Natural Product Inspired Organic Synthesis:

Enantiopure Heterocycles Modelled On Pramanicin



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

ABSTRACT

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This thesis is concerned with the synthesis of chiral pyrrolidinone scaffolds as mimics of the natural product pramanicin, and the evaluation of their antibacterial properties for use towards the development of potential novel antibacterial lead compounds.

Chapter 1 discusses the urgency of the antibiotic resistance problem as well as the current lack of new antibiotics in the drug pipeline. This dearth of new antibacterials is partly attributed to the combinatorial libraries used in the screening process which occupies a limited chemical space. By applying the natural product-inspired paradigm, it is hypothesised that a drug discovery process with a starting point based on a natural product, possessing intrinsic antibacterial properties, may provide insights to a novel class of antibacterials.

Chapter 2 describes the synthesis of three different scaffolds of oxygenated pyrrolidinones via a common bicyclic intermediate synthesised from L-pyroglutamic acid. The use of a mild and facile epoxidation condition utilising H_2O_2/tertiary amine afforded the epoxypyrrolidinones. α-Hydroxylation with "Davis oxaziridine" and a Ru-mediated dihydroxylation gave 2-hydroxypyrrolidinones and 2,3-dihydroxypyrrolidinones respectively. In all cases, a pendant Weinreb amide was used to introduce a variety of side-chains onto the parent pyrrolidinones. The enantioselective oxygenation of these scaffolds was accomplished as a result of the chiral [3.3.0] bicyclic intermediate.

Chapter 3 describes the attempted synthesis of oxygenated pyrrolidinones via tetramic acids. Although progress was thwarted by synthetic challenges discussed therein, a series of tetramic acids was synthesised. A synthetic sequence to install a 3-acyl moiety onto the parent tetramic acid core was accomplished via an *O*-acyl/*C*-acyl rearrangement using a series of carboxylic acids in the presence of excess DMAP. The use of a 3-acyltetramic acid with a pendant phosphorane permits a general synthetic route, compatible with a wide range of aldehydes, towards 3-enoyltetramic acids via Horner-Wadsworth-Emmons olefination. These tetramic acids are mimics of another class of bactericidal natural products which nonetheless allows the natural product-inspired paradigm to be investigated.

Finally, an analysis of the antibacterial properties of the synthesised compounds is discussed in Chapter 4. A cellular hole-plate bioassay with *E. coli* and *S. aureus* was chosen to provide a rapid assessment for active compounds. A correlation between the physicochemical properties and the observed activities of these active compounds suggested possible chemical modifications which could be undertaken in the future to improve their activities.

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"The Lord is my light…"

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ABBREVIATIONS

Ac	acetyl
acac	acetylacetonate
AHL	*N*-acylhomoserine lactones
Approx.	approximately
B. subtilis	*Bacillus subtilis*
BIOS	biologically oriented synthesis
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
Bu	butyl
COSY	correlation spectroscopy
CSA	camphorsulphonic acid
DABCO	1,4-diazabicyclo[2.2.2]octane
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DEPT	distortionless enhancement by polarisation transfer
DIBALH	di-*iso*-butylaluminium hydride
DIPEA	*N*,*N*-di-*iso*-propylethylamine
DIPT	di-*iso*-propyltartrate
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DMDO	dimethyldioxirane
DMF	dimethylformamide
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
E. coli	*Escherichia coli*
EDTA	ethylenediaminetetraacetate
ee	enantiomeric excess
Et	ethyl
eq.	equivalents
EXSY	exchange spectroscopy
h	hour
HMBC	heteronuclear multiple-bond correlation
HMDS	hexamethyldisilazane
HMPA	hexamethylphosphoramide
HMQC	heteronuclear multiple-quantum correlation
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectroscopy
HSQC	heteronuclear single-quantum correlation

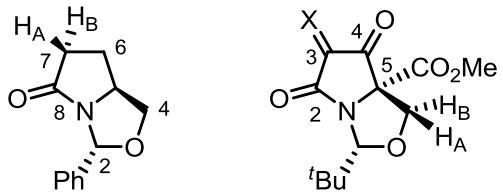
HTS	high-throughput screening
IR	infra-red
LDA	lithium diisopropylamide
LRMS	low resolution mass spectroscopy
m-CPBA	*meta*-chloroperbenzoic acid
Me	methyl
MIC	minimum inhibitory concentration
min	minute
MOM	methoxymethyl ether
mp	melting point
MRSA	methicillin-resistant *Staphylococcus aureus*
MS	molecular sieve
NDM-1	New Delhi metallo-beta-lactamase 1
HRMS	high resolution mass spectroscopy
HSQC	heteronuclear single-quantum correlation
NIS	*N*-iodosuccinimide
NMO	*N*-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance
NO	nitric oxide
nOe	nuclear Overhauser effect
nOeSY	nuclear Overhauser effect spectroscopy
P. aeruginosa	*Pseudomonas aeruginosa*
PCC	pyridinium chlorochromate
PMP	*p*-methoxyphenyl
ppm	parts per million
py	pyridine
Quant.	Quantitative
r.t.	room temperature
rac	racemic
ROESY	rotating frame nuclear Overhauser effect spectroscopy
S. aureus	*Staphylococcus aureus*
S. pneumonia	*Streptococcus pneumonia*
t	tertiary
TBAF	tetra-*n*-butylammonium fluoride
TBS	*tert*-butyldimethylsilyl
TES	triethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran

TLC	thin layer chromatography
TMS	trimethylsilyl
Tr	trityl (or triphenylmethyl)
UV	ultraviolet
VRE	vancomycin-resistant enterococcus
wt.	weight

NOMENCLATURE

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

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



Assignment of the respective NMR signals in the Experimental Section follows the numbering systems as drawn in the structure next to the data.

STEREOCHEMISTRY

Absolute stereochemistry

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

Relative stereochemistry

A dashed line is used to denote α-configuration, where the substituent lies behind the plane of the page, while a solid wedged line is used to denote β-configuration, where the substituent lies in front of the plane. A solid line is used to denote unknown stereochemistry, and a wavy line signifies the presence of a mixture of isomers at that chiral centre.

CHAPTER 1: INTRODUCTION

1.1 Background

1.1.1 Antibiotic discovery

The serendipitous discovery of penicillin in 1929 played an important role for a group of therapeutic drugs known as antibiotics.[1] Later in 1943, another antibiotic, streptomycin (an aminoglycoside), was discovered through the methodical testing of soil microbes when microbiologist Selman Waksman exploited the bacteria's innate ability to produce their own antibiotics.[2,3,4] This heralded the "golden age" of antibiotic discovery in the following twenty years where other classes of antibiotics were subsequently discovered.[5]

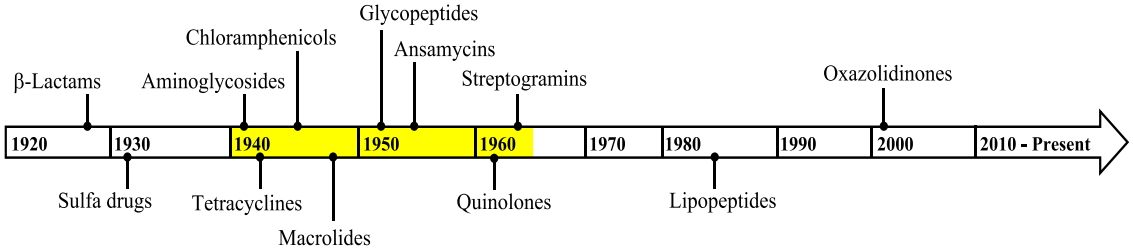


Figure 1.1. Timeline for discovery of different classes of antibiotics. Abstracted from K. Lewis *Nature* **2012**, *485*, 439-440 and adapted.

Most of the antibiotics discovered during this time were from the screening of soil-derived actinomycetes but this success was short-lived as its returns diminished owing to the rediscovery of known compounds, and the discovery platform was abandoned. Since the 1960s no new class of broad-spectrum antibiotics has been discovered,[6] and apart from natural product derived antibiotics, only two classes of synthetic antibiotics (quinolones[7] and oxazolidinone[8]) were developed.

Nonetheless, infectious disease ranks amongst the top causes of death in the world,[9] and since the introduction of the first antibiotic penicillin into the clinical setting in the 1940s, antibiotic

resistance has been observed to increase rapidly each time after a new antibiotic was introduced.

1.1.2 A brief introduction to known classes of antibacterial drugs

Throughout the timeline of antibiotic drug discovery, multiple classes of antibiotics have been discovered (see Section 1.1.1, *vide supra*). Antibiotics exhibit bactericidal activity by targeting various bacterial enzymatic targets, with common mechanisms of action being inhibition of bacterial cell wall synthesis, inhibition of bacterial protein synthesis, and inhibition of bacterial DNA replication and repair.[10]

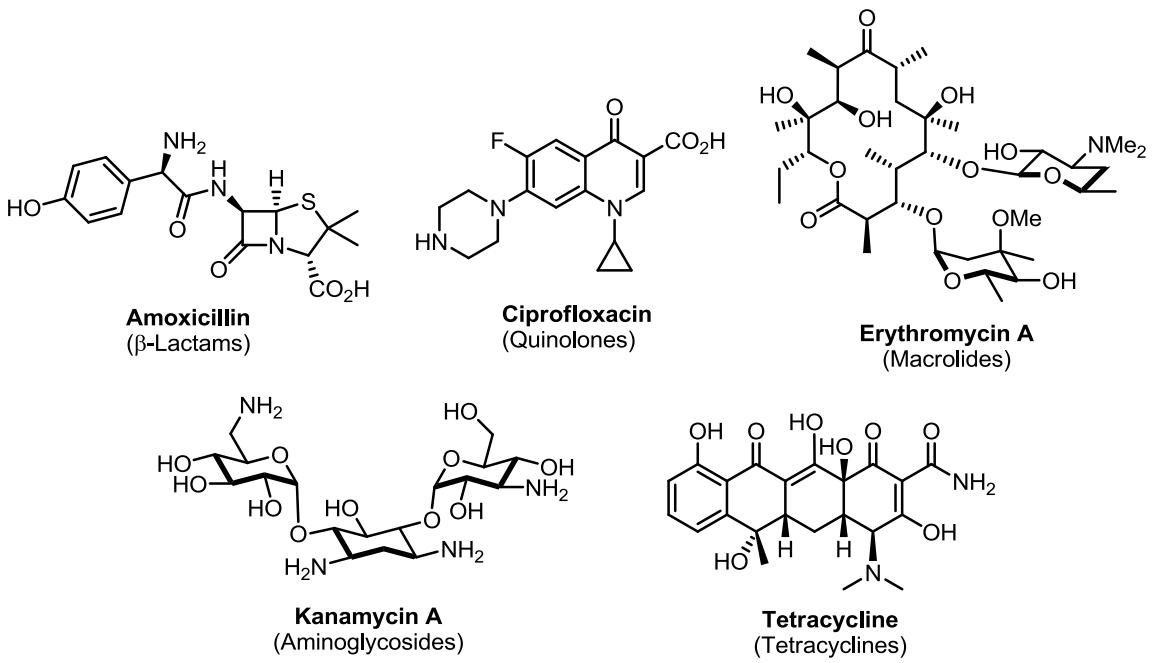


Figure 1.2. Examples of some clinical antibiotics and their classes in parentheses.

For example, β-lactams and glycopeptides are two antibiotic classes which inhibit cell wall synthesis. β-Lactams inhibit the bacterial enzyme, murein-transpeptidase, which catalyses the cross-linking of peptidoglycan chains making up the bacterial cell wall. This results in a weakened cell wall which ruptures due to the osmotic pressure within the cell, leading to bacterial death.[11,12] Conversely, a glycopeptide such as vancomycin acts via complexation to the terminal end of the peptidoglycan precursor, sterically hindering the binding of this

precursor to the bacterial enzymes involved in the polymerisation reaction required for cell wall biosynthesis.[13]

Antibiotics inhibiting bacterial protein synthesis generally target the bacterial ribosome. A bacterial ribosome is made up of two fragments consisting of a smaller 30S subunit and a larger 50S subunit. Aminoglycosides bind irreversibly to the 16S ribosomal RNA of the smaller ribosomal subunit,[14] while macrolides bind to the 23S ribosomal RNA of the larger ribosomal subunit containing the domain with the peptidyl transferase site, thereby disrupting elongation of the nascent peptide chain during peptide translation.[15]

Quinolones and fluoroquinolones inhibit bacterial DNA repair by targeting enzymes such as DNA gyrase (essential in controlling DNA topology during DNA replication) and topoisomerases (involved with DNA replication).[16,17]

The therapeutic efficacy of antibacterial drugs depends on their selectivity for prokaryotic cellular processes over eukaryotic cellular processes. Nonetheless, regardless of the antibiotics' mechanism of action, the ability to penetrate the bacteria cell wall has been considered an important property of antibacterial drugs. O'Shea and Moser have tabulated the average physicochemical parameters of known antibacterial classes as shown in Table 1.1, in an attempt to illustrate the different chemical space occupied by antibacterial drugs. (see Section 4.5, *vide infra*).[18]

Table 1.1: Average physicochemical parameters for known antibacterial classes tabulated by O'Shea and Moser.[18]

MW = average molecular weight; PSA = polar surface area; Relative PSA (%PSA) = (PSA/MSA) x 100; MSA = molecular surface area.

Antibiotic Class	**MW (Da)**	**clogP**	**clogD$_{7.4}$**	**PSA (Å^2)**	**Relative PSA**
Penicillins	412	1.4	-2.4	149	39%
Cephems	452	0.1	-3.0	210	51%
Carbapenems	397	-3.0	-5.8	159	43%
Sulfa drugs	273	0.6	-0.1	112	45%
Aminoglycosides	526	-2.9	-8.1	279	54%
Tetracyclines	481	-0.7	-3.6	184	40%
Macrolides	790	3.5	2.6	189	23%
Glycopeptides	1740	1.3	-1.8	586	37%

1.1.3 Antibiotic resistance

Currently, existing classes of antibiotics are usually based on similar molecular scaffolds and thus often have the same mechanism of action. For example, carbapenems, cephalosporins and penicillins are β-lactam antibiotics which act by disrupting the bacteria cell wall biosynthesis. The common mechanism of action of these drugs, coupled with their widespread use, is believed to have led to an increased selection pressure on these organisms and accelerated the emergence of resistance.[19]

Table 1.2: Some examples of the rapid onset of bacterial resistance of antibiotics.[20]

Name of antibiotic	Class of Antibiotic	Year first introduced/ approved by FDA	Year of first observed resistance
Penicillin	Penicillins (β-lactams)	1942	1945
Streptomycin	Aminoglycosides	1947	1947
Tetracycline	Tetracyclines	1952	1956
Methicillin	Penicillins	1959	1961
Cephalothin	Cephalosporins (β-lactams) (1st Generation)	1964	1966
Gentamicin	Aminoglycosides	1967	1970
Cefotaxime	Cephalosporins (3rd Generation)	1981	1983
Linezolid	Oxazolidinones	2000	2001

Antibiotic resistance has made the treatment of infectious diseases caused by bacteria a more pressing issue in recent decades, particularly with the persistence and emergence of the 'super-bugs', such as multidrug-resistant MRSA, VRE,[20] and NDM-1,[21] as well as the lack of new antibiotics from the pharmaceutical pipeline. The urgency of this world-wide concern could be seen from the theme of World Health Day 2011 being dedicated to address this issue. However, the problem of antibiotic resistance is a complex and multi-faceted one requiring not only efforts from research and discovery of new antibiotics, but also in areas such as, but not limited to, policies governing their administration and usage in humans and animals, medical infrastructure and good practices in clinical settings.[22] Today, this problem is mostly focused on the rapid spread of multidrug-resistant 'ESKAPE' organisms (*Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella* spp., *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.).[23]

Although it would be naïve to consider that the emergence of antibacterial resistance could be eradicated, every effort made to ameliorate the impacts of this pervasive problem would definitely prolong the lifetime of therapeutically valuable antibiotic drugs. For example, healthcare-associated infection is a common problem faced by hospitals and antibacterial resistance involved in such cases remains a serious health threat. A recent study of multidrug resistance and nosocomial bacteraemia has shown that although resistance itself does not contribute to a higher mortality rate as long as effective antibiotics remain for definitive therapy, it does however contribute to a longer hospitalisation period.[24] This suggests that if antibiotic resistance could be reduced, some pressure in the healthcare services could be relieved and this would lead to greater efficiency. In addition, this would also support advances in medicine by reducing infection during post-operative care.[22]

The resistance to one antibiotic of a class tends to result in resistance to its whole class, also known as the "resistance class effect".[25] In order to impede the onset of resistance, one plausible course of action would be to develop new antibiotics that are structurally different from existing ones, and either having multiple targets or having a different mechanism of action, preferably one that has yet to evolve to combat existing classes of antibacterials.

1.2 Different approaches towards discovery of new antibiotics

A number of approaches have been employed towards the discovery of new antibiotics, and a few of these approaches are outlined below.

1.2.1 Inadequacy of the combinatorial chemistry approach

During a genomics approach undertaken to identify potential novel targets by GlaxoSmithKline (GSK),[26] 160 genes were identified as essential bacterial targets after evaluating more than 300 genes for their potential as novel antibacterial targets over a span of seven years (1995-2001). Sixteen high-throughput screening (HTS) campaigns, mostly target based assays, gave hits and only five of these resulted in lead compounds; the remaining hits failed to afford compounds that met the lead criteria even after chemical modification.

Payne and co-workers[26] recognised that this low success rate was partly due to the limitations of existing chemical libraries that were synthesised using combinatorial chemistry which not only occupied a narrower chemical space, but also a different one. Generally, the compounds generated from early combinatorial libraries were not able to deliver the desired results, and this is evident from the dearth of new chemical entities arising from *de novo* combinatorial chemistry approaches to drug discovery. Although the use of combinatorial chemistry coupled with HTS is fast, the use of conventional solid-phase organic synthesis procedures also limits the complexity and diversity of the resulting libraries because it usually limits library

construction to commercially available starting materials, and in particular has relied heavily upon amide bond formation.[27] Thus these libraries are woefully inadequate to populate a sufficiently large and diverse area in the chemical space to provide genuinely valuable hits.[28,29] Furthermore, these libraries tend to be biased towards Lipinski's 'rule of five'[30] whereas known antibacterial drugs generally display a different set of pharmacodescriptors.[18]

Having identified the need to synthesise molecules with biological relevance, Payne and co-workers[26] preferred a cell based (phenotypic) assay for their screening as they found it relatively easier to determine the cellular target(s) of lead compounds compared to optimising the cell permeability of a known enzyme inhibitor. This is because the lead compounds found using cell based assay already have their cell permeability accounted for, and their targets could subsequently be determined, although this also comes with its own set of challenges.

1.2.2 Diversity-oriented synthesis (DOS) approach

To address the lack of diversity in available compound libraries, a diversity-oriented synthesis (DOS) approach was introduced. This approach aims to create libraries of structurally and functionally different compounds from a small number of precursors based on the four principles of appendage diversity, functional group diversity, stereochemical diversity and scaffold diversity; this approach not only introduces diversity, but also complexity as the molecules in these libraries now contain more stereocentres.

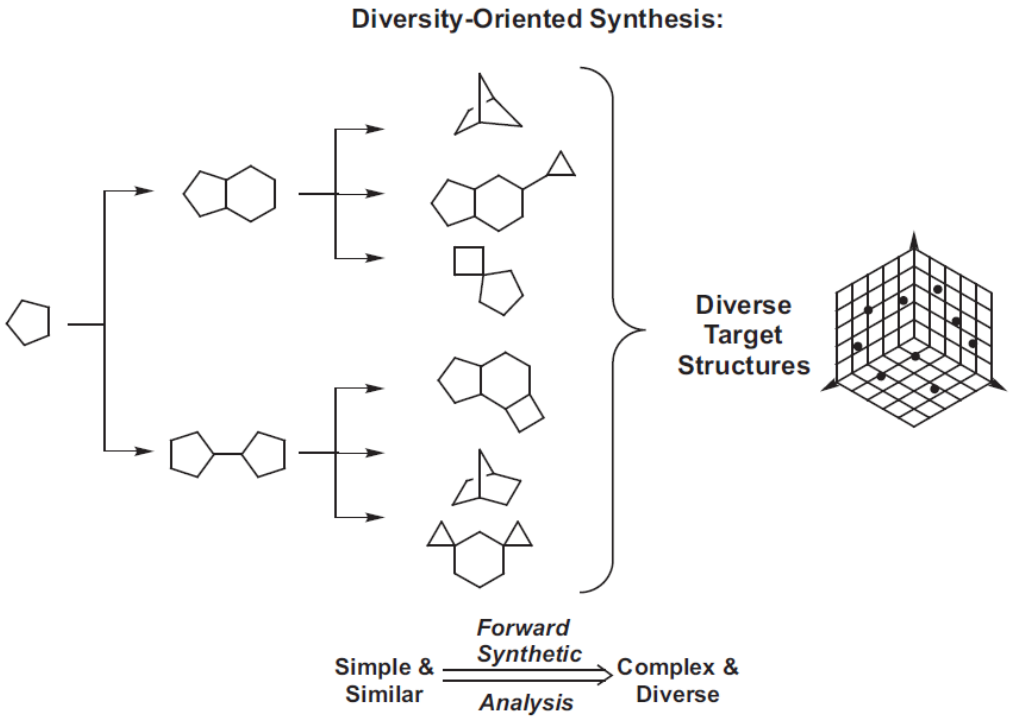
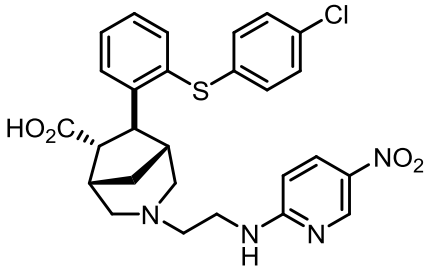


Figure 1.3. Illustration of the DOS concept with the library occupying a large chemical space. Abstracted from K. M. G. O'Connell, W. R. J. D. Galloway, D. R. Spring, in *Diversity-Oriented Synthesis: Basics and Applications in Organic Synthesis, Drug Discovery, and Chemical Biology*, ed. A. Trabocchi , Wiley, 2013, ch. 1, pp.1-26.

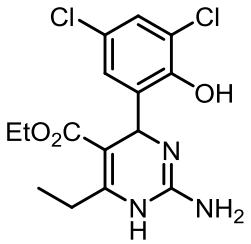
It is argued that the diversity generated in the process not only covers a larger chemical space, but would be more successful in phenotypic assays as they are not designed with any bias towards a particular cellular target.[31,32] It also has been shown that varying the molecular scaffold can provide greater structural diversity as compared to simply varying the peripheral attached functional groups, thus occupying a greater chemical space.[33,34]

This approach has been shown to have some potential towards antibiotics drug discovery as shown by Spring and co-workers. A DOS library consisting of 242 compounds possessing 18 molecular scaffolds was screened against three strains of *S. aureus* – a methicillin-susceptible *S. aureus* (MSSA), and two epidemic methicillin-resistant strains (EMRSA 15 and EMRSA 16), both of which are resistant to penicillins and erythromycin and are responsible for the majority of MRSA infections in the UK.

Table 1.3: Two antibacterial compounds indentified via DOS by Spring and co-workers[35]



(-)-Gemmacin



Emmacin

	MIC_{50} [μg mL^{-1}]		
	MSSA	EMRSA 15	EMRSA 16
(-)-Gemmacin	Not determined	8	16
Emmacin	2	9	9

The results revealed that the most potent compound in the library, which they named gemmacin, showed broad-spectrum gram-positive antibacterial activity *in vitro*, but was generally inactive against Gram-negative organisms. Gemmacin was found to have activity in an assay used to detect the generation of reactive oxygen species (IC_{50}= 0.35 mg/mL, in *Spodoptera frugiperda* cell line 21), suggesting its role as a cell membrane disrupter.[35] In a separate study, another compound named emmacin was discovered from a different DOS library and its mechanism of action was determined to be the prokaryotic-selective uncompetitive reversible inhibition of EMRSA 16 dihydrofolate reductase.[36,37]

Although both genomic and DOS approaches yielded hit compounds for antibacterial drug discovery, there is still a dearth of compounds in the antibiotics drug pipeline. Although other methodologies such as fragment-based drug design or computational methods have also been employed, it seems that probing molecules with biologically relevant chemical diversity is an important step towards overcoming the challenges involved with discovering a new

antibacterial drug having minimal side-effects on the human hosts and possessing activity across a broad range of pathogens.

1.2.3 Natural product inspired approach to drug discovery

Instead of designing molecular scaffolds *de novo*, Waldmann and co-workers[38] proposed a paradigm based on mimicking the structural framework of biologically active natural products. They argued that proteins can be viewed as macromolecules which consist of multiple individual sub-domains. The total number of such domains is limited and different biological targets could be considered as being made up of a combination of such domains. Furthermore, certain scaffolds of natural products produced by microbes could be considered to have a 'purposeful design' where organisms selectively make these compounds to confer an advantage for survival within its ecological niche. These compounds are produced to enable out-competition with other microbes and would likely possess intrinsic antibacterial properties.[1] In a way, these natural products are evolutionarily selected to bind with specific protein domains. Hence, mimics of these natural products might be expected to already have the required physicochemical properties for drug discovery.

The use of compound libraries, based on natural product templates with known biological activities, for screening in drug discovery is also known as "biologically oriented synthesis" (BIOS) approach. This concept focuses on the creation of a small compound library based on biologically relevant scaffolds, in contrast to DOS which aims to create structurally diverse and complex libraries.[39] However, BIOS approach cannot be applied to existing classes of antibiotics as that would result in similar compounds, bearing the same mechanisms of action, thus being susceptible to the same underlying mechanisms of resistance as mentioned previously.

Hence, one possibility to approach the discovery of new antibacterials is to apply the BIOS paradigm towards natural products possessing an intrinsic antibacterial activity, and with significantly different structures to that of known classes of antibiotics. Such molecular scaffolds could possibly provide a drug with a different mechanism of action. The use of such scaffolds as a starting point could provide better hit rates for new antibacterial lead compounds.

Although rare, there had been instances whereby newly identified natural products were found to be already used as synthetic therapeutics retrospectively. One such example is Tramadol, designed as a simplified version of morphine for use as an analgesic and used clinically in the late 1970s, has been recently isolated from the sub-Saharan *Nauclea latifolia* Sm. (Rubiaceae) plant.[40]

Other examples include 5-fluorouracils from the marine sponge *Phakellia fusca* which are previously known synthetic compounds and antitumour agents,[41] and clinically used benzodiazepines isolated from natural products at trace concentrations.[42]

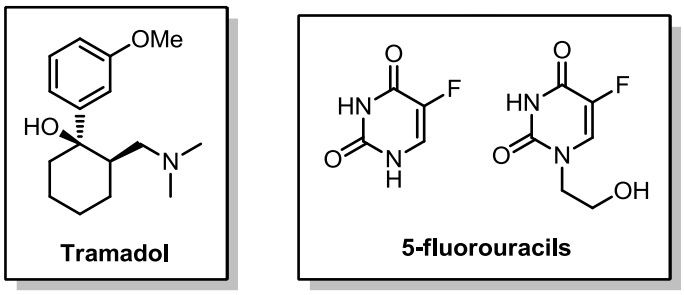


Figure 1.4. Tramadol, and 5-fluorouracils from marine sponge *Phakellia fusca*.

This suggests that the chemical space occupied by natural products, or natural product mimics, could potentially offer the previously untapped pool of lead compounds towards drug discovery efforts.

1.3 Pramanicin

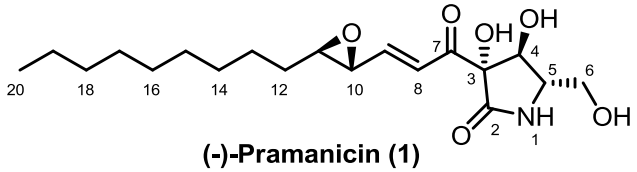
In the context of the above, one natural product of interest is pramanicin.

1.3.1 Isolation

Pramanicin was extracted from a sterile fungus (MF5868=BW283, Merck Microbial Resources Culture Collection), which in turn was isolated from an unidentified grass collected in New Jersey, as a crystalline white solid by Schwartz and co-workers in 1994.[43] This sterile fungus has subsequently been identified as a species of *Stagonospora*.[44] The isolation process reported therein was fairly straightforward and consisted of extraction, partitioning, and silica gel and Sepadex LH-20 (CH_2Cl_2/hexane/MeOH 10:10:2) chromatography.

1.3.2 Structure elucidation

Spectroscopic analysis carried out by Schwartz and co-workers revealed the structure of pramanicin (**1**) as having a long fatty acyl chain, containing an epoxide and enone functional group, with a polar poly-hydroxylated chiral lactam head group as shown below.[43]



(-)-Pramanicin (1)

Figure 1.5. Structure of (-)-pramanicin (**1**).

An initial analysis was conducted via mass spectrometry of **1** and its TMS derivatives. A comparison of the H_9-TMS and D_9-TMS derivative spectra of **1** gave the number of TMS groups present in each fragment. A molecular formula of $C_{19}H_{31}NO_6$ suggested five degree of unsaturation present in **1**. Mass spectral fragmentation suggested an *m/z* 242 fragment ($C_{10}H_{12}NO_6$) consisting of a polar head group (*m/z* 147, $C_5H_9NO_4$), and a $C_5H_3O_2$ piece

containing a carbonyl, C=C and an epoxide as there are three degrees of unsaturation in this fragment with no functional groups forming TMS derivatives.

A COSY experiment assisted with assigning the partial structure of the long fatty chain, as well as the C-3/C-4/C-5/C-6 fragment of **1**. The position of the epoxide on the side chain was confirmed via a ^{1}H-^{13}C HMBC experiment. The relative stereochemistry of the *trans* epoxide was determined from the small coupling constant (~ 2 Hz) between H-10 and H-11, and also from ROESY correlations observed between H9-H11, H10-H12 and H10-H13, with a lack of correlation between H10-H11. An enone functional group at C-7/C-8/C-9, suggested by UV (λ_{max} 245 nm, ε 14428) and IR (1708 cm^{-1}) spectroscopy, which was subsequently confirmed by a HMBC experiment, was found to link this long fatty chain to the polar head group bearing the C-3/C-4/C-5/C-6 fragment containing a N atom.

With two degrees of unsaturation remaining, the presence of a lactam was proposed from the observed IR absorption peak at 1688 cm^{-1}, together with a ^{13}C resonance having a chemical shift of δ174.94 ppm. The position of the lactam C=O was supported by the ^{13}C chemical shift of C-3 (δ88.09 ppm), suggesting it to be between two carbonyl groups, as well as the ^{1}H chemical shift of H-5 (δ3.47 ppm), suggesting it to be adjacent to an -NH- group rather than a carbonyl group. This amide bond was confirmed by a correlation between the proton at δ7.99 ppm with nitrogen at δ126.2 ppm via a ^{1}H-^{15}N HMQC experiment, as well as a $^{1}J_{HN}$ coupling constant of 92 Hz via a 1D ^{1}H-^{15}N HMQC experiment.

The ROESY correlations observed between H4-H6 and H4-C3(OH), suggested the relative stereochemistry of the lactam ring as shown in Figure 1.5. Furthermore, the lack of coupling between the lactam -NH- proton with H-5 supported this relative stereochemistry as the dihedral angle for one of its conformer was found to be approximately 90°.

Table 1.4: Chemical shift data, HMBC and ROESY correlations of pramanicin.[43]

Atom Number	**^{13}C NMR (substituition)**	**^{1}H NMR (multiplicity, *J* Hz)**	**^{1}H-^{13}C HMBC correlation**	**^{1}H-^{1}H ROESY correlation**
1 (NH)	-	Not observed	C-3, C-4, C-5	-
2	174.94 (C)	-	-	-
3	88.09 (C)	-	-	-
C-3 OH	-	6.3 (br s)	C-3, C-4, C-7	H-4
4	78.90 (CH)	4.15 (d, 7.2)	C-3, C-7	C-3 OH, H-6
C-4 OH	-	5.71 (br d, 5.3)	C-3, C-4, C-5	-
5	60.26 (CH)	3.47 (ddd, 2.8, 5.4, 7.2)	-	-
6	61.99 (CH_2)	3.55 (dd, 5.4, 11.7) 3.79 (dd, 2.8, 11.7)	-	-
C-6 OH	-	4.77 (t, 5.3)	-	-
7	197.88 (C)	-	-	-
8	127.84 (CH)	7.05 (dd, 0.7, 15.6)	C-7	-
9	145.13 (CH)	6.64 (dd, 7.0, 15.6)	C-7	H-11
10	57.79 (CH)	3.31 (ddd, 0.7, 1.9, ~7)	-	H-12, H-13
11	62.88 (CH)	2.93 (ddd, 1.9, 4.9, 6.2)	-	H-9
12	33.05 (CH_2)	1.60 (m)	-	-
13	26.97 (CH_2)	1.45 (m)	-	-
14-18	33.05 (CH_2) 30.66 (CH_2) 30.63 (CH_2) 30.51 (CH_2) 30.44 (CH_2)	1.29 (br s)	-	-
19	23.73 (CH_2)	1.29 (br s)	-	-
20	14.43 (CH_3)	0.89 (t, 6.8)	-	-

Chemical shifts of ^{1}H and ^{13}C NMR in ppm; ^{1}H NMR recorded at 400 MHz; CD_3OD used as solvent for ^{1}H and ^{13}C NMR experiments; DMSO-d_6 used as solvent to determine ^{1}H-^{13}C HMBC correlations for hydroxyl groups, recorded at 500 MHz.

The absolute stereochemistry of the side chain epoxide was only determined after the total synthesis of pramanicin was accomplished by Barrett and co-workers[45,46] (*vide infra*) when all the stereocentres of pramanicin were assigned by comparison of the NMR spectra and the optical rotation of their synthesised compound with the isolated natural product.

1.3.3 Bioactivity

Schwartz *et al.* reported the following antimicrobial activity of pramanicin using dish broth dilution assays whereby the MIC (minimum inhibitory concentration) is defined as the lowest concentration preventing visible growth (Table 1.5).[43]

Pramanicin was also reported to have significant cytotoxicity at concentrations of 20-100 μM towards Jurkat T lymphoblastic leukemia cells by Kutuk *et al.*.[47] In this study, mitochondria cytochrome *c* release, as well as caspase-9 and caspase-3 activation, was found to be dose-dependent on pramanicin and such pramanicin-induced apoptosis involved a few groups of mitogen-activated protein kinases (MAPKs) such as c-Jun N-terminal kinases (JNK) and p38 MAPKs.

Table 1.5: Antifungal and antibacterial activity of pramanicin.[43]

Organism	**Merck Culture Number**	**MIC of Pramanicin (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
Aspergillus fumigatus	MF 5668	100.0
Bacillus subtilis	MB 964	4.0

It is interesting to note that pramanicin not only has the above-mentioned antimicrobial activity, but also has some physiological activities in mammalian blood vessels. Kwan and co-workers reported that at a therapeutically effective concentration range of 4-100 μM, pramanicin is able to selectively cause endothelium-dependent vasorelaxation via the NO (Nitric oxide)-pathway but this was deemed by the authors as having minimal effects on the contractibility of smooth muscle cells.[48] The same group has shown that 100 μM of pramanicin is able to increase the intracellular concentration of Ca^{2+} via increased

permeability to Ca^{2+} ions which eventually causes endothelial cell injury, due to overloading of ions, leading to endothelial cell death.[49] They proposed that the partitioning of pramanicin into the lipid bilayers of the cell membrane causes increased permeability of ions such as Ca^{2+} but the mechanism of how this increased intracellular concentration of Ca^{2+} arise and the eventual cell injury still remains unclear.[48,50]

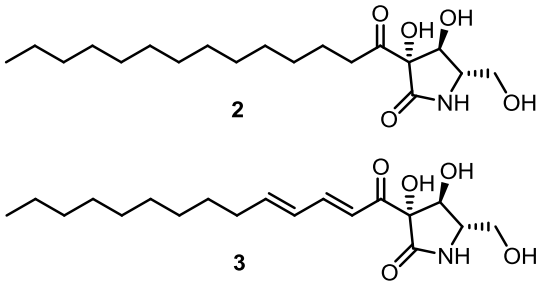


Figure 1.6. Analogues of pramanicin investigated by Kwan and co-workers.[50]

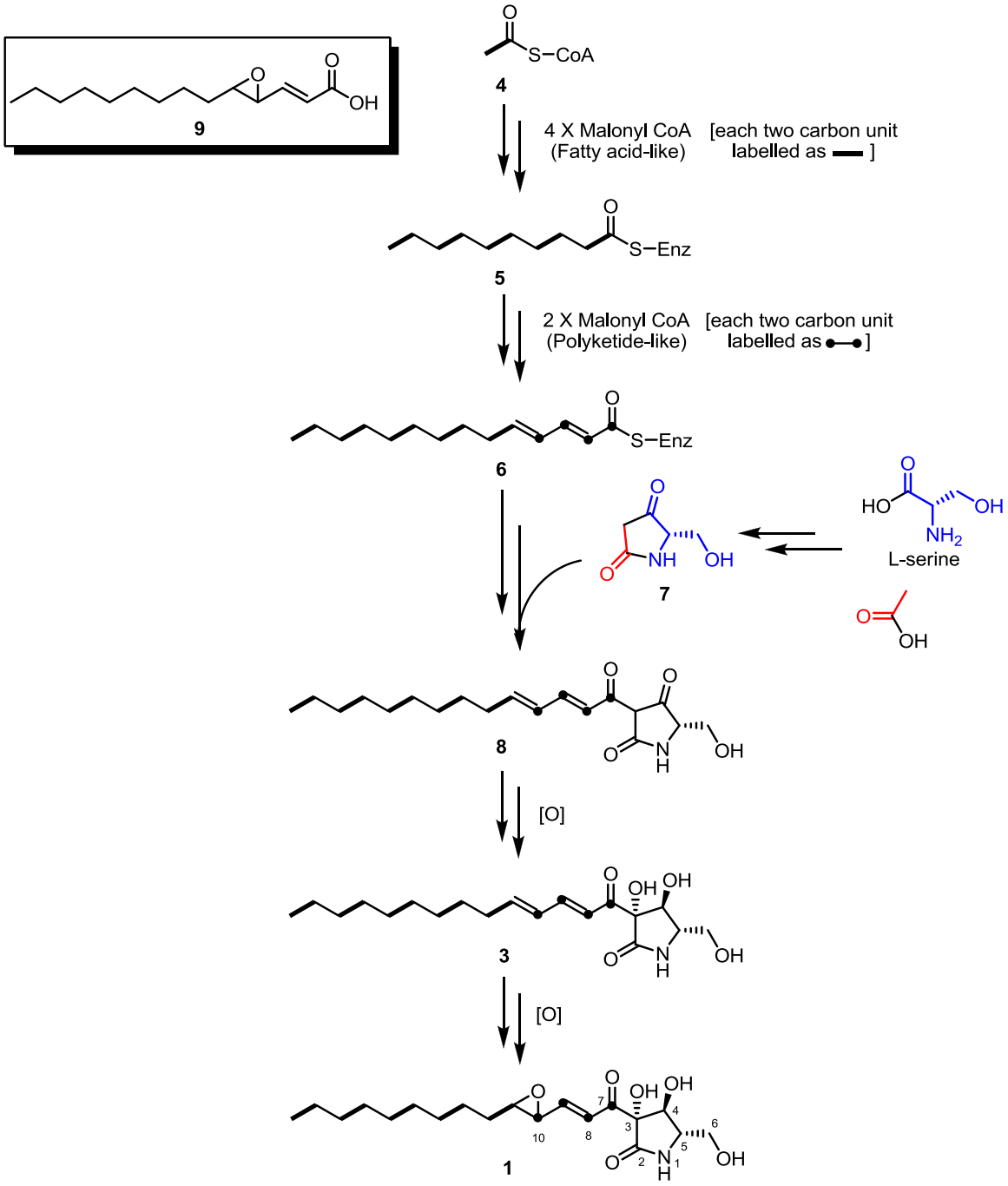
Further studies by Kwan and co-workers discovered that the epoxy group in pramanicin is optimal for vasorelaxation but not essential, as the other two analogues of pramanicin, **2** and **3**, retain partial activity.[50]

Extrapolation of these experimental data suggests that although pramanicin itself has modest antibacterial properties, it may be unsuitable as a drug given the potential toxicity to vascular endothelial cells.

1.3.4 Biosynthesis

Harrison and co-workers undertook some studies to elucidate the origin of the carbon skeleton of pramanicin (Scheme 1.1).[44,51,52] The biosynthesis of pramanicin is thought to proceed via a convergent synthesis of two fragments, mainly the hydrophobic tail and the polar lactam head. Incorporation of sodium [1-^{13}C]acetate into growth medium of *Stagonospora* sp. ATCC 74235 under conditions reported by Schwartz and co-workers[43] showed that the carbon

skeleton of the hydrophobic 4,5-epoxytetradec-2-enoyl tail (C-7 to C-20) is assembled from a starter acetate unit with six malonyl units in a 'head-to-tail' manner from a biosynthetic pathway similar to which fatty acids and polyketides are synthesised. On the other hand, the polar lactam head is assembled biosynthetically from an acetate unit and L-serine, with its three carbon atoms and nitrogen atom intact, to give a tetramic acid moiety. It subsequently undergoes a reduction of the C-4 keto-group and a hydroxylation at the C-3 position. The intermediacy of a tetramic acid moiety was concluded from the use of [1-^{13}C,1-$^{18}O_2$]serine, as the isolated pramanicin shows incorporation of ^{18}O into the C-4 hydroxyl group, suggesting that the C-O bond at position C4 was derived from serine. Although the precise sequence of the reduction of the C-4 keto-group, hydroxylation at the C-3 position, and epoxidation of the C-10, C-11 alkene was not determined in this study by Harrison and co-workers, they suggested that the epoxidation of the C-10, C-11 alkene is the last step in the biosynthetic pathway as **3** was isolated. This contradicted the earlier report by Schwartz and co-workers,[43] where the tail fragment **9** was isolated from the liquid fermentation broth of fungus MF5868, suggesting that the epoxidation reaction took place before the conjugation of the two fragments.



Scheme 1.1. Biosynthetic pathway towards pramanicin (**1**) as proposed by Harrison and co-workers.[52]

1.3.5 Mechanism of action

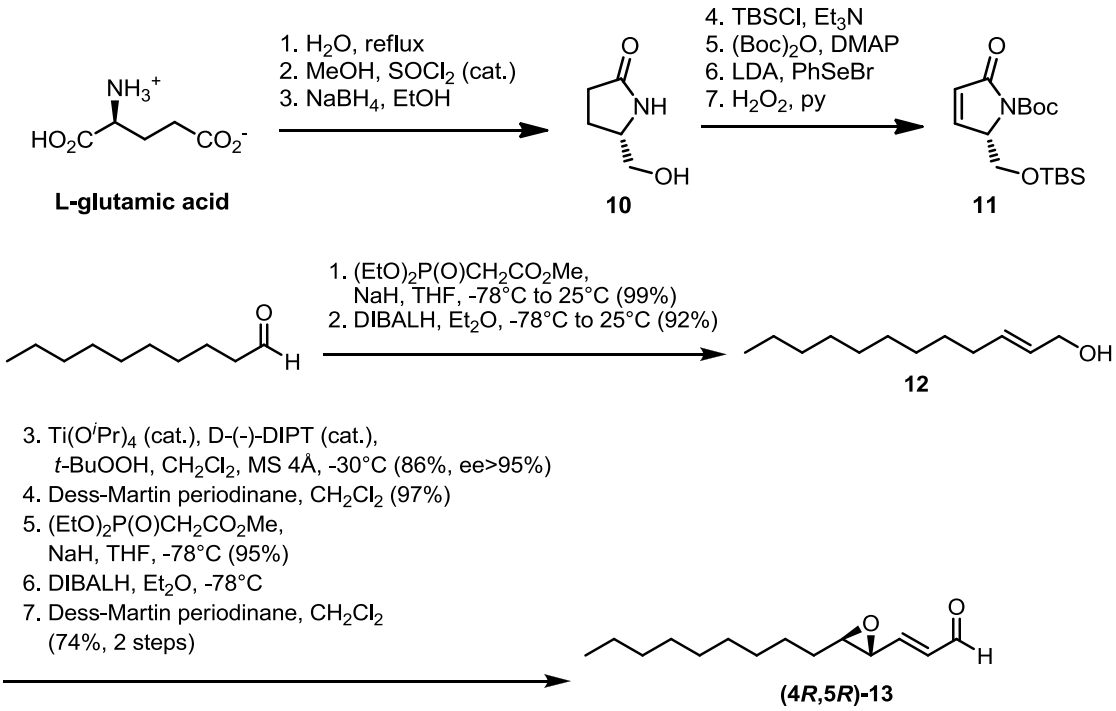
As of now, the mechanism of action of pramanicin that accounts for its antimicrobial activity has not been elucidated.

1.4 Total syntheses of pramanicin

1.4.1 Synthesis by Barrett

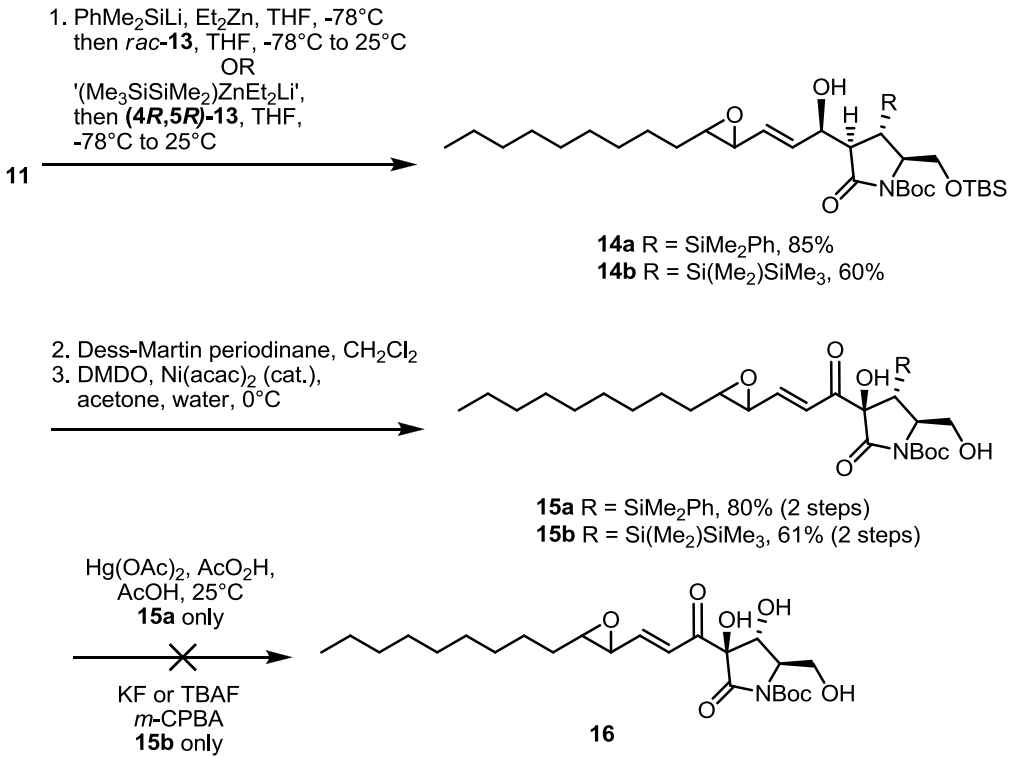
In 1998, Barrett and co-wokers[45] synthesised (+)-pramanicin via a one-pot Michael addition of an aminosilyl zincate species ($(Et_2N)Ph_2SiLi$) to an α,β-unsaturated lactam **11**, followed by quenching the resultant enolate with the α,β-unsaturated γ,δ-epoxy aldehyde **13** (Scheme 1.2). The stereochemistry of the C-3 and C-4 alcohols was introduced sequentially via a series of asymmetric induction by the lactam **11** during the Michael reaction - the C-4 alcohol was first introduced as a 'masked' hydroxyl group in the form of a bulky silyl substituent, which was converted to the desired secondary alcohol via a late-stage Fleming-Tamao oxidation with retention of stereochemistry, while the C-3 alcohol was subsequently installed via a Rubottom oxidation with *m*-CPBA *trans* to the bulky silyl group.

The convergent synthesis involves a three component one-pot Michael addition. The protected α,β-unsaturated lactam **11** was synthesised from L-glutamic acid; the (4*R*, 5*R*) α,β-unsaturated γ,δ-epoxy aldehyde **13** was synthesised from decanal using a double Wadsworth-Emmons strategy, with a stereoselective Sharpless asymmetric epoxidation of the intermediate allyl alcohol **12** to afford the epoxide with the desired stereochemistry (Scheme 1.2).



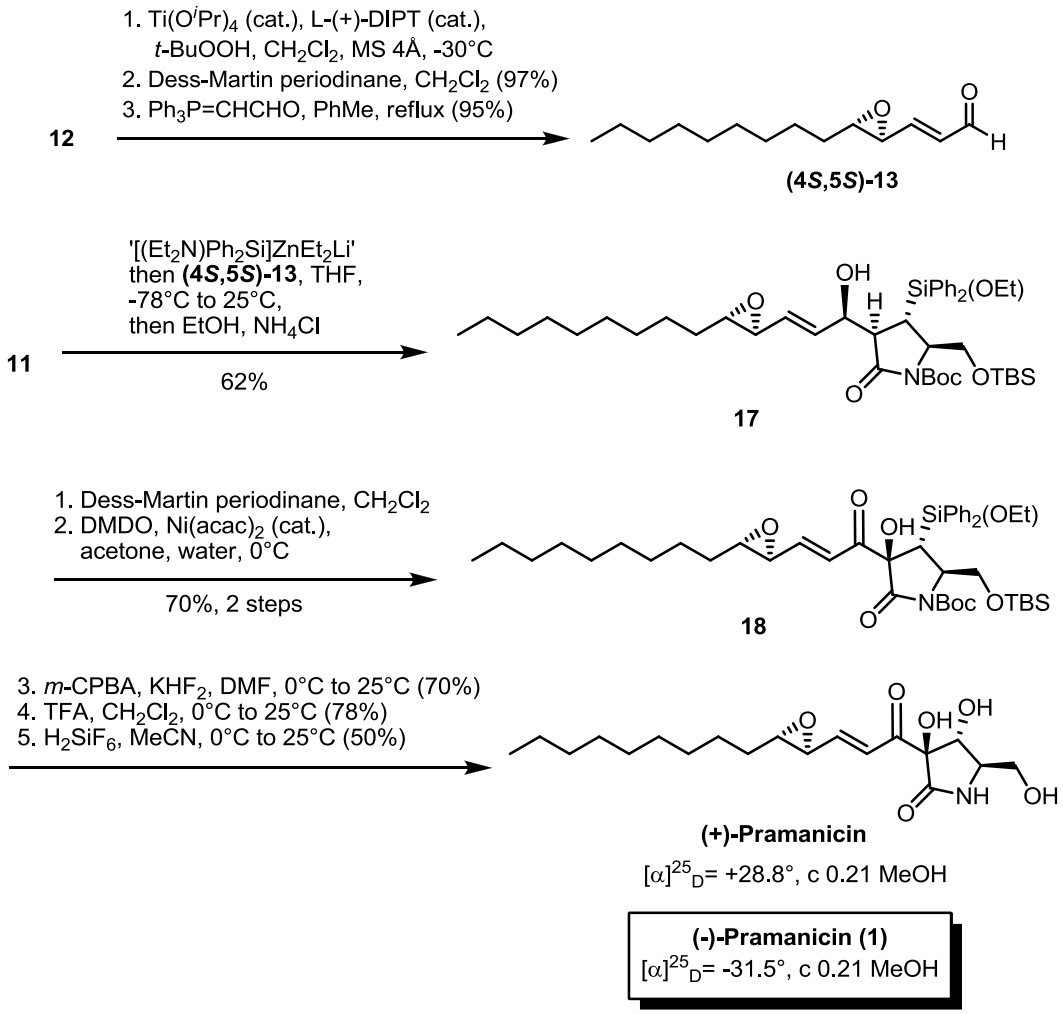
Scheme 1.2. Synthesis of precursors towards pramanicin.

Initial attempts of the Michael addition using the two above-mentioned components with lithium diethyl(dimethylphenylsilyl)zincate or lithium diethyl(pentamethyldisilyl)zincate gave the desired addition product **14a-b** respectively. However, a late-stage Fleming-Tamao oxidation to unmask the respective silyl group as an alcohol gave multiple degradation products despite using the mildest available condition with mercury(II) acetate in peracetic acid (Scheme 1.3).



Scheme 1.3. Initial attempt towards pramanicin utilising a dimethylphenylsilyl group.

Eventually, the use of an ethoxydiphenylsilyl group resulted in the desired unmasked alcohol with good yield, with the use of purified *m*-CPBA as highlighted by the authors (Scheme 1.4). This enantioselective synthesis of pramanicin using (4*S*, 5*S*) α,β-unsaturated γ,δ-epoxy aldehyde **13** was used to elucidate the stereochemistry of the epoxy side-chain relative to the lactam moiety. As this synthesised compound has an optical rotation that is approximately equal in magnitude but opposite in rotation to authentic pramanicin, this led the authors to conclude that the stereochemistry of natural pramanicin to be as that of (-)-pramanicin (**1**).



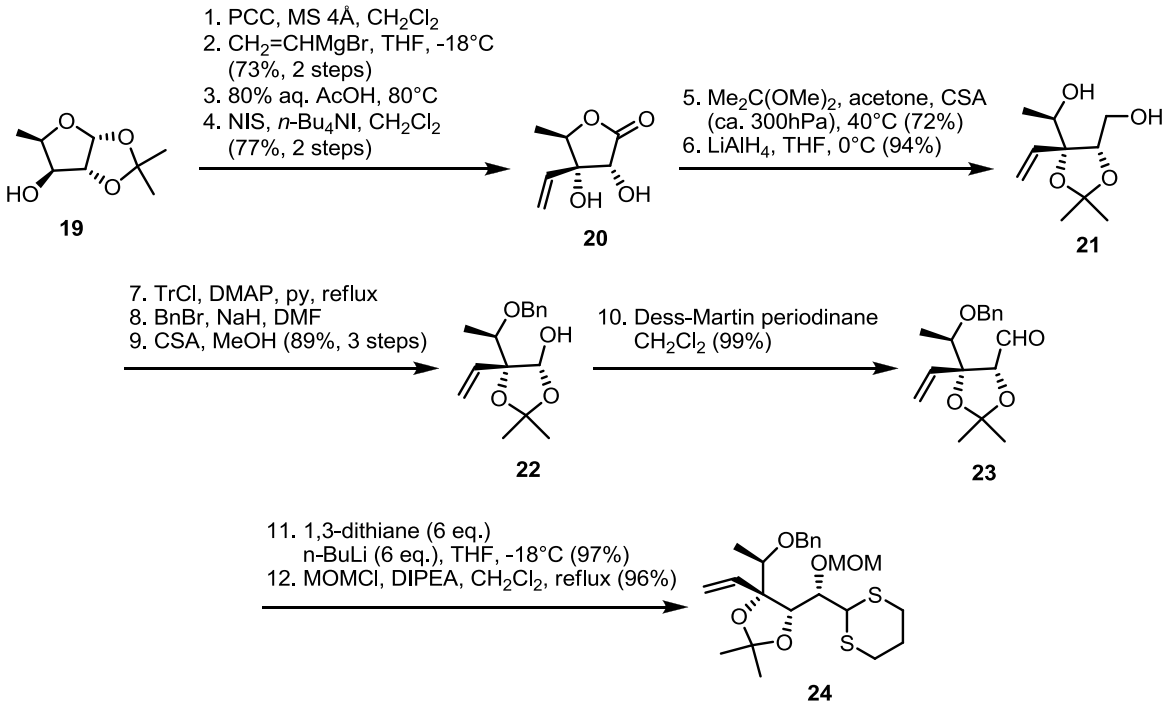
Scheme 1.4. Successful synthesis of (+)-pramanicin.

1.4.2 Synthesis by Tadano

In 2006, Tadano and co-workers[53] described the total synthesis of (-)-pramanicin (**1**) with an overall yield of 2% over 30 steps. They started from the known carbohydrate-based chiron 5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose **19** to access the poly-hydroxylated polar lactam head group, which was then coupled to an epoxy aldehyde fragment **31** via an aldol condensation reaction.

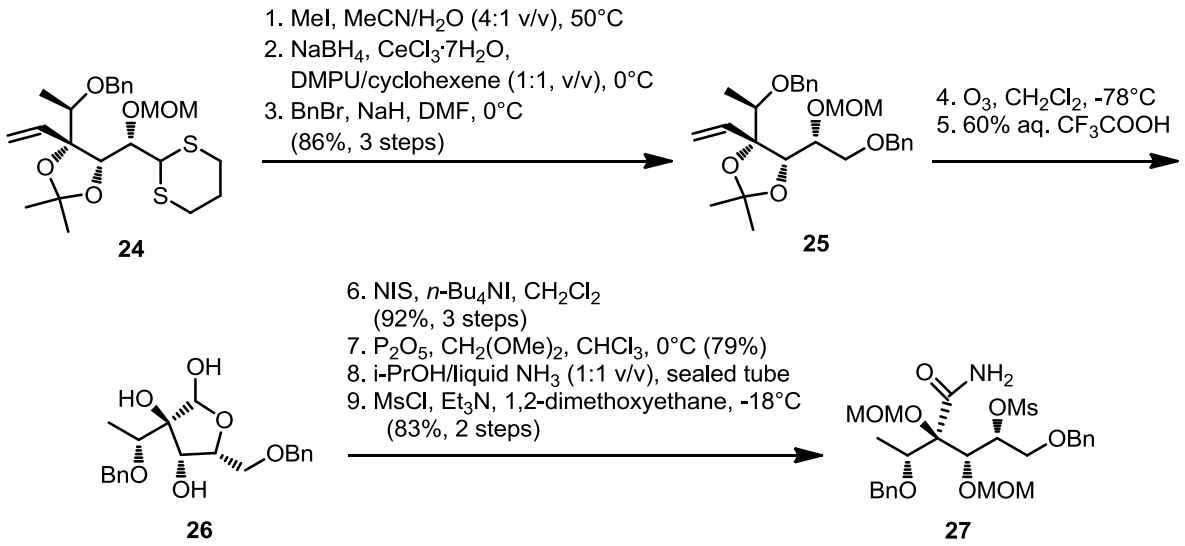
A multi-step sequence from furanose **19** gave the aldehyde **23**, which then underwent nucleophilic addition with 2-lithiated 1,3-dithiane to give **24** (Scheme1.5). The addition of the vinyl group and the 1,3-dithiane resulted in only one diastereomer of the desired product

respectively; the vinyl Grignard reagent attacked the convex face of the trioxabicyclo[3.3.0]octane, while the lithiated dithiane attacks the aldehyde from the less hindered β-face, consistent with a Li-ion associated five-membered chelation-controlled transition state.



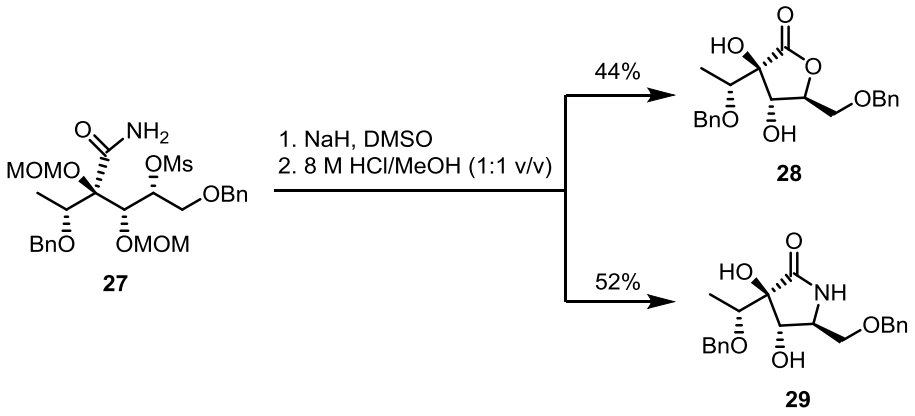
Scheme 1.5. Synthesis towards pramanicin beginning from furanose **19**.

Conversion of the 1,3-dithiane **24** to a benzyl ether was achieved, first by hydrolysis of the dithiane, followed by reduction of the masked aldehyde to a primary alcohol and then protection as a benzyl ether. Ozonolysis of the vinyl group, hydrolysis of the isopropylidene acetal and the MOM group, followed by a five-step sequence afforded the amide *O*-mesylate **27** key intermediate.



Scheme 1.6. Synthesis towards the key intermediate **27**.

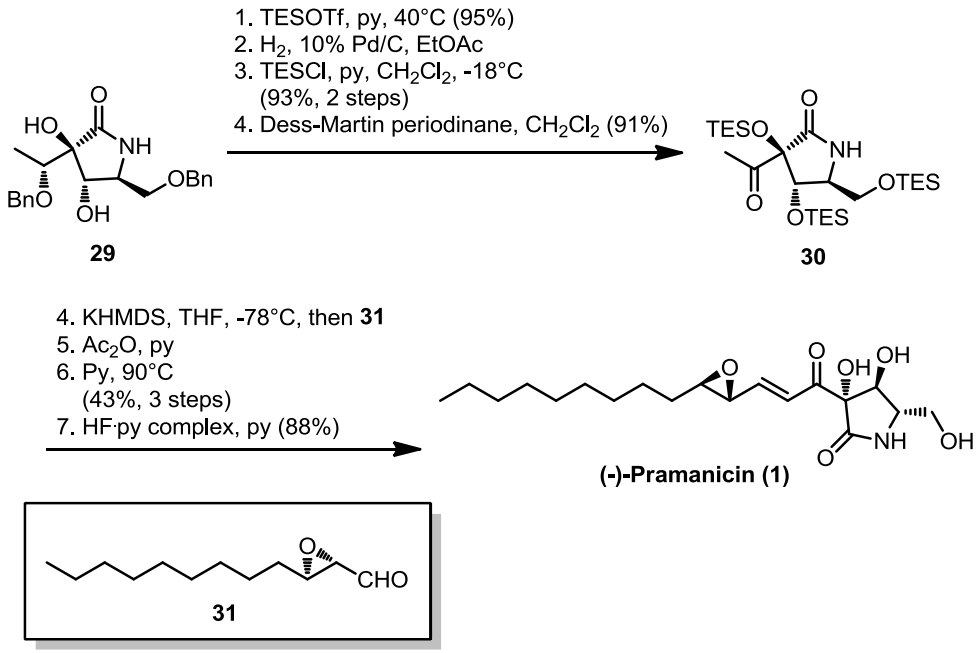
Treatment of the amide **27** with NaH gave two cyclisation products, one resulting from the attack of the amide anion, while the other from the attack of the imidate anion, such that the desired γ-lactam **29** and γ-lactone **28** were obtained in 52% and 44% yield respectively. Although other basic γ-lactamisation conditions were investigated (though not mentioned in the paper), the yield of the desired γ-lactam **29** could not be improved.



Scheme 1.7. Cyclisation of key intermediate **27**.

The secondary benzyl ether was converted to a methyl ketone which undergoes an aldol condensation reaction with (2*S*, 3*R*) α,β-epoxy aldehyde **31** (as synthesised using the

procedures described by Barrett *et al.*), followed by global silyl deprotection to give (-)-pramanicin.



Scheme 1.8. Late stage coupling of lactam head with epoxy-aldehyde **31**.

1.5 Past research endeavours by Moloney Group

1.5.1 Synthetic methodology towards a tetramic acid intermediate

Apart from pramanicin, there are a number of natural products exhibiting antibacterial activity and possessing a tetramic acid core that are of interest in the Moloney group. Past research endeavours of the group have focused on this natural product-inspired paradigm, mainly on compounds based on equisetin,[54] reutericyclin[55] and kibdelomycin.[56]

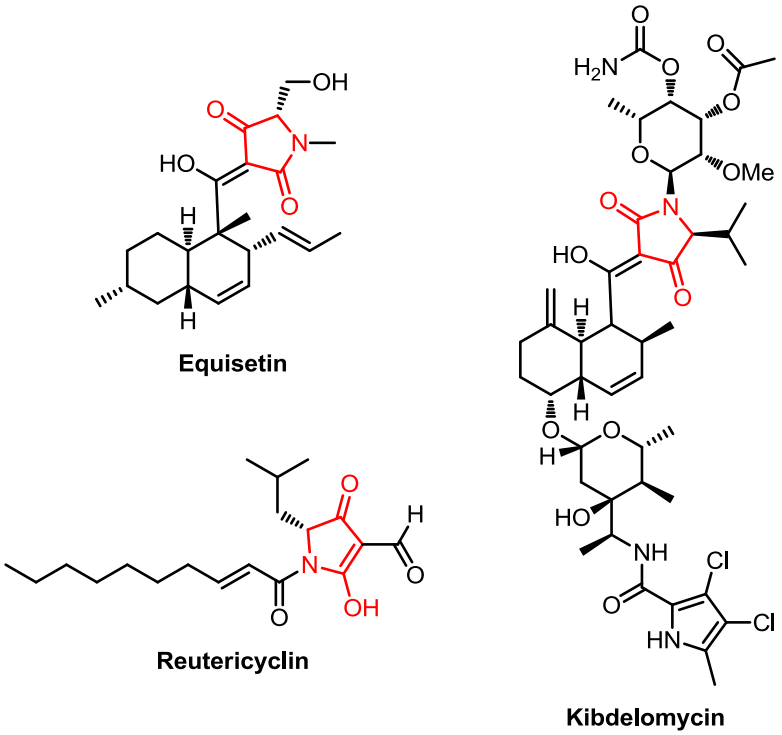
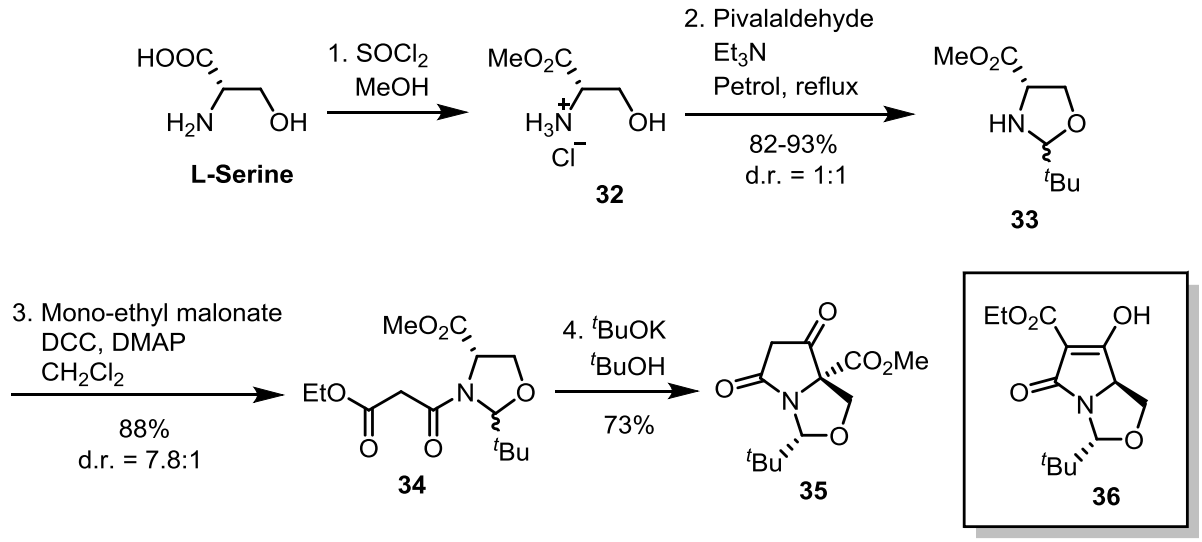


Figure 1.7. Examples of antibacterial natural products bearing a tetramic acid moiety.

A substrate-controlled approach as demonstrated by Seebach's "Self Regeneration of Stereocentres"[57] was utilised towards the synthesis of the chiral tetramic acid **35**, which serves as a template for further functionalisation towards mimics of such natural products. The oxazolidine **33** derived from L-serine was *N*-acylated with mono-ethyl malonate, afterwhich Dieckmann cyclisation gave a mixture of two tetramic acids **35** and **36**. Subsequent extraction using an aqueous 1 M NaH_2PO_4 solution removed **36** and gave the pure major tetramic acid **35** in 73% yield (Scheme 1.9).[58]

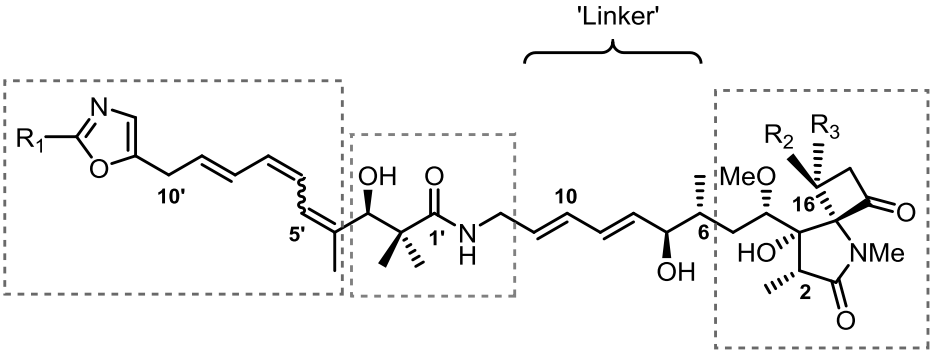


Scheme 1.9. Synthesis of tetramate intermediate **35** from L-serine.

There is significant representation of bactericidal tetramate-containing natural products present in the literature, but work by the Moloney group has revealed that the chiral bicyclic tetramic acid core **35** derived from either L-serine[59] or L-cysteine[60] alone does not possess any antibacterial activity. Nonetheless, the tetramate core based on L-threonine without a functionalised pendant attached showed significant antibacterial properties, albeit against Gram-positive *S. aureus* and *B. subtilis* but not the Gram-negative *E. coli*.[61]

1.5.2 Application towards oxazolomycin

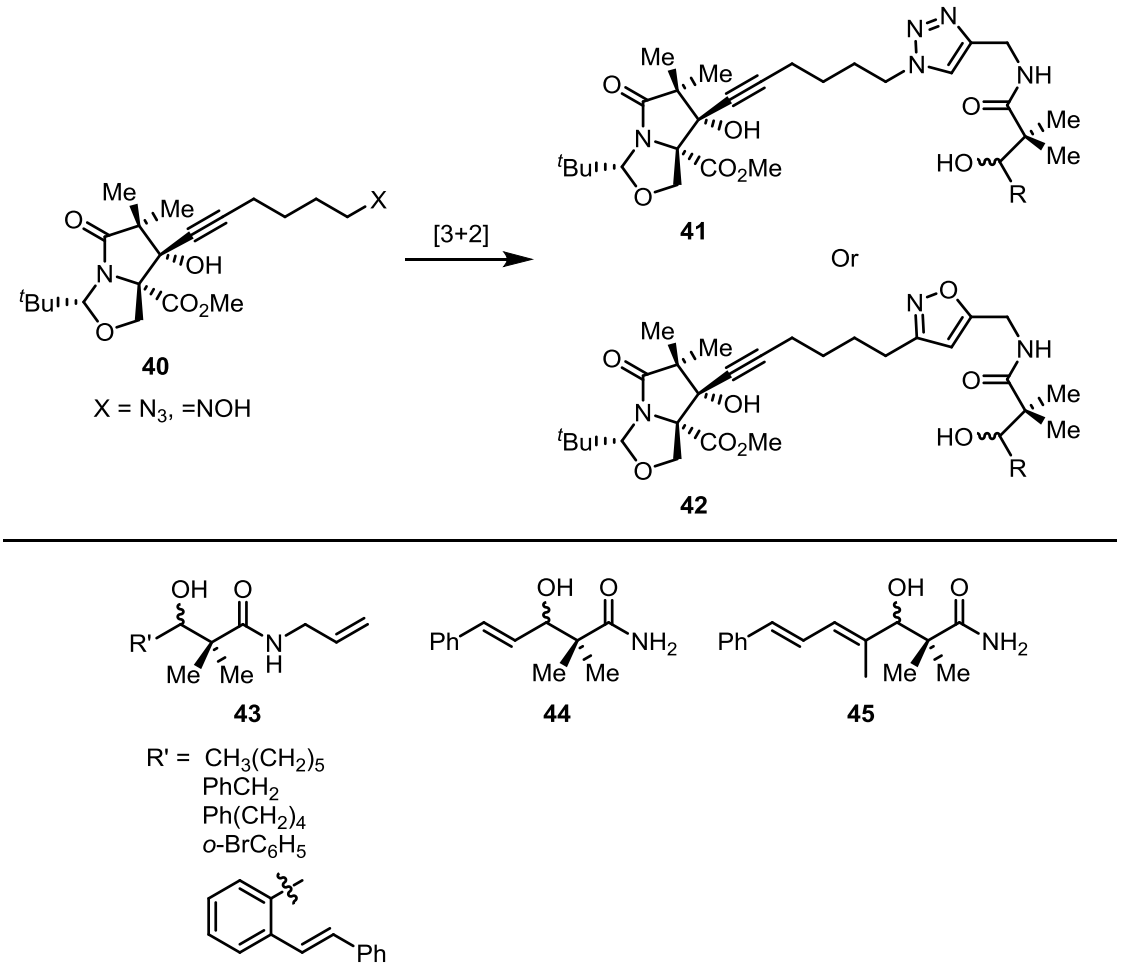
The natural product oxazolomycin[62,63] was envisioned to be constructed of three distinct portions – a triene tail, a 3-hydroxy-2,2-dimethylpropanamide mid-fragment which gives the molecule its U-shaped conformation, and a spiro lactam-lactone head group.



37 Oxazolomycin A :R_1 = H, R_2 = H, R_3 = H; 4'(*Z*), 6'(*Z*), 8'(*E*)
38 Oxazolomycin B :R_1 = H, R_2 = H, R_3 = H; 4'(*E*), 6'(*E*), 8'(*E*)
39 Oxazolomycin C :R_1 = H, R_2 = H, R_3 = H; 4'(*Z*), 6'(*E*), 8'(*E*)

Figure 1.8. Structure of oxazolomycins. [62, 63]

In a study, tetramic acid **40**, which is a mimic of the head group with a 'linker', was synthesised and coupled to various derivatives of the 3-hydroxy-2,2-dimethylpropanamide mid-fragment via [3+2] cycloaddition ("Click Chemistry"). This study has shown that the lactam head group alone does not exhibit antibacterial activity, and the coupled derivatives did not show activity towards *S. aureus* but displayed minimal activity towards *E. coli*.[64] In a separate study, it was found that derivatives of the 3-hydroxy-2,2-dimethylpropanamide fragment alone also did not exhibit much antibacterial activity.[65]



40 **41** **42** **43** **44** **45**

Figure 1.9. Top: [3+2] cycloaddition towards oxazolomycin mimics; Bottom: Derivatives of 3-hydroxy-2,2-dimethylpropanamide fragment.

However, the coupled compounds (**41** and **42**) exhibit a different selectivity towards the tested bacteria as compared to the natural product. As weak antibacterial activity was observed for the tested compounds (**41**-**45**), this suggests that the combination of the three structural fragments of oxazolomycin is required for antibacterial activity. Modifying either of the components would lead to a decrease in the potency, as well as bacterial selectivity, of the molecule.

1.5.3 Application towards streptolydigin

Functionalisation of the bicyclic tetramic acid core by addition of a 3-acyl pendant generally gave compounds with potent antibacterial activity. This has recently been demonstrated with a

library of 162 compounds, prepared by Moloney and co-workers[66] using the Dieckmann cyclisation methodology previously mentioned. The diversity about the tetramic acid core, consisting of 3-carboxamide, 3-acyl and 3-enamine, were introduced via one late-stage step at the end of the synthesis. These compounds were used to investigate a dual novel mechanism of action (MOA) exhibiting inhibitory activities against bacterial RNA polymerase (RNAP) and undecaprenyl pyrophosphate synthase (UPPS), and their design was inspired by the natural tetramic acid Streptolydigin (**46**) and Norvartis' structure-activity relationship (SAR) library against UPPS.

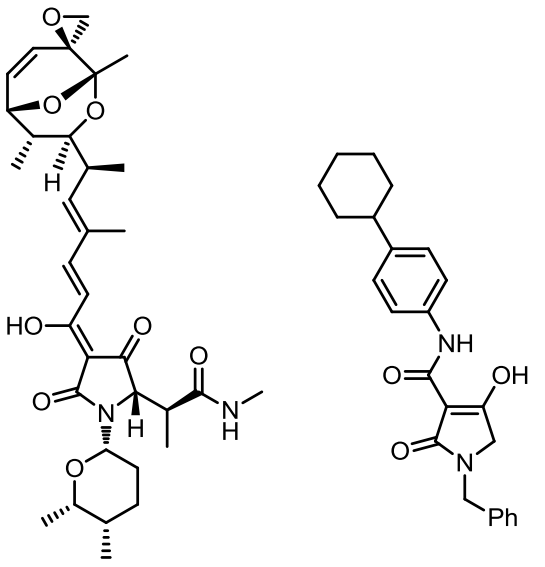


Figure 1.10. Structure of Streptolydigin (**46**) and one of Novartis' analogues (**47**), both containing the tetramic acid moiety.

The desired dual inhibition activity (against RNAP and UPPS) was observed from analogues bearing lipophilic aliphatic and bulky aromatic groups on the 3-carboxamide pendant of the bicyclic tetramic acid. A structure-activity relationship (SAR) analysis suggested that the oxygen atom on the 3-caboxamide/acyl group is important for such dual inhibition activity. Although some of these compounds were found to cause bacterial cell membrane disruption, none of them disrupted mammalian red blood cells and were inactive against the fungus *Candida albicans*, suggesting that these compounds are selective for bacteria.

Apart from their positive target-based assay results, these compounds also showed positive results in whole cell bacterial assays, in particular against the virulent Gram-positive strains which cause public health infections such as MRSA, VRE, erythromycin susceptible *S. pneumonia* and multi-drug resistant *S. pneumonia*. However, the activities were weaker against efflux-positive strains.

Eventually, a set of seven carboxamide compounds **48**-**51** with significant dual enzyme inhibition activity and of interest for further optimisation was identified. These compounds possessed similar physicochemical properties to the clinically used fluoroquinolones. Unfortunately, studies showed that these compounds bound to plasma protein, resulting in lower bioavailability and hence potentially lowering *in vivo* efficacy.

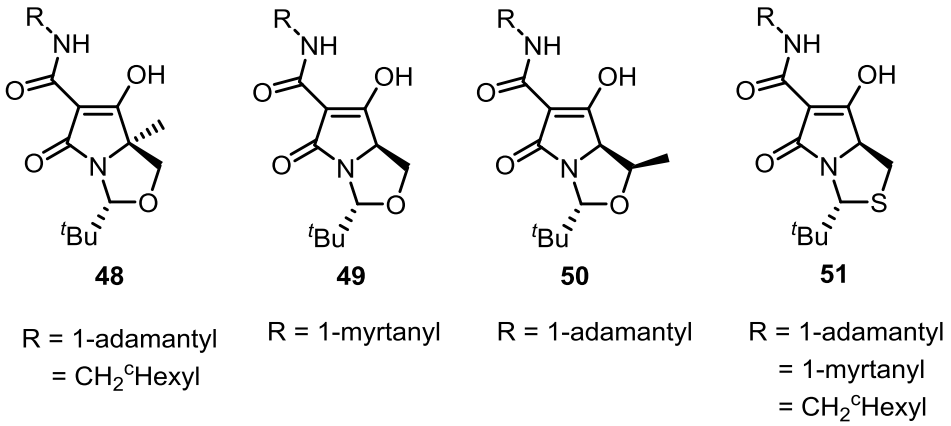


Figure 1.11. Seven carboxamide tetramic acid prepared by Moloney and co-workers.[64]

Nonetheless, the results obtained from these studies with natural product inspired tetramic acids demonstrated the feasibility of adopting this paradigm for the development of novel lead compounds as new antibiotics.

1.6 Aim

As an extension of our group's effort towards the development of novel antibiotics inspired by natural products, the aim of this thesis is to investigate the feasibility of using the natural product pramanicin towards the discovery of new antibacterials. This would be carried out by accessing different enantiopure heterocycles based on the polar lactam head of pramanicin to probe the effect of different oxygenation arrangements, via versatile synthetic routes with potential for variation of the R group to probe the effect of different side chains. (Figure 1.12). This not only includes synthetic methodologies compatible with a wide substrate scope, thus creating opportunities for future structure-activity relationship studies, but also incorporates other known bioactive scaffolds as intermediates in the synthetic route. The latter serves to further exemplify the natural product-inspired paradigm. The syntheses towards the desired oxygenated pyrrolidinones as pramanicin mimics would be described in greater detail in their respective chapters of this thesis.

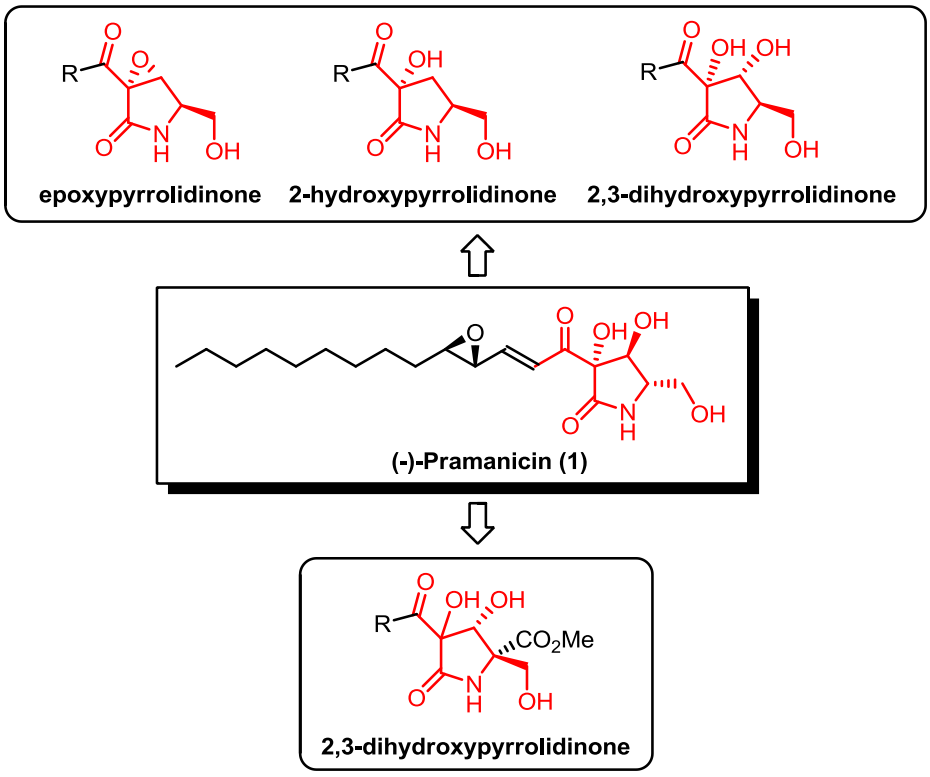


Figure 1.12. Different pramanicin mimics to be investigated in this thesis.

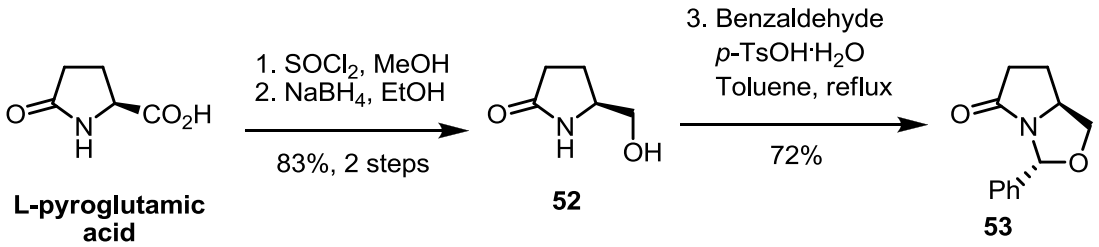
CHAPTER 2: PYROGLUTAMATE-DERIVED PRAMANICIN MIMICS

The approach towards natural product-inspired compounds for antibacterial drug discovery will focus on designing and synthesising mimics based on the lactam scaffold of the natural product pramanicin. Specifically, this chapter describes the synthesis of three oxygenated lactam scaffolds as potential lead compounds towards novel antibacterials. In this study, novel methodologies leading to the stereoselective synthesies of the oxygenated moiety of each of these scaffolds, as well as side-chain functionalisation of these scaffolds via a pendant exocyclic carbonyl group, will be reported.

2.1 Versatility of (2*R*, 5*S*)-1-aza-3-oxa-2-phenylbicyclo[3.3.0]octan-8-one scaffold

2.1.1 Synthesis from L-pyroglutamic acid

In order to access the targeted lactam scaffolds, it was envisaged that the bicyclic (2*R*, 5*S*)-1-aza-3-oxa-2-phenylbicyclo[3.3.0]octan-8-one **53** reported by Thottathil and co-workers would be a key intermediate for further functionalisation.[67] Thus lactam **53** was synthesised as a single diastereomer from L-pyroglutamic acid as shown in Scheme 2.1, following procedures reported in the literature.[67,68]



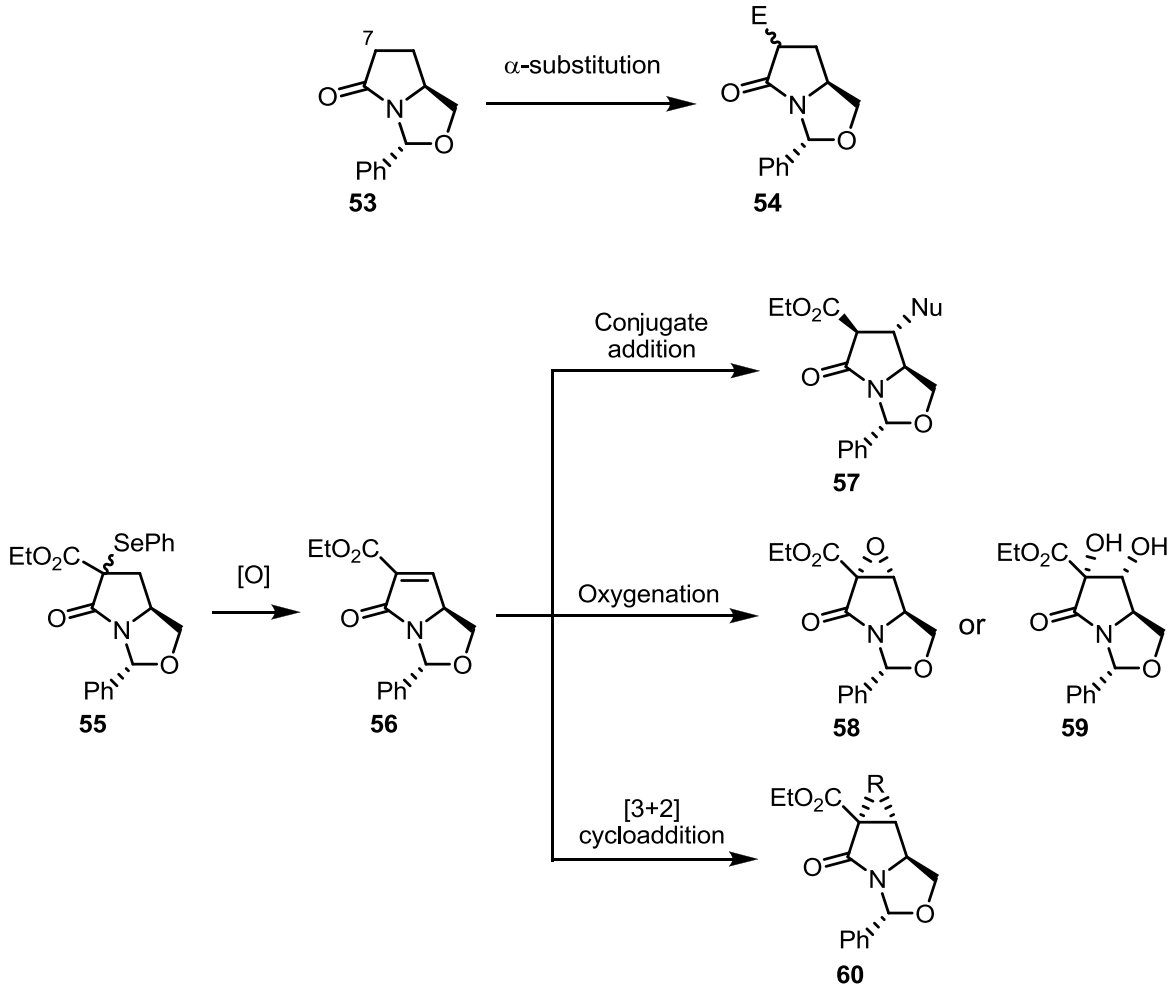
Scheme 2.1. Synthesis of (2*R*, 5*S*)-1-aza-3-oxa-2-phenylbicyclo[3.3.0]octan-8-one **53**.

In this route, L-pyroglutamic acid was firstly esterified using thionyl chloride with methanol as the solvent, followed by reduction of the ester to give (*S*)-5-(hydroxymethyl)-2-pyrrolidinone **52**. Simultaneous protection of the hydroxyl and amide groups as an *N*,*O*-acetal

using benzaldehyde was achieved by refluxing in toluene using a Dean-Stark apparatus, and the desired lactam **53** was obtained in 72% yield.

2.1.2 Previous work by the Moloney group

The versatility of the lactam scaffold **53** was previously demonstrated by the Moloney group. In their studies the bicyclic pyroglutamate-derived template **53** was further elaborated as shown in Scheme 2.2 to increase molecular complexity.



Scheme 2.2. Fuctionalisation of the chiral bicyclic pyroglutamate template.

The lactam enolate of **53** could undergo sequential functionalisation at the C-7 position[69] to introduce two different substituents. An introduction of a selenyl substituent at the C-7 position allows access to the α,β-unsaturated lactam **56**. Due to the nature of the chiral [3.3.0]

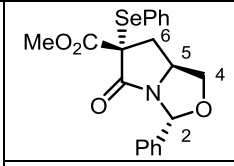
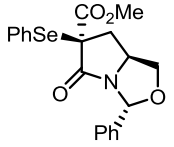
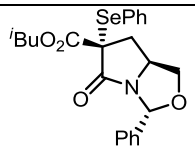
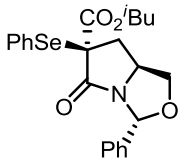
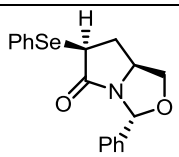
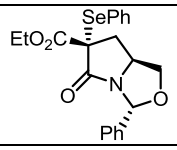
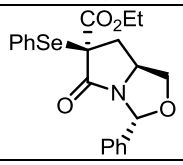
bicyclic system, functionalisation by conjugate addition with *C*-[70,71] or *N*-nucleophiles,[72,73] oxygenation[74] and cycloaddition[75] could be achieved stereoselectively. This clearly demonstrates the versatility of the (2*R*, 5*S*)-1-aza-3-oxa-2-phenylbicyclo[3.3.0]octan-8-one scaffold. Significantly, the concave-convex structure of the scaffold controls diastereoselectivity in its reactions, usually as a result of steric effects, but also in some cases due to stereoelectronic effects. Moreover, assessment of stereochemistry is readily achieved by NMR analysis as indicated below.

2.1.3 NMR characteristics of bicyclic lactam scaffold

In cases where the bicyclic lactam **53** has two different substituents at the C-7 position, the corresponding diastereomers could first be assigned via nOeSY, and then subsequently determined from a distinctive pattern in the chemical shifts of their H-6 protons.

For example, Cossy and co-workers[76] and Moloney and co-workers[69] have reported a series of compounds where one of the substituents at C-7 is a phenylselenyl group (Table 2.1). When the selenyl substituent is at the *endo* position of the bicycle, the chemical shift difference between the two diastereotopic H-6 protons is significantly greater than the chemical shift difference between the two diastereotopic H-6 protons when the selenyl substituent is at the *exo* position.

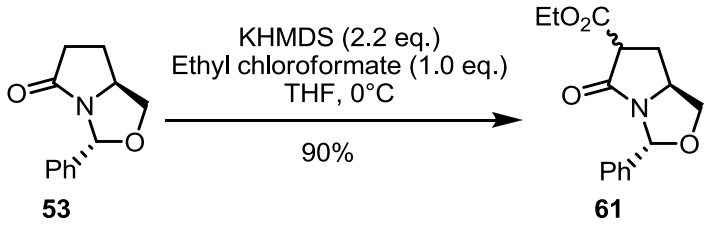
Table 2.1: Difference in chemical shift values to assign stereochemistry

		Chemical Shift (δ) Values of Protons (ppm)						
	Compound	**H2**	**H4**	**H4'**	**H5**	**H6**	**H6'**	**Δ(H6-H6') ppm**
Ref. 76.		6.24	4.11	3.86	3.18	2.93	2.60	0.33
		6.24	4.15-3.99	4.15-3.99	3.07	3.05	2.15	0.90
		6.21	4.08	4.02	3.07	2.92	2.56	0.36
		6.25	3.99-3.84	3.99-3.84	3.13-2.95	3.13-2.95	2.12	~0.92
Ref. 69.		6.27	4.16	3.4	3.65	2.50	2.50	0.00
		6.30	4.00-4.18	3.10	4.00-4.18	2.95-2.75	2.10-1.80	~0.90
		6.25	4.11-4.14	3.48	3.14-3.20	2.94	2.60	0.34
		6.27	4.06-4.11	3.12-3.15	4.06-4.11	3.06	2.14	0.92

2.2 Synthetic plan towards three oxygenated scaffolds of pramanicin mimics

In our studies, the three different oxygenated scaffolds of pramanicin mimics will be synthesised by exploiting the versatility of bicyclic intermediate **53**.

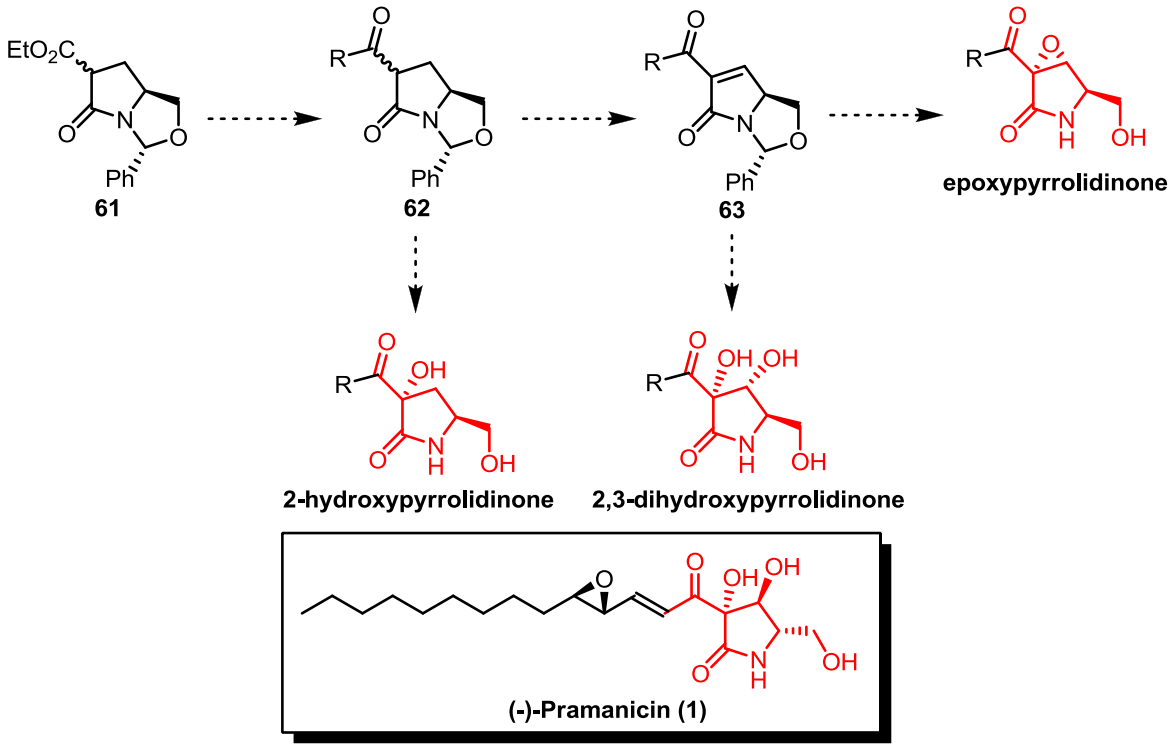
In order to achieve this, the ethyl ester **61**, described previously by Moloney and co-workers,[69] was synthesised with an adapted procedure using KHMDS and ethyl chloroformate.[77]



Scheme 2.3. Synthesis of ethyl ester **61**.

Although **61** was isolated as a 1:1 mixture of diastereomers at C-7, isomerisation occurs selectively to give the 7*S* diastereomer (*endo* ethyl ester) upon prolonged storage in the pure form in the freezer (-20°C) or at ambient temperatures. However, in solution ($CDCl_3$), it isomerised slowly back to a mixture of diastereomers.

The scheme below illustrates our synthetic plan for elaborating the intermediate **61** towards the three oxygenated pyrrolidinone scaffolds, by exploiting the versatility in which multiple functional groups could be introduced onto a single five-membered ring. The pendant ethyl ester provided access to various side chains. For example, α-oxygenation of **62** would permit installation of the tertiary alcohol in 2-hydroxypyrrolidinones while the α,β-unsaturated dicarbonyl **63** would be a precursor to epoxypyrrolidinones and 2,3-dihydroxypyrrolidinones. In all three cases, the oxygen atoms would be expected to be introduced in a stereoselective manner as a result of the chiral bicyclic [3.3.0] skeleton.



Scheme 2.4. Synthetic plan towards three oxygenated pyrrolidinone scaffold.

In the next part of this chapter, the use of this scaffold will be described, starting from the epoxypyrrolidinones, followed by 2-hydroxypyrrolidinones and 2,3-dihydroxypyrrolidinones, in increasing likeness towards the polar lactam head of pramanicin.

2.3 Epoxypyrrolidinones

The epoxypyrolidinone system is of interest as it not only bears similarities to the lactam head of pramanicin, but this motif is also found in other natural products. For example, epolactaene is a neuritogenic compound in human neuroblastoma cells isolated from a marine fungus *Penicillium sp.* BM1689-P.[78] Subsequent work done on epolactaene showed that it is an inhibitor of Hsp60 chaperone activity,[79] inhibitor of DNA polymerase,[80] and has apoptosis inducing effects on BALL-1 cells.[81] Other similar natural products include NG-391, which exhibits neurotrophic activity and an effect on neurite growth;[82] and L-755,807 which has been shown to be a non-peptide bradykinin inhibitor (Figure 2.1).[83]

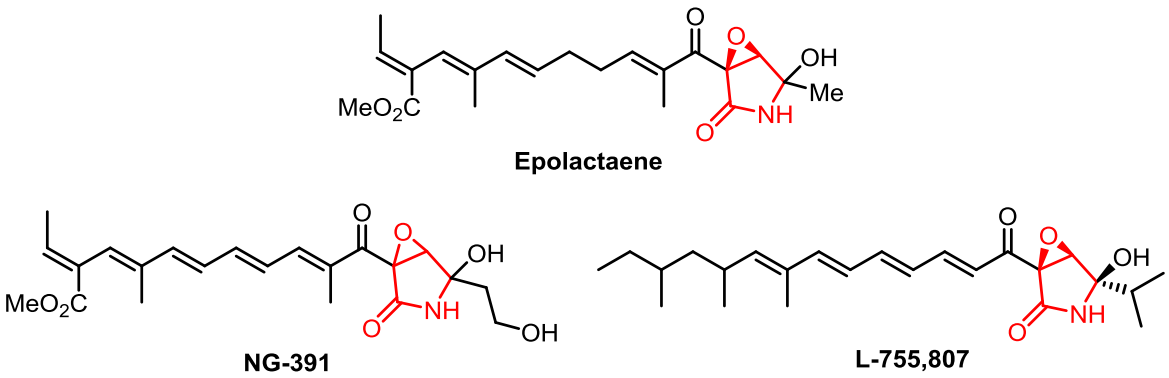


Figure 2.1. Structure of epolactaene, NG-391 and L-755,807, consisting of an epoxypyrrolidinone moiety.

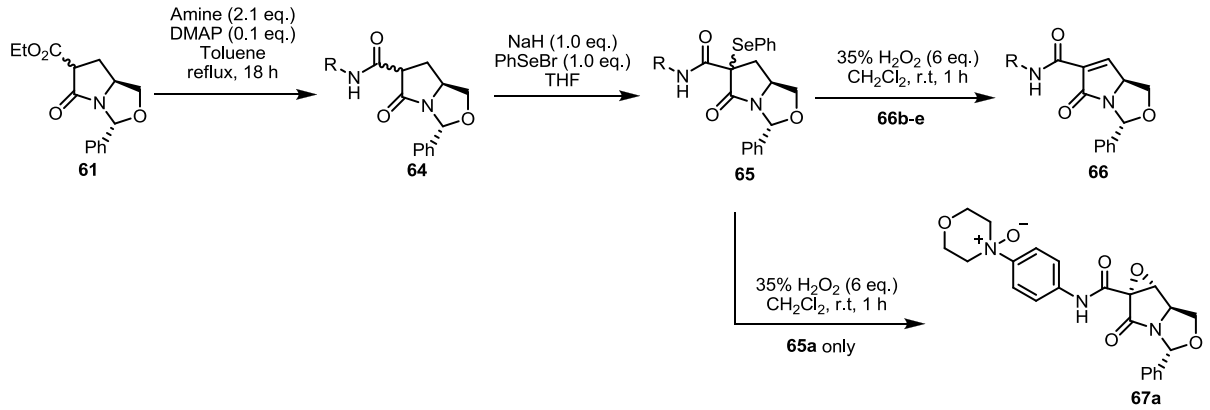
Although the antibacterial properties of the above natural products have not been studied in detail, their biologically relevant structures might be expected to further exemplify the paradigm of a natural-product inspired synthesis, and provide interesting bioactive chemical libraries.

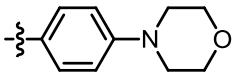
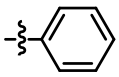
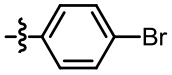
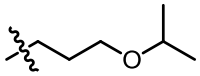
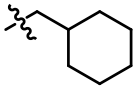
2.3.1 Initial work on carboxamido epoxypyrrolidinones

Initially, epoxypyrrolidinones with a pendant secondary amide were investigated, since such systems were known to be important for antibacterial activity in tetramate systems.[66]

Ethyl ester **61** was converted to the secondary amides **64a-e** by refluxing with the respective amines and catalytic DMAP in toluene. This provided the amides **64a-e** in good yields as an inseparable 1:1 mixture of diastereomers about the C-7 amide group. Selenylation of **64** gave **65** and a small amount of each selenyl diastereomer was isolated during column chromatography for characterisation purposes, afterwhich they were combined for the next reaction. The respective diastereomers of **65** could readily be assigned based on their characteristic H-6 chemical shift pattern (*vide supra*).

Table 2.2: Synthesis of carboxamido derivatives of epoxypyrrolidinones.



		Yield (%)			
		64	**65**	**66** (Crude Yield)	**67a***
a	R = 	67%	40%	N/A	40%
b	R= 	92%	87%	96%	
c	R = 	95%	77%	92%	
d	R= 	89%	78%	Quant.	
e	R = 	89%	87%	Quant.	

*The morpholino group of **67a** is oxidised to the *N*-oxide.

N/A = not applicable.

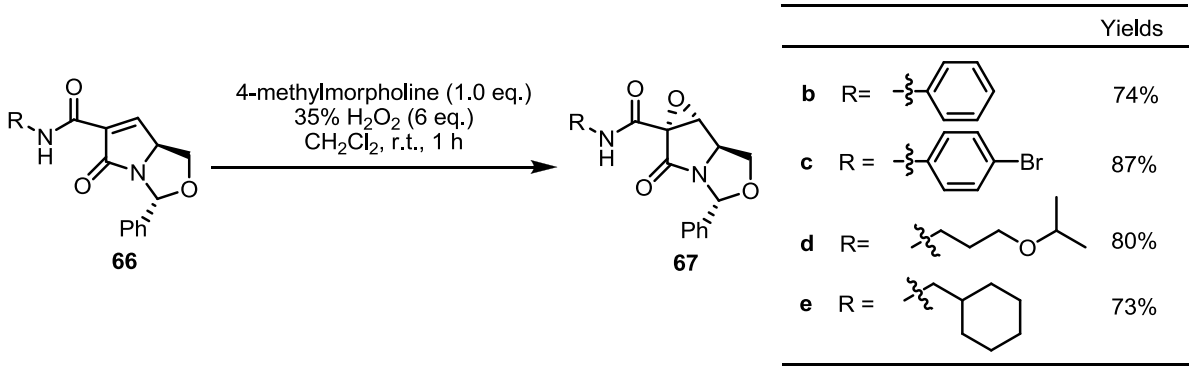
Oxidative elimination of the selenyl substituents gave the desired α,β-unsaturated lactams **66**. Surprisingly, treatment of **65a** under similar conditions gave the epoxide **67a** directly. Further analysis of **67a** suggests that the morpholino group had also been oxidised to the *N*-oxide. HRMS analysis confirmed the molecular mass of **67a**, while comparison of the ^{13}C carbon spectra of **67a** and its precursors **64a** and **65a** revealed a significant downfield shift of the carbon atom (C-10) adjacent to the N atom in the morpholino group of **67a**, suggesting the presence of an electron-withdrawing group, consistent with the formation of the *N*-oxide. However, the chemical shift of H-10 remained relatively unchanged, possibly due to the anisotropy effect from the benzene ring.

Table 2.3: Comparison of C-10 ^{13}C chemical shifts of morpholino derivatives.

Compound	C-10 ^{13}C chemical shifts (ppm)
64a (mixture of diastereomers)	49.69; 49.72
(7*R*)-**65a** (Se *endo*)	49.67
(7*S*)-**65a** (Se *exo*)	49.70
67a	61.23

On the basis of this fortuitous result, unsaturated lactams **66b-e** were treated with excess hydrogen peroxide in the presence of one equivalent of 4-methyl-morpholine and this gave the epoxy derivatives **67b-e** with good isolated yields (Table 2.4).

Table 2.4: Epoxidation of **66b-e** using 4-methyl-morpholine and H_2O_2.



The diastereoselectivity of the epoxidation reaction is generally about 10:1 based on their respective ^1H NMR spectra, in favour of the *exo*-epoxide. *Exo*-oxygen addition was confirmed from nOe analysis (Figure 2.2), as well as X-ray crystallography (Figure 2.3). This stereochemical outcome is consistent with the preferred nucleophilic attack of peroxy species at the less hindered face, similar to *exo*-alkylations at the less hindered face of the bicyclic system.[84] It is noteworthy that the H-6 signal is a singlet, indicating its orthogonality to H-5, and typically at a chemical shift of 4.6 ppm. This is also supported by the torsion angle between H-6 and H-5 which is found to be 85.8° from the X-ray crystal structure of **67e**.

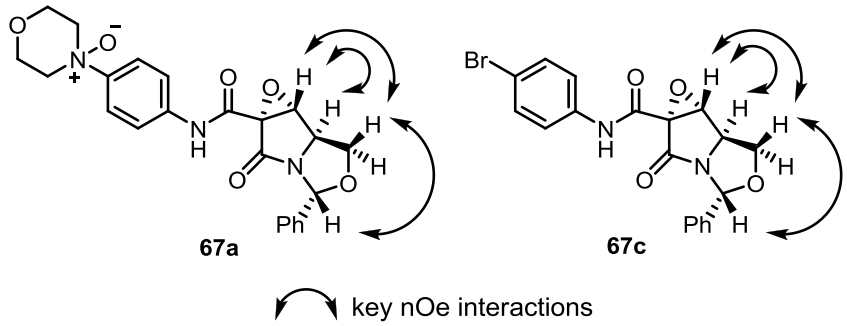


Figure 2.2. nOe analysis of **67a** and **67c**, showing key nOe interactions.

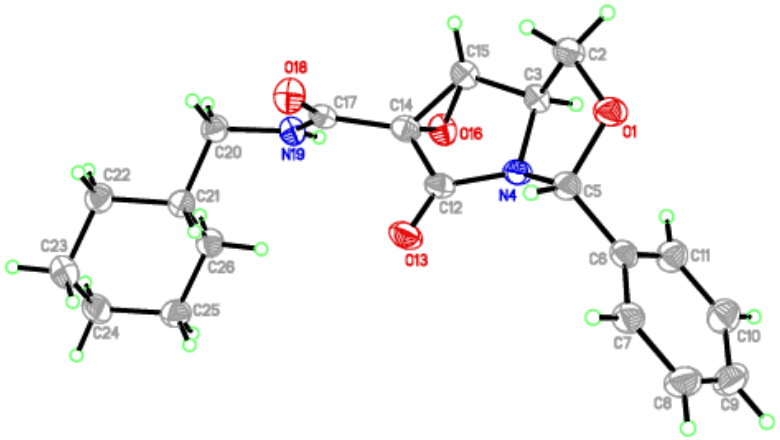
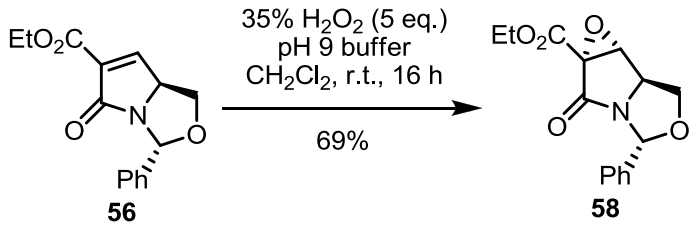


Figure 2.3. X-ray crystal structure of **67e**.

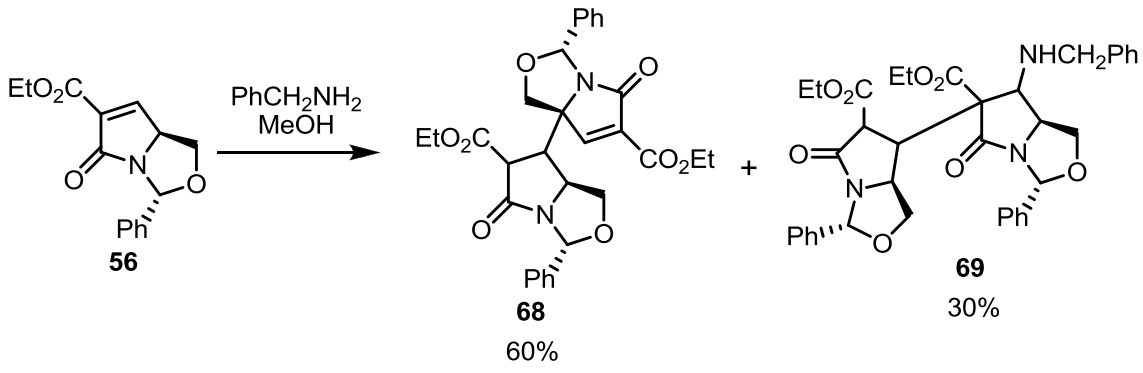
2.3.2 Further investigation of epoxidation condition

The serendipitous discovery of the mild and facile epoxidation condition mediated by 4-methyl-morpholine proved to be crucial towards the synthesis of epoxypyrrolidinones. Previous work by Moloney and co-workers[74] reported the difficulty associated with epoxidation of a similar 1,1-dicarbonyl α,β-unsaturated lactam **56**. Moloney and co-workers described the use of Herdeis conditions (*t*-BuOOH/*n*-Bu_4NF/K_2CO_3/DMF) gave a low 20% yield of the desired epoxide, while multiple alternative conditions utilising reagents such as NMO, alkaline hydrogen peroxide, hydrogen peroxide-Triton B, or hydrogen peroxide-*t*-BuLi were unsuccessful. Eventually, the use of 35% aq. hydrogen peroxide and a pH 9 (sodium tetraborate-HCl) buffer gave the epoxide after vigorous stirring of the biphasic reaction mixture overnight (Scheme 2.5). It was also reported that this reaction was slower at a lower pH and the sole product isolated at pH 3 was the dimer **68**.



Scheme 2.5. Epoxidation of **56**.[74]

A separate study by Moloney and co-workers also found that dimerisation of lactam **56** occured in the presence of a base such as benzylamine (Scheme 2.6).[73] As this lactam is prone to dimerisation under basic conditions, this may explain the failure of previous epoxidation attempts with hydrogen peroxide and a strong base.

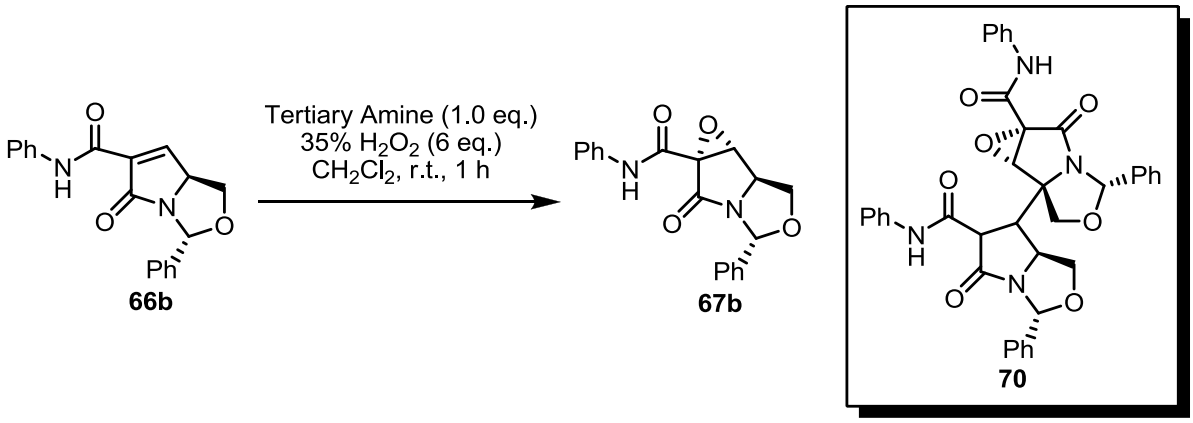


Scheme 2.6. Dimerisation of **56** in presence of benzylamine.[73]

In order to build on the epoxidation reaction as described in Table 2.4, the epoxidation of **66b** in the presence of amine bases other than 4-methyl-morpholine was investigated (Table 2.5). It is essential that a good, if not complete, conversion of the unsaturated lactam **66b** is achieved. This is because the retention factor of the epoxide **67b** is very close to that of **66b**, and residual **66b** tend to form side-products with very similar retention factors during purification with column chromatography.

Screening of different bases for the epoxidation reaction was carried out as shown in Table 2.5. The use of triethylamine resulted in a complex reaction mixture, but the desired epoxide **67b** was isolated in approximately 30% yield along with the dimerised product **70** in approximately 10% yield.

Table 2.5: Screening of bases for epoxidation of **66b**.



	pKa_H in H_2O	Reaction Duration	67b, Yield	Remarks
Et_3N	10.75	1 h	-	Complex ^1H NMR of crude spectrum
DABCO	2.97, 8.82	40 min	74%	-
4-methyl morpholine	7.38	1 h	Quant.	-
Pyridine	5.21	3 h	87%	1.0 eq of Pyridine and 6 eq. of H_2O_2 added after each hour

pKa_H in H_2O of H_2O_2 = 11.6.

Using DABCO gave the desired epoxide but the isolated yield was not as good as using 4-methyl-morpholine. Finally, the use of pyridine resulted in a sluggish reaction rate, with excess reagents required to achieve complete conversion of **66b**. This reaction presumably proceeded under a general base catalysis mechanism since the pK_{aH} value of the amine bases[85] suggest that they are unable to deprotonate hydrogen peroxide to form the peroxy anion (Table 2.5).

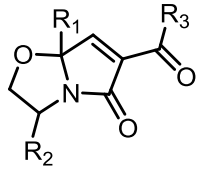
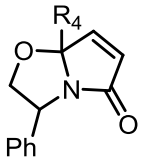
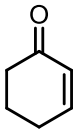
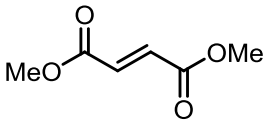
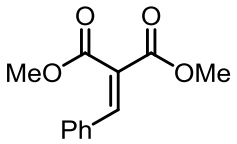
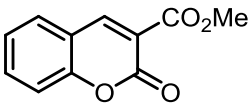
The observations from this screen agrees well with previous observations that such 1,1-dicarbonyl α,β-unsaturated lactams tend to be unstable under basic conditions, and that epoxidation tends to be slow below an optimal pH value.

It should be noted that Meyers and co-workers had previously reported the epoxidation of a closely related 1,1-dicarbonyl α,β-unsaturated lactam using only NMO in the absence of a metal catalyst.[86] Table 2.6 lists the substrates screened and only 1,1-dicarbonyl bicyclo[3.3.0]lactams underwent epoxidation, while the other substrates were unreactive under these conditions.

When these conditions were applied to **66b**, a complex reaction mixture was obtained, while the use of a biphasic mixture of aq. NMO and CH_2Cl_2 did not result in any conversion. Thus this suggested that **66b-e** (Table 2.4) were not epoxidised by NMO during the reaction as the conditions employed were too mild to form NMO *in situ* from 4-methyl-morpholine and hydrogen peroxide.[87]

Table 2.6: Epoxidation using NMO as described by Meyers *et al*.[86]

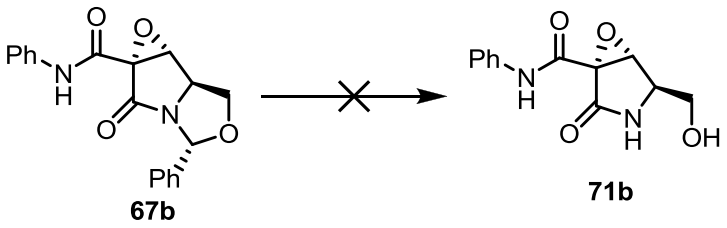
Reaction Condition: NMO (1.5 eq.), CH_2Cl_2, r.t., 3 h, with vigorous stirring.

Compound	Yields
	R_1, R_2 = Ph, R_3 = OMe : 90% R_1 = Me, R_2 = *i*-Pr, R_3 = OMe: 92% R_1 = Me, R_2 = Ph, R_3 = OBn: 99% R_1 = Me, R_2 = Ph, R_3 = O^{t}Bu: 92%
 R_4 = Ph, Me	No reaction
	No reaction
	No reaction
	No reaction
	No reaction

2.3.3 Unexpected failure of oxazolidinone hydrolysis

The final step of the planned synthesis required the hydrolysis of the oxazolidine. Following literature procedures,[74,88] the use of TFA in THF/H_2O did not lead to successful hydrolysis of the oxazolidine to the desired epoxy pyroglutaminol and only the starting material was recovered quantitatively (Table 2.7). This was unexpected as these conditions had previously been found to be robust for the hydrolysis of the oxazolidines with similar substrates.[72,89,90] The reason for the failure of this oxazolidine hydrolysis was subsequently investigated (see Section 2.3.5, *vide infra*).

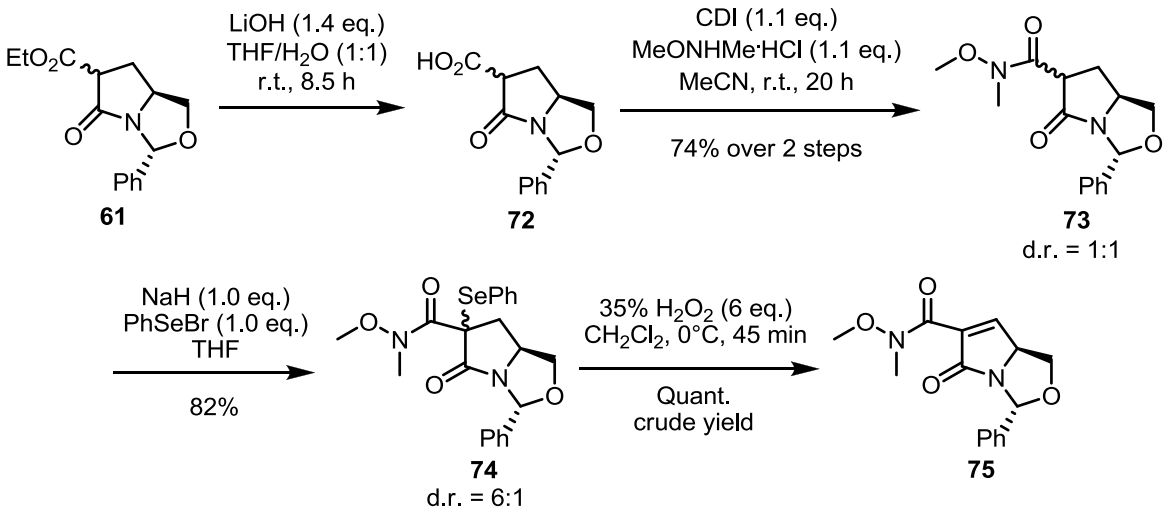
Table 2.7: Attempted hydrolysis of **67b**.



Entry	Condition	Remarks
1	TFA (4 eq.), THF/H_2O (2:1), r.t., 6.5 h	No conversion of **67b**; **67b** recovered.
2	TFA (4 eq.), MeOH, r.t., 23 h	No conversion of **67b**; **67b** recovered.
3	TFA (21 eq.), THF/H_2O (1:1), r.t., 19 h	LRMS analysis of crude mixture shows presence of **71b**; but ^{1}H NMR spectrum with MeCN-d_3 or MeOD-d_4 shows only **67b**.

2.3.4 Keto-derivatives of epoxypyrrolidinones

The route towards carboxamido epoxypyrrolidinones was thwarted by the unexpected stability of the oxazolidinone system as noted above. An antibacterial assay was conducted on compounds **67b-e**, but only modest activity was observed (see Chapter 4). These results prompted the pursuit of another set of epoxypyrrolidinones containing a pendant C-7 keto group.



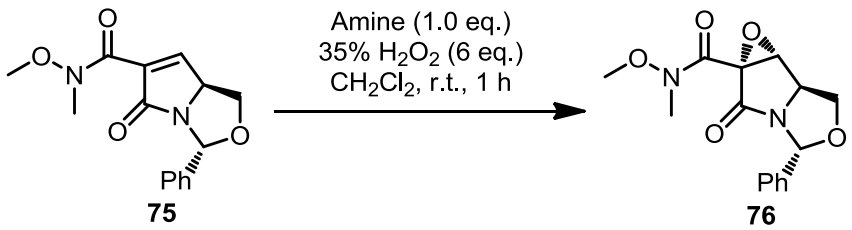
Scheme 2.7. Synthesis of common intermediate towards keto-epoxypyrrolidinones.

Ester hydrolysis of **61** followed by CDI-mediated coupling[91] gave the Weinreb amide **73** as an inseparable 1:1 mixture of diastereomers. Selenylation gave **74** (d.r. = 6:1), which was followed by oxidative elimination under standard conditions to give the α,β-unsaturated lactam **75**. It was found that oxidative elimination of **74** on a scale larger than 1 gram was best carried out at 0°C instead of ambient temperatures since this minimised the formation of unidentified by-products and gave overall cleaner conversion (Scheme 2.7).

The epoxidation of **75** required some optimisation as the use of 4-methyl-morpholine, which had been found to be the best tertiary amine for the epoxidation of **66b-e**, was sluggish. Given the previous observations whereby epoxidation tended to be slower below a certain optimal pH value, the use of pyridine as a base was omitted in the optimisation studies.

From the results shown in Table 2.8, triethylamine was found to give the best result as it gave the fastest reaction with only one addition of reagents (6 eq. of hydrogen peroxide/1 eq. of amine) required. This suggested that the tertiary carboxamido lactam **75** has a lower reactivity towards epoxidation as compared to the secondary carboxamido lactams **66b-e**. It is not clear why the use of DABCO required the addition of more reagents and a longer duration for reations time as compared to the use of 4-methyl-morpholine or triethylamine as base.

Table 2.8: Screening of bases for epoxidation of **75**.



75 **76**

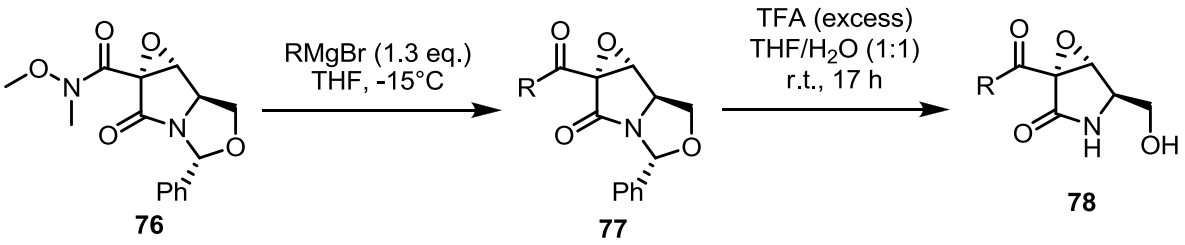
d.r. approx. 1:0.1

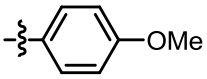
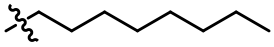
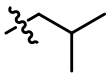
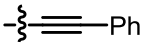
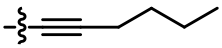
	pKa_H in H_2O	**Reaction Duration**	**76, Yield**	**Remarks**
4-Methylmorpholine	7.38	3 h	58%	1.0 eq of amine and 6 eq. of H_2O_2 added after each hour
DABCO	2.97, 8.82	6 h	68%	1.0 eq of amine and 6 eq. of H_2O_2 added four times over 6 h.
Et_3N	10.75	1 h	88%	-

pKa_H in H_2O of H_2O_2 = 11.6.

With **76** in hand, the desired keto-epoxypyrrolidinone derivatives were synthesised by reaction with the respective Grignard reagents to give the ketones **77a-g** in good yields.[92] Noteworthy was that the epoxy moiety remained intact. Synthesis of **77d** with *n*-BuLi instead of the Grignard reagent resulted in a low 18% yield of the isolated product, with unidentified side products as observed from TLC analysis. Oxazolidine hydrolysis using the acidic conditions employed previously gave the hydrolysed products **78a-g** in high yields (Table 2.9).

Table 2.9: Synthesis of keto-epoxypyrrolidinones **79a-g**.



		Yields	
		77	**78**
a	R = 	86%	94%
b	R = 	76%	87%
c	R= 	77%	71%
d	R= 	75%	96%
e	R= 	62%	80%
f	R= 	72%	77%
g	R= 	71%	86%

The stability of the epoxide in the presence of excess Grignard reagent could be explained from the steric requirements of the bicyclic [3.3.0] system. From the X-ray crystal structure (Appendix D) of **67e**, the two torsional angles of the bicycle (highlighted in red and blue respectively in Figure 2.4) are significantly different as a result of a trigonal pyramidal nitrogen atom and the tetrahedral carbon atom at the ring junctions. The lactam nitrogen tends to deviate slightly from a planar sp^2 configuration due to the ring strain present.[93]

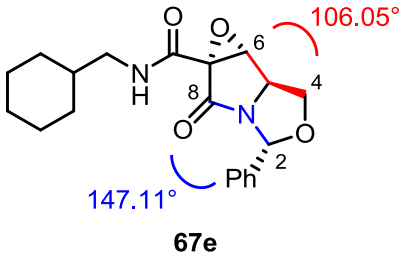


Figure 2.4. Torsional plane angle of the two edge of the bicyclic [3.3.0] **67e**.

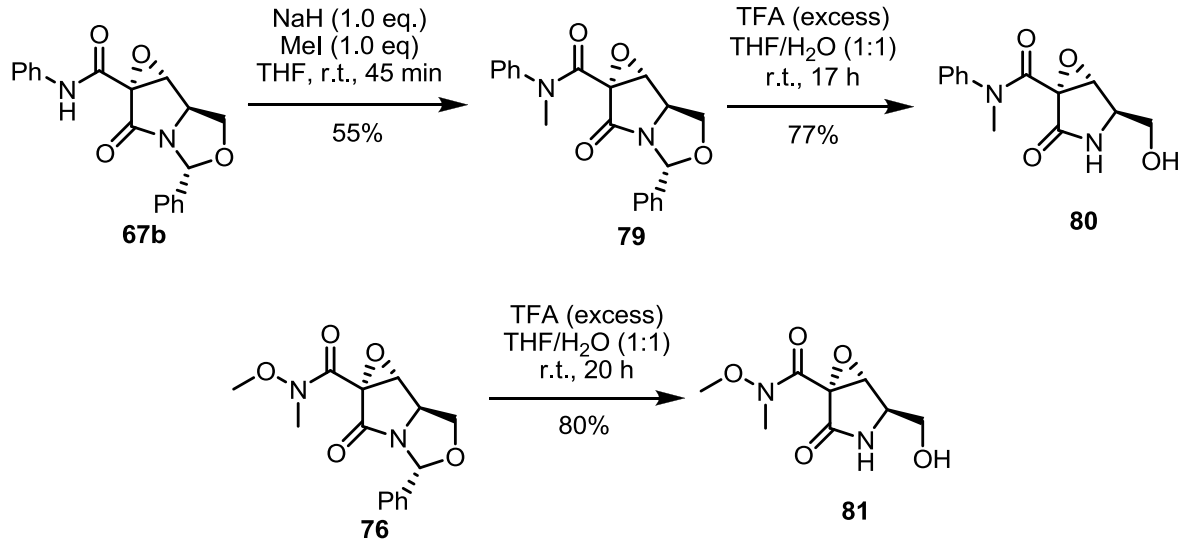
With this bicyclic conformation, the σ* approach to the epoxy C-O bond at C-6 is hindered by the C-4 methylene group, whereas the approach of nucleophile at C-7 is hindered by the presence of the two carbonyl groups.

Having successfully obtained the desired keto-epoxypyrrolidinones **78a-g**, investigations relating to the different epoxidation and oxazolidine hydrolysis behaviour or carboxamido pyrrolidinones and keto-lactams was undertaken.

2.3.5 Hydrolysis behavior of oxazolidines

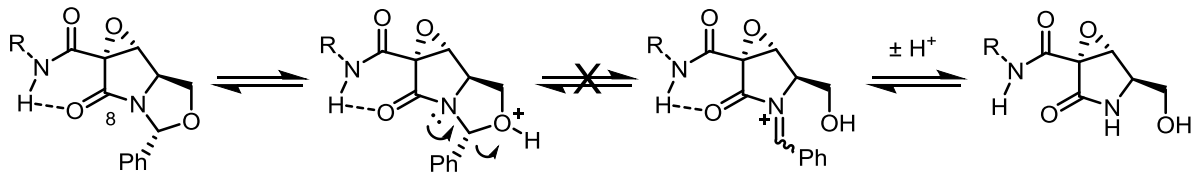
From the sections above, it was noted that the protected epoxyketopyrrolidinones **77a-g** underwent oxazolidine hydrolysis smoothly while the protected carboxamido epoxypyrrolidinone **67b** was stable to hydrolysis. Both types of compounds are similar structurally with the exception that **67b** possessed a hydrogen-bond donor on the pendant C-7 acyl chain.

In order to assess if this difference was responsible for the resistance to hydrolysis, further experiments as shown in Scheme 2.8 were undertaken. After **67b** was *N*-methylated to tertiary amide **79**, oxazolidine hydrolysis was successful and **80** was obtained. Similarly, oxazolidine hydrolysis was also successful with Weinreb amide **76**.



Scheme 2.8. Successful hydrolysis of protected tertiary carbamoyl epoxypyrrolidinones.

From these observations, it was proposed that the -NH- group of the secondary amide substituent may participate in an intramolecular hydrogen-bonding with the lactam carbonyl oxygen, which in turn reduced electron density about the lactam nitrogen atom and impeded the hydrolysis of the oxazolidine (Scheme 2.9).



Scheme 2.9. Reduced electron density of lactam nitrogen leading to stability of oxazolidines.

ATR IR spectra were obtained on neat samples and the data is shown in Figure 2.5.

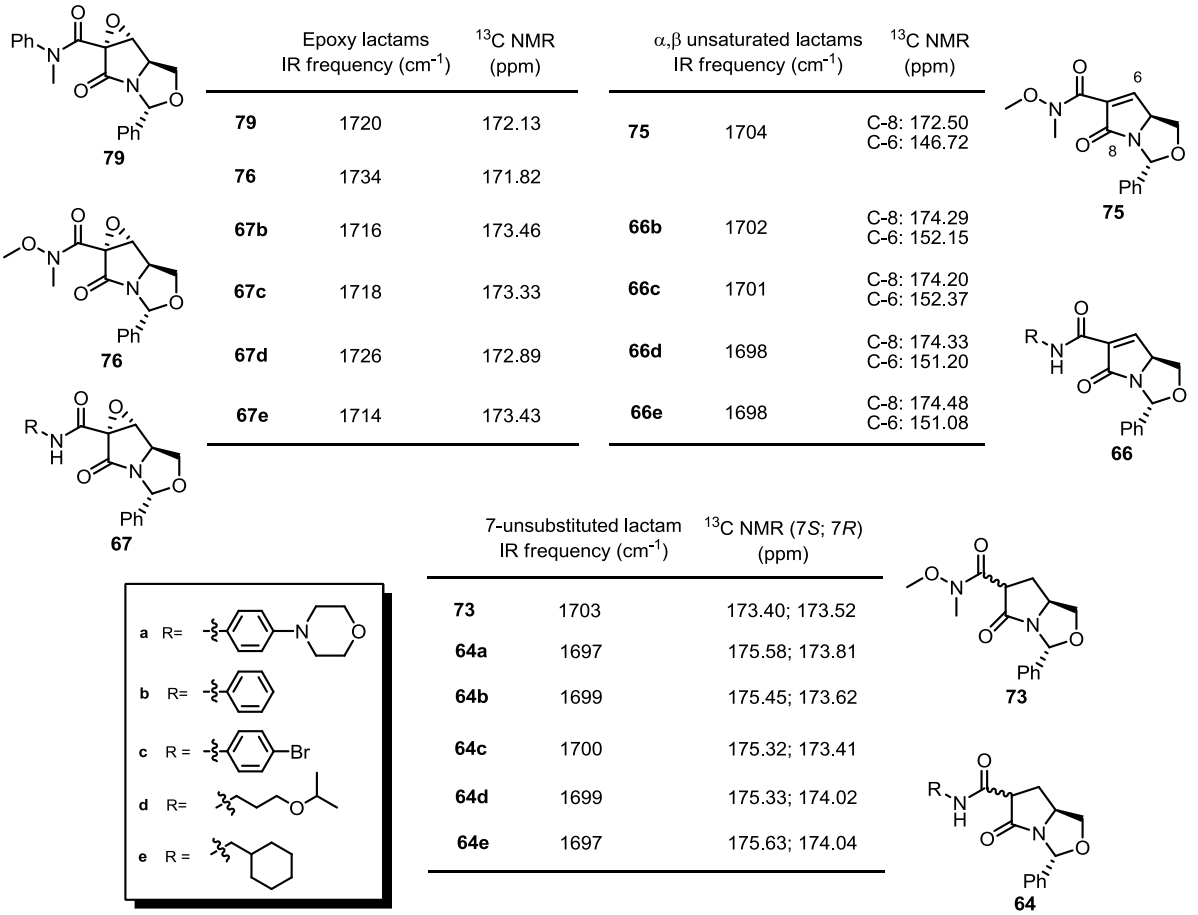


Figure 2.5. An analysis of IR stretching frequency and ^{13}C resonance frequency of lactam carbonyls to establish the presence of intramolecular hydrogen-bonding. $CDCl_3$ was used for all NMR experiments.

An analysis of the IR stretching frequency of the protected epoxypyrrolidinones (**67b-e**, **76**, **79**) and α,β-unsaturated lactams (**66b-e**, **75**) showed that compounds with a secondary amide generally have a reduced frequency of their C=O bands (that is shifted to a smaller wavenumber), suggesting it to be hydrogen-bonded.[94] The presence of such an interaction would also reduce the electron density at the carbon atom of a carbonyl group, resulting in their ^{13}C resonance frequency appearing more downfield as observed.

For the α,β-unsaturated lactams (**66b-e**, **75**), differences in their epoxidation rates were observed and this may be rationalised using similar arguments as above. Unsaturated lactams **66b-e**, having the intramolecular hydrogen-bonding, have reduced electron density at C-6,

making them better Michael acceptors, and as such a weaker base such as 4-methyl-morpholine is sufficient to effect the epoxidation reaction. Moreover, as **66b** is more reactive than **75**, it is more prone to dimerisation in the presence of a stronger base such as triethylamine.

Furthermore, the literature data reported for **56** correlates well with the observation that the reactivity of the α,β-unsaturated lactam was influenced by the carbonyl group at C-7 (Figure 2.6). Lactam **56** was found to be highly prone to dimerisation under basic conditions as the C-6 ^{13}C NMR chemical shift has a value of δ154 ppm, suggesting that it is a more reactive Michael acceptor than the amide derivatives **66b-e**. Without an *exo*-cyclic hydrogen bond donor, the lactam carbonyl group has a ^{13}C NMR chemical shift of δ171 ppm, which is comparable to **75**.

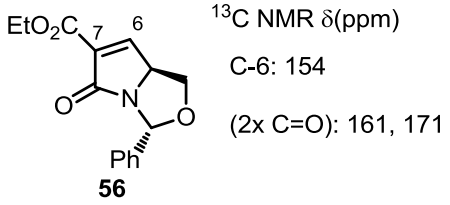


Figure 2.6. Literature NMR data for **56**.

The data of the 7-unsubstituted lactams **64a-e** showed that only one of the diastereomers exhibited such intramolecular hydrogen-bonding. In the absence of such hydrogen-bonding, the lactam ^{13}C resonance frequency of Weinreb amide **73** is around δ173 ppm. Although compounds **64a-e** were each isolated as an inseparable mixture of diastereomers, assignment of the NMR signals of the corresponding diastereomers was achieved by first identifying its H-6 protons, followed by HMBC correlations to their respective lactam carbonyl ^{13}C resonances. The difference in the chemical shifts of the H-6 protons resonances was compared with the analogous esters **61** to determine their stereochemistry about C-7 (Figure 2.7).

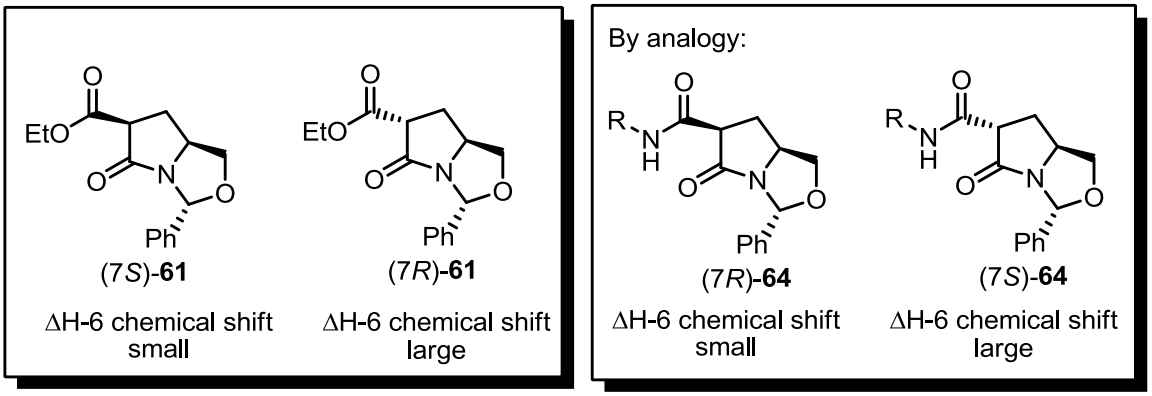


Figure 2.7. Stereochemistry of **61** determined and reported by M. J. Beard *et al. Tetrahedron* **1996**, *52*, 3719-3740. Stereochemistry of **64** assigned by analogy from the H-6 ^{1}H NMR signal.

This analysis suggested that the (7*S*)-**64a-e** (which corresponds to the secondary amide on the *exo* face) exhibited such hydrogen-bonding but not the *endo* (7*R*)-**64a-e** diastereomer. Intramolecular hydrogen-bonding is favoured by the *exo* amide when positions C-6 and C-7 are unsubstituted as in **64a-e**, probably due to the stereochemical constraints of the bicyclic ring. Nonetheless, hydrogen-bonding could be achieved by the *endo* amides when the epoxide is present at C-6 and C-7. It was noted that a stronger intramolecular hydrogen-bonding exists when the stereochemistry of the amide is *exo*. This was observed from the greater difference between the lactam ^{13}C resonance of **73** and **64a-e**, as compared to that between **79**, **76**, and **67b-e**. IR lactam stretching frequency of **64a-e** and **73** were not as indicative since the IR spectra were obtained with a mixture of diastereomers, leading to a broader IR stretching lactam carbonyl peak.

This intramolecular hydrogen-bonding may also occur, resulting in the lactam carbonyl (C-8) of **72** to resonate further downfield as compared to the carbonyl carbon of its carboxylic acid (C-9).

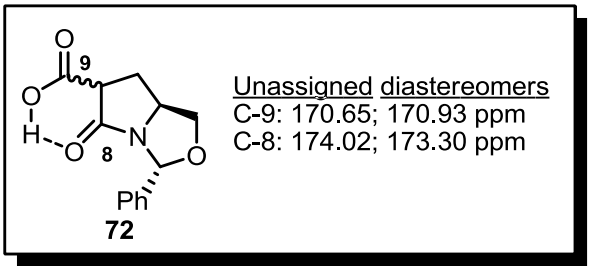
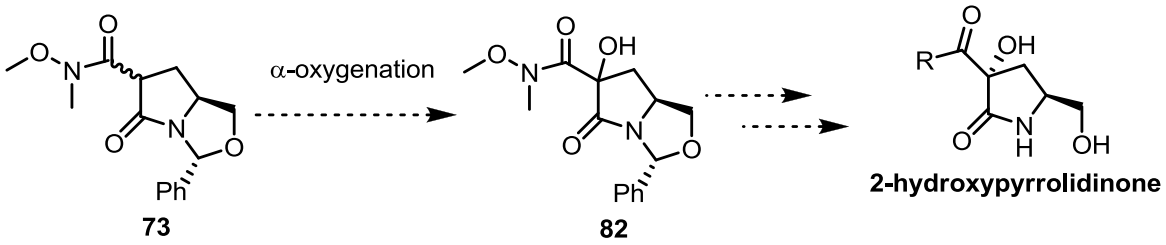


Figure 2.8. Lactam carbonyls of **72** resonate further downfield than its carboxylic acid carbonyls.

2.4 2-Hydroxypyrrolidinones

The second set of scaffold to be examined was the 2-hydroxypyrrolidinones. In the proposed synthesis to these compounds, α-oxygenation of the precursor **73** would be effected to yield the required tertiary alcohol stereoselectively.



Scheme 2.10. Proposed route towards 2-hydroxypyrrolidinones.

2.4.1 *O*-transfer reagents

Hydroxylation on bicyclic lactams has been reported by Hamada and co-workers[95] using an inorganic peroxy reagent MoOPH (MoO_5·Py·HMPA), also commonly known as Vedejs reagent,[96] or MoOPD (MoO_5·Py·DMPU). It was reported that LDA was the base that gave the best yield of the desired product and that hydroxylation generally gave *endo*-**83** as the major product (Table 2.10). This indicated that oxidant approached the lithium enolate from apparently the more concave face in a kinetically controlled process. The stereoelectronic "Cieplak effect" was proposed to account for this preferred endo-selectivity with small electrophiles due to the spatial orientation of the N lone pair (Figure 2.9).[97] It has been previously reported that electrophiles, except those that are small in size such as iodomethane

or a proton, generally approached the less hindered convex face of such bicyclic systems in a thermodynamically controlled process.[67,98,99] Interestingly, it was reported that the use of oxaziridines, as described by Davis and co-workers,[100] instead of the molybdenum complexes, gave no desired product.

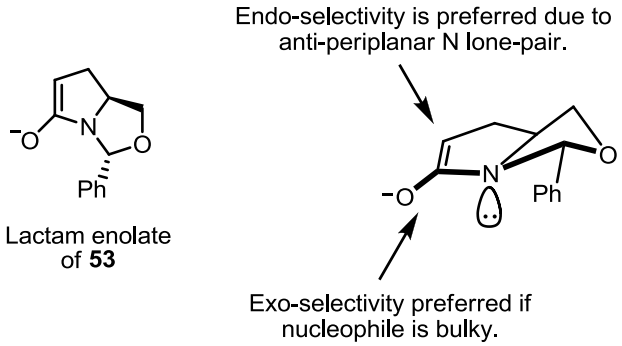
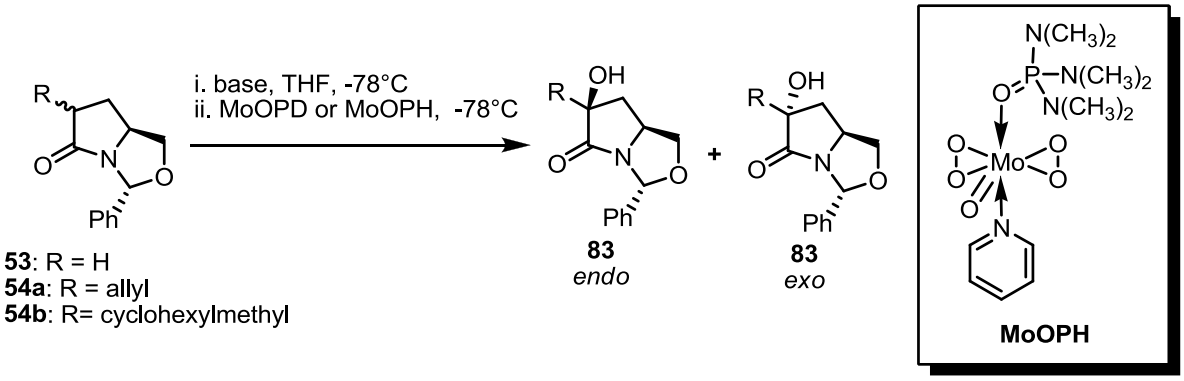


Figure 2.9. Stereoelectronic effects on diastereotopic selectivity of lactam enolate of **53**.

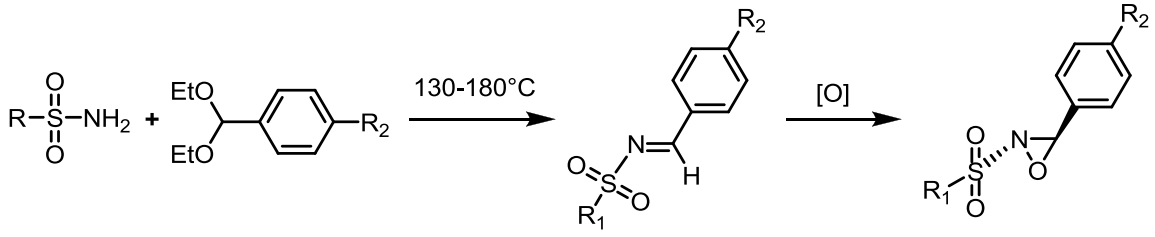
Table 2.10: α-Hydroxylation using Mo complexes.[95]



Entry	Compound	Base	Oxidising agent	Yield	endo:exo
1	**53**	LDA (1.5 eq.)	MoOPD (1.5 eq.)	78%	90:10
2	**53**	LDA (1.5 eq.)	MoOPH (1.5 eq.)	66%	92:8
3	**54a**	LDA (2 eq.)	MoOPD (2 eq.)	61%	100:0
4	**54b**	LDA (1.4 eq.)	MoOPD (2 eq.)	43%	92:8

The above utilised a substrate control for the asymmetric installation of the oxygen atom. In view of our need to obtain good stereoselectivity for the key α-hydroxylation step, the use of a bulky *O*-transfer reagent could further complement the use of the chiral bicyclic template, in a matched process. Davis and co-workers reported a class of oxaziridines which could be used

as an *O*-transfer reagent, in which aromatic diethyl acetals were heated with sulfonamides to give the corresponding sulfonimines, which were then oxidised with *m*-CPBA or oxone to give the racemic oxaziridines (Scheme 2.11).[100,101]



Scheme 2.11. Synthesis of Davis' oxaziridines.

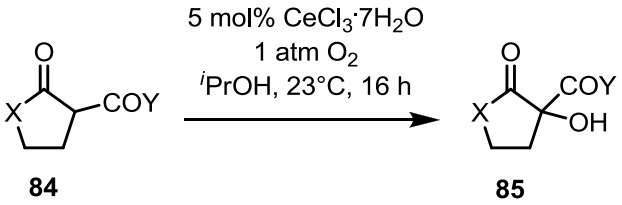
α-Hydroxylation of carbonyl compounds could be achieved via the nucleophilic attack of the respective enolates onto the oxaziridine followed by N-O bond cleavage, with subsequent generation of the sulfonimine as a by-product. Davis and co-workers reported that low reaction temperatures were crucial for the α-hydroxylation as that minimised the formation of a by-product between the enolate and the sulfonimine generated during the course of the reaction. The use of KHMDS as the base to generate the enolate was reported to give the highest product yields when used to generate the enolate. In addition, no over-oxidation of α-dicarbonyl by-product was detected, unlike when Vedejs' reagent was used.[102]

Dimethyloxirane (DMDO), an organic peroxy reagent, is also known to be a useful oxidising agent under mild reaction conditions. Smerz and Adam reported the application of DMDO towards the α-hydroxylation of 1,3-dicarbonyl compounds. The reaction, although mild, was slow and required the use of a Ni(II) catalyst (0.1 eq.). The order of reactivity of the 1,3-dicarbonyls was strongly dependent on the substitution pattern with diketones being the most reactive, followed by the keto esters and diesters; cyclic substrates were also more reactive than the corresponding acyclic ones.[103] Although the reaction conditions potentially produced acetone as the only by-product, this reagent may not be suitable for application on our

diamide **73** due to the potential low reactivity, as well as the possibility of trace amounts of the toxic Ni(II) present in the purified product which may confound the results from biological assays later on.

Alternative α-hydroxylation conditions include a report by Christoffers and co-workers[104] whereby the use of catalytic $CeCl_3 \cdot 7H_2O$ with molecular oxygen was used as the oxidant for the α-hydroxylation of a series of cyclic and acyclic β-dicarbonyl compounds (Table 2.11).

Table 2.11: Cerium(III) catalysed α-hydroxylation described by Christoffers *et al.* [104]



84 **85**

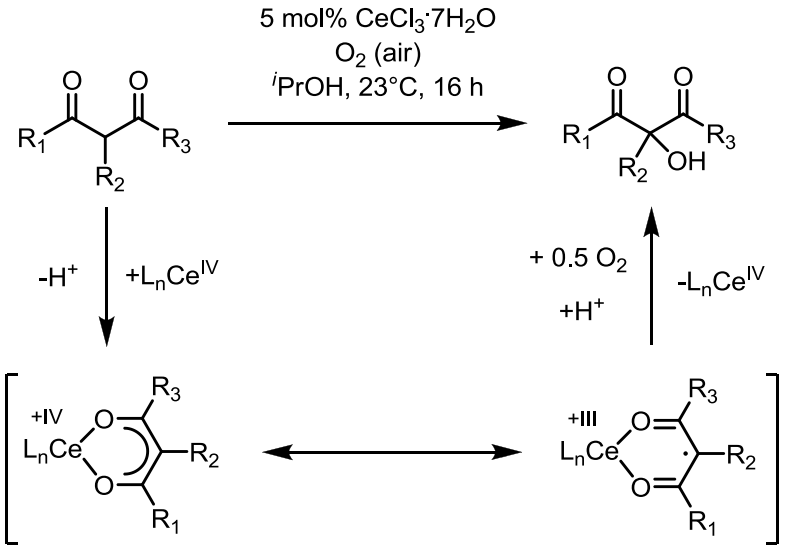
Compound	X	Y	Yield
84, **85**	CH_2	OEt	99%
	CH_2	O(L-menthyl)	99%
	NBn	Me	72%
	O	Me	78%

The cyclic substrates were hydroxylated in good yields while the acyclic β-dicarbonyl compounds (1,3-ketoesters) were hydroxylated in poorer yields of 13-45%. In addition to the lower yields obtained, a significant amount of the α-chlorinated by-product was isolated for some of the acyclic substrates.

Although it was observed that a reaction mixture containing high loadings of halogen counterions may result in the formation of α-halogenated by-product, a subsequent ^{18}O-labelling study established that the hydroxyl group installed originated from dioxygen and not from the capture of water present in the reaction mixture by β-dicarbonyl radical. Oxygen

uptake measurements performed by gravimetric and volumetric analysis revealed that 1 eq. of the β-dicarbonyl compound reacted with 0.5 eq. of dioxygen.[105]

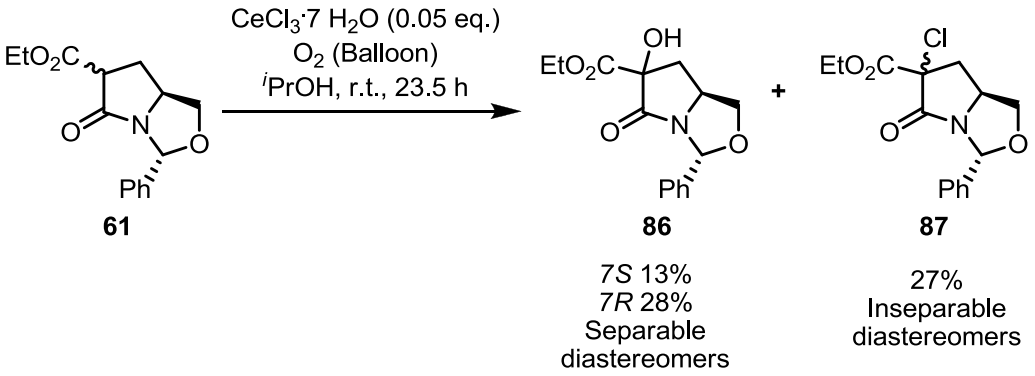
Thus, the proposed mechanism (Scheme 2.12) was suggested to involve the formation of a radical intermediate via a single electron transfer from the β-dicarbonyl ligand to the cerium metal centre. The β-dicarbonyl then forms a peroxy species with dioxygen which reoxidises two equivalents of Ce(III) to Ce(IV), while subsequently forming two equivalents of the hydroxylated compound.



Scheme 2.12. Oxidation mechanism proposed by Christoffers and co-workers.[104]

2.4.2 Synthesis of derivatives

An initial test reaction was carried out with lactam **61** using the procedure described by Christoffers and co-workers.[104]



Scheme 2.13. Attempt towards α-oxygenation of **61**.

The two diastereomers of the hydroxyl product **86** were isolated separately with a yield of 13% (7*S*) and 28% (7*R*). The stereochemistry of the hydroxyl-ester (7*R*)-**86** was determined via nOeSY (Figure 2.10), and the (7*S*)-**86** isomer was assigned via the analogous chemical shifts pattern of the H-6 protons known to be distinctive for such scaffolds (*vide supra*).

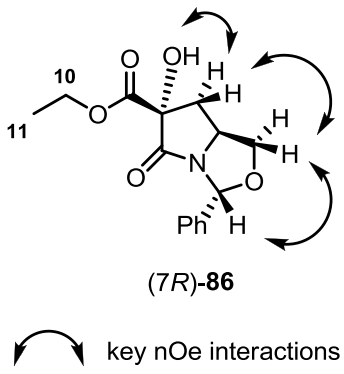
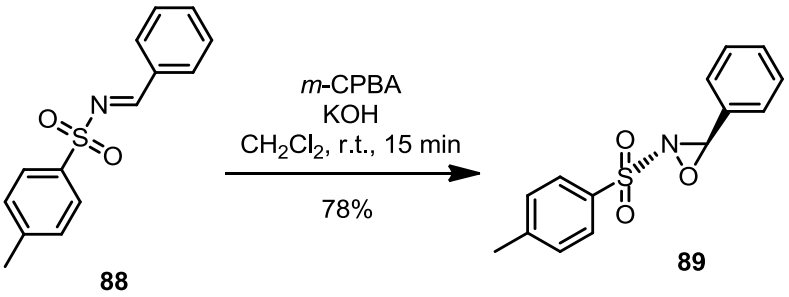


Figure 2.10. Key nOe interactions of (7*R*)-**86**.

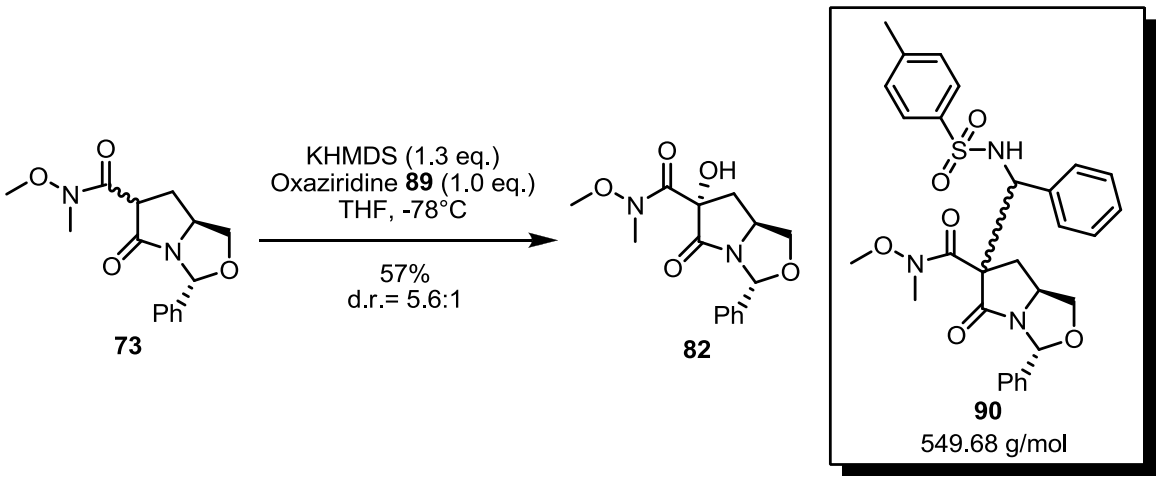
In addition, α-chlorinated **87** was isolated as a mixture of inseparable diastereomers. Given the low isolated yield of the desired product **86**, an alternative approach was required.

Oxaziridine **89** was chosen for use in the α-hydroxylation reaction as this racemic oxaziridine could be easily synthesised using a procedure reported by Ruano *et al.*[106] from commercially available (*E*)-*N*-benzylidene-4-methylbenzensulfonamide **88** (Scheme 2.14).



Scheme 2.14. Synthesis of oxaziridine **89**.

With oxaziridine **89** in hand, the α-hydroxylation reaction was attempted on Weinreb amide substrate **73** (Scheme 2.15). Although successfully formed, the purification of **82** proved to be challenging as there was a side product (with a complex ^{1}H NMR spectrum) that has a retention factor that was very close to product **82**. Analysis of this mixture by LRMS revealed peaks at *m/z* 550 and 572, which could possibly correspond to the $[M+H]^+$ and $[M+Na]^+$ ions of the addition by-product **90** respectively. The complex ^{1}H NMR spectrum further suggested that a diastereomeric mixture of **90** was obtained. The diastereomeric ratio of **82** (as determined from its ^{1}H NMR spectrum, based on integration of the corresponding H-2 resonances) was dependent on the purification process as it was observed that the earlier collected fractions of pure **82** contained more of the 7*R* (*exo* -OH) diastereomer while the later fractions contained more of the 7*S* (*endo* -OH) diastereomer.

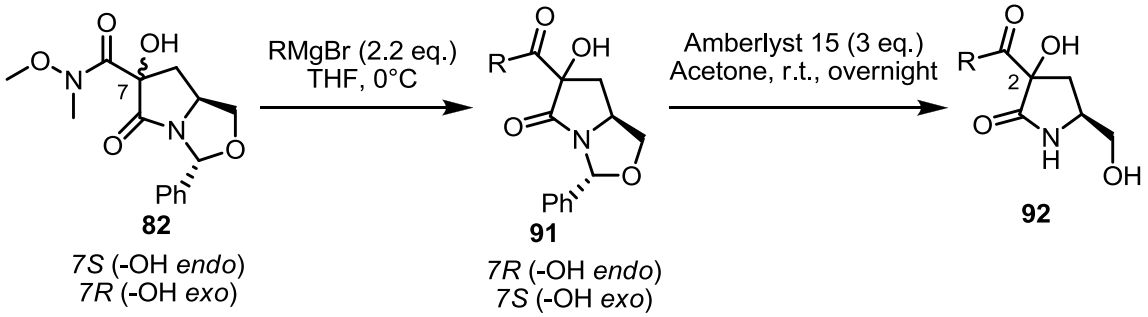


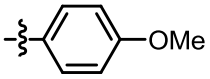
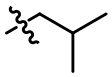
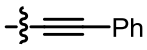
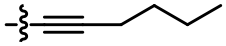
Scheme 2.15. α-oxygenation of **74**.

To obtain the desired 2-hydroxypyrrolidinones, Weinreb amide **82** was subjected to a similar sequence of Grignard reaction followed by oxazolidine hydrolysis.

Amide **82** with the unprotected tertiary alcohol was reacted with 2.2 eq. Grignard reagent to give the respective ketones **91a-g** in variable yields. As shown in Table 2.12, it was found that the efficiency of the conversion of **82** with the Grignard reagents is dependent on its stereochemistry at C-7. The conversion of (7*S*)-**82** (note that the *R,S* assignment changes due to the priority rule upon conversion to the ketone; the yields in parentheses are based on the corresponding diastereomer of starting material **82**) to the ketone was found to be lesser than (7*R*)-**82**.

Table 2.12: Synthesis of 2-hydroxypyrrolidinones **92**.



	d.r. of **82** used (-OH *exo*:*endo*)	**91**, Yield	**92**, Yield
a R = 	5.9:1	*7R* 9% (62%) *7S* 47% (55%)	- 83%
b R = 	3.3:1	*7R* 17% (74%) *7S* 35% (46%)	- 89%
c R= 	5.6:1	*7R* 7% (46%) *7S* 24% (28%)	- 97%
d R= 	12.5:1	*7R* 3% (41%) *7S* 35% (38%)	- 91%
e R= 	5.6:1	*7R* <4% (<26%)* *7S* 6% (7%)	- 90%
f R= 	3:1	*7R* 22% (89%) *7S* 49% (65%)	- 85%
g R= 	3:1	*7R* 16% (65%) *7S* 50% (66%)	- 93%

*Isolated with impurities.

Both diastereomers of **91a-g** could be separated during purification via flash column chromatography, and their stereochemistry were determined via nOeSY (Figure 2.11). As with this class of bicyclic pyroglutamates, the chemical shift difference between the two H-6 protons are significantly different between the two diastereomers. When the tertiary hydroxyl group is at the *endo* position of the bicyclic system, the chemical shift difference between the two H-6 protons is significantly greater than the chemical shift difference between the two H-6 protons when the hydroxyl group is at the *exo* position.

It is interesting to note that hydrolysis of oxazolidine (7*R*)-**91a** with Amberlyst 15 (3 eq.) at r.t. overnight gave no conversion and starting material was recovered quantitatively, while

hydrolysis under standard conditions with excess TFA in THF/H_2O gave a complex crude mixture. In contrast, (7*S*)-**91a-g** underwent hydrolysis with Amberlyst 15 smoothly to give high yields of (2*S*)-**92a-g**. Alternative hydrolysis conditions were employed (7*S*)-**91f-g** (TFA 20 eq., CH_2Cl_2, r.t., 2 h) and good yields of (2*S*)-**92f-g** were obtained.

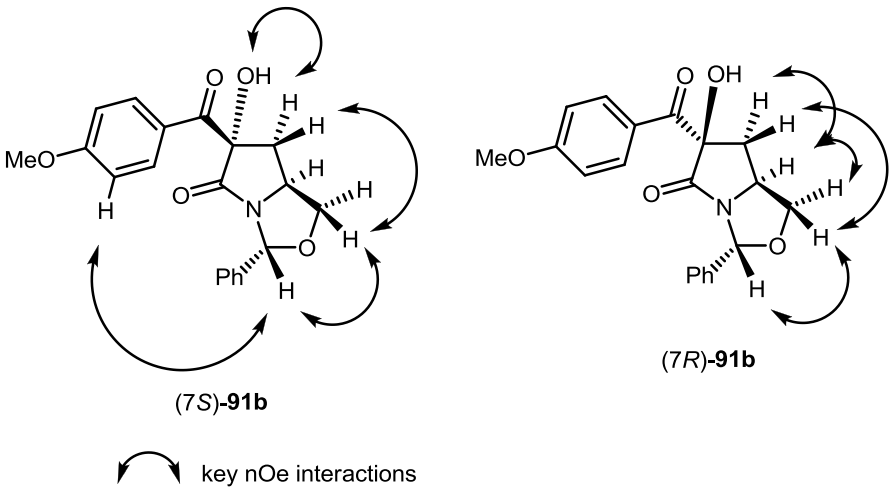


Figure 2.11. Key nOe interactions of separated diastereomers of **91b**.

There is an apparent trend that conversion of (7*S*)-**82** is the highest with alkynyl Grignard reagents, followed aryl and alkyl Grignard reagents, with the bulky isobutyl Grignard reagent having the lowest conversion, suggesting that the difference in steric requirements in the vicinity of the Weinreb amide are important. As shown in Figure 2.12, (7*S*)-**82** with the Weinreb moiety in the *endo* face is less accessible to nucleophilic attack by Grignard reagents, thus a less bulky alkynyl Grignard reagent is more favoured in the reaction while the bulky isobutyl Grignard reagent is less favoured.

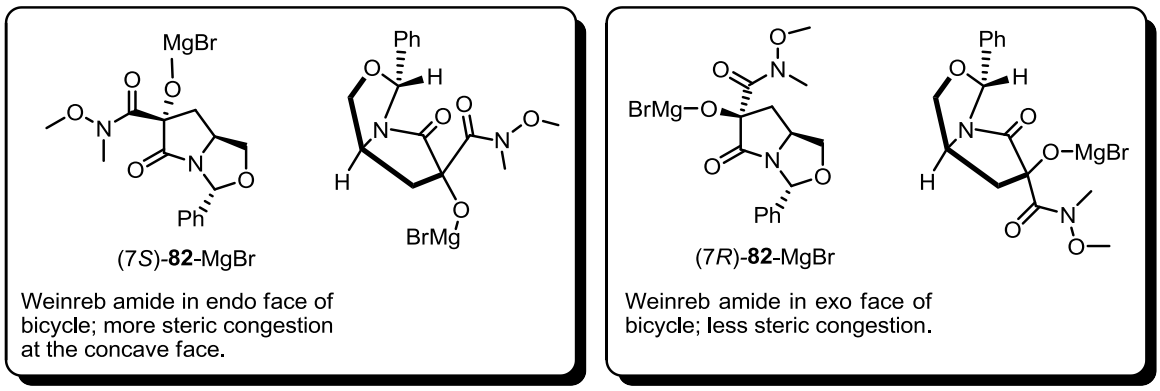
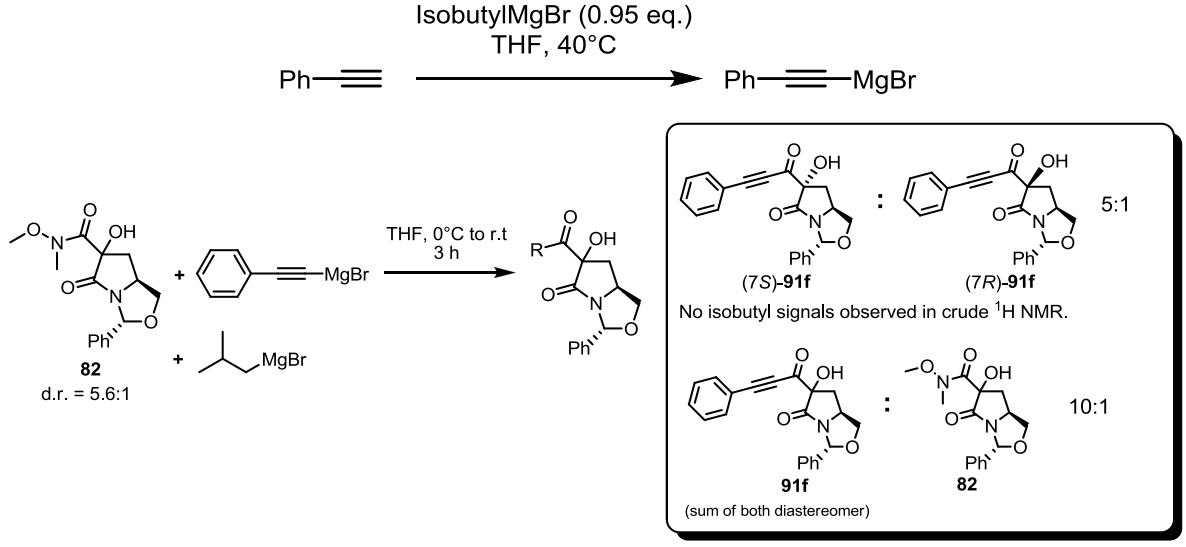


Figure 2.12. Possible explanation for the different conversion of **82** diastereomers.

A competition reaction of **82** using phenylethnyl magnesium bromide and isobutyl magnesium bromide was conducted (Scheme 2.16). The phenylethnyl magnesium bromide was freshly prepared from isobutyl magnesium bromide. Both Grignard reagents (2.2 eq.) were added simultaneously to **82**. Upon work-up, the crude ^{1}H NMR spectrum showed no isobutyl signals. Furthermore, comparing the H-2 signals, the ratio of (7*S*)-**91f** (*exo*-OH) to (7*R*)-**91f** (*endo*-OH) was 5:1, while the ratio of ynone **91f** to **82** was 10:1, suggesting good conversion to the ketone product. Since only ynone **91f** was observed in the ^{1}H NMR spectrum of the crude reaction mixture, it supports the reasoning that bulky Grignard reagents reacted slower with Weinreb amide **82**.



Scheme 2.16. Competition reaction of **82** between two different Grignard reagents.

This reasoning could also be substantiated from the X-ray crystal structure of an analogous compound (**96**, see Figure 2.15, *vide infra*), suggesting that a Bürgi-Dunitz trajectory towards the carbonyl carbon of Weinreb amide (7*S*)-**82** is sterically hindered due to the concave *endo*-face of the bicyclic ring.

2.4.3 Attempted synthesis of pyrrolidinones via reductive epoxide opening

Potentially, hydroxypyrrolidinones **92a-g** could be obtained from epoxypyrrolidinones **78a-g**. However, the stability of the epoxy group towards nucleophilic Grignard reagents suggested that an alternative to S_N2-type ring opening of the epoxide might be required for successful reduction.

There are literature precedents on palladium-catalysed reductive epoxide opening of α,β-epoxy carbonyl compounds with various hydride sources, and a proposed mechanism for the reaction is as shown in Figure 2.13. Depending on the type of Pd catalyst used, a deoxygenated by-product could be obtained as well.[107,108]

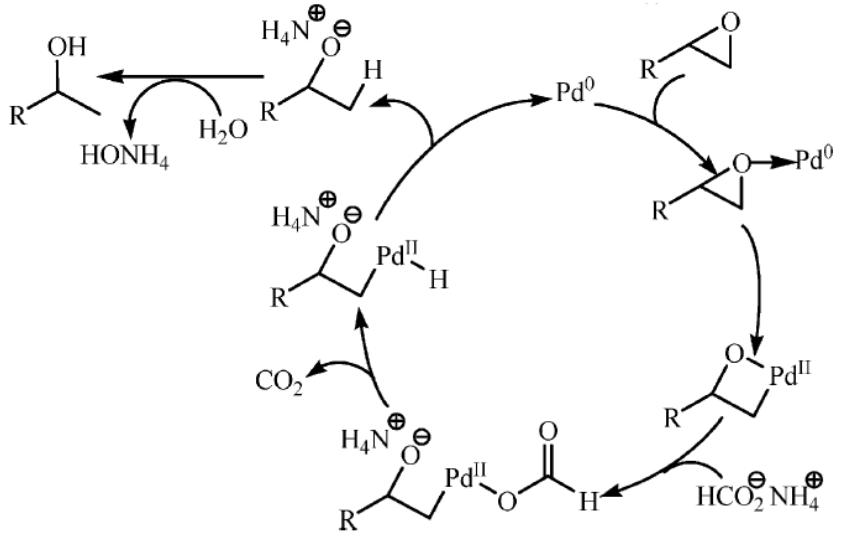
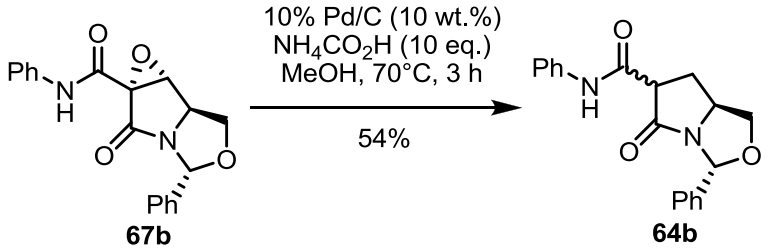


Figure 2.13. Proposed mechanism for reduction of epoxides by ammonium formate. Taken from E. Thiery, J. Le Bras, J. Muzart *Eur. J. Org. Chem.* **2009**, 961-985.

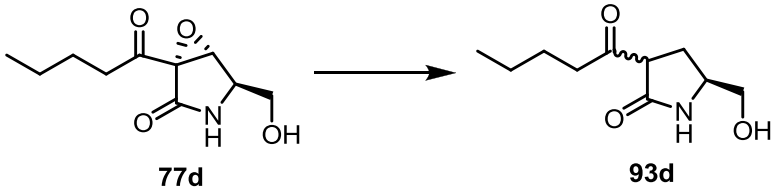
However, an attempt to react **67b** with excess ammonium formate as the hydride source (Scheme 2.17) did not yield any epoxide-opening product, but the deoxygenated **64b** was isolated in 54% yield instead.



Scheme 2.17. Deoxygenation of **67b** observed during attempted epoxide opening.

Further investigation with Rosemund catalyst ($Pd/BaSO_4$) using **77d** gave **93d** in 66% yield, while the conversion was slower with Lindlar's catalyst ($Pd/CaCO_3$ poisoned with Pb). In both cases, no desired epoxide-opening product was observed but the deoxygenated product was obtained instead.

Table 2.13: Deoxygenation of **77d** during attempted epoxide opening.



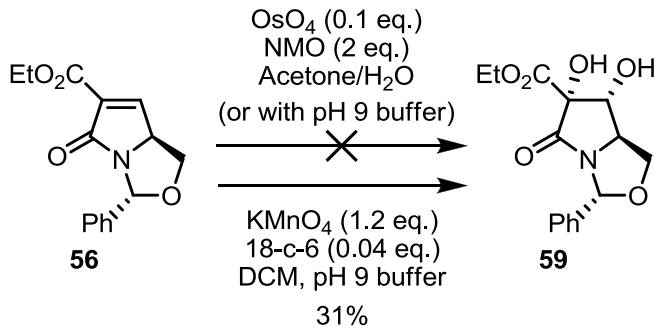
Entry	Condition	Remarks
1	5% $Pd/BaSO_4$ (10 wt%) H_2 (balloon), EtOH, r.t., 2 h	66% of **93d**.
2	5% $Pd/CaCO_3$ poisoned with Pb (10 wt%) Quinoline (1 μl), H_2 (balloon), EtOH, r.t., 2 h	**77d**:**93d** = 1:0.2 by ^1H NMR spectroscopy.

Should the epoxide ring-opening be successful under reductive conditions, it would have provided a shorter route towards hydroxypyrrolidinones. Nonetheless, the route utilising oxaziridine **89** described above (Scheme 2.15 and Table 2.12) was able to provide sufficient amounts of the desired compounds with good diastereopurity.

2.5 2,3-Dihydroxypyrrolidinones

2.5.1 Dihydroxylation

The required diol moiety in 2,3-dihydroxypyrrolidinones should be accessible from the α,β-unsaturated Weinreb amide **75**. Dihydroxylation on a similar bicyclic unsaturated substrate **56** has been reported by Moloney and co-workers (Scheme 2.18).[74,109] They found that the use of catalytic OsO_4 with NMO as the oxidant failed to give the desired diol **59**, but when $KMnO_4$ was employed in conjunction with 18-crown-6 ether the diol was obtained in 31% yield. However, this reported procedure entailed tedious work-up methods.



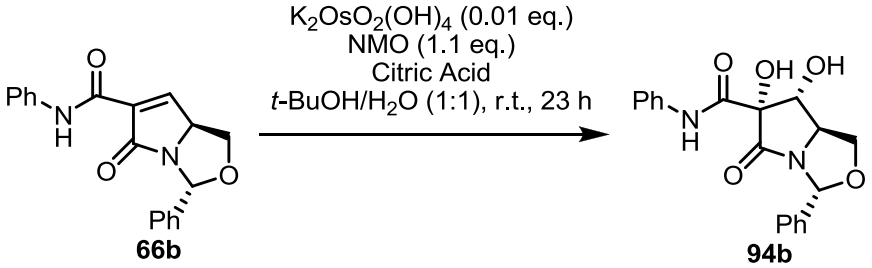
Scheme 2.18. Dihydroxylation of unsaturated lactam **56** reported by Moloney *et al*. [74, 109]

Sharpless and co-workers[110] described a modification to "standard" dihydroxylation conditions that is compatible both with electron-deficient alkenes such as α,β-unsaturated amides as well as tri-substituted alkenes. Citric acid was used as an additive and served a dual function – to provide the optimal pH in the reaction mixture by neutralising the 4-methyl-morpholine generated from NMO, as well as acting as a ligand for the osmium metal to prevent undesirable disproportionation of the active Os(VI) to Os(IV).

Dihydroxylation of **66b** under similar conditions to that reported above was attempted. Although Sharpless and co-workers reported that 2.0 eq. of citric acid was required for dihydroxylation of acyclic α,β-unsaturated amides while 0.75 eq. of citric acid was sufficient for a tertiary amide, the use of 2.0 eq. of citric acid for the dihydroxylation of **66b** gave

products with complicated ^{1}H NMR spectra. Conversion of **66b** with 1.0 eq. of citric acid was slow and the isolation of **94b** proved to be difficult due to the presence of multiple minor unidentified by-products (Table 2.14).

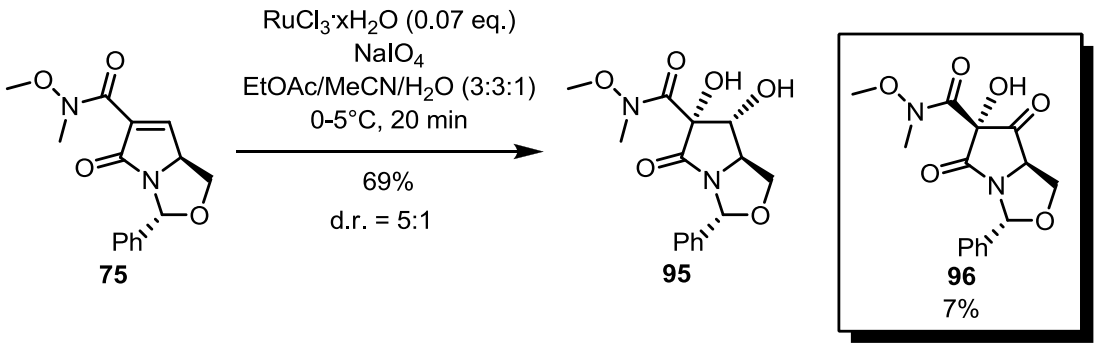
Table 2.14: Attempted Os-catalysed dihydroxylation.



66b **94b**

Entry	Eq. of Citric acid	Remarks
1	2.0	No **66b** present after 23 h. Isolated products have complex ^{1}H NMR spectrum.
2	1.0	Crude NMR spectrum shows mainly **67b**. 21% of **94b** as a single diastereomer with impurities.

Shing and co-workers[111] reported a ruthenium-mediated "flash dihydroxylation" procedure – named this way as simple substrates were dihydroxylated within 30 seconds, while a range of electron poor or hindered substrates were dihydroxylated within minutes. Gratifyingly, the Ru-mediated dihydroxylation worked well when applied to the intermediate **75**, giving the desired diol with an isolated yield of 69%, and a d.r. about the diol of 5:1 as determined from the ^{1}H NMR spectrum (based on the corresponding H-2 resonances). A nOeSY experiment was conducted on the diastereomeric mixture of **95**, and the nOe interactions observed for the major diastereomer suggested that the *exo*-diol was formed. This was in agreement with the stereoselectivity observed with such bicyclic [3.3.0] scaffolds.[70-75]



Scheme 2.19. Successful Ru-catalysed dihydroxylation of **75**.

A single diastereomer of tetramic acid **96** was also isolated during the synthesis of diol **95**, presumably arising from over-oxidation of **95**.[111] The Weinreb amide moiety was determined to be in the *endo* face of the bicycle from its X-ray crystal structure, and nOe correlations were observed between the lactam -OMe group with H-2 and H-4$_{endo}$.

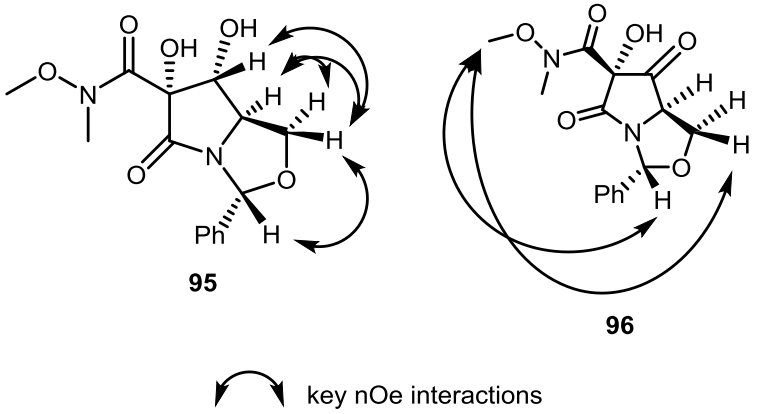


Figure 2.14. Key nOe interactions of the major diastereomer of **95**, and single diastereomer of **96**.

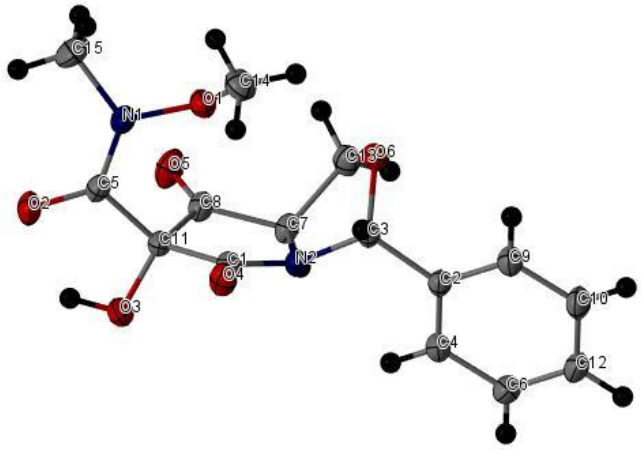
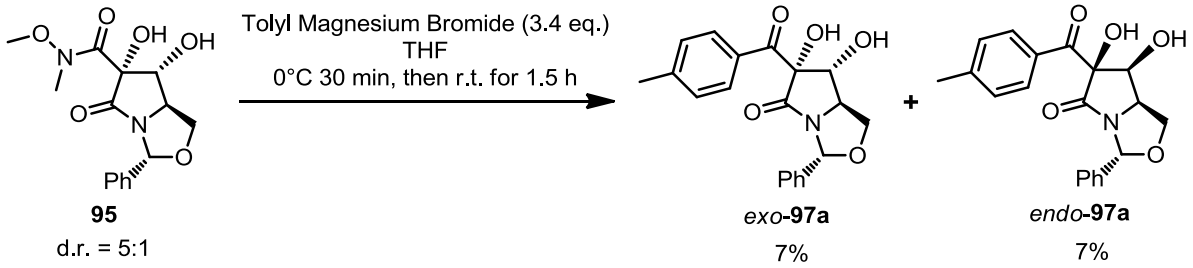


Figure 2.15. X-ray crystal structure of **96**.

2.5.2 Choice of diol protecting group

When the Grignard reaction was attempted with the unprotected diol **95**, a very low conversion of the desired product was obtained. In addition, very similar retention factor of the two diastereomers of **97a** made purification via flash column chromatography difficult (Scheme 2.20).



Scheme 2.20. Grignard reaction of unprotetcted diol **95**.

The stereochemistry of the respective diastereomers of **97a** was assigned by nOeSY experiment.

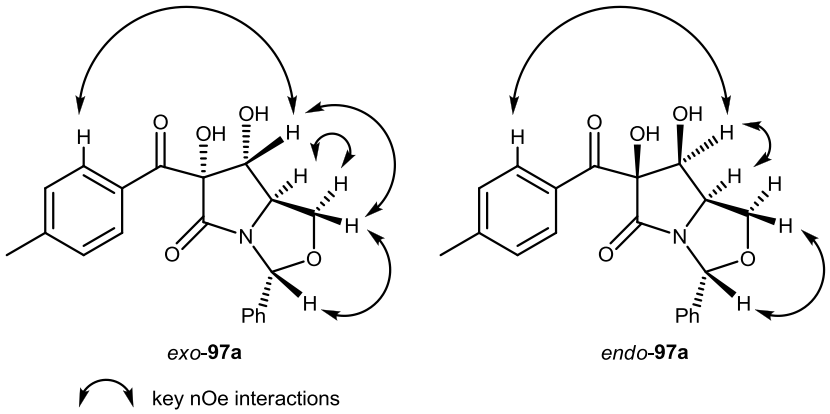
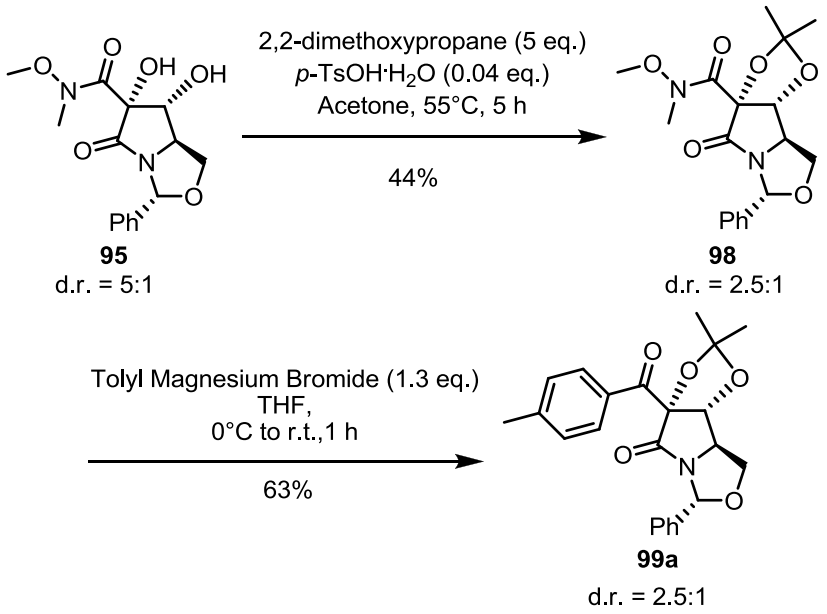


Figure 2.16. Key nOe interactions of **97a** diastereomers.

An acetonide was initially chosen as the protecting group for the diol **95** to improve the chemical yield, as it is achiral and would not add another stereogenic centre to the intermediates. Protection of the diol gave acetonide **98** in 44% yield, which when followed by

Grignard reaction of the Weinreb amide **98** gave the ketone **99a** in 63% yield as a mixture of diastereomers (*exo* diol: *endo* diol 1:0.4).

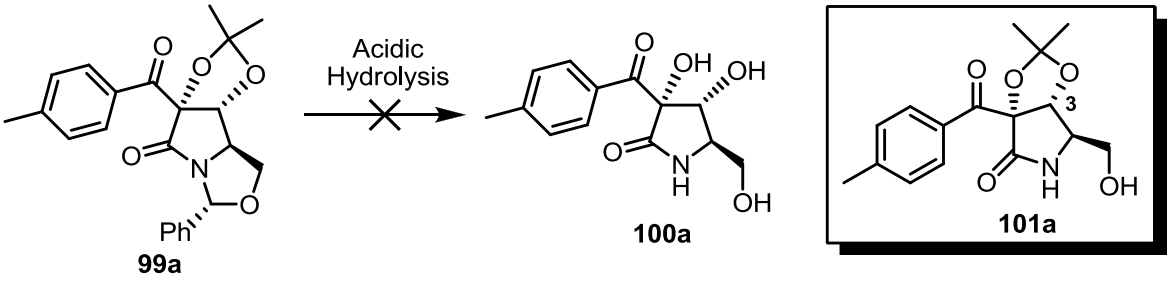


Scheme 2.21. Grignard reaction on acetonide protected diol **98**.

Although a single spot was observed from the TLC analysis of **99a**, its ^{1}H NMR spectrum contained two sets of H-6 signals, with some of the other signals being broad. A variable temperature ^{1}H and ^{13}C NMR experiment at 65°C (338K) improved the resolution of the broad peaks, but the ratio of these two forms remained essentially unchanged, suggesting their identity as diastereomers and not rotamers.

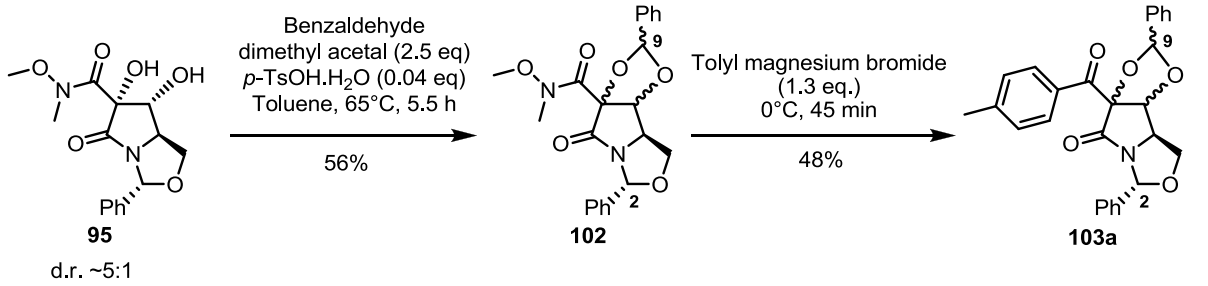
However, various acidic hydrolysis conditions failed to simultaneously deprotect both the oxazolidine and the acetonide to give the desired 2,3-dihydroxypyrrolidinone **100a**. The use of strong acidic conditions hydrolysed the oxazolidine ring but not the acetonide, and one diastereomer of **101a** was obtained in 25% yield.

Table 2.15: Atttempted hydrolysis of acetonide.



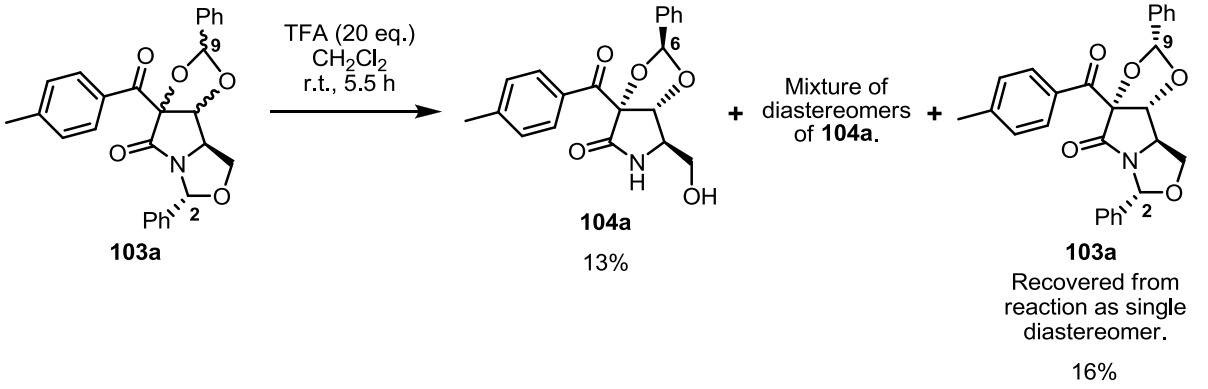
Entry	Condition	Remarks
1	Amberlyst 15 (5 eq.) Acetone/H_2O, r.t., 18 h	No conversion of **99a**, recovered from reaction.
2	TFA (10 eq.) Acetone/H_2O, r.t., 20 h	34% of **101a**. (d.r. 3.3:1, about diol). Recovered starting material.
3	TFA (10 eq.) THF/H_2O, 50°C., 5 h	TLC analysis shows mainly unreacted **99a** with minimal **101a**.
4	2 M aq. HCl (4 eq.) MeOH, r.t., 6 h	25% of **101a** (one diastereomer).

Given this problem, other more acid-labile diol protecting groups were investigated. When a phenylbenzylidene protecting group was investigated (Scheme 2.22), purification of **102** proved to be difficult as some unidentified by-products with similar retention factors to the desired product were observed. In addition, up to four diastereomers could be present due to the presence of a new stereogenic centre at the benzylidene C-9 further complicating the analysis. The material was nonetheless brought forward to ketone **103a**. As characterisation of **102** and **103a** proved to be difficult, the presence of the desired functional group introduced for each step was used to indicate the successful conversion achieved. For example, a new set of signals corresponding to H-9 was seen for **102**; a new tolyl signal was present for **103a**, as well as the presence of a ketone carbonyl group in the ^{13}C NMR spectrum. LRMS, HRMS and IR analysis also confirmed the presence of these relevant functional groups.



Scheme 2.22. Grignard reaction on phenylbenzylidene protected diol **102**.

Unfortunately, on attempted hydrolysis with TFA, only the oxazolidine was hydrolysed but not the phenylbenzylidene acetal (Scheme 2.23). After purification, **104a** and unreacted **103a** were isolated as a single diastereomer, each in 13% and 16% respectively.



Scheme 2.23. Attempted hydrolysis of phenylbenzylidene protecting group.

The relative stereochemistry of recovered **103a** was readily determined via nOeSY (Figure 2.17). For **104a**, nOeSY gave correlations between H6-NH, H6-H4, H3-H4 but the data was insufficient to assist with the stereochemical assignment, in particular the stereochemistry of the protected diol relative to the pyrrolidinone.

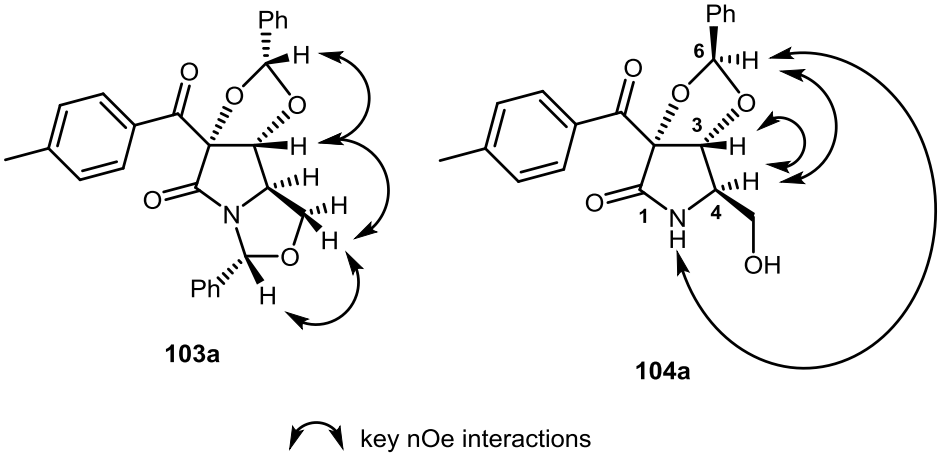
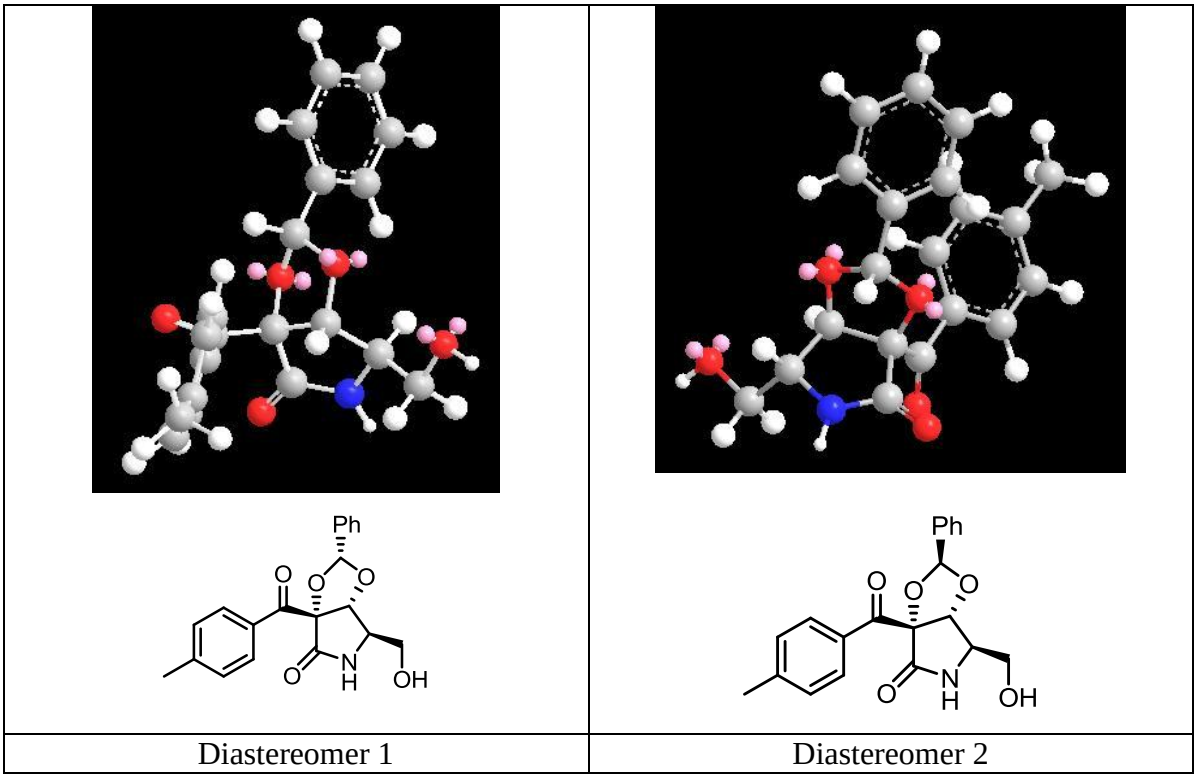


Figure 2.17. Key nOe interactions of **103a** and **104a**.

In order to determine the stereochemistry of **104a**, the most stable conformer of all four possible diastereomers of **104a** were generated (using MM2-minimised molecular model with ChemBio3D Ultra version 11). The nOe correlations observed between H6-NH, H6-H4 proved to be crucial as the inter-atomic distances of the calculated models suggest that only one diastereomer (Diastereomer 2, Table 2.16) could have the H-6 oriented in close proximity to -NH- and H-4. Thus, the stereochemistry of the acetal relative to the pyrrolidinone, and C-6 were assigned to be that as shown in Figure 2.17.



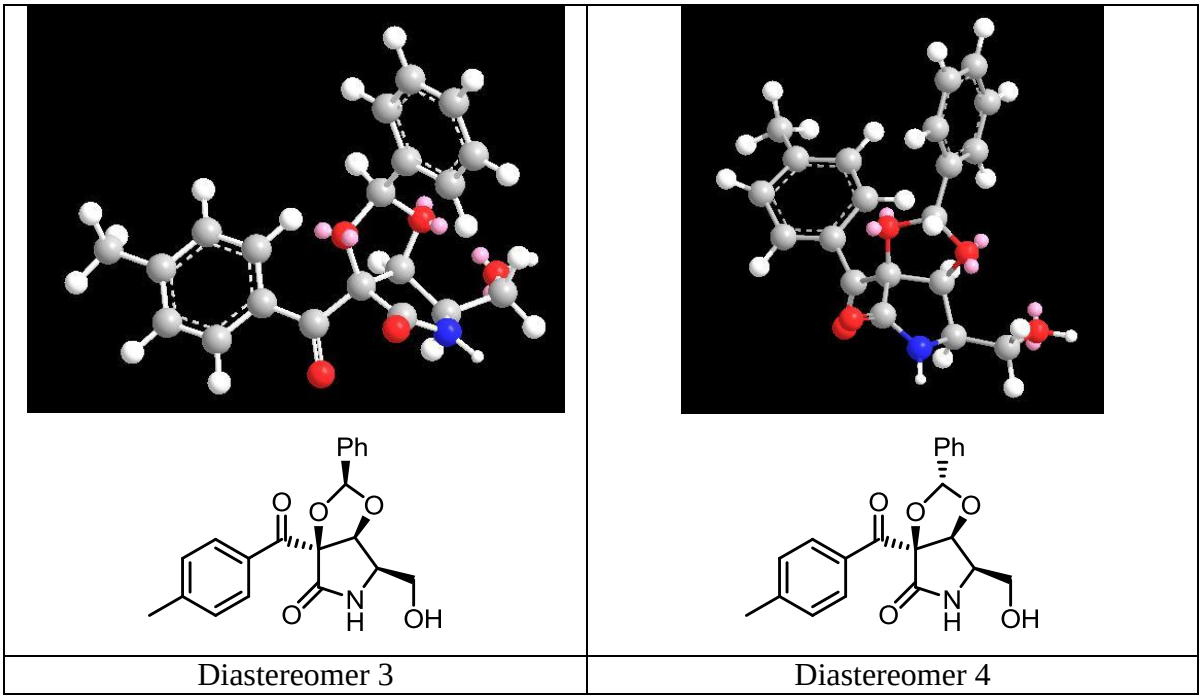
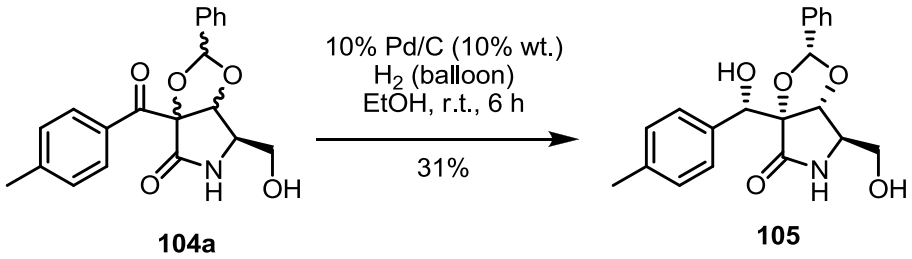


Figure 2.18. MM2-minimised molecular models of the four possible diastereomers of **104a** using ChemBio3D Ultra v.11.

Table 2.16: Determining the stereochemistry of **104a** from calculated inter-atomic distances of the generated molecular model.

	Calculated Dist. Between H-6 to -NH- (Å)	Calculated Dist. Between H-6 to H-4 (Å)
Diastereomer 1	5.7	4.3
Diastereomer 2	3.7	2.8
Diastereomer 3	5.6	5.0
Diastereomer 4	4.3	4.9

Given the stability of the phenylbenzylidene acetal towards Bronsted acid, deprotection under reductive conditions was investigated.



104a **105**

Scheme 2.24. Attempted hydrogenolysis of phenylbenzylidene protecting group.

When a mixture of diastereomers of **104a** was treated with hydrogen gas in the presence of Pd/C catalyst, 31% of pyroglutaminol **105** was isolated as a single diastereomer. The stereochemistry of the protected diol, benzylidene and newly formed hydroxyl group were assigned using nOe correlations (Figure 2.19). The stereoselective reduction of the ketone was reasoned with the aid of a MM2-minimised molecular model of **104a**. The molecular model suggests that one face of the ketone carbonyl is less hindered than the other – the face away from the acetal is less hindered, thus allowing a higher probability of hydride delivery from the Pd catalyst surface to give the alcohol **105** (Figure 2.19).

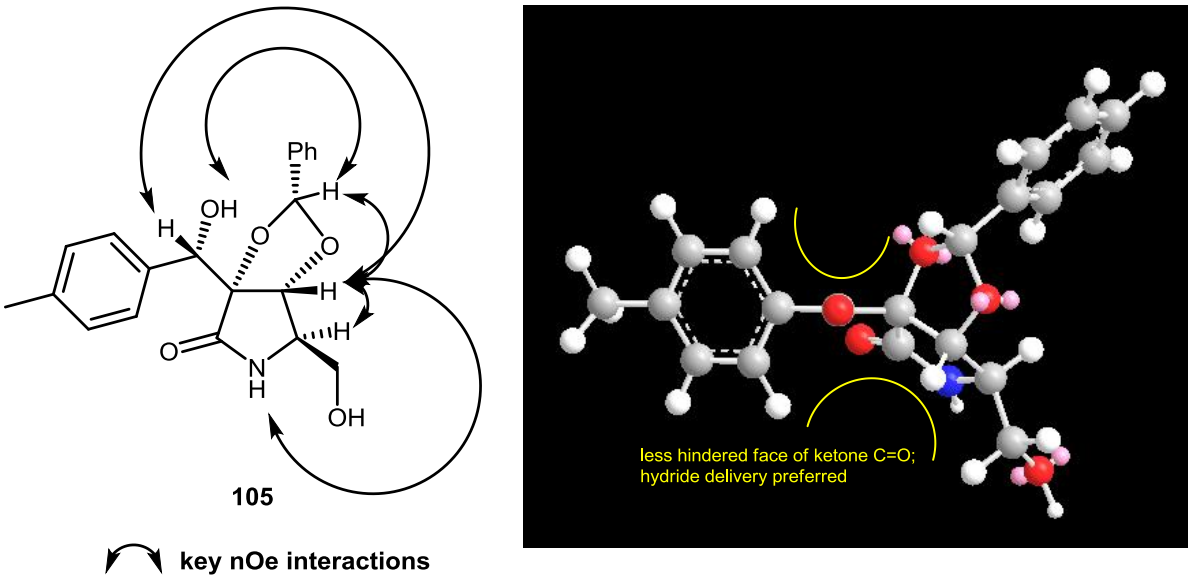
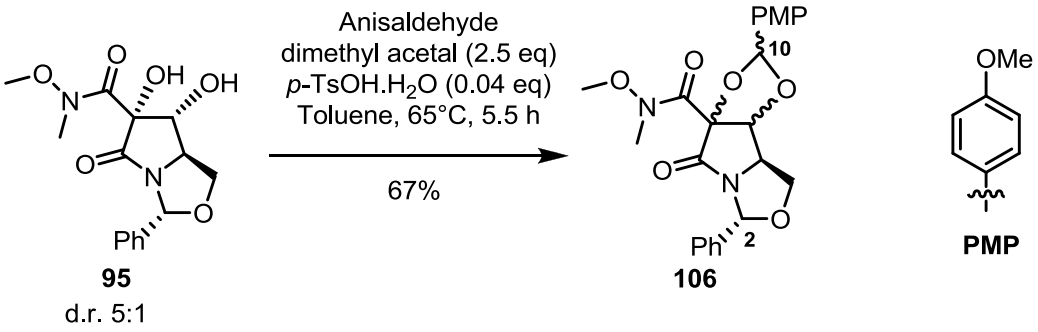


Figure 2.19. Key nOe interactions of **105**; MM2 minimised molecular model of ketone **104a** to reason the observed stereoselectivity in ketone reduction.

These results also seems to suggest that an acetal formed by the diol of such a 1,1-dicarbonyl-α,β-dihydroxy system might be more stable to acidic hydrolysis than expected. In view of the difficulties encountered with the deprotection of the acetal functionality, the more acid-labile *p*-methoxyphenyl (PMP) benzylidene diol protecting group[112] was sought and is described in the next section.

2.5.3 Synthesis of oxygenated derivatives

Protection of the diol with anisaldehyde dimethyl acetal gave **106** in 67% yield.



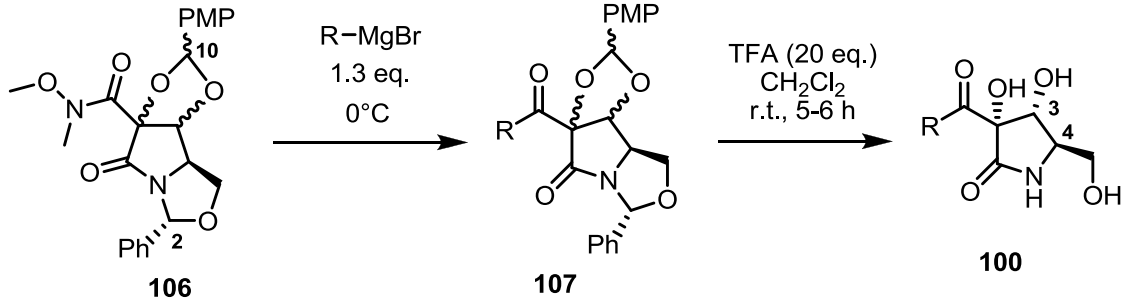
Scheme 2.25. Synthesis of *p*-methoxyphenyl (PMP) benzylidene protected diol **106**.

As four diastereomers of **106** could be present, assignment of the ^{1}H and ^{13}C NMR signals to the individual diastereomers proved difficult. However, the characteristic signals of **106** were clearly observed (i.e. ~6 ppm for H-2; ~5.7 ppm for H-10; ~3.3-3.8 ppm for -NMe, -OMe and Ar-OMe), together with the desired mass peaks from LRMS and HRMS analysis.

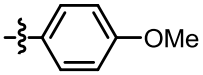
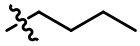
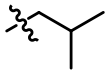
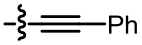
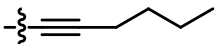
When **106** was reacted with the respective Grignard reagent, followed by treatment with TFA/CH_2Cl_2, global deprotection gave the desired 2,3-dihydroxypyrrolidinones **100a-g** in good yields (Table 2.17). However, a similar problem to that described earlier was encountered in that characterisation via NMR spectroscopy was difficult due to the complexity of the spectra for most derivatives of **107a-g** even though they were isolated as a single fraction during purification via flash column chromatography.

The modest yield obtained for these Grignard reactions may again be attributed to the steric bulk in the vicinity of the Weinreb amide due to the PMP benzylidene acetal. Some of the 2,3-dihydroxypyrrolidinones derivatives were isolated as a mixture of diastereomers (about the diol), and it was observed that the minor diastereomer has a larger J_{H3-H4} value and that the H-3 signal resonated further downfield as compared to the major diastereomer. As some of the derivatives were isolated as a pure diastereomer, it was reasoned that the major diastereomer obtained has the same stereochemistry as the pure *exo* diol diastereomer.

Table 2.17: Synthesis of 2,3-dihydroxypyrrolidinones **100**.

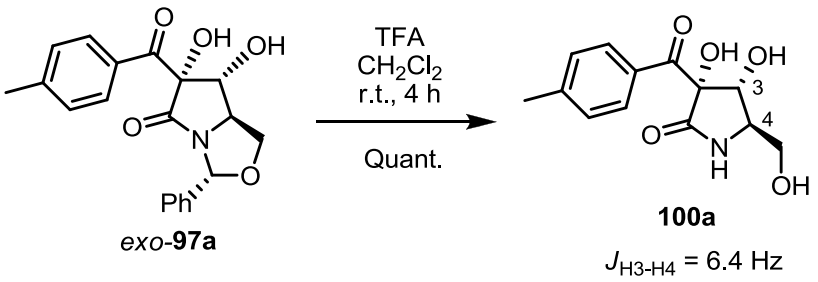


106 **107** **100**

	107, Yield	**100**, Yield	**100** - J_{H3-H4}
a R = 	45%	48%	6.4 Hz
b R = 	44%	52%	6.4 Hz
c R= 	30%	41%; d.r. 5:1	6.1 Hz (Major) 6.7 Hz (Minor)
d R= 	19%	58% (In another run, a mixture of diastereomers were obtained. d.r. 2:1)	6.1 Hz 6.1 Hz (major) 6.8 Hz (minor)
e R= 	27%	31%	6.1 Hz
f R= 	51%	48%; d.r. 3.3:1	6.7 Hz (major) 7.0 Hz (minor)
g R= 	34%*	72% (In another run, a mixture of diastereomers were obtained.)	6.8 Hz 6.8 Hz (major) 7.2 Hz (minor)

*nOeSY shows exo-diol but ~1:1 diastereomeric mixture about H-10

In order to ascertain the synthetic utility of this scheme, *exo*-**97a** prepared and isolated as a single diastereomer (see Scheme 2.20 above), was subjected to standard acidic hydrolysis conditions and **100a** thus obtained had the same J_{H3-H4} value of 6.4 Hz as that of **100a** obtained via the PMP protected intermediate, confirming their identity.



Scheme 2.26. Hydrolysis of previously obtained *exo*-**97a** for comparison of H3-H4 coupling constant.

In addition, X-ray crystallography of **100b** prepared via the PMP protected intermediate confirmed the *exo* stereochemistry.

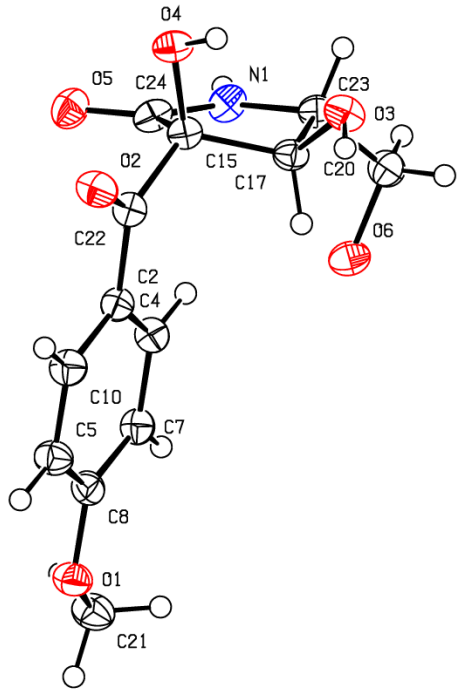


Figure 2.20. X-ray crystal structure of **100b**.

Although the overall yield of 2,3-dihydroxypyrrolidinones prepared via this route is low, sufficient samples for the assessment of their antibacterial properties were obtained. However, it is clear that should any of the derivatives possess antibacterial activity of interest, the synthetic route would require optimisation.

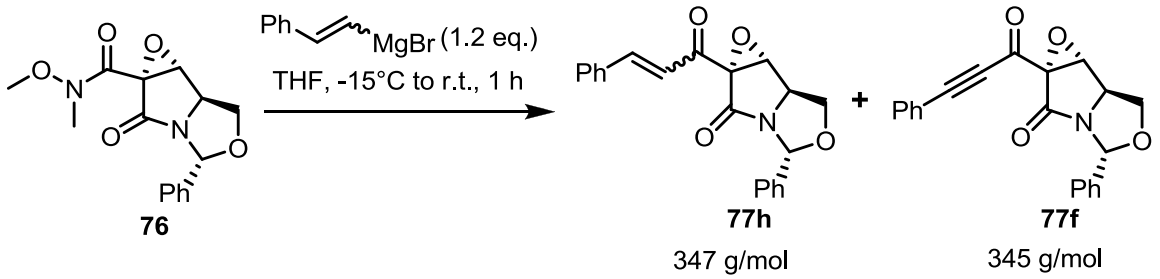
2.6 Enoyl derivatives of pyrrolidinones

Thus far, the synthesis of alkyl, aryl, and alkynyl keto-pyrrolidinones of the three different scaffolds has been described. The synthesis of alkenyl keto-pyrrolidinones proved to be particularly challenging and will be described in the following section.

2.6.1 Use of Grignard reagents from β-bromostyrene

It was anticipated that use of a vinyl Grignard reagent would provide a series of 2-enonyl pyrrolidinone derivatives, in an analogous fashion to that described earlier.

A vinyl Grignard reagent was therefore synthesised from β-bromostyrene (mixture of *E*/*Z* isomers) and magnesium metal following reaction conditions as described for the preparation of alkyl and aryl Grignard reagents previously. The synthesis of the Grignard reagent from β-bromostyrene appeared to be sluggish, even at 40°C as observed from the rate of disappearance of Mg metal. The Grignard reaction with **76** was also slow at -15°C as significant amounts of unreacted **76** was seen from TLC analysis after 30 min.



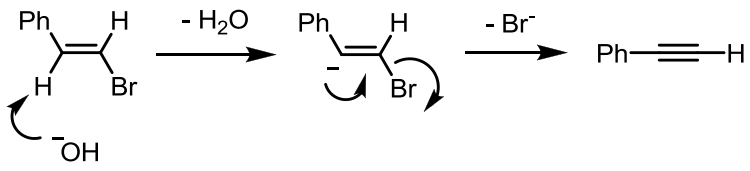
Scheme 2.27. Mixture of products obtained from Grignard reaction with styryl magnesium bromide.

A single spot fraction isolated during flash column chromatography after standard work-up procedures revealed the presence of a mixture of **77f** and **77h** (Scheme 2.27) – LRMS analysis of the isolated fraction showed peaks at *m*/*z* 368 and 370, with IR stretching frequency at 2197 cm^{-1}; ^{13}C NMR, HMQC and HMBC analysis showed two quaternary carbons at δ 97.15

and 85.78 ppm, all indicative of an alkyne functional group. From the ^{1}H NMR spectrum, the ratio of **77h** to **77f** was found to be 1:1.43, based on the H-6 singlets at δ4.32 and 4.44 ppm respectively (assigned via HSQC and HMBC).

The presence of the ynone **77f** was intriguing and may have been formed from the reaction of **76** with phenylethynyl Grignard reagent. Phenylethynyl magnesium bromide was likely to be a side-product in the preparation of the Grignard reagent as a result from using a small crystal of iodine to initiate the formation of the Grignard reagent. This observation was previously reported by Yoshino and co-workers during the preparation of styryl magnesium bromide with iodine as an additive.[113]

In addition, the degradation of β-bromostyrene to phenylacetylene in the presence of a strong base, NaOH and TBAB (*n*-$Bu_4N^+Br^-$), via a two-step E1cB mechanism has been described by Makosza and Chesnokov (Scheme 2.28).[114] Wolinsky *et al.* also reported that treatment of acyclic vinyl bromides with potassium *tert*-butoxide afforded acetylenes.[115] Thus it is plausible that as the basic styryl magnesium bromide was formed, deprotonation of the unreacted β-bromostyrene gave (bromoethynyl)benzene, which then formed phenylethynyl magnesium bromide.

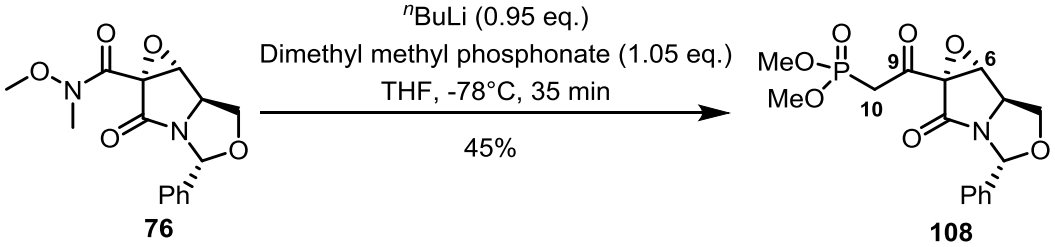


Scheme 2.28. Degradation of β-bromostyrene to phenylacetylene as proposed by Makosza and Chesnokov.[114]

The absence of an alkyne stretching frequency in the 2100-2200 cm^{-1} range of the β-bromostyrene reagent corroborated that the formation of the alkynyl motif occured during the synthesis of the Grignard reagent.

2.6.2 Horner-Wadsworth-Emmons (HWE) olefination strategy

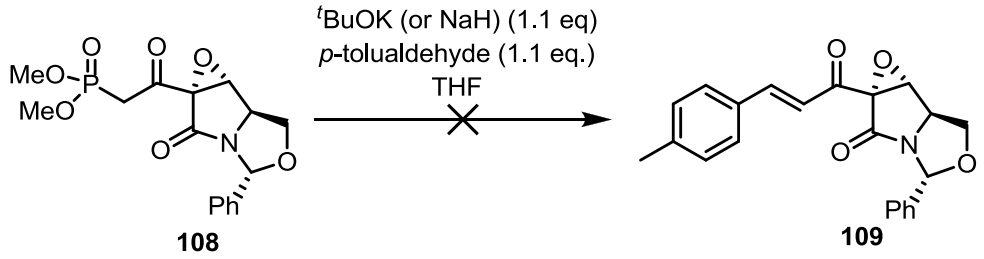
Conversion of the Weinreb amide to a phosphonate ester would allow for subsequent functionalisation to the desired enones via Horner-Wadsworth-Emmons (HWE) olefination from a common intermediate.



76 **108**

Scheme 2.29. Synthesis of phosphorane **108**.

Addition of the anion of dimethyl methyl phosphonate, generated with *n*-BuLi, to Weinreb amide **76** gave **108** in 45% yield. Characterisation with NMR and IR spectroscopy, and HRMS confirmed the identity of **108**. The epoxy and acetal moiety of **108** were intact as observed from the singlet at δ4.39 ppm (H-6) and δ6.32 ppm (H-2) respectively. The presence of the phosphonate ester could be observed from a ^{31}P NMR resonance at δ21.79 ppm. P-C coupling were also observed in the ^{1}H NMR spectrum (H-10', 3.31 ppm, $^{2}J_{PH}$ = 20.8 Hz; P-OMe, δ3.82 and δ3.76 ppm, $^{3}J_{PH}$ = 11.3 Hz) and the ^{13}C NMR spectrum (C-9, δ191.07 ppm, $^{2}J_{PC}$ = 5.7 Hz; C-10, δ39.93 ppm, $^{1}J_{PC}$ = 124 Hz; P-OMe, δ53.46 and δ53.21 ppm, $^{2}J_{PC}$ = 6.7 Hz). Strong IR bands at ῡ 1254 and 1026 cm^{-1} for P=O and C-O stretching respectively were also observed. However, when the phosphonate ester **108** was subjected to standard HWE conditions (which worked well for tetramic acid derivatives; see Chapter 3), none of the desired product **109** was obtained and only unreacted aldehyde and **108** were recovered.



108 **109**

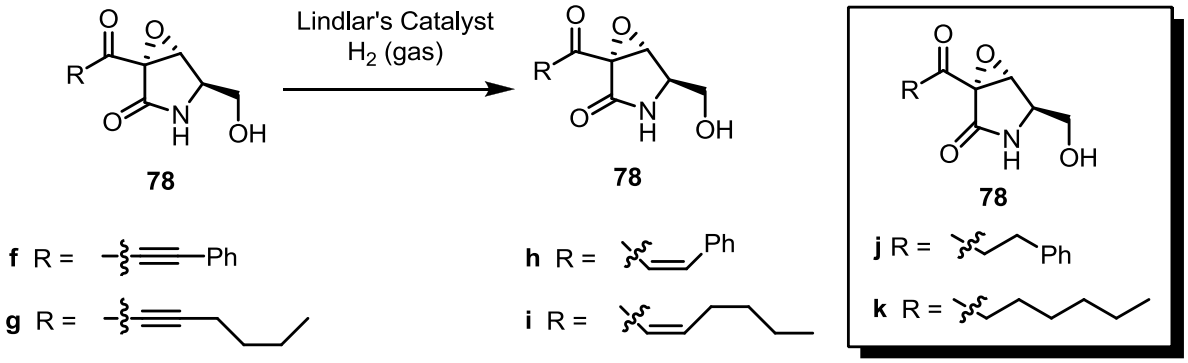
Scheme 2.30. Unsuccessful HWE olefination of phosphorane **108**.

In order to gain more insight into the reaction, a deuteration quenching study (NaH + D_2O quench) was carried out with phosphorane **108**. The deprotonation was first carried out at 0°C, and aliquots were drawn at 10 min and 30 min and quenched. Subsequently, the reaction flask was left to stir at room temperature, while aliquots were drawn at 60 min and 75 min. LRMS analysis of the aliquots showed the deuterated species at *m*/*z* 369 and 391 (as compared to the $[M+H]^+$ and $[M+Na]^+$ peaks at *m*/*z* 368 and 390 respectively). This suggests that the anion of **108** was formed but it failed to react with the aldehyde.

2.6.3 Reductive hydrogenation-isomerisation strategy

With the ynones (**78f-g**, **92f-g** and **100f-g**) in hand, the next option to be considered for the synthesis of enones would be to partially reduce the alkyne functionality to yield *cis*-enones, followed by isomerisation of the α,β-unsaturated bond to afford the more stable *trans*-enone. A sequence using Lindlar's catalyst/H_2 for the partial reduction of ynones followed by isomerisation of the *cis*-enones in the presence of catalytic amount of iodine was employed.[116] As the enones have a very similar retention factor to the ynone, LRMS analysis was used to monitor the reaction. Generally, the reaction was stopped when no peaks of the ynone were observed via LRMS analysis. The *E*:*Z* enone ratio was then determined from the respective alkene resonances in the ^{1}H NMR spectrum of the crude reaction mixture. The stereochemistry of the *E*-enones is evident from the large coupling constant (~ 16 Hz) of the two vinyl protons.

Table 2.18: Reductive hydrogenation of ynoyl epoxypyrrolidinones.



Entry	Starting Material	Condition	Remarks
1	**78f**	Lindlar's catalyst (10% wt.) Quinoline (1 μL) H_2 (balloon) EtOAc, r.t., 2.5 h	Slow rate of conversion to **78i**. **78f**: **78h** = 5:1.
2		Lindlar's catalyst (40% wt.) H_2 (balloon) EtOAc, r.t., 7 h	*E*:*Z* = 1:3.3. **78j** present as well.
3	**78g**	Lindlar's catalyst (10% wt.) Quinoline (1 μL) H_2 (balloon) EtOAc, r.t., 22 h	No enone present. 74% of **78k** isolated.
4		Lindlar's catalyst (40% wt.) H_2 (balloon) EtOAc, r.t., 1 h	No enone present, complete reduction to **78k**.
5		Lindlar's catalyst (13% wt.) Quinoline (1 μL) H_2 (balloon) EtOAc, r.t., 1 h	*E*:*Z* = 1:3.3. **78k** present as well.

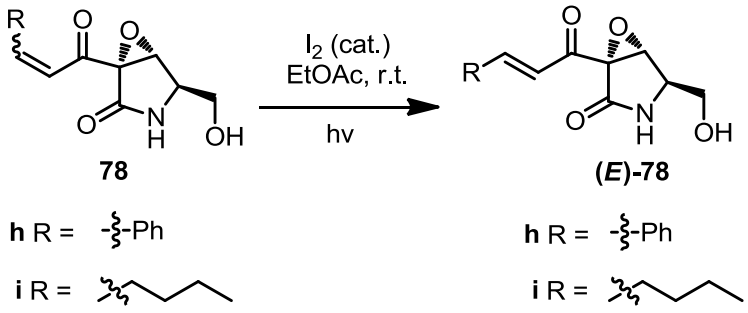
It is interesting to note that no peaks corresponding to the mass of the deoxygenated by-product was observed via LRMS analysis, unlike the results obtained for some epoxypyrrolidinones described earlier (see Section 2.4.3, *vide supra*). From the conditions used for the partial reduction of ynones above, the butyl ynone **78g** underwent reduction in the presence of quinoline rather easily, while the phenyl ynone **78f** underwent reduction in the presence of quinoline very slowly. This is presumably due to the extended conjugation (and

slight electron withdrawing effect) of the phenyl ring which decreased the electron density about the alkyne, resulting in the slower reduction rate.

Isomerisation of enoyl epoxypyrrolidinones were done on the mixture of (*E*)-, (*Z*)-enones, and the completely reduced ketones where stated, obtained after reductive hydrogenation of the ynoyl moiety. The quinoline, if present, in the reductive hydrogenation reaction was removed via a quick column chromatography prior to the isomerisation reaction.

Attempted isomerisation of the (*Z*)-enone to (*E*)-enone with catalytic amounts of iodine was initially carried out under ambient lighting but the isomerisation was found to be very sluggish. Irradiating the reaction mixture with a 500 Watt halogen lamp was found to accelerate the rate of isomerisation. Generally, the reaction mixture was irradiated between 5-6 hours, and minimal alkene signals corresponding to the (*Z*)-enone was observed from the ^{1}H NMR spectrum thereafter.

Table 2.19: Isomerisation of enoyl epoxypyrrolidinones.



78 (***E***)**-78**

h R = -§-Ph **h** R = -§-Ph

i R = **i** R =

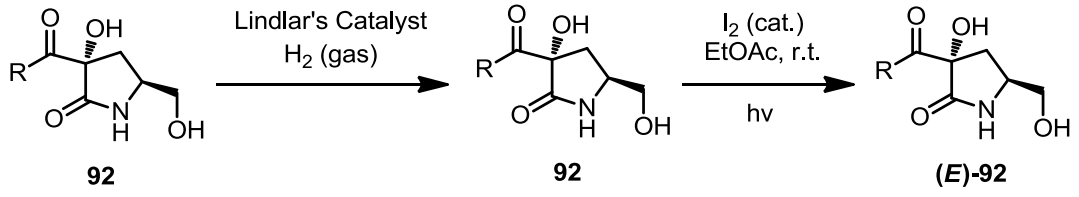
Entry	Starting Material	Condition	Remarks	Yield
1	**78h**	I_2 (cat.), EtOAc, r.t. Ambient fumehood light	Starting mixture *E*:*Z* = 1:3.3. After 21 h, *E*:*Z* = 1:1.	From entry 2, (*E*)-**78h** (50%) **78j** (30%)
2		I_2 (cat.), EtOAc 500W Halogen lamp	Carried on from above. After 2 h, *E*:*Z* = 6.7:1.	
3	**78i**	I_2 (cat.), EtOAc 500W Halogen lamp	After 5 h, no alkene signals of (*Z*)-**78i** seen.	(*E*)-**78i** (29%) **78k** (30%)

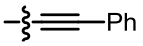
The completely reduced by-products **78j-k** have almost identical retention factor to their respective enones as observed from TLC analysis, and so the crude mixture obtained after isomerisation was purified using preparative HPLC (see Experimental Section). Although some (*Z*)-**78h** was observed in the ^{1}H NMR spectrum of the crude product after isomerisation, insufficient amounts were isolated for characterisation.

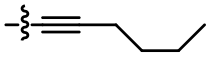
During the purification of **78i**, a small amount of **78e** (see Table 2.9, page 50, *vide supra*) was also isolated. This could arise from a slight excess of isobutyl magnesium bromide used in the synthesis of alkynyl Grignard reagent.

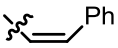
Applying a similar synthetic sequence to the 2-hydroxypyrrolidinones **92** gave the desired (*E*)-enones as shown in Table 2.20. No deoxygenated by-product was isolated during purification by preparative HPLC.

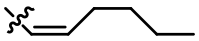
Table 2.20: Synthesis of enoyl 2-hydroxypyrrolidinones.



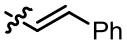
f R = 

g R = 

h R = 

i R = 

k R = 

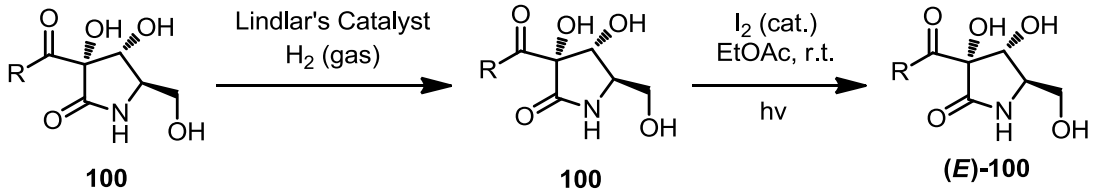
h R = 

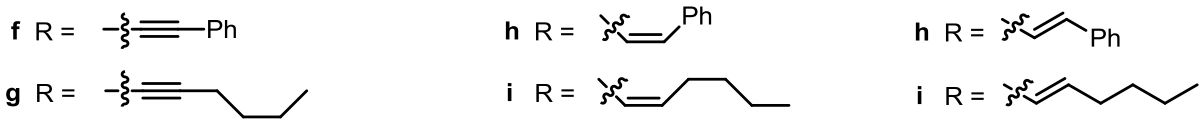
i R = 

Reductive hydrogenation of ynoyl 2,3-dihydroxypyrrolidinones.			
Entry	**Starting Material**	**Conditions**	**Remarks**
1	**92f**	Lindlar's catalyst (10% wt.) Quinoline (1 μL) H_2 (balloon) EtOAc, r.t., 2 h	No conversion to enone as determined by LRMS analysis after 2 h.
2		Lindlar's catalyst (15% wt.) H_2 (balloon) EtOAc, r.t., 4.5 h	*E*:*Z* = 1:5.
3	**92g**	Lindlar's catalyst (10% wt.) H_2 (balloon) EtOAc, r.t., 45 min	*E*:*Z* = 1:1.
Isomerisation of enoyl 2-hydroxypyrrolidinones.			
Entry	**Starting Material**	**Conditions**	**Remarks**
1	**92h**	I_2 (cat.), EtOAc, 5.5 h 500W Halogen lamp	No alkene signals of (*Z*)-**92h** seen. (*E*)-**92h** (35%)
2	**92i**	I_2 (cat.), EtOAc, 5 h 500W Halogen lamp	No alkene signals of (*Z*)-**92i** seen. (*E*)-**92i** (38%); **92k** (12%).

The desired (*E*)-enones of 2,3-dihydroxypyrrolidinones **100** were also similarly obtained as shown in Table 2.21. No deoxygenated or over-reduced by-products were isolated during purification by preparative HPLC.

Table 2.21: Reductive hydrogenation of ynoyl 2,3-dihydroxypyrrolidinones.





Reductive hydrogenation of ynoyl 2,3-dihydroxypyrrolidinones.			
Entry	**Starting Material**	**Condition**	**Remarks**
1	**100f**	Lindlar's catalyst (15% wt.) H_2 (balloon) EtOAc, r.t., 1 h 50 min	**100h** *E*:*Z* = 1:2.5.
2	**100g**	Lindlar's catalyst (10% wt.) H_2 (balloon) EtOAc, r.t., 2 h	**100i** *E*:*Z* = 1:1.
Isomerisation of enoyl 2,3-dihydroxypyrrolidinones.			
Entry	**Starting Material**	**Condition**	**Remarks**
1	**100h**	I_2 (cat.), EtOAc, 5.5 h 500W Halogen lamp	No alkene signals of (*Z*)-**100h** seen. (*E*)-**100h** (16%).
2	**100i**	I_2 (cat.), EtOAc, 5.5 h 500W Halogen lamp	No alkene signals of (*Z*)-**100i** seen. (*E*)-**100i** (17%)

The low yield obtained for **100h** and **100i** after preparative HPLC could be due to the by-products formed during the isomerisation stage. These by-products, observed in the respective HPLC traces, were isolated but their identities remained unknown as they existed as mixtures and were not present in sufficient amounts for thorough characterisation.

2.7 Summary for Chapter 2

In this chapter, the synthesis of three different scaffolds of pramanicin mimics from the versatile (2*R*, 5*S*)-1-aza-3-oxa-2-phenylbicyclo[3.3.0]octan-8-one common intermediate **53** was described.

In the synthesis towards epoxypyrrolidinones, the discovery of mild and facile epoxidation conditions utilising hydrogen peroxide and a tertiary amine proved to be advantageous, as the α,β-unsaturated intermediate is prone to dimerisation under basic conditions. The presence of weak intramolecular H-bonding within the scaffold was found to play a role in the reactivity of the α,β-unsaturated intermediate towards epoxidation, as well as the stability of the oxazolidine. This stability of **67b-e** towards oxazolidine hydrolysis led to the synthesis of analogous keto-epoxy derivatives **77a-g**, without such intramolecular H-bonding, which were successfully hydrolysed to the pyrrolidinones **78a-g**. Alkyl, aryl and alkynyl keto-derivatives were synthesised to demonstrate the scope of this route.

The synthesis of 2-hydroxypyrrolidinones was achieved using a similar Grignard reaction sequence of the Weinreb amide intermediate **82**, where the tertiary alcohol at C-2 was installed in a stereoselective manner using Davis oxaziridine.

The synthesis of 2,3-dihydroxypyrrolidinones was achieved with the required diol at C-2 and C-3 installed using a Ru-mediated dihydroxylation procedure. The choice of a PMP benzylidene as a diol protecting group was chosen because of its acid lability. However, interpretation and characterisation of intermediates **106** and **107a-g** using NMR spectroscopy was difficult due to the introduction of additional diastereomers due to the presence of a chiral PMP benzylidene. Nonetheless, the desired products **100a-g** were obtained as a single diastereomer in most cases.

Finally, the enoyl derivatives of all three scaffolds were successfully synthesised using a sequence consisting of partial reduction with Lindlar's catalyst/H_2 followed by isomerisation to the (*E*)-enones. Although low yields were obtained for some of these derivatives, sufficient

amounts were obtained to evaluate their antibacterial property and this will be described in Chapter 4.

CHAPTER 3: TETRAMIC ACID-DERIVED PRAMANICIN MIMICS

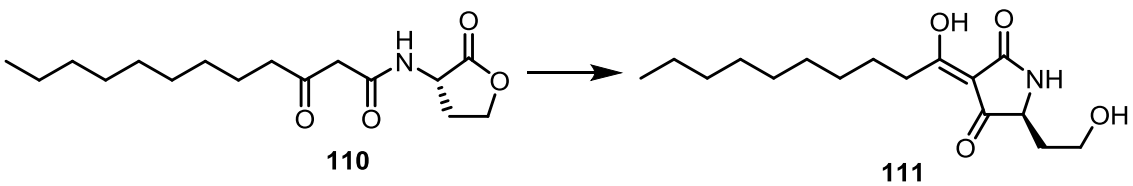
This chapter describes the attempted synthesis towards another set of oxygenated lactams as pramanicin mimics. Beginning from a known bicyclic tetramate derived from L-serine, early functionalisation was achieved by introduction of a 3-acyl side-chain. However, a biomimetic synthesis of 3-acyltetramic acids towards the desired 2,3-dihydroxypyrrolidinone scaffold was not successful.

3.1 Tetramic acids

3.1.1 Biological relevance of tetramic acids

In addition to the multiple natural products possessing a tetramic acid core exhibiting antibacterial activity (see Section 1.5.1, *vide supra*), tetramic acids are thought to play a role in the natural survival of microbes as well. Quorum sensing is a mechanism utilised by bacteria for intercellular communication. This occurs via small molecules, called autoinducers, which are synthesised within the bacterium and continuously released to its surroundings. The concentration of the autoinducers in a bacterial colony then correlates to the colony size, effecting population-dependent responses through collective changes in gene expression of the microbes upon reaching a threshold. In Gram-negative bacteria, *N*-acylhomoserine lactones (AHL) are responsible for quorum sensing.[117]

Janda and co-workers[118] found that one AHL **110** produced by *Pseudomonas aeruginosa* undergoes a non-enzymatic conversion to tetramic acid **111** in water. Similarly, synthetic 3-oxo-AHL with varying chain lengths also undergoes this rearrangement in a buffered solution.



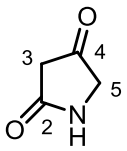
110 **111**

Scheme 3.1. Rearrangement of AHL **110** to tetramic acid **111**.[118]

Antibacterial evaluation of these tetramic acids reveals activity towards Gram-positive bacteria but not Gram-negative bacteria. Furthermore, the identification of AHL-hydrolysing enzymes in *Bacillus ceres* 240B1 suggested the presence of a "quorum-quenching" mechanism[119] which not only hinders population-dependent responses but also moderates the antibacterial effect of excess AHLs and prevents the formation of **111**. Hence, it is plausible that **110** not only acts as an autoinducer for *Pseudomonas aeruginosa*, but also as a defense against competing bacteria through the formation of bactericide **111**.[118]

3.1.2 General characteristics of tetramic acids

In its simplest form, a tetramic acid (2,4-pyrrolidinedione) **112** is a versatile building block for organic synthesis. It can undergo metallation or reaction with electrophiles at C-3, reaction with nucleophiles at C-4, and acylation on O-4 or C-3. The pK_a of the H-3 proton of unsubstituted tetramic acid is 6.5, while tetramic acids with an acyl substituent at C-3 have a pK_a of 3.0-3.5.[120]

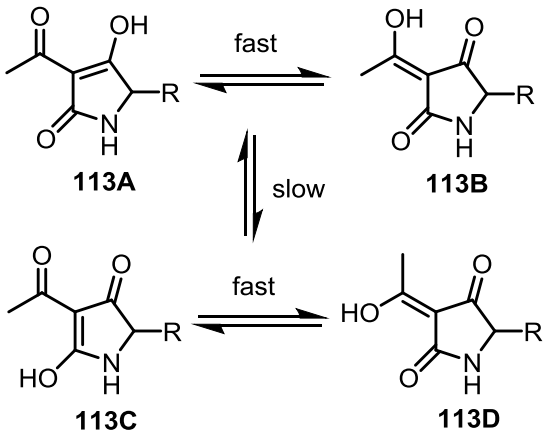


112

Figure 3.1. Tetramic acid (2,4-pyrrolidinedione).

Being more acidic, 3-acyltetramic acids exhibit extensive tautomeric behavior in solution. This phenomenon has been studied via NMR experiments with unsymmetrical 3-acetyltetramic acids. The two sets of rapidly interchanging internal tautomers (**113A** and **113B**; **113C** and **113D**) via proton transfer are usually observed as an averaged hybrid, as the rate of interconversion is too fast to be observed on the NMR time scale. However, the pair of slowly interchanging external tautomers via rotation of the acyl side-chain could be distinguished from NMR experiments. For example, the hydrogen-bonded ketone carbonyl of

113B resonates further downfield as compared to the free ketone carbonyl of **113D**. Similarly, the hydrogen-bonded lactam carbonyl of **113D** resonate further downfield as compared to **113B**.[121,122]



Scheme 3.2. Tautomerism of 3-acetyltetramic acid **113** in solution.

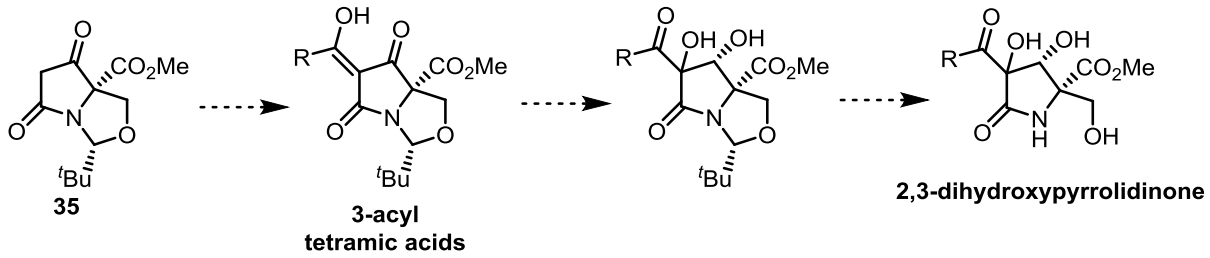
The 3-acyltetramic acid moiety is known for its metal chelating properties as a bidentate ligand. Tetramic acid **111** produced by *P. aeruginosa* not only possesses antibacterial properties but also binds iron with comparable affinity to known bacterial sideophores.[118] Metal chelation posed a problem when 3-acyltetramic acids were purified on silica gel. In addition to their high affinity towards silica gel, the metal chelates thus obtained exhibited significant signal broadening in their NMR spectra. However, the chelated metal could be removed by extraction with Na_2EDTA solution (0.05 M, pH ~4.7),[123,124] or by washing the 3-acyltetramic acids after silica gel chromatography with aq. HCl ($3 < pH < 5$).[125]

3.2 Synthetic plan towards tetramic acid-derived pramanicin mimics

The innate biological relevance of tetramic acids, together with the establishment of a tetramate intermediate in the biosynthetic pathway of pramanicin, makes it an appropriate molecular scaffold for application with the natural-product inspired synthesis paradigm. If successful, not only can it offer a different set of 2,3-dihydroxypyrrolidinones as pramanicin

mimics, but a set of tetramic acids potentially possessing antibacterial activity would be available as well.

The route as shown in Scheme 3.3 would be pursued in this thesis. Beginning with bicyclic tetramate **35**, early functionalisation by introduction of a 3-acyl pendant provides variation of the side-chain in these derivatives. A biomimetic sequence of ketone reduction and hydroxylation, followed by deprotection of the oxazolidine, would afford a different set of 2,3-dihydroxypyrrolidinones as before.

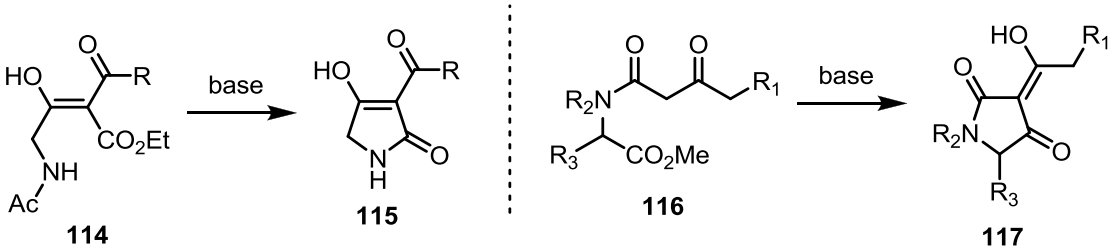


Scheme 3.3. Synthetic plan towards tetramate-derived pramanicin mimics.

Bicyclic tetramate **35** was synthesised following literature procedures from L-serine utilising Seebach's "Self Regeneration of Stereocentres" approach (see Scheme 1.9, page 27, *vide supra*). The enantiomeric excess (e.e.) of **35** determined using chiral HPLC was >90%. This is a minimum value as the minor enantiomer could not be detected below levels of 5% from spiking experiments.[58]

3.3 Side-chain elaboration of tetramic acid intermediates

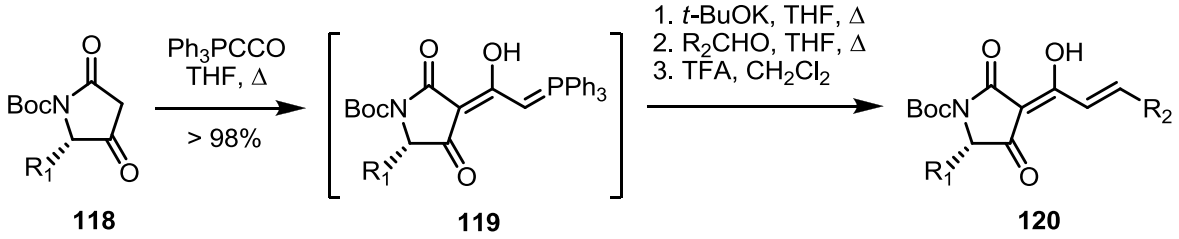
Multiple methods for the elaboration of a simple tetramic acid scaffold to 3-acyltetramic acids have been described. One approach utilises a Dieckmann-type cyclisation of β-ketoester derivatives towards tetramic acids, whereby the side-chain is incorporated prior to cyclisation.[126,127]

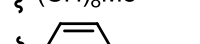
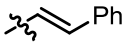
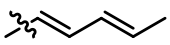
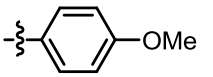


Scheme 3.4. Incorporation of side-chain prior to cyclisation to tetramic acids.

Direct C-3 acylation of tetramic acids with acid chlorides has been achieved with the use of a strong base (*t*-BuOK, NaH, LiH) in DMF.[128] Milder conditions includes the use of a Lewis acid such as $BF_3{\cdot}Et_2O$ with acid chlorides,[129] or a two-step sequence via *O*-acylation with carboxylic acids and DCC/DMAP followed by *O*-acyl/*C*-acyl migration mediated by triethylamine,[130] triethylamine with hydroxybenzotriazole,[131] or acetone cyanohydrins.[132]

Schobert and co-workers described the synthesis of 3-acyltetramic acids via the intermediate ylide **119** (Scheme 3.5). Tetramic acid **118** was treated with (triphenylphosphoranylidene)ketene (Ph_3PCCO), followed by Wittig olefination with aldehydes to afford 3-enoyltetramic acids.[133]

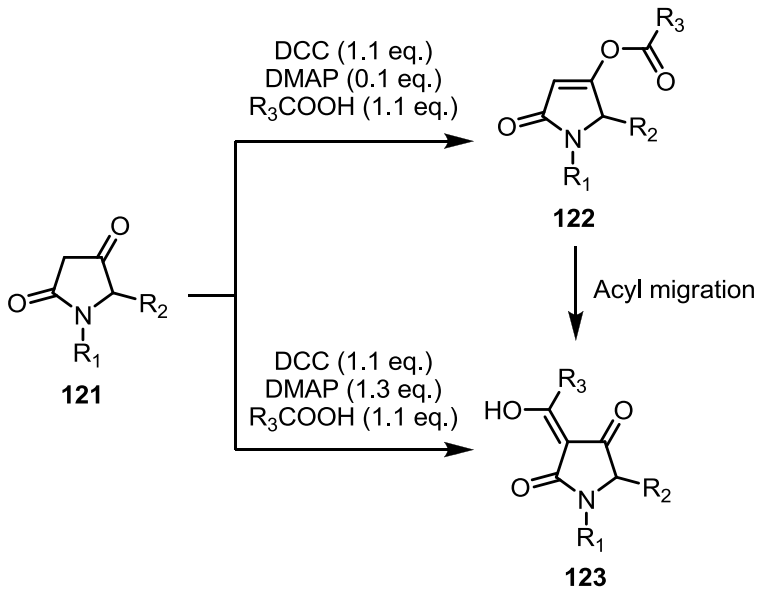


R_1	R_2	**120**
H		76%
H		84%
H		78%
p-(OH)Bn		66%
CH_2Ph		62%

Scheme 3.5. Synthesis of 3-acyltetramic acid using Ph_3PCCO by Schobert and co-workers.[133]

3.3.1 Acyltetramic acids via *O*-acyl/*C*-acyl migration

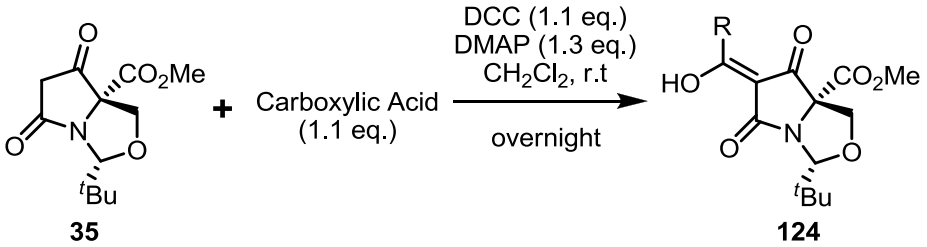
Recently, Jeong and Moloney[125] demonstrated that acylation of *N*-acyl tetramic acids with carboxylic acids in the presence of 0.1 eq. of DMAP (and 1.1 eq. DCC) gave the *O*-acyl tetramic acids while the use of 1.3 eq. of DMAP (and 1.1 eq. DCC) gave the *C*-acyl tetramic acids directly, a result of *in situ* acyl migration by excess DMAP.



Scheme 3.6. Different tetramic acid isomers obtained with catalytic or excess DMAP.[125]

Application of this latter reaction condition to **35** gave good yields with acetic acid, 4-pentenoic acid, chloroacetic acid and bromoacetic acid whereas a complex crude mixture resulted with vinylacetic acid.

Table 3.1: Synthesis of 3-acyltetramic acids via *O*-acyl/*C*-acyl rearrangement with excess DMAP.



35 **124**

		124, Yield
a	R= –Me	90%
b	R=	62%
c	R=	Complex crude NMR
d	R= $-CH_2Cl$	77%
e	R= $-CH_2Br$	54%

These compounds were found to give broad resonances in their respective ^{1}H NMR spectra after purification via column chromatography but the problem was remedied by washing the purified compound with dilute aq. HCl. The structure of **124** as drawn represents the major tautomer as determined from ^{13}C NMR spectroscopic analysis, where an intra-molecular hydrogen-bond between the exocyclic enol and lactam carbonyl resulted in a downfield shift of the lactam carbonyl resonance frequency. An example for **124a** is illustrated in Figure 3.2. The major tautomer of all 3-acyltetramic acids described in this chapter subsequently was similarly assigned.

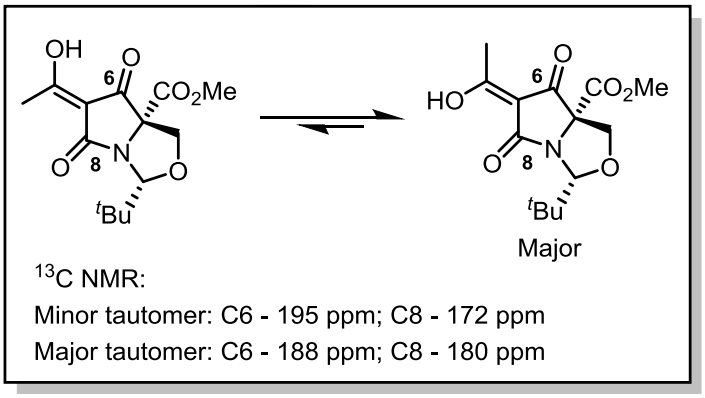


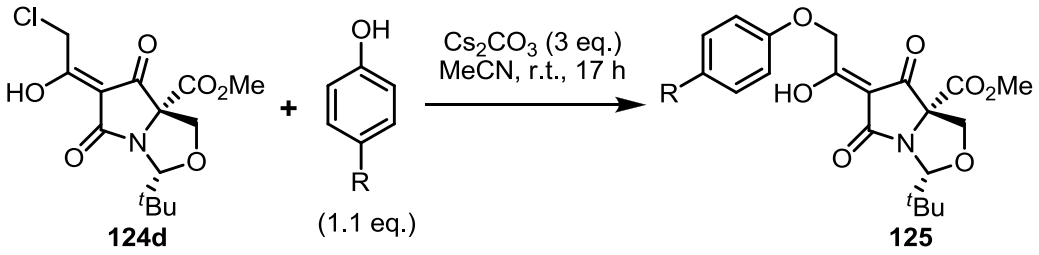
Figure 3.2. ^{13}C resonances of the carbonyl groups of **124a** indicating the presence of H-bonding.

The tautomeric behavior of these compounds, problems with broad resonances in their respective ^{1}H NMR spectra after purification via silica gel chromatography, and the absence of a singlet vinyl proton signal (~5.9-6.2 ppm) of the corresponding *O*-acyl tetramic acids,[125] suggested the presence of a *C*-acyl moiety. The identity of the *C*-acyl tetramic acids **124a-b**, **d-e** was also confirmed via HSQC and HMBC correlations.

3.3.2 Elaboration via nucleophilic substitution

Having these functionalised 3-acyltetramic acids **124** in hand, elaboration of the side-chain via a S_N2 nucleophilic substitution was investigated. Displacement of the α-chloro group of **124d** with phenols gave good yields of the aryl ethers **125a-c**.

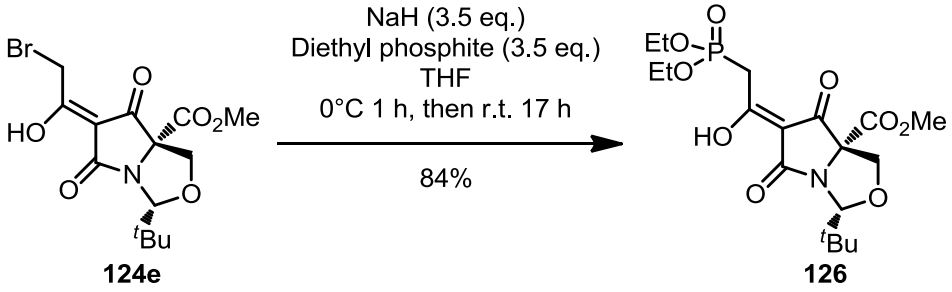
Table 3.2: Synthesis of *O*-aryl ether derivatives of 3-acyltetramic acids.



		125, Yield
a	R= –Me	66%
b	R= –OMe	69%
c	R= –Cl	73%

For **125c**, poor ^{1}H NMR spectral resolution was found when $CDCl_3$ and CD_2Cl_2 were used as solvents. Detailed analysis by COSY-EXSY at a low temperature of 278K not only gave well-resolved spectra, but also demonstrated the presence of a tautomeric mixture as reported for 3-acyltetramic acids (*vide supra*).

Similarly, displacement of the α-bromo group of **125e** with the anion generated from diethyl phosphite efficiently gave the phosphorane **126**.

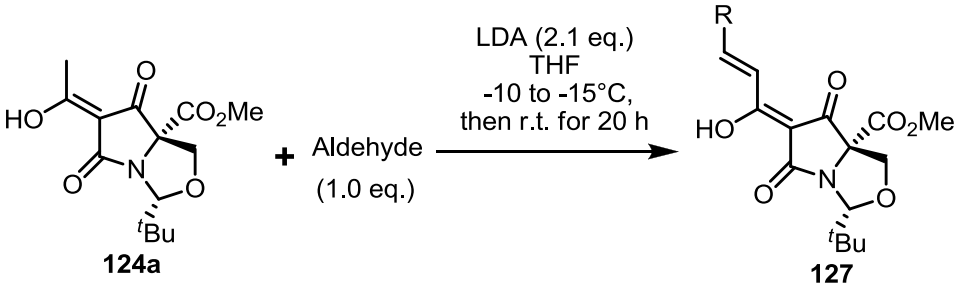


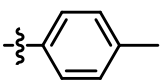
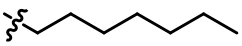
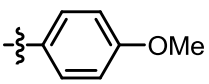
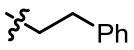
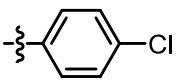
Scheme 3.7. Synthesis of phosphorane **126**.

3.3.3 Elaboration via Aldol condensation

Aldol condensation between **124a** with various aromatic and aliphatic aldehydes gave 3-enoyltetramic acids **127**, where the newly formed *trans*-double bond was evident from the large coupling constant (~ 16 Hz) of the two vinyl protons. The consistently poor yields of **127** suggested inefficient deprotonation during the reaction.

Table 3.3: Synthesis of 3-acyltetramic acids via aldol condensation.



	127, Yield		**127**, Yield
a R = 	12%	**d** R = 	18%
b R = 	14%	**e** R = 	11%
c R = 	31%		

A deuteration quenching study (LDA + D_2O quench) was conducted to confirm the extent of deprotonation of **124a**. However, a clean ^1H NMR spectrum of the product mixture could not be obtained and thus the ratio of D-**124a** to H-**124a** could not be ascertained.

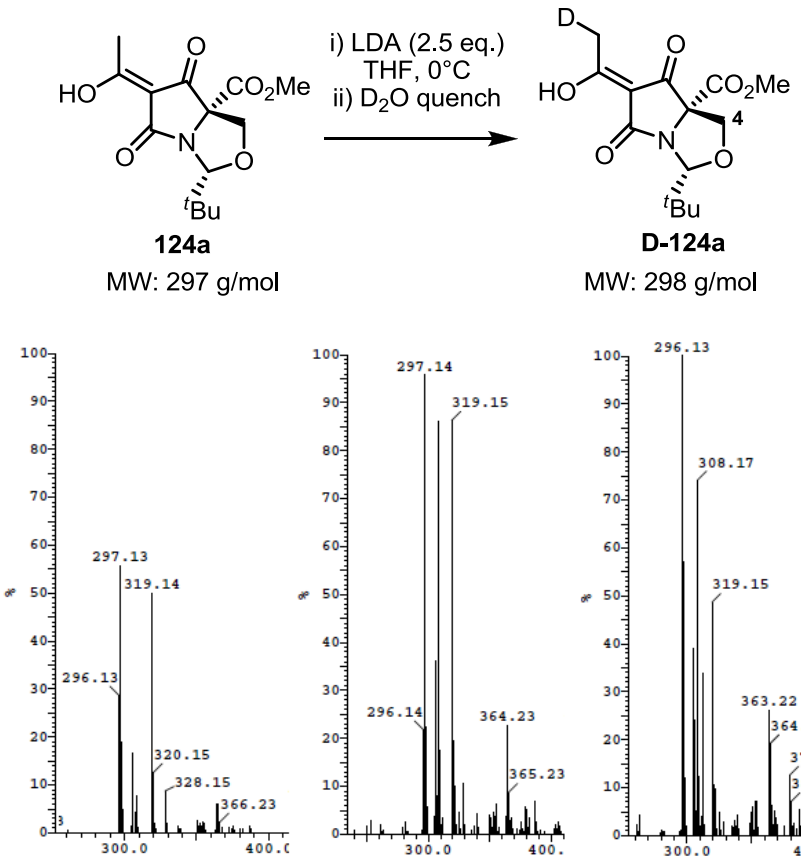
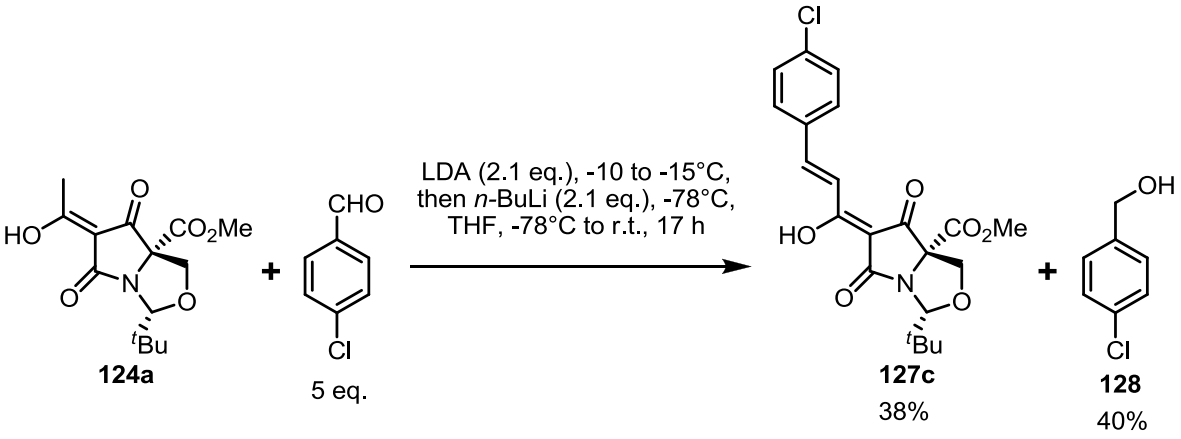


Figure 3.3. LRMS (ESI-) spectra of quenched aliquots at (from left) 0.5 h, 1 h, 2 h. The peak at *m/z* 296 corresponds to H-**124a**, while *m/z* 297 corresponds to D-**124a**.

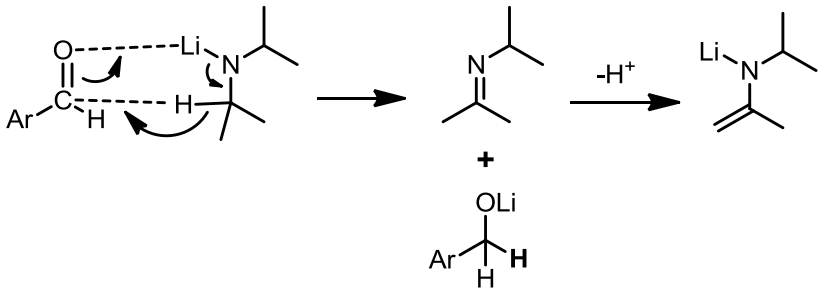
However, analysis of the product mixture with LRMS revealed that the proportion of dianion (as inferred by a peak at *m/z* 297 on the ESI- spectrum) increased during the first hour, but had decreased by the second hour (Figure 3.3). The incomplete deprotonation of **124a** in the presence of 2.5 eq. of LDA could possibly be the result of a "secondary amine effect".[134] Over the course of the deprotonation reaction, diisopropylamine formed from LDA would result in the partial reprotonation of the dianion formed. This may account for the low yields of the aldol condensation product observed.

In order to remove the effect of this "hidden proton", addition of *n*-BuLi was expected to remove the amine proton of diisopropylamine concomitantly generated from the initial step.



Scheme 3.8. Removal of "hidden proton" of DIPEA by addition of *n*-BuLi.

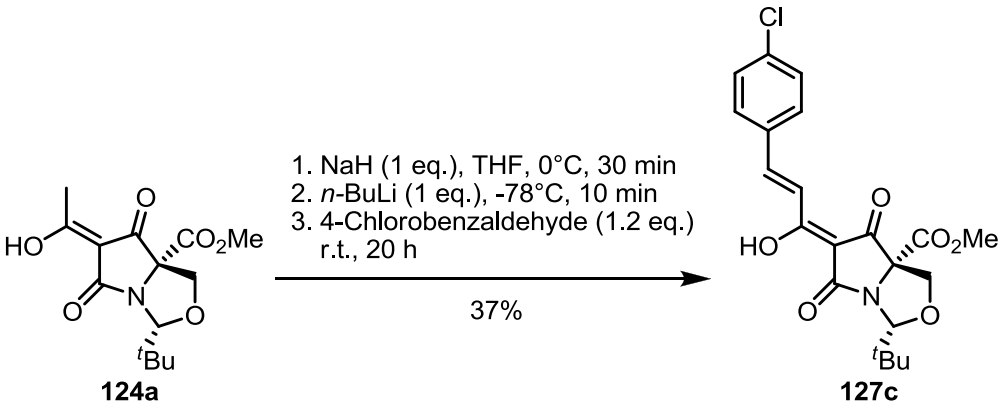
With the reaction condition as shown in Scheme 3.8, the yield for the desired 3-enonyltetramic acid **127c** did not improve significantly. In addition, the presence of excess LDA in the reaction mixture resulted in the reduction of the 4-chlorobenzaldehyde to 4-chlorobenzyl alcohol **128**. The isolated yield of **128** is consistent with the molar amount of excess LDA used.



Scheme 3.9. Reduction of aldehydes by LDA via β-hydride transfer.

The formation of 4-chlorobenzyl alcohol **128** via a hydride transfer from the β-carbon of LDA has been reported.[135,136] In some reports, the aza-enolate was postulated to participate as a competing nucleophile in possible side reactions (Scheme 3.9).[137]

To remove the complication with the use of excess LDA in the reaction mixture, the dianion of **124a** was formed using one equivalent of NaH, followed by another equivalent of *n*-BuLi.[138] Unfortunately, the lack of a significant improvement in product yield suggested that this strategy was not worth pursuing, and spurred the investigation of alternative methods for side-chain elaboration.

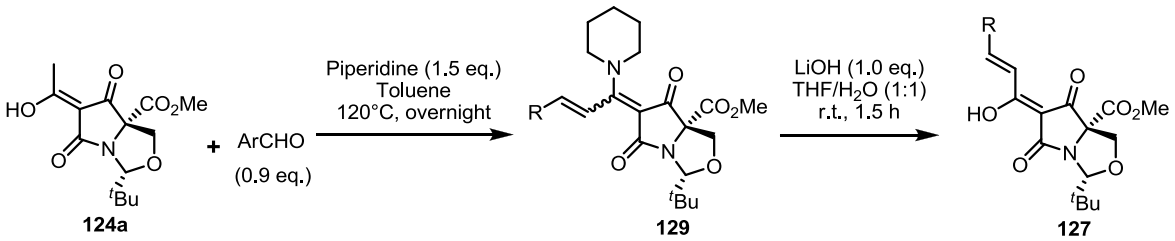


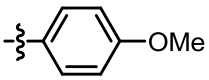
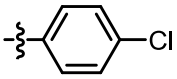
Scheme 3.10. Using two different bases to generate the dianion of **127a**.

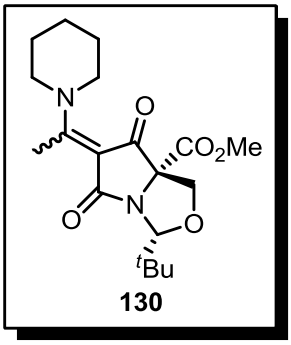
3.3.4 Elaboration via enamines

Following literature procedures,[139] heating **124a** with aromatic aldehydes and excess piperidine gave enamine **129** instead of the expected 3-acyltetramic acids. It was noted that the signals corresponding to the -CH_2- of the piperidine ring next to the nitrogen atom gave broad signals in ^{1}H and ^{13}C NMR spectra. Enamine **130** was isolated in 22% yield during the synthesis of **129c**. In addition, **130** was obtained with 82% yield when the reaction was carried out in the absence of aldehydes. Interestingly, the piperidyl enamines **129a-c** were not hydrolysed to 3-enonyltetramic acids **127a-c** under acidic conditions with aq. HCl, but were recovered quantitatively. Subsequently, it was found that hydrolysis occurred readily under basic conditions, presumably by an addition-elimination reaction of the hydroxyl ion, to give the desired products with good yields. The purity of the hydrolysed products after aqueous work-up was deemed acceptable as determined from their ^{1}H NMR spectra.

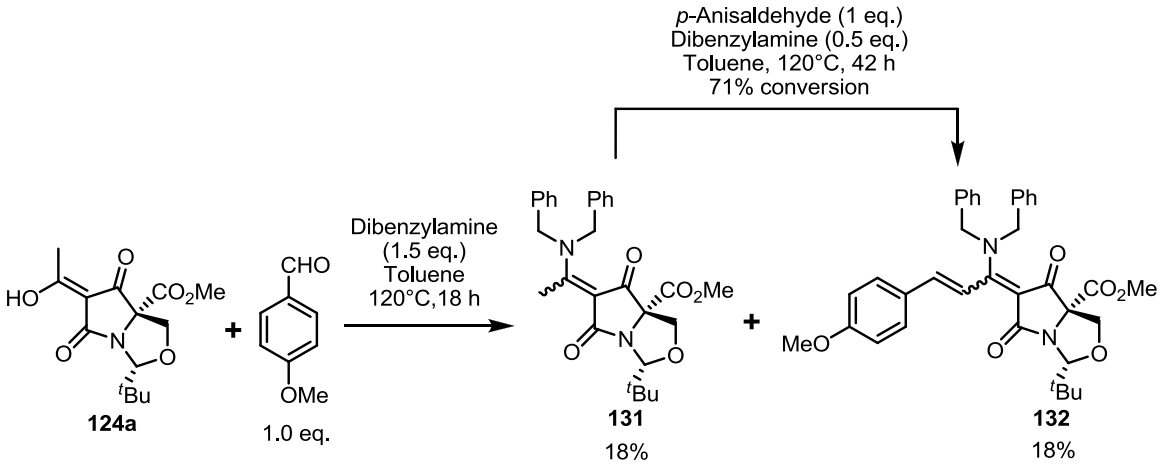
Table 3.4: 3-enoyltetramic acids via enamine intermediates.



	129, Yield	**127**, Yield
a R = 	55%	93%
b R = 	58%	Quant.
c R = 	51%	89%

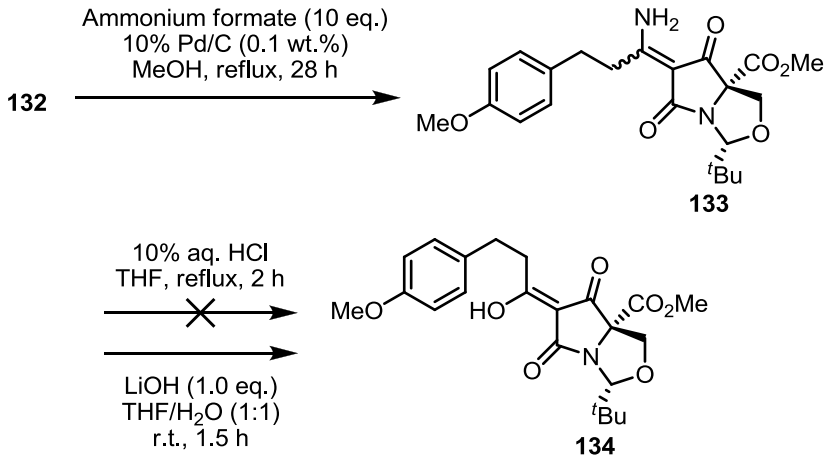


Initially, given the inability to hydrolyse the piperidyl enamine using acidic conditions, an alternative enamine was sought to allow the use of different deprotection conditions. In a separate route as shown in Scheme 3.11, condensation of **124a** with *p*-anisaldehyde with excess dibenzylamine gave a mixture of enamines **131** and **132**, with 18% yield each. The dibenzyl enamine **131** could be further converted to enamine **132** by heating with more *p*-anisaldehyde, with a conversion of 71% (based on ^{1}H NMR analysis). This suggested that the condensation reactions proceeded by the initial formation of the acetyl enamine **130**, rather than by formation of the enolate of **124a**.



Scheme 3.11. Synthesis of dibenzyl enamine intermediates.

Hydrogenolysis of the dibenzyl enamine gave the primary enamine **133**, the identity of which was inferred from the presence of a peak at *m/z* 415 (ESI-, corresponding to $[M-H]^-$) by LRMS analysis, and also from broad signals near δ10 ppm in its ^{1}H NMR spectrum, assumed to be the enamine -NH_2 protons. Similarly, basic hydrolysis gave **134** as indicated by LRMS and ^{1}H NMR spectrum (absence of enamine signals near 10 ppm).

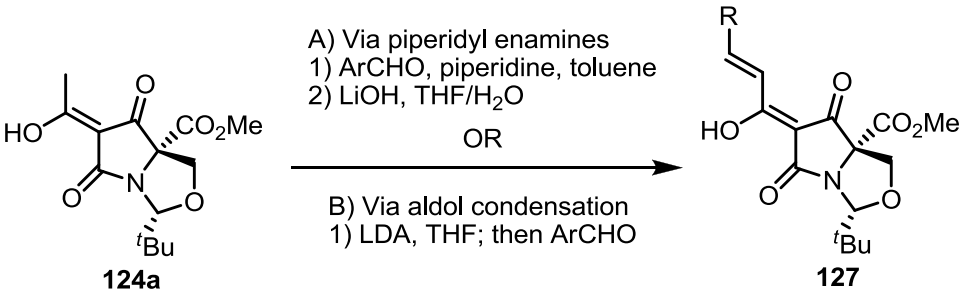


Scheme 3.12. Hydrolysis of dibenzyl enamine protecting group.

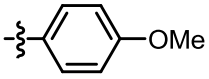
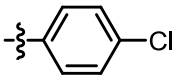
It should be noted that the stereochemistry about the exocyclic enamines (**129a-c**, **130**, **131**, **132**, **133**) C=C bond was not determined.

This route presents a more efficient approach than a single-step one involving formation of the dianion of **124a** with excess LDA followed by reaction with aldehydes which provided 3-enoyltetramic acids **127a-e** with yields of 12, 14, 31, 18 and 11% respectively (Table 3.5). However, the condensation reaction via an enamine intermediate is only compatible with non-enolisable aldehydes.

Table 3.5: Comparison of two routes towards 3-enoyltetramic acids from aromatic aldehydes.



124a **127**

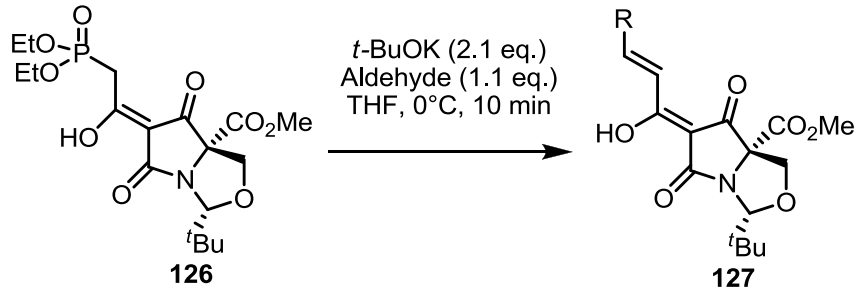
	127, Yield	
	Route A (2 steps)	**Route B**
a R = 	51%	12%
b R = 	58%	14%
c R = 	45%	31%

3.3.5 Elaboration via Horner-Wadsworth-Emmons olefination

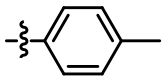
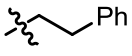
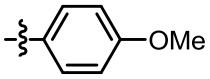
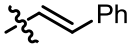
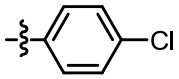
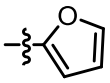
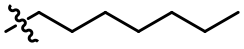
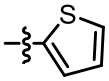
In light of the aforementioned synthetic routes towards the elaboration of the intermediate **124a**, a wider substrate scope could be achieved from the condensation of phosphorane **126** with various aldehydes via Horner-Wadsworth-Emmons (HWE) olefination. A similar synthetic sequence, between tetramic acid phosphorane with aldehydes, to yield aromatic dienoyl tetramic acids, has been previously reported by Rosen and co-workers.[140]

This route for side-chain elaboration towards functionalised tetramic acids gave 3-enonyltetramic acids **127a-h** in modest to good yields, and is by far the preferred route on the basis of fast reaction time and wide substrate scope (Table 3.6).

Table 3.6: Side-chain elaboration of **126** via HWE olefination.



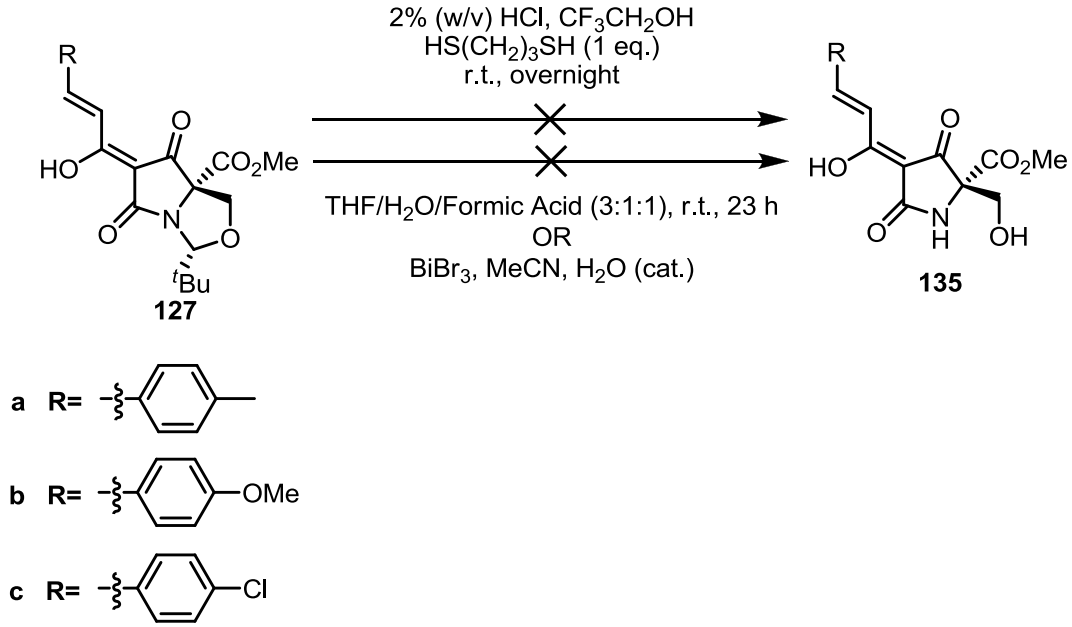
126 **127**

	127, Yield		**127**, Yield
a R = 	75%	**e** R= 	84%
b R= 	63%	**f** R= 	57%
c R= 	74%	**g** R= 	61%
d R= 	83%	**h** R= 	54%

3.4 Synthesis towards pramanicin mimics

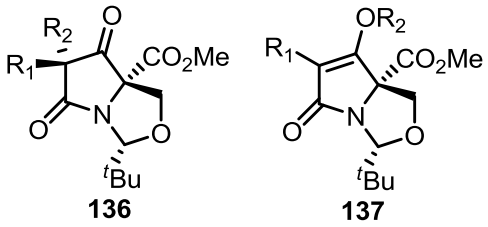
3.4.1 Stability of oxazolidine protecting group

An attempt to deprotect oxazolidine **127c** via Corey-Reichard protocol,[141] which was previously found to be universally successful for similar substrates,[72,89,142] gave a low and irreproducible yield of tetramate **135c**. No conversion of **127a-b** was observed under these reaction conditions even after 3-4 h, and the crude material obtained after stirring overnight gave complex and broad proton signals in the ^{1}H NMR spectrum.



Scheme 3.13. Attempted oxazolidine hydrolysis with various acidic conditions.

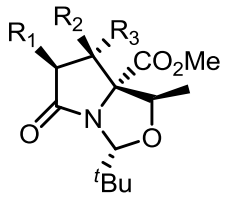
Furthermore, the use of other acidic conditions such as THF/H_2O/formic acid (3:1:1)[143] or $BiBr_3$/MeCN with catalytic H_2O[144] resulted in no hydrolysis of the oxazolidine. The basis for the stability of the oxazolidine observed for these tetramates is thought to be analogous to the stability of epoxypyrrolidinone **67b** (see Section 2.3.5, *vide supra*), since substrates that are successfully hydrolysed using Corey-Reichard protocol do not possess this exocyclic hydrogen bond donor (Figure 3.4).[59,61]



136

137

		Oxazolidine Hydrolysis Yield (%)	
R_1	R_2	**136**	**137**
H	H	41	-
H	$PhCH_2$	-	72
H	$C_5H_4NCH_2$	-	75
Me	H	66	-
Me	$C_5H_4NCH_2$	-	46
Ph	H	59	-
Ph	$PhCH_2$	79	-
Ph	$C_5H_6NOCH_2$	63	-

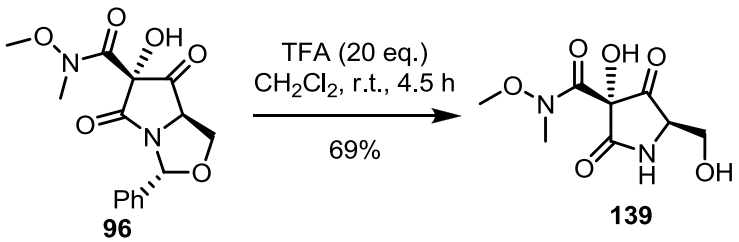


138

			Oxazolidine Hydrolysis Yield (%)
R_1	R_2	R_3	**138**
H	OH	Me	85
H	Me	OH	83
Me	OH	Me	89

Figure 3.4. Examples of substrates successfully hydrolysed using Corey-Reichard protocol.

This outcome suggests that the removal of the exocyclic enolic moiety would be crucial towards successful oxazolidine hydrolysis. This could be achieved either by hydroxylation at C-3, thus preventing the enolisation of the exocyclic ketone, or by reduction of the ketone at C-4, thus reducing the acidity of H-3 and preventing tautomerisation. This premise is supported by the successful oxazolidine hydrolysis of tetramic acid **96** using acidic conditions employed for oxazolidine hydrolysis of hydoxypyrrolidinones derivatives (see Section 2.5.1, *vide supra*).



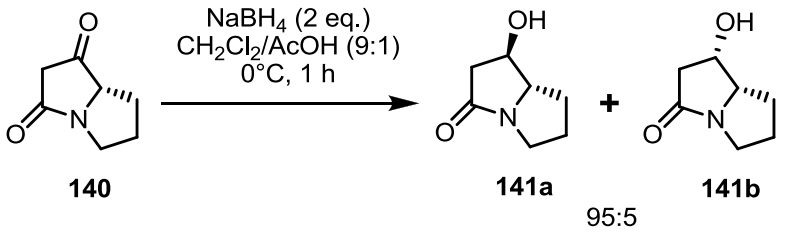
96 **139**

Scheme 3.14. Successful oxazolidine hydrolysis of tetramic acid **96** without an exocyclic enolic moiety at C-3.

Both these chemical transformations (C-3 hydroxylation and C-4 ketone reduction) have been mentioned earlier as biomimetic synthetic steps (see Section 1.3.4, *vide supra*) towards the desired pyrrolidinones, and their investigation described next.

3.4.2 Attempted reduction of tetramic acids

High stereoselectivity in reductions was achieved with tetramic acids bearing bulky *N*-substituents or at the C-5 position as demonstrated by Poncet and co-workers.[93] In the case of **140**, selective reduction of the [3.3.0] bicycle gave the hydroxyl-lactam **141** with a d.r. of 95:5 (Scheme 3.15).



Scheme 3.15. Stereoselective ketone reduction of tetramic acid **140**.

Of interest is that the favoured hydride approach was from the sterically hindered *endo* face of the bicycle; the lone pair electron density of the pyramidised N atom on one side of the bicycle results in an electrostatic repulsion when the hydride approaches from the less hindered *exo* face.

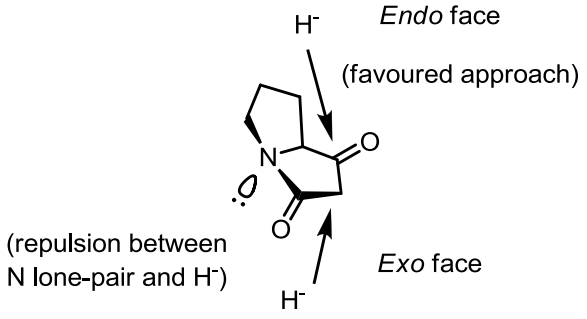
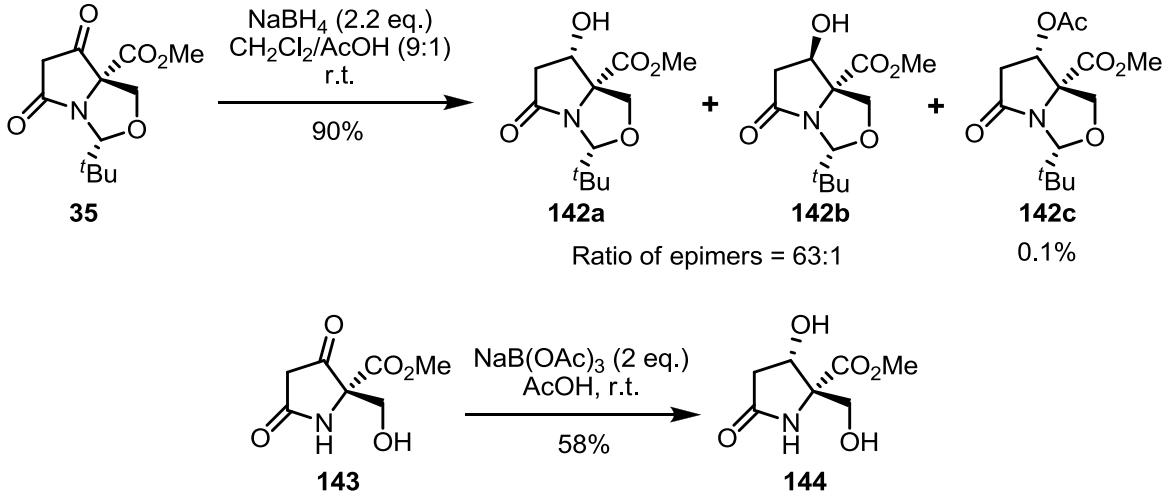


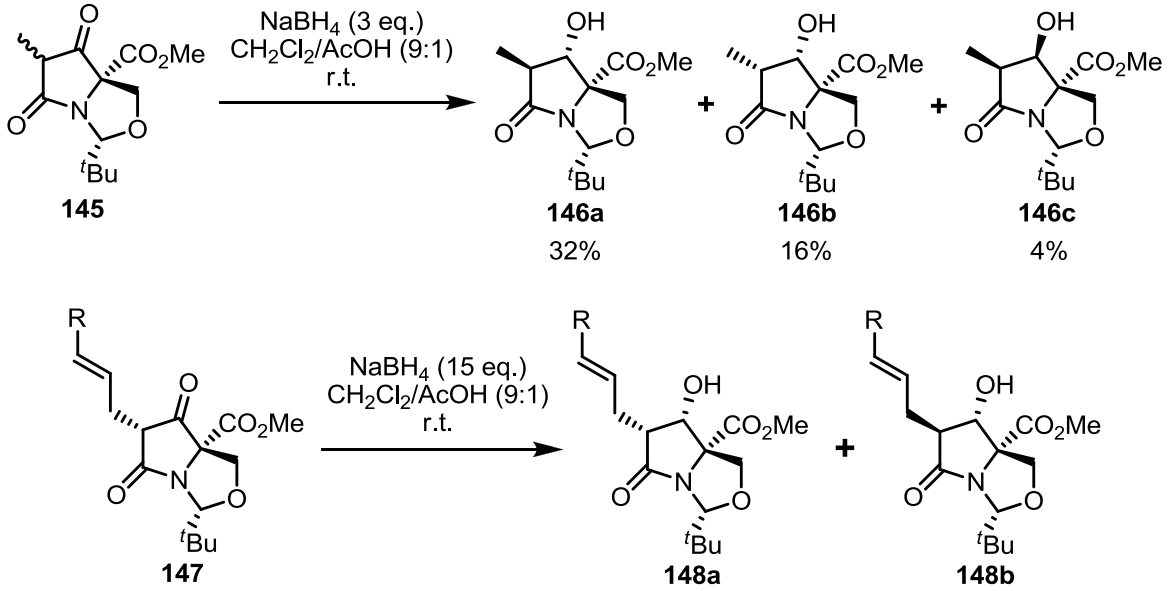
Figure 3.5. Electrostatic repulsion between N lone pair and hydride approaching form the *exo* face.[93]

This stereochemical outcome for the reduction of bicyclic tetramic acids have been previously employed by Moloney and co-workers. Reduction of 3-unsubstituted tetramic acids **35** or **143** with $NaBH_4$ in the presence of AcOH gave the corresponding alcohol with modest to good yields (Scheme 3.16).[145]



Scheme 3.16. Examples of successful ketone reduction of 3-unsubstituted tetramic acids.[145]

Reduction of 3-alkyl tetramic acids **145**[145] or **147**[146] with $NaBH_4$ under acidic conditions gave lower yields of the alcohol, with different epimers at C-3 isolated in both instances (Scheme 3.17).



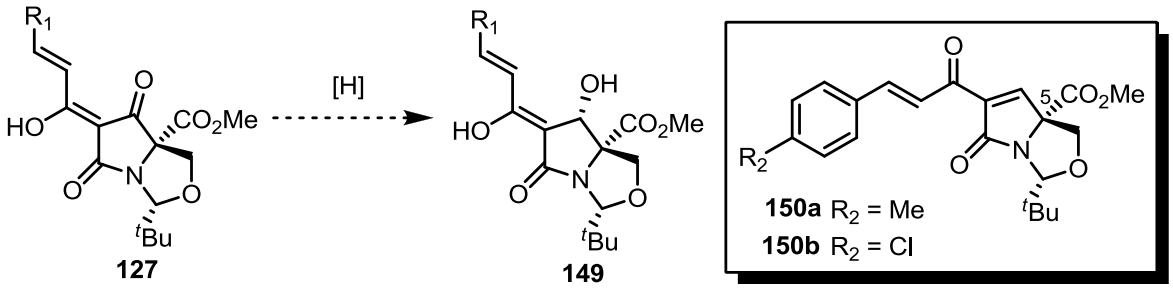
	148a	148b
R = Ph	24%	32%
R = H	20%	10%

Scheme 3.17. Examples of successful ketone reduction of 3-alkyltetramic acids.[145, 146]

However, applying these reduction conditions to the reduction of the ketone moiety in 3-enoyltetramic acids **127** failed to give the desired alcohol **149** in this current work.

With the use of acidic $NaBH_4$, small amounts of dienones **150a-b** were isolated (Table 3.7, entry 1 and 3). This suggested that the reduction of the ketone could have occurred, and a plausible mechanism for the formation this by-product entails the elimination of the hydroxyl group or possibly an acetate formed *in situ*. It was postulated that the complex mixture of by-products from these substrates could be due to the presence of the exocyclic enoyl moiety, contributing to a lack of chemoselectivity during reduction. In addition, the hydrogen-bonding experienced by the lactam carbonyl would decrease the lone pair electron density of the lactam nitrogen atom, diminishing the diastereoselectivity during ketone reduction. Although the use of an alternative milder 'Luche' condition with catalytic Ce(III) might improve the chemoselectivity of $NaBH_4$ (Table 3.7, entry 5-6),[147] unfortunately no conversion of the tetramic acid starting material was observed.

Table 3.7: Attempted reduction of **120**.



Entry	Starting Material	Condition	Remarks
1	**127a**	$NaBH_4$ (4.0 eq.) CH_2Cl_2/AcOH (9:1) -15°C to r.t, 3 h	**127a** added to prepared solution of $NaBH_4$-AcOH in CH_2Cl_2. No conversion after 3 h at -15°C; stir for another 3 h at r.t. Isolated **150a**.
2	**127a**	$NaBH(OAc)_3$ (2.2 eq.) CH_2Cl_2, r.t., 24 h	Isolated 6% **150a**. Unable to characterise other isolated by-products.
3	**127b**	$NaBH_4$ (2.0 eq.) THF, r.t., 5 h	Complicated crude mixture but ^{1}H NMR spectrum shows major signals belonging to **127b**.
4	**127c**	$NaBH_4$ (4.0 eq.) CH_2Cl_2/AcOH (9:1) r.t., 3 h	Incomplete conversion of **127a** with initial 2 eq. of $NaBH_4$; another 2 eq. of $NaBH_4$ added and stirred for 1 h. Isolated **150b** (17%); unable to characterise other fractions collected.
5	**127d**	$CeCl_3 \cdot 7H_2O$ (2.0 eq.) $NaBH_4$ (1.0 eq) MeOH -40°C to r.t., 4.5 h	No conversion of **127d**; recovered quantitatively.
6	**127d**	$CeCl_3 \cdot 7H_2O$ (4.0 eq.) $NaBH_4$ (4.0 eq.) MeOH, r.t., 3 h	No conversion of **127d**; recovered 65%.
7	**127d**	LiCl (2.0 eq.) $NaBH_4$ (2.0 eq.) MeOH, r.t., 3 h	No conversion of **127d**; recovered quantitatively.
8	**127d**	$NaBH_4$ (2.0 eq.) 0.5M methanolic NaOH solution r.t., 3 h	Complicated crude mixture with broad signals in ^{1}H NMR spectrum.

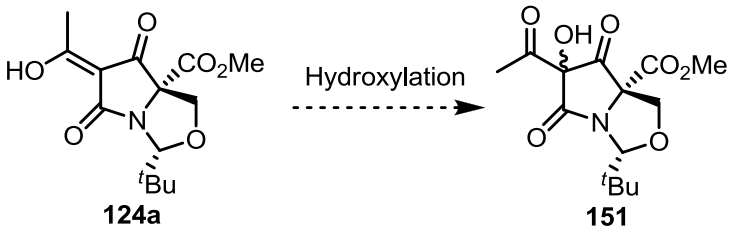
3.4.3 Attempted hydroxylation of 3-acyl tetramic acids

With the unsuccessful reduction of the ketone group, the α-hydroxylation of the tri-carbonyl system was investigated. Successful installation of the tertiary α-hydroxyl group would

interrupt tautomerism to the exocyclic enol, which should then facilitate the hydrolysis of the oxazolidine.

The required α-hydroxylation was envisaged to be formed via the epoxidation of the enol moiety with a mechanism similar to Rubottom oxidation. However, treatment of **124a** with *m*-CPBA or alkaline peroxide conditions failed to give any desired product and a complex crude reaction mixture was obtained (Table 3.8).

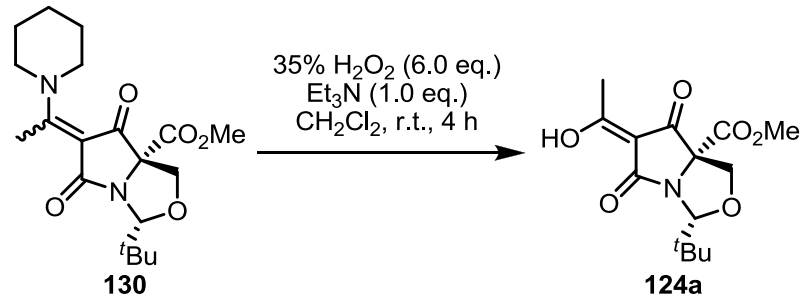
Table 3.8: Attempted α-hydroxylation of **124a** with peroxy reagents.



124a **151**

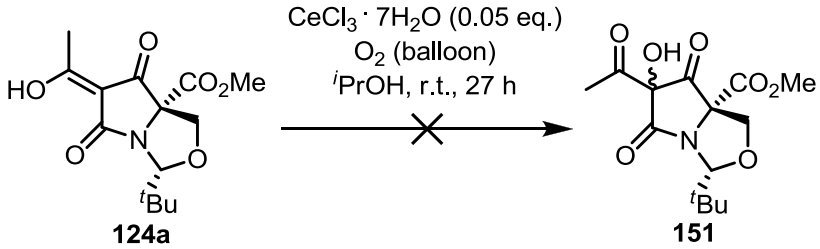
Entry	Condition	Remarks
1	*m*-CPBA (1.5 eq.) CH_2Cl_2, r.t., 2.5 h	Complex crude NMR with broad proton signals.
2	35% H_2O_2 (3 eq.), LiOH (3 eq.) CH_2Cl_2, r.t., 2.5 h	Complex crude NMR with broad proton signals.

Alternatively, epoxidation of the exocyclic C=C bond followed by hydrolysis would give the desired hydroxyl tri-carbonyl compound. As epoxidation failed with enol **124a**, an attempt to epoxidise piperidyl enamine **130** (a protected enol) under the conditions that worked well with the pyroglutamates (see Chapter 2, *vide supra*) also failed to give the desired product. Instead analysis of the crude NMR spectrum showed only the presence of 3-acetyltetramic acid **124a**. This suggests that epoxidation did not occur and the enamine was hydrolysed by water in the presence of a weak base.



Scheme 3.18. Attempted epoxidation of enamine **130**.

Disappointingly, the use of Christoffers' α-hydroxylation procedure[104] with catalytic Ce(III) and dioxygen returned 55% of unreacted **124a**, together with an isolated spot which gave a complex crude NMR spectrum which could not be identified.



Scheme 3.19. Attempted α-hydroxylation of **124a** with catalytic Ce(III) and dioxygen.

3.5 Summary for Chapter 3

In this chapter, tetramic acids were introduced as biologically relevant scaffolds. Their inclusion in the route towards the desired pramanicin mimics could potentially provide related sets of antibacterial derivatives.

The use of excess DMAP efficiently provided various 3-acyltetramic acids bearing a pendant suitable for further side-chain elaboration. The phosphorane **126** was utilised as a precursor for 3-enoyltetramic acids **127a-h** via HWE olefination, and these derivatives were used in the synthetic route towards the desired 2,3-dihydroxypyrrolidinones.

Unfortunately, further chemical modifications of 3-enoyltetramic acids **127** were unsuccessful – oxazolidine hydrolysis was impeded by intramolecular hydrogen-bonding; reduction of the ketone was possibly complicated by the presence of the exocyclic enoyl moiety; α-hydroxylation met with failure – and a comprehensive investigation of these failed attempts or an alternative route was not pursued due to time constraints. Nonetheless, a set of 3-acyltetramic acids were obtained from this synthetic endeavour, and the evaluation of their antibacterial properties will be discussed in Chapter 4.

CHAPTER 4: EVALUATION OF ANTIBACTERIAL PROPERTIES

The final chapter of this thesis reports the evaluation of the antibacterial properties of the pramanicin mimics derived from both pyroglutamates and tetramic acids, along with their respective synthetic intermediates en route, as described in the previous two chapters. A correlation of the antibacterial activity exhibited by these compounds with their calculated physicochemical properties, as well as comparison with known classes of antibacterial drugs, were undertaken to assess their potential as examples of a natural product-inspired approach towards novel antibacterial drugs.

4.1 Methods for antibacterial assay

Antibacterial assays were performed using the hole-plate method with either *Staphylococcus aureus* DS267 (Gram-positive) or *Escherichia coli* X580 (Gram-negative), using Cephalosporin C (CephC) as a standard. The samples were prepared as 70% DMSO/H_2O solutions, with serial dilution to the desired concentrations (4, 2, 1 and 0.5 mg/mL). A 100 μL aliquot of each solution was loaded into 10 mm wells in bioassay plates and incubated overnight at 37^{o}C. The diameters of the resultant inhibition zones were then measured (±0.5 mm) along two perpendicular axes, and these values averaged.[148,149,150]

Selected results are shown as bar charts in the respective sections for the purpose of discussion (see Appendix A.2 for complete data). The bar charts presented in this chapter show the activity as zone diameter of the samples at the stated concentrations, where a larger zone size signifies a stronger antibacterial activity. A "**H**" on top of a particular bar represents that the zone exists as a "halo", where an area of reduced bacteria density was observed instead of a distinct clear zone.



Figure 4.1. An example of an inoculated agar plate after incubation with the samples overnight.

Such a phenotypic assay was chosen as it provided quick results for preliminary evaluation of antibacterial properties of these compounds, giving also a direct method of assessing bacterial cell permeability as well. However, unlike broth assays, this assay was not able to provide MIC values of active compounds.

4.2 Pyroglutamate-derived mimics

Three groups of pramanicin mimics derived from pyroglutamates were described in Chapter 2. Their antibacterial activity, together with some of the intermediates in the synthesis, will be presented in the following sections.

For the purpose of discussion, compounds are grouped according to their structural moiety as follows: 7-unsubstituted carboxamido pyrrolidinones and carboxamido epoxypyrrolidinones (see Section 2.3.1); α,β-unsaturated lactams (see Section 2.3.1 and 2.3.4); 2-hydroxypyrrolidinones (see Section 2.4.2); and finally, 2,3-dihydroxypyrrolidinones (see Section 2.5.2 and 2.5.3).

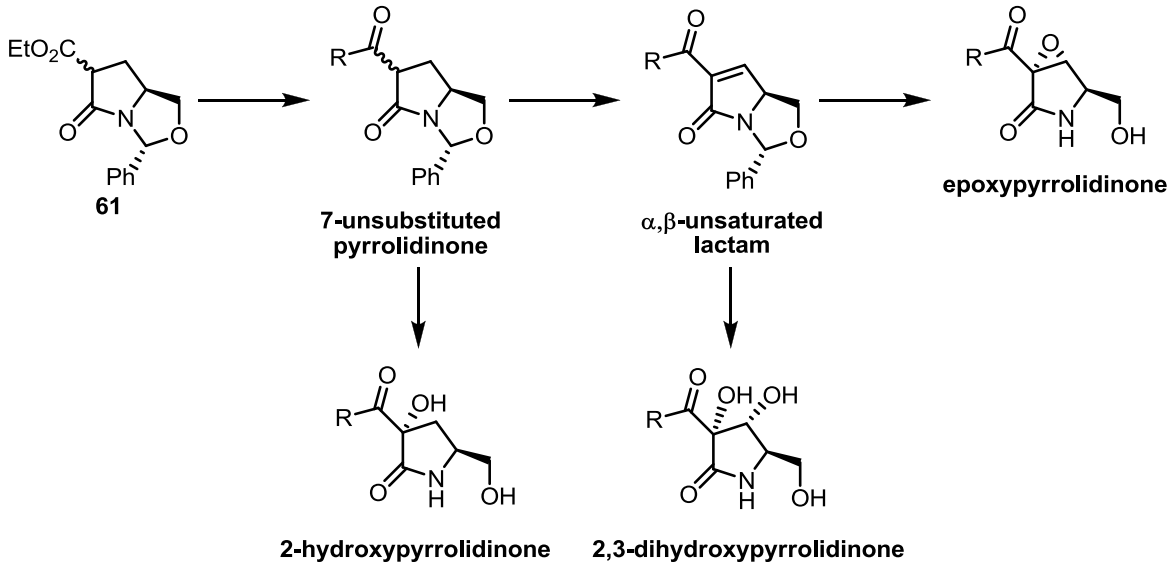


Figure 4.2. Synthetic overview of pyroglutamate-derived mimics.

4.2.1 7-Unsubstituted carboxamido pyrrolidinones and carboxamido epoxypyrrolidinones

The antibacterial property of pyrrolidinones bearing an exocyclic carboxamido moiety is summarised in Figure 4.3. In general, carboxamido pyrrolidinones (**64a-e**) and carboxamido epoxypyrrolidinones (**67a-e**, **76** and **79**) exhibited little or no antibacterial activity. The exceptions to this are **67a** and **67c**, which both show modest activity towards *E. coli*. Due to this lack of activity, as well as the difficulty involved with the oxazolidine hydrolysis (see Section 2.3.3, *vide supra*), this set of derivatives was not pursued further. Weinreb amide **73** described in the later part of Chapter 2 also did not exhibit antibacterial activity.

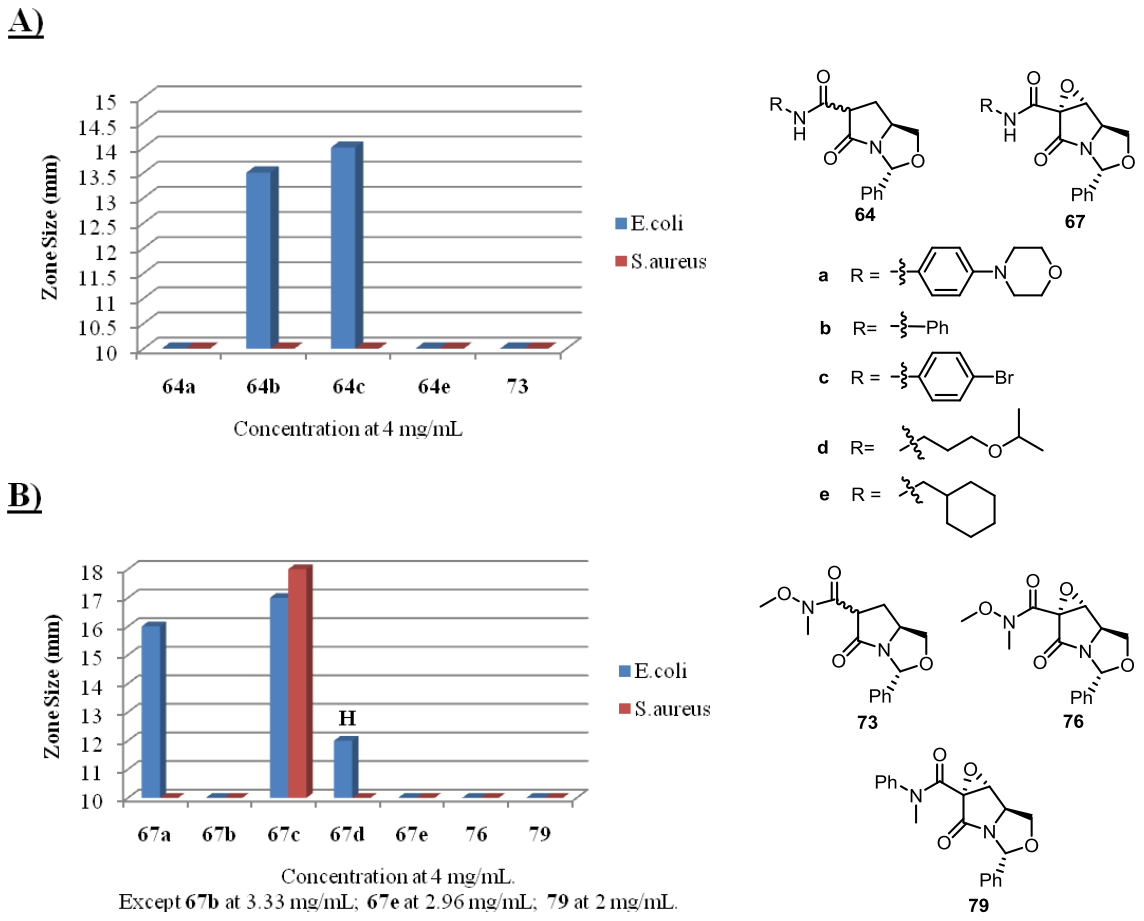


Figure 4.3. A) Bioactivity of 7-unsubstituted carboxamido pyrrolidinones; B) Bioactivity of carboxamido epoxypyrrolidinones. The mopholine ring of **67a** exists as an *N*-oxide.

4.2.2 α,β-Unsaturated lactams

In contrast, α,β-unsaturated lactams (**66b-e**, **75**) were found to have good activity towards both *S. aureus* and *E. coli* (Figure 4.4). The unsaturated lactams tested were used in crude form after aqueous workup from the oxidative elimination reaction, as further purification via silica gel column chromatography resulted in significant decomposition. However, the possibility that residual phenylselanol (PhSeOH) by-product might confound the bioactivity data was addressed by quickly purifying **66b** on silica gel to obtain a few milligrams of the pure product. A comparison between the activity of crude and purified **66b** showed that the antibacterial activities are very similar, suggesting that toxic selenium residues were unlikely to cause false positives in the bioassay.[151]

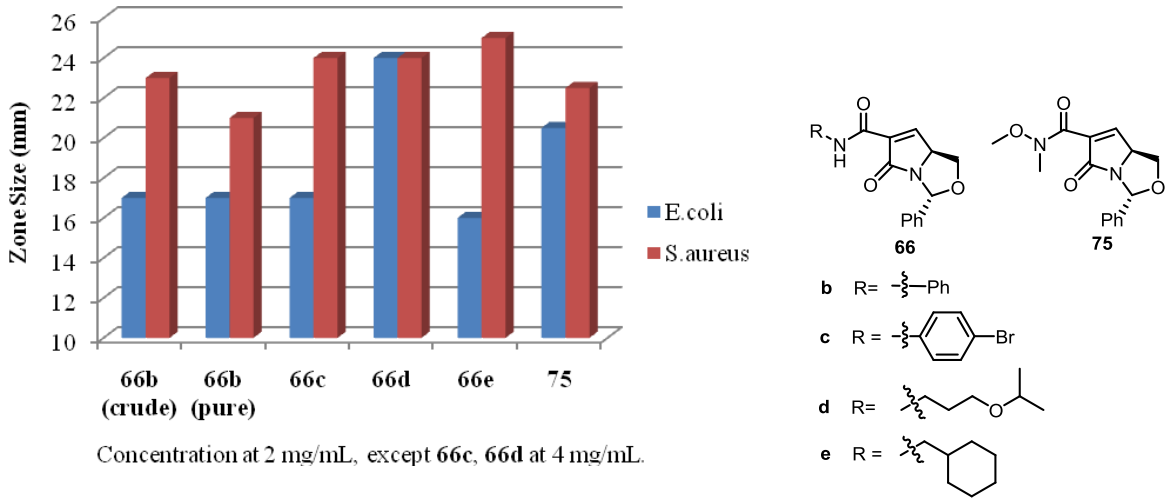


Figure 4.4. Bioactivity of α,β-unsaturated lactams.

4.2.3 Keto-epoxypyrrolidinones

Figure 4.5 summerises the antibacterial activity of the keto-epoxypyrrolidinones. In contrast to the carboxamido derivatives (Figure 4.3), the epoxyketones **77a-g** and 1,3-ketophosphorane **108** showed significant antibacterial activities. (Figure 4.5) Furthermore, oxazolidine deprotection was generally seen to result in compounds (amido alcohol **78**) which are selective for *E. coli* over *S. aureus*. Comparison of oxazolidine protected ketopyrrolidinones **77a-g** with **67a-e** therefore suggests that the epoxyketone moiety is important for conferring antibacterial activity.

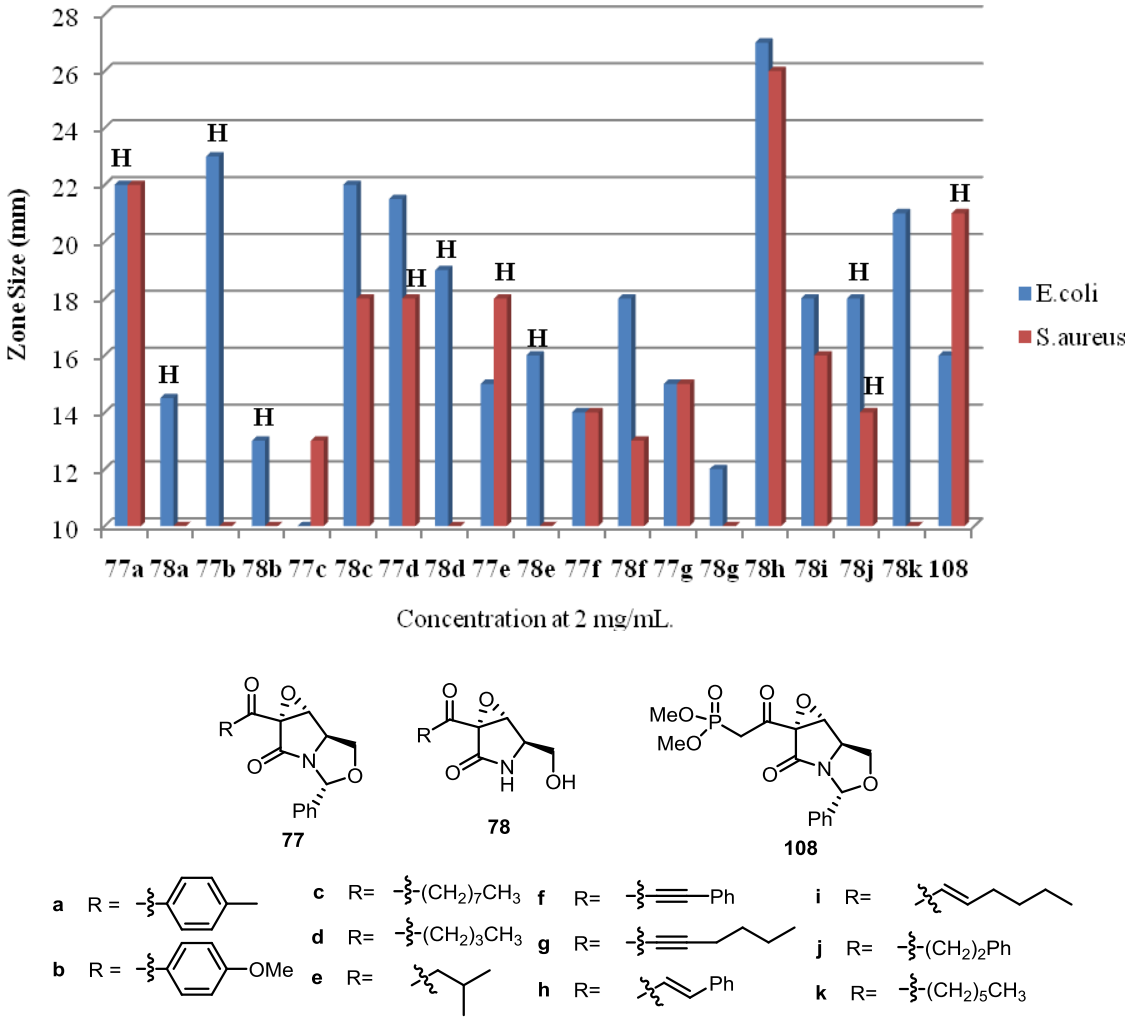


Figure 4.5. Bioactivity of keto-epoxypyrrolidinones.

The observed antibacterial activity of the compounds as shown in Figure 4.5 supports the biological relevance of this epoxyketone moiety, which was also found in other natural products (see Section 2.3, *vide supra*), justifying its incorporation into the investigation towards novel antibacterial lead compounds. Although the antibacterial mechanism of action of these compounds is as yet unknown, an example for the biological significance of the electrophilic epoxyketone moiety was found in a class of antitumour drug. Epoxomicin, a linear peptide epoxyketone natural product, was found to bind to proteasome with high selectivity. Proteasome inhibition occurs via a selective nucleophilic attack by the *N*-terminal

threonine residue on the epoxyketone, resulting in the irreversible formation of a morpholine ring (Figure 4.6).[152]

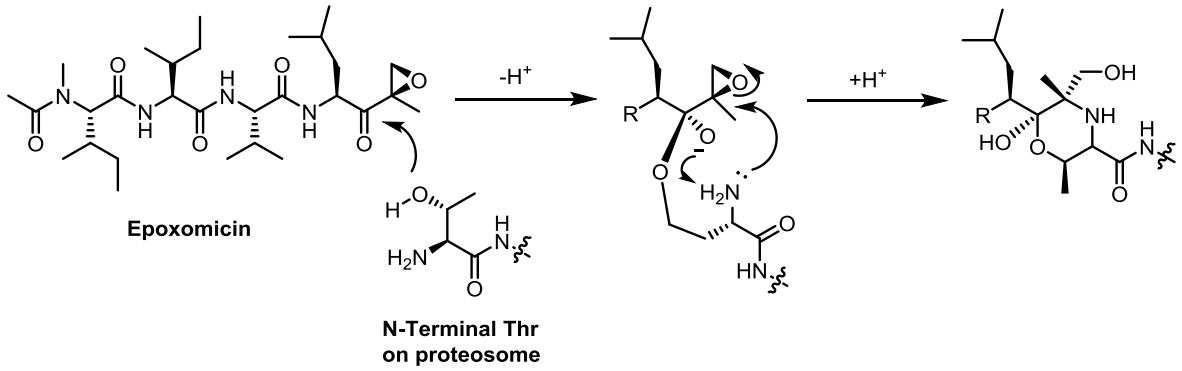


Figure 4.6. Proposed mechanism of epoxomicin with the *N*-terminal threonine residue on proteosome by Kim and Crews.[152]

4.2.4 2-Hydroxypyrrolidinones

Figure 4.7 summerises the antibacterial activities of the 2-hydroxypyrrolidinones. From these studies, it was found that 2-hydroxypyrrolidinones with a C-7 carboxamido substituent (**82**) or a C-7 carboxylate substituent (**86**) did not exhibit antibacterial activity, while 2-chloro-carboxylpyrrolidinone **87** exhibited some activity with *E. coli*.

In contrast, 2-hydroxy-ketopyrrolidinones (**91a-f**, **92a-i**, **92k**) generally exhibited antibacterial activity, and high selectivity, in particular towards *E. coli* over *S. aureus*. However, lower selectivity was observed for derivatives with Michael acceptor functionalities (for example ynones or enones) on their side-chain, due to better activity with *S.aureus*. For some derivatives, the minor hydroxyl diastereomer ((7*R*)-**91a-c**, (7*R*)-**91f**) exhibited greater activity towards *E.coli* as compared to the major hydroxyl diastereomer ((7*S*)-**91a-c**, (7*R*)-**91f**).

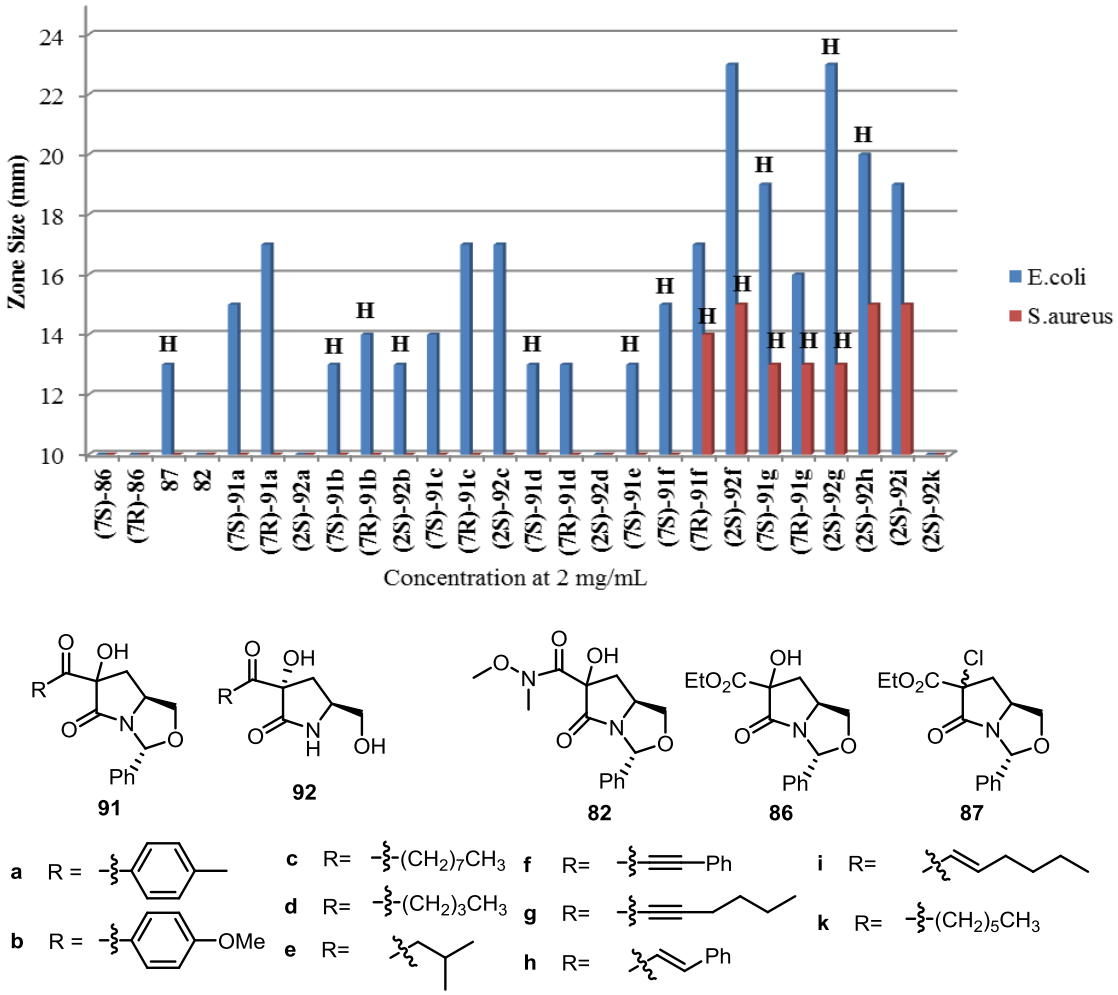


Figure 4.7. Bioactivity of 2-hydroxypyrrolidinones and 2-chloropyrrolidinone **87**.

4.2.5 2,3-Dihydroxypyrrolidinones

Analysis of the bioactivity of protected 2,3-dihydroxypyrrolidinones suggested that the exocyclic ketone at C-7, together with the presence of the free hydroxyl groups at C-6 and C-7, is again important (Figure 4.8). Absence of just the oxazolidine group (compare **101a** with **99a**) did not alter the antibacterial activity, while absence of the acetonide (compare **99a** with **97a**) resulted in a significant increase in antibacterial activity. From comparison of **101a** with **99a**, **103a** might be expected to have similar antibacterial activity after oxazolidine hydrolysis to **104a**. However, **104a** was observed to have significant activity with *E. coli*, perhaps due to the different stereochemistry of the phenylbenzylidene group.

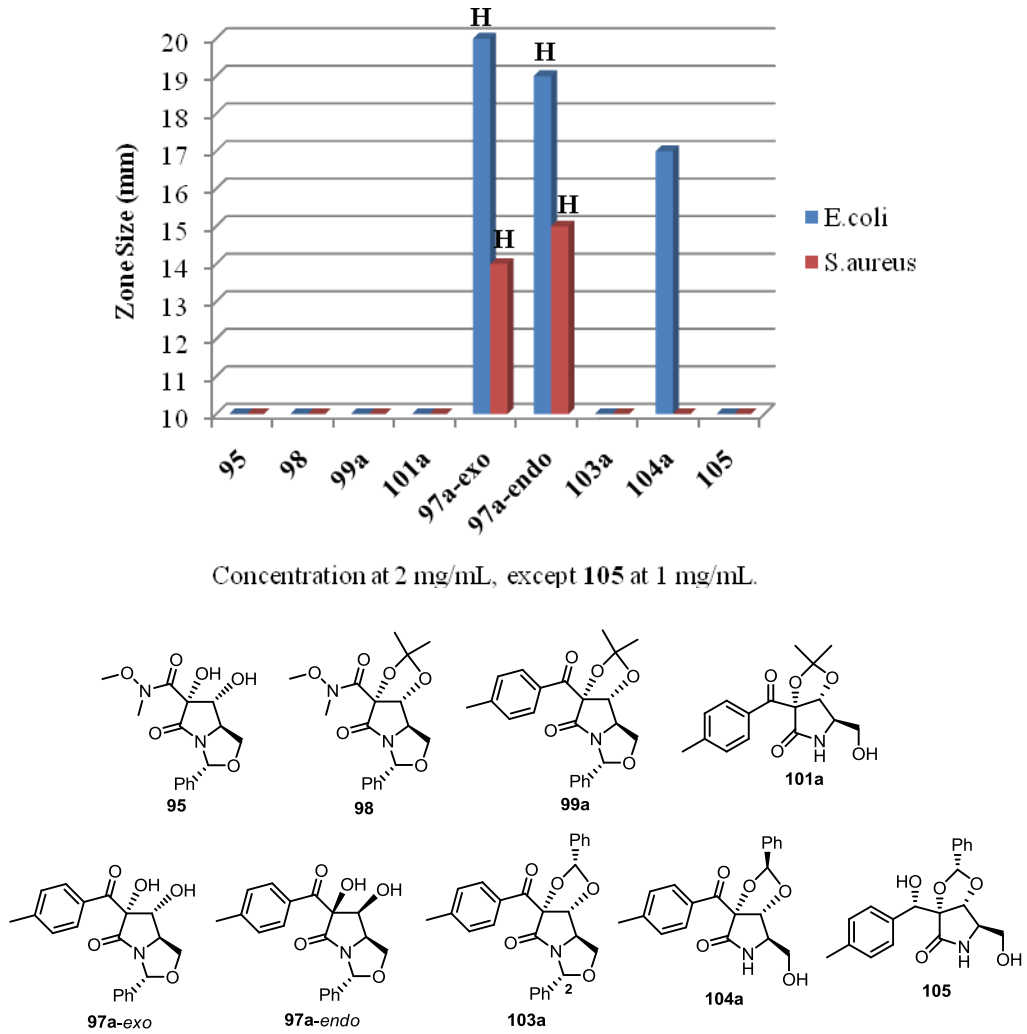


Figure 4.8. Bioactivity of protected 2,3-dihydroxypyrolidinones.

It was disappointing that the 2,3-dihydroxypyrrolidinones **100a-i**, possessing a scaffold that is most structurally similar to pramanicin did not show strong antibacterial effects. Only four out of the nine derivatives exhibited any antibacterial activity, with good antibacterial activity towards *E. coli* observed for **100c**. These results suggest that either a long hydrophobic side-chain (**100c**) or one that contains a Michael acceptor (**100f**, **100g-h**) plays an important role in the observation of antibacterial activity.

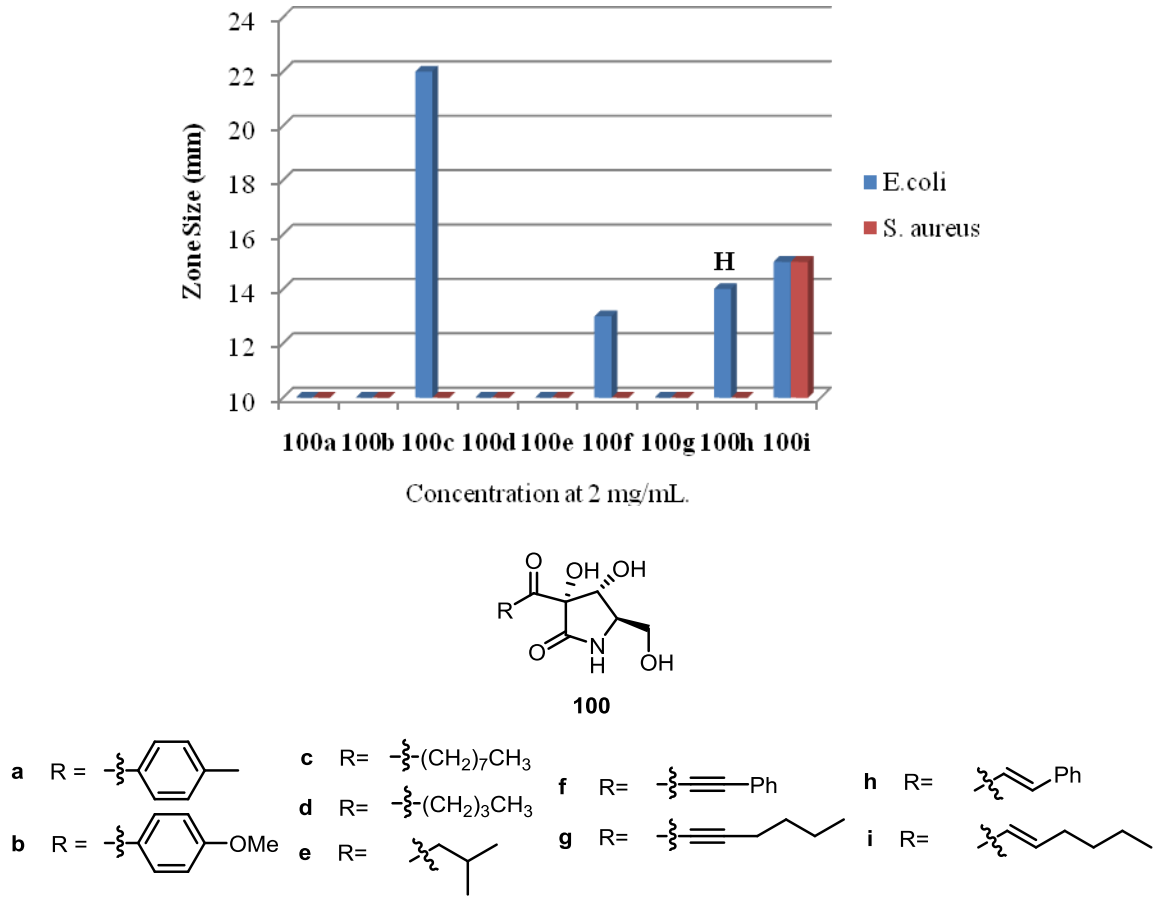


Figure 4.9. Bioactivity of deprotected 2,3-dihydroxypyrrolidinones.
100c tested as a diastereomeric mixture (about diol) of 1:0.2; **100f** tested as a diastereomeric mixture (about diol) of 1:0.3.

4.3 Tetramic acid-derived mimics

In a similar way, tetramic acid derivatives were grouped according to their structural moiety and are discussed sequentially as follows: enamine tetramic acids (see Section 3.3.4); 3-acyltetramic acids (see Section 3.3.1 and 3.3.2); 3-enoyltetramic acids (see Section 3.3.5); and finally, dienones (see Section 3.4.2).

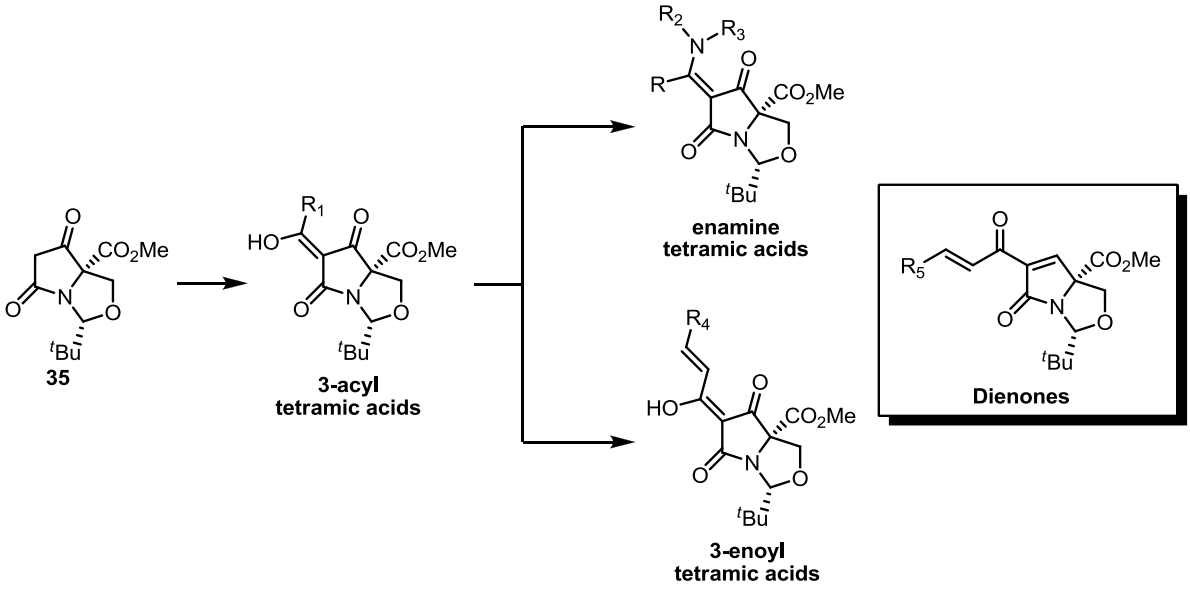


Figure 4.10. Synthetic overview of tetramic acid-derived mimics.

It was observed from the tested piperidyl enamines that an aromatic side-chain is required for antibacterial activity against *E. coli* (**129a-c**), and only minimal antibacterial activity was observed with a short acetyl side-chain (**130**). A change to a dibenzyl enamine (comparing **129b** with **132**) resulted in no activity (Figure 4.11).

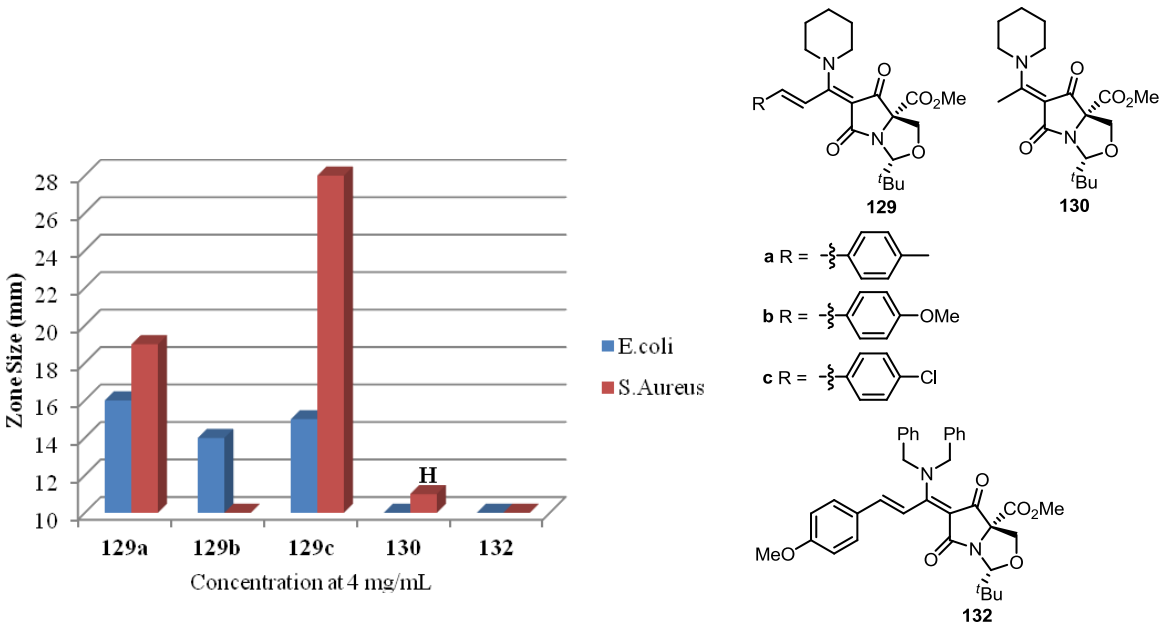


Figure 4.11. Bioactivity of enamine tetramic acids.

As shown in Figure 4.12 below, 3-acyltetramic acids with a long alkyl side-chain (**124b**) or a bromoacetyl side-chain (**124e**) gave significant antibacterial activity with *S. aureus*. Interestingly, the *O*-aryl ether derivatives **125b-c** gave modest antibacterial activity with *E. coli* but not with *S. aureus*. Phosphorane **126** did not exhibit any antibacterial activity on both bacteria species.

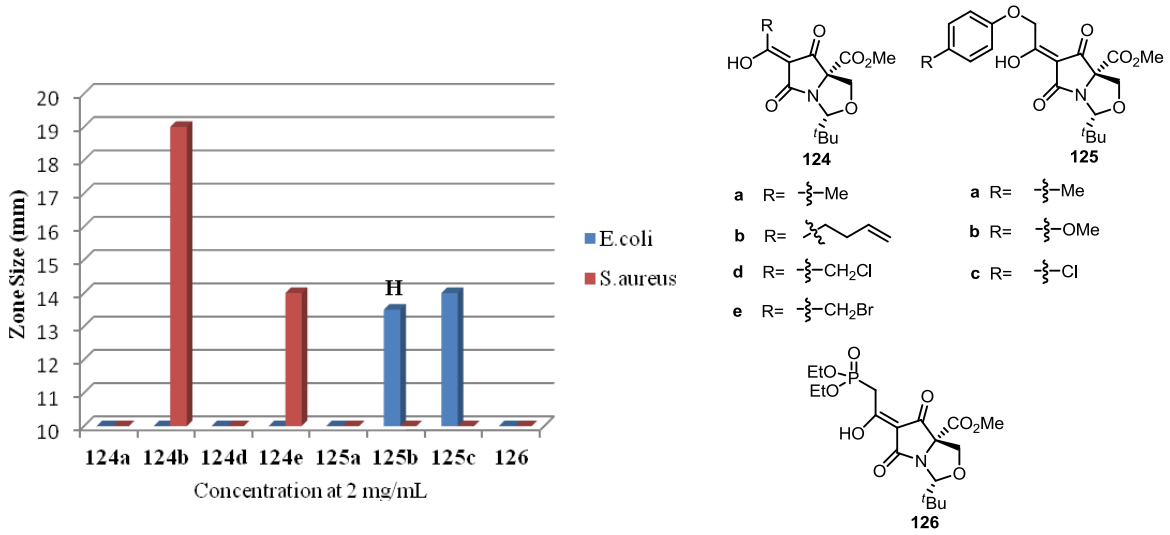


Figure 4.12. Bioactivity of 3-acyltetramic acids.

The 3-enoyltetramic acids evaluated in our studies showed that all tested derivatives **127a-h** were found to be highly selective towards *S. aureus* as compared to *E. coli* (Figure 4.13). Comparing **127a-h** with **124**, described in Figure 4.12, suggested that the incorporation of an additional conjugated C=C bond, together with a bulky hydrophobic side-chain enhanced the observed antibacterial activity.

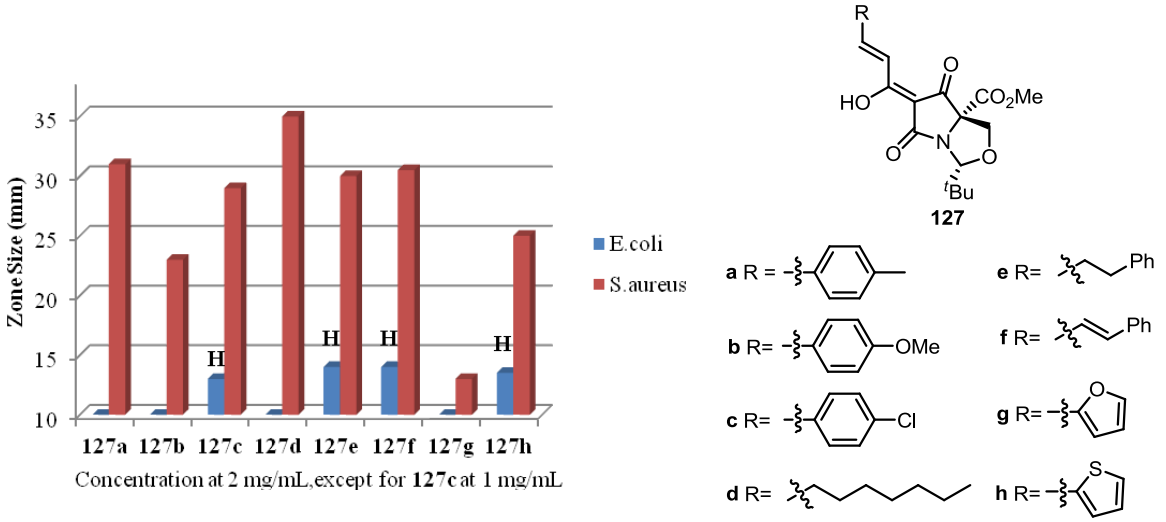


Figure 4.13. Bioactivity of 3-enoyltetramic acids.

As shown in Figure 4.14, dienones **150a-b** showed significant activity against both *E. coli* and *S. aureus*. This observed trend in antibacterial activities could be due to the presence of electrophilic motifs in the molecules, similar to **66b-e** and **75** as mentioned previously (see Section 4.3.2, *vide supra*).

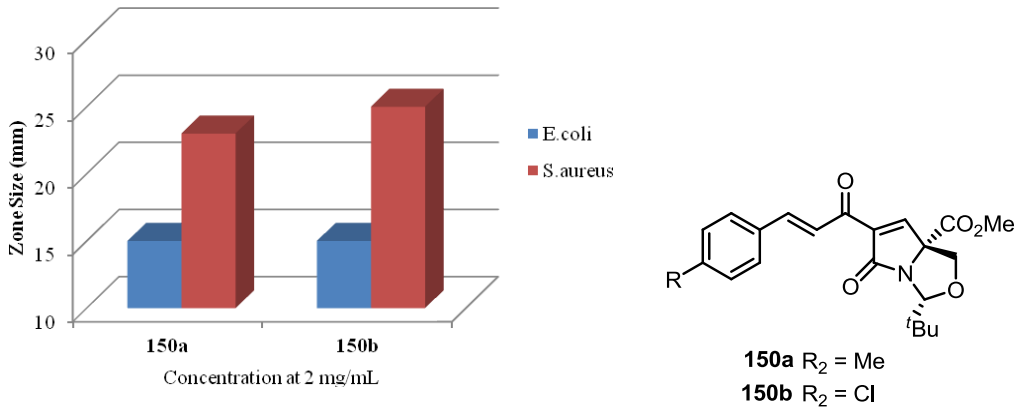


Figure 4.14. Bioactivity of bicyclic dienones **150a-b**.

Generally, tetramic acid derivatives described herein were found to be active towards Gram-positive *S. aureus* but possessed minimal activity towards Gram-negative *E. coli*. Similar observations were reported with the tetramic acids derived from AHL isolated from *P. aeruginosa* as investigated by Janda and co-workers (see Section 3.1.1),[118] as well as the tetramate systems previously investigated by Moloney and co-workers.[153,154]

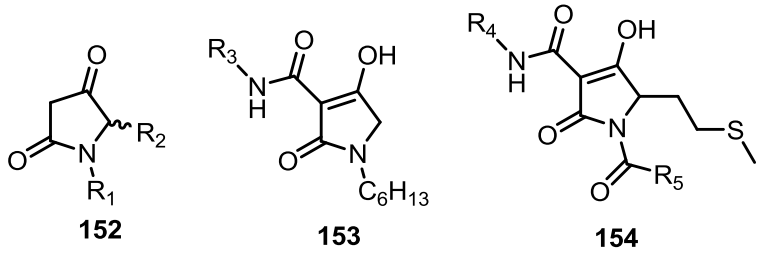


Figure 4.15. Tetramate libraries that are selective towards *S. aureus* compared with *E. coli* reported by Moloney and co-workers.[153, 154]

In summary, it was found that bicyclic tetramic acids with short side-chains (**124a, d-e**) did not possess significant antibacterial effect, while a bulky or hydrophobic side-chain at C-3 possessed enhanced antibacterial properties towards *S. aureus*. This was exemplified by piperidyl enamines **129a-c** and most of the 3-enoyltetramic acids (**127a-h**), all bearing bulky hydrophobic side-chains. These results suggest that a strategy that enables general side-chain functionalisation at C-3 would be advantageous towards the development of libraries of potential antibacterial lead compounds bearing a 3-acyltetramic acid moiety.

Although the enzymatic target(s) of these tetramic acids have not been identified, recent work by Moloney and co-workers[66] has shown that 3-acyltetramic acids, structurally similar to **127a-h**, which were inspired by the natural tetramic acid streptolydigin, possess dual mechanism of action towards bacterial RNA polymerase (RNAP) and undecaprenyl pyrophosphate synthase (UPPS). Another related natural product, such as kibdelomycin, is known to show DNA gyrase inhibitory activity.[56] Importantly, some of the 3-acyltetramic acids in that study are selective for bacterial cells over mammalian blood cell, suggesting that such systems might be suitable for exploitation in mammalian antibacterial chemotherapy.

4.4 Physiochemical properties and antibacterial activity of compounds

O'Shea and Moser[18] have demonstrated that the physicochemical properties of most known antibacterial drugs tend to be larger in molecular weight and are more polar as compared to other known drugs (Figure 4.16). A key reason for this is thought to be that most antibacterial drugs need to penetrate bacterial cell walls. This is compared with Lipinski's 'rule of five' (good absorption predicted if H-bond donors < 5; H-bond acceptors < 10; molecular weight < 500; clogP < 5) which is applicable for the prediction of good oral absorption in mammalian systems.

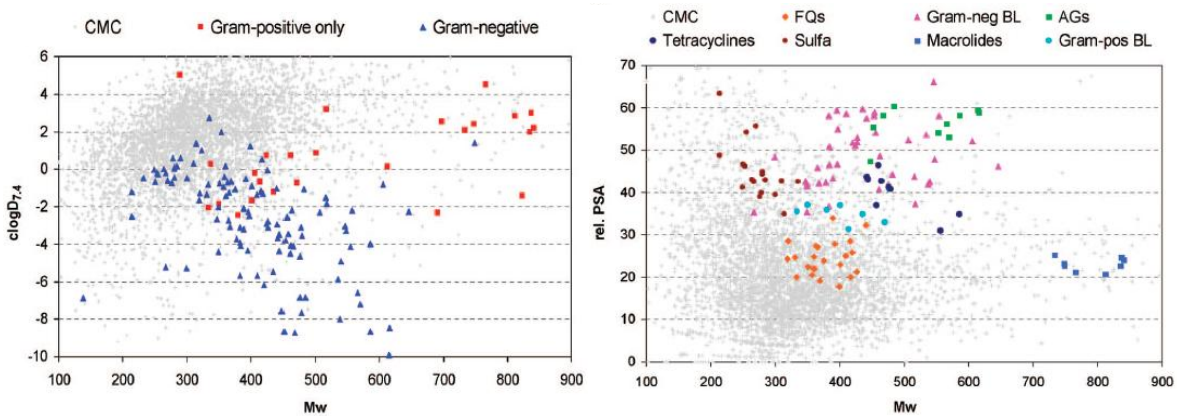


Figure 4.16. Correlation of physicochemical properties of known antibacterial drugs abstracted from R. O'Shea and H. E. Moser *J. Med. Chem.* **2008**, *51*, 2871-2878. Left: Plot of $clogD_{7.4}$ vs MW; Right: Plot of relative PSA (%PSA = PSA/MSA) vs MW.
MW = molecular weight; PSA = polar surface area; MSA = molecular surface area.
CMC = Comprehensive Medicical Chemistry; FQs = fluoroquinolones; BL = β-lactams; AGs = aminoglycosides.

In the context of physicochemical properties of our active compounds, compounds that are classified as 'active' towards the two bacterial strains tested were analysed. An 'active' compound was arbitrarily defined as one giving a zone size of more than 15 mm at a concentration of 1 mg/mL, regardless of it being distinct or a halo, when screened against either bacteria species, and plots of $clogD_{7.4}$ or %PSA against MW[155] (See Appendix B for calculated physicochemical data) are shown in Figure 4.17.

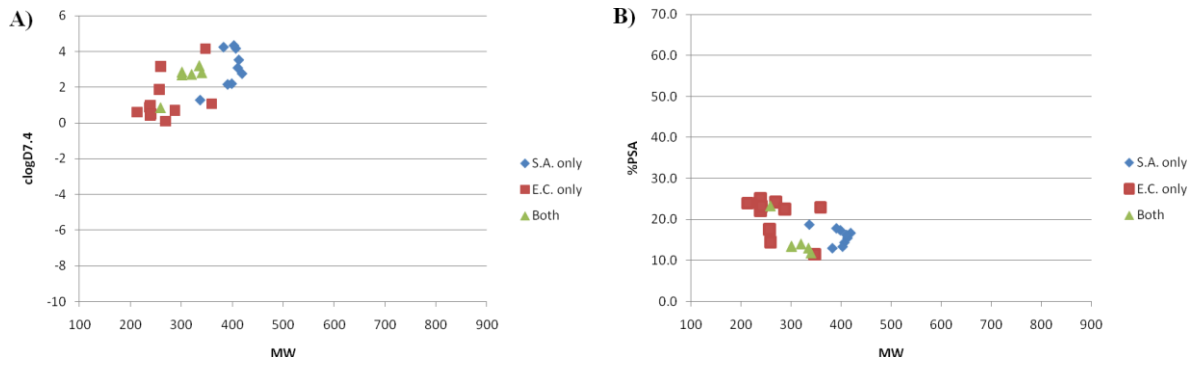


Figure 4.17. Correlation of physicochemical properties of 'active' compounds synthesised herein.
A) Plot of clog$D_{7.4}$ vs MW; B) Plot of %PSA vs MW.
"S.A. only" = compounds active towards *S. aureus*; "E.C. only" = compounds active towards *E. coli*; "Both" = compounds active towards both *S. aureus* and *E. coli*.

From these two plots, the 'active' compounds identified occupy a different space when compared to known antibacterial drugs as shown in Figure 4.16. Significantly, the 'active' compounds synthesised herein generally have a smaller molecular weight as well as being more hydrophobic, but of interest is that the active compound do seem to be tightly clustered in a range of clog$D_{7.4}$ and %PSA (*vide infra*).

It was observed that compounds active towards *E. coli* only are more polar than compounds active towards *S. aureus* only, as shown by lower clog$D_{7.4}$ or higher %PSA values (Figure 4.17). This trend is also observed when comparing the known antibacterial compounds plotted in Figure 4.16. Compounds active towards both *E. coli* and *S. aureus* generally have clog$D_{7.4}$ and %PSA values between the two aforementioned groups of compounds.

The observation that there may be a correlation between clog$D_{7.4}$, or %PSA, with antibacterial activity may be related to the ability of the compounds penetrating bacteria cell wall.[18] Antibacterial drugs are generally more polar than other drugs, and a certain degree of polarity is required for bactericidal molecules as the cell walls of both Gram-positive and Gram-

negative bacteria are charged.[156,157] Drug molecules are generally permeable across the cell wall of Gram-positive bacteria, made up of a rather porous peptidoglycans network.[158] However, a threshold appears to be present as very polar molecules may not be able to cross the lipophilic cytoplasmic membrane of the cell wall. Conversely, a larger polarity is required for molecules that traverse the cell wall of Gram-negative bacteria as non-specific influx is mainly via porins, water-filled transmembrane protein channels, present across its outer membrane.[159,160]

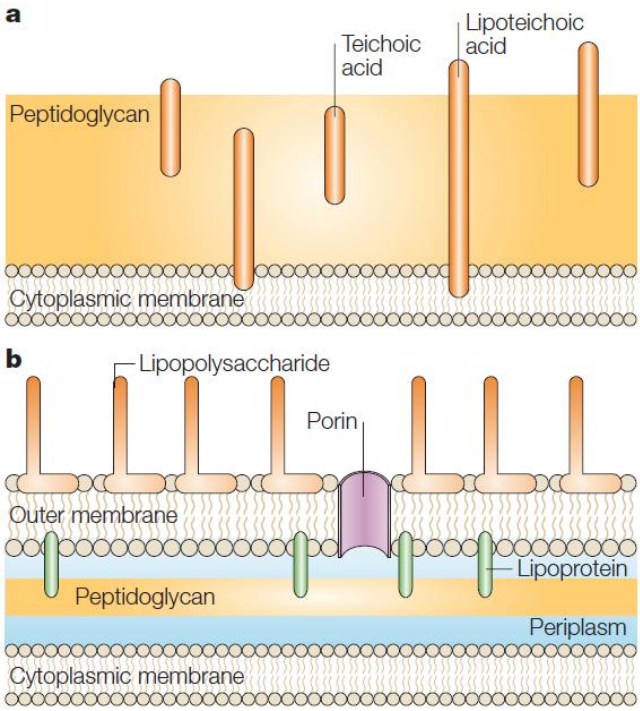


Figure 4.18. General cell wall structure of **a**) Gram-positive, and **b**) Gram-negative bacteria. Abstracted from M. T. Cabeen, C. Jacobs-Wagner *Nat. Rev. Microbiol.* **2005**, *3*, 601-610.

Optimal bioactivity for the different molecular scaffolds investigated was correlated with their respective clogP and %PSA ranges and the data are summarised in Table 4.2 below.

Table 4.2: clogP and %PSA ranges of compounds with optimal bioactivity.

Molecular Scaffold	clogP range	%PSA range
Epoxypyrrolidinones **77**	2.5-3.8	13-15%
Epoxypyrrolidinones **78**	-0.06-1.9	18-25%
2-Hydroxypyrrolidinones **91**	2.7-4.2	11-14%
2-Hydroxypyrrolidinones **92**	0.4-1.4	19-25%
2,3-Dihydroxypyrrolidinones **100**	-0.18-0.71	22-30%
Enamine tetramic acids **129**	4.1-4.8	11%
3-Enoyltetramic acids **127**	2.3-4.7	14%-20%

An investigation of the correlation between the polarity of the tested compounds with their antibacterial activity was made. A plot of $clogD_{7.4}$ (a descriptor of molecule polarity) against the antibacterial activity (zone sizes of compounds at concentrations of 1 mg/mL) of pyroglutamate-derived mimics and tetramic acid-derived mimics are shown respectively in Figure 4.19 below.

The optimal $clogD_{7.4}$ range lies between 2.0-4.5 for *S. aureus* (Gram-positive) selective tetramic acid-derived mimics (Figure 4.19 B), as discussed above.

As for the pyroglutamate-derived mimics (Figure 4.19 A), compounds falling within the two $clogD_{7.4}$ ranges (2.5-3.5 and 0.0-1.0) was observed to have significant antibacterial activity. On close inspection of the data, the peak in activity for compounds having a $clogD_{7.4}$ range between 2.5-3.5 coincides with good antibacterial activity with *S. aureus*. It was previously mentioned that more polar compounds are required to penetrate the Gram-negative bacteria cell wall via porins. Thus the activity of the compounds giving such significant *E. coli* activity posessing a $clogD_{7.4}$ range between 2.5-3.5 could be either due to inhibition of enzyme(s) located on the cell surface which are essential for bacterial growth, or utilising a non-porin entry pathway[161] into the bacteria cell.

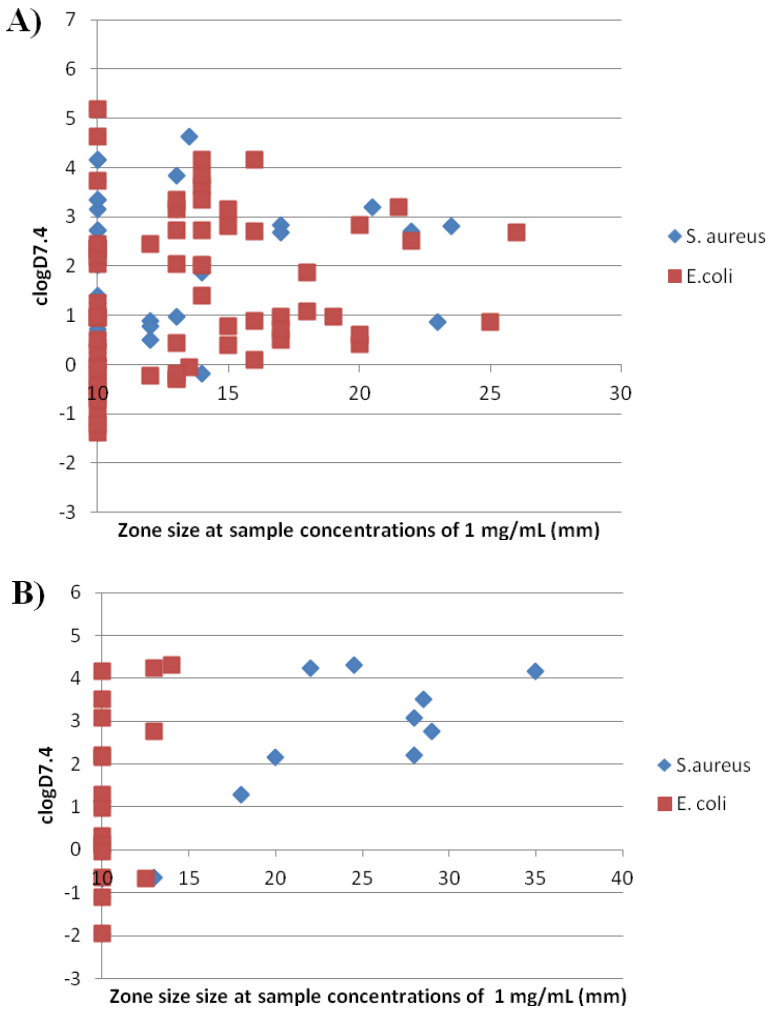


Figure 4.19. Correlation of $clogD_{7.4}$ (polarity) with the zone sizes (antibacterial activity) of A) pyroglutamate-derived, B) tetramic acid-derived compounds.

In addition to favourable calculated physicochemical properties of the tetramate derivatives, these compounds are also predicted to possess good oral absorption according to Lipinski's 'rule of five'. Even though these tetramates tend to deviate from the physicochemical properties of known antibacterial drugs, they were similar to other bioactive tetramate libraries studied by Moloney and co-workers (Figure 4.20).[59,66,162]

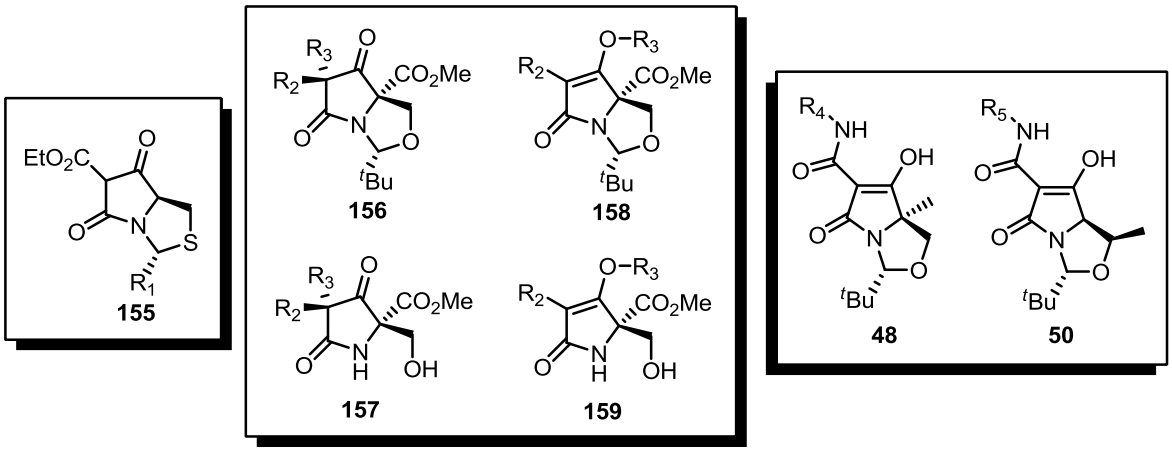


Figure 4.20. Tetramate libraries of active compounds reported by Moloney and co-workers with similar clogP and %PSA values to compounds synthesised herein.[59, 66, 162]

It is interesting to note that some pyrrolidinones with a long hydrophobic side-chain might have antibacterial activity due to their amphiphilic detergent-like structure, leading to non-specific disruption of the bacterial cell wall.[163,164] However, compounds such as **78g**, **92c**, 1**00c** were not active at all with *S. aureus*, suggesting that the aforementioned antibacterial activity is more likely to result from inhibition of specific bacterial target(s).

Of further interest is that electrophilic α,β-unsaturated lactams (**66b-e**, **75**) and dienenones (**150a-b**) were found to have consistent antibacterial activities against both *E. coli* and *S. aureus*. However, electrophilic compounds are usually not included in the drug discovery process. This is due to the possibility of indiscriminate covalent reaction with unintended cellular targets leading to toxicity, or their being predisposed to attack by cellular nucleophiles such as glutathione, resulting in short half-lives within the host.[165] Nonetheless, Scannevin and co-workers have discovered that certain electrophilic compounds such as fumarates could increase antioxidants levels in the cell, leading to cytoprotection during cellular stress.[166] Dimethyl fumarate, approved in 2013 and marketed by Biogen Idec as an immunomodulator under the name of Tecfidera, is expected to set a record in sales.[167] Thus, further investigation

with these compounds (**66b-e**, **75**, **150a-b**) would be required in order to assess their suitability as antibacterial lead compounds.

As mentioned previously, cell wall permeability may only be correlated with the polarity of a molecule. Thus, in light of the correlation of antibacterial activity with their physicochemical properties for these compound libraries, it would be prudent to assess the $clogD_{7.4}$/MW and %PSA/MW chemical spaces in conjunction with the privileged scaffolds described. This is because the observed antibacterial effects from this chosen phenotypic assay results from a combination of both bacterial cell wall permeability, as well as inhibition of enzymatic target(s).

Furthermore, the 'active' compounds (that is compounds with zone size of more than 15 mm at a concentration of 1 mg/mL) herein complied with Lipinski's 'rule of five' suggesting them to be suitable leads with good potential for oral absorption, a highly desirable property for new antibacterial drugs.

4.5 Summary for Chapter 4

In this chapter, the antibacterial properties of both pyroglutamate-derived and tetramic acid-derived pramanicin mimics were evaluated using hole-plate method.

Generally, the C-7 keto substituent of pyrrolidinones is important towards antibacterial activity when compared with pyrrolidinones having a C-7 carboxamido substituent. It was also observed that epoxypyrrolidinones after oxazolidine deprotection generally have selectivity for *E. coli* over *S. aureus*, whereas 2-hydroxypyrrolidinones, with or without oxazolidine deprotection, were generally selective for *E. coli* over *S. aureus*. Although the 2,3-dihydroxypyrrolidinones did not possess a strong antibacterial effect, most of the

derivatives investigated are selective for *E.coli* over *S. aureus*. Compounds with the α,β-unsaturated lactam motif were observed to be active against both *E. coli* and *S. aureus*. 3-Acyltetramic acids bearing bulky or hydrophobic side-chains at the C-3 position gave significant antibacterial activity towards *S. aureus* but generally only weak or no activity against *E. coli* was observed.

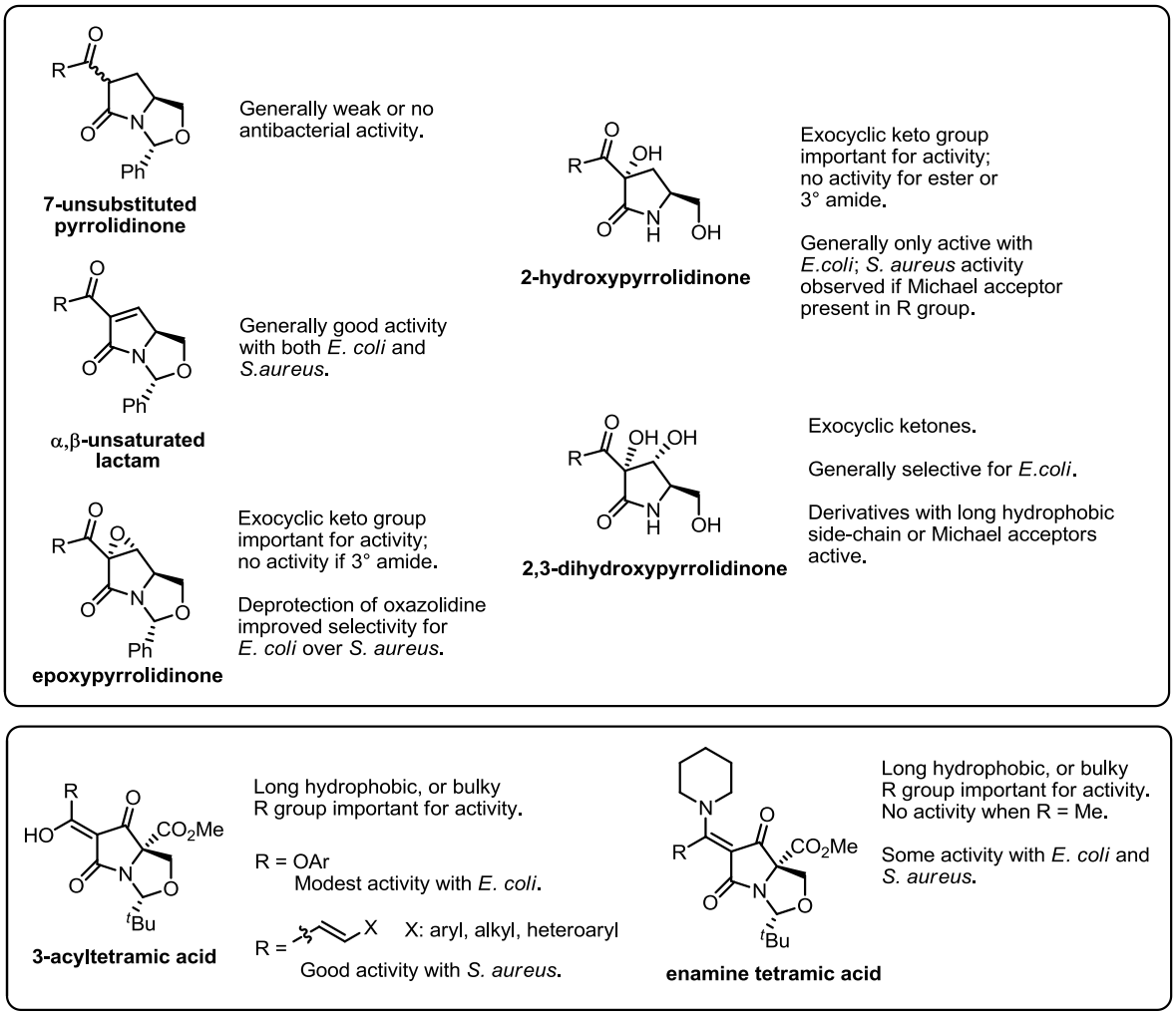


Figure 4.21. Summary of SAR of pyroglutamate-derived and tetramic acid-derived pramanicin mimics.

Overall, two sets of compounds, each having different selectivity for *E. coli* (Gram-negative) and *S. aureus* (Gram-positive), based on different natural products were obtained.

Analysis of the active compounds and their physicochemical properties gave insights to their cell permeability, and would assist with future studies relating to optimising their antibacterial properties. Although some of the compounds showing strong antibacterial activity possessed an amphiphilic detergent-like structure, selectivity towards either *E. coli* or *S. aureus* suggested that the observed activity was not a result of non-specific disruption of bacterial cell wall but indicated inhibition of specific bacterial target(s).

4.6 Conclusions

In light of these data, a natural product-inspired approach can provide novel molecular scaffolds suitable for investigation as new antibacterial drugs. Derivatives of three oxygenated pyrrolidinones scaffolds (epoxypyrrolidinones, 2-hydroxypyrrolidinones, 2,3-dihydroxypyrrolidinones) derived from pyroglutamates were described in Chapter 2. Chapter 3 described synthetic endeavours via tetramic acids to access a variant of pramanicin mimics. Although the synthesis towards oxygenated pyrrolidinones was unsuccessful, a series of 3-acyltetramic acids which was based on another class of natural products were obtained. Lastly, Chapter 4 describes the significant antibacterial properties observed with the chosen cell-based assay for the compounds synthesised herein, followed by a correlation with the physicochemical properties of these compounds.

The synthetic routes towards these two sets of pramanicin mimics allowed a wide range of side-chains to be attached to the parent molecular scaffold – functionalisation of the Weinreb amide for the oxygenated pyrrolidinones, and HWE olefination via a phosphorane for the tetramic acids – although some of these derivatives were obtained in low yields. However, these synthetic routes allow for the rapid functionalisation of the scaffolds, which could be used to access derivatives with higher molecular weight and polarity – a trend observed when comparing the physicochemical properties of our synthesised compounds with known

antibacterial drugs. This would hopefully provide compounds with enhanced antibacterial properties suitable for use as lead compounds towards the discovery of novel antibiotics.

CHAPTER 5: EXPERIMENTAL

5.1 General Procedures

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

^{13}C NMR spectra were recorded on a Bruker DQX400 spectrometer or Avance III (400 MHz) with UltraShield™ plus with cryoprobe at 100.6 MHz, or Bruker AVC500 spectrometer at 125.8 MHz, with proton decoupling. Chemical shifts (δ_C) are reported in parts per million (ppm) and are referenced to the residual protonated solvent peak. Assignment was aided by the use of edited HSQC and HMBC. HMBC experiments were performed on a Bruker AVC500 (500 MHz), or Avance III (400 MHz) spectrometer.

Infrared (IR) spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer, or Perkin Elmer Spectrum 100 FT-IR spectrometer, with an attached ATR module. Absorption maxima (υ_{max}) are reported in wavenumbers (cm^{-1}) and only selected peaks are reported.

Melting points were recorded using a Stuart Scientific SMP1 melting point apparatus or Büchi B-450 capillary melting point apparatus in open capillaries and are uncorrected.

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

field ionisation (FI). High resolution mass spectra (HRMS) were recorded on a Bruker microTOF (ESI), or Agilent accurate TOF-LC (1200 Series G1969A). The *m/z* values of major peaks are reported in Daltons and their intensities given as percentages of the base peaks.

Crystals for X-ray crystallography were grown from slow vapour diffusion at r.t. of petroleum ether into a solution of the requisite compound in CH_2Cl_2, or of CH_2Cl_2 into a solution of the requisite compound in MeOH.

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter or Jasco P-1030 polarimeter at the stated temperature, with concentrations *c* given in g/100mL $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}.

Thin layer chromatography (TLC) was performed using Merck aluminium foil backed sheets precoated with 0.2 mm Kieselgel 60 F_{254}. Product spots were visualised by quenching of UV fluorescence (λ_{max} 254 nm), iodine vapour, or by staining with an aqueous solution of $KMnO_4$ or a solution of 5% (w/v) phosphomolybdic acid in ethanol, followed by heating. Both dips were prepared according to J. Leonard, B. Lygo and G. Procter, "Advanced Practical Organic Chemistry", second edition, Blackie A & P, 1995. Retention factors (R_f) are quoted to the nearest 0.01. Flash column chromatography was carried out using Lancaster silica gel 60, 0.040-0.063 mm (230-400 mesh).

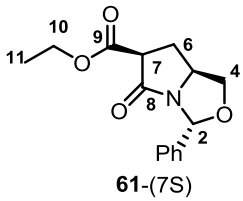
Reaction times are recorded in minutes (min), hours (h). Temperatures below 25°C were obtained using the following cold baths: 0°C ice/water, -10°C ice/methanol, -40°C dry ice/acetonitrile, -78°C dry ice/acetone. All reactions were carried out in oven-dried reaction flasks under inert (N_2) atmosphere unless otherwise stated. 'Petroleum ether' refers to that fraction of light petroleum ether boiling at 40-60°C and was used as received. All reagents were obtained either from Sigma Aldrich, Alfa Aesar, Lancaster Chemicals Ltd, or Matrix Scientific and used as supplied unless stated otherwise. *n*-BuLi was used as a solution in hexanes and was titrated against diphenylacetic acid before use.[168]

5.2 Experimental for Chapter 2

(2*R*, 5*S*)-7-Ethoxycarbonyl-8-oxo-2-phenyl-1-aza-3-oxa-bicyclo[3.3.0] octane, 61

Adapted procedure from N. K. Yee, Y. Dong, S. R. Kapadia, J. J. Song *J. Org. Chem.* **2002**, *67*, 8688-8691. Their respective spectroscopic data were consistent with those reported in the literature.[77]

A solution of 1 M solution of KHMDS in THF (7.63 mL, 7.63 mmol, 2.2 eq.) in a two-necked round-bottomed flask was cooled to 0°C. A solution of **53** (705 mg, 3.47 mmol, 1 eq.) in 10 mL of anhydrous THF was then added dropwise with stirring, and the resulting brown suspension was left to stir for a further 1 h at 0°C. A solution of ethyl chloroformate (0.33 mL, 3.47 mmol, 1 eq.) in 3 mL of anhydrous THF was added dropwise with stirring, and the reaction mixture was left to stir for 2 h at 0°C. The reaction was quenched with sat. aq. NH_4Cl and extracted with EtOAc. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents removed *in vacuo* to give the crude. Purification via silica column chromatography (Eluent 10% to 35% EtOAc/PE) gave the product as a pale yellow solid (764 mg, 80%) as a mixture of two diastereomers. It was found that **61** isomerises selectively to the 7*S* diastereomer on prolonged storage in pure form in the freezer (-20°C) or at ambient temperatures.



61-(7S)

δ_H (400 MHz, $CDCl_3$) 7.44 (d, *J* = 7.6 Hz, 2H, ArH), 7.30-7.38 (m, 3H, ArH), 6.32 (s, 1H, H-2), 4.20-4.34 (m, 3H, H-10 and H-4), 4.08-4.15 (m, 1H, H-5), 3.87 (t, *J* = 9.6 Hz, 1H, H-7), 3.68 (t, *J* = 8.1 Hz, 1H, H-4'), 2.56 (ddd, *J* = 7.3, 9.3, 13.4 Hz, 1H, H-6), 2.41 (ddd, *J* = 6.1, 9.9, 13.4 Hz, 1H, H-6'), 1.33 (t, *J* = 7.1 Hz, 3H, H-11); δ_C (100 MHz, $CDCl_3$) 172.18 (C-9), 169.14 (C-8), 138.31, 128.70, 128.45, 125.91 (ArC), 87.11 (C-2), 71.84 (C-4), 61.86 (C-10), 56.92 (C-5), 51.54 (C-7), 27.46 (C-6), 14.16 (C-11).

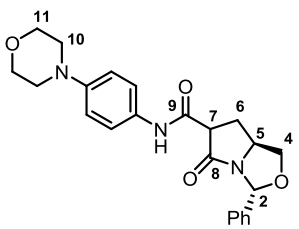
5.2.1 Experimental for epoxypyrrolidinones

The 7*R* and 7*S* diastereomers of **64a-e** and **65a-e** were assigned by comparing the chemical shifts values of H-6 and H-6' with those compounds reported by Cossy *et al.*[76] and Beard *et al.*[69]

General procedure for synthesis of amides **64a-e** from **61**

To a solution of the ester **61** (200 mg, 0.726 mmol, 1.0 eq.) in toluene (15 mL), was added the amine (2.1 eq.) and DMAP (0.1 eq.) and the resulting solution was heated at reflux for 18 h. The solution was then cooled to r.t., diluted with EtOAc (15 mL) and washed with 1% aq. HCl (15 mL). The aqueous layer was extracted with EtOAc and the combined organic layers were combined and washed with water and brine, dried with anhydrous $MgSO_4$ and filtered. The solvent was removed *in vacuo* and purification via silica gel flash column chromatography (Eluent: EtOAc/PE) gave amides **64a-e** as an inseparable mixture of diastereomers (at C-7).

(2*R*, 5*S*, 7*S*) and (2*R*, 5*S*, 7*R*)-1-Aza-7-(4'-morpholinophenylcarbamoyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 64a

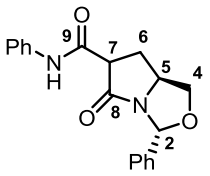


R_f = 0.11 and 0.19 (5% MeOH/EtOAc); brown oil; ν_{max} / cm^{-1} (film) 1697 (s, C=O), 1682 (s, C=O);

Diastereomer A (7*S*), diastereomer B (7*R*)

δ_H (400 MHz, $CDCl_3$) 9.56 (s, 1H, -NH-$_B$), 8.62 (s, 1H, -NH-$_A$), 7.35-7.51 (m, ArH), 6.84-6.90 (m, ArH), 6.33 (s, 1H, H-2_A), 6.30 (s, 1H, H-2_B), 4.35 (dd, *J* = 7.2, 8.3 Hz, 1H, H-4_B), 4.33 (dd, *J* = 6.3Hz, 7.8 Hz, 1H, H-4_A), 4.20-4.23 (m, 1H, H-5_A), 4.16 (m, 1H, H-5_B), 3.89 (Obscured by morpholinoaniline CH_2 signals, H-7_B), 3.84-3.87 (m, morpholinoaniline CH_2), 3.67 (dd, *J* = 5.0, 10.6 Hz, H-7_A), 3.58 (t, *J* = 8.3 Hz, 1H, H-4'$_B$), 3.49 (t, *J* = 8.5 Hz, 1H, H-4'$_A$), 3.09-3.13 (m, morpholinoaniline CH_2), 3.05 (ddd, *J* = 5.3, 8.1, 13.6 Hz , H-6_A), 2.74 (ddd, *J* = 7.3, 9.6, 13.4 Hz, H-6_B), 2.42 (ddd, *J* = 6.8, 10.4, 13.4 Hz, H-6'$_B$), 2.17 (ddd, *J* = 4.0, 10.6, 14.4 Hz, H-6'$_A$); δ_C (100 MHz, $CDCl_3$) 175.58 (C-8_A), 173.81 (C-8_B), 164.86 (C-9_B), 163.86 (C-9_A), 148.30, 148.23, 137.93, 130.48, 128.99, 128.61, 128.56, 125.96, 125.88, 121.17, 116.22, 116.18, 87.57 (C-2_A), 86.97 (C-2_B), 72.22 (C-4_B), 71.44 (C-4_A), 66.88 (C-11_A and 11_B), 57.53 (C-5_A), 56.20 (C-5_B), 51.27 (C-7_A), 50.05 (C-7_B), 49.72 (C-10_B), 49.69 (C-10_A), 26.85 (C-6_B), 23.56 (C-6_A); *m/z* (ESI+) 430.21 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{23}H_{25}N_3O_4Na$ ($[M+Na]^+$) 430.1737, found 430.1723.

(2*R*, 5*S*, 7*S*) and (2*R*, 5*S*, 7*R*)-1-Aza-3-oxa-8-oxo-2-phenyl-7-(phenylcarbamoyl)-bicyclo[3.3.0]octane, 64b

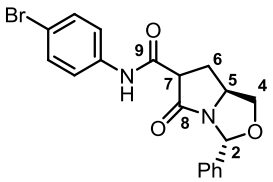


R_f = 0.47 and 0.32 (1:1 PE:EtOAc); brown oil; ν_{max} / cm^{-1} (film) 1699 (br, C=O), 1687 (br, C=O);

Diastereomer A (7*S*), diastereomer B (7*R*)

δ_H (500 MHz, $CDCl_3$) 9.72 (s, 1H, -NH-$_B$), 8.74 (s, 1H, -NH-$_A$), 7.34-7.60 (m, ArH), 7.11-7.14 (m ArH), 6.34 (s, 1H, H-2$_A$), 6.31 (s, 1H, H-2$_B$), 4.36 (dd, *J* = 6.3, 8.2 Hz, 1H, H-4'$_B$), 4.32 (dd, , *J* = 6.3, 8.2 Hz, 1H, H-4'$_A$), 4.31-4.38 (m, 1H, H-5$_B$), 4.16-4.31 (m, 1H, H-5$_A$), 3.90 (t, *J* = 9.9 Hz, 1H, H-7$_B$), 3.70 (dd, *J* = 5.4, 10.4 Hz, 1H, H-7$_A$), 3.59 (t, *J* = 8.4 Hz, 1H, H-4$_B$), 3.50 (t, *J* = 8.5 Hz, 1H, H-4$_A$), 3.08 (ddd, *J* = 5.4, 8.2, 13.6 Hz, 1H, H-6$_A$), 2.78 (ddd, *J* = 7.3, 9.5, 13.6 Hz, 1H, H-6$_B$), 2.44 (ddd, *J* = 6.9, 10.4, 13.6 Hz, 1H, H-6'$_B$), 2.20 (ddd, *J* = 4.1, 10.7, 14.5 Hz, 1H, H-6'$_A$); δ_C (125 MHz, $CDCl_3$) 175.45 (C-8$_A$), 173.62 (C-8$_B$), 165.14 (C-9$_B$), 165.02 (C-9$_A$), 137.88, 137.83, 137.59, 137.50, 129.01, 128.98, 128.61, 128.56, 128.45, 125.95, 125.85, 119.89, 119.80 87.59 (C-2$_A$), 86.98 (C-2$_B$), 72.23 (C-4$_B$), 71.42 (C-4$_A$), 57.45 (C-5$_A$), 56.14 (C-5$_B$), 51.30 (C-7$_A$), 50.11 (C-7$_B$), 26.79 (C-6$_B$), 23.28 (C-6$_A$); *m/z* (ESI-) 321.12 ([M-H]$^-$, 100%); HRMS (FI+) calculated for $C_{19}H_{18}N_2O_3$ ([M]$^+$) 322.1317 found 322.1314.

(2*R*, 5*S*, 7*R*) and (2*R*, 5*S*, 7*S*)-1-Aza-7-(4'-bromophenylcarbamoyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 64c



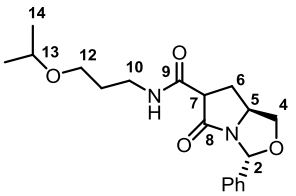
R_f = 0.25 and 0.53 (1:1 PE:EtOAc); brown oil; ν_{max} / cm^{-1} (film) 1700 (br, C=O), 1678 (br, C=O);

Diastereomer A (7*S*), diastereomer B (7*R*)

δ_H (500 MHz, $CDCl_3$) 9.80 (s, 1H, -NH-$_B$), 8.81 (s, 1H, -NH-$_A$), 7.35-7.50 (m,ArH), 6.33 (s, 1H, H-2$_A$), 6.30 (s, 1H, H-2$_B$), 4.37 (dd, *J* = 6.0, 8.2 Hz, 1H, H-4$_A$), 4.34 (dd, *J* = 6.3, 8.2 Hz, 1H, H-4$_B$), 4.24-4.30 (m, 1H, H-5$_B$), 4.16-4.23 (m, 1H, H-5$_A$), 3.88 (t, *J* = 10.1 Hz, 1H, H-7$_B$), 3.71 (dd, *J* = 5.7, 10.7 Hz, 1H, H-7$_A$), 3.59 (d, *J* = 8.5 Hz, 1H, H-4'$_B$), 3.51 (t, *J* = 8.8 Hz, 1H, H-4'$_A$), 3.05 (ddd, *J* = 5.4, 8.2, 13.9 Hz, 1H, H-6$_A$), 2.78 (ddd, *J* = 7.3, 9.5, 13.6 Hz, 1H, H-6$_B$), 2.42 (ddd, *J* = 6.9, 10.7, 13.6 Hz, 1H, H-6'$_B$), 2.21 (ddd, *J* = 3.8, 10.7, 14.2 Hz, 1H, H-6'$_A$); δ_C (125 MHz, $CDCl_3$) 175.32 (C-8$_A$), 173.41 (C-

8_B), 165.21 (C-9_B), 164.08 (C-9_A), 137.79, 137.69, 136.67, 136.57, 131.93 129.25, 129.07, 128.64, 128.59, 128.45, 128.47, 125.94, 125.84, 121.40, 121.33, 117.01, 116.91, 87.63 (C-2_A), 86.96 (C-2_B), 72.23 (C-4_B), 71.38 (C-4_A), 57.43 (C-5_A), 56.14 (C-5_B), 51.26 (C-7_A), 50.15 (C-7_B), 26.86 (C-6_B), 23.06 (C-6_A); HRMS (FI+) calculated for $C_{19}H_{17}BrN_2O_3$ ($[M]^+$) 400.0423, found 400.0439.

(2*R*, 5*S*, 7*R*) and (2*R*, 5*S*, 7*S*)-1-Aza-7-(3'-isopropoxypropylcarbamoyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 64d

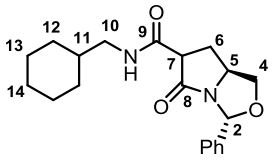


R_f = 0.38 and 0.26 (EtOAc); colourless oil; ν_{max} / cm^{-1} (film) 1699 (s, C=O), 1672 (br, C=O);

<u>Diastereomer A (7*S*), diastereomer B (7*R*)</u>

δ_H (500 MHz, $CDCl_3$) 7.68 (br s, 1H, -NH-$_B$), 7.31-7.45 (m, ArH), 7.00 (br s, 1H, -NH-$_A$), 6.28 (s, 2H, H-2_A and H-2_B), 4.30 (dd, *J* = 6.0, 8.2 Hz, 1H, H-4_B), 4.27 (dd, *J* = 6.3, 7.9 Hz, 1H, H-4_A), 4.16-4.22 (m, 1H, H-5_A), 4.08-4.14 (m, 1H, H-5_B), 3.58 (t, *J* = 9.8 Hz, 1H, H-7_B), 3.54-3.58 (m, 2H, H-13_A and H-13_B), 3.53-3.55 (m, 1H, H-4'$_B$), 3.50-3.47 (m, 5H, H-7_A and H-12_A; H-12_B), 3.44-3.46 (m, 1H, H-4'$_A$), 3.38-3.45 (m, 2H, H-10_B), 3.30- 3.37 (m, 2H, H-10_A), 2.99 (ddd, *J* = 4.7, 7.9, 13.2 Hz, 1H, H-6_A), 2.67 (ddd, *J* = 7.6, 9.8, 13.6 Hz, 1H, H-6_B), 2.38 (ddd, *J* = 6.3, 9.8, 13.6 Hz, 1H, H-6'$_B$), 2.10 (ddd, *J* = 4.4, 10.4, 14.5 Hz, 1H, H-6'$_A$), 1.75-1.83 (m, 4H, H-11_A and H-11_B), 1.18 (d, *J* = 3.5 Hz, 3H, H-14_B), 1.16 (d, *J* = 3.5 Hz, H-14_A; d, *J* = 3.5 Hz, H-14'$_B$; 6H), 1.14 (d, *J* = 3.5 Hz, 3H, H-14'$_A$); δ_C (125 MHz, $CDCl_3$) 175.33 (C-8_A), 174.02 (C-8_B), 167.02 (C-9_B), 166.09 (C-9_A), 138.16, 128.80, 128.72, 128.50, 128.46, 125.89, 87.40 (C-2_A), 86.91 (C-2_B), 72.03 (C-4_B), 71.71 (C-13_A), 71.63 (C-13_B), 71.57 (C-4_A), 66.15 (C-12_A), 65.96 (C-12_B), 57.64 (C-5_A), 56.24 (C-5_B), 51.11 (C-7_A), 49.67 (C-7_B), 38.14 (C-10_A), 37.54 (C-10_B), 29.61 (C-11_B), 29.43 (C-11_A), 26.69 (C-6_B), 24.28 (C-6_A), 22.06 (C-14_A), 22.03 (C-14_B); *m/z* (ESI+) 369.19 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{19}H_{26}N_2O_4Na$ 369.1785, found 369.1778.

(2*R*, 5*S*, 7*R*) and (2*R*, 5*S*, 7*S*)-1-Aza-7-(cyclohexylmethylcarbamoyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 64e



R_f = 0.20 and 0.28 (1:1 PE:EtOAc); White solid; mp = 138-141°C; ν_{max} / cm^{-1} (film) 1697 (br, C=O), 1656 (br, C=O);

Diastereomer A (7*S*), diastereomer B (7*R*)

δ_H (500 MHz, $CDCl_3$) 7.65 (br s, 1H, -NH-$_B$), 7.32-7.46 (m, ArH), 6.77 (br s, 1H, -NH-$_A$), 6.29 (s, 1H, H-2$_B$), 6.30 (s, 1H, H-2$_A$), 4.32 (dd, *J* = 6.0, 8.2 Hz, 1H, H-4$_B$), 4.28 (dd, *J* = 6.3, 8.2 Hz, 1H, H-4$_A$), 4.16-4.21 (m, 1H, H-5$_A$), 4.10-4.16 (m, 1H, H-5$_B$), 3.71 (t, *J* = 10.1 Hz, 1H, H-7$_B$), 3.56 (t, *J* = 8.2 Hz, 1H, H-4'$_B$), 3.50 (dd, *J* = 5.0, 10.4 Hz, 1H, H-7$_A$), 3.47 (t, *J* = 8.5 Hz, 1H, H-4'$_A$), 3.10-3.21 (m, 4H, H-10$_A$ and H-10$_B$), 3.01 (ddd, *J* = 4.7, 7.9, 12.9 Hz, 1H, H-6$_A$), 2.70 (ddd, *J* = 7.3, 9.8, 13.6 Hz, 1H, H-6$_B$), 2.37 (ddd, *J* = 6.6, 10.4, 13.6 Hz, 1H, H-6'$_B$), 2.12 (ddd, *J* = 4.4, 10.4, 14.5 Hz, 1H, H-6'$_A$), 1.64-1.77 (m, 10H, H-12$_A$, H-13$_A$, one of H-14$_A$; H-12$_B$, H-13$_B$, one of H-14$_B$), 1.45-1.55 (m, 2H, H-11$_A$; H-11$_B$), 1.10-1.29 (m, 6H, H-13'$_A$ and one of H-14$_A$; H-13'$_B$ and one of H-14$_B$), 0.86-1.00 (m, 4H, H-12'$_A$; H-12'$_B$); δ_C (125 MHz, $CDCl_3$) 175.63 (C-8$_A$), 174.04 (C-8$_B$), 167.02 (C-9$_B$), 166.02 (C-9$_A$), 138.04, 128.87, 128.76, 128.54, 128.50, 125.89, 125.82, 87.32 (C-2$_A$), 86.86 (C-2$_B$), 72.13 (C-4$_B$), 71.53 (C-4$_A$), 57.62 (C-5$_A$), 56.22 (C-5$_B$), 50.89 (C-7$_A$), 49.51 (C-7$_B$), 46.06 (C-10$_A$), 45.84 (C-10$_B$), 37.83 (C-11$_A$), 37.73 (C-11$_B$), 30.82 (C-12$_A$), 30.70 (C-12$_B$), 26.96 (C-6$_B$), 26.35 (C-14$_B$), 26.32 (C-14$_A$), 25.81 (C-13$_B$), 25.77 (C-13$_A$), 24.02 (C-6$_A$); *m/z* (ESI-) 341.20 ([M-H]$^-$, 100%); HRMS (ESI+) calculated for $C_{20}H_{26}N_2O_3Na$ 365.1836, found 365.1828.

General procedure for synthesis of selenyl derivatives **65a-e** from amides **64a-e**

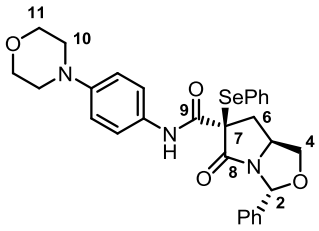
(NaH was pre-washed with dry Et_2O and used immediately for the reaction.)

Amides **64a-e** (1.0 eq.) in anhydrous THF (approx. concentration 0.10-0.13 M) was added dropwise to NaH (1.0 eq.) at 0°C and the mixture was warmed to r.t. and stirred for 10 min. PhSeBr (1.0 eq.) in anhydrous THF (approx. concentration 0.27-0.40 M) was then added dropwise to the mixture at r.t., and stirred for a further 30 min. The reaction mixture was then quenched with sat. aq. NH_4Cl and extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and

the solvents removed *in vacuo* to give the crude. Purification via flash column chromatography on silica gel (Eluent: EtOAc/PE) to give the two separate diastereomers.

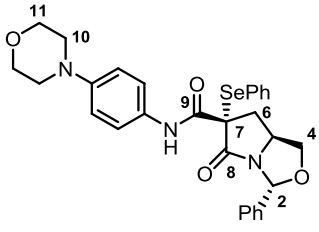
(2*R*, 5*S*, 7*R*)-1-Aza-7-(4'-morpholinophenylcabamoyl)-3-oxa-8-oxo-2-phenyl-7-(phenylselanyl)-bicyclo[3.3.0]octane, (7*R*)-65a

Scale of reaction: 125 mg (0.307 mmol), product: 68 mg, 40% (combination of both diastereomers);



R_f = 0.26 (1:1 PE:EtOAc); Brown oil; $[\alpha]_D^{20}$ = +135.8 (*c*= 0.55 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1697 (s, C=O), 1663 (s, C=O); δ_H (400 MHz, $CDCl_3$) 8.61 (br s, 1H, -NH-), 7.65-7.68 (m, 2H, ArH), 7.26-7.46 (m, 10H, ArH), 6.82-6.86 (m, 2H, ArH), 6.32 (s, 1H, H-2), 4.26 (t, *J* = 6.8 Hz, 1H, H-4), 4.05-4.11 (m, 1H, H-5), 3.87 (br t, *J* = 4.8 Hz, 4H, H-11), 3.61 (t, *J* = 8.5 Hz, 1H, H-4'), 3.35 (dd, *J* = 8.5, 15.4 Hz, 1H, H-6), 3.12 (br t, *J* = 4.8 Hz, 4H, H-10), 2.24 (dd, *J* = 3.1, 15.4 Hz, 1H, H-6'); δ_C (125 MHz, $CDCl_3$) 175.45 (C-8), 164.98 (C-9), 148.23, 137.93, 137.72, 131.49, 130.17, 130.29, 129.26, 128.90, 128.56, 127.13, 125.85, 120.96, 116.04 (ArC), 88.34 (C-2), 70.72 (C-4), 66.87 (C-11), 55.53 (C-5), 54.56 (C-7), 49.67 (C-10), 30.40 (C-6); *m/z* (ESI+) 586.14 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{29}H_{29}N_3O_4SeNa$ ($[M+Na]^+$) 586.1215, found 586.1207.

(2*R*, 5*S*, 7*S*)-1-Aza-7-(4'-morpholinophenylcabamoyl)-3-oxa-8-oxo-2-phenyl-7-(phenylselanyl)-bicyclo[3.3.0]octane, (7*S*)-65a

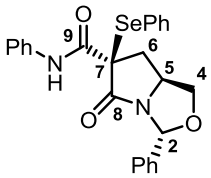


R_f = 0.4 (1:1 PE:EtOAc); brown oil; $[\alpha]_D^{20}$ = +32.3 (*c*= 0.50 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1697 (s, C=O), 1663 (s, C=O); δ_H (400 MHz, $CDCl_3$) 9.36 (br s, 1H, -NH-), 7.55-7.58 (m, 2H, ArH), 7.36-7.47 (m, 8H, ArH), 7.15-7.20 (m, 2H, ArH), 6.85-6.89 (m, 2H, ArH), 6.24 (s, 1H, H-2), 4.33 (dd, *J* = 6.1, 8.3 Hz, 1H, H-4), 4.00 (m, 1H, H-5), 3.87 (t, br, *J* = 4.8 Hz, 4H, H-11), 3.57 (t, *J* = 8.2 Hz, 1H, H-4'), 3.13 (br t, *J* = 4.8 Hz, 4H, H-10), 2.72 (d, *J* = 7.1 Hz, 2H, H-6 and H-6'); δ_C (125 MHz, $CDCl_3$) 173.29 (C-8), 166.02 (C-9), 148.13, 138.14, 137.57, 130.36, 130.35, 129.04, 129.03, 128.59, 126.26, 126.07, 120.94, 116.12 (ArC), 86.83 (C-2), 72.00 (C-4), 66.88 (C-11), 54.84 (C-5), 53.82 (C-

7), 49.70 (C-10), 36.06 (C-6); *m/z* (ESI+) 586.15 ([M+Na]$^+$, 100%); HRMS (ESI+) calculated for $C_{29}H_{29}N_3O_4SeNa$ ([M+Na]$^+$) 586.1215, found 586.1204.

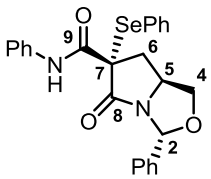
(2*R*, 5*S*, 7*R*)-1-Aza-3-oxa-8-oxo-2-phenyl-7-(phenylselanyl)-7-(phenylcarbamoyl)-bicyclo[3.3.0]octane, (7*R*)-65b

Scale of reaction: 150 mg (0.465 mmol), product: 237 mg, 87% (combination of both diastereomers);



R_f = 0.55 (2:1 PE:EtOAc); brown oil; $[\alpha]_D^{20}$ = +47.1 (*c*= 0.28 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1699 (br, C=O), 1669 (br, C=O); δ_H (500 MHz, $CDCl_3$) 8.74 (br s, 1H, -NH-), 7.67 (d, *J* = 8.2 Hz, 2H, ArH), 7.28-7.46 (m, 12H, ArH), 7.10 (t, *J* = 7.3 Hz, 1H, ArH), 6.33 (s, 1H, H-2), 4.27 (t, *J* = 8.7 Hz, 1H, H-4), 4.06-4.14 (m, 1H, H-5), 3.64 (t, *J*= 8.0 Hz, 1H, H-4'), 3.37 (dd, *J* = 8.4, 15.5Hz, 1H H-6), 2.26 (dd, *J* = 2.8, 15.5 Hz, 1H, H-6'); δ_C (125 MHz, $CDCl_3$) 175.43 (C-8), 165.35 (C-9),137.90, 137.74, 137.34, 130.37, 129.28, 128.92, 128.83, 128.57, 127.05, 125.85, 124.40, 119.67 (ArC), 88.38 (C-2), 70.68 (C-4), 55.51 (C-5), 54.43 (C-7), 30.22 (C-6); *m/z* (ESI+) 477.05 ([M-H]$^-$, 36%); HRMS (ESI+) calculated for $C_{25}H_{22}N_2NaO_3Se$ ([M+Na]$^+$) 501.0689, found 501.0690.

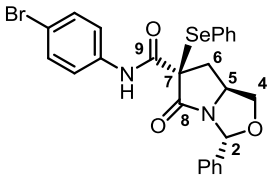
(2*R*, 5*S*, 7*S*)-1-Aza-3-oxa-8-oxo-2-phenyl-7-(phenylselanyl)-7-(phenylcarbamoyl)-bicyclo[3.3.0]octane, (7*S*)-65b



R_f = 0.45 (2:1 PE:EtOAc); brown oil; $[\alpha]_D^{20}$ = +74.0 (*c*= 1.0 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1699 (s, C=O), 1669 (s, C=O); δ_H (500 MHz, $CDCl_3$) 9.49 (br s, 1H, -NH-), 7.57 (d, *J* = 7.9 Hz , 2H, ArH), 7.36-7.50 (m, 7H, ArH), 7.32 (t, *J* = 8.5 Hz, 1H, ArH), 7.31 (t, *J* = 8.3 Hz , 2H, ArH), 7.18 (t, *J* = 7.7 Hz , 2H, ArH), 7.11 (t, *J* = 7.6 Hz , 1H, ArH), 6.24 (s, 1H, H-2), 4.34 (dd, *J* = 6.3, 8.5 Hz, 1H, H-4), 4.03 (apparent quintet, *J* = 6.8 Hz, 1H, H-5), 3.58 (t, *J*= 8.5 Hz, 1H, H-4'), 2.72 (d, *J* = 6.9 Hz, 2H, H-6 and H-6'); δ_C (125 MHz, $CDCl_3$) 173.17 (C-8), 166.46 (C-9), 138.15, 137.55, 137.48, 130.42, 129.08, 129.05, 128.90, 128.61, 126.22, 126.08, 124.31, 119.74 (ArC), 86.86 (C-2), 71.99 (C-4), 54.84 (C-5), 53.83 (C-7), 36.00 (C-6); *m/z* (ESI+) 477.05 ([M-H]$^-$, 100%); HRMS (ESI+) calculated for $C_{25}H_{22}N_2NaO_3Se$ ([M+Na]$^+$) 501.0689, found 501.0691.

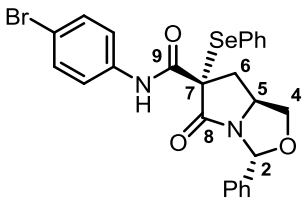
(2*R*, 5*S*, 7*R*)-1-Aza-7-(4'-bromophenylcarbamoyl)-3-oxa-8-oxo-2-phenyl-7-(phenylselanyl)-bicyclo[3.3.0]octane, (7*R*)-65c

Scale of reaction: 235 mg (0.586 mmol), product: 250 mg, 77% (combination of both diastereomers);



R_f = 0.49 (2:1 PE:EtOAc); light brown oil; $[\alpha]_D^{20}$ = +215.9 (*c*= 1.15 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1698 (s, C=O), 1668 (s, C=O); δ_H (500 MHz, $CDCl_3$) 8.74(s, 1H, -NH-), 7.64 (d, *J* = 8.2 Hz, 2H, ArH), 7.31-7.46 (m, 11H, ArH), 7.25-7.26 (m, 1H, ArH), 6.33 (s, 1H, H-2), 4.29 (dd, *J* = 6.3, 7.9 Hz, 1H, H-4), 4.06-4.11 (m, 1H, H-5), 3.68 (dd, *J* = 7.9, 9.5 Hz, 1H, H-4'), 3.31 (dd, *J* = 8.5, 15.4 Hz, 1H, H-6), 2.25 (dd, *J* = 2.8, 15.8 Hz, 1H, H-6'); δ_C (125 MHz, $CDCl_3$) 175.50 (C-8), 165.44 (C-9), 137.85, 137.82, 136.39, 131.79, 130.51, 129.29, 129.23, 128.97, 128.59, 128.44, 127.03, 125.84, 121.16, 116.95 (ArC), 88.49 (C-2), 70.60 (C-4), 55.41 (C-5), 53.90 (C-7), 29.88 (C-6); *m/z* (ESI-) 554.96 ($[M-H]^-$, 100%); HRMS (ESI-) calculated for $C_{25}H_{20}BrN_2O_3Se$ ($[M-H]^-$) 554.9827, found 554.9821.

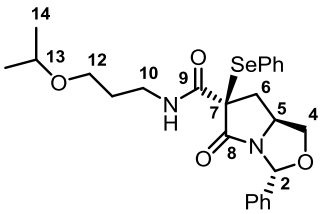
(2*R*, 5*S*, 7*S*)-1-Aza-7-(4'-bromophenylcarbamoyl)-3-oxa-8-oxo-2-phenyl-7-(phenylselanyl)-bicyclo[3.3.0]octane, -7*S*)-65c



R_f = 0.40 (2:1 PE:EtOAc); brown oil; $[\alpha]_D^{20}$ = +50.6 (*c*= 1.20 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1698 (s, C=O), 1668 (s, C=O); δ_H (500 MHz, $CDCl_3$) 9.50 (s, 1H, -NH-), 7.54 (d, *J* = 8.2 Hz, 2H, ArH), 7.33-7.47 (m, 10H, ArH), 7.18 (t, *J* = 7.6 Hz, 2H, ArH), 6.24 (s, 1H, H-2), 4.34 (dd, *J* = 6.3, 8.5 Hz, 1H, H-4), 4.04-4.10 (m, 1H, H-5), 3.58 (t, *J* = 8.4 Hz, 1H, H-4'), 2.70 (dd, *J* = 4.1, 7.6 Hz, 2H, H-6 and H-6'); δ_C (125 MHz, $CDCl_3$) 173.10 (C-8), 166.54 (C-9), 138.13, 137.44, 136.54, 131.86, 130.52, 129.11, 128.98, 128.64, 128.30, 126.11, 126.07, 121.24, 116.83 (ArC), 86.87 (C-2), 71.95 (C-4), 54.85 (C-5), 53.67 (C-7), 35.97 (C-6); *m/z* (ESI-) 554.95 ($[M-H]^-$, 100%); HRMS (ESI-) calculated for $C_{25}H_{20}BrN_2O_3Se$ ($[M-H]^-$) 554.9827, found 554.9825.

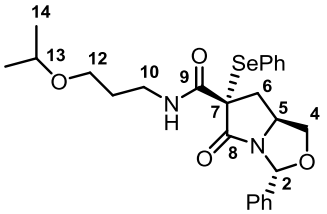
(2*R*, 5*S*, 7*R*)-1-Aza-7-(3'-isopropoxypropylcarbamoyl)-3-oxa-8-oxo-2-phenyl-7-(phenylselanyl)-bicyclo[3.3.0]octane, (7*R*)-65d

Scale of reaction: 190 mg (0.548 mmol), product: 216 mg, 78% (combination of both diastereomers);



R_f = 0.47 (1:1 PE:EtOAc); pale yellow oil; $[\alpha]_D^{20}$= +170.5 (*c*= 1.38 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1692 (s, C=O), 1668 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.66 (d, *J* = 8.2 Hz, 2H, ArH), 7.31-7.45 (m, 8H, ArH), 7.17 (t, *J* = 5.0 Hz, 1H, -NH-), 6.24 (s, 1H, H-2), 4.16 (dd, *J* = 6.3, 7.9 Hz, 1H, H-4), 4.01-4.07 (m, 1H, H-5), 3.52 (septet, *J* = 6.3 Hz, 1H, H-13), 3.39-3.43 (m, 2H, H-12), 3.28-3.35 (m, 4H, H-4', H-6, H-10), 2.13 (dd, *J* = 3.8, 15.1 Hz, 1H, H-6'), 1.66-1.71 (m, 2H, H-11), 1.14 (d, *J* = 6.0 Hz, 6H, H-14); δ_C (125 MHz, $CDCl_3$) 174.81 (C-8), 167.28 (C-9), 138.06, 137.18, 129.95, 129.26, 128.79, 128.48, 127.24, 125.91 (ArC), 87.93 (C-2), 71.68 (C-13), 71.07 (C-4), 65.73 (C-12), 55.97 (C-7), 54.81 (C-5), 37.23 (C-10), 36.10 (C-6), 29.67 (C-11), 22.09, 22.06 (C-14); *m/z* (ESI+) 525.17 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{25}H_{30}N_2O_4SeNa$ 525.1264, found 525.1259.

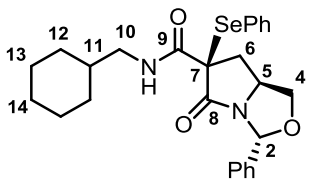
(2*R*, 5*S*, 7*S*)-1-Aza-7-(3'-isopropoxypropylcarbamoyl)-3-oxa-8-oxo-2-phenyl-7-(phenylselanyl)-bicyclo[3.3.0]octane, (7*S*)-65d



R_f = 0.18 (1:1 PE:EtOAc); pale yellow oil; $[\alpha]_D^{20}$= +102.8 (*c*= 1.44 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1696 (s, C=O), 1662 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.72 (t, *J* = 5.0 Hz, 1H, -NH-), 7.57 (t, *J* = 8.2 Hz, 2H, ArH), 7.35-7.42 (m, 6H, ArH), 7.18 (t, *J* = 7.6 Hz, 2H, ArH), 6.21 (s, 1H, H-2), 4.23 (dd, *J* = 6.3, 8.5Hz, 1H, H-4), 3.73-3.79 (m, 1H, H-5), 3.55 (septet, *J* = 6.0 Hz, 1H, H-13), 3.49 (d, *J* = 8.2 Hz, 1H, H-4'), 3.43 (t, *J* = 6.3 Hz, 2H, H-12), 3.30- 3.38 (m, 2H, H-10), 2.71 (dd, *J* = 6.9, 14.8 Hz, 1H, H-6), 2.64 (dd, *J* = 6.9, 14.8Hz, 1H, H-6'), 1.70-1.77 (m, 2H, H-11), 1.16 (d, *J* = 1.9 Hz, 3H, H-14), 1.15 (d, *J* = 1.9 Hz, 3H, H-14'); δ_C (125 MHz, $CDCl_3$) 173.60 (C-8), 168.24 (C-9), 137.92, 137.88, 137.73, 130.14, 129.01, 128.86, 128.48, 126.43, 126.04 (ArC), 86.77 (C-2), 71.95 (C-4), 71.59 (C-13), 65.59 (C-12), 54.81 (C-5), 53.76 (C-7), 37.23 (C-10), 36.10 (C-6), 29.67 (C-11), 22.09, 22.06 (C-14); *m/z* (ESI+) 525.15 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{25}H_{30}N_2O_4SeNa$ 525.1264, found 525.1249.

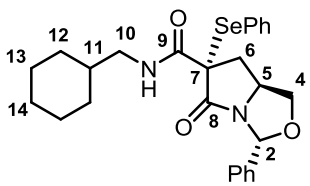
(2*R*, 5*S*, 7*R*)-1-Aza-7-(cyclohexylmethylcarbamoyl)-3-oxa-8-oxo-2-phenyl-7-(phenylselanyl)-bicyclo[3.3.0]octane, (7*R*)-65e

Scale of reaction: 200 mg (0.584 mmol), product: 253 mg, 87% (combination of both diastereomers);



R_f = 0.65 (1:1 PE:EtOAc); colourless oil; $[\alpha]_D^{20}$ = +176.8 (*c*= 1.44 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1693 (s, C=O) with broad shoulder; δ_H (500 MHz, $CDCl_3$) 7.65-7.67 (m, 2H, ArH), 7.32-7.46 (m, 8H, ArH), 6.95 (t, *J* = 5.7Hz, 1H, -NH-), 6.25 (s, 1H, H-2), 4.17 (dd, *J* = 6.3, 7.9 Hz, 1H, H-4), 4.02-4.07 (m, 1H, H-5), 3.33 (dd, *J* = 8.2, 8.8 Hz, 1H, H-4' and dd, *J* = 8.2, 14.8 Hz, 1H, H-6), 2.97-3.09 (m, 2H, H-10), 2.13 (dd, *J* = 3.8, 14.8 Hz, 1H, H-6'), 1.62-1.70 (m, 5H, H-12, H-13, one of H-14), 1.34-1.42 (m, 1H, H-11), 1.07-1.28 (m, 3H, H-13' and one of H-14), 0.83-0.91 (m, 2H, H-12'); δ_C (125 MHz, $CDCl_3$) 175.01 (C-8), 167.18 (C-9), 138.01, 137.21, 130.01, 129.28, 128.83, 128.51, 127.23, 125.85 (ArC), 87.88 (C-2), 71.04 (C-4), 55.84 (C-7), 55.81 (C-5), 46.20 (C-10), 37.65 (C-11), 31.48 (C-6), 30.68 (C-12), 26.29 (C-14), 25.76 (C-13); *m/z* (ESI-) 497.14 ($[M-H]^-$, 57%); HRMS (ESI+) calculated for $C_{26}H_{30}N_2O_3SeNa$ 521.1315, found 521.1309.

(2*R*, 5*S*, 7*S*)-1-Aza-7-(cyclohexylmethylcarbamoyl)-3-oxa-8-oxo-2-phenyl-7-(phenylselanyl)-bicyclo[3.3.0]octane, (7*S*)-65e



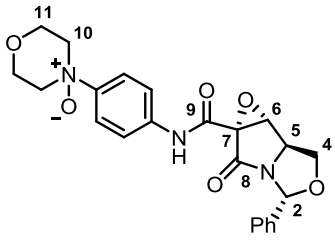
R_f = 0.44 (1:1 PE:EtOAc); colourless solid; mp = 121-124°C; $[\alpha]_D^{20}$ = +91.9 (*c*= 0.72 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1694 (s, C=O), 1662 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.65 (t, *J* = 5.0 Hz, 1H, -NH-), 7.57 (d, *J* = 8.2 Hz, 2H, ArH), 7.36-7.43 (m, 6H, ArH), 7.18 (t, *J* = 7.9 Hz, 2H, ArH), 6.22 (s, 1H, H-2), 4.25 (dd, *J* = 6.3, 8.2 Hz, 1H, H-4), 3.74-3.80 (m, 1H, H-5), 3.51 (t, *J* = 8.2 Hz, 1H, H-4'), 3.01-3.13 (m, 2H, H-10), 2.62-2.72 (m, 2H, H-6 and H-6'), 1.65-1.73 (m, 5H, H-12, H-13, one of H-14), 1.37-1.44 (m, 1H, H-11), 1.11-1.27 (m, 3H, H-13' and one of H-14), 0.86-0.95 (m, 2H, H-12'); δ_C (125 MHz, $CDCl_3$) 173.68 (C-8), 168.22 (C-9), 137.89, 137.69, 130.14, 128.99, 128.90, 128.51, 126.42, 126.04 (ArC), 86.74 (C-2), 72.03 (C-4), 54.85 (C-5), 53.75 (C-7), 45.91 (C-10), 37.62 (C-11), 36.09 (C-6), 30.82 (C-

12), 26.32 (C-14), 25.80 (C-13); *m/z* (ESI-) 497.15 ([M-H]$^-$, 96%); HRMS (ESI+) calculated for $C_{26}H_{30}N_2O_3SeNa$ 521.1315, found 521.1315.

Oxidative elimination-epoxidation of **65a**

35% (wt. in H_2O) H_2O_2 (75 μL, 0.848 mmol, 6 eq.) was added at r.t. to a solution of the selenyl amide **65a** (80 mg, 0.142 mmol, 1.0 eq.) in CH_2Cl_2 and the biphasic reaction mixture was stirred vigorously for 1 h. The reaction was quenched with sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents removed *in vacuo* to give the crude product. Purification via silica gel flash column chromatography (Eluent: 1% to 5% MeOH/EtOAc) gave **67a** (30 mg, 40%) as a white waxy solid.

(2*R*, 5*S*, 7*S*)-1-Aza-6,7-epoxy -7-(4'-morpholino-*N*-oxide-phenylcarbamoyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, **67a**



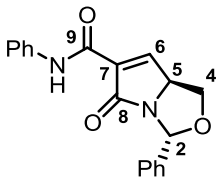
R_f = 0.15 (5% MeOH/CH_2Cl_2); white waxy solid; $[\alpha]_D^{20}$ = +188.5 (c= 1.23 in $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 10.26 (br s, 1H, -NH-), 8.00 (d, *J*= 8.5 Hz, 2H, morpholinoaniline ArH), 7.77 (d, *J* = 8.5 Hz, 2H, morpholinoaniline ArH), 7.31-7.46 (m, 5H, PhH), 6.38 (s, 1H, H-2), 4.70 (apparent t, *J* = 11.3 Hz, 2H, morpholine -CH_2-, H-11), 4.58 (s, 1H, H-6), 4.25-4.29 (m, 2H, H-5 and H-4), 3.86-3.94 (m, 4H, morpholine -CH_2-, H-11 and H-10), 3.67 (t, *J* = 11.3 Hz, 1H, H-4'), 3.11 (apparent d, *J* = 11.0 Hz, 2H, morpholine -CH_2-, H-10); δ_C (125 MHz, $CDCl_3$) 172.20 (C-8), 159.09 (C-9), 150.15, 136.55, 136.05, 128.14, 127.67, 124.76, 120.34, 119.58 (ArC), 87.13 (C-2), 66.74 (C-11), 64.56 (C-4), 62.37 (C-6), 61.23 (C-10), 57.21 (C-5), 56.11 (C-7); *m/z* (ESI-) 436.17 ([M-H]$^-$, 94%); HRMS (ESI+) calculated for $C_{23}H_{24}N_3O_6$ ([M+H]$^+$) 438.1660, found 438.1648.

General procedure for oxidative elimination of **66b-f**

35% (wt. in H_2O) H_2O_2 (6 eq.) was added at r.t. to a solution of the selenyl amides **65b-f** (1.0 eq.) in CH_2Cl_2 and the biphasic reaction mixture was stirred vigorously for 1 h. The reaction was quenched

with sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents removed *in vacuo* to give the crude product.

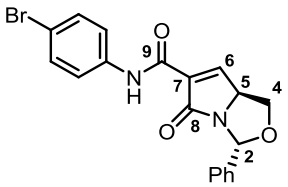
(2*R*, 5*S*)-1-Aza-3-oxa-8-oxo-2-phenyl-7-(phenylcarbamoyl)-bicyclo[3.3.0]oct-6-ene, 66b



Scale of reaction: 230 mg (0.482 mmol), product: 148 mg, 96%;

R_f = 0.26 (2:1 PE:EtOAc); yellow oil; $[\alpha]_D^{20}$ = +231.0 (*c*= 0.98 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1702 (s, C=O), 1672 (s, C=O); δ_H (500 MHz, $CDCl_3$) 10.20 (br s, 1H, -NH-), 8.14 (d, *J* = 1.9 Hz, 1H, H-6), 7.70 (d, *J* = 8.2 Hz, 2H, ArH), 7.55-7.57 (m, 2H, ArH), 7.35-7.46 (m, 5H, ArH), 7.16 (t, *J* = 7.3 Hz, 1H, ArH), 6.26 (s, 1H, H-2), 4.73 (dt, *J* = 1.6, 7.6 Hz, 1H, H-5), 4.41 (t, *J* = 7.6 Hz, 1H, H-4), 3.51 (t, *J* = 8.8 Hz, 1H, H-4'); δ_C (125 MHz, $CDCl_3$) 174.29 (C-8), 157.53 (C-9), 152.15 (C-6), 137.57, 137.32 (ArC), 133.40 (C-7), 129.12, 129.07, 128.69, 126.13, 124.85, 120.22 (ArC), 87.70 (C-2), 67.84 (C-4), 62.68 (C-5); *m/z* (ESI+) 343.13 ($[M+Na]^+$, 83%); HRMS (FI+) calculated for $C_{19}H_{16}N_2O_3$ 320.1161, found 320.1163.

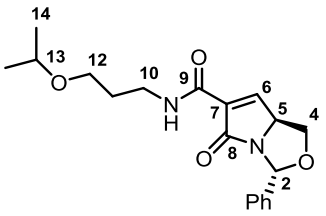
(2*R*, 5*S*)-1-Aza-7-(4'-bromophenylcarbamoyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]oct-6-ene, 66c



Scale of reaction: 100 mg (0.180 mmol), product: 66 mg, 92%;

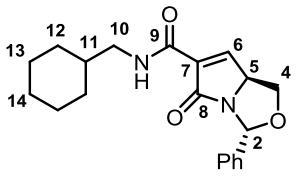
R_f = 0.24 (2:1 PE:EtOAc); yellow oil; $[\alpha]_D^{20}$ = +240.6 (*c*= 0.56 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1701 (s, C=O), 1672 (s, C=O); δ_H (500 MHz, $CDCl_3$) 10.24 (s, 1H, -NH-), 8.15 (d, *J* = 1.6 Hz, 1H, H-6), 7.40-7.62 (m, 9H, ArH), 6.25 (s, 1H, H-2), 4.74 (dt, *J* = 1.6, 7.9 Hz, 1H, H-5), 4.42 (t, *J* = 7.3 Hz, 1H, H-4), 3.52 (t, *J* = 8.5 Hz, 1H, H-4'); δ_C (125 MHz, $CDCl_3$) 174.20 (C-8), 157.53 (C-9), 152.37 (C-6), 137.44, 136.40 (ArC), 133.18 (C-7), 132.06, 129.15, 128.70, 126.10, 121.70, 117.48 (ArC), 87.71 (C-2), 67.79 (C-4), 62.74 (C-5); *m/z* (ESI+) 421.05 ($[M+Na]^+$, 100%), (ESI-) 397.04($[M-H]^-$, 100%); HRMS (FI+) calculated for $C_{19}H_{15}BrN_2O_3$ 398.0266, found 398.0266.

(2*R*, 5*S*)-1-Aza-7-(3'-isopropoxypropylcarbamoyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]oct-6-ene, 66d



Scale of reaction: 120 mg (0.239 mmol), product: 85 mg, quantitative; R_f = 0.50 (EtOAc); colourless oil; $[\alpha]_D^{20}$ = +163.6 (*c*= 1.15 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1698 (s, C=O), 1662 (s, C=O); δ_H(500 MHz, $CDCl_3$) 8.35 (br s, 1H, -NH-), 8.01 (d, *J* = 1.9 Hz, 1H, H-6), 7.52 (d, *J* = 6.9 Hz, 2H, ArH), 7.36-7.43 (m, 3H, ArH), 6.21 (s, 1H, H-2), 4.65 (dt, *J* = 1.6, 7.9 Hz, 1H, H-5), 4.35 (t, *J* = 7.6 Hz, 1H, H-4), 3.57 (quintet, *J* = 6.1 Hz, 1H, H-13), 3.49-3.53 (m, 4H, H-10 and H-12), 3.47 (t, *J* = 8.5 Hz, 1H, H-4'), 1.82-1.87 (m, 2H, H-11), 1.17 (d, *J* = 6.0 Hz, 6H, H-14); δ_C (125 MHz, $CDCl_3$) 174.33 (C-8), 159.77 (C-9), 151.20 (C-6), 137.82 (ArC), 133.32 (C-7), 128.94, 128.58, 126.10 (ArC), 87.55 (C-2), 71.71 (C-13), 67.74 (C-4), 65.74 (C-12), 62.45 (C-5), 36.85 (C-10), 29.69 (C-11), 22.03 (C-14); *m/z* (ESI+) 367.17 ($[M+Na]^+$, 97%); HRMS (FI+) calculated for $C_{19}H_{24}N_2O_4$ 344.1736, found 344.1739.

(2*R*, 5*S*)-1-Aza-7-(cyclohexylmethylcarbamoyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]oct-6-ene, 66e



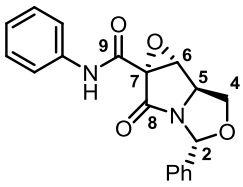
Scale of reaction: 35 mg (0.0704 mmol), product: 24 mg, quantitative; R_f = 0.16 (1:1 PE:EtOAc); colourless oil; $[\alpha]_D^{20}$ = +159.4 (*c*= 1.00 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1698 (s, C=O), 1663 (s, C=O); δ_H (500 MHz, $CDCl_3$) 8.28 (br s, 1H, -NH-), 8.02 (d, *J* = 1.9 Hz, 1H, H-6), 7.52-7.54 (m, 2H, ArH), 7.36-7.43 (m, 3H, ArH), 6.21 (s, 1H, H-2), 4.67 (dt, *J* = 1.6, 6.9 Hz, 1H, H-5), 4.37 (t, *J* = 7.6 Hz, 1H, H-4), 3.47 (t, *J* = 8.5 Hz, 1H, H-4'), 3.25 (t, *J* = 6.5 Hz, 2H, H-10), 1.66-1.79 (m, 5H, H-12, H-13, one of H-14), 1.51-1.60 (m, 1H, H-11), 1.12-1.28 (m, 3H, H-13' and one of H-14), 0.96-1.03 (m, 2H, H-12'); δ_C (125 MHz, $CDCl_3$) 174.48 (C-8), 159.77 (C-9), 151.08 (C-6), 137.74 (ArC), 133.36 (C-7), 129.01, 128.62, 126.12 (ArC), 87.59 (C-2), 67.82 (C-4), 62.57 (C-5), 45.31 (C-10), 37.79 (C-11), 30.81 (C-12), 26.30 (C-14), 25.79 (C-13); *m/z* (ESI-) 339.19 ($[M-H]^-$, 100%); HRMS (ESI+) calculated for $C_{20}H_{24}N_2O_3Na$ 363.1679, found 363.1666.

General procedure for epoxidation of α,β-unsaturated lactam **66b-e**

The α,β-unsaturated lactam **66b-e** used in this reaction was the crude obtained from the oxidative-elimination step.

35% (wt. in H_2O) H_2O_2 (6 eq.), followed by 4-methylmorpholine (1.0 eq.) was added to a solution of the α,β-unsaturated lactams **66b-e** (1.0 eq.) at r.t. The biphasic mixture was then stirred vigorously at r.t. for 1 h. The reaction was quenched with sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents removed *in vacuo* to give the crude product. Purification via silica gel flash column chromatography (Eluent: EtOAc/PE) afforded the epoxides.

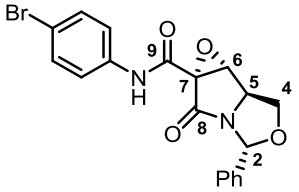
(2*R*, 5*S*, 7*S*)-1-Aza-6,7-epoxy-3-oxa-8-oxo-2-phenyl-7-(phenylcarbamoyl)-bicyclo[3.3.0]octane, 67b



Scale of reaction: 148 mg (0.462 mmol), product: 115 mg, 74%;

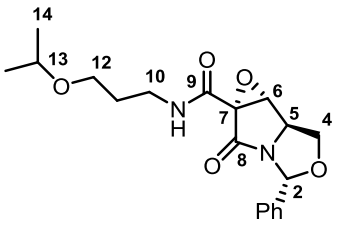
R_f = 0.56 (1:1 PE:EtOAc); pale yellow solid; mp = 202-206°C; $[\alpha]_D^{20}$ = +258.8 (*c*= 1.30 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1716 (s, C=O), 1685 (s, C=O); δ_H (500 MHz, $CDCl_3$) 10.05 (s, 1H, -NH-), 7.65 (d, *J* = 7.9 Hz, 2H, ArH), 7.35-7.41 (m, 7H, ArH), 7.19 (t, *J* = 7.3 Hz, 1H, ArH), 6.40 (s, 1H, H-2), 4.57 (s, 1H, H-6), 4.25-4.31 (m, 2H, H-5 and H-4), 3.67 (t, *J* = 11.3 Hz, 1H, H-4'); δ_C (125 MHz, $CDCl_3$) 173.46 (C-8), 159.67 (C-9), 137.25, 136.97, 129.21, 129.15, 128.71, 125.84, 125.37, 120.19 (ArC), 88.17 (C-2), 65.67 (C-4), 63.22 (C-6), 58.21 (C-5), 57.16 (C-7); *m/z* (ESI-) 335.12 ($[M-H]^-$, 100%); HRMS (ESI+) calculated for $C_{19}H_{17}N_2O_4$ 337.1183, found 337.1178.

(2*R*, 5*S*, 7*S*)-1-Aza-7-(4'-bromophenylcarbamoyl)-6,7-epoxy-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 67c



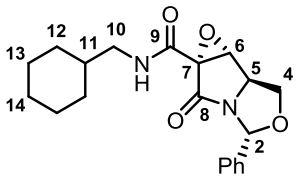
Scale of reaction: 29 mg (0.0726 mmol), product: 27 mg, 87%; R_f = 0.42 (2:1 PE:EtOAc); white solid; mp = 195-198°C; $[\alpha]_D^{20}$ = +213.9 (*c*= 1.08 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1718 (s, C=O), 1687 (s, C=O); δ_H (500 MHz, $CDCl_3$) 10.11 (s, 1H, -NH-), 7.55 (d, *J* = 8.8 Hz, 2H, ArH), 7.49 (d, *J* = 8.8 Hz, 2H, ArH), 7.36-7.41 (m, 5H, ArH), 6.39 (s, 1H, H-2), 4.57 (s, 1H, H-6), 4.25-4.30 (m, 2H, H-5 and H-4), 3.67 (t, *J* = 11.3 Hz, 1H, H-4'); δ_C (125 MHz, $CDCl_3$) 173.33 (C-8), 159.70 (C-9), 137.04, 135.97, 132.15, 129.13, 128.67, 125.75, 121.34, 118.01 (ArC), 88.11 (C-2), 65.55 (C-4), 63.24 (C-6), 58.14 (C-5), 57.02 (C-7); *m/z* (ESI-) 413.03 ($[M-H]^-$, 17%); HRMS (ESI+) calculated for $C_{19}H_{15}BrN_2NaO_4$ 437.0107, found 437.0108.

(2*R*, 5*S*, 7*S*)-1-Aza-6,7-epoxy-7-(3'-isopropoxypropylcarbamoyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 67d



Scale of reaction: 37 mg (0.107 mmol), product: 31 mg, 80%; R_f = 0.39 (EtOAc); white solid; mp = 132-136°C; $[\alpha]_D^{20}$ = +233.5 (*c*= 1.24 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1726 (s, C=O), 1656 (s, C=O); δ_H (500 MHz, $CDCl_3$) 8.29 (br s, 1H, -NH-), 7.32-7.39 (m, 5H, ArH), 6.36 (s, 1H, H-2), 4.42 (s, 1H, H-6), 4.18-4.24 (m, 2H, H-5 and H-4), 3.57-3.63 (m, 2H, H-4' and H-13), 3.47-3.55 (m, 4H, H-12 and H-10), 1.84 (quintet, *J* = 6.0 Hz, 2H, H-11), 1.20 (d, *J* = 6.3 Hz, 6H, H-14); δ_C (125 MHz, $CDCl_3$) 172.89 (C-8), 161.59 (C-9), 137.50, 128.89, 128.54, 125.75 (ArC), 88.00 (C-2), 71.89 (C-13), 66.35 (C-12), 65.57 (C-4), 62.71 (C-6), 58.07 (C-5), 57.03 (C-7), 38.20 (C-10), 29.22 (C-11), 22.03, 22.00 (C-14 and C-14'); *m/z* (ESI-) 359.18 ($[M-H]^-$, 98%); HRMS (ESI+) calculated for $C_{19}H_{25}N_2O_5$ 361.1758, found 361.1760.

(2*R*, 5*S*, 7*S*)-1-Aza-7-(cyclohexylmethylcarbamoyl)-6,7-epoxy-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 67e



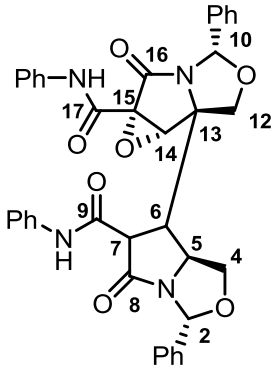
Scale of reaction: 55 mg (0.162 mmol), product: 42 mg, 73%; R_f = 0.59 (1:2 PE:EtOAc); white solid; mp = 182-186°C; $[\alpha]_D^{20}$ = +268.0 (*c*= 1.20 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1713 (s, C=O), 1678 (s, C=O); δ_H (500 MHz, $CDCl_3$) 8.20 (br s, 1H, -NH-), 7.33-7.40 (m, 5H, ArH), 6.36 (s, 1H, H-2), 4.47 (s, 1H, H-6), 4.20-4.26 (m, 2H, H-5 and H-4), 3.62 (t, *J* = 7.3 Hz, 1H, H-4'), 3.26-3.32 (m, 1H, H-10), 3.17-3.22 (m, 1H, H-10'), 1.68-1.79 (m, 5H, H-12, H-13, one of H-14), 1.53-1.61 (m, 1H, H-11), 1.14-1.31 (m, 3H, H-13' and one of H-14), 0.96-1.04 (m, 2H, H-12'); δ_C (125 MHz, $CDCl_3$) 173.43 (C-8), 161.72 (C-9), 137.33, 128.98, 128.58, 125.73 (ArC), 87.96 (C-2), 65.59 (C-4), 62.68 (C-6), 58.08 (C-5), 56.85 (C-7), 46.01 (C-10), 37.57 (C-11), 30.77 (C-12), 26.26 (C-14), 25.74 (C-14); *m/z* (ESI-) 355.18 ($[M-H]^-$, 97%); HRMS (ESI+) calculated for $C_{20}H_{25}N_2O_4$ 357.1809, found 357.1801.

Procedure for epoxidation of α,β-unsaturated lactam **66b** to **67b** using other tertiary amine or pyridine

35% (wt. in H_2O) H_2O_2 (58 μL, 0.651 mmol, 6 eq.) was added to a solution of the α,β-unsaturated lactam **66b** (35 mg, 0.109, 1.0 eq.) and tertiary amine – Et_3N or DABCO – or pyridine (1.0 eq.) at r.t. The biphasic mixture was then stirred vigorously at r.t. for 1 h. In the case with pyridine, 1.0 eq. of pyridine and 6 eq. of 35% (wt. in H_2O) H_2O_2 was added after each hour until the reaction is observed to be completed via TLC analysis after 3 h.The reaction was quenched with sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents removed *in vacuo* to give the crude product. Purification via silica gel flash column chromatography (Eluent: EtOAc/PE) afforded the epoxide **67b**.

Mono-epoxy dimer, 70

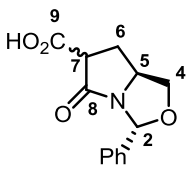
Mono-epoxy dimer **70** was isolated with ~10% yield when Et_3N was used in conjunction with H_2O_2 for the epoxidation of **66b** as described above.



R_f = 0.17 (2:1 PE:EtOAc); $[\alpha]_D^{20}$ = +59.4 (*c*= 0.25 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1718 (br s, C=O), 1683 (br s, C=O); δ_H (500 MHz, $CDCl_3$) 9.00 (br s, 1H, -NHCO-, C-9), 8.78 (br s, 1H, -NHCO-, C-17), 7.49-7.52 (m, 2H, ArH), 7.28-7.45 (m, 16H, ArH), 7.11-7.15 (m, 2H, ArH), 6.38 (s, 1H, H-2), 5.76 (s, 1H, H-10), 4.51 (s, 1H, H-14), 4.45 (dd, *J* = 6.3, 8.2 Hz, 1H, H-4), 4.37 (d, *J* = 8.8 Hz, 1H, H-12), 4.13-4.16 (m, 2H, H-5 and H-12'), 3.93 (d, *J* = 8.2 Hz, 1H, H-7), 3.67-3.71 (m, 2H, H-4' and H-6); δ_C (125 MHz, $CDCl_3$) 172.33 (C-8), 168.00 (C-16), 163.48 (C-9), 158.42 (C-17), 137.19, 136.99, 136.23, 134.34, 130.20, 129.11, 128.98, 128.72, 127.38, 125.72, 125.38, 124.97, 120.35, 120.13, 120.09 (ArC), 90.02 (C-10), 87.67 (C-2), 71.48 (C-4), 69.58 (C-13), 68.25 (C-12), 61.94 (C-15), 57.71(C-5), 56.59 (C-14), 52.50 (C-7), 44.03 (C-6); *m/z* (ESI-) 655.24 ([M-H]$^-$, 100%); HRMS (ESI+) calculated for $C_{38}H_{32}N_4NaO_7$ ([M+Na]$^+$) 679.2163, found 679.2165.

(2*R*, 5*S*, 7*S*) and (2*R*, 5*S*, 7*R*)-1-Aza-7-(carboxyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, (7*RS*)-72

LiOH (95.7 mg, 3.996 mmol, 1.1 eq.) was added to a solution of **61** (1 g, 3.632 mmol, 1.0 eq.) in 15 mL of THF/H_2O (1:1). The reaction mixture was stirred at r.t. for 6 h, and an additional 0.3 eq. of LiOH was added. After a total reaction time of 8.5 h, the reaction mixture was acidified with aq. 10% HCl dropwise (to about pH 1) and extracted with EtOAc. The combined organic layers were dried with $MgSO_4$, filtered and evaporated to give the crude. For the purpose of characterisation, a diastereomeric mixture **72-(7*RS*)** was isolated via column chromatography on silica gel (Eluent: 30% to 100% EtOAc/PE, then 5% MeOH/EtOAc). Subsequently, the reaction crude was used for the amide coupling reaction directly.



R_f = 0.08 (5% MeOH/EtOAc), tailing from baseline of TLC; colourless oil;

ν_{max} / cm^{-1} (film) 3429 (br,-OH), 1688 (br with a shoulder, C=O);

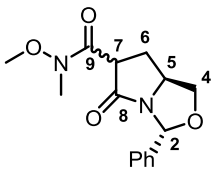
<u>Major diastereomer (a), minor diastereomer (b)</u>

δ_H (500 MHz, Acetone-d_6) 7.34-7.49 (m, 5H, Ph-H), 6.22 (s, 0.5H, H-2_a), 6.19 (s, 0.5H, H-2_b), 4.25-4.34 (m, 2H, H-4_a, H-4_b, H-5_a, H-5_b), 4.03 (t, *J* = 9.6 Hz, 0.5H, H-7_a), 3.69-3.73 (m, 2H, H-$4'_a$, H-7_b),

3.61 (t, *J* = 7.9 Hz, 0.5H, H-4'$_b$), 2.67-2.75 (m, 1H, H-6$_a$, H-6$_b$), 2.32-2.39 (m, 1H, H-6'$_a$, H-6'$_b$); δ_C (125 MHz, Acetone-d_6) 174.02 (C-8$_a$), 173.70 (C-8$_b$), 170.93 (C-9$_b$), 170.65 (C-9$_a$), 140.26, 140.01, 129.47, 129.36, 129.23, 129.21, 127.07, 126.98, 88.24 (C-2$_b$), 88.00 (C-2$_a$), 72.71 (C-4$_a$), 72.28 (C-4$_b$), 58.97 (C-5$_b$), 58.19 (C-5$_a$), 52.52 (C-7$_b$), 51.83 (C-7$_a$), 28.63 (C-6$_a$), 27.64 (C-6$_b$); *m/z* (ESI-) 246.07 ([M-H]$^-$, 100%); HRMS (ESI+) calculated for $C_{13}H_{13}NNaO_4$ ([M+Na]$^+$) 270.0737, found 270.0740.

(2*R*, 5*S*, 7*S*) and (2*R*, 5*S*, 7*R*)-1-Aza-7-(*N*,*O*-dimethylcarbamoyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, (7*RS*)-73

1,1'-carbonyl-diimidazole (647.8 mg, 3.995 mmol, 1.1 eq.) was added to a dry MeCN (15 mL) solution of crude **72-(7*RS*)** (assumed complete conversion for previous step) and the reaction mixture was stirred at r.t. for 1 h. *N*,*O*-dimethylhydroxylamine hydrochloride (389.7 mg, 3.995 mmol, 1.1 eq.) was then added and stirred at r.t for another 18 h. The reaction mixture was diluted with deionised water (pH 7-8), acidified with aq. 10% HCl dropwise (until pH 1-2) and extracted with EtOAc. The combined organic layers were dried with $MgSO_4$, filtered and evaporated to give the crude. Purification via silica gel flash column chromatography (Eluent: 10% to 60% EtOAc/PE) gave the product (780 mg) as a colourless oil in 74% yield over two steps.



R_f = 0.44 (EtOAc); colourless oil; ν_{max} / cm^{-1} (film) 1703 (s, C=O), 1654 (s, C=O);

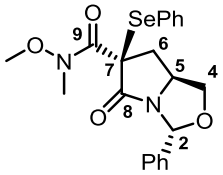
Diastereomer A (7*R*), diastereomer B (7*S*)

δ_H (500 MHz, $CDCl_3$) 7.43-7.47 (m, Ph-H), 7.30-7.39 (m, Ph-H), 6.31 (s, 1H, H-2$_A$), 6.28 (s, 1H, H-2$_B$), 4.53 (t, *J* = 9.3 Hz, 1H, H-7$_B$), 4.38-4.44 (m, 1H, H-5$_A$), 4.28-4.31 (m, 1H, H-4$_B$), 4.28-4.31 (m, 1H, H-7$_A$), 4.25 (dd, *J* = 6.5, 8.0 Hz, 1H, H-4$_A$), 4.13-4.18 (m, 1H, H-5$_B$), 3.88 (s, 3H, -O<u>Me</u>$_A$), 3.84 (s, 3H, -O<u>Me</u>$_B$), 3.75 (t, *J* = 8.2 Hz, 1H, H-4'$_B$), 3.51 (t, *J* = 8.2 Hz, 1H, H-4'$_A$), 3.28 (s, 3H, -NMe$_B$), 3.24 (s, 3H, -N<u>Me</u>$_A$), 2.67-2.71 (m, 1H, H-6$_A$), 2.44-2.53 (m, 2H, H-6$_B$ and H-6'$_B$), 2.09-2.15 (m, 1H, H-6'$_A$); δ_C (125 MHz, $CDCl_3$) 173.52 (C-8$_B$), 173.40 (C-8$_A$), 169.98 (C-9$_A$), 169.24 (C-9$_B$), 138.62, 138.04, 128.64, 128.45, 128.43, 126.03, 125.98, 87.31 (C-2$_A$), 87.19 (C-2$_B$), 71.91 (C-4$_B$), 71.71 (C-4$_A$), 62.08 (-O<u>Me</u>$_A$), 61.87 (-O<u>Me</u>$_B$), 58.40 (C-5$_A$), 57.19 (C-5$_B$), 48.41 (C-7$_A$), 47.66 (C-7$_B$), 32.36 (-N<u>Me</u>$_A$), 32.31 (-N<u>Me</u>$_B$), 27.92 (C-6$_A$), 26.75 (C-6$_B$); *m/z* (ESI+) 313.11 ([M+Na]$^+$, 31%); HRMS (ESI+) calculated for $C_{15}H_{18}N_2NaO_4$ ([M+Na]$^+$) 313.1159, found 313.1160.

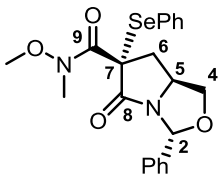
(2*R*, 5*S*, 7*R*)-1-Aza-7-(*N*,*O*-dimethylcarbamoyl)-3-oxa-8-oxo-2-phenyl-7-(phenylselanyl)-bicyclo[3.3.0]octane, (7*R*)-74 and **(2*R*, 5*S*, 7S)-1-Aza-7-(*N*,*O*-dimethylcarbamoyl)-3-oxa-8-oxo-2-phenyl-7-(phenylselanyl)- bicyclo[3.3.0]octane, (7*S*)-74**

(NaH was pre-washed with dry Et_2O and used immediately for the reaction.)

74 (600 mg, 2.067 mmol, 1.0 eq.) in anhydrous THF (7 mL) was added dropwise to pre-washed NaH (82.7 mg, 2.067 mmol, 1.0 eq., in 60% mineral oil) at 0°C and the mixture was warmed to r.t. and stirred for 30 min. PhSeBr (487.7 mg, 2.067 mmol, 1.0 eq.) in anhydrous THF (2 mL) was then added dropwise to the mixture at r.t., and stirred for a further 1 h. The reaction mixture was then quenched with sat. aq. NH_4Cl and extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents removed *in vacuo* to give the crude. Purification via silica gel flash column chromatography (Eluent: EtOAc/PE) gave the two separate diastereomers as a colourless oil (combined yield 82%).



R_f = 0.56 (1:1 EtOAc/PE); colourless oil; $[\alpha]_D^{20}$ = +240.1 (*c*= 0.89 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1694 (s, C=O), 1646 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.44-7.76 (m, 2H, Ph-H), 7.42-7.45 (m, 3H, Ph-H), 7.29-7.39 (m, 5H, Ph-H), 6.13 (s, 1H, H-2), 3.93-4.01 (m, 2H, H-5 and H-4), 3.36 (s, 3H, -OMe), 3.25 (s, 3H, -NMe), 2.97 (dd, *J* = 7.3, 13.9 Hz, 1H, H-6), 2.72 (t, *J* = 7.7 Hz, 1H, H-4'), 2.27 (dd, *J* = 5.2, 14.0 Hz, 1H, H-6'); δ_C (125 MHz, $CDCl_3$) 171.72 (C-8), 168.31 (C-9, br), 138.43, 138.12, 129.79, 129.17, 128.68, 128.36, 126.66, 125.96 (Ph-C), 86.99 (C-2), 70.55 (C-4), 62.33 (C-7, br), 60.76 (-OMe), 56.42 (C-5), 36.88 (C-6, br), 34.00 (-NMe); *m/z* (ESI+) 447.07 ($[M+H]^+$, 18%), 915.13 ($[2M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{21}H_{22}N_2NaO_4Se$ ($[M+Na]^+$) 469.0638, found 469.0631.

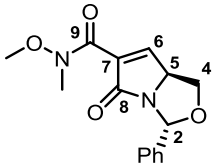


R_f = 0.36 (1:1 EtOAc/PE); colourless oil; $[\alpha]_D^{20}$ = -47.2 (*c*= 1.13 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1700 (s, C=O), 1649 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.65 (dd, *J* = 1.1, 8.0 Hz, 2H, Ph-H), 7.25-7.33 (m, 4H, Ph-H), 7.02-7.05 (m, 4H, Ph-H), 6.24 (s, 1H, H-2), 3.96 (t, *J* = 7.3 Hz, 1H, H-4), 3.72 (s, 3H, -OMe), 3.48 (t, *J* = 7.9 Hz, 1H, H-4'), 3.28 (s, 3H, -NMe), 3.05 (apparent quintet, *J* = 7.9 Hz, 1H, H-5), 2.86 (dd, *J* = 1.9, 6.3 Hz, 2H, H-6 and H-6');

δ_C (125 MHz, $CDCl_3$) 172.83 (C-8), 168.47 (C-9), 138.48, 137.73, 129.54, 128.88, 128.46, 128.19, 125.98, 125.94 (Ph-C), 86.92 (C-2), 71.66 (C-4), 63.00 (C-7), 60.12 (-O<u>Me</u>), 55.42 (C-5), 38.55 (C-6), 33.15 (-N<u>Me</u>); *m/z* (ESI+) 447.07 ($[M+H]^+$, 30%), 915.12 ($[2M+Na]^+$, 48%); HRMS (ESI+) calculated for $C_{21}H_{22}N_2NaO_4Se$ ($[M+Na]^+$) 469.0638, found 469.0630.

(2*R*, 5*S*)-1-Aza-7-(*N*,*O*-dimethylcarbamoyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]oct-6-ene, 75

35% (wt. in H_2O) H_2O_2 (837 μL, 9.43 mmol, 6 eq.) was added to a solution of the selenyl amide **74** (705 mg, 1.58 mmol, 1.0 eq.) in CH_2Cl_2 (20 mL) at 0°C and the biphasic reaction mixture was stirred vigorously at 0°C for 45 min. The reaction was quenched with sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents removed *in vacuo* to give the crude product as a colourless oil (460 mg, quantitative crude yield).

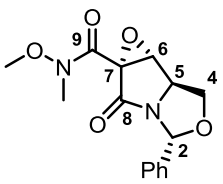


R_f = 0.24 (EtOAc); colourless oil; $[\alpha]_D^{20}$ = +107.6 (*c*= 1.03 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1704 (s, C=O), 1655 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.51-7.54 (m, 2H, Ph-H), 7.48 (br s, 1H, H-6), 7.34-7.42 (m, 3H, Ph-H), 6.22 (s, 1H, H-2), 4.64 (dt, *J* = 1.9, 7.3 Hz, 1H, H-5), 4.28 (t, *J* = 7.3 Hz, 1H, H-4), 3.73 (br, s, 3H, -O<u>Me</u>), 3.53 (t, *J* = 8.4 Hz, 1H, H-4'), 3.29 (s, 3H, -N<u>Me</u>); δ_C (125 MHz, $CDCl_3$) 172.50 (C-8), 162.86 (C-9, br), 146.72 (C-6, br), 138.19 (Ph-C), 137.06 (C-7), 128.76, 128.49, 126.20 (Ph-C), 87.77 (C-2), 67.66 (C-4), 63.24 (C-5), 61.65 (-O<u>Me</u>, br), 32.00 (-N<u>Me</u>, br); *m/z* (ESI+) 311.09 ($[M+Na]^+$, 27%), 599.16 ($[2M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{15}H_{16}N_2NaO_4$ ($[M+Na]^+$) 311.1002, found 311.1003.

(2*R*, 5*S*, 7*S*)-1-Aza-7-(*N*,*O*-dimethylcarbamoyl)-6,7-epoxy-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 76

Triethylamine (366 μL, 2.63 mmol, 1.0 eq.) was added dropwise to a solution of crude α,β-unsaturated lactam **75** (760 mg, 2.63 mmol, 1.0 eq.) and 35% (wt. in H_2O) H_2O_2 (1.39 mL, 15.7 mmol, 6 eq.) at 0°C. The biphasic mixture was then stirred vigorously at 0°C for 1 h. The reaction was quenched with sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents removed *in vacuo* to give the crude product. Purification via silica gel

flash column chromatography (Eluent: 10% to 60% EtOAc/PE) gave epoxide **76** (744 mg, 93%) as a white solid.



d.r. ~ 1:0.1

R_f = 0.53 (EtOAc); white solid; mp = 159-161°C; $[\alpha]_D^{20}$ = +110.7 (*c*= 1.13 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1734 (s, C=O), 1673 (s, C=O);

Major Diastereoisomer

δ_H (500 MHz, $CDCl_3$) 7.32-7.41 (m, 5H, Ph-H), 6.38 (s, 1H, H-2), 4.19-4.26 (m, 3H, H-4, H-5 and H-6), 3.78 (s, 3H, -OMe), 3.66 (t, *J* = 7.9 Hz, 1H, H-4'), 3.30 (s, 3H, -NMe); δ_C (125 MHz, $CDCl_3$) 171.82 (C-8), 162.35 (C-9), 137.67, 128.82, 128.51, 125.80 (Ph-C), 88.54 (C-2), 65.40 (C-4), 61.31 (-OMe), 61.20, 61.17 (C-6, C-7), 58.76 (C-5), 32.06 (-NMe); *m/z* (ESI+) 305.09 ($[M+H]^+$, 90%); HRMS (ESI+) calculated for $C_{15}H_{17}N_2O_5$ ($[M+H]^+$) 305.1132, found 305.1133.

General procedure for synthesis of Grignard reagents

Synthesis of aryl and alkyl Grignard reagents:

The respective Grignard reagent were prepared in excess: Magnesium flakes (100 mg, 4.114 mmol, 1.05 eq.) and I_2 (1 small crystal) were placed in a two-necked round-bottomed flask attached with a condenser. A solution of the bromide (1.0 eq.) in 10 mL THF was added to the magnesium dropwise slowly. Initiation of the reaction could be observed via the disappearance of the yellowish-brown colour of iodine. Once addition of the reagent has been completed, the mixture was heated at 45°C for 1-1.5 h. The solution was then cooled to r.t. and the required amount was used for the Grignard reaction directly.

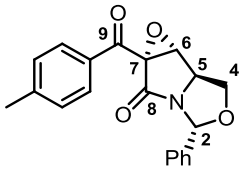
Synthesis of alkynyl Grignard reagents:

The respective alkynyl Grignard reagent were prepared in excess: A 2 M solution of isobutyl magnesium bromide in Et_2O (1.0 eq.) was added dropwise to a THF (5 mL) solution of the 1-alkyne (1.05 eq.) at 0°C. The suspension was then warmed to r.t. and then heated at 40°C for 1.25 h. The solution was used for the Grignard reaction directly at 40°C.

General procedures for Grignard reaction of epoxy Weinreb amide **76**

The Grignard reagent in THF (1.3 eq. based on amount of **76** used) was added to **76** (1.0 eq.) in THF at -15°C. The reaction was stirred at -15°C for 15-45 min (monitored via thin layer chromatography of the reaction mixture), afterwhich it was quenched with sat. aq. NH_4Cl and extracted with EtOAc. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents removed *in vacuo* to give the crude product. Purification via silica gel flash column chromatography (Eluent: EtOAc/PE) gave the respective product. For compound **77e**, a commercially available 2 M Et_2O solution of isobutyl magnesium bromide was used instead.

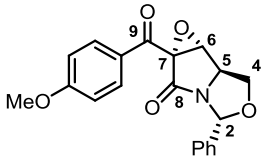
(2*R*, 5*S*, 7*S*)-1-Aza-6,7-epoxy-7-(4'methylphenylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 77a



Scale of reaction: 75 mg (0.246 mmol), product: 71 mg, 86%;

R_f = 0.56 (1:1 EtOAc/PE); colourless crystals, mp = 108-110°C; $[\alpha]_D^{20}$ = +121.3 (*c*= 1.0 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1723 (s, C=O), 1679 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.96 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.35-7.44 (m, 5H, Ph-H), 7.32 (d, *J* = 8.5 Hz, 2H, Ar-H), 6.43 (s, 1H, H-2), 4.27-4.32 (m, 3H, H-4, H-5, H-6), 3.77 (apparent quintet, *J* = 6.9 Hz, 1H, H-4'), 2.45 (s, 3H, -Ar-Me); δ_C (125 MHz, $CDCl_3$) 188.38 (C-9), 172.49 (C-8), 145.95, 137.76, 132.13, 129.73, 129.51, 128.84, 128.53, 125.85 (Ar-C, Ph-C), 88.48 (C-2), 65.74 (C-4), 63.68 (C-7), 61.67 (C-6), 58.82 (C-5), 21.89 (-Ar-Me); *m/z* (ESI+) 358.11 ($[M+Na]^+$, 29%), 693.23 ($[2M+Na]^+$, 81%); HRMS (ESI+) calculated for $C_{20}H_{17}NNaO_4$ ($[M+Na]^+$) 358.1050, found 358.1045.

(2*R*, 5*S*, 7*S*)-1-Aza-6,7-epoxy-7-(4'-methoxyphenylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 77b

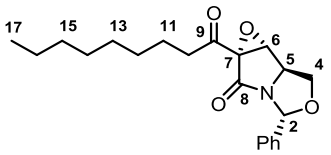


Scale of reaction: 150 mg (0.495 mmol), product: 131 mg, 76%;

R_f = 0.45 (1:1 EtOAc/PE); colourless oil; $[\alpha]_D^{20}$ = +209.3 (*c*= 0.99 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 1720 (s, C=O), 1672 (s, C=O); δ_H (500 MHz, $CDCl_3$) 8.06 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.38-7.44 (m, 5H, Ph-H), 6.99 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.42 (s, 1H, H-2), 4.26-4.32 (m, 3H, H-4, H-5, H-6), 3.90 (s, 3H, Ar-OMe), 3.76 (ddd, *J* = 0.6, 6.6, 12.3 Hz, 1H, H-4');

δ_C (125 MHz, $CDCl_3$) 187.01 (C-9), 172.63 (C-8), 164.83 (Ar<u>C</u>-OMe), 137.81 (PhC, no H), 132.18 (ArC), 128.83, 128.53 (PhC), 127.70 (ArC, no H), 125.85 (PhC), 114.10 (ArC), 88.46 (C-2), 65.78 (C-4), 63.68 (C-7), 61.61 (C-6), 58.82 (C-5), 55.61 (ArC-O<u>Me</u>); *m/z* (ESI+) 374.0 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{20}H_{17}NNaO_5$ ($[M+Na]^+$) 374.0999, found 374.0988.

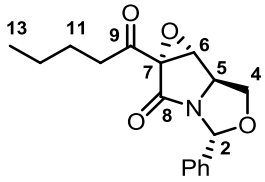
(2*R*, 5*S*, 7*S*)-1-aza-6,7-epoxy-7-(octylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 77c



Scale of reaction: 100 mg (0.329 mmol), product: 91 mg, 77%;

R_f = 0.53 (30% EtOAc/PE); colourless oil; $[\alpha]_D^{20}$ = +206.6 (*c*= 1.0 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1735 (br with shoulder, s, C=O); δ_H (500 MHz, $CDCl_3$) 7.32-7.41 (m, 5H, Ph-H), 6.39 (s, 1H, H-2), 4.19-4.24 (m, 3H, H-4, H-5, H-6), 3.56-3.61 (m, 1H, H-4'), 2.73 (t, *J* = 7.4 Hz, 2H, H-10), 1.56-1.67 (m, 2H, H-11), 1.25-1.35 (m, 10H, H-12, H-13, H-14, H-15, H-16), 0.89 (t, *J* = 6.9 Hz, 3H, H-17); δ_C (125 MHz, $CDCl_3$) 199.58 (C-9), 171.10 (C-8), 137.72, 128.82, 128.51, 125.78 (Ph-C), 88.31 (C-2), 65.63 (C-4), 62.93 (C-6), 61.87 (C-7), 58.26 (C-5), 40.77 (C-10), 31.76 (C-15), 29.25, 29.05, 28.97 (C-12, C-13, C-14), 22.81 (C-11), 22.62 (C-16), 14.07 (C-17); *m/z* (ESI+) 380.18 ($[M+Na]^+$, 57%), 737.38 ($[2M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{21}H_{27}NNaO_4$ ($[M+Na]^+$) 380.1832, found 380.1837.

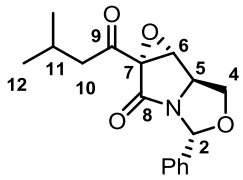
(2*R*, 5*S*, 7*S*)-1-aza-7-(butylcarbonyl)-6,7-epoxy-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 77d



Scale of reaction: 150 mg (0.493 mmol), product: 112 mg, 75%;

R_f = 0.38 (30% EtOAc/PE); colourless solid, mp = 96-98°C; $[\alpha]_D^{20}$ = +258.3 (*c*= 0.96 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 1735 (br with shoulder, s, C=O); δ_H (500 MHz, $CDCl_3$) 7.34-7.41 (m, 5H, Ph-H), 6.39 (s, 1H, H-2), 4.19-4.24 (m, 3H, H-4, H-5, H-6), 3.59 (ddd, *J* = 0.9, 7.6, 11.7 Hz, 1H, H-4'), 2.73 (t, *J* = 7.4 Hz, 2H, H-10), 1.61-1.66 (m, 2H, H-11), 1.33-1.40 (m, 2H, H-12), 0.93 (t, *J* = 7.4 Hz, 3H, H-13); δ_C (125 MHz, $CDCl_3$) 199.60 (C-9), 171.10 (C-8), 137.72, 128.83, 128.51, 125.78 (Ph-C), 88.31 (C-2), 65.63 (C-4), 62.94 (C-6), 61.89 (C-7), 58.27 (C-5), 40.43 (C-10), 24.87 (C-11), 22.11 (C-12), 13.78 (C-13); *m/z* (ESI+) 324.1 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{17}H_{19}NNaO_4$ ($[M+Na]^+$) 324.1206, found 324.1200.

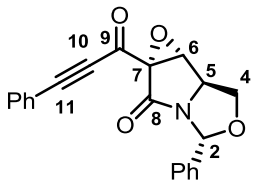
(2*R*, 5*S*, 7*S*)-1-Aza-6,7-epoxy-7-(isobutylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 77e



Scale of reaction: 110 mg (0.361 mmol), product: 68 mg, 62%;

R_f = 0.61 (1:1 EtOAc/PE); colourless crystals, mp = 116-118°C; $[\alpha]_D^{20}$ = +279.7 (*c*= 1.01 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 1732 (s, C=O, with shoulder towards shorter wavenumber); δ_H (500 MHz, $CDCl_3$) 7.32-7.40 (m, 5H, PhH), 6.38 (s, 1H, H-2), 4.18-4.24 (m, 3H, H-4, H-5, H-6), 3.58 (ddd, br, *J* = 0.9, 6.9, 12.3 Hz, 1H, H-4'), 2.65 (dd, *J* = 6.6, 17.0 Hz, 1H, H-10), 2.57 (dd, *J* = 6.6, 17.0 Hz, 1H, H-10'), 2.23 (doublet of septet, *J* = 6.8, 13.5 Hz, 1H, H-11), 0.98 (d, *J* = 6.6 Hz, 3H, H-12), 0.96 (d, *J* = 6.6 Hz, 3H, H-12'); δ_C (125 MHz, $CDCl_3$) 199.22 (C-9), 171.10 (C-8), 137.74, 128.82, 128.51, 125.78 (Ph-C), 88.31 (C-2), 65.65 (C-4), 62.80 (C-6), 61.99 (C-7), 58.25 (C-5), 49.26 (C-10), 24.03 (C-11), 22.58, 22.38 (C-12, C-12'); *m/z* (ESI+) 324.1 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{17}H_{19}NNaO_4$ ($[M+Na]^+$) 324.1206, found 324.1195.

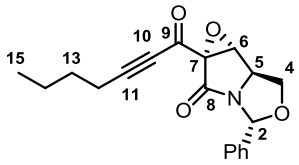
(2*R*, 5*S*, 7*S*)-1-Aza-6,7-epoxy-7-(phenylacetylenecarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 77f



Scale of reaction: 152 mg (0.500 mmol), product: 124 mg, 72%;

R_f = 0.34 (30% EtOAc/PE); white solid, mp = 154-156°C; $[\alpha]_D^{20}$ = +193.4 (*c*= 0.93 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 2195 (s, alkyne), 1728 (s, C=O), 1659 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.64-7.65 (m, 2H, Ph-H), 7.50-7.54 (m, 1H, Ph-H), 7.33-7.44 (m, 7H, Ph-H), 6.44 (s, 1H, H-2), 4.43 (s, 1H, H-6), 4.23-4.27 (m, 2H, H-4 and H-5), 3.65 (dt, *J* = 2.8, 11.3 Hz, 1H, H-4'); δ_C (125 MHz, $CDCl_3$) 175.05 (C-9), 169.62 (C-8), 137.70, 133.64, 131.71, 128.85, 128.76, 128.53, 125.80, 118.98 (Ph-C), 97.16 (C-11), 88.40 (C-2), 85.77 (C-10), 65.64 (C-4), 63.74 (C-6), 62.24 (C-7), 58.12 (C-5); *m/z* (ESI+) 368.0 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{21}H_{15}NNaO_4$ ($[M+Na]^+$) 368.0893, found 368.0882.

(2*R*, 5*S*, 7*S*)-1-Aza-6,7-epoxy-7-(butylacetylenecarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 77g



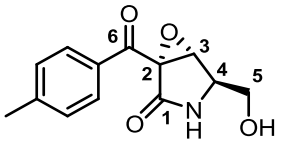
Scale of reaction: 152 mg (0.500 mmol), product: 115 mg, 71%;

R_f = 0.42 (30% EtOAc/PE); colourless oil; $[\alpha]_D^{20}$ = +246.68 (*c*= 0.99 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 2209 (s, alkyne), 1730 (s, C=O), 1666 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.32-7.40 (m, 5H, Ph-H), 6.39 (s, 1H, H-2), 4.33 (s, 1H, H-6), 4.19-4.25 (m, 2H, H-4 and H-5), 3.60 (dt, *J* = 6.9, 7.9 Hz, 1H, H-4'), 2.45 (t, *J* = 7.3 Hz, 2H, H-12), 1.58-1.63 (m, 2H, H-13), 1.42-1.49 (m, 2H, H-14), 0.94 (t, *J* = 7.3 Hz, 3H, H-15); δ_C (125 MHz, $CDCl_3$) 175.11 (C-9), 169.64 (C-8), 137.72, 128.82, 128.51, 125.78 (Ph-C), 101.25 (C-11), 88.34 (C-2), 78.56 (C-10), 65.64 (C-4), 63.39 (C-6), 62.22 (C-7), 58.09 (C-5), 29.37 (C-13), 21.90 (C-14), 18.98 (C-12), 13.43 (C-15); *m/z* (ESI+) 348.1 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{19}H_{19}NNaO_4$ ($[M+Na]^+$) 348.1206, found 348.1195.

General procedures for acidic oxazolidine hydrolysis to keto-epoxypyrrolidinones **78a-g**

TFA (0.4-0.6 mL, large excess) was added to a solution of **77a-g** in THF/H_2O (1:1) at r.t.. The reaction mixture was stirred at r.t. for 17-24 h and the solvents evaporated (toluene was used to azeotrope the water solvent under reduced pressure) to give the crude. Purification via silica gel flash column chromatography (Eluent: EtOAc/PE, 50% to 100%, then 10% MeOH/EtOAc) gave the respective product.

(2*S*, 4*R*)-2,3-Epoxy-4-hydroxymethyl-2-(4'-methylphenylcarbonyl)-1-oxo-pyrrolidinone, 78a

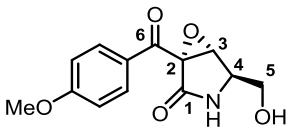


Scale of reaction: 71 mg (0.212 mmol), product: 49 mg, 94%;

R_f = 0.18 (EtOAc); white solid; mp = 182-186°C, white solid turns brown then melts; $[\alpha]_D^{20}$ = -38.4 (*c*= 0.63 in Acetonitrile); ν_{max} / cm^{-1} (film) 3496 (br, lactam –NH-), 3324 (br, -OH), 1707 (s, C=O), 1678 (s, C=O); δ_H (500 MHz, MeCN-d_3) 8.00 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.34 (d, *J* = 8.2 Hz, 2H, Ar-H), 6.38 (br s, 1H, -NH-), 4.31 (d, *J* = 2.5 Hz, 1H, H-3), 3.84 (dt, *J* = 1.4, 3.7 Hz, 1H, H-4), 3.72-3.80 (m, 2H, H-5), 3.41 (t, *J* = 5.7 Hz, 1H, -OH), 2.41 (s, 3H, Ar-Me); δ_C (125 MHz, MeCN-d_3) 190.49 (C-6), 171.66 (C-1), 146.65, 133.72, 130.41, 130.11

(Ar-C), 64.18 (C-2), 63.62 (C-3), 62.27 (C-5), 57.62 (C-4), 21.88 (Ar-Me); *m/z* (ESI+) 270.08 ($[M+Na]^+$, 25%), 517.15 ($[2M+Na]^+$, 97%); HRMS (ESI+) calculated for $C_{13}H_{13}N_2NaO_4$ ($[M+Na]^+$) 270.0737, found 270.0739.

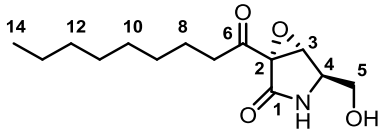
(2*S*, 4*R*)-2,3-Epoxy-4-hydroxymethyl-2-(4'-methoxyphenylcarbonyl)1-oxo-pyrrolidinone, 78b



Scale of reaction: 40 mg (0.114 mmol), product: 26 mg, 87%;

R_f = 0.25 (EtOAc); colourless solid, mp = 180-181°C; $[\alpha]_D^{20}$ = -26.8 (*c*= 0.91 in MeOH); ν_{max} / cm^{-1} (film) 3439 (br), 3213 (br), 1709 (s, C=O), 1660 (s, C=O); δ_H (500 MHz, MeCN-d_3) 8.12 (d, *J* = 8.8 Hz, 2H, ArH), 7.05 (d, *J* = 8.8 Hz, 2H, ArH), 6.44 (br s, 1H, -NH-), 4.33 (d, *J* = 2.5 Hz, 1H, H-3), 3.90 (s, 3H, Ar-OMe), 3.87 (dt, *J* = 1.6, 3.6 Hz, 1H, H-4), 3.75-3.82 (m, 2H, H-5 and H-5'), 3.46 (br s, 1H, -OH); δ_C (125 MHz, MeCN-d_3) 188.03 (C-6), 170.80 (C-1), 164.54, 131.46, 128.08, 114.00 (Ar-C), 63.05 (C-2), 62.43 (C-3), 61.23 (C-5), 56.54 (C-4), 55.47 (Ar-OMe); *m/z* (ESI+) 286.1 ($[M+Na]^+$, 80%), (ESI-) 262.1 ($[M-H]^-$, 100%); HRMS (ESI+) calculated for $C_{13}H_{13}NNaO_5$ ($[M+Na]^+$) 286.0686, found 286.0682.

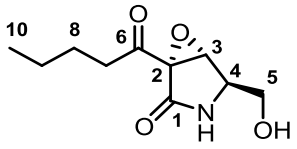
(2*S*, 4*R*)-2,3-Epoxy-4-hydroxymethyl-2-(octylcarbonyl)-1-oxo-pyrrolidinone, 78c



Scale of reaction: 71 mg (0.199 mmol), product: 38 mg, 71%;

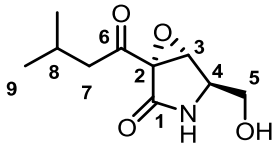
R_f = 0.34 (EtOAc); white solid; mp = 78-80°C; $[\alpha]_D^{20}$ = +115.7 (*c*= 0.98 in $CHCl_3$); ν_{max} / cm^{-1} (film) 3424 (br, lactam –NH-), 3274 (br, -OH), 1718 (s, C=O), 1673 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.18 (br s, 1H, -NH-), 4.13 (d, *J* = 2.2 Hz, 1H, H-3), 3.89 (very br, s, 1H, H-4), 3.83 (dd, *J* = 3.2, 12.3 Hz, 1H, H-5), 3.71 (dd, *J* = 3.5, 12.3 Hz, 1H, H-5'), 2.56 (ddd, *J* = 6.3, 8.5, 14.8 Hz, 1H, H-7), 2.47 (ddd, *J* = 6.3, 8.5, 14.8 Hz, 1H, H-7'), 1.50-1.60 (m, 2H, H-8), 1.26-1.32 (m, 10H, H-9, H-10, H-11, H-12, H-13), 0.88 (t, *J* = 6.9 Hz, 3H, H-14); δ_C (125 MHz, $CDCl_3$) 201.96 (C-6), 170.54 (C-1), 62.44 (C-3), 61.79 (C-5), 61.37 (C-2), 55.87 (C-4), 39.15 (C-7), 31.77 (C-13), 29.27, 29.08, 28.97, 22.61 (C9, C-10, C-11, C-12), 22.56 (C-8), 14.07 (C-14); *m/z* (ESI+) 292.15 ($[M+Na]^+$, 41%), 561.29 ($[2M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{14}H_{23}NNaO_4$ ($[M+Na]^+$) 292.1519, found 292.1522.

(2*S*, 4*R*)-2-(Butylcarbonyl)-2,3-epoxy-4-hydroxymethyl-1-oxo-pyrrolidinone, 78d



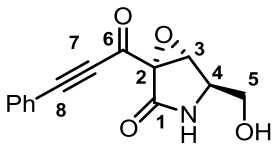
Scale of reaction: 112 mg (0.372 mmol), product: 76 mg, 96%; R_f = 0.24 (EtOAc); colourless oil; $[\alpha]_D^{20}$ = +147.4 (*c*= 0.85 in $CHCl_3$); ν_{max} / cm^{-1} (film) 3324 (br), 1720 (s, C=O), 1690 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.15 (br s, 1H, -NH-), 4.13 (d, *J* = 1.9 Hz, 1H, H-3), 3.89 (br t, *J* = 2.8 Hz, 1H, H-4), 3.83 (dd, *J* = 3.2, 12.3 Hz, 1H H-5), 3.72 (dd, *J* = 12.0, 3.5 Hz, 1H, H-5'), 2.58 (ddd, *J* = 6.3, 8.5, 18.0 Hz, 1H, H-7), 2.48 (ddd, *J* = 6.5, 8.4, 17.9 Hz, 1H, H-7'), 1.50-1.60 (m, 2H, H-8), 1.31 (sextet, *J* = 7.4 Hz, 2H, H-9), 0.90 (t, *J* = 7.3 Hz, 3H, H-10); δ_C (125 MHz, $CDCl_3$) 201.93 (C-6), 170.53 (C-1), 62.45 (C-3), 61.80 (C-5), 61.37 (C-2), 55.86 (C-4), 38.86 (C-7), 24.62 (C-8), 22.07 (C-9), 13.76 (C-10); *m/z* (ESI+) 236.1 ($[M+Na]^+$, 100%), (ESI-) 212.1 ($[M-H]^-$, 65%); HRMS (ESI+) calculated for $C_{10}H_{15}NNaO_4$ ($[M+Na]^+$) 236.0893, found 236.0893.

(2*S*, 4*R*)-2,3-Epoxy-4-hydroxymethyl-2-(isobutylcarbonyl)-1-oxo-pyrrolidinone, 78e



Scale of reaction: 60 mg (0.199 mmol), product: 34 mg, 80%; R_f = 0.26 (EtOAc); colourless solid, mp = 118-120°C; $[\alpha]_D^{20}$ = +135.8 (*c*= 0.93 in $CHCl_3$); ν_{max} / cm^{-1} (film) 3326 (br), 1719 (s, C=O), 1689 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.22 (br s, 1H, -NH-), 4.12 (d, *J* = 2.2 Hz, 1H, H-3), 3.89 (br t, *J* = 2.8 Hz, 1H, H-4), 3.82 (dd, *J* = 3.2, 12.3 Hz, 1H, H-5), 3.72 (dd, *J* = 3.5, 12.3 Hz, 1H, H-5'), 2.49 (dd, *J* = 6.5, 16.9 Hz, 1H, H-7), 2.32 (dd, *J* = 7.3, 16.7 Hz, 1H, H-7'), 2.16 (doublet of septet, *J* = 6.4, 13.4 Hz, 1H, H-8), 0.94 (d, *J* = 6.6 Hz, 3H, H-9), 0.91 (d, *J* = 6.6 Hz, 3H, H-9'); δ_C (125 MHz, $CDCl_3$) 201.70 (C-6), 170.53 (C-1), 62.25 (C-3), 61.77 (C-5), 61.51 (C-2), 55.87 (C-4), 47.63 (C-7), 23.88 (C-8), 22.55, 22.38 (C-9, C-9'); *m/z* (ESI) 212.1 ($[M-H]^-$, 100%); HRMS (ESI+) calculated for $C_{10}H_{15}NNaO_4$ ($[M+Na]^+$) 236.0893, found 236.0896.

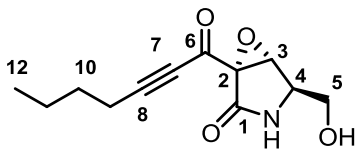
(2*S*, 4*R*)-2,3-Epoxy-4-hydroxymethyl-2-(phenylacetylenecarbonyl)-1-oxo-pyrrolidinone, 78f



Scale of reaction: 108 mg (0.313 mmol), product: 62 mg, 77%; R_f = 0.26 (EtOAc); colourless oil; $[\alpha]_D^{20}$ = +198.8 (*c*= 0.88 in $CHCl_3$); ν_{max} /

cm^{-1} (film) 3307 (br), 2192 (s, alkyne), 1702 (s, C=O), 1654 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.57 (d, *J* = 7.3 Hz, 2H, Ph-H), 7.44-7.47 (m, 2H, Ph-H and -NH-), 7.36 (t, *J* = 7.9 Hz, 2H, Ph-H), 4.38 (d, *J* = 1.9 Hz, 1H, H-3), 4.16 (br t, *J* = 5.4 Hz, 1H, -OH), 3.96-3.97 (m, 1H, H-4), 3.87-3.91 (m, 1H, H-5), 3.75-3.80 (m, 1H, H-5'), [poor resolution for H-5 and H-5']; δ_C (125 MHz, $CDCl_3$) 177.44 (C-6), 169.62 (C-1), 133.58, 131.50, 128.67, 118.96 (Ph-C), 96.06 (C-8), 85.14 (C-7), 63.23 (C-3), 61.74 (C-2), 61.57 (C-5), 55.88 (C-4); *m/z* (ESI+) 258.1 ($[M+H]^+$, 100%); HRMS (ESI+) calculated for $C_{14}H_{11}NNaO_4$ ($[M+Na]^+$) 280.0580, found 280.0575.

(2*S*, 4*R*)-2,3-Epoxy-4-hydroxymethyl-2-(butylacetylenecarbonyl)-1-oxo-pyrrolidinone, 78g



Scale of reaction: 92 mg (0.283 mmol), product: 58 mg, 86%; R_f = 0.30 (EtOAc); colourless oil; $[\alpha]_D^{20}$ = +172.7 (*c*= 0.96 in $CHCl_3$); ν_{max} / cm^{-1} (film) 3325 (br) 2960, 2935 (w, -NH-), 2211 (s, alkyne), 1705 (s, C=O; with a shoulder on the shorter frequency side); δ_H (500 MHz, $CDCl_3$) 7.57 (br s, 1H, -NH-), 4.29 (d, *J* = 1.9 Hz, 1H, H-3), 3.91 (br t, *J* = 3.8 Hz, 1H, H-4), 3.86 (dd, *J* = 3.2, 12.3 Hz, 1H, H-5), 3.73 (dd, *J* = 3.2, 12.3 Hz, 1H, H-5'), 2.40 (t, *J* = 7.1 Hz, 2H, H-9), 1.54-1.59 (m, 2H, H-10), 1.38-1.46 (m, 2H, H-11), 0.92 (t, *J* = 7.4 Hz, 3H, H-12); δ_C (125 MHz, $CDCl_3$) 177.74 (C-6), 169.98 (C-1, br), 100.34 (C-8), 77.77 (C-7), 62.98 (C-3), 61.57 (C-5), 61.26 (C-2), 55.92 (C-4), 29.36 (C-10), 21.89 (C-11), 18.86 (C-9), 13.41 (C-12); *m/z* (ESI+) 236.1 ($[M-H]^-$, 100%); HRMS (ESI+) calculated for $C_{12}H_{15}NNaO_4$ ($[M+Na]^+$) 260.0893, found 260.0890.

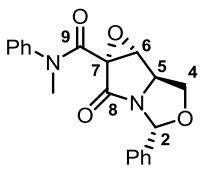
Evidence of weak intramolecular hydrogen-bonding interactions

Methylation of Secondary Amide **67b**

A solution of **67b** (35 mg, 0.104 mmol, 1.0 eq.) in dry THF (3 mL) was added to NaH (60% in mineral oil) (4.2 mg, 0.104 mmol, 1.0 eq.) at 0°C. The mixture was stirred at r.t. for 35 min. MeI (7 µL, 0.104 mmol, 1.0 eq.) was then added and the reaction mixture was stirred at r.t. for 45 min. The reaction was quenched with water, acidified with 10% aq. HCl dropwise and extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents removed *in vacuo* to give

the crude. Purification via silica gel flash column chromatography (Eluent: EtOAc/PE, 0% to 40%) gave the **79** (20 mg, 55%) as colourless oil.

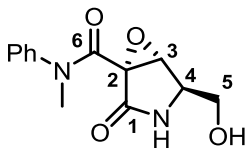
(2*R*, 5*S*, 7*S*)-1-Aza-6,7-epoxy-7-(*N*,*N'*-methylphenylcarbamoyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 79



R_f = 0.13 (1:1 EtOAc/PE); white solid, mp = 182-185°C; $[\alpha]_D^{20}$ = +81.2 (*c*= 0.99 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1720 (s, C=O), 1665 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.42-7.49 (m, 3H, Ph-H), 7.29-7.36 (m, 7H, Ph-H), 6.12 (s, 1H, H-2), 4.01 (s, 1H, H-6), 3.97 (dd, *J* = 6.9, 9.5 Hz, 1H, H-5), 3.90 (t, *J* = 6.9 Hz, 1H, H-4), 3.38 (s, 3H, -N<u>Me</u>-), 2.65 (dd, *J* = 8.2, 9.1 Hz, 1H, H-4'); δ_C (125 MHz, $CDCl_3$) 172.13 (C-8), 161.29 (C-9), 141.82, 137.71, 129.89, 128.74, 128.71, 128.42, 127.57, 125.76 (Ph-C), 88.17 (C-2), 64.91 (C-4), 63.46 (C-6), 61.87 (C-7), 58.53 (C-5), 37.91 (-N<u>Me</u>-); *m/z* (ESI+) 373.1 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{20}H_{18}N_2NaO_4$ ($[M+Na]^+$) 373.1159, found 373.1150.

Acidic oxazolidine hydrolysis of **76** and **79** as per general procedure for **77a-g**.

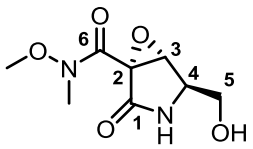
(2*S*, 4*R*)-2,3-Epoxy-4-hydroxymethyl-2-(*N*,*N'*-methylphenylcarbamoyl)-1-oxo-pyrrolidinone, 80



Scale of reaction: 20 mg (0.0562 mmol), product: 11 mg, 77%;

R_f = 0.11 (EtOAc); white solid, mp = 208-210°C; $[\alpha]_D^{20}$ = -20.6 (*c*= 0.53 in MeOH); ν_{max} / cm^{-1} (film) 3423 (br), 3195 (br), 1684 (s, C=O), 1651 (s, C=O); δ_H (500 MHz, MeOD-d_4) 7.47-7.52 (m, 3H, Ph-H), 7.37-7.39 (m, 2H, Ph-H), 4.13 (s, 1H, H-3), 3.48 (dd, *J* = 6.5, 8.0 Hz, 1H, H-4), 3.33 (s, 3H, -N<u>Me</u>-; overlapped with MeOD signal but shows the desired correlation in HSQC and HMBC), 2.91 (dd, *J* = 6.5, 10.9 Hz, 1H, H-5), 2.58 (dd, *J* = 8.0, 10.9 Hz, 1H, H-5'); δ_C (125 MHz, MeOD-d_4) 172.54 (C-1), 164.19 (C-6), 142.94, 131.22, 130.11, 129.36 (Ph-C), 63.13 (C-3), 62.13 (C-5), 61.65 (C-2), 57.63 (C-4), 38.44 (-N<u>Me</u>-); *m/z* (ESI+) 285.1 ($[M+Na]^+$, 100%), (ESI-) 261.0 ($[M-H]^-$, 100%); HRMS (ESI+) calculated for $C_{13}H_{14}N_2NaO_4$ ($[M+Na]^+$) 285.0846, found 285.0842.

(2*R*, 4*R*)-2,3-Epoxy-4-hydroxymethyl-2-(*N*,*O*-dimethylcarbamoyl)-oxo-pyrrolidinone, 81



d.r. ~ 1:0.1

Scale of reaction: 30 mg (0.0986 mmol), product: 17 mg, 80%;

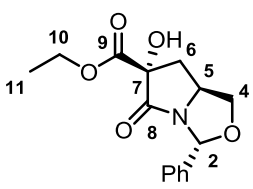
R_f = 0.24 (10% MeOH/EtOAc); white needle-like crystals, mp = 193-198°C; $[\alpha]_D^{20}$ = +14.2 (*c*= 0.48 in MeOH); ν_{max} / cm^{-1} (film) 3394 (br, lactam -NH-), 3239 (br, -OH), 1710 (s, C=O), 1659 (s, C=O); Major Diastereoisomer, at elevated temperature due to broad signals of rotamers - δ_H (400 MHz, Acetonitrile-d_3, 338 K) 6.18 (br s, 1H, -NH), 4.17 (d, *J* = 2.4 Hz, 1H, H-3), 3.72-3.74 (m, 1H, H-4), 3.70 (s, 3H, -O<u>Me</u>), 3.63 (dd, *J* = 5.7, 11.2 Hz, 1H, H-5), 3.57 (dd, *J* = 6.6, 11.2 Hz, 1H, H-5'), 3.21 (s, 3H, -N<u>Me</u>); δ_C (100 MHz, Acetonitrile-d_3, 338K) 170.81 (C-1), 164.21 (C-6), 63.16 (C-5, br), 62.15 (-O<u>Me</u>, br), 61.76 (C-2), 61.43 (C-3, br), 57.49 (C-4), 33.07 (-N<u>Me</u>, br); *m/z* (ESI+) 239.05 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_8H_{12}N_2NaO_5$ ($[M+Na]^+$) 239.0638, found 239.0638.

5.2.2 Experimental for 2-hydroxypyrrolidinones

<u>Initial investigation of Ce(III)-mediated α-hydroxylation of **61**</u>

61 (50 mg, 0.182 mmol, 1.0 eq.) was added to a solution of $CeCl_3.7H_2O$ (3.4 mg, 0.009 mmol, 0.05 eq.) in ^{i}PrOH (1 mL). The reaction mixture was first bubbled through with O_2 (balloon) to saturate the solvent, and left to stir with O_2 balloon attached. The reaction was stirred at r.t. for 25 h, and filtered over a pad of silica gel followed by washing with EtOAc. The solvent was evaporated to give the crude which was purified on via silica gel flash column chromatography (Eluent: 10% to 20% EtOAc/PE) to give the following:

(2*R*, 5*S*, 7*R*)-7-Ethoxycarbonyl-7-hydroxy-8-oxo-2-phenyl-1-aza-3-oxa-bicyclo[3.3.0]octane, (7*R*)-86

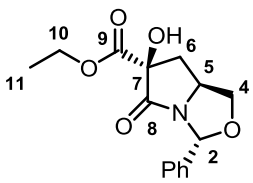


R_f = 0.35 (1:1 EtOAc:PE); colourless oil; $[\alpha]_D^{23}$ = +128.8 (*c*= 0.5 in MeCN); ν_{max} / cm^{-1} (film) 3422 (br,), 1744 (s, C=O), 1711 (s, C=O);

δ_H (400 MHz, $CDCl_3$) 7.45-7.48 (m, 2H, PhH), 7.33-7.40 (m, 3H, PhH), 6.29 (s, 1H, H-2), 4.34 (q, *J* = 7.1 Hz, 2H, H-10), 4.27-4.33 (m, 2H, H-5 and H-4), 3.64-3.70 (m, 1H, H-4'), 2.55 (dd, *J* = 4.8, 14.2 Hz, 1H, H-6), 2.47 (dd, *J* = 7.2, 14.2 Hz, 1H, H-6'), 1.35 (t, *J* = 7.1 Hz, 3H, H-

11); δ_C (100 MHz, $CDCl_3$) 172.62 (C-8), 171.03 (C-9), 137.83, 128.82, 128.50, 125.95 (PhC), 87.09 (C-2), 82.23 (C-7), 71.36 (C-4), 63.31 (C-10), 55.94 (C-5), 35.20 (C-6), 14.03 (C-11); *m/z* (ESI+) 314.25 ($[M+Na]^+$, 40%); HRMS (ESI+) calculated for $C_{15}H_{17}NNaO_5$ ($[M+Na]^+$) 314.0999, found 314.1000.

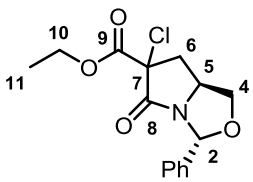
(2*R*, 5*S*, 7*S*)-7-Ethoxycarbonyl-7-hydroxy-8-oxo-2-phenyl-1-aza-3-oxa-bicyclo[3.3.0] octane, (7*S*)-86



R_f = 0.28 (1:1 EtOAc:PE); colourless oil; $[\alpha]_D^{23}$ = +98.8 (*c*= 0.80 in MeCN); ν_{max} / cm^{-1} (film) 3423 (br,), 1745 (s, C=O), 1712 (s, C=O); δ_H (400 MHz, $CDCl_3$) 7.35-7.44 (m, 5H, PhH), 6.33 (s, 1H, H-2), 4.24-4.35 (m, 4H, H-10, H-5 and H-4), 3.63 (t, *J* = 8.0 Hz, 1H, H-4'), 2.94 (dd, *J* = 6.7, 13.3 Hz, 1H, H-6), 2.12 (dd, *J* = 7.0, 13.3 Hz, 1H, H-6'), 1.30 (t, *J* = 7.1 Hz, 3H, H-11); δ_C (100 MHz, $CDCl_3$) 171.25 (C-8), 170.57 (C-9), 137.58, 128.94, 128.55, 126.02 (PhC), 86.99 (C-2), 82.10 (C-7), 72.26 (C-4), 63.01 (C-10), 55.28 (C-5), 37.45 (C-6), 14.01 (C-11); *m/z* (ESI+) 314.22 ($[M+Na]^+$, 70%); HRMS (ESI+) calculated for $C_{15}H_{17}NNaO_5$ ($[M+Na]^+$) 314.0999, found 314.1005.

Stereochemical assignment of **86-7*R*** and **86-7*S*** was done via nOeSY experiment and based on analogous structures from M. G. Moloney *et al*.[169]

(2*R*, 5*S*, 7*RS*)-7-Chloro-7-ethoxycarbonyl-8-oxo-2-phenyl-1-aza-3-oxa-bicyclo[3.3.0] octane, 87

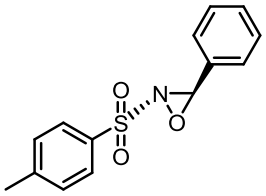


R_f = 0.63 (1:1 EtOAc:PE); courless oil; ν_{max} / cm^{-1} (film) 1758 (s, C=O), 1722 (s, C=O); δ_H (400 MHz, $CDCl_3$) 7.33-7.47 (m, 5H, PhH), 6.38 (s, 0.5H, H-2a), 6.32 (s, 0.5H, H-2b), 4.27-4.40 (m, 4H, H-4a, H-4b, H-5b, H-10a, H-10b), 4.13-4.20 (m, 0.5H, H-5a), 3.66-3.76 (m, 1H, H-4'a, H-4'b), 3.37 (dd, *J* = 6.5, 13.4 Hz, 0.5H, H-6a), 2.93 (dd, *J* = 6.7, 14.1 Hz, 0.5H, H-6b), 2.75 (dd, *J* = 6.2, 14.1 Hz, 0.5H, H-6'b), 2.37 (dd, *J* = 7.0, 13.4 Hz, 0.5H, H-6'a), 1.37 (t, *J* = 7.1 Hz, 1.5H, H-11b), 1.31 (t, *J* = 7.1 Hz, 1.5H, H-11a); δ_C (100 MHz, $CDCl_3$) 169.09 (C-8b), 167.86 (C-8a), 166.48 (C-9a), 166.14 (C-9b), 137.25, 137.23, 129.00, 128.98, 128.61, 128.56, 125.98 (PhC), 87.74 (C-2a), 87.10 (C-2b), 71.68 (C-4a), 71.00 (C-4b), 70.76

(C-7b), 70.43 (C-7a), 63.70, 63.63 (C-10), 56.12 (C-5b), 55.51 (C-5a), 41.17 (C-6a), 40.75 (C-6b), 14.00, 13.88 (C-11); *m/z* (ESI+) 332.19 ($[M+Na]^+$, 90%); HRMS (ESI+) calculated for $C_{15}H_{16}ClNNaO_4$ ($[M+Na]^+$) 332.0660, found 332.0677.

3-Phenyl-2-*p*-tolylsulfonyloxaziridine, 89

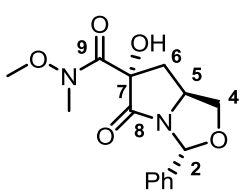
(*E*)-*N*-benzylidene-4-methylbenzensulfonamide (1 g, 3.856 mmol, 1.0 eq.) in CH_2Cl_2 (10 mL) was added to the white suspension of 77% *m*-CPBA (950.6 mg, 4.242 mmol, 1.1 eq.) and powdered KOH (757.3 mg, 13.50 mmol, 13.5 eq.) in 10 mL CH_2Cl_2. (prepared at 0°C on adding *m*-CPBA to KOH and warmed to r.t. prior use). After 15 min, the reaction crude is then filtered over Celite and the solvent evaporated to give the pure oxaziridine (824 mg, 78%).



R_f = 0.40 (20% EtOAc/PE); white solid, mp = 89-91°C {lit.[86] 87-89°C}; ν_{max} / cm^{-1} (film) 3068, 2930, 1350, 1168; δ_H (400 MHz, $CDCl_3$) 7.93 (d, *J* = 8.3 Hz, 2H, ArH), 7.40-7.47 (m, 7H, ArH), 5.45 (s, 1H), 2.50 (s, 3H); δ_C (100 MHz, $CDCl_3$) 146.36, 131.60, 131.35, 130.60, 130.03, 129.42, 128.71, 128.22, 76.33, 21.81; *m/z* (ESI-) 274.26 ($[M-H]^-$, 65%).

(2*R*, 5*S*, 7*S*) and (2*R*, 5*S*, 7*R*)-1-Aza-7-hydroxy-7-(*N*,*O*-dimethylcarbamoyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 82

To a 1 M THF solution of KHMDS (1.36 mL, 1.357 mmol, 1.3 eq.) at -78°C was added **73** (303 mg, 1.044 mmol, 1.0 eq.) in THF (4 mL). The solution was stirred at -78°C for 40 min, after which oxaziridine **89** (287.4 mg, 1.044 mmol, 1.0 eq.) in THF (3 mL) was added. After stirring at -78°C for 35 min, the reaction was quenched with sat. aq. NH_4Cl, extracted with EtOAc. The combined organic layers dried with $MgSO_4$, filtered and evaporated to give the crude, which was purified on silica gel (Eluent 0% to 10% EtOAc/CH_2Cl_2) to give the desired product (182 mg, 57%, d.r. = 1:0.2).



R_f = 0.54 (1:1 EtOAc:CH_2Cl_2); colourless solid; ν_{max} / cm^{-1} (film) 3404 (br, -OH), 1710 (s, C=O), 1655 (s, C=O); δ_H (400 MHz, MeCN-d_3) 7.36-7.49 (m, 5H, PhH), 6.20, 6.19 (s, 1H, H-2), 4.84, 4.82 (s, 1H, -OH), 4.33-4.40 (m, 0.75H, H-5), 4.28-4.32 (m, 1H, H-4), 4.06-4.13 (m, 0.25H, H-5), 3.68, 3.44 (s, 3H, -N<u>Me</u>), 3.54 (t, *J* = 8.2 Hz,

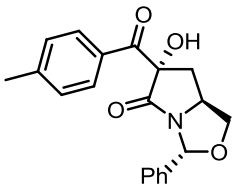
1H, H-4), 3.28, 3.23 (s, 3H, -OMe), 2.86 (dd, *J* = 7.9, 14.3 Hz, 0.6H, H-6b), 2.50 (dd, *J* = 5.5, 14.1 Hz, 0.4H, H-6a), 2.22 (dd, *J* = 7.6, 14.0 Hz, 0.4H, H-6a'), 2.00 (dd, *J* = 3.8, 14.3 Hz, 0.6H, H-6b'); δ_C (100 MHz, MeCN-d_3) 175.12, 173.31 (C-8), 171.28, 171.00 (C-9), 140.26, 139.87, 129.84, 129.75, 129.70, 129.66, 129.59, 129.51, 129.49, 129.48, 127.17, 127.09 (PhC), 89.57, 87.84 (C-2), 83.19, 81.94 (C-7), 73.01, 72.74 (C-4), 61.58, 61.08 (-NMe), 57.36, 56.78 (C-5), 37.32, 36.20 (C-6), 33.97, 33.61 (-OMe); *m/z* (ESI+) 329.24 ($[M+Na]^+$, 95%); HRMS (ESI+) calculated for $C_{15}H_{18}N_2NaO_5$ ($[M+Na]^+$) 329.1108, found 329.1121.

General procedures for Grignard reaction of hydroxy Weinreb amide **82**

Aryl, alkyl and alkenyl Grignard reagents are prepared as per the reaction with **76** (p. 165).

The Grignard reagent in THF (2.2 eq. based on amount of **82** used) was added to **82** (1.0 eq.) in THF at 0°C. The reaction was stirred at 0°C to r.t. over a period of 3-4 h, afterwhich it was quenched with sat. aq. NH_4Cl and extracted with EtOAc. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents removed *in vacuo* to give the crude product. Purification via silica gel flash column chromatography (Eluent: EtOAc/PE) gave the respective product as two separate diastereomers about C-7.

(2*R*, 5*S*, 7*S*)-1-Aza-7-hydroxy-7-(4'methylphenylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, (7*S*)-91a

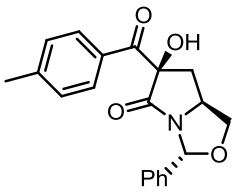


Scale of reaction: 87 mg (0.284 mmol), product: 45 mg, 47%;

R_f = 0.51 (1:1 EtOAc:PE); colourless crystals, mp = 145-146°C; $[\alpha]_D^{23}$ = +143.0 (*c*= 0.93 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3405 (br, -OH), 1709 (s, C=O), 1673 (s, C=O); δ_H (400 MHz, MeCN-d_3) 7.85 (d, *J* = 8.3 Hz, 2H, ArH), 7.48-7.53 (m, 2H, PhH), 7.38-7.47 (m, 3H, PhH), 7.36 (d, *J* = 8.0 Hz, 2H, ArH), 6.30 (s, 1H, H-2), 5.03 (s, 1H, -OH), 4.48 (ddt, *J* = 6.1, 6.9, 8.5 Hz, 1H, H-5), 4.33 (dd, *J* = 6.2, 8.4 Hz, 1H, H-4 exo), 3.69 (t, *J* = 8.5 Hz, 1H, H-4' endo), 2.68 (dd, *J* = 6.1, 14.4 Hz, 1H, H-6 endo), 2.42 (s, 3H, Ar-Me), 2.40 (dd, *J* = 7.0, 14.4 Hz, 1H, H-6'exo); δ_C (100 MHz, MeCN-d_3) 198.95 (C-8), 174.27 (C-9), 145.88, 139.54, 131.77, 130.78, 130.23, 129.98, 129.63, 127.23 (ArC), 88.38 (C-7), 88.12 (C-2), 72.72 (C-4), 58.02 (C-5),

39.08 (C-6), 21.77 (Ar-Me); *m/z* (ESI+) 360.27 ($[M+Na]^+$, 65%); HRMS (ESI+) calculated for $C_{20}H_{19}NNaO_4$ ($[M+Na]^+$) 360.1206, found 360.1222.

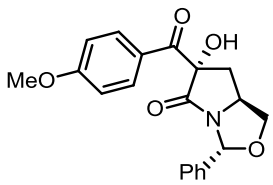
(2*R*, 5*S*, 7*R*)-1-Aza-7-hydroxy-7-(4'methylphenylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, (7*R*)-91a



Product: 9 mg, 9%;

R_f = 0.38 (1:1 EtOAc:PE); colourless oil; $[\alpha]_D^{23}$= +152.3 (*c*= 1.03 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3345 (br, -OH), 1702 (s, C=O), 1672 (s, C=O); δ_H (400 MHz, MeCN-d_3) 8.10 (d, *J* = 8.3 Hz, 2H, ArH), 7.35-7.42 (m, 5H, PhH), 7.29 (d, *J* = 8.1 Hz, 2H, ArH), 6.20 (s, 1H, H-2), 4.72 (s, 1H, -OH), 4.32 (dd, *J* = 6.1, 8.2 Hz, 1H, H-4), 4.14 (apparent dq, *J* = 6.5, 8.3 Hz, 1H, H-5), 3.63 (t, *J* = 8.3 Hz, 1H, H-4'), 3.13 (dd, *J* = 6.5, 13.1 Hz, 1H, H-6), 2.15 (s, 3H, Ar-Me), 2.12 (dd, *J* = 7.1, 13.2 Hz, 1H, H-6' partially obscured by Ar-Me); δ_C (100 MHz, MeCN-d_3) 197.86 (C-9), 173.89 (C-8), 145.59, 139.56, 132.38, 131.24, 129.93, 129.92, 129.56, 127.23, 127.01 (ArC), 89.29 (C-7), 88.12 (C-2), 73.43 (C-4), 56.34 (C-5), 40.05 (C-6), 21.74 (Ar-Me); *m/z* (ESI+) 360.26 ($[M+Na]^+$, 95%); HRMS (ESI+) calculated for $C_{20}H_{19}NNaO_4$ ($[M+Na]^+$) 360.1206, found 360.1218.

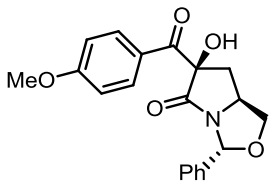
(2*R*, 5*S*, 7*S*)-1-Aza-7-hydroxy-7-(4'-methoxyphenylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, (7*S*)-91b



R_f = 0.32 (1:1 EtOAc:PE); colourless crystals, mp = 145-147°C;

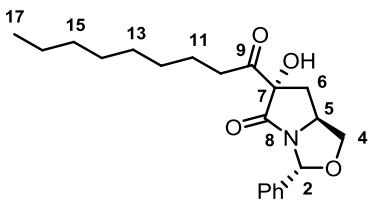
$[\alpha]_D^{23}$= +100.9 (*c*= 0.83 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3404 (br, -OH), 1707 (s, C=O), 1665 (s, C=O), 1599; δ_H (400 MHz, MeCN-d_3) 7.96 (d, *J* = 9.1 Hz, 2H, ArH), 7.48-7.54 (m, 2H, PhH), 7.36-7.47 (m, 3H, PhH), 7.05 (d, *J* = 9.1 Hz, 2H, ArH), 6.29 (s, 1H, H-2), 5.04 (s, 1H, -OH), 4.48 (ddt, *J* = 6.1, 7.0, 8.5 Hz, 1H, H-5), 4.34 (dd, *J* = 6.3, 8.3 Hz, 1H, H-4 exo), 3.88 (s, 3H, -OMe), 3.71 (t, *J* = 8.5 Hz, 1H, H-4' endo), 2.69 (dd, *J* = 6.1, 14.4 Hz, 1H, H-6 endo), 2.40 (dd, *J* = 7.0, 14.4 Hz, 1H, H-6'exo); δ_C (100 Mhz, MeCN-d_3) 197.41 (C-9), 174.36 (C-8), 165.04, 139.57, 133.28, 129.99, 129.65, 127.25, 126.80, 114.90 (ArC), 88.17 (C-7), 88.13 (C-2), 72.76 (C-4), 58.01 (C-5), 56.47 (-OMe), 39.30 (C-6); *m/z* (ESI+) 376.27 ($[M+Na]^+$, 55%); HRMS (ESI+) calculated for $C_{20}H_{19}NNaO_5$ ($[M+Na]^+$) 376.1155, found 376.1151.

(2*R*, 5*S*, 7*R*)-1-Aza-7-hydroxy-7-(4'-methoxyphenylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, (7*R*)-91b



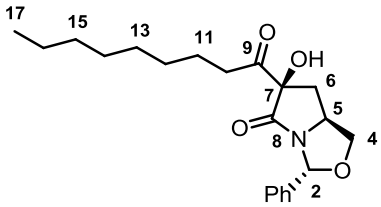
R_f = 0.24 (1:1 EtOAc:PE); colourless crystals, mp = 140-142°C; $[\alpha]_D^{23}$= +94.0 (*c*= 0.51 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3404 (br, -OH), 1707 (s, C=O), 1665 (s, C=O), 1599; δ_H (400 MHz, MeCN-d_3) 8.23 (d, *J* = 9.1 Hz, 2H, ArH), 7.29-7.46 (m, 5H, PhH), 6.98 (d, *J* = 9.1 Hz, 2H, ArH), 6.20 (s, 1H, H-2), 4.71 (s, 1H, -OH), 4.32 (dd, *J* = 6.0, 8.2 Hz, 1H, H-4 exo), 4.14 (ddt, *J* = 6.2, 7.0, 8.4 Hz, 1H, H-5), 3.86 (s, 3H, -OMe), 3.62 (t, *J* = 8.5 Hz, 1H, H-4' endo), 3.14 (dd, *J* = 6.5, 13.0 Hz, 1H, H-6 exo), 2.11 (dd, *J* = 7.1, 13.0 Hz, 1H, H-6' endo); δ_C (100 MHz, MeCN-d_3) 196.26 (C-9), 174.04 (C-8), 164.90, 139.58, 133.76, 129.91, 129.56, 127.54, 127.23, 114.53 (ArC), 89.26 (C-7), 88.12 (C-2), 73.46 (C-4), 56.37 (C-5), 56.35 (-OMe), 40.15 (C-6); *m/z* (ESI+) 376.26 ($[M+Na]^+$, 80%); HRMS (ESI+) calculated for $C_{20}H_{19}NNaO_5$ ($[M+Na]^+$) 376.1155, found 376.1165.

(2*R*, 5*S*, 7*S*)-1-Aza-7-hydroxy-7-(octylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, (7*S*)-91c



Scale of reaction: 90 mg (0.294 mmol), product: 25 mg, 24%; R_f = 0.53 (1:1 EtOAc:PE); colourless oil; $[\alpha]_D^{23}$= +106.6 (*c*= 0.91 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3403 (br, -OH), 1719 (s, C=O), 1703 (s, C=O); δ_H (400 MHz, MeCN-d_3) 7.38-7.47 (m, 5H, PhH), 6.12 (s, 1H, H-2), 4.67 (s, 1H, -OH), 4.30-4.39 (m, 2H, H-5, H-4), 3.63 (t, *J* = 8.1 Hz, 1H, H-4'), 2.69 (dt, *J* = 7.2, 18.1 Hz, 1H, H-10), 2.67 (dt, *J* = 7.2, 18.1 Hz, 1H, H-10'), 2.51 (dd, *J* = 5.4, 14.3 Hz, 1H, H-6), 2.24 (dd, *J* = 6.8, 14.2 Hz, 1H, H-6'), 1.54-1.59 (m, 2H, H-11), 1.26-1.35 (m, 10H, H-12, H-13, H-14, H-15, H-16), 0.89 (t, *J* = 7.0 Hz, 3H, H-17); δ_C (100 MHz, MeCN-d_3) 210.13 (C-9), 174.17 (C-8), 139.74, 129.90, 129.57, 127.19 (PhC), 88.65 (C-7), 87.82 (C-2), 72.71 (C-4), 57.67 (C-5), 38.47 (C-10), 36.75 (C-6), 32.61, 30.11, 29.61, 29.77, 24.10 (C-11), 23.39 (C-12, C-13, C-14, C-15, C-16), 14.38 (C-17); *m/z* (ESI+) 382.38 ($[M+Na]^+$, 70%); HRMS (ESI+) calculated for $C_{21}H_{29}NNaO_4$ ($[M+Na]^+$) 382.1989, found 382.1996.

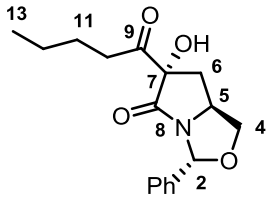
(2*R*, 5*S*, 7*R*)-1-Aza-7-hydroxy-7-(octylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, (7*R*)-91c



Product: 7 mg, 7%;

R_f = 0.38 (1:1 EtOAc:PE); colourless oil; $[\alpha]_D^{23}$ = +190.1 (*c*= 0.73 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3373 (br, -OH), 1694 (s, br with shoulder towards larger wavenumber, C=O); δ_H (400 MHz, MeCN-d_3) 7.37-7.43 (m, 5H, PhH), 6.16 (s, 1H, H-2), 4.48 (s, 1H, -OH), 4.32 (dd, *J* = 6.0, 8.2 Hz, 1H, H-4), 4.13-4.20 (m, 1H, H-5), 3.57 (t, *J* = 8.4 Hz, 1H, H-4'), 2.85 (dd, *J* = 6.8, 13.4 Hz, 1H, H-6), 2.67 (t, *J* = 7.2 Hz, 2H, H-10), 1.97 (dd, 1H, H-6', partially obscured by MeCN-d_3 signal), 1.48-1.55 (m, 2H, H-11), 1.19-1.34 (m, 10H, H-12, H-13, H-14, H-15, H-16), 0.87 (t, *J* = 7.0 Hz, 3H, H-17); δ_C (100 MHz, MeCN-d_3) 209.29 (C-9), 173.26 (C-8), 139.62, 129.93, 129.57, 127.19 (PhC), 88.96 (C-7), 88.01 (C-2), 73.61 (C-4), 56.40 (C-5), 37.91 (C-6), 37.33 (C-10), 32.55, 30.02, 29.88, 29.71 (C-13, C-14, C-15, C-16),24.02 (C-11), 23.36 (C-12), 14.38 (C-17); *m/z* (ESI+) 382.39 ($[M+Na]^+$, 70%); HRMS (ESI+) calculated for $C_{21}H_{29}NNaO_4$ ($[M+Na]^+$) 382.1989, found 382.1989.

(2*R*, 5*S*, 7*S*)-1-Aza-7-(butylcarbonyl)-7-hydroxy-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, (7*S*)-91d

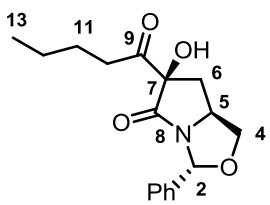


Scale of reaction: 108 mg (0.353 mmol), product: 37 mg, 35%

R_f = 0.53 (1:1 EtOAc:PE); colourless oil; $[\alpha]_D^{23}$ = +137.0 (*c*= 1.06 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3405 (br, -OH), 1719 (s, C=O), 1701 (s, C=O); δ_H (400 MHz, MeCN-d_3) 7.34-7.49 (m, 5H, PhH), 6.12 (s, 1H, H-2), 4.68 (s, 1H, -OH), 4.35-4.41 (m, 1H, H-5), 4.32 (dd, *J* = 6.1, 7.6 Hz, 1H, H-4), 3.63 (t, *J* = 8.1 Hz, 1H, H-4'), 2.71 (dt, *J* = 7.2, 18.1 Hz, 1H, H-10), 2.69 (dt, *J* = 7.2, 18.1 Hz, 1H, H-10'), 2.51 (dd, *J* = 5.5, 14.3 Hz, 1H, H-6), 2.24 (dd, *J* = 6.9, 14.3 Hz, 1H, H-6'), 1.52-1.60 (m, 2H, H-11), 1.28-1.39 (m, 2H, H-12), 0.92 (t, *J* = 7.3 Hz, 3H, H-13); δ_C (100 MHz, MeCN-d_3) 210.15 (C-9), 174.18 (C-8), 139.75, 129.91, 129.58, 127.20 (PhC), 88.67 (C-7), 87.82 (C-2), 72.71 (C-4), 57.67 (C-5), 38.20 (C-10), 36.76 (C-6), 26.11 (C-

11), 22.88 (C-12), 14.16 (C-13); *m/z* (ESI+) 326.30 ([M+Na]$^+$, 50%); HRMS (ESI+) calculated for $C_{17}H_{21}NNaO_4$ ([M+Na]$^+$) 326.1363, found 326.1371.

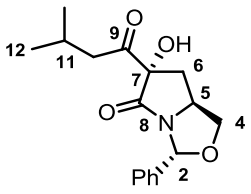
(2*R*, 5*S*, 7*R*)-1-Aza-7-(butylcarbonyl)-7-hydroxy-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, (7*R*)-91d



Product: 3 mg, 3%;

R_f = 0.39 (1:1 EtOAc:PE); colourless oil; $[\alpha]_D^{23}$= +156.2 (*c*= 0.66 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3383 (br, -OH), 1696 (s, br shoulder towards larger wavenumber, C=O); δ_H (400 MHz, MeCN-d_3) 7.34-7.44 (m, 5H, PhH), 6.16 (s, 1H, H-2), 4.49 (s, 1H, -OH), 4.32 (dd, *J* = 6.0, 8.2 Hz, 1H, H-4), 4.16 (apparent dq, *J* = 6.8, 8.5 Hz, 1H, H-5), 3.57 (t, *J* = 8.4 Hz, 1H, H-4'), 2.85 (dd, *J* = 6.8, 13.4 Hz, 1H, H-6), 2.68 (t, *J* = 7.2 Hz, 2H, H-10), 1.95 (dd, *J* = 6.9 Hz, 1H, H-6', partially obscured by MeCN-d_3 signal), 1.44-1.55 (m, 2H, H-11), 1.19-1.31 (m, 2H, H-12), 0.85 (t, *J* = 7.4 Hz, 3H, H-13); δ_C (100 MHz, MeCN-d_3) 209.36 (C-9), 173.27 (C-8), 139.61, 130.56, 129.93, 129.58, 127.20 (PhC), 88.96 (C-7), 88.01 (C-2), 73.60 (C-4), 56.40 (C-5), 37.95 (C-6), 37.08 (C-10), 26.13 (C-11), 22.83 (C-12), 14.08 (C-13); *m/z* (ESI+) 326.26 ([M+Na]$^+$, 65%); HRMS (ESI+) calculated for $C_{17}H_{21}NNaO_4$ ([M+Na]$^+$) 326.1363, found 326.1373.

(2*R*, 5*S*, 7*S*)-1-Aza-7-hydroxy-7-(isobutylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, (7*S*)-91e

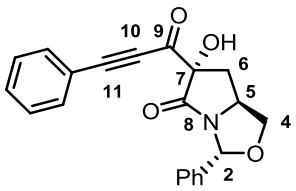


Scale of reaction: 86 mg (0.281 mmol), product: 5 mg, 6%;

R_f = 0.57 (1:1 EtOAc/PE); colourless oil; $[\alpha]_D^{23}$= +180.3 (*c*= 0.57 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3404 (br, -OH), 1718 (s, C=O), 1700 (s, C=O; δ_H (400 MHz, MeCN-d_3) 7.35-7.49 (m, 5H, PhH), 6.12 (s, 1H, H-2), 4.66 (s, 1H, -OH), 4.29-4.40 (m, 2H, H-5, H-4), 3.63 (t, *J* = 8.0 Hz, 1H, H-4'), 2.64 (dd, *J* = 6.7, 17.9 Hz, 1H, H-10), 2.56 (dd, *J* = 6.7, 17.9 Hz, 1H, H-10'), 2.50 (dd, *J* = 5.5, 14.3 Hz, 1H, H-6), 2.23 (dd, *J* = 6.9, 14.3 Hz, 1H, H-6'), 2.13-2.18 (m, 1H, H-11), 0.94 (d, *J* = 3.5 Hz, 3H, H-12), 0.92 (d, *J* = 3.5 Hz, 3H, H-12'); δ_C (100 MHz, MeCN-d_3) 209.63 (C-9), 174.08 (C-8), 139.77, 129.90, 129.58, 127.20 (PhC), 88.65 (C-7), 87.82 (C-2), 72.71 (C-4),

57.63 (C-5), 47.15 (C-10), 36.69 (C-6), 24.73 (C-11), 22.75, 22.68 (C-12, C-12'); *m/z* (ESI+) 326.31 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{17}H_{21}NNaO_4$ ($[M+Na]^+$) 327.1396, found 327.1415.

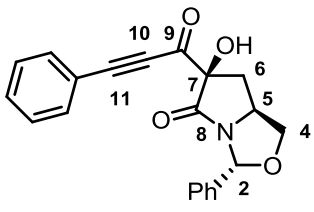
(2*R*, 5*S*, 7*S*)-1-Aza-7-hydroxy-7-(phenylacetylenecarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, (7*S*)-91f



Scale of reaction: 99 mg (0.323 mmol), product: 55 mg, 49%;

R_f = 0.54 (1:1 EtOAc:PE); white solid, mp = 154-156°C; $[\alpha]_D^{23}$= +151.1 (*c*= 1.12 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3405 (br, -OH), 2195 (s, alkyne), 1710 (s, C=O), 1675 (s, C=O); δ_H (400 MHz, MeCN-d_3) 7.66-7.71 (m, 2H, PhH), 7.57-7.63 (m, 1H, PhH), 7.36-7.56 (m, 7H, PhH), 6.24 (s, 1H, H-2), 4.85 (s, 1H, -OH), 4.46 (ddt, *J* = 5.6, 6.7, 8.1 Hz, 1H, H-5), 4.39 (dd, *J* = 6.1, 7.9 Hz, 1H, H-4), 3.73 (t, *J* = 8.3 Hz, 1H, H-4'), 2.83 (dd, *J* = 5.4, 14.7 Hz, 1H, H-6), 2.44 (dd, *J* = 7.0, 14.6 Hz, 1H, H-6'); δ_C (100 MHz, MeCN-d_3) 186.07 (C-9), 173.34 (C-8), 139.51, 134.26, 132.85, 130.09, 129.99, 129.62, 127.19, 119.78 (PhC), 97.87 (C-11), 89.92 (C-7), 88.13 (C-2), 85.99 (C-10), 72.96 (C-4), 57.73 (C-5), 36.98 (C-6); *m/z* (ESI+) 370.29 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{21}H_{18}NO_4$ ($[M+H]^+$) 348.1230, found 348.1224.

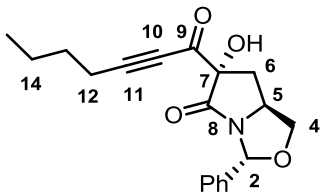
(2*R*, 5*S*, 7*R*)-1-Aza-7-hydroxy-7-(phenylacetylenecarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, (7*R*)-91f



Product: 25 mg, 22%;

R_f = 0.39 (1:1 EtOAc:PE); colourless oil; $[\alpha]_D^{23}$ = +42.9 (*c*= 1.08 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3404 (br, -OH), 2195 (s, alkyne), 1715 (s, C=O), 1666 (s, C=O); δ_H (400 MHz, MeCN-d_3) 7.52-7.58 (m, 1H, PhH), 7.39-7.48 (m, 6H, PhH), 7.28-7.34 (m, 3H, PhH), 6.25 (s, 1H, H-2), 4.74 (s, 1H, -OH), 4.30-4.46 (m, 2H, H-4, H-5), 3.66 (t, *J* = 7.8 Hz, 1H, H-4'), 3.07 (dd, *J* = 7.0, 14.0 Hz, 1H, H-6), 2.17 (dd, *J* = 6.6, 14.0 Hz, 1H, H-6'); δ_C (100 MHz, MeCN-d_3) 185.02 (C-9), 171.58 (C-8), 139.60, 134.15, 132.76, 129.99, 129.96, 129.58, 127.10, 119.60 (PhC), 97.05 (C-11), 90.10 (C-7), 87.91 (C-2), 85.08 (C-10), 73.72 (C-4), 56.45 (C-5), 37.17 (C-6); *m/z* (ESI+) 370.31 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{21}H_{18}NO_4$ ($[M+H]^+$) 348.1230, found 348.1232.

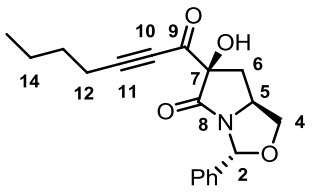
(2*R*, 5*S*, 7*S*)-1-Aza-7-hydroxy-7-(butylacetylenecarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, (7*S*)-91g



Scale of reaction: 100 mg (0.326 mmol), product: 53 mg, 50%;

R_f = 0.51 (1:1 EtOAc:PE); colourless oil; $[\alpha]_D^{23}$ = +176.9 (*c*= 1.28 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3416 (br, -OH), 2211 (s, alkyne), 1712 (s, C=O), 1680 (s, C=O); δ_H (400 MHz, MeCN-d_3) 7.37-7.49 (m, 5H, PhH), 6.15 (s, 1H, H-2), 4.71 (d, *J* = 0.9 Hz, 1H, -OH), 4.34-4.46 (m, 2H, H-5, H-4), 3.65 (t, *J* = 7.8 Hz, 1H, H-4'), 2.74 (dd, *J* = 4.9, 14.6 Hz, 1H, H-6), 2.51 (t, *J* = 7.0 Hz, 2H, H-12), 2.35 (dd, *J* = 7.1, 14.6 Hz, 1H, H-6'), 1.54-1.65 (m, 2H, H-13), 1.39-1.51 (m, 2H, H-14), 0.95 (t, *J* = 7.3 Hz, 3H, H-15); δ_C (100 MHz, MeCN-d_3) 185.89 (C-9), 173.23 (C-8), 139.55, 129.98, 129.61, 127.17 (PhC), 103.23 (C-11), 89.90 (C-7), 88.07 (C-2), 78.75 (C-10), 72.94 (C-4), 57.78 (C-5), 36.76 (C-6), 30.22 (C-13), 22.70 (C-14), 19.36 (C-12), 13.75 (C-15); *m/z* (ESI+) 350.30 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{19}H_{21}NNaO_4$ ($[M+Na]^+$) 350.1363, found 350.1356.

(2*R*, 5*S*, 7*R*)-1-Aza-7-hydroxy-7-(butylacetylenecarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, (7*R*)-91g



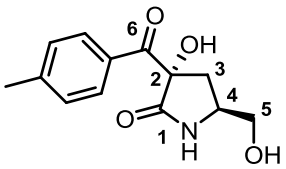
Product: 17 mg, 16%;

R_f = 0.37 (1:1 EtOAc:PE); colourless oil; $[\alpha]_D^{23}$ = +122.0 (*c*= 0.95 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3420 (br, -OH), 2210 (s, alkyne), 1718 (s, C=O), 1670 (s, C=O); δ_H (400 MHz, MeCN-d_3) 7.34-7.48 (m, 5H, PhH), 6.21 (s, 1H, H-2), 4.60 (s, 1H, -OH),4.37 (dd, *J* = 6.0, 8.0 Hz, 1H, H-4), 4.22 (apparent dq, *J* = 6.7, 8.7 Hz, 1H, H-5), 3.62 (t, *J* = 8.4 Hz, 1H, H-4'), 2.96 (dd, *J* = 7.0, 13.9 Hz, 1H, H-6), 2.38 (t, *J* = 7.1 Hz, 2H, H-12), 2.10 (dd, *J* = 6.6, 14.0 Hz, 1H, H-6'), 1.41-1.50 (m, 2H, H-13), 1.26-1.39 (m, 2H, H-14), 0.85 (t, *J* = 7.3 Hz, 3H, H-15); δ_C (100 MHz, MeCN-d_3) 185.08 (C-9), 171.68 (C-8), 139.64, 129.96, 129.60, 127.11 (PhC), 102.49 (C-11), 89.90 (C-7), 87.96 (C-2), 77.77 (C-10), 73.61 (C-4), 56.45 (C-5), 37.07 (C-6), 30.17 (C-13), 22.71 (C-14), 19.34 (C-12), 13.69 (C-15); *m/z* (ESI+) 350.31 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{19}H_{21}NNaO_4$ ($[M+Na]^+$) 350.1363, found 350.1372.

General procedures for acidic oxazolidine hydrolysis to 2-hydroxypyrrolidinones **(2*S*)-92a-g**

To a stirred solution of **(7*S*)-91** (1.0 eq.) in acetone (approx. concentration 0.06 M, with one small drop of water) at r.t was added Amberlyst 15 (3.0 eq.). After stirring at r.t. for 22-24 h, the reaction mixture was filtered and the resin rinsed with acetone. The solvent was evaporated to give the crude, which was purified via silica gel flash column chromatography (Eluent: EtOAc, then 5% MeOH/EtOAc) to give the respective product.

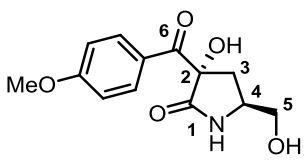
(2*S*, 4*R*)-2-Hydroxy-2-(4'-methylphenylcarbonyl)-4-hydroxymethyl-1-oxo-pyrrolidinone, (2*S*)-92a



Scale of reaction: 37 mg (0.110 mmol), product: 23 mg, 83%;

R_f = 0.19 (EtOAc); white solid, mp = 179-180°C; $[\alpha]_D^{23}$ = +17.4 (*c*= 0.95 in MeOH); ν_{max} / cm^{-1} (film) 3496 (br, -NH-), 3276 (br, -OH), 1685 (s, C=O), 1673 (s, C=O); δ_H (400 MHz, Acetone-d_6) 8.02 (d, *J* = 8.3 Hz, 2H, ArH), 7.41 (br s, 1H, -NH-), 7.30 (d, *J* = 7.9 Hz, 2H, ArH), 5.30 (s, 1H, -OH), 4.13 (t, *J* = 5.4 Hz, 1H, -CH_2OH), 3.90-4.01 (m, 1H, H-4), 3.72 (dd, *J* = 4.6, 10.9 Hz, 1H, H-5), 3.58 (dt, *J* = 4.6, 10.8 Hz, 1H, H-5'), 2.64 (dd, *J* = 6.6, 13.9 Hz, 1H, H-3), 2.40 (s, 3H, Ar-Me), 2.23 (dd, *J* = 7.3, 13.9 Hz, 1H, H-3'); δ_C (100 MHz, Acetone-d_6) 199.70 (C-6), 174.96 (C-1), 144.83, 132.25, 130.88, 129.74 (ArC), 83.54 (C-2), 64.85 (C-5), 54.49 (C-4), 38.03 (C-3), 21.58 (Ar-Me); *m/z* (ESI+) 272.22 ($[M+Na]^+$, 65%); HRMS (ESI+) calculated for $C_{13}H_{16}NO_4$ ($[M+H]^+$) 250.1074, found 250.1086.

(2*S*, 4*R*)-2-Hydroxy-4-hydroxymethyl-2-(4'-methoxyphenylcarbonyl)-1-oxo-pyrrolidinone, (2*S*)-92b

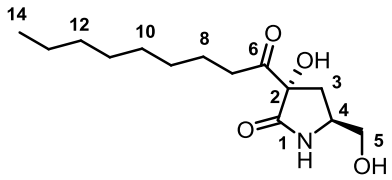


Scale of reaction: 15 mg (0.0424 mmol), product: 10 mg, 89%;

R_f = 0.14 (EtOAc); white solid, mp = 153-154°C; $[\alpha]_D^{23}$ = -23.5 (*c*= 0.56 in MeCN); ν_{max} / cm^{-1} (film) 3481 (br, -NH-), 3285 (br, -OH), 1687 (s, C=O), 1667 (s, C=O); δ_H (400 MHz, MeCN-d_3) 8.05 (d, *J* = 9.1 Hz, 2H, ArH), 7.00 (d, *J* = 9.1 Hz, 2H, ArH), 6.74 (br s, 1H, -NH-), 4.86 (s, 1H, -OH), 3.89-3.97 (m, 1H, H-4), 3.87 (s, 3H, -OMe), 3.66 (ddd, *J* =

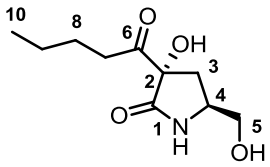
3.9, 5.1, 11.4 Hz, 1H, H-5), 3.46 (dt, *J* = 6.0, 11.6 Hz, 1H, H-5'), 3.13 (dd, *J* = 5.1, 6.4 Hz, 1H, -CH_2OH), 2.53 (dd, *J* = 7.0, 14.1 Hz, 1H, H-3), 2.18 (dd, *J* = 7.0, 14.3 Hz, 1H, H-3', partially obscured by water signal); δ_C (100 MHz, MeCN-d_3) 198.21 (C-6), 175.51 (C-1), 165.00, 133.19, 126.74, 114.77 (ArC), 83.18 (C-2), 64.30 (C-5), 56.42 (-OMe), 54.64 (C-4), 38.00 (C-3); *m/z* (ESI+) 288.23 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{13}H_{15}NNaO5$ ($[M+Na]^+$) 288.0842, found 288.0857.

(2*S*, 4*R*)-2-Hydroxy-4-hydroxymethyl-2-(octylcarbonyl)-1-oxo-pyrrolidinone, (2*S*)-92c



Scale of reaction: 15 mg (0.417 mmol), product: 11 mg, 97%; R_f = 0.28 (EtOAc); colourless crystals, mp = 101-103°C; $[\alpha]_D^{23}$ = +31.0 (*c*= 1.22 in MeOH); ν_{max} / cm^{-1} (film) 3298 (br), 1716 (s, C=O), 1689 (s, C=O); δ_H (400 MHz, MeCN-d_3) 6.61 (br s, 1H, -NH-), 4.42 (s, 1H, -OH), 3.77-3.83 (m, 1H, H-4), 3.61 (dt, *J* = 4.4, 11.3 Hz, 1H, H-5), 3.44 (dt, *J* = 6.1, 11.1 Hz, 1H, H-5'), 3.02 (dd, *J* = 5.1, 6.3 Hz, 1H, -CH_2OH), 2.68 (dt, *J* = 7.6, 17.9 Hz, 1H, H-7), 2.56 (dt, *J* = 7.2, 17.9 Hz, 1H, H-7'), 2.32 (dd, *J* = 6.3, 14.2 Hz, 1H, H-3), 2.03 (dd, *J* = 7.6, 14.2 Hz, 1H, H-3'), 1.50-1.57 (m, 2H, H-8), 1.25-1.31 (m, 10H, H-9, H-10, H-11, H-12, H-13), 0.89 (t, *J* = 7.0 Hz, 3H, H-14); δ_C (100 MHz, MeCN-d_3) 210.92 (C-6), 174.68 (C-1), 84.01 (C-2), 65.03 (C-5), 53.84 (C-4), 37.91 (C-7), 35.61 (C-3), 32.59, 30.09, 29.90, 29.79, 24.19 (C-8), 23.38 (C-9, C-10, C-11, C-12, C-13), 14.38 (C-14); *m/z* (ESI+) 294.30 ($[M+Na]^+$, 85%); HRMS (ESI+) calculated for $C_{14}H_{26}NO_4$ ($[M+H]^+$) 272.1856, found 272.1862.

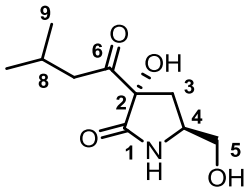
(2*S*, 4*R*)-2-(Butylcarbonyl)-2-hydroxy-4-hydroxymethyl-1-oxo-pyrrolidinone, (2*S*)-92d



Scale of reaction: 37 mg (0.122 mmol), product: 24 mg, 91%; R_f = 0.19 (EtOAc); colourless oil; $[\alpha]_D^{23}$ = +50.9 (*c*= 1.07 in MeOH); ν_{max} / cm^{-1} (film) 3311 (br), 1716 (s, C=O), 1690 (s, C=O); δ_H (400 MHz, MeCN-d_3) 6.81 (br s, 1H, -NH-), 4.57 (s, 1H, -OH), 3.77-3.84 (m, 1H, H-4), 3.61 (ddd, *J* = 4.1, 5.1, 11.2 Hz, 1H, H-5), 3.44 (dt, *J* = 6.3, 11.2 Hz, 1H, H-5'), 3.14 (dd, *J* = 5.1, 6.3 Hz, 1H, -CH_2OH), 2.69 (dt, *J* = 7.3, 17.9 Hz, 1H, H-7), 2.58 (dt, *J* = 7.3, 18.0 Hz, 1H, H-7'), 2.31 (dd, *J* = 6.2, 14.2 Hz, 1H, H-3), 2.04 (dd, *J* = 7.6, 14.2 Hz, 1H, H-3'), 1.47-1.58 (m, 2H, H-8), 1.24-1.36 (m, 2H, H-9), 0.89 (t, *J* = 7.4 Hz,

3H, H-10); δ_C (100 MHz, MeCN-d_3) 211.01 (C-6), 174.98 (C-1), 84.07 (C-2), 65.04 (C-5), 53.92 (C-4), 37.67 (C-7), 35.64 (C-3), 26.28 (C-)8, 22.89 (C-9), 14.15 (C-10); *m/z* (ESI+) 238.23 ($[M+Na]^+$, 40%); HRMS (ESI+) calculated for $C_{10}H_{18}NO_4$ ($[M+H]^+$) 216.1230, found 216.1241.

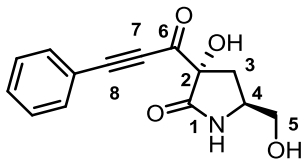
(2*S*, 4*R*)-2-Hydroxy-4-hydroxymethyl-2-(isobutylcarbonyl)-1-oxo-pyrrolidinone, (2*S*)-92e



Scale of reaction: 5 mg (0.0165 mmol), product: 3 mg, 90%;

R_f = 0.16 (EtOAc); colourless oil; $[\alpha]_D^{23}$ = +50.9 (*c*= 0.70 in MeOH); ν_{max} / cm^{-1} (film) 3295 (br), 1716 (s, C=O), 1690 (s, C=O); δ_H (400 MHz, MeCN-d_3) 6.66 (br s, 1H, -NH-), 4.46 (br s, 1H, -OH), 3.80-3.86 (m, 1H, H-4), 3.64 (dd, *J* = 4.2, 11.2 Hz, 1H, H-5), 3.47 (dd, *J* = 6.2, 11.2 Hz, 1H, H-5'), 3.12 (br s, 1H, -OH), 2.62 (dd, *J* = 6.7, 17.7 Hz, 1H, H-7), 2.49 (dd, *J* = 6.8, 17.7 Hz, 1H, H-7'), 2.34 (dd, *J* = 6.3, 14.2 Hz, 1H, H-3), 2.11-2.17 (m, 1H, H-8, partially obscured by water signal), 2.06 (dd, *J* = 6.3, 14.2 Hz, 1H, H-3'), 0.93 (d, *J* = 6.7 Hz, 3H, H-9), 0.92 (d, *J* = 6.7 Hz, 3H, H-9); δ_C (100 MHz, MeCN-d_3) 210.39 (C-6), 174.61 (C-1), 84.04 (C-2), 65.05 (C-5), 53.83 (C-4), 46.66 (C-7), 35.52 (C-3), 24.76 (C-8), 22.74, 22.67 (C-9); *m/z* (ESI-) 214.22 ($[M-H]^-$, 55%); HRMS (ESI+) calculated for $C_{10}H_{17}NNaO_4$ ($[M+Na]^+$) 238.1050, found 238.1057.

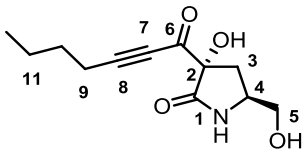
(2*S*, 4*R*)-2-Hydroxy-4-hydroxymethyl-2-(phenylacetylenecarbonyl)-1-oxo-pyrrolidinone, (2*S*)-92f



Scale of reaction: 82 mg (0.236 mmol), product: 52 mg, 85%;

R_f = 0.27 (EtOAc); white solid, mp = 158-160°C; $[\alpha]_D^{23}$ = -25.0 (*c*= 1.13 in MeOH); ν_{max} / cm^{-1} (film) 3259 (br), 2195 (s, alkyne), 1697 (s, C=O), 1678 (s, C=O); δ_H (400 MHz, MeCN-d_3) 7.63-7.70 (m, 2H, PhH), 7.53-7.60 (m, 1H, PhH), 7.44-7.51 (m, 2H, PhH), 6.78 (br s, 1H, -NH-), 4.61 (br s, 1H, -OH), 3.85-3.91 (m, 1H, H-4), 3.70 (ddd, *J* = 4.1, 5.0, 11.3 Hz, 1H, H-5), 3.52 (dt, *J* = 6.3, 11.3 Hz, 1H, H-5'), 3.13 (dd, *J* = 5.1, 6.4 Hz, 1H, -CH_2OH), 2.69 (ddd, *J* = 1.0, 6.7, 14.3 Hz, 1H, H-3), 2.19 (ddd, *J* = 0.8, 7.3, 14.3 Hz, 1H, H-3'); δ_C (100 MHz, MeCN-d_3) 187.33 (C-6), 173.62 (C-1), 134.24, 132.66, 130.00, 120.00 (PhC), 97.35 (C-8), 85.92 (C-7), 85.51 (C-2), 65.03 (C-5), 54.06 (C-4), 35.74 (C-3); *m/z* (ESI-) 258.20 ($[M-H]^-$, 70%); HRMS (ESI+) calculated for $C_{14}H_{14}NO_4$ ($[M+H]^+$) 260.0917, found 260.0911.

(2*S*, 4*R*)-2-Hydroxy-4-hydroxymethyl-2-(butylacetylenecarbonyl)-1-oxo-pyrrolidinone, (2*S*)-92g



Scale of reaction: 87 mg (0.266 mmol), product: 59 mg, 93%; R_f = 0.29 (EtOAc); colourless crystals, mp = 120-122°C; $[\alpha]_D^{23}$ = +18.5 (*c*= 1.14 in MeOH); ν_{max} / cm^{-1} (film) 3328 (peak with broad base), 2209 (s, alkyne), 1698 (s, C=O), 1686 (s, C=O); δ_H (400 MHz, MeCN-d_3) 6.71 (br s, 1H, -NH-), 4.45 (br s, 1H, -OH), 3.76-3.88 (m, 1H, H-4), 3.63 (dt, *J* = 4.3, 11.0 Hz, 1H, H-5), 3.47 (dt, *J* = 6.1, 10.9 Hz, 1H, H-5'), 3.05 (t, *J* = 5.6 Hz, 1H, -CH_2OH), 2.53 (dd, *J* = 6.8, 14.3 Hz, 1H, H-3), 2.47 (t, *J* = 7.0 Hz, 2H, H-9), 2.12 (dd, *J* = 7.3, 14.3 Hz, 1H, H-3'), 1.52-1.62 (m, 2H, H-10), 1.37-1.46 (m, 2H, H-11), 0.92 (t, *J* = 7.3 Hz, 3H, H-12); δ_C (100 MHz, MeCN-d_3) 187.27 (C-6), 173.54 (C-1), 102.59 (C-8), 85.30 (C-2), 78.59 (C-7), 65.48 (C-5), 54.04 (C-4), 35.88 (C-3), 30.26 (C-10), 22.67 (C-11), 19.34 (C-9), 13.74 (C-12); *m/z* (ESI+) 262.22 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{12}H_{18}NO_4$ ($[M+H]^+$) 240.1230, found 240.1241.

Competition reaction between isobutyl magnesium bromide and phenylethnyl magnesium bromide with Weinreb amide **82**

To a solution of **82** (40 mg, 0.131 mmol, 1.0 eq.) in THF (2 mL), commercially available isobutyl magnesium bromide (2 M in Et_2O) (0.14 ml, 0.287 mmol, 2.2 eq.) and phenylethnyl magnesium bromide (0.83 mL, 0.287 mmol, 2.2 eq.) were added via different syringes simultaneously. The reaction mixture was stirred at 0°C for 1 h, then 2 h at r.t. It was then quenched with sat. aq. NH_4Cl and extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents removed *in vacuo* to give the crude.

^{1}H NMR spectrum of the crude shows no isobutyl signals and the ratio between **(7*S*)-91f** and **(7*R*)-91f** was determined to be 1:0.2, while the ratio between **91f** and **82** was determined to be 1:0.1.

Attempted synthesis of pyrrolidinones via reductive epoxide opening using ammonium formate

To a solution of **67b** (25 mg, 0.0743 mmol, 1.0 eq.) in MeOH (5 ml) was added 10% Pd/C (2.5 mg; 10% wt.) and ammonium formate. The reaction mixture was stirred at 70°C for 3 h. The reaction

mixture was then cooled to r.t., filtered and the solvents evaporated to give the crude. Purification via silica gel flash column chromatography (Eluent: EtOAc/PE 10% to 100%, then 5% MeOH/EtOAc) gave the deoxygenated product **64b** (13 mg, 54%).

Attempted synthesis of pyrrolidinones via reductive epoxide opening using H_2 gas

(2*S*, 4*R*) and (2*R*, 4*R*)-2-(Butylcarbonyl)-4-hydroxymethyl-1-oxo-pyrrolidinone, 93d

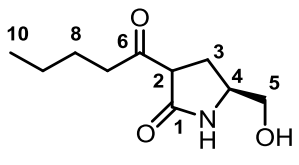
With Rosemund catalyst:

5% $Pd/BaSO_4$ (4 mg) was added to a solution of **77d** (39 mg, 0.183 mmol) in EtOH (4ml). H_2 gas (balloon) was bubbled through the suspension, stirring at r.t. for 2 h. Afterwhich, the suspension was filtered over Celite and the residue rinsed with EtOAc. The solvent was evaporated to give the crude and purification via silica gel flash column chromatography (Eluent: 20% to 100% EtOAc) gave **93d** (24 mg, 66%).

With Lindlar's catalyst:

5% $Pd/CaCO_3$ (poisoned with Pb) – Lindlar catalyst) (4 mg) was added to an EtOH (4 mL) solution of **77d** (38 mg, 0.178 mmol), followed by quinoline (1 μL, 0.00846 mmol). The reaction mixture was then stirred under H_2 (balloon) at r.t. for 2 h, filtered over Celite and the residue rinsed with EtOAc. The solvent was evaporated to give the crude.

^{1}H NMR spectrum of crude show **77d**: **93d** = 1:0.2.



R_f = 0.17 (EtOAc); white waxy solid, mp = 60-63°C; ν_{max} / cm^{-1} (film) 3331 (br, -NH-), 1683 (s, br with shoulder towards larger wavenumber, C=O); Proton spectrum shows a mixture of diastereomers, unassigned. δ_H (400 MHz, $CDCl_3$) 6.84, 6.80 (br s, 1H, -NH-), 3.79-3.85 (m) and 3.73-3.79 (m) (1H, H-4), 3.70 (dd, *J* = 3.3, 11.3 Hz, 1H, H-5), 3.57-3.66 (m, 1H, H-2), 3.53 (dd, *J* = 7.1, 11.2 Hz) and 3.46 (dd, *J* = 6.3, 11.4 Hz) (1H, H-5), 3.14 (br s, 1H, -OH), 2.92 (dt, *J* = 7.4, 17.8 Hz, 1H, H-7), 2.49-2.70 (m, 1.5H, H-7 and H-3), 2.22-2.27 (m, 1H, H-3), 1.87 (ddd, *J* = 5.1, 9.4, 13.2 Hz, 0.5H, H-3), 1.53-1.60 (m, 2H, H-8), 1.25-1.39 (m, 2H, H-9), 0.90 (t, *J* = 7.3 Hz, 3H, H-10); δ_C (100 MHz, $CDCl_3$) 206.50, 205.71 (C-6

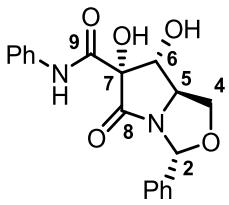
x2), 173.92, 173.31 (C-1x 2), 65.96, 65.66 (C-5 x2), 54.63, 54.49 (C-4 x2), 54.04, 53.95 (C-2 x2), 42.78, 42.52 (C-3 x2), 25.39 (C-8), 25.36, 24.96 (C-7), 24.15, 22.16 (C-9), 13.84 (C-10); *m/z* (ESI+) 200.17 ($[M+H]^+$, 85%); HRMS (ESI+) calculated for $C_{10}H_{17}NNaO_3$ ($[M+Na]^+$) 222.1101, found 222.1100.

5.2.3 Experimental for 2,3-dihydroxypyrrolidinones

Os-catalysed dihydroxylation

(2*R*, 5*S*, 6*R*, 7R)-1-Aza-6,7-dihydroxy-3-oxa-8-oxo-2-phenyl-7-(phenylcarbamoyl)-bicyclo[3.3.0]octane, 94b

66b (38 mg, 0.119 mmol, 1.0 eq.) and citric acid (23 mg, 0.119 mmol, 1.0 eq.) was dissolved in ^{t}BuOH/H_2O (1:1) (3 mL). Potassium osmate (VI) dihydrate (0.5 mg, 0.01 eq.), followed by NMO (16 mg, 0.137 mmol, 1.0 eq.) was then added to the reaction mixture. The mixture was then stirred at r.t. for 26 h. ^{t}BuOH was then removed *in vacuo* from the reaction mixture, followed by acidifying the aqueous layer with aq. 10% HCl and extraction with EtOAc. The combined organic layers were dried with $MgSO_4$, filtered and evaporated to give the crude. Purification via silica gel flash column chromatography (Eluent: 10% to 50% EtOAc/PE) gave the diol **94b** as a white solid (8.9 mg, 21% yield).

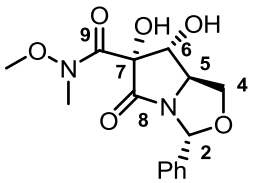


R_f = 0.16 (1:1 EtOAc:PE); white solid; mp = 215°C (decomposed); ν_{max} / cm^{-1} (film) 1706 (s, C=O), 1648 (s, C=O); δ_H (500 MHz, Acetone-d_6) 9.36 (br s, 1H, -NH-), 7.82 (d, *J* = 8.5 Hz, 2H, ArH), 7.34-7.50 (m, 7H, ArH), 7.14 (t, *J* = 7.6 Hz, 1H, ArH), 6.23 (s, 1H, H-2), 5.77 (s, 1H, -OH C-7), 5.42 (d, *J* = 6.0 Hz, 1H, -OH C-6), 4.80 (apparent t, *J* = 5.7 Hz, 1H, H-6), 4.42 (dd, *J* = 6.5, 8.4 Hz, 1H, H-4), 4.13-4.16 (m, 1H, H-5), 3.99 (t, *J* = 8.2 Hz, 1H, H-4'); δ_C (125 MHz, Acetone-d_6) 172.54 (C-8), 169.03 (C-9), 139.59, 139.18, 129.60, 129.53, 129.26, 127.10, 124.94, 120.49 (ArC), 87.83 (C-2), 84.96 (C-7), 74.86 (C-6), 70.54 (C-4), 64.38 (C-5); *m/z* (ESI-) 353.13 ($[M-H]^-$, 100%); HRMS (ESI+) calculated for $C_{19}H_{18}N_2NaO_5$ ($[M+Na]^+$) 377.1108, found 377.1109.

Ru-catalysed dihydroxylation

To a vigorously stirred solution of **75** (662 mg, 2.297 mmol, 1.0 eq.) in EtOAc/MeCN (1:1) (10 mL) at 0°C was added a solution of $RuCl_3.xH_2O$ (33 mg, 0.161 mmol, 0.07 eq.) and $NaIO_4$ (737 mg, 3.45 mmol, 1.5 eq.) in distilled water (5 mL). The biphasic mixture was stirred vigorously for 20 min and quenched with sat. aq. $Na_2S_2O_3$ and extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents removed *in vacuo* to give the crude. Purification via silica gel flash column chromatography (Eluent: 10% to 80% EtOAc/PE) gave diol **95** (513 mg, 69%) as a mixture diastereomers, and tetramic acid **96** (51 mg, 7%) as a single diastereomer.

(2*R*, 5*S*, 6*R*, 7*R*)-1-Aza-6,7-dihydroxy-7-(*N*,*O*-dimethylcarbamoyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 95



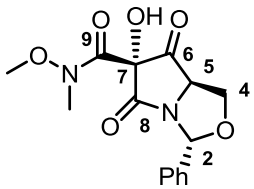
d.r. ~5:1

R_f = 0.49 (EtOAc); colourless oil; ν_{max} / cm^{-1} (film) 3386 (br, -OH), 1716 (s, C=O), 1659 (s, C=O);

Major diastereomer (*cis*-diol on *exo* face)

δ_H (400 MHz, MeCN-d_3) 7.34-7.51 (m, 5H, PhH), 6.21 (s, 1H, H-2), 4.81 (br s, 1H, -OH C-7), 4.45 (d, *J* = 5.1 Hz, 1H, H-6), 4.38 (ddd, *J* = 0.6, 6.5, 8.5 Hz, 1H, H-4*exo*), 4.00 (ddd, *J* = 5.1, 6.5, 7.8 Hz, 1H, H-5), 3.75 (t, *J* = 8.0 Hz, 1H, H-4'*endo*), 3.68 (s, 3H, -OMe), 3.62 (br s, 1H, -OH C-6) 3.29 (s, 3H, -NMe); δ_C (100 Mhz, MeCN-d_3) 171.55 (C-8), 169.86 (C-9), 139.34, 129.95, 129.63, 127.15 (PhC), 88.00 (C-2), 83.12 (C-7), 73.71 (C-6), 71.22 (C-4), 66.29 (C-5), 61.17 (-OMe), 33.61 (-NMe); *m/z* (ESI+) 345.27 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{15}H_{18}N_2NaO_6$ ($[M+Na]^+$) 345.1057, found 345.1069.

(2*R*, 5*S*, 7*R*)-1-Aza-2-(phenyl)-7-hydroxy-7-(*N*,*O*-dimethylcarbamoyl)-6,8-dioxo-3-oxabicyclo[3.3.0]-octane, 96



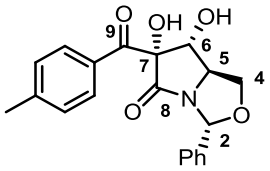
R_f = 0.71 (EtOAc); colourless crystals, mp = 141-143°C; $[\alpha]_D^{23}$ = +134.3 (*c*= 1.12 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3350 (br, -OH), 1786 (s, C=O), 1718 (s, C=O), 1677 (s, C=O); δ_H (400 MHz, MeCN-d_3) 7.36-7.53 (m, 5H, Ph), 6.69 (s, 1H, H-2), 5.34 (s, 1H, -OH), 4.74 (dd, *J* = 6.4, 8.8 Hz, 1H, H-5), 4.22 (apparent t, *J* = 8.7 Hz, 1H, H-

4), 4.01 (dd, *J* = 6.4, 8.5 Hz, 1H, H-4'), 3.65 (s, 3H, -OMe), 3.25 (s, 3H, -NMe); δ_C (100 MHz, MeCN-d_3) 201.32 (C-6), 171.46 (C-8), 166.54 (C-9), 138.11, 130.10, 129.77, 126.95 (PhC), 89.24 (C-2), 79.18 (C-7), 66.93 (C-4), 65.23 (C-5), 61.68 (-OMe), 33.58 (-NMe); *m/z* (ESI-) 319.57 ($[M-H]^-$, 100%); HRMS (ESI-) calculated for $C_{15}H_{15}N_2O_6$ ($[M-H]^-$) 319.0936, found 319.0937.

Grignard reaction of unprotected diol **95**

Tolyl magnesium bromide and the Grignard reaction were synthesised and carried out as described on p. 165. 3.4 eq. of the Grignard reagent was used and the reaction mixture was stirred at 0°C for 30 min, then r.t. for 1.5 h. 7% of each **97a** diastereomer was isolated.

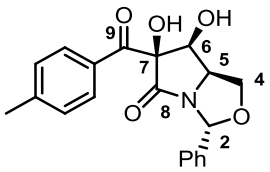
(2*R*, 5*S*, 6*R*, 7*S*)-1-Aza-6,7-dihydroxy-7-(4'methylphenylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, *exo* -97a



Scale of reaction: 52 mg (0.161 mmol), product: 4 mg, 7%;

R_f = 0.50 (1:1 EtOAc:PE); white waxy oil; $[\alpha]_D^{23}$ = +120.0 (*c*= 0.70 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3359 (br, -OH), 1728 (s, C=O), 1669 (s, C=O); δ_H (400 MHz, MeCN-d_3) 7.80 (d, *J* = 8.0 Hz, 2H, ArH), 7.39-7.53 (m, 5H, PhH), 7.36 (d, *J* = 8.0 Hz, 2H, ArH), 6.31 (s, 1H, H-2), 4.96 (br s, 1H, -OH), 4.72 (d, *J* = 5.9 Hz, 1H, H-6), 4.39 (ddd, *J* = 0.6, 6.4, 8.7 Hz, 1H, H-4*exo*), 4.17 (dt, *J* = 6.1, 7.2 Hz, 1H, H-5), 3.95 (dd, *J* = 7.3, 8.7 Hz, 1H, H-4*endo*), 3.88 (br s, 1H, -OH), 2.43 (s, 3H, Ar-Me); δ_C (100 MHz, MeCN-d_3) 198.03 (C-9), 172.51 (C-8), 146.04, 139.03, 132.19, 130.97, 130.20, 130.07, 129.66, 127.29 (ArC), 88.96 (C-7), 88.11 (C-2), 76.31 (C-6), 70.93 (C-4), 65.07 (C-5), 21.77 (Ar-Me); *m/z* (ESI+) 376.27 ($[M+Na]^+$, 35%); HRMS (ESI+) calculated for $C_{20}H_{19}NNaO_5$ ($[M+Na]^+$) 376.1155, found 376.1140.

(2*R*, 5*S*, 6*S*, 7*S*)-1-Aza-6,7-dihydroxy-7-(4'methylphenylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, *endo* -97a



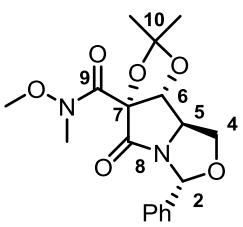
Product: 4 mg, 7%;

R_f = 0.38 (1:1 EtOAc:PE); white solid, mp = 163°C decomposes to brown liquid; $[\alpha]_D^{23}$ = +138.9 (*c*= 0.70 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3362 (br, -OH), 1709 (s, C=O), 1669 (s, C=O); δ_H (400 MHz, MeCN-d_3) 8.15 (d, *J* = 8.0 Hz, 2H, ArH), 7.33-7.39 (m, 5H, PhH), 7.31 (d, *J* = 8.0 Hz, 2H, ArH), 6.22 (s, 1H, H-2), 4.69 (d, *J* = 4.0 Hz, 1H, H-6), 4.37 (dt, *J* = 4.0, 7.0 Hz, 1H, H-5), 4.17 (dd, *J* = 6.8, 8.5 Hz, 1H, H-4*endo*), 4.07 (dd, *J* = 7.2, 8.4 Hz, 1H, H-4*exo*), 2.40 (s, 3H, Ar-Me); δ_C (100 MHz, MeCN-d_3) 196.26 (C-9), 173.30 (C-8), 145.86, 139.27, 133.06, 131.45, 129.88, 129.84, 129.55, 127.14 (ArC), 90.35 (C-7), 88.08 (C-2), 72.79 (C-6), 66.02 (C-4), 61.42 (C-5), 21.77 (Ar-Me); *m/z* (ESI+) 376.35 ($[M+Na]^+$, 50%); HRMS (ESI+) calculated for $C_{20}H_{19}NNaO_5$ ($[M+Na]^+$) 376.1155, found 376.1141.

Use of acetonide as diol protecting group towards 2,3-dihydroxypyrrolidinones

(2*R*, 5*S*, 6*R*, 7*R*)-1-Aza-6,7-(isopropylidene)dioxy-7-(*N*,*O*-dimethylcarbamoyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 98

To a solution of **96** (176 mg, 0.546 mmol, 1.0 eq.) in acetone (8 ml), were added 2,2-dimethoxypropane (0.34 mL, 2.730 mmol, 5 eq.), and *p*-TsOH·H_2O (4 mg, 0.0218 mmol, 0.04 eq.) at r.t.. The mixture was stirred at 55°C for 6 h. The reaction solvent was evaporated give the crude. Purification via silica gel flash column chromatography (Eluent: 10% to 50% EtOAc/PE) gave the acetonide as a colourless oil (86 mg, 44%), and unreacted **95** was recovered (33 mg, d.r. = 1:0.5).



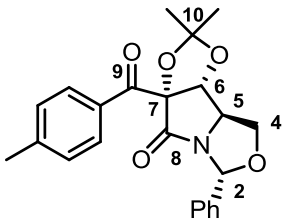
d.r. ~1:0.5

R_f = 0.35 (1:1 EtOAc/PE); colourless oil; ν_{max} / cm^{-1} (film) 1721 (s, C=O), 1687 (s, C=O); δ_H (400 MHz, MeCN-d_3, 303K) 7.34-7.50 (m, 5H, PhH), 6.33 (br s, 0.7H, H-2), 6.16 (s, 0.3H, H-2), 5.03 (br s, 0.7H, H-6), 4.95 (d, *J* = 4.9 Hz, 0.3H, H-6), 4.33 (t, *J* = 7.8 Hz, 1H, H-4), 4.20 (dd, *J* = 6.7, 8.1 Hz, 0.3H, H-4'), 4.05-4.15 (m, 1H, H-5), 3.69 (t, *J* = 8.3 Hz, 0.7H, H-4'), 3.64 (s, 2H, -OMe), 3.43 (br s, 1H, -OMe), 3.24 (br s, 3H, -NMe), 1.51 (s, 3H, -CMe), 1.44 (s, 2H, -CMe), 1.37 (s, 1H, -CMe); δ_C (100 MHz, MeCN-d_3, 303K) 172.26 (v br, C-8), 168.39 (v br, C-9), 139.66, 139.43, 129.97,

129.82, 129.71, 129.67, 129.56, 127.28, 126.78 (PhC), 115.43 (C-10), 92.00 (C-7), 88.42, 88.34 (C-2), 82.76, 78.92 (br, C-6), 69.50, 66.58 (C-4), 66.10 (br, C-5), 60.73, 60.00 (-OMe), 33.66 (v br, -NMe), 28.35, 27.88, 27.30, 25.79 (Me of acetonide); δ_H (400 MHz, MeCN-d_3, **338K**) 7.34-7.51 (m, 5H, PhH), 6.33 (s, 0.7H, H-2), 6.17 (s, 0.3H, H-2), 5.03 (d, *J* = 1.6 Hz, 0.7H, H-6), 4.95 (d, *J* = 4.8 Hz, 0.3H, H-6), 4.33 (t, *J* = 7.7 Hz, 1H, H-4), 4.21 (dd, *J* = 6.8, 8.3 Hz, 0.3H, H-4'), 4.05-4.14 (m, 1H, H-5), 3.72 (t, *J* = 8.3 Hz, 0.7H, H-4'), 3.66 (s, 2H, -OMe), 3.50 (s, 1H, -OMe), 3.28 (s, 3H, -NMe), 1.52 (s, 3H, -CMe), 1.46 (s, 2H, -CMe), 1.41 (s, 1H, -CMe); δ_C (100 MHz, MeCN-d_3, **338K**) 173.07, 170.97 (C-8), 168.63 (C-9), 139.98, 139.74, 130.10, 129.97, 129.88, 129.83, 129.73, 127.44, 126.99 (PhC), 117.43, 115.75 (C-10), 93.79, 92.34 (C-7), 88.83, 88.67 (C-2), 82.89, 79.47 (C-6), 69.65, 66.74 (C-4), 66.23, 61.83 (C-5), 61.01, 60.16 (-OMe), 34.31(v br, -NMe), 28.46, 27.82, 27.61, 26.15 (Me of acetonide); *m/z* (ESI+) 385.23 ($[M+Na]^+$, 60%); HRMS (ESI+) calculated for $C_{18}H_{22}N_2NaO_6$ ($[M+Na]^+$) 385.1370, found 385.1387.

(2*R*, 5*S*, 6*R*, 7*S*)-1-Aza-6,7-(isopropylidene)dioxy-7-(4'methylphenylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 99a

Tolyl magnesium bromide and the Grignard reaction were synthesised and carried out as described on p. 165. 1.3 eq. of the Grignard reagent was used and the reaction mixture was stirred at 0°C to r.t. over 1 h.



d.r. ~5:2

Scale of reaction: 70 mg (0.198 mmol), product: 48 mg, 63%;

R_f = 0.45 (30% EtOAc/PE); colourless oil; ν_{max} / cm^{-1} (film) 1719 (s, C=O), 1666 (s, C=O);

Major diastereomer

δ_H (400 MHz, MeCN-d_3) 8.13 (d, *J* = 8.3 Hz, 2H, ArH), 7.31-7.48 (m, 7H, PhH and ArH), 6.15 (s, 1H, H-2), 5.14 (s, 1H, H-6), 4.42 (dd, *J* = 6.6, 8.1 Hz, 1H, H-4), 4.07-4.25 (m, 1H, H-5), 3.80 (dd, *J* = 8.1, 10.0 Hz, 1H, H-4'), 2.43 (s, 3H, Ar-Me), 1.53 (s, 3H, C-Me), 1.13 (s, 3H, C-Me); δ_C (100 MHz, MeCN-d_3) 199.15 (C-9), 174.44 (C-8), 146.21, 139.64, 133.33, 131.90, 129.91, 129.64, 126.94 (ArC), 115.84 (C-10), 96.24 (C-7), 89.19 (C-2), 80.14 (C-6), 68.51 (C-4), 64.52 (C-5), 26.88, 25.73 (2x C-

Me), 21.84 (Ar-Me); *m/z* (ESI+) 416.39 ([M+Na]$^+$, 65%); HRMS (ESI+) calculated for $C_{23}H_{23}NNaO_5$ ([M+Na]$^+$) 416.1468, found 416.1453.

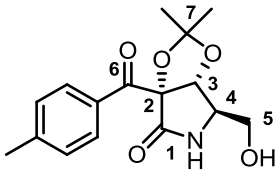
(2*S*, 3*R*, 4*R*)-2,3-(isopropylidene)dioxy-2-(4'-methylphenylcarbonyl)-4-hydroxymethyl-1-oxo-pyrrolidinone, 101a

Condition A:

TFA (5 μL, 0.635 mmol, 10 eq.) was added to a solution of **99a** (25 mg, 0.0635 mmol, 1.0 eq.) in THF (1.5 mL with a drop of water) at r.t.. The reaction mixture was stirred at r.t. for 20 h. Solvents were evaporated to give the crude, followed by purification via silica gel flash column chromatography (Eluent: EtOAc/PE, 10% to 50%) gave **101a** (6.5 mg, 34%, d.r. ~ 1:0.3 about the diol).

Condition B:

A solution of **99a** (17.5 mg, 0.0445 mmol, 1.0 eq.), 2 M aq. HCl (88 μL, 0.089 mmol, 4 eq.) and MeOH (1 mL) was stirred at r.t. for 6 h. Solvents were evaporated to give the crude, followed by purification via silica gel flash column chromatography (Eluent: EtOAc/PE, 10% to 50%) gave **101a** (3 mg, 25%, one diastereomer).

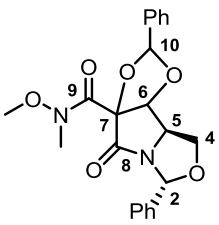


R_f = 0.21 (1:1 EtOAc:PE); colourless oil; $[\alpha]_D^{23}$ = -91.0 (*c*= 0.50 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3319 (br, -OH), 1708 (s, C=O), 1666 (s, C=O); δ_H (400 MHz, MeCN-d_3) 8.14 (d, *J* = 8.3 Hz, 2H, ArH), 7.33 (d, *J* = 8.0 Hz, 2H, ArH), 6.65 (br s, 1H, -NH-), 4.85 (s, 1H, H-3), 3.58-3.74 (m, 3H, H-4 and H-5), 2.42 (s, 3H, Ar-Me), 1.49 (s, 3H, CMe), 1.13 (s, 3H, CMe); δ_C (100 MHz, MeCN-d_3) 199.03 (C-6), 171.59 (C-1), 145.87, 133.57, 131.96, 129.74 (ArC), 114.45 (C-7), 92.89 (C-2), 82.19 (C-3), 63.51 (C-5), 60.45 (C-4), 26.92, 25.38 (2x -CMe), 21.81 (ArMe); *m/z* (ESI+) 328.26 ([M+Na]$^+$, 70%); HRMS (ESI+) calculated for $C_{16}H_{19}NNaO_5$ ([M+Na]$^+$) 328.1155, found 328.1164.

Use of phenyl benzylidene as diol protecting group towards 2,3-dihydroxypyrrolidinones

(2*R*, 5*S*)-1-Aza-6,7-(phenylidene)dioxy-7-(*N*,*O*-dimethylcarbamoyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 102

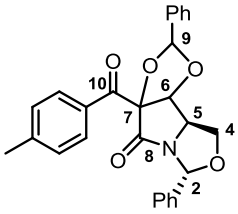
To a solution of **95** (136 mg, 0.422 mmol, 1.0 eq.) in toluene (6 mL), were added benzaldehyde dimethyl acetal (158 μL, 1.055 mmol, 2.5 eq.), and *p*-TsOH·H_2O (3.2 mg, 0.0169 mmol, 0.04 eq.) at r.t.. The mixture was stirred at 65°C for 5.5 h, and the solvents evaporated to give crude. Purification via silica gel flash column chromatography (Eluent: 10% to 45% EtOAc/PE) gave **102** (96.1 mg, 56%).



R_f = 0.47 and 0.54 (2:1 EtOAc:PE); colourless oil; ν_{max} / cm^{-1} (film) 1718 (s, C=O), 1683 (s, C=O); *m/z* (ESI+) 411.37 ($[M+H]^+$, 100%); HRMS (ESI+) calculated for $C_{22}H_{22}N_2NaO_6$ ($[M+Na]^+$) 433.1370, found 433.1384.

(2*R*, 5*S*)-1-Aza-6,7-(phenylidene)dioxy-7-(4'methylphenylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 103a

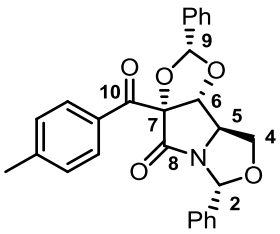
Tolyl magnesium bromide and the Grignard reaction were synthesised and carried out as described on p. 165. The Grignard reagent in THF (1.3 eq., 0.304 mmol) was added to **102** (96 mg, 0.234 mmol, 1.0 eq.) in THF (2 mL) at 0°C. The reaction mixture was stirred at 0°C for 45 min. It was quenched with sat. aq. NH_4Cl and extracted with EtOAc. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents removed *in vacuo* to give the crude product. Purification via silica gel flash column chromatography (Eluent: 5% to 45%, EtOAc/PE) gave **103a** (50 mg, 48%).



R_f = 0.46 (2:1 EtOAc:PE); colourless oil; ν_{max} / cm^{-1} (film) 1718 (s, C=O), 1666 (s, C=O); *m/z* (ESI+) 442.66 ($[M+H]^+$, 100%); HRMS (ESI+) calculated for $C_{27}H_{23}NNaO_5$ ($[M+Na]^+$) 464.1468, found 464.1475.

A single diastereomer (16%) was recovered from the acidic hydrolysis of a diasteromeric mixture of **103a** (*vide infra*).

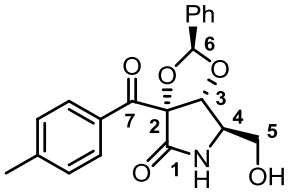
(2*R*, 5*S*, 6*R*, 7*S*, 9*R*)-1-Aza-6,7-(phenylidene)dioxy-7-(4'methylphenylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 103a



R_f = 0.46 (2:1 EtOAc:PE); colourless oil; $[\alpha]_D^{23}$ = +320.7 (*c*= 1.0 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 1720 (s, C=O), 1666 (s, C=O);δ_H (400 MHz, MeCN-d_3) 8.17 (d, *J* = 8.3 Hz, 2H, ArH), 7.46-7.57 (m, 5H, ArH), 7.29-7.45 (m, 7H, ArH), 6.31 (s, 1H, H-2), 6.13 (s, 1H, H-9), 5.20 (d, *J* = 4.4 Hz, 1H, H-6), 4.58 (dt, *J* = 4.4, 7.2 Hz, 1H, H-5), 4.23 (dd, *J* = 7.1, 8.3 Hz, 1H, H-4), 4.16 (dd, *J* = 7.4, 8.6 Hz, 1H, H-4'), 2.40 (s, 3H, Ar-Me); δ_C (100 MHz, MeCN-d_3) 195.14 (C-10), 171.38 (C-8), 146.63, 138.80, 136.79, 133.06, 131.70, 131.36, 130.09, 130.04, 139.66, 128.27, 127.18 (ArC), 108.25 (C-9), 98.15 (C-7), 88.36 (C-2), 81.52 (C-6), 66.21 (C-4), 59.52 (C-5), 21.87 (Ar-Me); *m/z* (ESI+) 442.64 ($[M+H]^+$, 100%); HRMS (ESI+) calculated for $C_{27}H_{23}NNaO_5$ ($[M+Na]^+$) 464.1468, found 464.1475.

(2*S*, 3*R*, 4*R*, 6*S*)-2,3-(phenylidene)dioxy-2-(4'-methylphenylcarbonyl)-4-hydroxymethyl-1-oxo-pyrrolidinone, 104a

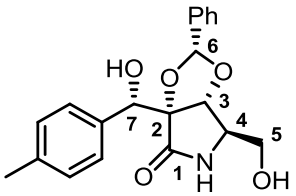
TFA (18 μL, 2.265 mmol, 20 eq.) was added to **103a** (50 mg, 0.113 mmol, 1 eq.) in CH_2Cl_2 (1.5 mL) and the resulting pale yellow solution was stirred at r.t. for 5.5 h. The solvents were removed *in vacuo* to give the crude which was purified via silica gel column chromatography (Eluent: 10% to 60% EtOAc/PE) to give a one fraction containing a diastereomeric mixture of **104a** (13 mg) and one pure diastereomer of **104a** (5 mg, 13%). One diastereomer of **103a** was recovered (16%).



R_f = 0.13 (1:1 EtOAc:PE); colourless oil; $[\alpha]_D^{23}$ = +23.4 (*c*= 0.63 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 1714 (s, C=O), 1669 (s, C=O); δ_H (400 MHz, MeCN-d_3) 8.09 (d, *J* = 8.3 Hz, 2H, ArH), 7.32-7.48 (m, 5H, ArH), 7.30 (d, *J* = 8.3 Hz, 2H, ArH), 6.97 (br s, 1H, -NH-), 6.15 (s, 1H, H-6), 4.97 (s, 1H, H-3), 3.81 (tt, *J* = 1.2, 6.4 Hz, 1H, H-4), 3.60-3.72 (m, 2H, H-5 and H-5') 3.26 (dt, *J* = 1.3, 5.7 Hz, 1H, -OH), 2.39 (s, 3H, Ar-Me); δ_C (100 MHz, MeCN-d_3) 196.51 (C-7), 170.62 (C-1), 145.96, 136.52, 133.46, 131.60, 130.98, 129.81, 129.47, 127.84 (ArC), 106.05 (C-6), 92.92 (C-2), 82.05 (C-3), 63.61 (C-4), 61.26 (C-5), 21.79 (Ar-Me); *m/z* (ESI+) 376.56 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{22}H_{19}NNaO_5$ ($[M+Na]^+$) 376.1155, found 376.1172.

(2*S*, 3*R*, 4*R*, 6*R*, 7*S*)-2,3-(phenylidene)dioxy-2-(hydroxymethyl-4'-methylphenylcarbonyl)-4-hydroxymethyl-1-oxo-pyrrolidinone, 105

10% Pd/C (1.5 mg) was added to a diastereomeric mixture of **104a** (13 mg, 0.0368 mmol) in EtOH (3 mL). The suspension was stirred at r.t. with bubbling of H_2 (balloon) for 6 h, afterwhich it was filtered over Celite and the residue rinsed with EtOAc. The solvent was evaporated to give the crude which was purified via silica gel flash column chromatography (Eluent: 10% to 60% EtOAc/PE) to give **105** (4 mg, 31%).

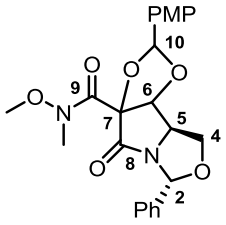


R_f = 0.63 (EtOAc); fine white crystals, mp = 156-158°C; $[\alpha]_D^{23}$ = +26.0 (*c*= 0.68 in MeOH); ν_{max} / cm^{-1} (film) 3329 (br), 1695 (s, C=O,); δ_H (400 MHz, MeCN-d_3) 7.39-7.45 (m, 5H, PhH), 7.33 (d, *J* = 8.1 Hz, 2H, ArH), 7.19 (d, *J* = 7.9 Hz, 2H, ArH), 6.37 (br s, 1H, -NH-), 5.98 (s, 1H, H-6), 5.25 (d, *J* = 3.3 Hz, 1H, H-7), 4.55 (s, 1H, H-3), 4.25 (d, *J* = 3.3 Hz, 1H, C7 -OH), 3.42-3.46 (m, 1H, H-4), 2.86 (t, *J* = 5.6 Hz, 1H, C5 -OH), 2.49 (ddd, *J* = 5.5, 6.9, 10.9 Hz, 1H, H-5), 2.43 (ddd, *J* = 5.5, 6.9, 10.9 Hz, 1H, H-5'), 2.34 (s, 3H, Ar-Me); δ_C (100 MHz, MeCN-d_3) 173.46 (C-1), 138.96, 137.82, 136.23, 130.71, 129.78, 129.40, 128.17, 127.81 (ArC), 107.28 (C-6), 90.00 (C-2), 79.27 (C-3), 73.22 (C-7), 62.64 (C-5), 60.27 (C-4), 21.22 (Ar-Me); *m/z* (ESI+) 354.65 ([M-H]$^-$, 100%); HRMS (ESI+) calculated for $C_{20}H_{21}NNaO_5$ ([M+Na]$^+$) 378.1312, found 378.1323.

Use of p-methoxyphenyl (PMP) benzylidene as diol protecting group towards 2,3-dihydroxypyrrolidinones

(2*R*, 5*S*)-1-Aza-6,7-(4'-methoxyphenylidene)dioxy-7-(*N*,*O*-dimethylcarbamoyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 106

To a solution of **95** (513 mg, 1.592 mmol 1.0 eq.) in toluene (12 mL), were added anisaldehyde dimethyl acetal (680 μL, 3.98 mmol, 2.5 eq.), and *p*-TsOH·H_2O (12.2 mg, 0.064 mmol, 0.04 eq.) at r.t.. The mixture was stirred at 65°C for 5.5 h, and the solvents evaporated to give crude. Purification via silica gel flash column chromatography (Eluent: 5% to 40% EtOAc/PE) gave **106** (468 mg, 67%) as a mixture of diastereomers.



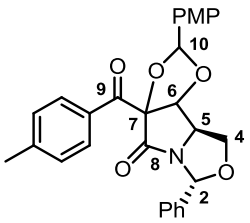
R_f = 0.37 and 0.45 (2:1 EtOAc:PE); ν_{max} / cm^{-1} (film) 1721 (s, C=O), 1683 (s, C=O); *m/z* (ESI+) 441.35 ($[M+H]^+$, 100%); HRMS (ESI+) calculated for $C_{23}H_{24}N_2NaO_7$ ($[M+Na]^+$) 463.1476, found 463.1484.

General procedures for Grignard reaction of Weinreb amide **106**

Aryl, alkyl and alkenyl Grignard reagents are prepared as per the reaction with **76** (p. 165).

The Grignard reagent in THF (1.3 eq. based on amount of **106** used) was added to **106** (1.0 eq.) in THF at 0°C. The reaction was stirred at 0°C to r.t. over a period of 45 min to 1 h, afterwhich it was quenched with sat. aq. NH_4Cl and extracted with EtOAc. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents removed *in vacuo* to give the crude product. Purification via silica gel flash column chromatography (Eluent: EtOAc/PE) gave the respective product as mixture of diastereomers about the protected diol.

(2*R*, 5*S*)-1-Aza-6,7-(4'-methoxyphenylidene)dioxy-7-(4'methylphenylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 107a

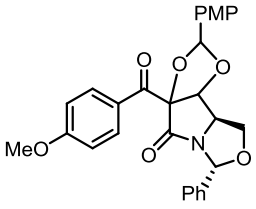


Scale of reaction: 77 mg (0.175 mmol), product: 37 mg, 45%;

R_f = 0.53 (30% EtOAc/PE); colourless oil; ν_{max} / cm^{-1} (film) 1718 (s, C=O), 1667 (s, C=O); δ_H (400 MHz, MeCN-d_3) 8.16 (d, *J* = 8.2 Hz, Ar-H), 8.14(d, *J* = 8.2 Hz, Ar-H), 8.06 (d, *J* = 8.3 Hz, Ar-H), 7.51-7.56 (m, ArH), 7.00 (d, *J* = 8.8 Hz, Ar-H), 6.93 (d, *J* = 8.8 Hz, Ar-H), 6.88 (d, *J* = 8.9 Hz, Ar-H), 6.32, 6.23, 6.17 (3x s, H-2), 6.20, 6.08, 5.95 (3x s, H-10), 5.30 (d, *J* = 1.2 Hz, H-6), 5.20 (s, H-6), 5.17 (d, *J* = 4.4 Hz, H-6), 4.56 (dt, *J* = 4.4, 7.2 Hz, H-5), 4.50 (dd, *J* = 6.5, 8.3 Hz, H-4), 4.43 (dd, *J* = 6.6, 8.1 Hz, H-4), 4.31 (ddd, *J* = 1.2, 6.5, 9.8 Hz, H-5), 4.19-4.27 (m, H-4), 4.14 (dd, *J* = 7.4, 8.6 Hz, H-4), 3.83, 3.79, 3.76 (3x s, 3H, Ar-O<u>Me</u>), 2.42, 2.40, 2.39 (3x s, 3H, Ar-<u>Me</u>); δ_C (100 MHz, MeCN-d_3) 198.47, 196.69, 195.22 (C-9), 173.60, 173.36, 171.52 (C-8), 162.31, 162.20, 162.17, 146.57, 146.43, 146.18, 139.50, 139.45, 138.79, 133.31, 133.05, 131.78, 131.67, 131.65, 130.05, 130.01, 129.91, 129.89, 129.83, 129.80, 129.63, 129.31, 128.72, 128.36, 127.73, 127.22, 127.16, 127.14, 126.94, 118.29, 114.96, 114.91, 114.80

(ArC), 108.22, 107.89, 106.57 (C-10), 97.98, 96.06, 95.75 (C-7), 89.45, 89.29, 88.32 (C-2), 81.33, 80.65, 80.20 (C-6), 69.21, 68.39, 66.17 (C-4), 64.77, 64.00, 59.48 (C-5), 56.11, 56.06, 56.03 (Ar-OMe), 21.85, 21.81 (Ar-Me); *m/z* (ESI+) 494.33 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{28}H_{25}NNaO_6$ ($[M+Na]^+$) 495.1607, found 495.1588.

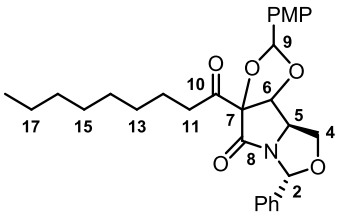
(2*R*, 5*S*)-1-Aza-6,7-(4'-methoxyphenylidene)dioxy-7-(4'methoxyphenylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 107b



Scale of reaction: 98 mg (0.222 mmol), product: 48 mg, 44%;

R_f = 0.22 (30% EtOAc/PE); colourless oil; ν_{max} / cm^{-1} (film) 1718 (s, C=O), 1661 (s, C=O); *m/z* (ESI+) 510.41 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{28}H_{25}NNaO_7$ ($[M+Na]^+$) 511.1556, found 511.1634.

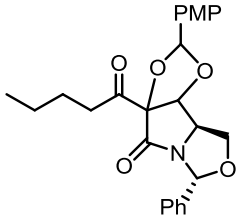
(2*R*, 5*S*)-1-Aza-6,7-(4'-methoxyphenylidene)dioxy-7-(octylcarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 107c



Scale of reaction: 128 mg (0.291 mmol), product: 43 mg, 30%;

R_f = 0.42 (30% EtOAc/PE); colourless oil; ν_{max} / cm^{-1} (film) 1726 (s, C=O), 1709 (s, C=O); *m/z* (ESI+) 494.48 ($[M+H]^+$, 100%); HRMS (ESI+) calculated for $C_{29}H_{36}NO_6$ ($[M+H]^+$) 494.2537, found 494.2543.

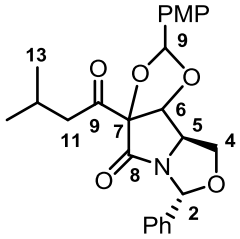
(2*R*, 5*S*)-1-Aza-7-(butylcarbonyl)-6,7-(4'-methoxyphenylidene)dioxy-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 107d



Scale of reaction: 135 mg (0.307 mmol), product: 33 mg, 19%;

R_f = 0.28 (30% EtOAc/PE); colourless oil; ν_{max} / cm^{-1} (film) 1724 (s, C=O), 1708 (s, C=O); *m/z* (ESI+) 438.42 ($[M+H]^+$, 100%); HRMS (ESI+) calculated for $C_{25}H_{27}NNaO_6$ ($[M+Na]^+$) 460.1731, found 460.1734.

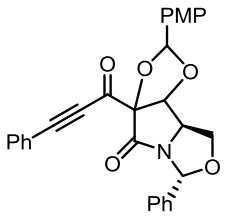
(2*R*, 5*S*)-1-Aza-7-(isobutylcarbonyl)-6,7-(4'-methoxyphenylidene)dioxy-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 107e



Scale of reaction: 140 mg (0.318 mmol), product: 37 mg, 27%;

R_f = 0.33 (30% EtOAc/PE); colourless oil; ν_{max} / cm^{-1} (film) 1707 (s, C=O, with shoulder towards longer wave number); *m/z* (ESI+) 438.39 ($[M+H]^+$, 25%); HRMS (ESI+) calculated for $C_{25}H_{27}NO_6$ ($[M+H]^+$) 438.1911, found 438.1927.

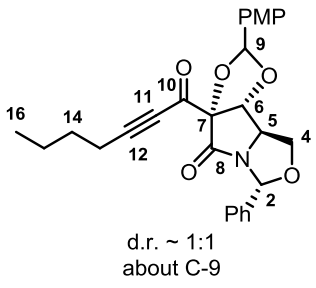
(2*R*, 5*S*)-1-Aza-6,7-(4'-methoxyphenylidene)dioxy-7-(phenylacetylenecarbonyl)-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 107f



Scale of reaction: 130 mg (0.295 mmol), product: 72 mg, 51%;

R_f = 0.68 (1:1 EtOAc/PE); pale yellow oil; ν_{max} / cm^{-1} (film) 2192 (s, alkyne), 1722 (s, C=O), 1661 (s, C=O); *m/z* (ESI+) 482.48 ($[M+H]^+$, 55%); HRMS (ESI+) calculated for $C_{29}H_{23}NNaO_6$ ($[M+Na]^+$) 504.1418, found 504.1431.

(2*R*, 5*S*, 6*R*, 7*S*)-1-Aza-7-(butylacetylenecarbonyl)-6,7-(4'-methoxyphenylidene)dioxy-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 107g



Scale of reaction: 135 mg (0.307 mmol), product: 48 mg, 34%;

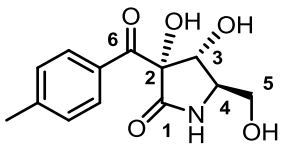
R_f = 0.22 (30% EtOAc/PE); colourless oil; ν_{max} / cm^{-1} (film) 2212 (s, alkyne), 1724 (s, C=O), 1665 (s, C=O); δ_H (400 MHz, MeCN-d_3) 7.49-7.54 (m, 2H, ArH), 7.37-7.48 (m, 5H, PhH), 6.92-7.01 (m, 2H, ArH), 6.28 (s, 0.5H, H-2), 6.25 (s, 0.5 H, H-2), 6.15 (s, 0.5 H, H-9), 6.11 (s, 0.5 H, H-9), 5.10 (d, *J* = 1.4 Hz, 0.5H, H-6), 4.97 (s, 0.5H, H-6), 4.48 (dd, *J* = 6.5, 8.4 Hz, 0.5H, H-4), 4.40 (dd, *J* = 6.7, 8.2 Hz, 0.5H, H-4), 4.19-4.31 (m, 1H, H-5), 3.81 and 3.80 (s, 3H, Ar-O<u>Me</u>), 3.68 (ddd, *J* = 4.6, 8.4, 9.7 Hz, 1H, H-4), 2.53 (dt, *J* = 7.0, 15.1 Hz, 2H, H-13), 1.52-1.66 (m, 2H, H-14), 1.35-1.50 (m, 2H, H-15), 0.86-0.96 (m, 3H, H-16); δ_C (100 MHz, MeCN-d_3) 184.23, 182.61 (C-10), 172.53, 172.40 (C-8), 162.29, 162.25 (ArC quart), 139.31, 139.26, 130.07, 129.98, 129.92, 129.65,

129.39, 127.94, 127.67, 127.21, 126.95, 114.92, 114.83 (ArC), 108.87, 106.87 (C-9), 104.32, 103.84 (C-12), 95.21, 94.91 (C-7), 89.35, 89.28 (C-2), 80.60, 80.46 (C-11), 79.64, 79.43 (C-6), 69.55, 68.60 (C-4), 64.83, 64.17 (C-5), 56.08, 55.33 (Ar-OMe), 30.22, 30.19 (C-14), 22.68 (C-15), 19.48, 19.47 (C-13), 13.76, 13.75 (C-16); *m/z* (ESI+) 462.38 ($[M+H]^+$, 15%); HRMS (ESI+) calculated for $C_{27}H_{28}NO_6$ ($[M+H]^+$) 462.1911, found 462.1917.

General procedures for acidic oxazolidine hydrolysis to 2,3-dihydroxypyrrolidinones **100a-g**

TFA (20 eq.) was added to a solution of **107a-g** in CH_2Cl_2 at r.t.. The reaction mixture was stirred at r.t. for 5-6.5 h and the solvents evaporated to give the crude. Purification via silica gel flash column chromatography (Eluent: 0% to 10% MeOH/EtOAc) gave the respective 2,3-dihydroxypyrrolidinones.

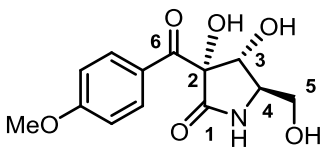
(2*S*, 3*R*, 4*R*)-2,3-Dihydroxy-2-(4'-methylphenylcarbonyl)-4-hydroxymethyl-1-oxo-pyrrolidinone, 100a



Scale of reaction: 35 mg (0.0742 mmol), product: 9 mg, 45%;

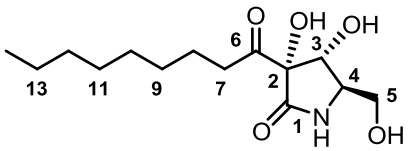
R_f = 0.23 (EtOAc); white solid, mp = 154-156°C; $[\alpha]_D^{23}$= -33.4 (*c*= 1.35 in MeOH); ν_{max} / cm^{-1} (film) 3452 (br, -NH-), 1692 (s, C=O), 1663 (s, C=O); δ_H (400 MHz, MeCN-d_3) 7.92 (d, *J* = 8.3 Hz, 2H, ArH), 7.32 (d, *J* = 8.3 Hz, 2H, ArH), 6.76 (br s, 1H, -NH-), 4.85 (br s, 1H, -OH), 4.55 (d, *J* = 6.4 Hz, 1H, H-3), 3.96 (br s, 1H, -OH), 3.80 (dd, *J* = 3.1, 11.8 Hz, 1H, H-5), 3.74 (br s, 1H, -OH), 3.61 (dd, *J* = 4.6, 11.8 Hz, 1H, H-5'), 3.53 (ddd, *J* = 2.2, 5.2, 7.3 Hz, 1H, H-4), 2.41 (s, 3H, Ar-Me); δ_C (100 MHz, MeCN-d_3) 198.62 (C-6), 173.05 (C-1), 146.21, 131.57, 130.75, 130.24 (ArC), 83.75 (C-2), 72.94 (C-3), 62.59 (C-4), 61.13 (C-5), 21.76 (Ar-Me); *m/z* (ESI+) 288.18 ($[M+Na]^+$, 85%); HRMS (ESI+) calculated for $C_{13}H_{15}NNaO_5$ ($[M+Na]^+$) 288.0842, found 288.0843.

(2*S*, 3*R*, 4*R*)-2,3-Dihydroxy-4-hydroxymethyl-2-(4'-methoxyphenylcarbonyl)-1-oxo-pyrrolidinone, 100b



Scale of reaction: 48 mg (0.0985 mmol), product: 15 mg, 52%; R_f = 0.14 (EtOAc); white solid, mp = 145-147°C; $[\alpha]_D^{23}$ = -27.3 (*c*= 1.21 in MeOH); ν_{max} / cm^{-1} (film) 3304 (br, -NH-), 1688 (s, C=O), 1677 (s, C=O); δ_H (400 MHz, MeCN-d_3) 8.03 (d, *J* = 9.0 Hz, 2H, ArH), 7.01 (d, *J* = 9.0 Hz, 2H, ArH), 6.81 (br s, 1H, -NH-), 4.55 (d, *J* = 6.4 Hz, 1H, H-3), 3.87 (s, 3H, -O<u>Me</u>), 3.81 (dd, *J* = 3.1, 11.8 Hz, 1H, H-5), 3.62 (dd, *J* = 4.6, 11.8 Hz, 1H, H-5'), 3.51-3.57 (m, 1H, H-4); δ_C (100 MHz, MeCN-d_3) 197.10 (C-6), 173.28 (C-1), 165.27, 133.23, 126.67, 114.89 (ArC), 83.47 (C-2), 73.06 (C-3), 62.67 (C-4), 61.14 (C-5), 56.47 (-O<u>Me</u>); *m/z* (ESI+) 304.21 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{13}H_{15}NNaO_6$ ($[M+Na]^+$) 304.0792, found 304.0800.

(2*S*, 3*R*, 4*R*)-2,3-Dihydroxy-4-hydroxymethyl-2-(octylcarbonyl)-1-oxo-pyrrolidinone, 100c



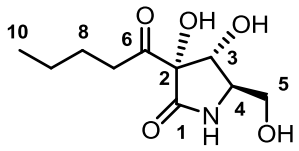
d.r. ~ 1:0.2 about diol

Scale of reaction: 42 mg (0.851 mmol), product: 10 mg, 41%; R_f = 0.18 (EtOAc); colourless oil; ν_{max} / cm^{-1} (film) 3317 (br, -OH), 1691 (s, C=O, with shoulder towards longer wave number);

<u>Major diastereomer</u>

δ_H (400 MHz, MeCN-d_3) 6.61 (br s, 1H, -NH-), 4.29 (d, *J* = 6.1 Hz, 1H, H-3), 3.76 (dd, *J* = 3.5, 11.6 Hz, 1H, H-5), 3.56 (dd, *J* = 5.5, 11.6 Hz, 1H, H-5'), 3.43 (dt, *J* = 3.5, 5.8 Hz, 1H, H-4), 2.53-2.72 (m, 2H, H-7), 1.48-1.60 (m, 2H, H-8), 1.21-1.36 (m, 10H, H-9, H-10, H-11, H-12, H-13), 0.88 (t, *J* = 7.3 Hz, 3H, H-14); δ_C (100 MHz, MeCN-d_3) 210.78 (C-6), 172.44 (C-1), 84.06 (C-2), 71.64 (C-3), 61.96 (C-5), 61.93 (C-4), 38.72 (C-7), 32.59, 30.11, 29.91, 29.80, 23.92 (C-8), 23.38 (C-9, C-10, C-11, C-12, C-13), 14.39 (C-14); *m/z* (ESI+) 288.37 ($[M+H]^+$, 35%); HRMS (ESI+) calculated for $C_{14}H_{25}NNaO_5$ ($[M+Na]^+$) 310.1625, found 310.1635.

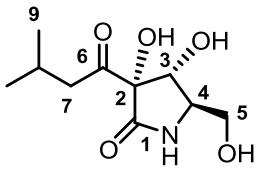
(2*S*, 3*R*, 4*R*)-2-(Butylcarbonyl)-2,3-dihydroxy-4-hydroxymethyl-1-oxo-pyrrolidinone, 100d



Scale of reaction: 30 mg (0.0686 mmol), product: 9 mg, 58%;

R_f = 0.20 (EtOAc); colourless oil; $[\alpha]_D^{23}$= +40.1 (*c*= 0.67 in MeOH); ν_{max} / cm^{-1} (film) 3291 (br, -NH-), 1680 (s, with shoulder towards longer wavelength, C=O); δ_H (400 MHz, MeCN-d_3) 6.68 (br s, 1H, -NH-), 4.30 (d, *J* = 6.1 Hz, 1H, H-3), 3.76 (dd, *J* = 3.5, 11.6 Hz, 1H, H-5), 3.57 (dd, *J* = 5.6, 11.6 Hz, 1H, H-5'), 3.45 (dt, *J* = 3.5, 5.8 Hz, 1H, H-4), 2.54-2.76 (m, 2H, H-7, partially obscured by broad water signal), 1.48-1.59 (m, 2H, H-8), 1.26-1.37 (m, 2H, H-9), 0.90 (t, *J* = 7.3 Hz, 3H, H-10); δ_C (100 MHz, MeCN-d_3) 210.76 (C-6), 172.56 (C-1), 84.10 (C-2), 71.64 (C-3), 62.01 (C-4), 61.96 (C-5), 38.44 (C-7), 26.03 (C-8), 22.90 (C-9), 14.15 (C-10); *m/z* (ESI+) 254.21 ($[M+Na]^+$, 90%); HRMS (ESI+) calculated for $C_{10}H_{17}NNaO_5$ ($[M+Na]^+$) 254.0999, found 254.1010.

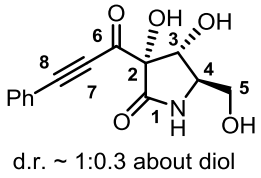
(2*S*, 3*R*, 4*R*)-2,3-Dihydroxy-4-hydroxymethyl-2-(isobutylcarbonyl)-1-oxo-pyrrolidinone, 100e



Scale of reaction: 37 mg (0.0846 mmol), product: 6 mg, 31%;

R_f = 0.18 (EtOAc); colourless oil; $[\alpha]_D^{23}$= +45.5 (*c*= 0.8 in MeOH); ν_{max} / cm^{-1} (film) 3355 (br, -OH), 1680 (s, C=O, with shoulder towards longer wave number); δ_H (400 MHz, MeCN-d_3) 6.68 (br s, 1H, -NH-), 4.29 (d, *J* = 6.0 Hz, 1H, H-3), 3.77 (dd, *J* = 3.6, 11.6 Hz, 1H, H-5), 3.57 (dd, *J* = 5.6, 11.7 Hz, 1H, H-5'), 3.44 (dt, *J* = 3.5, 5.8 Hz, 1H, H-4), 2.59 (dd, *J* = 6.8, 17.8 Hz, 1H, H-7), 2.48 (dd, *J* = 6.7, 17.8 Hz, 1H, H-7') (overlapping broad water signal at baseline), 2.09-2.19 (m, 1H, H-8), 0.91 (d, *J* = 6.7 Hz, 6H, H-9); δ_C (100 MHz, MeCN-d_3) 210.23 (C-6), 172.49 (C-1), 84.09 (C-2), 71.52 (C-3), 62.02 (C-4), 61.97 (C-5), 47.39 (C-7), 24.61 (C-8), 22.72 (C-9); *m/z* (ESI+) 232.23 ($[M+H]^+$, 20%); HRMS (ESI+) calculated for $C_{10}H_{17}NNaO_5$ ($[M+Na]^+$) 254.0999, found 254.1003.

(2*S*, 3*R*, 4*R*)-2,3-Dihydroxy-4-hydroxymethyl-2-(phenylacetylenecarbonyl)-1-oxo-pyrrolidinone, 100f



d.r. ~ 1:0.3 about diol

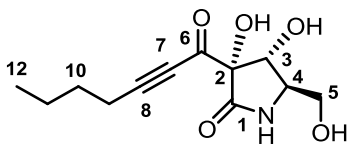
Scale of reaction: 70 mg (0.145 mmol), product: 19 mg, 48%;

R_f = 0.17 (EtOAc); white solid, mp = 149-150°C; ν_{max} / cm^{-1} (film) 3299 (br, -OH), 2193 (s, alkyne), 1699 (s, C=O), 1674 (s, C=O);

Major diastereomer

δ_H (400 MHz, MeCN-d_3) 7.63-7.71 (m, 2H, PhH), 7.54-7.62 (m, 1H, PhH), 7.44-7.52 (m, 2H, PhH), 6.69 (br s, 1H, -NH-), 4.74 (d, *J* = 6.7 Hz, 1H, H-3), 3.84 (dd, *J* = 3.0, 11.9 Hz, 1H, H-5), 3.64 (dd, *J* = 4.8, 11.9 Hz, 1H, H-5'), 3.50 (ddd, *J* = 3.0, 4.8, 6.7 Hz, 1H, H-4); δ_C (100 MHz, MeCN-d_3) 186.86 (C-6), 171.34 (C-1), 134.34, 132.73, 130.00, 119.95 (PhC), 97.73 (C-8), 86.09 (C-2), 85.87 (C-7), 72.16 (C-3), 61.71 (C-4), 61.30 (C-5); *m/z* (ESI+) 276.48 ($[M+H]^+$, 35%); HRMS (ESI+) calculated for $C_{14}H_{13}NNaO_5$ ($[M+Na]^+$) 298.0686, found 298.0698.

(2*S*, 3*R*, 4*R*)-2,3-Dihydroxy-4-hydroxymethyl-2-(butylacetylenecarbonyl)-1-oxo-pyrrolidinone, 100g



Scale of reaction: 45 mg (0.0975 mmol), product: 18 mg, 72%;

R_f = 0.21 (EtOAc); white solid, mp = 123-125°C; $[\alpha]_D^{23}$ = +37.7 (*c*= 1.33 in MeOH); ν_{max} / cm^{-1} (film) 3356 (br, -NH-), 2211 (s, alkyne), 1701 (s, C=O), 1682 (s, C=O); δ_H (400 MHz, MeCN-d_3) 6.67 (br s, 1H, -NH-), 4.57 (d, *J* = 6.8 Hz, 1H, H-3), 3.81 (dd, *J* = 3.2, 11.7 Hz, 1H, H-5), 3.57 (dd, *J* = 5.5, 11.7 Hz, 1H, H-5'), 3.46 (ddd, *J* = 3.1, 5.5, 6.8 Hz, 1H, H-4), 2.47 (t, *J* = 7.0 Hz, 2H, H-9) (overlapping broad water signal at baseline), 1.51-1.63 (m, 2H, H-10), 1.36-1.49 (m, 2H, H-11), 0.92 (t, *J* = 7.3 Hz, 3H, H-12); δ_C (100 MHz, MeCN-d_3) 186.67 (C-6), 171.36 (C-1), 103.00 (C-8), 85.69 (C-2), 78.77 (C-7), 72.39 (C-3), 61.85 (C-5), 61.79 (C-4), 30.27 (C-10), 22.66 (C-11), 19.38 (C-9), 13.77 (C-12); *m/z* (ESI+) 256.26 ($[M+H]^+$, 15%); HRMS (ESI+) calculated for $C_{12}H_{18}NO_5$ ($[M+H]^+$) 256.1179, found 256.1175.

5.2.4 Experimental for enoyl derivatives of pyrrolidinones

Enonyl derivatives from Grignard reagents from β-bromostyrene

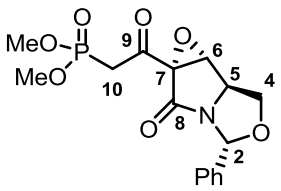
The Grignard reagent were prepared in excess: Magnesium flakes (70 mg, 2.879 mmol, 1.05 eq.) and I_2 (1 small crystal) were placed in a two-necked round-bottomed flask attached with a condenser. A solution of β-bromostyrene (352 µL, 2.742 mmol, 1.0 eq.) in 6 mL THF was added to the magnesium dropwise slowly. Initiation of the reaction could be observed via the disappearance of the yellowish-brown colour of iodine. Once addition of the reagent has been completed, the mixture was heated at 45°C for 1 h. The solution was then cooled to r.t. and the required amount was used for the Grignard reaction directly.

The Grignard reagent in THF (0.82 mL, 0.375 mmol, 1.2 eq.) was added to **76** (95 mg, 0.312 mmol, 1.0 eq.) in THF at -15°C. The reaction was stirred at -15°C for 1 h, afterwhich it was quenched with sat. aq. NH_4Cl and extracted with EtOAc. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents removed *in vacuo* to give the crude product. Purification via silica gel flash column chromatography (Eluent: EtOAc/PE) gave an inseparable mixture of **77h** and **77f**.

HWE olefination strategy towards enonyl derivatives

(2*R*, 5*S*, 7*S*)-1-Aza-6,7-epoxy-3-oxa-8-oxo-2-phenyl-7-[1'-(dimethyl-methylphosphonate)-carbamoyl]-bicyclo[3.3.0]octane, 108

n-BuLi, 1.6 M in hexanes (209 µL, 0.331 mmol, 0.95 eq.) was added dropwise to a THF solution (2 mL) of dimethyl methylphosphonate (40 µL, 0.365 mmol, 1.05 eq.) at -78°C. The solution was left to stir at -78°C for 30 min. After which, a solution of Weinreb amide **76** (106 mg, 0.348 mmol, 1.0 eq.) in dry THF (2 mL) was added dropwise to the mixture dropwise at -78°C, and left to stir at that temperature for 35 min. The reaction was then quenched with sat. aq. NH_4Cl and extracted with EtOAc. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude product. Purification via silica gel flash column chromatography (Eluent: 10% to 65%, EtOAc/PE) gave phosphorane **108** (55 mg, 45%) as a colourless oil.



R_f = 0.27 (EtOAc); colourless oil; $[\alpha]_D^{20}$ = +115.42 (*c*= 0.98 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 1717 (br s, with shoulder towards longer frequency, C=O), 1254 (s, P=O), 1026 (s, C-O); δ_H (500 MHz, $CDCl_3$) 7.33-7.41 (m, 5H, Ph-H), 6.35 (s, 1H, H-2), 4.39 (s, 1H, H-6), 4.15-4.23 (m, 3H, H-4, H-5, H-10), 3.95 (dd, *J* = 7.1, 8.7 Hz, 1H, H-4'), 3.82 (d, *J* = 11.3 Hz, 3H, P-OMe), 3.76 (d, *J* = 11.3 Hz, 3H, P-OMe), 3.31 (dd, *J* = 12.9, 20.8 Hz, 1H, H-10'); δ_C (125 MHz, $CDCl_3$) 191.07 (C-9, $^2J_{PC}$ = 5.7 Hz), 171.37 (C-8), 137.91, 128.82, 128.51, 125.82 (Ph-C), 88.43 (C-2), 65.44 (C-4), 65.35 (C-6), 62.30 (C-7), 58.79 (C-5), 53.46 (P-OMe, $^2J_{PC}$ = 6.7 Hz), 53.21 (P-OMe, $^2J_{PC}$ = 6.7 Hz), 39.93 (C-10, $^1J_{PC}$ = 124 Hz); δ_P (200 MHz, $CDCl_3$) 21.76; *m/z* (ESI+) 390.0 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{16}H_{18}NNaO_7P$ ($[M+Na]^+$) 390.0713, found 390.0709.

(2*R*, 5*S*, 7*S*)-1-Aza-6,7-epoxy-7-[4'-methoxyphenyl-(*E*)-vinylenecarbonyl]-3-oxa-8-oxo-2-phenyl-bicyclo[3.3.0]octane, 109

108 (50 mg, 0.136 mmol, 1.0 eq.) in dry THF (2 mL), was added to solid ^{t}BuOK (16.8 mg, 0.150 mmol, 1.1 eq.) or NaH (60% in mineral oil) (6.0 mg, 0.150 mmol, 1.1 eq.) at 0°C and the suspension was stirred at 0°C for 30 min. *p*-tolualdehyde (18 µL, 0.150 mmol, 1.1 eq.) in dry THF (1 mL) was then added. The reaction mixture was stirred at 0°C for 10 min, and then at r.t. for 1 h. The reaction was quenched with sat. aq. NH_4Cl, acidified with 10% aq. HCl and extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude. The crude gave a clean ^{1}H NMR spectrum of a mixture of only **108** and *p*-tolualdehyde.

Deuteration quenching study of **108**

108 (40 mg, 0.109 mmol, 1.0 eq.) in dry THF (3 mL), was added to solid NaH (60% in mineral oil) (5.2 mg, 0.131 mmol, 1.1 eq.) at 0°C and stirred at 0°C for 30 min. During which, aliquots were drawn at 10 min and 30 min, each quenched in D_2O and extracted with EtOAc and the organic layer submitted for LRMS analysis. The mixture was then continued to stir at r.t. for another 45 min, during which aliquots were drawn at 60 min and 75 min and treated as above. LRMS analysis of the aliquots

shows the deuterated species at 369 and 391 *m/z* (as compared to the $[M+H]^+$ and $[M+Na]^+$ peaks at 368 and 390 *m/z* respectively).

General procedure for partial reductive hydrogenation-isomerisation of ynones **78f-g**, **92f-g** and **100f-g**

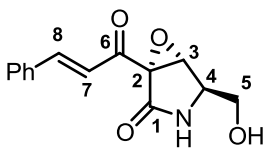
Reductive hydrogenation with Lindlar's catalyst, 5% $Pd/CaCO_3$ (poisoned with Pb):

To an EtOAc solution (approx. 0.02-0.05 M) of the ynone (between 0.07-0.1 mmol scale) was added Lindlar's catalyst (10-15% wt. of ynone). Quinoline (1 μL) was added for the reduction of all butylethynyl derivatives.The suspension was stirred at r.t. with H_2 gas (balloon) bubbled through the mixture and the progress of the reaction was monitored using LRMS analysis. The suspension was then filtered over Celite, rinsed with EtOAc and the solvent removed in vacuo to give the crude.

Isomerisation of enones:

The crude from the above reduction was dissolved in EtOAc (approx. 0.02 M), with one drop of dilute I_2/EtOAc solution added. The solution irradiated using a 500W halogen lamp with stirring for 5-6 h. The reaction was quenched with aq. $Na_2S_2O_3$ solution and extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude. Purification of the crude was done via reverse-phase preparative HPLC on an Agilent 1200 series system with Phenomenex® Luna C18(2) column (10.0 × 100 mm, 5 μm) using the elution gradient with a MeCN-H_2O mobile phase at a flow rate of 5 mL/min and detection at λ210 and 254 nm (see Appendix B for the respective elution conditions).

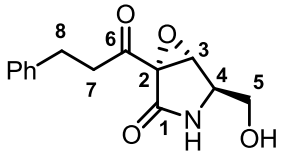
(2*S*, 4*R*)-2,3-Epoxy-4-hydroxymethyl-2-[(*E*)-phenylvinylenecarbonyl]-1-oxo-pyrrolidinone, (*E*)-78h



Preparative HPLC retention time: 20.37 min; white solid, mp = 71-73°C; $[\alpha]_D^{23}$ = +45.2 (*c*= 1.0 in MeOH); ν_{max} / cm^{-1} (film) 3330 (br, -OH), 1711 (s, C=O), 1671 (s, C=O); δ_H (400 MHz, MeCN-d_3) 7.84 (d, *J* = 16.2 Hz, 1H, H-8), 7.66-7.68 (m, 2H, PhH), 7.40-7.50 (m, 3H, PhH), 7.07 (d, *J* = 16.2 Hz, 1H, H-7), 6.36 (br s, 1H, -NH-), 4.25 (d, *J* = 2.4 Hz, 1H, H-3), 3.77-3.82 (m, 1H, H-4), 3.69 (dd, *J* = 3.7, 11.8 Hz, 1H, H-5), 3.64

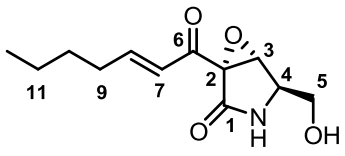
(dd, *J* = 4.4, 11.8 Hz, 1H, H-5'), 3.28 (br s, 1H, -OH); δ_C (100 MHz, MeCN-d_3) 190.28 (C-6), 170.66 (C-1), 145.90 (C-8), 135.32, 132.18, 130.10, 129.77 (PhC), 122.95 (C-7), 63.46 (C-3), 62.93 (C-2), 62.46 (C-5), 56.79 (C-4); *m/z* (ESI+) 282.50 ($[M+Na]^+$, 90%); HRMS (ESI+) calculated for $C_{14}H_{13}NNaO_4$ ($[M+Na]^+$) 282.0737, found 282.0746.

(2*S*, 4*R*)-2,3-Epoxy-2-(ethylphenylcarbonyl)-4-hydroxymethyl-1-oxo-pyrrolidinone, 78j



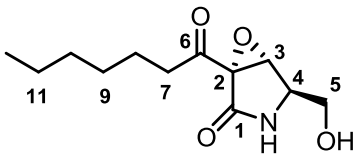
Preparative HPLC retention time: 22.21 min; colourless oil; $[\alpha]_D^{23}$= +90.1 (*c*= 0.25 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3336 (br, -OH), 1724 (s, C=O), 1692 (s, C=O); δ_H (400 MHz, MeCN-d_3) 7.25-7.32 (m, 2H, PhH), 7.16-7.24 (m, 3H, PhH), 6.30 (br s, 1H, -NH-), 4.09 (d, *J* = 2.5 Hz, 1H, H-3), 3.70-3.73 (m, 1H, H-4), 3.61 (dd, *J* = 4.0, 11.7 Hz, 1H, H-5), 3.55 (dd, *J* = 4.7, 11.7 Hz, 1H, H-5'), 2.85-2.96 (m, 4H, H-7 and H-8); δ_C (100 MHz, MeCN-d_3) 200.96 (C-6), 170.24 (C-1), 142.03, 129.45, 129.34, 127.08 (PhC), 63.44 (C-3), 62.48 (C-5), 62.38 (C-2), 56.48 (C-4), 42.09, 29.38 (C-7 and C-8); *m/z* (ESI+) 284.51 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{14}H_{15}NNaO_4$ ($[M+Na]^+$) 284.0893, found 284.0897.

(2*S*, 4*R*)-2,3-Epoxy-4-hydroxymethyl-2-[(*E*)-butylvinylenecarbonyl]-1-oxo-pyrrolidinone, (*E*)-78i



Retention time: 21.67 min; colourless oil; $[\alpha]_D^{23}$= +127.3 (*c*= 0.83 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3318 (br, -OH), 1713 (br s, C=O; with shoulder towards smaller wavenumber); δ_H (400 MHz, MeCN-d_3) 7.12 (dt, *J* = 7.0, 15.8 Hz, 1H, H-8), 6.39 (dt, *J* = 1.6, 15.8 Hz, 1H, H-7), 6.31(br s, 1H, -NH-), 4.14 (d, *J* = 2.4 Hz, 1H, H-3), 3.73-3.76 (m, 1H, H-4), 3.65 (dd, *J* = 3.8, 11.7 Hz, 1H, H-5), 3.59 (dd, *J* = 4.5, 11.7 Hz, 1H, H-5'), 2.26 (apparent dq, *J* = 1.6, 7.1 Hz, 2H, H-9), 1.40-1.50 (m, 2H, H-10), 1.28-1.39 (m, 2H, H-11), 0.91 (t, *J* = 7.3 Hz, 3H, H-12); δ_C (100 MHz, MeCN-d_3) 190.15 (C-6), 170.72 (C-1), 152.42 (C-8), 126.81 (C-7), 63.24 (C-3), 62.70 (C-2), 62.43 (C-5), 56.76 (C-4), 33.02 (C-9), 30.75 (C-10), 22.97 (C-11), 14.10 (C-12); *m/z* (ESI+) 240.51 ($[M+H]^+$, 100%); HRMS (ESI+) calculated for $C_{12}H_{17}NNaO_4$ ($[M+Na]^+$) 262.1050, found 262.1053.

(2*S*, 4*R*)-2-(Hexylcarbonyl)-2,3-epoxy-4-hydroxymethyl-1-oxo-pyrrolidinone, 78k

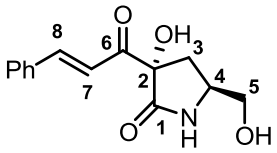


Retention time: 27.61 min; colourless oil; $[\alpha]_D^{23}$= +109.7 (*c*= 0.5 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3333 (br, -OH), 1723 (s, C=O), 1692 (s, C=O); δ_H (400 MHz, MeCN-d_3) 6.30 (br s, 1H, -NH-), 4.11 (d, *J* = 2.4 Hz, 1H, H-3), 3.70-3.73 (m, 1H, H-4), 3.62 (dd, *J* = 4.0, 11.7 Hz, 1H, H-5), 3.56 (dd, *J* = 4.7, 11.7 Hz, 1H, H-5'), 2.58 (apparent t, *J* = 7.4 Hz, 2H, H-7), 1.50-1.60 (m, 2H), 1.24-1.35 (m, 6H) (H-8, H-9, H10, H11), 0.88 (t, *J* = 7.3 Hz, 3H, H-12); δ_C (100 MHz, MeCN-d_3) 201.88 (C-6), 170.41 (C-1), 63.35 (C-3), 62.50 (C-5), 62.42 (C-2), 56.52 (C-4), 40.59 (C-7), 32.31, 29.40, 23.50, 23.23 (C-8, C-9, C-10, C-11), 13.97 (C-12); *m/z* (ESI+) 242.56 ($[M+H]^+$, 100%); HRMS (ESI+) calculated for $C_{12}H_{19}NNaO_4$ ($[M+Na]^+$) 264.1206, found 264.1209.

(2*S*, 4*R*)-2,3-Epoxy-4-hydroxymethyl-2-(isobutylcarbonyl)-1-oxo-pyrrolidinone, 78e

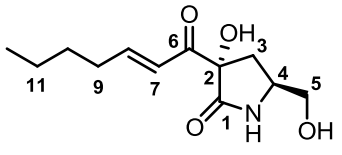
Retention time: 13.97 min

(2*S*, 4*R*)-2-Hydroxy-4-hydroxymethyl-2-[(*E*)-phenylvinylenecarbonyl]-1-oxo-pyrrolidinone, (*E*)-92h



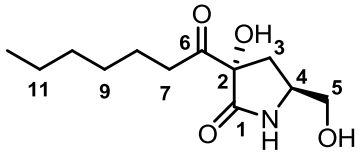
Retention time: 17.204 min; white crystals, mp = 107-109°C; $[\alpha]_D^{23}$= -47.2 (*c*= 1.16 in MeOH); ν_{max} / cm^{-1} (film) 3278 (br, -OH), 1687 (br s, C=O); δ_H (400 MHz, MeCN-d_3) 7.77 (d, *J* = 15.9 Hz, 1H, H-8), 7.64-7.71 (m, 2H, PhH), 7.39-7.51 (m, 3H, PhH), 7.32 (d, *J* = 15.9 Hz, 1H, H-7), 6.84 (br s , 1H, -NH-), 4.75 (br s , 1H, -OH), 3.86-3.91 (m, 1H, H-4), 3.69 (dd, *J* = 3.8, 11.4 Hz, 1H, H-5), 3.51 (dd, *J* = 5.5, 11.4 Hz, 1H, H-5'), 2.50 (dd, *J* = 6.1, 14.4 Hz, 1H, H-3), 2.14 (dd, *J* = 7.8, 14.4 Hz, 1H, H-3'); δ_C (100 MHz, MeCN-d_3) 198.60 (C-6), 174.84 (C-1), 145.48 (C-8), 135.46, 132.02, 130.06, 129.78 (PhC), 121.15 (C-7), 83.70 (C-2), 64.68 (C-5), 53.96 (C-4), 35.59 (C-3); *m/z* (ESI+) 262.53 ($[M+H]^+$, 98%); HRMS (ESI+) calculated for $C_{14}H_{16}NO_4$ ($[M+H]^+$) 262.1074, found 262.1076.

(2*S*, 4*R*)-2-Hydroxy-4-hydroxymethyl-2-[(*E*)-butylvinylenecarbonyl]-1-oxo-pyrrolidinone, (*E*)-92i



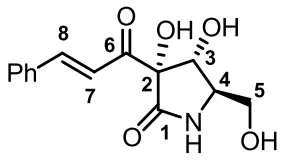
Retention time: 17.98 min; colourless oil; $[\alpha]_D^{23}$ = +45.3 (*c*= 1.07 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3303 (br, -OH), 1702 (br s, C=O; with shoulder towards smaller wavenumber); δ_H (400 MHz, MeCN-d_3) 7.07 (dt, *J* = 7.0, 15.5 Hz, 1H, H-8), 6.76 (br s , 1H, -NH-), 6.56 (dt, *J* = 1.6, 15.5 Hz, 1H, H-7), 4.60 (br s, 1H, -OH), 3.79-3.90 (m, 1H, H-4), 3.64 (dd, *J* = 3.9, 11.3 Hz, 1H, H-5), 3.45 (dd, *J* = 5.9, 11.3 Hz, 1H, H-5'), 2.35 (dd, *J* = 6.4, 14.3 Hz, 1H, H-3), 2.24-2.29 (m, 2H, H-9) (partially obscured by water signal), 2.06 (dd, *J* = 7.6, 14.3 Hz, 1H, H-3'), 1.41-1.51 (m, 2H, H-10), 1.28-1.40 (m, 2H, H-11), 0.91 (t, *J* = 7.3 Hz, 3H, H-12); δ_C (100 MHz, MeCN-d_3) 198.28 (C-6), 174.80 (C-1), 151.75 (C-8), 124.49 (C-7), 83.30 (C-2), 64.82 (C-5), 54.03 (C-4), 35.73 (C-3), 32.98 (C-9), 30.78 (C-10), 23.01 (C-11), 14.12 (C-12); *m/z* (ESI+) 264.58 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{12}H_{19}NNaO_4$ ($[M+Na]^+$) 264.1206, found 264.1206.

(2*S*, 4*R*)-2-(Hexylcarbonyl)-2-hydroxy-4-hydroxymethyl-1-oxo-pyrrolidinone, 92k



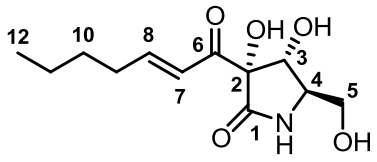
Retention time: 22.20 min; colourless oil; $[\alpha]_D^{23}$ = +75.0 (*c*= 0.84 in CH_2Cl_2); ν_{max} / cm^{-1} (film) 3289 (br, -OH), 1715 (br s, C=O), 1690 (br s, C=O); δ_H (400 MHz, MeCN-d_3) 6.67 (br s, 1H, -NH-), 4.47 (br s, 1H, -OH), 3.74-3.87 (m, 1H, H-4), 3.61 (dd, *J* = 4.2, 11.2 Hz, 1H, H-5), 3.44 (dd, *J* = 6.3, 11.2 Hz, 1H, H-5'), 2.68 (dd, *J* = 7.3, 17.9 Hz, 1H, H-7), 2.57 (dd, *J* = 7.3, 17.9 Hz, 1H, H-7'), 2.32 (dd, *J* = 6.3, 14.2 Hz, 1H, H-3), 2.04 (dd, *J* = 7.6, 14.2 Hz, 1H, H-3'), 1.47-1.60 (m, 2H), 1.26-1.32 (m, 6H) (H-8, H-9, H-10, H-11), 0.88 (t, *J* = 7.3 Hz, 3H, H-12); δ_C (100 MHz, MeCN-d_3) 211.01 (C-6), 174.76 (C-1), 84.05 (C-2), 65.09 (C-5), 53.90 (C-4), 37.96 (C-7), 35.67 (C-3), 32.36, 29.48, 24.17, 23.25 (C-8, C-9, C-10, C-11), 14.35 (C-12); *m/z* (ESI+) 244.58 ($[M+H]^+$, 85%); HRMS (ESI+) calculated for $C_{12}H_{22}NO_4$ ($[M+H]^+$) 244.1543, found 244.1544.

(2*S*, 3*R*, 4*R*)-2,3-Dihydroxy-4-hydroxymethyl-2-[(*E*)-phenylvinylenecarbonyl]-1-oxo-pyrrolidinone, (*E*)-100h



Retention time: 10.91 min; white solid, mp = decomposes at 175°C; $[\alpha]_D^{23}$ = -36.2 (*c*= 1.09 in MeOH); ν_{max} / cm^{-1} (film) 3250 (br, -OH), 1706 (C=O), 1697(C=O); δ_H (400 MHz, MeCN-d_3) 7.79 (d, *J* = 15.9 Hz, 1H, H-8), 7.66-7.73 (m, 2H, PhH), 7.40-7.50 (m, 3H, PhH), 7.23 (d, *J* = 15.9 Hz, 1H, H-7), 6.63 (br s, 1H, -NH-), 4.41 (d, *J* = 6.2 Hz, 1H, H-3), 3.81 (dd, *J* = 3.4, 11.7 Hz, 1H, H-5), 3.62 81 (dd, *J* = 5.4, 11.6 Hz, 1H, H-5'), 3.50 (ddd, *J* = 3.4, 5.4, 6.2 Hz, 1H, H-4); δ_C (100 MHz, MeCN-d_3) 198.28 (C-6), 172.3 (C-1), 145.58 (C-8), 135.41, 132.10, 130.07, 129.82 (PhC), 121.17 (C-7), 83.73 (C-2), 71.89 (C-3), 62.22 (C-4), 62.11 (C-5); *m/z* (ESI+) 278.65 ($[M+H]^+$, 100%); HRMS (ESI+) calculated for $C_{14}H_{16}NO_5$ ($[M+H]^+$) 278.1023, found 278.1024.

(2*S*, 3*R*, 4*R*)-2,3-Dihydroxy-4-hydroxymethyl-2-[(*E*)-butylvinylenecarbonyl]-1-oxo-pyrrolidinone, (*E*)-100i

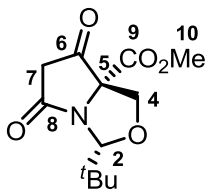


Retention time: 15.31 min; colourless oil; $[\alpha]_D^{23}$ = +37.2 (*c*= 0.68 in MeOH); ν_{max} / cm^{-1} (film) 3346 (br, -OH), 1701 (br s, C=O with shoulder towards smaller wavenumber); δ_H (400 MHz, MeCN-d_3) 7.09 (dt, *J* = 7.0, 15.5 Hz, 1H, H-8), 6.54 (br s, 1H, -NH-), 6.50 (dt, *J* = 1.6, 15.6 Hz, 1H, H-7), 4.54 (s, 1H, -OH C2), 4.31 (dd, *J* = 6.3, 8.6 Hz, 1H, H-3), 3.73-3.83 (m, 1H, H-5), 3.58 (ddd, *J* = 5.1, 6.6, 11.7 Hz, 1H, H-5'), 3.51 (d, *J* = 8.6 Hz, 1H, -OH C3), 3.42-3.48 (m, 1H, H-4), 3.05 (dd, *J* = 5.1, 6.6 Hz, 1H, -OH C5), 2.27 (apparent dq, *J* = 1.6, 7.1 Hz, 2H, H-9), 1.41-1.55 (m, 2H, H-10), 1.28-1.41 (m, 2H, H-11), 0.91 (t, *J* = 7.3 Hz, 3H, H-12); δ_C (100 MHz, MeCN-d_3) 197.71 (C-6), 172.24 (C-1), 152.07 (C-8), 124.62 (C-7), 83.33 (C-2), 71.77 (C-3), 62.20 (C-4), 61.87 (C-5), 33.04 (C-9), 30.79 (C-10), 23.02 (C-11), 14.11 (C-12); *m/z* (ESI+) 258.86 ($[M+H]^+$, 75%); HRMS (ESI+) calculated for $C_{12}H_{20}NO_5$ ($[M+H]^+$) 258.1336, found 258.1334.

5.3 Experimental for Chapter 3

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-3-oxabicyclo[3.3.0]-octane, 35

Compound **35** is a known compound and was synthesised according to literature procedures.[58] The respective spectroscopic data was consistent with those reported in the literature.



Orange crystalline solid, {lit.[58] orange crystalline solid, mp = 115-120°C}; $[\alpha]_D^{23}$=+95.2 (c 2.05 $CHCl_3$), {lit.[58] $[\alpha]_D^{20}$= +100.3 (c 2.02 $CHCl_3$)}; δ_H (400 MHz, $CDCl_3$) 5.09 (s, 1H, H-2), 4.81 (d, *J* = 9.0 Hz, 1H, H-4), 3.83 (s, 3H, H-10), 3.74 (d, *J* = 21.2 Hz, 1H, H-7), 3.62 (d, *J* = 9.0 Hz, 1H, H-4'), 3.17 (d, *J* = 21.2 Hz, 1H, H-7'), 0.91 (-^{t}Bu); δ_C (100 MHz, $CDCl_3$) 198.29 (C-6), 172.30 (C-9), 166.84 (C-8), 98.27 (C-2), 80.51 (C-5), 67.89 (C-4), 53.72 (C-10), 44.79 (C-7), 35.59 (-<u>C</u>-^{t}Bu), 24.64 (-^{t}Bu).

The structures of all subsequent 3-acyltetramic acid drawn correspond to the major tautomeric form present, unless otherwise stated.

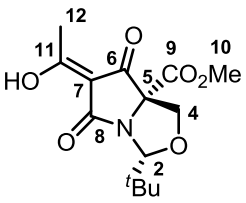
<u>Acyltetramic acid via *O*-acyl/*C*-acyl migtation</u>

<u>General procedure for synthesis of 3-acyltetramic acids **124**</u>

The respective acid (1.1 eq.) was added dropwise to a CH_2Cl_2 solution (approx. 0.25 M) of tetramic acid **35** (1.0 eq.), DMAP (1.3 eq.) and DCC (1.1 eq.). The reaction mixture was then stirred at r.t. overnight (17-20 h). The suspension was filtered and the solvents evaporated to give the crude which was purified via flash column chromatography on silica gel. The product thus obtained was washed with a solution of sat. NH_4Cl and 10% aq. HCl, dried with anhydrous $MgSO_4$, filtered and the solvents removed to afford the 3-acyl tetramic acids **124a-b**, **d-e**.

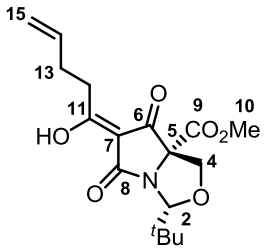
The structures as drawn correspond to the major tautomeric form present.

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-1'-methylvinylidene]-3-oxabicyclo[3.3.0]-octane, 124a



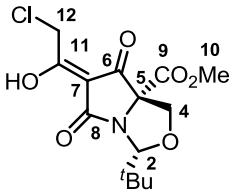
Scale of reaction: 300 mg (1.176 mmol), product: 315 mg, 90%; R_f = 0.45 (10% MeOH/EtOAc); $[\alpha]_D^{20}$ = +66.0 (c= 1.20 in $CHCl_3$); orange oil; ν_{max} / cm^{-1} (film) 1756 (s, C=O), 1719 (s, C=O), 1660 (s, C=O); δ_H (400 MHz, $CDCl_3$) 4.83 (s, 1H, H-2), 4.81 (d, *J* = 8.8 Hz, 1H, H-4), 3.77 (s, 3H, -CO_2Me), 3.48 (d, *J* = 8.8 Hz, 1H, H-4'), 2.47 (s, 3H, H-12), 0.91 (s, 9H, -^{t}Bu); δ_C (125 MHz, $CDCl_3$) 188.39 (C-11), 187.96 (C-6), 179.84 (C-8), 167.42 (C-9), 101.73 (C-7), 98.20 (C-2), 78.39 (C-5), 68.16 (C-4), 53.30 (-CO_2Me), 35.15 (-C-^{t}Bu), 24.60 (-^{t}Bu), 19.93 (C-12); *m/z* (ESI-) 296.09 ([M-H]$^-$, 94%); HRMS (ESI-) calculated for $C_{14}H_{18}NO_6$ ([M-H]$^-$) 296.1140, found 296.1142.

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-1'-(but-3-ene)vinylidene]- 3-oxabicyclo[3.3.0]-octane, 124b



Scale of reaction: 150 mg (0.588 mmol), product: 122 mg, 62%; R_f = 0.26 (EtOAc); $[\alpha]_D^{20}$ = +75.9 (c= 1.28 in $CHCl_3$); orange oil; ν_{max} / cm^{-1} (film) 1756 (s, C=O), 1718 (s, C=O), 1659 (s, C=O); δ_H (400 MHz, $CDCl_3$) 5.75-5.85 (m, 1H, H-14), 5.05 (dt, *J* = 8, 21 Hz, 2H, H-15), 4.85 (s, 1H, H-2), 4.82 (d, *J* = 8.8 Hz, 1H, H-4), 3.78 (s, 3H, -CO_2Me), 3.49 (d, *J* = 8.8 Hz, 1H, H-4'), 2.96 (apparent t, *J* = 8 Hz, 2H, H-12), 2.40-2.46 (m, 2H, H-13), 0.92 (s, 9H, -^{t}Bu); δ_C (100 MHz, $CDCl_3$) 191.12 (C-11), 187.72 (C-6), 180.11 (C-8), 167.40 (C-9), 135.59 (C-4), 116.32 (C-15), 101.33 (C-7), 98.23 (C-2), 78.31 (C-5), 68.18 (C-4), 53.29 (-CO_2Me), 35.14 (-C-^{t}Bu), 32.29 (C-12), 29.47 (C-13), 24.61 (-^{t}Bu); *m/z* (ESI-) 336.13 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{17}H_{22}NO_6$ ([M-H]$^-$) 336.1453, found 336.1453.

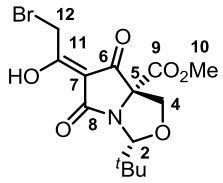
(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-1'-(chloromethyl)vinylidene]-3-oxabicyclo[3.3.0]-octane, 124d



Scale of reaction: 280 mg (1.097 mmol), product: 280 mg, 77%;

R_f = 0.56 (5% MeOH/EtOAc); $[\alpha]_D^{20}$ = +49.8 (c= 1.20 in $CHCl_3$); pale orange oil; ν_{max} / cm^{-1} (film) 1754 (s, C=O), 1720 (s, C=O), 1662 (s, C=O); δ_H (500 MHz, $CDCl_3$) 12.05 (br, s, enol –OH), 4.86 (s, 1H, H-2), 4.83 (d, *J* = 9.1 Hz, 1H, H-4), 4.63 (d, *J* = 13.6 Hz, 1H, H-12), 4.53 (d, *J* = 13.6 Hz, 1H, H-12'), 3.80 (s, 3H, -CO_2Me), 3.53 (d, *J* = 9.1 Hz, 1H, H-4'), 0.93 (s, 9H, -^{t}Bu); δ_C (125 MHz, $CDCl_3$) 186.69 (C-6), 181.91 (C-11), 179.40 (C-8), 166.74 (C-9), 101.53 (C-7), 98.42 (C-2), 78.43 (C-5), 68.01 (C-4), 53.48 (-CO_2Me), 38.84 (C-12), 35.16 (-C-^{t}Bu), 24.57 (-^{t}Bu); *m/z* (ESI-) 330.08 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{14}H_{17}ClNO_6$ ([M-H]$^-$) 330.0750, found 330.0750.

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-1'-(bromomethyl)vinylidene]-3-oxabicyclo[3.3.0]-octane, 124e



Scale of reaction: 500 mg (1.959 mmol), product: 397 mg, 54%;

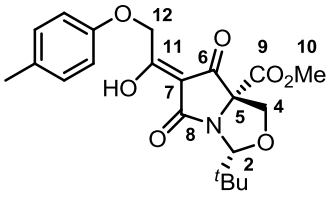
R_f = 0.65 (5% MeOH/EtOAc); $[\alpha]_D^{20}$ = +39.9 (c= 1.0 in $CHCl_3$); yellow oil; ν_{max} / cm^{-1} (film) 1753 (s, C=O), 1720 (s, C=O), 1661 (s, C=O); δ_H (500 MHz, $CDCl_3$) 4.88 (s, 1H, H-2), 4.84 (d, *J* = 9.1 Hz, 1H, H-4), 4.41 (d, *J* = 10.7 Hz, 1H, H-12), 4.35 (d, *J* = 10.7 Hz, 1H, H-12'), 3.80 (s, 3H, -CO_2Me), 3.54 (d, *J* = 8.8 Hz, 1H, H-4'), 0.93 (s, 9H, -^{t}Bu); δ_C (125 MHz, $CDCl_3$) 186.95 (C-6), 181.70 (C-11), 179.11 (C-8), 166.84 (C-9), 101.43 (C-7), 98.40 (C-2), 78.47 (C-5), 68.06 (C-4), 53.48 (-CO_2Me), 35.19 (-C-^{t}Bu), 24.60 (-^{t}Bu), 23.21 (C-12); *m/z* (ESI-) 374.00 ([M-H]$^-$, 68%); HRMS (ESI-) calculated for $C_{14}H_{17}BrNO_6$ ([M-H]$^-$) 374.0245, found 374.0242.

General procedures for synthesis of *O*-aryl ether tetramic acids **125a-c**

Cs_2CO_3 (3.0 eq.) and the corresponding phenol (1.1 eq.) was added to **124d** (1.0 eq.) dissolved in MeCN. The solution was stirred at r.t. for 17 h, afterwhich the solvents were evaporated and water and EtOAc were added to the residue. The aqueous layer was acidified with dilute 10% aq. HCl and extracted with EtOAc. The combined organic layers were dried with $MgSO_4$, filtered and evaporated

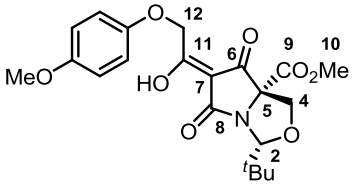
to give the crude. Purification via flash column chromatography on silica gel (Eluent: EtOAc/Petroleum Ether) gave the product, which was then washed with a solution of aq. sat. NH_4Cl and 10% aq. HCl, dried with anhydrous $MgSO_4$, filtered and the solvents evaporated.

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-1'-(4''-methylphenoxy)methyl-allylidene]-3-oxabicyclo[3.3.0]-octane, 125a



Scale of reaction: 60 mg (0.181 mmol), product: 48 mg, 66%; R_f = 0.37 (EtOAc); $[\alpha]_D^{20}$ = +61.3 (c= 1.10 in $CHCl_3$); pale orange oil; ν_{max} / cm^{-1} (film) 1755 (s, C=O), 1717 (s, C=O), 1661 (s, C=O), 1286, 1176, 1047 (s, C-O-C); δ_H (500 MHz, $CDCl_3$) 7.10 (d, *J* = 8.2 Hz, 2H, ArH), 6.85 (d, *J* = 8.2 Hz, 2H, ArH), 5.26 (d, *J* = 17.0 Hz, 1H, H-12), 5.18 (d, *J* = 17.0 Hz, 1H, H-12'), 4.87 (s, 1H, H-2), 4.86 (d, *J* = 9.1 Hz, 1H, H-4), 3.82 (s, 3H, -CO_2Me), 3.55 (d, *J* = 9.1 Hz, 1H, H-4'), 2.30 (s, 3H, Ar-Me), 0.94 (s, 9H, -^{t}Bu); δ_C (125 MHz, $CDCl_3$) 187.27 (C-6), 185.92 (C-11), 179.83 (C-8), 166.95 (C-9), 155.55, 131.47, 130.07, 114.60 (Ar-C), 100.80 (C-7), 98.35 (C-2), 78.28 (C-5), 68.06 (C-4), 65.21 (C-12), 53.43 (C-10), 35.16 (-C-^{t}Bu), 24.59 (-^{t}Bu), 20.47 (Ar-Me); *m/z* (ESI-) 402.14 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{21}H_{24}NO_7$ ([M-H]$^-$) 402.1558, found 402.1561.

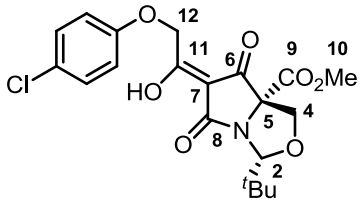
(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-1'-(4''-methoxyphenoxy)methyl-allylidene]-3-oxabicyclo[3.3.0]-octane, 125b



Scale of reaction: 55 mg (0.166 mmol), product: 48 mg, 69%; R_f = 0.33 (EtOAc); $[\alpha]_D^{20}$ = +24.6 (c= 0.97 in $CHCl_3$); yellow oil; ν_{max} / cm^{-1} (film) 1754 (s, C=O), 1716 (s, C=O), 1661 (s, C=O), 1285, 1178 (br), 1045 (br s, C-O-C); δ_H (500 MHz, $CDCl_3$) 6.90 (d, *J* = 9.1 Hz, 2H, ArH), 6.83 (d, *J* = 9.1 Hz, 2H, ArH), 5.23 (d, *J* = 17.3 Hz, 1H, H-12), 5.14 (d, *J* = 17.0 Hz, 1H, H-12'), 4.87 (s, 1H, H-2), 4.85 (d, *J* = 8.8 Hz, 1H, H-4), 3.81 (s, 3H, -CO_2Me), 3.77 (s, 3H, Ar-OMe), 3.54 (d, *J* = 8.8 Hz, 1H, H-4'), 0.94 (s, 9h, -^{t}Bu); δ_C (125 MHz, $CDCl_3$) 187.26 (C-6), 185.94 (C-11), 179.81 (C-8), 166.94 (C-9), 154.79, 151.78, 116.03, 114.70 (Ar-C), 100.81 (C-7), 98.35 (C-2), 78.28 (C-5), 68.06 (C-4), 65.96 (C-12),

55.65 (Ar-O<u>Me</u>), 53.43 (C-10), 35.15 (-<u>C</u>-^{t}Bu), 24.58 (-^{t}Bu); *m/z* (ESI-) 418.13 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{21}H_{24}NO_7$ ([M-H]$^-$) 418.1507, found 418.1513.

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-1'-(4''-chlorophenoxy)methyl-allylidene]-3-oxabicyclo[3.3.0]-octane, 125c



Scale of reaction: 45 mg (0.136 mmol), product: 42 mg, 73%; R_f = 0.38 (EtOAc); $[\alpha]_D^{20}$ = +20.4 (c= 0.95 in $CHCl_3$); yellow oil; ν_{max} / cm^{-1} (film) 1754 (s, C=O), 1716 (s, C=O), 1662 (s, C=O), 1284, 1173, 1045 (s, C-O-C); δ_H (400 MHz, $CDCl_3$) 11.64 (s, br, enol –OH), 7.26 (d, *J* = 8.5 Hz, 2H, ArH), 6.89 (d, *J* = 9.0 Hz, 2H, ArH), 5.28 (d, *J* = 17.2 Hz, 1H, H-12), 5.20 (d, *J* = 13.5 Hz, 1H, H-12'), 4.87 (s, 1H, H-2), 4.86 (d, *J* = 9.0 Hz, 1H, H-4), 3.82 (s, 3H, -CO_2<u>Me</u>), 3.55 (d, *J* = 9.0 Hz, 1H, H-4'), 0.94 (s, 9H, -^{t}Bu); δ_H (400 MHz, Acetone-d_6) 10.08 (br s,enol -OH), 7.34 (d, *J* = 9.0 Hz, 2H, ArH), 7.01 (d, *J* = 9.0 Hz, 2H, ArH), 5.35 (s, 2H, H-12), 4.90 (s, 1H, H-2), 4.77 (d, *J* = 8.7 Hz, 1H, H-4), 3.82 (s, 3H, -CO_2<u>Me</u>), 3.77 (d, *J* = 8.7 Hz, 1H, H-4'), 0.93 (s, 9H, -^{t}Bu); [broad signals in ^{13}C]

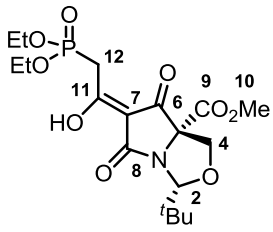
δ_H (700 MHz, CD_2Cl_2, 278K) 7.30 (d, *J* = 8.9 Hz, 2H, ArH), 6.92 (d , *J* = 8.5 Hz, 2H, ArH), 5.27 (d, *J* = 17.2 Hz, 1H, H-12), 5.22 (d, *J* = 17.2 Hz, 1H, H-12'), 4.90 (s, 1H, H-2), 4.83 (d, *J* = 9.1 Hz, 1H, H-4), 3.81 (s, 3H, -CO_2<u>Me</u>), 3.58 (d, *J* = 9.0 Hz, 1H, H-4'), 0.95 (s, 9H, -^{t}Bu); δ_C (175 MHz, CD_2Cl_2, 278K) 187.20 (C-6), 184.88 (C-11), 179.78 (C-8), 167.04 (C-9), 156.32, 129.52, 126.73, 116.00 (Ar-C), 101.04 (C-7), 98.24 (C-2), 78.28 (C-5), 67.98 (C-4), 65.19 (C-12), 53.49 (C-10; obscured by CD_2Cl_2 signals but HMBC correlation with C-9 seen), 35.06 (-<u>C</u>-^{t}Bu), 24.35 (-^{t}Bu); *m/z* (ESI-) 422.09 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{20}H_{21}ClNO_7$ ([M-H]$^-$) 422.1012, found 422.1010.

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-1'-(diethyl-methylphosphonate)-allylidene]-3-oxabicyclo[3.3.0]-octane, 126

NaH used was pre-washed with dry Et_2O.

A suspension of NaH (87.1 mg, 2.186 mmol, 3.5 eq.; 60% in mineral oil) in dry THF (3 mL) was cooled to 0°C. Diethyl phosphate (284 µL, 2.186 mmol, 3.5 eq.) was then added dropwise and the resulting suspension was stirred at 0°C for 30 min. **124e** (235 mg, 0.625 mmol, 1.0 eq.) in THF (5 mL)

was then added slowly dropwise. The reaction mixture was stirred at 0°C for 1 h, then at r.t. for 16 h. Reaction was quenched with water and extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude. Purification via flash column chromatography on silica gel (Eluent: EtOAc/PE, 10% to 100%, then 5% MeOH/EtOAc) gave **126** (228 mg, 84%) as an orange oil, which was then washed with sat. NH_4Cl and 10% aq. HCl to remove chelated metals from the 3-acyltetramic acid moiety.



R_f = 0.11 (5% MeOH/EtOAc); orange oil; $[\alpha]_D^{20}$ = +40.8 (*c*= 0.88 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1754 (s, C=O), 1718 (s, C=O), 1658 (s, C=O), 1265 (s, P=O), 1047, 1020, 956 (s, RO-P); δ_H (500 MHz, $CDCl_3$) 4.86 (s, 1H, H-2), 4.83 (d, *J* = 8.8 Hz, 1H, H-4), 4.16 (apparent quintet, *J* = 7.3 Hz, 4H, -O<u>C</u>$H_2$$CH_3$), 3.78 (s, 3H, -$CO_2$<u>Me</u>), 3.55 (d, *J* = 24.0 Hz, 2H, H-12), 3.50 (d, *J* = 8.8 Hz, 1H, H-4'), 1.31 (dt, *J* = 6.9 Hz, 2.8 Hz, 6H, -OCH_2<u>C</u>H_3), 0.93 (s, 9H, -^{t}Bu); δ_C (125 MHz, $CDCl_3$) 187.38 (C-6), 181.25 (C-11, $^2J_{PC}$ = 9.5 Hz), 179.49 (C-8), 167.14 (C-9), 102.53 (C-7, $^3J_{PC}$ = 6.7 Hz), 98.32 (C-2), 78.46 (C-5), 68.09 (C-4), 63.05 (-O<u>C</u>$H_2$$CH_3$, $^2J_{PC}$ = 6.7 Hz), 53.30 (C-10), 35.13 (-<u>C</u>-^{t}Bu), 32.36 (C-12, $^1J_{PC}$ = 127.8 Hz), 24.58 (-^{t}Bu), 16.24 (-OCH_2<u>C</u>H_3, $^3J_{PC}$ = 6.7 Hz); δ_P (200 MHz, $CDCl_3$) 19.21; *m/z* (ESI-) 432.11 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{18}H_{27}NO_9P$([M-H]$^-$) 432.1429, found 432.1431.

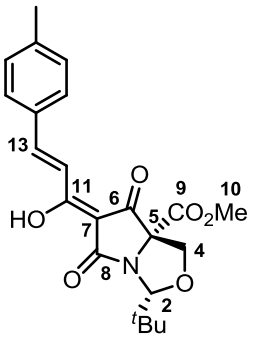
<u>Elaboration via Aldol condensation</u>

<u>General Procedure for Aldol Condensation of Tetramic Acid **124a** with Aldehydes</u>

A solution of **124a** (1.0 eq.) in anhydrous THF was added to 1.8 M LDA solution (2.1 eq.), at -10°C and the solution was stirred at -10°C for 1 h. A solution of aldehyde (1.0 eq.) in anhydrous THF was then added dropwise to the reaction mixture at -10°C (Overall approx. concentration 0.07-0.1 M, based on **124a**). The reaction mixture was allowed to warm to r.t. and left to stir at r.t. for 20 h. The reaction mixture was then quenched with sat. aq. NH_4Cl and extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude. Purification via flash column chromatography on silica gel (Eluent: EtOAc/PE, 10% to 100%) gave

the product as a yellow or orange oil. The product **127** was then washed with 10% aq. HCl to remove chelated metals from the 3-acyltetramic acid moiety to give a well resolved ^{1}H NMR spectrum.

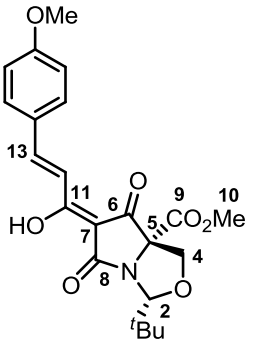
(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-3-(*E*)-(4'-methylphenyl)allylidene]-3-oxabicyclo[3.3.0]-octane, 127a



Scale of reaction: 120 mg (0.404 mmol), product: 20 mg, 12%;

R_f = 0.70 (5% MeOH/EtOAc); $[\alpha]_D^{20}$ = +66.8 (*c*= 1.00 in $CHCl_3$); bright yellow oil; ν_{max} / cm^{-1} (film) 1753 (s, C=O), 1707 (s, C=O), 1652 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.98 (d, *J* = 16.1 Hz, 1H, H-13), 7.68 (d, *J* = 16.1 Hz, 1H, H-12), 7.57 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.24 (d, *J* = 7.9 Hz, 2H, Ar-H), 4.90 (s, 1H, H-2), 4.87 (d, *J* = 8.8 Hz, 1H, H-4), 3.81 (s, 3H, -CO_2Me), 3.55 (d, *J* = 8.8 Hz, 1H, H-4'), 2.41 (s, 3H, Ar-Me), 0.96 (s, 9H, -^{t}Bu); δ_C (125 MHz, $CDCl_3$) 187.99 (C-6), 180.72 (C-8), 176.99 (C-11), 167.71 (C-9), 147.34 (C-13), 142.92, 131.42, 129.92, 129.64, 129.47 (ArC), 116.12 (C-12), 99.76 (C-7), 98.27 (C-2), 78.31 (C-5), 68.34 (C-4), 53.26 (C-10), 35.20 (-C-^{t}Bu), 24.66 (-^{t}Bu), 21.69 (Ar-Me); *m/z* (ESI-) 398.17 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{22}H_{24}NO_6$ ([M-H]$^-$) 398.1609, found 398.1603.

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-3-(*E*)-(4'-methoxyphenyl)allylidene]-3-oxabicyclo[3.3.0]-octane, 127b

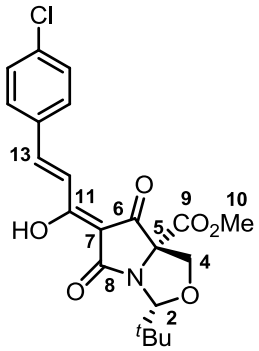


Scale of reaction: 130 mg (0.437 mmol), product: 26 mg, 14%;

R_f = 0.58 (5% MeOH/EtOAc); $[\alpha]_D^{20}$ = +74.3 (*c*= 1.04 in $CHCl_3$); bright orange oil; ν_{max} / cm^{-1} (film) 1752 (s, C=O), 1705 (s, C=O), 1651 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.97 (d, *J* = 15.8 Hz, 1H, H-13), 7.63 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.60 (d, *J* = 15.8 Hz, 1H, H-12), 6.94 (d, *J* = 8.8 Hz, 2H, Ar-H), 4.89 (s, 1H, H-2), 4.86 (d, *J* = 8.8 Hz, 1H, H-4), 3.87 (s, 3H, Ar-OMe), 3.81 (s, 3H, -CO_2Me), 3.55 (d, *J* = 8.8 Hz, 1H, H-4'), 0.96 (s, 9H, -^{t}Bu); δ_C (125 MHz, $CDCl_3$) 187.99 (C-6), 180.92 (C-8), 177.06 (C-11), 167.81 (C-9), 162.96 (ArC-OMe), 147.19 (C-13), 131.74, 131.52, 126.92, 114.69 (ArC), 114.59 (C-12), 99.31 (C-7), 98.24 (C-2), 78.30 (C-5), 68.37 (C-4), 55.51 (Ar-

OMe), 53.23 (C-10), 35.19 (-C-^{t}Bu), 24.66 (-^{t}Bu); *m/z* (ESI-) 414.16 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{22}H_{24}NO_7$ ([M-H]$^-$) 414.1558, found 414.1560.

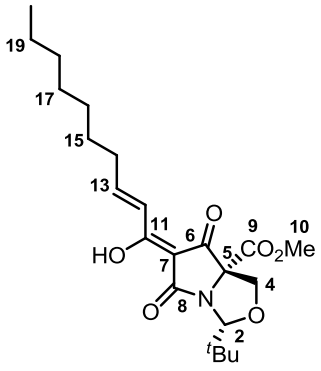
(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-3-(*E*)-(4'-chlorophenyl)allylidene]-3-oxabicyclo[3.3.0]-octane, 127c



Scale of reaction: 90 mg (0.303 mmol), product: 40 mg, 31%;

R_f = 0.49 (5% MeOH/EtOAc); $[\alpha]_D^{20}$ = +63.6 (*c*= 0.5 in $CHCl_3$); bright yellow oil; ν_{max} / cm^{-1} (film) 1755 (s, C=O), 1711 (s, C=O), 1655 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.92 (d, *J* = 16.0 Hz, 1H, H-13), 7.70 (d, *J* = 16.0 Hz, 1H, H-12), 7.59 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.41 (d, *J* = 8.5 Hz, 2H, Ar-H), 4.90 (s, 1H, H-2), 4.87 (d, *J* = 8.9 Hz, 1H, H-4), 3.81 (s, 3H, -CO_2Me), 3.55 (d, *J* = 8.9 Hz, 1H, H-4'), 0.96 (s, 9H, -^{t}Bu); δ_C (125 Mhz, $CDCl_3$) 188.01 (C-6), 180.38 (C-8), 176.30 (C-11), 167.56 (C-9), 145.31 (C-13), 137.93, 132.55, 130.36, 129.48 (ArC), 117.70 (C-12), 100.35 (C-7), 98.32 (C-2), 78.34 (C-5), 68.29 (C-4), 53.31 (C-10), 35.21 (-C-^{t}Bu), 24.64 (-^{t}Bu); *m/z* (ESI-) 418.21 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{21}H_{21}ClNO_6$ ([M-H]$^-$) 418.1062, found 418.1063.

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-3-(*E*)-heptylallylidene]-3-oxabicyclo[3.3.0]-octane, 127d

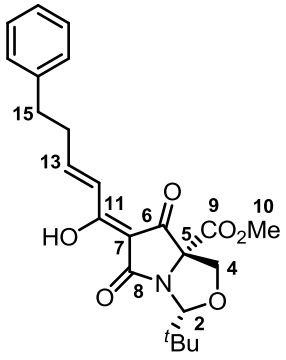


Scale of reaction: 130 mg (0.437 mmol), product: 32 mg, 18%;

R_f = 0.42 (EtOAc); $[\alpha]_D^{20}$ = +64.9 (*c*= 1.23 in $CHCl_3$); orange oil; ν_{max} / cm^{-1} (film) 1755 (s, C=O), 1714 (s, C=O), 1640 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.36 (d, *J* = 15.4 Hz, 1H, H-13), 7.07 (d, *J* = 15.4 Hz, 1H, H-12), 4.87 (s, 1H, H-2), 4.84 (d, *J* = 8.8 Hz, 1H, H-4), 3.79 (s, 3H, -CO_2Me), 3.51 (d, *J* = 8.8 Hz, 1H, H-4'), 2.36 (apparent quartet, *J* = 6.9 Hz, 2H, H-14), 1.49-1.53 (m, 2H, H-15), 1.25-1.36 (m, 8H, H-16, H-17, H-18, H-19), 0.94 (s, 9H, -^{t}Bu), 0.89 (t, *J* = 6.9 Hz, 3H, H-20); δ_C (125 MHz, $CDCl_3$) 187.85 (C-6), 180.77 (C-8), 177.08 (C-11), 167.63 (C-9), 154.33 (C-13), 120.92 (C-12), 101.54 (C-7), 98.25 (C-2), 78.25 (C-5), 68.28 (C-4), 53.24 (C-10), 35.18 (-C-^{t}Bu), 33.53 (C-14), 31.65, 29.21, 29.00 (C-16, C-17, C-18), 27.95 (C-15),

24.63 (-^{t}Bu), 22.57 (C-19), 14.04 (C-20); *m/z* (ESI-) 406.24 ([M-H]$^-$, 100%); HRMS (ESI+) calculated for $C_{22}H_{33}NNaO_6$ ([M+Na]$^+$) 430.2200, found 430.2196.

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-3-(*E*)-ethylphenylallylidene]-3-oxabicyclo[3.3.0]-octane, 127e



Scale of reaction: 130 mg (0.437 mmol), product: 20 mg, 11%;

R_f = 0.39 (EtOAc); $[\alpha]_D^{20}$ = +72.5 (*c*= 0.91 in $CHCl_3$); orange oil; ν_{max} / cm^{-1} (film) 1754 (s, C=O), 1711 (s, C=O), 1641 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.36 (d, *J* = 15.8 Hz, 1H, H-13), 7.28-7.33 (m, 2H, Ar-H), 7.19-7.24 (m, 3H, Ar-H), 7.12 (d, *J* = 15.8 Hz, 1H, H-12), 4.87 (s, 1H, H-2), 4.84 (d, *J* = 8.8 Hz, 1H, H-4), 3.80 (s, 3H, -CO_2<u>Me</u>), 3.51 (d, *J* = 8.8 Hz, 1H, H-4'), 2.83-2.86 (m, 2H, H-15), 2.67-2.71 (m, 2H, H-14), 0.94 (s, 9H, -^{t}Bu); δ_C (125 MHz, $CDCl_3$) 187.85 (C-6), 180.62 (C-8), 176.77 (C-11), 167.58 (C-9), 152.46 (C-13), 140.29, 128.59, 128.57, 128.29, 126.32 (ArC), 121.43 (C-12), 99.43 (C-7), 98.27 (C-2), 78.25 (C-5), 68.25 (C-4), 53.27 (C-10), 35.30 (-<u>C</u>-^{t}Bu), 35.08 (C-14), 34.17 (C-15), 24.63 (-^{t}Bu); *m/z* (ESI-) 412.20 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{23}H_{27}NNaO_6$ ([M+Na]$^+$) 436.1731, found 436.1727.

<u>Deuteration quenching study of **124a**</u>

A solution of **124a** (20 mg, 0.0673 mmol, 1.0 eq.) in anhydrous THF (3 mL) was added to 1.8 M LDA solution (94 μL, 2.5 eq.), at 0°C and the solution was stirred at 0°C. After 0.5 h, 1 h and 2 h respectively, 0.5 ml aliquots of the reaction mixture was quenched with D_2O. The organic layer was washed with sat. aq. NH_4Cl and 10% aq. HCl, and the aqueous layer extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude. LRMS (ESI-) analysis of the aliquots gave peaks at 296 and 297 *m/z*, corresponding to H-**124a** and D-**124a** respectively, which were used compare both species.

Use of *n*-BuLi to overcome the "secondary amine effect"

A 1.8 M solution of LDA (372 µL, 0.671 mmol, 2.1 eq.) was added to a solution of **124a** (95 mg, 0.320 mmol, 1.0 eq.) in dry THF (4 mL) at -15°C and stirred for 45 min. The solution was then cooled to -78°C. A 1.4 M hexane solution of ^{n}BuLi (479 µL, 0.671 mmol, 2.1 eq.) was then added dropwise and stirred at -78°C for 10 min. A solution of 4-chlorobenzaldehyde (224.1 mg, 1.598 mmol, 5 eq.) in dry THF (1 mL) was then added. The solution was allowed to warm to r.t. overnight and left to stir for 17 h. The reaction was quenched with sat. aq. NH_4Cl and extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude. Purification via flash column chromatography on silica gel (Eluent: 10% to 100% EtOAc/PE, then 5% MeOH/EtOAc) gave **127c** (51 mg, 38%) and 4-chlorobenzyl alcohol (92 mg, 40%).

Use of NaH to prevent reduction of 4-chlorobenzaldehyde

A solution of **124a** (85 mg, 0.286mmol, 1.0eq.) in dry THF (3.5 mL) was added to NaH (11.4 mg, 0.286 mmol, 1.0eq., 60% in mineral oil) at 0°C. The mixture was stirred at 0°C for 10 min and then cooled to -78°C. A 1.4 M solution of ^{n}BuLi (204 µL, 0.286 mmol, 1.0 eq.) was then added dropwise and stirred at -78°C for 10 min. A solution of 4-chlorobenzaldehyde (48.1 mg, 0.343 mmol, 1.2 eq.) in dry THF (1.5 mL) was then added. The solution was allowed to warm to r.t. overnight and left to stir for 20 h. The reaction was quenched with sat. aq. NH_4Cl and extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude. Purification via silica gel flash column chromatography (Eluent: 10% to 100%, EtOAc/PE) gave **127c** (44 mg, 37%).

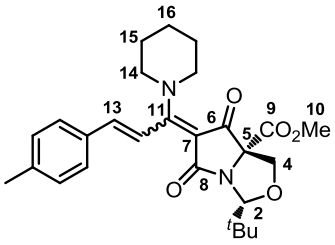
Elaboration via enamines

General procedure for synthesis of piperidyl enamines **129a-c**

A solution of **124a**, the corresponding aromatic aldehyde (0.9 eq.) and piperidine (1.5 eq.) in toluene was heated at reflux (120°C) overnight. The reaction mixture was then cooled to r.t., the solvents evaporated and the crude was purified via flash column chromatography on silica gel. The product

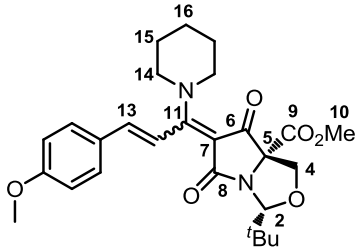
thus obtained was washed with a solution of sat. aq. NH_4Cl and 10% aq. HCl, dried with anhydrous $MgSO_4$, filtered and the solvents removed to afford the pure product.

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[1-piperidyl-3-(*E*)-(4'-methylphenyl)allylidene]-3-oxabicyclo[3.3.0]-octane, 129a



Scale of reaction: 110 mg (0.370 mmol), product: 95 mg, 55%; R_f = 0.31 (EtOAc); $[\alpha]_D^{20}$ = +321.2 (c=1.05 in $CHCl_3$); dark yellow oil; ν_{max} / cm^{-1} (film) 1745 (s, C=O), 1693 (s, C=O), 1634 (s, C=O); δ_H (500 MHz, Acetone-d_6) 7.58 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.34 (d, *J* = 15.8 Hz, 1H, H-13), 7.27 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.02 (d, *J* = 15.8 Hz, 1H, H-12), 4.79 (s, 1H, H-2), 4.69 (d, *J* = 8.2 Hz, 1H, H-4), 3.92 (broad unresolved signal, 1H, one of H-14), 3.74 (s, 6H, -CO_2Me and one of H-14 and H-14'; base of singlet is broad), 3.57 (d, *J* = 8.5 Hz, 1H, H-4'), 2.38 (s, 3H, Ar-Me), 1.96 (broad unresolved signal, 2H, one of H-15 and one of H-15'), 1.80 (broad unresolved signal, 4H, H-16, one of H-15 and one of H-15'), 0.89 (s, 9H, -^{t}Bu); δ_C (125 MHz, Acetone-d_6) 187.27 (C-6), 177.58 (C-8), 170.89 (C-9), 168.09 (C-11), 148.12 (C-13), 141.64, 133.62, 130.42, 129.41 (ArC), 119.73 (C-12), 98.64 (C-2), 95.91 (C-7), 78.29 (C-5), 69.44 (C-4), 56.41 (C-14 or C-14'), 52.81 (-CO_2Me), 52.66 (C-14 or C-14'), 35.97 (-C-^{t}Bu), 27.35 (C-15), 25.38 (-^{t}Bu), 24.22 (C-16), 21.46 (Ar-Me); *m/z* (ESI+) 467.28 ($[M+H]^+$, 100%); HRMS (ESI+) calculated for $C_{27}H_{34}N_2NaO_5$ 489.2360, found 489.2367.

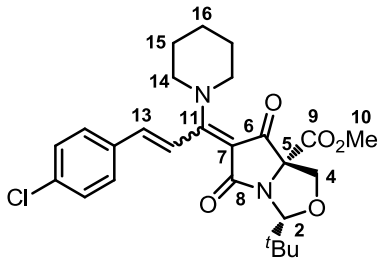
(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[1-piperidyl-3-(*E*)-(4'-methoxyphenyl)allylidene]-3-oxabicyclo[3.3.0]-octane, 129b



Scale of reaction: 80 mg (0.269 mmol), product: 75 mg, 58%; R_f = 0.32 (EtOAc); $[\alpha]_D^{20}$ = +400.9 (c= 0.96 in $CHCl_3$); orange oil; ν_{max} / cm^{-1} (film) 1745 (s, C=O), 1693 (s, C=O), 1634 (s, C=O); δ_H (400 MHz, Acetone-d_6) 7.64 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.34 (d, *J* = 15.7 Hz, 1H, H-13), 6.98 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.93 (d, *J* = 15.7 Hz, 1H, H-12), 4.78 (s, 1H, H-2), 4.69 (d, *J* = 8.6 Hz, 1H, H-4), 3.85 (s, 3H, Ar-OMe), 3.79 (s, 3H, -

CO_2Me), 3.78-3.80 (broad unresolved signals, 2H, H), 3.57 (d, *J* = 8.6Hz, 1H, H-4'), 1.93 (broad unresolved signals, 2H), 1.78 (broad unresolved signals, 2H), 0.88 (s, 9H, -^{t}Bu); δ_C (125 MHz, Acetone-d_6) 187.26 (C-6), 177.73 (C-8), 170.95 (C-9), 168.28 (C-11), 162.74 (ArC), 148.47 (C-13), 131.26, 128.96 (ArC), 117.95 (C-12), 115.22 (ArC), 98.61 (C-2), 95.57 (C-7), 78.28 (C-5), 69.44 (C-4), 55.84 (Ar-O<u>Me</u>), 55.63 (C-14 or C-14'), 52.79 (-CO_2<u>Me</u>), 52.60 (C-14 or C-14'), 35.97 (-<u>C</u>-^{t}Bu), 27.35 (C-15), 25.36 (-^{t}Bu), 24.25 (C-16); HRMS (FI+) calculated for $C_{27}H_{34}N_2O_6$ 482.2417, found 482.2437.

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[1-piperidyl-3-(*E*)-(4'-chlorophenyl)allylidene]-3-oxabicyclo[3.3.0]-octane, 129c

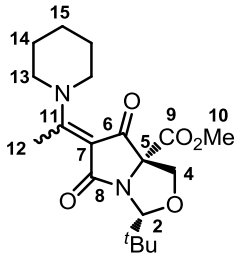


Scale of reaction: 85 mg (0.286 mmol), product: 71 mg, 51%; R_f = 0.30 (EtOAc); $[\alpha]_D^{20}$ = +344.2 (c= 0.28 in $CHCl_3$); orange oil; ν_{max} / cm^{-1} (film) 1747 (s, C=O), 1693 (s, C=O), 1637 (s, C=O); δ_H (400 MHz, $CDCl_3$) 7.46 (d, *J* = 8.6Hz, 2H, Ar-H), 7.36 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.22 (d, *J* = 15.9 Hz, 1H, H-13), 6.74 (d, *J* = 15.9 Hz, 1H, H-12), 4.87 (s, 1H, H-2), 4.84 (d, *J* = 8.6 Hz, 1H, H-4), 3.79 (s, 3H, -CO_2Me), 3.78-3.80 (broad unresolved signals, 2H, H-14 or H-14'), 3.64 (broad unresolved signals, 2H, H-14 or H-14'), 3.54 (d, *J* = 8.6 Hz, 1H, H-4'), 1.80 (broad unresolved signals, 6H, H-15, H-15', H-16), 0.91 (s, 9H, -^{t}Bu); δ_C (100 MHz, $CDCl_3$) 187.58 (C-6), 177.06 (C-8), 169.63 (C-9), 166.57 (C-11), 146.03 (C-13), 136.63, 133.11, 129.52, 129.22 (Ar<u>C</u>), 119.78 (C-12), 98.16 (C-2), 96.39 (C-7), 77.57 (C-5), 68.91 (C-4), 56.10 (C-14 or C-14'), 52.89 (-CO_2<u>Me</u>), 52.15 (C-14 or C-14'), 35.34 (-<u>C</u>-^{t}Bu), 26.61 (C-15), 24.86 (-^{t}Bu), 23.46 (C-16); *m/z* (ESI+) 487.23 ($[M+H]^+$, 100%); HRMS (FI+) calculated for $C_{26}H_{31}ClN_2O_5$ 486.1921, found 486.2147.

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[1-piperidyl-1'-methyl-vinylidene]-3-oxabicyclo[3.3.0]-octane, 130

A solution of **124a** (140 mg, 0.471 mmol, 1.0 eq.) and piperidine (68.9 μL, 0.706 mmol, 1.5 eq.) in toluene was heated at reflux (120°C) overnight. The reaction mixture was then cooled to r.t., the

solvents evaporated and the crude was purified via flash column chromatography (Eluent: EtOAC/PE 10% to 70%) on silica gel to afford **130** (140 mg, 82%).



R_f = 0.19 (EtOAc); $[\alpha]_D^{20}$ = +272.7 (c= 0.91 in $CHCl_3$); orange oil; ν_{max} / cm^{-1} (film) 1745 (s, C=O), 1693 (s, C=O), 1634 (s, C=O); δ_H (500 MHz, $CDCl_3$) 4.84 (s, 1H, H-2), 4.79 (d, *J* = 8.5 Hz, 1H, H-4), 3.79 (s, 3H, -CO_2Me), 3.69-3.73 (m, 2H, one of H-13 and one of H-13'), 3.62-3.67 (m, 1H, one of H-13), 3.52-3.57 (m, 1H, one of H-13'), 3.45 (d, *J* = 8.6 Hz, 1H, H-4'), 2.59 (s, 3H, H-12), 1.90-1.94 (m, 1H, one of H-14), 1.68-1.83 (m, 5H, one of H-14, H-14' and H-15), 0.92 (s, 9H, -^{t}Bu); δ_C (125 MHz, $CDCl_3$) 186.89 (C-6), 177.42 (C-8), 170.38 (C-11), 169.58 (C-9), 98.18 (C-2), 97.11 (C-7), 77.12 (C-5; in the middle of $CDCl_3$ signals but correlation observed in HMBC), 68.85 (C-4), 56.43 (C-13), 52.82 (-CO_2Me), 50.48 (C-13'), 35.28 (-C-^{t}Bu), 26.77 (C-14), 26.23 (C-14'), 24.82 (-^{t}Bu), 23.40 (C-15), 18.85 (C-12); *m/z* (ESI+) 365.23 ($[M+H]^+$, 100%); HRMS (ESI+) calculated for $C_{19}H_{28}N_2NaO_5$ ($[M+Na]^+$) 387.1890, found 387.1883.

Procedure for hydrolysis of piperidine enamines **129a-c** to give 3-enoyl tetramic acids **127a-c**

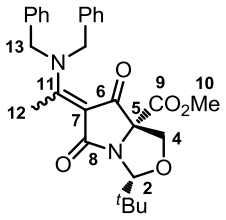
Solid LiOH (1.0 eq.) was added to a solution of **129a-c** (1.0 eq., 50 mg scale) in THF/H_2O (1:1) (approx. concentration 0.02-0.05 M) and the deep yellow solution was stirred at r.t for 1.0-1.5 h. (The solution begins as a deep yellow solution and becomes pale yellow in colour when the reaction is completed). The solution was then acidified with 10% aq. HCl and saturated with aq. sat. NH_4Cl, followed by extraction with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents evaporated to give the desired 3-enoyl tetramic acids **127a-c** with sufficiently good purity as determined by ^{1}H NMR. (See above for characterisation of **127a-c**).

Synthesis of *N, N'*-dibenzyl enamines **131** and **132**

A solution of **124a** (90 mg, 0.303 mmol, 1.0 eq.), *p*-anisaldehyde (36.6 μL, 0.303 mmol, 1.0 eq.), and dibenzylamine (87.3 μL, 0.454 mmol, 1.5 mmol) in toluene (5 mL) was heated to 120°C for 22 h. The solution was cooled to r.t., diluted with EtOAc and washed with aq. 5% HCl. The organic layer was then dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude.

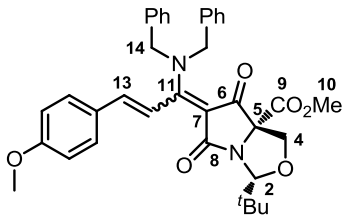
Purification via flash column chromatography on silica gel (Eluent: EtOAc/PE, 5% to 35%) gave **131** (25.5 mg, 18%) and **132** (32 mg, 18%).

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[1-(*N*,*N*'-dibenzyl)-1'-methyl-vinylidene]-3-oxabicyclo[3.3.0]-octane, 131



R_f = 0.45 (1:1 PE:EtOAc); bright yellow oil; $[\alpha]_D^{20}$ = +348.7 (*c*= 1.28 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1746 (s, C=O), 1695 (s, C=O), 1639 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.30-7.39 (m, 6H, ArH), 7.12-7.18 (m, 4H, ArH), 5.15 (d, *J* = 14.8 Hz, 1H, H-13), 4.93 (s, 1H, H-2), 4.82 (d, *J* = 8.4 Hz, 1H, H-4), 4.77 (d, *J* = 16.1 Hz, 1H, H-13a), 4.73 (d, *J* = 14.8 Hz, 1H, H-13'), 4.50 (d, *J* = 16.4 Hz, 1H, H-13a'), 3.77 (s, 3H, -CO_2Me), 3.47 (d, *J* = 8.8 Hz, 1H, H-4'), 2.68 (s, 3H, H-12), 0.94 (s, 9H, -^{t}Bu); δ_C (125 MHz, $CDCl_3$) 187.76 (C-6), 176.39 (C-8), 173.39 (C-11), 169.37 (C-9), 134.38, 133.47, 129.33, 129.10, 128.69, 128.61, 128.22 126.39 (ArC), 98.81 (C-7), 98.08 (C-2), 77.06 (C-5, obscured by $CDCl_3$ signals but correlation seen on HSQC), 68.85 (C-4), 61.00 (C-13), 53.71 (C-13a), 52.89 (C-10), 35.34 (-<u>C</u>-^{t}Bu), 24.84 (-^{t}Bu), 19.77 (C-12); *m/z* (ESI+) 499.23 ($[M+Na]^+$, 100%); HRMS (ESI+) calculated for $C_{28}H_{33}N_2O_5$ ($[M+H]^+$) 477.2384, found 477.2380.

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[1-(*N*,*N*'-dibenzyl)-3-(*E*)-(4'-methoxyphenyl)allylidene]-3-oxabicyclo[3.3.0]-octane, 132



R_f = 0.36 (1:1 PE:EtOAc); bright yellow oil; $[\alpha]_D^{20}$ = +312.7 (*c*= 1.07 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1746 (s, C=O), 1695 (s, C=O), 1638 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.47 (d, *J* = 8.8 Hz, 2H, ArH), 7.31-7.36 (m, 7H, ArH and H-13), 7.15 (d, *J* = 6.9 Hz, 4H, ArH), 6.89 (d, *J* = 8.8 Hz, 2H, ArH), 6.84 (d, *J* = 15.4 Hz, 1H, H-12), 4.96 (s, 1H, H-2), 4.93 (d, *J* = 14.8 Hz, 2H, H-14), 4.86 (d, *J* = 8.5 Hz, 1H, H-4), 4.58-5.60 (broad signal, 2H, H-14'), 3.84 (s, 3H, Ar-O<u>Me</u>), 3.78 (s, 3H, -CO_2Me), 3.56 (d, *J* = 8.5 Hz, 1H, H-4'), 0.94 (s, 9H, -^{t}Bu); δ_C (125 MHz, $CDCl_3$) 187.86 (C-6), 176.82 (C-8), 169.66 (C-9), 162.10 (Ar<u>C</u>-OMe), 149.44 (C-13), 134.12 (broad), 130.52, 128.97, 128.39 (broad), 127.41 (ArC), 116.92 (C-12), 114.42 (ArC), 98.05 (C-2), 97.14 (C-7), 77.62 (C-5),

69.01 (C-4), 55.43 (Ar-O<u>Me</u>), 52.86 (-CO_2<u>Me</u>), 35.40 (-<u>C</u>-^{t}Bu), 24.88 (-^{t}Bu); *m/z* (ESI+) 617.29 ($[M+Na]^+$, 100%), 595.32 ($[M+H]^+$, 65%); HRMS (ESI+) calculated for $C_{36}H_{39}N_2O_6$ ($[M+H]^+$) 595.2803, found 595.2797.

In a separate experiment, **131** (26 mg, 0.055 mmol, 1.0 eq.), *p*-anisaldehyde (6.6 μL, 0.055 mmol, 1.0 eq.), dibenzylamine (5.2 μL, 0.027 mmol, 0.5 eq.) was heated in toluene (4 mL) at 120°C for 42 h. The solution was cooled to r.t., diluted with EtOAc and washed with 5% aq. HCl. The organic layer was then dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude. ^{1}H NMR shows **131**: **132** = 1:2.5, based on H-4 signals of the product.

<u>Debenzylation of **132** and basic hydrolysis of **133**</u>

Debenzylation:

To a solution of **132** (30 mg, 0.0504 mmol, 1.0 eq.) in MeOH (5 mL), was added 10% Pd/C (10% wt. of **132**) and ammonium formate (31.8 mg, 0.504 mmol, 10.0 eq.). The reaction mixture was stirred at r.t. for 19 h. As there is no conversion based on TLC analysis, the reaction mixture was then heated at 60°C for another 28 h, cooled to r.t. and filtered over Celite. The filtrate was evaporated and CH_2Cl_2 was added to the residue, followed by filtration to remove the precipitated solids. The filtrate was evaporated to give the crude. Purification via flash column chromatography on silica gel (Eluent: EtOAc/petroleum ether, 20% to 100%, then 5% MeOH/EtOAc) gave 15 mg of the presumed -NH_2 enamine **133**. Analysis via low resolution mass spectrometry gave the desired mass peak.

Basic hydrolysis:

LiOH (1 mg) was added to the presumed enamine **133** (15 mg, 0.036 mmol) in THF/H_2O (1:1) (3 mL). The reaction mixture was stirred at r.t. for 2 h. The reaction mixture was then acidified with 10% aq. HCl, saturated with brine and extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude which was then purified via silica gel flash column chromatography (Eluent: EtOAc/petroleum ether, 20% to 100%, then 5% MeOH/EtOAc) to give the product **134** (<5 mg). Analysis via low resolution mass

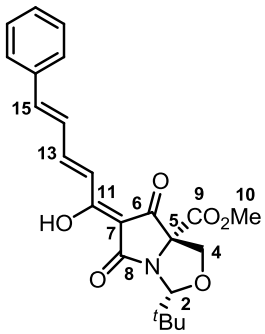
spectrometry gave the desired mass peak; ^{1}H NMR suggests enamine hydrolysed due to absence of resonance signals ~10 ppm.

Elaboration via Horner-Wadsworth-Emmons olefination

General procedure for Horner-Wadsworth-Emmons olefination towards **127a-h**

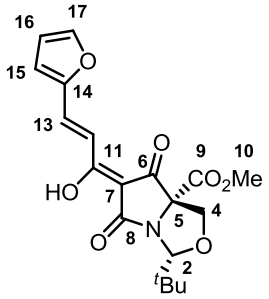
Phosphorane **126** (1.0 eq.; approximately 0.09 mmol scale) in dry THF (1.5 mL), was added to solid ^{t}BuOK (2.1 eq.) at 0°C and the suspension was stirred at 0°C for 30 min. The corresponding aldehyde (1.1 eq.) in dry THF (1.5 mL) was then added. The reaction mixture was stirred at 0°C for 10 min, quenched with aq. sat. NH_4Cl, acidified with aq. 10% HCl and extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude. Purification via flash column chromatography on silica gel (Eluent: EtOAc/PE, then 5% MeOH/EtOAc) gave the product, which was then washed with sat. aq. NH_4Cl and 10% aq. HCl. (See above for characterisation of **127a-e**.)

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-7-[(*Z*)-1-hydroxy-1'-(*E*,*E*-phenyldienyl)vinylidene]- 6,8-dioxo-3-oxabicyclo[3.3.0]-octane, 127f



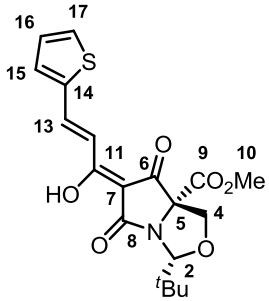
R_f = 0.31 (EtOAc); $[\alpha]_D^{20}$ = +51.8 (*c*= 0.88 in $CHCl_3$); orange oil; ν_{max} / cm^{-1} (film) 1752 (s, C=O), 1705 (s, C=O), 1651 (s, C=O); δ_H (500 MHz, $CDCl_3$) 7.78 (dd, *J* = 15.1 Hz, 10.1 Hz, 1H, H-13), 7.51-7.57 (m, 2H, ArH), 7.36-7.48 (m, 3H, ArH), 7.24 (d, *J* = 15.1 Hz, 1H, H-12), 6.95-7.03 (m, 2H, H-14 and H-15), 4.89 (s, 1H, H-2), 4.86 (d, *J* = 8.8 Hz, 1H, H-4), 3.81 (s, 3H, -CO_2Me), 3.54 (d, *J* = 8.8 Hz, 1H, H-4'), 0.96 (s, 9H, -^{t}Bu); δ_C (125 MHz, $CDCl_3$) 186.64 (C-6), 179.68 (C-8), 175.45 (C-11), 166.74 (C-9), 146.41 (C-13), 143.86 (C-15), 129.10, 128.00, 127.09, 126.82 (ArC), 125.91 (C-14), 119.66 (C-12), 98.72 (C-7), 97.25 (C-2), 77.28 (C-5), 67.34 (C-4), 52.25 (C-10), 34.20 (-C- ^{t}Bu), 23.66 (-^{t}Bu); *m/z* (ESI-) 410.17 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{23}H_{24}NO_6$ ([M-H]$^-$) 410.1609, found 410.1603.

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-7-[(*Z*)-1-hydroxy-3-(*E*)-(2'-furan)allylidene]-6-8-dioxo-3-oxabicyclo[3.3.0]-octane, 127g



R_f = 0.32 (EtOAc); dark yellow oil; $[\alpha]_D^{20}$ = +102.9 (*c*= 0.50 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1752 (s, C=O), 1704 (s, C=O), 1651 (s, C=O), 1614, 1589, 1537, 1483, 1387, 1368 (s, furan C=C); δ_H (500 MHz, $CDCl_3$) 7.72 (d, *J* = 15.4 Hz, 1H, H-13), 7.61 (d, *J* = 1.6 Hz, 1H, H-17), 7.54 (d, *J* = 15.4 Hz, 1H, H-12), 6.86 (d, *J* = 3.5 Hz, 1H, H-15), 6.56 (dd, *J* = 1.6, 3.5 Hz, 1H, H-16), 4.89 (s, 1H, H-2), 4.86 (d, *J* = 9.1 Hz, 1H, H-4), 3.80 (s, 3H, -CO_2Me), 3.54 (d, *J* = 9.1 Hz, 1H, H-4'), 0.95 (s, 9H, -^{t}Bu); δ_C (125 MHz, $CDCl_3$) 187.71 (C-6), 180.62 (C-8), 176.26 (C-11), 167.71 (C-9), 151.28 (C-14), 147.06 (C-17), 132.11 (C-13), 118.81 (C-15), 114.81 (C-12), 113.38 (C-16), 99.84 (C-7), 98.24 (C-2), 78.28 (C-5), 68.33 (C-4), 53.24 (C-10), 35.20 (-C-^{t}Bu), 24.65 (-^{t}Bu); *m/z* (ESI+) 398.13 ($[M+Na]^+$, 63%), 374.13 ($[M-H]^-$, 47%); HRMS (ESI+) calculated for $C_{19}H_{21}NNaO_7$ ($[M+Na]^+$) 398.1210, found 398.1215.

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-7-[(*Z*)-1-hydroxy-3-(*E*)-(2'-thiophene)allylidene]-6,8-dioxo-3-oxabicyclo[3.3.0]-octane, 127h



R_f = 0.39 (EtOAc); dark yellow oil; $[\alpha]_D^{20}$ = +148.2 (*c*= 0.48 in $CHCl_3$); ν_{max} / cm^{-1} (film) 1752 (s, C=O), 1705 (s, C=O), 1651 (s, C=O), 1604, 1570, 1503, 1482, 1383, 1355 (s, thiophene C=C); δ_H (500 MHz, $CDCl_3$) 8.09 (d, *J* = 15.8 Hz, 1H, H-13), 7.57 (d, *J* = 5.0 Hz, 1H, H-17), 7.48 (d, *J* = 15.4 Hz, 1H, H-12), 7.45 (d, *J* = 3.5 Hz, 1H, H-15), 7.13 (dd, *J* = 3.8, 5.0 Hz, 1H, H-16), 4.89 (s, 1H, H-2), 4.86 (d, *J* = 8.8 Hz, 1H, H-4), 3.81 (s, 3H, -CO_2Me), 3.54 (d, *J* = 8.8 Hz, 1H, H-4'), 0.95 (s, 9H, -^{t}Bu); δ_C (125 MHz, $CDCl_3$) 187.85 (C-6), 180.64 (C-8), 176.24 (C-11), 167.68 (C-9), 139.98 (C-14), 139.32 (C-13), 133.70 (C-15), 132.11 (C-17), 128.82 (C-16), 115.82 (C-12), 99.64 (C-7), 98.27 (C-2), 78.31 (C-5), 68.34 (C-4), 53.27 (C-10), 35.19(-C-^{t}Bu), 24.65 (-^{t}Bu); *m/z* (ESI-) 390.11 ($[M-H]^-$, 55%); HRMS (ESI-) calculated for $C_{19}H_{20}NO_6S$ ($[M-H]^-$) 390.1017, found 390.1025.

Synthesis towards pramanicin mimics

Hydrolysis of *tert*-butyl oxazolidine

Condition A:

1,3-propanedithiol (9.5 μL, 0.0953 mmol, 1.0 eq.) was added to a solution of **127c** in 2%(w/v) trifluoroethanol (5 mL). The reaction mixture was stirred at r.t. overnight and the solvent was evaporated to give the crude as an orange oil. Purification via silica gel flash column chromatography (Eluent: EtOAc to 5% MeOH/EtOAc) to gave a low and irreproducible yield of **135c**.

Condition B:

127a (55 mg, 0.138 mmol, 1.0 eq.) in a 3:1:1 mixture of THF/H_2O/formic acid (2.5 mL) was stirred at r.t. for 23 h. The reaction mixture was then diluted with sat. aq. $NaHCO_3$ portionwise till pH 7 and extracted with EtOAc. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to gave the unreacted starting material quantitatively.

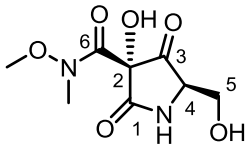
Condition C:

$BiBr_3$ (6.4 mg, 0.0143, 0.15 eq.) was added to a solution of **127c** (40 mg, 0.0953mmol, 1.0 eq.) in MeCN (2 mL), followed by 10 μL of deionised water was added. The reaction mixture was stirred at r.t for 24 h (pale yellow precipitate in pale yellow solution) by washing with 10% aq. HCl and extracted with EtOAc. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to gave the unreacted starting material quantitatively.

Successful oxazolidine hydrolysis of tetramic acid **96**

(2*R*, 4*R*)-2-Hydroxy-2-(*N*,*O*-dimethylcarbamoyl)pyrrolidine-1,3-dione, 139

TFA (98 μL, 1.303 mmol, 20 eq.) was added to a solution of **96** (21 mg, 0.0652 mmol, 1.0 eq.) in CH_2Cl_2 (2.5 mL) at r.t.. The reaction mixture was stirred at r.t. for 4.5 h, afterwhich the solvents were evaporated to give the crude. Purification via silica gel flash column chromatography (Eluent: 50% to 100% EtOAc/PE, then 10% MeOH/EtOAc) gave tetramic acid **139** as a white solid (10.5 mg, 69%).



R_f = 0.14 (EtOAc); white solid, m.pt. = 129-131°C; $[\alpha]_D^{23}$= +38.6 (*c*= 1.24 in MeOH); ν_{max} / cm^{-1} (film) 3350 (br, -OH), 1780 (s, C=O), 1687 (s, C=O), 1666 (s, C=O); δ_H (400 MHz, MeCN-d_3) 7.46 (br s, 1H, -NH-), 5.20 (br s, 1H, -OH C-2), 4.34 (dd, *J* = 4.1, 7.7 Hz, 1H, H-4), 3.87 (dd, *J* = 4.1, 11.5 Hz, 1H, H-5), 3.61 (s, 3H, -O<u>Me</u>), 3.62-3.58 (m, 1H, H-5'; obscured by -O<u>M</u>e singlet), 3.21 (s, 3H, -N<u>Me</u>); δ_C (100 MHz, MeCN-d_3) 202.93 (C-3), 169.93 (C-1), 167.08 (C-6), 75.77 (C-2), 64.69 (C-4), 61.97 (C-5), 61.47 (-O<u>Me</u>), 33.65 (-N<u>Me</u>); *m/z* (ESI+) 255.17 ([M+Na]$^+$, 90%); HRMS (ESI+) calculated for $C_8H_{12}N_2NaO_6$ ([M+Na]$^+$) 255.0588, found 255.0592.

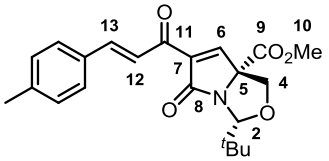
<u>Attempted reduction of 3-enoyltetramic acid **127**</u>

Condition A:

A CH_2Cl_2/AcOH solution (2.5 mL:0.4 mL) was cooled to -15°C. $NaBH_4$ (10.6 mg, 0.280 mmol, 4.0 eq.) was added followed by **127a** (28 mg, 0.070 mmol, 1.0 eq.) in 1 mL of CH_2Cl_2 dropwise. The reaction mixture was allowed to warm to r.t. from -15°C over 3 h and was quenched by addition of water. Brine and aq. sat. $NaHCO_3$ was added followed by extraction with EtOAc. The combined organic layers were dried with $MgSO_4$, filtered and evaporated to give the crude. Purification via silica gel flash column chromatography (Eluent: EtOAc/PE, 10% to 100%, then 10% MeOH/EtOAc) gave dienone **150a**, with other complex mixture of unidentified by-products.

Following the same procedure with **127c**, **150b** (5 mg, 17%) was obtained.

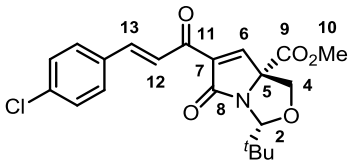
(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-7-[4'-methoxyphenyl-(*E*)-vinylenecarbonyl]-8-oxo-3-oxabicyclo[3.3.0]-oct-6-ene, 150a



R_f = 0.57 (PE:EtOAc 2:1); $[\alpha]_D^{20}$= +70.8 (c= 0.24 in $CHCl_3$); yellow oil; ν_{max} / cm^{-1} (film) 1746 (m, C=O), 1716 (s, C=O), 1665 (m, C=O); δ_H (500 MHz, $CDCl_3$) 7.84 (d, *J* = 12.0 Hz, 2H, H-12 and H-13), 7.83 (s, 1H, H-6), 7.58 (d, *J* = 7.9 Hz, 2H, ArH), 7.21 (d, *J* = 7.9 Hz, 2H, ArH), 4.86 (d, *J* = 8.5 Hz, 1H, H-4), 4.81 (s, 1H, H-2), 3.79 (s, 3H, -CO_2<u>Me</u>), 3.40 (d, *J* = 8.8 Hz, 1H, H-4'), 2.40 (s, 3H, Ar-<u>Me</u>), 1.00 (s, 9H, -^{t}Bu); δ_C (125 MHz, $CDCl_3$) 183.60 (C-11), 174.14 (C-8), 168.12 (C-9), 151.67 (C-6), 146.03 (C-

13), 141.89 (ArC), 139.06 (C-7), 131.77, 129.68, 129.14 (ArC), 121.54 (C-12), 97.44 (C-2), 75.18 (C-5), 70.53 (C-4), 53.42 (C-10), 35.37 (-<u>C</u>- ^{t}Bu), 24.84 (- ^{t}Bu), 21.60 (Ar-<u>Me</u>); *m/z* (ESI+) 384.0 ($[M+H]^+$, 60%); HRMS (ESI+) calculated for $C_{22}H_{25}NNaO_5$ ($[M+Na]^+$) 406.1625, found 406.1618.

(2*R*, 5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-7-[4'-chlorophenyl-(*E*)-vinylenecarbonyl]-8-oxo-3-oxabicyclo[3.3.0]-oct-6-ene, 150b



R_f = 0.58 (PE:EtOAc 2:1); $[\alpha]_D^{20}$= +107.3 (c= 0.22 in $CHCl_3$); yellow oil; ν_{max} / cm^{-1} (film) 1715 (s, C=O; but with shoulder), 1667 (m, C=O); δ_H (500 MHz, $CDCl_3$) 7.87 (s, 1H, H-6), 7.85 (d, *J* = 15.1 Hz, 1H, H-12), 7.78 (d, *J* = 15.1Hz, 1H, H-13), 7.61 (d, *J* = 8.5 Hz, 2H, ArH), 7.38 (d, *J* = 8.5 Hz, 2H, ArH), 4.86 (d, *J* = 8.5 Hz, 1H, H-4), 4.81 (s, 1H, H-2), 3.80 (s, 3H, -CO_2<u>Me</u>), 3.40 (d, *J* = 8.5 Hz, 1H, H-4'), 1.00 (s, 9H, - ^{t}Bu); δ_C (125 MHz, $CDCl_3$) 183.37 (C-11), 174.06 (C-8), 167.98 (C-9), 152.15 (C-6), 144.21 (C-13), 138.78 (C-7), 137.10, 132.96, 130.17, 129.23 (ArC), 122.90 (C-12), 97.48 (C-2), 75.24 (C-5), 70.51 (C-4), 53.46 (C-10), 35.37 (-<u>C</u>- ^{t}Bu), 24.82 (- ^{t}Bu); *m/z* (ESI+) 404.12 ($[M+H]^+$, 71%); HRMS (ESI+) calculated for $C_{21}H_{22}ClNNaO_5$ ($[M+Na]^+$) 426.1079, found 426.1062.

Condition B:

A mixture of $NaBH(OAc)_3$ (58.4 mg, 0.275 mmol, 2.2 eq.) in CH_2Cl_2 (1 mL) was cooled to 0°C. **127a** (50 mg, 0.125 mmol, 1.0 eq.) in CH_2Cl_2 (2.5 ml) was then added dropwise. The reaction mixture was then stirred at r.t. for 24 h. The solvent was removed under reduced pressure and partitioned between EtOAc and aq. sat. $NaHCO_3$. The aqueous layer was extracted with EtOAc and the combined organic layers were dried with $MgSO_4$, filtered and evaporated to give the crude. Purification via silica gel flash column chromatography (Eluent: EtOAc/PE, 10% to 100%, then 10% MeOH/EtOAc) gave dienone **150a** (4 mg, 8%) with other complex mixture of unidentified by-products.

Condition C:

$CeCl_3 \cdot 7H_2O$ (54.8 mg, 0.147 mmol, 2.0 eq.) was added to a solution of **127d** (30 mg, 0.0736 mmol, 1.0 eq.) in MeOH (3 mL) at r.t.. The mixture was stirred at r.t. until all the salt has dissolved. The solution

was then cooled to -40°C, and $NaBH_4$ (3.9 mg, 0.103 mmol, 1.4 eq.) was added and stirred at -40°C for 30 min. The reaction mixture was allowed to warm to r.t. over 4 h. The solvent was then evaporated and the residue partitioned between water and EtOAc. The aqueous layer was acidified with 10% aq. HCl and extracted with EtOAc. The combined organic layers were dried with $MgSO_4$, filtered and evaporated to give unreacted **127d** (27 mg).

Condition D:

127d (20 mg, 0.0491 mmol, 1.0 eq.) was dissolved in 0.5 M methanolic NaOH solution (1 mL). This solution was then added to $NaBH_4$ (3.7 mg, 0.0982 mmol, 2.0 eq.) in 0.5 M methanolic NaOH solution (1 mL). The reaction mixture is then stirred at r.t. for 3 h. The reaction was quenched with acidified with 10% aq. HCl (efferverscence observed), and partitioned between water and EtOAc. The combined organic layers were dried with $MgSO_4$, filtered and evaporated to give the crude. ^{1}H NMR spectrum of the crude mixture is complicated, with broad signals.

Attempted α-hydroxylation of **117a** with peroxy reagents

Condition A:

70% *m*-CPBA (58.5 mg, 0.237 mmol, 1.5 eq.) was added to a solution of **124a** (47 mg, 0.158 mmol, 1.0 eq.) in CH_2Cl_2 (3 mL) and stirred at r.t. for 2.5 h. The reaction was then quenched with sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layers were dried with $MgSO_4$, filtered and evaporated to give the crude. ^{1}H NMR spectrum of the crude mixture is complicated, with broad signals.

Condition B:

124a (50 mg, 0.168 mmol, 1.0eq.) was dissolved in CH_2Cl_2 (3 mL), 35% H_2O_2 (44 µL, 0.504 mmol, 3.0 eq.) and LiOH (12.1 mg, 0.504 mmol, 3.0 eq.) were added and the biphasic mixture was stirred vigorously at r.t. for 2.5 h. The reaction mixture was acidified with 10% aq. HCl and extracted with EtOAc. The combined organic layers were dried with $MgSO_4$, filtered and evaporated to give the

crude. ^{1}H NMR spectrum of the crude mixture is complicated, with broad signals. LRMS analysis of the crude also shows no mass peak corresponding to the desired product.

Attempted α-hydroxylation of enamine **123** with peroxy reagents

Et_3N (6 μL, 0.0412 mmol, 1.0 eq.) was added to a mixture of piperidyl enamine **130** (15 mg, 0.0412 mmol, 1.0 eq.) in CH_2Cl_2 (3 mL), and 35% (wt. in H_2O) H_2O_2 (22 μL, 0.247 mmol, 6.0 eq.) at r.t.. The biphasic mixture was then stirred vigorously at r.t. for 4 h. The mixture was acidified with 10% aq. HCl and extracted with CH_2Cl_2. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude. ^{1}H NMR spectrum of the crude shows only **124a**.

Attempted α-hydroxylation of **117a** with Ce(III)

$CeCl_3.7H_2O$ (3.4 mg, 0.0092 mmol, 0.05 eq.) was added to a solution of **124a** (55 mg, 0.185 mmol, 1.0 eq.) in ^{i}PrOH (2 mL). The reaction mixture was first bubbled through with O_2 (balloon) to saturate the solvent, and left to stir with O_2 balloon attached. The reaction was stirred at r.t. for 27 h, and filtered over a pad of silica gel followed by washing with EtOAc. The solvent was evaporated to give the crude which was purified on via silica gel flash column chromatography (Eluent: EtOAc then 5%MeOH/EtOAc) to return **124a** (30 mg), and an isolated spot which gave a complex crude NMR spectrum and could not be identified.

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APPENDIX A: HOLE-PLATE ANTIBACTERIAL ASSAY

Agar plates for the bioassay were prepared by Wendy Sobey, and the testing for some of the compounds were carried out by Tharindi Panduwawala. Samples were tested on agar plates inoculated with *E. coli* X580 and *S. aureus* DS267 respectively. A 70% DMSO/H_2O solution of each compound to be tested was prepared in concentrations of 4, 2, 1, 0.5 mg/mL via serial dilution of the most concentrated sample. A 100 µL aliquot of each solution was loaded into a 10 mm well, and the plates were covered and incubated at 37°C between 16 and 20 h. The diameters of the resultant inhibition zones were then measured (±0.5 mm). A set of calibration data, using Cephalosporin C (CephC) as a standard, was also collected simultaneously with each run of bioassays.

A.1 Calibration data for hole-plate antibacterial assay

An example of a calibration data set is illustrated below with the respective calibration curves.

Table A.1: An example of calibration data for one of the bioassays.

Conc. of stock soln. (µg/mL)	**Vol. of CephC stock (µL/well)**	**Vol. of water (µL/well)**	**Mass of CephC (µg/well)**	**Moles of CephC (nmol/well)**	**Zone size (mm)**	**Log (nmol CephC)**
S. aureus						
1000	20	80	20	48.14	Not active	1.683
	40	60	40	96.29	13.5	1.984
	60	40	60	144.44	16.0	2.160
	80	20	80	192.59	19.0	2.285
	100	0	100	240.73	21.0	2.382
E. coli						
10	20	80	0.2	0.4815	Not active	-0.3174
	40	60	0.4	0.9629	19	-0.0164
	60	40	0.6	1.4444	22	0.1597
	80	20	0.8	1.9259	25	0.2846
	100	0	1	2.4073	30	0.3815
100	20	80	2	4.8146	31	0.6826
	40	60	4	9.6293	36	0.9836
	60	40	6	12.4439	38	1.1597
	80	20	8	19.2585	42	1.2846
	100	0	10	24.0732	44	1.3815

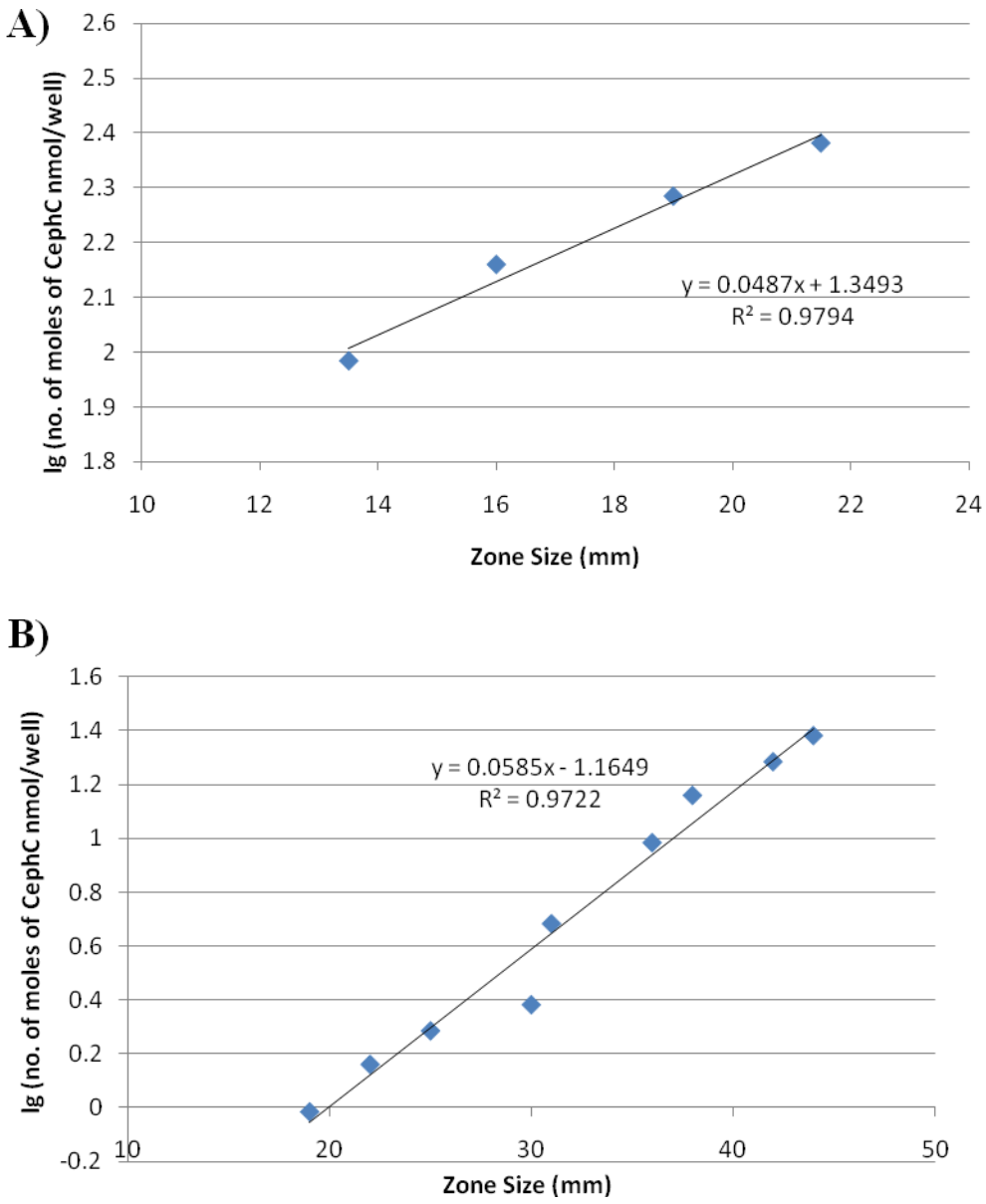


Figure A.1. Calibration curve for A) *S. aureus*; B) *E. coli*.

A.2 Full data from hole-plate antibacterial assay

Efficiency ratio (Eff_R) is an attempt to compare the bactericidal effect of a sample to an equivalent amount of CephC standard that would have caused the same zone size, and is defined as Eff_R = (No. of moles of sample/ Equivalent No. of moles of CephC). The equivalent amount of CephC standard that would have caused the same zone size was calculated using the respective calibration curves shown in Figure A.1. No Eff_R would be calculated if the sample did not show a distinct zone, or a "halo" (abbreviated as (H)). N.A. = not active.

Compound	**Conc. (mg/mL)**	**Zone Size *S. aureus* (mm)**	**Eff_R**	**Zone Size *E.coli.* (mm)**	**Eff_R**	**MW**	**clogP**	**$clogD_{7.4}$**	**PSA**	**MSA**	**CMR**	**%PSA**
7-unsubstituted carboxamido pyrrolidinones												
64a	4	N.A.	-	N.A.	-	407.47	4.65	4.16	93.14	649.92	110.13	14.3
64b	4	N.A.	-	13.5	1219.3	322.36	2.7	2.7	58.64	447.91	89.92	13.1
64c	4	N.A.	-	14	909.8	401.26	3.47	3.47	58.64	468.5	97.44	12.5
64e	4	N.A.	-	N.A.	-	342.44	2.8	2.8	58.64	526.75	94.01	11.1
73	4	N.A.	-	N.A.	-	290.32	1.06	1.06	59.08	422.3	74.51	14.0
Carboxamido epoxypyrrolidinones												
67a	4	N.A.	-	16	657.6	437.45	-2.67	-2.67	107.28	587.45	123.3	18.3
67b	3.33	N.A.	-	N.A.	-	336.35	2.36	2.36	71.17	443.35	88.76	16.1
67c	4	18	4.504	17	1401.7	415.24	3.12	3.12	71.17	463.94	96.38	15.3
67d	4	N.A.	-	12 (H)		360.41	1.12	1.12	80.4	538.65	92.28	14.9
67e	2.96	N.A.	-	N.A.	-	356.42	2.45	2.45	71.17	522.21	92.95	13.6
76	2	N.A.	-	N.A.	-	216.19	-2.03	-2.03	91.4	301.37	46.42	30.3
79	2	N.A.	-	N.A.	-	262.26	-0.53	-0.53	82.17	360.71	64.85	22.8

Compound	**Conc. (mg/mL)**	**Zone Size *S. aureus* (mm)**	**Eff_R**	**Zone Size *E.coli.* (mm)**	**Eff_R**	**MW**	**clogP**	**$clogD_{7.4}$**	**PSA**	**MSA**	**CMR**	**%PSA**
α,β-Unsaturated lactams												
66b (crude)	2	23	1.177	17	908.487	320.35	2.71	2.71	58.64	419.85	90.54	14.0
	1	22	0.706	16	533.567							
	0.5	20	0.508	15	313.371							
66b (pure)	2	21	1.694	17	908.487							
	1	20	1.016	16	533.567							
	0.5	19	0.609	15	313.371							
66c	4	24	3.039	17	1483.29	399.24	3.48	3.48	58.64	440.19	98.16	13.3
	2	23	1.700	-								
66d	4	24	3.523	24	669.726	344.41	1.48	1.48	67.87	515.37	94.06	13.2
	2	22	2.204	-								
66e	2	25	1.132	16	422.489	340.42	2.81	2.81	58.64	498.67	94.73	11.8
	1	23.5	0.704	15	247.107							
	0.5	22	0.438	13.5	156.317							

Compound	**Conc. (mg/mL)**	**Zone Size *S. aureus* (mm)**	**Eff_R**	**Zone Size *E.coli.* (mm)**	**Eff_R**	**MW**	**clogP**	**$clogD_{7.4}$**	**PSA**	**MSA**	**CMR**	**%PSA**
Keto-epoxypyrrolidinones												
77a	2	22	1.468	22 (H)	-	335.36	3.19	3.19	59.14	456.55	89.34	13.0
	1	20.5	0.958	21.5 (H)	-							
	0.5	19	0.629	21 (H)	-							
78a	4	N.A.	-	17 (H)	-	247.25	0.44	0.44	78.93	339.64	62.31	23.2
	2	N.A.	-	14.5 (H)	-							
	1	N.A.	-	13 (H)	-							
77b	4	N.A.	-	24 (H)	-	351.36	2.52	2.52	68.37	472.51	90.76	14.5
	2	N.A.	-	23 (H)	-							
	1	N.A.	-	22 (H)	-							
78b	4	N.A.	-	14 (H)	-	263.25	-0.23	-0.23	88.16	355.77	63.74	24.8
	2	N.A.	-	13 (H)	-							
	1	N.A.	-	12 (H)	-							
77c	2	13	6.833	N.A.	-	357.45	4.62	4.62	59.14	564.63	96.27	10.5
	1	13.5 (H)	-	N.A.	-							
	0.5	N.A.	-	N.A.	-							
78c	2	21	4.367	22.5	247.605	269.34	1.87	1.87	78.93	448.09	69.24	17.6
	1	18	3.724	22	207.697							
	0.5	14	3.795	18	187.585							
77d	2	18 (H)	-	21.5	503.760	301.34	2.84	2.84	59.14	442.25	77.86	13.4
	1	17 (H)	-	20	354.196							
	0.5	17 (H)	-	18	279.006							
78d	4	N.A.	-	21 (H)	-	213.23	0.1	0.1	78.93	325.92	50.84	24.2
	2	N.A.	-	19 (H)	-							
	1	N.A.	-	16 (H)	-							

Compound	**Conc. (mg/mL)**	**Zone Size *S. aureus* (mm)**	**Eff_R**	**Zone Size *E.coli.* (mm)**	**Eff_R**	**MW**	**clogP**	**$clogD_{7.4}$**	**PSA**	**MSA**	**CMR**	**%PSA**
Keto-epoxypyrrolidinones *cont.*												
77e	4	19 (H)	-	17	2801.58	301.34	2.68	2.68	59.14	440.39	77.81	13.4
	2	18 (H)	-	15	2206.85							
	1	17 (H)	-	26 (H)	-							
78e	4	N.A.	-	17 (H)	-	213.23	-0.06	-0.06	78.93	323.7	50.78	24.4
	2	N.A.	-	16 (H)	-							
	1	N.A.	-	13.5 (H)	-							
77f	4	14.5	10.34	14.5	1497.96	345.35	3.63	3.63	59.14	443.55	89.84	13.3
	2	14	5.437	14	803.102							
	1	14	2.719	14	401.551							
78f	4	16	11.93	22	706.190	257.25	0.88	0.88	78.93	327.03	62.81	24.1
	2	13	8.076	18	617.009							
	1	12 (H)	-	16	407.813							
77g	4	16	9.430	16	1289.72	325.36	3.83	3.83	59.14	460.41	86.48	12.8
	2	15	5.216	15	741.423							
	1	13	3.193	14	426.222							
78g	4	14	15.83	14	2338.05	237.26	1.08	1.08	78.93	343.66	59.45	23.0
	2	N.A.	-	12	1545.34							
	1	N.A.	-	18 (H)	-							
(*E*)-78h	2	26	0.403	27	103.656	259.26	0.86	0.86	78.93	339.68	67.42	23.2
	1	23	0.403	25	73.886							
	0.5	19	0.508	20	89.647							
(*E*)-78i	2	16	4.404	18	553.913	239.27	0.98	0.98	78.93	356.69	61.13	22.1
	1	13	4.406	17	330.683							
	0.5	N.A.	-	15	235.712							

Compound	**Conc. (mg/mL)**	**Zone Size *S. aureus* (mm)**	**Eff_R**	**Zone Size *E.coli.* (mm)**	**Eff_R**	**MW**	**clogP**	**$clogD_{7.4}$**	**PSA**	**MSA**	**CMR**	**%PSA**
Keto-epoxypyrrolidinones *cont.*												
78j	2	14 (H)	-	18 (H)	-	261.28	0.78	0.78	78.93	370.05	66.33	21.3
	1	12 (H)	-	15 (H)	-							
	0.5	N.A.	-	13 (H)	-							
78k	4	14	13.87	25	317.556	241.29	0.98	0.98	78.93	387.03	60.04	20.4
	2	N.A.	-	21	322.694							
	1	N.A.	-	19	230.017							
108	2	21 (H)	-	16	1442.52	367.29	0.72	0.72	94.67	489.72	83.99	19.3
	1	N.A.	-	13.5	1273.04							
	0.5	N.A.	-	12	895.078							
2-Hydroxypyrrolidinones												
(7*S*)-86	4	N.A.	-	N.A.	-	291.3	0.95	0.95	76.07	415.03	72.42	18.3
	2	N.A.	-	N.A.	-							
	1	N.A.	-	N.A.	-							
(7*R*)-86	2	N.A.	-	N.A.	-	291.3	0.95	0.95	76.07	415.03	72.42	18.3
	1	N.A.	-	N.A.	-							
87	4	N.A.	-	14 (H)	-	309.75	2.31	2.31	55.84	419.69	75.47	13.3
	2	N.A.	-	13 (H)	-							
	1	N.A.	-	N.A.	-							
82	4	N.A.	-	13 (H)		306.32	0.24	0.24	79.31	434.74	75.86	18.2
	2	N.A.	-	N.A.	-							
	1	N.A.	-	N.A.	-							

Compound	Conc. (mg/mL)	Zone Size *S. aureus* (mm)	Eff_R	Zone Size *E.coli.* (mm)	Eff_R	MW	clogP	$clogD_{7.4}$	PSA	MSA	CMR	%PSA
2-Hydroxypyrrolidinones *cont.*												
(7*S*)-91a	4	N.A.	-	16	3140.90	337.38	2.72	2.72	66.84	473.47	91.76	14.1
	2	N.A.	-	15	1971.17							
	1	N.A.	-	13 (H)	-							
(7*R*)-91a	4	N.A.	-	20	1265.48	337.38	2.72	2.72	66.84	473.47	91.76	14.1
	2	N.A.	-	17	1251.19							
	1	N.A.	-	14	1237.07							
(2*S*)-92a	4	N.A.	-	14 (H)	-	249.27	-0.03	-0.03	86.63	356.52	64.73	24.3
	2	N.A.	-	N.A.	-							
	1	N.A.	-	N.A.	-							
(7*S*)-91b	4	N.A.	-	14 (H)	-	353.37	2.05	2.05	76.07	489.55	93.18	15.5
	2	N.A.	-	13 (H)	-							
	1	N.A.	-	13 (H)	-							
(7*R*)-91b	4	13	11.66	13	5929.67	353.37	2.05	2.05	76.07	489.55	93.18	15.5
	2	N.A.	-	14 (H)	-							
	1	N.A.	-	N.A.	-							
(2*S*)-92b	4	N.A.	-	14 (H)	-	265.27	-0.7	-0.7	95.86	372.33	66.15	25.7
	2	N.A.	-	13 (H)	-							
	1	N.A.	-	N.A.	-							
(7*S*)-91c	2	N.A.	-	14	2322.09	359.47	4.15	4.15	66.84	581.86	98.68	11.5
	1	N.A.	-	14	1161.05							
	0.5	N.A.	-	14	580.52							
(7*R*)-91c	4	N.A.	-	17	2348.60	359.47	4.15	4.15	66.84	581.86	98.68	11.5
	2	N.A.	-	16	1174.30							
	1	N.A.	-	16	736.97							

Compound	Conc. (mg/mL)	Zone Size *S. aureus* (mm)	Eff_R	Zone Size *E.coli.* (mm)	Eff_R	MW	clogP	$clogD_{7.4}$	PSA	MSA	CMR	%PSA
2-Hydroxypyrrolidinones *cont.*												
(2*S*)-92c	2	N.A.	-	17	1555.61	271.36	1.4	1.4	86.63	465.38	71.65	18.6
	1	N.A.	-	14 (H)	-							
	0.5	N.A.	-	N.A.	-							
(7*S*)-91d	4	N.A.	-	14	5503.21	303.36	2.37	2.37	66.84	459.34	80.28	14.6
	2	N.A.	-	13 (H)	-							
	1	N.A.	-	N.A.	-							
(7*R*)-91d	4	N.A.	-	17	2783.02	303.36	2.37	2.37	66.84	459.34	80.28	14.6
	2	N.A.	-	13	3453.71							
	1	N.A.	-	N.A.	-							
(2*S*)-92d	4	N.A.	-	13 (H)	-	215.25	-0.38	-0.38	86.63	343.14	53.25	25.2
	2	N.A.	-	N.A.	-							
	1	N.A.	-	N.A.	-							
(7*S*)-91e	4	13 (H)	-	15 (H)	-	303.36	2.21	2.21	66.84	458.66	80.23	14.6
	2	N.A.	-	13 (H)	-							
	1	N.A.	-	N.A.	-							
(7*S*)-91f	4	N.A.	-	18 (H)	-	347.37	3.16	3.16	66.84	460.85	92.25	14.5
	2	N.A.	-	15 (H)	-							
	1	N.A.	-	13 (H)	-							
(7*R*)-91f	4	15	9.937	20	953.603	347.37	3.16	3.16	66.84	460.85	92.25	14.5
	2	N.A.	-	17	753.248							
	1	N.A.	-	15	510.867							
(2*S*)-92f	4	15 (H)	-	24	694.424	259.26	0.41	0.41	86.63	344.82	65.22	25.1
	2	15 (H)	-	23	404.385							
	1	N.A.	-	20	319.422							

Compound	Conc. (mg/mL)	Zone Size *S. aureus* (mm)	Eff_R	Zone Size *E.coli.* (mm)	Eff_R	MW	clogP	$clogD_{7.4}$	PSA	MSA	CMR	%PSA
2-Hydroxypyrrolidinones *cont.*												
(7*S*)-91g	2	13 (H)	-	19 (H)	-	327.38	3.36	3.35	66.84	477.76	88.89	14.0
	1	N.A.	-	13 (H)	-							
	0.5	N.A.	-	N.A.	-							
(7*R*)-91g	4	13	14.00	19	1178.44	327.38	3.36	3.35	66.84	477.76	88.89	14.0
	2	13 (H)	-	16	930.846							
	1	N.A.	-	14	631.318							
(2*S*)-92g	4	13 (H)	-	24 (H)	-	239.27	0.61	0.61	86.63	361.88	61.86	23.9
	2	13 (H)	-	23 (H)	-							
	1	N.A.	-	20 (H)	-							
(2*S*)-(*E*)-92h	2	15	5.082	20 (H)	-	261.28	0.39	0.39	86.63	357.08	69.84	24.3
	1	N.A.	-	15 (H)	-							
	0.5	N.A.	-	14 (H)	-							
(2*S*)-(*E*)-92i	2	15	5.503	19	-	241.29	0.51	0.51	86.63	374.28	63.54	23.1
	1	12	5.505	17	-							
	0.5	N.A.	-	15 (H)	-							
(2*S*)-92k	4	N.A.	-	13 (H)	-	243.3	0.51	0.51	86.63	404.93	62.45	21.4
	2	N.A.	-	N.A.	-							
	1	N.A.	-	N.A.	-							
Protected 2,3-dihydroxyprrolidinones												
95	4	N.A.	-	N.A.	-	322.32	-0.45	-0.45	99.54	442.29	76.96	22.5
	2	N.A.	-	N.A.	-							
	1	N.A.	-	N.A.	-							
98	2	N.A.	-	N.A.	-	362.38	1.25	1.25	77.54	524.64	89.17	14.8
	1	N.A.	-	N.A.	-							
	0.5	N.A.	-	N.A.	-							

Compound	Conc. (mg/mL)	Zone Size *S. aureus* (mm)	Eff_R	Zone Size *E.coli.* (mm)	Eff_R	MW	clogP	$clogD_{7.4}$	PSA	MSA	CMR	%PSA
Protected 2,3-dihydroxyprrolidinones *cont.*												
99a	4	N.A.	-	N.A.	-	393.44	3.73	3.73	65.07	563.65	105.06	11.5
	2	N.A.	-	N.A.	-							
	1	N.A.	-	N.A.	-							
101a	2	N.A.	-	N.A.	-	305.33	0.98	0.98	84.86	447.05	78.03	19.0
	1	N.A.	-	N.A.	-							
	0.5	N.A.	-	N.A.	-							
***exo*-97a**	4	17 (H)	-	23 (H)	-	353.37	2.03	2.03	87.07	481.29	92.85	18.1
	2	14 (H)	-	20 (H)	-							
	1	N.A.	-	14	584.885							
***endo*-97a**	2	15 (H)	-	19 (H)	-	353.37	2.03	2.03	87.07	481.29	92.85	18.1
	1	14 (H)	-	14 (H)	-							
	0.5	N.A.	-	N.A.	-							
103a	4	N.A.	-	N.A.	-	441.48	5.19	5.19	65.07	601.88	119.74	10.8
	2	N.A.	-	N.A.	-							
	1	N.A.	-	N.A.	-							
104a	4	13	11.93	20	526.175	353.37	2.45	2.45	84.86	484.25	92.71	17.5
	2	N.A.	-	17	447.817							
	1	N.A.	-	12	543.338							
105	1	N.A.	-	N.A.	-	355.39	2.45	2.45	84.86	485.7	92.71	17.5

Compound	Conc. (mg/mL)	Zone Size *S. aureus* (mm)	Eff_R	Zone Size *E.coli.* (mm)	Eff_R	MW	clogP	$clogD_{7.4}$	PSA	MSA	CMR	%PSA
Deprotected 2,3-dihydroxyprrolidinones												
100a	2	N.A.	-	N.A.	-	265.27	-0.72	-0.72	106.86	364.45	65.82	29.3
	1	N.A.	-	N.A.	-							
	0.5	N.A.	-	N.A.	-							
100b	2	N.A.	-	N.A.	-	281.26	-1.39	-1.39	116.09	380.84	67.25	30.5
	1	N.A.	-	N.A.	-							
	0.5	N.A.	-	N.A.	-							
100c	4	12	18.49	25	266.645	287.36	0.71	0.71	106.86	474	72.75	22.5
	2	N.A.	-	22	226.936							
	1	N.A.	-	17	275.343							
100d	4	N.A.	-	N.A.	-	231.25	-1.07	-1.07	106.86	350.48	54.35	30.5
	2	N.A.	-	N.A.	-							
	1	N.A.	-	N.A.	-							
100e	4	N.A.	-	N.A.	-	231.25	-1.22	-1.22	106.86	348.4	54.29	30.7
	2	N.A.	-	N.A.	-							
	1	N.A.	-	N.A.	-							
100f	4	N.A.	-	15	1639.14	275.26	-0.28	-0.28	106.86	352.34	66.32	30.3
	2	N.A.	-	13 (H)	-							
	1	N.A.	-	13 (H)	-							
100g	4	N.A.	-	N.A.	-	255.27	-0.08	-0.08	106.86	368.82	62.96	29.0
	2	N.A.	-	N.A.	-							
	1	N.A.	-	N.A.	-							
100h	2	N.A.	-	14 (H)	-	277.28	-0.3	-0.3	106.86	364.8	70.93	29.3
	1	N.A.	-	13 (H)	-							
	0.5	N.A.	-	N.A.	-							
100i	2	15	5.161	15	876.813	257.29	-0.18	-0.18	106.86	382.12	64.64	28.0
	1	14	3.251	13 (H)	-							
	0.5	13	2.049	N.A.	-							

Compound	**Conc. (mg/mL)**	**Zone Size *S. aureus* (mm)**	**Eff_R**	**Zone Size *E.coli.* (mm)**	**Eff_R**	**MW**	**clogP**	**$clogD_{7.4}$**	**PSA**	**MSA**	**CMR**	**%PSA**
Enamine tetramic acids												
129a	4	19	3.344	16	1645.36	466.58	4.74	4.74	76.15	712.48	131.53	10.7
129b	4	N.A.	-	14	1838.23	482.58	4.07	4.07	85.38	729.36	132.95	11.7
129c	4	28	0.625	15	1649.08	486.99	4.83	4.83	76.15	697.74	131.29	10.9
130	4	11 (H)	-	N.A.	-	364.44	2.25	2.25	76.15	570.22	96.31	13.4
132	4	N.A.	-	N.A.	-	594.71	6.67	6.67	85.38	879.63	170.04	9.7
3-Acyltetramic acids												
124a	2	N.A.	-	N.A.	-	297.31	1.22	0.32	93.14	435.52	72.44	21.4
	1	N.A.	-	N.A.	-							
	0.5	N.A.	-	N.A.	-							
124b	4	20	4.731	N.A.	-	337.37	2.33	1.29	93.14	496.62	86.21	18.8
	2	19	2.736	N.A.	-							
	1	18	1.582	N.A.	-							
124d	2	N.A.	-	N.A.	-	331.75	1.53	-1.11	93.14	448.21	77.03	20.8
	1	N.A.	-	N.A.	-							
	0.5	N.A.	-	N.A.	-							
124e	2	14	5.434	N.A.	-	376.2	1.71	-0.64	93.14	451.71	80.02	20.6
	1	13	3.246	N.A.	-							
	0.5	N.A.	-	N.A.	-							
125a	2	N.A.	-	N.A.	-	403.43	3.02	0.13	102.37	588.69	103.28	17.4
	1	N.A.	-	N.A.	-							
	0.5	N.A.	-	N.A.	-							
125b	2	N.A.	-	13.5 (H)		419.43	2.35	-0.66	111.6	604.55	104.71	18.5
	1	N.A.	-	12.5 (H)								
	0.5	N.A.	-	N.A.	-							

Compound	**Conc. (mg/mL)**	**Zone Size *S. aureus* (mm)**	**Eff_R**	**Zone Size *E.coli.* (mm)**	**Eff_R**	**MW**	**clogP**	**$clogD_{7.4}$**	**PSA**	**MSA**	**CMR**	**%PSA**
3-Acyltetramic acids *cont.*												
125c	2	N.A.	-	14	430.654	423.85	3.11	-0.04	102.37	572.33	103.05	17.9
	1	N.A.	-	N.A.	-							
	0.5	N.A.	-	N.A.	-							
126	2	N.A.	-	N.A.	-	433.39	0.83	-1.96	128.67	641.25	101.48	20.1
	1	N.A.	-	N.A.	-							
	0.5	N.A.	-	N.A.	-							
3-Enoyltetramic acids												
127a	4	32	1.239	N.A.	-	399.44	3.04	2.2	102.37	589.61	109.08	17.4
	2	31	0.693	N.A.	-							
	1	28	0.485	N.A.	-							
127b	4	25	2.611	N.A.	-	415.44	3.71	3.01	93.14	574.08	107.66	16.2
	2	23	1.634	N.A.	-							
127c	4	33	0.293	15	1912.74	419.86	3.8	2.76	93.14	558.43	107.43	16.7
	1	29	0.151	13 (H)								
	0.5	19	0.464	N.A.	-							
127d	2	35	0.105	N.A.	-	407.51	4.65	4.16	93.14	649.92	110.13	14.3
	1	35	0.052	N.A.	-							
	0.5	33	0.038	N.A.	-							
127e	2	30	0.450	14 (H)		413.47	4	3.52	93.14	603.49	111.82	15.4
	1	28.5	0.280	N.A.	-							
	0.5	25	0.233	N.A.	-							
127f	2	30.5	0.263	14 (H)		411.45	3.72	3.08	93.14	574.23	112.94	16.2
	1	28	0.206	N.A.	-							
	0.5	25	0.175	N.A.	-							

Compound	**Conc. (mg/mL)**	**Zone Size *S. aureus* (mm)**	**Eff_R**	**Zone Size *E.coli.* (mm)**	**Eff_R**	**MW**	**clogP**	**$clogD_{7.4}$**	**PSA**	**MSA**	**CMR**	**%PSA**
3-Enoyltetramic acids *cont.*												
127g	2	13	6.507	N.A.	-	375.38	2.26	0.97	106.28	513.35	95.01	20.7
	1	N.A.	-	N.A.	-							
	0.5	N.A.	-	N.A.	-							
127h	2	25	0.737	13.5 (H)	-	391.44	3.11	2.16	93.14	521.86	99.51	17.8
	1	20	0.898	N.A.	-							
	0.5	13.5 (H)	-	N.A.	-							
Bicyclic dienones												
150a	4	24	2.505	16	2763.52	383.44	4.23	4.23	72.91	564.09	105.69	12.9
	2	23	1.430	15	1734.33							
	1	22	0.816	13	1366.16							
150b	4	26	1.825	16	2623.79	403.86	4.32	4.32	72.91	548.05	105.46	13.3
	2	25	1.042	15	1646.64							
	1	24.5	0.556	14	1628.06							

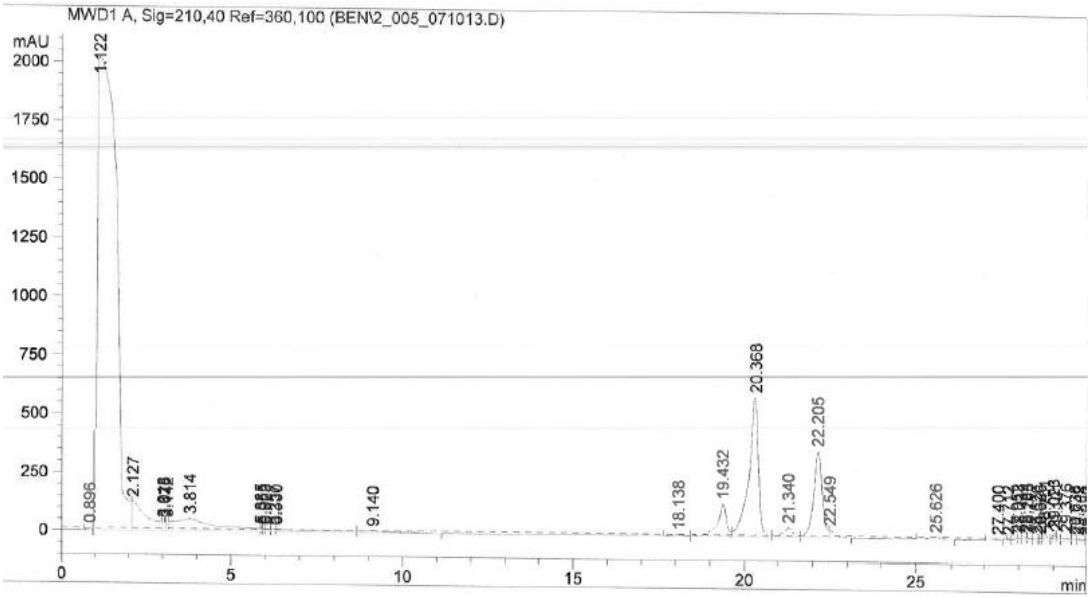
APPENDIX B: PREPARATIVE HPLC TRACES

All crude mixture purified using reverse-phase preparative HPLC are loaded as a DMSO solution.

(*E*)-78h and 78j

Elution condition:

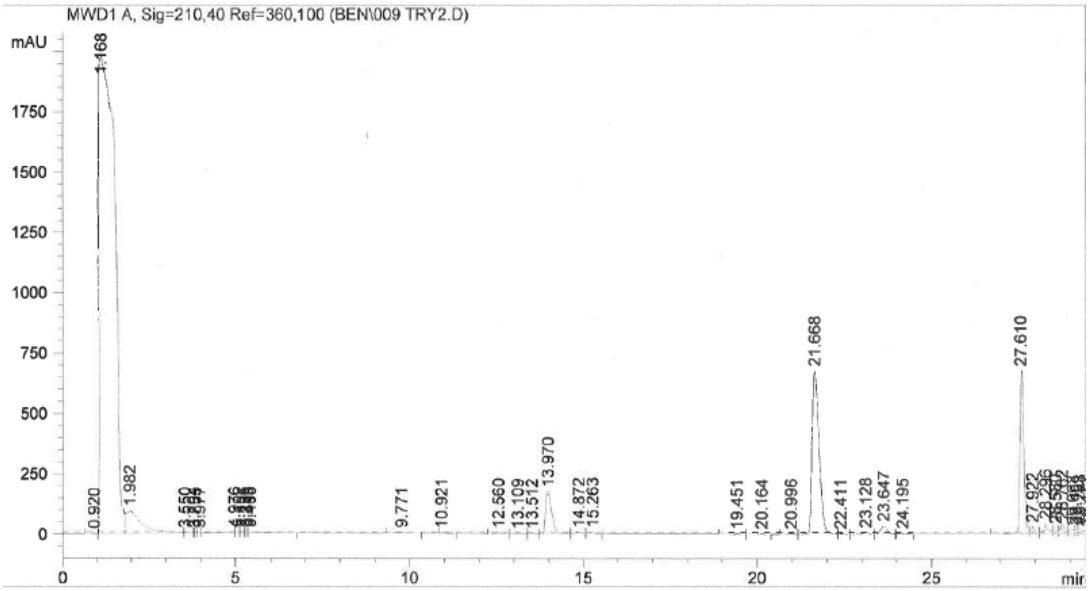
2 min at 5% MeCN/H_2O, a gradient from 5% to 25% MeCN/H_2O in 18 min, 5 min at 25% MeCN/H_2O, a gradient from 25% to 80% MeCN/H_2O in 5 min, followed by 80% MeCN/H_2O for 5 min.



(*E*)-78i and 78k

Elution condition:

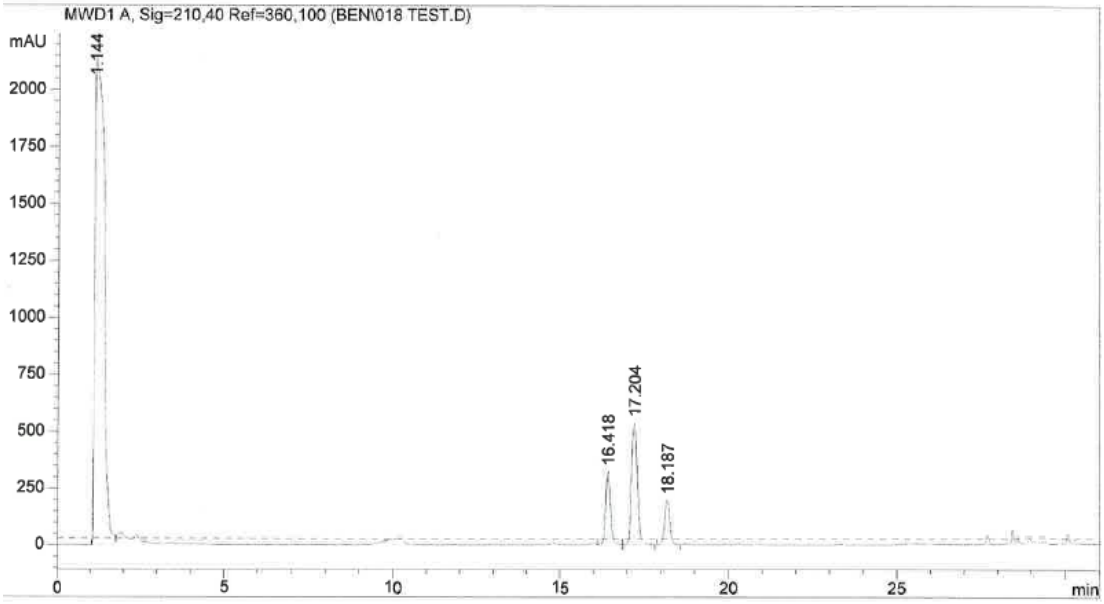
2 min at 5% MeCN/H_2O, a gradient from 5% to 25% MeCN/H_2O in 18 min, 5 min at 25% MeCN/H_2O, a gradient from 25% to 80% MeCN/H_2O in 5 min, followed by 80% MeCN/H_2O for 5 min.



(*E*)-92h

Elution condition:

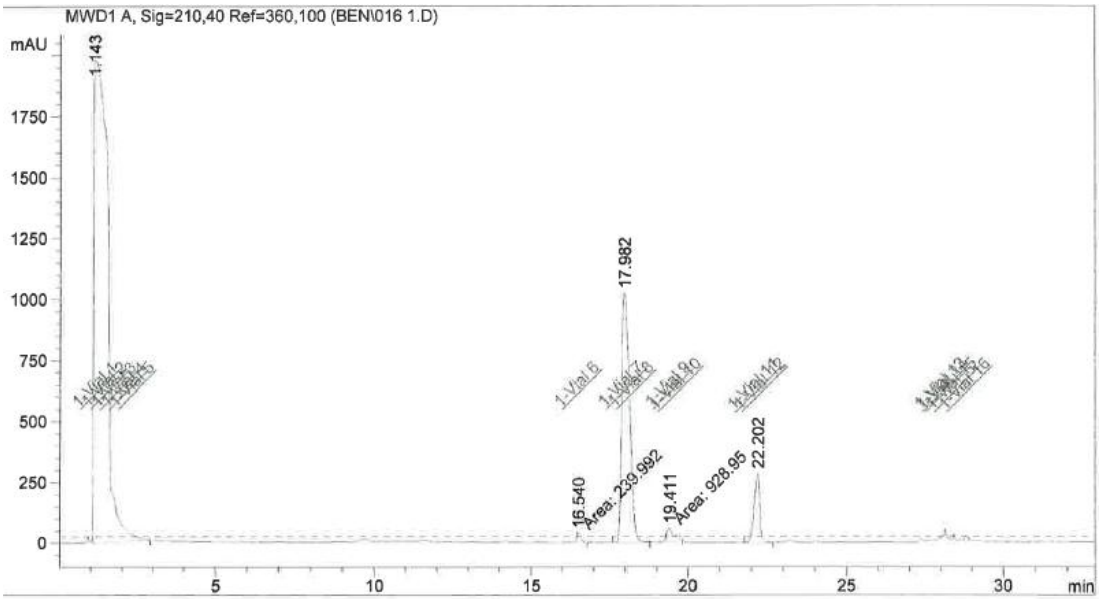
1 min at 5% MeCN/H_2O, a gradient from 5% to 25% MeCN/H_2O in 19 min, 5 min at 25% MeCN/H_2O, a gradient from 25% to 80% MeCN/H_2O in 5 min, followed by 80% MeCN/H_2O for 5 min.



(*E*)-92i and 92k

Elution condition:

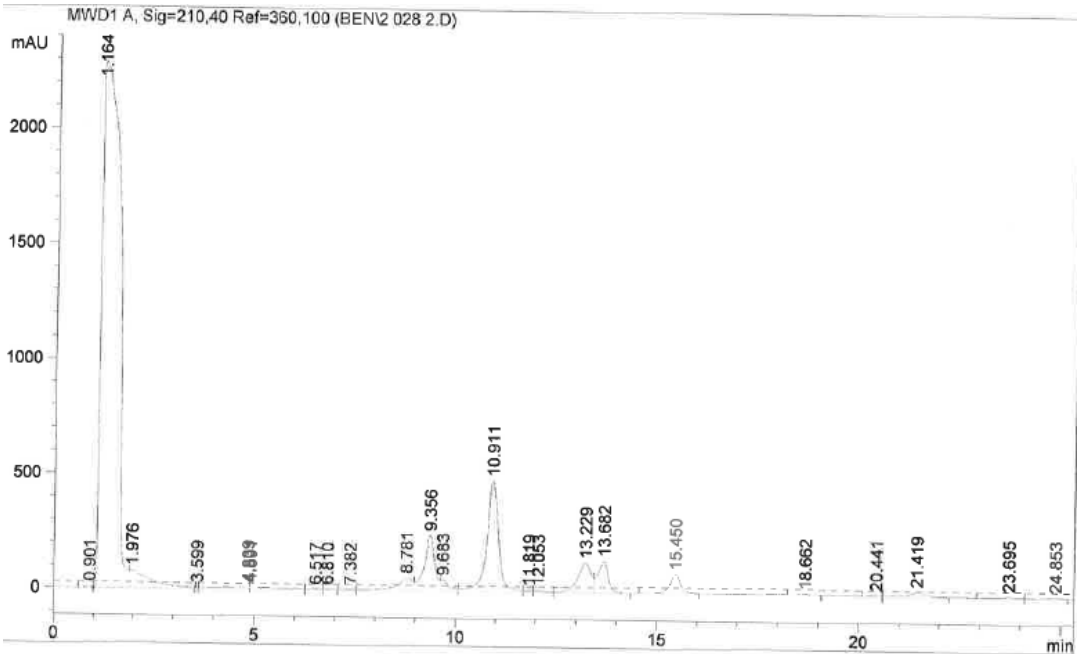
1 min at 5% MeCN/H_2O, a gradient from 5% to 25% MeCN/H_2O in 19 min, 5 min at 25% MeCN/H_2O, a gradient from 25% to 80% MeCN/H_2O in 5 min, followed by 80% MeCN/H_2O for 5 min.



(*E*)-100h

Elution condition:

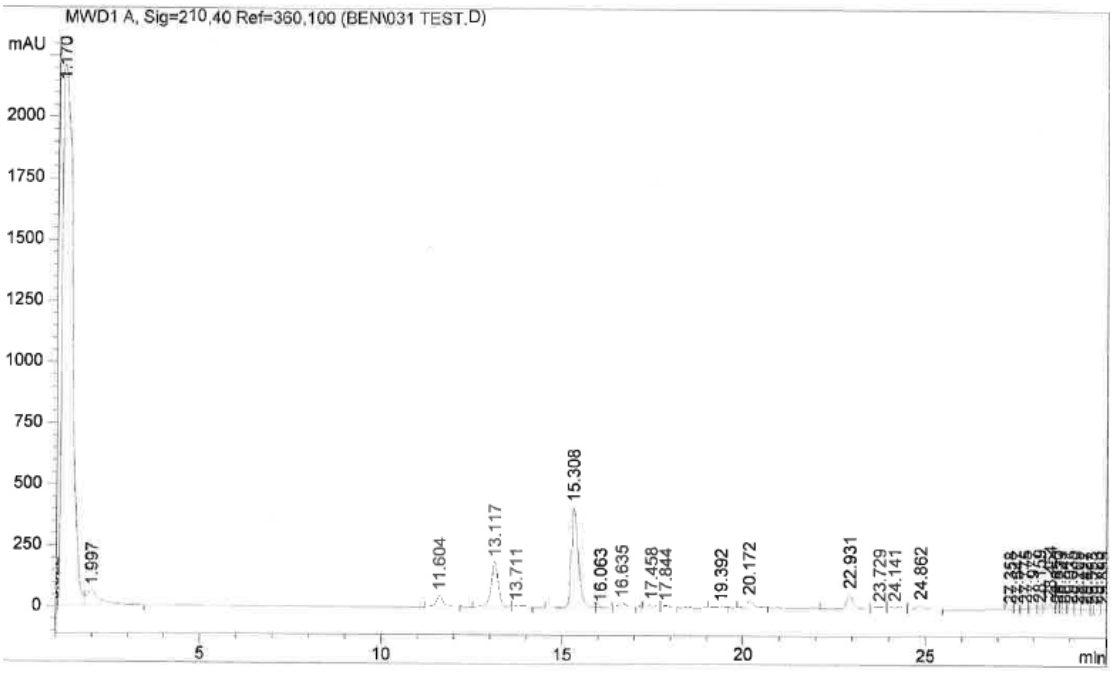
1 min at 5% MeCN/H_2O, a gradient from 10% to 25% MeCN/H_2O in 24 min, 2 min at 25% MeCN/H_2O, a gradient from 25% to 80% MeCN/H_2O in 3 min, followed by 80% MeCN/H_2O for 4 min.



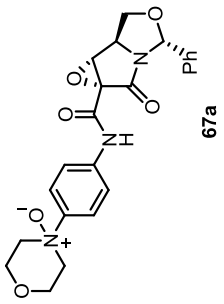
(*E*)-100i

Elution condition:

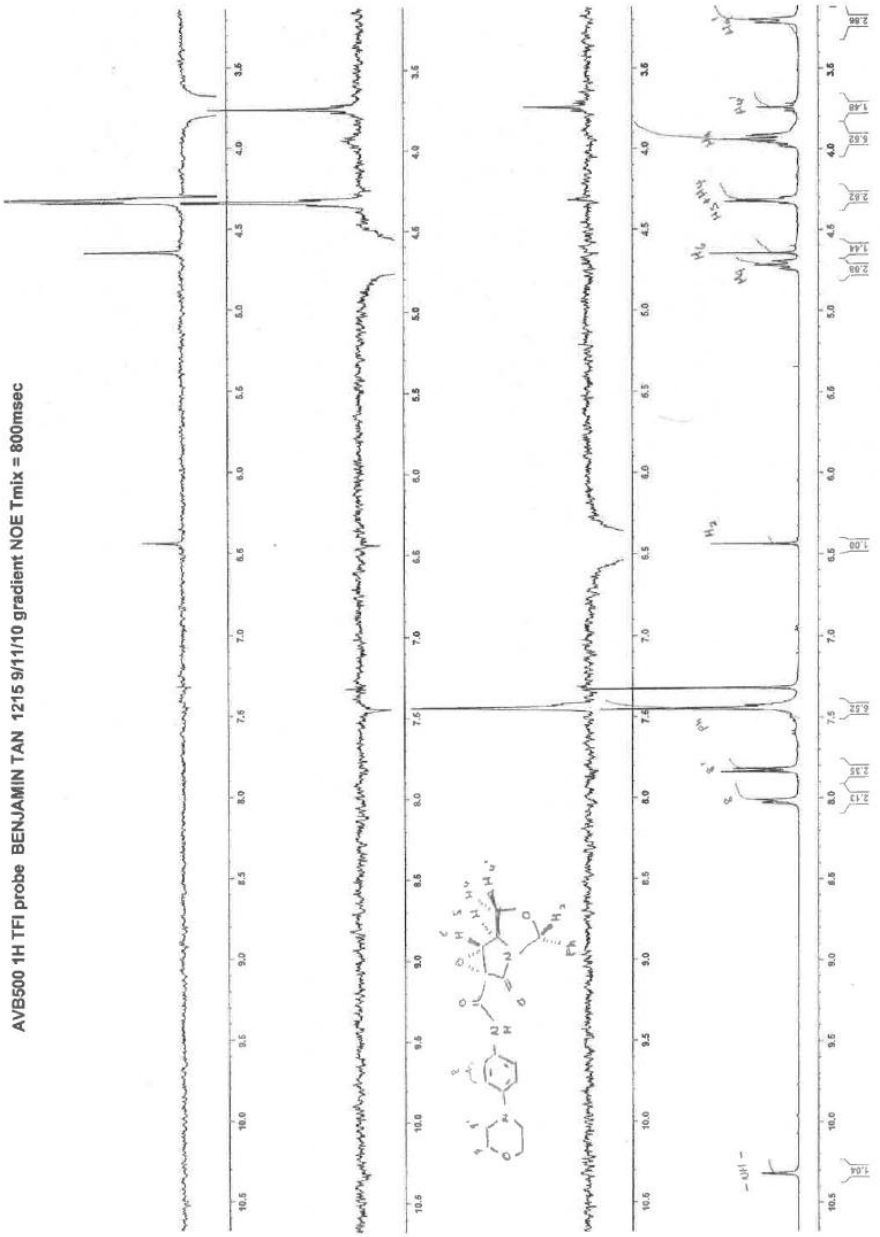
1 min at 5% MeCN/H_2O, a gradient from 5% to 25% MeCN/H_2O in 19 min, 5 min at 25% MeCN/H_2O, a gradient from 25% to 80% MeCN/H_2O in 5 min, followed by 80% MeCN/H_2O for 5 min.

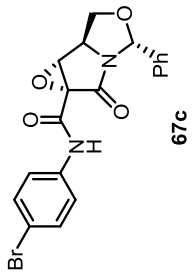


APPENDIX C: nOe SPECTRA

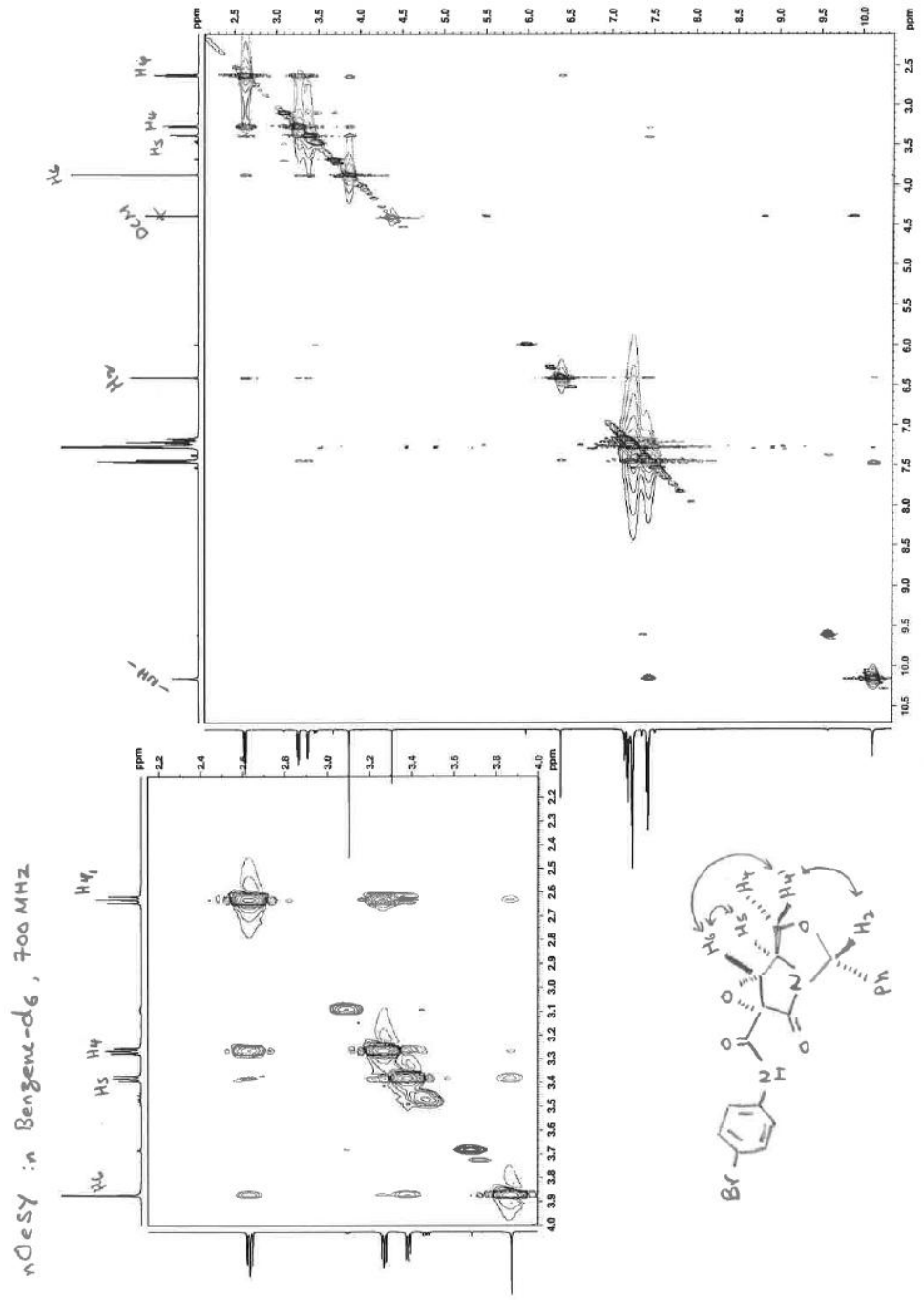


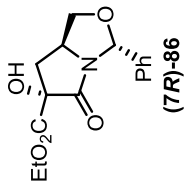
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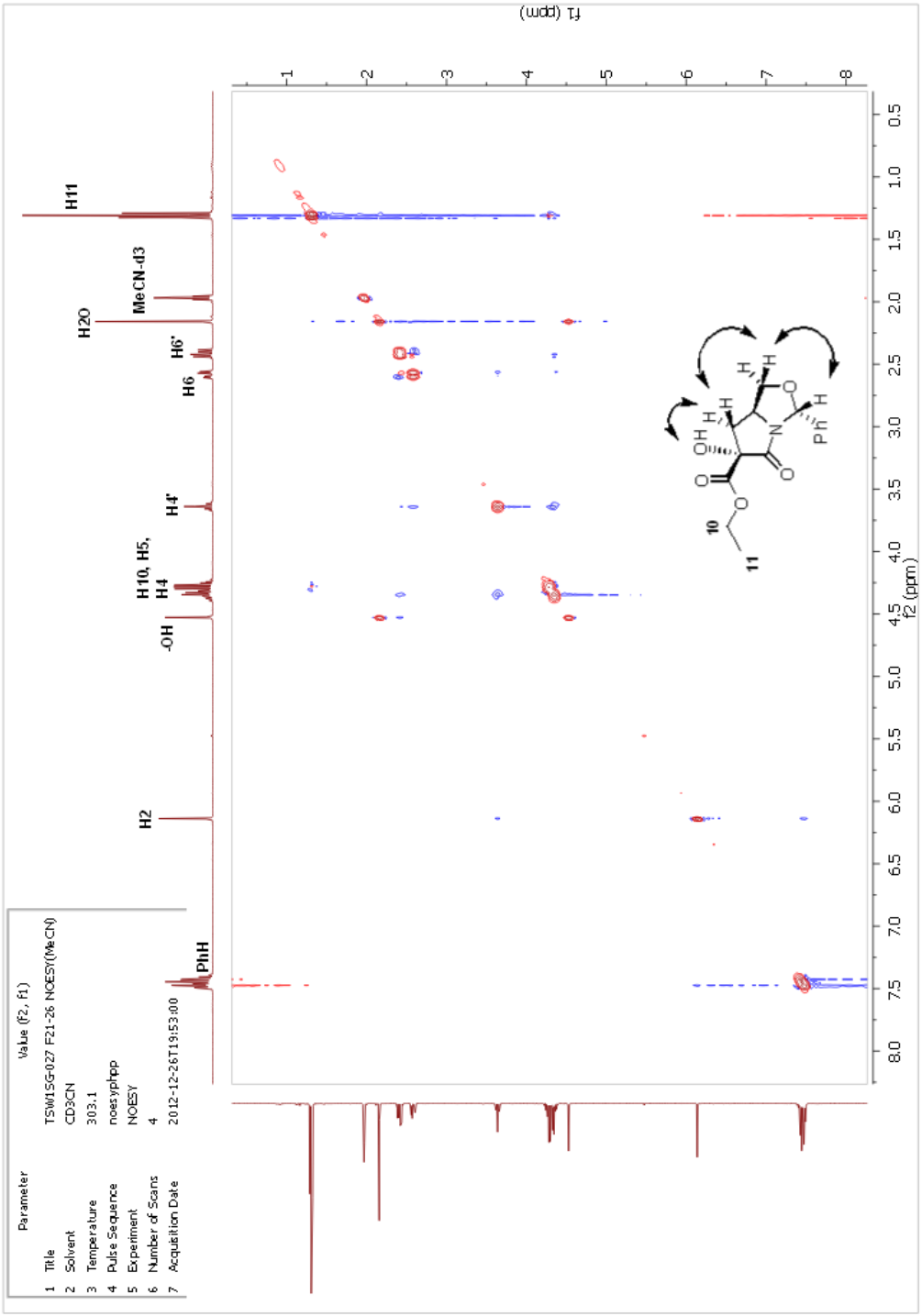


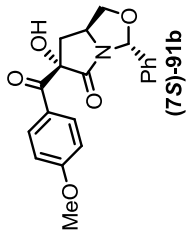
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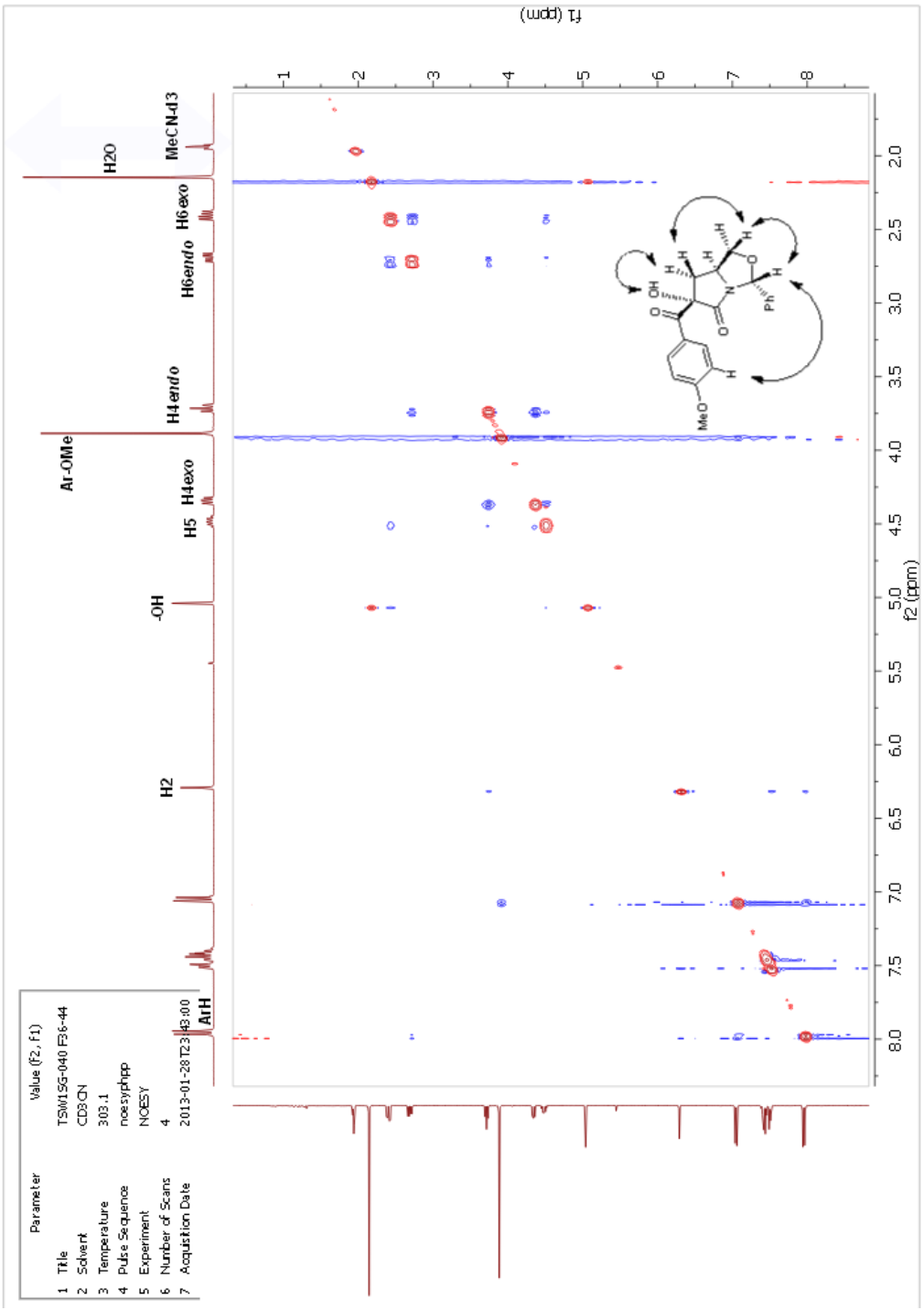

OH
EtO2C
O
N
O
Ph

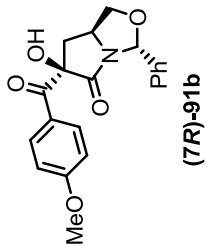
(7*R*)-86


f1 (ppm)
f2 (ppm)
H11
MeCN-d3
H20
H6'
H6
H4'
H10, H5,
H4
-OH
H2
PhH
Parameter
Value (F2, F1)
1 Title TSW15G-027 F21-26 NOESY(MeCN)
2 Solvent CD3CN
3 Temperature 303.1
4 Pulse Sequence noesyphpp
5 Experiment NOESY
6 Number of Scans 4
7 Acquisition Date 2012-12-26T19:53:00
10
11

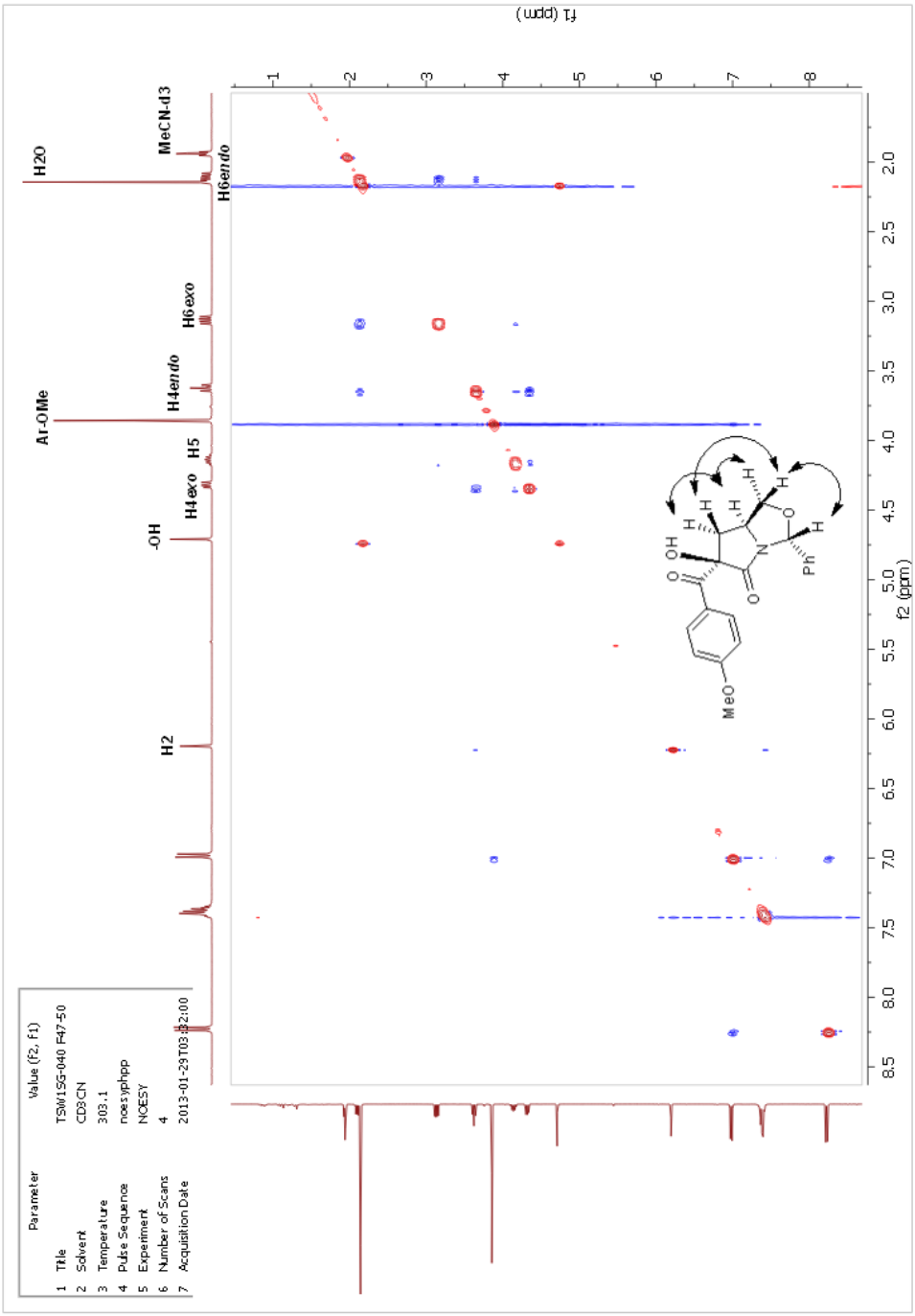

OH
O
N
O
Ph
O
MeO

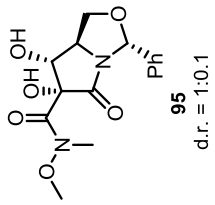
(7S)-91b


f1 (ppm)
f2 (ppm)
MeCN-d3
H2O
H6exo
H6endo
H4endo
Ar-OMe
H4exo
H5
-OH
H2
ArH
Parameter
Value (F2, F1)
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2 Solvent CD3CN
3 Temperature 303.1
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5 Experiment NOESY
6 Number of Scans 4
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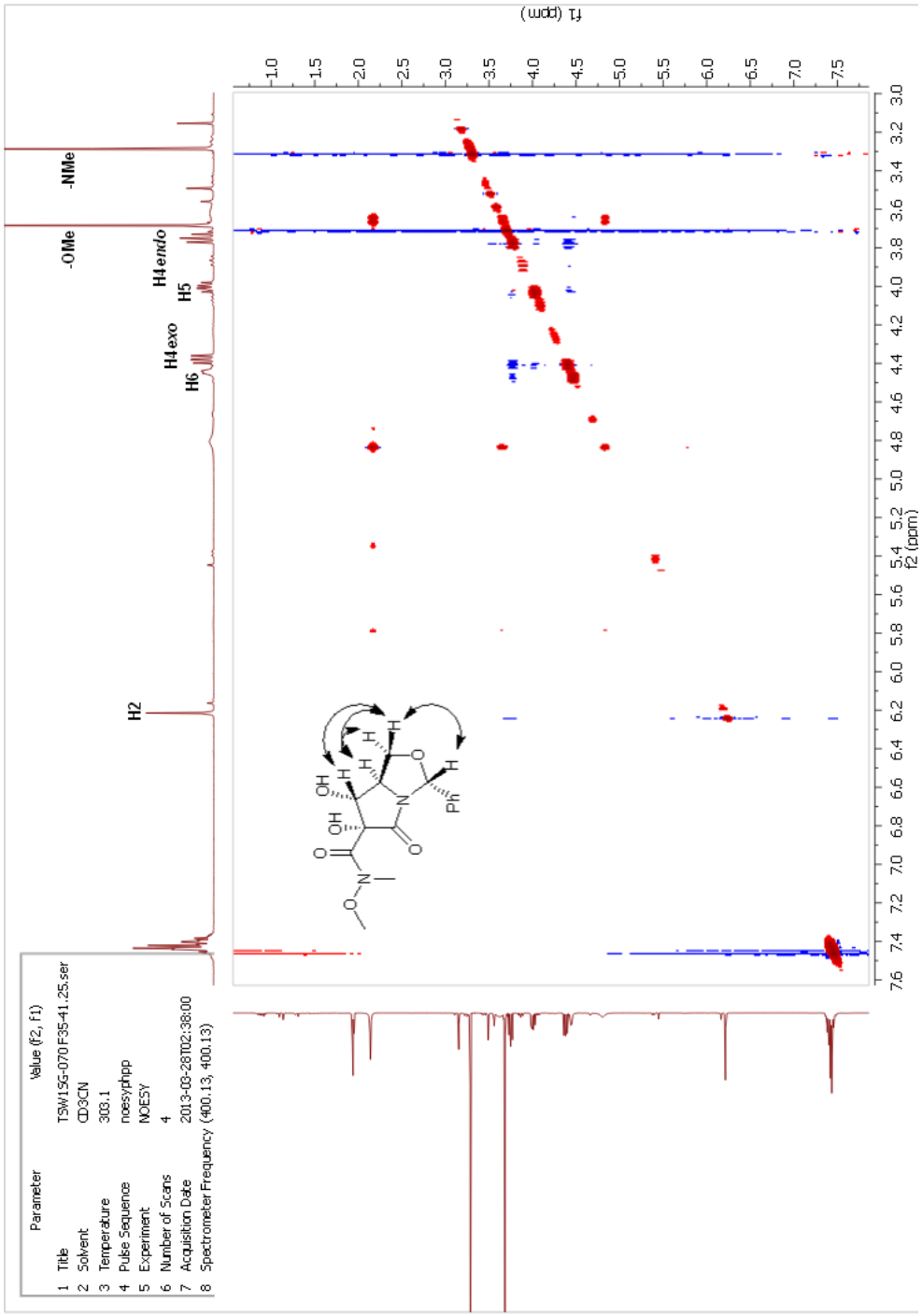

OH
O
O
N
O
Ph
MeO

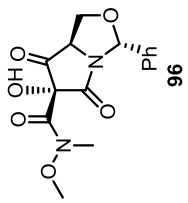
(7*R*)-91b


f1 (ppm)
H2O
MeCN-d3
H6endo
H6exo
H1endo
Ar-OMe
H5
H4exo
-OH
H2
f2 (ppm)
Parameter
Value (f2, f1)
1 Title
TSW15G-040 F47-50
2 Solvent
CD3CN
3 Temperature
303.1
4 Pulse Sequence
noesyphpp
5 Experiment
NOESY
6 Number of Scans
4
7 Acquisition Date
2013-01-29T03:32:00

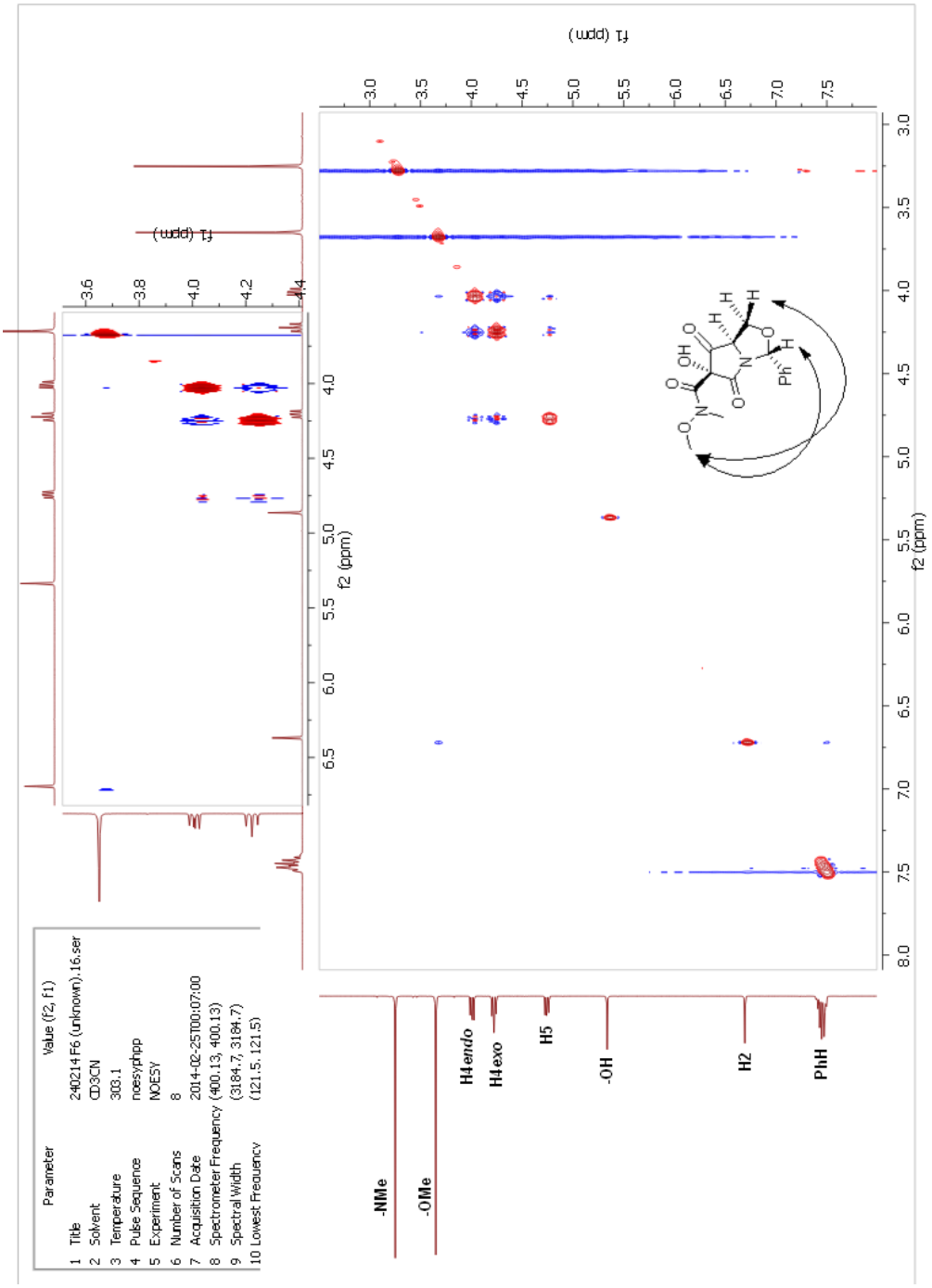


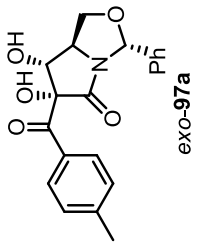
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d.r. = 1:0.1


-NMe
-OMe
H4endo
H5
H4exo
H6
H2
f1 (ppm)
f2 (ppm)
Parameter | Value (f2, f1)
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2 Solvent | CD3CN
3 Temperature | 303.1
4 Pulse Sequence | noesyphpp
5 Experiment | NOESY
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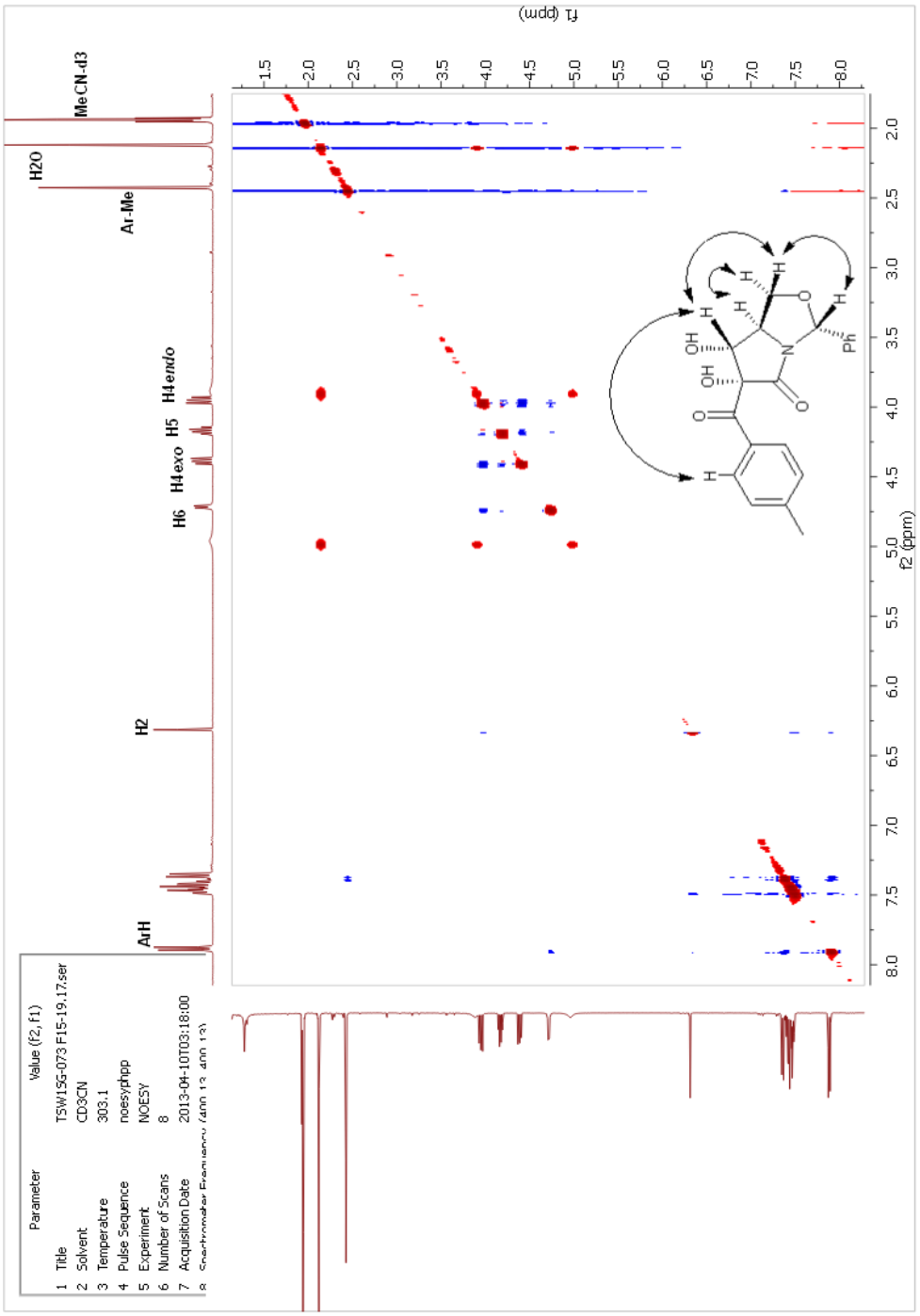

OH
O
O
O
N
O
N
Ph

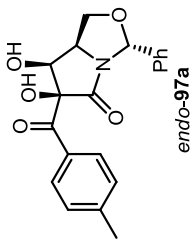
96


f1 (ppm)
f2 (ppm)
-NMe
-OMe
H4endo
H4exo
H5
-OH
H2
PhH
Parameter
Value (f2, f1)
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240214 F6 (unknown).16.ser
2 Solvent
CD3CN
3 Temperature
303.1
4 Pulse Sequence
noesyphpp
5 Experiment
NOESY
6 Number of Scans
8
7 Acquisition Date
2014-02-25T00:07:00
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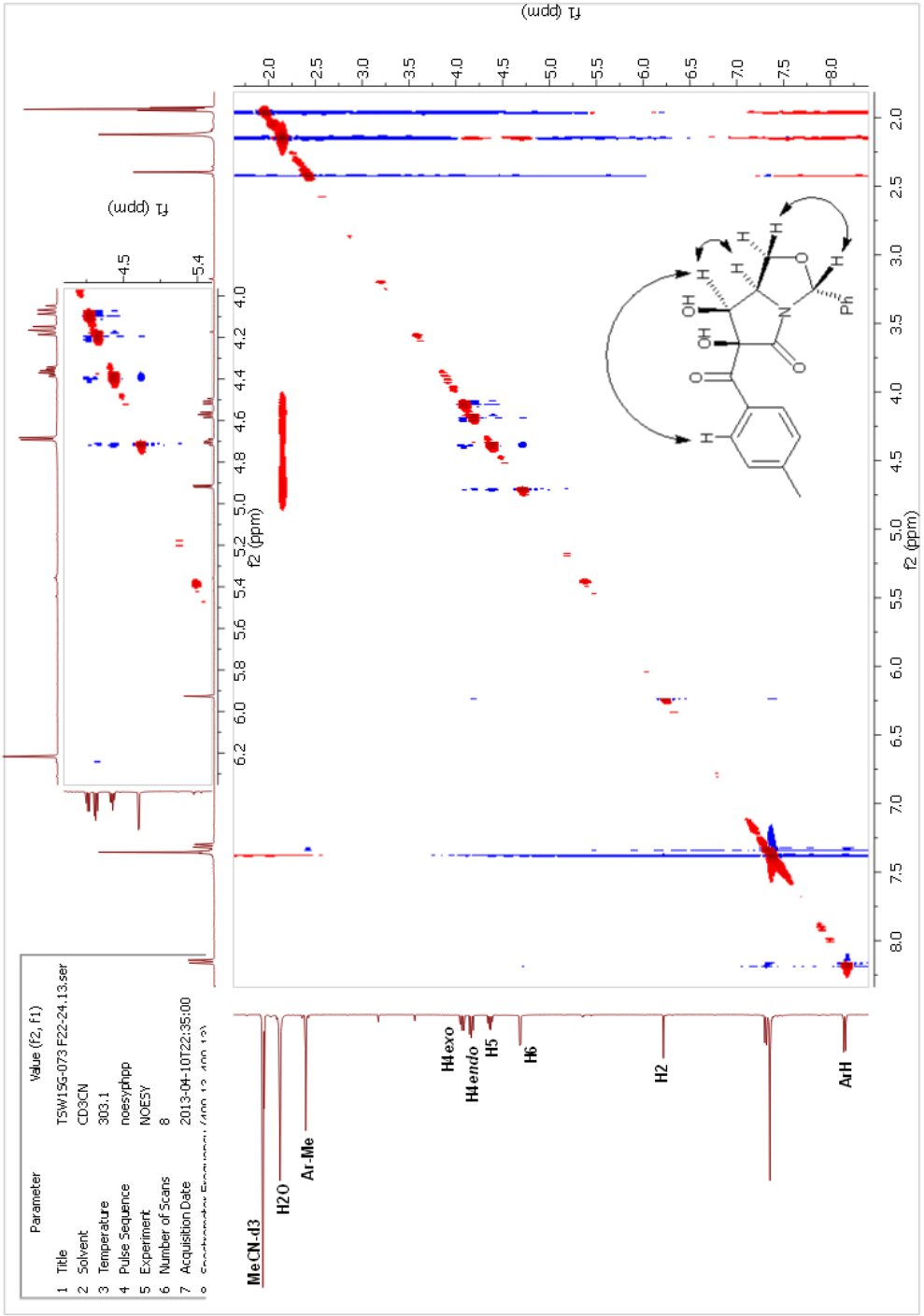

OH
OH
O
N
O
O
Ph

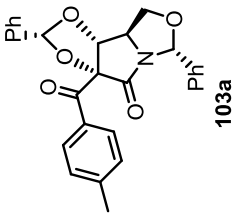
exo-**97a**

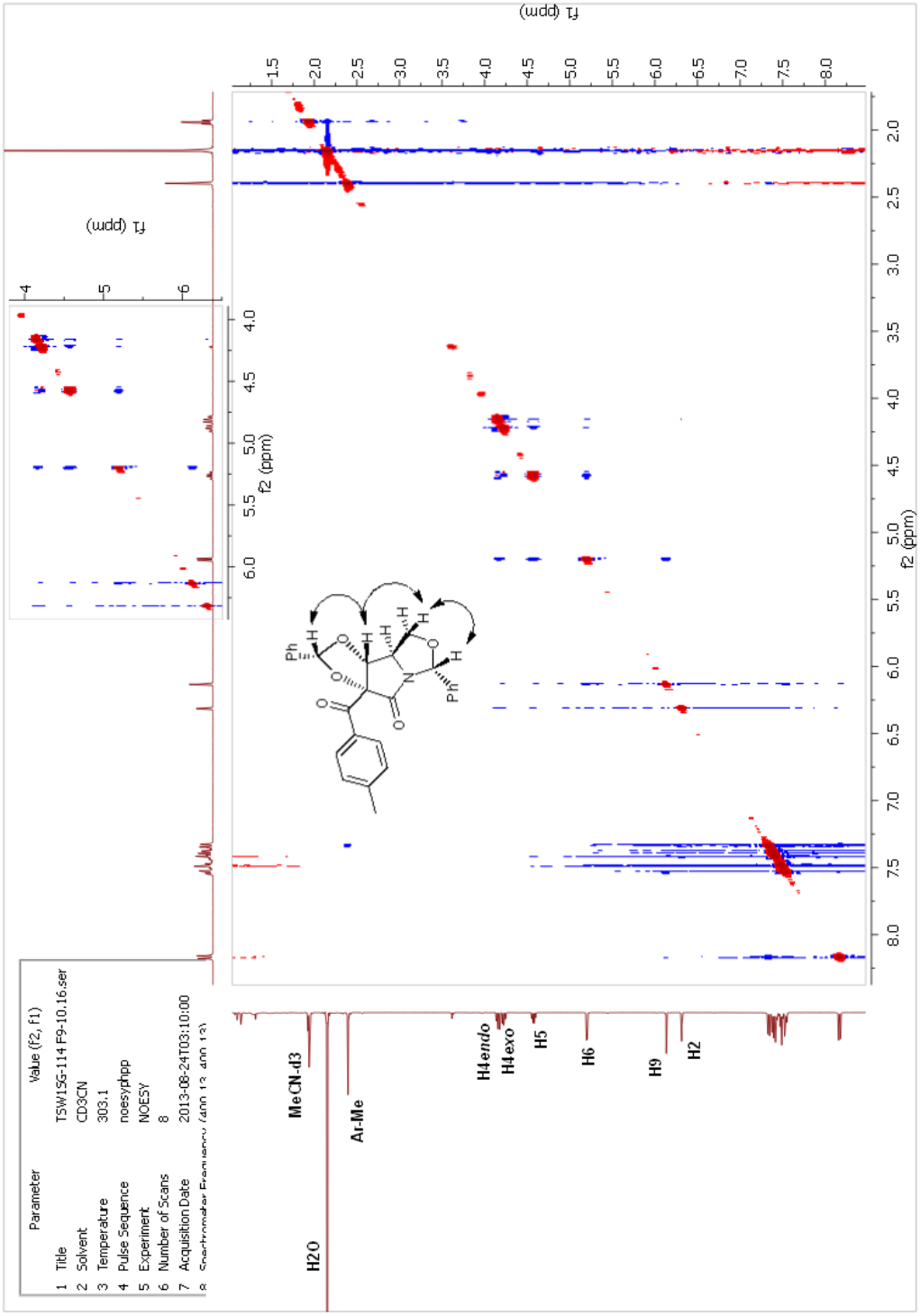

MeCN-d3
H20
Ar-Me
H4endo
H5
H4exo
H6
H2
ArH
f1 (ppm)
f2 (ppm)
Parameter
Value (f2, f1)
1 Title
TSW1SG-073 F15-19.17.ser
2 Solvent
CD3CN
3 Temperature
303.1
4 Pulse Sequence
noesyphpp
5 Experiment
NOESY
6 Number of Scans
8
7 Acquisition Date
2013-04-10T03:18:00

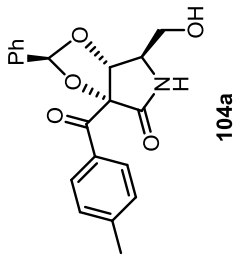

OH
OH
O
O
N
O
Ph

endo-**97a**

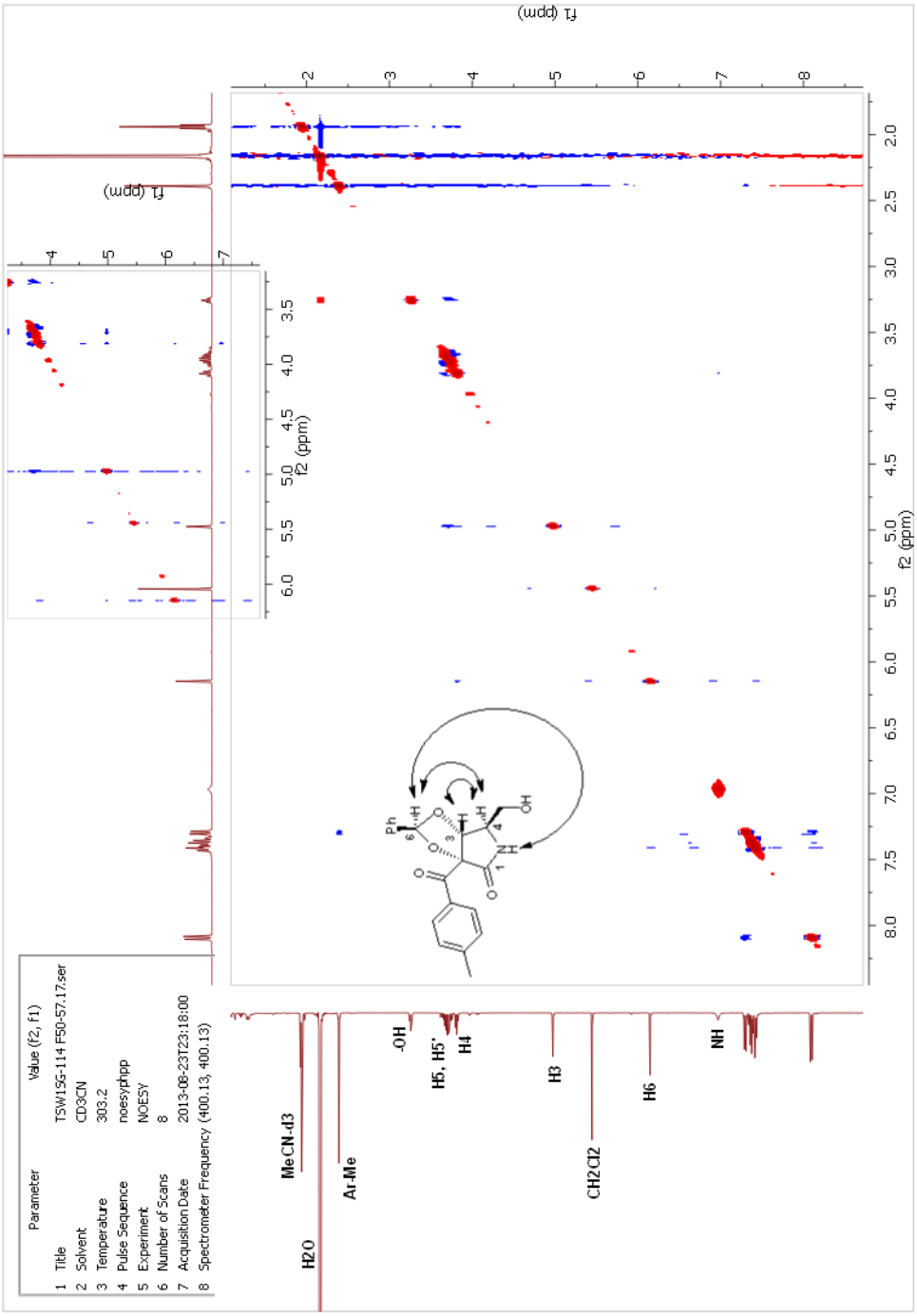

f1 (ppm)
f2 (ppm)
MeCN-d3
H2O
Ar-Me
H4exo
H4endo
H5
H6
H2
ArH
Parameter
Value (f2, f1)
1 Title
TSW15G-073 F22-24.13.ser
2 Solvent
CD3CN
3 Temperature
303.1
4 Pulse Sequence
noesyphpp
5 Experiment
NOESY
6 Number of Scans
8
7 Acquisition Date
2013-04-10T22:35:00

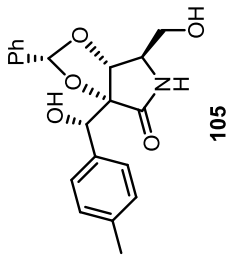

Ph
Ph
103a


f1 (ppm)
f2 (ppm)
MeCN-d3
H2O
Ar-Me
H1endo
H1exo
H5
H6
H9
H2
Parameter
Value (f2, f1)
1 Title
TSW1SG-114 F9-10.16.ser
2 Solvent
CD3CN
3 Temperature
303.1
4 Pulse Sequence
noesyphpp
5 Experiment
NOESY
6 Number of Scans
8
7 Acquisition Date
2013-08-24T03:10:00

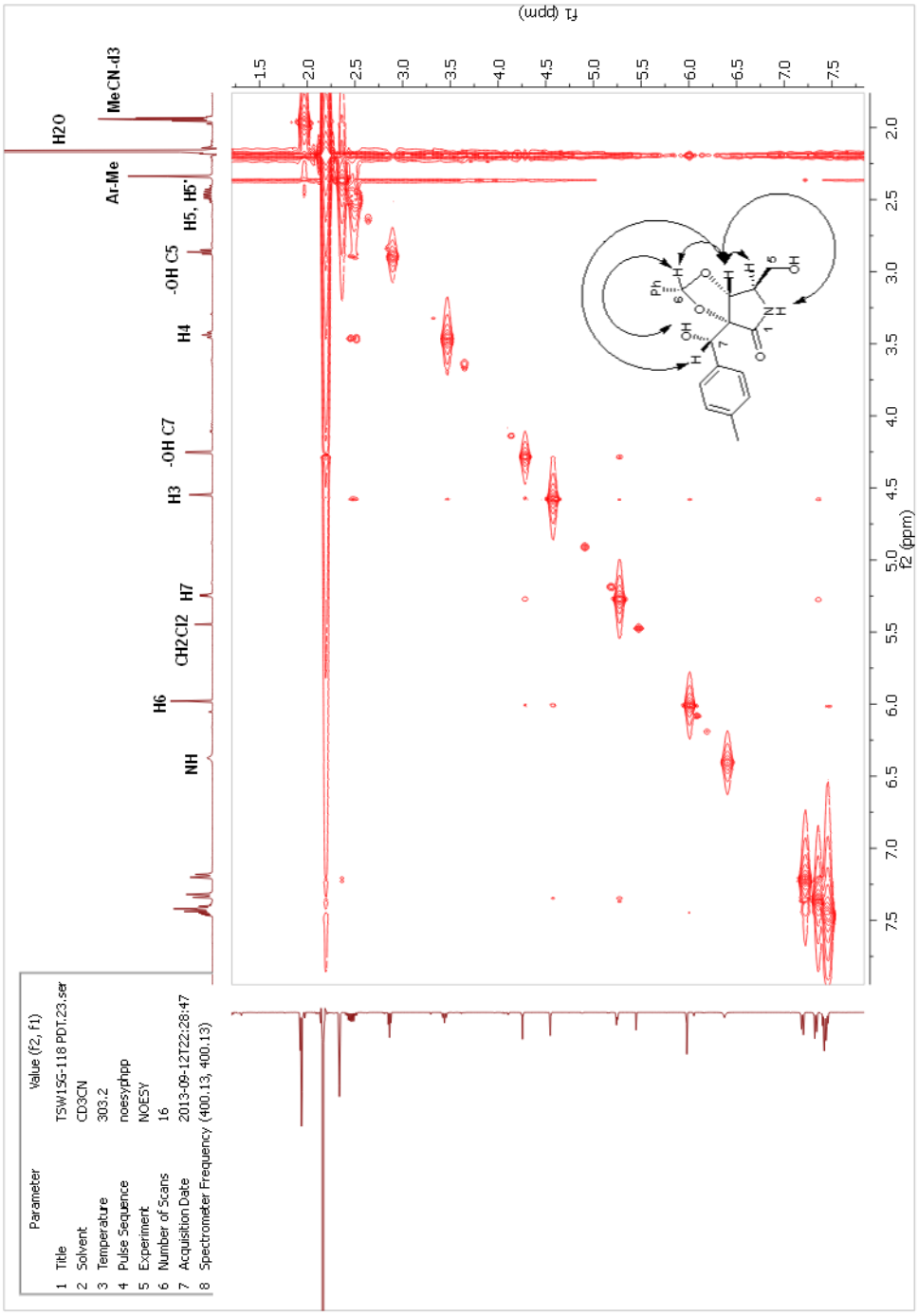

Ph
O
O
O
N
H
OH
O

104a


f1 (ppm)
f2 (ppm)
Parameter Value (f2, f1)
1 Title TSW15G-114 F50-57.17.ser
2 Solvent CD3CN
3 Temperature 303.2
4 Pulse Sequence noesyphpp
5 Experiment NOESY
6 Number of Scans 8
7 Acquisition Date 2013-08-23T23:18:00
8 Spectrometer Frequency (400.13, 400.13)
H2O
MeCN-d3
Ar-Me
-OH
H5, H5'
H4
H3
CH2Cl2
H6
NH


Ph
O
O
OH
OH
N
H
O

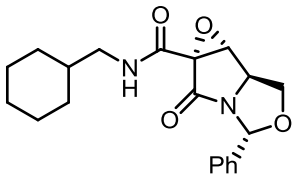
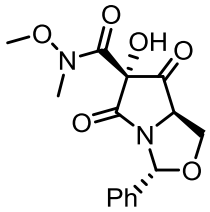
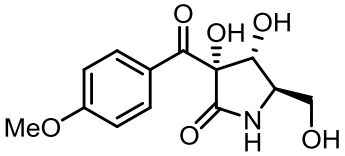
105


f1 (ppm)
H2O
MeCN-d3
Ar-Me
H5, H5'
-OH C5
H4
-OH C7
H3
CH2Cl2
H7
H6
NH
f2 (ppm)
Parameter
Value (f2, f1)
1 Title
TSW15G-118 PDT.23.ser
2 Solvent
CD3CN
3 Temperature
303.2
4 Pulse Sequence
noesyphpp
5 Experiment
NOESY
6 Number of Scans
16
7 Acquisition Date
2013-09-12T22:28:47
8 Spectrometer Frequency
(400.13, 400.13)

APPENDIX D: X-RAY CRYSTALLOGRAPHY DATA

Crystallographic data for compounds as shown below are provided on a CD attached to the inside of the back cover.

Table D.1: Contents of CD appendix.

Compound Number	Structure	X-Ray file name
67e		6362
96		2035
100b		094