showed some activity at 4 mg/mL, while its analogous methoxycarbonyl **81a** was inactive. The alkenyl analogues, which were already potent inhibitors, also displayed enhanced activities, with a three- to four-fold increase of zone size after decarboxylation. The cysteine analogues were again superior, and the most active analogue **110b** was 27-fold more potent than the standard CephC (Table 5.1).

Table 5.1. Relative potencies of alkenyl analogues with respect to the standard CephC, with and without the methoxycarbonyl group.



96a-c (Z=CO_2Me)
110a-c (Z=H)

Compound	X, Y	Z=CO_2Me	Z=H
96a, 110a	O, H	1.6	4.4
96b, 110b	S, H	7.9	27
96c, 110c	O, Me	2.2	5.6

Figure 5.7 illustrates the antibacterial activity of analogues that mimic the natural products pramanicin and oxazolomycin. With regards to *S. aureus*, the results clearly show that lipohilic aliphatic side-chains are important for conferring activity, as dienoyls **114,122,123** and the saturated analogues **124-126** exhibited potent inhibitions. The activity was also dependent on the length of the acyl chain, and notably the highest activities were observed not for **123** and **126**, which had the same length as the side-chain of pramanicin, but for the shorter ones **122** and **125**. It was of surprise to find that the dienoyl derivatives were slightly less potent than the analogous saturated compounds. In this respect, R. Schobert and co-workers have reported that a high degree of unsaturation at the side-chain is not a prerequisite for the antibacterial activity of acyltetramates. They found that **403** (Figure 5.5 B), a structural analogue of the natural ravenic acid, was equally or more active against Gram-positive bacteria than the natural product.[264]

The most active compound of these series, saturated undecylidene **125**, had a relative potency of 85 with respect to CephC.

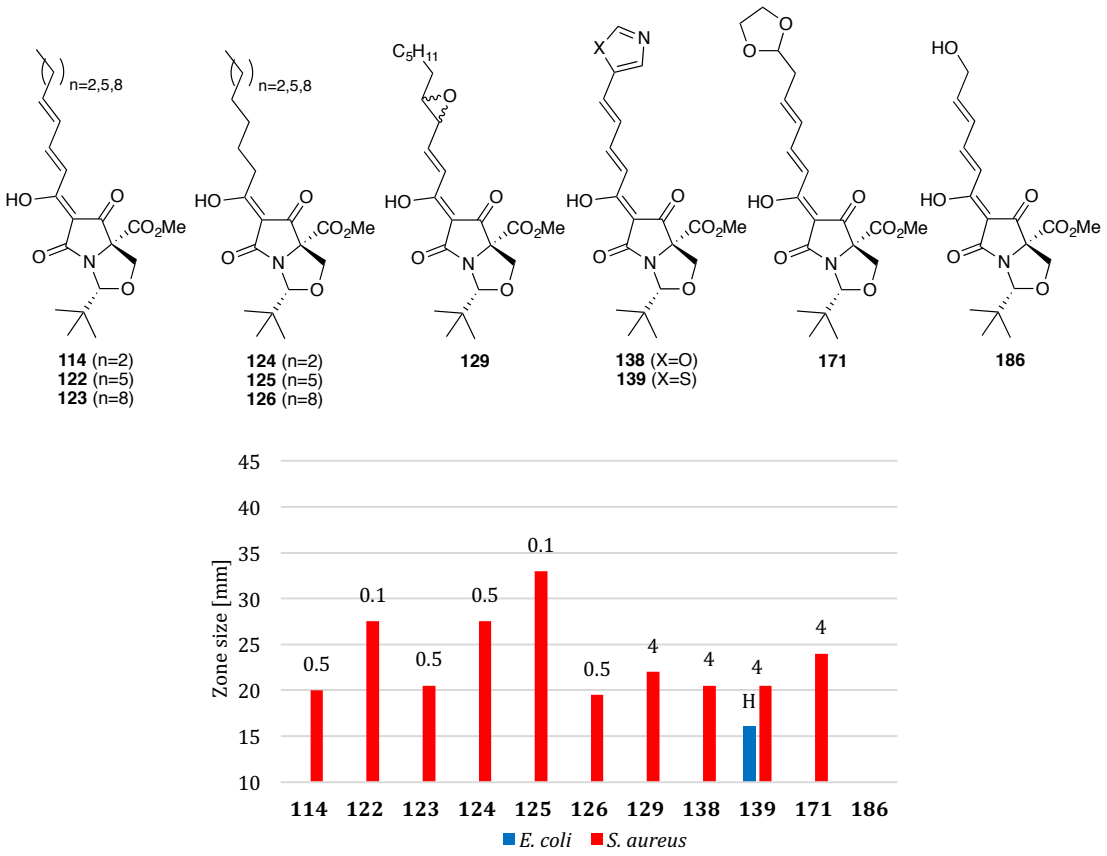


Figure 5.7. Antibacterial activity of mimics of pramanicin and oxazolomycin at 0.1-4 mg/mL against *E. coli* and *S. aureus*.

Another unexpected result was that epoxide **129** had significantly weaker activity than the analogous diene **122**. Substitution of the aliphatic chains with more polar oxazole, thiazole and dioxolane rings also led to a significant reduction in potency. Interestingly, the thiazole analogue, which exhibited the lowest Gram-positive activity, was also the only one to show some weak Gram-negative activity against *E. coli*.

These results are in accordance with those reported by S. W. B. Tan *et al.*, who studied the antibacterial activity of several *C*(7)-acyltetramates and found that the most active ones against *S. aureus*, but not *E. coli*, where the enoyls bearing a long hydrophobic side-chain.[154]

Enol ether **127** was also tested and found to be inactive at 4 mg/mL (Figure 5.8). This outcome seems to indicate that the presence of the tetramate moiety is essential for the antibacterial activity of these analogues. However, the tetramate core or a simple tricarbonyl system such as **44a** or **81a** respectively are not sufficient for activity, and appropriate functionalisation of the acyl chain is required to obtain potent compounds.

In an attempt to further increase the potency of the dienones, **122** was subjected to decarboxylation. However, in this case the Gram-positive activity was not favoured and instead a slight decrease was observed (Figure 5.8).

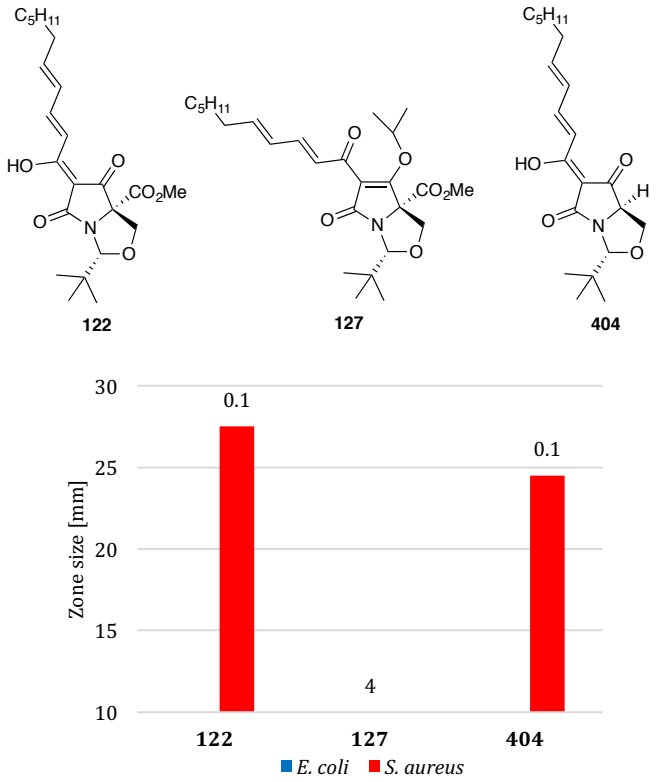


Figure 5.8. Antibacterial activity of dienone **122** and derivatives at 0.1-4 mg/mL against *E. coli* and *S. aureus*.

Of interest is the high selectivity of these compounds for Gram-positive *S. aureus*, with only a few showing weak activities against Gram-negative *E. coli*. This could be attributed to either them interfering with an enzyme or biological process that is not found or is not essential for the growth and survival of *E. coli*, or to poor cell penetration or efficient efflux pump removal. On this subject,

S. B. Singh and co-workers have reported that the *C*(7)-acyltetramate kibdelomycin also exhibits selective Gram-positive antibacterial activity.[265] Even though this natural product was shown to inhibit DNA gyrase and topoisomerase enzymes from both *S. aureus* and *E. coli*, this inhibition only correlated with whole-cell activity of the former, and the efficacy of the latter was probably limited by poor cell membrane uptake or by the active efflux pump.

5.1.2.3 Compounds from Chapter 2: broth dilution assay

Most of the synthesised *C*(7)-acyltetramates were tested in collaboration with SeaLife Pharma to determine their MIC values against *E. coli*, MRSA and *S. pneumoniae*, and the results for active compounds are shown in Table 5.2. Consistent with the results from the hole-plate method, no or little Gram-negative activity was observed. On the contrary, for the Gram-positive strains MRSA and *S. pneumoniae* low MIC values were obtained, in particular for decarboxylated alkenyl analogues **110a-c** and aliphatic **114,122-126**, with MIC values as low as 1 μg/mL (2.5 μM) against MRSA. These values are in fact comparable to those of known drugs,[266,267] and considering that MIC values of <16 μg/mL are acceptable as starting points for pharmaceutical development,[268] these compounds could be considered as promising hits.

Table 5.2. Antibacterial activity of selected compounds against Gram-negative *Escherichia coli* and Gram-positive MRSA and *Streptococcus pneumoniae*.

Comp	MIC [μg/mL]			Comp	MIC [μg/mL]		
	E. coli	MRSA	*S. pneumoniae*		*E. coli*	MRSA	*S. pneumoniae*
94b	n.a.[1]	>250	n.a.	**114**	n.a.	>31	>31
95b	n.a.	>250	>500	**122**	n.a.	<1	<1
96a	n.a.	>16	>16	**123**	n.a.	<2	<2
96b	n.a.	>2	>2	**124**	n.a.	<2	>6
96c	n.a.	>31	>16	**125**	n.a.	<2	<2
109	250	250	n.d.[2]	**126**	n.a.	<2	<2
110a	n.a.	7.81	3.91	**138**	250	125	125
110b	n.a.	0.98	0.45	**139**	250	62.5	62.5
110c	250	7.81	3.91	**171**	250	125	125

[1]n.a. = not active.

[2]n.d. = not determined.

In order to evaluate whether the hole-plate method provides valuable results, which could be used to rank and prioritise compounds for drug discovery, the correlation between zone sizes obtained from this method and MIC values determined at SeaLife Pharma was analysed. For this purpose, MIC values and relative potencies were compared and are represented in Figure 5.9. The relative potencies were chosen instead of zone sizes as a means to normalise all the results, given that different compounds had been tested at different concentrations depending on their potency. Overall, analogues with higher relative potencies gave lower MIC values, with an approximate logarithmic relationship. These results validate the effectiveness of the hole-plate method, which despite not providing accurate MIC values, represents a valuable quick method to rank analogues.

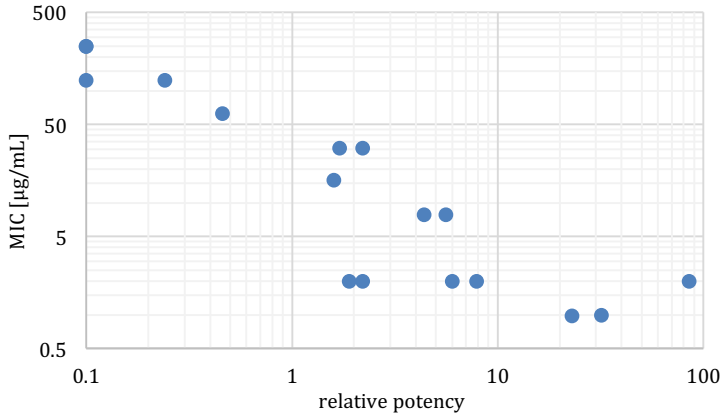


Figure 5.9. Correlation between MIC values determined at SeaLife Pharma and relative potencies obtained from the hole-plate method.

Some of the analogues were also tested for antibacterial activity against Gram-positive *Enterococcus faecalis* and Gram-negative *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, and antifungal activity against *Candida albicans*, *Candida krusei* and *Aspergillus fumigatus*. Table 5.3 shows the results for the ones that showed some activity (full data in Appendix F). The results were again consistent with these compounds possessing selective activity against Gram-positive but not Gram-negative bacteria, as they only showed inhibition of *E. faecalis*. With regards to the antifungal activity, only analogues **114,122-126**, which are the ones with hydrophobic aliphatic acyl chains, showed some inhibition, and one in particular was undecylidene **125**.

Table 5.3. Antibacterial and antifungal activity of selected compounds determined by SeaLife Pharma.

Comp	*E. faecalis*	*K. pneumoniae*	*P. aeruginosa*	*C. albicans*	*C. krusei*	*A. fumigatus*
94b	250	n.a.*	n.a.	n.a.	n.a.	n.a.
95b	250	n.a.	n.a.	n.a.	n.a.	n.a.
96a	16	n.a.	n.a.	n.a.	n.a.	n.a.
96b	2	n.a.	n.a.	n.a.	n.a.	n.a.
96c	16	n.a.	n.a.	n.a.	n.a.	n.a.
114	16	n.a.	n.a.	250	250	250
122	1	n.a.	250	250	250	62
123	2	n.a.	n.a.	500	500	n.a.
124	2	n.a.	500	500	500	200
125	2	n.a.	n.a.	250	250	8
126	2	n.a.	n.a.	250	250	250

*n.a. = not active.

In collaboration with SeaLife Pharma, the selectivity of the analogues to prokaryotic cells over mammalian cells was analysed by means of their EC_{50} values against HaCaT, an immortalised keratinocyte human cell line (Table 5.4).

Table 5.4. Cytotoxicity of selected analogues. EC_{50} values against human HaCaT, MIC values against *S. aureus* and therapeutic ratio, calculated as EC_{50} (HaCaT) over MIC (*S. aureus*).

Compound	HaCaT EC_{50} [µg/mL]	*S. aureus* MIC [µg/mL]	Therapeutic ratio
94b	250	250	1.0
95b	250	250	1.0
96a	62.5	16	3.9
96b	75	2	38
96c	62.5	31	2.0
114	175	31	5.6
122	87.5	1	88
123	150	2	75
124	25	2	12
125	45	2	22
126	24	2	12

The therapeutic window was estimated as the ratio of EC_{50} (HaCaT) to MIC (*S. aureus*), where higher values would indicate compounds likely to be less toxic. The best compounds in this regard were the cysteine alkenyl **96b** and dienoyls **122** and **123**, with therapeutic ratios of 38-88. Even though it had been disappointing to find that saturated analogues **124-126** had similar antibacterial properties to the dienoyls, this data suggests that that they might present bigger toxicities. The dienoyl analogues, and in particular undecylidene **122**, seem to be the most promising hits from the *C*(7)-acyltetramate series, with an excellent compromise between high potency and low toxicity.

5.1.2.4 Compounds from Chapter 3

Most of the tetramates and pyroglutamates from the first small library of reduced compounds displayed no or weak antibacterial activities (Figure 5.10). Only the decarboxylated tetramates **207a-c**, and in particular the one derived from cysteine, showed some inhibition at 4 mg/mL. This would be consistent with (i) the tetramate itself is not sufficient for antibacterial activity, and further functionalisation is required, (ii) decarboxylation of the bicyclic system can be beneficial and (iii) the cysteine analogues seem to possess slightly higher activity.

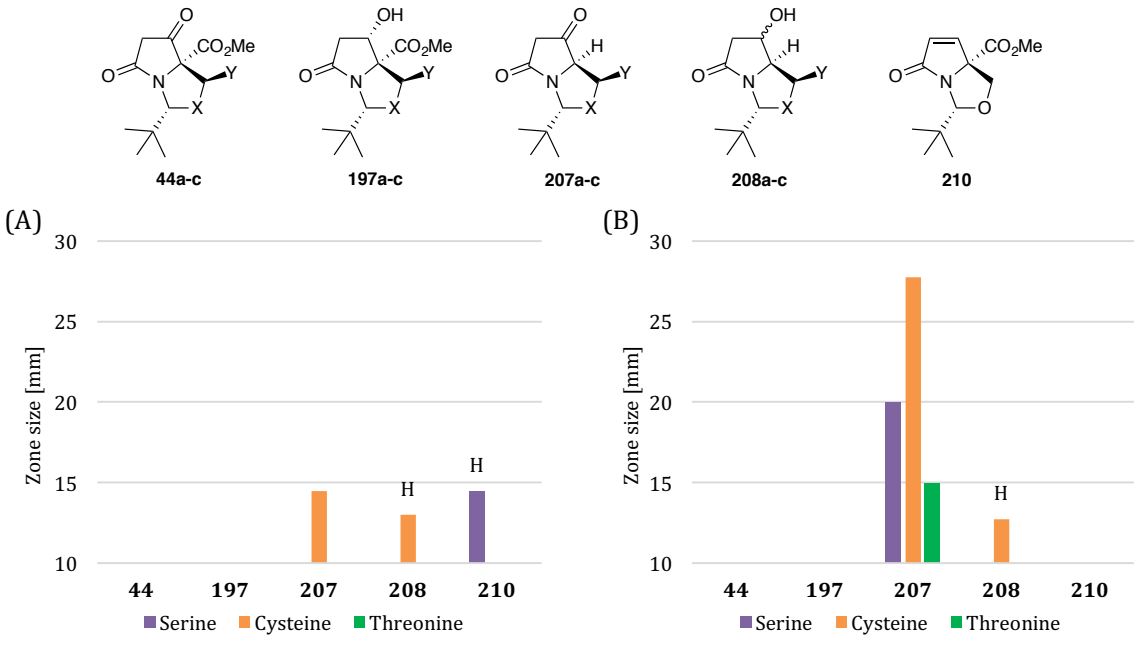


Figure 5.10. Antibacterial activity of reduced analogues at 4 mg/mL against (A) *E. coli* and (B) *S. aureus*.

The *C*(7)-acylpyroglutamates exhibited more promising antibacterial properties. These analogues precipitated in the wells when tested at 4 mg/mL, thus they were diluted to 1 mg/mL and the resulting zone sizes are presented in Figure 5.11. While the *C*(7)-acyltetramates **77a**,**79** and the enol ether derivatives **116I**,**117** did not have significant antibacterial activity, it would seem that hydrogenation to the ether or iodide analogues is beneficial. Ethyl ester **248** and Weinreb amide **264** both displayed inhibition of *E. coli* and *S. aureus* at 1 mg/mL.

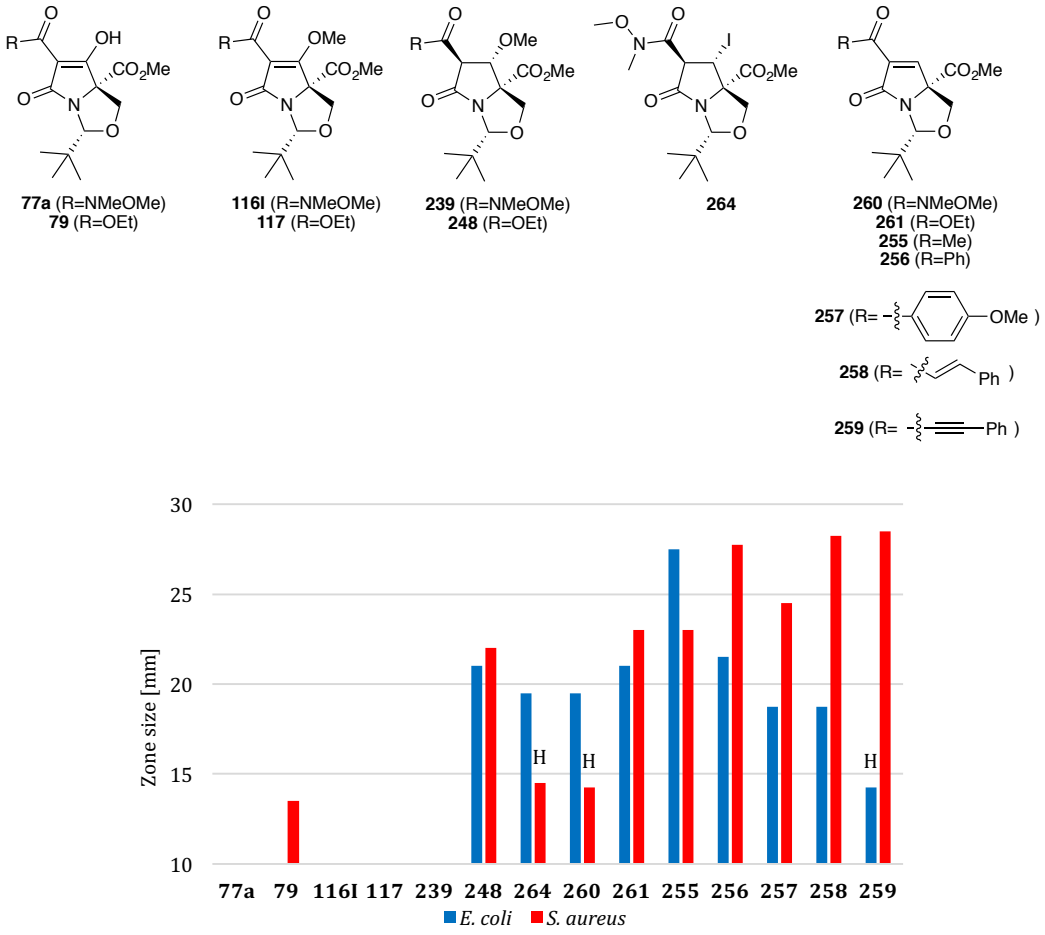


Figure 5.11. Antibacterial activity of *C*(7)-acylpyroglutamates at 4 mg/mL (**77a**,**79**,**116I**,**117**,**239**) and at 1 mg/mL (**248**,**264**,**255-261**) against *E. coli* and *S. aureus*.

The enone analogues also exhibited potent activities, both for *E. coli* and *S. aureus*. However, the trends observed for both strains differed, and among the keto-derivatives interestingly acetyl **255**, the most active compound against *E. coli*, was the least active against *S. aureus*, and the opposite was true for alkynyl **259.**

The activity of these pyroglutamates and enones against both strains of bacteria, in contrast to the results from the tetramate systems, might indicate that either they act via a different mechanism of action, or that they possess appropriate physicochemical properties to effectively cross the bacterial cell membrane. Noteworthy, the Gram-positive results from the ketone derivatives differ from the analogous tricarbonyl compounds. While the former showed activities which did not depend considerably on the side-chain, for the latter only the alkenyl analogues were active.

Despite the promising activity of these analogues, their electrophilic α,β-unsaturated carbonyl moiety might compromise their suitability as hit compounds. Such electrophilic compounds are often excluded from drug discovery processes due to potential low selectivity leading to toxicity, given that they may react with a nucleophilic residue present in several cellular targets.[269] However, the prevalence and pharmacological advantages of such covalent drugs has been discussed[270] and further investigation of these compounds might be worthy.

5.1.2.5 Compounds from Chapter 4

The antibacterial properties of all the pyroglutamate analogues prepared as described in Chapter 4, including the bicyclic systems, the *N*,*O*-acetal deprotected esters and carboxylic acids and the β-lactones, were also examined using the hole-plate method. Most of them were inactive against both *E. coli* and *S. aureus*, and the ones that showed inhibition are shown in Figure 5.12. Noteworthy, most of the β-lactones exhibited some antibacterial activity, although weak, which was higher for *E. coli* (only halo zones were observed for **336** and **340** against *S. aureus*).

The weak activities observed for these pyroglutamate systems clearly suggest that further optimisation of the structures is needed to obtain hit compounds. The selectivity towards *E. coli*, which diverges from the results of the tetramate and reduced analogues, could indicate that their inhibition results from a different mode of action, and for the β-lactones it might involve nucleophilic attack at the β-lactone carbonyl.

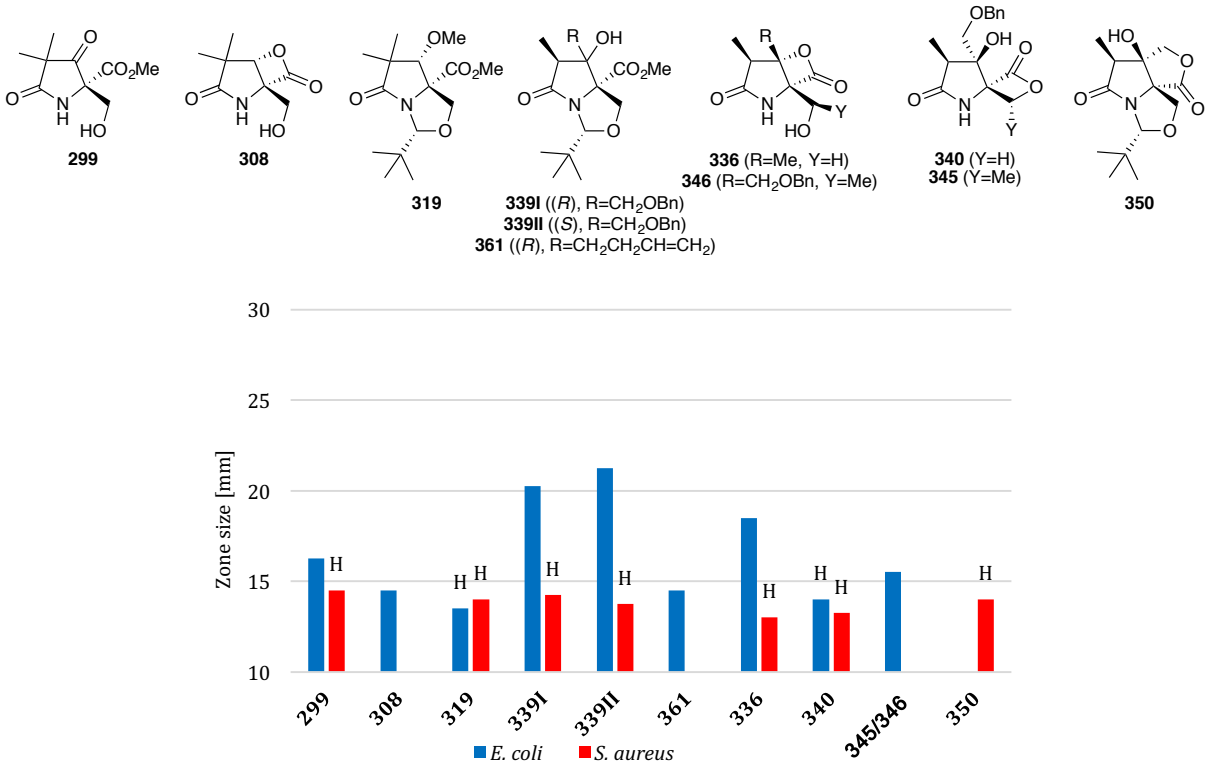


Figure 5.12. Antibacterial activity of selected compounds from Chapter 4 at 4 mg/mL against *E. coli* and *S. aureus*.

5.1.3 Physicochemical properties

P. D. Leeson compared the physicochemical property profiles of the oral drugs launched until 2002 and reported that anti-infective drugs tend to have higher molecular weight and lower lipophilicity than other drugs.[271] He attributed this divergence to the need for antibiotic drugs to act in non-mammalian systems, and to penetrate through the bacterial cell wall. Another analysis by R. O'Shea further recognised that drugs designed for Gram-positive or Gram-negative bacteria have different requirements, where compounds with activity against the latter generally have even larger molecular weights and are more polar.[272] This is probably driven by the additional outer membrane in Gram-negative bacteria, which serves as a barrier for small molecules. This membrane contains protein channels (porins), which are responsible for the influx of several classes of antibiotics.[273] These channels are water-filled, thus they only allow the penetration of hydrophilic molecules.

In order to understand SAR of the synthesised analogues, their physicochemical properties were analysed. clogP was chosen as an indication of lipophilicity, and molecular surface area (MSA) as a measure of steric effects,[135] and these were calculated using Marvin (ChemAxon). Compounds were classified depending on their activity against one or both strains of bacteria, and the ones active against *S. aureus* were separated between 'weak' and 'active', where the former were arbitrarily defined as those which had a relative potency <1. As observed in Figure 5.13 B, there seems to be a trend for the most active compounds against *S. aureus* from Chapter 2 to be more hydrophobic and with larger MSA than those active against *E. coli*, which are tightly clustered.

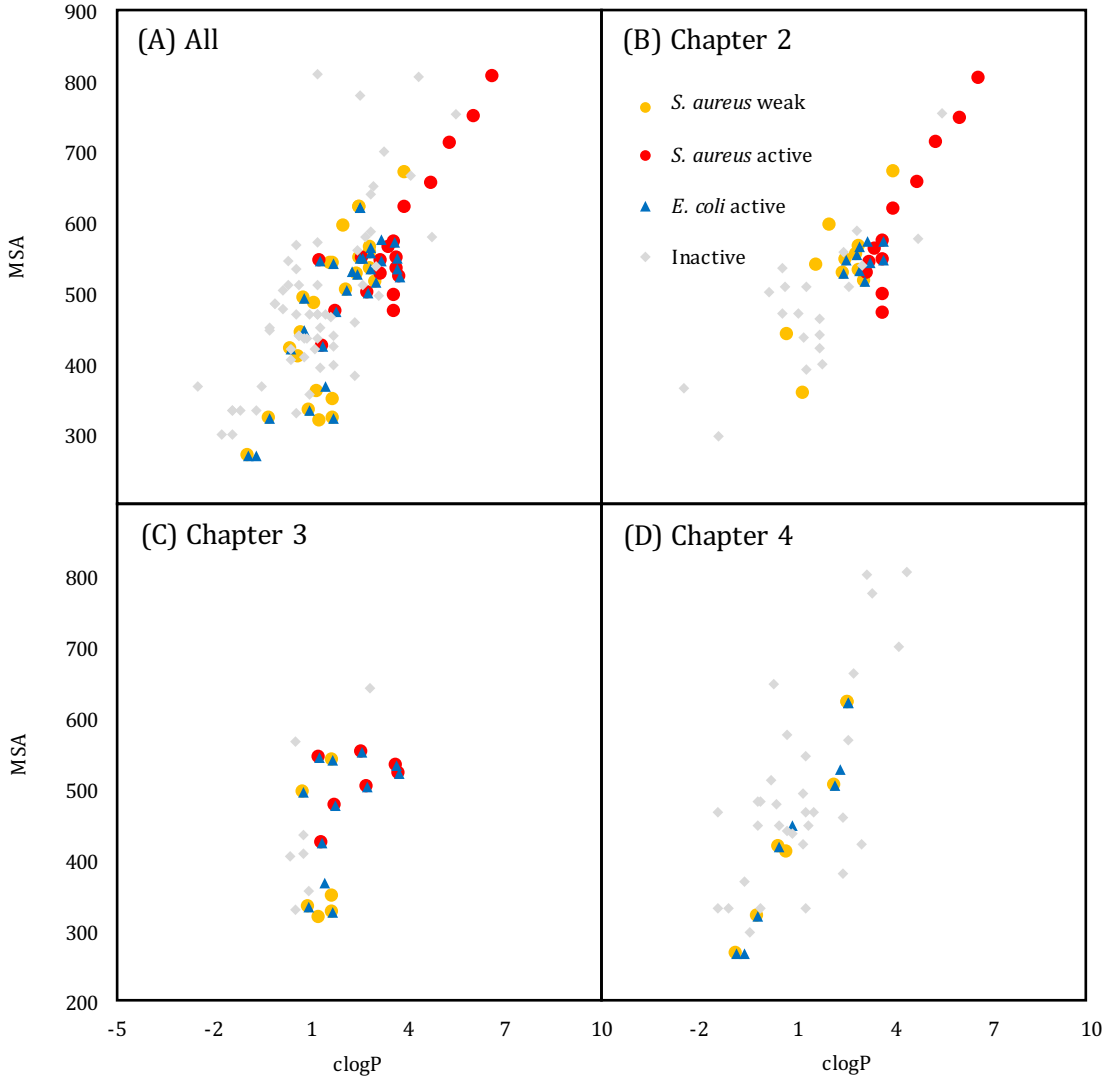


Figure 5.13. Plot of MSA against clogP of synthesised analogues, determined using Calculator Plugins in Marvin 16.7.25.0, 2016, ChemAxon (http://www.chemaxon.com).

Y.-C. Jeong *et al.* studied several *C*(7)-acyltetramates and found that those with clogP >3 and MSA >620 tended to be more easily transported by the efflux pump in Gram-negative *H. influenziae*,[135] and in fact the most active tetramates synthesised herein (with selective Gram-positive activity) had clogP >3 and MSA >500. Thus, the observed lack of activity of these systems against Gram-negative bacteria could be ascribed to efflux pump (and/or lack of cell permeability).[274–276]

With regards to the pyroglutamate analogues from Chapter 4, some of them possessed similar clogP and MSA values to active tetramates, and yet displayed no antibacterial activity. This observation would reinforce the need of the enolisable tetramate for the biological activity of the most active compounds from Chapter 2. Noteworthy, the β-lactones, which had shown some Gram-negative activity, occupy a different physicochemical space with smaller clogP and MSA values ($-0.9 < \text{clogP} < 0.8$, $260 < \text{MSA} < 450$).

It is also worth noting that the plots in Figure 5.13 indicate an approximate linear trend of clogP and MSA values. In this work, the preparation of compounds has been driven by synthetic accessibility instead of rational design of compound structures based on physicochemical properties. Future work could involve the design of tetramates and pyroglutamates which cover a wider chemical space, where a decrease of polarity does not lead to a significant increase of MSA and vice versa.

In terms of the physicochemical properties of the synthesised analogues in comparison to other known antibacterial drugs, they resemble those of fluoroquinolones (Figure 5.14). Even though antibacterials are known to generally not follow Lipinski's 'rule of five', most of the compounds described herein, including the most active ones, do actually obey the criteria, with only a few possessing clogP values larger than 5. Therefore, they could be suitable hits with the required properties for oral absorption.

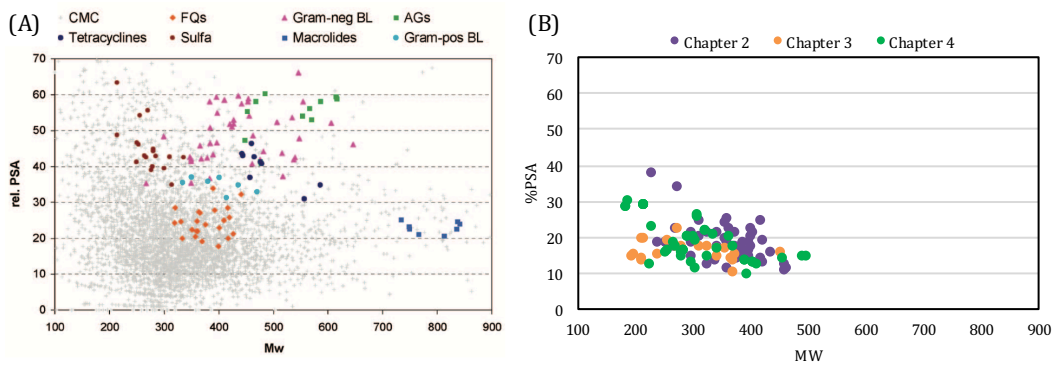


Figure 5.14. Plot of %PSA against MW of (A) known antibacterial drugs and (B) synthesised analogues. CMC = comprehensive medicinal chemistry; FQ = fluoroquinolone; BL = β-lactam; AG = aminoglycoside. Adapted with permission from O'Shea, R.; Moser, H. E. J. Med. Chem. **2008**, *51* (10), 287. Copyright (2008) American Chemical Society.

Even though the target(s) of these compounds still remains to be elucidated, there are reported studies on the biological mechanism of action of systems analogous to the most active compounds from Chapter 2. As discussed in Section 1.6.3, previous work in the Moloney group revealed that some *C*(7)-acyltetramates inspired from streptolydigin possessed dual mechanism of action against bacterial RNAP and UPPS.[135] S. Peukert and co-workers have also reported carboxamide tetramic acids possessing inhibitory properties against UPPS, which correlated with their antibacterial activities against Gram-positive bacteria. Some naturally occurring tetramates such as kibdelomycin[265] and amycolamicin[277] have been reported to possess bacterial DNA gyrase and topoIV inhibition properties, and recent work in the Moloney group has shown some tetramates derived from cysteine which also had inhibition properties against these enzymes.[278] Yet another example of a *C*(7)-acyltetramate exhibiting selective Gram-positive antibacterial activity was reported by T. Watanabe *et al.*, who found that signermycin B was capable of inhibiting the histidine kinase WalK, responsible for regulating bacterial cell wall metabolism and growth.[279]

5.2 Anticancer properties

5.2.1 High-content screening for drug discovery

Since the rise of the genomic era in 1990, drug targets, typically proteins with key roles in disease pathogenesis, have been an important focus in drug discovery. This approach is known as target-

based screening or target discovery.[280] Many advances have been made in the field, including new tools to identify new targets, high-throughput screening assays to identify compounds that interact with them, and structure-based tools to aid the lead identification and optimisation processes.[281] This approach offers several advantages, such as the rational design of drug structures. Additionally, knowledge of a drug target is beneficial for the toxicology studies performed during preclinical development.[282] However, it has also been recognised that the limitations of target discovery have led to a low success rate of drug approval.[283] Invalidated targets for disease is one of the main reasons for attrition of the compounds during clinical studies, which are found to lack drug efficacy.[282] Moreover, it has been indicated that the number of druggable targets might be more restricted than what it was originally thought.[284]

The alternative strategy to drug discovery is the phenotypic approach, where the effects that a compound induce in cells, tissues or whole organisms are measured. Phenotypic assays have the benefit that the identified drugs are more likely to possess molecular mechanisms of action relevant to the disease.[285] However, subsequent determination of the relevant target(s) of the active molecules, which would aid in the structural optimisation of the drug candidates, can be challenging.

In the field of oncology, a recent approach to elucidating the biological mode of action of novel small molecules is high-content screening (HCS). HCS enables the measurement of several parameters at a single cell level, which can facilitate the target prediction of a drug candidate[286,287] or the identification of proteins involved in biological processes.[288] Despite the promise of this technique, its use in industry and academia has been limited by long assay development times, the need for specialised equipment and complex data analysis. A report by S. Singh *et al.* on the use of HCS in academia[289] revealed that these are in fact "relatively low content", and 60-80% of the studies used only 1-2 features, with typically one of them being cell count. In some cases, several potential features had been analysed prior to selection. The performance of higher content screens requires the use of sophisticated multidimensional data analysis.

Some methods that have been successful are machine learning[290–293] and profiling of samples.[289] In the former, algorithms are trained with cells that represent the phenotype of interest, and in the latter a profile or signature is defined as a set of numerical values that represent a certain condition, and samples are classified into different profiles.

5.2.2 Development of a HCS platform to profile the mechanism of action of small molecules

5.2.2.1 Objectives

With several tetramates and pyroglutamates in hand, which could be considered simple mimics of natural products that have been reported to possess anticancer properties, it was of interest to determine whether the synthesised analogues had anticancer activity. Furthermore, the design of a screening platform which could also give information on their mode of action would be advantageous.

A high-throughput phenotypic screen of the compounds would allow for a quick evaluation of anticancer activity. Additionally, instead of measuring a single endpoint, which would provide a cell count for viability calculations, high-content imaging of several cell features could provide valuable information. It was hypothesised that a set of approved drugs with known mechanisms of action could be used in the same screen, as a means of comparison with new analogues. Known drugs having the same or similar mode of action might have features that follow common patterns, which could be identified via data analysis. These could then be used as a 'fingerprint' of that MOA, to which the synthesised compounds could be compared.

This work was performed in collaboration with the Department of Oncology and the Target Discovery Institute (TDI), and additional compounds were donated by several chemists in the Department of Chemistry, University of Oxford. Most of the compounds were either mimics or analogues of natural products, or fragments prepared during their total syntheses. A set of 114

FDA-approved drugs obtained from the National Cancer Institute were used, and the full list is indicated in Appendix G.

5.2.2.2 Protocol

The screening protocol that was followed is outlined in Figure 5.15, and Table 5.5 indicates the four cancer cell lines that were tested. 384-Well plates were used, and for each cell line an initial validation screen was performed before screening the compounds. The validation involved optimising parameters such as cell number and concentration of positive control.

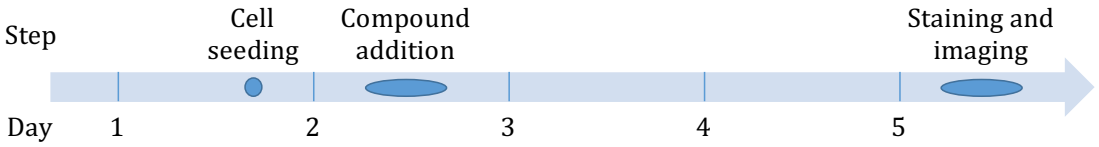


Figure 5.15. HTS protocol.

Table 5.5. Cell lines used for the HTS and optimised cell number for seeding.

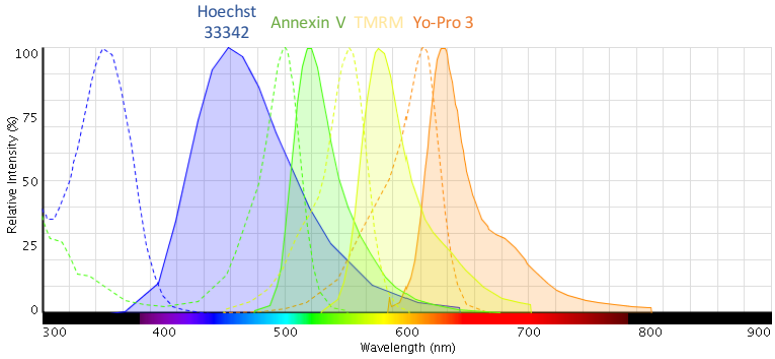
Cell line	Cell type	Tissue	Properties	Optimised cell number / well
HeLa	Cervical cancer	Cervix.	Adherent, epithelial	800
H460	Non-small cell lung cancer	Lung, pleural effusion.		1000
MCF7	Breast cancer	Mammary gland, breast. Derived from metastatic site: pleural effusion.		1200
PC3	Prostate cancer	Prostate. Derived from metastatic site: bone.		2750

Cells were seeded in the afternoon of Day 1 and incubated at 37 °C overnight, whereupon they adhered to the bottom of the wells. A specific number of cells was added to each well, which was optimised to achieve a cell confluence of 70-80% for the negative control on Day 5, which would be optimal for imaging. Given that the doubling patterns and the morphology of each cell line are different, optimisation of the cell number for each of the four cell lines was required. For this

purpose, in the validation screen the plate was seeded with cell numbers ranging from 300 to 3000, and those giving the closest confluence to 75% on Day 5 were chosen (Table 5.5).

Compound solutions were added during Day 2 at four different concentrations: 10 μM, 1 μM, 100 nM and 10 nM, each of which was ran in duplicates. The compound solutions were prepared by serial dilution from a 10 mM stock solution in DMSO, with a final concentration of DMSO of 0.1%. A solution of 0.1% DMSO in cell medium was used as a negative control, and 15 nM Docetaxel (DCT) was employed as the positive control.

Table 5.6. Stains used for the HTS.



Stain	Colour	Cellular localisation	Excitation / emission [nm]
Hoechst 33342	Blue	Nucleus. Any dsDNA.	350 / 461
Annexin V (Alexa Fluor® 488 conjugate)	Green	Binds to phosphatidylserine on the cell surface during apoptosis.	495 / 518
TMRM	Yellow-orange	Mitochondria. Accumulates in active mitochondria.	548 / 574
Yo-Pro®-3 iodide	Red	Nucleus. Permeant in cells with damaged nuclear membrane.	612 / 631
Brightfield		Whole cell image.	Light

Cells were incubated with the compounds for three days, after which they were stained and imaged. Four live cell stains were chosen, which indicated different cell localisation/population and emitted at different wavelengths (Table 5.6). Hoechst 33342, which stains any double-stranded DNA, would give an indication of total cell number and it could be used for viability

calculations. TMRM emits in the yellow-orange channel. Healthy cells having active mitochondria with intact membrane potentials give a bright signal, but upon loss of potential, accumulation of TMRM ceases and the signal disappears. Annexin V conjugated to Alexa Fluor® 488, a green-fluorescent dye, is used to detect the externalisation of phosphatidylserine to extracellular surface, which is one of the earliest indicators of apoptosis. Yo-Pro 3, which emits in the red channel, stains the nucleus and is only permeant in cells with damaged nuclear membranes, thus it is an indicator of dead or dying cells. With these stains, cells could be divided in four distinct populations: healthy (blue and yellow), early apoptotic (blue and green), late apoptotic (blue, green and red) and necrotic (blue and red).

5.2.2.3 Image analysis

A high-content imager (Operetta®, Perkin-Elmer) was used to measure the cellular fluorescence in five channels, one for each stain and the brightfield, at three fields per well. The number of fields was chosen as the best balance between obtaining a representative area of the wells in a reasonable amount of time. Imaging three fields involved a reading time of one hour per plate. For this reason, in Day 2 the addition of compounds of a certain concentration was performed every two hours (as these were done in duplicates), starting with the lowest concentration.

Figure 5.16 and Figure 5.17 show examples of single field images for negative and positive controls respectively. As expected, wells treated with 15 nM DCT had a significantly lower number of cells, and most of these were in late apoptosis or necrosis (Figure 5.18). On the other hand, wells treated with 0.1% DMSO had a higher number of cells and most of them were healthy.

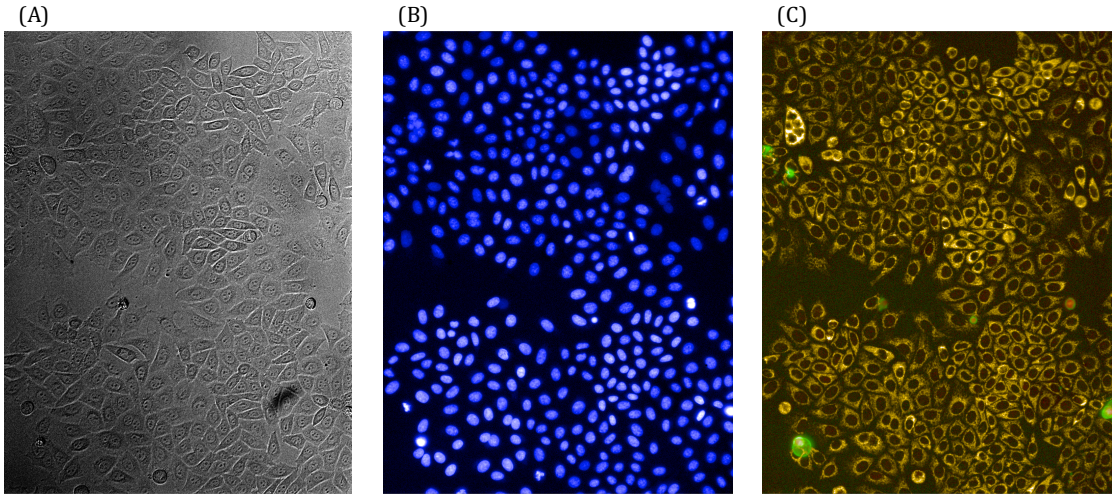


Figure 5.16. Example of high-content images from a well with HeLa cells treated with negative control (0.1% DMSO). (A) Brightfield; (B) blue channel (Hoechest 33342); (C) yellow (TMRM), green (Annexin V) and red (Yo-Pro 3) channels.

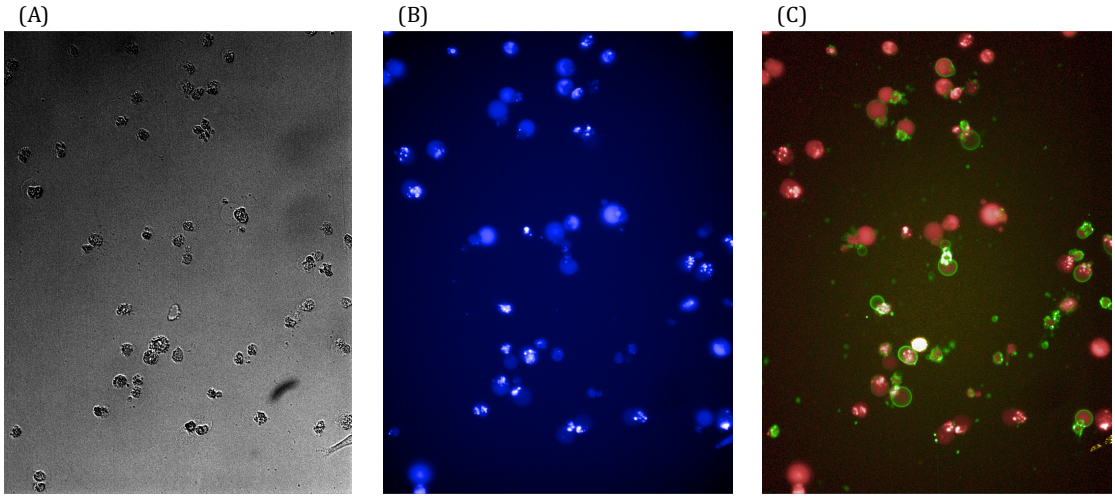


Figure 5.17. Example of high-content images from a well with HeLa cells treated with positive control (15 nM DCT). (A) Brightfield; (B) blue channel (Hoechest 33342); (C) yellow (TMRM), green (Annexin V) and red (Yo-Pro 3) channels.

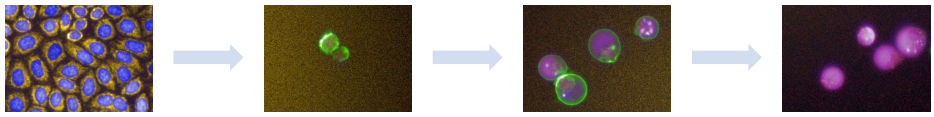


Figure 5.18. Examples of HeLa cells from different populations. Images include four channels (blue, yellow, green and red). Nuclei stained in both blue and red are shown in purple.

The images obtained were then analysed using Columbus™ (Perkin Elmer), an image data storage and analysis system. The Ebner group at the TDI had previously incorporated several tools in the software, employing machine learning techniques, which were capable of extracting several measurements of the images using the information from the five channels. These measurements, or features, included, other than the intensity of each stain, cell characteristics such as size, shape, texture and cell fragmentation. This analysis gave a total of 460 features for each cell.

With four cell lines, four concentrations of compounds in duplicates, thousands of cells per well and 460 features per cell, the amount of data generated by the screen was high dimensional.

5.2.2.4 Data analysis

Cytotoxicity of the compounds was determined using the information from the Hoechst stain (blue channel) only, which stained the nuclei of cells from all populations and gave an indication of cell viability for each compound concentration. Active compounds at a certain concentration were considered as those which had a cell number of less than 70% compared to cells treated with negative control (0.1% DMSO).

Drugs from the FDA-approved set with known anticancer activity, such as microtubule stabilisers paclitaxel[294] and cabazitaxel,[295] DNA biosynthesis inhibitors daunorubicin[296] and doxorubicin,[297] and proteasome inhibitors bortezomib[48] and carfilzomib[298] showed activity at the four concentrations tested (full data in Appendix G), which partly served as a validation of the screen. Moreover, there was generally a high window between the average cell numbers of the positive and negative controls: 7.4-9.4 for HeLa and H460 cell lines, 3.3-5.7 for MCF7 and 1.6-2.8 for PC3. The higher values for HeLa and H460 could derive from a lower standard deviation of the controls; while HeLa and H460 cells grew uniformly on the bottom of the flask, MCF7 and PC3 cells tended to form 'clamps' and sometimes grew in several layers on top of each other. In particular for PC3 cells, it was observed that on the one hand the cell number for the negative control was underestimated due to the formation of several layers of cells, which were not segmented by

Columbus, and on the other hand the number for the positive control was overestimated due to significant fragmentation of the nuclei, which was detected as distinct cells.

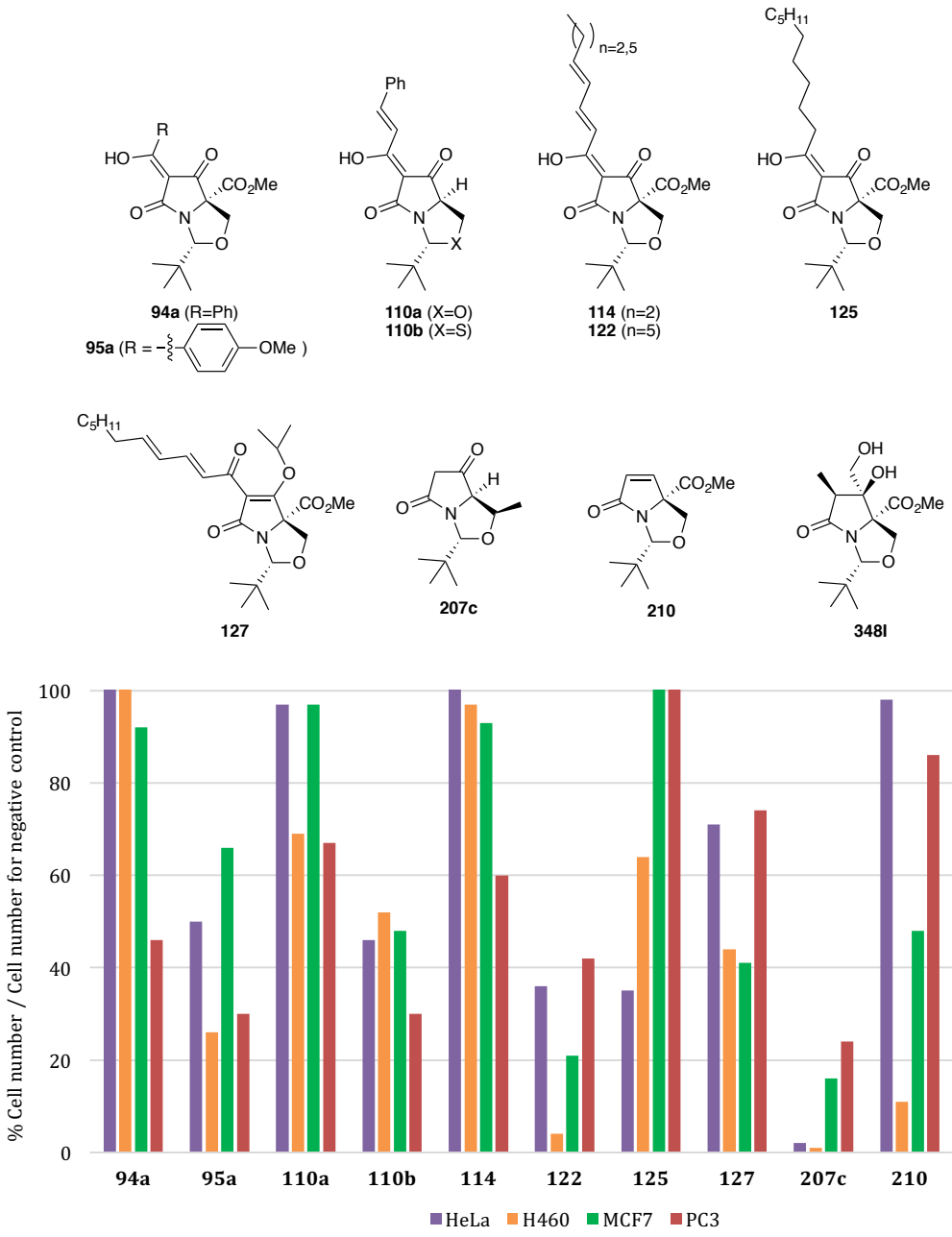


Figure 5.19. Cytotoxicity of selected compounds at 10 μM against four cancer cell lines determined from the Hoechst stain in the high-throughput screen.

The compounds that were active against at least one cell line at the maximum concentration (10 μM) are indicated in Figure 5.19. Amongst the *C*(7)-acyltetramates, decarboxylated alkenyl **110b** and dienoyl **122** were again the most active ones, as well as aryl **95a**. Interestingly, though,

while protected tetramate **127** had shown no antibacterial properties, it did exhibit anticancer activity. This result could indicate that the antibacterial and anticancer properties of these compounds originate from different pharmacophores, and for the latter, the enolisable tetramate seems not to be necessary.

It was disappointing to find that none of the β-lactones exhibited anticancer activity. This could be due to either lack of cell permeability, no inhibition of the proteasome, or both. Future work could involve studying the permeability of these analogues, for instance via a Caco-2 assay, as well as their ability to inhibit the proteasome, using the purified enzyme. These results would aid in the design of structurally optimised β-lactones.

The information from the other stains and features could provide a better understanding of the effect of the active compounds on the cells, as well as an indication of whether the inactive ones caused any changes in the cells, despite not leading to cell death or arrest. Due to the high dimensionality of the data generated, collaboration with computational biologists was required, and analysis of the data has been initiated by Dr Francesca Buffa and Dr Sheng Yu at the Department of Oncology, University of Oxford. Some preliminary results have shown that the data set can be analysed using a sparse principal component analysis (SPCA),[299] a technique commonly used for statistical analysis and dimensionality reduction. In a principal component analysis,[300] linear combinations of the original variables, or features, are created, which are known as principal components (PC), and these are ranked so that the first PC is the one that accounts for the most data variability. In SPCA, features that do not contribute significantly to the variance of the data are eliminated.

Unfortunately, this work is still ongoing, but some preliminary results have shown that for HeLa, the first seven PCs could explain 65% of the variance, and the top contributing features of the screen were chosen as those which had the highest coefficient in these seven PCs. These features

will be used to cluster the compounds, and it is hoped that FDA-approved drugs with a same or similar MOA will appear in the same cluster.

5.2.3 Determination of IC_{50} values

In collaboration with the CRUK Oxford Centre Preclinical Validation Facility, the active compounds from the HTS were analysed to determine IC_{50} values against five cell lines: HeLa, H460, MCF7, SKOV-3 and KMS-12BM (Table 5.7). The protocol followed was similar to the one from the high-throughput screen: seeding cells overnight, incubating with compounds for 3 days and staining/reading. In this case two stains were used: Hoechst as an indicator of total cell number, and propidium iodide as an indicator of cell death. While Hoechst would be sufficient for the determination of IC_{50} values, propidium iodide could show whether the compounds are killing the cells or inhibiting their growth, and this information would aid in the determination of the mode of action.

Table 5.7. Additional cell lines used for the determination of IC_{50} values.

Cell line	Cell type	Tissue	Properties
SKOV-3	Ovarian cancer	Ovary: ascites	Adherent, epithelial
KMS-12BM	Multiple myeloma	Bone marrow	Suspension

As observed in Figure 5.20, the anticancer activity of most of the active compounds from the HTS was confirmed, which provided further evidence for the validity of the screen. However, the majority of the IC_{50} values were in the 10-20 μM range, which is a significantly higher concentration than that desired for hit structures. Therefore, optimisation of the compound structures will be required to find hits for anticancer drug discovery.

For the structural optimisation of these analogues, elucidation of the mechanism of action would be of use. In this respect, in addition to the expected results from the data analysis of the HCS, the active analogues will be assayed against known targets such as for proteasome inhibition or caspase 3/7 activation; this work is ongoing.

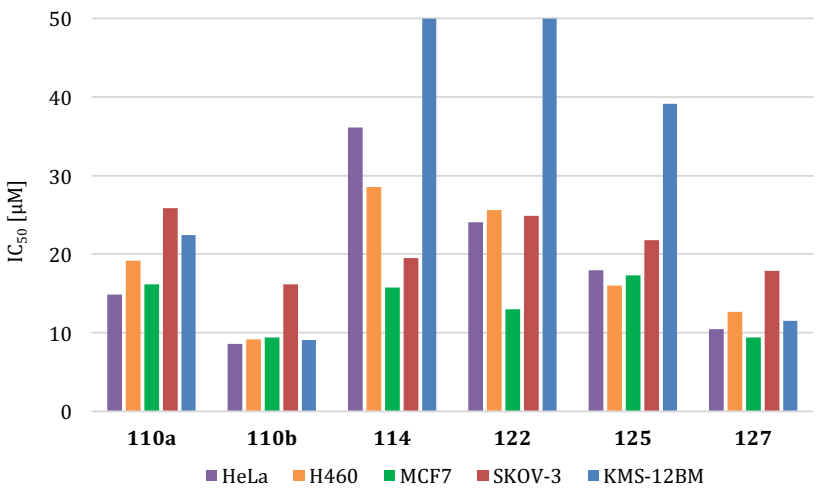


Figure 5.20. IC_{50} values of active compounds against HeLa, H460, MCF7, SKOV-3 and KMS-12BM cell lines, determined at the Department of Oncology, University of Oxford.

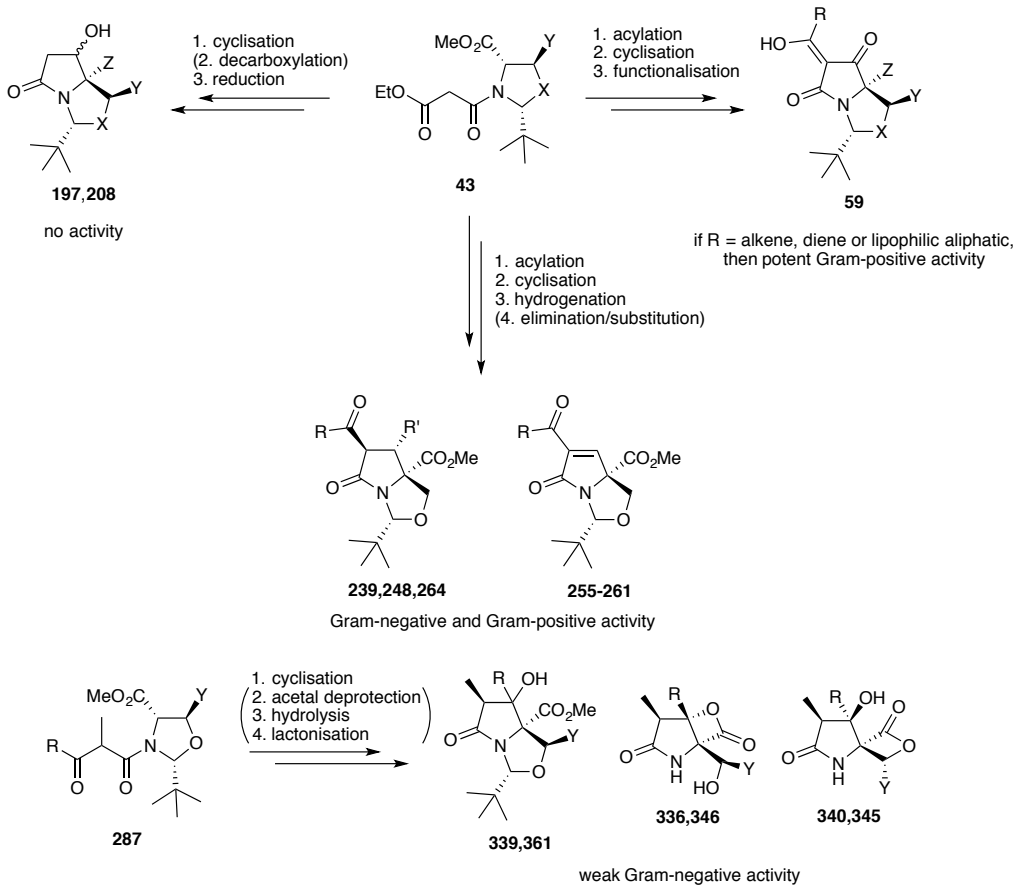
In spite of the lack of promising anticancer activity of the synthesised analogues, other compounds obtained from other chemists which were also tested in the HTS showed a decrease of cell number compared to the negative control down to 100 nM. The activity of these compounds was confirmed with IC_{50} values of 80-200 nM, which further validated the screen. Therefore, this HTS represents a quick and efficient method to evaluate the anticancer properties of small molecules, where the active ones can be further analysed to determine more precise IC_{50} values. Moreover, using the data from the other features in the HCS could give an insight into their mode of action, and this information could be used to select specific assays against the target(s).

5.3 Summary of Chapter 5

In this chapter, the antibacterial (Scheme 5.1) and anticancer properties of the synthesised tetramates and pyroglutamates were evaluated.

Antibacterial assays using the hole-plate method showed that, amongst the first library of simple *C*(7)-acyltetramates, the only active ones were those with alkenyl acyl chains. This activity was lost when deprotecting the *N*,*O*-acetal, but decarboxylation resulted in enhanced inhibition. For the further functionalised analogues, aliphatic dienoyls and saturated derivatives were potent

inhibitors of *S. aureus*, but substitution with more polar heterocycles was detrimental. This activity was confirmed with broth dilution assays, which showed that the most active compounds had MIC values <2 μg/mL.



Scheme 5.1. Summary of key analogues synthesised and their antibacterial activities.

While the tetramate core and the reduced tetramates displayed no antibacterial activity, pyroglutamates derived from hydrogenation of methylated *C*(7)-acyltetramates, and enones formed after elimination, were active against both Gram-positive and Gram-negative bacteria. With regards to the pyroglutamate systems synthesised in Chapter 4, only weak activities against Gram-negative bacteria were observed, and in particular for the β-lactones.

Analysis of the physicochemical properties of the compounds revealed that those with selective activity against Gram-positive *S. aureus* tended to have larger clogP and MSA values, while the ones with Gram-negative activity were more polar and smaller. This observation might be related

to cell permeability. Moreover, the active compounds had physicochemical properties similar to clinical fluoroquinolones, and generally followed Lipinski's 'rule of five'.

For the evaluation of anticancer properties, a high-content screen was designed which stained cells with four different dyes and divided them in four distinct populations. This HCS has the potential of giving an insight into the mode of action of compounds via comparison with known drugs, in addition to giving information on cell viability. Amongst the tetramate and pyroglutamate systems, a small number of analogues were found to be active, and these were further analysed to determine IC_{50} values, which were in the 10-20 μM range.

Overall, the promising activity of some of the synthesised analogues demonstrates the suitability of tetramates as 3D-templates to prepare novel antibiotics. Furthermore, this work also confirms the value of natural products as starting points for drug discovery, where the preparation of simpler mimics inspired by their complex structures can lead to potent hit compounds.

CHAPTER 6: EXPERIMENTAL SECTION

6.1 General techniques

All reagents were obtained from Sigma Aldrich, Alfa Aesar, Apollo Scientific or Acros Organics and used without further purification. All reactions were conducted in oven-dried glassware under an inert atmosphere of nitrogen unless not using anhydrous solvents. Anhydrous solvents were dried by pre-storing them over activated 3 Å molecular sieves before being passed through an activated alumina column on a solvent tower under N_2 pressure. 'Petrol' refers to light petroleum ether of boiling point 40-60 °C and was used as purchased. Solvents were evaporated at 40 °C under reduced pressure on a Büchi R-114 rotatory evaporator attached to a Vacuubrand CVC2 pump and a pressure control system. Concentrations (c) in the general procedures refer to the limiting reagent and are given in mmol/mL.

Temperatures below 25 °C were obtained using the following cooling baths: 0 °C ice/water, -10 °C ice/acetone, -15 °C dry ice/ethylene glycol and -78 °C dry ice/acetone.

Analytical thin layer chromatography (TLC) was carried out on Merck aluminium foil backed sheets precoated with 0.2 mm Kielselgel 60 F_{254}. The eluent used is specified in each case. The spots were visualised by UV irradiation (λ 254 nm) and by staining with a $KMnO_4$ solution followed by heating. Retention factors (R_f) are quoted to the nearest 0.01. *Flash* column chromatography was performed on Kielselgel 60 silica gel (230-400 mesh particle size). The eluents are specified in each case.

Optical rotations were recorded at 25 °C on a Perkin-Elmer 241 polarimeter or Unipol L 2000 using the D line of sodium (589 nm) and a path length of 1 dm. Concentrations (c) are given in g/100 mL and specific rotations ($[\alpha]_D^{20}$) are quoted in 10^{-1} deg cm^2 g^{-1}.

Melting points were measured using a Stuart Scientific SMP1 melting point instrument and are uncorrected.

Infrared spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer equipped with an attached Pike Miracle attenuated total reflectance (ATR) module. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}) and only selected peaks are reported.

^{1}H NMR spectra were recorded on the following instruments: Bruker DPX200 (200 MHz), AVIII HD 400 (400 MHz) and AVII 500 (500 MHz). Chemical shifts (δ_H) are reported in parts per million (ppm) downfield from TMS and are referenced to the residual ^{1}H solvent peak. Coupling constants (*J*) are quoted in Hz. Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, sept = septet, n = nonet and m = multiplet), coupling constant, and assignment. Two-dimensional COSY spectra were recorded on Bruker AVIII HD 400 (400 MHz) and AVII 500 (500 MHz) spectrometers, and NOE experiments were performed on a Bruker AVII 500 (500 MHz).

^{13}C NMR spectra were recorded on a Bruker AVIII HD 400 at 100.6 MHz and AVII 500 at 125.8 MHz with proton decoupling. Chemical shifts (δ_C) are reported in ppm downfield from TMS and are referenced to the residual ^{13}C solvent peak. Assignments of the spectra were made with HSQC and HMBC experiments, which were performed on a Bruker AVII 500 spectrometer.

Low resolution mass spectra (*m/z*) were recorded on a Fison Platform spectrometer using electrospray ionisation (ESI). Selected 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), on an Agilent 7200 Q-TOF (CI) or on a Water GCT (EI or FI). LC/MS spectra were recorded on a Bruker 9.4T FT-ICR-MS (ESI).

Crystals for X-ray crystallography were grown from slow vapour diffusion of petrol into a solution of the compound in EtOAc at room temperature.

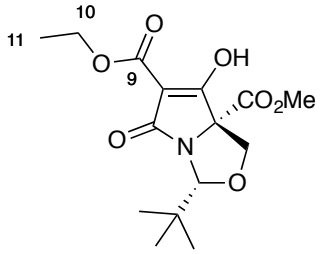
The enantiomeric excess (ee) of the products was determined by chiral stationary phase HPLC in a Dionex P680 chromatography with a Dionex UVD170U detector (Daicel Chiralpak OJ-H and AD-H columns).

In the cases where the products exist as mixtures of tautomers, the structures as drawn correspond to the major tautomeric form present. The ratio of tautomers was calculated from the ^{1}H NMR spectrum in $CDCl_3$. In the cases where the products exist as mixtures of diastereomers, the ratio was calculated from the ^{1}H NMR spectrum.

6.2 Experimental for Chapter 2

Synthetic protocol and characterisation of previously reported compounds **42a-c,**[160,164,301] **43a-c,**[135,136,160] **44a-c,** [135,136,160] **80,**[302,303] **83',**[165] **89,**[166] **149,**[304] **150,**[305] **142,**[191] **156,**[193] **162,**[306] **413,**[307] **170**,[196] **180**[308] and **180'**,[309] which were prepared using the reported methods, are included in Appendix A.

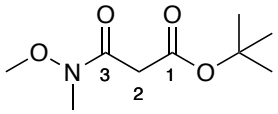
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-ethoxycarbonyl-6-hydroxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (79)



To a solution of tetramic acid **44a** (92 mg, 0.36 mmol) and DMAP (103 mg, 0.840 mmol) in anhydrous DCM (7 mL) was added ethyl chloroformate (45 μL, 0.46 mmol). The solution was stirred for 24 h at room temperature and was then concentrated under reduced pressure. EtOAc was then added and the organic layer was extracted with 1 M aqueous NaH_2PO_4. The aqueous phase was acidified with 2 M aqueous HCl and extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give ethyl ester **79** (75 mg, 0.23 mmol, 64%) as an orange solid; m.p. 62 °C. R_f (20% MeOH in EtOAc) 0.69; $[\alpha]_D^{20}$ +81.6 (*c* 0.7, $CHCl_3$); ν_{max}/cm^{-1} 2960 (C-H), 1725 (C=O), 1663 (C=O); δ_H (400 MHz, $CDCl_3$) 0.88 (9H, s, C$(CH_3)_3$), 1.35 (3H, t, *J* 7.1, C(11)H_3), 3.46 (1H, d, *J* 8.4, C(4)H_AH_B), 3.77 (3H, s, CO_2CH_3), 4.34 (2H, q, *J* 7.1, C(10)H_2), 4.71 (1H, s, C(2)*H*), 4.81 (1H, d, *J* 8.4, C(4)H_AH_B); δ_C (100 MHz, $CDCl_3$)

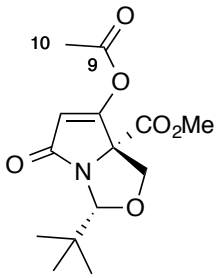
14.2 (*C*(11)), 24.7 (C(*C*H$_3$)$_3$), 35.2 (*C*(CH$_3$)$_3$), 53.6 (CO$_2$*C*H$_3$), 61.9 (*C*(10)), 69.0 (*C*(4)), 74.4 (*C*(5)), 97.6 (*C*(2)), 98.3 (*C*(7)), 167.0 (*C*O$_2$CH$_3$), 167.2 (*C*(9)), 172.5 (*C*(8)), 184.4 (*C*(6)); *m/z* (ESI$^+$) 328.1 (MH$^+$, 82%), 350.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 350.1210; $C_{15}H_{21}NNaO_7$ (MNa$^+$) requires 350.1210.

tert-Butyl 3-(methoxy(methyl)amino)-3-oxopropanoate (85)



Pyridine (50 µL, 0.62 mmol) was added dropwise to a solution of *tert*-butyl acetoacetate (30 µL, 29 mg, 0.18 mmol) and magnesium bromide (52 mg, 0.28 mmol) in anhydrous DCM (200 µL) at 0 °C. The mixture was stirred at 0 °C for 15 min and carbamic chloride **80** (68 mg, 0.55 mmol) in anhydrous DCM (400 µL) was added dropwise. The suspension was stirred at rt for 24 h and acidified with 2M HCl at 0 °C. The aqueous phase was extracted with Et$_2$O, dried over anhydrous Na$_2$SO$_4$ and concentrated *in vacuo*. The residue was purified by *flash* column chromatography (25% EtOAc in petrol) to give Weinreb amide **85** (37 mg, 0.18 mmol, quant.) as a colourless oil. R$_f$ (25% EtOAc in petrol) 0.17; ν_{max}/cm^{-1} 2979 (C-H), 1734 (C=O), 1666 (C=O); δ_H (400 MHz, CDCl$_3$) 1.48 (9H, s, C(C*H*$_3$)$_3$), 3.22 (3H, s, NC*H*$_3$), 3.41 (2H, s, C*H*$_2$), 3.72 (3H, s, OC*H*$_3$); δ_C (100 MHz, CDCl$_3$) 28.1 (C(*C*H$_3$)$_3$), 32.4 (N*C*H$_3$), 41.6 (*C*(2)), 61.4 (NO*C*H$_3$), 81.8 (*C*(CH$_3$)$_3$), 166.7 (*C*(1)), 168.0 (*C*(3)); HRMS (CI$^+$) found 204.1231, $C_9H_{18}NO_9$ (MH$^+$) requires 204.1230.

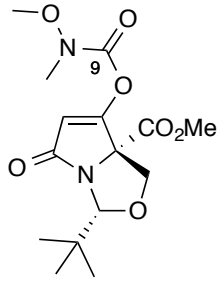
(2*R*,5*R*)-6-Acetoxy-1-aza-2-(*tert*-butyl)-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (86)



Pyridine (100 µL, 1.24 mmol) was added to a solution of tetramic acid **44a** (102 mg, 0.398 mmol) and magnesium bromide (113 mg, 0.615 mmol) in anhydrous DCM (600 µL) at 0 °C. The suspension was stirred at this temperature for 15 min and acetyl chloride (85 µL, 1.2 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 24 h and then acidified with 2 M aqueous HCl at 0 °C. The aqueous phase was extracted with Et$_2$O, dried over anhydrous Na$_2$SO$_4$ and concentrated *in vacuo*. The residue was purified by *flash* column

chromatography (10% MeOH in EtOAc) to give the *O*-acylated product **86** (104 mg, 0.350 mmol, 88%) as a 7:1 inseparable mixture with starting material as a yellow oil, instead of the desired *C*-acyltetramate **81a**. R_f (10% MeOH in EtOAc) 0.78; ν_{max}/cm^{-1} 2959 (C-H), 1796 (C=O), 1749 (C=O), 1718 (C=O), 1624 (C=C); δ_H (400 MHz, $CDCl_3$) 0.94 (9H, s, C(CH_3)$_3$), 2.28 (3H, s, C(10)H_3), 3.51 (1H, d, *J* 8.8, C(4)H_AH$_B$), 3.82 (3H, s, CO_2CH_3), 4.75 (1H, s, C(2)*H*), 4.84 (1H, d, *J* 8.8, C(4)H$_A$$H_B$), 6.09 (1H, s, C(7)*H*); δ_C (100 MHz, $CDCl_3$) 21.0 (*C*(10)), 24.7 (C(*C*H$_3$)$_3$), 35.2 (*C*(CH$_3$)$_3$), 53.4 (CO_2CH_3), 70.1 (*C*(4)), 75.4 (*C*(5)), 96.8 (*C*(2)), 108.9 (*C*(7)), 163.6 (*C*(6)), 165.6 (*C*O_2CH$_3$), 167.9 (*C*(9)), 176.9 (*C*(8)); *m/z* (ESI$^+$) 298.0 (MH$^+$, 100%), 320.0 (MNa$^+$, 44%); HRMS (ESI$^+$) found 320.1110, $C_{14}H_{19}NNaO_6$ (MNa$^+$) requires 320.1105.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-6-((methoxy(methyl)carbamoyl)oxy)-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (87)

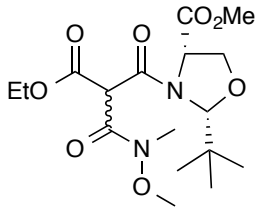


Pyridine (220 µL, 2.73 mmol) was added to a solution of tetramic acid **44a** (226 mg, 0.884 mmol) and magnesium bromide (251 mg, 1.36 mmol) in anhydrous DCM (400 µL) at 0 °C. The suspension was stirred at this temperature for 15 min and a solution of methoxy(methyl)carbamic chloride **80** (329 mg, 2.66 mmol) in anhydrous DCM (400 µL) was added dropwise. The reaction mixture was stirred at room temperature for 24 h and then acidified with 2 M aqueous HCl at 0 °C. The aqueous phase was extracted with Et_2O, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the *O*-acylated product **87** (289 mg, 0.844 mmol, 95%) as a yellow solid, instead of the desired *C*-acyltetramate **77a**; m.p. 73 °C. R_f (50% EtOAc in petrol) 0.60; $[\alpha]_D^{20}$ +113.6 (*c* 0.6, $CHCl_3$); ν_{max}/cm^{-1} 2959 (C-H), 1750 (C=O), 1719 (C=O), 1631 (C=C); δ_H (500 MHz, $CDCl_3$) 0.92 (9H, s, C(CH_3)$_3$), 3.21 (3H, s, NCH_3), 3.53 (1H, d, *J* 8.6, C(4)H_AH$_B$), 3.70 (3H, s, NOCH_3), 3.79 (3H, s, CO_2CH_3), 4.73 (1H, s, C(2)*H*), 4.83 (1H, d, *J* 8.6, C(4)H$_A$$H_B$), 6.02 (1H, s, C(7)*H*); δ_C (125 MHz, $CDCl_3$) 24.8 (C(*C*H$_3$)$_3$), 35.3 (*C*(CH$_3$)$_3$), 35.6 (N*C*H$_3$), 53.4 (CO_2CH_3), 62.0 (NO*C*H$_3$), 70.4 (*C*(4)), 75.5 (*C*(5)), 96.9 (*C*(2)), 107.5 (*C*(7)), 150.8 (*C*(9)), 164.3 (*C*(6)), 168.1 (*C*O_2CH$_3$), 177.0 (*C*(8)); *m/z* (ESI$^+$) 343.0 (MH$^+$, 100%), 365.0 (MNa$^+$, 58%); HRMS (ESI$^+$) found 365.1323, $C_{15}H_{22}N_2NaO_7$ (MNa$^+$) requires 365.1319.

General procedure A: Acylation of *N*-acyloxazolidines and thiazolidines

Magnesium bromide (1.5 eq) and dry pyridine (1.5 eq) were added dropwise to a solution of *N*-acyloxazolidine or thiazolidine (1.0 eq) in anhydrous DCM (*c* 0.5) at 0 °C. The suspension was stirred at this temperature for 15 min and a solution of methoxy(methyl)carbamic chloride **80** (1.5 eq) in anhydrous DCM was added dropwise. The reaction mixture was stirred at room temperature for 24 h and then acidified with 2 M aqueous HCl at 0 °C. The aqueous phase was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by *flash* column chromatography to give the tricarbonyl oxazolidine or thiazolidine.

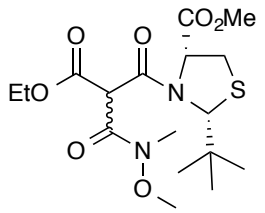
(2*R*,5*S*)-2-(*tert*-Butyl)-1-((*R*)-1-ethoxycarbonyl-1-(methoxy(methyl)carbamoyl))acetyl-5-methoxycarbonyl-1,3-oxazolidine (92a) and (2*R*,5*S*)-2-(*tert*-Butyl)-1-((*S*)-1-ethoxycarbonyl-1-(methoxy(methyl)carbamoyl))acetyl-5-methoxycarbonyl-1,3-oxazolidine (92a')



General procedure A (from **43a**); yield 70% (1.80 g); yellow oil; 1.5:1 mixture of diastereomers. R_f (10% EtOAc in DCM) 0.21 + 0.26; ν_{max}/cm^{-1} 2979 (C-H), 2959 (C-H), 1747 (C=O), 1680 (C=O); δ_H (400 MHz, $CDCl_3$) major diastereomer: 0.88 (9H, s, C(CH_3)$_3$), 1.28 (3H, t, *J* 7.2, $CO_2CH_2CH_3$), 3.20 (3H, s, NCH_3), 3.66 (3H, s, NOCH_3), 3.75 (3H, s, CO_2CH_3), 3.86-3.95 (1H, m, C(4)H_AH_B), 4.30 (2H, q, *J* 7.2, $CO_2CH_2CH_3$), 4.50-4.53 (2H, m, C(4)H_AH_B + C(5)H), 4.91 (1H, s, C(7)H), 5.35 (1H, s, C(2)H); minor diastereomer: 0.88 (9H, s, C(CH_3)$_3$), 1.28 (3H, t, *J* 7.0, $CO_2CH_2CH_3$), 3.21 (3H, s, NCH_3), 3.72 (3H, s, NOCH_3), 3.76 (3H, s, CO_2CH_3), 3.86-3.95 (1H, m, C(4)H_AH_B), 4.24-4.30 (2H, m, $CO_2CH_2CH_3$), 4.38-4.40 (1H, m, C(5)H), 4.50-4.53 (1H, m, C(4)H_AH_B), 4.99 (1H, s, C(7)H), 5.31 (1H, s, C(2)H); δ_C (100 MHz, $CDCl_3$) major diastereomer: 14.2 ($CO_2CH_2CH_3$), 25.7 (C(CH_3)$_3$), 32.7 (NCH_3), 37.5 (C(CH_3)$_3$), 52.8 (CO_2CH_3), 59.0 (C(7)), 59.5 (C(5)), 61.3 (NOCH_3), 62.2 ($CO_2CH_2CH_3$), 67.9 (C(4)), 97.0 (C(2)), 165.1 ($CO_2CH_2CH_3$), 165.7, 166.0 (CON, C(8)), 169.8 (CO_2CH_3); minor diastereomer: 14.2 ($CO_2CH_2CH_3$), 25.7 (C(CH_3)$_3$), 32.9 (NCH_3), 37.3 (C(CH_3)$_3$), 52.9 (CO_2CH_3), 58.5

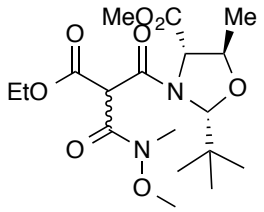
(*C*(7)), 59.5 (*C*(5)), 61.7 (NO*C*H$_3$), 62.6 (CO$_2$*C*H$_2$CH$_3$), 67.7 (*C*(4)), 97.2 (*C*(2)), 165.1 (*C*O$_2$CH$_2$CH$_3$), 165.6, 165.8 (*C*ON, *C*(8)), 169.7 (*C*O$_2$CH$_3$); *m/z* (ESI$^+$) 389.2 (MH$^+$, 100%), 411.2 (MNa$^+$, 61%); HRMS (ESI$^+$) found 411.1746, $C_{17}H_{28}N_2NaO_8$ (MNa$^+$) requires 411.1738.

(2*R*,5*S*)-2-(*tert*-Butyl)-1-((*R*)-1-ethoxycarbonyl-1-(methoxy(methyl)carbamoyl))acetyl-5-methoxycarbonyl-1,3-thiazolidine (92b) and (2*R*,5*S*)-2-(*tert*-Butyl)-1-((*S*)-1-ethoxycarbonyl-1-(methoxy(methyl)carbamoyl))acetyl-5-methoxycarbonyl-1,3-thiazolidine (92b')



General procedure A (from **43b**); yield 80% (1.41 g); orange oil; 2:1 mixture of diastereomers. R_f (10% EtOAc in DCM) 0.23; ν_{max}/cm^{-1} 2958 (C-H), 1745 (C=O), 1671 (C=O); δ_H (500 MHz, $CDCl_3$, 2.5:1 mixture of rotamers, major A and minor B) major diastereomer: 0.98 (9H, s, C(C*H*$_3$)$_3$ B), 1.00 (9H, s, C(C*H*$_3$)$_3$ A), 1.28-1.31 (3H, m, CO$_2$CH$_2$C*H*$_3$), 3.21 (3H, s, NC*H*$_3$ A), 3.26-3.35 (2H, m, C(4)H_AH_B), 3.39 (3H, s, NC*H*$_3$ B), 3.64 (3H, s, NOC*H*$_3$), 3.75 (3H, s, CO$_2$C*H*$_3$ B), 3.78 (3H, s, CO$_2$C*H*$_3$ A), 4.25-4.30 (2H, m, CO$_2$C*H*$_2$CH$_3$), 4.46 (1H, s, C(2)*H* B), 4.65 (1H, s, C(7)*H* A), 4.77 (1H, t, *J* 8.0, C(5)*H* A), 5.14-5.17 (2H, m, C(5)*H* B + C(7)*H* B), 5.62 (1H, s, C(2)*H* A); minor diastereomer: 0.98 (9H, s, C(C*H*$_3$)$_3$ B), 1.00 (9H, s, C(C*H*$_3$)$_3$ A), 1.28-1.31 (3H, m, CO$_2$CH$_2$C*H*$_3$), 3.19 (3H, s, NC*H*$_3$ A), 3.26-3.35 (2H, m, C(4)H_AH_B), 3.39 (3H, s, NC*H*$_3$ B), 3.70 (3H, s, NOC*H*$_3$), 3.74 (3H, s, CO$_2$C*H*$_3$ B), 3.78 (3H, s, CO$_2$C*H*$_3$ A), 4.25-4.30 (2H, m, CO$_2$C*H*$_2$CH$_3$), 4.54 (1H, s, C(2)*H* B), 4.65 (1H, s, C(7)*H* A), 4.77 (1H, t, *J* 8.0, C(5)*H* A), 5.06-5.08 (1H, m, C(5)*H* B), 5.06-5.22 (1H, m C(7)*H* B), 5.58 (1H, s, C(2)*H* A); δ_C (125 MHz, $CDCl_3$) major diastereomer: 14.2 (CO$_2$CH$_2$*C*H$_3$), 27.0 (C(*C*H$_3$)$_3$), 33.0 (N*C*H$_3$), 34.6 (*C*(4)), 39.8 (*C*(CH$_3$)$_3$), 53.1 (CO$_2$*C*H$_3$), 57.9 (*C*(7)), 61.2 (NO*C*H$_3$), 62.4 (CO$_2$*C*H$_2$CH$_3$), 64.6 (*C*(5)), 73.4 (*C*(2)), 164.7 (*C*O$_2$CH$_2$CH$_3$), 165.2 (*C*(8)), 165.5 (*C*ON), 170.7 (*C*O$_2$CH$_3$); minor diastereomer: 14.1 (CO$_2$CH$_2$*C*H$_3$), 27.1 (C(*C*H$_3$)$_3$), 32.5 (N*C*H$_3$), 34.0 (*C*(4)), 39.4 (*C*(CH$_3$)$_3$), 52.6 (CO$_2$*C*H$_3$), 57.7 (*C*(5)), 61.6 (NO*C*H$_3$), 62.6 (CO$_2$*C*H$_2$CH$_3$), 64.1 (*C*(7)), 73.8 (*C*(2)), 164.7 (*C*O$_2$CH$_2$CH$_3$), 165.4 (*C*(8)), 165.7 (*C*ON), 170.6 (*C*O$_2$CH$_3$); *m/z* (ESI$^+$) 405.2 (MH$^+$, 15%), 427.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 405.16796, $C_{17}H_{29}N_2O_7S$ (MH$^+$) requires 405.16900.

(2*R*,4*R*,5*S*)-2-(*tert*-Butyl)-1-((*R*)-1-ethoxycarbonyl-1-(methoxy(methyl)carbamoyl))acetyl-5-methoxycarbonyl-4-methyl-1,3-oxazolidine (92c) and
(2*R*,4*R*,5*S*)-2-(*tert*-Butyl)-1-((*S*)-1-ethoxycarbonyl-1-(methoxy(methyl)carbamoyl))acetyl-5-methoxycarbonyl-4-methyl-1,3-oxazolidine (92c')



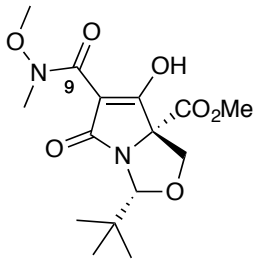
General procedure A (from **43c**); yield 76% (2.90 g); orange oil; 1.4:1 mixture of diastereomers. R_f (10% EtOAc in DCM) 0.15; ν_{max}/cm^{-1} 2979 (C-H), 2959 (C-H), 1745 (C=O), 1677 (C=O); δ_H (500 MHz, $CDCl_3$) major diastereomer: 0.91 (9H, s, C(CH_3)$_3$), 1.29 (3H, t, *J* 7.0, $CO_2CH_2CH_3$), 1.34 (3H, d, *J* 6.0, C(4)CH_3), 3.20 (3H, s, NCH_3), 3.66 (3H, s, NOCH_3), 3.76 (3H, s, CO_2CH_3), 4.11 (1H, d, *J* 3.5, C(5)*H*), 4.22-4.30 (m, 2H, $CO_2CH_2CH_3$), 4.74-4.86 (1H, m, C(4)*H*), 4.76 (1H, s, C(7)*H*), 5.50 (1H, s, C(2)*H*); minor diastereomer: 0.90 (9H, s, C(CH_3)$_3$), 1.29 (3H, t, *J* 7.0, $CO_2CH_2CH_3$), 1.34 (3H, d, *J* 6.0, C(4)CH_3), 3.22 (3H, s, NCH_3), 3.72 (3H, s, NOCH_3), 3.78 (3H, s, CO_2CH_3), 4.02 (1H, d, *J* 3.3, C(5)*H*), 4.22-4.30 (m, 2H, $CO_2CH_2CH_3$), 4.74-4.86 (1H, m, C(4)*H*), 4.90 (1H, s, C(7)*H*), 5.48 (1H, s, C(2)*H*); δ_C (100 MHz, $CDCl_3$) major diastereomer: 14.2 ($CO_2CH_2CH_3$), 20.1 (C(4)CH_3), 25.9 (C(CH_3)$_3$), 32.8 (NCH_3), 38.2 ($C(CH_3)_3$), 52.9 (CO_2CH_3), 58.5 (*C*(7)), 61.2 (NOCH_3), 62.3 ($CO_2CH_2CH_3$), 65.6 (*C*(5)), 76.4 (*C*(4)), 96.4 (*C*(2)), 164.9 ($CO_2CH_2CH_3$), 165.7, 165.8 (*C*ON, *C*(8)), 169.9 (CO_2CH_3); minor diastereomer: 14.2 ($CO_2CH_2CH_3$), 19.8 (C(4)CH_3), 25.9 (C(CH_3)$_3$), 33.0 (NCH_3), 37.9 ($C(CH_3)_3$), 52.9 (CO_2CH_3), 58.3 (*C*(7)), 61.7 (NOCH_3), 62.6 ($CO_2CH_2CH_3$), 65.3 (*C*(5)), 76.0 (*C*(4)), 96.4 (*C*(2)), 164.9 ($CO_2CH_2CH_3$), 165.4, 165.8 (*C*ON, *C*(8)), 169.9 (CO_2CH_3); *m/z* (ESI$^+$) 403.2 (MH^+, 13%), 425.2 (MNa^+, 100%); HRMS (ESI$^+$) found 403.20705, $C_{18}H_{31}N_2O_8$ (MH^+) requires 403.20749.

General procedure B: Dieckmann cyclisation of tricarbonyl oxazolidines and thiazolidines

1,8-Diazabicycloundec-7-ene (1.1 eq) was added to a solution of tricarbonyl oxazolidine or thiazolidine (1.0 eq) in anhydrous THF (*c* 0.2) and stirred at room temperature for 24 h. The reaction mixture was then cooled to 0 °C and diluted with water. The mixture was partitioned between Et_2O and water, the aqueous layer was acidified with 2 M aqueous HCl, extracted with

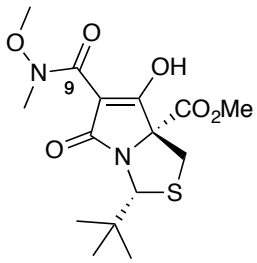
EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the Weinreb amide.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-7-(methoxy(methyl)carbamoyl)-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (77a)



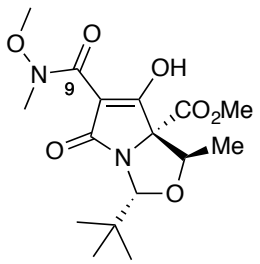
General procedure B (from **92a**); yield 84% (4.61 g); orange solid; m.p. 100-102 °C. R_f (20% MeOH in EtOAc) 0.31; $[\alpha]_D^{20}$ +117.9 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2959 (C-H), 1745 (C=O), 1708 (C=O), 1642 (C=O), 1579 (C=C); δ_H (400 MHz, $CDCl_3$) 0.91 (9H, s, C(CH_3)$_3$), 3.50 (1H, d, *J* 8.8, C(4)H_AH$_B$), 3.51 (3H, s, NCH_3), 3.78 (3H, s, CO_2CH_3), 3.80 (3H, s, NOCH_3), 4.77 (1H, s, C(2)*H*), 4.83 (1H, d, *J* 8.8, C(4)H$_A$$H_B$); δ_C (125 MHz, $CDCl_3$) 24.8 (C(*C*H$_3$)$_3$), 35.3 (*C*(CH$_3$)$_3$), 35.7 (N*C*H$_3$), 53.4 (CO_2*C*H$_3$), 62.4 (NO*C*H$_3$), 69.0 (*C*(4)), 76.1 (*C*(5)), 88.4 (*C*(7)), 98.1 (*C*(2)), 166.5 (*C*(9)), 168.0 (CO_2CH$_3$), 180.7 (*C*(8)), 185.7 (*C*(6)); *m/z* (ESI$^+$) 343.1 (MH$^+$, 40%), 365.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 343.14926, $C_{15}H_{23}N_2O_7$ (MH$^+$) requires 343.14998.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-7-(methoxy(methyl)carbamoyl)-8-oxo-3-thiabicyclo[3.3.0]-oct-6-ene (77b)



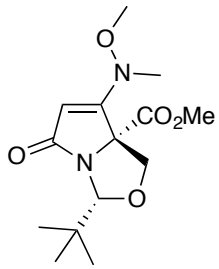
General procedure B (from **92b**); yield 84% (1.26 g); orange oil. R_f (20% MeOH in EtOAc) 0.39; $[\alpha]_D^{20}$ +150.4 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2956 (C-H), 1750 (C=O), 1697 (C=O), 1651 (C=O), 1568 (C=C); δ_H (400 MHz, $CDCl_3$) 0.95 (9H, s, C(CH_3)$_3$), 2.87 (1H, d, *J* 11.6, C(4)H_AH$_B$), 3.49 (3H, s, NCH_3), 3.75 (1H, d, *J* 11.6, C(4)H$_A$$H_B$), 3.76 (3H, s, CO_2CH_3), 3.77 (3H, s, NOCH_3), 5.03 (1H, s, C(2)*H*); δ_C (125 MHz, $CDCl_3$) 26.6 (C(*C*H$_3$)$_3$), 33.7 (*C*(4)), 35.9 (N*C*H$_3$), 36.8 (*C*(CH$_3$)$_3$), 53.4 (CO_2*C*H$_3$), 62.4 (NO*C*H$_3$), 73.7 (*C*(2)), 81.3 (*C*(5)), 85.0 (*C*(7)), 166.5 (*C*(9)), 168.2 (CO_2CH$_3$), 179.9 (*C*(8)), 185.6 (*C*(6)); *m/z* (ESI$^+$) 359.1 (MH$^+$, 54%), 381.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 359.12673, $C_{15}H_{23}N_2O_6S$ (MH$^+$) requires 359.12713.

(2*R*,4*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-7-(methoxy(methyl)carbamoyl)-4-methyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (77c)



General procedure B (from **92c**, with 5 eq of DBU); yield 79% (512 mg); orange oil. R_f (20% MeOH in EtOAc) 0.33; $[\alpha]_D^{20}$ +109.6 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2959 (C-H), 1750 (C=O), 1713 (C=O), 1644 (C=O), 1580 (C=C); δ_H (400 MHz, $CDCl_3$) 0.90 (9H, s, C(C*H*$_3$)$_3$), 1.08 (3H, d, *J* 6.7, C(4)C*H*$_3$), 3.51 (3H, s, NC*H*$_3$), 3.78 (3H, s, CO_2CH_3), 3.81 (3H, s, NOC*H*$_3$), 4.89 (1H, s, C(2)*H*), 5.14 (1H, q, *J* 6.7, C(4)*H*); δ_C (125 MHz, $CDCl_3$) 14.7 (C(4)*C*H$_3$), 24.9 (C(*C*H$_3$)$_3$), 35.4 (*C*(CH$_3$)$_3$), 35.6 (N*C*H$_3$), 53.4 (CO_2CH_3), 62.3 (NO*C*H$_3$), 74.8 (*C*(4)), 78.8 (*C*(5)), 91.5 (*C*(7)), 95.3 (*C*(2)), 166.3 (*C*(9)), 168.4 (*C*O$_2$CH$_3$), 179.0 (*C*(8)), 185.7 (*C*(6)); *m/z* (ESI$^+$) 357.2 (MH$^+$, 46%), 379.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 379.14727, $C_{16}H_{24}N_2NaO_7$ (MNa$^+$) requires 379.14757.

(2*R*,5*S*)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-6-(methoxy(methyl)amino)-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (93)



A solution of Weinreb amide **77a** (41 mg, 0.12 mmol) in $CDCl_3$ (1 mL) was stirred at room temperature for 30 d and evaporated *in vacuo*. The residue was purified by *flash* column chromatography (50% EtOAc in petrol) to give enamine **93** (21 mg, 0.070 mmol, 59%) as an off-white solid; m.p. 86 °C. R_f (50% EtOAc in petrol) 0.24; $[\alpha]_D^{20}$ +148.0 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2957 (C-H), 1746 (C=O), 1702 (C=O), 1603 (C=C); δ_H (500 MHz, $CDCl_3$) 0.89 (9H, s, C(C*H*$_3$)$_3$), 2.98 (3H, s, NC*H*$_3$), 3.43 (1H, d, *J* 8.7, C(4)*H*$_A$H$_B$), 3.51 (3H, s, NOC*H*$_3$), 3.77 (3H, s, CO_2CH_3), 4.67 (1H, s, C(2)*H*), 4.79 (1H, d, *J* 8.7, C(4)H$_A$*H*$_B$), 4.87 (*C*(7)); δ_C (125 MHz, $CDCl_3$) 24.9 (C(*C*H$_3$)$_3$), 35.3 (*C*(CH$_3$)$_3$), 38.8 (N*C*H$_3$), 53.1 (CO_2CH_3), 61.0 (NO*C*H$_3$), 69.8 (*C*(4)), 74.8 (*C*(5)), 95.9 (*C*(7)), 96.9 (*C*(2)), 167.0 (*C*(6)), 169.1 (*C*O$_2$CH$_3$), 178.1 (*C*(8)); *m/z* (ESI$^+$) 299.2 (MH$^+$, 100%), 321.2 (MNa$^+$, 48%); HRMS (ESI$^+$) found 299.16017, $C_{14}H_{23}N_2O_5$ (MH$^+$) requires 299.16015.

General procedure C: Reaction of Weinreb amide with Grignard reagents

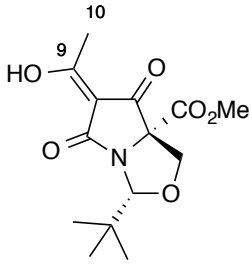
The Grignard reagent was prepared in excess.

Synthesis of alkyl, aryl and alkenyl Grignard reagents: The bromide (3.7 mmol, 1.0 eq) and 2 drops of 1,2-dibromoethane were added dropwise to magnesium turnings (100 mg, 4.11 mmol, 1.1 eq) in anhydrous THF (10 mL). The mixture was heated at 45 °C for 1 h and then cooled to room temperature.

Synthesis of alkynyl Grignard reagents: Isobutylmagnesium bromide (2 M in Et_2O, 1.0 eq) was added dropwise to a solution of the 1-alkyne (1.1 eq) in anhydrous THF (*c* 0.5) at 0 °C. The suspension was then warmed to room temperature and heated at 40 °C for 1.5 h. The solution was used for the Grignard reaction directly at 40 °C.

The prepared Grignard reagent (2.4 eq) was added to the Weinreb amide (1.0 eq) in anhydrous THF (*c* 0.2) dropwise at -15 °C. The reaction mixture was stirred at -15 °C for 1 h. The solution was quenched with saturated aqueous NH_4Cl, acidified with 2 M aqueous HCl and extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, concentrated *in vacuo* and purified via *flash* column chromatography. The product thus obtained was dissolved in EtOAc, washed with 2 M aqueous HCl, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give the *C*(7)-acyltetramate.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*)-1-hydroxyethylidene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (81a)[154]



Method 1: DMAP (50 mg, 0.41 mmol) was added to a solution of the *O*-acylated product **86** (76 mg, 0.25 mmol) in anhydrous DCM (600 µL). The mixture was stirred at room temperature for 4 h and the solvent was then evaporated under reduced pressure. The crude product was purified by *flash* column chromatography (10% MeOH in EtOAc) to give an orange solid which was washed with 2 M aqueous HCl, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give the *C*-acyltetramate **81a** (64 mg, 0.22 mmol, 85%).

Method 2: General procedure C (from **77a**, with 3 M MeMgBr in Et_2O); yield 82% (66 mg).

Orange oil; 4.5:1 mixture of tautomers. R_f (EtOAc) 0.11; $[\alpha]_D^{20}$ +73.2 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2959 (C-H), 1754 (C=O), 1718 (C=O), 1657 (C=O), 1604 (C=C); δ_H (500 MHz, $CDCl_3$) major tautomer: 0.93 (9H, s, C(CH_3)$_3$), 2.49 (3H, s, C(10)H_3), 3.50 (1H, d, *J* 9.0, C(4)H_AH$_B$), 3.79 (3H, s, CO_2CH_3), 4.83 (1H, d, *J* 9.0, C(4)H$_A$$H_B$), 4.85 (1H, s, C(2)*H*); minor tautomer: 0.93 (9H, s, C(CH_3)$_3$), 2.57 (3H, s, C(10)H_3), 3.50 (1H, d, *J* 9.0, C(4)H_AH$_B$), 3.80 (3H, s, CO_2CH_3), 4.84 (1H, d, *J* 9.0, C(4)H$_A$$H_B$), 4.89 (1H, s, C(2)*H*); δ_C (125 MHz, $CDCl_3$) major tautomer: 20.1 (*C*(10)), 24.8 (C(*C*H$_3$)$_3$), 35.3 (*C*(CH$_3$)$_3$), 53.5 (CO_2CH_3), 68.3 (*C*(4)), 78.6 (*C*(5)), 98.4 (*C*(2)), 101.9 (*C*(7)), 167.6 (CO_2CH_3), 180.0 (*C*(8)), 188.1 (*C*(6)), 188.6 (*C*(9)); minor tautomer: 20.0 (*C*(10)), 24.9 (C(*C*H$_3$)$_3$), 35.5 (*C*(CH$_3$)$_3$), 53.6 (CO_2CH_3), 68.3 (*C*(4)), 76.6 (*C*(5)), 98.5 (*C*(2)), 104.6 (*C*(7)), 167.5 (CO_2CH_3), 172.5 (*C*(8)), 190.2 (*C*(9)), 195.7 (*C*(6)); *m/z* (ESI$^+$) 298.1 (MH$^+$, 65%), 320.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 320.10993, $C_{14}H_{19}NNaO_6$ (MNa$^+$) requires 320.11046.

δ_H (500 MHz, CD_2Cl_2, 3.5:1 mixture of tautomers) major tautomer: 0.91 (9H, s, C(CH_3)$_3$), 2.47 (3H, s, C(10)H_3), 3.50 (1H, d, *J* 9.0, C(4)H_AH$_B$), 3.76 (3H, s, CO_2CH_3), 4.77 (1H, d, *J* 8.5, C(4)H$_A$$H_B$), 4.85 (1H, s, C(2)*H*); minor tautomer: 0.90 (9H, s, C(CH_3)$_3$), 2.54 (3H, s, C(10)H_3), 3.50 (1H, d, *J* 9.0, C(4)H_AH$_B$), 3.77 (3H, s, CO_2CH_3), 4.78 (1H, d, *J* 9.0, C(4)H$_A$$H_B$), 4.86 (1H, s, C(2)*H*); δ_C (125 MHz, CD_2Cl_2) major tautomer: 20.3 (*C*(10)), 25.0 (C(*C*H$_3$)$_3$), 35.6 (*C*(CH$_3$)$_3$), 53.8 (CO_2CH_3), 68.7 (*C*(4)), 79.0 (*C*(5)), 98.7 (*C*(2)), 102.3 (*C*(7)), 168.1 (CO_2CH_3), 180.5 (*C*(8)), 188.4 (*C*(6)), 189.0 (*C*(9)); minor tautomer: 20.2 (*C*(10)), 25.1 (C(*C*H$_3$)$_3$), 35.7 (*C*(CH$_3$)$_3$), 53.9 (CO_2CH_3), 68.7 (*C*(4)), 77.0 (*C*(5)), 98.8 (*C*(2)), 104.9 (*C*(7)), 168.0 (CO_2CH_3), 172.7 (*C*(8)), 190.6 (*C*(9)), 196.1 (*C*(6)).

δ_H (500 MHz, C_6D_6, 4.8:1 mixture of tautomers) major tautomer: 1.02 (9H, s, C(CH_3)$_3$), 1.92 (3H, s, C(10)H_3), 3.08 (1H, d, *J* 9.0, C(4)H_AH$_B$), 3.27 (3H, s, CO_2CH_3), 4.78 (1H, s, C(2)*H*), 4.87 (1H, d, *J* 8.5, C(4)H$_A$$H_B$); minor tautomer: 1.07 (9H, s, C(CH_3)$_3$), 1.97 (3H, s, C(10)H_3), 3.04 (1H, d, *J* 9.0, C(4)H_AH$_B$), 3.26 (3H, s, CO_2CH_3), 4.84 (1H, d, *J* 9.0, C(4)H$_A$$H_B$), 4.98 (1H, s, C(2)*H*); δ_C (125 MHz, C_6D_6) major tautomer: 19.1 (*C*(10)), 24.9 (C(*C*H$_3$)$_3$), 35.4 (*C*(CH$_3$)$_3$), 52.8 (CO_2CH_3), 68.3 (*C*(4)), 78.9 (*C*(5)), 98.4 (*C*(2)), 102.1 (*C*(7)), 167.7 (CO_2CH_3), 180.4 (*C*(8)), 187.7 (*C*(6)), 188.1 (*C*(9)); minor

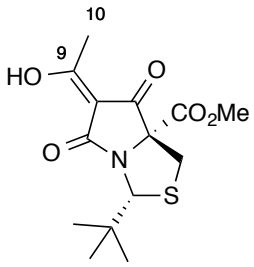
tautomer: 19.0 (*C*(10)), 25.1 (C(*C*H$_3$)$_3$), 35.6 (*C*(CH$_3$)$_3$), 52.9 (CO$_2$*C*H$_3$), 68.2 (*C*(4)), 77.0 (*C*(5)), 98.6 (*C*(2)), 104.9 (*C*(7)), 167.7 (*C*O$_2$CH$_3$), 171.9 (*C*(8)), 189.9 (*C*(9)), 196.3 (*C*(6)).

δ_H (500 MHz, DMSO-d$_6$) 0.82 (9H, s, C(C*H*$_3$)$_3$), 2.38 (3H, s, C(10)*H*$_3$), 3.49 (1H, d, *J* 8.5, C(4)*H*$_A$H$_B$), 3.66 (3H, s, CO$_2$C*H*$_3$), 4.55 (1H, d, *J* 8.5, C(4)H$_A$*H*$_B$), 4.73 (1H, s, C(2)*H*); δ_C (125 MHz, DMSO-d$_6$) 22.4 (*C*(10)), 24.7 (C(*C*H$_3$)$_3$), 34.9 (*C*(CH$_3$)$_3$), 52.8 (CO$_2$*C*H$_3$), 67.8 (*C*(4)), 76.8 (*C*(5)), 97.2 (*C*(2)), 101.3 (*C*(7)), 168.9 (*C*O$_2$CH$_3$), 176.7 (*C*(8)), 187.7 (*C*(9)), 188.5 (*C*(6)).

δ_H (500 MHz, CD$_3$OD) 0.91 (9H, s, C(C*H*$_3$)$_3$), 2.49 (3H, s, C(10)*H*$_3$), 3.57 (1H, d, *J* 9.0, C(4)*H*$_A$H$_B$), 3.76 (3H, s, CO$_2$C*H*$_3$), 4.72 (1H, d, *J* 8.5, C(4)H$_A$*H*$_B$), 4.86 (1H, s, C(2)*H*); δ_C (125 MHz, CD$_3$OD) 20.0 (*C*(10)), 25.2 (C(*C*H$_3$)$_3$), 36.1 (*C*(CH$_3$)$_3$), 53.7 (CO$_2$*C*H$_3$), 69.0 (*C*(4)), 79.3 (*C*(5)), 99.4 (*C*(2)), 103.4 (*C*(7)), 169.3 (*C*O$_2$CH$_3$), 179.4 (*C*(8)), 189.7 (*C*(9)), 191.0 (*C*(6)).

δ_H (500 MHz, CD$_3$CN) 0.89 (9H, s, C(C*H*$_3$)$_3$), 2.45 (3H, s, C(10)*H*$_3$), 3.57 (1H, d, *J* 8.5, C(4)*H*$_A$H$_B$), 3.74 (3H, s, CO$_2$C*H*$_3$), 4.68 (1H, d, *J* 9.0, C(4)H$_A$*H*$_B$), 4.85 (1H, s, C(2)*H*); δ_C (125 MHz, CD$_3$CN) 20.2 (*C*(10)), 25.0 (C(*C*H$_3$)$_3$), 35.7 (*C*(CH$_3$)$_3$), 54.0 (CO$_2$*C*H$_3$), 68.7 (*C*(4)), 79.3 (*C*(5)), 99.0 (*C*(2)), 102.8 (*C*(7)), 168.8 (*C*O$_2$CH$_3$), 181.0 (*C*(8)), 189.0 (*C*(6)), 189.7 (*C*(9)).

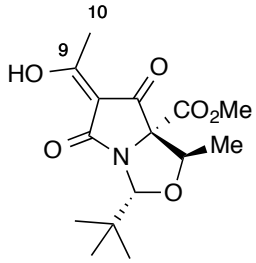
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*)-1-hydroxyethylidene]-5-methoxycarbonyl-6,8-dioxo-3-thiabicyclo[3.3.0]-octane (81b)



General procedure C (from **77b**, with 3 M MeMgBr in Et$_2$O); yield 63% (79 mg); orange solid; m.p. 99-101 °C; 5.6:1 mixture of tautomers. R$_f$ (EtOAc) 0.17; $[\alpha]_D^{20}$ +196.6 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2957 (C-H), 1757 (C=O), 1717 (C=O), 1656 (C=O), 1611 (C=C); δ_H (500 MHz, CDCl$_3$) major tautomer: 0.97 (9H, s, C(C*H*$_3$)$_3$), 2.47 (3H, s, C(10)*H*$_3$), 2.85 (1H, d, *J* 11.5, C(4)*H*$_A$H$_B$), 3.77 (1H, d, *J* 11.5, C(4)H$_A$*H*$_B$), 3.78 (3H, s, CO$_2$C*H*$_3$), 5.10 (1H, s, C(2)*H*); minor tautomer: 0.97 (9H, s, C(C*H*$_3$)$_3$), 2.56 (3H, s, C(10)*H*$_3$), 2.84 (1H, d, *J* 11.5, C(4)*H*$_A$H$_B$), 3.77 (1H, d, *J* 11.5, C(4)H$_A$*H*$_B$), 3.79 (3H, s, CO$_2$C*H*$_3$), 5.17 (1H, s, C(2)*H*); δ_C (100 MHz, CDCl$_3$) major tautomer: 20.0 (*C*(10)), 26.6 (C(*C*H$_3$)$_3$), 33.4 (*C*(4)), 36.9 (*C*(CH$_3$)$_3$), 53.5 (CO$_2$*C*H$_3$), 73.6 (*C*(2)), 83.1 (*C*(5)), 100.4 (*C*(7)), 167.6 (*C*O$_2$CH$_3$),

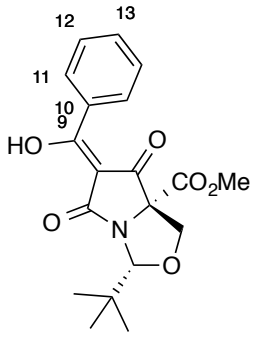
177.4 (*C*(8)), 187.6 (*C*(9)), 188.9 (*C*(6)); minor tautomer: 19.6 (*C*(10)), 26.6 (C(*C*H_3)$_3$), 33.6 (*C*(4)), 36.9 (*C*(CH_3)$_3$), 53.7 (CO_2*C*H_3), 73.8 (*C*(2)), 81.0 (*C*(5)), 103.0 (*C*(7)), 167.2 (*C*O_2CH_3), 170.3 (*C*(8)), 189.1 (*C*(9)), 196.5 (*C*(6)); *m/z* (ESI$^+$) 314.1 (MH^+, 52%), 336.1 (MNa^+, 100%); HRMS (ESI$^+$) found 336.08717, $C_{14}H_{19}NNaO_5S$ (MNa^+) requires 336.08761.

(2*R*,4*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*)-1-hydroxyethylidene]-5-methoxycarbonyl-4-methyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (81c)



General procedure C (from **77c**, with 3 M MeMgBr in Et_2O); yield 71% (150 mg); orange oil; 3.2:1 mixture of tautomers. R_f (EtOAc) 0.15; $[\alpha]_D^{20}$ +137.0 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2961 (C-H), 1756 (C=O), 1712 (C=O), 1659 (C=O), 1607 (C=C); δ_H (500 MHz, $CDCl_3$) major tautomer: 0.92 (9H, s, C(CH_3)$_3$), 1.05 (3H, d, *J* 6.8, C(4)CH_3), 2.48 (3H, s, C(10)H_3), 3.78 (3H, s, CO_2CH_3), 4.96 (1H, s, C(2)*H*), 5.14 (1H, q, *J* 6.8, C(4)*H*); minor tautomer: 0.91 (9H, s, C(CH_3)$_3$), 1.04 (3H, d, *J* 6.8, C(4)CH_3), 2.56 (3H, s, C(10)H_3), 3.79 (3H, s, CO_2CH_3), 4.98 (1H, s, C(2)*H*), 5.14 (1H, q, *J* 6.8, C(4)*H*); δ_C (100 MHz, $CDCl_3$) major tautomer: 14.7 (C(4)*C*H_3), 20.0 (*C*(10)), 24.8 (C(*C*H_3)$_3$), 35.4 (*C*(CH_3)$_3$), 53.4 (CO_2*C*H_3), 75.0 (*C*(4)), 81.5 (*C*(5)), 95.8 (*C*(2)), 103.4 (*C*(7)), 168.0 (*C*O_2CH_3), 179.5 (*C*(8)), 187.3 (*C*(9)), 188.6 (*C*(6)); minor tautomer: 14.9 (C(4)*C*H_3), 19.9 (*C*(10)), 24.9 (C(*C*H_3)$_3$), 35.5 (*C*(CH_3)$_3$), 53.5 (CO_2*C*H_3), 74.7 (*C*(4)), 79.7 (*C*(5)), 95.9 (*C*(2)), 106.1 (*C*(7)), 167.8 (*C*O_2CH_3), 172.2 (*C*(8)), 189.2 (*C*(9)), 196.0 (*C*(6)); *m/z* (ESI$^+$) 312.2 (MH^+, 19%), 334.1 (MNa^+, 100%); HRMS (ESI$^+$) found 334.12585, $C_{15}H_{21}NNaO_6$ (MNa^+) requires 334.12611.

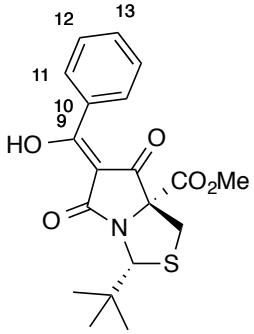
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*)-hydroxy(phenyl)methylene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (94a)



General procedure C (from **77a**, with bromobenzene); yield 84% (196 mg); orange oil. R_f (EtOAc) 0.32; $[\alpha]_D^{20}$ +157.2 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2960 (C-H), 1753 (C=O), 1711 (C=O), 1652 (C=O), 1591 (C=C), 1557 (C=C); δ_H (500 MHz, $CDCl_3$) 0.97 (9H, s, C(CH_3)$_3$), 3.55 (1H, d, *J* 9.0, C(4)H_AH_B), 3.82 (3H, s, CO_2CH_3), 4.86 (1H, d, *J* 9.0, C(4)H_AH_B), 4.93 (1H, s, C(2)*H*), 7.50 (2H,

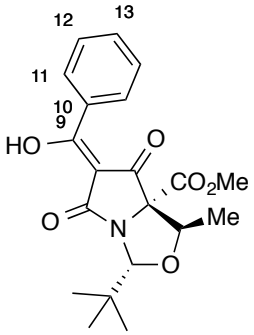
t, *J* 7.5, C(12)*H*), 7.64 (1H, t, *J* 7.5, C(13)*H*), 8.18 (2H, d, *J* 7.5, C(11)*H*); δ_C (100 MHz, $CDCl_3$) 24.8 (C(CH_3)$_3$), 35.4 (*C*(CH_3)$_3$), 53.5 (CO_2CH_3), 68.7 (*C*(4)), 77.8 (*C*(5)), 98.8 (*C*(2)), 99.7 (*C*(7)), 128.4 (*C*(12)), 130.2 (*C*(11)), 131.0 (*C*(10)), 134.9 (*C*(13)), 167.7 (CO_2CH_3), 182.6 (*C*(8)), 183.8 (*C*(9)), 186.4 (*C*(6)); *m/z* (ESI^+) 360.1 (MH^+, 100%), 382.1 (MNa^+, 66%); HRMS (ESI^+) found 382.12526, $C_{19}H_{21}NNaO_6$ (MNa^+) requires 382.12611.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*)-hydroxyl(phenyl)methylene]-5-methoxycarbonyl-6,8-dioxo-3-thiabicyclo[3.3.0]-octane (94b)



General procedure C (from **77b**, with bromobenzene); yield 76% (133 mg); orange oil. R_f (EtOAc) 0.31; $[\alpha]_D^{20}$ +262.1 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2956 (C-H), 1754 (C=O), 1708 (C=O), 1651 (C=O), 1592 (C=C), 1558 (C=C); δ_H (500 MHz, $CDCl_3$) 1.02 (9H, s, C(CH_3)$_3$), 2.90 (1H, d, *J* 11.5, C(4)H_AH_B), 3.80 (1H, d, *J* 11.5, C(4)H$_A$$H_B$), 3.81 (3H, s, CO_2CH_3), 5.20 (1H, s, C(2)*H*), 7.50 (2H, t, *J* 7.5, C(12)*H*), 7.63 (1H, t, *J* 7.5, C(13)*H*), 8.16 (2H, d, *J* 7.5, C(11)*H*); δ_C (100 MHz, $CDCl_3$) 26.7 (C(*C*H_3)$_3$), 33.7 (*C*(4)), 36.9 (*C*(CH_3)$_3$), 53.6 (CO_2CH_3), 74.2 (*C*(2)), 82.4 (*C*(5)), 98.2 (*C*(7)), 128.4 (*C*(12)), 130.1 (*C*(11)), 131.1 (*C*(10)), 134.7 (*C*(13)), 167.7 (CO_2CH_3), 179.9 (*C*(8)), 183.0 (*C*(9)), 187.2 (*C*(6)); *m/z* (ESI^+) 376.1 (MH^+, 96%), 398.1 (MNa^+, 100%); HRMS (ESI^+) found 398.10285, $C_{19}H_{21}NNaO_5S$ (MNa^+) requires 398.10326.

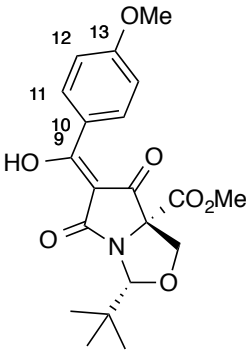
(2*R*,4*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*)-hydroxy(phenyl)methylene]-5-methoxycarbonyl-4-methyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (94c)



General procedure C (from **77c**, with bromobenzene); yield 74% (63 mg); orange oil. R_f (EtOAc) 0.33; $[\alpha]_D^{20}$ +188.2 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2961 (C-H), 1755 (C=O), 1712 (C=O), 1653 (C=O), 1593 (C=C), 1559 (C=C); δ_H (400 MHz, $CDCl_3$) 0.95 (9H, s, C(CH_3)$_3$), 1.07 (3H, d, *J* 6.8, C(4)CH_3), 3.80 (3H, s, CO_2CH_3), 5.03 (1H, s, C(2)*H*), 5.17 (1H, q, *J* 6.8, C(4)*H*), 7.50 (2H, t, *J* 7.5, C(12)*H*), 7.63 (1H, t, *J* 7.5, C(13)*H*), 8.16 (2H, d, *J* 7.5, C(11)*H*); δ_C (100 MHz, $CDCl_3$) 14.6 (C(4)*C*H_3), 24.9 (C(*C*H_3)$_3$), 35.4 (*C*(CH_3)$_3$), 53.4 (CO_2CH_3), 75.3 (*C*(4)), 80.7 (*C*(5)), 96.1 (*C*(2)),

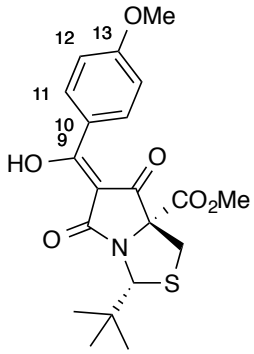
101.2 (*C*(7)), 128.4 (*C*(12)), 130.2 (*C*(11)), 131.0 (*C*(10)), 134.8 (*C*(13)), 168.0 (*C*$O_2$$CH_3$), 182.0 (*C*(8)), 182.6 (*C*(9)), 186.8 (*C*(6)); *m/z* (ESI$^+$) 374.2 (MH^+, 100%), 396.2 (MNa^+, 77%); HRMS (ESI$^+$) found 374.15920, $C_{20}H_{24}NO_6$ (MH^+) requires 374.15981.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*)-hydroxy(4-methoxyphenyl)methylene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (95a)



General procedure C (from **77a**, with 4-bromoanisole); yield 83% (69 mg); orange oil. R_f (EtOAc) 0.28; $[\alpha]_D^{20}$ +143.0 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2960 (C-H), 1754 (C=O), 1703 (C=O), 1652 (C=O), 1603 (C=C), 1586 (C=C); δ_H (500 MHz, $CDCl_3$) 0.96 (9H, s, C(CH_3)$_3$), 3.54 (1H, d, *J* 8.9, C(4)H_AH$_B$), 3.82 (3H, s, CO_2CH_3), 3.90 (3H, s, C(13)OCH_3), 4.86 (1H, d, *J* 8.9, C(4)H$_A$$H_B$), 4.92 (1H, s, C(2)*H*), 6.98 (2H, d, *J* 9.0, C(12)*H*), 8.34 (2H, d, *J* 9.0, C(11)*H*); δ_C (125 MHz, $CDCl_3$) 24.8 (C(*C*H$_3$)$_3$), 35.4 (*C*(CH$_3$)$_3$), 53.5 (CO_2*C*H$_3$), 55.8 (C(13)O*C*H$_3$), 68.8 (*C*(4)), 77.6 (*C*(5)), 98.6 (*C*(7)), 98.8 (*C*(2)), 113.9 (*C*(12)), 123.5 (*C*(10)), 133.2 (*C*(11)), 165.4 (*C*(13)), 168.0 (*C*$O_2$$CH_3$), 182.8 (*C*(9)), 183.3 (*C*(8)), 186.6 (*C*(6)); *m/z* (ESI$^+$) 390.2 (MH^+, 100%), 412.1 (MNa^+, 46%); HRMS (ESI$^+$) found 390.15407, $C_{20}H_{24}NO_7$ (MH^+) requires 390.15473.

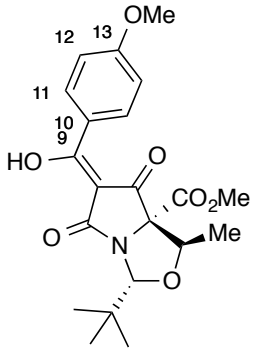
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*)-hydroxy(4-methoxyphenyl)methylene]-5-methoxycarbonyl-6,8-dioxo-3-thiabicyclo[3.3.0]-octane (95b)



General procedure C (from **77b**, with 4-bromoanisole); yield 75% (144 mg); yellow solid; m.p. 58-60 °C. R_f (EtOAc) 0.24; $[\alpha]_D^{20}$ +280.7 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2956 (C-H), 1753 (C=O), 1700 (C=O), 1649 (C=O), 1584 (C=C), 1551 (C=C), 1508 (C=C); δ_H (500 MHz, $CDCl_3$) 1.01 (9H, s, C(CH_3)$_3$), 2.90 (1H, d, *J* 11.5, C(4)H_AH$_B$), 3.81 (3H, s, CO_2CH_3), 3.81 (1H, d, *J* 11.5, C(4)H$_A$$H_B$), 3.90 (3H, s, C(13)OC$H_3$), 5.19 (1H, s, C(2)*H*), 6.97 (2H, d, *J* 9.0, C(12)*H*), 8.32 (2H, d, *J* 9.0, C(11)*H*); δ_C (100 MHz, $CDCl_3$) 26.7 (C(*C*H$_3$)$_3$), 33.9 (*C*(4)), 36.9 (*C*(CH$_3$)$_3$), 53.5 (CO_2*C*H$_3$), 55.8 (C(13)O*C*H$_3$), 74.2 (*C*(2)), 82.3 (*C*(5)), 97.1 (*C*(7)), 113.9 (*C*(12)), 123.6 (*C*(10)), 133.0 (*C*(11)), 165.2 (*C*(13)), 168.0 (*C*$O_2$$CH_3$), 180.6 (*C*(8)), 182.2 (*C*(9)), 187.3 (*C*(6));

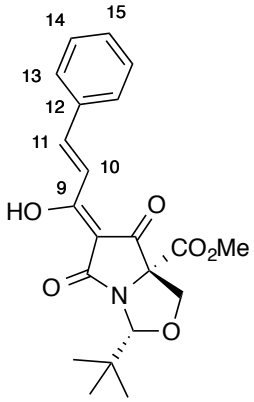
m/z (ESI+) 406.1 (MH^+, 79%), 428.1 (MNa^+, 100%); HRMS (ESI+) found 428.11344, $C_{20}H_{23}NNaO_6S$ (MNa^+) requires 428.11383.

(2*R*,4*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*)-hydroxy(4-methoxyphenyl)methylene]-5-methoxycarbonyl-4-methyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (95c)



General procedure C (from **77c**, with 4-bromoanisole); yield 60% (57 mg); orange oil. R_f (EtOAc) 0.30; $[\alpha]_D^{20}$ +180.3 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2960 (C-H), 1753 (C=O), 1699 (C=O), 1650 (C=O), 1585 (C=C), 1553 (C=C); δ_H (400 MHz, $CDCl_3$) 0.94 (9H, s, C(CH_3)$_3$), 1.06 (3H, d, *J* 6.7, C(4)CH_3), 3.80 (3H, s, CO_2CH_3), 3.88 (3H, s, C(13)OCH_3), 5.01 (1H, s, C(2)*H*), 5.16 (1H, q, *J* 6.7, C(4)*H*), 6.97 (2H, d, *J* 9.2, C(12)*H*), 8.31 (2H, d, *J* 9.2, C(11)*H*); δ_C (100 MHz, $CDCl_3$) 14.6 (C(4)*C*H_3), 24.9 (C(*C*H_3)$_3$), 35.4 (*C*(CH$_3$)$_3$), 53.4 (CO_2*C*H_3), 55.7 (C(13)O*C*H_3), 75.3 (*C*(4)), 80.5 (*C*(5)), 96.0 (*C*(2)), 100.1 (*C*(7)), 113.9 (*C*(12)), 123.5 (*C*(10)), 133.1 (*C*(11)), 165.3 (*C*(13)), 168.3 (CO_2CH_3), 181.7 (*C*(9)), 182.6 (*C*(8)), 186.9 (*C*(6)); *m/z* (ESI+) 404.2 (MH^+, 64%), 426.1 (MNa^+, 100%); HRMS (ESI-) found 402.15611, $C_{21}H_{24}NO_7$ ($M\text{-}H^-$) requires 402.15583.

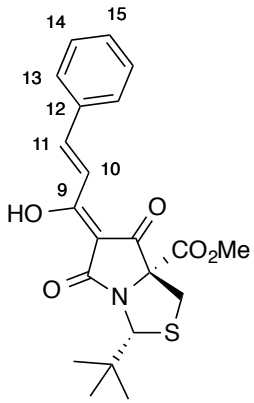
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*,2*E*)-1-hydroxy-3-phenylallylidene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (96a)



General procedure C (from **77a**, with β-bromostyrene); yield 79% (271 mg); orange oil; 3.6:1 mixture of tautomers. R_f (EtOAc) 0.47; $[\alpha]_D^{20}$ +78.4 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2959 (C-H), 1752 (C=O), 1708 (C=O), 1653 (C=O), 1622 (C=C), 1580 (C=C), 1562 (C=C); δ_H (500 MHz, $CDCl_3$) major tautomer: 0.95 (9H, s, C(CH_3)$_3$), 3.55 (1H, d, *J* 8.9, C(4)H_AH_B), 3.80 (3H, s, CO_2CH_3), 4.86 (1H, d, *J* 8.9, C(4)H_AH_B), 4.90 (1H, s, C(2)*H*), 7.41-7.47 (3H, m, C(14)*H* + C(15)*H*), 7.66 (2H, dd, *J* 7.8, 1.8, C(13)*H*), 7.72 (1H, d, *J* 16.0, C(10)*H*), 7.98 (1H, d, *J* 16.0, C(11)*H*); minor tautomer: 0.95 (9H, s, C(CH_3)$_3$), 3.53 (1H, d, *J* 8.5, C(4)H_AH_B), 3.81 (3H, s, CO_2CH_3), 4.87 (1H, d, *J* 8.5, C(4)H_AH_B), 4.94 (1H, s, C(2)*H*), 7.41-7.47 (3H, m, C(14)*H* + C(15)*H*),

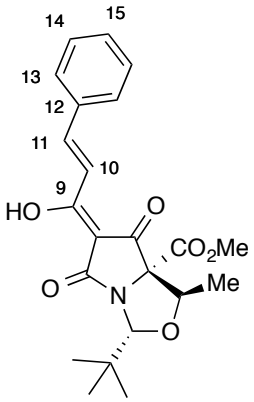
7.67-7.69 (2H, m, C(13)*H*), 7.90 (1H, d, *J* 16.0, C(10)*H*), 8.01 (1H, d, *J* 16.0, C(11)*H*); δ_C (125 MHz, $CDCl_3$) major tautomer: 24.8 (C(CH_3)$_3$), 35.4 (*C*(CH_3)$_3$), 53.5 (CO_2CH_3), 68.5 (*C*(4)), 78.5 (*C*(5)), 98.5 (*C*(2)), 100.3 (*C*(7)), 117.4 (*C*(10)), 129.3 (*C*(14)), 129.5 (*C*(13)), 132.0 (*C*(15)), 134.2 (*C*(12)), 147.2 (*C*(11)), 167.8 (CO_2CH_3), 176.9 (*C*(9)), 180.7 (*C*(8)), 188.1 (*C*(6)); minor tautomer: 24.9 (C(CH_3)$_3$), 35.5 (*C*(CH_3)$_3$), 53.6 (CO_2CH_3), 68.4 (*C*(4)), 78.5 (*C*(5)), 98.6 (*C*(2)), 102.5 (*C*(7)), 116.7 (*C*(10)), 129.5 (*C*(14)), 129.7 (*C*(13)), 132.2 (*C*(15)), 134.3 (*C*(12)), 148.0 (*C*(11)), 167.7 (CO_2CH_3), 172.9 (*C*(8)), 177.4 (*C*(9)), 196.9 (*C*(6)); *m/z* (ESI$^+$) 386.1 (MH$^+$, 40%), 408.1 (MNa$^+$, 80%); HRMS (ESI$^+$) found 386.15902, $C_{21}H_{24}NO_6$ (MH$^+$) requires 386.15981.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*,2*E*)-1-hydroxy-3-phenylallylidene]-5-methoxycarbonyl-6,8-dioxo-3-thiabicyclo[3.3.0]-octane (96b)



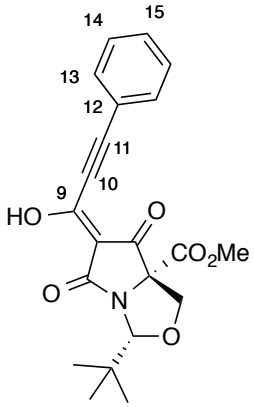
General procedure C (from **77b**, with β-bromostyrene); yield 72% (125 mg); orange solid; m.p. 137-139 °C; 3.8:1 mixture of tautomers. R_f (EtOAc) 0.48; $[\alpha]_D^{20}$ +292.8 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2956 (C-H), 1755 (C=O), 1707 (C=O), 1652 (C=O), 1626 (C=C), 1582 (C=C), 1564 (C=C); δ_H (500 MHz, $CDCl_3$) major tautomer: 1.00 (9H, s, C(CH_3)$_3$), 2.91 (1H, d, *J* 11.5, C(4)H_AH_B), 3.80 (3H, s, CO_2CH_3), 3.81 (1H, d, *J* 11.5, C(4)H_AH_B), 5.18 (1H, s, C(2)*H*), 7.40-7.47 (3H, m, C(14)*H* + C(15)*H*), 7.66 (2H, dd, *J* 7.8, 1.8, C(13)*H*), 7.72 (1H, d, *J* 16.0, C(10)*H*), 7.95 (1H, d, *J* 16.0, C(11)*H*); δ_C (100 MHz, $CDCl_3$) major tautomer: 26.6 (C(CH_3)$_3$), 33.6 (*C*(4)), 36.9 (*C*(CH_3)$_3$), 53.5 (CO_2CH_3), 73.8 (*C*(2)), 83.1 (*C*(5)), 98.8 (*C*(7)), 117.5 (*C*(10)), 129.3 (*C*(14)), 129.4 (*C*(13)), 131.9 (*C*(15)), 134.3 (*C*(12)), 146.7 (*C*(11)), 167.8 (CO_2CH_3), 176.3 (*C*(9)), 178.1 (*C*(8)), 188.9 (*C*(6)); *m/z* (ESI$^+$) 402.1 (MH$^+$, 95%), 424.1 (MNa$^+$, 87%); HRMS (ESI$^+$) found 424.11816, $C_{21}H_{23}NNaO_5S$ (MNa$^+$) requires 424.11891.

(2*R*,4*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*,2*E*)-1-hydroxy-3-phenylallylidene]-5-methoxycarbonyl-4-methyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (96c)



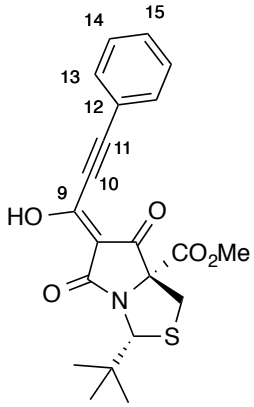
General procedure C (from **77c**, with β-bromostyrene); yield 71% (69 mg); yellow solid; m.p. 78-80 °C; 3.5:1 mixture of tautomers. R_f (EtOAc) 0.41; $[\alpha]_D^{20}$ +67.3 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2960 (C-H), 1754 (C=O), 1709 (C=O), 1652 (C=O), 1625 (C=C), 1581 (C=C), 1564 (C=C); δ_H (500 MHz, $CDCl_3$) major tautomer: 0.94 (9H, s, C(CH_3)$_3$), 1.08 (1H, d, *J* 7.0, C(4)CH_3), 3.78 (3H, s, CO_2CH_3), 4.99 (1H, s, C(2)*H*), 5.16 (1H, q, *J* 7.0, C(4)*H*), 7.38-7.46 (3H, m, C(14)*H* + C(15)*H*), 7.65 (2H, dd, *J* 8.0, 1.5, C(13)*H*), 7.72 (1H, d, *J* 16.0, C(10)*H*), 7.95 (1H, d, *J* 16.0, C(11)*H*); δ_C (125 MHz, $CDCl_3$) major tautomer: 14.7 (C(4)*C*H$_3$), 24.8 (C(*C*H$_3$)$_3$), 35.4 (*C*(CH$_3$)$_3$), 53.3 (CO_2CH_3), 75.1 (*C*(4)), 81.4 (*C*(5)), 95.8 (*C*(2)), 101.8 (*C*(7)), 117.4 (*C*(10)), 129.2 (*C*(14)), 129.4 (*C*(13)), 131.9 (*C*(15)), 134.2 (*C*(12)), 146.8 (*C*(11)), 168.1 (CO_2CH_3), 175.8 (*C*(9)), 180.1 (*C*(8)), 188.4 (*C*(6)); *m/z* (ESI$^+$) 400.2 (MH$^+$, 35%), 422.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 400.17522, $C_{22}H_{26}NO_6$ (MH$^+$) requires 400.17546.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*)-1-hydroxy-3-phenylprop-2-yn-1-ylidene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (97a)



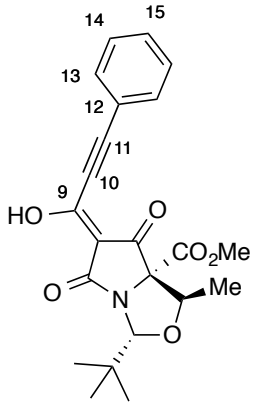
General procedure C (from **77a**, with phenylacetylene); yield 81% (47 mg); orange oil. R_f (EtOAc) 0.43; $[\alpha]_D^{20}$ +122.7 (*c* 0.6, DCM); ν_{max}/cm^{-1} 2959 (C-H), 2209 (C≡C), 1755 (C=O), 1719 (C=O), 1664 (C=O), 1601 (C=C), 1579 (C=C); δ_H (400 MHz, $CDCl_3$) 0.94 (9H, s, C(CH_3)$_3$), 3.56 (1H, d, *J* 8.8, C(4)H_AH$_B$), 3.80 (3H, s, CO_2CH_3), 4.86 (1H, d, *J* 8.8, C(4)H$_A$$H_B$), 4.89 (1H, s, C(2)*H*), 7.41 (2H, t, *J* 7.4, C(14)*H*), 7.51 (1H, t, *J* 7.4, C(15)*H*), 7.74 (2H, d, *J* 7.4, C(13)*H*); δ_C (100 MHz, $CDCl_3$) 24.8 (C(*C*H$_3$)$_3$), 35.4 (*C*(CH$_3$)$_3$), 53.5 (CO_2CH_3), 68.4 (*C*(4)), 78.5 (*C*(5)), 98.5 (*C*(2)), 105.7, 119.5, 126.2 (*C*(7), *C*(10), *C*(11)), 128.9 (*C*(14)), 129.5 (*C*(12)), 132.1 (*C*(15)), 133.9 (*C*(13)), 160.9 (*C*(9)), 167.5 (CO_2CH_3), 179.4 (*C*(8)), 186.1 (*C*(6)); *m/z* (ESI$^+$) 384.1 (MH$^+$, 100%), 406.1 (MNa$^+$, 56%); HRMS (ESI$^+$) found 384.14302, $C_{21}H_{22}NO_6$ (MH$^+$) requires 384.14416.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*)-1-hydroxy-3-phenylprop-2-yn-1-ylidene]-5-methoxycarbonyl-6,8-dioxo-3-thiabicyclo[3.3.0]-octane (97b)



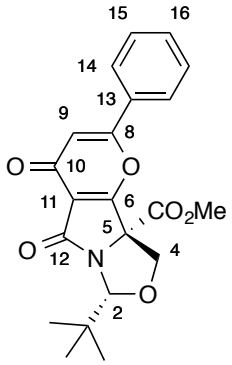
General procedure C (from **77b**, with phenylacetylene); yield 66% (29 mg); orange oil. R_f (EtOAc) 0.46; $[\alpha]_D^{20}$ +309.9 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2956 (C-H), 2208 (C≡C), 1754 (C=O), 1662 (C=O), 1579 (C=C); δ_H (400 MHz, $CDCl_3$) 1.00 (9H, s, C(CH_3)$_3$), 2.92 (1H, d, *J* 11.2, C(4)H_AH_B), 3.80 (3H, s, CO_2CH_3), 3.81 (1H, d, *J* 11.2, C(4)H_AH_B), 5.14 (1H, s, C(2)*H*), 7.42 (2H, t, *J* 7.2, C(14)*H*), 7.48-7.51 (1H, m, C(15)*H*), 7.74 (2H, d, *J* 7.2, C(13)*H*); δ_C (125 MHz, $CDCl_3$) 26.6 (C(*C*H$_3$)$_3$), 33.5 (*C*(4)), 36.9 (*C*(CH$_3$)$_3$), 53.6 (CO_2CH_3), 73.8 (*C*(2)), 83.0 (*C*(5)), 106.5, 119.6, 126.1 (*C*(7), *C*(10), *C*(11)), 128.9 (*C*(14)), 129.5 (*C*(12)), 132.0 (*C*(15)), 133.9 (*C*(13)), 160.1 (*C*(9)), 167.5 (CO_2CH_3), 176.9 (*C*(8)), 186.9 (*C*(6)); *m/z* (ESI$^+$) 400.1 (MH$^+$, 46%), 422.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 400.12056, $C_{21}H_{22}NO_5S$ (MH$^+$) requires 400.12132.

(2*R*,4*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*)-1-hydroxy-3-phenylprop-2-yn-1-ylidene]-5-methoxycarbonyl-4-methyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (97c)



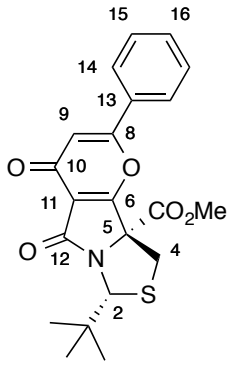
General procedure C (from **77c**, with phenylacetylene); yield 81% (41 mg); orange oil. R_f (EtOAc) 0.48; $[\alpha]_D^{20}$ +150.3 (*c* 0.5, DCM); ν_{max}/cm^{-1} 2959 (C-H), 2208 (C≡C), 1740 (C=O), 1662 (C=O), 1576 (C=C); δ_H (400 MHz, $CDCl_3$) 0.93 (9H, s, C(CH_3)$_3$), 1.09 (3H, d, *J* 6.8, C(4)CH_3), 3.78 (3H, s, CO_2CH_3), 4.99 (1H, s, C(2)*H*), 5.16 (1H, q, *J* 6.8, C(4)*H*), 7.41 (2H, t, *J* 7.6, C(14)*H*), 7.48-7.52 (1H, m, C(15)*H*), 7.74 (2H, d, *J* 7.6, C(13)*H*); δ_C (100 MHz, $CDCl_3$) 14.7 (C(4)*C*H$_3$), 24.8 (C(*C*H$_3$)$_3$), 35.4 (*C*(CH$_3$)$_3$), 53.4 (CO_2CH_3), 75.1 (*C*(4)), 81.4 (*C*(5)), 95.9 (*C*(2)), 107.1, 119.6, 126.2 (*C*(7), *C*(10), *C*(11)), 128.9 (*C*(14)), 129.6 (*C*(12)), 131.9 (*C*(15)), 133.9 (*C*(13)), 159.7 (*C*(9)), 167.8 (CO_2CH_3), 178.8 (*C*(8)), 186.4 (*C*(6)); *m/z* (ESI$^+$) 398.2 (MH$^+$, 100%); HRMS (ESI$^+$) found 398.15909, $C_{22}H_{24}NO_6$ (MH$^+$) requires 398.15981.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-3,7-dioxa-10,12-dioxo-8-phenyltricyclo[7.3.0.0^{6,11}]-dodec-6(11),8-diene (98a)



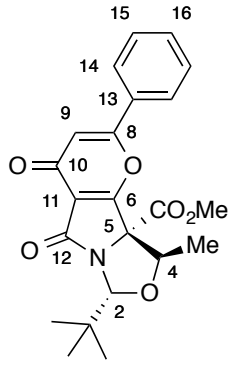
Degradation product of **97a**; orange solid; m.p. 230-232 °C. R_f (EtOAc) 0.78; $[\alpha]_D^{20}$ +58.3 (*c* 1.1, DCM); ν_{max}/cm^{-1} 2959 (C-H), 1734 (C=O), 1663 (C=O); δ_H (400 MHz, $CDCl_3$) 0.95 (9H, s, C(CH_3)$_3$), 3.67 (1H, d, *J* 8.6, C(4)H_AH$_B$), 3.83 (3H, s, CO_2CH_3), 4.88 (1H, s, C(2)*H*), 5.00 (1H, d, *J* 8.6, C(4)H$_A$$H_B$), 6.80 (1H, s, C(9)*H*), 7.48-7.56 (3H, m, C(15)*H* + C(16)*H*), 7.66-7.69 (2H, m, C(14)*H*); δ_C (100 MHz, $CDCl_3$) 24.8 (C(*C*H$_3$)$_3$), 35.3 (*C*(CH$_3$)$_3$), 54.1 (CO_2*C*H$_3$), 69.4 (*C*(4)), 72.8 (*C*(5)), 97.4 (*C*(2)), 114.2 (*C*(9)), 116.0 (*C*(11)), 126.1 (*C*(14)), 129.5 (*C*(15)), 129.6 (*C*(13)), 132.4 (*C*(16)), 163.9 (*C*(8)), 166.9 (*C*O$_2$CH$_3$), 169.7 (*C*(12)), 173.0 (*C*(10)), 174.6 (*C*(6)); *m/z* (ESI$^+$) 384.1 (MH$^+$, 23%), 406.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 384.14299, $C_{21}H_{22}NO_6$ (MH$^+$) requires 384.14416.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-7-oxa-10,12-dioxo-8-phenyl-3-thiatricyclo[7.3.0.0^{6,11}]-dodec-6(11),8-diene (98b)



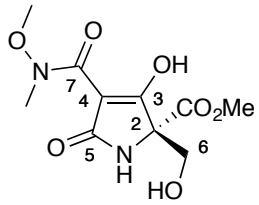
Degradation product of **97b**; yellow oil. R_f (50% EtOAc in petrol) 0.38; $[\alpha]_D^{20}$ +138.5 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2956 (C-H), 1734 (C=O), 1666 (C=O); δ_H (500 MHz, $CDCl_3$) 1.01 (9H, s, C(CH_3)$_3$), 2.98 (1H, d, *J* 11.2, C(4)H_AH$_B$), 3.84 (3H, s, CO_2CH_3), 3.88 (1H, d, *J* 11.2, C(4)H$_A$$H_B$), 5.11 (1H, s, C(2)*H*), 6.83 (1H, s, C(9)*H*), 7.50-7.53 (2H, m, C(15)*H*), 7.55-7.58 (1H, m, C(16)*H*), 7.68-7.70 (2H, m, C(14)*H*); δ_C (125 MHz, $CDCl_3$) 26.7 (C(*C*H$_3$)$_3$), 33.3 (*C*(4)), 37.0 (*C*(CH$_3$)$_3$), 54.1 (CO_2*C*H$_3$), 71.9 (*C*(2)), 77.3 (*C*(5)), 114.2 (*C*(11)), 114.3 (*C*(9)), 126.1 (*C*(14)), 129.5 (*C*(15)), 129.7 (*C*(13)), 132.5 (*C*(16)), 163.8 (*C*(8)), 166.9 (*C*O$_2$CH$_3$), 167.8 (*C*(12)), 173.0 (*C*(10)), 174.8 (*C*(6)); *m/z* (ESI$^+$) 400.0 (MH$^+$, 26%), 422.0 (MNa$^+$, 100%); HRMS (ESI$^+$) found 400.12068, $C_{21}H_{22}NO_5S$ (MH$^+$) requires 400.12132.

(2*R*,4*R*,5*R*)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-4-methyl-3,7-dioxa-10,12-dioxo-8-phenyltricyclo[7.3.0.0^{6,11}]-dodec-6(11),8-diene (98c)



Degradation product of **97c**; colourless oil. R_f (50% EtOAc in petrol) 0.44; $[\alpha]_D^{20}$ +121.4 (*c* 1.1, DCM); ν_{max}/cm^{-1} 2960 (C-H), 1737 (C=O), 1665 (C=O); δ_H (400 MHz, $CDCl_3$) 0.93 (9H, s, C(C*H*$_3$)$_3$), 1.04 (3H, d, *J* 6.7, C(4)C*H*$_3$), 3.83 (3H, s, CO_2C*H*$_3$), 4.95 (1H, s, C(2)*H*), 5.30 (1H, q, *J* 6.7, C(4)*H*), 6.83 (1H, s, C(9)*H*), 7.49-7.58 (3H, m, C(15)*H* + C(16)*H*), 7.67-7.70 (2H, m, C(14)*H*); δ_C (100 MHz, $CDCl_3$) 15.1 (C(4)*C*H$_3$), 24.8 (C(*C*H$_3$)$_3$), 35.4 (*C*(CH$_3$)$_3$), 54.0 (CO_2*C*H$_3$), 74.2 (*C*(4)), 76.1 (*C*(5)), 94.8 (*C*(2)), 114.2 (*C*(9)), 117.4 (*C*(11)), 126.1 (*C*(14)), 129.5 (*C*(15)), 129.6 (*C*(13)), 132.5 (*C*(16)), 163.8 (*C*(8)), 167.2 (CO_2*C*H$_3$), 169.4 (*C*(12)), 172.9 (*C*(10)), 173.6 (*C*(6)); *m/z* (ESI$^+$) 398.2 (MH$^+$, 34%), 420.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 398.15906, $C_{22}H_{24}NO_6$ (MH$^+$) requires 398.15981.

Methyl (*R*)-3-hydroxy-2-hydroxymethyl-4-(methoxy(methyl)carbamoyl)-5-oxo-2,5-dihydro-1*H*-pyrrole-2-carboxylate (100)

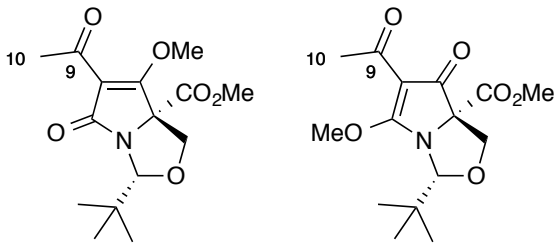


Weinreb amide **77a** (20 mg, 0.058 mmol) was treated with propane-1,3-dithiol (12 μL, 0.12 mmol) followed by a freshly prepared solution of 1.5% HCl in 2,2,2-trifluoroethanol (970 μL). The solution was stirred at room temperature for 17 h and concentrated *in vacuo*. The crude residue was dissolved in methanol, washed with petrol and concentrated under reduced pressure to give *N,O*-acetal deprotected amide **100** (10 mg, 0.036 mmol, 63%) as a light yellow oil, which could not be further purified. $[\alpha]_D^{20}$ +44.2 (*c* 0.7, MeOH); ν_{max}/cm^{-1} 3325 (N-H, O-H), 2954 (C-H), 1737 (C=O), 1682 (C=O), 1618 (C=C); δ_H (500 MHz, CD_3OD) 2.98 (3H, s, NC*H*$_3$), 3.74 (3H, s, CO_2C*H*$_3$), 3.85 (1H, d, *J* 11.3, C(6)*H*$_A$H$_B$), 3.90 (3H, s, NOC*H*$_3$), 4.07 (1H, d, *J* 11.3, C(6)H$_A$*H*$_B$); δ_C (125 MHz, CD_3OD) 35.7 (N*C*H$_3$), 53.3 (CO_2*C*H$_3$), 61.9 (NO*C*H$_3$), 65.0 (*C*(6)), 73.4 (*C*(2)), 91.6 (*C*(4)), 169.5 (CO_2*C*H$_3$), 167.1 (*C*(7)), 178.3 (*C*(5)), 187.1 (*C*(3)); *m/z* (ESI$^+$) 275.0 (MH$^+$, 61%), 297.0 (MNa$^+$, 100%); HRMS (ESI$^+$) found 297.06902, $C_{10}H_{14}N_2NaO_7$ (MNa$^+$) requires 297.06932.

General procedure D: Alkylation of *C*(7)-acyltetramates[187]

Diethyl azodicarboxylate (1.1 eq) was added to a solution of *C*(7)-acyltetramate (1.0 eq), the required alcohol (1.1 eq) and triphenylphosphine (1.1 eq) in anhydrous THF (*c* 0.1) at 0 °C. The mixture was stirred at room temperature for 13-24 h and then concentrated under reduced pressure. The crude residue was purified by *flash* column chromatography to give the enol ether.

(2*R*,5*R*)-7-Acetyl-1-aza-2-(*tert*-butyl)-6-methoxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (103) and (2*R*,5*R*)-7-Acetyl-1-aza-2-(*tert*-butyl)-8-methoxy-5-methoxycarbonyl-3-oxa-6-oxobicyclo[3.3.0]-oct-7-ene (103')



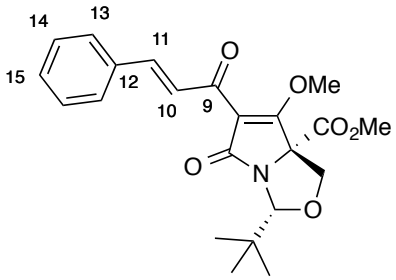
General procedure D (from **81a**, with methanol, for 19 h).

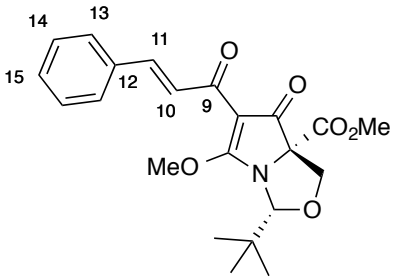
Major isomer (**103**): Yield 56% (270 mg); yellow oil. R_f (5% EtOAc in DCM) 0.43; $[\alpha]_D^{20}$ +164.3 (*c* 0.6, DCM); ν_{max}/cm^{-1} 2959 (C-H), 1751 (C=O), 1713 (C=O), 1685 (C=O), 1605 (C=C); δ_H (500 MHz, $CDCl_3$) 0.90 (9H, s, C(CH_3)$_3$), 2.56 (3H, s, C(10)H_3), 3.46 (1H, d, *J* 8.6, C(4)H_AH$_B$), 3.80 (3H, s, CO_2CH_3), 4.02 (3H, s, C(6)OCH_3), 4.73 (1H, s, C(2)*H*), 4.82 (1H, d, *J* 8.6, C(4)H$_A$$H_B$); δ_C (100 MHz, $CDCl_3$) 24.8 (C(*C*H$_3$)$_3$), 30.7 (*C*(10)), 35.3 (*C*(CH$_3$)$_3$), 53.6 (CO_2*C*H$_3$), 63.4 (C(6)O*C*H$_3$), 69.5 (*C*(4)), 74.1 (*C*(5)), 97.7 (*C*(2)), 110.6 (*C*(7)), 168.0 (*C*O$_2$CH$_3$), 175.3 (*C*(6)), 175.6 (*C*(8)), 195.3 (*C*(9)); *m/z* (ESI$^+$) 312.1 (MH$^+$, 30%), 334.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 312.14337, $C_{15}H_{22}NO_6$ (MH$^+$) requires 312.14416.

Minor isomer (**103'**): Yield 19% (90 mg); yellow oil. R_f (5% EtOAc in DCM) 0.22; $[\alpha]_D^{20}$ +17.1 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2962 (C-H), 1751 (C=O), 1698 (C=O), 1656 (C=O), 1531 (C=C); δ_H (500 MHz, $CDCl_3$) 0.89 (9H, s, C(CH_3)$_3$), 2.44 (3H, s, C(10)H_3), 3.59 (1H, d, *J* 9.0, C(4)H_AH$_B$), 3.76 (3H, s,

CO_2CH_3), 4.26 (3H, s, C(8)OCH_3), 4.62 (1H, s, C(2)H), 4.83 (1H, d, J 9.0, C(4)H_AH_B); δ_C (125 MHz, $CDCl_3$) 24.8 (C(CH_3)$_3$), 30.4 (C(10)), 35.6 (C(CH_3)$_3$), 53.4 (CO_2CH_3), 64.0 (C(8)OCH_3), 68.0 (C(4)), 78.2 (C(5)), 98.6 (C(2)), 102.2 (C(7)), 166.4 (CO_2CH_3), 184.3 (C(8)), 189.4 (C(6)), 193.1 (C(9)); *m/z* (ESI$^+$) 312.1 (MH$^+$, 44%), 334.1 (MNa$^+$, 34%); HRMS (ESI$^+$) found 312.14432, $C_{15}H_{22}NO_6$ (MH$^+$) requires 312.14416.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-cinnamoyl-6-methoxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (104) and (2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-cinnamoyl-8-methoxy-5-methoxycarbonyl-3-oxa-6-oxobicyclo[3.3.0]-oct-7-ene (104')

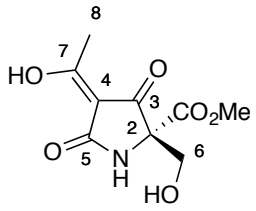




General procedure D (from **96a**, with methanol, for 24 h); yield 50% (41 mg); yellow solid; 4:1 mixture of isomers. R_f (30% EtOAc in petrol) 0.44; ν_{max}/cm^{-1} 2958 (C-H), 1752 (C=O), 1710 (C=O), 1666 (C=O), 1650 (C=O), 1607 (C=C), 1576 (C=C); δ_H (500 MHz, $CDCl_3$) major iosmer (**104**): 0.93 (9H, s, C(CH_3)$_3$), 3.54 (1H, d, J 8.5, C(4)H_AH_B), 3.83 (3H, s, CO_2CH_3), 4.04 (3H, s, C(6)OCH_3), 4.77 (1H, s, C(2)H), 4.86 (1H, d, J 8.5, C(4)H_AH_B), 7.35-7.40 (3H, m, C(14)H + C(15)H), 7.45 (1H, d, J 15.9, C(10)H), 7.61-7.64 (2H, m, C(13)H), 7.68 (1H, d, J 15.9, C(11)H); minor iosmer (**104'**): 0.92 (9H, s, C(CH_3)$_3$), 3.67 (1H, d, J 9.0, C(4)H_AH_B), 3.83 (3H, s, CO_2CH_3), 4.04 (3H, s, C(8)OCH_3), 4.67 (1H, s, C(2)H), 4.88 (1H, d, J 9.0, C(4)H_AH_B), 7.35-7.40 (3H, m, C(14)H + C(15)H), 7.61-7.64 (2H, m, C(13)H), 7.66 (1H, d, J 16.0, C(11)H), 7.74 (1H, d, J 16.0, C(10)H); δ_C (125 MHz, $CDCl_3$) major iosmer (**104**): 24.8 (C(CH_3)$_3$), 35.3 (C(CH_3)$_3$), 53.6 (CO_2CH_3), 63.1 (C(6)OCH_3), 69.6 (C(4)), 74.3 (C(5)), 97.7 (C(2)), 110.3 (C(7)), 125.7 (C(10)), 129.0 (C(13)), 129.0 (C(14)), 130.9 (C(15)), 134.6 (C(12)), 144.6 (C(11)), 168.2 (CO_2CH_3), 175.4 (C(6)), 175.8 (C(8)), 186.5 (C(9)); minor iosmer (**104'**): 24.9 (C(CH_3)$_3$), 35.6 (C(CH_3)$_3$), 53.5 (CO_2CH_3), 64.0 (C(8)OCH_3), 68.1 (C(4)), 78.2 (C(5)), 98.8 (C(2)),

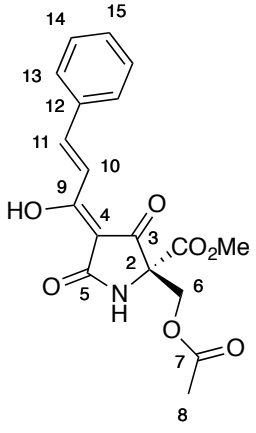
102.2 (*C*(7)), 125.6 (*C*(10)), 128.7, 128.9 (*C*(13), *C*(14)), 130.4 (*C*(15)), 135.2 (*C*(12)), 142.2 (*C*(11)), 166.4 (CO_2CH_3), 189.5 (*C*(6)), 183.9 (*C*(9)), 185.0 (*C*(8)); *m/z* (ESI$^+$) 400.2 (MH^+, 100%), 422.2 (MNa^+, 29%); HRMS (ESI$^+$) found 400.17506, $C_{22}H_{26}NO_6$ (MH^+) requires 400.17546.

Methyl (*R*)-4-((*Z*)-1-hydroxyethylidene)-2-hydroxymethyl-3,5-dioxopyrrolidine-2-carboxylate (105)



To a solution of **103** (214 mg, 0.687 mmol) in 2,2,2-trifluoroethanol (3.4 ml) were added propane-1,3-dithiol (80 µL, 0.80 mmol) and HCl (160 µL, 1.92 mmol). The mixture was heated at 50 °C for 18 h and then concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (EtOAc to 20% MeOH in EtOAc) to give alcohol **105** (117 mg, 0.510 mmol, 74%) as an orange oil. R_f (20% MeOH in EtOAc) 0.13; $[\alpha]_D^{20}$ +47.0 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3326 (N-H, O-H), 2956 (C-H), 1743 (C=O), 1711 (C=O), 1656 (C=O), 1604 (C=C); δ_H (500 MHz, CD_3OD) 2.42 (3H, s, C(8)H_3), 3.75 (3H, s, CO_2CH_3), 3.93 (1H, d, *J* 11.5, C(6)H_AH_B), 4.02 (1H, d, *J* 11.5, C(6)H_AH_B); δ_C (125 MHz, CD_3OD) 20.0 (*C*(8)), 53.5 (CO_2CH_3), 64.3 (*C*(6)), 75.5 (*C*(2)), 101.9 (*C*(4)), 168.4 (CO_2CH_3), 176.5 (*C*(5)), 187.7 (*C*(7)), 191.2 (*C*(3)); *m/z* (ESI$^+$) 230.1 (MH^+, 13%), 252.1 (MNa^+, 100%); HRMS (ESI$^+$) found 252.04779, $C_9H_{11}NNaO_6$ (MNa^+) requires 252.04786.

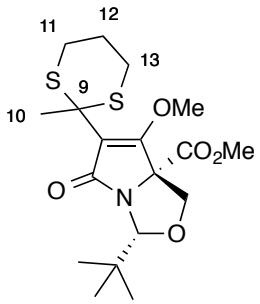
Methyl (*R*)-2-(acetoxymethyl)-4-((*Z*,2*E*)-1-hydroxy-3-phenylallylidene)-3,5-dioxopyrrolidine-2-carboxylate (107)



To a solution of **104** (61 mg, 0.15 mmol) in 2,2,2-trifluoroethanol (770 µl) were added propane-1,3-dithiol (16 µL, 0.16 mmol) and HCl (12 µL, 0.14 mmol). The mixture was heated at 50 °C for 13 h, then concentrated *in vacuo* and diluted with brine. The aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was dissolved in anhydrous DCM (1.5 mL), and pyridine (19 µL, 0.24 mmol) and acetic anhydride (22 µL, 0.23 mmol) were added. The mixture was stirred at room temperature for 15 h and concentrated *in vacuo*. The crude

residue was purified by *flash* column chromatography (EtOAc to 5% MeOH in EtOAc) and washed with 2 M aqueous HCl to give acetyl **107** (18 mg, 0.049 mmol, 33%) as an orange oil. R_f (5% MeOH in EtOAc) 0.30; $[\alpha]_D^{20}$ +28.3 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3339 (N-H), 2956 (C-H), 1750 (C=O), 1712 (C=O), 1665 (C=O), 1627 (C=C), 1582 (C=C), 1568 (C=C); δ_H (500 MHz, CD_3OD) 2.01 (3H, s, C(8)H_3), 3.79 (3H, s, CO_2CH_3), 4.48 (1H, d, *J* 11.5, C(6)H_AH_B), 4.64 (1H, d, *J* 11.5, C(6)H_AH_B), 7.45-7.49 (3H, m, Ph), 7.68-7.72 (2H, m, Ph), 7.74 (1H, d, *J* 16.0, C(10)*H*), 7.98 (1H, d, *J* 16.0, C(11)*H*); δ_C (125 MHz, CD_3OD) 20.4 (*C*(8)), 54.0 (CO_2CH_3), 65.1 (*C*(6)), 72.7 (*C*(2)), 100.1 (*C*(4)), 118.1 (*C*(10)), 130.1, 130.3, 132.7, 135.7 (Ph), 146.9 (*C*(11)), 167.9 (CO_2CH_3), 171.8 (*C*(7)), 176.4 (*C*(9)), 176.5 (*C*(5)), 189.4 (*C*(3)); *m/z* (ESI^-) 358.0 ($M-H^-$, 72%); HRMS (ESI^+) found 360.10776, $C_{18}H_{18}NO_7$ (MH^+) requires 360.10778.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-methoxy-5-methoxycarbonyl-7-(2-methyl-1,3-dithian-2-yl)-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (108)

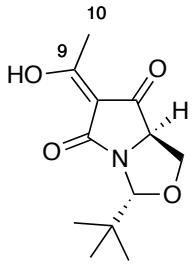


To a solution of **103** (46 mg, 0.15 mmol) in 2,2,2-trifluoroethanol (740 µl) were added propane-1,3-dithiol (20 µL, 0.20 mmol) and HCl (40 µL, 0.48 mmol). The mixture was stirred at rt for 3 h and then concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (DCM) to give dithiane **108** (36 mg, 0.091 mmol, 62%) as a yellow solid; m.p. 112-114 °C. R_f (30% EtOAc in petrol) 0.43; $[\alpha]_D^{20}$ +181.9 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2957 (C-H), 1745 (C=O), 1710 (C=O), 1634 (C=C); δ_H (500 MHz, $CDCl_3$) 0.91 (9H, s, C(CH_3)$_3$), 1.87-1.96 (1H, m, C(12)H_AH_B), 1.97 (3H, s, C(10)H_3), 2.00-2.07 (1H, m, C(12)H_AH_B), 2.79-2.86 (3H, m, C(11)H_AH_B + C(13)H_AH_B), 2.99 (1H, ddd, *J* 14.2, 10.9, 3.4, C(11)H_AH_B or C(13)H_AH_B), 3.54 (1H, d, *J* 8.0, C(4)H_AH_B), 3.78 (3H, s, C(6)OCH_3), 3.82 (3H, s, CO_2CH_3), 4.73 (1H, s, C(2)*H*), 4.94 (1H, d, *J* 8.0, C(4)H_AH_B); δ_C (125 MHz, $CDCl_3$) 24.5 (*C*(12)), 24.8 (C(CH_3)$_3$), 27.7 (*C*(10)), 28.4, 29.1 (*C*(11), *C*(13)), 35.2 (*C*(CH_3)$_3$), 45.7 (*C*(9)), 53.5 (CO_2CH_3), 59.0 (C(6)OCH_3), 69.7 (*C*(4)), 72.7 (*C*(5)), 96.5 (*C*(2)), 117.7 (*C*(7)), 167.6 (*C*(6)), 169.0 (CO_2CH_3), 174.8 (*C*(8)); *m/z* (ESI^+) 402.1 (MH^+, 46%), 424.1 (MNa^+, 100%); HRMS (ESI^+) found 402.13945, $C_{18}H_{28}NO_5S_2$ (MH^+) requires 402.14034.

General procedure E: Decarboxylation of *C*(7)-acyltetramates[161]

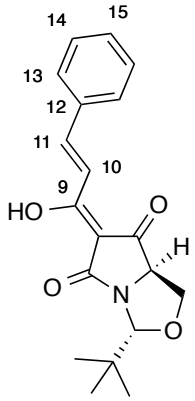
C(7)-Acyltetramate (1.0 eq) and $LiOH{\cdot}H_2O$ (5.0 eq) in a 4:1:1 mixture of THF/MeOH/H_2O (*c* 0.07) were heated at reflux for 8 h and then cooled to room temperature. The mixture was diluted with brine, acidified with 2 M aqueous HCl, extracted with EtOAc, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in MeCN and heated at reflux for 5 h, then cooled to room temperature. The mixture was diluted with brine, acidified with 2 M aqueous HCl, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by *flash* column chromatography and the product thus obtained was dissolved in EtOAc, washed with 2 M aqueous HCl, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give the decarboxylated *C*(7)-acyltetramate.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*)-1-hydroxyethylidene]-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (109)



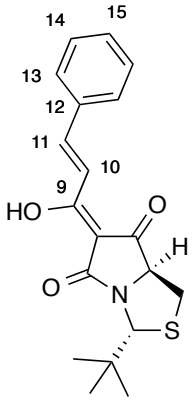
General procedure E (from **81a**); yield 77% (20 mg); orange oil; 2.4:1 mixture of tautomers. R_f (10% MeOH in EtOAc) 0.48; $[\alpha]_D^{20}$ +135.2 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2960 (C-H), 1716 (C=O), 1651 (C=O), 1605 (C=C); δ_H (500 MHz, $CDCl_3$) major tautomer: 0.95 (9H, s, C(CH_3)$_3$), 2.46 (3H, s, C(10)H_3), 3.43 (1H, dd, *J* 9.5, 8.0, C(4)H_AH_B), 4.14 (1H, dd, *J* 9.5, 7.2, C(5)*H*), 4.23-4.29 (1H, m, C(4)H_AH_B), 4.93 (1H, s, C(2)*H*); minor tautomer: 0.95 (9H, s, C(CH_3)$_3$), 2.51 (3H, s, C(10)H_3), 3.42 (1H, dd, *J* 12.0, 11.0, C(4)H_AH_B), 4.23-4.29 (2H, m, C(4)H_AH_B + C(5)*H*), 4.95 (1H, s, C(2)*H*); δ_C (125 MHz, $CDCl_3$) major tautomer: 19.8 (*C*(10)), 24.8 (C(*C*H_3)$_3$), 36.0 (*C*(CH_3)$_3$), 66.8 (*C*(4)), 67.3 (*C*(5)), 95.8 (*C*(2)), 103.1 (*C*(7)), 178.9 (*C*(8)), 186.6 (*C*(9)), 191.9 (*C*(6)); minor tautomer: 20.6 (*C*(10)), 24.8 (C(*C*H_3)$_3$), 36.1 (*C*(CH_3)$_3$), 64.6 (*C*(5)), 66.8 (*C*(4)), 95.9 (*C*(2)), 105.9 (*C*(7)), 172.0 (*C*(8)), 190.4 (*C*(9)), 197.9 (*C*(6)); *m/z* (ESI$^-$) 238.1 (M-H$^-$, 100%); HRMS (ESI$^-$) found 238.10849, $C_{12}H_{16}NO_4$ (M-H$^-$) requires 238.10848.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*,2*E*)-1-hydroxy-3-phenylallylidene]-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (110a)



General procedure E (from **96a**); yield 71% (24 mg); orange oil; 2.3:1 mixture of tautomers. R_f (EtOAc) 0.32; $[\alpha]_D^{20}$ +199.5 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2959 (C-H), 1707 (C=O), 1649 (C=O), 1626 (C=C), 1581 (C=C), 1564 (C=C); δ_H (500 MHz, $CDCl_3$) major tautomer: 0.99 (9H, s, C(CH_3)$_3$), 3.49 (1H, dd, *J* 9.5, 8.5, C(4)H_AH$_B$), 4.20 (1H, dd, *J* 9.5, 7.2, C(5)*H*), 4.27-4.31 (1H, m, C(4)H$_A$$H_B$), 4.99 (1H, s, C(2)*H*), 7.40-7.45 (3H, m, C(14)*H* + C(15)*H*), 7.65-7.70 (2H, m, C(13)*H*), 7.75 (1H, d, *J* 16.0, C(10)*H*), 7.93 (1H, d, *J* 16.0, C(11)*H*); minor tautomer: 0.99 (9H, s, C(CH_3)$_3$), 3.45-3.49 (1H, m, C(4)H_AH$_B$), 4.27-4.30 (2H, m, C(4)H$_A$$H_B$ + C(5)*H*), 5.03 (1H, s, C(2)*H*), 7.40-7.45 (3H, m, C(14)*H* + C(15)*H*), 7.65-7.70 (2H, m, C(13)*H*), 7.88 (1H, d, *J* 16.0, C(10)*H*), 7.96 (1H, d, *J* 16.0, C(11)*H*); δ_C (125 MHz, $CDCl_3$) major tautomer: 24.9 (C(*C*H$_3$)$_3$), 36.1 (*C*(CH$_3$)$_3$), 67.0 (*C*(4)), 67.3 (*C*(5)), 96.0 (*C*(2)), 101.7 (*C*(7)), 117.7 (*C*(10)), 129.2 (*C*(14)), 129.3 (*C*(13)), 131.7 (*C*(15)), 134.4 (*C*(12)), 146.1 (*C*(11)), 175.5 (*C*(9)), 179.6 (*C*(8)), 191.9 (*C*(6)); minor tautomer: 24.9 (C(*C*H$_3$)$_3$), 36.3 (*C*(CH$_3$)$_3$), 65.3 (*C*(5)), 66.9 (*C*(4)), 96.2 (*C*(2)), 103.8 (*C*(7)), 117.1 (*C*(10)), 129.2, 129.5 (*C*(13), *C*(14)), 131.8 (*C*(15)), 134.4 (*C*(12)), 146.8 (*C*(11)), 172.2 (*C*(8)), 176.7 (*C*(9)), 200.5 (*C*(6)); *m/z* (ESI$^-$) 326.1 (M-H$^-$, 37%); HRMS (ESI$^-$) found 326.13989, $C_{19}H_{20}NO_4$ (M-H$^-$) requires 326.13978.

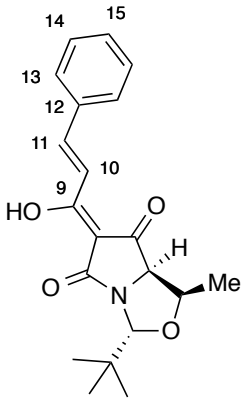
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*,2*E*)-1-hydroxy-3-phenylallylidene]-6,8-dioxo-3-thiabicyclo[3.3.0]-octane (110b)



General procedure E (from **96b**); yield 73% (21 mg); orange solid; m.p. 132 °C; 3.0:1 mixture of tautomers. R_f (EtOAc) 0.51; $[\alpha]_D^{20}$ +315.2 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2963 (C-H), 1705 (C=O), 1649 (C=O), 1627 (C=C), 1582 (C=C), 1567 (C=C); δ_H (500 MHz, $CDCl_3$) major tautomer: 1.04 (9H, s, C(CH_3)$_3$), 2.81 (1H, t, *J* 10.5, C(4)H_AH$_B$), 3.22 (1H, dd, *J* 10.5, 6.8, C(4)H$_A$$H_B$), 4.33 (1H, dd, *J* 10.5, 6.8, C(5)*H*), 5.16 (1H, s, C(2)*H*), 7.40-7.45 (3H, m, C(14)*H* + C(15)*H*), 7.64-7.69 (2H, m, C(13)*H*), 7.75 (1H, d, *J* 16.0, C(10)*H*), 7.89 (1H, d, *J* 16.0, C(11)*H*); minor tautomer: 1.04 (9H, s, C(CH_3)$_3$), 2.80 (1H, t, *J* 10.5, C(4)H_AH$_B$), 3.22 (1H, dd, *J* 10.5, 6.8, C(4)H$_A$$H_B$), 4.42 (1H, dd, *J* 10.5, 6.8,

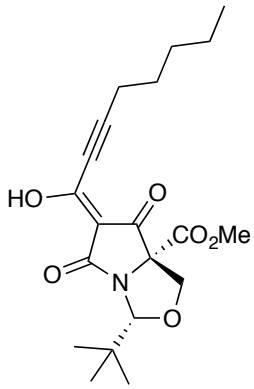
C(5)*H*), 5.23 (1H, s, C(2)*H*), 7.40-7.45 (3H, m, C(14)*H* + C(15)*H*), 7.64-7.69 (2H, m, C(13)*H*), 7.89 (1H, d, *J* 16.0, C(10)*H*), 7.93 (1H, d, *J* 16.0, C(11)*H*); δ_C (125 MHz, $CDCl_3$) major tautomer: 26.4 (C(CH_3)$_3$), 32.8 (*C*(4)), 37.6 (*C*(CH_3)$_3$), 71.0 (*C*(2)), 71.3 (*C*(5)), 100.5 (*C*(7)), 117.8 (*C*(10)), 129.2 (*C*(14)), 129.2 (*C*(13)), 131.5 (*C*(15)), 134.5 (*C*(12)), 145.5 (*C*(11)), 174.7 (*C*(9)), 176.0 (*C*(8)), 192.1 (*C*(6)); minor tautomer: 26.5 (C(CH_3)$_3$), 33.0 (*C*(4)), 37.8 (*C*(CH_3)$_3$), 69.1 (*C*(5)), 71.2 (*C*(2)), 102.6 (*C*(7)), 117.1 (*C*(10)), 129.2, 129.4 (*C*(13), *C*(14)), 131.7 (*C*(15)), 134.5 (*C*(12)), 146.3 (*C*(11)), 169.0 (*C*(8)), 175.8 (*C*(9)), 200.7 (*C*(6)); *m/z* (ESI$^-$) 342.0 (M-H$^-$, 72%); HRMS (ESI$^-$) found 342.11715, $C_{19}H_{20}NO_3S$ (M-H$^-$) requires 342.11694.

(2*R*,4*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*,2*E*)-1-hydroxy-3-phenylallylidene]-4-methyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (110c)



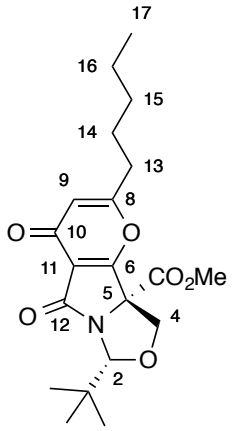
General procedure E (from **96c**); yield 74% (20 mg); orange solid; m.p. 84 °C; 1.9:1 mixture of tautomers. R_f (EtOAc) 0.38; $[\alpha]_D^{20}$ +197.4 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2968 (C-H), 1706 (C=O), 1649 (C=O), 1626 (C=C), 1581 (C=C), 1565 (C=C); δ_H (500 MHz, $CDCl_3$) major tautomer: 0.98 (9H, s, C(C*H*$_3$)$_3$), 1.04 (3H, d, *J* 6.5, C(4)C*H*$_3$), 4.28 (1H, d, *J* 7.0, C(5)*H*), 4.59 (1H, dq, *J* 7.0, 6.5, C(4)*H*), 5.07 (1H, s, C(2)*H*), 7.40-7.45 (3H, m, C(14)*H* + C(15)*H*), 7.65-7.70 (2H, m, C(13)*H*), 7.76 (1H, d, *J* 16.0, C(10)*H*), 7.91 (1H, d, *J* 16.0, C(11)*H*); minor tautomer: 0.98 (9H, s, C(C*H*$_3$)$_3$), 1.02 (3H, d, *J* 6.5, C(4)C*H*$_3$), 4.37 (1H, d, *J* 7.0, C(5)*H*), 4.57 (1H, dq, *J* 7.0, 6.5, C(4)*H*), 5.10 (1H, s, C(2)*H*), 7.40-7.45 (3H, m, C(14)*H* + C(15)*H*), 7.65-7.70 (2H, m, C(13)*H*), 7.87 (1H, d, *J* 16.0, C(10)*H*), 7.95 (1H, d, *J* 16.0, C(11)*H*); δ_C (125 MHz, $CDCl_3$) major tautomer: 15.3 (C(4)*C*H$_3$), 24.9 (C(*C*H$_3$)$_3$), 36.3 (*C*(CH_3)$_3$), 70.1 (*C*(5)), 73.6 (*C*(4)), 93.8 (*C*(2)), 103.5 (*C*(7)), 117.7 (*C*(10)), 129.2 (*C*(14)), 129.3 (*C*(13)), 131.6 (*C*(15)), 134.5 (*C*(12)), 145.8 (*C*(11)), 174.3 (*C*(9)), 179.0 (*C*(8)), 192.0 (*C*(6)); minor tautomer: 15.6 (C(4)*C*H$_3$), 24.9 (C(*C*H$_3$)$_3$), 36.4 (*C*(CH_3)$_3$), 68.2 (*C*(5)), 73.2 (*C*(4)), 94.1 (*C*(2)), 105.7 (*C*(7)), 117.2 (*C*(10)), 129.2 (*C*(14)), 129.4 (*C*(13)), 131.7 (*C*(15)), 134.5 (*C*(12)), 146.5 (*C*(11)), 171.8 (*C*(8)), 175.6 (*C*(9)), 200.6 (*C*(6)); *m/z* (ESI$^+$) 342.1 (MH$^+$, 100%), 364.1 (MNa$^+$, 79%); HRMS (ESI$^+$) found 342.16997, $C_{20}H_{24}NO_4$ (MH$^+$) requires 342.16998.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*)-1-hydroxyoct-2-yn-1-ylidene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (113)



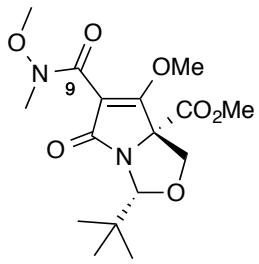
General procedure C (from **77a**, with 1-heptyne); yield 73% (53 mg); orange oil. R_f (EtOAc) 0.41. The product degraded within hours, before it could be fully characterised.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-3,7-dioxa-10,12-dioxo-8-pentyltricyclo[7.3.0.0^{6,11}]-dodec-6(11),8-diene (115)



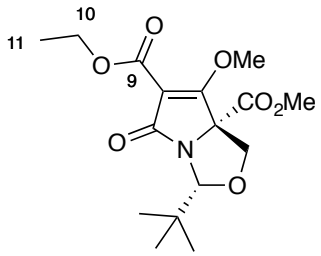
Degradation product of **113**; orange oil. R_f (EtOAc) 0.82; $[\alpha]_D^{20}$ +83.9 (*c* 0.7, DCM); ν_{max}/cm^{-1} 2958 (C-H), 1739 (C=O), 1670 (C=O); δ_H (500 MHz, $CDCl_3$) 0.90 (3H, t, *J* 7.0, C(17)H_3), 0.94 (9H, s, C(CH_3)$_3$), 1.31-1.34 (4H, m, C(15)H_2 + C(16)H_2), 1.58-1.65 (2H, m, C(14)H_2), 2.55 (1H, t, *J* 7.5, C(13)H_AH$_B$), 2.55 (1H, t, *J* 7.5, C(13)H$_A$$H_B$), 3.54 (1H, d, *J* 8.6, C(4)H_AH$_B$), 3.81 (3H, s, CO_2CH_3), 4.84 (1H, s, C(2)*H*), 4.93 (1H, d, *J* 8.6, C(4)H$_A$$H_B$), 6.20 (1H, s, C(9)*H*); δ_C (100 MHz, $CDCl_3$) 14.0 (*C*(17)), 22.4 (*C*(16)), 24.8 (C(*C*H$_3$)$_3$), 26.4 (*C*(14)), 30.9 (*C*(15)), 33.1 (*C*(13)), 35.4 (*C*(CH$_3$)$_3$), 53.9 (CO_2CH_3), 69.2 (*C*(4)), 72.6 (*C*(5)), 97.3 (*C*(2)), 115.8 (*C*(11)), 116.6 (*C*(9)), 166.8 (*C*O$_2$CH$_3$), 169.9 (*C*(8)), 169.9 (*C*(12)), 173.1 (*C*(10)), 174.8 (*C*(6)); *m/z* (ESI$^+$) 378.2 (MH$^+$, 25%), 400.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 378.19009, $C_{20}H_{28}NO_6$ (MH$^+$) requires 378.19111.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-methoxy-5-methoxycarbonyl-7-(methoxy(methyl)carbamoyl)-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (116l)



General procedure D with DIAD instead of DEAD (from **77a**, with methanol, for 13 h); yield 80% (342 mg); colourless oil. R_f (25% EtOAc in DCM) 0.41; $[\alpha]_D^{20}$ +74.6 (*c* 1.1, DCM); ν_{max}/cm^{-1} 2959 (C-H), 1752 (C=O), 1719 (C=O), 1650 (C=O); δ_H (500 MHz, $CDCl_3$, 4:1 mixture of rotamers, major A and minor B) 0.89 (9H, s, C(CH_3)$_3$), 3.29 (3H, s, NCH_3 A), 3.33 (3H, s, NCH_3 B), 3.51 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.71 (3H, s, NOCH_3 A), 3.79 (3H, s, CO_2CH_3), 3.87 (3H, s, NOCH_3 B), 3.93 (3H, s, C(6)OCH_3 A), 4.02 (3H, s, C(6)OCH_3 B), 4.70 (1H, s, C(2)H), 4.79 (1H, d, *J* 8.5, C(4)H$_A$$H_B$); δ_C (125 MHz, $CDCl_3$) 24.7 (C(CH$_3$)$_3$), 32.5 (NCH$_3$), 35.2 (C(CH$_3$)$_3$), 53.4 (CO_2CH$_3$), 60.2 (C(6)OCH$_3$), 61.9 (NOCH$_3$), 69.5 (C(4)), 74.4 (C(5)), 97.3 (C(2)), 105.0 (C(7)), 163.0 (C(9)), 168.3 (CO_2CH$_3$), 169.7 (C(6)), 174.9 (C(8)); *m/z* (ESI$^+$) 357.1 (MH$^+$, 100%), 379.1 (MNa$^+$, 51%); HRMS (ESI$^+$) found 357.16506, $C_{16}H_{25}N_2O_7$ (MH$^+$) requires 357.16563.

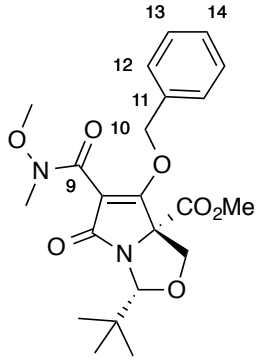
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-ethoxycarbonyl-6-methoxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (117)



To a solution of tetramic acid **44a** (432 mg, 1.69 mmol) and DMAP (465 mg, 3.81 mmol) in anhydrous DCM (34 mL) was added ethyl chloroformate (195 µL, 2.04 mmol). The solution was stirred at room temperature for 19 h, acidified with 2 M aqueous HCl and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant residue was dissolved in anhydrous THF (17 mL), and triphenylphosphine (488 mg, 1.86 mmol) and methanol (75 µL, 1.9 mmol) were added. DEAD (292 µL, 1.86 mmol) was then added dropwise at 0 °C and the mixture was stirred at room temperature for 24 h before concentrating it under reduced pressure. The crude product was purified by *flash* column chromatography to give methylated ethyl ester **117** (198 mg, 0.580 mmol, 34%) as a light yellow oil. R_f (40% EtOAc in petrol) 0.52; $[\alpha]_D^{20}$ +106.6 (*c* 0.7, $CHCl_3$); ν_{max}/cm^{-1} 2959 (C-H), 1742 (C=O), 1717 (C=O), 1643 (C=O); δ_H (400 MHz, $CDCl_3$) 0.88 (9H, s, C(CH_3)$_3$), 1.35 (3H, t, *J* 7.1, C(11)H_3),

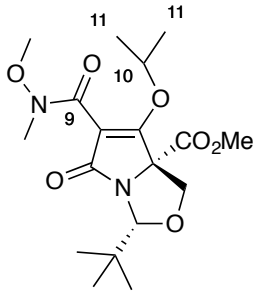
3.47 (1H, d, *J* 8.5, C(4)H_AH_B), 3.78 (3H, s, CO_2CH_3), 4.06 (3H, s, C(6)OCH_3), 4.32 (2H, q, *J* 7.1, C(10)H_2), 4.72 (1H, s, C(2)*H*), 4.81 (1H, d, *J* 8.5, C(4)H_AH_B); δ_C (100 MHz, $CDCl_3$) 14.3 (*C*(11)), 24.7 (C(CH_3)$_3$), 35.2 (*C*(CH_3)$_3$), 53.5 (CO_2CH_3), 61.9 (*C*(10)), 62.0 (C(6)OCH_3), 69.5 (*C*(4)), 74.1 (*C*(5)), 97.6 (*C*(2)), 103.6 (*C*(7)), 162.1 (*C*(9)), 167.9 (CO_2CH_3), 174.0 (*C*(6)), 174.1 (*C*(8)); *m/z* (ESI$^+$) 342.2 (MH^+, 54%), 364.2 (MNa^+, 100%); HRMS (ESI$^+$) found 342.15484, $C_{16}H_{24}NO_7$ (MH^+) requires 342.15473.

(2*R*,5*R*)-1-Aza-6-benzyloxy-2-(*tert*-butyl)-5-methoxycarbonyl-7-(methoxy(methyl)carbamoyl)-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (116II)



General procedure D (from **77a**, with benzyl alcohol, for 18 h); yield 71%; yellow oil. R_f (20% EtOAc in DCM) 0.57; $[\alpha]_D^{20}$ +29.2 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2957 (C-H), 1751 (C=O), 1718 (C=O), 1646 (C=O); δ_H (400 MHz, $CDCl_3$, 2:1 mixture of rotamers, major A and minor B) 0.89 (9H, s, C(CH_3)$_3$), 2.88 (3H, s, NCH_3 B), 3.25 (3H, s, NCH_3 A), 3.42 (3H, s, NOCH_3 A), 3.57 (1H, d, *J* 8.6, C(4)H_AH_B), 3.77 (3H, s, CO_2CH_3), 3.80 (3H, s, NOCH_3 B), 4.68 (1H, s, C(2)*H*), 4.82 (1H, d, *J* 8.6, C(4)H_AH_B), 5.22 (1H, d, *J* 11.8, C(10)H_AH_B A), 5.31 (1H, d, *J* 11.8, C(10)H_AH_B A), 5.22 (1H, d, *J* 12.4, C(10)H_AH_B B), 5.31 (1H, d, *J* 12.4, C(10)H_AH_B B), 7.22-7.24 (2H, m, C(12)*H*), 7.34-7.37 (3H, m, C(13)*H* + C(14)*H*); δ_C (100 MHz, $CDCl_3$) 24.7 (C(*C*H_3)$_3$), 32.3 (N*C*H_3), 35.2 (*C*(CH_3)$_3$), 53.2 (CO_2CH_3), 61.3 (NO*C*H_3), 68.0 (*C*(4)), 74.5 (*C*(10)), 74.6 (*C*(5)), 97.2 (*C*(2)), 105.7 (*C*(7)), 127.9 (*C*(12)), 128.7 (*C*(13)), 129.0 (*C*(14)), 134.5 (*C*(11)), 162.6 (*C*(9)), 168.2 (CO_2CH_3), 169.0 (*C*(6)), 174.7 (*C*(8)); *m/z* (ESI$^+$) 433.1 (MH^+, 58%), 455.1 (MNa^+, 100%); HRMS (ESI$^+$) found 433.19625, $C_{22}H_{29}N_2O_7$ (MH^+) requires 433.19693.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-7-(methoxy(methyl)carbamoyl)-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (116III)



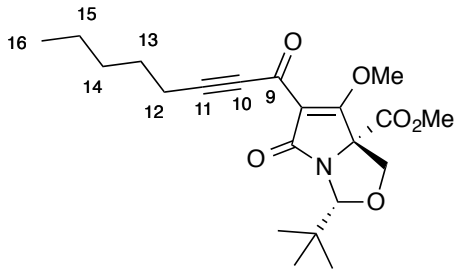
General procedure D (from **77a**, with 2-propanol, for 24 h); yield 69%; yellow oil. R_f (20% EtOAc in DCM) 0.57; $[\alpha]_D^{20}$ +57.4 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2959 (C-H), 1752 (C=O), 1716 (C=O), 1641 (C=O); δ_H (400 MHz, $CDCl_3$, 3:1 mixture of rotamers, major A and minor B) 0.90 (9H, s, C(CH_3)$_3$), 1.20 (3H, d, *J* 6.0, C(11$_A$)H_3), 1.28-1.34 (3H, m, C(11$_B$)H_3), 3.29 (3H, s, NCH_3 A), 3.34 (3H, s, NCH_3 B), 3.51 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.73 (3H, s, NOCH_3 A), 3.79 (3H, s, CO_2CH_3), 3.82 (3H, s, NOCH_3 B), 4.72 (1H, s, C(2)*H*), 4.72-4.77 (1H, m, C(10)*H*), 4.81 (1H, d, *J* 8.5, C(4)H$_A$$H_B$); δ_C (100 MHz, $CDCl_3$) 21.1 (*C*(11$_A$)), 22.2 (*C*(11$_B$)), 24.7 (C(*C*H$_3$)$_3$), 32.4 (N*C*H$_3$), 35.2 (*C*(CH$_3$)$_3$), 53.2 (CO_2*C*H$_3$), 61.8 (NO*C*H$_3$), 69.4 (*C*(4)), 74.9 (*C*(5)), 76.8 (*C*(10)), 97.1 (*C*(2)), 104.4 (*C*(7)), 163.5 (*C*(9)), 168.1 (*C*O_2CH$_3$), 168.3 (*C*(6)), 175.4 (*C*(8)); *m/z* (ESI$^+$) 407.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 385.19639, $C_{18}H_{29}N_2O_7$ (MH$^+$) requires 385.19693.

General procedure F: Grignard reaction of enol-protected Weinreb amide with alkynyl Grignard reagents

Synthesis of the alkynyl Grignard reagent: Isobutylmagnesium bromide (2 M in Et_2O, 1.0 eq) was added dropwise to a solution of the 1-alkyne (1.1 eq) in anhydrous THF (*c* 0.7) at 0 °C. The suspension was then warmed to room temperature and heated at 40 °C for 5 h. The solution was used for the Grignard reaction directly at 40 °C.

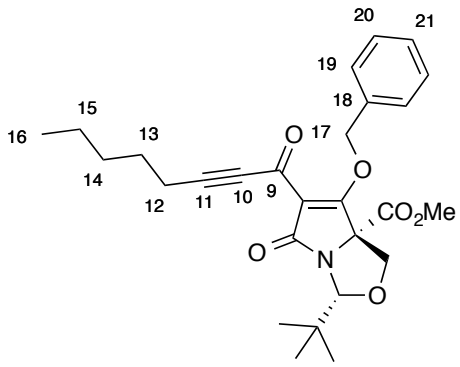
The prepared Grignard reagent (2.5 eq) was added to enol-protected Weinreb amide (1.0 eq) in anhydrous THF (*c* 0.2) dropwise at -15 °C. The reaction mixture was stirred at -15 °C for 1 h. The solution was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, concentrated *in vacuo* and purified by *flash* column chromatography to give the *C*(7)-alkynone product.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-methoxy-5-methoxycarbonyl-7-(oct-2-ynoyl)-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (118I)



General procedure F (from **116I**, with 1-heptyne); yield 27% (13 mg); yellow oil. R_f (DCM) 0.48; $[\alpha]_D^{20}$ +120.0 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2958 (C-H), 2213 (C≡C), 1752 (C=O), 1720 (C=O), 1637 (C=O), 1604 (C=C); δ_H (500 MHz, $CDCl_3$) 0.90 (3H, t, *J* 7.0, C(16)H_3), 0.90 (9H, s, C(CH_3)$_3$), 1.33 (2H, tq, *J* 7.5, 7.0, C(15)H_2), 1.39-1.45 (2H, m, C(14)H_2), 1.63 (2H, tt, *J* 7.5, 7.0, C(13)H_2), 2.44 (2H, t, *J* 7.0, C(12)H_2), 3.47 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.80 (3H, s, CO_2CH_3), 4.05 (3H, s, C(6)OCH_3), 4.75 (1H, s, C(2)*H*), 4.82 (1H, d, *J* 8.5, C(4)H$_A$$H_B$); δ_C (125 MHz, $CDCl_3$) 14.0 (*C*(16)), 19.6 (*C*(12)), 22.3 (*C*(15)), 24.8 (C(*C*H$_3$)$_3$), 27.5 (*C*(13)), 31.2 (*C*(14)), 35.3 (*C*(CH$_3$)$_3$), 53.6 (CO_2CH_3), 63.5 (C(6)O*C*H$_3$), 69.5 (*C*(4)), 73.8 (*C*(5)), 81.9 (*C*(10)), 97.8 (*C*(2)), 99.7 (*C*(11)), 110.2 (*C*(7)), 168.0 (*C*O$_2$CH$_3$), 172.3 (*C*(9)), 174.1 (*C*(8)), 175.6 (*C*(6)); *m/z* (ESI$^+$) 392.1 (MH$^+$, 42%), 414.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 392.20660, $C_{21}H_{30}NO_6$ (MH$^+$) requires 392.20676.

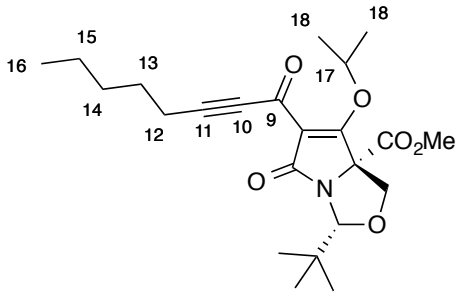
(2*R*,5*R*)-1-Aza-6-benzyloxy-2-(*tert*-butyl)-5-methoxycarbonyl-7-(oct-2-ynoyl)-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (118II)



General procedure F (from **116II**, with 1-heptyne); yield 35% (18 mg); yellow oil. R_f (20% EtOAc in petrol) 0.58; $[\alpha]_D^{20}$ +105.6 (*c* 0.7, DCM); ν_{max}/cm^{-1} 2957 (C-H), 2210 (C≡C), 1752 (C=O), 1721 (C=O), 1634 (C=O), 1598 (C=C); δ_H (500 MHz, $CDCl_3$) 0.90 (9H, s, C(CH_3)$_3$), 0.91 (3H, t, *J* 7.2, C(16)H_3), 1.29-1.37 (2H, m, C(15)H_2), 1.38-1.44 (2H, m, C(14)H_2), 1.62 (2H, tt, *J* 7.5, 7.0, C(13)H_2), 2.44 (2H, t, *J* 7.0, C(12)H_2), 3.47 (1H, d, *J* 8.6, C(4)H_AH$_B$), 3.71 (3H, s, CO_2CH_3), 4.75 (1H, s, C(2)*H*), 4.81 (1H, d, *J* 8.6, C(4)H$_A$$H_B$), 5.19 (1H, d, *J* 12.0, C(17)H_AH$_B$), 5.50 (1H, d, *J* 12.0, C(17)H$_A$$H_B$), 7.22-7.24 (2H, m, C(19)*H*), 7.35-7.37 (3H, m, C(20)*H* + C(21)*H*); δ_C (125 MHz, $CDCl_3$) 14.0 (*C*(16)), 19.7 (*C*(12)), 22.3 (*C*(15)), 24.8 (C(*C*H$_3$)$_3$), 27.5 (*C*(13)), 31.2 (*C*(14)), 35.3 (*C*(CH$_3$)$_3$), 53.4 (CO_2CH_3), 69.6 (*C*(4)), 74.2 (*C*(5)), 77.8 (*C*(17)), 81.9

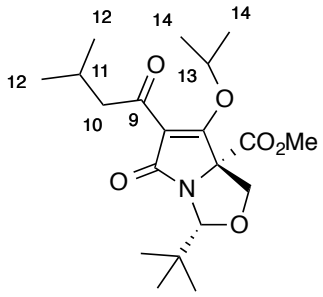
(*C*(10)), 97.8 (*C*(2)), 100.0 (*C*(11)), 110.6 (*C*(7)), 128.3 (*C*(19)), 128.9 (*C*(20)), 129.3 (*C*(21)), 134.1 (*C*(18)), 168.0 (CO_2CH_3), 172.6 (*C*(9)), 174.1 (*C*(8)), 174.6 (*C*(6)); *m/z* (ESI+) 468.3 (MH^+, 66%), 490.2 (MNa^+, 100%); HRMS (ESI+) found 468.23692, $C_{27}H_{34}NO_6$ (MH^+) requires 468.23806.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-7-(oct-2-ynoyl)-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (118III)



General procedure F (from **116III**, with 1-heptyne); yield 59% (657 mg); light yellow solid; m.p. 63-65 °C. R_f (DCM) 0.43; $[\alpha]_D^{20}$ +117.3 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2958 (C-H), 2211 (C≡C), 1754 (C=O), 1720 (C=O), 1634 (C=O), 1587 (C=C); δ_H (500 MHz, $CDCl_3$) 0.90 (3H, t, *J* 7.2, C(16)H_3), 0.91 (9H, s, C(CH_3)$_3$), 1.17 (3H, d, *J* 6.0, C(18_A)H_3), 1.30-1.37 (2H, m, C(15)H_2), 1.32 (3H, d, *J* 6.0, C(18_B)H_3), 1.39-1.45 (2H, m, C(14)H_2), 1.63 (2H, tt, *J* 7.5, 7.0, C(13)H_2), 2.45 (2H, t, *J* 7.0, C(12)H_2), 3.45 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.79 (3H, s, CO_2CH_3), 4.77 (1H, s, C(2)*H*), 4.82 (1H, d, *J* 8.5, C(4)H$_A$$H_B$), 5.20 (1H, sept, *J* 6.0, C(17)*H*); δ_C (125 MHz, $CDCl_3$) 14.0 (*C*(16)), 19.7 (*C*(12)), 21.1 (*C*(18_A)), 22.3 (*C*(15)), 22.3 (*C*(18_B)), 24.8 (C(*C*H$_3$)$_3$), 27.5 (*C*(13)), 31.2 (*C*(14)), 35.3 (*C*(CH$_3$)$_3$), 53.4 (CO_2CH_3), 69.6 (*C*(4)), 74.4 (*C*(5)), 79.7 (*C*(17)), 81.9 (*C*(10)), 97.8 (*C*(2)), 99.3 (*C*(11)), 109.4 (*C*(7)), 168.1 (CO_2CH_3), 172.7 (*C*(9)), 173.9 (*C*(8)), 174.6 (*C*(6)); *m/z* (ESI+) 420.2 (MH^+, 61%), 442.2 (MNa^+, 100%); HRMS (ESI+) found 420.23707, $C_{23}H_{34}NO_6$ (MH^+) requires 420.23806.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-7-(3-methylbutanoyl)-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (119III)



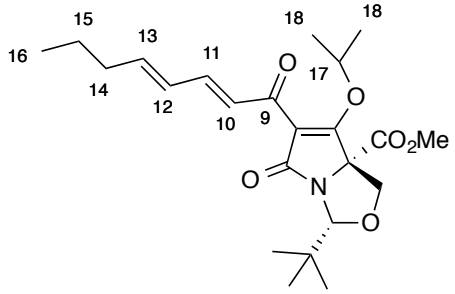
Side-product from the reaction of isopropoxy Weinreb amide **116III** with alkynyl Grignard reagents; yellow oil. R_f (DCM) 0.40; $[\alpha]_D^{20}$ +104.2 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2958 (C-H), 1754 (C=O), 1712 (C=O), 1681 (C=O), 1588 (C=C); δ_H (400 MHz, $CDCl_3$) 0.90 (9H, s, C(CH_3)$_3$), 0.93 (3H, d, *J* 6.7, C(12_A)H_3), 0.95 (3H, d, *J* 6.7, C(12_B)H_3), 1.10 (3H, d, *J* 6.1, C(14_A)H_3), 1.30 (3H, d, *J* 6.1, C(14_B)H_3), 2.19 (1H, n, *J* 6.7, C(11)*H*), 2.77 (1H, dd, *J* 17.7,

6.7, C(10)H_AH_B), 3.02 (1H, dd, *J* 17.7, 6.7, C(10)H_AH_B), 3.45 (1H, d, *J* 8.4, C(4)H_AH_B), 3.77 (3H, s, CO_2CH_3), 4.73 (1H, s, C(2)*H*), 4.80 (1H, d, *J* 8.4, C(4)H_AH_B), 5.16 (1H, sept, *J* 6.1, C(13)*H*); δ_C (100 MHz, $CDCl_3$) 21.0 (*C*(14_A)), 22.3 (*C*(14_B)), 22.7 (*C*(12_B)), 22.8 (*C*(12_A)), 24.1 (*C*(11)), 24.8 (C(*C*H_3)$_3$), 35.3 (*C*(CH_3)$_3$), 51.3 (*C*(10)), 53.3 (CO_2CH_3), 69.6 (*C*(4)), 74.7 (*C*(5)), 78.9 (*C*(13)), 97.6 (*C*(2)), 110.1 (*C*(7)), 168.2 (CO_2CH_3), 173.1 (*C*(6)), 176.2 (*C*(8)), 198.2 (*C*(9)); *m/z* (ESI$^+$) 382.2 (MH^+, 18%), 404.2 (MNa^+, 74%); HRMS (ESI$^+$) found 382.22260, $C_{20}H_{32}NO_6$ (MH^+) requires 382.22241.

General procedure G: Isomerisation of alkynones

Triphenylphosphine (1.0 eq, unless otherwise specified) was added to a solution of alkynone (1.0 eq) in anhydrous toluene (*c* 0.2). The reaction mixture was stirred at room temperature for 24 h, concentrated *in vacuo* and purified by *flash* column chromatography to give the dienone.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-7-((2*E*,4*E*)-octa-2,4-dienoyl)-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (121)

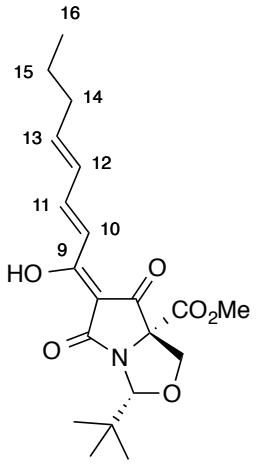


General procedure G (from **118III**); yield 88% (574 mg); yellow oil. R_f (DCM) 0.39; $[\alpha]_D^{20}$ +129.7 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2960 (C-H), 1754 (C=O), 1712 (C=O), 1652 (C=O), 1591 (C=C); δ_H (500 MHz, C_6D_6) 0.71 (3H, t, *J* 7.4, C(16)H_3), 0.87 (3H, d, *J* 6.0, C(18_A)H_3), 1.02 (3H, d, *J* 6.0, C(18_B)H_3), 1.11 (9H, s, C(CH_3)$_3$), 1.13 (2H, sext, *J* 7.4, C(15)H_2), 1.76 (2H, q, *J* 7.4, C(14)H_2), 3.05 (1H, d, *J* 8.4, C(4)H_AH_B), 3.29 (3H, s, CO_2CH_3), 4.84 (1H, d, *J* 8.4, C(4)H_AH_B), 4.88 (1H, s, C(2)*H*), 5.41 (1H, sept, *J* 6.0, C(17)*H*), 5.76 (1H, dt, *J* 15.0, 7.4, C(13)*H*), 5.99 (1H, dd, *J* 15.0, 11.0, C(12)*H*), 7.17 (1H, d, *J* 15.0, C(10)*H*), 7.59 (1H, dd, *J* 15.0, 11.0, C(11)*H*); δ_C (125 MHz, C_6D_6) 13.8 (*C*(16)), 20.7 (*C*(18_A)), 22.0 (*C*(18_B)), 22.0 (*C*(15)), 25.1 (C(*C*H_3)$_3$), 35.3 (*C*(14)), 35.5 (*C*(CH_3)$_3$), 52.6 (CO_2CH_3), 69.7 (*C*(4)), 75.2 (*C*(5)), 78.5 (*C*(17)), 97.8 (*C*(2)), 110.5 (*C*(7)), 128.4 (*C*(10)), 129.9 (*C*(12)), 144.1 (*C*(11)), 146.1 (*C*(13)), 168.6 (CO_2CH_3), 173.5 (*C*(6)), 176.3 (*C*(8)), 186.7 (*C*(9)); *m/z* (ESI$^+$) 420.3 (MH^+, 47%), 442.2 (MNa^+, 100%); HRMS (ESI$^+$) found 420.23708, $C_{23}H_{34}NO_6$ (MH^+) requires 420.23806.

General procedure H: Deprotection of isopropoxy enol ethers

$NaHCO_3$ (60 eq) was added to a biphasic solution of the enol ether (1.0 eq) and Aliquat 336 (1.1 eq) in 1:1 toluene/H_2O (*c* 0.05). The mixture was heated at 80 °C for 6 h, quenched with saturated aqueous NH_4Cl, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by *flash* column chromatography to give the deprotected tetramate.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*,2*E*,4*E*)-1-hydroxyocta-2,4-dien-1-ylidene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (114)



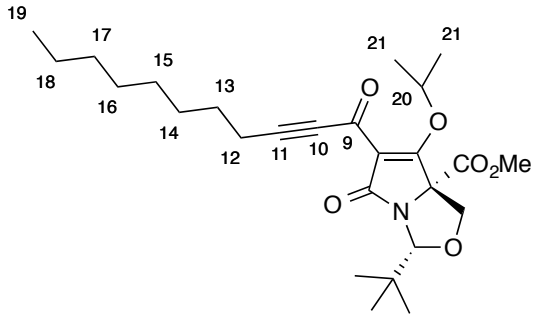
Method 1: BBr_3 (1 M in DCM, 150 µL, 0.150 mmol) was added dropwise to a solution of isopropoxy **121** (63 mg, 0.15 mmol) in anhydrous DCM (1.5 mL). The solution was stirred at room temperature for 1 h, quenched with H_2O and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (EtOAc), and the product obtained was dissolved in EtOAc, washed with 2 M aqueous HCl, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give dienoyltetramate **114** (21 mg, 0.055 mmol, 37%).

Method 2: General procedure H (from **121**); yield 56% (29 mg).

Orange oil; 3.5:1 mixture of tautomers. R_f (EtOAc) 0.42; $[\alpha]_D^{20}$ +73.4 (*c* 0.4, $CHCl_3$); ν_{max}/cm^{-1} 2960 (C-H), 1752 (C=O), 1712 (C=O), 1654 (C=O), 1612 (C=C), 1557 (C=C); δ_H (500 MHz, $CDCl_3$) major tautomer: 0.91-0.94 (3H, m, C(16)H_3), 0.93 (9H, s, C(CH_3)$_3$), 1.48 (2H, sext, *J* 7.5, C(15)H_2), 2.22 (2H, q, *J* 7.5, C(14)H_2), 3.50 (1H, d, *J* 8.9, C(4)H_AH_B), 3.77 (3H, s, CO_2CH_3), 4.82 (1H, s, C(2)*H*), 4.85 (1H, d, *J* 8.9, C(4)H_AH_B), 6.32-6.40 (2H, m, C(12)*H* + C(13)*H*), 7.03 (1H, d, *J* 15.2, C(10)*H*), 7.55-7.60 (1H, m, C(11)*H*); δ_C (125 MHz, $CDCl_3$) major tautomer: 13.8 (*C*(16)), 21.9 (*C*(15)), 24.8 (C(*C*H_3)$_3$), 35.3 (*C*(CH_3)$_3$), 35.6 (*C*(14)), 53.4 (CO_2CH_3), 68.5 (*C*(4)), 78.4 (*C*(5)), 98.4 (*C*(2)), 99.6 (*C*(7)), 119.0

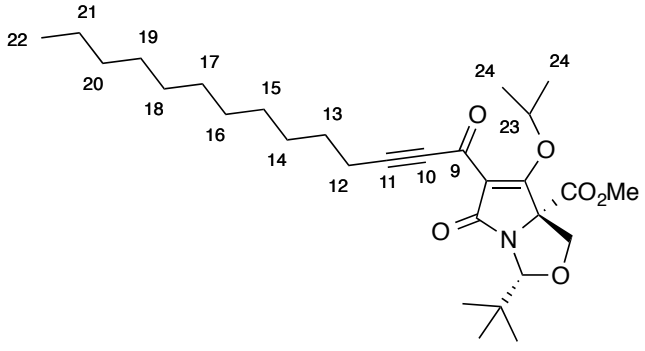
(*C*(10)), 129.8 (*C*(12)), 148.3 (*C*(11)), 150.4 (*C*(13)), 167.9 (*C*$O_2$$CH_3$), 177.4 (*C*(9)), 180.9 (*C*(8)), 188.0 (*C*(6)); *m/z* (ESI$^+$) 378.2 (MH^+, 100%), 400.2 (MNa^+, 66%), *m/z* (ESI$^-$) 376.1 ($M\text{-}H^-$, 100%); HRMS (ESI$^-$) found 376.17788, $C_{20}H_{26}NO_6$ ($M\text{-}H^-$) requires 376.17656.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxo-7-(undec-2-ynoyl)bicyclo[3.3.0]-oct-6-ene (405)



General procedure F (from **116III**, with 1-decyne); yield 54% (156 mg); yellow oil. R_f (DCM) 0.42; $[\alpha]_D^{20}$ +99.3 (*c* 0.7, DCM); ν_{max}/cm^{-1} 2957 (C-H), 2929 (C-H), 2211 (C≡C), 1754 (C=O), 1721 (C=O), 1634 (C=O), 1586 (C=C); δ_H (400 MHz, $CDCl_3$) 0.84 (3H, t, *J* 6.9, C(19)H_3), 0.87 (9H, s, C(CH_3)$_3$), 1.13 (3H, d, *J* 6.0, C(21$_A$)H_3), 1.21-1.27 (8H, m, C(15-18)H_2), 1.29 (3H, d, *J* 6.0, C(21$_B$)H_3), 1.35-1.42 (2H, m, C(14)H_2), 1.65 (2H, quint, *J* 7.2, C(13)H_2), 2.42 (2H, t, *J* 7.2, C(12)H_2), 3.42 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.75 (3H, s, CO_2CH_3), 4.73 (1H, s, C(2)*H*), 4.78 (1H, d, *J* 8.5, C(4)H$_A$$H_B$), 5.17 (1H, sept, *J* 6.0, C(20)*H*); δ_C (125 MHz, $CDCl_3$) 14.2 (*C*(19)), 19.6 (*C*(12)), 21.0 (*C*(21$_A$)), 22.2 (*C*(21$_B$)), 22.7 (*C*(18)), 24.7 (C(*C*H$_3$)$_3$), 27.7 (*C*(13)), 29.0 (*C*(14)), 29.1, 29.2 (*C*(15), *C*(16)), 31.9 (*C*(17)), 35.2 (*C*(CH$_3$)$_3$), 53.3 (CO_2CH_3), 69.5 (*C*(4)), 74.3 (*C*(5)), 79.6 (*C*(20)), 81.8 (*C*(10)), 97.7 (*C*(2)), 99.2 (*C*(11)), 109.3 (*C*(7)), 168.0 (*C*$O_2$$CH_3$), 172.5 (*C*(9)), 173.8 (*C*(6)), 174.5 (*C*(8)); *m/z* (ESI$^+$) 462.3 (MH^+, 67%), 484.3 (MNa^+, 100%); HRMS (ESI$^+$) found 462.28353, $C_{26}H_{40}NO_6$ (MH^+) requires 462.28501.

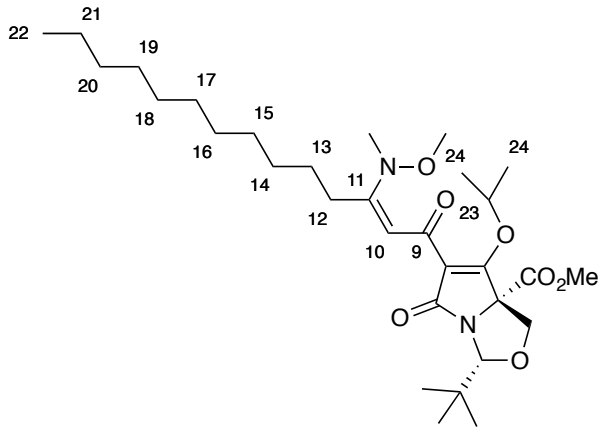
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxo-7-(tetradec-2-ynoyl)bicyclo[3.3.0]-oct-6-ene (406)



General procedure F (from **116III**, with 1-tridecyne); yield 47% (141 mg); light yellow oil. R_f (20% EtOAc in petrol) 0.52; $[\alpha]_D^{20}$ +93.3 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2925 (C-H), 2211 (C≡C), 1754 (C=O), 1721

(C=O), 1633 (C=O), 1583 (C=C); δ_H (400 MHz, $CDCl_3$) 0.85 (3H, t, *J* 6.8, C(22)H_3), 0.90 (9H, s, C(CH_3)$_3$), 1.15 (3H, d, *J* 6.0, C(24$_A$)H_3), 1.22-1.29 (14H, m, C(15-21)H_2), 1.31 (3H, d, *J* 6.0, C(24$_B$)H_3), 1.41 (2H, quint, *J* 7.2, C(14)H_2), 1.61 (2H, quint, *J* 7.2, C(13)H_2), 2.44 (2H, t, *J* 7.2, C(12)H_2), 3.44 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.78 (3H, s, CO_2CH_3), 4.75 (1H, s, C(2)*H*), 4.80 (1H, d, *J* 8.5, C(4)H$_A$$H_B$), 5.19 (1H, sept, *J* 6.0, C(23)*H*); δ_C (100 MHz, $CDCl_3$) 14.2 (*C*(22)), 19.7 (*C*(12)), 21.1 (*C*(24$_A$)), 22.3 (*C*(24$_B$)), 22.8 (*C*(21)), 24.8 (C(*C*H$_3$)$_3$), 27.8 (*C*(13)), 29.1 (*C*(14)), 29.2, 29.5, 29.6, 29.7, 29.7 (*C*(15-19)), 32.0 (*C*(20)), 35.3 (*C*(CH$_3$)$_3$), 53.3 (CO_2*C*H$_3$), 69.6 (*C*(4)), 74.4 (*C*(5)), 79.7 (*C*(23)), 81.8 (*C*(10)), 97.8 (*C*(2)), 99.3 (*C*(11)), 109.4 (*C*(7)), 168.1 (*C*O_2CH$_3$), 172.6 (*C*(9)), 173.9 (*C*(6)), 174.6 (*C*(8)); *m/z* (ESI$^+$) 526.3 (MNa$^+$, 31%); HRMS (ESI$^+$) found 504.33205, $C_{29}H_{46}NO_6$ (MH$^+$) requires 504.33196.

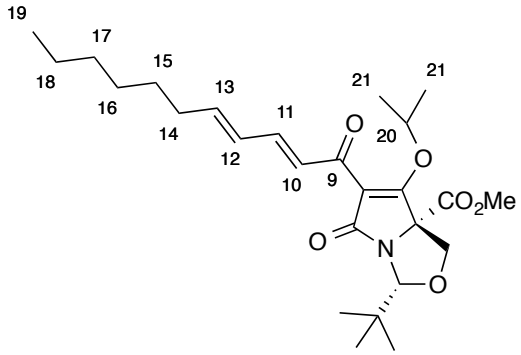
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-7-((*E*)-3-(methoxy(methyl)amino)tetradec-2-enoyl)-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (407)



Side-product (24%, 79 mg) from the Grignard reaction of isopropoxy Weinreb amide **116III** and 1-tridecyne; colourless oil. R_f (20% EtOAc in petrol) 0.51; $[\alpha]_D^{20}$ +114.1 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2925 (C-H), 1753 (C=O), 1708 (C=O), 1635 (C=O), 1585 (C=C); δ_H (500 MHz, CD_2Cl_2) 0.86-0.89 (3H, m, C(22)H_3), 0.88 (9H, s, C(CH_3)$_3$), 1.12 (3H, d, *J* 6.0, C(24$_A$)H_3), 1.26 (3H, d, *J* 6.0, C(24$_B$)H_3), 1.25-1.33 (14H, m, C(15-21)H_2), 1.40 (2H, tt, *J* 7.5, 6.5, C(14)H_2), 1.46-1.52 (2H, m, C(13)H_2), 2.79 (1H, dt, *J* 12.4, 7.4, C(12)H_AH$_B$), 2.94 (1H, dt, *J* 12.4, 8.1, C(12)H$_A$$H_B$), 3.20 (3H, s, NC$H_3$), 3.47 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.67 (3H, s, NOCH_3), 3.76 (3H, s, CO_2CH_3), 4.68 (1H, s, C(2)*H*), 4.75 (1H, d, *J* 8.5, C(4)H$_A$$H_B$), 5.13 (1H, sept, *J* 6.0, C(23)*H*), 5.73 (1H, s, C(10)*H*); δ_C (125 MHz, CD_2Cl_2) 14.4 (*C*(22)), 21.5 (*C*(24$_A$)), 22.6 (*C*(24$_B$)), 23.3 (*C*(21)), 25.0 (C(*C*H$_3$)$_3$), 28.7 (*C*(13)), 28.9 (*C*(12)), 29.9, 30.0, 30.1, 30.2, 30.2, 30.3 (*C*(14-19)), 32.5 (*C*(20)), 35.6 (*C*(CH$_3$)$_3$), 37.2 (N*C*H$_3$), 53.4 (CO_2*C*H$_3$), 60.4 (NO*C*H$_3$), 70.2 (*C*(4)), 74.8 (*C*(5)),

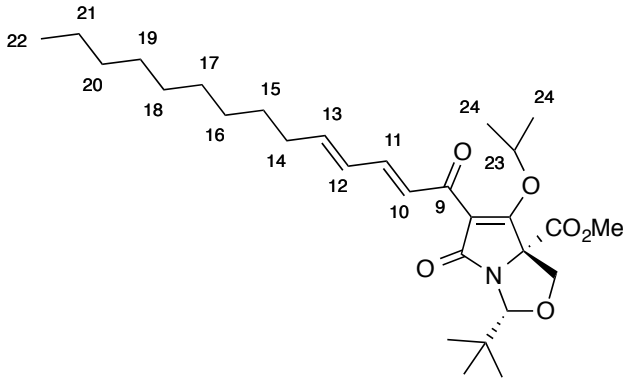
77.4 (*C*(23)), 96.0 (*C*(10)), 97.7 (*C*(2)), 113.7 (*C*(7)), 164.7 (*C*(11)), 169.4 (*C*$O_2$$CH_3$), 169.8 (*C*(6)), 177.4 (*C*(8)), 183.7 (*C*(9)); *m/z* (ESI+) 565.4 (MH^+, 100%); HRMS (ESI+) found 565.38398, $C_{31}H_{53}N_2O_7$ (MH^+) requires 565.38473.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxo-7-((2*E*,4*E*)-undeca-2,4-dienoyl)bicyclo[3.3.0]-oct-6-ene (127)



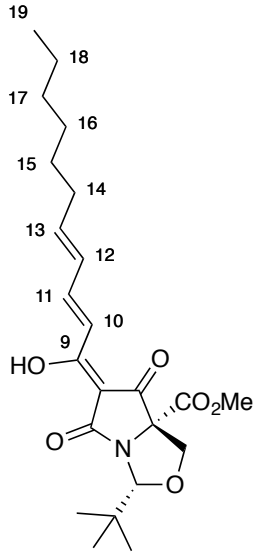
General procedure G (from **405**); yield 81% (129 mg); orange oil. R_f (10% EtOAc in petrol) 0.28; $[\alpha]_D^{20}$ +126.0 (*c* 0.4, DCM); ν_{max}/cm^{-1} 2957 (C-H), 2929 (C-H), 1755 (C=O), 1713 (C=O), 1663 (C=O), 1626 (C=C), 1591 (C=C); δ_H (500 MHz, C_6D_6) 0.87 (3H, d, *J* 6.0, C(21$_A$)*H*$_3$), 0.89 (3H, t, *J* 7.2, C(19)*H*$_3$), 1.02 (3H, d, *J* 6.0, C(21$_B$)*H*$_3$), 1.07-1.17 (6H, m, C(16-18)*H*$_2$), 1.12 (9H, s, C(C*H*$_3$)$_3$), 1.21-1.26 (2H, m, C(15)*H*$_2$), 1.82 (2H, q, *J* 6.8, C(14)*H*$_2$), 3.04 (1H, d, *J* 8.4, C(4)*H*$_A$H$_B$), 3.29 (3H, s, CO_2CH_3), 4.85 (1H, d, *J* 8.4, C(4)H$_A$*H*$_B$), 4.89 (1H, s, C(2)*H*), 5.43 (1H, sept, *J* 6.0, C(20)*H*), 5.80 (1H, dt, *J* 15.2, 6.8, C(13)*H*), 6.03 (1H, dd, *J* 15.2, 11.0, C(12)*H*), 7.20 (1H, d, *J* 15.2, C(10)*H*), 7.61 (1H, dd, *J* 15.2, 11.0, C(11)*H*); δ_C (100 MHz, C_6D_6) 14.2 (*C*(19)), 20.7 (*C*(21$_A$)), 22.0 (*C*(21$_B$)), 22.9 (*C*(18)), 25.1 (C(*C*H$_3$)$_3$), 28.9, 29.2, 32.0 (*C*(15-17)), 33.4 (*C*(14)), 35.5 (*C*(CH$_3$)$_3$), 52.5 (CO_2CH_3), 69.7 (*C*(4)), 75.2 (*C*(5)), 78.5 (*C*(20)), 97.9 (*C*(2)), 110.5 (*C*(7)), 128.0 (*C*(10)), 129.7 (*C*(12)), 144.2 (*C*(11)), 146.3 (*C*(13)), 168.6 (*C*$O_2$$CH_3$), 173.6 (*C*(6)), 176.3 (*C*(8)), 186.7 (*C*(9)); *m/z* (ESI+) 462.3 (MH^+, 100%), 484.3 (MNa^+, 79%); HRMS (ESI+) found 462.28530, $C_{26}H_{40}NO_6$ (MH^+) requires 462.28501.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxo-7-((2*E*,4*E*)-tetradeca-2,4-dienoyl)bicyclo[3.3.0]-oct-6-ene (408)



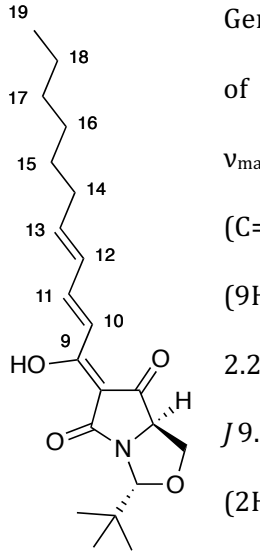
General procedure G (from **406**); yield 75% (80 mg); orange oil. R_f (DCM) 0.32; $[\alpha]_D^{20}$ +120.3 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2957 (C-H), 2926 (C-H), 1755 (C=O), 1714 (C=O), 1663 (C=O), 1626 (C=C), 1591 (C=C); δ_H (400 MHz, C_6D_6) 0.87 (3H, d, *J* 6.0, C(24$_A$)H_3), 0.93 (3H, t, *J* 7.0, C(22)H_3), 1.02 (3H, d, *J* 6.0, C(24$_B$)H_3), 1.11 (9H, s, C(CH_3)$_3$), 1.13-1.35 (14H, m, C(15-21)H_2), 1.84 (2H, q, *J* 6.8, C(14)H_2), 3.04 (1H, d, *J* 8.4, C(4)H_AH$_B$), 3.29 (3H, s, CO_2CH_3), 4.85 (1H, d, *J* 8.4, C(4)H$_A$$H_B$), 4.89 (1H, s, C(2)*H*), 5.43 (1H, sept, *J* 6.0, C(23)*H*), 5.82 (1H, dt, *J* 15.2, 6.8, C(13)*H*), 6.04 (1H, dd, *J* 15.2, 11.2, C(12)*H*), 7.20 (1H, d, *J* 15.2, C(10)*H*), 7.64 (1H, dd, *J* 15.2, 11.2, C(11)*H*); δ_C (100 MHz, C_6D_6) 14.4 (*C*(22)), 20.6 (*C*(24$_A$)), 22.0 (*C*(24$_B$)), 23.1 (*C*(21)), 25.1 (C(*C*H$_3$)$_3$), 28.9, 29.6, 29.8, 29.9, 30.0, 32.3 (*C*(15-20)), 33.4 (*C*(14)), 35.5 (*C*(CH$_3$)$_3$), 52.6 (CO_2CH_3), 69.6 (*C*(4)), 75.2 (*C*(5)), 78.5 (*C*(23)), 97.8 (*C*(2)), 110.5 (*C*(7)), 128.0 (*C*(10)), 129.7 (*C*(12)), 144.2 (*C*(11)), 146.4 (*C*(13)), 168.6 (CO_2CH_3), 173.5 (*C*(6)), 176.3 (*C*(8)), 186.7 (*C*(9)); *m/z* (ESI$^+$) 504.4 (MH$^+$, 100%), 526.3 (MNa$^+$, 43%); HRMS (ESI$^+$) found 504.33218, $C_{29}H_{46}NO_6$ (MH$^+$) requires 504.33196.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*,2*E*,4*E*)-1-hydroxyundeca-2,4-dien-1-ylidene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (122)



General procedure H (from **127**); yield 48% (14 mg); orange oil; 1.9:1 mixture of tautomers. R_f (50% EtOAc in petrol) 0.16; $[\alpha]_D^{20}$ +62.9 (*c* 0.9, $CHCl_3$); ν_{max}/cm^{-1} 2960 (C-H), 1753 (C=O), 1712 (C=O), 1651 (C=O), 1613 (C=C), 1558 (C=C); δ_H (500 MHz, $CDCl_3$) major tautomer: 0.88 (3H, t, *J* 6.9, C(19)H_3), 0.94 (9H, s, C(CH_3)$_3$), 1.24-1.35 (6H, m, C(16-18)H_2), 1.42-1.48 (2H, m, C(15)H_2), 2.22-2.27 (2H, m, C(14)H_2), 3.51 (1H, d, *J* 8.9, C(4)H_AH_B), 3.78 (3H, s, CO_2CH_3), 4.83 (1H, d, *J* 8.9, C(4)H_AH_B), 4.86 (1H, s, C(2)*H*), 6.32-6.40 (2H, m, C(12)*H* + C(13)*H*), 7.03 (1H, d, *J* 15.3, C(10)*H*), 7.58 (1H, dd, *J* 15.3, 10.2, C(11)*H*); δ_C (125 MHz, $CDCl_3$) major tautomer: 14.2 (*C*(19)), 22.7 (*C*(18)), 24.8 (C(*C*H_3)$_3$), 28.6 (*C*(15)), 29.0 (*C*(16)), 29.9 (*C*(17)), 33.7 (*C*(14)), 35.4 (*C*(CH_3)$_3$), 53.4 (CO_2CH_3), 68.5 (*C*(4)), 78.4 (*C*(5)), 98.4 (*C*(2)), 99.6 (*C*(7)), 118.9 (*C*(10)), 129.7 (*C*(12)), 148.4 (*C*(11)), 150.8 (*C*(13)), 167.9 (*C*O_2CH_3), 177.4 (*C*(9)), 181.0 (*C*(8)), 188.0 (*C*(6)); *m/z* (ESI$^-$) 418.1 (M-H$^-$, 100%); HRMS (ESI$^+$) found 420.23812, $C_{23}H_{34}NO_6$ (MH$^+$) requires 420.23806.

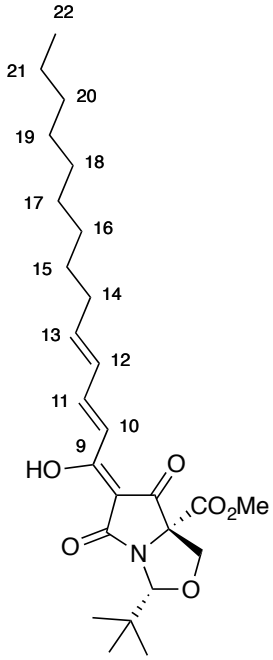
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*,2*E*,4*E*)-1-hydroxyundeca-2,4-dien-1-ylidene]-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (404)



General procedure E (from **122**); yield 51% (18 mg); orange oil; 2.2:1 mixture of tautomers. R_f (80% EtOAc in petrol) 0.18; $[\alpha]_D^{20}$ +136.4 (*c* 0.5, $CHCl_3$); ν_{max}/cm^{-1} 2957 (C-H), 2927 (C-H), 1708 (C=O), 1650 (C=O), 1613 (C=O), 1557 (C=C); δ_H (500 MHz, $CDCl_3$) major tautomer: 0.86-0.90 (3H, m, C(19)H_3), 0.97 (9H, s, C(CH_3)$_3$), 1.25-1.34 (6H, m, C(16-18)H_2), 1.45 (2H, tt, *J* 7.0, 6.5, C(15)H_2), 2.23 (2H, td, *J* 7.0, 6.0, C(14)H_2), 3.45 (1H, dd, *J* 9.5, 8.3, C(4)H_AH_B), 4.15 (1H, dd, *J* 9.5, 7.0, C(5)*H*), 4.26 (1H, dd, *J* 8.3, 7.0, C(4)H_AH_B), 4.95 (1H, s, C(2)*H*), 6.32-6.40 (2H, m, C(12)*H* + C(13)*H*), 7.07 (1H, d, *J* 15.5, C(10)*H*), 7.53 (1H, dd, *J* 15.5, 10.0, C(11)*H*); δ_C (125 MHz, $CDCl_3$) major tautomer: 14.2 (*C*(19)), 22.7 (*C*(18)), 24.9 (C(*C*H_3)$_3$), 28.6 (*C*(15)), 29.0 (*C*(16)), 31.8 (*C*(17)), 33.6 (*C*(14)), 36.1 (*C*(CH_3)$_3$), 67.0 (*C*(4)), 67.3

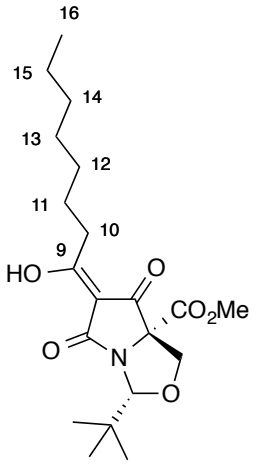
(*C*(5)), 96.0 (*C*(2)), 101.1 (*C*(7)), 119.2 (*C*(10)), 129.7 (*C*(12)), 147.2 (*C*(11)), 149.7 (*C*(13)), 176.0 (*C*(9)), 179.9 (*C*(8)), 191.8 (*C*(6)); *m/z* (ESI$^-$) 360.2 (M-H$^-$, 100%); HRMS (ESI$^-$) found 360.21797, $C_{21}H_{30}NO_4$ (M-H$^-$) requires 360.21803.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*,2*E*,4*E*)-1-hydroxytetradeca-2,4-dien-1-ylidene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (123)



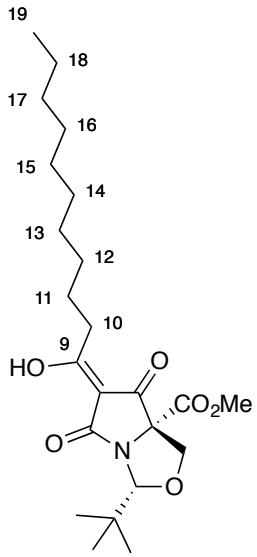
General procedure H (from **408**); yield 41% (30 mg); orange oil; 2.6:1 mixture of tautomers. R_f (70% EtOAc in petrol) 0.26; $[\alpha]_D^{20}$ +76.6 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2924 (C-H), 1754 (C=O), 1710 (C=O), 1654 (C=O), 1613 (C=C), 1556 (C=C); δ_H (500 MHz, $CDCl_3$) major tautomer: 0.88 (3H, t, *J* 6.6, C(22)H_3), 0.93 (9H, s, C(CH_3)$_3$), 1.20-1.33 (12H, m, C(16-21)H_2), 1.40-1.48 (2H, m, C(15)H_2), 2.24 (2H, q, *J* 6.3, C(14)H_2), 3.51 (1H, d, *J* 8.8, C(4)H_AH_B), 3.78 (3H, s, CO_2CH_3), 4.83 (1H, d, *J* 8.8, C(4)H_AH_B), 4.86 (1H, s, C(2)*H*), 6.32-6.40 (2H, m, C(12)*H* + C(13)*H*), 7.03 (1H, d, *J* 15.2, C(10)*H*), 7.58 (1H, dd, *J* 15.2, 9.7, C(11)*H*); δ_C (125 MHz, $CDCl_3$) major tautomer: 14.3 (*C*(22)), 22.8 (*C*(21)), 24.8 (C(*C*H$_3$)$_3$), 28.6 (*C*(15)), 29.3, 29.4, 29.5, 29.6 (*C*(16-19)), 32.0 (*C*(20)), 33.7 (*C*(14)), 35.4 (*C*(CH$_3$)$_3$), 53.4 (CO_2CH_3), 68.5 (*C*(4)), 78.5 (*C*(5)), 98.4 (*C*(2)), 99.6 (*C*(7)), 118.9 (*C*(10)), 129.7 (*C*(12)), 148.3 (*C*(11)), 150.8 (*C*(13)), 167.9 (*C*O$_2$CH$_3$), 177.4 (*C*(9)), 180.9 (*C*(8)), 188.0 (*C*(6)); *m/z* (ESI$^+$) 462.3 (MH$^+$, 19%), 484.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 462.28491, $C_{26}H_{40}NO_6$ (MH$^+$) requires 462.28501.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*)-1-hydroxyoctylidene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (124)



General procedure C (from **77a**, with 1-bromoheptane); yield 60% (124 mg); orange oil; 3.4:1 mixture of tautomers. R_f (EtOAc) 0.40; $[\alpha]_D^{20}$ +67.4 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2958 (C-H), 2930 (C-H), 1758 (C=O), 1718 (C=O), 1657 (C=O), 1602 (C=C); δ_H (400 MHz, $CDCl_3$) major tautomer: 0.84-0.89 (3H, m, C(16)H_3), 0.92 (9H, s, C(CH_3)$_3$), 1.24-1.30 (8H, m, C(12-15)H_2), 1.66 (2H, quint, *J* 7.6, C(11)H_2), 2.83 (2H, app td, *J* 7.6, 2.5, C(10)H_2), 3.48 (1H, d, *J* 8.9, C(4)H_AH$_B$), 3.78 (3H, s, CO_2CH_3), 4.81 (1H, d, *J* 8.9, C(4)H$_A$$H_B$), 4.84 (1H, s, C(2)$H$); δ_C (100 MHz, $CDCl_3$) major tautomer: 14.2 (*C*(16)), 22.7 (*C*(15)), 24.8 (C(*C*H$_3$)$_3$), 26.0 (*C*(11)), 28.9, 29.3 ((*C*(12), *C*(13)), 31.7 (*C*(14)), 33.2 (*C*(10)), 35.3 (*C*(CH$_3$)$_3$), 53.4 (CO_2CH_3), 68.4 (*C*(4)), 78.4 (*C*(5)), 98.4 (*C*(2)), 101.2 (*C*(7)), 167.6 (CO_2CH_3), 180.4 (*C*(8)), 187.9 (*C*(6)), 192.6 (*C*(9)); *m/z* (ESI$^+$) 382.2 (MH^+, 81%), 404.3 (MNa^+, 100%), *m/z* (ESI$^-$) 380.2 ($M-H^-$, 100%); HRMS (ESI$^-$) found 380.20918, $C_{20}H_{30}NO_6$ (M-H$^-$) requires 380.20786.

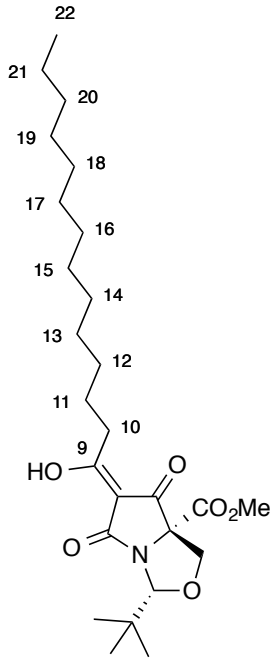
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*)-1-hydroxyundecylidene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (125)



General procedure C (from **77a**, with 1-bromodecane); yield 61% (119 mg); orange oil; 6:1 mixture of tautomers. R_f (EtOAc) 0.35; $[\alpha]_D^{20}$ +64.6 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2926 (C-H), 2856 (C-H), 1758 (C=O), 1719 (C=O), 1658 (C=O), 1602 (C=C); δ_H (400 MHz, $CDCl_3$) major tautomer: 0.86 (3H, t, *J* 6.8, C(19)H_3), 0.91 (9H, s, C(CH_3)$_3$), 1.24-1.37 (14H, m, C(12-18)H_2), 1.65 (2H, quint, *J* 7.5, C(11)H_2), 2.81-2.84 (2H, m, C(10)H_2), 3.48 (1H, d, *J* 8.8, C(4)H_AH$_B$), 3.77 (3H, s, CO_2CH_3), 4.81 (1H, d, *J* 8.8, C(4)H$_A$$H_B$), 4.83 (1H, s, C(2)$H$); δ_C (100 MHz, $CDCl_3$) major tautomer: 14.2 (*C*(19)), 22.8 (*C*(18)), 24.8 (C(*C*H$_3$)$_3$), 26.0 (*C*(11)), 29.3, 29.3, 29.4, 29.5, 29.6 (*C*(12-16), 32.0 (*C*(17)), 33.2 (*C*(10)), 35.3 (*C*(CH$_3$)$_3$), 53.4 (CO_2CH_3), 68.5 (*C*(4)), 78.4 (*C*(5)), 98.3 (*C*(2)), 101.1 (*C*(7)), 167.6 (CO_2CH_3), 180.4 (*C*(8)), 187.9 (*C*(6)), 192.6 (*C*(9)); *m/z* (ESI$^+$)

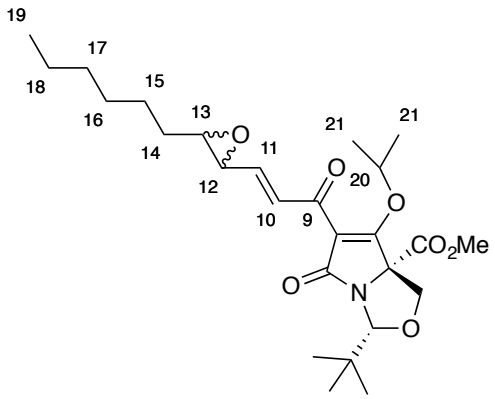
424.3 (MH^+, 100%), 446.3 (MNa^+, 76%), *m/z* (ESI$^-$) 422.2 (M-H$^-$, 100%); HRMS (ESI$^-$) found 422.25451, $C_{23}H_{36}NO_6$ (M-H$^-$) requires 422.25371.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*)-1-hydroxytetradecylidene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (126)



General procedure C (from **77a**, with 1-bromotridecane); yield 66% (102 mg); orange oil; 4.3:1 mixture of tautomers. R_f (EtOAc) 0.46; $[\alpha]_D^{20}$ +62.0 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2924 (C-H), 2854 (C-H), 1758 (C=O), 1719 (C=O), 1658 (C=O), 1603 (C=C); δ_H (400 MHz, $CDCl_3$) major tautomer: 0.87 (3H, t, *J* 7.0, C(22)H_3), 0.92 (9H, s, C(CH_3)$_3$), 1.24-1.30 (18H, m, C(13-21)H_2), 1.33-1.39 (2H, m, C(12)H_2), 1.66 (2H, quint, *J* 7.5, C(11)H_2), 2.82-2.85 (2H, m, C(10)H_2), 3.49 (1H, d, *J* 8.9, C(4)H_AH_B), 3.78 (3H, s, CO_2CH_3), 4.82 (1H, d, *J* 8.9, C(4)H_AH_B), 4.84 (1H, s, C(2)H); δ_C (100 MHz, $CDCl_3$) major tautomer: 14.3 (*C*(22)), 22.8 (*C*(21)), 24.8 (C(*C*H$_3$)$_3$), 26.0 (*C*(11)), 29.3, 29.3, 29.5, 29.5, 29.7, 29.8, 29.8, 29.8 (*C*(12-19), 32.1 (*C*(20)), 33.2 (*C*(10)), 35.3 (*C*(CH$_3$)$_3$), 53.4 (CO_2CH_3), 68.4 (*C*(4)), 78.5 (*C*(5)), 98.4 (*C*(2)), 101.2 (*C*(7)), 167.6 (CO_2CH_3), 180.4 (*C*(8)), 187.9 (*C*(6)), 192.7 (*C*(9)); *m/z* (ESI$^-$) 464.3 (M-H$^-$, 100%); HRMS (ESI$^-$) found 464.30132, $C_{26}H_{42}NO_6$ (M-H$^-$) requires 464.30066.

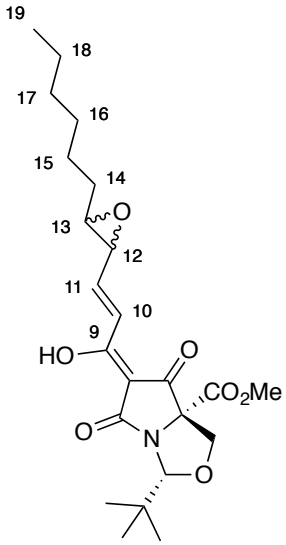
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-((*E*)-3-((2*R*,3*R*)-3-hexyloxiran-2-yl)acryloyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene ((*R*,*R*)-128) and
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-((*E*)-3-((2*S*,3*S*)-3-hexyloxiran-2-yl)acryloyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene ((*S*,*S*)-128)



*m*CPBA (80%, 88 mg, 0.41 mmol) was added portionwise to a solution of diene **127** (64 mg, 0.14 mmol) in anhydrous DCM (700 μL) at 0 °C. The mixture was stirred at room temperature for 5 h and quenched with saturated aqueous $Na_2S_2O_3$. The aqueous phase was extracted with DCM, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (10% EtOAc in petrol) to give a 1:1 unseparable diastereomeric mixture of epoxide **128** (43 mg, 0.089 mmol, 64%) as a light yellow oil. R_f (10% EtOAc in petrol) 0.19; ν_{max}/cm^{-1} 2960 (C-H), 2931 (C-H), 1754 (C=O), 1711 (C=O), 1671 (C=O), 1618 (C=C), 1585 (C=C); δ_H (500 MHz, $CDCl_3$, 1:1 mixture of diastereomers A and B) 0.88 (6H, t, *J* 6.8, C(19)H_3 A + B), 0.91 (9H, s, C(CH_3)$_3$ A), 0.91 (9H, s, C(CH_3)$_3$ B), 1.09 (3H, d, *J* 6.0, C(21$_A$)H_3 A), 1.09 (3H, d, *J* 6.0, C(21$_A$)H_3 B), 1.25-1.37 (12H, m, C(16-18)H_2 A + B), 1.31 (3H, d, *J* 6.0, C(21$_B$)H_3 A), 1.32 (3H, d, *J* 6.0, C(21$_B$)H_3 B), 1.40-1.49 (4H, m, C(15)H_2 A + B), 1.56-1.62 (4H, m, C(14)H_2 A + B), 2.94-2.97 (2H, m, C(13)*H* A + B), 3.27-3.29 (2H, m, C(12)*H* A + B), 3.48 (1H, d, *J* 8.5, C(4)H_AH$_B$ A), 3.48 (1H, d, *J* 8.5, C(4)H_AH$_B$ B), 3.78 (3H, s, CO_2CH_3 A), 3.79 (3H, s, CO_2CH_3 B), 4.74 (2H, s, C(2)*H* A + B), 4.82 (1H, d, *J* 8.5, C(4)H$_A$$H_B$ A), 4.82 (1H, d, *J* 8.5, C(4)H$_A$$H_B$ B), 5.16 (1H, sept, *J* 6.0, C(20)*H* A), 5.17 (1H, sept, *J* 6.0, C(20)*H* B), 6.59 (1H, dd, *J* 15.6, 8.0, C(11)*H* A), 6.65 (1H, dd, *J* 15.6, 7.3, C(11)*H* B), 7.16 (1H, dd, *J* 15.6, 0.5, C(10)*H* A), 7.23 (1H, dd, *J* 15.6, 0.5, C(10)*H* B); δ_C (125 MHz, $CDCl_3$) 14.2 (*C*(19) A + B), 20.9 (*C*(21$_A$) A), 21.0 (*C*(21$_A$) B), 22.3 (*C*(21$_B$) A), 22.3 (*C*(21$_B$) B), 22.7 (*C*(18) A + B), 24.8 (C(*C*H$_3$)$_3$ A + B), 25.9 (*C*(15) A + B), 29.2 (*C*(16) A + B), 31.8 (*C*(17) A + B), 32.1 (*C*(14) A), 32.1 (*C*(14) B), 35.3 (*C*(CH$_3$)$_3$ A + B), 53.4 (CO_2CH_3 A + B), 56.9 (*C*(12) A), 57.1 (*C*(12) B), 61.6 (*C*(13) A), 62.0 (*C*(13) B), 69.6 (*C*(4) A + B), 74.9 (*C*(5) A), 74.9 (*C*(5) B), 79.2 (*C*(20) A), 79.3 (*C*(20) B), 97.7

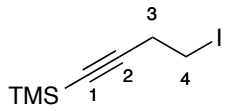
(*C*(2) A), 97.7 (*C*(2) B), 109.0 (*C*(7) A), 109.0 (*C*(7) B), 130.3 (*C*(10) A), 130.7 (*C*(10) B), 144.5 (*C*(11) A), 144.7 (*C*(11) B), 168.1 (*C*$O_2$$CH_3$ A + B), 174.2 (*C*(6) A), 174.3 (*C*(6) B), 176.1 (*C*(8) A), 176.2 (*C*(8) A), 185.5 (*C*(9) A), 185.8 (*C*(9) B); *m/z* (ESI$^+$) 478.3 (MH^+, 93%), 500.3 (MNa^+, 100%); HRMS (ESI$^+$) found 478.27963, $C_{26}H_{40}NO_7$ (MH^+) requires 478.27993.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*,2*E*)-3-((2*R*,3*R*)-3-hexyloxiran-2-yl)-1-hydroxyallylidene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane ((*R*,*R*)-129) and (2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*,2*E*)-3-((2*S*,3*S*)-3-hexyloxiran-2-yl)-1-hydroxyallylidene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane ((*S*,*S*)-129)



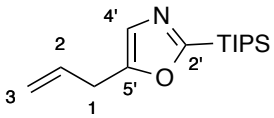
General procedure H (from **128**); yield 77% (43 mg); orange oil. R_f (80% EtOAc in petrol) 0.32; ν_{max}/cm^{-1} 2958 (C-H), 2930 (C-H), 1753 (C=O), 1715 (C=O), 1645 (C=O), 1616 (C=C), 1574 (C=C); δ_H (400 MHz, $CDCl_3$, 1:1 mixture of diastereomers A and B) 0.85-0.90 (6H, m, C(19)H_3 A + B), 0.93 (18H, s, C(CH_3)$_3$ A + B), 1.23-1.36 (12H, m, C(16-18)H_2 A + B), 1.42-1.48 (4H, m, C(15)H_2 A + B), 1.60-1.66 (4H, m, C(14)H_2 A + B), 2.95-3.00 (2H, m, C(13)*H* A + B), 3.31-3.34 (2H, m, C(12)*H* A + B), 3.51 (1H, d, *J* 8.8, C(4)$H_A$$H_B$ A), 3.52 (1H, d, *J* 8.8, C(4)$H_A$$H_B$ B), 3.79 (3H, s, CO_2CH_3 A), 3.79 (3H, s, CO_2CH_3 B), 4.84 (2H, d, *J* 8.8, C(4)$H_A$$H_B$ A + B), 4.87 (2H, s, C(2)*H* A + B), 6.94 (1H, dd, *J* 15.6, 7.6, C(11)*H* A), 6.95 (1H, dd, *J* 15.6, 7.6, C(11)*H* B), 7.34 (2H, d, *J* 15.6, C(10)*H* A + B); δ_C (125 MHz, $CDCl_3$) 14.2 (*C*(19) A + B), 22.6 (*C*(18) A + B), 24.8 (C(*C*H_3)$_3$ A + B), 25.9 (*C*(15) A + B), 29.1 (*C*(16) A + B), 31.8 (*C*(17) A + B), 32.1 (*C*(14) A + B), 35.3 (*C*(CH$_3$)$_3$ A + B), 53.5 (CO_2*C*H_3 A), 53.5 (CO_2*C*H_3 B), 57.0 (*C*(12) A), 57.0 (*C*(12) B), 62.3 (*C*(13) A), 62.4 (*C*(13) B), 68.3 (*C*(4) A + B), 78.4 (*C*(5) A + B), 98.5 (*C*(2) A), 98.5 (*C*(2) B), 100.4 (*C*(7) A + B), 122.6 (*C*(10) A), 122.7 (*C*(10) B), 148.5 (*C*(11) A + B), 167.5 (*C*$O_2$$CH_3$ A), 167.6 (*C*$O_2$$CH_3$ B), 175.2 (*C*(9) A + B), 180.3 (*C*(8) A + B), 187.8 (*C*(6) A + B); *m/z* (ESI$^-$) 434.2 (M-H$^-$, 100%); HRMS (ESI$^-$) found 434.21883, $C_{23}H_{32}NO_7$ (M-H$^-$) requires 434.21843.

(4-Iodobut-1-ynyl)trimethylsilane (146)



Sodium iodide (276 mg, 1.84 mmol) was added to a solution of tosylate **150** (267 mg, 0.900 mmol) in acetone (3 mL) and the mixture was stirred at 60 °C for 5 h. The solution was then diluted with Et_2O and the solid formed was filtered. The filtrates were washed with aqueous saturated $Na_2S_2O_3$ and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (petrol) to give iodide **146** (165 mg, 0.654 mmol, 73%) as a colourless oil. R_f (petrol) 0.39; ν_{max}/cm^{-1} 2959 (C-H), 2175 (C≡C); δ_H (400 MHz, $CDCl_3$) 0.16 (9H, s, Si(CH_3)$_3$), 2.79 (2H, t, *J* 7.6, C(3)H_2), 3.21 (2H, t, *J* 7.6, C(4)H_2); δ_C (100 MHz, $CDCl_3$) 0.0 (Si(*C*H_3)$_3$), 1.1 (*C*(4)), 25.1 (*C*(3)), 86.8 (*C*(1)), 105.1 (*C*(2)); HRMS (FI$^+$) found 251.9837, $C_7H_{13}ISi$ (M^+) requires 251.9831.

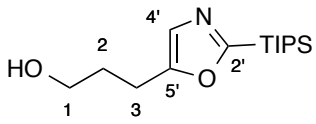
5-Allyl-2-(triisopropylsilyl)oxazole (152)



CuCN (495 mg, 5.52 mmol) and LiCl (485 mg, 11.4 mmol) were stirred at 130 °C under vacuum for 5 h. After cooling to room temperature, anhydrous THF (10 mL) was added and stirring was continued for further 30 min. To a solution of TIPS-protected oxazole **142** (1.08 g, 4.80 mmol) in anhydrous THF (12 mL) was added *n*-BuLi (1.6 M in hexanes, 3.3 mL, 5.3 mmol) dropwise at -78 °C. After stirring at this temperature for 30 min, the CuCN·2LiCl solution was added by cannula (with another 2 mL of anhydrous THF) and the mixture was stirred at -78 °C for 2 h. Allyl bromide (630 μL, 7.28 mmol) was then added and the solution was stirred at -78 °C for 30 min and at room temperature for 2 h. The reaction mixture was quenched with saturated aqueous NH_4Cl, extracted with Et_2O and dried over anhydrous Na_2SO_4. The crude product was purified by *flash* column chromatography (5% EtOAc in petrol) to give a 13:1 inseparable mixture of alkene **152** (1.21 g, 4.48 mmol, 93%) and remaining starting material as a light yellow oil. R_f (5% EtOAc in petrol) 0.38; ν_{max}/cm^{-1} 2944 (C-H), 2867 (C-H), 1464 (C=C); δ_H (400 MHz, $CDCl_3$) 1.12 (18H, d, *J* 7.4, SiCH(CH_3)$_2$), 1.39 (3H, sept, *J* 7.4, SiC*H*(CH$_3$)$_2$), 3.45 (2H, app dq, *J* 6.4, 1.2, C(1)H_2), 5.11-5.13 (1H, m, C(3)H_AH$_B$), 5.14-5.16 (1H, m, C(3)H$_A$$H_B$), 5.87-5.97 (1H, m, C(2)*H*), 6.83 (1H, t, *J* 1.2, C(4')*H*); δ_C (100 MHz, $CDCl_3$) 11.1 (Si*C*H(CH$_3$)$_2$), 18.5

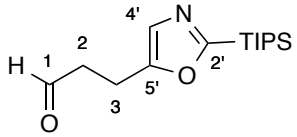
(SiCH(CH$_3$)$_2$), 30.2 (C(1)), 117.5 (C(3)), 123.0 (C(4')), 133.0 (C(2)), 152.6 (C(5')), 167.9 (C(2')); *m/z* (ESI$^+$) 266.2 (MH$^+$, 100%); HRMS (ESI$^+$) found 266.19354, $C_{15}H_{28}NOSi$ (MH$^+$) requires 266.19347.

3-(2-(Triisopropylsilyl)oxazol-5-yl)propan-1-ol (153)



9-BBN (0.5 M in THF, 24 mL, 12 mmol) was added dropwise to a solution of alkene **152** (1.14 g, 3.98 mmol) in anhydrous THF (16 mL) at 0 °C and the solution was stirred at room temperature for 13 h. The mixture was then cooled to 0 °C and water (2.2 mL), aqueous NaOH (2 M, 20 mL, 40 mmol) and H_2O_2 (35% w/w, 3.4 mL, 40 mmol) were added dropwise. The biphasic mixture was stirred at room temperature for 6 h, diluted with brine and extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (30% EtOAc in DCM) to give alcohol **153** (1.02 g, 3.59 mmol, 90%) as a colourless oil. R_f (20% EtOAc in petrol) 0.33; ν_{max}/cm^{-1} 3340 (O-H), 2944 (C-H), 2867 (C-H), 1464 (C=C); δ_H (400 MHz, $CDCl_3$) 1.11 (18H, d, *J* 7.4, SiCH(CH_3)$_2$), 1.37 (3H, sept, *J* 7.4, SiCH(CH$_3$)$_2$), 1.90 (2H, tt, *J* 7.4, 6.4, C(2)H_2), 1.96 (1H, br s, OH), 2.79 (2H, t, *J* 7.4, C(3)H_2), 3.68 (2H, t, *J* 6.4, C(1)H_2), 6.81 (1H, s, C(4')H); δ_C (100 MHz, $CDCl_3$) 11.1 (SiCH(CH$_3$)$_2$), 18.5 (SiCH(CH$_3$)$_2$), 22.1 (C(3)), 30.8 (C(2)), 61.8 (C(1)), 122.5 (C(4')), 154.3 (C(5')), 167.7 (C(2')); *m/z* (ESI$^+$) 284.2 (MH$^+$, 100%); HRMS (ESI$^+$) found 284.20412, $C_{15}H_{30}NO_2Si$ (MH$^+$) requires 284.20403.

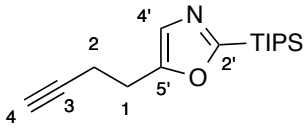
3-(2-(Triisopropylsilyl)oxazol-5-yl)propanal (154)



To a solution of alcohol **153** (346 mg, 1.22 mmol) and $NaHCO_3$ (115 mg, 1.37 mmol) in anhydrous DCM (6.1 mL) was added Dess-Martin periodinane (767 mg, 1.81 mmol) portionwise at 0 °C. After stirring at room temperature for 5 h, a 1:1 mixture of saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$ was added and the organic layer was extracted with Et_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (5% EtOAc in DCM) to give aldehyde **154** (303 mg, 1.08 mmol, 89%) as a yellow oil. R_f (5% EtOAc in

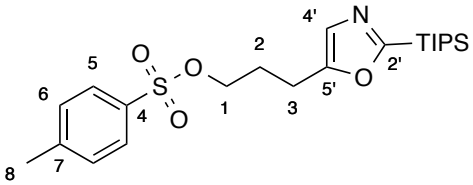
DCM) 0.36; ν_{max}/cm^{-1} 2944 (C-H), 2867 (C-H), 1728 (C=O), 1465 (C=C); δ_H (400 MHz, $CDCl_3$) 1.10 (18H, d, *J* 7.4, SiCH(CH_3)$_2$), 1.36 (3H, sept, *J* 7.4, SiC*H*(CH_3)$_2$), 2.80 (2H, t, *J* 7.6, C(2)H_2), 3.02 (2H, t, *J* 7.6, C(3)H_2), 6.83 (1H, s, C(4')*H*), 9.82 (1H, s, C(1)*H*); δ_C (100 MHz, $CDCl_3$) 11.0 (Si*C*H(CH_3)$_2$), 18.5 (SiCH(CH_3)$_2$), 18.5 (*C*(3)), 41.7 (*C*(2)), 122.8 (*C*(4')), 152.7 (*C*(5')), 168.0 (*C*(2')), 200.3 (*C*(1)); *m/z* (ESI^+) 282.1 (MH^+, 5%); HRMS (ESI^+) found 304.1703, $C_{15}H_{27}NNaO_2Si$ (MNa^+) requires 304.17033.

5-(But-3-yn-1-yl)-2-(triisopropylsilyl)oxazole (130)



Potassium carbonate (300 mg, 2.17 mmol) and a solution of the Bestmann reagent **156** (250 mg, 1.30 mmol) in MeOH (1.5 mL) were added to a solution of aldehyde **154** (295 mg, 1.05 mmol) in MeOH (9 mL). After stirring at room temperature for 14 h, the suspension was cooled to 0 °C, quenched with saturated aqueous $NaHCO_3$ and extracted with Et_2O. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (5% EtOAc in petrol) to give alkyne **130** (267 mg, 0.963 mmol, 92%) as a colourless oil. R_f (5% EtOAc in petrol) 0.24; ν_{max}/cm^{-1} 3313 (C-H), 2944 (C-H), 2867 (C-H), 2123 (C≡C), 1465 (C=C); δ_H (400 MHz, $CDCl_3$) 1.12 (18H, d, *J* 7.4, SiCH(CH_3)$_2$), 1.38 (3H, sept, *J* 7.4, SiC*H*(CH_3)$_2$), 1.97 (1H, t, *J* 2.6, C(4)*H*), 2.53 (2H, td, *J* 7.4, 2.6, C(2)H_2), 2.93 (2H, td, *J* 7.4, 0.8, C(1)H_2), 6.90 (1H, t, *J* 0.8, C(4')*H*); δ_C (100 MHz, $CDCl_3$) 11.1 (Si*C*H(CH_3)$_2$), 17.7 (*C*(2)), 18.5 (SiCH(CH_3)$_2$), 25.2 (*C*(1)), 69.4 (*C*(4)), 82.7 (*C*(3)), 123.1 (*C*(4')), 152.7 (*C*(5')), 167.9 (*C*(2')); *m/z* (ESI^+) 278.2 (MH^+, 100%); HRMS (ESI^+) found 278.19331, $C_{16}H_{28}NOSi$ (MH^+) requires 278.19347.

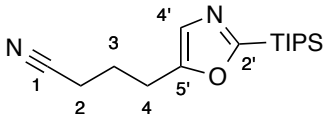
3-(2-(Triisopropylsilyl)oxazol-5-yl)propyl 4-methylbenzenesulfonate (158)



To a solution of alcohol **153** (93 mg, 0.33 mmol) in anhydrous DCM (1.6 mL) were added triethylamine (75 µL, 0.54 mmol), DMAP (5 mg, 0.04 mmol) and *p*-toluenesulfonyl chloride (90 mg, 0.47 mmol). The reaction mixture was stirred at room temperature for 3 h, diluted with water and extracted with Et_2O. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude

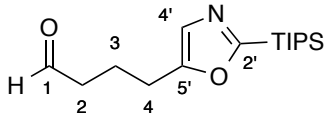
product was purified by *flash* column chromatography (DCM to 5% EtOAc in DCM) to give tosylate **158** (117 mg, 0.267 mmol, 82%) as a colourless oil. R_f (5% EtOAc in DCM) 0.57; ν_{max}/cm^{-1} 2943 (C-H), 2865 (C-H), 1176 (S=O); δ_H (500 MHz, $CDCl_3$) 1.10 (18H, d, *J* 7.5, SiCH(CH_3)$_2$), 1.36 (3H, sept, *J* 7.5, SiC*H*(CH_3)$_2$), 2.00 (2H, tt, *J* 7.5, 6.0, C(2)H_2), 2.45 (3H, s, C(8)H_3), 2.76 (2H, t, *J* 7.5, C(3)H_2), 4.05 (2H, t, *J* 6.0, C(1)H_2), 6.75 (1H, s, C(4')*H*), 7.34 (2H, d, *J* 8.2, C(6)*H*), 7.78 (2H, d, *J* 8.2, C(5)*H*); δ_C (125 MHz, $CDCl_3$) 11.1 (Si*C*H(CH_3)$_2$), 18.5 (SiCH(*C*H_3)$_2$), 21.8 (*C*(3)), 21.8 (*C*(8)), 27.3 (*C*(2)), 69.3 (*C*(1)), 122.9 (*C*(4')), 128.0 (*C*(5)), 130.0 (*C*(6)), 133.1 (*C*(7)), 145.0 (*C*(4)), 152.8 (*C*(5')), 168.0 (*C*(2')); *m/z* (ESI$^+$) 438.2 (MH$^+$, 100%), 460.3 (MNa$^+$, 4%); HRMS (ESI$^+$) found 438.21277, $C_{22}H_{36}NO_4SSi$ (MH$^+$) requires 438.21288.

4-(2-(Triisopropylsilyl)oxazol-5-yl)butanenitrile (159)



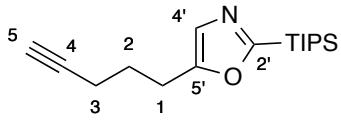
Triphenylphosphine (1.67 g, 6.37 mmol) and acetone cyanohydrin (580 µL, 6.35 mmol) were added to a solution of alcohol **153** (723 mg, 2.55 mmol) in anhydrous THF (10 mL). After stirring at room temperature for 10 min, the solution was cooled to 0 °C and diethyl azodicarboxylate (1.0 mL, 6.4 mmol) in anhydrous THF (3 mL) was added dropwise. The mixture was stirred at 0 °C for 1 h and for further 18 h at room temperature. The solvent was then evaporated and the crude product was purified by *flash* column chromatography (10% to 20% EtOAc in petrol) to give nitrile **159** (737 mg, 2.52 mmol, 99%) as a light yellow oil. R_f (20% EtOAc in petrol) 0.30; ν_{max}/cm^{-1} 2944 (C-H), 2867 (C-H), 2247 (C≡N), 1464 (C=C); δ_H (500 MHz, $CDCl_3$) 1.12 (18H, d, *J* 7.5, SiCH(CH_3)$_2$), 1.39 (3H, sept, *J* 7.5, SiC*H*(CH_3)$_2$), 2.03 (2H, quint, *J* 7.5, C(3)H_2), 2.38 (2H, t, *J* 7.5, C(2)H_2), 2.88 (2H, td, *J* 7.5, 1.0, C(4)H_2), 6.89 (1H, t, *J* 1.0, C(4')*H*); δ_C (125 MHz, $CDCl_3$) 11.1 (Si*C*H(CH_3)$_2$), 16.6 (*C*(2)), 18.5 (SiCH(*C*H_3)$_2$), 24.0 (*C*(3)), 24.5 (*C*(4)), 119.0 (*C*(1)), 123.4 (*C*(4')), 152.1 (*C*(5')), 168.4 (*C*(2')); *m/z* (ESI$^+$) 293.2 (MH$^+$, 100%), 315.2 (MNa$^+$, 14%); HRMS (ESI$^+$) found 293.20420, $C_{16}H_{29}N_2OSi$ (MH$^+$) requires 293.20437.

4-(2-(Triisopropylsilyl)oxazol-5-yl)butanal (160)



DIBAL-H (1 M in toluene, 3.7 mL, 3.7 mmol) was added dropwise to a solution of nitrile **159** (363 mg, 1.24 mmol) in anhydrous DCM (12 mL) at -78 °C. After stirring at this temperature for 3 h, EtOAc was added followed by a saturated aqueous solution of Rochelle's salt. After stirring at room temperature for 1 h, the aqueous layer was extracted with EtOAc, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (20% EtOAc in petrol) to give aldehyde **160** (286 mg, 0.969 mmol, 78%) as a colourless oil. R_f (20% EtOAc in petrol) 0.34; ν_{max}/cm^{-1} 2944 (C-H), 2867 (C-H), 1726 (C=O), 1464 (C=C); δ_H (500 MHz, $CDCl_3$) 1.13 (18H, d, *J* 7.5, SiCH(CH_3)$_2$), 1.40 (3H, sept, *J* 7.5, SiC*H*(CH$_3$)$_2$), 2.00 (2H, quint, *J* 7.2, C(3)H_2), 2.50 (2H, td, *J* 7.2, 1.2, C(2)H_2), 2.76 (2H, td, *J* 7.2, 0.7, C(4)H_2), 6.86 (1H, s, C(4')*H*), 9.78 (1H, t, *J* 1.2, C(1)*H*); δ_C (100 MHz, $CDCl_3$) 11.1 (Si*C*H(CH$_3$)$_2$), 18.5 (SiCH(*C*H$_3$)$_2$), 20.4 (*C*(3)), 24.9 (*C*(4)), 43.0 (*C*(2)), 122.8 (*C*(4')), 153.6 (*C*(5')), 168.0 (*C*(2')), 201.6 (*C*(1)); *m/z* (ESI^+) 296.2 (MH^+, 13%); HRMS (ESI^+) found 296.20417, $C_{16}H_{30}NO_2Si$ (MH^+) requires 296.20403.

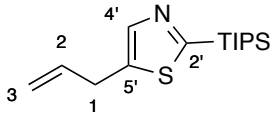
5-(Pent-4-yn-1-yl)-2-(triisopropylsilyl)oxazole (132)



Potassium carbonate (324 mg, 1.68 mmol) and a solution of the Bestmann reagent **156** (278 mg, 1.44 mmol) in MeOH (2 mL) were added to a solution of aldehyde **160** (342 mg, 1.16 mmol) in MeOH (10 mL). After stirring at room temperature for 16 h, the suspension was cooled to 0 °C, quenched with saturated aqueous $NaHCO_3$ and extracted with Et_2O. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (5% EtOAc in petrol) to give alkyne **132** (291 mg, 0.999 mmol, 86%) as a colourless oil. R_f (5% EtOAc in petrol) 0.27; ν_{max}/cm^{-1} 3312 (C-H), 2944 (C-H), 2867 (C-H), 2120 (C≡C), 1464 (C=C); δ_H (400 MHz, $CDCl_3$) 1.12 (18H, d, *J* 7.4, SiCH(CH_3)$_2$), 1.38 (3H, sept, *J* 7.4, SiC*H*(CH$_3$)$_2$), 1.88 (2H, quint, *J* 7.2, C(2)H_2), 1.98 (1H, t, *J* 2.6, C(5)*H*), 2.24 (2H, td, *J* 7.2, 2.6, C(3)H_2), 2.82 (2H, td, *J* 7.2, 0.8, C(1)H_2), 6.84 (1H, t, *J* 0.8, C(4')*H*); δ_C (100 MHz, $CDCl_3$) 11.1 (Si*C*H(CH$_3$)$_2$),

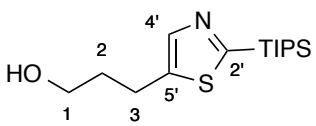
18.0 (*C*(3)), 18.5 (SiCH(*C*H$_3$)$_2$), 24.6 (*C*(2)), 26.7 (*C*(1)), 69.2 (*C*(5)), 83.5 (*C*(4)), 122.8 (*C*(4')), 153.8 (*C*(5')), 167.7 (*C*(2')); *m/z* (ESI$^+$) 292.2 (MH$^+$, 100%); HRMS (ESI$^+$) found 292.20919, $C_{17}H_{30}NOSi$ (MH$^+$) requires 292.20912.

5-Allyl-2-(triisopropylsilyl)thiazole (409)



CuCN (782 mg, 8.73 mmol) and LiCl (752 mg, 17.7 mmol) were stirred at 130 °C under vacuum for 5 h. After cooling to room temperature, anhydrous THF (15 mL) was added and stirring was continued for further 30 min. To a solution of protected thiazole **162** (1.92 g, 7.94 mmol) in anhydrous THF (20 mL) was added *n*-BuLi (1.6 M in hexanes, 5.5 mL, 8.8 mmol) dropwise at -78 °C. After stirring at this temperature for 30 min, the CuCN·2LiCl solution was added by cannula (with another 5 mL of anhydrous THF) and the mixture was stirred at -78 °C for 2 h. Allyl bromide (1.0 mL, 12 mmol) was then added and the solution was stirred at -78 °C for 30 min and at room temperature for 2 h. The reaction mixture was quenched with saturated aqueous NH_4Cl, extracted with Et_2O and dried over anhydrous Na_2SO_4. The crude product was purified by *flash* column chromatography (5% EtOAc in petrol) to give alkene **409** (1.92 g, 6.83 mmol, 86%) as an orange oil. R_f (5% EtOAc in petrol) 0.37; ν_{max}/cm^{-1} 2943 (C-H), 2866 (C-H), 1462 (C=C); δ_H (400 MHz, $CDCl_3$) 1.13 (18H, d, *J* 7.5, SiCH(C*H*$_3$)$_2$), 1.42 (3H, sept, *J* 7.5, SiC*H*(CH$_3$)$_2$), 3.63 (2H, d, *J* 6.6, C(1)*H*$_2$), 5.09-5.19 (2H, m, C(3)*H*$_2$), 5.98 (1H, ddt, *J* 16.8, 10.0, 6.6, C(2)*H*), 7.80 (1H, s, C(4')*H*); δ_C (100 MHz, $CDCl_3$) 11.8 (Si*C*H(CH$_3$)$_2$), 18.7 (SiCH(*C*H$_3$)$_2$), 31.2 (*C*(1)), 116.8 (*C*(3)), 136.1 (*C*(2)), 139.5 (*C*(5')), 143.3 (*C*(4')), 169.4 (*C*(2')); *m/z* (ESI$^+$) 282.1 (MH$^+$, 100%); HRMS (ESI$^+$) found 282.17045, $C_{15}H_{28}NSSi$ (MH$^+$) requires 282.17062.

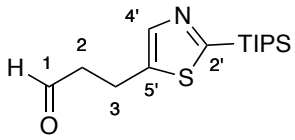
3-(2-(Triisopropylsilyl)thiazol-5-yl)propan-1-ol (163)



9-BBN (0.5 M in THF, 41 mL, 20 mmol) was added dropwise to a solution of alkene **409** (1.92 g, 6.83 mmol) in anhydrous THF (27 mL) at 0 °C and the solution was stirred at room temperature for 13 h. The mixture was then cooled to 0 °C and water (3.8 mL), aqueous NaOH (2 M, 34 mL, 68 mmol) and H_2O_2 (35% w/w, 5.8 mL,

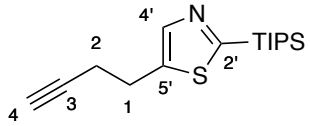
68 mmol) were added dropwise. The biphasic mixture was stirred at room temperature for 6 h, diluted with brine and extracted with EtOAc, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by *flash* column chromatography (20% EtOAc in DCM) to give alcohol **163** (1.69 g, 5.65 mmol, 83%) as a yellow oil. R_f (20% EtOAc in DCM) 0.22; ν_{max}/cm^{-1} 3348 (O-H), 2942 (C-H), 2866 (C-H), 1462 (C=C); δ_H (400 MHz, $CDCl_3$) 1.12 (18H, d, *J* 7.5, SiCH(C*H*$_3$)$_2$), 1.41 (3H, sept, *J* 7.5, SiC*H*(CH$_3$)$_2$), 1.90 (1H, br s, O*H*), 1.95 (2H, tt, *J* 7.6, 6.4, C(2)*H*$_2$), 2.99 (2H, t, *J* 7.6, C(3)*H*$_2$), 3.69 (2H, t, *J* 6.4, C(1)*H*$_2$), 7.80 (1H, s, C(4')*H*); δ_C (100 MHz, $CDCl_3$) 11.7 (Si*C*H(CH$_3$)$_2$), 18.6 (SiCH(*C*H$_3$)$_2$), 23.2 (*C*(3)), 34.5 (*C*(2)), 61.7 (*C*(1)), 141.3 (*C*(5')), 143.1 (*C*(4')), 168.9 (*C*(2')); *m/z* (ESI$^+$) 300.2 (MH$^+$, 100%); HRMS (ESI$^+$) found 300.18109, $C_{15}H_{30}NOSSi$ (MH$^+$) requires 300.18119.

3-(2-(Triisopropylsilyl)thiazol-5-yl)propanal (410)



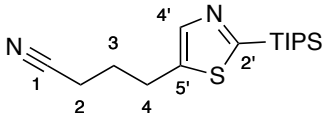
To a solution of alcohol **163** (358 mg, 1.19 mmol) and $NaHCO_3$ (111 mg, 1.32 mmol) in anhydrous DCM (6 mL) was added Dess-Martin periodinane (766 mg, 1.81 mmol) portionwise at 0 °C. After stirring at room temperature for 5 h, a 1:1 mixture of saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$ was added and the organic layer was extracted with Et_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (2% EtOAc in DCM) to give aldehyde **410** (242 mg, 0.813 mmol, 68%) as a yellow oil. R_f (2% EtOAc in DCM) 0.24; ν_{max}/cm^{-1} 2943 (C-H), 2865 (C-H), 2722 (C-H), 1727 (C=O), 1462 (C=C); δ_H (400 MHz, $CDCl_3$) 1.12 (18H, d, *J* 7.5, SiCH(C*H*$_3$)$_2$), 1.41 (3H, sept, *J* 7.5, SiC*H*(CH$_3$)$_2$), 2.87 (2H, td, *J* 7.4, 1.0, C(2)*H*$_2$), 3.23 (2H, td, *J* 7.4, 0.8, C(3)*H*$_2$), 7.82 (1H, t, *J* 0.8, C(4')*H*), 9.83 (1H, t, *J* 1.0, C(1)*H*); δ_C (100 MHz, $CDCl_3$) 11.8 (Si*C*H(CH$_3$)$_2$), 18.6 (SiCH(*C*H$_3$)$_2$), 19.5 (*C*(3)), 45.3 (*C*(2)), 139.7 (*C*(5')), 143.4 (*C*(4')), 169.6 (*C*(2')), 200.4 (*C*(1)); *m/z* (ESI$^+$) 298.1 (MH$^+$, 4%); HRMS (ESI$^+$) found 298.16538, $C_{15}H_{28}NOSSi$ (MH$^+$) requires 298.16554.

5-(But-3-yn-1-yl)-2-(triisopropylsilyl)thiazole (131)



Potassium carbonate (192 mg, 1.39 mmol) and a solution of the Bestmann reagent **156** (160 mg, 0.835 mmol) in MeOH (2.0 mL) were added to a solution of aldehyde **410** (204 mg, 0.685 mmol) in MeOH (4.9 mL). After stirring at room temperature for 15 h, the suspension was cooled to 0 °C, quenched with saturated aqueous $NaHCO_3$ and extracted with Et_2O. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (5% EtOAc in petrol) to give alkyne **131** (164 mg, 0.557 mmol, 81%) as a colourless oil. R_f (5% EtOAc in petrol) 0.26; ν_{max}/cm^{-1} 3311 (C-H), 2943 (C-H), 2866 (C-H), 2122 (C≡C), 1463 (C=C); δ_H (400 MHz, $CDCl_3$) 1.12 (18H, d, *J* 7.6, SiCH(CH_3)$_2$), 1.42 (3H, sept, *J* 7.6, SiC*H*(CH$_3$)$_2$), 2.02 (1H, t, *J* 2.6, C(4)*H*), 2.54 (2H, td, *J* 7.4, 2.6, C(2)H_2), 3.12 (2H, t, *J* 7.4, C(1)H_2), 7.87 (1H, s, C(4')*H*); δ_C (100 MHz, $CDCl_3$) 11.8 (Si*C*H(CH$_3$)$_2$), 18.6 (SiCH(*C*H$_3$)$_2$), 21.2 (*C*(2)), 26.3 (*C*(1)), 69.9 (*C*(4)), 82.9 (*C*(3)), 139.4 (*C*(5')), 143.5 (*C*(4')), 169.4 (*C*(2')); *m/z* (ESI$^+$) 294.2 (MH$^+$, 100%); HRMS (ESI$^+$) found 294.17069, $C_{16}H_{28}NSSi$ (MH$^+$) requires 294.17062.

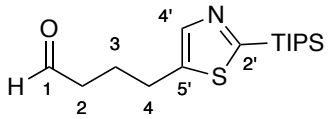
4-(2-(Triisopropylsilyl)thiazol-5-yl)butanenitrile (411)



Triphenylphosphine (2.29 g, 8.74 mmol) and acetone cyanohydrin (800 μL, 8.76 mmol) were added to a solution of alcohol **163** (1.05 g, 3.50 mmol) in anhydrous THF (12 mL). After stirring at room temperature for 5 min, the solution was cooled to 0 °C and diethyl azodicarboxylate (1.4 mL, 8.9 mmol) in anhydrous THF (5 mL) was added dropwise. The mixture was stirred for 1 h at 0 °C and for further 18 h at room temperature. The solvent was then evaporated and the crude product was purified by *flash* column chromatography (DCM) to give nitrile **411** (1.00 g, 3.25 mmol, 93%) as a light yellow oil. R_f (DCM) 0.35; ν_{max}/cm^{-1} 2943 (C-H), 2866 (C-H), 2247 (C≡N), 1462 (C=C); δ_H (500 MHz, $CDCl_3$) 1.13 (18H, d, *J* 7.5, SiCH(CH_3)$_2$), 1.42 (3H, sept, *J* 7.5, SiC*H*(CH$_3$)$_2$), 2.05 (2H, quint, *J* 7.2, C(3)H_2), 2.39 (2H, t, *J* 7.2, C(2)H_2), 3.07 (2H, td, *J* 7.2, 0.7, C(4)H_2), 7.83 (1H, t, *J* 0.7, C(4')*H*); δ_C (125 MHz, $CDCl_3$) 11.7 (Si*C*H(CH$_3$)$_2$), 16.6 (*C*(2)), 18.6 (SiCH(*C*H$_3$)$_2$), 25.7 (*C*(4)), 27.3 (*C*(3)), 119.1 (*C*(1)), 138.8 (*C*(5')),

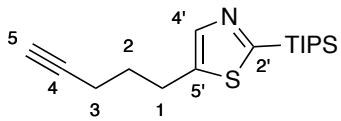
143.7 (*C*(4')), 170.0 (*C*(2')); *m/z* (ESI+) 309.2 (MH+, 100%), 331.1 (MNa+, 3%); HRMS (ESI+) found 309.18152, $C_{16}H_{29}N_2SSi$ (MH^+) requires 309.18152.

4-(2-(Triisopropylsilyl)thiazol-5-yl)butanal (412)



DIBAL-H (1 M in cyclohexanes, 9.1 mL, 9.1 mmol) was added dropwise to a solution of nitrile **411** (939 mg, 3.04 mmol) in anhydrous DCM (30 mL) at -78 °C. After stirring at this temperature for 3 h, EtOAc was added followed by a saturated aqueous solution of Rochelle's salt. After stirring at room temperature for 1 h, the aqueous layer was extracted with Et_2O, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (15% EtOAc in petrol) to give aldehyde **412** (566 mg, 1.82 mmol, 60%) as a yellow oil. R_f (15% EtOAc in petrol) 0.29; ν_{max}/cm^{-1} 2943 (C-H), 2865 (C-H), 2718 (C-H), 1725 (C=O), 1462 (C=C); δ_H (500 MHz, $CDCl_3$) 1.13 (18H, d, *J* 7.5, SiCH(CH_3)$_2$), 1.42 (3H, sept, *J* 7.5, SiC*H*(CH_3)$_2$), 2.02 (2H, quint, *J* 7.2, C(3)H_2), 2.52 (2H, td, *J* 7.2, 1.2, C(2)H_2), 2.94 (2H, t, *J* 7.2, C(4)H_2), 7.80 (1H, s, C(4')*H*), 9.78 (1H, t, *J* 1.2, C(1)*H*); δ_C (125 MHz, $CDCl_3$) 11.8 (Si*C*H(CH_3)$_2$), 18.6 (SiCH(CH_3)$_2$), 24.1 (*C*(3)), 26.2 (*C*(4)), 43.1 (*C*(2)), 140.6 (*C*(5')), 143.3 (*C*(4')), 169.3 (*C*(2')), 201.7 (*C*(1)); *m/z* (ESI+) 312.1 (MH^+, 7%); HRMS (ESI+) found 312.18133, $C_{16}H_{30}NOSSi$ (MH^+) requires 312.18119.

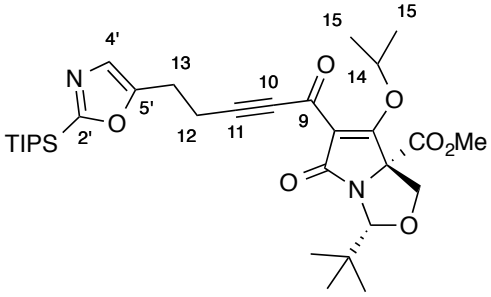
5-(Pent-4-yn-1-yl)-2-(triisopropylsilyl)thiazole (133)



Potassium carbonate (505 mg, 3.65 mmol) and a solution of the Bestmann reagent **156** (421 mg, 2.19 mmol) in MeOH (6 mL) were added to a solution of aldehyde **412** (566 mg, 1.82 mmol) in MeOH (12 mL). After stirring at room temperature for 15 h, the suspension was cooled to 0 °C, quenched with saturated aqueous $NaHCO_3$ and extracted with Et_2O. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (5% EtOAc in petrol) to give alkyne **133** (322 mg, 1.05 mmol, 58%) as a colourless oil. R_f (5% EtOAc in petrol) 0.24; ν_{max}/cm^{-1} 3311 (C-H), 2943 (C-H), 2866 (C-H), 2120

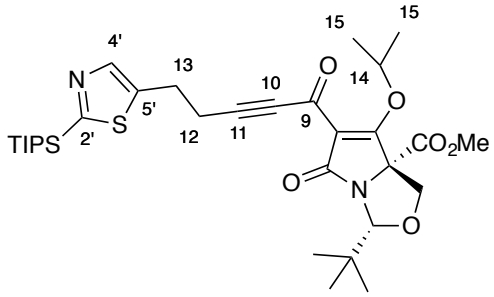
(C≡C), 1462 (C=C); δ_H (400 MHz, $CDCl_3$) 1.12 (18H, d, *J* 7.2, SiCH(CH_3)$_2$), 1.42 (3H, sept, *J* 7.2, SiCH(CH$_3$)$_2$), 1.90 (2H, quint, *J* 7.5, C(2)H_2), 1.98-2.01 (1H, m, C(5)H), 2.25 (2H, td, *J* 7.5, 2.4, C(3)H_2), 2.82 (2H, t, *J* 7.5, C(1)H_2), 7.81 (1H, s, C(4')H); δ_C (100 MHz, $CDCl_3$) 11.8 (SiCH(CH$_3$)$_2$), 18.0 (C(3)), 18.6 (SiCH(CH$_3$)$_2$), 25.7 (C(1)), 30.3 (C(2)), 69.3 (C(5)), 83.5 (C(4)), 140.7 (C(5')), 143.3 (C(4')), 169.0 (C(2')); *m/z* (ESI$^+$) 308.2 (MH$^+$, 100%); HRMS (ESI$^+$) found 308.18636, $C_{17}H_{30}NSSi$ (MH$^+$) requires 308.18627.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxo-7-(5-(2-(triisopropylsilyl)oxazol-5-yl)pent-2-ynoyl)bicyclo[3.3.0]-oct-6-ene (134)



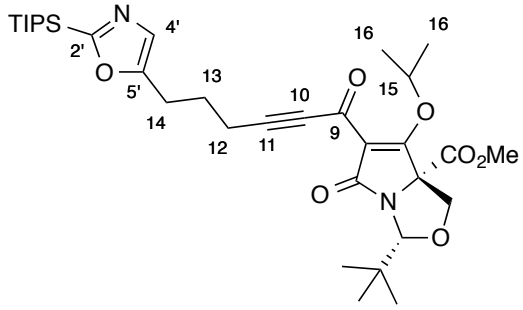
General procedure F (from **116III**, with **130**); yield 58% (93 mg); light yellow oil. R_f (20% EtOAc in petrol) 0.26; $[\alpha]_D^{20}$ +65.1 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2947 (C-H), 2868 (C-H), 2215 (C≡C), 1755 (C=O), 1721 (C=O), 1635 (C=O), 1587 (C=C); δ_H (500 MHz, $CDCl_3$) 0.89 (9H, s, C(CH_3)$_3$), 1.09 (18H, d, *J* 7.5, SiCH(CH_3)$_2$), 1.13 (3H, d, *J* 6.1, C(15$_A$)H_3), 1.29 (3H, d, *J* 6.1, C(15$_B$)H_3), 1.40 (3H, sept, *J* 7.5, SiCH(CH$_3$)$_2$), 2.79 (2H, t, *J* 7.5, C(12)H_2), 3.04 (2H, t, *J* 7.5, C(13)H_2), 3.43 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.77 (3H, s, CO$_2$CH_3), 4.74 (1H, s, C(2)H), 4.80 (1H, d, *J* 8.5, C(4)H$_A$$H_B$), 5.18 (1H, sept, *J* 6.1, C(14)H), 6.90 (1H, s, C(4')H); δ_C (125 MHz, $CDCl_3$) 11.0 (SiCH(CH$_3$)$_2$), 18.5 (SiCH(CH$_3$)$_2$), 18.7 (C(12)), 21.1 (C(15$_A$)), 22.3 (C(15$_B$)), 24.4 (C(13)), 24.7 (C(CH$_3$)$_3$), 35.3 (C(CH$_3$)$_3$), 53.3 (CO$_2$$CH_3$), 69.5 ($C$(4)), 74.4 ($C$(5)), 80.0 ($C$(14)), 82.1 ($C$(10)), 96.1 ($C$(11)), 97.8 ($C$(2)), 109.1 ($C$(7)), 123.2 ($C$(4')), 152.1 ($C$(5')), 168.0 ($CO_2CH_3$), 168.1 ($C$(2')), 172.1 ($C$(9)), 174.4 ($C$(6)), 174.5 ($C$(8)); *m/z* (ESI$^+$) 601.4 (MH$^+$, 100%), 623.3 (MNa$^+$, 14%); HRMS (ESI$^+$) found 601.33007, $C_{32}H_{49}N_2O_7Si$ (MH$^+$) requires 601.33035.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxo-7-(5-(2-(triisopropylsilyl)thiazol-5-yl)pent-2-ynoyl)bicyclo[3.3.0]-oct-6-ene (135)



General procedure F (from **116III**, with **131**); yield 58% (67 mg); light yellow oil. R_f (25% EtOAc in petrol) 0.42; $[\alpha]_D^{20}$ +70.2 (*c* 0.6, $CHCl_3$); ν_{max}/cm^{-1} 2945 (C-H), 2867 (C-H), 2215 (C≡C), 1755 (C=O), 1722 (C=O), 1636 (C=O), 1585 (C=C); δ_H (400 MHz, $CDCl_3$) 0.89 (9H, s, C(CH_3)$_3$), 1.10 (18H, d, *J* 7.6, SiCH(CH_3)$_2$), 1.13 (3H, d, *J* 6.1, C(15$_A$)H_3), 1.29 (3H, d, *J* 6.1, C(15$_B$)H_3), 1.39 (3H, sept, *J* 7.6, SiC*H*(CH$_3$)$_2$), 2.80 (2H, t, *J* 7.6, C(12)H_2), 3.22 (2H, t, *J* 7.6, C(13)H_2), 3.44 (1H, d, *J* 8.8, C(4)H_AH$_B$), 3.77 (3H, s, CO$_2$CH_3), 4.74 (1H, s, C(2)*H*), 4.80 (1H, d, *J* 8.8, C(4)H$_A$$H_B$), 5.19 (1H, sept, *J* 6.1, C(14)*H*), 7.86 (1H, s, C(4')*H*); δ_C (100 MHz, $CDCl_3$) 11.7 (Si*C*H(CH$_3$)$_2$), 18.6 (SiCH(*C*H$_3$)$_2$), 21.1 (*C*(15$_A$)), 22.1 (*C*(12)), 22.2 (*C*(15$_B$)), 24.7 (C(*C*H$_3$)$_3$), 25.4 (*C*(13)), 35.3 (*C*(CH$_3$)$_3$), 53.4 (CO$_2$*C*H$_3$), 69.5 (*C*(4)), 74.4 (*C*(5)), 79.9 (*C*(14)), 82.4 (*C*(10)), 96.2 (*C*(11)), 97.8 (*C*(2)), 109.1 (*C*(7)), 138.8 (*C*(5')), 143.6 (*C*(4')), 168.0 (*C*O$_2$CH$_3$), 169.6 (*C*(2')), 172.1 (*C*(9)), 174.3, 174.5 (*C*(6), *C*(8)); *m/z* (ESI$^+$) 617.3 (MH$^+$, 100%); HRMS (ESI$^+$) found 617.30709, $C_{32}H_{49}N_2O_6SSi$ (MH$^+$) requires 617.30751.

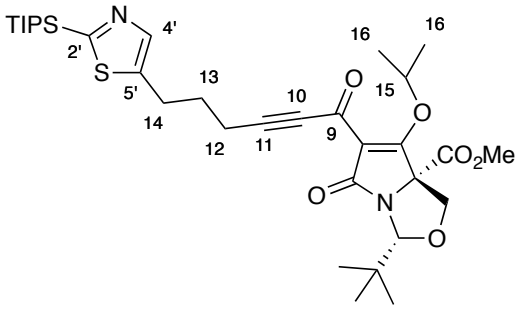
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxo-7-(6-(2-(triisopropylsilyl)oxazol-5-yl)hex-2-ynoyl)bicyclo[3.3.0]-oct-6-ene (136)



General procedure F (from **116III**, with **132**); yield 54% (81 mg); light yellow oil. R_f (20% EtOAc in petrol) 0.24; $[\alpha]_D^{20}$ +58.2 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2947 (C-H), 2868 (C-H), 2215 (C≡C), 1755 (C=O), 1721 (C=O), 1635 (C=O), 1587 (C=C); δ_H (500 MHz, $CDCl_3$) 0.91 (9H, s, C(CH_3)$_3$), 1.12 (18H, d, *J* 7.5, SiCH(CH_3)$_2$), 1.17 (3H, d, *J* 6.0, C(16$_A$)H_3), 1.33 (3H, d, *J* 6.0, C(16$_B$)H_3), 1.38 (3H, sept, *J* 7.5, SiC*H*(CH$_3$)$_2$), 1.99 (2H, quint, *J* 7.2, C(13)H_2), 2.51 (2H, t, *J* 7.2, C(12)H_2), 2.90 (2H, td, *J* 7.2, 2.7, C(14)H_2), 3.46 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.79 (3H, s, CO$_2$CH_3),

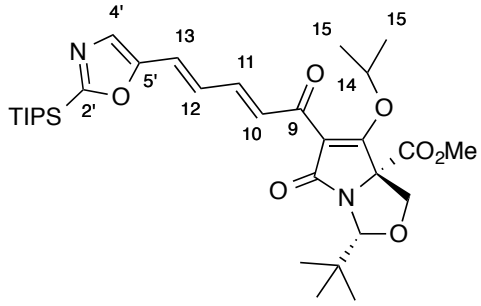
4.76 (1H, s, C(2)*H*), 4.82 (1H, d, *J* 8.5, C(4)H_AH_B), 5.24 (1H, sept, *J* 6.0, C(15)*H*), 6.87 (1H, s, C(4')*H*); δ_C (125 MHz, $CDCl_3$) 11.1 (Si*C*H(CH_3)$_2$), 18.5 (SiCH(*C*H_3)$_2$), 19.0 (*C*(12)), 21.1 (*C*(16$_A$)), 22.3 (*C*(16$_B$)), 24.6 (*C*(14)), 24.8 (C(*C*H_3)$_3$), 25.9 (*C*(13)), 35.3 (*C*(CH_3)$_3$), 53.4 (CO_2*C*H_3), 69.6 (*C*(4)), 74.5 (*C*(5)), 80.0 (*C*(15)), 82.3 (*C*(10)), 97.4 (*C*(11)), 97.8 (*C*(2)), 109.2 (*C*(7)), 123.0 (*C*(4')), 153.5 (*C*(5')), 167.8 (*C*(2')), 168.1 (*C*$O_2$$CH_3$), 172.4 (*C*(9)), 174.3 (*C*(6)), 174.6 (*C*(8)); *m/z* (ESI$^+$) 615.4 (MH^+, 100%), 637.3 (MNa^+, 14%); HRMS (ESI$^+$) found 615.34560, $C_{33}H_{51}N_2O_7Si$ (MH^+) requires 615.34600.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxo-7-(6-(2-(triisopropylsilyl)thiazol-5-yl)hex-2-ynoyl)bicyclo[3.3.0]-oct-6-ene (137)



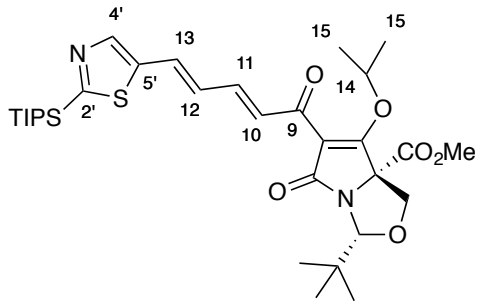
General procedure F (from **116III**, with **133**); yield 46% (93 mg); light yellow oil. R_f (20% EtOAc in petrol) 0.30; $[\alpha]_D^{20}$ +58.3 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2945 (C-H), 2867 (C-H), 2213 (C≡C), 1755 (C=O), 1721 (C=O), 1635 (C=O), 1583 (C=C); δ_H (400 MHz, $CDCl_3$) 0.88 (9H, s, C(C*H*$_3$)$_3$), 1.10 (18H, d, *J* 7.6, SiCH(C*H*$_3$)$_2$), 1.15 (3H, d, *J* 6.1, C(16$_A$)*H*$_3$), 1.30 (3H, d, *J* 6.1, C(16$_B$)*H*$_3$), 1.39 (3H, sept, *J* 7.6, SiC*H*(CH_3)$_2$), 1.99 (2H, quint, *J* 6.9, C(13)*H*$_2$), 2.50 (2H, t, *J* 6.9, C(12)*H*$_2$), 3.05-3.10 (2H, m, C(14)*H*$_2$), 3.44 (1H, d, *J* 8.8, C(4)*H*$_A$H$_B$), 3.76 (3H, s, CO_2C*H*$_3$), 4.74 (1H, s, C(2)*H*), 4.80 (1H, d, *J* 8.8, C(4)H$_A$*H*$_B$), 5.22 (1H, sept, *J* 6.1, C(15)*H*), 7.81 (1H, s, C(4')*H*); δ_C (100 MHz, $CDCl_3$) 11.7 (Si*C*H(CH_3)$_2$), 18.6 (SiCH(*C*H_3)$_2$), 18.9 (*C*(12)), 21.1 (*C*(16$_A$)), 22.3 (*C*(16$_B$)), 24.7 (C(*C*H_3)$_3$), 25.7 (*C*(13)), 29.5 (*C*(14)), 35.2 (*C*(CH_3)$_3$), 53.3 (CO_2*C*H_3), 69.5 (*C*(4)), 74.4 (*C*(5)), 79.9 (*C*(15)), 82.4 (*C*(10)), 97.4 (*C*(11)), 97.7 (*C*(2)), 109.2 (*C*(7)), 140.3 (*C*(5')), 143.4 (*C*(4')), 167.8 (*C*(2')), 168.0 (*C*$O_2$$CH_3$), 169.0 (*C*(9)), 174.2 (*C*(6)), 174.5 (*C*(8)); *m/z* (ESI$^+$) 631.4 (MH^+, 100%); HRMS (ESI$^+$) found 631.32288, $C_{33}H_{51}N_2O_6SSi$ (MH^+) requires 631.32316.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxo-7-((2*E*,4*E*)-5-(2-(triisopropylsilyl)oxazol-5-yl)penta-2,4-dienoyl)bicyclo[3.3.0]-oct-6-ene (164)



General procedure G (from **134**); yield 94% (53 mg); yellow oil. R_f (20% EtOAc in petrol) 0.27; $[\alpha]_D^{20}$ +118.2 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2956 (C-H), 2868 (C-H), 1755 (C=O), 1713 (C=O), 1657 (C=O), 1588 (C=C); δ_H (500 MHz, $CDCl_3$) 0.92 (9H, s, C(CH_3)$_3$), 1.10 (3H, d, *J* 6.0, C(15$_A$)H_3), 1.15 (18H, d, *J* 7.5, SiCH(CH_3)$_2$), 1.32 (3H, d, *J* 6.0, C(15$_B$)H_3), 1.42 (3H, sept, *J* 7.5, SiC*H*(CH$_3$)$_2$), 3.49 (1H, d, *J* 9.0, C(4)H_AH$_B$), 3.78 (3H, s, CO$_2$CH_3), 4.75 (1H, s, C(2)*H*), 4.83 (1H, d, *J* 9.0, C(4)H$_A$$H_B$), 5.24 (1H, sept, *J* 6.0, C(14)*H*), 6.80 (1H, d, *J* 15.0, C(13)*H*), 6.92 (1H, dd, *J* 15.0, 11.2, C(12)*H*), 7.13 (1H, d, *J* 15.0, C(10)*H*), 7.21 (1H, s, C(4')*H*), 7.41 (1H, dd, *J* 15.0, 11.2, C(11)*H*); δ_C (125 MHz, $CDCl_3$) 11.1 (Si*C*H(CH$_3$)$_2$), 18.5 (SiCH(*C*H$_3$)$_2$), 20.9 (*C*(15$_A$)), 22.3 (*C*(15$_B$)), 24.8 (C(*C*H$_3$)$_3$), 35.3 (*C*(CH$_3$)$_3$), 53.3 (CO$_2$*C*H$_3$), 69.6 (*C*(4)), 74.9 (*C*(5)), 79.1 (*C*(14)), 97.7 (*C*(2)), 109.6 (*C*(7)), 125.3 (*C*(13)), 127.9 (*C*(12)), 128.3 (*C*(4')), 129.7 (*C*(10)), 142.6 (*C*(11)), 151.2 (*C*(5')), 168.2 (*C*O$_2$CH$_3$), 170.4 (*C*(2')), 174.0 (*C*(6)), 176.5 (*C*(8)), 186.1 (*C*(9)); *m/z* (ESI$^+$) 601.4 (MH$^+$, 100%), 623.2 (MNa$^+$, 21%); HRMS (ESI$^+$) found 601.33025, $C_{32}H_{49}N_2O_7Si$ (MH$^+$) requires 602.33035.

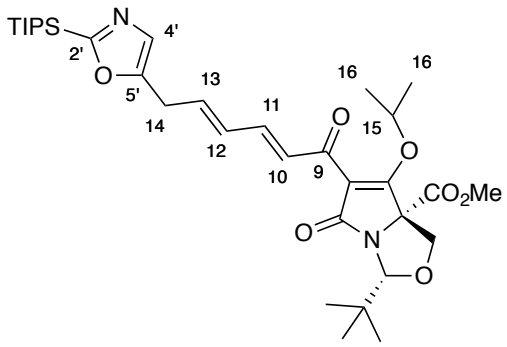
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxo-7-((2*E*,4*E*)-5-(2-(triisopropylsilyl)thiazol-5-yl)penta-2,4-dienoyl)bicyclo[3.3.0]-oct-6-ene (165)



General procedure G (from **135**); yield 85% (57 mg); orange oil. R_f (25% EtOAc in petrol) 0.50; $[\alpha]_D^{20}$ +130.9 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2946 (C-H), 2867 (C-H), 1754 (C=O), 1712 (C=O), 1656 (C=O), 1585 (C=C); δ_H (400 MHz, $CDCl_3$) 0.91 (9H, s, C(CH_3)$_3$), 1.11 (3H, d, *J* 6.1, C(15$_A$)H_3), 1.13 (18H, d, *J* 7.6, SiCH(CH_3)$_2$), 1.32 (3H, d, *J* 6.1, C(15$_B$)H_3), 1.44 (3H, sept, *J* 7.6, SiC*H*(CH$_3$)$_2$), 3.49 (1H, d, *J* 8.4, C(4)H_AH$_B$), 3.79 (3H, s, CO$_2$CH_3), 4.75 (1H, s, C(2)*H*), 4.83 (1H, d,

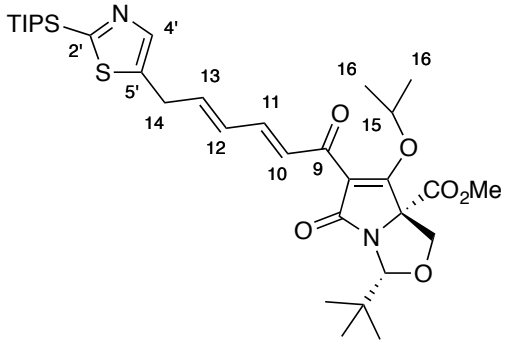
J 8.4, C(4)H_AH_B), 5.22 (1H, sept, *J* 6.1, C(14)*H*), 6.79 (1H, dd, *J* 15.2, 11.0, C(12)*H*), 7.05 (1H, d, *J* 15.2, C(10)*H*), 7.15 (1H, d, *J* 15.2, C(13)*H*), 7.40 (1H, dd, *J* 15.2, 11.0, C(11)*H*), 8.06 (1H, s, C(4')*H*); δ_C (125 MHz, $CDCl_3$) 11.7 (Si*C*H(CH_3)$_2$), 18.5 (SiCH(*C*H_3)$_2$), 20.9 (*C*(15_A)), 22.3 (*C*(15_B)), 24.8 (C(*C*H_3)$_3$), 35.3 (*C*(CH_3)$_3$), 53.3 (CO_2*C*H_3), 69.6 (*C*(4)), 74.8 (*C*(5)), 79.1 (*C*(14)), 97.7 (*C*(2)), 109.5 (*C*(7)), 130.5 (*C*(12)), 130.9 (*C*(13)), 129.3 (*C*(10)), 139.1 (*C*(5')), 142.8 (*C*(11)), 146.4 (*C*(4')), 168.2 (*C*O_2CH_3), 172.5 (*C*(2')), 173.9 (*C*(6)), 176.3 (*C*(8)), 186.3 (*C*(9)); *m/z* (ESI$^+$) 617.3 (MH^+, 100%); HRMS (ESI$^+$) found 617.30709, $C_{32}H_{49}N_2O_6SSi$ (MH^+) requires 617.30751.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxo-7-((2*E*,4*E*)-6-(2-(triisopropylsilyl)oxazol-5-yl)hexa-2,4-dienoyl)bicyclo[3.3.0]-oct-6-ene (166)



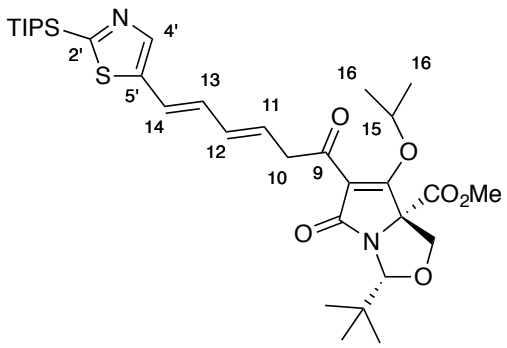
General procedure G (from **136**); yield 31% (59 mg); orange oil. R_f (15% EtOAc in petrol) 0.13; $[\alpha]_D^{20}$ +68.6 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2956 (C-H), 2868 (C-H), 1755 (C=O), 1713 (C=O), 1665 (C=O), 1629 (C=C), 1593 (C=C); δ_H (500 MHz, $CDCl_3$) 0.90 (9H, s, C(CH_3)$_3$), 1.09 (3H, d, *J* 6.0, C(16_A)H_3), 1.12 (18H, d, *J* 7.5, SiCH(CH_3)$_2$), 1.30 (3H, d, *J* 6.0, C(16_B)H_3), 1.38 (3H, sept, *J* 7.5, SiC*H*(CH_3)$_2$), 3.48 (1H, d, *J* 8.5, C(4)H_AH_B), 3.59 (2H, d, *J* 6.5, C(14)H_2), 3.78 (3H, s, CO_2CH_3), 4.73 (1H, s, C(2)*H*), 4.82 (1H, d, *J* 8.5, C(4)H_AH_B), 5.17 (1H, sept, *J* 6.0, C(15)*H*), 6.26 (1H, dt, *J* 15.0, 6.5, C(13)*H*), 6.36 (1H, dd, *J* 15.0, 10.5, C(12)*H*), 6.86 (1H, s, C(4')*H*), 6.88 (1H, d, *J* 15.2, C(10)*H*), 7.28 (1H, dd, *J* 15.2, 10.5, C(11)*H*); δ_C (125 MHz, $CDCl_3$) 11.1 (Si*C*H(CH_3)$_2$), 18.5 (SiCH(*C*H_3)$_2$), 20.9 (*C*(16_A)), 22.3 (*C*(16_B)), 24.8 (C(*C*H_3)$_3$), 29.5 (*C*(14)), 35.3 (*C*(CH_3)$_3$), 53.3 (CO_2*C*H_3), 69.6 (*C*(4)), 74.8 (*C*(5)), 79.0 (*C*(15)), 97.7 (*C*(2)), 109.5 (*C*(7)), 123.4 (*C*(4')), 128.6 (*C*(10)), 131.5 (*C*(12)), 139.1 (*C*(13)), 143.2 (*C*(11)), 151.2 (*C*(5')), 168.2 (*C*O_2CH_3), 168.4 (*C*(2')), 173.7 (*C*(6)), 176.3 (*C*(8)), 186.9 (*C*(9)); *m/z* (ESI$^+$) 615.4 (MH^+, 94%), 637.4 (MNa^+, 13%); HRMS (ESI$^+$) found 615.34581, $C_{33}H_{51}N_2O_7Si$ (MH^+) requires 615.34600.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxo-7-((2*E*,4*E*)-6-(2-(triisopropylsilyl)thiazol-5-yl)hexa-2,4-dienoyl)bicyclo[3.3.0]-oct-6-ene (167)



General procedure G (from **137**); yield 25% (26 mg); yellow oil. R_f (15% EtOAc in petrol) 0.19; $[\alpha]_D^{20}$ +74.7 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2959 (C-H), 2867 (C-H), 1755 (C=O), 1713 (C=O), 1665 (C=O), 1629 (C=C), 1592 (C=C); δ_H (500 MHz, $CDCl_3$) 0.91 (9H, s, C(CH_3)$_3$), 1.10 (3H, d, *J* 6.0, C(16$_A$)H_3), 1.13 (18H, d, *J* 7.5, SiCH(CH_3)$_2$), 1.31 (3H, d, *J* 6.0, C(16$_B$)H_3), 1.42 (3H, sept, *J* 7.5, SiC*H*(CH$_3$)$_2$), 3.48 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.77 (2H, d, *J* 6.5, C(14)H_2), 3.78 (3H, s, CO$_2$CH_3), 4.74 (1H, s, C(2)*H*), 4.82 (1H, d, *J* 8.5, C(4)H$_A$*H*$_B$), 5.18 (1H, sept, *J* 6.0, C(15)*H*), 6.29-6.40 (2H, m, C(12)*H* + C(13)*H*), 6.91 (1H, d, *J* 15.0, C(10)*H*), 7.29 (1H, dd, *J* 15.0, 10.2, C(11)*H*), 7.81 (1H, s, C(4')*H*); δ_C (125 MHz, $CDCl_3$) 11.8 (Si*C*H(CH$_3$)$_2$), 18.6 (SiCH(*C*H$_3$)$_2$), 20.9 (*C*(16$_A$)), 22.3 (*C*(16$_B$)), 24.8 (C(*C*H$_3$)$_3$), 30.5 (*C*(14)), 35.3 (*C*(CH$_3$)$_3$), 53.3 (CO$_2$*C*H$_3$), 69.6 (*C*(4)), 74.9 (*C*(5)), 79.0 (*C*(15)), 97.7 (*C*(2)), 109.5 (*C*(7)), 128.6 (*C*(10)), 130.8 (*C*(12)), 138.0 (*C*(5')), 142.0 (*C*(13)), 143.2 (*C*(11)), 143.6 (*C*(4')), 168.2 (*C*O$_2$CH$_3$), 170.1 (*C*(2')), 173.7 (*C*(6)), 176.3 (*C*(8)), 186.8 (*C*(9)); *m/z* (ESI$^+$) 631.3 (MH$^+$, 100%); HRMS (ESI$^+$) found 631.32277, $C_{33}H_{51}N_2O_6SSi$ (MH$^+$) requires 631.32316.

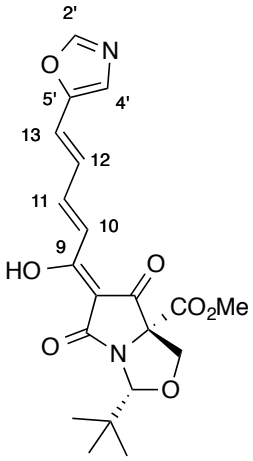
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxo-7-((3*E*,5*E*)-6-(2-(triisopropylsilyl)thiazol-5-yl)hexa-3,5-dienoyl)bicyclo[3.3.0]-oct-6-ene (169)



Side-product (29%, 30 mg) from the isomerisation of alkynone **137**, isolated together with other impurities; orange oil. R_f (15% EtOAc in petrol) 0.27; ν_{max}/cm^{-1} 2948 (C-H), 2868 (C-H), 1753 (C=O), 1711 (C=O), 1683 (C=O), 1588 (C=C); δ_H (500 MHz, $CDCl_3$) 0.91 (9H, s, C(CH_3)$_3$), 1.11 (3H, d, *J* 6.0, C(16$_A$)H_3), 1.13 (18H, d, *J* 7.5, SiCH(CH_3)$_2$), 1.30 (3H, d, *J* 6.0, C(16$_B$)H_3), 1.42 (3H, sept, *J* 7.5, SiC*H*(CH$_3$)$_2$), 3.45 (1H,

d, *J* 8.5, C(4)H_AH_B), 3.76-3.81 (1H, m, C(10)H_AH_B), 3.77 (3H, s, CO_2CH_3), 3.93 (1H, dd, *J* 18.7, 7.2, C(10)H_AH_B), 4.75 (1H, s, C(2)*H*), 4.82 (1H, d, *J* 8.5, C(4)H_AH_B), 5.20 (1H, sept, *J* 6.0, C(15)*H*), 5.96 (1H, dt, *J* 15.0, 7.2, C(11)*H*), 6.27 (1H, dd, *J* 15.0, 10.0, C(12)*H*), 6.59 (1H, dd, *J* 15.5, 10.0, C(13)*H*), 6.67 (1H, d, *J* 15.5, C(14)*H*), 7.90 (1H, s, C(4')*H*); δ_C (125 MHz, $CDCl_3$) 11.7 (Si*C*H$(CH_3)_2$), 18.6 (SiCH$(CH_3)_2$), 21.1 (*C*(16_A)), 22.3 (*C*(16_B)), 24.8 (C$(CH_3)_3$), 35.3 (*C*$(CH_3)_3$), 46.1 (*C*(10)), 53.4 (CO_2CH_3), 69.6 (*C*(4)), 74.8 (*C*(5)), 79.5 (*C*(15)), 97.7 (*C*(2)), 109.1 (*C*(7)), 121.2 (*C*(14)), 127.2 (*C*(11)), 132.4 (*C*(13)), 133.8 (*C*(12)), 140.0 (*C*(5')), 144.0 (*C*(4')), 168.0 (CO_2CH_3), 169.4 (*C*(2')), 174.2 (*C*(6)), 176.0 (*C*(8)), 195.5 (*C*(9)); *m/z* (ESI+) 631.3 (MH^+, 100%); HRMS (ESI+) found 631.32277, $C_{33}H_{51}N_2O_6SSi$ (MH^+) requires 631.32316.

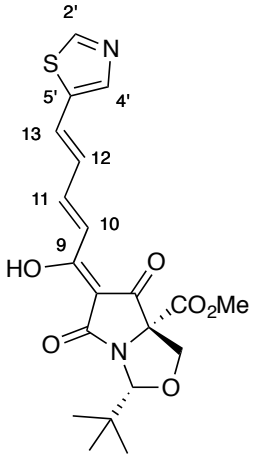
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*,2*E*,4*E*)-1-hydroxy-5-(oxazol-5-yl)penta-2,4-dien-1-ylidene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (138)



$NaHCO_3$ (690 mg, 8.21 mmol) was added to a biphasic solution of enol ether **164** (81 mg, 0.14 mmol) and Aliquat 336 (61 mg, 0.15 mmol) in 1:1 toluene/H_2O (2.7 mL). The mixture was heated at 80 °C for 6 h, quenched with saturated aqueous NH_4Cl, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure The residue was dissolved in THF (2.7 mL), and aqueous HCl (1 M, 1.1 mL, 1.1 mmol) was added. The mixture was stirred at room temperature for 1 h, diluted with brine, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (80% EtOAc in petrol to 5% MeOH in EtOAc), and the product thus obtained was dissolved in EtOAc, washed with 2 M aqueous HCl, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give deprotected tetramate **138** (33 mg, 0.083 mmol, 61%) as an orange solid; m.p. 98 °C; 3:1 mixture of tautomers. R_f (5% MeOH in EtOAc) 0.20; $[\alpha]_D^{20}$ +49.3 (*c* 0.4, $CHCl_3$); ν_{max}/cm^{-1} 2960 (C-H), 2917 (C-H), 1752 (C=O), 1706 (C=O), 1654 (C=O), 1632 (C=C), 1603 (C=C), 1571 (C=C); δ_H (500 MHz, $CDCl_3$) major tautomer: 0.94 (9H, s, C(CH_3)$_3$), 3.53 (1H, d, *J* 8.9, C(4)H_AH_B), 3.80 (3H, s, CO_2CH_3), 4.85 (1H, d, *J* 8.9, C(4)H_AH_B),

4.88 (1H, s, C(2)*H*), 6.86 (1H, d, *J* 15.5, C(13)*H*), 6.98 (1H, dd, *J* 15.5, 11.0, C(12)*H*), 7.25 (1H, s, C(4')*H*), 7.27 (1H, d, *J* 15.2, C(10)*H*), 7.66 (1H, dd, *J* 15.2, 11.0, C(11)*H*), 7.93 (1H, s, C(2')*H*); δ_C (125 MHz, $CDCl_3$) major tautomer: 24.8 ($C(CH_3)_3$), 35.4 ($C(CH_3)_3$), 53.5 (CO_2CH_3), 68.5 (*C*(4)), 78.5 (*C*(5)), 98.5 (*C*(2)), 100.4 (*C*(7)), 122.6 (*C*(10)), 126.3 (*C*(13)), 127.7 (*C*(2')), 128.1 (*C*(12)), 128.8 (*C*(4')), 145.5 (*C*(11)), 149.4 (*C*(5')), 167.8 (CO_2CH_3), 175.9 (*C*(9)), 180.5 (*C*(8)), 188.0 (*C*(6)); *m/z* (ESI$^-$) 401.1 (M-H$^-$, 100%); HRMS (ESI$^-$) found 401.13568, $C_{20}H_{21}N_2O_7$ (M-H$^-$) requires 401.13542.

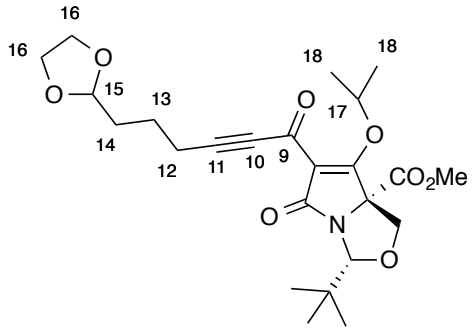
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*,2*E*,4*E*)-1-hydroxy-5-(thiazol-5-yl)penta-2,4-dien-1-ylidene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (139)



$NaHCO_3$ (423 mg, 5.04 mmol) was added to a biphasic solution of enol ether **165** (51 mg, 0.083 mmol) and Aliquat 336 (36 mg, 0.089 mmol) in 1:1 toluene/H_2O (1.7 mL). The mixture was heated at 80 °C for 6 h, quenched with saturated aqueous NH_4Cl, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in THF (1.7 mL), and aqueous HCl (1 M, 660 µL, 0.660 mmol) was added. The mixture was stirred at room temperature for 1 h, diluted with brine, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (80% EtOAc in petrol to 5% MeOH in EtOAc), and the product thus obtained was dissolved in EtOAc, washed with 2 M aqueous HCl, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give deprotected tetramate **139** (22 mg, 0.053 mmol, 65%) as an orange solid; m.p. 152 °C; 4:1 mixture of tautomers. R_f (5% MeOH in EtOAc) 0.26; $[\alpha]_D^{20}$ +69.7 (*c* 0.5, $CHCl_3$); ν_{max}/cm^{-1} 2959 (C-H), 1752 (C=O), 1705 (C=O), 1654 (C=O), 1617 (C=C), 1601 (C=C), 1556 (C=C); δ_H (500 MHz, $CDCl_3$) major tautomer: 0.94 (9H, s, C(CH_3)$_3$), 3.52 (1H, d, *J* 9.0, C(4)H_AH_B), 3.79 (3H, s, CO_2CH_3), 4.84 (1H, d, *J* 9.0, C(4)H_AH_B), 4.87 (1H, s, C(2)*H*), 6.81 (1H, dd, *J* 15.0, 11.0, C(12)*H*), 7.22 (1H, d, *J* 15.0, C(10)*H*), 7.22 (1H, d, *J* 15.0, C(13)*H*), 7.67 (1H, dd, *J* 15.0, 11.0, C(11)*H*), 7.95 (1H, s, C(4')*H*), 8.79 (1H, s, C(2')*H*); δ_C (125 MHz,

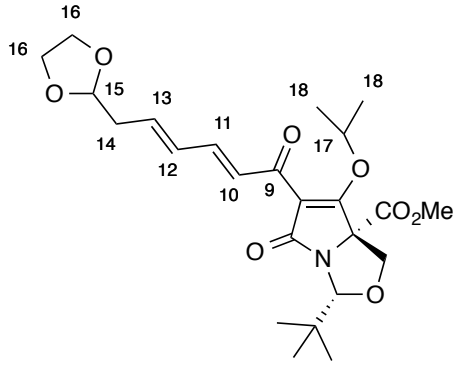
$CDCl_3$) major tautomer: 24.8 (C(CH$_3$)$_3$), 35.4 (C(CH$_3$)$_3$), 53.4 (CO$_2$$CH_3$), 68.4 ($C$(4)), 78.4 ($C$(5)), 98.4 ($C$(2)), 100.3 ($C$(7)), 121.9 ($C$(10)), 130.0 ($C$(12)), 132.7 ($C$(13)), 145.3 ($C$(4')), 145.7 ($C$(11)), 154.4 ($C$(2')), 136.7 ($C$(5')), 167.8 ($CO_2CH_3$), 176.0 ($C$(9)), 180.5 ($C$(8)), 187.9 ($C$(6)); *m/z* (ESI$^-$) 417.1 (M-H$^-$, 100%); HRMS (ESI$^-$) found 417.11263, $C_{20}H_{21}N_2O_6S$ (M-H$^-$) requires 417.11258.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-(6-(1,3-dioxolan-2-yl)hex-2-ynoyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (414)



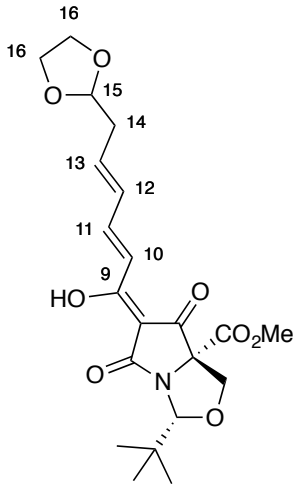
General procedure F (from **116III**, with **170**); yield 38% (137 mg); light yellow oil. R_f (30% EtOAc in petrol) 0.17; $[\alpha]_D^{20}$ +81.1 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2958 (C-H), 2211 (C≡C), 1753 (C=O), 1720 (C=O), 1633 (C=O), 1586 (C=C); δ_H (500 MHz, $CDCl_3$) 0.90 (9H, s, C(CH_3)$_3$), 1.16 (3H, d, *J* 6.0, C(18$_A$)H_3), 1.31 (3H, d, *J* 6.0, C(18$_B$)H_3), 1.74-1.83 (4H, m, C(13)H_2 + C(14)H_2), 2.52 (2H, t, *J* 7.0, C(12)H_2), 3.45 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.78 (3H, s, CO$_2$CH_3), 3.82-3.85 (2H, m, C(16)H_2), 3.94-3.97 (2H, m, C(16)H_2), 4.75 (1H, s, C(2)H), 4.81 (1H, d, *J* 8.5, C(4)H$_A$$H_B$), 4.88 (1H, t, *J* 4.5, C(15)H), 5.19 (1H, sept, *J* 6.0, C(17)H); δ_C (125 MHz, $CDCl_3$) 19.6 (C(12)), 21.1 (C(18$_A$)), 22.3 (C(13)), 22.3 (C(18$_B$)), 24.8 (C(CH$_3$)$_3$), 32.9 (C(14)), 35.3 (C(CH$_3$)$_3$), 53.3 (CO$_2$$CH_3$), 65.0 ($C$(16)), 69.5 ($C$(4)), 74.4 ($C$(5)), 79.8 ($C$(17)), 82.0 ($C$(10)), 97.8 ($C$(2)), 98.3 ($C$(11)), 104.1 ($C$(15)), 109.3 ($C$(7)), 168.1 ($CO_2CH_3$), 172.5 ($C$(9)), 174.0 ($C$(6)), 174.6 ($C$(8)); *m/z* (ESI$^+$) 464.2 (MH$^+$, 100%), 486.2 (MNa$^+$, 44%); HRMS (ESI$^+$) found 464.22780, $C_{24}H_{34}NO_8$ (MH$^+$) requires 464.22789.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-((2*E*,4*E*)-6-(1,3-dioxolan-2-yl)hexa-2,4-dienoyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (415)



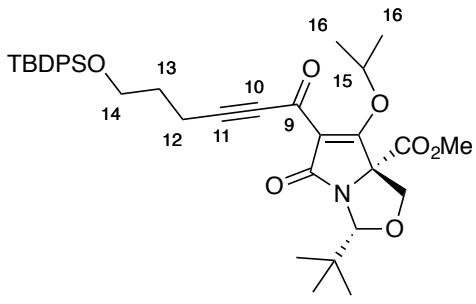
General procedure G (from **414**); yield 73% (100 mg); yellow oil. R_f (40% EtOAc in petrol) 0.48; $[\alpha]_D^{20}$ +12.4 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2959 (C-H), 1753 (C=O), 1711 (C=O), 1663 (C=O), 1627 (C=C), 1592 (C=C); δ_H (500 MHz, $CDCl_3$) 0.91 (9H, s, C(CH_3)$_3$), 1.09 (3H, d, *J* 6.1, C(18$_A$)H_3), 1.30 (3H, d, *J* 6.1, C(18$_B$)H_3), 2.55-2.58 (2H, m, C(14)H_2), 3.48 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.78 (3H, s, CO_2CH_3), 3.85-3.88 (2H, m, C(16)H_2), 3.96-3.99 (2H, m, C(16)H_2), 4.73 (1H, s, C(2)*H*), 4.82 (1H, d, *J* 8.5, C(4)H$_A$$H_B$), 4.95 (1H, t, *J* 4.5, C(15)*H*), 5.15 (1H, sept, *J* 6.1, C(17)*H*), 6.21 (1H, dt, *J* 15.5, 7.4, C(13)*H*), 6.39 (1H, dd, *J* 15.5, 11.0, C(12)*H*), 6.84 (1H, d, *J* 15.2, C(10)*H*), 7.26 (1H, dd, *J* 15.2, 11.0, C(11)*H*); δ_C (125 MHz, $CDCl_3$) 20.9 (*C*(18$_A$)), 22.3 (*C*(18$_B$)), 24.8 (C(*C*H$_3$)$_3$), 35.3 (*C*(CH$_3$)$_3$), 38.1 (*C*(14)), 53.3 (CO_2CH_3), 65.2 (*C*(16)), 69.6 (*C*(4)), 74.8 (*C*(5)), 78.8 (*C*(17)), 97.6 (*C*(2)), 103.2 (*C*(15)), 109.6 (*C*(7)), 128.1 (*C*(10)), 132.4 (*C*(12)), 139.1 (*C*(13)), 143.9 (*C*(11)), 168.3 (*C*O$_2$CH$_3$), 173.3 (*C*(6)), 176.3 (*C*(8)), 187.1 (*C*(9)); *m/z* (ESI$^+$) 464.2 (MH$^+$, 100%), 486.2 (MNa$^+$, 74%); HRMS (ESI$^+$) found 464.22791, $C_{24}H_{34}NO_8$ (MH$^+$) requires 464.22789.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*,2*E*,4*E*)-6-(1,3-dioxolan-2-yl)-1-hydroxyhexa-2,4-dien-1-ylidene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (171)



General procedure H (from **415**); yield 56% (51 mg); orange oil; 3:1 mixture of tautomers. R_f (EtOAc) 0.20; $[\alpha]_D^{20}$ +73.1 (*c* 0.6, $CHCl_3$); ν_{max}/cm^{-1} 2960 (C-H), 1752 (C=O), 1709 (C=O), 1654 (C=O), 1612 (C=C), 1554 (C=C); δ_H (400 MHz, $CDCl_3$) major tautomer: 0.93 (9H, s, C(CH_3)$_3$), 2.61-2.64 (2H, m, C(14)H_2), 3.51 (1H, d, *J* 8.9, C(4)H_AH$_B$), 3.78 (3H, s, CO_2CH_3), 3.86-3.92 (2H, m, C(16)H_2), 3.97-4.00 (2H, m, C(16)H_2), 4.83 (1H, d, *J* 8.9, C(4)H$_A$$H_B$), 4.86 (1H, s, C(2)*H*), 4.98 (1H, t, *J* 4.4, C(15)*H*), 6.35 (1H, dt, *J* 15.4, 7.2, C(13)*H*), 6.47 (1H, dd, *J* 15.4, 10.8, C(12)*H*), 7.07 (1H, d, *J* 15.3, C(10)*H*), 7.57 (1H, dd, *J* 15.3, 10.8, C(11)*H*); δ_C (125 MHz, $CDCl_3$) 24.8 (C(CH_3)$_3$), 35.4 (*C*(CH$_3$)$_3$), 38.2 (*C*(14)), 53.4 (CO_2CH_3), 65.3 (*C*(16)), 68.5 (*C*(4)), 78.5 (*C*(5)), 98.4 (*C*(2)), 99.9 (*C*(7)), 102.9 (*C*(15)), 120.1 (*C*(10)), 132.5 (*C*(12)), 142.2 (*C*(13)), 147.3 (*C*(11)), 167.8 (CO_2CH_3), 177.1 (*C*(9)), 180.8 (*C*(8)), 187.9 (*C*(6)); *m/z* (ESI$^-$) 420.1 (M-H$^-$, 100%); HRMS (ESI$^-$) found 420.16671, $C_{21}H_{26}NO_8$ (M-H$^-$) requires 420.16639.

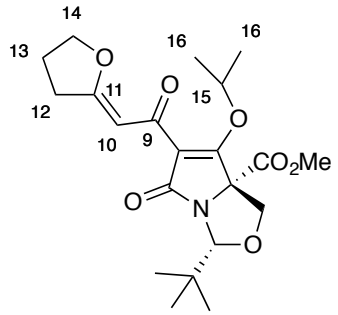
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-(6-((*tert*-butyldiphenylsilyl)oxy)hex-2-ynoyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (181)



General procedure F (from **116III**, with **180**); yield 46% (154 mg); light yellow oil. R_f (10% EtOAc in petrol) 0.24; $[\alpha]_D^{20}$ +68.9 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2957 (C-H), 2212 (C≡C), 1754 (C=O), 1720 (C=O), 1633 (C=O), 1587 (C=C); δ_H (500 MHz, $CDCl_3$) 0.91 (9H, s, C(CH_3)$_3$), 1.05 (9H, s, OSiC(CH_3)$_3$), 1.16 (3H, d, *J* 6.0, C(16$_A$)H_3), 1.32 (3H, d, *J* 6.0, C(16$_B$)H_3), 1.88 (2H, tt, *J* 7.3, 6.0, C(13)H_2), 2.63 (2H, t, *J* 7.3, C(12)H_2), 3.45 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.76 (2H, t, *J* 6.0, C(14)H_2), 3.79 (3H, s, CO_2CH_3), 4.76 (1H, s, C(2)*H*), 4.82 (1H, d, *J* 8.5, C(4)H$_A$$H_B$), 5.21 (1H, sept, *J* 6.0, C(15)*H*), 7.36-7.44 (6H, m, Ph), 7.64-7.67 (4H, m, Ph); δ_C (125 MHz, $CDCl_3$) 16.4 (*C*(12)), 19.4 (OSi*C*(CH$_3$)$_3$),

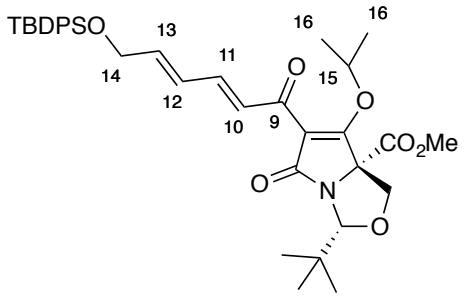
21.2 (*C*(16$_A$)), 22.3 (*C*(16$_B$)), 24.8 (C(*C*H$_3$)$_3$), 27.0 (OSiC(*C*H$_3$)$_3$), 30.9 (*C*(13)), 35.3 (*C*(CH$_3$)$_3$), 53.4 (CO$_2$*C*H$_3$), 62.5 (*C*(14)), 69.6 (*C*(4)), 74.4 (*C*(5)), 79.8 (*C*(15)), 81.8 (*C*(10)), 97.8 (*C*(2)), 98.7 (*C*(11)), 109.3 (*C*(7)), 127.8, 129.8, 133.8, 135.7 (Ph), 168.1 (*C*O$_2$CH$_3$), 172.5 (*C*(9)), 174.1 (*C*(6)), 174.6 (*C*(8)); *m/z* (ESI$^+$) 646.4 (MH$^+$, 100%), 668.3 (MNa$^+$, 28%); HRMS (ESI$^+$) found 646.32001, $C_{37}H_{48}NO_7Si$ (MH$^+$) requires 646.31946.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-(2-(dihydrofuran-2(3H)-ylidene)acetyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (182)



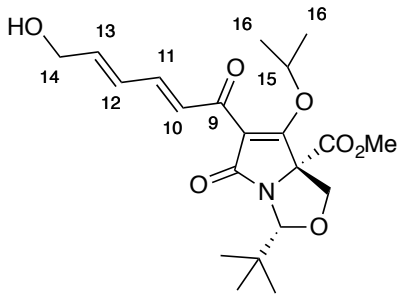
TBAF (1 M in THF, 130 μL, 0.130 mmol) was added to a solution of alkynone **181** (21 mg, 0.033 mmol) in anhydrous THF (660 μL). The solution was stirred for 1 h, diluted with brine and extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (30% EtOAc in petrol) to give dihydrofuran **182** (9 mg, 0.02 mmol, 67%) as a yellow oil, instead of the desired alcohol. R_f (30% EtOAc in petrol) 0.39; $[\alpha]_D^{20}$ +94.4 (*c* 0.8, $CHCl_3$); ν_{max}/cm^{-1} 2958 (C-H), 2918 (C-H), 1751 (C=O), 1709 (C=O), 1655 (C=O), 1574 (C=C); δ_H (500 MHz, $CDCl_3$) 0.90 (9H, s, C(C*H*$_3$)$_3$), 1.10 (3H, d, *J* 6.1, C(16$_A$)*H*$_3$), 1.28 (3H, d, *J* 6.1, C(16$_B$)*H*$_3$), 2.12 (2H, tt, *J* 8.0, 7.2, C(13)*H*$_2$), 3.13 (1H, dtd, *J* 19.0, 8.0, 1.5, C(12)*H*$_A$H$_B$), 3.19 (1H, dtd, *J* 19.0, 8.0, 1.5, C(12)H$_A$*H*$_B$), 3.47 (1H, d, *J* 8.5, C(4)*H*$_A$H$_B$),), 3.77 (3H, s, CO$_2$C*H*$_3$), 4.25-4.29 (2H, m, C(14)*H*$_2$), 4.72 (1H, s, C(2)*H*), 4.80 (1H, d, *J* 8.5, C(4)H$_A$*H*$_B$), 5.19 (1H, sept, *J* 6.1, C(15)*H*), 6.36 (1H, t, *J* 1.5, C(10)*H*); δ_C (125 MHz, $CDCl_3$) 21.0 (*C*(16$_A$)), 22.3 (*C*(16$_B$)), 23.7 (*C*(13)), 24.8 (C(*C*H$_3$)$_3$), 31.9 (*C*(12)), 35.3 (*C*(CH$_3$)$_3$), 53.2 (CO$_2$*C*H$_3$), 69.7 (*C*(4)), 72.2 (*C*(14)), 74.6 (*C*(5)), 77.8 (*C*(15)), 97.5 (*C*(2)), 100.0 (*C*(10)), 111.3 (*C*(7)), 168.6 (*C*O$_2$CH$_3$), 171.4 (*C*(6)), 176.7 (*C*(8)), 179.3 (*C*(11)), 186.1 (*C*(9)); HRMS (CI$^+$) found 408.2025, $C_{21}H_{30}NO_7$ (MH$^+$) requires 408.2017.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-((2*E*,4*E*)-6-((*tert*-butyldiphenylsilyl)oxy)hexa-2,4-dienoyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (183)



General procedure G (from **181**); yield 85% (130 mg); yellow solid; m.p. 62 °C. R_f (10% EtOAc in petrol) 0.33; $[\alpha]_D^{20}$ +95.1 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2958 (C-H), 1754 (C=O), 1711 (C=O), 1664 (C=O), 1632 (C=C), 1590 (C=C); δ_H (500 MHz, $CDCl_3$) 0.93 (9H, s, C(CH_3)$_3$), 1.08 (9H, s, OSiC(CH_3)$_3$), 1.11 (3H, d, *J* 6.0, C(16$_A$)H_3), 1.32 (3H, d, *J* 6.0, C(16$_B$)H_3), 3.50 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.79 (3H, s, CO_2CH_3), 4.31-4.33 (2H, m, C(14)H_2), 4.76 (1H, s, C(2)*H*), 4.84 (1H, d, *J* 8.5, C(4)H$_A$$H_B$), 5.17 (1H, sept, *J* 6.0, C(15)*H*), 6.27 (1H, dt, *J* 15.0, 4.2, C(13)*H*), 6.61-6.68 (1H, m, C(12)*H*), 6.92 (1H, d, *J* 15.2, C(10)*H*), 7.34 (1H, dd, *J* 15.2, 11.5, C(11)*H*), 7.36-7.46 (6H, m, Ph), 7.65-7.68 (4H, m, Ph); δ_C (125 MHz, $CDCl_3$) 19.4 (OSi*C*(CH$_3$)$_3$), 20.9 (*C*(16$_A$)), 22.3 (*C*(16$_B$)), 24.8 (C(*C*H$_3$)$_3$), 26.9 (OSiC(*C*H$_3$)$_3$), 35.3 (*C*(CH$_3$)$_3$), 53.3 (CO_2CH_3), 63.8 (*C*(14)), 69.6 (*C*(4)), 74.9 (*C*(5)), 78.8 (*C*(15)), 97.7 (*C*(2)), 109.6 (*C*(7)), 127.4 (*C*(12)), 127.9 (Ph), 128.2 (*C*(10)), 129.9, 133.3, 135.6 (Ph), 143.9 (*C*(11)), 144.0 (*C*(13)), 168.3 (CO_2CH_3), 173.3 (*C*(6)), 176.4 (*C*(8)), 187.1 (*C*(9)); HRMS (CI$^+$) found 646.3202, $C_{37}H_{48}NO_7Si$ (MH^+) requires 646.3195.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-((2*E*,4*E*)-6-hydroxyhexa-2,4-dienoyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (184)

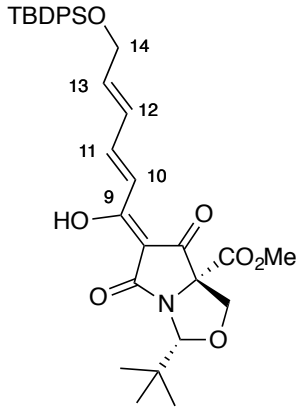


Method 1: To a solution of TBDPS ether **183** (17 mg, 0.026 mmol) in anhydrous THF (530 μL) was added TBAF (1 M in THF, 105 μL, 0.105 mmol). After stirring at room temperature for 1 h, the dark brown solution was diluted with brine, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by *flash* column chromatography (50% EtOAc in petrol) to give alcohol **184** (3 mg, 0.007 mmol, 25%) as a yellow oil.

Method 2: PPTS (170 mg, 0.676 mmol) was added to a solution of TBS ether **187** (165 mg, 0.316 mmol) in anhydrous MeOH (3.2 mL). The mixture was stirred at room temperature for 5 h, diluted with brine and extracted wih EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give alcohol **184** (130 mg) as a yellow oil, which was used in the following step without further purification.

R_f (60% EtOAc in petrol) 0.38; $[\alpha]_D^{20}$ +74.7 (*c* 0.4, $CHCl_3$); ν_{max}/cm^{-1} 2962 (C-H), 1752 (C=O), 1710 (C=O), 1663 (C=O), 1589 (C=C); δ_H (500 MHz, $CDCl_3$) 0.92 (9H, s, C(CH_3)$_3$), 1.11 (3H, d, *J* 6.0, C(16$_A$)H_3), 1.31 (3H, d, *J* 6.0, C(16$_B$)H_3), 3.49 (1H, d, *J* 8.6, C(4)H_AH$_B$), 3.79 (3H, s, CO_2CH_3), 4.31 (2H, d, *J* 4.5, C(14)H_2), 4.75 (1H, s, C(2)*H*), 4.83 (1H, d, *J* 8.6, C(4)H$_A$$H_B$), 5.18 (1H, sept, *J* 6.0, C(15)*H*), 6.32 (1H, dt, *J* 15.0, 4.5, C(13)*H*), 6.52 (1H, dd, *J* 15.0, 11.0, C(12)*H*), 6.94 (1H, d, *J* 15.0, C(10)*H*), 7.31 (1H, dd, *J* 15.0, 11.0, C(11)*H*); δ_C (125 MHz, $CDCl_3$) 21.0 (*C*(16$_A$)), 22.3 (*C*(16$_B$)), 24.8 (C(*C*H$_3$)$_3$), 35.3 (*C*(CH$_3$)$_3$), 53.3 (CO_2CH_3), 63.0 (*C*(14)), 69.6 (*C*(4)), 74.9 (*C*(5)), 79.0 (*C*(15)), 97.7 (*C*(2)), 109.5 (*C*(7)), 128.7 (*C*(12)), 129.0 (*C*(10)), 143.1 (*C*(11) + *C*(13)), 168.2 (CO_2CH_3), 173.7 (*C*(6)), 176.3 (*C*(8)), 186.9 (*C*(9)); *m/z* (ESI$^-$) 406.3 (M-H$^-$, 100%); HRMS (ESI$^+$) found 430.18358, $C_{21}H_{29}NNaO_7$ (MNa$^+$) requires 430.18362.

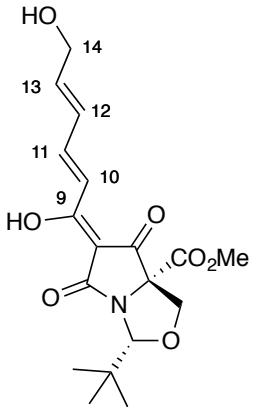
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*,2*E*,4*E*)-6-((*tert*-butyldiphenylsilyl)oxy))-1-hydroxyhexa-2,4-dien-1-ylidene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (185)



General procedure H (from **183**); yield 57% (23 mg); orange solid; m.p. 70 °C; 4.5:1 mixture of tautomers. R_f (80% EtOAc in petrol) 0.16; $[\alpha]_D^{20}$ +31.4 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2961 (C-H), 1754 (C=O), 1710 (C=O), 1654 (C=O), 1615 (C=C), 1557 (C=C); δ_H (400 MHz, $CDCl_3$) major tautomer: 0.94 (9H, s, C(CH_3)$_3$), 1.08 (9H, s, OSiC(CH_3)$_3$), 3.52 (1H, d, *J* 9.2, C(4)H_AH$_B$), 3.80 (3H, s, CO_2CH_3), 4.35-4.39 (2H, m, C(14)H_2), 4.84 (1H, d, *J* 9.2, C(4)H$_A$$H_B$), 4.88 (1H, s, C(2)*H*), 6.38 (1H, dt, *J* 15.2, 4.0, C(13)*H*), 6.68-6.76 (1H, m, C(12)*H*), 7.13 (1H, d, *J* 15.2, C(10)*H*), 7.36-7.47 (6H, m, Ph), 7.60-7.68 (5H, m, C(11)*H* + Ph);

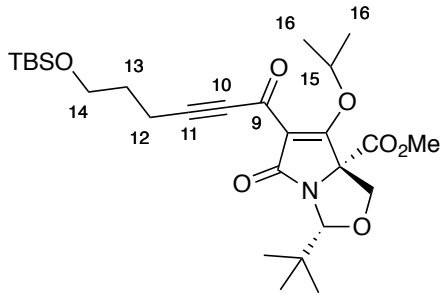
δ_C (125 MHz, $CDCl_3$) major tautomer: 19.4 (OSi*C*(CH_3)$_3$), 24.8 (C(*C*H_3)$_3$), 26.9 (OSiC(*C*H_3)$_3$), 35.4 (*C*(CH_3)$_3$), 53.4 (CO_2*C*H_3), 63.8 (*C*(14)), 68.5 (*C*(4)), 78.4 (*C*(5)), 98.4 (*C*(2)), 99.9 (*C*(7)), 120.3 (*C*(10)), 127.6 (*C*(12)), 128.0, 130.0, 133.1, 135.6 (Ph), 146.9 (*C*(13)), 147.2 (*C*(11)), 167.8 (*C*O_2CH_3), 177.2 (*C*(9)), 180.0 (*C*(8)), 188.1 (*C*(6)); *m/z* (ESI$^+$) 626.2 (MH^+, 17%); *m/z* (ESI$^-$) 602.3 ($M\text{-}H^-$, 100%); HRMS (ESI$^+$) found 604.27239, $C_{34}H_{42}NO_7Si$ (MH^+) requires 604.27251.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-[(*Z*,2*E*,4*E*)-1,6-dihydroxyhexa-2,4-dien-1-ylidene]-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (186)



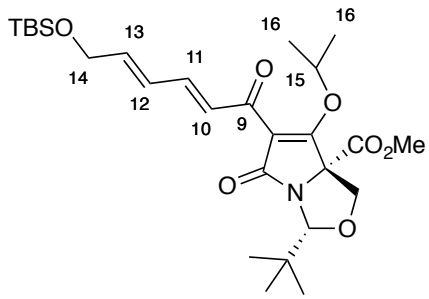
TBAF (1 M in THF, 125 μL, 0.125 mmol) was added to a solution of TBDPS ether **185** (38 mg, 0.062 mmol) in anhydrous THF (2.4 mL), and the reaction mixture was stirred at room temperature for 1 h. Brine was then added, and the aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (EtOAc to 15% MeOH in EtOAc), and the product collected was washed with 2 M aqueous HCl, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give alcohol **186** (6 mg, 0.02 mmol, 15%) as an orange solid; m.p. 100 °C; 2.8:1 mixture of tautomers. R_f (15% MeOH in EtOAc) 0.16; $[\alpha]_D^{20}$ +64.3 (*c* 0.5, $CHCl_3$); ν_{max}/cm^{-1} 2961 (C-H), 2918 (C-H), 1752 (C=O), 1712 (C=O), 1655 (C=O), 1613 (C=C), 1557 (C=C); δ_H (500 MHz, $CDCl_3$) major tautomer: 0.94 (9H, s, C(C*H*$_3$)$_3$), 3.51 (1H, d, *J* 8.9, C(4)*H*$_A$H$_B$), 3.79 (3H, s, CO_2CH_3), 4.37-4.38 (2H, m, C(14)*H*$_2$), 4.83 (1H, d, *J* 8.9, C(4)H$_A$*H*$_B$), 4.87 (1H, s, C(2)*H*), 6.43 (1H, dt, *J* 15.2, 4.7, C(13)*H*), 6.60 (1H, dd, *J* 15.2, 11.2, C(12)*H*), 7.13 (1H, d, *J* 15.3, C(10)*H*), 7.60 (1H, dd, *J* 15.3, 11.2, C(11)*H*); δ_C (125 MHz, $CDCl_3$) major tautomer: 24.8 (C(*C*H_3)$_3$), 35.4 (*C*(CH_3)$_3$), 53.4 (CO_2*C*H_3), 62.9 (*C*(14)), 68.5 (*C*(4)), 78.4 (*C*(5)), 98.4 (*C*(2)), 100.1 (*C*(7)), 121.0 (*C*(10)), 128.5 (*C*(12)), 145.9 (*C*(13)), 146.4 (*C*(11)), 167.8 (*C*O_2CH_3), 176.9 (*C*(9)), 180.7 (*C*(8)), 188.0 (*C*(6)); *m/z* (ESI$^-$) 364.1 ($M\text{-}H^-$, 100%); HRMS (ESI$^-$) found 364.13988, $C_{18}H_{22}NO_7$ ($M\text{-}H^-$) requires 364.14018.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-(6-((*tert*-butyldimethylsilyl)oxy)hex-2-ynoyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (181')



General procedure F (from **116III**, with **180'**); yield 81% (48 mg); white solid; m.p. 108 °C. R_f (10% EtOAc in petrol) 0.18; $[\alpha]_D^{20}$ +82.1 (c 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2960 (C-H), 2205 (C≡C), 1740 (C=O), 1725 (C=O), 1631 (C=O), 1565 (C=C); δ_H (400 MHz, $CDCl_3$) 0.03 (6H, s, Si(CH_3)$_2$), 0.87 (9H, s, OSiC(CH_3)$_3$), 0.89 (9H, s, C(CH_3)$_3$), 1.14 (3H, d, *J* 6.1, C(16$_A$)H_3), 1.30 (3H, d, *J* 6.1, C(16$_B$)H_3), 1.81 (2H, tt, *J* 7.0, 6.0, C(13)H_2), 2.54 (2H, t, *J* 7.0, C(12)H_2), 3.44 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.71 (2H, t, *J* 6.0, C(14)H_2), 3.77 (3H, s, CO_2CH_3), 4.80 (1H, s, C(2)*H*), 4.82 (1H, d, *J* 8.5, C(4)H$_A$$H_B$), 5.19 (1H, sept, *J* 6.1, C(15)*H*); δ_C (100 MHz, $CDCl_3$) -5.3 (OSi(*C*H$_3$)$_2$), 16.2 (*C*(12)), 18.4 (OSi*C*(CH$_3$)$_3$), 21.0 (*C*(16$_A$)), 22.3 (*C*(16$_B$)), 24.7 (C(*C*H$_3$)$_3$), 26.0 (OSiC(*C*H$_3$)$_3$), 30.8 (*C*(13)), 35.3 (*C*(CH$_3$)$_3$), 53.3 (CO_2*C*H$_3$), 61.4 (*C*(14)), 69.5 (*C*(4)), 74.4 (*C*(5)), 79.7 (*C*(15)), 81.8 (*C*(10)), 97.7 (*C*(2)), 98.7 (*C*(11)), 109.3 (*C*(7)), 168.1 (*C*O$_2$CH$_3$), 172.5 (*C*(9)), 173.9 (*C*(6)), 174.5 (*C*(8)); HRMS (CI$^+$) found 522.2891, $C_{27}H_{44}NO_7Si$ (MH$^+$) requires 522.2882.

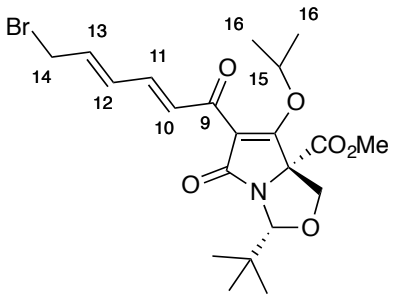
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-((2*E*,4*E*)-6-((*tert*-butyldimethylsilyl)oxy)hexa-2,4-dienoyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (187)



General procedure G (from **181'**); yield 67% (32 mg); orange solid; m.p. 96 °C. R_f (10% EtOAc in petrol) 0.26; $[\alpha]_D^{20}$ +86.7 (c 0.6, $CHCl_3$); ν_{max}/cm^{-1} 2957 (C-H), 1754 (C=O), 1712 (C=O), 1664 (C=O), 1632 (C=C), 1591 (C=C); δ_H (400 MHz, $CDCl_3$) 0.07 (6H, s, Si(CH_3)$_2$), 0.91 (9H, s, C(CH_3)$_3$)), 0.91 (9H, s, OSiC(CH_3)$_3$), 1.09 (3H, d, *J* 6.1, C(16$_A$)H_3), 1.32 (3H, d, *J* 6.1, C(16$_B$)H_3), 3.48 (1H, d, *J* 8.6, C(4)H_AH$_B$), 3.78 (3H, s, CO_2CH_3), 4.28-4.32 (2H, m, C(14)H_2), 4.74 (1H, s, C(2)*H*), 4.82 (1H, d, *J* 8.6, C(4)H$_A$$H_B$), 5.15 (1H, sept, *J* 6.1, C(15)*H*), 6.27 (1H, dt, *J* 15.2, 4.4, C(13)*H*), 6.51 (1H, ddt, *J* 15.2, 11.2, 1.6, C(12)*H*), 6.89 (1H, d, *J* 15.2, C(10)*H*), 7.32 (1H, dd, *J* 15.2, 11.2, C(11)*H*); δ_C (100 MHz, $CDCl_3$) -5.3

(OSi(CH$_3$)$_2$), 18.5 (OSiC(CH$_3$)$_3$), 20.9 (C(16$_A$)), 22.3 (C(16$_B$)), 24.8 (C(CH$_3$)$_3$), 26.1 (OSiC(CH$_3$)$_3$), 35.3 (C(CH$_3$)$_3$), 53.3 (CO$_2$$CH_3$), 63.1 ($C$(14)), 69.6 ($C$(4)), 74.8 ($C$(5)), 78.8 ($C$(15)), 97.6 ($C$(2)), 109.6 ($C$(7)), 127.5 ($C$(12)), 128.1 ($C$(10)), 143.7 ($C$(11)), 144.4 ($C$(13)), 168.2 ($CO_2CH_3$), 173.4 ($C$(6)), 176.3 ($C$(8)), 187.1 ($C$(9)); HRMS (CI$^+$) found 522.2888, $C_{27}H_{44}NO_7Si$ (MH$^+$) requires 522.2882.

(2*R*,5*R*)-1-Aza-7-((2*E*,4*E*)-6-bromohexa-2,4-dienoyl)-2-(*tert*-butyl)-6-isopropoxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (189)



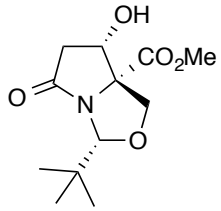
Crude alcohol **184** (130 mg) in anhydrous DCM (600 μL) was added to a solution of tetrabromomethane (276 mg, 0.833 mmol) and triphenylphosphine (299 mg, 1.14 mmol) in anhydrous DCM (1 mL) at 0 °C. After stirring at 0 °C for 10 min, the solution was concentrated under reduced pressure. The residue was purified by *flash* column chromatography (20% EtOAc in petrol) to give bromide **189** (104 mg, 0.222 mmol, 70% over two steps) as a yellow oil. R_f (20% EtOAc in petrol) 0.36; $[\alpha]_D^{20}$ +67.4 (*c* 0.6, CHCl$_3$); ν_{max}/cm^{-1} 2962 (C-H), 1753 (C=O), 1709 (C=O), 1662 (C=O), 1622 (C=C), 1590 (C=C); δ_H (500 MHz, CDCl$_3$) 0.92 (9H, s, C(CH_3)$_3$), 1.10 (3H, d, *J* 6.0, C(16$_A$)H_3), 1.32 (3H, d, *J* 6.0, C(16$_B$)H_3), 3.48 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.79 (3H, s, CO$_2$CH_3), 4.04 (2H, d, *J* 8.0, C(14)H_2), 4.74 (1H, s, C(2)H), 4.83 (1H, d, *J* 8.5, C(4)H$_A$$H_B$), 5.20 (1H, sept, *J* 6.0, C(15)H), 6.32 (1H, dt, *J* 15.0, 8.0, C(13)H), 6.48 (1H, dd, *J* 15.0, 11.0, C(12)H), 7.01 (1H, d, *J* 15.2, C(10)H), 7.26 (1H, dd, *J* 15.2, 11.0, C(11)H); δ_C (125 MHz, CDCl$_3$) 20.9 (C(16$_A$)), 22.3 (C(16$_B$)), 24.8 (C(CH$_3$)$_3$), 31.5 (C(14)), 35.3 (C(CH$_3$)$_3$), 53.4 (CO$_2$$CH_3$), 69.6 ($C$(4)), 74.9 ($C$(5)), 79.2 ($C$(15)), 97.7 ($C$(2)), 109.3 ($C$(7)), 130.3 ($C$(10)), 132.8 ($C$(12)), 138.3 ($C$(13)), 141.6 ($C$(11)), 168.1 ($CO_2CH_3$), 174.1 ($C$(6)), 176.3 ($C$(8)), 186.4 ($C$(9)); *m/z* (ESI$^+$) 492.2 (MNa$^+$, 19%); HRMS (ESI$^+$) found 492.09909, $C_{21}H_{28}BrNNaO_6$ (MNa$^+$) requires 492.10032.

6.3 Experimental for Chapter 3

General procedure I: Reduction of tetramic acids[182]

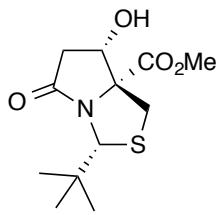
$NaBH_4$ (2.2 eq) was added portionwise at 0 °C to a solution of tetramic acid (1.0 eq) and acetic acid (9.0 eq) in anhydrous DCM (*c* 0.2). The reaction mixture was stirred at 0 °C for 15 min and then at room temperature for 2 h (unless otherwise specified). The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography to give the desired alcohol.

(2*R*,5*R*,6*S*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-octane (197a)[182]



General procedure I (from **44a**); yield quant. (129 mg); white solid; m.p. 146-148 °C (lit.[182] m.p. 129-130.5 °C); R_f (EtOAc) 0.49; $[\alpha]_D^{20}$ +73.8 (*c* 1.1, $CHCl_3$); ν_{max}/cm^{-1} 3392 (O-H), 2959 (C-H), 1743 (C=O), 1691 (C=O); δ_H (500 MHz, $CDCl_3$) 0.86 (9H, s, C(CH_3)$_3$), 2.81 (1H, dd, *J* 15.5, 8.0, C(7)H_AH_B), 3.15 (1H, dd, *J* 15.5, 10.5, C(7)H_AH_B), 3.56 (1H, d, *J* 8.5, C(4)H_AH_B), 3.82 (3H, s, CO_2CH_3), 4.56 (1H, dd, *J* 10.5, 8.0, C(6)*H*), 4.86 (1H, s, C(2)*H*), 4.88 (1H, d, *J* 8.5, C(4)H_AH_B); δ_C (100 MHz, $CDCl_3$) 24.9 (C(CH_3)$_3$), 36.2 (C(CH_3)$_3$), 43.2 (C(7)), 52.9 (CO_2CH_3), 73.7, 73.8 (C(4), C(6)), 77.6 (C(5)), 96.4 (C(2)), 170.9 (CO_2CH_3), 175.0 (C(8)); *m/z* (ESI$^+$) 258.1 (MH^+, 100%), 280.1 (MNa^+, 21%); HRMS (ESI$^+$) found 280.1169, $C_{12}H_{19}NNaO_5$ (MNa^+) requires 280.1155.

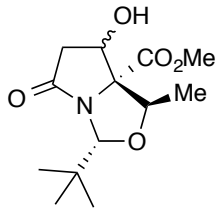
(2*R*,5*R*,6*S*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-8-oxo-3-thiabicyclo[3.3.0]-octane (197b)



General procedure I (from **44b**); yield quant. (101 mg); white solid; m.p. 75-77 °C. R_f (50% EtOAc in DCM) 0.38; $[\alpha]_D^{20}$ +18.6 (*c* 1.0, DCM); ν_{max}/cm^{-1} 3380 (O-H), 2956 (C-H), 1744 (C=O), 1686 (C=O); δ_H (500 MHz, $CDCl_3$) 0.92 (9H, s, C(CH_3)$_3$), 2.63 (1H, d, *J* 4.2, O*H*), 2.74 (1H, dd, *J* 15.8, 8.0, C(7)H_AH_B), 2.98 (1H, dd, *J* 15.8, 10.5, C(7)H_AH_B), 3.03 (1H, d, *J* 11.5, C(4)H_AH_B), 3.84 (3H, s, CO_2CH_3), 3.90 (1H, d, *J* 11.5,

C(4)H_AH_B), 4.43 (1H, ddd, *J* 10.5, 8.0, 4.2, C(6)*H*), 5.04 (1H, s, C(2)*H*); δ_C (125 MHz, $CDCl_3$) 26.6 (C(CH_3)$_3$), 38.0, 38.7 (*C*(4), *C*(CH_3)$_3$), 40.9 (*C*(7)), 53.1 (CO_2CH_3), 72.8, *C*(2)), 76.6 (*C*(6)), 82.0 (*C*(5)), 171.2 (CO_2CH_3), 173.8 (*C*(8)); *m/z* (ESI$^+$) 274.1 (MH^+, 52%), 296.1 (MNa^+, 100%); HRMS (ESI$^+$) found 296.0936, $C_{12}H_{19}NNaO_4S$ (MNa^+) requires 296.0927.

(2*R*,4*R*,5*R*,6*R*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-4-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane ((*R*)-197'c) and (2*R*,4*R*,5*R*,6*S*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-4-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane ((*S*)-197c)

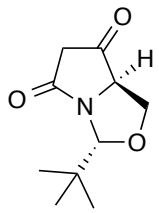


General procedure I (from **44c**, for 2 d); yield 51% (105 mg); white solid; 5.3:1 mixture of diastereomers. R_f (50% EtOAc in DCM) 0.29; ν_{max}/cm^{-1} 3399 (O-H), 2959 (C-H), 1743 (C=O), 1689 (C=O); δ_H (400 MHz, $CDCl_3$) major diastereomer (**(*S*)-197c**): 0.87 (9H, s, C(CH_3)$_3$), 1.24 (3H, d, *J* 6.8, C(4)CH_3), 2.80 (1H, dd, *J* 15.6, 8.2, C(7)H_AH_B), 2.86 (1H, br s, O*H*), 3.11 (1H, dd, *J* 15.6, 10.4, C(7)H_AH_B), 3.81 (3H, s, CO_2CH_3), 4.57-4.64 (1H, m, C(6)*H*), 4.84 (1H, s, C(2)*H*), 5.01 (1H, q, *J* 6.8, C(4)*H*); δ_C (100 MHz, $CDCl_3$) major diastereomer (**(*S*)-197c**): 16.1 (C(4)*C*H_3), 25.2 (C(*C*H_3)$_3$), 36.4 (*C*(CH_3)$_3$), 43.0 (*C*(7)), 52.8 (CO_2CH_3), 69.7, *C*(6)), 75.5 (*C*(4)), 79.3 (*C*(5)), 94.1 (*C*(2)), 171.4 (CO_2CH_3), 174.9 (*C*(8)); *m/z* (ESI$^+$) 272.1 (MH^+, 45%), 294.1 (MNa^+, 100%); HRMS (ESI$^+$) found 294.1315, $C_{13}H_{21}NNaO_5$ (MNa^+) requires 294.1312.

General procedure J: Decarboxylation of tetramic acids[161]

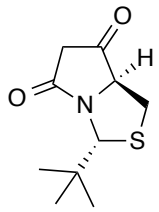
Tetramic acid (1.0 eq) and NaOH (2.0 eq) in a 4:1:1 mixture of THF/MeOH/H_2O were heated at reflux for 8 h and then cooled to room temperature. The mixture was acidified with 2 M aqueous HCl, extracted with EtOAc, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in MeCN and heated at reflux for 5 h, then cooled to room temperature. The mixture was acidified with 2 M aqueous HCl, extracted with EtOAc, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography and the product thus obtained was dissolved in EtOAc, washed with 2 M aqueous HCl, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give the decarboxylated tetramic acid.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (207a)[136]



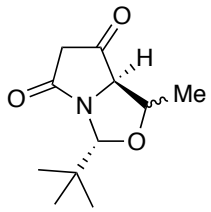
General procedure J (from **44a**); yield 66% (78 mg); white solid; m.p. 147-149 °C (lit.[136] m.p. 148-152 °C). R_f (EtOAc) 0.09; $[\alpha]_D^{20}$ +130.0 (*c* 0.8, DCM); ν_{max}/cm^{-1} 2964 (C-H), 1653 (C=O); δ_H (400 MHz, $CDCl_3$) 0.95 (9H, s, C(CH_3)$_3$), 3.14 (1H, dd, *J* 21.8, 1.2, C(7)H_AH_B), 3.44 (1H, d, *J* 21.8, C(7)H$_A$$H_B$), 3.55 (1H, dd, *J* 9.2, 8.2, C(4)H_AH_B), 4.25 (1H, dd, *J* 8.2, 7.3, C(4)H$_A$$H_B$), 4.33-4.37 (1H, m, C(5)*H*), 5.16 (1H, s, C(2)*H*); δ_C (100 MHz, $CDCl_3$) 24.8 (C(*C*H$_3$)$_3$), 36.4 (*C*(CH$_3$)$_3$), 44.7 (*C*(7)), 66.6 (*C*(4)), 68.8 (*C*(5)), 96.4 (*C*(2)), 172.7 (*C*(8)), 202.8 (*C*(6)); *m/z* (ESI$^+$) 198.1 (MH$^+$, 53%), 220.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 198.11237, $C_{10}H_{16}NO_3$ (MH$^+$) requires 198.11247.

(2*R*,5*S*)-1-Aza-2-(*tert*-butyl)-6,8-dioxo-3-thiabicyclo[3.3.0]-octane (207b)



General procedure J (from **44b**); yield 32% (33 mg); off-white solid; m.p. 142-144 °C. R_f (50% EtOAc in DCM) 0.50; $[\alpha]_D^{20}$ +71.2 (*c* 0.8, DCM); ν_{max}/cm^{-1} 2960 (C-H), 1769 (C=O), 1699 (C=O); δ_H (400 MHz, $CDCl_3$) 1.00 (9H, s, C(CH_3)$_3$), 2.84 (1H, t, *J* 10.4, C(4)H_AH_B), 3.13 (1H, dd, *J* 22.0, 1.5, C(7)H_AH_B), 3.19 (1H, dd, *J* 10.4, 7.0, C(4)H$_A$$H_B$), 3.31 (1H, d, *J* 22.0, C(7)H$_A$$H_B$), 4.42-4.46 (1H, m, C(5)*H*), 5.30 (1H, s, C(2)*H*); δ_C (100 MHz, $CDCl_3$) 26.4 (C(*C*H$_3$)$_3$), 33.3 (*C*(4)), 37.8 (*C*(CH$_3$)$_3$), 43.0 (*C*(7)), 71.5 (*C*(2)), 72.4 (*C*(5)), 170.1 (*C*(8)), 203.5 (*C*(6)); *m/z* (ESI$^+$) 214.1 (MH$^+$, 91%), 236.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 214.08959, $C_{10}H_{16}NO_2S$ (MH$^+$) requires 214.08963.

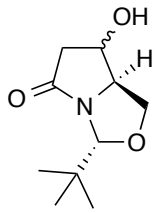
(2*R*,4*R*,5*R*)-1-Aza-2-(*tert*-butyl)-4-methyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (207c)[160] and (2*R*,4*S*,5*R*)-1-Aza-2-(*tert*-butyl)-4-methyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (209)



General procedure J (from **44c**); yield 47% (153 mg); white solid; 2.9:1 mixture of diastereomers. R_f (50% EtOAc in DCM) 0.46; ν_{max}/cm^{-1} 2960 (C-H), 1775 (C=O), 1647 (C=O); δ_H (500 MHz, C_6D_6) major diastereomer (**207c**): 0.60 (3H, d, *J* 6.5, C(4)C*H*$_3$), 0.92 (9H, s, C(CH_3)$_3$), 2.39-2.43 (2H, m, (7)H_AH_B), 3.73 (1H, dd, *J* 6.5, 1.0, C(5)*H*), 3.94 (1H, quint, *J* 6.5, C(4)*H*), 5.06 (1H, s, C(2)*H*); minor diastereomer

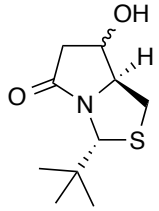
(**209**): 0.94 (9H, s, C(CH_3)$_3$), 1.09 (3H, d, *J* 6.0, C(4)CH_3), 2.39-2.43 (2H, m, (7)H_AH_B), 3.07 (1H, dq, *J* 9.0, 6.0, C(4)*H*), 3.15 (1H, dd, *J* 9.0, 1.0, C(5)*H*), 5.20 (1H, s, C(2)*H*); δ_C (125 MHz, C_6D_6) major diastereomer (**207c**): 15.5 (C(4)*C*H$_3$), 24.9 (C(*C*H$_3$)$_3$), 36.3 (*C*(CH$_3$)$_3$), 44.7 (*C*(7)), 71.7 (*C*(5)), 73.6 (*C*(4)), 94.4 (*C*(2)), 172.7 (*C*(8)), 203.0 (*C*(6)); minor diastereomer (**209**) 17.3 (C(4)*C*H$_3$), 25.0 (C(*C*H$_3$)$_3$), 36.0 (*C*(CH$_3$)$_3$), 43.9 (*C*(7)), 73.8, 74.2 (*C*(4), *C*(5)), 95.7 (*C*(2)), 172.3 (*C*(8)), 202.0 (*C*(6)); *m/z* (ESI$^+$) 212.2 (MH$^+$, 81%), 234.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 212.12803, $C_{11}H_{18}NO_3$ (MH$^+$) requires 212.12812.

(2*R*,5*R*,6*R*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-3-oxa-8-oxobicyclo[3.3.0]-octane ((*R*)-208'a) and (2*R*,5*R*,6*S*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-3-oxa-8-oxobicyclo[3.3.0]-octane ((*S*)-208a)



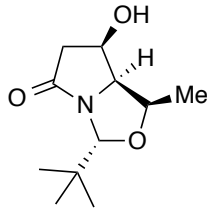
General procedure I (from **207a**); yield 99% (112 mg); white solid; 3.2:1 mixture of diastereomers. R_f (EtOAc) 0.58; ν_{max}/cm^{-1} 3399 (O-H), 2960 (C-H), 1682 (C=O); δ_H (400 MHz, $CDCl_3$) major diastereomer (**(*S*)-208a**): 0.91 (9H, s, C(CH_3)$_3$), 2.80 (1H, dd, *J* 17.2, 6.8, C(7)H_AH_B), 2.86 (1H, dd, *J* 17.2, 8.0, C(7)H$_A$$H_B$), 3.22 (1H, br s, O*H*), 3.32 (1H, dd, *J* 9.4, 8.2, C(4)H_AH_B), 3.99 (1H, ddd, *J* 9.4, 6.4, 3.2, C(5)*H*), 4.27 (1H, dd, *J* 8.2, 6.4, C(4)H$_A$$H_B$), 4.35-4.42 (1H, m, C(6)*H*), 4.87 (1H, s, C(2)*H*); minor diastereomer (**(*R*)-208'a**): 0.90 (9H, s, C(CH_3)$_3$), 2.43 (1H, dd, *J* 17.2, 1.2, C(7)H_AH_B), 3.06 (1H, dd, *J* 17.2, 6.0, C(7)H$_A$$H_B$), 3.30 (1H, br s, O*H*), 4.05 (2H, d, *J* 7.6, C(4)H_AH_B), 4.21 (1H, td, *J* 7.6, 5.2, C(5)*H*), 4.52-4.58 (1H, m, C(6)*H*), 4.91 (1H, s, C(2)*H*); δ_C (100 MHz, $CDCl_3$) major diastereomer (**(*S*)-208a**): 24.9 (C(*C*H$_3$)$_3$), 36.6 (*C*(CH$_3$)$_3$), 43.9 (*C*(7)), 69.0 (*C*(5)), 69.1 (*C*(6)), 70.2 (*C*(4)), 95.1 (*C*(2)), 176.6 (*C*(8)); minor diastereomer (**(*R*)-208'a**): 25.0 (C(*C*H$_3$)$_3$), 37.0 (*C*(CH$_3$)$_3$), 45.0 (*C*(7)), 65.3 (*C*(4)), 65.4 (*C*(5)), 66.5 (*C*(6)), 94.6 (*C*(2)), 176.6 (*C*(8)); *m/z* (ESI$^+$) 200.1 (MH$^+$, 31%), 222.1 (MNa$^+$, 93%); HRMS (ESI$^+$) found 200.12804, $C_{10}H_{18}NO_3$ (MH$^+$) requires 200.12812.

(2*R*,5*S*,6*R*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]-octane ((*R*)-208'b) and (2*R*,5*S*,6*S*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]-octane ((*S*)-208b)



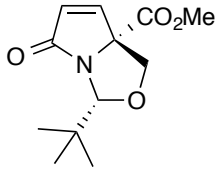
General procedure I (from **207b**); yield 86% (32 mg); white solid; 1.9:1 mixture of diastereomers. R_f (50% EtOAc in DCM) 0.21; ν_{max}/cm^{-1} 3369 (O-H), 2962 (C-H), 1674 (C=O); δ_H (500 MHz, $CDCl_3$) major diastereomer (**(*R*)-208'b**): 0.96 (9H, s, C(CH_3)$_3$), 2.46 (1H, dd, *J* 17.2, 3.0, C(7)H_AH$_B$), 2.93 (1H, dd, *J* 17.2, 6.5, C(7)H$_A$$H_B$), 2.97 (1H, dd, *J* 10.8, 7.5, C(4)H_AH$_B$), 3.21 (1H, dd, *J* 10.8, 7.5, C(4)H$_A$$H_B$), 4.33 (1H, td, *J* 7.5, 5.5, C(5)*H*), 4.58 (1H, ddd, *J* 6.5, 5.5, 3.0, C(6)*H*), 5.05 (1H, s, C(2)*H*); minor diastereomer (**(*S*)-208b**): 0.98 (9H, s, C(CH_3)$_3$), 2.51 (1H, t, *J* 10.2, C(4)H_AH$_B$), 2.61 (1H, dd, *J* 18.0, 3.5, C(7)H_AH$_B$), 2.83 (1H, dd, *J* 18.0, 7.5, C(7)H$_A$$H_B$), 3.11 (1H, dd, *J* 10.2, 6.0, C(4)H$_A$$H_B$), 4.08 (1H, ddd, *J* 10.2, 6.0, 2.0, C(5)*H*), 4.29 (1H, ddd, *J* 7.5, 3.5, 2.0, C(6)*H*), 5.01 (1H, s, C(2)*H*); δ_C (100 MHz, $CDCl_3$) major diastereomer (**(*R*)-208'b**): 26.5 (C(*C*H$_3$)$_3$), 30.6 (*C*(4)), 38.2 (*C*(CH$_3$)$_3$), 42.4 (*C*(7)), 66.7 (*C*(6)), 68.8 (*C*(5)), 70.8 (*C*(2)), 174.1 (*C*(8)); minor diastereomer (**(*S*)-208b**): 26.5 (C(*C*H$_3$)$_3$), 35.0 (*C*(4)), 37.6 (*C*(CH$_3$)$_3$), 41.8 (*C*(7)), 69.5 (*C*(6)), 71.0 (*C*(2)), 73.8 (*C*(5)), 175.0 (*C*(8)); *m/z* (ESI$^+$) 216.1 (MH$^+$, 97%), 238.1 (MNa$^+$, 90%); HRMS (ESI$^+$) found 216.10514, $C_{10}H_{18}NO_2S$ (MH$^+$) requires 216.10528.

(2*R*,4*R*,5*S*,6*R*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-4-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane (208'c)



General procedure I (from **207c**); yield 92% (63 mg); white solid; m.p. 186-188 °C. R_f (50% EtOAc in DCM) 0.40; $[\alpha]_D^{20}$ +139.7 (*c* 1.0, DCM); ν_{max}/cm^{-1} 3303 (O-H), 2950 (C-H), 1670 (C=O); δ_H (500 MHz, $CDCl_3$) 0.92 (9H, s, C(CH_3)$_3$), 1.24 (3H, d, *J* 6.5, C(4)CH_3), 2.50 (1H, dd, *J* 18.0, 6.5, C(7)H_AH$_B$), 2.92 (1H, dd, *J* 18.0, 9.0, C(7)H$_A$$H_B$), 4.06 (1H, dd, *J* 7.5, 5.5, C(5)*H*), 4.35 (1H, qd, *J* 6.5, 5.5, C(4)*H*), 4.92-4.97 (1H, m, C(6)*H*), 4.94 (1H, s, C(2)*H*); δ_C (125 MHz, $CDCl_3$) 16.2 (C(4)*C*H$_3$), 25.1 (C(*C*H$_3$)$_3$), 35.9 (*C*(CH$_3$)$_3$), 42.1 (*C*(7)), 65.6 (*C*(6)), 66.0 (*C*(5)), 74.7 (*C*(4)), 93.7 (*C*(2)), 178.1 (*C*(8)); *m/z* (ESI$^+$) 214.1 (MH$^+$, 27%), 236.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 214.14350, $C_{11}H_{20}NO_3$ (MH$^+$) requires 214.14377.

(2*R*,5*S*)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (210)

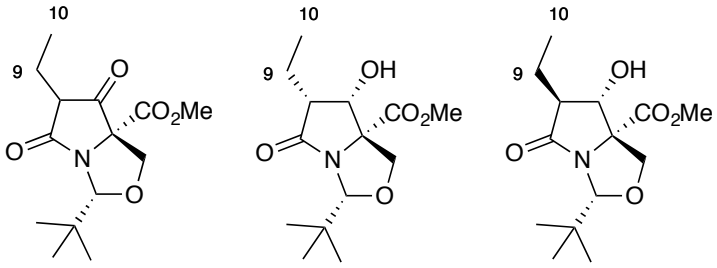


A solution of diethyl azodicarboxylate (1.7 eq) in anhydrous THF was added dropwise to a solution of alcohol **197a** (1.0 eq), triphenylphosphine (4.0 eq) and the acid indicated in Table 6.1 (1.05 eq) in anhydrous THF (*c* 0.1). The reaction mixture was stirred at room temperature for 2 h, poured into 0.05 M aqueous NaH_2PO_4, extracted with EtOAc and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the crude product was purified by *flash* column chromatography (DCM to 10% EtOAc in DCM) to give alkene **210** as a white solid, instead of the desired alcohol **197'a**; m.p. 77-79 °C. R_f (20% EtOAc in DCM) 0.68; $[\alpha]_D^{20}$ +232.0 (*c* 0.9, DCM); ν_{max}/cm^{-1} 2959 (C-H), 1744 (C=O), 1711 (C=O); δ_H (400 MHz, $CDCl_3$) 0.95 (9H, s, C(CH_3)$_3$), 3.31 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.76 (3H, s, CO_2CH_3), 4.70 (1H, s, C(2)*H*), 4.79 (1H, d, *J* 8.5, C(4)H$_A$$H_B$), 6.16 (1H, d, *J* 5.7, C(6)*H*), 7.09 (1H, d, *J* 5.7, C(7)*H*); δ_C (100 MHz, $CDCl_3$) 25.0 (C(*C*H$_3$)$_3$), 35.5 (*C*(CH$_3$)$_3$), 53.3 (CO_2CH_3), 70.9 (*C*(4)), 78.1 (*C*(5)), 97.1 (*C*(2)), 130.0 (*C*(6)), 146.7 (*C*(7)), 169.3 (*C*O$_2$CH$_3$), 177.8 (*C*(8)); *m/z* (ESI$^+$) 240.1 (MH$^+$, 20%), 262.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 240.12289, $C_{12}H_{18}NO_4$ (MH$^+$) requires 240.12303.

Table 6.1. Acids used for the attempted inversion of configuration of alcohol **197a**.

Entry	Acid	Yield
1	Benzoic acid	Quant. (416 mg)
2	4-Nitrobenzoic acid	81% (38 mg)
3	Acetic acid	75% (34 mg)

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-ethyl-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (211), (2*R*,5*R*,6*S*,7*R*)-1-Aza-2-(*tert*-butyl)-7-ethyl-6-hydroxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-octane (212') and **(2*R*,5*R*,6*S*,7*S*)-1-Aza-2-(*tert*-butyl)-7-ethyl-6-hydroxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-octane (212)**



$NaBH_4$ (20 mg, 0.53 mmol) was added portionwise at 0 °C to a solution of acetyltetramate **81a** (75 mg, 0.25 mmol) and acetic acid (130 μL, 2.27 mmol) in anhydrous DCM (1.2 mL). The reaction mixture was stirred at 0 °C for 15 min and at room temperature for 2 h, quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc, washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give the first crude product. The aqueous phase was acidified with 2 M aqueous HCl, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the second crude product. The first crude product was purified by *flash* column chromatography (50% EtOAc in petrol) to give alcohols **212** (7 mg, 0.02 mmol, 10%) and **212'** (7 mg, 0.02 mmol, 10%) as colourless oils. The second crude product was purified by *flash* column chromatography (50% EtOAc in petrol to EtOAc) to give tetramate **211** (17 mg, 0.060 mmol, 24%) as an orange oil.

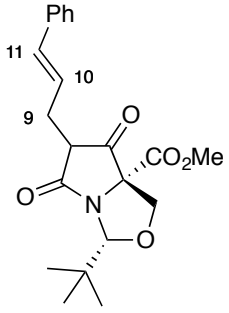
Tetramate (**211**): R_f (EtOAc) 0.15; $[\alpha]_D^{20}$ +64.7 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2962 (C-H), 1749 (C=O), 1643 (C=O); δ_H (400 MHz, $CDCl_3$) 0.90 (9H, s, C(CH_3)$_3$), 0.95 (3H, t, *J* 7.4, C(10)H_3), 1.84-1.91 (2H, m, C(9)H_2), 3.47 (1H, d, *J* 9.0, C(4)H_AH$_B$), 3.54 (1H, t, *J* 5.3, C(7)*H*), 3.81 (3H, s, CO_2CH_3), 4.80 (1H, d, *J* 9.0, C(4)H$_A$$H_B$), 5.06 (1H, s, C(2)*H*); δ_C (100 MHz, $CDCl_3$) 11.1 (*C*(10)), 17.5 (*C*(9)), 24.8 (C(*C*H$_3$)$_3$), 35.7 (*C*(CH$_3$)$_3$), 53.8 (CO_2CH_3), 55.0 (*C*(7)), 68.0 (*C*(4)), 79.1 (*C*(5)), 98.2 (*C*(2)), 167.1 (*C*O$_2$CH$_3$),

175.1 (*C*(8)), 201.4 (*C*(6)); *m/z* (ESI$^-$) 282.1 (M-H$^-$, 100%); HRMS (ESI$^-$) found 282.13430, $C_{14}H_{20}NO_5$ (M-H$^-$) requires 282.13470.

Alcohol *R* (**212'**): R_f (50% EtOAc in petrol) 0.32; $[\alpha]_D^{20}$ +51.2 (*c* 0.7, $CHCl_3$); ν_{max}/cm^{-1} 3400 (O-H), 2959 (C-H), 1742 (C=O), 1688 (C=O); δ_H (500 MHz, $CDCl_3$) 0.89 (9H, s, C(CH_3)$_3$), 1.10 (3H, t, *J* 7.5, C(10)H_3), 1.91 (2H, quint, *J* 7.5, C(9)H_2), 2.59 (1H, d, *J* 5.4, O*H*), 2.70 (1H, dt, *J* 9.0, 7.5, C(7)*H*), 3.40 (1H, d, *J* 8.6, C(4)H_AH_B), 3.80 (3H, s, CO_2CH_3), 4.53-4.55 (1H, m, C(6)*H*), 4.85 (1H, s, C(2)*H*), 4.90 (1H, d, *J* 8.6, C(4)H$_A$*H*$_B$); δ_C (125 MHz, $CDCl_3$) 11.3 (*C*(10)), 21.2 (*C*(9)), 24.9 (C(*C*H$_3$)$_3$), 36.2 (*C*(CH$_3$)$_3$), 52.8 (CO_2CH_3), 53.2 (*C*(7)), 74.1 (*C*(4)), 75.4 (*C*(5)), 79.3 (*C*(6)), 96.3 (*C*(2)), 171.0 (CO_2CH_3), 176.5 (*C*(8)); *m/z* (ESI$^+$) 286.2 (MH$^+$, 100%), 308.2 (MNa$^+$, 91%); HRMS (ESI$^+$) found 286.16497, $C_{14}H_{24}NO_5$ (MH$^+$) requires 286.16490.

Alcohol *S* (**212**): R_f (50% EtOAc in petrol) 0.44; $[\alpha]_D^{20}$ +30.6 (*c* 0.6, $CHCl_3$); ν_{max}/cm^{-1} 3430 (O-H), 2960 (C-H), 1745 (C=O), 1691 (C=O); δ_H (500 MHz, $CDCl_3$) 0.87 (9H, s, C(CH_3)$_3$), 1.06 (3H, t, *J* 7.5, C(10)H_3), 1.54-1.63 (1H, m, C(9)H_AH_B), 1.80-1.89 (1H, m, C(9)H$_A$*H*$_B$), 2.61 (1H, s, O*H*), 3.07 (1H, dt, *J* 10.4, 6.4, C(7)*H*), 3.49 (1H, d, *J* 8.7, C(4)H_AH_B), 3.82 (3H, s, CO_2CH_3), 4.16 (1H, d, *J* 10.4, C(6)*H*), 4.87 (1H, s, C(2)*H*), 4.91 (1H, d, *J* 8.7, C(4)H$_A$*H*$_B$); δ_C (125 MHz, $CDCl_3$) 12.6 (*C*(10)), 19.7 (*C*(9)), 25.1 (C(*C*H$_3$)$_3$), 35.7 (*C*(CH$_3$)$_3$), 52.7 (CO_2CH_3), 52.9 (*C*(7)), 72.7 (*C*(6)), 74.6 (*C*(4)), 77.7 (*C*(5)), 97.1 (*C*(2)), 171.1 (CO_2CH_3), 180.1 (*C*(8)); *m/z* (ESI$^+$) 308.2 (MNa$^+$, 11%); HRMS (ESI$^+$) found 308.14704, $C_{14}H_{23}NNaO_5$ (MNa$^+$) requires 308.14684.

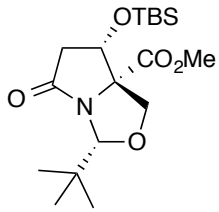
(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7-cinnamyl-5-methoxycarbonyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (213)



General procedure I (from **96a**, for 3 h); yield 45% (12 mg); yellow solid; m.p. 85 °C. R_f (10% MeOH in EtOAc) 0.20; $[\alpha]_D^{20}$ +51.0 (*c* 0.6, $CHCl_3$); ν_{max}/cm^{-1} 2960 (C-H), 1748 (C=O), 1643 (C=O); δ_H (500 MHz, $CDCl_3$) 0.91 (9H, s, C(CH_3)$_3$), 2.76 (2H, m, C(9)H_2), 3.38 (1H, d, *J* 9.0, C(4)H_AH_B), 3.73 (1H, m, C(7)*H*), 3.82 (3H, s, CO_2CH_3), 4.74 (1H, d, *J* 9.0, C(4)H$_A$*H*$_B$), 5.04 (1H, s, C(2)*H*), 6.15 (1H, dt, *J* 15.6, 7.4, C(10)*H*), 6.50 (1H, d, *J* 15.6, C(11)*H*), 7.15-7.37 (5H, m, Ph); δ_C (125 MHz,

$CDCl_3$) 24.8 (C(CH_3)$_3$), 27.4 (*C*(9)), 35.7 (*C*(CH_3)$_3$), 53.8 (CO_2CH_3), 54.5 (*C*(7)), 67.9 (*C*(4)), 79.2 (*C*(5)), 98.3 (*C*(2)), 124.2 (*C*(10)), 126.4, 127.8, 128.7 (Ph), 134.5 (*C*(11)), 136.8 (Ph), 167.0 (CO_2CH_3), 174.5 (*C*(8)), 200.7 (*C*(6)); *m/z* (ESI$^-$) 370.1 (M-H$^-$, 100%); HRMS (ESI$^+$) found 372.18067, $C_{21}H_{26}NO_5$ (MH$^+$) requires 372.18055.

(2*R*,5*R*,6*S*)-1-Aza-2-(*tert*-Butyl)-6-((*tert*-butyldimethylsilyl)oxy)-5-methoxycarbonyl-8-oxo-3-oxabicyclo[3.3.0]-octane (217)

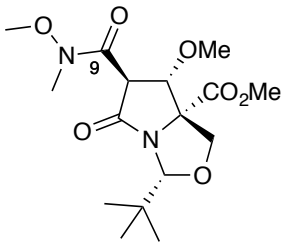


TBS chloride (575 mg, 3.81 mmol) was added to a solution of alcohol **197a** (498 mg, 1.94 mmol) and imidazole (533 mg, 7.83 mmol) in anhydrous THF (10 mL) at 0 °C. After stirring at room temperature for 14 h, TLC showed the presence of starting material left, and imidazole (267 mg, 3.92 mmol) and TBS chloride (296 mg, 1.96 mmol) were added. The reaction mixture was stirred for another 6 h, quenched with H_2O, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (DCM) and the remaining TBSOH was azeotropically removed with toluene to give silyl ether **217** (623 mg, 1.68 mmol, 87%) as a white solid; m.p. 146 °C. R_f (DCM) 0.51; $[\alpha]_D^{20}$ +49.6 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2954 (C-H), 1744 (C=O), 1715 (C=O); δ_H (400 MHz, $CDCl_3$) 0.03 (3H, s, SiCH_3), 0.03 (3H, s, SiCH_3), 0.84 (9H, s, SiC(CH_3)$_3$), 0.86 (9H, s, C(CH_3)$_3$), 2.68 (1H, dd, *J* 15.4, 7.7, C(7)H_AH$_B$), 3.15 (1H, dd, *J* 15.4, 10.5, C(7)H$_A$$H_B$), 3.52 (1H, d, *J* 8.6, C(4)H_AH$_B$), 3.77 (3H, s, CO_2CH_3), 4.44 (1H, dd, *J* 10.5, 7.7, C(6)*H*), 4.88 (1H, s, C(2)*H*), 4.88 (1H, d, *J* 8.6, C(4)H$_A$$H_B$); δ_C (100 MHz, $CDCl_3$) -5.0 (SiCH_3), -4.9 (SiCH_3), 18.0 (Si*C*(CH$_3$)$_3$), 24.9 (C(*C*H$_3$)$_3$), 25.6 (SiC(*C*H$_3$)$_3$), 36.3 (*C*(CH$_3$)$_3$), 44.1 (*C*(7)), 52.5 (CO_2CH_3), 74.0 (*C*(4)), 75.0 (*C*(6)), 77.4 (*C*(5)), 96.2 (*C*(2)), 170.1 (CO_2CH_3), 174.7 (*C*(8)); *m/z* (ESI$^+$) 372.2 (MH$^+$, 36%), 394.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 372.21921, $C_{18}H_{34}NO_5Si$ (MH$^+$) requires 372.22008.

General procedure K: Hydrogenation of *C*(7)-acyltetramates

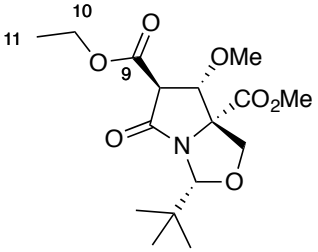
A suspension of the *C*(7)-acyltetramate (1.0 eq) and PtO_2 (0.2 eq) in EtOAc (*c* 0.02) was stirred at room temperature under an atmosphere of H_2 for 3 h. The mixture was filtered through Celite and concentrated under reduced pressure to give the hydrogenated product.

(2*R*,5*R*,6*S*,7*S*)-1-Aza-2-(*tert*-butyl)-6-methoxy-5-methoxycarbonyl-7-(methoxy(methyl)carbamoyl)-3-oxa-8-oxobicyclo[3.3.0]-octane (239)



General procedure K (from **116l**); yield quant. (440 mg); white solid; m.p. 178 °C. R_f (50% EtOAc in petrol) 0.24; $[\alpha]_D^{20}$ -39.0 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2960 (C-H), 1748 (C=O), 1718 (C=O), 1668 (C=O); δ_H (500 MHz, $CDCl_3$) 0.87 (9H, s, C(CH_3)$_3$), 3.29 (3H, s, NCH_3), 3.30 (3H, s, C(6)OCH_3), 3.72 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.81 (3H, s, NOCH_3), 3.83 (3H, s, CO_2CH_3), 4.61 (1H, d, *J* 10.2, C(6)*H*), 4.73 (1H, d, *J* 10.2, C(7)*H*), 4.86 (1H, s, C(2)*H*), 4.98 (1H, d, *J* 8.5, C(4)H$_A$$H_B$); δ_C (125 MHz, $CDCl_3$) 25.0 (C(*C*H$_3$)$_3$), 32.4 (N*C*H$_3$), 36.2 (*C*(CH$_3$)$_3$), 52.9 (CO_2CH_3), 54.8 (*C*(7)), 59.1 (C(6)O*C*H$_3$), 62.1 (NO*C*H$_3$), 73.7 (*C*(4)), 74.7 (*C*(5)), 84.5 (*C*(6)), 96.2 (*C*(2)), 167.5 (*C*(9)), 170.1 (CO_2CH_3), 170.4 (*C*(8)); *m/z* (ESI$^+$) 359.2 (MH$^+$, 59%), 381.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 359.18165, $C_{16}H_{27}N_2O_7$ (MH$^+$) requires 359.18128.

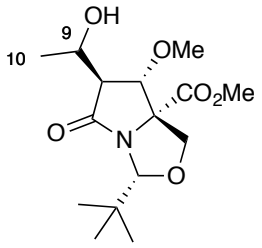
(2*R*,5*R*,6*S*,7*S*)-1-Aza-2-(*tert*-butyl)-7-ethoxycarbonyl-6-methoxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-octane (248)



General procedure K (from **117**); yield quant. (66 mg); white solid; m.p. 132 °C. R_f (20% EtOAc in petrol) 0.29; $[\alpha]_D^{20}$ -6.6 (*c* 0.7, $CHCl_3$); ν_{max}/cm^{-1} 2957 (C-H), 1738 (C=O), 1718 (C=O); δ_H (400 MHz, $CDCl_3$) 0.85 (9H, s, C(CH_3)$_3$), 1.32 (3H, t, *J* 7.2, C(11)H_3), 3.31 (3H, s, C(6)OCH_3), 3.69 (1H, d, *J* 8.7, C(4)H_AH$_B$), 3.80 (3H, s, CO_2CH_3), 4.23 (1H, d, *J* 10.6, C(7)*H*), 4.25 (1H, dq, *J* 10.8, 7.2, C(10)H_AH$_B$), 4.31 (1H, dq, *J* 10.8, 7.2, C(10)H$_A$$H_B$), 4.45 (1H, d, *J* 10.6, C(6)*H*), 4.87 (1H, s, C(2)*H*), 4.98 (1H, d, *J* 8.7, C(4)H$_A$$H_B$); δ_C (100 MHz, $CDCl_3$) 14.3 (*C*(11)), 24.8 (C(*C*H$_3$)$_3$), 36.2 (*C*(CH$_3$)$_3$), 52.9 (CO_2CH_3), 58.2 (*C*(7)), 59.1 (C(6)O*C*H$_3$), 62.2 (*C*(10)), 73.6 (*C*(4)), 74.4 (*C*(5)), 84.9

(*C*(6)), 96.1 (*C*(2)), 167.9 (*C*(9)), 169.2 (*C*(8)), 169.7 (*C*$O_2$$CH_3$); *m/z* (ESI$^+$) 344.2 ($MH^+$, 21%), 366.2 ($MNa^+$, 100%); HRMS (ESI$^+$) found 344.17060, $C_{16}H_{26}NO_7$ (MH^+) requires 344.17038.

(2*R*,5*R*,6*S*,7*S*)-1-Aza-2-(*tert*-butyl)-7-(1-hydroxyethyl)-6-methoxy-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-octane (249)

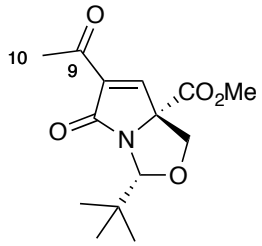


General procedure K (from **103**); yield 57% (33 mg); white solid; m.p. 121 °C. R_f (50% EtOAc in petrol) 0.27; $[\alpha]_D^{20}$ -7.4 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 3467 (O-H), 2975 (C-H), 2960 (C-H), 1740 (C=O), 1702 (C=O); δ_H (400 MHz, $CDCl_3$) 0.85 (9H, s, C(CH_3)$_3$), 1.27 (3H, d, *J* 6.4, C(10)H_3), 3.13 (1H, dd, *J* 10.7, 7.1, C(7)*H*), 3.30 (3H, s, C(6)OCH_3), 3.56 (1H, d, *J* 8.8, C(4)$H_A$$H_B$), 3.69 (1H, d, *J* 10.7, C(6)*H*), 3.80 (3H, s, CO_2CH_3), 3.90-3.94 (1H, m, *C*(9)*H*), 3.97-3.98 (1H, m, O*H*), 4.86 (1H, s, C(2)*H*), 4.97 (1H, d, *J* 8.8, C(4)$H_A$$H_B$); δ_C (100 MHz, $CDCl_3$) 20.9 (*C*(10)), 24.8 (C(*C*H_3)$_3$), 36.2 (*C*(CH_3)$_3$), 52.9 (CO_2*C*H_3), 56.5 (*C*(7)), 59.2 (C(6)O*C*H_3), 67.6 (*C*(9)), 73.9 (*C*(4)), 74.7 (*C*(5)), 86.0 (*C*(6)), 95.6 (*C*(2)), 170.0 (*C*$O_2$$CH_3$), 176.0 (*C*(8)); *m/z* (ESI$^+$) 316.2 (MH^+, 18%), 338.2 (MNa^+, 100%); HRMS (ESI$^+$) found 338.15733, $C_{15}H_{25}NNaO_6$ (MNa^+) requires 338.15741.

General procedure L: Grignard addition to Weinreb amide 239 and elimination

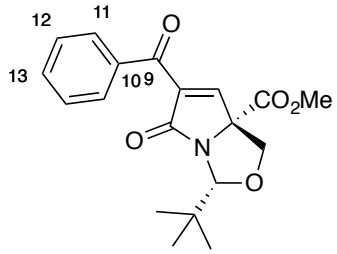
The Grignard reagent (1.2 eq), prepared following General procedure C if not commercially available, was added to a solution of Weinreb amide **239** (1.0 eq) in anhydrous THF (*c* 0.2) at -15 °C. The solution was stirred at the same temperature for 1 h, then quenched with aqueous saturated NH_4Cl, extracted with Et_2O, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in DCM (*c* 0.1), stirred with *para*-toluenesulfonic acid monohydrate (0.5 eq) for 24 h and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography to give the enone product.

(2*R*,5*S*)-7-Acetyl-1-aza-2-(*tert*-butyl)-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (255)



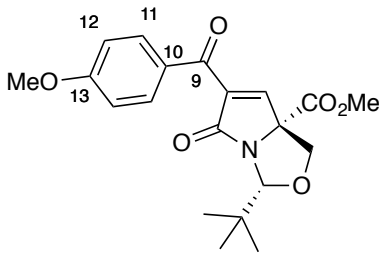
General procedure L (with 3 M MeMgBr in Et_2O); yield 48% (18 mg); yellow oil. R_f (20% EtOAc in petrol) 0.20; $[\alpha]_D^{20}$ +187.6 (*c* 0.5, $CHCl_3$); ν_{max}/cm^{-1} 2960 (C-H), 1715 (C=O), 1695 (C=O), 1610 (C=C); δ_H (400 MHz, $CDCl_3$) 0.95 (9H, s, C(CH_3)$_3$), 2.56 (3H, s, C(10)H_3), 3.33 (1H, d, *J* 8.6, C(4)H_AH$_B$), 3.77 (3H, s, CO_2CH_3), 4.74 (1H, s, C(2)*H*), 4.81 (1H, d, *J* 8.6, C(4)H$_A$$H_B$), 7.70 (1H, s, C(6)*H*); δ_C (100 MHz, $CDCl_3$) 24.9 (C(*C*H$_3$)$_3$), 29.3 (*C*(10)), 35.5 (*C*(CH$_3$)$_3$), 53.6 (CO_2*C*H$_3$), 70.6 (*C*(4)), 75.2 (*C*(5)), 97.6 (*C*(2)), 138.7 (*C*(7)), 151.1 (*C*(6)), 168.1 (*C*O$_2$CH$_3$), 173.9 (*C*(8)), 193.3 (*C*(9)); *m/z* (ESI$^+$) 282.1 (MH$^+$, 18%), 304.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 282.13377, $C_{14}H_{20}NO_5$ (MH$^+$) requires 282.13360.

(2*R*,5*S*)-1-Aza-7-benzoyl-2-(*tert*-butyl)-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (256)



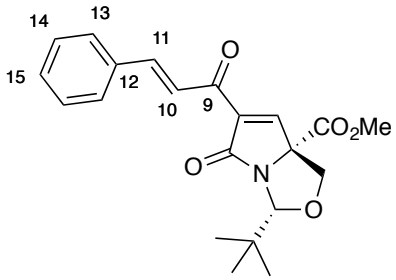
General procedure L (with 3 M PhMgBr in Et_2O); yield 68% (31 mg); white solid; m.p. 86 °C. R_f (20% EtOAc in petrol) 0.25; $[\alpha]_D^{20}$ +148.1 (*c* 0.6, $CHCl_3$); ν_{max}/cm^{-1} 3062 (C-H), 2958 (C-H), 1747 (C=O), 1714 (C=O), 1655 (C=O), 1615 (C=C); δ_H (400 MHz, $CDCl_3$) 0.96 (9H, s, C(CH_3)$_3$), 3.48 (1H, d, *J* 8.6, C(4)H_AH$_B$), 3.81 (3H, s, CO_2CH_3), 4.79 (1H, s, C(2)*H*), 4.86 (1H, d, *J* 8.6, C(4)H$_A$$H_B$), 7.42 (1H, s, C(6)*H*), 7.48 (2H, t, *J* 7.6, C(12)*H*), 7.63 (1H, tt, *J* 7.6, 1.2, C(13)*H*), 7.85-7.88 (2H, m, C(11)*H*); δ_C (100 MHz, $CDCl_3$) 24.9 (C(*C*H$_3$)$_3$), 35.5 (*C*(CH$_3$)$_3$), 53.6 (CO_2*C*H$_3$), 70.6 (*C*(4)), 75.9 (*C*(5)), 97.5 (*C*(2)), 128.8 (*C*(12)), 129.7 (*C*(11)), 134.3 (*C*(13)), 135.9 (*C*(10)), 139.9 (*C*(7)), 148.5 (*C*(6)), 168.5 (*C*O$_2$CH$_3$), 173.5 (*C*(8)), 188.5 (*C*(9)); *m/z* (ESI$^+$) 366.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 344.14936, $C_{19}H_{22}NO_5$ (MH$^+$) requires 344.14925.

(2*R*,5*S*)-1-Aza-2-(*tert*-butyl)-7-(4-methoxybenzoyl)-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (257)



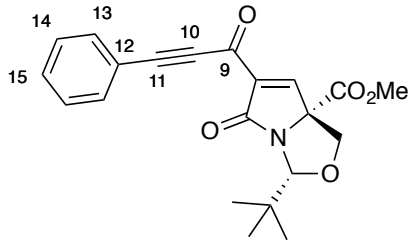
General procedure L (with *p*-MeOPhMgBr); yield 50% (21 mg); yellow solid; m.p. 126 °C. R_f (20% EtOAc in petrol) 0.11; $[\alpha]_D^{20}$ +156.5 (*c* 0.5, $CHCl_3$); ν_{max}/cm^{-1} 2958 (C-H), 1750 (C=O), 1710 (C=O), 1644 (C=O), 1599 (C=C); δ_H (400 MHz, $CDCl_3$) 0.97 (9H, s, C(CH_3)$_3$), 3.47 (1H, d, *J* 8.6, C(4)H_AH_B), 3.81 (3H, s, CO_2CH_3), 3.87 (3H, s, C(13)OCH_3), 4.78 (1H, s, C(2)*H*), 4.85 (1H, d, *J* 8.6, C(4)H$_A$$H_B$), 6.95 (2H, d, *J* 8.9, C(12)*H*), 7.37 (1H, s, C(6)*H*), 7.87 (2H, d, *J* 8.9, C(11)*H*); δ_C (100 MHz, $CDCl_3$) 25.0 (C(CH_3)$_3$), 35.5 (*C*(CH_3)$_3$), 53.6 (CO_2CH_3), 55.7 (C(13)O*C*H$_3$), 70.6 (*C*(4)), 76.0 (*C*(5)), 97.5 (*C*(2)), 114.1 (*C*(12)), 129.0 (*C*(10)), 132.2 (*C*(11)), 140.5 (*C*(7)), 147.6 (*C*(6)), 164.6 (*C*(13)), 168.6 (*C*O_2CH_3), 173.9 (*C*(8)), 186.9 (*C*(9)); *m/z* (ESI$^+$) 374.2 (MH$^+$, 38%), 396.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 374.16002, $C_{20}H_{24}NO_6$ (MH$^+$) requires 374.15981.

(2*R*,5*S*)-1-Aza-2-(*tert*-butyl)-7-cinnamoyl-5-methoxycarbonyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (258)



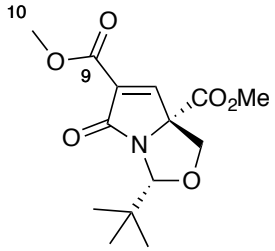
General procedure L (with PhCH=CHMgBr); yield 49% (19 mg); yellow oil. R_f (20% EtOAc in petrol) 0.22; $[\alpha]_D^{20}$ +162.1 (*c* 0.3, $CHCl_3$); ν_{max}/cm^{-1} 3026 (C-H), 2958 (C-H), 1747 (C=O), 1713 (C=O), 1666 (C=O), 1612 (C=C); δ_H (400 MHz, $CDCl_3$) 0.99 (9H, s, C(CH_3)$_3$), 3.39 (1H, d, *J* 8.6, C(4)H_AH_B), 3.78 (3H, s, CO_2CH_3), 4.80 (1H, s, C(2)*H*), 4.85 (1H, d, *J* 8.6, C(4)H$_A$$H_B$), 7.37-7.43 (3H, m, C(14)*H* + C(15)*H*), 7.66-7.68 (2H, m, C(13)*H*), 7.84-7.85 (3H, m, C(6)*H* + C(10)*H* + C(11)*H*); δ_C (100 MHz, $CDCl_3$) 25.0 (C(*C*H_3)$_3$), 35.5 (*C*(CH_3)$_3$), 53.6 (CO_2CH_3), 70.7 (*C*(4)), 75.3 (*C*(5)), 97.6 (*C*(2)), 122.7 (*C*(10)), 129.1 (*C*(14)), 129.2 (*C*(13)), 131.3 (*C*(15)), 134.6 (*C*(12)), 139.1 (*C*(7)), 146.0 (*C*(11)), 152.0 (*C*(6)), 168.2 (*C*O_2CH_3), 174.2 (*C*(8)), 183.7 (*C*(9)); *m/z* (ESI$^+$) 370.2 (MH$^+$, 42%), 392.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 370.16495, $C_{21}H_{24}NO_5$ (MH$^+$) requires 370.16490.

(2*R*,5*S*)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-3-oxa-8-oxo-7-(3-phenylpropioloyl)bicyclo[3.3.0]-oct-6-ene (259)



General procedure L (with PhC≡CMgBr); yield 42% (17 mg); orange solid; m.p. 110 °C. R_f (20% EtOAc in petrol) 0.28; $[\alpha]_D^{20}$ +154.5 (*c* 0.5, $CHCl_3$); ν_{max}/cm^{-1} 2955 (C-H), 2204 (C≡C), 1725 (C=O), 1639 (C=O), 1613 (C=C); δ_H (400 MHz, $CDCl_3$) 0.97 (9H, s, C(CH_3)$_3$), 3.41 (1H, d, *J* 8.6, C(4)H_AH$_B$), 3.79 (3H, s, CO_2CH_3), 4.80 (1H, s, C(2)*H*), 4.86 (1H, d, *J* 8.6, C(4)H$_A$$H_B$), 7.41 (2H, t, *J* 7.4, C(14)*H*), 7.50 (1H, tt, *J* 7.4, 1.2, C(15)*H*), 7.66-7.68 (2H, m, C(13)*H*), 7.89 (1H, s, C(6)*H*); δ_C (100 MHz, $CDCl_3$) 25.0 (C(*C*H_3)$_3$), 35.5 (*C*(CH$_3$)$_3$), 53.7 (CO_2CH_3), 70.6 (*C*(4)), 75.0 (*C*(5)), 87.3 (*C*(10)), 95.3 (*C*(11)), 97.6 (*C*(2)), 119.5 (*C*(12)), 128.9 (*C*(14)), 131.6 (*C*(15)), 133.6 (*C*(13)), 138.0 (*C*(7)), 153.2 (*C*(6)), 168.1 (CO_2CH_3), 170.5 (*C*(9)), 171.9 (*C*(8)); *m/z* (ESI$^+$) 368.2 (MH$^+$, 27%), 390.0 (MNa$^+$, 100%); HRMS (ESI$^+$) found 368.14939, $C_{21}H_{22}NO_5$ (MH$^+$) requires 368.14925.

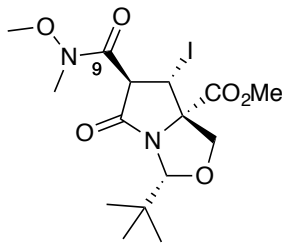
(2*R*,5*S*)-1-Aza-2-(*tert*-butyl)-5,7-dimethoxycarbonyl-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (262)



A solution of Weinreb amide **239** (27 mg, 0.075 mmol) and *p*-toluenesulfonic acid monohydrate (29 mg, 0.15 mmol) in DCM (750 μL) was stirred at room temperature for 24 h, then diluted with brine and extracted with Et_2O. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by *flash* column chromatography (25% to 50% EtOAc in petrol) to give methyl ester **262** (4 mg, 0.02 mmol, 19%) as a colourless oil, instead of the desired Weinreb amide **260**. R_f (50% EtOAc in petrol) 0.48; $[\alpha]_D^{20}$ +134.6 (*c* 0.4, $CHCl_3$); ν_{max}/cm^{-1} 2958 (C-H), 1731 (C=O), 1628 (C=C); δ_H (500 MHz, $CDCl_3$) 0.95 (9H, s, C(CH_3)$_3$), 3.36 (1H, d, *J* 8.5, C(4)H_AH$_B$), 3.77 (3H, s, CO_2CH_3), 3.88 (3H, s, C(10)H_3), 4.75 (1H, s, C(2)*H*), 4.82 (1H, d, *J* 8.5, C(4)H$_A$$H_B$), 7.77 (1H, s, C(6)*H*); δ_C (125 MHz, $CDCl_3$) 24.9 (C(*C*H_3)$_3$), 35.5 (*C*(CH$_3$)$_3$), 52.7 (*C*(10)), 53.6 (CO_2CH_3), 70.5 (*C*(4)), 75.2 (*C*(5)), 97.7 (*C*(2)), 132.8 (*C*(7)),

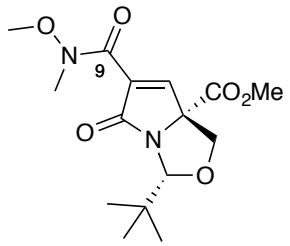
152.9 (*C*(6)), 161.1 (*C*(9)), 168.1 (*C*$O_2$$CH_3$), 172.2 (*C*(8)); *m/z* (ESI$^+$) 298.1 (MH^+, 33%), 320.2 (MNa^+, 100%); HRMS (ESI$^+$) found 298.12884, $C_{14}H_{20}NO_6$ (MH^+) requires 298.12851.

(2*R*,5*S*,6*S*,7*S*)-1-Aza-2-(*tert*-butyl)-6-iodo-5-methoxycarbonyl-7-(methoxy(methyl)carbamoyl)-3-oxa-8-oxobicyclo[3.3.0]-octane (264)



TMSI (25 µL, 0.18 mmol) was added to a solution of methyl ether **239** (53 mg, 0.15 mmol) in anhydrous DCM (740 µL). The solution was stirred at room temperature for 1 h, then diluted with H_2O and extracted with Et_2O. The combined organic exctracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by *flash* column chromatography (30% EtOAc in petrol) to give iodide **264** (39 mg, 0.086 mmol, 59%) as a white solid; m.p. 146 °C (deg). R_f (30% EtOAc in petrol) 0.28; $[\alpha]_D^{20}$ +1.6 (*c* 1.3, $CHCl_3$); ν_{max}/cm^{-1} 2951 (C-H), 1755 (C=O), 1708 (C=O), 1668 (C=O); δ_H (500 MHz, $CDCl_3$) 0.87 (9H, s, C(CH_3)$_3$), 3.32 (3H, s, NCH_3), 3.62 (1H, d, *J* 8.9, C(4)H_AH$_B$), 3.82 (3H, s, NOCH_3), 3.88 (3H, s, CO_2CH_3), 4.89 (1H, s, C(2)*H*), 4.89 (1H, d, *J* 12.2, C(7)*H*), 4.95 (1H, d, *J* 8.9, C(4)H$_A$$H_B$), 4.96 (1H, d, *J* 12.2, C(6)*H*); δ_C (125 MHz, $CDCl_3$) 17.4 (*C*(7)), 24.9 (C(*C*H$_3$)$_3$), 32.5 (N*C*H$_3$), 36.4 (*C*(CH$_3$)$_3$), 53.1 (CO_2*C*H$_3$), 59.1 (*C*(6)), 62.2 (NO*C*H$_3$), 73.4 (*C*(4)), 75.7 (*C*(5)), 96.9 (*C*(2)), 165.9 (*C*(9)), 170.2 (*C*$O_2$$CH_3$), 170.3 (*C*(8)); *m/z* (ESI$^+$) 455.0 (MH^+, 31%), 477.0 (MNa^+, 100%); HRMS (ESI$^+$) found 455.06721, $C_{15}H_{24}IN_2O_6$ (MH^+) requires 455.06736.

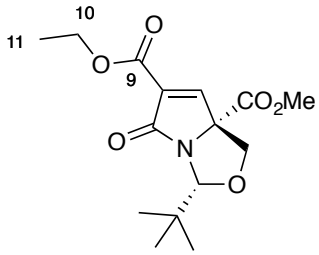
(2*R*,5*S*)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-7-(methoxy(methyl)carbamoyl)-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (260)



Triethylamine (79 µL, 0.57 mmol) was added to a solution of iodide **264** (39 mg, 0.086 mmol) in anhydrous DCM (1.1 mL). The mixture was stirred at room temperature for 20 h, diluted with brine and extracted with Et_2O. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (25% to 60% EtOAc in petrol) to give alkene **260** (13 mg, 0.041 mmol, 48%) as

a white solid; m.p. 213 °C (deg). R_f (60% EtOAc in petrol) 0.24; $[\alpha]_D^{20}$ +184.5 (*c* 0.4, $CHCl_3$); ν_{max}/cm^{-1} 2955 (C-H), 1741 (C=O), 1702 (C=O), 1668 (C=O), 1635 (C=C); δ_H (500 MHz, $CDCl_3$) 0.96 (9H, s, C(CH_3)$_3$), 3.29 (3H, s, NCH_3), 3.41 (1H, d, *J* 8.6, C(4)H_AH$_B$), 3.70 (3H, s, NOCH_3), 3.78 (3H, s, CO_2CH_3), 4.73 (1H, s, C(2)*H*), 4.80 (1H, d, *J* 8.6, C(4)H$_A$$H_B$), 7.26 (1H, s, C(6)*H*); δ_C (125 MHz, $CDCl_3$) 25.0 (C(*C*H$_3$)$_3$), 32.3 (N*C*H$_3$), 35.5 (*C*(CH$_3$)$_3$), 53.5 (CO_2CH_3), 61.8 (NO*C*H$_3$), 70.5 (*C*(4)), 76.2 (*C*(5)), 97.4 (*C*(2)), 137.7 (*C*(7)), 144.9 (*C*(6)), 162.3 (*C*(9)), 168.7 (CO_2CH_3), 173.3 (*C*(8)); *m/z* (ESI$^+$) 327.2 (MH$^+$, 100%), 349.0 (MNa$^+$, 56%); HRMS (ESI$^+$) found 327.15507, $C_{15}H_{23}N_2O_6$ (MH$^+$) requires 327.15506.

(2*R*,5*S*)-1-Aza-2-(*tert*-butyl)-7-ethoxycarbonyl-5-methoxycarbonyl-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (261)

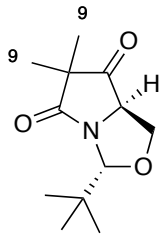


TMSI (10 μL, 0.070 mmol) was added to a solution of methyl ether **248** (20 mg, 0.058 mmol) in anhydrous DCM (300 μL). The solution was stirred at room temperature for 1 h, then diluted with H_2O and extracted with Et_2O. The combined organic exctracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude residue was dissolved in anhydrous DCM (600 μL), and triethylamine (24 μL, 0.17 mmol) was added. The mixture was then stirred at room temperature for 20 h, diluted with brine and extracted with Et_2O. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (10% to 20% EtOAc in petrol) to give alkene **261** (14 mg, 0.044 mmol, 75%) as a yellow oil. R_f (25% EtOAc in petrol) 0.24; $[\alpha]_D^{20}$ +159.2 (*c* 0.4, $CHCl_3$); ν_{max}/cm^{-1} 2960 (C-H), 1730 (C=O), 1627 (C=C); δ_H (400 MHz, $CDCl_3$) 0.94 (9H, s, C(CH_3)$_3$), 1.35 (3H, t, *J* 7.1, C(11)H_3), 3.35 (1H, d, *J* 8.6, C(4)H_AH$_B$), 3.77 (3H, s, CO_2CH_3), 4.33 (2H, q, *J* 7.1, C(10)H_2), 4.74 (1H, s, C(2)*H*), 4.81 (1H, d, *J* 8.6, C(4)H$_A$$H_B$), 7.75 (1H, s, C(6)*H*); δ_C (100 MHz, $CDCl_3$) 14.3 (*C*(11)), 24.9 (C(*C*H$_3$)$_3$), 35.5 (*C*(CH$_3$)$_3$), 53.6 (CO_2CH_3), 61.9 (*C*(10)), 70.5 (*C*(4)), 75.1 (*C*(5)), 97.6 (*C*(2)), 132.9 (*C*(7)), 152.5 (*C*(6)), 160.7 (*C*(9)), 168.2 (CO_2CH_3), 172.3 (*C*(8)); *m/z* (ESI$^+$) 334.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 334.12605, $C_{15}H_{21}NNaO_6$ (MNa$^+$) requires 334.12611.

6.4 Experimental for Chapter 4

Synthetic protocol and characterisation of previously reported compounds **294-297,**[136,139,242] **52,**[139] **303,**[139] **416,**[241] **330,**[241] **417,**[310] **422-424**[141,311]**, 374,**[249] **375**[250] and **381**,[251] which were prepared using the reported methods, are included in Appendix A.

(2*R*,5*R*)-1-Aza-2-(*tert*-butyl)-7,7-dimethyl-3-oxa-6,8-dioxobicyclo[3.3.0]-octane (298)

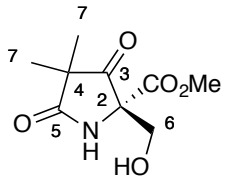


Side-product (4%, 150 mg) from the Dieckmann cyclisation of oxazolidine **296**; light yellow solid; m.p. 46 °C. R_f (DCM) 0.46; $[\alpha]_D^{20}$ -140.1 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2972 (C-H), 1769 (C=O), 1707 (C=O); δ_H (400 MHz, $CDCl_3$) 0.95 (9H, s, C(CH_3)$_3$), 1.20 (3H, s, C(9$_A$)H_3), 1.39 (3H, s, C(9$_B$)H_3), 3.44 (1H, dd, *J* 9.2, 8.2, C(4)H_AH$_B$), 4.27 (1H, dd, *J* 8.2, 7.2, C(4)H$_A$$H_B$), 4.36 (1H, dd, *J* 9.2, 7.2, C(5)*H*), 5.12 (1H, s, C(2)*H*); δ_C (100 MHz, $CDCl_3$) 18.6 (*C*(9$_A$)), 24.0 (*C*(9$_B$)), 24.8 (C(*C*H$_3$)$_3$), 36.4 (*C*(CH$_3$)$_3$), 51.4 (*C*(7)), 66.0 (*C*(5)), 67.1 (*C*(4)), 96.3 (*C*(2)), 179.8 (*C*(8)), 209.8 (*C*(6)); HRMS (CI$^+$) found 226.1443, $C_{12}H_{20}NO_3$ (MH$^+$) requires 226.1438.

General procedure M: *N,O*-Acetal deprotection of tetramic acids and pyrrolidinones[179]

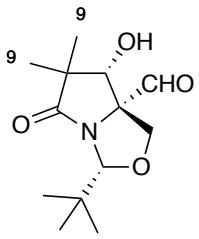
Tetramic acid or pyrrolidinone (1.0 eq) was treated with 1,3-propanedithiol (1.5-5 eq) followed by a freshly prepared solution of 1.5% HCl in 2,2,2-trifluoroethanol (*c* 0.06). The reaction mixture was heated at 50 °C (unless otherwise specified) for 12-28 h and then evaporated under reduced pressure. The crude product was purified by *flash* column chromatography to give the unprotected alcohol.

Methyl (*R*)-2-hydroxymethyl-4,4-dimethyl-3,5-dioxopyrrolidine-2-carboxylate (299)



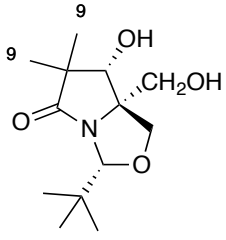
General procedure M (from **52**, 4.0 eq of 1,3-propanedithiol, for 15 h); yield 78% (249 mg); white solid; m.p. 84 °C. R_f (EtOAc) 0.48; $[\alpha]_D^{20}$ -20.0 (*c* 0.5, MeOH); ν_{max}/cm^{-1} 3543, 3338 (N-H, O-H), 2980 (C-H), 1775 (C=O), 1701 (C=O), 1691 (C=O); δ_H (400 MHz, CD_3OD) 1.19 (3H, s, C(7_A)H_3), 1.22 (3H, s, C(7_B)H_3), 3.76 (3H, s, CO_2CH_3), 3.78 (1H, d, *J* 11.6, C(6)H_AH_B), 4.15 (1H, d, *J* 11.6, C(6)H_AH_B); δ_C (100 MHz, CD_3OD) 19.8 (*C*(7_A)), 22.2 (*C*(7_B)), 47.4 (*C*(4)), 53.7 (CO_2CH_3), 63.7 (*C*(6)), 76.0 (*C*(2)), 168.3 (CO_2CH_3), 180.5 (*C*(5)), 209.6 (*C*(3)); *m/z* (ESI+) 216.1 (MH^+, 27%), 238.1 (MNa^+, 100%); HRMS (ESI+) found 216.08647, $C_9H_{14}NO_5$ (MH^+) requires 216.08665.

(2*R*,5*R*,6*S*)-1-Aza-2-(*tert*-butyl)-5-formyl-6-hydroxy-7,7-dimethyl-3-oxa-8-oxobicyclo[3.3.0]-octane (304)



Side-product (10%, 200 mg) from the reduction of tetramate **52**; white solid; m.p. 212 °C. R_f (40% EtOAc in petrol) 0.38; $[\alpha]_D^{20}$ +17.9 (*c* 0.8, MeOH); ν_{max}/cm^{-1} 3394 (O-H), 2967 (C-H), 1729 (C=O), 1684 (C=O); δ_H (400 MHz, $(CD_3)_2CO$) 0.83 (9H, s, C(CH_3)$_3$), 1.17 (3H, s, C(9_A)H_3), 1.30 (3H, s, C(9_B)H_3), 3.47 (1H, dd, *J* 8.4, 1.4, C(4)H_AH_B), 4.35 (1H, d, *J* 5.2, C(6)*H*), 4.77 (1H, d, *J* 8.4, C(4)H_AH_B), 4.78 (1H, s, C(2)*H*), 5.16 (1H, d, *J* 5.2, O*H*), 9.86 (1H, d, *J* 1.4, C*H*O); δ_C (100 MHz, $(CD_3)_2CO$) 21.7 (*C*(9_B)), 23.4 (*C*(9_A)), 25.5 (C(CH_3)$_3$), 36.1 (*C*(CH_3)$_3$), 50.2 (*C*(7)), 72.2 (*C*(4)), 78.2 (*C*(5)), 81.7 (*C*(6)), 96.5 (*C*(2)), 183.3 (*C*(8)), 199.9 (*C*HO); *m/z* (ESI+) 256.2 (MH^+, 47%); HRMS (ESI+) found 256.15445, $C_{13}H_{22}NO_4$ (MH^+) requires 256.15433.

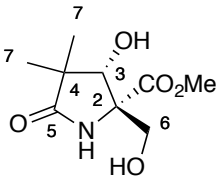
(2*R*,5*R*,6*S*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-hydroxymethyl-7,7-dimethyl-3-oxa-8-oxobicyclo[3.3.0]-octane (305)



Side-product (1%, 15 mg) from the reduction of tetramate **52**; white solid; m.p. 178 °C. R_f (40% EtOAc in petrol) 0.17; $[\alpha]_D^{20}$ +58.2 (*c* 0.5, $CHCl_3$); ν_{max}/cm^{-1} 3350 (O-H), 3282 (O-H), 2967 (C-H), 1676 (C=O); δ_H (400 MHz,

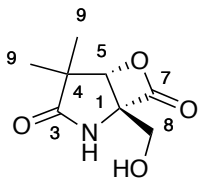
$CDCl_3$) 0.93 (9H, s, C(CH_3)$_3$), 1.18 (3H, s, C(9_A)H_3), 1.29 (3H, s, C(9_B)H_3), 3.20-3.23 (1H, m, C(5)CH_2O*H*), 3.23 (1H, d, *J* 8.2, C(4)H_AH_B), 3.84-3.89 (2H, m, C(5)CH_AH_B + C(6)O*H*), 3.97 (1H, dd, *J* 11.2, 2.8, C(5)CH_AH_B), 4.04 (1H, d, *J* 6.2, C(6)*H*), 4.32 (1H, d, *J* 8.2, C(4)H_AH_B), 4.76 (1H, s, C(2)*H*); δ_C (100 MHz, $CDCl_3$) 21.2 (*C*(9_B)), 24.5 (*C*(9_A)), 25.8 (C(*C*H_3)$_3$), 35.2 (*C*(CH_3)$_3$), 50.0 (*C*(7)), 63.0 (C(5)*C*H_2), 70.1 (*C*(5)), 74.6 (*C*(4)), 81.1 (*C*(6)), 96.8 (*C*(2)), 183.6 (*C*(8)); *m/z* (ESI$^+$) 258.2 (MH^+, 89%), 280.2 (MNa^+, 100%); HRMS (ESI$^+$) found 258.17004, $C_{13}H_{24}NO_4$ (MH^+) requires 258.16998.

Methyl (2*R*,3*S*)-3-hydroxy-2-hydroxymethyl-4,4-dimethyl-5-oxopyrrolidine-2-carboxylate (306)



General procedure M (from **303**, 4.0 eq of 1,3-propanedithiol, for 15 h); yield quant. (219 mg); white solid; m.p. 139-141 °C. R_f (10% MeOH in EtOAc) 0.42; $[\alpha]_D^{20}$ -16.6 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3347, 3217 (N-H, O-H), 2971 (C-H), 1716 (C=O), 1677 (C=O); δ_H (400 MHz, CD_3OD) 1.07 (3H, s, C(7_A)H_3), 1.15 (3H, s, C(7_B)H_3), 3.67 (1H, d, *J* 11.2, C(6)H_AH_B), 3.75 (3H, s, CO_2CH_3), 4.01 (1H, s, C(3)*H*), 4.06 (1H, d, *J* 11.2, C(6)H_AH_B); δ_C (100 MHz, CD_3OD) 19.6 (*C*(7_A)), 25.3 (*C*(7_B)), 45.5 (*C*(4)), 52.7 (CO_2CH_3), 66.0 (*C*(6)), 71.9 (*C*(2)), 78.5 (*C*(3)), 172.9 (*C*O_2CH_3), 183.7 (*C*(5)); *m/z* (ESI$^+$) 218.1 (MH^+, 100%), 240.1 (MNa^+, 80%); HRMS (ESI$^+$) found 218.10245, $C_9H_{16}NO_5$ (MH^+) requires 218.10230.

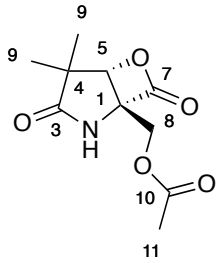
(1*R*,5*S*)-1-Hydroxymethyl-4,4-dimethyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (308)



Sodium hydroxide (43 mg, 1.1 mmol) was added to a solution of ester **306** (187 mg, 0.862 mmol) in 1:1:1 MeOH/THF/H_2O (8.4 mL). The mixture was stirred at room temperature for 1 h, acidified with 1 M aqueous HCl and concentrated *in vacuo* to give a white solid. Triethylamine (240 µL, 1.72 mmol) and BOP chloride (440 mg, 1.73 mmol) were added to a suspension of the crude acid in anhydrous DCM (17 mL). After stirring at room temperature for 19 h, brine was added and the aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (EtOAc) to give fused-β-lactone **308**

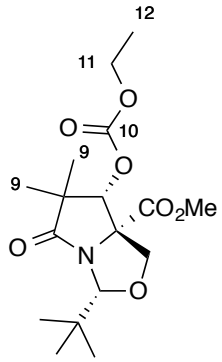
(71 mg, 0.38 mmol, 45%) as a white solid; m.p. 163 °C. R_f (EtOAc) 0.36; $[\alpha]_D^{20}$ -82.8 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3306, 3217 (N-H, O-H), 2930 (C-H), 1833 (C=O), 1686 (C=O); δ_H (400 MHz, CD_3OD) 1.20 (3H, s, C(9_A)H_3), 1.23 (3H, s, C(9_B)H_3), 3.74 (1H, d, *J* 12.2, C(8)H_AH_B), 4.00 (1H, d, *J* 12.2, C(8)H_AH_B), 4.81 (1H, s, C(5)*H*); δ_C (100 MHz, CD_3OD) 17.0 (*C*(9_B)), 24.3 (*C*(9_A)), 44.2 (*C*(4)), 58.7 (*C*(8)), 77.8 (*C*(1)), 81.8 (*C*(5)), 170.4 (*C*(7)), 182.5 (*C*(3)); *m/z* (ESI$^-$) 184.1 (M-H$^-$, 100%); HRMS (ESI$^-$) found 184.0610, $C_8H_{10}NO_4$ (M-H$^-$) requires 184.06153.

((1*R*,5*S*)-4,4-Dimethyl-3,7-dioxo-6-oxa-2-azabicyclo[3.2.0]heptan-1-yl)methyl acetate (310)



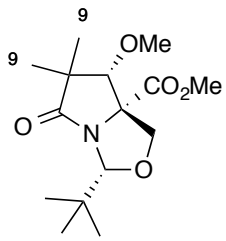
Pyridine (4 µL, 0.05 mmol) and acetic anhydride (5 µL, 0.05 mmol) were added to a suspension of β-lactone **308** (6 mg, 0.03 mmol) in anhydrous DCM (300 µL). After stirring at room temperature for 13 h, the product was concentrated under reduced pressure to give pure acetylated β-lactone **310** (7 mg, 0.03 mmol, quant.) as a white solid; m.p. 153 °C. R_f (50% EtOAc in DCM) 0.48; $[\alpha]_D^{20}$ -40.8 (*c* 0.4, $CHCl_3$); ν_{max}/cm^{-1} 3171 (N-H), 2971 (C-H), 1843 (C=O), 1746 (C=O), 1706 (C=O); δ_H (500 MHz, $CDCl_3$) 1.23 (3H, s, C(9_A)H_3), 1.31 (3H, s, C(9_B)H_3), 2.13 (3H, s, C(11)H_3), 4.46 (1H, d, *J* 12.5, C(8)H_AH_B), 4.50 (1H, d, *J* 12.5, C(8)H_AH_B), 4.67 (1H, s, C(5)*H*), 6.63 (1H, br s, N*H*); δ_C (125 MHz, $CDCl_3$) 16.8 (*C*(9_B)), 20.7 (*C*(11)), 24.2 (*C*(9_A)), 42.9 (*C*(4)), 59.4 (*C*(8)), 73.4 (*C*(1)), 81.3 (*C*(5)), 166.8 (*C*(7)), 170.3 (*C*(10)), 179.6 (*C*(3)); *m/z* (ESI$^+$) 228.1 (MH$^+$, 10%), 250.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 228.08668, $C_{10}H_{14}NO_5$ (MH$^+$) requires 228.08665.

(2*R*,5*R*,6*S*)-1-Aza-2-(*tert*-butyl)-6-(ethoxycarbonyl)oxy-5-methoxycarbonyl-7,7-dimethyl-3-oxa-8-oxobicyclo[3.3.0]-octane (312)



Diethyl azodicarboxylate (1.7 eq) was added dropwise to a solution of alcohol **303** (1.0 eq), triphenylphosphine (4 eq) and either benzoic acid, *p*-nitro benzoic acid or chloroacetic acid (1.0 eq) in anhydrous THF (*c* 0.1 M) at 0 °C. The solution was stirred at room temperature for 18-24 h and then concentrated *in vacuo*. Purification by *flash* column chromatography gave the undesired carbonate ester **312** as a colourless oil, instead of the desired inversion product. R_f (20% EtOAc in petrol) 0.34; $[\alpha]_D^{20}$ -30.3 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2960 (C-H), 1757 (C=O), 1723 (C=O); δ_H (500 MHz, C_6D_6) 0.87 (3H, t, *J* 7.2, C(12)H_3), 1.07 (9H, s, C(CH_3)$_3$), 1.16 (3H, s, C(9$_A$)H_3), 1.47 (3H, s, C(9$_B$)H_3), 3.30 (3H, s, CO_2CH_3), 3.32 (1H, d, *J* 9.1, C(4)H_AH_B), 3.81 (1H, dq, *J* 10.6, 7.2, C(11)H_AH_B), 3.90 (1H, dq, *J* 10.6, 7.2, C(11)H_AH_B), 4.85 (1H, s, C(6)*H*), 4.90 (1H, s, C(2)*H*), 5.01 (1H, d, *J* 9.1, C(4)H_AH_B); δ_C (125 MHz, C_6D_6) 14.0 (*C*(12)), 20.5 (*C*(9$_B$)), 24.7 (*C*(9$_A$)), 25.4 (C(*C*H$_3$)$_3$), 35.9 (*C*(CH$_3$)$_3$), 48.7 (*C*(7)), 52.2 (CO_2CH_3), 64.8 (*C*(11)), 73.9 (*C*(5)), 76.2 (*C*(4)), 83.3 (*C*(6)), 97.4 (*C*(2)), 154.8 (*C*(10)), 170.0 (CO_2CH_3), 180.0 (*C*(8)); *m/z* (ESI$^+$) 358.3 (MH^+, 46%), 380.2 (MNa^+, 100%); HRMS (ESI$^+$) found 380.16790, $C_{17}H_{27}NNaO_7$ (MNa^+) requires 380.16797.

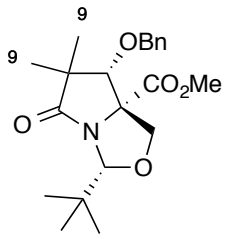
(2*R*,5*R*,6*S*)-1-Aza-2-(*tert*-butyl)-6-methoxy-5-methoxycarbonyl-7,7-dimethyl-3-oxa-8-oxobicyclo[3.3.0]-octane (319)



NaH (60% w/w, 20 mg, 0.51 mmol) was added portionwise to a solution of alcohol **303** (97 mg, 0.34 mmol) and methyl iodide (24 µL, 0.38 mmol) in anhydrous THF (3.4 mL) at 0 °C. The solution was stirred at 0 °C for 30 min and at room temperature for 4 h. H_2O was then added and the aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give methyl ether **319** (104 mg, 0.347 mmol, quant.) as a white solid; m.p. 57 °C. R_f (20% EtOAc in petrol) 0.41; $[\alpha]_D^{20}$ +8.9 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2975 (C-H), 2954 (C-H), 1741 (C=O), 1712 (C=O); δ_H (400 MHz, $CDCl_3$) 0.88 (9H, s, C(CH_3)$_3$), 1.22 (3H, s, C(9$_A$)H_3), 1.32 (3H, s, C(9$_B$)H_3), 3.37 (1H, d, *J* 8.4, C(4)H_AH_B), 3.39 (3H, s, C(6)OCH_3), 3.55 (1H, s, C(6)*H*), 3.78 (3H, s,

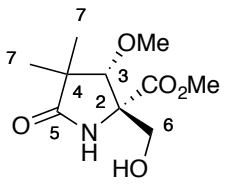
CO_2CH_3), 4.82 (1H, s, C(2)*H*), 4.97 (1H, d, *J* 8.4, C(4)H_AH_B); δ_C (100 MHz, $CDCl_3$) 20.1 (*C*(9_B)), 25.1 (C(*C*H_3)$_3$), 25.5 (*C*(9_A)), 35.7 (*C*(CH_3)$_3$), 49.6 (*C*(7)), 52.5 (CO_2CH_3), 60.3 (C(6)O*C*H_3), 74.8 (*C*(5)), 75.8 (*C*(4)), 90.5 (*C*(6)), 96.8 (*C*(2)), 170.5 (*C*O_2CH_3), 182.1 (*C*(8)); *m/z* (ESI$^+$) 300.2 (MH^+, 66%), 322.2 (MNa^+, 100%); HRMS (CI$^+$) found 300.1801, $C_{15}H_{26}NO_5$ (MH^+) requires 300.1805.

(2*R*,5*R*,6*S*)-1-Aza-6-benzyloxy-2-(*tert*-butyl)-5-methoxycarbonyl-7,7-dimethyl-3-oxa-8-oxobicyclo[3.3.0]-octane (320)



NaH (60% w/w, 20 mg, 0.50 mmol) was added portionwise to a solution of alcohol **303** (88 mg, 0.31 mmol) and benzyl bromide (47 µL, 0.40 mmol) in anhydrous THF (3.5 mL) at 0 °C. The solution was stirred at 0 °C for 30 min and at room temperature for 17 h. H_2O was then added and the aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (petrol to 20% EtOAc in petrol) to give benzyl ether **320** (106 mg, 0.281 mmol, 91%) as a colourless oil. R_f (20% EtOAc in petrol) 0.42; $[\alpha]_D^{20}$ -18.0 (*c* 1.0, DCM); ν_{max}/cm^{-1} 2958 (C-H), 1714 (C=O); δ_H (400 MHz, $CDCl_3$) 0.89 (9H, s, C(CH_3)$_3$), 1.12 (3H, s, C(9_A)*H*$_3$), 1.38 (3H, s, C(9_B)*H*$_3$), 3.33 (1H, d, *J* 8.4, C(4)H_AH_B), 3.76 (4H, s, C(6)*H* + CO_2CH_3), 4.44 (1H, d, *J* 11.8, OCH_AH_BPh), 4.67 (1H, d, *J* 11.8, OCH_AH_BPh), 4.82 (1H, s, C(2)*H*), 4.96 (1H, d, *J* 8.4, C(4)H_AH_B), 7.26-7.38 (5H, m, Ph); δ_C (100 MHz, $CDCl_3$) 20.5 (*C*(9_B)), 25.1 (C(*C*H_3)$_3$), 25.1 (*C*(9_A)), 35.8 (*C*(CH_3)$_3$), 49.6 (*C*(7)), 52.6 (CO_2CH_3), 73.6 (O*C*H_2Ph), 74.8 (*C*(5)), 75.7 (*C*(4)), 87.0 (*C*(6)), 96.8 (*C*(2)), 127.8, 128.3, 128.6, 137.0 (Ph), 170.7 (*C*O_2CH_3), 182.1 (*C*(8)); *m/z* (ESI$^+$) 376.2 (MH^+, 35%), 398.2 (MNa^+, 100%); HRMS (ESI$^+$) found 376.21183, $C_{21}H_{30}NO_5$ (MH^+) requires 376.21185.

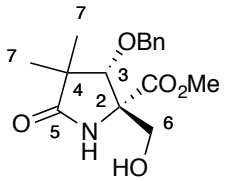
Methyl (2*R*,3*S*)-2-hydroxymethyl-3-methoxy-4,4-dimethyl-5-oxopyrrolidine-2-carboxylate (321)



General procedure M (from **319**, 4.0 eq of 1,3-propanedithiol, for 13 h); yield 95% (89 mg); white solid; m.p. 132 °C. R_f (10% MeOH in EtOAc) 0.48; $[\alpha]_D^{20}$ -36.4 (*c* 1.1, MeOH); ν_{max}/cm^{-1} 3339 (N-H, O-H), 2953 (C-H), 1738 (C=O),

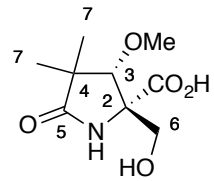
1684 (C=O); δ_H (400 MHz, CD_3OD) 1.09 (3H, s, C(7_A)H_3), 1.21 (3H, s, C(7_B)H_3), 3.47 (3H, s, C(3)OCH_3), 3.74 (1H, d, *J* 11.4, C(6)H_AH_B), 3.74 (4H, s, CO_2CH_3 + C(3)*H*), 4.05 (1H, d, *J* 11.4, C(6)H_AH_B); δ_C (100 MHz, CD_3OD) 19.7 (*C*(7_A)), 26.0 (*C*(7_B)), 45.5 (*C*(4)), 52.7 (CO_2CH_3), 61.0 (C(3)O*C*H_3), 66.2 (*C*(6)), 71.3 (*C*(2)), 88.5 (*C*(3)), 172.5 (CO_2CH_3), 183.1 (*C*(5)); *m/z* (ESI^+) 232.0 (MH^+, 37%), 254.0 (MNa^+, 100%); HRMS (ESI^+) found 232.11828, $C_{10}H_{18}NO_5$ (MH^+) requires 232.11795.

Methyl (2*R*,3*S*)-3-benzyloxy-2-hydroxymethyl-4,4-dimethyl-5-oxopyrrolidine-2-carboxylate (322)



General procedure M (from **320**, 4.0 eq of 1,3-propanedithiol, for 12 h); yield quant. (81 mg); colourless oil. R_f (EtOAc) 0.24; $[\alpha]_D^{20}$ -39.1 (*c* 0.5, DCM); ν_{max}/cm^{-1} 3338 (N-H, O-H), 2951 (C-H), 1743 (C=O), 1698 (C=O); δ_H (400 MHz, CD_3OD) 1.13 (3H, s, C(7_A)H_3), 1.14 (3H, s, C(7_B)H_3), 3.70 (3H, s, CO_2CH_3), 3.75 (1H, d, *J* 11.6, C(6)H_AH_B), 3.99 (1H, s, C(3)*H*), 4.07 (1H, d, *J* 11.6, C(6)H_AH_B), 4.59 (1H, d, *J* 11.6, OCH_AH_BPh), 4.72 (1H, d, *J* 11.6, OCH_AH_BPh), 7.27-7.37 (5H, m, Ph); δ_C (100 MHz, CD_3OD) 20.3 (*C*(7_B)), 25.7 (*C*(7_A)), 45.6 (*C*(4)), 52.8 (CO_2CH_3), 66.1 (*C*(6)), 71.4 (*C*(2)), 75.3 (O*C*H_2Ph), 85.7 (*C*(3)), 128.9, 129.4, 129.4, 139.2 (Ph), 172.6 (CO_2CH_3), 183.2 (*C*(5)); *m/z* (ESI^+) 308.1 (MH^+, 14%), 330.1 (MNa^+, 100%); HRMS (ESI^+) found 308.14931, $C_{16}H_{22}NO_5$ (MH^+) requires 308.14925.

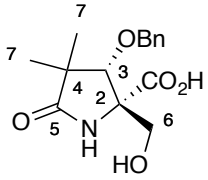
(2*R*,3*S*)-2-Hydroxymethyl-3-methoxy-4,4-dimethyl-5-oxopyrrolidine-2-carboxylic acid (323)



NaOH (20 mg, 0.49 mmol) was added to a solution of ester **321** (82 mg, 0.35 mmol) in 1:1:1 MeOH/THF/H_2O (3.6 mL). The reaction mixture was stirred at room temperature for 24 h, diluted with brine and acidified with 2 M aqueous HCl. The aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give acid **323** (65 mg, 0.30 mmol, 85%) as a colourless oil. $[\alpha]_D^{20}$ -32.8 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3304 (N-H, O-H), 2937 (C-H), 1669 (C=O); δ_H (400 MHz, CD_3OD) 1.13 (3H, s, C(7_A)H_3), 1.21 (3H, s, C(7_B)H_3), 3.50 (3H, s, C(3)OCH_3), 3.76 (1H, d, *J* 11.5,

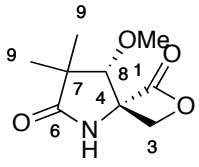
C(6)H_AH_B), 3.76 (1H, s, C(3)*H*), 4.04 (1H, d, *J* 11.5, C(6)H_AH_B); δ_C (100 MHz, CD_3OD) 19.8 (*C*(7_A)), 25.9 (*C*(7_B)), 45.6 (*C*(4)), 61.0 (C(3)O*C*H_3), 66.2 (*C*(6)), 70.9 (*C*(2)), 88.3 (*C*(3)), 173.7 (*C*O_2H), 183.2 (*C*(5)); *m/z* (ESI^+) 218.1 (MH^+, 51%), 240.1 (MNa^+, 100%); *m/z* (ESI^-) 216.1 ($M\text{-}H^-$, 100%); HRMS (ESI^-) found 216.08728, $C_9H_{14}NO_5$ ($M\text{-}H^-$) requires 216.08775.

(2*R*,3*S*)-3-Benzyloxy-2-hydroxymethyl-4,4-dimethyl-5-oxopyrrolidine-2-carboxylic acid (324)



Sodium hydroxide (6 mg, 0.1 mmol) was added to a solution of ester **322** (26 mg, 0.084 mmol) in 1:1:1 MeOH/THF/H_2O (840 μL). The mixture was stirred at room temperature for 24 h, diluted with H_2O and washed with DCM. The aqueous layer was acidified with 1 M aqueous HCl, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give acid **324** (23 mg, 0.078 mmol, 93%) as white solid; m.p. 66 °C. $[\alpha]_D^{20}$ -31.1 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3293 (O-H), 2928 (C-H), 1665 (C=O); δ_H (400 MHz, CD_3OD) 1.13 (3H, s, C(7_A)H_3), 1.17 (3H, s, C(7_B)H_3), 3.77 (1H, d, *J* 11.6, C(6)H_AH_B), 4.00 (1H, s, C(3)*H*), 4.06 (1H, d, *J* 11.6, C(6)H_AH_B), 4.59 (1H, d, *J* 11.6, OCH_AH_BPh), 4.79 (1H, d, *J* 11.6, OCH_AH_BPh), 7.28-7.38 (5H, m, Ph); δ_C (100 MHz, CD_3OD) 19.0 (*C*(7_B)), 24.3 (*C*(7_A)), 44.3 (*C*(4)), 64.8 (*C*(6)), 69.7 (*C*(2)), 73.9 (O*C*H_2Ph), 84.2 (*C*(3)), 127.4, 127.6, 127.9, 137.9 (Ph), 172.4 (*C*O_2H), 181.9 (*C*(5)); *m/z* (ESI^+) 294.1 (MH^+, 30%), 316.1 (MNa^+, 100%), *m/z* (ESI^-) 292.1 ($M\text{-}H^-$, 100%); HRMS (ESI^-) found 292.11899, $C_{15}H_{18}NO_5$ ($M\text{-}H^-$) requires 292.11905.

(4*R*,8*S*)-8-Methoxy-7,7-dimethyl-2-oxa-5-azaspiro[3.4]octane-1,6-dione (325)



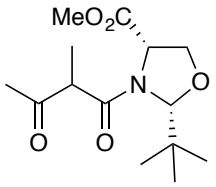
HATU (228 mg, 0.600 mmol) and DIPEA (100 μL, 0.574 mmol) were added to a solution of acid **323** (65 mg, 0.30 mmol) in anhydrous THF (10 mL) at 0 °C. The mixture was stirred at room temperature for 36 h and quenched with brine. The aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by *flash* column chromatography (40% to 50% EtOAc in petrol) to give spiro-β-lactone **325** (16 mg, 0.081 mmol, 27%) as a 2.5:1 inseparable mixture with

tetramethylurea **327**. R_f (EtOAc) 0.51; ν_{max}/cm^{-1} 3228 (N-H), 2973 (C-H), 1827 (C=O), 1714 (C=O); δ_H (500 MHz, CD_3OD) 1.25 (3H, s, C(9$_A$)H_3), 1.28 (3H, s, C(9$_B$)H_3), 3.58 (3H, s, C(8)OCH_3), 4.10 (1H, s, C(8)H), 4.43 (1H, d, *J* 6.0, C(3)H_AH_B), 4.51 (1H, d, *J* 6.0, C(3)H_AH_B); δ_C (125 MHz, CD_3OD) 20.2 (*C*(9$_A$)), 24.2 (*C*(9$_B$)), 45.4 (*C*(7)), 60.4 (C(8)O*C*H$_3$), 70.6 (*C*(3)), 77.2 (*C*(4)), 88.8 (*C*(8)), 171.3 (*C*(1)), 182.0 (*C*(6)); *m/z* (ESI$^+$) 200.2 (MH$^+$, 51%), 222.0 (MNa$^+$, 100%); HRMS (ESI$^+$) found 200.09190, $C_9H_{14}NO_4$ (MH$^+$) requires 200.09173.

General procedure N: *N*-Acylation of oxazolidines[136]

To a solution of oxazolidine (1.0 eq) in anhydrous DCM (*c* 0.5) were added DMAP (0.05 eq) and DCC (1.05 eq). The mixture was cooled to 0 °C and the required β-carbonyl carboxylic acid (1.05 eq) was added. After stirring 30 min at 0 °C and 5 h at room temperature, the reaction mixture was filtered and washed with DCM. The combined filtrates were concentrated *in vacuo* and purified by *flash* column chromatography to give the *N*-acyloxazolidine.

(2*R*,5*S*)-2-(*tert*-Butyl)-5-methoxycarbonyl-1-(2-methyl-3-oxobutanoyl)-1,3-oxazolidine (331)

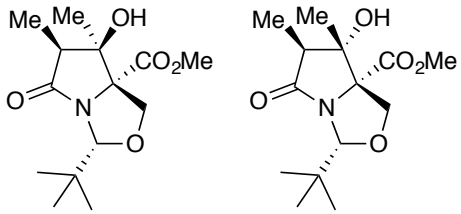


General procedure N (from **42a**, with **330**); yield 43% (506 mg); yellow solid; m.p. 93 °C. R_f (5% EtOAc in DCM) 0.22; $[\alpha]_D^{20}$ -16.6 (*c* 0.5, $CHCl_3$); ν_{max}/cm^{-1} 2957 (C-H), 1744 (C=O), 1730 (C=O), 1656 (C=O); δ_H (400 MHz, $CDCl_3$) 0.94 (9H, s, C(CH_3)$_3$), 1.31 (3H, d, *J* 6.8, C(7)CH_3), 2.28 (3H, s, C(6)CH_3), 3.62 (1H, q, *J* 6.8, C(7)H), 3.81 (3H, s, CO_2CH_3), 4.06 (1H, t, *J* 8.8, C(4)H_AH_B), 4.52-4.58 (2H, m, C(4)H_AH_B + C(5)H), 5.35 (1H, s, C(2)H); δ_C (125 MHz, $CDCl_3$) 12.9 (C(7)*C*H$_3$), 25.9 (C(*C*H$_3$)$_3$), 27.7 (C(6)*C*H$_3$), 37.9 (*C*(CH$_3$)$_3$), 53.0 (CO_2CH_3), 53.7 (*C*(7)), 59.6 (*C*(5)), 68.2 (*C*(4)), 97.0 (*C*(2)), 170.0 (*C*O$_2$CH$_3$), 172.5 (*C*(8)), 203.0 (*C*(6)); *m/z* (ESI$^+$) 286.1 (MH$^+$, 19%), 308.1 (MNa$^+$, 100%); HRMS (ESI$^+$) found 286.16519, $C_{14}H_{24}NO_5$ (MH$^+$) requires 286.16490.

General procedure O: Aldol cyclisation of *N*-acyloxazolidines[141]

Sodium methoxide (1.05 eq) was added to a solution of *N*-acyloxazolidine (1.0 eq) in anhydrous MeOH (*c* 0.2), and stirring was continued at room temperature for 18 h. The reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with Et_2O. The organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography to give the bicyclic pyrrolidinone.

(2*R*,5*R*,6*R*,7*S*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-6,7-dimethyl-3-oxa-8-oxobicyclo[3.3.0]-octane (332I) and (2*R*,5*R*,6*S*,7*S*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-6,7-dimethyl-3-oxa-8-oxobicyclo[3.3.0]-octane (332II)



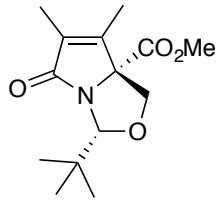
General procedure O (from **331**).

Major diastereomer (**(*R*)-332I**): Yield 55% (355 mg); white solid; m.p. 162 °C. R_f (40% EtOAc in petrol) 0.27; $[\alpha]_D^{20}$ +18.8 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 3405 (O-H), 2955 (C-H), 1733 (C=O), 1692 (C=O); δ_H (500 MHz, $CDCl_3$) 0.87 (9H, s, C(CH_3)$_3$), 1.09 (3H, d, *J* 7.2, C(7)CH_3), 1.26 (3H, s, C(6)CH_3), 2.24 (1H, br s, O*H*), 3.12 (1H, q, *J* 7.2, C(7)*H*), 3.78 (3H, s, CO_2CH_3), 4.29 (1H, d, *J* 8.9, C(4)H_AH_B), 4.52 (1H, d, *J* 8.9, C(4)H_AH_B), 4.92 (1H, s, C(2)*H*); δ_C (125 MHz, $CDCl_3$) 6.6 (C(7)*C*H$_3$), 22.2 (C(6)*C*H$_3$), 25.1 (C(*C*H$_3$)$_3$), 36.7 (*C*(CH$_3$)$_3$), 48.9 (*C*(7)), 52.8 (CO_2CH_3), 68.3 (*C*(4)), 79.4 (*C*(5)), 80.2 (*C*(6)), 96.7 (*C*(2)), 172.0 (CO_2CH_3), 179.0 (*C*(8)); *m/z* (ESI$^+$) 286.2 (MH$^+$, 44%), 308.1 (MNa$^+$, 22%); HRMS (ESI$^+$) found 286.16494, $C_{14}H_{24}NO_5$ (MH$^+$) requires 286.16490.

Minor diastereomer (**(*S*)-332II**): Yield 23% (150 mg); white solid; m.p. 179 °C. R_f (40% EtOAc in petrol) 0.20; $[\alpha]_D^{20}$ +15.9 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 3356 (O-H), 2959 (C-H), 1733 (C=O), 1686 (C=O); δ_H (500 MHz, $CDCl_3$) 0.89 (9H, s, C(CH_3)$_3$), 1.08 (3H, d, *J* 7.5, C(7)CH_3), 1.23 (3H, s, C(6)CH_3),

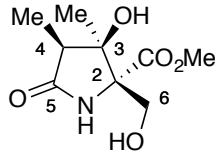
2.31 (1H, br s, O*H*), 3.44 (1H, q, *J* 7.5, C(7)*H*), 3.81 (3H, s, CO_2CH_3), 3.96 (1H, d, *J* 9.5, C(4)H_AH_B), 4.62 (1H, d, *J* 9.5, C(4)H_AH_B), 4.90 (1H, s, C(2)*H*); δ_C (125 MHz, $CDCl_3$) 7.9 (C(7)*C*H_3), 20.2 (C(6)*C*H_3), 25.1 (C(*C*H_3)$_3$), 36.7 (*C*(CH_3)$_3$), 49.1 (*C*(7)), 52.8 (CO_2CH_3), 69.1 (*C*(4)), 78.8 (*C*(5)), 80.7 (*C*(6)), 96.2 (*C*(2)), 171.9 (CO_2CH_3), 177.0 (*C*(8)); *m/z* (ESI^+) 286.2 (MH^+, 100%), 308.1 (MNa^+, 22%); HRMS (ESI^+) found 286.16497, $C_{14}H_{24}NO_5$ (MH^+) requires 286.16490.

(2*R*,5*S*)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-6,7-dimethyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (333)



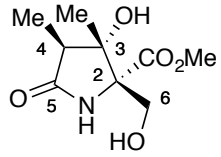
Side-product (9%, 54 mg) from the aldol cyclisation of oxazolidine **331**; colourless oil. R_f (10% EtOAc in petrol) 0.45; $[\alpha]_D^{20}$ +259.5 (*c* 0.5, $CHCl_3$); ν_{max}/cm^{-1} 2957 (C-H), 1742 (C=O), 1708 (C=O); δ_H (500 MHz, $CDCl_3$) 0.93 (9H, s, C(CH_3)$_3$), 1.78-1.80 (3H, m, C(7)CH_3), 1.82-1.84 (3H, m, C(6)CH_3), 3.19 (1H, d, *J* 8.2, C(4)H_AH_B), 3.75 (3H, s, CO_2CH_3), 4.66 (1H, s, C(2)*H*), 4.79 (1H, d, *J* 8.2, C(4)H_AH_B); δ_C (125 MHz, $CDCl_3$) 9.0 (C(7)*C*H_3), 12.3 (C(6)*C*H_3), 24.9 (C(*C*H_3)$_3$), 35.4 (*C*(CH_3)$_3$), 53.1 (CO_2CH_3), 70.6 (*C*(4)), 78.1 (*C*(5)), 96.9 (*C*(2)), 131.4 (*C*(7)), 150.4 (*C*(6)), 170.3 (CO_2CH_3), 179.3 (*C*(8)); *m/z* (ESI^+) 268.1 (MH^+, 66%), 290.1 (MNa^+, 100%); HRMS (ESI^+) found 268.15448, $C_{14}H_{22}NO_4$ (MH^+) requires 268.15433.

Methyl (2*R*,3*R*,4*S*)-3-hydroxy-2-hydroxymethyl-3,4-dimethyl-5-oxopyrrolidine-2-carboxylate (334I)



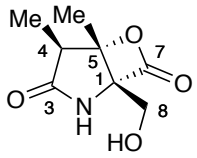
General procedure M (from **332I**, 1.5 eq of 1,3-propanedithiol, for 12 h); yield quant. (357 mg); colourless oil. R_f (10% MeOH in EtOAc) 0.37; $[\alpha]_D^{20}$ -41.8 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3342 (O-H), 2981 (C-H), 2945 (C-H), 1729 (C=O), 1684 (C=O); δ_H (400 MHz, CD_3OD) 1.05 (3H, d, *J* 7.2, C(4)CH_3), 1.26 (3H, s, C(3)CH_3), 2.41 (1H, q, *J* 7.2, C(4)*H*), 3.77 (3H, s, CO_2CH_3), 3.83 (1H, d, *J* 11.3, C(6)H_AH_B), 4.18 (1H, d, *J* 11.3, C(6)H_AH_B); δ_C (100 MHz, CD_3OD) 7.3 (C(4)*C*H_3), 22.9 (C(3)*C*H_3), 47.9 (*C*(4)), 53.0 (CO_2CH_3), 64.1 (*C*(6)), 75.5 (*C*(2)), 79.4 (*C*(3)), 173.7 (CO_2CH_3), 181.0 (*C*(5)); *m/z* (ESI^+) 218.1 (MH^+, 23%), 240.1 (MNa^+, 54%); HRMS (ESI^+) found 240.08436, $C_9H_{15}NNaO_5$ (MNa^+) requires 240.08534.

Methyl (2*R*,3*S*,4*S*)-3-hydroxy-2-hydroxymethyl-3,4-dimethyl-5-oxopyrrolidine-2-carboxylate (334II)



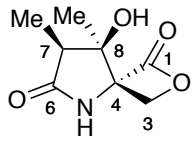
General procedure M (from **332II**, 1.5 eq of 1,3-propanedithiol, for 16 h); yield 92% (70 mg); white solid; m.p. 166 °C. R_f (10% MeOH in EtOAc) 0.30; $[\alpha]_D^{20}$ -31.6 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3373 (O-H or N-H), 3334 (O-H or N-H), 2960 (C-H), 1743 (C=O), 1661 (C=O); δ_H (400 MHz, CD_3OD) 1.12 (3H, d, *J* 7.6, C(4)CH_3), 1.28 (3H, s, C(3)CH_3), 2.47 (1H, q, *J* 7.6, C(4)*H*), 3.61 (1H, d, *J* 10.8, C(6)H_AH_B), 3.76 (3H, s, CO_2CH_3), 4.06 (1H, d, *J* 10.8, C(6)H_AH_B); δ_C (100 MHz, CD_3OD) 11.7 (C(4)*C*H_3), 19.0 (C(3)*C*H_3), 50.3 (*C*(4)), 52.7 (CO_2CH_3), 65.1 (*C*(6)), 76.5 (*C*(2)), 79.4 (*C*(3)), 172.4 (CO_2CH_3), 180.8 (*C*(5)); *m/z* (ESI^+) 218.1 (MH^+, 11%), 240.1 (MNa^+, 29%); HRMS (ESI^+) found 218.10251, $C_9H_{16}NO_5$ (MH^+) requires 218.10230.

(1*R*,4*S*,5*S*)-1-Hydroxymethyl-4,5-dimethyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (336)



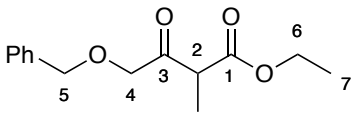
Sodium hydroxide (16 mg, 0.39 mmol) was added to a solution of ester **334II** (64 mg, 0.29 mmol) in 1:1:1 MeOH/THF/H_2O (3 mL). The mixture was stirred at room temperature for 2 h, acidified with 2 M aqueous HCl and concentrated *in vacuo*. The residue was dissolved in anhydrous DCM (5.9 mL), and triethylamine (82 μL, 0.59 mmol) and BOP chloride (152 mg, 0.597 mmol) were added. After stirring at room temperature for 15 h, brine was added and the aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (EtOAc) to give fused-β-lactone **336** (8 mg, 0.04 mmol, 21%) as a white solid; m.p. 133 °C. R_f (EtOAc) 0.34; $[\alpha]_D^{20}$ -56.3 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3391 (O-H or N-H), 3229 (O-H or N-H), 2929 (C-H), 1825 (C=O), 1666 (C=O); δ_H (500 MHz, CD_3OD) 1.23 (3H, d, *J* 8.0, C(4)CH_3), 1.68 (3H, s, C(5)CH_3), 2.77 (1H, q, *J* 8.0, C(4)*H*), 3.81 (1H, d, *J* 12.6, C(8)H_AH_B), 3.95 (1H, d, *J* 12.6, C(8)H_AH_B); δ_C (125 MHz, CD_3OD) 13.2 (C(4)*C*H_3), 15.7 (C(5)*C*H_3), 46.5 (*C*(4)), 57.0 (*C*(8)), 79.1 (*C*(1)), 87.5 (*C*(5)), 170.4 (*C*(7)), 180.4 (*C*(3)); *m/z* (ESI^-) 184.1 ($M\text{-}H^-$, 39%); HRMS (ESI^+) found 186.07628, $C_8H_{12}NO_4$ (MH^+) requires 186.07608.

(4*R*,7*S*,8*R*)-8-Hydroxy-7,8-dimethyl-2-oxa-5-azaspiro[3.4]octane-1,6-dione (337)



Sodium hydroxide (21 mg, 0.53 mmol) was added to a solution of ester **334I** (90 mg, 0.41 mmol) in 1:1:1 MeOH/THF/H_2O (3.9 mL). The mixture was stirred at room temperature for 2 h, acidified with 2 M aqueous HCl and concentrated *in vacuo*. To a solution of the residue in anhydrous THF (14 mL) at 0 °C were added HATU (329 mg, 0.865 mmol) and DIPEA (288 μL, 1.65 mmol), and the mixture was stirred at room temperature for 32 h. Brine was then added and the aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (30% to 80% EtOAc in petrol) to give spiro-β-lactone **337** (27 mg, 0.037 mmol, 9%) as a yellow oil, as a 1:4 inseparable mixture with tetramethylurea **327**. R_f (EtOAc) 0.41; ν_{max}/cm^{-1} 2944 (C-H), 2885 (C-H), 1832 (C=O), 1720 (C=O); δ_H (500 MHz, CD_3OD) 1.10 (3H, d, *J* 7.2, C(7)CH_3), 1.42 (3H, s, C(8)CH_3), 2.48 (1H, q, *J* 7.2, C(7)*H*), 4.39 (1H, d, *J* 6.3, C(3)H_AH_B), 5.67 (1H, d, *J* 6.3, C(3)H_AH_B); δ_C (125 MHz, CD_3OD) 7.3 (C(7)*C*H_3), 20.9 (C(8)*C*H_3), 47.9 (*C*(7)), 67.7 (*C*(3)), 77.5 (*C*(8)), 81.8 (*C*(4)), 172.6 (*C*(1)), 180.1 (*C*(6)); HRMS (CI^+) found 186.0761, $C_8H_{12}NO_4$ (MH^+) requires 186.0761.

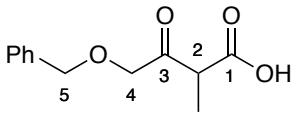
Ethyl 4-benzyloxy-2-methyl-3-oxobutanoate (418)[312]



To ester **417** (8.44 g, 35.7 mmol) in anhydrous THF (36 mL) was added potassium *tert*-butoxide (4.00 g, 35.6 mmol) portionwise at 0 °C. After stirring at room temperature for 45 min, the yellow solution was cooled again to 0 °C and methyl iodide (2.4 mL, 39 mmol) was added dropwise. After stirring at room temperature for another 6 h, water was added and extracted with Et_2O. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give ester **418** (8.10 g, 32.4 mmol, 91%) as a yellow oil. R_f (10% EtOAc in petrol) 0.24; ν_{max}/cm^{-1} 2985 (C-H), 1719 (C=O); δ_H (400 MHz, $CDCl_3$) 1.21 (3H, t, *J* 7.0, C(7)H_3), 1.34 (3H, d, *J* 7.2, C(2)CH_3), 3.71 (1H, q, *J* 7.2, C(2)*H*), 4.10-4.16 (2H, m, C(6)H_2), 4.18 (2H, s, C(4)H_2), 4.58 (2H, s, C(5)H_2), 7.30-7.38 (5H, m, Ph); δ_C (100 MHz, $CDCl_3$) 12.4 (C(2)*C*H_3), 14.1 (*C*(7)), 49.2 (*C*(2)), 61.5 (*C*(6)), 73.6 (*C*(5)), 74.4 (*C*(4)),

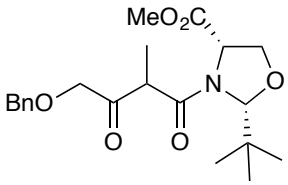
127.9, 128.2, 128.6, 137.1 (Ph), 170.5 (*C*(1)), 204.5 (*C*(3)); *m/z* (ESI+) 273.1 (MNa^+, 100%); HRMS (ESI+) found 273.10965, $C_{14}H_{18}NaO_4$ (MNa^+) requires 273.10973.

4-Benzyloxy-2-methyl-3-oxobutanoic acid (419)[140]



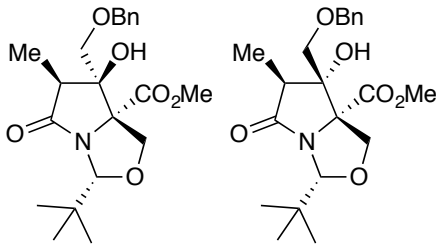
Ethyl ester **418** (8.06 g, 32.2 mmol) was stirred with aqueous NaOH (1 M, 35 mL, 35 mmol) and H_2O (54 mL) at room temperature. After 36 h, the aqueous phase was washed with Et_2O, acidified with 2 M aqueous HCl and extracted with EtOAc. The organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give acid **419** (4.48 g, 20.1 mmol, 63%) as a white solid; m.p. 68 °C (lit.[140] m.p. 28-29 °C). ν_{max}/cm^{-1} 3141 (O-H), 2985 (C-H), 2937 (C-H), 1756 (C=O), 1724 (C=O); δ_H (400 MHz, $CDCl_3$) 1.37 (3H, d, *J* 7.2, C(2)CH_3), 3.80 (1H, q, *J* 7.2, C(2)*H*), 4.19 (2H, s, C(4)H_2), 4.58 (2H, s, C(5)H_2), 7.31-7.38 (5H, m, Ph); δ_C (100 MHz, $CDCl_3$) 12.4 (C(2)*C*H_3), 48.8 (*C*(2)), 73.7 (*C*(5)), 74.3 (*C*(4)), 128.0, 128.3, 128.7, 136.9 (Ph), 175.8 (*C*(1)), 204.2 (*C*(3)); *m/z* (ESI+) 245.0 (MNa^+, 100%); HRMS (ESI+) found 245.07857, $C_{12}H_{14}NaO_4$ (MNa^+) requires 245.07843.

(2*R*,5*S*)-1-(4-(Benzyloxy)-2-methyl-3-oxobutanoyl)-2-(*tert*-butyl)-5-methoxycarbonyl-1,3-oxazolidine (338)[140]



General procedure N (from **42a**, with **419**); yield 38% (2.26 g); colourless oil. R_f (20% EtOAc in petrol) 0.32; $[\alpha]_D^{20}$ -79.2 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2956 (C-H), 1731 (C=O), 1657 (C=O); δ_H (400 MHz, $CDCl_3$) 0.85 (9H, s, C(CH_3)$_3$), 1.43 (3H, d, *J* 6.8, C(7)CH_3), 3.25 (1H, t, *J* 7.8, C(4)$H_A$$H_B$), 3.76 (3H, s, CO_2CH_3), 3.78 (1H, q, *J* 6.8, C(7)*H*), 4.03 (1H, d, *J* 16.4, C(6)C$H_A$$H_B$), 4.13-4.17 (1H, m, C(4)$H_A$$H_B$), 4.15 (1H, d, *J* 16.4, C(6)C$H_A$$H_B$), 4.49 (2H, s, OC$H_2$Ph), 4.76-4.78 (1H, m, C(5)*H*), 5.32 (1H, s, C(2)*H*), 7.28-7.31 (2H, m, Ph), 7.33-7.38 (3H, m, Ph); δ_C (100 MHz, $CDCl_3$) 13.6 (C(7)*C*H_3), 25.7 (C(*C*H_3)$_3$), 37.6 (*C*(CH$_3$)$_3$), 50.7 (*C*(7)), 52.7 (CO_2*C*H_3), 60.2 (*C*(5)), 67.9 (*C*(4)), 74.2 (O*C*H_2Ph), 74.7 (C(6)*C*H_2), 96.2 (*C*(2)), 128.4, 128.7, 128.9, 136.5 (Ph), 170.5 (*C*O_2CH$_3$), 174.0 (*C*(8)), 204.5 (*C*(6)); *m/z* (ESI+) 392.2 (MH^+, 74%), 414.2 (MNa^+, 100%); HRMS (ESI+) found 392.20552, $C_{21}H_{30}NO_6$ (MH^+) requires 392.20676.

(2*R*,5*R*,6*R*,7*S*)-1-Aza-6-((benzyloxy)methyl)-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-7-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane (339I) and **(2*R*,5*R*,6*S*,7*S*)-1-Aza-6-((benzyloxy)methyl)-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-7-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane (339II)**[140]



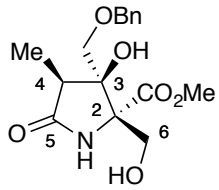
General procedure O (from **338**).

Major diastereomer (**(*R*)-339I**): Yield 59% (1.20 g); white solid; m.p. 116 °C (lit.[140] m.p. 100-102 °C). R_f (30% EtOAc in petrol) 0.36; $[\alpha]_D^{20}$ +27.8 (*c* 0.6, $CHCl_3$); ν_{max}/cm^{-1} 3421 (O-H), 2961 (C-H), 1746 (C=O), 1704 (C=O); δ_H (500 MHz, $CDCl_3$) 0.87 (9H, s, C(CH_3)$_3$), 1.10 (3H, d, *J* 7.0, C(7)CH_3), 2.91 (1H, d, *J* 1.0, O*H*), 3.20 (1H, q, *J* 7.0, C(7)*H*), 3.41 (1H, d, *J* 9.7, C(6)CH_AH$_B$), 3.46 (1H, d, *J* 9.7, C(6)CH$_A$$H_B$), 3.66 (3H, s, CO_2CH_3), 4.15 (1H, d, *J* 9.0, C(4)H_AH$_B$), 4.45 (1H, d, *J* 11.5, OCH_AH$_B$Ph), 4.54 (1H, d, *J* 11.5, OCH$_A$$H_B$Ph), 4.60 (1H, d, *J* 9.0, C(4)H$_A$$H_B$), 4.89 (1H, s, C(2)*H*), 7.29-7.39 (5H, m, Ph); δ_C (125 MHz, $CDCl_3$) 7.4 (C(7)*C*H$_3$), 25.1 (C(*C*H$_3$)$_3$), 36.5 (*C*(CH$_3$)$_3$), 46.0 (*C*(7)), 52.7 (CO_2CH_3), 68.4 (*C*(4)), 71.4 (C(6)*C*H$_2$), 74.0 (O*C*H$_2$Ph), 78.2 (*C*(5)), 81.4 (*C*(6)), 96.3 (*C*(2)), 128.1, 128.4, 128.7, 136.9 (Ph), 171.7 (CO_2CH_3), 178.0 (*C*(8)); *m/z* (ESI$^+$) 392.2 (MH$^+$, 57%), 414.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 392.20681, $C_{21}H_{30}NO_6$ (MH$^+$) requires 392.20676.

Minor diastereomer (**(*S*)-339II**): Yield 29% (592 mg); white solid; m.p. 52 °C. R_f (30% EtOAc in petrol) 0.26; $[\alpha]_D^{20}$ +22.3 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3392 (O-H), 2957 (C-H), 1744 (C=O), 1693 (C=O); δ_H (500 MHz, $CDCl_3$) 0.89 (9H, s, C(CH_3)$_3$), 1.04 (3H, d, *J* 7.5, C(7)CH_3), 3.16 (1H, s, O*H*), 3.43 (2H, s, C(6)CH_2), 3.51 (1H, q, *J* 7.5, C(7)*H*), 3.67 (1H, d, *J* 9.5, C(4)H_AH$_B$), 3.79 (3H, s, CO_2CH_3), 4.50 (1H, d, *J* 11.5, OCH_AH$_B$Ph), 4.58 (1H, d, *J* 11.5, OCH$_A$$H_B$Ph), 4.74 (1H, d, *J* 9.5, C(4)H$_A$$H_B$), 4.78 (1H, s, C(2)*H*), 7.29-7.39 (5H, m, Ph); δ_C (125 MHz, $CDCl_3$) 7.8 (C(7)*C*H$_3$), 25.1 (C(*C*H$_3$)$_3$), 36.3 (*C*(CH$_3$)$_3$), 47.4 (*C*(7)), 52.6 (CO_2CH_3), 69.3 (C(6)*C*H$_2$), 70.5 (*C*(4)), 74.1 (O*C*H$_2$Ph), 78.5 (*C*(5)), 80.5 (*C*(6)),

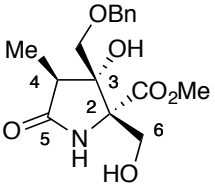
95.5 (*C*(2)), 128.2, 128.6, 128.9, 136.4 (Ph), 171.2 (*C*O_2CH_3), 175.7 (*C*(8)); *m/z* (ESI$^+$) 392.2 (MH^+, 63%), 414.2 (MNa^+, 100%); HRMS (ESI$^+$) found 392.20660, $C_{21}H_{30}NO_6$ (MH^+) requires 392.20676.

Methyl (2*R*,3*R*,4*S*)-3-((benzyloxy)methyl)-3-hydroxy-2-hydroxymethyl-4-methyl-5-oxopyrrolidine-2-carboxylate (352I)[140]



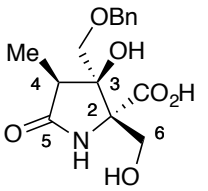
General procedure M (from **339I**, 1.5 eq of 1,3-propanedithiol, for 24 h, at rt); yield quant. (269 mg); white solid, m.p. 55 °C. R_f (10% MeOH in EtOAc) 0.46; $[\alpha]_D^{20}$ -51.8 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3324 (O-H, N-H), 2948 (C-H), 1731 (C=O), 1690 (C=O); δ_H (400 MHz, CD_3OD) 1.02 (3H, d, *J* 7.2, C(4)CH_3), 2.84 (1H, q, *J* 7.2, C(4)*H*), 3.35 (1H, d, *J* 9.6, C(3)CH_AH_B), 3.56 (3H, s, CO_2CH_3), 3.59 (1H, d, *J* 9.6, C(3)CH_AH_B), 3.83 (1H, d, *J* 11.2, C(6)H_AH_B), 4.21 (1H, d, *J* 11.2, C(6)H_AH_B), 4.41 (1H, d, *J* 11.9, OCH_AH_BPh), 4.50 (1H, d, *J* 11.9, OCH_AH_BPh), 7.26-7.35 (5H, m, Ph); δ_C (100 MHz, CD_3OD) 7.4 (C(4)*C*H_3), 43.2 (*C*(4)), 53.0 (CO_2CH_3), 64.7 (*C*(6)), 70.9 (C(3)*C*H_2), 73.6 *C*(2), 74.2 (O*C*H_2Ph), 81.6 (*C*(3)), 128.7, 128.7, 129.4, 139.1 (Ph), 173.1 (*C*O_2CH_3), 180.8 (*C*(5)); *m/z* (ESI$^+$) 324.1 (MH^+, 30%), 346.1 (MNa^+, 100%); HRMS (ESI$^+$) found 324.14436, $C_{16}H_{22}NO_6$ (MH^+) requires 324.14416.

Methyl (2*R*,3*S*,4*S*)-3-((benzyloxy)methyl)-3-hydroxy-2-hydroxymethyl-4-methyl-5-oxopyrrolidine-2-carboxylate (352II)



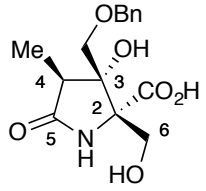
General procedure M (from **339II**, 5 eq of 1,3-propanedithiol, for 24 h, at rt); yield 81% (167 mg); white solid; m.p. 151 °C. R_f (10% MeOH in EtOAc) 0.45; $[\alpha]_D^{20}$ -34.5 (*c* 0.8, MeOH); ν_{max}/cm^{-1} 3361 (O-H, N-H), 2951 (C-H), 1688 (C=O); δ_H (400 MHz, CD_3OD) 1.18 (3H, d, *J* 7.6, C(4)CH_3), 2.65 (1H, q, *J* 7.6, C(4)*H*), 3.58 (1H, d, *J* 9.2, C(3)CH_AH_B), 3.64 (1H, d, *J* 9.2, C(3)CH_AH_B), 3.74 (1H, d, *J* 10.8, C(6)H_AH_B), 3.75 (3H, s, CO_2CH_3), 4.11 (1H, d, *J* 10.8, C(6)H_AH_B), 4.49 (1H, d, *J* 11.8, OCH_AH_BPh), 4.55 (1H, d, *J* 11.8, OCH_AH_BPh), 7.27-7.37 (5H, m, Ph); δ_C (100 MHz, CD_3OD) 10.6 (C(4)*C*H_3), 48.2 (*C*(4)), 52.8 (CO_2CH_3), 63.9 (*C*(6)), 70.7 (C(3)*C*H_2), 74.4 (O*C*H_2Ph), 75.4 *C*(2), 80.6 (*C*(3)), 128.8, 128.9, 129.3, 139.0 (Ph), 172.3 (*C*O_2CH_3), 180.4 (*C*(5)); *m/z* (ESI$^+$) 324.1 (MH^+, 56%), 346.1 (MNa^+, 83%); HRMS (ESI$^+$) found 324.14444, $C_{16}H_{22}NO_6$ (MH^+) requires 324.14416.

(2*R*,3*R*,4*S*)-3-((Benzyloxy)methyl)-3-hydroxy-2-hydroxymethyl-4-methyl-5-oxopyrrolidine-2-carboxylic acid (420I)[140]



NaOH (14 mg, 0.34 mmol) was added to a solution of methyl ester **352I** (95 mg, 0.29 mmol) in 1:1:1 MeOH/THF/H_2O (3 mL). The reaction mixture was stirred at room temperature for 24 h, then diluted with brine and washed with EtOAc. The aqueous layer was acidified with 2 M aqueous HCl, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give acid **420I** (83 mg, 0.27 mmol, 93%) as a white solid; m.p. 94 °C (lit.[140] m.p. 90-100 °C). $[\alpha]_D^{20}$ -44.2 (*c* 0.5, MeOH); ν_{max}/cm^{-1} 3321 (O-H, N-H), 2919 (C-H), 2955 (C-H), 1691 (C=O); δ_H (400 MHz, CD_3OD) 1.05 (3H, d, *J* 7.4, C(4)CH_3), 2.82 (1H, q, *J* 7.4, C(4)*H*), 3.46 (1H, d, *J* 10.0, C(3)CH_AH$_B$), 3.64 (1H, d, *J* 10.0, C(3)CH$_A$$H_B$), 3.88 (1H, d, *J* 11.2, C(6)H_AH$_B$), 4.22 (1H, d, *J* 11.2, C(6)H$_A$$H_B$), 4.43 (1H, d, *J* 11.9, OCH_AH$_B$Ph), 4.55 (1H, d, *J* 11.9, OCH$_A$$H_B$Ph), 7.23-7.37 (5H, m, Ph); δ_C (100 MHz, CD_3OD) 7.8 (C(4)*C*H$_3$), 43.9 (*C*(4)), 64.8 (*C*(6)), 72.0 (C(3)*C*H$_2$), 73.4 (*C*(2)), 74.2 (O*C*H$_2$Ph), 81.4 (*C*(3)), 128.6, 128.7, 129.3, 139.3 (Ph), 174.3 (*C*O$_2$H), 180.9 (*C*(5)); *m/z* (ESI$^-$) 308.1 (M-H$^-$, 100%); HRMS (ESI$^-$) found 308.11430, $C_{15}H_{18}NO_6$ (M-H$^-$) requires 308.11396.

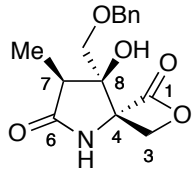
(2*R*,3*S*,4*S*)-3-((Benzyloxy)methyl)-3-hydroxy-2-hydroxymethyl-4-methyl-5-oxopyrrolidine-2-carboxylic acid (420II)



NaOH (16 mg, 0.40 mmol) was added to a solution of methyl ester **352II** (101 mg, 0.312 mmol) in 1:1:1 MeOH/THF/H_2O (3.1 mL). The reaction mixture was stirred at room temperature for 2 h, then diluted with brine and acidified with 2 M aqueous HCl. The aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give acid **420II** (97 mg, 0.31 mmol, quant.) as a white solid; m.p. 146 °C. $[\alpha]_D^{20}$ -32.9 (*c* 0.7, MeOH); ν_{max}/cm^{-1} 3306 (O-H, N-H), 2924 (C-H), 1684 (C=O); δ_H (500 MHz, CD_3OD) 1.19 (3H, d, *J* 7.6, C(4)CH_3), 2.67 (1H, q, *J* 7.6, C(4)*H*), 3.61 (1H, d, *J* 9.7, C(3)CH_AH$_B$), 3.68 (1H, d, *J* 9.7, C(3)CH$_A$$H_B$), 3.74 (1H, d, *J* 11.0, C(6)H_AH$_B$), 4.07 (1H, d, *J* 11.0, C(6)H$_A$$H_B$), 4.51 (1H, d, *J* 11.7, OCH_AH$_B$Ph), 4.56 (1H, d, *J* 11.7, OCH$_A$$H_B$Ph), 7.26-7.29 (1H, m, Ph), 7.31-7.37 (4H, m, Ph); δ_C (125 MHz, CD_3OD) 10.8 (C(4)*C*H$_3$), 48.6 (*C*(4)), 64.1 (*C*(6)), 70.9

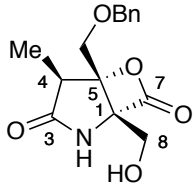
(C(3)CH_2), 74.5 (OCH_2Ph), 75.1 C(2), 80.4 (C(3)), 128.8, 128.9, 129.4, 139.1 (Ph), 173.3 (CO_2H), 180.6 (C(5)); *m/z* (ESI$^-$) 308.1 (M-H$^-$, 100%); HRMS (ESI$^+$) found 310.12862, $C_{15}H_{20}NO_6$ (MH$^+$) requires 310.12851.

(4*R*,7*S*,8*R*)-8-((Benzyloxy)methyl)-8-hydroxy-7-methyl-2-oxa-5-azaspiro[3.4]octane-1,6-dione (340)



HATU (70 mg, 0.18 mmol) and DIPEA (64 μL, 0.37 mmol) were added to a solution of acid **420I** (28 mg, 0.091 mmol) in anhydrous THF (3 mL) at 0 °C. The mixture was stirred at room temperature for 33 h and quenched with brine. The aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by *flash* column chromatography (50% EtOAc in petrol) to give spiro-β-lactone **340** (23 mg, 0.080 mmol, 88%) as a white solid; m.p. 198 °C (deg). R_f (EtOAc) 0.56; $[\alpha]_D^{20}$ -27.1 (*c* 0.3, MeOH); ν_{max}/cm^{-1} 3448 (O-H or N-H), 3242 (O-H or N-H), 2902 (C-H), 2864 (C-H), 1813 (C=O), 1733 (C=O), 1675 (C=C); δ_H (500 MHz, CD_3OD) 1.09 (3H, d, *J* 7.3, C(7)CH_3), 2.54 (1H, q, *J* 7.3, C(7)*H*), 3.70 (1H, d, *J* 10.0, C(8)CH_AH$_B$), 3.73 (1H, d, *J* 10.0, C(8)CH$_A$$H_B$), 4.28 (1H, d, *J* 6.0, C(3)H_AH$_B$), 4.51 (1H, d, *J* 11.5, OCH_AH$_B$Ph), 4.55 (1H, d, *J* 11.5, OCH$_A$$H_B$Ph), 4.86 (1H, d, *J* 6.0, C(3)H$_A$$H_B$), 7.27 (1H, tt, *J* 7.0, 1.5, Ph), 7.31-7.38 (4H, m, Ph); δ_C (125 MHz, CD_3OD) 8.2 (C(7)CH_3), 44.8 (C(7)), 69.4 (C(3)), 74.2 (C(8)CH_2), 74.9 (OCH_2Ph), 79.6 (C(8)), 80.7 C(4), 128.8, 128.9, 129.3, 139.0 (Ph), 172.6 (C(1)), 179.5 (C(6)); *m/z* (ESI$^+$) 314.1 (MNa$^+$, 49%); HRMS (ESI$^+$) found 314.09986, $C_{15}H_{17}NNaO_5$ (MNa$^+$) requires 314.09989.

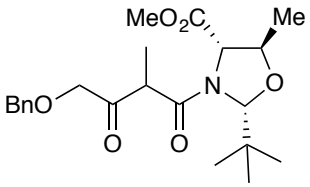
(1*R*,4*S*,5*S*)-5-((Benzyloxy)methyl)-1-hydroxymethyl-4-methyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (341)



Triethylamine (25 μL, 0.18 mmol) and BOPCl (49 mg, 0.19 mmol) were added to a solution of acid **420II** (27 mg, 0.088 mmol) in anhydrous DCM (1.8 mL). After stirring at room temperature for 14 h, the solution was diluted with brine, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by *flash* column chromatography (70% EtOAc in petrol) to give

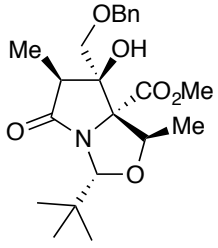
fused-β-lactone **341** (16 mg, 0.056 mmol, 64%) as a white solid; m.p. 96 °C. R_f (70% EtOAc in petrol) 0.20; $[\alpha]_D^{20}$ -4.2 (*c* 0.4, MeOH); δ_H (500 MHz, CD_3OD) 1.26 (3H, d, *J* 8.0, C(4)CH_3), 2.82 (1H, q, *J* 8.0, C(4)*H*), 3.82 (1H, d, *J* 12.5, C(8)H_AH_B), 4.06 (1H, d, *J* 11.7, C(5)CH_AH_B), 4.07 (1H, d, *J* 12.5, C(8)H_AH_B), 4.09 (1H, d, *J* 11.7, C(5)H_AH_B), 4.60 (1H, d, *J* 11.7, OCH_AH_BPh), 4.65 (1H, d, *J* 11.7, OCH_AH_BPh), 7.27-7.36 (5H, m, Ph); δ_C (125 MHz, CD_3OD) 12.4 (C(4)*C*H_3), 45.1 (*C*(4)), 57.2 (*C*(8)), 68.3 (C(5)*C*H_2), 74.9 (O*C*H_2Ph), 79.1 (*C*(1)), 87.6 *C*(5), 128.9, 128.9, 129.5, 139.0 (Ph), 170.0 (*C*(7)), 180.0 (*C*(3)); *m/z* (ESI+) 292.1 (MH^+, 33%), 314.1 (MNa^+, 100%); HRMS (ESI+) found 292.11812, $C_{15}H_{18}NO_5$ (MH^+) requires 292.11795.

(2*R*,4*R*,5*S*)-1-(4-(Benzyloxy)-2-methyl-3-oxobutanoyl)-2-(*tert*-butyl)-5-methoxycarbonyl-4-methyl-1,3-oxazolidine (342)



General procedure N (from **42c**, with **419**); yield 28% (1.71 g); white solid; m.p. 124 °C. R_f (30% EtOAc in petrol) 0.18; $[\alpha]_D^{20}$ -13.0 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2975 (C-H), 2962 (C-H), 1748 (C=O), 1650 (C=O); δ_H (500 MHz, $CDCl_3$) 0.90 (9H, s, C(CH_3)$_3$), 1.33-1.36 (6H, m, C(4)CH_3 + C(7)CH_3), 3.62 (1H, q, *J* 6.9, C(7)*H*), 3.72 (3H, s, CO_2CH_3), 4.09 (1H, d, *J* 4.3, C(5)*H*), 4.28 (2H, s, OCH_2Ph), 4.58 (1H, d, *J* 11.9, C(6)CH_AH_B), 4.63 (1H, d, *J* 11.9, C(6)CH_AH_B), 4.73-4.78 (1H, m, C(4)*H*), 5.41 (1H, s, C(2)*H*), 7.29-7.36 (5H, m, Ph); δ_C (125 MHz, $CDCl_3$) 13.2 (C(7)*C*H_3), 20.4 (C(4)*C*H_3), 26.1 (C(*C*H_3)$_3$), 38.3 (*C*(CH_3)$_3$), 49.6 (*C*(7)), 53.0 (CO_2CH_3), 65.9 (*C*(5)), 72.9 (O*C*H_2Ph), 73.5 (C(6)*C*H_2), 76.2 (*C*(4)), 96.5 (*C*(2)), 128.1, 128.3, 128.6, 137.3 (Ph), 169.6 (*C*O_2CH_3), 171.9 (*C*(8)), 202.5 (*C*(6)); *m/z* (ESI+) 428.2 (MNa^+, 100%); HRMS (ESI+) found 428.20411, $C_{22}H_{31}NNaO_6$ (MNa^+) requires 428.20436.

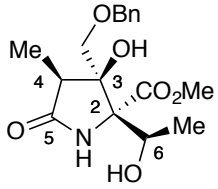
(2*R*,4*R*,5*R*,6*R*,7*S*)-1-Aza-6-((benzyloxy)methyl)-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-4,7-dimethyl-3-oxa-8-oxobicyclo[3.3.0]-octane (343)



General procedure O (from **342**); yield 82% (1.29 g); white solid; m.p. 146 °C. R_f (30% EtOAc in petrol) 0.38; $[\alpha]_D^{20}$ -13.7 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 3353 (O-H), 2959 (C-H), 1745 (C=O), 1690 (C=O); δ_H (500 MHz, $CDCl_3$) 0.91 (9H, s, C(CH_3)$_3$), 1.05 (3H, d, *J* 7.0, C(7)CH_3), 1.66 (3H, d, *J* 6.5, C(4)CH_3), 2.60 (1H, s,

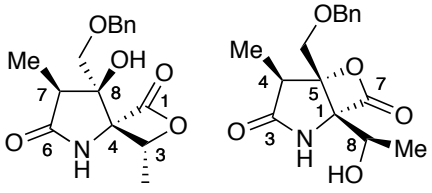
O*H*), 3.24 (1H, q, *J* 7.0, C(7)*H*), 3.47 (1H, d, *J* 10.0, C(6)CH_AH$_B$), 3.56 (1H, d, *J* 10.0, C(6)CH$_A$$H_B$), 3.60 (3H, s, CO_2CH_3), 4.44 (1H, d, *J* 11.5, OCH_AH$_B$Ph), 4.55 (1H, d, *J* 11.5, OCH$_A$$H_B$Ph), 4.72 (1H, q, *J* 6.5, C(4)*H*), 5.03 (1H, s, C(2)*H*), 7.27-7.32 (3H, m, Ph), 7.33-7.37 (2H, m, Ph); δ_C (125 MHz, $CDCl_3$) 6.7 (C(7)*C*H$_3$), 15.7 (C(4)*C*H$_3$), 25.8 (C(*C*H$_3$)$_3$), 37.5 (*C*(CH$_3$)$_3$), 45.6 (*C*(7)), 52.7 (CO_2*C*H$_3$), 70.1 (C(6)*C*H$_2$), 73.7 (O*C*H$_2$Ph), 77.6 (*C*(5)), 79.0 (*C*(4)), 85.5 (*C*(6)), 96.6 (*C*(2)), 127.8, 128.1, 128.6, 137.1 (Ph), 171.6 (CO_2CH_3), 180.5 (*C*(8)); *m/z* (ESI$^+$) 428.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 428.20403, $C_{22}H_{31}NNaO_6$ (MNa$^+$) requires 428.20546.

Methyl (2*R*,3*R*,4*S*)-3-((benzyloxy)methyl)-3-hydroxy-2-((*R*)-1-hydroxyethyl)-4-methyl-5-oxopyrrolidine-2-carboxylate (421)



General procedure M (from **343**, 5 eq of 1,3-propanedithiol, for 28 h, at rt); yield quant. (314 mg); colourless oil. R_f (EtOAc) 0.30; $[\alpha]_D^{20}$ -51.1 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3337 (O-H, N-H), 2942 (C-H), 1690 (C=O); δ_H (400 MHz, CD_3OD) 1.05 (3H, d, *J* 7.4, C(4)CH_3), 1.25 (3H, d, *J* 6.5, C(6)CH_3), 2.85 (1H, q, *J* 7.4, C(4)*H*), 3.44 (1H, d, *J* 9.4, C(3)CH_AH$_B$), 3.55 (3H, s, CO_2CH_3), 3.80 (1H, d, *J* 9.4, C(3)CH$_A$$H_B$), 4.18 (1H, q, *J* 6.5, C(6)*H*), 4.43 (1H, d, *J* 11.8, OCH_AH$_B$Ph), 4.50 (1H, d, *J* 11.8, OCH$_A$$H_B$Ph), 7.26-7.36 (5H, m, Ph); δ_C (100 MHz, CD_3OD) 7.9 (C(4)*C*H$_3$), 19.1 (C(6)*C*H$_3$), 43.4 (*C*(4)), 52.6 (CO_2*C*H$_3$), 70.8 (*C*(6)), 72.4 (C(3)*C*H$_2$), 74.1 (O*C*H$_2$Ph), 74.2 *C*(2), 81.7 (*C*(3)), 128.7, 128.7, 129.4, 139.1 (Ph), 173.2 (*C*O$_2$CH$_3$), 181.2 (*C*(5)); *m/z* (ESI$^+$) 338.2 (MH$^+$, 14%), 360.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 360.14172, $C_{17}H_{23}NNaO_6$ (MNa$^+$) requires 360.14286.

(3*R*,4*R*,7*S*,8*R*)-8-((Benzyloxy)methyl)-8-hydroxy-3,7-dimethyl-2-oxa-5-azaspiro[3.4]octane-1,6-dione (345) or (1*R*,4*S*,5*S*)-5-((Benzyloxy)methyl)-1-((*R*)-1-hydroxyethyl)-4-methyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (346)



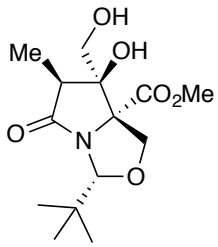
Method 1: NaOH (18 mg, 0.45 mmol) was added to a solution of methyl ester **421** (123 mg, 0.365 mmol) in 1:1:1 MeOH/THF/H_2O (3.6 mL). The reaction mixture was stirred at room temperature for 42 h, then diluted with brine and acidified with 2 M aqueous HCl. The aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. HATU (158 mg, 0.416 mmol) and DIPEA (71 μL, 0.41 mmol) were added to a solution of the crude acid in anhydrous THF (6.8 mL) at 0 °C. The mixture was stirred at room temperature for 36 h, diluted with brine, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (40% to 50% EtOAc in petrol) to give β-lactone **345** or **346** (21 mg, 0.069 mmol, 18%).

Method 2: NaOH (3 mg, 0.07 mmol) was added to a solution of methyl ester **421** (21 mg, 0.061 mmol) in 1:1:1 MeOH/THF/H_2O (600 μL). The reaction mixture was stirred at room temperature for 42 h, then diluted with brine and acidified with 2 M aqueous HCl. The aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in anhydrous DCM (820 μL), and triethylamine (11 μL, 0.079 mmol) and BOPCl (23 mg, 0.088 mmol) were added. After stirring at room temperature for 15 h, the solution was diluted with brine, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by *flash* column chromatography (40% to 60% EtOAc in petrol) to give β-lactone **345** or **346** (3 mg, 0.01 mmol, 16%).

White solid; m.p. >250 °C (deg). R_f (80% EtOAc in petrol) 0.37; $[\alpha]_D^{20}$ -8.7 (*c* 0.5, MeOH); ν_{max}/cm^{-1} 3267 (O-H or N-H), 2874 (C-H), 1821 (C=O), 1708 (C=O); δ_H (500 MHz, CD_3OD) 1.09 (3H, d, *J* 7.0, C(7/4)CH_3), 1.47 (3H, d, *J* 6.5, C(3/8)CH_3), 2.53 (1H, q, *J* 7.0, C(7/4)*H*), 3.69 (1H, d, *J* 10.1, C(8/5)CH_AH$_B$), 3.72 (1H, d, *J* 10.1, C(8/5)CH$_A$$H_B$), 4.52 (1H, d, *J* 11.7, OCH_AH$_B$Ph), 4.55 (1H, d, *J* 11.7, OCH$_A$$H_B$Ph), 4.25 (1H, q, *J* 6.5, C(3/8)*H*), 7.25-7.38 (5H, m, Ph); δ_C (125 MHz, CD_3OD) 8.4 (C(7/4)*C*H$_3$), 15.2 (C(3/8)*C*H$_3$), 44.8 (*C*(7/4)), 74.1 (C(8/5)*C*H$_2$), 74.8 (O*C*H$_2$Ph), 77.5 (*C*(3/8)), 79.5 (*C*(8/5), 81.1 (*C*(4/1)), 128.8, 128.9, 129.4, 139.0 (Ph), 172.8 (*C*(1/7)), 180.2 (*C*(6/3));

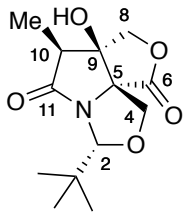
m/z (ESI+) 306.1 (MH^+, 45%); HRMS (ESI+) found 306.13358, $C_{16}H_{20}NO_5$ (MH^+) requires 306.13360.

(2*R*,5*R*,6*R*,7*S*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-6-hydroxymethyl-5-methoxycarbonyl-7-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane (348I)



A suspension of benzyloxy **339I** (206 mg, 0.525 mmol) and 10% Pd/C (59 mg, 0.056 mmol) in methanol (7 mL) was stirred under H_2 for 14 h. The mixture was filtered through Celite and purified by *flash* column chromatography (40% to 60% EtOAc in petrol) to give alcohol **348I** (147 mg, 0.488 mmol, 93%) as a colourless oil. R_f (60% EtOAc in petrol) 0.20; $[\alpha]_D^{20}$ +37.1 (*c* 0.7, $CHCl_3$); ν_{max}/cm^{-1} 3437 (O-H), 2958 (C-H), 1696 (C=O); δ_H (400 MHz, $CDCl_3$) 0.86 (9H, s, C(CH_3)$_3$), 1.08 (3H, d, *J* 7.2, C(7)CH_3), 2.87 (1H, t, *J* 5.8, C(6)CH_2O*H*), 3.07 (1H, q, *J* 7.2, C(7)*H*), 3.56-3.62 (3H, m, C(6)CH_2 + C(6)O*H*), 3.80 (3H, s, CO_2CH_3), 4.21 (1H, d, *J* 8.9, C(4)H_AH_B), 4.52 (1H, d, *J* 8.9, C(4)H_AH_B), 4.88 (1H, s, C(2)*H*); δ_C (100 MHz, $CDCl_3$) 7.3 (C(7)*C*H_3), 25.1 (C(*C*H_3)$_3$), 36.5 (*C*(CH_3)$_3$), 45.8 (*C*(7)), 53.1 (CO_2CH_3), 64.6 (C(6)*C*H_2), 68.9 (*C*(4)), 78.5 (*C*(5)), 82.7 (*C*(6)), 96.5 (*C*(2)), 172.9 (*C*O_2CH_3), 178.6 (*C*(8)); *m/z* (ESI+) 302.1 (MH^+, 69%), 324.1 (MNa^+, 23%); HRMS (ESI+) found 302.15992, $C_{14}H_{24}NO_6$ (MH^+) requires 302.15981.

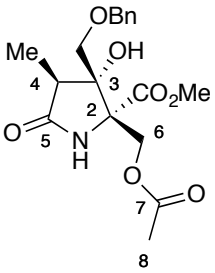
(2*R*,5*R*,9*R*,10*S*)-1-Aza-2-(*tert*-butyl)-9-hydroxy-10-methyl-3,7-dioxa-6,11-dioxotricyclo[6.3.0.0^{5,9}]-undecane (350)



Side-product from the hydrogenation of benzyloxy **339I**; off-white solid; m.p. 227 °C. R_f (60% EtOAc in petrol) 0.47; $[\alpha]_D^{20}$ +27.9 (*c* 0.7, MeOH); ν_{max}/cm^{-1} 3406 (O-H), 2954 (C-H), 1794 (C=O), 1689 (C=O); δ_H (500 MHz, $CDCl_3$) 1.03 (9H, s, C(CH_3)$_3$), 1.22 (3H, d, *J* 7.0, C(10)CH_3), 3.04 (1H, br s, C(9)O*H*), 3.08 (1H, q, *J* 7.0, C(10)*H*), 4.06 (1H, d, *J* 9.8, C(8)H_AH_B), 4.20 (1H, d, *J* 9.0, C(4)H_AH_B), 4.33 (1H, d, *J* 9.8, C(8)H_AH_B), 4.36 (1H, d, *J* 9.0, C(4)H_AH_B), 4.98 (1H, s, C(2)*H*); δ_C (125 MHz, $CDCl_3$) 6.7 (C(10)*C*H_3), 25.6 (C(*C*H_3)$_3$), 36.4 (*C*(CH_3)$_3$), 47.4 (*C*(10)), 68.0 (*C*(4)), 71.0 (*C*(8)), 72.9 (*C*(5)), 82.2 (*C*(9)), 99.3

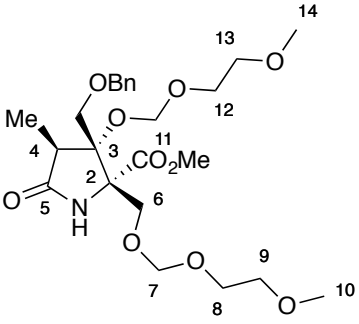
(*C*(2)), 173.5 (*C*(6)), 179.7 (*C*(11)); *m/z* (ESI+) 292.1 (MNa^+, 70%); HRMS (ESI-) found 268.11908, $C_{13}H_{18}NO_5$ ($M\text{-}H^-$) requires 268.11905.

Methyl (2*R*,3*S*,4*S*)-2-(acetoxymethyl)-3-((benzyloxy)methyl)-3-hydroxy-4-methyl-5-oxopyrrolidine-2-carboxylate (353)[140]



Dry pyridine (13 μL, 0.16 mmol) and acetic anhydride (15 μL, 0.16 mmol) were added to a solution of alcohol **352II** (10 mg, 0.032 mmol) in anhydrous DCM (300 μL). The mixture was stirred at room temperature for 5 h and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (70% EtOAc in petrol) to give monoacetyl **353** (10 mg, 0.027, 84%) as a white solid; m.p. 185 °C (lit.[140] m.p. 115-120 °C). R_f (70% EtOAc in petrol) 0.22; $[\alpha]_D^{20}$ -32.6 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 3300 (O-H, N-H), 2923 (C-H), 1731 (C=O), 1690 (C=O); δ_H (500 MHz, $CDCl_3$) 1.15 (3H, d, *J* 7.5, C(4)CH_3), 2.05 (3H, s, C(8)H_3), 2.77 (1H, q, *J* 7.5, C(4)*H*), 3.54 (1H, d, *J* 9.7, C(3)CH_AH_B), 3.68 (1H, d, *J* 9.7, C(3)CH_AH_B), 3.79 (3H, s, CO_2CH_3), 3.80 (1H, s, O*H*), 4.04 (1H, d, *J* 11.0, C(6)H_AH_B), 4.57 (1H, d, *J* 11.5, OCH_AH_BPh), 4.60 (1H, d, *J* 11.5, OCH_AH_BPh), 4.71 (1H, d, *J* 11.0, C(6)H_AH_B), 6.61 (1H, s, N*H*), 7.31-7.40 (5H, m, Ph); δ_C (125 MHz, $CDCl_3$) 10.6 (C(4)CH_3), 20.8 (*C*(8)), 46.1 (*C*(4)), 53.1 (CO_2CH_3), 66.2 (*C*(6)), 69.0 (C(3)CH_2), 71.4 *C*(2), 74.2 (OCH_2Ph), 80.3 (*C*(3)), 128.3, 128.4, 128.8, 136.8 (Ph), 170.2 (CO_2CH_3), 170.3 (*C*(7)), 177.1 (*C*(5)); HRMS (CI+) found 366.1552, $C_{18}H_{24}NO_7$ (MH^+) requires 366.1547.

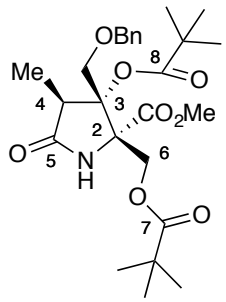
Methyl (2*R*,3*S*,4*S*)-3-((benzyloxy)methyl)-3-((2-methoxyethoxy)methoxy)-2-(((2-methoxyethoxy)methoxy)methyl)-4-methyl-5-oxopyrrolidine-2-carboxylate (356)



To a solution of alcohol **352II** (24 mg, 0.074 mmol) in anhydrous DCM (750 μL) at 0 °C were added dropwise DIPEA (39 μL, 0.22 mmol) and MEMCl (13 μL, 0.11 mmol). The solution was stirred at room temperature and the same amount of DIPEA and MEMCl was added every 10 h until no starting material or mono-protected product were observed by TLC. The reaction mixture was quenched with brine and the

aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (70% to 80% EtOAc in petrol) to give double MEM-protected **356** (18 mg, 0.036 mmol, 49%) as a colourless oil. R_f (EtOAc) 0.28; $[\alpha]_D^{20}$ -35.8 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3367 (N-H), 2929 (C-H), 2881 (C-H), 1736 (C=O), 1713 (C=O), 1687 (C=C); δ_H (500 MHz, CD_3OD) 1.17 (3H, d, *J* 7.0, C(4)CH_3), 2.66 (1H, q, *J* 7.0, C(4)*H*), 3.14 (2H, t, *J* 4.8, C(13)H_2), 3.24 (3H, s, C(14)H_3), 3.34-3.37 (1H, m, C(12)H_AH_B), 3.38 (3H, s, C(10)H_3), 3.41-3.46 (2H, m, C(3)CH_AH_B + C(12)H$_A H_B$), 3.53-3.58 (3H, m, C(3)CH$_A H_B$ + C(9)H_2), 3.67-3.72 (2H, m, C(8)H_2), 3.74 (3H, s, CO_2CH_3), 3.95 (1H, d, *J* 10.0, C(6)H_AH_B), 4.32 (1H, d, *J* 10.0, C(6)H$_A H_B$), 4.43 (1H, d, *J* 11.5, OCH_AH_BPh), 4.47 (1H, d, *J* 11.5, OCH$_A H_B$Ph), 4.49 (1H, d, *J* 11.0, C(11)H_AH_B), 4.67 (1H, d, *J* 6.7, C(7)H_AH_B), 4.69 (1H, d, *J* 6.7, C(7)H$_A H_B$), 5.03 (1H, d, *J* 11.0, C(11)H$_A H_B$), 7.29-7.35 (5H, m, Ph); δ_C (125 MHz, CD_3OD) 8.0 (C(4)CH_3), 45.4 (*C*(4)), 52.9 (CO_2CH_3), 58.9 (*C*(14)), 59.1 (*C*(10)), 68.0 (*C*(8)), 68.4 (*C*(6)), 68.6 (*C*(12)), 70.9 (C(3)CH_2), 72.6 (*C*(11)), 72.6 (*C*(13)), 72.9 (*C*(9)), 74.6 (OCH_2Ph), 75.8 (*C*(2)), 79.2 (*C*(3)), 97.4 (*C*(7)), 129.0, 129.4, 129.5, 138.6 (Ph), 171.7 (CO_2CH_3), 179.0 (*C*(5)); *m/z* (ESI$^+$) 522.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 522.23028, $C_{24}H_{37}NNaO_{10}$ (MNa$^+$) requires 522.23097.

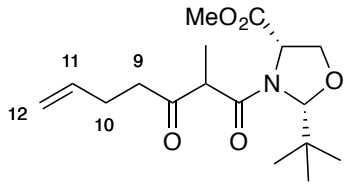
Methyl (2*R*,3*S*,4*S*)-3-((benzyloxy)methyl)-4-methyl-5-oxo-3-(pivaloyloxy)-2-((pivaloyloxy)methyl)pyrrolidine-2-carboxylate (358)



Triethylamine (9 μL, 0.06 mmol) and pivaloyl chloride (8 μL, 0.06 mmol) were added to a solution of alcohol **352** (18 mg, 0.056 mmol) in anhydrous DCM (570 μL) at 0 °C. After stirring at room temperature for 14 h, the solution was diluted with brine and extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated *in vacuo* and purified by *flash* column chromatography (20% EtOAc in petrol) to give double pivalate ester **358** (12 mg, 0.024 mmol, 43%) as a white solid; m.p. 131 °C. R_f (20% EtOAc in petrol) 0.17; $[\alpha]_D^{20}$ -40.6 (*c* 0.8, MeOH); ν_{max}/cm^{-1} 3455 (N-H), 2963 (C-H), 1764 (C=O), 1735 (C=O), 1717 (C=O), 1701 (C=O); δ_H (500 MHz, $CDCl_3$) 1.15 (9H, s, C(7)C(CH_3)$_3$), 1.23 (3H, d, *J* 7.5, C(4)CH_3), 1.29 (9H, s, C(8)C(CH_3)$_3$), 3.03 (1H, br s, N*H*), 3.15 (1H, q, *J* 7.5, C(4)*H*), 3.62 (1H, d, *J* 9.5, C(3)CH_AH_B), 3.66 (1H,

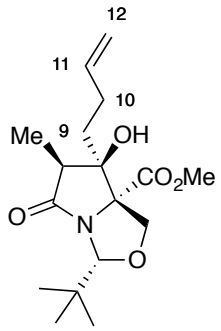
d, *J* 9.5, C(3)CH_AH_B), 3.75 (3H, s, CO_2CH_3), 4.40 (1H, d, *J* 11.5, OCH_AH_BPh), 4.46 (1H, d, *J* 12.0, C(6)H_AH_B), 4.56 (1H, d, *J* 11.5, OCH_AH_BPh), 4.86 (1H, d, *J* 12.0, C(6)H_AH_B), 7.25-7.37 (5H, m, Ph); δ_C (125 MHz, $CDCl_3$) 9.2 (C(4)CH_3), 26.6 (C(8)C$(CH_3)_3$), 27.4 (C(7)C$(CH_3)_3$), 38.8 (C(7)C$(CH_3)_3$), 42.5 (C(8)C$(CH_3)_3$), 45.9 (C(4)), 52.8 (CO_2CH_3), 61.9 (C(6)), 69.9 (C(3)CH_2), 72.6 (C(2)), 74.3 (OCH_2Ph), 77.4 (C(3)), 128.3, 128.5, 128.8, 136.5 (Ph), 169.2 (CO_2CH_3), 174.0 (C(5)), 177.0 (C(7)), 182.0 (C(8)); *m/z* (ESI^+) 514.2 (MNa^+, 79%); HRMS (ESI^+) found 514.24086, $C_{26}H_{37}NNaO_8$ (MNa^+) requires 514.24114.

(2*R*,5*S*)-2-(*tert*-Butyl)-5-methoxycarbonyl-1-(2-methyl-3-oxohept-6-enoyl)-1,3-oxazolidine (360)



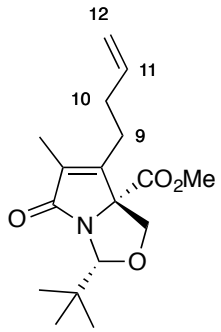
General procedure N (from **42a**, with **424**); yield 41% (1.60 g); light yellow oil. R_f (30% EtOAc in petrol) 0.30; $[\alpha]_D^{20}$ -4.2 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 3080 (C-H), 2958 (C-H), 1747 (C=O), 1725 (C=O), 1659 (C=C); δ_H (400 MHz, $CDCl_3$) 0.92 (9H, s, C$(CH_3)_3$), 1.29 (3H, d, *J* 6.7, C(7)CH_3), 2.30-2.38 (2H, m, C(10)H_2), 2.60-2.77 (2H, m, C(9)H_2), 3.62 (1H, q, *J* 6.7, C(7)*H*), 3.79 (3H, s, CO_2CH_3), 4.03 (1H, t, *J* 7.8, C(4)H_AH_B), 4.50-4.58 (2H, m, C(4)H_AH_B + C(5)*H*), 4.93-4.98 (1H, m, C(12)H_AH_B), 4.98-5.05 (1H, m, C(12)H_AH_B), 5.32 (1H, s, C(2)*H*), 5.74-5.85 (1H, m, C(11)*H*); δ_C (100 MHz, $CDCl_3$) 12.9 (C(7)CH_3), 25.9 (C$(CH_3)_3$), 27.7 (C(10)), 37.8 (C$(CH_3)_3$), 39.4 (C(9)), 53.1 (C(7)), 53.1 (CO_2CH_3), 59.6 (C(5)), 68.1 (C(4)), 96.9 (C(2)), 115.3 (C(12)), 137.2 (C(11)), 169.9 (CO_2CH_3), 172.5 (C(8)), 204.2 (C(6)); *m/z* (ESI^+) 348.2 (MNa^+, 74%); HRMS (ESI^+) found 348.17846, $C_{17}H_{27}NNaO_5$ (MNa^+) requires 348.17814.

(2*R*,5*R*,6*R*,7*S*)-1-Aza-6-(but-3-en-1-yl)-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-7-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane (361)



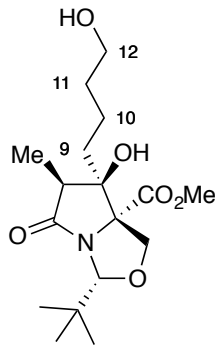
General procedure O (from **360**); yield 80% (1.28 g); white solid; m.p. 168 °C. R_f (30% EtOAc in petrol) 0.38; $[\alpha]_D^{20}$ +5.6 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 3403 (O-H), 3082 (C-H), 2951 (C-H), 1733 (C=O), 1690 (C=O), 1642 (C=C); δ_H (400 MHz, $CDCl_3$) 0.87 (9H, s, C(CH_3)$_3$), 1.10 (3H, d, *J* 7.2, C(7)CH_3), 1.47 (1H, ddd, *J* 14.8, 10.8, 4.6, C(9)H_AH$_B$), 1.77 (1H, ddd, *J* 14.8, 10.8, 6.4, C(9)H$_A$$H_B$), 2.05-2.25 (2H, m, C(10)H_2), 3.22 (1H, q, *J* 7.2, C(7)*H*), 3.78 (3H, s, CO_2CH_3), 4.26 (1H, d, *J* 8.6, C(4)H_AH$_B$), 4.59 (1H, d, *J* 8.6, C(4)H$_A$$H_B$), 4.89 (1H, s, C(2)*H*), 4.98-5.02 (1H, m, C(12)H_AH$_B$), 5.02-5.08 (1H, m, C(12)H$_A$$H_B$), 5.78 (1H, ddt, *J* 17.0, 10.2, 6.6, C(11)*H*); δ_C (100 MHz, $CDCl_3$) 7.0 (C(7)*C*H$_3$), 25.1 (C(*C*H$_3$)$_3$), 27.7 (*C*(10)), 34.6 (*C*(9)), 36.7 (*C*(CH$_3$)$_3$), 49.2 (*C*(7)), 52.9 (CO_2*C*H$_3$), 68.3 (*C*(4)), 79.1 (*C*(5)), 83.1 (*C*(6)), 96.2 (*C*(2)), 115.8 (*C*(12)), 137.8 (*C*(11)), 172.0 (*C*O$_2$CH$_3$), 178.3 (*C*(8)); *m/z* (ESI$^+$) 326.2 (MH$^+$, 54%), 348.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 326.19623, $C_{17}H_{28}NO_5$ (MH$^+$) requires 326.19620.

(2*R*,5*S*)-1-Aza-6-(but-3-en-1-yl)-2-(*tert*-butyl)-5-methoxycarbonyl-7-methyl-3-oxa-8-oxobicyclo[3.3.0]-oct-6-ene (425)



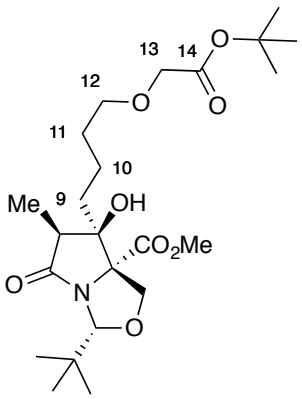
Side-product (9%, 132 mg) from the aldol cyclisation of oxazolidine **360**; white solid; m.p. 84 °C. R_f (30% EtOAc in petrol) 0.67; $[\alpha]_D^{20}$ +189.1 (*c* 1.0, $CHCl_3$); ν_{max}/cm^{-1} 2956 (C-H), 1733 (C=O), 1706 (C=O); δ_H (400 MHz, $CDCl_3$) 0.92 (9H, s, C(CH_3)$_3$), 1.80 (3H, s, C(7)CH_3), 2.00-2.07 (1H, m, C(10)H_AH$_B$), 2.10-2.24 (2H, m, C(9)H_AH$_B$ + C(10)H$_A$$H_B$), 2.45-2.51 (1H, m, C(9)H$_A$$H_B$), 3.19 (1H, d, *J* 8.3, C(4)H_AH$_B$), 3.73 (3H, s, CO_2CH_3), 4.64 (1H, s, C(2)*H*), 4.78 (1H, d, *J* 8.3, C(4)H$_A$$H_B$), 4.99-5.06 (2H, m, C(12)H_2), 5.71 (1H, ddt, *J* 16.8, 10.2, 6.4, C(11)*H*); δ_C (100 MHz, $CDCl_3$) 9.2 (C(7)*C*H$_3$), 24.9 (C(*C*H$_3$)$_3$), 26.7 (*C*(9)), 31.4 (*C*(10)), 35.3 (*C*(CH$_3$)$_3$), 53.0 (CO_2*C*H$_3$), 70.9 (*C*(4)), 77.8 (*C*(5)), 96.5 (*C*(2)), 116.4 (*C*(12)), 132.3 (*C*(7)), 136.5 (*C*(11)), 153.2 (*C*(6)), 170.4 (*C*O$_2$CH$_3$), 179.0 (*C*(8)); *m/z* (ESI$^+$) 330.2 (MNa$^+$, 49%); HRMS (ESI$^+$) found 330.16754, $C_{17}H_{25}NNaO_4$ (MNa$^+$) requires 330.16758.

(2*R*,5*R*,6*R*,7*S*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-6-(4-hydroxybutyl)-5-methoxycarbonyl-7-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane (362)



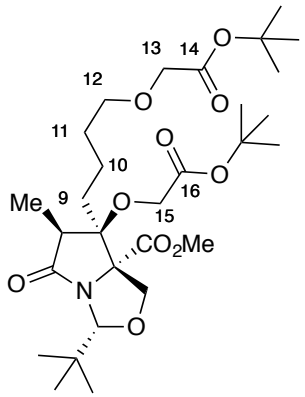
BH_3·THF (1 M in THF, 2.6 mL, 2.6 mmol) was added dropwise to a solution of alkene **361** (282 mg, 0.866 mmol) in anhydrous THF (3.5 mL) at 0 °C. The reaction mixture was stirred at room temperature for 11 h and cooled to 0 °C. H_2O (480 μL), aqueous NaOH (2 M, 4.3 mL, 8.6 mmol) and H_2O_2 (35% w/w, 740 μL, 8.64 mmol) were then added dropwise and the suspension was stirred at room temperature for another 6 h. Brine was added and the aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (50% to 60% EtOAc in petrol) to give alcohol **362** (230 mg, 0.670 mmol, 77%) as a white solid; m.p. 190 °C. R_f (80% EtOAc in petrol) 0.21; $[\alpha]_D^{20}$ +5.9 (*c* 1.0, MeOH); ν_{max}/cm^{-1} 3445 (O-H), 3262 (O-H), 3082 (C-H), 2955 (C-H), 1735 (C=O), 1693 (C=O); δ_H (400 MHz, CD_3OD) 0.85 (9H, s, C(CH_3)$_3$), 1.05 (3H, d, *J* 7.1, C(7)CH_3), 1.29-1.51 (5H, m, C(9)H_AH$_B$ + C(10)H_2 + C(11)H_2), 1.68-1.76 (1H, m, C(9)H$_A$$H_B$), 3.19 (1H, q, *J* 7.1, C(7)*H*), 3.53 (2H, t, *J* 6.3, C(12)H_2), 3.78 (3H, s, CO_2CH_3), 4.26 (1H, d, *J* 8.6, C(4)H_AH$_B$), 4.54 (1H, d, *J* 8.6, C(4)H$_A$$H_B$), 4.80 (1H, s, C(2)*H*); δ_C (100 MHz, CD_3OD) 7.5 (C(7)*C*H$_3$), 20.8 (*C*(10)), 25.4 (C(*C*H$_3$)$_3$), 33.9 (*C*(11)), 36.2 (*C*(9)), 37.4 (*C*(CH$_3$)$_3$), 50.8 (*C*(7)), 53.2 (CO_2*C*H$_3$), 62.6 (*C*(12)), 69.2 (*C*(4)), 80.8 (*C*(5)), 83.6 (*C*(6)), 97.0 (*C*(2)), 173.5 (*C*O$_2$CH$_3$), 180.9 (*C*(8)); *m/z* (ESI$^+$) 366.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 366.18872, $C_{17}H_{29}NNaO_6$ (MNa$^+$) requires 366.18871.

(2*R*,5*R*,6*R*,7*S*)-1-Aza-6-(4-(2-(*tert*-butoxy)-2-oxoethoxy)butyl)-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-7-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane (363)



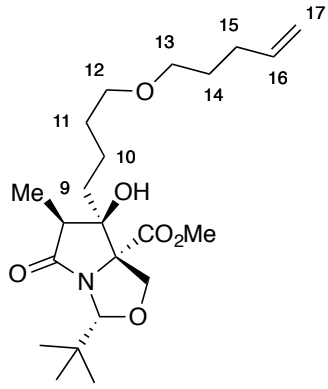
NaH (60% w/w, 4.0 mg, 0.10 mmol) was added to a solution of alcohol **362** (20 mg, 0.059 mmol) in anhydrous THF (300 μL) at 0 °C. The suspension was stirred at this temperature for 20 min, and *tert*-butyl bromoacetate (13 μL, 0.068 mmol) was added. The reaction mixture was stirred at room temperature for 16 h, quenched with aqueous saturated NH_4Cl, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (10% to 30% EtOAc in petrol) to give monosubsituted product **363** (14 mg, 0.031 mmol, 52%) as a white solid; m.p. 132 °C. R_f (30% EtOAc in petrol) 0.18; $[\alpha]_D^{20}$ +10.6 (*c* 0.4, $CHCl_3$); ν_{max}/cm^{-1} 3405 (O-H), 2974 (C-H), 1732 (C=O), 1692 (C=O); δ_H (500 MHz, $CDCl_3$) 0.88 (9H, s, C(CH_3)$_3$), 1.11 (3H, d, *J* 7.1, C(7)CH_3), 1.42-1.53 (3H, m, C(9)H_AH$_B$ + C(10)H_AH$_B$ + C(11)H_AH$_B$), 1.47 (9H, s, OC(CH_3)$_3$), 1.62-1.66 (2H, m, C(10)H$_A$$H_B$ + C(11)H$_A$$H_B$), 1.78-1.84 (1H, m, C(9)H$_A$$H_B$), 3.00 (1H, s, C(6)O*H*), 3.19 (1H, q, *J* 7.1, C(7)*H*), 3.46-3.55 (2H, m, C(12)H_2), 3.78 (3H, s, CO_2CH_3), 3.91 (1H, d, *J* 16.7, C(13)H_AH$_B$), 3.96 (1H, d, *J* 16.7, C(13)H$_A$$H_B$), 4.32 (1H, d, *J* 8.6, C(4)H_AH$_B$), 4.56 (1H, d, *J* 8.6, C(4)H$_A$$H_B$), 4.90 (1H, s, C(2)*H*); δ_C (125 MHz, $CDCl_3$) 7.3 (C(7)*C*H$_3$), 19.1 (*C*(10)), 25.1 (C(*C*H$_3$)$_3$), 28.3 (OC(*C*H$_3$)$_3$), 28.9 (*C*(11)), 34.0 (*C*(9)), 36.6 (*C*(CH$_3$)$_3$), 49.5 (*C*(7)), 52.7 (CO_2*C*H$_3$), 68.3 (*C*(4)), 68.4 (*C*(13)), 70.3 (*C*(12)), 79.2 (*C*(5)), 82.3 (O*C*(CH$_3$)$_3$), 82.3 (*C*(6)), 96.1 (*C*(2)), 170.5 (*C*(14)), 172.4 (*C*O$_2$CH$_3$), 178.7 (*C*(8)); *m/z* (ESI$^+$) 480.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 480.25661, $C_{23}H_{39}NNaO_8$ (MNa$^+$) requires 480.25679.

(2*R*,5*R*,6*R*,7*S*)-1-Aza-6-(2-(*tert*-butoxy)-2-oxoethoxy)-6-(4-(2-(*tert*-butoxy)-2-oxoethoxy)butyl)-2-(*tert*-butyl)-5-methoxycarbonyl-7-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane (364)



Side-product (15%, 5 mg) from the substitution of alcohol **362** with *tert*-butyl bromoacetate; white solid; m.p. 64 °C. R_f (30% EtOAc in petrol) 0.38; $[\alpha]_D^{20}$ +20.1 (*c* 0.8, $CHCl_3$); ν_{max}/cm^{-1} 2961 (C-H), 1744 (C=O), 1721 (C=O); δ_H (500 MHz, $CDCl_3$) 0.87 (9H, s, C(CH_3)$_3$), 1.18 (3H, d, *J* 7.2, C(7)CH_3), 1.45 (9H, s, OC(CH_3)$_3$), 1.47 (9H, s, OC(CH_3)$_3$), 1.43-1.47 (3H, m, C(9)H_AH$_B$ + C(10)H_2), 1.53-1.60 (2H, m, C(11)H_2), 1.90-1.95 (1H, m, C(9)H$_A$$H_B$), 3.19 (1H, q, *J* 7.2, C(7)*H*), 3.46 (1H, dt, *J* 9.0, 6.4, C(12)H_AH$_B$), 3.51 (1H, dt, *J* 9.0, 6.4, C(12)H$_A$$H_B$), 3.77 (3H, s, CO_2CH_3), 3.90 (1H, d, *J* 16.4, C(13)H_AH$_B$), 3.91 (1H, d, *J* 15.5, C(15)H_AH$_B$), 3.93 (1H, d, *J* 16.4, C(13)H$_A$$H_B$), 4.01 (1H, d, *J* 15.5, C(15)H$_A$$H_B$), 4.36 (1H, d, *J* 8.6, C(4)H_AH$_B$), 4.59 (1H, d, *J* 8.6, C(4)H$_A$$H_B$), 4.91 (1H, s, C(2)*H*); δ_C (125 MHz, $CDCl_3$) 8.9 (C(7)*C*H$_3$), 20.0 (*C*(10)), 25.1 (C(*C*H$_3$)$_3$), 28.2 (OC(*C*H$_3$)$_3$), 28.3 (OC(*C*H$_3$)$_3$), 29.9 (*C*(11)), 32.7 (*C*(9)), 36.5 (*C*(CH$_3$)$_3$), 49.1 (*C*(7)), 52.9 (CO_2CH_3), 63.2 (*C*(15)), 68.8 (*C*(4)), 68.8 (*C*(13)), 71.0 (*C*(12)), 79.8 (*C*(5)), 81.7 (O*C*(CH$_3$)$_3$), 82.1 (O*C*(CH$_3$)$_3$), 87.8 (*C*(6)), 96.4 (*C*(2)), 168.3 (*C*(16)), 169.8 (*C*(14)), 172.1 (*C*O$_2$CH$_3$), 178.5 (*C*(8)); *m/z* (ESI$^+$) 594.4 (MNa$^+$, 100%); HRMS (ESI$^+$) found 594.32390, $C_{29}H_{49}NNaO_{10}$ (MNa$^+$) requires 594.32570.

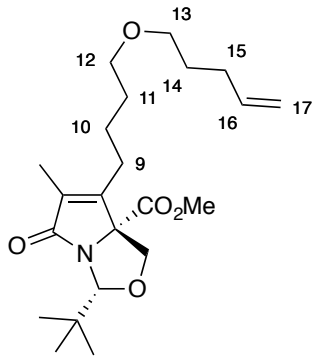
(2*R*,5*R*,6*R*,7*S*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-7-methyl-3-oxa-8-oxo-6-(4-(pent-4-en-1-yloxy)butyl)bicyclo[3.3.0]-octane (365)



NaH (60% w/w, 12 mg, 0.30 mmol) was added to a solution of alcohol **362** (21 mg, 0.060 mmol) and tetrabutylammonium iodide (2 mg, 0.006 mmol) in anhydrous THF (600 µL) at 0 °C. The suspension was stirred at this temperature for 10 min and 5-bromo-1-pentene (36 µL, 0.30 mmol) was added. The reaction mixture was then stirred at room temperature for 18 h and quenched with saturated aqueous NH_4Cl. The aqueous layer was extracted with Et_2O, dried over anhydrous Na_2SO_4 and concentrated

under reduced pressure. The crude product was purified by *flash* column chromatography (10% to 30% EtOAc in petrol) to give alkene **365** (6 mg, 0.01 mmol, 25%) as a white solid; m.p. 93 °C. R_f (30% EtOAc in petrol) 0.29; $[\alpha]_D^{20}$ +14.4 (*c* 0.3, $CHCl_3$); ν_{max}/cm^{-1} 3423 (O-H), 2954 (C-H), 2926 (C-H), 1727 (C=O), 1692 (C=O); δ_H (500 MHz, $CDCl_3$) 0.87 (9H, s, C(CH_3)$_3$), 1.09 (3H, d, *J* 7.0, C(7)CH_3), 1.40-1.48 (3H, m, C(10)H_2 + C(9)H_AH_B), 1.54-1.59 (2H, m, C(11)H_2), 1.63-1.72 (3H, m, C(14)H_2 + C(9)H$_A$$H_B$), 2.08-2.13 (2H, m, C(15)H_2), 2.67 (1H, s, OH), 3.18 (1H, q, *J* 7.0, C(7)H), 3.40-3.44 (4H, m, C(12)H_2 + C(13)H_2), 3.77 (3H, s, CO_2CH_3), 4.27 (1H, d, *J* 8.7, C(4)H_AH_B), 4.57 (1H, d, *J* 8.7, C(4)H$_A$$H_B$), 4.89 (1H, s, C(2)$H$), 4.97 (1H, dq, *J* 10.2, 1.5, C(17)H_AH_B), 5.02 (1H, dq, *J* 17.0, 1.5, C(17)H$_A$$H_B$), 5.80 (1H, ddt, *J* 17.0, 10.2, 6.7, C(16)H); δ_C (125 MHz, $CDCl_3$) 7.2 (C(7)CH$_3$), 20.5 (C(10)), 25.1 (C(CH$_3$)$_3$), 28.9 (C(14)), 29.7 (C(11)), 30.4 (C(15)), 34.7 (C(9)), 36.6 (C(CH$_3$)$_3$), 49.3 (C(7)), 52.8 (CO_2CH$_3$), 68.4 (C(4)), 70.6 (C(13)), 70.7 (C(12)), 79.2 (C(5)), 83.0 (C(6)), 96.2 (C(2)), 115.0 (C(17)), 138.2 (C(16)), 172.2 (CO_2CH$_3$), 178.5 (C(8)); *m/z* (ESI$^+$) 412.2 (MH$^+$, 31%), 434.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 412.26951, $C_{22}H_{38}NO_6$ (MH$^+$) requires 412.26936.

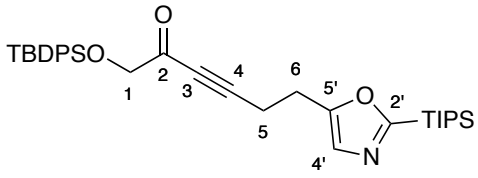
(2*R*,5*S*)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-7-methyl-3-oxa-8-oxo-6-(4-(pent-4-en-1-yloxy)butyl)bicyclo[3.3.0]-oct-6-ene (366)



Side-product (25%, 6 mg) from the substitution of alcohol **362** with 5-bromo-1-pentene; colourless oil. R_f (30%) 0.28; $[\alpha]_D^{20}$ +155.0 (*c* 0.6, $CHCl_3$); ν_{max}/cm^{-1} 2953 (C-H), 2865 (C-H), 1742 (C=O), 1709 (C=O); δ_H (500 MHz, $CDCl_3$) 0.92 (9H, s, C(CH_3)$_3$), 1.32-1.39 (1H, m, C(10)H_AH_B), 1.47-1.58 (3H, m, C(10)H$_A$$H_B$ + C(11)H_2), 1.65 (2H, tt, *J* 8.0, 6.5, C(14)H_2), 1.81 (3H, s, C(7)CH_3), 2.05-2.12 (3H, m, C(9)H_AH_B + C(15)H_2), 2.37-2.43 (1H, m, C(9)H$_A$$H_B$), 3.19 (1H, d, *J* 8.3, C(4)H_AH_B), 3.37-3.41 (4H, m, C(12)H_2 + C(13)H_2), 3.73 (3H, s, CO_2CH_3), 4.65 (1H, s, C(2)H), 4.80 (1H, d, *J* 8.3, C(4)H$_A$$H_B$), 4.96 (1H, ddt, *J* 10.2, 2.1, 1.5, C(17)H_AH_B), 5.01 (1H, dq, *J* 17.0, 1.5, C(17)H$_A$$H_B$), 5.81 (1H, ddt, *J* 17.0, 10.2, 6.6, C(16)H); δ_C (125 MHz, $CDCl_3$) 9.2 (C(7)CH$_3$), 24.4 (C(10)), 24.9 (C(CH$_3$)$_3$), 27.1 (C(9)), 29.0 (C(14)), 29.8 (C(11)), 30.4 (C(15)), 35.3 (C(CH$_3$)$_3$), 53.0 (CO_2CH$_3$), 70.2 (C(12)), 70.5 (C(13)), 70.9 (C(4)), 77.9 (C(5)), 96.5 (C(2)), 114.9 (C(17)), 131.9 (C(7)), 138.4 (C(16)), 154.1 (C(6)), 170.4 (CO_2CH$_3$),

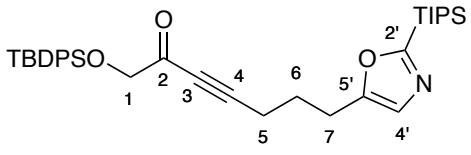
179.1 (*C*(8)); *m/z* (ESI+) 416.2 (MNa^+, 100%); HRMS (ESI+) found 416.24052, $C_{22}H_{35}NNaO_5$ (MNa^+) requires 416.24074.

1-((*tert*-Butyldiphenylsilyl)oxy)-6-(2-(triisopropylsilyl)oxazol-5-yl)hex-3-yn-2-one (376)



To a solution of alkyne **130** (53 mg, 0.19 mmol) in anhydrous THF (400 μL) was added *n*-BuLi (1.6 M in hexanes, 140 μL, 0.224 mmol) dropwise at -78 °C, and the solution was stirred at this temperature for 30 min. Weinreb amide **375** (90 mg, 0.25 mmol) in anhydrous THF (370 μL) was then added dropwise, and the reaction mixture was stirred at -78 °C for 20 min and at 0 °C for 2 h. The reaction was quenched with saturated aqueous NH_4Cl and the aqueous layer was extracted with Et_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (5% to 10% EtOAc in petrol) to give alkynone **376** (64 mg, 0.11 mmol, 58%) as a light yellow oil. R_f (10% EtOAc in petrol) 0.18; ν_{max}/cm^{-1} 3072 (C-H), 2943 (C-H), 2865 (C-H), 2214 (C≡C), 1698 (C=O), 1678 (C=C); δ_H (400 MHz, $CDCl_3$) 1.09 (9H, s, SiC(CH_3)$_3$), 1.11 (18H, d, *J* 7.4, SiCH(CH_3)$_2$), 1.37 (3H, sept, *J* 7.4, SiC*H*(CH_3)$_2$), 2.69 (2H, t, *J* 7.4, C(5)H_2), 2.95 (2H, t, *J* 7.4, C(6)H_2), 4.28 (2H, s, C(1)H_2), 6.88 (1H, s, C(4')*H*), 7.36-7.46 (6H, m, Ph), 7.65-7.68 (4H, m, Ph); δ_C (100 MHz, $CDCl_3$) 11.1 (Si*C*H(CH_3)$_2$), 18.3 (*C*(5)), 18.5 (SiCH(*C*H_3)$_2$), 19.5 (Si*C*(CH_3)$_3$), 24.3 (*C*(6)), 26.8 (SiC(*C*H_3)$_3$), 70.7 (*C*(1)), 79.5 (*C*(4)), 94.6 (*C*(3)), 123.2 (*C*(4')), 128.0, 130.1, 132.8, 135.7 (Ph), 151.8 (*C*(5')), 168.3 (*C*(2')), 185.8 (*C*(2)); *m/z* (ESI+) 574.4 (MH^+, 100%), 596.4 (MNa^+, 9%); HRMS (ESI+) found 574.31624, $C_{34}H_{48}NO_3Si_2$ (MH^+) requires 574.31672.

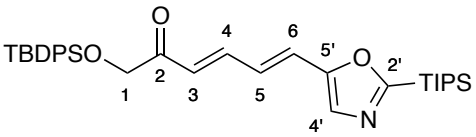
1-((*tert*-Butyldiphenylsilyl)oxy)-7-(2-(triisopropylsilyl)oxazol-5-yl)hept-3-yn-2-one (377)



To a solution of alkyne **132** (51 mg, 0.17 mmol) in anhydrous THF (340 μL) was added *n*-BuLi (1.6 M in hexanes, 130 μL, 0.208 mmol) dropwise at -78 °C, and the

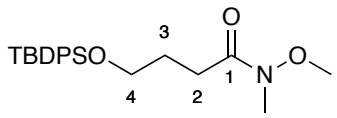
solution was stirred at this temperature for 30 min. Weinreb amide **375** (85 mg, 0.24 mmol) in anhydrous THF (350 μL) was then added dropwise and the reaction mixture was stirred at -78 °C for 20 min and at 0 °C for 2 h. The reaction was quenched with saturated aqueous NH_4Cl and the aqueous layer was extracted with Et_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (5% to 10% EtOAc in petrol) to give alkynone **377** (73 mg, 0.12 mmol, 72%) as a light yellow oil. R_f (10% EtOAc in petrol) 0.16; ν_{max}/cm^{-1} 3072 (C-H), 2943 (C-H), 2865 (C-H), 2112 (C≡C), 1698 (C=O), 1677 (C=C); δ_H (400 MHz, $CDCl_3$) 1.09 (9H, s, SiC(CH_3)$_3$), 1.12 (18H, d, *J* 7.4, SiCH(CH_3)$_2$), 1.38 (3H, sept, *J* 7.4, SiC*H*(CH_3)$_2$), 1.91 (2H, quint, *J* 7.1, C(6)H_2), 2.40 (2H, t, *J* 7.1, C(5)H_2), 2.78 (2H, td, *J* 7.1, 0.8, C(7)H_2), 4.30 (2H, s, C(1)H_2), 6.82 (1H, t, *J* 0.8, C(4')*H*), 7.36-7.44 (6H, m, Ph), 7.66-7.69 (4H, m, Ph); δ_C (100 MHz, $CDCl_3$) 11.1 (Si*C*H(CH_3)$_2$), 18.5 (SiCH(*C*H_3)$_2$), 18.6 (*C*(5)), 19.4 (Si*C*(CH_3)$_3$), 24.7 (*C*(7)), 25.9 (*C*(6)), 26.8 (SiC(*C*H_3)$_3$), 70.6 (*C*(1)), 79.6 (*C*(4)), 95.9 (*C*(3)), 122.9 (*C*(4')), 128.0, 130.1, 132.8, 135.6 (Ph), 153.1 (*C*(5')), 167.9 (*C*(2')), 186.2 (*C*(2)); *m/z* (ESI$^+$) 588.3 (MH$^+$, 100%), 610.2 (MNa$^+$, 9%); HRMS (ESI$^+$) found 588.33208, $C_{35}H_{50}NO_3Si_2$ (MH$^+$) requires 588.33237.

(3*E*,5*E*)-1-((*tert*-Butyldiphenylsilyl)oxy)-6-(2-(triisopropylsilyl)oxazol-5-yl)hexa-3,5-dien-2-one (378)



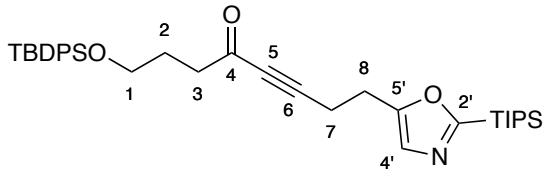
General procedure G (from **376**, with 2 eq of triphenylphosphine); yield 41% (7 mg); orange oil. R_f (10% EtOAc in petrol) 0.21; ν_{max}/cm^{-1} 3072 (C-H), 2944 (C-H), 2866 (C-H), 1705 (C=O), 1686 (C=C); δ_H (500 MHz, $CDCl_3$) 1.12 (9H, s, SiC(CH_3)$_3$), 1.16 (18H, d, *J* 7.5, SiCH(CH_3)$_2$), 1.43 (3H, sept, *J* 7.5, SiC*H*(CH_3)$_2$), 4.36 (2H, s, C(1)H_2), 6.69 (1H, d, *J* 15.3, C(3)*H*), 6.74-6.83 (2H, m, C(5)*H* + C(6)*H*), 7.23 (1H, s, C(4')*H*), 7.36-7.46 (7H, m, C(4)*H* + Ph), 7.66-7.68 (4H, m, Ph); δ_C (125 MHz, $CDCl_3$) 11.1 (Si*C*H(CH_3)$_2$), 18.5 (SiCH(*C*H_3)$_2$), 19.5 (Si*C*(CH_3)$_3$), 26.9 (SiC(*C*H_3)$_3$), 69.6 (*C*(1)), 125.5 (*C*(6)), 125.7 (*C*(3)), 125.7 (*C*(5)), 128.0 (Ph), 128.2 (*C*(4')), 130.1, 132.9, 135.7 (Ph), 142.4 (*C*(4)), 151.1 (*C*(5')), 170.4 (*C*(2')), 198.3 (*C*(2)); *m/z* (ESI$^+$) 574.3 (MH$^+$, 100%); HRMS (ESI$^+$) found 574.31668, $C_{34}H_{48}NO_3Si_2$ (MH$^+$) requires 574.31672.

4-((*tert*-Butyldiphenylsilyl)oxy)-*N*-methoxy-*N*-methylbutanamide (382)[252]



TBDPSCl (650 μL, 2.50 mmol) was added dropwise to a solution of alcohol **381** (333 mg, 2.26 mmol) and imidazole (206 mg, 3.02 mmol) in anhydrous DCM (4.5 mL) at 0 °C, and stirring was continued at room temperature for 1 h. The reaction mixture was then diluted with water and the aqueous phase was extracted with DCM, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by *flash* column chromatography (10% to 20% EtOAc in petrol) to give silyl ether **382** (803 mg, 2.08 mmol, 94%) as a colourless oil. R_f (20% EtOAc in petrol) 0.25; ν_{max}/cm^{-1} 3071 (C-H), 2932 (C-H), 2857 (C-H), 1666 (C=O); δ_H (400 MHz, $CDCl_3$) 1.06 (9H, s, SiC(CH_3)$_3$), 1.87-1.94 (2H, m, C(3)H_2), 2.57 (2H, t, *J* 7.4, C(2)H_2), 3.18 (3H, s, NCH_3), 3.67 (3H, s, NOCH_3), 3.74 (2H, t, *J* 6.0, C(4)H_2), 7.36-7.44 (6H, m, Ph), 7.66-7.69 (4H, m, Ph); δ_C (100 MHz, $CDCl_3$) 19.4 (Si*C*(CH_3)$_3$), 27.0 (SiC(*C*H_3)$_3$), 27.6 (*C*(3)), 28.5 (*C*(2)), 32.3 (N*C*H_3), 61.3 (NO*C*H_3), 63.3 (*C*(4)), 127.7, 129.7, 134.0, 135.7 (Ph), 174.6 (*C*(1)); *m/z* (ESI$^+$) 408.2 (MNa$^+$, 100%); HRMS (ESI$^+$) found 408.19547, $C_{22}H_{31}NNaO_3Si$ (MNa$^+$) requires 408.19654.

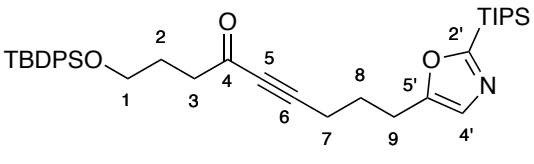
1-((*tert*-Butyldiphenylsilyl)oxy)-8-(2-(triisopropylsilyl)oxazol-5-yl)oct-5-yn-4-one (383)



To a solution of alkyne **130** (60 mg, 0.22 mmol) in anhydrous THF (800 μL) was added *n*-BuLi (1.6 M in hexanes, 300 μL, 0.480 mmol) dropwise at -78 °C, and the solution was stirred at this temperature for 30 min. A solution of Weinreb amide **382** (209 mg, 0.543 mmol) in anhydrous THF (350 μL) was then added dropwise and the reaction mixture was stirred at -78 °C for 20 min and at 0 °C for 2 h. The reaction was quenched with saturated aqueous NH_4Cl and the aqueous layer was extracted with Et_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (4% to 8% EtOAc in petrol) to give alkynone **383** (120 mg, 0.199 mmol, 92%) as a light yellow oil. R_f (10% EtOAc in petrol) 0.20; ν_{max}/cm^{-1} 3071 (C-H), 2943 (C-H), 2866 (C-H),

2214 (C≡C), 1676 (C=O); δ_H (400 MHz, $CDCl_3$) 1.04 (9H, s, SiC(CH_3)$_3$), 1.12 (18H, d, *J* 7.4, SiCH(CH_3)$_2$), 1.39 (3H, sept, *J* 7.4, SiC*H*(CH_3)$_2$), 1.85-1.92 (2H, m, C(2)H_2), 2.64 (2H, t, *J* 7.3, C(3)H_2), 2.71 (2H, t, *J* 7.4, C(7)H_2), 2.98 (2H, t, *J* 7.4, C(8)H_2), 3.66 (2H, t, *J* 6.0, C(1)H_2), 6.91 (1H, s, C(4')*H*), 7.32-7.43 (6H, m, Ph), 7.63-7.66 (4H, m, Ph); δ_C (100 MHz, $CDCl_3$) 11.1 (Si*C*H(CH_3)$_2$), 18.2 (*C*(7)), 18.5 (SiCH(*C*H_3)$_2$), 19.3 (Si*C*(CH_3)$_3$), 24.5 (*C*(8)), 27.0 (SiC(*C*H_3)$_3$), 27.0 (*C*(2)), 42.3 (*C*(3)), 62.9 (*C*(1)), 81.5 (*C*(6)), 91.5 (*C*(5)), 123.3 (*C*(4')), 127.8, 129.8, 133.8, 135.7 (Ph), 152.0 (*C*(5')), 168.3 (*C*(2')), 187.8 (*C*(4)); *m/z* (ESI$^+$) 602.4 (MH$^+$, 100%); HRMS (ESI$^+$) found 602.34758, $C_{36}H_{52}NO_3Si_2$ (MH$^+$) requires 602.34802.

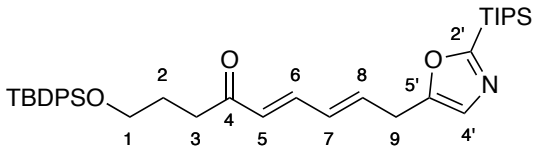
1-((*tert*-Butyldiphenylsilyl)oxy)-9-(2-(triisopropylsilyl)oxazol-5-yl)non-5-yn-4-one (384)



To a solution of alkyne **132** (89 mg, 0.31 mmol) in anhydrous THF (600 μL) was added *n*-BuLi (1.6 M in hexanes, 430 μL, 0.690 mmol) dropwise at -78 °C, and the solution was stirred at this temperature for 30 min. A solution of Weinreb amide **382** (300 mg, 0.779 mmol) in anhydrous THF (600 μL) was then added dropwise and the reaction mixture was stirred at -78 °C for 20 min and at 0 °C for 2 h. The reaction was quenched with saturated aqueous NH_4Cl and the aqueous layer was extracted with Et_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (4% to 8% EtOAc in petrol) to give alkynone **384** (177 mg, 0.287 mmol, 94%) as a colourless oil. R_f (10% EtOAc in petrol) 0.16; ν_{max}/cm^{-1} 3072 (C-H), 2943 (C-H), 2866 (C-H), 2212 (C≡C), 1675 (C=O); δ_H (400 MHz, $CDCl_3$) 1.05 (9H, s, SiC(CH_3)$_3$), 1.13 (18H, d, *J* 7.4, SiCH(CH_3)$_2$), 1.39 (3H, sept, *J* 7.4, SiC*H*(CH_3)$_2$), 1.89-1.97 (4H, m, C(2)H_2 + C(8)H_2), 2.41 (2H, t, *J* 7.1, C(7)H_2), 2.69 (2H, t, *J* 7.3, C(3)H_2), 2.82 (2H, t, *J* 7.1, C(9)H_2), 3.68 (2H, t, *J* 6.0, C(1)H_2), 6.85 (1H, s, C(4')*H*), 7.35-7.44 (6H, m, Ph), 7.64-7.66 (4H, m, Ph); δ_C (100 MHz, $CDCl_3$) 11.1 (Si*C*H(CH_3)$_2$), 18.4 (*C*(7)), 18.5 (SiCH(*C*H_3)$_2$), 19.3 (Si*C*(CH_3)$_3$), 24.7 (*C*(9)), 26.1 (*C*(8)), 26.9 (SiC(*C*H_3)$_3$), 27.0 (*C*(2)), 42.3 (*C*(3)), 62.8 (*C*(1)), 81.6 (*C*(6)), 92.6 (*C*(5)), 122.9 (*C*(4')), 127.8, 129.7, 133.8, 135.6 (Ph), 153.2

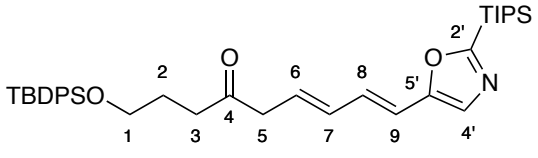
(*C*(5')), 167.9 (*C*(2')), 187.9 (*C*(4)); *m/z* (ESI+) 616.4 (MH^+, 100%), 638.3 (MNa^+, 24%); HRMS (ESI+) found 616.36347, $C_{37}H_{54}NO_3Si_2$ (MH^+) requires 616.36367.

(5*E*,7*E*)-1-((*tert*-Butyldiphenylsilyl)oxy)-9-(2-(triisopropylsilyl)oxazol-5-yl)nona-5,7-dien-4-one (385)



General procedure G (from **384**, with 2.5 eq of triphenylphosphine); yield 50% (26 mg); yellow oil. R_f (10% EtOAc in petrol) 0.13; ν_{max}/cm^{-1} 3071 (C-H), 2943 (C-H), 2866 (C-H), 1690 (C=O), 1667 (C=C); δ_H (400 MHz, $CDCl_3$) 1.05 (9H, s, SiC(CH_3)$_3$), 1.13 (18H, d, *J* 7.4, SiCH(CH_3)$_2$), 1.40 (3H, sept, *J* 7.4, SiC*H*(CH$_3$)$_2$), 1.88 (2H, tt, *J* 7.4, 6.0, C(2)H_2), 2.68 (2H, t, *J* 7.4, C(3)H_2), 3.59 (2H, d, *J* 4.8, C(9)H_2), 3.69 (2H, t, *J* 6.0, C(1)H_2), 6.11 (1H, d, *J* 15.6, C(5)*H*), 6.16-6.27 (2H, m, C(7)*H* + C(8)*H*), 6.88 (1H, s, C(4')*H*), 7.11-7.18 (1H, m, C(6)*H*), 7.34-7.44 (6H, m, Ph), 7.63-7.66 (4H, m, Ph); δ_C (125 MHz, $CDCl_3$) 11.1 (Si*C*H(CH$_3$)$_2$), 18.5 (SiCH(*C*H$_3$)$_2$), 19.4 (Si*C*(CH$_3$)$_3$), 27.0 (SiC(*C*H$_3$)$_3$), 27.3 (*C*(2)), 29.4 (*C*(9)), 37.1 (*C*(3)), 63.2 (*C*(1)), 123.5 (*C*(4')), 127.8 (Ph), 129.6 (*C*(5)), 129.8 (Ph), 131.2 (*C*(7)), 134.0, 135.7 (Ph), 137.8 (*C*(8)), 141.7 (*C*(6)), 151.2 (*C*(5')), 168.4 (*C*(2')), 200.7 (*C*(4)); *m/z* (ESI+) 616.4 (MH^+, 100%); HRMS (ESI+) found 616.36388, $C_{37}H_{54}NO_3Si_2$ (MH^+) requires 616.36367.

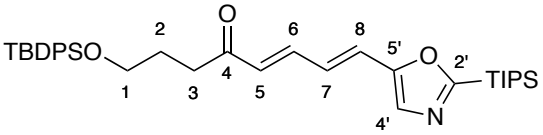
(6*E*,8*E*)-1-((*tert*-Butyldiphenylsilyl)oxy)-9-(2-(triisopropylsilyl)oxazol-5-yl)nona-6,8-dien-4-one (386)



Side-product (29%, 15 mg) from the isomerisation of alkynone **384**; yellow oil. R_f (10% EtOAc in petrol) 0.22; ν_{max}/cm^{-1} 3071 (C-H), 2944 (C-H), 2866 (C-H), 1717 (C=O); δ_H (500 MHz, $CDCl_3$) 1.04 (9H, s, SiC(CH_3)$_3$), 1.15 (18H, d, *J* 7.5, SiCH(CH_3)$_2$), 1.41 (3H, sept, *J* 7.5, SiC*H*(CH$_3$)$_2$), 1.83 (2H, tt, *J* 7.3, 6.0, C(2)H_2), 2.59 (2H, t, *J* 7.3, C(3)H_2), 3.25 (2H, d, *J* 7.3, C(5)H_2), 3.67 (2H, t, *J* 6.0, C(1)H_2), 5.95 (1H, dt, *J* 15.0, 7.3, C(6)*H*), 6.22 (1H, dd, *J* 15.0, 10.8, C(7)*H*), 6.35 (1H, d, *J* 15.5, C(9)*H*), 6.71 (1H, dd, *J* 15.5, 10.8, C(8)*H*), 7.03 (1H, s, C(4')*H*), 7.36-7.43 (6H, m, Ph), 7.63-7.65 (4H, m, Ph); δ_C (125 MHz, $CDCl_3$) 11.1 (Si*C*H(CH$_3$)$_2$),

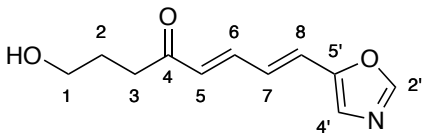
18.5 (SiCH(CH$_3$)$_2$), 19.4 (SiC(CH$_3$)$_3$), 26.6 (C(2)), 27.0 (SiC(CH$_3$)$_3$), 39.0 (C(3)), 47.0 (C(5)), 63.0 (C(1)), 116.6 (C(9)), 125.0 (C(4')), 127.8 (Ph), 127.8 (C(6)), 129.6 (C(8)), 129.8 (Ph), 133.6 (C(7)), 133.9, 135.7 (Ph), 151.8 (C(5')), 168.5 (C(2')), 208.3 (C(4)); *m/z* (ESI$^+$) 616.4 (MH$^+$, 100%); HRMS (ESI$^+$) found 616.36333, $C_{37}H_{54}NO_3Si_2$ (MH$^+$) requires 616.36367.

(5*E*,7*E*)-1-((*tert*-Butyldiphenylsilyl)oxy)-8-(2-(triisopropylsilyl)oxazol-5-yl)octa-5,7-dien-4-one (387)



General procedure G (from **383**, with 2.5 eq of triphenylphosphine); yield 90% (73 mg); yellow oil. R_f (10% EtOAc in petrol) 0.25; ν_{max}/cm^{-1} 3071 (C-H), 2944 (C-H), 2866 (C-H), 1686 (C=O), 1664 (C=C); δ_H (400 MHz, $CDCl_3$) 1.06 (9H, s, SiC(CH_3)$_3$), 1.16 (18H, d, *J* 7.4, SiCH(CH_3)$_2$), 1.43 (3H, sept, *J* 7.4, SiCH(CH$_3$)$_2$), 1.90 (2H, tt, *J* 7.4, 6.0, C(2)H_2), 2.71 (2H, t, *J* 7.4, C(3)H_2), 3.71 (2H, t, *J* 6.0, C(1)H_2), 6.33 (1H, d, *J* 15.4, C(5)H), 6.72-6.82 (2H, m, C(7)H + C(8)H), 7.21 (1H, s, C(4')H), 7.26 (1H, ddd, *J* 15.4, 9.1, 1.4, C(6)H), 7.35-7.42 (6H, m, Ph), 7.64-7.67 (4H, m, Ph); δ_C (100 MHz, $CDCl_3$) 11.1 (SiCH(CH$_3$)$_2$), 18.5 (SiCH(CH$_3$)$_2$), 19.4 (SiC(CH$_3$)$_3$), 27.0 (SiC(CH$_3$)$_3$), 27.4 (C(2)), 37.6 (C(3)), 63.2 (C(1)), 124.6 (C(8)), 127.5 (C(7)), 127.8 (Ph), 127.9 (C(4')), 129.8 (Ph), 130.6 (C(5)), 133.9, 135.7 (Ph), 141.3 (C(6)), 151.1 (C(5')), 170.2 (C(2')), 200.3 (C(4)); *m/z* (ESI$^+$) 602.4 (MH$^+$, 100%), 624.4 (MNa$^+$, 13%); HRMS (ESI$^+$) found 602.34784, $C_{36}H_{52}NO_3Si_2$ (MH$^+$) requires 602.34802.

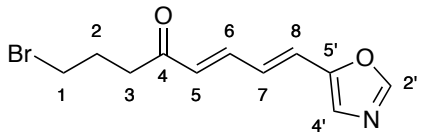
(5*E*,7*E*)-1-Hydroxy-8-(oxazol-5-yl)octa-5,7-dien-4-one (388)



TBAF (1 M in THF, 195 μL, 0.195 mmol) was added to a solution of silyl ether **387** (56 mg, 0.092 mmol) in anhydrous THF (1.8 mL). The solution was stirred at room temperature for 1 h, quenched with saturated aqueous NH_4Cl, extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography (50% EtOAc in petrol to 5% MeOH in EtOAc) to give alcohol **388** (9 mg, 0.04 mmol, 49%) as an orange solid; m.p. 106 °C. R_f (5% MeOH in EtOAc) 0.44; ν_{max}/cm^{-1} 3277

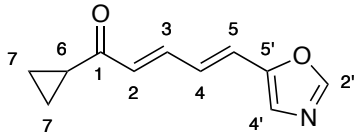
(O-H), 3105 (C-H), 2955 (C-H), 1678 (C=O), 1653 (C=C), 1630 (C=C), 1614 (C=C); δ_H (400 MHz, $CDCl_3$) 1.91 (2H, tt, *J* 6.9, 6.0, C(2)H_2), 2.02 (1H, br s, O*H*), 2.75 (2H, t, *J* 6.9, C(3)H_2), 3.69 (2H, t, *J* 6.0, C(1)H_2), 6.34 (1H, d, *J* 15.4, C(5)*H*), 6.74 (1H, d, *J* 15.5, C(8)*H*), 6.83 (1H, dd, 15.5, 10.6, C(7)*H*), 7.16 (1H, s, C(4')*H*), 7.27 (1H, dd, *J* 15.4, 10.6, C(6)*H*), 7.88 (1H, s, C(2')*H*); δ_C (100 MHz, $CDCl_3$) 26.9 (*C*(2)), 37.9 (*C*(3)), 62.4 (*C*(1)), 124.1 (*C*(8)), 127.1 (*C*(4')), 128.0 (*C*(7)), 131.0 (*C*(5)), 141.3 (*C*(6)), 149.5 (*C*(5')), 151.5 (*C*(2')), 200.5 (*C*(4)); HRMS (CI^+) found 208.0973, $C_{11}H_{14}NO_3$ (MH^+) requires 208.0968.

(5*E*,7*E*)-1-Bromo-8-(oxazol-5-yl)octa-5,7-dien-4-one (389)



Methanesulfonyl chloride (4 μL, 0.05 mmol) was added to a solution of alcohol **388** (9 mg, 0.04 mmol) and Et_3N (12 μL, 0.086 mmol) in anhydrous THF (430 μL) at 0 °C. The reaction mixture was stirred at room temperature for 30 min, LiBr (16 mg, 0.18 mmol) was added, stirred for another 21 h and quenched with H_2O. The aqueous phase was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (40% EtOAc in petrol) to give bromide **389** (4 mg, 0.02 mmol, 35%) as an orange oil. R_f (40% EtOAc in petrol) 0.28; ν_{max}/cm^{-1} 3126 (C-H), 2961 (C-H), 1677 (C=O), 1593 (C=C); δ_H (400 MHz, $CDCl_3$) 2.21 (2H, tt, *J* 7.0, 6.3, C(2)H_2), 2.81 (2H, t, *J* 7.0, C(3)H_2), 3.49 (2H, t, *J* 6.3, C(1)H_2), 6.33 (1H, d, *J* 15.4, C(5)*H*), 6.77 (1H, d, *J* 15.5, C(8)*H*), 6.84 (1H, dd, 15.5, 10.3, C(7)*H*), 7.17 (1H, s, C(4')*H*), 7.28 (1H, dd, *J* 15.4, 10.3, C(6)*H*), 7.89 (1H, s, C(2')*H*); δ_C (125 MHz, $CDCl_3$) 26.9 (*C*(2)), 33.7 (*C*(1)), 38.9 (*C*(3)), 124.2 (*C*(8)), 127.2 (*C*(4')), 128.0 (*C*(7)), 130.9 (*C*(5)), 141.3 (*C*(6)), 149.5 (*C*(5')), 151.6 (*C*(2')), 198.7 (*C*(4)); HRMS (EI^+) found 269.0044, $C_{11}H_{12}BrNO_3$ (M^+) requires 269.0051.

(2*E*,4*E*)-1-Cyclopropyl-5-(oxazol-5-yl)penta-2,4-dien-1-one (390)

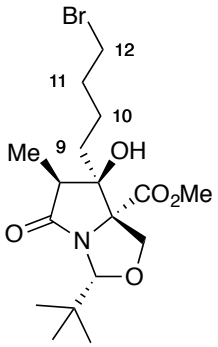


NaH (60% w/w, 1 mg, 0.02 mmol) was added to a solution of alcohol **362** (5 mg, 0.01 mmol) and tetrabutylammonium iodide

(0.7 mg, 0.002 mmol) in anhydrous THF (100 μL) at 0 °C. The suspension was stirred at this temperature for 10 min and a solution of bromide **389** (4 mg, 0.02 mmol) in anhydrous THF (300 μL) was added. The reaction mixture was then stirred at room temperature for 14 h and quenched with saturated aqueous NH_4Cl. The aqueous layer was extracted with Et_2O, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (40% EtOAc in petrol to EtOAc) to give recovered alcohol **362** and cyclopropyl **390** (2 mg, 0.01, 61%) as a yellow oil, instead of the desired substitution product.

Cyclopropyl **390**: R_f (40% EtOAc in petrol) 0.61; ν_{max}/cm^{-1} 3126 (C-H), 2963 (C-H), 1668 (C=O), 1645 (C=C), 1623 (C=C), 1594 (C=C); δ_H (500 MHz, $CDCl_3$) 0.94-0.98 (2H, m, C(7)H_AH_B), 1.11-1.15 (2H, m, C(7)H_AH_B), 2.14 (1H, tt, *J* 7.8, 4.5, C(6)*H*), 6.46 (1H, d, *J* 15.3, C(2)*H*), 6.75 (1H, d, *J* 15.5, C(5)*H*), 6.86 (1H, dd, 15.5, 11.1, C(4)*H*), 7.16 (1H, s, C(4')*H*), 7.30 (1H, dd, *J* 15.3, 11.1, C(3)*H*), 7.88 (1H, s, C(2')*H*); δ_C (125 MHz, $CDCl_3$) 11.6 (*C*(7)), 20.1 (*C*(6)), 123.8 (*C*(5)), 126.9 (*C*(4')), 128.2 (*C*(4)), 131.3 (*C*(2)), 140.4 (*C*(3)), 149.7 (*C*(5')), 151.5 (*C*(2')), 200.0 (*C*(1)); *m/z* (ESI$^+$) 190.0 (MH^+, 100%); HRMS (ESI$^+$) found 190.08643, $C_{11}H_{12}NO_2$ (MH^+) requires 190.08626.

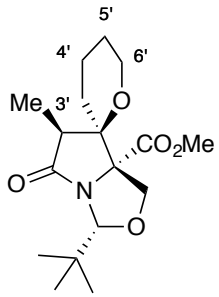
(2*R*,5*R*,6*R*,7*S*)-1-Aza-6-(4-bromobutyl)-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-7-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane (391)



To a solution of alcohol **362** (30 mg, 0.089 mmol) in anhydrous DCM (890 μL) were added tetrabromomethane (68 mg, 0.20 mmol) and triphenylphosphine (52 mg, 0.20 mmol) at 0 °C. The mixture was stirred at 0 °C for 10 min and at room temperature for 1 h, and the solvent was removed *in vacuo*. The crude product was purified by *flash* column chromatography (10% to 30% EtOAc in petrol) to give bromide **391** (28 mg, 0.069 mmol, 78%) as a white solid; m.p. 136 °C. R_f (30% EtOAc in petrol) 0.27; $[\alpha]_D^{20}$ +7.2 (*c* 0.4, $CHCl_3$); ν_{max}/cm^{-1} 3430 (O-H), 2956 (C-H), 1731 (C=O), 1691 (C=O); δ_H (400 MHz, $CDCl_3$) 0.86 (9H, s, C(CH_3)$_3$), 1.10 (3H, d, *J* 7.2, C(7)CH_3), 1.34-1.49 (2H, m, C(9)H_AH_B + C(10)H_AH_B), 1.54-1.72 (2H, m, C(9)H_AH_B + C(10)H_AH_B), 1.76-1.86 (2H, m, C(11)H_2), 2.26 (1H, s, O*H*), 3.23 (1H, q, *J* 7.2, C(7)*H*), 3.38 (2H, t, *J* 6.6, C(12)H_2),

3.79 (3H, s, CO_2CH_3), 4.27 (1H, d, *J* 8.8, C(4)H_AH_B), 4.57 (1H, d, *J* 8.8, C(4)H_AH_B), 4.88 (1H, s, C(2)*H*); δ_C (100 MHz, $CDCl_3$) 7.0 (C(7)*C*H_3), 21.9 (*C*(10)), 25.1 (C(*C*H_3)$_3$), 33.1 (*C*(12)), 33.2 (*C*(11)), 34.8 (*C*(9)), 36.7 (*C*$(CH_3)_3$), 49.2 (*C*(7)), 53.0 (CO_2*C*H_3), 68.3 (*C*(4)), 79.1 (*C*(5)), 82.7 (*C*(6)), 96.1 (*C*(2)), 172.0 (*C*O_2CH_3), 178.3 (*C*(8)); *m/z* (ESI$^+$) 406.2 (MH^+, 100%), 428.2 (MNa^+, 64%); HRMS (ESI$^+$) found 406.12235, $C_{17}H_{29}NO_5Br$ (MH^+) requires 406.12236.

(*R*)-Spiro[(2*R*,5*R*,7*S*)-1-aza-2-(*tert*-butyl)-5-methoxycarbonyl-7-methyl-3-oxa-8-oxobicyclo[3.3.0]-octane-6,2'-tetrahydro-2*H*-pyran] (393)



5-Phenyl-1-pentanol (6 μL, 0.04 mmol) was added to a suspension of NaH (60% w/w, 2 mg, 0.05 mmol) and tetrabutylammonium iodide (2 mg, 0.004 mmol) in anhydrous THF (100 μL) at 0 °C. After stirring at this temperature for 10 min, a solution of bromide **391** (16 mg, 0.038 mmol) in anhydrous THF (300 μL) was added. The mixture was stirred at room temperature for 12 h and quenched with saturated aqueous NH_4Cl. The aqueous layer was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by *flash* column chromatography (1% to 20% EtOAc in petrol) to give spiro-cycle **393** (9 mg, 0.03 mmol, 74%) as a colourless oil, instead of the desired substitution product. R_f (20% EtOAc in petrol) 0.41; $[\alpha]_D^{20}$ +5.9 (*c* 0.6, $CHCl_3$); ν_{max}/cm^{-1} 2955 (C-H), 1719 (C=O); δ_H (500 MHz, $CDCl_3$) 0.89 (9H, s, C(CH_3)$_3$), 1.09 (3H, d, *J* 7.2, C(7)CH_3), 1.44-1.58 (4H, m, C(3')H_AH_B + C(4')H_AH_B + C(5')H_2), 1.64-1.72 (2H, m, C(3')H_AH_B + C(4')H_AH_B), 2.96 (1H, q, *J* 7.2, C(7)*H*), 3.29 (1H, td, *J* 11.4, 3.6, C(6')H_AH_B), 3.76 (3H, s, CO_2CH_3), 3.81-3.86 (1H, m, C(6')H_AH_B), 3.84 (1H, d, *J* 8.0, C(4)H_AH_B), 4.76 (1H, d, *J* 8.0, C(4)H_AH_B), 4.78 (1H, s, C(2)*H*); δ_C (125 MHz, $CDCl_3$) 7.2 (C(7)*C*H_3), 19.8 (*C*(4')), 24.7 (*C*(5')), 25.1 (C(*C*H_3)$_3$), 30.1 (*C*(3')), 35.9 (*C*$(CH_3)_3$), 50.9 (*C*(7)), 52.5 (CO_2*C*H_3), 65.2 (*C*(6')), 69.4 (*C*(4)), 80.4 (*C*(5)), 82.0 (*C*(6)), 95.1 (*C*(2)), 172.1 (*C*O_2CH_3), 177.9 (*C*(8)); *m/z* (ESI$^+$) 326.2 (MH^+, 53%), 348.2 (MNa^+, 100%); HRMS (ESI$^+$) found 326.19600, $C_{17}H_{28}NO_5$ (MH^+) requires 326.19620.

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APPENDICES

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