The Synthesis of Hybridised Tetramic Acids for Antibacterial Activity



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ABSTRACT

The Synthesis of Hybridised Tetramic Acids for Antibacterial Activity

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Tetramic acids are a naturally occurring class of antibacterial compounds which have been widely assessed in the literature. Hybridised tetramic acids are those which contain the tetramic acid bonded to another antibacterially active compound with the aim of possessing dual-inhibition functionality. This thesis describes the synthesis and biological evaluation of hybridised tetramic acids.

Chapter I reviews the methods by which drug discovery is employed in the search for novel antibiotics and the need for the discovery of antibiotics possessing novel modes of action. Previously employed tetramic acid syntheses and points of structural manipulation have been reviewed. A discussion of hybrids and a review of hybrids found in the literature has been produced.

Chapter II discusses the synthesis of tetramic acids conjugated to other antibacterial compounds through various linkers. Tetramic acids were successfully conjugated with coumarins, imidazoles, and imidazolines and attempts at synthesising various other conjugates were made. Chapter II also discusses the structural features of the tetramic acids which were troublesome when attempted to purify the hybrid compounds.

Chapter III investigates the synthesis of tetramic acid hybrids by fused hybridisation strategy. To achieve this, attempts were made at synthesising various spirocyclic tetramic acids however, isomerisation rendered the ability to characterise and biologically evaluate these compounds difficult.

The synthesised compounds were evaluated for their antibacterial activity against multidrug resistant pathogens and disappointingly, all but one showed no antibacterial activity.

Acknowledgements

Firstly, I would like to thank my supervisor, Professor Mark Moloney for giving me the opportunity to join his research group. Despite my research providing numerous difficulties and headaches, I have been the recipient of endless support and encouragement throughout – without which I would not be writing this. I cannot thank Mark enough for the unwavering guidance and enthusiasm he has provided.

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Abbreviations

Ac	acetyl
aq.	aqueous
Ar	aromatic
Bn	benzyl
BOC	*tert*-butyloxycarbonyl
Bu	butyl
^{t}Bu	*tert*-butyl
C	Celcius
CLIP-HSQMBC	clean in-phase heteronuclear single quantum multiple bond correlation
COSY	Correlation Spectroscopy
d	day
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N,N'*-dicyclohexylcarbodiimide
DEAD	diethyl azodicarboxylate
DCM	dichloromethane
DMAP	4-dimethylamino pyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide

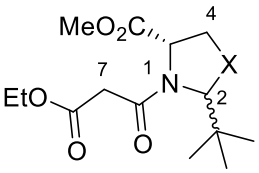
DNA	deoxyribose nucleic acid
dppf	(diphenylphosphino)ferrocene
E	trans
EtOAc	ethyl acetate
EC_{50}	half maximal effective concentration
E. coli	*Escherichia coli*
ent	enantiomer
ESI	electrospray ionisation
eq	equivalents
Et	ethyl
FBDD	fragment based drug design
FT-IR	fourier-transform infra-red
h	hour
Hal	halogen
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single-quantum correlation
HTS	high-throughput screen
IR	infra-red
LRMS	low resolution mass spectrometry

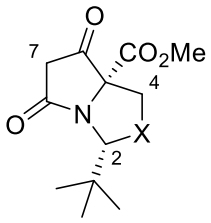
M	metal
m-CPBA	*meta*-chloroperbenzoic acid
Me	methyl
MeCN	acetonitrile
MIC	minimum inhibitory concentration
min	minute
M.p	melting point
MRSA	methicillin resistant *Staphylococcus aureus*
Mw	molecular weight
n.a.	not active
NMR	nuclear magnetic resonance
Ph	phenyl
ppm	parts per million
R_f	retention factor
rt	room temperature
SAR	structure activity relationship
sat.	saturated
S_N2	nucleophilic substitution
S_NAr	nucleophilic aromatic substitution

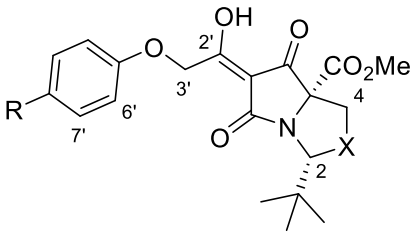
tert	tertiary
TA	tetramic acid
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethyl silyl

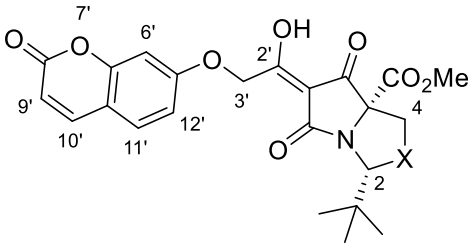
Nomenclature

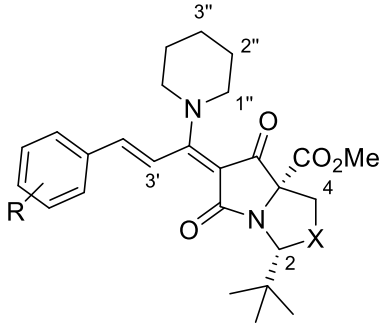
The following non-systematic number systems have been used throughout this thesis to provide direct comparability of spectroscopic data.

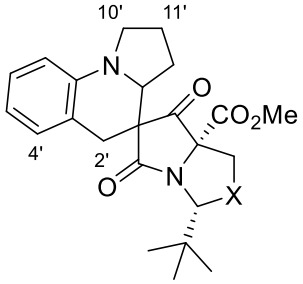












Chapter I: Introduction

Antibiotic discovery

The first antibiotic was reported by Paul Ehrlich early in the 20th Century. Ehrlich discovered that certain chemical dyes would colour some bacterial cells and not others.[1] This observation led to the conclusion that bacteria could be killed selectively over other cells and the discovery of the first antibiotic Arsphenamine, used in the treatment of syphilis, soon followed.[2]

Penicillin, first discovered by Alexander Fleming, proved to be extremely important in the history of antibiotics. However, it was not until some years after the discovery that penicillin was isolated and purified, and marked the first antibiotic drug.[3] In 1943, Selman Waksman had devised a technique to identify natural products possessing antibacterial activity and thus discovered Streptomycin.[4] This approach was applied widely within the scientific community and led to the 'Golden Era' of antibiotic discovery, highlighted in Figure **1.1** below.[5]

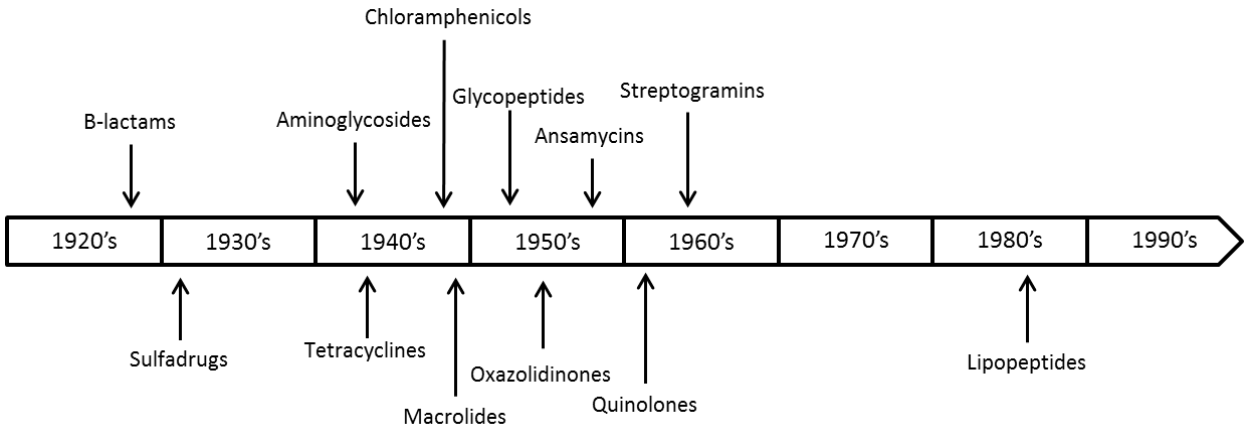


Figure 1.1 Antibiotic discovery timeline. The so called 'Golden-Era' occurred between the 1940s and 1960s.

There was a significant discovery void between the mid 1960's and the mid 1980's, principally arising from the view that the problem of infection had been solved, along with the rising interest in the development of cancer therapies. Lipopeptides were the first class to be discovered after this void period and they were used to treat Gram-positive bacterial infections. Since then, only Bedaquiline was discovered as a novel antibiotic, however this possessed a narrow spectrum of activity, and was used only to treat tuberculosis.[6] Further discovery of novel antibiotics in the post 'golden-era' period has been very limited with most clinically introduced antibiotics being modifications of existing antibiotics.[6]

Complications have arisen from the lack of discovery of novel antibiotics, and by the emergence of resistance, which was predicted by Alexander Fleming in his Nobel Prize acceptance speech.[7] Bacteria have developed resistance to all introduced classes of antibiotics and this resistance is beginning have serious implications to healthcare around the globe. Many infections are becoming increasingly difficult to treat which threatens to send us back into a pre-antibiotic era.[8]

Antibiotic resistance

Bacterial resistance to antibiotic drugs was first observed in the 1930s.[9] More recently, resistance has become widespread due to many factors including increased consumption, use in livestock, international travel and the over-prescription of antibiotics. Worryingly, between the years 2000 and 2010, the use of antibiotics has been estimated to have increased by 35 %.[10] This increased exposure to antibiotics is likely to lead to the more rapid development of antibacterial resistance. 'Last-resort antibiotic' consumption has also increased, and compounds such as carbapenems and polymixins have seen a 40 % and 13 % increase in use respectively. These 'last-resort' drugs are now showing a reduced effect due to antibacterial resistance.[10]

In the European Union alone, around 25,000 people die each year from infections due to multi-drug resistant bacteria.[11] Estimates predict that by 2050, there could be 10 million deaths per year worldwide directly attributed to antibiotic resistance.[12] Additionally, routine surgeries which create open or deep wounds require the use antibiotics to prevent infections and would be of higher risk with the emergence of resistant bacteria.[13]

Antibiotic resistance mechanisms

Resistance primarily occurs through random mutations leading to the administered antibiotic becoming ineffective, and importantly, this resistance can be transferred to other bacteria through horizontal or vertical gene transfer.[14]

Vertical gene transfer is the direct transfer of genetic material from parents to offspring, either sexually or asexually. In the context of antibiotics, those bacteria that mutate for resistance can pass on the resistant genes to their offspring through replication. Once these bacteria start to multiply, the number of bacteria resistant to an antibiotic rapidly increases until an entire colony of bacteria contains resistance to a particular antibiotic.[15]

Horizontal gene transfer in bacteria is very efficient and may proceed with many mechanisms; these include conjugation, transformation, and transduction. A brief summary of these mechanisms are outlined below and graphically represented in Figure **1.2**.[14]

Conjugation occurs through self-replicating plasmids and requires cell-to-cell contact between bacteria, transferring DNA between them. The transfer of plasmids containing DNA conferring antibacterial resistance has been widely observed, and in various ecosystems, from bacteria in soil and water, to food and healthcare relevant pathogens.[14] In humans, this is particularly prevalent and once antibiotic resistance plasmids have been established, they rapidly spread across strains.[16] Plasmid transfer has led to worldwide spread of various

antibiotic resistance genes to a multitude of antibacterial classes such as quinolones, tetracyclines, β-lactams, and many more.[17]

Transformation is a direct genetic exchange between bacteria, be it the same strain or different, where extracellular DNA uptake occurs. This extracellular DNA is then integrated and expressed by the host. By this process, bacteria can exchange antibiotic resistance genes between one another, ultimately leading to a colony of antibiotic resistant bacteria of the same or even different strain.[14] *In vitro* experiments have been performed to track the transformation of antibiotic resistance genes. Sparling *et al.* showed that genes containing resistance to streptomycin, rifampicin, erythromycin, nalidixic acid, and kanamycin could be transferred into *Neisseria gonorrhoea*.[18]

Transduction involves the transfer of genes through bacteriophages. These phages can transfer genes that are advantageous to their hosts, promoting their own survival and dissemination.[14,19] The DNA transferred can be either chromosomal DNA or plasmids. The transfer of antibiotic resistance genes has been well documented for various bacterial strains.[20–22] Certain bacteriophages have been reported to be compatible with a wide host range, across multiple species.[23]

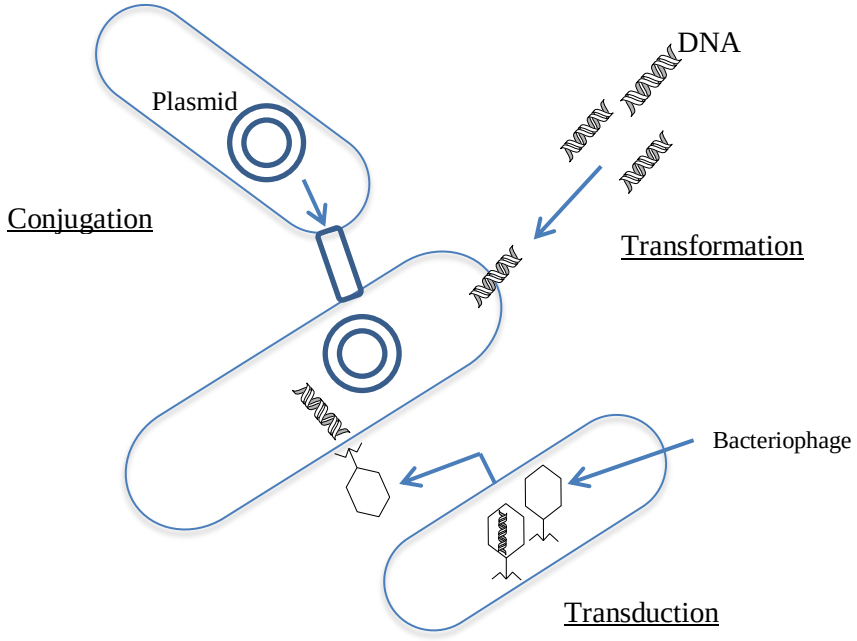


Figure 1.2. Graphical representation of conjugation, transformation and transduction, the mechanisms for horizontal gene transfer.

Methods of drug discovery

The approaches which can be applied to drug discovery takes many shapes and numerous methods have been developed, and include the use of natural products, high-throughput screening, target-based discovery and more recently, computational drug design. These are detailed below, discussed in the context of antibacterial drug development.

Natural product inspired drug discovery

Many naturally occurring compounds have been employed as antibacterial drugs; aminoglycosides, glycopeptides, lipopeptides, macrolides, streptogramin, tetracyclines, and β-lactams all originated from natural products. A review by Butler et al. in 2013 highlighted the importance of natural products in drug discovery even today, despite the focus on more modern techniques. Butler reported that of the 22 novel antibiotics to have been marketed

since 2000, 12 were natural products, or natural-product derived.[24] Despite this, larger pharmaceutical companies have placed less emphasis on the discovery of novel compounds based on natural product structures, although this results more from commercial and IP reasons rather than scientific ones.

Newman et al. conducted a review of all antibacterial compounds approved between 1981 and 2010 and classified them as being natural products, natural product-derived, or synthetic compounds. 74 % of antibiotics fell under the class of either natural product or natural product-derived, highlighting the huge importance of natural products in antibacterial drug discovery.[25]

Target-oriented synthesis and HTS

Drug discovery in the modern era often utilises high-throughput screening, and relies on taking a large number of small molecules and assessing their ability to bind to a chosen enzyme target, selected for its causative role in infection. The synthesis of compounds derived from these 'hits' is then conducted, giving a library of analogues. Alternatively, phenotypic screening against a whole cell or organism can also be employed, without direct knowledge of the biological target.[26]

However, it is now more widely appreciated that the approach of screening against one particular target often results in low chemical space coverage.[27] This is particularly important since, in addition to its bacterial cell killing ability, an antibiotic also needs to permeate bacterial cell walls, and be suitable for oral administration in humans. This information is not always considered when a single protein inhibitor is being designed and therefore the success rate of HTS to develop antibiotics has been particularly low.[28] A compound collection from SmithKline-Beecham comprising over half a million compounds was screened against many

antibacterial targets. From these screens only 5 lead compounds were discovered, which was unsustainably low when considering the large effort invested.[28]

It has been suggested that the low success rate could be due to the lack of chemical diversity in many compound libraries. Significant overlap is often observed in these compound libraries as they are prepared using known, simple chemical techniques, often employing the same or at least similar commercially available starting materials.[28]

Fragment-based drug design (FBDD)

Fragment-based drug design works similarly to a typical HTS, but the key difference is that the fragments are much smaller and are designed to be more complementary to key sites in the biological target (Figure **1.3**).[29] The fragments are then linked, and structural elaboration results in lead compounds. HTS hits can often be low quality and it can be difficult for a chemist to determine important components of the hit.

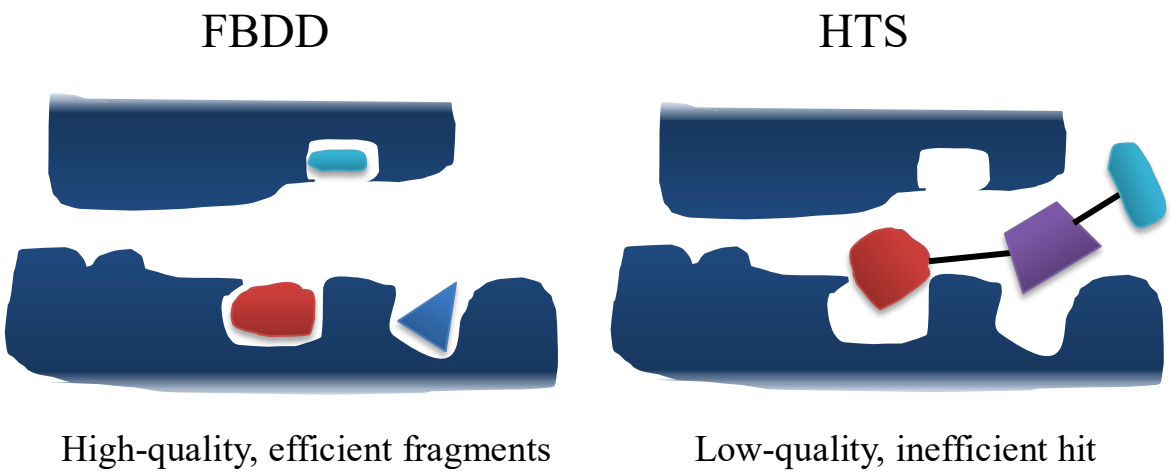


Figure 1.3. Comparison of FBDD and HTS

Knowledge of the target is essential to make high-affinity interactions between the fragments and receptor, often this is done computationally using crystal structures.[30] By crystallising a

natural inhibitor in the receptor, the active site can be elucidated. Subsequent identification of the amino acid residues in the active site can reveal areas for potential interaction.[31] For example, if a lysine (terminal NH_3^+) residue occupies the active site, a favourable functional group for a fragment would contain a negative charge, such as a carboxylate. Crystal structures can sometimes be misleading, since in solution, proteins are flexible and may often adopt multiple conformations. In crystal structures, the protein is static and the structure determined may not be the most favourable conformation.[32]

Fragment-based drug design (FBDD)	**High-throughput screening (HTS)**
Emphasises efficiency	Emphasises potency
Typical library size of $10^2 – 10^3$	Typical library size 10^5+
M_w range ~150 – 300	M_w range ~ 250 – 600
Hit activity range is mM – low µM	Hit activity range is low µM to nM
Clearly defined binding interactions, large ratio of atoms are involved in binding	Often contain functional groups unused in target, low ratio of atoms involved in binding

Table 1.1. The key differences between HTS and FBDD.

Fragments often comply with the 'rule of three'. Similar to Lipinski's guidelines, it is thought that fragments should have particular properties to be seen as suitable for lead discoveries. Properties of 40 fragment hits against a range of targets were examined and the results indicated on average that fragments were: $M_w < 300$, hydrogen bond donors ≤ 3, hydrogen bond acceptors ≤ 3, cLogP = 3.[33]

Tetramic acids

The tetramic acid skeleton **1** is a pyrrolidine-2,4-dione ring system, however in nature it is commonly found as the acylated derivative **2** (Figure **1.4**). Natural products containing the tetramic acid moiety display a wide range of biological activity, including antiviral, antitumor and antibacterial properties.[34] Their presence in many natural products makes them an attractive prospect for drug discovery programs.



1 **2**

Figure 1.4. Tetramic acid skeleton **1** and acylated tetramic acid **2**.

Antibacterial natural products containing tetramic acids

A large number of natural products possessing antibacterial activity containing tetramic acids have been isolated, which show considerable structural diversity around the ring nucleus. Examples in include Equisetin **3**, which was first isolated in 1974 from *Fusarium Equisetin*, a white mould.[35] Equisetin **3** displays activity against Gram-positive bacteria *Bacillus subtilis* and MRSA with MIC values of 8 μg/mL^{-1} and 16 μg/mL^{-1} respectively, which represents appreciable activity.[36] Kibdelomycin **4** was first reported in 2011 by Singh et al. and displayed potent, broad-spectrum activity against Gram-positive bacteria.[37] MIC values were calculated against *E.faecium, S. aureus* & *S. pneumoniae* and showed values of 1, 0.25 and 0.25 μg/mL^{-1} respectively. No activity was observed when assessed against Gram-negative bacteria *E. coli* and *P. aeruginosa.*[38]

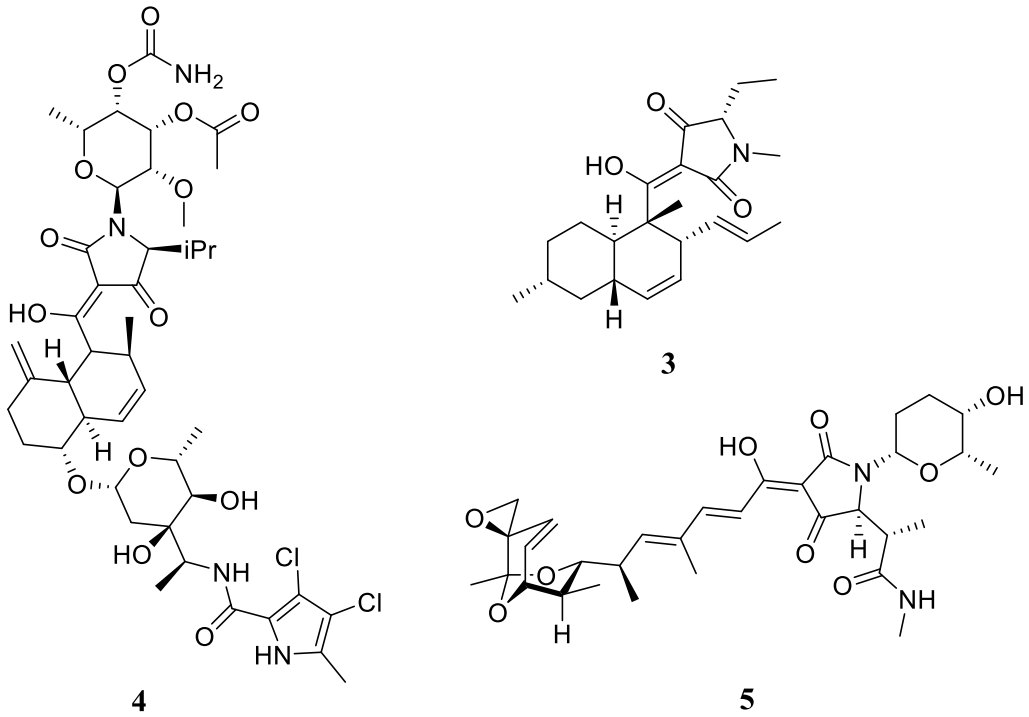


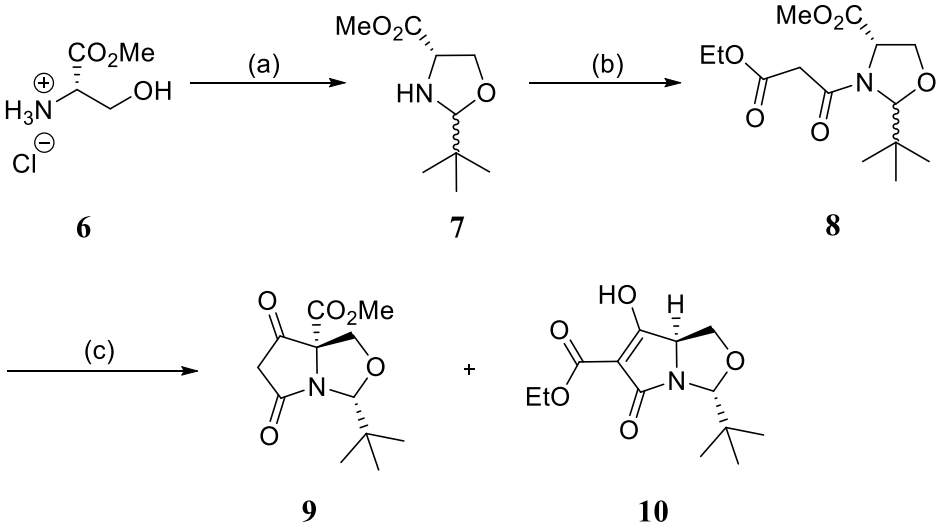
Figure 1.5. Equisetin **3**, Kibdelomycin **4** and Streptolydigin **5**, antibacterial natural products containing a tetramic acid.

Streptolydigin **5** is another natural product which contains the tetramic acid moiety and possesses potent antibacterial activity. First isolated in 1956, Streptolydigin **5** inhibits *Clostridium* and *Streptococcus* bacterial species with MICs observed at 0.04 μg/mL^{-1}.[39] Several other natural products possessing antibacterial activity and the tetramic acid moiety have also been reported. These include Altersetin,[40] Reutericyclin,[41] Tirandalydigin,[42] Tirandamycins,[43] Vancoresmycin,[44] Virgineone,[45] and Zopfiellamides A and B.[46]

Previous work in the Moloney group

Synthesis of the tetramic acid core

The Moloney group has successfully identified a convenient asymmetric synthesis of chiral tetramic acids with the potential for diversity at numerous sites around the skeletal structure (Scheme **1.1**).



Scheme 1.1. Synthesis of tetramic acid **9** from L-serine methyl ester HCl**.** (a) NEt_3, pivaldehyde, petrol, reflux, 18 h (b) Ethyl hydrogen malonate, DCC, DMAP, DCM, 5 h. (c) KO^tBu, ^tBuOH, reflux, 3h.

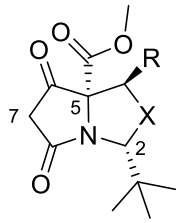
L-Serine ester hydrochloride **6** was reacted with pivaldehyde in presence of NEt_3 in petrol to yield oxazolidine **7**. The mixture of *cis/trans* oxazolidine isomers was then acylated using ethyl hydrogen malonate, DCC and DMAP in DCM to yield a mixture of *N*-acylated

oxazolidine isomers **8**. Subsequent Dieckmann cyclisation using KO^{t}Bu in ^{t}BuOH yielded the desired tetramic acid **9** along with side-product **10**.[47]

The antibacterial activity of tetramic acids **9** and **10** was assessed. It was shown that the tetramic acid cores alone do not possess any intrinsic antibacterial activity against Gram-positive or Gram-negative bacteria. However, it has been shown that with side-chain functionalisation, the tetramic acids can possess significant antibacterial activity and thus, the tetramic acid provides a useful template for antibacterial drug discovery.[48,49]

Functionalisation of the tetramic acid core

Appealingly, the tetramic acid core **9** is amenable to functionalisation through various points and many of these have been exploited within the Moloney group.



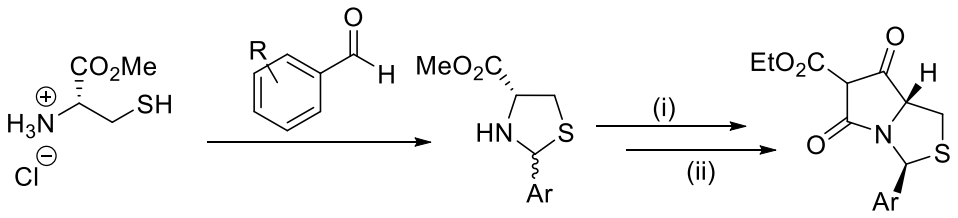
9

9o R = H, X = O, **9s** R = H, X = S, **9t** R = Me, X = O

Functionalisation has been shown possible at C-2, C-5, and C-7 positions and is outlined below.

Functionalisation at C-2

To functionalise the tetramic acid at C2, research in the Moloney group focused on condensing L-cysteine methyl ester hydrochloride **10** with various aromatic aldehydes, compared to the previously used pivaldehyde (Scheme **1.2**).[50] Such change was found not to be possible for serine, due to the lower stability of the corresponding oxazolidine.



10 **11a-g** **12a-g**

Scheme 1.2. Synthesis of C-2 functionalised tetramic acids. (i) Ethyl hydrogen malonate, DCC, DMAP, DCM, 5h. (ii) KO^{t}Bu, THF, reflux, 3h.

With thiazolidines **11a-g** in hand, the tetramic acids **12a-g** were synthesised by application of previously used methodology. *N*-Acylation with ethyl hydrogen malonate in the presence of DCC and DMAP with subsequent Dieckmann cyclisation with KO^{t}Bu and THF (replacing ^{t}BuOH from the original synthesis). Noteworthy is the formation of the 2-*S*, 5-*R* diastereomer as opposed to the 2-*R*, 5-*R* enantiomer observed in the synthesis of the tertiary butyl tetramic acids **9**. Table **1.2** below details the aromatic aldehydes incorporated into the tetramic acid core.[50]

Ar =	Yield (%)
12a = C_6H_5	43
12b = *p*-BrC_6H_4	45
12c = *p*-FC_6H_4	50

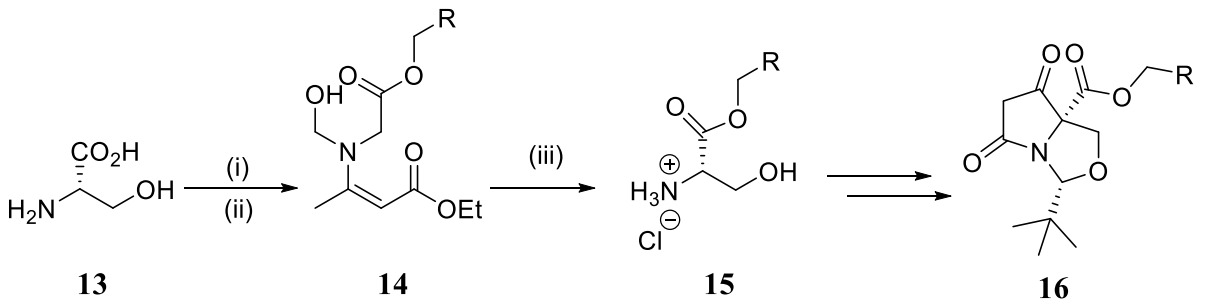
12d = *p*-$NO_2C_6H_4$	52
12e = 2-Cl-4-FC_6H_3	49
12f = *m*-BrC_6H_4	78
12g = 2-C_4H_3O	40

Table 1.2. Various synthesised tetramates functionalised at C2.

Recent, unpublished work in the Moloney group has also successfully condensed halogen substituted pyridine aldehydes at the C-2 position. In addition to this, further structural elaboration has been possible through cross-coupling reactions between **12b** and a variety of substrates (Zhang, unpublished results).

Functionalisation at C-5

C-5 Functionalisation to expand the range of available serine esters has only been achieved recently and required manipulation and protection of L-serine. Inspired by work by Maclaren *et al.* where L-serine was functionalised with various benzyl halides through S_N2 reaction with the carboxylic acid present in L-serine,[51] various C-5 substituted tetramates have been synthesised. Initially, L-serine was condensed with ethyl acetoacetate in presence of N,N,N,N-tetramethyl guanidine base in DMF. After stirring and protection of the amine group, alkyl or benzyl bromide was subsequently added to yield the enamine amino acid esters **14** (Scheme **1.3**).

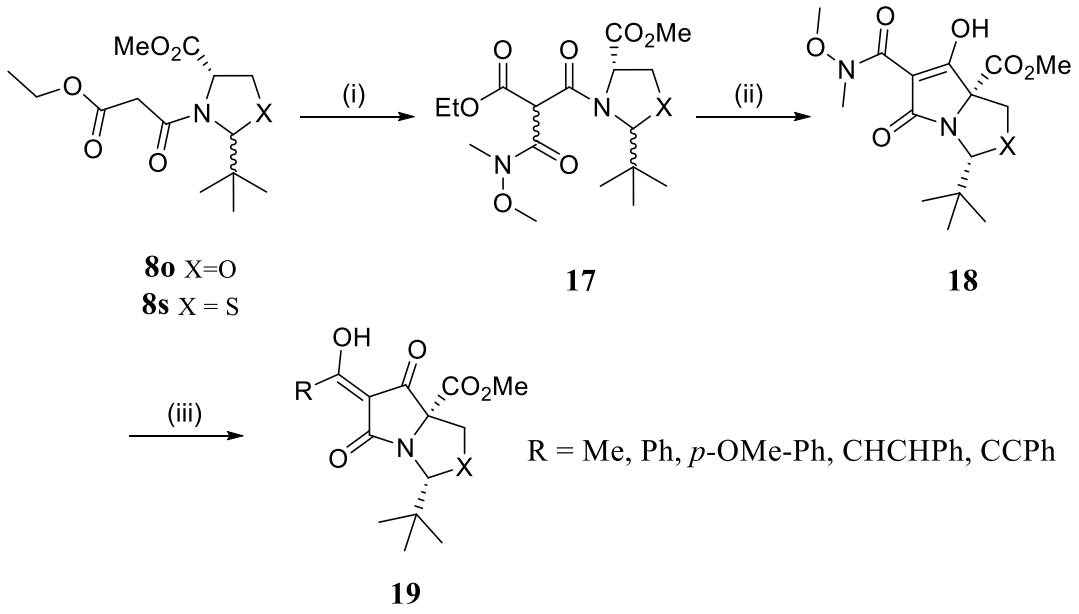


Scheme 1.3. Synthesis of C5 functionalised tetramic acids. (i) Ethyl acetoacetate, N,N,N,N-tetramethyl guanidine, benzyl bromide, DMF. (ii) Methanolic HCl.

Subsequent deprotection with methanolic HCl yielded the substituted amino acid esters **15** as the hydrochloride salt. Repeating previously described steps in Scheme **1.1** and Scheme **1.2** to **15** yielded the C-5 functionalised tetramic acids **16**.[52]

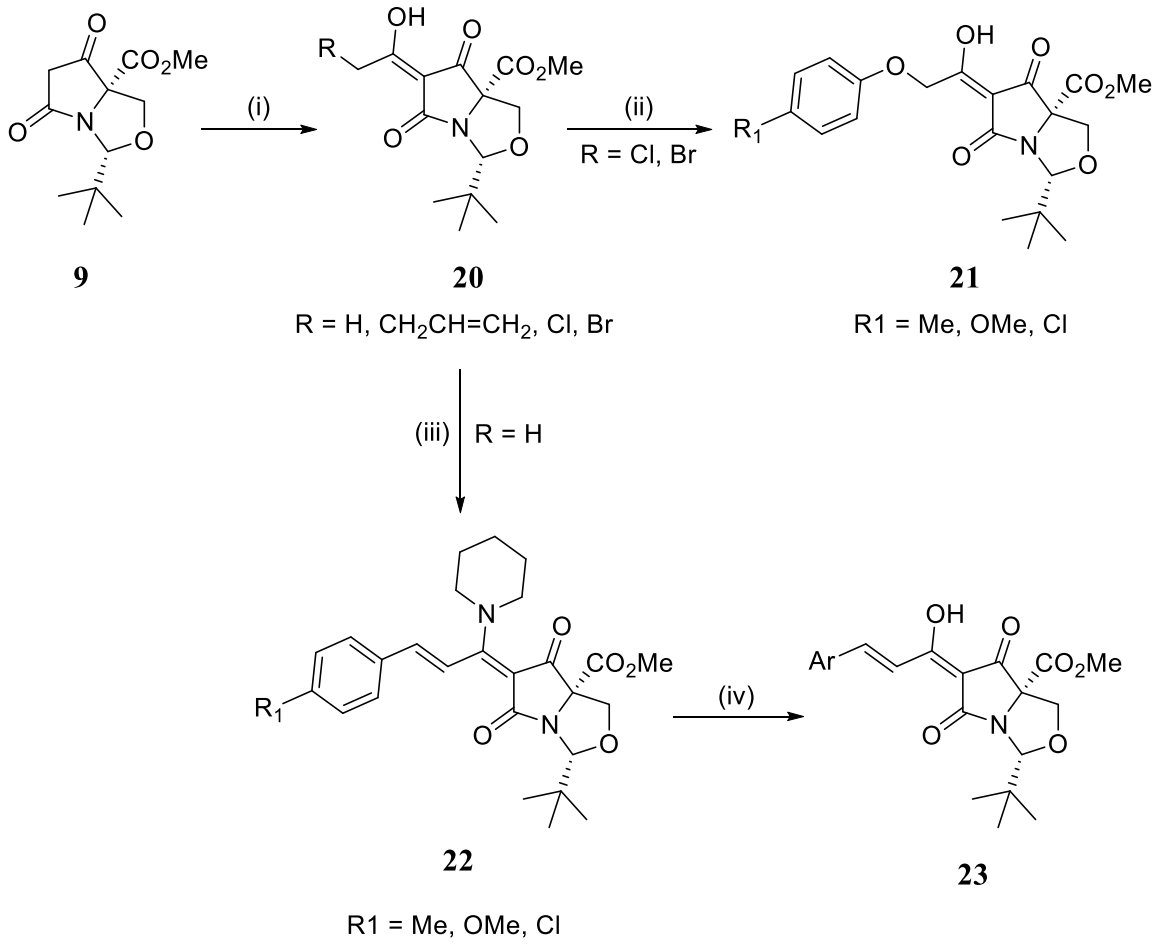
Functionalisation at C-7

Manipulation of either the tetramic acid core **9** or the *N*-acylated oxazolidine/thiazolidine **8** can lead to C-7 functionalisation, with toleration of a wide range of structural diversity. Reaction of the oxazolidine/thiazolidines **8o**/**8s** with methoxy(methyl)carbamic chloride yields the corresponding Weinreb amide **17**, which can be subject to Dieckmann cyclisation in presence of DBU to yield the Weinreb amide tetramic acids **18**. Further reaction with a range of Grignard reagents leads to C-7 functionalised tetramic acids **19** (Scheme **1.4**).[53]



Scheme 1.4. Synthesis of C-7 functionalised tetramic acids through Weinreb amide manipulation. (i) Methoxy(methyl)carbamic chloride, pyridine, $MgBr_2$, rt, 24h. (ii) DBU, THF, rt, 24h. (iii) RMgBr, THF, -15 °C, 1 h.

Manipulation of C-7 from the tetramic acid core **9** has led to a large range of functionally diverse compounds. Initial coupling with the corresponding carboxylic acid yields 7-acyl tetramic acids **20**. Further elaboration was achieved through two routes; S_N2 displacement of halogenated **20** with substituted phenols, yielding compounds **21**, or enamine generation using piperidine, followed by condensation with aromatic aldehydes to yield enamines **22**, which were hydrolysed to the enol compounds **23** (Scheme **1.5**).



Scheme 1.5. Synthesis of C-7 functionalised tetramic acids through manipulation at C-7. (i) RCH_2COOH, DCC, DMAP, DCM, rt. (ii) $R_1C_6H_4OH$, Cs_2CO_3, MeCN, rt. (iii) RC_6H_4CHO, piperidine, toluene, reflux. (iv) LiOH, THF-H_2O (1:1), rt, 1.5h.

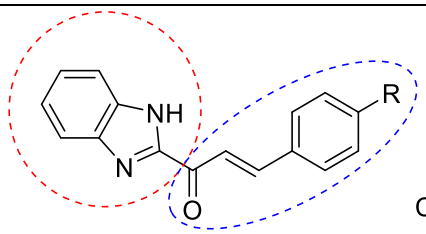
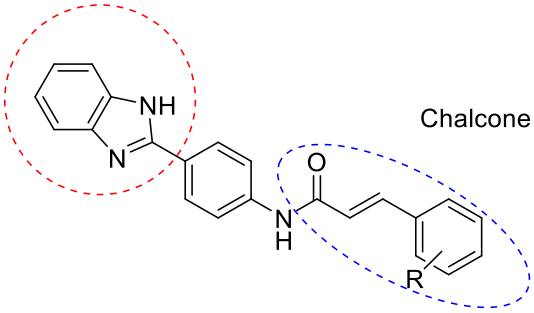
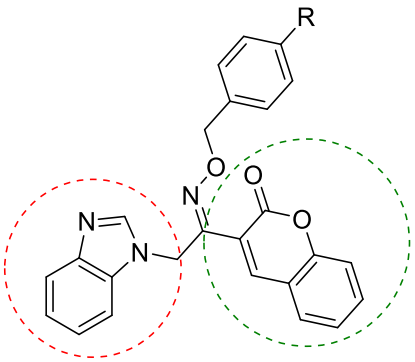
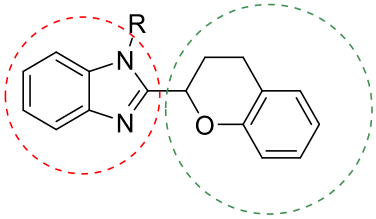
Multiple methods for the functionalisation of tetramic acids have been developed in the Moloney group, as described above. The large range of functional groups tolerated in the structural elaboration of tetramic acids provides useful methodology in the further development of tetramic acid containing compounds. This project aims to utilise some of these methodologies to generate novel tetramic acid containing compounds which possess antibacterial properties.

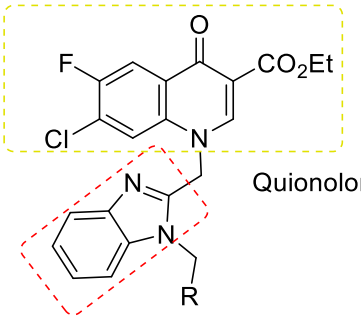
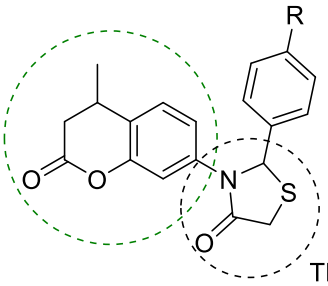
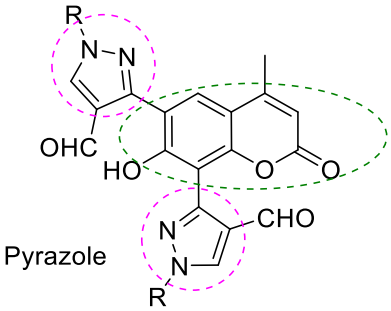
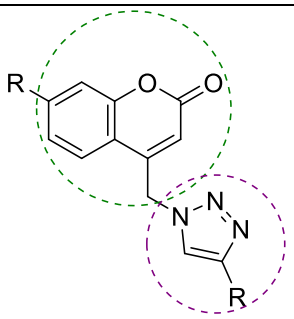
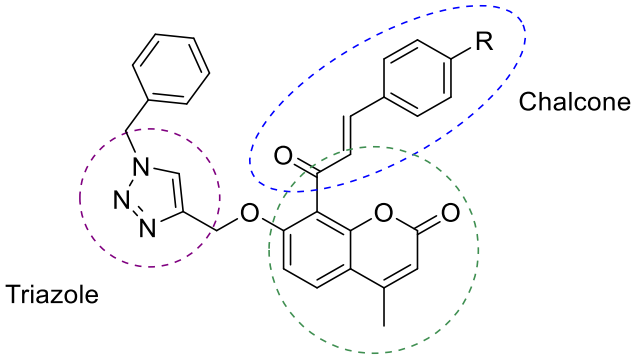
Hybrid compounds as a strategy for drug development

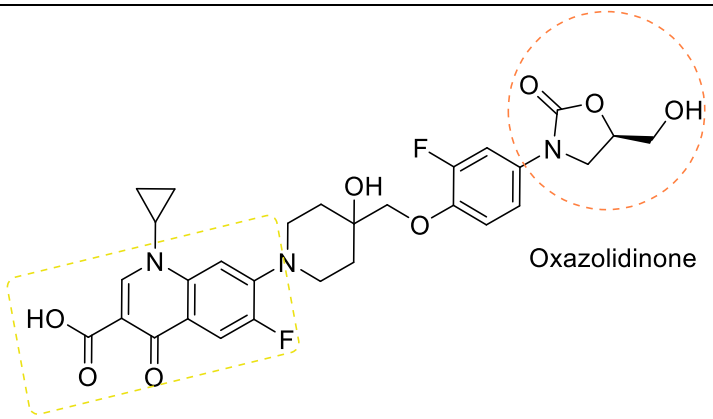
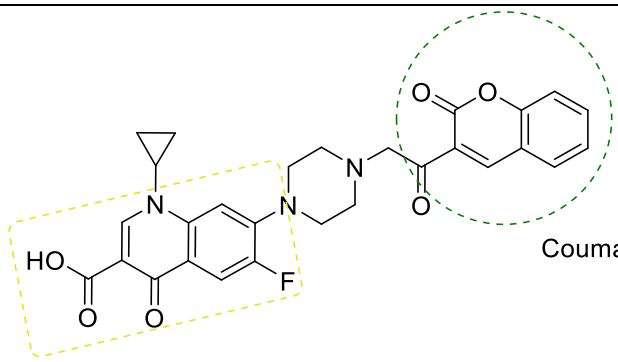
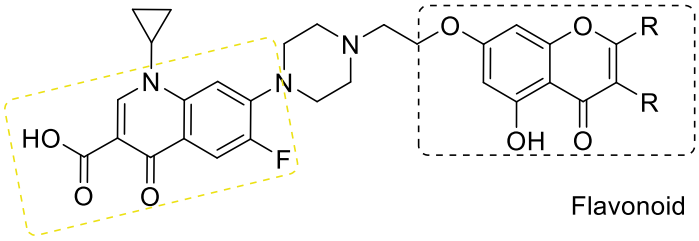
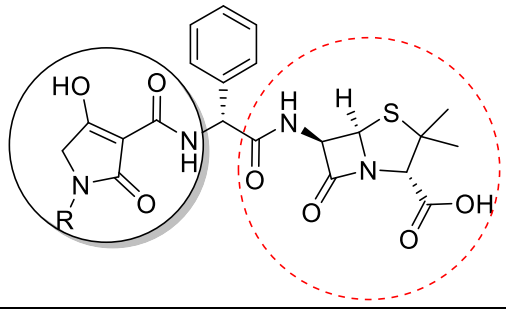
Hybrid antibiotics offer a promising strategy to treat infectious disease and also address the onset of antibiotic resistance.[54,55] Two or more pharmacophores known to possess antibacterial activity are covalently linked with the aim of inhibiting multiple bacterial cellular targets in the one drug. Hybrids can also be designed to consist of structures known to possess strong cell permeability (such as siderophores) with the aim of increasing the activity of the other covalently linked antibiotic.[56] By linking the active moieties, pharmacokinetic behaviour becomes more predictable than combination therapy, an approach in which two or more compounds are co-administered; this approach also requires experimentation to identify the ideal dosage of each individual drug as interactions can be antagonistic, additive or synergistic.[57] Additionally, drugs can often have unfavourable interactions with each other and this needs to be considered when formulating combinations.

Somewhat counter-intuitively, hybrids are best categorised based on the component linking the pharmacophore frameworks, known as the linker. Those possessing long linkers, distinctly separating the pharmacophores, are known as "conjugates" (or "cleavable conjugates" if the linker is not stable to metabolism), while those in which the frameworks are very near to one another are named "fused" and those that are linked through common functionality in the sub-units are known as "merged".[56] This classification can become problematic in any particular type of hybrid but summarised in Table **1.3** are some classes of hybrid antibacterials found in the literature which combine two known biologically active moieties, along with their reported bioactivity.

Antibacterial hybrid classes

Hybrid class	Gram-positive		Gram-negative	
	Bacterium	Activity	Bacterium	Activity
Benzimidazoles				
 Chalcone	*n/a*	n/a	*KA*	3.2 µg/mL *
 Chalcone	*SA*	14 mm ▲	*EC*	14 mm ▲
 Coumarin	*BS*	0.95 µg/mL *	*EC*	3.1 µg/mL *
 Coumarin	*SA*	1.0 µg/mL *	*ST*	10 µg/mL *

 Quionolone	*SA*	4.0 µg/mL *	*PA*	1.0 µg/mL *
Coumarins				
 Thiazolidinone	*SA*	20 mm ▲	*EC*	20 mm ▲
 Pyrazole	*SA*	25 µg/mL *	*PA*	6.3 µg/mL *
 Triazole	*EF*	8.0 µg/mL *	*EC*	>260 µg/mL *
	*SA*	35 mm ▲	*EC*	33 mm ▲

Ciprofloxacin (Fluoroquinolones)				
 Oxazolidinone	*CD*	0.13 µg/mL *	n/a	n/a
 Coumarin	*SA*	0.19 µg/mL *	*EC*	0.013 µg/mL *
 Flavonoid	*SA*	0.29 µg/mL *	*EC*	0.71 µg/mL *
Tetramic acids				
 Ampicillin	*SA*	0.20 µg/mL *	*EC*	1.6 µg/mL *

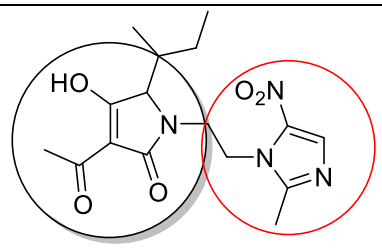
 Metronidazole	*CD*	0.25 µg/mL *	*n/a*	n/a

Table 1.3. Classes of antibacterial hybrids identified from the literature. * = MIC, ▲ = zone inhibition. *BS = B. subtilis, CD = C. difficile, EC = E. coli, EF = E. faecalis, KA = K. aerogenes, PA = P. aeruginosa, SA = S. aureus, ST = S. typhimurium, TM = T. maritimum*.

Benzimidazoles in drug discovery

Benzimidazoles are an important pharmacophore in both the history of drug development and current drug discovery efforts. Benzimidazoles possess a large range of biological activities including antihelminthic, antifungal, and antibacterial properties.[58] Examples of clinically used benzimidazole containing drugs are shown in Figure **1.6**. Interestingly, Ledipasvir, although not an antibiotic, possesses both a benzimidazole and imidazole functionality, and was the best-selling small molecule drug of 2015,[59] providing clear evidence for the feasibility of benzimidazoles as an effective moiety in drug development.

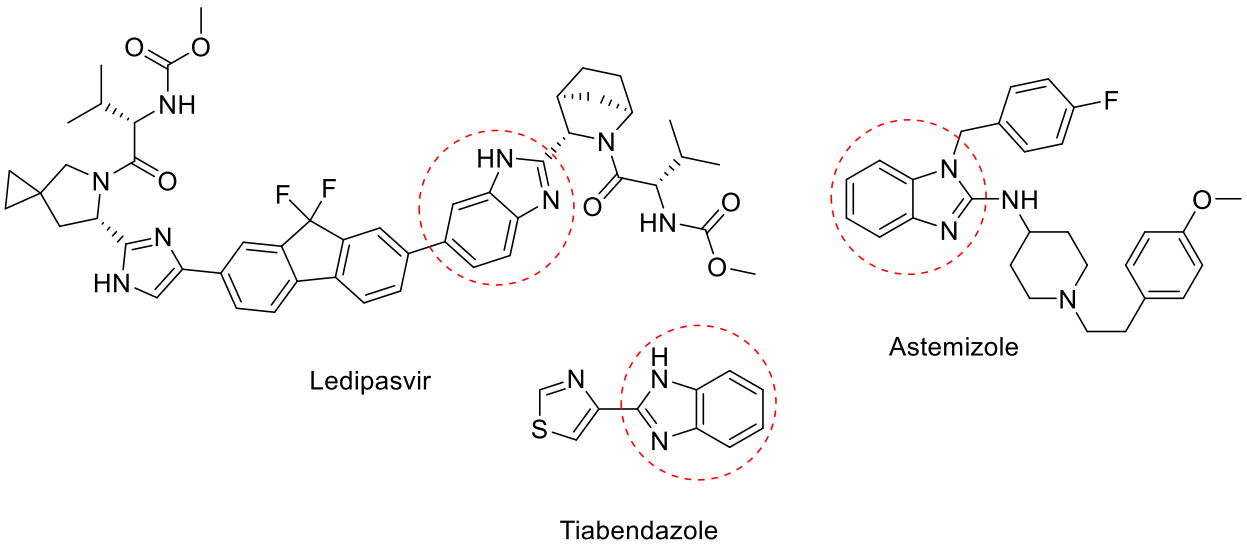
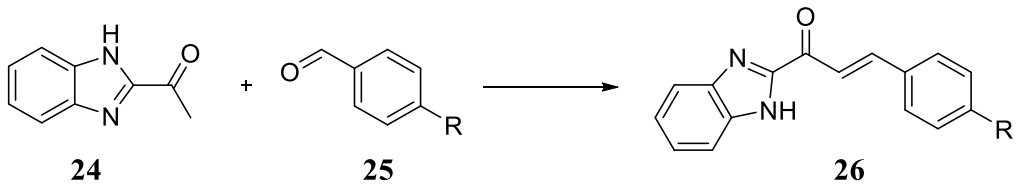


Figure 1.6. Marketed drugs containing benzimidazoles: Ledipasvir, Tiabenzidole, and Astemizole.

Benzimidazole – chalcone hybrids

The first benzimidazole chalcone hybrid (Table **1.3**) was reported by Selvakumar.[60] Coumarin was chosen as the partnering hybrid component as it has been shown numerous times in the literature to possess antiviral, antitubercular, antileishmanial, anticancer, antimalarial, and importantly, antibacterial activity.[61] Moreover, coumarins contain few reactive functional groups, making for synthetic simplicity; an aldol reaction between synthesised acyl-benzimidazole **24** and various benzaldehydes **25** yielded a range of chalcone benzimidazole hybrids **26** (Figure **1.7**).



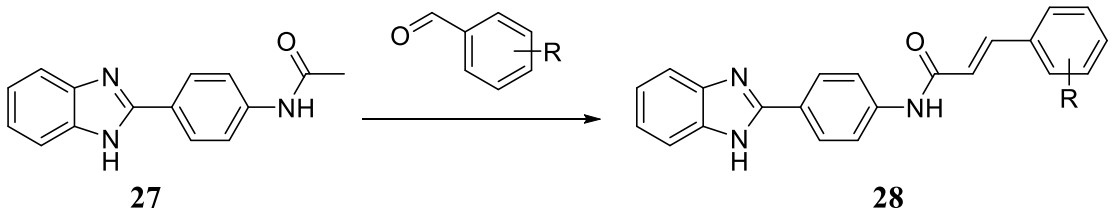
24 **25** **26**

Figure 1.7. Synthesis of benzimidazole chalcone hybrids by aldol condensation.

Antimicrobial evaluation revealed that several compounds displayed activity against Gram-negative bacteria *K. aerogenes* and *E. coli* in an agar diffusion assay with potency assessed against negative DMSO and positive Ciprofloxacin controls. MIC values were determined and the most active in the series possessed an MIC of 3.2 $\mu g/mL^{-1}$ compared to 0.2 $\mu g/mL^{-1}$ for Ciprofloxacin. Despite being 16-fold less active than the control, the activity is still promising, good enough for onward development, and provides justification for the concept of hybridisation.

Khadabadi *et al.* also synthesised benzimidazole chalcone hybrids. Unlike the work described above, these hybrids contained an aniline linker which slightly separated the pharmacophores.

Synthesis of these conjugates was carried out by taking aniline substituted **27** and reacting with acetyl chloride. Aldol condensation with various benzaldehydes then yielded the desired product **28** (Figure **1.8**).



27 **28**

Figure 1.8. Synthesis of aniline linked benzimidazole chalcone hybrids by aldol condensation.

The most active compound against Gram-positive bacteria possessed an inhibition zone of 14 mm against *S. aureus* compared to 17 mm in the control (Streptomycin). In the Gram-negative screen, the most active compound showed an inhibition zone of 14 mm against *E. coli* versus 22 mm for Streptomycin. Both activities are weaker than, but comparable to, that of the clinically used drug Streptomycin, which again offers promise for the strategy of benzimidazole-chalcone hybridisation.

Benzimidazole – coumarin hybrids

Coumarins display a wide range of biological activities including anti-inflammatory, anticancer, antiviral and antimicrobial properties. They are also present in a range of clinically-used drugs, including antibiotics, and thus are relevant in drug discovery.[62] More detail on coumarin compounds and their relevance in hybrids is discussed later in this chapter. Like chalcones, coumarins are attractive synthetically due to their easy accessibility from simple starting materials. Table **1.3**, entry C displays a benzimidazole coumarin hybrid designed by Sashidhara *et al.* S_N2 displacement of bromoacetylcoumarin **29** by benzimidazole

yielded the hybrid **30**, which was subsequently modified by reaction with hydroxylamine, and further S_N2 displacement of various phenyl substituted benzyl chlorides followed, yielding a range of benzimidazole coumarin hybrids **31** (Figure **1.9**).

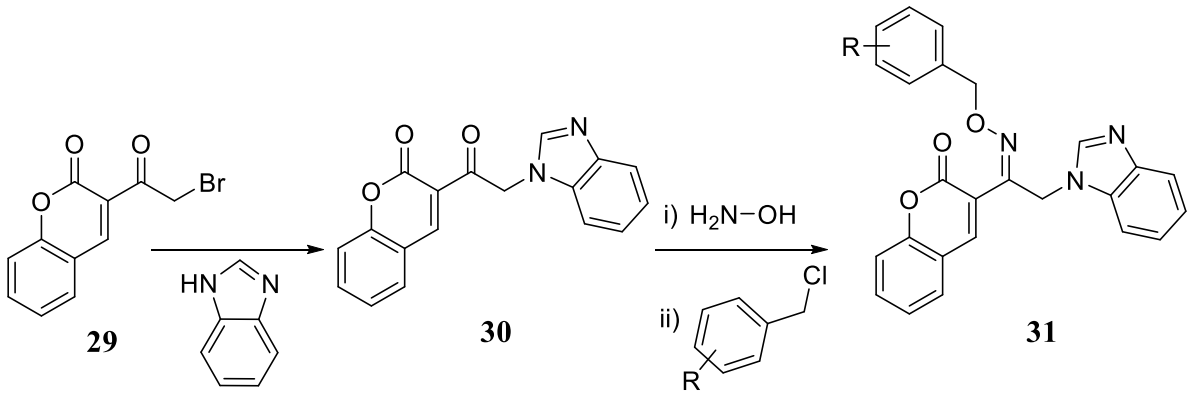
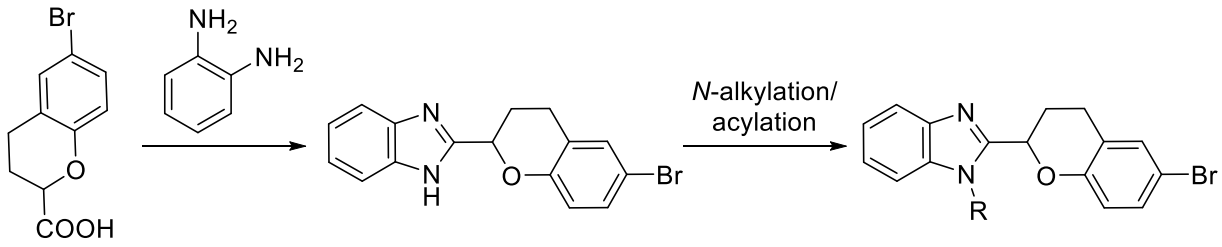


Figure 1.9. Synthesis of oxime linked benzimidazole coumarin hybrids.

The antibacterial activity of the synthesised hybrids was assessed against various strains of Gram-positive and Gram-negative bacteria in a microdilution assay method. MIC values were calculated against a range of clinically used antibiotics possessing different modes of action, namely Ampicillin, Kanamycin, Tetracycline and Ciprofloxacin. The most active compound in the series possessed an MIC of 0.95 µg/mL^{-1} against *B. subtilis*, a Gram-positive strain of bacteria. By comparison, the calculated MIC's for the Ampicillin, Kanamycin, Tetracycline and Ciprofloxacin controls were 25.0, 6.25, 6.25 and 0.60 µg/mL^{-1} respectively. In the Gram-negative screen, the most active compound displayed an MIC of 3.12 µg/mL^{-1}, compared to the 1.56, 3.15, 6.25 and 12.5 µg/mL^{-1} values for the controls.[63]

Benzimidazole-coumarin hybrids compared favourably to the clinically relevant antibiotics, performing better than three of the four controls in both the Gram-positive and Gram-negative screen. These results suggest significant promise to the strategy of hybridisation in antibacterial drug discovery.

Raut *et al.* also synthesised a range of benzimidazole coumarin hybrids however, these compounds possessed no linker between the pharmacophores. Condensation of compound **32** with *o*-phenylenediamine yielded hybrids **33**, which could be further modified to form *N*-alkylated or *N*-acylated hybrids **34** (Figure **1.10**).



32 **33** **34**

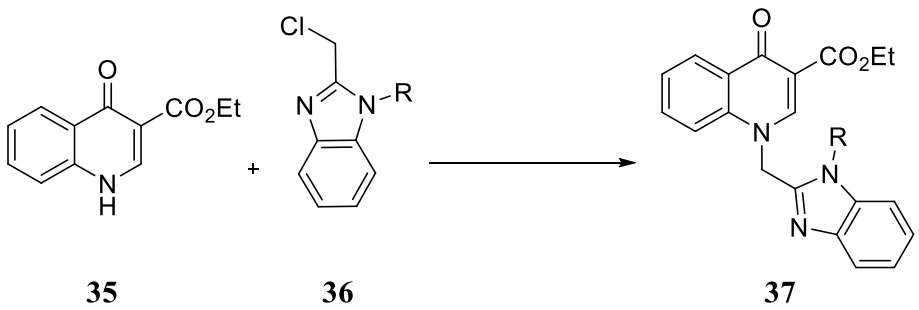
Figure 1.10. Synthesis of benzimidazole coumarin hybrids.

The activity of the synthesised compounds was assessed against Gram-positive and Gram-negative bacteria strains *S. aureus* and *S. typhimurium*; the most active compounds possessed MIC values of 1.0 and 10.0 μg/mL^{-1} respectively. The positive control antibiotic Cephalexin showed an MIC value of 0.1 μg/mL^{-1} in both screens. Synthesised hybrids were found to be not as active; however they did display good antibacterial activity and provide a platform to continue development in benzimidazole coumarin hybrids.[64]

Benzimidazole-quinolone hybrids

Quinolones are a common pharmacophore in antibacterial drug discovery and represent a class of clinically widely-used antibiotics. Quinolones will be discussed in further detail later in this chapter. Zhou *et al.* synthesised a range of benzimidazole quinolone hybrids and evaluated their antimicrobial activity against Gram-positive and Gram-negative bacteria *S.*

aureus and *P. aeruginosa* respectively. The hybrids **37** were accessed by S_N2 displacement of chloro-substituted benzimidazoles **36** by synthesised quinolones **35** (Figure **1.11**).



35 **36** **37**

Figure 1.11. Synthesis of benzimidazole quinolone hybrids by S_N2 reaction.

Activity against both Gram-positive and Gram-negative bacteria was favourable when compared to the Ciprofloxacin and Norfloxacin controls. In the most active hybrid, an MIC of 4.0 μg/mL^{-1} against *S. aureus* was observed compared to 0.5 and 64 μg/mL^{-1} in the respective controls. In the screen against the Gram-negative *P. aeruginosa*, an MIC of 1.0 μg/mL^{-1} was recorded compared to 32.0 and 4.0 μg/mL^{-1} in the controls.[65]

Coumarins in drug discovery

Coumarins represent an important biologically active moiety in drug discovery and development, and can be found in a range of clinically used drugs. Clorobiocin, Coumermycin and Novobiocin (Figure **1.12**) represent drugs from a class of antibiotics named aminocoumarins which act by inhibition of DNA gyrase.[66]

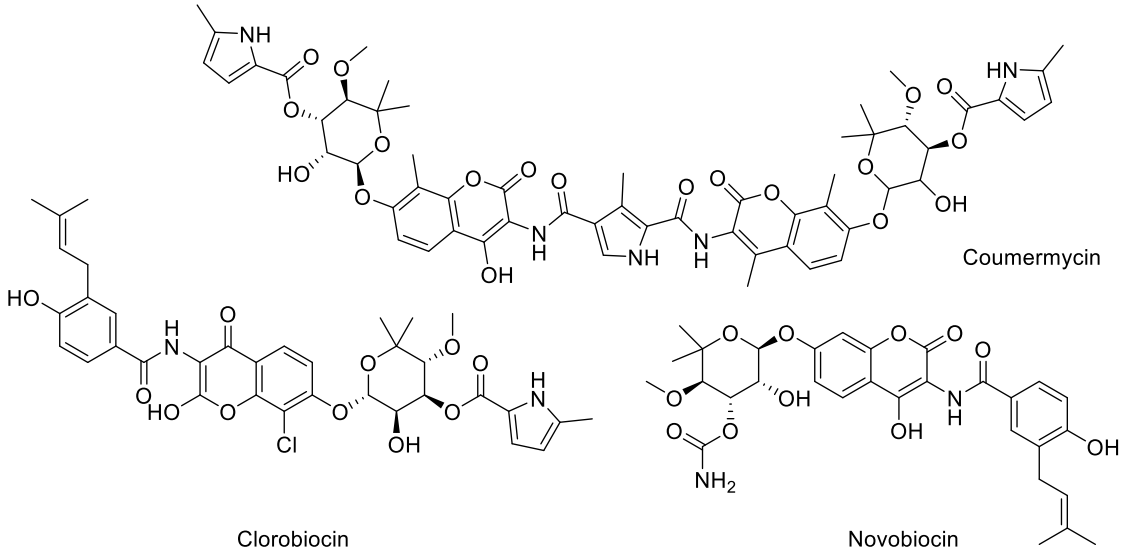


Figure 1.12. Aminocoumarin antibiotics Clorobiocin, Coumermycin and Novobiocin.

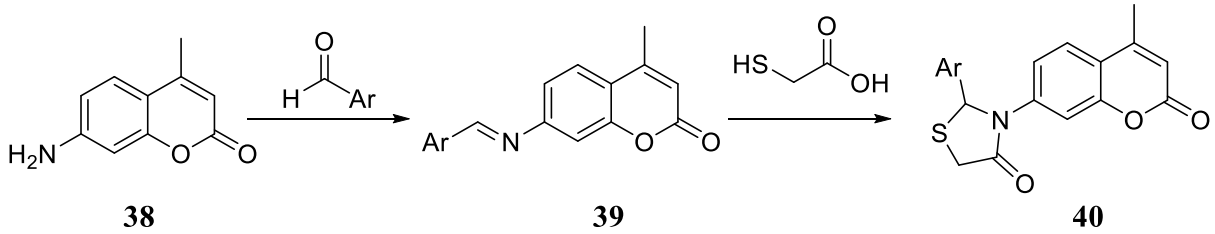
In addition to comprising a class of antibiotics, coumarins are also present in many anticoagulants (such as Warfarin) as they are vitamin K antagonists.[67] A strong presence in the clinic and indeed the literature justifies their investigation in the search for novel antibiotics and hybridisations.[61,68]

Coumarin-thiazolidinone hybrids

Thiazolidinones have displayed a vast range of biological activities, from anti-convulsant and antiantihypertensive activity to anticancer and antimicrobial acitivity.[69] In light of this, they represent an important pharmacophore in the discovery for novel biologically active compounds.

Veeresh *et al.* synthesised a range of thiazolidinone-coumarin fused hybrids and their antibacterial activity was assessed. The most active compounds in the series displayed inhibition zones of 20 mm against both Gram-positive and Gram-negative bacteria, *S.aureus* and *E.coli* respectively. Synthesis of the hybrids was conducted in two steps by condensing 7-amino-4-methylcoumarin **38** and various aromatic aldehydes to give intermediate imines **39**,

which were reacted with thioglycolic acid to generate the thiazolidinone heterocycle, yielding a range of coumarin-thiazolidinone hybrids **40** (Figure **1.13**).[70]



38 **39** **40**

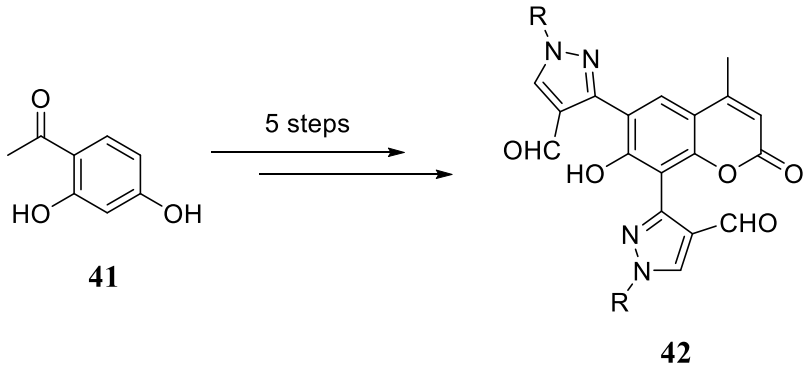
Figure 1.13. Synthesis of coumarin-thiazolidinone hybrids.

Coumarin-pyrazole hybrids

Coumarins have also been coupled to various aromatic heterocycles containing multiple nitrogen atoms.

For example, pyrazoles have been reported numerous times in the literature as potent antimicrobial compounds[71–73] and a clinically used antibiotic named Cefoselis[74] contains the pyrazole functionality, highlighting their relevance in the discovery of antibacterial compounds.

R. Nagamallu *et al.* synthesised a range of pyrazole-coumarin hybrids which possessed antimicrobial activity against both Gram-negative and Gram-positive bacteria. The most active compound in the series displayed an MIC of 25 μg/mL^{-1} against *S.aureus* and interestingly, a more potent 6.3 μg/mL^{-1} against *E.coli*. Synthesis of the pyrazole-coumarin hybrids was achieved in 5 steps from simple starting materials **41** and ultimately yielding bis-pyrazoles **42** (Figure **1.14**).[75]



41 **42**

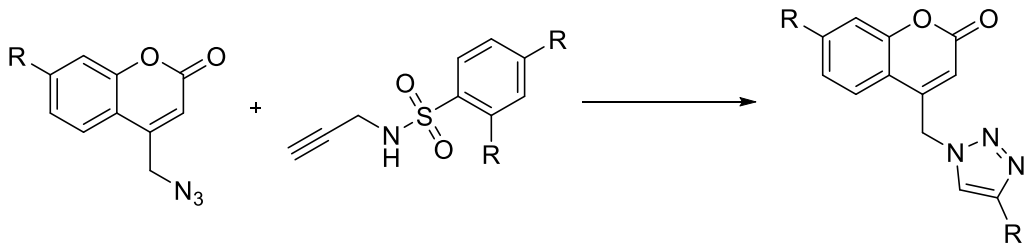
Figure 1.14. Synthesis of bispyrazole-coumarin hybrids.

Coumarin-triazole hybrids

Triazoles are a nitrogen-containing heterocyclic class of compounds which have been shown in the literature to possess broad spectrum antimicrobial activity.[76,77] Additionally, Tazobactam is a clinically administered antibiotic containing triazole functionality, and this further justifies the exploration of triazoles as antimicrobial compounds.

Kraljevic *et al.* investigated the effects of hybridising triazoles with coumarins and identified a range of active compounds against Gram-positive bacteria (no activity was displayed against Gram-negative bacteria). The most active compound in the series possessed an MIC of 8 $\mu g/mL^{-1}$ against *E.faecalis* - despite being 16-fold less active than the ciprofloxacin control, this represents appreciable activity suitable for optimisation.[78]

Synthesis of the triazole hybrids **45** was achieved by 'Click' chemistry, employing a copper catalysed Huisgen 1,3-dipolar cycloaddition between coumarin containing azides **43** and alkynes **44** (Figure **1.15**).[78]



43 **44** **45**

Figure 1.15. Synthesis of coumarin-triazole hybrids.

Triazoles have also been synthesised containing both coumarin and chalcone moieties in an attempt to combine three known antimicrobial functionalities. Dongamanti *et al.* successfully synthesised a range of compounds containing the three moieties and their activity was assessed against Gram-positive and Gram-negative bacteria. In the Gram-positive screen against *S.aureus*, the most active compound possessed an inhibition zone of 35 mm compared to 30 mm for the Amoxicillin control. In the Gram-negative screen against *E.coli*, the most active compound displayed a similarly potent activity of 33 mm against 30 mm in the Amoxicillin control. These activities are clear evidence for the value of combining multiple pharmacophores in a single covalently linked compound.[79]

Synthetically, the hybrids were accessed by an initial aldol condensation between coumarin **46** and an aryl aldehyde, followed by S_N2 reaction with 3-bromopropyne to generate the coumarin-chalcone intermediate **47**, which was subsequently modified to allow a Click reaction with azides yielding the hybrids **48** (Figure **1.16**).

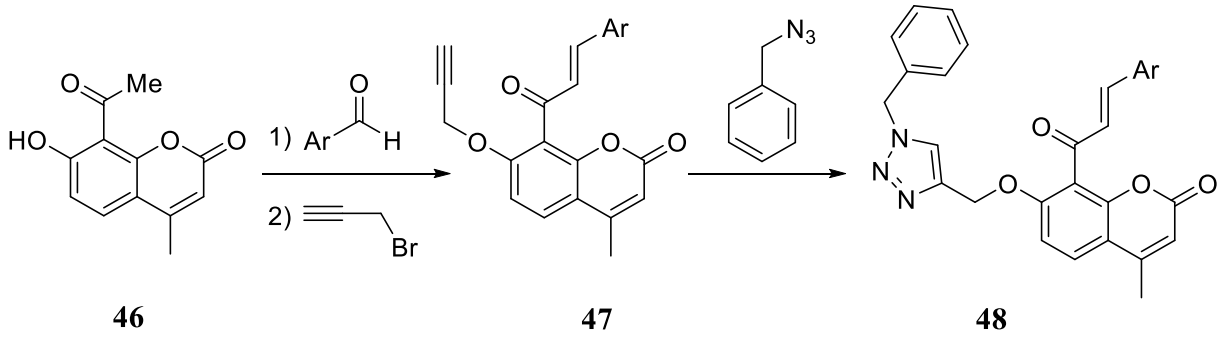


Figure 1.16. Synthesis of coumarin-chalcone-triazole hybrids.

Ciprofloxacin

Since their discovery in the 1960s, quinolones have become a major class of clinically employed antibiotics. Several drugs such as Norfloxacin[80], Levofloxacin[81], and Ciprofloxacin[82] have emerged as successful antibiotics since the discovery of the first quinolone drug, Nalidixic acid[83] (Figure **1.17**). Ciprofloxacin possesses very broad-spectrum antibacterial activity, particularly against Gram-negative bacteria, which renders it an exciting prospect in the generation of novel hybrid antibiotics.

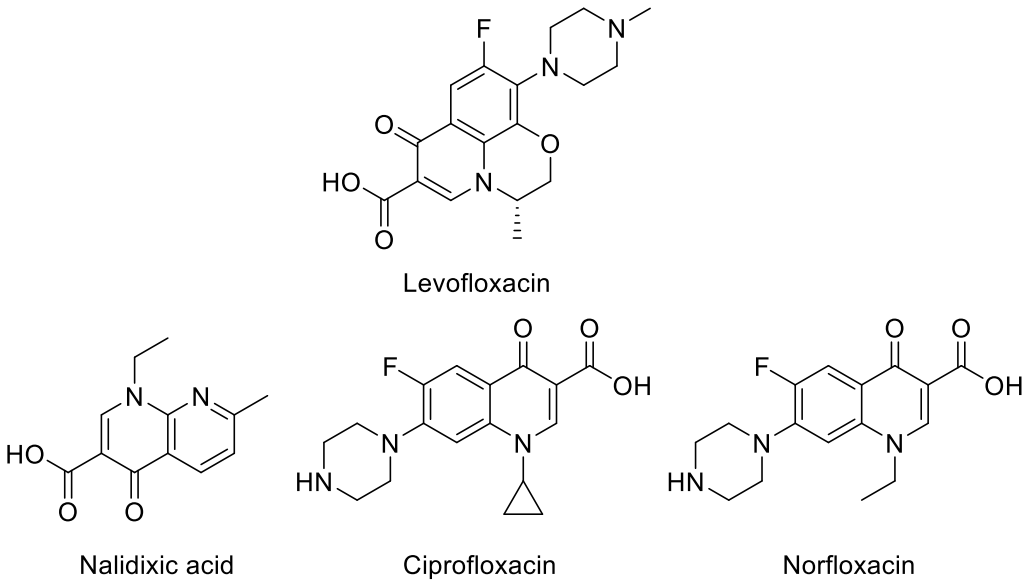
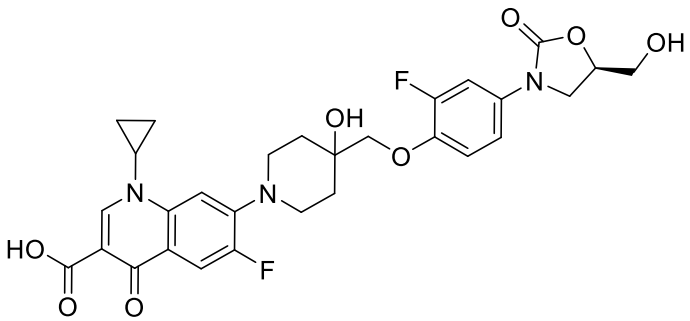


Figure 1.17 Examples of quinolone drugs.

Several attempts at the hybridisation of ciprofloxacin have been made and some examples are detailed below.

Ciprofloxacin-oxazolidinone hybrids

Oxazolidinones are the latest class of antibiotics to be discovered possessing a novel mode of action and are represented in the clinic by Linezolid and Tedizolid.[84] Hybridisation with Ciprofloxacin has been achieved and is thus far a success; Cadazolid **49** is produced by Actelion Pharmaceuticals and is currently in phase III Clinical trials (Figure **1.18**).[85]



49

Figure 1.18. Structure of Cadazolid.

Cadazolid combines ciprofloxacin and the oxazolidinone pharmacophore and has been developed specifically to treat *C.difficile* infections. Activity against *C.difficile* is very potent with an MIC of 0.125 μg/mL^{-1}, including against strains of bacteria resistant to linezolid and fluoroquinolones – the respective components in the hybrid.[55,86,87] Cadazolid represents a triumph in the concept of hybrid antibiotics given its progress into late stage clinical evaluation.

Ciprofloxacin-benzothiazole hybrids

Benzothiazoles, structurally related to previously discussed benzimidazoles and thiazolidinones, are another important class of compounds in drug discovery. They are present in a range of clinically-relevant drugs, for example Frentizole and Zopolrestat, and have been shown in the literature to possess a whole range of biological activity as anti-virals, anti-diabetics and antimicrobials.[88,89]

Sharma *et al.* successfully hybridised Ciprofloxacin with an amino-substituted benzothiazole to generate a range of antibacterially active compounds. In their series, the most active compound displayed activity of 3.0 μg/mL^{-1} and 6.0 μg/mL^{-1} against *S.aureus* and *E.coli* respectively. Synthetically, the hybrids were accessed through an S_N2 reaction between the secondary amine of ciprofloxacin **51** and chloro-substituted benzothiazoles **52** to yield the hybrids **53** (Figure **1.19**).

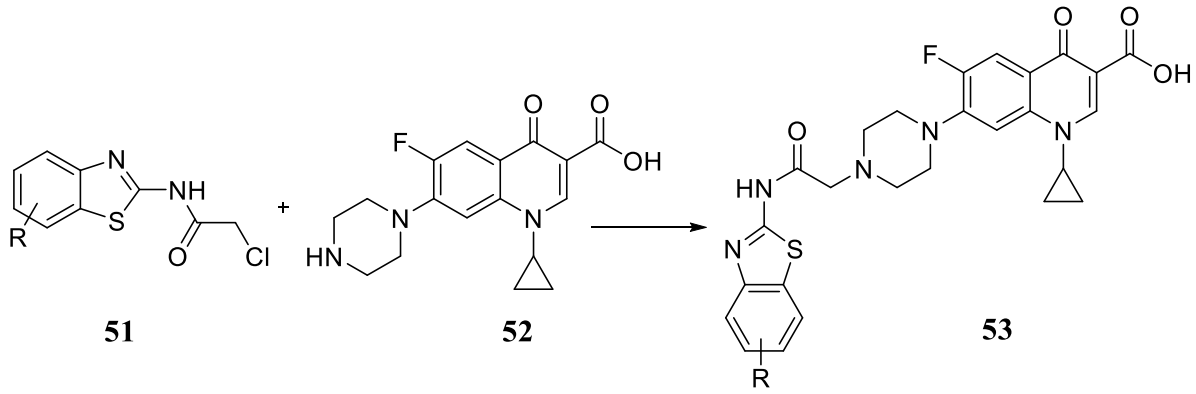


Figure 1.19. Synthesis of ciprofloxacin-benzothiazole hybrids.

Ciprofloxacin-coumarin hybrids

Coumarins, as discussed earlier in this chapter, are important biologically active compounds and are amenable to hybridisation. The combination of ciprofloxacin and coumarin moieties has been achieved by Samadi *et al.* which displayed very potent antimicrobial activity against

both Gram-positive and Gram-negative bacteria. In the most active compound, Gram-positive *S.aureus* was inhibited with an MIC of 0.19 μg/mL^{-1} and Gram-negative *E.coli* was inhibited with an MIC of 0.013 μg/mL^{-1}. The activities observed were as potent as the most active controls, and this outcome encourages the discovery of more, novel hybrid antibacterials.[90]

Similar to the synthesis of ciprofloxacin-benzothiazole hybrids, accessing ciprofloxacin-coumarin hybrids was achieved by initially synthesising bromo-substituted coumarins **54**, and subsequent S_N2 displacement by the ciprofloxacin amine **52** yielding the ciprofloxacin-coumarin fused compounds **55** (Figure **1.20**).[90]

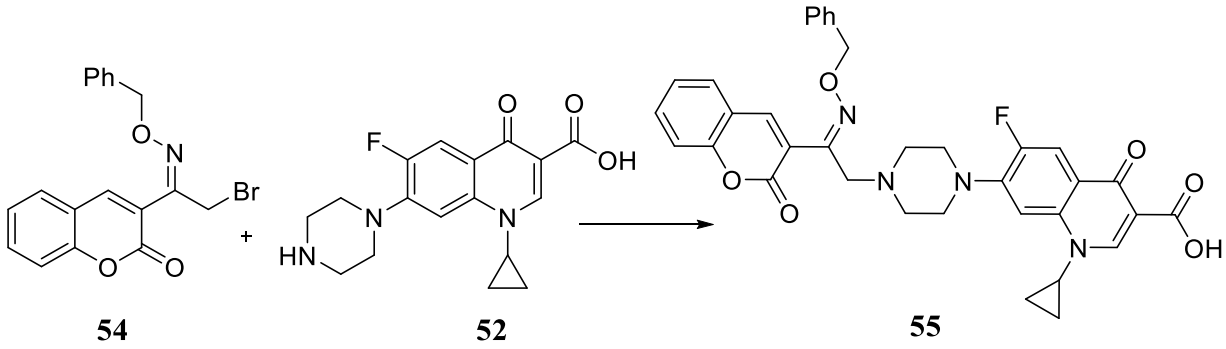
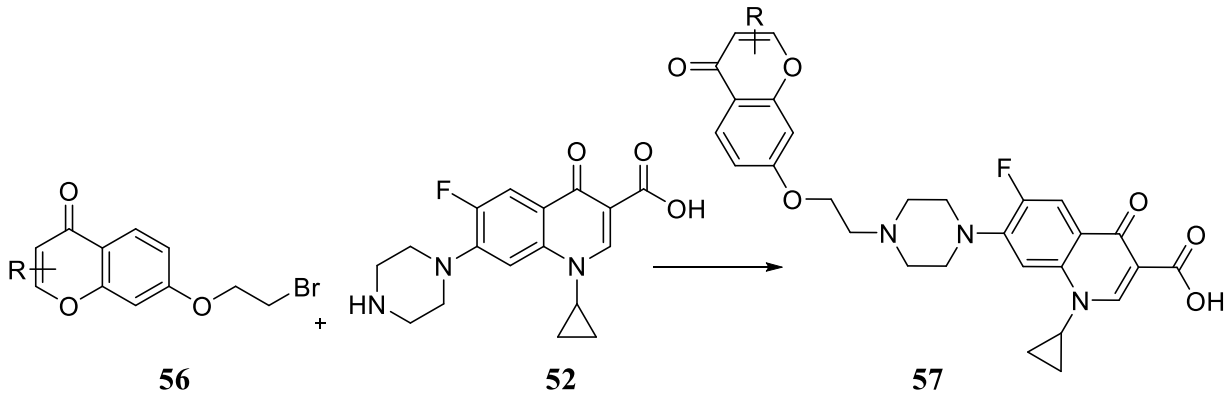


Figure 1.20. Synthesis of Ciprofloxacin-coumarin hybrids.

Ciprofloxacin-flavonoid hybrids

Flavonoids have been described numerous times in the literature as biologically active compounds displaying anti-inflammatory, anti-oxidant, anti-cancer and antibacterial activity.[91] Zhu *et al.* synthesised a range of ciprofloxacin-flavonoid hybrids which displayed potent antimicrobial activity. Against *S.aureus*, the most active compound in the series exhibited an MIC of 0.29 μg/mL^{-1} compared to 6.8 μg/mL^{-1} in the Ciprofloxacin control. In the Gram-negative screen against *E.coli*, an MIC of 0.71 μg/mL^{-1} was observed which again outperformed the control which possessed an MIC of 5.7 μg/mL^{-1}.

As in the previous hybridisations containing Ciprofloxacin, the flavonoid hybrids were synthesised through an S_N2 reaction on halogen-substituted flavonoids **56** by the Ciprofloxacin secondary amine to yield the flavonoid hybrids **57** (Figure **1.21**).[92]



56 **52** **57**

Figure 1.21. Synthesis of Ciprofloxacin-flavonoid hybrids.

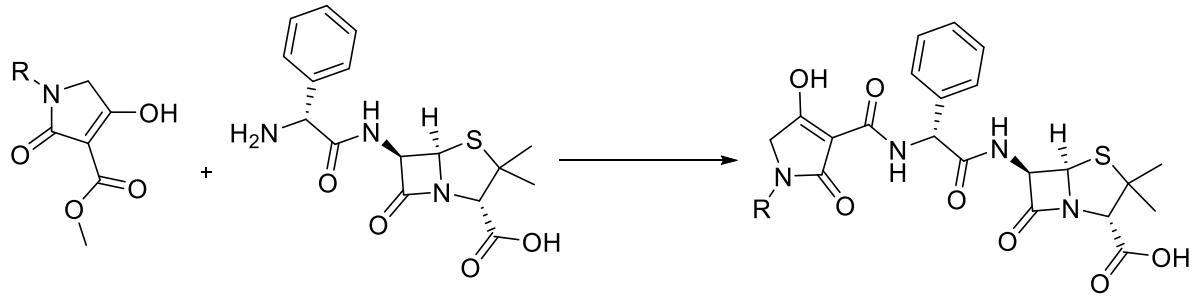
Tetramic acids

Tetramic acids, discussed in detail early in this chapter, have also been subject to hybridisation attempts with Lee *et al.* successfully synthesising tetramic acid-ampicillin and tetramic acid-metronidazole conjugates.

Tetramic acid-ampicillin hybrids

Ampicillin is a commonly used β-lactam antibiotic derived from penicillin, possessing potent broad-spectrum activity against Gram-positive and Gram-negative bacteria. Additionally, it was the first β-lactam antibiotic to show Gram-negative activity.[93] Lee *et al.* synthesised tetramic acid-ampicillin fused hybrids and assessed their activity against Gram-positive and Gram-negative bacteria. Against *S.aureus*, their most active compound displayed an MIC of 0.20 μg/mL^{-1} which was the same as the Ampicillin control. In the Gram-negative screen against *E.coli*, their most active compound had an MIC of 1.6 μg/mL^{-1} which compares favourably to 3.1 μg/mL^{-1} for the Ampicillin control.

Synthetically, the hybrids were accessed by microwave irradiation of the tetramic acid **58** with ampicillin **59** in an amination reaction to yield the hybrids **60** (Figure **1.22**).[94]



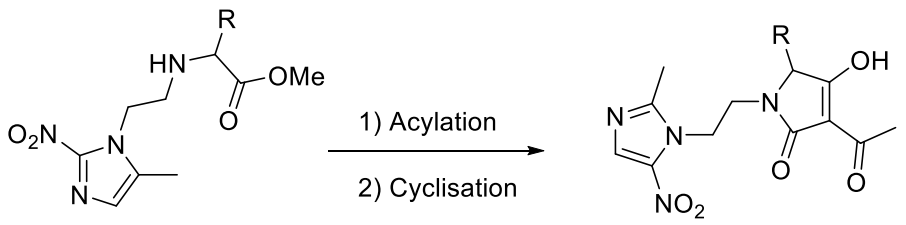
58 **59** **60**

Figure 1.22. Synthesis of tetramic acid-ampicillin hybrids.

Tetramic acid-metronidazole hybrids

Metronidazole is a clinically administered member of the nitroimidazole class of antibiotics. It is commonly used in the treatment of anaerobic bacteria and shows activity against both Gram-positive and Gram-negative strains.[95] In the tetramic acid-metronidazole hybrids prepared by Lee *et al*., the most active compound displayed an MIC of 0.25 μg/mL^{-1} against *C.difficile*, a Gram-positive bacterial strain. This activity was comparable to the MIC value of 0.13 μg/mL^{-1} for the metronidazole control.

To access the tetramic acid-metronidazole hybrids, Lee *et al.* initially synthesised mentronidazole substituted amino acid esters **61**. Subsequent *N*-acylation and Dieckmann cyclisation yielded the hybrids **62** (Figure **1.23**).[96]



61 **62**

Figure 1.23. Synthesis of tetramic acid-metronidazole hybrids.

The tetramic acid is a functional group present in many naturally occurring antibacterially active compounds, and has been widely studied in the literature for its ability to act as an antibacterial moiety. While extensive SAR analysis has provided a range of antibacterially active compounds,[49,97] this hybridisation with other active antibacterials has recently shown promise.[94,98]

Given this reported success with tetramate hybrids, examination of the same strategy with bicyclic tetramates was of interest, and this thesis describes attempts to synthesise tetramic

acid hybrids using fused and conjugated hybridisation strategy, and to examine their antibacterial properties.

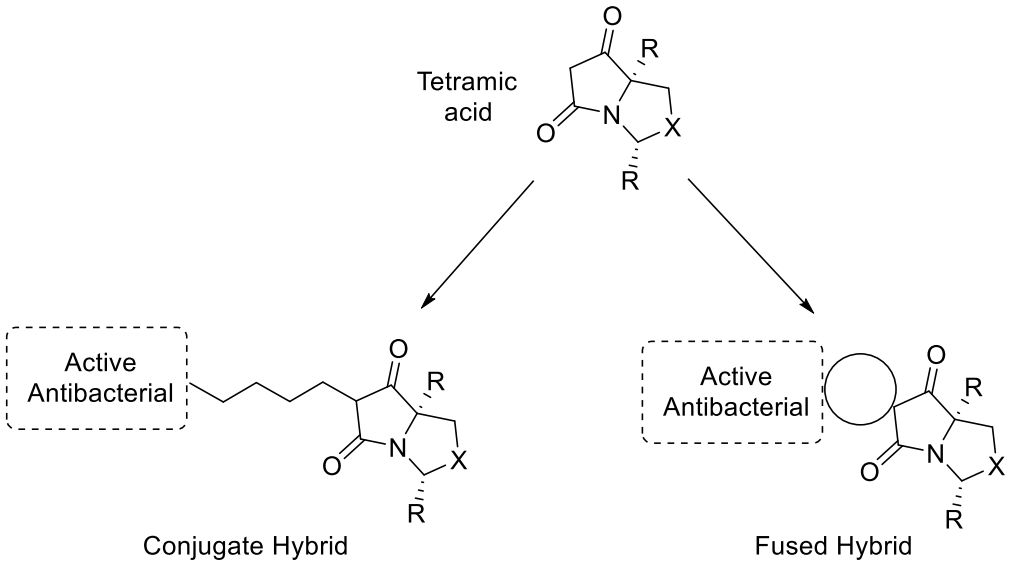


Figure 1.24. Project plan to synthesise tetramic acid hybrids containing different linkers.

This project aimed to utilise previously developed methodology to functionalise the tetramic acid core at the C-2, C-5, and C-7 positions. Variation at each of these positions can provide SAR data on the tetramic acids. C-7 functionalisation was explored in the most detail and the introduction of functional groups amenable to the rapid generation of hybrid tetramic acids was developed, allowing a small library of compounds to be synthesised possessing acyl or spirocyclic functionality at the C-7 position. It was hypothesised that functionalisation at C-7 with other, known antibacterial compounds would provide a hybrid tetramic acid.

Chapter II: Tetramic Acid Hybrids

This chapter describes the novel research for the functionalisation of the tetramic acid core by manipulation at the C-7 position. Further structural elaboration was performed to yield a range of hybrid coumarin-tetramic acids and imidazole-tetramic acids, which were subsequently biologically evaluated. Also described here are the attempts made to generate hybrids with clinically-used antibiotics.

Coumarin hybrids

Aminocoumarins are a class of clinically relevant antibiotics which display good Gram-positive and Gram-negative activity, making them an attractive series of compounds to form hybrids with. Coumarins have also been employed in hybridisation strategies numerous times in the literature. Veeresh *et al.* described coumarin-thiazolidinone hybrids **40**, displaying good antibacterial activity against *S. aureus* and *E. coli* (zone inhibition 20 mm for both).[70] Pyrazoles have also been covalently linked with coumarins to form hybrids, Nagamallu *et al.* successfully synthesised hybrid coumarin-pyrazoles **42**, which inhibited Gram-positive (*S.aureus*) and Gram-negative (*E.coli*) bacteria, with MIC values of 25 μg/mL^{-1} and 6.3 μg/mL^{-1} respectively.[72] Furthermore, Kraljevic *et al.* described the synthesis triazoles **45** displaying activity against Gram-positive bacteria, possessing an MIC value of 8 μg/mL^{-1} against *E. faecalis* (Figure **2.1**).[78] The plethora of literature successfully applying coumarins to hybridisation strategies offered promise in the development of novel antibacterial compounds. Therefore, the synthesis of tetramic acid – coumarin hybrids was undertaken.

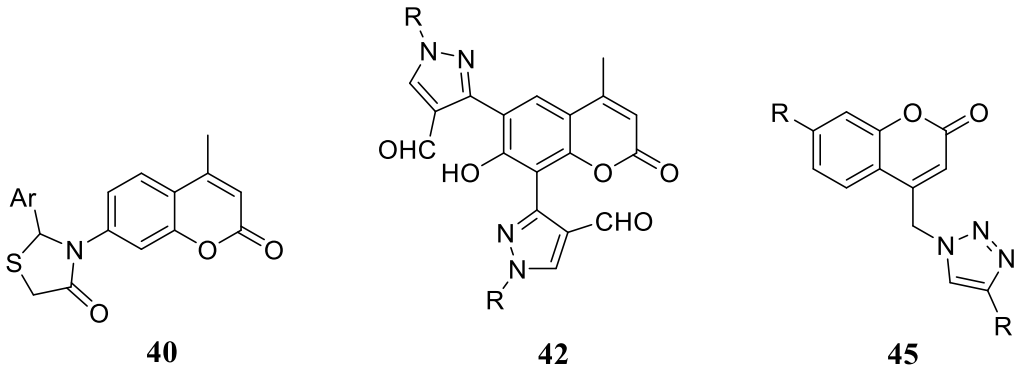


Figure 2.1. Examples of coumarins hybridised with thiazolidines, pyrazoles and triazoles

Tetramic acid – coumarin hybrids

Using established methodology in the Moloney group, the tetramic acid cores **9o/9s** were synthesised (Figure **2.2**).

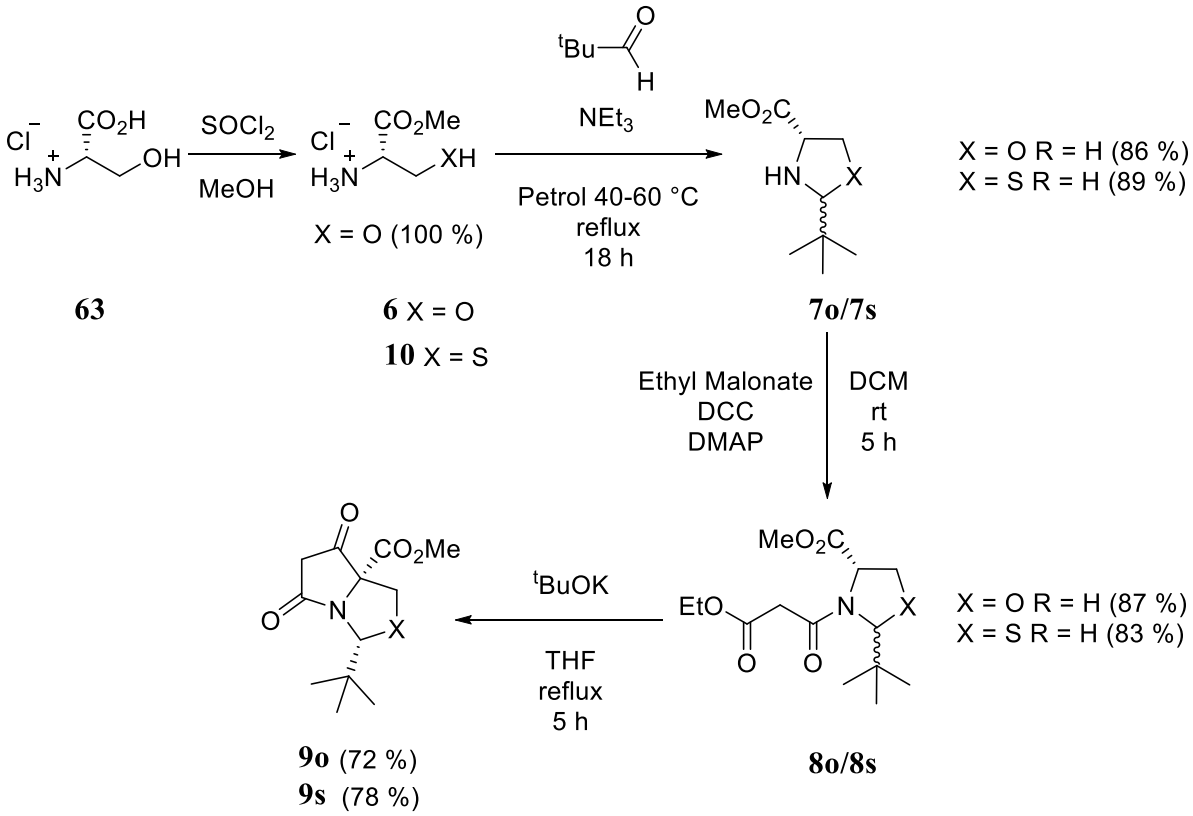
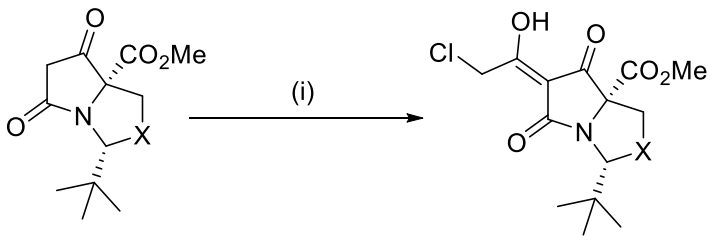


Figure 2.2. Synthesis of the tetramic acid cores **9**

Cysteine methyl ester **10** is commercially available; however serine methyl ester **6** was synthesised. Serine **63** was dissolved in MeOH and thionyl chloride was subsequently added. Reaction at 40 °C for 3 h yielded serine methyl ester hydrochloride in quantitative yield. With serine and cysteine methyl ester hydrochloride in hand, cyclisation with pivaldehyde and NEt_3 in petrol using Dean-Stark conditions was performed. After 18 h, reaction yielded oxazolidine **7o** and thiazolidine **7s** in good yields, as a mixture of *cis*- and *trans*- isomers (1.6:1 *cis:trans* for thiazolidine and 1.7:1 *cis:trans* for oxazolidine). *N*-Acylation of the oxazolidine and thiazolidine was achieved by coupling with ethyl hydrogen malonate, using DCC and DMAP in DCM. Stirring at room temperature for 5 h yielded compounds **8o** and **8s** in 83 % and 87 % yield as a mixture of *cis*- and *trans*- isomers in a 1.6:1 *cis:trans* ratio for *N*-acyl thiazolidine and 1.5:1 *cis:trans* ratio for the *N*-acyl oxazolidine. Cyclisation to form the bicyclic tetramic acids **9o** and **9s** was achieved by heating KO^tBu at reflux in dry THF for 3 h. Acidification of the resulting mixture using 2M HCl, and separation with ethyl acetate, yielded the oxazolidine and thiazolidine tetramic acids **9o** and **9s** in 72 % and 78 % yield respectively. With the tetramic acid cores in hand, further manipulation at the C7 position was performed to yield functionalised tetramic acids **64o** and **64s** (Scheme **2.1**).

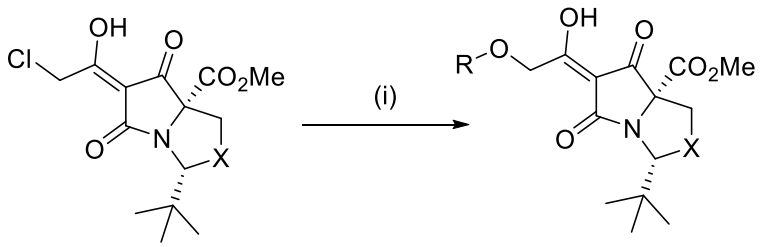


9o/9s **64o/64s**

Scheme 2.1. Functionalisation of the tetramic acid core at the C-7 position (i) Chloroacetic acid, DCC, DMAP, DCM, rt, 18 h. X = S, 30 %. X = O, 36 %.

Tetramic acids **9** were reacted with chloroacetic acid in the presence of DCC and DMAP in DCM to yield the α-chloroketone tetramic acid **64** in 30 % yield for X = O and 36 % yield for X = S. Purification of the acylated tetramic acids **64** was achieved using column chromatography, however multiple runs through the column were necessary to achieve a clean NMR spectrum – this was due to the co-elution of **64** with various, unidentified side products.

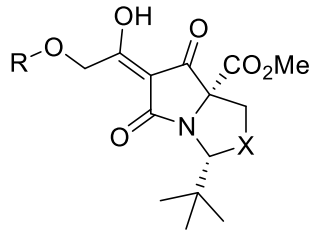
Important to this synthesis was the inclusion of the α-chloro ketone, which is a favourable substrate for S_N2 reactions. This strategy allowed for rapid library generation using a range of phenolic coumarin compounds (Scheme **2.2**), but with a very short linking tether.



64o/64s **65o/65s**

Scheme 2.2. Synthesis of tetramic acid - coumarin hybrids by S_N2 reaction. (i) Phenol, Cs_2CO_3, MeCN, 18 h - 48 h, 16 - 58 %.

Chloro-substituted tetramic acids **64** were dissolved in dry MeCN, followed by addition of the phenolic coumarin and Cs_2CO_3. The reaction mixture was then heated to reflux for 18 h until reaction was complete by LRMS. TLC analysis revealed that the chloro-substituted starting materials **64** and the desired tetramic acid – coumarin products **65o** and **65s** had the same R_f, rendering TLC ineffective in monitoring the progress of the reaction and thus, mass spectrometry was employed to monitor reaction progress. Various tetramic acid – coumarin hybrids were successfully synthesised and are outlined below in Table **2.1**.



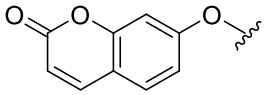
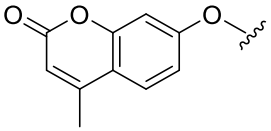
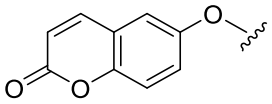
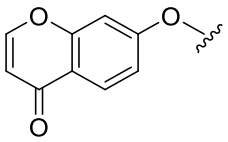
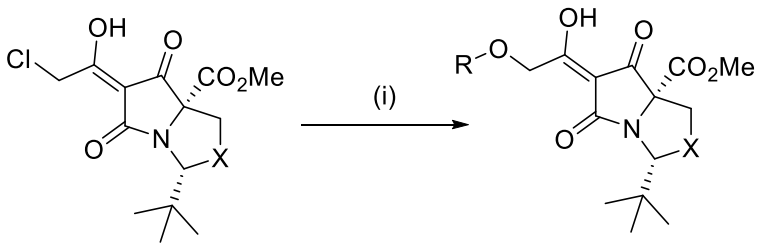
	R =	X = O Yield (%)	X = S Yield (%)
66o/s		55	16
67o/s		43	35
68o/s		37	27
69o/s		16	33

Table 2.1. Synthesis of tetramic acid - coumarin hybrids

The suitability of non-phenolic coumarins using this route was also investigated. Table **2.2** below indicates the synthesis of tetramic acid – coumarin hybrids in which the nucleophilic oxygen is no longer directly attached to an aromatic ring.



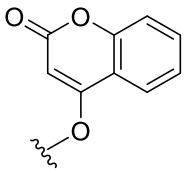
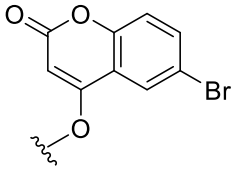
	R =	X = O Yield (%)	X = S Yield (%)
70o/s		58	0
71o/s		0	0

Table 2.2. Non-phenolic tetramic acid - coumarin hybrid synthesis. (i) 4-hydroxycoumarin, Cs_2CO_3, MeCN, 18 h, reflux.

The reaction conditions employed in this reaction mirrored the previously used conditions for the phenolic coumarins. It was reasoned that due to the delocalisation of the oxygen lone pair in the non-phenolic coumarins (Figure **2.3**), the acidity of the proton would remain relatively high and Cs_2CO_3 would be sufficient for deprotonation of the hydroxyl group.

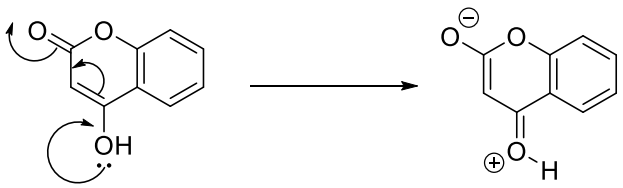


Figure 2.3. Delocalisation of oxygen lone-pair increasing acidity of the hydroxy proton.

Compound **70o** was isolated in good yield, comparable to the phenolic coumarins. It was noticed however, that decomposition in solution was rapid and the ^{1}H NMR spectrum quickly became unresolved. Decomposition of this product might be expected to proceed by the mechanism outlined below in Figure **2.4**, although this was not examined in detail.

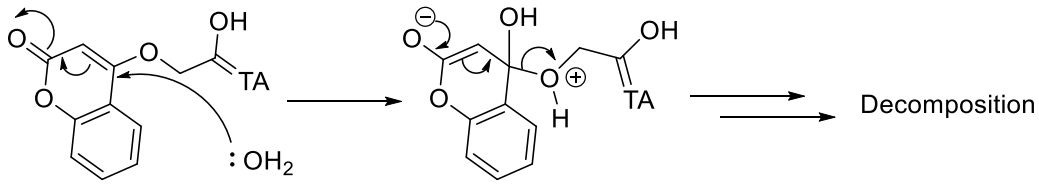
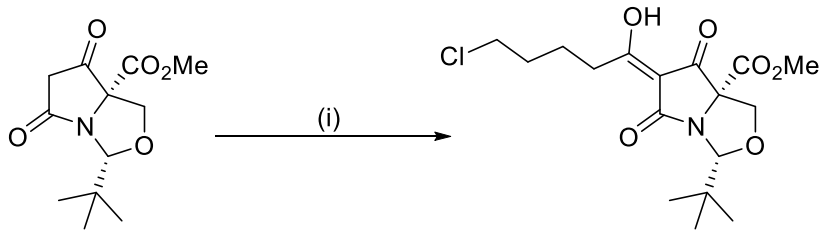


Figure 2.4. Potential decomposition mechanism of tetramic acid - non-phenolic coumarin hybrids. (TA = tetramic acid).

Bromo-substituted coumarin **71** was of interest for its further application for metal-mediated coupling processes, but attempted formation did not yield any desired product; this reaction was not examined further.

Synthesis of hybrids with a longer linker

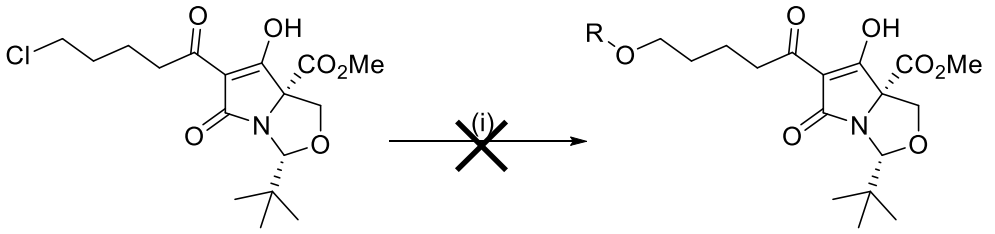
In order to assess the importance of the length of linker, the synthesis of tetramic acid – coumarin hybrids with longer linkers was attempted. In hybrids, the linker between the two active pharmacophores has been shown to be important for the activity of the hybrid.[99,100] To synthesise the hybrids with increased linker length, tetramic acid **9o** was reacted with 5-chlorovaleric acid in presence of DCC and DMAP to yield the chloro-substituted tetramic acid **72**; this would increase the number of carbons between the tetramic acid and the coumarin from one (Table **2.2**) to four (Table **2.3**). The desired product was obtained in low but acceptable yield of 33 % (Scheme **2.3**).



9o **72**

Scheme 2.3. Synthesis of chloro-substituted tetramic acid **72**. (i) 5-chlorovaleric acid, DCC, DMAP, DCM, rt, 48 h, 33 %.

Compound **72** was expected to be susceptible to S_N2 displacement, and was then reacted with various coumarins in an attempt to synthesise the tetramic acid – coumarin hybrids in Table **2.3**.



72 **73/74**

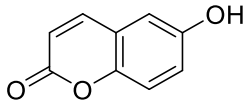
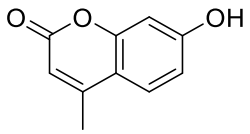
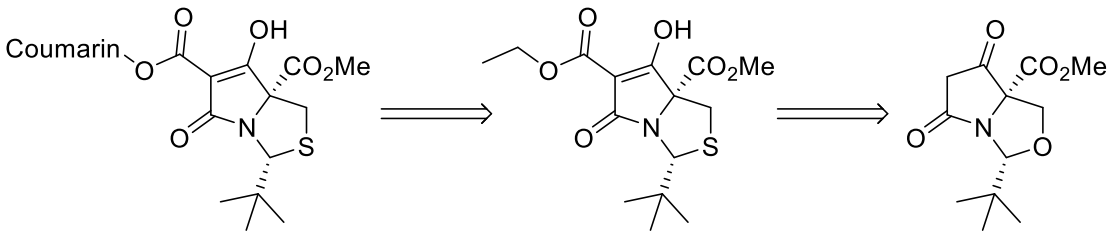
	R =	Yield (%)
73		0
74		0

Table 2.3. Attempted synthesis of tetramic acid - coumarin hybrids **73/74**. (i) Coumarin, Cs_2CO_3, MeCN, reflux, 48 h.

Synthesis of the tetramic acid – coumarin hybrids with increased linker length was unsuccessful using previously employed S_N2 conditions. By TLC and mass spectrometry, starting material was consumed and the desired product was observed by mass spectrometry, albeit as a small peak. However, isolation of the new TLC spot did not yield the desired product in either case. Reaction time was increased from the usual 18 h to 48 h, in order to achieve complete consumption of starting material, presumably due to the inherently decreased reactivity of primary alkyl halides compared to alpha-halo ketones. The reason for this problematic reactivity was not clear, since elimination was not found to be a competing reaction.

Synthesis of hybrids with an ester linker

The effect of replacing a ketone with an ester group at the C-7 position was examined. Retrosynthetic analysis of the desired hybrids **75** (Figure **2.5**) identified a possible synthetic route towards ester-substituted tetramic acid hybrids, using an approach which had been developed earlier.

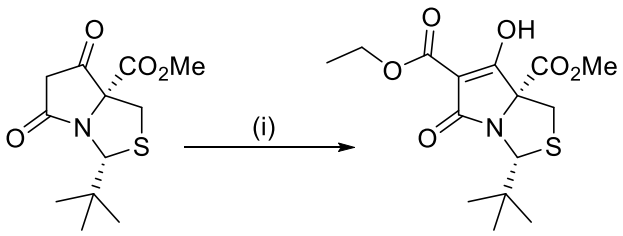


75 **76** **9s**

Figure 2.5. Retrosynthetic analysis of desired ester-substituted tetramic acid hybrids **75**.

In the analysis, tetramic acid core **9s** would be esterified using previously employed chemistry in the Moloney group. With **76** in hand, transesterification with various phenolic coumarins would yield the ester-substituted tetramic acid hybrids, which would allow investigation of the role of the functional group linking the two active pharmacophores.

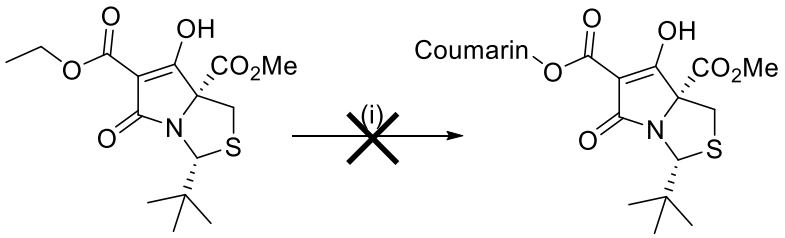
Initially, the ester substituted tetramic acid **76** was synthesised following procedures described by Moloney *et al.* (Scheme **2.4**).[101]



9s **76**

Scheme 2.4. Synthesis of ester-substituted tetramic acid **76**. (i) Ethyl chloroformate, DMAP, DCM, rt, 18 h, 24 %.

C-7 Ester-substituted **76** was synthesised by reaction of tetramic acid **9s** in the presence of DMAP in 24 % yield. With **76** in hand, transesterification reactions with two phenolic coumarins were attempted (Scheme **2.5**).



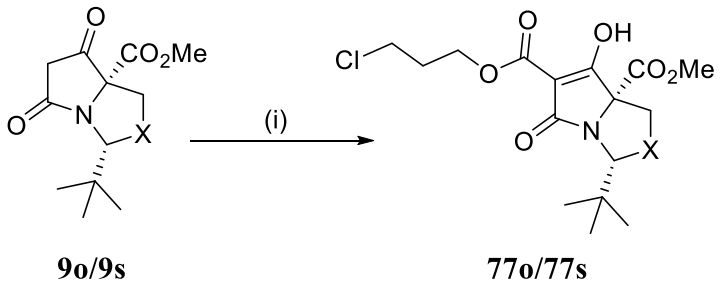
76 **75**

Scheme 2.5. Attempted transesterification of tetramic acid **76** with phenolic coumarins to yield hybrids **75**. (i) Phenolic coumarin, toluene, reflux, 18 h.

However, the synthesis of the tetramic acid – coumarin hybrids **75** was unsuccessful. Initial attempts involved refluxing the ethyl ester tetramic acid **76** in toluene with the phenolic coumarin. After 18 h at reflux, TLC and mass spectrometry revealed only starting materials and no evidence of product formation was observed. The reaction was repeated in the presence of $ZnCl_2$ acting as a Lewis acid, but no change in reactivity was observed and only starting materials were recovered. However, the lack of reactivity could result from a reaction requiring interaction of bulky coumarin and tetramate components; this is likely to be complicated by the very acidic C7 proton. Considering this, other transesterification attempts were not made.

Synthesis of hybrids with a longer ester linker

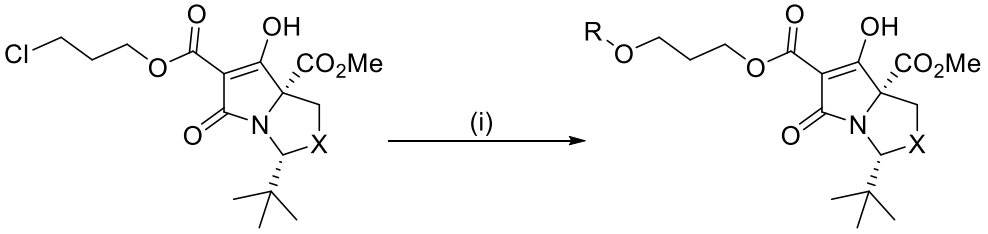
Other attempts to synthesise tetramic acid – coumarin hybrids with longer and different linkers were made. By repeating conditions previously employed, compounds **77**, which contained an ester group at C-7 instead of a keto group, and a longer carbon chain linker were synthesised (Scheme **2.6**).



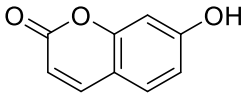
9o/9s **77o/77s**

Scheme 2.6. Synthesis of chloro-substituted tetramic acids **77o/77s**. (i) 3-chloropropyl chloroformate, DMAP, DCM, rt, 18 h, 24 % (X=S), 51 % (X=O).

Tetramic acids **9** were reacted with 3-chloropropyl chloroformate in the presence of DMAP at rt overnight to give the C-7-ester substituted tetramic acids **77o** and **77s** in 51 % yield and 24 % yield respectively. Characterisation of the chloro-substituted tetramic acids **77** indicated a high level of tautomerism in the NMR spectrum; no proton at C-7 was observed so it was reasoned that two enolic forms were present but not the keto form. With the ester linked chloro-substituted tetramic acids **77o** and **77s** in hand, previously employed S_N2 conditions were attempted with various phenolic coumarins (Table **2.4**).



77o/77s **78** X=O **79** X=S

78/79	R =	X = O (%)	X = S (%)
78a/79a		17	-

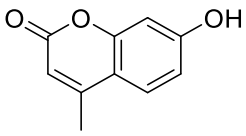
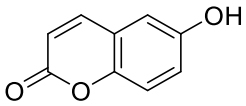
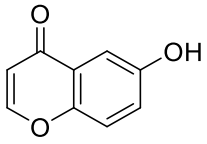
78b/79b		18	32
78c/79c		32	12
78d/79d		0	0

Table 2.4. Synthesis of tetramic acid - coumarin hybrids **78** and **79** containing a longer linker and ester substitution at C-7. (i) Phenolic coumarin, Cs_2CO_3, MeCN, reflux, 48 h.

The synthesis of tetramic acid – coumarin hybrids with a longer ester linker was successful in most cases, but not for the tetramic acid – flavone compounds **78d/79d**. Yields for the synthesis of the tetramic acid – coumarin compounds were low but comparable to the previously synthesised systems. The low yield is likely due to the small scale of reaction and multiple acid washes required to generate a clean NMR spectrum. Noteworthy is the reaction time for these systems, which was slower than for alpha-halo ketones (48 h compared to 18 h).

Purification of tetramic acid – coumarin hybrids.

Difficulties in the isolation and purification of the tetramate products were found in most cases. As the reaction is performed in base, the tetramic acid product is deprotonated and therefore liquid-liquid separation did not transfer the desired product into the organic phase (Figure **2.6**). To overcome this, the aqueous layer was acidified with 2M HCl which re-

protonated the tetramic acid, allowing transfer into the organic phase and subsequent purification by column chromatography.

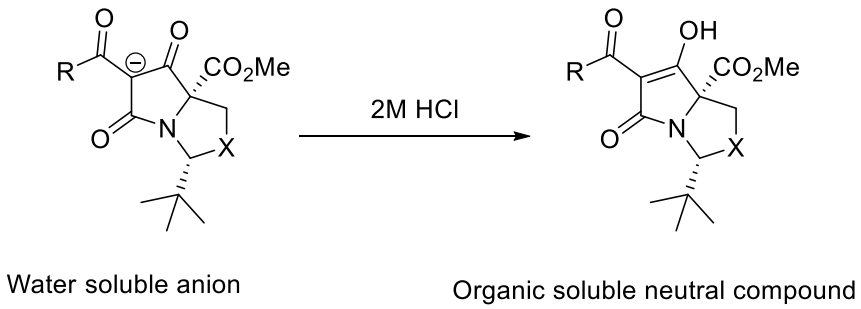


Figure 2.6. Acidification of tetramic acid - coumarin hybrids

Column chromatography was effective in the purification of the tetramic acid – coumarin hybrid compounds. However, ^{1}H NMR analysis immediately after chromatography revealed a non-resolved spectrum (Compound **78a**, Table **2.5**). Multiple 2M HCl acid washes after column chromatography were necessary to achieve a well-resolved NMR spectrum. It was thought that the broadness of the NMR peaks was due to the high affinity to metals of the tetramic acids, a phenomenon documented in the literature but not investigated in detail.[34,102] An investigation into the metal binding abilities of the synthesised tetramic acids is reported later in this chapter.

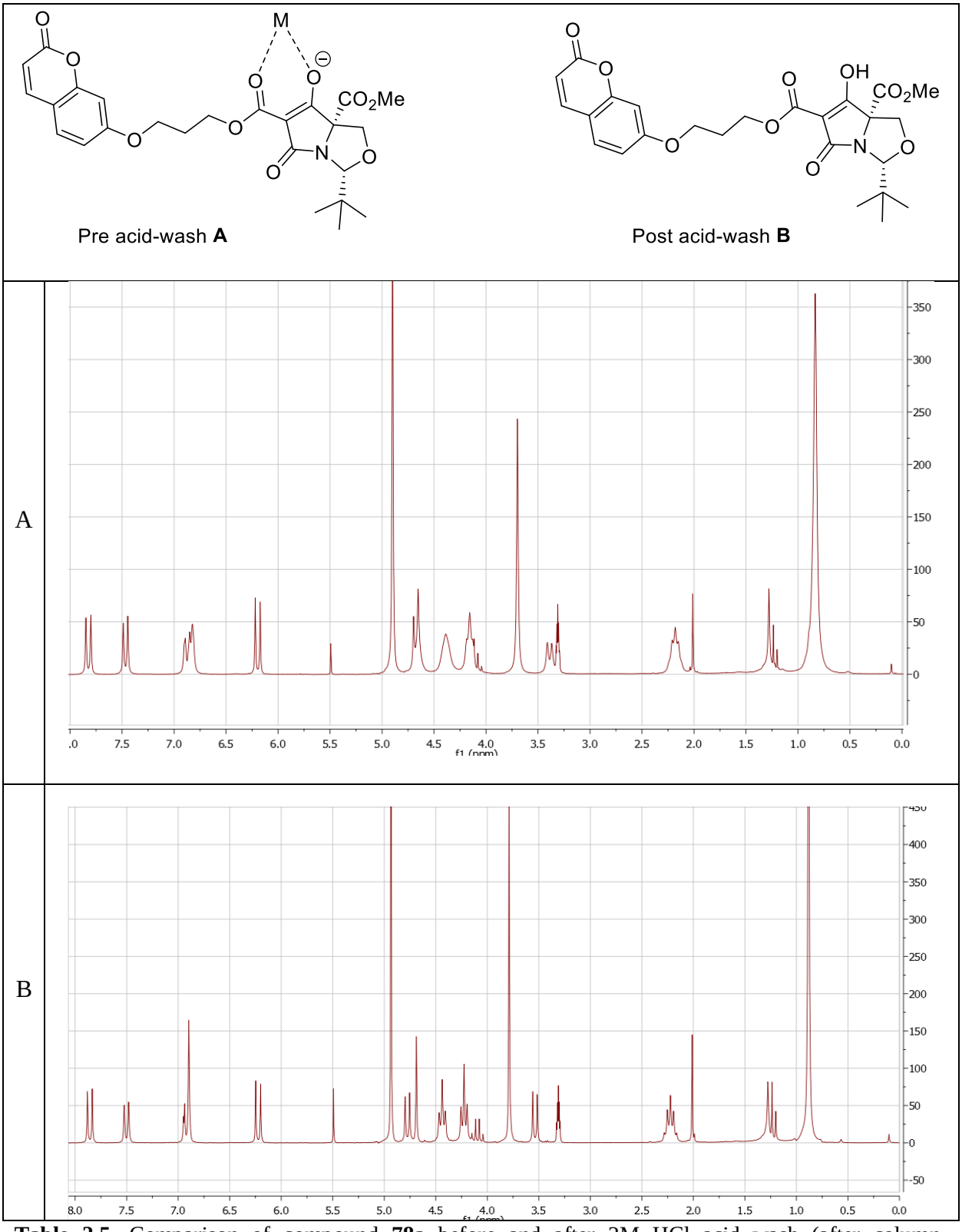
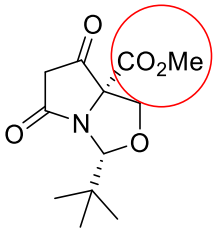


Table 2.5. Comparison of compound **78a** before and after 2M HCl acid wash (after column chromatography, NMR solvent MeOD, 200 MHz).

As can be seen in Table **2.5** above, the NMR spectrum before acid washing (entry **A**) shows significant broadening of the NMR peaks. It is also important to note that the peaks which have broadened the most compared to entry **B** (post acid-wash) are those closest in proximity to the acid centre, thought to be responsible for the metal chelation properties of tetramic acids. Broadening is most obvious for the peaks around 2.25, 4.25 and 4.45 ppm, which correspond to the three CH_2 protons in the linker chain that are close to the acidic centre. The alkene CH peaks at around 6.2 and 7.8 ppm do not show any broadening and these peaks are distant from the acidic centre, adding further evidence that the chelation centre is at the enolic site. Not only have the peaks broadened before relative to after the acid-wash, but changes in ppm are also observed. For most peaks, the change in ppm is subtle; however the C4 protons have noticeably shifted. Pre acid-wash, one of the C4 protons resides at 3.39 ppm however in entry **B** the chemical shift of this proton has shifted to 3.54 ppm. Considering these observations, it is clear that the acid-wash after purification by column chromatography is essential to obtain a well resolved NMR spectrum.

Tetramic acid – coumarin hybrids with a novel tetramic acid

Having successfully synthesised a small compound library of tetramic acid – coumarin hybrids, it was decided to vary the tetramic ester component of the hybrid (Figure **2.6**).



9o

Figure 2.6. Tetramic acid **9o**, red circle highlighting the point of desired functionalisation

To achieve this, conditions were employed that were recently described by the Moloney group.[52] As the methyl ester is essentially inert once incorporated in the bicyclic ring, precluding transesterification, it was necessary to introduce the novel ester functionality before forming the bicyclic tetramic acid (Scheme **2.7**). Most methods for the synthesis of serine esters use a BOC protected serine starting material, but we were interested in the development of a more scalable process.

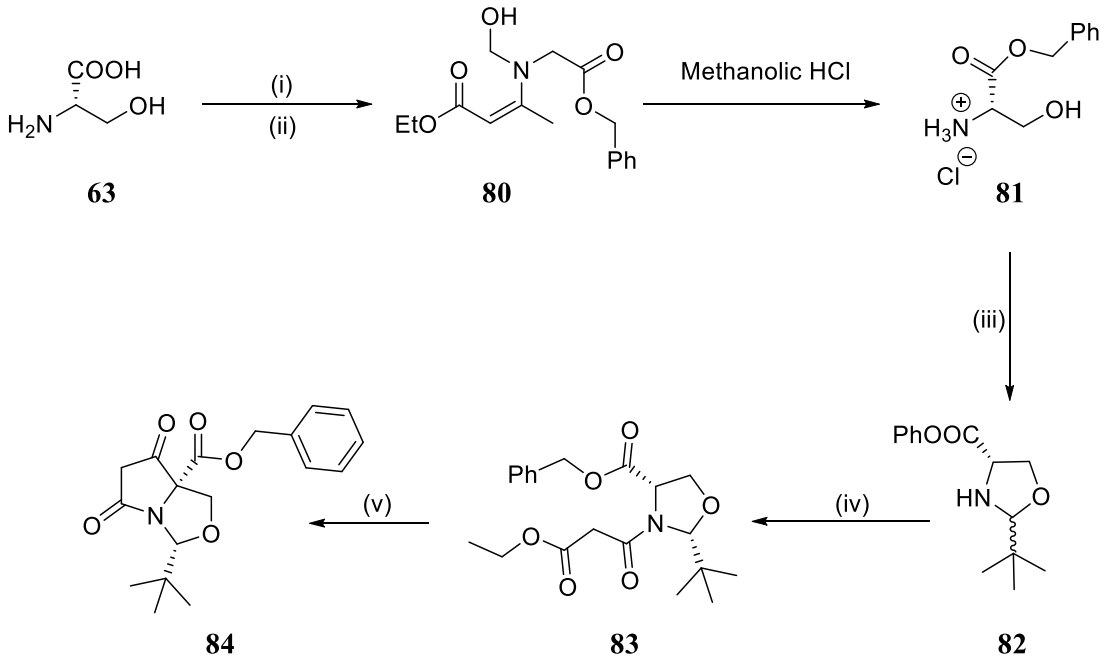
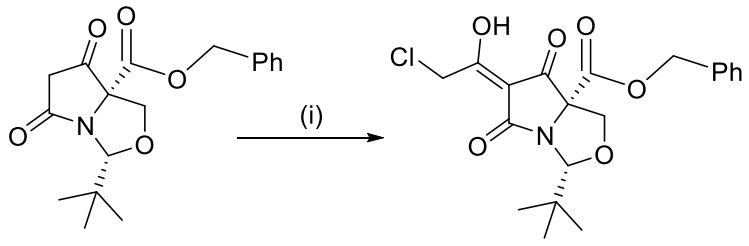


Figure 2.7. Synthesis of benzyl ester tetramic acid **84**. (i) Ethyl acetoacetate, N,N,N,N-tetramethyl guanidine, DMF, rt, 16 h, quantitative yield. (ii) Benzyl bromide, rt, 24 h, quantitative yield. (iii) Pivaldehyde, NEt_3, petrol 40 – 60 °C, reflux, 18 h, 86 %. (iv) Ethyl hydrogen malonate, DCC, DMAP, DCM, rt, 6 h, 35 % (as a single diastereomer). (v) KO^tBu, THF (dry), reflux, 3 h, 81 %.

Initially, L-serine is dissolved in DMF and *N*,*N*,*N*,*N*-tetramethyl guanidine is added, followed by ethyl acetoacetate, which acts as a protecting group to the nucleophilic primary amine of L-serine. After stirring for 16 h to allow the formation of the enamine, benzyl bromide was

added and performed an S_N2 reaction with the carboxylic acid group to yield compound **80** in quantitative yield. Unfortunately, this approach was not suitable for L-Cysteine as the thiol group, which is more nucleophilic than the alcohol, since no product was observed.

With the benzyl substituted enamine in hand, the protecting group had to be removed so as to allow oxazolidine formation in the subsequent steps. To do this, a solution of methanol HCl was prepared by mixing acetyl chloride with MeOH. The resulting solution was added dropwise to the enamine-protected serine benzyl ester **80**. After stirring for 5 min, the solvent was removed and yielded L-serine benzyl ester hydrochloride **81** in 76 % yield. With the L-serine benzyl ester **81** in hand, cyclisation with pivaldehyde to form the oxazolidine was performed yielding the oxazolidine **82** in 86 % yield. *N*-Acylation followed, using DCC, DMAP and ethyl hydrogen malonate to give the *N*-acylated oxazolidine **83** in 35 % yield (isolated as a single diaseteroisomer). Cyclisation of **83** with KO^{t}Bu in dry THF yielded the benzyl ester tetramic acid core **84** in 81 % yield. The benzyl ester tetramic acid **84** was then reacted with chloroacetic acid to yield the chloro-substituted tetramic acid **85**, which is set up for the S_N2 displacement reactions successfully employed previously (Scheme **2.7**).

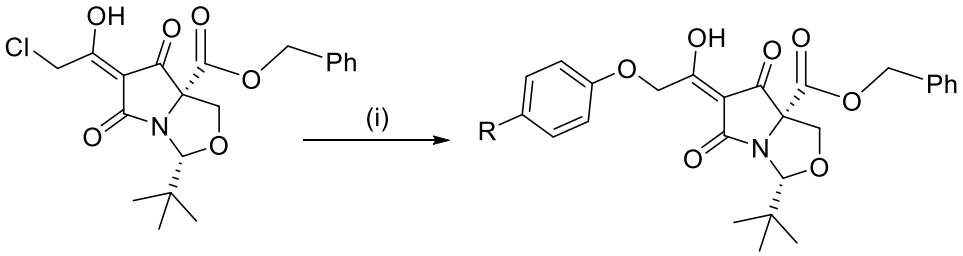


84 **85**

Scheme 2.7. Synthesis of chloro-substituted benzyl ester tetramic acid **85**. (i) Chloroacetic acid, DCC, DMAP, DCM, rt, 18 h, 22 % mass recovered.

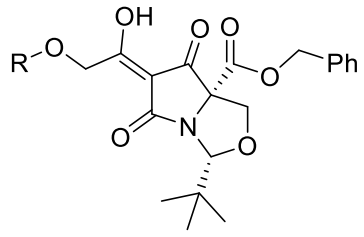
Chloroacetic acid was successfully coupled at the C7 position of the tetramic acid core using the DCC, DMAP conditions, yielding the chloro-substituted tetramic acid in 22 % recovered

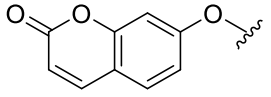
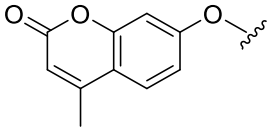
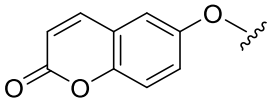
mass. Purification by column chromatography proved troublesome as a pure NMR spectrum of **85** was unobtainable, even after three runs through a column. However, there was sufficient evidence of product formation from NMR, LRMS and TLC to attempt to generate the tetramic acid – coumarin hybrids (Scheme **2.8**, Table **2.6**).



85 **86**

Scheme 2.8. Synthesis of benzyl ester tetramic acid - coumarin hybrids **86**. (i) Hydroxycoumarin, Cs_2CO_3, MeCN, reflux, 18 h.



	R =	Yield (%)
86a		30
86b		27
86c		22

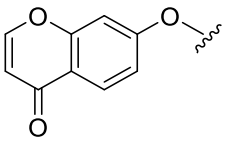
86d		0

Table 2.6. Yields of synthesised benzyl ester tetramic acid - coumarin hybrids **86**.

S_N2 displacement of the chloro-substituted tetramic acid yielded the coumarin hybrids **86a** – **86d** in low yields; this is likely due to the small scale of reaction and the numerous acid washes necessary to achieve a clean NMR spectrum. Reaction of the tetramic acid with the flavone to produce compound **86d** did not produce clean material and no signs of product formation were observed which correlates with the observation seen previously in Table **2.4**.

Tetramic acid – imidazole hybrids

Imidazoles are present in many clinically used antibiotics and have been reported in the literature numerous times as antibacterial agents.[103] Nitroimidazoles are a class of imidazole containing antibiotics commonly used to treat anaerobic and parasitic bacterial infections.[104] Examples of nitroimidazoles include metronidazole, tinidazole, and nimorazole and among many others (Figure **2.8**).

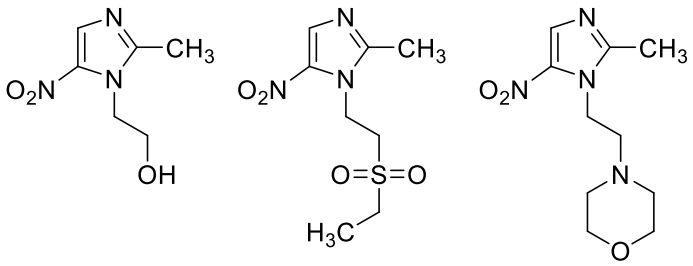
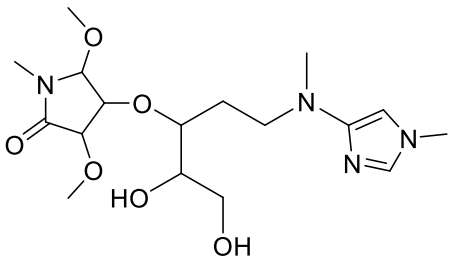


Figure 2.8. Examples of nitroimidazole antibiotics Metronidazole, Tinidazole and Nimorazole.

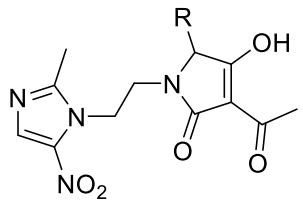
Naik *et al.* identified an imidazole antibiotic isolated from the marine sponge *Iricinia fusca*. The compound **87** (Figure **2.9**) displayed activity against the Gram-positive bacteria *S. aureus* with an MIC value of 280 µM. [105]



87

Figure 2.9. Compound **87**, an antimicrobial isolated from *Iricinia fusca.*

Tetramic acid – metronidazole hybrid antibiotics have been synthesised by Lee *et al.* Potent activity was reported to be observed against Gram-positive bacteria, with the hybrid compound displaying an MIC of 0.25 μg/mL^{-1} against *C. difficile* (Figure **2.10**).

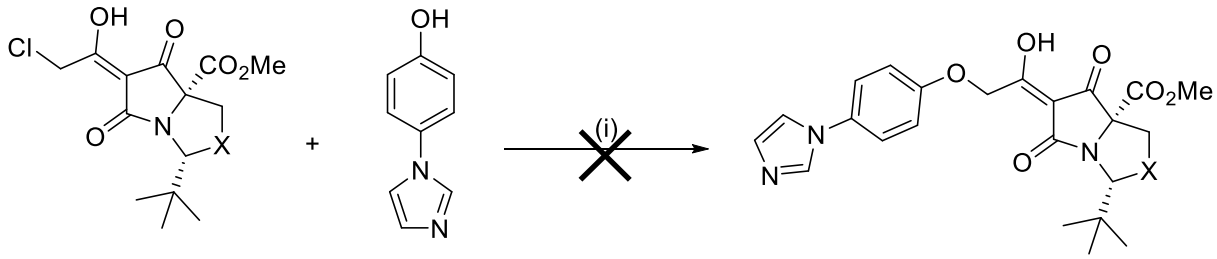


62

Figure 2.10. Tetramic acid - imidazole hybrid **62**.

Synthesis of tetramic acid – imidazole hybrids

Owing to the success of imidazoles as antibiotics, the synthesis of tetramic acid – imidazole hybrids was attempted. As the S_N2 displacement of the chloroketo tetramic acid **64o/64s** was successful in rapidly generating tetramic acid – coumarin hybrids, it was reasoned that the same approach could be applied to imidazoles. Commercially available phenolic imidazole **88** was coupled with the tetramic acid to yield the hybrid compounds **89** (Scheme **2.9**).

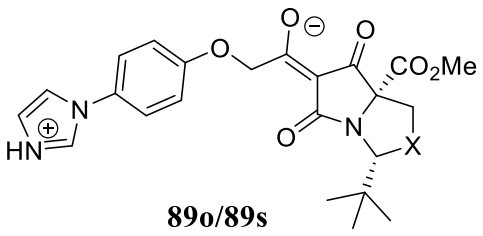


64o/64s **88** **89o/89s**

Scheme 2.9. Synthesis of tetramic acid - imidazole hybrids **89**. (i) 1.5 eq phenol, Cs_2CO_3, MeCN, reflux, 18 h.

Repeating the conditions previously employed to synthesise the tetramic acid – coumarin hybrids did not result in successful isolation of the product, despite mass detection in the LRMS and consumption of the tetramic acids **64** by TLC. In comparison of the R_f values of previously synthesised compounds to that of the tetramic acid – imidazole hybrid, the imidazole did not behave in a similar way. The R_f of the tetramic acid – coumarin hybrids in 5% MeOH in EtOAc is usually between 0.55 and 0.65, however the R_f of the tetramic acid – imidazole is 0.00 and the product did not move from baseline. This resulted in an inability to purify the tetramic acid – imidazole hybrid.

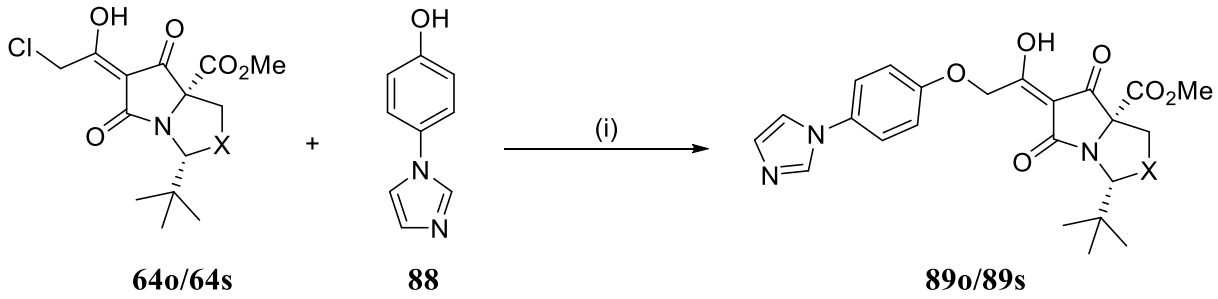
Another distinguishing feature of the tetramic acid – imidazole hybrids is the ionisation mode in which the product is detected by mass spectrometry. For the tetramic acid – coumarin compounds, the product is detected in the negative ionisation mode ($[M-H]^-$) and is not observed in the positive ionisation spectrum. In the tetramic acid – imidazole hybrids, the mass of the product is detected in both the positive ($[M+H]^+$) and the negative ionisation modes ($[M-H]^-$). Unlike in the tetramic acid – coumarin hybrids, the imidazole containing hybrids possess a basic nitrogen. It was hypothesised that the tetramic acid – imidazole hybrids exists as a zwitterion, as depicted in Figure **2.11** below, making it very polar.



89o/89s

Figure 2.11. Zwitterionic form of tetramic acid - imidazole hybrid **89**.

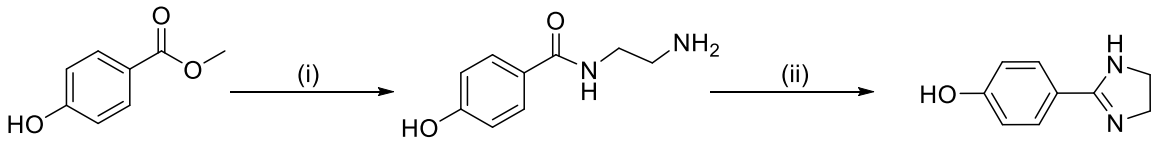
The observation of the mass ion in both negative and positive ionisation modes provides evidence for the zwitterion. In order to account for this chemical difference between the tetramic acid – coumarin hybrids and the tetramic acid – imidazole hybrids, the reaction conditions for hybrid generation had to be altered. Initially, the equivalents of the phenol were adapted. The tetramic acid and phenol were reacted in a 1:1 ratio, whereas previously 1.5 equivalents of the phenol were used (Scheme **2.10**).



Scheme 2.10. Synthesis of tetramic acid - imidazole hybrids **89**. (i) 1.0 eq phenol, Cs_2CO_3, MeCN, reflux, 18 h, quantitative yield.

Following the reaction, the solvent was removed *in vacuo* and the resulting residue was analysed by NMR spectroscopy. Complete conversion of the tetramic acid was achieved and the tetramic acid – imidazole hybrids **89** were isolated in quantitative yield, without the need for further purification.

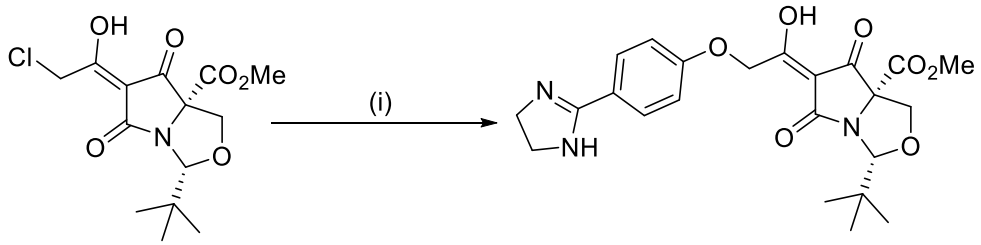
In order to further investigate and confirm the influence of the basic nitrogen on the chemical behaviour of these hybrids, a structurally related imidazoline – tetramic acid hybrid was synthesised. As the phenolic substitution of the chloro-substituted tetramic acid **64** proved reliable, a phenolic imidazoline compound was desired (Scheme **2.11**).



90 **91** **92**

Scheme 2.11. Synthesis of phenolic imidazoline **92**. (i) Methyl-4-hydroxybenzoate (**90**), ethylene diamine, reflux, 6 h. (ii) Ethanol:H_2O 1:2, reflux, 30 min, 35 %.

Klimesová *et al.* reported the synthesis of imidazoline **92** by refluxing methyl-4-hydroxybenzoate **90** in ethylene diamine at reflux.[106] NMR analysis after 6 h revealed formation of both **91** and **92**. In order to drive the cyclisation reaction, the mixture was heated to reflux in a solution of ethanol:H_2O (1:2) for 30 minutes. After this time, a solid precipitate formed which was filtered, washed with ethanol, and dried to yield the phenolic imidazoline **92** in 35 % yield. With the imidazoline in hand, the synthesis of a tetramic acid – imidazoline hybrid was attempted (Scheme **2.12**) using 1.0 equivalent of phenol.



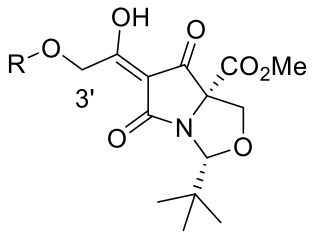
64o **93**

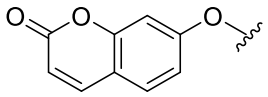
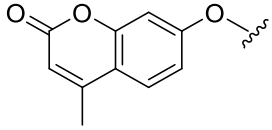
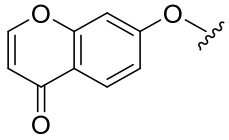
Scheme 2.12. Synthesis of tetramic acid – imidazoline hybrid **93**. (i) Phenol **92**, Cs_2CO_3, MeCN, reflux, 18 h, 85 %.

S_N2 displacement of the chloro-substituted tetramic acid with the phenolic imidazoline proceeded in 85 % yield. TLC analysis in 19:1 EtOAc:MeOH revealed that the tetramic acid

– imidazoline hybrid possessed an R_f value of 0.00, as seen with the imidazole hybrids **89**. This provided further evidence that tetramic acid hybrids containing a basic nitrogen had zwitterionic character, making handling the compound troublesome due to their insolubility in organic solvents.

As there are two nucleophilic sites in the phenolic imidazole and imidazoline which could displace the α-chloro function, it was necessary to confirm that the S_N2 reaction proceeded through oxygen and not nitrogen. By comparing the proton NMR shifts of C3' to those of previously synthesised compounds, and those in the literature,[97] a determination of which atom was modified could be made (Table **2.7**).



R =	C3'-H shift (ppm)
	5.17
	5.14
	5.22

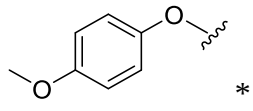
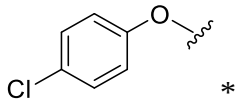
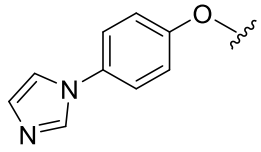
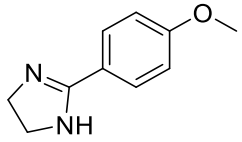
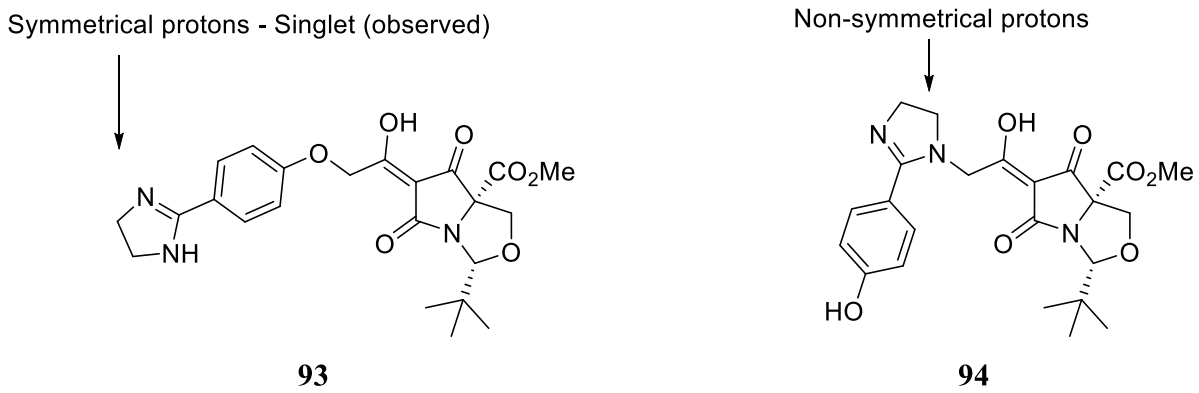
 *	5.18
 *	5.24
	5.11
	5.14

Table 2.7. Proton NMR shift comparison of C3'-H. * = values taken from literature source.[97]

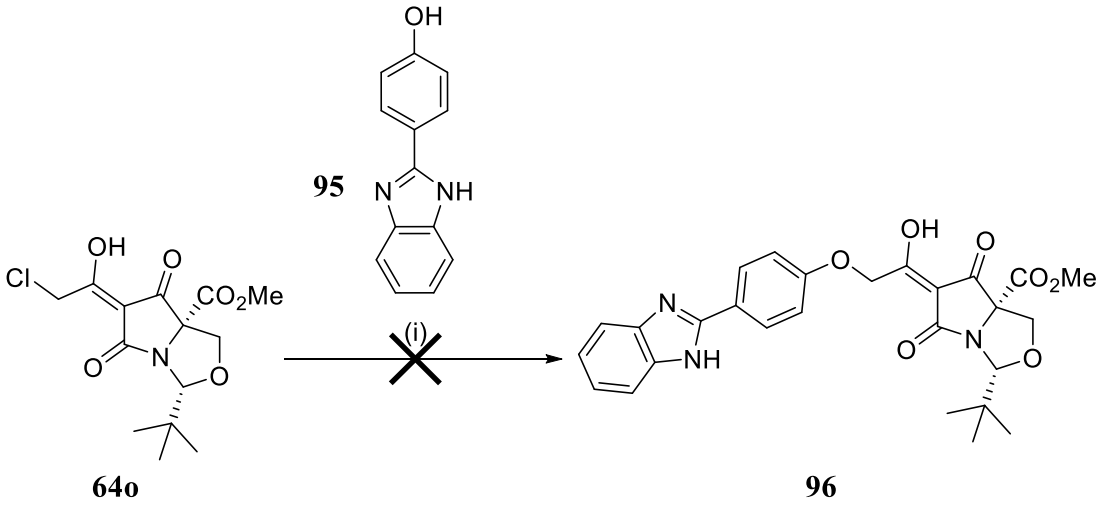
As can be seen from Table **2.7** above, the proton shift of C3' in the tetramic acid – imidazole/imidazoline hybrids is consistent with previously synthesised phenoxy-substituted tetramic acids, in which oxygen is the only potential nucleophile. Further evidence for nucleophilic oxygen is the 4H singlet in the imidazoline tetramic acid, corresponding to C11'. The singlet nature of the protons results from chemical equivalence, which can only be true if there is an element of symmetry in the phenolic imidazoline. Attack through nitrogen would break this symmetry and result in the generation of multiplicity, which is not observed by NMR spectroscopy (Figure **2.12**).



93 **94**

Figure 2.12. Symmetrical nature of **93**, consistent with NMR data. Structure **94** inconsistent with NMR analysis.

The synthesis of a structurally related tetramic acid – benzimidazole hybrid was also attempted. Benzimidazoles are found in many clinically administered drugs and have been employed in numerous hybridisation strategies as antibacterials as outlined in Chapter I.[60,63,64] Commercially available phenolic benzimidazole **95** was reacted with the α-chloro substituted tetramic acid **64o**, using conditions previously used to synthesise imidazole/imidazoline hybrids (Scheme **2.13**)

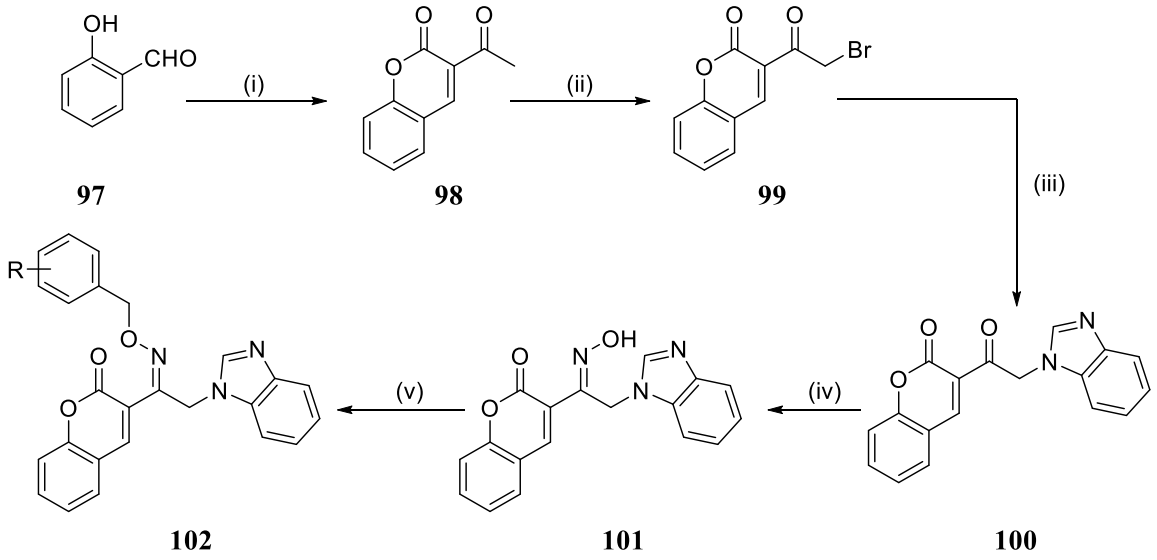


Scheme 2.13. Attempted synthesis of tetramic acid - benzimidazole hybrid **96**. (i) **95**, Cs_2CO_3, MeCN, reflux, 18 h.

Attempted S_N2 displacement using phenolic benzimidazole **95** was unsuccessful. Starting material was consumed by TLC and LRMS, but no product **96** was isolated.

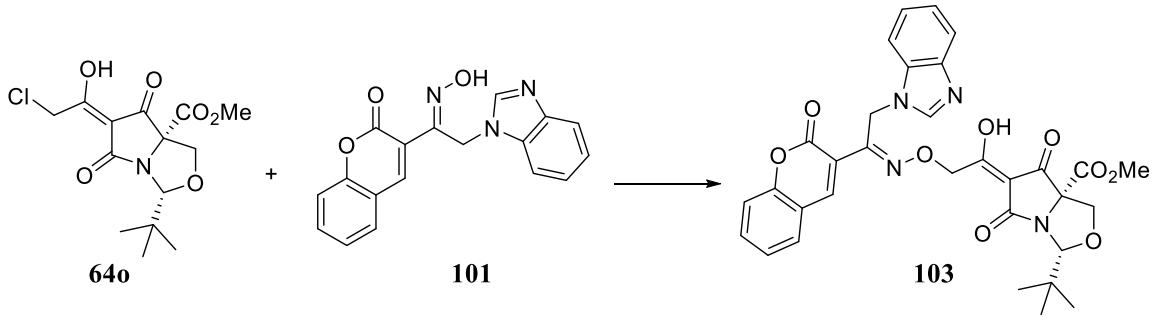
Synthesis of tetramic acid – coumarin – benzimidazole hybrids

Benzmidazole – coumarin hybrids were synthesised by Singh *et al.*[63] and displayed potent antibacterial activity against both Gram-positive and Gram-negative bacteria. Their work (Scheme **2.14**) inspired the attempted generation of a tetramic acid – benzimidazole – coumarin compound, possessing three known pharmacophores.



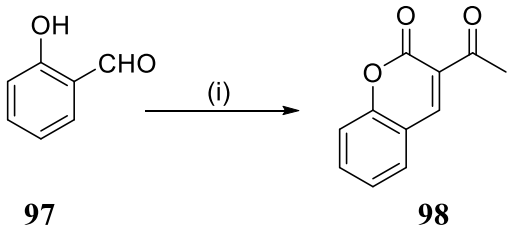
Scheme 2.14. Singh's synthesis of benzimidazole - coumarin antibacterials **102**. (i) Ethyl acetoacetate, piperidine. (ii) Bromine. (iii) Benzimidazole. (iv) Hydroxylamine hydrochloride. (v) Benzyl halide.

Initially, salicaldehyde **97** is reacted with ethyl acetoacetate and piperidine to yield compound **98**. Subsequent bromination in chloroform resulted in the formation of compound **99**. S_N2 displacement of the bromine with benzimidazole yielded **100**, which was further functionalised with hydroxylamine hydrochloride to produce the oxime **101**. Oxime **101** was a sufficiently good nucleophile to react with benzyl halides and yielded the benzimidazole – coumarin hybrids **102**. As the oxime was able to successfully react with benzyl halides, it was reasoned that the oxime would be able to displace the α-chloro substituted tetramic acid **64** to yield a hybrid compound containing three pharmacophores (Scheme **2.15**).



Scheme 2.15. Synthetic plan to generate a tetramic acid - benzimidazole - coumarin hybrid.

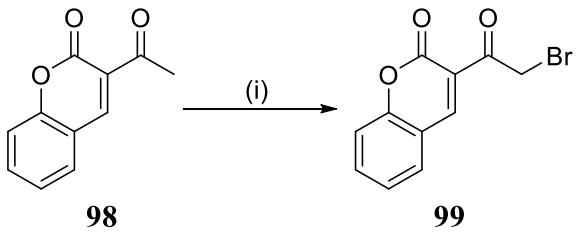
In accordance with the synthetic plan, the synthesis of acyl-coumarin **98** was undertaken. By repeating conditions reported in the literature, the reaction was carried out as described in Scheme **2.16**.



Scheme 2.16. Synthesis of 3-acyl coumarin **98**. (i) Ethyl acetoacetate, piperidine, 10 min, 52 %.

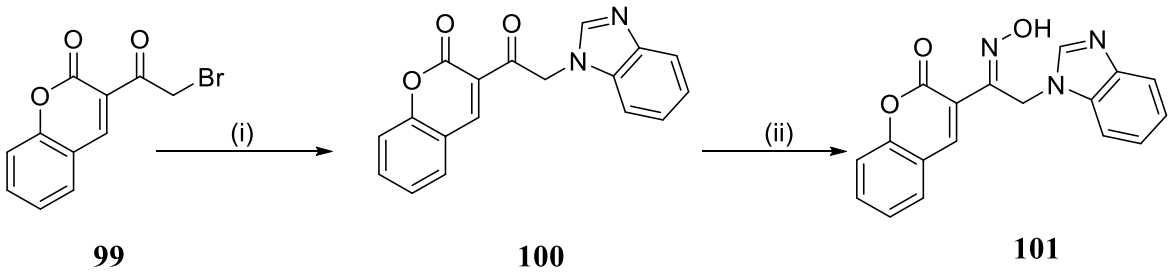
Initially, this reaction was performed as reported by Secci *et al.* in which EtOH was used as a solvent.[107] Under these conditions, no Knoevenagel condensation product was observed. However, other conditions were attempted in which no solvent was required: salicylaldehyde and ethyl acetoacetate were mixed in solvent free conditions, with two drops of piperidine added.[108] After solid formation was observed, crushing and stirring for 5 minutes in dilute HCl, followed by filtration yielded the acyl coumarin **98** in 52 % yield. With acyl coumarin

98 in hand, bromination was attempted to yield the α-bromo ketone **99**, susceptible to S_N2 displacement (Scheme **2.17**).



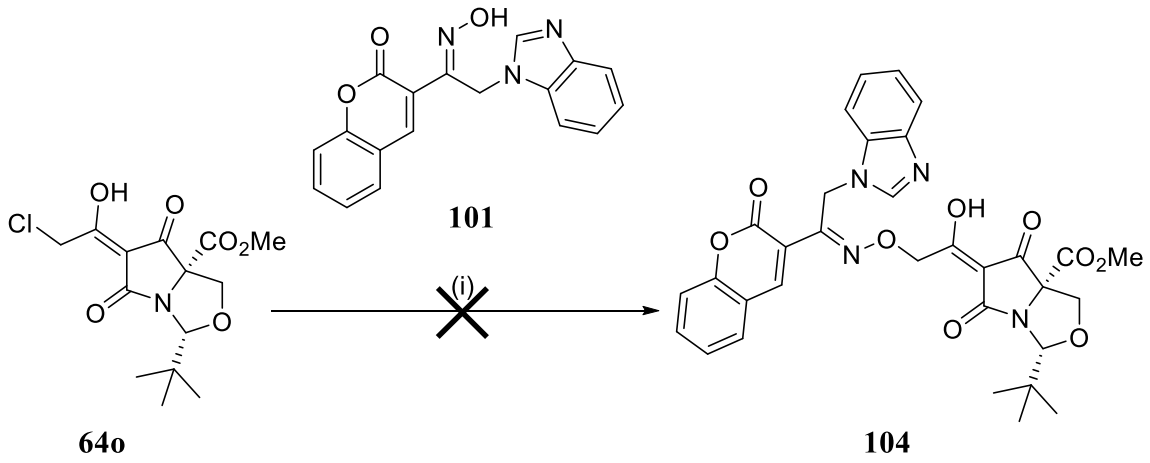
Scheme 2.17. Bromination of acyl coumarin **99**. (i) Br_2, $CHCl_3$ (dry), gentle heating, 15 min, 60 %.

Coumarin **98** was dissolved in dry chloroform and bromine was added. Gentle heating was applied to the reaction mixture until the evolution of HBr gas ceased. The reaction mixture was then cooled in an ice bath until a solid precipitate formed, which was obtained by vacuum filtration. Subsequent recrystallisation from acetic acid yielded the bromoacetyl coumarin **99**. Next step was the S_N2 reaction with benzimidazole to displace the bromine and yield the hybrid compound **100**. Following hybrid generation, reaction with hydroxylamine hydrochloride would yield oxime **101** (Scheme **2.18**).



Scheme 2.18. Generation of benzimidazole - coumarin oxime **101**. (i) Benzimidazole, MeCN, rt, 18 h, 79 %. (ii) Hydroxylamine hydrochloride, EtOH, reflux, 4 h, 22 %.

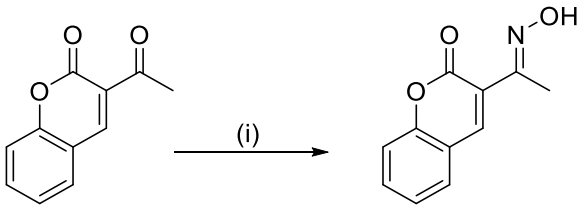
Reaction of **99** with benzimidazole in MeCN at rt for 18 h yielded the benzimidazole – coumarin **100**. Subsequent reaction with hydroxylamine hydrochloride in ethanol for 4 h yielded the oxime **101** in 22 % yield. With the oxime in hand, displacement with the α-chlorotetramic acid was attempted (Scheme **2.19**).



Scheme 2.19. Attempted synthesis of tetramic acid - benzimidazole - coumarin hybrid. (i) Benzimidazole - coumarin **101**, Cs_2CO_3, MeCN, reflux, 18 h.

Reaction between oxime **101** and tetramic acid **64o** yielded none of the desired product, despite consumption of starting material by TLC and MS. NMR analysis revealed an uninterpretable spectrum. Conditions where the base was altered were attempted however using KO^tBu and K_2CO_3 resulted in no change in reaction outcome.

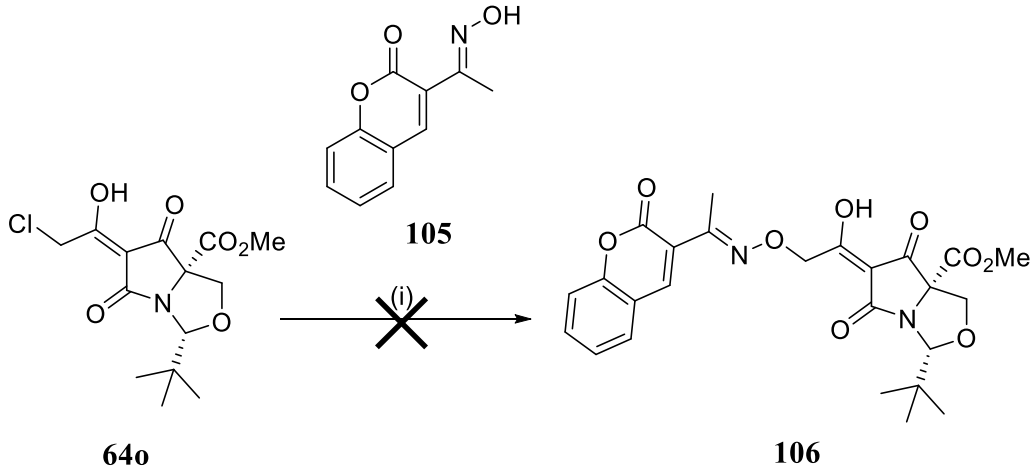
Despite unsuccessful generation of the three-component hybrid, the conversion of ketones to oximes inspired a novel attempt at synthesis of the tetramic acid – coumarin hybrids. Synthesis of acyl coumarin **98** followed by reaction with hydroxylamine hydrochloride would yield a tetramic acid – coumarin hybrid precursor **105**, containing an oxime linker (Scheme **2.20**) unlike the phenoxy linkers previously employed.



98 **105**

Scheme 2.20. Synthesis of oxime coumarin **105**. (i) Hydroxylamine hydrochloride, EtOH, 4 h, 11 %.

Acyl coumarin **98** was dissolved in EtOH and reacted with hydroxylamine hydrochloride to yield the target oxime **105** in 11 % yield. The R_F value of the starting material and product were similar and thus, co-elution occurred when purifying by column chromatography. With the oxime in hand, S_N2 conditions were utilised in an attempt to synthesise a tetramic acid – coumarin hybrid (Scheme **2.21**).



64o **106**

Scheme 2.21. Attempted synthesis of oxime linked tetramic acid - coumarin hybrid **106**. (i) Oxime coumarin **105**, Cs_2CO_3, MeCN, reflux, 18 h.

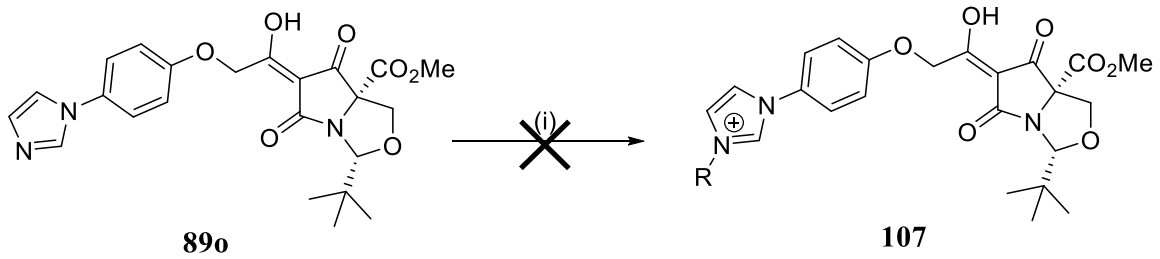
The attempted synthesis of **106** by repeating S_N2 conditions previously used was unsuccessful. TLC and LRMS analysis revealed consumption of the starting material

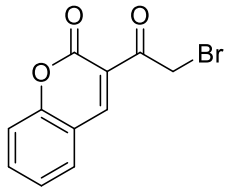
however no product was isolated, nor was there any evidence of product formation from TLC or LRMS.

Further functionalisation of tetramic acid – imidazole hybrids

The synthesised tetramic acid – imidazole/imidazole hybrids were chemically difficult to handle due to their zwitterionic behaviour, owing to the presence of a basic nitrogen in these structures. However, not only is the nitrogen atom basic, it is also nucleophilic. Literature seArChes of imidazole reactivities revealed that alkylation of both nitrogens contained within the imidazole was possible, which would result in a positively charged compound (commonly, charged imidazoles are employed as ionic liquids).[109] The generation of a positively charged hybrid was of particular interest as Richter *et al.* showed that, at physiological pH, positively charged nitrogen containing antibacterials displayed significantly improved Gram-negative activity by showing improved accumulation in the Gram-negative bacteria *E.coli*.[110] This enhanced ability to accumulate in the bacteria was attributed to the cationic behaviour of the synthesised compounds, which were able to pass through a particular porin – neutral and negatively charged compounds were unable to pass through this porin.

Synthetically, the charged imidazoles in the literature were accessed by reacting imidazole with base and performing a substitution reaction with alkyl halides – these conditions were repeated with the tetramic acid – imidazole hybrids (Scheme **2.22**).



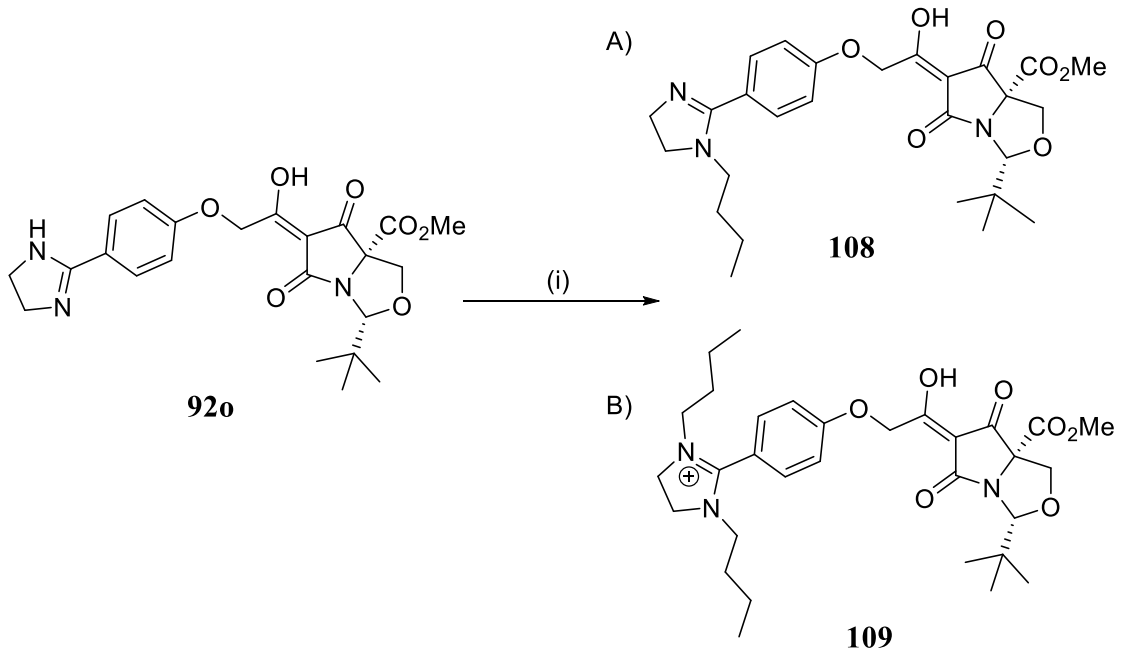
R-Hal =	Yield (%)
Methyl iodide	0
Butyl bromide	0
Benzyl bromide	0
	0

Scheme 2.22. Attempted synthesis of charged tetramic acid - imidazole hybrids **107**. (i) R-Hal, MeCN, rt, 24 h.

Alkylation of the tetramic acid – imidazole was attempted with various alkyl halides, namely methyl iodide, butyl bromide and benzyl bromide. Reaction with these halides yielded none of the desired product by LRMS or NMR. In addition to the simple alkyl halides, the alkylation reaction was attempted with the bromo-substituted acyl coumarin, which would have yielded a positively charged tetramic acid – imidazole – coumarin hybrid compound, however this was also unsuccessful and only starting material was recovered.

Further functionalisation of tetramic acid – imidazoline hybrids

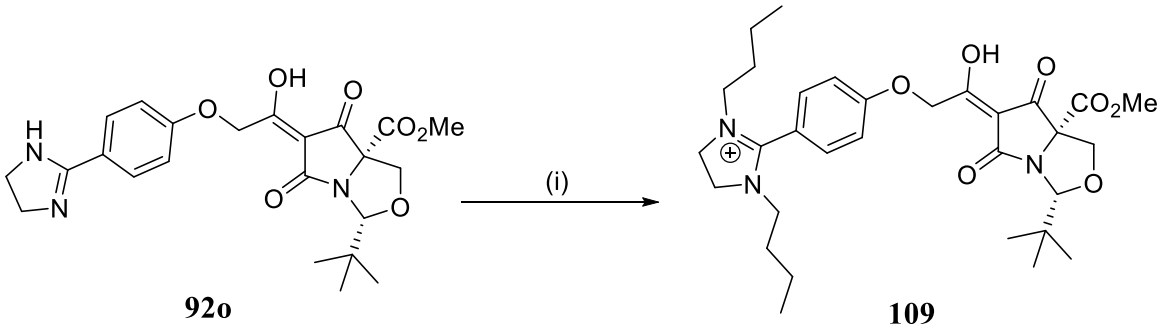
Despite unsuccessful alkylation of the imidazoles, alkylation was attempted with the imidazoline systems **92**. The tetramic acid – imidazoline series would however behave differently as there are two nucleophilic nitrogen atoms instead of the one of the imidazoles. Initially, the conditions previously used were employed using just one equivalent of the alkyl halide (Scheme **2.23**).



Scheme 2.23. Attempted synthesis of alkylated imidazolines **108**. (i) Butyl bromide, MeCN, rt, 18 h. A) 0 %, B) 50 %

Using just one equivalent of butyl bromide, the expected product from the reaction was compound **108**, the mono-alkylated tetramic acid – imidazoline hybrid. However, upon analysis by LRMS, the mass ion for the expected product was not observed. Instead, the mass ion for the double alkylated imidazoline **109** was observed, along with unreacted starting material. This preference for double alkylation suggests that once alkylated, the imidazoline

subsequently has increased nucleophilicity. Following this observation, the reaction conditions were adapted in an attempt to double alkylate the imidazoline, which would in turn generate a positively charged tetramic acid – imidazoline hybrid (Scheme **2.24**).



92o **109**

R-Hal =	Yield (%)
Methyl iodide	0
Butyl bromide	Quantitative
Benzyl bromide	0

Scheme 2.24. Synthesis of alkylated imidazoline cations. (i) R-Hal, MeCN, 18 h, rt.

Alkylation of the tetramic acid – imidazoline was unsuccessful upon coupling with benzyl bromide or methyl iodide; starting material was consumed but LRMS and NMR were not consistent with the desired product. However, reaction with butyl bromide yielded the desired double-alkylated imidazoline in quantitative yield.

Attempted synthesis of a tetramic acid – metronidazole hybrid

Lee *et al.* synthesised a range of tetramic acid – metronidazole hybrids which displayed potent antibacterial activity, particularly against Gram-negative bacteria. In their work, the 3-

acyl tetramic acid unit contained in reutericyclin **111** (a naturally occurring antibacterial compound) was coupled with metronidazole **110** at the tetramic acid nitrogen (Figure **2.13**).

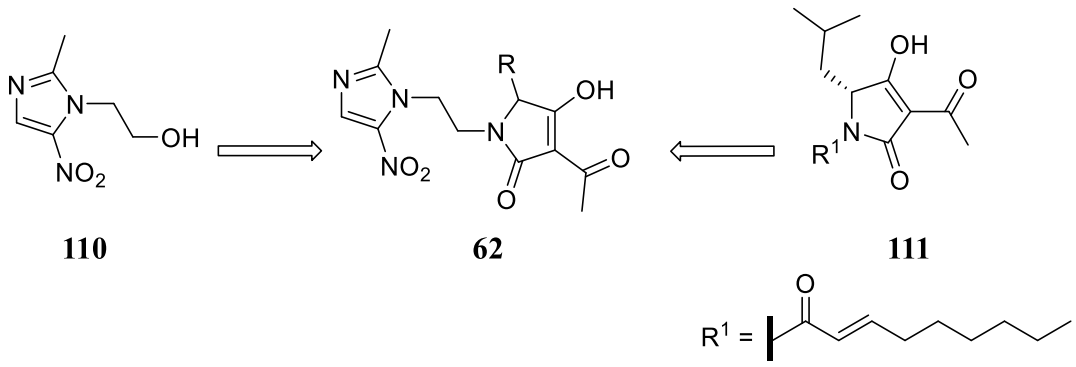
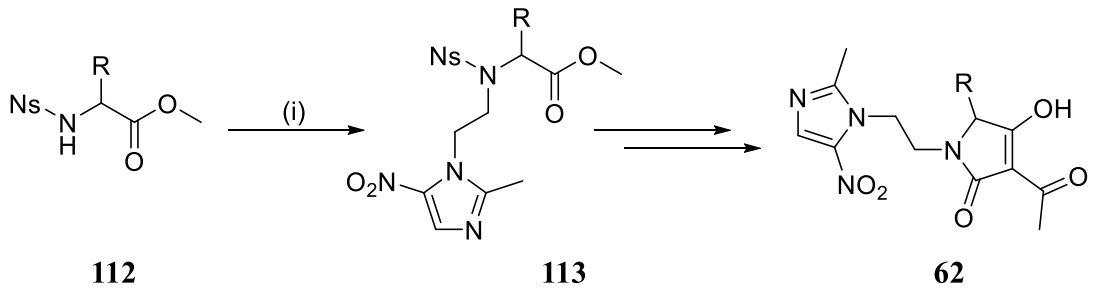


Figure 2.13. Lee *et al.* synthetic plan for the generation of tetramic acid - metronidazole hybrids.

Synthetically, the hybrids were accessed by performing a Mitsunobu reaction between the respective amino acid methyl ester **112** and metronidazole **110**. Subsequent cyclisation yielded the tetramic acid hybrids **62** (Scheme **2.25**, Table **2.8**).



Scheme 2.25. Synthesis of tetramic acid - metronidazole hybrids by Lee *et al.* (i) Metronidazole **110**, PPh_3, DEAD, THF.

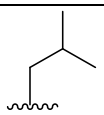
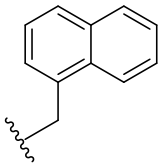
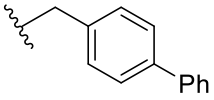
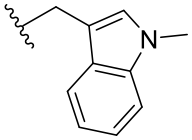
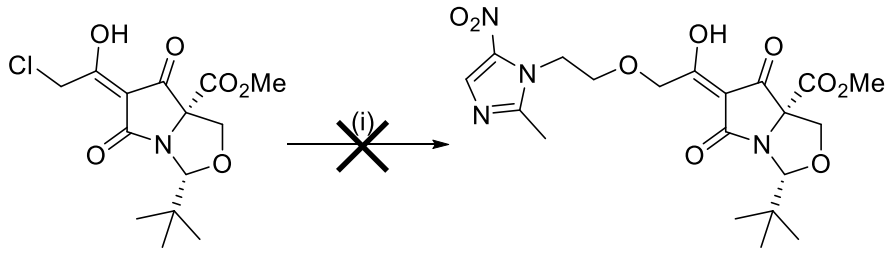
62 R =	MIC (μg/mL^{-1})*	MIC (μg/mL^{-1})▲
 **62a**	64	0.5
 **62b**	128	0.5
 **62c**	32	1.0
 **62d**	256	1.5

Table 2.8. Antibacterial activity of tetramic acid - metronidazole hybrids **62a-d**. * = *Actinomycetes viscosus* (gram-positive). ▲ = *Bacteroides* sp. (gram-negative).

The synthesised tetramic acid – metronidazole hybrids have potent antibacterial activity against Gram-negative bacteria. Also, activity against Gram-positive bacteria was observed but the hybrids are clearly selective towards Gram-negative bacteria. SAR can also be drawn from this series, so that for example substitution of the isopropyl group has little effect on the potency. The successful synthesis of these hybrid systems inspired an attempt to synthesise tetramic acid – metronidazole hybrids using the chloro-substituted tetramic acid **64o**. As

before, using an alcohol as a nucleophile to displace the chlorine atom was attempted (Scheme **2.26**).



64o **114**

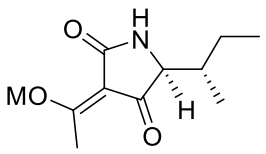
Scheme 2.26. Attempted synthesis of tetramic acid - metronidazole hybrid **114**. (i) Metronidazole, base, MeCN, reflux, 24 h.

Synthesis of tetramic acid – metronidazole hybrid **114** was however unsuccessful. Cs_2CO_3 base was initially used to undergo S_N2 displacement however; instead of a phenolic alcohol group the metronidazole contains an aliphatic alcohol, which possesses a higher pKa. Therefore NaH, a much stronger base than Cs_2CO_3, was also attempted but yielded no desired product and only starting material was observed by TLC and LRMS.

Metal chelation of tetramic acids

Tenuazonic acid (Figure **2.14**), a 3-acyl tetramic acid, and its salts have been extensively investigated since its isolation and discovery in the late 1950's. Tenuazonic acid itself was discovered as the magnesium (II) salt, and various mixtures of different tenuazonic acid salts were subsequently discovered, such as the Na^+, Ca^{2+} and Fe^{3+} salts, and in various

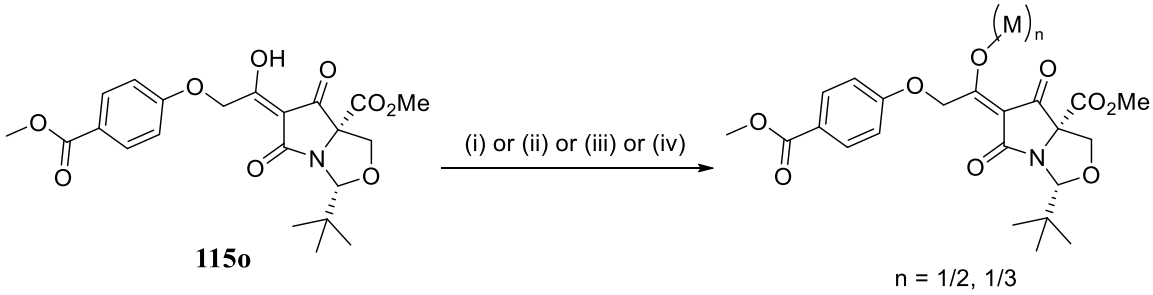
stoichiometric ratios. The discovery of these multiple metal complexes highlights the affinity of 3-acyl tetramic acids to metal ions.



M = Na^{+}, 1/2 Ca^{2+}, 1/2 Mg^{2+}, 1/3 Fe^{3+}

Figure 2.14. Tenuazonic acid, a 3-acyl tetramic acid isolated as a metal salt.

To investigate if the synthesised tetramic acids behaved chemically similarly to those found in the literature, an investigation into the metal binding ability of tetramic acids was undertaken. Compound **115o** (7.5 mg) was dissolved in MeOD and its NMR spectrum was recorded. Following this, 4 further solutions of tetramic acid **115** (7.5 mg) in MeOD and a source of magnesium (II), zinc (II), iron (II) and iron (III) dissolved in 0.1 mL of D_2O was added into each one separately (Scheme **2.27**).



Scheme 2.27. Metal complexation of tetramic acids. (i) $MgSO_4$ (0.5 eq) in 0.1 mL D2O , (ii) $ZnSO_4.7H_2O$ (0.5 eq) in 0.1 mL D2O, (iii) $FeSO_4.7H_2O$ (0.5 eq) in 0.1 mL D2O, (iv) $Fe_2(NO_3)_3.9H_2O$ (0.33 eq) in 0.1 mL D_2O**.**

Following addition of metal into the tetramic acid solution, colour changes and precipitation occurred depending on the metal being added. For zinc and magnesium, no colour change was observed and no precipitate was formed (Figure **2.15**). However, when iron (II) was added, the solution turned immediately dark orange but without precipitate. Upon addition of iron (III), a deep, dark red solution formed along with instant precipitation (Figure **2.15**). Crystallisation of the iron precipitate was attempted from MeOH, EtOH and DCM, and various mixtures thereof; however no single crystal was obtained.

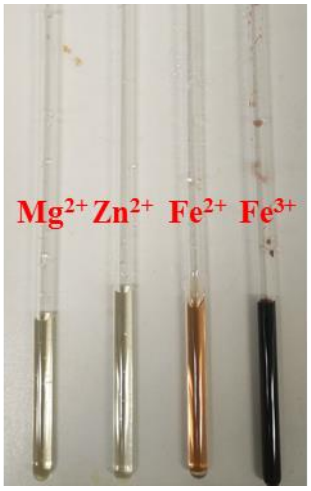


Figure 2.15. Addition of metal to 3-acyl tetramic acid solutions

Observations of solutions before and after metal addition clearly show the high affinity of the tetramic acid to iron. NMR analysis also revealed a high affinity of the tetramic acid to iron (Figure **2.16**).

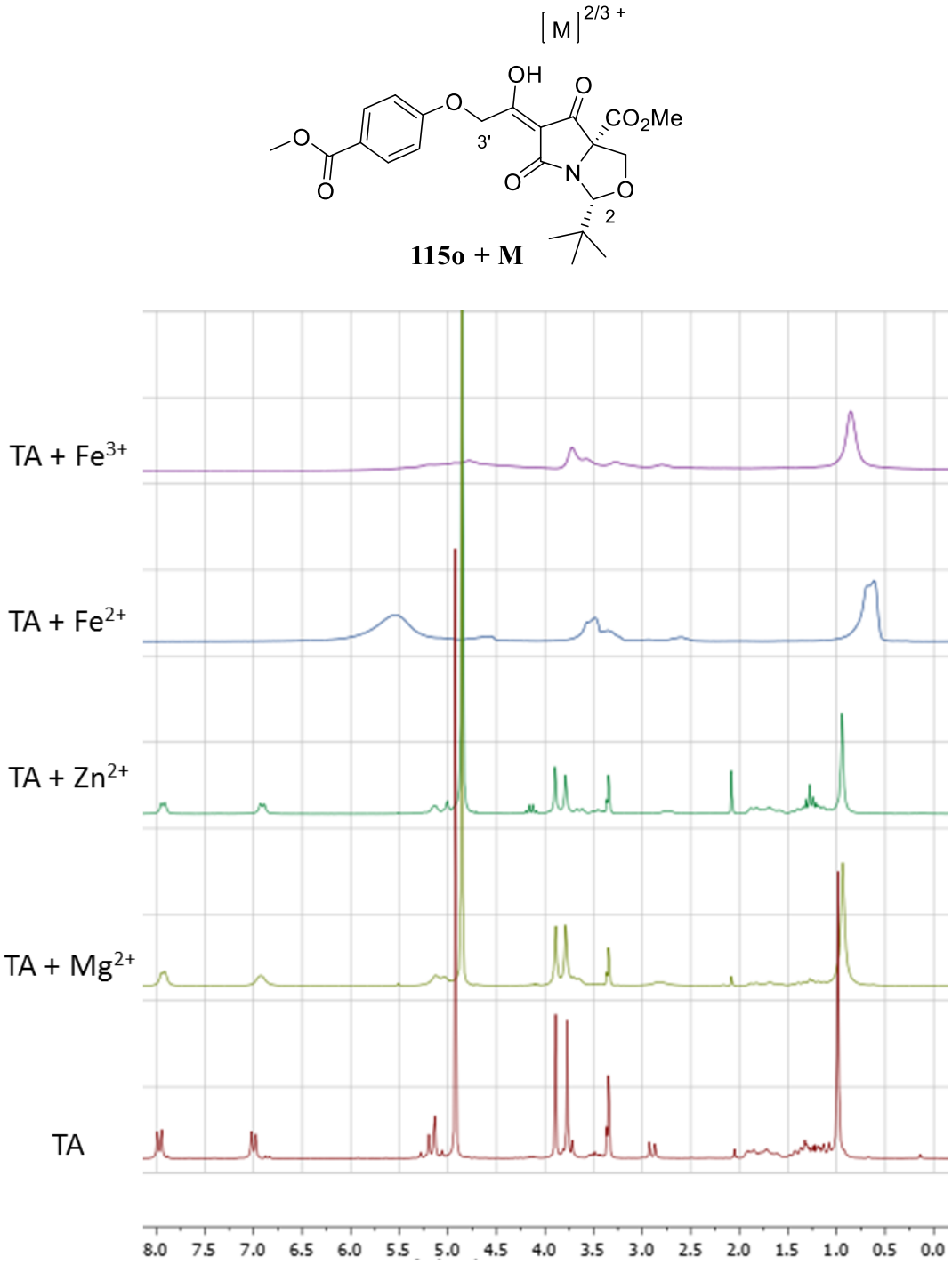


Figure 2.16. Comparison of NMR between Tetramic acid **115o** (TA) and TA + Magnesium, Zinc and Iron.

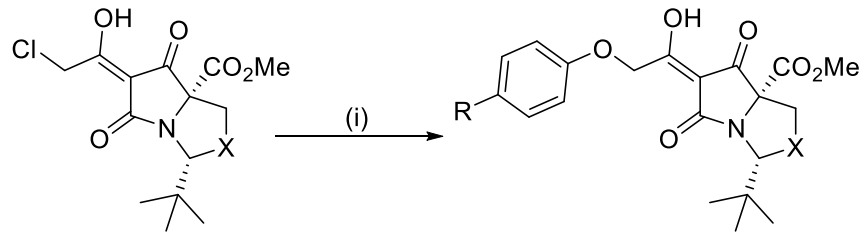
Upon addition of Mg^{2+} and Zn^{2+}, there is a slight broadening of the NMR peaks, noticeable by observation of the aromatic protons at ~7.00 and ~8.00 ppm, protons at C-2 and C-3' at ~5.20 ppm, and the methyl ester protons at ~3.60 and 3.90 ppm. Proton at C4a at ~ 2.8 ppm

becomes noticeably broader and loses its doublet appearance. The broadening of the NMR peaks indicates an affinity to the magnesium and zinc metals. Upon addition of both iron (II) and iron (III), the spectrum becomes no longer interpretable as there is complete broadening right across the spectrum.

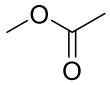
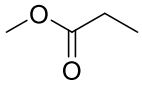
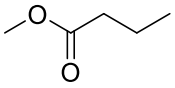
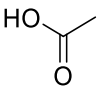
To further investigate the ability of 3-acyl tetramic acids to bind metal, ESI mass-spectrometry was utilised. When compound **115** below was mixed with iron III, mass ion peaks were observed for 3 TA + Fe (1097) and 3 TA + 3 Fe (1207), indicating metal complex formation. **115o** mixed with magnesium revealed mass ions of 998 and 1435, corresponding to 2 TA + 3 Mg and 3 TA + 2 Mg respectively. This outcome confirms the ability of the metal cation to bind to 2 or 3 ligands, as required. The formation of zinc complexes with tetramic acids were not observed by mass spectrometry however, literature reports indicate the possibility of such complexes.

Synthesis of tetramic acid hybrid precursors

In order to facilitate the synthesis of tetramic acid hybrids containing clinically relevant antibiotics as the second pharmacophore, a large range of variously-substituted tetramic acids were synthesised. Extending the chemistry successful for the synthesis of many tetramic acid – coumarin and tetramic acid – imidazole hybrids, various substituted phenols were reacted with the chloro-substituted tetramic acid **64** to yield a tetramic acid with a functional group able to undergo further structural elaboration (Scheme **2.28**).



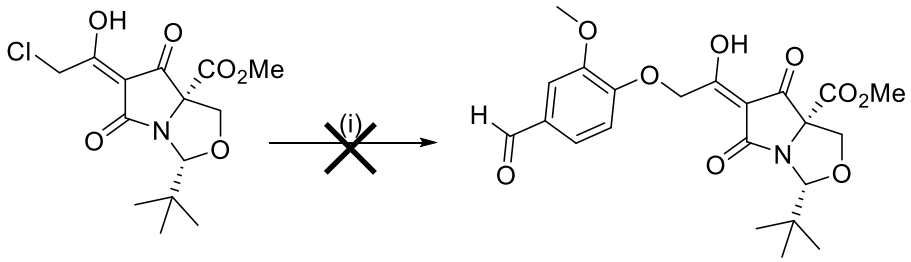
64o/64s **115o/s - 124o/s**

	R =	Yield (%) X = O	Yield (%) X = S
115o		71	-
116o/116s		77	40
117o/117s		82	85
118o/118s		56	0
119o/119s		86	64
120o/120s		86	63
121o/121s		69	51
122o	3-OMe	53	-
123o/123s		0	0
124o/124s		0	0

Scheme 2.24. Synthesis of functionalised tetramic acids. (i) Phenol, Cs_2CO_3, MeCN, reflux, 6 - 48 h.

The synthesis of functionalised tetramic acids **115 – 124** using previously employed S_N2 chemistry was successful. It was envisaged that having a library with such a diverse range of functionality would enable access to different pharmacophores. The yields of all compounds were comparable for those that were successfully synthesised, however the time to reach complete consumption of starting material varied depending on the functional group in the 4-position. Those that contained electron withdrawing groups needed to be reacted longer than those without, presumably due to their lower nucleophilicity. Yields were generally lower for the X=S system, except for case **117**, where the yield **117o** is lower at 82 % (85 % for **117s**), and some reactions yielded no clean, isolated product for X = S.

Compounds **123** and **124** were not isolated despite LRMS and TLC showing consumption of starting material and signs of product formation, therefore it was likely that the product decomposed on the column. To investigate whether other aromatic aldehydes would also decompose due to column chromatography, the synthesis of another aromatic aldehyde was attempted (Scheme **2.29**).



64o **125**

Scheme 2.29. Attempted synthesis of an aromatic aldehyde tetramic acid **125**. (i) Vanillin, Cs_2CO_3, MeCN, reflux, 18 h.

Repeating the conditions used previously, reaction of tetramic acid **64o** with vanillin yielded no desired product after column chromatography, despite signs of product formation by

LRMS and TLC. In this system, aromatic aldehydes cannot be isolated due to problems with purification, despite evidence for their formation in the crude reaction mixture.

Attempted synthesis of tetramic acid – ampicillin/amoxicillin hybrids

Lee *et al.* reported the synthesis of various tetramic acid – ampicillin hybrids. To access these systems, the amine group of ampicillin **59** was reacted with ester-substituted tetramic acids **126** in a microwave-assisted amination reaction (Figure **2.17**).[94]

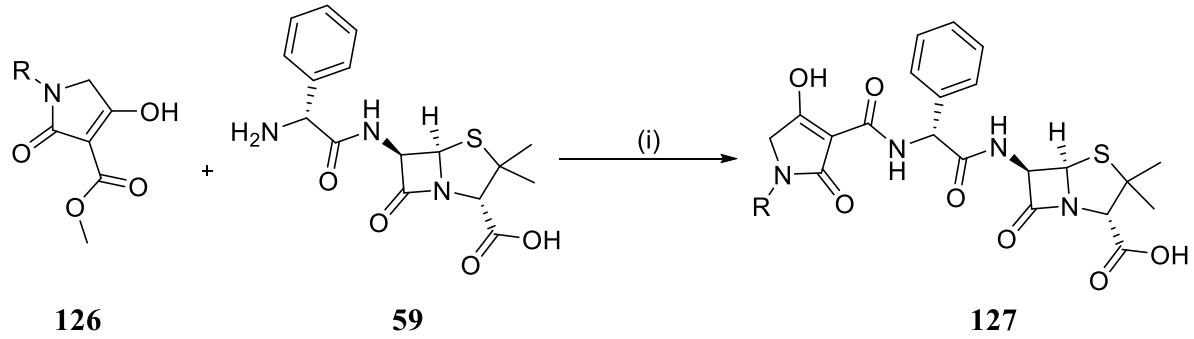
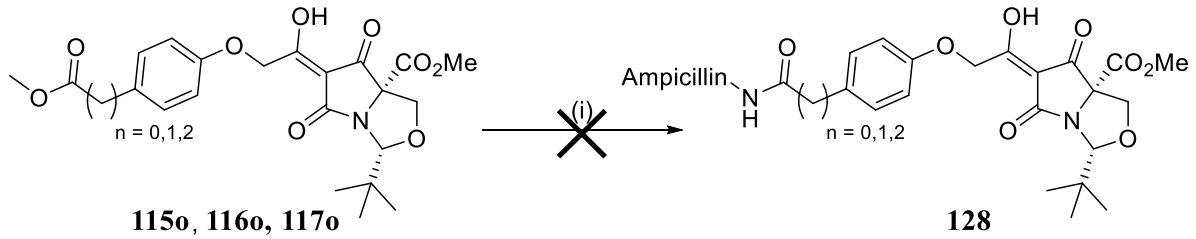


Figure 2.17. Lee *et al.* synthesis of tetramic acid - ampicillin hybrids **127**. (i) DMF, 100 °C, 5 mins.

Hybrids **127** displayed potent antibacterial activity against bacteria with MIC values of 0.2 and 1.6 μg/mL^{-1} against Gram-positive *S. aureus* and Gram-negative *E. coli* respectively. Following the same synthetic procedures as Lee *et al.* with the synthesised tetramic acids **115**, **116** and **117** would yield the tetramic acid – ampicillin hybrids **128** (Table **2.9**).



115o, **116o, 117o** **128**

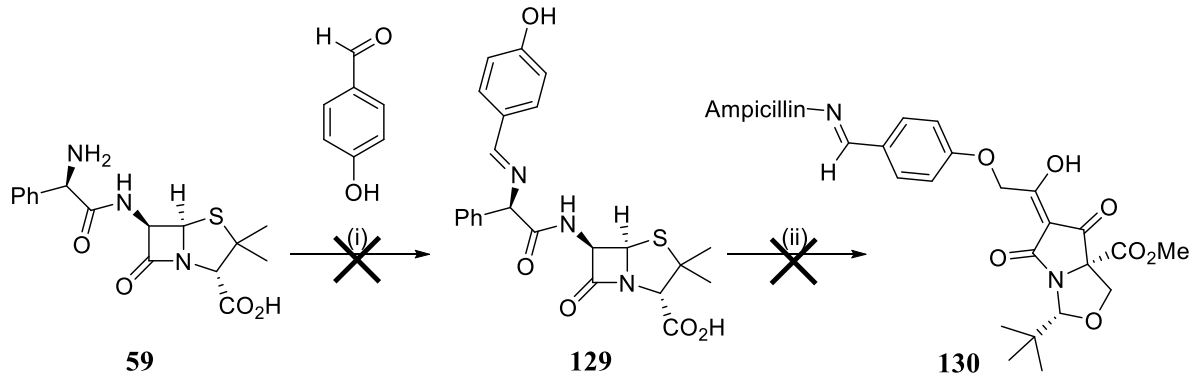
	Reaction time (mins)	Temperature	Yield (%)
n = 0	5	100 °C	0
	10	115 °C	0
	15	150 °C	0
n = 1	5	100 °C	0
	10	115 °C	0
	15	150 °C	0
n = 2	5	100 °C	0
	10	115 °C	0
	15	150 °C	0

Table 2.5. Attempted synthesis of tetramic acid - ampicillin hybrids **128** by microwave assisted amination reaction. (i) Ampicillin, DMF.

Reactions of the ester substituted tetramic acids with ampicillin repeating conditions used by Lee *et al.* were unsuccessful, with only starting material observed by LRMS and TLC after the reaction. In an attempt to induce reactivity, the reaction time was increased from 5 mins to 10 mins and subsequently 15 mins, whilst also increasing the temperature from 100 °C to 115 °C and 150 °C. However, in all cases, only starting material was recovered and no evidence of product formation was observed. Reactions with cysteine-derived tetramic acids **115s, 116s**

and **117s** were not attempted in light of these results. Microwave-assisted amination has been shown to be sensitive to the substrates employed in the reaction, as detailed by Lee *et al.* in their reported synthesis of these hybrid compounds.[94]

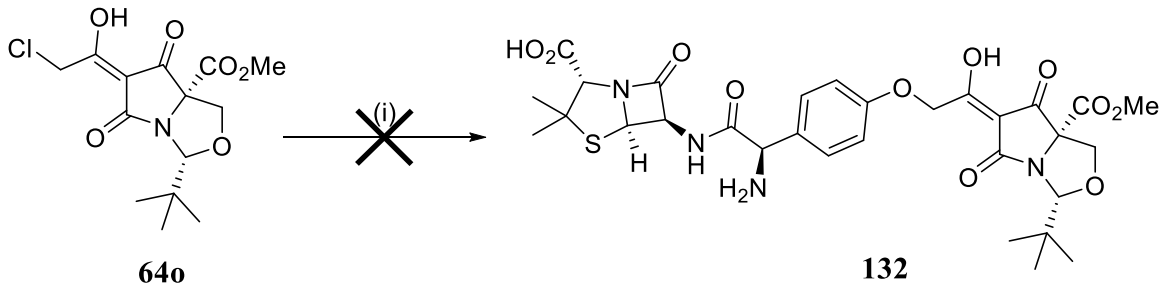
Other attempts to synthesise tetramic acid – ampicillin hybrids were also made, using a reductive amination approach. It was envisaged that reaction of the amine group in ampicillin **59** with 4-hydroxybenzaldehyde would generate an ampicillin substituted phenol **129**, which could undergo S_N2 reaction used previously in the synthesis of tetramic acid hybrids (Scheme **2.30**).



Scheme 2.30. Attempted synthesis of tetramic acid - ampicillin hybrids through imine formation and subsequent S_N2 displacement. (i) 4-hydroxybenzaldehyde, EtOH, reflux, 3 h. (ii) Tetramic acid **64o**, MeCN, 18 h, reflux.

Attempts to synthesise the tetramic acid – ampicillin hybrids **130** through imine formation and subsequent S_N2 reaction that has been previously employed in generating hybrids was unsuccessful.

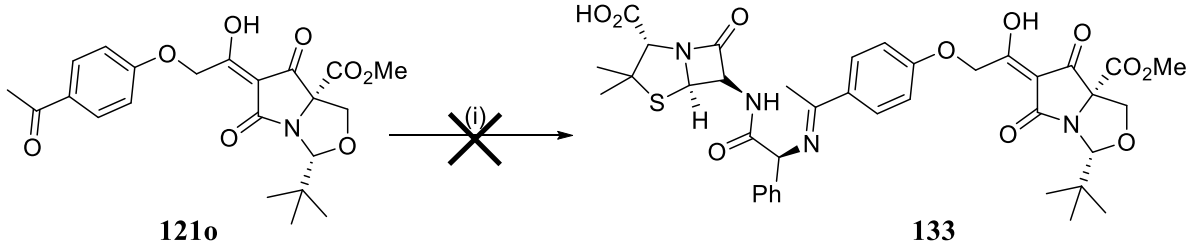
Amoxicillin **131**, a penicillin antibiotic containing phenolic oxygen, was also reacted with the tetramic acid **64o** in an attempt to couple the antibiotic to the tetramic acid directly through an S_N2 reaction (Scheme **2.31**).



64o **132**

Scheme 2.31. Attempted synthesis of tetramic acid - amoxicillin hybrids **132**. (i) Amoxicillin, Cs_2CO_3, MeCN, reflux, 18 h.

In another attempt to generate a tetramic acid – ampicillin hybrid, compound **121o** was reacted with ampicillin directly in the hope of generating an imine by condensation of the free amine of ampicillin with the ketone carbonyl (Scheme **2.32**).



121o **133**

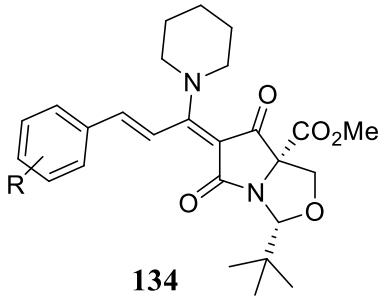
Scheme 2.32. Attempted synthesis of tetramic acid - ampicillin hybrid **133**. (i) EtOH, reflux, 18 h.

Compound **133** was not isolated, nor was there any evidence for the formation of the product by LRMS or TLC, and only starting materials were observed. This could be due to the steric

hindrance of both ampicillin and starting material **121o** reducing the ability of ampicillin to successfully condense onto the ketone. The aldehyde analogue **123** would be more susceptible to imine formation, however isolation of the aldehyde product was not possible due to decomposition (discussed earlier in this chapter).

Attempted synthesis of enamine linked tetramic acid – ampicillin hybrids

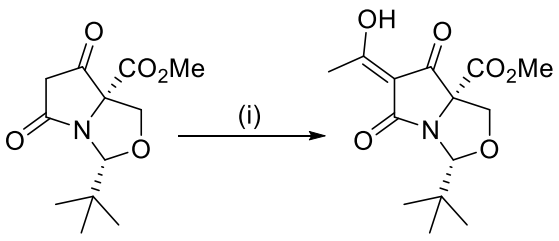
Previous work in the Moloney group identified the synthesis of enamine-linked tetramic acids (Figure **2.18**, **134**).[97] It was reasoned that with the appropriate R group substitution, the enamine linked tetramic acids could be a precursor to tetramic acid hybrids.



134

Figure 2.18. General structure of enamine-substituted tetramic acids **134**.

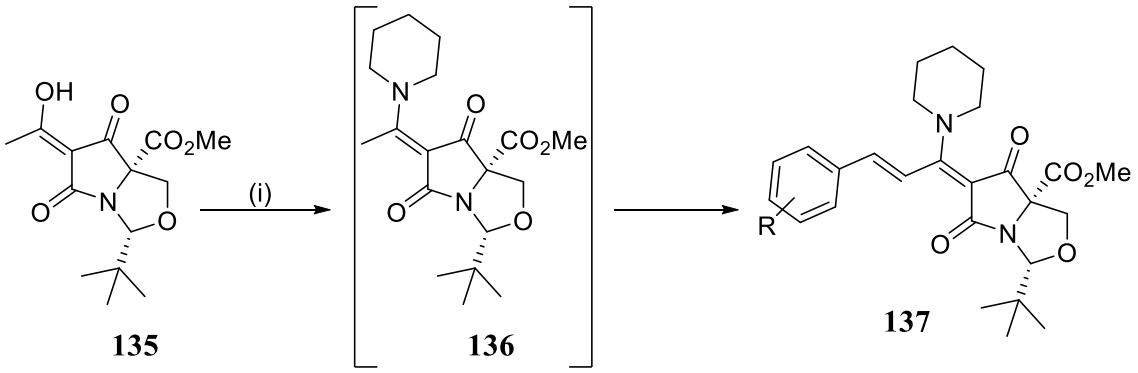
Synthetically, to access the enamine tetramic acids, the tetramic acid core **9o** was reacted with acetic acid (Scheme **2.33**).



9o **135**

Scheme 2.33. Synthesis of tetramic acid **135**. (i) Acetic acid, DCC, DMAP, DCM, rt, 18 h, 39 %.

Repeating conditions used previously, coupling of acetic acid onto the tetramic acid core **9o** in presence of DCC and DMAP yielded the acylated tetramic acid **135** in 39 % yield. To further functionalise the acylated tetramic acid **135** and generate an enamine linked aromatic compound, literature reported conditions were employed (Scheme **2.34**).

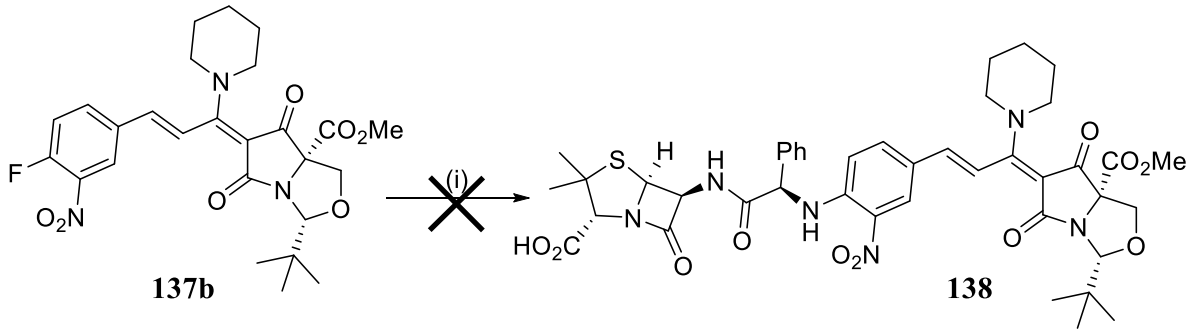


R =	Yield (%)
3,4,5-trifluoro – **137a**	32
3-nitro, 4-fluoro – **137b**	35

Scheme 2.34. Synthesis of fluoro-substituted enamine linked tetramic acids **137a** and **137b** (i) Corresponding benzaldehyde, piperidine, toluene, reflux, 18 h.

Synthesis of enamine-linked tetramic acids **137** was successful for both the trifluoro benzaldehyde and the 4-fluoro-3-nitro-substituted benzaldehyde. Reaction proceeds by initial stirring in presence of piperidine to generate the enamine intermediate **136**. Subsequent addition of the corresponding benzaldehyde results in an aldol condensation reaction, yielding the enamine containing tetramic acids **137** in low but functional yields.

With the enamine tetramic acids in hand, S_NAr conditions were employed in an attempt to generate a tetramic acid – ampicillin hybrid. It was envisaged that the free amine of the ampicillin could undergo nucleophilic substitution with the fluoro-substituted aromatic tetramic acids, facilitated by electron withdrawing groups ortho to the fluorine leaving group (Scheme **2.35**).



Scheme 2.35. Attempted synthesis of enamine-linked tetramic acid - ampicillin hybrids **138**. (i) K_2CO_3, DMF, reflux, 48 h.

Reaction of enamine **137b** with ampicillin yielded no desired product **138**. TLC and LRMS analysis revealed only starting material. Reaction with the 3,4,5-trifluoro substituted enamine tetramic acid also yielded no desired hybrid product. For reasons mentioned earlier in this chapter, no further attempts at generating tetramic acid – ampicillin hybrids were made.

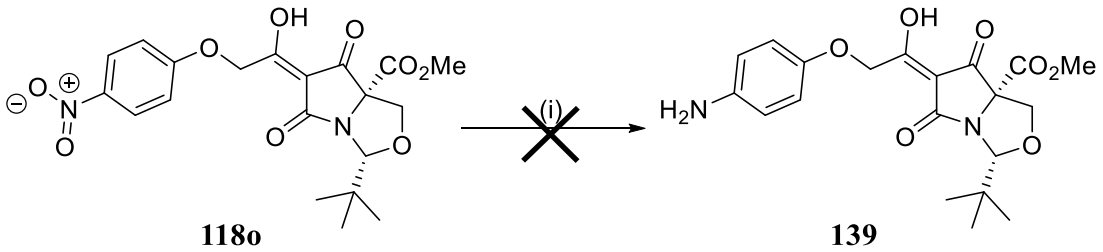
Ampicillin, amoxicillin, and penicillins in general are very functionally dense and therefore the range of suitable chemistry is limited. This could explain why the formation of the tetramic acid – penicillin derivatives was unsuccessful.

Reduction of nitrile and nitro aromatic tetramic acids

In order to generate a reactive intermediate tetramic acid, it was necessary to convert the relatively inert nitro and nitrile groups of compounds **118** and **119** respectively, into more

reactive functional groups. To do this, it was envisaged that reduction of the nitrile and nitro groups of compounds **118** and **119** would provide a reactive aniline substituent which could undergo further manipulation to generate hybrid systems with electrophilic pharmacophores.

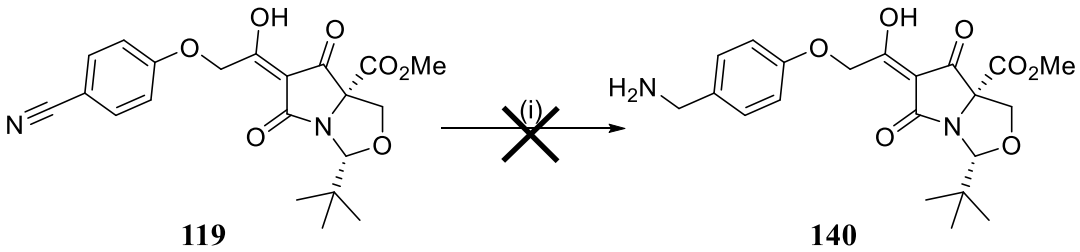
Initially, an attempt to reduce the nitro compound **118** was made using $NaBH_4$ in presence chArCoal, according to literature precedent (Scheme **2.36**).[111]



118o **139**

Scheme 2.36. Attempted reduction of nitro-substituted tetramic acid **118o**. (i) $NaBH_4$, H_2O:THF (1:0.5), charcoal, rt.

No evidence for the formation of the aniline substituted tetramic acid **139** was observed by LRMS or TLC. Reduction of the nitrile substituted tetramic acid was also attempted, using similar conditions to those employed in the attempted reduction of the nitro compound but in presence of $NiCl_2$ catalyst according to literature precedent (Scheme **2.37**).[112]



119 **140**

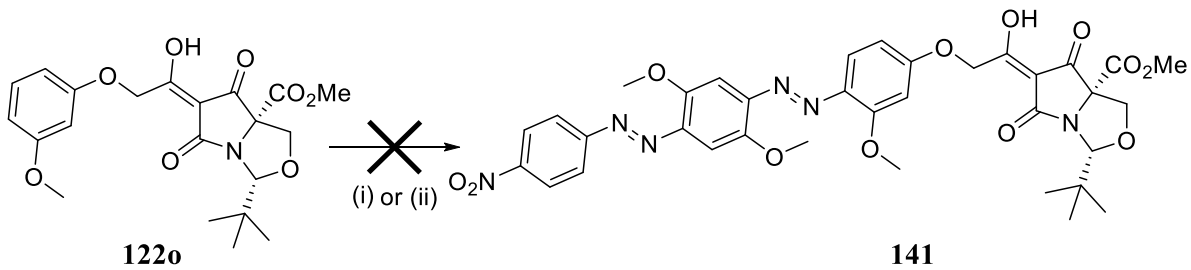
Scheme 2.37. Attempted reduction of tetramic acid **119o**. (i) $NaBH_4$, $NiCl_2$, EtOH, rt.

As seen in the reduction of the nitro-substituted tetramic acid, reduction of the nitrile was unsuccessful – no product formation was observed and only starting materials were recovered.

Even if product formation was observed, in both cases the resulting amine provides a basic nitrogen and thus, the zwitterionic behaviour observed in the imidazole hybrid systems (discussed earlier in this chapter) would become relevant. This would make purification of the resulting amine compounds difficult and significantly reducing their ability to act as a nucleophile, rendering further manipulation not possible. Therefore, no further attempts at generating amine substituted tetramic acids were undertaken.

Synthesis of azo-linked tetramic acids

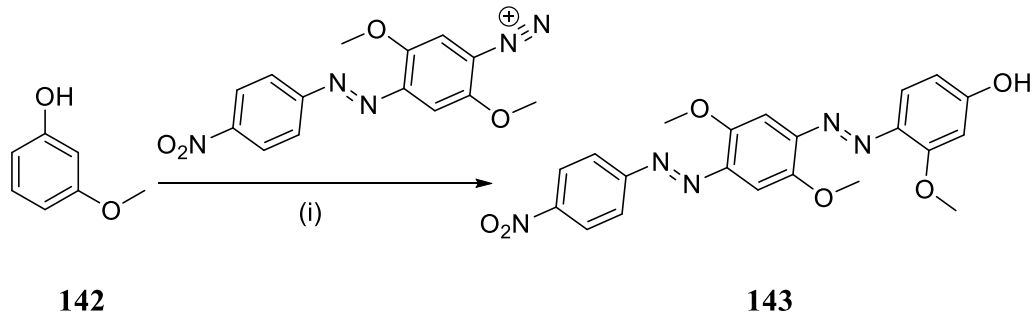
From the 3-methoxy substituted tetramic acid **122o**, it was envisaged that further manipulation could be achieved through azo coupling reactions. The 3-methoxy group was positioned so as to direct azo coupling to the 4-position to reduce the number of isomers obtained from the reaction. An azo coupling was attempted with Fast Black K salt (removing the need to pre-form the diazonium salt) following methodology developed in the Moloney group (Scheme **2.38**).[113]



122o **141**

Scheme 2.38. Attempted azo coupling of tetramic acid **122o** and fast black K salt. (i) Fast black K salt, EtOH, 5 °C, 5 h. (ii) Fast black K salt, NaOAc, EtOH, 5 °C, 18 h.

Azo coupling of Fast Black K salt with the 3-methoxy tetramic acid was unsuccessful. Initially, the reaction was attempted in ethanol at 5 °C for 5 h, following the literature protocol, however this yielded no product and only starting materials were observed by LRMS and TLC. Also reported in the literature is the use of NaOAc to facilitate azo coupling reactions and thus the reaction was attempted again in the presence of NaOAc, but this too yielded no desired product. To confirm reaction conditions, azo coupling using the same method was attempted on 3-methoxy phenol (Scheme **2.39**).



142 **143**

Scheme 2.39. Azo coupling of 3-methoxy phenol with fast black K salt to confirm reaction conditions. (i) Fast black K salt, EtOH, 5 °C, 18 h, 81 %.

In contrast to the reaction of the 3-methoxy substituted tetramic acid, reaction of 3-methoxy phenol **142** with Fast Black K salt in the presence of ethanol at 5°C yielded the azo-coupled compound **143** in 81 % yield. This suggests that the presence of the tetramic acid in Scheme **2.38** imparts a lack of reactivity towards diazonium salts. This could be due to the strong ability of the tetramic acid core to bind to cationic species. In this case, the positively charged diazonium salt could form a complex with the tetramic acid reducing reactivity.

Noteworthy is the presence of multiple isomers in the formation of azo-coupled compound **143**. Unlike the tetramic acid system, there is no significant steric hindrance around the alcohol or methoxy groups, resulting in a lack of selectivity towards a particular position on

the aromatic ring. Furthermore, the azo functional groups can exist as either E or Z isomers, further complicating the characterisation of compound **143**. Despite being unable to separate and characterise each individual isomer, evidence by NMR, LRMS, HRMS and IR confirmed the successful synthesis of **143**.

Antibacterial screening

The synthesised tetramic acid hybrids, and their respective tetramic acid hybrid precursors, were screened for antibacterial activity (Table **2.10**).

Compound (Appendix)	*E. coli* ^	*K. pneumoniae* ^	*P. aerogenosa* ^	MRSA *
1	n.a	n.a	n.a	n.a
2	n.a	n.a	n.a	n.a
3	n.a	n.a	n.a	n.a
4	n.a	n.a	n.a	n.a
5	n.a	n.a	n.a	n.a
6	n.a	n.a	n.a	n.a
7	n.a	n.a	n.a	n.a
8	n.a	n.a	n.a	n.a
9	n.a	n.a	n.a	n.a
10	n.a	n.a	n.a	n.a
11	n.a	n.a	n.a	n.a
12	n.a	n.a	n.a	n.a
13	n.a	n.a	n.a	n.a
14	n.a	n.a	n.a	n.a

15	n.a	n.a	n.a	n.a
16	n.a	n.a	n.a	n.a
17	n.a	n.a	n.a	n.a
18	n.a	n.a	n.a	n.a
19	n.a	n.a	n.a	n.a
20	n.a	n.a	n.a	n.a
21	n.a	n.a	n.a	n.a
22	n.a	n.a	n.a	n.a
23	n.a	n.a	n.a	n.a
24	n.a	n.a	n.a	n.a
25	n.a	n.a	n.a	n.a
26	n.a	n.a	n.a	n.a
27	n.a	n.a	n.a	n.a
28	n.a	n.a	n.a	n.a
29	n.a	n.a	n.a	n.a
30	n.a	n.a	n.a	n.a
31	n.a	n.a	n.a	n.a
32	n.a	n.a	n.a	n.a
33	n.a	n.a	n.a	n.a
34	n.a	n.a	n.a	n.a
35	n.a	n.a	n.a	n.a
36	n.a	n.a	n.a	n.a
37	n.a	n.a	n.a	n.a
38	n.a	n.a	n.a	32.25 ug/mL^{-1}

39	n.a	n.a	n.a	n.a
40	n.a	n.a	n.a	n.a
41	n.a	n.a	n.a	n.a
42	n.a	n.a	n.a	n.a

Table 2.10. Biological evaluation of synthesised tetramic acids and tetramic acid hybrids in chapter II. n.a = not active. * = Gram-positive ^ = Gram-negative.

All synthesised hybrids and the corresponding tetramic acid components displayed no antibacterial activity except for one, Table **2.10** entry **38**, which displayed an MIC value of 32.25 µg/mL^{-1} against MRSA. When comparing the active compound **38** (Table **2.10**) to the serine derived analogue **42** (Figure **2.19**), it is clear that sulphur plays an important role in the displayed biological activity. In addition to the presence of sulphur, both ester substitution at C-7 and length of linker between the pharmacophores appear to be of significance. However, as the synthesis of keto compound was unsuccessful, the importance of linker length and C-7 substitution in isolation cannot be determined.

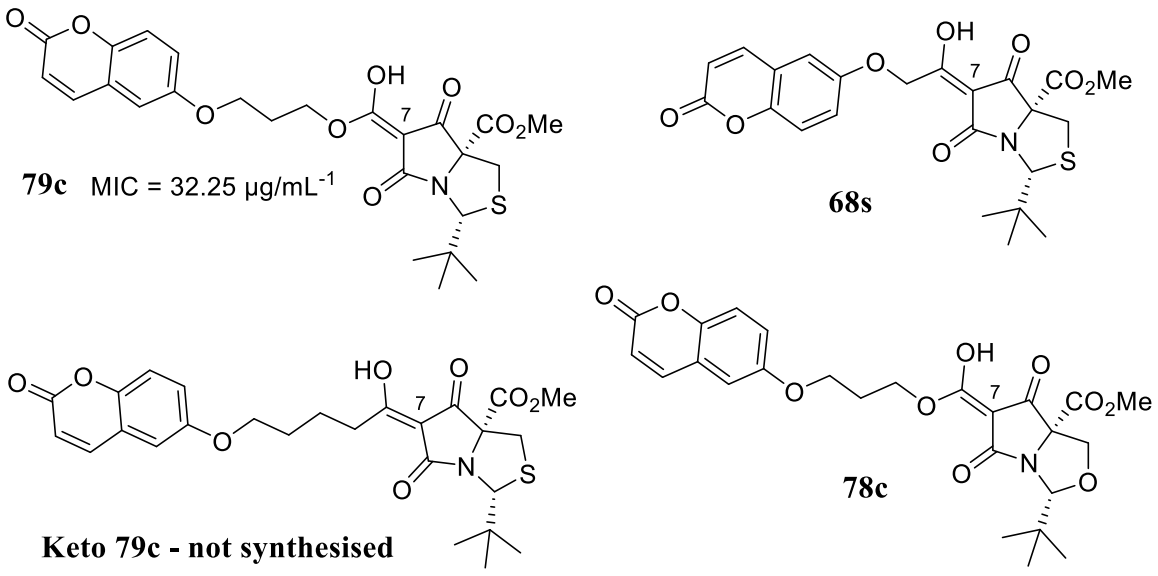


Figure 2.19. SAR analysis of bioassayed compounds in chapter II.

Summary

A large library of tetramic acid hybrids has been synthesised. C-7 manipulation of the tetramic acid cores with a chloro-substituted side chain provided a tetramic acid susceptible to substitution reactions. Conjugation of tetramic acids with coumarins has been achieved using this method to yield tetramic acid – coumarin hybrid compounds. The linker between these compounds has been varied to investigate the role of the linker on antibacterial activity. Tetramic acid – imidazole/imidazoline hybrids have also been synthesised using this methodology. Further manipulation of the imidazoline was achieved by alkylation of the nitrogen. Varying the tetramic acid core has also been investigated, with a benzyl ester substituted tetramic acid being conjugated to coumarins having been synthesised.

Purification of the tetramic acid compounds initially proved troublesome due to the ability of the tetramic acid to bind to divalent metal cations. To overcome this, 2M HCl washes were employed which successfully provided a more resolved NMR spectrum. Tetramic acid hybrids which contained a basic nitrogen displayed zwitterionic behaviour and rendered column chromatography ineffective in purification.

The synthesis of tetramic acid hybrids conjugated to clinically employed antibiotics was unsuccessful. Attempts were made to conjugate the tetramic acid core to a range of penicillins and also metronidazole. Azo coupling was successfully achieved on the model system however, produced no desired product when attempted on the tetramic acid, highlighting the impact the tetramic acid has on reactivity.

Antibacterial evaluation of the synthesised compounds in Chapter II revealed only one antibacterially active compound possessing an MIC value of 32 $\mu g/mL^{-1}$.

Future Work

To further probe the effect of increasing linker length on antibacterial activity, a systematic investigation into linker length could be made. Chapter II describes the synthesis of tetramic acid – coumarin hybrids possessing linkers of carbon length one and four, however the synthesis of linkers longer than this were not attempted. The synthesis of tetramic acids containing linkers of carbon chain lengths 7, 10, and 12 etc. for both the keto-linked and ester-linked tetramic acids would provide an insight into the role of linker length on antibacterial activity (Figure **2.20**). The resulting SAR would guide the next iteration of compounds to determine the optimum linker length.

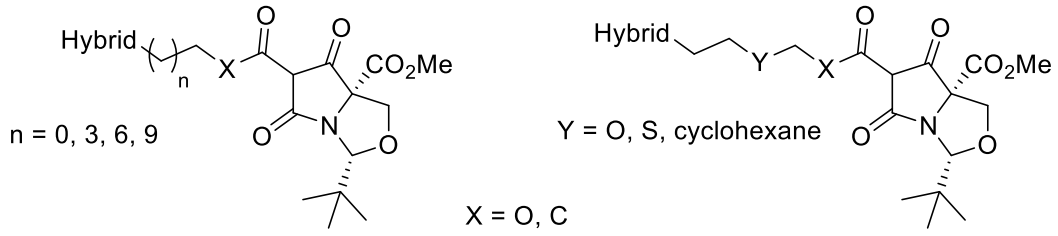


Figure 2.20. Proposed synthesis of tetramic acid hybrids containing longer and functionally different linkers.

Furthermore, an investigation into the type of linker could be made. Thus far, and proposed above, the linker contains only straight carbon chains, however the synthesis of tetramic acid hybrids containing different functionality within the linker were not attempted. The introduction of ether, thioether, or cyclic hydrocarbons into the linker would provide an insight into the importance of the linker's chemical composition. Combined with the chain length variation, functionality variation would provide useful SAR and guide further development of the hybrid series.

Chapter III: Spirocyclic Tetramic Acids

Introduction

Several drugs on the market possess spirocyclic functionality, and the spirocyclic motif is becoming increasingly investigated in medicinal chemistry due to their structural novelty and unique 3D structure.[114] Multiple efforts to develop methods for their synthesis have been made and examples include alkylations[115,116], transition metal based spiro formation[117,118] and rearrangements[119,120]. One approach of synthesising spirocycles which has been developed exploits the *tert-amino* effect (Figure **3.1**).[121,122]

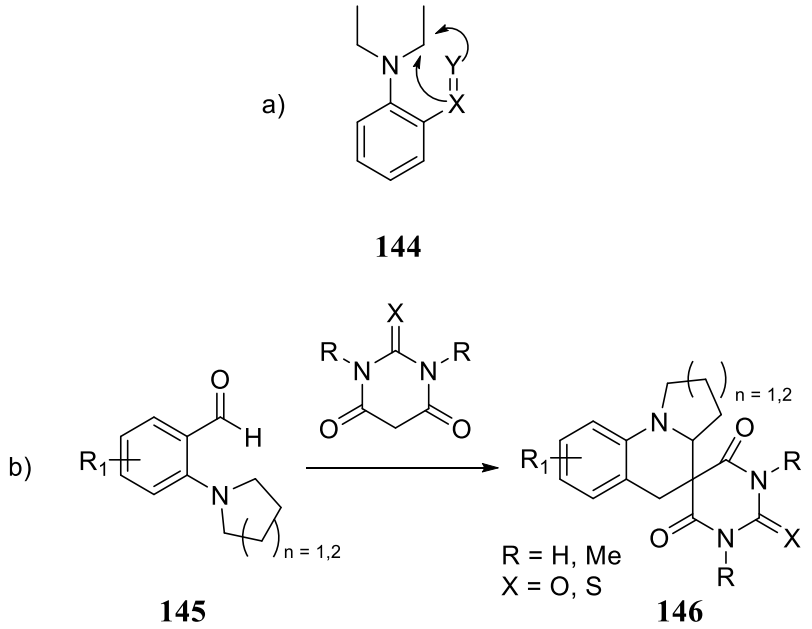


Figure 3.1. a) *tert-amino* effect displayed in an *ortho*-substituted N-dialkylaniline b) Spirocyclic generation by exploiting the *tert-amino* effect.

Spirocyclic compounds following the general scheme shown in Figure **3.1b** have been synthesised and allow a range of chemical functionality at the aldehyde, the *tertiary* amine and the barbiturate nucleophile positions.[121] Recent publications by AstraZeneca have reported the synthesis of antibacterial agents using this approach and highlight the biological

value of clinically relevant compounds which possess spiro-centres introduced by the *tert-amino* effect (Figure **3.1**). Compound ETX0914 (Figure **3.2**) entered clinical trials as a bacterial type II topoisomerase inhibitor, possessing an MIC of 200 nM against *H. influenza*.[123]

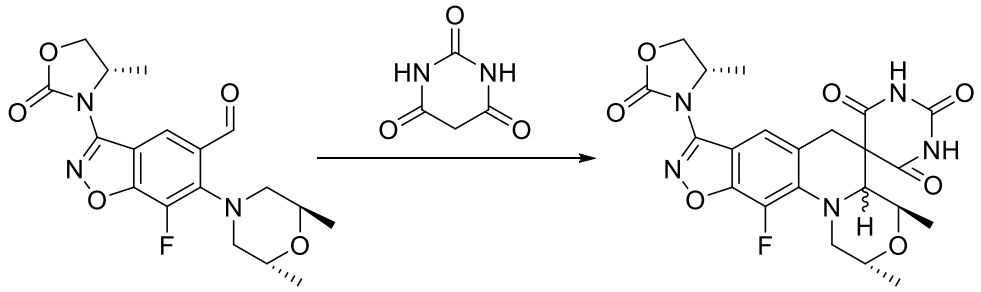
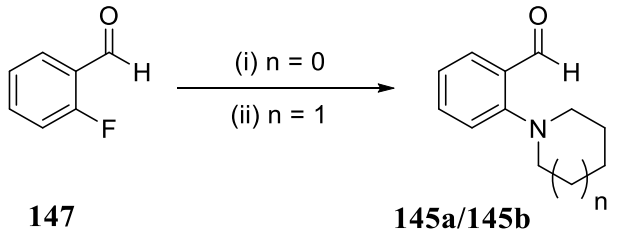


Figure 3.2. Spirocyclic generation step in the synthesis of ETX0914.

In their work, initially, a Knövenagel condensation occurs between the nucleophilic barbiturate and the aldehyde **147**, followed by an intramolecular 1,5-hydride shift to yield the spirocycle. Noteworthy is the resulting 9:1 mixture of diastereoisomers upon cyclisation. The product mixture was separated by chiral supercritical fluid chromatography (SFC) and the major product was the most thermodynamically favoured.[123] Given the success of this strategy both from a synthetic perspective and in the generation of a range of antibacterially active compounds, an attempt to replace the barbiturate unit with a tetramic acid one was made. This would provide access to a fused hybrid system (see Chapter I).

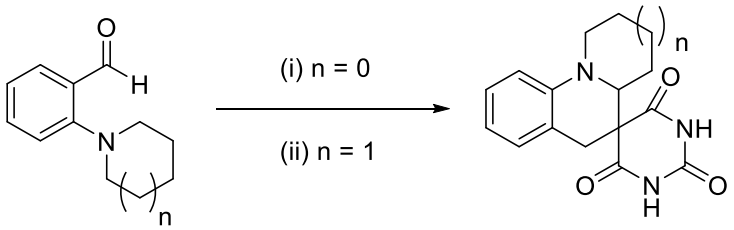
Initially, a range of 2-aminobenzaldehydes were synthesised utilising nucleophilic aromatic substitution using conditions reported by Rabong et al. shown in (Scheme **3.1**). The *ortho*-carbonyl activates the fluorine towards nucleophilic attack due to mesomeric electron withdrawing effects. [122]



147 **145a/145b**

Scheme 3.1. Synthesis of 2-aminobenzaldehydes. (i) Pyrrolidine, K_2CO_3, DMF, reflux, 5 h, 76 %. (ii) Piperidine, K_2CO_3, DMF, reflux, 24 h, 56 %.

Spirocycle generation was then attempted by heating to 40 ° C for 5 h using a range of known, simple barbiturates to generate a range of spirocycles to confirm the reaction conditions, and assess the polarity of compounds prepared from this process for further development (Scheme **3.2**). [121]



145a/145b **149a/149b**

Scheme 3.2. Synthesis of barbiturate spirocycles. (i) Barbituric acid, MeOH, 40°C, 58 %. (ii) Barbituric acid, MeOH, 40°C, 49 %.

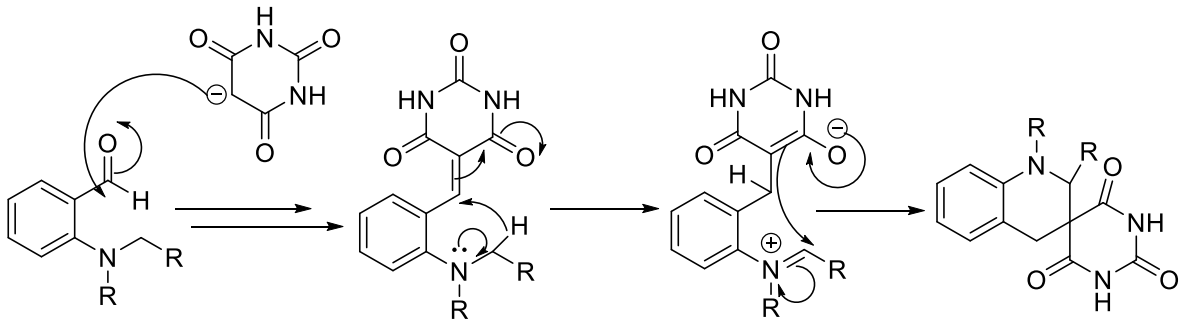
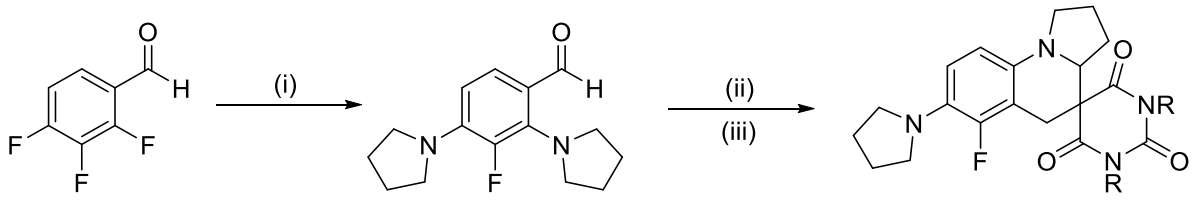


Figure 3.3. Mechanism of spirocycle formation by 1,5-hydride shift reaction.

Yields for the simple spirocycles were moderate and gave compounds which were stable and to extend its suitability further, a more functionalised aldehyde was synthesised to investigate the potential scope of the chemistry. Taking 2,3,4-trifluorobenzaldehyde **150** and reacting with over two equivalents of pyrrolidine at reflux for 24 h in DMF led to the formation of the trisubstituted aldehyde **151** with a single fluorine remaining on the aromatic ring (Scheme **3.3**) in 51 % yield, comparable to those previously synthesised in Scheme **3.10**.



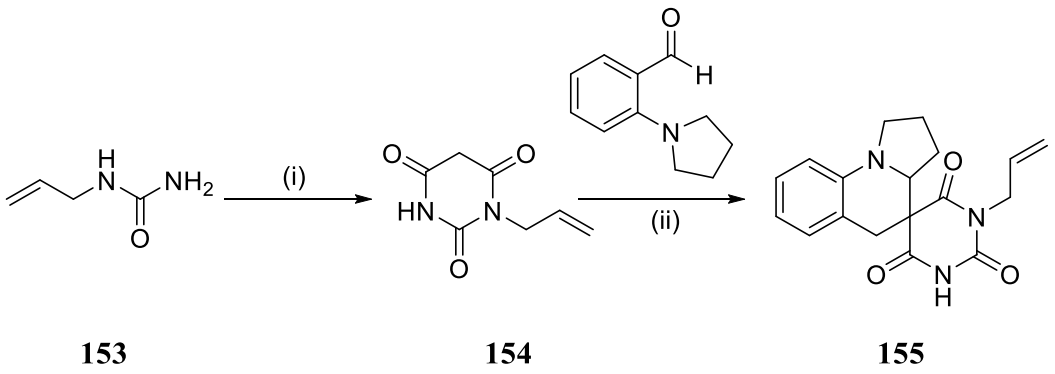
150 **151** **152a** R = H
152b R = Me

Scheme 3.3. Synthesis of a tri-substituted benzaldehyde. (i) Pyrrolidine, K_2CO_3, DMF, reflux, 24 h, 51 %. (ii) Barbituric acid, MeOH, 40 °C, 5 h, 10 %. (iii) N-Methyl barbituric acid, MeOH, 40 °C, 6 h, 31 %.

With compound **151** in hand, generation of a barbiturate spirocycle was attempted by mixing in MeOH and heating to 40 °C; compound **152a** was synthesised in 10 % yield and compound **152b** was synthesised in 31 % yield. The yield is significantly reduced in comparison to less functionalised aldehydes (Scheme **3.1**, 92 – 99 %), and careful purification of **152** by column chromatography was required to obtain a clean NMR spectrum due to difficult separation.

Thus far, only spirocycles containing a symmetrical barbituric acid component had been synthesised, but of interest was use of differentially substituted barbiturates which would both break the symmetry and introduce a useful functional moiety for further elaboration. *N*-

Allylbarbiturate **154** was synthesised using methodology previously used in the Moloney group[124] (Scheme **3.4**); initially compound **153** was reacted with ethanoic anhydride and propanedioic acid at 90 °C for 5 h and then converted to the corresponding spirocycle **155** in 53 % yield.



Scheme 3.4. Synthesis of N-allylbarbiturate. (i) Ethanoic anhydride, propanedioic acid, acetic acid, 90 °C, 5 h, 24 %. (ii) 2-pyrrolidinylbenzaldehyde, MeOH, 5 h, 53 %.

Compound **155** was isolated as a 1:1 mixture of diastereoisomers (by NMR), indicating that the 1,5-hydride shift shows no selectivity; whether this occurred upon formation or upon later epimerisation was not established (Figure **3.3).** Diastereoselection in these processes does not appear to have been widely discussed in the literature.

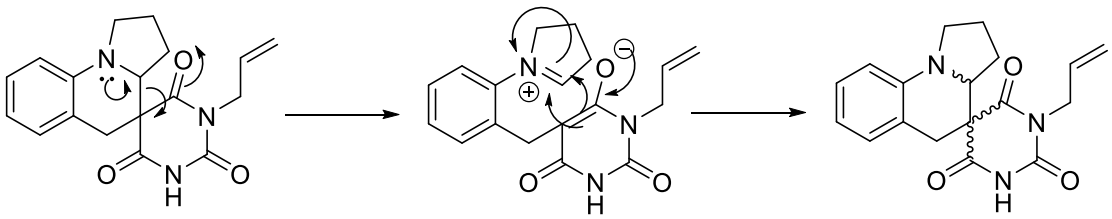
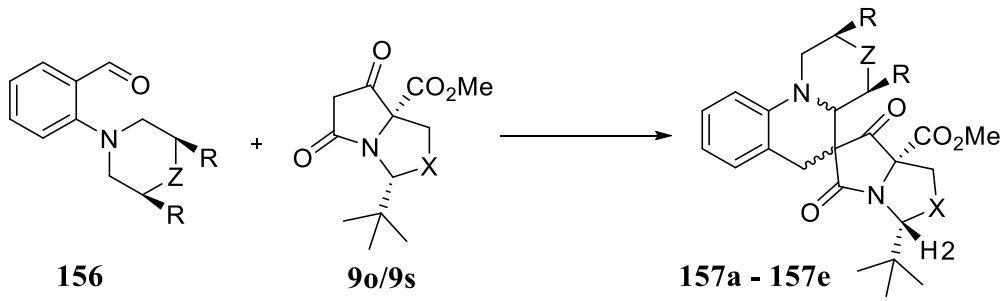


Figure 3.3. Mechanism of isomerisation in non-symmetrical barbiturate spirocycles

Incorporation of the tetramate into the spirocycles.

These initial attempts to create spirocyclic compounds showed that addition of a non-symmetrical nucleophile to the benzaldehyde was possible and proceeded in good yield; however, this also highlighted that diastereomeric products could be obtained. Extension of this approach to tetramates, known to possess antibacterial activity, as the nucleophile for the spirocyclic generation was the next aim. Previous preliminary work in the Moloney group had showed that synthesis of spiro-tetramates using this methodology was possible using a range of cyclic amino aldehydes (Table **3.1**).[125]

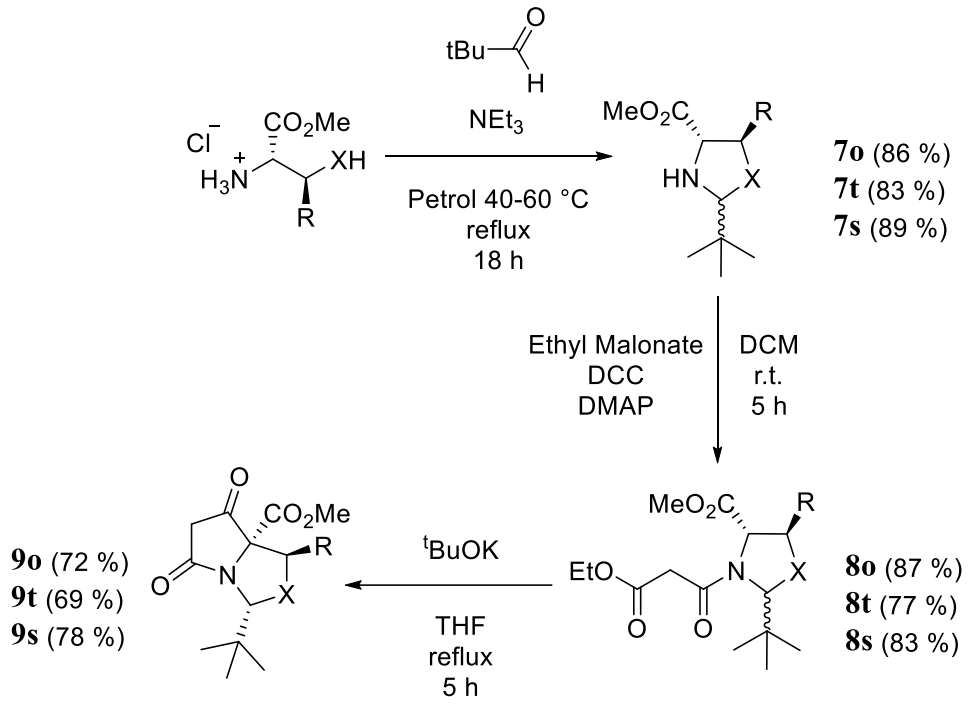


156 **9o/9s** **157a - 157e**

R	Z	X	Product	Yield (%)	No. Isomers
Me	O	O	**157a**	18	4
H	CH_2	O	**157b**	37	3
H	-	O	**157c**	58	4 (1 in Toluene-d_6)
H	O	O	**157d**	7	2
Me	O	S	**157e**	23	3

Table 3.1. Previously synthesised spiro-tetramates

The required bicyclic tetramate cores were synthesised by literature methods from L-serine, L-cysteine and L-threonine as shown in Scheme **3.5**.[47]



Scheme 3.5. Synthesis of tetramate cores.

Consistent with the synthesis of *N*-allylbarbiturate spirocycle in Scheme **3.4**, the non-symmetrical nature of the tetramic acid led to the formation of multiple diastereomers. In the case of compound **157c**, it was possible to observe a single diastereomer using the NMR solvent Toluene-d_6. The stereochemistry of this compound was investigated using CLIP-HSQMBC NMR and X-Ray analysis, and was assigned as RRSR. In a rate of isomerisation experiment, it was found that compound **157c** (the only compound to show a single diastereomer) equilibrated to a mixture of 3 diastereomers over a period of 7 days by NMR using H-2 (labelled above in Table **3.1**) as a reference proton (Table **3.2**); this confirmed that the process was reversible and was likely to proceed in a process similar to that of Figure **3.3**.

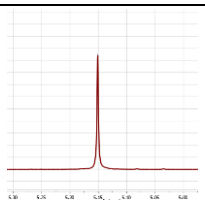
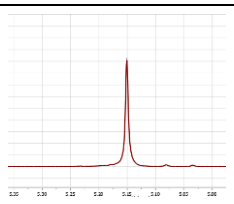
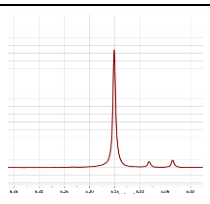
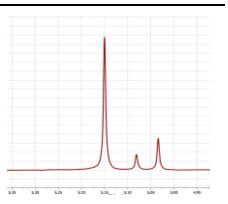
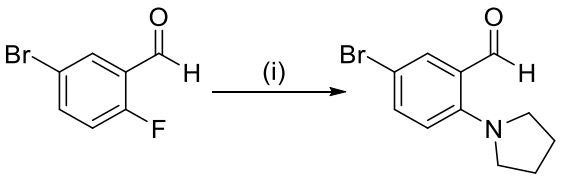
Days	0	1	3	7
C2-H				

Table 3.2 Isomerisation of compound **157c** over 7 days.

Synthesis of aryl-functionalised spirocycles

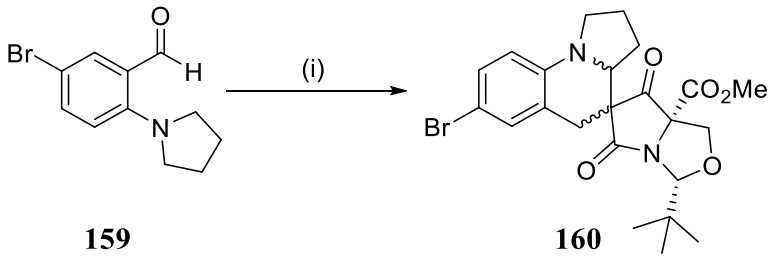
Following this previous work, more functionalised spirocycles were examined. It was decided that synthesis of a bromo-substituted aldehyde **159** would allow downstream functionalisation through Suzuki cross-coupling reactions. Conversion of compound **158** to **159** was attempted using previous S_NAr conditions and was achieved in 73 % yield (Scheme **3.6**).



158 **159**

Scheme 3.6. Synthesis of bromo-substituted benzaldehye **159.** (i) Pyrrolidine, K_2CO_3, DMF, reflux, 6 h, 73 %.

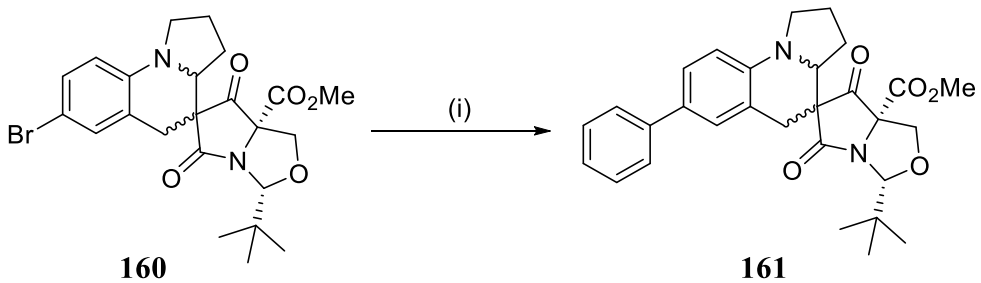
With compound **159** in hand, the synthesis of spiro-tetramates was attempted using conditions previously used in the Moloney group.[125] Both the benzaldehyde and serine tetramate were mixed in MeOH and heated to reflux for 6 h to yield bromo-substituted spiro-tetramate **160** (Scheme **3.7**).



159 **160**

Scheme 3.7. Synthesis of bromo-substituted spiro-tetramate. (i) Serine tetramic acid **9o**, MeOH, reflux, 6 h, 62 %.

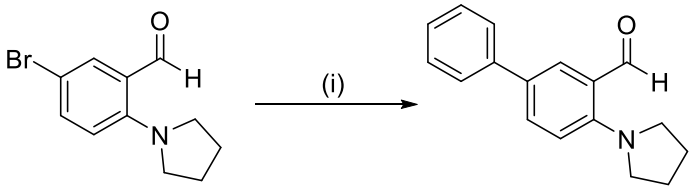
Compound **160** was synthesised in 62 % yield but as an inseparable mixture of 4 diastereoisomers; this was a surprise, since the reversible nature of the reaction and the sterically encumbered nature of the system suggested that the formation of such a gross mixture would be unlikely. Unfortunately, the stereochemistry of the major isomer could not be determined due to signal overlap in the NMR spectrum. Despite the presence of multiple diastereoisomers, a reaction to functionalise at the bromine position was attempted using Suzuki conditions.[126] Phenyl boronic acid was chosen due to its availability and simplicity, and was successfully coupled to spiro-tetramate **160**, using $Pd(dppf)Cl_2$ as a catalyst at reflux for 24 h, to yield compound **161** in 38 % yield (Scheme **3.8**).



160 **161**

Scheme 3.8. Cross coupling reaction of **160** with phenylboronic acid. (i) Phenyl boronic acid, $Pd(dppf)Cl_2$, THF, reflux, 24 h, 38 %.

Of interest was whether incorporation of the phenyl ring to aldehyde **159** before cyclisation would affect the diastereomeric ratio of compound **161**. By simply reversing the order of reactions this possibility was investigated. Compound **159** was subject to identical conditions used in Scheme **3.8** and phenyl substituted aldehyde **162** was synthesised in 63 % yield (Scheme **3.9**).



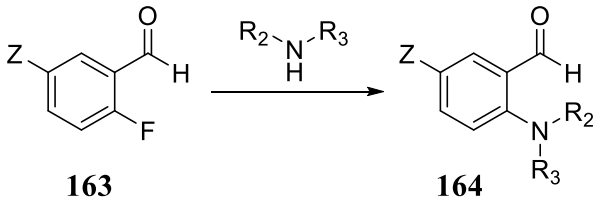
159 **162**

Scheme 3.9. Synthesis of phenyl-substituted benzaldehyde **162**. (i) Phenyl boronic acid, $Pd(dppf)Cl_2$, THF, reflux, 24 h, 63 %.

With compound **162** in hand, spirocyclic generation was attempted and compound **161** was synthesised in 34 % yield, comparable to other spirocyclic reactions. Once again, **161** was also isolated as a mixture of 4 diastereoisomers, demonstrating that reversing the order of reaction has no effect on diastereomeric ratios.

Exploration of 2-aminobenzaldehyde substituents

Previously, various substitutions were attempted on the phenyl ring moiety however, the aminobenzaldehyde was relatively unexplored (pyrrolidine, piperidine and morpholine only). Moreover, only cyclic amines were incorporated into the spirocyclic product; this led to the generation of a range of aliphatic 2-aminobenzaldehydes to probe the effect of changing from cyclic amines to aliphatic. Application of the conditions used in Scheme **3.6**, multiple aliphatic substituted amino benzaldehydes **164** were synthesised, including examples containing functionalisation on the aromatic ring (Table **3.3**, entry i and j).



163 **164**

Entry	Aldehyde	R_2	R_3	Yield (%)	Reaction time (h)
a	Z = H	iPr	iPr	-	-
b	Z = H	Bn	Bn	7 (recovered mass)	29
c	Z = H	Me	iPr	16	48
d	Z = H	Et	Et	18	24
e	Z = H	Me	$CH(Ph)_2$	-	-
f	Z = H	Et	iPr	1	72
g	Z = H	Et	Bn	25	48
h	Z = H	Me	Bn	12	48
i	$Z = NO_2$	Et	Et	50	24
j	Z = Br	Et	Et	47	24

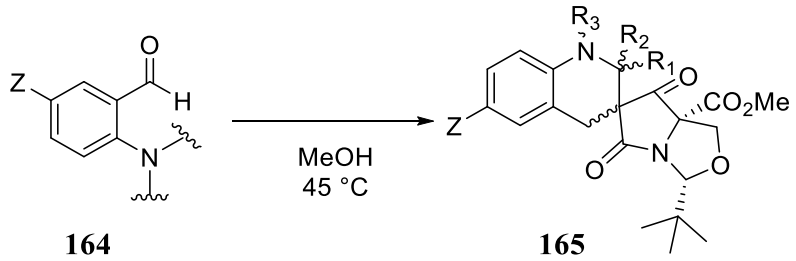
Table 3.3. Synthesis of various aliphatic 2-aminobenzaldehydes

Yields when compared to the cyclic aminobenzaldehydes (73 % for pyrrolidinyl) are in most cases significantly reduced. The only compounds with comparable yields are those possessing an electron withdrawing group para- to fluorine, most likely increasing the electrophilicity of the carbon attached to fluorine due to delocalisation. Additionally, cyclic compounds are much more spatially defined due to the ring imposing a restriction of rotation about the C-C

bonds whereas the aliphatic amines are more free to rotate, and this could impose an unfavourable entropic penalty upon generating the 2-aminobenzaldehyde.

Steric effects could be the reason for the formation of no product (Table **3.3** entries **a** and **e**). By comparing entries **a** and **d**, the isopropyl functional group possesses a larger steric bulk than an ethyl group, and this increase leads to a loss in reactivity. This was shown to be true when an attempt to synthesise a 2-aminobenzaldehyde with both an isopropyl group and an ethyl group (Table **3.3**, entry **f**) was made, which was achieved in only 1 % yield. Moving from diethyl, to ethyl and isopropyl, then diisopropyl saw yields reduce from 18 %, to 1 % with final loss of reactivity respectively. Methyl and isopropyl substituted amine retains relatively good reactivity at 16 % (Table **3.3**, entry **c**) presumably due to the reduction in steric bulk from ethyl to methyl. Generally, reaction times tend to increase for more sterically hindered amines whilst also leading to a general reduction in yield. With the various 2-aminobenzaldehydes in hand, spirocyclic generation was attempted using tetramate **9o** (Table **3.4**).

Of interest was the inclusion of an electron withdrawing group para- to the amino group of the aldehydes (see entries **i** and **j**). If equilibration proceeded through the mechanism highlighted in Figure **3.3**, then this might prevent or slow down the rate of isomerisation.



164 **165**

Table 3.4 Entry	Tetramate	Z	R_1	R_2	R_3	Yield (%)	No. of isomers
a	X = O	H	Me	Me	iPr	-	-
b	X = O	H	H	Ph	Bn	43	4
c	X = O	H	Me	Me	Me	-	-
d	X = O	H	H	Me	Et	7	2 (3:1 mixture)
e	X = O	H	Ph	Ph	Me	-	-
f	X = O	H	H/Me	Me/H	iPr/Et	-	-
g	X = O	H	H/Me	Ph/Me	Et/Bn	-	-
h	X = O	H	H	Ph	Me	-	-
i	X = O	NO_2	H	Me	Et	-	-
j	X = O	Br	H	Me	Et	-	-

Table 3.4. Synthesis of tetramate spirocycles **165** using aliphatic 2-aminobenzaldehydes **164**.

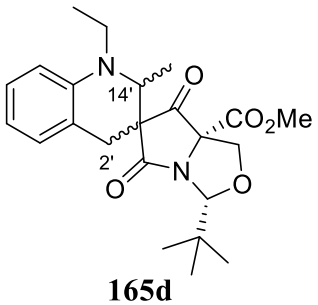
As can be seen in Table **3.4**, most attempts at the synthesis of aliphatic amino substituted tetramate spirocycles were unsuccessful. Entry **b** shows the spirocyclic tetramate product as a mixture of 4 isomers in 43 % yield. Characterisation of the major was not possible due to signal overlap and degradation in Toluene-d_6. Table **3.4,** entry **d**, the only other successful spirocyclic generation, was isolated as a mixture of 2 isomers, both of which were characterised by CLIP-HSQMBC, discussed in more detail below. Attempts to reduce isomerisation by inclusion of an electron-withdrawing group para to amine substituents did not even give a spirocycle (entries **i** and **j**).

Purification of spirocyclic tetramates

Of all the synthesised spirocyclic tetramates, only one (**157c**) was characterised as a single diastereoisomer, and once isolated, this equilibrated to 3 isomers within days. This equilibration proved to be a serious problem with this compound set. To obtain clean compound **157c,** the crude reaction mixture was cooled in an ice bath and the RRSR isomer crystallised out of methanol as a conglomerate. Key to this method of purification is the low solubility of the spirocycles in methanol. Crystallisation by cooling was ineffective for purifying other synthesised spirocyclic tetramates (Table **3.4**, entries **b** & **d**) and thus removal of solvent by rotary evaporation was necessary to yield spirocycles, even though this gave them as a mixture of multiple diastereoisomers. To purify compound **165d**, it was necessary to perform flash column chromatography to obtain a clean product, however it was likely that this would not favour the isolation of a single isomer. To confirm this, pure compound **157c** as the RRSR isomer was run through a column in the same conditions used to purify compound **165d**. NMR in deuterated toluene (which previously revealed a single isomer) clearly revealed that compound **157c** had equilibrated to a mixture of 4 diastereoisomers.

Characterisation of 165d

Characterisation of compound **165d** was possible using a combination of molecular modelling and CLIP-HSQMBC; this is an NMR technique which identifies long-range proton-carbon couplings with the coupling constant dependant on the dihedral angle (Karplus equation).[127] To characterise the product **165d** (Table **3.4**), CLIP-HSQMBC was used to both identify the stereogenic carbon and assign the stereochemistry of the major, and by extension, the minor isomer.



165d

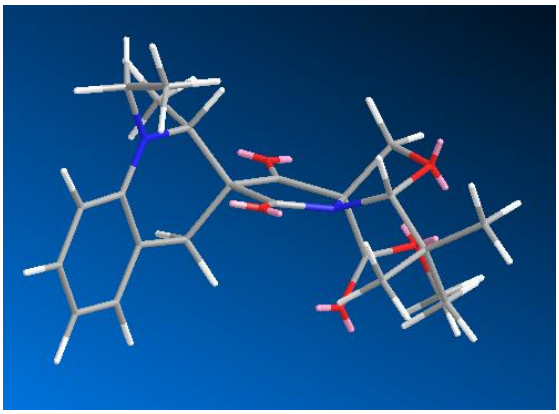
Figure 3.4. Compound **165d**.

Initially, coupling constants were recorded between protons 14', 2'$_A$ and 2'$_B$ (labelled above in Figure **3.4**) and the carbonyl carbons of the ketone and amide in the bicyclic ring (Table **3.5**). Measurement of the coupling constants would reveal which carbon is the stereogenic centre and provide insight into the geometry of the molecule.

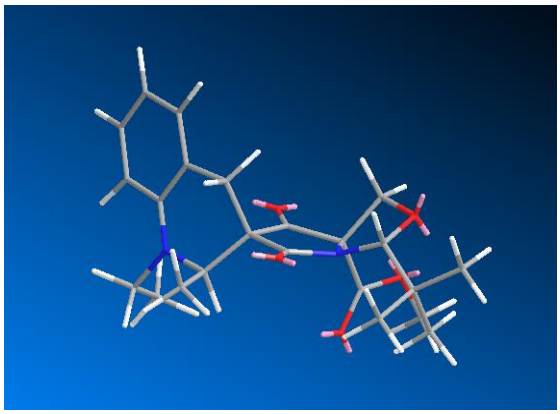
Major Isomer (by NMR)	^3J value C=O$_{Ketone}$	^3J value C=O$_{Amide}$	Minor Isomer (by NMR)	^3J value C=O$_{Ketone}$	^3J value C=O$_{Amide}$
H-14'	< 1 Hz	< 1 Hz	H-14'	< 1 Hz	< 1 Hz
H-2'$_A$	9.1 Hz	2.3 Hz	H-2'$_A$	~ 3 Hz	9.2 Hz
H-2'$_B$	2.0 Hz	< 1 Hz	H-2'$_B$	< 1 Hz	2.4 Hz

Table 3.5. Measured coupling constants in compound **165d.**

A coupling constant of 9.1 Hz between H-2'$_A$ and C=O$_{Ketone}$ in the major isomer suggests a *trans*- relationship between the pair, as predicted by the Karplus equation in which the ^3J values depend on the spatial relationship of the respective atoms. In the minor isomer, a coupling constant of 9.2 Hz is observed between H-2'$_A$ and the C=O$_{Amide}$, which shows a *trans*- relationship between this proton and the C=O$_{Amide}$, contrary to the major isomer. This reversal of coupling constants is evidence for the isomerisation occurring around the spirocyclic carbon and not 14'. Using this information, it was possible to determine the stereochemistry of the major isomer using molecular modelling (ChemDraw3D) to determine the dihedral angles; this allowed the major isomer to be assigned as RRRR and the minor as RRSR. The measured angles are recorded below in Table **3.6** for clarity.



Major Isomer (RRRR)



Minor Isomer (RRSR)

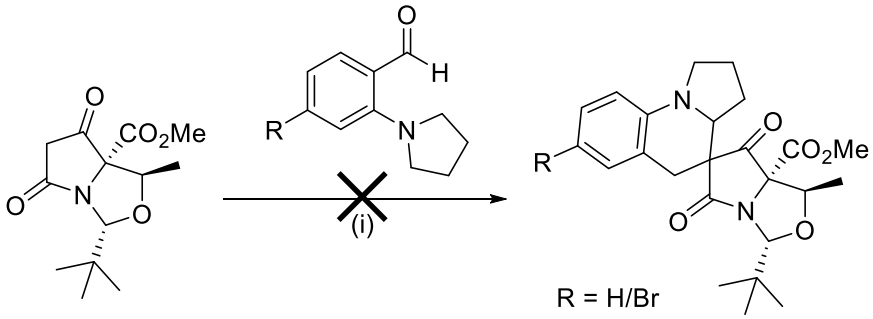
Major Isomer (by NMR)	Dihedral Angle C=O$_{Ketone}$	Dihedral Angle C=O$_{Amide}$	Minor Isomer (by NMR)	Dihedral Angle C=O$_{Ketone}$	Dihedral Angle C=O$_{Amide}$
H-14'	50.7 °	68.3°	H-14'	68.0 °	51.0 °
H-2'$_A$	168.0 °	49.7 °	H-2'$_A$	58.7 °	169.0 °
H-2'$_B$	57.6 °	60.7 °	H-2'$_B$	59.6 °	50.7 °

Table 3.6. Measured dihedral angles of both isomers using molecular modelling.

Overall, CLIP-HSQMBC proved to be very useful in the characterisation of a mixture of diastereoisomers by providing measurement of coupling constants between carbon and protons. Subsequent molecular modelling revealed the major isomer of compound **165d** as RRRR. Noteworthy is that compound **165d** decomposed over a period of days in the NMR and so was unstable in Toluene-d_6.

Varying the tetramate

Investigation into a range of aliphatic 2-aminobenzaldehydes revealed there was not much scope for variation due to steric and electronic demands for the success of the reactions. Considering this, it was decided to vary the tetramate component of the spirocycle. Initially, spirocyclisation was attempted using the threonine-derived spirocycle (Scheme **3.10**).

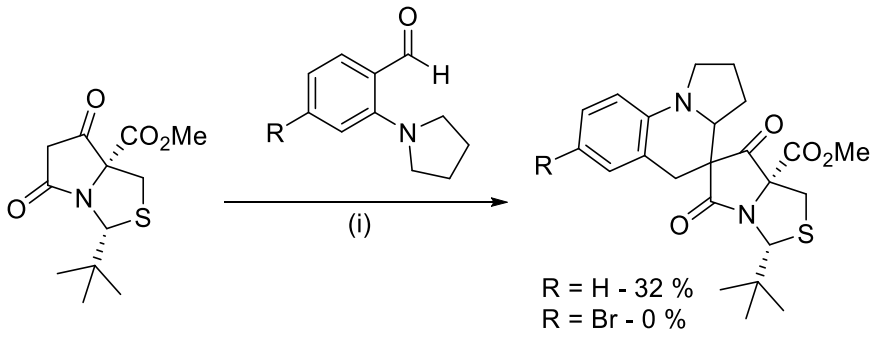


9t **167** R = H
168 R = Br

Scheme 3.10. Attempted synthesis of a threonine tetramate spirocycles **9t**. (i) 2-aminobenzaldehyde, MeOH, 45 °C, 6 h.

Spirocyclic generation was attempted on the 2-pyrrolidinyl benzaldehyde as only a single isomer had been earlier observed. Reactions both with and without the bromo-substituted aldehyde were unsuccessful. It is likely the additional methyl group imposes a significant

steric impact on the system and slows reactivity. It was decided to attempt the cyclisation on cysteine tetramate systems; this was done using analogous chemistry (Scheme **3.11**).



64s

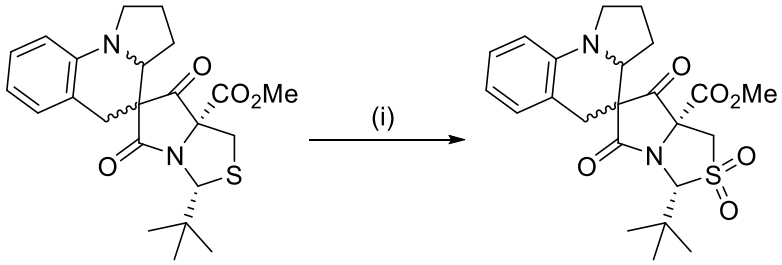
169 R=H

170 R=Br

Scheme 3.11. Attempts to synthesise a cysteine tetramate spirocycle. (i) 2-aminobenzaldehyde, MeOH, 45 °C, 6 h, **169** 32 %, **170** 0 %.

Synthesis of unsubstituted cysteine tetramate **169** was successful and was obtained as a mixture of 4 diastereomers in 32 % yield, although lower than the analogous reaction with serine-derived tetramate. Characterisation of the isomers was not possible due to signal overlap in the spectrum. There was no product obtained from the bromo-substituted aldehyde, revealing a messy spectrum with no signs of product **170**.

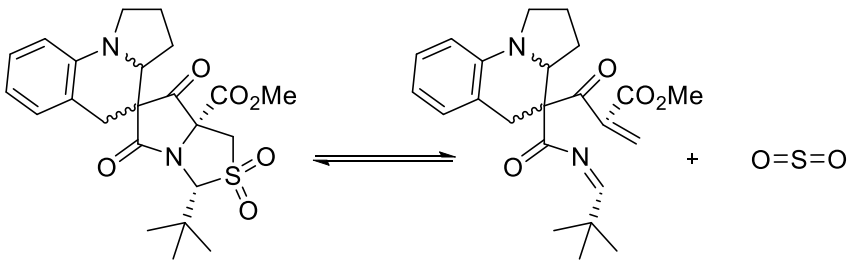
To assess the effect of a more polar tetramate component, oxidation of the sulfur in the cysteine system was attempted on the mixture of diastereomers to see whether the equilibrium could be driven towards one isomer (Scheme **3.12**).



169 **171**

Scheme 3.12. Oxidation of cysteine derived spirocycle **169**. (i) mCPBA 5.0 eq, $CHCl_3$, 24 h, rt.

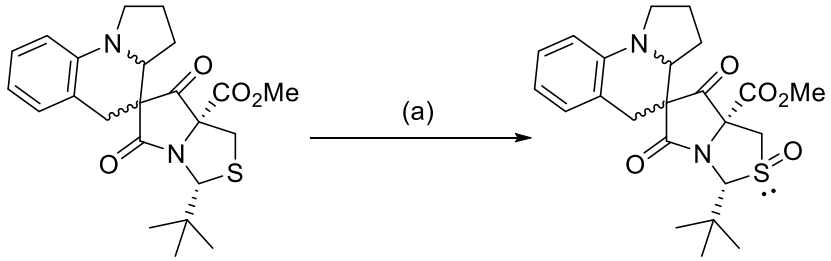
Initially, an excess of 5.0 equivalents of mCPBA in chloroform at r.t. for 24 h was used to oxidise to the sulfone repeating conditions used by Gupta.[128] This reaction was unsuccessful despite positive results from the crude LRMS. It is possible that cheletropic extrusion resulted in the degradation of the sulfone and thus no expected product was obtained after purification by column chromatography (Figure **3.5**).



171 **172**

Figure 3.5. Proposed cheletropic extrusion of **171**

Instead, we turned our attention to synthesising the sulfoxide equivalent, not susceptible to cheletropic extrusion yet still more polar than the cysteine tetramate. The same reaction conditions used previously were employed but in a 1:1 stoichiometric ratio (Scheme **3.13**).



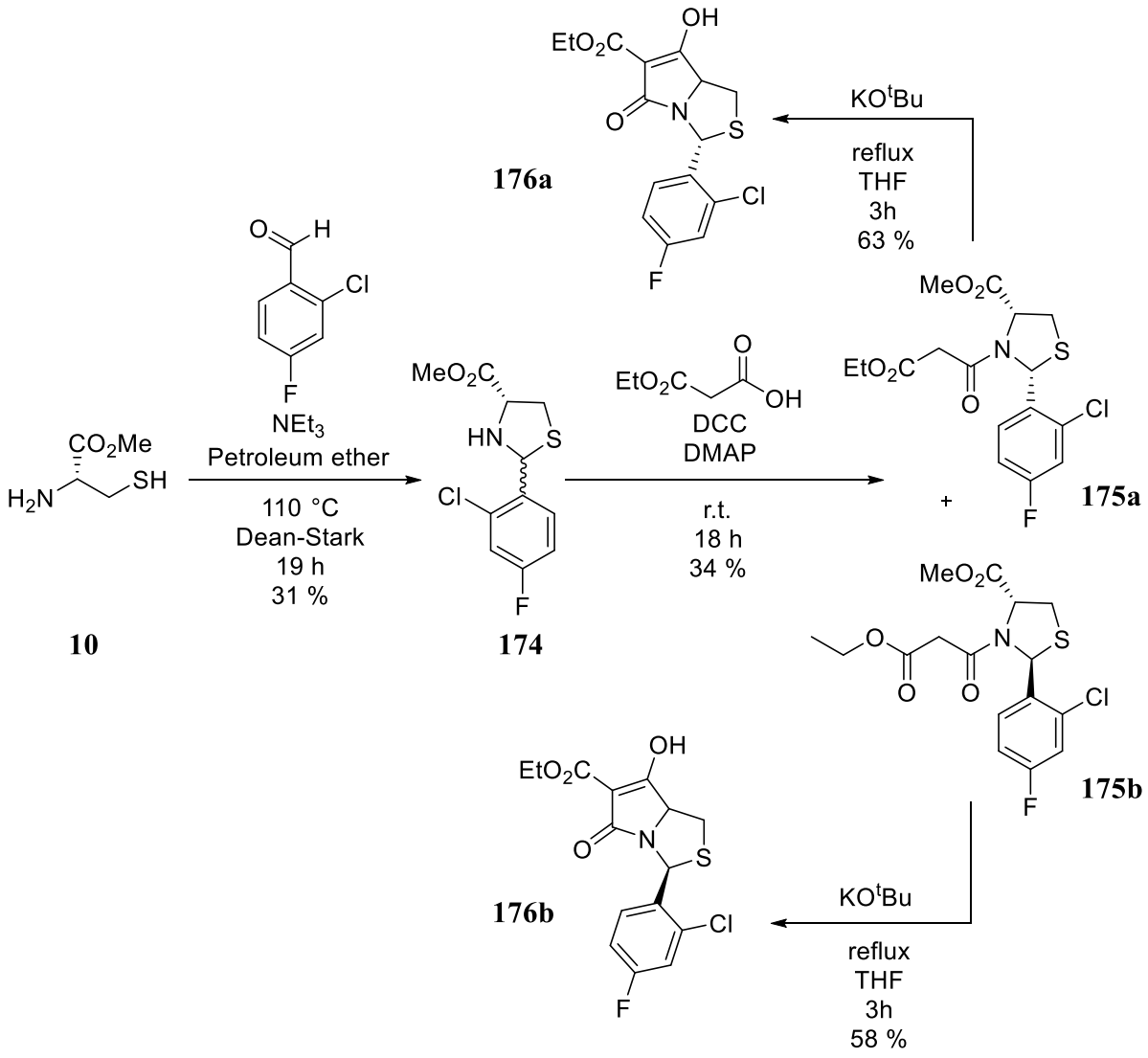
169 **173**

Figure 3.13. Mono-oxidation of **173**. (a) mCPBA, $CHCl_3$, 24 h, rt, 78 %.

Compound **173** was synthesised in 78 % yield as a 1.0 : 1.0 : 2.2 : 2.6 : 3.4 : 5.6 : 10.6 : 12 mixture of 8 diastereomers. Clearly, some diastereomers are more prevalent than others but separation of such a complex mixture was clearly impracticable.

Exploration of aryl tetramates

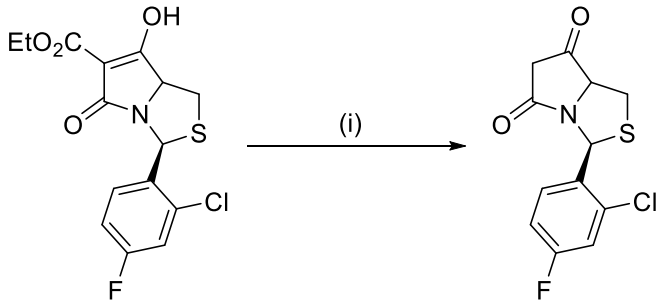
Observation of isomeric equilibration in every system examined resulted in the need to change the approach, to try to find a system where one diastereomer could exist without degradation. Replacement of the ^{t}Bu group would significantly alter the geometry of the compound and so substituting for a different moiety could alter the diastereomeric ratios observed in the spirocycles. Initially, synthesis of aryl tetramates was done using published methodology (Scheme **3.14**).[129]



Scheme 3.14. Synthesis of aryl-substituted tetramic acids.

Commercially available cysteine methyl ester **10** was reacted with 2-chloro-4-fluoro benzaldehyde (chosen due to its synthetic simplicity) for 19 h using Dean-Stark conditions to yield a diastereomeric mixture of thiazolidines **174**. Next, thiazolidines were acylated with ethyl hydrogen malonate by coupling with DCC and catalytic amount of DMAP at r.t. for 18 h yielding a separable mixture of **175a** and **175b**. **175a** and **175b** were refluxed separately in THF in the presence of potassium *tert*-butoxide for 3 h to yield tetramates **176a** and **176b** in 63 % and 58 % yield respectively.

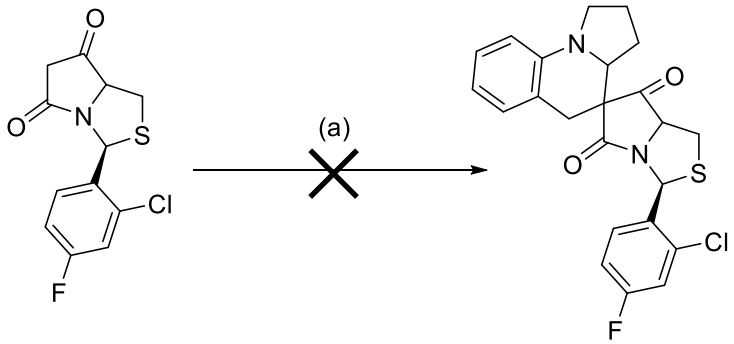
In contrast to the ^{t}Bu system, cyclisation from the *N*-acyl thiazolidines with KO^{t}Bu yielded the ethyl ester rather than the methyl ester. In the cyclisation step, two Dieckmann cyclisations can occur. Thus, decarboxylation is necessary to obtain a tetramate suitable for spirocyclisation. To do this, **175b** was dissolved in acetonitrile and a drop of H_2O was added. The mixture was refluxed for 6 h to yield decarboxylated cysteine tetramate **177** in 93 % yield (Scheme **3.15**).



176b **177**

Scheme 3.15. Decarboxylation of aryl-substituted tetramate **176b**. (i) Wet MeCN, reflux, 6 h, 93 %.

With **177** in hand, spirocyclisation was attempted with 2-pyrrolidinyl benzaldehyde using previously successful conditions (Scheme **3.16**).



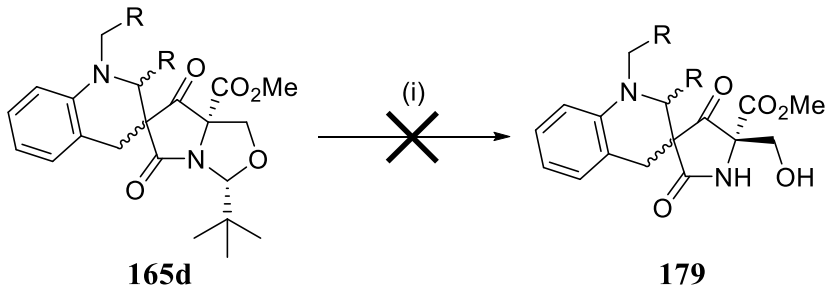
177 **178**

Scheme 3.16. Attempted spirocyclic generation of aryl-substituted tetramate **178**. (a) 2-pyrrolidinylbenzaldehyde, MeOH, 40 °C.

However, no product **178** was observed from reacting tetramate **177** and 2-pyrrolidinyl benzaldehyde. This reaction was not followed up further.

Increasing polarity of the spirocycles

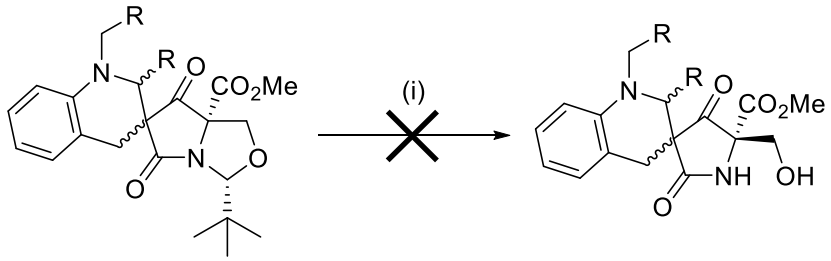
To assess biological activity, compounds must readily dissolve in a relevant solvent (usually H_2O or DMSO). Synthesised spirocyclic tetramates were insoluble in both water and DMSO, and only partially soluble in MeOH. To increase the polarity of the systems, deprotection of the oxazolidine ring was attempted (Scheme **3.17**).



165d **179**

Scheme 3.17. Attempted synthesis of deprotected tetramate spirocycles **179**. (i) TFA, THF/H_2O, rt, 40 h.

Initial attempts involved stirring TFA in THF/H_2O at r.t. over a period of 40 h to remove the acid labile ^{t}Bu group. Introducing an NH and OH whilst simultaneously removing very hydrophobic ^{t}Bu would increase the polarity and likely increase the solubility in H_2O/DMSO. Unfortunately, deprotection using TFA was unsuccessful in any system. Following this, another method was attempted which employed HCl, trifluoroethanol and propanedithiol stirring in the presence of spirocycles at rt for 18 h (Scheme **3.18**), but this too was unsuccessful.



165d **179**

Scheme 3.18. Attempted synthesis of deprotected tetramate spirocycles **179**. (i) $HS(CH_2)_3SH$, HCl, CF_3CH_2OH, rt, 18 h.

Earlier attempted syntheses of aryl-substituted tetramates would also serve to increase the polarity, by replacement of the tBu with a substituted aryl ring. However, once synthesised these compounds would not cyclise to a spirotetramate and thus a more polar, hydrophilic tetramate could not be synthesised.

Successfully synthesised spirocycles, albeit as mixtures of diasteroisomers, were submitted for biological evaluation, but no data could be collected due to two factors; the spirocycles were insoluble in the solvents required for the assay (DMSO/water) and it was reported that the samples were found to degrade very rapidly.

Summary

Spirocyclic tetramic acids were investigated as a method to synthesise fused hybrid compounds. A range of barbiturate spirocycles were synthesised to confirm reaction conditions and provide insight into the diastereoselectivity and reversibility of the 1,5-hydride shift reaction. S_NAr reaction conditions were employed to synthesise a large range of 2-amino benzaldehydes – a precursor in the 1,5-hydride shift reaction.

Reaction of the tetramic acid cores with the synthesised 2-amino benzaldehydes produced spirocyclic tetramic acid compounds. In all cases, multiple diastereomers were observed by NMR which made characterisation of the compounds difficult. CLIP-HSQMBC was employed to characterise **165d** as a mixture of two diastereomers - RRRR as the major isomer and RRSR as the minor isomer.

Further manipulation of a bromo-substituted tetramic acid spirocycle was successfully achieved through a Suzuki coupling reaction, however this yielded the spirocyclic product as a mixture of diastereomers. Oxidation to the sulfoxide substituted tetramic acid was also successful but resulted in a mixture of 8 diastereomers being formed.

An investigation into varying the tetramic acid core was made by synthesising a C-2 phenyl substituted tetramic acid. Reaction of this tetramic acid with a 2-amino benzaldehyde yielded no desired product. Furthermore, deprotection of the spirocyclic tetramic acid to yield a more soluble compound was unsuccessful.

Lack of solubility in suitable solvents and reported degradation resulted in the antimicrobial evaluation of these compounds not being investigated.

Future Work

Investigations into varying the tetramic acid core prior to generating the spirocycle could be made. In all cases, isomerisation and equilibration into a mixture of diastereomers was observed, which proved troublesome when characterising the compounds. By altering the steric or electronic properties of the tetramic acid, the rate of isomerisation and ratio of diastereomers could be altered. For example, if the methyl ester moiety of the tetramic acid was replaced with a bulkier ester, the diastereomeric ratio could be altered, which could aid in the characterisation and stability of the spirocyclic tetramic acids.

In addition to altering the tetramic acid component, functional group changes could be made to the 2-amino benzaldehyde component. Polarity proved troublesome when attempting to purify the spirocycles, and when attempting to determine their antibacterial properties. Synthesised spirocycles were not soluble in assay-suitable solvents such as DMSO and H_2O. If the polarity was adjusted by introduction of polar functionality, such that the spirocycles were soluble in assay relevant solvents, SAR could be drawn from the results and could guide the next series of compounds to be synthesised.

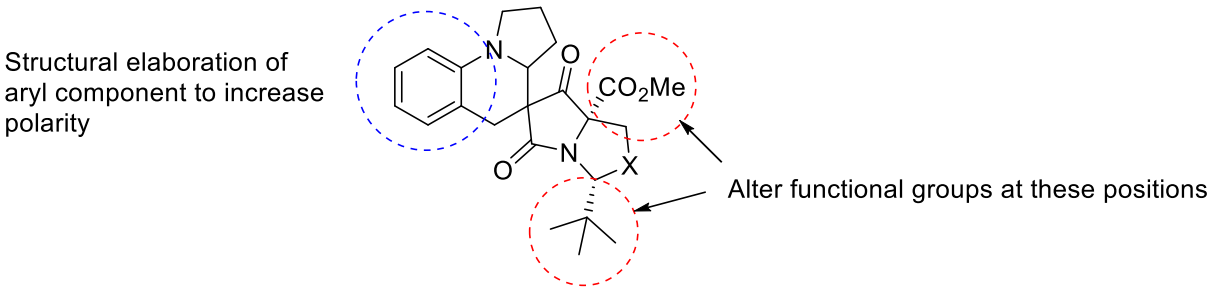


Figure 3.6. Points of potential structural manipulation to enhance polarity or selectivity towards a diastereomer.

Chapter IV: Experimental

General techniques

Commercial reagents were obtained from Acros Organics, Alfa Aesar, Apollo Scientific, Fisher Scientific, Fluorochem or Sigma Aldrich and were used without further purification. All reactions were performed in oven-dried glassware and under a N_2 atmosphere unless non-anhydrous solvents were used. Anhydrous solvents were obtained from an MBraun MB SPS5solvent purification system. 'Petrol' refers to light petroleum ether of boiling point 40-60 °C and was used as purchased. Solvents were evaporated at 40 °C under reduced pressure on a Büchi R-114 rotatory evaporator attached to a Vacuubrand CVC2 pump attached to a pressure control system.

Analytical thin layer chromatography (TLC) was carried out on Merck aluminium foil backed sheets coated with 0.2 mm Kielselgen 60 F_{254}. The eluent used is specified in each case. TLC spots were visualised by UV irradiation (λ 254 nm) and if necessary, staining with a $KMnO_4$ solution followed by heating using a heat gun. Retention factors (R_f) are quoted to two decimal places. *Flash* column chromatography was performed on Kielselgel 60 silica gel (230 – 400 mesh particle size).

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

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

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

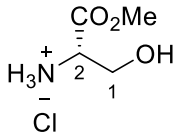
^{1}H NMR spectra were recorded on the following instruments: Bruker DPX200 (200 MHz), AVIII HD 400 (400 MHz), AVC 500 (500 MHz) and AV600 (600 MHz). Chemical shifts (δ_H) are reported in parts per million (ppm) downfield from TMS and are referenced to the residual ^{1}H solvent peak. Coupling constants (*J*) are reported in Hz and accurate to 0.5 Hz. Data are reported as follows: Chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, dd = double doublet, dt = double triplet, m = multiplet), coupling constant, and proton assignment. Two-dimensional COSY and HSQC data were recorded on a Bruker AVIII HD 400 (400 MHz) and AVC 500 (500 MHz). CLIP-HSQMBC data were recorded on Bruker AV600 (600 MHz).

^{13}C NMR spectra were recorded on a Bruker AVIII HD 400 at 100 MHz, AVC 500 at 125 MHz and AV600 at 150 MHz with proton decoupling. Chemical shifts (δ_C) are reported in ppm downfield from TMS and are referenced to the residual ^{13}C solvent peak. Assignments of the spectra were made with HSQC experiments, which were performed on a Bruker AVIII HD 400. Low resolution mass spectra (m/z) were recorded on a Fison Platform spectrometer using electrospray ionisation (ESI). Selected peaks are reported in Daltons and their intensities are given as percentages of the base peak. High resolution mass spectra (HRMS) were recorded on a Bruker microTOF (ESI), on an Agilent 7200 Q-TOF (CI) or on a Waters GCT (EI).

In the cases where the products exist as a mixture of diasteromers or tautomers, the ratio present was calculated from the ^{1}H NMR spectrum.

Experimental for Chapter II

Serine methyl ester hydrochloride 6o

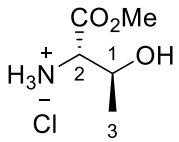


To stirring MeOH (30 mL) at 0 °C was added $SOCl_2$ (5.20 mL, 71.4 mmol) dropwise followed by L-serine (5.0 g, 47.6 mmol) portion wise. The mixture was heated to 40°C and stirred at this temperature for 3 h. Solvent was removed under reduced pressure to give L-serine methyl ester hydrogen chloride as a white solid. Yield 7.40 g, quantitative.

M.p: 161 °C (Lit. 161 - 162 °C)[130]; **^{1}H NMR (400 MHz, D_2O):** 3.83 (3H, s, CO_2***Me***), 3.97 (1H, dd, *J* = 12.5, 3.4 Hz, C1-***H_A***H_B), 4.08 (1H, dd, *J* = 12.5, 4.2 Hz, C1-H_A***H_B***), 4.17 (1H, t, *J* = 4.2 Hz, C2-***H***); **^{13}C NMR (100 MHz, D_2O):** 53.7 (CO_2**Me**), 54.7 (C-5), 59.2 (C-4), 168.9 (**C**O_2Me); **m/z (ESI$^+$):** 120.1 ($[M+H]^+$); $[\alpha]_D^{25}$ = + 4.1 (c = 1.0 in MeOH) (Lit. + 3.9, c = 4.0 MeOH).[131]

Data are in agreement with those found in the literature.[131]

Threonine methyl ester hydrochloride 6t



To stirring MeOH (30 mL) at 0 °C was added $SOCl_2$ (4.6 mL, 63.0 mmol) dropwise followed by L-threonine (5.0 g, 42.0 mmol) portion wise. The mixture was heated to 40°C and stirred

at this temperature for 3 h. Solvent was removed under reduced pressure to give L-threonine methyl ester hydrogen chloride as a white solid. Yield 7.09 g, quantitative.

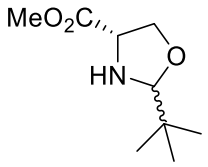
M.p: 140 °C (Lit. 140 – 142 °C)[132]; **^{1}H NMR (400 MHz, D_2O):** 1.30 (3H, d, *J* = 6.6 Hz, C1-***Me***), 3.82 (3H, s, CO_2***Me***), 4.08 (1H, d, *J* = 3.7 Hz, C2-***H***), 4.39 (1H, qd, *J* = 6.7, 3.8 Hz, C1-***H***); **^{13}C NMR (100 MHz, D_2O):** 18.7 (CH**C**H_3), 53.6 (CO_2**C**H_3), 58.3 (**C**HCH_3), 65.1 (NH_2**C**H), 169.1 (**C**O_2Me); **m/z (ESI$^+$):** 134.1 ($[M+H]^+$); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = -35.5 (c = 1.0 in H_2O).

Data are in agreement with those found in the literature.[132]

General procedure A. Synthesis of oxazolidines/thiazolidines.

Amino acid methyl ester (1.0 eq), the corresponding aldehyde (1.2 eq) and NEt_3 (1.5 eq) were added to Petrol (100 – 150 mL). Reaction mixture was heated to 110 °C for 18 h using Dean-Stark apparatus for continuous removal of water. Once complete, reaction mixture was filtered and the precipitate washed with Et_2O. Filtrates were combined and concentrated *in vacuo* to yield the oxazolidine/thiazolidine.

Methyl (4*S*)-2-(tert-butyl)oxazolidine-4-carboxylate 7o



Synthesised according to general procedure A.

Colourless oil.

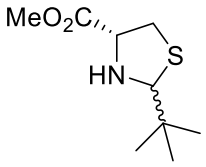
Yield 7.66 g, 86 %. Isolated as a mixture of two diastereomers, *cis:trans* 1.7:1 (by NMR).

R_f: 0.60 (4:1 EtOAc:DCM); **^{1}H NMR (400 MHz, $CDCl_3$) Major (*cis*):** 0.98 (9H, s, (C***H*****$_3$**)$_3$), 3.65 – 3.71 (1H, m, C4-***H*****$_A$**H_B), 3.75 (3H, s, O***Me***), 3.87 – 3.99 (2H, m, C5-***H*** & CH_A***H*****$_B$**),

4.07 (1H, s, C2-***H***); **Minor (*trans*):** 0.90 (9H, s, (C***H*****$_3$**)$_3$), 3.65 – 3.71 (1H, m, C4-***H*****$_A$**H$_B$), 3.73 (3H, s, O***Me***), 3.89 – 3.95 (1H, m, C5-***H***), 4.10 (1H, t, *J* = 7.3 Hz, C5-H$_A$***H*****$_B$**), 4.31 (1H, s, C2-***H***); **^{13}C NMR (100 MHz, CDCl$_3$): Major (*cis*):** 25.3 (C(**C**H$_3$)$_3$). 33.3 (**C**(CH$_3$)$_3$), 52.7 (CO$_2$**Me**), 59.5 (C5), 68.4 (C4), 99.9 (C2), 173.0 (**C**O$_2$Me); **Minor (*trans*):** 25.0 (C(**C**H$_3$)$_3$), 34.7 (**C**(CH$_3$)$_3$), 52.5 (CO$_2$**Me**), 59.5 (C5), 69.0 (C4), 99.3 (C2), 173.2 (**C**O$_2$Me).

Data are in agreement with those found in the literature.[47]

Methyl (4*R*)-2-(tert-butyl)thiazolidine-4-carboxylate 7s



Synthesised according to general procedure A.

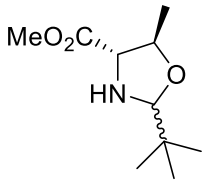
Colourless oil.

Yield 5.04 g, 89 %. Isolated as a mixture of two diastereomers, *cis:trans* 1.6:1 (by NMR).

R$_f$: 0.6 (4:1 EtOAc:DCM); **^{1}H NMR (400 MHz, CDCl$_3$) Major isomer:** 1.05 (9H, s, (C***H*****$_3$**)$_3$), 2.66 (1H, t, *J* = 10.0 Hz, C5-***H***), 3.24 (1H, dd, *J* = 10.2, 6.7 Hz, C4-***H*****$_A$**H$_B$) , 3.76 (3H, s, O***Me***), 3.76 – 3.83 (1H, m, C4-H$_A$***H*****$_B$**), 4.44 (1H, s, C2-***H***); **Minor isomer**: 0.96 (9H, s, (C***H*****$_3$**)$_3$), 3.00 (1H, dd, *J* = 10.6, 5.6 Hz, C5-***H***), 3.10 (1H, dd, *J* = 10.6, 6.4 Hz, C4-***H*****$_A$**H$_B$, 3.74 (3H, s, O***Me***) 4.12 (1H, dd, *J* = 6.3, 5.6 Hz, C4-H$_A$***H*****$_B$**), 4.51 (1H, s, C2-***H***); **^{13}C NMR (100 MHz, CDCl$_3$) Major isomer:** 26.7 (C(**C**H$_3$)$_3$), 34.0 (**C**(CH$_3$)$_3$), 37.5 (C4), 52.5 (CO$_2$**Me**), 65.5 (C5), 81.8 (C2), 171.8 (**C**O$_2$Me); **Minor isomer:** 26.6 (C(**C**H$_3$)$_3$), 35.9 (**C**(CH$_3$)$_3$), 37.0 (C4), 52.5 (CO$_2$**Me**), 65.1 (C5), 79.8 (C2), 172.5 (**C**O$_2$Me); **m/z (ESI$^+$):** 204.1 ([M+H]$^+$).

Data are in agreement with those found in the literature.[47]

Methyl (4*S*,5*R*)-2-(tert-butyl)-5-methyloxazolidine-4-carboxylate 7t



Synthesised according to general procedure A

Colourless oil.

Yield 7.01 g, 83 %. Isolated as two isomers, *cis:trans* 1.2:1 (by NMR).

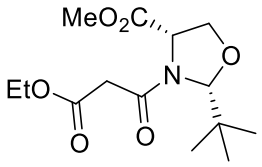
R_f: 0.65 (3:1 EtOAc:DCM); **^{1}H NMR (400 MHz, $CDCl_3$) Major isomer:** 0.97 (9H, s, (C***H*₃**)₃), 1.33 (3H, d, *J* = 6.1 Hz, C4-***Me***), 3.36 (1H, d, *J* =7.4 Hz, C5-***H***), 3.77 (3H, s, O***Me***), 3.78 – 3.87 (1H, m, C4-***H***), 4.37 (1H, s, C2-***H***); **Minor isomer:** 0.90 (9H, s, (C***H*₃**)₃), 1.37 (3H, d, *J* = 6.0 Hz, C4-***Me***), 3.46 (1H, d, *J* = 7.1 Hz, C5-***H***), 3.75 (3H, s. O***Me***), 3.78 – 3.87 (1H, m, C4-***H***), 4.29 (1H, s, C2-***H***); **^{13}C NMR (100 MHz, $CDCl_3$) Major isomer:** 20.0 (C4-**Me**), 25.3 (C(**C**H₃)₃), 33.7 (**C**(CH₃)₃), 52.6 (CO₂**Me**), 67.2 (C5), 77.4 (C4), 98.3 (C2), 172.0 (**C**O₂Me); **Minor isomer:** 19.0 (C4-**Me**), 24.9 (C(**C**H₃)₃), 34.8 (**C**(CH₃)₃), 52.5 (CO₂**Me**), 65.9 (C5), 76.3 (C4), 98.9 (C2), 172.8 (**C**O₂Me); **m/z (ESI$^+$):** 202.1 ([M+H]$^+$), 224.1 ([M+Na]$^+$).

Data are in agreement with those found in the literature.[47]

General procedure B. *N*-Acylation of oxazolidines/thiazolidines.

To a solution of oxazolidine (1.0 eq) in anhydrous DCM was added DMAP (0.05 eq) and DCC (1.05 eq). Reaction mixture was cooled to 0°C, ethyl hydrogen malonate (1.05 eq) was added dropwise and then mixture was stirred at room temperature for 5h. A white precipitate forms which was filtered and washed with DCM. Filtrate was concentrated *in vacuo* and purified by flash column chromatography to yield the N-Acylated oxazolidine/thiazolidine.

Methyl(2*R*,4*S*)-2-(tert-butyl)-3-(3-ethoxy-3-oxopropanoyl)oxazolidine-4-carboxylate 8o



Synthesised according to general procedure B.

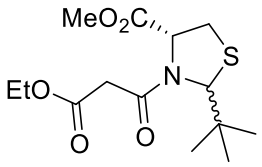
Colourless oil.

Yield 10.73 g, 87 %.

R_f: 0.75 (1:1 Petrol:EtOAc); **^{1}H NMR (200 MHz, $CDCl_3$):** 0.90 (9H, s, C(C***H*****$_3$**)$_3$), 1.29 (3H, t, *J* = 7.1 Hz, CH_2C***H*****$_3$**), 3.50 (1H, d, *J* = 15.4 Hz, C7-***H*****$_A$**H_B), 3.70 (1H, d, *J* = 15.6 Hz, C7-H_A***H*****$_B$**), 3.79 (3H, s, CO_2***Me***), 4.00 (1H, t, *J* = 7.8 Hz, C4-***H*****$_A$**H_B), 4.21 (2H, q, *J* = 7.1 Hz, C***H*****$_2$**CH_3), 4.53 – 4.58 (1H, m, C4-Ha***Hb***), 4.69 – 4.73 (1H, m, C5-***H***), 5.31 (1H, s, C2-***H***); **^{13}C NMR (100 MHz, $CDCl_3$):** 14.1 (CH_2**C**H_3), 25.6 (C(**C**H_3)$_3$), 37.3 (**C**(CH_3)$_3$), 43.1 (C7), 52.9 (CO_2**C**H_3), 59.5 (C4), 61.7 (**C**H_2CH_3), 67.7 (C5), 96.7 (C2), 167.5 (C8), 167.5 (**C**O_2Me), 169.9 (C6); **m/z (ESI$^+$):** 302.2 ($[M+H]^+$), 324.1 ($[M+Na]^+$).

Data are in agreement with those found in the literature.[47]

Methyl(4*R*)-2-(tert-butyl)-3-(3-ethoxy-3-oxopropanoyl)thiazolidine-4-carboxylate 8s



Synthesised according to general procedure B.

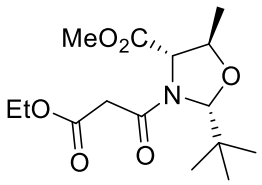
Colourless oil.

Yield 6.53 g, 83 %. Isolated as a mixture of diastereomers.

R_f: 0.70 (1:1 Petrol:EtOAc); **^{1}H NMR (200 MHz, $CDCl_3$) Major isomer:** 0.98 (9H, s, C(C***H*****$_3$**$)_3$), 1.27 (3H, t, *J* = 7.1 Hz, CH_2C***H*****$_3$**), 3.25 – 3.61 (4H, m, C4-***H*****$_A$*****H*****$_B$** & C7-***H*****$_A$*****H*****$_B$**) 3.80 (3H, s, CO_2***Me***), 4.19 (2H, q, *J* = 7.1 Hz, C***H*****$_2$**CH_3), 4.88 (1H, t, *J* = 8.0 Hz, C5-***H***), 5.53 (1H, s, C2-***H***); **Minor isomer:** 1.06 (9H, s, C(C***H*****$_3$**$)_3$), 1.27 (3H, t, *J* = 7.1 Hz, CH_2C***H*****$_3$**), 3.25 – 3.61 (4H, m, C4-***H*****$_A$*****H*****$_B$** & C7-***H*****$_A$*****H*****$_B$**), 3.78 (3H, s, CO_2***Me***), 4.19 (2H, q, *J* = 7.1 Hz, C***H*****$_2$**CH_3), 4.79 (1H, s, C2-***H***), 5.11 (1H, t, *J* = 9.5 Hz, C5-***H***); **^{13}C NMR (100 MHz, $CDCl_3$) Major isomer:** 14.1 (CH_2**C**H_3), 26.9 (C(**C**$H_3)_3$), 34.2 (C4), 39.4 (**C**$(CH_3)_3$), 53.0 (CO_2**Me**), 61.7 (**C**H_2CH_3), 64.2 (C5), 73.3 (C2), 166.8 – 167.0 (C8 & **C**O_2Me), 170.8 (C6); **Minor isomer:** 27.2 (C(**C**$H_3)_3$), 32.9 (C4), 40.1 (**C**$(CH_3)_3$), 42.4 (C7), 52.6 (CO_2**Me**), 61.7 (**C**H_2CH_3), 64.2 (C5), 74.7 (C2), 166.8 – 167.0 (C8 & **C**O_2Me), 170.6 (C6); **m/z (ESI$^+$):** 318.1 ($[M+H]^+$).

Data are in agreement with those found in the literature.[47]

Methyl(2*R*,4*S*,5*R*)-2-(tert-butyl)-3-(3-ethoxy-3-oxopropanoyl)-5-methyloxazolidine-4-carboxylate 8t



Synthesised according to general procedure B.

Orange oil.

Yield 4.61 g, 42 %.

^{1}H NMR (400 MHz, $CDCl_3$): 0.90 (9H, s, C(C***H*****$_3$**$)_3$), 1.28 (3H, t, *J* = 7.1 Hz, CH_2C***H*****$_3$**), 1.36 (3H, d, *J* = 6.2 Hz, C4-C***H*****$_3$**), 3.43 (1H, d, *J* = 15.4 Hz, C7-***H*****$_A$**H_B), 3.57 (1H, d, *J* = 15.2 Hz,

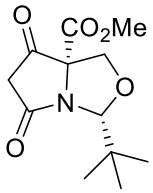
C7-H_A***H_B***), 3.79 (3H, s, CO_2***Me***), 4.20 (2H, q, *J* = 7.1 Hz, C***H_2***CH_3), 4.27-4.32 (1H, m, C5-***H***), 4.73-4.80 (1H, m, C4-***H***), 5.45 (1H, s, C2-***H***); **^{13}C NMR (100 MHz, $CDCl_3$):** 14.2 (CH_2**C**H_3), 20.3 (C4-**C**H_3), 26.0 (C(**C**$H_3)_3$), 38.0 (**C**$(CH_3)_3$), 43.0 (C7), 65.6 (CO_2**C**H_3), 76.3 (C4), 96.3 (C2), 167.4 (C6), 167.5 (C8), 170.1 (**C**O_2Me); **m/z (ESI$^+$):** 316.2 ($[M+H]^+$), 653.2 ($[2M+Na]^+$).

Data are in agreement with those found in the literature.[47]

General procedure C. Dieckmann cyclisation to form tetramic acids.

A solution of *N*-acyl thiazolidine/oxazolidine (1.0 eq) and KO^{t}Bu (1.05 eq) were dissolved in anhydrous THF and the reaction mixture was heated at reflux for 3 h. Upon completion, the mixture was separated between diethyl ether and H_2O, aqueous phase was acidified using 2M HCl and extracted with ethyl acetate. The organic layers were combined and washed with a 1M solution of NaH_2PO_4, brine, and then dried over anhydrous Na_2SO_4. The organic layer was concentrated *in vacuo* to yield the tetramic acid.

Methyl(3*R*,7a*R*)-3-(tert-butyl)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 9o



Synthesised according to general procedure C.

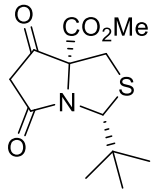
Yellow solid.

Yield 3.64 g, 72 %

R_f: 0.35 (3:1 EtOAc:MeOH); **M.p:** 124 – 126 °C (Lit. 115 – 120 °C)[47]; **^{1}H NMR (400 MHz, $CDCl_3$):** 0.90 (9H, s, CH(C$\boldsymbol{H_3}$)$_3$), 3.17 (1H, d, *J* = 21.2 Hz, C7-$\boldsymbol{H_A}$H$_B$), 3.62 (1H, d, *J* = 9.0 Hz, C4-$\boldsymbol{H_A}$H$_B$), 3.74 (1H, dd, *J* = 21.2, 0.8 Hz, C7-H$_A$$\boldsymbol{H_B}$), 3.83 (3H, s, CO_2***Me***), 4.80 (1H, d, *J* = 9.0 Hz, C4-H$_A$$\boldsymbol{H_B}$), 5.08 (1H, d, *J* = 0.8 Hz, C2-***H***); **^{13}C NMR (100 MHz, $CDCl_3$):** 24.7 (C(**C**H$_3$)$_3$), 35.6 (**C**(CH$_3$)$_3$), 44.8 (C7), 53.7 (CO_2**C**H$_3$), 63.2 (C4), 80.6 (C5), 96.7 (C2), 166.3 (**C**O_2CH$_3$), 172.4 (C8), 198.4 (C6); **m/z (ESI$^-$):** 254.1 ([M-H]$^-$); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = +109.6 (c = 1.1 in $CHCl_3$) (Lit. +100.3, c = 2.2 in $CHCl_3$).[47]

Data are in agreement with those found in the literature.[47]

Methyl-(3*R*,7a*R*)-3-(tert-butyl)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]thiazole-7a(5H)-carboxylate 9s



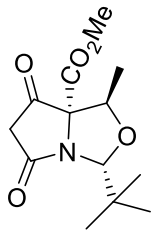
Synthesised according to general procedure C.

Yield 2.06 g, 78 %.

R_f: 0.40 (3:1 EtOAc:MeOH); **M.p:** 111-113 °C (Lit. 114 – 116 °C)[133]; **^{1}H NMR (400 MHz, $CDCl_3$):** 0.95 (9H, s, (C$\boldsymbol{H_3}$)$_3$), 3.01 (1H, d, *J* = 11.5 Hz, C4-$\boldsymbol{H_A}$H$_B$), 3.13 (1H, d, *J* = 21.4 Hz, C7-$\boldsymbol{H_A}$H$_B$), 3.62 (1H, d, *J* = 21.4 Hz, C7-H$_A$ $\boldsymbol{H_B}$), 3.78 (1H, d, *J* = 11.6 Hz, C4-H$_A$$\boldsymbol{H_B}$), 3.82 (3H, s, O***Me***), 5.32 (1H, s, C2-***H***); **^{13}C NMR (100 MHz, $CDCl_3$):** 26.4 (C(**C**H$_3$)$_3$ 33.9 (C4), 37.2 (**C**(CH$_3$)$_3$), 42.9 (C7), 53.9 (CO_2**C**H$_3$), 73.7 (C2), 84.9 (C5), 167.1 (**C**O_2CH$_3$), 170.6 (C8), 199.5 (C6); **m/z (ESI$^-$):** 270.1 ([M-H]$^-$); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = + 147.3 (c = 1.0 in DCM) (Lit. + 153.3, c = 1.0 in DCM)[133].

Data are in agreement with those found in the literature.[133]

Methyl-(1*R*,3*R*,7a*R*)-3-(tert-butyl)-1-methyl-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 9t



Synthesised according to general procedure C.

Yellow solid.

Yield 2.32 g, 59 %.

R_f: 0.35 (1:6 MeOH:EtOAc); **M.p:** 129 - 130 °C (Lit. 132 – 134 °C)[133]; **^{1}H NMR (400 MHz, $CDCl_3$):** 0.89 (9H, s, (C***H*₃**)₃), 1.04 (3H, d, *J* = 6.7 Hz, C4-***Me***), 3.14 (1H, d, *J* = 21.7 Hz, C7-***H*$_A$**H$_b$), 3.64 (1H, d, *J* = 21.7 Hz, C7-H$_A$***H*$_B$**), 3.80 (3H, s, O***Me***), 5.08 (1H, q, *J* = 6.7Hz, C4-***H***), 5.12 (1H, s, C2-***H***); **^{13}C NMR (100 MHz, $CDCl_3$):** 15.1 (C4-**C**H$_3$), 24.7 (C(**C**H$_3$)$_3$), 35.5 (**C**(CH$_3$)$_3$), 45.2 (C7), 53.6 (CO$_2$**C**H$_3$), 75.0 (C4), 83.6 (C5), 95.9 (C2), 167.1 (**C**O$_2$CH$_3$), 172.9 (C8), 199.4 (C6); **m/z (ESI$^+$):** 268.1 ([M-H]$^-$); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = +53.1 (c = 1.0 in DCM) (Lit. + 50.0, c = 1.0 in DCM).[133]

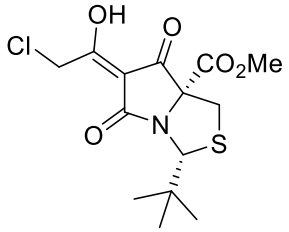
Data are in agreement with those found in the literature.[133]

General procedure D. C7-Acylation of tetramic acids by DCC coupling.

Tetramic acid (1.0 eq), DCC (1.1 eq) and DMAP (1.3 eq) were dissolved in anhydrous DCM and cooled to 0 °C. The corresponding carboxylic acid (1.1 eq) was added portion wise over 30 min. Once added, the mixture was allowed to warm to rt and was stirred for 18 – 24 h. Once complete, the reaction mixture was filtered, and the precipitate was washed with DCM.

The combined filtrates were concentrated *in vacuo* and the resulting residue was purified by column chromatography to yield the C7-acylated tetramic acid.

Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(2-chloro-1-hydroxyethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]thiazole-7a(5H)-carboxylate 64s



Synthesised according to general procedure D.

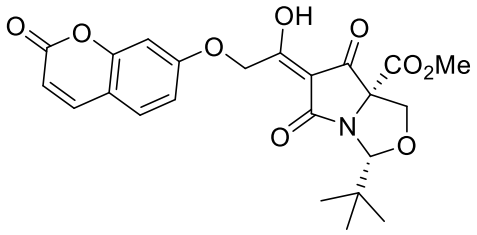
Dark orange gummy solid.

Yield 476 mg, 30 %.

R_f: 0.60 (1:19 EtOAc:MeOH); **^{1}H NMR (400 MHz, $CDCl_3$):** 0.97 (9H, s, C(C**H_3**)$_3$), 2.87 (1H, d, *J* = 11.5 Hz, C4-***H_A***H$_B$), 3.64 – 3.88 (4H, m, CO_2***Me*** & C4-H$_A$***H_B***), 4.50 – 4.64 (2H, m, C3'-***H_AH_B***), 5.10 (1H, s, C2-H); **^{13}C NMR (100 MHz, $CDCl_3$):** 26.5 (C(**C**H$_3$)$_3$), 29.0 (**C**(CH$_3$)$_3$), 33.1 (C4), 36.8 (C3'), 53.6 (CO_2**Me**), 73.6 (C2), 79.2 (C5), 100.3 (C7), 176.7 (C8), 187.8 (C2'), 189.2 (C6); **ν_{max}/cm^{-1}:** 1750 (C=O), 1619 (br, C=C & C=O); **m/z (ESI$^-$):** 346.0 ([M-H]$^-$); **HRMS (ESI$^-$):** Found 346.0522, $C_{14}H_{17}ClNO_5S^-$ requires 346.0521; **$[\alpha]_D^{25}$:** +115 (c = 1.0 in MeOH).

Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-((2-oxo-2H-chromen-7-yl)oxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate

66o

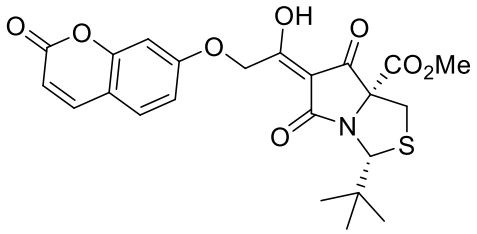


Chloro-substituted tetramate **64o** (69 mg, 0.208 mmol), 7-hydroxycoumarin (51 mg, 0.313 mmol) and Cs_2CO_3 (136 mg, 0.416 mmol) were dissolved in dry acetonitrile (5 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as an orange solid. Yield 52 mg, 55 %.

R_f: 0.55 (1:19 MeOH:EtOAc); **M.p:** 105 – 106 °C; **^{1}H NMR (400 MHz, MeOD):** 0.90 (9H, s, C(C***H*****$_3$**)$_3$), 3.47 (1H, d, *J* = 8.5 Hz, C4-***H*****$_A$**H$_B$), 3.76 (3H, s, CO_2***Me***), 4.72 (1H, d, *J* = 8.5 Hz, C4-H$_A$***H*****$_B$**), 4.77 (1H, s, C2-***H***), 5.07 (1H, d, *J* = 17.0 Hz, C3'-***H*****$_A$**H$_B$), 5.22 (1H, d, *J* = 17.0 Hz, C3'-H$_A$***H*****$_B$**), 6.22 (1H, d, *J* = 9.5 Hz, C9'-***H***), 6.83 (1H, d, *J* = 2.2 Hz, C6'-***H***), 6.93 (1H,dd, *J* = 8.7, 2.2 Hz, C12'-***H***), 7.51 (1H, d, *J* = 8.6 Hz, C11'-***H***), 7.86 (1H, d, *J* = 9.5 Hz, C10'-***H***); **^{13}C NMR (100 MHz, MeOD):** 25.4 (C(**C**H$_3$)$_3$), 36.2 (**C**(CH$_3$)$_3$), 53.2 (CO_2**Me**), 70.0 (C4), 72.6 (C3'), 98.9 (C2), 100.1 (C7), 102.8 (C6'), 112.3 (q**C**), 113.3 (C9'), 114.1 (C12'), 130.3 (C11'), 145.7 (C10'), 156.9 (qC), 163.3 (C8'), 163.6 (q**C**), 171.6 (**C**O_2Me), 182.0 (C8), 189.4 (C6), 191.8 (C2'); **ν_{max}/cm^{-1}:** 2980, 1692 (C=O), 1609 (C=O), 1557, 1508,

1456; **m/z (ESI$^-$):** 456.1 ([M-H]$^-$); **HRMS (ESI$^+$):** Found 480.1266, $C_{23}H_{23}NO_9Na^+$ requires 480.1265; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$**:** + 93.7 (c = 1.0 in MeOH).

Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-((2-oxo-2H-chromen-7-yl)oxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]thiazole-7a(5H)-carboxylate 66s

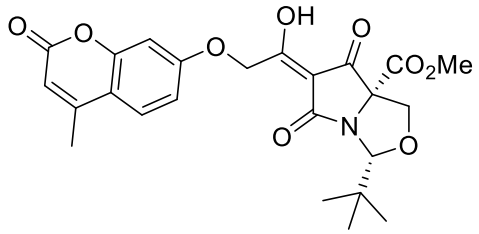


Chloro-substituted **64s** (46 mg, 0.132 mmol), 7-hydroxycoumarin (32 mg, 0.198 mmol) and Cs_2CO_3 (86 mg, 0.264 mmol) were dissolved in dry acetonitrile (5 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as an orange oil. 10 mg, 16 %.

R_f: 0.60 (1:19 MeOH:EtOAc); **^{1}H NMR (400 MHz, MeOD):** 0.95 (9H, s, C(C$\boldsymbol{H_3}$)$_3$), 2.87 (1H, d, *J* = 11.2 Hz, C4-$\boldsymbol{H_A}$H$_B$), 3.72 (1H, d, *J* = 11.2 Hz, C4-H$_A$$\boldsymbol{H_B}$), 3.75 (3H, s, CO$_2$***Me***), 5.10 (1H, s, C2-***H***), 5.05 (1H, d, *J* = 16.9 Hz, C3'-$\boldsymbol{H_A}$H$_B$), 5.20 (1H, d, *J* = 17.0 Hz, C3'-H$_A$$\boldsymbol{H_B}$), 6.23 (1H, d, *J* = 9.4 Hz, C9'-***H***), 6.84 (1H, d, *J* = 2.3 Hz, C12'-***H***), 6.94 (1H, dd, *J* = 8.7, 2.3 Hz, C6'-***H***), 7.52 (1H, d, *J* = 8.6 Hz, C7'-***H***), 7.87 (1H, d, *J* = 9.4 Hz, C8'-***H***); **^{13}C NMR (125 MHz, MeOD):** 27.1 (C(**C**H$_3$)$_3$), 34.8 (C4), 37.8 (**C**(CH$_3$)$_3$), 53.2 (CO$_2$**Me**), 72.6

(C3'), 74.4 (C2), 83.4 (C5), 98.3 (C7), 102.9 (C6'), 113.3 (C9'), 114.1 (qC), 114.2 (C12'), 130.3 (C11'), 145.8 (C10'), 157.0 (qC), 163.4 (C8'), 163.8 (qC), 171.6 (**C**CO_2Me), 179.8 (C8), 189.1 (C6), 192.2 (C2'); **ν_{max}/cm^{-1}:** 3056, 2954, 1734 (C=O), 1689 (C=O), 1611 (C=O); **m/z (ESI$^-$):** 472.0 ([M-H]$^-$); **HRMS (ESI$^-$):** Found 472.1069, $C_{23}H_{22}NO_8S^-$ requires 472.1072; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{25}}$**:** +81.5 (c = 1.0 in MeOH).

Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 67o

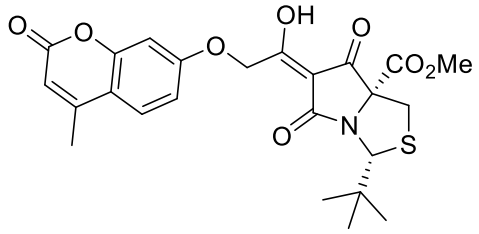


Chloro-substituted **64o** (60 mg, 0.181 mmol), 4-methylumbelliferone (47.9 mg, 0.272 mmol) and Cs_2CO_3 (118 mg, 0.362 mmol) were dissolved in dry acetonitrile (5 mL). Reaction mixture was heated to reflux for 24 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as an orange solid. 37 mg, 43 %.

R_f = 0.50 (19:1 EtOAc:MeOH); **M.p:** 113-115 °C; **^{1}H NMR (400 MHz, MeOD):** 0.90 (9H, s, C(C***H*****$_3$**)$_3$), 2.42 (3H, s, C***H*****$_3$**), 3.46 (1H, d, *J* = 8.5 Hz, C4-***H*****$_A$**H$_B$), 3.75 (3H, s, CO_2***Me***), 4.71 (1H, d, *J* = 8.4 Hz, C4-H$_A$***H*****$_B$**), 4.76 (1H, s, C2-***H***), 5.04 (1H, d, *J* = 17.1 Hz, C3'-***H*****$_A$**H$_B$), 5.19 (1H, d, *J* = 17.1 Hz, C3'-H$_A$***H*****$_B$**), 6.12 (1H, s, C9'-***H***), 6.79 (1H, d, *J* = 2.3 Hz, C6'-***H***),

6.94 (1H, dd, *J* = 8.8, 2.2 Hz, C12'-***H***), 7.63 (1H, d, *J* = 8.8 Hz, C11'-***H***); **^{13}C NMR (100 MHz, MeOD):** 18.7 (**C**CH_3), 25.4 (C(**C**H_3)$_3$), 36.2 (**C**$(CH_3)_3$), 53.2 (CO_2**Me**), 70.0 (C4), 72.5 (C3'), 78.6 (C5), 98.9 (C2), 100.2 (C7), 102.9 (C6'), 112.1 (C9'), 113.9 (C12'), 114.9 (qC), 127.2 (C11'), 155.6 (qC), 156.2 (qC), 163.5 (qC), 171.6 (**C**O_2Me), 181.9 (C8), 189.4 (C6), 191.7 (C2'); **ν_{max}/cm^{-1}:** 2980, 1690 (C=O), 1612 (C=O), 1460, 1392; **m/z (ESI$^-$):** 470.1 ([M-H]$^-$); **HRMS (ESI):** Found 472.1601, $C_{24}H_{26}NO_9^+$ requires 472.1602; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$**:** +66.1 (c = 1.0 in MeOH).

Methyl(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]thiazole-7a(5H)-carboxylate 67s

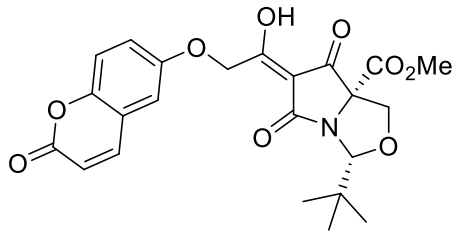


Chloro-substituted **64s** (60 mg, 0.181 mmol), 4-methylumbelliferone (47.9 mg, 0.272 mmol) and Cs_2CO_3 (118 mg, 0.362 mmol) were dissolved in dry acetonitrile (5 mL). Reaction mixture was heated to reflux for 24 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as an orange solid. Yield 26 mg, 35 %.

R_f: 0.55 (19:1 EtOAc:MeOH); **M.p:** 119 - 120 °C; **^{1}H NMR (400 MHz, MeOD):** 0.95 (9H, s, C(C***H*****$_3$**)$_3$), 2.42 (3H, s, C***H*****$_3$**), 2.86 (1H, d, *J* = 11.1 Hz, C4-***H***$_A$H$_B$), 3.71 (1H, d, *J* = 11.2 Hz,

C4-H_A***H_B***), 3.75 (3H, s, CO_2***Me***), 5.09 (1H, s, C2-***H***), 5.05 (1H, d, *J* = 16.8 Hz, C3'-***H_A***H_B), 5.18 (1H, d, *J* = 16.8 Hz, C3'-H_A***H_B***), 6.13 (1H, s, C9'-***H***), 6.81 (1H, m, C6'-***H***), 6.95 (1H, m, C12'-***H***), 7.64 (1H, d, *J* = 8.7 Hz, C7'-***H***); **^{13}C NMR (100 MHz, MeOD):** 18.7 (CH_3), 27.1 (C(**C**H_3)$_3$), 34.9 (C4), 37.7 (**C**(CH_3)$_3$), 53.3 (CO_2***Me***), 72.5 (C3'), 74.5 (C2), 83.4 (C5), 98.4 (C7), 102.9 (C6'), 112.1 (C9'), 113.9 (C12'), 114.9 (qC), 127.2 (C11'), 155.7 (qC), 156.2 (qC), 163.5 (qC), 171.6 (**C**O_2Me), 179.6 (C8), 189.1 (C6), 192.1 (C2'); **ν_{max}/cm^{-1}:** 1688 (C=O), 1612 (C=O), 1457; **m/z (ESI$^-$):** 486.0 ([M-H]$^-$); **HRMS (ESI$^+$):** Found 488.1385, $C_{24}H_{26}NO_8S^+$ requires 488.1374; $[\alpha]_D^{25}$**:** +77.4 (c = 1.0 in MeOH).

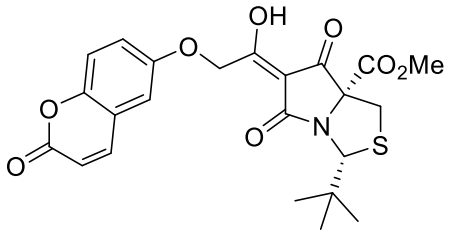
Methyl(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-((2-oxo-2H-chromen-6-yl)oxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 68o



Chloro-substituted **64o** (80 mg, 0.242 mmol), 6-hydroxycoumarin (59 mg, 0.362 mmol) and Cs_2CO_3 (158 mg, 0.484 mmol) were dissolved in dry acetonitrile (5 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as yellow solid. Yield 41 mg, 37 % yield.

R_f: 0.60 (1:19 MeOH:EtOAc); **M.p:** 98 – 100 °C; **^{1}H NMR (MeOD, 400 MHz):** 0.90 (9H, s, C(C***H*****$_3$**$)_3$), 3.50 (1H, d, *J* = 8.4 Hz, C4-***H*****$_A$**H_B), 3.76 (3H, s, CO_2***Me***), 4.73 (1H, d, *J* = 8.5 Hz, C4-H_A***H*****$_B$**), 4.78 (1H, s, C2-***H***), 5.06 (1H, d, *J* = 17.2 Hz, C3'-***H*****$_A$**H_B), 5.21 (1H, d, *J* = 17.2 Hz, C3'-H_A***H*****$_B$**), 6.41 (1H, d, *J* = 9.5 Hz, C8'-***H***), 7.09 – 7.30 (3H, m, Ar-***H***), 7.89 (1H, d, *J* = 9.5 Hz, C7'-***H***); **^{13}C NMR (MeOD, 100 MHz):** 25.4 (C(**C**$H_3)_3$), 36.2 (**C**$(CH_3)_3$), 53.3 (CO_2**Me**), 69.9 (C4), 99.0 (C2), 112.6 (C8'), 117.3 (ArC), 118.5 (ArC), 120.7 (C6''), 121.5 (ArC), 145.6 (C7'), 149.7 (q**C**), 156.7 (q**C**), 163.1 (C9'), 171.1 (**C**O_2Me), 181.3 (C8), 189.7 (C6), 191.0 (C2'); **ν_{max}/cm^{-1}:** 1720 (C=O), 1689 (C=O), 1622 (C=O), 1570 (C=O); **m/z (ESI$^-$):** 456.0 ([M-H]$^-$); **HRMS (ESI$^+$):** Found 458.1447, $C_{23}H_{24}NO_9^+$ requires 458.1446; **$[\alpha]_D^{25}$:** +81.5 (c = 1.0 in MeOH).

Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-((2-oxo-2H-chromen-6-yl)oxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]thiazole-7a(5H)-carboxylate 68s

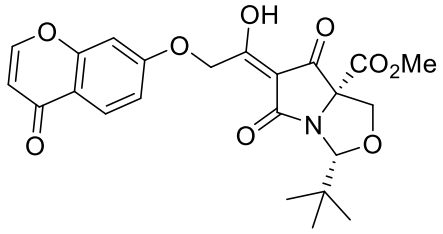


Chloro-substituted **64s** (72mg, 0.207 mmol), 6-hydroxycoumarin (50 mg, 0.311 mmol) and Cs_2CO_3 (135 mg, 0.414 mmol) were dissolved in dry acetonitrile (5 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as an orange solid. Yield 27 mg, 27 %.

R_f: 0.55 (1:19 MeOH:EtOAc); **M.p:** 107 – 108 °C; **^{1}H NMR (MeOD, 400 MHz):** 0.95 (9H, s, C(C**H**$_3$)$_3$), 2.86 (1H, d, *J* = 11.2 Hz, C4-***H***$_A$H$_B$), 3.71 (1H, d, *J* = 11.2 Hz, C4-H$_A$***H***$_B$), 3.74 (3H, s, CO$_2$***Me***), 5.09 (1H, s, C2-***H***), 5.00 (1H, d, *J* = 17.0 Hz, C3'-***H***$_A$H$_B$), 5.16 (1H, d, *J* = 17.1 Hz, C3'-H$_A$***H***$_B$), 6.40 (1H, d, *J* = 9.5 Hz, C8'-***H***), 7.09 (1H, d, *J* = 2.5 Hz, C6'-***H***), 7.19 – 7.27 (2H, m, C11'-***H*** & C12'-***H***), 7.88 (1H, d, *J* = 9.5 Hz, C7'-***H***); **^{13}C NMR (MeOD, 100 MHz):** 27.1 (C(**C**H$_3$)$_3$), 34.8 (C4), 37.7 (**C**(CH$_3$)$_3$), 53.3 (CO$_2$**Me**), 72.6 (C3'), 74.4 (C2), 83.4 (C5), 98.5 (C7), 112.6 (C6'), 117.3 (C8'), 118.5 (ArC), 120.7 (C6''), 121.5 (ArC), 145.7 (C7'), 149.7 (C10''), 156.8 (C5'), 163.1 (C9'), 171.5 (**C**O$_2$Me), 179.5 (C8), 189.6 (C6), 191.9 (C2'); **m/z (ESI$^-$):** 472.1 ([M-H]$^-$); **HRMS (ESI$^+$):** Found 474.1217, $C_{23}H_{24}NO_8S^+$ requires 474.1218; **ν_{max}/cm^{-1}:** 2955, 1685 (C=O), 1568 (C=O), 1456 (C=O), 1261. **$[\alpha]_D^{25}$:** +204.6 (c = 1.0 in MeOH).

Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-((4-oxo-4H-chromen-7-yl)oxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate

69o

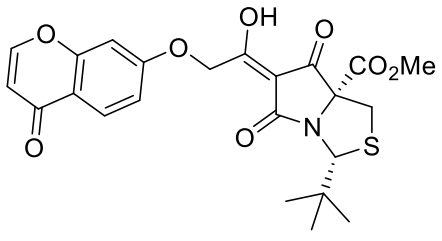


Chloro-substituted **64o** (110 mg, 0.332 mmol), 7-hydroxy-4-chromone (81 mg, 0.498 mmol) and Cs_2CO_3 (215 mg, 0.664 mmol) were dissolved in dry acetonitrile (5 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of

ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as a yellow solid. 24 mg, 16 %.

R_f: 0.60 (1:19 MeOH:EtOAc); **M.p:** 111 – 112 °C; **^{1}H NMR (400 MHz, MeOD):** 0.90 (9H, s, C(C**H$_3$**)$_3$), 3.48 (1H, d, *J* = 8.5 Hz, C4-***H$_A$***H$_B$), 3.75 (3H, s, CO_2***Me***), 4.72 (1H, d, *J* = 8.5 Hz, C4-H$_A$***H$_B$***), 4.77 (1H, s, C2-***H***), 5.12 (1H, d, *J* = 17.1 Hz, C3'-***H$_A$***H$_B$), 5.27 (1H, d, *J* = 17.1 Hz, C3'-H$_A$***H$_B$***), 6.28 (1H, d, *J* = 6.0 Hz, C9'-***H***), 6.97 (1H, d, *J* = C6'-***H***), 7.09 (1H, d, *J* = 9.0, 2.3 Hz, C11'-***H***), 8.02 (1H, d, *J* = 8.9 Hz, C10'-***H***), 8.08 (1H, d, *J* = 6.0 Hz, C8'-***H***); **^{13}C NMR (100 MHz, MeOD):** 25.4 (C(**C**H$_3$)$_3$), 36.2 (**C**(CH$_3$)$_3$), 53.2 (CO_2**Me**), 69.9 (C4), 72.4 (C3'), 98.9 (C2), 100.5 (C7), 102.6 (C6'), 113.0 (C9'), 116.6 (C11'), 119.3 (qC), 127.6 (C10'), 158.0 (C8'), 159.9 (qC), 165.3 (qC), 171.4 (**C**O_2Me), 179.4 (C8), 188.9 (C6), 191.4 (C2'); **m/z (ESI$^-$):** 456.0 ([M-H]$^-$); **HRMS (ESI$^+$):** Found 458.1444, $C_{23}H_{24}NO_9^+$ requires 458.1446; **ν_{max}/cm^{-1}:** 2959, 1742 (C=O), 1688 (C=O), 1623 (C=O), 1444; **$[\alpha]_D^{25}$:** +82.6 (c = 0.5 in MeOH).

Methyl(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-((4-oxo-4H-chromen-7-yl)oxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]thiazole-7a(5H)-carboxylate 69s

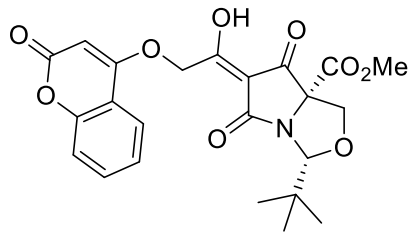


Chloro-substituted **64s** (57 mg, 0.164 mmol), 7-hydroxy-4-chromone (40 mg, 0.246 mmol) and Cs_2CO_3 (107 mg, 0.328 mmol) were dissolved in dry acetonitrile (5 mL). Reaction mixture was heated to reflux for 24 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic

layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as a yellow oil. Yield 26 mg, 33 %.

R_f: 0.60 (1:19 MeOH:EtOAc); **^{1}H NMR (400 MHz, MeOD):** 0.95 (9H, s, C(C***H*****$_3$**)$_3$), 2.88 (1H, d, *J* = 11.2 Hz, C4-***H*****$_A$**H$_B$), 3.72 (1H, d, *J* = 11.2 Hz, C4-H$_A$***H*****$_B$**), 3.75 (3H, s, CO_2***Me***), 5.10 (1H, s, C2-***H***), 5.11 (1H, d, *J* = 16.9 Hz, C3'-***H*****$_A$**H$_B$), 5.17 (1H, d, *J* = 16.9 Hz, C3'-H$_A$***H*****$_B$**), 6.28 (1H, d, *J* = 5.9 Hz, C9'-***H***), 6.97 (1H, d, *J* = 2.2 Hz, C6'-***H***), 7.09 (1H, dd, *J* = 8.9, 2.3 Hz, C12'-***H***), 8.02 (1H, d, *J* = 8.9 Hz, C11'-***H***), 8.08 (1H, d, *J* = 6.0 Hz, C8'-***H***); **^{13}C NMR (100 MHz, MeOD):** 27.1 (C(**C**H$_3$)$_3$), 34.8 (C4), 37.7 (**C**(CH$_3$)$_3$), 53.0 (CO_2**Me**), 74.4 (C2), 83.3 (C5), 98.5 (C7), 102.6 (C6'), 113.0 (C9'), 116.6 (C11'), 119.3 (qC), 127.6 (C10'), 158.0 (C8'), 159.9 (qC), 165.3 (qC), 171.5 (**C**O_2Me), 179.4 (C8), 188.6 (C6), 191.9 (C2'); **ν_{max}/cm^{-1}:** 2956, 1742 (C=O), 1686 (C=O), 1624 (C=O), 1444; **m/z (ESI$^-$):** 472.0 ([M-H]$^-$); **HRMS (ESI$^+$):** Found 474.1215, $C_{23}H_{24}NO_8S^+$ requires 474.1217; **$[\alpha]_D^{25}$:** +185.8 (c = 1.0 in MeOH).

Methyl(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-((2-oxo-2H-chromen-4-yl)oxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 70o

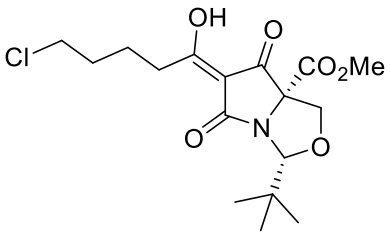


Chloro-substituted **64o** (94 mg, 0.280 mmol), 4-hydroxycoumarin (46 mg, 0.280 mmol) and Cs_2CO_3 (111 mg, 0.340 mmol) were dissolved in dry acetonitrile (5 mL). Reaction mixture

was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as a yellow oil. Yield 74 mg, 58 %.

R_f: 0.60 (1:19 MeOH:EtOAc); **^{1}H NMR (200 MHz, MeOD):** 0.90 (9H, s, C(C***H*****$_3$)$_3$), 3.47 (1H, d, *J* = 8.4 Hz, C4-***H*****$_A$**H$_B$), 3.75 (3H, s, CO_2***Me***), 4.72 (1H, d, *J* = 8.4 Hz, C4-H$_A$***H*****$_B$**), 4.77 (1H, s, C2-***H***), 5.24 (1H, d, *J* = 16.6 Hz, C3'-***H*****$_A$**H$_B$), 5.37 (1H, d, *J* = 16.6 Hz, C3'-H$_A$***H*****$_B$**), 6.84 – 6.94 (1H, m, Ar***H***), 7.41 – 7.51 (1H, m, Ar***H***), 7.82 – 8.06 (2H, m, Ar***H***); **ν_{max}/cm^{-1}:** 2850 – 3300, 1686 (C=O), 1620 (C=O), 1564; **m/z (ESI$^-$):** 456.0 ([M-H]$^-$); **HRMS (ESI$^+$):** Found 458.1444, $C_{23}H_{24}NO_9^+$ requires 458.1446; **$[\alpha]_D^{25}$:** +67.8 (c = 0.4 in MeOH).

Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(5-chloro-1-hydroxypentylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 72



Synthesised according to general procedure D.

Orange oil.

Yield 686 mg, 33 %.

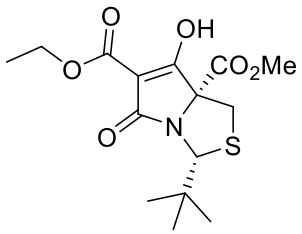
R_f: 0.50 (1:9 MeOH:EtOAc); **^{1}H NMR (400 MHz, MeOD):** 0.91 (9H, s, C(C***H*****$_3$)$_3$), 1.82 – 1.86 (4H, m, C10-***H*** & C11-***H***), 2.89 – 2.96 (2H, m, C9-***H***), 3.56 – 3.61 (3H, m, C4-***H*****$_A$**H$_B$ &

C12-***H***), 3.76 (3H, s, CO_2***Me***), 4.72 (1H, d, *J* = 8.7 Hz, C4-H_A***H_B***), 4.86 (1H, s, C2-***H***); **^{13}C NMR (100 MHz, MeOD):** 24.2 & 33.0 (C10 & C11), 25.2 (C(**C**H_3)$_3$), 33.1 (C9), 36.1 (**C**$(CH_3)_3$), 45.1 (C12), 53.8 (CO_2***Me***), 69.0 (C4), 79.2 (C5), 99.4 (C2), 103.1 (C7), 169.2 (**C**O_2Me), 179.6 (C8), 190.7 (C6), 192.3 (C2'); **ν_{max}/cm^{-1}:** 2959, 1755 (C=O), 1717 (C=O), 1658 (C=O), 1602, 1286; **m/z (ESI$^-$):** 374.0 ($[M-H]^-$); **HRMS (ESI$^+$):** Found 374.1366, $C_{17}H_{25}NO_6Cl^-$ requires 374.1365; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$**:** +59.6 (c = 1.0 in MeOH).

General procedure E. C7-acylation using chloroformate.

Tetramic acid **9o/9s**, corresponding chloroformate, and DMAP were dissolved in anhydrous DCM. The reaction mixture was stirred at rt for 18 - 24 h until complete consumption of starting materials by LRMS. Once complete, the solvent was removed *in vacuo* and the resulting mixture was dissolved in EtOAc then washed with 2M HCl. The organic layers were combined and concentrated *in vacuo* to yield the crude product, which was purified by *flash* column chromatography. Once purified, the product was dissolved in EtOAc, washed with 2M HCl, then a saturated solution of ammonium chloride. The organic layers were combined, dried with $MgSO_4$ and concentrated *in vacuo* to yield the title compound.

6-ethyl-7a-methyl(3*R*,7a*R*)-3-(tert-butyl)-7-hydroxy-5-oxo-1H,3H-pyrrolo[1,2-c]thiazole-6,7a(5H)-dicarboxylate 76



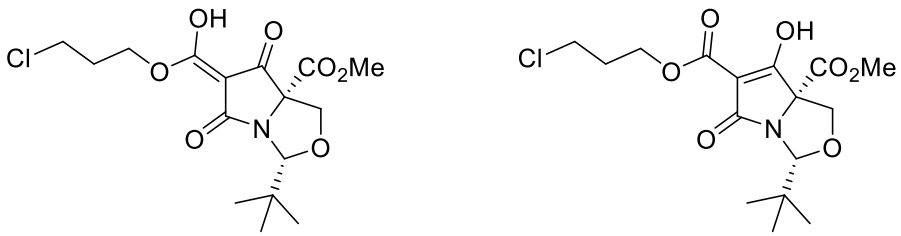
Synthesised according to general procedure E.

Orange oil.

Yield 134 mg, 24 %.

R_f: 0.35 (1:19 MeOH:EtOAc); **^{1}H NMR (400 MHz, $CDCl_3$):** 1.00 (9H, s, C(C***H*****$_3$**)$_3$), 1.39 (3H, t, *J* = 7.1 Hz, CH_2C***H*****$_3$**), 2.84 (1H, d, *J* = 11.4 Hz, C4-***H*****$_A$**H_B), 3.75 (1H, d, *J* = 11.4 Hz, C4-H_A***H*****$_B$**), 3.80 (3H, s, CO_2***Me***), 4.38 (2H, q, *J* = 7.1 Hz, C***H*****$_2$**CH_3), 4.99 (1H, s, C2-***H***); **^{13}C NMR (100 MHz, $CDCl_3$):** 15.9 (CH_2**C**H_3), 26.3 (C(**C**H_3)$_3$), 33.8 (**C**(CH_3)$_3$), 36.4 (**C**(CH_3)$_3$), 53.2 (CO_2***Me***), 66.4 (**C**H_2CH_3), 71.1 (C2), 78.8 (C5), 94.1 (C7), 163.9 (**C**O_2Et), 170.0 (**C**O_2Me), 176.2 (C8), 185.9 (C6); **ν_{max}/cm^{-1}:** 1750 (C=O), 1702 (C=O), 1617 (C=O); **m/z (ESI$^-$):** 342.0 ([M-H]$^-$); **HRMS (ESI$^-$):** Found 342.1016, $C_{15}H_{20}NO_6S^-$ requires 342.1017; **$[\alpha]_D^{25}$:** +145.0 (c = 1.0 in DCM).

Methyl-(3*R*,7a*R*,*Z*)-3-(tert-butyl)-6-((3-chloropropoxy)(hydroxy)methylene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 77o



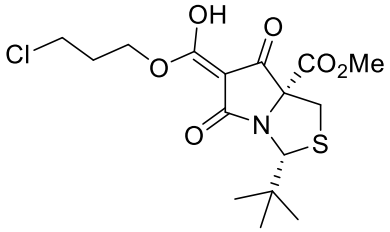
Synthesised according to general procedure E.

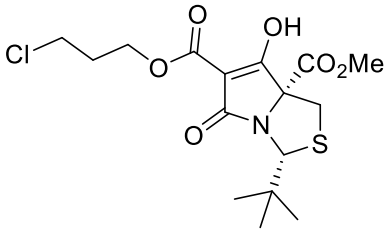
Serine tetramate **9o** (317 mg, 1.24 mmol), 3-chloropropyl chloroformate (0.22 mL, 1.86 mmol) and DMAP (334 mg, 2.73 mmol) were dissolved in anhydrous DCM (15 mL). Once purified, the product was dissolved in EtOAc, washed with 2M HCl then a saturated solution of ammonium chloride. Organic layers were combined, dried with $MgSO_4$ and concentrated *in vacuo* to yield the title compound as an orange oil. Yield 135 mg, 29 %.

Product was isolated as a mixture of two enolic forms. **R_f:** 0.45 (1:19 MeOH:EtOAc); **^{1}H NMR (400 MHz, MeOD) (Major enolic form):** 0.89 (9H, s, C(C***H*****$_3$**)$_3$), 2.15 (2H, p, *J* = 6.3 Hz, C11-***H***), 3.51 (1H, d, *J* = 8.5 Hz, C4-***H*****$_A$**H$_B$), 3.71 (2H, t, *J* = 6.4 Hz, C12'-***H***), 3.77 (3H, s, CO_2***Me***), 4.30 – 4.36 (2H, m, C10-***H***), 4.71 (1H, s, C2-***H***), 4.76 (1H, d, *J* = 8.5 Hz, C4-H$_A$***H*****$_B$**); **(Minor enolic form):** 0.88 (9H, s, C(C***H*****$_3$**)$_3$), 2.07 – 2.14 (2H, m, C11-***H***), 3.46 (1H, d, *J* = 8.6 Hz, C4-***H*****$_A$**H$_B$), 3.63 – 3.70 (2H, m, C12-***H***), 3.77 (3H, s, CO_2***Me***), 4.22 – 4.29 (2H, m, C10-***H***), 4.62 (1H, s, C2-***H***), 4.76 (1H, d, *J* = 8.5 Hz, C4-H$_A$***H*****$_B$**); **^{13}C NMR (100 MHz, MeOD) (Major enolic form):** 25.3 (C(**C**H$_3$)$_3$), 32.8 (C11), 36.1 (**C**(CH$_3$)$_3$), 42.3 (C12), 53.7 (CO_2***Me***), 61.9 (C10), 70.0 (C4), 76.4 (C5), 97.0 (C7), 98.6 (C2), 169.8 (qC), 178.4 (qC), 185.3 (qC); **(Minor enolic form):** 25.2 (C(**C**H$_3$)$_3$), 32.7 (C11), 36.0 (**C**(CH$_3$)$_3$), 41.8 (C12), 55.3 (CO_2**Me**), 65.7 (C10), 70.6 (C4), 76.9 (C5), 97.0 (C7), 97.9 (C2), 170.0 (qC), 177.3

(qC), 182.3 (qC); **ν_{max}/cm^{-1}:** 1748 (C=O), 1717 (C=O), 1700 (C=O); **m/z (ESI$^-$):** 374.1 ([M-H]$^-$); **HRMS (ESI$^+$):** Found 374.1366, $C_{17}H_{24}O_6NCl^-$ requires 374.1365.

Methyl-(3*R*,7a*R*,*Z*)-3-(tert-butyl)-6-((3-chloropropoxy)(hydroxy)methylene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]thiazole-7a(5H)-carboxylate 77s





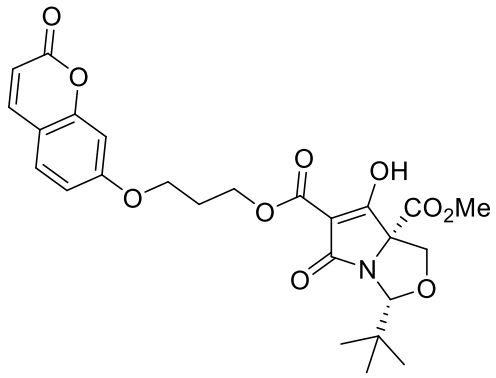
Synthesised according to general procedure D.

Cysteine tetramate **9s** (1.73 g, 6.38 mmol), 3-chloropropyl chloroformate (1.54 mL, 12.8 mmol) and DMAP (1.72 g, 14.0 mmol) were dissolved in anhydrous DCM (25 mL). Once purified, the product was dissolved in EtOAc, washed with 2M HCl then a saturated solution of ammonium chloride. Organic layers were combined, dried with $MgSO_4$ and concentrated *in vacuo* to yield the title compound as an orange oil. Yield 598 mg, 24 %.

Product was isolated as a mixture of two enolic forms. **R_f:** 0.45 (1:19 MeOH:EtOAc); **^{1}H NMR (400 MHz, MeOD) (Major enolic form):** 0.95 (9H, s, C(C***H*****$_3$**)$_3$), 2.16 (2H, p, *J* = 6.3 Hz, C11-***H***), 2.92 (1H, d, *J* = 11.3 Hz, C4-***H*****$_A$**H$_B$), 3.63 – 3.73 (2H, m, C12-***H*** & C4-H$_A$***H*****$_B$**), 3.78 (3H, s, CO_2***Me***), 4.35 (2H, t, *J* = 6.1 Hz, C10-***H***), 4.98 (1H, s, C2-***H***); (**Minor enolic form);** 0.93 (9H, s, C(C***H*****$_3$**)$_3$), 1.95 (2H, p, *J* = 6.3 Hz, C11-***H***), 2.86 (1H, d, *J* = 11.3 Hz, C4-***H*****$_A$**H$_B$), 3.63 – 3.73 (5H, m, C10-***H*** & C12-***H*** & C4-H$_A$***H*****$_B$**), 3.77 (CO_2***Me***), 4.87 (1H, s, C2-***H***), **^{13}C NMR (100 MHz, MeOD) (Major enolic form):** 25.6 (C(**C**H$_3$)$_3$), 31.4 (C11), 32.5 (C4), 36.3 (**C**(CH$_3$)$_3$), 40.9 (C12), 52.5 (CO_2**Me**), 60.8 (C10), 72.0 (C2), 79.3 (C5), 95.0 (C7), 163.2 (qC), 168.0 (qC), 173.9 (qC), 182.7 (qC); **(Minor enolic form);** 25.6 (C(**C**H$_3$)$_3$), 32.7 (C4), 35.1 (C11), 36.3 (**C**(CH$_3$)$_3$), 41.0 (C12), 52.3 (CO_2**Me**), 58.1 (C10), 71.3 (C2), 80.3

(C5), 95.0 (C7), 164.0 (qC), 168.6 (qC), 175.6 (qC), 182.7 (qC); **ν_{max}/cm^{-1}:** 2959, 1747 (C=O), 1717 (C=O), 1653 (C=O), 1559; **m/z (ESI$^-$):** 390.0 ([M-H]$^-$); **HRMS (ESI)$^+$:** Found 390.0783, $C_{16}H_{21}ClNO_6S^-$ requires 390.0773.

7a-methyl 6-(3-((2-oxo-2H-chromen-7-yl)oxy)propyl)-(3*R*,7a*R*)-3-(tert-butyl)-7-hydroxy-5-oxo-1H,3H-pyrrolo[1,2-c]oxazole-6,7a(5H)-dicarboxylate 78a

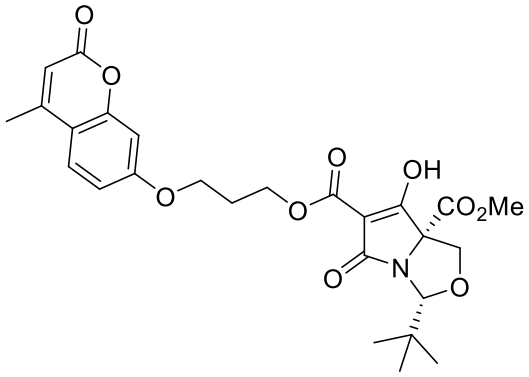


Chloro-substituted **77o** (128 mg, 0.342 mmol), Umbelliferone (83 mg, 0.514 mmol) and Cs_2CO_3 (334 mg, 1.026 mmol) were dissolved in dry acetonitrile (10 mL). Reaction mixture was heated to reflux for 48 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as an orange oil. Yield 29 mg, 17 %.

R_f = 0.25 (19:1 EtOAc:MeOH); **^{1}H NMR (400 MHz, MeOD):** 0.88 (9H, s, C(C***H*****$_3$**)$_3$), 2.22 (2H, p, *J* = 6.1 Hz, C4'-***H***), 3.54 (1H, d, *J* = 8.6 Hz, C4-***H*****$_A$**H$_B$), 3.79 (3H, s, CO_2***Me***), 4.22 (2H, t, *J* = 6.0 Hz) & 4.40 – 4.47 (2H, m) (C3'-***H*** & C5'-***H***), 4.69 (1H, s, C2-***H***), 4.77 (1H, d, *J* = 8.6 Hz, C4-H$_A$***H*****$_B$**), 6.22 (1H, d, *J* = 9.4 Hz, C11'-***H***), 6.89 – 6.94 (2H, m, C8'-***H*** & C14'-***H***), 7.50 (1H, d, *J* = 8.5 Hz, C13'-***H***), 7.86 (1H, d, *J* = 9.5 Hz, C12'-***H***); **^{13}C NMR (100 MHz, MeOD):** 25.2 (C(**C**H$_3$)$_3$), 29.4 (C4'), 36.1 (**C**(CH$_3$)$_3$), 53.9 (CO_2**Me**), 62.4 & 66.6 (C3'

& C5'), 70.0 (C4), 75.6 (C5), 98.6 (C2), 99.6 (C7), 102.4 & 114.1 (C8' & C14'), 113.4 (C11'), 130.4 (C13'), 145.7 (C12'), 157.0 (qC), 163.3 (qC), 163.8 (qC), 164.3 (qC), 169.0 (**C**CO_2Me), 176.6 (C8), 182.9 (C1'); **ν_{max}/cm^{-1}:** 2959, 1730 (C=O), 1614 (C=O), 1433, 1400, 1282; **m/z (ESI$^-$):** 500.1 ([M-H]$^-$); **HRMS (ESI$^+$):** Found 502.1708, $C_{25}H_{28}NO_{10}^+$ requires 507.1708; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$**:** +69.7 (c = 1.0 in MeOH).

7a-methyl 6-(3-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)propyl) (3*R*,7a*R*)-3-(tert-butyl)-7-hydroxy-5-oxo-1H,3H-pyrrolo[1,2-c]oxazole-6,7a(5H)-dicarboxylate 78b

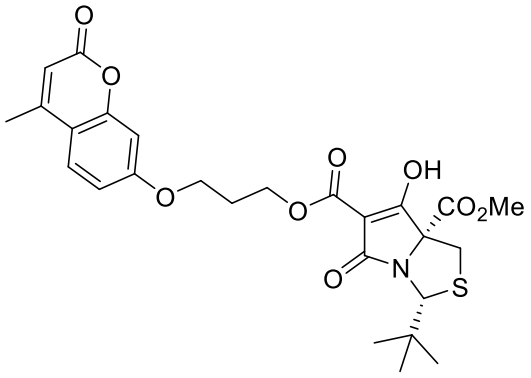


Chloro-substituted **77o** (150 mg, 0.399 mmol), 4-methylumbelliferone (105 mg, 0.599 mmol) and Cs_2CO_3 (390 mg, 1.197 mmol) were dissolved in dry acetonitrile (10 mL). Reaction mixture was heated to reflux for 48 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as an orange oil. 37 mg, 18 %.

R_f = 0.25 (19:1 EtOAc:MeOH); **^{1}H NMR (400 MHz, MeOD):** 0.88 (9H, s, C(C$\boldsymbol{H_3}$)$_3$), 2.20 (2H, p, *J* = 6.2 Hz, C4'-***H***), 2.41 (3H, d, *J* = 1.2 Hz, C12'-***Me***), 3.48 (1H, d, *J* = 8.5 Hz, C4-$\boldsymbol{H_A}H_B$), 3.75 (3H, s, CO_2***Me***), 4.21 (2H, t, *J* = 6.1 Hz) & 4.35 – 4.41 (2H, m) (C3'-***H*** & C5'-***H***), 4.69 (1H, s, C2-***H***), 4.74 (1H, d, *J* = 8.5 Hz, C4-$H_A\boldsymbol{H_B}$), 6.11 (1H, d, *J* = 1.3 Hz, C11'-***H***),

6.85 (1H, d, *J* = 2.4 Hz, C8'-***H***), 6.93 (1H, dd, *J* = 8.8, 2.4 Hz, C14'-***H***), 7.61 (1H, d, *J* = 8.8 Hz, C13'-***H***); **^{13}C NMR (100 MHz, MeOD):** 18.7 (C12'-**Me**), 25.3 (C(**C**H_3)$_3$), 29.6 (C4'), 36.1 (**C**(CH_3)$_3$), 53.6 (CO_2**Me**), 61.6 & 66.6 (C3' & C5'), 70.0 (C4), 76.6 (C5), 96.5 (C7), 98.6 (C2), 102.4 (C8'), 112.0 (C11'), 113.9(C14'), 114.7 (qC), 127.2 (C13'), 155.7 (qC), 156.3 (qC), 163.5 (qC), 163.7 (qC), 165.1 (qC), 170.1 (**C**O_2Me), 178.8 (C8), 185.9 (C2'); **ν_{max}/cm^{-1}:** 2959, 1703 (br, C=O), 1613 (C=O), 1435, 1390, 1283; **m/z (ESI$^-$):** 514.1 ([M-H]$^-$) **HRMS (ESI$^+$):** Found 516.1864, $C_{26}H_{30}NO_{10}^+$ requires 516.1864; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$**:** +71.1 (c = 1.0 in MeOH).

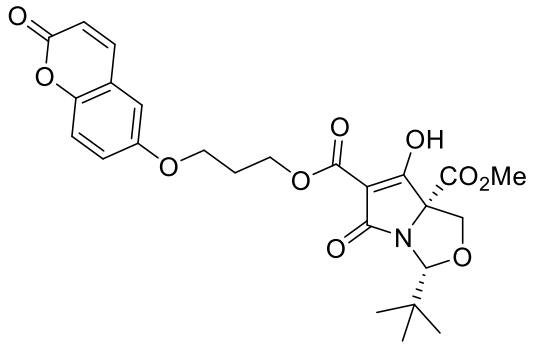
7a-methyl 6-(3-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)propyl) (3*R*,7a*R*)-3-(tert-butyl)-7-hydroxy-5-oxo-1H,3H-pyrrolo[1,2-c]thiazole-6,7a(5H)-dicarboxylate 79b



Chloro-substituted **77s** (151 mg, 0.385 mmol), 4-methylumbelliferone (102 mg, 0.578 mmol) and Cs_2CO_3 (377 mg, 1.16 mmol) were dissolved in dry acetonitrile (10 mL). Reaction mixture was heated to reflux for 48 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as pale orange oil. 66 mg, 32 %.

R_f = 0.25 (19:1 EtOAc:MeOH); **^{1}H NMR (400 MHz, MeOD):** 0.92 (9H, s, C(C***H*****$_3$**)$_3$), 2.20 (2H, p, *J* = 6.2 Hz, C4'-***H***), 2.41 (3H, d, *J* = 1.1 Hz, C12'-(C***H*****$_3$**), 2.85 (1H, d, *J* = 11.3 Hz, C4-***H*****$_A$**H$_B$), 3.67 (1H, d, *J* = 11.2 Hz, C4-H$_A$***H*****$_B$**), 3.74 (3H, s, CO_2***Me***), 4.20 (2H, t, *J* = 6.1 Hz) & 4.36-4.40 (2H, m) (C3' & C5'), 4.97 (1H, s, C2'-***H***), 6.11 – 6.12 (1H, m, C11'-***H***), 6.84 (1H, d, *J* = 2.4 Hz, C8'-***H***), 6.93 (1H, dd, *J* = 8.8, 2.2 Hz, C14'-***H***), 7.61 (1H, d, *J* = 8.7 Hz, C13'-***H***); **^{13}C NMR (100 MHz, MeOD):** 18.7 (C12'-**C**H$_3$), 27.0 (C(**C**H$_3$)$_3$), 29.6 (C4'), 34.2 (C4), 37.7 (**C**(CH$_3$)$_3$), 53.6 (CO_2**Me**), 61.6 & 66.7 (C3' & C5'), 73.7 (C2-***H***), 81.4 (C5), 94.2 (C7), 102.4 (C8'), 112.0 (C11'), 113.8 (C14'), 114.7(C12'), 127.2 (C13'), 155.6 (qC), 156.3 (qC), 163.5 (qC), 163.7 (qC), 170.1 (**C**O_2Me), 176.8 (C8), 186.2 (C2'); **ν_{max}/cm^{-1}:** 2955, 1703 (br, C=O), 1613 (C=O), 1434, 1391; **m/z (ESI$^-$):** 530.0 ([M-H]$^-$); **HRMS (ESI)$^+$:** Found 532.1636, $C_{26}H_{30}NO_9S^+$ requires 523.1636; **$[\alpha]_D^{25}$:** +124.4 (c = 1.0 in MeOH).

7a-methyl 6-(3-((2-oxo-2H-chromen-6-yl)oxy)propyl) (3*R*,7a*R*)-3-(tert-butyl)-7-hydroxy-5-oxo-1H,3H-pyrrolo[1,2-c]oxazole-6,7a(5H)-dicarboxylate 78c

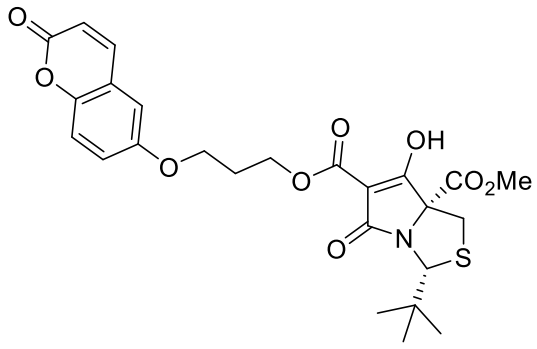


Chloro-substituted **77o** (185 mg, 0.492 mmol), 6-hydroxycoumarin (120 mg, 0.738 mmol) and Cs_2CO_3 (480 mg, 1.48 mmol) were dissolved in dry acetonitrile (15 mL). Reaction mixture was heated to reflux for 48 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude

product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as a yellow oil. 80 mg, 32 %.

R_f = 0.25 (19:1 EtOAc:MeOH); **^{1}H NMR (400 MHz, MeOD):** 0.89 (9H, s, C(C$\boldsymbol{H_3}$)$_3$), 2.20 (2H, p, *J* = 6.1 Hz, C4'-$\boldsymbol{H_A}$H$_B$), 3.52 (1H, d, *J* = 8.6 Hz, C4-H$_A$$\boldsymbol{H_B}$), 3.78 (3H, s, CO_2***Me***), 4.19 (2H, t, *J* = 6.1 Hz) & 4.37 – 4.45 (2H, m) (C3'-***H*** & C5'-***H***), 4.69 (1H, s, C2-***H***), 4.77 (1H, d, *J* = 8.6 Hz, C4-H$_A$$\boldsymbol{H_B}$), 6.41 (1H, d, *J* = 9.5 Hz, C10'-***H***), 7.15 – 7.21 (2H, m, Ar-***H***), 7.24 – 7.28 (1H, m, Ar-***H***), 7.93 (1H, d, *J* = 9.5 Hz, C9'-***H***); **^{13}C NMR (100 MHz, MeOD):** 25.2 (C(**C**H$_3$)$_3$), 29.6 (C4'), 36.1 (**C**(CH$_3$)$_3$), 53.8 (CO_2**Me**), 62.2 & 66.5 (C3' & C5'), 70.0 (C4), 75.9 (C5), 98.6 (C2), 112.2 (ArC), 117.3 (C10'), 118.6 (ArC), 120.8 (qC), 121.5 (ArC), 145.7 (C9'), 149.6 (qC), 157.0 (qC), 163.1 (qC), 164.6 (qC), 169.4 (**C**O_2Me), 177.3 (C8), 183.9 (C6), 186.0 (C2'); **ν_{max}/cm^{-1}:** 2959, 1724 (br, C=O), 1626 (C=O), 1571, 1439, 1262 **m/z (ESI$^-$):** 500.1 ([M-H]$^-$); **HRMS (ESI$^+$):** Found 502.1707, $C_{25}H_{28}NO_{10}^+$ requires 502.1708; **$[\alpha]_D^{25}$:** +57.7 (c = 1.0 in MeOH).

7a-methyl 6-(3-((2-oxo-2H-chromen-6-yl)oxy)propyl) (3*R*,7a*R*)-3-(tert-butyl)-7-hydroxy-5-oxo-1H,3H-pyrrolo[1,2-c]oxazole-6,7a(5H)-dicarboxylate 79c

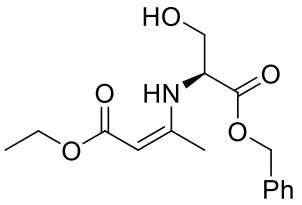


Chloro-substituted **77s** (145 mg, 0.370 mmol), 6-hydroxycoumarin (90mg, 0.555 mmol) and Cs_2CO_3 (362 mg, 1.11 mmol) were dissolved in dry acetonitrile (10 mL). Reaction mixture was heated to reflux for 48 h and monitored by TLC. Upon completion, reaction mixture was

concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as a yellow oil. 23 mg, 12 %.

R_f = 0.30 (19:1 EtOAc:MeOH); **^{1}H NMR (400 MHz, MeOD):** 0.93 (9H, s, C(C**H$_3$**)$_3$), 2.01 (2H, p, *J* = 6.2 Hz, C4'-***H***), 2.85 (1H, d, *J* = 11.2 Hz, C4-***H$_A$***H$_B$), 3.68 (1H, d, *J* = 11.2 Hz, C4-H$_A$***H$_B$***), 3.74 (3H, s, CO_2Me), 3.74 – 3.77 (2H, m) & 4.13 (2H, t, *J* = 6.2 Hz) (C3'-***H*** & C4'-***H***), 5.01 (1H, s, C2-***H***), 6.42 (1H, d, *J* = 9.5 Hz, C10'-***H***), 7.15 – 7.21 (2H, m, Ar-***H***), 7.24 – 7.29 (1H, m, Ar-***H***), 7.92 (1H, d, *J* = 9.5 Hz, C9'-***H***); **^{13}C NMR (100 MHz, MeOD):** 27.0 (C(**C**H$_3$)$_3$), 33.2 (C4'), 34.4 (C4), 37.7 (**C**(CH$_3$)$_3$), 53.5 (CO_2**Me**), 59.4 & 66.4 (C3' & C5'), 73.8 (C2), 81.9 (C5), 92.7 (C7), 112.1 (ArC), 117.3 (C10'), 118.6 (ArC), 120.8 (qC), 121.3 (ArC), 145.6 (C9'), 149.5 (qC), 157.2 (qC), 163.1 (qC), 170.6 (**C**O_2Me), 177.9 (C8), 187.4 (C6); **ν_{max}/cm^{-1}:** 2970, 1709 (br, C=O), 1571, 1439, 1262; **m/z (ESI$^-$):** 516.1 ([M-H]$^-$); **HRMS (ESI$^-$):** Found 516.1864, $C_{26}H_{30}O_{10}N^-$ requires 516.1864, $[\alpha]_D^{25}$**:** +58.8 (c = 1.6 in MeOH).

Ethyl (*S*,*Z*)-3-((1-(benzyloxy)-3-hydroxy-1-oxopropan-2-yl)amino)but-2-enoate 80

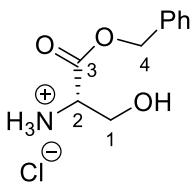


L-Serine (4.105 g, 39.1 mmol) was dissolved in dry DMF (30 mL) and N,N,N,N-tetramethylguanidine (4.90 mL, 39.1 mmol) was added at 0°C. After stirring for 10 mins, ethylacetoacetate (4.94 mL, 39.1 mmol) was added and the mixture was stirred at rt After 18 h, benzyl bromide (4.64 mL, 39.1 mmol) was and the reaction mixture was stirred for a further 24 h at rt. Upon completion, reaction was separated between EtOAc and saturated $NaHCO_3$ solution. Organic layers were combined and washed with water and saturated $NaHCO_3$ solution. Organic layers were then concentrated *in vacuo* to yield the title compound as a gummy, pale yellow solid. Yield 11.96 g, quantitative.

Mp. 91 – 93 °C (Lit. 91 – 95 °C)[52]; **^{1}H NMR (400 MHz, $CDCl_3$):** 1.22 (3H, t, *J* = 7.1 Hz, C5-***H***), 1.86 (3H, d, *J* = 0.6 Hz, C2-***H***), 3.87 – 3.97 (2H, m, C2'-***H***), 4.07 (2H, qd, *J* = 7.1, 0.9 Hz, C4-***H***), 4.24 (1H, dt, *J* = 9.6, 4.9 Hz, C1'-***H***), 4.55 (1H, d, *J* = 0.8 Hz, C3'-***H***), 5.14 – 5.22 (2H, m, C3'-***H***), 7.30 – 7.34 (5H, m, Ar-***H***), 8.95 (1H, d, *J* = 9.5 Hz, N***H***); **m/z (ESI$^+$):** 308.2 ($[M+H]^+$), 330.2 ($[M+Na]^+$); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$: +117.6 (c = 1.0 in DCM) (Lit. + 115.1, c = 1.0 in DCM).[52]

Data are in agreement with those found in the literature.[52]

Serine benzyl ester hydrochloride 81

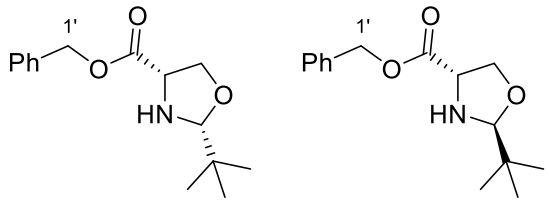


Protected serine **80** (11.96 g, 39.0 mmol) was added to a solution of methanolic HCl (150 mL) (made following general procedure H). Reaction mixture was stirred for 10 min and the solvent was removed *in vacuo*. Resulting white solid was washed with DCM to yield serine benzyl ester hydrochloride. Yield 6.83 g, 76 %.

M.p: 173 °C (Lit. 180 – 182 °C)[52]; **^{1}H NMR (200 MHz, D_2O):** 3.97 (1H, dd, *J* = 12.5, 3.5 Hz, C1-***H***$_A$H$_B$), 4.08 (1H, dd, *J* = 12.5, 4.2 Hz, C1-H$_A$***H$_B$***), 4.27 (1H, t, *J* = 3.8 Hz, C2-***H***), 5.29 (2H, s, C4-***H***), 7.41 – 7.44 (5H, m, Ar***H***); **m/z (ESI$^+$):** 196.2 ([M+H]$^+$); $[\alpha]_D^{25}$**:** -2.4 (c = 1.0 in MeOH) (Lit. -2.1, c = 1.0 in MeOH)

Data are in agreement with those found in the literature.[52]

Benzyl (4*S*)-2-(tert-butyl)oxazolidine-4-carboxylate 82



Major Minor

Synthesised according to general procedure A.

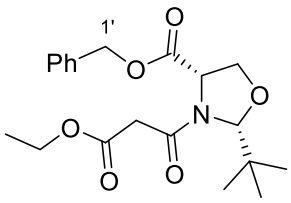
L-serine benzyl ester (6.1 g, 26.3 mmol) was dissolved in petroleum ether (150 mL), then NEt_3 (3.7 mL, 39.5 mmol) and pivaldehyde (3.4 mL, 31.6 mmol) was added. The mixture was heated at reflux with continuous removal of water using dean-stark apparatus for 20 h. White precipitate was then filtered and washed with diethyl ether. Combined filtrates were concentrated under reduced pressure to yield the title compound as a colourless oil. Isolated as a mixture of two diastereomers. Yield 5.97 g, 86 %.

^{1}H NMR (200 MHz, $CDCl_3$) Major isomer: 1.00 (9H, s, C(C***H$_3$***)$_3$), 3.67 – 3.81 (1H, m, C4-***H$_A$***H$_B$), 3.88 – 4.03 (2H, m, C4-H$_A$***H$_B$*** & C2-***H***), 4.09 – 4.18 (1H, m, C5-***H***), 5.11 – 5.27 (2H,

m, C1'-***H***), 7.33 – 7.39 (5H, m, Ar***H***); **Minor isomer:** 0.92 (9H, s, C(C***H₃***)₃, minor isomer), 3.81 (1H, m, C4-***H***$_A$H$_B$), 3.88 – 4.03 (1H, m, C4-H$_A$***H$_B$***), 4.09 – 4.18 (1H, m, C5-***H***), 4.36 (1H, s, C2-***H***, minor isomer), 5.11 – 5.27 (2H, m, C1'-***H***), 7.33 – 7.39 (5H, m, Ar***H***); **m/z (ESI$^+$):** 264.2 ([M+H]$^+$)

Data are in agreement with those found in the literature.[52]

Benzyl(2*R*,4*S*)-2-(tert-butyl)-3-(3-ethoxy-3-oxopropanoyl)oxazolidine-4-carboxylate 83

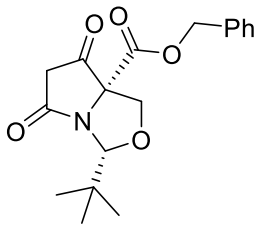


To a solution of oxazolidine **82** (5.95 g, 22.6 mmol) in anhydrous DCM was added DMAP (276 mg, 2.26 mmol) and DCC (5.13 g, 24.9 mmol). Mixture was cooled to 0°C, Ethyl hydrogen malonate (2.93 mL, 24.9 mmol) was added dropwise and then mixture was stirred at room temperature for 18h. A white precipitate forms which was filtered and washed with DCM. Filtrate was concentrated *in vacuo* and purified by flash column chromatography to yield the title compound as a colourless oil. Yield 3.00 g, 35 %.

Rf = 0.25 (3:7 EtOAc:Petrol); **^{1}H NMR (400 MHz, CDCl$_3$):** 0.86 (9H, s, C(C***H₃***)$_3$), 1.26 (3H, t, *J* = 7.2 Hz, CH$_2$C***H₃***), 3.47 (1H, d, *J* = 15.3 Hz, C7-***H***$_A$H$_B$), 3.66 (1H, d, *J* = 15.5 Hz, C7-H$_A$***H$_B$***), 4.02 (1H, t, *J* = 8.0 Hz, C4-***H***$_A$H$_B$), 4.18 (2H, app qd, *J* = 7.1, 3.2 Hz, C***H₂***CH$_3$), 4.55 (1H, d, *J* = 8.8 Hz, C4-H$_A$***H$_B$***), 4.73 (1H, m, C5-***H***), 5.17 (1H, d, *J* = 12.1 Hz, C1'-***H***$_A$H$_B$), 5.24 (1H, d, *J* = 12.1 Hz, C1'-H$_A$***H$_B$***), 5.31 (1H, s, C2-***H***), 7.34 – 7.38 (5H, m, Ar***H***); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$**:** -61.6 (c = 1.0 in DCM) (Lit. – 57.4, c = 1.0 in DCM)[52]

Data are in agreement with those found in the literature.[52]

Benzyl-(3*R*,7a*R*)-3-(tert-butyl)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 84



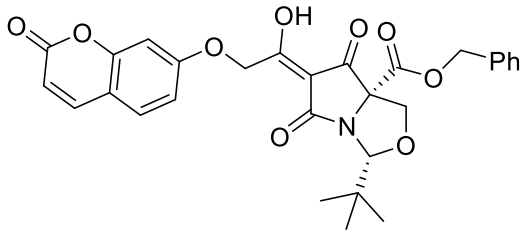
A solution of N-acyl oxazolidine (2.98 g, 7.89 mmol) and KO^{t}Bu (929 mg, 8.28 mmol) in anhydrous THF (25 mL) was heated at reflux for 4 h. The reaction mixture was then separated between diethyl ether and H_2O, aqueous phase was acidified using 2M HCl and extracted with ethyl acetate. Organic layers were washed with a 1M solution of NaH_2PO_4 and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the benzyl ester tetramic acid as a gummy solid. Yield 2.11 g, 81 %.

R_f: 0.10 (EtOAc); **^{1}H NMR (400 MHz, $CDCl_3$):** 0.85 (9H, s, C(C$\boldsymbol{H_3}$)$_3$), 3.14 (1H, d, *J* = 21.1 Hz, C7-$\boldsymbol{H_A}H_B$), 3.64 (1H, d, *J* = 9.0 Hz, C4-$\boldsymbol{H_A}H_B$), 3.69 (1H, dd, *J* = 21.1, 0.8 Hz, C7-$H_A\boldsymbol{H_B}$), 4.81 (1H, d, *J* = 9.0 Hz, C4-$H_A\boldsymbol{H_B}$), 5.07 (1H, d, *J* = 0.8 Hz, C2-***H***), 5.18 (1H, d, *J* = 12.1 Hz, C1'-$\boldsymbol{H_A}H_B$), 5.25 (1H, d, *J* = 12.1 Hz, C1'-$H_A\boldsymbol{H_B}$); **m/z (ESI$^-$):** 330.1 ([M-H]$^-$); $\boldsymbol{[\alpha]_D^{25}}$**:** +51.1 (c = 1.0 in DCM) (Lit. + 53.5, c = 1.0 in DCM)[52].

Data are in agreement with those found in the literature.[52]

Benzyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-((2-oxo-2H-chromen-7-yl)oxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate

86a

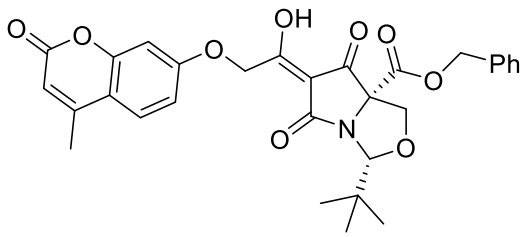


Chloro-substituted **85** (89 mg, 0.219 mmol), umbelliferone (58 mg, 0.328 mmol) and Cs_2CO_3 (143 mg, 0.437 mmol) were dissolved in dry acetonitrile (5 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as an orange solid. 35 mg, 30 %.

R_f = 0.60 (19:1 EtOAc:MeOH); **M.p:** 94 – 95 °C; **^{1}H NMR (400 MHz, MeOD):** 0.84 (9H, s, C(C***H*****$_3$**)$_3$), 3.49 (1H, d, *J* = 8.6 Hz, C4-***H*****$_A$**H$_B$), 4.74 (1H, d, *J* = 8.6 Hz, C4-H$_A$***H*****$_B$**), 4.76 (1H, s, C2-***H***), 5.07 (1H, d, *J* = 17.0 Hz, C3'-***H*****$_A$**H$_B$), 5.14 – 5.25 (3H, m, C3'-H$_A$***H*****$_B$** & OC***H*****$_2$**Ph), 6.21 (1H, d, *J* = 9.5 Hz, C9'-***H***), 6.81 (1H, d, *J* = 2.3 Hz, C6'-***H***), 6.91 (1H, dd, *J* = 8.6, 2.3 Hz, C12'-***H***), 7.28 – 7.39 (5H, m, Ar***H***), 7.46 (1H, d, *J* = 8.7 Hz, C11'-***H***), 7.84 (1H, d, *J* = 9.5 Hz, C10'-***H***); **^{13}C NMR (100 MHz, MeOD):** 25.5 (C(**C**H$_3$)$_3$), 36.2 (**C**(CH$_3$)$_3$), 68.5 (O**C**H$_2$Ph), 70.0 (C4), 72.6 (C3'), 78.7 (C5'), 99.1 (C2), 100.2 (C7), 102.9 (C6'), 113.3 (C9'), 114.1 (qC), 114.1 (C12'), 129.3 (ArC), 129.4 (ArC), 129.5 (ArC), 130.3 (C11'), 136.9 (qC), 145.7 (C10'), 156.9 (qC), 163.3 (qC), 170.9 (**C**O$_2$CH$_2$Ph), 182.0 (C8), 189.4 (C2'), 191.9

(C6); **ν_{max}/cm^{-1}:** 2980, 1732 (C=O), 1697 (C=O), 1613 (C=O), 1459; **$[\alpha]_D^{25}$:** +46.8 (c = 0.5 in MeOH).

Benzyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 86b

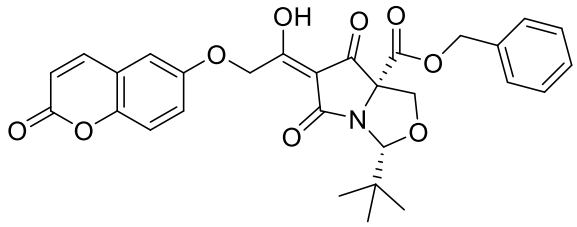


Chloro-substituted **85** (90 mg, 0.220 mmol), 4-methylumbelliferone (58 mg, 0.330 mmol) and Cs_2CO_3 (143 mg, 0.440 mmol) were dissolved in dry acetonitrile (5 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as an orange solid. 32 mg, 27 %.

R_f = 0.60 (19:1 EtOAc:MeOH); **M.p:** 92 – 93 °C; **^{1}H NMR (400 MHz, MeOD):** 0.84 (9H, s, C(C***H*****$_3$**)$_3$), 2.40 (3H, d, *J* = 1.1 Hz, C***H*****$_3$**), 3.49 (1H, d, *J* = 8.5 Hz, C4-***H*****$_A$**H$_B$), 4.74 (1H, d, *J* = 8.5 Hz, C4-H$_A$***H*****$_B$**), 4.76 (1H, s, C2-***H***), 5.06 (1H, d, *J* = 17.0 Hz, C3'-***H*****$_A$**H$_B$), 5.14 – 5.24 (3H, m, C3'-H$_A$***H*****$_B$** & OC***H*****$_2$**Ph), 6.11 (1H, d, *J* = 1.4 Hz, C9'-***H***), 6.79 (1H, d, *J* = 2.3 Hz, C6'-***H***), 6.92 (1H, dd, *J* = 8.8, 2.2 Hz, C12'-***H***), 7.26 – 7.39 (5H, m, Ar-***H***), 7.59 (1H, d, *J* = 8.8 Hz, C11'-***H***); **^{13}C NMR (100 MHz, MeOD):** 18.7 (CH$_3$), 25.5 (C(**C**H$_3$)$_3$), 36.2 (**C**(CH$_3$)$_3$), 68.4 (O**C**H$_2$Ph), 70.0 (C4), 72.7 (C3'), 78.7 (C5'), 99.0 (C2), 100.2 (C7), 102.9

(C6'), 112.1 (C9'), 113.8 (C12'), 114.9 (qC), 127.1 (C11'), 129.3 (ArC), 129.4 (ArC), 129.5 (ArC), 136.9 (qC), 155.6 (qC), 156.2 (qC), 163.5 (qC), 170.9 (**C**O_2CH_2Ph), 182.1 (C8), 189.4 (C2'), 191.9 (C6); **ν_{max}/cm^{-1}:** 2980, 1690 (C=O), 1612 (C=O), 1458; **m/z (ESI$^-$):** 546.1 ([M-H]$^-$); **HRMS (ESI$^-$):** Found 570.1733, $C_{24}H_{29}NO_9Na^+$ requires 570.1735; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$**:** +44.9 (c = 1.0 in MeOH).

Benzyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-((2-oxo-2H-chromen-6-yl)oxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 86c

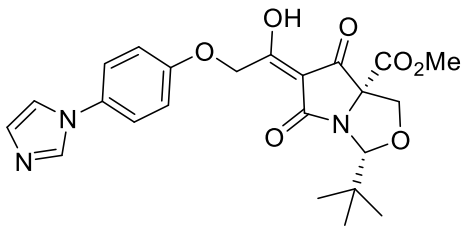


Chloro-substituted **85** (92 mg, 0.226 mmol), 6-hydroxycoumarin (55 mg, 0.339 mmol) and Cs_2CO_3 (146 mg, 0.448 mmol) were dissolved in dry acetonitrile (5 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as an orange solid. 31 mg, 22 %.

R_f = 0.60 (19:1 EtOAc:MeOH); **M.p:** 88 – 89 °C; **^{1}H NMR (400 MHz, MeOD):** 0.85 (9H, s, (C(C$\boldsymbol{H_3}$)$_3$)), 3.48 (1H, d, *J* = 8.6 Hz, C4-$\boldsymbol{H_A}H_B$), 4.74 (1H, d, *J* = 8.6 Hz, C4-$H_A\boldsymbol{H_B}$), 4.76 (1H, s, C2-***H***), 5.03 (1H, d, *J* = 17.0 Hz, C3'-$\boldsymbol{H_A}H_B$), 5.12 – 5.27 (3H, m, C3'-$H_A\boldsymbol{H_B}$ & OC$\boldsymbol{H_2}$Ph), 6.38 (1H, d, *J* = 9.5 Hz, C8'-***H***), 7.18 – 7.39 (8H, m, C6'-***H***, C11'-***H***, C12'-***H*** & $OCH_2\boldsymbol{Ph}$),

7.81 (1H, d, *J* = 9.6 Hz, C7'-***H***); **^{13}C NMR (100 MHz, MeOD):** 24.2 (C(**C**H_3)$_3$), 35.0 (**C**(CH$_3$)$_3$), 67.1 (O**C**H$_2$Ph), 68.7 (C4), 71.5 (C3'), 77.2 (C5), 97.7 (C2), 98.9 (C7), 111.2 (ArC), 115.8 (C8'), 117.1 (ArC), 119.3 (qC), 120.2 (ArC), 128.2 (OCH$_2$**Ph**), 128.2 (OCH$_2$**Ph**), 128.3 (OCH$_2$**Ph**), 135.6 (qC), 144.4 (qC), 148.3 (C7'), 155.6 (qC), 161.9 (qC), 169.7 (**C**O$_2$CH$_2$Ph), 180.8 (C8), 188.9 (C2'), 190.6 (C6); **ν_{max}/cm^{-1}:** 2980, 1718 (C=O), 1622 (C=O), 1570, 1459; **m/z (ESI$^-$):** 532.1 ([M-H]$^-$); **HRMS (ESI$^-$):** Found 532.1610, $C_{29}H_{26}NO_9^-$ requires 532.1610; **$[\alpha]_D^{25}$:** +47.4 (c = 1.0 in MeOH).

Methyl(3*R*,7a*R*,*E*)-6-(2-(4-(1H-imidazol-1-yl)phenoxy)-1-hydroxyethylidene)-3-(tert-butyl)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 89o

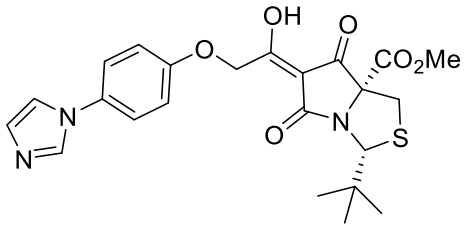


Chloro-substituted **64o** (52 mg, 0.157 mmol), 4-(imidazole-1-yl) phenol (25 mg, 0.157 mmol) and Cs_2CO_3 (77 mg, 0.236 mmol) were dissolved in dry acetonitrile (10 mL). Reaction mixture was heated to reflux for 48 h and monitored by TLC. Upon completion, reaction mixture was filtered and concentrated *in vacuo* to yield the title compound as a yellow solid. Yield 71 mg, quantitative.

^{1}H NMR (MeOD, 400 MHz): 0.90 (9H, s, C(C***H*****$_3$**)$_3$), 3.45 (1H, d, *J* = 8.5 Hz, C4-***H*****$_A$**H$_B$), 3.74 (3H, s, CO$_2$***Me***), 4.71 (1H, d, *J* = 8.5 Hz, C4-H$_A$***H*****$_B$**), 4.76 (1H, s, C2-***H***), 5.05 (1H, d, *J* = 17.0 Hz, C3'-***H*****$_A$**H$_B$), 5.17 (1H, d, *J* = 17.0 Hz, C3'-H$_A$***H*****$_B$**), 7.05 (2H, d, *J* = 9.0 Hz, C6'-***H***), 7.11 (1H, s, imidazole-***H***), 7.43 (2H, d, *J* = 8.9 Hz, C7'-***H***), 7.46 (1H, s, imidazole-***H***), 8.00 (1H, s, NC***H***N); **^{13}C NMR (MeOD, 100 MHz):** 25.5 (C(**C**H$_3$)$_3$), 36.3 (**C**(CH$_3$)$_3$), 53.2 (CO$_2$***Me***), 70.1 (C4), 72.7 (C3'), 78.6 (C5), 98.9 (C2), 100.0 (C7), 116.9 (C6'), 120.1 (**C**imidazole), 123.9 (C7'), 129.9 (**C**imidazole), 131.9 (C8'), 137.0 (N**C**HN), 159.6 (C4'),

171.8 (**C**CO_2Me), 182.0 (C8), 190.2 (C2'), 191.7 (C6); **ν_{max}/cm^{-1}:** 1731 (C=O); 1689 (C=O), 1612 (C=O); **m/z (ESI$^-$):** 454.1 ([M-H]$^-$); **HRMS (ESI$^+$):** Found 456.1763, $C_{23}H_{26}N_3O_7^+$ requires 456.1765;

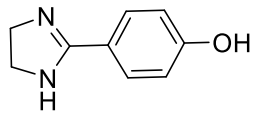
Methyl(3*R*,7a*R*,*E*)-6-(2-(4-(1H-imidazol-1-yl)phenoxy)-1-hydroxyethylidene)-3-(tert-butyl)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]thiazole-7a(5H)-carboxylate 89s



Chloro-substituted **64s** (158 mg, 0.454 mmol), 4-(imidazole-1-yl) phenol (73 mg, 0.454 mmol) and Cs_2CO_3 (148 mg, 0.454 mmol) were dissolved in dry acetonitrile (10 mL). Reaction mixture was heated to reflux for 48 h and monitored by TLC. Upon completion, reaction mixture was filtered and concentrated *in vacuo* to yield the title compound as a yellow solid. Yield 214 mg, quantitative.

^{1}H NMR (MeOD, 400 MHz): 0.95 (9H, s, C(C***H*****$_3$**)$_3$), 2.85 (1H, d, *J* = 11.2 Hz, C4-***H*****$_A$**H$_b$), 3.71 (1H, d, *J* = 11.3 Hz, C4-H$_A$***H*****$_B$**), 3.73 (3H, s, CO_2***Me***), 5.05 (1H, d, *J* = 17.1 Hz, C3'-***H*****$_A$**H$_B$), 5.10 (1H, s, C2-***H***), 5.07 (1H, d, *J* = 17.1 Hz, C3'-***H*****$_A$**H$_B$), 5.20 (1H, d, *J* = 17.1 Hz, C3'-H$_A$***H*****$_B$**), 7.04 (2H, d, *J* = 8.9 Hz, C6'-***H***), 7.11 (1H, broad s, imidazole-***H***), 7.42 (2H, d, *J* = 9.0 Hz, C7'-***H***), 7.45 (1H, broad s, imidazole-***H***), 7.99 (1H, broad s, NC***H***N); **^{13}C NMR (MeOD, 100 MHz):** 27.1 (C(**C**H$_3$)$_3$), 34.7 (**C**(CH$_3$)$_3$), 35.0 (C4), 53.3 (CO_2***Me***), 72.7 (C2), 74.5 (C3'), 83.4 (C5), 98.2 (C7), 116.9 (C6'), 120.1 (**C**imidazole), 120.5 (C8'), 123.9 (C7'), 129.9 (**C**imidazole), 137.0 (N**C**HN), 159.5 (C5'), 171.8 (**C**CO_2Me), 179.8 (C8), 190.0 (C2'), 192.1 (C6); **ν_{max}/cm^{-1}:** 1518, 1637, 1692 (C=O), 1731 (C=O); **m/z (ESI$^+$):** 472.1 ([M+H]$^+$); **HRMS (ESI$^+$):** Found 456.1763, $C_{23}H_{26}N_3O_7^+$ requires 456.1765.

4-(4,5-dihydro-1H-imidazol-2-yl)phenol 92

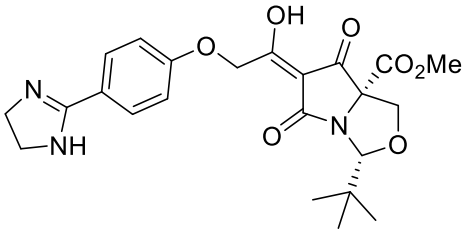


A stirred solution of methyl-4-hydroxybenzoate (20 g, 131 mmol) in ethylenediamine (27 mL, 394 mmol) was heated to reflux for 6 h. Reaction mixture was concentrated *in vacuo*, dissolved in a 2:1 H_2O:EtOH solution and heated to reflux for a further 30 mins. Upon completion determined by NMR, solid precipitate was filtered and washed with cold EtOH to yield the title compound as a white solid. Yield 7.41 g, 35 %.

M.p: >250°C; **^{1}H NMR (200 MHz,DMSO-*d*$_6$):** 3.56 (4H, s, C7-***H*** & C8-***H***), 6.70 (2H, d, *J* = 8.7 Hz, C2-***H***), 7.61 (2H, d, *J* = 8.7 Hz, C3-***H***); **m/z (ESI$^+$):** 163.0 ([M+H]$^+$).

Data are in agreement with those found in the literature.[134]

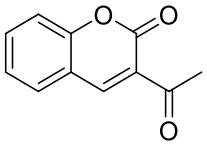
Methyl(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(2-(4-(4,5-dihydro-1H-imidazol-2-yl)phenoxy)-1-hydroxyethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 93



Chloro-substituted **64o** (98 mg, 0.296 mmol), phenol **92** (48 mg, 0.296 mmol) and Cs_2CO_3 (101 mg, 0.296 mmol) were dissolved in dry acetonitrile (10 mL). Reaction mixture was heated to reflux for 48 h and monitored by TLC. Upon completion, reaction mixture was filtered and concentrated *in vacuo* to yield the title compound as a yellow solid. Yield 214 mg, quantitative.

M.p: 188 – 189 °C; **^{1}H NMR (500 MHz, MeOD):** 0.90 (9H, s, C(C***H*****$_3$**)$_3$), 3.45 (1H, d, *J* = 8.5 Hz, C4-***H*****$_A$**H$_B$), 3.72 (4H, s, imidazoline-***H***), 3.74 (3H, s, CO$_2$***Me***), 4.71 (1H, d, *J* = 8.5 Hz, C4-H$_A$***H*****$_B$**), 4.76 (1H, s, C2-***H***), 5.04 (1H, d, *J* = 17.1 Hz, C3'-***H*****$_A$**H$_B$), 5.17 (1H, d, *J* = 17.1 Hz, C3'-H$_A$***H*****$_B$**), 6.95 (2H, d, *J* = 8.9 Hz, C5'-***H***), 7.70 (2H, d, *J* = 8.9 Hz, C6'-***H***); **^{13}C NMR (125 MHz, MeOD):** 25.4 (C(**C**H$_3$)$_3$), 36.3 (**C**(CH$_3$)$_3$), 50.2 (C imidazoline) 53.2 (**C**O$_2$Me), 70.0 (C4), 72.4 (C2'), 78.5 (C5), 98.9 (C2), 100.0 (C7), 115.6 (C5'), 123.5 (qC), 129.9 (C6'), 162.4 (qC), 167.1 (qC), 171.8 (**C**O$_2$Me), 182.1 (C8), 190.0 (C6), 191.8 (C1'); **m/z (ESI$^+$):** 458.2 ([M+H]$^+$); **HRMS (ESI$^+$):** Found 458.1916, $C_{23}H_{28}N_3O_7^+$ requires 458.1922; **$[\alpha]_D^{25}$:** +53.5 (c = 1.0 in MeOH).

3-acetyl-2H-chromen-2-one 98

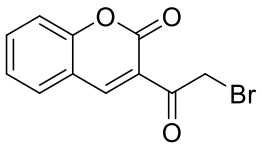


Salicaldehyde (1 g, 8.19 mmol) and ethylacetoacetate (1.04 mL, 8.19 mmol) were mixed in a RBF with stirring and 2-3 drops of piperidine were added which caused solution to immediately turn yellow. After stirring for 5 mins, a solid precipitate formed and was crushed. Dilute HCl was added and solid was filtered by vacuum filtration to yield the title compound as a pale-yellow solid. 800 mg, 52 %.

M.p: 118 - 119 °C (Lit. 119 – 121 °C)[135]; **^{1}H NMR (CDCl$_3$):** 2.73 (3H, s, C***H*****$_3$**), 7.30 – 7.41 (2H, m, Ar-***H***), 7.61 – 7.71 (2H, m, Ar-***H***), 8.52 (1H, s, C=C***H***);

Data are in agreement with those found in the literature.[136]

3-(2-bromoacetyl)-2H-chromen-2-one 99

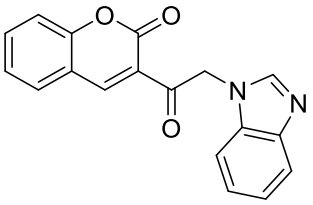


3-acetylcoumarin (1.75 g, 9.30 mmol) was dissolved in $CHCl_3$ and Br_2 (0.48 mL, 9.30 mmol) dissolved in a small volume of $CHCl_3$ was slowly added. Reaction mixture was stirred and heated intermittently until evolution of HBr gas ceased. Solid precipitate was filtered by vacuum filtration and washed with cold $CHCl_3$ to yield the title compound as an off white solid (1.31 g, 53 %).

M.p: 162 - 164°C (Lit. 162 – 164 °C)[135]; **^{1}H NMR ($CDCl_3$, 200 MHz):** 4.75 (2H, s, C***H*₂***), 7.40 (2H, dd, *J* = 8.3, 0.8 Hz, C6-***H*** & C9-***H***), 7.66 – 7.76 (2H, m, C7-***H*** & C8-***H***).

Data are in agreement with those found in the literature.[137]

3-(2-(1H-benzo[d]imidazol-1-yl)acetyl)-2H-chromen-2-one 100



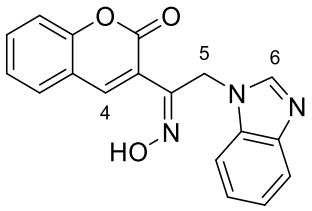
To a stirred solution of 3-(2-bromoacetyl)-2*H*-chromen-2-one (640 mg, 2.40 mmol) in acetonitrile was added benzimidazole (283 mg, 2.40 mmol). Reaction mixture was stirred at room temperature for 4 h until completion, monitored by TLC. Upon completion, the reaction mixture was quenched with a saturated solution of $NaHCO_3$ and extracted with EtOAc. Organic layers were combined, dried over Na_2SO_4 and solvent was removed *in vacuo*.

Subsequent residue was purified by flash column chromatography to yield the title compound as a yellow solid. Yield 576 mg, 79 %.

R_f: 0.60 (1:19 MeOH:$CHCl_3$); **M.p:** 149 – 150 °C (Lit. 150 – 152 °C)[63]; **^{1}H NMR (DMSO-d_6):** 5.88 (2H, s, C***H***$_2$), 7.18 – 7.23 (2H, m, Ar-***H***), 7.56 (1H, d, *J* = 8.4 Hz, Ar-***H***), 7.66 – 7.70 (1H, m, Ar-***H***), 7.82 (1H, ddd, *J* = 8.7, 7.4, 1.6 Hz, Ar-***H***), 8.03 (1H, dd, *J* = 7.8, 1.6 Hz, Ar-***H***), 8.18 (1H, s, C=C***H***), 8.83 (1H, s, N=C***H***); **m/z (ESI$^+$):** 305.2 ([M+H]$^+$).

Data are in agreement with those found in the literature.[63]

(*E*)-3-(2-(1H-benzo[d]imidazol-1-yl)-1-(hydroxyimino)ethyl)-2H-chromen-2-one 101

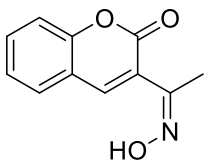


Benzimidazole – coumarin **100** (165 mg, 0.543 mmol) and hydroxylamine hydrochloride (45 mg, 0.651 mmol) were dissolved in EtOH (5 mL) and the reaction was heated to reflux for 4 h. Upon completion by TLC, solvent was removed *in vacuo*. Resulting residue was purified by column chromatography to yield the title compound as a yellow solid. Yield 104 mg, 60 %.

M.p: 178 – 180 °C (Lit. 178 – 179 °C)[63]; **^{1}H NMR (200 MHz, DMSO-d_6):** 5.54 (2H, s, C5-***H***), 7.10 – 7.23 (2H, m, Ar-***H***), 7.25 – 7.47 (4H, m, Ar-***H***), 7.53 – 7.71 (2H, m, Ar-***H***), 8.06 (1H, s, C4-***H***), 8.11 (1H, s, C6-***H***); **m/z (ESI$^+$):** 320.0 ([M+H]$^+$).

Data are in agreement with those found in the literature.[63]

(*Z*)-3-(1-(hydroxyimino)ethyl)-2H-chromen-2-one 105

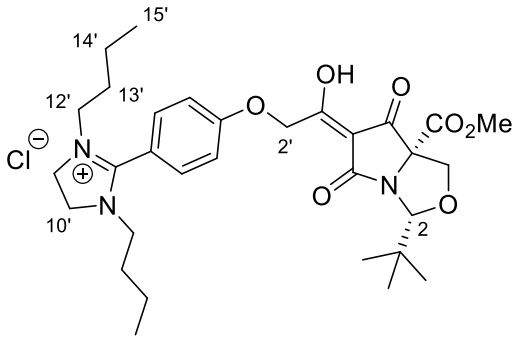


3-acetyl-2H-chromen-2-one **98** (512 mg, 2.72 mmol) was dissolved in EtOH (10 mL) and hydroxylamine hydrochloride (227 mg, 3.26 mmol) was added. Reaction mixture was heated to reflux for 4 h until complete consumption of starting material was observed. Once complete, solvent was removed *in vacuo* and the resulting residue was purified by column chromatography to yield the title compound as a yellow solid. Yield 62 mg, 11 %.

M.p: 168 – 169 °C (Lit 170 °C)[138]; **^{1}H NMR (200 MHz, $CDCl_3$):** 2.29 (3H, s, C***H*$_3$**), 7.29 – 7.38 (2H, m, Ar-***H***), 7.54 (2H, dd, *J* = 7.6, 0.7 Hz, Ar***H***), 7.88 (1H, d, *J* = 0.7 Hz, C=C***H***); **m/z (ESI$^+$):** 204.1 ([M+H]$^+$).

Data are in agreement with those found in the literature.[138]

1,3-dibutyl-2-(4-((*E*)-2-((3*R*,7a*R*)-3-(tert-butyl)-7a-(methoxycarbonyl)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazol-6(5H)-ylidene)-2-hydroxyethoxy)phenyl)-4,5-dihydro-1H-imidazol-3-ium chloride 109

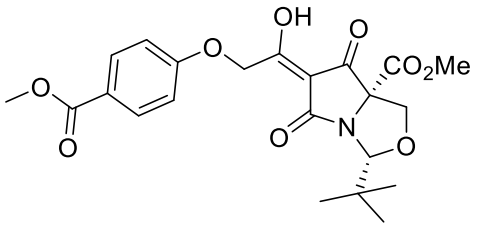


Imidazoline tetramate **93** (99 mg, 0.217 mmol) was dissolved in MeCN (5 mL) and butyl bromide (60 mg, 0.434 mmol) was added. Reaction was stirred at rt for 18 h until complete

consumption of starting materials was observed, which was monitored by LRMS. Upon completion, solvent was removed *in vacuo* to yield the title compound as the chloride salt. Yield 130 mg, quantitative.

R_f: 0.00 (1:4 MeOH:EtOAc); **M.p:** 146 – 147 °C; **^{1}H NMR (500 MHz, MeOD):** 0.86 (6H, t, *J* = 7.5 Hz, C15'-***H***), 0.90 (9H, s, C(C***H*****$_3$**)$_3$), 1.23 (4H, dt, *J* = 16.2, 7.4 Hz, C14'***H***), 1.50 – 1.62 (4H, m, C13'-***H***), 3.25 (4H, t, *J* = 7.5 Hz, *J* = 7.5 Hz, C12'-***H***), 3.44 (1H, d, *J* = 8.5 Hz, C4-***H*****$_A$**H$_B$), 3.74 (3H, s, CO_2***Me***), 3.78 (2H, s, C10'-***H*** & C11'-***H***), 4.05 (2H, s, C10'-***H*** & C11'-***H***), 4.71 (1H, d, *J* = 8.5 Hz, C4-H$_A$***H*****$_B$**), 4.76 (1H, s, C2-***H***), 5.12 (2H, dd, *J* = 17.0 Hz, C2'-***H***), 6.97 (1H, d, *J* = 8.9 Hz, Ar***H***), 7.17 (1H, d, *J* = 8.8 Hz, Ar***H***), 7.41 (1H, d, *J* = 8.8 Hz, Ar***H***), 7.72 (1H, d, *J* = 8.9 Hz, Ar***H***); **^{13}C NMR (125 Hz, MeOD):** 13.8 (C15'), 20.6 (C14'), 25.4 (C(**C**H$_3$)$_3$), 30.3 (C13'), 36.3 (**C**(CH$_3$)$_3$), 47.5 – 48.2 (C10' & C11' & C12'), 53.1 (CO_2**Me**), 70.0 (C4), 72.4 (C2), 78.6 (C5), 98.9 (C2), 100.1 (C7), 115.8 (ArC), 117.2 (ArC), 122.0 (qC), 130.1 (ArC), 131.0 (ArC), 163.0 (qC), 167.9 (qC), 171.7 (**C**O_2Me), 182.1 (C8), 189.9 (C2'), 191.8 (C6); **ν_{max}/cm^{-1}:** 1738 (C=O), 1691 (C=O), 1614 (br), 1445, 1222; **m/z (ESI$^+$):** 570.3 ([M+H]$^+$); **HRMS (ESI$^+$):** Found 570.3166, $C_{31}H_{44}O_7N_3^+$ requires 570.3174; **$[\alpha]_D^{25}$:** +26.3 (c = 1.0 in MeOH).

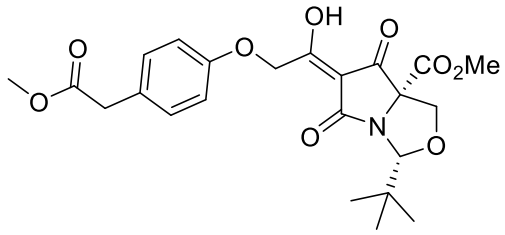
Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-(4-(methoxycarbonyl)phenoxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 115o



Chloro-substituted **64o** (226 mg, 0.683 mmol), methyl paraben (114 mg, 0.751 mmol) and Cs_2CO_3 (667 mg, 2.05 mmol) were dissolved in dry acetonitrile (10 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as yellow oil. Yield 305 mg, 71 %.

R_f: 0.60 (1:19 MeOH:EtOAc); **M.p:** 149 – 151 °C; **^{1}H NMR (400 MHz, *Acetone-d_6*):** 0.89 (9H, s, C(C***H*****$_3$**)$_3$), 3.45 (1H, d, *J* = 8.6 Hz, C4-***H*****$_A$**H$_B$), 3.73 (CO_2***Me***), 3.81 (CO_2***Me***), 4.66 (1H, d, *J* = 8.4 Hz, C4-H$_A$***H*****$_B$**), 4.75 (1H, s, C2-***H***), 5.11 (2H, app q, *J* = 17.0 Hz, C2'-***H***), 6.97 (2H, d, *J* = 8.9 Hz, C5'-***H***), 7.89 (2H, d, *J* = 8.9 Hz, C6'-***H***), 6.01 (1H, s, O***H***); **^{13}C NMR (100 MHz, *Acetone-d_6*):** 25.2 (C(**C**H$_3$)$_3$), 35.8 (**C**(CH$_3$)$_3$), 52.0 (CO_2**Me**), 53.0 (CO_2**Me**), 69.4 (C4), 70.3 (C2'), 78.0 (C5), 98.4 (C2), 100.3 (C7), 115.2 (C5'), 123.7 (ArC), 132.1 (C6'), 162.9 (**C**O_2Me), 166.7 (**C**O_2Me), 170.1 (ArC), 180.0 (C8), 188.1 (C1'), 189.6 (C6); **ν_{max}/cm^{-1}:** 3181 (broad, OH), 1691, 1604 (broad, s, C=O); **m/z** (ESI$^-$): 446.1 ([M-H$^-$]$^-$); **HRMS** (ESI$^-$): found 446.1451, $C_{22}H_{24}NO_9^-$ requires 446.1457; $[\boldsymbol{\alpha}]_D^{25}$**:** + 83.8 (c = 0.5 in MeOH).

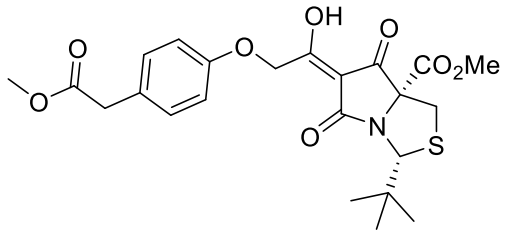
Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-(4-(2-methoxy-2-oxoethyl)phenoxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 116o



Chloro-substituted **64o** (50 mg, 0.150 mmol), methyl 4-hydroxyphenylacetate (25.1 mg, 0.150 mmol) and Cs_2CO_3 (148 mg, 0.450 mmol) were dissolved in dry acetonitrile (5 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as yellow oil. Yield 22 mg, 32 %.

^{1}H NMR (400 MHz, $CDCl_3$): 0.75 (9H, s, C(C$\boldsymbol{H_3}$)$_3$), 3.51 (3H, s, CO_2**Me**), 3.61 (3H, s, CO_2**Me**), 3.40 – 3.43 (2H, broad s, C9'-$\boldsymbol{H_AH_B}$), 3.39 (1H, d, *J* = 8.9 Hz, C4-$\boldsymbol{H_A}H_B$), 4.58 (1H, d, *J* = 8.6 Hz, C4-$H_A\boldsymbol{H_B}$), 4.66 (1H, s, C2-H), 4.90 – 5.08 (2H, m, C3'-$\boldsymbol{H_AH_B}$), 6.73 (2H, d, *J* = 8.4 Hz, C6'-***H***), 7.02 (2H, d, *J* = 8.0 Hz, C7'-***H***); **^{13}C NMR (100 MHz, $CDCl_3$):** 22.9 (C(**C**H$_3$)$_3$), 34.8 (**C**(CH$_3$)$_3$), 39.4 (C9'), 51.0 (CO_2**Me**), 52.1 (CO_2**Me**), 68.2 (C4), 72.7 (C2'), 78.5 (C5), 97.7 (C2), 100.1 (C7), 114.4 (C6'), 114.7 (qC), 114.9 (qC), 126.9 (qC), 130.0 (C7'), 157.4 (qC), 168.9 (qC), 172.8 (qC); 186.9 (C2'), 188.0 (C6); **ν_{max}/cm^{-1}:** 1600 – 1620 (br, C=O), 1741 (C=O); **m/z (ESI$^-$):** 460.1 ([M-H]$^-$); **HRMS (ESI$^-$):** Found 460.1614, $C_{23}H_{26}NO_9^-$ requires 460.1613; $\boldsymbol{[\alpha]_D^{25}}$**:** +75.9 (c = 1.0 in MeOH).

Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-(4-(2-methoxy-2-oxoethyl)phenoxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]thiazole-7a(5H)-carboxylate 116s

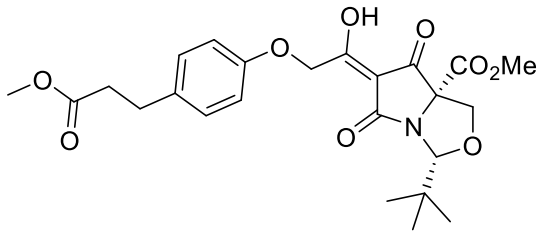


Chloro-substituted tetramate **64s** (52 mg, 0.144 mmol), methyl 4-hydroxyphenylacetate (48 mg, 0.288 mmol) and Cs_2CO_3 (234 mg, 0.720 mmol) were dissolved in dry acetonitrile (10 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as an orange gummy solid. Yield 26 mg, 38 %.

R_f: 0.60 (1:19 MeOH:EtOAc); **^{1}H NMR (400 MHz, $CDCl_3$):** 0.81 (9H, s, C(C***H*****$_3$**)$_3$), 2.80 (1H, d, *J* = 11.3 Hz, C4-***H*****$_A$**H$_B$), 3.41 (2H, s, C9'-***H*****$_A$*****H*****$_B$**), 3.50 (3H, s, CO_2***Me***), 3.53 (1H, d, *J* = 11.3 Hz, C4-H$_A$***H*****$_B$**), 3.60 (3H, s, CO_2***Me***), 4.96 (1H, s, C2-***H***), 5.09 (1H, d, *J* = 17.0 Hz, C3'-***H*****$_A$**H$_B$), 5.14 (1H, d, *J* = 17.0 Hz, C3'-H$_A$***H*****$_B$**), 6.73 (2H, d, *J* = 8.2 Hz, C6'-***H***), 7.02 (2H, d, *J* = 8.1 Hz, C7'-***H***); **^{13}C NMR (100 MHz, $CDCl_3$):** 25.6 (C(**C**H$_3$)$_3$), 36.3 (**C**(CH$_3$)$_3$), 39.4 (C-9'), 51.1 (CO_2**Me**), 52.4 (CO_2**Me**), 66.9 (C-3'), 73.0 (C-2), 81.9 (C-5), 100.0 (C-7), 114.4 (ArC), 127.3 (ArC), 130.1 (ArC), 157.2 (ArC), 168.0 (**C**O$_2$Me), 172.8 (**C**O$_2$Me), 175.3 (C8), 185.8 (C2'), 188.5 (C6); **m/z (ESI$^-$):** 476.0 ([M-H]$^-$); **HRMS (ESI$^-$):** Found 478.1532,

$C_{22}H_{24}NO_8S^-$ requires 478.1530; **ν_{max}/cm^{-1}:** 1740 (C=O_{Ester}), 1612 (br, C=O); **$[\alpha]_D^{25}$:** + 113.2 (c = 0.5 in MeOH).

Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-(4-(3-methoxy-3-oxopropyl)phenoxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 117o

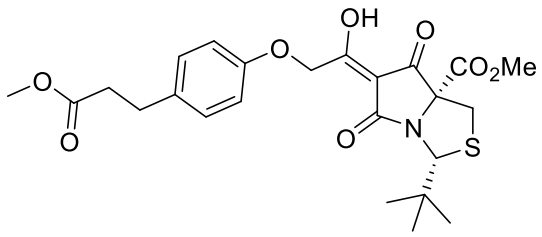


Chloro-substituted **64o** (50 mg, 0.150 mmol), Methyl 3-(4-hydroxyphenyl)propionate (54 mg, 0.300 mmol) and Cs_2CO_3 (158 mg, 0.760 mmol) were dissolved in dry acetonitrile (5 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as yellow oil. 26 mg, 36 % yield.

R_f: 0.35 (EtOAc); **^{1}H NMR (400 MHz, $CDCl_3$):** 0.74 (9H, s, C(C***H*****$_3$**)$_3$), 2.41 (2H, td, *J* = 7.6, 4.3 Hz, C9'-***H***), 2.67 (2H, app q, *J* = 7.4 Hz, C10'-***H***), 3.46 (3H, s, CO_2***Me***) 3.48 (1H, d, *J* = 8.6 Hz, C4-***H*****$_A$**H$_B$), 3.60 (3H, s, CO_2***Me***), 4.57 (1H, d, *J* = 8.6 Hz, C4-H$_A$***H*****$_B$**), 4.66 (1H, s, C2-***H***), 5.01 (2H, m, C3'-***H*****$_A$*H*****$_B$**), 6.68 (2H, d, *J* = 8.1 Hz, C6'-***H***), 6.95 (2H, d, *J* = 8.3 Hz, C7'-***H***); **^{13}C NMR (100 MHz, $CDCl_3$):** 23.9 (C(**C**H$_3$)$_3$), 29.7 (C-10'), 34.8 (**C**(CH$_3$)$_3$), 35.4 (C9'), 50.6 (CO_2**Me**), 52.3 (CO_2**Me**), 68.1 (C4), 76.9 (C5), 97.8 (C2), 101.9 (C7), 114.4 (C6'),

114.7 (qC), 129.0 (C7'), 133.6 (qC), 156.7 (qC), 158.0 (qC), 168.2 (**C**CO_2Me), 168.3 (**C**CO_2Me), 177.6 (C8), 187.5 (C2'), 187.6 (C6); **m/z (ESI)$^-$:** 474.1 ([M-H]$^-$); **HRMS (ESI)$^-$:** Found 474.1767, $C_{24}H_{28}NO_9^-$ requires 474.1770; **ν_{max}/cm^{-1}:** 1610 – 1640 (br, C=O), 1741 (C=O_{Amide}); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$**:** + 46.4 (c = 1.0 in MeOH).

Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-(4-(3-methoxy-3-oxopropyl)phenoxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]thiazole-7a(5H)-carboxylate 117s

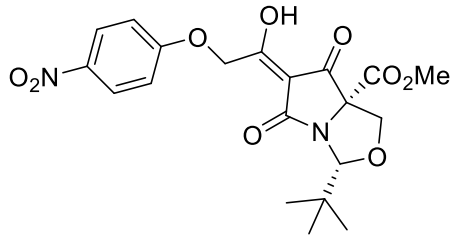


Chloro-substituted **64s** (200 mg, 0.576 mmol), Methyl 3-(4-hydroxyphenyl)propionate (207mg, 1.15 mmol) and Cs_2CO_3 (563 mg, 1.73 mmol) were dissolved in dry acetonitrile (10 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as a yellow oil. Yield 241 mg, 85 %.

R_f: 0.60 (1:19 MeOH:EtOAc); **^{1}H NMR (400 MHz, $CDCl_3$):** 0.98 (9H, s, C(C***H***$_3$)$_3$), 2.59 (2H, t, *J* = 7.8 Hz, C9'-H), 2.86-2.92 (3H, m, C4-***H***$_A$H$_B$ & C10'-***H***), 3.66 (3H, s, CO_2Me), 3.76 – 3.83 (4H, m, C4-H$_A$***H***$_B$ + CO_2**Me**), 5.11 (1H, s, C2-***H***), 5.17 (2H, m, C3'-***H***), 6.86 (2H, d, *J* = 8.3 Hz, C6'-***H***), 7.12 (2H, d, *J* = 8.1 Hz, C7'-***H***); **^{13}C NMR (100 MHz, $CDCl_3$):** 26.6 (C(**C**H$_3$)$_3$), 30.2 (C10'), 33.2 (C4), 36.0 (C9'), 36.9 (**C**(CH$_3$)$_3$), 51.8 (CO_2**Me**), 53.7 (CO_2**Me**), 65.0 (C3'), 73.7 (C2), 83.0 (C5), 99.6 (qC), 100.1 (qC), 114.9 (C6'), 129.6 (C7'), 134.3,

156.3 (ArC), 167.2 (**C**CO_2Me), 173.5 (**C**CO_2Me), 177.2 (C8), 184.7 (C1'), 188.2 (C6); **ν_{max}/cm^{-1}:** 1741 (C=O), 1610 – 1640 (br, C=O); **m/z (ESI$^-$):** 490.1 ([M-H]$^-$); **HRMS** (ESI$^-$): Found 490.1689, $C_{24}H_{28}NO_8S^+$ requires 490.1687; **$[\alpha]_D^{25}$:** +115.7 (c = 1.0 in MeOH).

Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-(4-nitrophenoxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 118o

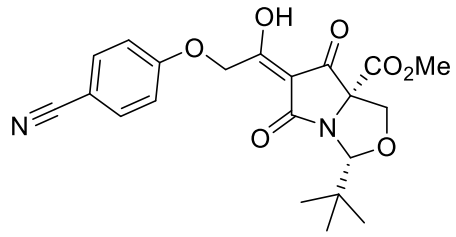


Chloro-substituted **64o** (115 mg, 0.347 mmol), 4-nitrophenol (53 mg, 0.382 mmol) and Cs_2CO_3 (339 mg, 1.04 mmol) were dissolved in dry acetonitrile (10 mL). Reaction mixture was heated to reflux for 24 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as a yellow oil. Yield 84 mg, 56 %.

^{1}H NMR (400 MHz, Acetone-*d6*): 0.75 (9H, s, (C(C***H*****$_3$**)$_3$), 3.27 (1H, d, *J* = 8.4 Hz, C4-***H*****$_A$**H$_B$), 3.58 (3H, s, CO_2***Me***), 4.52 (1H, d, *J* = 8.4 Hz, C4-H$_A$***H*****$_B$**), 4.60 (1H, s, C2-***H***), 5.01 (1H, d, *J* = 17.1 Hz, C3'-***H*****$_A$**H$_B$), 5.14 (1H, d, *J* = 17.1 Hz, C3'-***H*****$_A$**H$_B$), 5.27 (1H, d, *J* = 17.1 Hz, C3'-H$_A$***H*****$_B$**), 6.92 (2H, d, *J* = 9.3 Hz, C6'-***H***), 8.03 (2H, d, *J* = 9.3 Hz, C7'-***H***); **^{13}C NMR (100 MHz, Acetone-*d6*):** 24.4 (C(**C**H$_3$)$_3$, 35.0 (**C**(CH$_3$)$_3$), 52.0 (CO_2**Me**), 68.7 (C4), 70.8 (C3'), 77.1 (C5), 97.5 (C2), 98.7 (C7), 115.0 (C6'), 125.5 (C7'), 141.4 (C8'), 163.5 (**C**O_2Me),

164.0 (C5'), 179.7 (C8), 186.7 (C2'), 189.1 (C6); **m/z (ESI$^-$):** 433.0 ([M-H]$^-$); **HRMS (ESI$^-$):** Found 433.1247. $C_{20}H_{21}N_2O_9^-$ requires 433.1253; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$: +51.7 (c = 1.0 in MeOH).

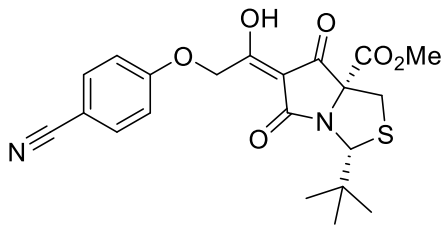
Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(2-(4-cyanophenoxy)-1-hydroxyethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 119o



Chloro-substituted **64o** (354 mg, 1.07 mmol), 4-cyanophenol (191 mg, 1.60 mmol) and Cs_2CO_3 (1.04 g, 3.20 mmol) were dissolved in dry acetonitrile (15 mL). Reaction mixture was heated to reflux for 24 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as a yellow oil. Yield 380 mg, 86 %.

^{1}H NMR ($CDCl_3$, 400 MHz): 0.93 (9H, s, C(C$\boldsymbol{H_3}$)$_3$), 3.55 (1H, d, *J* = 8.9 Hz, C4-$\boldsymbol{H_A}H_B$), 3.81 (3H, s, $CO_2\boldsymbol{Me}$), 4.83 – 4.88 (2H, m, C2-***H*** & C4-$H_A\boldsymbol{H_B}$), 5.22 – 5.32 (2H, m, C3'-$\boldsymbol{H_AH_B}$), 7.00 (2H, d, *J* = 8.5 Hz, C6'-***H***), 7.60 (2H, d, *J* = 8.7 Hz, C7'-***H***); **^{13}C NMR ($CDCl_3$, 100 MHz):** 24.6 (C(**C**H_3)$_3$), 35.2 (**C**(CH_3)$_3$), 53.5 ($CO_2\boldsymbol{Me}$), 65.2 (C3'), 68.1 (C4), 78.4 (C5), 98.4 (C2), 101.0 (C7), 105.5 (C8'), 115.4 (C6'), 118.8 (**C**N), 134.2 (C7'), 160.8 (C5'), 166.7 (**C**O_2Me), 179.9 (C8), 186.9 (C2'), 189.2 (C6); **ν_{max}/cm^{-1}:** 2226 (CN); 1753 (C=O), 1716, 1660 (C=O), 1603 (C=C); **m/z (ESI$^-$):** 413.0 ([M-H]$^-$); **HRMS (ESI$^-$):** Found 413.1351, $C_{21}H_{22}N_2O_7^-$ requires 413.1354; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$: +47.2 (c = 1.0 in MeOH).

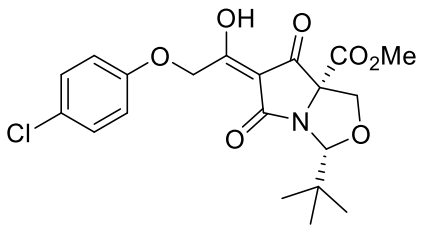
Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(2-(4-cyanophenoxy)-1-hydroxyethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]thiazole-7a(5H)-carboxylate 119s



Chloro-substituted tetramate **64s** (133 mg, 0.380 mmol), 4-cyanophenol (90 mg, 0.760 mmol) and Cs_2CO_3 (619 mg, 1.90 mmol) were dissolved in dry acetonitrile (10 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as an orange oil. Yield 106 mg, 65 %.

R_f: 0.60 (1:19 MeOH:EtOAc); **^{1}H NMR ($CDCl_3$, 400 MHz):** 0.97 (9H, s, C(C$\boldsymbol{H_3}$)), 2.98 (1H, d, *J* = 11.3 Hz, C4-$\boldsymbol{H_A}H_B$), 3.70 (1H, d, *J* = 11.3 Hz, C4-$H_A\boldsymbol{H_B}$), 3.77 (3H, s, CO_2***Me***), 5.11 (1H, s, C2-***H***), 5.32 (2H, app d, *J* = 3.4 Hz, C3'-$\boldsymbol{H_AH_B}$), 7.08 (2H, d, *J* = 8.0 Hz, C6'-***H***), 7.66 (2H, d, *J* = 8.0 Hz, C7'-***H***); **^{13}C NMR ($CDCl_3$, 100 MHz):** 25.6 (C(**C**H_3)), 32.6 (C4), 36.3 (**C**$(CH_3)_3$), 52.4 (CO_2***Me***), 67.7 (C3'), 72.9 (C2), 81.7 (C5), 99.9 (C7), 104.1 (C8'), 115.4 (C6'), 118.5 (CN), 133.8 (C7'), 158.1 (C5'), 168.0 (CO_2Me), 175.4 (C8), 185.3 (C2'), 187.8 (C6); **ν_{max}/cm^{-1}:** 2227 (CN), 1751 (C=O), 1630, 1605; **m/z (ESI$^-$):** 429.0 ([M-H])$^-$; **HRMS (ESI$^-$):** Found 429.1120, $C_{21}H_{22}N_2O_6S^-$ requires 429.1126; $\boldsymbol{[\alpha]_D^{25}}$**:** +107.6 (c = 0.5 in MeOH).

Methyl(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(2-(4-chlorophenoxy)-1-hydroxyethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 120o

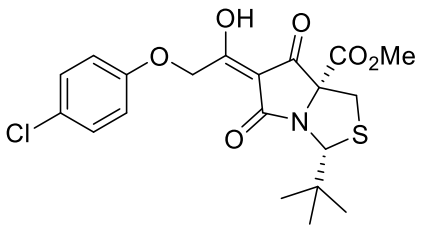


Chloro-substituted tetramate **64o** (98 mg, 0.296 mmol), 4-chlorophenol (76 mg, 0.592 mmol) and Cs_2CO_3 (384 mg, 0.888 mmol) were dissolved in dry acetonitrile (10 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as an orange oil. Yield 125 mg, 86 %.

R_f: 0.55 (1:19 MeOH:EtOAc); **^{1}H NMR (400 MHz, $CDCl_3$):** 0.93 (9H, s, C(C***H*****$_3$)$_3$), 3.54 (1H, d, *J* = 9.0 Hz, C4-***H*****$_A$H$_B$), 3.82 (3H, s, CO_2***Me***), 4.85 (1H, d, *J* = 9.0 Hz, C4-H$_A$***H*****$_B$**), 4.87 (1H, s, C2-***H***), 5.24 (1H, dd, *J* = 17.1 Hz, C2'-***H***), 6.88 (2H, d, *J* = 8.9 Hz, C5'-***H***), 7.25 (2H, d, *J* = 8.9 Hz, C6'-***H***); **m/z (ESI$^+$):** 422.1 ([M-H]$^-$); **$[\alpha]_D^{25}$:** +19.7 (c = 1.0 in $CHCl_3$)(Lit. +20.4 in $CHCl_3$).[97]

Data is in agreement with that found in the literature.[97]

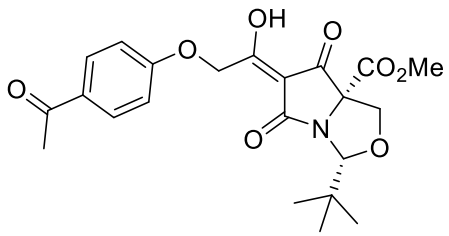
Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(2-(4-chlorophenoxy)-1-hydroxyethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]thiazole-7a(5H)-carboxylate 120s



Chloro-substituted tetramate **64s** (133 mg, 0.402 mmol), 4-chlorophenol (103 mg, 0.803 mmol) and Cs_2CO_3 (520 mg, 1.21 mmol) were dissolved in dry acetonitrile (10 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as an orange oil. Yield 108 mg, 63 %.

R_f: 0.55 (1:19 MeOH:EtOAc); **^{1}H NMR ($CDCl_3$, 400 MHz):** 0.98 (9H, s, C(C***H*****$_3$**)$_3$), 2.88 (1H, d, *J* = 11.6 Hz, C4-***H*****$_A$**H$_B$), 3.75 – 3.81 (4H, m, C4-H$_A$***H*****$_B$** & CO_2***Me***), 5.10 (1H, s, C2-***H***), 5.14 – 5.25 (2H, m, C3'-***H***), 6.87 (2H, d, *J* = 8.4 Hz, C6'-***H***), 7.24 (2H, d, *J* = 8.4 Hz, C7'-***H***); **^{13}C NMR ($CDCl_3$, 100 MHz);** 26.5 (C(**C**H$_3$)$_3$), 33.1 (C4), 36.8 (**C**(CH$_3$)$_3$), 53.5 (CO_2**Me**), 73.6 (C2), 82.9 (C5), 99.6 (C7), 116.1 (C6'), 127.0 (C8'), 129.6 (C7'), 156.3 (C5'), 167.0 (**C**O_2Me), 177.1 (C8), 183.9 (C2'), 188.1 (C6); **ν_{max}/cm^{-1}:** 1753 (C=O), 1712 (C=O), 1665 (C=O), 1284; **m/z (ESI$^-$):** 446.1 ([M-H]$^-$); **HRMS (ESI$^-$):** Found 438.0791, $C_{20}H_{22}ClNO_6S^-$ requires 438.0784; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$: +112.0 (c = 1.0 in MeOH).

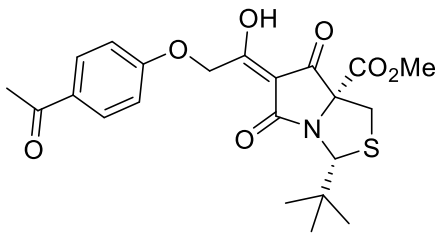
Methyl-(3*R*,7a*R*,*E*)-6-(2-(4-acetylphenoxy)-1-hydroxyethylidene)-3-(tert-butyl)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 121o



Chloro-substituted tetramate **64o** (438 mg, 1.32 mmol), 4-hydroxyacetophenone (360 mg, 2.64 mmol) and Cs_2CO_3 (1.72 g, 5.28 mmol) were dissolved in dry acetonitrile (20 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as an orange oil. Yield 371 mg, 69 %.

R_f: 0.55 (1:19 MeOH:EtOAc); **^{1}H NMR (400 MHz, $CDCl_3$):** 0.93 (9H, s, C(C$\boldsymbol{H_3}$)$_3$), 2.55 (3H, s, COMe), 3.56 (1H, d, *J* = 8.9 Hz, C4-$\boldsymbol{H_a}H_b$), 4.43 – 4.48 (2H, m, C4-$H_A\boldsymbol{H_B}$ & C2-***H***), 5.19 (1H, d, *J* = 17.0 Hz, C3'-$\boldsymbol{H_A}H_B$), 5.36 (1H, d, *J* = 17.0 Hz, C3'-$H_A\boldsymbol{H_B}$), 6.97 (2H, d, *J* = 8.3 Hz, C6'-***H***), 7.94 (2H, d, *J* = 8.4 Hz, C7-***H***); **^{13}C NMR (100 MHz, $CDCl_3$):** 24.6 (C(**C**H_3-)$_3$, 26.4 (CO**Me**), 35.2 (**C**$(CH_3)_3$), 53.6 (CO_2**Me**), 65.0 (C3'), 68.1 (C4), 78.4 (C5), 98.4 (C2), 100.9 (C7), 114.4 (C6'), 130.7 (C7'), 131.5 (qC), 161.3 (qC), 166.8 (**C**O_2Me), 179.9 (C8), 184.8 (C2'), 187.2 (C6), 196.6 (**C**OMe); **ν_{max}/cm^{-1}:** 1754 (C=O), 1717 (C=O), 1672 (C=O), 1598 (C=C); **m/z (ESI$^-$):** 430.1 ([M-H]$^-$); **HRMS (ESI$^-$):** Found 432.1655, $C_{22}H_{26}NO_8^+$ requires 432.1653; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$: +52.3 (c = 1.0 in MeOH).

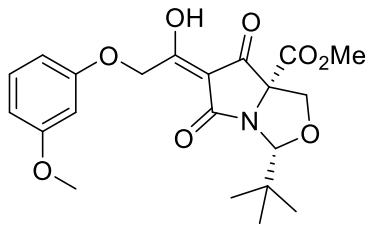
Methyl-(3*R*,7a*R*,*E*)-6-(2-(4-acetylphenoxy)-1-hydroxyethylidene)-3-(tert-butyl)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]thiazole-7a(5H)-carboxylate 121s



Chloro-substituted tetramate **64s** (103 mg, 0.297 mmol), 4-hydroxyacetophenone (81 mg, 0.594 mmol) and Cs_2CO_3 (290 mg, 0.891 mmol) were dissolved in dry acetonitrile (10 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as an orange oil. Yield 133 mg, 51 %.

R_f: 0.60 (1:19 MeOH:EtOAc); **^{1}H NMR (400 MHz, $CDCl_3$):** 0.99 (9H, s, $C(CH_3)_3$), 2.56 (3H, s, COMe), 2.90 (1H, d, *J* = 12.0 Hz, C4-***H***$_A$H$_B$), 3.77 – 3.83 (4H, m, C4-H$_A$***H***$_B$& CO_2Me), 5.11 (1H, s, C2-***H***), 5.21 – 5.33 (2H, m, C3'-***H***), 5.21 – 5.33 (2H, m, C3'-***H***$_A$***H***$_B$), 6.98 (2H, d, *J* = 8.6 Hz, C6'-***H***), 7.94 (2H, d, *J* = 8.5 Hz, C7'-***H***); **^{13}C NMR (100 MHz, $CDCl_3$):** 26.5 ($C(\mathbf{C}H_3)_3$), 33.0 (C4), 36.8 ($\mathbf{C}(CH_3)_3$), 53.6 ($CO_2\mathbf{Me}$), 64.7 (C3'), 73.6 (C2), 82.9 (C5), 99.7 (C7), 114.4 (C6'), 130.7 (C7'), 131.5 (ArC), 161.3 (ArC), 166.9 ($\mathbf{C}O_2Me$), 177.1 (C8), 183.4 (C2'), 188.1 (C6), 196.7 (**C**OMe); **ν_{max}/cm^{-1}**: 1756 (C=O), 1715 (C=O), 1675 (C=O), 1600 (C=C); **m/z (ESI$^+$):** 446.1 ([M-H]$^-$); **HRMS (ESI)$^-$:** Found 446.1270 ([M-H]$^-$), $C_{22}H_{24}NO_7S^-$ requires 446.1279; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$: +126.6 (c = 1.0 in MeOH).

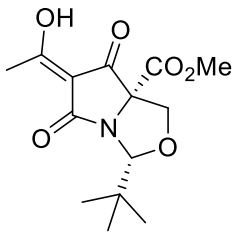
Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxy-2-(3-methoxyphenoxy)ethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 122o



Chloro-substituted tetramate **64o** (80 mg, 0.242 mmol), 3-methoxyphenol (33 mg, 0.266 mmol) and Cs_2CO_3 (619 mg, 0.484 mmol) were dissolved in dry acetonitrile (5 mL). Reaction mixture was heated to reflux for 18 h and monitored by TLC. Upon completion, reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and washed with 2M HCl. Organic layers were concentrated *in vacuo* and purified by flash column chromatography. Crude product was dissolved in EtOAc, washed with 2M HCl, and then a saturated solution of ammonium chloride. Organic layers were combined and concentrated *in vacuo* to yield the title compound as an orange oil. Yield 60 mg, 59 %.

R_f: 0.60 (1:19 MeOH:EtOAc); **^{1}H NMR (400 MHz, MeOD):** 0.93 (9H, s, C(C***H*****$_3$**)$_3$), 3.52 (1H, d, *J* = 8.9 Hz, C4-***H*****$_A$**H$_B$), 3.80 (3H, s, CO_2***Me***), 3.87 (3H, s, O***Me***), 4.82 – 4.86 (2H, m, C4-H$_A$***H*****$_B$** & C2-***H***), 5.25 – 5.35 (2H, m, C3'-***H***), 6.46 – 6.59 (2H, m, Ar-***H***), 7.10 – 7.21 (2H, m, Ar***H***); **^{13}C NMR (100 MHz, MeOD):** 24.7 (C(**C**H$_3$)$_3$), 35.3 (**C**(CH$_3$)$_3$), 53.6 (CO_2***Me***), 56.0 (O***Me***), 66.5 (C3'), 68.3 (C4), 98.4 (C2), 112.3 (ArC), 115.1 (ArC), 120.8 (ArC), 123.2 (ArC), 147.1 (qC), 150.0 (qC), 167.0 (**C**O_2Me), 179.7 (C2'), 185.9 (C8); **ν_{max}/cm^{-1}:** 1754 (C=O), 1718 (C=O), 1660 (C=O), 1504; **m/z (ESI$^-$):** 418.0 ([M-H]$^-$); **HRMS (ESI$^+$):** Found 420.1653, $C_{21}H_{26}NO_8^+$ requires 420.1653; $[\alpha]_D^{25}$: +66.4 (c = 1.0 in MeOH).

Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-(1-hydroxyethylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 135



Synthesised according to general procedure D

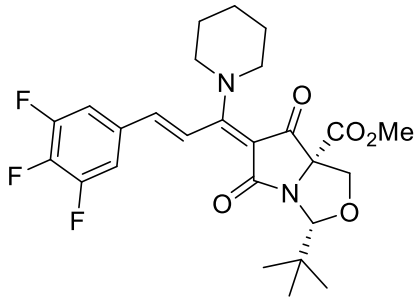
Yellow solid.

Yield 524 mg, 39 %.

^{1}H NMR (400 MHz, $CDCl_3$): 0.93 (9H, s, C(C***H*****$_3$**)$_3$), 2.49 (3H, s, C9-C***H*****$_3$**), 3.50 (1H, d, *J* = 8.9 Hz, C4-***H*****$_A$**H$_B$), 3.79 (3H, s, CO_2***Me***), 4.83 (1H, d, *J* = 9.0 Hz, C4-H$_A$***H*****$_B$**), 4.85 (1H, s, C2-***H***); **m/z (ESI$^-$)**: 296.1 ([M-H]$^-$).

Data are in agreement with those found in the literature..[97]

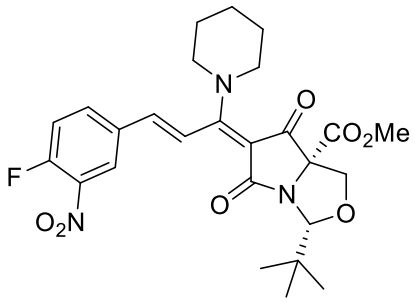
Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-5,7-dioxo-6-((*E*)-1-(piperidin-1-yl)-3-(3,4,5-trifluorophenyl)allylidene)dihydro-1H,3H-pyrrolo[1,2-c]oxazole-7a(5H)-carboxylate 137a



7-Acyl tetramate **135** (200 mg, 0.673 mmol) was dissolved in toluene (10 mL), then 3,4,5-trifluorobenzaldehyde (97 mg, 0.606 mmol) and piperidine (0.1 mL, 1.00 mol) were added. Reaction mixture was then heated to reflux for 18 h until completion by TLC. Once complete, solvent was removed *in vacuo* and the resulting residue was purified by column chromatography to yield the final compound as a yellow oil. Yield 90 mg, 35 %.

R_f: 0.30 (EtOAc); **^{1}H NMR (400 MHz, Acetone-d_6):** 0.74 (9H, s, C(C$\boldsymbol{H_3}$)$_3$), 1.40 – 1.76 (6H, m, C2''-***H*** & C3''-***H***), 2.66 – 2.74 (4H, m, C1''-***H***), 3.42 (1H, d, *J* = 8.4 Hz, C4-$\boldsymbol{H_a}$H$_b$), 3.58 (CO_2***Me***), 4.53 (1H, d, *J* = 8.4 Hz, C4-H$_a$$\boldsymbol{H_b}$), 4.63 (1H, app d, *J* = 1.7 Hz, C2-***H***), 6.90 (1H, d, *J* = 15.7 Hz, C3'-***H***), 7.02 (1H, d, *J* = 15.7 Hz, C4'-***H***), 7.05 – 7.17 (2H, m, C9'-***H*** & C10'-***H***), 7.43 (1H, m, C6'-***H***); **^{13}C NMR (100 MHz, Acetone-d_6):** 24.0, 25.6, 26.4 (C2'' & C3'' & C4''), 24.4 (C(**C**H$_3$)$_3$): 35.0 (**C**(CH$_3$)$_3$): 51.9 (CO_2**Me**), 52.1 (C1'' & C5''), 68.5 (C4), 77.4 (C5), 97.8 (C2), 95.1 (*qC*), 112.5 (ArC), 120.2 (C3'), 123.1 (C4'), 142.4 (qC), 144.5 (qC), 166.6 (qC), 169.8 (qC), 186.6 (C6); **ν_{max}/cm^{-1}:** 1747 (C=O), 1685 (C=O), 1624 (C=C); **HRMS (CI$^+$):** Found 507.2098, $C_{26}H_{30}F_3N_2O_5^+$ requires 507.2101; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$**:** Sample too dark.

Methyl-(3*R*,7a*R*,*E*)-3-(tert-butyl)-6-((*E*)-3-(4-fluoro-3-nitrophenyl)-1-(piperidin-1-yl)allylidene)-5,7-dioxodihydro-1H,3H-pyrrolo[1,2-c]thiazole-7a(5H)-carboxylate 137b

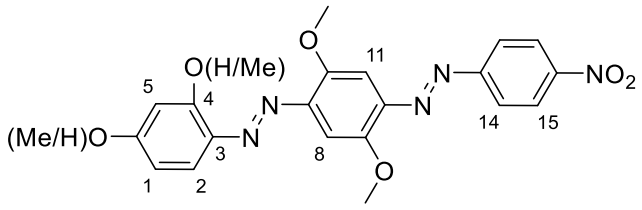


7-Acyl tetramate **135** (153 mg, 0.515 mmol) was dissolved in toluene (10 mL), and then 4-fluoro-3-nitrobenzaldehyde (128 mg, 0.773 mmol) and piperidine (77 µL, 1.00 mol) were added. Reaction mixture was then heated to reflux for 18 h until completion by TLC. Once complete, solvent was removed *in vacuo* and the resulting residue was purified by column chromatography to yield the final compound as a yellow oil. Yield 90 mg, 35 %.

R_f: 0.30 (EtOAc); **^{1}H NMR (500 MHz, Acetone-d_6):** 0.87 (9H, s, (C(C***H***$_3$)$_3$)), 1.60 – 1.82 (6H, m, broad unresolved signals, C2''-***H***, C3''-***H***, C4''-***H***), 3.12 (4H, m, C1''-***H***, C5''-***H***), 3.56 (1H, d, *J* = 8.4 Hz, C4-***H***$_A$H$_B$), 3.71 (3H, s, CO$_2$***Me***), 4.66 (1H, d, *J* = 8.5 Hz, C4-H$_A$***H***$_B$), 4.76 (1H, s, C2-***H***), 7.03 (1H, d, *J* = 15.7 Hz, C3'-***H***), 7.27 (1H, d, *J* = 8.7 Hz, C9'-H), 7.31 (1H, d, *J* = 15.7 Hz, C4'-***H***), 7.80 (1H, dd, *J* = 8.7, 2.2 Hz, C8'-***H***), 8.04 (1H, d, *J* = 2.2 Hz, C5'-***H***); **^{13}C NMR (125 MHz, Acetone-d_6):** 24.2, 24.6, 26.5 (C2'',3'',4''), 25.3 (C(**C**H$_3$)$_3$), 35.9 (**C**(CH$_3$)$_3$), 52.8 (C1'',5''), 69.4 (C4), 77.9 (C5), 95.8 (C7), 98.6 (C2), 119.9 (C3'), 121.7 (C4'), 126.9 (C5'), 127.9 (qC), 133.8 (qC), 142.4 (qC), 145.8 (C9'), 148.1 (qC), 167.7 (qC), 170.8 (qC), 177.5 (C8), 187.4 (C6); **ν_{max}/cm^{-1}:** 1749 (C=O), 1686 (C=O), 1620 (C=C); **HRMS (CI$^+$):** Found 516.2131, $C_{26}H_{31}FN_3O_7^+$ requires 516.2141; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$**:** Sample too dark.

4-((*E*)-(2,5-dimethoxy-4-((*E*)-(4-nitrophenyl)diazenyl)phenyl)diazenyl)-3-methoxyphenol

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3-methoxyphenol (100 mg, 0.806 mmol) was dissolved in EtOH (10 mL) and the reaction mixture was cooled to 0 °C. Once cooled, Fast Black K Salt (337 mg, 0.806 mmol) was added and the mixture was stirred at 5 °C for 18 h. Once complete by LRMS, the reaction mixture was concentrated *in vacuo* to yield the title compound as a dark black solid.

Isolated as a mixtures of isomers

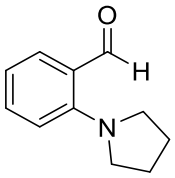
R_f: 0.70 (3:2 EtOAC:Petrol); **M.p:** >250 °C; **^{1}H NMR (400 MHz, $CDCl_3$) Major isomer:** 3.99 (3H, s, O***Me***), 4.00 (3H, s, O***Me***), 4.11 (3H, s, O***Me***), 5.97 (1H, d, *J* = 1.9 Hz, C5-***H***), 6.42 (1H, dd, *J* = 9.8, 1.9 Hz, C1-***H***), 7.26 (1H, d, *J* = 9.6 Hz, C2-***H***), 7.39 (1H, s) & 7.47 (1H, s) (C8-***H*** & C11-***H***), 7.98 (2H, d, *J* = 9.0 Hz, C14-***H***), 8.35 (2H, d, *J* = 9.1 Hz, C15-***H***); **Minor isomer:** 3.87 (3H, s, O***Me***), 4.03 (3H, s, O***Me***), 4.12 (3H, s, O***Me***), 6.28 (1H, d, *J* = 2.6 Hz, C5-***H***), 6.54 (1H, dd, *J* = 9.3, 2.7 Hz, C1-***H***), 7.51 (1H, d, *J* = 9.2 Hz, C2-***H***), 7.47 (1H, s) & 7.63 (1H, s) (C8-***H*** & C11-***H***), 8.03 (2H, d, *J* = 9.0 Hz, C14-***H***), 8.36 – 8.38 (2H, m, C15-***H***); **^{13}C NMR (100 MHz, $CDCl_3$):** 56.3 (O**Me**), 56.6 (O**Me**), 57.1 (O**Me**), 97.8, 98.6 (C8 & C11), 106.1 (C5), 123.3 (C14), 123.6 (qC), 124.9 (C15), 126.3 (C2), 131.7 (qC), 138.7 (C2), 142.1 (qC), 155.5 (qC), 160.4 (qC), 186.7 (qC); **ν_{max}/cm^{-1}:** 2980, 1521, 1338, 1267; **m/z (ESI$^+$):** 438.2 ($[M+H]^+$); **HRMS (ESI$^+$):** Found 438.1409, $C_{21}H_{20}N_5O_6^+$ requires 438.1408;

Experimental for Chapter III

General procedure F. Synthesis of 2-aminobenzaldehydes.

To a stirring solution of K_2CO_3 (1.15 eq) in DMF was added 2-fluorobenzaldehyde (1.0 eq). Amine (1.15 eq) was added dropwise and the mixture heated to reflux until complete by TLC analysis. Solution was then allowed to cool to room temperature, diluted with H_2O and extracted with $CHCl_3$. Organic layers were washed with a saturated solution of NH_4Cl, solvent was removed and residue purified by *flash* chromatography to yield the 2-aminobenzaldehyde.

2-(pyrrolidin-1-yl)benzaldehyde 145a



Synthesised according to general procedure F.

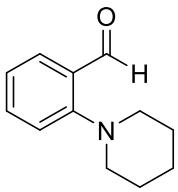
Brown oil.

Yield 377 mg, 76 %.

R_f: 0.20 (1:19 EtOAc:PE); **^{1}H NMR (400 MHz, $CDCl_3$):** 1.98 (4H, m, C3'-***H*** & C4'-***H***), 3.35 (4H, m, C2'-***H*** & C5'-***H***), 6.81 (2H, m, C5-***H***, 7.38 (1H, m, C4-***H***), 7.70 (1H, m, C6-***H***), 10.08 (1H, s, C***H***O); **^{13}C NMR (100 MHz, $CDCl_3$):** 26.0 (C3' & C4'), 52.8 (C2' & C5'), 114.6 (C3), 116.5 (C5), 123.0 (C1), 133.2 (C6), 134.3 (C4), 150.0 (C2), 190.2 (C=O); **m/z (ESI^+):** 176.1 ($[M+H]^+$).

Data are in agreement with those found in the literature.[121]

2-(piperidin-1-yl)benzaldehyde 145b



Synthesised according to general procedure F.

Yellow oil.

Yield 516 mg, 57 %.

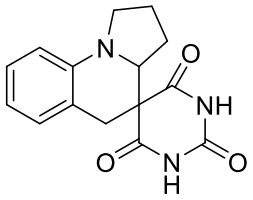
^{1}H NMR (400 MHz, $CDCl_3$): 1.57 – 1.64 (2H, m, C4'-***H***), 1.73 – 1.79 (4H, m, C3'-***H***, C5'-***H***), 3.05 (4H, t, *J* = 5.0 Hz, C2'-***H***, C6'-***H***), 7.04 – 7.10 (2H, m, C3-***H***, C5-***H***), 7.47 – 7.52 (1H, m, *C4*-***H***), 7.79 (1H, dd, *J* = 7.7, 1.7 Hz, C6-***H***), 10.30 (1H, s, C***H***O); **m/z (ESI$^+$):** 190.2 ($[M+H]^+$).

Data are in agreement with those found in the literature.[121]

General procedure G. Synthesis of spirocyclic barbiturates.

Barbiturate (1.0 eq) was dissolved in MeOH and 2-aminobenzaldehyde (1.1 eq) was added. The reaction mixture was heated to 45 °C for 5 – 6 hours. Once complete, the reaction mixture was concentrated *in vacuo* and the resulting residue was purified by *flash* column chromatography to yield the spirocyclic barbiturate.

1',2',3',3a'-tetrahydro-2H,5'H-spiro[pyrimidine-5,4'-pyrrolo[1,2-a]quinoline]-2,4,6(1H,3H)-trione 149a



Synthesised according to general procedure G.

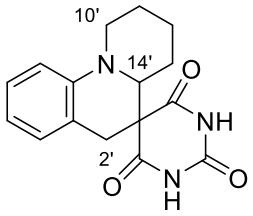
Yellow solid.

Yield 65 mg, 58 %.

R_f: 0.40 (1:3 EtOAc:Petrol); **M.p:** 244 - 246 °C (Lit. 252 °C)[121]; **^{1}H NMR (400 MHz, $CDCl_3$):** 1.69 (1H, dq, *J* = 11.7, 8.9 Hz, C12'-***H***$_A$H$_B$), 1.93 – 2.07 (2H, m, C11'-***H***$_A$***H***$_B$), 2.15 (1H, m, C12'-H$_A$***H***$_B$), 3.06 (1H, d, *J* = 16.5 Hz, C2'-***H***$_A$H$_B$), 3.27 (1H, q, *J* = 7.9 Hz, C10'-***H***$_A$H$_B$), 3.42 (1H, d, *J* = 16.5 Hz, C2'-H$_A$***H***$_B$), 3.55 (1H, td, *J* = 8.3, 3.7 Hz, C10'-H$_A$***H***$_B$), 3.77 (1H, dd, *J* = 9.0, 6.2 Hz, C13'-***H***), 6.53 – 6.60 (2H, m, C5'-***H***, C7'-***H***), 7.00 (1H, d, *J* = 7.4 Hz, C4'-***H***), 7.05 (1H, t, *J* = 7.8 Hz, C6'-***H***); **m/z (ESI$^-$):** 284.1 ([M-H]$^-$).

Data are in agreement with those found in the literature.[121]

2,3,4,4a-tetrahydro-1H,2'H,6H-spiro[pyrido[1,2-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione 149b



Synthesised according to general procedure G.

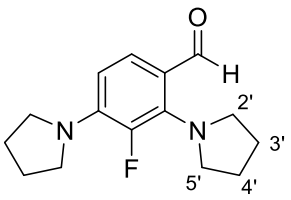
Yellow solid.

Yield 127 mg, 85 %.

R_f: 0.40 (1:3 EtOAc:Petrol); **M.p:** 149 – 150 °C (Lit. 155 – 157 °C)[122]; **^{1}H NMR (400 MHz, $CDCl_3$):** 1.35 – 1.86 (6H, m, C11'-***H*** & C12'-***H*** & C13'-***H***), 2.73 – 2.82 (1H, m, C10'-$\boldsymbol{H_A}H_B$), 3.15 (1H, d, *J* = 16.7 Hz, C2'-$\boldsymbol{H_A}H_B$), 3.23 (1H, d, *J* = 16.8 Hz, C2'-$H_A\boldsymbol{H_B}$), 3.42 (1H, dd, *J* = 11.3, 2.4 Hz, C14'-***H***), 4.01 – 4.07 (1H, m, C10'-$H_A\boldsymbol{H_B}$), 6.68 (1H, td, *J* = 7.4, 1.1 Hz, Ar***H***), 6.82 – 6.85 (1H, m, Ar***H***), 6.95 (1H, dd, *J* = 7.5, 1.5 Hz, Ar***H***), 7.04 – 7.09 (1H, m, Ar***H***); 8.54 (2H, broad s, NH); **m/z (ESI^+):** 300.2 ($[M+H]^+$).

Data are in agreement with those found in the literature.[122]

3-fluoro-2,4-di(pyrrolidine-1-yl)benzaldehyde 151

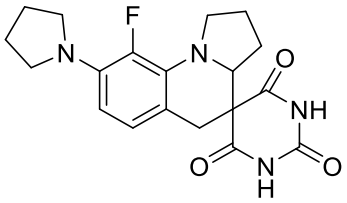


To a stirring solution of K_2CO_3 (1.33 g, 9.65 mmol) in DMF (15 mL) was added 2,3,4-trifluorobenzaldehyde (0.5 mL, 4.38 mmol). Pyrrolidine (0.69 mL, 9.65 mmool) was added

dropwise and the mixture heated to reflux until complete by TLC analysis. Solution was then allowed to cool to room temperature, diluted with H_2O and extracted with $CHCl_3$. Organic layers were washed with a saturated solution of NH_4Cl, then the solvent was removed and residue purified by *flash* chromatography to yield the title compound as a yellow oil. Yield 585 mg, 51 %.

^{1}H NMR (200 MHz, $CDCl_3$): 1.92 – 2.00 (8H, m, C3'-***H***, C4'-***H***), 3.30 – 3.39 (4H, m, C2'-***H***$_A$H$_B$, C5'-***H***$_A$H$_B$), 3.47 – 3.61 (4H, m, C2'-H$_A$***H***$_B$, C5'-H$_A$***H***$_B$), 6.38 (1H, ddd, *J* = 8.8, 7.9, 0.8 Hz C5-***H***), 7.47 (1H, dd, *J* = 8.8, 1.2 Hz, C6'-***H***), 10.05 (1H, s, C***H***O); **^{13}C NMR (100 MHz, $CDCl_3$)**: 25.6, 25.6, 26.1 (C2' & C3'), 50.1, 53.6 (C1' & C4'), 109.8 (C5), 122.4 (qC), 125.9 (C6), 144.7 (qC), 143.1 (qC), 145.9 (qC), 189.9 (C=O); **m/z (ESI$^+$):** 263.2 ($[M+H]^+$), 285.2 ($[M+Na]^+$); **HRMS (ESI$^+$):** Found 263.1552, $C_{15}H_{20}ON_2F^+$ requires 263.1554.

9'-fluoro-8'-(pyrrolidin-1-yl)-1',2',3',3a'-tetrahydro-2H,5'H-spiro[pyrimidine-5,4'-pyrrolo[1,2-a]quinoline]-2,4,6(1H,3H)-trione 152a



Synthesised according to general procedure G.

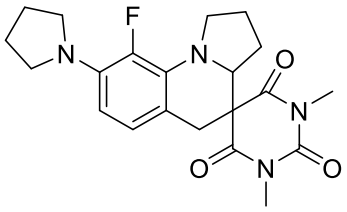
Yellow solid.

Yield 29 mg, 10 %.

R$_f$: 0.70 (1:3 EtOAc:Petrol); **M.p:** 204 – 205 °C; **^{1}H NMR (400 MHz, $CDCl_3$):** 1.72 – 1.89 (2H, m, C11'-***H***$_A$H$_B$, C12'-***H***$_A$H$_B$), 1.89 – 2.01 (5H, m, C11'-H$_A$***H***$_B$, C2''-***H***, C3''-***H***), 2.22 –

2.33 (1H, m, C12'-H_A***H_B***), 2.99 (1H, d, *J* = 16.3 Hz, C2'-***H_A***H_B), 3.30 - 3.40 (4H, m, C1''-***H***, C4''-***H***), 3.40 – 3.45 (1H, m, C10'-***H_A***H_B), 3.50 (1H, d, *J* = 16.3 Hz, C2'-H_A***H_B***), 3.86 (1H, dd, *J* = 7.6 Hz, 3.4 Hz, C13'-***H***), 3.93 – 4.04 (1H, m, C10'-H_A***H_B***), 6.22 (1H, t, *J* = 8.2 Hz, C5'-***H***), 6.69 (1H, d, *J* = 8.3 Hz, C4'-***H***), 8.24 (1H, s, N***H***), 8.57 (1H, s, N***H***); **^{13}C NMR (100 MHz, $CDCl_3$):** 23.7 (C11'), 25.1 (C2'',3''), 27.9 (C12'), 35.2 (C2'), 50.0 (C1'), 52.0 (C10'), 66.0 (C13'), 106.1 (C5'), 122.8 (C4'), 132.8 (qC), 137.0 (qC), 148.7 (qC), 157.2 (qC), 168.5 (qC), 171.3 (qC); **ν_{max}/cm^{-1}:** 1688 (C=O), 1697 (C=O), 1701 (C=O), 3411 (C-H); **m/z (ESI$^+$):** 373.2 ($[M+H]^+$); **HRMS (ESI$^+$):** Found 373.1671, $C_{19}H_{22}FN_4O_3^+$ requires 373.1670.

9'-fluoro-1,3-dimethyl-8'-(pyrrolidin-1-yl)-1',2',3',3a'-tetrahydro-2H,5'H-spiro[pyrimidine-5,4'-pyrrolo[1,2-a]quinoline]-2,4,6(1H,3H)-trione 152b



Synthesised according to general procedure G.

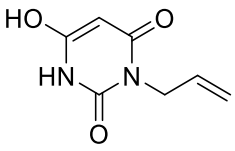
Off-white solid.

Yield 40 mg, 31 %.

R_f: 0.40 (1:3 EtOAc:Petrol); **M.p:** 189 – 190 °C **^{1}H NMR (400 MHz, $CDCl_3$):** 1.51 – 1.73 (2H, m, C11'-***H_A***H_B & C12'-***H_A***H_B), 1.83 – 1.99 (5H, m, C11'-H_A***H_B*** & C2''-***H*** & C3''-***H***), 2.19 (1H, m, C12'-H_A***H_B***), 2.94 (1H, d, *J* = 16.3 Hz, C2'-***H_A***H_B), 3.24 (3H, s, N***Me***), 3.30 – 3.44 (5H, m, C1''-***H*** & C4''-***H*** & C10'-***H_A***H_B), 3.38 (3H, s, N***Me***), 3.54 (1H, d, *J* = 16.4 Hz, C2'-H_A***H_B***), 3.84 (1H, dd, *J* = 7.5, 3.1 Hz, C13'-***H***), 3.98 (1H, m, C10'-H_A***H_B***), 6.22 (1H, t, *J* = 8.3 Hz, C5'-***H***), 6.68 (1H, d, *J* = 8.3 Hz, C4'-***H***); **^{13}C (100 MHz, $CDCl_3$):** 23.5 (C11'), 25.1 (C2'' & C3''), 27.8 (C12'), 28.5 (N**Me**), 29.0 (N**Me**), 35.8 (C2'), 49.2 (C1'), 50.2 (C1'' &

C4''), 52.1 (C10'), 66.6 (C13'), 106.0 (C5'), 112.6 (qC), 122.6 (C4'), 133.0 (qC), 136.8 (qC), 141.3 (qC), 143.6 (qC), 151.4 (qC); **ν_{max}/cm^{-1}:** 1703 (C=O), 1697 (C=O), 1618; **m/z (ESI$^+$):** 401.2 ([M+H]$^+$); **HRMS (ESI$^+$):** Found 401.4619, $C_{21}H_{26}FN_4O_3^+$ requires 401.4625.

3-allyl-6-hydroxypyrimidine-2,4(1H,3H)-dione 154

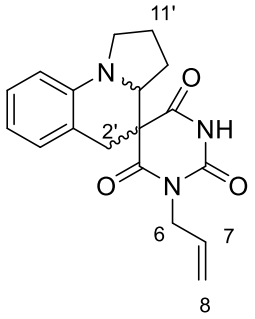


To a solution of N-allylurea (1.0 g, 10.0 mmol) in acetic acid (30 mL) was added malonic acid (1.05 g, 10.00 mmol) at rt. The reaction mixture was added acetic anhydride (20 mL, 21.2 mmol) at 60 °C over a period of 30 min. Once addition was complete, reaction mixture was heated at 90 °C for 5 h. Solvent was removed *in vacuo* and to the resulting suspension was added EtOAc dropwise to precipitate the title compound as an off white solid. Yield 411 mg, 24 %.

M.p: 155 – 156 °C (Lit. 158 °C)[139]; **^{1}H NMR (200 MHz, MeOD):** 4.40 (2H, dt, *J* = 5.6, 1.5 Hz, C1'-***H***), 5.15 (1H, dd, *J* = 10.2, 1.4 Hz, C3'-***H***$_A$H$_B$), 5.23 (1H, dd, *J* = 17.2, 1.4 Hz, C3'-H$_A$***H$_B$***), 5.27 (1H, q, *J* = 1.4 Hz, C2'-***H***), 5.74 – 5.95 (1H, m, C2'-***H***); **m/z (ESI$^-$):** 167.1 ([M-H]$^-$).

Data are in agreement with those found in the literature.[139]

1-allyl-1',2',3',3a'-tetrahydro-2H,5'H-spiro[pyrimidine-5,4'-pyrrolo[1,2-a]quinoline]-2,4,6(1H,3H)-trione 155



Synthesised according to general procedure G.

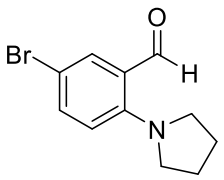
Off white solid.

Yield 51 mg, 53 %. Isolated as a mixture of two diastereomers.

R_f: 0.40 (1:3 EtOAc:Petrol); **M.p:** 186 – 187 °C; **^{1}H NMR (400 MHz, $CDCl_3$):** 1.48 – 1.66 (1H, m, C12'-$\boldsymbol{H_A}H_B$), 1.94 – 2.07 (2H, m C11'-$\boldsymbol{H_AH_B}$), 2.07 – 2.19 (1H, m, C12'- $H_A\boldsymbol{H_B}$), 3.03 (1H, d, *J* = 16.0 Hz, C2'-$\boldsymbol{H_A}H_B$, major isomer), 3.05 (1H, broad d, *J* = 16.0 Hz, C2'-$\boldsymbol{H_A}H_B$, minor isomer), 3.31 (1H, dd, *J* = 8.8, 7.1 Hz, C10'-$\boldsymbol{H_A}H_B$), 3.55 (1H, d, *J* = 16.0 Hz, C2'-$H_A\boldsymbol{H_B}$), 3.58 – 3.63 (1H, m, C10'- $H_A\mathbf{H_B}$), 3.91 (1H, ddd, *J* = 9.4, 6.1, 3.7, C13'-***H***), 4.34 (1H, m, C6-$\boldsymbol{H_A}H_B$), 4.52 (1H, dd, *J* = 6.1, 1.5 Hz, C6-$H_A\boldsymbol{H_b}$), 5.15 – 5.36 (2H, m, C8-***H***), 5.71 – 5.93 (1H, m, C7-***H***), 6.60 (1H, dd, *J* = 8.1, 4.7 Hz, (C7'-***H***), 6.67 (1H, td, *J* = 7.4, 2.9 Hz, C5'-***H***), 7.03 (1H, d, *J* = 7.4 Hz, C4'-***H***), 7.17 (1H, m, C6'-***H***); **^{13}C NMR (100 MHz, $CDCl_3$):** 23.3 (C11'), 27.9 (C12'), 36.7 (C2', major isomer), 37.1 (C2', minor isomer), 43.1 (C6, minor isomer), 43.7 (C6, major isomer), 47.7 (C10, minor isomer), 47.8 (C10, major isomer), 48.8 (C1', minor isomer), 49.3 (C1', major isomer), 63.7 (C13'), 111.2 (C7', minor isomer), 111.4 (C11', major isomer), 116.2 (C5', minor isomer), 116.3 (C5', major isomer), 117.8 (qC, minor isomer), 117.8 (qC, major isomer), 119.2 (C8, minor isomer), 119.3 (C8, major isomer), 127.6 (C6'), 128.2 (C4'), 131.0 (qC), 131.1 (qC), 143.2 (qC), 149.6 (qC),

166.5 (C=O, major isomer), 166.7 (C=O, minor isomer), 170.6 (C=O, major isomer), 170.9 (C=O, minor isomer); **m/z (ESI$^-$):** 324.1 ([M-H]$^-$), **HRMS (ESI$^+$):** Found 169.0611, $C_7H_9N_2O_3^+$ requires 169.0608.

5-bromo-2-(pyrrolidin-1-yl)benzaldehyde 159



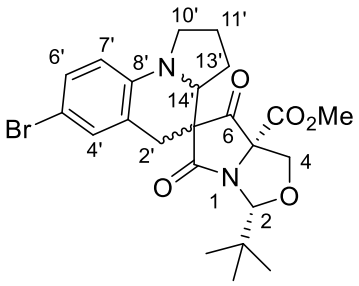
Synthesised according to general procedure F.

Brown oil.

Yield 478 mg, 73 %.

R_f: 0.25 (1:19 EtOAc:Petrol); **^{1}H NMR (200 MHz, $CDCl_3$):** 1.99 (4H, m, C3'-***H*** & C4'-***H***), 3.34 (4H, m, C2'-***H*** & C5'-***H***), 6.71 (1H, d, *J* = 9.1 Hz, C3-***H***), 7.41 (1H, dd, *J* = 9.1, 2.6 Hz, C4-***H***), 7.78 (1H, d, *J* = 2.6 Hz, C6-***H***), 10.01 (1H, s, C***H***O); **^{13}C NMR (100 Hz, $CDCl_3$):** 26.0 (C3' & C4'), 52.9 (C2' & C5'), 108.2 (C5), 116.5 (C3), 123.9 (C1), 134.6 (C6), 136.7 (C4), 148.9 (C2), 188.8 (C=O); **ν_{max}/cm^{-1}**: 1739 (C=O), 1559 & 1458 (C=C), 1152 (C-N); **m/z (ESI$^+$):** 254.0, 256.0 ([M+H$^+$]); **HRMS (ESI$^+$):** Found 254.0175, 256.0155, $C_{11}H_{12}BrNO$ requires: 254.0175, 256.0155.

Methyl-(3a*R*,3'*R*,4*S*,7a'*R*)-7-bromo-3'-(tert-butyl)-5',7'-dioxo-1,2,3,3a-tetrahydro-1'H,3'H,5H,5'H-spiro[pyrrolo[1,2-a]quinoline-4,6'-pyrrolo[1,2-c]oxazole]-7a'(7'H)-carboxylate 160

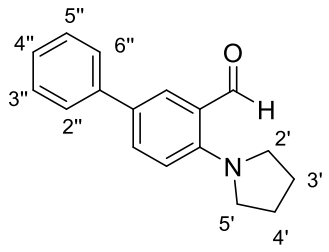


5-bromo-2-(pyrrolidin-1-yl)benzaldehyde (72.7 mL, 1.1 eq) and serine tetramate (100 mg, 1.0 eq) were refluxed in MeOH (6 mL) for 6 h. Upon completion by TLC, solution was cooled to 0°C overnight and solvent removed by rotary evaporation to yield a brown gummy solid. Yield 393 mg, 62 %.

Isolated as three diastereoisomers in solution.

R_f: 0.80 (1:3 EtOAc:Petrol); **^{1}H NMR (400 MHz, *benzene*-d_6) Major isomer:** 0.81 (1H, m, C13'-***H*$_A$**H$_B$), 1.02 (9H, s, C(C***H*$_3$**)$_3$), 1.33 (1H, m, C11'-***H*$_A$**H$_B$), 1.62 (1H, m, C13'-H$_A$***H*$_B$**), 1.82 (1H, m, C11'-H$_A$***H*$_B$**), 2.84-2.90 (1H, m, C10'-***H*$_A$**H$_B$), 3.05 (1H, m, C10'-H$_A$***H*$_B$**), 3.12 (1H, d, *J* = 8.9 Hz, C4-***H*$_A$**H$_B$), 3.19 (1H, d, *J* = 19.3 Hz, C2'-***H*$_A$**H$_B$), 3.26 (3H, s, O***Me***), 3.45 (1H, m, C2'-H$_A$***H*$_B$**), 3.67 (1H, dd, *J* = 10.4, 5.6 Hz, C14'-***H***), 4.74 (1H, d, *J* = 8.9 Hz, C4-H$_A$***H*$_B$**), 5.12 (1H, s, C2-***H***), 6.11 (1H, d, *J* = 8.8 Hz, C7'-***H***), 7.09 (1H, d, *J* = 2.5 Hz, C4'-***H***), 7.27 (1H, dd, *J* = 8.7, 2.7 Hz, C6'-***H***); **^{13}C NMR (100 MHz, *benzene*-d_6) Major isomer:** 23.4 (C13'), 24.6 (C(**C**H$_3$)$_3$), 28.5 (C11'), 35.3 (**C**(CH$_3$)$_3$), 36.9 (C2'), 47.2 (C10'), 51.9 (C7), 53.0 (CO$_2$**C**H$_3$), 60.0 (C14'), 67.6 (C4), 78.1 (C5), 98.7 (C2), 107.5 (C5'), 112.4 (C7'), 117.6 (C3'), 130.9 (C4'), 131.1 (C6'), 142.7 (C8'), 166.8 (**C**O$_2$CH$_3$), 177.9 (C8), 199.4 (C6); **ν_{max}/cm^{-1}**: 1742 (C=O), 1718 (C=O), 1112 (C-O); **m/z (ESI$^+$):** 491.2 ([M+H]$^+$), 493.0 ([M+H]$^+$); **HRMS (ESI$^+$):** Found 491.1174, $C_{23}H_{28}BrN_2O_5^+$ requires: 491.1176.

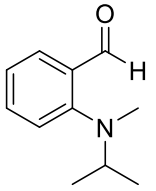
4-(pyrrolidin-1-yl)-[1,1'-biphenyl]-3-carbaldehyde 162



5-bromo-2-(pyrrolidin-1-yl)benzaldehyde (430 mg, 1.68 mmol) and phenylboronic acid (310 mg, 2.52 mmol) were dissolved in anhydrous THF (20 mL). To the solution was added $Pd(dppf)Cl_2$ (62.8 mg, 0.216 mmol) followed by Cs_2CO_3 (1.65 g in 2 mL H_2O, 5.07 mmol). Mixture was stirred at r.t. for 40 min then heated to reflux for 48 h. Once complete by TLC, the mixture was allowed to cool to r.t. and filtered through a pad of celite with the aid of EtOAc. Solvent was removed by rotary evaporation and the residue taken up into EtOAc, washed with brine (1 x 15 mL) and a saturated solution of sodium bicarbonate (2 x 15 mL). Organic layers were concentrated *in vacuo* and residue purified by flash chromatography to yield the title compound as a yellow oil. Yield 267 mg, 63 %.

R_f: 0.75 (1:19 EtOAc:PE); **^{1}H NMR (200 Hz, $CDCl_3$):** 2.02 (4H, m, C3'-***H_AH_B***, C4'-***H_AH_B***), 3.42 (4H, m, C2'-***H_AH_B***, C5'-***H_AH_B***), 6.92 (1H, d, *J* = 8.8 Hz, C3-***H***), 7.32 (1H, m, C4''-***H***), 7.43 (2H, m, C3''-***H***, C5''-***H***), 7.58 (2H, m, C2''-***H***, C6''-***H***), 7.67 (1H, dd, *J* = 8.8, 2.4 Hz, C4-***H***), 7.96 (1H, d, *J* = 2.4 Hz, C6-***H***), 10.17 (1H, s, C***H***O); **^{13}C NMR (100 Hz, $CDCl_3$):** 26.0 (C3' & C4'), 52.8 (C2' & C5'), 115.2 (C3), 123.0 (qC), 126.2 (C2'' & C6''), 126.6 (C4''), 128.8 (C3'' & C5''), 129.3 (qC), 131.2 (C6), 132.8 (C4), 140.0 (qC), 149.3 (qC), 190.2 (**C**HO); **ν_{max}/cm^{-1}**: 1738 (C=O), 1541, 1494, 1449 (C=C); **HRMS (EI$^+$):** Found 251.1298 ($[M]^+$), $C_{17}H_{17}NO$ requires 251.1310.

2-(isopropyl(methyl)amino)benzaldehyde 164c



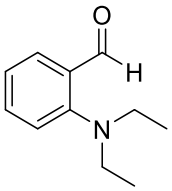
Synthesised according to general procedure F.

Dark orange oil.

Yield 138 mg, 16 %.

R_f: 0.50 (4:1 DCM:Hexane);**^{1}H NMR (400 MHz, $CDCl_3$):** 1.14 (6H, d, *J* = 6.6 Hz, CH(C***H*****$_3$**)$_2$), 2.72 (3H, S, NC***H*****$_3$**), 3.46 (1H, hep, *J* = 6.5 Hz, C***H***$(CH_3)_2$), 7.00 (1H, m, C5-***H***), 7.07 (1H, m, C3-***H***), 7.45 (1H, ddd, *J* = 8.2, 7.2, 1.8 Hz, C4-***H***), 7.77 (1H, dd, *J* = 7.7, 1.8 Hz, C6-***H***), 10.19 (1H, s, C***H***O); **^{13}C NMR (100 MHz, $CDCl_3$):** 18.8 (CH(**C**$H_3)_2$), 32.3 (NCH_3), 59.0 (**C**H$(CH_3)_2$), 120.2 (C3), 121.2 (C5), 128.8 (C1), 129.5 (C6), 134.3 (C4), 156.1 (C2) 191.5 (**C**HO); **ν_{max}/cm^{-1}:** 1684.4 (C=O). 1454, 1519, 1595; **HRMS (ESI$^+$):** Found 178.1226, $C_{11}H_{16}NO^+$ requires 178.1226;

2-(diethylamino)benzaldehyde 164d



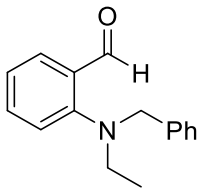
Synthesised according to general procedure F.

Brown oil.

Yield 150 mg, 18 %.

R_f: 0.50 (4:1 DCM:Hexane); **^{1}H NMR (400 MHz, $CDCl_3$):** 1.05 (6H, t, *J* = 7.1 Hz, CH_2CH_3), 3.18 (4H, q, *J* = 7.1 Hz, CH_2CH_3), 7.08 (1H, t, *J* = 7.6 Hz, C5-***H***), 7.15 (1H, dd, *J* = 8.2, 1.0 Hz, C3-***H***), 7.49 (1H, ddd, *J* = 8.2, 7.2, 1.8 Hz, C4-***H***), 7.80 (1H, dd, *J* = 7.7, 1.7 Hz, C6-***H***), 10.35 (1H, s, C***H***O); **^{13}C NMR (100 MHz, $CDCl_3$):** 12.5 (CH_2CH_3), 49.1 (CH_2CH_3), 121.8 (C3), 122.4 (C5), 129.0 (C6), 130.9 (C1), 134.4 (C4), 154.7 (C2), 192.3 (CHO); **ν_{max}/cm^{-1}:** 1683 (C=O), 1450, 1482 & 1594 (C=C). **HRMS (CI$^+$):** Found 178.1234, $C_{11}H_{16}NO^+$ requires 178.1226.

2-(benzyl(ethyl)amino)benzaldehyde 164g



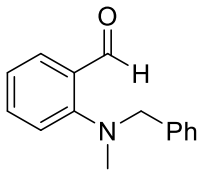
Synthesised according to general procedure F.

Yellow oil.

Yield 574 mg, 25 %

R_f: 0.70 (4:1 DCM:Hexane); **^{1}H NMR (400 MHz, $CDCl_3$):** 1.07 (3H, t, *J* = 7.1 Hz, CH_2CH_3), 3.18 (2H, q, *J* = 7.1 Hz, CH_2CH_3), 4.32 (2H, s, CH_2Ph), 7.11 (2H, m, Ar-***H***), 7.53 (4H, m, Ar-***H***), 7.82 (1H, dd, *J* = 7.7, 1.8 Hz, C6-***H***), 7.89 (2H, m, Ar-***H***), 10.47 (1H, s, C***H***O); **^{13}C NMR (100 MHz, $CDCl_3$):** 11.9 (CH_2CH_3), 49.7 (CH_2CH_3), 58.5 (CH_2Ph), 122.1 (ArC), 122.7 (ArC), 127.4 (ArC), 128.4 (ArC), 128.5 (ArC), 129.1 (ArC), 129.2 (ArC), 129.8 (ArC), 130.4 (ArC), 134.5 (ArC), 137.9 (ArC), 154.6 (C2), 191.9 (CHO); **ν_{max}/cm^{-1}:** 1739 (C=O); 1453, 1481, 1595; **m/z (ESI$^+$):** 240.2 ($[M+H]^+$); **HRMS (ESI$^+$):** Found 240.1384, $C_{16}H_{18}NO^+$ requires 240.1383.

2-(benzyl(methyl)amino)benzaldehyde 164h



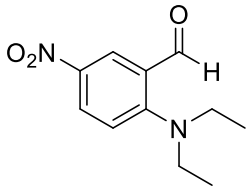
Synthesised according to general procedure F.

Yellow oil.

Yield 246 mg, 12 %.

R_f: 0.70 (4:1 DCM:Hexane); **^{1}H NMR (400 MHz, $CDCl_3$):** 2.77 (3H, s, C***H*****$_3$**), 4.29 (2H, s, C***H*****$_2$**), 7.03 (2H, m, C5-***H*** & Ar-***H***), 7.22 (3H, m, Ar-***H***), 7.28 (1H, m, Ar-***H***), 7.43 (1H, m, Ar-***H***), 7.77 (1H, dd, *J* = 7.7, 1.7 Hz, C6-***H***), 10.35 (1H, s, C***H***O); **^{13}C NMR (100 MHz, $CDCl_3$):** 42.4 (**C**H_3), 62.5 (**C**H_2), 119.6 (C3), 121.7 (ArC), 127.5 (ArC), 128.1 (C2' & C6'), 128.6 (C3' & C5'), 128.2 (C1), 130.3 (ArC), 134.8 (ArC), 137.5 (C1'), 155.8 (C2), 191.4 (**C**HO); **ν_{max}/cm^{-1}:** 1712 (C=O), 1453, 1484, 1596; **m/z (ESI$^+$):** 226.0 ([M+H$^+$]); **HRMS (ESI$^+$):** Found 226.1226, $C_{15}H_{16}NO^+$ requires 226.1226.

2-(diethylamino)-5-nitrobenzaldehyde 164i



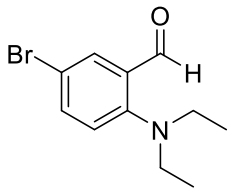
Synthesised according to general procedure F.

Yellow solid.

Yield 326 mg, 50 %.

R_f: 0.55 (4:1 DCM:Hexane); **^{1}H NMR (400 MHz, $CDCl_3$):** 1.23 (6H, t, *J* = 7.1 Hz, CH_2CH_3), 3.44 (4H, q, *J* = 7.1 Hz, CH_2CH_3), 7.02 (1H, d, *J* = 9.3 Hz, C3-***H***), 8.20 (1H, dd, *J* = 9.3, 2.9 Hz, C4-***H***), 8.59 (1H, d, *J* = 2.8 Hz, C6-***H***), 10.01 (1H, s, C***H***O); **^{13}C NMR (100 MHz, $CDCl_3$):** 12.7 ($CH_2\mathbf{C}H_3$), 48.1 ($\mathbf{C}H_2CH_3$), 118.2 (C3), 125.3 (qC), 128.5 (C6), 128.7 (C4), 139.4 (qC), 156.9 (C2), 188.5 (**C**HO); **ν_{max}/cm^{-1}:** 1739 (C=O), 1435, 1572, 1592 (C=$C_{Aromatic}$), 1366 (NO_2); **m/z (ESI$^+$):** 223.0 ($[M+H]^+$); **HRMS (ESI$^+$):** Found 223.1079, $C_{11}H_{15}N_2O_3^+$ requires 223.1077.

5-bromo-2-(diethylamino)benzaldehyde 164j



Synthesised according to general procedure F.

Yellow oil.

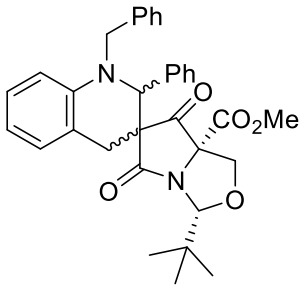
Yield 304 mg, 47 %.

R_f: 0.75 (4:1 DCM:Hexane); **^{1}H NMR (400 MHz, $CDCl_3$):** 1.05 (6H, t, *J* = 7.1 Hz, CH_2CH_3), 3.17 (4H, q, *J* = 7.1 Hz, CH_2CH_3), 7.03 (1H, d, *J* = 8.7 Hz, C3-***H***), 7.56 (1H, dd, *J* = 8.7, 2.6 Hz, C4-***H***), 7.89 (1H, d, *J* = 2.6 Hz, C6-***H***), 10.24 (1H, s, C***H***O); **^{13}C NMR (100 MHz, $CDCl_3$):** 12.4 ($CH_2\mathbf{C}H_3$), 49.1 ($\mathbf{C}H_2CH_3$), 115.6 (C5), 123.7 (C3), 131.8 (C6), 132.1 (C1), 137.0 (C4), 153.5 (C2), 190.7 (**C**HO); **ν_{max}/cm^{-1}:** 1739 (C=O), 1467, 1585, 1680; **HRMS (CI$^+$):** Found 256.0333, $C_{11}H_{15}BrNO^+$ requires 256.0332.

General procedure H. Synthesis of spirocyclic tetramic acids.

Tetramic acid (1.0 eq) was dissolved in MeOH and 2-aminobenzaldehyde (1.1 eq) was added. The reaction mixture was heated to reflux for 6 hours. Upon completion by TLC, the precipitate was filtered and the filtrate was concentrated *in vacuo.* The resulting residue was purified by *flash* column chromatography to yield the spirocyclic tetramic acid.

Methyl(3*R*,7a*R*)-1'-benzyl-3-(tert-butyl)-5,7-dioxo-2'-phenyl-1',4'-dihydro-1H,2'H,3H,5H-spiro[pyrrolo[1,2-c]oxazole-6,3'-quinoline]-7a(7H)-carboxylate 165b



Synthesised according to general procedure H.

Yellow oil.

Yield 46 mg, 43 %. Isolated as a mixture of 4 diasteromers.

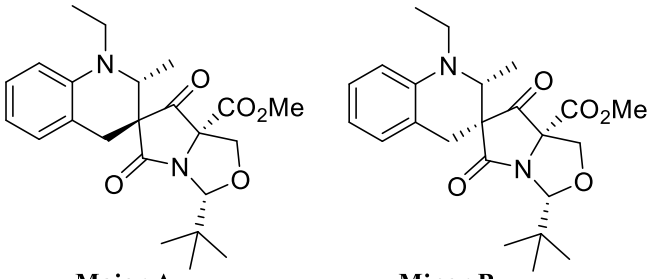
R_f: 0.70 (1:3 EtOAc:PE); **^{1}H NMR (500 Hz, C_6D_6) Major isomer:** 1.13 (9H, s, C(C***H*****$_3$**)$_3$), 2.73 (1H, dd, *J* = 17.3, 1.8 Hz, C2'-***H*****$_A$**H$_B$), 3.22 (1H, d, *J* = 9.5 Hz, C4-***H*****$_A$**H$_B$) 3.64 (1H, d, *J* = 17.3 Hz, C2'-H$_A$***H*****$_B$**), 4.18 (1H, d, *J* = 17.8 Hz, C11'-***H*****$_A$**H$_B$), 4.51 (1H, d, *J* = 17.9 Hz, C11'-H$_A$***H*****$_B$**), 5.17 (1H, s, C2-***H***), 5.60 (1H, d, *J* = 1.7 Hz, C14'-***H***), 6.67 (1H, d, *J* = 8.2 Hz, Ar-***H***), 6.75 – 7.38 (m, Ar-***H***), 7.43 (2H, dd, *J* = 7.5, 2.0 Hz, Ar-***H***); **^{13}C NMR (125 Hz, C_6D_6) Major isomer:** 24.8 (C(**C**H$_3$)$_3$), 27.6 (C2'), 35.2 (**C**(CH$_3$)$_3$), 52.6 (CO_2**Me**), 52.7 (C4), 53.4 (C11'), 63.6 (C14'), 98.4 (C2), 110.6 (ArC), 117.3 (ArC), 126.1 (ArC), 126.7 – 128.6 (ArC), 129.2 (ArC), 138.2 (ArC), 144.3 (ArC), 167.2 (**C**O_2Me), 175.0 (C8), 199.1 (C6);

ν_{max}/cm^{-1}: 1744 (C=O), 1712 (C=O), 1602; **m/z (ESI$^+$):** 539.2 ([M+H]$^+$); **HRMS (ESI$^+$):** Found 539.2537, $C_{33}H_{35}N_2O_5^+$ requires 539.2540.

Methyl-(2'*R*,3*R*,6*R*,7a*R*)-3-(tert-butyl)-1'-ethyl-2'-methyl-5,7-dioxo-1',4'-dihydro-1H,2'H,3H,5H-spiro[pyrrolo[1,2-c]oxazole-6,3'-quinoline]-7a(7H)-carboxylate (Major)

Methyl-(2'*R*,3*R*,6*S*,7a*R*)-3-(tert-butyl)-1'-ethyl-2'-methyl-5,7-dioxo-1',4'-dihydro-1H,2'H,3H,5H-spiro[pyrrolo[1,2-c]oxazole-6,3'-quinoline]-7a(7H)-carboxylate (Minor)

165d



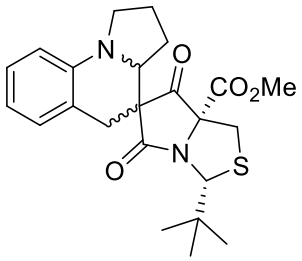
Serine tetramate (50 mg, 0.39 mmol) and 2-diethylaminebenzaldehyde (76 mg, 0.43 mmol) were dissolved in MeOH (5 mL). Reaction mixture was heated to reflux for 6 h until shown complete by TLC analysis. Upon completion, solvent was removed *in vacuo* to yield the title compound as a mixture of 2 diastereomers (Major A, Minor B). Yield 11 mg, 7 %.

R_f: 0.90 (1:19 MeOH:EtOAc); **^{1}H NMR (600 Hz, $CDCl_3$) Major isomer A:** 1.00 (3H, t, *J* = 7.1 Hz, $NCH_2C\boldsymbol{H_3}$), 1.13 (9H, s, $C(C\boldsymbol{H_3})_3$), 1.20 (3H, d, *J* = 6.6 Hz, C14-***H***), 2.60 (1H, d, *J* = 17.4 Hz, C2'-***H'***), 3.04 (1H, t, *J* = 7.2 Hz, $NC\boldsymbol{H_A}H_BCH_3$), 3.12 (1H, d, *J* = 9.0 Hz, C4-$\boldsymbol{H_A}H_B$), 3.24 (1H, t, *J* = 6.9 Hz, $NCH_A\boldsymbol{H_B}CH_3$), 3.32 (3H, s, $CO_2\boldsymbol{Me}$), 3.58 (1H, d, *J* = 17.4 Hz, C2'-***H***), 4.40 (1H, qd, *J* = 6.6, 1.3 Hz, C14'-***Me***), 4.79 (1H, d, *J* = 9.0 Hz, C4-$H_A\boldsymbol{H_B}$), 5.23 (1H, s, C2-***H***), 6.68-6.73 (2H, m, Ar-***H***), 6.87 (1H, td, *J* = 7.4, 1.1 Hz, Ar-***H***), 7.12-7.19 (m, Ar-***H***). **Minor isomer B:** 1.04 (3H, t, *J* = 7.1 Hz, $NCH_2C\boldsymbol{H_3}$), 1.10 (9H, s, $C(C\boldsymbol{H_3})_3$), 1.16 (3H, d, *J* =

4.1 Hz, C14-***Me***), 2.68 (1H, d, *J* = 17.4 Hz, C2'-***H'***), 3.01 (1H, t, *J* = 7.2 Hz, NC***H***$_A$H$_B$CH$_3$), 3.19 (1H, d, *J* = 9.0 Hz, C4-***H***$_A$H$_B$), 3.30 (3H, s, CO$_2$***Me***), 3.65 (1H, d, *J* = 17.1 Hz, C2'-***H***), 4.56 (1H, qd, *J* = 6.6, 1.2 Hz, C14'-***H***), 4.85 (1H, d, *J* = 9.0 Hz, C4-H$_A$***H$_B$***), 5.14 (1H, s, C2-***H***), 6.65-6.74 (m, Ar-***H***), 6.80 – 6.94 (m, Ar-***H***), 7.13-7.20 (m, Ar-***H***); **^{13}C NMR (150 Hz, CDCl$_3$) Major isomer:** 13.6 (CH$_2$**C**H$_3$), 15.0 (C14**C**H$_3$), 24.8 (C(**C**H$_3$)$_3$), 26.5 (C2'), 45.3 (**C**H$_2$CH$_3$), 52.6 (CO$_2$**C**H$_3$), 55.9 (C14'), 69.4 (C4), 78.3 (C5), 98.6 (C2), 112.0 (ArC), 116.9 (ArC), 129.4 (ArC), 142.0 (ArC), 167.8 (**C**O$_2$Me), 177.5 (C8), 199.2 (C6); **m/z (ESI$^+$):** 415.0 ([M+H]$^+$) **HRMS (ESI$^+$):** Found 415.2225, $C_{23}H_{31}N_2O_5^+$ requires 415.2227.

See appendix for characterisation by CLIP-HSQMBC NMR analysis.

Methyl-(3'*R*,7a'*R*)-3'-(tert-butyl)-5',7'-dioxo-1,2,3,3a-tetrahydro-1'H,3'H,5H,5'H-spiro[pyrrolo[1,2-a]quinoline-4,6'-pyrrolo[1,2-c]thiazole]-7a'(7'H)-carboxylate 169



Synthesised according to general procedure H.

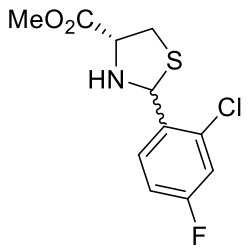
Brown solid.

Yield 51 mg, 32 %. Isolated as a mixture of four diastereomers.

R$_f$: 0.80 (EtOAc); **^{1}H NMR (400 Hz, benzene–*d*$_6$): Major isomer:** 0.92 (1H, m, C13'-***H$_A$***H$_B$), 1.00 (9H, s, C(C***H$_3$***)$_3$), 1.40 (2H, m, C11'-***H$_A$H$_B$***), 1.71 (dtd, 1H, *J* = 11.6, 6.0, 2.2 Hz, C13'-H$_A$***H$_B$***), 2.63 (1H, d, *J* = 11.5 Hz, C4-***H$_A$***H$_B$), 2.93 (1H, m, C10'-***H$_A$***H$_B$), 3.07 (3H, s, OC***H$_3$***), 3.11 (1H, m, C10'-H$_A$***H$_B$***), 3.29 (1H, d, *J* = 16.0 Hz, C2'-***H$_A$***H$_B$), 3.40 (1H, d, *J* = 15.9 Hz, C2'-H$_A$***H$_B$***), 3.71 (1H, d, *J* = C4-H$_A$***H$_B$***), 3.82 (1H, dd, *J* = 10.0, 4.0 Hz, C14'-***H***), 5.55 (1H, s, C2-***H***), 6.48 (1H, dd, *J* = 8.2, 1.2 Hz, C7'-***H***), 6.70 (1H, t, *J* = 7.4 Hz, C5'-***H***),

6.94 (1H, d, *J* = 7.6 Hz, C4'-***H***), 7.20 (1H, t, *J* = 7.6 Hz, C6'-***H***); **^{13}C NMR (100 MHz, benzene-d_6) Major isomer:** 23.9 (C13'), 26.6 (C(**C**H_3)$_3$), 28.9 (C11'), 34.4 (**C**(CH_3)$_3$), 38.1 (C2'), 47.7 (C10'), 51.2 (C7), 53.0 (OCH_3), 60.7 (C14'), 74.4 (C2), 82.9 (C5), 111.3 (C7'), 116.2 (C5'), 128.5 (C6'), 128.9 (C4'), 144.2 (C8'), 167.7 (**C**O_2CH_3), 176.9 (C8), 200.8 (C6); **ν_{max}/cm^{-1}**: 1745 (C=O), 1711 (C=O), 1606 (C=O), 1507 (C=C), 1462 (C=C); **m/z (ESI$^+$):** 429.2 ([M+H]$^+$), 451.2 ([M+Na]$^+$); **HRMS (ESI$^+$):** Found 429.1848, $C_{23}H_{29}N_2O_4S^+$ requires: 429.1843.

Methyl(R)-2-(2-chloro-4-fluorophenyl)thiazolidine-4-carboxylate 174



Synthesised according to general procedure A.

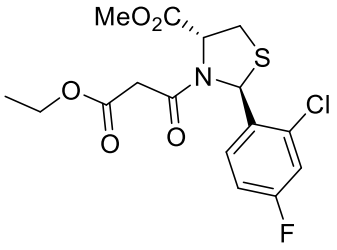
Colourless oil.

Yield 604 mg, 31 %. Isolated as a 1:1.8 mixture of *cis:trans* isomers.

R_f: 0.60 (1:3 EtOAc:Petrol); **^{1}H NMR (200 MHz, $CDCl_3$) Major isomer:** 3.05–3.17 (1H, m, C4-***H_A***H_B), 3.35 (1H, dd, *J* = 10.6, 6.4 Hz, C4-H_A***H_B***), 3.82 (3H, s, CO_2***Me***), 4.22 (1H, t, *J* = 6.6 Hz, C5-***H***), 6.03 (1H, s, C2-***H***), 6.91 – 7.02 (1H, m, Ar***H***), 7.07–7.17 (1H, m, Ar***H***), 7.57 (1H, dd, *J* = 8.8, 6.3 Hz, Ar***H***); **Minor isomer:** 3.05–3.17 (1H, m, C4-***H_A***H_B), 3.47 (1H, dd, *J* = 10.3, 6.9 Hz, C4-H_A***H_B***), 3.81 (3H, s, CO_2***Me***), 4.01 (1H, dd, J = 9.1, 6.9 Hz, C5-***H***), 5.90 (1H, s, C2-***H***), 6.91 – 7.02 (1H, m, Ar***H***), 7.07–7.17 (1H, m, Ar***H***), 7.74 (1H, dd, *J* = 8.7, 6.0 Hz, Ar***H***); **m/z (ESI$^+$):** 276.0 ([M+H]$^+$).

Data are in agreement with those found in the literature.[50]

Methyl-(2*S*,4*R*)-2-(2-chloro-4-fluorophenyl)-3-(3-ethoxy-3-oxopropanoyl)thiazolidine-4-carboxylate 175 b



Synthesised according to general procedure B.

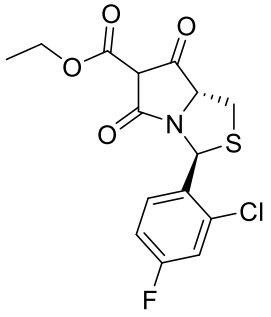
White solid.

Yield 375 mg, 63 %.

R_f: 0.30 (2:5 EtOAc:Petrol); **M.p:** 32 – 33 °C (Lit. 32 – 33 °C)[50]; **^{1}H NMR (200 MHz, $CDCl_3$); mixture of conformers;** 1.21 (3H, t, *J* = 7.2 Hz, $OCH_2C\boldsymbol{H_3}$, major conformer), 1.29 (3H, t, *J* = 7.2 Hz, $OCH_2C\boldsymbol{H_3}$, minor conformer), 3.04 – 3.52 (4H, m, C4-$\boldsymbol{H_AH_B}$), C2'-$\boldsymbol{H_AH_B}$), 3.79 (3H, s, $CO_2\boldsymbol{Me}$, major conformer), 3.85 (3H, s, $CO_2\boldsymbol{Me}$, minor conformer), 4.08 (2H, q, *J* = 7.1 Hz, $OC\boldsymbol{H_2}CH_3$, major conformer), 4.21 (2H, q, *J* = 7.2 Hz, $OC\boldsymbol{H_2}CH_3$), minor conformer), 5.21 (1H, dd, *J* = 4.5, 2.3 Hz, C5-***H***, major conformer), 5.30 (1H, dd, *J* = 6.6, 1.3 Hz, C5-***H***, minor conformer), 6.42 (1H, s, C2-***H***, major conformer), 6.45 (1H, s, C2-***H***, minor conformer), 6.87 – 7.36 (3H, m, Ar-***H***); **m/z (ESI$^+$):** 412.0 ($[M+Na]^+$).

Data are in agreement with those found in the literature.[50]

Ethyl-(3*S*,7a*R*)-3-(2-chloro-4-fluorophenyl)-5,7-dioxotetrahydro-1H,3H-pyrrolo[1,2-c]thiazole-6-carboxylate 176b

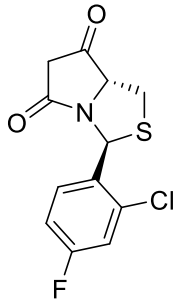


To a stirring solution of 2*S*, 5*R* N-acyl thiazolidine (1.0 eq) in anhydrous THF (30 mL/g) was added KO^{t}Bu (1.2 eq). Reaction mixture was then heated at reflux for 3 h. The reaction mixture was then separated between diethyl ether and H_2O, aqueous phase was acidified using 2M HCl and extracted with ethyl acetate. Organic layers were concentrated *in vacuo* and residue was purified by column chromatography. Products were dissolved in DCM and washed with 5 % citric acid. Organic layer was dried, filtered and concentrated to yield the title compound as a yellow solid. Yield 185 mg, 58 %.

^{1}H NMR (200 MHz, $CDCl_3$): 1.39 (3H, t, *J* = 7.1 Hz, $CH_2C\boldsymbol{H_3}$), 2.99 (1H, dd, *J* = 11.1, 8.9 Hz, C4-$\boldsymbol{H_A}H_B$), 3.28 (1H, dd, *J* = 11.0, 6.8 Hz, C4-$H_A\boldsymbol{H_B}$), 4.40 (2H, q, *J* = 7.1 Hz, $C\boldsymbol{H_2}CH_3$), 4.91 (1H, dd, *J* = 8.9, 6.8 Hz, C5-***H***), 6.45 (1H, s, C2-***H***), 6.97 (1H, td, *J* = 8.2, 2.6 Hz, C5'-***H***), 7.14 (1H, dd, *J* = 8.3, 2.6 Hz, C3'-***H***), 7.39 (1H, dd, *J* = 8.7, 5.9 Hz, C6'-***H***); **m/z (ESI$^-$):** 356.0 ([M-H]$^-$); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = -337.1 (c = 0.30 in MeOH).

Data are in agreement with those found in the literature.[50]

(3*S*,7a*R*)-3-(2-chloro-4-fluorophenyl)dihydro-3H,5H-pyrrolo[1,2-c]thiazole-5,7(6H)-dione 177



To a stirred solution of tetramic acid **176b** (0.52 mmol) in acetonitrile (5 mL) was added one drop of distilled water. Reaction was heated to reflux for 6 h until complete by TLC. Solvent was removed *in vacuo* to yield the title compound as a dark orange oil. Yield 138 mg, 93 %.

R_f: 0.15 (1:6 MeOH:EtOAc); **^{1}H NMR (200 MHz, $CDCl_3$):** 3.09 (1H, dd, *J* = 10.8, 9.6 Hz, C4-$\boldsymbol{H_A}H_B$), 3.19 – 3.34 (2H, m, C4-$H_A\boldsymbol{H_B}$, C7-$\boldsymbol{H_A}H_B$), 3.46 (1H, d, *J* = 22.1 Hz, C7-$H_A\boldsymbol{H_B}$), 4.78 (1H, dd, *J* = 9.6, 7.0 Hz, C5-***H***), 6.75 (1H, s, C2-***H***), 6.99 (1H, td, *J* = 8.2, 2.5 Hz, C5'-***H***), 7.17 (1H, dd, *J* = 8.2 Hz, 2.5 Hz, C3'-***H***), 7.30 (1H, dd, *J* = 8.7, 5.8 Hz, C6'-***H***); **^{13}C NMR (100 MHz, $CDCl_3$):** 33.2 (C4), 43.4 (C7), 60.4 (C2), 72.4 (C5), 100.0 (C1'), 114.4 (C6'), 117.8 (C5'), 126.5 (C3'), 133.5 (C2'), 163.4 (C4'), 169.0 (C8), 202.1 (C6); **ν_{max}/cm^{-1}:** 2981 (C-H), 1696 (C=O_{Ketone}), 1649 (C=O_{Amide}), 1232 (C-F), 728.3 (C-Cl); **m/z (ESI$^-$):** 284.0 ([M-H]$^-$); **HRMS (ESI$^-$):** Found 283.9954, $C_{12}H_8ClFNO_2S^-$ requires 283.9954; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = -257.0 (c = 0.25 in $CHCl_3$).

Attempted syntheses

Attempted deprotection of spirocyclic tetramic acids with TFA.

Spirocyclic tetramic acid (1.0 eq) was dissolved in a 1:1 mixture of THF and H_2O. To the stirred solution was added TFA (excess) dropwise. The reaction mixture was stirred at rt for 18 h until consumption of starting materials was observed by TLC. Once complete, the reaction mixture was extracted with EtOAc, organic layers combined and concentrated *in vacuo*. Resulting residue was purified by *flash* column chromatography. No fractions containing the expected product were obtained.

Attempted hybridisation of enamine tetramic acids with ampicillin.

Enamine tetramic acid **137b** (100 mg, 0.197 mmol), ampicillin sodium salt (82 mg, 0.217 mmol), and K_2CO_3 (61 mg, 0.395 mmol) were dissolved in DMF (5 mL). The reaction mixture was heated to reflux for 72 h until consumption of starting materials was observed by TLC. Once complete, the reaction mixture was separated between H_2O and EtOAc, the organic layers combined and concentrated *in vacuo* however no signs of product formation were observed. The aqueous layer was acidified using citric acid and extracted with EtOAc and concentrated *in vacuo* however no signs of product formation were observed.

Attempted microwave assisted hybridisation of tetramic acids and ampicillin.

Tetramic acid (**115o, 116o, 117o**) was dissolved in DMF in a microwave tube and ampicillin (1.0 eq) was added. The reaction mixture was microwave irradiated for 5, 10, and 15-minute intervals at a temperature of 100, 115, and 150 °C respectively. The reaction was monitored by TLC and LRMS however no product formation was observed and only starting material was recovered.

Attempted synthesis of amoxicillin – tetramic acid hybrids.

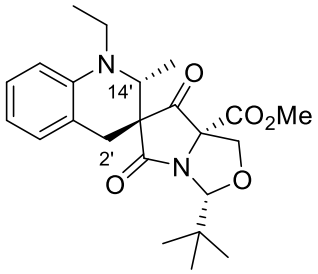
Tetramic acid **9o** (91 mg, 0.275 mmol) was dissolved in MeCN (5 mL) then amoxicillin (200 mg, 0.550 mmol) and Cs_2CO_3 (1.10 mmol) were added. The reaction mixture was heated to reflux for 24 h and the reaction was monitored by LRMS and TLC, which revealed no consumption of starting materials.

Attempted synthesis of metronidazole – tetramic acid hybrids.

Metronidazole (127 mg, 0.740 mmol) was dissolved in MeCN (10 mL) and NaH (44 mg, 1.11 mmol) was added. Reaction mixture was stirred for 10 min and then tetramic acid **9o** (245 mg, 0.740 mmol) was added portion wise. The eaction mixture was heated to reflux for 24 h and monitored by TLC. LRMS and TLC confirmed that starting materials were present and no product formation was observed.

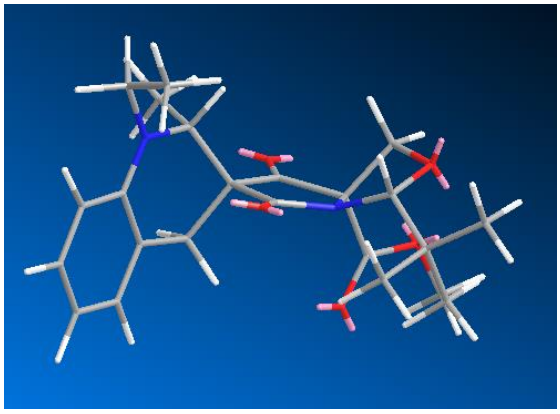
Appendix

Characterisation of 165d by CLIP-HSQMBC.



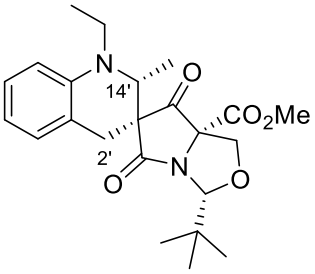
165d Major isomer

Major Isomer (by NMR)	^3J value $C{=}O_{Ketone}$	^3J value $C{=}O_{Amide}$
H-14’	< 1 Hz	< 1 Hz
H-2’$_A$	9.1 Hz	2.3 Hz
H-2’$_B$	2.0 Hz	< 1 Hz



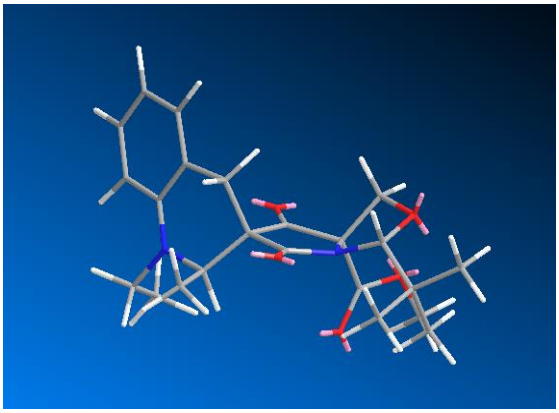
Major Isomer (RRRR)

Major Isomer (by NMR)	Dihedral Angle $C{=}O_{Ketone}$	Dihedral Angle $C{=}O_{Amide}$
H-14’	50.7 °	68.3°
H-2’$_A$	168.0 °	49.7 °
H-2’$_B$	57.6 °	60.7 °



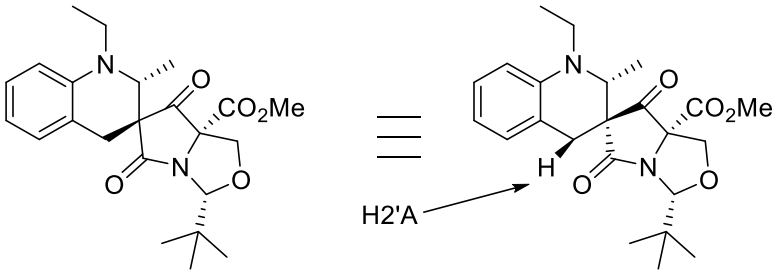
165d Minor isomer

Minor Isomer (by NMR)	^3J value C=O$_{Ketone}$	^3J value C=O$_{Amide}$
H-14’	< 1 Hz	< 1 Hz
H-2’$_A$	~ 3 Hz	9.2 Hz
H-2’$_B$	< 1 Hz	2.4 Hz

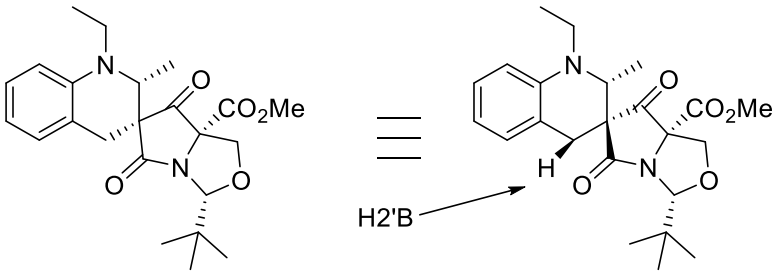


Minor Isomer (RR<u>S</u>R)

Minor Isomer (by NMR)	Dihedral Angle C=O$_{Ketone}$	Dihedral Angle C=O$_{Amide}$
H-14’	68.0 °	51.0 °
H-2’$_A$	58.7 °	169.0 °
H-2’$_B$	59.6 °	50.7 °

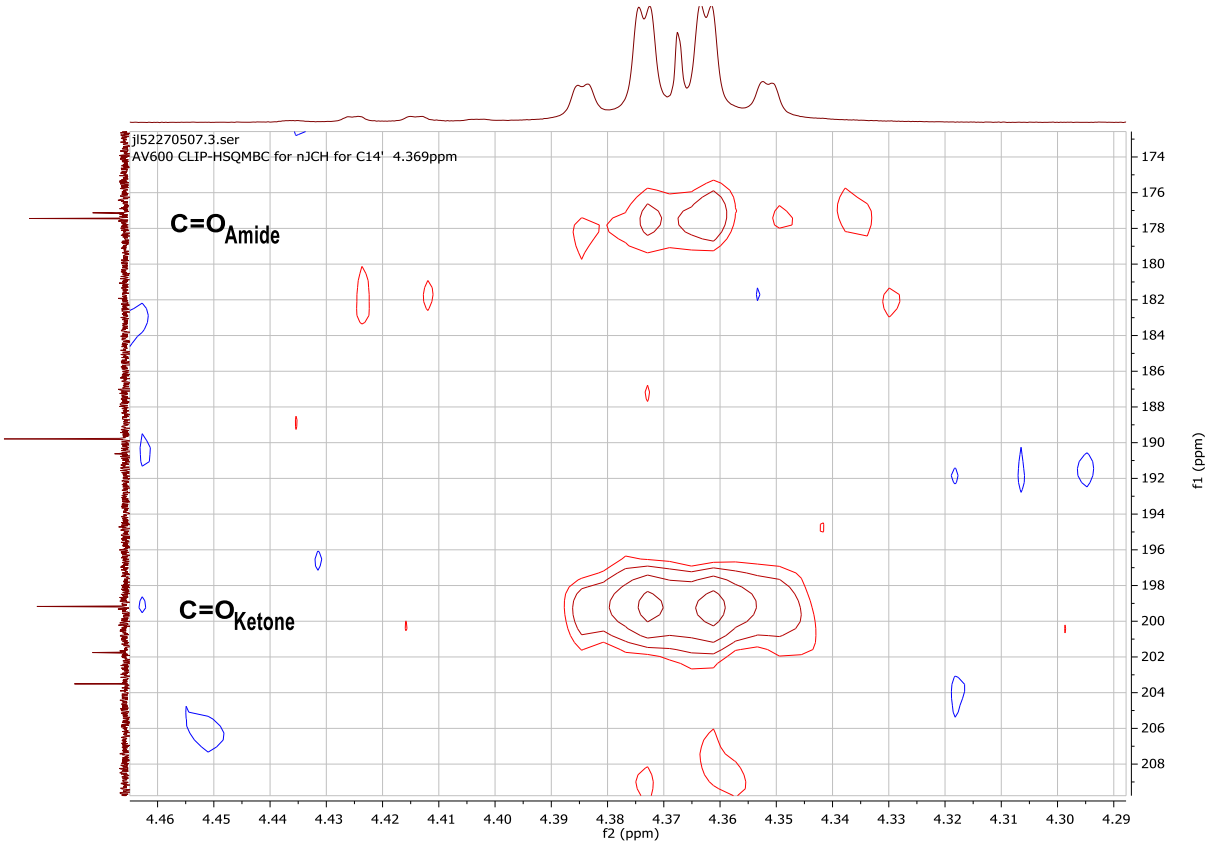


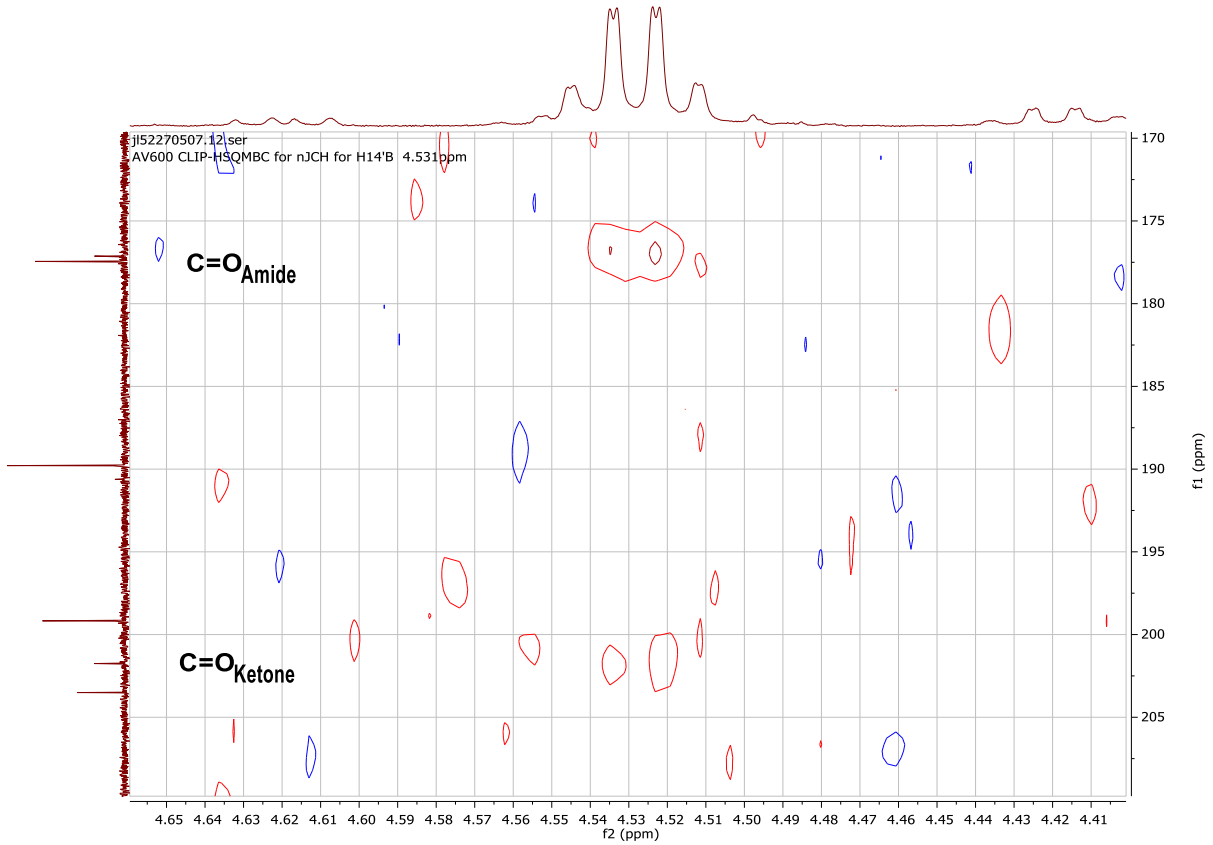
Major isomer



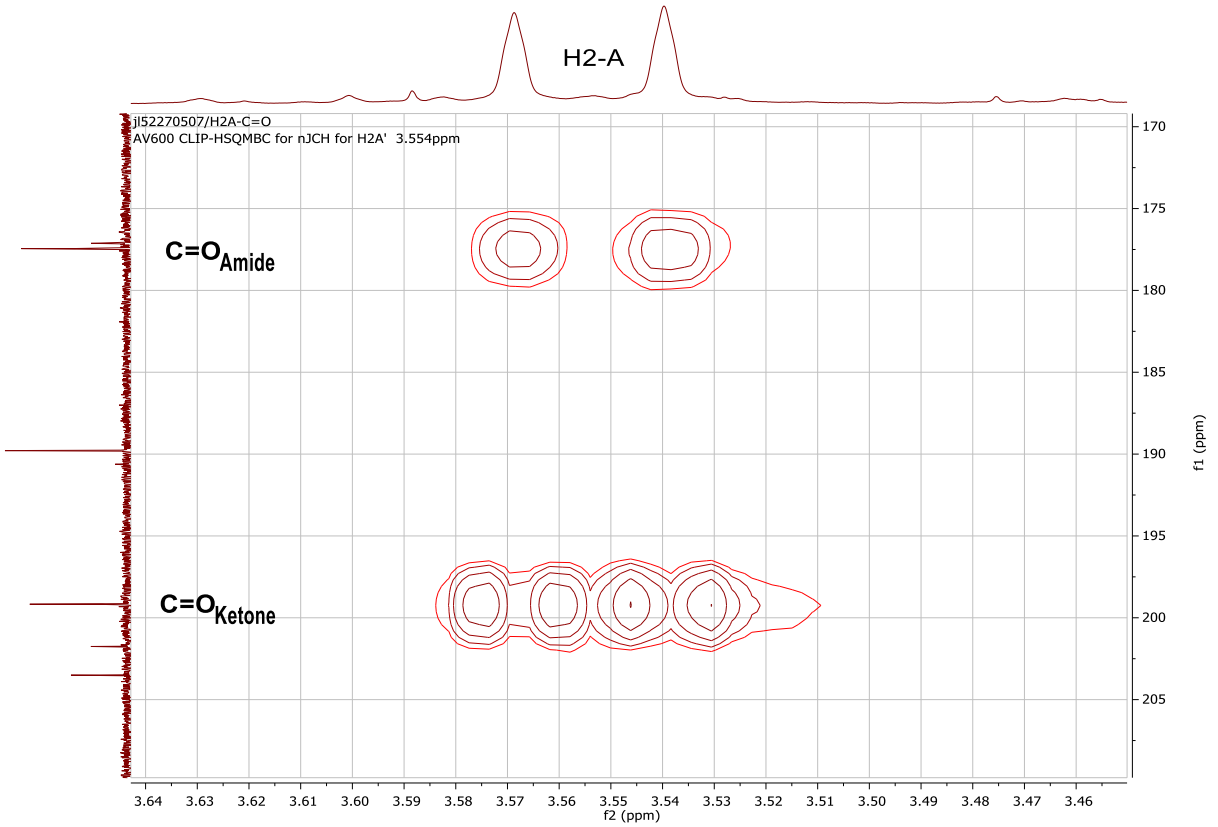
Minor isomer

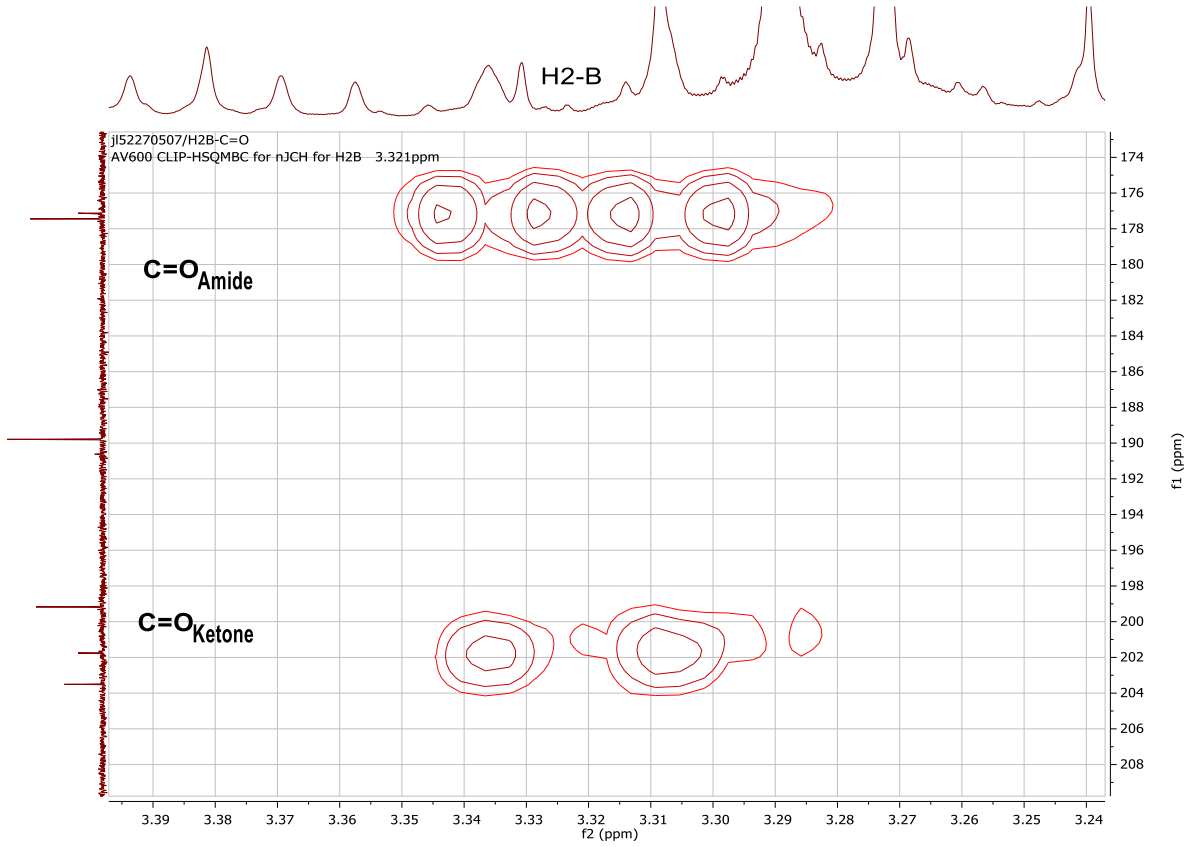
H-14 CLIP-HSQMBC Coupling.



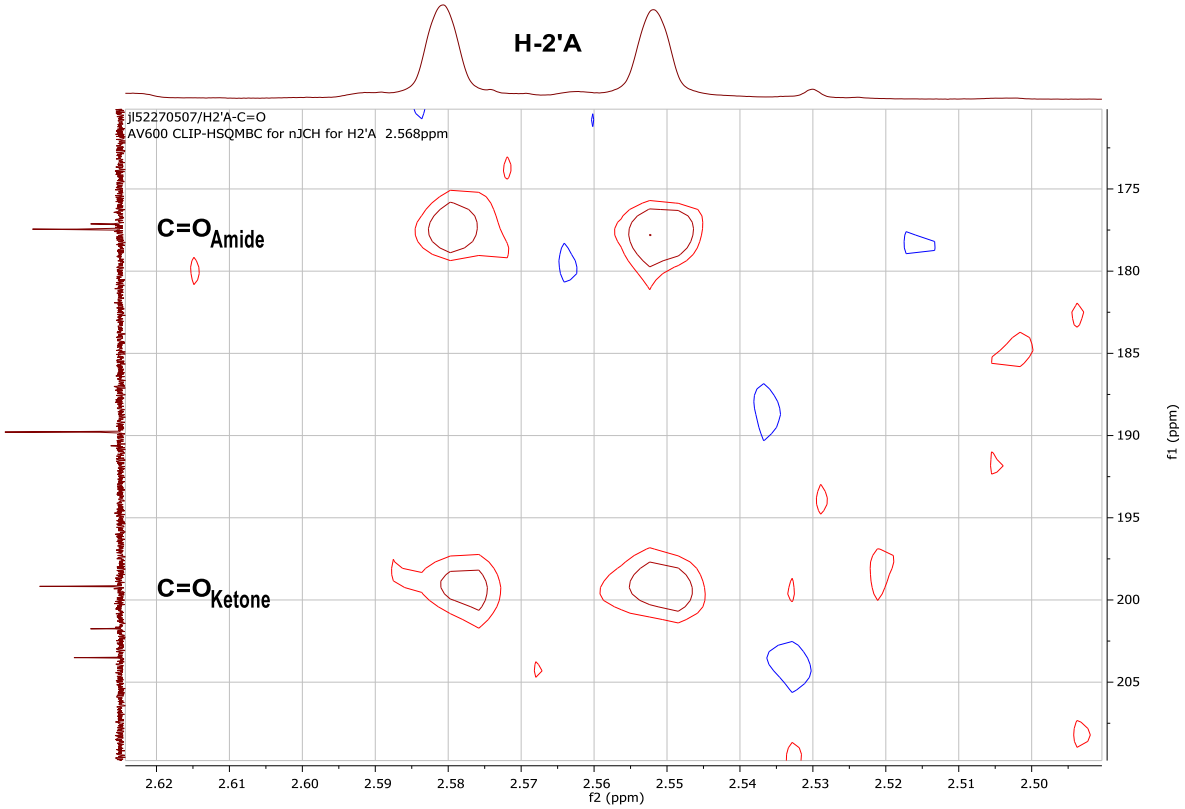


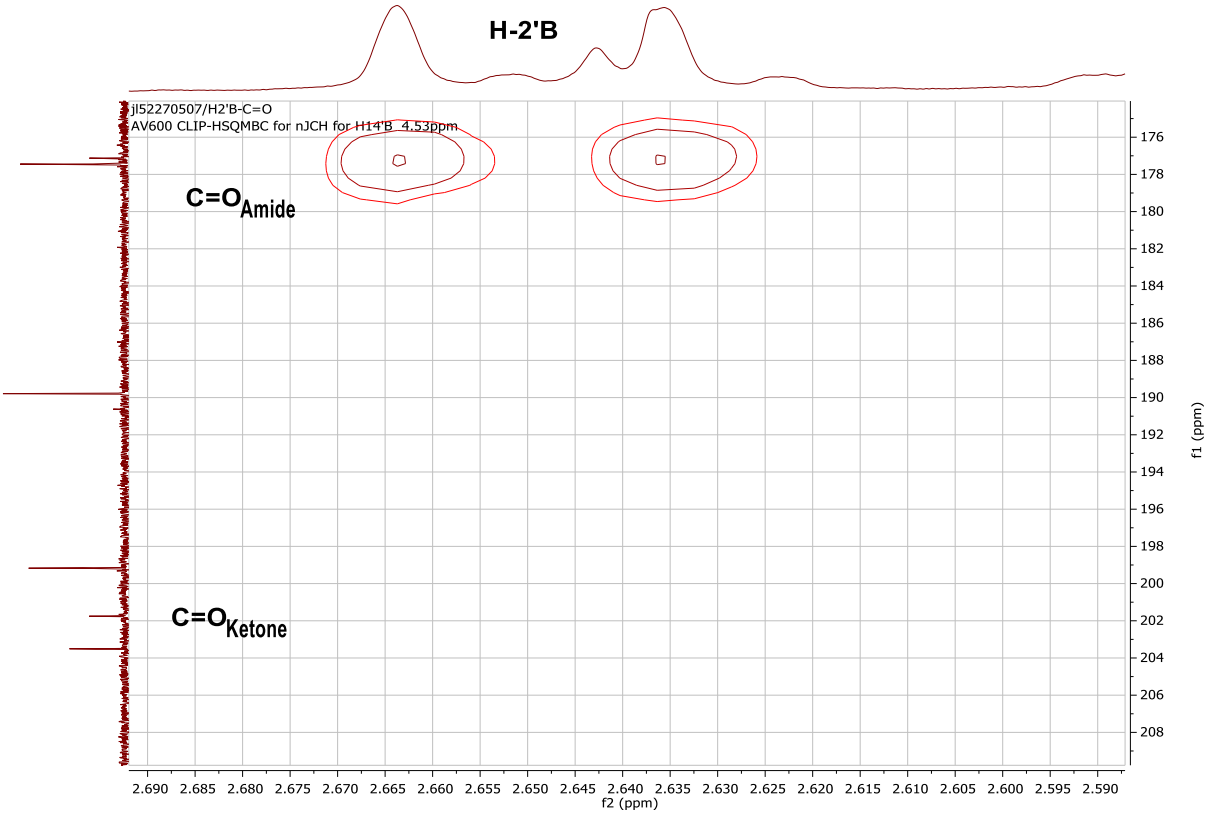
H2A & H2B CLIP-HSQMBC Coupling



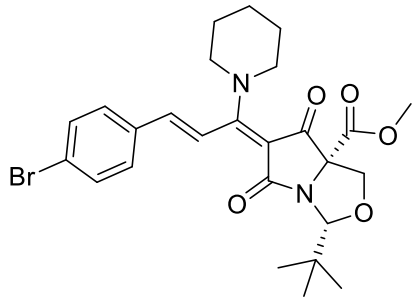
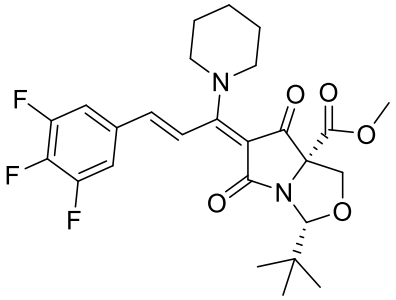
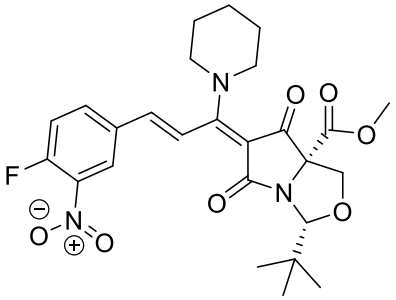
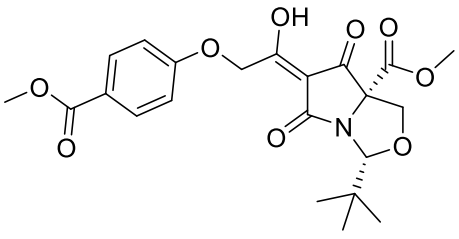
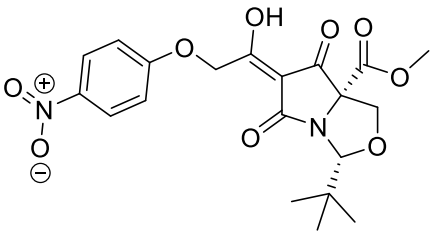


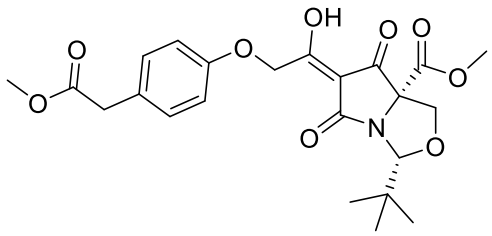
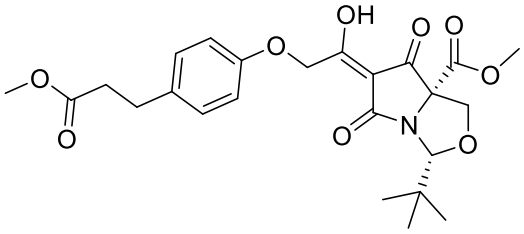
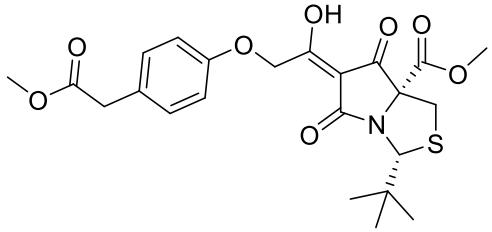
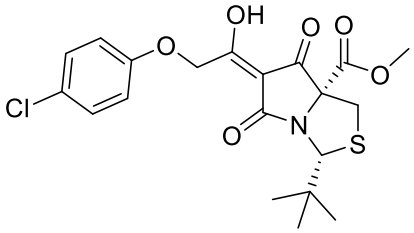
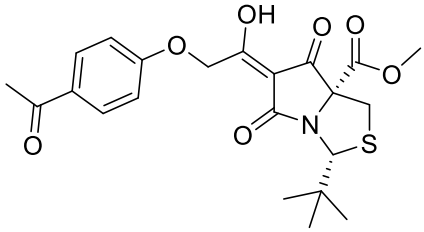
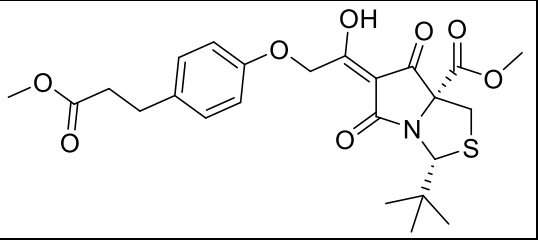
H2'A & H2'B CLIP-HSQMBC Coupling

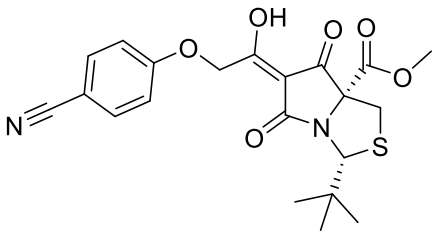
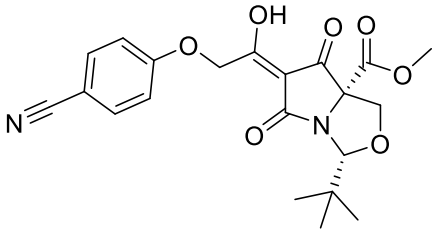
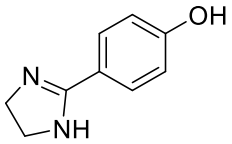
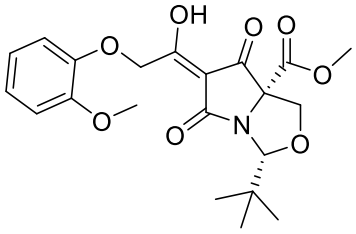
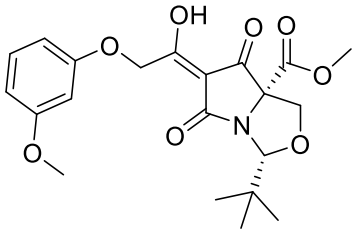
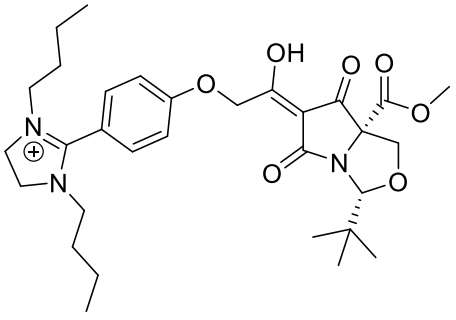


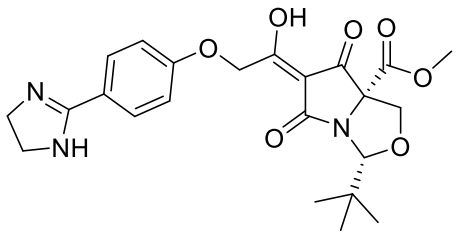
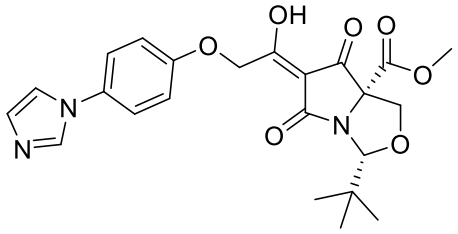
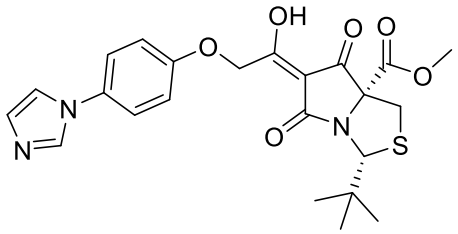
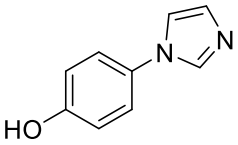
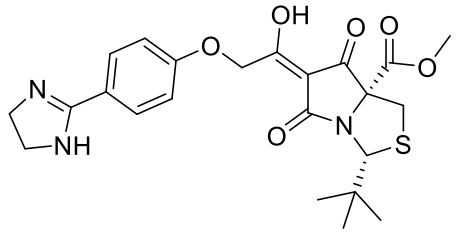
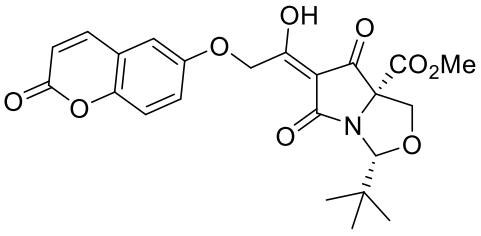
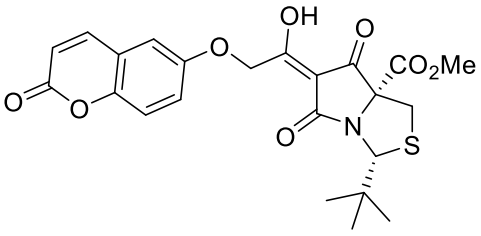


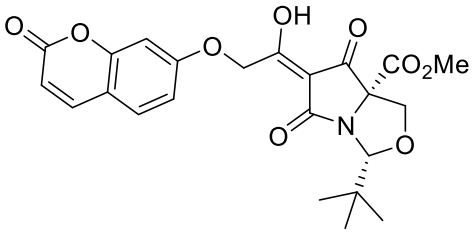
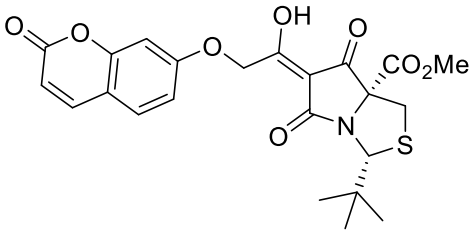
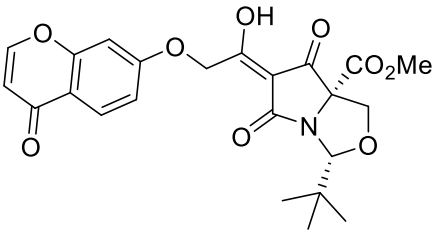
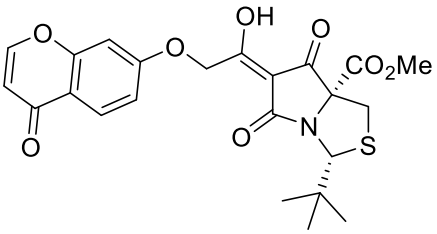
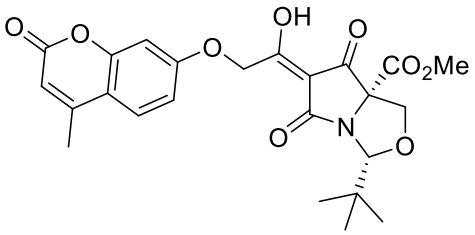
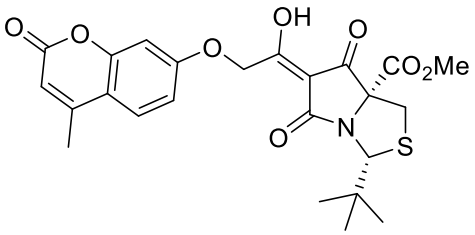
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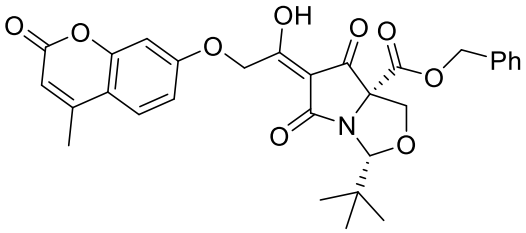
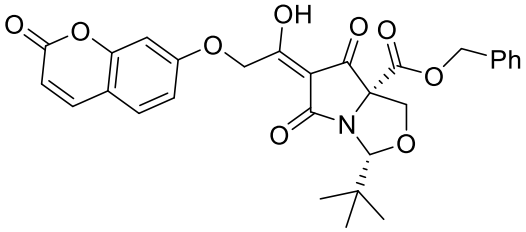
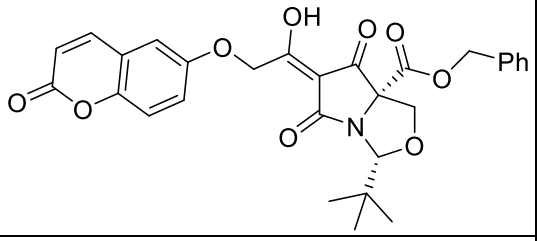
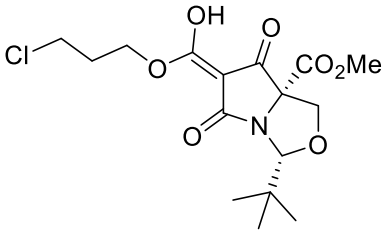
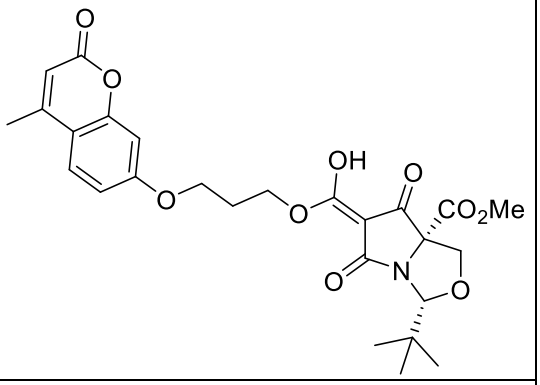
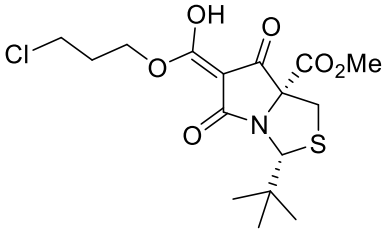
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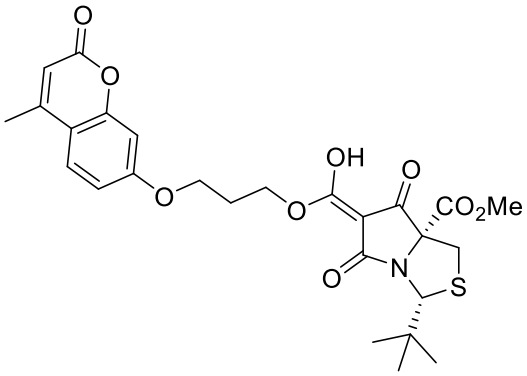
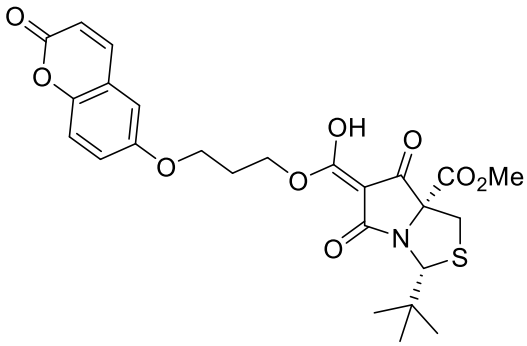
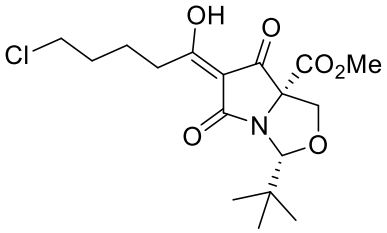
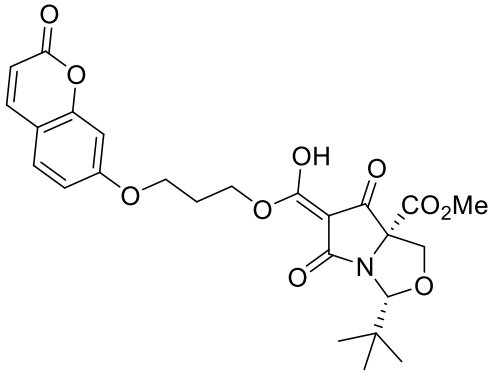
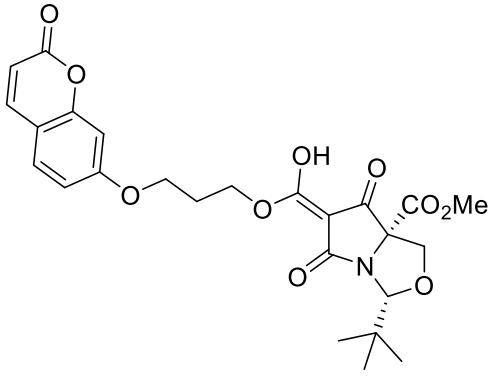
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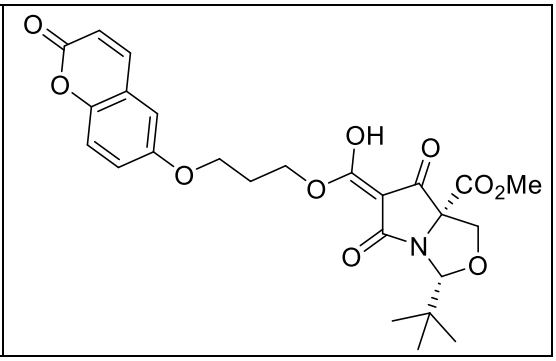
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