

Natural Product Guided Antibacterial Drug Discovery:

Tetramates As Core Scaffolds

A thesis submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Organic Chemistry

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ABSTRACT

Natural product guided antibacterial drug discovery: tetramates as core scaffolds

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This thesis describes the synthesis and biological evaluation of a library of compounds containing the tetramic acid core in search of novel antibacterial drug candidates.

Chapter 1 discusses the need for new antibiotics due to the emergence of virulent bacterial strains resistant to clinically available drugs and the hiatus in the discovery of new replacement antibitoics that has become a global threat to human health. Different platforms for antibacterial drug discovery and the re-emergence of natural products-based approach that has gained importance in the quest for novel antibiotics are discussed. In this regard, the intrinsic antibacterial activity of natural products containing a tetramate core structure and the strategies developed to synthesise the core scaffold are described.

Chapter 2 discusses the use of L-serine and L-cysteine in tetramic acid synthesis and the application of L-cysteine-derived thiazolidine templates suitable for stereoselective ring closing reactions to obtain the tetramic acid core with scope for further functionalization.

Chapters 3 and 4 describe a range of synthetic routes for appropriate substitutions of the tetramate core for compound library generation. Elaboration of the tetramate core via carboxamide tetramate synthesis, Suzuki-Miyaura cross-coupling reactions, glycosylations and their aglycone analogue synthesis, etherification, tetramate-pyroglutamate systems, Buchwald aminations/amidations, cycloadditions and β-lactam hybrids as possible chemical modifications of the tetramate core structure are discussed.

Chapter 5 describes the antibacetiral activity and physicochemical properties of the library of compounds synthesised. A preliminary evaluation of their antibiotic activity was conducted against *S. aureus* and *E. coli* using the hole-plate method. MICs of the tetramates synthesised were determined against several Gram-negative strains; *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and Gram-positive strains; MRSA, *Enterococcus faecalis and Streptococcus pneumoniae*, in whole-cell bioassays. Physicochemical properties of the compound library were analysed to map the chemical space occupied by tetramates with potent antibacterial activity. Enzyme inhibition studies were conducted to identify possible modes of action that contribute to whole-cell antibiotic activity and in this regard, the inhibition of enzymes *S. aureus* topoisomerase IV, *S. aureus* RNA polymerase, *E. coli* RNA polymerase, *E. coli* gyrase and *M. tuberculosis* gyrase are discussed. Since plasma protein binding of compounds is an important factor that determines the bioavailability of antibiotics and their clinical outcome, a study of the binding affinity of these drug candidates to Human Serum Albumin (HSA) by both whole-cell bioassay and NMR spectroscopy-based protein binding experiments are discussed. Finally, a brief note on the potential of tetramic acids to function as proteasome inhibitors in anticancer chemotherapy is included at the end of this chapter.

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ABBREVIATIONS

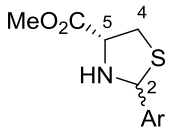
Ac	acetyl
APA	aminopenicillanic acid
Approx.	approximately
Ar	aromatic
B. subtilis	*Bacillus subtilis*
B. licheniformis	*Bacillus licheniformis*
Bn	benzyl
BIOS	biology-oriented synthesis
Boc	*tert*-butyloxycarbonyl
Bu	butyl
t-Bu	*tert*-butyl
CFX	ciprofloxacin
$cLogD_{7.4}$	calculated distribution coefficient between octanol and water at pH 7.4
cLogP	calculated partition coefficient between octanol and water
COSY	correlation spectroscopy
COD	cyclooctadiene
Cp*	pentamethylcyclopentadienyl
Cy	cyclohexyl
dba	dibenzylideneacetone
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DEPT	distortionless enhancement by polarisation transfer
DFT	density functional theory
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DNP	dictionary of natural products
DOS	diversity-oriented synthesis
dppf	(diphenylphosphino)ferrocene
E. coli	*Escherichia coli*
E. faecalis	*Enterococcus faecalis*
E. faecium	*Enterococcus faecium*
e.e.	enantiomeric excess

ent	enantiomer
ESI	electrospray ionisation
Et	ethyl
EtOAc	ethyl acetate
eq.	equivalents
FBDD	fragment-based drug discovery
FI	field ionization
Fmoc	fluorenylmethyloxycarbonyl
Fsp^3	Fraction of sp^3 hybridized carbon count over total carbon count
h	hour
HMBC	heteronuclear multiple-bond correlation
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HSA	human serum albumin
HSQC	heteronuclear single-quantum correlation
HTS	high-thoughput screening
IC_{50}	concentration of a compound required to achieve 50 % inhibition in a cellular or biochemical assay
ICP-MS	inductively coupled plasma mass spectrometry
IPA	isopropyl alcohol
IR	infra-red
K. pneumoniae	*Klebsiella pneumoniae*
LC-MS	liquid chromatography-mass spectrometry
LRMS	low resolution mass spectrometry
m-CPBA	*meta*-chloroperbenzoic acid
Me	methyl
MIC	minimum inhibitory concentration
min	minute
mp	melting point
MRSA	methicillin resistant *Staphylococcus aureus*
MSA	molecular surface area
MW	molecular weight
NDM-1	New Delhi metallo-beta-lactamase I
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy

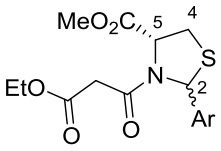
p-NBn	*para*-nitrobenzyl
PPB	plasma protein binding
P. aeruginosa	*Pseudomonas aeruginosa*
ppm	parts per million
PSA	polar surface area
quant.	quantitative
R_f	retention factor
rt	room temperature
RNA	ribonucleic acid
RNAP	RNA polymerase
Ro5	rule of 5
RuAAC	ruthenium-catalysed azide alkyne cycloaddition
RuPhos	2-dicyclohexylphosphino-2',6'-diisopropoxybiphenyl
S. aureus	*Staphylococcus aureus*
S. pneumoniae	*Streptococcus pneumoniae*
S. pyogenes	*Streptococcus pyogenes*
SAR	structure activity relationship
SCONP	structural classification of natural products
SCRFK	scalar cross-relaxation of the first kind
T3P	Propylphosphonic anhydride solution
TBAF	tetra-*n*-butylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TEG	tetraethylene glycol
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
L-Trp	L-tryptophan
UPPS	undecaprenyl pyrophosphate synthase
VRE	vancomycin-resistant *Enterococcus*
w/v	weight/volume
XantPhos	4,5-bis(diphenylphosphino)-9,9-dimethylxanthene
XPhos	2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl

NOMENCLATURE

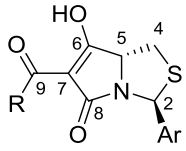
The following non-systematic numbering systems have been used throughout this thesis for thiazolidines, *N*-acylthiazolidines, bicyclic tetramates and their oxazolidine analogues.



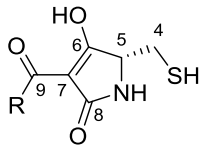
Thiazolidine



***N*-Acylthiazolidine**



Bicyclic tetramate



Monocyclic tetramate

Assignment of the respective NMR signals in the Experimental section is according to the numbering system specified on the structure drawn next to the data.

In the characterization of compounds,

- *N*-Acylthiazolidines: where the major and minor conformers have distinct chemical shift values, they have been specified as either 'major conformer' or 'minor conformer' in compound characterisation.
- Carboxamide tetramates: where the major and minor tautomeric forms have distinct chemical shift values, they have been specified as either AB (major tautomeric form) or CD (minor tautomeric form) in compound characterisation.

Nomenclature used for the bicyclic tetramates in this thesis is consistent with that published in the literature by the Moloney group.

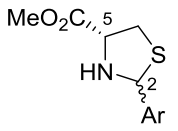
STEREOCHEMISTRY

Absolute stereochemistry

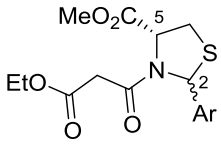
Cahn-Ingold-Prelog convention has been used throughout this thesis to describe stereogenic centers as *R* and *S*, while *E* and *Z* descriptors have been used to describe the geometry of double bonds. A 'hashed wedged bond' is used to denote a substituent behind the plane of the page and a 'wedged bond' is used to denote a substituent in front of the plane of the page. The absolute stereochemistry specified for the bicyclic tetramates through out this thesis is that of the major enantiomer (supported by experimental evidence for selected samples and assumed to be consistent for the series).

Relative stereochemistry

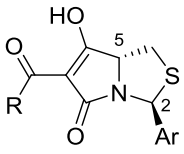
The terms '*cis*' and '*trans*' refer to the relative stereochemistry between H-2 and H-5 in thiazolidines, *N*-acylthiazolidines and the bicyclic tetramates. A wavy bond signifies the presence of a mixture of isomers at that chiral center.



Thiazolidine



***N*-Acylthiazolidine**



Bicyclic tetramate

CHAPTER 1 : INTRODUCTION

1.1 The discovery of antibiotics

The 20th century marked the discovery and application of antibiotics, one of the most successful therapeutic agents to be introduced into clinical use. The first antibiotics were of synthetic origin, discovered by a systematic screening approach of libraries of chemical compounds.[1] Introduced by Paul Ehrlich in 1909, these became the key focus in the search for more antibiotics in the early days of antibiotic research, which also led to the discovery of sulfonamides.[1–3]

However, the serendipitous discovery of penicillin in 1928 proved to be a landmark event, and paved the way for natural-product based antibacterial drug discovery, superseding Ehrlich's approach of chemical library screening. In 1943, Streptomycin was discovered in the laboratory of Selman Waksman in his study of soil-inhabiting microbes for their production of metabolites with antibiotic activity. This 'Waksman approach', which exploited the ability of microorganisms to synthesise antibiotics to ensure their own survival among other pathogens, became the cornerstone of drug search strategies and led to what is considered to be the 'golden era' of antibiotic drug discovery[2] (Figure 1.1).

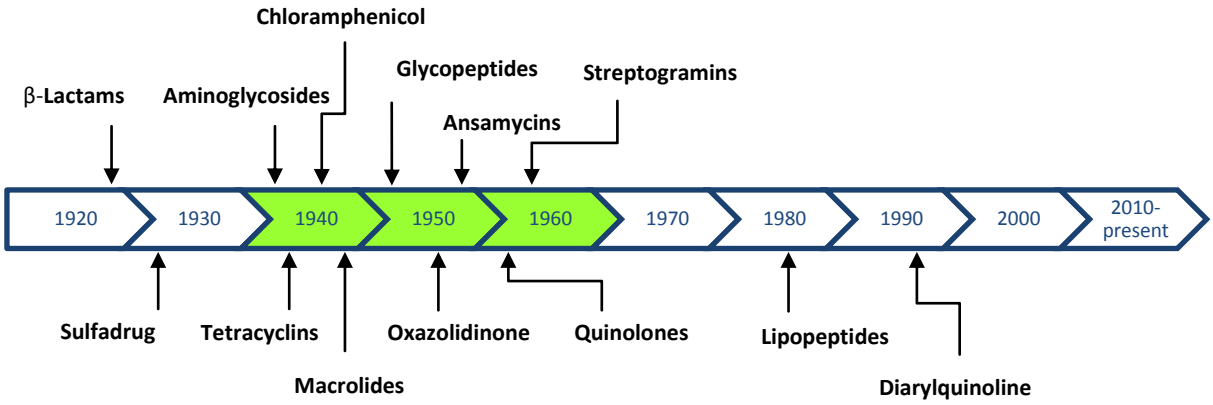


Figure 1.1. Timeline for the discovery of the major classes of antibiotics that were later introduced to the clinic. The 'golden era' is highlighted. Adapted by permission from Macmillan Publishers Ltd: Nature (Lewis, K., *Nature*, **2012**, *485*, 439-440), copyright 2012.

However, the success achieved from 1940-1960 with the 'Walksman approach' ultimately proved not to be sustainable. The continuous screening of soil-dwelling microbes for antibiotics led to the re-discovery of known compounds and hence this platform became less successful and in time was abandoned. No new class of broad spectrum antibiotics has been discovered since the 1960s. In 1986, Daptomycin (a lipopeptide), a first-in-class antibiotic targeting Gram-positive bacteria was discovered subsequent to a considerable void in the drug discovery timeline.[4] In 1997, Bedaquiline (a diarylquinoline) was discovered as a narrow-spectrum antibiotic to treat tuberculosis.[4,5] Apart from sulfa drugs, oxazolidinones, quinolones and now diarylquinolines, all other classes of antibiotics were natural product-derived. In the post 'golden era', the discovery of new classes of antibiotics has been very slow and most new antibiotics introduced to the market were derivatives of old classes of antibiotics via chemical modification.[4]

Now, with the once-treatable infections becoming difficult to cure, the decreasing effectiveness of antibiotics has become a global threat due to the widespread bacterial resistance to clinically approved drugs.[6] This, coupled with the hiatus in the discovery of new replacement antibiotics, is a portent of a possible medical catastrophe that will force us back to the pre-antibiotic era of widespread and recurring infectious disease and epidemics.

1.2 Antibiotic resistance

The observation of antibiotic resistance dates back to the 1940s, though it was not widespread at that time.[1] Over the years, resistance has grown from being a minor issue to a broad threat, owing to the increased consumption of antibiotics and slow discovery of new molecular scaffolds with novel modes of action to counter rapidly developing antibiotic resistance. According to the U.S. Centers for Disease Control and Prevention (CDC, 2013), antibiotic resistance is responsible for more than 2 million infections and approximately 23,000 deaths

each year in the United States.[6] In 2009, the European Center for Disease Control and prevention (ECDC) estimated that 25,000 deaths a year occurred from antibiotic resistant infections,[6] indicating the severity of the threat imposed on human health by the rapid onset of antibiotic resistance by pathogenic microbes.

Table 1.1. The classes of antibiotics, their year of discovery, introduction to the clinic and reported observation of resistance. Adapted by permission from Macmillan Publishers Ltd: Nature Reviews Drug Discovery (Lewis, K., *Nat. Rev. Drug. Discov.*, **2013**, *12* (5), 371-387), copyright 2013 (*in vitro* data in support of resistance observed, where resistance was observed prior to introduction).

Antibiotic class; example	Year of discovery	Year of introduction	Year resistance observed
Sulfa drugs; prontosil	1932	1936	1942
β-Lactams; penicillin	1928	1938	1945
Aminoglycosides; streptomycin	1943	1946	1946
Chloramphenicols; chloramphenicol	1946	1948	1950
Macrolides; erythromycin	1948	1951	1955
Tetracyclins; chlortetracyclin	1944	1952	1950
Rifamycin; rifampicin	1957	1958	1962
Glycopeptides; vancomycin	1953	1958	1960
Quinolones; ciproflaxacin	1961	1968	1968
Streptogramins; Streptogramins B	1963	1998	1964
Oxazolidinones; linezolid	1955	2000	2001
Lipopeptides; daptomycin	1986	2003	1987
Fidaxomycin	1948	2011	1977
Diarylquinolines; bedaquiline	1997	2012	2006

Antibiotics target bacterial cell-wall biosynthesis, protein synthesis, nucleic acid metabolism or DNA replication and repair mechanisms to exert their effect on target organisms (modes of action of known antibiotics are discussed in Chapter 5). When bacteria are exposed to

antibiotics, they develop strategies to combat the effect of antibacterials for survival, thereby developing resistance.[7,8] The most common mechanisms by which bacteria acquire resistance to antibiotics are by enzymatic modification or degradation of the antibacterial compound, mutations of the target sites, decreased exposure to antibiotics (decreased uptake by change in cell wall/membrane permeability and increased efflux) or circumventing a metabolic pathway driven by antibiotic-sensitive enzymes.[7,8]

Even though bacterial resistance has been a continuing problem since antibiotics were introduced clinically (Table 1.1), it is the escalation and prevalence in diversity of resistant organisms that has recently become a growing threat to global public health.[7] According to the World Health Organization, in 2014, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were listed as the three agents of the greatest concern associated with both hospital and community acquired infections.[6] The multidrug-resistant 'superbugs' such as MRSA, VRE, *E. coli* and *Klebsiella* spp. carrying NDM-1 are among some of the notable global examples that render treatment challenging and costly.[6,9,10] Since multidrug-resistant strains are no longer localized to hospitals, but are found in communities, society is at a larger risk of succumbing to infections that are incurable. Today, the problem of drug resistance is exacerbated and more focus is shifted to multidrug-resistant ESKAPE organisms (*Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella* spp., *Acinetobacter baumannii*, *Pseudomonas aeruginosa, Enterobacter* spp.) where some strains are resistant to all currently available antibiotics.[9,11]

While the rapid rise in antibiotic resistance over the past few years can be related to the increased consumption of antibiotics for human use, applications also in agriculture and animal husbandry are significant and must not be ignored.[12,13] Between 2000 and 2010, the consumption of antibiotics has increased by 36 %.[12] The increase in demand for 'last resort antibiotics' such as carbapenems (by 40 %) and polymixins (by 13 %) is of concern as some

antibiotics that were once effective are now proving to have little effect.[12] Further, international travel contributes to the spread of such resistant strains, and the spread of strains carrying NDM-1 is one such example,[6,14] highlighting that antibiotic resistance is not a regional problem but a global issue. Impeding the onset of antibiotic resistance is a global responsibility that needs to be addressed at an international level.[14] While implementing good antibiotic stewardship, society needs to, more than ever, find alternative drugs for infections before all the antibiotics in clinical use become ineffective. In our quest for new antibiotics, one approach to overcome antibiotic resistance would be to develop new scaffolds that possess novel and/or multiple modes of action to address resistance prevalent with current antibiotics.

1.3 Platforms for antibiotic drug discovery

Drug discovery is a challenging task, and over the years, scientists have tried different approaches that would deliver new antibiotics. Even though the first class of antibiotics, sulfa drugs, were discovered by screening a compound library, since then the prevalent model for drug discovery was based on natural products tested in whole-cell screens. Medicinal chemistry then played an important role in the optimization of any identified activities, making them more useful with lower doses and with a more expanded target spectrum. During the time span where no new scaffolds were introduced to the clinic, more innovative drug discovery approaches involving target-based screening emerged. However, while this aided both in a better understanding of protein-drug interactions and the concept of rational drug design, more than twenty years since its implementation, no new antibiotics have been discovered through this platform[3] (Figure 1.2). Some of the platforms used in drug discovery are discussed below.

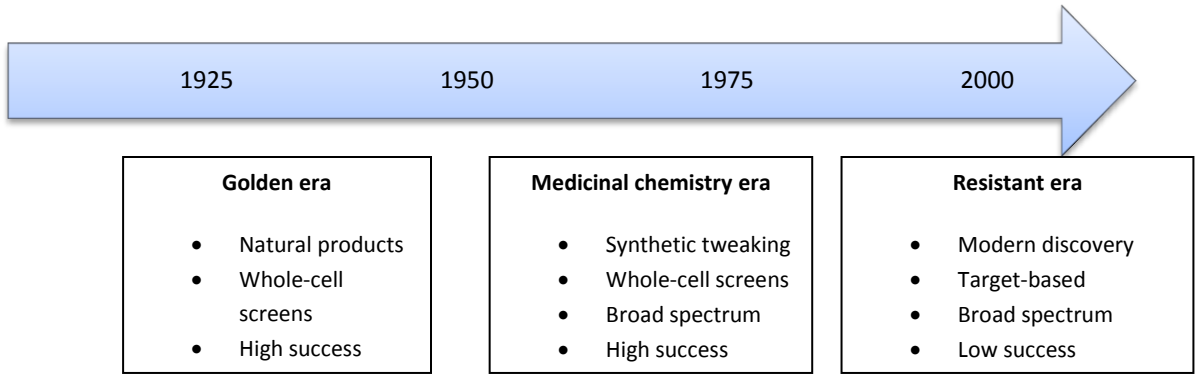


Figure 1.2. Models of antibiotic drug discovery and development with respect to the prevalent methods of each era. Adapted by permission from Macmillan Publishers Ltd: Nature (Brown, E.D. and Wright, G.D., *Nature*, **2016**, *529*, 336-343), copyright 2016.

1.3.1 Target-oriented synthesis and combinatorial chemistry (and the emergence of a genomics-derived, target-based HTS approach)

In target-oriented synthesis, small molecules are screened against a preselected protein target to identify hits. The small molecules can either be natural products or libraries of drug candidates in pharma.[15] Once a target compound is identified, retrosynthetic analysis is used to plan its synthesis from a structurally simple starting material.[15] In target-oriented synthesis, only a single target structure with known or predicted properties is synthesised and thus, only one precise region in chemical space could be accessed.[16] This was often a limiting factor in designing an antibiotic that could not only permeate the bacterial cell wall but also be orally administered to humans.

In combinatorial chemistry, a dense yet wider region of chemical space is explored that could either be inspired by a natural product or a known drug. The goal is to synthesise chemical analogues of the target structure with known or predicted properties.[16] This approach became very popular as it facilitated the synthesis of large libraries of compounds in a short time span that could be screened against protein targets or whole-cells.

However, it also became evident that the bacterial cellular targets of the clinically available antibiotics were confined to a short list, and thus, pathogenic strains could easily develop resistance.[17] The development of a genomics-derived approach helped identify those underexploited, uniquely bacterial targets from a genome sequence and screen a library of compounds in search of novel leads in an effort to overcome the resistance problem.[17] Thus, pharmaceutical companies were keen to apply this new approach to identify possible leads from combinatorial chemical libraries.

Between 1995 and 2001, the SmithKline-Beecham compound collection comprising of 260,000 to 530,000 compounds were screened against antibacterial targets in 67 HTS campaigns, but only 5 leads were discovered.[17] The low return in this approach was also experienced by 34 other different companies.[18]

According to Payne and co-workers, one main reason why the compound libraries of many pharmaceutical companies failed in generating promising results was the lack of chemical diversity.[17] The compound collections of different companies had considerable overlap as they were prepared using a combinatorial chemistry approach with similar starting material and chemical synthesis methodologies that are applicable in combinatorial chemistry. As a result, these compound libraries occupied a narrow and different chemical space compared to marketed drugs and optimization of leads into a marketable antibiotic required a large and long term effort. Thus, it seemed that a broader molecular diversity and better understanding of physicochemical properties was necessary to select good hits worthy of optimization.[17]

1.3.2 Diversity-oriented synthesis (DOS) approach

A diversity-oriented approach to library design was introduced with the aim of accessing chemical structure space more broadly. The DOS approach uses forward-synthetic planning to generate more complex and diverse products from simple and similar starting materials.[16] A

key difference is that the selected molecular skeletons are more spherical or three-dimensional as opposed to the relatively flat molecules generated from the combinatorial chemistry approach.[16]

In DOS, synthetic routes are limited to about five steps to maximize synthetic efficiency. Thus, tandem reactions are designed where the product of one reaction is the substrate for the subsequent step, adding complexity at each step, thereby increasing complexity of the molecule in a very efficient manner.[19]

Structural diversity can be introduced in DOS libraries via skeletal diversity (the presence of many distinct scaffolds), appendage diversity (variations in substituents around the skeleton), functional group diversity (variation in the functional groups present) and stereochemical diversity (synthesising different stereoisomers to access different binding patterns at the target).[19–21]

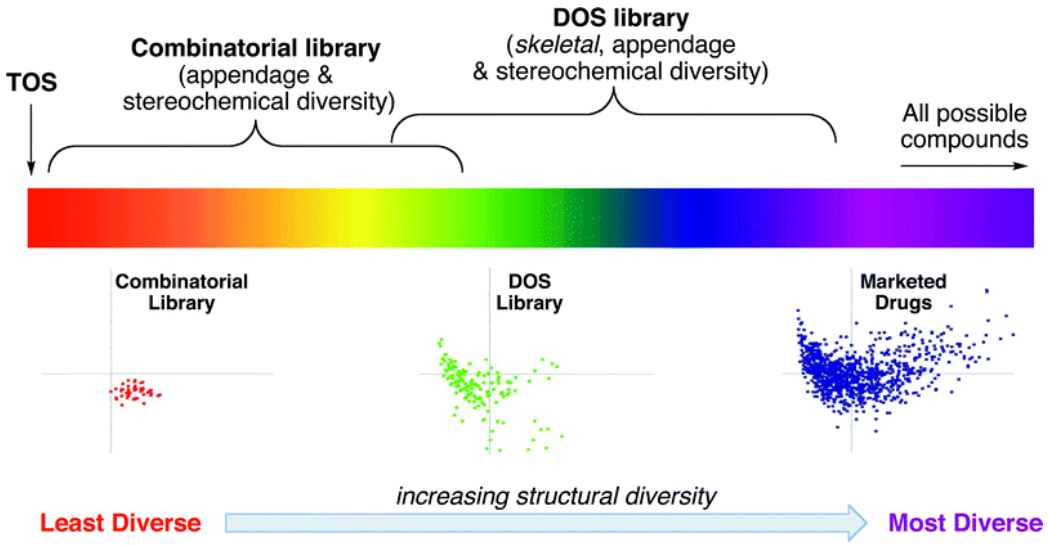


Figure 1.3. The 'molecular diversity spectrum', ranging from a target-oriented synthesis of a specific molecule to the synthesis of all possible compounds. Combinatorial chemistry approach and DOS will produce compound collections between these two extremes. The 2D molecular diversity spectrum is created using principal component analysis of different physicochemical and topological chemical descriptor properties, with two principal components plotted on x and y axes (for details, see reference) Reproduced from Galloway, W.R.J.D., Bender, A., Welch, M. and Spring, D.R., *Chem. Commun*., **2009**, 2446-2462 with permission of the Royal Society of Chemistry.

Figure 1.3 illustrates the ability of a DOS library to cover a broader chemical space compared to the other two approaches. The underpinning rationale of the DOS approach is that the more diverse the chemical space, the higher are the chances of identifying drug-like leads. This gives the ability to access untapped or unexplored chemical space in the discovery of biologically active small molecules.

1.3.3 Fragment-based drug discovery (FBDD)

While the DOS approach involves an unbiased library design where the target protein is not preselected, fragment-based drug discovery (FBDD) uses the identification of suitable fragments by screening against a preselected target protein as a key starting point. This fragment is then elaborated into lead compounds (Figure 1.4).[22]

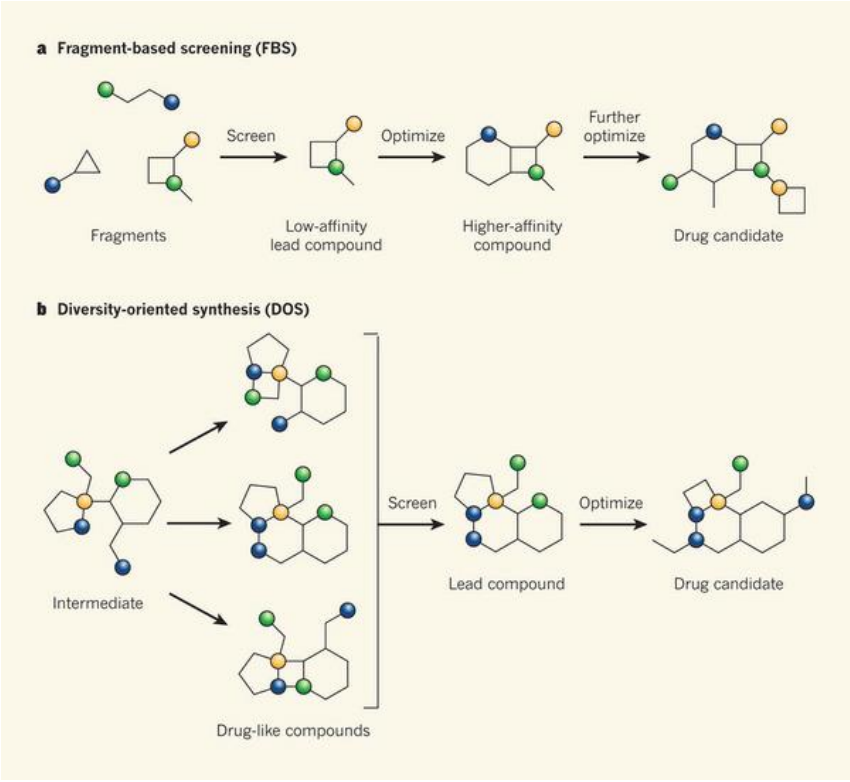


Figure 1.4. A comparison of (a) Fragment-based approach and (b) Diversity-oriented approach in generating lead compounds for drug discovery. Reprinted by permission from Macmillan Publishers Ltd: Nature (Hajduk, P.J., Galloway, W.R.J.D. and Spring, D.R, *Nature*, **2011**, *470*, 42-43), copyright 2011.

The starting fragments are low-molecular mass molecules which can be optimized into potent small molecule ligand. These fragments typically obey the 'Rule of Three' where the molecular mass is < 300 Da, and there are up to 3 hydrogen bond donors, up to 3 hydrogen bond acceptors and a cLogP of ≤ 3.[23] This acts as a good starting point in selecting fragments, since it provides ample scope for derivatisation without leading to excessive molecular weight. These validated fragment hits are then chemically elaborated. Hence, it is important that the fragments have functionality that will facilitate chemical modification.

It is vital to identify drugs that are not only active at a target biomolecule, but also have the correct physicochemical properties for bacterial cell penetration and oral bioavailability. The optimized drug candidates in this approach are tailored constructions of fragments that are soluble and of low molecular mass. Therefore, there is greater potential of discovering the smallest possible structure with very high target interaction. Thus, proponents of this strategy believe that FBDD is a better approach for the efficient synthesis of drug-like molecules.[22,23]

1.3.4 Natural product guided drug discovery

Natural products have proven to be a powerful source of leads in the field of drug discovery. During the history of the discovery of the major classes of antibiotics, natural products have provided the starting point for a majority of the major antibiotic classes; β-lactams, aminoglycosides, macrolides, tetracyclins, rifamycins, glycopeptides, streptogramins and lipopeptides.

Nonetheless, the combinatorial chemistry approach gained wide popularity in drug discovery and as a result, there was a decline in the focus on natural products. However, given that only one *de novo* combinatorial compound 'sorafenib' (an antitumor drug) was approved as a drug

during the 25 plus years time frame, the focus is being shifted back to designing drugs containing the 'structural aspects' of natural products.[24,25]

In a review paper published by Butler et al. in 2013, it was reported that out of 22 new antibiotics which have been launched since 2000, 2 are natural products and 10 are natural product-derived.[24] Further, out of the antibiotics reported to be undergoing clinical trial at that time, 19 had new structural templates out of which 11 were related to natural products.[26,27] This outcome was even more remarkable given the decreased emphasis of pharma on the natural product-based drug discovery approach. More recently, in 2015, the discovery of 'teixobactin' was reported,[28] which too is a natural product belonging to a new class of antibiotics, and may lead to a resurgence of interest using this particular approach.

Newman et al. have classified the 104 small molecule antibacterial drugs introduced between 1981 and 2010 based on their source of discovery.[25] Their data can be put into a graphical representation as below in Figure 1.5, where 75 % are either natural products or natural product inspired antibiotics.

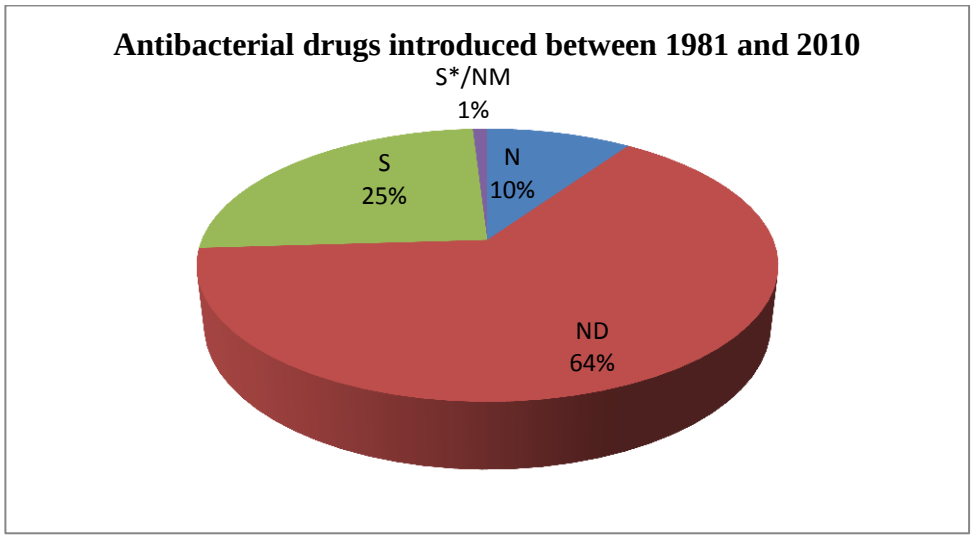


Figure 1.5. Antibacterial drugs introduced between 1981-2010 categorized based on their source; N = Natural product, ND = Natural product-derived, S = Synthetic, S*/NM = Synthetic source but originally modelled on a natural product parent to give a natural product mimic.

The intrinsic utility of natural products as an attractive source of drug leads can be related to their use by organisms as a defence mechanism for survival in the environment. Moreover, since this activity also comes with built-in bacterial cell wall permeability, they are very likely to offer a more viable starting point for antibacterial drug discovery.[29]

1.3.4.1 Biology-oriented synthesis (BIOS)

Inspired by the higher activity of natural products, Waldmann et al. introduced the concept of library design around the core structure (scaffold) of natural products, instead of total synthesis. This approach afforded the ability to generate 'biologically relevant libraries' that have more "drug-likeness" and therefore generate more leads.[30] The idea was centred around the identification of compound classes from biologically relevant structural space as validated starting points.

Thus, a 'structural classification of natural products' (SCONP) was introduced by Waldmann and co-workers that charts the chemical space explored by nature, using the dictionary of natural products (DNP) as the basis for the analysis.[31] These natural product structures were subjected to iterative deconstructions to arrive at a smaller scaffold known as the 'parent scaffold' and a natural product scaffold tree was constructed.[31] This scaffold tree provides guidance for the design of natural product derived compound libraries; if the parent scaffold can be related to an active natural product, then appropriate functionalization might also give biological activity.

Natural products interact with protein targets. Proteins can be regarded as an assembly of domains. The 3D structure of proteins is determined by the arrangement of the secondary structures such as α helices and β sheets introducing characteristic fold types of each protein domain. The domain folds are more conserved since a given fold type can be formed from different amino acid sequences.[19] Thus, there are only a limited number of domains with

which ligands can interact. However, since the different amino acid sequences underlying the domain structure can determine the ligands that can be bound, similar domain structures can bind a diverse set of ligands. Thus, the Waldmann group put forward a second approach to library design known as 'protein structure similarity clustering' (PSSC), where the ligand that binds to a member of a protein cluster might also bind to other members of the same protein cluster.[32]

For efficient ligand-protein interactions, the ligand structure needs to be complementary to the binding pocket of the protein. If the ligand or framework for a certain domain family is known, then the core scaffold of the ligand can be used as a starting point to generate a library of similar ligands by varying the functionality in the periphery of the scaffold. The library is then assayed to identify hits that can then be optimized into lead structures. Since this approach taps into the biologically relevant structure space, a high hit rate with reduced library size can be expected.[30] Since the design of libraries of such ligands is inspired by natural products, it is given the term 'biology-oriented synthesis' (BIOS).[19,30] However, even though BIOS was initially developed by the analysis of natural product structures, the concept behind this approach is accessing biologically relevant structure space rather than natural occurrence. Thus, BIOS can even be extended to gain inspiration from drugs that are not natural products.[32]

Applying the BIOS strategy, the Waldmann group has synthesised several libraries derived from natural products, e.g. a library of macroline-derived compounds containing the cycloocta[*b*]indole scaffold developed in search of inhibitors for *Mycobacterium tuberculosis* protein tyrosine phosphatase-B (MptpB)[33,34] (Figure 1.6).

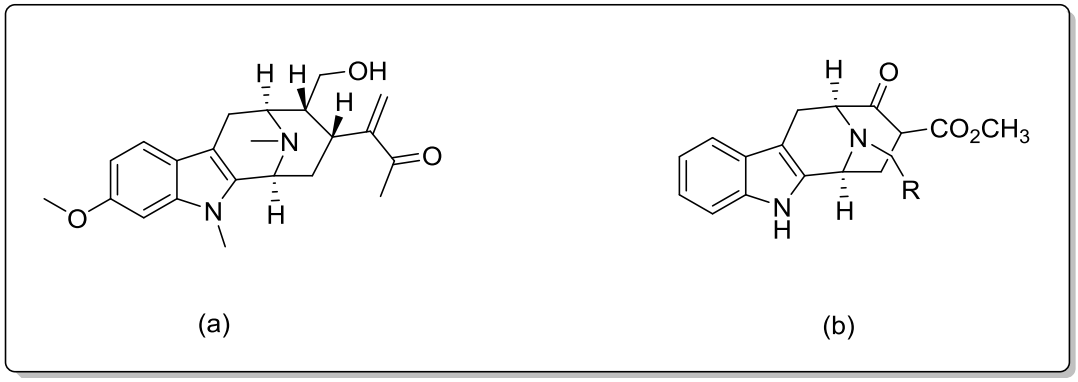


Figure 1.6. (a) Macroline and (b) Macroline-derived compound library from BIOS approach.

1.4 Tetramic acids

Tetramic acids attracted much interest in the 1960s when many biologically active natural products bearing the tetramic acid ring system were isolated.[35] Tetramic acid is a pyrrolidine-2,4-dione ring system **1** that in nature is commonly found as a 3-acyl derivative or sometimes as the 4-*O*-alkyl ether derivative. The structural diversity and complexity of tetramic acids vary from simple heterocycles to more complex systems. They display a very broad range of biological activities such as antibiotic, antiviral and antitumor properties making them attractive scaffolds in natural product-derived drug discovery. The naturally occurring tetramic acids can be subdivided based on the substituents on the core structure and some examples with antibiotic activity are listed below.

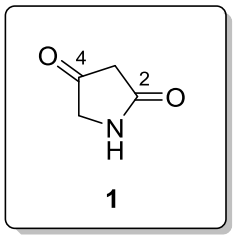


Figure 1.7. Pyrrolidine-2,4-dione ring system **1**.

1.4.1 3-Acyl tetramic acids

One of the simplest 3-acyl tetramic acids to be isolated was L-tenuazonic acid **2**. Although originally isolated from a culture filtrate of *Alternaria tenuis*, this compound was later isolated from several other fungal strains.[35–37] Despite its antibiotic activity, among a broad spectrum of biological activities, L-tenuazonic acid had limited use due to high toxicity.[35]

Epicoccarines **3A** and **3B** were isolated from the *Epiccocum* sp. Epicoccarine A showed activity against *Mycobacterium vaccae* (MIC = 6.25 μg mL^{-1}), while Epicoccarine B showed only moderate activity against Gram-positive bacteria.[38]

Reutericyclin **4** was isolated from *Lactobacillus reuteri*, that exhibits potent antibiotic activity against a range of Gram-positive strains with MIC as low as 0.06 μg mL^{-1}. The cellular target of reutericyclin is the cytoplasmic membrane where it acts as a proton ionophore, thereby dissipating the transmembrane ΔpH.[39,40]

Vancoresmycin **5**, a tetramate derivative with a highly oxygenated long alkyl chain was isolated from the broth of *Amycolatopsis* sp. The absolute configuration for this compound is not yet determined. Vancoresmycin shows potent antibacterial activity against Gram-positive strains *Staphylococcus aureus* (MIC ≤ 0.04 μg mL^{-1}), *Streptococcus pneumoniae* (MIC ≤ 0.04 μg mL^{-1}) and *Enterococcus faecalis* (MIC ≤ 0.6 μg mL^{-1}).[41]

Virgineone **6**, a 3-acyl tetramate with a terminal glycone was isolated from *Lachnum* spp. and is reported to possess antifungal activity and also antibacterial activity against Gram-positive *S. aureus* including several MRSA isolates (MIC = 7.2 μg mL^{-1}), while its aglycone counterpart **7** demonstrated slightly higher potency with MIC ranging from 2.8-5.5 μg mL^{-1}.[42]

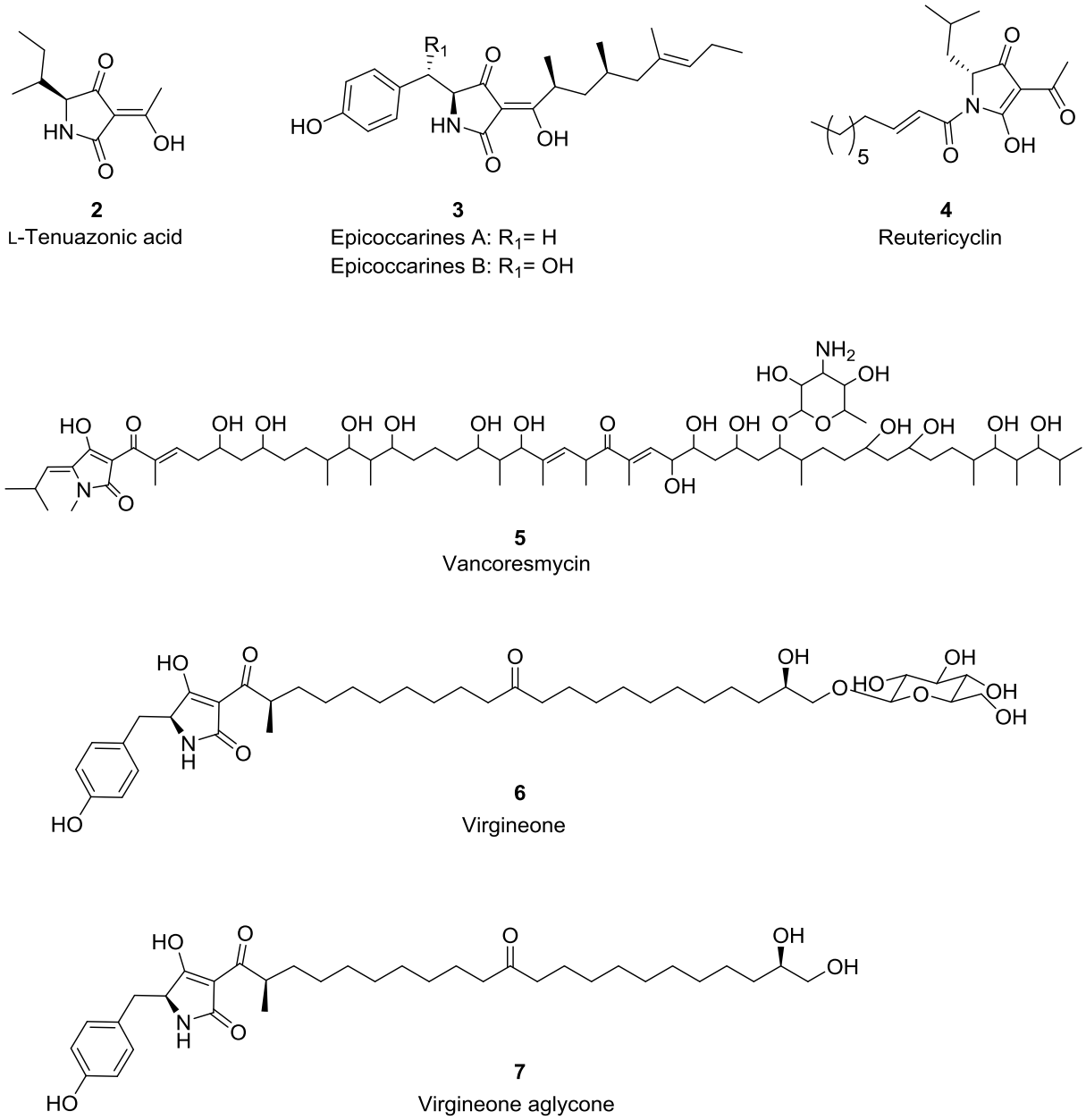


Figure 1.8. Examples of 3-acyl tetramates.

1.4.2 3-Decalinoyltetramic acids

Equisetin **8** was the first tetramate to be isolated in this class from *Fusarium equisetin.* It was found to display a broad range of biological activities including antimicrobial, HIV inhibitory and mammalian DNA intercalation.[37,43]

Altersetin **9**, isolated from endophytic *Alternaria* spp. showed good antibiotic activity against *S. aureus*, *S. pyogenes*, *S. pneumoniae* and *E. faecalis* with MICs in the range of 0.12-2 µg mL^{-1}. Altersetin showed moderate *in vivo* efficacy in a *S. aureus* infected mouse model in concentrations of 10 or 25 mg/kg. Below 10 mg/kg, altersetin showed no *in vivo* efficacy.[44]

Zopfiellamides **10A** and **10B** were isolated from the marine fungus *Zopfiella latipes* and found to have moderate antibacterial activity against a range of Gram-positive strains including *Arthrobacter citreus*, *Bacillus brevis*, *B. subtilis*, *B. licheniformis*, *Corynebacterium insidiosum*, *Micrococcus luteus*, *Mycobacterium phlei*, *Streptomyces* sp. and the Gram-negative *Acinetobacter calcoaceticus* with MICs between 2-10 µg mL^{-1} for Zopfiellamide A, while Zopfiellamide B was comparatively less active.[45]

In 2012, the isolation of Signermycin B **11** from *Streptomyces* sp. and its antibacterial activity was reported. This tetramate exerted activity against Gram-positive *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Streptococcus mutans* including MRSA and vancomycin resistant *E. faecalis* with MIC values in the range of 3.13-6.25 µg mL^{-1}. Signermycin B is reported to target the dimerization domain of Histidine Kinase WalK involved in cell wall metabolism and growth.[46]

Kibdelomycin **12**, isolated from *Kibdelosporangium* sp. was only discovered in 2011. It is reported to display broad spectrum Gram-positive antibacterial activity and inhibition of DNA synthesis.[47] It shows potent activity against *S. aureus*, *S. pneumoniae* and *E. faecium* including several of their drug-resistant strains with MICs of 0.25-1 µg mL^{-1}. However, in the presence of 50 % human serum, this activity was greatly reduced.[47] This tetramate is also reported to show activity against the Gram-negative *Acinetobacter baumannii* (MIC = 0.125 µg mL^{-1}).[48] Kibdelomycin belongs to a novel class of bacterial type II topoisomerase inhibitors which shows inhibition of topoisomerase IV and DNA gyrase. It demonstrated more potent inhibition of *S. aureus* gyrase supercoiling (IC_{50} = 9 nM) and *E. coli* DNA gyrase

ATPase activity (IC_{50} = 11 nM). Inhibition of topo IV was much weaker, as is observed for the known novobiocin antibiotic. Kibdelomycin showed *in vivo* efficacy at a 6.25 mg/kg twice-daily dose in a *C. difficile* infected hamster model with complete elimination of detectable *C. difficile* counts in cecal contents.[47,49]

A kibdelomycin analogue, amycolamicin **13** was isolated from actinomycete *Amycolatopsis* sp. It is an inhibitor of bacterial type II topoisomerases and shows potent antibacterial activity against several drug-resistant Gram-positive strains (MIC = 0.125-2 µg mL^{-1}) and some Gram-negative strains such as *E. coli*, *K. pneumoniae* and *P. aeruginosa* (MIC = 8 µg mL^{-1}).[50]

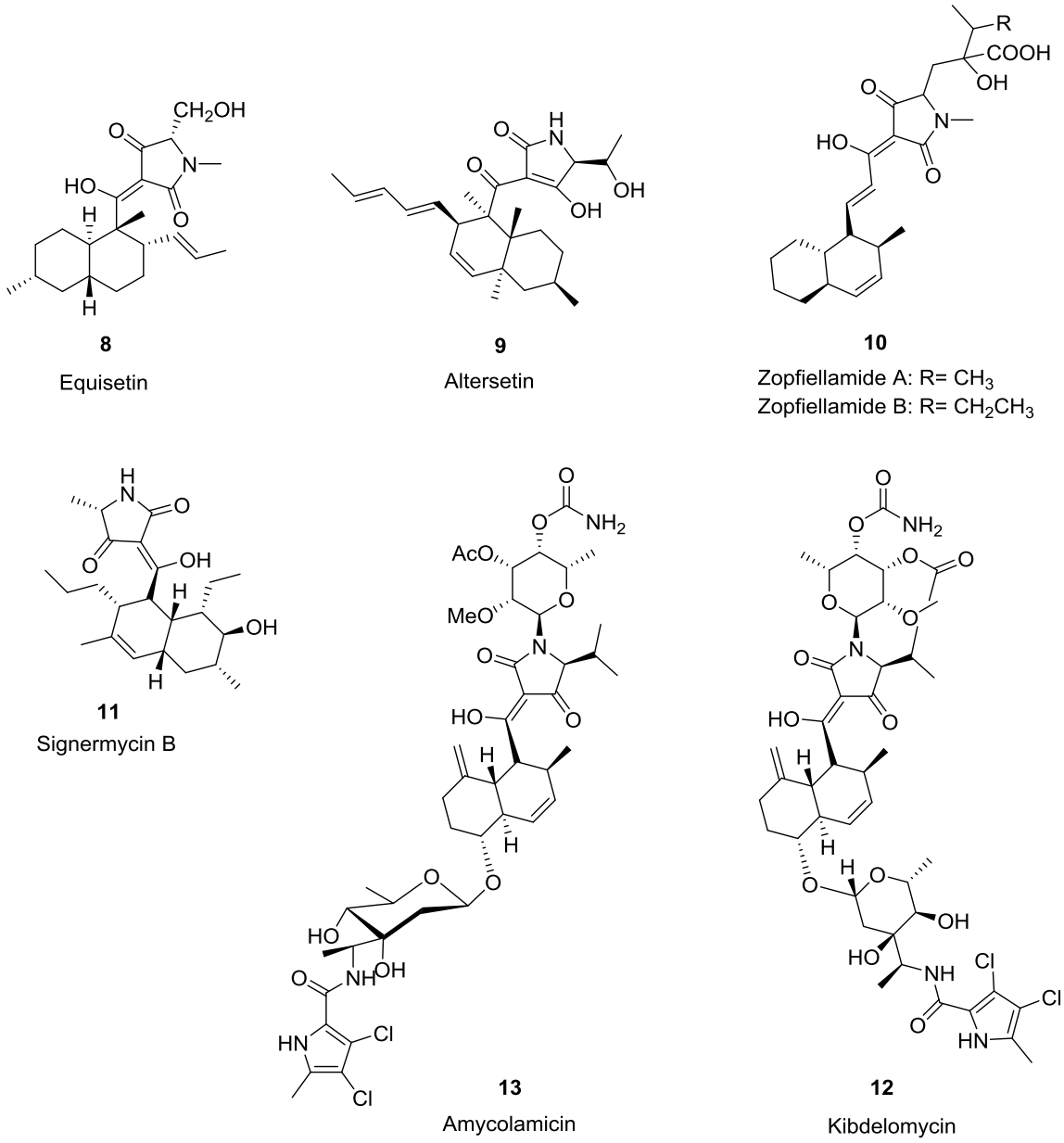


Figure 1.9. Examples of 3-decalinoyltetramic acids.

1.4.3 Dienoyltetramic acids

Streptolydigin **14** was isolated from *Streptomyces lydicus* in 1955. It is a glycosylated acyl tetramate linked to an epoxide containing bicyclic ketal via a diene chain. Notable antibacterial activity against a broad spectrum of Gram-positive strains was observed; including *Clostridium* and *Streptococcus* sp. with MICs as low as 0.04 μg mL^{-1}.

Streptolydigin exerts its antibacterial activity by inhibiting RNA polymerase enzyme, and thus interfering with RNA chain elongation during transcription. The enzyme-binding site of the tetramate is different to that of the rifamycin binding site, which is also a known inhibitor of RNAP. Thus, due to the unique biochemical interactions, cross resistance is minimal.[51] An evaluation of *in vivo* efficacy showed that the subcutaneous administration of streptolydigin **14** at doses of 100-330 mg/kg/day ptotected mice infected with *S. hemolyticus*, *S. pneumoniae* and *P. multocida*.[51]

Tirandamycin **15** are structural analogues of streptolydigin. The first of these was isolated in the 1970s and since then, a series of tirandamycins have been isolated from terrestrial and marine *Streptomyces* strains. These lack the sugar moiety and the acetamide substituent on the tetramate ring compared to streptolydigin, which is possibly responsible for the reduced potency observed for tirandamycins. It inhibits RNA polymerase.[52] Tirandalydigin **16** is another dienoyl tetramate isolated from *Streptomyces* sp. and possesses structural features common to both streptolydigin and tirandamycins.[53]

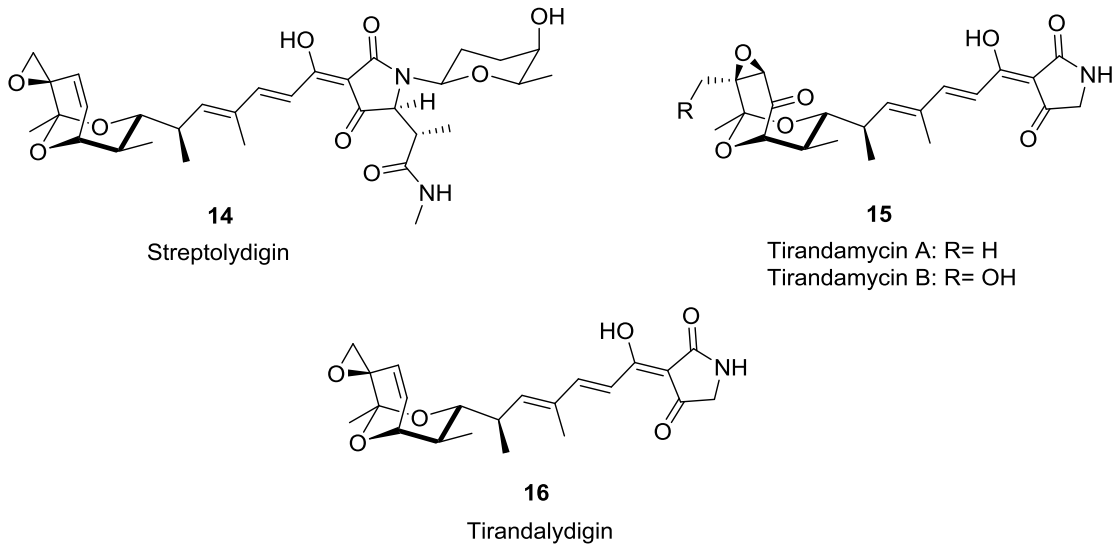
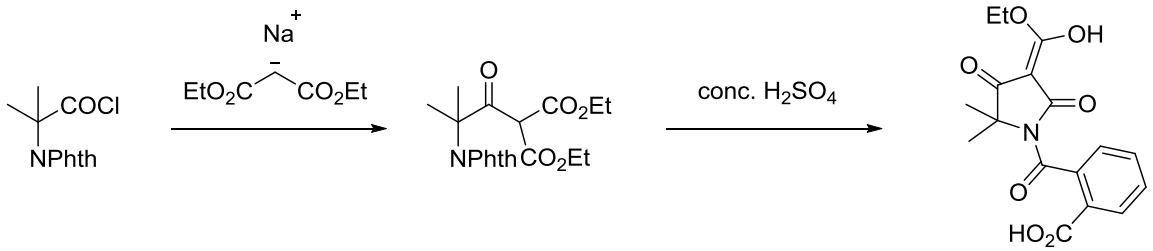


Figure 1.10. Examples of dienoyltetramic acids.

1.5 Synthetic routes to tetramic acids

1.5.1 Gabriel's method of ring closure by C-N bond formation

The first synthetic route to a tetramic acid derivative was reported by S. Gabriel in 1914 using phthalimidoisobutyryl chloride **17** and diethyl sodiomalonate as reactants.[35] Ring closure by C-N bond formation led to the tetramic acid derivative **19**, Scheme 1.1.

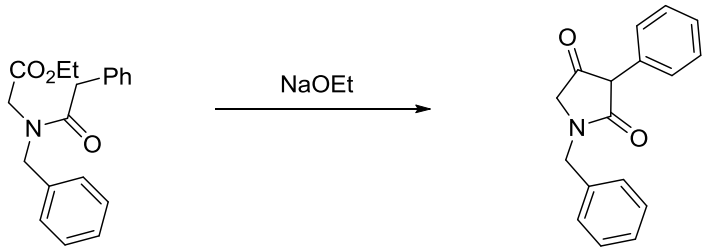


17 **18** **19**

Scheme 1.1. Synthesis of tetramic acid derivative by S. Gabriel.

1.5.2 Ring closure by Dieckmann cyclisation

In 1950, King and McMillan applied Dieckmann cyclisation to access tetramic acid derivative **21** from *N*-acyl-α-amino ester **20**, Scheme 1.2.[54]

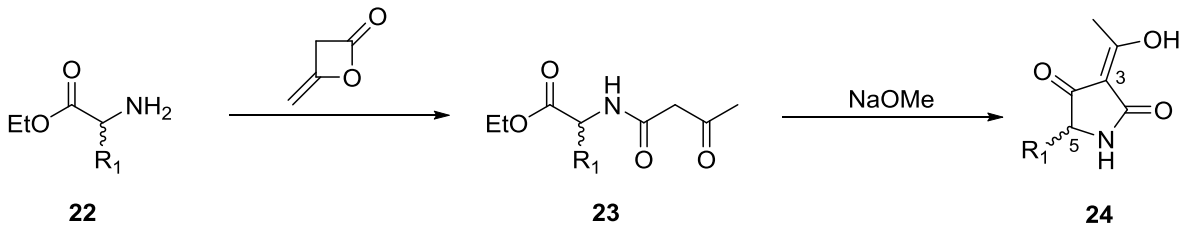


20 **21**

Scheme 1.2. An example of ring closure by Dieckmann cyclisation.

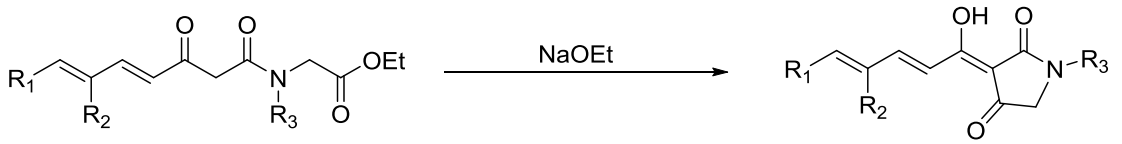
1.5.3 3-Acyltetramic acids *via* Lacey's modified Dieckmann cyclisation

The application of Dieckmann cyclisation was advanced by Lacey in 1954 to synthesise 3-acyltetramic acids from readily available α-amino esters. It is a convenient two-step synthesis where the amino ester **22** is treated with diketene to produce the amide **23**, which then leads to the corresponding 3-acyltetramic acid **24** via Dieckmann cyclisation, Scheme 1.3. This method has become widely used in the synthesis of tetramic acids due to the possible variation in substitution at C-5 from the α-amino esters, even though variation at C-3 is restricted by the availability of the substituted diketene. Further, the use of basic conditions can lead to racemization of stereogenic centres with acidic hydrogens.[55]



Scheme 1.3. 3-Acyltetramic acids via Lacey's modified Dieckmann cyclisation.

This method has been adopted to synthesize L-tenuazonic acid **2** from ethyl L-isoleucinate and diketene, but with racemization of the stereogenic center at C-5. Rinehart investigated the applicability of this method for the synthesis of 3-dienoyltetramic acids **27** and **28** from their precursors **25** and **26** in their study of streptolydigin **14** analogues, Scheme 1.4.[56] Racemic tirandamycin **15A** has also been synthesised by Bartlett at al. using the Lacey-Dieckmann approach.[57]



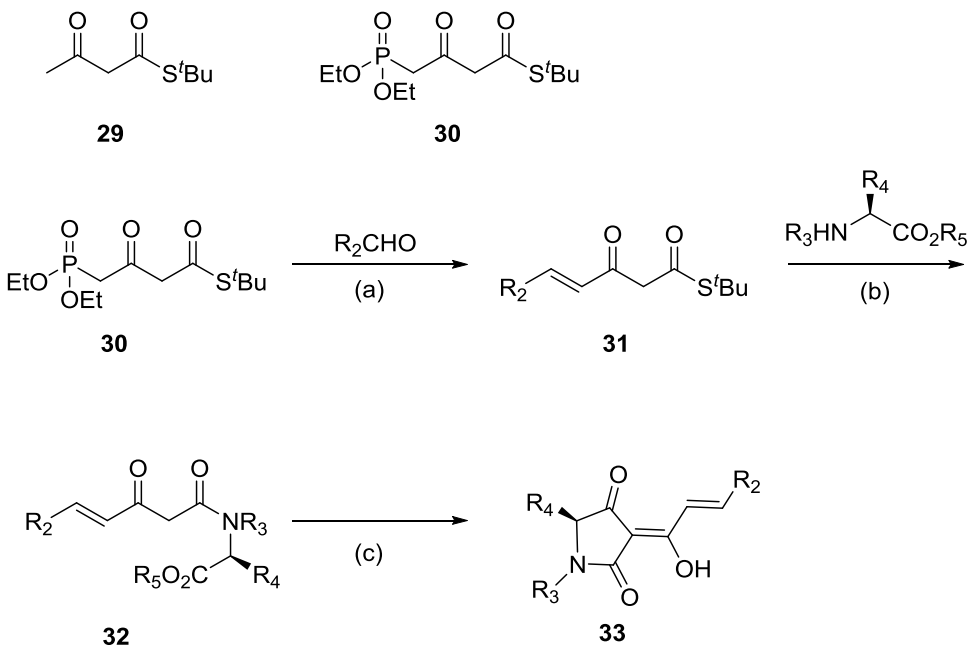
25: R_1=Me, R_2=H, R_3=Bnz
26: R_1=*i*-Pr, R_2=Me, R_3=Bnz

27: R_1=Me, R_2=H, R_3=Bnz
28: R_1=*i*-Pr, R_2=Me, R_3=Bnz

Scheme 1.4. Rinehart's synthesis of 3-dienoyltetramic acids.

1.5.4 3-Acyltetramic acids by Ley and co-workers

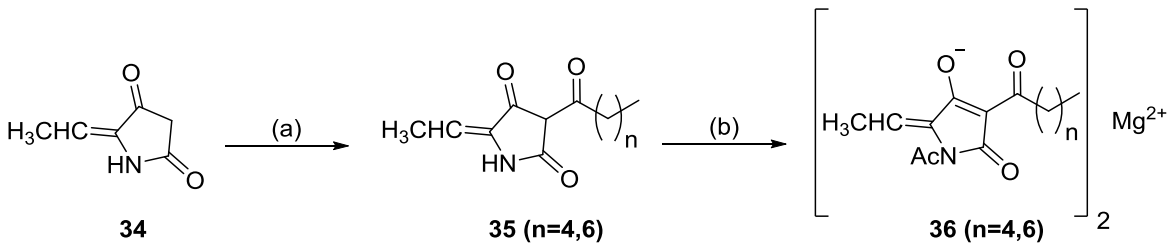
In 1992, Ley and co-workers reported a stereo-controlled synthesis of 3-acyltetramates, which was a significant advancement of the Lacey-Dieckmann approach. Using *t*-butyl-3-oxobutanethionate **29** and its 4-diethylphosphono derivative **30**, a range of β-ketoamides were synthesised as suitable precursors for 3-acyltetramic acids. Horner-Wadsworth-Emmons coupling reaction of **30** with various carbonyl compounds was employed to generate various *E*-substituted products **31**. Amination of **31** afforded the corresponding β-ketoamides **32**. Cyclisation of these β-ketoamides in the presence of TBAF or KO^{t}Bu resulted in tetramic acids **33** with complete retention of configuration, Scheme 1.5.[58]



Scheme 1.5. Stereo-controlled synthesis of 3-acyl tetramates. (a) NaH, 0 °C; (b) AgO_2CCF_3; (c) TBAF or KO^{t}Bu.

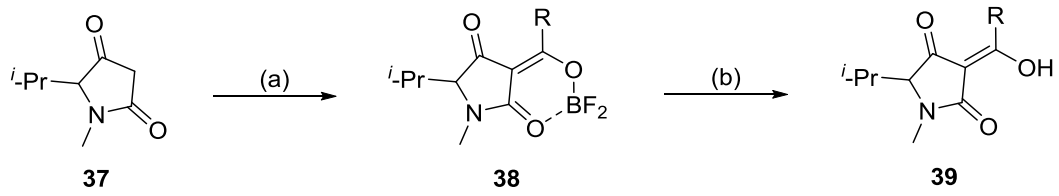
1.5.5 C-3 Acylation of the tetramate ring

Since a vast number of tetramates have a 3-acyl substitution, synthesis of the pyrrolidine-2,4-dione ring followed by acylation at C-3 seemed to be a more reliable method for the synthesis of more complex tetramates. This technique was first exploited by Kohl in 1974 for the synthesis of Magnesidin **36**.[59] The tetramate ring **34** was formed through Lacey-Dieckmann cyclisation from DL-threonine, which was then acylated at C-3 using an acyl chloride, Scheme 1.6.



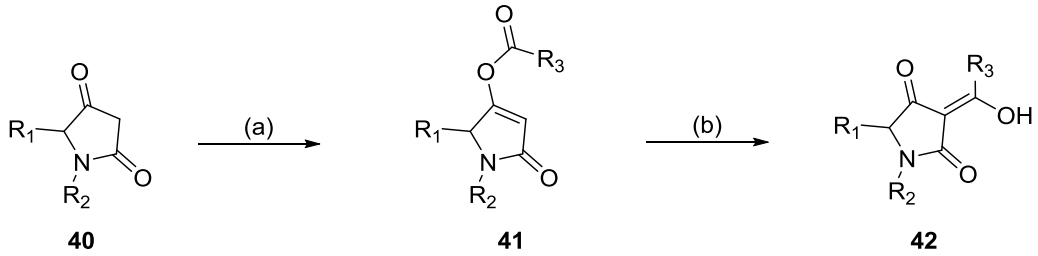
Scheme 1.6. Synthesis of Magnesidin **36** by Kohl et al. (a) $CH_3(CH_2)_nCOCl$, $BF_3.OEt_2$; (b) (i) 0.1 N NaOH; (ii) $MgCl_2$; (iii) Ac_2O, NaOAc.

This method was further investigated by Jones and co-workers, where C-3 acylation was successfully achieved with $BF_3.OEt_2$ followed by neutral workup conditions. The tetramic acids were isolated as their boron difluoride complexes **38** in good yield, which on treatment with methanol yielded tetramic acids **39**, Scheme 1.7.[60]



Scheme 1.7. Jones's method of C-3 acylation (a) RCOCl, $BF_3.OEt_2$; (b) MeOH.

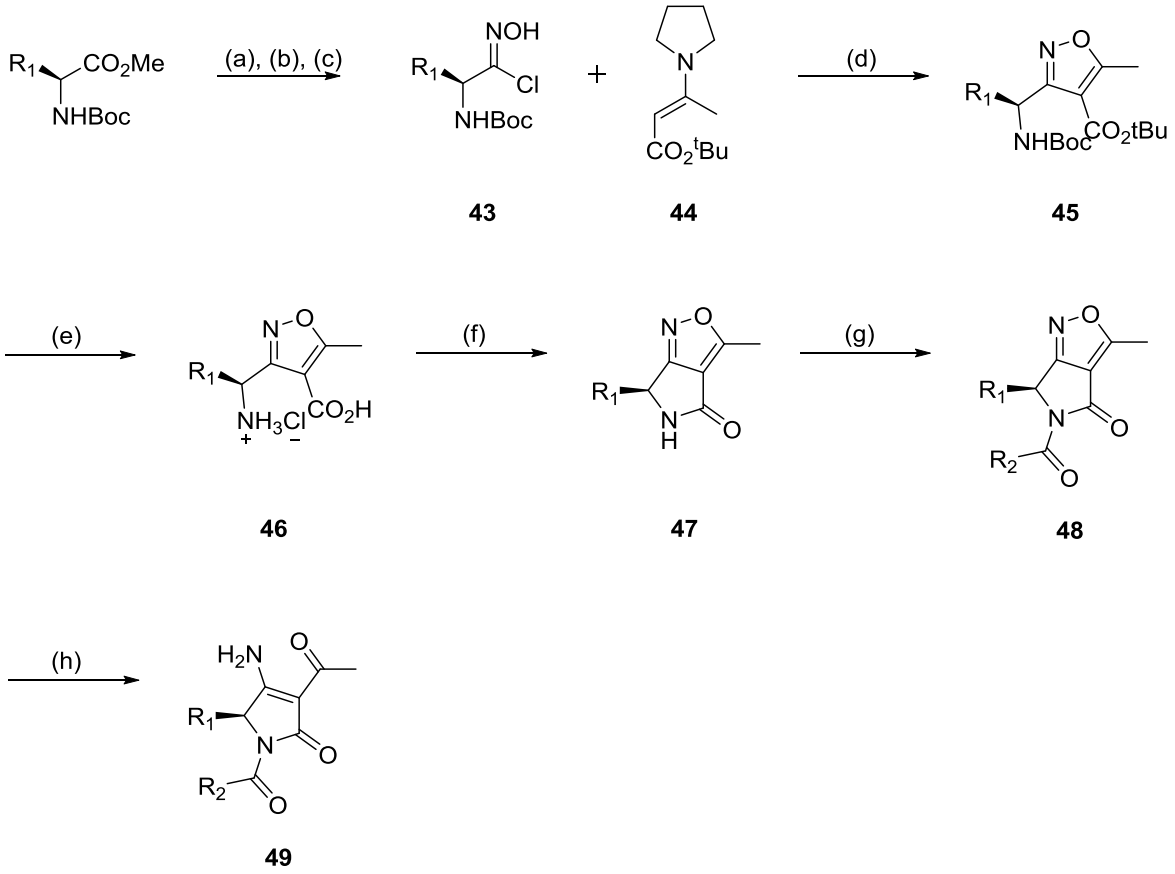
In 1987, Yoshi and co-workers described a base-mediated 'one pot' acylation procedure that gives 3-acyltetramates in excellent yields.[35] The tetramic acids **40** were acylated at *O*-4 to yield tetramate esters **41**. Treatment of these esters with Et_3N resulted in their rearrangement to the required 3-acyltetramates **42**, Scheme 1.8.



Scheme 1.8. Yoshi's approach to C-3 acylation (a) R_3CO_2H, DCC, DMAP; (b) Et_3N.

More recently, Jones and co-workers reported a strategy leading to 3-acyltetramic acids using pyrroloisoxazoles as masked tetramic acids and templates for peripheral elaboration in their efforts to develop reutericyclin analogues.[61] Commercially available methyl esters of amino acids (*S*-valine, *S*-leucine, *S*-phenylglycine) were converted to their C-chloro-oximes **43** which undergoes cycloaddition to **44** via a nitrile oxide generated *in situ* to afford isoxazole **45**. The simultaneous deprotection of *N*-Boc amine and ester hydrolysis led to **46**, which can

then be cyclised to the lactam **47**. These pyrroloisoxazoles can be *N*-acylated and subsequent N-O bond cleavage of the pyrroloisoxazole nucleus leads to the 3-acyltetramic acid derivatives **49**, Scheme 1.9.[61]



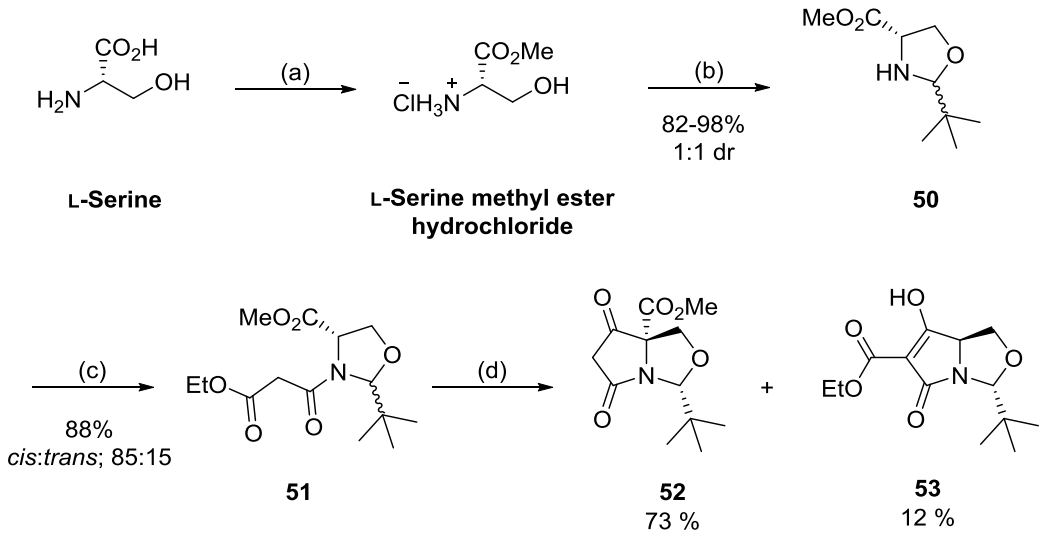
Scheme 1.9. Jones's approach to 3-acyl tetramic acids. (a) DIBAL-H, toluene, -78 °C; (b) $H_2NOH.HCl$, NaOAc, aq. EtOH, 2-8 °C; (c) NCS, $CHCl_3$, reflux, 18 h; (d) Et_3N, $CHCl_3$, reflux; (e) TFA, 20 °C, 2M HCl; (f) T3P, EtOAc, 0-20 °C, 17 h; (g) BuLi, THF, -78 °C; R_2COCl; (h) H_2 (1 atm), Pd/C, EtOH or $Mo(CO)_6$, aq. MeCN; 2M HCl.

1.6 Tetramic acids: Moloney group's research work

1.6.1 Synthesis of the tetramate core

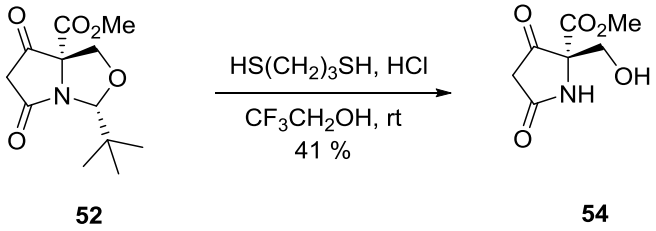
The Moloney group successfully developed an alternative approach towards the synthesis of chiral tetramic acids inspired by Seebach's "Self-regeneration of Stereocenters".[62,63] The L-serine-derived oxazolidine **50** was acylated with monoethyl malonate to give the *N*-acyl

derivative **51**. Subsequent Dieckmann cyclisation yielded tetramic acids **52** and **53**, which were separable in an aqueous workup, isolating **52** as the major product of cyclisation in 73 % yield, while **53** was isolated in 12 % yield, Scheme 1.10.[64]



Scheme 1.10. Synthesis of the tetramic acid template **52** from L-serine (a) $SOCl_2$, MeOH, reflux, 1h; (b) Et_3N, pivaldehyde, petrol, reflux, 18 h; (c) Monoethyl malonate, DCC, DMAP, CH_2Cl_2, 3-5 h; (d) KO^{t}Bu, ^{t}BuOH, reflux, 3h.

The *N*,*O*-acetal deprotection of the bicyclic tetramate **52** thus obtained could be achieved using the Corey-Reichard conditions to yield the monocyclic tetramate **54**, Scheme 1.11.[65]



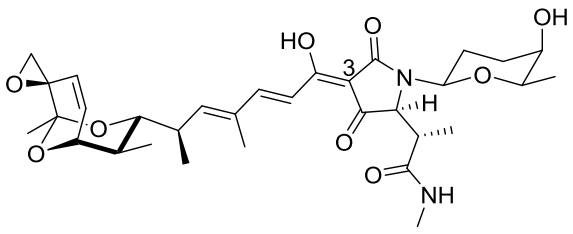
Scheme 1.11. The deprotection of *N*,*O*-acetal to yield monocyclic tetramate core **54**.

An assay to test the bioactivity of both the L-serine derived bicyclic and monocyclic tetramate core has shown that the tetramic acid core alone does not possess antibacterial activity against Gram-negative *E. coli* or Gram-positive *S. aureus*, but appropriate functionalisation leads to

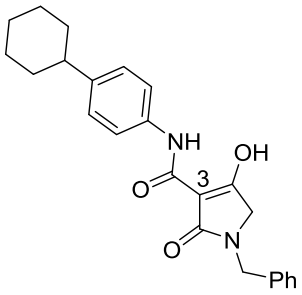
significantly improved activity.[65] Thus, research work by the Moloney group has shown that the tetramate core acts as a suitable template in antibacterial drug discovery.[65–67]

1.6.2 Application towards tetramate library generation

Utilizing the synthetic route to the tetramate core based on Dieckmann cyclisation outlined above, the Moloney group was able to generate a library of 162 analogues based on monocyclic, bicyclic and tricyclic tetramate templates.[66] Bicyclic tetramate templates were synthesized from L-serine, α-methyl-DL-serine, L-threonine and L-cysteine to generate diversity around the tetramate acid core. The design of the library was inspired by Novartis' structure activity relationship (SAR) study for undecaprenyl pyrophosphate synthase (UPPS) enzyme and the chemical structure of natural product streptolydigin **14**.



14

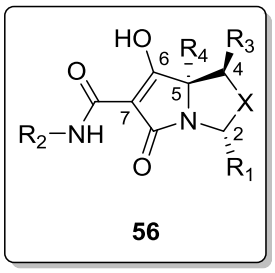


Novartis' analogue
55

Figure 1.11. Streptolydigin **14** and Novartis' analogue **55** containing the tetramic acid core.

Since it was evident that the C-3 substitution of both **14** and **55** were very different, the library was synthesised to probe the effect of various C-7 substitutions, namely, 7-carboxamide, 7-acyl and 7-enamine using the template **56**. These functionalisations at C-7 were introduced on the tetramate core via a late-stage synthetic step, enabling the generation of a wide range of diversity appropriate for library design, while the substituent at C-2 was limited to mainly '^{t}Bu'.

Activity studies indicated that several bicyclic carboxamides of type **56** demonstrated dual enzyme inhibition of both UPPS and RNAP. This was an interesting outcome since these compounds with a single core scaffold could inhibit two enzymes involved in different metabolic pathways, and this might help deter the onset of resistance.[66] Interestingly, the carboxamide pendant of these tetramates with dual enzyme inhibition contained either a lipophilic aliphatic or a bulky aromatic group. A similar observation had been reported in Novartis' SAR study of UPPS binding analogues.[68] Appropriately substituted bicyclic 7-acyltetramic acids also displayed dual enzyme inhibition, albeit with less effectiveness against UPPS. However, 7-enamine derivatives did not exhibit enzyme inhibitory activity, unlike their structurally similar 7-carboxamides and 7-acyltetramate analogues. This suggests that the oxygen atom of the amide/acyl group might be important for favourable ligand-receptor interactions. It was also noted that the enzyme inhibitory activity was only weakly affected by the substitutions at C-4 (H or Me), C-5 (H or Me) and the heteroatom X (S or O).



56

Figure 1.12. Bicyclic carboxamide tetramates.

Whole-cell antibacterial activity of the tetramates were assessed against clinically relevant bacterial strains. Broad spectrum activity was observed against various Gram-positive strains such as MRSA, vancomycin susceptible *S. aureus* and *E. faecalis*, VRE, erythromycin susceptible *S. pneumoniae* and multidrug resistant *S. pneumoniae*. Activity was also observed against the Gram-negative *Haemophilus influenzae*, but not against Gram-negative *E. coli* including an efflux-negative strain, possibly suggesting impermeability to the outer cell membrane.[66]

An evaluation of the capacity to disrupt cell membranes showed that some of these analogues could disrupt bacterial cell membranes, but none of the analogues disrupted mammalian red blood cells (IC_{50} > 100 μM).

Based on the antibacterial activity with multiple enzyme inhibition and physicochemical properties (similar to clinical fluoroquinolones), the Moloney group identified a series of carboxamide tetramates suitable for future drug design and some examples are given in Figure 1.13. Unfortunately, there was a significant reduction in efficacy when tested in a whole-cell assay in the presence of serum (MIC > 64 μg mL^{-1}), indicating high plasma protein binding (PPB) leading to low therapeutic concentrations, thus limiting their application in oral and injectable use; PPB generally correlates with lipophilicity.[66] The compounds were also ineffective in an *in vivo* testing in a mouse model infected with *S. aureus* and it has been suspected that the lack of efficacy could be due to high PPB.[66] However, this has not been investigated in full.

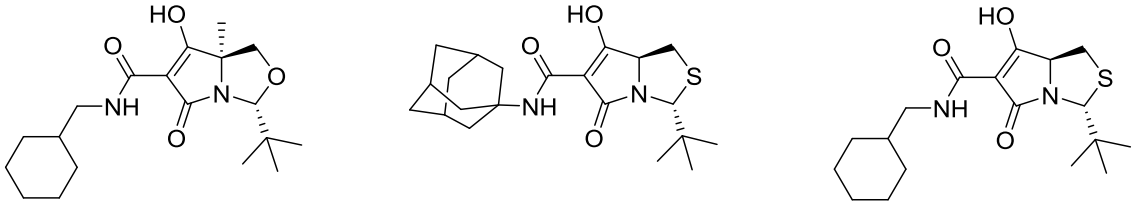


Figure 1.13. Carboxamide tetramic acids prepared by the Moloney group.

1.6.3 Application of tetramic acid chemistry towards other natural products

1.6.3.1 Application towards oxazolomycins

Oxazolomycins **60-62** (Figure 1.14) are structurally novel antibiotic and antiviral agents. They comprise of a polar spirocyclic γ-lactam-β-lactone head group linked to a mid-fragment containing a 3-hydroxy-2,2-dimethylpropanamide moiety. The discovery of inthomycin and

clathrynamide also bearing the 3-hydroxy-2,2-dimethylpropanamide group highlights a possible pharmacological significance attributable to this smaller motif.

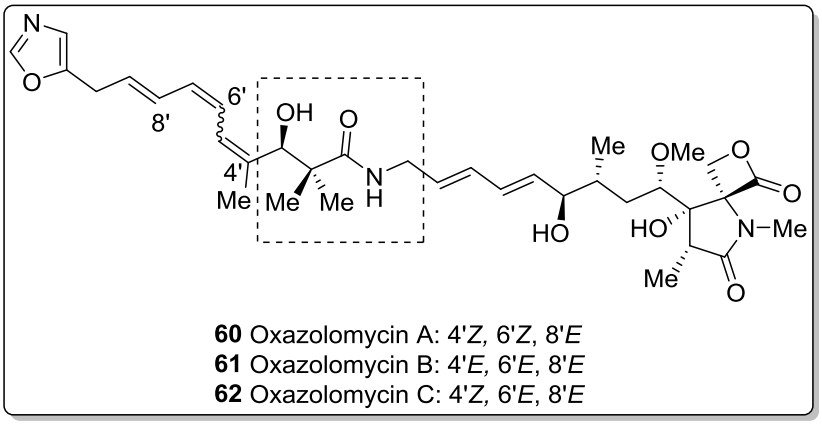


Figure 1.14. Structure of oxazolomycins.

The Moloney group synthesised various derivatives of the 3-hydroxy-2,2-dimethylpropanamide mid fragment **63-67** (Figure 1.15) and evaluated their bioactivity. The antibacterial activity exhibited by these fragments against *S. aureus* (MIC = 4.0 mg mL^{-1}) and *E. coli* (MIC = 0.5-1.0 mg mL^{-1}) were found to be rather weak. However, it was shown that this *gem*-dimethyl amide motif is likely to induce a turn in the preferred conformation of oxazolomycins and offers a template that can be optimized for antibacterial activity with appropriate substitution.[69]

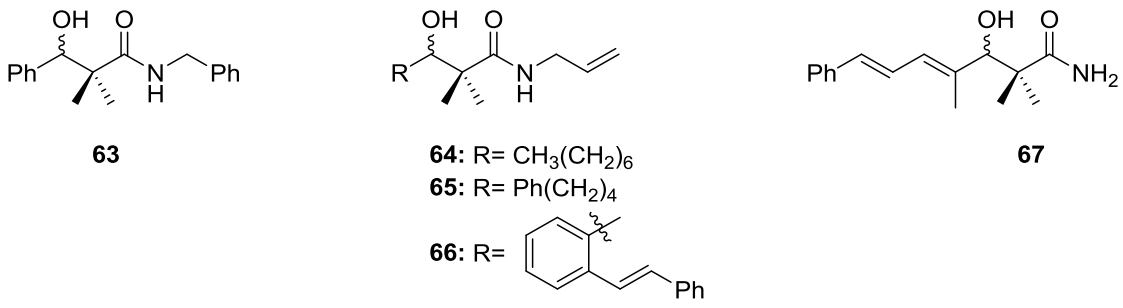


Figure 1.15. Examples of 3-hydroxy-2,2-dimethylpropanamide derivatives synthesised by Moloney and co-workers.

Following on from this work, it was envisaged that a mimic of oxazolomycin derived from the conjugation of the smaller components with a suitable linker might improve bioactivity.[70] Thus, the 3-hydroxy-2,2-dimethylpropanamide motif was linked to a bicyclic tetramate-derived head group **68** and **70** using a click-type approach (Figure 1.16).

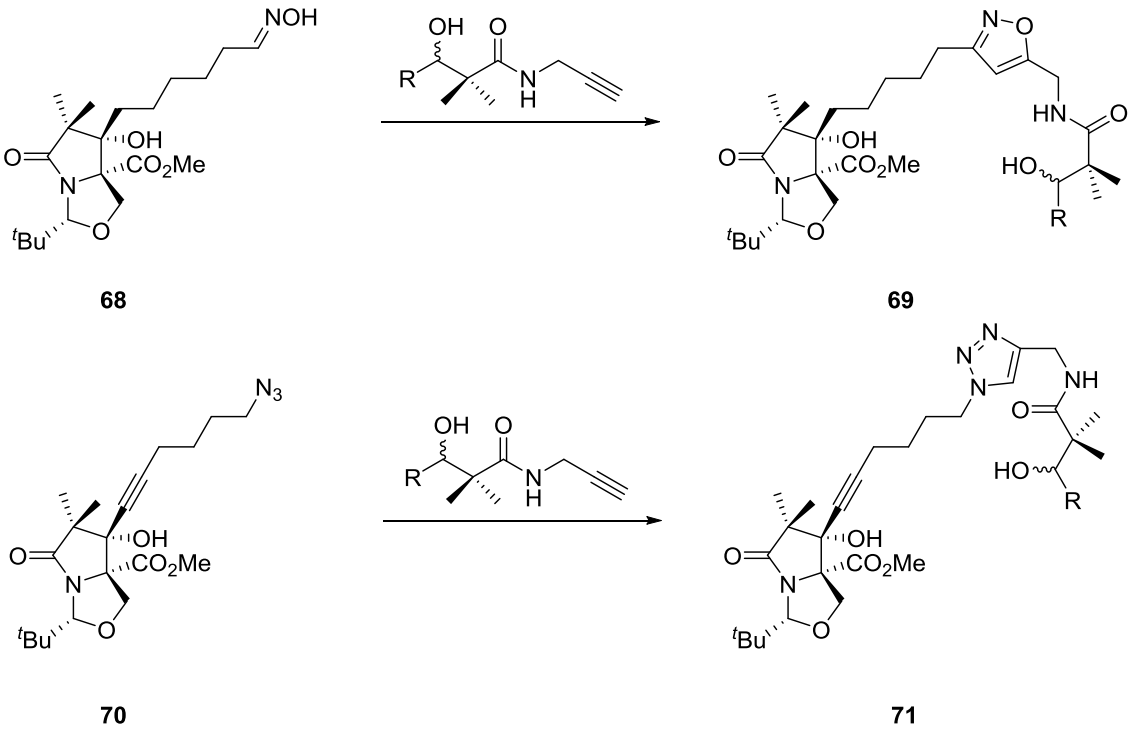


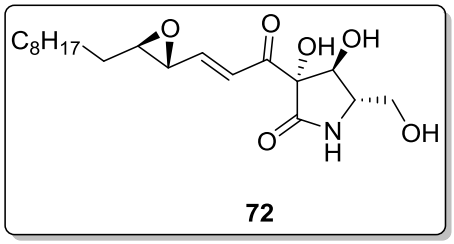
Figure 1.16. 'Click' chemistry approach towards oxazolomycin mimics.

However, while no inhibition of *S. aureus* was observed, these compounds of type **69** and **71** displayed only weak activity against *E. coli*. This was a different pattern of bioactivity compared to the parent natural product oxazolomycins which are active against *S. aureus* but not *E. coli.*[70]

1.6.3.2 Application towards pramanicin

Pramanicin **72** is a natural product that has a broad range of biological activities including antifungal, antibacterial and antitumor properties (Figure 1.17). Due to their structural similarity to pyroglutamate and tetramate systems, the Moloney group has been interested in

the possibility of employing such related oxygenated lactam systems for antibacterial drug discovery.[71] (Unpublished data on tetramate-derived pramanicin analogues; S.W.B. Tan, M.G. Moloney)



72

Figure 1.17. Structure of Pramanicin.

1.7 Aim

The past research work by the Moloney group mainly developed tetramates derived from oxazolidines with a hydrophobic ^{t}Bu group at C-2 due to the stability conferred by this bulky substituent. As a result, in library generation, side chain modifications were limited to that of the C-7 pendant group. The aim of the work related in this thesis is to investigate the feasibility of incorporating diversity at both C-7 and C-2 for library generation in search of novel antibiotics (Figure 1.18).

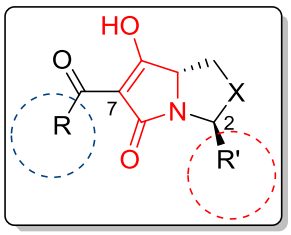


Figure 1.18. Functionalized tetramate core (X=S or O).

The tetramate core would be derived from L-cysteine (or L-serine) with a range of aromatic substituents at C-2 that can also offer opportunity for late stage functionalization. Following on from the success achieved with carboxamide tetramates, this research will also focus on variations of the carboxamide pendant at C-7. The ability to elaborate substitution at two ends

of the tetramate core would be beneficial in the development of drug candidates with optimized physicochemical properties. As an extension of our efforts in the development of natural product inspired antibiotics, the feasibility of a hybridisation approach in which tetramates conjugated with a known antibiotic scaffold will also be explored. The synthesis and evaluation of bioactivity of the compound library will be discussed in detail in subsequent chapters.

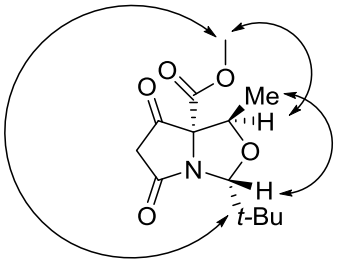
CHAPTER 2 : CYSTEINE-DERIVED TETRAMATE SYSTEMS: STEREOSELECTIVITY

This chapter will discuss the synthesis of the tetramate core derived from L-cysteine with a range of C-2 substituents and the stereoselectivity of the cyclisation process leading to the bicyclic tetramate system, which acts as a template for library generation. During the stereochemical assignment of the intermediate thiazolidine systems, an interesting observation was made on their behaviour in solution, which is discussed in Section 2.6. Some content in this chapter have been reproduced from Panduwawala, T.D., Iqbal, S., Tirfoin, R. and Moloney, M.G., *Org. Biomol. Chem*., **2016**, *14*, 4464-44778 with permission from the Royal Society of Chemistry and reprinted with permission from Panduwawala, T.D., Josa-Cullere, L., Kuprov, I., Odell, B., Moloney, M.G. and Claridge, T.W.C., *J. Org. Chem*., **2016**, *81* (10), 4142-4148, copyright (2016) American Chemical Society.

2.1 Previous work by the Moloney Group

The Moloney group has thoroughly explored the synthesis of oxazolidine-derived tetramates starting from L-serine[64], L-threonine[72] and thiazolidine-derived tetramates from L-cysteine[66] with pivaldehyde as the condensing species, Scheme 2.1. In this synthetic route, methyl ester hydrochlorides of amino acids L-serine, L-threonine and L-cysteine were condensed with *t*-BuCHO by refluxing in petrol using a Dean-Stark apparatus to yield oxazolidines **50a**, **50b** and thiazolidine **50c** as a mixture of diastereomers with a *cis*/*trans* relationship between the C-2 and C-5 substituents of the ring. The oxa/thiazolidines were then *N*-acylated under the DCC coupling conditions[73] to yield malonamides **51a-c** in good yield. These malonamides thus obtained were either predominantly (**51a**: *cis*:*trans*; 85:15) or exclusively the *cis*-2,5 diastereomer (**51b-c**) and this stereochemistry was confirmed by NOE analysis.

Dieckmann cyclisation of malonamides **51a-c** in the presence of KO^{t}Bu gave bicyclic tetramates of type **52** and **53**. The major products **52a-c** are formed via ring closure from the C-5 enolate onto the ethyl ester. This places the bulky C-2 '*t*-Bu' group on the *exo*-face of the bicyclic ring system with a '*cis*' relationship between the C-2 and C-5 substituents across the bicyclic system.[64,66,72] The enantiopurity of the major product from Dieckmann cyclisation was determined for tetramate **52a** derived from L-serine and an ee of >90 % was reported.[64] For **52b**, NOE analysis is shown in Figure 2.1.



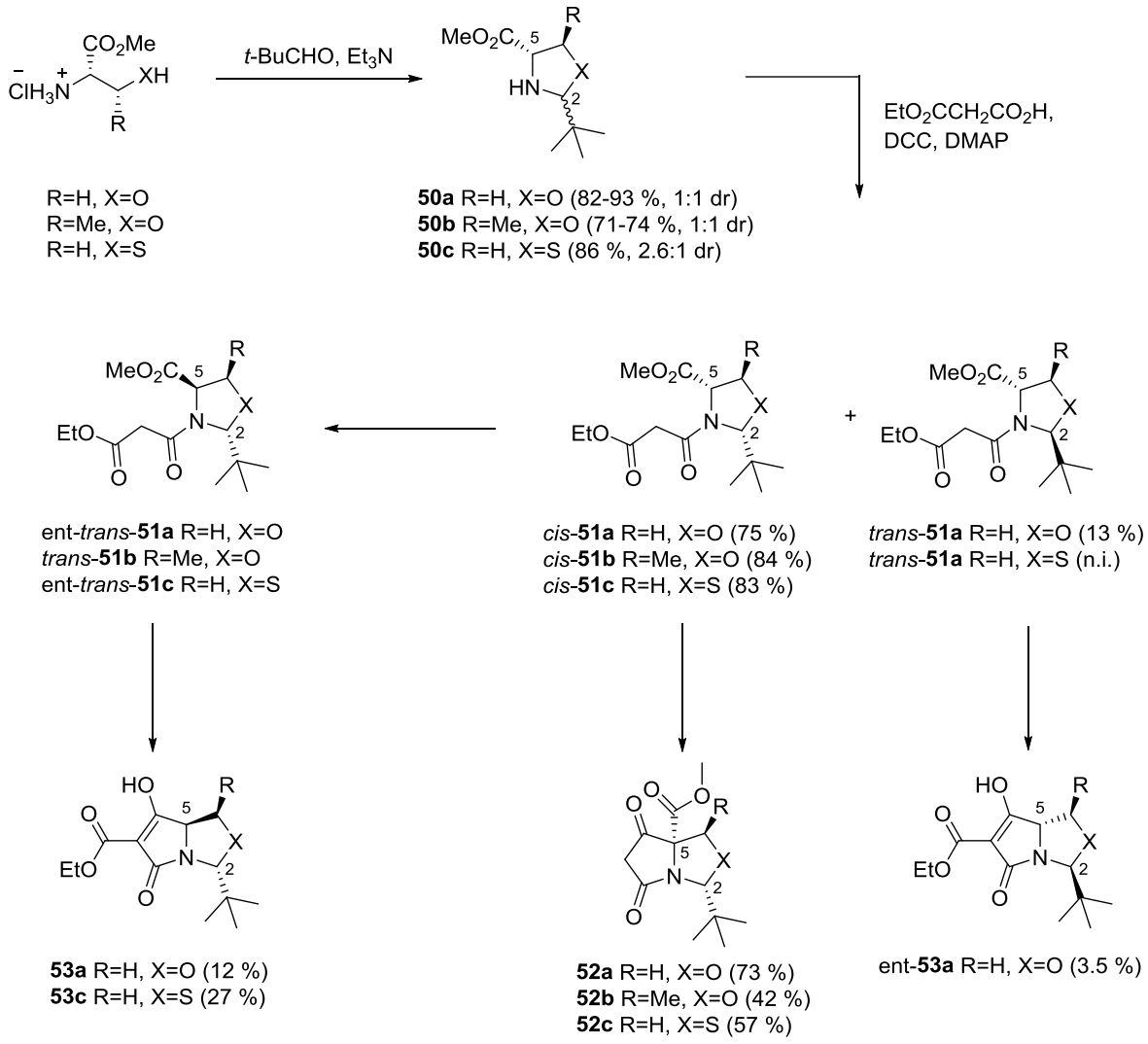
52b

Figure 2.1. NOE correlation data for tetramate template **52b**. Adapted from Tetrahedron: *Asymmetry*, **2010**, *21*, Anwar, M., Cowley, A. R. and Moloney, M.G., Novel chiral pyrrolidinone scaffolds derived from threonine with antibacterial activity, 1758-1770, Copyright (2010), with permission from Elsevier.

However, under the basic reaction conditions, some epimerisation at C-5 of *cis*(2,5)-**51a-c** can lead to ent-*trans*(2,5)-**51a-c**. This gives rise to an alternative mode of cyclisation by closure of the more stabilized malonamide side chain enolate onto the methyl ester at C-5, forming the minor product **53a-c**. As studied for the systems derived from L-serine,[64] the *trans*-malonamide *trans*(2,5)-**51a** can cyclise in a similar fashion from the side chain enolate onto the C-5 methyl ester to yield ent-**53a** as a by-product in 3.5 % yield, again placing the bulky *t*-Bu group on the less hindered face of the bicyclic tetramate.

Even though this synthetic approach led to tetramates with high enantiopurity, this work was limited to the use of pivaldehyde as the condensing species, leading to very hydrophobic tetramates with limited synthetic scope at C-2. Thus, we set out to expand the scope of this

approach by using aromatic aldehydes including those that allow for late-stage functionalization to improve molecular polarity and aqueous solubility, thereby generating tetramate templates more suitable for drug development.

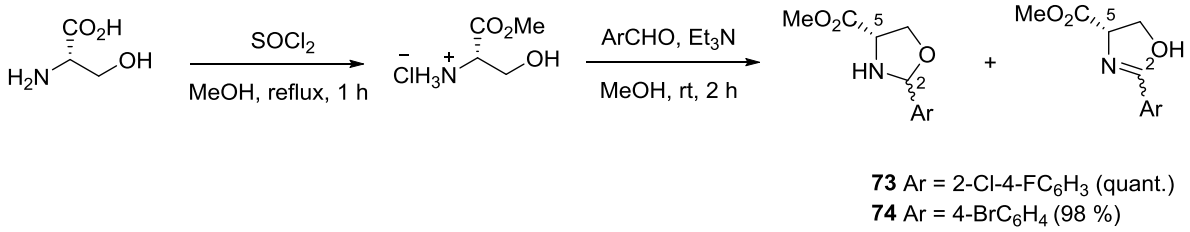


Scheme 2.1. Synthesis of tetramic acid templates derived from L-serine, L-threonine and L-cysteine. *cis*- ans *trans*- diastereomers are described with respect to H-2 and H-5. (n.i. = not isolated).

2.2 Towards the synthesis of oxazolidine-derived tetramates with C-2 aryl substitution

In this route, L-serine was first esterified to give methyl ester using thionyl chloride in methanol as the solvent. The protection of the hydroxyl and amine groups as an *N,O*-acetal

was achieved using aromatic aldehydes as the condensing species to yield substituted oxazolidines **73** and **74**, following a procedure reported in the literature,[74] Scheme 2.2.



Scheme 2.2. Synthesis of oxazolidines **73-74**.

The ^{1}H NMR spectra of these oxazolidines in $CDCl_3$ and CD_2Cl_2 at ambient temperature indicated the presence of three species. While two of them could be assigned to the *cis*- and *trans*- diastereomers with respect to C-2 and C-5 substituents from NOE analysis, the other could be assigned to the open chain form based on the azomethine proton chemical shift. This suggested a three component ring-chain tautomeric equilibrium between the *cis-trans* diastereomers and their open chain form as in Figure 2.2.

The chemical shift data were in excellent agreement with those reported in the literature for similar oxazolidine systems[74] and the open chain form (B) was observed to be the predominant form. The data are listed in Table 2.1.

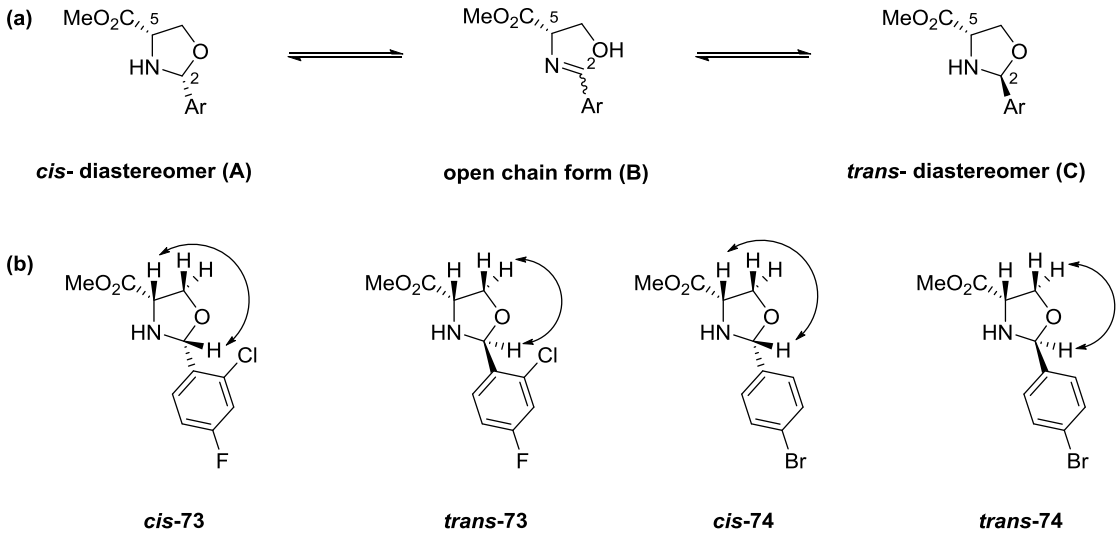
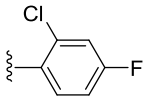
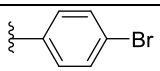


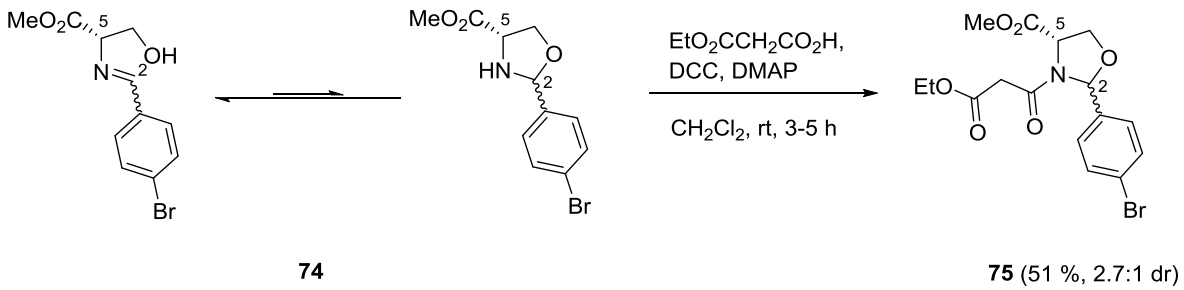
Figure 2.2. (a) Ring-chain tautomerism of oxazolidines, (b) Key NOE interactions of **73-74**.

Table 2.1. Chemical shift values for H-2 and relative population of ring-chain tautomers.

Compound No.	Structure Ar =	δ_H for H-2 (ppm)			A:B:C ratio
		cis (A)	open chain (B)	*trans* (C)	
73		5.61	8.68	5.95	1.5:2.6:1
74		5.27	8.28	5.64	1.2:3.3:1

The tautomeric equilibrium thus observed also appeared to be solvent dependent, where the more polar solvent preferred the open chain form. For compound **73**, in $CDCl_3$ at room temperature, the ratio between A:B:C forms was 1.5:2.6:1 with the open chain form being more predominant, while in CD_3OD, the open chain form was observed exclusively.

It was interesting to note that when the C-2 substituent on the oxazolidine ring is a bulky *t*-Bu group, the ring form was observed exclusively and the open chain form was not observed by NMR spectroscopy.[64] However, replacing this rather hydrophobic *t*-Bu group with planar aromatic systems compromises on the stability of the oxazolidine ring, resulting in it being the less favoured form. Nevertheless, if the secondary amine can be substituted, the oxazolidine could be 'locked' in its ring form, thereby driving the equilibrium in its favour. Thus, oxazolidine **74** was taken through as its tautomeric mixture and acylated with monoethyl malonate under the DCC coupling conditions to yield *N*-acylated oxazolidine **75** in its ring form, Scheme 2.3.



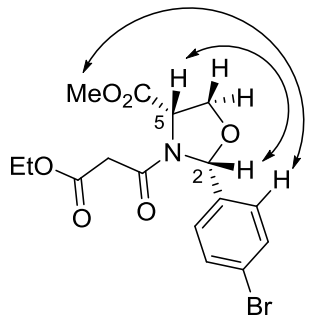
74 **75** (51 %, 2.7:1 dr)

Scheme 2.3. Synthesis of *N*-acyloxazolidine **75**.

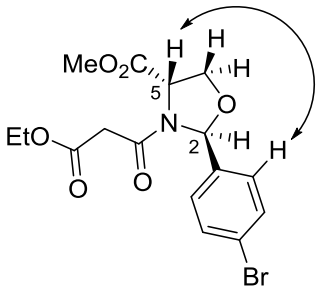
The malonamide **75** was obtained as a mixture of *cis*- and *trans*- diastereomers in 2.7:1 ratio with the *cis*- isomer being predominant and was stable to column chromatography. A shift in the diastereomeric ratio was not observed over a time span of 1 month in $CDCl_3$ at room temperature. Further, the *cis*- and *trans*-diastereomers of **75** were separated by column chromatography and analysed by NMR spectroscopy in $CDCl_3$ at room temperature. Both diastereomers showed no interconversion over time. Thus, the irreversible formation of **75**, where its *cis*- and *trans*- diastereomeric ratio does not reflect the equilibrium distribution of *cis*- and *trans*- oxazolidine **74**, is an example of Curtin-Hammett principle. Each diastereomer existed as a rotameric pair and the relative stereochemistry of C-2 and C-5 substituents across the ring was confirmed by NOE experiments (Figure 2.3). NMR resonances for H-2s were distinct and for both rotamers, H-2 of '*trans*' appears to be more downfield than '*cis*' diastereomer as listed in Table 2.2.

Table 2.2. H-2 chemical shifts for **75**.

Diastereomer	**δ_H for H-2 (ppm)**	
	Major rotamer	**Minor rotamer**
***cis*-75**	6.04	6.16
***trans*-75**	6.29	6.42



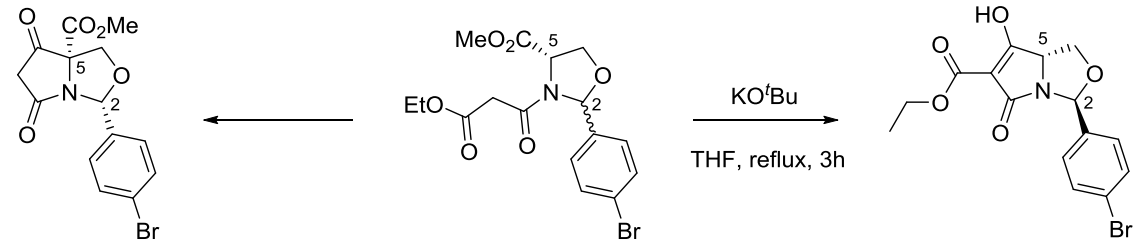
***cis*-75**



***trans*-75**

Figure 2.3. Key NOE interactions of **75**.

This mixture of diastereomers was then cyclised under basic conditions to give the bicyclic tetramate **76** while **77** was not isolated even though its corresponding mass could be detected by mass spectrometry in the crude reaction mixture, Scheme 2.4. This is in contrast to the observed reactivity of the malonamide **51a** with a '*t*-Bu' at C-2, where the major product of cyclisation was the methyl ester **52a**. However, during acidic work up of the cyclisation reaction, the product proved to be unstable. Thus, the crude reaction mixture was directly purified by column chromatography on silica gel run with 1 % Et_3N, but the desired product **76** was isolated in only 8 % yield (the absolute configuration of **76** is assigned in analogy to the research work discussed in Section 2.3, *vide infra*).

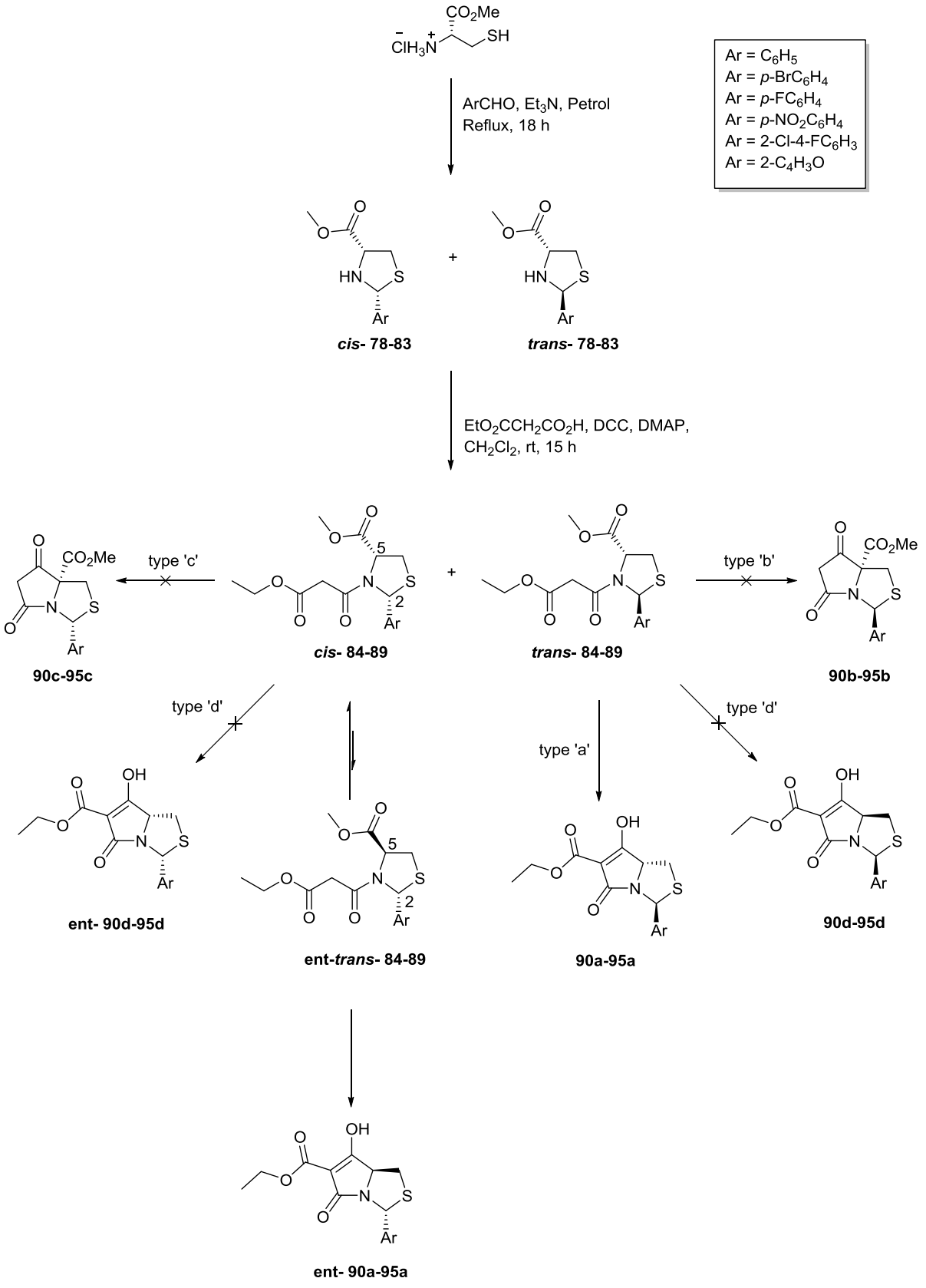


Scheme 2.4. Synthesis of bicyclic tetramate **76**.

Thus, it was evident that the bicyclic tetramate **76** with C-2 aryl substitution lacked the stability possessed by the analogous tetramates with C-2 *t*-Bu substitution. Hence, serine-derived tetramates did not prove to be an attractive route to introduce diversity at C-2. Therefore, we looked to employ cysteine to generate more stable tetramates via thiazolidine intermediates.[75]

2.3 Synthesis of thiazolidine-derived tetramates with C-2 aryl substitution

A schematic representation of the synthesis of thiazolidine-derived bicyclic tetramates with C-2 aryl substituents is given below in Scheme 2.5.



Scheme 2.5. Synthesis of thiazolidine-derived bicyclic tetramates. Experimental conditions for Dieckmann cyclisation; KO^{t}Bu, THF, reflux, 3 h.

2.3.1 Synthesis of thiazolidines with C-2 aryl substitution

Firstly, the condensation of commercially available L-cysteine methyl ester hydrochloride with the relevant aromatic aldehydes was achieved by refluxing in petrol with a Dean-Stark apparatus to give thiazolidines **78-83** in good yield (76-94 %). These thiazolidines were obtained as a mixture of *cis*- and *trans*- diastereomers in a ratio of between 0.8:1 and 2:1 and their relative stereochemistry was assigned by NOE analysis (Figure 2.4). The diastereomers could be readily distinguished by the difference in the chemical shift of H-2, where H-2 resonance of the *trans*- diastereomer appeared more downfield than that of the *cis*-diastereomer (Table 2.3). In contrast to the oxazolidine analogues **74** and **75**, the open chain tautomer was not observed for the thiazolidines. Further, these thiazolidines were stable to column purification unlike their oxazolidine counterparts.

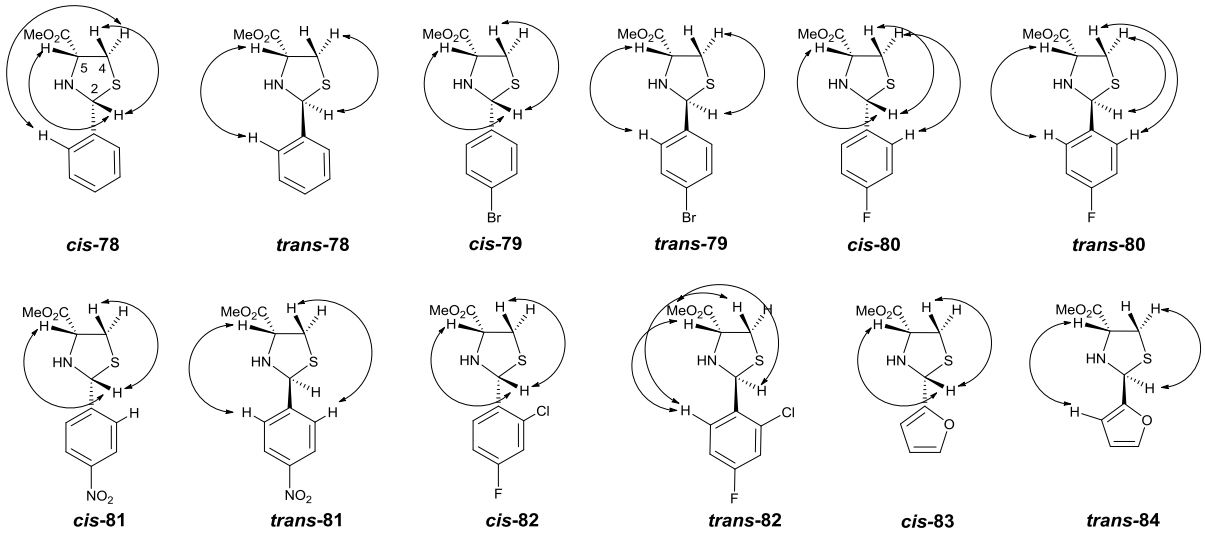
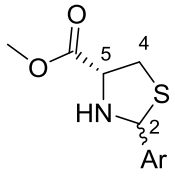
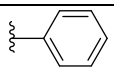
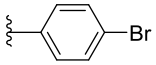
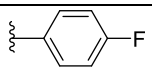
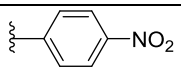
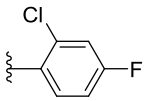
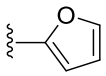


Figure 2.4. Key NOE interactions of thiazolidines **78-83**. Adapted from Panduwawala, T.D., Iqbal, S., Tirfoin, R. and Moloney, M.G., *Org. Biomol. Chem.*, **2016**, *14*, 4464-44778 with permission from the Royal Society of Chemistry.

Table 2.3. Yield (%) and chemical shift data of H-2 for thiazolidines **78-83.** Adapted from Panduwawala, T.D., Iqbal, S., Tirfoin, R. and Moloney, M.G., *Org. Biomol. Chem*., **2016**, *14*, 4464-44778 with permission from the Royal Society of Chemistry.

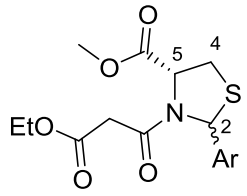
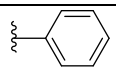
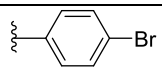
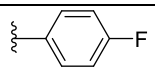
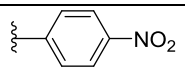
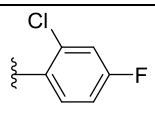
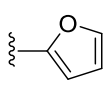
Compound No.		**δ_H (ppm) for H-2**		***cis*:*trans* ratio**	**% Yield**
		cis	***trans***		
78		5.57	5.82	1.7:1	91
79		5.49	5.76	1.3:1	94
80		5.53	5.79	1.7:1	90
81		5.69	5.87	0.8:1	94
82		5.89	6.02	0.8:1	76
83		5.48	5.68	0.9:1	85

2.3.2 Acylation of thiazolidines with C-2 aryl substitution

The subsequent acylation of these thiazolidines to the corresponding malonamides **84-89** by DCC coupling proceeded with improved yield (85-99 %) with an extended reaction time of 15 h and the malonamides were obtained as a mixture of *cis*- and *trans*- diastereomers. Their relative stereochemistry was readily assigned by NOE experiments (Figure 2.5). On analysing the ratio of diastereomers of malonamides **84-89**, it was evident that the diastereomeric ratio of thiazolidines **78-83** was conserved through to acylation. This is in contrast to the oxazolidine systems where there was a shift in the *cis*-*trans* equilibrium in favour of the *cis*-diastereomer.

The malonamide diastereomers appeared as a rotameric pair with characteristic chemical shift values observed for H-2. The major rotamer of *trans*- diastereomer was more downfield than that of the *cis*- diastereomer (with the exception of **89**), while this pattern was reversed for the minor rotamer (Table 2.4). Since the *cis:trans* ratio is unchanged through to acylation, it could be assumed that the *cis*-malonamide derives from *cis*-thiazolidine and *trans*-malonamide derives from *trans*-thiazolidine. This outcome was further confirmed by analysing the diastereomeric ratio of the unreacted thiazolidines **79**, **80** and **82** recovered from the acylation process, where their diastereomeric ratio was found to be the same as that of the corresponding starting thiazolidines **79**, **80** and **82**.

Table 2.4. Yield (%) and chemical shift data of H-2 for *N*-acylthiazolidines **84-89**. Adapted from Panduwawala, T.D., Iqbal, S., Tirfoin, R. and Moloney, M.G., *Org. Biomol. Chem.*, **2016**, *14*, 4464-44778 with permission from the Royal Society of Chemistry.

Compound No.		**δ_H (ppm) for H-2**				***cis*:*trans* ratio**	**% Yield**
		cis		***trans***			
		Major rotamer	**Minor rotamer**	**Major rotamer**	**Minor rotamer**		
84		6.10	6.28	6.14	6.24	1.6:1	99
85		6.11	6.27	6.14	6.24	1.3:1	99
86		6.13	6.30	6.17	6.29	1.5:1	87
87		6.22	6.27	6.23	6.24	0.9:1	85
88		6.34	6.46	6.42	6.45	0.8:1	97
89		6.18	6.56	6.16	6.32 - 6.35 (obscur-ed by H4')	0.8:1	89

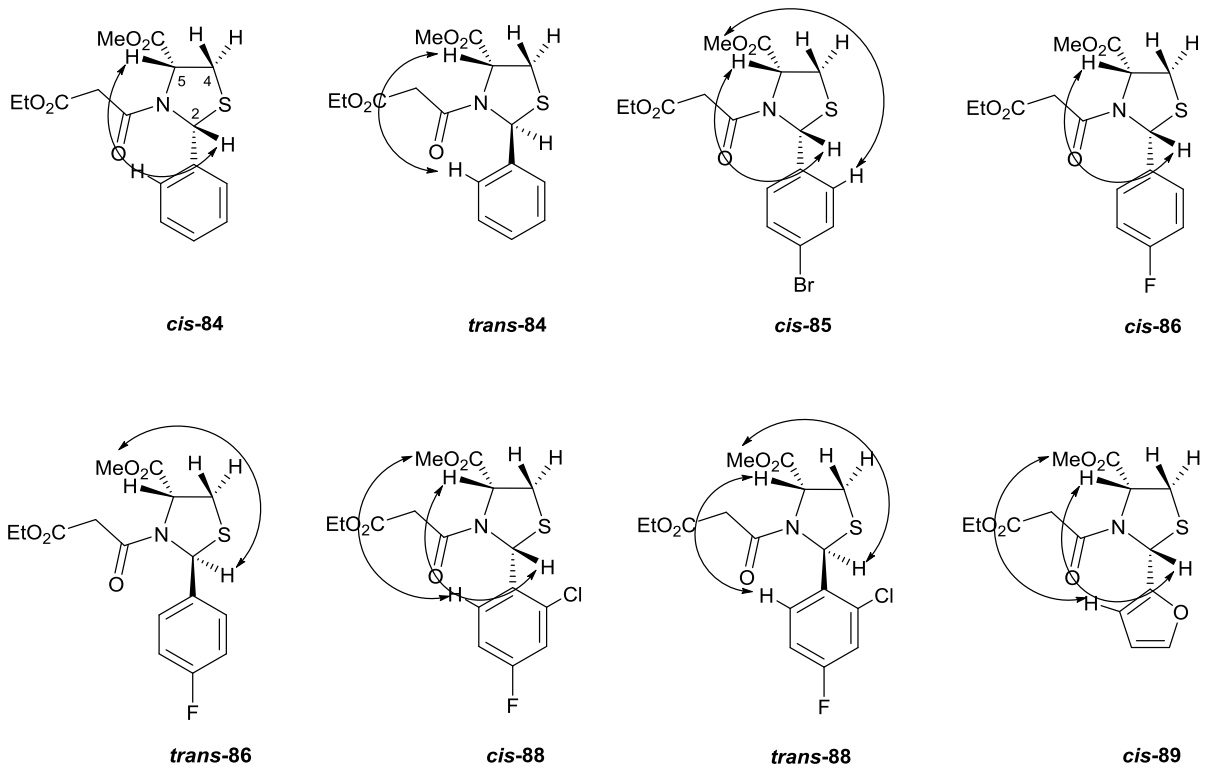


Figure 2.5. Key NOE interactions of *N*-acylthiazolidines **84-89**. Adapted from Panduwawala, T.D., Iqbal, S., Tirfoin, R. and Moloney, M.G., *Org. Biomol. Chem*., **2016**, *14*, 4464-44778 with permission from the Royal Society of Chemistry.

2.3.3 Cyclisation of *N*-acylthiazolidines to yield bicyclic tetramates with C-2 aryl substitution

Dieckmann cyclisation of *N*-acylthiazolidines **84-89** upon reaction with KO^{t}Bu gave bicyclic tetramates **90-95** with complete enolization as observed by ^{1}H NMR spectra and as a single diastereomer with a *trans*- relationship between H-2 and H-5 across the bicyclic ring systems as confirmed by NOE analysis (Figure 2.6). This indicated that ring closure led to tetramates of type '**a**' and not type '**d**', Scheme 2.5 (page 41, *vide supra*). Further, the formation of the methyl ester tetramate of type '**c**' was detected by mass spectrometry, but not isolated except in the cyclisation of *cis*-**88**, where it was isolated in < 1 % yield indicating that this is not the predominant product of cyclisation. Formation of its diastereomer of type '**b**' was not detected in the cyclisation of *trans*-**88**.

This mode of cyclisation is in contrast to that observed for oxazolidine/thiazolidine-derived tetramates with '*t*-Bu' substitution at C-2, where the major product of cyclisation was the methyl ester type '**c**'. This is a result of the ring closure of the less stable C-5 enolate onto the ethyl ester, placing the bulky '*t*-Bu' group on the less hindered *exo*- face of the bicyclic ring. When the C-2 substituent is a smaller aryl system that is not sterically demanding, cyclisation proceeds via ring closure of the more stable enolate onto the C-5 methyl ester to yield ethyl ester tetramates of type '**a**'.

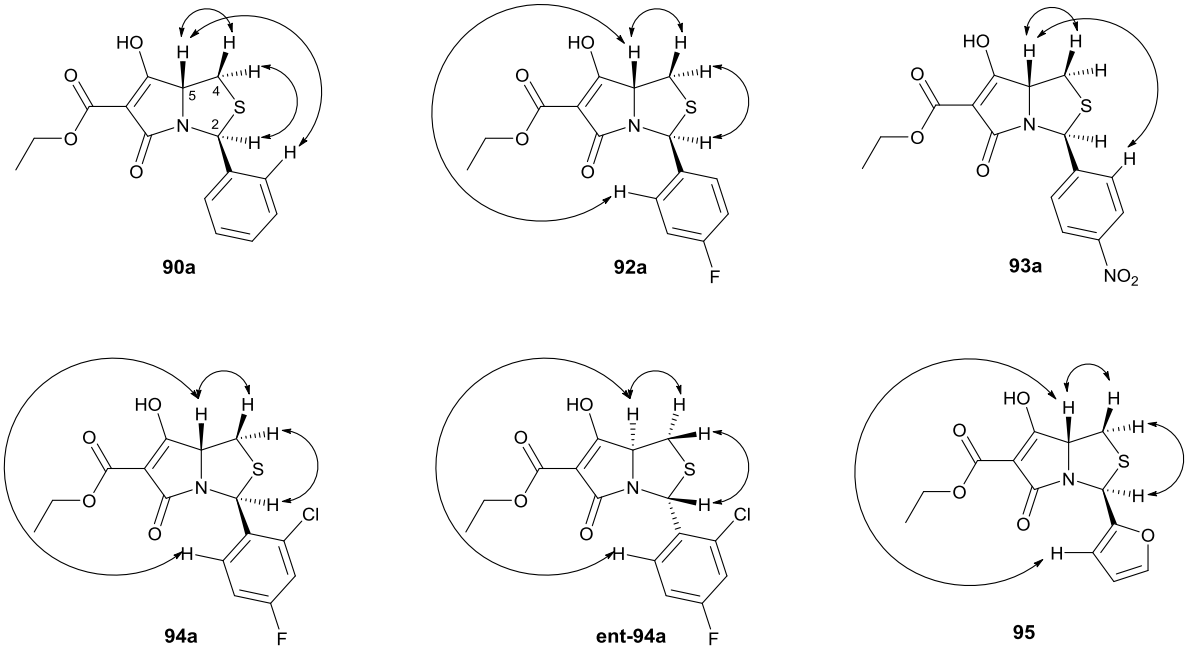


Figure 2.6. NOE analysis of tetramates **90a-95a**. Adapted from Panduwawala, T.D., Iqbal, S., Tirfoin, R. and Moloney, M.G., *Org. Biomol. Chem*., **2016**, *14*, 4464-44778 with permission from the Royal Society of Chemistry.

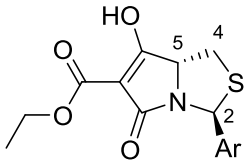
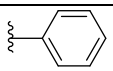
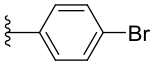
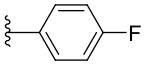
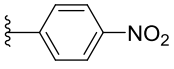
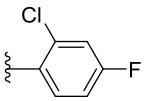
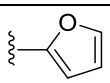
Since the *cis*- and *trans*- malonamides were not separable by column chromatography in most cases, the mixture of diastereomers was used for the cyclisation. Hence, it is possible that *trans*-malonamide cyclises to give tetramates **90a-95a** while *cis*-malonamide epimerises at C-5 under the basic conditions of the cyclisation process and then cyclises to give ent- **90a-95a** (as shown in Scheme 2.5, page 41, *vide supra*). This would then lead to a compromise in the

enantiopurity of the tetramates. Thus, a detailed study of the cyclisation process was conducted.

2.4 Determination of the enantiopurity of thiazolidine-derived tetramates with C-2 aryl substitution

In order to determine the enantiomeric excess of the products obtained from the Dieckmann cyclisation process, the racemic analogue of tetramate **92a** was synthesised under the same reaction conditions. This was then compared with **92a** obtained from the enantiopure series by chiral HPLC and the e.e. was found to be 80 %. Similarly, the e.e. values for **91a**, **94a** and **95a** were found to be in the range of 76-83 %. Thus, it was evident that there is an attrition in the e.e. when the diastereomeric mixtures of malonamides are cyclised.

Table 2.5. Chemical shift data of H-2, yield (%) and e.e. for tetramates **90a-95a** (nd = not determined).

Compound No.		**δ_H (ppm) for H-2**	**% Yield**	**% e.e.**
90a		6.25	43	nd
91a		6.43	45	76
92a		6.34	50	80
93a		6.43	52	nd
94a		6.45	49	83
95a		6.30	40	80

Thus, for further investigation, *cis*- and *trans*- malonamides of **88** were successfully separated by column chromatography (other malonamides except **89** were not similarly separable). The relative stereochemistry of both *cis*-**88** and *trans*-**88** was confirmed by NOE experiments (Figure 2.5) and absolute configuration of *trans*-**88** was confirmed by X-ray crystallography (Figure 2.7). Each diastereomer of **88** was then separately cyclised under the standard reaction conditions.

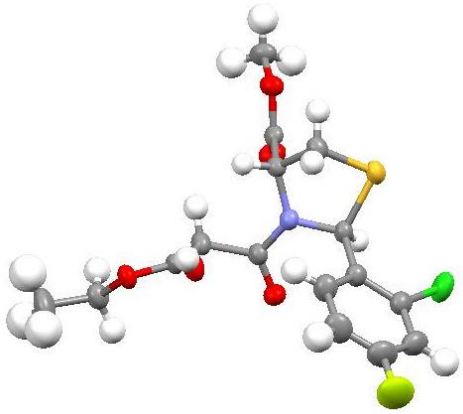


Figure 2.7. X-ray crystal structure of *trans*-**88**.

The cyclisation of *trans*-**88** gave product **94a** in 58 % yield while *cis*-**88** gave product ent-**94a** in only 6 % yield. NOE analysis suggested that both the cyclised products have the same relative stereochemistry (Figure 2.6). Further, their different retention times on chiral HPLC and $[\alpha]_D$ values of opposite sign indicated that they are enantiomers (Table 2.6). Thus, it is evident that *trans*-**88** cyclises efficiently with higher yield than *cis*-**88**, with a yield ratio of 10:1. Since **94a** obtained from the cyclisation of the *cis*- and *trans*- diastereomeric mixture had an e.e. of 83 % with the enantiomeric ratio being 10.7:1, this attrition in e.e. could be accounted for in relation to the yield ratio obtained for the cyclisation of *cis*- and *trans*-diastereomers.

Further evidence to support the difference in yield for the cyclisation of *cis*- and *trans*-malonamides was obtained from the recovered unreacted malonamide from the Dieckmann

cyclisation step for compounds **85**, **86** and **88**. A comparison of their chemical shifts to the respective *cis*- and *trans*- diastereomers showed the presence of *cis*- but not *trans*- diastereomer in the unreacted malonamide, which further confirms that *trans*- cyclises preferentially over its *cis*- diastereomer.

Table 2.6. Chiral HPLC retention times (solvent= IPA:Hexane;20:80) and optical rotation data for compound series **94a**.

Compound No.	**Retention time (min)**		$[\alpha]_D^{25}$
	Peak 1	**Peak 2**	
(±)-94a	11.810	16.806	-
94a (83 % e.e.)	11.512	15.917	-264.0
(-)-94a	11.317	-	-331.8
(+)-94a	-	15.903	+309.5

In support of the absolute configuration proposed for the major enantiomer **(-)-94a**, an X-ray crystal structure was obtained for the carboxamide tetramate derived from **(-)-94a** (Figure 2.8).

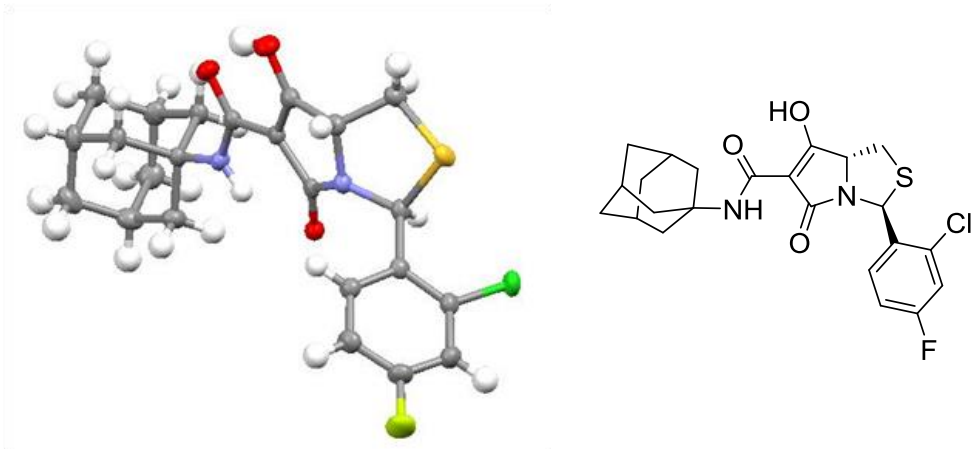


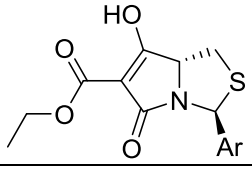
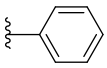
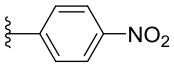
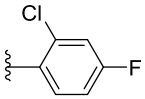
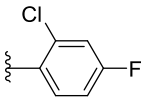
Figure 2.8. X-ray crystal structure of carboxamide tetramate derived from **(-)-94a**. Absolute structure parameter (Flack x parameter) = -0.005(9).

2.5 Purification of thiazolidine-derived tetramate esters

The purification of bicyclic tetramate esters by silica gel column chromatography resulted in broad signals for the proton resonances in ^{1}H NMR spectra. This observation was in agreement with other reports on 3-acyltetramic acids and 3-acyltetronic acids, where signal broadening was a result of the formation of metal chelates during column chromatography on silica gel containing trace amounts of metal impurities such as Mg^{2+}, Fe^{2+} and mainly Ca^{2+}.[76–78] Washing the product with 0.5 M aqueous HCl was suggested as a solution to obtain metal-free tetramates with improved signal sharpness and resolution.[76] However, treatment of tetramate esters **90a-95a** with 0.5 M HCl was not an attractive approach to obtain their metal-free form due to the acid-mediated hydrolysis of the ethyl ester. Therefore, the purification of these tetramate esters was modified so that the silica gel column was run with 1 % Et_3N in the eluent, and the purified product was then washed with 5 % citric acid to remove any residual Et_3N.

A similar result was observed for tetramates purified by preparative thin layer chromatography with silica supports, where the peaks were slightly broader than that of the metal-free form with significant chemical shift differences observed for H-2 and H-5 (Table 2.7). Moreover, metal chelated tetramates had different melting points, optical rotation data and retention times on chiral HPLC as observed for **94a** and ent-**94a** in comparison to their metal-free form (data in Appendix C). Analysis by mass spectrometry for tetramate **91a** showed the presence of peaks that could be attributable to $[M-H]+Ca^{2+}$ and $[M-H]+Mg^{2+}$ indicating the possibility of these tetramates to chelate to such divalent metal cations (Figure 2.9).

Table 2.7. ^{1}H chemical shift values of H-2 and H-5 for the metal-chelated and metal-free forms of tetramates (in CD_3OD, 400 MHz). Adapted from Panduwawala, T.D., Iqbal, S., Tirfoin, R. and Moloney, M.G., *Org. Biomol. Chem.*, **2016**, *14*, 4464-44778 with permission from the Royal Society of Chemistry.

Compound No.		**δ_H (ppm) Metal-chelated tetramate**		**δ_H (ppm) Metal-free tetramate**	
		H-2	**H-5**	**H-2**	**H-5**
90a		6.36 (s)	4.12-4.26 (m)	6.25	4.83 (app t)
93a		6.43 (s)	4.13-4.32 (m)	6.32 (s)	4.95 (dd)
(-)-94a		6.52 (s)	4.42 (app br. t)	6.39 (s)	5.05 (dd)
(+)-94a		6.52 (s)	4.43 (app br. t)	6.39 (s)	5.06 (dd)

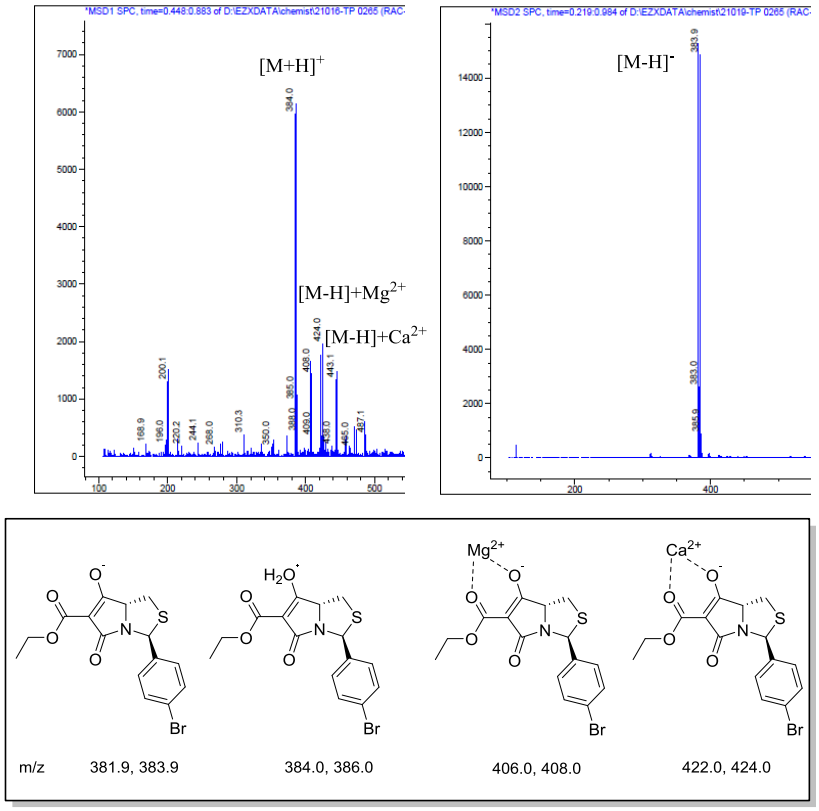


Figure 2.9. Mass spectrometry analysis (ESI) for tetramate **91a** indicating possible metal chelation. Adapted from Panduwawala, T.D., Iqbal, S., Tirfoin, R. and Moloney, M.G., *Org. Biomol. Chem.*, **2016**, *14*, 4464-44778 with permission from the Royal Society of Chemistry.

2.6 Scalar cross-relaxation of the first kind observed in thiazolidines

In the course of analysing the 2D NOESY spectra to assign the relative stereochemistry of the diastereomeric mixtures of oxazolidines **73-74** and thiazolidines **78-83**, anomalous strong positive cross-peaks were observed between NHs, H-2 and H-5 in addition to the conventional negative cross-peaks (Figure 2.10). While the negative cross-peaks between the CH protons were assigned to the through-space nuclear Overhauser effect, the reason for positive cross-peaks had to be investigated. In compounds with labile protons, where their chemical exchange is possible, such positive cross-peaks can clearly be observed for the correlated protons. However, of interest was the presence of strong positive cross-peaks between protons that cannot possibly exchange, which is indicative of another phenomenon: scalar cross-relaxation of the first kind (SCRFK).[79]

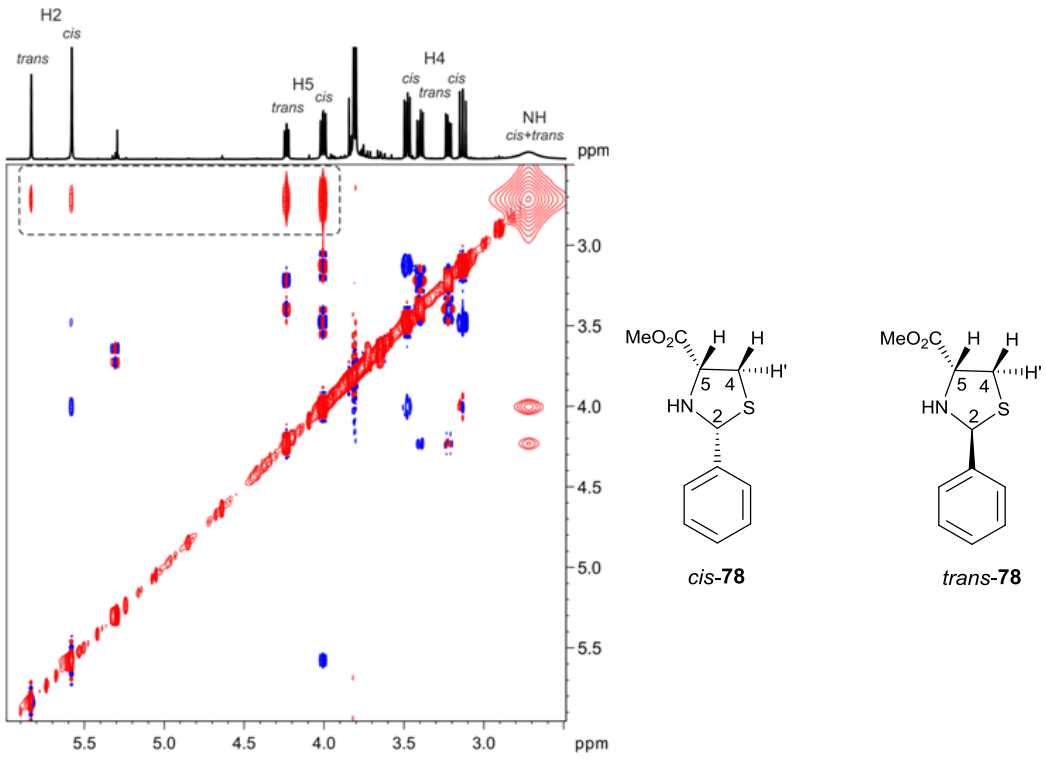


Figure 2.10. ^{1}H-^{1}H NOESY spectrum of **78** (*cis:trans*; 1.6:1) in $CDCl_3$ at 298 K showing anomalous positive cross-peaks between the N-H and its neighbouring H-2 and H-5 (boxed region). Reprinted with permission from Panduwawala, T.D., Josa-Cullere, L., Kuprov, I., Odell, B., Moloney, M.G. and Claridge, T.W.C., *J. Org. Chem.*, **2016**, *81* (10), 4142-4148, copyright (2016) American Chemical Society.

In order to observe SCRFK, there needs to be a modulation of the coupling constants of the *J*-coupled protons (NH and H-2, NH and H-5) on a millisecond timescale so that the rate of longitudinal scalar cross-relaxation becomes significant (Equation 1).[80]

$$\left\langle \hat{S}_Z^{(1)} \middle| \hat{\hat{R}}_{\mathrm{SRFK}} \middle| \hat{S}_Z^{(2)} \right\rangle = \frac{\Delta_J^2 \tau_{\mathrm{ex}}}{2} \frac{1}{1+\left(\omega_1 - \omega_2\right)^2 \tau_{\mathrm{ex}}^2} \approx \frac{\Delta_J^2 \tau_{\mathrm{ex}}}{2} \qquad \text{(Equation 1)}$$

where Δ_J = size of modulation of coupling constant, τ_{ex} = correlation time of the exchange process, $(\omega_1\text{-}\omega_2)$ = frequency separation between the *J*-coupled protons in the ^{1}H spectrum. Equation 1 shows that the rate of cross-relaxation is increased with a larger modulation of the coupling constant (Δ_J) and a smaller chemical shift difference between the coupled protons.

The NOESY spectrum for **78** could be reproduced by a theoretical calculation using the *Spinach* spin-simulation programme when SCRFK was included between NH, H-2 and H-5.[79] Without this term, only the conventional NOEs were observed.[79]

In a previous publication on aziridines,[80] SCRFK was theoretically shown to arise from a conformational transition involving nitrogen inversion, which leads to a modulation of the *J*-coupling between the N-H and the vicinal C-H protons. The high energy barrier for inversion in aziridines (~70 kJ mol^{-1}) was sufficient to give *J*-modulation rates appropriate to invoke SCRFK.[80] However, energy barriers for nitrogen inversion in thiazolidines of type **78-83** could not be found in literature reports although the free energy of activation for nitrogen inversion in *N*-methylthiazolidine is reported to be 35.9 k mol^{-1}.[81,82] DFT calculations of the nitrogen inversion energy barriers for the two diastereomers of thiazolidine **78** (*cis*-**78** = 19.7 kJ mol^{-1} and *trans*-**78** = 25.4 kJ mol^{-1})[79] shows that they are lower than that of aziridines, and as such, the nitrogen inversion rates for thiazolidines would be too high to give rise to SCRFK. Moreover, thiazolidine **78** was studied at a temperature range of 193-298 K, but the ^{1}H NMR spectra did not show the presence of invertomers at lower temperatures. This was in

contrast to aziridines where invertomers were observed in ^{1}H NMR at 193 K.[80] This suggests that nitrogen inversion is possibly not responsible, but another mechanism operates to bring about *J*-coupling modulation leading to SCRFK in these thiazolidines.

An examination of the NOESY spectra for similar thiazolidines **78-83** indicated a correlation between the presence of a broad NH resonance in coalescence and the appearance of positive cross-peaks between NH, H-2 and H-5. When the NH resonances of *cis*- and *trans*-diastereomers were well resolved, only NOEs and no anomalous positive cross-peaks were observed. This was the case for thiazolidine **78** at 193 K, where the ^{1}H spectrum recorded showed a well resolved NH coupling structure. The corresponding 2D NOESY spectrum showed only the classical NOEs and chemical exchange peaks between the NHs of the two diastereomers (Figure 2.11), indicating these undergo slow intermolecular exchange.

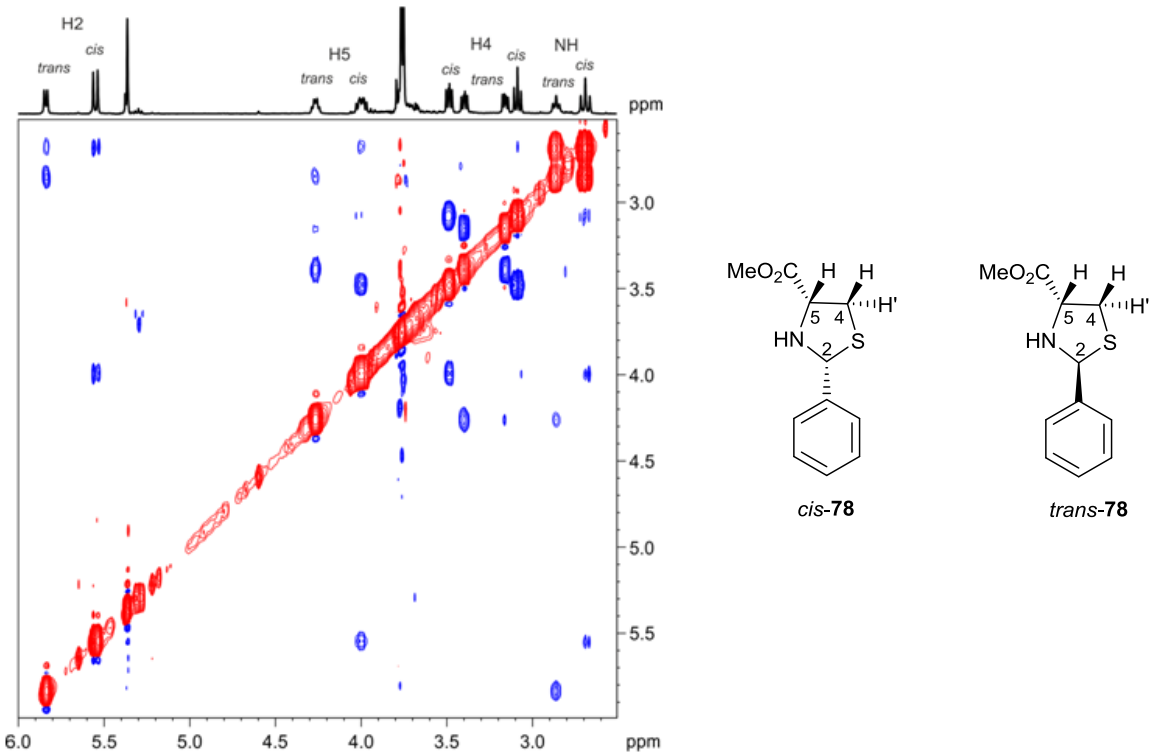


Figure 2.11. ^{1}H-^{1}H NOESY spectrum of **78** (*cis:trans*; 1.6:1) in CD_2Cl_2 at 193 K showing only NOEs (blue) and NH chemical exchange peaks (red), ($^{3}J_{NH\text{-}H2}$ = $^{3}J_{NH\text{-}H5}$ = 13.0 Hz, *trans*: $^{3}J_{NH\text{-}H2}$ = $^{3}J_{NH\text{-}H5}$ = 9.0 Hz.). Reprinted with permission from Panduwawala, T.D., Josa-Cullere, L., Kuprov, I., Odell, B., Moloney, M.G. and Claridge, T.W.C., *J. Org. Chem.*, **2016**, *81* (10), 4142-4148, copyright (2016) American Chemical Society.

This proton exchange process is a plausible mechanism that can give rise to SCRFK where the protonation-deprotonation at the nitrogen centre can lead to *J*-coupling modulation of $^{3}J_{NH-H2}$ and $^{3}J_{NH-H5}$, where the exchange process could be bimolecular or acid-catalysed (Figure 2.12).

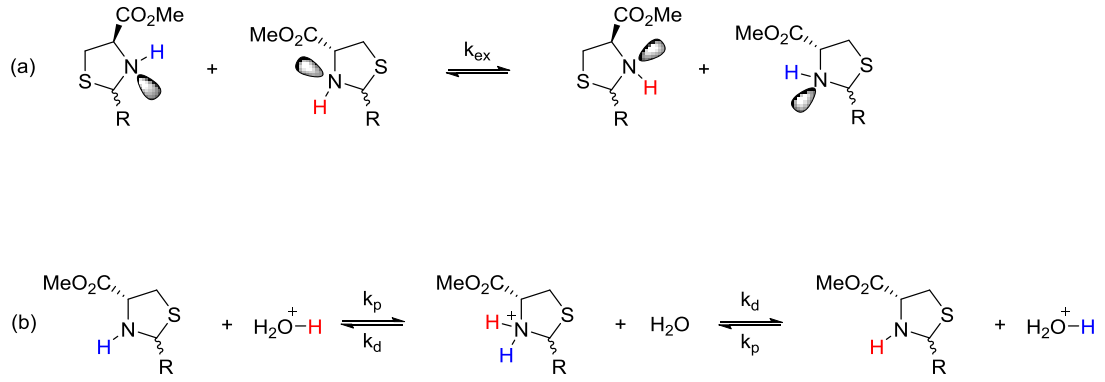


Figure 2.12. Possible proton exchange mechanisms in thiazolidines (a) bimolecular proton exchange (b) acid-catalysed proton exchange. Adapted with permission from Panduwawala, T.D., Josa-Cullere, L., Kuprov, I., Odell, B., Moloney, M.G. and Claridge, T.W.C., *J. Org. Chem.*, **2016**, *81* (10), 4142-4148, copyright (2016) American Chemical Society.

This exchange process could lead to modulation of the scalar coupling since the incoming proton spin-state is uncorrelated with the spin-state of the protons vicinal to the nitrogen centre. Further, this leads to exchange-decoupling where there is a loss of coupling structure and the NH resonance broadens. This behaviour was clearly demonstrated in a variable temperature study (213-298 K) for **78**, where the well-resolved coupling structures of NHs is gradually lost and broadened with increasing temperature, which eventually collapses to one broad NH resonance at 298 K.[79] Estimates of NH exchange rates for thiazolidine **78** could be obtained from line shape simulations of the data from variable temperature experiments which were then used to simulate NOESY spectra for **78** using the *Spinach* spin-simulation programme.[79] At rates above ~200 s^{-1}, scalar cross-relaxation peaks could be reproduced as that was experimentally observed for **78**.[79] It is also noteworthy that NH-H5 cross peaks appeared more intense than NH-H2 despite the influence of identical exchange processes and

similar *J*-coupling constants. This is a result of the smaller chemical shift difference for NH-H5 compared to NH-H2, which affects SCRFK as expressed in Equation 1.

Further investigation into this phenomenon revealed that, where SCRFK is not observed, the effect could be triggered by (1) acidification or (2) increasing concentration.

(1) Thiazolidine **78** dissolved in acid-free CD_2Cl_2 showed no cross-peaks related to SCRFK at ambient temperature, but adding trace amounts of deuterated acetic acid (0.01 % v/v) reintroduced positive-cross peaks. The effect of acid on the exchange process was further investigated where thiazolidine **78** treated with pH 5 buffer gave rise to SCRFK cross-peaks while that treated with pH 7 did not, indicating that this exchange process is indeed acid-catalysed.[79]

(2) The influence of concentration on SCRFK was studied for **78** dissolved in acid-free CD_2Cl_2 over a concentration range of 8 mM-1.8 M. Broadening of the NH resonances was observed with the loss of coupling structure with increasing concentration, indicative of an increase in exchange rate. Moreover, scalar cross-relaxation cross-peaks were only observed at higher concentrations as expected from the ^{1}H spectra, suggesting the involvement of bimolecular exchange.[79]

Scalar cross-relaxation cross-peaks were also observed in a range of solvents such as $CDCl_3$, CD_2Cl_2, C_6D_6, toluene-d_8 but was not observed in DMSO-d_6 possibly due to hydrogen bonding that interferes with the NH proton exchange process.[79]

Thus, the experimental evidence obtained for SCRFK highlights the salient point that NH proton exchange process is responsible for scalar cross-relaxation peaks observed between NHs, H-2 and H-5 in NOESY spectra with the NH proton on-off rate being temperature dependent and the NH exchange mechanism being bimolecular and acid-catalysed as proposed in Figure 2.12.

2.7 Summary

In this chapter, the synthesis of the C-2 aryl substituted bicyclic tetramate core and the stereoselectivity of the cyclisation process were discussed. Tetramates derived from L-serine with aromatic substituents at C-2 were unstable. Replacing this choice of amino acid with L-cysteine afforded stable bicyclic tetramates with aromatic substituents at C-2. Dieckmann cyclisation leading to these bicyclic tetramates was highly chemoselective and diastereoselective with a '*trans*' relationship observed for H-2 and H-5 of the tetramate core, placing the aromatic ring on '*exo*' face of the bicyclic ring system. Further, Dieckmann cyclisation proceeded with good enantioselectivity, the minor attrition in e.e. arising from the less efficient epimerisation at C-5 of the *cis*-malonamide followed by Dieckmann ring closure. Of interest was that this highly chemoselective cyclisation proceeds in a different direction to that observed for the *t*-Bu series, highlighting the influence of the C-2 substituent on the chemoselectivity of the Dieckmann cyclisation process. In addition, during the course of assigning the relative stereochemistry of the intermediate thiazolidines and oxazolidines, an interesting phenomenon 'SCRFK' arising from the modulation of the coupling constants of *J*-coupled protons N-H/H-2 and N-H/H-5 was observed as a result of the bimolecular NH proton exchange process.

CHAPTER 3 : FUNCTIONALIZED TETRAMATES - I

Functionalized tetramates can be derived from side chain modifications at C-2 and C-7 leading to tetramate libraries. The synthesis of the core tetramate esters was outlined in Chapter 2 and this chapter will discuss the functionalization of these tetramate ester templates at C-7 (Figure 3.1).

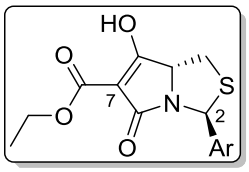


Figure 3.1. Tetramates for C-7 functionalization.

3.1 Functionalized tetramic acids

As discussed in Section 1.4, tetramic acid is a pyrrolidine-2,4-dione ring system **1** that exists mainly as a 3-acyl derivative or sometimes as a 4-*O*-alkyl ether derivative in natural products. The tetramic acid core **1** (Figure 3.2) can undergo reactions with nucleophiles at the cyclic ketone C-4, electrophilic substitution at C-3 and acylation at C-3 or O-4.[35] The latter leads to the mostly commonly found 3-acyl derivatives and less abundant 4-*O*-alkyl ether derivative respectively.

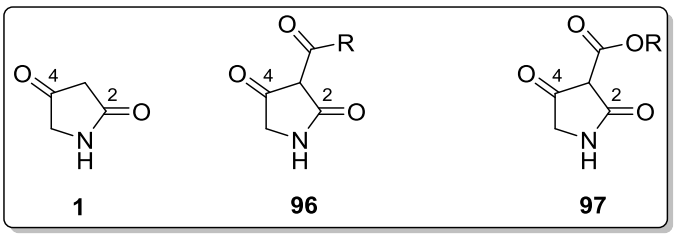


Figure 3.2. Unsubstituted and substituted tetramates.

As depicted in the name of this class of compounds, they are inherently acidic at H-3 and vary with the C-3 substitution type. While the unsubstituted form is only weakly acidic with the

pKa of H-3 being 6.4, the 3-acyl substituted tetramates **96** have a pKa of 3.0-3.5 and 3-alkoxycarbonyl tetramates **97** a pKa of 2.3-2.5.[35]

For C-3 substituted tetramates, ^{1}H NMR spectra indicate complete enolization and may exhibit several tautomeric forms **98A**-**D** in solution as represented in Figure 3.3.

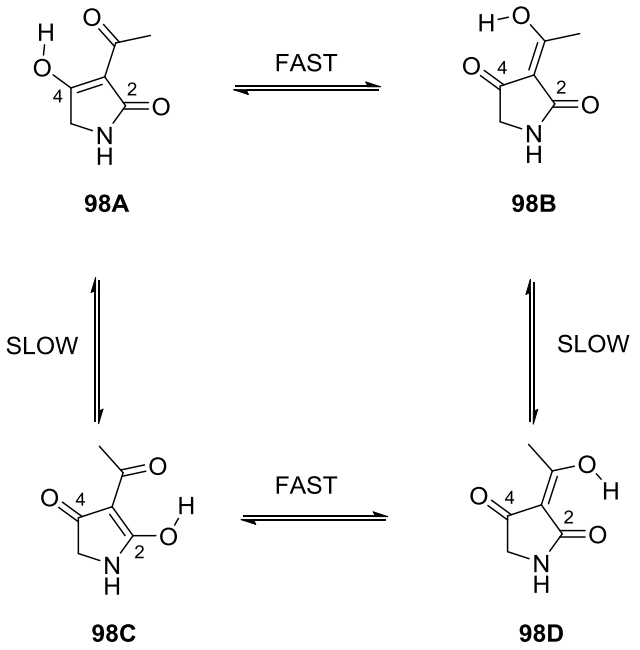


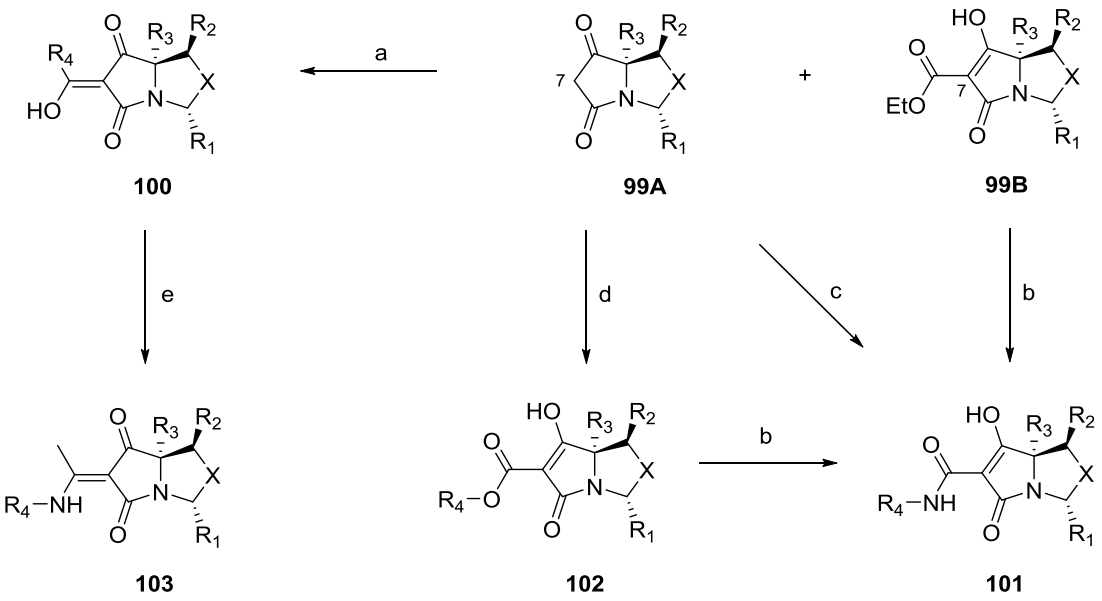
Figure 3.3. Tautomeric behaviour of 3-acyltetramic acids in solution. Adapted with permission from Royles, B. J. L., *Chem. Rev.* **1995**, *95* (6), 1981-2001. Copyright (1995) American Chemical Society.

Tautomer pairs A-B and C-D are internal tautomers that are rapidly interchanging through proton transfer. This internal tautomerisation process is too fast for the separate tautomers to be detected on NMR time-scale and as a result, we observe an averaged-out signal. However, the external tautomerism observed for the *endo*-enolic pair A-C and *exo*-enolic pair B-D via bond rotation of the acyl side chain occurs more slowly and the separate tautomers of each pair can be detected by NMR spectroscopy. Thus, the ratios of the external tautomers could be quantified by analysis of ^{1}H and ^{13}C NMR spectra.[35] Further, ^{13}C chemical shift data for the C-2, C-4 and 3-acyl carbonyl functional groups proved to be useful for the deduction of the tautomeric forms observed in solution. The enolic carbon C-4 of **98A** would resonate at a

lower frequency compared to the keto group at C-4 of **98C**, while the H-bonded carbonyl at C-4 of **98B** would resonate more downfield compared to the free carbonyl group at C-4 in **98D**.[35]

3.2 Previous work by the Moloney Group

Functionalization of the bicyclic tetramic acid template at C-7 (analogous to C-3 of the tetramic acid core **1**) was previously demonstrated by the Moloney group, where the effect of various 7-acyl, 7-carboxamide and 7-enamine substitutions on antibacterial activity and enzyme inhibition was analysed.[66]

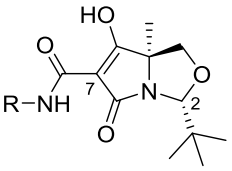


Scheme 3.1. General reaction scheme to access C-7 functionalized tetramates from the bicyclic tetramate templates **99A** and **99B**; (a) R_4CO_2H (1.1 eq), DCC (1.1 eq), DMAP (1.1 eq), CH_2Cl_2, rt, overnight; (b) RNH_2, toluene, reflux, 4h; (c) for R_4=Ph, Phenyl isocyanate (1.1 eq), DMAP (1.3 eq), rt; (d) R_4=Bu, butyl chloroformate (1.2 eq), DMAP (2.2 eq), CH_2Cl_2, rt; (e) amine (1.1 eq), toluene, reflux, 4 h; from **100** (where R_4=Me).

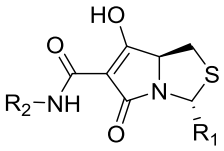
Following the synthetic routes from Scheme 3.1, several functionalized tetramic acids were developed, examples for which are given in Figure 3.4. As discussed in Section 1.6.2 (page 27-29), enzyme inhibition studies of UPPS and RNA polymerase showed that dual enzyme

inhibition could be achieved for carboxamide tetramates with either a lipophilic aliphatic or a bulky aromatic group. Appropriately substituted bicyclic 7-acyltetramic acids too displayed dual enzyme inhibition, although less effectively against UPPS. Interestingly, enamines did not exhibit enzyme inhibitory activity, unlike their structurally similar 7-carboxamides and 7-acyltetramate analogues, highlighting the need for the oxygen atom of the amide/acyl group that might be important for favourable ligand-receptor interactions. Further, the need for a substituent at C-2 was highlighted by the compromised activity against both enzymes observed for **58D** compared to its *t*-Bu analogue **58C**.[66]

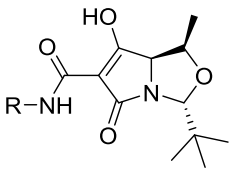
Similarly, an assessment of whole-cell antibacterial activity against clinically relevant bacteria has shown that carboxamide tetramates showed excellent bioactivity along with some 3-acyltetramic acids, while the enamines were not active. However, the loss of bioactivity in the presence of human serum albumin was of concern. The carboxamide tetramates being smaller and less lipophilic among other compounds synthesised in the Moloney drug discovery programme, with appropriate lipophilicity and drug-likeness, these carboxamides could be developed to reduce affinity to plasma proteins, thereby optimising tetramates for oral and injectable use.[66]



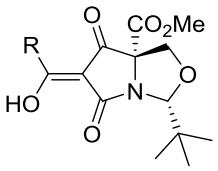
(±)-57A; R=CH_2Cy
(±)-57B; R=1-adamantyl
(±)-57C; R=C_9H_{19}
(±)-57D; R=4-phenoxyphenyl



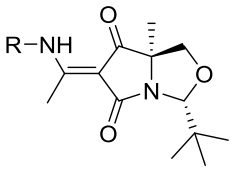
58A; R_1=*t*-Bu, R_2=1-adamantyl
58B; R_1=*t*-Bu, R_2=myrtanyl
58C; R_1=*t*-Bu, R_2=C_9H_{19}
58D; R_1=H, R_2=C_9H_{19}
59A; R_1=*t*-Bu, R_2=CH_2Cy



104A; R=1-adamantyl
104B; R=myrtanyl



105A; R=$C_{11}H_{23}$
105B; R=$C_{13}H_{27}$



(±)-106A; R=1-adamantyl

Figure 3.4. Representative analogues of C-7 functionalized tetramates.

All of the tetramates above comprised of a hydrophobic *t*-Bu group and the synthesis of tetramate libraries with less hydrophobic substituents at C-2 had not been explored before. Since the ability to synthesise bicyclic tetramates derived from thiazolidines with varied substitution at C-2 as described in Chapter 2 was possible, the scope to develop tetramates with reduced lipophilicity and improved drug-likeness in our efforts to develop drug candidates for oral use was expanded (Physicochemical properties of these compounds and their antibacterial activity will be discussed in Chapter 5).

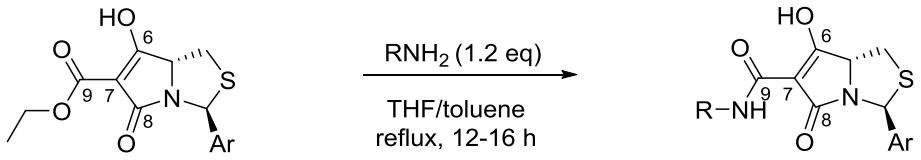
3.3 Synthesis of carboxamide tetramates derived from thiazolidines with C-2 aryl substitution

The carboxamide functional group is commonly found in synthetic and natural products. In an analysis of chemical functionalities of the known drugs in the Comprehensive Medicinal Chemistry (CMC) database,[83] the carboxamide functionality was found in 27 % of known

drugs, highlighting its importance in medicinal chemistry. This is not surprising as they are relatively chemically stable, and have both hydrogen-bond accepting and donating properties.

3.3.1 Synthetic route to access carboxamide tetramates

The tetramate template obtained from the Dieckmann cyclisation outlined in Chapter 2 resulted in an alkoxy carbonyl functional group at C-7 as the major product of cyclisation, which is in contrast to those systems with *t*-Bu at C-2. Thus, the bicyclic tetramate template **107** provided an electrophilic site susceptible to nucleophilic attack, to generate carboxamide tetramates of type **108** as shown in Scheme 3.2.



107 **108**

Scheme 3.2. General reaction scheme to access C-7 functionalized carboxamide tetramates **108** from the bicyclic tetramate ester template **107**.

Ethyl esters are not considered as activated species for amide bond formation and the extended conjugation observed for the tri-carbonyl system in tetramate **107** meant that the reactivity of the ester would be further compromised and elevated temperatures may be required to drive the reaction. Since the tetramate esters were quite polar, THF was chosen as an inert solvent to aid solubility. However, the reaction did not go to completion by refluxing in THF for 16 h. Thus, toluene was used as a co-solvent together with THF to achieve the desired solubility and elevated temperatures needed for the aminolysis of ester **107** to yield carboxamide **108**.

Following this approach, a library of carboxamide tetramates was synthesised in good yield using a range of primary amines to access a broad chemical space, and the results are listed in Tables 3.1-3.4 (the chemical space occupied by these compounds and their antibacterial activity will be discussed in Chapter 5).

While carboxamide synthesis proceeded in good yield without the need for a base catalyst, our efforts to improve the yield of **109** and **120** proved that the use of catalytic DMAP[71] or DBU[84] led to no improvement in yield. More recently, L-proline was used as a catalyst in the transamidation of carboxamides with improved yield.[85] However, this effect could not be translated to carboxamide synthesis of **109** from the corresponding tetramate ester and amine.

Table 3.1. C-7 carboxamide tetramates **109-114** from 1-adamantylamine.

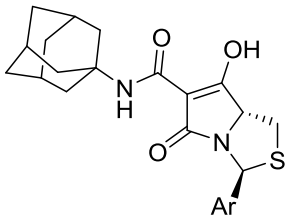
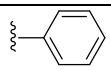
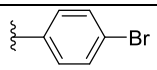
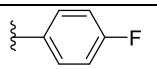
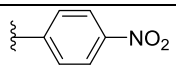
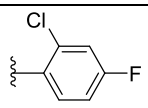
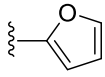
Carboxamide tetramate	Compound number	Ar =	% Yield	Tautomeric ratio (AB:CD)
	**109**		45	2.7:1
	110		70	2.6:1
	111		56	2.7:1
	112		40	2.4:1
	113		60	2.5:1
	114		92	3:1

Table 3.2. C-7 carboxamide tetramates **115-118** from 4-cyclohexylaniline.

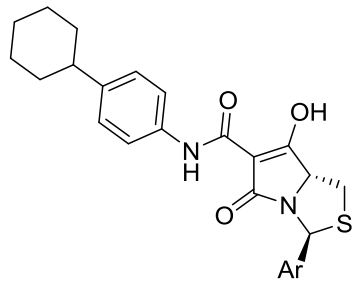
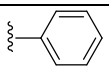
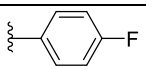
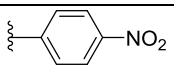
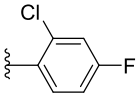
Carboxamide tetramate	Compound number	Ar =	% Yield	Tautomeric ratio (AB:CD)
	**115**		54	15:1
	116		45	13:1
	117		59	12.5:1
	118		73	15:1

Table 3.3. C-7 carboxamide tetramates **119-122** from 4-chloro-2-methylaniline.

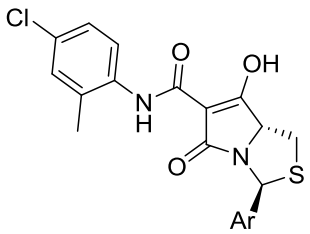
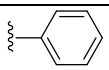
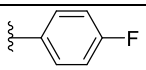
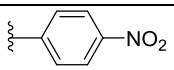
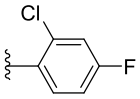
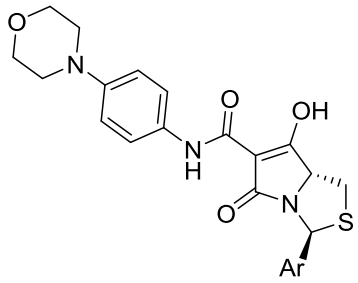
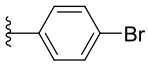
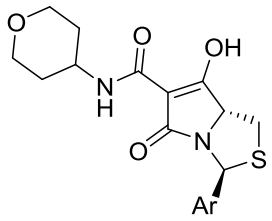
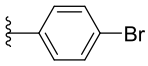
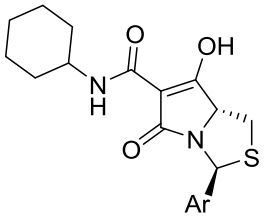
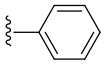
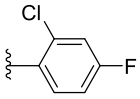
Carboxamide tetramate	Compound number	Ar =	% Yield	Tautomeric ratio
	**119**		53	only 1 form
	120		40	"
	121		37	"
	122		35	"

Table 3.4. C-7 carboxamide tetramates **123-124** from 4-cyclohexylaniline, **125** from 4-aminotetrahydropyran and **126-127** from cyclohexylamine.

Carboxamide tetramate	Compound number	Ar =	% Yield	Tautomeric ratio
	**123**		69	only 1 form
	124		75	"
	**125**		70	5.2:1
	**126**		49	3.1:1
	127		40	3.1:1

3.3.2 Stereochemistry across the bicyclic system of carboxamide tetramates

As anticipated, the stereochemical relationship of the bicyclic tetramate esters was conserved in the amide systems, where a *trans*- relationship is observed between H-2 and H-5, as shown by NOE analysis in Figure 3.5.

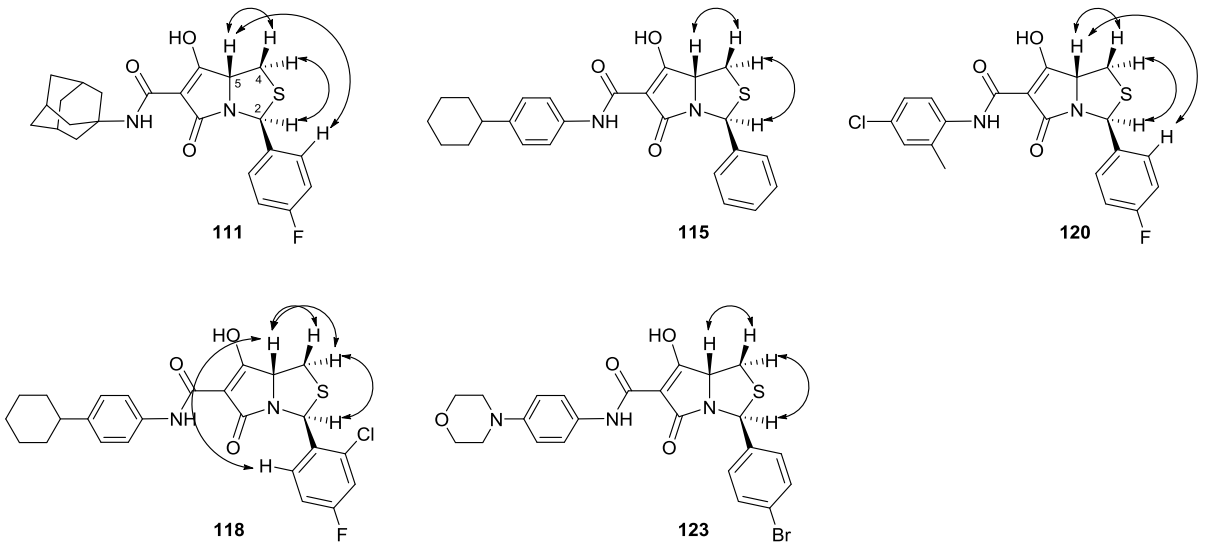


Figure 3.5. NOE analysis of selected carboxamide tetramates.

3.3.3 Assignment of carbonyl functional groups of carboxamide tetramates

The carbonyl functional groups of carboxamide tetramates were assigned by HMBC correlation data and are exemplified in Figure 3.6. C-6 correlates with H-4s and H-5, while C-8 correlates with H-2 and H-5. For compounds **125** and **126**, C-9 correlates with the β-CH of the amine with respect to C-9, confirming the assignment of the carbonyl functional groups.

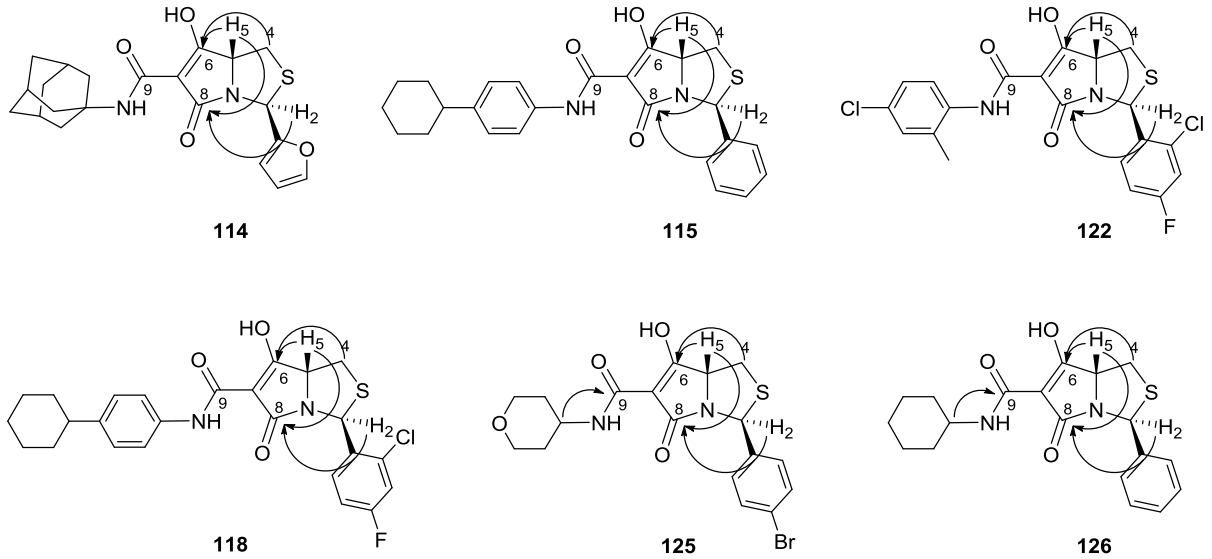


Figure 3.6. HMBC correlation data of selected carboxamide tetramates.

Moreover, under the experimental conditions used for the synthesis of carboxamide tetramates, it is unlikely that the more polar functional group isomer **128** would be formed by reaction of amine at C-6 (Figure 3.7).

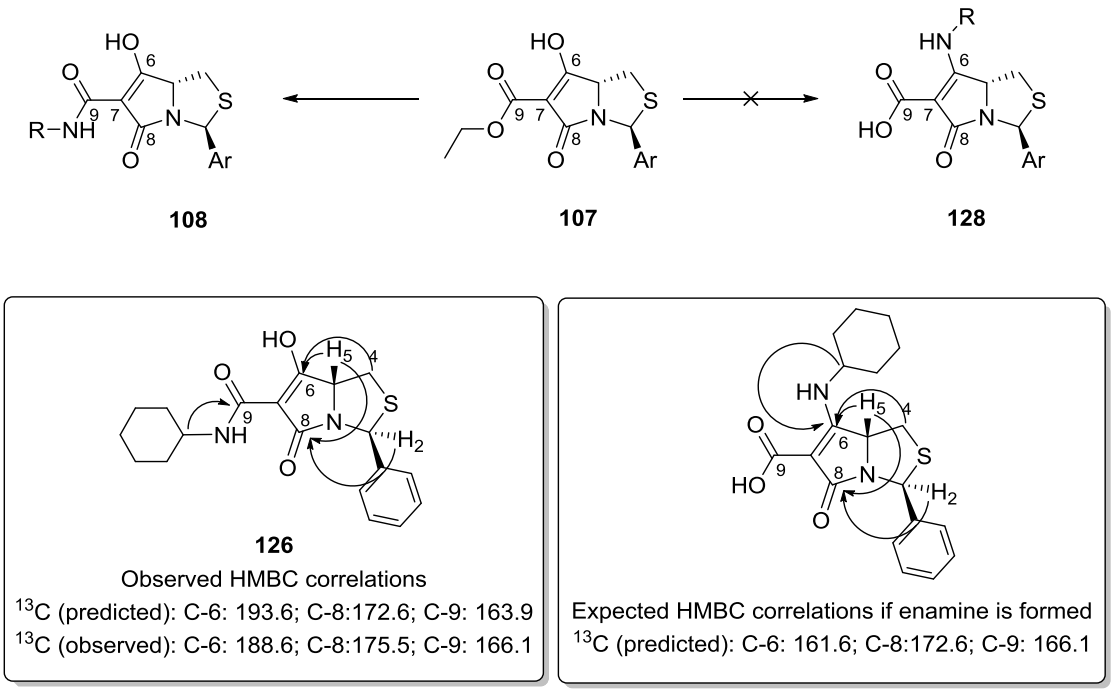


Figure 3.7. HMBC correlation data for the selected example **126** confirming carboxamide formation. ^{13}C predicted chemical shift values were obtained from ACD/I-Lab, version 15.01, Advanced Chemistry Development, Inc. (ACD/Labs), Toronto, ON, Canada, www.acdlabs.com, 2015.

HMBC correlation data and ^{13}C chemical shift values of **125-127** were useful in confirming that the product formed is indeed the amide **108** and not enamine **128**. This is exemplified in Figure 3.7 for tetramate **126**. Moreover, X-ray crystal structure obtained for **113** confirmed the formation of carboxamide tetramate (Figure 3.8).

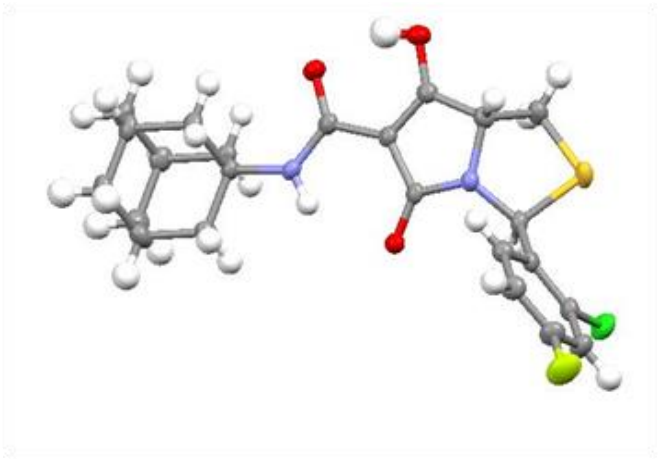


Figure 3.8. X-ray crystal structure of **113**.

3.3.4 Tautomerism in carboxamide tetramates

As discussed in Section 3.1, the internal tautomeric pairs (A and B, C and D) are not distinguished by NMR analysis and are observed as an averaged hybrid, while the external tautomeric pairs (AB and CD) have distinct chemical shift differences. Since the tautomeric forms are closely associated with C-6, C-8 and C-9 of the carboxamide tetramates, an analysis of their ^{13}C chemical shift differences was of interest.

Table 3.5. ^{13}C chemical shifts of tautomers of selected examples of carboxamide tetramates.

Compound Number	Solvent	^{13}C chemical shifts (ppm)			Tautomeric form
		C-6	C-8	C-9	
109	$CDCl_3$	187.8	172.2	165.8	major
		191.1	178.0	166.4	minor
110	$CDCl_3$	188.4	172.4	165.9	major
		191.1	178.2	166.4	minor
113	$CDCl_3$	188.7	172.0	166.0	major
		191.0	177.8	166.5	minor
	MeOD	189.5	175.2	166.9	only 1 form (hybrid)
118	CD_2Cl_2	185.2	171.7	164.3	major
		191.5	178.2	165.4	minor
122	CD_2Cl_2	184.7	171.7	164.3	only 1 form
125	CD_2Cl_2	186.6	172.5	165.9	major
		191.6	178.8	166.5	minor

Interestingly, the ^{13}C chemical shifts of C-6, C-8 and C-9 of the minor tautomers were invariably more downfield than their major tautomers, with the minimum $\Delta\delta c$ observed for the *exo*-cyclic amide C-9. The tautomeric behaviour is also solvent dependent, as depicted in the chemical shift values of **113** in $CDCl_3$ and CD_3OD. In methanol, only one set of resonances is observed for each carbon, and appears to be that of an averaged hybrid structure due to the facile equilibrium of the tautomeric forms. Similarly, only one set of resonances was observed in other polar solvents such as acetone-d_6, DMSO-d_6 and CD_3CN. While it was not possible to elucidate the tautomeric pairs solely based on the chemical shift data above, NOESY correlation data obtained for **118** in dry CD_2Cl_2 revealed that the major tautomer is

118A based on the NOE observed from H-5 to proximal enol O-H, which would not have been possible in other tautomeric forms B, C and D (Figure 3.9). This conclusion is supported by X-ray crystallographic structure determination of tetramate **113** (Figure 3.8, page 69, *vide supra*), which was found to exist in the *endo*-enolic form A. Since the chemical shift pattern observed for the major/minor tautomers are similar for all compounds, the carboxamide tetramates described in this chapter and beyond are drawn in their major tautomeric form A.

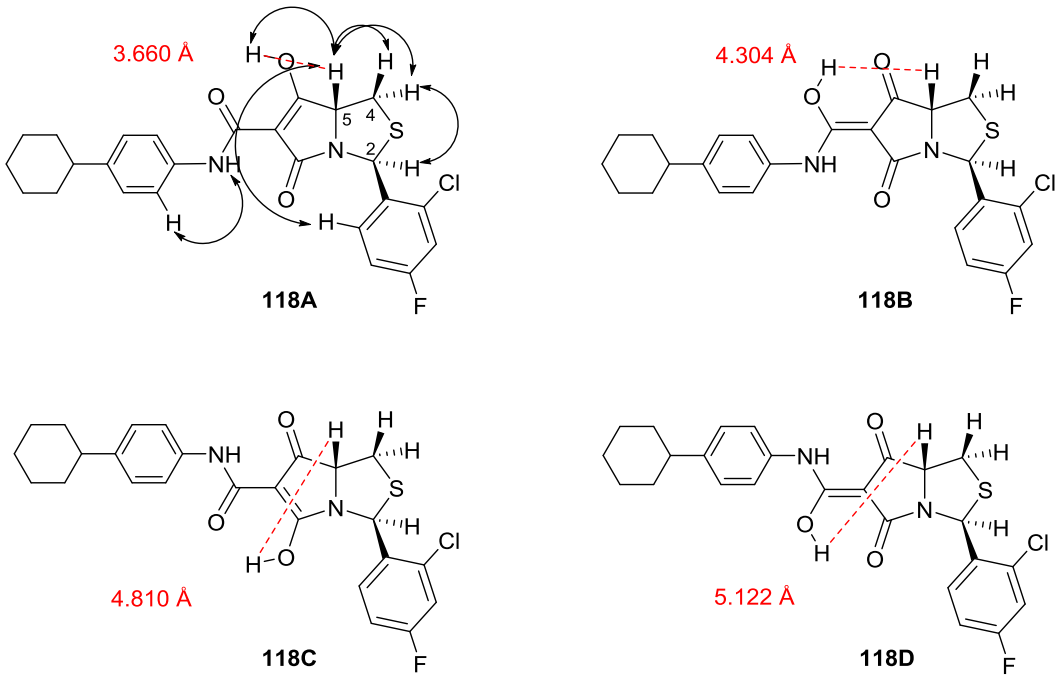


Figure 3.9. NOESY correlation data for **118** suggesting tautomer A as the major tautomer. The distance between H-5 and enol O-H for each tautomer was calculated for the MM2-energy minimized molecular model using Chem3D v.15.

Minimum energy conformations were generated for the four tautomeric forms A-D for compounds **109-127** using MM2 method of Chem3D version 15 (data provided in Appendix E) and three examples are given in Table 3.6. The calculations provided a comparison of the thermodynamic stability of tautomer A in comparison to other forms, which is the tautomeric form observed for **113** by X-ray crystallography and the major tautomeric form of **118** by NOESY analysis.

Table 3.6. MM2-minimized energy of tautomeric forms A-D for selected examples **113, 118** and **122**.

Compound Number	Tautomeric ratio	Minimum Energy of tautomeric forms (kcal/mol)			
		A	**B**	**C**	**D**
113	2.5:1	18.9332	39.8570	26.8331	39.3211
118	15:1	6.9345	31.2785	13.0366	30.7538
122	only 1 form	0.7964	25.5440	7.0510	26.2300

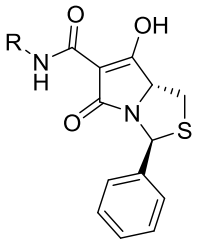
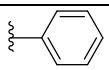
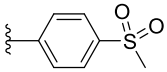
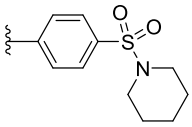
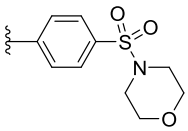
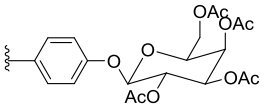
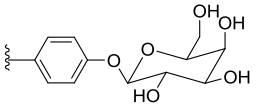
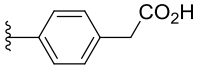
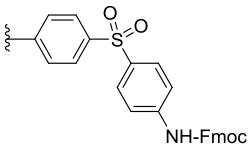
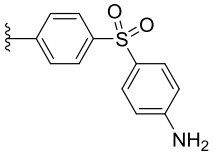
The ratio of minimum energies of external tautomers **A** and **C** ($E_{min}C/E_{min}A$) for **113** is 1.42. For tetramates **118** and **122**, it is 1.88 and 8.85 respectively. This could provide insight into the population difference observed for **113**, **118** and **122** and its relationship to the corresponding energy ratio, where **113** with a smaller $E_{min}C/E_{min}A$ ratio also has a smaller tautomeric population difference.

3.3.5 Elaboration of the carboxamide tetramate library

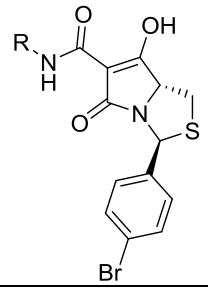
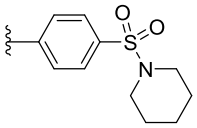
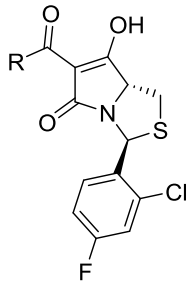
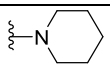
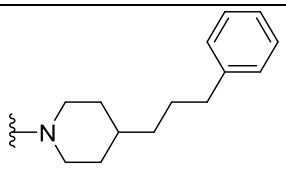
3.3.5.1 Further synthesis of carboxamide tetramates from 90a, 91a and 94a

In order to obtain a more diverse range of substituents at the C-7 side chain, several other secondary and tertiary carboxamide tetramates were prepared from tetramate esters **90a**, **91a** and **94a** as listed in Table 3.7, using a range of primary and secondary amines. This enabled the incorporation of a range of functional groups such as sulfones, sulfonamides, carboxylic acids and carbohydrates to probe an expanded chemical space and compare their bioactivity with the simple unsubstituted phenyl system **129** (Chemical space occupied by these tetramates is discussed in Chapter 5).

Table 3.7. Carboxamide tetramates **129-137, 138** and **139-140** derived from tetramate esters **90a**, **91a** and **94a**. (*Yield quoted for the syntheses of **134** via methanolysis of **133** and **137** via deprotection of **136**)

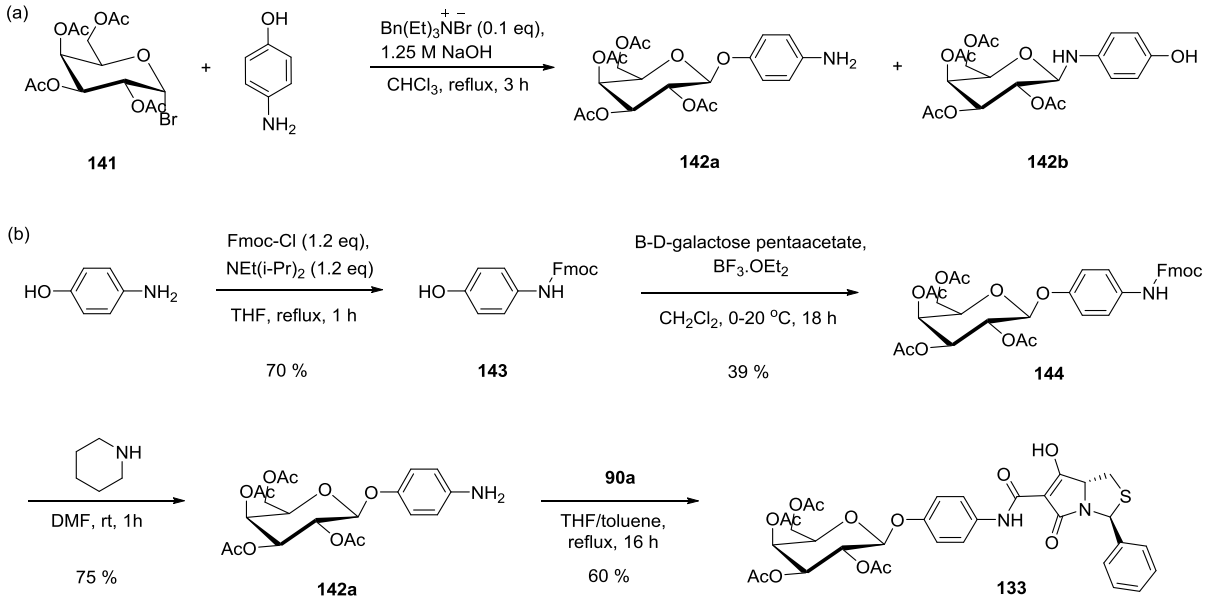
Carboxamide tetramate	Compound number	R =	% Yield
	**129**		30
	130		74
	131		75
	132		72
	133		60
	134		89*
	135		24
	136		30
	137		40*

Continued...

	**138**		40
	**139**		30
	140		36

3.3.5.2 Synthesis of carboxamide tetramates 133-134

The design of carboxamide **133** was inspired by naturally occurring tetramates kibdelomycin **12** and amycolamicin **13**. Incorporation of a glycosylated subunit in **133** was achieved using a glycosylated amine derivative for which the synthesis is outlined in Scheme 3.3. It has been previously reported that the desired glycosylated amine **142a** could be synthesised in 87 % yield from 4-aminophenol and bromogalactose **141** under the reaction conditions given in Scheme 3.3 (a).[86] However, this result could not be replicated as a complicated NMR spectrum was obtained suggesting the formation of two inseparable products in 3:1 ratio, which was assumed to be **142a** and **142b**. Thus, instead of 4-aminophenol, Fmoc protected amine **143** was used for glycosylation, Scheme 3.3 (b).



Scheme 3.3. Synthesis of glycosylated tetramate **133**.

Since the Fmoc protecting group is base sensitive, the glycosylation conditions using NaOH in (a) could not be employed for **143**. A Lewis acid-mediated glycosylation of benzyl alcohol has been reported previously using $BF_3.OEt_2$.[87,88] Thus, these conditions were applied to synthesise **144** with equatorial substitution of the aryl group confirmed by the *trans*-diaxial $^3J_{H1\text{-}H2}$ coupling constant (8.0 Hz) and its comparison to starting β-D-galactose pentaacetate (Figure 3.10). Base-mediated deprotection of the amine gave the desired product **142a** which was treated with tetramate **90a** to yield carboxamide **133** in 60 % yield.

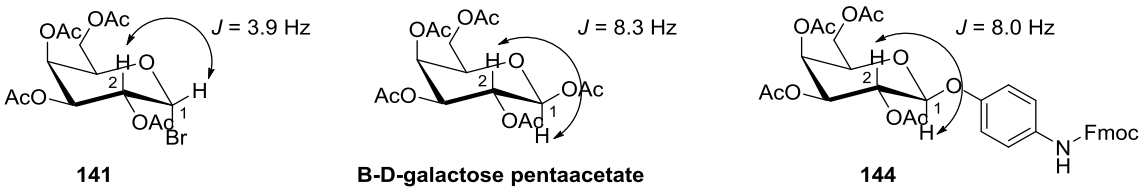
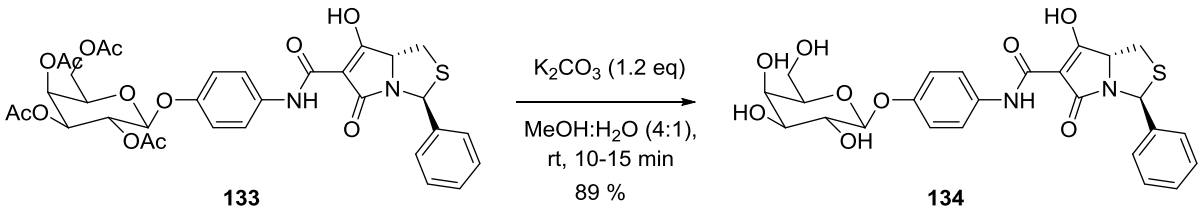


Figure 3.10. Equatorial substitution of aryl group in **144**.

Methanolysis of tetramate **133** under basic conditions afforded **134**, thereby incorporating a very polar sugar group on the C-7 side chain, Scheme 3.4.

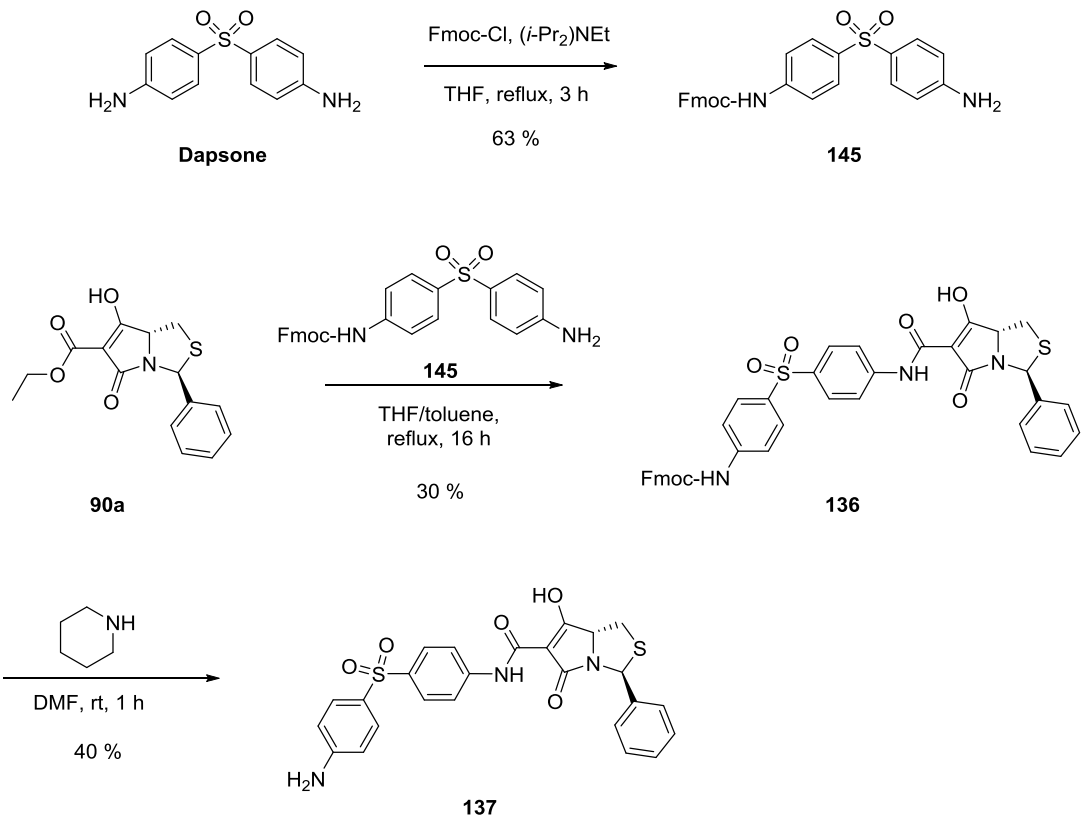


Scheme 3.4. Synthesis of **134** by methanolysis of acylated glycosyl unit of **133**.

3.3.5.3 Synthesis of carboxamide tetramates 133-134

Dapsone (diaminodiphenyl sulfone), which is a sulfone drug, is an antibiotic that has a mode of action similar to that of sulfonamides. Their antibacterial activity is a result of the inhibition of dihydropteroate synthetase through competition with *p*-aminobenzoate (PABA), thereby inhibiting the synthesis of dihydrofolic acid.[89] The incorporation of dapsone on to the tetramate system via the synthetic route outlined in Scheme 3.5 was investigated.

Fmoc protection of dapsone with 1 eq of Fmoc-Cl resulted in the monoprotected product **145**, while protection of both amine groups was not observed. Aminolysis of tetramate ester **90a** with amine **145** resulted in carboxamide tetramate **136**, which on subsequent deprotection gave **137** in 40 % yield.



Scheme 3.5. Synthesis of tetramates **136-137**.

3.4 Purification of carboxamide tetramates

Similar to the tetramate esters discussed in Chapter 2, carboxamide tetramates purified by silica gel column chromatography also gave broad signal resonances in the ^{1}H NMR spectra, consistent with metal chelation. Hence, these carboxamides were routinely purified on silica column run with 1 % Et_3N in the eluent. Residual Et_3N was removed by washing with 5 % citric acid, giving the purified product with sharp and well resolved ^{1}H spectra (Figure 3.11). Alternatively, it was also possible to purify these carboxamides on silica column run without Et_3N in the eluent, and subsequently washed with 2 M HCl to give the metal-free form.

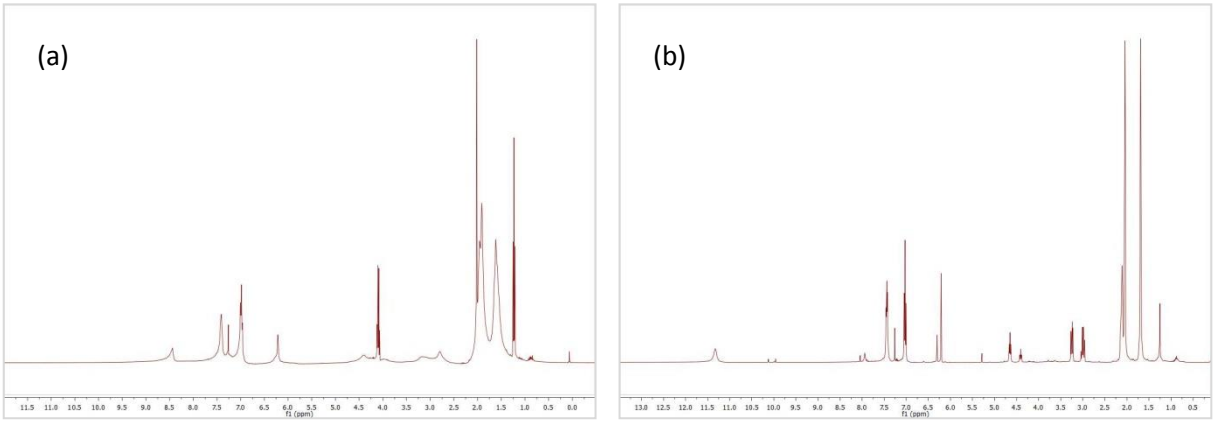


Figure 3.11. ^{1}H NMR spectra of tetramate **111** post-column purification, (a) before acid wash and (b) after acid wash.

Since peak broadening was a recurrent observation post-column purification, the metals present and their content in the metal-chelated tetramate and acid-washed tetramate (assumed metal-free form) for compound **109** were analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, from the Department of Earth Sciences, University of Oxford). Na, Mg, Ca and Zn were found in high abundance in the metal-chelated tetramate, which have been significantly removed by washing with 2M HCl (Table 3.8).

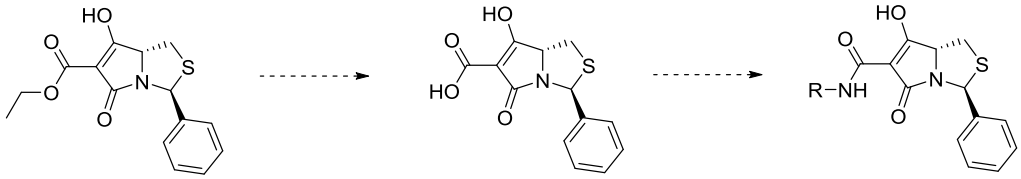
Table 3.8. Metal content observed in metal-chelated and acid-washed form (assumed metal-free) of carboxamide **109**.

Compound 109	**Metal content (ppm)**			
	Mg	**Ca**	**Zn**	**Na**
Metal-chelated	7026.1	20483.7	71.2	3385.6
Acid-washed (assumed metal-free)	15.9	24.8	9.9	18.8

3.5 Attempted functionalizations of the C-7 side chain of tetramates

While aminolysis of tetramate esters to produce carboxamide tetramates was an attractive approach to synthesise tetramate libraries, several other routes to C-7 side chain modifications were also explored, and the results are discussed below.

3.5.1 Carboxamide tetramates via DCC coupling



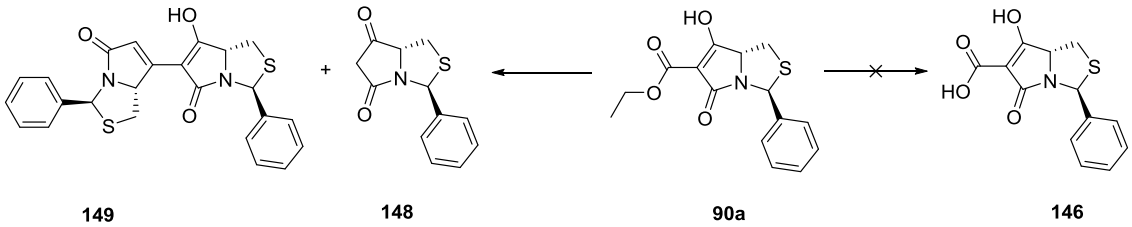
Scheme 3.6. Proposed synthetic route to carboxamide tetramates via DCC coupling.

As represented in Scheme 3.6, the proposed route towards the synthesis of carboxamide tetramate **147** required the intermediate carboxylic acid **146** generated from the hydrolysis of tetramate ester **90a**. Several acid-catalysed and base-mediated conditions were screened to obtain **146** from **90a**. However, the tetramate ester was susceptible to decarboxylation and controlled ester hydrolysis to yield **146** was not achieved.

3.5.1.1 Hydrolysis of the tetramate ester

Attempts to hydrolyze the β-keto ester resulted in the decarboxylated product **148**, under both acidic and basic reaction conditions. Acidic conditions using TFA was most promising to drive the reaction to completion. However, this resulted in **148** in 10 % yield along with the dehydrated dimer **149** (mass observed by LRMS, complicated NMR) in 20 % yield. A similar outcome leading to dimerisation has been reported before for monocyclic tetramates under milder conditions.[90]

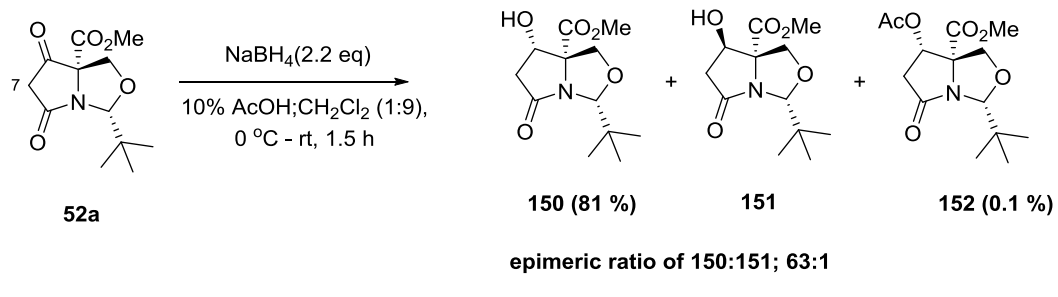
Table 3.9. Attempted hydrolysis of **90a.**



Entry	Conditions	Remarks
1	$LiOH.H_2O$ (1.1 eq), THF/H_2O (1:1), rt, 2 h	Only **90a** detected in crude.
2	$LiOH.H_2O$ (4 eq), THF/H_2O (1:1), reflux, overnight	Reaction did not go to completion to give **148**
3	KOH (5 eq), CH_3CN/H2O (1:1), reflux, overnight	Reaction did not go to completion to give **148**
4	TFA (2 eq), THF/H_2O (1:1), rt, overnight	Reaction did not go to completion to give **148**
5	TFA (4 eq), THF/H_2O (1:1), reflux, 3 h	Reaction went to completion giving **148** (10 %) and **149** (20 %)

3.5.2 C-7 Functionalization via reduction of tetramate systems

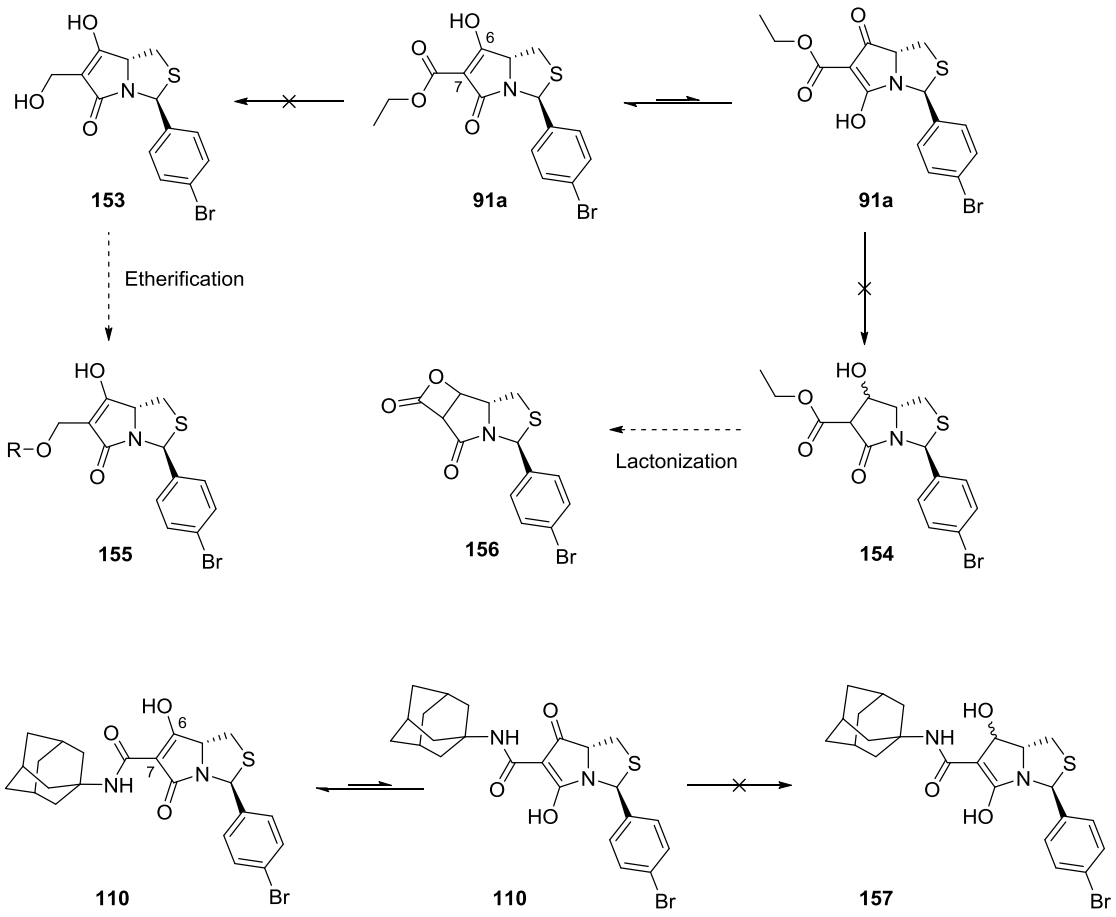
Reduction of bicyclic tetramic acids has previously been explored by Moloney and co-workers.[91] For 7-unsubstituted tetramic acid **52a**, reduction of keto group was achieved by $NaBH(OAc)_3$ generated *in situ* to give a mixture of epimeric alcohols with high stereoselectivity, Scheme 3.7.[91]



Scheme 3.7. Reduction of 7-unsubstituted tetramates

The proposed synthetic route to functionalized tetramates involved the reduction of ethyl ester at C-7 or keto-group at C-6 of tetramate ester **91a** giving alcohols **153** and **154** respectively, Scheme 3.8. While **154** may result in lactonization to yield **156**, alcohol **153** could be used for C-7 side chain functionalization to yield **155** via etherification with various organohalides and phenols/alcohols using Mitsunobu conditions.[92] Similarly, the proposed reduction of carboxamide tetramate **110** would give alcohol **157**.

However, since the work reported herein involves a 7-alkoxy tetramic acid or 7-carboxamide tetramic acid which is enolized at C-6, the conditions reported for **52a** were not successful to give either of alcohol **154** or **157**. This was rather disappointing as it would be reasonable to anticipate a shift in the facile tautomeric equilibrium towards the keto form at C-6 as reduction proceeds. Similarly, reduction of the ethyl ester **91a** to yield **153** was also not observed under the experimental conditions listed in Table 3.10.



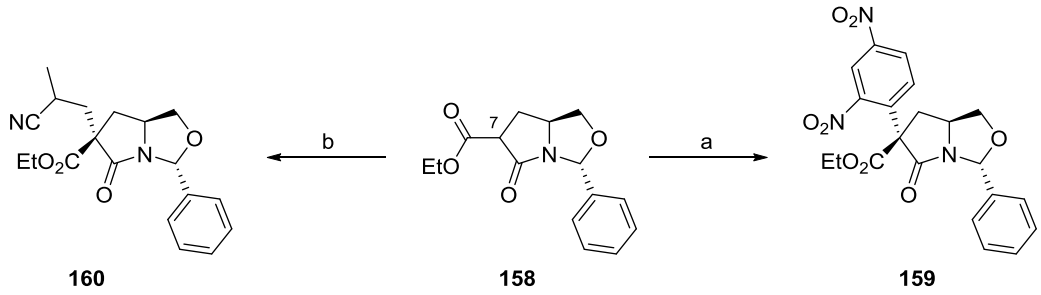
Scheme 3.8. Proposed synthetic route towards C-7 functionalization via reduction.

Table 3.10. Conditions used in attempted reduction of **91a** and **110**.

Entry	Compound Number	Conditions	Remarks
1	**91a** and **110**	$NaBH_4$ (2.2 eq) , EtOH, 0-20 °C, 20 h	Only starting material detected in crude NMR
2	**91a**	$LiBEt_3H$ (1.0 M in THF) (2.2 eq), THF, 0-20 °C, 30 min-12 h	Only **91a** detected in crude NMR
3	**110**	$NaBH_4$, 10 % AcOH in CH_2Cl_2, 0 °C- rt, 2 h	Only **110** detected in crude NMR

3.5.3 C-7 Functionalization via electrophilic α-substitution of tetramate systems

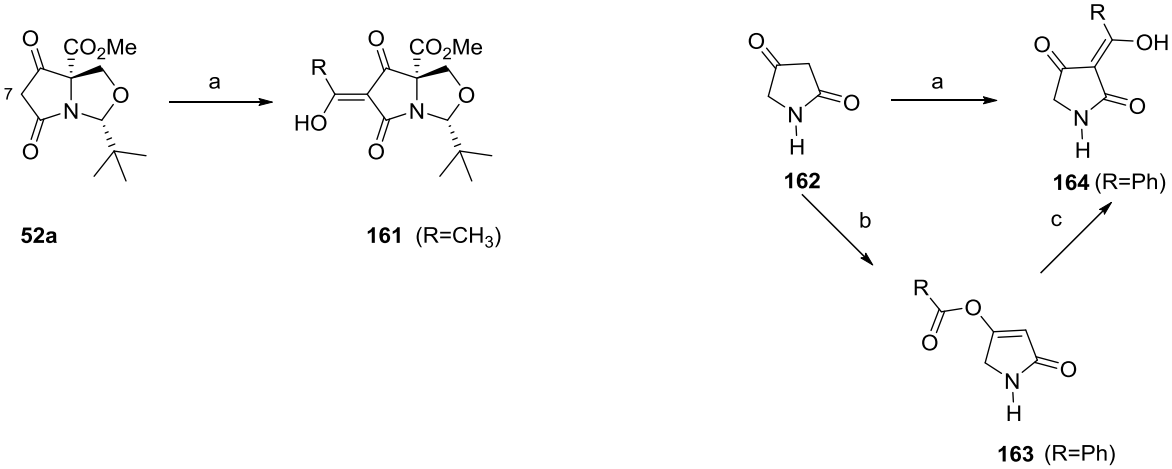
Electrophilic substitutions of pyroglutamate-derived [3.3.0] bicyclic system **158** were studied by Moloney and co-workers for the synthesis of spirocyclic lactams, Scheme 3.9.[93] Bicyclic systems **159** and **160** can then act as precursors for the synthesis of spirocyclic lactams.



160 **158** **159**

Scheme 3.9. Electrophilic α-substitution at C-7. (a) NaH, $FC_6H_3(NO_2)_2$, THF; (b) NaH, Br $CH(CH_3)CN$, THF

Another substitution possible at C-7 is acylation, and this has been widely employed by Moloney and co-workers to generate 7-acyltetramates. While catalytic amounts of DMAP leads to *O*-acyl tetramate **163**, using an excess leads to acyl migration resulting in the desired *C*-acyl tetramates **161** and **164**, Scheme 3.10.[78,94]

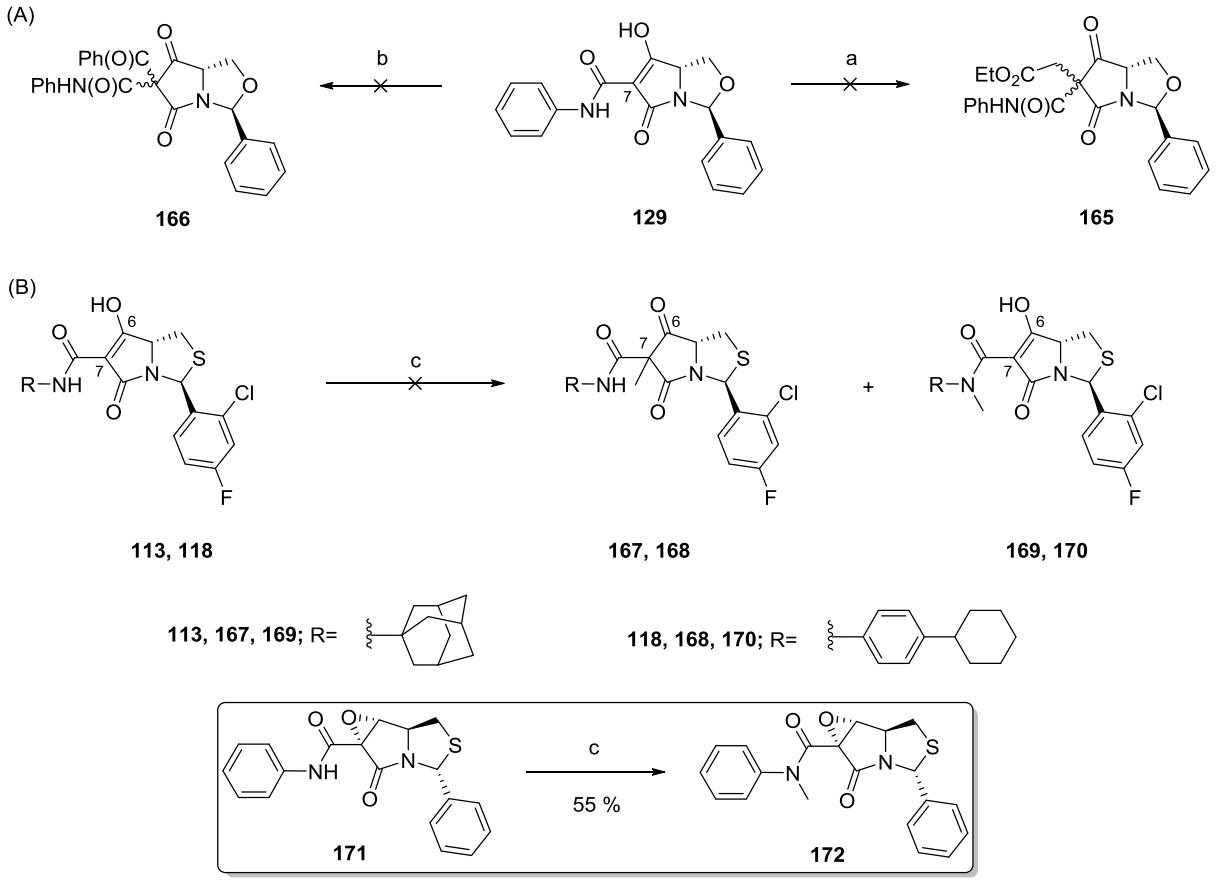


52a **161** (R=CH_3)

Scheme 3.10. C-7 Acylation (a) RCO_2H (1.1 eq), DCC (1.1 eq), DMAP (1.3 eq), CH_2Cl_2, rt; (b) RCO_2H (1.1 eq), DCC (1.1 eq), DMAP (0.1eq), CH_2Cl_2, rt; (c) DMAP (1.3 eq), CH_2Cl_2, rt.

Following on from these procedures, electrophilic α-substitutions of carboxamide tetramate **129** to yield **165** and **166** were investigated (Table 3.11, Entries a-b), but the desired products were not formed. Similarly, reaction of tetramates with much less sterically demanding CH_3I was also not successful in α-methylation of **113** and **118** to yield **167** and **168** respectively (Table 3.11, Entry c). Further, methylation of the amide functional group of **113** and **118** to yield the corresponding tertiary amides **169** and **170** was also not observed, even though this has been observed in a similar pyroglutamate-derived system **171** under the same reaction conditions.[71] The lack of success achieved with reduction at C-6 and electrophilic substitutions at C-7 indicates the low reactivity of these conjugated tricarbonyl systems compared to C-7 unsubstituted tetramates (e.g. **52a**) and pyroglutamate-derived systems **158** and **171** discussed above.

Table 3.11. Electrophilic α-substitutions at C-7.



Entry	Compound Number	Conditions	Remarks
a	**129**	NaH (1.5 eq), Ethyl bromoacetate (1.2 eq), THF, 0-20 °C, 18 h	Mass of product **165** not observed. **129** detected by LRMS
b	**129**	$PhCO_2H$ (1.1 eq), DCC (1.1 eq), DMAP (1.3 eq), CH_2Cl_2, rt, 18 h	**129** isolated. Mass of product **166** not observed
c	**113** and **118**	NaH (2 eq), CH_3I (1 eq), THF, 0-20 °C, 24 h	Only starting material observed by LRMS and ^1H NMR

3.6 Summary

In this chapter, the strategies for the functionalization of the tetramate core at C-7 in library generation was discussed. The selective reactivity of the ester functional group with primary and secondary amines afforded a range of carboxamide tetramates **109**-**127** and **129**-**140**. These tetramates exist in tautomeric equilibrium, with the *endo*-enolic form at C-6 being the major tautomeric form as observed by X-ray crystallography and NOESY correlation data. The lack of reactivity of the tetramate ester **91a** and amides **110**, **113**, **118** and **129** towards reduction, electrophilic α-substitution at C-7 via alkylation/acylation and methylation of secondary amides is indicative of the low reactivity of these conjugated tricarbonyl compounds compared to other β-dicarbonyl tetramate systems.

CHAPTER 4 : FUNCTIONALIZED TETRAMATES - II

The main point of functionalization of the [3.3.0] bicyclic tetramate scaffold in library generation thus far has been the C-7 side chain. Due to the limited synthetic feasibility, tetramate library synthesis with diversity at C-2 had not been explored before. The compound library synthesised previously comprised of a bulky, hydrophobic '*t*-Bu' group at C-2 as this was a synthetic requirement. The loss of antibacterial activity of these compounds in the presence of human serum albumin (HSA) was attributed to their hydrophobic nature that results in a higher binding affinity to HSA. Thus, increasing hydrophilicity at the C-2 end was of interest. This chapter describes the synthesis of a range of functionalized tetramates with diversity at C-2 (Figure 4.1), as potential lead compounds towards novel antibacterials. In this study, both late-stage side-chain functionalization of tetramates via elaborated C-2 pendant groups and the incorporation of functionalized aldehydes in the early stages of tetramate synthesis have been explored for the generation of tetramate libraries.

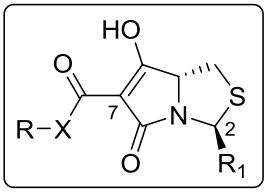
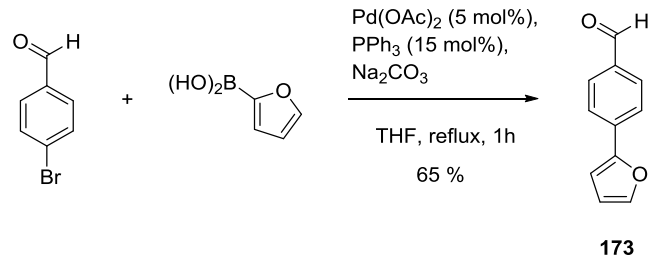


Figure 4.1. Tetramates for C-2 functionalization (X=O, NH)

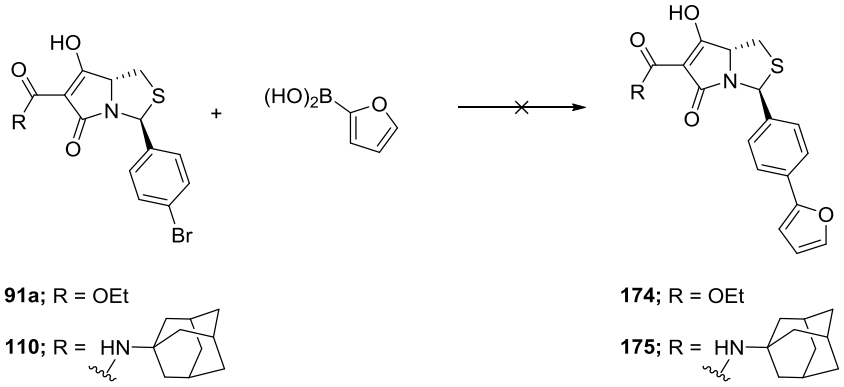
4.1 Elaboration via Suzuki-Miyaura (SM) cross-coupling reactions

In a test reaction, SM cross-coupling of 4-bromobenzaldehyde with 2-furanylboronic acid yielded **173** in 65 % yield, Scheme 4.1. However, attempts to introduce pendant groups at C-2 of the tetramate scaffold via SM cross-coupling failed under the conditions shown in Table 4.1, and only the starting tetramate was isolated by column purification for both **91a** and **110**.



Scheme 4.1. SM cross-coupling of 4-bromobenzaldehyde to give **173**.

Table 4.1. Attempted SM cross-coupling on thiazolidine-derived tetramates **91a** and **110**.

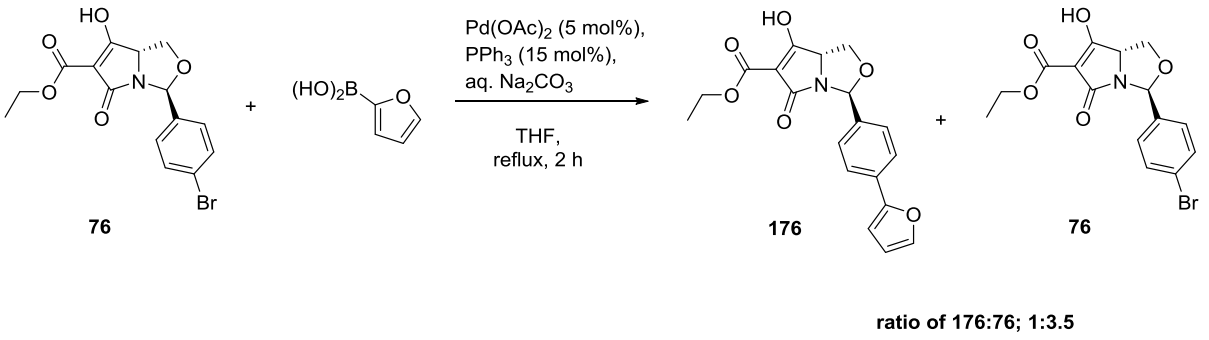


Entry	Reactant	Conditions	Remarks
1	**91a**	$Pd(OAc)_2$ (5 mol%), PPh_3 (15 mol %), aq. Na_2CO_3, THF, reflux, 48 h	**174** was not detected by LRMS. **91a** was isolated by column purification.
2	**110**	$Pd(OAc)_2$ (5 mol%), PPh_3 (15 mol %), aq. Na_2CO_3, THF, reflux, 48 h	**175** was not detected by LRMS. **110** was isolated by column purification.
3	**91a**	$Pd(dppf)Cl_2$ (5 mol%), $AsPh_3$ (15 mol %), aq. Na_2CO_3, THF, reflux, 48 h	**91a** was isolated by column purification.
Several other bases and solvent conditions were tested: (base: K_2CO_3, Cs_2CO_3, K_3PO_4, NaOH; solvent: DME, 1,4-Dioxane, DMSO)			No product formation was observed.

The results obtained earlier by ICP-MS for metal chelation of tetramates (Section 3.4, page 78, *vide supra*) suggest that these tetramates with a 1,3-bicarbonyl system are good ligands for metal chelation. Further, tetramates **91a** and **110** are derived from cysteine, where sulfur

too can chelate to metals. Chelation to Pd(0)/Pd(II) at the bicarbonyl system or thioether could lead to catalyst deactivation and hence interfere with the catalytic process. This would result in failed cross-coupling reactions as observed for **91a** and **110**.

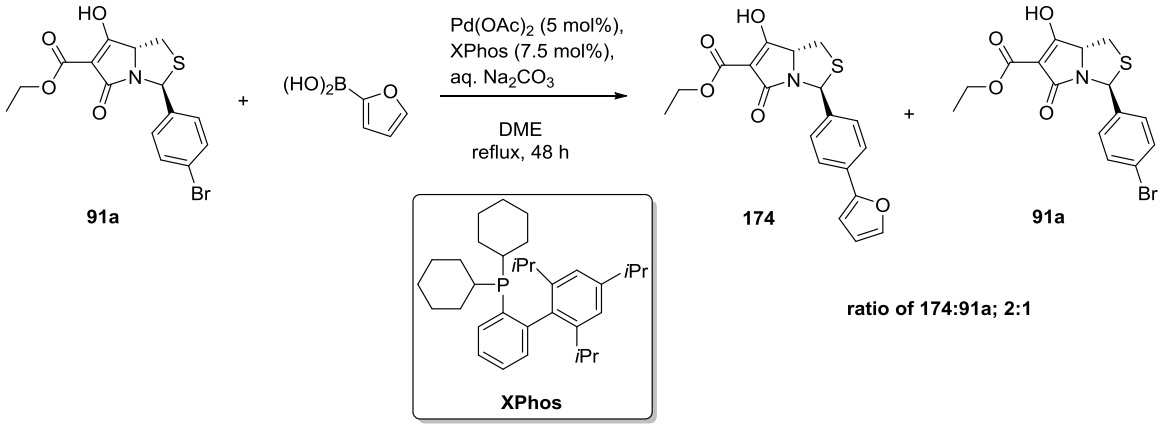
Hence, the same cross-coupling reaction was tested on the oxazolidine-derived analogue **76**, Scheme 4.2. After a reaction time of 2 h, the formation of product **176** could be observed by LRMS data, which was confirmed by HRMS data. Analysis of ^{1}H NMR spectrum indicated the presence of product **176** and unreacted tetramate **76** in a ratio of 1:3.5. Thus, the failure of SM cross-coupling on thiazolidine-derived tetramic acids **91a** and **110** could be attributed to catalyst deactivation by cyclic thioether, and alternative reaction conditions were necessary.



Scheme 4.2. SM cross-coupling of oxazolidine-derived tetramate **76** to give product **176**.

The use and impact of dialkylbiaryl phosphine ligands in SM cross-coupling reactions have been discussed previously by Buchwald et al,[95] where the enhanced activity and expanded substrate scope of palladium catalysts derived from these ligands has been attributed to their electronic and steric properties. These structural features affect the rates of oxidative addition, transmetallation and reductive elimination steps in the catalytic process. While successful SM cross-coupling on thiophene systems have been documented using PPh_3 and $AsPh_3$ listed in Table 4.1,[96] no reports on systems containing non-aromatized thioethers such as those of the thiazolidine-derived tetramates were found in the literature. However, a more recent report on

Suzuki cross-coupling on biotin-derived systems[97] together with a review by Buchwald et al.[95] prompted the application of dialkylbiaryl phosphines for SM cross-coupling on thiazolidine-derived tetramates, and when XPhos was used, this proved to be successful, with the desired product being formed along with some unreacted starting material, Scheme 4.3.



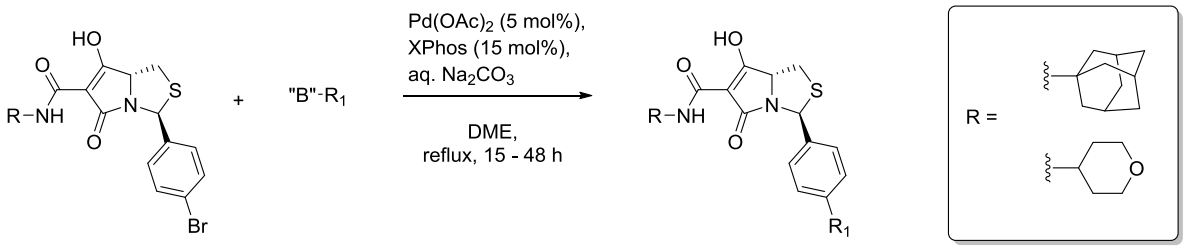
Scheme 4.3. SM cross-coupling of thiazolidine-derived tetramate **91a** to give product **174**.

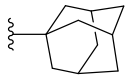
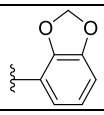
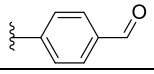
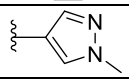
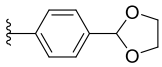
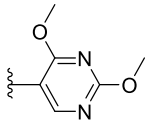
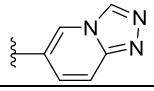
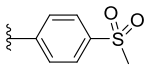
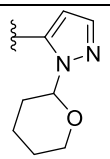
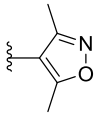
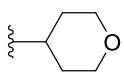
It is reported that the active form of the catalyst for SM cross-coupling involving dialkylbiaryl phosphine ligands is the monoligated $L_1Pd(0)$ species rather than the more highly coordinated $L_2Pd(0)$.[95,98] However, a L:Pd=1.5:1 was not sufficient to drive the reaction to completion even after a reaction time of 48 h. Analysis of ^{1}H NMR data indicated the presence of product **174** and unreacted tetramate **91a** in a ratio of 2:1. Increasing the L:Pd ratio to 3:1 led to completion of the reaction and was successfully applied to the synthesis of a range of carboxamide tetramates derived from **110** and **125**, with elaborated pendant groups at C-2, Table 4.2.

The exact reason for the observed catalyst deactivation by thioether when PPh_3 is used as a ligand, which is not observed when PPh_3 is replaced with XPhos is not known. However, it seems likely that the replacement of two Ph groups with alkyl groups in XPhos would lead to increased electron density at phosphorous, and therefore, higher electron density at Pd(0) in

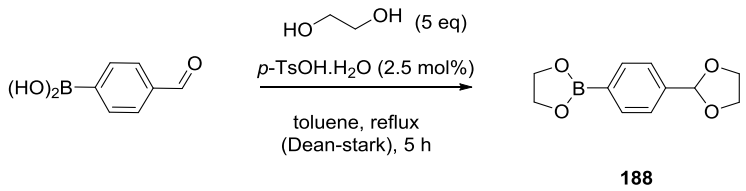
the monoligated $L_1Pd(0)$ species. The outcome would be an increase in the rate of oxidative addition.[95] Further, the lower phenyl ring (*i*Pr substituted) of XPhos allows for stabilizing Pd-arene interactions.[95] The higher electron density at Pd(0) could also reduce the affinity for chelation by thioethers, thereby preventing catalyst deactivation.

Table 4.2. SM cross-coupling of carboxamide tetramates **110** and **125**.



Carboxamide tetramate (R=)	Compound Number	R_1	% Yield	Compound Number	R_1	% Yield
	**175**		72	**182**		70
	177		74	**183**		78
	178		80	**184**		53
	179		45	**185**		70
	180		78	**186**		60
	181		74			
	**187**		30			

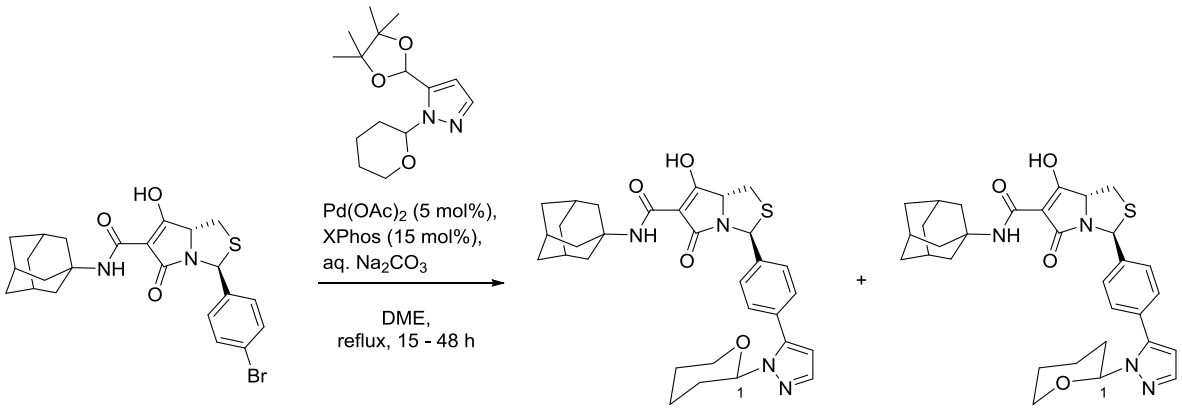
The boron reagents used for the above transformations were commercially available acids or their pinacol esters, except for **178** where the boron reagent was a glycol ester **188** obtained from the corresponding boronic acid, according to literature procedure,[99] Scheme 4.4.



188

Scheme 4.4. Synthesis of boronic acid glycol ester **188** used for SM cross-coupling to obtain **178**.

Carboxamide tetramate **186** was synthesised from the corresponding commercially available 1-(tetrahydropyran-2-yl)-1H-pyrazole-5-boronic acid pinacol ester obtained as a racemic mixture. As a consequence, SM cross-coupling of this with carboxamide tetramate **110** gave product **186** as a mixture of diastereomers, Scheme 4.5. The equatorial substitution of the tetrahydropyran ring was confirmed by the $^{3}J_{(H1\text{-}H2)}$-coupling constant of H-1 (dd, J=10.1, 2.3 Hz).



110 **186** (1:1 dr)

Scheme 4.5. Synthesis of carboxamide tetramate **186**.

4.2 Elaboration via C-2 pendant glycones

Several naturally occurring tetramic acids bearing sugar groups have been isolated; virgineone,[42] kibdelomycin,[47] amycolamicin[50] and streptolydigin[51] are some examples. While the exact role of the sugar moiety of these tetramates is not known, it may play an important role in pharmacokinetics, giving improved bioavailability with increased solubility and transportation. Further, sugar groups may also help modulate toxicity and aid in bacterial target interaction.[100] Thus, investigating the effect of glycosylated tetramates on antibacterial activity was of interest and their syntheses are discussed below.

4.2.1 Tetramates with phenyl glycosides of monosaccharide galactose

Bicyclic tetramates with C-2 pendant glycones were derived from β-D-galactose pentaacetate as outlined in Scheme 4.6. The $BiBr_3$-mediated, quantitative synthesis of acetobromo-α-D-galactose **141** from β-D-galactose pentaacetate was according to a literature procedure.[101] The axial bromination at the anomeric carbon, with inversion of configuration was confirmed by the $^3J_{(H1\text{-}H2)}$-coupling constant of H-1 (d, J = 4.1 Hz) and clearly different to the $^3J_{(H1\text{-}H2)}$-coupling constant of H-1 (d, J = 8.3 Hz) of β-D-galactose pentaacetate where -OAc substitution at C-1 is equatorial (Figure 4.2). Acetobromo-α-D-galactose **141** thus obtained was pure and could be used in subsequent reactions without further purification.

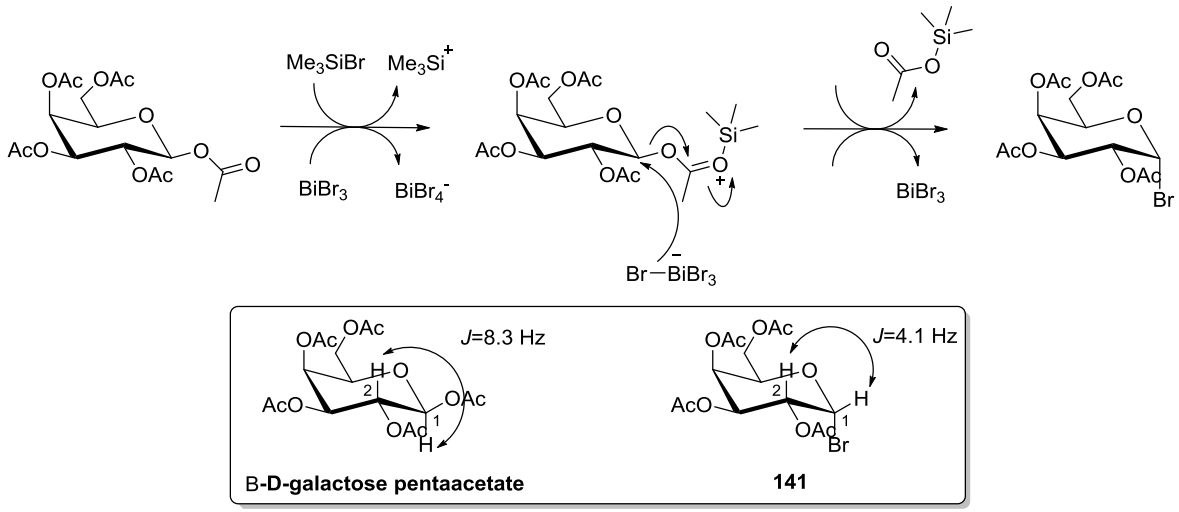
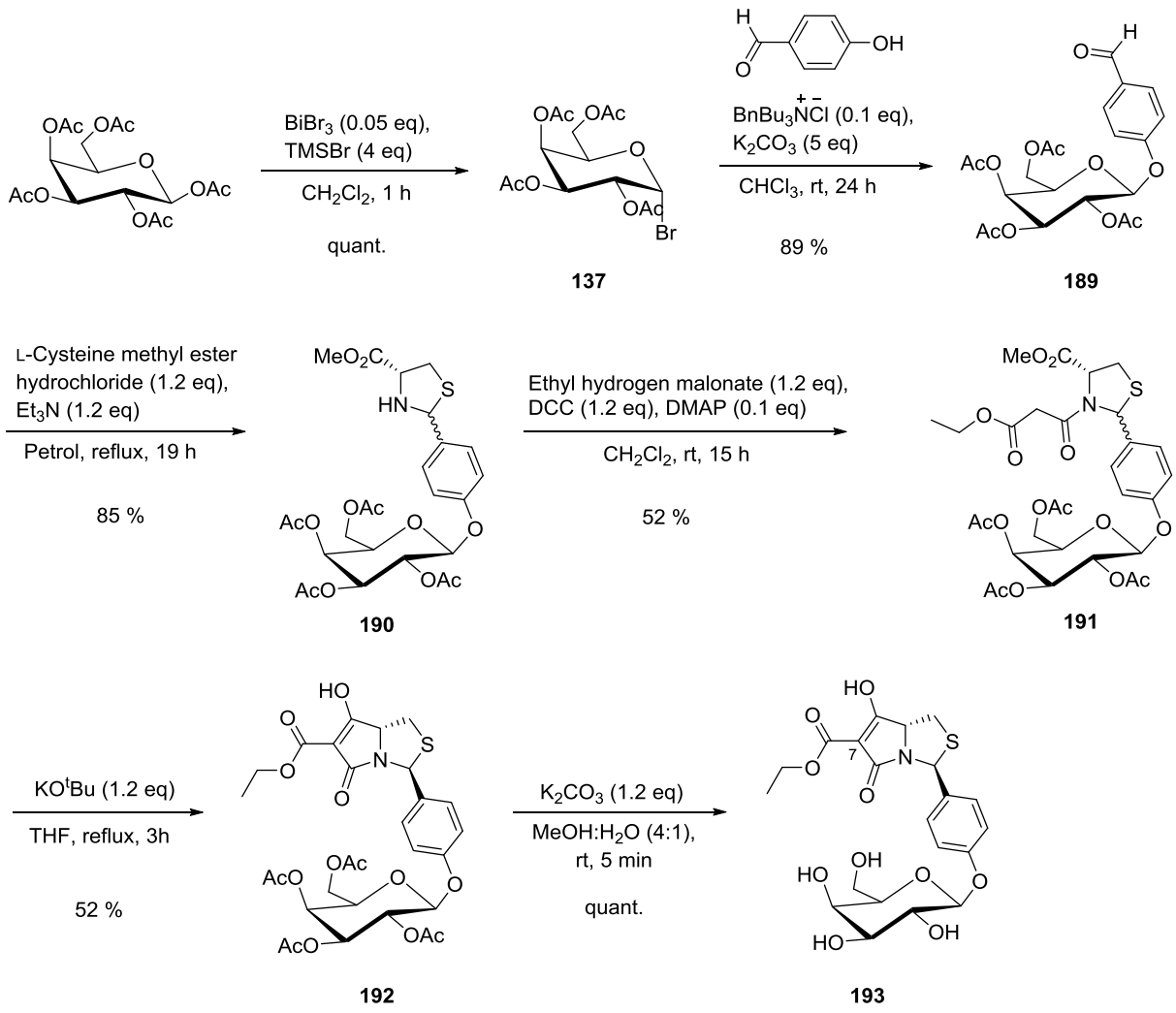


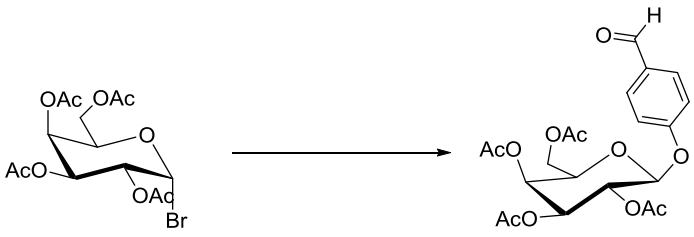
Figure 4.2. Proposed mechanism for axial bromination at the anomeric carbon.[101]



Scheme 4.6. Synthesis of tetramates **192** and **193** with C-2 pendant glycones derived from galactose.

Solid-liquid phase transfer-catalysed aryl glycosylation of 4-hydroxybenzaldehyde with acetobromo-α-D-galactose **141** gave the desired aldehyde **189**, according to a modified literature procedure.[102] The β-anomer was obtained exclusively from aryl glycosylation, where the equatorial substitution of the phenoxide at the anomeric carbon was confirmed by ^{3}J-coupling constant of the anomeric C-H (d, J = 8.0 Hz). Experimental conditions employing $BnNBu_3Cl$ as a phase transfer catalyst and $CHCl_3$ as a solvent provided improved yield, Table 4.3.

Table 4.3. Phase transfer catalysed aryl glycosylation to yield **189**.



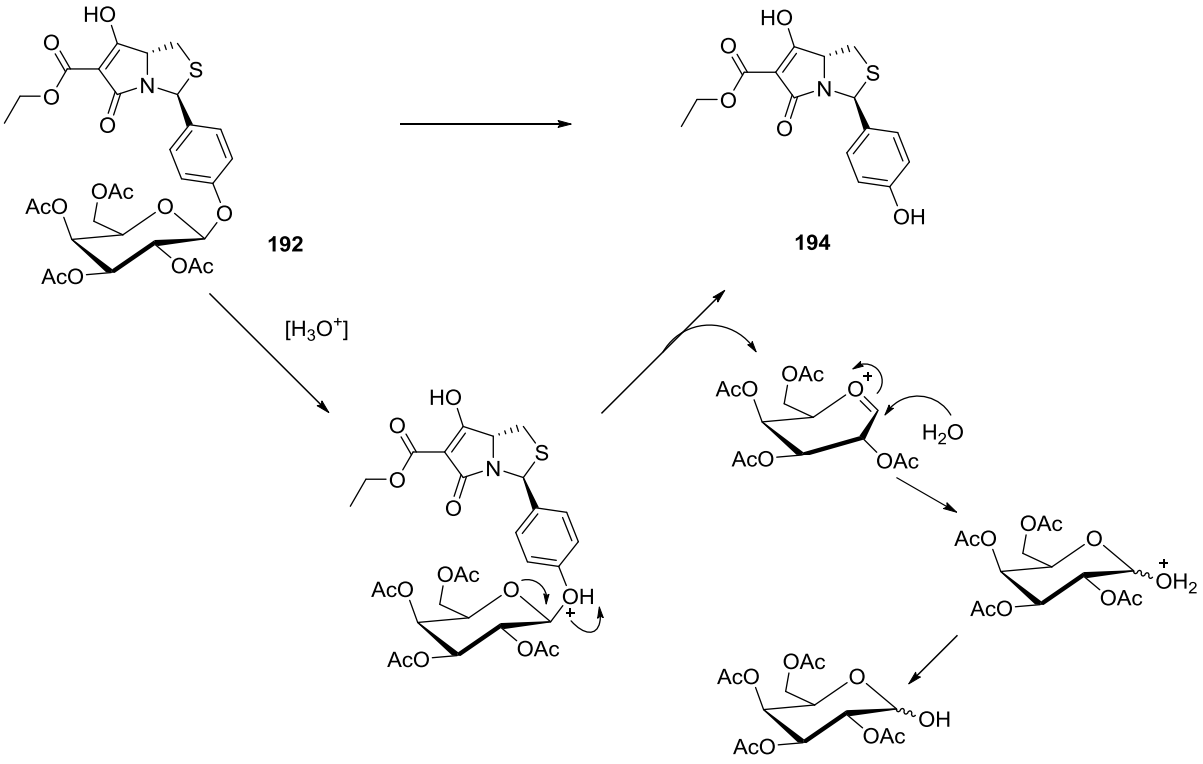
141 **189**

Entry	Conditions	% Yield
1	4-Hydroxybenzaldehyde (1.5 eq), Bu_4NBr (0.2 eq), sat. K_2CO_3:CH_2Cl_2 (1:1), rt, 24 h	20
2	4-Hydroxybenzaldehyde (1.2 eq), $BnBu_3NCl$ (0.2 eq), powdered K_2CO_3 (5 eq), $CHCl_3$, rt, 24 h	89

The glycosylated aldehyde **189** was then condensed with L-cysteine methyl ester hydrochloride to yield thiazolidine **190** in 85 % yield with a *cis*:*trans* diastereomeric ratio of 1.6:1. *N*-Acylation of **190** gave malonamide **191** in 52 % yield, which upon Dieckmann cyclisation afforded glycosylated tetramate **192** with the expected mass observed by LRMS. However, upon work-up, the aglycone **194** was isolated, which was indicative of an acid-mediated deglycosylation of the tetramate, Scheme 4.7. Hence, in order to obtain tetramate

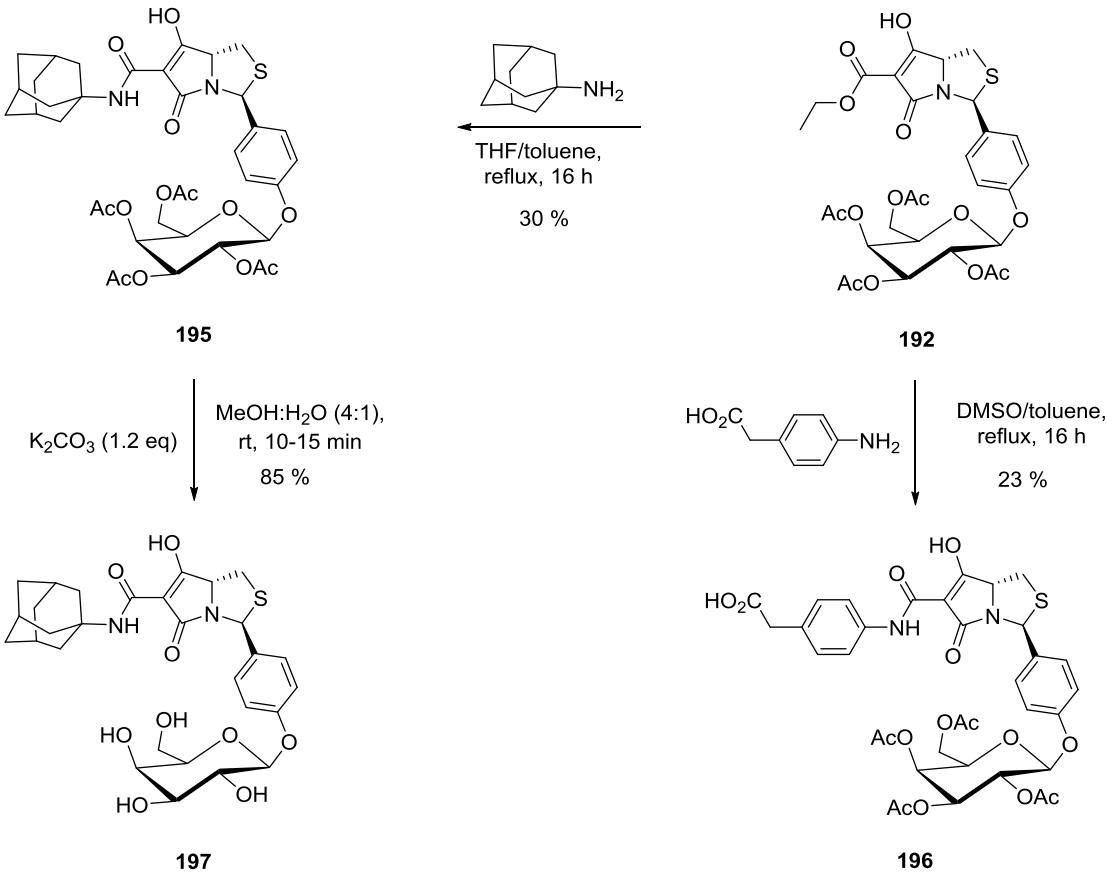
192 with the sugar group intact, the crude obtained from Dieckmann cyclisation was column purified directly without acidic work-up.

The tetramate ester **192** thus obtained was converted to the deacylated sugar-substituted tetramate **193** via base-catalysed methanolysis in quantitative yield with a highly polar substitution at C-2 (Scheme 4.6, page 93, *vide supra*). It was also interesting to note that neither hydrolysis nor transesterification of the ethyl ester at C-7 was observed under these reaction conditions.



Scheme 4.7. Acid-mediated deglycosylation of **192**.

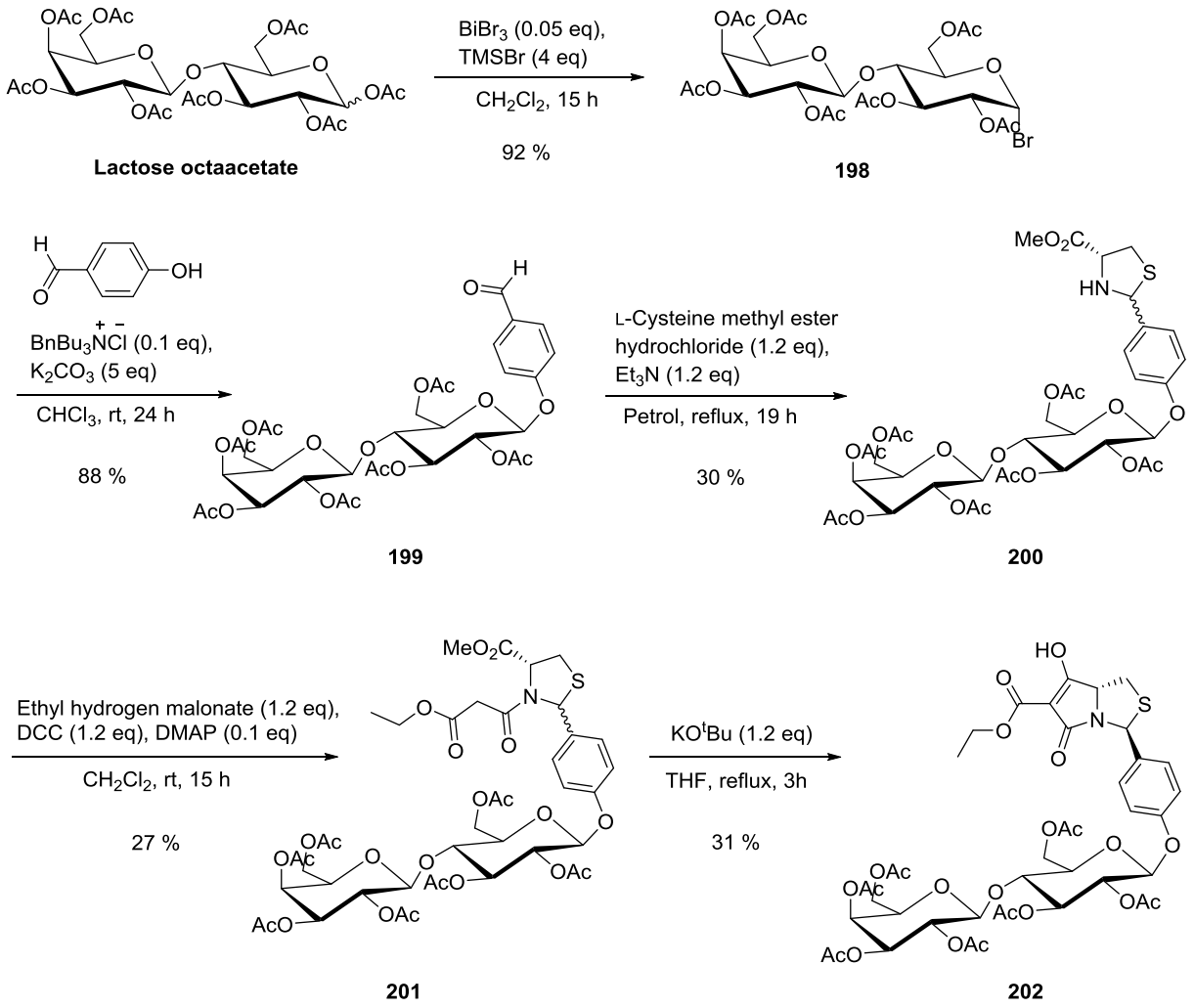
Aminolysis of tetramate ester **192** afforded carboxamide tetramates **195** and **196** with the acyl groups of the sugar moiety intact. Methanolysis of **195** resulted in the very polar carboxamide tetramate **197**, Scheme 4.8.



Scheme 4.8. Synthesis of carboxamide tetramates **195, 196** and **197**.

4.2.2 Tetramates with phenyl glycosides of disaccharide lactose

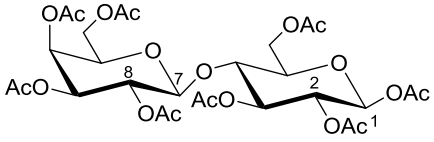
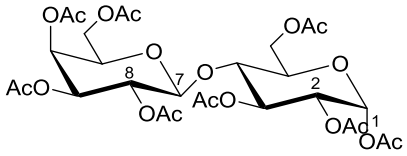
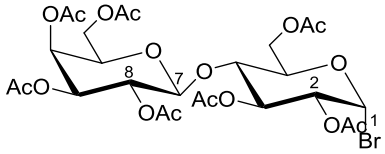
A tetramate with a disaccharide pendant group was derived from commercially available lactose octaacetate. Lactose substituted tetramate was synthesised via a synthetic route similar to that described for the monosaccharide analogue in Section 4.1.2.1 and is outlined in Scheme 4.9.



Scheme 4.9. Synthesis of tetramate **202** with C-2 pendant glycone derived from lactose.

Commercially available lactose octaacetate was obtained as a 2.9:1 mixture of α- and β-anomers at the glucopyranosyl end, confirmed by ^{3}J-coupling constants of anomeric C-Hs of the disaccharide. $BiBr_3$-catalysed bromination of the octaacetate afforded **198** as the α-anomer in 92 % yield, once again confirmed by ^{3}J-coupling constants of anomeric C-Hs, Table 4.4. Solid-liquid phase transfer catalysed aryl glycosylation gave aldehyde **199**, which was condensed with L-cysteine methyl ester hydrochloride to give thiazolidine **200**. Subsequent acylation and cyclisation afforded tetramate **202** with a disaccharide pendant group at C-2.

Table 4.4. Chemical shifts and ^{3}J-coupling constants of lactose octaacetate anomers and their brominated analogue.

Compound	Structure	δ_{H1} (ppm)	$^{3}J_{(H1-H2)}$ (Hz)	δ_{H7} (ppm)	$^{3}J_{(H7-H8)}$ (Hz)
β-anomer		5.68	8.3	4.48	8.1
α-anomer		6.26	3.7	4.49	7.8
198		6.51	4.0	4.50	7.8

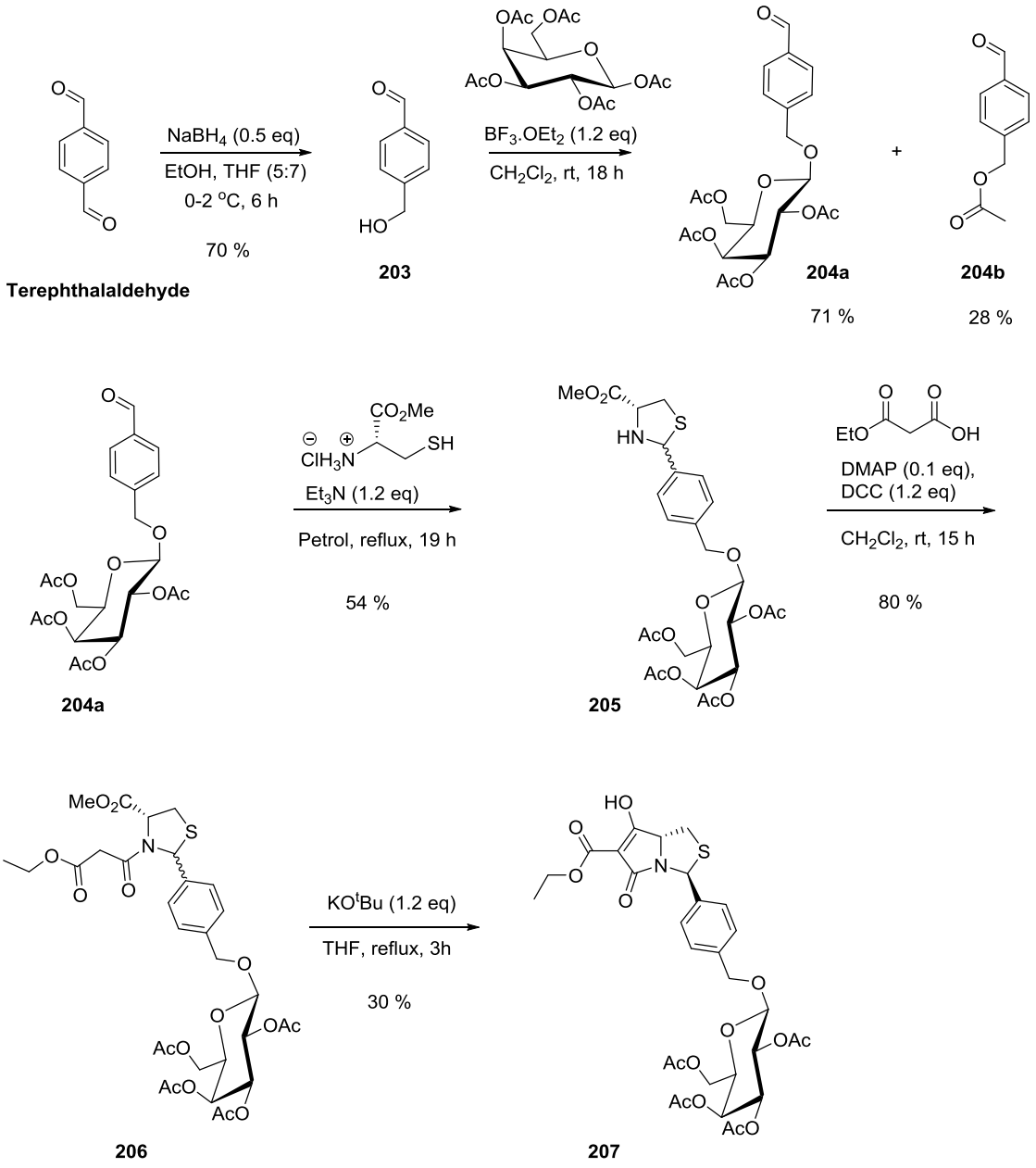
4.2.3 Tetramates with benzyl glycosides of monosaccharide galactose

Due to the acid-labile nature of phenyl glycosides and hence, the difficulty associated with the acidic workup procedure, an alternative route to the incorporation of glycones via benzyl alcohol was explored, Scheme 4.10.

The controlled reduction of terephthalaldehyde with $NaBH_4$ afforded 4-(hydroxymethyl)benzaldehyde **203** in 70 % yield according to a modified literature procedure.[103] The controlled reduction of one aldehyde group was facilitated by maintaining the reaction temperature between 0-2 °C.

Lewis acid-catalysed glycosylation of **203** with β-D-galactose pentaacetate resulted in the glycosylated aldehyde **204a** as the β-anomer in 71 % yield along with the by-product **204b** in 28 % yield. Following the synthetic route to access the bicyclic tetramate core, condensation of aldehyde **204a** with amino ester yielded thiazolidine **205**. This was then acylated and

cyclized under basic conditions to afford benzyl glycosylated tetramate **207**, which was stable to acidic work-up.



Scheme 4.10. Synthesis of tetramate **207** with C-2 pendant glycone derived from galactose.

4.3 Tetramates with analogous aglycones

In order to assess the requirement for glycosyl groups for antibacterial activity, the analogous aglycone tetramates **194**, **216** and **218a** were synthesised (Figure 4.3).

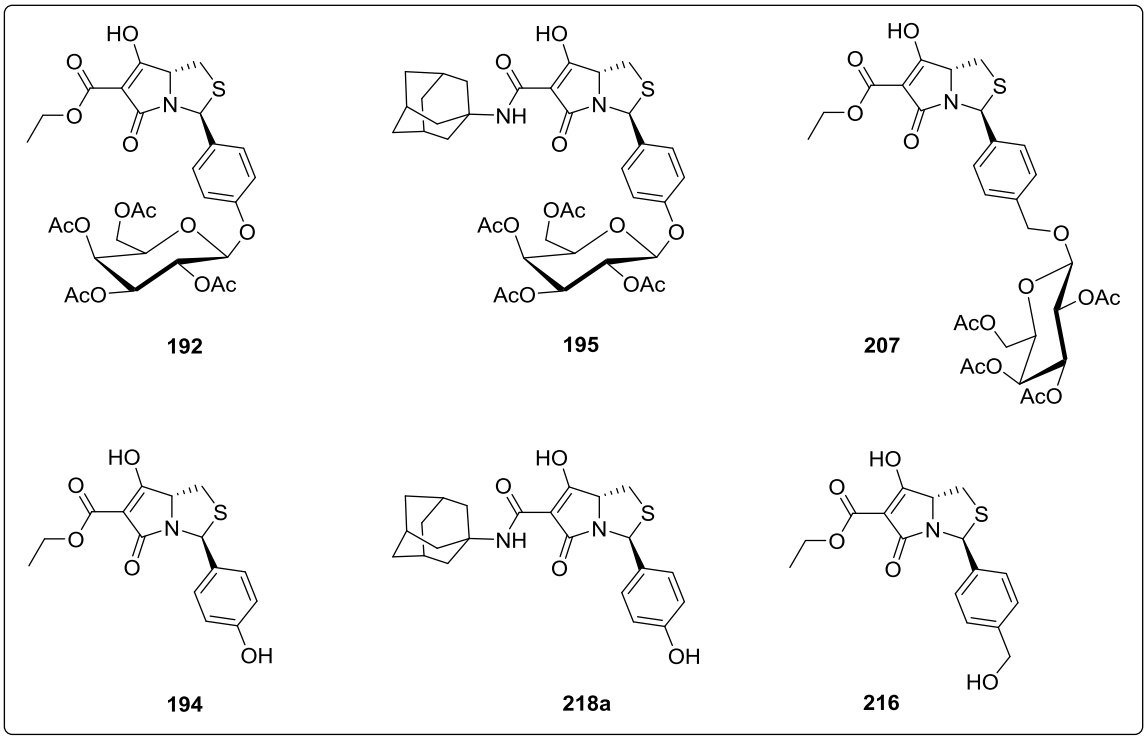
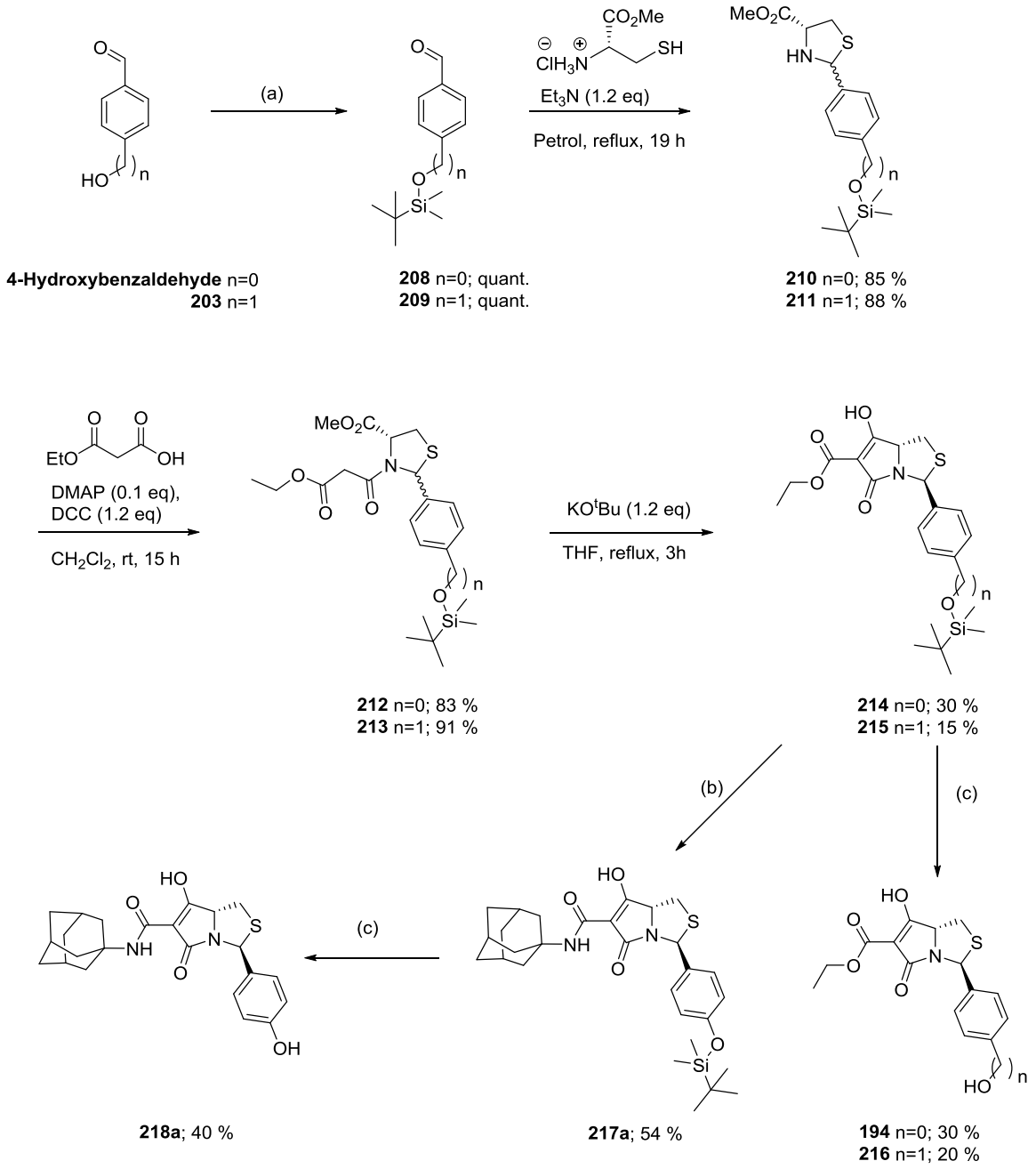


Figure 4.3. Glycosylated tetramates and their aglycosyl analogues.

Tetramates **194** and **218a** were derived from the silyl ether of 4-hydroxybenzaldehyde while tetramate **216** was derived from the silyl ether of 4-(hydroxymethyl)benzaldehyde following the procedure outlined in Scheme 4.11 to access the bicyclic tetramate core.



4-Hydroxybenzaldehyde n=0
203 n=1

208 n=0; quant.
209 n=1; quant.

210 n=0; 85 %
211 n=1; 88 %

212 n=0; 83 %
213 n=1; 91 %

214 n=0; 30 %
215 n=1; 15 %

218a; 40 %

217a; 54 %

194 n=0; 30 %
216 n=1; 20 %

Scheme 4.11. Synthesis of aglycone analogues **194**, **216** and **218a**. (a) for n=0: TBDMSCl (1.5 eq), Et_3N (1.5 eq), CH_2Cl_2, rt, 2 h; for n=1: TBDMSCl (1.5 eq), imidazole (1.5 eq), CH_2Cl_2, rt, 6 h; (b) 1-adamantylamine (1.2 eq), THF/toluene, reflux, 16 h; (c) for n=0: KF (2 eq), tetraethyleneglycol/THF, rt, 30 min; for n=1: KF (2 eq), tetraethyleneglycol/THF, 80 °C, 5 h.

Several conditions were investigated for the deprotection of silyl ether **217a** to obtain tetramate **218a**, Table 4.5. While TBAF was a successful desilylating agent, it was not possible to obtain **218a** in high purity after column purification, possibly due to the elution of

TBAF along with the product. It is also possible that the basicity of fluoride ion in an aprotic solvent[104] could lead to deprotonation of the tetramic acid, which then elutes as a counterion along with Bu_4N^+, compromising the purity of the product. However, washing the product with 2M HCl was not successful in removing the alkyl impurity. Hence alternative conditions using KF as a fluoride source were employed (Table 4.5).

Table 4.5. Conditions for silyl ether deprotection of **217a**.

Entry	Conditions	Remarks
1	TBAF (1.5 eq), THF, rt, 40 min	Reaction went to completion as observed by LRMS. Product **218a** was not pure post-column purification
2	KF (1.5 eq), THF, rt , 2 h	No progress of reaction observed by LRMS and TLC
3	KF (1.5 eq), TEG/THF, rt, 30 min	Product observed by LRMS and purified by column chromatography

It was interesting to note that KF was only effective in the presence of tetraethyleneglycol (TEG) as a solvent, and using other polar solvents such as THF in the absence of TEG did not lead to desilylation. An explanation for the role of bis-terminal hydroxyl polyethers such as TEG in the deprotection of silyl ethers has been proposed by Yan and co-workers[105] as demonstrated in Figure 4.4, and involves appropriate coordination of the nucleophilic fluoride.

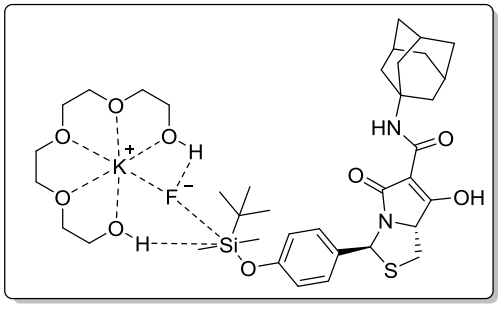


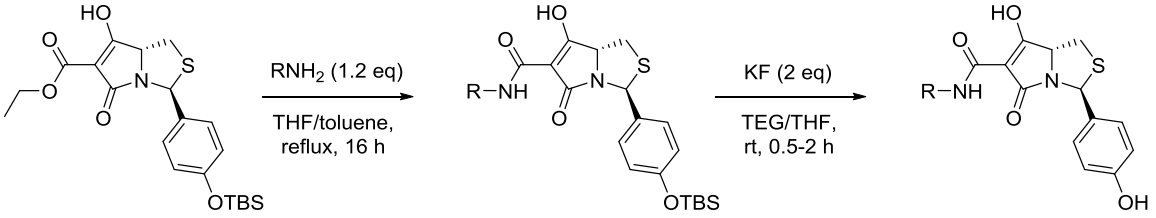
Figure 4.4. The role of TEG in silyl ether deprotection.

While KF is not soluble in THF and hence rendered ineffective in desilylation, the presence of TEG aids in solubility of the alkali metal salt with the polyether chelating to K^+. The 'free' fluoride counter anion is H-bonded to one terminal hydroxyl group controlling the basicity of F^-, while the other terminal OH group activates the electrophile. Thus, the use of KF with TEG was a promising alternative to obtaining silyl ether deprotected tetramates **194**, **216** and **218a** in high purity.

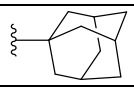
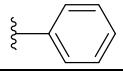
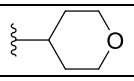
4.4 Elaboration via Williamson ether synthesis

The synthetic feasibility of incorporating phenolic groups at the C-2 end of the bicyclic tetramate core as discussed in Section 4.1.2.4 provided opportunity for the synthesis of carboxamide tetramates such as **218a** with more polar substituents at C-2. Following the synthetic route outlined in Scheme 4.11 (page 101, *vide supra*), two other carboxamide tetramates **218b** and **218c** were also synthesised and are summarised in Table 4.6.

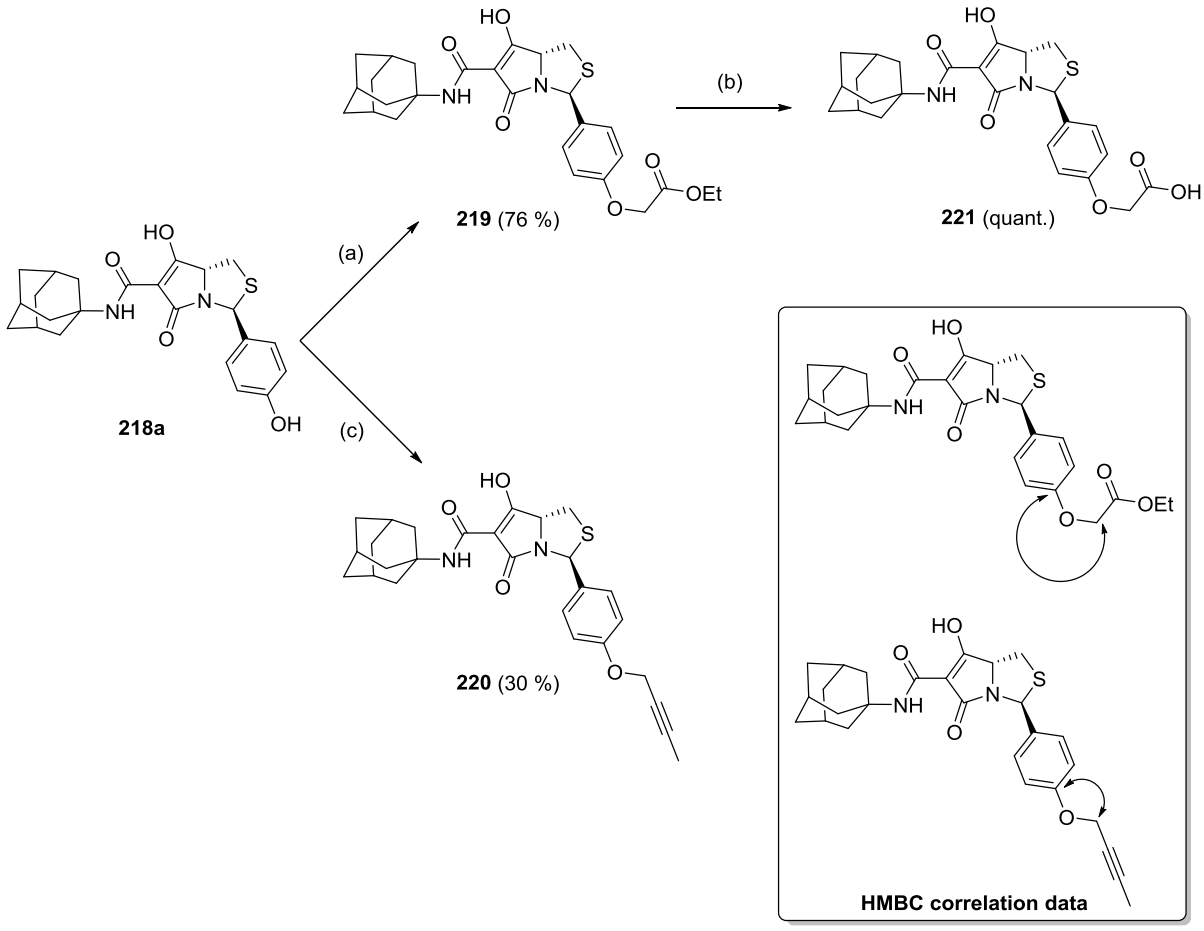
Table 4.6. Synthesis of carboxamide tetramates **218a-c**.



214 **217a-c** **218a-c**

Compound Entry	R=	% Yield	
		217	**218**
a		54	40
b		27	45
c		30	45

The presence of a phenolic group at the C-2 position provided a platform for further functionalization of carboxamide tetramates via Williamson ether synthesis as outlined in Scheme 4.12. In the presence of K_2CO_3, nucleophilic substitution of ethyl bromoacetate and 1-bromo-2-butyne by phenoxide of **218a** gave carboxamide tetramates **219** and **220** respectively. HMBC correlation data confirmed the substitution at the phenol to give the expected products **219** and **220**, while substitution at the enol was not observed. Ester hydrolysis of **219** afforded the carboxylic acid bearing tetramate **221** with improved hydrophilicity, while carboxamide **220** provided potential for further modification with improved hydrophilicity (Section 4.7.2, page 115, *vide infra*).



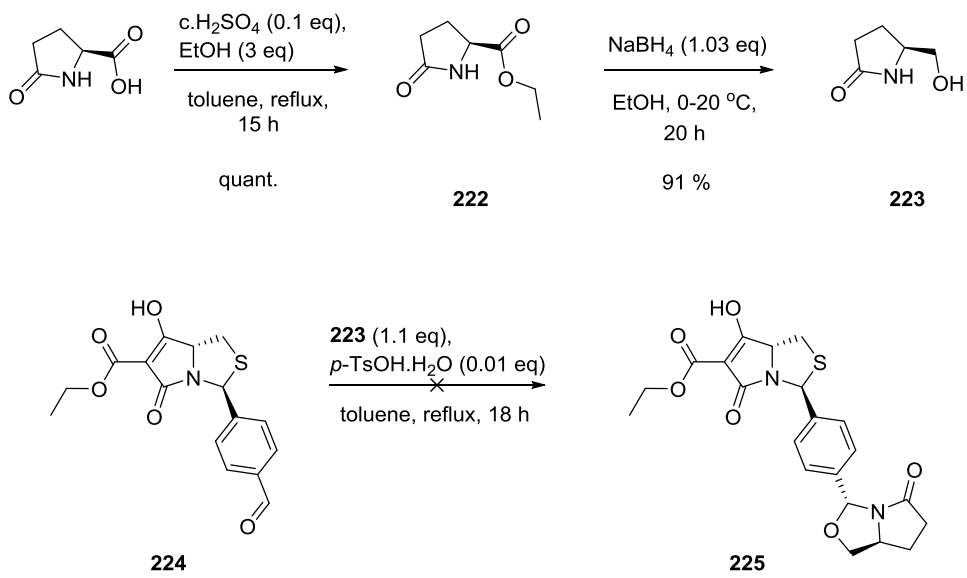
Scheme 4.12. Etherification of carboxamide tetramate **218a**. (a) Ethyl bromoacetate (1.5 eq), K_2CO_3 (2.1 eq), THF, rt, 18 h; (b) $LiOH.H_2O$ (2.0 eq), $THF:H_2O$ (1:1), rt, 2 h; (c) 1-Bromo-2-butyne (1.5 eq), K_2CO_3 (2.1 eq), THF, rt, 18 h.

4.5 Elaboration via pyroglutamates

The pyroglutamate core is found in antibiotic natural products such as the oxazolomycins **60-62** and pramanicin **72**. The Moloney group has previously explored the biomimetic synthesis of the pyroglutamate core found in these natural products in their efforts to synthesise antibacterial agents.[70,71,93,106]

The synthesis of a pyroglutamate-tetramate hybrid system has not been looked at before. The incorporation of the pyroglutamate core to the tetramate scaffold would provide a platform on which two pharmacophores can be conjugated to produce hybrid structures. However, in the context of this research, the incorporation of the pyroglutamate core derived from pyroglutamic acid was studied as a means of improving hydrophilicity of carboxamide tetramates via C-2 functionalisation.

Lactam **223** was synthesised from L-pyroglutamic acid according to Scheme 4.13, following procedures reported in the literature.[107,108] In this route, L-pyroglutamic acid was esterified with ethanol, followed by reduction of the ester to give (*S*)-5-(hydroxymethyl)-2-pyrrolidinone **223** in 91 % yield. The proposed route to incorporate the pyroglutamate core on to the tetramate was via the condensation of tetramate **224** with lactam **223** using a Dean-Stark apparatus, Scheme 4.13.



Scheme 4.13. Attempted synthesis of tetramate **225**.

Due to the surprising failure of this reaction, the pyroglutamate core **223** was incorporated at an early stage in the synthesis of tetramate ester **225**. As represented in Scheme 4.14, the condensation of **223** with terephthalaldehyde by refluxing in toluene using a Dean-Stark apparatus yielded a mixture of products **226a** and **226b** in 1:2.4 ratio as observed by ^{1}H NMR analysis and confirmed by HRMS data. The lactam-functionalized aldehyde **226b** was isolated by column chromatography as a single diastereomer. This stereochemical assignment was in agreement with previous reports on *N*,*O*-acetal formation of **223** with benzaldehyde.[107] Aldehyde **226b** was in turn condensed with amino ester to yield thiazolidine **227** as an inseparable 1:1 *cis*- and *trans*- diastereomeric mixture. Upon acylation with ethyl hydrogen malonate, *trans*- diastereomer **228** was isolated and cyclised to afford the desired bicyclic tetramate ester **225** in 70 % yield. Subsequently, the tetramate ester was converted to carboxamide **229** with 1-adamantylamine.

The stereochemical assignment of the tetramate system was confirmed by NOE experiments (Figure 4.5). The relative stereochemistry of H-2 and H-5 of the [3.3.0] bicyclic tetramate system was shown to be '*trans*', as would be expected from the stereochemical analysis

described in Chapter 2. Similarly, the relative stereochemistry of H-11 and H-14 of the pyroglutamate-derived [3.3.0] bicyclic system was also observed to be '*trans*' in accordance with other pyroglutamate-derived bicyclic systems reported in the literature.[71,93]

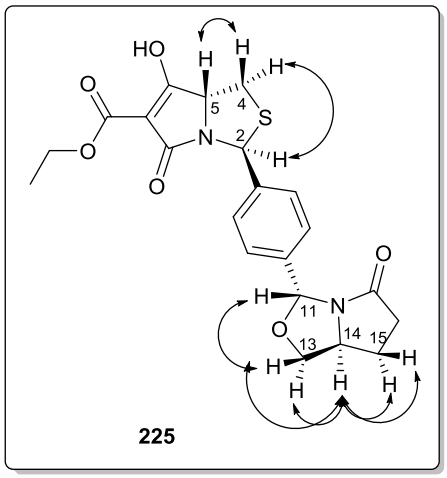
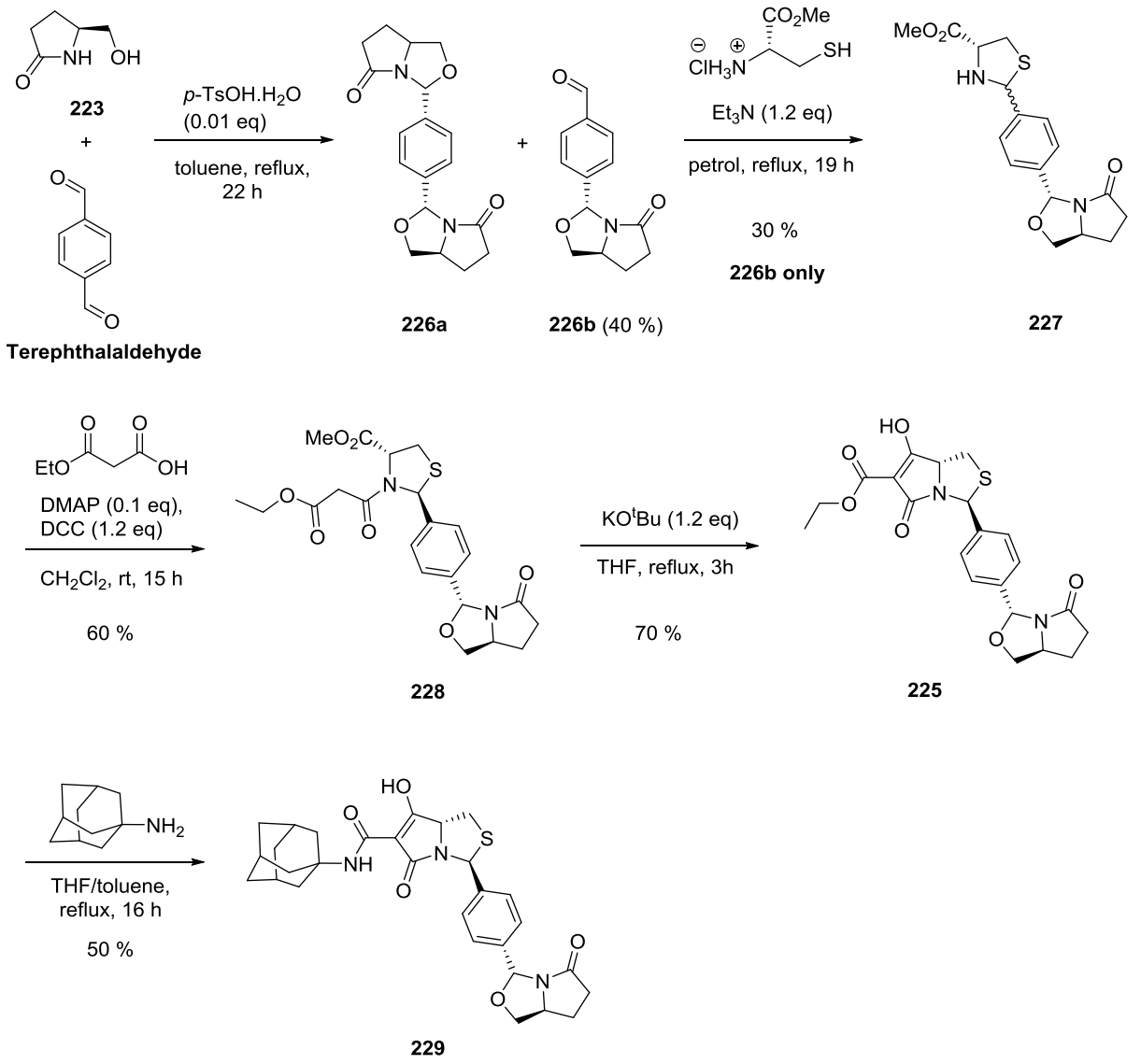


Figure 4.5. NOE analysis of tetramate ester **225**.



Scheme 4.14. Synthesis of tetramates **225** and **229**.

4.6 Elaboration via Buchwald-Hartwig amination reactions

Carey et al. have analysed the reactions used for the preparation of small molecule drug candidates (<550 MW) within the process chemistry R&D departments of GlaxoSmithKline, AstraZeneca and Pfizer.[109] In their analysis of 128 drug candidates in early development, the predominant reaction category was found to be heteroatom alkylation and arylation, which accounted for 19 % of the total reactions analysed. Out of the heteroatom

alkylation/arylations, *N*-substitutions were more frequent due to the development of Pd-catalyzed aryl C-N bond formation led by Buchwald and Hartwig, highlighting their wide application.[109] Herein, the application of C-N bond formation in the synthesis of functionalized tetramates is discussed.

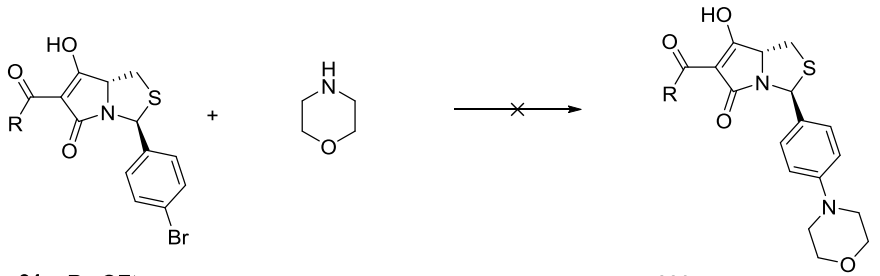
4.6.1 Attempted Buchwald-Hartwig amination of tetramates

Functionalization of tetramates via aryl C-N cross-coupling at the C-2 end via Buchwald-Hartwig amination was of interest and the incorporation of saturated *N*-heterocycles and lactams to expand the library of tetramates with improved physicochemical properties was examined. Due to the recent advances in catalysts based on dialkylbiarylphosphine ligands in Pd-catalysed aminations, C-N cross-coupling has found extensive practical application.[110,111] Dialkylbiaryphosphine ligand XPhos, which was a promising ligand in SM cross-coupling reactions on thiazolidine-derived tetramates discussed in Section 4.1 (page 86-91), is being widely used in Buchwald aminations. Hence, C-N cross-coupling of tetramates **91a** and **110** with morpholine in the presence of XPhos was attempted. Due to the unexpected failure of this reaction, several other ligands used in C-N cross-coupling were explored. A review by Surry et al. stated RuPhos to be the most generally useful ligand for amination with 2° amines.[111] Hence in addition to XPhos, dialkylbiarylphosphine ligand RuPhos and the chelating ligand Xantphos were also employed in our attempts to incorporate amines via Buchwald C-N cross-coupling reactions.

The monoligated $L_1Pd(0)$ complex is the catalytically active species in C-N cross-coupling.[111] Thus, when $Pd_2(dba)_3$ or single component precatalyst such as RuPhos Pd G2 is employed, where the catalyst is in the active Pd(0) oxidation state, it can be used directly without reduction. However, when $Pd(OAc)_2$ was used, reduction of Pd(II) to Pd(0) was necessary

prior to cross-coupling. Water-mediated catalyst pre-activation for C-N cross-coupling has previously been reported by Fors and co-workers.[112] Thus, following on from this report, $Pd(OAc)_2$ and ligand (XPhos) were heated with H_2O for 1.5 min before the amine and tetramate were added to the reaction flask. The catalyst-ligand combinations employed for Pd-catalysed amination of tetramate systems are listed in Table 4.7. These reactions were carried out in the presence of K_2CO_3 or Cs_2CO_3 as the base and refluxed in solvents such as t-BuOH, 1,4-dioxane, DME or toluene/DME. However, despite the various reaction conditions attempted, Buchwald-Hartwig amination of the tetramate system was not successful as a method for late-stage functionalization.

Table 4.7 Attempted Buchwald-Hartwig amination of tetramates **91a** and **110**.



91a; R= OEt
110; R= NH-Adamantyl

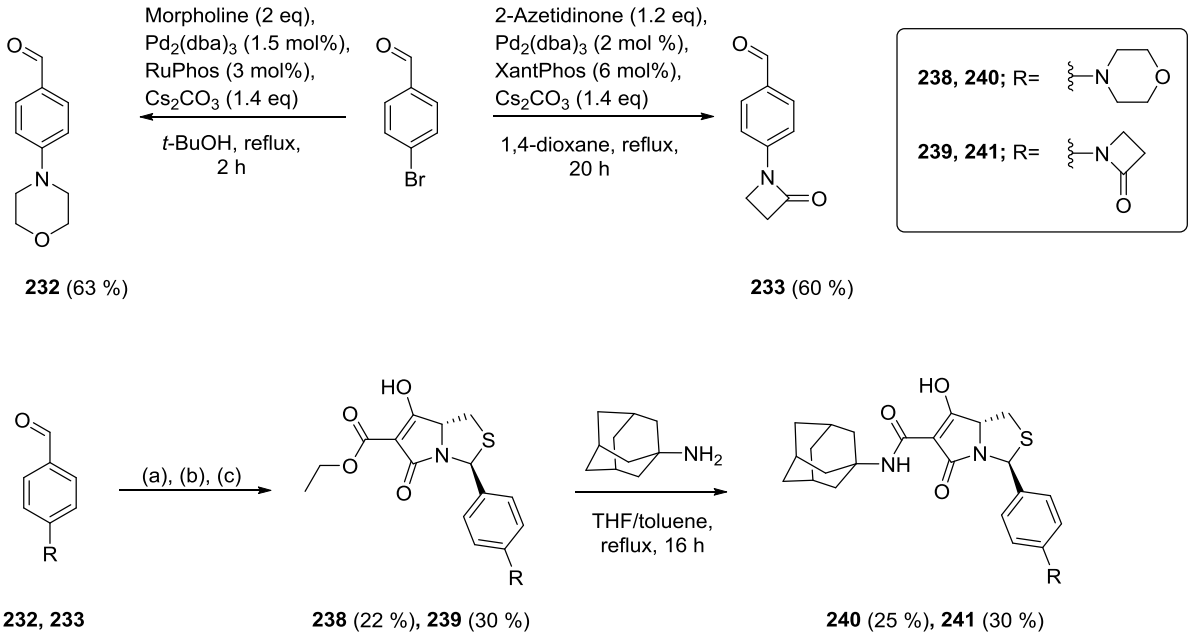
230; R= OEt
231; R= NH-Adamantyl

Ligand / Catalyst	$Pd_2(dba)_3$	$Pd(OAc)_2$	RuPhos Pd G2
XPhos	X **110** isolated	X **110** isolated	
RuPhos	X **110** isolated		X **110** isolated
XantPhos	X **91a** isolated		

4.6.2 Synthesis of C-2 amine/amide functionalized tetramates

Despite the failure in the application of C-N cross-coupling as a late-stage functionalization of tetramates, a study of the effect of amine/lactam substitution of the tetramate system on

antibacterial activity was of interest. Therefore, the starting aldehyde was functionalized via Buchwald amination/amidation which was then used to synthesise the tetramate core *de novo*, as illustrated in Scheme 4.15.



Scheme 4.15. Synthesis of tetramates **238-241** via Buchwald amination. (a) L-cysteine methyl ester hydrochloride, Et_3N, petrol, reflux (Dean-Stark), 19 h; (b) ethyl hydrogen malonate, DCC, DMAP, CH_2Cl_2, rt, 15 h; (c) KO^tBu, THF, reflux, 3 h.

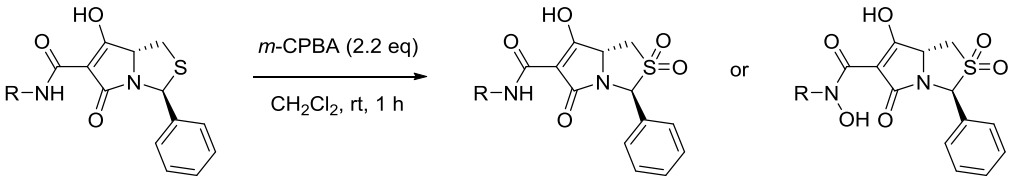
4.6.3 A brief note on the oxidation of carboxamide tetramates

The oxidation of sulfur of the bicyclic tetramate core would lead to sulfone synthesis, which would be an attractive alternative reactant for Buchwald-Hartwig cross-coupling reactions in order to probe possible interference from cyclic thioethers such as those on the thiazolidine-derived tetramate core on Pd-catalysed amination reactions. Further, oxidation would also lead to improved polarity of the compound, and is an attractive method to optimize physicochemical properties where improved polarity is required.

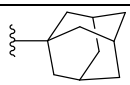
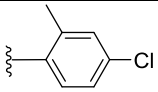
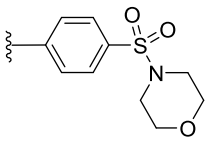
As a test reaction, sulfone synthesis was attempted on tetramates **109** and **119**. Controlled oxidation of these carboxamide tetramates with *m*-CPBA to obtain sulfones of type 'a'; **109a**

and **119a** was not successful. Instead, the mass corresponding to the hydroxamide **109b** and **119b** was observed by LRMS analysis (putative hydroxamide structure drawn similar to reported oxidation product of the corresponding amide [113]). The increased polarity of these compounds rendered purification by flash column chromatography difficult. For the carboxamide tetramate with extended conjugation of the lone pair electrons of amide **132**, the undesired product 'b' could be avoided. Upon purification by column chromatography, the desired product of type 'a' was obtained in rather low yield, Table 4.8. Therefore, it would be interesting to explore alternative oxidising agents such as $KMnO_4$ and oxone which have also been used in the synthesis of penicillanic acid sulfones.[114,115]

Table 4.8. Synthesis of sulfones derived from carboxamide tetramates.



109, 119, 132 **109a, 119a, 132a** **109b, 119b, 132b**

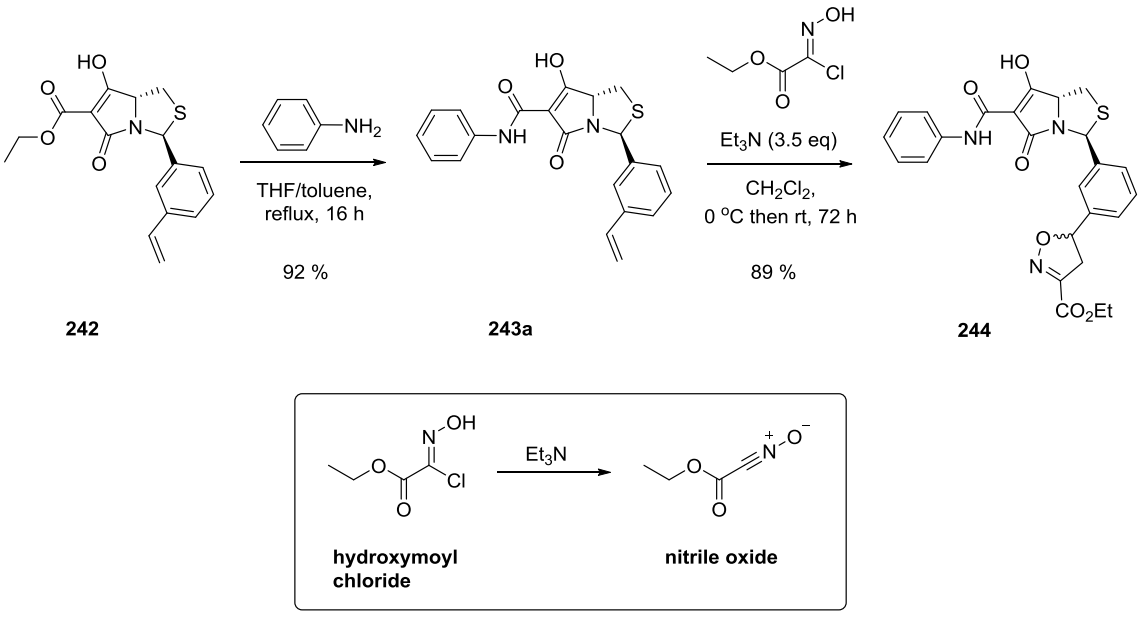
Entry	Compound Entry	R=	Remarks
1	**109, 109a-b**		Only **109b** observed by LRMS
2	**119, 119a-b**		Only **109b** observed by LRMS
3	**132, 132a**		**132a** isolated in 24 % yield

4.7 Elaboration via cycloaddition

4.7.1 Elaboration via 1,3-dipolar cycloaddition: 2-Isoxazolines via nitrile oxide-alkene cycloaddition

In view of biological activities, 2-isoxazolines are reported to possess good antibacterial activity and are being used as stable bioisosteric replacements for the amide bond and oxazolidinone ring.[116,117] Due to the convenient synthesis of 2-isoxazolines from readily available precursors and their ability to serve as templates for the construction of new systems, nitrile oxide cycloadditions have garnered considerable importance in organic synthesis.[118]

The synthetic route to the incorporation of 2-isoxazoline ring on carboxamide tetramate **243a** is outlined in Scheme 4.16. The isoxazoline ring was generated by a [3+2] cycloaddition of the '1,3-dipole' nitrile oxide and 'dipolarophile' tetramate **243a** according to literature procedures, but with prolonged reaction time.[119,120] The nitrile oxide required for cycloaddition was generated *in situ* via commercially available hydroxymoyl chloride in the presence of Et_3N.



Scheme 4.16. Synthesis of 2-isoxazoline **244**.

The interplay of steric and electronic substituent effects is responsible for the regioselectivity of cycloaddition.[121] Attachment of the dipolarophilic carbon of greater spatial requirement, which in the case of tetramate **243a** is the monosubstituted end of the alkene, with the oxygen of the nitrile oxide, is the sterically more favoured outcome. However, this regioselectivity of cycloaddition of the nitrile oxide to tetramate alkene **243a** leading to the 5-substituted isoxazoline product **244** and not the 4-substituted isoxazoline **245** could not be confirmed by HMBC correlation data, where a correlation between H-4 and *C*O_2Et was not observed as indicated in Figure 4.6. Thus, the cycloadduct is assigned to be **244** based on steric effects according to literature reports.[121]

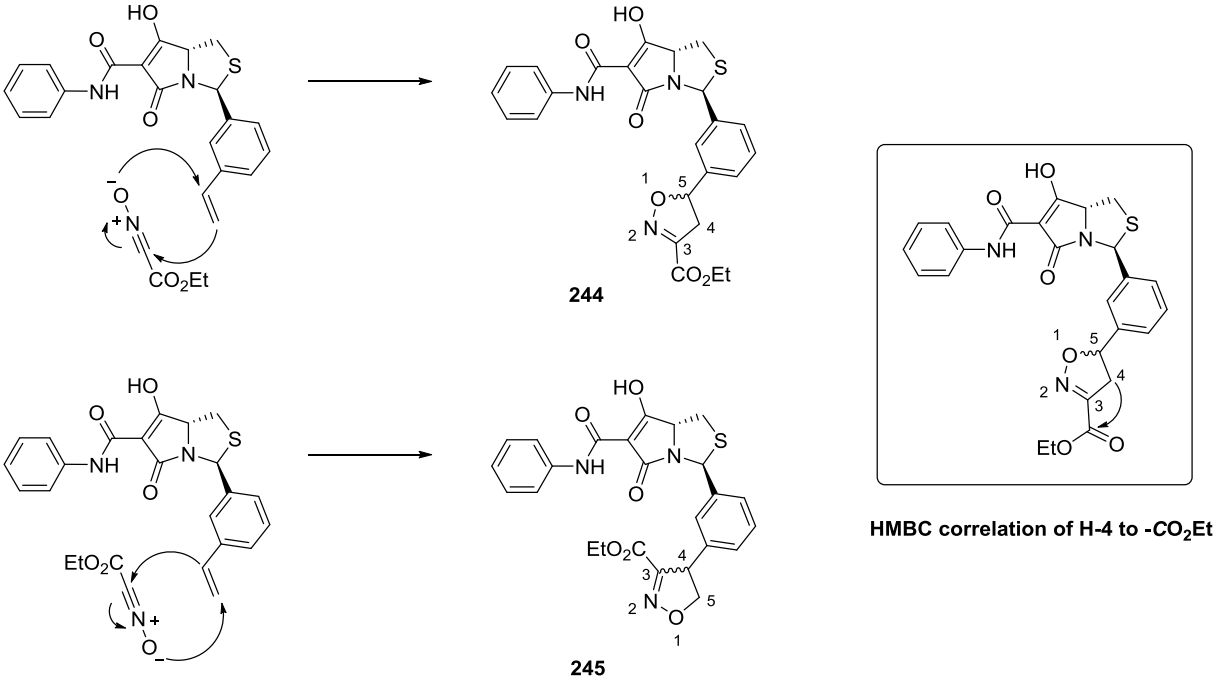


Figure 4.6. Regioselectivity of nitrile oxide cycloaddition to alkene.

Cycloaddition of nitrile oxide to alkene led to the generation of a new stereocenter at C-5 in product **244**, as a consequence of which a 1:1 diastereomeric mixture of cycloadducts **244a** and **244b** was obtained (Figure 4.7).

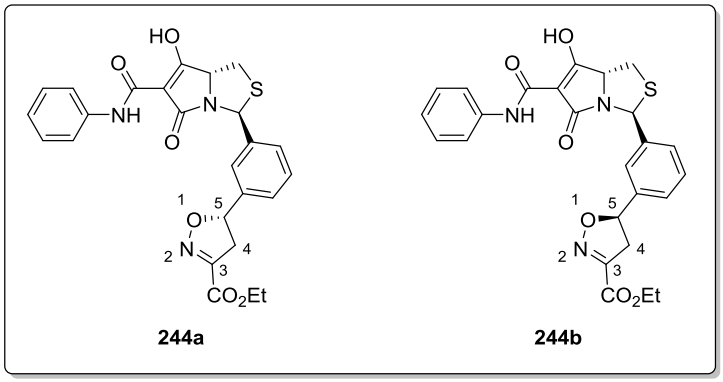
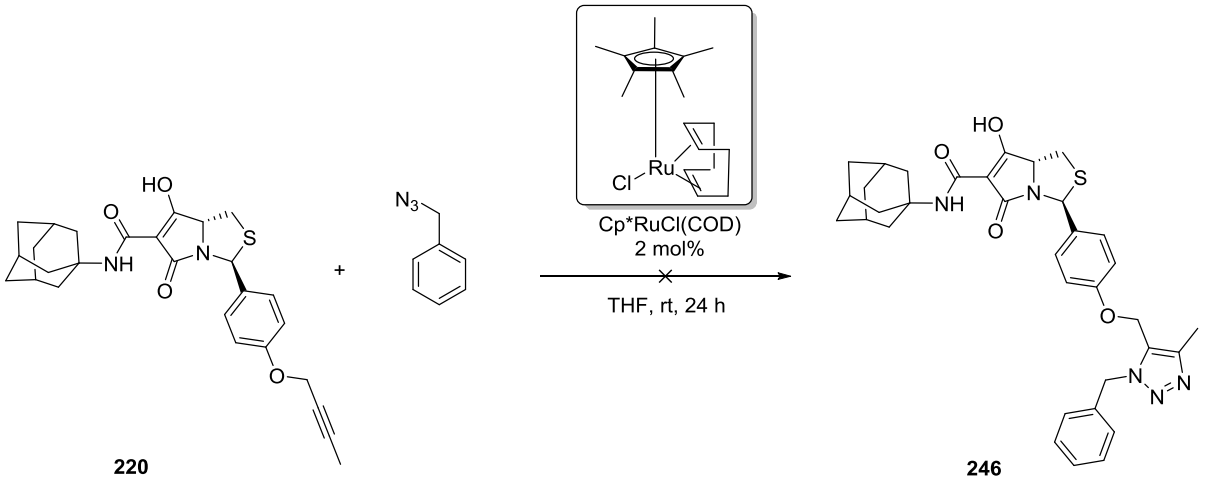


Figure 4.7. Diastereomers of tetramate **244**.

4.7.2 Elaboration via transition metal catalysed cycloaddition: attempted synthesis of triazoles via azide-alkyne cycloaddition

The triazole moiety is a common pharmacophore found in many medicinal drugs such as antifungal, anticancer, antibacterial and antiparasitic agents.[122] The aromatic, electron rich structure of triazoles imparts the ability to bind with a variety of enzymes and receptors in biological systems via non-covalent interactions offering a broad range of biological activities. They are bioisosteres of various groups such as amides, oxazoles, pyrazoles and imidazoles, and further, act as linkers in combining two pharmacophore fragments, leading to novel compounds via a 'click' chemistry approach. Hence, triazoles are widely employed in the synthesis of medicinal compounds.[122,123]

To introduce triazoles to tetramate systems, an approach involving the cycloaddition of benzyl azide with internal alkyne **220** was investigated. Ruthenium-catalyzed 1,3-dipolar azide-alkyne cycloaddition (RuAAC) proceeds with the regioselectivity favouring 1,5-disubstituted 1,2,3-triazoles.[124] When internal alkynes are engaged in cycloaddition, the outcome would be a 1,4,5-trisubstituted 1,2,3-triazole. Thus, benzyl azide cycloaddition to internal alkyne **220** would yield the expected tetramate **246** with a trisubstituted 1,2,3-triazole as represented in Scheme 4.17.



Scheme 4.17. Attempted synthesis of 1,2,3-triazole **246**.

Cp*RuCl(COD) was used as the catalyst for the cycloaddition of benzyl azide with internal alkyne following literature procedure.[124] However, the expected product **246** was not observed after a reaction time of 24 h and the reactant **220** was isolated by column chromatography.

4.8 Elaboration via 6-aminopenicillanic acid (6-APA): A hybrid systems approach

Hybrid or heterodimer antibiotics have gained popularity in the recent past as an attractive approach to the development of novel antibiotics that can target bacterial strains, especially those that are resistant to the parent antibiotics. Therefore, the synthesis of such hybrid antibiotics comprising of the tetramate scaffold that is covalently linked to a compound of a known antibiotic class was of interest.

4.8.1 A brief introduction to hybrid antibiotics

An approach involving a combination of drugs has been proposed as an attractive strategy to combat the growing antibiotic resistance in pathogenic bacteria to clinically available antibiotics. Two such strategies involving combinations of antibiotics are; 1) combination therapy and 2) hybrid antibiotics. Combination therapy involves the use of two separate chemical entities in combination to bring about a synergistic effect,[125] while hybrid/heterodimer antibiotics involve two pharmacophores that are covalently linked into one chemical entity suitable for monotherapy.[126]

A small number of combination therapies have proven to be successful in the clinic. The well known examples are Augmentin (a combination of β-lactam class antibiotic amoxicillin and lactamase inhibitor clavulanate) marketed by GSK and Bactrim (synergistic combination of trimethoprim and sulfamethoxazole) marketed by Roche. Combination therapy can be a combination of compounds where one compound 1) prevents the degradation of the primary antibiotic such as in the case of Augmentin, or 2) is itself an antibiotic that targets a similar or different pathway to the other as in Bactrim. However, the different pharmacokinetics of the drugs in combination would make drug formulation challenging. Further, this strategy is not effective against multi-drug resistant strains that exhibit resistance against both classes of antibiotics in combination. Moreover, the limited number of antibiotic classes in clinical use in turn limits the scope of this approach.[126]

Hybrid antibiotics have emerged as an attractive alternative to combination therapy where the covalent link between the two pharmacophores makes the pharmacokinetics of the single molecular entity more predictable. The rational design of a hybrid antibiotic can lead to the inhibition of both bacterial targets which would help in overcoming resistance against both drugs.[126] The covalent connection between the two drug molecules can be one that is cleavable leading to a prodrug approach or one which is chemically stable leading to a hybrid

structure with a dual mode of action. Another advantage of the hybrid antibiotic approach is the ability to use the penetration capacity of one drug motif for the transportation of the other, thereby increasing its bioavailability and cell-penetration.[126]

The most widely represented class of antibiotics in hybrid systems is the fluoroquinolone class that targets both topoisomerase IV and DNA gyrase. Fluoroquinolones have successfully been hybridized with oxazolidinones,[127,128] rifamycins,[129] tetracyclins[130] and aminoglycosides[131,132]. Similarly, hybrid systems of the aminoglycoside class of antibiotics have been synthesised with β-lactams (cephalosporins),[133] oxazolidinones and chloramphenicols.[134] These hybrid systems exhibit either enhanced (synergistic) or diminished (antagonistic) antibiotic activity compared to the respective parent antibiotics. Three hybrid molecules MCB-3837 (fluoroquinolone-oxazolidinone), TD-1792 (cephalosporin-vancomycin) and CBR-2092 (rifamycin-fluoroquinolone) have progressed into human clinical trials.[126]

Interestingly, studies on the 'click' conjugate product of ciproflaxacin and neomycin B prepared by Pokrovskaya and co-workers provided evidence for the delay in resistance development against both Gram-(+)ve and Gram-(-)ve bacteria in comparison to the parent antibiotic separately or their 1:1 mixture. This finding validates the application of a hybridization approach to drug discovery to combat rapid resistance development.[131]

4.8.2 Synthesis of tetramate-penicillanic acid hybrid systems

The history of the β-lactam antibiotics dates back to 1928 when penicillin was discovered by Alexander Fleming. Since then, many derivatives of penicillin and different classes of β-lactams have emerged with broad-spectrum and extended-spectrum antibiotic activity. Cephalosporins, carbapenems and monobactams are such classes bearing the β-lactam

moiety.[135,136] The mode of action of β-lactam antibiotics is linked to the biosynthesis of the bacterial cell wall, where they inhibit essential enzymes such as transpeptidases and carboxypeptidases involved in peptidoglycan synthesis.[137]

The discovery of a biosynthetic route for the preparation of the penicillin nucleus 6-aminopenicillanic acid (6-APA) in 1957, opened doors to the development of a range of semi-synthetic penicillins. The first semi-synthetic penicillin to reach clinical practice was phenethicillin, a close analogue of penicillin V. This was followed by a series of penicillin derivatives such as ampicillin and methicillin among many others.[135] While methicillin was the first semi-synthetic penicillin stable to staphylococcal β-lactamase with antibiotic activity against penicillin resistant *S. aureus*, these semi-synthetic derivatives also had improved oral absorption and a broader spectrum of antibiotic activity compared to the naturally occurring penicillins.[135,136]

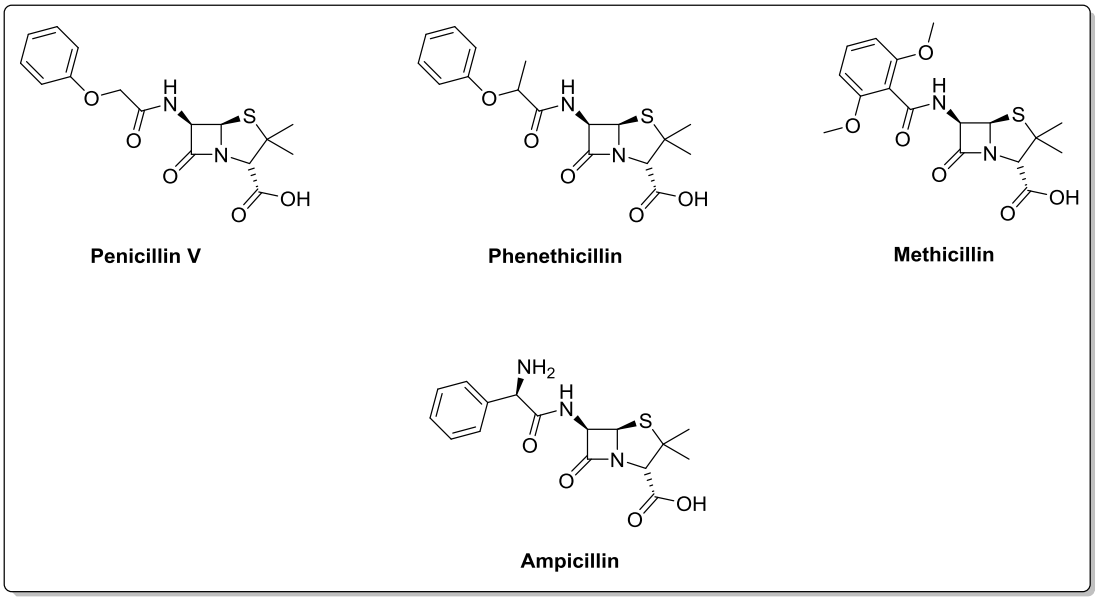


Figure 4.8. Examples of penicillin antibiotics.

Our first attempt at developing conjugated β-lactams with tetramic acid was via Buchwald amination of 2-azetidinone to yield monobactam-tetramate hybrid **241** (Figure 4.9), the synthesis for which was discussed in Section 4.6.2 (page 110-111, *vide supra*).

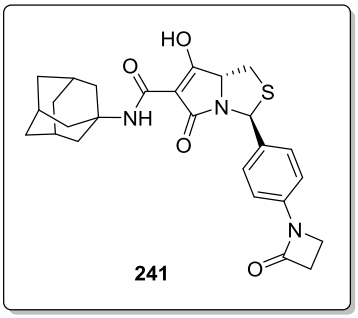
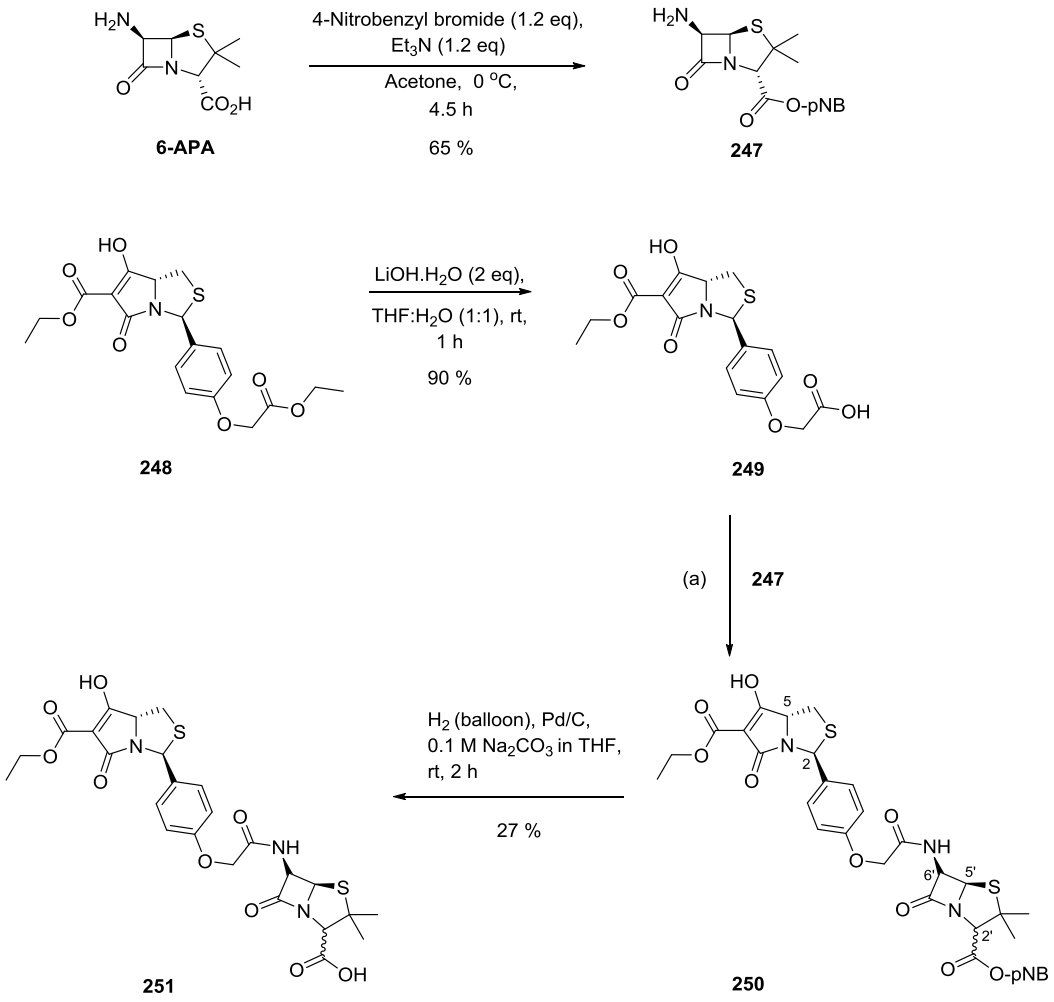


Figure 4.9. Carboxamide tetramate conjugated to 2-azetidinone to give a monobactam-tetramate hybrid **241.**

The synthesis of penicillin-tetramate hybrid system was inspired by the utility of 6-APA in the development of penicillin derivatives and its synthesis is outlined below in Scheme 5.2.



Scheme 4.18. Synthesis of penicillin-tetramate hybrid system **251**; (a) Table 4.9.

Carboxylic acid protection of 6-APA with 4-nitrobenzyl bromide afforded the benzyl ester **247** in 65 % yield. Ester hydrolysis of tetramate **248** with LiOH gave carboxylic acid **249** in 90 % yield, with the ethyl ester of the tetramate core intact. The hybridisation of tetramate **249** with 6-APA derived **247** via amide coupling was explored under both DCC-mediated and T3P-mediated coupling conditions to obtained **250** and these are listed in Table 4.9. Upon hydrogenolysis of **250**, the desired deprotected tetramate hybrid **251** was obtained.

Table 4.9. Conditions for amide coupling to yield **250**.

Entry	Conditions	% Yield	Remarks
1	DCC (1.1 eq), DMAP (0.1 eq), CH_2Cl_2, rt, 15 h	38	A mixture of four diastereomers was obtained. **250a:(250b+250d):250c**; 1:0.4:1.3
2	T3P (2 eq), Pyridine (3.4 eq), EtOAc, 0 °C, 15 h	32	A mixture of four diastereomers was obtained. **250a:(250b+250d):250c**; 1:0.3:0.6

DCC mediated amide coupling of carboxylic acid **249** with the amine **247** afforded penicillin-tetramate hybrid **250** as a mixture of four diastereomers (Figure 4.10 (a)). Diastereomers **250a** and **250b** could be accounted for as arising from the enantiomers of tetramate **249**, where **250a** would be the major diastereomer of the two, in analogy with stereoselectivity studies discussed in Chapter 2. It was postulated that the other two diastereomers would be a result of epimerisation at C-2' of **250a** and **250b**, leading to **250c** and **250d** respectively. Analysis by NOESY spectroscopy confirmed this outcome, where the spatial correlation of H-2' and H-5' was different for **250a** and **250c** (Figure 4.10 (b)). The presence of **250c** as the major diastereomer suggests significant epimerisation at C-2' of **250a**. A similar NOE analysis of the minor diastereomers **250b** and **250d** was not possible as the ^{1}H signals were often obscured by the major diastereomers, except for H-2 of the tetramate, where the ^{1}H signals for the minor diastereomers could be seen to be overlapping (Figure 4.11 (a)). Similarly, peak

resolution by LC-MS was not successful for **250** and only two resolved peaks were observed for **251** (Figure 4.11 (b)). Thus, the relative populations of the diastereomers given above in Table 4.9 are approximate values based on the integration of ^{1}H peaks of H-2. It has been reported that amide bond formation can be facilitated with low epimerization in epimerization-prone substrates by using T3P and pyridine.[138] However, under these reaction conditions, epimerization at C-2' could not be completely avoided, but was significantly reduced. Tetramate **250** was obtained as a mixture of diastereomers, but now with **250a** being the major diastereomer. Thus, in this regard, 7-aminocephalosporanic acid would be a better alternative to obtain tetramate-β-lactam hybrid systems free of epimerisation under the above reaction conditions.

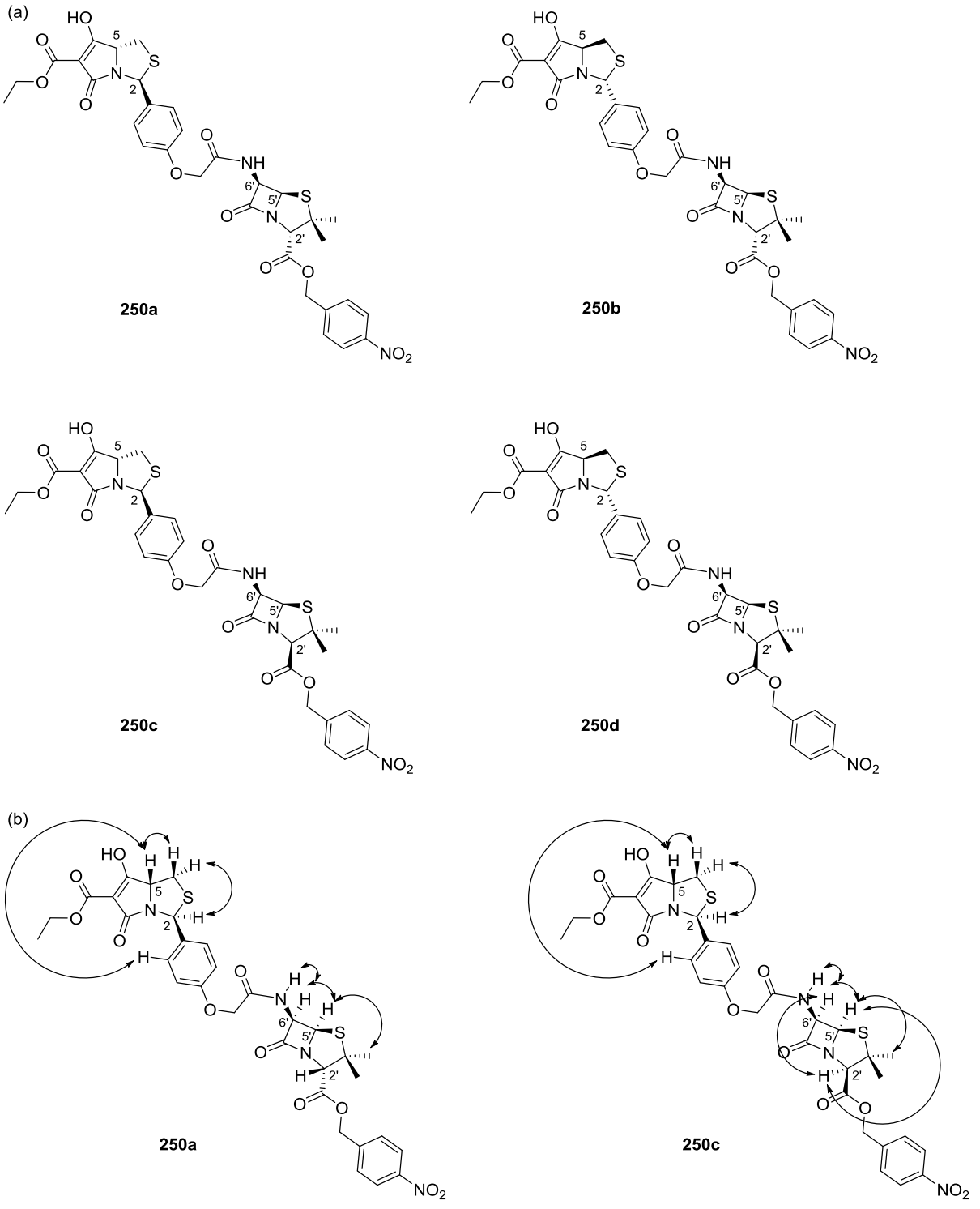


Figure 4.10. (a) Diastereomers of tetramate-penicillanic acid hybrid system **250** and (b) NOE correlation data for tetramate **250**.

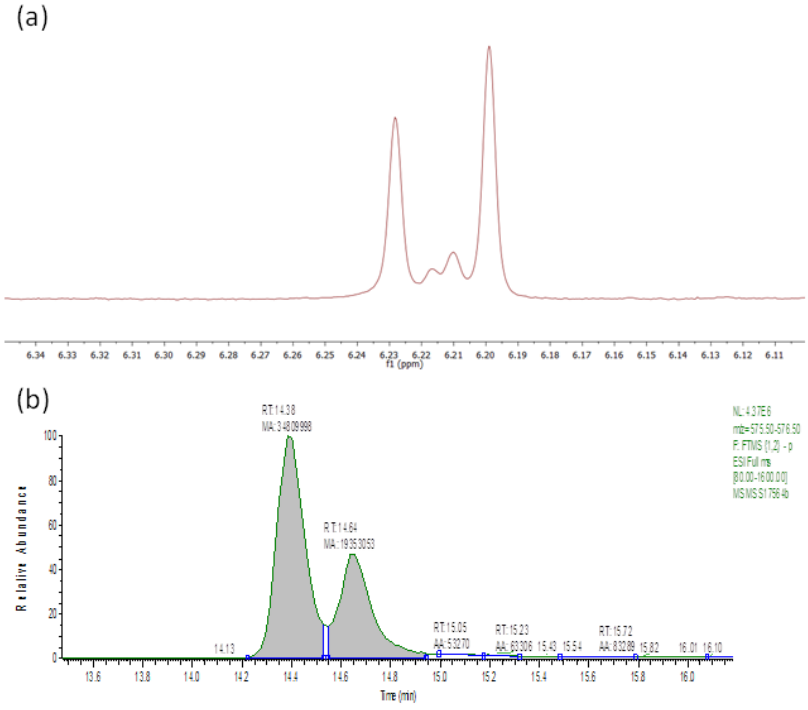


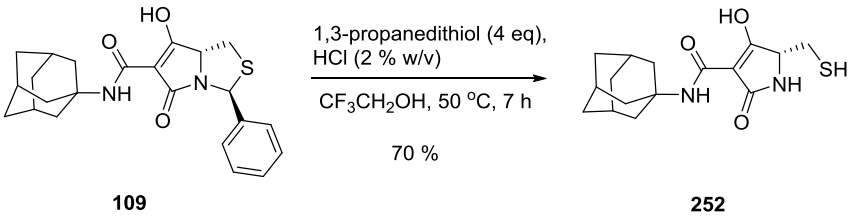
Figure 4.11. (a) ^{1}H NMR of **250** showing the peaks for H-2 of the four diastereomers. (b) peak resolution by LC-MS for **251**.

4.9 Deprotection of *N,S*-acetal of bicyclic tetramates

In comparison to *N,O*-acetal systems of oxazolidines, *N,S*-acetals of thiazolidines have shown higher stability to acids.[139] The deprotection of thiazolidines often require harsh conditions such as heating under reflux with 5M HCl for 72 h.[140] However, Onoda et al. have shown that Boc-protected thiazolidines can be deprotected at the *N,S*-acetal in good yield with 15 eq of TFA in water-saturated CH_2Cl_2 under ambient conditions within 2 h.[141]

Deprotection of carboxamide tetramate **109** was investigated in the presence of TFA in CH_2Cl_2. After a reaction time of 24 h, only the reactant **109** was observed by LRMS data. Analysis of ^{1}H NMR spectrum of the crude mixture showed only the tetramate **109** confirming the above outcome. However, the presence of water accelerated the deprotection, although complete deprotection was not observed in 24 h (**109**:aldehyde ratio observed in

crude ^{1}H NMR spectrum was 10:1). Interestingly, the deprotection of thiazolidine via Corey-Reichard protocol[142] was successful where the acid-catalysed transfer of the N/S bridging group of **109** to 1,3-propanedithiol led to the deprotected carboxamide tetramate **252** in 70 % yield as illustrated in Scheme 4.19. This observation highlights the need for stronger conditions for the deprotection of thiazolidine-derived carboxamide tetramates.



109 **252**

Scheme 4.19. Deprotection of thiazolidine.

4.10 Summary

In this chapter, both late-stage side-chain functionalization of tetramates via elaborated C-2 pendant groups and the *de novo* synthesis of tetramates with functionalized aldehydes have been explored for the generation of tetramate libraries.

Suzuki-Miyaura cross-coupling of tetramates **110** and **125** with a range of boronic acids/esters generated a library of tetramates **175-187** with varying C-2 pendant groups. SM cross-coupling was successful with XPhos, but not with PPh_3 and $AsPh_3$ as ligands.

Strategies for the incorporation of glycone pendant groups inspired by tetramate natural products were investigated. Tetramates with a phenyl glycosidic link to monosaccharide **192**, **193**, **195-197** and disaccharide **202** were synthesised. Further, tetramate **207** was synthesised with a benzyl glycosidic link to monosaccharide with improved stability. Aglycone analogues **194**, **216**, **218a-c** were synthesised in order to compare their biological activity.

Williamson etherification of aglycones afforded functionalized ethers **219**-**221** with potential for further modification. Further, functionalized tetramates with tetramate-pyroglutamate hybrids **225** and **229** were synthesised.

Buchwald amination as a strategy for late stage functionalization of **110** was not successful, but tetramates **238**-**241** with amine/amide substitutions at C-2 were obtained via functionalised aldehydes incorporated in the early stages of tetramate synthesis. This strategy also afforded a tetramate-monobactam hybrid in compounds **239** and **241**.

Elaboration via nitrile oxide-alkene cycloaddition afforded tetramate **244** with a 5-substituted isoxazoline. Ruthenium-catalysed azide-alkyne cycloaddition (RuAAC) of **220** with benzyl azide was not successful in generating the desired triazole **246**.

The synthesis of hybrid systems of tetramates with a known antibiotic was investigated via the coupling of tetramate **249** with **247** derived from 6-aminopenicillanic acid. The product **250** was obtained as a mixture of 4 diastereomers. Hydrogenolysis of **250** afforded the tetramate-penicillanic acid hybrid **251**.

Finally, the *N*,*S*-acetal of tetramate **109** was stable to deprotection in acid under ambient conditions, but could be deprotected under Corey-Reichard conditions to yield the monocyclic tetramate **252**.

CHAPTER 5 : EVALUATION OF ANTIBIOTIC ACTIVITY

The final chapter of this thesis reports the evaluation of the antibacterial properties of alkoxycarbonyl tetramates and carboxamide tetramates described in the previous chapters. A correlation of calculated physicochemical properties with their antibacterial activity was undertaken and compared to that of known antibiotic classes. Possible modes of action for our library of tetramates are discussed along with those reported in the literature for isolated natural product tetramates. An NMR-based ligand displacement assay was conducted to determine the binding affinity of some tetramates for the plasma protein Human Serum Albumin (HSA). Finally, a brief note on the potential of tetramic acids to function as proteasome inhibitors in anticancer chemotherapy is included at the end of this chapter.

5.1 Known classes of antibacterial drugs

Antibiotics bring about their biological activity in target organisms by interfering with one or more processes that is essential to the survival or growth of bacteria. Such pathways that are often the targets of antibacterials are bacterial cell wall biosynthesis, protein synthesis and DNA replication.[4,8]

β-Lactams and glycopeptides are two classes of antibiotics that target cell wall biosynthesis. The bacterial cell wall consists of peptidoglycan (murein) layers that are cross-linked. This cross-linking is catalysed by transpeptidases, which recognize the D-Ala-D-Ala subunit of the murein side chain for cross-linking. β-Lactams, which are structurally analogous to this carboxy terminal portion (D-Ala-D-Ala) of the murein subunit binds to the active site of the transpeptidase enzyme thereby interfering with the cross-linking process.[8] This results in a weakened bacterial cell wall which ruptures due to osmotic pressure within the cell, leading to cell death.[8,135,143] Glycopeptides act by hydrogen bonding with the peptidoglycan precursor,

forming stable complexes. The now sterically hindered substrate is unable to align with the active site of the enzyme peptidoglycan polymerase and as a result, the synthesis of the peptidoglycan backbone is interrupted.[144]

Inhibitors of protein synthesis target the bacterial ribosome which is made up of two subunits; 30S and 50S. Aminoglycosides bind to the 30S subunit of the bacterial ribosome and compromise the translational accuracy, resulting in the misreading of certain mRNAs and premature termination of peptide chains. This perturbation of the elongation of the nascent peptide chain leads to the synthesis of aberrant proteins.[145] Chloramphenicols bind to bacterial ribosomes and interrupt the formation of peptide bonds by inhibiting peptidyl transferase.[7] Macrolides prevent protein synthesis by binding to the tRNA binding site of the 50S ribosomal subunit while tetracyclins function at the 30S subunit and prevent the association of aminoacyl-tRNA with the ribosome.[7,146] Streptogramins and oxazolidinones are two other classes of antibiotics that exert their effect by inhibiting protein synthesis.[147,148]

Inhibitors of nucleic acid synthesis act by inhibiting enzymes involved in RNA or DNA synthesis. Rifamycins inhibit the initiation of transcription by binding to bacterial RNA polymerase, while quinolones target DNA topoisomerases namely DNA gyrase and DNA topoisomerase IV which are responsible for the supercoiling and decatenation of DNA.[7,149]

While there is no generally applicable theory to differentiate antibiotic drugs from non-drugs, an analysis of the physicochemical property space of the known antibacterial drug classes has been beneficial in the understanding of the chemical space occupied by the clinically available antibiotics. To this end, O'Shea and Moser have analysed the major antibiotic classes and calculated the mean values for physicochemical properties as listed in Table 5.1.[150]

Table 5.1. Average physicochemical properties of known antibacterial classes. (MW=molecular weight, %PSA=(PSA/MSA)*100, where PSA= polar surface area, MSA= molecular surface area) Adapted with permission from O'Shea, R.; Moser, H. E. *J. Med. Chem.* **2008**, *51* (10), 2871–2878. Copyright (2008) American Chemical Society.

Antibiotic class	MW (Da)	clogP	clogD$_{7.4}$	%PSA	H-bond donor	H-bond acceptor
Inhibitors of cell wall biosynthesis						
Glycopeptides	1740	1.3	-1.8	37	22.8	37.2
Penicillins	413	1.4	-2.4	39	2.8	8.5
Cephems	452	0.1	-3.0	51	4.1	10.8
Carbapenems	397	-3.0	-5.8	43	4.5	9.0
Inhibitors of protein synthesis						
Aminoglycosides	526	-2.9	-8.1	54	14.8	15.4
Macrolides	790	3.5	2.6	23	3.6	15.0
Tetracyclins	481	-0.7	-3.6	40	7.1	10.5
Inhibitors of nucleic acid metabolism						
Fluoroquinolones	371	1.3	-0.8	25	2.1	6.5
Sulfa drugs	273	0.6	-0.1	45	3.1	6.2

In 1996, Lipinski's study introduced a systematic approach to correlate physicochemical properties of drugs with oral bioavailability named as 'the rule of 5' (Ro5).[151] This study predicted that poor absorption and permeation (passive) is more likely when there are more than 5 H-bond donors and 10 H-bond acceptors, MW > 500 and clogP > 5. However, looking at the analysis in Table 5.1, it is evident that some antibacterials deviate from this generalisation and occupy chemical space beyond the Ro5, and this has been demonstrated by Leeson and co-workers.[152] Bacterial organisms are diverse and for whole-cell bioactivity, antibiotics should have the ability to penetrate the bacterial cell wall and exert its effect in non-human systems. Thus, their physicochemical properties may not comply with those set out in the Ro5.[152] In a more recent study by Mugumbate and co-workers, physicochemical properties were analysed for antibacterials categorized by the protein target class. Their study

shows that antibacterials that target riboprotein generally violate the Ro5, while compounds that target other bacterial proteins generally comply with the Ro5.[153]

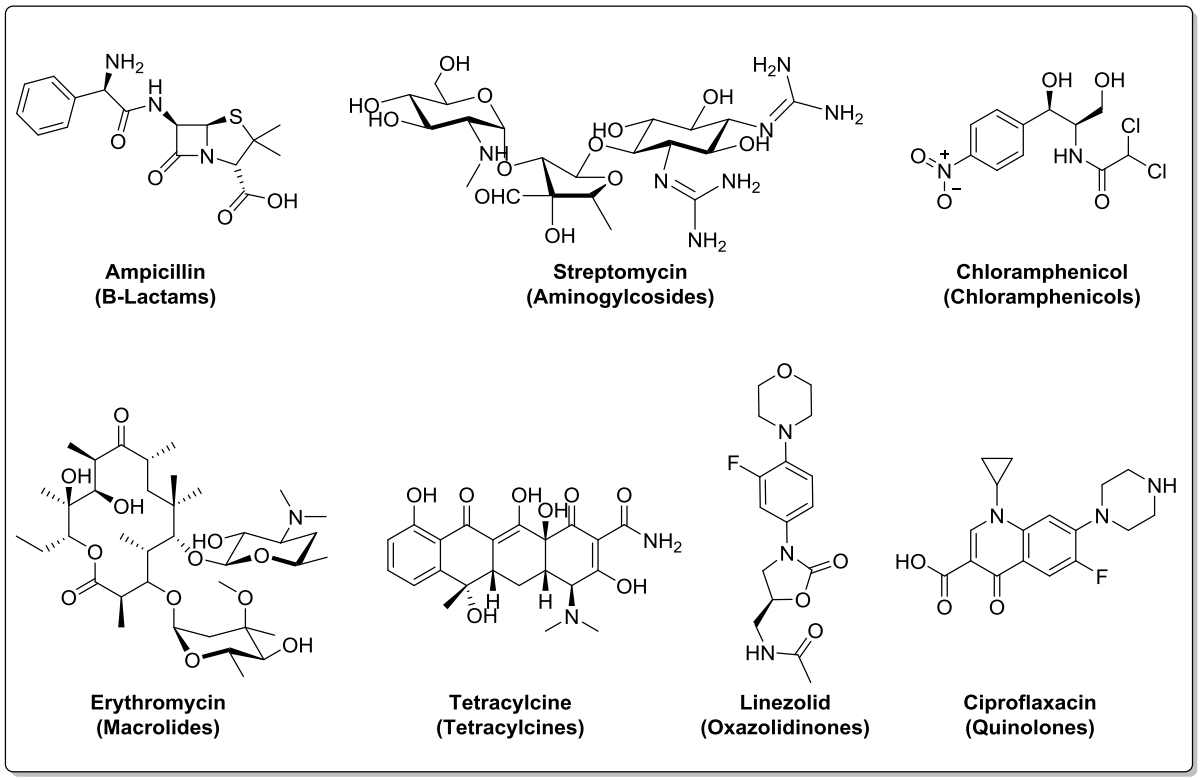


Figure 5.1. Examples of some antibiotics and their antibiotic class.

5.2 Tetramates with known antibiotic activity

The antibiotic activity of some tetramates isolated from natural sources were discussed in Chapter 1, under Section 1.4 (page 14-19). Physicochemical and structure properties were calculated for those tetramates with antibiotic activity and are listed in Table 5.2. Their physicochemical properties are compared with those of known classes of antibiotics in Figure 5.2.

Table 5.2. Physicochemical and structure properties of some tetramates**.** Calculator Plugins were used for physicochemical and structure property prediction and calculation, Marvin (16.4.18.0), 2016, ChemAxon (http://www.chemaxon.com).

Tetramate	MW	clogP	clogD$_{7.4}$	PSA	MSA	%PSA	H-bond donor	H-bond acceptor
Reutericyclin	349	5.21	1.68	74.7	578.3	12.9	1	4
Epicoccarine A	386	4.69	2.36	86.6	604.5	14.4	3	4
Vancoresmycin	1344	2.55	0.31	422.6	2174.4	19.4	17	22
Virgineone	750	4.82	1.57	223.3	1191.7	18.7	8	12
Virgineone aglycone	588	6.59	3.34	144.2	983.4	14.7	5	7
Equisetin	376	3.03	0.44	77.8	589.0	13.2	2	4
Altersetin	428	4.14	0.82	86.6	657.7	13.2	3	4
Zopfiellamide A	446	3.15	-1.84	115.1	658.2	17.5	3	6
Zopfiellamide B	460	3.60	-1.35	115.1	688.2	16.7	3	6
Signermycin	390	3.22	0.81	86.6	614.1	14.1	3	4
Kibdelomycin	940	3.67	0.73	258.5	1282.5	20.2	6	12
Amycolamycin	940	3.67	0.73	258.5	1280.0	20.2	6	12
Streptolydigin	601	2.09	-0.01	147.2	867.1	17.0	3	9
Tirandamycin A	417	1.61	-0.47	114.5	578.65	19.8	2	7
Tirandamycin B	433	0.56	-1.52	134.7	588.6	22.9	3	8

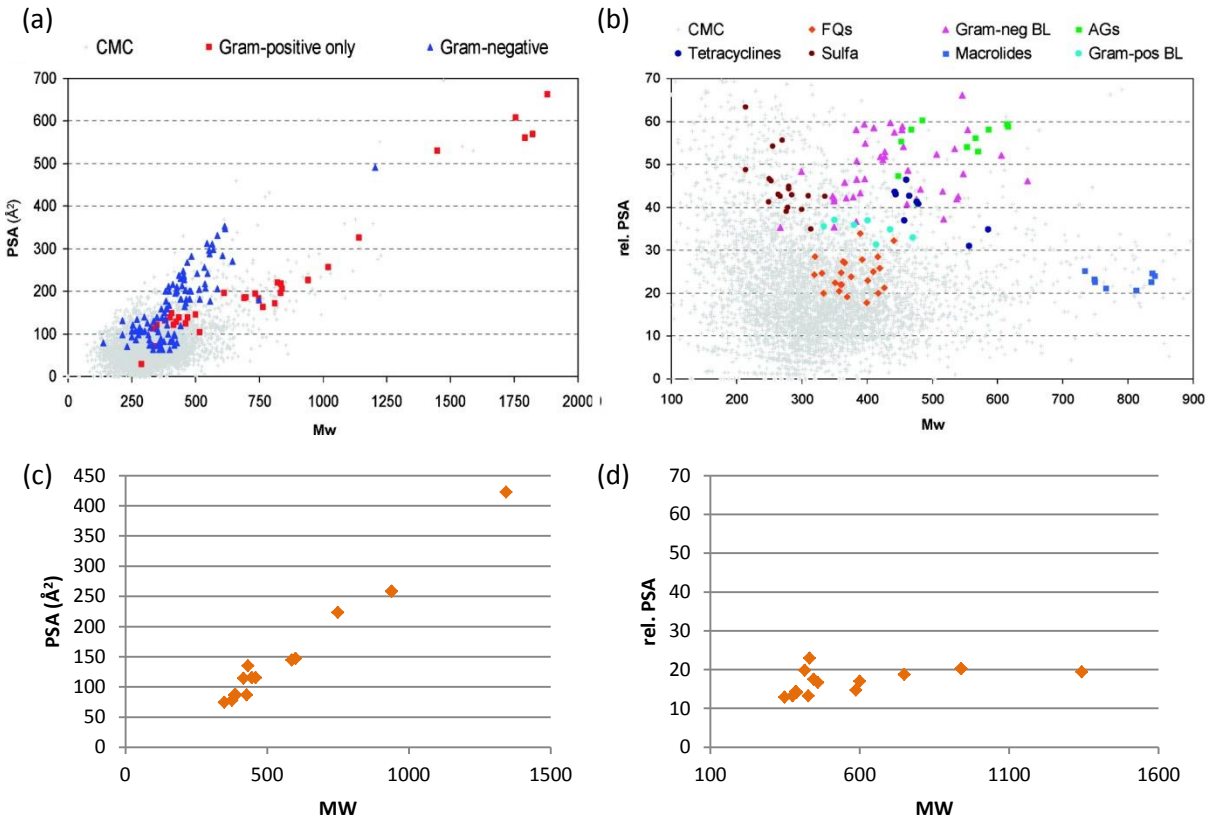


Figure 5.2. Physicochemical properties of known classes of antibiotics and naturally occurring tetramates. (a) Plot of PSA vs MW for Gram-(+)ve only and Gram-(-)ve antibacterials; (b) Plot of rel. PSA vs MW for major antibacterial classes; (c) Plot of PSA vs MW for naturally occurring tetramates with antibacterial activity; (d) Plot of rel. PSA vs MW for naturally occurring tetramates with antibacterial activity. CMC=comprehensive medicinal chemistry, FQ=fluoroquinolones, BL=β-lactams, AG=aminoglycosides. Parts (a) and (b) of this figure are reprinted with permission from O'Shea R. Moser, H.E., *J. Med. Chem*, **2008**, *51 (10)*, 2871-2878. Copyright (2008) American Chemical Society.

Tetramic acids are generally more potent against Gram-positive bacterial strains and their physicochemical properties (PSA vs MW) follow a similar trend to that observed for other known antibacterials that are active against Gram-positive strains as represented in Figure 5.2 (a) and (c). The major classes of antibiotics occupy a vast chemical space as depicted in Figure 5.2(b) and naturally occurring tetramic acid antibacterials show close resemblance to fluoroquinolones where they occupy chemical space with rel. PSA of 13-25 % with most of the compounds clustered in the MW range of 300-600 Da.

5.3 Evaluation of antibacterial activity of tetramates

5.3.1 Preliminary evaluation of antibacterial activity: hole-plate method

Whole-cell antibacterial assays were performed using the hole-plate method with Gram-positive *Staphylococcus aureus* DS267 or Gram-negative *Escherichia coli* X580, using Cephalosporin C as a standard. The samples were prepared as 4 mg/mL solutions of 70 % DMSO in MeOH. A 100 µL aliquot of each sample solution to be tested was loaded into 10 mm wells in agar plates and incubated overnight at 37 °C. The assays were repeated in triplicates (technical replicates) and the diameters of the resultant inhibition zones were measured (±1 mm) along two perpendicular axes and then averaged to obtain the zone of inhibition. Where the compounds tested showed potent antibacterial activity at 4 mg/mL, with a zone of inhibition of >25 mm, the compound solutions were diluted to the desired concentrations to obtain a zone of inhibition of ≤25 mm. A 'control/blank' was run with solvent alone, to ensure the solvent made no contribution to the zone of inhibition. The relative potency was estimated by reference to standards prepared with cephalosporin C,[154,155] Appendix G.

The results for the biological activities of compounds at given concentrations are represented in bar charts, where a larger zone diameter signifies a stronger antibacterial activity. The symbol 'H' denotes a 'halo' zone of inhibition where an area of reduced bacterial density was observed instead of a clear zone. Error bars represent standard error (where standard error = standard deviation/√(sample size)). Thus, using the hole-plate method, a quick preliminary evaluation of antibacterial activity of compounds against whole cells was obtained and an example is given in Figure 5.3.



Figure 5.3. An example of agar plates after incubation with the samples overnight, showing clear zones of inhibition around the wells.

5.3.2 Evaluation of antibacterial activity of tetramate esters and carboxamide tetramates: hole-plate method

The synthesis of the bicyclic tetramate core derived from cysteine was described in Chapter 2. The derivatisation of these tetramate esters to carboxamide tetramates via C-7 functionalization was discussed in Chapter 3. In the section that follows, assessment of the antibacterial activity of these compounds via the hole-plate method will be discussed.

In general, tetramate esters **90a**-**95a** showed little or no antibacterial activity against both *S. aureus* and *E. coli*, with the exception of **94a**, which showed a weak, but discernible zone of inhibition against both bacterial strains at a concentration of 4 mg/mL (zone of inhibition (mm) observed with **94a** for *S. aureus* = 12 and *E. coli* = 13 (H)).

Converting these tetramate esters to their carboxamide analogues led to a significant improvement in their antibacterial activity against *S. aureus*, but no activity was observed with *E. coli*. This suggests that these tetramates either do not have a mode of action whereby they interfere with a biological process essential for Gram-negative bacterial growth and survival or their uptake through the cell membrane or the influence of an active efflux pump limits the efficacy of tetramates against Gram-negative organisms.[156] C-7 functionalized

carboxamide tetramates on the other hand showed promising antibacterial activity and examples of tetramates and their antibacterial activity are represented in Figures 5.4 - 5.7.

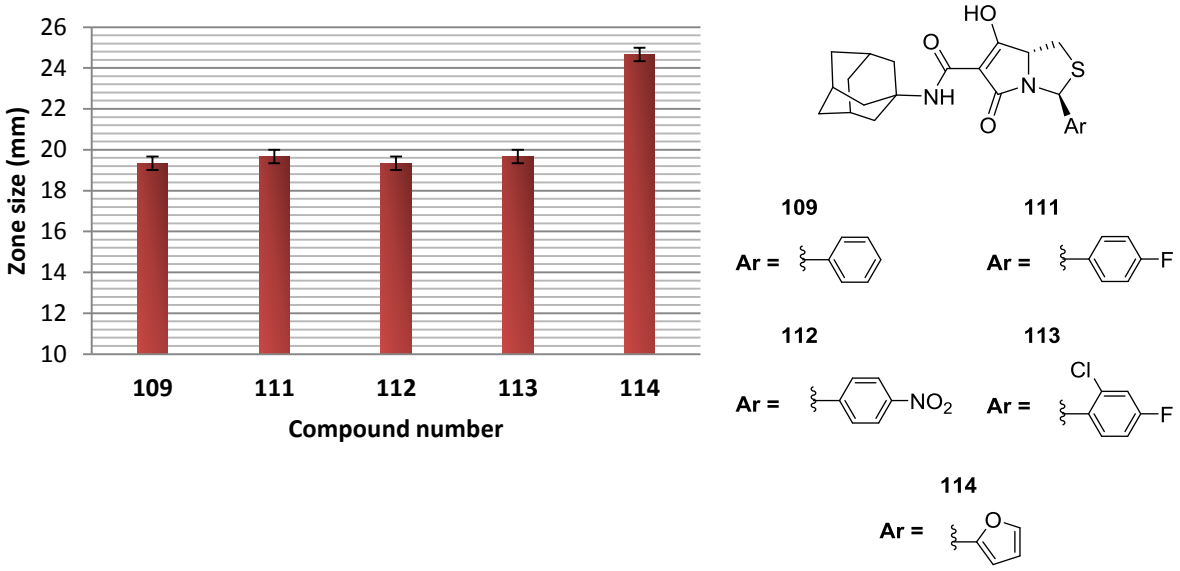


Figure 5.4. Bioactivity of carboxamide tetramates (concentration at 1 µg/mL, except **114** at 50 µg/mL).

The presence of an adamantyl carboxamide side chain on the tetramate core resulted in a significant enhancement of antibacterial activity of compounds as illustrated in Figure 5.4. Tetramates were active at a concentration of 1 µg/mL with the exception of compound **114** with a 2-furanyl substituent, which exhibited no antibacterial activity at a concentration of 1 µg/mL, and the zone size reported was obtained at a concentration of 50 µg/mL.

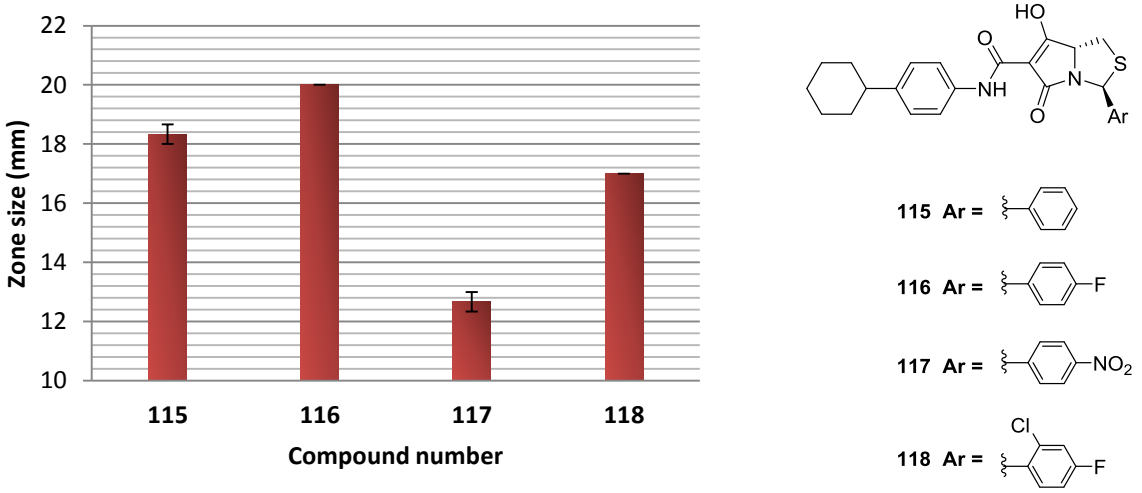


Figure 5.5. Bioactivity of carboxamide tetramates (concentration at 10 µg/mL, except **115** at 2 µg/mL and **117** at 50 µg/mL).

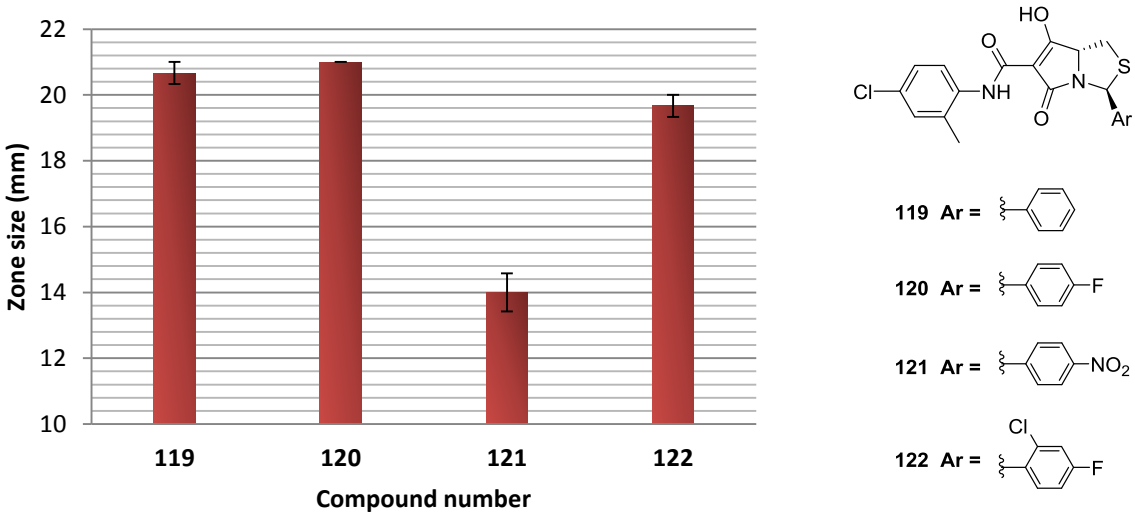


Figure 5.6. Bioactivity of carboxamide tetramates (concentration at 25 µg/mL, except **120** at 50 µg/mL and **121** at 100 µg/mL).

Tetramates devoid of the adamantyl side chain as in compounds **115-118** with a 4-cyclohexylphenyl group or compounds **119-122** with a 4-chloro-2-methylphenyl group demonstrated good antibacterial activity, but were much less potent compared to their adamantyl analogues as can be seen in the graphical representations above in Figures 5.5 and 5.6. While these tetramates required higher concentrations to bring about similar levels of potency *in vitro*, it was evident that the 4-cyclohexylphenyl group in compounds **115-118** (Figure 5.5) conferred more antibacterial activity compared to their analogous tetramates **119-122** (Figure 5.6).

The decrease in activity observed for **123**, **124** and **129** further confirms the requirement for a bulky pendant group at C-7 of the bicyclic tetramate core for potent antibacterial activity (Figure 5.7).

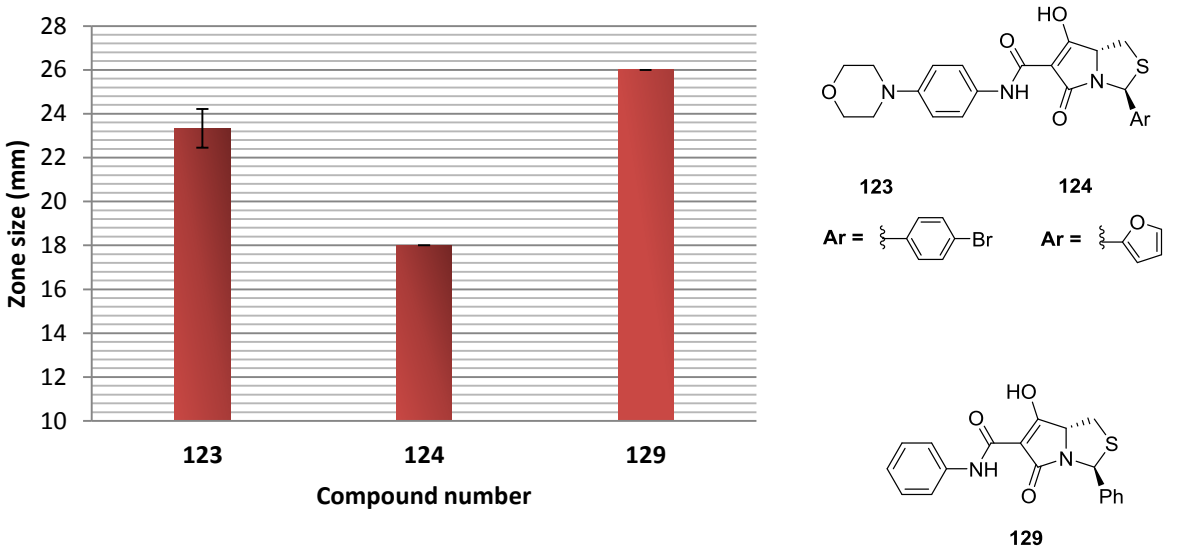
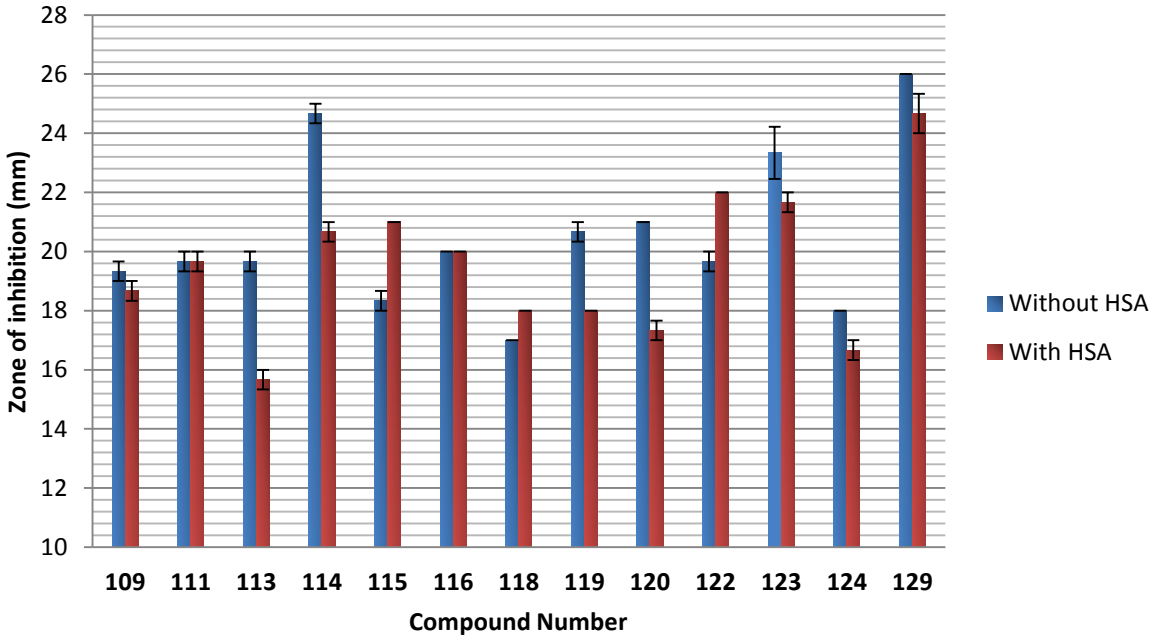


Figure 5.7. Bioactivity of carboxamide tetramates (concentration at 4 mg/mL, except **129** at 0.5 mg/mL).

Since the initial compound library synthesized by Moloney and co-workers with a ^{t}Bu substituent at C-2 of the tetramate core showed no antibiotic activity in the presence of Human Serum Albumin (HSA) discussed in Section 3.2,[66] it was of interest to employ the hole-plate method as a preliminary evaluation of the antibacterial activity observed in the presence of HSA.

Similar to the procedure outlined above for the whole-cell bioassay (page 134), a 50 µL aliquot of each sample solution was loaded into 10 mm wells in agar plates along with a 50 µL aliquot of an aqueous stock solution of HSA (8 mg/mL) and incubated overnight at 37 °C. The final concentration of HSA in the well was 4 mg/mL, which is 10 % of the physiological serum albumin concentration.[66,157] The assays were repeated in triplicates (technical replicates) and the diameters of the resultant inhibition zones were measured (±1 mm) along two perpendicular axes and then averaged to obtain the zone of inhibition. These data were compared with the bioactivity of each sample in the absence of HSA and selected examples are represented in Figure 5.8.



Compound Number	109	111	113	114	115	116	118	119	120	122	123	124	129
Concentration of compound without HSA (µg/mL)	1	1	1	50	2	10	10	25	50	25	4000	4000	500
Concentration of compound with HSA (µg/mL)	5	5	5	50	50	50	50	100	250	250	4000	4000	500

Figure 5.8. Bioactivity of carboxamide tetramates with/without HSA. In the presence of HSA, increased sample concentrations were necessary for compounds **109-122** in order to obtain similar levels of antibacterial activity to that exhibited when HSA is not present.

The data represented in Figure 5.8 suggest that there is a reduction in potency observed when the test compounds are incubated in the presence of HSA. While **109-113** showed good antibacterial activity at 1 µg/mL in the absence of HSA, there was a complete loss of activity in the presence of 4 mg/mL of HSA (where the compound:HSA ratio is approximately 1:30). These data suggest that carboxamides possibly bind to HSA, leading to a reduction in their effective concentration in solution. However, by using this preliminary evaluation, it is not possible to conclude whether the binding to HSA is a result of site specific association or non-specific hydrophobic interactions.[158] Nevertheless, of interest was the recovery of bioactivity

of the tetramates **109-113** by increasing the concentration of the test compounds to 5 µg/mL (compound:HSA ratio is approximately 1:6), suggesting that carboxamide tetramates are potent antibacterial leads in the presence of HSA. Similarly, tetramates **115-122** exhibited no antibacterial activity at their original concentrations when tested in the presence of HSA, but their activity was regained by increasing the compound concentrations as represented in Figure 5.8. Interestingly, tetramates **114**, **123**, **124** and **129** which were less potent compared to the other tetramates discussed above were able to retain their potency in the presence of HSA without significant compromise.

As the samples were prepared in 70 % DMSO in MeOH to aid solubility, followed by mixing with albumin solution resulted in a final solvent composition of 35:15:50 of DMSO:MeOH:H_2O in the well (35 % DMSO). It is known that higher concentrations of DMSO can lead to protein denaturation,[159,160] and this might have interfered with the above analysis. Carboxamides **109-113** showed good antibiotic activity at 1 µg/mL, and these samples could be dissolved in a minimum amount of DMSO in H_2O at such low concentrations. Thus, the antibiotic activity of carboxamides **109-113** in the presence of HSA was tested where the final solvent composition was 2 % DMSO in H_2O. The zones of inhibition measured were in agreement with the data obtained in the presence of 35 % DMSO, suggesting that the antibiotic activity observed for the tetramates in the presence of HSA was not a result of the compound unbound to denatured HSA.

5.3.3 Evaluation of Minimum Inhibitory Concentrations (MICs) of tetramate esters and carboxamide tetramates: broth dilution method

Broth assays were performed to determine MICs of compounds and are illustrated in Figures 5.9 and 5.10 for tetramate esters and carboxamides respectively (MICs were obtained in collaboration with Sealife Pharma). MIC data represents the lowest concentrations of

compounds required to completely inhibit visible bacterial growth over 24 h of incubation. Thus, a lower MIC value indicates higher potency. A value of zero indicates no activity. (The complete data set is provided in Appendix G).

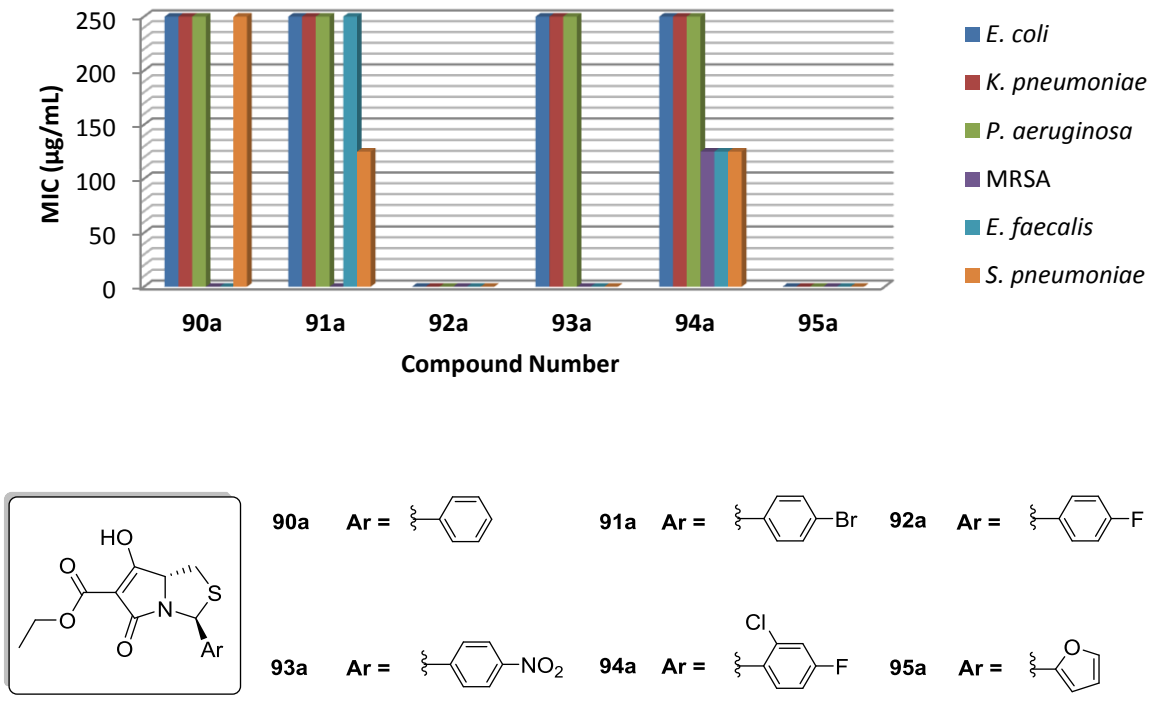
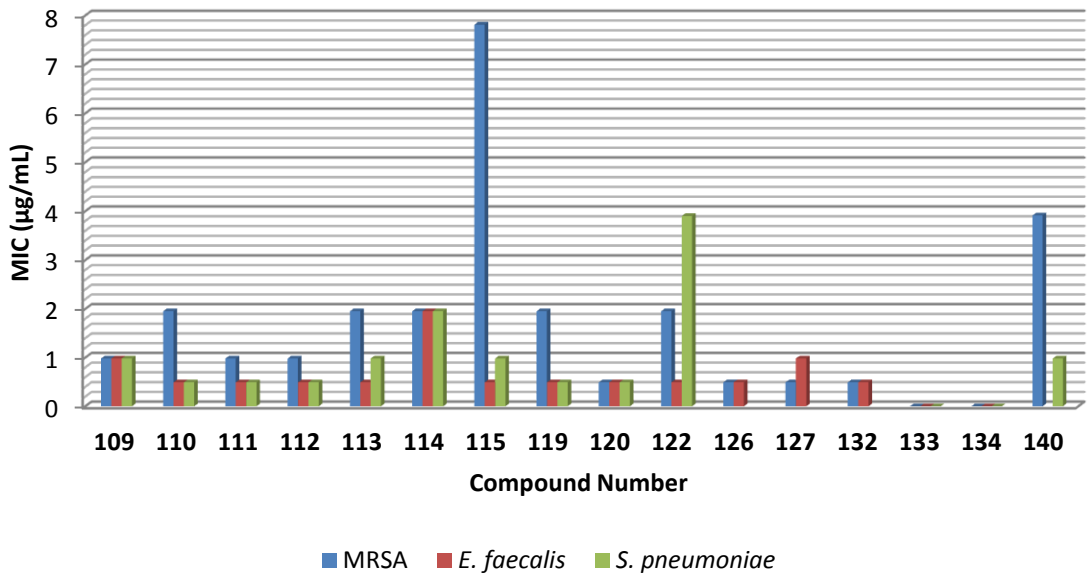


Figure 5.9. MIC of tetramates **90a-95a** against Gram-positive strains MRSA, *Enterococcus faecalis* and *Streptococcus pneumoniae* and Gram-negative strains *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

The antibacterial activity of tetramates **90a-95a** were rather weak, consistent with the observations from the preliminary assay via the hole-plate method. Compounds **92a** and **95a** showed no bioactivity against both Gram-(-)ve and Gram-(+)ve strains. The MICs of tetramates **90a**, **91a**, **93a** and **94a** against the Gram-(-)ve strains were 250 µg/mL, while slightly stronger activity was observed against Gram-(+)ve strains with MICs of 125 µg/mL as represented in Figure 5.9.

The carboxamide tetramates discussed in Chapter 3 in general showed either weak (MIC = 250 µg/mL) or no activity against Gram-(-)ve strains. This was in agreement with the data

obtained from the hole-plate method, where inhibition of Gram-(-)ve *E. coli* was not observed. However, some exhibited promising antibacterial activity against Gram-(+)ve strains and the MICs for selected examples are represented in Figure 5.10.



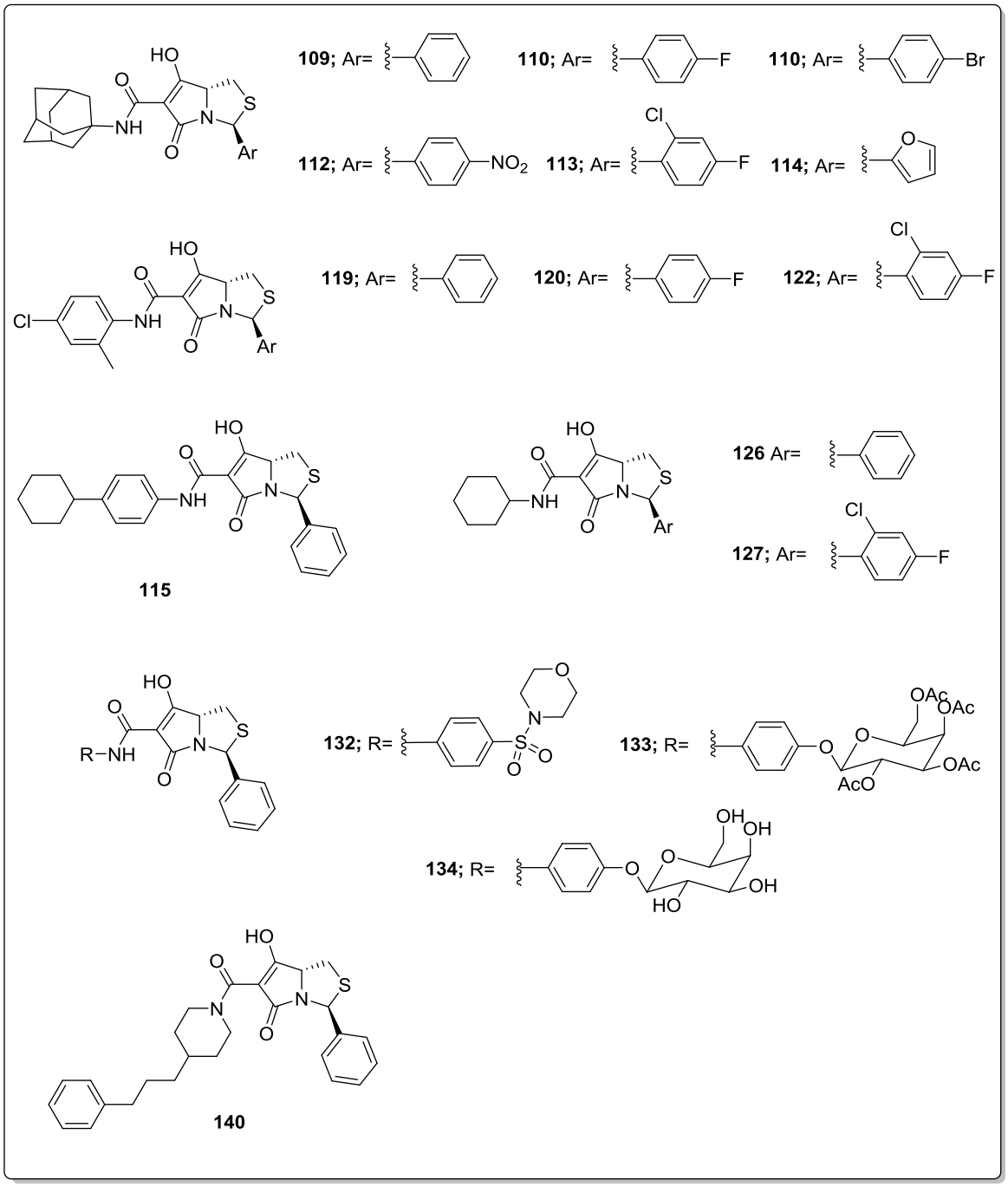


Figure 5.10. MICs of tetramates **109-140** against Gram-positive strains MRSA, *Enterococcus faecalis* and *Streptococcus pneumoniae* (MIC against *S. pneumoniae* for compounds **126**, **127**, **132** and against *E. faecalis* for **140** are not available).

The most potent carboxamide tetramates showed an MIC of 0.49 µg/mL, which is a remarkable improvement in activity compared to their tetramate ester analogues, highlighting the need for a carboxamide pendant group for whole-cell antibacterial activity. However, the presence of more polar carboxamides at C-7 led to a loss of activity as observed for **133** and

134. Although the magnitude of the activity threshold an antibacterial drug candidate should possess to be progressed from a whole-cell screen is not defined, according to O'Neill and co-workers, at a preliminary evaluation stage, a MIC value of 16-32 μg/mL against a test organism is considered a suitable minimum.[161] A comparison of these MIC values of tetramates with that of other known antibiotics (Table 5.3) suggest that compounds **109-140** listed in Figure 5.10 are good starting points in the development of novel antibiotics with tetramates as core scaffolds.

Table 5.3. Bioactivity range for some known antibiotics against MRSA strains (28 strains).[162] Adapted from O'Neill, A. J.; Bostock, J. M.; Moita, A. M.; Chopra, I. Antimicrobial Activity and Mechanisms of Resistance to Cephalosporin P1, an Antibiotic Related to Fusidic Acid. *J. Antimicrob. Chemother.* **2002**, *50* (6), 839–848, by permission of Oxford University Press.

Antibiotic class	Antibiotic	MIC (μg/mL)
β-lactam	Ceftriaxone	128 - >256
	Cefotaxime	64 - 128
	Cefepime	64 - >256
	Oxacillin	256 - 512
Oxazolidinone	Linezolid	1 - 4
Glycopeptide	Vancomycin	2 - 4

The selectivity of these tetramates to prokaryotic cells over mammalian cells was analysed via a primary cytotoxicity screening against HaCaT cell line (cytotoxicity studies were conducted in collaboration with Sealife Pharma, data provided in Appendix G). According to O'Neill and co-workers, compounds that are worth progressing from this stage should possess ≥10-fold higher antibacterial activity over cytotoxicity.[161] Thus, based on bioactivity and cytotoxicity data, the following carboxamide tetramates were chosen as promising leads for antibacterial drug discovery (Figure 5.11).

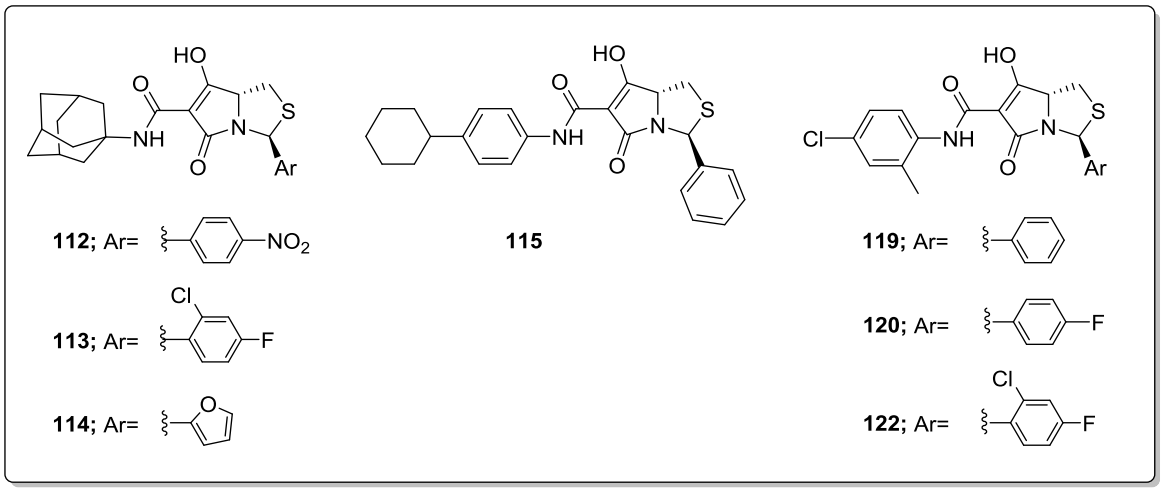


Figure 5.11. Selected promising leads for antibacterial drug discovery.

5.3.4 Evaluation of Minimum Inhibitory Concentrations (MICs) of tetramates with C-2 functionalization: broth dilution method

The synthesis of derivatives of tetramates via C-2 functionalization was discussed in Chapter 4. These compounds were assayed for antibacterial activity against Gram-(-)ve bacterial strains *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and Gram-(+)ve strains MRSA, *Enterococcus faecalis* and *Streptococcus pneumoniae*. Promising activity against Gram-(-)ve strains was not observed. Their activity against Gram-(+)ve strains are discussed in this section. (The complete data set is provided in Appendix G).

5.3.4.1 Tetramates synthesised via SM-cross coupling

Antibacterial activity of tetramates **175-187** synthesised via SM-cross coupling was evaluated and MIC values of selected examples are represented in Figure 5.12. Their activity is compared with that of **109** where R=H.

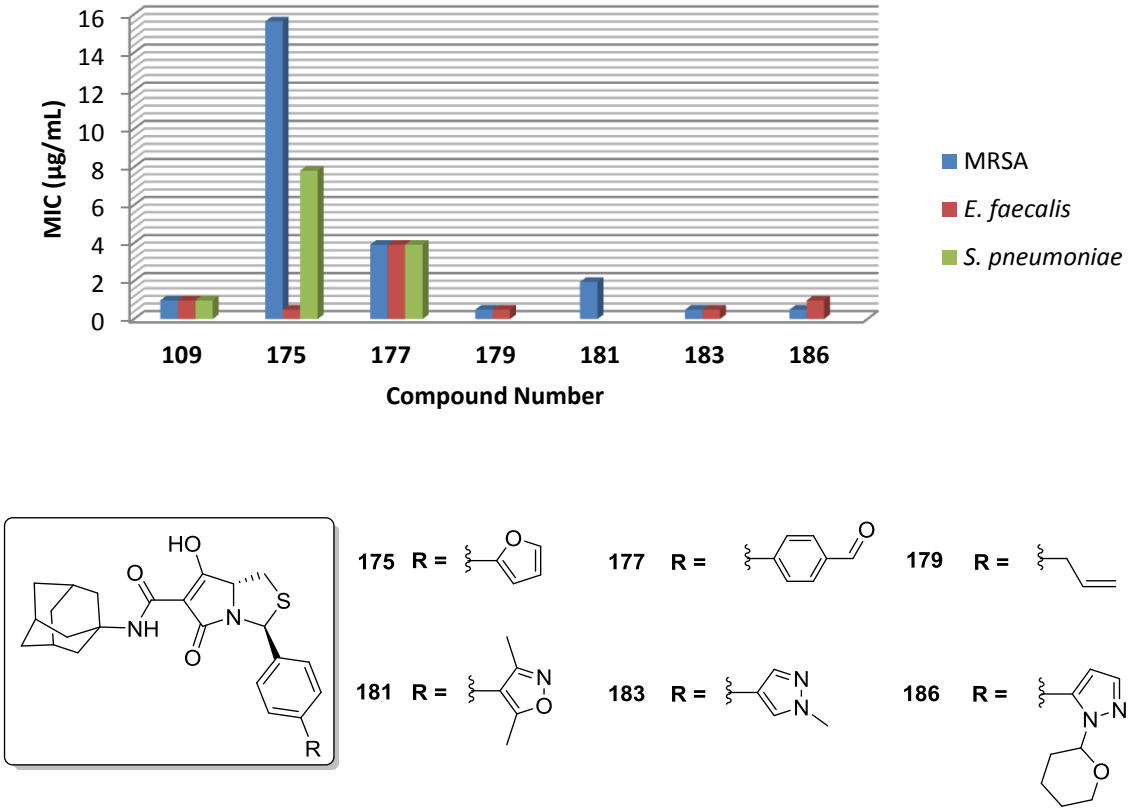


Figure 5.12. MICs of tetramates synthesised via SM cross-coupling. Activity reported against Gram-positive strains MRSA, *Enterococcus faecalis* and *Streptococcus pneumoniae* (MIC against *S. pneumoniae* for compounds **179-186** are not available) and compared with **109** (R=H).

Despite the lack of activity of carboxamide tetramates against Gram-(-)ve bacterial strains, potent antibacterial activity was observed against Gram-(+)ve strains MRSA, *Enterococcus* and *Streptococcus pneumoniae*. Compounds **179**, **183** and **186** displayed improved antibacterial activity against MRSA while **175**, **179** and **183** showed improved activity against *E. faecalis* compared to **109**.

5.3.4.2 Tetramates with pendant glycones

Antibacterial activity of glycosylated tetramates were evaluated against both Gram-(+)ve and Gram-(-)ve strains. While these compounds were either inactive or only weakly active (MIC

= 250 µg/mL) against Gram-(-)ve strains tested, they displayed potent antibacterial activity against Gram-(+)ve strains MRSA, *Enterococcus faecalis* and *Streptococcus pneumoniae*.

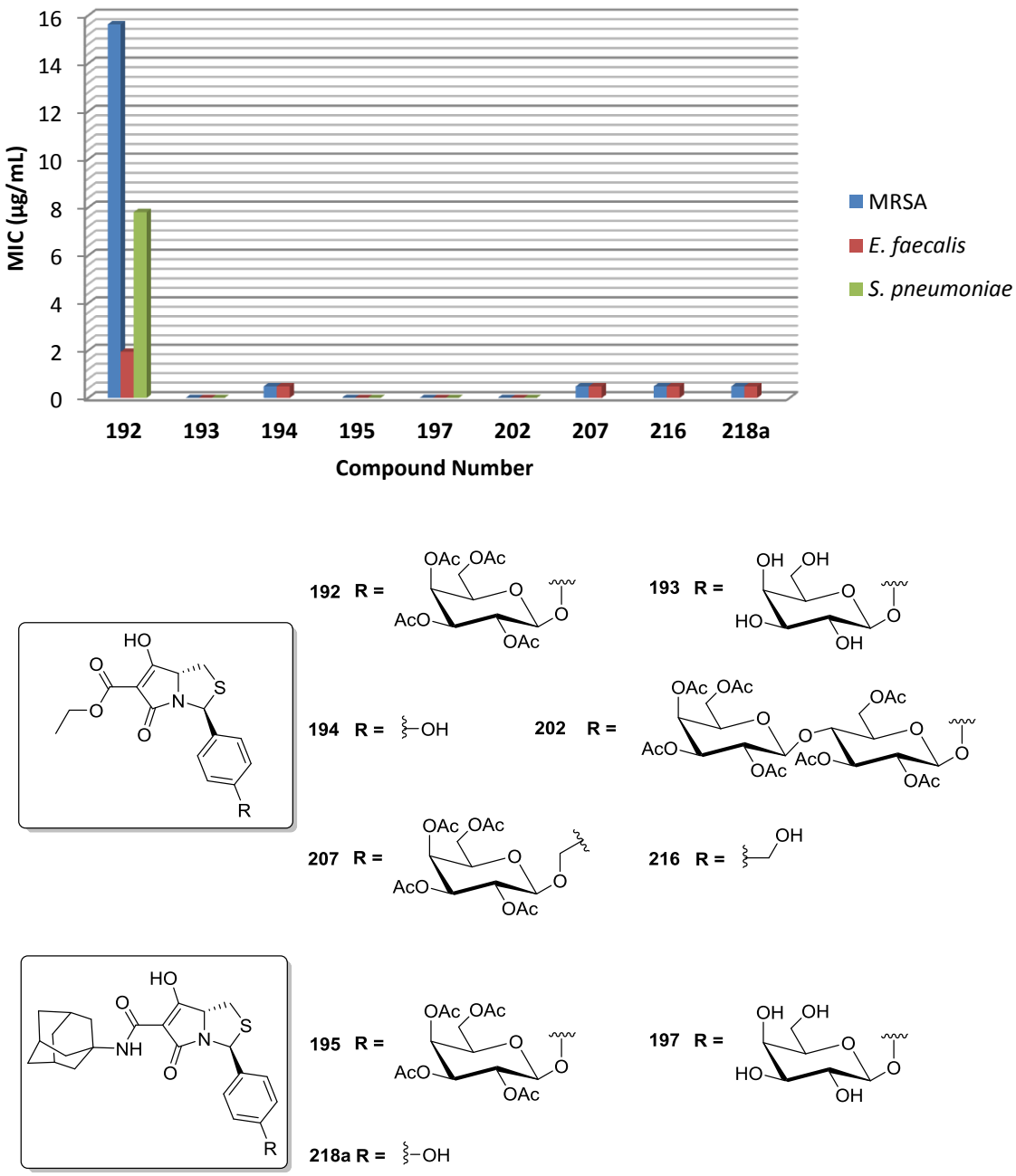


Figure 5.13. Antibacterial activity of glycosylated tetramates and their aglycone analogues (MIC against *S. pneumoniae* for compounds **194**, **207-218a** are not available).

Contrary to the trend in antibiotic activity observed for other tetramate esters **90a-95a** (represented in Figure 5.9, page 139, *vide supra*), tetramate ester **192** proved to be more active than its adamantyl analogue **195**. While **192** showed good antibacterial activity against Gram-

(+)ve strains, carboxamide **195** was inactive against all three strains. Further, increasing the polarity at C-2 as in **193** via deacylation of **192** conferred no bioactivity. Introduction of a disaccharide analogue also led to the complete loss of antibiotic activity of tetramate ester **202**. Further, it was interesting to note that the aglycone analogue **194** was slightly more potent than the glycosylated tetramate **192**. A similar trend was observed for carboxamide tetramate **195** where its aglycone analogue **218a** showed a remarkable improvement in activity.

In the case of glycosyl tetramate **207**, in which an acylated galactose is bound to the tetramate via a benzyl ether linkage, improved antibiotic activity was observed compared to **192**. Both **207** and its aglycone analogue **216** displayed similar levels of antibacterial activity.

Even though the exact role of the glycosidic residue is not known to us, it is postulated that it may assist in improving bioavailability, modulating toxicity and bacterial target interaction.[100] However, the important conclusions derived from glycosylated tetramate esters is that the presence of a glycone does not lead to a significant compromise in bioactivity as has been demonstrated for **192** and **207**. However, a significant increase in polarity at the C-2 end leads to a detrimental reduction in the antibiotic activity as observed with **193**.

Converting tetramate ester **192** to its carboxamide analogues **195** (data in Figure 5.13, page 145, *vide supra*) and **196** (data in Appendix G) led to a loss in activity. However, the absence of the glycone moiety in these carboxamide tetramates restored antibacterial activity as observed for **218a**, indicating that the presence of a glycone is not favourable for antibiotic activity of carboxamide tetramates. This outcome for amides is in contrast to that observed for tetramate esters.

5.3.4.3 Tetramates via Williamson ether synthesis

The synthetic feasibility of tetramates with phenolic substitution at C-2 and their etherification was discussed in Chapter 4, Sections 4.3-4.4 (page 100-104). The results from the evaluation of their antibacterial activity are reported herein with promising activity against Gram-positive strains and are compared with tetramate **218a**.

Etherification of **218a** with ethyl bromoacetate to give **219** resulted in a 64-fold reduction in antibacterial activity. Ethyl ester hydrolysis of **219** to give carboxylic acid **221** (Section 4.4, page 104, *vide supra*) resulted in a further 8-fold reduction in potency (MIC of **221** against MRSA was 250 μg/mL). Etherification of **218a** with 1-bromo-2-butyne afforded **220** without much compromise in antibiotic activity. It was also interesting to note that replacement of the adamantyl side chain with either phenyl (**218b**) or tetrahydropyranyl (**218c**) groups led to a reduction in potency, with no antibacterial activity being observed for the latter.

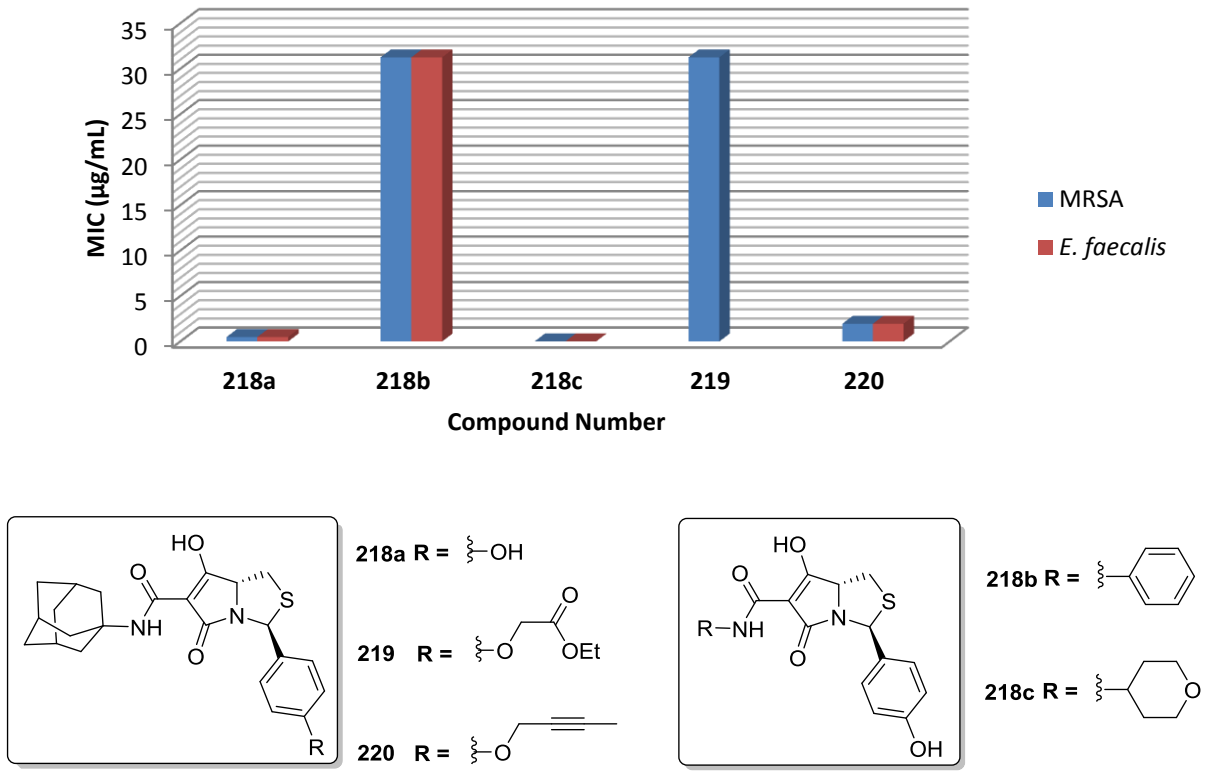


Figure 5.14. Antibacterial activity of tetramates **218-220** (MIC against *Enterococcus faecalis* for compounds **218c** and **219** are not available).

5.3.4.4 Tetramates with lactams, amines and isoxazoline

Tetramates with lactams derived from the pyroglutamate core and lactams and amines derived from Buchwald amination reactions were discussed in Sections 4.5-4.6 (page 105-111). The synthesis of isoxazoline via 1,3-dipolar cycloaddition of nitrile oxide-alkene was discussed in Section 4.7.1 (page 113-115). The synthesis of tetramates with β-lactams derived from 6-APA to generate hybrid systems was discussed in Section 4.8.2 (page 118-123). In this section, results from the biological evaluation of these compounds for antibacterial activity are presented.

5.3.4.4.1 Tetramates with lactams/amines derived from pyroglutamate and Buchwald C-N cross-coupling reactions

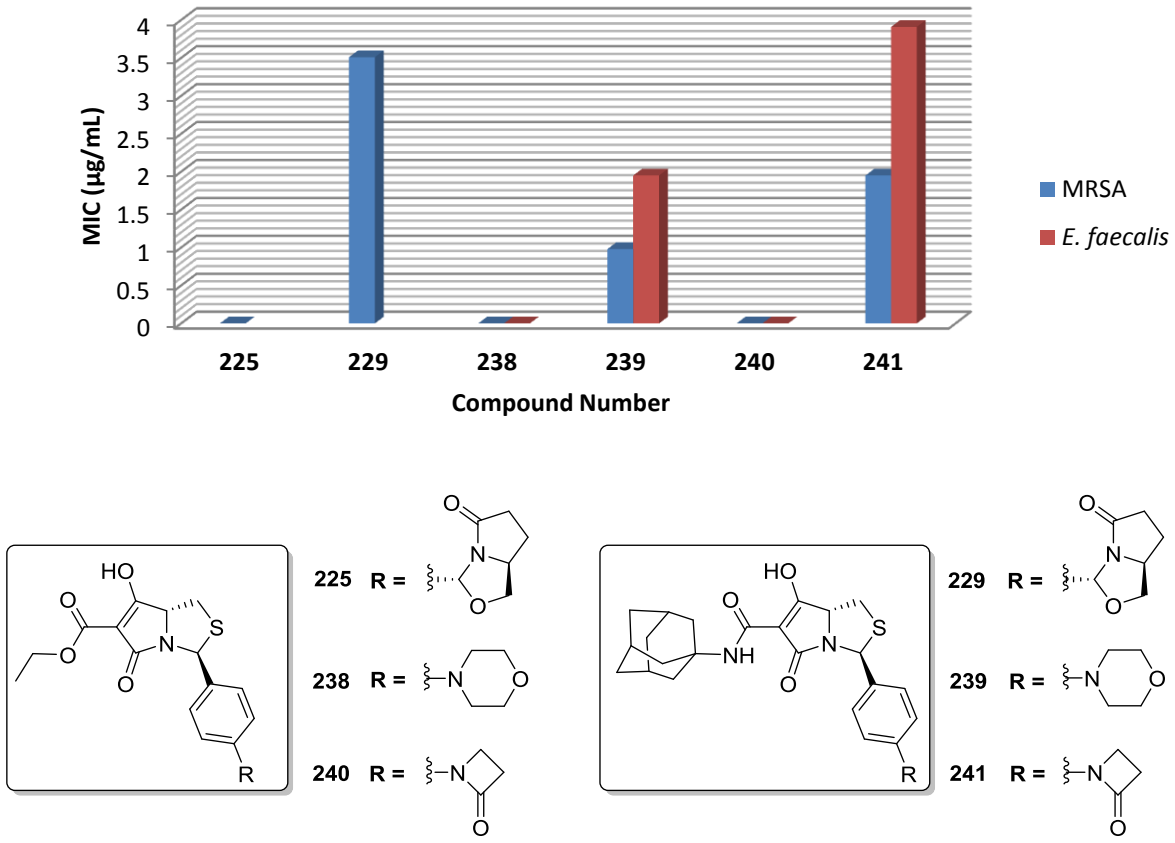


Figure 5.15. Antibacterial activity of tetramates **225** and **229** derived from pyroglutamic acid and **238-241** derived from Buchwald C-N cross-coupling (MIC against *Enterococcus faecalis* for compounds **225** and **229** are not available).

In general, carboxamide tetramates **229**, **239** and **241** with the adamantyl side chain showed good antibacterial activity when compared to their tetramate esters which showed no activity against the Gram-(+)ve bacterial strains tested. This observation is in agreement with the general trend observed for tetramate esters and their carboxamide analogues, highlighting the need for a bulky, hydrophobic C-7 pendant group. Of the carboxamides, morpholine substituted tetramate **239** was the most active while pyroglutamate substituted tetramate **229** was the least potent.

5.3.4.4.2 Tetramates with isoxazoline and its precursors

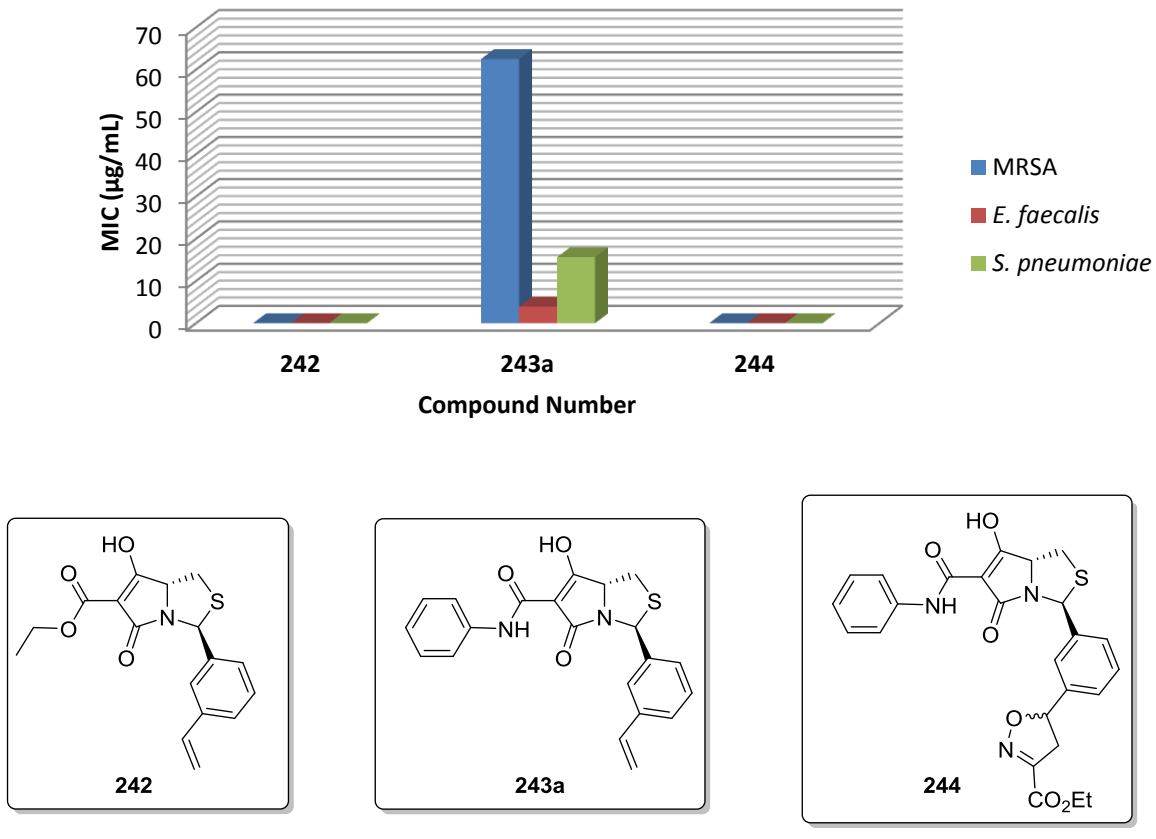


Figure 5.16. Antibacterial activity of cycloadduct **244** via 1,3-dipolar cycloaddition and its precursors against Gram-positive strains MRSA, *Enterococcus faecalis* and *Streptococcus pneumoniae.*

Tetramate ester **242** exhibited no antibacterial activity against all three Gram-(+)ve strains listed in Figure 5.16. Upon aminolysis, improved biological activity was observed for **243a**. Cycloaddition of nitrile oxide to **243a** generated isoxazoline **244** with compromised antibacterial activity.

5.3.4.4.3 Tetramate-penicillanic acid hybrids and their precursors

The synthesis of tetramate-penicillanic acid hybrid system derived from 6-APA was outlined in Section 4.8.2 (page 118-123). Compounds **248**-**251** were assayed against Gram-(-)ve strain *E. coli* and Gram-(+)ve strains MRSA and *Enterococcus faecalis*. No antibacterial activity was observed for both hybrids and precursors. Thus, hybridizing the tetramate core with a known antibiotic did not lead to improved efficacy *in vitro*. However, this conclusion is derived bearing in mind that it was the tetramate ester used for hybridization and not a carboxamide analogue, whereas carboxamides generally possess better biological activity as discussed above.

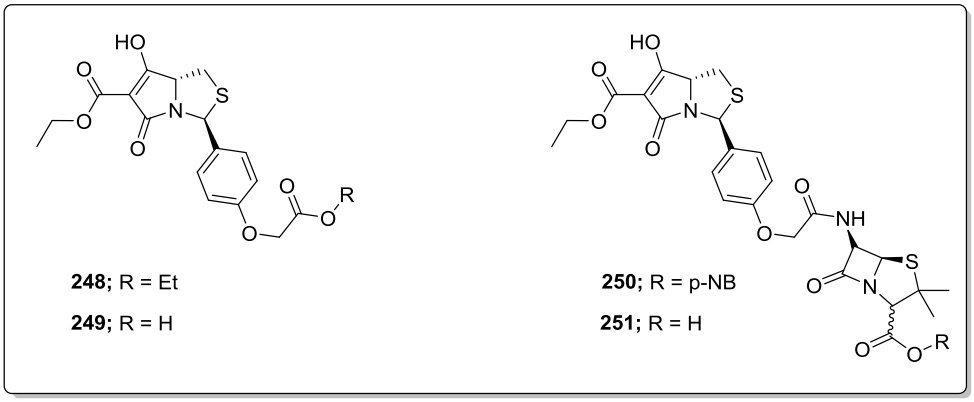


Figure 5.17. Tetramate-penicillanic acid hybrids (**250**, **251**) and their precursors (**248**, **249**).

5.3.4.5 *N,S*-Acetal deprotected carboxamide tetramates

Antibacterial activity of *N,S*-acetal deprotected tetramate was evaluated against *S. aureus* and *E. coli* using the hole-plate method. The activity of compound **252** was also tested in the

presence of HSA. The data obtained are represented in Figure 5.18 and compared with tetramate **109**.

It is evident that in the absence of a C-2 substituent, antibacterial activity of tetramate **252** against Gram-positive *S. aureus* is significantly reduced. To obtain a similar zone of inhibition to that of **109**, a 1000-fold increase in concentration of **252** was necessary. Interestingly, upon deprotection, an increase in activity against Gram-negative *E. coli* could be observed. At a concentration of 4 mg/mL, **252** showed activity, while **109** was completely inactive against *E. coli*. It is noteworthy that *N,S*-acetal deprotected tetramate **252** does not exhibit significant binding to albumin as its antibacterial activity is retained when treated with HSA. This improvement in activity observed for *E. coli* could be due at least in part to its increased polarity, which in turn leads to increased bacterial cell membrane permeability.

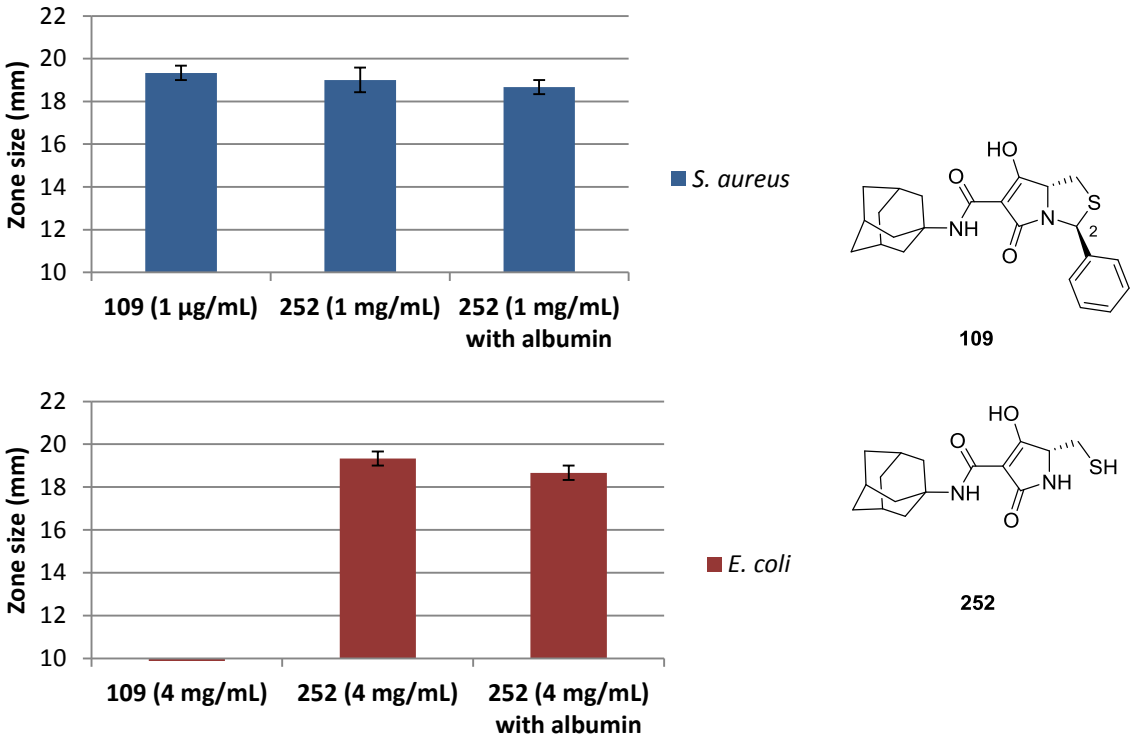


Figure 5.18. Antibacterial activity of tetramate **252** with/without HSA compared with **109**.

5.4 Physicochemical properties and antibacterial activity

Leeson and co-workers have demonstrated that the physicochemical properties of antiinfectives deviate from other classes of drugs, where they possess a higher mean molecular weight and polarity.[152] This is probably due to the fact that an antibiotic needs to exert its effect on non-mammalian systems coupled with cell wall penetration of target cells. Physicochemical properties of antibacterial drugs were further categorised by O'Shea and Moser, based on the cell wall structure of bacteria; Gram-positive or Gram-negative, as listed in Table 5.4.[150]

Table 5.4. Average physicochemical properties of antibacterial drugs by targeted bacterial cell type. Adapted with permission from O'Shea, R.; Moser, H. E. *J. Med. Chem.* **2008**, *51* (10), 2871–2878. Copyright (2008) American Chemical Society.

Parameter	Only Gram-positive activity	Only Gram-negative activity
MW	813	414
clogP	2.1	-0.1
$clogD_{7.4}$	-0.2	-2.8
rel. PSA	30	42

The cell walls of Gram-negative bacteria contain an outer cell membrane in addition to peptidoglycan layer and the inner membrane, which acts as barrier for cell wall penetration by small molecules. Membrane proteins (porins) serve as entry routes for compounds with certain size exclusion limits. These protein channels are water-filled and consequently facilitate the uptake of hydrophilic compounds. Thus, antibiotics targeting Gram-negative strains are generally more hydrophilic as represented in the lower clogP/$clogD_{7.4}$ values and higher rel. PSA.[150]

For the comparison of physicochemical properties of the tetramate compound library discussed in this thesis and their antibacterial activity, 'activity' was defined as MIC of ≤ 8 μg/mL[150] against at least one Gram-(+)ve bacterial strain. A plot of MW, $clogD_{7.4}$ and rel.

PSA is represented in Figure 5.19. clogD$_{7.4}$ was used as a descriptor of lipophilicity of a molecule over clogP, as tetramic acids would be ionized under physiological pH.

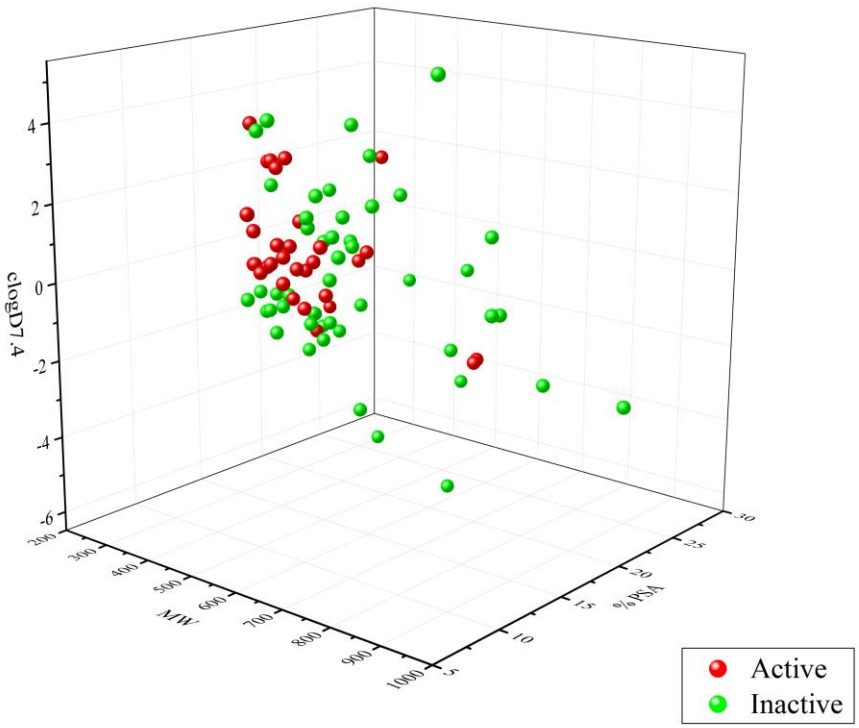


Figure 5.19. Correlation of physicochemical properties with activity. Plot of MW, clogD$_{7.4}$, and rel. PSA. Calculator Plugins were used for physicochemical and structure property prediction and calculation, Marvin (16.4.18.0), 2016, ChemAxon (http://www.chemaxon.com). 'Activity' is defined as MIC of ≤ 8 μg/mL against at least one Gram-(+)ve bacterial strain. All compounds were either inactive or weakly active (MIC>125 μg/mL) against Gram-(-)ve strains and are not represented in this plot.

With reference to Figure 5.19, compounds that are active occupy physicochemical space with MW of 300-600, clogD$_{7.4}$ of -2.5 to 4 and rel. PSA of 11-22 %, while 'active' tetramate esters **192** and **207** with glycone pendants appear as outliers due to higher MW in the region of 650-670. Compounds that are most hydrophilic (that is, with lower clogD$_{7.4}$ and higher rel. PSA) did not exhibit antibacterial activity against Gram-positive strains. Despite the mean MW of known Gram-positive antibacterial agents being around 800,[150] for tetramates, such high MW led to compromised activity. It is interesting to note that, even though several polar tetramates that fit in to the chemical space of antibacterials with Gram-negative activity were

synthesised, the expected activity was not observed. This possibly suggests that even if these compounds were successful in penetrating the cell wall, they could be expelled from the cell via efflux pumps or that these compounds do not show specific bacterial enzyme inhibition or other mode of action for a phenotypic outcome. Nevertheless, antibacterial activity of *N*,*S*-acetal deprotected tetramate **252** observed against *E. coli* suggests a possible route towards improved Gram-negative activity of tetramates. While the physicochemical properties of known antibiotics differ vastly, the tetramates synthesised as discussed in this thesis show close resemblance to fluoroquinolones, Figure 5.20.

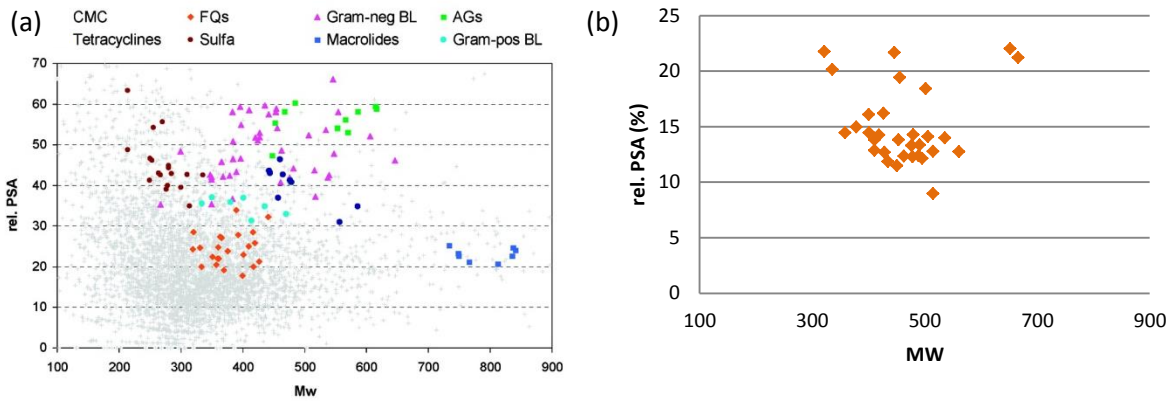


Figure 5.20. (a) Plot of rel. PSA (%) vs MW for major classes of antibacterials, (b) Plot of rel. PSA (%) vs MW for active tetramates. CMC=comprehensive medicinal chemistry, FQ=fluoroquinolones, BL=β-lactams, AG=aminoglycosides. Part (a) is reprinted with permission from O'Shea R. Moser, H.E., *J. Med. Chem*, **2008**, *51 (10)*, 2871-2878. Copyright (2008) American Chemical Society.

Lipinski's Rule of Five (Ro5) provides a guideline for oral drug-likeness of compounds. It states that values of clogP<5, MW<500, H-bond acceptors<10 and H-bond donors<5 lead to improved passive absorption or permeation. If a compound fails to meet two or more of these requirements, the Ro5 is violated.[151] As tetramates with antibacterial activity also possess desired physicochemical properties stated in Lipinsky's Ro5, these compounds have the potential to be developed as oral drug candidates (the complete data set for physicochemical properties is provided in Appendix J).

Lovering and co-workers introduced Fsp^3 (Fsp^3 = sp^3 hybridized carbon count/ total carbon count) as a descriptor of complexity of a molecule.[163] Greater three-dimensionality results in greater specificity and selectivity, thereby reducing undesired off-target interactions of molecules. Thus, increased complexity could be used to reduce toxicity of drug candidates.[164] In their study, Lovering and co-workers have demonstrated that complexity measured in Fsp^3, increased for molecules in clinical progression and drugs have an average Fsp^3 of 0.47.[163] This study was complemented by Ritchie and co-workers where an increase in aromatic rings led to increased compound attrition.[165] Thus, introducing complexity as an inherent part of the scaffold can be a promising starting point.[164] As more complex molecules can be made with increased saturation, more diverse chemical space can be accessed, and this can lead to improved chances of finding antibacterial compounds. In this regard, the bicyclic tetramate core can be considered an attractive scaffold in library generation, where Fsp^3 of the tetramate scaffold in Figure 5.21 is 0.43.

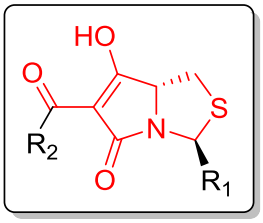


Figure 5.21. Bicyclic tetramate core (Fsp^3 = 0.43).

For the biologically active tetramates for which cytotoxicity against HaCaT mammalian cell line was determined (in collaboration with Sealife Pharma), the mean Fsp^3 calculated for tetramates with low toxicity was higher than those with higher toxicity levels, Figure 5.22. While some compounds showed >60-fold antibacterial activity over cytotoxicity, for the purpose of this comparison, compounds with low toxicity were defined as those that possess ≥10-fold[161] antibacterial activity (against at least one bacterial strain) over cytotoxicity.

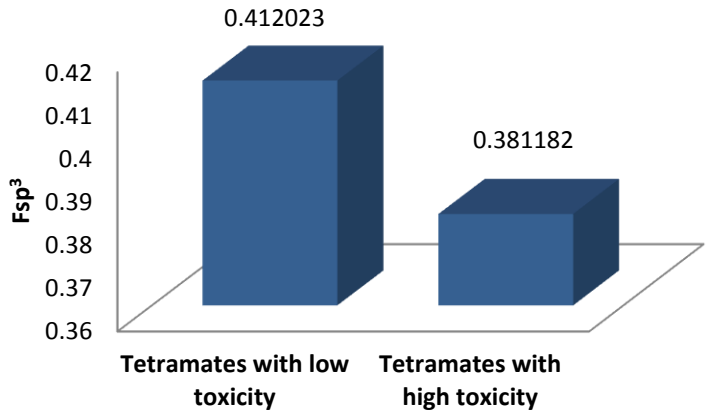


Figure 5.22. Mean Fsp^3 values for tetramates categorized into two classes as 'low toxicity' and 'high toxicity' based on toxicity observed against HaCaT mammalian cell line. Compounds with low toxicity are defined as those that possess ≥10-fold antibacterial activity (against at least one bacterial strain) over cytotoxicity, while compounds with high toxicity are defined as those that possess <10-fold antibacterial activity over cytotoxicity. Fsp^3 is a descriptor of complexity of a molecule, where a higher Fsp^3 value suggests higher molecular complexity, (Fsp^3 = sp^3 hybridized carbon count/ total carbon count). The graph depicts that tetramates with lower toxicity have a higher mean Fsp^3 value and thus, higher molecular complexity.

Electrophilic compounds are generally considered a liability in the drug discovery paradigm due to their potential for covalent off-target interactions that can lead to drug-related toxicity even though covalent drugs are being widely used.[166] Thus, compounds with electrophilic functional groups such as those with Michael acceptors are disfavoured as they are prone to nucleophilic displacement that can lead to alkylation of proteins, DNA and also react with non-specific thiols such as glutathiones, leading to toxicity.[166] Since the tetramates discussed herein contain such α,β-unsaturated carbonyl functional groups in the tetramate core that can act as Michael acceptors, they could be susceptible to unintended nuceophilic attack. Nevertheless, in a preliminary evaluation, no reaction of tetramate **113** with benzyl amine or L-cysteine methyl ester was observed under ambient conditions and further investigation would provide valuable information on their potential for off-target interactions.

5.5 Mode of action of tetramates

Naturally occurring tetramates such as kibdelomycin **12** and amycolamycin **13** are inhibitors of bacterial type II topoisomerases; topoisomerase IV and DNA gyrase.[47,50] Streptolydigin **14** and tirandamycin **15** are inhibitors of RNA polymerase (RNAP).[51,52] In recent work by Moloney and co-workers, tetramates synthesised have shown inhibition of both RNAP and undecaprenyl pyrophosphate synthase (UPPS).[66]

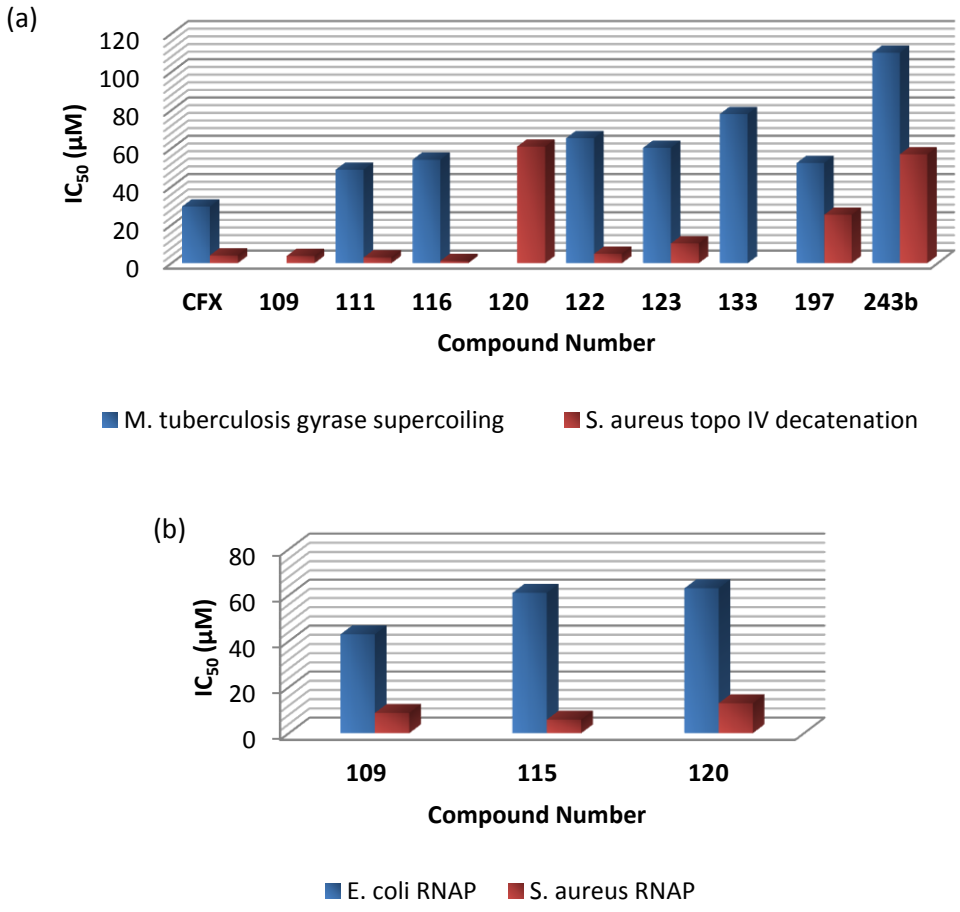
While some tetramates from the compound library discussed in this thesis exhibited promising biological activity in a phenotypic screening such as whole-cell bioassays, it was also interesting to elucidate possible modes of action that could bring about such a phenotypic outcome. In this regard, inhibition of possible bacterial target enzymes by some bicyclic tetramates was studied for *S. aureus* topoisomerase IV and RNAP. Further enzyme inhibition was also studied for *E. coli* gyrase, *E. coli* RNAP and *M. tuberculosis* gyrase. However, inhibition of *E. coli* gyrase was not observed and the data for inhibition of other enzymes are discussed below for selected examples.

Bacterial topoisomerase IV (topo IV) and DNA gyrase are enzymes that are essential to the cell. The primary function of topoisomerase IV is the decatenation of daughter chromosomes that are multiply linked, during the final stages of DNA replication.[156] The role of DNA gyrase is catalysis of negative supercoiling of DNA during DNA replication.[167]

The compounds discussed herein were initially tested at a fixed concentration of 100 μM for the inhibition of DNA gyrase and topo IV enzymes. The percentage of DNA supercoiled or decatenated in the presence of each test compound was calculated. A lower percentage of supercoiling or decatenation indicated a higher level of inhibition of the target enzymes. The data were compared with that of a known inhibitor of type II topoisomerase, ciproflaxacin, which is a fluoroquinolone. Of the compounds assayed, IC_{50} values of some of the more

potent inhibitors were determined and are represented in Figure 5.23 (a). (Topo IV and gyrase enzyme inhibition studies were conducted in collaboration with Prof. A. Maxwell at John Innes Centre and Inspiralis Limited, Norwich research park. The complete data set is provided in Appendix H).

Tetramates **109**, **115** and **120** were tested for their inhibition of *S. aureus* and *E. coli* RNAP, an essential enzyme for transcription. Bacterial RNAP is an attractive target in antibiotic chemotherapy as the bacterial RNAP subunit sequences are highly conserved while being different to that of eukaryotic RNAP subunit sequences. This permits broad-spectrum activity and therapeutic selectivity.[168] The data obtained for RNAP enzyme inhibition are represented in Figure 5.23 (b). (Data for RNAP inhibition were obtained in collaboration with Dr. Richard H. Ebright at Waksman Institute of Microbiology, Rutgers University).



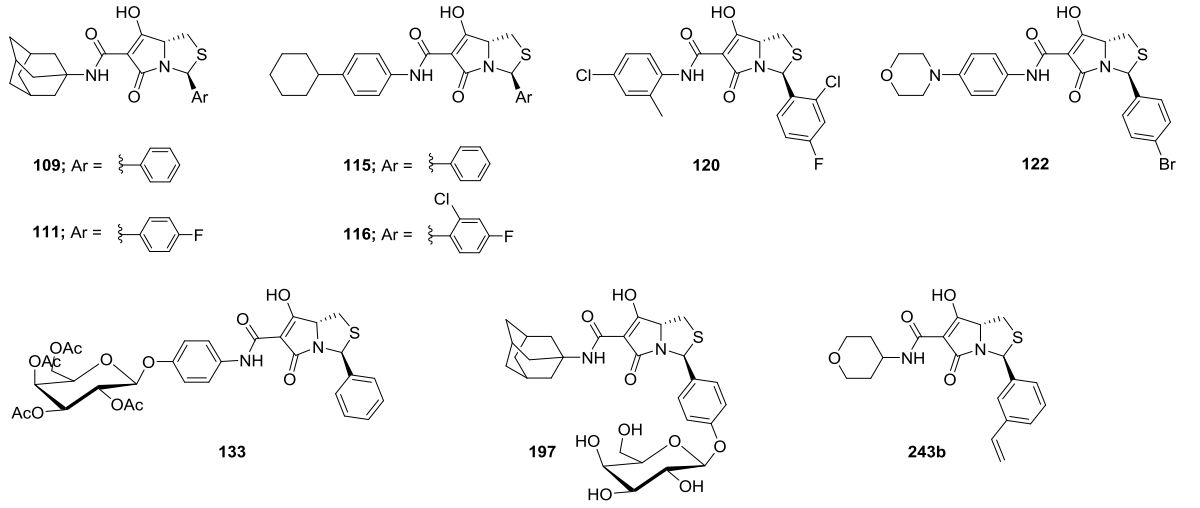


Figure 5.23. (a) IC_{50} of tetramates against *M. tuberculosis* gyrase and *S. aureus* topoisomerase IV. CFX = ciproflaxacin. Compounds **109** and **120** were weak inhibitors of *M. tuberculosis* gyrase and **133** was a weak inhibitor of *S. aureus* topo IV (IC_{50} not determined). (b) IC_{50} of tetramates against *S. aureus* RNAP and *E. coli* RNAP.

A comparison of MICs of tetramates against MRSA and IC_{50} obtained for the inhibition of *S. aureus* RNAP and topo IV indicates that the compounds that are the most potent *in vitro* also exhibit high efficacy in enzyme inhibition, suggesting that the inhibition of RNAP or topo IV are possible modes of action that inhibit bacterial growth as observed in whole-cell bioassays. Further, compounds **109**, **115** and **120** were inhibitors of both *S. aureus* topo IV and RNAP enzymes, which is indicative of a dual mode of action. However, not all compounds that showed high efficacy in enzyme inhibition assays demonstrated high efficacy in whole-cell assays as observed for compounds **133**, **197** and **243b**, possibly due to low cell permeability or elimination via efflux mechanisms, leading to reduced bioavailability in the bacterial cell. The same could be said for compounds **109**, **115** and **120** which inhibit *E. coli* RNAP but do not show antibacterial activity against *E. coli* in whole-cell bioassays. In general *E. coli* RNAP proved to be less sensitive to tetramates than *S. aureus* RNAP. Similarly, tetramates were stronger inhibitors of *S. aureus* topo IV than *M. tuberculosis* gyrase, except in the case of **133**, which was a stronger inhibitor of *M. tuberculosis* gyrase.

5.6 Binding to Human Serum Albumin (HSA)

Human serum albumin is a predominant protein in plasma with a blood concentration of about 700 μM.[157] It acts as a depot for a range of compounds which can be beneficial for the transportation of those compounds with poor solubility in plasma. Binding of antibiotics to HSA is considered undesirable as this would lead to a decrease in free drug concentration in blood, compromising their efficacy.[158,161] Thus, optimizing drug design to minimize protein binding is recommended.[158] However, reports that contradict the need for minimized protein binding have emerged where it is argued that attempts to optimize physicochemical properties of drugs to reduce binding to HSA can result in an increase in clearance rate of drugs from the body, thereby reducing the residence time of the drug.[169,170] Therefore, high plasma protein binding (PPB) need not be used as a sole criterion in compound selection. Nevertheless, determining PPB of drug candidates is still of interest in understanding their potency in plasma.[169] In this regard, equilibrium dialysis and ultrafiltration are frequently used *in vitro* methods of determining total PPB of antibiotics.[171] While the interaction of a drug with HSA could either be due to hydrophobic effects or site-specific target interactions, it is the latter that can be pharmacologically challenging, especially one that involves covalent binding.[158]

Section 5.3.2 (page 136-138, *vide supra*) included a study of the antibacterial activity of tetramates in the presence of HSA using the hole-plate method. In the section that follows, the quantification of site specific interaction of tetramates using a NMR spectroscopy-based approach will be discussed.

5.6.1 NMR spectroscopy-guided approach to evaluating site specific plasma protein binding: a ligand displacement assay

HSA is made up of three domains I, II and III and each domain comprises of two subdomains A and B.[157] Sudlow and co-workers have identified two specific binding sites on HSA for

acidic drugs, namely, site I (in domain IIA) and site II (in domain IIIA).[172,173] To probe the binding of tetramates to HSA, the competitive displacement of the site II-selective albumin binding amino acid L-tryptophan (L-Trp)[157,174,175] was examined and quantified by NMR spectroscopy (unpublished data; Moloney and co-workers).

The experiments were run using Car-Purcell-Meiboom-Gill (CPMG) acquisition technique that gives an enhanced S/N ratio of an NMR spectrum. As a result, interference from protein signals in the ^{1}H NMR could be eliminated. HSA and L-Trp were used in equimolar quantities (100 μM) where complete binding of L-Trp to HSA was observed. The 1:1 HSA:L-Trp solution was treated with the competitor ligand (tetramate) in 10-fold excess, and the displaced L-Trp was quantified. An example of a NMR spectrum obtained for the displaced L-Trp is given in Figure 5.24.

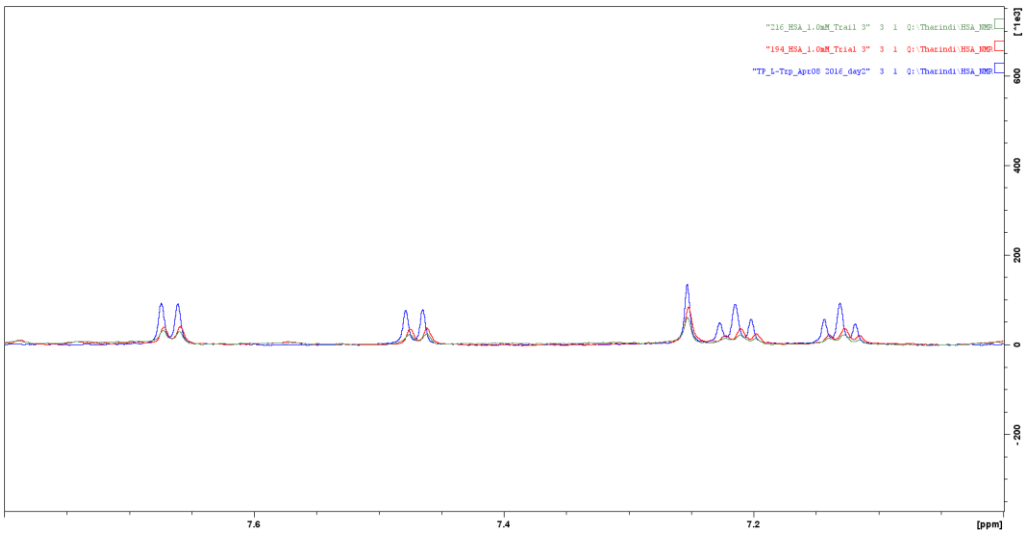


Figure 5.24. NMR spectrum obtained for the displacement of L-Trp by **194** and **216** (Blue = L-Trp only, Red = **194**, Green = **216**)

Figure 5.25 gives a graphical representation of the % displacement of L-Trp by bioactive tetramates tested at 1.0 mM.

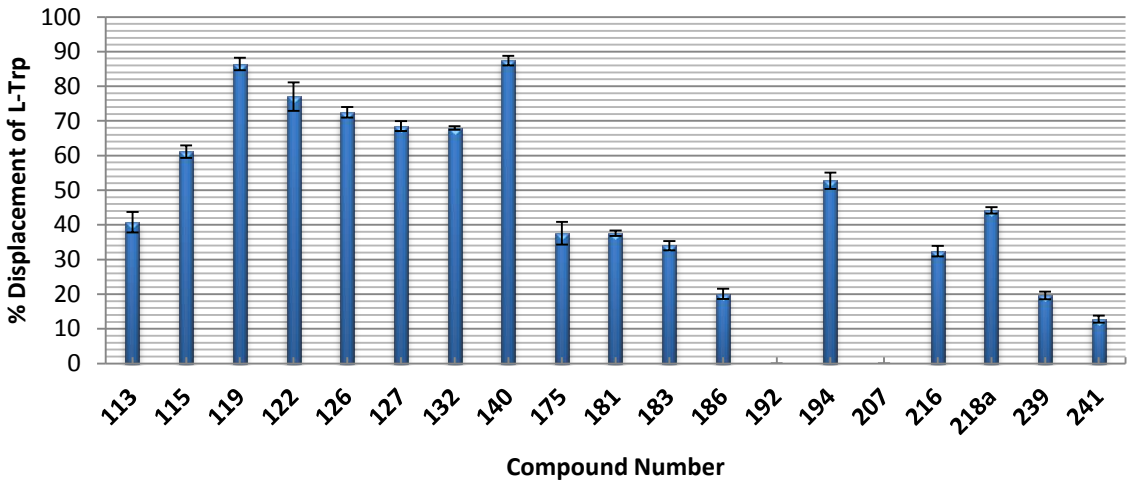


Figure 5.25. Displacement of L-Trp by tetramates (concentration of HSA=100 μM, L-Trp=100 μM, tetramate=1.0 mM).

Compounds **192** and **207** did not displace L-Trp at 1.0 mM. Increasing their concentrations to 4.0 mM resulted in the displacement of the reporter ligand L-Trp with 28 % displacement observed for **192** and 7 % displacement observed for **207**, indicating their low affinity for site II of HSA.

In general, drugs with high lipophilicity are strong binders of HSA. Perola and co-workers, in their study of benzimidazole urea lead series, have identified that compounds with clogP<2.1 and PSA>120 Å bind strongly to HSA, as reflected in the shift in MIC values, known as the 'serum shift'.[176] Brown and co-workers have shown that compounds with clogD>2 tend to have significant serum shifts.[177] These conclusions indicate an increase in PPB with hydrophobicity. However, no apparent correlation was observed in a comparison of $clogD_{7.4}$ with % displacement of L-Trp for tetramates, Figure 5.26. In fact, compounds that were the most polar, did not show the least % displacement of L-Trp. This outcome was rather expected, as a comparison of physicochemical properties (clogP, clogD, PSA) relating to hydrophobicity of drugs and their PPB would provide an analysis of the possible binding due to hydrophobic effects. Since, in the ligand displacement assay discussed herein, where site-

specific binding is quantified, the specific binding interaction of tetramates with HSA would not correlate with clogD values. This is in agreement with a similar outcome reported for cephalosporin cefpiramide, which has a high serum shift despite its polarity.[177] Cephalosporins do not follow the general trend of increased PPB associated with hydrophobicity, but rather, site-specific interaction at site I of HSA.[178]

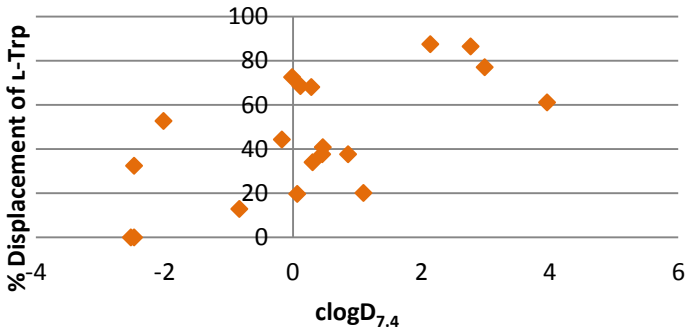


Figure 5.26. A plot of $clogD_{7.4}$ vs % displacement of L-Trp for tetramates.

In this ligand displacement assay, L-Trp is used as the reporter ligand, which binds with specificity to site II of HSA. Therefore, the data gathered is a representation of the affinity of tetramates to site II of HSA quantified by the competitive displacement of the said reporter ligand. Thus, a complete picture of the binding of tetramates to HSA; tetramates bound to site I of HSA or non-specific, off-target site interactions on HSA is not captured, providing scope for further development.

5.7 A brief note on proteasome inhibition activity of tetramates

The proteasome regulates protein degradation and removal of abnormal proteins in cells. Proteasome activity required for tumor cells is higher than that for normal cells, which makes malignant cells more sensitive to proteasome inhibition.[179,180] Thus, the ubiquitine-proteasome system (UPS) has been a target of anticancer therapy.[180,181] Interestingly,

tetramates with inhibition of 20S proteasome assembly have been reported by Izumikawa and co-workers.[179]

Carboxamide tetramates **109** and **120** from our compound library were tested for 20S proteasome inhibition and the data are represented in Figure 5.27, which indicates the potential of the bicyclic tetramate core in antitumor therapy.

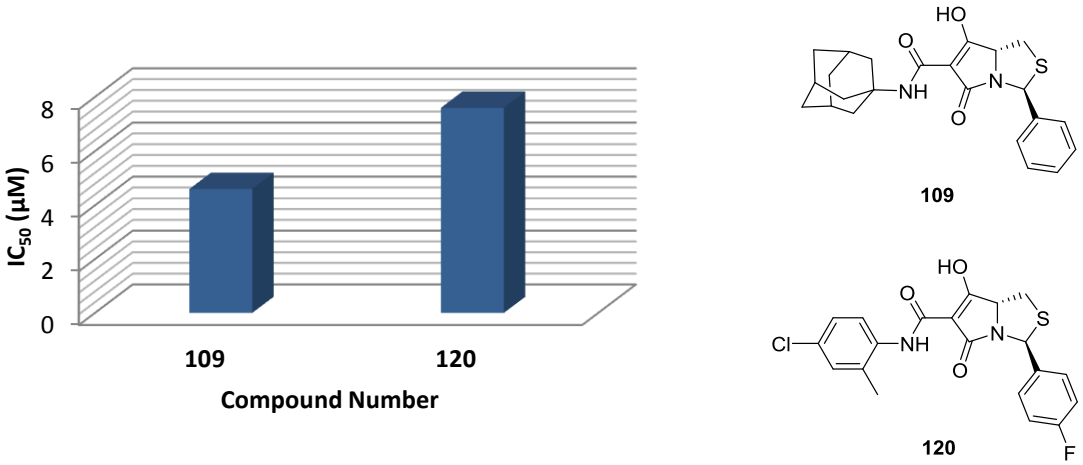


Figure 5.27. Proteasome inhibition by tetramates **109** (IC_{50} = 4.6 µM) and **120** (IC_{50} = 7.6 µM). (Determined in collaboration with Dr. Jetze J. Tepe, Department of Chemistry, Michigan State University).

5.8 Summary

In this chapter, the antibacterial activity of tetramic acids was discussed along with their mode of action and affinity to HSA.

Generally, tetramate esters were inactive against both Gram-positive and Gram-negative bacterial strains. The introduction of a carboxamide pendant group at C-7 of the tetramate core conferred antibacterial activity against Gram-positive strains. The exemption to this trend was the tetramate glycones, where the presence of a carboxamide pendant group led to compromised activity. The most active carboxamide tetramates comprised of a hydrophobic adamantyl group at C-7, while the introduction of more polar substituents led to reduced or no activity.

Antibacterial activity observed for various C-2 functionalized tetramates demonstrated the ability to accommodate a range of substituents while retaining antibacterial activity, providing scope for physicochemical property optimization. Carboxamide tetramates obtained via SM cross-coupling, glycosylation, etherification, pyroglutamate-derived lactams and Buchwald amination reactions provide such examples. Of interest was the bioactivity observed for galactose-derived glycosyl tetramate ester and their aglycone analogues, with this series of compounds being the only tetramates esters that demonstrated good antibiotic activity. However, it is noteworthy that incorporating very polar substituents can lead to detrimental reduction in activity as observed for **193** and **221**. Further, hybridization approach to drug discovery involving a tetramate ester and penicillanic acid core failed to generate a hybrid system with antibiotic activity. The bioactivity data obtained for **252** highlights the need for a C-2 pendant group for improved bioactivity against Gram-positive strains, while its absence is advantageous for Gram-negative activity. An analysis of physicochemical properties indicates that bioactive tetramates generally occupy physicochemical space with MW of 300-600, $clogD_{7.4}$ of -2.5 to 4 and rel. PSA of 11-22. With regards to cytotoxicity, compounds with low toxicity had a higher Fsp^3 value compared to those with high toxicity.

Results from enzyme inhibition assays suggest that *S. aureus* topo IV and RNAP are possible targets for tetramates, which could be the mode of action for the bacterial growth inhibition observed against MRSA. Further, tetramates also showed inhibition of *E. coli* RNAP and *M. tuberculosis* gyrase enzymes.

Evaluation of antibacterial activity of tetramates in the presence of HSA via the hole-plate method suggests possible binding of tetramates to albumin, as observed by the shift in sample test concentration although significant compromise was not observed in some others. Site-specific interaction of bioactive tetramates with HSA at site II was determined by a NMR-

based ligand displacement assay where glycosylated tetramates **192** and **207** showed the least affinity.

On an additional note, proteasome inhibition by tetramates highlights the potential of this tetramate scaffold to be developed for anticancer therapy.

CHAPTER 6 : EXPERIMENTAL

6.1 General techniques

^{1}H NMR spectra were recorded on Bruker DPX200 (200 MHz), AVIII HD 400 (400 MHz) or AVII 500 (500 MHz) spectrometers. Chemical shifts (δ_H) are reported in parts per million (ppm) and are referenced to the residual ^{1}H solvent peak. The abbreviations used to describe multiplicities are as follows; s (single), br. s (broad singlet), d (doublet), dd (double doublet), t (triplet), td (triple doublet), q (quartet), m (multiplet) and br (broad). Coupling constants (*J*) are given in Hertz (Hz). Two-dimensional COSY (correlation spectroscopy) spectra were obtained on Brucker AVIII HD 400 (400 MHz) or AVII 500 (500 MHz) spectrometers. 2D-NOESY and 1D-NOE experiments were performed on a Brucker AVII 500 (500 MHz) spectrometer.

^{13}C NMR spectra were recorded on a Bruker AVIII HD 400 spectrometer at 100.6 MHz or AVII 500 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 ^{13}C solvent peak. Assignment of carbon peaks was routinely made with the assistance of the results from DEPT-135 sequences and two-dimensional HSQC spectra obtained on Brucker AVIII HD 400 or AVII 500 and HMBC experiments recorded on Bruker AVII 500 spectrometers.

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

Low resolution mass spectra (*m/z*) were recorded on a Waters LCT Premier spectrometer using electrospray ionisation (ESI). High resolution mass spectra were recorded on a Bruker μTOF (ESI) spectrometer by the internal service at the Department of Chemistry, University

of Oxford. The *m/z* values of major peaks are reported in Daltons and their intensities given as percentages of the base peak.

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

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter or Jasco P-2000 polarimeter with a water jacketed 10 cm cell at the stated temperature (25 °C). Specific rotations are reported in 10^{-1} deg cm^2 g^{-1} and concentrations (*c*) in g/100 mL. Wavelength of light used was 589 nm.

The enantiomeric excess (e.e.) of the products were determined by chiral stationary phase HPLC in a Dionex P680 chromatograph with a Dionex UVD170U detector (Diacel Chiralpak AD-H)

Crystals for X-ray crystallography were grown from vapour diffusion of petroleum ether into a solution of the requisite compound in EtOAc at room temperature or from a solution of the requisite sample in MeOH at 4 °C.

Thin layer chromatography (TLC) was performed using Merck aluminium foil backed sheets pre-coated with 0.2 mm Kieselgel 60 $F_{254.}$ Product spots were visualized by quenching of UV fluorescence (λ_{max} 254 nm) or by staining with an aqueous solution of $KMnO_4$ followed by heating. Retention factors (R_f) are quoted to the nearest 0.01. Flash column chromatography was carried out using Merck Geduran Si 60 (40-63 μm) mesh silica gel.

Reaction times are recorded in minutes (min) or hours (h). Reactions were carried out in oven dried reaction flasks, using dry solvents, under inert (N_2) atmosphere. Solvents were evaporated at 40 ºC under reduced pressure on a Buchi RE 111 Rotavapour attached to a Vacuubrand CVC2 pump and pressure control system. 'Petroleum ether' refers to light petroleum ether of boiling point 40-60 ºC and was used as purchased from commercial

sources. All reagents were obtained from Sigma Aldrich, Alfa Aesar, Apollo Scientific or Acros Organics and used as obtained from suppliers.

6.2 Experimental for Chapter 2

Synthesis of tetramic acids were carried out according to published procedures. Racemic samples of compounds **79**, **80**, **82**, **85**, **86**, **88**, **91a**, **92a** and **94a** were prepared as for those from enantiopure starting material. (Compound characterization of racemic analogues are furnished in Appendix A).

6.2.1 General procedure: Esterification of L-serine and DL-cysteine[182]

To a suspension of the amino acid (1.0 eq) in MeOH (2 mL/mmol) at 0 °C , $SOCl_2$ (1.2 eq) was added drop-wise under continuous stirring and warmed to rt. The mixture was then heated under reflux for 1-3 h. The reaction mixture was concentrated *in vacuo* to obtain the respective amino ester.

6.2.2 General procedure: Synthesis of oxazolidines 73-74 [74]

L-Serine methyl ester hydrochloride (1.0 eq) was dissolved in methanol (5 mL/mmol). Aromatic aldehyde (1.0 eq) and Et_3N (2.0 eq) were then added and the mixture was stirred at rt for 2 h. The solvent was evaporated off and Et_2O (30 mL/mmol) was added to the residue and stirred for 30 min. The mixture was dried over Na_2SO_4, filtered and concentrated *in vacuo* to give oxazolidines **73-74** in high purity and was used without further purification in the subsequent step.

6.2.3 General procedure: Synthesis of thiazolidines 78-83 [64,75,183]

To L-cysteine methyl ester hydrochloride (1.0 eq) in petrol (25 mL/1 g), Et_3N (1.2 eq) and aldehyde (1.2 eq) were added. The mixture was heated under reflux, with continuous removal

of water using a Dean Stark apparatus, for 19 h. The reaction mixture was cooled to rt, filtered and washed with Et_2O. The combined filtrates were concentrated *in vacuo* and the residue was purified by silica gel flash column chromatography (eluent: EtOAc/petrol) to give the required thiazolidines **78-83** as an inseparable mixture of diastereomers.

6.2.4 General procedure: Synthesis of *N*-acylated oxazolidine 75 and *N*-acylated thiazolidines 84-89 [75,184]

A solution of ethyl hydrogen malonate (1.2 eq) in CH_2Cl_2 (2.5 mL/mmol) was added to a stirred solution of oxazolidine/thiazolidine (1.0 eq), DCC (1.2 eq) and DMAP (0.1 eq) in CH_2Cl_2 (5 mL/mmol) at 0 °C. The mixture was stirred at 0 °C for 15 min and then at rt for 15 h (for thiazolidines) or 4 h (for oxazolidine). The reaction mixture was filtered to remove dicyclohexylurea and the residue was washed with CH_2Cl_2. The combined filtrates were concentrated *in vacuo* and purified by silica gel flash column chromatography (eluent: EtOAc/petrol) to give *N*-acylated oxazolidine **75** and *N*-acylated thiazolidines **84-89**.

6.2.5 General procedure: Synthesis of bicyclic tetramates[75,185]

6.2.5.1 Synthesis of bicyclic tetramate 76

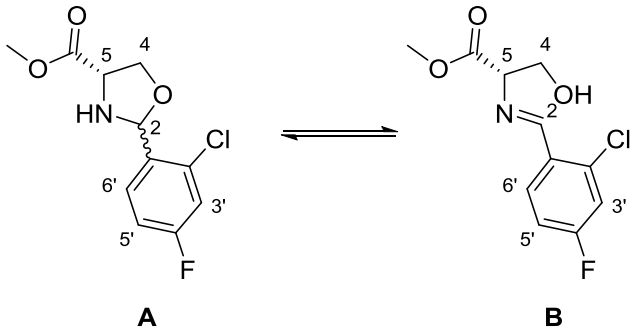
To a solution of the *N*-acylated oxazolidine (1.0 eq) in THF (5 mL/mmol), KO^{t}Bu (1.0 eq) was added and the mixture heated under reflux for 3 h. It was then cooled to rt and concentrated *in vacuo*. The crude was purified directly by silica gel flash column chromatography (eluent; EtOAc/MeOH/1 % Et_3N) to give **76**.

6.2.5.2 Synthesis of bicyclic tetramates 90a-95a

To a solution of the *N*-acylated thiazolidine (1.0 eq) in THF (5 mL/mmol), KO^{t}Bu (1.2 eq) was added and heated at reflux for 3 h. It was then cooled to rt and concentrated *in vacuo*. The crude residue was partitioned between Et_2O and water. The aqueous layer was separated,

acidified with 2M HCl (to pH 1) and extracted with EtOAc. The aqueous layer was re-acidified and re-extracted with EtOAc. The combined EtOAc extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (eluent: EtOAc/MeOH/1 % Et_3N). The product was then dissolved in CH_2Cl_2 and washed with 5% citric acid. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield the desired bicyclic tetramates **90a-95a**.

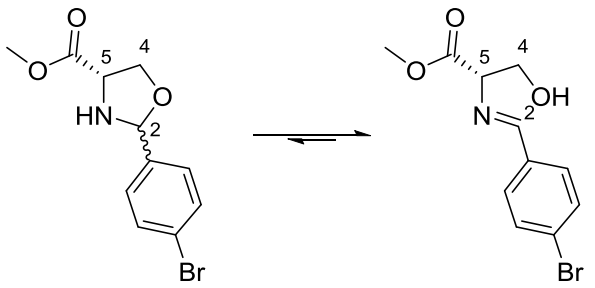
Methyl (2*RS*,5*S*)-2-(2-chloro-4-fluorophenyl)-1,3-oxazolidine-5-carboxylate (A) and Methyl (*E/Z*)-*N*-((2-chloro-4-fluorophenyl)methylidene)-L-serinate (B), 73



Yield (0.69 g, quant.); yellow solid; 1:1 ring (A) and open chain (B) tautomers. R_f = 0.28 (EtOAc/petrol 1:2); ν_{max}/cm^{-1} (neat) 1223 (C-F), 1736 (C=O) 3310 (OH/NH); Ring tautomer (A): 1.5:1 *cis* and *trans* diastereomers: δ_H (400 MHz, $CDCl_3$) major isomer (*cis*): 2.92 (1H, br. s., NH), 3.77 (3H, s, CO_2CH_3), 4.00 - 4.10 (3H, m, $H4_A$ + $H4_B$ + H5), 5.61 (1H, s, H2), 6.93 - 7.05 (1H, m, H5'), 7.08 - 7.13 (1H, m, H3'), 7.64 (1H, dd, *J* = 8.8, 6.1 Hz, H6'); minor isomer (*trans*): 2.92 (1H, br. s., NH), 3.78 (3H, s, CO_2CH_3), 3.89 (1H, dd, *J* = 8.2, 5.8 Hz, $H4_A$), 4.00 - 4.10 (1H, m, H5), 4.19 - 4.23 (1H, m, $H4_B$), 5.95 (1H, s, H2), 6.93 - 7.05 (1H, m, H5'), 7.08 - 7.13 (1H, m, H3'), 7.55 (1H, dd, *J* = 8.7, 6.2 Hz, H6'); δ_C (100.6 MHz, $CDCl_3$) major isomer (*cis*): 52.7 (CO_2CH_3), 59.8 (C5), 68.5 (C4), 89.7 (C2), 114.4 (d, *J* = 21.5 Hz, C5′), 116.9 (d, *J* = 24.6 Hz, C3′), 128.7 (d, *J* = 8.7 Hz, C6′), 131.4 (d, *J* = 4.0 Hz, C1′), 133.9 (d, *J* = 10.3 Hz, C2′), 162.5 (d, *J* = 251.1 Hz, C4′), 172.6 (CO_2CH_3); minor isomer (*trans*): 52.5 (CO_2CH_3), 59.0 (C5), 68.4 (C4), 89.2 (C2), 113.8 (d, *J* = 21.5 Hz, C5′), 117.1 (d, *J* = 24.6 Hz, C3′), 128.4 (d, *J* = 8.7 Hz, C6′), 133.0 (d, *J* = 3.2 Hz, C1′), 134.0 (d, *J* = 10.3 Hz, C2′), 162.4 (d, *J* = 253.5 Hz, C4′), 172.5 (CO_2CH_3); Open chain tautomer (B): δ_H (400 MHz, $CDCl_3$): 2.92 (1H, br. s., OH), 3.75

(3H, s, CO_2CH_3), 3.93 - 4.10 (2H, m, $H4_A$ + $H4_B$), 4.19 - 4.23 (1H, m, H5), 6.93 - 7.05 (1H, m, H5'), 7.08 - 7.13 (1H, m, H3'), 8.10 (1H, dd, *J* = 8.8, 6.4 Hz, H6'), 8.69 (1H, s, H2); δ_C (100.6 MHz, $CDCl_3$): 52.3 (CO_2CH_3), 63.6 (C4), 73.8 (C5), 114.7 (d, *J* = 21.5 Hz, C5′), 116.9 (d, *J* = 25.4 Hz, C3′), 130.3 (d, *J* = 9.5 Hz, C6′), 128.9 (d, *J* = 4.0 Hz, C1′), 136.5 (d, *J* = 10.3 Hz, C2′), 161.0 (C2), 164.1 (d, *J* = 255.9 Hz, C4′), 170.7 (CO_2CH_3); *m/z* (ESI^+) 260 ($[M+H]^+$ 100 %); HRMS (ESI^+); $C_{11}H_{12}O_3NClF$ $[M+H]^+$; found 260.04812, requires 260.04843.

Methyl (2*RS*,5*S*)-2-(4-bromophenyl)-1,3-oxazolidine-5-carboxylate (A) and Methyl (*E/Z*)-*N*-((4-bromophenyl)methylidene)-L-serinate (B), 74

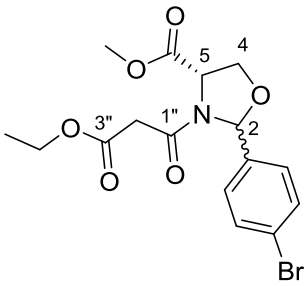


A **B**

Yield (0.76 g, 98 %); yellow oil; 1:1.5 ring (A) and open chain (B) tautomers. R_f = 0.32 (EtOAc/petrol 1:2); ν_{max}/cm^{-1} (neat) 1733 (C=O); Ring tautomer (A): 1.2:1 *cis* and *trans* diastereomers: δ_H (400 MHz, CD_2Cl_2) major isomer (*cis*): 2.65 (1H, br. s., NH), 3.76 (3H, s, CO_2CH_3), 3.96 - 4.11 (3H, m, $H4_A$ + $H4_B$ + H5), 5.27 (1H, s, H2), 7.36 - 7.41 (2H, m, Ar-C*H*), 7.47 - 7.54 (2H, m, Ar-C*H*); minor isomer (*trans*): 2.65 (1H, br. s., NH), 3.75 (3H, s, CO_2CH_3), 3.83 (1H, dd, *J* = 8.0, 5.5 Hz, $H4_A$), 3.96 - 4.03 (1H, m, H5), 4.04 - 4.11 (1H, m, $H4_B$), 5.64 (1H, s, H2), 7.36 - 7.41 (2H, m, Ar-C*H*), 7.47 - 7.54 (2H, m, Ar-C*H*); δ_C (100.6 MHz, CD_2Cl_2) major isomer (*cis*): 53.1 (CO_2CH_3), 60.4 (C5), 69.2 (C4), 93.3 (C2), 122.8, 137.8 (Ar-C), 128.7, 132.1 (Ar-CH), 173.3 (CO_2CH_3); minor isomer (*trans*): 52.9 (CO_2CH_3), 59.6 (C5), 68.5 (C4), 92.4 (C2), 123.3, 139.3 (Ar-C), 128.7, 131.9 (Ar-CH), 173.3 (CO_2CH_3); Open chain tautomer (B): δ_H (400 MHz, CD_2Cl_2): 2.65 (1H, br. s., OH), 3.73 (3H, s, CO_2CH_3), 3.89 - 4.03 (2H, m, $H4_A$ + $H4_B$), 4.14 (1H, dd, *J* = 6.1, 4.9 Hz, H5), 7.57 (2H, d, *J* = 8.6 Hz, H2'), 7.65 (2H, d, *J* = 8.6 Hz, H3'), 8.28 (1H, s, H2); δ_C (100.6 MHz, CD_2Cl_2): 52.7 (CO_2CH_3), 64.1 (C4), 74.5 (C5), 126.3, 135.1 (Ar-C), 130.5, 132.4 (Ar-CH), 164.7 (C2), 171.3 (CO_2CH_3); *m/z* (ESI^+) 286 ($[M+H]^+$ 11 %)

and 288 ([M+H]$^+$ 11 %); HRMS (ESI$^+$); $C_{11}H_{13}O_3NBr$ [M+H]$^+$; found 286.00690 and 288.00482, requires 286.00733 and 288.00529.

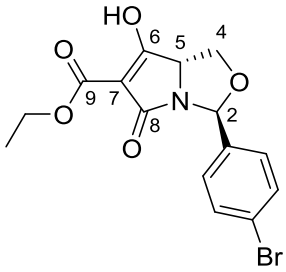
Methyl (2*RS*,5*S*)-2-(4-bromophenyl)-1-(3-ethoxy-3-oxopropanoyl)-1,3-oxazolidine-5-carboxylate, 75



Yield (0.95 g, 51 %); yellow oil; 2.7:1 *cis* and *trans* diastereomers; R_f = 0.31 (*trans*), 0.43 (*cis*) (EtOAc/petrol 1:1); ν_{max}/cm^{-1} (neat) 1666 (C=O), 1741 (C=O); δ_H (400 MHz, $CDCl_3$) major isomer (*cis*, a mixture of two conformers) major conformer: 1.19 - 1.31 (3H, m, OCH_2CH_3), 3.01 (1H, d, *J* = 15.8 Hz, H2"$_A$), 3.04 (1H, d, *J* = 15.8 Hz, H2"$_B$), 3.80 (3H, s, CO_2CH_3), 4.11 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.22 - 4.29 (2H, m, H4), 4.86 (1H, app t, *J* = 5.5 Hz, H5), 6.04 (1H, s, H2), 7.57 (2H, d, *J* = 8.7 Hz, Ar-C*H*), 7.60 (2H, d, *J* = 8.7 Hz, Ar-C*H*); minor conformer: 1.19 - 1.31 (3H, m, OCH_2CH_3), 3.39 (1H, d, *J* = 15.4 Hz, H2"$_A$), 3.50 (1H, d, *J* = 15.4 Hz, H2"$_B$), 3.80 (3H, s, CO_2CH_3), 4.18 (2H, q, *J* = 7.2 Hz, OCH_2CH_3), 4.22 - 4.29 (1H, m, H4), 4.48 (1H, dd, *J* = 9.1, 1.8 Hz, H4), 4.77 (1H, dd, *J* = 6.6, 1.8 Hz, H5), 6.16 (1H, s, H2), 7.40 (2H, d, *J* = 8.5 Hz, Ar-C*H*), 7.47 (2H, d, *J* = 8.5 Hz, Ar-C*H*); δ_C (100.6 MHz, $CDCl_3$) major isomer (*cis*, a mixture of two conformers) major conformer: 13.9 (OCH_2CH_3), 42.1 (C2"), 52.7 (CO_2CH_3), 58.4 (C5), 61.5 (OCH_2CH_3), 68.5 (C4), 90.6 (C2), 124.4, 135.4 (Ar-C), 129.7, 132.2 (Ar-CH), 164.0 (C1"), 166.4 (C3"), 169.9 (C6); minor conformer: 13.9 (OCH_2CH_3), 42.5 (C2"), 53.0 (CO_2CH_3), 58.6 (C5), 61.7 (OCH_2CH_3), 69.6 (C4), 90.8 (C2), 123.1, 135.9 (Ar-C), 129.1, 131.3 (Ar-CH), 164.8 (C1"), 166.9 (C3"), 169.9 (CO_2CH_3); δ_H (400 MHz, $CDCl_3$) minor isomer (*trans*, a mixture of two conformers) major conformer: 1.23 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 3.04 (1H, d, *J* = 15.4 Hz, H2"$_A$), 3.12 (1H, d, *J* = 15.4 Hz, H2"$_B$), 3.81 (3H, s, CO_2CH_3), 4.06 - 4.15 (3H, m, OCH_2CH_3 + H4), 4.19 - 4.26 (1H, m, H4), 4.83 (1H, dd, *J* = 6.7, 2.8 Hz, H5), 6.29 (1H, s, H2), 7.28 (2H, d, *J* = 8.3 Hz, Ar-C*H*), 7.58 (2H, d, *J* = 8.3 Hz, Ar-C*H*); minor conformer: 1.31 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 3.39 - 3.43 (2H, m, H2"), 3.87 (3H, s, CO_2CH_3), 4.19 - 4.26 (3H, m,

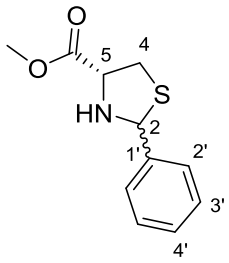
OC*H*$_2$CH$_3$ + H4), 4.30 - 4.35 (1H, m, H4), 4.77 (1H, app d, *J* = 5.6 Hz, H5), 6.42 (1H, s, H2), 7.31 (2H, d, *J* = 8.3 Hz, Ar-C*H*), 7.50 (2H, d, *J* = 8.3 Hz, Ar-C*H*); δ_C (100.6 MHz, $CDCl_3$) minor isomer (*trans*, a mixture of two conformers) major conformer: 14.0 (OCH$_2$*C*H$_3$), 42.4 (C2"), 52.8 (CO$_2$*C*H$_3$), 58.7 (C5), 61.6 (O*C*H$_2$CH$_3$), 67.4 (C4), 89.8 (C2), 124.4, 135.4 (Ar-C), 128.7, 132.4 (Ar-*C*H), 163.7 (C1"), 166.0 (C3"), 169.6 (*C*O$_2$CH$_3$); minor conformer: 14.1 (OCH$_2$*C*H$_3$), 42.8 (C2"), 53.4 (CO$_2$*C*H$_3$), 59.0 (C5), 61.8 (O*C*H$_2$CH$_3$), 69.0 (C4), 90.2 (C2), 123.0, 136.3 (Ar-C), 128.2, 131.6 (Ar-*C*H), 164.2 (C1"), 167.1 (C3"), 170.4 (*C*O$_2$CH$_3$); *m/z* (ESI$^+$) 422 ([M+Na]$^+$ 100%) and 424 ([M+Na]$^+$ 100%); HRMS (ESI$^+$); $C_{16}H_{19}O_6NBr$ [M+H]$^+$; found 400.03808 and 402.03594, requires 400.03903 and 402.03698.

(-)-(2*S*,5*S*)-1-Aza-2-(4-bromophenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-oxabicyclo[3.3.0]oct-6-ene, 76



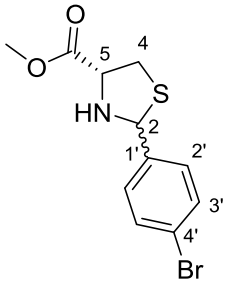
Yield (0.19 g, 8 %); yellow solid, mp 136-138 °C; R_f = 0.29 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -13.2, (*c* = 0.33, MeOH); ν_{max}/cm^{-1} (neat) 1639 (C=C), 1697 (C=O), 1738 (C=O); δ_H (400 MHz, Methanol-*d*$_4$): 1.23 - 1.30 (3H, m, OCH$_2$C*H*$_3$), 3.66 - 3.77 (1H, m, H4$_A$), 3.86 - 4.01 (2H, m, H4$_B$ + H5), 4.13 - 4.28 (2H, m, OC*H*$_2$CH$_3$), 6.24 (1H, s, H2), 7.38 (2H, d, *J* = 8.3 Hz, Ar-H), 7.48 (2H, d, *J* = 8.3 Hz, Ar-H); δ_C (100.6 MHz, Methanol-*d*$_4$): 15.2 (OCH$_2$*C*H$_3$), 60.2 (O*C*H$_2$CH$_3$), 65.4 (C5), 67.6 (C4), 89.6 (C2), 92.6 (C7), 123.2, 140.4 (Ar-C), 129.6, 132.5 (Ar-*C*H), 167.8 (C8), 183.7 (C9), 196.3 (C6); *m/z* (ESI$^-$) 366.0 and 368.0 ([M-H]$^-$, 100%); HRMS (ESI$^-$); $C_{15}H_{13}O_5NBr$ [M-H]$^-$; found 365.99948 and 367.99725, requires 365.99826 and 367. 99621.

Methyl (2*RS*,5*R*)-2-phenyl-1,3-thiazolidine-5-carboxylate, 78



Yield (2.37 g, 91 %); colourless oil; inseparable 1.7:1 *cis* and *trans* diastereomers; R_f = 0.48 (EtOAc/petrol 1:3); ν_{max}/cm^{-1} (neat) 1736 (s, C=O), 3314 (m, N-H); δ_H (400 MHz, $CDCl_3$) major isomer (*cis*): 2.71 (1H, br. s., NH), 3.12 (1H, dd, *J* = 10.3, 9.1 Hz, $H4_A$), 3.47 (1H, dd, *J* = 10.3, 7.1 Hz, $H4_B$), 3.80 (3H, s, CO_2CH_3), 3.99 (1H, app t, *J* = 7.9 Hz, H5), 5.57 (1H, s, H2), 7.27 - 7.41 (3H, m, H3′+ H4′), 7.52 - 7.56 (2H, m, H2′); minor isomer (*trans*): 2.71 (1H, br. s., NH), 3.20 (1H, dd, *J* = 10.5, 5.9 Hz, $H4_A$), 3.38 (1H, dd, *J* = 10.5, 7.1 Hz, $H4_B$), 3.78 (3H, s, CO_2CH_3), 4.22 (1H, app t, *J* = 6.4 Hz, H5), 5.82 (1H, s, H2), 7.27 - 7.41 (3H, m, H3′ + H4′), 7.47 - 7.51 (2H, m, H2′); δ_C (100.6 MHz, $CDCl_3$) major isomer (*cis*): 39.2 (C4), 52.6 (CO_2CH_3), 64.3 (C5), 70.9 (C2), 127.5 (C2′), 128.5 (C4′), 128.7 C3′), 138.2 (C1'), 171.6 (CO_2CH_3); minor isomer (*trans*): 38.2 (C4), 52.6 (CO_2CH_3), 65.6 (C5), 72.6 (C2), 127.0 (C2′), 127.9 (C4'), 128.7 (C3′), 141.2 (C1'), 172.2 (CO_2CH_3); *m/z* (ESI^+) 224 ($[M+H]^+$, 100%); HRMS (ESI^+); $C_{11}H_{13}NNaO_2S$ $[M+Na]^+$; found 246.0563, requires 246.0559.

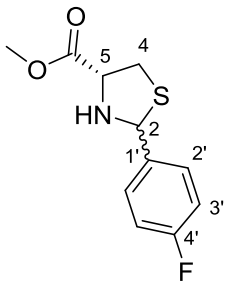
Methyl (2*RS*,5*R*)-2-(4-bromophenyl)-1,3-thiazolidine-5-carboxylate, 79



Yield (5.60 g, 94 %); colourless oil; inseparable 1.3:1 *cis* and *trans* diastereomers; R_f = 0.23 (EtOAc/petrol 1:4); ν_{max}/cm^{-1} (neat) 1737 (C=O), 3313 (N-H); δ_H (400 MHz, $CDCl_3$) major isomer (*cis*): 2.64 (1H, br. s., NH), 3.10 (1H, dd, *J* = 10.3, 8.8 Hz, $H4_A$), 3.45 (1H, dd, *J* = 10.3, 7.1 Hz, $H4_B$), 3.79 (3H, s, CO_2CH_3), 3.97 (1H, dd, *J* = 8.8, 7.1 Hz, H5), 5.49 (1H, s, H2), 7.32 - 7.51 (4H, m, H2′ + H3'); minor isomer (*trans*): 2.64 (1H, br. s., NH), 3.15 (1H, dd, *J* = 10.5, 6.1 Hz, $H4_A$), 3.36 (1H, dd, *J* = 10.5, 7.1 Hz, $H4_B$), 3.78 (3H, s, CO_2CH_3), 4.13 (1H, app t, *J* = 6.5 Hz, H5), 5.76 (1H, s, H2), 7.32 - 7.51 (4H, m, H2′ + H3'); δ_C (100.6 MHz, $CDCl_3$) major isomer (*cis*): 39.0 (C4), 52.5 (CO_2CH_3), 65.3 (C5), 71.6 (C2), 122.4 (C4′), 129.0 (C2′), 131.6 (C3′), 137.0 (C1′), 171.3 (CO_2CH_3); minor isomer (*trans*): 37.9 (C4), 52.4

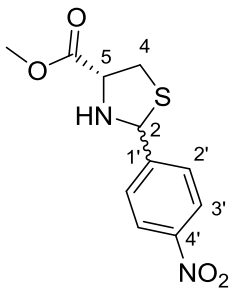
(CO_2CH_3), 64.0 (C5), 69.8 (C2), 121.5 (C4′), 128.5 (C2′), 131.0 (C3′), 140.5 (C1′), 171.9 (CO_2CH_3); *m/z* (ESI^+) 302, 304 ($[M+H]^+$ 100%); HRMS (ESI^+); $C_{11}H_{13}O_2NBrS$ $[M+H]^+$; found 301.98412 and 303.98175, requires 301.98449 and 303.98244.

Methyl (2*RS*,5*R*)-2-(4-fluorophenyl)-1,3-thiazolidine-5-carboxylate, 80



Yield (2.54 g, 90 %); colourless oil; inseparable 1.7:1 *cis* and *trans* diastereomers; R_f = 0.35 (EtOAc/petrol 1:3); ν_{max}/cm^{-1} (neat) 1225 (C-F), 1741 (C=O), 3311 (N-H); δ_H (400 MHz, $CDCl_3$) major isomer (*cis*): 2.66 (1H, br. s., NH), 3.12 (1H, dd, *J* = 10.3, 8.8 Hz, $H4_A$), 3.47 (1H, dd, *J* = 10.3, 7.1 Hz, $H4_B$), 3.81 (3H, s, CO_2CH_3), 3.98 (1H, dd, *J* = 8.8, 7.1 Hz, H5), 5.53 (1H, s, H2), 6.98 - 7.09 (2H, m, H3′), 7.51 (2H, dd, *J* = 8.6, 5.4 Hz, H2′); minor isomer (*trans*): 2.66 (1H, br. s., NH), 3.17 - 3.23 (1H, m, $H4_A$), 3.36 - 3.42 (1H, m, $H4_B$), 3.80 (3H, s, CO_2CH_3), 4.18 (1H, app t, *J* = 6.5 Hz, H5), 5.79 (1H, s, H2), 6.98 - 7.09 (2H, m, H3′), 7.47 (2H, dd, *J* = 8.6, 5.4 Hz, H2′); δ_C (100.6 MHz, $CDCl_3$) major isomer (*cis*): 39.2 (C4), 52.6 (CO_2CH_3), 65.4 (C5), 71.8 (C2), 115.5 (d, *J* = 21.5 Hz, C3′), 129.3 (d, *J* = 8.7 Hz, C2′), 133.9 (d, *J* = 3.2 Hz, C1′), 162.8 (d, *J* = 247.2 Hz, C4′), 171.5 (CO_2CH_3); minor isomer (*trans*): 38.0 (C4), 52.6 (CO_2CH_3), 64.1 (C5), 69.9 (C2), 115.1 (d, *J* = 21.5 Hz, C3′), 128.7 (d, *J* = 8.0 Hz, C2′), 136.9 (d, *J* = 3.2 Hz, C1′), 162.3 (d, *J* = 246.4 Hz, C4′), 172.1 (CO_2CH_3); *m/z* (ESI^+) 264 ($[M+Na]^+$, 56%); HRMS (ESI^+); $C_{11}H_{12}FNNaO_2S$ $[M+Na]^+$; found 264.0460, requires 264.0465.

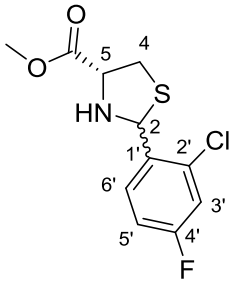
Methyl (2*RS*,5*R*)-2-(4-nitrophenyl)-1,3-thiazolidine-5-carboxylate, 81



Yield (2.95 g, 94 %); yellow oil; 0.8:1 inseparable *cis* and *trans* diastereomers; R_f = 0.20 (EtOAc/petrol 1:3); ν_{max}/cm^{-1} (neat) 1348 ($ArNO_2$), 1516 ($ArNO_2$), 1730 (C=O), 3316 (N-H); δ_H (400 MHz, $CDCl_3$)

major isomer (*trans*): 2.88 (1H, br. s., NH), 3.08 - 3.16 (1H, m, $H4_A$), 3.36 (1H, dd, *J* = 10.6, 6.7 Hz, $H4_B$), 3.78 (3H, s, CO_2CH_3), 4.07 (1H, app t, *J* = 6.7 Hz, H5), 5.87 (1H, s, H2), 7.61 (2H, d, *J* = 8.8 Hz, H2′), 8.12 (2H, d, *J* = 8.8 Hz, H3′); minor isomer (*cis*): 2.88 (1H, br. s., NH), 3.08 - 3.16 (1H, m, $H4_A$), 3.46 (1H, dd, *J* = 10.4, 7.0 Hz, $H4_B$), 3.78 (3H, s, CO_2CH_3), 4.00 (1H, dd, *J* = 8.8, 7.0 Hz, H5), 5.59 (1H, s, H2), 7.67 (2H, d, *J* = 8.8 Hz, H2′), 8.18 (2H, d, *J* = 8.8 Hz, H3′); δ_C (100.6 MHz, $CDCl_3$) major isomer (*trans*): 38.1 (C4), 52.6 (CO_2CH_3), 64.1 (C5), 69.0 (C2), 123.5 (C3′), 127.5 (C2′), 145.5 (C1′), 149.5 (C4′), 171.7 (CO_2CH_3); minor isomer (*cis*): 39.0 (C4), 52.6 (CO_2CH_3), 65.3 (C5), 70.9 (C2), 123.7 (C3′), 128.4 (C2′), 147.1 (C4′), 147.7 (C1′), 171.2 (CO_2CH_3); *m/z* (ESI^+) 269 ($[M+H]^+$, 100%); HRMS (ESI^+); $C_{11}H_{12}N_2NaO_4S$ $[M+Na]^+$; found 291.0405, requires 291.0410.

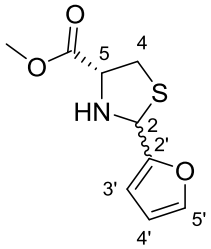
Methyl (2*RS*,5*R*)-2-(2-chloro-4-fluorophenyl)-1,3-thiazolidine-5-carboxylate, 82



Yield (2.45 g, 76 %); colourless oil; 0.8:1 inseparable *cis* and *trans* diastereomers; R_f = 0.58 (EtOAc/petrol 1:3); ν_{max}/cm^{-1} (neat) 1226 (C-F), 1737 (C=O), 3321 (N-H); δ_H (400 MHz, $CDCl_3$) major isomer (*trans*): 2.67 (1H, br. s., NH), 3.04 - 3.14 (1H, m, $H4_A$), 3.33 (1H, dd, *J* = 10.6, 6.5 Hz, $H4_B$), 3.81 (3H, s, CO_2CH_3), 4.21 (1H, app t, *J* = 6.6 Hz, H5), 6.02 (1H, s, H2), 6.96 (1H, app td, *J* = 8.3, 2.5 Hz, H5′), 7.08 - 7.16 (1H, m, H3′), 7.57 (1H, dd, *J* = 8.7, 6.1 Hz, H6′); minor isomer (*cis*) 2.67 (1H, br. s., NH), 3.04 - 3.14 (1H, m, $H4_A$), 3.46 (1H, dd, *J* = 10.4, 7.0 Hz, $H4_B$), 3.80 (3H, s, CO_2CH_3), 4.00 (1H, dd, *J* = 9.1, 7.0 Hz, H5), 5.89 (1H, s, H2), 7.03 (1H, app td, *J* = 8.3, 2.6 Hz, H5′), 7.08 - 7.16 (1H, m, H3′), 7.74 (1H, dd, *J* = 8.7, 6.1 Hz, H6′); δ_C (100.6 MHz, $CDCl_3$) major isomer (*trans*): 37.3 (C4), 52.6 (CO_2CH_3), 64.7 (C5), 66.5 (C2), 113.8 (d, *J* = 20.7 Hz, C5′), 117.0 (d, *J* = 24.6 Hz, C3′), 127.8 (d, *J* = 8.7 Hz, C6′), 133.5 (d, *J* = 10.3 Hz, C2′), 136.1 (d, *J* = 4.0 Hz, C1′), 161.6 (d, *J* = 249.5 Hz, C4′), 171.9 (CO_2CH_3); minor isomer (*cis*): 38.8 (C4), 52.6 (CO_2CH_3), 65.3 (C5), 67.6 (C2), 114.5 (d, *J* = 21.5 Hz, C5′), 117.1 (d, *J* = 24.6 Hz, C3′), 129.7 (d, *J* = 8.7 Hz, C6′),

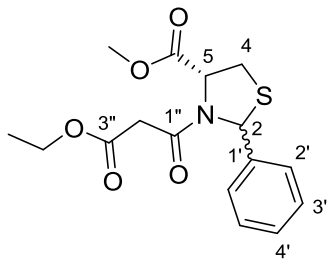
131.9 (d, *J* = 4.0 Hz, C1′), 134.5 (d, *J* = 10.3 Hz, C2′), 162.1 (d, *J* = 251.9 Hz, C4′), 171.4 (*C*O_2CH_3); *m/z* (ESI$^+$) 276 ([M+H]$^+$ 100%); HRMS (ESI$^+$); $C_{11}H_{11}ClFNNaO_2S$ [M+Na]$^+$; found 298.0062, requires 298.0075.

Methyl (2*RS*,5*R*)-2-(furan-2-yl)-1,3-thiazolidine-5-carboxylate, 83



Yield (3.17 g, 85 %); yellow oil; 0.9:1 inseparable *cis* and *trans* diastereomers; R_f = 0.53 (EtOAc/petrol 1:4); ν_{max}/cm^{-1} (neat) 1736 (C=O), 3317 (N-H); δ_H (400 MHz, $CDCl_3$) major isomer (*trans*): 2.90 (1H, br. s., NH), 2.91 - 2.97 (1H, m, $H4_A$), 3.23 - 3.29 (1H, m, $H4_B$), 3.62 (3H, s, CO_2CH_3), 4.09 (1H, app t, *J* = 6.7 Hz, H5), 5.68 (1H, s, H2), 6.15 - 6.17 (1H, m, H4′), 6.18 - 6.20 (1H, m, H3′), 7.24 - 7.26 (1H, m, H5′); minor isomer (*cis*): 2.90 (1H, br. s., NH), 2.91 - 2.97 (1H, m, $H4_A$), 3.23 - 3.29 (1H, m, $H4_B$), 3.63 (3H, s, CO_2CH_3), 3.81 (1H, dd, *J* = 9.3, 6.9 Hz, H5), 5.48 (1H, s, H2), 6.20 - 6.22 (1H, m, H4′), 6.28 - 6.29 (1H, m, H3′), 7.29 - 7.31 (1H, m, H5′); δ_C (100.6 MHz, $CDCl_3$) major isomer (*trans*): 37.9 (C4), 51.9 (CO_2CH_3), 63.2 (C2), 63.6 (C5), 106.6 (C3′), 109.6 (C4'), 141.9 (C5′), 153.2 (C2'), 171.1 (*C*O_2CH_3); minor isomer (*cis*): 37.3 (C4), 51.9 (CO_2CH_3), 63.9 (C2), 64.8 (C5), 107.4 (C3′), 109.9 (C4′), 142.2 (C5′), 150.1 (C2′), 170.5 (*C*O_2CH_3); *m/z* (ESI$^+$) 214 ([M+H]$^+$ 100%); HRMS (ESI$^+$); $C_9H_{11}NNaO_3S$ [M+Na]$^+$; found 236.0351, requires 236.0352.

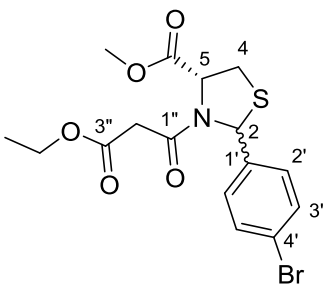
Methyl (2*RS*,5*R*)-1-(3-ethoxy-3-oxopropanoyl)-2-phenyl-1,3-thiazolidine-5-carboxylate, 84



Yield (2.54 g, 99 %); colourless oil; 1.6:1 inseparable *cis* and *trans* diastereomers; R_f = 0.61 (EtOAc/petrol 1:1); ν_{max}/cm^{-1} (neat) 1658 (C=O), 1734 (C=O); δ_H (400 MHz, $CDCl_3$) major isomer (*cis*, a mixture of two conformers): 1.09 - 1.21 (3H, m, OCH_2CH_3), 2.98 - 3.55 (4H, m, $H4_A$, $H4_B$, $H2''_A$, $H2''_B$), 3.70 (3H, s, CO_2CH_3 minor conformer), 3.73 (3H, s, CO_2CH_3 major conformer), 4.03 (2H, q, *J* = 7.1

Hz, OC*H*$_2$CH$_3$ major conformer), 4.07 - 4.14 (2H, m, OC*H*$_2$CH$_3$ minor conformer), 4.98 (1H, app t, *J* = 6.7 Hz, H5 major conformer), 5.02 (1H, dd, *J* = 6.2, 2.8 Hz, H5 minor conformer), 6.10 (1H, s, H2 major conformer), 6.28 (1H, s, H2 minor conformer), 7.13 - 7.34 (3H, m, H3′ + H4′), 7.45 (2H, d, *J* = 7.3 Hz, H2' minor conformer), 7.60 (2H, d, *J* = 7.6 Hz, H2' major conformer); minor isomer (*trans*, a mixture of two conformers): 1.09 - 1.21 (3H, m, OCH$_2$C*H*$_3$), 2.98 - 3.55 (4H, m, H4$_A$, H4$_B$, H2″$_A$, H2″$_B$), 3.69 (3H, s, CO$_2$C*H*$_3$ major conformer), 3.74 (3H, s, CO$_2$C*H*$_3$ minor conformer), 3.98 (2H, q, *J* = 7.1 Hz, OC*H*$_2$CH$_3$ major conformer), 4.07 - 4.14 (2H, m, OC*H*$_2$CH$_3$ minor conformer), 5.17 (1H, app d, *J* = 5.6 Hz, H5 minor conformer), 5.23 (1H, app d, *J* = 6.6 Hz, H5 major conformer), 6.14 (1H, s, H2 major conformer), 6.24 (1H, s, H2 minor conformer), 7.13 - 7.34 (5H, m, H2′ + H3′ + H4′); δ_C (100.6 MHz, CDCl$_3$) major isomer (*cis*, a mixture of two conformers): 13.5, 13.7 (OCH$_2$*C*H$_3$), 31.5, 33.3 (C4), 41.6, 42.4 (C2″), 52.2, 52.5 (CO$_2$*C*H$_3$), 60.9, 61.1 (O*C*H$_2$CH$_3$), 63.3, 64.2 (C5), 65.7, 66.5 (C2), 126.0, 126.5 (C2′), 127.4, 128.0 (C4′), 127.7, 128.5 (C3′), 138.5, 139.5 (C1′), 164.8, 165.1 (C1″), 166.2, 166.6 (C3″), 169.7, 169.8 (*C*O$_2$CH$_3$); minor isomer (*trans*, a mixture of two conformers): 13.5, 13.6 (OCH$_2$*C*H$_3$), 30.5, 32.5 (C4), 41.8, 42.8 (C2″), 52.2, 52.9 (CO$_2$*C*H$_3$), 60.8, 61.1 (O*C*H$_2$CH$_3$), 63.5, 63.8 (C5), 64.5, 65.1 (C2), 124.3, 124.5 (C2′), 127.0, 127.9 (C4′), 128.0, 128.7 (C3′), 141.5, 141.8 (C1′), 164.2, 164.8 (C1″), 165.8, 166.7 (C3″), 169.0, 169.8 (*C*O$_2$CH$_3$); *m/z* (ESI$^+$) 338 ([M+H]$^+$, 100%,), 360.0 ([M+Na]$^+$, 55%); HRMS (ESI$^+$); C$_{16}$H$_{19}$NNaO$_5$S [M+Na]$^+$; found 360.0873, requires 360.0876.

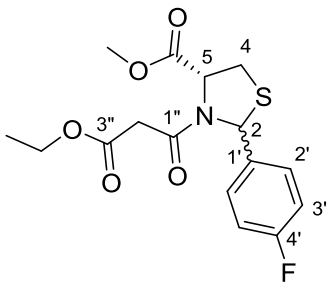
Methyl (2*RS*,5*R*)-2-(4-bromophenyl)-1-(3-ethoxy-3-oxopropanoyl)-1,3-thiazolidine-5-carboxylate, 85



Yield (2.06 g, 99 %); colourless oil; 1.3:1 inseparable *cis* and *trans* diastereomers; R_f = 0.17 (EtOAc/petrol 1:3); ν_{max}/cm^{-1} (neat) 1660 (C=O), 1738 (C=O); δ_H (400 MHz, CDCl$_3$) major isomer (*cis*, a mixture of two conformers): 1.19 - 1.31 (3H, m,

OCH_2CH_3), 3.07 - 3.55 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$), 3.81 (3H, s, CO_2CH_3 minor conformer), 3.83 (3H, s, CO_2CH_3 major conformer), 4.03 - 4.24 (2H, m, OCH_2CH_3), 5.04 (1H, app t, *J* = 7.0 Hz, H5), 6.11 (1H, s, H2 major conformer), 6.27 (1H, s, H2 minor conformer), 7.39 - 7.45 (4H, m, H2' + H3' minor conformer), 7.48 - 7.59 (4H, m, H2' + H3' major conformer); minor isomer (*trans*, a mixture of two conformers): 1.19 - 1.31 (3H, m, OCH_2CH_3), 3.07 - 3.55 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$), 3.78 (3H, s, CO_2CH_3 major conformer), 3.84 (3H, s, CO_2CH_3 minor conformer), 4.03 - 4.24 (2H, m, OCH_2CH_3), 5.18 (1H, app d, *J* = 5.1 Hz, H5 minor conformer), 5.26 - 5.33 (1H, m, H5 major conformer), 6.14 (1H, s, H2 major conformer), 6.24 (1H, s, H2 minor conformer), 7.11 (2H, d, *J* = 8.6 Hz, H2′ major conformer), 7.16 (2H, d, *J* = 8.3 Hz, H2′ minor conformer), 7.39 - 7.45 (2H, m, H3' minor conformer), 7.48 - 7.59 (2H, m, H3' major conformer); δ_C (100.6 MHz, $CDCl_3$) major isomer (*cis*, a mixture of two conformers): 13.9, 14.1 (OCH_2CH_3), 32.0, 33.9 (C4), 42.0, 42.9 (C2″), 52.7, 53.1 (CO_2CH_3), 61.6, 61.8 (OCH_2CH_3), 63.7, 64.6 (C5), 65.7, 66.5 (C2), 121.9, 122.6 (C4′), 128.2, 128.8 (C2′), 131.3, 132.1 (C3′), 137.7, 138.9 (C1′), 165.0, 165.3 (C1″), 166.5, 166.9 (C3″), 170.0, 170.1 (CO_2CH_3); minor isomer (*trans*, a mixture of two conformers): 13.9, 14.0 (OCH_2CH_3), 31.0, 33.0 (C4), 42.3, 43.2 (C2″), 52.8, 53.4 (CO_2CH_3), 61.5, 61.8 (OCH_2CH_3), 64.0, 64.2 (C5), 64.4, 65.1 (C2), 121.3, 122.4 (C4′), 126.5, 126.6 (C2′), 131.6, 132.3 (C3′), 140.9, 141.1 (C1′), 164.6, 165.2 (C1″), 166.1, 167.2 (C3″), 169.2, 170.0 (CO_2CH_3); *m/z* (ESI^+) 416, 418 ($[M+H]^+$ 100%); HRMS (ESI^+); $C_{16}H_{19}O_5NBrS$ $[M+H]^+$; found 416.01454 and 418.01234, requires 416.01618 and 418.01414.

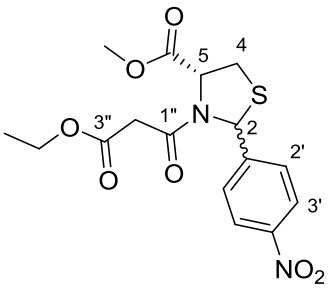
Methyl (2*RS*,5*R*)-1-(3-ethoxy-3-oxopropanoyl)-2-(4-fluorophenyl)-1,3-thiazolidine-5-carboxylate, 86



Yield (2.32 g, 87 %); colourless oil; 1.5:1 inseparable *cis* and *trans* diastereomers; R_f = 0.61 (EtOAc/petrol 1:1); ν_{max}/cm^{-1} (neat) 1224 (C-F), 1662 (C=O), 1741 (C=O); δ_H (400 MHz, $CDCl_3$)

major isomer (*cis*, a mixture of two conformers): 1.19 - 1.31 (3H, m, OCH_2CH_3), 3.07 - 3.55 (4H, m, $H4_A$, $H4_B$, $H2''_A$, $H2''_B$), 3.81 (3H, s, CO_2CH_3 minor conformer), 3.83 (3H, s, CO_2CH_3 major conformer), 4.05 - 4.24 (2H, m, OCH_2CH_3), 5.03 - 5.08 (1H, m, H5), 6.13 (1H, s, H2 major conformer), 6.30 (1H, s, H2 minor conformer), 6.95 - 7.01 (2H, m, H3′ minor conformer), 7.04 - 7.10 (2H, m, H3′ major conformer), 7.51 (2H, dd, J = 8.6, 5.4 Hz, H2′ minor conformer), 7.67 (2H, dd, J = 8.6, 5.1 Hz, H2′ major conformer); minor isomer (*trans*, a mixture of two conformers): 1.19 - 1.31 (3H, m, OCH_2CH_3), 3.07 - 3.55 (4H, m, $H4_A$, $H4_B$, $H2''_A$, $H2''_B$), 3.78 (3H, s, CO_2CH_3 major conformer), 3.84 (3H, s, CO_2CH_3 minor conformer), 4.05 - 4.24 (2H, m, OCH_2CH_3), 5.19 (1H, app d, J = 5.1 Hz, H5 minor conformer), 5.28 - 5.31 (1H, m, H5 major conformer), 6.17 (1H, s, H2 major conformer), 6.29 (1H, s, H2 minor conformer), 6.95 - 7.01 (2H, m, H3′ minor conformer), 7.04 - 7.10 (2H, m, H3′ major conformer), 7.21 (2H, dd, J = 8.7, 5.0 Hz, H2′ major conformer), 7.24 - 7.29 (2H, m, H2′ minor conformer); δ_C (100.6 MHz, $CDCl_3$) major isomer (*cis*, a mixture of two conformers): 13.9, 14.0 (OCH_2CH_3), 32.0, 33.8 (C4), 42.1, 43.0 (C2″), 52.7, 53.1 (CO_2CH_3), 61.6, 61.7 (OCH_2CH_3), 63.7, 64.6 (C5), 65.6, 66.4 (C2), 115.0 (d, J = 21.5 Hz, C3′ minor conformer), 115.9 (d, J = 21.5 Hz, C3′ major conformer), 128.4 (d, J = 8.0 Hz, C2′ major conformer), 129.0 (d, J = 8.7 Hz, C2′ minor conformer), 135.6 (d, J = 2.4 Hz, C1′), 162.6 (d, J = 248.8 Hz, C4′), 165.0, 165.4 (C1″), 166.6, 167.0 (C3″), 170.1, 170.2 (CO_2CH_3); minor isomer (*trans*, a mixture of two conformers): 13.9, 14.0 (OCH_2CH_3), 30.9, 33.0 (C4), 42.3, 43.3 (C2″), 52.8, 53.4 (CO_2CH_3), 61.5, 61.7 (OCH_2CH_3), 63.9, 64.2 (C5), 64.3, 65.0 (C2), 115.3 (d, J = 22.3 Hz, C3′ minor conformer), 116.2 (d, J = 21.5 Hz, C3′ major conformer), 126.5 (d, J = 8.7 Hz, C2′ minor conformer), 126.7 (d, J = 8.0 Hz, C2′ major conformer), 137.9 (d, J = 2.4 Hz, C1′), 162.5 (d, J = 248.8 Hz, C4′), 164.6, 165.2 (C1″), 166.1, 167.2 (C3″), 169.3 (CO_2CH_3); *m/z* (ESI^+) 378 ($[M+Na]^+$, 100%); HRMS (ESI^+); $C_{16}H_{18}FNNaO_5S$ $[M+Na]^+$; found 378.0779, requires 378.0782.

Methyl (2*RS*,5*R*)-1-(3-ethoxy-3-oxopropanoyl)-2-(4-nitrophenyl)-1,3-thiazolidine-5-carboxylate, 87

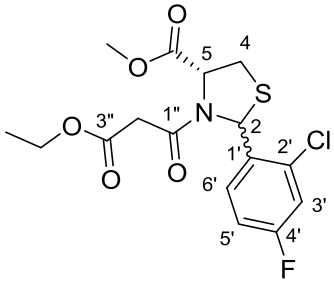


Yield (2.21 g, 85 %); orange oil; 0.9:1 inseparable *cis* and *trans* diastereomers; R_f = 0.48 (EtOAc/petrol 1:1); ν_{max}/cm^{-1} (neat) 1347 ($ArNO_2$), 1520 ($ArNO_2$), 1663 (C=O), 1740 (C=O); δ_H (400 MHz, $CDCl_3$): major isomer (*trans*, mixture of two conformers); 1.07 - 1.21 (3H, m, OCH_2CH_3), 2.98 - 3.54 (4H, m, $H4_A$, $H4_B$, $H2''_A$, $H2''_B$), 3.69 (3H, s, CO_2CH_3, major conformer), 3.76 (3H, s, CO_2CH_3, minor conformer), 3.93 - 4.15 (2H, m, OCH_2CH_3), 5.21 (1H, app d, *J* = 4.7 Hz, H5 major conformer), 5.26 (1H, app d, *J* = 6.9 Hz, H5 minor conformer), 6.23 (1H, s, H2 major conformer), 6.24 (1H, s, H2 minor conformer), 7.37 - 7.43 (2H, m, H2′), 8.05 (2H, d, *J* = 8.8 Hz, H3′ major conformer), 8.14 (2H, d, *J* = 8.6 Hz, H3′ minor conformer); minor isomer (*cis*, mixture of two conformers); 1.07 - 1.21 (3H, m, OCH_2CH_3), 2.98 - 3.54 (4H, m, $H4_A$, $H4_B$, $H2''_A$, $H2''_B$), 3.74 (3H, s, CO_2CH_3 minor conformer), 3.76 (3H, s, CO_2CH_3 major conformer), 3.93 - 4.15 (2H, m, OCH_2CH_3), 4.92 (1H, app t, *J* = 7.3 Hz, H5 major conformer), 5.07 (1H, dd, *J* = 5.5, 3.3 Hz, H5 minor conformer), 6.22 (1H, s, H2 major conformer), 6.27 (1H, s, H2 minor conformer), 7.64 (2H, d, *J* = 8.8 Hz, H2′ minor conformer), 7.85 (2H, d, *J* = 8.6 Hz, H2′ major conformer), 8.05 (2H, d, *J* = 8.8 Hz, H3′ minor conformer), 8.14 (2H, d, *J* = 8.6 Hz, H3′ major conformer); δ_C (100.6 MHz, $CDCl_3$) major isomer (*trans*, a mixture of two conformers): 13.6, 13.7 (OCH_2CH_3), 30.7, 32.7 (C4), 41.9, 42.6 (C2″), 53.0, 53.2 (CO_2CH_3), 61.2, 61.5 (OCH_2CH_3), 63.7, 64.0 (C5), 63.7, 64.3 (C2), 123.5, 124.1 (C3′), 125.4, 125.7 (C2′), 146.6, 147.2, 148.7, 149.3 (Ar-C), 164.9 (C1″), 165.7, 166.9 (C3″), 168.8, 169.6 (CO_2CH_3); minor isomer (*cis*, a mixture of two conformers): 13.6, 13.7 (OCH_2CH_3), 31.6, 33.9 (C4), 41.6, 42.4 (C2″), 52.5, 53.3 (CO_2CH_3), 61.3, 61.4 (OCH_2CH_3), 63.6, 64.5 (C5), 64.9, 66.1 (C2), 123.2, 123.7 (C3′), 127.2, 127.5 (C2′), 146.2, 146.6, 147.0, 147.3 (Ar-C), 165.1, 165.2 (C1″), 166.1, 166.5 (C3″), 169.8 (CO_2CH_3); *m/z* (ESI^+) 383 ($[M+H]^+$, 100%) and

405 ([M+Na]$^+$, 63%); HRMS (ESI$^+$); $C_{16}H_{18}N_2NaO_7S$ [M+Na]$^+$; found 405.0717, requires 405.0727.

(-)-Methyl (2*S*,5*R*)-2-(2-chloro-4-fluorophenyl)-1-(3-ethoxy-3-oxopropanoyl)-1,3-thiazolidine-5-carboxylate, *trans*-88 and

(+)-Methyl (2*R*,5*R*)-2-(2-chloro-4-fluorophenyl)-1-(3-ethoxy-3-oxopropanoyl)-1,3-thiazolidine-5-carboxylate, *cis*-88



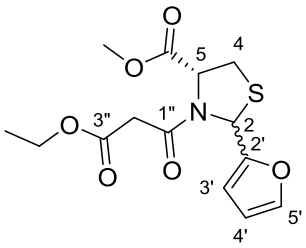
Yield (3.06 g, 97 %); 0.8:1 separable *cis* and *trans* diastereomers; Major isomer (*trans*, a mixture of two conformers): white crystalline solid, mp 32-34 ^oC; R_f = 0.16 (EtOAc/petrol 1:3); 100 % ee (2*S*,5*R*); $[\alpha]_D^{25}$ = -258.0 (*c* = 1.02, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1223 (C-F), 1662 (C=O), 1739 (C=O); δ_H (400 MHz, $CDCl_3$) 1.21 (3H, t, *J* = 7.2 Hz, OCH_2CH_3 major conformer), 1.29 (3H, t, *J* = 7.2 Hz, OCH_2CH_3 minor conformer), 3.04 - 3.52 (4H, m, $H4_A$, $H4_B$, $H2''_A$, $H2''_B$), 3.79 (3H, s, CO_2CH_3 major conformer), 3.85 (3H, s, OCH_3 minor conformer), 4.08 (2H, q, *J* = 7.2 Hz , OCH_2CH_3 major conformer), 4.16 - 4.26 (2H, m, OCH_2CH_3 minor conformer), 5.19 - 5.23 (1H, m, H5 minor conformer), 5.28 - 5.33 (1H, m, H5 major conformer), 6.42 (1H, s, H2 major conformer), 6.45 (1H, s, H2 minor conformer), 6.92 (1H, app td, *J* = 8.3, 2.4 Hz, H5′ minor conformer), 7.02 (1H, app td, *J* = 8.2, 2.6 Hz, H5′ major conformer), 7.10 - 7.18 (2H, m, H3′ minor conformer + H6' major conformer), 7.20 (1H, dd, *J* = 8.2, 2.6 Hz, H3′ major conformer), 7.32 (1H, dd, *J* = 8.7, 6.0 Hz, H6′ minor conformer); δ_C (100.6 MHz, $CDCl_3$): 13.9, 14.0 (OCH_2CH_3), 30.5, 32.4 (C4), 42.0, 43.0 (C2″), 52.8, 53.5 (CO_2CH_3), 61.5, 61.8 (OCH_2CH_3), 62.0, 62.9 (C2), 64.2, 64.5 (C5), 113.7 (d, *J* = 21.5 Hz, C5′ minor conformer), 114.5 (d, *J* = 21.5 Hz, C5′ major conformer), 117.4 (d, *J* = 25.4 Hz, C3′ minor conformer), 118.0 (d, *J* = 24.6 Hz, C3′ major conformer), 125.7 (d, *J* = 8.7 Hz, C6′ minor conformer), 126.4 (d, *J* = 8.7 Hz, C6′ major conformer), 132.5 (d, *J* = 10.3 Hz, C2′), 134.6 (d, *J* = 3.2 Hz, C1′ minor conformer), 134.7 (d, *J* = 3.2 Hz, C1′ major conformer), 161.6 (d, *J* = 249.5 Hz, C4′ minor conformer),

162.1 (d, *J* = 251.9 Hz, C4′ major conformer), 164.5, 165.0 (C1″), 165.9, 167.4 (C3″), 169.1, 170.0 (*C*O_2CH_3); *m/z* (ESI$^+$) 390 ([M+H]$^+$ 100%), 412.0 ([M+Na]$^+$ 76%); HRMS (ESI$^+$); $C_{16}H_{17}ClFNNaO_5S$ [M+Na]$^+$; found 412.0390, requires 412.0392. The e.e. was determined by HPLC using a Chiralpak AD-H column [*n*-hexane/*i*-PrOH (96:4)]; flow rate 1.0 mL/min; τ_{major} = 25.46 min.

Minor isomer (*cis*, a mixture of two conformers): colourless oil; R_f = 0.24 (EtOAc/petrol 1:3); $[\alpha]_D^{25}$ = +124.4 (*c* = 1.0, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1226 (C-F), 1666 (C=O), 1742 (C=O); δ_H (400 MHz, $CDCl_3$): 1.16 - 1.25 (3H, m, OCH_2CH_3), 3.03 - 3.51 (4H, m, $H4_A$, $H4_B$, $H2''_A$, $H2''_B$), 3.80 (3H, s, CO_2CH_3 major conformer), 3.84 (3H, s, CO_2CH_3 minor conformer), 4.04 - 4.17 (2H, m, OCH_2CH_3), 4.86 (1H, dd, *J* = 9.4, 6.2 Hz, H5 major conformer), 5.04 (1H, app t, *J* = 5.1 Hz, H5 minor conformer), 6.34 (1H, s, H2 major conformer), 6.46 (1H, s, H2 minor conformer), 6.91 (1H, app td, *J* = 8.4, 2.6 Hz, H5′ minor conformer), 7.03 (1H, app td, *J* = 8.3, 2.6 Hz, H5′ major conformer), 7.12 (1H, dd, *J* = 8.2, 2.6 Hz, H3′), 7.79 (1H, dd, *J* = 8.7, 6.0 Hz, H6′ minor conformer), 8.24 (1H, dd, *J* = 8.7, 6.0 Hz, H6′ major conformer); δ_C (100.6 MHz, $CDCl_3$): 13.8 (OCH_2CH_3), 31.0, 33.6 (C4), 41.6, 42.6 (C2″), 52.6, 53.1 (CO_2CH_3), 61.5 (OCH_2CH_3), 62.7, 64.3 (C2), 64.0, 65.0 (C5), 114.1 (d, *J* = 21.5 Hz, C5′ minor conformer), 114.5 (d, *J* = 20.7 Hz, C5′ major conformer), 116.5 (d, *J* = 24.6 Hz, C3′ minor conformer), 117.2 (d, *J* = 25.4 Hz, C3′ major conformer), 128.2 (d, *J* = 8.7 Hz, C6′ minor conformer), 128.8 (d, *J* = 8.7 Hz, C6′ major conformer), 132.5 (d, *J* = 11.1 Hz, C2′), 133.6 (d, *J* = 3.2 Hz, C1′), 161.5 (d, *J* = 249.5 Hz, C4′ minor conformer), 162.0 (d, *J* = 251.1 Hz, C4′ major conformer), 165.0, 165.1 (C1″), 166.1, 166.6 (C3″), 170.2, 170.3 (*C*O_2CH_3); *m/z* (ESI$^+$) 390 ([M+H]$^+$ 100%), 412.0 ([M+Na]$^+$ 75%); HRMS (ESI$^+$); $C_{16}H_{17}ClFNNaO_5S$ [M+Na]$^+$; found 412.0391, requires 412.0392.

(-)-Methyl (2*S*,5*R*)-1-(3-ethoxy-3-oxopropanoyl)-2-(furan-2-yl)-1,3-thiazolidine-5-carboxylate, *trans*-89 and

(+)-Methyl (2*R*,5*R*)-1-(3-ethoxy-3-oxopropanoyl)-2-(furan-2-yl)-1,3-thiazolidine-5-carboxylate, *cis*-89

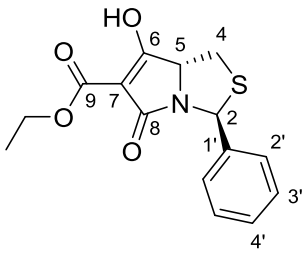


Yield (4.05 g, 89 %); 0.8:1 separable *cis* and *trans* diastereomers; Major isomer (*trans*, a mixture of two conformers): yellow solid, mp 64-66 ^{o}C; R_f = 0.23 (EtOAc/petrol 1:2); $[\alpha]_D^{25}$ = -271.7 (*c* = 0.23, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1664 (C=O), 1735 (C=O); δ_H (400 MHz, $CDCl_3$) 1.23 - 1.29 (3H, m, OCH_2CH_3), 3.23 (1H, app d, *J* = 12.5 Hz, $H4_A$/ $H4_B$ major conformer), 3.31 (1H, d, *J* = 15.5 Hz, $H2''_A$/$H2''_B$ major conformer), 3.38 - 3.40 (2H, m, $H2''_A$ + $H2''_B$ minor conformer), 3.45 - 3.49 (2H, m, $H4_A$/ $H4_B$ minor conformer + $H2''_A$/$H2''_B$ major conformer), 3.58 (1H, dd, *J* = 12.3, 6.9 Hz, $H4_A$/ $H4_B$ major conformer), 3.69 (1H, dd, *J* = 12.0, 6.0 Hz, $H4_A$/ $H4_B$ minor conformer), 3.77 (3H, s, CO_2CH_3 major conformer), 3.83 (3H, s, CO_2CH_3 minor conformer), 4.12 - 4.21 (2H, m, OCH_2CH_3), 5.05 (1H, app d, *J* = 5.9 Hz, H5 minor conformer), 5.18 (1H, app d, *J* = 6.8 Hz, H5 major conformer), 6.16 (1H, s, H2 major conformer), 6.23 - 6.25 (1H, m, H3' major conformer), 6.26 - 6.29 (2H, m, H3' + H4' minor conformer), 6.32 - 6.35 (2H, m, H2 minor conformer + H4' major conformer), 7.29 - 7.33 (1H, m, H5' minor conformer), 7.38 - 7.42 (1H, m, H5' major conformer); δ_C (100.6 MHz, $CDCl_3$): 13.2 (OCH_2CH_3), 31.2, 33.1 (C4), 41.1, 42.4 (C2″), 51.8, 52.6 (CO_2CH_3), 58.0, 58.5 (C2), 60.6, 60.7 (OCH_2CH_3), 62.1, 62.5 (C5), 105.2, 106.4 (C3'), 109.7, 109.9 (C4'), 141.1, 142.5 (C5'), 151.8, 152.0 (C2'), 164.1 (C1″), 165.7, 166.3 (C3″), 168.8, 169.5 (CO_2CH_3); *m/z* (ESI^+) 328 ($[M+H]^+$ 100%), 350 ($[M+Na]^+$ 26 %); HRMS (ESI^+); $C_{14}H_{17}NNaO_6S$ $[M+Na]^+$; found 350.0677, requires 350.0669.

Minor isomer (*cis*, a mixture of two conformers): yellow oil; R_f = 0.35 (EtOAc/petrol 1:2); $[\alpha]_D^{25}$ = +41.3 (*c* = 0.15, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1665 (C=O), 1736 (C=O); δ_H (500 MHz, $CDCl_3$) 1.27 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 3.22 - 3.35 (2H, m, $H4_A$ + $H4_B$ major

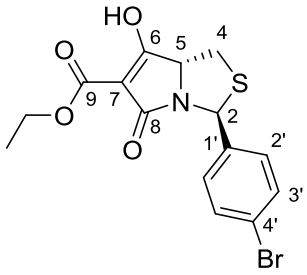
conformer), 3.38 (1H, d, *J* = 15.4 Hz, H2″$_A$/H2"$_B$ major conformer), 3.41 - 3.46 (2H, m, H4$_A$/ H4$_B$ + H2″$_A$/H2"$_B$ minor conformer), 3.49 - 3.54 (1H, m, H2″$_A$/H2"$_B$ minor conformer), 3.56 (1H, d, *J* = 15.5 Hz, H2″$_A$/H2"$_B$ major conformer), 3.64 (1H, dd, *J* = 11.4, 4.9 Hz, H4$_A$/H4$_B$ minor conformer), 3.76 (3H, s, CO_2CH_3), 4.13 - 4.25 (4H, m, OCH_2CH_3), 4.91 (1H, dd, *J* = 9.0, 6.8 Hz, H5 major conformer), 4.96 (1H, dd, *J* = 6.8, 4.9 Hz, H5 minor conformer), 6.18 (1H, s, H2 major conformer), 6.26 - 6.29 (1H, m, H4' minor conformer), 6.34 (1H, dd, *J* = 3.2, 1.9 Hz, H4' major conformer), 6.44 (1H, d, *J* = 3.2 Hz, H3' minor conformer), 6.56 (1H, s, H2 minor conformer), 6.76 (1H, d, *J* = 3.2 Hz, H3' major conformer), 7.32 - 7.34 (1H, m, H5' minor conformer), 7.39 - 7.43 (1H, m, H5' major conformer); δ_C (100.6 MHz, $CDCl_3$): 14.0 (OCH_2CH_3), 32.0, 33.6 (C4), 41.8, 42.4 (C2″), 52.7, 53.1 (CO_2CH_3), 58.7, 59.29 (C2), 61.7 (OCH_2CH_3), 62.7, 63.4 (C5), 108.6, 109.1 (C3'), 110.3, 110.7 (C4'), 142.5, 143.2 (C5'), 151.2, 152.0 (C2'), 164.6 (C1″), 166.7, 166.9 (C3″), 169.9 (CO_2CH_3); *m/z* (ESI$^+$) 328 ([M+H]$^+$ 100 %), 350 ([M+Na]$^+$ 60 %); HRMS (ESI$^+$); $C_{14}H_{17}NNaO_6S$ [M+Na]$^+$; found 350.06688, requires 350.06688.

(-)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-6-hydroxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 90a



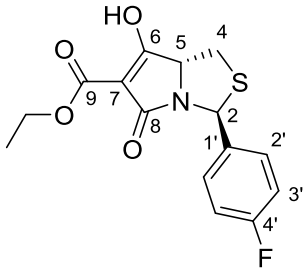
Yield (1.02 g, 43 %); yellow solid, mp 88-90 ^oC; R_f = 0.55 (EtOAc/MeOH 6:1); $[\alpha]_D^{25}$ = -220.8 (*c* = 1.0, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1617 (C=C), 1656 (C=O), 1712 (C=O); δ_H (400 MHz, $CDCl_3$): 1.30 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 2.90 (1H, dd, *J* = 11.0, 8.1 Hz, H4$_A$), 3.19 (1H, dd, *J* = 11.0, 7.1 Hz, H4$_B$), 4.30 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.67 (1H, app t, *J* = 7.6 Hz, H5), 6.25 (1H, s, H2), 7.17 - 7.22 (1H, m, H4′), 7.23 - 7.28 (2H, m, H3′), 7.35- 7.41 (2H, m, H2′); δ_C (100.6 MHz, $CDCl_3$): 14.1 (OCH_2CH_3), 32.5 (C4), 61.6 (OCH_2CH_3), 62.2 (C2), 65.2 (C5), 98.8 (C7), 126.4 (C2′), 128.0 (C4′), 128.5 (C3′), 139.9 (C1′), 166.9 (C9), 168.8 (C8), 185.8 (C6); *m/z* (ESI$^-$) 304 ([M-H]$^-$, 100%); HRMS (ESI$^-$); $C_{15}H_{14}NO_4S$ [M-H]$^-$; found 304.0639, requires 304.0649.

(-)-(2*S*,5*R*)-1-Aza-2-(4-bromophenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 91a



Yield (0.50 g, 45 %); yellow solid, mp 134-136 ^{o}C; R_f = 0.26 (EtOAc/MeOH 9:1); 76 % ee (2*S*,5*R*); $[\alpha]_D^{25}$ = -190.5 (*c* = 1.0, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1656 (C=O), 1711 (C=O); δ_H (400 MHz, CD_2Cl_2): 1.36 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 3.02 (1H, dd, *J* = 11.3, 8.3 Hz, $H4_A$), 3.29 (1H, dd, *J* = 11.3, 7.1 Hz, $H4_B$), 4.37 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.76 (1H, app t, *J* = 7.6 Hz, H5), 6.22 (1H, s, H2), 7.35 (1H, d, *J* = 8.3 Hz, H2′), 7.49 (2H, d, *J* = 8.3 Hz, H3′); δ_C (100.6 MHz, CD_2Cl_2): 14.5 (OCH_2CH_3), 33.3 (C4), 62.3 (OCH_2CH_3), 62.3 (C2), 65.9 (C5), 98.5 (C7), 122.3 (C4′), 128.8 (C2′), 132.1 (C3′), 140.5 (C1′), 167.4 (C9), 170.3 (C8), 187.3 (C6); *m/z* (ESI$^-$) 382, 384 ($[M-H]^-$ 100%); HRMS (ESI$^-$); $C_{15}H_{13}O_4NBrS$ $[M-H]^-$; found 381.97563 and 383.97343, requires 381.97541 and 383.97337. The e.e. was determined by HPLC using a Chiralpak AD-H column [*n*-hexane/*i*-PrOH (80:20)]; flow rate 1.0 mL/min; τ_{major} = 15.73 min, τ_{minor} = 26.27 min.

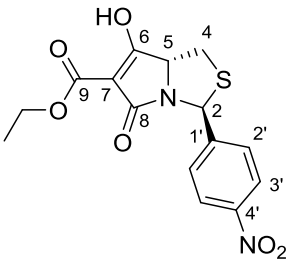
(-)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-2-(4-fluorophenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 92a



Yield (0.46 g, 50 %); yellow solid; R_f = 0.55 (EtOAc/MeOH 6:1); 80 % ee (2*S*,5*R*); $[\alpha]_D^{25}$ = -192.4, (*c* = 0.47, MeOH); ν_{max}/cm^{-1} (neat) 1224 (C-F), 1658 (C=O), 1710 (C=O); For **92a** purified by preparative TLC, δ_H (400 MHz, Methanol-d_4): 1.28 (3H, t, *J* = 7.0 Hz, OCH_2CH_3), 2.95 (1H, dd, *J* = 11.0, 7.3 Hz, $H4_A$), 3.20 (1H, dd, *J* = 11.0, 7.6 Hz, $H4_B$), 4.15 - 4.24 (2H, m, OCH_2CH_3 + H5), 6.34 (1H, s, H2), 7.04 (2H, app t, *J* = 8.8 Hz, H3′), 7.47 (2H, dd, *J* = 8.6, 5.4 Hz, H2′); δ_C (125.8 MHz, Methanol-d_4): 15.2 (OCH_2CH_3), 34.9 (C4), 59.7 (OCH_2CH_3), 64.6 (C2), 70.6 (C5), 91.9 (C7), 116.0 (d, *J* = 21.9 Hz, C3′), 129.6 (d, *J* = 8.6 Hz, C2′), 139.8 (C1′), 163.7 (d, *J* = 244.8 Hz, C4′), 167.3 (C9), 180.2 (C8), 195.1 (C6); *m/z* (ESI$^-$) 322 ($[M-H]^-$, 100 %); HRMS (ESI$^-$); $C_{15}H_{13}FNO_4S$ $[M-H]^-$

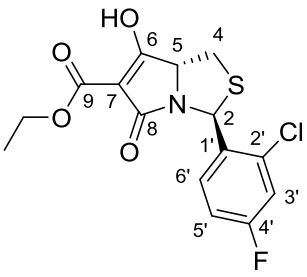
; found 322.0552, requires 322.0555. The e.e. was determined by HPLC using a Chiralpak AD-H column [*n*-hexane/*i*-PrOH (75:25)]; flow rate 1.0 mL/min; τ_{major} = 9.11 min, τ_{minor} = 12.88 min.

(-)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-6-hydroxy-2-(4-nitrophenyl)-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 93a



Yield (0.32 g, 52 %); yellow solid, mp 92 ^{o}C; R_f = 0.52 (EtOAc/MeOH 6:1); $[\alpha]_D^{25}$ = -222.2 (*c* = 0.35, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1344 ($ArNO_2$), 1517 ($ArNO_2$), 1660 (C=O), 1711 (C=O); δ_H (400 MHz, CD_2Cl_2): 1.36 (3H, t, *J* = 7.2 Hz, OCH_2CH_3), 3.05 (1H, dd, *J* = 11.1, 8.5 Hz, $H4_A$), 3.31 (1H, dd, *J* = 11.1, 7.2 Hz, $H4_B$), 4.38 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.79 (1H, app t, *J* = 7.6 Hz, H5), 6.32 (1H, s, H2), 7.63 (2H, d, *J* = 8.4 Hz, H2′), 8.19 (2H, d, *J* = 8.4 Hz, H3′); δ_C (125.8 MHz, CD_2Cl_2): 14.5 (OCH_2CH_3), 33.5 (C4), 62.2 (C2), 62.4 (OCH_2CH_3), 65.9 (C5), 99.7 (C7), 124.4 (C3′), 127.9 (C2′), 148.3 (C1′), 148.3 (C4′), 167.4 (C9), 169.2 (C8), 186.6 (C6); *m/z* (ESI$^-$) 349 ([M-H]$^-$, 100%); HRMS (ESI$^-$); $C_{15}H_{13}N_2O_6S$ [M-H]$^-$; found 349.0500, requires 349.0510.

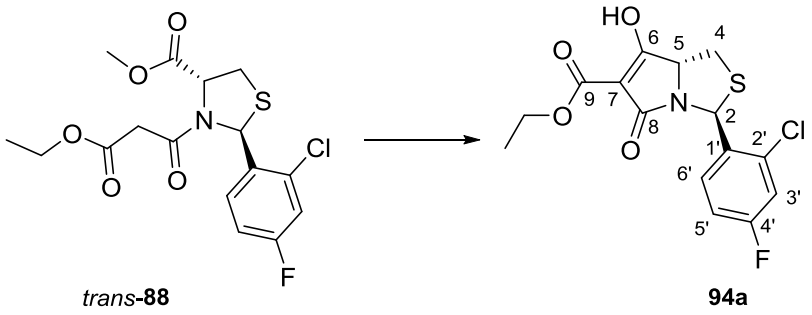
(-)-(2*S*,5*R*)-1-Aza-2-(2-chloro-4-fluorophenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 94a



Yield (0.48 g, 49 %); yellow solid, mp 128 ^{o}C; R_f = 0.57 (EtOAc/MeOH 6:1); 83 % ee (2*S*,5*R*); $[\alpha]_D^{25}$ = -264.0 (*c* = 0.20, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1233 (C-F), 1621 (C=C), 1658 (C=O), 1715 (C=O); δ_H (400 MHz, $CDCl_3$): 1.38 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 2.99 (1H, dd, *J* = 11.0, 8.8 Hz, $H4_A$), 3.28 (1H, dd, *J* = 11.0, 7.0 Hz, $H4_B$), 4.39 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.91 (1H, dd, *J* = 8.8, 7.0 Hz, H5), 6.45 (1H, s, H2), 6.97 (1H, app td, *J* = 8.3, 2.6 Hz, H5′), 7.13 (1H, dd, *J* = 8.3, 2.6 Hz, H3′), 7.38 (1H, dd, *J* = 8.3, 5.9 Hz, H6′); δ_C (100.6 MHz, $CDCl_3$): 14.1 (OCH_2CH_3), 32.4 (C4), 59.4

(C2), 61.8 (O*C*$H_2$$CH_3$), 66.0 (C5), 98.8 (C7), 114.2 (d, *J* = 21.5 Hz, C5′), 117.4 (d, *J* = 25.4 Hz, C3′), 127.3 (d, *J* = 9.5 Hz, C6′), 133.2 (d, *J* = 10.3 Hz, C2′), 134.3 (d, *J* = 3.2 Hz, C1′), 161.9 (d, *J* = 250.3 Hz, C4′), 167.0 (C9), 167.9 (C8), 185.8 (C6); *m/z* (ESI$^-$) 356 ([M-H]$^-$, 100%); HRMS (ESI$^-$); $C_{15}H_{12}ClFNO_4S$ [M-H]$^-$; found 356.0172, requires 356.0165. The e.e. was determined by HPLC using a Chiralpak AD-H column [*n*-hexane/*i*-PrOH (80:20)]; flow rate 1.0 mL/min; τ_{major} = 11.51 min, τ_{minor} = 15.92 min.

(-)-(2*S*,5*R*)-1-Aza-2-(2-chloro-4-fluorophenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 94a

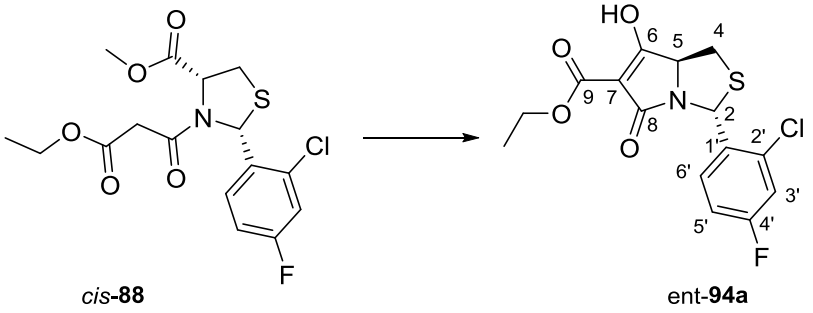


trans-**88** → **94a**

Yield (30 mg, 58 %); yellow solid, mp 176-178 ^oC; R_f = 0.57 (EtOAc/MeOH 6:1); ν_{max}/cm^{-1} (neat) 1236 (C-F), 1650 (C=O), 1712 (C=O); For **94a** purified by preparative TLC: 100 % e.e. (2*S*,5*R*); $[\alpha]_D^{25}$ = -240.4 (*c* = 0.27, MeOH); δ_H (400 MHz, Methanol-d_4): 1.28 (3H, t, *J* = 7.0 Hz, OCH$_2$C*H*$_3$), 2.95 (1H, dd, *J* = 11.0, 7.8 Hz, H4$_A$), 3.20 (1H, dd, *J* = 11.0, 7.3 Hz, H4$_B$), 4.14 - 4.23 (2H, m, OC*H*$_2$CH$_3$), 4.42 (1H, app t, *J* = 7.6 Hz, H5), 6.52 (1H, s, H2), 7.07 (1H, app td, *J* = 8.5, 2.5 Hz, H5′), 7.21 (1H, dd, *J* = 8.6, 2.5 Hz, H3′), 7.52 (1H, dd, *J* = 8.7, 6.0 Hz, H6′); δ_C (125.8 MHz, Methanol-d_4): 15.2 (OCH$_2$*C*H$_3$), 34.2 (C4), 59.7 (O*C*H$_2$CH$_3$), 62.3 (C2), 71.8 (C5), 91.5 (C7), 115.3 (d, *J* = 21.0 Hz, C5′), 117.9 (d, *J* = 25.8 Hz, C3′), 129.2 (d, *J* = 8.6 Hz, C6′), 134.1 (d, *J* = 10.5 Hz, C2′), 138.3 (d, *J* = 2.9 Hz, C1′), 163.2 (d, *J* = 248.0 Hz, C4′), 167.2 (C9), 179.7 (C8), 195.0 (C6); *m/z* (ESI$^-$) 356 ([M-H]$^-$, 100%); HRMS (ESI$^-$); $C_{15}H_{12}ClFNO_4S$ [M-H]$^-$; found 356.0170, requires 356.0165. The e.e. was determined by HPLC using a Chiralpak AD-H column [*n*-hexane/*i*-PrOH (90:10)]; flow rate 1.0 mL/min; τ = 8.85 min. For **94a** purified by flash column chromatography followed by acid wash: 98 % e.e. (2*S*,5*R*); $[\alpha]_D^{25}$ = -331.8 (*c* = 0.27, $CHCl_3$); δ_H (400 MHz, Methanol-d_4): 1.32 (3H, t, *J* = 7.1 Hz, OCH$_2$C*H*$_3$), 3.04 (1H, dd, *J* =

11.0, 8.9 Hz, $H4_A$), 3.29 - 3.40 (1H, m, $H4_B$, obscured by solvent peak), 4.31 (2H, q, *J* = 7.0 Hz, OCH_2CH_3), 5.05 (1H, dd, *J* = 8.8, 6.9 Hz, H5), 6.39 (1H, s, H2), 7.10 (1H, app td, *J* = 8.5, 2.6 Hz, H5′), 7.25 (1H, dd, *J* = 8.5, 2.6 Hz, H3′), 7.58 (1H, dd, *J* = 8.7, 6.0 Hz, H6′). The e.e. was determined by HPLC using a Chiralpak AD-H column [*n*-hexane/*i*-PrOH (80:20)]; flow rate 1.0 mL/min; τ_{major} = 11.32 min, τ_{minor} = 15.85 min.

(+)-(2*R*,5*S*)-1-Aza-2-(2-chloro-4-fluorophenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, ent-94a

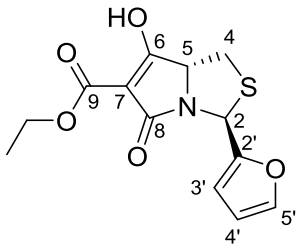


cis-**88** ent-**94a**

Yield (33 mg, 6 %); yellow solid; R_f = 0.57 (EtOAc/MeOH 6:1); ν_{max}/cm^{-1} (neat) 1232 (C-F), 1656 (C=O), 1709 (C=O); for ent-**94a** purified by preparative TLC; 100 % e.e. (2*R*,5*S*); $[\alpha]_D^{25}$ = +230.3 (*c* = 0.38, MeOH); δ_H (400 MHz, Methanol-d_4): 1.28 (3H, t, *J* = 7.0 Hz, OCH_2CH_3), 2.95 (1H, dd, *J* = 10.7, 8.0 Hz, $H4_A$), 3.20 (1H, *J* = 10.7, 7.5 Hz, $H4_B$), 4.19 (2H, q, *J* = 7.0 Hz, OCH_2CH_3), 4.43 (1H, app t, *J* = 7.2 Hz, H5), 6.52 (1H, s, H2), 7.07 (1H, app td, *J* = 8.5, 2.6 Hz, H5′), 7.21 (1H, dd, *J* = 8.5, 2.6 Hz, H3′), 7.51 (1H, dd, *J* = 8.7, 6.0 Hz, H6′); δ_C (125.8 MHz, Methanol-d_4): 15.2 (OCH_2CH_3), 34.2 (C4), 59.7 (OCH_2CH_3), 62.3 (C2), 71.8 (C5), 91.5 (C7), 115.3 (d, *J* = 21.9 Hz, C5′), 117.9 (d, *J* = 25.8 Hz, C3′), 129.2 (d, *J* = 8.6 Hz, C6′), 134.1 (d, *J* = 10.5 Hz, C2′), 138.3 (d, *J* = 2.9 Hz, C1′), 163.2 (d, *J* = 248.0 Hz, C4′), 167.3 (C9), 179.7 (C8), 195.0 (C6); *m/z* (ESI$^-$) 356 ($[M-H]^-$, 100%); HRMS (ESI$^-$); $C_{15}H_{12}ClFNO_4S$ $[M-H]^-$; found 356.0172, requires 356.0165. The e.e. was determined by HPLC using a Chiralpak AD-H column [*n*-hexane/*i*-PrOH (90:10)]; flow rate 1.0 mL/min; τ = 11.07 min. For ent-**94a** purified by flash column chromatography followed by acid wash: 96 % e.e. (2*R*,5*S*); $[\alpha]_D^{25}$ = +309.5 (*c* = 0.20, $CHCl_3$); δ_H (400 MHz, Methanol-d_4): 1.32 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 3.03 (1H, dd, *J* = 10.9, 8.9

Hz, $H4_A$), 3.29 - 3.40 (1H, m, $H4_B$, obscured by solvent peak), 4.31 (2H, q, *J* = 7.0 Hz, OCH_2CH_3), 5.06 (1H, dd, *J* = 8.9, 7.0 Hz, H5), 6.39 (1H, s, H2), 7.10 (1H, app td, *J* = 8.4, 2.6 Hz, H5′), 7.25 (1H, dd, *J* = 8.5, 2.6 Hz, H3′), 7.57 (1H, dd, *J* = 8.8, 6.0 Hz, H6′). The e.e. was determined by HPLC using a Chiralpak AD-H column [*n*-hexane/*i*-PrOH (80:20)]; flow rate 1.0 mL/min; τ_{major} = 15.90 min, τ_{minor} = 12.34 min.

(-)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-2-(2-furanyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 95a



Yield (1.53 g, 40 %); brown oil; R_f = 0.30 (EtOAc/MeOH 9:1); 80 % ee (2*S*,5*R*); $[\alpha]_D^{25}$ = -153.3 (*c* = 0.06, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1614 (C=C), 1663 (C=O), 1709 (C=O); δ_H (400 MHz, CD_2Cl_2): 1.36 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 3.08 (1H, dd, *J* = 11.0, 6.9 Hz, $H4_A$), 3.47 (1H, dd, *J* = 11.0, 8.1 Hz, $H4_B$), 4.37 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.83 (1H, app t, *J* = 7.5 Hz, H5), 6.30 (1H, s, H2), 6.33 - 6.35 (2H, m, H3′+ H4'), 7.40- 7.43 (1H, m, H5′); δ_C (125.8 MHz, CD_2Cl_2): 14.5 (OCH_2CH_3), 32.9 (C4), 56.3 (C2), 62.3 (OCH_2CH_3), 65.2 (C5), 99.2 (C7), 107.5 (C3'), 110.9 (C4'), 143.5 (C5′), 153.4 (C2′), 167.4 (C9), 168.8 (C8), 187.2 (C6); *m/z* (ESI^-) 294 ($[M-H]^-$, 50 %); HRMS (ESI^-); $C_{13}H_{12}NO_5S$ $[M-H]^-$; found 294.0446, requires 294.0442. The e.e. was determined by HPLC using a Chiralpak AD-H column [*n*-hexane/*i*-PrOH (85:15)]; flow rate 1.0 mL/min; τ_{major} = 23.42 min, τ_{minor} = 31.26 min.

6.3 Experimental for Chapter 3

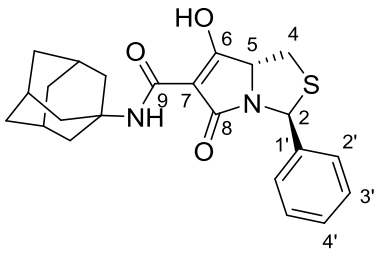
6.3.1 General procedure: Synthesis of carboxamide tetramates 109-140

To tetramic acid (1.0 eq) dissolved in THF/toluene or DMSO/toluene (for **135** only) (1:9, 10 mL/mmol), amine (1.5 eq) was added. The solution was heated at reflux for 16 h, cooled to rt and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (eluent: EtOAc/MeOH/1 % Et_3N). The product was dissolved in

CH_2Cl_2 and washed with 5% citric acid. The organic fractions were dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield the bicyclic carboxamide tetramate.

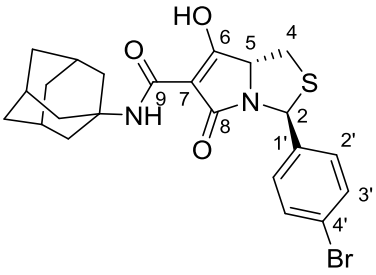
Where the major and minor tautomeric forms of carboxamide tetramates have distinct chemical shift values, they have been specified as either AB (major tautomeric form) or CD (minor tautomeric form) in compound characterisation. It is assumed that the e.e. of the bicyclic tetramate ester is retained through to aminolysis and hence, the e.e. of carboxamide tetramates derived from tetramates esters were not explicitly determined.

(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-6-hydroxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 109



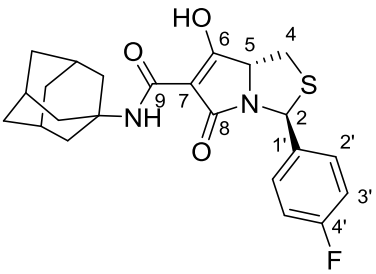
Yield (0.19 g, 38 %); yellow solid, mp 94-96 °C; 2.7:1 AB:CD tautomers; R_f = 0.78 (EtOAc/MeOH 20:1); $[\alpha]_D^{25}$ = -132.0 (*c* = 0.20, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1623 (C=C), 1649 (C=O), 1691 (C=O), 3313 (N-H/O-H); δ_H (400 MHz, $CDCl_3$): 1.60 (6H, broad signal, Adamantyl-C*H*$_2$), 1.95 (6H, broad signal, Adamantyl-C*H*$_2$), 2.04 (3H, broad signal, Adamantyl-C*H*), 2.84 - 2.98 (1H, m, H4$_A$), 3.10 - 3.20 (1H, m, Hz, H4$_B$), 4.35 (1H, app t, *J* = 7.8 Hz, H5 CD), 4.58 (1H, app t, *J* = 7.7 Hz, H5 AB), 6.17 (1H, s, H2 AB), 6.26 (1H, s, H2 CD), 7.15 - 7.21 (1H, m, H4′), 7.22 - 7.29 (2H, m, H3′), 7.31 - 7.41 (2H, m, H2′), 7.85 (1H, br. s., NH/OH CD), 12.19 (1H, br. s., NH/OH AB); δ_C (100.6 MHz, $CDCl_3$): 29.2 (Adamantyl-*C*H), 32.4 (C4 AB), 32.6 (C4 CD), 35.7 (Adamantyl-*C*H$_2$ CD), 35.9 (Adamantyl-*C*H$_2$ AB), 41.4 (Adamantyl-*C*H$_2$), 52.9 (Adamantyl-C AB), 54.3 (Adamantyl-C CD), 61.9 (C2 AB), 62.4 (C2 CD), 67.2 (C5 AB), 70.5 (C5 CD), 85.3 (C7 CD), 94.9 (C7 AB), 126.1 (C2′ CD), 126.2 (C2′ AB), 127.8 (C4′ CD), 127.9 (C4′ AB), 128.4 (C3′ CD), 128.5 (C3′ AB), 140.2 (C1′ AB), 140.5 (C1′ CD), 165.8 (C9 AB), 166.4 (C9 CD), 172.2 (C8 AB), 178.0 (C8 CD), 187.8 (C6 AB), 191.1 (C6 CD); *m/z* (ESI$^-$) 409 ([M-H]$^-$, 100%); HRMS (ESI$^-$); $C_{23}H_{25}N_2O_3S$ [M-H]$^-$; found 409.1574, requires 409.1591.

(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(4-bromophenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 110



Yield (2.80 g, 70 %); brown foam, 2.6:1 AB:CD tautomers, mp 108-110 °C; R_f = 0.72 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -159.5 (*c* = 0.25, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1625 (C=C), 1648 (C=O), 1688 (C=O), 3317 (O-H/N-H); δ_H (400 MHz, $CDCl_3$): 1.69 (6H, br. signal, Adamantyl-C*H*$_2$), 2.05 (6H, br. signal, Adamantyl-C*H*$_2$), 2.11 (3H, br. signal, Adamantyl-C*H*), 2.96 - 3.06 (1H, m, H4$_A$), 3.20 - 3.27 (1H, m, H4$_B$), 4.39 (1H, app t, *J* = 7.8 Hz, H5 CD), 4.63 (1H, app t, *J* = 7.7 Hz, H5 AB), 6.18 (1H, s, H2 AB), 6.27 (1H, s, H2 CD), 7.31- 7.36 (2H, m, Ar-C*H*), 7.40 (1H, br. s., NH AB), 7.46 (2H, d, *J* = 8.3 Hz, Ar-C*H*), 7.94 (1H, br. s., NH CD), 10.78 (1H, br. s., OH); δ_C (100.6 MHz, $CDCl_3$): 29.2 (Adamantyl-*C*H), 32.5 (C4 AB), 32.8 (C4 CD), 35.8 (Adamantyl-*C*H$_2$ CD), 36.0 (Adamantyl-*C*H$_2$ AB), 41.5 (Adamantyl-*C*H$_2$ AB), 41.5 (Adamantyl-*C*H$_2$ CD), 53.1 (Adamantyl-C AB), 54.5 (Adamantyl-C CD), 61.5 (C2 AB), 62.1 (C2 CD), 67.3 (C5 AB), 70.5 (C5 CD), 85.3 (C7 CD), 94.5 (C7 AB), 121.9 (C4' CD), 122.0 (C4' AB), 128.1 (C2' CD), 128.2 (C2' AB), 131.57 (C3' CD), 131.62 (C3' AB), 139.4 (C1' AB), 139.6 (C1' CD), 165.9 (C9 AB), 166.4 (C9 CD), 172.4 (C8 AB), 178.2 (C8 CD), 188.4 (C6 AB), 191.1 (C6 CD); *m/z* (ESI^-) 487, 489 ($[M-H]^-$, 100 %); HRMS (ESI^-); $C_{23}H_{24}N_2O_3BrS$ $[M-H]^-$; found 487.07009 and 489.06794, requires 487.06965 and 489.06760.

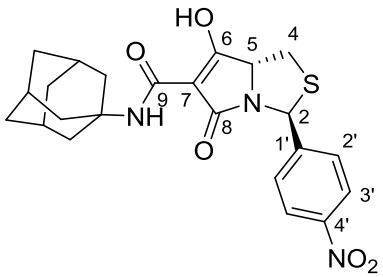
(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(4-fluorophenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 111



Yield (0.19 g, 56 %); brown oil; 2.7:1 AB:CD tautomers; R_f = 0.76 (EtOAc/MeOH 20:1); $[\alpha]_D^{25}$ = -177.5, (*c* = 0.20, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1229 (C-F), 1625 (C=C), 1649 (C=O), 1690 (C=O), 3313 (N-H/O-H); δ_H (400 MHz, $CDCl_3$): 1.70 (6H, br.

signal, Adamantyl-C*H*$_2$), 2.05 (6H, br. signal, Adamantyl-C*H*$_2$), 2.12 (3H, br. signal, Adamantyl-C*H*), 2.96 - 3.05 (1H, m, H4$_A$), 3.26 (1H, dd, *J* = 11.3, 7.1 Hz, H4$_B$), 4.42 (1H, app t, *J* = 7.6 Hz, H5 CD), 4.66 (1H, app t, *J* = 7.8 Hz, H5 AB), 6.22 (1H, s, H2 AB), 6.31 (1H, s, H2 CD), 7.04 (2H, app t, *J* = 7.8 Hz, H3′), 7.38 - 7.51 (2H, m, H2′), 7.95 (1H, br. s., NH/OH CD), 11.34 (1H, br. s., NH/OH AB); δ_C (100.6 MHz, $CDCl_3$): 29.2 (Adamantyl-*C*H), 32.5 (C4 AB), 32.8 (C4 CD), 35.8 (Adamantyl-*C*H$_2$ CD), 36.0 (Adamantyl-*C*H$_2$ AB), 41.50 (Adamantyl-*C*H$_2$ AB), 41.54 (Adamantyl-*C*H$_2$ CD), 53.1 (Adamantyl-C AB), 54.5 (Adamantyl-C CD), 61.5 (C2 AB), 62.0 (C2 CD), 67.4 (C5 AB), 70.5 (C5 CD), 94.7 (C7), 115.38 (d, *J* = 21.5 Hz, C3′ CD), 115.43 (d, *J* = 21.5 Hz, C3′ AB), 128.1 (d, *J* = 8.0 Hz, C2′ CD), 128.2 (d, *J* = 8.0 Hz, C2′ AB), 136.1 (d, *J* = 3.2 Hz, C1′ AB), 136.3 (d, *J* = 3.2 Hz, C1′ CD), 162.3 (d, *J* = 246.4 Hz, C4′ CD), 162.4 (d, *J* = 247.2 Hz, C4′ AB), 166.0 (C9 AB), 166.5 (C9 CD), 172.4 (C8 AB), 178.1 (C8 CD), 188.3 (C6 AB), 191.1 (C6 CD); *m/z* (ESI$^-$) 427 ([M-H]$^-$, 100%); HRMS (ESI$^-$); $C_{23}H_{24}FN_2O_3S$ [M-H]$^-$; found 427.1502, requires 427.1497.

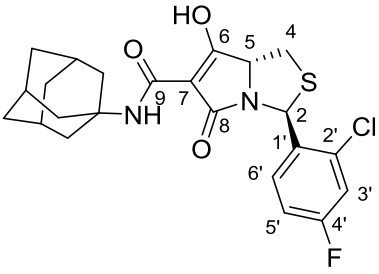
(-)-(2*S*,5*R*)-7-(Adamantyaminolcarbonyl)-1-aza-6-hydroxy-2-(4-nitrophenyl)-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 112



Yield (0.53 g, 35 %); brown oil; 2.4:1 AB:CD tautomers; R_f = 0.76 (EtOAc/MeOH 20:1); $[\alpha]_D^{25}$ = -93.8, (*c* = 0.50, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1347 (sym $ArNO_2$), 1522 (asym $ArNO_2$), 1643 (C=O, br with shoulder towards lower wavenumber), 1682 (C=O), 3333 (N-H/O-H); δ_H (400 MHz, $CDCl_3$): 1.68 (6H, br. signal, Adamantyl-C*H*$_2$), 2.00 - 2.08 (6H, m, Adamantyl-C*H*$_2$), 2.08 - 2.16 (3H, m, Adamantyl-C*H*), 2.99 - 3.09 (1H, m, H4$_A$), 3.21 - 3.30 (1H, m, H4$_B$), 4.40 (1H, app t, *J* = 7.8 Hz, H5 CD), 4.64 (1H, app t, *J* = 7.3 Hz, H5 AB), 6.27 (1H, s, H2 AB), 6.36 (1H, s, H2 CD), 7.55 - 7.65 (2H, m, H2′), 8.15 - 8.21 (2H, m, H3′), 7.38 (1H, br. s., NH AB), 7.97 (1H, br. s., NH CD), 10.82 (1H, br. s., OH); δ_C (100.6 MHz, $CDCl_3$): 29.2 (Adamantyl-*C*H), 32.7 (C4

AB), 32.9 (C4 CD), 35.7 (Adamantyl-*C*H_2 CD), 35.9 (Adamantyl-*C*H_2 AB), 41.4 (Adamantyl-*C*H_2 AB), 41.5 (Adamantyl-*C*H_2 CD), 53.3 (Adamantyl-C AB), 54.6 (Adamantyl-C CD), 61.3 (C2 AB), 61.8 (C2 CD), 67.6 (C5 AB), 70.6 (C5 CD), 85.1 (C7 CD), 93.7 (C7 AB), 123.77 (C3' CD), 123.79 (C3′ AB), 127.2 (C2′ CD), 127.3 (C2′ AB), 147.4 (C1' CD), 147.5 (C1′ AB), 147.6 (C4′ AB), 147.8 (C4′ CD), 166.0 (C9 AB), 166.4 (C9 CD), 172.6 (C8 AB), 178.3 (C8 CD), 189.1 (C6 AB), 190.8 (C6 CD); *m/z* (ESI$^-$) 454 ([M-H]$^-$, 12%); HRMS (ESI$^-$); $C_{23}H_{24}N_3O_5S$ [M-H]$^-$; found 454.1438, requires 454.1442.

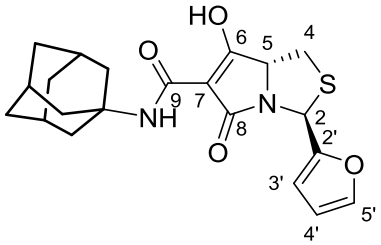
(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(2-chloro-4-fluorophenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 113



Yield (0.15 g, 60 %); yellow solid, mp 120°C; 2.5:1 AB:CD tautomers; R_f = 0.78 (EtOAc/MeOH 20:1); $[\alpha]_D^{25}$ = -147.5, (*c* = 0.20, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1234 (C-F), 1626 (C=C), 1649 (C=O), 1692 (s, C=O), 3318 (N-H/O-H); δ_H (400 MHz, $CDCl_3$): 1.63 - 1.75 (6H, br. signal, Adamantyl-C*H*$_2$), 2.00 - 2.09 (6H, br. signal, Adamantyl-C*H*$_2$), 2.09 - 2.18 (3H, br. signal, Adamantyl-C*H*), 2.96 – 3.04 (1H, m, $H4_A$), 3.20 - 3.28 (1H, m, $H4_B$), 4.57 (1H, dd, *J* = 8.6, 7.6 Hz, H5 CD), 4.79 (1H, dd, *J* = 8.8, 6.9 Hz, H5 AB), 6.42 (1H, s, H2 AB), 6.49 (1H, s, H2 CD), 6.98 (1H, app td, *J* = 8.3, 2.3 Hz, H5′), 7.13 (1H, dd, *J* = 8.3, 2.3 Hz, H3′), 7.36 - 7.44 (1H, m, H6′), 7.97 (1H, br. s., NH/OH CD), 11.69 (1H, br. s., NH/OH AB); δ_C (100.6 MHz, $CDCl_3$): 29.2 (Adamantyl-*C*H), 32.2 (C4 AB), 32.3 (C4 CD), 35.8 (Adamantyl-*C*H_2 CD), 36.0 (Adamantyl-*C*H_2 AB), 41.48 (Adamantyl-*C*H_2 AB), 41.54 (Adamantyl-*C*H_2 CD), 53.3 (Adamantyl-C AB), 54.6 (Adamantyl-C CD), 59.1 (C2 AB), 59.7 (C2 CD), 68.4 (C5 AB), 71.5 (C5 CD), 85.0 (C7 CD), 94.0 (C7 AB), 114.2 (d, *J* = 21.5 Hz, C5′ CD), 114.3 (d, *J* = 21.5 Hz, C5′ AB), 117.29 (d, *J* = 25.4 Hz, C3′ AB), 117.31 (d, *J* = 25.4 Hz, C3′ CD), 127.2 (d, *J* = 9.5 Hz, C6′ CD), 127.4 (d, *J* = 8.7 Hz, C6′ AB), 133.0 (d, *J* = 10.3 Hz, C2′ CD), 133.1 (d, *J* = 10.3 Hz,

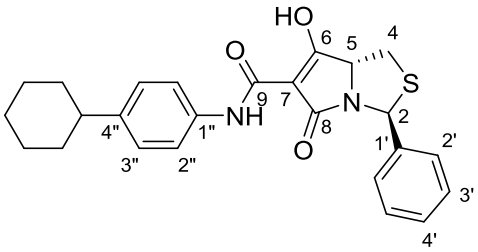
C2′ AB), 134.6 (d, *J* = 4.0 Hz, C1′ AB), 134.9 (d, *J* = 4.0 Hz, C1′ CD), 161.8 (d, *J* = 249.5 Hz, C4′ CD), 161.9 (d, *J* = 250.3 Hz, C4′ AB), 166.0 (C9 AB), 166.5 (C9 CD), 172.0 (C8 AB), 177.8 (C8 CD), 188.7 (C6 AB), 191.0 (C6 CD); *m/z* (ESI⁻) 461 ([M-H]⁻, 15%); HRMS (ESI⁻); $C_{23}H_{23}ClFN_2O_3S$ [M-H]⁻; found 461.1108, requires 461.1107.

(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(2-furanyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 114



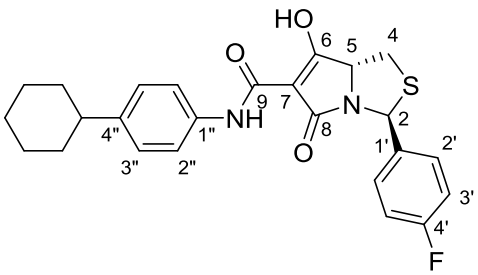
Yield (0.28 g, 92 %); brown soild, mp 88-90 °C; 3:1 AB:CD tautomers; R_f = 0.76 (EtOAc/MeOH 20:1); $[\alpha]_D^{25}$ = -141.7 (*c* = 0.06, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1627 (C=C), 1649 (C=O), 1692 (C=O), 3318 (N-H/O-H); δ_H (500 MHz, CD_2Cl_2): 1.70 (6H, br. signal, Adamantyl-C*H*$_2$), 2.06 (6H, br. signal, Adamantyl-C*H*$_2$), 2.10 (3H, br. signal, Adamantyl-C*H*), 3.06 (1H, dd, *J* = 11.0, 7.1 Hz, H4$_A$), 3.41 (1H, dd, *J* = 11.0, 8.1 Hz, H4$_B$), 4.42 (1H, app t, *J* = 7.4 Hz, H5 CD), 4.69 (1H, app t, *J* = 7.5 Hz, H5 AB), 6.26 (1H, s, H2 AB), 6.32 - 6.35 (3H, m, H2 CD + H3' + H4'), 7.35 (1H, br. s., NH/OH AB), 7.39 - 7.44 (1H, m, H5′), 7.94 (1H, br. s., NH/OH CD); δ_C (125.8 MHz, CD_2Cl_2): 30.0 (Adamantyl-*C*H), 32.6 (C4 AB), 33.1 (C4 CD), 36.4 (Adamantyl-*C*H$_2$, CD), 36.6 (Adamantyl-*C*H$_2$, AB), 42.07 (Adamantyl-*C*H$_2$ AB), 42.13 (Adamantyl-*C*H$_2$ CD), 53.6 (Adamantyl-C, obscured by CD_2Cl_2 signal but HMBC corrleation seen with adamantyl-CH$_2$), 56.1 (C2 AB), 56.6 (C2 CD), 67.2 (C5 AB), 70.5 (C5 CD), 85.4 (C7 CD), 95.0 (C7 AB), 107.4 (C3′), 110.9 (C4′), 143.4 (C5'), 153.5 (C2′ AB), 153.7 (C2′ CD), 166.6 (C9 AB), 167.1 (C9 CD), 172.8 (C8 AB), 178.7 (C8 CD), 189.5 (C6 AB), 192.0 (C6 CD); *m/z* (ESI⁻) 399 ([M-H]⁻, 62 %); HRMS (ESI⁻); $C_{21}H_{23}N_2O_4S$ [M-H]⁻; found 399.13827, requires 399.13840.

(-)-(2*S*,5*R*)-1-Aza-7-(4-cyclohexylphenylaminocarbonyl)-6-hydroxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 115



Yield (0.41 g, 54 %); yellow foam, mp 88 °C; 15:1 AB:CD tautomers; R_f = 0.73 (EtOAc/MeOH 20:1); $[\alpha]_D^{25}$ = -159.0 (*c* = 0.20, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1630 (C=C), 1651 (C=O), 1692 (C=O), 3281 (N-H/O-H); δ_H (400 MHz, CD_2Cl_2): 1.20 - 1.33 (1H, m, Cy-C*H*$_2$), 1.34 - 1.46 (4H, m, Cy-C*H*$_2$), 1.70 - 1.79 (1H, m, Cy-C*H*$_2$), 1.80 - 1.92 (4H, m, Cy-C*H*$_2$), 2.41 - 2.59 (1H, m, Cy-C*H*), 3.04 (1H, dd, *J* = 11.2, 8.7 Hz, H4$_A$), 3.32 (1H, dd, *J* = 11.2, 6.9 Hz, H4$_B$), 4.56 (1H, app t, *J* = 7.8 Hz, H5 CD), 4.89 (1H, app t, *J* = 7.7 Hz, H5 CD), 6.26 (1H, s, H2 AB), 6.35 (1H, s, H2 CD), 7.20 (2H, d, *J* = 8.6 Hz, H3″), 7.29 - 7.35 (1H, m, H4′), 7.36 - 7.42 (2H, m, H3′), 7.46 - 7.53 (4H, m, H2′ and H2″), 7.98 (1H, br. s., OH), 9.28 (1H, br. s., NH); δ_C (125.8 MHz, CD_2Cl_2): 26.7 (Cy-*C*H$_2$), 27.4 (Cy-*C*H$_2$), 33.0 (C4), 35.0 (Cy-*C*H$_2$), 44.6 (Cy-*C*H), 62.2 (C2 AB), 63.1 (C2 CD), 67.2 (C5 AB), 71.8 (C5 CD), 99.3 (C7), 121.0 (C2″), 126.9 (C2′), 127.9 (C3″), 128.7 (C4′), 129.2 (C3′), 134.8 (C1″), 140.9 (C1′), 145.8 (C4″), 164.4 (C9 AB), 165.4 (C9 CD), 172.1 (C8 AB), 178.6 (C8 CD), 184.9 (C6 AB), 191.7 (C6 CD); *m/z* (ESI^-) 433 ($[M-H]^-$, 34%); HRMS (ESI^-); $C_{25}H_{25}N_2O_3S$ $[M-H]^-$; found 433.1598, requires 433.1591.

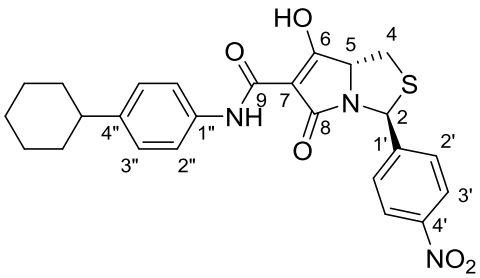
(-)-(2*S*,5*R*)-1-Aza-7-(4-cyclohexylphenylaminocarbonyl)-2-(4-fluorophenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 116



Yield (0.17 g, 45 %); brown oil; 13:1 AB:CD tautomers; R_f = 0.66 (EtOAc 100 %); $[\alpha]_D^{25}$ = -220.5 (*c* = 0.20, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1228 (C-F), 1636 (C=C), 1648 (C=O), 1689 (C=O), 3290, 3307 (N-H/O-H); δ_H (400 MHz, CD_2Cl_2): 1.21 - 1.32 (1H, m, Cy-C*H*$_2$), 1.33 - 1.48 (4H, m, Cy-C*H*$_2$), 1.70 - 1.79 (1H, m, Cy-C*H*$_2$), 1.80 - 1.91 (4H, m, Cy-C*H*$_2$), 2.45 - 2.56 (1H, m, Cy-C*H*), 3.04

(1H, dd, *J* = 11.2, 8.7 Hz, $H4_A$), 3.32 (1H, dd, *J* = 11.2, 6.9 Hz, $H4_B$), 4.53 (1H, app t, *J* = 7.8 Hz, H5 CD), 4.87 (1H, app t, *J* = 7.7 Hz, H5 AB), 6.23 (1H, s, H2 AB), 6.33 (1H, s, H2 CD), 7.08 (2H, app t, *J* = 8.6 Hz, H3′), 7.20 (2H, d, *J* = 8.3 Hz, H3″), 7.43 - 7.52 (4H, m, H2′ and H2″), 9.26 (1H, s, NH), 9.45 (1H, br. s., OH); δ_C (100.6 MHz, CD_2Cl_2): 26.7 (Cy-*C*H_2), 27.4 (Cy-*C*H_2), 33.0 (C4), 35.0 (Cy-*C*H_2), 44.6 (Cy-*C*H), 61.7 (C2), 67.1 (C5), 99.3 (C7), 116.0 (d, *J* = 21.5 Hz, C3′), 121.0 (C2″), 127.9 (C3″), 128.9 (d, *J* = 8.0 Hz, C2′), 134.7 (C1″), 136.7 (d, *J* = 3.2 Hz, C1′), 145.8 (C4″), 163.1 (d, *J* = 246.4 Hz, C4′), 164.3 (C9), 172.2 (C8), 185.0 (C6); *m/z* (ESI^-) 451 ([M-H]$^-$, 5%); HRMS (ESI^-); $C_{25}H_{24}FN_2O_3S$ [M-H]$^-$; found 451.1501, requires 451.1497.

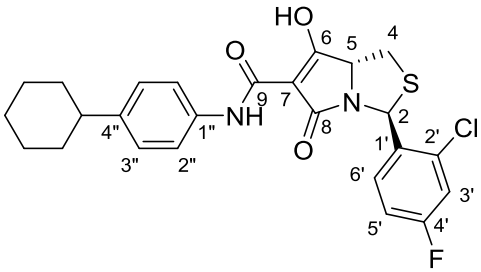
(-)-(2*S*,5*R*)-1-Aza-7-(4-cyclohexylphenylaminocarbonyl)-6-hydroxy-2-(4-nitrophenyl)-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 117



Yield (76 mg, 59 %); brown solid, mp 108-110 °C; 12.5:1 AB:CD tautomers; R_f = 0.46 (EtOAc 100 %); $[\alpha]_D^{25}$ = -89.5 (*c* = 0.20, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1347 (sym $ArNO_2$), 1522 (asym $ArNO_2$), 1630 (C=C), 1651 (C=O), 1695 (C=O), 3323 (N-H/O-H); δ_H (400 MHz, CD_2Cl_2): 1.18 - 1.32 (1H, m, Cy-C*H*$_2$), 1.33 - 1.48 (4H, m, Cy-C*H*$_2$), 1.69 - 1.78 (1H, m, Cy-C*H*$_2$), 1.78 - 1.90 (4H, m, Cy-C*H*$_2$), 2.46- 2.54 (1H, m, Cy-C*H*), 3.10 (1H, dd, *J* = 11.3, 8.6 Hz, $H4_A$), 3.34 (1H, dd, *J* = 11.3, 7.1 Hz, $H4_B$), 4.53 (1H, app t, *J* = 7.8 Hz, H5 CD), 4.87 (1H, dd, *J* = 8.3, 7.1 Hz, H5 AB), 6.32 (1H, s, H2 AB), 6.42 (1H, s, H2 CD), 7.21 (2H, d, *J* = 8.3, H3″), 7.47 (2H, d, *J* = 8.3 Hz, H2″), 7.66 (2H, d, *J* = 8.8 Hz, H2′), 7.89 (1H, br. s., OH), 8.22 (2H, d, *J* = 8.8 Hz, H3′), 9.20 (1H, br. s., NH); δ_C (125.8 MHz, CD_2Cl_2): 26.7 (Cy-*C*H_2), 27.4 (Cy-*C*H_2), 33.2 (C4 AB), 33.6 (C4 CD), 35.0 (Cy-*C*H_2), 44.6 (Cy-*C*H), 61.6 (C2 AB), 62.5 (C2 CD), 67.2 (C5 AB), 71.6 (C5 CD), 86.3 (C7 CD), 99.0 (C7 AB), 121.0 (C2″), 124.4 (C3′), 127.9 (C2′), 127.9 (C3″), 134.6 (C1″), 146.0 (C4″), 148.0 (C1′), 148.3 (C4′), 164.3 (C9

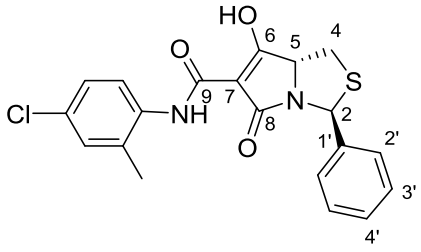
AB), 165.3 (C9 CD), 172.4 (C8 AB), 178.7 (C8 CD), 185.4 (C6 AB), 191.5 (C6 CD); *m/z* (ESI$^-$) 478 ([M-H]$^-$, 12%); HRMS (ESI$^-$); $C_{25}H_{24}N_3O_5S$ [M-H]$^-$; found 478.1437, requires 478.1442.

(-)-(2*S*,5*R*)-1-Aza-2-(2-chloro-4-fluorophenyl)-7-(4-cyclohexylphenylaminocarbonyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 118



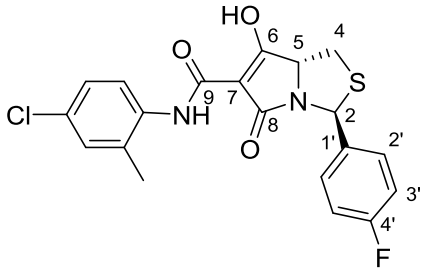
Yield (0.41 g, 73 %); yellow foam, mp 100-102 °C; 15:1 AB:CD tautomers; R_f = 0.57 (EtOAc 100 %); $[\alpha]_D^{25}$ = -184.8 (*c* = 0.40, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1234 (C-F), 1654 (C=O), 1694 (C=O); δ_H (400 MHz, CD_2Cl_2): 1.18 - 1.32 (1H, m, Cy-C*H*$_2$), 1.33 - 1.48 (4H, m, Cy-C*H*$_2$), 1.68 - 1.77 (1H, m, Cy-C*H*$_2$), 1.78 - 1.91 (4H, m, Cy-C*H*$_2$), 2.41 - 2.57 (1H, m, Cy-C*H*), 3.05 (1H, dd, *J* = 11.0, 9.1 Hz, H4$_A$), 3.32 (1H, dd, *J* = 11.0, 6.9 Hz, H4$_B$), 4.67 (1H, app t , *J* = 7.5 Hz, H5 CD), 5.00 (1H, dd, *J* = 9.1, 6.9 Hz, H5 AB), 6.43 (1H, s, H2 AB), 6.43 (1H, s, H2 CD), 7.05 (1H, app td, *J* = 8.3, 2.7 Hz, H5′), 7.16 - 7.23 (3H, m, H3′ + H3″), 7.44 - 7.51 (3H, m, H6′ + H2″), 9.22 (1H, br. s., NH), 10.04 (1H, br. s., OH); δ_C (125.8 MHz, CD_2Cl_2): 26.7 (Cy-*C*H$_2$), 27.4 (Cy-*C*H$_2$), 32.8 (C4), 35.0 (Cy-*C*H$_2$), 44.6 (Cy-*C*H), 59.3 (C2 AB), 60.3 (C2 CD), 67.8 (C5 AB), 72.4 (C5 CD), 86.2 (C7 CD), 98.9 (C7 AB), 115.0 (d, *J* = 20.7 Hz, C5′), 117.8 (d, *J* = 25.4 Hz, C3′), 121.0 (C2″), 127.9 (C3″), 128.2 (d, *J* = 9.5 Hz, C6′), 133.5 (d, *J* = 10.3 Hz, C2′), 134.7 (C1″), 135.1 (d, *J* = 2.4 Hz, C1′), 145.9 (C4″), 162.6 (d, *J* = 249.9 Hz, C4′), 164.3 (C9 AB), 165.4 (C9 CD), 171.7 (C8 AB), 178.2 (C8 CD), 185.2 (C6 AB), 191.5 (C6 CD); *m/z* (ESI$^-$) 485 ([M-H]$^-$, 100%); HRMS (ESI$^-$); $C_{25}H_{23}ClFN_2O_3S$ [M-H]$^-$; found 485.1125, requires 485.1107.

(-)-(2*S*,5*R*)-1-Aza-7-(4-chloro-2-methylphenylaminocarbonyl)-6-hydroxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 119



Yield (0.13 g, 40 %); yellow solid, mp 88 °C; R_f = 0.63 (EtOAc/MeOH 12:1); $[\alpha]_D^{25}$ = -145.0 (*c* = 0.20, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1636 (C=C), 1691 (C=O), 3210, 3266 (N-H/O-H); δ_H (400 MHz, $CDCl_3$): 2.34 (3H, s, C*H*$_3$), 3.06 (1H, dd, *J* = 11.1, 8.4 Hz, $H4_A$), 3.34 (1H, dd, *J* = 11.1, 7.0 Hz, $H4_B$), 4.50 (1H, br. s., OH), 4.90 (1H, dd, *J* = 8.4, 7.0 Hz, H5), 6.31 (1H, s, H2), 7.17-7.23 (2H, m, Ar-C*H*), 7.29 - 7.35 (1H, m, H4'), 7.36 - 7.42 (2H, m, H3'), 7.47 - 7.52 (2H, m, H2'), 8.04 (1H, d, *J* = 9.4 Hz, Ar-C*H*), 9.39 (1H, br. s., NH); δ_C (100.6 MHz, CD_2Cl_2): 17.9 (*C*H$_3$), 32.9 (C4), 62.1 (C2), 67.2 (C5), 99.8 (C7), 123.0, 127.0, 130.8 (Ar-*C*H), 126.8 (C2'), 128.8 (C4'), 129.2 (C3'), 130.1, 130.8, 134.4 (Ar-C), 140.2 (C1'), 164.1 (C9), 172.1 (C8), 184.3 (C6); *m/z* (ESI^-) 399 ($[M-H]^-$, 100%); HRMS (ESI^-); $C_{20}H_{16}ClN_2O_3S$ $[M-H]^-$; found 399.0576, requires 399.0576.

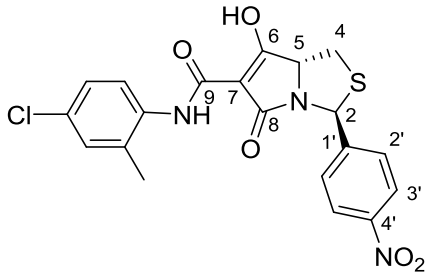
(-)-(2*S*,5*R*)-1-Aza-7-(4-chloro-2-methylphenylaminocarbonyl)-2-(4-fluorophenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 120



Yield (82 mg, 40 %); brown solid, mp 126 °C; R_f = 0.57 (EtOAc/MeOH 12:1); $[\alpha]_D^{25}$ = -165.14 (*c* = 0.39, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1226 (C-F), 1631 (C=C), 1655 (C=O), 1690 (C=O); δ_H (400 MHz, CD_2Cl_2): 2.31 (3H, s, C*H*$_3$), 3.05 (1H, dd, *J* = 11.0, 8.9 Hz, $H4_A$), 3.33 (1H, dd, *J* = 11.0, 6.9 Hz, $H4_B$), 4.83 - 4.92 (1H, m, H5), 6.23 (1H, s, H2), 7.09 (2H, app t, *J* = 8.4 Hz, H3′), 7.16 - 7.24 (2H, m, Ar-C*H*), 7.49 (2H, dd, *J* = 8.3, 5.3 Hz, H2′), 8.04 (1H, d, *J* = 9.3 Hz, Ar-C*H*), 9.39 (1H, br. s., NH), 11.00 (1H, br. s., OH); δ_C (125.8 MHz, $CDCl_3$): 17.6 (*C*H$_3$), 32.4 (C4), 61.1 (C2), 66.4 (C5), 99.2 (C7), 115.7 (d, *J* = 21.9 Hz, C3′), 122.7, 126.7, 130.4 (Ar-*C*H), 128.3 (d, *J* = 8.6 Hz, C2′), 130.0, 130.1, 133.4 (Ar-C), 135.5 (d, *J* = 3.8 Hz, C1′),

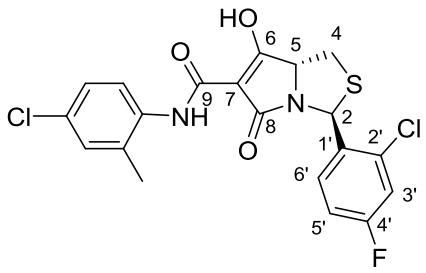
162.6 (d, *J* = 248.0 Hz, C4′), 163.6 (C9), 171.7 (C8), 184.1 (C6); *m/z* (ESI$^-$) 417 ([M-H]$^-$, 76%); HRMS (ESI$^-$); $C_{20}H_{15}ClFN_2O_3S$ [M-H]$^-$; found 417.0484, requires 417.0481.

(-)-(2*S*,5*R*)-1-Aza-7-(4-chloro-2-methylphenylaminocarbonyl)-6-hydroxy-2-(4-nitrophenyl)-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 121



Yield (56 mg, 37 %); brown solid, mp 190 °C; R_f = 0.62 (EtOAc/MeOH 12:1); $[\alpha]_D^{25}$ = -94.0 (*c* = 0.20, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1347 (sym $ArNO_2$), 1522 (asym $ArNO_2$), 1634 (C=C), 1693 (C=O); δ_H (400 MHz, CD_2Cl_2): 2.32 (3H, s, C*H*$_3$), 3.11 (1H, dd, *J* = 11.2, 8.4 Hz, H4$_A$), 3.36 (1H, dd, *J* = 11.2, 7.0 Hz, H4$_B$), 4.92 (1H, dd, *J* = 8.4, 7.0 Hz, H5), 5.02 (1H, br. s., OH), 6.32 (1H, s, H2), 7.18 - 7.24 (2H, m, Ar-C*H*), 7.67 (2H, d, *J* = 8.8 Hz, H2′), 8.03 (1H, d, *J* = 8.6 Hz, Ar-C*H*), 8.23 (2H, d, *J* = 8.8 Hz, H3′), 9.32 (1H, br. s., NH); δ_C (125.8 MHz, CD_2Cl_2): 17.9 (*C*H$_3$), 33.2 (C4), 61.5 (C2), 67.2 (C5), 99.7 (C7), 123.3, 127.1, 130.9 (Ar-*C*H), 124.5 (C3′), 127.9 (C2′), 130.5, 131.0, 134.2 (Ar-C), 147.9 (C1′), 148.3 (C4′), 164.2 (C9), 172.4 (C8), 184.9 (C6); *m/z* (ESI$^-$) 444 ([M-H]$^-$, 69%); HRMS (ESI$^-$); $C_{20}H_{15}ClN_3O_5S$ [M-H]$^-$; found 444.0426, requires 444.0426.

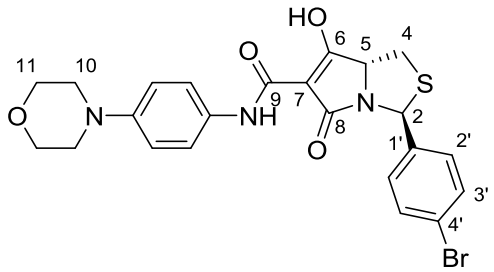
(-)-(2*S*,5*R*)-1-Aza-2-(2-chloro-4-fluorophenyl)-7-(4-chloro-2-methylphenylaminocarbonyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 122



Yield (60 mg, 35 %); brown solid, mp 108-110 °C; R_f = 0.63 (EtOAc/MeOH 12:1); $[\alpha]_D^{25}$ = -218.0 (*c* = 0.20, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1235 (C-F), 1635 (C=C), 1697 (C=O); δ_H (400 MHz, CD_2Cl_2): 2.31 (3H, s, C*H*$_3$), 3.06 (1H, dd, *J* = 11.0, 9.3 Hz, H4$_A$), 3.34 (1H, dd, *J* = 11.0, 6.7 Hz, H4$_B$), 5.04 (1H, dd, *J* = 9.3, 6.7 Hz, H5), 6.42 (1H, s, H2), 6.54 (1H, br. s., OH), 7.06 (1H, app td, *J* = 8.3, 2.7 Hz, H5′),

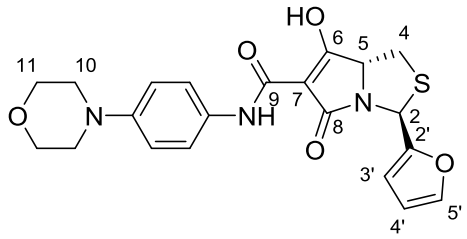
7.16 - 7.24 (3H, m, H3′ + Ar-C*H*), 7.48 (1H, dd, *J* = 8.8, 5.9 Hz, H6′), 8.04 (1H, d, *J* = 8.3 Hz, Ar-C*H*), 9.35 (1H, br. s., NH); δ_C (125.8 MHz, CD_2Cl_2): 17.9 (*C*H_3), 32.8 (C4), 59.2 (C2), 67.9 (C5), 99.6 (C7), 115.1 (d, *J* = 21.5 Hz, C5′), 117.8 (d, *J* = 24.6 Hz, C3′), 123.2, 127.1, 130.8 (Ar-*C*H), 128.1 (d, *J* = 8.7 Hz, C6′), 130.3, 130.9, 134.3 (Ar-C), 133.5 (d, *J* = 10.3 Hz, C2′), 135.0 (d, *J* = 4.0 Hz, C1′), 162.6 (d, *J* = 249.9 Hz, C4′), 164.3 (C9), 171.7 (C8), 184.7 (C6); *m/z* (ESI⁻) 451 ([M-H]⁻, 100%); HRMS (ESI⁻); $C_{20}H_{14}Cl_2FN_2O_3S$ [M-H]⁻; found 451.0080, requires 451.0092.

(-)-(2*S*,5*R*)-1-Aza-2-(4-bromophenyl)-6-hydroxy-7-(4-morpholinophenylaminocarbonyl)-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 123



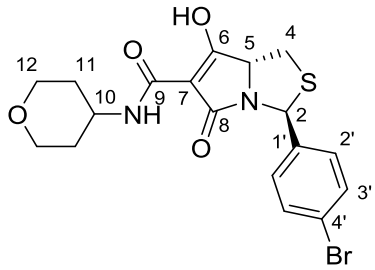
Yield (0.15 g, 24 %); brown solid, mp 116 °C; R_f = 0.29 (EtOAc/MeOH 12:1); $[\alpha]_D^{25}$ = -154.5 (*c* = 0.22, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1627 (C=C), 1686 (C=O); δ_H (400 MHz, CD_2Cl_2): 3.04 (1H, dd, *J* = 11.2, 8.4 Hz, $H4_A$), 3.12 (4H, br. t, *J* = 4.7 Hz, H10), 3.30 (1H, dd, *J* = 11.2, 7.1 Hz, $H4_B$), 3.82 (4H, br. t, *J* = 4.7 Hz, H11), 4.80 (1H, br signal, H5), 6.22 (1H, s, H2), 6.90 (2H, d, *J* = 8.8 Hz, morpholinophenyl Ar-C*H*), 7.37 (2H, d, *J* = 8.3 Hz, H2′), 7.45 (2H, d, *J* = 8.8 Hz, morpholinophenyl Ar-C*H*), 7.51 (2H, d, *J* = 8.3 Hz, H3′), 8.08 (1H, br. s., NH/OH), 9.20 (1H, br. s., NH/OH); δ_C (125.8 MHz, CD_2Cl_2): 33.1 (C4), 49.9 (C10), 61.9 (C2), 67.3 (C5 + C11), 98.9 (C7), 116.4, 122.3 (morpholinophenyl Ar-*C*H), 122.5 (C4′), 128.8 (C2′), 129.3, 149.4 (morpholinophenyl Ar-C), 132.3 (C3'), 140.1 (C1′), 164.1 (C9), 172.4 (C8), 185.3 (C6); *m/z* (ESI⁻) 514.0, 516.0 ([M-H]⁻, 100%); HRMS (ESI⁻); $C_{23}H_{21}O_4N_3BrS$ [M-H]⁻; found 514.04451 and 516.04235, requires 514.04416, 516.04212.

(-)-(2*S*,5*R*)-1-Aza-2-(2-furanyl)-6-hydroxy-7-(4-morpholinophenylaminocarbonyl)-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 124



Yield (0.11 g, 85 %); brown solid, mp 110 °C; R_f = 0. 47 (EtOAc/MeOH 12:1); $[\alpha]_D^{25}$ = -153.3 (*c* = 0.06, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1630 (C=C), 1688 (C=O), 3274 (N-H/O-H); δ_H (400 MHz, CD_2Cl_2): 3.06 - 3.11 (1H, m, $H4_A$, obscured by H10), 3.12 (4H, br. t, *J* = 4.8 Hz, H10), 3.46 (1H, dd, *J* = 11.0, 8.1 Hz, $H4_B$), 3.82 (4H, br. t, *J* = 4.7 Hz, H11), 4.85 (1H, broad signal, H5), 6.30 (1H, s, H2), 6.34 - 6.40 (2H, m, H3' + H4'), 6.90 (2H, d, *J* = 9.0 Hz, morpholinophenyl Ar-C*H*), 7.41 - 7.49 (3H, m, H5' + morpholinophenyl Ar-C*H*), 8.38 (1H, br. s., NH/OH), 9.18 (1H, br. s., NH/OH); δ_C (125.8 MHz, CD_2Cl_2): 32.5 (C4), 49.9 (C10), 55.7 (C2), 66.4 (C5), 67.3 (C11), 98.4 (C7), 107.7 (H3'), 111.0 (H4'), 116.4, 122.3 (morpholinophenyl Ar-*C*H), 129.3, 149.4 (Morpholinophenyl Ar-C), 143.6 (C5'), 153.2 (C2′), 164.1 (C9), 172.2 (C8), 186.1 (C6); *m/z* (ESI^-) 426 ($[M-H]^-$, 100%); HRMS (ESI^-); $C_{21}H_{20}O_5N_3S$ $[M-H]^-$; found 426.11310, requires 426.11291.

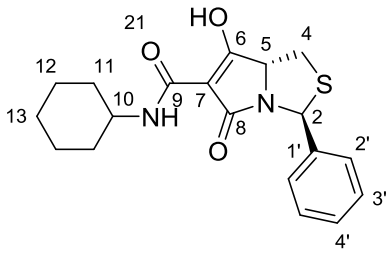
(-)-(2*S*,5*R*)-1-Aza-2-(4-bromophenyl)-6-hydroxy-8-oxo-7-(tetrahydro-2*H*-pyran-4-aminocarbonyl)-3-thia-bicyclo[3.3.0]oct-6-ene, 125



Yield (0.63 g, 55 %); yellow solid, mp 90-92 °C, 5.2:1 AB:CD tautomers; R_f = 0.37 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -171.9 (*c* = 0.385, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1623 (C=C), 1647 (C=O), 1688 (C=O), 3319 (O-H/N-H); δ_H (400 MHz, CD_2Cl_2): 1.50 - 1.71 (2H, m, H11), 1.83 - 1.98 (2H, m, H11), 3.01 (1H, dd, *J* = 11.3, 8.3 Hz, $H4_A$), 3.27 (1H, dd, *J* = 11.3, 7.1 Hz, $H4_B$), 3.41 - 3.52 (2H, m, H12), 3.88 - 3.99 (2H, m, H12), 4.00 - 4.11 (1H, m, H10), 4.42 (1H, app t, *J* = 7.8 Hz, H5 CD), 4.75 (1H, app t, *J* = 7.7 Hz, H5 AB), 6.18 (1H, s, H2 AB), 6.27 (1H, s, H2 CD), 6.89 (2H, br. s., NH + OH), 7.35 (2H, d, *J* = 8.3 Hz, H2'), 7.50 (2H, d, *J* = 8.3 Hz, H3'); δ_C (125.8 MHz, CD_2Cl_2): 33.0 (C4), 33.3 (C11), 45.9 (C10 AB), 47.7 (C10 CD), 62.0 (C2 AB), 62.7 (C2 CD), 66.9 (C12), 67.4 (C5 AB), 71.4 (C5

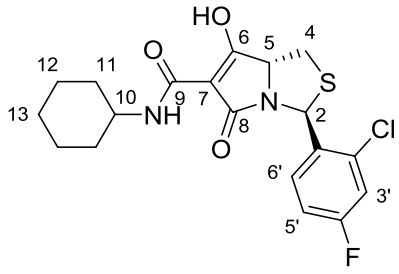
CD), 85.5 (C7 CD), 97.2 (C7 AB), 122.4 (C4'), 128.8 (C2'), 132.2 (C3'), 140.3 (C4'), 165.9 (C9 AB), 166.5 (C9 CD), 172.5 (C8 AB), 178.8(C8 CD), 186.6 (C6 AB), 191.6 (C6 CD); *m/z* (ESI$^-$) 437, 439 ([M-H]$^-$, 100 %); HRMS (ESI$^-$); $C_{18}H_{18}N_2O_4BrS$ [M-H]$^-$; found 437.01407 and 439.01190, requires 437.01761 and 439.01557.

(-)-(2*S*,5*R*)-1-Aza-7-(cyclohexylaminocarbonyl)-6-hydroxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 126



Yield (0.29 g, 49 %); brown solid, mp 54 °C; R_f = 0.77 (EtOAc/MeOH 96:4); $[\alpha]_D^{25}$ = -261.5 (*c* = 0.20, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1619 (C=C), 1645 (C=O), 1686 (C=O), 3324 (N-H/O-H); δ_H (500 MHz, Methanol-d_4) 1.23 - 1.47 (5H, m, 2 x H11, 2 x H12, H13), 1.57 - 1.67 (1H, m, H13), 1.70 - 1.82 (2H, m, H12), 1.86 - 1.96 (2H, m, H11), 3.01 (1H, dd, *J* = 11.0, 8.4 Hz, $H4_A$), 3.27 - 3.30 (1H, m, $H4_B$, obscured by solvent peak), 3.79 - 3.89 (1H, m, H10), 4.82 (1H, app t, *J* = 7.7 Hz, H5), 6.24 (1H, s, H2), 7.26 - 7.29 (1H, m, H4'), 7.32 - 7.37 (2H, m, H3'), 7.44 - 7.48 (2H, m, H2'); δ_C (125.8 MHz, Methanol-d_4): 25.8 (C12), 26.6 (C13), 33.4 (C4), 33.7 (C11), 49.8 (C10), 63.5 (C2), 69.4 (C5), 95.3 (C7), 127.6 (C2'), 129.1 (C4'), 129.7 (C3'), 142.3 (C1'), 166.1 (C9), 175.5 (C8), 188.6 (C6); *m/z* (ESI$^-$) 357 ([M-H]$^-$, 100 %); HRMS (ESI$^+$); $C_{19}H_{23}O_3N_2S$ [M+H]$^+$; found 359.14265, requires 359.14239.

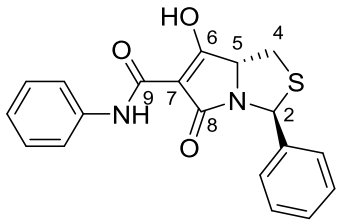
(-)-(2*S*,5*R*)-1-Aza-2-(2-chloro-4-fluorophenyl)-7-(cyclohexylaminocarbonyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 127



Yield (0.16 g, 40 %); yellow foam; mp 80-82 °C; R_f = 0.70 (EtOAc/MeOH 96:4); $[\alpha]_D^{25}$ = -291.6 (*c* = 0.27, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1620 (C=C), 1646 (C=O), 1689 (C=O), 3326 (NH/OH); δ_H (400 MHz, Methanol-d_4) 1.23 - 1.47 (5H, m, 2

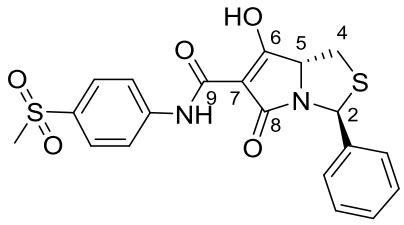
x H11, 2 x H12, H13), 1.57 - 1.67 (1H, m, H13), 1.70 - 1.82 (2H, m, H12), 1.86 - 1.96 (2H, m, H11), 3.03 (1H, dd, *J* = 10.8, 8.8 Hz, $H4_A$), 3.27 - 3.33 (1H, m, $H4_B$, obscured by solvent peak), 3.79 - 3.89 (1H, m, H10), 4.97 (1H, dd, *J* = 8.8, 7.2 Hz, H5), 6.40 (1H, s, H2), 7.11 (1H, app td, *J* = 8.4, 2.5 Hz, H5'), 7.26 (1H, dd, *J* = 8.6, 2.5 Hz, H3'), 7.58 (1H, dd, *J* = 8.6, 6.0 Hz, H6'); δ_C (125.8 MHz, Methanol-d_4): 25.8 (C12), 26.5 (C13), 33.0 (C4), 33.7 (C11), 49.6 (C10), 60.5 (C2), 70.2 (C5), 94.6 (C7), 115.6 (d, *J* = 21.9 Hz, C5′), 118.1 (d, *J* = 24.8 Hz, C3′), 129.3 (d, *J* = 9.5 Hz, C6′), 134.1 (d, *J* = 10.5 Hz, C2′), 136.8 (d, *J* = 3.8 Hz, C1′), 163.5 (d, *J* = 248.9 Hz, C4′), 166.1 (C9), 175.2 (C8), 189.2 (C6); *m/z* (ESI^-) 409 ($[M-H]^-$, 100 %); HRMS (ESI^-); $C_{19}H_{19}O_3N_2ClFS$ $[M-H]^-$; found 409.07999, requires 409.07835.

(-)-(2*S*,5*R*)-1-Aza-6-hydroxy-8-oxo-2-phenyl-7-(phenylaminocarbonyl)-3-thiabicyclo[3.3.0]oct-6-ene, 129



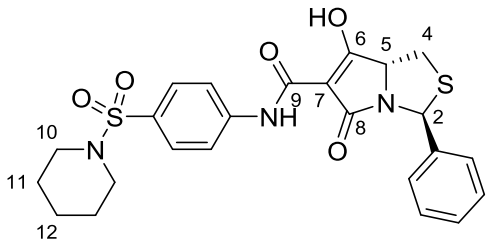
Yield (0.16 g, 55 %); brown solid, mp 156-160 °C; R_f = 0.51 (EtOAc/MeOH 98:2); $[\alpha]_D^{25}$ = -72.5 (*c* = 1.0, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1633 (C=C), 1651 (C=O), 1691 (C=O), 3289 (N-H/O-H); δ_H (400 MHz, CD_2Cl_2): 3.05 (1H, dd, *J* = 11.1, 8.6 Hz, $H4_A$), 3.33 (1H, dd, *J* = 11.1, 6.9 Hz, $H4_B$), 4.90 (1H, app t, *J* = 7.7 Hz, H5), 6.28 (1H, s, H2), 7.13 - 7.19 (1H, m, Ar-C*H*), 7.30 - 7.42 (5H, m, Ar-C*H*), 7.46 - 7.52 (2H, m, Ar-C*H*), 7.57 - 7.63 (2H, m, Ar-C*H*), 9.42 (1H, br. s., OH/NH), 9.69 (1H, br. s., OH/NH); δ_C (125.8 MHz, CD_2Cl_2): 33.1 (C4), 62.4 (C2), 67.6 (C5), 98.4 (C7), 120.9, 125.3, 126.9, 128.7, 129.2, 129.6 (Ar-*C*H), 137.4, 141.0 (Ar-C), 164.6 (C9), 172.8 (C8), 185.7 (C6); *m/z* (ESI^-) 351 ($[M-H]^-$, 100 %); HRMS (ESI^-); $C_{19}H_{15}O_3N_2S$ $[M-H]^-$; found 351.08051, requires 351.08089.

(-)-(2*S*,5*R*)-1-Aza-6-hydroxy-7-(4-(methylsulfonyl)phenylaminocarbonyl)-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 130



Yield (0.26 g, 40 %); yellow foam; mp 126-130 °C; R_f = 0.62 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -195.9 (*c* = 0.19, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1144, 1291 (S=O), 1621 (C=C), 1667 (C=O), 1687 (C=O); δ_H (500 MHz, $CDCl_3$): 3.03 - 3.09 (1H, m, $H4_A$, obscured by C*H*$_3$), 3.05 (3H, s, C*H*$_3$), 3.34 (1H, dd, *J* = 11.2, 7.2 Hz, $H4_B$), 4.91 (1H, dd, *J* = 8.3, 7.2 Hz, H5), 6.29 (1H, s, H2), 6.83 (1H, br. s, OH), 7.31 - 7.36 (1H, m, Ar-C*H*), 7.37 - 7.42 (2H, m, Ar-C*H*), 7.48 - 7.53 (2H, m, Ar-C*H*), 7.80 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.93 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 9.70 (1H, s, NH); δ_C (125.8 MHz, $CDCl_3$): 32.2 (C4), 44.6 (*C*H$_3$), 61.7 (C2), 66.2 (C5), 99.3 (C7), 120.1, 126.4, 128.4, 128.8, 128.9 (Ar-C*H*), 136.0, 139.4, 141.5 (Ar-*C*), 163.9 (C9), 171.2 (C8), 184.1 (C6); *m/z* (ESI^-) 429 ($[M-H]^-$, 100 %); HRMS (ESI^-); $C_{20}H_{17}O_5N_2S_2$ $[M-H]^-$; found 429.05888, requires 429.05844.

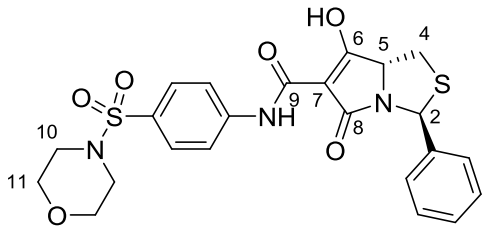
(-)-(2*S*,5*R*)-1-Aza-6-hydroxy-8-oxo-2-phenyl-7-(4-(piperidine-1-sulfonyl)phenylaminocarbonyl)-3-thiabicyclo[3.3.0]oct-6-ene, 131



Yield (0.24 g, 25 %); light brown foam; mp 126-130 °C; R_f = 0.35 (EtOAc: MeOH; 98:2); $[\alpha]_D^{25}$ = -192.3 (*c* = 0.16, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1162, 1338 (S=O), 1631 (C=C), 1651 (C=O), 1689 (C=O); δ_H (400 MHz, $CDCl_3$): 1.36 - 1.48 (2H, m, H12), 1.65 (4H, br. quin, *J* = 5.6 Hz, H11), 2.99 (4H, br. t, *J* = 5.4 Hz, H10), 3.06 (1H, dd, *J* = 11.3, 8.4 Hz, $H4_A$), 3.34 (1H, dd, *J* = 11.3, 7.1 Hz, $H4_B$), 4.92 (1H, dd, *J* = 8.4, 7.1 Hz, H5), 6.29 (1H, s, H2), 7.30 - 7.36 (1H, m, Ar-C*H*), 7.37 - 7.42 (2H, m, Ar-C*H*), 7.48 - 7.52 (2H, m, Ar-C*H*), 7.61 (1H, br. s., OH), 7.74 (2H, d, *J* = 9.8 Hz, Ar-C*H*), 7.75 (2H, d, *J* = 9.8 Hz, Ar-C*H*), 9.63 (1H, s, NH); δ_C (125.8 MHz, $CDCl_3$): 23.5 (C12), 25.1 (C11), 32.2 (C4), 46.9 (C10), 61.8 (C2), 66.2 (C5), 99.3 (C7), 119.8, 126.4, 128.4, 128.8,

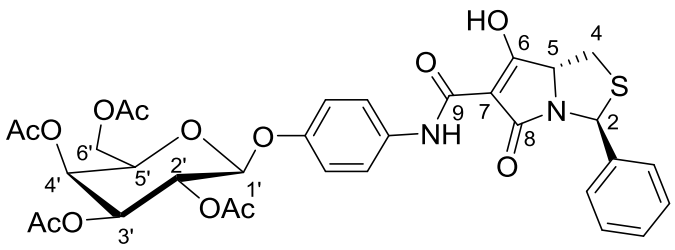
129.0 (Ar-C*H*), 131.9, 139.4, 140.5 (Ar-*C*), 163.9 (C9), 171.2 (C8), 184.1 (C6); *m/z* (ESI^-) 498 ($[M-H]^-$, 26 %); HRMS (ESI^-); $C_{24}H_{24}O_5N_3S_2$ $[M-H]^-$; found 498.11756, requires 498.11629.

(-)-(2*S*,5*R*)-1-Aza-6-hydroxy-7-(4-(morpholinosulfonyl)phenylaminocarbonyl)-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 132



Yield (0.14 g, 30 %); light brown solid; mp 118-120 °C; R_f = 0.47 (EtOAc/MeOH 96:4); $[\alpha]_D^{25}$ = -203.4 (*c* = 0.23, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1162, 1347 (S=O), 1629 (C=C), 1655 (C=O), 1688 (C=O); δ_H (400 MHz, $CDCl_3$): 3.00 (4H, br. t, *J* = 4.7 Hz, H10), 3.06 (1H, dd, *J* = 11.1, 8.6 Hz, $H4_A$), 3.34 (1H, dd, *J* = 11.1, 7.1 Hz, $H4_B$), 3.75 (4H, br. t, *J* = 4.7 Hz, H11), 4.92 (1H, dd, *J* = 8.6, 7.1 Hz, H5), 6.28 (1H, s, H2), 7.30 - 7.36 (1H, m, Ar-C*H*), 7.36 - 7.42 (2H, m, Ar-C*H*), 7.47 - 7.53 (2H, m, Ar-C*H*), 7.75 (2H, d, *J* = 9.0 Hz, Ar-C*H*), 7.78 (2H, d, *J* = 9.0 Hz, Ar-C*H*), 8.55 (1H, br. s, OH), 9.68 (1H, s, NH); δ_C (100.6 MHz, $CDCl_3$): 32.2 (C4), 45.9 (C10), 61.7 (C2), 66.0 (C11), 66.2 (C5), 99.3 (C7), 119.9, 126.4, 128.4, 128.8, 129.2 (Ar-C*H*), 130.5, 139.4, 141.0 (Ar-*C*), 163.9 (C9), 171.2 (C8), 184.0 (C6); *m/z* (ESI^-) 500 ($[M-H]^-$, 100 %); HRMS (ESI^-); $C_{23}H_{22}O_6N_3S_2$ $[M-H]^-$; found 500.09556, requires 500.09555.

(-)-(2*S*,5*R*)-1-Aza-6-hydroxy-8-oxo-2-phenyl-7-(4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)phenylaminocarbonyl)-3-thiabicyclo[3.3.0]oct-6-ene, 133

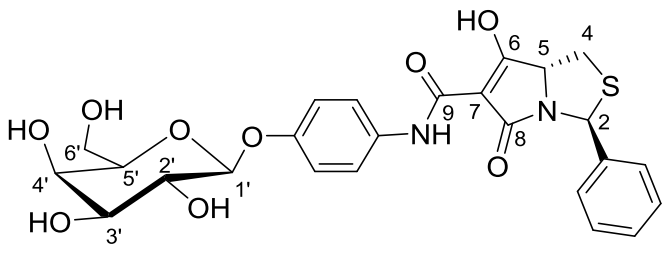


Yield (0.17 g, 60 %); yellow foam, mp 130-132 °C; R_f = 0.72 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -148.7 (*c* = 0.20, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1213 (C-O), 1647 (C=O, br with shoulder towards smaller wave number), 1689 (C=O), 1747 (C=O); δ_H (500

MHz, CD_2Cl_2): 1.99 (3H, s, C*H*$_3$-C(=O)-O-), 2.04 (3H, s, C*H*$_3$-C(=O)-O-), 2.06 (3H, s, C*H*$_3$-C(=O)-O-), 2.17 (3H, s, C*H*$_3$-C(=O)-O-), 3.03 (1H, dd, *J* = 11.1, 7.8 Hz, H4$_A$), 3.26 (1H, dd, *J* = 11.1, 7.4 Hz, H4$_B$), 4.04 - 4.09 (1H, m, H5'), 4.15 (1H, dd, *J* = 11.3, 6.0 Hz, H6'$_A$), 4.21 (1H, dd, *J* = 11.3, 7.2 Hz, H6'$_B$), 4.58 (1H, br signal, H5), 5.01 (1H, d, *J* = 8.0 Hz, H1'), 5.10 (1H, dd, *J* = 10.5, 3.6 Hz, H3'), 5.39 (1H, dd, *J* = 10.5, 8.0 Hz, H2'), 5.44 (1H, dd, *J* = 3.4, 0.9 Hz, H4'), 6.33 (1H, s, H2), 6.97 (2H, d, *J* = 9.0 Hz, Ar-C*H*), 7.26 - 7.32 (1H, m, Ar-C*H*), 7.33 - 7.40 (2H, m, Ar-C*H*), 7.45 - 7.49 (2H, m, Ar-C*H*), 7.51 (2H, d, *J* = 9.0 Hz, Ar-C*H*); δ_C (125.8 MHz, CD_2Cl_2): 20.9, 21.0, 21.0, 21.1 (4x*C*H$_3$-C(=O)-O-), 33.7 (C4), 62.0 (C6'), 63.4 (C2), 67.5 (C4'), 68.7 (C5), 69.1 (C2'), 71.4 (C3'), 71.7 (C5'), 100.7 (C1'), 118.1, 121.8, 126.8, 128.3, 129.0 (Ar-*C*H), 134.4, 142.0, 153.6 (Ar-C), 164.7 (C9), 169.9, 170.5, 170.7, 170.8 (CH$_3$-*C*(=O)-O-), 175.8 (C8), 189.0 (C6); *m/z* (ESI$^-$) 697 ([M-H]$^-$, 100 %); HRMS (ESI$^-$); $C_{33}H_{33}O_{13}N_2S$ [M-H]$^-$; found 697.16989, requires 697.16979.

(-)-(2*S*,5*R*)-1-Aza-7-(4-(β-D-galactopyranosyloxy)phenylaminocarbonyl)-6-hydroxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 134

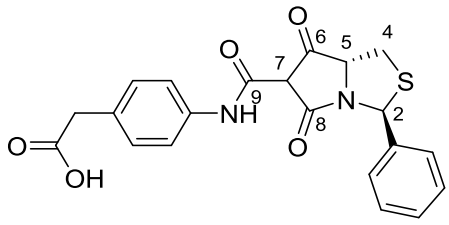
Tetramate **133** (20 mg, 0.03 mmol, 1.0 eq) was dissolved in MeOH (2 mL) and aq. K_2CO_3 (4.8 mg in 0.5 mL of H_2O, 0.034 mmol, 1.2 eq) was added. The reaction was stirred at rt for 10-15 min and upon completion, solvents were removed *in vacuo*. The residue was treated with MeOH, filtered and concentrated *in vacuo* to obtain **134**.



Yield (15 mg, 89 %); yellow solid, mp >250 °C; R_f = 0.10 (EtOAc/MeOH 3:1); $[\alpha]_D^{25}$ = -221.7 (*c* = 0.12, H_2O); ν_{max}/cm^{-1} (neat) 1215 (C-O), 1630 (C=C, br with shoulder towards larger wave number), 1664 (C=O), 3233 (O-H); δ_H (500 MHz, D_2O): 2.98 (1H, dd, *J* = 10.8, 8.5 Hz, H4$_A$), 3.24 (1H, dd, *J* = 10.8, 7.6 Hz, H4$_B$), 3.68 - 3.82 (5H, m, H2', H3', H5', 2xH6'), 3.95 (1H, app d, *J* = 3.0 Hz, H4'), 4.48 (1H, app t, *J* = 7.6 Hz, H5), 4.97 (1H,

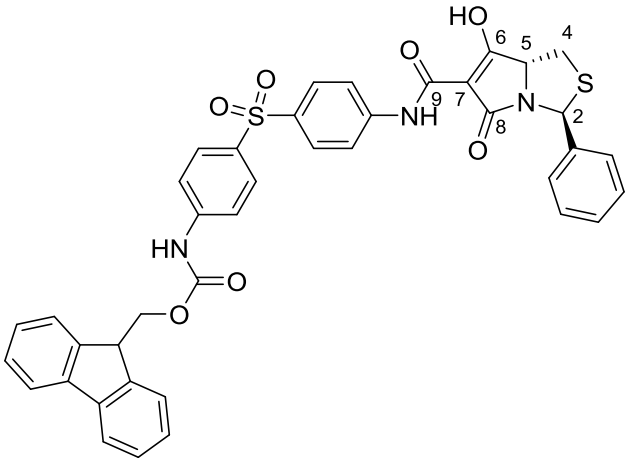
d, *J* = 7.6 Hz, H1'), 6.29 (1H, s, H2), 7.08 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.30 - 7.50 (7H, m, Ar-C*H*); δ_C (125.8 MHz, D_2O): 32.6 (C4), 60.7 (C6'), 62.8 (C2), 68.5 (C4'), 69.4 (C5), 70.5, 72.5, 75.3 (C2', C3', C5'), 93.0 (C7), 101.0 (C1'), 117.0, 123.4, 125.9, 128.0, 128.9 (Ar-*C*H), 132.6, 141.4, 153.3 (Ar-C), 165.0 (C9), 179.0 (C8), 193.6 (C6); *m/z* (ESI$^-$) 529 ([M-H]$^-$, 100 %); HRMS (ESI$^+$); $C_{25}H_{26}O_9N_2NaS$ $[M+Na]^+$; found 553.12523, requires 553.12512.

(-)-(2*S*,5*R*)-1-Aza-7-(4-(carboxymethyl)phenylaminocarbonyl)-6,8-dioxo-2-phenyl-3-thia-bicyclo[3.3.0]octane, 135



Yield (20 mg, 15 %); yellow solid, mp 200-202 °C; R_f = 0.14 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -93.9 (*c* = 0.093, (DMSO); ν_{max}/cm^{-1} (neat) 1660 (C=O), 1704 (C=O), 3060, 3248 (O-H/N-H); δ_H (500 MHz, Methanol-d_4): 2.98 (1H, dd, *J* = 11.0, 8.0 Hz, $H4_A$), 3.38 (1H, dd, *J* = 11.0, 7.0 Hz, $H4_B$), 3.60 (2H, s, -C$H_2$$CO_2$H), 4.93 (1H, dd, *J* = 8.0, 7.0 Hz, H5), 5.18 (1H, s, H7), 6.17 (1H, s, H2), 7.18 (2H, d, *J* = 8.7 Hz, Ar-C*H*), 7.25 - 7.29 (1H, m, Ar-C*H*), 7.31 (2H, d, *J* = 8.7 Hz, Ar-C*H*), 7.33 - 7.38 (2H, m, Ar-C*H*) 7.46 - 7.49 (2H, m, Ar-C*H*); δ_C (125.8 MHz, Methanol-d_4): 35.4 (C4), 41.4 (-*C*$H_2$$CO_2$H), 63.3 (C2), 70.2 (C5), 89.9 (C7), 120.9, 127.5, 129.0, 129.7, 131.6 (Ar-*C*H), 131.9, 140.7, 143.0 (Ar-C), 162.8 (C6 + C9), 175.7 (*C*O_2H), 180.4 (C8); *m/z* (ESI/FI) molecular ion not detected.

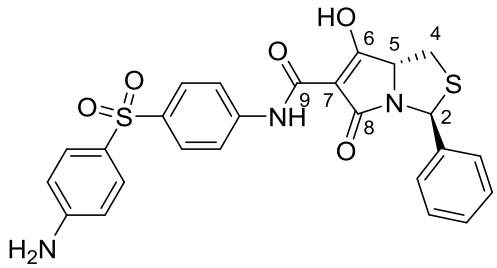
(-)-(2*S*,5*R*)-1-Aza-7-((4-((4-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)phenyl)sulfonyl)phenyl)aminocarbonyl)-6-hydroxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 136



Yield (0.32 g, 30 %); yellow solid, mp 170 °C; 1.3:1 tautomers; R_f = 0.59 (EtOAc/MeOH 98:2); $[\alpha]_D^{25}$ = -118.3 (*c* = 0.21, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1148, 1364 (S=O), 1629 (C=C), 1649 (C=O), 1690 (C=O), 1736 (C=O), 3302 (N-H/O-H); δ_H (500 MHz, Acetone-d_6) major tautomer: 3.13 - 3.25 (1H, m, $H4_A$), 3.45 (1H, dd, *J* = 10.9, 6.8 Hz, $H4_B$), 4.30 (1H, t, *J* = 6.5 Hz, OCH_2CH-), 4.54 (2H, d, *J* = 6.6 Hz, OCH_2CH-), 5.14 - 5.20 (1H, m, H5), 6.25 (1H, s, H2), 7.22 - 7.44 (8H, m, Ar-C*H*), 7.45 - 7.56 (3H, m, Ar-C*H*), 7.68 - 7.76 (4H, m, Ar-C*H*), 7.84 - 7.91 (4H, m, Ar-C*H*), 7.92 - 7.97 (2H, m, Ar-C*H*), 9.31 (1H, br. s, NH/OH), 9.91 (1H, br. s, NH/OH); minor tautomer: 2.99 - 3.05 (1H, m, $H4_A$), 3.40 (1H, dd, *J* = 11.0, 6.6 Hz, $H4_B$), 4.30 (1H, t, *J* = 6.5 Hz, OCH_2CH-), 4.55 (2H, d, *J* = 6.6 Hz, OCH_2CH-), 5.01 (1H, app t, *J* = 7.5 Hz, H5), 6.20 (1H, s, H2), 7.22 - 7.44 (8H, m, Ar-C*H*), 7.45 - 7.56 (3H, m, Ar-C*H*), 7.68 - 7.76 (4H, m, Ar-C*H*), 7.84 - 7.91 (4H, m, Ar-C*H*), 7.92 - 7.97 (2H, m, Ar-C*H*), 9.31 (1H, br. s, NH/OH), 9.91 (1H, br. s, NH/OH); δ_C (125.8 MHz, Acetone-d_6) major tautomer: 32.9 (C4), 47.9 (OCH_2CH-), 62.5 (C2), 67.4 (OCH_2CH-), 67.8 (C5), 94.4 (C7), 119.1, 119.2, 121.0, 126.0, 127.3, 127.4, 128.1, 128.7, 129.3, 129.5, 129.7, 129.8 (Ar-*C*H), 129.6, 130.2, 136.5, 138.6, 142.3, 144.9, 146.2 (Ar-C), 154.2 ($HNCO_2$), 164.4 (C9), 172.6 (C8), 185.0 (C6); minor tautomer: 35.4 (C4), 47.9 (OCH_2CH-), 62.9 (C2), 67.4 (OCH_2CH-), 69.6 (C5), 94.4 (C7), 119.1, 119.2, 121.0, 126.0, 127.3, 127.4, 128.1, 128.7, 129.3, 129.5, 129.7, 129.8 (Ar-*C*H), 129.6, 130.2, 136.5, 138.6, 142.3, 144.9, 146.2 (Ar-C), 154.2 ($HNCO_2$), 164.4 (C9), 176.6 (C8), 185.0 (C6); *m/z* (ESI/FI) molecular ion not detected.

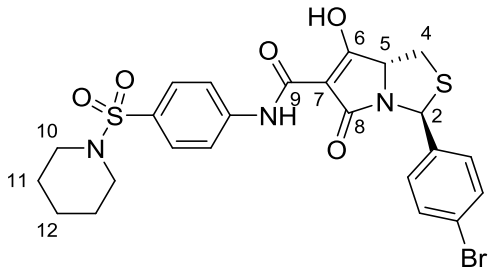
(-)-(2*S*,5*R*)-7-((4-((4-Aminophenyl)sulfonyl)phenyl)aminocarbonyl)-1-aza-6-hydroxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 137

Tetramate **136** (0.22 g, 0.31 mmol, 1.0 eq) was dissolved in DMF (2 mL) and piperidine (0.03 mL, 0.31 mmol, 1.0 eq) was added. The reaction was stirred at rt for 1 h until complete deprotection was observed by TLC. The crude was purified by silica gel flash column chromatography to obtain **137** (eluent: 100 % EtOAc to EtOAc/MeOH 9:1 with 1 % Et_3N).



Yield (62 mg, 40 %); brown solid, mp 152-156 °C; R_f = 0.56 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -209.4 (*c* = 0.20, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1146, 1366 (S=O), 1627 (C=C), 1654 (C=O), 1687 (C=O); δ_H (500 MHz, Acetone-d_6): 3.18 (1H, dd, *J* = 11.0, 8.9 Hz, $H4_A$), 3.45 (1H, dd, *J* = 11.0, 6.8 Hz, $H4_B$), 5.16 (1H, app t, *J* = 7.7 Hz, H5), 6.25 (1H, s, H2), 6.74 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.29 - 7.34 (1H, m, Ar-C*H*), 7.35 - 7.41 (2H, m, Ar-C*H*), 7.52 - 7.56 (2H, m, Ar-C*H*), 7.63 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.84 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.88 (2H, d, *J* = 8.8 Hz, Ar-C*H*); δ_C (125.8 MHz, Acetone-d_6): 32.9 (C4), 62.5 (C2), 67.8 (C5), 99.7 (C7), 114.3, 120.6, 127.4, 128.9, 129.2, 129.5, 130.5 (Ar-*C*H), 129.0, 140.1, 141.9, 142.0, 154.1 (Ar-C), 164.3 (C9), 172.7 (C8), 184.8 (C6); *m/z* (ESI^-) 506 ($[M-H]^-$, 47 %); HRMS (ESI^+); $C_{25}H_{22}N_3O_5S_2$ $[M+H]^+$; found 508.09978, requires 508.09954.

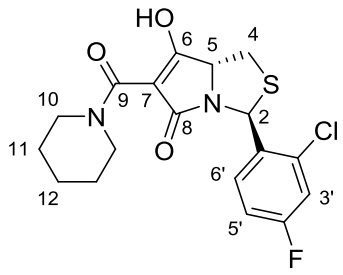
(-)-(2*S*,5*R*)-1-Aza-2-(4-bromophenyl)-6-hydroxy-8-oxo-7-(4-(piperidine-1-sulfonyl)phenylaminocarbonyl)-3-thiabicyclo[3.3.0]oct-6-ene, 138



Yield (0.57 g, 40 %); brown foam; mp 148-150 °C; R_f = 0.70 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -263.4 (*c* = 0.15, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1162,1337 (S=O), 1631 (C=C), 1650 (C=O), 1690 (C=O); δ_H (500 MHz, Acetone-d_6): 1.35 - 1.47 (2H, m, H12), 1.61 (4H, br. quin, *J* = 5.7 Hz, H11), 2.96 (4H,

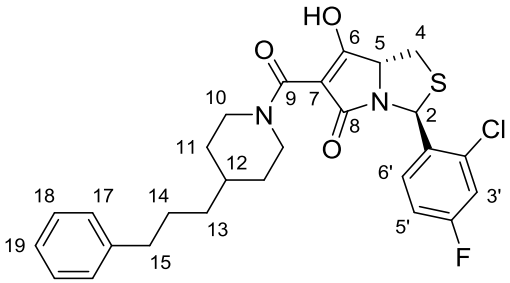
br. t, *J* = 5.4 Hz, H10), 3.21 (1H, dd, *J* = 11.0, 8.8 Hz, $H4_A$), 3.49 (1H, dd, *J* = 11.0, 7.0 Hz, $H4_B$), 3.78 (2H, br. s, N-H + O-H), 5.21 (1H, app t, *J* = 7.7 Hz, H5), 6.25 (1H, s, H2), 7.53 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.59 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.76 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.92 (2H, d, *J* = 8.8 Hz, Ar-C*H*); δ_C (125.8 MHz, Acetone-d_6): 24.2 (C12), 26.0 (C11), 32.9 (C4), 47.8 (C10), 61.9 (C2), 67.7 (C5), 99.9 (C7), 120.5, 129.6, 130.0, 132.5 (Ar-C*H*), 122.4, 132.7, 141.3, 142.1 (Ar-*C*), 164.3 (C9), 172.7 (C8), 184.8 (C6); *m/z* (ESI^-) 576, 578 ($[M-H]^-$, 100 %); HRMS (ESI^+); $C_{24}H_{25}O_5N_3BrS_2$ $[M+H]^+$; found 578.04135 and 580.03904 requires 578.04135 and 580.03930.

(-)-(2*S*,5*R*)-1-Aza-2-(2-chloro-4-fluorophenyl)-6-hydroxy-8-oxo-7-(piperidine-1-carbonyl)-3-thiabicyclo[3.3.0]oct-6-ene, 139



Yield (0.10 g, 30 %); light brown foam; mp 80-84 °C; R_f = 0.54 (EtOAc/MeOH 4:1); $[\alpha]_D^{25}$ = -320.5 (*c* = 0.15, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1649 (C=O), 1685 (C=O); δ_H (500 MHz, CD_2Cl_2): 1.60 - 1.74 (6H, m, H11, H12), 3.04 (1H, dd, *J* = 11.2, 8.4 Hz, $H4_A$), 3.27 (1H, dd, *J* = 11.2, 7.1 Hz, $H4_B$), 3.75 - 3.91 (4H, m, H10), 4.68 (1H, app t, *J* = 7.7 Hz, H5), 6.51 (1H, s, H2), 7.03 (1H, app td, *J* = 8.4, 2.5 Hz, H5'), 7.16 (1H, dd, *J* = 8.4, 2.5 Hz, H3'), 7.45 (1H, dd, *J* = 8.7, 6.0 Hz, H6'); δ_C (125.8 MHz, CD_2Cl_2): 24.6, 26.8 (C12, C11), 33.5 (C4), 47.9 (C10), 61.2 (C2), 69.8 (C5), 90.0 (C7), 114.8 (d, *J* = 21.9 Hz, C5′), 117.7 (d, *J* = 24.8 Hz, C3′), 128.2 (d, *J* = 9.5 Hz, C6′), 133.5 (d, *J* = 10.5 Hz, C2′), 135.8 (d, *J* = 3.8 Hz, C1′), 162.4 (d, *J* = 249.9 Hz, C4′), 166.1 (C9), 175.9 (C8), 192.7 (C6); *m/z* (ESI^-) 395 ($[M-H]^-$, 100 %); HRMS (ESI^+); $C_{18}H_{19}O_3N_2ClFS$ $[M+H]^+$; found 397.07851, requires 397.07835.

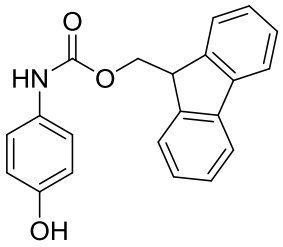
(-)-(2*S*,5*R*)-1-Aza-2-(2-chloro-4-fluorophenyl)-6-hydroxy-8-oxo-7-(4-(3-phenylpropyl)piperidine-1-carbonyl)-3-thiabicyclo[3.3.0]oct-6-ene, 140



Yield (0.15 g, 36 %); yellow foam; mp 78-80 °C; R_f = 0.34 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -175.8 (*c* = 0.27, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1686 (C=O); δ_H (500 MHz, CD_2Cl_2): 1.13 - 1.35 (3H, m, H11, H15), 1.53 - 1.69 (3H, m, H12, H14), 1.75 - 1.87 (2H, m, H11), 2.56 - 2.63 (1H, m, H15), 2.65 - 2.80 (1H, m, H13), 2.88 - 2.98 (2H, m, H10), 3.03 (1H, dd, *J* = 11.3, 8.1 Hz, $H4_A$), 3.25 (1H, dd, *J* = 11.3, 7.3 Hz, $H4_B$), 3.32 - 3.38 (1H, m, H13), 4.55 - 4.68 (3H, m, H5, H10), 6.50 (1H, s, H2), 7.02 (1H, app td, *J* = 8.4, 2.6 Hz, H5'), 7.13 - 7.20 (4H, m, H3', H17, H19), 7.23 - 7.29 (2H, m, H18), 7.45 (1H, dd, *J* = 8.8, 6.1 Hz, H6'); δ_C (125.8 MHz, CD_2Cl_2): 29.1 (C14), 33.0 (C11), 33.5 (C4), 36.1 (C12), 36.5 (C15), 44.9 (C13), 47.0 (C10), 61.3 (C2), 69.9 (C5), 91.2 (C7), 114.8 (d, *J* = 21.0 Hz, C5′), 117.7 (d, *J* = 24.8 Hz, C3′), 126.2 (C19), 128.2 (d, *J* = 8.6 Hz, C6′), 128.8 (C18), 128.9 (C17), 133.5 (d, *J* = 10.5 Hz, C2′), 136.0 (d, *J* = 2.9 Hz, C1′), 143.2 (C16), 162.4 (d, *J* = 249.9 Hz, C4′), 166.2 (C9), 175.9 (C8), 191.9 (C6); *m/z* (ESI^-) 513 ($[M-H]^-$, 100 %); HRMS (ESI^+); $C_{27}H_{29}O_3N_2ClFS$ $[M+H]^+$; found 515.15651, requires 515.15660.

9-Fluorenylmethyl (4-hydroxyphenyl)carbamate, 143

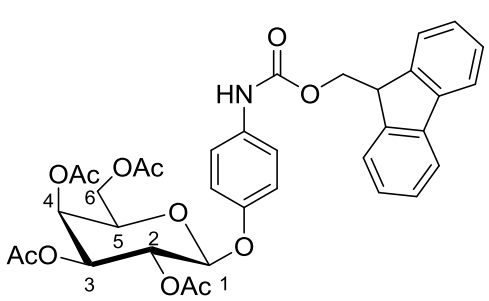
To 4-aminophenol (1.0 g, 9.13 mmol, 1.0 eq) in THF (20 mL), DIPEA (1.91 mL, 11.0 mmol, 1.2 eq) and Fmoc chloride (2.85 g, 11.0 mmol, 1.2 eq) were added and stirred at reflux for 1 h. The reaction was cooled to rt, and concentrated *in vacuo*. The residue was dissolved in 10 mL of CH_2Cl_2 and treated with 25 mL of sat. NH_4Cl. The precipitate formed was filtered and washed with sat. NH_4Cl (2x15 mL) to obtain **143**.



Yield (2.12 g, 70 %); brown solid, mp 230-232 °C; R_f = 0.47 (EtOAc/petrol 1:2); ν_{max}/cm^{-1} (neat) 1697 (C=O), 3337 (O-H); δ_H (400 MHz, Dimethyl sulfoxide-d_6): 4.28 (1H, t, *J* = 6.6 Hz, OCH_2CH-), 4.43 (2H, br. d, *J* = 5.9 Hz, OCH_2CH-), 6.69 (2H, d, *J* = 7.3 Hz, Ar-C*H*), 7.26 (2H, br. signal, Ar-C*H*), 7.34 (2H, app t, *J* = 7.1 Hz, Ar-C*H*), 7.42 (2H, app t, *J* = 7.1 Hz, Ar-C*H*), 7.74 (2H, br. d, *J* = 6.4 Hz, Ar-C*H*), 7.90 (2H, d, *J* = 7.6 Hz, Ar-C*H*), 9.15 (1H, br. s., NH/OH), 9.42 (1H, br. s., NH/OH); δ_C (100.6 MHz, Dimethyl sulfoxide-d_6): 46.7 (OCH_2CH-), 65.4 (OCH_2CH-), 115.1, 120.2, 120.2, 125.1, 127.1, 127.7 (Ar-*C*H), 130.5, 140.8, 143.9, 152.9 (Ar-C), 153.6 ($HNCO_2$); *m/z* (ESI^+) 354 ($[M+Na]^+$, 75 %); HRMS (ESI^+); $C_{21}H_{17}O_3NNa$ $[M+Na]^+$; found 354.10922, requires 354.11006.

(+)-9-Fluorenylmethyl (4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)phenyl)carbamate, 144

Aryl glycosylation of **143** was according to a modified literature procedure.[87] β-D-Galactose pentaacetate (0.87 g, 2.23 mmol, 1.0 eq) and **143** (0.89 g, 2.68 mmol, 1.2 eq) in CH_2Cl_2 (10 mL) was cooled to 0 °C. $BF_3.OEt_2$ (0.33 mL, 2.68 mmol, 1.2 eq) was added dropwise under N_2. The reaction flask was warmed to rt and stirred for 18 h. The reaction mixture was quenched with sat. $NaHCO_3$ (10 mL) and left to stir for 30 min. The product was extracted with CH_2Cl_2, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography to obtain **144** (eluent: EtOAc/petrol).

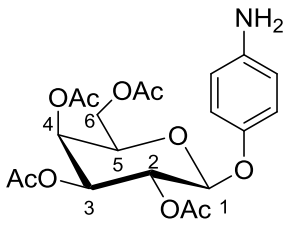


Yield (0.57 g, 39 %); brown solid; R_f = 0.47 (EtOAc/petrol 1:2); $[\alpha]_D^{25}$ = +35.4 (*c* = 0.23, $CHCl_3$) ν_{max}/cm^{-1} (neat) 1216 (C-O), 1745 (C=O); δ_H (400 MHz, CD_2Cl_2): 1.99 (3H, s, CH_3-C(=O)-O-), 2.03 (3H, s, CH_3-C(=O)-O-), 2.06 (3H, s, CH_3-C(=O)-O-), 2.17 (3H, s, CH_3-C(=O)-O-), 4.02 - 4.24 (3H, m, $H6_A$, $H6_B$, H5), 4.28 (1H, t, *J* = 6.6 Hz,

OCH_2CH-), 4.52 (2H, d, *J* = 6.6 Hz, OCH_2CH-), 5.00 (1H, d, *J* = 8.0 Hz, H1), 5.11 (1H, dd, *J* = 10.5, 3.4 Hz, H3), 5.39 (1H, dd, *J* = 10.5, 8.0 Hz, H2), 5.44 (1H, app d, *J* = 2.7 Hz, H4), 6.95 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.33 (4H, app t, *J* = 7.4 Hz, Ar-C*H*), 7.42 (2H, app t, *J* = 7.4 Hz, Ar-C*H*), 7.63 (2H, d, *J* = 7.4 Hz, Ar-C*H*), 7.80 (2H, d, *J* = 7.4 Hz, Ar-C*H*); δ_C (100.6 MHz, CD_2Cl_2): 20.9, 21.0, 21.0, 21.1 (CH_3-C(=O)-O-), 47.7 (OCH_2CH-), 62.0 (C6), 67.2 (OCH_2CH-), 67.6 (C4), 69.1 (C2), 71.4 (C3), 71.7 (C5), 100.7 (C1), 118.3, 120.5, 120.8, 125.5, 127.6, 128.3 (Ar-CH) 141.9, 144.4, 153.6, 154.1 (Ar-C), 169.9, 170.6, 170.7, 170.8 (CH_3-C(=O)-O-); *m/z* (ESI^+) 684 ($[M+Na]^+$, 44 %); HRMS (ESI^+); $C_{35}H_{35}O_{12}NNa$ $[M+Na]^+$; found 684.20315, requires 684.20515.

(+)-4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)aniline, 142a

144 (0.46 g, 0.7 mmol, 1.0 eq) was dissolved in DMF (4 mL) and piperidine (0.07 mL, 0.7 mmol, 1.0 eq) was added. The reaction was stirred at rt for 1 h until complete deprotection was observed by TLC. The crude was purified by silica gel flash column chromatography to obtain **142a** (eluent: EtOAc/petrol).

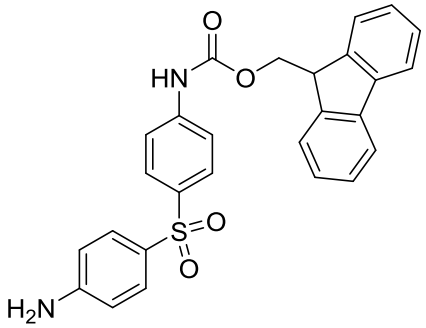


Yield (0.23 g, 75 %); yellow oil; R_f = 0.18 (EtOAc/petrol 1:1); $[\alpha]_D^{25}$ = +3.84 (*c* = 0.43, $CHCl_3$) {lit. $[\alpha]_D^{22}$ = +5.83 (*c* = 2.0, $CHCl_3$)}[86]; ν_{max}/cm^{-1} (neat) 1216 (C-O), 1743 (C=O), 3368, 3452 (N-H); δ_H (500 MHz, CD_2Cl_2): 1.98 (3H, s, CH_3-C(=O)-O-), 2.03 (3H, s, CH_3-C(=O)-O-), 2.06 (3H, s, CH_3-C(=O)-O-), 2.17 (3H, s, CH_3-C(=O)-O-), 3.60 (2H, br. s., NH), 3.96 - 4.04 (1H, m, H5), 4.14 (1H, dd, *J* = 11.4, 6.0 Hz, $H6_A$), 4.20 (1H, dd, *J* = 11.4, 7.1 Hz, $H6_B$), 4.88 (1H, d, *J* = 8.0 Hz, H1), 5.07 (1H, dd, *J* = 10.4, 3.4 Hz, H3), 5.31 - 5.36 (1H, m, H2, obscured by solvent peak), 5.42 (1H, dd, *J* = 3.4, 0.9 Hz, H4), 6.60 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 6.82 (2H, d, *J* = 8.8 Hz, Ar-C*H*); δ_C (125.8 MHz, CD_2Cl_2): 20.9, 21.0, 21.0, 21.1 (CH_3-C(=O)-O-), 62.0 (C6), 67.6 (C4), 69.2 (C2), 71.4 (C3), 71.6 (C5), 101.7 (C1), 116.1,

119.4 (Ar-*C*H) 143.5, 150.3 (Ar-C), 169.9, 170.6, 170.7, 170.7 (CH_3-*C*(=O)-O-); *m/z* (ESI^+) 462 ($[M+Na]^+$, 20 %); HRMS (ESI^+); $C_{20}H_{25}O_{10}NNa$ $[M+Na]^+$; found 462.13580, requires 462.13707.

9-Fluorenylmethyl (4-((4-aminophenyl)sulfonyl)phenyl)carbamate 145

To 4-aminophenylsulfone (1.0 g, 4.0 mmol, 1.0 eq) in THF (20 mL), DIPEA (0.77 mL, 4.4 mmol, 1.1 eq) and Fmoc chloride (1.15 g, 4.4 mmol, 1.1 eq) were added and stirred at reflux for 3 h. The reaction mixture was cooled to rt, treated with sat. NH_4Cl (20 mL) and the product was extracted with EtOAc. The organic fractions were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography to obtain **145** (eluent: EtOAc/petrol).



Yield (1.18 g, 63 %); white foam, mp 144 °C; R_f = 0.61 (EtOAc/petrol 2:1); ν_{max}/cm^{-1} (neat) 1145, 1315 (S=O), 1722 (C=O), 3335, 3375, 3478 (N-H); δ_H (400 MHz, $CDCl_3$): 4.23 (1H, t, *J* = 6.6 Hz, OCH_2CH-), 4.54 (2H, d, *J* = 6.6 Hz, OCH_2CH-), 6.60 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.21 (1H, br. s, NH), 7.30 (2H, td, *J* = 7.5, 1.2 Hz, Ar-C*H*), 7.37 - 7.47 (4H, m, Ar-C*H*), 7.59 (2H, d, *J* = 7.5 Hz, Ar-C*H*), 7.65 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.74 - 7.80 (4H, m, Ar-C*H*); δ_C (100.6 MHz, $CDCl_3$): 46.9 (OCH_2CH-), 67.0 (OCH_2CH-), 114.1, 118.3, 120.0, 124.8, 127.1, 127.8, 128.4, 129.5 (Ar-*C*H), 129.6, 136.8, 141.3, 141.8, 143.4, 150.9 (Ar-C), 152.9 ($HNCO_2$); *m/z* (ESI^+) 471 ($[M+H]^+$, 100 %); HRMS (ESI^+); $C_{27}H_{23}O_4N_2S$ $[M+H]^+$; found 471.13716, requires 471.13730.

Attempted hydrolysis of ethyl ester **90a** under basic conditions

To **90a** (100 mg, 0.33 mmol, 1.0 eq) dissolved in THF/CH_3CN (2 mL), the relevant base in 2 mL of H_2O was added and stirred under the experimental conditions listed in the table below. The reaction mixture was acidified with 10 % HCl to pH 1 and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo*.

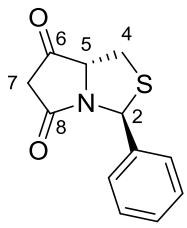
Entry	Reaction Condition	Remarks
1	LiOH.H_2O (1.1 eq), THF/H_2O (1:1), rt, 2 h	Only **90a** detected in crude.
2	LiOH.H_2O (4 eq), THF/H_2O (1:1), reflux, overnight	Reaction did not go to completion to give **148**
3	KOH (5 eq), CH_3CN/H2O (1:1), reflux, overnight	Reaction did not go to completion to give **148**

Attempted hydrolysis of ethyl ester **90a** under acidic conditions

To **90a** (100 mg, 0.33 mmol, 1.0 eq) in 3:1 THF:H_2O (4 mL), TFA (0.05 mL, 0.66 mmol, 2 eq) was added and the reaction stirred at rt overnight. The reaction mixture was concentrated *in vacuo* to obtain a mixture of **90a** and **148**.

(2*S*, 5*R*)-1-Aza-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 148

To **90a** (500 mg, 1.64 mmol, 1.0 eq) in 3:1 THF:H_2O (12 mL), TFA (0.50 mL, 6.56 mmol, 4 eq) was added and the reaction stirred at reflux for 3 h. The progress of the reaction was monitored by LRMS and upon completion, it was cooled to rt and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography to obtain **148** (eluent: EtOAc/petrol 1:1 to EtOAc 100 %).



Yield (36 mg, 10 %); colouless oil; R_f = 0.57 (EtOAc/MeOH 9:1); δ_H (200 MHz, $CDCl_3$): 3.03 - 3.48 (4H, m, H4, H7), 4.63 (1H, app t, *J* = 8.2 Hz, H5), 6.61 (1H, s, H2), 7.28 - 7.53 (5H, m, Ar-C*H*); *m/z* (ESI^-) 232 ($[M-H]^-$, 20 %); HRMS (ESI^-); $C_{12}H_{10}O_2NS$ $[M-H]^-$; found 232.04401, requires 232.04377.

Attempted reduction of **91a** and **110**

Condition 1 (For **91a** and **110**): Tetramate (50 mg, 1.0 eq) was dissolved in EtOH (1-2 mL) and cooled to 0 °C. To the stirred solution, $NaBH_4$ (1.03 eq) was added portionwise. The reaction was left to stir for 30 min at 0 °C and at rt for 20 h. Glacial acetic acid was added dropwise until pH 5 and then filtered through a Celite pad, eulting with EtOAc. The filtrate was concentrated *in vacuo* to obtain unreacted starting material.

Condition 2: Tetramate **91a** (20 mg, 0.052 mmol, 1.0 eq) was dissolved in THF (1-2 mL) and cooled to 0 °C. To the stirred solution, $LiBEt_3H$ (1.0 M in THF; 0.12 mL, 0.12 mmol, 2.2 eq) was added dropwise and reaction was left to stir for 30 min at 0 °C and then at rt for 12 h. The mixture was quenched with NH_4Cl (0.1 mL) and sat. $NaHCO_3$ (0.4 mL). The aqueous layer was extracted with EtOAc and the organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield unreacted starting material.

Condition 3: To **110** (50 mg, 0.10 mmol, 1.0 eq) in 10 % AcOH in CH_2Cl_2 (1 mL) at 0 °C, $NaBH_4$ (8.5 mg, 0.22 mmol, 2.2 eq) was added portionwise. The reaction was left to stir for 30 min at 0 °C and at rt for 1.5 h. Solvent was removed *in vacuo* and the residue was partitioned between Et_2O and sat. $NaHCO_3$. The aqueous layer was extracted twice with EtOAc and the combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield unreacted starting material.

Attempted C-7 functionalization via electrophilic α-substitution of tetramate systems

Attempted synthesis of **165**

To a suspension of NaH (3.4 mg, 0.086 mmol, 2.0 eq) in THF cooled to 0 °C, **129** (15 mg, 0.043 mmol, 1.0 eq) in THF (2 mL) was added and the reaction stirred for 30 min. Ethyl bromoaceetate (5.8 μL, 0.052 mmol, 1.2 eq) was then added and the reaction left to stir overnight for 18 h at rt. The reaction was quenched with ice and diluted with Et_2O. The aqueous layer was extracted with EtOAc and the combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo* to obtain unreacted strating material.

Attempted synthesis of **166**

To **129** (50 mg, 0.14 mmol, 1.0 eq) in CH_2Cl_2 (2 mL) cooled to 0 °C, DMAP (23 mg, 0.18 mmol, 1.3 eq), DCC (35 mg, 0.17 mmol, 1.2 eq) and phenylacetic acid (23 mg, 0.17 mmol, 1.2 eq) were added and stirred overnight for 18 h at rt. The reaction mixture was filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography to obtain unreacted **129**.

Attempted synthesis of **167-170** from **113** and **118**

A solution of the tetramate **113**/**118** (25 mg, 1.0 eq) in THF (3 mL) was added to NaH (2.0 eq) at 0 °C and the mixture was stirred at rt for 35 min. MeI (1 eq) was then added and stirred at rt for 24 h. The reaction was quenched with ice, acidified with 10 % HCl added dropwise and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo* to obtain unreacted starting material.

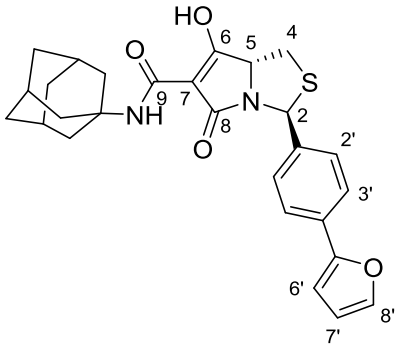
6.4 Experimental for Chapter 4

Carboxamide tetramates: where the major and minor tautomeric forms have distinct chemical shift values, they have been specified as either AB (major tautomeric form) or CD (minor tautomeric form) in compound characterisation.

6.4.1 General procedure: Synthesis of carboxamide tetramates 175-186 from 110 and 187 from 125 via Suzuki-Miyaura cross-coupling reactions

Suzuki-Miyaura cross-coupling reactions were carried out according to a modified literature procedure.[97] Tetramic acid (1.0 eq), the appropriate boronic acid (3 eq), $Pd(OAc)_2$ (0.05 eq) XPhos (0.15 eq), aq. Na_2CO_3 (2 M, 6.3 eq) and 1,2-dimethoxyethane (6.3 mL/mmol) were placed in a sealed flask and degassed with N_2. The suspension was refluxed for 15-48 h as required with the progress of the reaction monitored by TLC and LRMS. The crude mixture was filtered through a Celite plug and the filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography (eluent: EtOAc/petrol to EtOAc/MeOH/1 % Et_3N). The product isolated was dissolved in CH_2Cl_2 and washed with 5% citric acid. The organic fractions were dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield the desired bicyclic carboxamide tetramate.

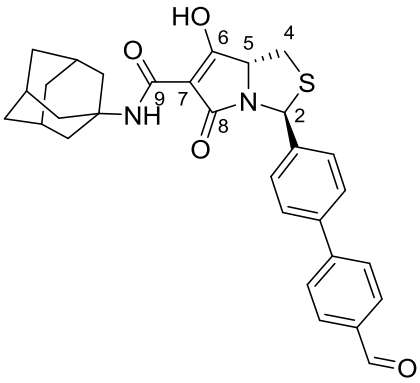
(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(4-(2-furanyl)phenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 175



Yield (0.35 g, 72 %); brown foam, 2.7:1 AB:CD tautomers, mp 130-132 °C; R_f = 0.63 (EtOAc/MeOH 98:2); $[\alpha]_D^{25}$ = -240.7 (*c* = 0.25, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1625 (C=C), 1648 (C=O), 1689 (C=O), 3315 (O-H/N-H); δ_H (400 MHz, $CDCl_3$): 1.70 (6H, br. signal, Adamantyl-C*H*$_2$), 2.06 (6H, br. signal, Adamantyl-C*H*$_2$), 2.12 (3H, br. signal, Adamantyl-C*H*), 2.98 - 3.09 (1H, m, $H4_A$), 3.27

(1H, dd, *J* = 11.1, 7.2 Hz, $H4_B$), 4.45 (1H, app t, *J* = 7.7 Hz, H5 CD), 4.69 (1H, app t, *J* = 7.7 Hz, H5 AB), 6.27 (1H, s, H2 AB), 6.37 (1H, s, H2 CD), 6.47 (1H, dd, *J* = 3.4, 1.7 Hz, H7'), 6.65 (1H, app d, *J* = 3.4 Hz, H6'), 7.43 (1H, br. s, NH AB), 7.45 - 7.50 (3H, m, H2' + H8'), 7.65 (2H, d, *J* = 8.3 Hz, H3'), 7.94 (1H, br. s, NH CD), 11.75 (1H, br. s, OH); δ_C (100.6 MHz, $CDCl_3$): 29.3 (Adamantyl-*C*H), 32.4 (C4 AB), 32.7 (C4 CD), 35.8 (Adamantyl-*C*H_2 CD), 36.0 (Adamantyl-*C*H_2 AB), 41.5 (Adamantyl-*C*H_2 AB), 41.6 (Adamantyl-*C*H_2 CD), 53.1 (Adamantyl-C AB), 54.5 (Adamantyl-C CD), 61.9 (C2 AB), 62.5 (C2 CD), 67.3 (C5 AB), 70.6 (C5 CD), 85.3 (C7 CD), 94.9 (C7 AB), 105.3 (C6'), 111.6 (C7'), 123.9 (C3'), 126.7 (C2' CD), 126.8 (C2' AB), 130.5 (C4' CD), 130.6 (C4' AB), 139.2 (C1' AB), 139.5 (C1' CD), 142.2 (C8'), 153.4 (C5'), 166.0 (C9 AB), 166.5 (C9 CD), 172.4 (C8 AB), 178.3 (C8 CD), 188.2 (C6 AB), 191.2 (C6 CD); *m/z* (ESI$^-$) 475 ([M-H]$^-$, 100 %); HRMS (ESI$^-$); $C_{27}H_{27}N_2O_4S$ [M-H]$^-$; found 475.16905, requires 475.16860.

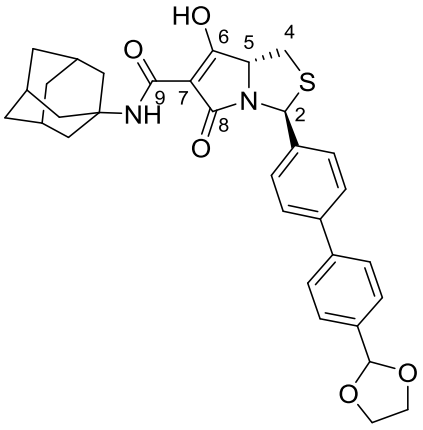
(-)-(2*S*,5*R*)-7-(Adamantanylaminocarbonyl)-1-aza-2-(4'-(formyl)-[1,1'-biphenyl]-4-yl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 177



Yield (0.31 g, 74 %); brown foam, 2.6:1 AB:CD tautomers, mp 150-152 °C; R_f = 0.49 (EtOAc: MeOH; 96:4); $[\alpha]_D^{25}$ = -216.1 (*c* = 0.19, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1623 (C=C), 1646 (C=O), 1687 (C=O), 3315 (O-H/N-H); δ_H (400 MHz, $CDCl_3$): 1.57 - 1.80 (6H, m, Adamantyl-C*H*$_2$), 2.02 - 2.09 (6H, m, Adamantyl-C*H*$_2$), 2.10 - 2.20 (3H, m, Adamantyl-C*H*), 2.99 – 3.10 (1H, m, $H4_A$), 3.29 (1H, dd, *J* = 11.3, 7.1 Hz, $H4_B$), 4.48 (1H, app t, *J* = 7.7 Hz, H5 CD), 4.72 (1H, app t, *J* = 7.8 Hz, H5 AB), 6.30 (1H, s, H2 AB), 6.40 (1H, s, H2 CD), 7.43 (1H, br. s, NH), 7.54 - 7.60 (2H, m, Ar-C*H*), 7.60 - 7.64 (2H, m, Ar-C*H*), 7.73 (2H, d, *J* = 8.3 Hz, Ar-C*H*), 7.95 (2H, d, *J* = 8.3 Hz, Ar-C*H*), 10.05 (1H, s, C*H*O), 11.00 (1H, br. s, OH); δ_C (100.6 MHz, $CDCl_3$): 29.3 (Adamantyl-*C*H), 32.6 (C4 AB),

32.9 (C4 CD), 35.8 (Adamantyl-*C*H_2 CD), 36.0 (Adamantyl-*C*H_2 AB), 41.5 (Adamantyl-*C*H_2 AB), 41.6 (Adamantyl-*C*H_2 CD), 53.1 (Adamantyl-C AB), 54.5 (Adamantyl-C CD), 61.7 (C2 AB), 62.3 (C2 CD), 67.5 (C5 AB), 70.7 (C5 CD), 85.3 (C7 CD), 94.7 (C7 AB), 127.0, 127.1, 127.6, 130.2 (Ar-*C*H), 135.3, 139.5, 140.7, 146.4 (Ar-C), 166.0 (C9 AB), 166.5 (C9 CD), 172.4 (C8 AB), 178.2 (C8 CD), 188.4 (C6 AB), 191.1 (C6 CD), 191.8 (*C*HO); *m/z* (ESI$^-$) 513 ([M-H]$^-$, 100 %); HRMS (ESI$^-$); $C_{30}H_{29}N_2O_4S$ [M-H]$^-$; found 513.19140, requires 513.18535.

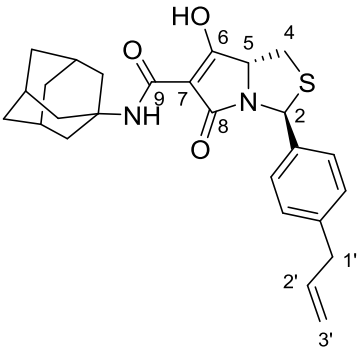
(-)-(2*S*,5*R*)-7-(Adamantanylaminocarbonyl)-1-aza-2-(4'-(1,3-dioxolan-2-yl)-[1,1'-biphenyl]-4-yl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 178



Yield (92 mg, 80 %); yellow oil; 3:1 AB:CD tautomers; R_f = 0.56 (EtOAc/MeOH 96:4); $[\alpha]_D^{25}$ = -182.5 (*c* = 0.47, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1622 (C=C), 1647 (C=O), 1687 (C=O), 3316 (O-H/N-H); δ_H (400 MHz, $CDCl_3$) 1.71 (6H, br. signal, Adamantyl-C*H*$_2$), 2.01 - 2.16 (9H, m, Adamantyl-C*H*$_2$ + Adamantyl-C*H*), 3.02 (1H, dd, *J* = 11.1, 8.4 Hz, H4$_A$), 3.29 (1H, dd, *J* = 11.1, 7.0 Hz, H4$_B$), 4.00 - 4.07 (2H, m, dioxolane-C*H*$_2$), 4.08 - 4.15 (2H, m, dioxolane-C*H*$_2$), 4.49 (1H, app t, *J* = 7.8 Hz, H5 CD), 4.75 (1H, dd, *J* = 8.3, 7.1 Hz, H5 AB), 5.82 (1H, s, dioxolane-C*H*), 6.27 (1H, s, H2 AB), 6.36 (1H, s, H2 CD), 7.38 (1H, br. s, NH AB), 7.50 - 7.55 (4H, m, Ar-C*H*), 7.58 - 7.64 (4H, m, Ar-C*H*), 7.94 (1H, br. s, NH CD), 9.75 (1H, br. s, OH); δ_C (100.6 MHz, $CDCl_3$): 30.1 (Adamantyl-*C*H), 33.1 (C4 AB), 33.5 (C4 CD), 36.4 (Adamantyl-*C*H_2 CD), 36.6 (Adamantyl-*C*H_2 AB), 42.1 (Adamantyl-*C*H_2 AB), 42.2 (Adamantyl-*C*H_2 CD), 53.5 (Adamantyl-C AB), 54.4 (Adamantyl-C CD), 62.4 (C2 AB), 62.9 (C2 CD), 65.9 (dioxolane-*C*H_2), 68.0 (C5 AB), 71.6 (C5 CD), 96.0 (C7), 104.0 (dioxolane-C*H*), 127.4, 127.5, 127.6, 127.9 (Ar-*C*H), 138.0, 140.6, 141.0, 141.8 (Ar-C), 166.6 (C9), 172.9 (C8), 188.4 (C6 AB),

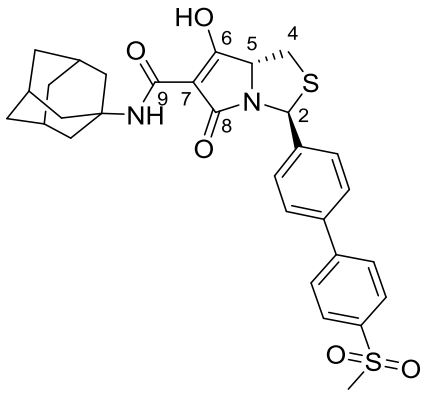
191.1 (C6 CD); *m/z* (ESI$^-$) 557 ([M-H]$^-$, 50 %); HRMS (ESI$^-$); $C_{32}H_{33}N_2O_5S$ [M-H]$^-$; found 557.21398, requires 557.21157.

(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)- 2-(4-allylphenyl)-1-aza-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 179



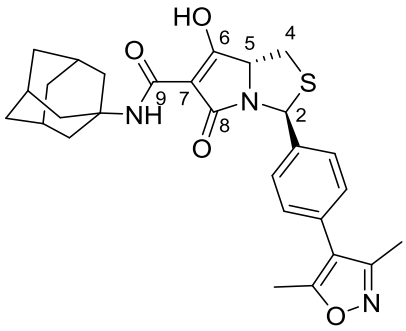
Yield (25 mg, 45 %); brown oil, 2.5:1 AB:CD tautomers; R_f = 0.58 (EtOAc/Petrol 1:1); $[\alpha]_D^{25}$ = -175.4 (*c* = 0.53, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1621 (C=C), 1646 (C=O), 1686 (C=O), 3315 (O-H/N-H); δ_H (200 MHz, $CDCl_3$); 1.70 (6H, br. signal, Adamantyl-C*H*$_2$), 2.06 (6H, br. signal, Adamantyl-C*H*$_2$), 2.12 (3H, br. signal, Adamantyl-C*H*), 2.92 - 3.08 (1H, m, H4$_A$), 3.26 (1H, dd, *J* = 11.1, 7.2 Hz, H4$_B$), 3.38 (2H, app d, *J* = 5.9 Hz, H1'), 4.44 (1H, app t, *J* = 7.8 Hz, H5 CD), 4.68 (1H, app t, *J* = 7.7 Hz, H5 AB), 5.01 - 5.15 (2H, m, H3'), 5.83 - 6.06 (1H, m, H2'), 6.24 (1H, s, H2 AB), 6.34 (1H, s, H2 CD), 7.18 (2H, d, *J* = 8.1 Hz, Ar-C*H*), 7.40 (2H, d, *J* = 8.1 Hz, Ar-C*H*), 7.93 (1H, br. s., NH/OH CD), 10.40 (3H, br. s., NH+OH AB and NH/OH CD); δ_C (100.6 MHz, $CDCl_3$): 29.3 (Adamantyl-*C*H), 32.5 (C4 AB), 32.7 (C4 CD), 35.8 (Adamantyl-*C*H$_2$ CD), 36.0 (Adamantyl-*C*H$_2$ AB), 39.8 (C1'), 41.5 (Adamantyl-*C*H$_2$ AB), 41.6 (Adamantyl-CH$_2$ CD), 53.1 (Adamantyl-C AB), 54.5 (Adamantyl-C CD), 61.9 (C2 AB), 62.4 (C2 CD), 67.3 (C5 AB), 70.6 (C5 CD), 85.4 (C7 CD), 95.0 (C7 AB), 116.0 (C3'), 126.4, 128.8 (Ar-*C*H CD), 126.5, 128.8 (Ar-*C*H AB), 137.1 (C2'), 138.1, 140.0 (Ar-C AB), 138.4, 139.9 (Ar-C CD), 166.0 (C9 AB), 166.5 (C9 CD), 172.3 (C8 AB), 178.2 (C8 CD), 188.0 (C6 AB), 191.3 (C6 CD); *m/z* (ESI$^-$) 449 ([M-H]$^-$, 100 %); HRMS (ESI$^+$); $C_{26}H_{31}N_2O_3S$ [M+H]$^+$; found 451.20477, requires 451.20499.

(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-6-hydroxy-2-(4'-(methylsulfonyl)-[1,1'-biphenyl]-4-yl)-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 180



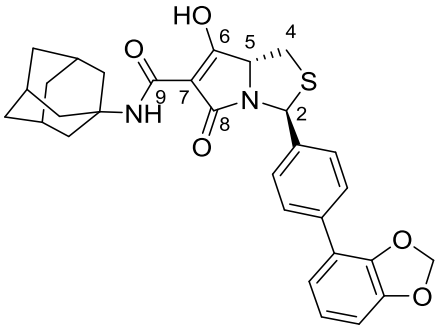
Yield (0.16 g, 53 %); brown foam; 3:1 AB:CD tautomers; mp 154-156 °C; R_f = 0.55 (EtOAc/MeOH 94:6); $[\alpha]_D^{25}$ = -218.6 (*c* = 0.15, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1149, 1305 (S=O), 1622 (C=C), 1646 (C=O), 1685 (C=O), 3314 (N-H/O-H); δ_H (400 MHz, CD_2Cl_2) 1.70 (6H, br. signal, Adamantyl-C*H*$_2$), 2.01 - 2.16 (9H, m, Adamantyl-C*H*$_2$ + Adamantyl-C*H*), 3.03 (1H, dd, *J* = 11.3, 8.5 Hz, $H4_A$), 3.07 (3H, s, C*H*$_3$), 3.29 (1H, dd, *J* = 11.3, 7.1 Hz, $H4_B$), 4.47 (1H, app t, *J* = 7.8 Hz, H5 CD), 4.74 (1H, dd, *J* = 8.5, 7.1 Hz, H5 AB), 6.28 (1H, s, H2 AB), 6.37 (1H, s, H2 CD), 7.17 (1H, br. s, OH), 7.36 (1H, br. s, NH AB), 7.58 (2H, d, *J* = 8.3 Hz, Ar-C*H*), 7.64 (2H, d, *J* = 8.3 Hz, Ar-C*H*), 7.79 (2H, d, *J* = 8.5 Hz, Ar-C*H*), 7.94 (1H, br. s, NH CD), 7.98 (2H, d, *J* = 8.5 Hz, Ar-C*H*); δ_C (125.8 MHz, CD_2Cl_2): 30.0 (Adamantyl-*C*H), 33.2 (C4), 36.6 (Adamantyl-*C*H$_2$), 42.1 (Adamantyl-*C*H$_2$), 45.0 (*C*H$_3$), 53.5 (Adamantyl-C), 62.3 (C2 AB), 62.9 (C2 CD), 68.1 (C5 AB), 71.6 (C5 CD), 85.7 (C7 CD), 95.8 (C7 AB), 127.5, 127.6, 128.2, 128.4 (Ar-C*H*), 139.3, 140.0, 142.2, 146.4 (Ar-C CD), 139.4, 140.0, 141.9, 146.4 (Ar-C AB), 166.6 (C9 AB), 167.2 (C9 CD), 172.9 (C8 AB), 179.0 (C8 CD), 188.5 (C6 AB), 191.5 (C6 CD); *m/z* (ESI^-) 563 ($[M-H]^-$, 21 %); HRMS (ESI^-); $C_{30}H_{31}O_5N_2S_2$ $[M-H]^-$; found 563.16921, requires 563.16799.

(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(4-(3,5-dimethylisoxazol-4-yl)phenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 181



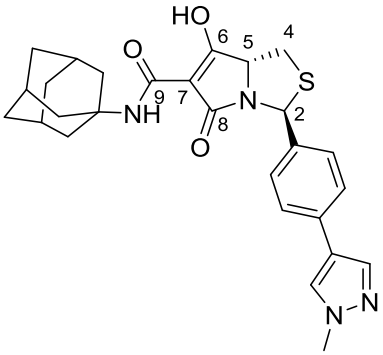
Yield (0.23 g, 72 %); yellow solid; 2.5:1 AB:CD tautomers; mp 130-134 °C; R_f = 0.60 (EtOAc/MeOH 96:4); $[\alpha]_D^{25}$ = -195.5 (*c* = 0.23, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1624 (C=C, br with shoulder towards higher wavenumber), 1686 (C=O), 3311 (N-H/O-H); δ_H (400 MHz, $CDCl_3$) 1.68 (6H, br. signal, Adamantyl-C*H*$_2$), 2.00 - 2.07 (6H, m, Adamantyl-C*H*$_2$), 2.08 - 2.16 (3H, m, Adamantyl-C*H*), 2.24 (3H, s, C*H*$_3$), 2.38 (3H, s, C*H*$_3$), 2.95 - 3.07 (1H, m, H4$_A$), 3.24 - 3.32 (1H, m, H4$_B$), 4.47 (1H, app t, *J* = 7.8 Hz, H5 CD), 4.72 (1H, dd, *J* = 8.6, 6.9 Hz, H5 AB), 6.25 (1H, s, H2 AB), 6.35 (1H, s, H2 CD), 7.23 (2H, d, *J* = 8.3 Hz, Ar-C*H*), 7.41 (1H, s, NH AB), 7.48 - 7.55 (2H, m, Ar-C*H*), 7.93 (1H, s, NH CD), 11.16 (1H, br. s, OH); δ_C (100.6 MHz, $CDCl_3$): 10.7 (*C*H$_3$), 11.4 (*C*H$_3$), 29.2 (Adamantyl-*C*H), 32.6 (C4 AB), 32.8 (C4 CD), 35.7 (Adamantyl-*C*H$_2$ CD), 35.9 (Adamantyl-*C*H$_2$ AB), 41.4 (Adamantyl-*C*H$_2$ AB), 41.5 (Adamantyl-*C*H$_2$ CD), 53.0 (Adamantyl-C AB), 53.4 (Adamantyl-C CD), 61.6 (C2 AB), 62.1 (C2 CD), 67.5 (C5 AB), 70.7 (C5 CD), 85.2 (C7 CD), 94.7 (C7 AB), 116.0, 130.2, 139.6, 158.5, 165.2 (Ar-C, AB), 116.0, 130.1, 139.9, 158.5, 165.2 (Ar-C CD), 126.6, 129.1 (Ar-*C*H CD), 126.8, 129.2 (Ar-*C*H AB), 165.9 (C9 AB), 166.4 (C9 CD), 172.2 (C8 AB), 178.0 (C8 CD), 188.1 (C6 AB), 191.0 (C6 CD); *m/z* (ESI$^-$) 504 ([M-H]$^-$, 100 %); HRMS (ESI$^-$); $C_{28}H_{30}O_4N_3S$ [M-H]$^-$; found 504.19749, requires 504.19625.

(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(4-(benzo[*d*][1,3]dioxol-4-yl)phenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 182



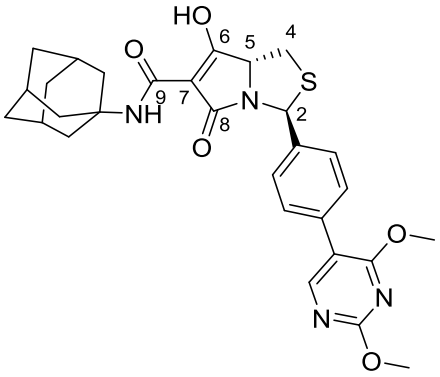
Yield (75 mg, 60 %); yellow solid, mp 130-132 °C; 3:1 AB:CD tautomers; R_f = 0.72 (EtOAc; 100 %); $[\alpha]_D^{25}$ = -199.7 (*c* = 0.18, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1621 (C=C), 1646 (C=O), 1686 (C=O), 3310 (N-H/O-H); δ_H (400 MHz, $CDCl_3$) 1.65 - 1.74 (6H, m, Adamantyl-C*H*$_2$), 2.02 - 2.08 (6H, m, Adamantyl-C*H*$_2$), 2.09 - 2.16 (3H, m, Adamantyl-C*H*), 2.98 - 3.05 (1H, m, H4$_A$), 3.24 - 3.32 (1H, m, H4$_B$), 4.47 (1H, app t, *J* = 7.8 Hz, H5 CD), 4.74 (1H, dd, *J* = 8.1, 7.3 Hz, H5 AB), 6.00 (2H, s, C*H*$_2$), 6.25 (1H, s, H2 AB), 6.34 (1H, s, H2 CD), 6.87 - 6.90 (1H, m, Ar-C*H*), 7.05 - 7.09 (2H, m, Ar-C*H*), 7.37 (1H, br. s, NH, AB), 7.50 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.51 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.93 (1H, br. s, NH CD), 9.01 (1H, br. s, OH); δ_C (125.8 MHz, $CDCl_3$): 30.0 (Adamantyl-*C*H), 33.1 (C4 AB), 33.4 (C4 CD), 36.4 (Adamantyl-*C*H$_2$ CD), 36.6 (Adamantyl-*C*H$_2$ AB), 42.1 (Adamantyl-*C*H$_2$ AB), 42.2 (Adamantyl-*C*H$_2$ CD), 55.0 (Adamantyl-C), 62.3 (C2 AB), 62.9 (C2 CD), 68.0 (C5 AB), 71.5 (C5 CD), 85.7 (C7 CD), 96.1 (C7 AB), 102.0 (*C*H$_2$), 107.9, 109.0, 121.1, 127.3, 127.5 (Ar-*C*H), 135.3, 140.0, 141.1, 147.9, 148.8 (Ar-C), 166.6 (C9 AB), 167.2 (C9 CD), 172.8 (C8 AB), 179.0 (C8 CD), 188.3 (C6 AB), 191.6 (C6 CD); *m/z* (ESI^-) 529 ($[M-H]^-$, 100 %); HRMS (ESI^+); $C_{30}H_{31}O_5N_2S$ $[M+H]^+$; found 531.19469, requires 531.19482.

(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-6-hydroxy-2-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 183



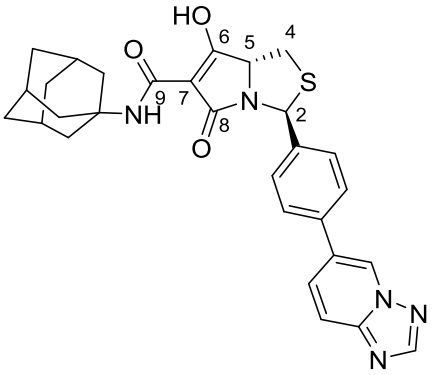
Yield (0.24 g, 78 %); light brown foam, mp 140-142 °C; 2.7:1 AB:CD tautomers; R_f = 0.72 (EtOAc/MeOH 4:1); $[\alpha]_D^{25}$ = -293.5 (*c* = 0.17, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1618 (C=C), 1645 (C=O), 1684 (C=O), 3310 (N-H/O-H); δ_H (400 MHz, $CDCl_3$) 1.69 (6H, br. signal, Adamantyl-C*H*$_2$), 2.04 (6H, br. signal, Adamantyl-C*H*$_2$), 2.11 (3H, br. signal, Adamantyl-C*H*), 3.00 (1H, dd, *J* = 11.0, 8.6 Hz, H4$_A$), 3.26 (1H, dd, *J* = 11.0, 7.2 Hz, H4$_B$), 3.93 (3H, s, C*H*$_3$), 4.44 (1H, app t, *J* = 7.7 Hz, H5 CD), 4.69 (1H, app t, *J* = 7.8 Hz, H5 AB), 6.24 (1H, s, H2 AB), 6.34 (1H, s, H2 CD), 7.42 (1H, br. s, NH AB), 7.44 (2H, d, *J* = 10.8 Hz, Ar-C*H*), 7.44 (2H, d, *J* = 10.8 Hz, Ar-C*H*), 7.59 (1H, s, Ar-C*H*), 7.74 (1H, s, Ar-C*H*), 7.92 (1H, s, NH CD), 10.70 (1H, br. s, OH); δ_C (125.8 MHz, $CDCl_3$): 29.2 (Adamantyl-*C*H), 32.4 (C4 AB), 32.7 (C4 CD), 35.8 (Adamantyl-*C*H$_2$ CD), 36.0 (Adamantyl-*C*H$_2$ AB), 39.0 (*C*H$_3$), 41.4 (Adamantyl-*C*H$_2$ AB), 41.5 (Adamantyl-*C*H$_2$ CD), 53.4 (Adamantyl-*C* AB), 54.4 (Adamantyl-*C* CD), 61.8 (C2 AB), 62.4 (C2 CD), 67.3 (C5 AB), 70.6 (C5 CD), 85.3 (C7 CD), 95.0 (C7 AB), 125.6, 126.8, 126.9, 136.7 (Ar-*C*H AB), 125.5, 126.7, 126.9, 136.7 (Ar-*C*H CD), 122.5, 132.3, 138.5 (Ar-C CD), 122.5, 132.4, 138.2 (Ar-C AB), 165.9 (C9 AB), 166.5 (C9 CD), 172.3 (C8 AB), 178.2 (C8 CD), 188.0 (C6 AB), 191.2 (C6 CD); *m/z* (ESI^-) 489 ($[M-H]^-$, 100 %); HRMS (ESI^+); $C_{27}H_{31}O_3N_4S$ $[M+H]^+$; found 491.21116, requires 491.21114.

(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(4-(2,4-dimethoxypyrimidin-5-yl)phenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 184



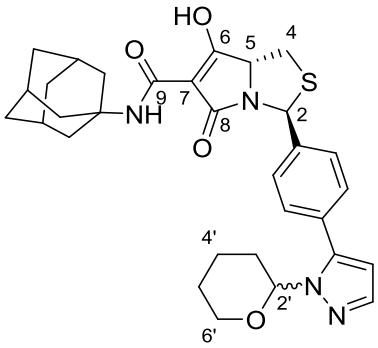
Yield (0.18 g, 56 %); light brown foam, mp 106-108 °C; 2.5:1 AB:CD tautomers; R_f = 0.66 (EtOAc/MeOH 96:4); $[\alpha]_D^{25}$ = -290.3 (*c* = 0.15, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1624 (C=C), 1647 (C=O), 1687 (C=O), 3313 (N-H/O-H); δ_H (400 MHz, $CDCl_3$) 1.68 - 1.74 (6H, m, Adamantyl-C*H*$_2$), 2.02 - 2.10 (6H, m, Adamantyl-C*H*$_2$), 2.11 - 2.20 (3H, m, Adamantyl-C*H*), 2.98 - 3.10 (1H, m, H4$_A$), 3.30 (1H, dd, *J* = 11.0, 7.1 Hz, H4$_B$), 4.02 (3H, s, OC*H*$_3$), 4.04 (3H, s, OC*H*$_3$), 4.47 (1H, app t, *J* = 7.8 Hz, H5 CD), 4.71 (1H, dd, *J* = 8.3, 7.1 Hz, H5 AB), 6.28 (1H, s, H2 AB), 6.38 (1H, s, H2 CD), 6.40 (1H, br. s, OH), 7.43 (1H, br. s, NH AB), 7.47 - 7.56 (4H, m, Ar-C*H*), 7.94 (1H, s, NH CD), 8.24 - 8.27 (1H, m, Ar-C*H*, overlapping singlet peaks for AB and CD tautomers); δ_C (125.8 MHz, $CDCl_3$): 29.3 (Adamantyl-*C*H), 32.6 (C4 AB), 32.9 (C4 CD), 35.8 (Adamantyl-*C*H$_2$ CD), 36.0 (Adamantyl-*C*H$_2$ AB), 41.5 (Adamantyl-*C*H$_2$ AB), 41.6 (Adamantyl-*C*H$_2$ CD), 53.2 (Adamantyl-C AB), 54.1 (O*C*H$_3$), 54.5 (Adamantyl-C CD), 54.9 (O*C*H$_3$), 61.9 (C2 AB), 62.4 (C2 CD), 67.5 (C5 AB), 70.7 (C5 CD), 85.4 (C7 CD), 94.8 (C7 AB), 126.5, 129.0, 157.5 (Ar-*C*H CD), 126.6, 129.1, 157.5 (Ar-*C*H AB), 115.7, 133.1, 139.8, 164.6, 168.1 (Ar-C CD), 115.7, 133.0, 140.1, 164.6, 168.1 (Ar-C CD), 166.0 (C9 AB), 166.6 (C9 CD), 172.4 (C8 AB), 178.2 (C8 CD), 188.3 (C6 AB), 191.2 (C6 CD); *m/z* (ESI$^-$) 547 ([M-H]$^-$, 100 %); HRMS (ESI$^+$); $C_{29}H_{33}O_5N_4S$ $[M+H]^+$; found 549.21610, requires 549.21662.

(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-6-hydroxy-8-oxo-3-thia-2-(4-([1,2,4])triazolo[1,5-*a*]pyridin-6-yl)phenyl)-bicyclo[3.3.0]oct-6-ene, 185



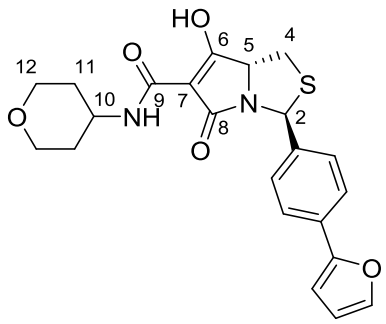
Yield (0.12 g, 70 %); light brown solid, mp 220 °C; 3:1 AB:CD tautomers; R_f = 0.68 (EtOAc/MeOH 4:1); $[\alpha]_D^{25}$ = -217.8 (*c* = 0.19, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1620 (C=C, br with shoulder towards higher wavenumber), 1684 (C=O), 3307 (N-H/O-H); δ_H (400 MHz, CD_2Cl_2) 1.70 (6H, broad signal, Adamantyl-C*H*$_2$), 2.01 - 2.16 (9H, m, Adamantyl-C*H*$_2$ + Adamantyl-C*H*), 3.04 (1H, dd, *J* = 11.0, 8.6 Hz, H4$_A$), 3.30 (1H, dd, *J* = 11.0, 7.1 Hz, H4$_B$), 4.48 (1H, app t, *J* = 7.0 Hz, H5 CD), 4.75 (1H, app t, *J* = 7.7 Hz, H5 AB), 6.28 (1H, s, H2 AB), 6.37 (1H, s, H2 CD), 7.37 (1H, br. s, NH AB), 7.59 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.62 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.79 - 7.81 (2H, m, Ar-C*H*), 7.95 (1H, br. s, NH CD), 8.32 (1H, s, Ar-C*H*), 8.82 (1H, s, Ar-C*H*); δ_C (125.8 MHz, CD_2Cl_2): 30.1 (Adamantyl-*C*H), 33.2 (C4), 36.6 (Adamantyl-*C*H$_2$), 42.1 (Adamantyl-*C*H$_2$), 53.6 (Adamantyl-C, obscured by CD_2Cl_2 signals, but HMBC correlation seen with adamantyl-C*H*$_2$), 62.3 (C2 AB), 62.8 (C2 CD), 68.1 (C5 AB), 71.6 (C5 CD), 95.8 (C7), 117.1, 126.5, 127.9, 127.9, 130.4, 154.9 (Ar-*C*H), 128.4, 136.6, 141.7, 150.4 (Ar-C), 166.6 (C9 AB), 167.2 (C9 CD), 173.0 (C8), 188.5 (C6 AB), 191.5 (C6 CD); *m/z* (ESI$^-$) 526 ([M-H]$^-$, 100 %); HRMS (ESI$^+$); $C_{29}H_{30}O_3N_5S$ $[M+H]^+$; found 528.20630, requires 528.20748.

(2*S*,2'*RS*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-6-hydroxy-8-oxo-2-(4-(1-(tetrahydro-2*H*-pyran-2'-yl)-*1H*-pyrazol-5-yl)phenyl)-3-thiabicyclo[3.3.0]oct-6-ene, 186



Yield (0.14 g, 60 %); yellow foam; 1:1 dr, each diastereomer exists as 2.6:1 AB:CD tautomers; R_f = 0.63 (EtOAc/MeOH 9:1); ν_{max}/cm^{-1} (neat) 1622 (C=C), 1646 (C=O), 1686 (C=O), 3315 (N-H/O-H); since the diastereomers are overlapping, the ^1H resonances are reported together as multiplets, δ_H (500 MHz, CD_2Cl_2) 1.50 - 1.61 (2H, m, H4' + H5'), 1.68 - 1.74 (7H, broad signal, Adamantyl-C*H*$_2$ + H5'), 1.76 - 1.82 (1H, m, H3'), 1.99 - 2.16 (10H, m, Adamantyl-C*H*$_2$ + Adamantyl-C*H* + H4'), 2.48 - 2.57 (1H, m, H3'), 3.00 - 3.06 (1H, m, H4$_A$), 3.26 - 3.33 (1H, m, H4$_B$), 3.56 - 3.64 (1H, m, H6'), 4.05 - 4.11 (1H, m, H6'), 4.45 - 4.51 (1H, m, H5 CD), 4.73 - 4.78 (1H, m, H5 AB), 4.88 (1H, br. s, OH), 5.15 - 5.20 (1H, m, H2'), 6.27 (1H, s, H2 AB), 6.33 - 6.35 (1H, m, Ar-C*H*), 6.36 (1H, s, H2 CD), 7.36 (1H, br. s, NH AB), 7.50 - 7.58 (5H, m, Ar-C*H*), 7.94 (1H, br. s, NH CD); δ_C (125.8 MHz, CD_2Cl_2): 23.6 (C4'), 25.5 (C5'), 30.1 (Adamantyl-*C*H), 30.2 (C3'), 33.2, 33.3 (C4 AB), 33.5, 33.6 (C4 CD), 36.4 (Adamantyl-*C*H$_2$ CD), 36.6 (Adamantyl-*C*H$_2$ AB), 42.1 (Adamantyl-*C*H$_2$ AB), 42.2 (Adamantyl-*C*H$_2$ CD), 53.6 (Adamantyl-C, obscured by CD_2Cl_2 signals, but HMBC correlation seen with adamantyl-C*H*$_2$), 62.2, 62.3 (C2 AB), 62.8, 62.9 (C2 CD), 68.1 (C5 AB), 68.2 (C6'), 71.6 (C5 CD), 84.8 (C2'), 85.6 (C7 CD), 95.8 (C7 AB), 107.1, 127.2, 129.7, 139.6 (Ar-C*H* AB), 107.1, 127.1, 129.7, 139.6 (Ar-C*H* CD), 107.1, 127.2, 129.7, 139.6 (Ar-C*H* AB), 130.8, 141.8, 144.2 (Ar-C AB), 130.7, 142.1, 144.2 (Ar-C CD), 166.6 (C9 AB), 167.2 (C9 CD), 172.9 (C8 AB), 179.0 (C8 CD), 188.5 (C6 AB), 191.5 (C6 CD); *m/z* (ESI$^-$) 559 ([M-H]$^-$, 100 %); HRMS (ESI$^+$); $C_{31}H_{37}O_4N_4S$ [M+H]$^+$; found 561.25289, requires 561.25300.

(-)-(2*S*,5*R*)-1-Aza-2-(4-(2-furanyl)phenyl)-6-hydroxy-8-oxo-7-(tetrahydro-2*H*-pyran-4-aminocarbonyl)-3-thia-bicyclo[3.3.0]oct-6-ene, 187

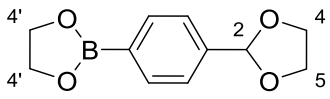


Yield (70 mg, 30 %); yellow oil, 5.3:1 AB:CD tautomers; R_f = 0.45 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -181.7 (*c* = 0.12, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1624 (C=C with shoulder towards higher wavenumber), 1687 (C=O), 3317 (O-H/N-H); δ_H (400 MHz, CD_2Cl_2) major tautomer (AB): 1.51 - 1.73 (2H, m, H11), 1.83 - 1.99 (2H, m, H11), 2.98 - 3.07 (1H, m, $H4_A$), 3.29 (1H, dd, *J* = 11.1, 7.2 Hz, $H4_B$), 3.47 (2H, app t, *J* = 11.6, 2.2 Hz, H12), 3.88 - 3.99 (2H, m, H12), 4.01 - 4.12 (1H, m, H10), 4.48 (1H, app t, *J* = 7.8 Hz, H5 CD), 4.80 (1H, dd, *J* = 8.0, 7.2 Hz, H5 AB), 6.24 (1H, s, H2 AB), 6.33 (1H, s, H2 CD), 6.51 (1H, dd, *J* = 3.4, 1.8 Hz, Ar-C*H*), 6.71 (1H, dd, *J* = 3.4, 0.8 Hz, Ar-C*H*), 7.45 - 7.51 (3H, m, Ar-C*H*), 7.67 (2H, d, *J* = 8.4 Hz, Ar-C*H*), 8.84 (1H, br. s, NH/OH); δ_C (100.6 MHz, CD_2Cl_2): 33.0 (C4), 33.3 (C11), 45.9 (C10 AB), 47.6 (C10 CD), 62.3 (C2 AB), 63.0 (C2 CD), 66.9 (C12), 67.5 (C5 AB), 71.6 (C5 CD), 97.5 (C7), 106.0, 112.3, 124.4, 127.3, 142.9 (Ar-*C*H), 131.2, 140.1, 154.0 (Ar-C), 165.9 (C9 AB), 166.6 (C9 CD), 172.4 (C8 AB), 178.5 (C8 CD), 186.5 (C6 AB), 191.3 (C6 CD); *m/z* (ESI^-) 425 ($[M-H]^-$, 100 %); HRMS (ESI^-); $C_{22}H_{21}N_2O_5S$ $[M-H]^-$; found 425.11872, requires 425.11767.

2-(4-(1,3-dioxolan-2-yl)phenyl)-1,3,2-dioxaborolane, 188

The synthesis of **188** was according to a modified literature procedure.[99] To 4-formylphenyl boronic acid (0.5 g, 3.33 mmol, 1.0 eq) in toluene (30 mL), ethylene glycol (0.93 mL, 16.7 mmol, 5.0 eq) and *p*-toluenesulfonic acid (15.8 mg, 0.08 mmol, 0.025 eq) was added and refluxed with a Dean-Stark apparatus. The reaction was not complete in 5 h and was refluxed overnight. The reaction flask was cooled to rt and concentrated under reduced pressure.The residue was dissolved in 5 mL of CH_2Cl_2 and treated with 1.0 g of $MgSO_4$. The mixture was

stirred at rt for 1 h and filtered washing with CH_2Cl_2. The filtrate was concentrated *in vacuo* to obtain **188**.



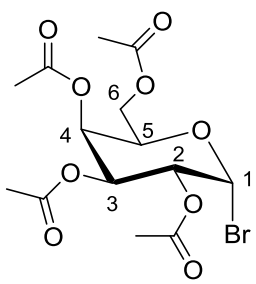
Yield (0.69 g, 94 %); white solid, mp 102-104 °C {lit. mp = 104-106 °C}[99]; R_f = 0.44 (EtOAc/petrol 2:1); ν_{max}/cm^{-1} (neat) 1070, 1093 (C-O), 1334 (B-O), 2915, 2984 (C-H); δ_H (400 MHz, CD_2Cl_2): 3.98 - 4.05 (2H, m, H4/H5), 4.06 - 4.13 (2H, m, H4/H5), 4.36 (4H, s, H4'), 5.78 (1H, s, H2), 7.48 (2H, d, *J* = 8.2 Hz, Ar-C*H*), 7.81 (2H, d, *J* = 8.2 Hz, Ar-C*H*); δ_C (100.6 MHz, CD_2Cl_2): 65.9 (C4, C5), 66.7 (C4'), 104.0 (C2), 126.5, 135.3 (Ar-*C*H) 130.5, 141.9 (Ar-C); *m/z* HRMS (FI^+); $C_{11}H_{13}BO_4$ $[M]^+$; found 220.0910, requires 220.0909.

6.4.2 Procedures for the synthesis of tetramates with glycones

<u>$BiBr_3$ catalysed bromination of β-D-galactose pentaacetate</u>[101]

To a stirred solution of β-D-galactose pentaacetate (0.3 g, 0.77 mmol, 1.0 eq) and $BiBr_3$ (17 mg, 0.039 mmol, 0.05 eq) in 3 mL of CH_2Cl_2 was added under N_2, TMSBr (0.41 mL, 3.08 mmol, 4.0 eq). The reaction was stirred at rt and monitored by TLC. After 2 h, upon completion of reaction, the reaction mixture was poured into cold sat. $NaHCO_3$ and extracted twice with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to obtain **141**.

(+)-2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide, 141



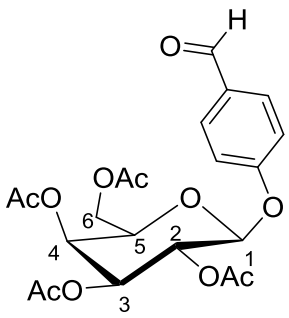
Yield (0.32 g, quant.); white solid, mp 82-84 °C {lit. mp = 84-85 °C}[101]; R_f = 0.39 (EtOAc/Petrol 1:3); $[\alpha]_D^{25}$ = +200.7 (*c* = 0.5, $CHCl_3$) {lit. $[\alpha]_D$ = +217 (*c* = 1, $CHCl_3$)}[101]; ν_{max}/cm^{-1} (neat) 1220 (C-O), 1749 (C=O); δ_H (400 MHz, $CDCl_3$): 2.02 (3H, s, C*H*$_3$-C(=O)-O-), 2.07 (3H, s, C*H*$_3$-C(=O)-O-), 2.12 (3H, s, C*H*$_3$-C(=O)-O-), 2.17 (3H, s, C*H*$_3$-C(=O)-O-), 4.12 (1H, dd, *J* = 11.4, 6.8, $H6_A$), 4.19 (1H, dd, *J* = 11.4, 6.4, $H6_B$), 4.49 (1H, app t, *J* = 6.6, H5), 5.06 (1H, dd,

J = 10.6, 4.1 Hz, H2), 5.41 (1H, dd, *J* = 10.6, 3.2 Hz, H3), 5.53 (1H, dd, *J* = 3.2, 1.2 Hz, H4), 6.70 (1H, d, *J* = 4.1 Hz, H1); δ_C (125.8 MHz, $CDCl_3$): 20.5, 20.6, 20.6, 20.7 (*C*H_3-C(=O)-O-), 60.8 (C6), 66.9 (C4), 67.7 (C2), 68.0 (C3), 71.0 (C5), 88.1 (C1), 169.7, 169.9, 170.1, 170.3 (CH_3-*C*(=O)-O-); *m/z* (ESI^+ and FI^+) 331 ($[M\text{-}Br]^+$, 100 %); molecular ion not detected.

Solid-liquid phase transfer catalaysed aryl glycosylation[102]

To **141** (1.0 g, 2.44 mmol, 1.0 eq), 4-hydroxybenzaldehyde (0.36 g, 2.93 mmol, 1.2 eq) and benzyltributylammonium chloride (0.17 g, 0.49 mmol, 0.2 eq) in $CHCl_3$ (5 mL), was added powdered K_2CO_3 (1.69 g, 12.2 mmol, 5 eq) and the mixture stirred at rt for 24 h. The reaction mixture was neutralised with 10 % HCl and the organic layer was separated. The organic layer was washed with sat. $NaHCO_3$ and brine, then dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (eluent: EtOAc/petrol) to furnish **189**.

(-)-4-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyloxy)benzaldehyde, 189

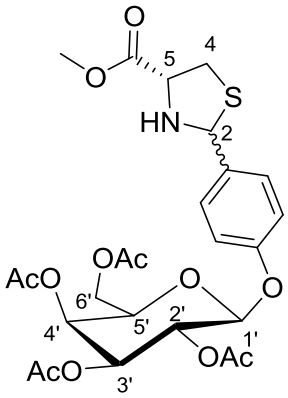


Yield (0.95 g, 86 %); white solid, mp 132 °C {lit. mp = 140-141 °C}[102]; R_f = 0.18 (EtOAc/petrol 1:2); $[\alpha]_D^{25}$ = -1.87 (*c* = 1.0, $CHCl_3$) ν_{max}/cm^{-1} (neat) 1212 (C-O), 1694 (C=O), 1746 (C=O); δ_H (400 MHz, $CDCl_3$): 2.02 (3H, s, C*H*$_3$-C(=O)-O-), 2.06 (6H, s, 2xC*H*$_3$-C(=O)-O-), 2.18 (3H, s, C*H*$_3$-C(=O)-O-), 4.10 - 4.26 (3H, m, $H6_A$ + $H6_B$ + H5), 5.14 (1H, dd, *J* = 10.3, 3.4 Hz, H3), 5.18 (1H, d, *J* = 8.0 Hz, H1), 5.48 (1H, app d, *J* = 3.4 Hz, H4), 5.52 (1H, dd, *J* = 10.3, 8.0 Hz, H2), 7.11 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.85 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 9.92 (1H, s, C*H*O); δ_C (100.6 MHz, $CDCl_3$): 20.5, 20.6, 20.6, 20.7 (*C*H_3-C(=O)-O-), 61.3 (C6), 66.7 (C4), 68.3 (C2), 70.6 (C3), 71.3 (C5), 98.5 (C1), 116.7, 131.8 (Ar-*C*H), 161.2 (Ar-C), 169.3, 170.0, 170.1, 170.3 (CH_3-*C*(=O)-O-), 190.7 (*C*HO); *m/z* (ESI^+) 475

([M+Na]$^+$, 100 %); HRMS (ESI$^+$); $C_{21}H_{24}O_{11}Na$ [M+Na]$^+$; found 475.11966, requires 475.12108.

Methyl (2*RS*,5*R*)-2-(4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)phenyl)-1,3-thiazolidine-5-carboxylate, 190

Thiazolidine **190** was derived from the condensation of L-cysteine methyl ester hydrochloride and aldehyde **189** following the procedure for thiazolidine synthesis outlined in general procedure 6.2.3.

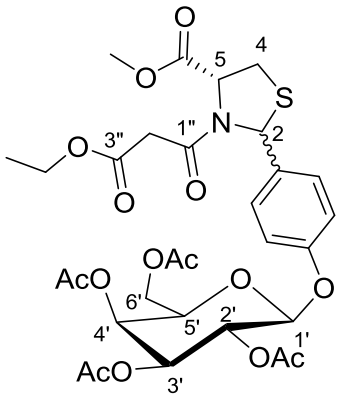


Yield (0.14 g, 70 %); colourless oil; 1.6:1 *cis* and *trans* diastereomers; R_f = 0.4 (EtOAc/petrol 1:1); ν_{max}/cm^{-1} (neat) 1223 (C-O), 1745 (C=O), 3314 (N-H); δ_H (400 MHz, $CDCl_3$) major isomer (*cis*): 1.97 (3H, s, C*H*$_3$-C(=O)-O-), 2.01 (3H, s, C*H*$_3$-C(=O)-O-), 2.02 (3H, s, C*H*$_3$-C(=O)-O-), 2.14 (3H, s, C*H*$_3$-C(=O)-O-), 2.60 (1H, br. s., NH), 3.06 (1H, dd, *J* = 10.3, 8.8 Hz, H4$_A$), 3.41 (1H, dd, *J* = 10.3, 7.1 Hz, H4$_B$), 3.76 (3H, s, -CO_2C*H*$_3$), 3.93 (1H, dd, *J* = 8.8, 7.1 Hz, H5), 4.01 - 4.20 (3H, m, H6'$_A$ + H6'$_B$ + H5'), 5.03 (1H, d, *J* = 8.1 Hz, H1'), 5.05 - 5.11 (1H, m, H3', obscured by H3' of minor isomer), 5.39 - 5.46 (2H, m, H2' + H4'), 5.47 (1H, s, H2), 6.96 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.42 (2H, d, *J* = 8.6 Hz, Ar-C*H*); minor isomer (*trans*): 1.97 (3H, s, C*H*$_3$-C(=O)-O-), 2.01 (3H, s, C*H*$_3$-C(=O)-O-), 2.02 (3H, s, C*H*$_3$-C(=O)-O-), 2.13 (3H, s, C*H*$_3$-C(=O)-O-), 2.60 (1H, br. s., NH), 3.15 (1H, dd, *J* = 10.5, 5.8 Hz, H4$_A$), 3.34 (1H, dd, *J* = 10.5, 7.1 Hz, H4$_B$), 3.74 (3H, s, -CO_2C*H*$_3$), 4.01 - 4.20 (4H, m, H6'$_A$ + H6'$_B$ + H5' + H5), 5.00 (1H, d, *J* = 7.8 Hz, H1'), 5.05 - 5.11 (1H, m, H3', obscured by H3' of major isomer), 5.39 - 5.46 (2H, m, H2' + H4'), 5.73 (1H, s, H2), 6.92 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.38 (2H, d, *J* = 8.6 Hz, Ar-C*H*); δ_C (100.6 MHz, $CDCl_3$): major isomer (*cis*): 20.4, 20.5, 20.5, 20.5 (*C*H$_3$-C(=O)-O-), 39.0 (C4), 52.4 (-CO_2*C*H$_3$), 61.2 (C6'), 65.3 (C5), 66.7 (C4'), 68.4 (C2'), 70.6 (C3'), 70.9 (C5'), 71.8 (C2), 99.3 (C1'), 116.8, 128.7 (Ar-*C*H), 132.9, 156.9 (Ar-C), 169.2, 169.9, 170.0, 170.1 (CH$_3$-

C(=O)-O-), 171.4 (-*C*O_2CH_3); minor isomer (*trans*): 20.4, 20.5, 20.5, 20.5 (*C*H_3-C(=O)-O-), 37.9 (C4), 52.4 (-CO_2*C*H_3), 61.2 (C6'), 64.0 (C5), 66.7 (C4'), 68.4 (C2'), 70.0 (C2), 70.6 (C3'), 70.9 (C5'), 99.4 (C1'), 116.6, 128.1 (Ar-*C*H), 135.9, 156.4 (Ar-C), 169.2, 169.9, 170.0, 170.1 (CH_3-*C*(=O)-O-), 172.0 (-*C*O_2CH_3); *m/z* (ESI^+) 570 ($[M+H]^+$ 35 %); HRMS (ESI^+); $C_{25}H_{32}O_{12}NS$ $[M+H]^+$; found 570.16190, requires 570.16397.

Methyl (2*RS*,5*R*)-1-(3-ethoxy-3-oxopropanoyl)-2-(4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)phenyl)-1,3-thiazolidine-5-carboxylate, 191

N-Acylthiazolidine **191** was derived from **190** following the procedure for *N*-acylation outlined in general procedure 6.2.4.



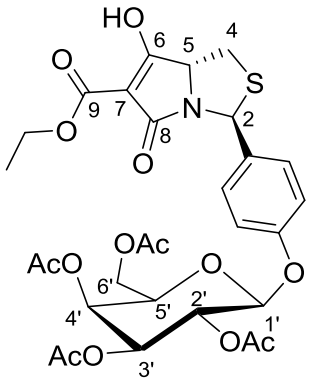
Yield (68 mg, 52 %); colourless oil; 1.1:1 *cis* and *trans* diastereomers; R_f = 0.18 (EtOAc/petrol 1:1); ν_{max}/cm^{-1} (neat) 1223 (C-O), 1663 (C=O), 1745 (C=O); δ_H (500 MHz, $CDCl_3$) major isomer (*cis*, a mixture of two conformers): 1.20 - 1.31 (3H, m, OCH_2CH_3), 2.01 (3H, s, C*H*$_3$-C(=O)-O-), 2.05 - 2.11 (6H, m, 2xC*H*$_3$-C(=O)-O-), 2.18 (3H, s, C*H*$_3$-C(=O)-O-), 3.07 - 3.46 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$), 3.82 (3H, s, CO_2CH_3 major conformer), 3.83 (3H, s, CO_2CH_3 minor conformer), 4.03 - 4.24 (5H, m, OCH_2CH_3, $H6'_A$ + $H6'_B$ + H5'), 4.99 - 5.08 (2H, m, H5 + H1'), 5.11 (1H, dd, *J* = 10.6, 3.1 Hz, H3'), 5.43 - 5.52 (2H, m, H2' + H4'), 6.12 (1H, s, H2 major conformer), 6.28 (1H, s, H2 minor conformer), 6.91 - 6.96 (2H, m, Ar-C*H* minor conformer), 6.98 - 7.04 (2H, m, Ar-C*H* major conformer), 7.46 (2H, d, *J* = 8.5 Hz, Ar-C*H* minor conformer); 7.62 (2H, d, *J* = 8.7 Hz, Ar-C*H* major conformer); minor isomer (*trans*, a mixture of two conformers): 1.20 - 1.31 (3H, m, OCH_2CH_3), 2.01 (3H, s, C*H*$_3$-C(=O)-O-), 2.05 - 2.11 (6H, m, 2xC*H*$_3$-C(=O)-O-), 2.18 (3H, s, C*H*$_3$-C(=O)-O-), 3.07 - 3.46 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$), 3.79 (3H, s, CO_2CH_3 major conformer), 3.85 (3H, s, CO_2CH_3 minor conformer), 4.03 - 4.24 (5H, m, OCH_2CH_3 + $H6'_A$ + $H6'_B$ + H5'), 4.99 - 5.08 (1H, m, H1'

obscured by H5 of major isomer), 5.11 (1H, dd, *J* = 10.6, 3.1 Hz, H3'), 5.18 (1H, app d, *J* = 5.4 Hz, H5 minor conformer), 5.28 - 5.31 (1H, m, H5 major conformer), 5.43 - 5.52 (2H, m, H2' + H4'), 6.16 (1H, s, H2 major conformer), 6.28 (1H, s, H2 minor conformer), 6.91 - 6.96 (2H, m, Ar-C*H* minor conformer), 6.98 - 7.04 (2H, m, Ar-C*H* major conformer), 7.16 (2H, d, *J* = 8.7 Hz, Ar-C*H* major conformer); 7.22 (2H, d, *J* = 8.6 Hz, Ar-C*H* minor conformer); δ_C (125.8 MHz, $CDCl_3$): major isomer (*cis*, a mixture of two conformers): 14.0, 14.2 (OCH_2CH_3), 20.6, 20.6, 20.6, 20.7 (CH_3-C(=O)-O-), 33.1, 33.8 (C4), 42.1, 43.1 (C2″), 52.7, 53.4 (CO_2CH_3), 61.2, 61.3 (C6'), 61.6, 61.7 (OCH_2CH_3), 63.8, 64.6 (C5), 65.8, 66.7 (C2), 66.8 (C4'), 68.5 (C2'), 70.7, 70.8 (C3'), 70.9, 71.1 (C5'), 99.3, 99.5 (C1'), 116.6, 117.2, 127.9, 128.6 (Ar-*C*H), 133.5, 134.5, 156.6, 157.0 (Ar-C), 165.0, 165.4 (C1''), 166.7, 167.1 (C3''), 169.3, 169.4, 170.06, 170.08, 170.15, 170.18, 170.20, 170.23, 170.3, 170.4 (CO_2CH_3 and CH_3-*C*(=O)-O-); minor isomer (*trans*, a mixture of two conformers): 14.0, 14.1 (OCH_2CH_3), 20.6, 20.6, 20.6, 20.7 (CH_3-C(=O)-O-), 31.0, 32.0 (C4), 42.4, 43.3 (C2″), 52.8, 53.4 (CO_2CH_3), 61.2, 61.3 (C6'), 61.5, 61.8 (OCH_2CH_3), 64.0, 64.2 (C5), 64.5, 65.2 (C2), 66.8 (C4'), 68.5 (C2'), 70.7, 70.8 (C3'), 70.9, 71.1 (C5'), 99.3, 99.6 (C1'), 116.9, 117.4, 126.1, 126.3 (Ar-*C*H), 136.6, 137.0, 156.3, 156.8 (Ar-C), 164.6, 165.3 (C1''), 166.3, 167.3 (C3''), 169.30, 169.33, 170.06, 170.08, 170.15, 170.18, 170.20, 170.23, 170.3, 170.4 (CO_2CH_3 and CH_3-*C*(=O)-O-); *m/z* (ESI^+) 706 ($[M+Na]^+$ 43 %); HRMS (ESI^+); $C_{30}H_{37}O_{15}NNaS$ $[M+Na]^+$; found 706.17535, requires 706.17761.

(-)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-6-hydroxy-8-oxo-2-(4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)phenyl)-3-thiabicyclo[3.3.0]oct-6-ene, 192

Tetramate **192** was obtained from **191** following the procedure for Dieckmann cyclisation outlined in general procedure 6.2.5.1. The product isolated by flash column chromatography (eluent: 100 % EtOAc to EtOAc/MeOH/1 % Et_3N) was then dissolved in CH_2Cl_2 and washed

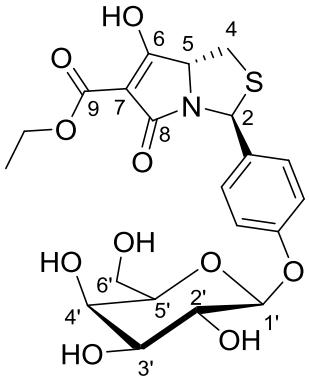
with 5% citric acid. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield **192**.



Yield (0.66 g, 52 %); yellow solid, mp 114 °C; R_f = 0.45 (EtOAc/ MeOH 9:1); $[\alpha]_D^{25}$ = -152.6 (*c* = 0.27, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1217 (C-O), 1613 (C=C), 1746 (C=O, br with shoulder towards smaller wave number); δ_H (400 MHz, $CDCl_3$): 1.39 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 2.02 (3H, s, CH_3-C(=O)-O-), 2.07 (3H, s, CH_3-C(=O)-O-), 2.07 (3H, s, CH_3-C(=O)-O-), 2.19 (3H, s, CH_3-C(=O)-O-), 2.99 (1H, dd, *J* = 11.0, 8.3 Hz, $H4_A$), 3.29 (1H, dd, *J* = 11.0, 7.0 Hz, $H4_B$), 4.03 - 4.09 (1H, m, H5'), 4.15 (1H, dd, *J* = 11.4, 5.8 Hz, $H6'_A$), 4.19 (1H, dd, *J* = 11.4, 7.1 Hz, $H6'_B$), 4.40 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.75 (1H, app t, *J* = 7.6 Hz, H5), 5.03 (1H, d, *J* = 8.1 Hz, H1'), 5.11 (1H, dd, *J* = 10.5, 3.4 Hz, H3'), 5.44 - 5.52 (2H, m, H2' + H4'), 6.27 (1H, s, H2), 6.97 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.43 (2H, d, *J* = 8.8 Hz, Ar-C*H*); δ_C (125.8 MHz, CD_2Cl_2): 14.5 (OCH_2CH_3), 20.9, 21.0, 21.1, 21.1 (CH_3-C(=O)-O-), 33.4 (C4), 62.0 (C6'), 62.2 (OCH_2CH_3), 62.5 (C2), 66.1 (C5), 67.5 (C4'), 69.0 (C2'), 71.3 (C3'), 71.8 (C5'), 100.2 (C1'), 117.5, 128.3 (Ar-CH), 136.0, 157.3 (Ar-C), 167.6 (C9), 169.2 (C8), 169.9, 170.5, 170.7, 170.8 (CH_3-C(=O)-O-), 186.7 (C6); *m/z* (ESI^+) 674 ($[M+Na]^+$, 15 %); HRMS (ESI^+); $C_{29}H_{33}O_{14}NNaS$ $[M+Na]^+$; found 674.15101, requires 674.15140.

(-)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-2-(4-(β-D-galactopyranosyloxy)phenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 193

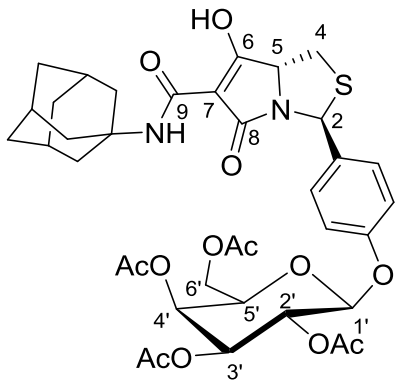
Tetramate **192** (28 mg, 0.04 mmol, 1.0 eq) was dissolved in MeOH (2 mL) and aq. K_2CO_3 (6.6 mg in 0.5 mL of H_2O, 0.048 mmol, 1.2 eq) was added. The reaction was stirred at rt for 5 min and upon completion, solvents were removed *in vacuo* to afford **193**. (An acidic work-up was not possible due to the acid-labile glycosidic linkage).



Yield (25 mg, quant.); yellow solid, mp >260 °C; R_f = 0.07 (EtOAc/MeOH 3:1); $[\alpha]_D^{25}$ = - 215.1 (*c* = 0.18, DMSO); ν_{max}/cm^{-1} (neat) 1227 (C-O), 1627 (C=C, br. with shoulder towards higher wavenumber), 1689 (C=O), 3246 (O-H); δ_H (400 MHz, D_2O): 1.28 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 3.00 (1H, dd, *J* = 11.1, 7.9 Hz, $H4_A$), 3.27 (1H, dd, *J* = 11.1, 7.5 Hz, $H4_B$), 3.73 - 3.84 (4H, m, $H6'_A$ + $H6'_B$ + H3'+ H2'), 3.85 - 3.91 (1H, m, H5'), 4.01 (1H, app d, *J* = 2.5 Hz, H4'), 4.18 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.45 (1H, app t, *J* = 7.6 Hz, H5), 5.06 (1H, d, *J* = 7.2 Hz, H1'), 6.31 (1H, s, H2), 7.14 (2H, d, *J* = 8.7 Hz, Ar-C*H*), 7.47 (2H, d, *J* = 8.7 Hz, Ar-C*H*); δ_C (100.6 MHz, D_2O): 13.9 (OCH_2CH_3), 32.9 (C4), 59.7 (OCH_2CH_3), 60.8 (C6'), 62.6 (C2), 68.5 (C4'), 69.2 (C5), 70.6, 72.6 (C2', C3'), 75.4 (C5'), 91.1 (C7), 100.8 (C1'), 116.7, 127.6 (Ar-*C*H), 136.1, 156.3 (Ar-C), 166.2 (C9), 178.7 (C8), 194.4 (C6); *m/z* (ESI^-) 482 ($[M-H]^-$, 100 %); HRMS (ESI^+); $C_{21}H_{25}O_{10}NNaS$ $[M+Na]^+$; found 506.10689, requires 506.10914.

(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-6-hydroxy-8-oxo-2-(4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)phenyl)-3-thiabicyclo[3.3.0]oct-6-ene, 195

Carboxamide tetramate **195** was obtained by aminolysis of **192** with 1-adamantylamine according to the general procedure 6.3.1 for the synthesis of carboxamides with THF/toluene as solvent.

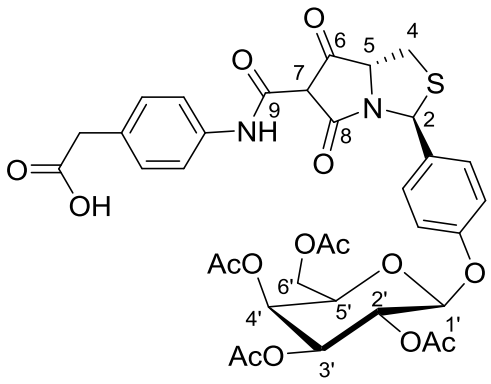


Yield (22 mg, 30 %); yellow foaming solid, mp 142-146 °C; 2.9:1 AB:CD tautomers; R_f = 0.38 (EtOAc/petrol 4:1); $[\alpha]_D^{25}$ = -148.2 (*c* = 0.74, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1227 (C-O), 1626 (C=C), 1650 (C=O), 1690 (C=O), 1753 (C=O); δ_H (400 MHz, CD_2Cl_2): 1.69 (6H, br. signal, Adamantyl-CH_2), 1.98 (3H, s, CH_3-C(=O)-O-), 2.04 (12H, br. signal, Adamantyl-CH_2 + 2xCH_3-C(=O)-O-), 2.09 (3H, br. signal, Adamantyl-C*H*), 2.16 (3H, s, CH_3-C(=O)-O-),

2.98 (1H, dd, *J* = 11.0, 8.6 Hz, $H4_A$), 3.25 (1H, dd, *J* = 11.0, 7.1 Hz, $H4_B$), 3.47 (1H, br. s., OH), 4.04 - 4.22 (3H, m, $H6'_A$ + $H6'_B$ + H5'), 4.41 (1H, app t, *J* = 6.9 Hz, H5 CD), 4.69 (1H, app t, *J* = 7.6 Hz, H5 AB), 5.05 (1H, d, *J* = 8.0 Hz, H1'), 5.10 (1H, dd, *J* = 10.5, 3.4 Hz, H3'), 5.40 (1H, dd, *J* = 10.5, 8.0 Hz, H2'), 5.44 (1H, app d, *J* = 3.4 Hz, H4'), 6.18 (1H, s, H2 AB), 6.27 (1H, s, H2 CD), 6.99 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.34 (1H, br. s., NH AB), 7.40 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.91 (1H, br. s., NH CD); δ_C (100.6 MHz, CD_2Cl_2): 20.8, 20.9, 21.0, 21.0 (*C*H_3-C(=O)-O-), 30.0 (Adamantyl-*C*H), 33.1 (C4), 36.5 (Adamantyl-*C*H_2), 42.0 (Adamantyl-*C*H_2), 53.3 (Adamantyl-C), 62.0 (C6'), 62.0 (C2), 67.5 (C4'), 67.8 (C5), 69.0 (C2'), 71.3 (C3'), 71.7 (C5'), 96.0 (C7), 100.1 (C1'), 117.4, 128.3 (Ar-*C*H), 136.1, 157.2 (Ar-C), 166.5 (C9), 169.8, 170.5, 170.66, 170.69 (CH_3-*C*(=O)-O-), 172.7 (C8), 188.1 (C6); *m/z* (ESI^-) 755 ([M-H]$^-$, 29 %); HRMS (ESI^-); $C_{37}H_{43}O_{13}N_2S$ [M-H]$^-$; found 755.24963, requires 755.24913.

(-)-(2*S*,5*R*)-1-Aza-7-((4-carboxymethylphenyl)aminocarbonyl)-6,8-dioxo-2-(4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)phenyl)-3-thia-bicyclo[3.3.0]octane, 196

Carboxamide tetramate **196** was obtained by aminolysis of **192** with 4-aminophenylacetic acid according to the general procedure 6.3.1 for the synthesis of carboxamides with DMSO/toluene as solvent.

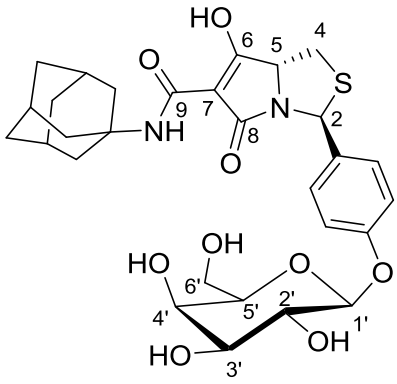


Yield (58 mg, 23 %); brown solid, mp 148-150 °C; R_f = 0.20 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -40.4 (*c* = 0.14, DMSO); ν_{max}/cm^{-1} (neat) 1228 (C-O), 1747 (C=O); δ_H (400 MHz, CD_2Cl_2): 1.99 (3H, s, C*H*$_3$-C(=O)-O-), 2.04 (3H, s, C*H*$_3$-C(=O)-O-), 2.04 (3H, s, C*H*$_3$-C(=O)-O-), 2.17 (3H, s, C*H*$_3$-C(=O)-O-), 2.94 (1H, dd, *J* = 10.6, 8.7 Hz, $H4_A$), 3.31 (1H, dd, *J* = 10.6, 6.5 Hz, $H4_B$), 3.61 (-C*H*$_2$CO$_2$H), 4.05 - 4.23 (3H, m, 2xH6' + H5'), 4.86 (1H, app t, *J* = 7.3 Hz, H5), 5.06 (1H, d, *J* = 8.1 Hz, H1'), 5.11 (1H, dd, *J* = 10.5, 3.4 Hz, H3'), 5.23 (1H, s, H7), 5.40 (1H, dd, *J* = 10.5, 8.1 Hz, H2'),

5.44 (1H, app d, *J* = 3.4 Hz, H4'), 6.13 (1H, s, H2), 6.98 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.09 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.24 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.40 (2H, d, *J* = 8.6 Hz, Ar-C*H*); δ_C (125.8 MHz, CD_2Cl_2): 20.9, 21.0, 21.1, 21.1 (*C*H_3-C(=O)-O-), 35.1 (C4), 40.3 (-*C*H_2CO_2H), 62.0 (C6'), 62.7 (C2), 67.5 (C4'), 69.1 (C5), 69.1 (C2'), 71.3 (C3'), 71.8 (C5'), 91.9 (C7), 100.3 (C1'), 117.5, 120.4, 128.3, 131.1 (Ar-*C*H), 130.5, 136.9, 139.3, 157.2 (Ar-C), 170.0, 170.6, 170.8, 170.9 (CH_3-*C*(=O)-O-), 174.0 (*C*O_2H), 177.6 (C8); *m/z* (ESI/FI) molecular ion not detected.

(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(4-(β-D-galactopyranosyloxy)phenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 197

Tetramate **195** (31 mg, 0.04 mmol, 1.0 eq) was dissolved in MeOH (2 mL) and aq. K_2CO_3 (6.6 mg in 0.5 mL of H_2O, 0.048 mmol, 1.2 eq) was added. The reaction was stirred at rt for 10-15 min and upon completion, solvents were removed *in vacuo*. The residue was dissolved in MeOH, filtered and concentrated *in vacuo* to afford **197**. (An acidic work-up was not possible due to the acid-labile glycosidic linkage).



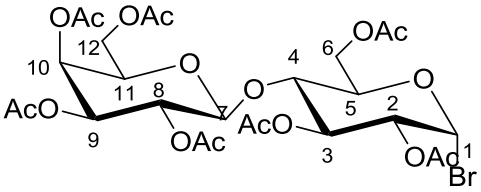
Yield (20 mg, 85 %); yellow solid, mp >260 °C; R_f = 0.08 (EtOAc/MeOH 3:1); $[\alpha]_D^{25}$ = -130.8 (*c* = 0.11, H_2O); ν_{max}/cm^{-1} (neat) 1228 (C-O), 1607 (br with shoulder towards higher wavenumber), 3339 (O-H); δ_H (500 MHz, Methanol-*d4*): 1.71 (6H, br. signal, Adamantyl-CH_2), 2.05 (9H, br. signal, Adamantyl-CH_2 + Adamantyl-C*H*), 2.93 (1H, dd, *J* = 11.0, 6.9 Hz, $H4_A$), 3.17 (1H, dd, *J* = 11.0, 7.8 Hz, $H4_B$), 3.60 - 3.66 (1H, m, H3'), 3.70 - 3.82 (4H, m, $H6'_A$ + $H6'_B$ + H5' + H2'), 3.94 (1H, app d, *J* = 3.0 Hz, H4'), 4.21 (1H, app t, *J* = 7.3 Hz, H5), 4.90 (1H, d, *J* = 7.9 Hz, H1'), 6.25 (1H, s, H2), 7.08 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.39 (2H, d, *J* = 8.6 Hz, Ar-C*H*); δ_C (125.8 MHz, Methanol-*d4*): 31.0 (Adamantyl-*C*H), 34.4 (C4), 37.7 (Adamantyl-*C*H_2), 43.3 (Adamantyl-*C*H_2), 51.9 (Adamantyl-C), 62.3 (C6'), 64.7 (C2),

70.1 (C4'), 70.4 (C5), 72.3 (C2'), 74.7 (C3'), 76.9 (C5'), 94.6 (C7), 102.9 (C1'), 117.7, 128.8 (Ar-*C*H), 137.6, 158.4 (Ar-C), 167.4 (C9), 181.0 (C8), 194.0 (C6); *m/z* (ESI$^-$) 587 ([M-H]$^-$, 38 %); HRMS (ESI$^-$); $C_{29}H_{35}O_9N_2S$ [M-H]$^-$; found 587.20687, requires 587.20687.

$BiBr_3$ catalysed bromination of lactose octaacetate[101]

To a stirred solution of lactose octaacetate (2.0 g, 2.95 mmol, 1.0 eq) and $BiBr_3$ (66 mg, 0.15 mmol, 0.05 eq) in 20 mL of CH_2Cl_2 was added under N_2, TMSBr (1.56 mL, 11.8 mmol, 4.0 eq). The reaction was stirred at rt and after 15 h, upon completion of reaction, the reaction mixture was poured into cold sat. $NaHCO_3$ and extracted twice with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to obtain **198**.

(+)-4-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-D-glucopyranosyl bromide, 198

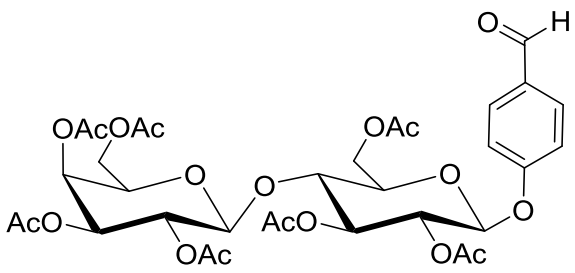


Yield (1.95 g, 92 %); white solid, mp 98 °C; R_f = 0.33 (EtOAc/petrol 1:1); $[\alpha]_D^{25}$ = +94.1 (*c* = 1.0, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1211 (C-O), 1743 (C=O); δ_H (400 MHz, CD_2Cl_2): 1.94 (3H, s, C*H*$_3$-C(=O)-O-), 2.02 - 2.06 (9H, s, C*H*$_3$-C(=O)-O-), 2.07 (3H, s, C*H*$_3$-C(=O)-O-), 2.11 (3H, s, C*H*$_3$-C(=O)-O-), 2.14 (3H, s, C*H*$_3$-C(=O)-O-), 3.79 - 3.93 (2H, m, H5 + H11), 4.04 - 4.23 (4H, m, H6 + H12 + H4), 4.41 - 4.54 (1H, m, H6/H12), 4.50 (1H, d, *J* = 7.9 Hz, H7), 4.74 (1H, dd, *J* = 9.9, 4.0 Hz, H2), 4.93 (1H, dd, *J* = 10.4, 3.2 Hz, H9), 5.10 (1H, dd, *J* = 10.4, 7.9 Hz, H8), 5.33 (1H, app d, *J* = 3.2 Hz, H10), 5.53 (1H, app t, *J* = 9.6 Hz, H3), 6.51 (1H, d, *J* = 4.0 Hz, H1); δ_C (100.6 MHz, CD_2Cl_2): 20.4, 20.6, 20.7 (*C*H$_3$-C(=O)-O-), 60.8, 61.0 (C6, C12), 66.6 (C10), 69.0 (C8), 69.5 (C3), 70.7 (C5/C11), 70.8 (C2), 70.9 (C9), 72.9 (C4), 74.9 (C5/C11), 86.3 (C1), 110.7 (C7); *m/z* (ESI$^+$) 721,723 ([M+Na]$^+$, 78 %); HRMS (ESI$^+$); $C_{26}H_{35}O_{17}BrNa$ [M+Na]$^+$; found 721.09288 and 723.09099, requires 721.09498 and 723.09294.

Solid-liquid phase transfer catalaysed aryl glycosylation[102]

To **198** (2.63 g, 3.76 mmol, 1.0 eq), 4-hydroxybenzaldehyde (0.55 g, 4.51 mmol, 1.2 eq) and benzyltributylammonium chloride (0.23 g, 0.75 mmol, 0.2 eq) in $CHCl_3$ (15 mL), was added powdered K_2CO_3 (1.69 g, 12.2 mmol, 5 eq) and the mixture stirred at rt for 24 h. The reaction mixture was neutralised with 10 % HCl and the organic layer was separated. The organic layer was washed with sat. $NaHCO_3$ and brine, then dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (eluent: EtOAc/petrol) to furnish **199**.

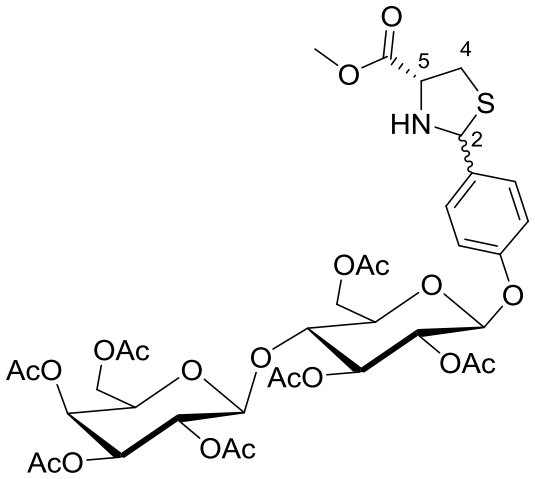
(+)-4-(4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-D-glucopyranosyloxy)benzaldehyde, 199



Yield (2.45 g, 88 %); white foam, mp 98-104 °C; R_f = 0.28 (EtOAc/petrol 1:1); $[\alpha]_D^{25}$ = +14.9 (*c* = 1.0, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1699 (C=O), 1744 (C=O); δ_H (400 MHz, $CDCl_3$): 1.95 (3H, s, C*H*$_3$-C(=O)-O-), 2.01 - 2.10 (15H, m, 5xC*H*$_3$-C(=O)-O-), 2.13 (3H, s, C*H*$_3$-C(=O)-O-), 3.78 - 3.93 (2H, m, lactose-C*H*), 4.01 - 4.25 (4H, m, lactose-C*H*$_2$ + lactose-C*H*), 4.36 - 4.54 (1H, m, lactose-C*H*$_2$), 4.51 (1H, d, *J* = 7.8 Hz, lactose-C*H*), 4.93 - 5.00 (1H, m, lactose-C*H*), 5.07 - 5.21 (4H, m, lactose-C*H*), 5.25 - 5.30 (1H, m, lactose-C*H*), 7.06 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.82 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 9.89 (1H, s, C*H*O); δ_C (100.6 MHz, $CDCl_3$) 20.4, 20.4, 20.5, 20.6, 20.7, 20.7, 20.9 (*C*H$_3$-C(=O)-O-), 60.7, 61.3 (lactose-*C*H$_2$), 68.2, 69.0, 70.6, 70.7, 71.2, 72.6, 72.9, 76.0, 97.6, 101.0 (lactose-*C*H), 116.6, 131.7 (Ar-C*H*), 127.4, 139.3 (Ar-C), 169.0, 169.1, 169.4, 169.6, 169.9, 170.0, 170.3 (CH$_3$-*C*(=O)-O-), 190.6 (1H, s, *C*HO); *m/z* (ESI$^+$) 763 ([M+Na]$^+$, 4 %); HRMS (ESI$^+$); $C_{33}H_{40}O_{19}Na$ [M+Na]$^+$; found 763.20569, requires 763.20560.

Methyl (2*RS*,5*R*)-2-(4-(4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-D-glucopyranosyloxy)phenyl)-1,3-thiazolidine-5-carboxylate, 200

Thiazolidine **200** was derived from the condensation of L-cysteine methyl ester hydrochloride and aldehyde **199** following the procedure for thiazolidine synthesis outlined in general procedure 6.2.3.

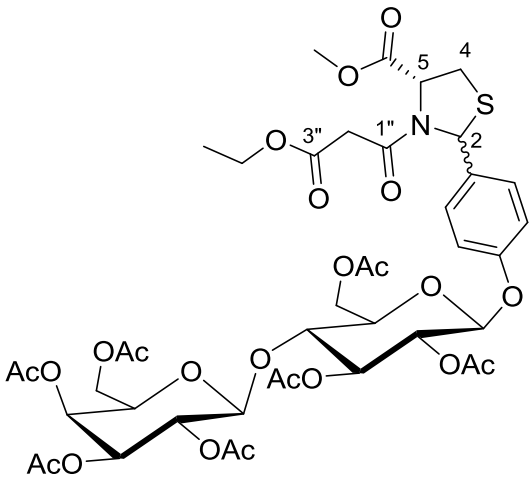


Yield (2.05 g, 30 %); white foam; 1.7:1 *cis* and *trans* diastereomers; R_f = 0.37 (EtOAc/petrol 2:1); ν_{max}/cm^{-1} (neat) 1738 (C=O); δ_H (400 MHz, $CDCl_3$) major isomer (*cis*): 1.95 (3H, s, C*H*$_3$-C(=O)-O-), 2.02 - 2.07 (12H, m, 4xC*H*$_3$-C(=O)-O-), 2.10 - 2.16 (6H, m, 2xC*H*$_3$-C(=O)-O-), 2.58 (1H, br. s., NH), 3.09 (1H, dd, *J* = 10.3, 8.8 Hz, H4$_A$), 3.44 (1H, dd, *J* = 10.3, 7.1 Hz, H4$_B$), 3.73 - 3.77 (1H, m, lactose-C*H*), 3.79 (3H, s, CO_2CH_3), 3.84 - 3.91 (1H, m, lactose-C*H*), 4.01 - 4.20 (3H, m, H5 + lactose-C*H*$_2$), 4.44 - 4.58 (3H, m, lactose-C*H* + lactose-C*H*$_2$), 4.66 - 4.83 (2H, m, lactose-C*H*), 4.90 - 5.40 (4H, m, lactose-C*H*), 5.46 - 5.55 (2H, H2 + lactose-C*H*), 6.95 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.43 (2H, d, *J* = 8.6 Hz, Ar-C*H*); minor isomer (*trans*): 1.95 (3H, s, C*H*$_3$-C(=O)-O-), 2.02 - 2.07 (12H, m, 4xC*H*$_3$-C(=O)-O-), 2.10 - 2.16 (6H, m, 2xC*H*$_3$-C(=O)-O-), 2.58 (1H, br. s., NH), 3.16 - 3.21 (1H, m, H4$_A$), 3.33 - 3.40 (1H, m, H4$_B$), 3.73 - 3.77 (1H, m, lactose-C*H*), 3.79 (3H, s, CO_2CH_3), 3.84 - 3.91 (1H, m, lactose-C*H*), 4.01 - 4.20 (3H, m, H5 + lactose-C*H*$_2$), 4.44 - 4.58 (3H, m, lactose-C*H* + lactose-C*H*$_2$), 4.66 - 4.83 (2H, m, lactose-C*H*), 4.90 - 5.40 (4H, m, lactose-C*H*), 5.46 - 5.55 (2H, H2 + lactose-C*H*), 6.91 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.39 (2H, d, *J* = 8.8 Hz, Ar-C*H*); δ_C (125.8 MHz, $CDCl_3$) major isomer (*cis*): 20.4, 20.6, 20.7, 20.7, 20.8, 20.8, 21.0 (*C*H$_3$-C(=O)-O-), 39.1 (C4), 52.6 (CO_2CH_3), 60.8, 61.8 (lactose-*C*H$_2$), 66.6 (lactose-*C*H), 68.1 (C5), 69.1 (lactose-*C*H), 69.5 (C2), 70.5, 70.9, 71.0, 71.3, 72.7, 76.3, 89.9, 100.9 (lactose-*C*H), 116.9, 128.8 (Ar-C*H*), 132.9, 156.9 (Ar-C), 169.0, 169.6, 170.1, 170.2,

170.3, 170.3, 170.5 (CH_3-*C*(=O)-O-), 171.5 (*C*O_2CH_3); minor isomer (*trans*): 20.4, 20.6, 20.7, 20.7, 20.8, 20.8, 21.0 (*C*H_3-C(=O)-O-), 38.0 (C4), 52.5 (CO_2*C*H_3), 60.4, 61.7 (lactose-*C*H_2), 66.5 (lactose-*C*H), 68.1 (C5), 69.0 (lactose-*C*H), 69.5 (C2), 70.6, 70.9, 71.0, 71.4, 72.8, 76.1, 89.9, 101.0 (lactose-*C*H), 116.7, 128.2 (Ar-C*H*), 135.9, 156.4 (Ar-C), 169.1, 169.6, 170.0, 170.1, 170.3, 170.3, 170.5 (CH_3-*C*(=O)-O-), 172.1 (*C*O_2CH_3); *m/z* (ESI^+) 858 ($[M+H]^+$, 89 %); HRMS (ESI^+); $C_{37}H_{48}NO_{20}S$ $[M+H]^+$; found 858.24967, requires 858.24959.

Methyl (2*RS*,5*R*)-1-(3-ethoxy-3-oxopropanoyl)-2-(4-(4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-D-glucopyranosyloxy)phenyl)-1,3-thiazolidine-5-carboxylate, 201

N-Acylthiazolidine **201** was derived from **200** following the procedure for *N*-acylation outlined in general procedure 6.2.4.



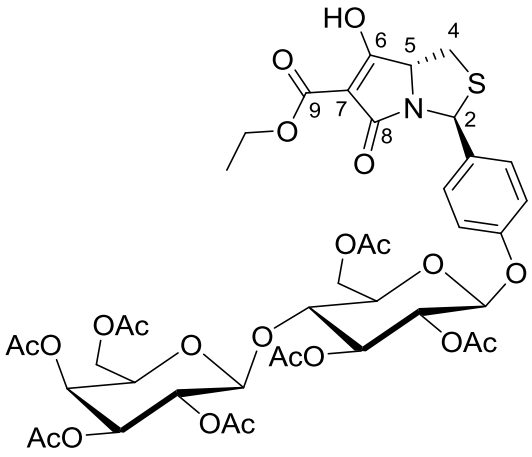
Yield 27 % (0.60 g); white solid; 1.2:1 *cis* and *trans* diastereomers; R_f = 0.32 (EtOAc/petrol 3:2); ν_{max}/cm^{-1} (neat) 1661 (C=O), 1742 (C=O); δ_H (400 MHz, $CDCl_3$) major isomer (*cis*, a mixture of two conformers): 1.19 - 1.31 (3H, m, OCH_2CH_3), 1.96 - 2.18 (21H, m, 7 x CH_3-C(=O)-O- major conformer + 21H, m, 7 x CH_3-C(=O)-O- minor conformer), 3.04 - 3.55 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$), 3.75 - 3.81 (1H, m, lactose-C*H*), 3.83 (3H, s, CO_2CH_3 major conformer), 3.85 (3H, s, CO_2CH_3 minor conformer), 3.86 - 3.93 (2H, m, lactose-C*H*), 4.05 - 4.19 (4H, m, OCH_2CH_3 + lactose-CH_2), 4.43 - 4.62 (3H, m, lactose-C*H* + lactose-CH_2), 4.92 - 5.42 (6H, m, H5 + 5 x lactose-C*H*), 6.11 (1H, s, H2 major conformer), 6.27 (1H, s, H2 minor conformer), 6.87 - 6.94 (2H, m, Ar-C*H* minor conformer), 6.95 - 7.02 (2H, m, Ar-C*H* major conformer), 7.45 (2H, d, *J* = 8.6 Hz, Ar-C*H* minor conformer), 7.61 (2H, d, *J* = 8.8 Hz, Ar-C*H* major conformer); minor isomer (*trans*, a mixture of two conformers): 1.19 - 1.31 (3H, m, OCH_2CH_3), 1.96 - 2.18 (21H, m, 7 x CH_3-C(=O)-O-

major conformer + 21H, m, 7 x C*H*$_3$-C(=O)-O- minor conformer), 3.04 - 3.55 (4H, m, H4$_A$ + H4$_B$ + H2″$_A$ + H2″$_B$), 3.75 - 3.81 (4H, m, CO$_2$C*H*$_3$ + lactose-C*H*), 3.86 - 3.93 (2H, m, lactose-C*H*), 4.05 - 4.19 (4H, m, OC*H*$_2$CH$_3$ + lactose-C*H*$_2$), 4.43 - 4.62 (3H, m, lactose-C*H* + lactose-C*H*$_2$), 4.92 - 5.42 (6H, m, H5 + 5 x lactose-C*H*), 6.15 (1H, s, H2 major conformer), 6.27 (1H, s, H2 minor conformer), 6.87 - 6.94 (2H, m, Ar-C*H* minor conformer), 6.95 - 7.02 (2H, m, Ar-C*H* major conformer), 7.15 (2H, d, *J* = 8.6 Hz, Ar-C*H* major conformer), 7.22 (2H, d, *J* = 8.8 Hz, Ar-C*H* minor conformer);

δ_C (125.8 MHz, $CDCl_3$) major isomer (*cis*, a mixture of two conformers): 14.0, 14.1 (OCH$_2$*C*H$_3$), 20.3, 20.4, 20.5, 20.6, 20.6, 20.8, 21.0 (*C*H$_3$-C(=O)-O-), 32.0, 33.0 (C4), 42.1, 43.0 (C2″), 52.7, 53.2 (CO$_2$*C*H$_3$), 60.4, 60.7, 60.8, 61.5, 61.6, 61.7, 61.8, 61.9, 61.9, 62.4 (O*C*H$_2$CH$_3$, lactose-C*H*$_2$, obscured by *trans* isomer), 63.8, 64.0 (C5), 65.7, 65.8 (C2), 66.6, 66.7, 68.6, 69.0, 70.7, 70.9, 71.3, 71.5, 72.6, 72.7, 72.7, 72.8, 72.8, 73.3, 76.1, 76.1, 98.4, 98.6, 101.1, 102.0 (lactose-*C*H), 116.5, 117.1, 127.9, 128.6 (Ar-*C*H), 134.2, 134.5, 156.9, 157.1 (Ar-C), 165.0, 165.4 (C1″), 166.7, 167.1 (C2″), 169.1, 170.0, 169.6, 169.7, 170.0, 170.1, 170.2, 170.3, 170.3 (*C*O$_2$CH$_3$ + CH$_3$-*C*(=O)-O-); minor isomer (*trans*, a mixture of two conformers): 14.0, 14.1 (OCH$_2$*C*H$_3$), 20.3, 20.4, 20.5, 20.6, 20.6, 20.8, 21.0 (*C*H$_3$-C(=O)-O-), 31.0, 33.0 (C4), 42.4, 43.3 (C2″), 52.8, 53.4 (CO$_2$*C*H$_3$), 60.4, 60.7, 60.8, 61.5, 61.6, 61.7, 61.8, 61.9, 61.9, 62.4 (O*C*H$_2$CH$_3$, lactose-C*H*$_2$, obscured by *cis* isomer), 64.2, 64.6 (C5), 64.6, 65.2 (C2), 66.6, 66.7, 68.6, 69.0, 70.9, 70.9, 71.3, 71.4, 72.6, 72.7, 72.7, 72.8, 72.8, 73.3, 76.0, 76.1, 98.4, 98.6, 101.01, 101.1 (lactose-*C*H), 116.9, 117.4, 126.1, 126.3 (Ar-*C*H), 136.6, 137.0, 156.7, 157.1 (Ar-C), 164.5, 165.3 (C1″), 166.2, 167.3 (C2″), 169.0, 169.3, 169.5, 170.0, 170.1, 170.2, 170.3, 170.3 (*C*O$_2$CH$_3$ + CH$_3$-*C*(=O)-O-); *m/z* (ESI/FI) molecular ion not detected.

(-)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-6-hydroxy-8-oxo-2-(4-(4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-D-glucopyranosyloxy)phenyl)-3-thiabicyclo[3.3.0]oct-6-ene, 202

Tetramate **202** was obtained from **201** following the procedure for Dieckmann cyclisation outlined in general procedure 6.2.5.1. The product isolated by flash column chromatography (eluent: 100 % EtOAc to EtOAc/MeOH/1 % Et_3N) was then dissolved in CH_2Cl_2 and washed with 5% citric acid. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield **202**.

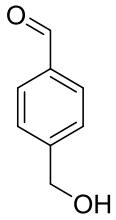


Yield (0.15 g, 31 %); yellow solid, mp 144-146 °C; R_f = 0.38 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -96.8 (*c* = 0.16, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1215 (C-O), 1620 (C=O), 1659 (C=O), 1744 (C=O); δ_H (400 MHz, CD_2Cl_2): 1.36 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 1.95 (3H, s, CH_3-C(=O)-O-), 2.03 (3H, s, CH_3-C(=O)-O-), 2.05 (6H, s, CH_3-C(=O)-O-), 2.06 (3H, s, CH_3-C(=O)-O-), 2.08 (3H, s, CH_3-C(=O)-O-), 2.12 (3H, s, CH_3-C(=O)-O-), 3.00 (1H, dd, *J* = 11.3, 8.3 Hz, $H4_A$), 3.28 (1H, dd, *J* = 11.3, 7.1 Hz, $H4_B$), 3.73 - 3.82 (1H, m, lactose-C*H*), 3.87 - 3.95 (2H, m, lactose-C*H*), 4.04 - 4.20 (3H, m, lactos-CH_2), 4.37 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.46 - 4.55 (2H, m, lactose-C*H* + lactose-CH_2), 4.76 (1H, app t, *J* = 7.6 Hz, H5), 4.94 - 5.01 (1H, m, lactose-C*H*), 5.04 - 5.10 (2H, m, lactose-C*H*), 5.12 - 5.17 (1H, m, lactose-C*H*), 5.22 - 5.28 (1H, m, lactose-C*H*), 5.36 (1H, dd, *J* = 3.4, 1.0 Hz, lactose-C*H*), 6.23 (1H, s, H2), 6.96 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.39 (2H, d, *J* = 8.6 Hz, Ar-C*H*); δ_C (125.8 MHz, CD_2Cl_2): 14.5 (OCH_2CH_3), 20.8, 20.9, 21.0, 21.2 (CH_3-C(=O)-O-), 33.4 (C4), 61.5 (lactose-CH_2), 62.2 (OCH_2CH_3), 62.3 (lactose-CH_2), 62.4 (C2), 66.0 (C5), 67.3, 69.5, 71.5, 71.7, 72.9, 73.6, 76.5, 99.5, 101.6 (lactose-CH), 117.4, 128.3 (Ar-CH), 136.0, 157.1 (Ar-C), 167.6 (C9), 169.2 (C8), 169.7, 170.5, 170.3, 170.5, 170.6, 170.8 (CH_3-C(=O)-O-), 186.6 (C6); *m/z* (ESI⁻)

938 ([M-H]$^-$, 100 %); HRMS (ESI$^-$); $C_{41}H_{48}O_{22}NS$ [M-H]$^-$; found 938.2412, requires 938.23942.

4-(Hydroxymethyl)benzaldehyde, 203

The reduction of dialdehyde was according to a modified literature procedure.[103] Terephthalaldehyde (2.0 g, 15 mmol, 1.0 eq) in EtOH:THF (5:7, 60 mL) was cooled to -5 °C. $NaBH_4$ (0.28 g, 7.5 mmol, 0.5 eq) was added with continuous stirring over 30 min, while maintaining the temperature at -5 °C. The mixture was stirred for 6 h at 0-2 °C and then neutralized with 2M HCl to pH 5. Solvents were removed *in vacuo* and H_2O was added to the residue. The product was extracted twice with EtOAc and the combined organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography to furnish **203**.

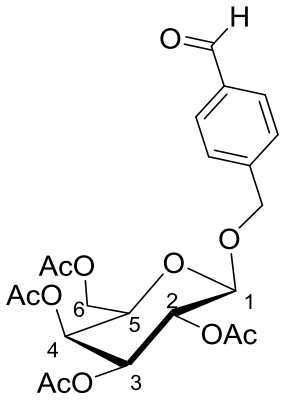


Yield (0.81 g, 70 %); white crystalline solid, mp 40 °C {lit. mp = 40 °C}[103]; R_f = 0.28 (EtOAc/petrol 1:2); ν_{max}/cm^{-1} (neat) 1684 (C=O), 3369 (OH); δ_H (400 MHz, $CDCl_3$) 2.29 (1H, br. s, OH), 4.80 (2H, s, OC*H*$_2$), 7.53 (2H, d, *J* = 8.1 Hz, Ar-C*H*), 7.87 (2H, d, *J* = 8.1 Hz, Ar-C*H*), 9.99 (1H, s, C*H*O); δ_C (125.8 MHz, $CDCl_3$): 64.5 (O*C*H$_2$), 126.9, 130.0 (Ar-C*H*), 135.6, 147.8 (Ar-C), 192.1 (*C*HO); *m/z* (ESI) molecular ion not detected.

(-)-4-((2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyloxy)methyl)benzaldehyde, 204a

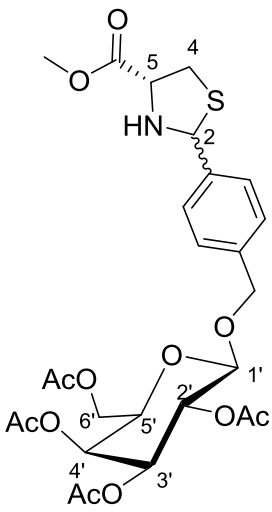
Glycosylation of **203** was according to a modified literature procedure.[87] β-D-Galactose pentaacetate (5.5 g, 14.1 mmol, 1.5 eq) and **203** (1.28 g, 9.39 mmol, 1.0 eq) in CH_2Cl_2 (50 mL) was cooled to 0 °C and $BF_3.OEt_2$ (1.73 mL, 14.1 mmol, 1.5 eq) was added dropwise under N_2. The reaction flask was warmed to rt and stirred for 18 h. The reaction mixture was quenched with sat. $NaHCO_3$ (20 mL) and left to stir for 30 min. The product was extracted

with CH_2Cl_2, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography to obtain **204a** (eluent: EtOAc/petrol).



Yield (3.09 g, 71 %); white crystalline solid, mp 88-90 °C; R_f = 0.12 (EtOAc/petrol 1:2); $[\alpha]_D^{25}$ = -30.3 (*c* = 1.25, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1217 (C-O), 1699 (C=O), 1747 (C=O); δ_H (400 MHz, $CDCl_3$): 1.99 (3H, s, C*H*$_3$-C(=O)-O-), 2.05 (3H, s, C*H*$_3$-C(=O)-O-), 2.06 (3H, s, C*H*$_3$-C(=O)-O-), 2.17 (3H, s, C*H*$_3$-C(=O)-O-), 3.93 (1H, app td, *J* = 6.6, 1.0 Hz, H5), 4.15 (1H, d, *J* = 11.3, 6.6 Hz, H6$_A$), 4.21 (1H, d, *J* = 11.3, 6.6 Hz, H6$_B$), 4.58 (1H, d, *J* = 7.9 Hz, H1), 4.71 (1H, d, *J* = 13.2 Hz, OC*H*$_2$), 4.98 - 5.05 (2H, m, OC*H*$_2$ + H3), 5.32 (1H, dd, *J* = 10.3, 7.9 Hz, H2), 5.41 (1H, dd, *J* = 3.4, 1.0 Hz, H4), 7.46 (2H, d, *J* = 8.1 Hz, Ar-C*H*), 7.87 (2H, d, *J* = 8.1 Hz, Ar-C*H*), 10.01 (1H, s, C*H*O); δ_C (100.6 MHz, $CDCl_3$): 20.5, 20.6, 20.7, 20.8 (*C*H$_3$-C(=O)-O-), 61.2 (C6), 66.9 (C4), 68.7 (C2), 70.0 (O*C*H$_2$), 70.7 (C3), 70.8 (C5), 100.4 (C1), 127.5, 129.9 (Ar-*C*H), 135.9, 143.8 (Ar-C), 169.4, 170.1, 170.2, 170.4 (CH$_3$-*C*(=O)-O-), 191.8 (*C*HO); *m/z* (ESI$^+$) 489 ([M+Na]$^+$, 100 %); HRMS (ESI$^+$); $C_{22}H_{26}O_{11}Na$ [M+Na]$^+$; found 489.13711, requires 489.13673.

Methyl (2*RS*,5*R*)-2-(4-((2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)methyl)phenyl)-1,3-thiazolidine-5-carboxylate, 205



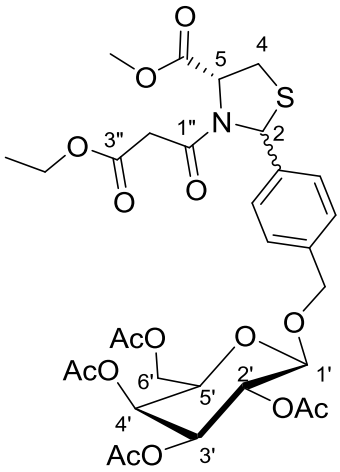
Thiazolidine **205** was derived from the condensation of L-cysteine methyl ester hydrochloride and aldehyde **204a** following the procedure for thiazolidine synthesis outlined in general procedure 6.2.3.

Yield (2.08 g, 54 %); yellow foam; 1.7:1 *cis* and *trans* diastereomers; R_f = 0.53 (EtOAc/petrol 2:1); ν_{max}/cm^{-1} (neat) 1217 (C-O), 1742 (C=O); δ_H (500 MHz, $CDCl_3$) major isomer (*cis*) 1.98 (3H, s, C*H*$_3$-

C(=O)-O-), 2.03 (3H, s, C*H*$_3$-C(=O)-O-), 2.07 (3H, s, C*H*$_3$-C(=O)-O-), 2.16 (3H, s, C*H*$_3$-C(=O)-O-), 2.66 (1H, br. t, *J* = 9.4 Hz, NH), 3.12 (1H, dd, *J* = 10.3, 9.1 Hz, H4$_A$), 3.48 (1H, dd, *J* = 10.3, 7.2 Hz, H4$_B$), 3.82 (3H, s, -CO$_2$C*H*$_3$), 3.86 - 3.91 (1H, m, H5'), 3.95 - 4.03 (1H, m, H5), 4.12 - 4.24 (2H, m, H6'), 4.49 - 4.54 (1H, m, H1'), 4.60 - 4.66 (1H, m, OC*H*$_2$), 4.88 - 4.93 (1H, m, OC*H*$_2$), 4.96 - 5.02 (1H, m, H3'), 5.25 - 5.32 (1H, m, H2'), 5.37 - 5.40 (1H, m, H4'), 5.56 (1H, d, *J* = 9.3 Hz, H2), 7.30 (2H, d, *J* = 8.1 Hz, Ar-C*H*), 7.51 (2H, d, *J* = 8.1 Hz, Ar-C*H*); minor isomer (*trans*): 1.98 (3H, s, C*H*$_3$-C(=O)-O-), 2.02 (3H, s, C*H*$_3$-C(=O)-O-), 2.07 (3H, s, C*H*$_3$-C(=O)-O-), 2.16 (3H, s, C*H*$_3$-C(=O)-O-), 2.86 (1H, br. s., NH), 3.21 (1H, dd, *J* = 10.7, 6.0 Hz, H4$_A$), 3.40 (1H, dd, *J* = 10.7, 7.1 Hz, H4$_B$), 3.80 (3H, s, -CO$_2$C*H*$_3$), 3.86 - 3.91 (1H, m, H5'), 4.12 - 4.24 (3H, m, H5 + H6'), 4.49 - 4.54 (1H, m, H1'), 4.60 - 4.66 (1H, m, OC*H*$_2$), 4.88 - 4.93 (1H, m, OC*H*$_2$), 4.96 - 5.02 (1H, m, H3'), 5.25 - 5.32 (1H, m, H2'), 5.37 - 5.40 (1H, m, H4'), 5.82 (1H, s, H2), 7.25 (2H, d, *J* = 8.1 Hz, Ar-C*H*), 7.47 (2H, d, *J* = 8.1 Hz, Ar-C*H*); δ_C (125.8 MHz, $CDCl_3$): major isomer (*cis*): 20.5, 20.6, 20.7, 20.8 (*C*H$_3$-C(=O)-O-), 39.2 (C4), 52.6 (-CO$_2$*C*H$_3$), 61.3 (C6'), 65.5 (C5), 67.0 (C4'), 68.8 (C2'), 70.1 (O*C*H$_2$), 70.7 (C5'), 70.9 (C3'), 72.2 (C2), 99.8 (C1'), 127.6, 127.9 (Ar-*C*H), 137.3, 137.9 (Ar-C), 169.4, 170.1, 170.2, 170.4 (CH$_3$-*C*(=O)-O-), 171.6 (-*C*O$_2$CH$_3$); minor isomer (*trans*): 20.5, 20.6, 20.7, 20.8 (*C*H$_3$-C(=O)-O-), 38.1 (C4), 52.6 (-CO$_2$*C*H$_3$), 61.3 (C6'), 64.2 (C5), 66.7 (C4'), 68.48 (C2'), 70.2 (O*C*H$_2$), 70.4 (C2), 70.7 (C5'), 70.9 (C3'), 99.7 (C1'), 127.0, 127.8 (Ar-*C*H), 136.4, 141.1 (Ar-C), 169.4, 170.1, 170.2, 170.4 (CH$_3$-*C*(=O)-O-), 172.1 (-*C*O$_2$CH$_3$); *m/z* (ESI$^+$) 584 ([M+H]$^+$ 100 %); HRMS (ESI$^+$); $C_{26}H_{34}O_{12}NS$ [M+H]$^+$; found 584.17932, requires 584.17962.

Methyl (2*RS*,5*R*)-1-(3-ethoxy-3-oxopropanoyl)-2-(4-((2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)methyl)phenyl)-1,3-thiazolidine-5-carboxylate, 206

N-Acylthiazolidine **206** was derived from **205** following the procedure for *N*-acylation outlined in general procedure 6.2.4.

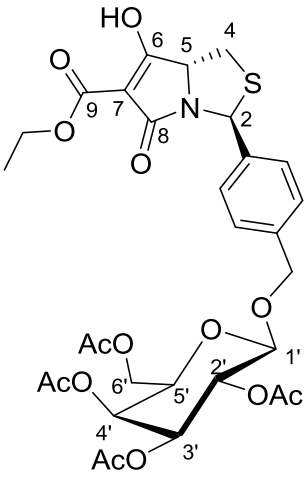


Yield (1.93 g, 80 %); white foam; 1.3:1 *cis* and *trans* diastereomers; R_f = 0.42 (EtOAc/petrol 2:1); ν_{max}/cm^{-1} (neat) 1216 (C-O), 1662 (C=O), 1741 (C=O); δ_H (500 MHz, $CDCl_3$) major isomer (*cis*, a mixture of two conformers): 1.21 - 1.31 (3H, m, OCH_2CH_3), 1.99 (3H, s, CH_3-C(=O)-O-), 2.04 (3H, s, CH_3-C(=O)-O-), 2.07 (3H, s, CH_3-C(=O)-O-), 2.17 (3H, s, CH_3-C(=O)-O-), 3.07 - 3.57 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$), 3.83 (3H, s, CO_2CH_3 minor conformer), 3.85 (3H, s, CO_2CH_3 major conformer), 3.87 - 3.94 (1H, m, H5'), 4.09 - 4.27 (4H, m, OCH_2CH_3 + H6'), 4.50 - 4.66 (2H, m, H1' + OCH_2), 4.86 - 4.93 (1H, m, OCH_2), 4.97 - 5.05 (1H, m, H3'), 5.07 (1H, app t, *J* = 7.1 Hz, H5), 5.24 - 5.32 (1H, m, H2'), 5.37 - 5.42 (1H, m, H6'), 6.16 (1H, s, H2 major conformer), 6.35 (1H, s, H2 minor conformer), 7.22 - 7.27 (2H, m, Ar-C*H* minor conformer), 7.31 - 7.33 (2H, m, Ar-C*H* major conformer), 7.51 (2H, d, *J* = 7.9 Hz, Ar-C*H* minor conformer), 7.66 (2H, d, *J* = 8.1 Hz, Ar-C*H* major conformer); minor isomer (*trans*, a mixture of two conformers): 1.21 - 1.31 (3H, m, OCH_2CH_3), 1.99 (3H, s, CH_3-C(=O)-O-), 2.05 (3H, s, CH_3-C(=O)-O-), 2.08 (3H, s, CH_3-C(=O)-O-), 2.16 (3H, s, CH_3-C(=O)-O-), 3.07 - 3.57 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$), 3.80 (3H, s, CO_2CH_3 major conformer), 3.86 (3H, s, CO_2CH_3 minor conformer), 3.87 - 3.94 (1H, m, H5'), 4.09 - 4.27 (4H, m, OCH_2CH_3 + H6'), 4.50 - 4.66 (2H, m, H1' + OCH_2), 4.86 - 4.93 (1H, m, OCH_2), 4.97 - 5.05 (1H, m, H3'), 5.20 (1H, app d, *J* = 5.7 Hz, H5 minor conformer), 5.24 - 5.32 (2H, m, H5 major conformer + H2'), 5.37 - 5.42 (1H, m, H6'), 6.19 (1H, s, H2 major conformer), 6.32 (1H, s, H2 minor conformer), 7.21 (2H, d, *J* = 8.2 Hz, Ar-C*H* major conformer), 7.22 - 7.27 (4H, m, Ar-C*H* minor conformer), 7.31 - 7.33 (2H, m, Ar-

C*H* major conformer); δ_C (125.8 MHz, $CDCl_3$): major isomer (*cis*, a mixture of two conformers): 14.0, 14.1 ($OCH_2\mathit{C}H_3$), 20.6, 20.7, 20.7, 20.8 ($\mathit{C}H_3$-C(=O)-O-), 32.1, 33.1 (C4), 42.2, 43.0 (C2″), 52.7, 53.4 ($CO_2\mathit{C}H_3$), 61.3 (C6'), 61.7, 62.0 ($O\mathit{C}H_2CH_3$), 63.8, 64.7 (C5), 66.1, 66.9 (C2), 67.1 (C4'), 68.8 (C2'), 70.2, 70.3 ($O\mathit{C}H_2$), 70.7, 70.8 (C3'), 70.9 (C5'), 99.8, 100.0 (C1'), 126.6, 127.3, 127.6, 128.2 (Ar-*C*H), 136.5, 137.4, 138.5, 139.6 (Ar-C), 165.0, 165.5 (C1"), 166.7, 167.1 (C3"), 169.3, 169.4, 169.4, 170.1, 170.1, 170.2, 170.2, 170.3, 170.4 ($\mathit{C}O_2CH_3$ + CH_3-*C*(=O)-O-); minor isomer (*trans*, a mixture of two conformers): 14.0, 14.1 ($OCH_2\mathit{C}H_3$), 20.6, 20.7, 20.7, 20.8 ($\mathit{C}H_3$-C(=O)-O-), 31.0, 33.9 (C4), 42.5, 43.3 (C2″), 52.8, 53.5 ($CO_2\mathit{C}H_3$), 61.2, 61.3 (C6'), 61.6, 61.8 ($O\mathit{C}H_2CH_3$), 64.0, 64.3 (C5), 64.8, 65.5 (C2), 67.1 (C4'), 68.8 (C2'), 70.0, 70.3 ($O\mathit{C}H_2$), 70.7, 70.9 (C3'), 70.8, 70.9 (C5'), 99.8, 100.2 (C1'), 124.9, 125.1, 127.9, 128.3 (Ar-*C*H), 136.1, 137.4, 141.5, 141.9 (Ar-C), 164.6, 165.3 (C1"), 166.3, 167.3 (C3"), 169.3, 169.4, 169.4, 170.1, 170.1, 170.2, 170.2, 170.3, 170.4 ($\mathit{C}O_2CH_3$ + CH_3-*C*(=O)-O-); *m/z* (ESI^+) 698 ($[M+H]^+$ 100 %); HRMS (ESI^+); $C_{31}H_{40}O_{15}NS$ $[M+H]^+$; found 698.21035, requires 698.21132.

(-)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-6-hydroxy-8-oxo-2-(4-((2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)methyl)phenyl)-3-thiabicyclo[3.3.0]oct-6-ene, 207

Tetramate **207** was obtained from **206** following the procedure for Dieckmann cyclisation outlined in general procedure 6.2.5.2.



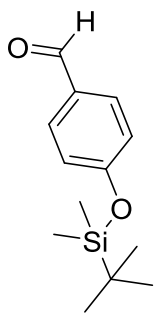
Yield (0.44 g, 30 %); yellow foam, mp 110-114 °C; R_f = 0.20 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -143.0 (*c* = 0.17, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1217 (C-O), 1618 (C=O), 1660 (C=O), 1744 (C=O, br with shoulder towards smaller wave number); δ_H (400 MHz, $CDCl_3$): 1.37 (3H, t, *J* = 7.1 Hz, $OCH_2C\mathit{H}_3$), 1.95 (3H, s, $C\mathit{H}_3$-C(=O)-O-), 2.01 (3H, s, $C\mathit{H}_3$-C(=O)-O-), 2.04 (3H, s, $C\mathit{H}_3$-C(=O)-O-), 2.14

(3H, s, C*H*$_3$-C(=O)-O-), 3.02 (1H, dd, *J* = 11.1, 8.3 Hz, H4$_A$), 3.30 (1H, dd, *J* = 11.1, 7.1 Hz, H4$_B$), 3.91 (1H, td, *J* = 6.5, 1.1 Hz, H5'), 4.13, 4.18 (2H, ABq, J_{AB} = 11.4, 6.8 Hz, H6'), 4.38 (2H, q, *J* = 7.1 Hz, OC*H*$_2$CH$_3$), 4.54 (1H, d, *J* = 8.0 Hz, H1'), 4.62 (1H, d, *J*= 12.3 Hz, OC*H*$_2$), 4.80 (1H, app t, *J* = 7.7 Hz, H5), 4.87 (1H, d, *J* = 12.3 Hz, OC*H*$_2$), 4.98 (1H, dd, *J* = 10.5, 3.5 Hz, H3'), 5.18 (1H, dd, *J* = 10.5, 8.0 Hz, H2'), 5.37 (1H, dd, *J* = 3.5, 1.1 Hz, H4'), 6.27 (1H, s, H2), 7.29 (2H, d, *J* = 8.1 Hz, Ar-C*H*), 7.44 (2H, d, *J* = 8.1 Hz, Ar-C*H*); δ_C (125.8 MHz, CD_2Cl_2): 14.5 (OCH$_2$*C*H$_3$), 20.9, 21.0, 21.1, 21.1 (*C*H$_3$-C(=O)-O-), 33.4 (C4), 62.0 (C6'), 62.3 (O*C*H$_2$CH$_3$), 62.6 (C2), 65.9 (C5), 67.7 (C4'), 69.3 (C2'), 70.8 (OC*H*$_2$), 71.4 (C3'), 71.5 (C5'), 99.7 (C7), 100.5 (C1'), 127.0, 128.5 (Ar-*C*H), 137.5, 140.9 (Ar-C), 167.6 (C9), 169.0 (C8), 169.9, 170.5, 170.7, 170.8 (CH$_3$-*C*(=O)-O-), 186.6 (C6); *m/z* (ESI$^-$) 664 ([M-H]$^-$, 100 %); HRMS (ESI$^-$); $C_{30}H_{34}O_{14}NS$ [M-H]$^-$; found 664.17073, requires 664.17055.

6.4.3 Procedures for the synthesis of aglycone analogues

4-((*tert*-butyldimethylsilyl)oxy)benzaldehyde, 208

To 4-hydroxybenzaldehyde (2.0 g, 16.4 mmol, 1.0 eq) and Et_3N (3.42 mL, 24.6 mmol, 1.5 eq) in CH_2Cl_2 (40 mL) at 0 °C, a solution of TBDMSCl in CH_2Cl_2 (3.7 g, 24.6 mmol, 1.5 eq) was added portionwise. The reaction mixture was stirred at rt for 2 h and quenched with water. The organic layer was separated and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (eluent: EtOAc/petrol).

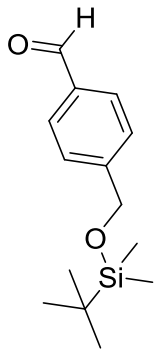


Yield (3.86 g, quant.); yellow oil, R_f = 0.74 (EtOAc/petrol 1:9); ν_{max}/cm^{-1} (neat) 1102 (Si-O), 1257 (Si-C), 1698 (C=O); δ_H (400 MHz, $CDCl_3$): 0.24 (6H, s, Si(C*H*$_3$)$_2$), 0.99 (9H, s, SiC(C*H*$_3$)$_3$), 6.94 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.78 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 9.87 (1H, s, C*H*O); δ_C (100.6 MHz, $CDCl_3$): -4.4

($Si(CH_3)_2$), 18.2 ($SiC(CH_3)_3$), 25.5 ($SiC(CH_3)_3$), 120.4, 131.8 (Ar-*C*H), 130.3, 161.4 (Ar-C), 190.8 (*C*HO); *m/z* (ESI^+) 237 ($[M+H]^+$, 100 %); HRMS (ESI^+); $C_{13}H_{21}O_2Si$ $[M+H]^+$; found 237.13038, requires 237.13053.

4-(((*tert*-Butyldimethylsilyl)oxy)methyl)benzaldehyde, 209

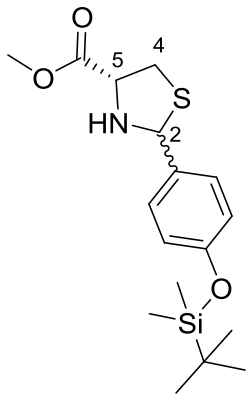
To **203** (0.81 g, 5.96 mmol, 1.0 eq) in CH_2Cl_2 (20 mL), imidazole (0.61 g, 8.94 mmol, 1.5 eq) was added and stirred at rt for 30 min. TBDMSCl (1.35 g, 8.94 mmol, 1.5 eq) was then added portionwise and the reaction mixture was stirred at rt for 6 h. The reaction was quenched with water and the product extracted with CH_2Cl_2. The organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (eluent: EtOAc/petrol).



Yield (1.47 g, 99 %); colourless oil; R_f = 0.56 (EtOAc/petrol 1:9); ν_{max}/cm^{-1} (neat) 1107 (Si-O), 1255 (Si-C), 1703 (C=O); δ_H (400 MHz, $CDCl_3$): 0.13 (6H, s, $Si(CH_3)_2$), 0.96 (9H, s, $SiC(CH_3)_3$), 4.83 (2H, s, OCH_2), 7.50 (2H, d, *J* = 8.1 Hz, Ar-C*H*), 7.86 (2H, d, *J* = 8.1 Hz, Ar-C*H*), 10.01 (1H, s, C*H*O); δ_C (100.6 MHz, $CDCl_3$): -5.4 ($Si(CH_3)_2$), 18.4 ($SiC(CH_3)_3$), 25.9 ($SiC(CH_3)_3$), 64.4 (OCH_2), 126.2, 129.8 (Ar-*C*H), 135.3, 148.7 (Ar-C), 192.1 (*C*HO); *m/z* (ESI) molecular ion not detected.

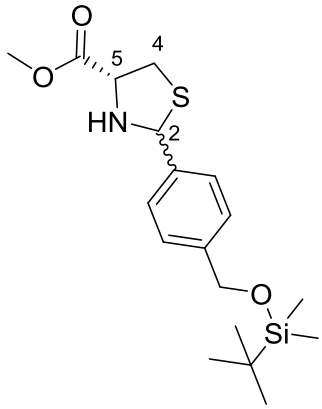
Methyl (2*RS*,5*R*)-2-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-1,3-thiazolidine-5-carboxylate, 210

Thiazolidine **210** was derived from the condensation of L-cysteine methyl ester hydrochloride and aldehyde **208** following the procedure for thiazolidine synthesis outlined in general procedure 6.2.3.



Yield (4.81 g, 85 %); yellow oil; 2:1 *cis* and *trans* diastereomers; R_f = 0.20 (EtOAc/petrol 1:9); ν_{max}/cm^{-1} (neat) 1254 (Si-C), 1742 (C=O), 3309 (N-H); δ_H (400 MHz, $CDCl_3$) major isomer (*cis*): 0.19 (6H, s, Si(CH_3)$_2$), 0.97 (9H, s, SiC(CH_3)$_3$), 2.60 (1H, app t, *J* = 12.4, NH), 3.08 (1H, dd, *J* = 10.3, 9.1 Hz, H4$_A$), 3.42 (1H, dd, *J* = 10.3, 7.1 Hz, H4$_B$), 3.77 (3H, s, CO_2CH_3), 3.89 - 3.99 (1H, m, H5), 5.49 (1H, d, *J* = 12.2 Hz, H2), 6.81 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.38 (2H, d, *J* = 8.6 Hz, Ar-C*H*); minor isomer (*trans*): 0.17 (6H, s, Si(CH_3)$_2$), 0.97 (9H, s, SiC(CH_3)$_3$), 2.76 (1H, br. s., NH), 3.20 (1H, dd, *J* = 10.5, 5.5 Hz, H4$_A$), 3.36 (1H, dd, *J* = 10.5, 7.1 Hz, H4$_B$), 3.75 (3H, s, CO_2CH_3), 4.21(1H, app t, *J* = 6.1 Hz, H5), 5.72 (1H, s, H2), 6.78 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.34 (2H, d, *J* = 8.6 Hz, Ar-C*H*); δ_C (100.6 MHz, $CDCl_3$): major isomer (*cis*): -4.6 (Si(*C*H_3)$_2$), 18.0 (Si*C*(CH$_3$)$_3$), 25.5 (SiC(*C*H_3)$_3$), 39.0 (C4), 52.4 (CO_2*C*H_3), 65.3 (C5), 72.2 (C2), 120.0, 128.5 (Ar-*C*H), 130.6, 155.8 (Ar-C), 171.5 (*C*O_2CH_3); minor isomer (*trans*): -4.6 (Si(*C*H_3)$_2$), 18.0 (Si*C*(CH$_3$)$_3$), 25.5 (SiC(*C*H_3)$_3$), 37.9 (C4), 52.3 (CO_2*C*H_3), 64.1 (C5), 70.5 (C2), 119.7, 128.1 (Ar-*C*H), 133.3, 155.2 (Ar-C), 172.1 (*C*O_2CH_3); *m/z* (ESI$^+$) 354 ([M+H]$^+$ 100 %); HRMS (ESI$^+$); $C_{17}H_{28}O_3NSSi$ [M+H]$^+$; found 354.15499, requires 354.15537.

Methyl (2*RS*,5*R*)-2-(4-(((*tert*-butyldimethylsilyl)oxy)methyl)phenyl)-1,3-thiazolidine-5-carboxylate, 211



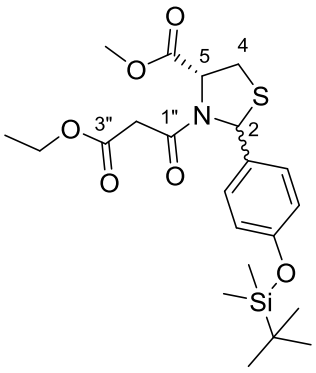
Thiazolidine **211** was derived from the condensation of L-cysteine methyl ester hydrochloride and aldehyde **209** following the procedure for thiazolidine synthesis outlined in general procedure 6.2.3.

Yield (1.89 g, 88 %); colourless oil; 1.6:1 *cis* and *trans* diastereomers; R_f = 0.54 (EtOAc/petrol 1:4); ν_{max}/cm^{-1} (neat) 1253 (Si-C), 1742 (C=O), 3311 (N-H); δ_H (400 MHz, $CDCl_3$) major isomer (*cis*): 0.11 (6H, s,

Si(CH_3)$_2$), 0.95 (9H, s, SiC(CH_3)$_3$), 2.36 (1H, br. s, NH), 3.12 (1H, dd, *J* = 10.3, 9.1 Hz, H4$_A$), 3.47 (1H, dd, *J* = 10.3, 7.2 Hz, H4$_B$), 3.82 (3H, s, CO_2CH_3), 4.00 (1H, dd, *J* = 9.1, 7.2 Hz, H5), 4.75 (2H, s, OCH_2), 5.57 (1H, d, *J* = 12.2 Hz, H2), 7.34 (2H, d, *J* = 8.3 Hz, Ar-C*H*), 7.49 (2H, d, *J* = 8.3 Hz, Ar-C*H*); minor isomer (*trans*): 0.10 (6H, s, Si(CH_3)$_2$), 0.94 (9H, s, SiC(CH_3)$_3$), 2.36 (1H, br. s., NH), 3.22 (1H, dd, *J* = 10.7, 5.8 Hz, H4$_A$), 3.40 (1H, dd, *J* = 10.7, 7.1 Hz, H4$_B$), 3.80 (3H, s, CO_2CH_3), 4.24 (1H, dd, *J* = 7.1, 5.8 Hz, H5), 4.73 (2H, s, OCH_2), 5.82 (1H, s, H2), 7.29 (2H, d, *J* = 8.3 Hz, Ar-C*H*), 7.45 (2H, d, *J* = 8.3 Hz, Ar-C*H*); δ_C (125.8 MHz, $CDCl_3$): major isomer (*cis*): -5.3 (Si(*C*H_3)$_2$), 18.4 (Si*C*(CH$_3$)$_3$), 25.9 (SiC(*C*H$_3$)$_3$), 39.3 (C4), 52.6 (CO_2CH_3), 64.6 (O*C*H$_2$), 65.6 (C5), 72.5 (C2), 126.3, 127.3 (Ar-*C*H), 136.7, 142.1 (Ar-C), 171.6 (*C*O$_2$CH$_3$); minor isomer (*trans*): -5.3 (Si(*C*H$_3$)$_2$), 15.3 (Si*C*(CH$_3$)$_3$), 25.9 (SiC(*C*H$_3$)$_3$), 38.1 (C4), 52.5 (CO_2CH_3), 64.3 (C5), 64.6 (O*C*H$_2$), 70.7 (C2), 126.1, 126.8 (Ar-*C*H), 139.6, 141.3 (Ar-C), 172.2 (*C*O$_2$CH$_3$); *m/z* (ESI$^+$) 368 ([M+H]$^+$ 43 %); HRMS (ESI$^+$); $C_{18}H_{30}O_3NSSi$ [M+H]$^+$; found 368.17144, requires 368.17102.

Methyl (2*RS*,5*R*)- 2-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-1-(3-ethoxy-3-oxopropanoyl)-1,3-thiazolidine-5-carboxylate, 212

N-Acylthiazolidine **212** was derived from **210** following the procedure for *N*-acylation outlined in general procedure 6.2.4.

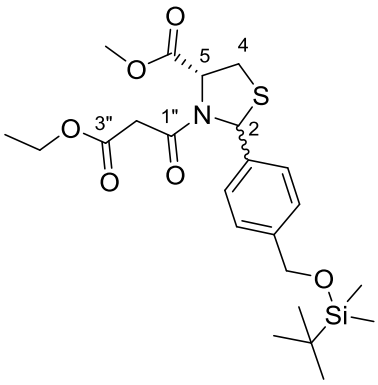


Yield (5.2 g, 83 %); yellow oil; 2:1 *cis* and *trans* diastereomers; R_f = 0.15 (EtOAc/petrol 1:4); ν_{max}/cm^{-1} (neat) 1252 (Si-C), 1662 (C=O), 1742 (C=O); δ_H (400 MHz, $CDCl_3$) major isomer (*cis*, a mixture of two conformers): 0.16 and 0.19 (6H, s, Si(CH_3)$_2$), 0.95 and 0.97 (9H, s, SiC(CH_3)$_3$), 1.19 - 1.28 (3H, m, OCH_2CH_3), 3.05 - 3.47 (4H, m, H4$_A$ + H4$_B$ + H2″$_A$ + H2″$_B$), 3.82 (3H, s, CO_2CH_3), 4.06 - 4.17 (2H, m, OCH_2CH$_3$), 4.99 - 5.04 (1H, m, H5 minor conformer), 5.07 (1H, app t, *J* = 6.5 Hz, H5 major conformer), 6.08 (1H, s, H2 major conformer), 6.33 (1H, s, H2 minor

conformer), 6.78 - 6.87 (2H, m, Ar-C*H*, major conformer), 6.93 (2H, d, *J* = 8.6 Hz, Ar-C*H*, minor conformer), 7.37 (2H, d, *J* = 8.6 Hz, Ar-C*H*, minor conformer), 7.51 (2H, d, *J* = 8.6 Hz, Ar-C*H*, major conformer); minor isomer (*trans*, a mixture of two conformers: 0.16 and 0.19 (6H, s, Si(CH_3)$_2$), 0.95 and 0.97 (9H, s, SiC(CH_3)$_3$), 1.19 - 1.28 (3H, m, OCH$_2$CH_3), 3.05 - 3.47 (4H, m, H4$_A$ + H4$_B$ + H2″$_A$ + H2″$_B$), 3.77 (3H, s, CO$_2$CH_3), 4.06 - 4.17 (2H, m, OCH_2CH$_3$), 5.16 (1H, app d, *J* = 5.9 Hz, H5 minor conformer), 5.25 - 5.29 (1H, m, H5 major conformer), 6.12 (1H, s, H2 major conformer), 6.30 (1H, s, H2 minor conformer), 6.74 (2H, d, *J* = 8.6 Hz, Ar-C*H*, minor conformer), 6.78 - 6.87 (2H, m, Ar-C*H*, major conformer), 7.07 (2H, d, *J* = 8.6 Hz, Ar-C*H*, major conformer), 7.13 (2H, d, *J* = 8.6 Hz, Ar-C*H*, minor conformer); δ_C (100.6 MHz, CDCl$_3$): major isomer (*cis*, a mixture of two conformers): -4.5 (Si(*C*H$_3$)$_2$), 13.9, 14.1 (OCH$_2$*C*H$_3$), 18.1 (Si*C*(CH$_3$)$_3$), 25.5 (SiC(*C*H$_3$)$_3$), 32.0, 33.1 (C4), 42.0, 43.4 (C2″), 52.6, 53.3 (CO$_2$*C*H$_3$), 61.5, 61.7 (O*C*H$_2$CH$_3$), 63.6, 64.5 (C5), 66.1, 66.5 (C2), 119.5, 120.4, 127.9, 128.5 (Ar-*C*H), 132.0, 155.9 (Ar-*C*), 165.5 (C1"), 166.8 (C3"), 170.2 (*C*O$_2$CH$_3$); minor isomer (*trans*, a mixture of two conformers): -4.5 (Si(*C*H$_3$)$_2$), 13.9, 14.1 (OCH$_2$*C*H$_3$), 18.1 (Si*C*(CH$_3$)$_3$), 25.5 (SiC(*C*H$_3$)$_3$), 31.0, 33.8 (C4), 42.3, 42.9 (C2″), 52.7, 53.4 (CO$_2$*C*H$_3$), 60.3, 61.4 (O*C*H$_2$CH$_3$), 63.9, 64.1 (C5), 64.8, 65.4 (C2), 119.7, 120.5, 126.0, 126.2 (Ar-*C*H), 134.2, 155.8 (Ar-*C*), 165.4 (C1"), 166.3 (C3"), 169.4 (*C*O$_2$CH$_3$); *m/z* (ESI$^+$) 468 ([M+H]$^+$ 100 %); HRMS (ESI$^+$); $C_{22}H_{34}O_6NSSi$ [M+H]$^+$; found 468.18630, requires 468.18706.

Methyl (2*RS*,5*R*)- 2-(4-(((*tert*-butyldimethylsilyl)oxy)methyl)phenyl)-1-(3-ethoxy-3-oxopropanoyl)-1,3-thiazolidine-5-carboxylate, 213

N-Acylthiazolidine **213** was derived from **211** following the procedure for *N*-acylation outlined in general procedure 6.2.4.

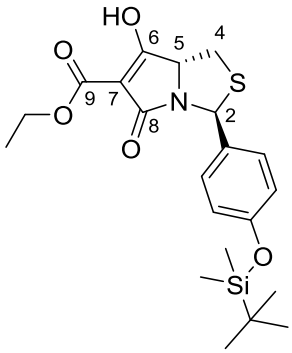


Yield (2.25 g, 91 %); colourless oil; inseparable 1.7:1 *cis* and *trans* diastereomers; R_f = 0.20 (EtOAc/petrol 1:3); ν_{max}/cm^{-1} (neat) 1250 (Si-C), 1662 (C=O), 1742 (C=O); δ_H (500 MHz, $CDCl_3$) major isomer (*cis*, a mixture of two conformers): 0.09 and 0.11 (6H, s, Si(CH_3)$_2$), 0.94 and 0.95 (9H, s, SiC(CH_3)$_3$), 1.20 - 1.30 (3H, m, OCH$_2$CH_3), 3.07 - 3.58 (4H, m, H4$_A$ + H4$_B$ + H2″$_A$ + H2″$_B$), 3.81 and 3.85 (3H, s, CO$_2$CH_3), 4.06 - 4.25 (2H, m, OCH_2CH$_3$), 4.70 and 4.75 (OCH_2), 5.08 (1H, app t, *J* = 6.8 Hz, H5), 6.14 and 6.37 (1H, s, H2), 7.21 - 7.27 (2H, m, Ar-C*H* minor conformer), 7.32 - 7.38 (2H, m, Ar-C*H* major conformer), 7.48 (2H, d, *J* = 8.2 Hz, Ar-C*H* minor conformer), 7.62 (2H, d, *J* = 8.2 Hz, Ar-C*H* major conformer); minor isomer (*trans*, a mixture of two conformers: 0.09 and 0.11 (6H, s, Si(CH_3)$_2$), 0.94 and 0.95 (9H, s, SiC(CH_3)$_3$), 1.20 - 1.30 (3H, m, OCH$_2$CH_3), 3.07 - 3.58 (4H, m, H4$_A$ + H4$_B$ + H2″$_A$ + H2″$_B$), 3.80 and 3.86 (3H, s, CO$_2$CH_3), 4.06 - 4.25 (2H, m, OCH_2CH$_3$), 4.70 and 4.74 (OCH_2), 5.20 (1H, app d, *J* = 5.9 Hz, H5 minor conformer), 5.29 - 5.33 (1H, m, H5 major conformer), 6.18 and 6.33 (1H, s, H2), 7.19 (2H, d, *J* = 8.2 Hz, Ar-C*H* major conformer), 7.21 - 7.27 (4H, m, Ar-C*H* minor conformer), 7.32 - 7.38 (2H, m, Ar-C*H* major conformer); δ_C (125.8 MHz, $CDCl_3$): major isomer (*cis*, a mixture of two conformers): -5.3 (Si(*C*H$_3$)$_2$), 14.0 (OCH$_2$*C*H$_3$), 18.4 (Si*C*(CH$_3$)$_3$), 25.9 (SiC(*C*H$_3$)$_3$), 32.1, 33.8 (C4), 42.1, 43.4 (C2″), 52.7, 53.1 (CO$_2$*C*H$_3$), 61.6, 61.8 (O*C*H$_2$CH$_3$), 63.8, 64.7 (C5), 64.5, 64.6 (OCH_2), 66.2, 67.0 (C2), 125.8, 126.4, 126.6, 127.0 (Ar-*C*H), 137.2, 138.3, 141.2, 142.1 (Ar-*C*), 164.4, 165.6 (C1"), 166.8, 167.2 (C3"), 170.2, 170.3 (*C*O$_2$CH$_3$); minor isomer (*trans*, a mixture of two conformers): -5.3 (Si(*C*H$_3$)$_2$), 14.0, 14.2 (OCH$_2$*C*H$_3$), 18.4 (Si*C*(CH$_3$)$_3$), 25.9 (SiC(*C*H$_3$)$_3$), 31.0, 33.1 (C4), 42.4, 43.0 (C2″), 52.8, 53.4 (CO$_2$*C*H$_3$), 61.5, 61.8 (O*C*H$_2$CH$_3$), 64.0, 64.3 (C5), 64.4, 64.6 (OCH_2), 65.0, 65.6 (C2), 124.6, 124.9, 126.2, 126.8 (Ar-*C*H), 140.4, 140.6, 140.8, 142.0 (Ar-*C*), 164.9, 165.4 (C1"), 166.3, 167.2 (C3"), 169.5, 170.2 (*C*O$_2$CH$_3$); *m/z*

(ESI$^+$) 482 ([M+H]$^+$ 100 %); HRMS (ESI$^+$); $C_{23}H_{36}O_6NSSi$ [M+H]$^+$; found 482.20171, requires 482.20271.

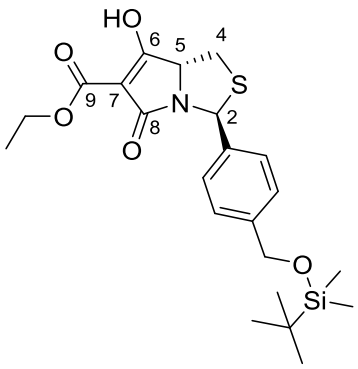
(-)-(2*S*,5*R*)-1-Aza-2-(4-(*tert*-butyldimethylsilyl)oxyphenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 214

Tetramate **214** was obtained from **212** following the procedure for Dieckmann cyclisation outlined in general procedure 6.2.5.2.



Yield (0.14 g, 30 %); yellow solid, mp 118-122 °C; R_f = 0.42 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -105.3 (*c* = 1.0, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1261 (Si-C), 1610 (C=C), 1651 (C=O), 1685 (C=O), 3311 (O-H); δ_H (400 MHz, CD_2Cl_2): 0.20 (6H, s, Si(C*H*$_3$)$_2$), 0.98 (9H, s, SiC(C*H*$_3$)$_3$), 1.36 (3H, t, *J* = 7.1 Hz, OCH$_2$C*H*$_3$), 2.99 (1H, dd, *J* = 11.0, 8.3 Hz, H4$_A$), 3.30 (1H, dd, *J* = 11.1, 7.0 Hz, H4$_B$), 4.36 (2H, q, *J* = 7.1 Hz, OC*H*$_2$CH$_3$), 4.77 (1H, app t, *J* = 7.7 Hz, H5), 6.22 (1H, s, H2), 6.82 (2H, d, *J* = 8.3 Hz, Ar-C*H*), 7.32 (2H, d, *J* = 8.3 Hz, Ar-C*H*); δ_C (100.6 MHz, CD_2Cl_2): -4.2 (Si(*C*H$_3$)$_2$), 14.6 (OCH$_2$*C*H$_3$), 18.6 (Si*C*(CH$_3$)$_3$), 26.0 (SiC(*C*H$_3$)$_3$), 33.4 (C4), 62.1 (O*C*H$_2$CH$_3$), 62.5 (C2), 66.2 (C5), 120.6, 128.1 (Ar-*C*H), 133.8, 156.1 (Ar-C), 167.5 (C9), 186.8 (C6); *m/z* (ESI$^-$) 434 ([M-H]$^-$, 100 %); HRMS (ESI$^-$); $C_{21}H_{28}O_5NSSi$ [M-H]$^-$; found 434.14657, requires 434.14629.

(-)-(2*S*,5*R*)-1-Aza-2-(4-(((*tert*-butyldimethylsilyl)oxy)methyl)phenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 215



Tetramate **215** was obtained from **213** following the procedure for Dieckmann cyclisation outlined in general procedure 6.2.5.2.

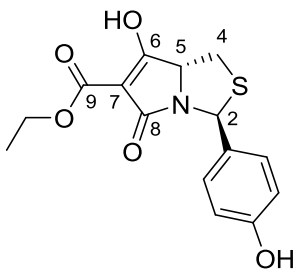
Yield (0.31 g, 15 %); yellow foam, mp 94-96 °C; R_f = 0.47 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -172.2 (*c* = 0.17, MeOH); ν_{max}/cm^{-1} (neat) 1610 (C=C), 1689 (C=O, br with shoulder towards

smaller wavenumber); δ_H (400 MHz, Methanol-d_4): 0.07 (6H, s, Si(CH_3)$_2$), 0.91 (9H, s, SiC(CH_3)$_3$), 1.31 (3H, t, J = 7.0 Hz, OCH$_2$CH_3), 2.98 (1H, dd, J = 10.5, 8.8 Hz, H4$_A$), 3.23 - 3.33 (1H, m, H4$_B$, obscured by solvent peak), 4.29 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 4.59 (OCH_2), 4.87 (1H, app t, J = 7.3 Hz, H5), 6.22 (1H, s, H2), 7.35 (2H, d, J = 8.0 Hz, Ar-C*H*), 7.45 (2H, d, J = 8.0 Hz, Ar-C*H*); δ_C (100.6 MHz, Methanol-d_4): -5.6 (Si(*C*H$_3$)$_2$), 14.8 (OCH$_2$*C*H$_3$), 19.3 (Si*C*(CH$_3$)$_3$), 26.5 (SiC(*C*H$_3$)$_3$), 33.7 (C4), 61.7 (O*C*H$_2$CH$_3$), 63.4 (C2), 64.9 (O*C*H$_2$), 67.6 (C5), 99.5 (C7), 127.5, 128.2 (Ar-*C*H), 141.1, 142.7 (Ar-C), 164.9 (C9), 172.8 (C8), 184.9 (C6); *m/z* (ESI$^-$) 448 ([M-H]$^-$, 100 %); HRMS (ESI$^-$); $C_{22}H_{30}O_5NSSi$ [M-H]$^-$; found 448.16259, requires 448.16194.

6.4.3.1 General procedure for silyl ether deprotection of 214, 215 and 217a-c to yield 194, 216 and 218a-c

The deprotection of silyl ethers was according to a modified literature procedure.[105] To tetramate (1.0 eq) dissolved in THF (2 mL/mmol), tetraethylene glycol (5 eq) and KF (2 eq) were added. The reaction was stirred at rt for 0.5 - 2 h (for the deprotection of **215**, the reaction mixture was heated at 80 °C for 5 h). Solvent was evapourated *in vacuo* and the residue was purified by silica gel flash column chromatography (eluent: EtOAc/MeOH/1% Et$_3$N). The product isolated was dissolved in CH_2Cl_2 (a few drops of MeOH was needed to aid solubility) and washed with 0.1 M HCl. The organic fraction was dried over Na_2SO_4, filtered and concentrated *in vacuo* to obtain the required product.

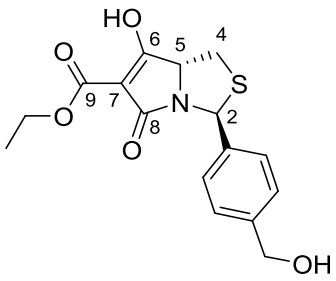
(-)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-6-hydroxy-2-(4-(hydroxy)phenyl)-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 194



Yield (88 mg, 30 %); yellow solid, mp 172 °C; R_f = 0.21 (EtOAc/ MeOH 9:1); $[\alpha]_D^{25}$ = -188.9 (c = 0.13, MeOH); ν_{max}/cm^{-1} (neat) 1611 (C=C), 1642 (C=O), 1686 (C=O), 3369 (O-H); δ_H (200 MHz,

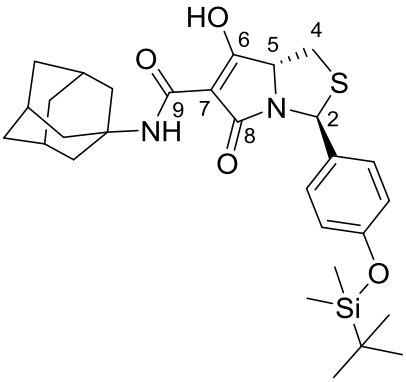
Acetone-d_6): 1.29 (3H, t, J = 7.1 Hz, OCH_2CH_3), 3.08 (1H, dd, J = 11.0, 8.0 Hz, $H4_A$), 3.38 (1H, dd, J = 11.0, 7.0 Hz, $H4_A$), 4.30 (2H, q, J = 7.1 Hz, OCH_2CH_3), 4.96 (1H, app t, J = 7.6 Hz, H5), 6.18 (1H, s, H2), 6.80 (2H, d, J = 8.5 Hz, Ar-C*H*), 7.33 (2H, d, J = 8.5 Hz, Ar-C*H*); δ_C (125.8 MHz, Acetone-d_6): 14.6 (OCH_2CH_3), 33.4 (C4), 61.4 (OCH_2CH_3), 63.1 (C2), 66.6 (C5), 99.3 (C7), 116.0, 128.8 (Ar-*C*H), 133.0, 158.1 (Ar-C), 166.3 (C9), 169.9 (C8), 186.0 (C6); *m/z* (ESI⁻) 320 ([M-H]⁻, 73 %); HRMS (ESI⁻); $C_{15}H_{14}O_5NS$ [M-H]⁻; found 320.06016, requires 320.05982.

(-)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-6-hydroxy-2-(4-((hydroxy)methyl)phenyl)-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 216



Yield (24 mg, 20 %); white foam, mp 78-80 °C; R_f = 0.18 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -273.7 (c = 0.11, MeOH); ν_{max}/cm^{-1} (neat) 1609 (C=C), 1690 (C=O, br with shoulder towards smaller wavenumber), 3350 (O-H); δ_H (200 MHz, Acetone-d_6): 1.29 (3H, t, J = 7.1 Hz, OCH_2CH_3), 3.12 (1H, dd, J = 11.1, 8.0 Hz, $H4_A$), 3.39 (1H, dd, J = 11.1, 7.0 Hz, $H4_A$), 4.30 (2H, q, J = 7.1 Hz, OCH_2CH_3), 4.62 (OCH_2), 5.00 (1H, app t, J = 7.6 Hz, H5), 6.25 (1H, s, H2), 7.34 (2H, d, J = 8.3 Hz, Ar-C*H*), 7.45 (2H, d, J = 8.3 Hz, Ar-C*H*); δ_C (125.8 MHz, Acetone-d_6): 14.6 (OCH_2CH_3), 33.4 (C4), 61.5 (OCH_2CH_3), 63.2 (C2), 64.4 (OCH_2), 66.6 (C5), 99.4 (C7), 127.2, 127.5 (Ar-*C*H), 140.8, 143.2 (Ar-C), 166.3 (C9), 169.7 (C8), 186.0 (C6); *m/z* (ESI⁻) 334 ([M-H]⁻, 100 %); HRMS (ESI⁻); $C_{16}H_{16}O_5NS$ [M-H]⁻; found 334.07552, requires 334.07547.

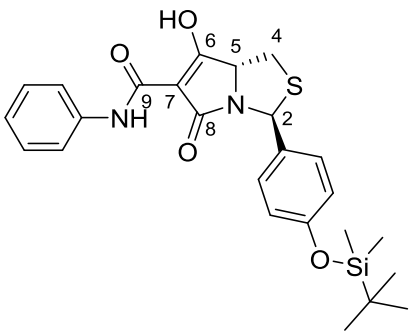
(-)-(2*S*,5*R*)-1-Aza-2-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 217a



Carboxamide tetramate **217a** was obtained by aminolysis of **214** with 1-adamantylamine according to the general procedure 6.3.1 for the synthesis of carboxamides with THF/toluene as solvent.

Yield (0.46 g, 54 %); brown foaming solid, mp 158-160 °C; R_f = 0.78 (EtOAc/petrol 1:1); $[\alpha]_D^{25}$ = -144.1 (*c* = 0.25, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1262 (Si-C), 1627 (C=C), 1648 (C=O), 1688 (C=O), 3309 (O-H); δ_H (400 MHz, CD_2Cl_2): 0.20 (6H, s, Si(C*H*$_3$)$_2$), 0.98 (9H, s, SiC(C*H*$_3$)$_3$), 1.70 (6H, br. signal, Adamantyl-C*H*$_2$), 2.06 (6H, br. signal, Adamantyl-C*H*$_2$), 2.10 (3H, br.signal, Adamantyl-C*H*), 2.97 (1H, dd, *J* = 11.1, 8.6 Hz, H4$_A$), 3.25 (1H, dd, *J* = 11.1, 7.1 Hz, H4$_B$), 4.70 (1H, br. signal, H5), 6.18 (1H, s, H2), 6.81 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.31 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 9.74 (1H, br. s., NH/OH); δ_C (125.8 MHz, CD_2Cl_2): -4.2 (Si(*C*H$_3$)$_2$), 18.6 (Si*C*(CH$_3$)$_3$), 26.0 (SiC(*C*H$_3$)$_3$), 30.1 (Adamantyl-*C*H), 33.1 (C4), 36.6 (Adamantyl-*C*H$_2$), 42.1 (Adamantyl-*C*H$_2$), 53.4 (Adamantyl-C), 62.2 (C2), 67.8 (C5), 96.2 (C7), 120.6, 128.2 (Ar-*C*H), 133.9, 156.1 (Ar-C), 166.6 (C9), 172.7 (C8), 188.0 (C6); *m/z* (ESI$^-$) 539 ([M-H]$^-$, 100 %); HRMS (ESI$^-$); $C_{29}H_{39}O_4N_2SSi$ [M-H]$^-$; found 539.24045, requires 539.23943.

(-)-(2*S*,5*R*)-1-Aza-2-(4-(*tert*-butyldimethylsilyl)oxyphenyl)-6-hydroxy-8-oxo-7-phenylaminocarbonyl-3-thiabicyclo[3.3.0]oct-6-ene, 217b



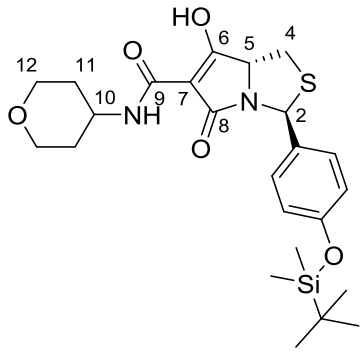
Carboxamide tetramate **217b** was obtained by aminolysis of **214** with aniline according to the general procedure 6.3.1 for the synthesis of carboxamides with THF/toluene as solvent.

Yield (0.35 g, 27 %); yellow foam, mp 80-84 °C; R_f = 0.68

(EtOAc/MeOH 98:2); $[\alpha]_D^{25}$ = -174.6 (*c* = 0.19, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1261 (Si-C), 1651 (C=O, br with shoulder towards smaller wave number), 1693 (C=O); δ_H (400 MHz, CD_2Cl_2): 0.21 (6H, s, Si(CH_3)$_2$), 0.99 (9H, s, SiC(CH_3)$_3$), 3.02 (1H, dd, *J* = 11.1, 8.8 Hz, H4$_A$), 3.31 (1H, dd, *J* = 11.1, 7.0 Hz, H4$_B$), 4.86 (1H, app t, *J* = 7.7 Hz, H5), 6.24 (1H, s, H2), 6.84 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.16 (1H, app t, *J* = 7.4 Hz, Ar-C*H*), 7.32 - 7.40 (4H, m, Ar-C*H*), 7.58 (2H, d, *J* = 7.8 Hz, Ar-C*H*), 9.20 (1H, br. s, NH/OH), 9.38 (1H, br. s, NH/OH); δ_C (100.6 MHz, CD_2Cl_2): -4.4 (Si(*C*H$_3$)$_2$), 18.2 (Si*C*(CH$_3$)$_3$), 25.6 (SiC(*C*H$_3$)$_3$), 32.3 (C4), 61.5 (C2), 66.3 (C5), 120.2, 120.3, 125.0, 127.8, 129.1 (Ar-*C*H), 132.3, 136.4, 155.7 (Ar-C), 163.8 (C9), 171.5 (C8), 184.2 (C6); *m/z* (ESI^-) 481 ([M-H]$^-$, 100 %); HRMS (ESI^-); $C_{25}H_{29}O_4N_2SSi$ [M-H]$^-$; found 481.16199, requires 481.16228.

(-)-(2*S*,5*R*)-1-Aza-2-(4-(*tert*-butyldimethylsilyl)oxyphenyl)-6-hydroxy-8-oxo-7-(tetrahydro-2*H*-pyran-4-aminocarbonyl)-3-thiabicyclo[3.3.0]oct-6-ene, 217c

Carboxamide tetramate **217c** was obtained by aminolysis of **214** with 4-aminotetrahydropyran according to the general procedure 6.3.1 for the synthesis of carboxamides with THF/toluene as solvent.

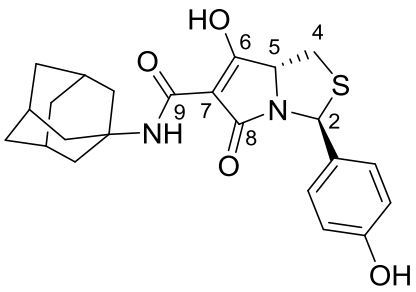


Yield (0.33 g, 30 %); yellow foam, 5.4:1 AB:CD tautomers, mp 76-80 °C; R_f = 0.49 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -161.3 (*c* = 0.25, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1263 (Si-C), 1650 (C=O, br with shoulder towards smaller wavenumber), 1692 (C=O), 3324 (O-H/N-H); δ_H (400 MHz, $CDCl_3$): 0.18 (6H, s, Si(CH_3)$_2$), 0.97 (9H, s, SiC(CH_3)$_3$), 1.49 - 1.70 (2H, m, H11), 1.82 - 1.99 (2H, m, H11), 2.97 (1H, dd, *J* = 11.0, 8.6 Hz, H4$_A$), 3.26 (1H, dd, *J* = 11.0, 7.1 Hz, H4$_B$), 3.44 - 3.52 (2H, m, H12), 3.87 - 4.10 (3H, m, H10 + H12), 4.45 (1H, app t, *J* = 7.7 Hz, H5 CD), 4.75 (1H, app t, *J* = 7.7 Hz, H5 AB), 6.19 (1H, s, H2 AB), 6.29 (1H, s, H2 CD), 6.80 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.33 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 10.87 (1H, br. s, NH/OH); δ_C (100.6 MHz, $CDCl_3$): -4.5 (Si(*C*H$_3$)$_2$), 18.1

(Si*C*$(CH_3)_3$), 25.6 (SiC(*C*$H_3)_3$), 32.3 (C4), 32.6 (C11), 45.1 (C10), 61.5 (C2 AB), 62.3 (C2 CD), 66.3 (C12), 66.6 (C5 AB), 70.6 (C5 CD), 97.0 (C7), 120.0, 127.7 (Ar-*C*H), 132.5, 155.6 (Ar-C), 165.1 (C9 AB), 165.9 (C9 CD), 171.7 (C8 AB), 179.3 (C8 CD), 185.4 (C6 AB), 191.4 (C6 CD); *m/z* (ESI⁻) 489 ([M-H]⁻, 100 %); HRMS (ESI⁻); $C_{24}H_{33}O_5N_2SSi$ [M-H]⁻; found 489.18805, requires 489.18740.

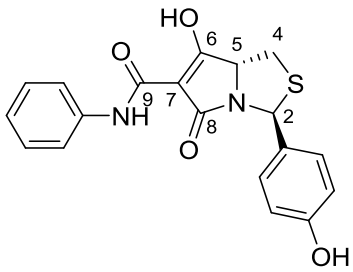
(-)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-6-hydroxy-2-(4-hydroxyphenyl)-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 218a

The synthesis of **218a** from **217a** was according to the procedure for silyl ether deprotection outlined in general procedure 6.4.3.1.



Yield (0.16 g, 40 %); yellow solid, mp 220 °C; R_f = 0.49 (EtOAc/MeOH 96:4); $[\alpha]_D^{25}$ = -249.1 (*c* = 0.15, DMSO); ν_{max}/cm^{-1} (neat) 1612 (C=C), 1637 (C=O), 1676 (C=O), 3333 (O-H); δ_H (500 MHz, Dimethyl sulfoxide-d_6): 1.63 (6H, br. signal, Adamantyl-CH_2), 1.97 (6H, br. signal, Adamantyl-CH_2), 2.04 (3H, br. signal, Adamantyl-C*H*), 2.96 (1H, dd, *J* = 11.0, 8.7 Hz, $H4_A$), 3.28 (1H, dd, *J* = 11.0, 6.9 Hz, $H4_B$), 4.90 (1H, app t, *J* = 7.7 Hz , H5), 6.03 (1H, s, H2), 6.72 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.24 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.66 (1H, br. s., NH/OH), 9.45 (1H, br. s., NH/OH); δ_C (125.8 MHz, Dimethyl sulfoxide-d_6): 28.8 (Adamantyl-*C*H), 33.9 (C4), 35.7 (Adamantyl-*C*H_2), 41.1 (Adamantyl-*C*H_2), 51.3 (Adamantyl-C), 61.2 (C2), 67.0 (C5), 96.4 (C7), 115.0, 127.7 (Ar-*C*H), 131.1, 157.0 (Ar-C), 163.3 (C9), 172.9 (C8), 183.5 (C6); *m/z* (ESI⁻) 425 ([M-H]⁻, 100 %); HRMS (ESI⁻); $C_{23}H_{25}O_4N_2S$ [M-H]⁻; found 425.15378, requires 425.15295.

(-)-(2*S*,5*R*)-1-Aza-6-hydroxy-2-(4-hydroxyphenyl)-8-oxo-7-phenylaminocarbonyl-3-thiabicyclo[3.3.0]oct-6-ene, 218b

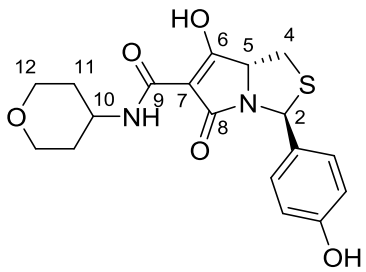


The synthesis of **218b** from **217b** was according to the procedure for silyl ether deprotection outlined in general procedure 6.4.3.1.

Yield (75 mg, 45 %); yellow solid, mp 100-104 °C; R_f = 0.64 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -257.9 (*c* = 0.15, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1648 (C=O, br with shoulder towards smaller wave number), 1676 (C=O), 3303 (N-H/O-H); δ_H (500 MHz, Methanol-d_4): 3.02 (1H, dd, *J* = 11.0, 8.4 Hz, $H4_A$), 3.33 (1H, dd, *J* = 11.0, 7.0 Hz, $H4_B$), 4.97 (1H, dd, *J* = 8.4, 7.0 Hz, H5), 6.17 (1H, s, H2), 6.77 (2H, d, *J* = 8.5 Hz, Ar-C*H*), 7.12 (1H, app t, *J* = 7.4 Hz Ar-C*H*), 7.30 - 7.36 (4H, m, Ar-C*H*), 7.60 (2H, d, *J* = 7.7 Hz, Ar-C*H*); δ_C (125.8 MHz, Methanol-d_4): 33.3 (C4), 63.1 (C2), 68.4 (C5), 99.7 (C7), 116.4, 121.4, 125.8, 129.2, 130.2 (Ar-*C*H), 132.6, 138.7, 158.7 (Ar-C), 164.2 (C9), 174.4 (C8), 185.0 (C6); *m/z* (ESI^-) 367 ($[M-H]^-$, 74 %); HRMS (ESI^-); $C_{19}H_{15}O_4N_2S$ $[M-H]^-$; found 367.07512, requires 367.07580.

(-)-(2*S*,5*R*)-1-Aza-2-(4-hydroxyphenyl)-6-hydroxy-8-oxo-7-(tetrahydro-2*H*-pyran-4-aminocarbonyl)-3-thia-bicyclo[3.3.0]oct-6-ene, 218c

The synthesis of **218c** from **217c** was according to the procedure for silyl ether deprotection outlined in general procedure 6.4.3.1.



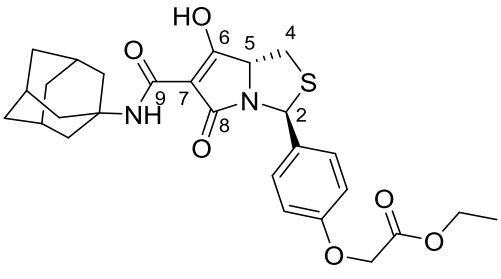
Yield (0.11 g, 45 %); yellow solid, mp 200 °C; R_f = 0.47 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -237.9 (*c* = 0.14, DMSO); ν_{max}/cm^{-1} (neat) 1639 (C=O), 1691 (C=O), 3295 (O-H); δ_H (500 MHz, DMSO-d_6): 1.42 - 1.54 (2H, m, H11), 1.68 - 1.81 (2H, m, H11), 2.95 (1H, dd, *J* = 11.0, 8.4 Hz, $H4_A$), 3.27 (1H, dd, *J* = 11.0, 6.9 Hz, $H4_B$), 3.35 - 3.43 (2H, m, H12), 3.76 - 3.84 (2H, m, H12), 3.86 - 3.98 (1H, m, H10), 4.82 (1H, app t, *J* =

7.6 Hz, H5), 6.06 (1H, s, H2), 6.72 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.24 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 8.03 (1H, br. s, NH/OH), 9.45 (1H, br. s, NH/OH); δ_C (125.8 MHz, DMSO-d_6): 32.3 (C4), 32.4 (C11), 44.4 (C10), 61.6 (C2), 65.7 (C12), 67.2 (C5), 95.4 (C7), 115.0, 127.7 (Ar-*C*H), 131.4, 157.0 (Ar-C), 162.8 (C9), 173.4 (C8), 184.1 (C6); *m/z* (ESI$^-$) 375 ([M-H]$^-$, 100 %); HRMS (ESI$^-$); $C_{18}H_{19}N_2O_5S$ [M-H]$^-$; found 375.10159, requires 375.10202.

6.4.4 General procedure for the synthesis of *O*-aryl ether tetramic acids 219 and 220

To tetramate (1.0 eq) dissolved in THF, K_2CO_3 (2.1 eq) and the relevant alkyl halide (1.5 eq) were added and the reaction stirred at rt for 18 h. Upon completion, solvents were removed *in vacuo* and the residue was treated with H_2O and EtOAc. The aqueous layer was acidified with 10 % HCl and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo* to give the crude, which was purified by silica gel flash column chromatography (eluent: EtOAc/petrol to EtOAc/MeOH/1 % Et_3N). The product isolated was dissolved in CH_2Cl_2 and washed with 5 % citric acid. The organic fraction was dried over Na_2SO_4, filtered and concentrated *in vacuo* to obtain the required ether.

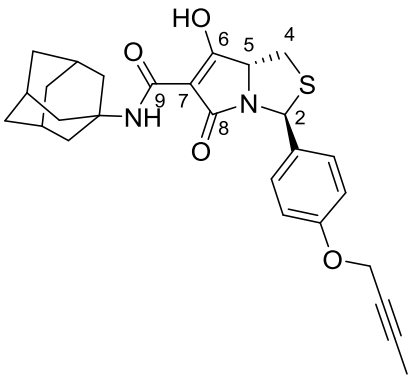
(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(4-(ethoxy-2-oxoethoxy)phenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 219



Yield (0.22 g, 70 %); yellow oil; 3.3:1 AB:CD tautomers; R_f = 0.75 (EtOAc/MeOH 98:2); $[\alpha]_D^{25}$ = -184.6 (*c* = 0.18, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1624 (C=C), 1648 (C=O), 1688 (C=O), 1737 (C=O), 3311 (N-H/O-H); δ_H (500 MHz, $CDCl_3$) 1.30 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 1.70 (6H, br. signal, Adamantyl-CH_2), 2.05 (6H, br. signal, Adamantyl-CH_2), 2.12 (3H, br. signal, Adamantyl-C*H*), 3.00 (1H, dd, *J* = 11.3, 8.9 Hz, $H4_A$), 3.25 (1H, dd, *J* = 11.3, 7.1 Hz, $H4_B$), 4.27 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.37 - 4.46 (1H, m, H5 CD),

4.61 (2H, s, OC*H*$_2$), 4.66 (1H, app t, *J* = 7.7 Hz, H5 AB), 6.20 (1H, s, H2 AB), 6.30 (1H, s, H2 CD), 6.88 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.40 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.98 (2H, br. s, N-H + O-H); δ_C (125.8 MHz, $CDCl_3$): 14.1 ($OCH_2\mathit{C}H_3$), 29.3 (Adamantyl-*C*H), 32.5 (C4), 36.0 (Adamantyl-*C*H_2), 41.5 (Adamantyl-*C*H_2), 53.1 (Adamantyl-C), 61.4 ($O\mathit{C}H_2CH_3$), 61.6 (C2 AB), 62.2 (C2 CD), 65.5 ($O\mathit{C}H_2$), 67.3 (C5 AB), 70.6 (C5 CD), 85.4 (C7 CD), 93.7 (C7 AB), 114.7, 127.9 (Ar-C*H*), 133.5, 157.6 (Ar-C), 166.0 (C9 AB), 166.5 (C9 CD), 168.7 ($\mathit{C}O_2CH_2CH_3$), 172.3 (C8 AB), 178.2 (C8 CD), 188.0 (C6 AB), 191.2 (C6 CD); *m/z* (ESI$^-$) 511 ([M-H]$^-$, 100 %); HRMS (ESI$^+$); $C_{27}H_{33}O_6N_2S$ $[M+H]^+$; found 513.20572, requires 513.20538.

(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(4-(but-2-yn-1-yloxy)phenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 220

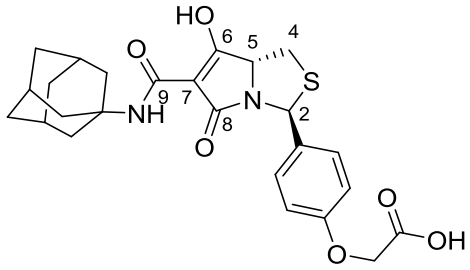


Yield (62 mg, 30 %); brown oil; 2.8:1 AB:CD tautomers; R_f = 0.66 (EtOAc/MeOH 98:2); $[\alpha]_D^{25}$ = -209.4 (*c* = 0.14, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1622 (C=C), 1648 (C=O), 1684 (C=O), 3306 (O-H/N-H); δ_H (400 MHz, $CDCl_3$): 1.64 - 1.74 (6H, m, Adamantyl-C*H*$_2$), 1.86 (3H, t, *J* = 2.3 Hz, -C≡C-C*H*$_3$), 2.02 - 2.08 (6H, m, Adamantyl-C*H*$_2$), 2.09 - 2.18 (3H, m, Adamantyl-C*H*), 2.99 (1H, dd, *J* = 11.3, 8.5 Hz, H4$_A$), 3.26 (1H, dd, *J* = 11.3, 7.0 Hz, H4$_B$), 4.43 (1H, app t, *J* = 7.7 Hz, H5 CD), 4.64 (2H, q , *J* = 2.3 Hz, OC*H*$_2$-C≡C-), 4.68 (1H, dd, *J* = 8.5, 7.0 Hz, H5 AB), 6.21 (1H, s, H2 AB), 6.30 (1H, s, H2 CD), 6.94 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.40 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 9.79 (2H, br. s., NH + OH); δ_C (125.8 MHz, $CDCl_3$): 3.7 (-C≡C-$\mathit{C}H_3$), 29.3 (Adamantyl-*C*H), 32.5 (C4 AB), 32.8 (C4 CD), 35.8 (Adamantyl-*C*H_2 CD), 36.0 (Adamantyl-*C*H_2 AB), 40.9 (Adamantyl-*C*H_2 CD), 41.5 (Adamantyl-*C*H_2 AB), 53.1 (Adamantyl-C), 56.4 ($O\mathit{C}H_2$-C≡C-), 61.6 (C2 AB), 62.2 (C2 CD), 67.3 (C5 AB), 70.6 (C5 CD), 73.8 (-C≡C-), 83.8 (-C≡C-), 85.4 (C7 CD), 95.1 (C7 AB),

114.8, 127.7 (Ar-*C*H), 132.8, 157.6 (Ar-C), 166.0 (C9 AB), 166.5 (C9 CD), 172.2 (C8 AB), 178.1 (C8 CD), 187.9 (C6 AB), 191.2 (C6 CD); *m/z* (ESI^-) 477 ($[M-H]^-$, 100 %); HRMS (ESI^-); $C_{27}H_{29}N_2O_4S$ $[M-H]^-$; found 477.18466, requires 477.18535.

(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(4-(carboxymethoxy)phenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 221

To tetramate **219** (1.51 g, 2.95 mmol, 1.0 eq) dissolved in THF:H_2O (1:1) was added LiOH.H_2O (0.25 g, 5.9 mmol, 2.0 eq) and the reaction stirred at rt for 2 h. Upon completion, the aqueous layer was acidified with 10 % HCl and extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to give **221**.

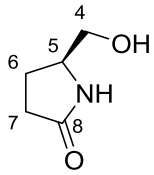


Yield (0.14 g, 98 %); yellow solid, mp 128-130 °C; R_f = 0.06 (EtOAc/MeOH 3:1); $[\alpha]_D^{25}$ = -187.9 (*c* = 0.17, MeOH); ν_{max}/cm^{-1} (neat) 1608 (C=C), 1645 (C=O), 1682 (C=O), 1737 (C=O); δ_H (400 MHz, Methanol-d_4) 1.74 (6H, br. signal, Adamantyl-CH_2), 2.09 (9H, br. signal, Adamantyl-CH_2 + Adamantyl-C*H*), 2.98 (1H, dd, *J* = 11.1, 8.2 Hz, $H4_A$), 3.24 - 3.30 (1H, m, $H4_B$, obscured by solvent peak), 4.66 (2H, s, OCH_2), 4.79 (1H, app t, *J* = 7.6 Hz, H5), 6.18 (1H, s, H2), 6.92 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.40 (2H, d, *J* = 8.6 Hz, Ar-C*H*); δ_C (125.8 MHz, Methanol-d_4): 31.0 (Adamantyl-*C*H), 33.3 (C4), 37.3 (Adamantyl-*C*H_2), 42.8 (Adamantyl-*C*H_2), 54.1 (Adamantyl-C), 63.1 (C2), 66.0 (O*C*H_2), 69.3 (C5), 95.5 (C7), 115.8, 129.0 (Ar-C*H*), 135.1, 159.4 (Ar-*C*), 166.8 (C9), 172.7 (*C*$O_2CH_2CH_3$), 175.6 (C8), 189.0 (C6); *m/z* (ESI^-) 483 ($[M-H]^-$, 100 %); HRMS (ESI^+); $C_{25}H_{29}O_6N_2S$ $[M+H]^+$; found 485.17412, requires 485.17408.

6.4.5 Procedures for the synthesis of pyroglutamate substituted tetramic acids

(*S*)-5-(Hydroxymethyl)-2-pyrrolidinone, 223

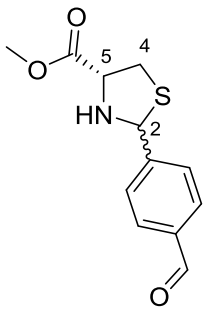
Compound **223** was synthesised according published procedures and the NMR spectroscopic data were consistent with those reported in the literature.[108,186]



δ_H (400 MHz, $CDCl_3$): 1.74 - 1.86 (1H, m, H6), 2.11 - 2.24 (1H, m, H6), 2.28 - 2.45 (2H, m, H7), 3.47 (1H, dd, *J* = 11.4, 7.0 Hz, H4), 3.69 (1H, dd, *J* = 11.4, 3.2 Hz, H4), 3.77 - 3.85 (1H, m, H5), 4.92 (1H, br.s., OH), 7.56 (1H, br.s., NH); δ_C (100.6 MHz, $CDCl_3$): 22.4 (C6), 30.1 (C7), 56.3 (C5), 65.3 (C4), 179.4 (C8); *m/z* (ESI^+) 138 ($[M+Na]^+$, 4 %); HRMS (ESI^+); $C_5H_9O_2NNa$ $[M+Na]^+$; found 138.05243, requires 138.05255.

Methyl (2*RS*,5*R*)- 2-(4-formylphenyl)-1,3-thiazolidine-5-carboxylate, 224a

Thiazolidine **224a** was derived from the condensation of L-cysteine methyl ester hydrochloride and terephthalaldehyde following the procedure for thiazolidine synthesis outlined in general procedure 6.2.3

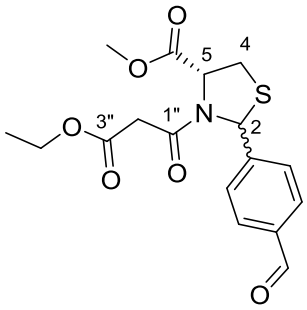


Yield (0.35 g, 24 %); yellow oil; 1:1 *cis* and *trans* diastereomers; R_f = 0.23 (EtOAc/petrol 1:2); ν_{max}/cm^{-1} (neat) 1697 (C=O), 1737 (C=O), 3310 (N-H); δ_H (200 MHz, $CDCl_3$): *cis* isomer: 2.88 (1H, br. s., NH), 3.07 - 3.21 (1H, m, $H4_A$), 3.49 (1H, dd, *J* = 10.4, 7.1 Hz, $H4_B$), 3.81 (3H, s, CO_2CH_3), 4.02 (1H, dd, *J* = 8.9, 7.1 Hz, H5), 5.60 (1H, s, H2), 7.57 - 7.73 (2H, m, Ar-C*H*), 7.80 - 7.94 (2H, m, Ar-C*H*), 10.01 (C*H*O); *trans* isomer: 2.88 (1H, br. s., NH), 3.07 - 3.21 (1H, m, $H4_A$), 3.39 (1H, dd, *J* = 10.6, 6.9 Hz, $H4_B$), 3.80 (3H, s, CO_2CH_3), 4.14 (1H, app t, *J* = 6.6 Hz, H5), 5.88 (1H, s, H2), 7.57 - 7.73 (2H, m, Ar-C*H*), 7.80 - 7.94 (2H, m, Ar-C*H*), 9.98 (C*H*O); δ_C (100.6 MHz, $CDCl_3$) *cis* isomer: 39.1 (C4), 52.7 (CO_2CH_3), 65.5 (C5), 71.7 (C2), 128.1, 130.0 (Ar-C*H*), 136.5, 148.7 (Ar-C), 171.3 (CO_2CH_3), 191.6 (*C*HO); *trans* isomer: 38.1 (C4),

52.7 (CO_2CH_3), 64.2 (C5), 69.7 (C2), 127.3, 129.8 (Ar-C*H*), 135.7, 144.8 (Ar-C), 171.9 (CO_2CH_3), 191.7 (*C*HO); *m/z* (ESI^+) 252 ($[M+H]^+$, 100 %); HRMS (ESI^+); $C_{12}H_{14}NO_3S$ $[M+H]^+$; found 252.06894, requires 252.06889.

Methyl (2*RS*,5*R*)-1-(3-ethoxy-3-oxopropanoyl)-2-(4-formylphenyl)-1,3-thiazolidine-5-carboxylate 224b

N-Acylthiazolidine **224b** was derived from **224a** following the procedure for *N*-acylation outlined in general procedure 6.2.4.

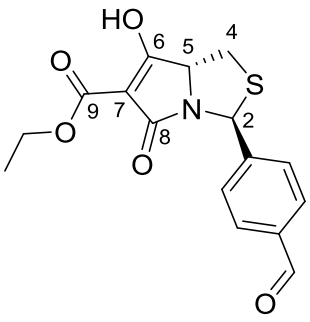


Yield (0.33 g, 70 %); yellow oil; 0.9:1 *cis* and *trans* diastereomers; R_f = 0.33 (EtOAc/petrol 1:1); ν_{max}/cm^{-1} (neat) 1660 (C=O), 1697 (C=O), 1737 (C=O); δ_H (400 MHz, $CDCl_3$) *cis* isomer (a mixture of two conformers): 1.06 - 1.39 (3H, m, OCH_2CH_3), 3.05 - 3.60 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$), 3.83 and 3.84 (3H, s, CO_2CH_3), 4.00 - 4.27 (2H, m, OCH_2CH_3), 5.04 (1H, app t, *J* = 7.2 Hz, H5 major conformer) and 5.09 (1H, dd, *J* = 6.2, 2.9 Hz, H5 minor conformer), 6.22 (1H, s, H2 major conformer), 6.34 (1H, s, H2 minor conformer), 7.68 (2H, d, *J* = 7.6 Hz, Ar-C*H* minor conformer), 7.80 - 7.94 (4H, m, Ar-C*H* major conformer + 2H, m, Ar-C*H* minor conformer), 9.95 (1H, s, C*H*O major conformer) and 9.96 (1H, s, C*H*O minor conformer); *trans* isomer (a mixture of two conformers): 1.06 - 1.39 (3H, m, OCH_2CH_3), 3.05 - 3.60 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$), 3.79 (3H, s, CO_2CH_3 minor conformer), 3.85 (3H, s, CO_2CH_3 major conformer), 4.00 - 4.27 (2H, m, OCH_2CH_3), 5.24 (1H, app d, *J* = 5.6 Hz, H5 minor conformer), 5.33 (1H, app d, *J* = 6.9 Hz, H5 major conformer), 6.25 (1H, s, H2 major conformer), 6.30 (1H, s, H2 minor conformer), 7.40 (2H, d, *J* = 7.8 Hz, Ar-C*H* major conformer), 7.44 (2H, d, *J* = 7.8 Hz, Ar-C*H* minor conformer), 7.80 - 7.94 (2H, m, Ar-C*H* major conformer + 2H, m, Ar-C*H*, minor conformer), 10.01 (1H, s, C*H*O); δ_C (125.8 MHz, $CDCl_3$) *cis* isomer (a mixture of two conformers): 13.9, 14.0 (OCH_2CH_3), 32.0, 33.8 (C4), 42.0, 42.8 (C2″), 52.8, 53.2 (CO_2CH_3),

61.7, 61.8 (O*C*$H_2$$CH_3$), 63.8, 64.8 (C5), 64.5, 66.8 (C2), 127.0, 127.5, 129.8, 130.3 (Ar-*C*H), 135.6, 135.9, 145.5, 146.6 (Ar-*C*), 165.2, 165.3 (C1″), 166.4, 166.9 (C2″), 169.9, 170.0 (*C*$O_2$$CH_3$), 191.5, 191.8 (*C*HO); *trans* isomer (a mixture of two conformers): 13.9, 14.0 (OCH$_2$*C*H_3), 33.0, 34.1 (C4), 42.3, 43.1 (C2″), 52.8, 53.5 (CO_2*C*H_3), 61.5, 61.6 (O*C*$H_2$$CH_3$), 64.0, 64.2 (C5), 65.2, 65.7 (C2), 125.3, 125.5, 130.1, 130.6 (Ar-*C*H), 136.2, 136.3, 148.2, 148.7 (Ar-*C*), 164.8, 165.2 (C1″), 166.0, 167.2 (C2″), 168.7, 169.1 (*C*$O_2$$CH_3$), 191.2, 191.6 (*C*HO); *m/z* (ESI$^+$) 388 ([M+Na]$^+$, 100%); HRMS (ESI$^+$); $C_{17}H_{19}O_6NNaS$ [M+Na]$^+$; found 388.08226, requires 388.08253.

(-)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-2-(4-formylphenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 224

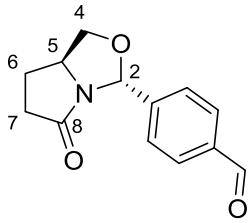
Tetramate **224** was obtained from **224b** following the procedure for Dieckmann cyclisation outlined in general procedure 6.2.5.2.



Yield (57 mg, 25 %); yellow oil; R_f = 0.24 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -193.2 (*c* = 0.19, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1665 (C=O), 1697 (C=O); δ_H (200 MHz, CD_2Cl_2): 1.37 (3H, t, *J* = 7.1 Hz, OCH$_2$C*H*$_3$), 3.04 (1H, dd, *J* = 11.2, 8.3 Hz, H4$_A$), 3.31 (1H, dd, *J* = 11.2, 7.2 Hz, H4$_B$), 4.38 (2H, q, *J* = 7.1 Hz, OC*H*$_2$CH$_3$), 4.81 (1H, dd, *J* = 8.3, 7.2 Hz, H5), 6.32 (1H, s, H2), 7.61 (2H, d, *J* = 8.3 Hz, Ar-C*H*), 7.87 (2H, d, *J* = 8.3 Hz, Ar-C*H*), 10.0 (1H, s, C*H*O); δ_C (100.6 MHz, CD_2Cl_2): 14.5 (OCH$_2$*C*H$_3$), 33.5 (C4), 62.3 (O*C*H$_2$CH$_3$), 62.5 (C2), 66.1 (C5), 127.5, 130.7 (Ar-*C*H), 136.7, 147.7 (Ar-C), 167.4 (C9), 186.6 (C6), 192.1 (*C*HO); *m/z* (ESI$^-$) 332 ([M-H]$^-$, 100 %); HRMS (ESI$^-$); $C_{16}H_{14}O_5NS$ [M-H]$^-$; found 332.06001, requires 332.05982.

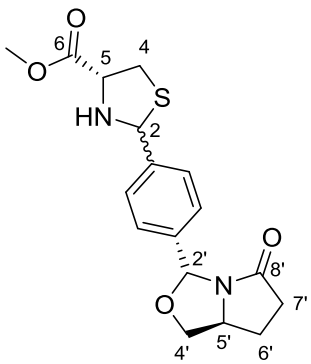
(+)-(2*R*, 5*S*)-1-Aza-2-(4-formylphenyl)-3-oxa-8-oxo-bicyclo[3.3.0]octane, 226b

N,*O*-Acetal **226b** was synthesised according to a modified literature procedure.[107] A mixture of **223** (0.46 g, 4.0 mmol, 1.0 eq), terephthalaldehyde (1.07 g, 8.0 mmol, 2.0 eq) and *p*-toluenesulphonic acid (8 mg, 0.04 mmol, 0.01 eq) in toluene (25 mL) was refluxed with a Dean-Stark apparatus for 22 h. After cooling to rt, EtOAc (10 mL) and water (10 mL) were added. The aqueous layer was separated and extracted with EtOAc. The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (eluent: EtOAc/petrol).



Yield (0.37 g, 40 %); colourless oil; R_f = 0.28 (EtOAc/petrol 2:1); $[\alpha]_D^{25}$ = +245.7 (*c* = 1.52, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1699 (C=O); δ_H (400 MHz, $CDCl_3$): 1.91-2.05 (1H, m, H6), 2.35-2.45 (1H, m, H6), 2.52 - 2.62 (1H, m, H7), 2.83 (1H, ddd, *J* = 17.5, 10.0, 9.2 Hz, H7), 3.51 (1H, app t, *J* = 8.1 Hz, H4), 4.07 - 4.16 (1H, m, H5), 4.25 (1H, dd, *J* = 8.1, 6.4 Hz, H4), 6.35 (1H, s, H2), 7.62 (2H, d, *J* = 8.5 Hz, Ar-C*H*), 7.87 (2H, d, *J* = 8.5, Ar-C*H*), 10.01 (1H, s, C*H*O); δ_C (100.6 MHz, $CDCl_3$): 22.9 (C6), 33.2 (C7), 58.6 (C5), 71.8 (C4), 86.4 (C2), 126.6, 129.8 (Ar-*C*H), 136.4, 145.3 (Ar-C), 178.3 (C8), 191.8 (*C*HO); *m/z* (ESI^+) 254 ($[M+Na]^+$, 50 %); HRMS (ESI^+); $C_{13}H_{13}NO_3NNa$ $[M+Na]^+$; found 254.07888, requires 254.07876.

Methyl (2*RS*,5*R*)-2-(4-((2'*R*, 5'*S*)-1'-aza-3'-oxa-8'-oxo-bicyclo[3.3.0]octan-2'-yl)phenyl)-1,3-thiazolidine-5-carboxylate, 227



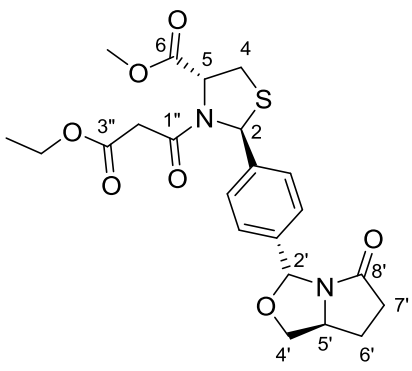
Thiazolidine **227** was derived from the condensation of L-cysteine methyl ester hydrochloride and aldehyde **226b** following the procedure for thiazolidine synthesis outlined in general procedure 6.2.3.

Yield (1.54 g, 30 %); colourless oil; 1:1 *cis* and *trans*

diastereomers; R_f = 0.24 (EtOA/petrol 3:1); ν_{max}/cm^{-1} (neat) 1704 (C=O), 1734 (C=O), 3305 (N-H); δ_H (400 MHz, $CDCl_3$) *cis* diastereomer: 1.63 - 1.99 (1H, m, H6), 2.28 - 2.41 (1H, m, H6), 2.44 - 2.59 (1H, m, H7), 2.71 - 2.92 (1H, m, H7), 3.02 - 3.18 (1H, m, $H4_A$), 3.29 - 3.51 (2H, m, $H4_B$ + H4'), 3.72 (3H, s, CO_2CH_3), 3.95 (1H, app t, *J* = 7.8 Hz, H5), 4.03 - 4.23 (2H, m, H5' + H4'), 5.51 (1H, s, H2), 6.26 (1H, s, H2'), 7.34 - 7.51 (4H, m, Ar-C*H*); *trans* diastereomer: 1.63 - 1.99 (1H, m, H6), 2.28 - 2.41 (1H, m, H6), 2.44 - 2.59 (1H, m, H7), 2.71 - 2.92 (1H, m, H7), 3.02 - 3.18 (1H, m, $H4_A$), 3.29 - 3.51 (2H, m, $H4_B$ + H4'), 3.72 (3H, s, CO_2CH_3), 4.03 - 4.23 (3H, m, H5 + H5' + H4'), 5.78 (1H, s, H2), 6.28 (1H, s, H2'), 7.34 - 7.51 (4H, m, Ar-C*H*); δ_C (100.6 MHz, $CDCl_3$) *cis* diastereomer: 23.0 (C6'), 33.2 (C7'), 39.1 (C4), 53.3 (CO_2CH_3), 58.6 (C5'), 65.4 (C5), 71.5 (C4'), 72.1 (C2), 86.6 (C2'), 126.2, 127.4 (Ar-*C*H), 138.4, 139.3 (Ar-C), 171.4 (CO_2CH_3), 178.0 (C8); *trans* diastereomer: 23.0 (C6'), 33.2 (C7'), 38.0 (C4), 52.2 (CO_2CH_3), 58.6 (C5'), 64.1 (C5), 70.3 (C4'), 71.5 (C2), 86.6 (C2'), 125.9, 126.9 (Ar-*C*H), 138.4, 141.6 (Ar-*C*), 172.1 (CO_2CH_3), 178.0 (C8); *m/z* (ESI^+) 349 ($[M+H]^+$, 100 %); HRMS (ESI^+); $C_{17}H_{21}O_4N_2S$ $[M+H]^+$; found 349.12180, requires 349.12165.

(-)-Methyl (2*S*,5*R*)-2-(4-((2'*R*, 5'*S*)-1'-aza-3'-oxa-8'-oxo-bicyclo[3.3.0]octan-2'-yl)phenyl)-1-(3-ethoxy-3-oxopropanoyl)-1,3-thiazolidine-5-carboxylate, 228

N-Acylthiazolidine **228** was derived from **227** following the procedure for *N*-acylation outlined in general procedure 6.2.4.

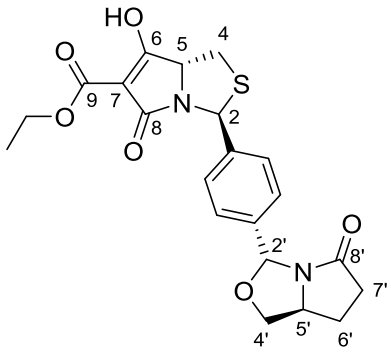


Yield (0.67 g, 60 %); white foam; R_f = 0.16 (EtOAc/petrol 3:1); $[\alpha]_D^{25}$ = -258.0 (*c* = 1.02, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1660 (C=O), 1698 (C=O), 1738 (C=O); δ_H (400 MHz, $CDCl_3$) a mixture of two conformers: 1.21 (3H, t, *J* = 7.2 Hz, OCH_2CH_3 major conformer), 1.28 (3H, t, *J* = 7.2 Hz, OCH_2CH_3 minor conformer), 1.88 - 2.01 (1H, m, H6'), 2.33 - 2.44 (1H, m, H6'), 2.47 - 2.61 (1H, m, H7'), 2.73 - 2.87 (1H, m, H7'), 3.03 - 3.53 (5H, m,

$H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$ + H4'), 3.78 (3H, s, CO_2CH_3 major conformer), 3.83 (3H, s, CO_2CH_3 minor conformer), 4.05 - 4.27 (4H, m, OCH_2CH_3 + H5' + H4'), 5.19 (1H, app d, *J* = 5.4 Hz, H5 minor conformer), 5.27 (1H, app d, *J* = 6.4 Hz, H5 major conformer), 6.18 (1H, s, H2 major conformer), 6.30 (1H, s, H2 minor conformer), 6.26 (1H, s, H2' major conformer), 6.28 (1H, s, H2' minor conformer), 7.20 (2H, d, *J* = 8.3 Hz, Ar-C*H* major conformer), 7.25 (2H, d, *J* = 8.3 Hz, Ar-C*H* minor conformer), 7.38 (2H, d, *J* = 8.3 Hz, Ar-C*H* minor conformer), 7.46 (2H, d, *J* = 8.3 Hz, Ar-C*H* major conformer); δ_C (100.6 MHz, $CDCl_3$) a mixture of two conformers: 13.9, 14.0 (OCH_2CH_3), 22.8, 23.0 (C6'), 30.9, 31.9 (C4), 33.3 (C7'), 42.4, 43.3 (C2″), 52.7, 53.4 (CO_2CH_3), 58.5, 58.8 (C5'), 61.4, 61.7 (OCH_2CH_3), 63.9, 64.2 (C5), 64.7, 65.4 (C2), 71.5, 71.8 (C4'), 86.6, 86.7 (C2'), 124.8, 125.0, 126.5, 126.9 (Ar-CH), 139.3, 139.4, 142.1, 142.3 (Ar-C), 164.5, 165.2 (C1″), 166.2, 167.1 (C3″), 169.3, 170.2 (CO_2CH_3), 177.9, 178.0 (C8); *m/z* (ESI^+) 485 ($[M+Na]^+$ 100 %); HRMS (ESI^+); $C_{22}H_{26}O_7N_2NaS$ $[M+Na]^+$; found 485.13478, requires 485.13529.

(-)-(2*S*,5*R*)-1-Aza-2-(4-((2'*R*, 5'*S*)-1'-aza-3'-oxa-8'-oxo-bicyclo[3.3.0]octan-2'-yl)phenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 225

Tetramate **225** was obtained from **228** following the procedure for Dieckmann cyclisation outlined in general procedure 6.2.5.2.

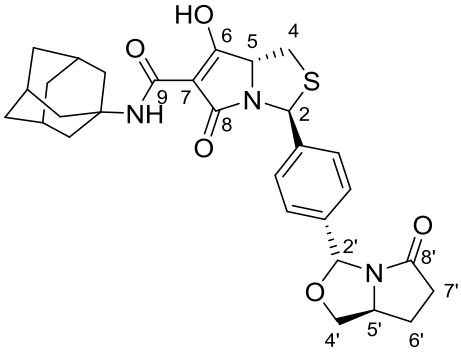


Yield (0.39 g, 70 %); white solid, mp >250 °C; R_f = 0.30 (EtOAc/MeOH 4:1); $[\alpha]_D^{25}$ = -104.8 (*c* = 0.15, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1681 (C=O), 1691 (C=O), 1712 (C=O); δ_H (400 MHz, CD_2Cl_2): 1.36 (3H, t, *J* = 7.2 Hz, OCH_2CH_3), 1.87 - 1.98 (1H, m, H6'), 2.36 (1H, dddd, *J* = 13.5, 10.0, 7.6, 3.7 Hz, H6'), 2.49 (1H, ddd, *J* = 17.3, 10.0, 3.7 Hz, H7'), 2.71 - 2.83 (1H, m, H7'), 3.01 (1H, dd, *J* = 11.1, 8.2 Hz, $H4_A$), 3.28 (1H, dd, *J* = 11.1, 7.2 Hz, $H4_B$), 3.46 (1H, app t, *J* = 8.1 Hz, H4'), 4.08 - 4.16 (1H, m, H5'), 4.21 (1H, dd, *J* = 8.1, 6.2 Hz, H4'),

4.37 (2H, q, *J* = 7.2 Hz, OC*H*$_2$CH$_3$), 4.78 (1H, app t, *J* = 7.6 Hz, H5), 6.23 (1H, s, H2'), 6.27 (1H, s, H2), 7.43 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.44 (2H, d, *J* = 8.8 Hz, Ar-C*H*); δ_C (100.6 MHz, CD_2Cl_2): 14.5 (OCH$_2$*C*H$_3$), 23.5 (C6'), 33.3 (C4), 33.9 (C7'), 59.4 (C5'), 62.2 (O*C*H$_2$CH$_3$), 62.6 (C2), 66.0 (C5), 72.3 (C4'), 87.3 (C2'), 126.8, 126.9 16.0 (Ar-C*H*), 139.7, 141.4 (Ar-C), 167.4 (C8), 178.6 (C8'), 186.5 (C6); *m/z* (ESI$^-$) 429 ([M-H]$^-$, 100 %); HRMS (ESI$^-$); $C_{21}H_{21}O_6N_2S$ [M-H]$^-$; found 429.11295, requires 429.11258.

(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(4-((2'*R*, 5'*S*)-1'-aza-3'-oxa-8'-oxo-bicyclo[3.3.0]octan-2'-yl)phenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 229

Carboxamide tetramate **229** was obtained by aminolysis of **225** with 1-adamantylamine according to the general procedure 6.3.1 for the synthesis of carboxamides with THF/toluene as solvent.



Yield (44 mg, 50 %); white foam, mp 152 °C; R_f = 0.47 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -121.8 (*c* = 0.13, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1625 (C=C), 1648 (C=O), 1691 (C=O), 3306 (N-H); δ_H (400 MHz, DMSO-d_6): 1.63 (6H, br. signal, Adamantyl-C*H*$_2$), 1.87 - 1.98 (7H, m, Adamantyl-C*H*$_2$ + H6'), 2.04 (3H, br. signal, Adamantyl-C*H*), 2.23 - 2.32 (1H, m, H6'), 2.42 (1H, ddd, *J* = 17.2, 10.0, 4.0 Hz, H7'), 2.72 (1H, ddd, *J* = 17.2, 10.0, 8.8 Hz, H7'), 3.01 (1H, dd, *J* = 11.0, 8.4 Hz, H4$_A$), 3.28 (1H, dd, *J* = 11.0, 7.1 Hz, H4$_B$), 3.44 (1H, app t, *J* = 7.6 Hz, H4'), 4.08 - 4.21 (2H, m, H4' + H5'), 4.90 (1H, app br. t, *J* = 7.2 Hz, H5), 6.07 (1H, s, H2'), 6.14 (1H, s, H2), 7.36 (2H, d, *J* = 8.3 Hz, Ar-C*H*), 7.45 (2H, d, *J* = 8.3 Hz, Ar-C*H*), 7.69 (1H, br. s., NH/OH); δ_C (100.6 MHz, DMSO-d_6): 22.0 (C6'), 28.8 (Adamantyl-*C*H), 32.0 (C4), 32.8 (C7'), 35.8 (Adamantyl-*C*H$_2$), 41.2 (Adamantyl-*C*H$_2$), 51.2 (Adamantyl-C), 58.6 (C5'), 61.3 (C2), 67.1 (C5), 71.0 (C4'), 86.3 (C2'), 126.2, 126.3 (Ar-C*H*), 138.9, 141.5 (Ar-C), 163.3 (C9), 173.4 (C8), 178.0 (C8'); *m/z*

(ESI$^-$) 534 ([M-H]$^-$, 20 %); HRMS (ESI$^-$); $C_{29}H_{32}N_3O_5S$ [M-H]$^-$; found 534.2078, requires 534.20682.

6.4.6 Procedures for the synthesis of C-2 amine/amide functionalized tetramates

Attempted Buchwald-Hartwig amination reactions of **91a** and **110**

Condition 1[112]: $Pd(OAc)_2$ (1 mol %) and XPhos (3 mol %) were placed in a round-bottomed flask and degassed with N_2 three times.. To this, *tert*-butanol and H_2O (4 mol %) were added and degassed with N_2. The solution was heated to 110 °C for 1.5 min. In a separate reaction flask, tetramic acid **110** (100 mg, 1.0 eq), morpholine (1.5 eq) and Cs_2CO_3 (2.5 eq) were treated with *tert*-butanol (4 mL) and degassed with N_2. To this reaction vessel, the activated catalyst was transferred and the solution was heated at 110 °C. No product was observed by LRMS after a reaction time of 48 h. The reaction flask was cooled to rt and EtOAc and H_2O were added. The organic layer was separated and the aqueuos layer was re-extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4, filtered through a Celite pad and concentrated *in vacuo*. Starting material was isolated by colum purification.

Condition 2: Tetramic acid **110**/**91a** (50-100 mg, 1.0 eq), morpholine (1.5 eq), Cs_2CO_3 (2.5 eq), $Pd_2(dba)_3$ (5 mol %), XPhos/RuPhos/XantPhos (15 mol %) and 1,2-dimethoxyethane were placed in sealed flask and degassed with N_2. The reaction mixture was heated at 80 °C for 48 h. The reaction flask was cooled to rt, diluted with EtOAc and filtered through a Celite pad. The filtrate was concentrated *in vacuo* and starting material was isolated by column purification. (In order to obtain elevated temperatures, other solvents such as *tert*-butanol and DME/toluene were used, but no product was formed).

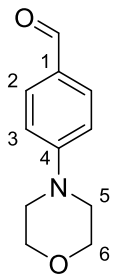
Condition 3[187]: Tetramic acid **110** (70 mg, 1.0 eq), RuPhos Pd G2 (5 mol %), Cs_2CO_3 (2.5 eq) and *tert*-butanol were placed in a sealed round-bottomed flask and degassed with N_2. Morpholine (1.5 eq) was added via syringe and heated to reflux for 24 h. The reaction mixture

was cooled to rt, diluted with EtOAc and filtered through a Celite plug. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography to obtain unreacted starting material.

Procedures for the synthesis of C-2 amine/amide functionalized tetramates from functionalized aldehydes via Buchwald amination/amidation

4-(Morpholin-4-yl)benzaldehyde, 232

4-Bromobenzaldehyde (1.8 g, 9.72 mmol, 1.0 eq), $Pd_2(dba)_3$ (0.13 g, 0.15 mmol, 1.5 mol %), RuPhos (0.14 g, 0.29 mmol, 3 mol %), Cs_2CO_3 (4.43 g, 13.6 mmol, 1.4 eq) and *tert*-butanol (40 mL) were placed in a sealed round-bottomed flask and degassed with N_2. Morpholine (2 eq) was added via syringe and heated to reflux for 2 h. The reaction mixture was cooled to rt, diluted with EtOAc and filtered through a Celite plug. The filtrate was concentrated *in vacuo* and the residue was purified by silica gel flash column chromatography (eluent: EtOAc/petrol) to obtain **232**.

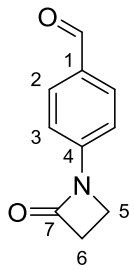


Yield (1.17 g, 63 %); yellow crystalline solid, mp 70 °C; R_f = 0.26 (EtOAc/petrol 1:3); ν_{max}/cm^{-1} (neat) 1682 (C=O); δ_H (400 MHz, $CDCl_3$) 3.33 - 3.39 (4H, m, H5), 3.84 - 3.90 (4H, m, H6), 6.93 (2H, d, *J* = 8.8 Hz, H3), 7.79 (2H, d, *J* = 8.8 Hz, H2), 9.81 (1H, s, C*H*O); δ_C (100.6 MHz, $CDCl_3$): 47.1 (C5), 66.4 (C6), 113.3 (C3), 127.5 (C1), 131.7 (C2), 155.0 (C4), 190.4 (*C*HO); *m/z* (ESI^+) 192 ($[M+H]^+$, 50 %); HRMS (ESI^+); $C_{11}H_{14}O_2N$ $[M+H]^+$; found 192.10154, requires 192.10191.

4-(2-Oxoazetidin-1-yl)benzaldehyde, 233

The synthesis of **233** is according to a modified literature procedure.[188] 4-Bromobenzaldehyde (1.6 g, 8.76 mmol, 1.0 eq), 2-azetidinone (0.75 g, 10.5 mmol, 1.2 eq), $Pd_2(dba)_3$ (0.16 g, 0.18 mmol, 2 mol %), XantPhos (0.30 g, 0.53 mmol, 6 mol %), Cs_2CO_3 (4.0 g, 12.3 mmol, 1.4 eq)

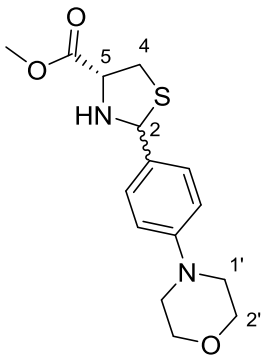
and 1,4-dioxane (40 mL) were placed in a sealed round-bottomed flask and degassed with N_2. The reaction mixture was heated to reflux for 20 h. The reaction mixture was cooled to rt, diluted with EtOAc and filtered through a Celite plug. The filtrate was concentrated *in vacuo* and the residue was purified by silica gel flash column chromatography (eluent: EtOAc/petrol) to obtain **233**.



Yield (0.92 g, 60 %); yellow solid; mp 108 °C; R_f = 0.28 (EtOAc/petrol 1:2); ν_{max}/cm^{-1} (neat) 1690 (C=O), 1747 (C=O); δ_H (400 MHz, $CDCl_3$) 3.20 (2H, t, *J* = 4.7 Hz, H5), 3.72 (2H, t, *J* = 4.7 Hz, H6), 7.48 (2H, d, *J* = 8.6 Hz, H3), 7.87 (2H, d, *J* = 8.6 Hz, H2), 9.92 (1H, s, C*H*O); δ_C (125.8 MHz, $CDCl_3$): 36.6 (C5), 38.4 (C6), 116.2 (C3), 131.3 (C2), 132.0 (C1), 143.2 (C4), 164.9 (C7), 190.8 (*C*HO); *m/z* (ESI^+) 176 ($[M+H]^+$, 38 %); HRMS (ESI^+); $C_{10}H_{10}O_2N$ $[M+H]^+$; found 176.07069, requires 176.07061.

Methyl (2*RS*,5*R*)-2-(4-morpholinophenyl)-1,3-thiazolidine-5-carboxylate, 234

Thiazolidine **234** was derived from the condensation of L-cysteine methyl ester hydrochloride and aldehyde **232** following the procedure for thiazolidine synthesis outlined in general procedure 6.2.3.

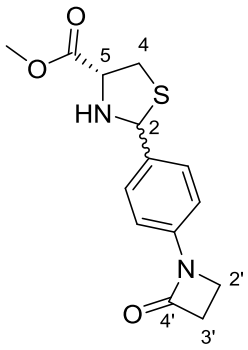


Yield (1.46 g, 78 %); yellow oil; 2.2:1 *cis* and *trans* diastereomers; R_f = 0.29 (EtOAc/petrol 1:1); ν_{max}/cm^{-1} (neat) 1737 (C=O), 3303 (N-H); δ_H (400 MHz, $CDCl_3$) major isomer (*cis*): 2.61 (1H, app t, *J* = 12.3 Hz, NH), 3.11 (1H, dd, *J* = 10.3, 8.8 Hz, $H4_A$), 3.14 - 3.20 (4H, m, H1'), 3.46 (1H, dd, *J* = 10.3, 7.1 Hz, $H4_B$), 3.81 (3H, s, CO_2CH_3), 3.83 - 3.89 (4H, m, H2'), 3.93 - 4.02 (1H, m, H5), 5.52 (1H, d, *J* = 12.3 Hz, H2), 6.85 - 6.93 (2H, m, Ar-C*H*), 7.37 - 7.47 (2H, m, Ar-C*H*); minor isomer (*trans*): 2.75 (1H, br. s., NH), 3.14 - 3.20 (4H, m, H1'), 3.24 (1H, dd, *J* = 10.6, 5.5 Hz, $H4_A$), 3.40 (1H, dd, *J* = 10.6,

7.2 Hz, $H4_B$), 3.80 (3H, s, CO_2CH_3), 3.83 - 3.89 (4H, m, H2'), 4.25 (1H, app t, *J* = 6.0 Hz, H5), 5.76 (1H, s, H2), 6.85 - 6.93 (2H, m, Ar-C*H*), 7.37 - 7.47 (2H, m, Ar-C*H*); δ_C (100.6 MHz, $CDCl_3$): major isomer (*cis*): 39.3 (C4), 49.0 (C1'), 52.6 (CO_2CH_3), 65.5 (C5), 66.8 (C2'), 72.4 (C2), 115.4, 128.4 (Ar-C*H*), 129.0, 151.5 (Ar-C), 171.7 (CO_2CH_3); minor isomer (*trans*): 38.0 (C4), 49.0 (C1'), 52.5 (CO_2CH_3), 64.2 (C5), 66.8 (C2'), 70.7 (C2), 115.3, 127.9 (Ar-C*H*), 131.9, 151.0 (Ar-C), 172.3 (CO_2CH_3); *m/z* (ESI^+) 309 ($[M+H]^+$, 100%); HRMS (ESI^+); $C_{15}H_{21}N_2O_3S$ $[M+H]^+$; found 309.12665, requires 309.12674.

Methyl (2*RS*,5*R*)-2-(4-(2-oxoazetidin-1-yl)phenyl)-1,3-thiazolidine-5-carboxylate, 235

Thiazolidine **235** was derived from the condensation of L-cysteine methyl ester hydrochloride and aldehyde **233** following the procedure for thiazolidine synthesis outlined in general procedure 6.2.3.

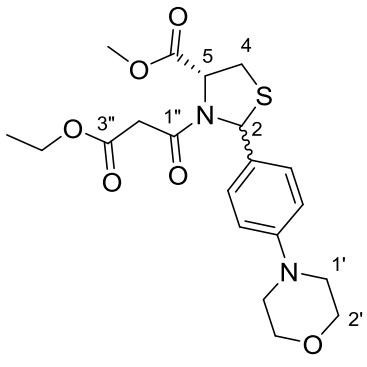


Yield (0.87 g, 58 %); white solid; 1.7:1 *cis* and *trans* diastereomers; R_f = 0.23 (EtOAc/petrol 1:1); ν_{max}/cm^{-1} (neat) 1738 (C=O), 3300 (N-H); δ_H (500 MHz, $CDCl_3$) major isomer (*cis*): 2.61 (1H, br. s, NH), 3.06 - 3.17 (3H, m, $H4_A$, H2'), 3.47 (1H, dd, *J* = 10.4, 7.1 Hz, $H4_B$), 3.61 - 3.66 (2H, m, H3'), 3.82 (3H, s, CO_2CH_3), 3.99 (1H, app t, *J* = 7.9 Hz, H5), 5.53 (1H, s, H2), 7.36 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.50 (2H, d, *J* = 8.6 Hz, Ar-C*H*); minor isomer (*trans*): 2.81 (1H, br. s., NH), 3.06 - 3.17 (2H, m, H2'), 3.22 (1H, dd, *J* = 10.6, 5.7 Hz, $H4_A$), 3.40 (1H, dd, *J* = 10.6, 7.2 Hz, $H4_B$), 3.61 - 3.66 (2H, m, H3'), 3.81 (3H, s, CO_2CH_3), 4.21 (1H, app t, *J* = 6.5 Hz, H5), 5.79 (1H, s, H2), 7.33 (2H, d, *J* = 8.5 Hz, Ar-C*H*), 7.47 (2H, d, *J* = 8.5 Hz, Ar-C*H*); δ_C (125.8 MHz, $CDCl_3$): major isomer (*cis*): 36.2 (C2'), 38.1 (C1'), 39.3 (C4), 52.6 (CO_2CH_3), 65.5 (C5), 72.2 (C2), 116.3, 128.4 (Ar-C*H*), 133.4, 138.7 (Ar-C), 164.5 (C4'), 171.6 (CO_2CH_3); minor isomer (*trans*): 36.2 (C2'), 38.1 (C1'), 38.1 (C4), 52.6 (CO_2CH_3), 64.2 (C5), 70.4 (C2), 116.1, 127.9 (Ar-C*H*), 136.4, 138.1

(Ar-C), 164.4 (C4'), 172.2 (CO_2CH_3); *m/z* (ESI^+) 293 ($[M+H]^+$, 100%); HRMS (ESI^+); $C_{14}H_{17}N_2O_3S$ $[M+H]^+$; found 293.09541, requires 293.09544.

Methyl (2*RS*,5*R*)-1-(3-ethoxy-3-oxopropanoyl)-2-(4-morpholinophenyl)-1,3-thiazolidine-5-carboxylate, 236

N-Acylthiazolidine **236** was derived from **234** following the procedure for *N*-acylation outlined in general procedure 6.2.4.

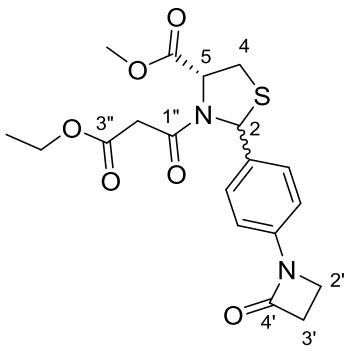


Yield (1.54 g, 78 %); yellow oil; 2.6:1 *cis* and *trans* diastereomers; R_f = 0.42 (*trans*), 0.51 (cis) (EtOAc/petrol 2:1); ν_{max}/cm^{-1} (neat) 1659 (C=O), 1738 (C=O); δ_H (400 MHz, $CDCl_3$): major isomer (*cis*, mixture of two conformers); 1.20 - 1.32 (3H, m, OCH_2CH_3), 3.06 - 3.57 (8H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$ + H1'), 3.81 - 3.88 (7H, m, CO_2CH_3 + H1'), 4.07 - 4.26 (2H, m, OCH_2CH_3), 5.07 (1H, app t, *J* = 6.5 Hz, H5), 6.08 (1H, s, H2 major conformer) and 6.30 (1H, s, H2 minor conformer), 6.81 - 6.93 (2H, m, Ar-C*H*), 7.40 - 7.45 (2H, m, Ar-C*H*, minor conformer), 7.55 (2H, d, *J* = 8.6 Hz, Ar-C*H*, major conformer); minor isomer (*trans*, mixture of two conformers); 1.20 - 1.32 (3H, m, OCH_2CH_3), 3.06 - 3.57 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$), 3.81 - 3.88 (7H, m, CO_2CH_3 + H2'), 4.07 - 4.26 (2H, m, OCH_2CH_3), 5.18 (1H, app d, *J* = 5.6 Hz, H5 minor conformer), 5.26 - 5.31 (1H, m, H5 major conformer), 6.13 (1H, s, H2 major conformer), 6.29 (1H, s, H2 minor conformer), 6.81 - 6.93 (2H, m, Ar-C*H*), 7.11 (2H, d, *J* = 8.8 Hz, Ar-C*H* major conformer), 7.19 (2H, d, *J* = 8.8 Hz, Ar-C*H* minor conformer); δ_C (100.6 MHz, $CDCl_3$) major isomer (*cis*, mixture of two conformers): 13.9 (OCH_2CH_3), 31.9, 33.5 (C4), 41.9, 43.2 (C2″), 48.5, 49.0 (C1'), 52.5, 53.3 (CO_2CH_3), 61.3, 61.5 (OCH_2CH_3), 63.8, 64.0 (C5), 66.0, 66.6 (C2), 66.6 (C2'), 115.3, 115.4, 127.4, 128.2 (Ar-CH), 130.0, 151.1 (Ar-C), 165.4 (C1″), 166.7 (C3″), 170.1 (CO_2CH_3); minor isomer (*trans*, mixture of two conformers): 13.9 (OCH_2CH_3), 30.8, 33.6 (C4), 42.2, 42.8 (C2″), 48.5, 48.8

(C1'), 52.6, 52.9 (CO_2CH_3), 61.2, 61.3 (OCH_2CH_3), 63.5, 64.3 (C5), 64.7, 65.3 (C2), 66.6 (C2'), 114.9, 115.5, 125.6, 125.8 (Ar-*C*H), 132.2, 151.0 (Ar-C), 165.3 (C1″), 166.3 (C3″), 169.4 (CO_2CH_3); *m/z* (ESI^+) 423 ($[M+H]^+$, 100%); HRMS (ESI^+); $C_{20}H_{27}N_2O_6S$ $[M+H]^+$; found 423.15772, requires 423.15843.

Methyl (2*RS*,5*R*)-1-(3-ethoxy-3-oxopropanoyl)-2-(4-(2-oxoazetidin-1-yl)phenyl)-1,3-thiazolidine-5-carboxylate, 237

N-Acylthiazolidine **237** was derived from **235** following the procedure for *N*-acylation outlined in general procedure 6.2.4.

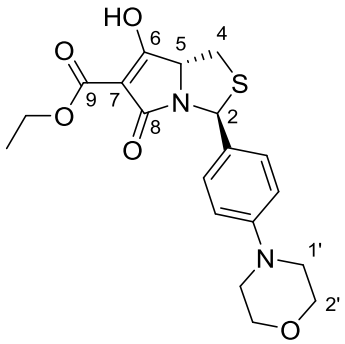


Yield (0.97 g, 81 %); colourless oil; 1.1:1 *cis* and *trans* diastereomers; R_f = 0.23 (*trans*), 0.31 (*cis*) (EtOAc/petrol 2:1); ν_{max}/cm^{-1} (neat) 1659 (C=O), 1734 (C=O); δ_H (400 MHz, $CDCl_3$): major isomer (*cis*, mixture of two conformers); 1.20 - 1.31 (3H, m, OCH_2CH_3), 3.06 - 3.67 (8H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$ + H2' + H3'), 3.81 (3H, m, CO_2CH_3 minor conformer), 3.84 (3H, m, CO_2CH_3 major conformer), 4.07 - 4.24 (2H, m, OCH_2CH_3), 5.07 (1H, app t, *J* = 6.7 Hz, H5), 6.11 (1H, s, H2 major conformer), 6.30 (1H, s, H2 minor conformer), 7.28 - 7.33 (2H, m, Ar-C*H* minor conformer), 7.34 - 7.41 (2H, m, Ar-C*H* major conformer), 7.50 (2H, d, *J* = 8.6 Hz, Ar-C*H* minor conformer), 7.65 (2H, d, *J* = 8.6 Hz, Ar-C*H* major conformer); minor isomer (*trans*, mixture of two conformers); 1.20 - 1.31 (3H, m, OCH_2CH_3), 3.06 - 3.67 (8H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$ + H2' + H3'), 3.79 (3H, m, CO_2CH_3 major conformer), 3.85 (3H, m, CO_2CH_3 minor conformer), 4.07 - 4.24 (2H, m, OCH_2CH_3), 5.20 (1H, app d, *J* = 5.4 Hz, H5 minor conformer), 5.27 - 5.32 (1H, m, H5 major conformer), 6.15 (1H, s, H2 major conformer), 6.28 (1H, s, H2 minor conformer), 7.20 (2H, *J* = 8.3 Hz, Ar-C*H* major conformer, 7.24 - 7.28 (2H, m, Ar-C*H* minor conformer), 7.28 - 7.33 (2H, m, Ar-C*H* minor conformer), 7.34 - 7.41 (2H, m, Ar-C*H* major conformer); δ_C (125.8 MHz, $CDCl_3$) major isomer (*cis*, mixture of two

conformers): 14.0, 14.1 ($OCH_2\mathit{C}H_3$), 32.1, 33.8 (C4), 36.2, 36.3 (C2'), 38.0, 38.1 (C3'), 42.1, 43.0 (C2″), 52.8, 53.2 ($CO_2\mathit{C}H_3$), 61.6, 61.7 ($O\mathit{C}H_2CH_3$), 63.7, 64.6 (C5), 66.0, 66.8 (C2), 115.9, 116.6, 127.5, 128.4 (Ar-*C*H), 134.0, 134.8, 138.7, 138.7 (Ar-C), 164.3, 164.5 (C4'), 165.2, 165.5 (C1″), 166.7, 167.1 (C3″), 170.2, 171.6 ($\mathit{C}O_2CH_3$); minor isomer (*trans*, mixture of two conformers): 14.0, 14.1 ($OCH_2\mathit{C}H_3$), 31.0, 33.1 (C4), 36.1, 36.3 (C2'), 38.0, 38.1 (C3'), 42.4, 43.3 (C2″), 52.8, 53.4 ($CO_2\mathit{C}H_3$), 61.5, 61.8 ($O\mathit{C}H_2CH_3$), 64.0, 64.2 (C5), 64.7, 65.4 (C2), 116.3, 116.8, 125.7, 125.9 (Ar-*C*H), 137.0, 137.6, 138.6, 138.7 (Ar-C), 164.3, 164.5 (C4'), 164.9, 165.3 (C1″), 166.3, 167.2 (C3″), 169.4, 170.2 ($\mathit{C}O_2CH_3$); *m/z* (ESI^+) 407 ($[M+H]^+$, 85 %); HRMS (ESI^+); $C_{19}H_{23}N_2O_6S$ $[M+H]^+$; found 407.12658, requires 407.12713.

(-)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-6-hydroxy-2-(morpholinophenyl)-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 238

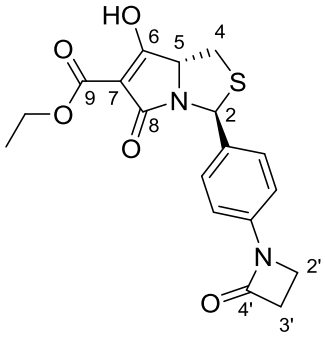
Tetramate **238** was obtained from **236** following the procedure for Dieckmann cyclisation outlined in general procedure 6.2.5.2.



Yield (0.24 g, 22 %); brown oil; R_f = 0.42 (EtOAc/MeOH 84:16); $[\alpha]_D^{25}$ = -88.2 (*c* = 0.20, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1611 (C=C), 1652 (C=O), 1688 (C=O); δ_H (500 MHz, CD_2Cl_2): 1.37 (3H, t, *J* = 7.1 Hz, $OCH_2C\mathit{H}_3$), 2.99 (1H, dd, *J* = 11.1, 8.3 Hz, $H4_A$), 3.14 (4H, br. t, *J* = 4.7 Hz, H1'), 3.28 (1H, dd, *J* = 11.1, 7.0 Hz, $H4_B$), 3.82 (4H, br. t, *J* = 4.7 Hz, H2'), 4.37 (2H, q, *J* = 7.1 Hz, $OC\mathit{H}_2CH_3$), 4.79 (1H, app t, *J* = 7.7 Hz, H5), 6.21 (1H, s, H2), 6.89 (2H, d, *J* = 8.6 Hz, Ar-C*H*), 7.35 (2H, d, *J* = 8.6 Hz, Ar-C*H*); δ_C (125.8 MHz, CD_2Cl_2): 14.5 ($OCH_2\mathit{C}H_3$), 33.3 (C4), 49.7 (C1'), 62.2 ($O\mathit{C}H_2CH_3$), 62.6 (C2), 65.9 (C5), 67.3 (C2'), 99.8 (C7), 115.9, 127.8 (Ar-*C*H), 131.9, 151.8 (Ar-C), 167.6 (C9), 168.9 (C8), 186.5 (C6); *m/z* (ESI^-) 389 ($[M-H]^-$, 87 %); HRMS (ESI^+); $C_{19}H_{23}N_2O_5S$ $[M+H]^+$; found 391.13205, requires 391.13222.

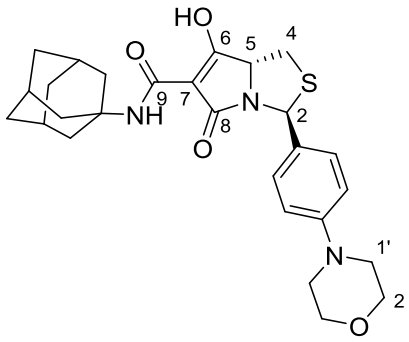
(-)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-6-hydroxy-2-(4-(2-oxoazetidin-1-yl)phenyl)-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 239

Tetramate **239** was obtained from **237** following the procedure for Dieckmann cyclisation outlined in general procedure 6.2.5.2.



Yield (0.23 g, 30 %); yellow solid, mp 124-126 °C; R_f = 0.32 (EtOAc/MeOH 84:16); $[\alpha]_D^{25}$ = -182.5 (*c* = 0.15, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1610 (C=C), 1658 (C=O), 1739 (C=O, br with shoulder towards lower wavenumber); δ_H (500 MHz, CD_2Cl_2): 1.37 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 3.01 (1H, dd, *J* = 11.2, 8.2 Hz, $H4_A$), 3.08 (2H, t, *J* = 4.6 Hz, H2'), 3.29 (1H, dd, *J* = 11.2, 7.1 Hz, $H4_B$), 3.61 (4H, t, *J* = 4.6 Hz, H3'), 4.38 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.79 (1H, app t, *J* = 7.5 Hz, H5), 6.24 (1H, s, H2), 7.33 (2H, d, *J* = 8.5 Hz, Ar-C*H*), 7.43 (2H, d, *J* = 8.5 Hz, Ar-C*H*); δ_C (125.8 MHz, CD_2Cl_2): 14.5 (OCH_2CH_3), 33.3 (C4), 36.8 (C2'), 38.7 (C3'), 62.3 (OCH_2CH_3), 62.6 (C2), 65.9 (C5), 99.7 (C7), 116.6, 127.8 (Ar-*C*H), 136.1, 139.1 (Ar-C), 165.0 (C4'), 167.6 (C9), 169.0 (C8), 186.6 (C6); *m/z* (ESI^-) 373 ($[M-H]^-$, 100 %); HRMS (ESI^-); $C_{19}H_{23}N_2O_5S$ $[M-H]^-$; found 373.08701, requires 373.08637.

(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-6-hydroxy-2-(4-morpholinophenyl)-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 240



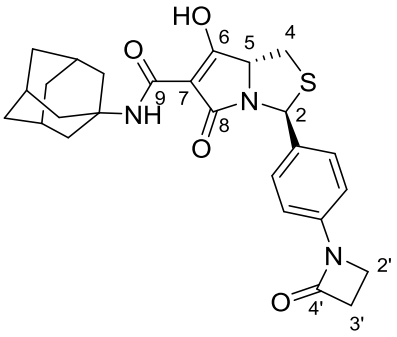
Carboxamide tetramate **240** was obtained by aminolysis of **238** with 1-adamantylamine according to the general procedure 6.3.1 for the synthesis of carboxamides with THF/toluene as solvent.

Yield (0.23 g, 25 %); brown solid, mp 120-124 °C; 3:1 AB:CD tautomers; R_f = 0.70 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -144.1 (*c* = 0.20, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1613 (C=C), 1647 (C=O), 1685 (C=O), 3310 (N-

H/O-H); δ_H (500 MHz, CD_2Cl_2) 1.69 - 1.81 (6H, m, Adamantyl-C*H*$_2$), 2.06 - 2.20 (9H, m, Adamantyl-C*H*$_2$, Adamantyl-C*H*), 2.96 - 3.06 (1H, m, H4$_A$), 3.18 (4H, br. t, *J* = 4.7 Hz, H1'), 3.25 - 3.32 (1H, m, H4$_B$), 3.87 (4H, br. t, *J* = 4.7 Hz, H2'), 4.01 (2H, br. s, NH + OH AB), 4.47 (1H, app t, *J* = 7.9 Hz, H5 CD), 4.75 (1H, dd, *J* = 8.3, 7.2 Hz, H5 AB), 6.20 (1H, s, H2 AB), 6.28 (1H, s, H2 CD), 6.90 - 6.98 (2H, m, Ar-C*H*), 7.39 (2H, d, *J* = 8.7 Hz, Ar-C*H*), 7.93 (1H, br. s, NH/OH, CD); δ_C (125.8 MHz, CD_2Cl_2): 30.1 (Adamantyl-*C*H), 33.1 (C4 AB), 33.4 (C4 CD), 36.4 (Adamantyl-*C*H$_2$ CD), 36.6 (Adamantyl-*C*H$_2$ AB), 42.1 (Adamantyl-*C*H$_2$ AB), 42.2 (Adamantyl-*C*H$_2$ CD), 49.8 (C1'), 53.4 (Adamantyl-C AB), 54.9 (Adamantyl-C CD), 62.3 (C2 AB), 62.9 (C2 CD), 67.2 (C2'), 67.8 (C5 AB), 71.5 (C5 CD), 85.7 (C7 CD), 96.4 (C7 AB), 116.0, 127.9 (Ar-*C*H), 127.8, 151.6 (Ar-C), 166.6 (C9 AB), 167.2 (C9 CD), 172.7 (C8 AB), 178.9 (C8 CD), 187.9 (C6 AB), 191.6 (C6 CD); *m/z* (ESI$^+$) 496 ([M+H]$^+$, 75 %); HRMS (ESI$^+$); $C_{27}H_{34}O_4N_3S$ [M+H]$^+$; found 496.22607, requires 496.22645.

(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-6-hydroxy-2-(4-(2-oxoazetidin-1-yl)phenyl)-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 241

Carboxamide tetramate **241** was obtained by aminolysis of **239** with 1-adamantylamine according to the general procedure 6.3.1 for the synthesis of carboxamides with THF/toluene as solvent.



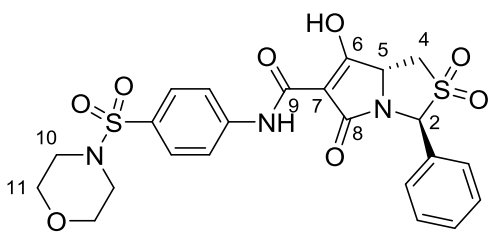
Yield (47 mg, 30 %); brown solid, mp 148-150 °C; 3:1 AB:CD tautomers; R_f = 0.62 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -240.0 (*c* = 0.19, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1622 (C=C), 1647 (C=O), 1687 (C=O), 1745 (C=O), 3315 (N-H/O-H); δ_H (500 MHz, CD_2Cl_2) 1.65 - 1.76 (6H, m, Adamantyl-C*H*$_2$), 2.02 - 2.16 (9H, m, Adamantyl-C*H*$_2$ + Adamantyl-C*H*), 2.96 - 3.03 (1H, m, H4$_A$), 3.08 (2H, t, *J* = 4.5 Hz, H2'), 3.20 - 3.28 (1H, m, H4$_B$), 3.60 (2H, t, *J* = 4.5 Hz, H3'), 4.42 (1H, app t, *J* = 7.7 Hz, H5 CD), 4.69 (1H, app t, *J* = 7.7 Hz, H5 AB), 4.98 (2H, br. s, NH + OH), 6.19 (1H, s, H2

AB), 6.28 (1H, s, H2 CD), 7.30 - 7.34 (2H, m, Ar-C*H*), 7.40 - 7.44 (2H, m, Ar-C*H*); δ_C (125.8 MHz, CD_2Cl_2): 30.1 (Adamantyl-*C*H), 33.1 (C4 AB), 33.4 (C4 CD), 36.4 (Adamantyl-*C*H_2 CD), 36.6 (Adamantyl-*C*H_2 AB), 36.8 (C2'), 38.7 (C3'), 42.1 (Adamantyl-*C*H_2 AB), 42.2 (Adamantyl-*C*H_2 CD), 53.5 (Adamantyl-C AB), 55.0 (Adamantyl-C CD), 62.3 (C2 AB), 62.9 (C2 CD), 67.9 (C5 AB), 71.4 (C5 CD), 85.7 (C7 CD), 96.0 (C7 AB), 116.0, 127.8 (Ar-*C*H, AB), 116.0, 127.7 (Ar-*C*H CD), 136.3, 139.0 (Ar-C AB), 136.6, 138.9 (Ar-C CD), 165.0 (C4'), 166.6 (C9 AB), 167.2 (C9 CD), 172.9 (C8 AB), 179.0 (C8 CD), 188.3 (C6 AB), 191.6 (C6 CD); *m/z* (ESI^-) 478 ($[M-H]^-$, 25 %); HRMS (ESI^-); $C_{26}H_{28}O_4N_3S$ $[M-H]^-$; found 478.18107, requires 478.18060.

6.4.7 Procedure for the synthesis of sulfone 132a

(2*S*,5*R*)-1-Aza-6-hydroxy-7-(4-(morpholinosulfonyl)phenylaminocarbonyl)-3,3,8-trioxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 132a

To a solution of **132** (39 mg, 0.078 mmol, 1.0 eq) in CH_2Cl_2 (2 mL), 70 % *m*-CPBA (43 mg, 0.17 mmol, 2.2 eq) was added and stirred at rt for 1 h. The reaction was quenched with sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic fractions were dried over Na_2SO_4, filtered and solvent removed *in vacuo* to give the crude residue, which was purified by flash column chromatography (eluent: EtOAc/petrol/1 % Et_3N). The product isolated was dissolved in CH_2Cl_2 (a few drops of MeOH was added to aid solubility) and washed with 0.1 M HCl. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford **132a**.



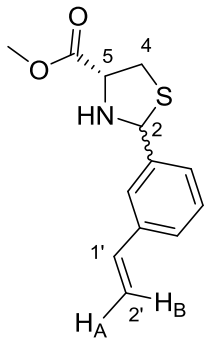
Yield (10 mg, 24 %); light brown solid, mp 188 °C; R_f = 0.33 (EtOAc/MeOH 96:4); ν_{max}/cm^{-1} (neat) 1161, 1345 (S=O), 1628 (C=C), 1654 (C=O), 1695 (C=O); δ_H (400 MHz, CD_2Cl_2): 2.97 (4H, br. t, *J* = 4.7 Hz, H10), 3.28 (1H, dd, *J* = 13.2, 8.0 Hz, $H4_A$), 3.58 (1H, dd, *J* = 13.2, 8.3 Hz, $H4_B$), 3.72

(4H, br. t, *J* = 4.7 Hz, H11), 4.76 (1H, br. s., NH/OH), 5.06 (1H, br. signal, H5), 6.00 (1H, s, H2), 7.45 - 7.53 (5H, m, Ar-C*H*), 7.74 (2H, d, *J* = 8.0 Hz, Ar-C*H*), 7.83 (2H, d, *J* = 8.0 Hz, Ar-C*H*), 9.59 (1H, br. s., NH/OH); *m/z* (ESI^-) 532 ($[M-H]^-$, 100 %); HRMS (ESI^-); $C_{23}H_{22}O_8N_3S_2$ $[M-H]^-$; found 532.08584, requires 532.08538.

6.4.8 Procedures for the synthesis of functionalized tetramates via cycloaddition

Methyl (2*RS*,5*R*)-2-(3-vinylphenyl)-1,3-thiazolidine-5-carboxylate, 242a

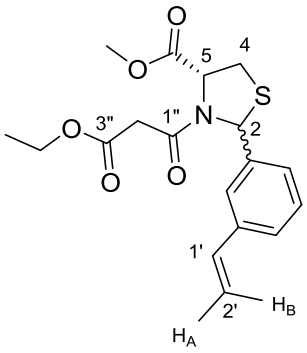
Thiazolidine **242a** was derived from the condensation of L-cysteine methyl ester hydrochloride and 3-vinylbenzaldehyde following the procedure for thiazolidine synthesis outlined in general procedure 6.2.3.



Yield (5.60 g, 84 %); colourless oil; 1.4:1 *cis* and *trans* diastereomers; R_f = 0.43 (EtOAc/petrol 1:4); ν_{max}/cm^{-1} (neat) 1737 (C=O), 3305 (N-H); δ_H (400 MHz, $CDCl_3$) major isomer (*cis*): 2.70 (1H, app t, *J* = 12.2, NH), 3.14 (1H, dd, *J* = 10.3, 9.1 Hz, $H4_A$), 3.49 (1H, dd, *J* = 10.3, 7.1 Hz, $H4_B$), 3.83 (3H, s, CO_2CH_3), 3.97 - 4.07 (1H, m, H5), 5.26 - 5.33 (1H, m, $H2'_A$), 5.58 (1H, d, *J* = 12.2 Hz, H2), 5.75 - 5.86 (1H, m, $H2'_B$), 6.69 - 6.79 (1H, m, H1'), 7.28 - 7.60 (4H, m, Ar-C*H*); minor isomer (*trans*): 2.89 (1H, br. s., NH), 3.23 (1H, dd, *J* = 10.8, 5.9 Hz, $H4_A$), 3.41 (1H, dd, *J* = 10.8, 7.1 Hz, $H4_B$), 3.82 (3H, s, CO_2CH_3), 4.20 - 4.28 (1H, m, H5), 5.26 - 5.33 (1H, m, $H2'_A$), 5.75 - 5.86 (2H, m, H2 + $H2'_B$), 6.69 - 6.79 (1H, m, H1'), 7.28 - 7.60 (4H, m, Ar-C*H*); δ_C (100.6 MHz, $CDCl_3$): major isomer (*cis*): 39.1 (C4), 52.5 (CO_2CH_3), 65.5 (C5), 72.4 (C2), 114.5 (C2'), 125.3, 126.4, 126.8, 128.8 (Ar-*C*H), 136.3 (C1'), 138.0, 138.3 (Ar-C), 171.5 (CO_2CH_3); minor isomer (*trans*): 38.0 (C4), 52.5 (CO_2CH_3), 64.2 (C5), 70.6 (C2), 114.2 (C2'), 124.8, 125.6, 126.3, 128.5 (Ar-*C*H), 136.5 (C1'), 137.7, 141.4 (Ar-C), 172.1 (CO_2CH_3); *m/z* (ESI^+) 250 ($[M+H]^+$ 100 %); HRMS (ESI^+); $C_{13}H_{16}O_2NS$ $[M+H]^+$; found 250.08899, requires 250.08963.

Methyl (2*RS*,5*R*)-1-(3-ethoxy-3-oxopropanoyl)-2-(3-vinylphenyl)-1,3-thiazolidine-5-carboxylate, 242b

N-Acylthiazolidine **242b** was derived from **242a** following the procedure for *N*-acylation outlined in general procedure 6.2.4.

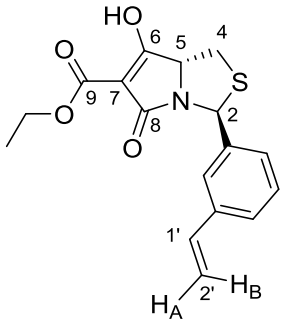


Yield (4.40 g, 96 %); colourless oil; 1.2:1 *cis* and *trans* diastereomers; R_f = 0.18 (EtOAc/petrol 1:3); ν_{max}/cm^{-1} (neat) 1661 (C=O), 1738 (C=O); δ_H (500 MHz, $CDCl_3$) major isomer (*cis*, a mixture of two conformers): 1.08 - 1.40 (3H, m, OCH_2CH_3), 3.08 - 3.59 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$), 3.81 and 3.85 (3H, s, CO_2CH_3), 4.06 - 4.34 (2H, m, OCH_2CH_3), 5.04 - 5.09 (1H, m, H5), 5.22 - 5.26 and 5.29 - 5.34 (1H, m, $H2'_A$), 5.76 (1H, app d, *J* = 17.5, $H2'_B$ minor conformer), 5.85 (1H, app d, *J* = 17.5, $H2'_B$ major conformer), 6.15 and 6.32 (1H, s, H2), 6.64 - 6.76 (1H, m, H1'), 7.03 - 7.89 (4H, m, Ar-C*H*); minor isomer (*trans*, a mixture of two conformers): 1.08 - 1.40 (3H, m, OCH_2CH_3), 3.08 - 3.59 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$, + $H2''_B$), 3.80 and 3.85 (3H, s, CO_2CH_3), 4.06 - 4.34 (2H, m, OCH_2CH_3), 5.22 - 5.26 and 5.29 - 5.34 (2H, m, H5 + $H2'_A$), 5.77 (1H, app d, *J* = 17.5, $H2'_B$ minor conformer), 5.78 (1H, app d, *J* = 17.5, $H2'_B$ major conformer), 6.18 and 6.37 (1H, s, H2), 6.64 - 6.76 (1H, m, H1'), 7.03 - 7.89 (4H, m, Ar-C*H*); δ_C (125.8 MHz, $CDCl_3$): major isomer (*cis*, a mixture of two conformers): 14.0 (OCH_2CH_3), 32.0, 33.0 (C4), 41.1, 42.1 (C2″), 52.7, 53.1 (CO_2CH_3), 61.6 (OCH_2CH_3), 63.8,64.7 (C5), 66.2, 67.0 (C2), 114.0, 115.0 (C2'), 122.6, 122.8, 124.0, 124.1, 124.2, 124.8, 125.5, 125.7, 126.0, 126.2, 126.5, 127.2, 128.1, 128.7, 129.2, 129.4 (Ar-*C*H), 136.2, 136.7 (C1'), 138.0, 138.3, 140.2, 143.3 (Ar-C), 165.0, 165.5 (C1"), 166.7, 167.5 (C3"), 170.1, 170.3 (CO_2CH_3); minor isomer (*trans*, a mixture of two conformers): 14.0 (OCH_2CH_3), 31.0, 33.0 (C4), 42.4, 43.3 (C2″), 52.8, 53.4 (CO_2CH_3), 61.5, 61.8 (OCH_2CH_3), 64.0, 64.2 (C5), 65.0, 65.6 (C2), 114.2, 115.2 (C2'), 122.6, 122.8, 124.0, 124.1, 124.2, 124.8, 125.5, 125.7, 126.0,

126.2, 126.5, 127.2, 128.1, 128.7, 129.2, 129.4 (Ar-*C*H), 136.0, 136.6 (C1'), 137.9, 138.6, 142.1, 142.2 (Ar-*C*), 164.5, 165.4 (C1''), 166.3, 167.3 (C3''), 169.1, 169.4 (CO_2CH_3); *m/z* (ESI^+) 364 ($[M+H]^+$ 20 %); HRMS (ESI^+); $C_{18}H_{22}O_5NS$ $[M+H]^+$; found 364.12079, requires 364.12132.

(-)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thia-2-(3-vinylphenyl)-bicyclo[3.3.0]oct-6-ene, 242

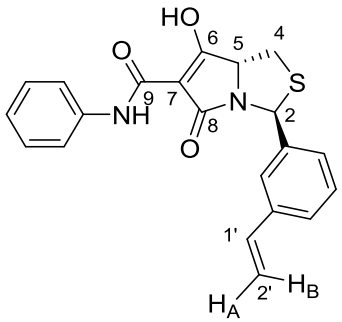
Tetramate **242** was obtained from **242b** following the procedure for Dieckmann cyclisation outlined in general procedure 6.2.5.2.



Yield (1.67 g, 55 %); yellow foaming solid, mp 84-88 °C; R_f = 0.41 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -231.7 (*c* = 1.0, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1616 (C=C), 1654 (C=O), 1704 (C=O); δ_H (400 MHz, CD_2Cl_2): 1.36 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 3.01 (1H, dd, *J* = 11.0, 8.3 Hz, $H4_A$), 3.30 (1H, dd, *J* = 11.0, 7.0 Hz, $H4_B$), 4.36 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.78 (1H, app t, *J* = 7.6 Hz, H5), 5.28 (1H, dd, *J* = 11.0, 0.7 Hz, $H2'_A$), 5.79 (1H, dd, *J* = 17.6, 0.7 Hz, $H2'_B$), 6.27 (1H, s, H2), 6.73 (1H, dd, *J* = 17.6, 11.0 Hz, H1'), 7.29 - 7.50 (4H, m, Ar-C*H*); δ_C (100.6 MHz, CD_2Cl_2): 14.6 (OCH_2CH_3), 33.4 (C4), 62.0 (OCH_2CH_3), 62.7 (C2), 66.3 (C5), 99.0 (C7), 114.9 (C2′), 124.6, 126.2, 129.3, (Ar-*C*H), 137.0 (C1'), 138.5, 141.5 (Ar-C), 167.3 (C9), 169.8 (C8), 186.8 (C6); *m/z* (ESI^-) 330 ($[M-H]^-$, 100 %); HRMS (ESI^-); $C_{17}H_{16}O_4NS$ $[M-H]^-$; found 330.08071, requires 330.08055.

(-)-(2*S*,5*R*)-1-Aza-6-hydroxy-8-oxo-7-phenylaminocarbonyl-3-thia-2-(3-vinylphenyl)-bicyclo[3.3.0]oct-6-ene, 243a

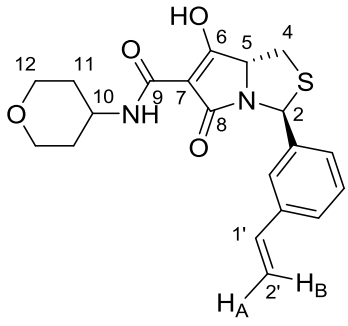
Carboxamide tetramate **243a** was obtained by aminolysis of **242** with aniline according to the general procedure 6.3.1 for the synthesis of carboxamides with THF/toluene as solvent.



Yield (0.89 g, 92 %); brown foam, mp 80-84 °C; R_f = 0.60 (EtOAc/MeOH 98:2); $[\alpha]_D^{25}$ = -146.0 (*c* = 0.2, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1633 (C=C), 1650 (C=O), 1690 (C=O), 3278 (N-H/O-H); δ_H (400 MHz, CD_2Cl_2): 3.06 (1H, dd, *J* = 11.1, 8.7 Hz, $H4_A$), 3.34 (1H, dd, *J* = 11.1, 6.9 Hz, $H4_B$), 4.92 (1H, dd, *J* = 8.7, 6.9 Hz, H5), 5.29 (1H, dd, *J* = 11.0, 0.7 Hz, $H2'_A$), 5.80 (1H, dd, *J* = 17.6, 0.7 Hz, $H2'_B$), 6.26 (1H, s, H2), 6.74 (1H, dd, *J* = 17.6, 11.0 Hz, H1'), 7.14 - 7.19 (1H, m, Ar-C*H*), 7.32- 7.41 (5H, m, Ar-C*H*), 7.49 - 7.54 (1H, m, Ar-C*H*), 7.55 - 7.61 (2H, m, Ar-C*H*), 9.34 (1H, br. s., NH/OH); δ_C (100.6 MHz, CD_2Cl_2): 32.9 (C4), 62.1 (C2), 67.1 (C5), 99.5 (C7), 115.0 (C2′), 120.8, 124.7, 125.4, 126.3, 126.4, 129.4, 129.6 (Ar-*C*H), 136.9 (C1'), 137.2, 138.6, 141.1 (Ar-*C*), 164.4 (C9), 172.1 (C8), 184.8 (C6); *m/z* (ESI^-) 377 ($[M-H]^-$, 100 %); HRMS (ESI^-); $C_{21}H_{17}O_3N_2S$ $[M-H]^-$; found 377.09604, requires 377.09654.

(-)-(2*S*,5*R*)-1-Aza-6-hydroxy-8-oxo-7-(tetrahydro-2*H*-pyran-4-aminocarbonyl)-3-thia-2-(3-vinylphenyl)-bicyclo[3.3.0]oct-6-ene, 243b

Carboxamide tetramate **243b** was obtained by aminolysis of **242** with 4-aminotetrahydropyran according to the general procedure 6.3.1 for the synthesis of carboxamides with THF/toluene as solvent.

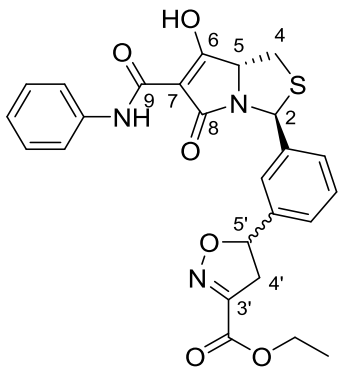


Yield (0.23 g, 66 %); yellow solid, mp 64-66 °C; R_f = 0.53 (EtOAc/MeOH 12:1); $[\alpha]_D^{25}$ = -205.4 (*c* = 0.74, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1622 (C=C), 1646 (C=O), 1687 (C=O), 3322 (N-H/O-H); δ_H (400 MHz, CD_2Cl_2): 1.52 - 1.67 (2H, m, H11), 1.84 - 1.96 (2H, m, H11), 3.01 (1H, dd, *J* = 11.0, 8.3 Hz, $H4_A$), 3.28 (1H, dd, *J* = 11.0, 7.1 Hz, $H4_B$), 3.47 (2H, app tt, *J* = 11.5, 2.1 Hz, H12), 3.89 - 3.97 (2H, m, H12), 4.00 - 4.11 (1H, m, H10), 4.74 (1H, br. signal, H5), 5.28 (1H, dd, *J* = 11.0, 0.7 Hz, $H2'_A$), 5.79 (1H, dd, *J* = 17.6, 0.7 Hz, $H2'_B$), 6.24 (1H, s, H2), 6.73 (1H, dd, *J* = 17.6, 11.0 Hz, H1'), 7.29

- 7.51 (4H, m, Ar-C*H*), 8.30 (2H, br. s., NH + OH); δ_C (125.8 MHz, CD_2Cl_2): 33.1 (C4), 33.3 (C11), 46.1 (C10), 62.4 (C2), 66.8 (C12), 67.9 (C5), 97.5 (C7), 114.9 (C2′), 124.6, 126.2, 126.3, 129.4 (Ar-*C*H), 136.9 (C1'), 138.5, 141.6 (Ar-C), 166.0 (C9), 172.7 (C8), 186.4 (C6); *m/z* (ESI^-) 385 ($[M-H]^-$, 100 %); HRMS (ESI^-); $C_{20}H_{21}O_4N_2S$ $[M-H]^-$; found 385.12290, requires 385.12275.

(2*S*,5*R*,5'*RS*)-1-Aza-2-(3-(3'-ethoxycarbonyl-4',5'-dihydroisoxazole)phenyl)-6-hydroxy-8-oxo-7-phenylaminocarbonyl-3-thiabicyclo[3.3.0]oct-6-ene, 244

The synthesis of **244** was according to a modified literature procedure.[120] Ethyl 2-chloro-2(hydroxyimino)acetate (0.88 g, 5.82 mmol, 3 eq) and **243a** (0.73 g, 1.94 mmol, 1.0 eq) were mixed in CH_2Cl_2 (4 mL) and cooled to 0 °C. Et_3N (0.95 mL, 6.79 mmol, 3.5 eq) was added slowly and the mixture was stirred at rt for 72 h until complete conversion to product was observed. The reaction mixture was diluted with EtOAc and H_2O and the organic fraction was separated. The aqueous layer was re-extracted with EtOAc and the combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification of the crude residue by silica gel flash column chromatography (eluent: EtOAc/petrol to EtOAc/MEOH/1% Et_3N) afforded **244**. The product was dissolved in CH_2Cl_2 and washed with 5 % citric acid. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to obtain **244**.



Yield (0.85 g, 89 %); yellow foam; 1:1 diastereomers; R_f = 0.68 (EtOAc/MeOH 96:4); ν_{max}/cm^{-1} (neat) 1655 (C=O), 1693 (C=O), 1723 (C=O), 3280 (N-H/O-H); δ_H (500 MHz, $CDCl_3$): 1.33 - 1.42 (6H, m, 2 x OCH_2CH_3), 3.06 (2H, app d, *J* = 14.7 Hz, 2 x $H4_A$), 3.19 - 3.27 (2H, m, 2 x $H4'_A$), 3.67 (1H, app d, *J* = 11.5 Hz, $H4'_B$), 3.71 (1H, app d, *J* = 11.5 Hz, $H4'_B$), 4.32 - 4.41 (4H, m, 2 x OCH_2CH_3), 4.50 (2H, br. signal, 2 x H5), 4.57 - 4.65 (2H, m, 2 x $H4_B$), 5.78 - 5.85 (2H, m,

2 x H5'), 6.26 (2H, br. s., NH/OH), 6.94- 7.00 (2H, m, 2 x H2), 7.15 - 7.22 (2H, m, Ar-C*H*), 7.35 - 7.41 (6H, m, Ar-C*H*), 7.45 - 7.50 (6H, m, Ar-C*H*), 7.59 - 7.65 (4H, m, Ar-C*H*), 9.41 (2H, br. s., NH/OH); δ_C (125.8 MHz, $CDCl_3$): 13.9, 14.10 ($OCH_2\mathit{C}H_3$), 33.6 (C4), 41.6 (C4'), 57.3 (C5), 62.3, 63.6 ($O\mathit{C}H_2CH_3$), 82.1 (C2), 84.5 (C5'), 98.9 (C7), 120.4, 123.4, 125.2, 126.2, 126.7, 129.2, 129.8 (Ar-*C*H), 136.0, 136.5, 140.7 (Ar-C), 151.1, 151.2 (*C*=N), 160.4 ($\mathit{C}O_2CH_2CH_3$), 163.4 (C9), 168.7 (C8), 182.6 (C6); *m/z* (ESI^-) 492 ($[M-H]^-$, 50 %); HRMS (ESI^-); $C_{25}H_{22}O_6N_3S$ $[M-H]^-$; found 492.12299, requires 492.12348.

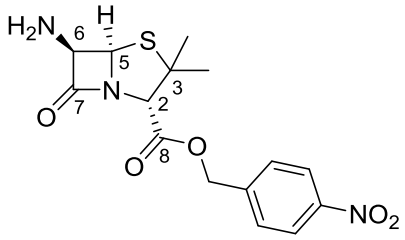
Attempted synthesis of tetramate **246** via Ru-catalysed azide-alkyne cycloaddition (RuAAC)

Cp*RuCl(COD) (1 mg, 3 μmol, 2 mol%) was added to a reaction flask. The flask was sealed and flushed with N_2 three times. THF (1.5 mL) was added followed by tetramate **220** (72 mg, 0.15 mmol, 1 eq) and benzyl azide (0.5 M in CH_2Cl_2, 0.3 mL, 0.15 mmol, 1 eq). The reaction mixture was stirred at rt and monitored by LRMS and TLC. After a reaction time of 24 h, no product was observed. On column purification of the crude, starting material was isolated.

6.4.9 Procedures for the synthesis of tetramic acid-penicillanic acid hybrids

(+)-4-Nitrobenzyl (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate, 247

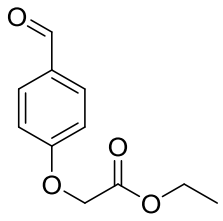
The synthesis of **247** was according to a modified literature procedure.[189] 6-Aminopenicillanic acid (2.0 g, 9.3 mmol, 1.0 eq) in acetone (6 mL) was cooled to 0 °C and treated with Et_3N (2.62 mL, 18.8 mmol, 2.02 eq). The mixture was stirred for 30 min at 0 °C and *p*-nitrobenzylbromide (2.2 g, 10.2 mmol, 1.1 eq) was added portionwise. The resulting reaction mixture was stirred at 0 °C for 4 h before being poured into Et_2O. The precipitate was removed by filtration and the solvents were removed *in vacuo* to obtain the crude. Purification of the crude by silica gel flash column chromatography (eluent: EtOAc/petrol) afforded **247**.



Yield (1.82 g, 56 %); yellow oil; R_f = 0.20 (EtOAc/petrol 1:1); $[\alpha]_D^{25}$ = +163.4 (*c* = 0.84, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1745 (C-O), 1773 (C=O), 3333, 3391 (N-H); δ_H (500 MHz, $CDCl_3$): 1.44 (3H, s, C*H*$_3$), 1.63 (3H, s, C*H*$_3$), 1.81 (2H, br. s, NH), 4.45 (1H, s, H2), 4.58 (1H, br. signal, H6), 5.25 (1H, d, *J* = 13.1 Hz, OC*H*$_2$), 5.29 (1H, d, *J* = 13.1 Hz, OC*H*$_2$), 5.50 (1H, d, *J* = 4.3 Hz, H5), 7.54 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 8.23 (2H, d, *J* = 8.8 Hz, Ar-C*H*); δ_C (125.8 MHz, $CDCl_3$): 27.0 (*C*H$_3$), 31.6 (*C*H$_3$), 62.8 (C6), 63.8 (C3), 65.7 (O*C*H$_2$), 69.9 (C2), 70.0 (C5), 123.9, 128.9 (Ar-*C*H), 141.8, 147.9 (Ar-C), 167.7 (C8), 177.8 (C7); *m/z* (ESI^+) 352 ($[M+H]^+$, 87 %); HRMS (ESI^+); $C_{15}H_{18}O_5N_3S$ $[M+H]^+$; found 352.09580, requires 352.09617.

Ethyl 2-(4-formylphenoxy)acetate, 248a[190]

Aldehyde 248a was synthesised according to a literature procedure.[190] A solution of 4-hydroxybenzaldehyde (3.95 g, 32.3 mmol, 1.0 eq) in acetonitrile (15 mL) was treated with powdered K_2CO_3 (6.7 g, 48.5 mmol, 1.5 eq) and NaI (0.73 g, 4.85 mmol, 0.15 eq). The mixture was heated at reflux under N_2 for 30 min. Ethyl bromoacetate (4.48 mL, 40.4 mmol, 1.25 eq) was added dropwise and the reaction mixture was heated at reflux for a further 18 h. The reaction mixture was cooled to rt and diluted with water and CH_2Cl_2. The organic phase was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by silica gel flash column chromatography afforded **248a**.

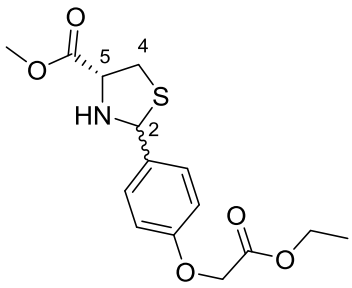


Yield (5.07 g, 75 %); colourless oil; R_f = 0.48 (EtOAc/petrol 1:3); ν_{max}/cm^{-1} (neat) 1687 (C=O), 1752 (C=O); δ_H (400 MHz, $CDCl_3$) 1.29 (3H, t, *J* = 7.2 Hz, OCH$_2$C*H*$_3$), 4.27 (2H, q, *J* = 7.2 Hz, OC*H*$_2$CH$_3$), 4.70 (2H, s, PhOC*H*$_2$), 7.00 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.83 (2H, d, *J* = 8.8 Hz, Ar-

C*H*), 9.88 (1H, s, C*H*O); δ_C (125.8 MHz, $CDCl_3$): 14.1 ($OCH_2\mathit{C}H_3$), 61.6 ($O\mathit{C}H_2CH_3$), 65.1 ($PhO\mathit{C}H_2$), 114.8.2, 131.9 (Ar-*C*H), 130.7, 162.5 (Ar-C), 168.0 ($\mathit{C}O_2CH_2CH_3$), 190.6 (*C*HO); *m/z* (ESI^+) 209 ($[M+H]^+$, 15 %); HRMS (ESI^+); $C_{11}H_{13}O_4$ $[M+H]^+$; found 209.08122, requires 209.08084.

Methyl (2*RS*,5*R*)-2-(4-(2-ethoxy-2-oxoethoxy)phenyl)-1,3-thiazolidine-5-carboxylate, 248b

Thiazolidine **248b** was derived from the condensation of L-cysteine methyl ester hydrochloride and aldehyde **248a** following the procedure for thiazolidine synthesis outlined in general procedure 6.2.3.

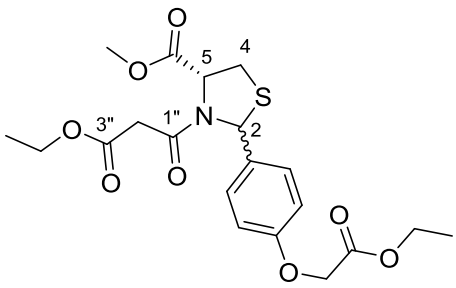


Yield (6.52 g, 83 %); colourless oil; 2:1 *cis* and *trans* diastereomers; R_f = 0.25 (EtOAc/petrol 1:2); ν_{max}/cm^{-1} (neat) 1735 (C=O), 3306 (N-H); δ_H (400 MHz, $CDCl_3$) major isomer (*cis*): 1.25 - 1.33 ($OCH_2C\mathit{H}_3$), 2.45 - 2.83 (1H, m, NH), 3.10 (1H, dd, *J* = 10.3, 9.1 Hz, $H4_A$), 3.45 (1H, dd, *J* = 10.3, 7.1 Hz, $H4_B$), 3.80 (3H, s, $CO_2C\mathit{H}_3$), 3.96 (1H, app t, *J* = 8.0 Hz, H5), 4.18 - 4.30 (2H, m, $OC\mathit{H}_2CH_3$), 4.62 ($PhOC\mathit{H}_2$), 5.51 (1H, s, H2), 6.90 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.46 (2H, d, *J* = 8.8 Hz, Ar-C*H*); minor isomer (*trans*): 1.25 - 1.33 ($OCH_2C\mathit{H}_3$), 2.45 - 2.83 (1H, m, NH), 3.21 (1H, dd, *J* = 10.6, 5.6 Hz, $H4_A$), 3.38 (1H, dd, *J* = 10.6, 7.2 Hz, $H4_B$), 3.78 (3H, s, $CO_2C\mathit{H}_3$), 4.18 - 4.30 (3H, m, $OC\mathit{H}_2CH_3$ + H5), 4.60 ($PhOC\mathit{H}_2$), 5.76 (1H, s, H2), 6.86 (2H, d, *J* = 8.8 Hz, Ar-C*H*), 7.42 (2H, d, *J* = 8.8 Hz, Ar-C*H*); δ_C (100.6 MHz, $CDCl_3$): major isomer (*cis*): 14.1 ($OCH_2\mathit{C}H_3$), 39.2 (C4), 52.6 ($CO_2\mathit{C}H_3$), 61.4 ($O\mathit{C}H_2CH_3$), 65.4 (C5), 65.4 ($PhO\mathit{C}H_2$), 72.1 (C2), 114.7, 128.8 (Ar-C*H*), 131.2, 158.0 (Ar-C), 168.7 ($\mathit{C}O_2CH_2CH_3$), 171.5 ($\mathit{C}O_2CH_3$); minor isomer (*trans*): 14.1 ($OCH_2\mathit{C}H_3$), 38.0 (C4), 52.5 ($CO_2\mathit{C}H_3$), 61.3 ($O\mathit{C}H_2CH_3$), 64.1 (C5), 65.4 ($PhO\mathit{C}H_2$), 70.3 (C2), 114.5, 128.3 (Ar-C*H*), 134.1, 157.5 (Ar-C), 168.7

($CO_2CH_2CH_3$), 172.2 (CO_2CH_3); *m/z* (ESI^+) 326 ($[M+H]^+$, 100%); HRMS (ESI^+); $C_{15}H_{20}NO_5S$ $[M+H]^+$; found 326.10535, requires 326.10567.

Methyl (2*RS*,5*R*)-2-(4-(2-ethoxy-2-oxoethoxy)phenyl)-1-(3-ethoxy-3-oxopropanoyl)-1,3-thiazolidine-5-carboxylate, 248c

N-Acylthiazolidine **248c** was derived from **248b** following the procedure for *N*-acylation outlined in general procedure 6.2.4.

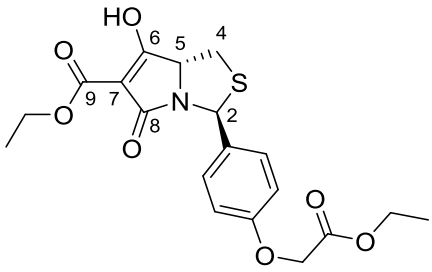


Yield (7.59 g, 86 %); colourless oil; 1.8:1 *cis* and *trans* diastereomers; R_f = 0.20 (EtOAc/petrol; 2:3); ν_{max}/cm^{-1} (neat) 1660 (C=O), 1734 (C=O); δ_H (400 MHz, $CDCl_3$): major isomer (*cis*, mixture of two conformers): 1.18 - 1.33 (6H, m, 2 x OCH_2CH_3), 3.06 - 3.56 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + H2″), 3.79 (3H, m, CO_2CH_3 minor conformer) and 3.82 (3H, m, CO_2CH_3 major conformer), 4.04 - 4.16 (2H, m, OCH_2CH_3), 4.27 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.57 (2H, s, $PhOCH_2$ minor conformer) and 4.61 (2H, s, $PhOCH_2$ major conformer), 5.05 (1H, app t, *J* = 6.7 Hz, H5), 6.10 (1H, s, H2 major conformer) and 6.31 (1H, s, H2 minor conformer), 6.80 - 6.85 (2H, m, Ar-CH minor conformer), 6.86 - 6.93 (2H, m, Ar-CH major conformer), 7.45 (2H, d, *J* = 8.8 Hz, Ar-CH minor conformer), 7.59 (2H, d, *J* = 8.6 Hz, Ar-CH major conformer); minor isomer (*trans*, mixture of conformers): 1.18 - 1.33 (6H, m, 2 x OCH_2CH_3), 3.06 - 3.56 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + H2″), 3.78 (3H, m, CO_2CH_3 major conformer) and 3.83 (3H, m, CO_2CH_3 minor conformer), 4.04 - 4.16 (2H, m, OCH_2CH_3), 4.27 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.57 (2H, s, $PhOCH_2$ minor conformer) and 4.61 (2H, s, $PhOCH_2$ major conformer), 5.17 (1H, app d, *J* = 5.6 Hz, H5 minor conformer), 5.27 (1H, app d, *J* = 5.9 Hz, H5 major conformer), 6.14 (1H, s, H2 major conformer) and 6.29 (1H, s, H2 minor conformer), 6.80 - 6.85 (2H, m, Ar-CH minor conformer), 6.86 - 6.93 (2H, m, Ar-CH major conformer), 7.14 (2H, d, *J* = 8.6 Hz, Ar-CH major conformer), 7.21 (2H, d, *J* = 8.6 Hz, Ar-

C*H* minor conformer); δ_C (125.8 MHz, $CDCl_3$) major isomer (*cis*, mixture of two conformers): 14.0, 14.1 (OCH_2CH_3), 32.0, 33.7 (C4), 42.1, 42.9 (C2″), 52.7, 53.4 (CO_2CH_3), 61.4, 61.5 (OCH_2CH_3), 63.6, 64.6 (C5), 65.3, 65.4 ($PhOCH_2$), 65.9, 66.5 (C2), 114.7, 115.0, 127.9, 128.7 (Ar-*C*H), 131.8, 132.7, 157.5, 157.9 (Ar-C), 164.5, 165.5 (C1″), 166.7, 167.1 (C3″), 168.6, 168.8 ($CO_2CH_2CH_3$), 170.2, 171.6 (CO_2CH_3); minor isomer (*trans*, mixture of two conformers): 13.9, 14.1 (OCH_2CH_3), 30.9, 33.0 (C4), 42.3, 43.3 (C2″), 52.7, 53.4 (CO_2CH_3), 61.4, 61.5 (OCH_2CH_3), 63.9, 64.2 (C5), 64.5, 65.3 (C2), 65.3, 65.5 ($PhOCH_2$), 114.3, 115.2, 126.1, 126.3 (Ar-*C*H), 134.8, 135.3, 157.2, 157.8 (Ar-C), 164.9, 165.3 (C1″), 166.2, 167.2 (C3″), 168.5, 168.8 ($CO_2CH_2CH_3$), 169.4, 170.2 (CO_2CH_3); *m/z* (ESI^+) 440 ($[M+H]^+$, 56 %); HRMS (ESI^+); $C_{20}H_{26}NO_8S$ $[M+H]^+$; found 440.13712, requires 440.13736.

(-)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-2-(4-(2-ethoxy-2-oxoethoxy)phenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 248

Tetramate **248** was obtained from **248c** following the procedure for Dieckmann cyclisation outlined in general procedure 6.2.5.2.

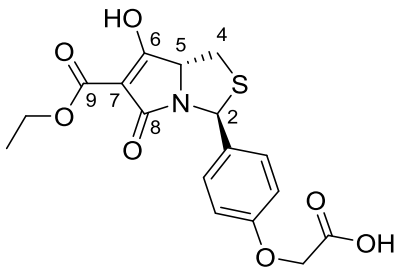


Yield (1.85 g, 30 %); yellow oil; R_f = 0.25 (EtOAc/MeOH 9:1); $[\alpha]_D^{25}$ = -136.6, (*c* = 0.67, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1610 (C=C), 1657 (C=O), 1710 (C=O, br with shoulder towards higher wavenumber); δ_H (400 MHz, CD_2Cl_2): 1.28 (3H, t, *J* = 7.1 Hz, $PhOCH_2CO_2CH_2CH_3$), 1.36 (3H, t, *J* = 7.2 Hz, OCH_2CH_3), 2.99 (1H, dd, *J* = 11.2, 8.2 Hz, $H4_A$), 3.29 (1H, dd, *J* = 11.2, 7.1 Hz, $H4_B$), 4.23 (2H, q, *J* = 7.1 Hz, $PhOCH_2CO_2CH_2CH_3$), 4.37 (2H, q, *J* = 7.2 Hz, OCH_2CH_3), 4.62 (2H, s, $PhOCH_2$), 4.78 (1H, app t, *J* = 7.7 Hz, H5), 6.23 (1H, s, H2), 6.87 (2H, d, *J* = 8.7 Hz, Ar-C*H*), 7.39 (2H, d, *J* = 8.7 Hz, Ar-C*H*); δ_C (125.8 MHz, CD_2Cl_2): 14.5 ($PhOCH_2CO_2CH_2CH_3$), 14.6 (OCH_2CH_3), 33.4 (C4), 61.4 (OCH_2CH_3), 61.6 ($PhOCH_2CO_2CH_2CH_3$), 62.9 (C2), 65.9 ($PhOCH_2$), 66.5 (C5), 99.4 (C7), 115.4, 128.7 (Ar-*C*H), 135.1, 158.8 (Ar-C), 166.3 (C9), 169.4

($PhOCH_2CO_2CH_2CH_3$), 169.7 (C8), 185.9 (C6); *m/z* (ESI^-) 406 ($[M-H]^-$, 100 %); HRMS (ESI^-); $C_{19}H_{20}NO_7S$ $[M-H]^-$; found 406.09679, requires 406.09660.

(-)-(2*S*,5*R*)-1-Aza-2-(4-(carboxymethoxy)phenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 249

To tetramate **248** (1.13 g, 2.79 mmol, 1.0 eq) dissolved in THF:H_2O (1:1) was added LiOH.H_2O (0.23 g, 5.58 mmol, 2 eq) and the reaction mixture stirred at rt for 1 h. Upon completion, the aqueous layer was acidifeid with 10 % HCl and extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to give **249**.



Yield (0.95 g, 90 %); yellow solid, mp 110-112 °C; R_f = 0.075 (EtOAc/MeOH 3:1); $[\alpha]_D^{25}$ = -157.3 (*c* = 0.30, MeOH); ν_{max}/cm^{-1} (neat) 1609 (C=C), 1660 (C=O), 1708 (C=O), 2980 (C(O)O-H); δ_H (400 MHz, Methanol-d_4): 1.31 (3H, t, *J* = 7.2 Hz, OCH_2CH_3), 2.99 (1H, dd, *J* = 11.0, 8.1 Hz, $H4_A$), 3.26 - 3.33 (1H, m, $H4_B$, obscured by solvent peak), 4.28 (2H, q, *J* = 7.2 Hz, OCH_2CH_3), 4.66 (2H, s, $PhOCH_2$), 4.78 (1H, app t, *J* = 7.6 Hz, H5), 6.21 (1H, s, H2), 6.91 (2H, d, *J* = 8.7 Hz, Ar-C*H*), 7.40 (2H, d, *J* = 8.7 Hz, Ar-C*H*); δ_C (125.8 MHz, CD_2Cl_2): 14.8 (OCH_2CH_3), 33.9 (C4), 61.4 (OCH_2CH_3), 63.5 (C2), 66.0 ($PhOCH_2$), 68.1 (C5), 98.3 (C7), 115.7, 129.0 (Ar-*C*H), 135.3, 159.3 (Ar-C), 165.4 (C9), 172.7 ($PhOCH_2CO_2H$), 174.2 (C8), 186.6 (C6); *m/z* (ESI^-) 378 ($[M-H]^-$, 100 %); HRMS (ESI^-); $C_{17}H_{16}NO_7S$ $[M-H]^-$; found 378.06556, requires 378.06530.

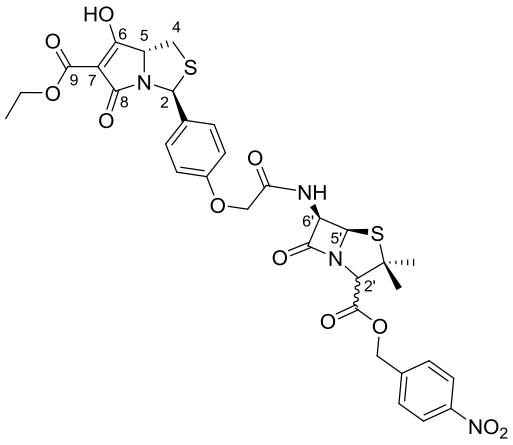
Procedures for tetramic acid-penicillanic acid coupling

Procedure 1: Tetramic acid **249** (0.54 g, 1.42 mmol, 1.01 eq) was added to a stirred solution of **247** (0.49 g, 1.40 mmol, 1.0 eq), DCC (0.32 g, 1.54 mmol, 1.1 eq) and DMAP (17 mg, 0.14

mmol, 0.1 eq) in CH_2Cl_2:DMF (9:1, 7 mL) at 0 °C. The mixture was stirred at 0 °C for 15 min and then at rt for 15 h. The reaction mixture was filtered to remove dicyclohexylurea and the residue was washed with CH_2Cl_2. The combined filtrates were concentrated *in vacuo* and purified by silica gel flash column chromatography (eluent: EtOAc/Methanol/1 % Et_3N). The product isolated was dissolved in CH_2Cl_2 and washed with 5 % citric acid. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield **250** as a mixture of four inseparable diastereomers.

Procedure 2[138]: Tetramic acid **249** (25 mg, 66 μmol, 1.0 eq), amine **247** (25 mg, 73 μmol, 1.1 eq), pyridine (18.2 μL, 0.22 mmol, 3.4 eq) and EtOAc were placed in a sealed round-bottomed flask. The mixture was cooled between -10 °C and -20 °C and T3P (50 wt% in DMF, 0.084 mL, 0.13 mmol, 2 eq) was added by syringe. The resulting solution was stirred at 0 °C for 15 h. The reaction mixture was poured into sat. $NaHCO_3$ and extracted twice with CH_2Cl_2. The combined organic fractions were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (eluent: EtOAc/Methanol/1 % Et_3N). The product isolated was dissolved in CH_2Cl_2 and washed with 5 % citric acid. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield **250** as a mixture of four inseparable diastereomers.

(2*S*,5*R*)-1-Aza-2-(4-((((2'*RS*,5'*R*,6'*R*)-3',3'-dimethyl-2'-(((4-nitrophenyl)methoxy)cabonyl)-7'-oxo-4'-thia-1'-azabicyclo[3.2.0]heptan-6'-yl)amino)-2-oxoethoxy)phenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 250



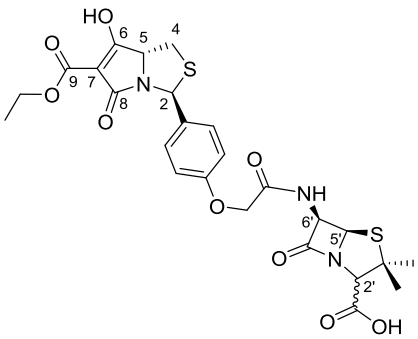
Yield (0.38 g, 38 %); yellow foam; R_f = 0.49 (EtOAc/MeOH 9:1); ν_{max}/cm^{-1} (neat) 1609 (C=C), 1660 (C=O, br with shoulder towards smaller wavenumber), 1739 (C=O), 1783 (C=O), 3335 (NH/OH); Characterization is reported for the two major diastereomers 250a (2'*S*) and 250c (2'*R*) obtained from procedure 1.

δ_H (400 MHz, CD_2Cl_2) Diastereomer 250a: 1.36 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 1.44 (3H, s, CH_3), 1.58 (3H, s, CH_3), 2.96 - 3.04 (1H, m, H4), 3.25 - 3.32 (1H, m, H4), 3.74 (1H, s, H2'), 4.37 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.53 - 4.57 (2H, m, OCH_2-*p*NB), 4.72 - 4.79 (1H, m, H5), 5.26 - 5.30 (2H, m, $PhOCH_2$), 5.59 (1H, d, *J* = 4.2 Hz, H5'), 5.72 (1H, dd, *J* = 9.2, 4.2 Hz, H6'), 6.23 (1H, s, H2), 6.90 - 6.96 (2H, m, Ar-C*H*), 7.28 (1H, d, *J* = 9.2 Hz, NH), 7.36 - 7.45 (2H, m, Ar-C*H*), 7.52 - 7.61 (2H, m, Ar-C*H*), 8.18 - 8.26 (2H, m, Ar-C*H*); δ_C (125.8 MHz, CD_2Cl_2): 14.5 (OCH_2CH_3), 27.2, 32.5 (CH_3), 33.4 (C4), 58.8 (C6'), 59.5 (C3'), 62.3 (OCH_2CH_3), 62.4 (C2), 65.9 (C5), 66.3 ($PhOCH_2$), 68.1 (OCH_2-*p*NB), 68.4 (C5'), 73.5 (C2'), 99.7 (C7), 115.4, 124.4, 128.6, 129.5 (Ar-*C*H), 134.8, 142.6, 148.5, 157.4 (Ar-C), 167.6 (C9), 168.9 (C8), 169.6 (CO_2*p*NB), 170.6 (C7'), 173.5 ($PhOCH_2CONH$-), 186.5 (C6); Diastereomer 250b: 1.13 (3H, s, CH_3), 1.36 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 1.47 (3H, s, CH_3), 2.96 - 3.04 (1H, m, H4), 3.25 - 3.32 (1H, m, H4), 3.74 (1H, s, H2'), 4.37 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 4.53 - 4.57 (2H, m, OCH_2-*p*NB), 4.67 (1H, dd, *J* = 9.2, 4.3 Hz, H6'), 4.72 - 4.79 (1H, m, H5), 5.17 (1H, d, *J* = 4.3 Hz, H5'), 5.26 - 5.30 (2H, m, $PhOCH_2$), 6.20 (1H, s, H2), 6.90 - 6.96 (2H, m, Ar-C*H*), 7.16 (1H, d, *J* = 9.2 Hz, NH), 7.36 - 7.45 (2H, m, Ar-C*H*), 7.52 - 7.61 (2H, m, Ar-C*H*), 8.18 - 8.26 (2H, m, Ar-C*H*); δ_C (125.8 MHz, CD_2Cl_2): 14.5 (OCH_2CH_3), 27.0, 27.2

(*C*H_3), 33.4 (C4), 53.2 (C2'), 57.1 (C6'), 59.5 (C3'), 62.3 (O*C*H_2CH$_3$), 62.4 (C2), 65.9 (C5), 66.3 (PhO*C*H_2), 66.8 (C5'), 68.1 (O*C*H_2-*p*NB), 99.7 (C7), 115.3, 124.4, 128.6, 129.7 (Ar-*C*H), 134.8, 142.8, 148.5, 157.6 (Ar-C), 167.6 (C9), 168.9 (C8), 169.6 (*C*O_2*p*NB), 170.6 (C7'), 173.5 (PhOCH$_2$*C*ONH-), 186.5 (C6); *m/z* (ESI$^-$) 711 ([M-H]$^-$, 43 %); HRMS (ESI$^-$); $C_{32}H_{31}N_4O_{11}S_2$ [M-H]$^-$; found 711.14417, requires 711.14362.

(2'*RS*,5'*R*,6'*R*)-6'-(2-(4-((2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-en-2-yl)phenoxy)acetamido)-3',3'-dimethyl-7'-oxo-4'-thia-1'-azabicyclo[3.2.0]heptane-2'-carboxylic acid, 251

250 (0.1 g, 0.14 mmol, 1.0 eq) was dissolved in a solution of $NaHCO_3$ (0.1 M, 2.8 mL, 0.28 mmol, 2 eq) in THF (4 mL). 10 % Pd/C (10 mg) was then added and the mixture hydrogenated at rt for 2 h at 1 atm. The suspension was filtered through a Celite pad and the solids were rinsed with water. The combined filtrate was extracted with Et_2O and the aqueous layer was concentrated *in vacuo* to yield **251**.



Yield (22 mg, 27 %); yellow foam, R_f = 0.09 (EtOAc/MeOH 3:1); ν_{max}/cm^{-1} (neat) 1581 (br peak extending to higher wavenumber), 1737 (C=O), 3306 (C(O)O-H); Characterization reported for the two major diastereomers 251a (2'*S*) and 251c (2'*R*).

δ_H (500 MHz, D_2O): Diastereomer 251a: 1.24 (3H, t, *J* = 7.1 Hz, OCH$_2$C*H*$_3$), 1.50 (3H, s, C*H*$_3$), 1.56 (3H, s, C*H*$_3$), 2.93 - 3.00 (1H, m, H4), 3.19 - 3.27 (1H, m, H4), 3.46 (1H, s, H2'), 4.11 - 4.18 (2H, m, OC*H*$_2$CH$_3$), 4.30 (1H, d, *J* = 6.6 Hz, H6'), 4.38 - 4.46 (1H, m, H5), 4.65 - 4.72 (2H, m, PhOC*H*$_2$), 5.04 (1H, d, *J* = 6.6 Hz, H5'), 6.29 (1H, s, H2), 6.95 - 7.08 (2H, m, Ar-C*H*), 7.40 - 7.46 (2H, m, Ar-C*H*); δ_C (125.8 MHz, D_2O): 13.7 (OCH$_2$*C*H$_3$), 26.4, 26.7 (C*H*$_3$), 32.7 (C4), 59.1 (C3'), 59.6 (C6'), 59.7 (O*C*H$_2$CH$_3$), 62.6 (C2), 66.0 (C5'), 66.7

(PhOC*H*$_2$), 69.1 (C5), 74.9 (C2'), 91.1 (C7), 115.0, 127.5 (Ar-*C*H), 134.9, 156.8 (Ar-C), 166.2 (C9), 171.4, 171.5, 175.4 (PhOCH$_2$*C*ONH + *C*O$_2$H + C7'), 178.7 (C8), 194.5 (C6); δ_H (500 MHz, D$_2$O): Diastereomer 251b: 1.20 (3H, s, C*H*$_3$), 1.24 (3H, t, *J* = 7.1 Hz, OCH$_2$C*H*$_3$), 1.51 (3H, s, C*H*$_3$), 2.93 - 3.00 (1H, m, H4), 3.19 - 3.27 (1H, m, H4), 3.70 (1H, s, H2'), 4.11 - 4.18 (2H, m, OC*H*$_2$CH$_3$), 4.38 - 4.46 (1H, m, H5), 4.63 (1H, d, *J* = 6.6 Hz, H6'), 4.65 - 4.72 (2H, m, PhOC*H*$_2$), 5.11 (1H, d, *J* = 6.6 Hz, H5'), 6.27 (1H, s, H2), 6.95 - 7.08 (2H, m, Ar-C*H*), 7.40 - 7.46 (2H, m, Ar-C*H*); δ_C (125.8 MHz, D$_2$O): 13.7 (OCH$_2$*C*H$_3$), 26.4, 26.7 (C*H*$_3$), 32.8 (C4), 53.1 (C2'), 57.5 (C6'), 59.1 (C3'), 59.7 (O*C*H$_2$CH$_3$), 62.5 (C2), 64.7 (C5'), 66.6 (PhOC*H*$_2$), 69.1 (C5), 91.1 (C7), 114.9, 127.5 (Ar-*C*H), 135.0, 156.7 (Ar-C), 166.2 (C9), 171.4, 171.5, 174.9 (PhOCH$_2$*C*ONH + *C*O$_2$H + C7'), 178.7 (C8), 194.5 (C6); *m/z* (ESI$^-$) 576 ([M-H]$^-$, 54 %); HRMS (ESI$^-$); $C_{25}H_{26}N_3O_9S_2$ [M-H]$^-$; found 576.11246, requires 576.11159.

6.4.10 Procedure for *N,S*-acetal deprotection

Attempted *N,S*-acetal deprotection with TFA

TFA (56 µL, 0.73 mmol, 15 eq) was added to tetramic acid **109** (20 mg, 0.049 mmol, 1 eq) dissolved in CH_2Cl_2 (1.5 mL). The reaction mixture was stirred at rt for 24 h. The solvents were removed *in vacuo* to obtain unreacted starting material.

Attempted *N,S*-acetal deprotection with TFA in the presence of water

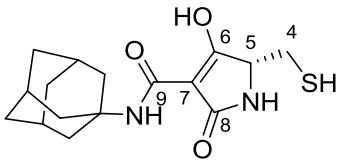
TFA (56 µL, 0.73 mmol, 15 eq) was added to tetramic acid **109** (20 mg, 0.049 mmol, 1 eq) dissolved in THF (1.5 mL THF + 0.1 mL H_2O). The reaction mixture was stirred at rt for 24 h. The solvents were removed *in vacuo* to obtain **109** along with aldehyde (**109**:aldehyde; 10:1).

N,S-Acetal deprotection via Corey-Reichard protocol[142]

109 (84 mg, 0.20 mmol, 1 eq) was added to a solution of 2 % HCl in trifluoroethanol (3.3 mL). 1,3-propanedithiol (0.082 mL, 0.82 mmol, 4 eq) was added to this solution and heated at

50 °C for 7 h. The reaction flask was cooled and solvents removed *in vacuo*. The crude residue was purified by silica gel flash column chromatography (eluent: EtOAc/ Methanol/1 % citric acid). The product isolated was dissolved in CH_2Cl_2 and washed with 5 % citric acid. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to obtain **252**.

(-)-(5*R*)-*N*-(Adamantan-1-yl)-6-hydroxy-5-(mercaptomethyl)-2-oxo-2,5-dihydro-1*H*-pyrrole-3-carboxamide, 252



Yield (46 mg, 70 %); yellow solid, mp 84 °C; 2.1:1 AB:CD tautomers; R_f = 0.068 (EtOAc/MeOH 4:1); $[\alpha]_D^{25}$ = -39.0 (*c* = 0.4, $CHCl_3$); ν_{max}/cm^{-1} (neat) 1620 (C=C), 1650 (C=O), 1682 (C=O), 3294 (N-H/O-H); δ_H (400 MHz, $CDCl_3$): 1.66 - 1.76 (6H, m, Adamantyl-C*H*$_2$), 2.04 - 2.09 (6H, m, Adamantyl-C*H*$_2$), 2.12 (3H, br. signal, Adamantyl-C*H*), 2.59 - 2.74 (1H, m, H4$_A$), 2.99 - 3.12 (1H, m, Hz, H4$_B$), 3.92 (1H, dd, *J* = 8.0, 3.6 Hz, H5 CD), 4.19 (1H, dd, *J* = 7.5, 3.6 Hz, H5 AB), 5.78 (1H, br. s., NH/OH/SH), 6.78 (2H, br. s., NH/OH/SH), 7.44 (1H, br. s., NH/OH/SH AB), 7.80 (1H, br. s., NH/OH/SH CD); δ_C (125.8 MHz, $CDCl_3$): 26.2 (C4 AB), 26.5 (C4 CD), 29.3 (Adamantyl-*C*H), 35.9 (Adamantyl-*C*H$_2$ CD), 36.1 (Adamantyl-*C*H$_2$ AB), 41.6 (Adamantyl-*C*H$_2$ AB), 41.7 (Adamantyl-*C*H$_2$ CD), 52.8 (Adamantyl-C AB), 54.2 (Adamantyl-C CD), 59.0 (C5 AB), 63.1 (C5 CD), 85.2 (C7 CD), 96.4 (C7 AB), 166.0 (C9 AB), 166.9 (C9 CD), 171.4 (C8 AB), 177.4 (C8 CD), 186.6 (C6 AB), 191.1 (C6 CD); *m/z* (ESI$^-$) 321 ([M-H]$^-$, 100%); HRMS (ESI$^+$); $C_{16}H_{23}N_2O_3S$ [M+H]$^+$; found 323.14259, requires 323.14239.

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APPENDICES

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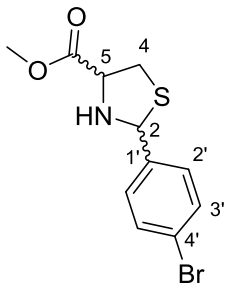
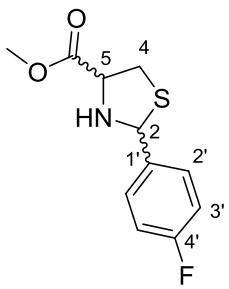
APPENDIX A: CHARACTERISATION OF RACEMIC ANALOGUES

(±)-Methyl (2*RS*,5*R*)-2-(4-bromophenyl)-1,3-thiazolidine-5-carboxylate, (±)-79



Yield (1.25 g, 50 % from L-cysteine); colourless oil; 1.4:1 *cis* and *trans* diastereomers; R_f = 0.23 (EtOAc/petrol 1:4); ν_{max}/cm^{-1} (film) 1737 (C=O), 3311 (N-H); δ_H (400 MHz, $CDCl_3$) major isomer (*cis*): 2.61 (1H, br. s., NH), 3.11 (1H, dd, *J* = 10.3, 8.8 Hz, $H4_A$), 3.46 (1H, dd, *J* = 10.3, 7.1 Hz, $H4_B$), 3.80 (3H, s, CO_2CH_3), 3.98 (1H, dd, *J* = 8.8, 7.1 Hz, H5), 5.50 (1H, s, H2), 7.34 - 7.51 (4H, m, H2′ + H3'); minor isomer: 2.61 (1H, br. s., NH), 3.16 (1H, dd, *J* = 10.5, 6.1 Hz, $H4_A$), 3.37 (1H, dd, *J* = 10.5, 7.1 Hz, $H4_B$), 3.79 (3H, s, CO_2CH_3), 4.14 (1H, app t, *J* = 6.6 Hz, H5), 5.76 (1H, s, H2), 7.34 - 7.51 (4H, m, H2′ + H3'); δ_C (100.6 MHz, $CDCl_3$) major isomer: 39.1 (C4), 52.6 (CO_2CH_3), 65.4 (C5), 71.7 (C2), 122.5 (C4′), 129.1 (C2′), 131.7 (C3′), 137.1 (C1′), 171.4 (CO_2CH_3); minor isomer: 38.0 (C4), 52.5 (CO_2CH_3), 64.1 (C5), 69.9 (C2), 121.6 (C4′), 128.6 (C2′), 131.4 (C3′), 140.5 (C1′), 172.0 (CO_2CH_3); *m/z* (ESI^+) 302, 304 ($[M+H]^+$ 100%); HRMS (ESI^+); $C_{11}H_{13}O_2NBrS$ $[M+H]^+$; found 301.98483 and 303.98253, requires 301.98449 and 303.98244.

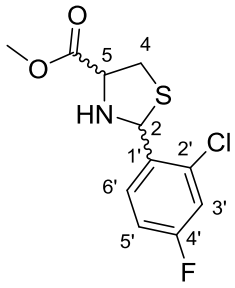
(±)-Methyl (2*RS*,5*R*)-2-(4-fluorophenyl)-1,3-thiazolidine-5-carboxylate, (±)-80



Yield (0.42 g, 43 % from L-cysteine); colourless oil; 1.7:1 *cis* and *trans* diastereomers; R_f = 0.35 (EtOAc/petrol 1:3); ν_{max}/cm^{-1} (neat) 1223 (C-F), 1741 (C=O), 3315 (N-H); δ_H (200 MHz, $CDCl_3$) major isomer (*cis*): 2.57 (1H, br. s., NH), 3.05 - 3.26 (1H, m, $H4_A$), 3.33 - 3.53 (1H, m, $H4_B$), 3.81 (3H, s, CO_2CH_3), 3.99 (1H, dd, *J* = 8.8, 7.1 Hz, H5), 5.53 (1H, s, H2), 6.96 - 7.12 (2H, m, H3′), 7.43 - 7.56 (2H, m, H2′); minor isomer (*trans*): 2.57 (1H, br. s., NH), 3.05 - 3.26 (1H, m, $H4_A$), 3.33 - 3.53 (1H, m, Hz, $H4_B$), 3.80 (3H, s, CO_2CH_3), 4.14 -

4.23 (1H, m, H5), 5.79 (1H, s, H2), 6.96 – 7.12 (2H, m, H3′), 7.43 - 7.56 (2H, m, H2′); δ_C (100.6 MHz, $CDCl_3$) major isomer (cis): 39.2 (C4), 52.6 ($CO_2\mathit{C}H_3$), 65.4 (C5), 71.8 (C2), 115.5 (d, *J* = 21.5 Hz, C3′), 129.3 (d, *J* = 8.7 Hz, C2′), 133.9 (d, *J* = 3.2 Hz, C1′), 162.8 (d, *J* = 248.0 Hz, C4′), 171.5 ($\mathit{C}O_2CH_3$); minor isomer (*trans*): 38.0 (C4), 52.6 ($CO_2\mathit{C}H_3$), 64.1 (C5), 69.9 (C2), 115.1 (d, *J* = 21.5 Hz, C3′), 128.7 (d, *J* = 8.0 Hz, C2′), 136.9 (d, *J* = 3.2 Hz, C1′), 162.3 (d, *J* = 246.4 Hz, C4′), 172.1 ($\mathit{C}O_2CH_3$); *m/z* (ESI^+) 242 ($[M+H]^+$, 100%); HRMS (ESI^+); $C_{11}H_{13}FNO_2S$ $[M+H]^+$; found 242.0642, requires 242.0646.

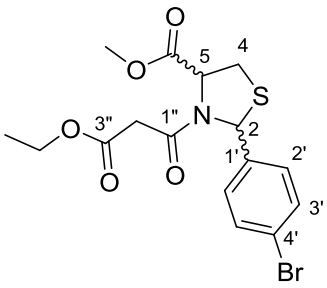
(±)-Methyl (2*RS*,5*R*)-2-(2-chloro-4-fluorophenyl)-1,3-thiazolidine-5-carboxylate, (±)-82



Yield (1.15 g, 51 % from L-cysteine); colourless oil; 0.9:1 *cis* and *trans* diastereomers; R_f = 0.18 (EtOAc/petrol 1:5); ν_{max}/cm^{-1} (neat) 1227 (C-F), 1738 (C=O), 3324 (N-H); δ_H (400 MHz, $CDCl_3$) major isomer (*trans*): 2.66 (1H, br. s., NH), 3.06 - 3.14 (1H, m, $H4_A$), 3.33 (1H, dd, *J* = 10.6, 6.5 Hz, $H4_B$), 3.81 (3H, s, $CO_2C\mathit{H}_3$), 4.21 (1H, app t, *J* = 6.6 Hz, H5), 6.02 (1H, s, H2), 6.95 (2H, app td, *J* = 8.3, 2.5 Hz, H5′), 7.08 - 7.15 (2H, m, H3′), 7.57 (2H, dd, *J* = 8.7, 6.1 Hz, H6′); minor isomer (*cis*): 2.66 (1H, br. s., NH), 3.06 - 3.14 (1H, m, $H4_A$), 3.45 (1H, dd, *J* = 10.4, 7.0 Hz, $H4_B$), 3.80 (3H, s, $CO_2C\mathit{H}_3$), 3.96 - 4.04 (1H, m, H5), 5.88 (1H, s, H2), 7.03 (2H, app td, *J* = 8.3, 2.6 Hz, H5′), 7.08 - 7.15 (2H, m, H3′), 7.74 (2H, dd, *J* = 8.7, 6.1 Hz, H6′); δ_C (100.6 MHz, $CDCl_3$): major isomer (*trans*): 37.3 (C4), 52.6 ($CO_2\mathit{C}H_3$), 64.7 (C5), 66.5 (C2), 113.8 (d, *J* = 21.5 Hz, C5′), 117.0 (d, *J* = 25.4 Hz, C3′), 127.8 (d, *J* = 8.7 Hz, C6′), 133.5 (d, *J* = 10.3 Hz, C2′), 136.1 (d, *J* = 4.0 Hz, C1′), 161.6 (d, *J* = 249.5 Hz, C4′), 171.9 ($\mathit{C}O_2CH_3$); minor isomer (*cis*): 38.7 (C4), 52.6 ($CO_2\mathit{C}H_3$), 65.3 (C5), 67.6 (C2), 114.5 (d, *J* = 20.7 Hz, C5′), 117.1 (d, *J* = 24.6 Hz, C3′), 129.7 (d, *J* = 8.7 Hz, C6′), 131.9 (d, *J* = 3.2 Hz, C1′), 134.5 (d, *J* = 10.3 Hz, C2′), 162.1 (d, *J* = 251.1 Hz, C4′), 171.4 ($\mathit{C}O_2CH_3$); *m/z*

(ESI$^+$) 276 ([M+H]$^+$ 100%); HRMS (ESI$^+$); $C_{11}H_{11}ClFNNaO_2S$ [M+Na]$^+$; found 298.0079, requires 298.0075.

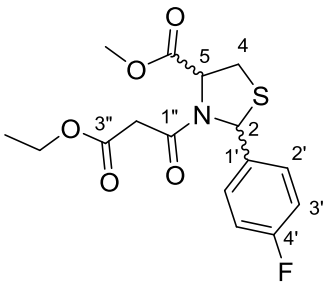
(±)-Methyl (2*RS*,5*R*)-2-(4-bromophenyl)-1-(3-ethoxy-3-oxopropanoyl)-1,3-thiazolidine-5-carboxylate, (±)-85



Yield (1.44 g, 83 %); colourless oil; 1.2:1 *cis* and *trans* diastereomers; R_f = 0.17 (EtOAc/petrol 1:3); ν_{max}/cm^{-1} (film) 1660 (C=O), 1739 (C=O); δ_H (400 MHz, $CDCl_3$) major isomer (a mixture of two conformers): 1.19 - 1.31 (3H, m, OCH_2CH_3), 3.07 - 3.55 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$), 3.82 (3H, s, CO_2CH_3 minor conformer), 3.83 (3H, s, CO_2CH_3 major conformer), 4.05 - 4.25 (2H, m, OCH_2CH_3), 5.04 (1H, app t, *J* = 7.1 Hz, H5), 6.11 (1H, s, H2 major conformer), 6.27 (1H, s, H2 minor conformer), 7.39 - 7.45 (2H, m, H2'), 7.48 - 7.59 (2H, m, H3'); minor isomer (a mixture of two conformers): 1.19 - 1.31 (3H, m, OCH_2CH_3), 3.07 - 3.55 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$), 3.79 (3H, s, CO_2CH_3 major conformer), 3.85 (3H, s, CO_2CH_3 minor conformer), 4.05 - 4.25 (2H, m, OCH_2CH_3), 5.15 - 5.21 (1H, m, H5 minor conformer), 5.26 - 5.33 (1H, m, H5 major conformer), 6.14 (1H, s, H2 major conformer), 6.24 (1H, s, H2 minor conformer), 7.11 (2H, d, *J* = 8.6 Hz, H2′ major conformer), 7.17 (2H, d, *J* = 8.3 Hz, H2′ minor conformer), 7.39 - 7.45 (2H, m, H3' minor conformer), 7.48 - 7.59 (2H, m, H3' major conformer); δ_C (100.6 MHz, $CDCl_3$) major isomer (a mixture of two conformers): 14.0, 14.1 (OCH_2CH_3), 32.0, 33.9 (C4), 42.1, 42.9 (C2″), 52.8, 53.2 (CO_2CH_3), 61.6, 61.8 (OCH_2CH_3), 63.7, 64.6 (C5), 65.7, 66.6 (C2), 122.0, 122.7 (C4′), 128.2, 128.9 (C2′), 131.3, 132.1 (C3′), 137.7, 138.9 (C1′), 165.0, 165.3 (C1″), 166.5, 166.9 (C3″), 170.0, 170.1 (CO_2CH_3); minor isomer (a mixture of two conformers): 13.9, 14.0 (OCH_2CH_3), 31.0, 33.0 (C4), 42.3, 43.2 (C2″), 52.8, 53.4 (CO_2CH_3), 61.5, 61.8 (OCH_2CH_3), 64.0, 64.2 (C5), 64.4, 65.1 (C2), 121.3, 122.4 (C4′), 126.5, 126.6 (C2′), 131.6, 132.3 (C3′), 140.9, 141.1 (C1′), 164.6, 165.2 (C1″), 166.1, 167.2

(C3″), 169.2, 170.0 (CO_2CH_3); *m/z* (ESI^+) 416, 418 ($[M+H]^+$ 47 %); HRMS (ESI^+); $C_{16}H_{19}O_5NBrS$ $[M+H]^+$; found 416.01562 and 418.01352, requires 416.01618 and 418.01414.

(±)-Methyl (2*RS*,5*R*)-1-(3-ethoxy-3-oxopropanoyl)-2-(4-fluorophenyl)-1,3-thiazolidine-5-carboxylate, (±)-86

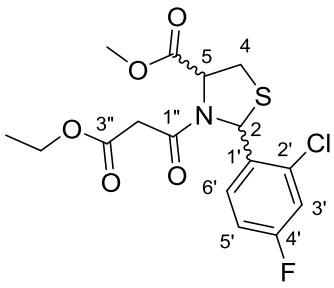


Yield (0.43 g, 81 %); colourless oil; 1.5:1 *cis* and *trans* diastereomers; R_f = 0.61 (EtOAc/petrol 1:1); ν_{max}/cm^{-1} (neat) 1224 (C-F), 1662 (C=O), 1740 (C=O); δ_H (400 MHz, $CDCl_3$) major isomer (*cis*, a mixture of two conformers): 1.19 - 1.31 (3H, m, OCH_2CH_3), 3.07 - 3.55 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$), 3.81 (3H, s, CO_2CH_3 minor conformer), 3.83 (3H, s, CO_2CH_3 major conformer), 4.05 - 4.24 (2H, m, OCH_2CH_3), 5.03 - 5.08 (1H, m, H5), 6.13 (1H, s, H2 major conformer), 6.30 (1H, s, H2 minor conformer), 6.95 - 7.01 (2H, m, H3′ minor conformer), 7.04 - 7.10 (2H, m, H3′ major conformer), 7.51 (2H, dd, *J* = 8.6, 5.4 Hz, H2′ minor conformer), 7.67 (2H, dd, *J* = 8.6, 5.2 Hz, H2′ major conformer); minor isomer (*trans*, a mixture of two conformers): 1.19 - 1.31 (3H, m, OCH_2CH_3), 3.07 - 3.55 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$), 3.78 (3H, s, CO_2CH_3 major conformer), 3.84 (3H, s, CO_2CH_3 minor conformer), 4.05 - 4.24 (2H, m, OCH_2CH_3), 5.19 (1H, app d, *J* = 5.4 Hz, H5 minor conformer), 5.28 - 5.31 (1H, m, H5 major conformer), 6.17 (1H, s, H2 major conformer), 6.29 (1H, s, H2 minor conformer), 6.95 - 7.01 (2H, m, H3′ minor conformer), 7.04 - 7.10 (2H, m, H3′ major conformer), 7.21 (2H, dd, *J* = 8.6, 5.2 Hz, H2′ major conformer), 7.24 - 7.29 (2H, m, H2′ minor conformer); δ_C (100.6 MHz, $CDCl_3$) major isomer (*cis*, a mixture of two conformers): 13.9, 14.0 (OCH_2CH_3), 32.0, 33.8 (C4), 42.1, 43.0 (C2″), 52.8, 53.1 (CO_2CH_3), 61.5, 61.6, 61.8 (OCH_2CH_3), 63.7, 64.6 (C5), 65.7, 66.4 (C2), 115.0 (d, *J* = 21.5 Hz, C3′ minor conformer), 115.9 (d, *J* = 21.5 Hz, C3′ major conformer), 128.4 (d, *J* = 8.0 Hz, C2′ major conformer), 129.0 (d, *J* = 8.7 Hz, C2′ minor conformer), 135.6 (d, *J* = 2.4 Hz, C1′), 162.6 (d, *J* = 248.8 Hz, C4′), 165.0, 165.4 (C1″),

166.6, 167.0 (C3″), 170.2, 170.2 (CO_2CH_3); minor isomer (*trans*, a mixture of two conformers): 13.9, 14.0 (OCH_2CH_3), 30.9, 33.0 (C4), 42.3, 43.3 (C2″), 52.8, 53.4 (CO_2CH_3), 61.5, 61.6, 61.8 (OCH_2CH_3), 64.0, 64.2 (C5), 64.4, 65.1 (C2), 115.3 (d, *J* = 22.3 Hz, C3′ minor conformer), 116.2 (d, *J* = 21.5 Hz, C3′ major conformer), 126.5 (d, *J* = 8.7 Hz, C2′ minor conformer), 126.7 (d, *J* = 8.7 Hz, C2′ major conformer), 137.9 (d, *J* = 2.4 Hz, C1′), 162.5 (d, *J* = 248.0 Hz, C4′), 164.6, 165.2 (C1″), 166.1, 167.2 (C3″), 169.3, 170.1 (CO_2CH_3); *m/z* (ESI^+) 356 ($[M+H]^+$, 100%) 378 ($[M+Na]^+$, 94%); HRMS (ESI^+); $C_{16}H_{18}FNNaO_5S$ $[M+Na]^+$; found 378.0797, requires 378.0782.

(±)-Methyl (2*S*,5*R*)-2-(2-chloro-4-fluorophenyl)-(3-ethoxy-3-oxopropanoyl)-1,3-thiazolidine-5-carboxylate, (±)-*trans*-88 and

(±)-Methyl (2*R*,5*R*)-2-(2-chloro-4-fluorophenyl)-(3-ethoxy-3-oxopropanoyl)-1,3-thiazolidine-5-carboxylate, (±)-*cis*-88

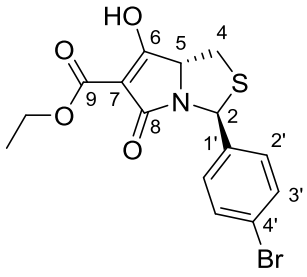


Yield (0.73 g, 94 %); 0.7:1 separable *cis* and *trans* diastereomers; Major isomer (*trans*, a mixture of two conformers): white solid, mp 82-84 ^oC; R_f = 0.16 (EtOAc/petrol 1:3); ν_{max}/cm^{-1} (neat) 1225 (C-F), 1668 (C=O), 1743 (C=O); δ_H (400 MHz, $CDCl_3$): 1.23 (3H, t, *J* = 7.1 Hz, OCH_2CH_3 major conformer), 1.31 (3H, t, *J* = 7.2 Hz, OCH_2CH_3 minor conformer), 3.06 - 3.48 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$), 3.81 (3H, s, CO_2CH_3 major conformer), 3.87 (3H, s, CO_2CH_3 minor conformer), 4.10 (2H, q, *J* = 7.2 Hz, OCH_2CH_3 major conformer), 4.18 - 4.27 (2H, m, OCH_2CH_3 minor conformer), 5.22 (1H, dd, *J* = 5.0, 1.6 Hz, H5 minor conformer), 5.30 - 5.34 (1H, m, H5 major conformer), 6.44 (1H, s, H2 major conformer), 6.47 (1H, s, H2 minor conformer), 6.94 (1H, app td, *J* = 8.4, 2.6 Hz, H5′ minor conformer), 7.03 (1H, *J* = 8.3, 2.6 Hz, H5′ major conformer), 7.12 - 7.18 (2H, m, H3′ minor conformer + H6' major conformer), 7.21 (1H, dd, *J* = 8.2, 2.6 Hz, H3′ major conformer), 7.33 (1H, dd, *J* = 8.6, 5.9 Hz, H6′ minor conformer); δ_C (100.6 MHz,

$CDCl_3$): 13.9, 14.0 ($OCH_2\mathit{C}H_3$), 30.6, 32.5 (C4), 42.1, 43.1 (C2″), 52.9, 53.5 ($CO_2\mathit{C}H_3$), 61.6, 61.9 ($O\mathit{C}H_2CH_3$), 62.0, 62.9 (C2), 64.3, 64.6 (C5), 113.8 (d, *J* = 20.7 Hz, C5′ minor conformer), 114.5 (d, *J* = 21.5 Hz, C5′ major conformer), 117.5 (d, *J* = 24.6 Hz, C3′ minor conformer), 118.0 (d, *J* = 24.6 Hz, C3′major conformer), 125.7 (d, *J* = 8.7 Hz, C6′ minor conformer), 126.4 (d, *J* = 8.7 Hz, C6′ major conformer), 132.6 (d, *J* = 10.3 Hz, C2′), 134.6 (d, *J* = 3.2 Hz, C1′ minor conformer), 134.7 (d, *J* = 3.2 Hz, C1′ major conformer), 161.6 (d, *J* = 249.5 Hz, C4′ minor conformer), 162.1 (d, *J* = 251.9 Hz, C4′ major conformer), 164.5, 165.1 (C1″), 166.0, 167.5 (C3″), 169.2, 170.1 ($\mathit{C}O_2CH_3$); *m/z* (ESI^+) 390 ($[M+H]^+$ 100%), 412.0 ($[M+Na]^+$ 65%); HRMS (ESI^+); $C_{16}H_{17}ClFNNaO_5S$ $[M+Na]^+$; found 412.0398, requires 412.0392. Minor isomer (*cis*, a mixture of two conformers): colourless oil; R_f = 0.24 (EtOAc/petrol 1:3); ν_{max}/cm^{-1} (neat) 1224 (C-F), 1664 (C=O), 1740 (C=O); δ_H (400 MHz, $CDCl_3$): 1.21 - 1.30 (3H, m, $OCH_2C\mathit{H}_3$), 3.07 - 3.54 (4H, m, $H4_A$ + $H4_B$ + $H2''_A$ + $H2''_B$), 3.85 (3H, s, $CO_2C\mathit{H}_3$ major conformer), 3.89 (3H, s, $CO_2C\mathit{H}_3$ minor conformer), 4.09 - 4.22 (2H, m, $OC\mathit{H}_2CH_3$), 4.91 (1H, dd, *J* = 9.2, 6.5 Hz, H5 major conformer), 5.06 (1H, app t, *J* = 5.0 Hz, H5 minor conformer), 6.38 (1H, s, H2 major conformer), 6.52 (1H, s, H2 minor conformer), 6.95 (1H, app t, *J* = 8.3 Hz, H5′ minor conformer), 7.08 (1H, app td, *J* = 8.3, 2.4 Hz, H5′ major conformer), 7.17 (1H, dd, *J* = 8.1, 2.4 Hz, H3′), 7.84 (1H, dd, *J* = 8.7, 6.0 Hz, H6′ minor conformer), 8.28 (1H, dd, *J* = 8.7, 6.0 Hz, H6′ major conformer); δ_C (100.6 MHz, $CDCl_3$): 14.0 ($OCH_2\mathit{C}H_3$), 31.2, 33.8 (C4), 41.8, 42.8 (C2″), 52.8, 53.3 ($CO_2\mathit{C}H_3$), 61.7 ($O\mathit{C}H_2CH_3$), 62.9, 64.5 (C2), 64.2, 65.2 (C5), 114.3 (d, *J* = 20.7 Hz, C5′ minor conformer), 114.7 (d, *J* = 20.7 Hz, C5′ major conformer), 116.7 (d, *J* = 25.4 Hz, C3′ minor conformer), 117.4 (d, *J* = 25.4 Hz, C3′ major conformer), 128.3 (d, *J* = 8.0 Hz, C6′ minor conformer), 128.9 (d, *J* = 8.7 Hz, C6′ major conformer), 132.7 (d, *J* = 10.3 Hz, C2′), 133.7 (d, *J* = 4.0 Hz, C1′), 162.2 (d, *J* = 251.9 Hz, C4′), 165.1, 165.3 (C1″), 166.2, 166.8 (C3″), 170.3, 170.4

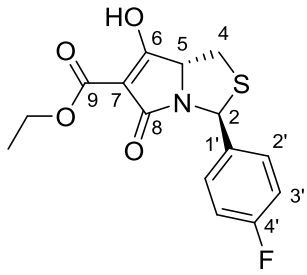
(CO_2CH_3); *m/z* (ESI$^+$) 390 ([M+H]$^+$ 100%), 412.0 ([M+Na]$^+$ 71%); HRMS (ESI$^+$); $C_{16}H_{17}ClFNNaO_5S$ [M+Na]$^+$; found 412.0393, requires 412.0392.

(±)-(2*S*,5*R*)-1-Aza-2-(4-bromophenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, (±)-91a



Yield (47 mg, 39 %); yellow solid, mp 148 °C; R_f = 0.26 (EtOAc: MeOH; 9:1); ν_{max}/cm^{-1} (neat) 1618 (C=C), 1657 (C=O), 1712 (C=O); δ_H (500 MHz, CD_2Cl_2): 1.37 (3H, t, *J* = 7.2 Hz, OCH_2CH_3), 3.02 (1H, dd, *J* = 11.2, 8.2 Hz, $H4_A$), 3.29 (1H, dd, *J* = 11.2, 7.1 Hz, $H4_B$), 4.38 (2H, q, *J* = 7.2 Hz, OCH_2CH_3), 4.76 (1H, app t, *J* = 7.6 Hz, H5), 6.22 (1H, s, H2), 7.35 (2H, d, *J* = 8.3 Hz, H2′), 7.49 (2H, d, *J* = 8.5 Hz, H3′); δ_C (125.8 MHz, CD_2Cl_2): 14.5 (OCH_2CH_3), 33.4 (C4), 62.3 (OCH_2CH_3), 62.4 (C2), 65.8 (C5), 99.7 (C7), 122.4 (C4′), 128.8 (C2′), 132.2 (C3′), 140.3 (C1′), 167.5 (C9), 169.1 (C8), 186.6 (C6); *m/z* (ESI$^-$) 382.0, 384.0 ([M-H]$^-$, 100%); HRMS (ESI$^-$); $C_{15}H_{13}O_4NBrS$ [M-H]$^-$; found 381.97573 and 383.97348, requires 381.97541 and 383. 97337.

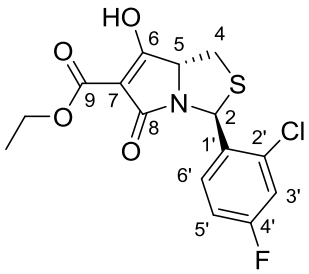
(±)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-2-(4-fluorophenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, (±)-92a



Yield (0.12 g, 53 %); yellow solid; R_f = 0.55 (EtOAc/MeOH 6:1); ν_{max}/cm^{-1} (neat) 1224 (C-F), 1658 (C=O), 1709 (C=O); For **(±)-9c** purified by preparative TLC, δ_H (400 MHz, Methanol-d_4): 1.28 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 2.95 (1H, dd, *J* = 11.0, 7.3 Hz, $H4_A$), 3.20 (1H, dd, *J* = 11.0, 7.6 Hz, $H4_B$), 4.14 - 4.24 (3H, m, OCH_2CH_3 + H5), 6.34 (1H, s, H2), 7.04 (2H, app t, *J* = 8.8 Hz, H3′), 7.47 (2H, dd, *J* = 8.6, 5.4 Hz, H2′); δ_C (125.8 MHz, Methanol-d_4): 15.2 (OCH_2CH_3), 34.9 (C4), 59.7 (OCH_2CH_3), 64.6 (C2), 70.6 (C5), 91.9 (C7), 116.0 (d, *J* = 21.9 Hz, C3′), 129.6 (d, *J* = 8.6 Hz, C2′), 139.8 (C1′), 163.7 (d, *J* = 245.1 Hz, C4′), 167.3

(C9), 180.2 (C8), 195.1 (C6); *m/z* (ESI$^-$) 322 ([M-H]$^-$, 100%); HRMS (ESI$^-$); $C_{15}H_{13}FNO_4S$ [M-H]$^-$; found 322.0569, requires 322.0555.

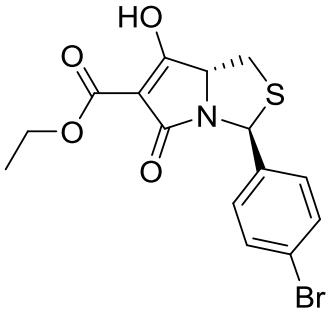
(±)-(2*S*,5*R*)-1-Aza-2-(2-chloro-4-fluorophenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, (±)-94a



Yield (74 mg, 45 %); yellow solid, mp 96-98 °C; R_f = 0.57 (EtOAc/MeOH 6:1); ν_{max}/cm^{-1} (neat) 1230 (C-F), 1658 (C=O), 1707 (C=O); δ_H (500 MHz, CD_2Cl_2): 1.37 (3H, t, *J* = 7.2 Hz, OCH_2CH_3), 3.01 (1H, dd, *J* = 11.0, 8.8 Hz, H4$_A$), 3.30 (1H, dd, *J* = 11.0, 6.9 Hz, H4$_B$), 4.39 (2H, q, *J* = 7.2 Hz, OCH_2CH_3), 4.93 (1H, dd, *J* = 8.8, 6.9 Hz, H5), 6.41 (1H, s, H2), 7.03 (1H, app td, *J* = 8.4, 2.6 Hz, H5′), 7.17 (1H, dd, *J* = 8.4, 2.6 Hz, H3′), 7.42 (1H, dd, *J* = 8.4, 6.0 Hz, H6′); δ_C (125.8 MHz, CD_2Cl_2): 14.5 (OCH_2CH_3), 33.0 (C4), 59.9 (OCH_2CH_3), 62.4 (C2), 66.6 (C5), 99.5 (C7), 114.9 (d, *J* = 21.9 Hz, C5′), 117.8 (d, *J* = 25.8 Hz, C3′), 128.0 (d, *J* = 8.6 Hz, C6′), 133.6 (d, *J* = 10.5 Hz, C2′), 135.4 (d, *J* = 3.8 Hz, C1′), 162.6 (d, *J* = 249.9 Hz, C4′), 167.5 (C9), 168.3 (C8), 186.5 (C6); *m/z* (ESI$^-$) 356 ([M-H]$^-$, 100%); HRMS (ESI$^+$); $C_{15}H_{14}O_4NClFS$ [M+H]$^+$; found 358.03120, requires 358.03106.

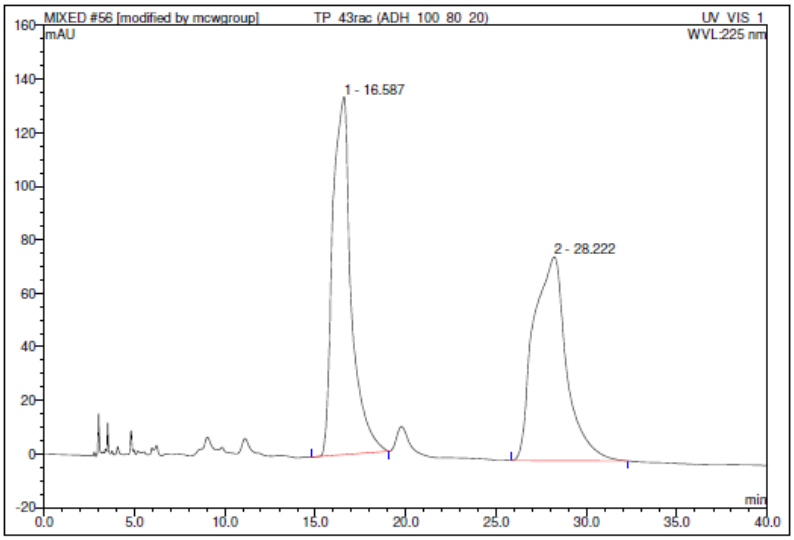
APPENDIX B: CHROMATOGRAMS OBTAINED ON CHIRAL HPLC

(±)-(2*S*,5*R*)-1-Aza-2-(4-bromophenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, (±)-91a



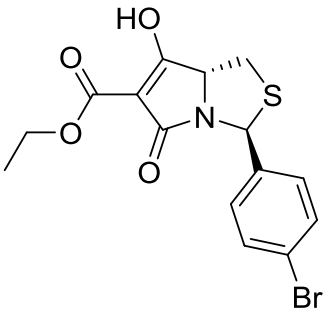
56 TP_43rac (ADH_100_80_20)

Sample Name:	**TP_43rac (ADH_100_80_20)**	*Injection Volume:*	**15.0**
Vial Number:	**GA2**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**225**
Control Program:	**A_100_80_20**	*Bandwidth:*	**1**
Quantif. Method:	**Standard**	*Dilution Factor:*	**1.0000**
Recording Time:	**4/9/2015 10:01**	*Sample Weight:*	**1.0000**
Run Time (min):	**40.00**	*Sample Amount:*	**1.0000**



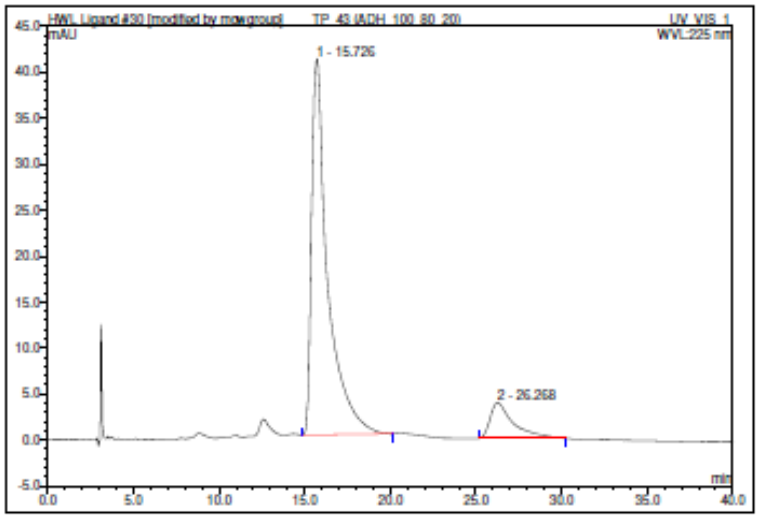
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	16.59	n.a.	133.665	159.221	49.84	n.a.	BMB*
2	28.22	n.a.	76.102	160.233	50.16	n.a.	BMB*
Total:			209.767	319.454	100.00	0.000	

(2*S*,5*R*)-1-Aza-2-(4-bromophenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 91a



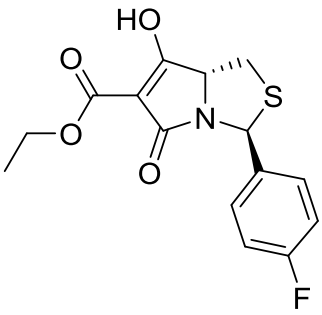
30 TP_43 (ADH_100_80_20)

Sample Name:	TP_43 (ADH_100_80_20)	*Injection Volume:*	12.0
Vial Number:	GA9	*Channel:*	UV_VIS_1
Sample Type:	unknown	*Wavelength:*	225
Control Program:	A_100_80_20	*Bandwidth:*	1
Quantif. Method:	Standard	*Dilution Factor:*	1.0000
Recording Time:	18/8/2015 12:12	*Sample Weight:*	1.0000
Run Time (min):	40.00	*Sample Amount:*	1.0000



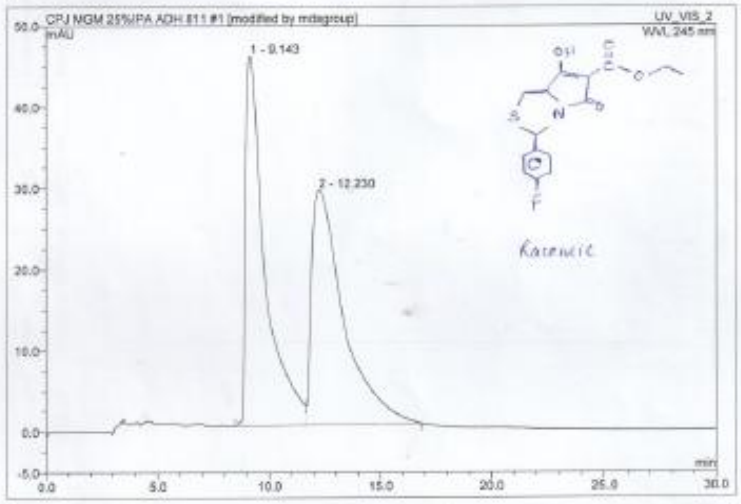
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	15.73	n.a.	40.898	44.631	88.10	n.a.	BMB*
2	26.27	n.a.	3.876	6.027	11.90	n.a.	BMB*
Total:			44.773	50.658	100.00	0.000	

(±)-(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-2-(4-fluorophenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, (±)-92a



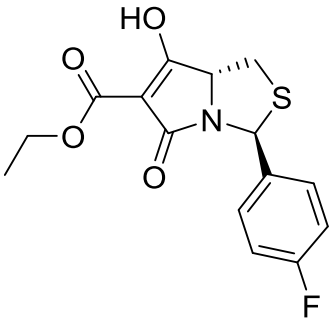
1 CPJ MGM 25%IPA ADH 8/11

Sample Name:	CPJ MGM 25%IPA ADH 8/11	Injection Volume:	50.0
Vial Number:	GE1	Channel:	UV_VIS_2
Sample Type:	unknown	Wavelength:	245.0
Control Program:	program_new	Bandwidth:	1
Quantif. Method:	method	Dilution Factor:	1.0000
Recording Time:	8/11/2013 14:10	Sample Weight:	1.0000
Run Time (min):	30.00	Sample Amount:	1.0000



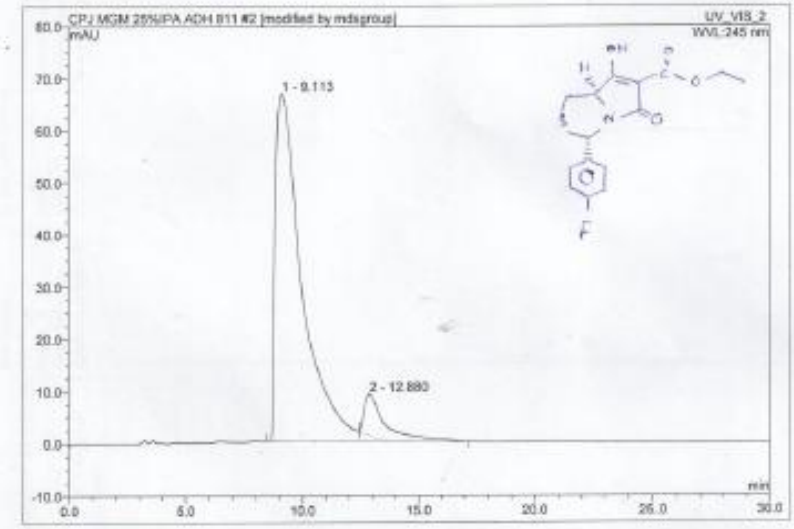
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	9.14	n.a.	45.523	45.834	48.89	n.a.	BM
2	12.23	n.a.	28.881	47.909	51.11	n.a.	MB
Total:			74.404	93.744	100.00	0.000	

(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-2-(4-fluorophenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 92a



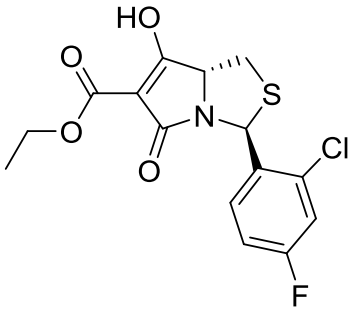
2 CPJ MGM 25%IPA ADH 8/11

Sample Name:	CPJ MGM 25%IPA ADH 8/11	*Injection Volume:*	50.0
Vial Number:	GE2	*Channel:*	UV_VIS_2
Sample Type:	unknown	*Wavelength:*	245.0
Control Program:	program_new	*Bandwidth:*	1
Quantif. Method:	method	*Dilution Factor:*	1.0000
Recording Time:	8/11/2013 14:42	*Sample Weight:*	1.0000
Run Time (min):	30.00	*Sample Amount:*	1.0000



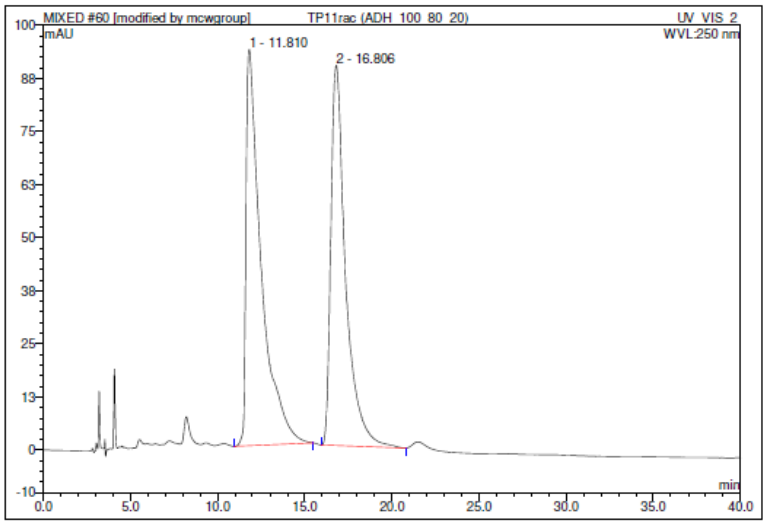
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	9.11	n.a.	66.547	87.277	90.05	n.a.	BM *
2	12.88	n.a.	8.966	9.639	9.95	n.a.	MB*
Total:			75.513	96.916	100.00	0.000	

(±)-(2*S*,5*R*)-1-Aza-2-(2-chloro-4-fluorophenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, (±)-94a



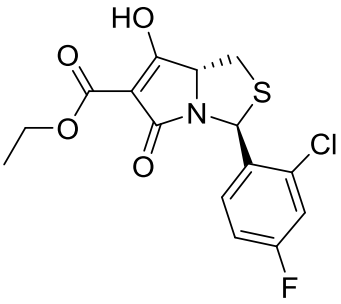
60 TP11rac (ADH_100_80_20)

Sample Name:	**TP11rac (ADH_100_80_20)**	*Injection Volume:*	**15.0**
Vial Number:	**RC13**	*Channel:*	**UV_VIS_2**
Sample Type:	**unknown**	*Wavelength:*	**250**
Control Program:	**A_100_80_20**	*Bandwidth:*	**1**
Quantif. Method:	**Standard**	*Dilution Factor:*	**1.0000**
Recording Time:	**27/10/2015 9:28**	*Sample Weight:*	**1.0000**
Run Time (min):	**40.00**	*Sample Amount:*	**1.0000**



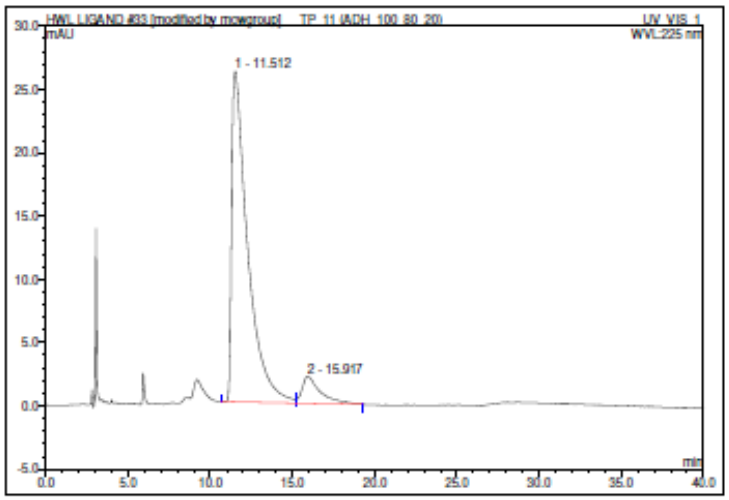
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	11.81	n.a.	93.235	94.188	51.75	n.a.	BMB*
2	16.81	n.a.	89.493	87.835	48.25	n.a.	BMB*
Total:			182.728	182.022	100.00	0.000	

(2*S*,5*R*)-1-Aza-2-(2-chloro-4-fluorophenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 94a



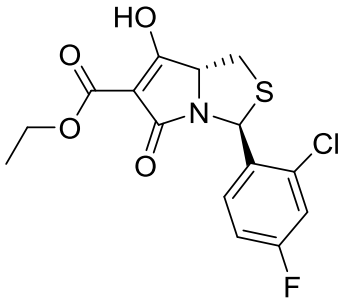
33 TP_11 (ADH_100_80_20)

Sample Name:	TP_11 (ADH_100_80_20)	*Injection Volume:*	12.0
Vial Number:	GA10	*Channel:*	UV_VIS_1
Sample Type:	unknown	*Wavelength:*	225
Control Program:	A_100_80_20	*Bandwidth:*	1
Quantif. Method:	Standard	*Dilution Factor:*	1.0000
Recording Time:	18/8/2015 12:53	*Sample Weight:*	1.0000
Run Time (min):	40.00	*Sample Amount:*	1.0000



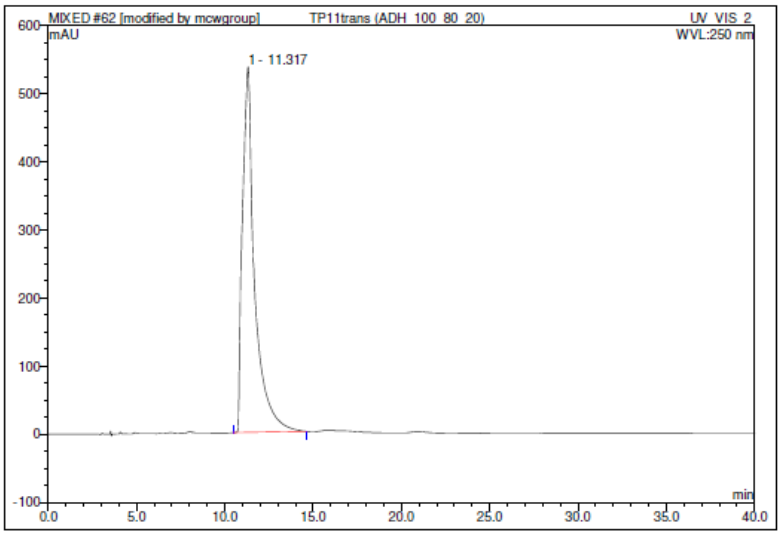
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	11.51	n.a.	26.147	29.483	91.45	n.a.	BM *
2	15.92	n.a.	2.148	2.758	8.55	n.a.	MB*
Total:			28.295	32.241	100.00	0.000	

(-)-(2*S*,5*R*)-1-Aza-2-(2-chloro-4-fluorophenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, (-)-94a *from column chromatography*



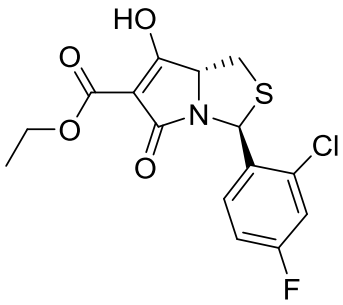
62 TP11trans (ADH_100_80_20)

Sample Name:	**TP11trans (ADH_100_80_20)**	*Injection Volume:*	**15.0**
Vial Number:	**RC11**	*Channel:*	**UV_VIS_2**
Sample Type:	**unknown**	*Wavelength:*	**250**
Control Program:	**A_100_80_20**	*Bandwidth:*	**1**
Quantif. Method:	**Standard**	*Dilution Factor:*	**1.0000**
Recording Time:	**27/10/2015 10:58**	*Sample Weight:*	**1.0000**
Run Time (min):	**40.00**	*Sample Amount:*	**1.0000**



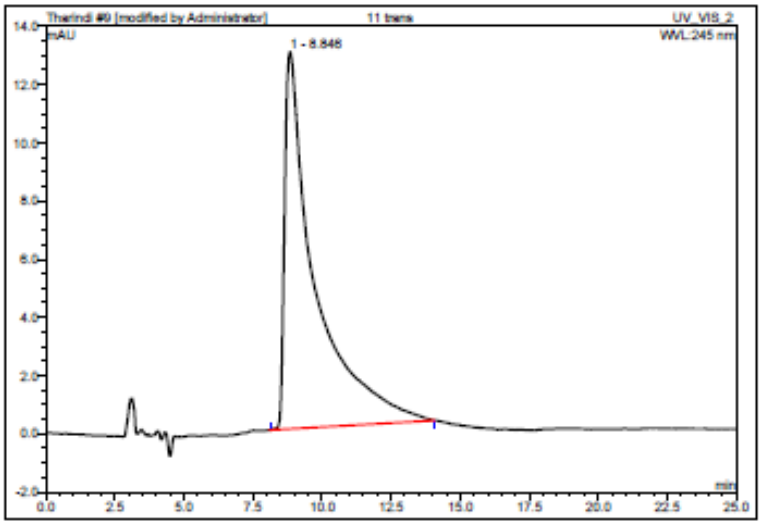
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	11.32	n.a.	536.320	425.671	100.00	n.a.	BMB*
Total:			536.320	425.671	100.00	0.000	

(-)-(2*S*,5*R*)-1-Aza-2-(2-chloro-4-fluorophenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, (-)-94a *from preparative TLC*



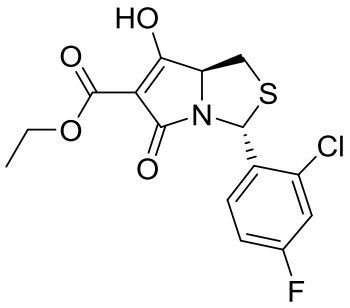
9 11 trans

Sample Name:	**11 trans**	*Injection Volume:*	**10.0**
Vial Number:	**GB6**	*Channel:*	**UV_VIS_2**
Sample Type:	**unknown**	*Wavelength:*	**245**
Control Program:	**col a 1% 1 ml 30min**	*Bandwidth:*	**1**
Quantif. Method:	**Ph-pent**	*Dilution Factor:*	**1.0000**
Recording Time:	**2/12/2013 14:38**	*Sample Weight:*	**1.0000**
Run Time (min):	**25.00**	*Sample Amount:*	**1.0000**



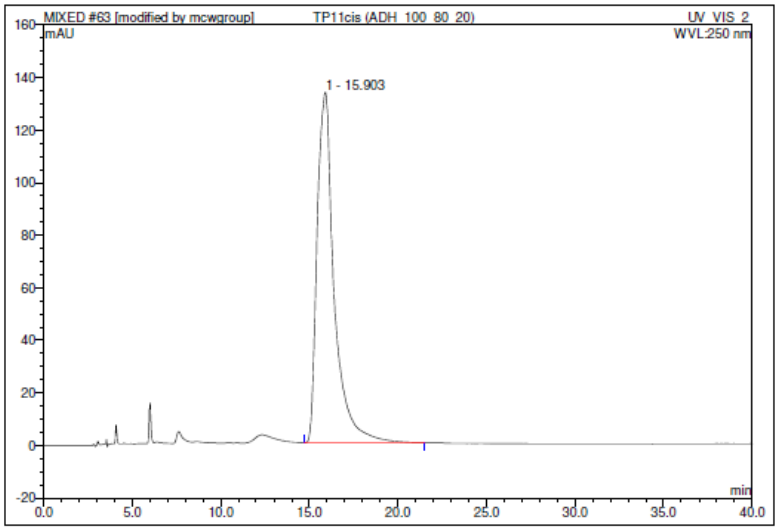
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	8.85	n.a.	12.947	16.409	100.00	n.a.	BMB
Total:			12.947	16.409	100.00	0.000	

(+)-(2*R*,5*S*)-1-Aza-2-(2-chloro-4-fluorophenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, (+)-94a (or ent-94a) *from column chromatography*



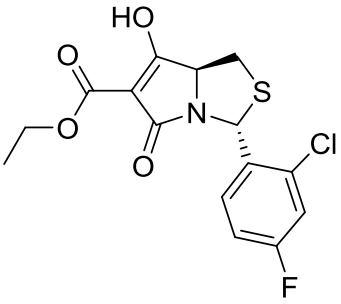
63 TP11cis (ADH_100_80_20)

Sample Name:	**TP11cis (ADH_100_80_20)**	*Injection Volume:*	**15.0**
Vial Number:	**RC10**	*Channel:*	**UV_VIS_2**
Sample Type:	**unknown**	*Wavelength:*	**250**
Control Program:	**A_100_80_20**	*Bandwidth:*	**1**
Quantif. Method:	**Standard**	*Dilution Factor:*	**1.0000**
Recording Time:	**27/10/2015 11:38**	*Sample Weight:*	**1.0000**
Run Time (min):	**40.00**	*Sample Amount:*	**1.0000**



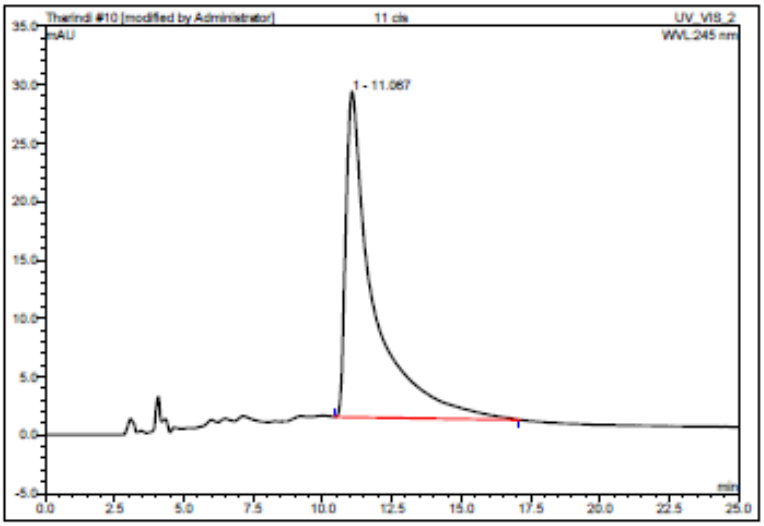
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	15.90	n.a.	133.334	148.907	100.00	n.a.	BMB*
Total:			133.334	148.907	100.00	0.000	

(+)-(2*R*,5*S*)-1-Aza-2-(2-chloro-4-fluorophenyl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, (+)-94a (or ent-94a) *from preparative TLC*



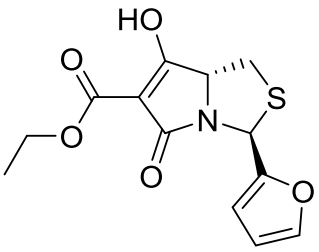
10 11 cis

Sample Name:	**11 cis**	*Injection Volume:*	**10.0**
Vial Number:	**GC6**	*Channel:*	**UV_VIS_2**
Sample Type:	**unknown**	*Wavelength:*	**245**
Control Program:	**col a 1% 1 ml 30min**	*Bandwidth:*	**1**
Quantif. Method:	**Ph-pent**	*Dilution Factor:*	**1.0000**
Recording Time:	**2/12/2013 15:04**	*Sample Weight:*	**1.0000**
Run Time (min):	**25.00**	*Sample Amount:*	**1.0000**



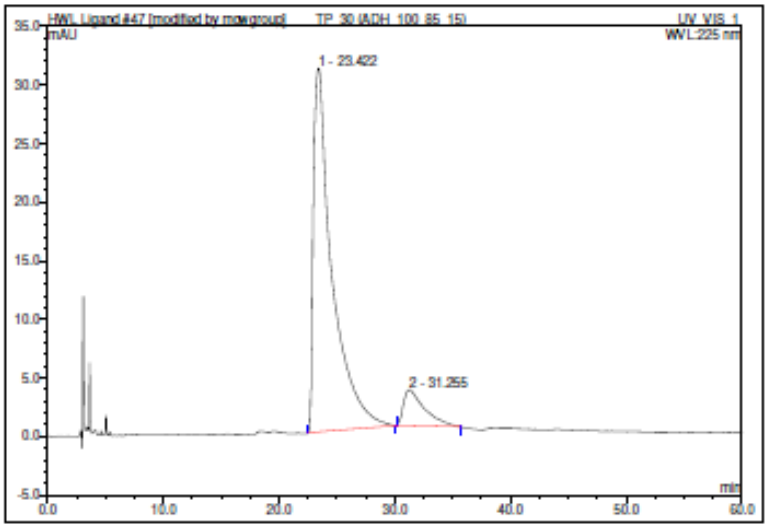
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	11.07	n.a.	27.876	32.175	100.00	n.a.	BMB*
Total:			27.876	32.175	100.00	0.000	

(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-2-(2-furanyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 95a



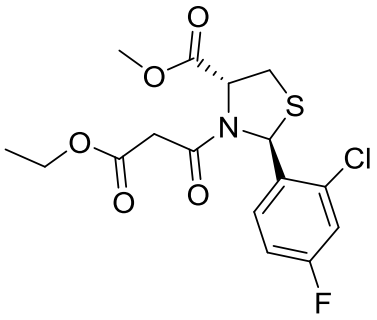
47 TP_30 (ADH_100_85_15)

Sample Name:	TP_30 (ADH_100_85_15)	*Injection Volume:*	15.0
Vial Number:	GA13	*Channel:*	UV_VIS_1
Sample Type:	unknown	*Wavelength:*	225
Control Program:	A_100_85_15	*Bandwidth:*	1
Quantif. Method:	Standard	*Dilution Factor:*	1.0000
Recording Time:	20/8/2015 15:26	*Sample Weight:*	1.0000
Run Time (min):	60.00	*Sample Amount:*	1.0000



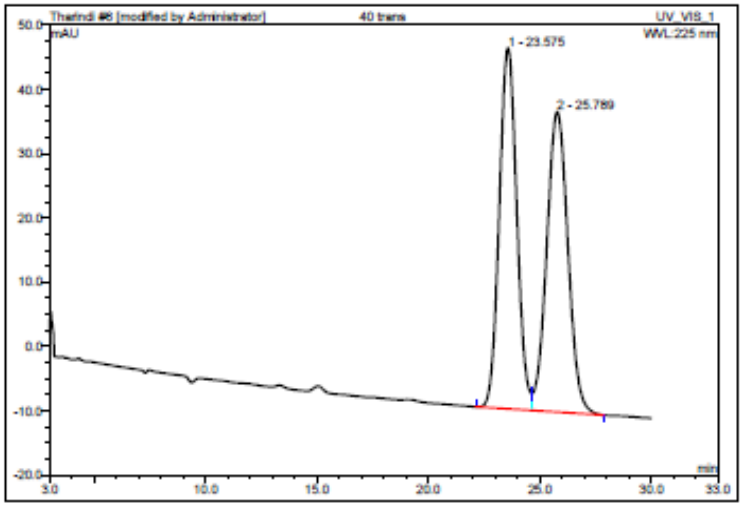
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	23.42	n.a.	31.015	57.903	90.03	n.a.	BMB*
2	31.25	n.a.	3.052	6.414	9.97	n.a.	BMB*
Total:			34.066	64.317	100.00	0.000	

(±)-Methyl (2*S*,5*R*)-2-(2-chloro-4-fluorophenyl)-(3-ethoxy-3-oxopropanoyl)-1,3-thiazolidine-5-carboxylate, (±)-*trans*-88



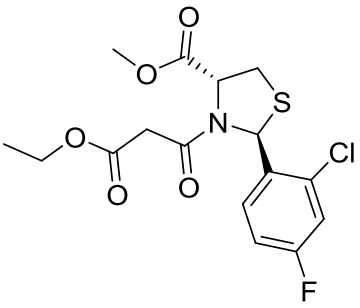
6 40 trans

Sample Name:	40 trans	*Injection Volume:*	10.0
Vial Number:	GB5	*Channel:*	UV_VIS_1
Sample Type:	unknown	*Wavelength:*	225
Control Program:	col a 1% 1 ml 30min	*Bandwidth:*	1
Quantif. Method:	Ph-pent	*Dilution Factor:*	1.0000
Recording Time:	2/12/2013 11:47	*Sample Weight:*	1.0000
Run Time (min):	30.00	*Sample Amount:*	1.0000



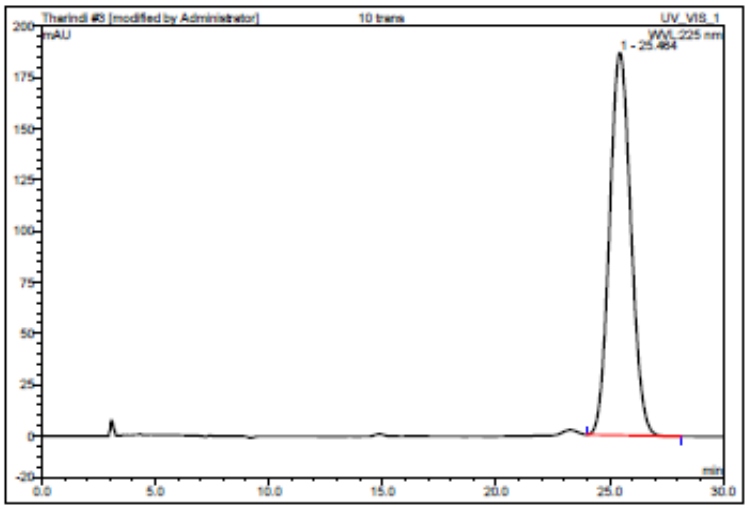
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	23.57	n.a.	56.133	51.034	49.75	n.a.	BM
2	25.79	n.a.	46.729	51.551	50.25	n.a.	MB
Total:			102.863	102.585	100.00	0.000	

(-)-Methyl (2*S*,5*R*)-2-(2-chloro-4-fluorophenyl)-(3-ethoxy-3-oxopropanoyl)-1,3-thiazolidine-5-carboxylate, (-)-*trans*-88



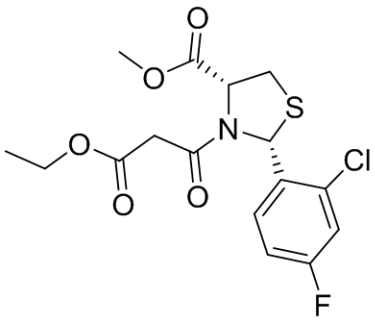
3 10 trans

Sample Name:	10 trans	Injection Volume:	10.0
Vial Number:	GB4	Channel:	UV_VIS_1
Sample Type:	unknown	Wavelength:	225
Control Program:	col a 1% 1 ml 30min	Bandwidth:	1
Quantif. Method:	Ph-pent	Dilution Factor:	1.0000
Recording Time:	29/11/2013 16:08	Sample Weight:	1.0000
Run Time (min):	30.00	Sample Amount:	1.0000



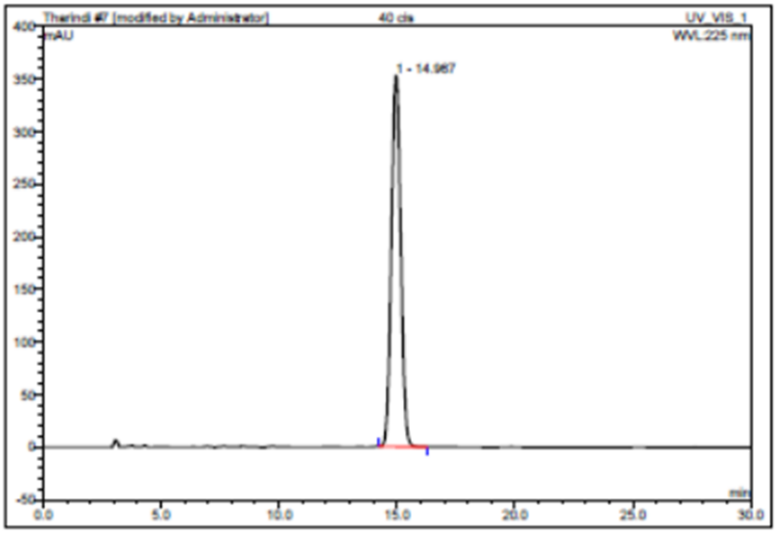
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	25.46	n.a.	186.726	203.132	100.00	n.a.	BMB*
Total:			186.726	203.132	100.00	0.000	

(±)-Methyl (2*R*,5*R*)-2-(2-chloro-4-fluorophenyl)-(3-ethoxy-3-oxopropanoyl)-1,3-thiazolidine-5-carboxylate, (±)-*cis*-88 (enantiomers were not separable)



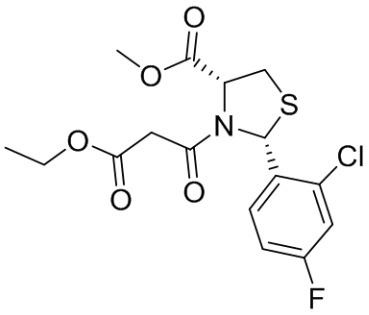
7 40 cis

Sample Name:	**40 cis**	*Injection Volume:*	**10.0**
Vial Number:	**GC5**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**225**
Control Program:	**col a 1% 1 ml 30min**	*Bandwidth:*	**1**
Quantit. Method:	**Ph-pent**	*Dilution Factor:*	**1.0000**
Recording Time:	**2/12/2013 13:14**	*Sample Weight:*	**1.0000**
Run Time (min):	**30.00**	*Sample Amount:*	**1.0000**



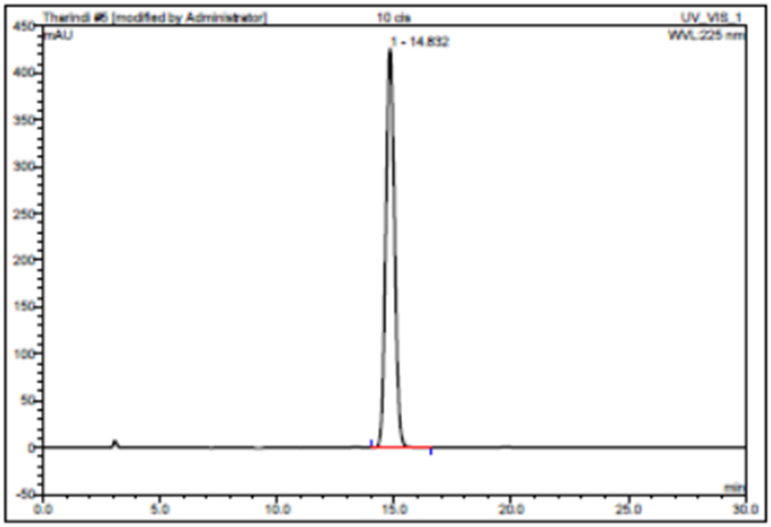
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	14.97	n.a.	352.987	162.311	100.00	n.a.	BMB*
Total:			352.987	162.311	100.00	0.000	

(+)-Methyl (2*R*,5*R*)-2-(2-chloro-4-fluorophenyl)-(3-ethoxy-3-oxopropanoyl)-1,3-thiazolidine-5-carboxylate, (+)-*cis*-88



5 10 cis

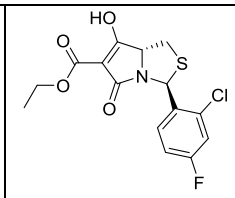
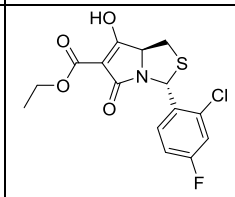
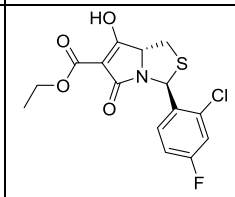
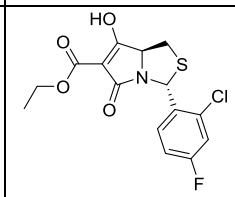
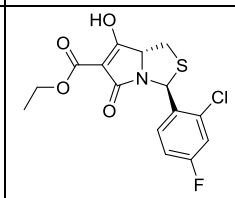
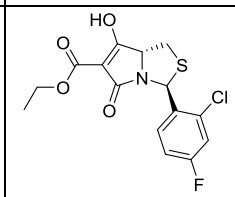
Sample Name:	**10 cis**	*Injection Volume:*	**10.0**
Vial Number:	**GA5**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**225**
Control Program:	**col a 1% 1 ml 30min**	*Bandwidth:*	**1**
Quantif. Method:	**Ph-pent**	*Dilution Factor:*	**1.0000**
Recording Time:	**29/11/2013 17:13**	*Sample Weight:*	**1.0000**
Run Time (min):	**30.00**	*Sample Amount:*	**1.0000**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	14.83	n.a.	425.984	183.282	100.00	n.a.	BMB
Total:			425.984	183.282	100.00	0.000	

APPENDIX C: PHYSICAL PROPERTIES OF TETRAMATE 94a

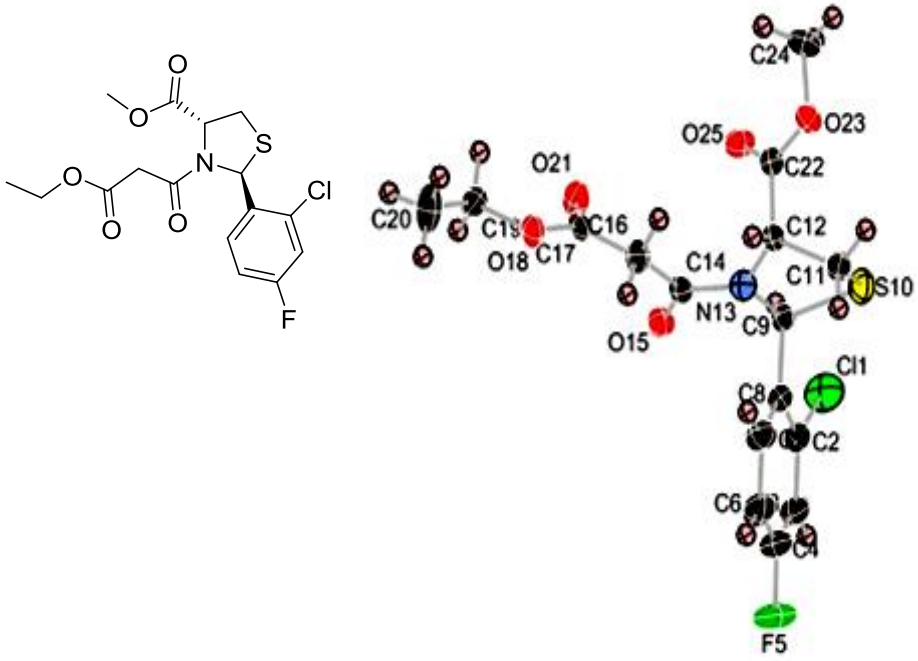
(*Method of purification; **(a):** Prep TLC with Si support, **(b):** flash column chromatography with silica support (run with 1 % Et_3N) followed by an acid wash with 5 % citric acid.

Tetramate		**Method of purification***	**Solvent system for HPLC**	**Retention time (min)**		**% e.e.**	$[\alpha]_D^{25}$	**mp (°C)**
				Peak 1	**Peak 2**			
94a (from *trans*-**88**)		**a**	IPA:Hex; 10:90	8.846	-	100	-240.4	>250
ent-**94a** (from *cis*-**88**)		**a**	IPA:Hex; 10:90	-	11.067	100	+230.3	>250
94a (from *trans*-**88**)		**b**	IPA:Hex; 20:80	11.317 (major)	15.849 (minor)	98	-331.8	176-178
ent-**94a** (from *cis*-**88**)		**b**	IPA:Hex; 20:80	12.342 (minor)	15.903 (major)	96	+309.5	-
94a (from cis/trans-**88** mixture)		**b**	IPA:Hex; 20:80	11.512 (major)	15.917 (minor)	83	-264.0	128
(±)-94a		**b**	IPA:Hex; 20:80	11.810	16.806	-	-	148

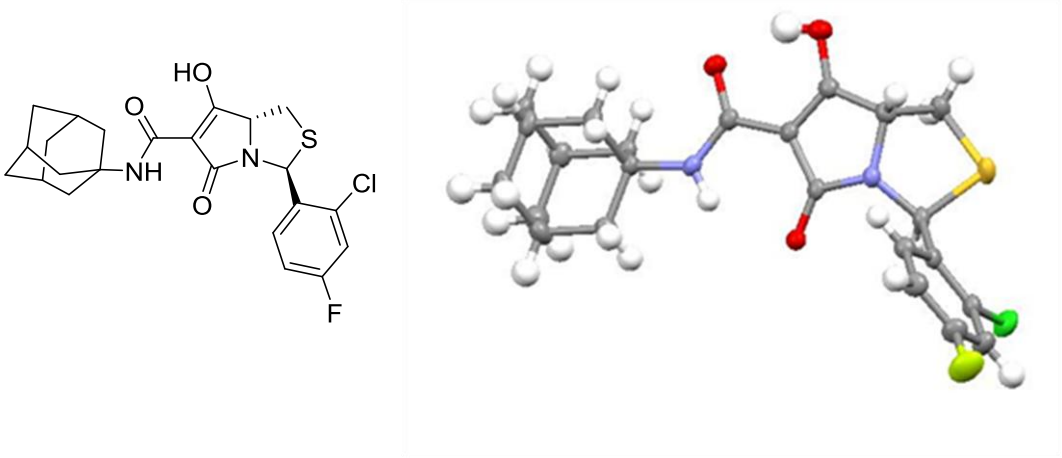
APPENDIX D: X-RAY CRYSTALLOGRAPHY DATA FOR *trans*-88, 113 AND NOESY SPECTRUM OF 118

X-Ray crystallography data

(-)-Methyl (2*S*,5*R*)-2-(2-chloro-4-fluorophenyl)-(3-ethoxy-3-oxopropanoyl)-1,3-thiazolidine-5-carboxylate, (-)-*trans*-88

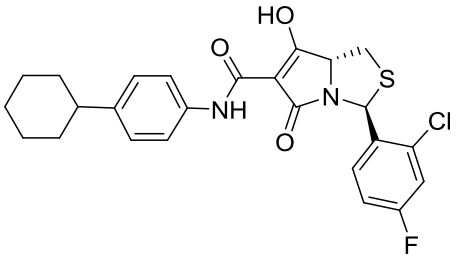


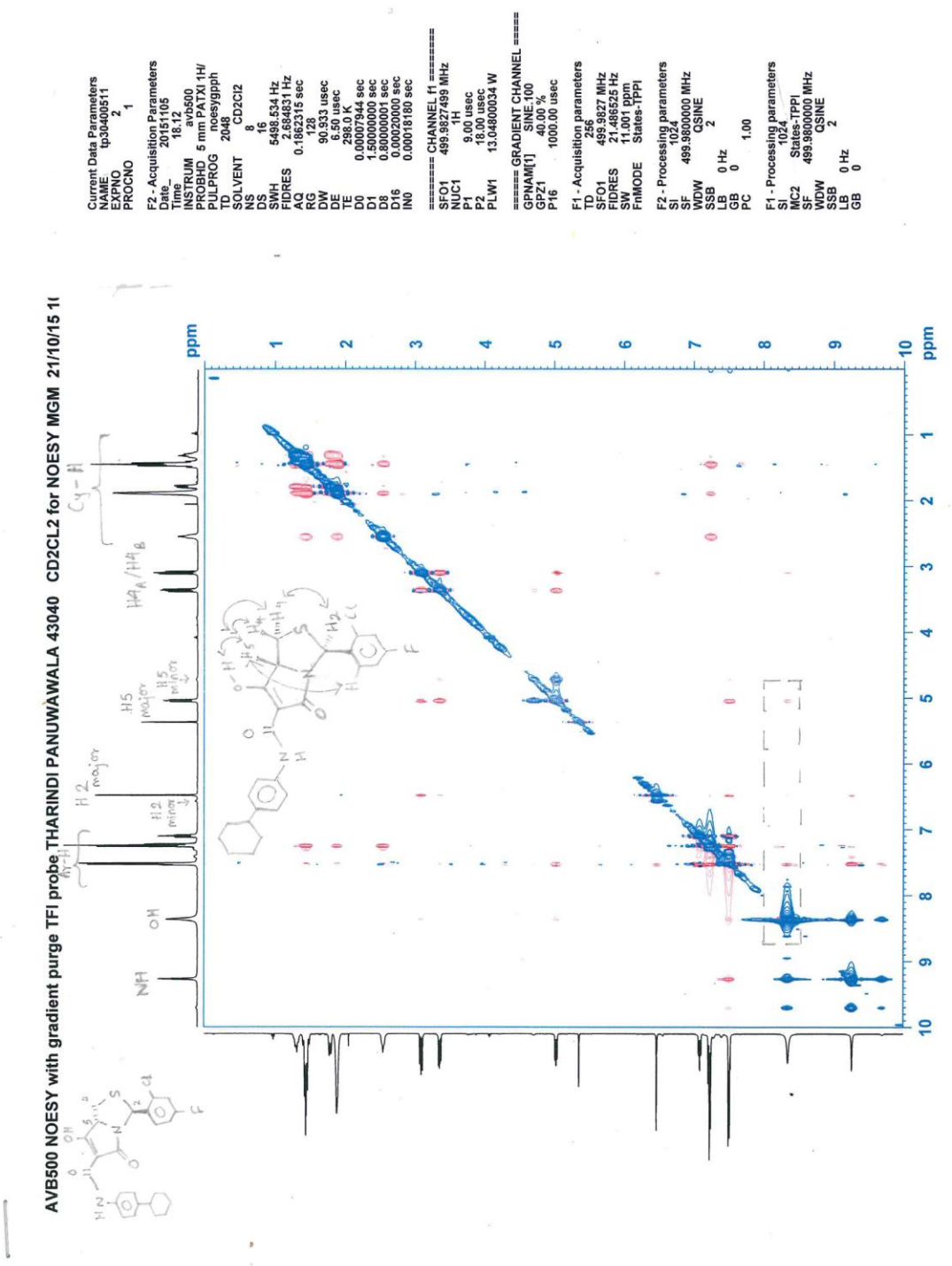
(-)-(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(2-chloro-4-fluorophenyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, (-)-113



The .cif files are submitted separately on the CD.

NOESY spectrum of **118**





APPENDIX E: MM2 ENERGY CALCULATIONS FOR CARBOXAMIDES 109-127

Minimum energies for each tautomeric form were calculated using MM2 method of Chem3D version 15.

Table E.1. Minimum energies for tautomers A-D of tetramates **109-127**.

Tetramate	Energy (kcal/mol)			
	A	**B**	**C**	**D**
109	16.0804	35.7000	22.6440	34.9795
110	16.7081	36.3741	24.2766	35.7101
111	16.0565	35.7281	22.4102	35.0636
112	15.4563	34.8543	32.5050	34.1261
113	18.9332	39.8570	26.8331	39.3211
114	24.2832	43.2331	31.7748	42.6276
115	2.4254	27.1156	8.8662	26.4327
116	2.3728	27.1569	8.6121	26.5199
117	4.0037	26.4523	17.2149	25.8123
118	6.9345	31.2785	13.0366	30.7538
119	1.8602	22.5116	8.4903	21.9308
120	1.8348	22.5793	8.2859	22.0365
121	0.2847	20.3548	10.2257	19.7059
122	0.7964	25.5440	7.0510	26.2300
123	17.6489	42.2161	23.9121	41.5968
124	25.2820	49.0382	32.6301	48.4710
125	7.6126	28.1248	13.8858	27.4426
126	3.0170	23.7384	9.6134	23.0097
127	7.5538	27.9121	13.7305	27.3651

APPENDIX F: INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS) FOR METAL DETECTION

Compound 109	**Metal (ng/g)**									
	Li7 (LR)	**B11 (LR)**	**Na23 (LR)**	**Mg24 (LR)**	**Rb85 (LR)**	**Sr88 (LR)**	**Y89 (LR)**	**Zr90 (LR)**	**Nb93 (LR)**	**Mo97 (LR)**
Acid washed (Avg)	Below DL	Below DL	18,754.99	15,863.64	Below DL	296.03	Below DL	2959.60	Below DL	651.56
Metal-chelated (Avg)	Below DL	Below DL	3,385,564.10	7,039,607.83	Below DL	9988.28	624.16	1957.61	Below DL	96.28
	Ru101 (LR)	**Rh103 (LR)**	**Pd105 (LR)**	**Ag107 (LR)**	**Cd111 (LR)**	**In115 (LR)**	**Sn118 (LR)**	**Sb121 (LR)**	**Te128 (LR)**	**Cs133 (LR)**
Acid washed (Avg)	Below DL	Below DL	880.38	44.72	Below DL	Below DL	3968.59	43.22	Below DL	Below DL
Metal-chelated (Avg)	Below DL	Below DL	1597.71	Below DL	Below DL	Below DL	86.46	Below DL	Below DL	808.77
	Ba138 (LR)	**La139 (LR)**	**Ce140 (LR)**	**Pr141 (LR)**	**Nd143 (LR)**	**Sm147 (LR)**	**Eu151 (LR)**	**Gd157 (LR)**	**Tb159 (LR)**	**Dy161 (LR)**
Acid washed (Avg)	35.92	Below DL	5.64	Below DL	6.04	Below DL	Below DL	Below DL	Below DL	Below DL
Metal-chelated (Avg)	1303.43	1092.41	449.46	249.85	962.36	186.46	29.76	151.34	24.32	138.35

Compound 109	**Metal (ng/g)**									
	Ho165 (LR)	**Er166 (LR)**	**Tm169 (LR)**	**Yb172 (LR)**	**Lu175 (LR)**	**Hf178 (LR)**	**Ta181 (LR)**	**W184 (LR)**	**Re187 (LR)**	**Os190 (LR)**
Acid washed (Avg)	Below DL	Below DL	Below DL	Below DL	Below DL	29.32	Below DL	52.44	Below DL	Below DL
Metal-chelated (Avg)	24.57	68.19	6.66	62.81	10.93	15.05	Below DL	Below DL	Below DL	Below DL
	Ir193 (LR)	**Pt195 (LR)**	**Au197 (LR)**	**Hg200 (LR)**	**Tl205 (LR)**	**Pb208 (LR)**	**Bi209 (LR)**	**Th232 (LR)**	**U238 (LR)**	**Al27 (MR)**
Acid washed (Avg)	2067.40	Below DL	Below DL	10547.90	Below DL	133.01	Below DL	259.39	154.64	11,435.19
Metal-chelated (Avg)	Below DL	Below DL	Below DL	7829.05	Below DL	207.83	24.95	265.58	210.46	17,885.66
	Si28 (MR)	**P31 (MR)**	**S32 (MR)**	**Ca44 (MR)**	**Sc45 (MR)**	**Ti47 (MR)**	**V51 (MR)**	**Cr52 (MR)**	**Mn55 (MR)**	**Fe56 (MR)**
Acid washed (Avg)	Below DL	11,675.02	77,109,829.82	24,763.37	Below DL	608.87	1062.15	1945.30	Below DL	34,756.06
Metal-chelated (Avg)	Below DL	12,375.36	84,926,735.11	20,483,701.53	Below DL	1101.77	1006.78	1282.95	3088.25	38,189.35

Compound 109	**Metal (ng/g)**									
	Co59 (MR)	**Ni60 (MR)**	**Cu63 (MR)**	**Zn66 (MR)**	**Ga69 (MR)**	**Ge72 (MR)**	**K39 (HR)**	**Ge72 (HR)**	**As75 (HR)**	**Se78 (HR)**
Acid washed (Avg)	291.10	2627.81	2586.13	9955.93	Below DL	24.65	Below DL	45.65	Below DL	12,234.66
Metal-chelated (Avg)	220.79	2784.16	2916.85	71,155.08	Below DL	Below DL	26,703.53	Below DL	38.46	21,974.08

APPENDIX G: EVALUATION OF ANTIBACTERIAL ACTIVITY

Hole-plate antibacterial assay

The assessment of the antibiotic activiy of tetramic acids against *Staphylococcus aureus* DS267, a Gram-positive bacterium and *Escherichia coli* X580, a Gram-negative bacterium, was made using the hole plate method with Cephalosporin C as a standard. The agar plates for the bio assay were prepared by Wendy Sobey at the Department of Chemistry.

Bioassay for drug candidates in the absence of serum albumin

The samples were prepared as 4 mg/mL solutions of 70 % DMSO in MeOH, with serial dilution to the desired concentrations where necessary. A 100 µL aliquot of each sample solution to be tested was loaded into 10 mm wells in agar plates and incubated overnight (18 h) at 37 °C. The assays were repeated in triplicates and the diameters of the resultant inhibition zones were measured (±1 mm) along two perpendicular axes and then averaged to obtain the zone of inhibition. A 'blank' was run with solvent alone, to ensure the solvent made no contribution to the zone of inhibition. The relative potency was estimated by reference to standards prepared with cephalosporin C.

Bioassay for drug candidates in the presence of serum albumin

The albumin binding assay for drug candidates were carried out, in the presence of human serum albumin (HSA) at 10 % the albumin concentration in human serum. A stock solutions of 8 mg/mL of HSA was prepared by dissolving in H_2O. Solutions of the required concentration of compounds to be tested were prepared in 70 % DMSO in MeOH. A 50 µL aliquot of each sample solution was loaded into 10 mm wells in agar plates along with a 50 µL aliquot of an aqueous stock solution of HSA (8 mg/mL) and incubated overnight at 37 °C. The final concentration of HSA in the well was 4 mg/mL, which is 10 % of the physiological

serum albumin concentration. The assays were repeated in triplicates and the diameters of the resultant inhibition zones were measured (±1 mm) along two perpendicular axes and then averaged to obtain the zone of inhibition. These data were compared with the bioactivity of each sample in the absence of HSA.

Calibration data for hole-plate antibacterial assay

Table G.1. An example of calibration data for a bioassay (na= not active).

Ceph C stock solution (µg/mL)	Ceph C stock solution (µL/well)	Water (µL/well)	Cephalosprin C		Zone size (mm)	log nmoles/well
			µg/well	nmoles/ well		
S. aureus						
1000	20	80	20	48.1	na	1.68
	40	60	40	96.3	13	1.98
	60	40	60	144.4	16	2.16
	80	20	80	192.6	18	2.28
	100	0	100	240.7	19	2.38
E. coli						
10	20	80	0.2	0.481	17	-0.318
	40	60	0.4	0.963	22	-0.016
	60	40	0.6	1.444	25	0.160
	80	20	0.8	1.926	26	0.285
	100	0	1	2.407	27	0.381
100	20	80	2	4.815	30	0.683
	40	60	4	9.629	33	0.984
	60	40	6	12.444	35	1.095
	80	20	8	19.259	36	1.285
	100	0	10	24.073	38	1.382

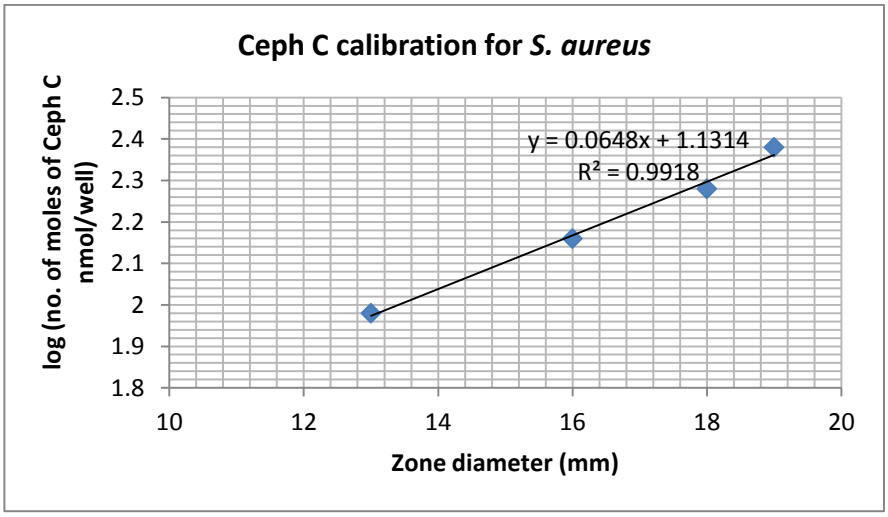


Figure G.1. Calibration curve for *S. aureus*

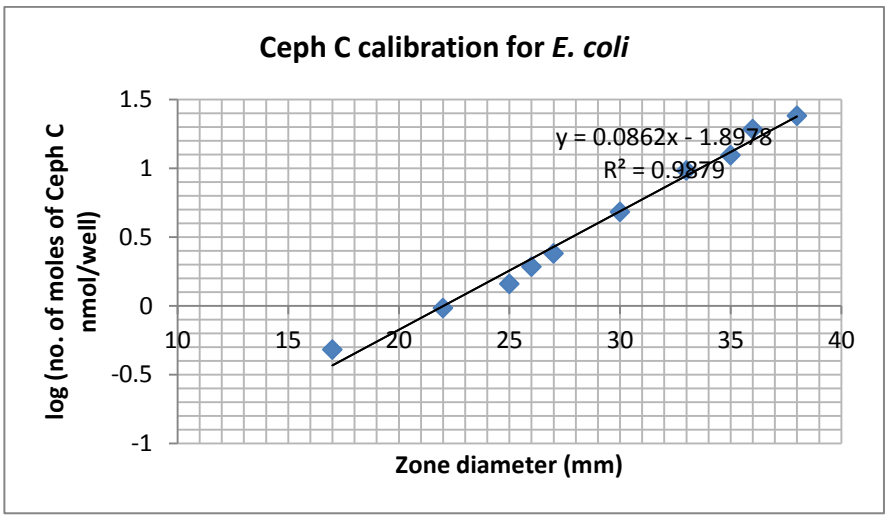


Figure G.2. Calibration curve for *E. coli*

Data from hole-plate antibacterial assay

The bactericidal effect of a given sample was compared with that of the Ceph C standard by determining the concentration of Ceph C required to confer the same zone of inhibition as the test compound. This is expressed as the 'relative potency' (rel. potency), where rel. potency = equivalent no. of moles of Ceph C/no. of moles of sample. The equivalent no. of moles of Ceph C for a given zone size was calculated from the calibration curves in Figures G.1-2.

Table G.2. Antibacterial activity of tetramates agaisnt *S. aureus* determined by hole-plate method (na = not active).

Compou-nd	**Concent-ration (µg/mL)**	**Zone size (mm)** *S. aureus*				**Rel. potency**
		Trial 1	**Trial 2**	**Trial 3**	**Average**	
90a	4000	na	na	na	na	-
91a	4000	na	na	na	na	-
92a	4000	na	na	na	na	-
93a	4000	na	na	na	na	-
94a	4000	12	12	12	12	0.072
95a	4000	na	na	na	na	-
109	1	20	19	19	19.3	988.14
111	1	20	19	20	19.7	1095.00
112	1	19	19	20	19.3	1096.60
113	1	20	19	20	19.7	1181.98
114	50	25	24	25	24.7	43.16
115	2	18	18	19	18.3	450.51
116	10	20	20	20	20	120.93
117	50	12	13	13	12.7	8.62
118	10	17	17	17	17	83.11
119	25	21	21	20	20.7	47.52
120	50	21	21	21	21	25.97
121	100	15	14	13	14	4.86
122	25	19	20	20	19.7	46.26
123	4000	25	23	22	23.3	0.56
124	4000	18	18	18	18	0.21
129	500	26	26	26	26	4.61
252	1000	20	18	19	19	0.74

Table G.3. Antibacterial activity of tetramates against *E. coli* determined by hole-plate method (other tetramates were not active).

Compou-nd	Concent-ration (μg/mL)	Zone size (mm) *E. coli*				Rel. potency
		Trial 1	Trial 2	Trial 3	Average	
252	4000	20	19	19	19.3	4.70×10^{-4}

Data from hole-plate antibacterial assay with HSA tabulated with the data obtained in the absence of HSA

Table G.4. Antibacterial activity of tetramates agaisnt *S. aureus* compared in the presence/absence of HSA by hole-plate method.

Compound	Concentration (μg/mL)	HSA		Zone size (mm) *S. aureus*			
		with	without	Trial 1	Trial 2	Trial 3	Avg.
109	1		✓	20	19	19	19.3
	5	✓		19	19	18	18.7
111	1		✓	20	19	20	19.7
	5	✓		20	19	20	19.7
112	1		✓	19	19	20	19.3
	5	✓		14	14	14	14
113	1		✓	20	19	20	19.7
	5	✓		16	16	15	15.7
114	50		✓	25	24	25	24.7
	50	✓		21	20	21	20.7
115	2		✓	18	18	19	18.3
	50	✓		21	21	21	21
116	10		✓	20	20	20	20
	50	✓		20	20	20	20
117	50		✓	12	13	13	12.7

	100	✓		20 (H)	20 (H)	20 (H)	20 (H)
118	10		✓	17	17	17	17
	50	✓		18	18	18	18
119	25		✓	21	21	20	20.7
	100	✓		18	18	18	18
120	50		✓	21	21	21	21
	250	✓		17	18	17	17.3
121	100		✓	15	14	13	14
	500	✓		15	15	14	14.7
122	25		✓	19	20	20	19.7
	250	✓		22	22	22	22
123	4000		✓	25	23	22	23.3
	4000	✓		21	22	22	21.7
124	4000		✓	18	18	18	18
	4000	✓		16	17	17	16.7
129	500		✓	26	26	26	26
	500	✓		24	24	26	24.7
252	1000		✓	20	18	19	19
	1000	✓		19	19	18	18.7

Table G.5. Antibacterial activity of tetramate **252** against *E.coli* compared in the presence/absence of HSA by hole-plate method.

Compound	**Concentration (µg/mL)**	**HSA**		**Zone size (mm)** *E. coli*			
		with	**without**	**Trial 1**	**Trial 2**	**Trial 3**	**Avg.**
252	4000		✓	20	19	19	19.3
	4000	✓		19	18	19	18.7

Data from broth dilution assay to detremine MICs

Broth assays were performed to determine MICs of compounds in collaboration with Sealife Pharma. The assay method followed at Sealife Pharama is as follows.

The extracts were tested in a primary 96 well plate screening assay, according to SOP 0906. The substances were diluted in "MHB" for bacterial screening to a stock solution of 1000 µg/mL, serial diluted and overlaid with a microbe solution in a concentration of 10^4 CFU/ml. The plates were incubated for 24 h at 35 °C.

Bacterial strains against which the antibacterial activity of the compounds were tested:

Gram-negative	**Gram-positive**
Escherichia coli	Multi-drug resistant *Staphylococcus aureus* (MRSA)
Pseudomonas aeruginosa	*Enterococcus feacalis*
Klebsiella pneumoniae	*Streptococcus pneumoniae*

Compounds tested were either only weakly active or not active against Gram-negative strains. Toxicity studies on some candidates were done on HaCat mammalian cell line.

Table G.6. MICs of tetramates determined by broth dilution method (na = not active, cont.= contaminated)

Compound	MIC (μg/mL)						Toxicity (μg/mL)
	Escherichia coli	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	**MRSA**	***Enterococcus feacalis***	***Streptococcus pneumoniae***	
90a	250	250	250	na	na	250	200
91a	250	250	250	na	250	125	125
92a	na	na	na	na	na	na	150
93a	250	250	250	na	na	na	65
94a	250	250	250	125	125	125	80
95a	na	na	na	na	na	na	62
109	na	na	na	0.98	0.98	0.98	1
110	na	na	na	1.95	0.49	0.49	3
111	na	na	na	1.95	0.49	0.49	5
112	na	na	na	1.95	0.49	0.49	20
113	na	na	na	1.95	0.49	0.98	15.6
114	na	na	na	1.95	1.95	1.95	65
115	na	na	na	7.81	0.49	0.98	16
116	na	na	na	na	na	na	35
117	na	na	na	na	na	na	6

118	na	na	na	na	na	na	31
119	na	na	na	1.95	0.49	1.95	16
120	na	na	na	0.49	0.49	0.49	10
121	na	na	na	31.25	0.98	31.25	31
122	na	na	na	1.95	0.49	3.9	16
123	na	na	na	na	na	na	250
124	na	na	na	na	na	na	
125	na	na	na	125	62.5	125	50
126	na	na	-	0.49	0.49	-	-
127	na	na	-	0.49	0.98	-	-
129	250	250	250	na	125	125	16
130	na	na	-	na	cont	-	-
131	na	na	-	250	cont	-	-
132	250	250	-	0.49	0.49	-	-
133	na	na	na	na	na	na	200
134	na	na	na	na	na	na	125
135	250	250	-	250	250	-	-
136	na	-	-	na	-	-	-
137	na	-	-	na	-	-	-

138	na	-	-	na	-	na	-
139	250	-	-	125	-	250	-
140	na	-	-	3.91	-	0.98	-
175	na	na	na	15.63	0.49	7.81	30
177	na	na	na	3.91	3.91	3.91	1
178	na	na	na	62.5	15.63	15.63	3.9
179	na	na	-	0.49	0.49	-	-
180	na	na	-	31.25	cont.	-	-
181	na	na	-	1.95	cont.	-	-
182	na	na	-	na	na	-	-
183	na	na	-	0.49	0.49	-	-
184	na	na	-	na	na	-	-
185	na	-	-	na	na	-	-
186	na	na	-	0.49	0.98	-	-
187	na	na	na	na	na	na	150
192	na	na	na	15.63	1.95	7.81	125
193	na	na	na	na	na	na	250
194	250	250	-	0.49	0.49	-	-
195	na	na	na	na	na	na	50

196	250	250	-	na	na	-	-
197	na	na	na	na	na	na	31
202	na	na	na	na	na	na	31
207	250	250	-	0.49	0.49	-	-
216	250	250	-	0.49	0.49	-	-
218a	na	250	-	0.49	0.49	-	-
218b	na	250	-	31.25	31.25	-	-
218c	250	250	-	na	na	-	-
219	na	na	-	31.25	cont.	-	-
220	na	na	-	1.95	1.95	-	-
221	na	na	-	250	cont.	-	-
224	na	na	na	na	na	na	45
225	na	na	-	na	cont.	-	-
229	na	na	-	3.91	cont.	-	-
238	na	-	-	250	-	-	-
239	na	-	-	na	-	-	-
240	na	-	-	0.98	1.95	-	-
241	na	-	-	1.95	3.91	-	-
242	na	na	na	na	na	na	

243a	na	na	na	62.5	3.91	15.63	31.25
243b	na	na	na	na	na	na	62
244	na	na	na	na	na	na	40
248	na	na	na	na	na	na	-
249	na	na	na	na	na	na	-
250	na	na	na	na	na	na	-
251	na	na	na	na	na	na	-

APPENDIX H: ENZYME INHIBITION STUDIES

Enzyme inhibition assays were run in collaboration with Prof. A. Maxwell at John Innes Center & Inspiralis Limited. The assay method is as follows.

Assay Set Up

In all assays, the activity of the enzyme was determined prior to the testing of the compounds and 1 U defined as the amount of enzyme required to just fully supercoil or decatenate the substrate. Compounds were tested at 100 μM. Final DMSO concentration in the assays was 1% (v/v).

***E. coli* gyrase supercoiling**

1 U of DNA gyrase was incubated with 0.5 μg of relaxed pBR322 DNA in a 30 μl reaction at 37 °C for 30 minutes under the following conditions: 35 mM Tris.HCl (pH 7.5), 24 mM KCl, 4 mM $MgCl_2$, 2 mM DTT, 1.8 mM Spermidine, 1 mM ATP, 6.5% (w/v) glycerol and 0.1 mg/ml BSA.

Each reaction was stopped by the addition of 30 μl chloroform/iso-amyl alcohol (26:1) and 20 μl Stop Dye (40% sucrose, 100 mM Tris.HCl (pH 7.5), 10 mM EDTA, 0.5 μg/ml bromophenol blue), before being loaded on a 1.0% TAE (Tris.acetate 0.04 mM, EDTA 0.002 mM) gel run at 80V for 2 h.

***M .tuberculosis* gyrase supercoiling**

1 U of *M. tuberculosis* gyrase (final concentration in assay 13nM) was incubated with 0.5 μg of relaxed pBR322 DNA in a 30 μl reaction at 37 °C for 30 minutes under the following conditions: 50 mM HEPES. KOH (pH 7.9), 6 mM magnesium acetate, 4 mM DTT, 1 mM ATP, 100 mM potassium glutamate, 2 mM spermidine and 0.05 mg/ml albumin.

Each reaction was stopped by the addition of 30 µl chloroform/iso-amyl alcohol (26:1) and 20 µl Stop Dye (40% sucrose, 100 mM Tris.HCl (pH 7.5), 10 mM EDTA, 0.5 µg/ml bromophenol blue), before being loaded on a 1.0% TAE (Tris.acetate 0.04 mM, EDTA 0.002 mM) gel run at 80V for 2 h.

S.aureus topo IV decatenation

1 U of *S. aureus* topo IV was incubated with 200 ng kDNA DNA in a 30 µl reaction at 37 °C for 30 minutes under the following conditions: 50 mM Tris.HCl (7.5), 5 mM $MgCl_2$, 5 mM DTT, 1.5 mM ATP, 350 mM potassium glutamate and 0.05 mg/ml BSA.

Each reaction was stopped by the addition of 30 µl chloroform/iso-amyl alcohol (26:1) and 20 µl Stop Dye (40% sucrose, 100 mM Tris.HCl (pH 7.5), 10 mM EDTA, 0.5 µg/ml bromophenol blue), before being loaded on a 1.0% TAE (Tris.acetate 0.04 mM, EDTA 0.002 mM) gel run at 80V for 2 h.

Data acquisition and analysis

Bands were visualised by ethidium staining for 10 minutes, destained for 10 minutes in water and analysed by gel documentation equipment (Syngene, Cambridge, UK) and quantitated using Syngene Gene Tools software. Raw gel data (fluorescent band volumes) collected from Syngene, GeneTools gel analysis software were converted to a % of the 100% control (the fully supercoiled or decatenated DNA band). These were analyzed using SigmaPlot Version 12.5 (2014).

Table H.1. Results for enzyme inhibition of *E. coli* gyrase, *M. tuberculosis* gyrase and *S. aureus* topo IV.

CFX (ciproflaxacin) was used as a standard. No inhibition of *E. coli* gyrase supercoiling was observed.

Compound	*M. tuberculosis* gyrase supercoiling			*S. aureus* topo IV decatenation		
	% Supercoiled Assay 2	% Supercoiled Assay 1	Avg.	% Decatenated Assay 1	% Decatenated Assay 2	Avg.
Enzyme alone	100	100	100	100	100	100
DMSO	82.51	105.38	93.95	100.0	98.65	99.32
CFX	2.49	3.44	2.96	0.00	0.00	0.00
90a	95.35	120.35	107.85	100.0	105.05	102.53
91a	98.08	103.65	100.86	6.93	6.29	6.61
92a	96.80	112.48	104.64	98.11	86.60	92.36
93a	69.81	64.63	67.22	33.31	22.77	28.04
94a	94.02	95.86	94.94	62.13	69.59	65.86
95a	73.77	81.10	77.44	17.26	11.02	14.14
109	28.10	25.30	26.70	26.12	6.66	16.39
110	19.04	19.79	19.42	6.26	8.67	7.47
111	12.50	13.24	12.87	16.11	8.70	12.40
112	21.34	21.50	21.42	10.46	13.77	12.12
113	31.14	22.18	26.66	10.40	10.31	10.36
114	13.84	17.85	15.85	4.07	2.00	3.04
115	9.69	31.78	20.74	18.15	7.61	12.88
116	13.43	18.17	15.80	15.21	11.42	13.31
117	20.54	15.58	18.06	17.06	3.70	10.38
118	24.64	15.52	20.08	25.48	16.29	20.88
119	19.82	8.76	14.29	8.19	7.25	7.72
120	43.06	19.52	31.29	24.33	19.31	21.82
121	16.82	9.70	13.26	7.82	3.11	5.47
122	19.38	4.13	11.75	8.85	8.50	8.67

123	2.50	13.03	7.77	11.99	6.70	9.34
124	12.13	7.96	10.04	6.93	3.35	5.14
129	51.32	52.83	52.07	21.99	16.02	19.00
133	7.68	6.69	7.19	50.30	52.16	51.23
134	75.79	87.88	81.84	110.56	100.83	105.70
175	32.05	27.91	29.98	12.37	31.43	21.90
192	57.04	70.43	63.73	65.47	73.23	69.35
193	84.21	82.44	83.33	106.54	99.17	102.85
195	20.32	15.10	17.71	31.93	26.94	29.44
197	0	0	0	4.70	3.60	4.15
242	70.35	81.22	75.79	17.69	21.32	2.00
243b	7.52	36.16	21.84	9.24	14.11	11.67

Table H.2. IC_{50} values for *M. tuberculosis* gyrase and *S. aureus* topo IV inhibition (nd = not determined).

Compound Number	**IC_{50} (μM)**	
	***M. tuberculosis* gyrase supercoiling**	***S. aureus* topo IV decatenation**
CFX	29.6	4.11
109	nd	3.8
111	48.9	3.0
116	54.1	1.22
120	nd	61.0
122	65.4	4.9
123	60.3	10.3
133	78.0	nd
197	52.4	25.3
243b	110.0	56.9

Table H.3. IC_{50} values for *E. coli* RNAP and *S. aureus* RNAP inhibition. (Determined in Collaboration with Dr. Richard H. Ebright at Waksman Institute of Microbiology, Rutgers University).

Compound number	***E. coli* RNAP**	***S. aureus* RNAP**
109	43	8.8
115	61	5.9
120	63	13.0

APPENDIX I: BINDING TO HSA; A LIGAND-DISPLACEMENT ASSAY BASED ON NMR SPECTROSCOPY

The binding of tetramates to HSA was determined by quantifying the amount of ligand (L-Trp) displaced in a competitive ligand displacement assay. All ^{1}H spectra were obtianed using zg30 (normal ^{1}H spectrum, ns=64), noesygppr1d (water suppressed, ns=64) and CPMG method for HSA studies (water suppressed with background filter for proteins, ns=256) on Bruker AVIIIHD 600 (600 MHz) NMR spectrometer and processed using TOPSPIN (Bruker) v3 software.

Preparation of stock solutions

The following stock solutions were prepared in deuterated solvents.

- 50 µL of 250 mM stock solution of the tetramate under study in DMSO
- 1 mL of 100 mM stock solution of L-tryptophan in DMSO
- 100 mL of 50 mM phosphate buffer solution in D_2O
- 2 mL of 200 µM stock solution of HSA in phosphate buffer solution

Preparation of 1:1 L-trp:HSA solution to ensure complete binding of ligand to HSA

A 1:1 solution of HSA and L-tryptophan were prepared by dissolving 1 µL of 100 mM L-trp in a solution of 499 µL of 200 µM HSA and 500 µL of phosphate buffer, where the final concentrations of HSA and L-trp in solution were 100 µM each. This 1:1 L-trp:HSA solution was analysed by NMR spectroscopy to ensure all of L-trp is bound to HSA (i.e. no free L-trp ligand is observed in the NMR spectrum).

Preparation of 100 µM L-trp as a reference for quantification

A 100 µM solution of L-trp was prepared by dissolving 1 µL of 100 mM stock solution of L-trp in 999 µL of phosphate buffer.

Displacement of L-trp bound to HSA by a competotior ligand (tetramate)

Before each displacement experiment, the purity of each compound was checked by ^{1}H NMR spectroscopy (1.0 mM of the compound in phosphate buffer).

Preparation of sample for the displacement assay: 4 µL of 250 mM competitor ligand was added to a 1:1 solution of HSA and L-trp according to the table below, where the concentrations of each component in the final 1 mL solution were as follows:

- HSA: 100 µM
- L-Trp: 100 µM
- Competitor ligand (tetramate): 1.0 mM

100 µM HSA	**100 mM L-Trp**	**Phosphate buffer**	**250 mM Competitor ligand (tetramate)**
500 µL	1 µL	495 µL	4 µL

The sample was then analysed by NMR spectroscopy. All experiments were carried out in triplicates.

The amount of L-trp displaced was quantified using 'lastscale for calibration' with the spectrum obtained for 100 µM L-Trp alone as the reference. All integrations were made with respect to the ^{1}H signal of L-trp at 7.01 ppm.

The % displacment of L-trp was calculated using the following formula,

$$\%\ \text{Displacement of L} - \text{Trp} = \frac{ITrp\ displaced}{ITrp} \times 100$$

where,

ITrp displaced = integral of L-trp displaced from HSA by competitor ligand

ITrp = integral of L-trp signal alone

Table I.1. % Displacement of L-Trp by tetramates

Compound	% Displacement of L-Trp			
	Trial 1	**Trial 2**	**Trial 3**	**Avg.**
113	45.15	35.08	42.03	40.75
115	58.82	64.60	59.96	61.13
119	84.41	90.08	84.83	86.44
122	70.32	84.41	76.27	77.00
126	74.19	73.89	69.43	72.50
127	75.04	66.73	63.76	68.51
132	69.01	67.62	67.26	67.96
140	88.55	84.60	89.05	87.40
175	44.09	34.59	34.11	37.60
181	36.71	36.95	39.13	37.60
183	31.63	34.02	36.30	33.98
186	23.01	19.31	17.95	20.09
192	0	0	0	0
194	54.88	48.04	55.26	52.73
207	0	0	0	0
216	32.73	29.57	34.96	32.42
218a	43.55	43.00	46.08	44.21
239	19.99	17.56	21.33	19.63
241	13.56	10.80	14.07	12.81

APPENDIX J: PHYSICOCHEMICAL AND STRUCTURE PROPERTIES

Physicochemical and structure properties calculated for the tetramate library are listed in Table J.1. Calculator Plugins were used for physicochemical and structure property prediction and calculation, Marvin (16.4.18.0), 2016 ChemAxon (http://www.chemaxon.com).

Table J.1. Physicochemical and structure properties of the tetramate library.

Compound	MW	clogP	clogD7.4	PSA	MSA	%PSA	H-bond donor count	H-bond acceptor count
90a	305.4	1.79	-1.69	66.84	390.3	17.12529	1	3
91a	384.2	2.56	-0.95	66.84	410.28	16.29131	1	3
92a	323.3	1.94	-1.58	66.84	397.35	16.82144	1	4
93a	350.4	1.73	-1.78	109.98	397.35	27.67837	1	5
94a	357.8	2.54	-0.98	66.84	412.32	16.21071	1	4
95a	295.3	0.85	-2.66	79.98	362.65	22.05432	1	3
109	410.5	2.57	0.28	69.64	542.39	12.83947	2	3
110	489.4	3.33	0.7	69.64	562.99	12.36967	2	3
111	428.5	2.71	0.12	69.64	549.61	12.6708	2	4
112	455.5	2.51	-0.15	112.78	580.84	19.41671	2	5
113	463	3.31	0.47	69.64	565.09	12.3237	2	4
114	400.5	1.63	-0.98	82.78	515.13	16.06973	2	3
115	434.6	4.61	3.96	69.64	587.77	11.84817	2	3
116	452.5	4.75	3.82	69.64	594.51	11.71385	2	4

117	479.6	4.55	3.57	112.78	626.99	17.98753	2	5
118	487	5.35	4.16	69.64	611.07	11.3964	2	4
119	400.9	3.61	2.77	69.64	482.25	14.44064	2	3
120	418.9	3.75	2.64	69.64	489.6	14.22386	2	4
121	445.9	3.35	2.39	112.78	520.6	21.66347	2	5
122	453.3	4.36	2.99	69.64	505.17	13.78546	2	4
123	516.4	3.15	2.17	82.11	589.75	13.92285	2	5
124	427.5	1.44	0.46	95.25	541.85	17.57867	2	5
125	439.3	1.2	-1.55	78.87	488.94	16.13081	2	4
126	358.46	2.27	-0.01	69.64	482.97	14.41912	2	3
127	410.9	3.02	0.12	69.64	505.13	13.78655	2	4
129	352.4	2.49	1.9	69.64	434	16.04608	2	3
130	430.5	1.33	0.29	103.78	528.49	19.63708	2	5
131	499.6	2.4	1.36	107.02	645.64	16.5758	2	5
132	501.6	1.33	0.29	116.25	631.18	18.41788	2	6
133	698.7	1.68	0.91	193.3	909.07	21.26349	2	9
134	530.6	-0.08	-0.85	169.02	651.78	25.93206	6	9
135	410.1	2.13	-1.66	106.94	503.73	21.22963	3	5
136	729.8	6.49	5.43	142.11	916.35	15.50827	3	7
137	507.6	2.62	1.58	129.8	619.13	20.9649	3	6
138	578.5	3.16	1.87	107.02	665.71	16.07607	2	5
139	396.9	2.29	-0.67	60.85	478.85	12.70753	1	4
140	515	5.05	2.14	60.85	678.38	8.969899	1	4

175	476.6	3.27	0.86	82.78	623.28	13.28135	2	3
177	514.6	3.92	1.63	86.71	679.05	12.76931	2	4
178	558.7	4.2	1.91	88.1	747.73	11.78233	2	4
179	450.6	3.66	1.35	69.64	606.88	11.47509	2	3
180	564.7	3.05	0.74	103.78	744.08	13.94743	2	5
181	505.6	2.91	0.46	95.67	679.37	14.08216	2	4
182	530.6	3.84	1.5	88.1	687.28	12.81865	2	5
183	490.6	2.64	0.31	87.46	655.48	13.34289	2	4
184	548.66	3.25	0.82	113.88	730.56	15.58804	2	7
185	527.6	3.4	1.08	99.83	675.42	14.78043	2	5
186	560.7	3.46	1.1	96.69	759.82	12.72538	2	5
187	426.5	1.14	-1.37	92.01	547.43	16.80763	2	4
192	651.6	0.99	-2.52	190.5	865.53	22.00964	1	9
193	483.5	-0.78	-4.28	166.22	608.11	27.33387	5	9
194	321.4	1.49	-2.01	87.07	400.5	21.74032	2	4
195	756.8	1.76	-0.73	193.3	1018.15	18.98541	2	9
196	756.7	1.32	-3.08	230.6	977.83	23.58283	2	11
197	588.7	-0.01	-2.49	169.02	759.97	22.24035	6	9
202	939.9	0.54	-2.96	287.86	1264.01	22.77355	1	14
207	665.7	1.02	-2.47	190.5	899.02	21.18974	1	9
216	335.4	1.03	-2.47	87.07	432.12	20.1495	2	4
218a	426.5	2.26	-0.17	89.87	554.98	16.19338	3	4
218b	368.4	2.19	1.47	89.87	446.54	20.12586	3	4

218c	376.4	0.13	-2.41	99.1	480.67	20.61706	3	5
219	512.6	2.39	-0.09	105.17	696.78	15.09372	2	5
220	478.6	3.47	0.99	78.87	642.1	12.28313	2	4
221	484.6	1.89	-4	116.17	628.51	18.4834	3	6
224	333.4	1.51	-2	83.91	417.21	20.11217	1	4
225	430.5	1.36	-2.14	96.38	553.25	17.4207	1	5
229	535.7	2.13	-0.31	99.18	709.13	13.98615	2	5
238	390.5	1.54	-1.81	79.31	525.28	15.09861	1	5
239	374.4	0.83	-2.66	87.15	479	18.19415	1	4
240	495.6	2.45	0.07	82.11	676.54	12.13675	2	5
241	479.6	1.61	-0.83	89.95	630.41	14.26849	2	4
242	331.4	2.53	-0.95	66.84	422.6	15.81637	1	3
243a	378.5	3.23	2.66	69.64	466.01	14.94389	2	3
243b	386.5	1.17	-1.19	78.87	500.55	15.75667	2	4
244	493.5	3.43	2.73	117.53	619.6	18.96869	2	6
248	407.4	1.62	-1.89	102.37	545.62	18.76214	1	5
249	379.4	1.11	-5.8	113.37	476.53	23.79074	2	6
250	712.8	2.39	-1.11	194.92	906.66	21.49869	2	9
251	577.6	0.58	-6.33	162.78	723.61	22.49554	3	8
252	322.4	0.46	-1.68	78.43	430.1	18.23529	4	3