Synthesis of the Acylpyridones: Natural and Unnatural Products

by

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Doctoral Thesis

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Abstract

The 3-acyl-4-hydroxy-pyridin-2-one core is a common feature observed in a number of natural products. This thesis describes the design and the development of an isoxazolopyridone as a masked form of this core and elaboration at the sites C-3' (Me), C-7 and N-5 of the related isoxazolopyridone. The polar nature of the heterocyclic trione makes these compounds difficult to work with, particularly with regards to purification and handling. A strategy is applied to disguise this polar nature, by masking the 4-hydroxy and 3-acyl functions as an isoxazole.

The isoxazolopyridone building block is available from diaminopropionic acid or β-alanine. A 1,3-dipolar cycloaddition of a nitrile oxide with a pyrrolidine enamine resulted in an isoxazole. In the case of the β-alanine series, a photolytic dehydrochlorination reaction was employed to introduce the C6-C7 unsaturation. The isoxazolopyridone building block was then elaborated at the sites, C-3' (Me), N-5 and C-7 to work towards the development of natural products and non-natural analogues. The use of anion-type aldol reaction was applied to construct a number of side chains at C-3' (Me). The use of Suzuki cross-coupling reactions, with a palladium-based catalyst for C-C coupling with a 7-iodoisoxazolopyridone was developed. The construction of the hydroxamic acid functionality at N-5 was investigated with little success. The unmasking of the isoxazolopyridone revealed the heterocyclic trione in the final step of the synthesis, by cleavage of the N-O bond followed by diazotization to prove its validity as a synthetic strategy.

Acknowledgements

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Abbreviations

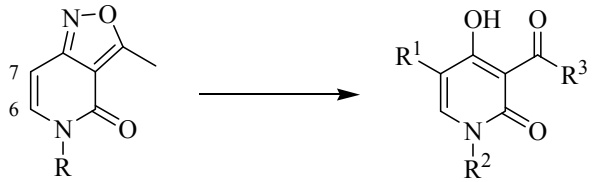
Ad	adenylation domain
ATP	adenosine triphosphate
n-BuLi	*n*-butyllithium
^{13}C	carbon-13
$CHCl_3$	chloroform
DCM	dichloromethane
DIBAL-H	diisobutylaluminium hydride
DMDO	dimethyldioxirane
DMF	*N*,*N*-dimethylformamide
Et	ethyl
GC-MS	gas chromatography-mass spectrometry
^{1}H	proton
HMDS	hexamethydisilazane
HPLC	high performance liquid chromatography
IR	infra red
LDA	lithium diisopropylamide
LC-MS	liquid chromatography mass spectrometry
m-CPBA	meta-chloroperoxybenzoic acid
MeOH	methanol
NADPH	nicotinamide adenine dinucleotide phosphate
NMR	nuclear magnetic resonance
Ph	phenyl
SAM	*S*-adenosyl methionine
TFA	trifluoroacetic acid
THF	tetrahydrofuran

TIPSCl	triisopropylsilyl chloride
TLC	thin layer chromatography
TMSCl	trimethylsilyl chloride
TsOH	*para*-toluenesulfonic acid

Contents

Introduction

1.1 The 3-acyl-4-hydroxypyridone unit

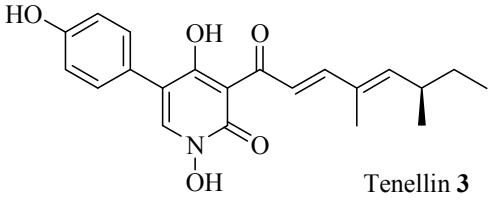


Isoxazolopyridone as masked version **1** Acylpyridone unit **2**

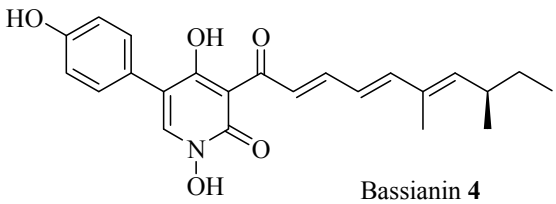
A range of fungi have been found to produce compounds with the core 3-acyl-4-hydroxy-pyridone nucleus as in **2**, which constitutes a structurally diverse group of biologically active natural products.[1] Isoxazolopyridone **1** is a simplified masked version of the highly polar acylpyridone **2** that is central to the synthetic strategy employed in this thesis. Interest in the acylpyridones has been due to their wide spectrum of biological activity, which includes antifungal, antibiotic and antiviral.[1] As a result, these compounds have become the subject of biosynthetic studies and total synthesis. Examples of natural products with such activity include tenellin **3**, bassianin **4**, ilicicolin **5** and funiculosin **6**.[1] Tenellin **3** is a 2-pyridone isolated from the imperfect fungus *Beauveria bassiana* as a yellow pigment; it is structurally related to other 2-pyridones and 3-acyltetramic acids. The fungus was originally isolated by an Italian microbiologist Agostino Bassi[2] and was demonstrated to show insect pathogenic properties causing white muscardine disease in domestic silkworm. It was later found to be effective towards the Colorado potato beetle. It is also used in a number of commercial products such as the insecticide Mycotrol.

New synthetic methodology will be described in this thesis towards the synthesis of tenellin and its analogues. Synthetic interest arises not only due to its biological activity, but also because of the number of diverse functionalities it contains. Other key members of the tenellin family of secondary metabolites will also be discussed along with their biosynthetic origins.

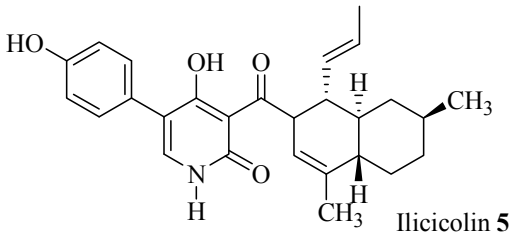
The synthetic strategy applied in this thesis towards tenellin could also be implemented to allow direct access to a number of 2-pyridones isolated from other insect pathogenic fungi. The polar nature of the acylpyridone unit **2** makes these compounds difficult to work with, particularly with regards to purification and handling. A strategy was devised to disguise this polar nature, by masking the acylpyridone unit in the form of isoxazolo[4,3-*c*]pyridone **1**, which is then used as the building block. The R^1 position in the acylpyridone **2** is normally occupied by an aryl group, such as 4-hydroxyphenyl in tenellin **3**. The R^3 position frequently consists of sequences of alternating double and single C-C bonds known as polyenes. The R^2 position is often simple; methyl, hydrogen or even hydroxyl.



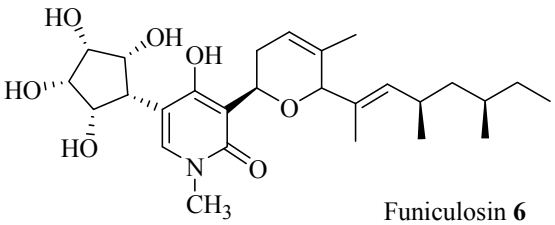
Tenellin **3**



Bassianin **4**



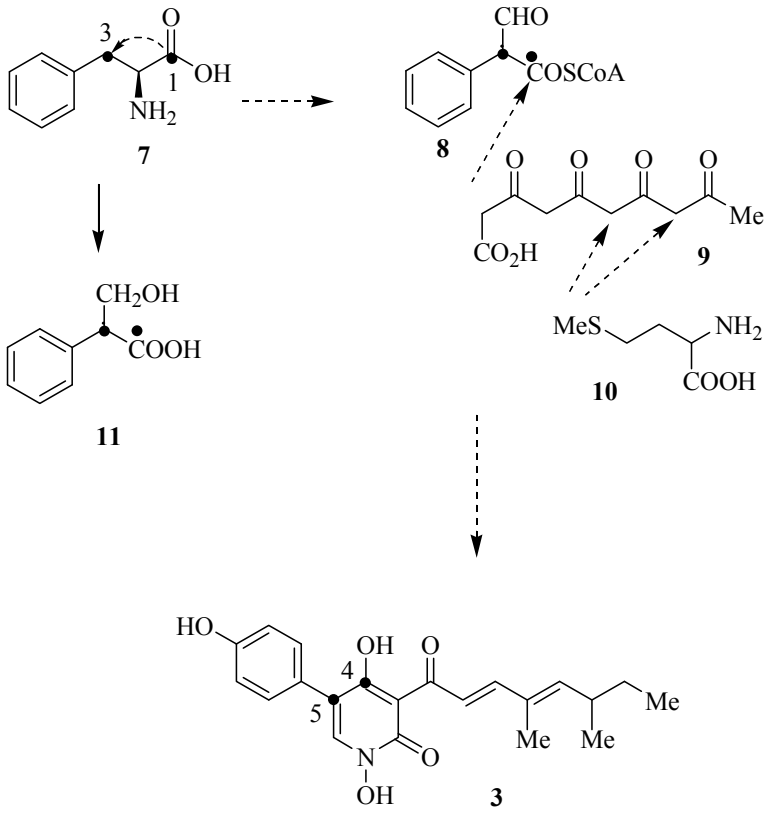
Ilicicolin **5**



Funiculosin **6**

1.2 Biosynthesis of the 3-acyl-4-hydroxypyridone natural products

Bassianin **4** and tenellin **3** have been isolated from the imperfect fungi *Beauveria bassiana* and *Beauveria tenella* as yellow pigments.[3] They contain the 5-(4-hydroxyphenyl)-pyridin-2-one ring system with the acylated moiety at C-3. The proposed biosynthetic pathway suggests that tenellin **3** is assembled from three key precursors, polyketide **9**, phenylalanine **7,** and methionine **10** (Scheme 1).[3]

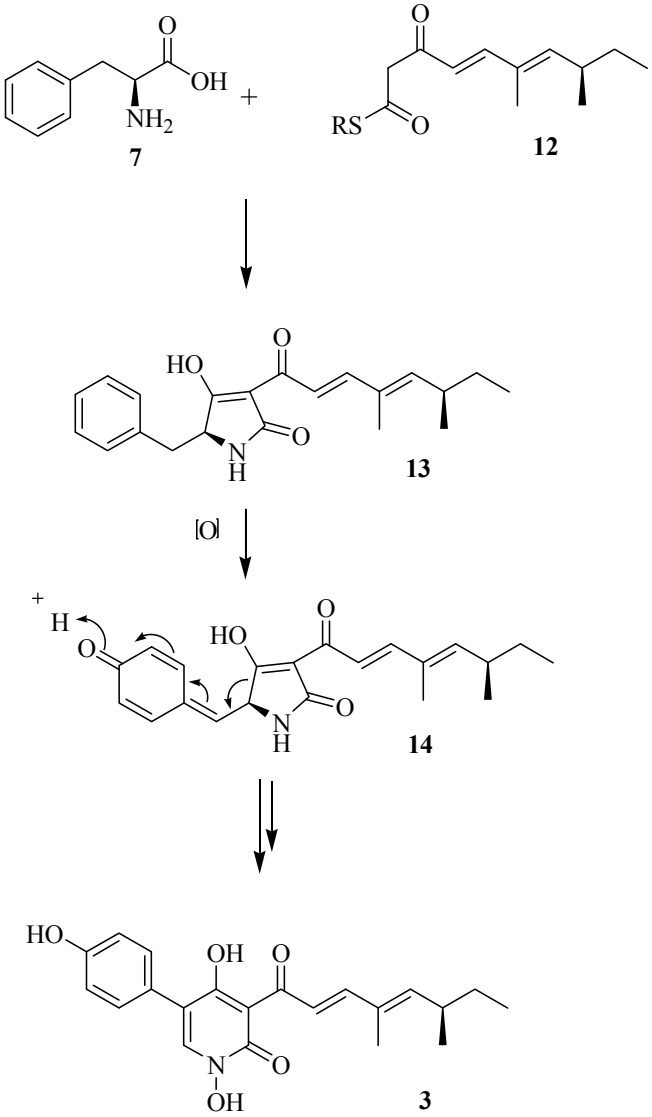


Scheme 1. Proposed biosynthesis of tenellin **3** by Leete *et al.*

The biosynthetic route for tenellin **3** shows the presence of a possible intermediate α-formylphenylacetyl coenzyme A **8**, which undergoes a condensation reaction with polyketide **9** to form the key carbon skeleton.[3] The migration of the carboxylic group of phenylalanine **7** leads to its eventual position at C-4 in tenellin **3**, and the additional methyl groups required are provided by methionine **10**.

During 1975 Leete *et al.* showed interest in the rearrangement of the carboxylic group of phenylalanine in the biosynthesis of tenellin and they proved that the rearrangement of the carboxylic group of phenylalanine in this case is intramolecular;[4] a similar type of rearrangement was observed in the biosynthesis of tropic acid **11**.[5] This was confirmed through numerous feeding experiments using [1,3-$^{13}C_2$]phenylalanine, wherein the majority of the labeled molecules contained two ^{13}C atoms. Administration of DL-[1,3-$^{13}C_2$]phenylalanine into cultures of *B. bassiana* for 7 days afforded tenellin **3**, and after purification ^{13}C NMR spectroscopy revealed coupling between the two adjacent C4 and C5 signals due to spin spin coupling, suggesting these are derived from C1 and C3 carbons of phenylalanine, therefore supporting the type of rearrangement to be intramolecular.

During 1977 Wright *et al.* proposed a hypothesis for the biosynthesis of tenellin **3** using an aromatic amino acid L-phenylalanine and polyketide derived chains (Scheme 2).[6] It was also suggested, with evidence from the feeding experiments that tyrosine does not contribute towards the aryl ring although phenylalanine does.

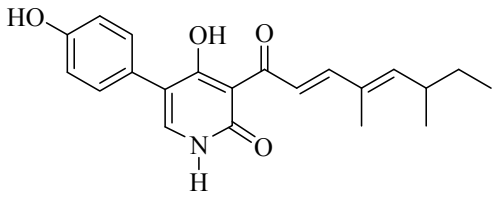


Scheme 2. Wright *et al.* proposed hypothesis for the biosynthesis of tenellin **3**

The hypothesis suggests phenylalanine **7** is fused with a polyketide chain **12** to form the acyltetramic acid **13**, which then undergoes oxidation at the *para* position of the aryl ring to construct a quinoid intermediate **14**.[6] Rearrangement of this intermediate directs the formation of the pyridone ring and ultimately furnishes the target natural product tenellin **3**. This hypothesis was later tested by Moore and co workers,[1] who prepared the acyltetramic acid intermediate **13** (Scheme 3), and conducted feeding experiments using isotopically labeled **13** with ^{13}C. The feeding experiments conducted include the addition of [4-^{13}C]-acyltetramic acid **13** at a concentration of 2.0 mM and in a minimum volume

of ethanol to *B. bassiana*. Together with the HPLC and ^{13}C NMR results clearly indicating no observable isotope enrichment at C-4 of tenellin, it was concluded that the feeding experiments demonstrated the acyltetramic acid **13** was not an intermediate in the biosynthesis of tenellin **3** and therefore does not support the hypothesis.

Although this acyltetramic acid intermediate **13** was not identified from the cultures of *B. bassiana*, a minor metabolite **17** was isolated after HPLC analysis to be a precursor of tenellin, which lacks the hydroxylamine moiety.[1]



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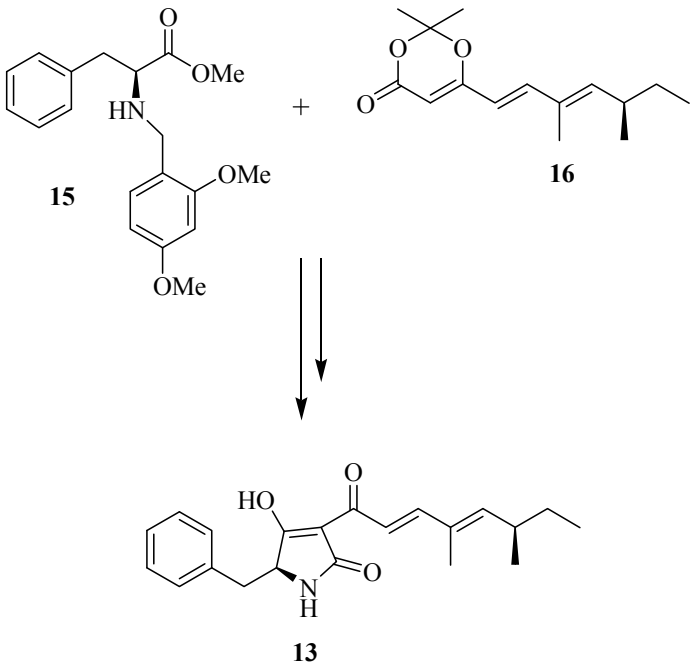
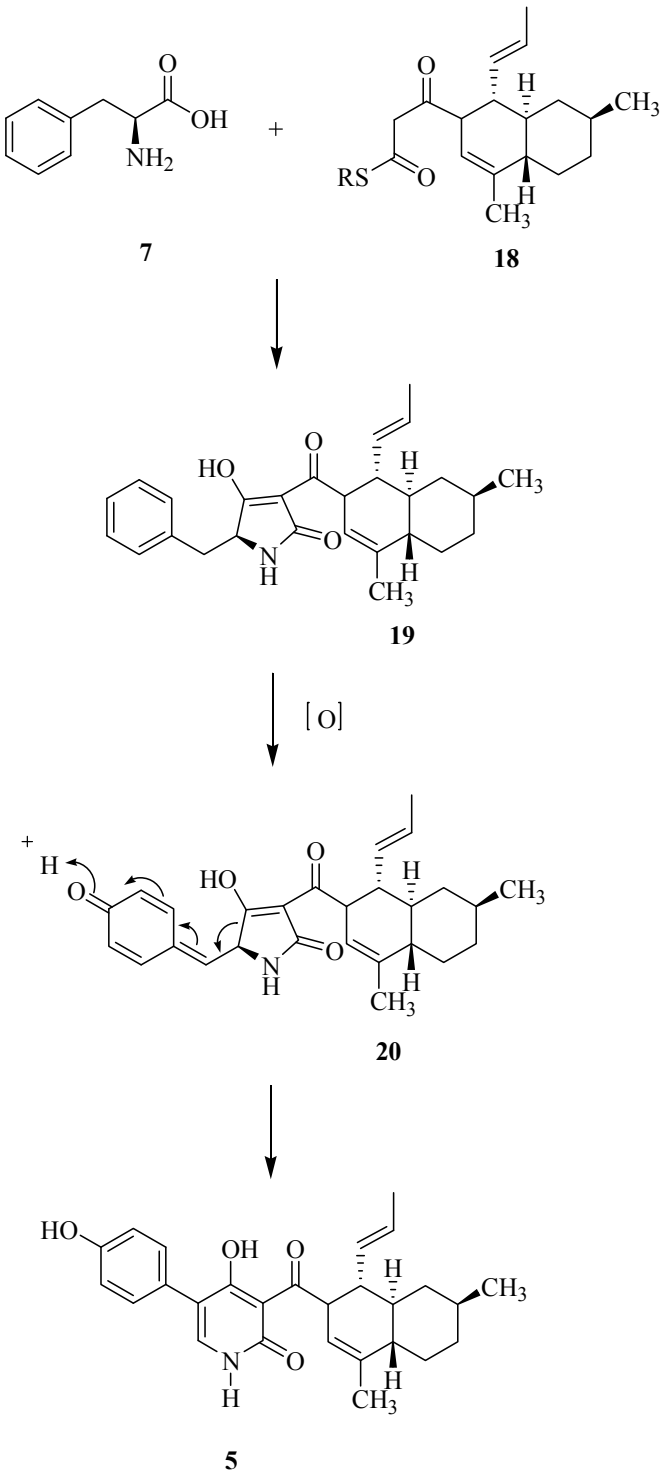
The acyltetramic acid intermediate **13** was prepared *via* a condensation reaction between the protected L-phenylalanine methyl ester **15** and complementary side chain **16** under acidic conditions followed by a Dieckmann cyclisation (Scheme 3).[1]



Scheme 3. Preparation of the acyltetramic acid intermediate **13**

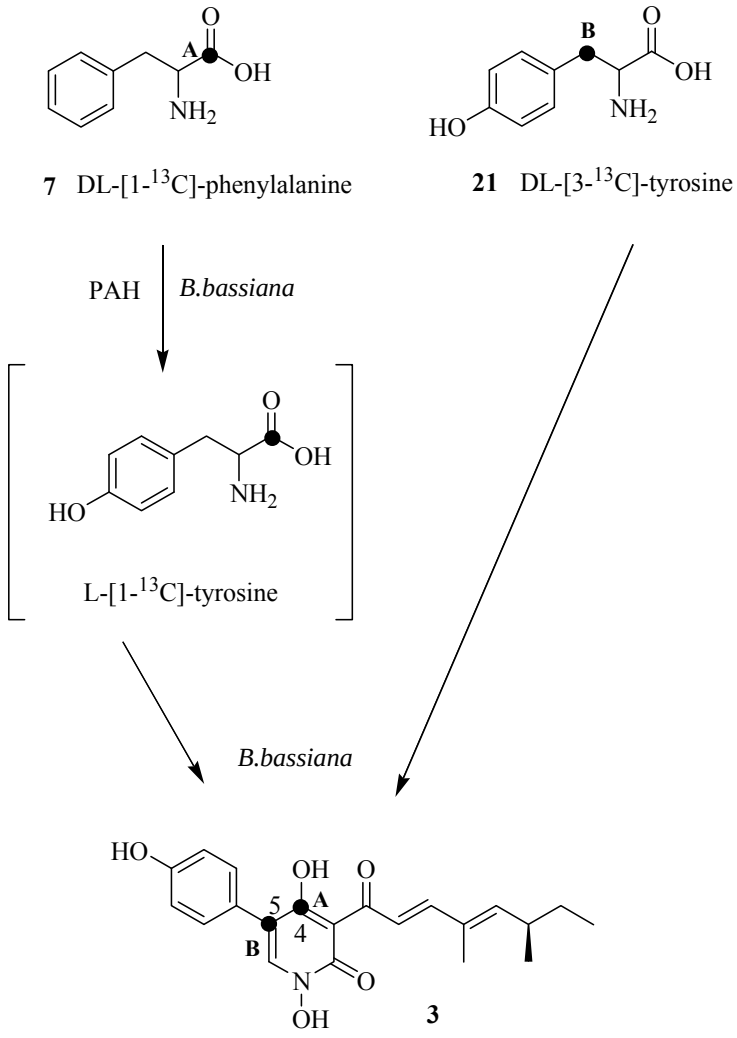
The amine protection in compound **15** was necessary for the successful Dieckmann cyclisation. Deprotection of the amine followed to yield the acyltetramic acid intermediate **13**.

A similar hypothesis was proposed for the biosynthesis of ilicicolin by Wright *et al.* (Scheme 4).[6] The biosynthetic proposal involves phenylalanine **7** interacting with the bicyclic carbocycle **18** to afford an acyltetramic acid intermediate **19**. The bicyclic carbocycle **18** is formed through cyclisation of a linear polyketide chain. The proposed biosynthetic pathway from here continues similarly to the proposed tenellin **3** biosynthesis. A number of feeding experiments were carried out by Tanabe and co-workers using ^{13}C-labeled phenylalanine, ^{15}N-labeled phenylalanine and ^{13}C-labeled acetates in *Cylindrocladium ilicicola*.[7] The results of these experiments suggest phenylalanine as a possible intermediate in the biosynthesis of ilicicolin **5**.



Scheme 4. Biosynthesis of ilicicolin **5** proposed by Wright *et al.*

The role of tyrosine in the biosynthesis of tenellin **3** was later reinvestigated, as DL-[3-^{13}C]-tyrosine **21** was introduced to a culture of *B. bassiana* at a concentration of 3.0 mM.[1] Tenellin was successfully isolated and purified by HPLC, and ^{13}C NMR spectroscopy was used to show ^{13}C enrichment at the C-5 position of tenellin **3**. Following the success of this, DL-[1-^{13}C]-phenylalanine **7** was also introduced to a culture of *B. bassiana* at a concentration of 3.5 mM. ^{13}C NMR spectroscopy illustrated ^{13}C enrichment at the C-4 position of tenellin **3**. It seems in this case phenylalanine is converted into tyrosine due to the presence of the enzyme phenylalanine hydroxylase (PAH) in *B. bassiana* before forming the natural product tenellin **3** (Scheme 5).[1]

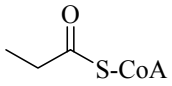


Scheme 5

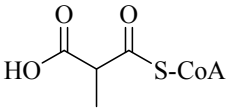
Both phenylalanine and tyrosine have thus been incorporated into cultures of *B.bassiana* to produce tenellin **3**. It seems that phenylalanine is incorporated 20% more efficiently than tyrosine.[1] This could be due to many factors, one of which may be the different transport rates of the amino acids across membranes. The new evidence from the tyrosine feeding experiments may suggest a *para*-hydroxyphenyl acyltetramic acid pretenellin A **27** (Scheme 7), to be a more suitable intermediate than the acyltetramic acid intermediate **13** proposed this far in the biosynthesis of tenellin **3**. It is likely the phenolic hydroxyl is introduced by tyrosine rather than a late stage oxidation of the aryl ring in the biosynthesis.

In previous discussions, the biosynthesis of tenellin has been considered to be derived from a polyketide pathway, and evidence from the isotope feeding experiments has shown amino acids such as phenylalanine and tyrosine to be suitable co-precursors, and demonstrated that the amino acids undergo a late stage rearrangement.[1] Recent biosynthetic studies during 2007 have shown that the polyketide synthase and nonribosomal peptide synthetase (PKS-NRPS) are involved in the biosynthesis of the 2-pyridone natural products.[8] The NRPS are large multifunctional proteins, which work in conjunction with the PKS synthetase. These are also large multi-enzyme complexes with active sites that consist of modular units (modules). Each protein contains one or more modules. Each module contains multiple domains (active sites) with defined functions. The modules determine the length of carbon backbone of complex polyketides. The large multifunctional proteins assemble acyl units in a programmed fashion to build natural products. The functionality of the natural products is controlled by each individual functional domain. The PKS-NRPS allows identification of various domains for a given peptide sequence and predicts the starter and extender precursors at each domain. In general acetyl-CoA and malonyl-CoA are the starter and extender in the fungal polyketide pathway. For example, in the modular polyketide synthases of 6-deoxyerythronolide B,[9, 10] each protein contains a module with multiple domains (Scheme 6). The modules are responsible for growing a chain of carbon-carbon bond. Module one contains a loading module as a starter unit, and towards the end there is a termination module, which is to release the product. In this example, the starter group,

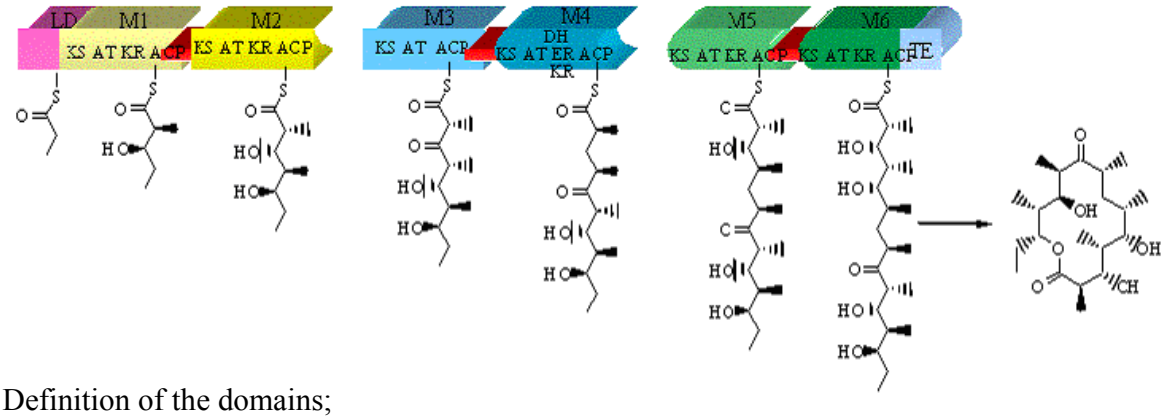
propionyl CoA is loaded onto the ACP domain catalysed by the AT domain. There follows a condensation reaction with the extender, methylmalonyl bound also to an ACP domain, to release CO_2 and HS-ACP in module one. The KR domain is responsible for the reduction of the carbonyl group to an alcohol. A similar sequence follows in modules 2-6, however in module 4, dehydration (DH domain) and further reduction (ER domain) reactions take place to leave the saturated bond. Finally, in the last step the polyketide chain is released to cyclise to the 6-deoxyerythronolide B.[9]



Propionyl CoA



Methylmalonyl CoA



Definition of the domains;

AT: acyl-transferases - loading of starter, extender and intermediate acyl units

ACP: acyl carrier proteins - holds the growing chain as a thioester

KS: β-keto-acyl synthases - catalyses the polyketide chain extension

KR: β-keto reductases - responsible for the reduction to an alcohol

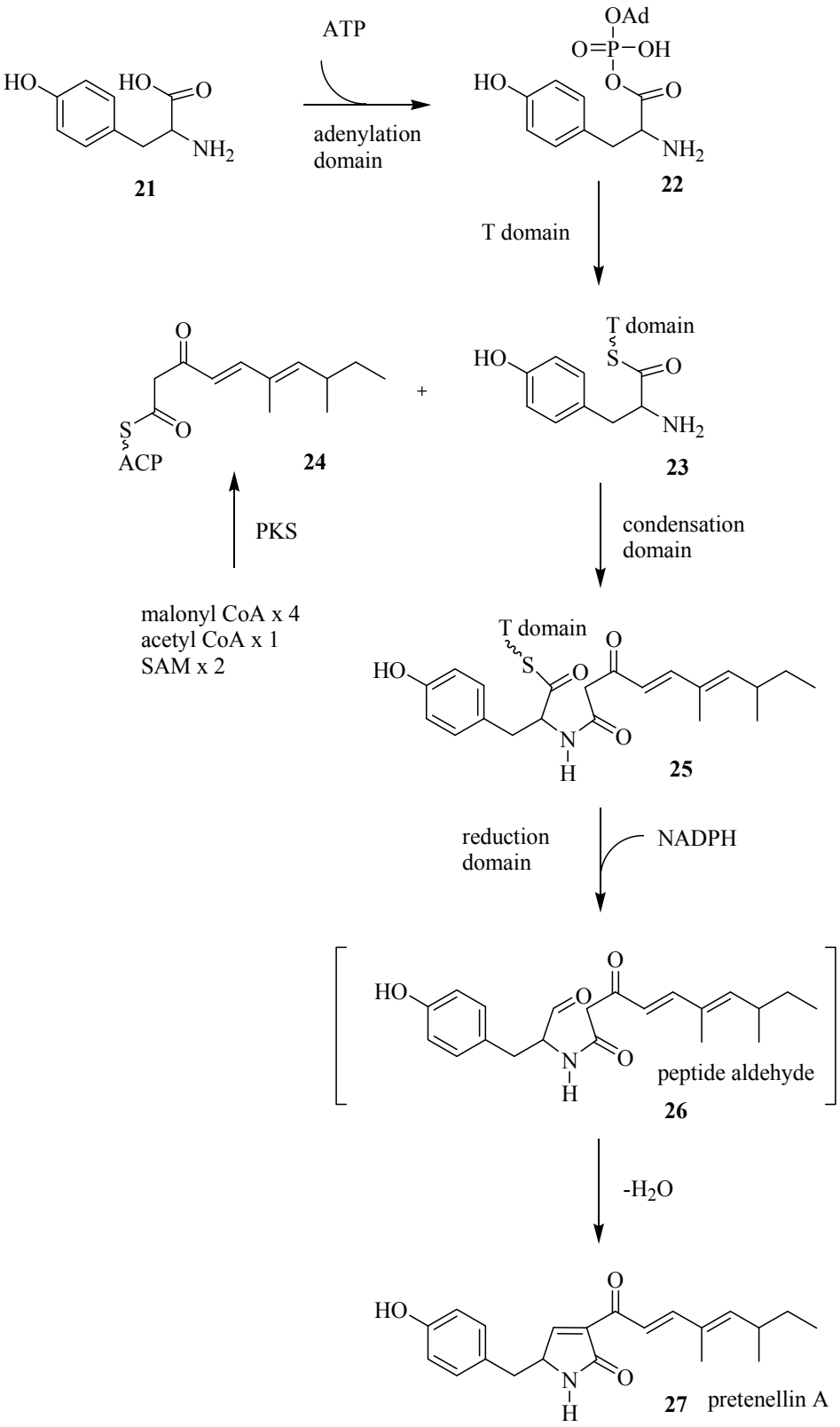
DH: dehydratases - eliminates water to give an unsaturated thioester

ER: enoyl reductases - catalyses the final reduction to full saturation

TE: thiolesterase – release of the polyketide chain

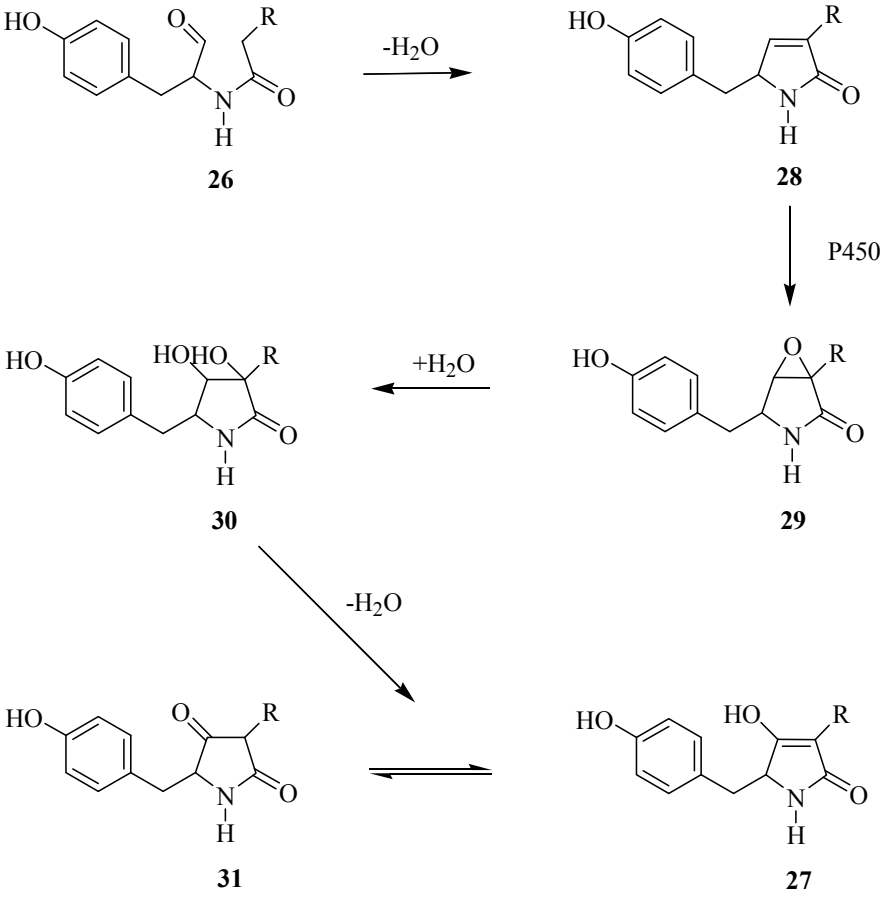
Scheme 6. Modular polyketide synthases (PKSs)[9]

Previous studies have identified some PKS-NRPS genes responsible for the biosynthesis of a number of pyridones.[11, 12] Further studies are in search for similar PKS-NRPS genes involved in tenellin biosynthesis.[13] Cox *et al* have proposed the biosynthesis of pretenellin A **27** (Scheme 7) to be the product of the tenS gene encoding tenellin synthetase (TENS) PKS-NRPS protein.[13] The pathway is followed as the desired amino acid is chosen by the NRPS section (adenylation domain) and is believed to be tyrosine **21**, which is then adenylated to an active ester **22** followed by transfer onto the T (thiolation) domain. The amine bound to the T domain **23** and the polyketide bound to the ACP (acyl carrier protein) domain **24** undergo condensation (catalysed by the C domain) to afford the *N*-β-ketoacyl amino thioester **25** (bound to the T domain). The polyketide backbone is formed from acetates and malonates from the PKS portion of the synthetase. The reduction domain could release the T domain to afford the peptide aldehyde **26**, which would be followed by cyclisation to the acyltetramic acid pretenellin A **27**.



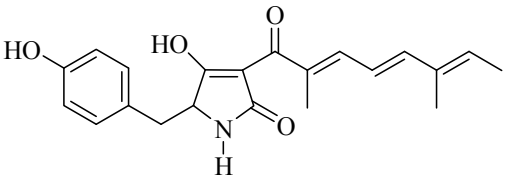
Scheme 7. Proposed biosynthesis of pretenellin A **27**

The timing of the cyclisation step to pretenellin A remains unclear, i.e. whether it takes place before or after the release from the synthetase, and the mechanistic aspects of this step are yet to be fully understood.[8] The proposed mechanistic pathway (Scheme 8) suggests the peptide aldehyde **26** could cyclise readily to the intermediate **28** by a dehydration reaction. Previously, a similar pathway to the acyltetramic acids has been reported in the case of fusarin.[8] In the case of fusarin biosynthesis, the cyclic intermediate **28** is proposed to undergo an epoxidation reaction catalysed by cytochrome P450 to **29**. Such compounds can undergo hydrolysis to the 1,2 diol intermediate **30**, and dehydration of the diol would lead to the acyltetramic acid core **31**. Eventually tautomerisation of the keto form would afford compound **27**. Following this pathway, it is possible a series of cyclodehydration, oxidation, hydration and dehydrations reactions could explain the cyclisation step to afford pretenellin A **27**.

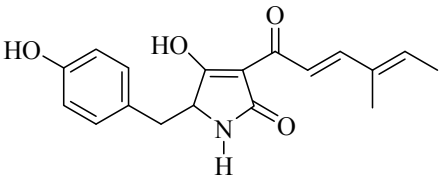


Scheme 8 Proposed mechanism for the ring closure to pretenellin

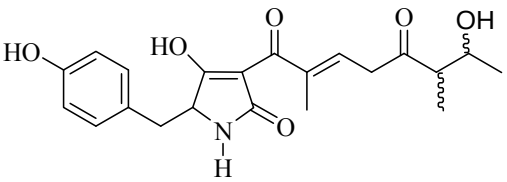
Further studies by Cox and co-workers during 2008 focused their attention on the role of TENS PKS-NRPS in tenellin biosynthesis.[13] The authors identified three new precursors of tenellin synthesised from the large multi-enzyme protein complexes, as TENS PKS-NRPS from *B.bassiana* was expressed in *Aspergillus oryzae*. Previous reports have used *Aspergillus oryzae* to identify roles of PKS genes.[14] Prototenellin A **32** and B **33** were isolated as the major metabolites and identified without great difficulty, whilst prototenellin C **34** proved difficult to isolate due to instability during purification. These are all structurally related and differ in the polyketide side chain. The structures of prototenellin A **32** and B **33** were confirmed by LCMS and NMR analysis, however prototenellin C **34** has not been fully characterised but is suggested to be the illustrated structure. Prototenellin C **34** was confirmed to contain a more oxygenated side chain than tenellin, and these compounds show ^{1}H NMR signals corresponding to the presence of the *para*-hydroxyphenyl substituent. In a second experiment the reaction conditions were altered by the addition of a biosynthetic gene cluster of TENS PKS-NRPS since the previous experiment failed to produce the tenellin side chain, and in this case pretenellin A **27** was isolated as the major metabolite.[13] These experimental data have revealed key aspects for the programming for fungal PKS-NRPS proteins in the biosynthesis of tenellin **3**.



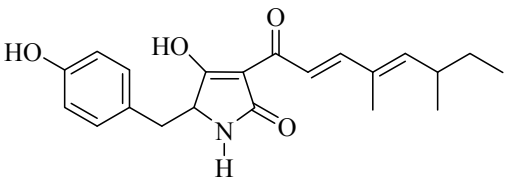
Prototenellin A **32**



Prototenellin B **33**

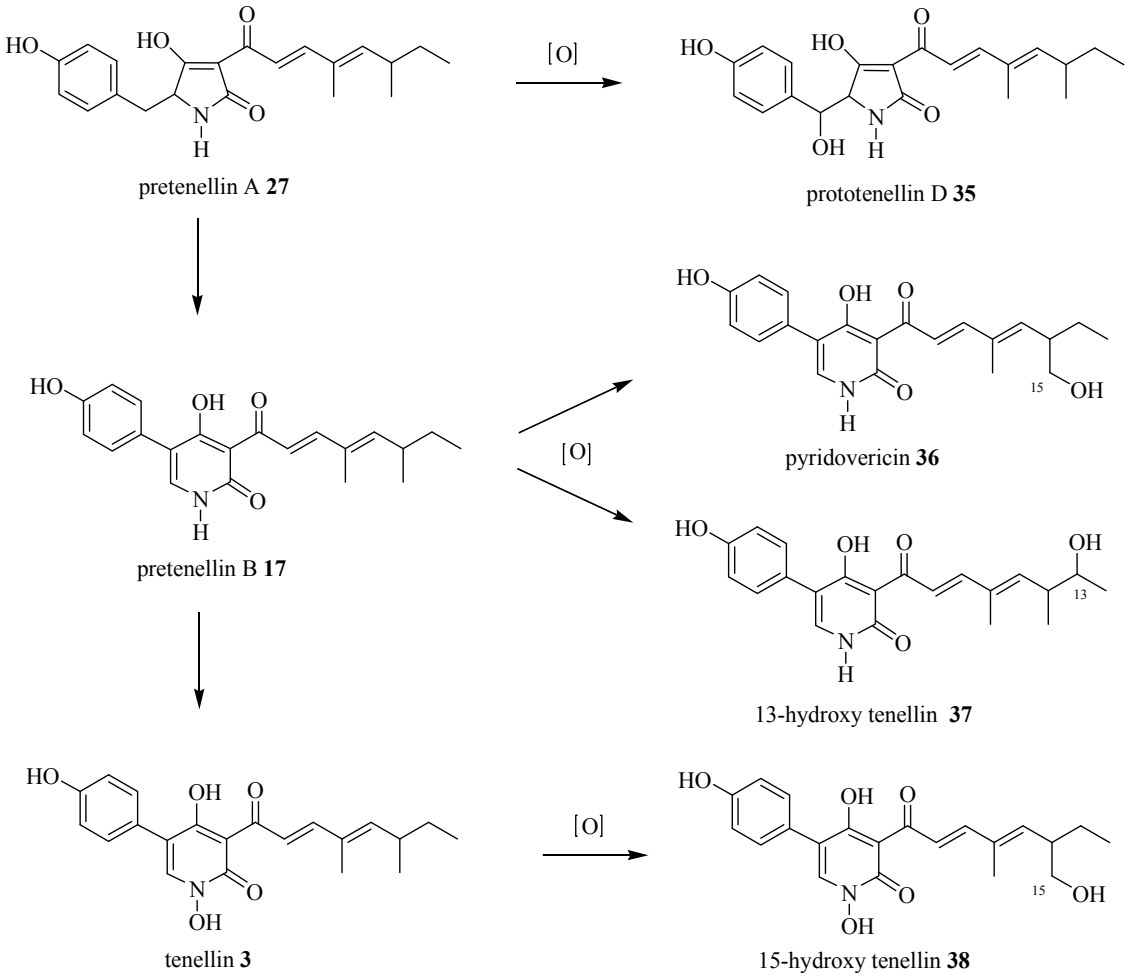


Prototenellin C **34**



Pretenellin A **27**

Biosynthetic studies towards tenellin **3** by Cox and co-workers have focused their attention on the possible routes to tenellin **3** and analogues from pretenellin A **27**.[15, 16] Strains of *B.bassiana* were grown in liquid culture for 10 days followed by extraction using suitable solvent. The organic extracts were analysed using liquid chromatography mass spectrometry (LCMS) and structures elucidated by ^{1}H and ^{13}C NMR spectroscopy, which revealed the presence of a number of metabolites (Scheme 9).[15] The major metabolite isolated was tenellin **3**, and others include pretenellin A **27**, prototenellin D **35** and 15-hydroxy tenellin **38**. The minor metabolites include the isolation of pretenellin B **17**, pyridovericin **36** and 13-hydroxy tenellin **37**, demonstrating the late stage side chain oxidations. Further experiments were conducted in cell free extracts of *B.bassiana* containing TENS PKS-NRPS with purified pretenellin A and prototenellin D.[15] These extracts were grown for 10 days and are incapable of tenellin **3** biosynthesis but contain the cytochrome P450 proteins. The purified pretenellin A **27** and prototenellin D **35** were incubated in separate batches of the cell free extracts and then isolated through extraction with ethyl acetate. The metabolites isolated were purified by HPLC and in the case of pretenellin A **27**, pyridovericin **36** and 13-hydroxy tenellin **37** were both observed.[15] However in the case of prototenellin D no metabolites were detected and the cell free extract was unstable.



Scheme 9. Proposed biosynthetic relationship of compounds isolated

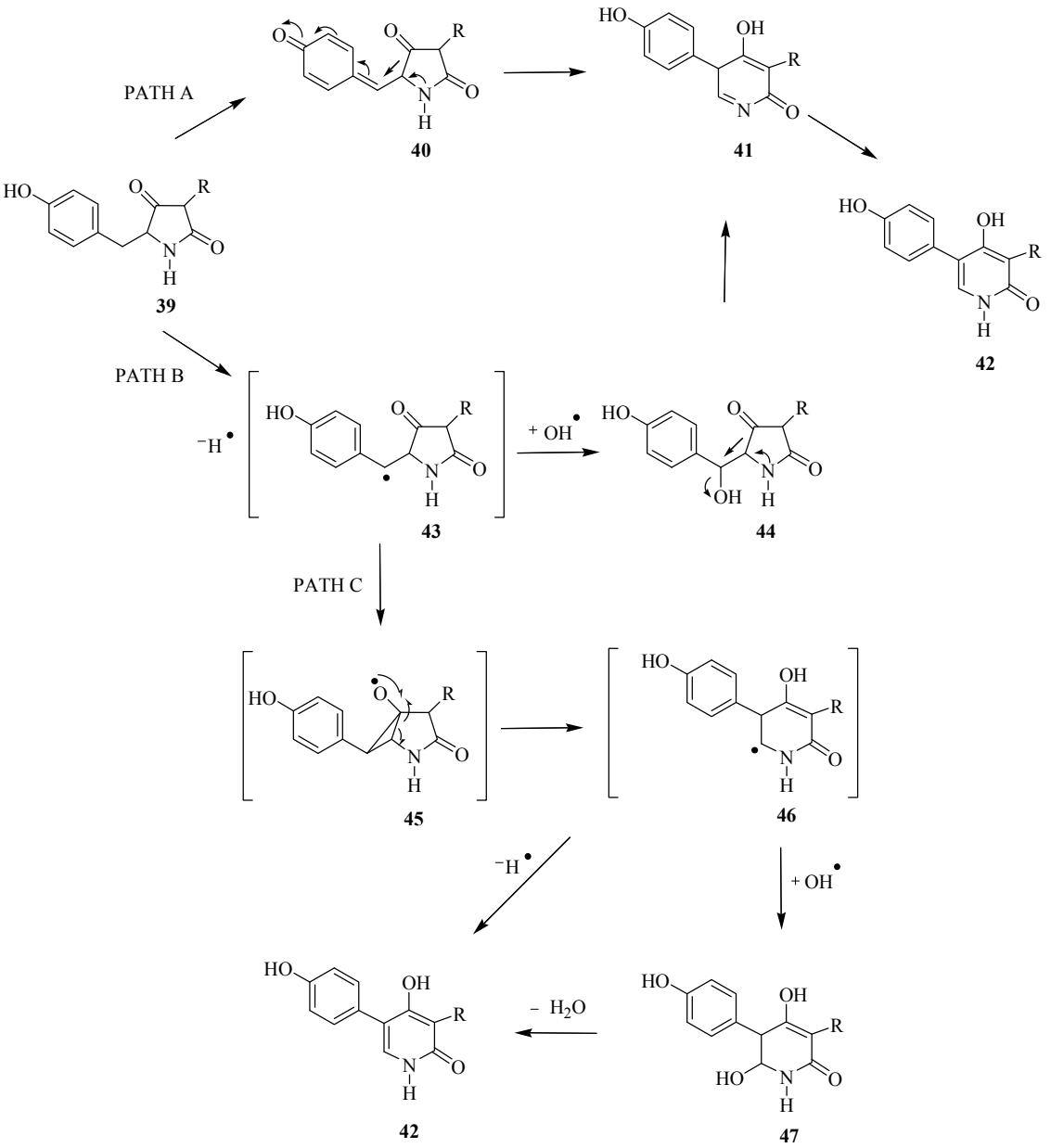
Previous hypotheses of tenellin biosynthesis 'evolved' around the acyltetramic acid intermediates, which undergo an oxidation reaction at the *para* position of the aryl ring to construct a quinoid intermediate, followed by a rearrangement to tenellin **3**. However in this case two possible steps from the pretenellin A **27** are considered, ring expansion followed by *N*-hydroxylation catalysed by cytochrome P450 oxidases **(**Scheme 9).[15] From the experimental results obtained it appears the ring expansion of the pretenellin A **27** is catalysed by the cytochrome P450 oxidases to produce tenellin **3** and derivatives. A second oxidative step must follow to yield the 15-hydroxytenellin **38** suggesting the presence of an additional P450 enzyme in the *B.bassiana*. The presence of excess P450 enzyme would also account for the selective hydroxylation of the polyketide side chain

and would explain the presence of the other hydroxylated pyridones. From the experimental results, protenellin D **35** formed from pretenellin A **27** may be a possible precursor of tenellin **3**, however current experimental data to support this remains weak. It is currently described as a "shunt" compound as it failed to produce any 2-pyridones whilst incubated with the cell free extracts of *B.bassiana*.[15]

There have been several possible mechanisms suggested for the ring expansion of acyltetramic acid intermediates to 2-pyridones.[8] The first proposed oxidative ring expansion follows path A (Scheme 10),[15] which has been discussed previously to follow the quinomethide mechanism. Path B follows a radical route, which involves a hydrogen atom abstraction at the benzylic position forming intermediate **43**, followed by the addition of a hydroxyl radical to give the alcohol **44**. Ring expansion then follows to leave the imine **41** as in path A, followed by tautomerisation to the 2-pyridone **42**. Path C again follows a radical route, and similarly a hydrogen atom is abstracted from the benzylic position forming intermediate **43**; cytochromes P450 are known to be responsible for such hydrogen atom abstraction.[15] A radical-induced ring expansion follows *via* a short lived radical intermediate **45**. Ring expansion follows to a possible radical intermediate **46**, which can either pick up a hydroxyl radical leading to the alcohol **47** and elimination of water to leave the 2-pyridone **42**, or in one step to yield the 2-pyridone **42** through the loss of another hydrogen atom. A similar mechanism has been proposed for aspyridone.[17]

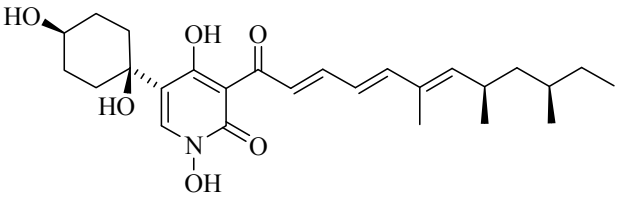
The possible ring expansion of the acyltetramic acid intermediates to 2-pyridones is proposed by Cox *et al* to most likely to follow path C.[15] It is unlikely that path A would be followed, as this could not account for compounds which lack the *para*-substituted phenol, as observed in other 2-pyridones such as leporin B. It would not be possible to form the quinomethide **40** in the absence of the *para*-substituted phenol. If Path B is likely then it would suggest protenellin D as a true intermediate, but this is currently described as a "shunt" compound. Therefore Path C seems in favour for the ring expansion to 2-pyridones, and it is also known that cytochrome P450 hydroxylation

proceeds by H-atom abstraction, which also supports this route. Nevertheless further investigation is required to prove the proposed hypothesis.



Scheme 10. Proposed ring expansions of the acyltetramic acid to 2-pyridones

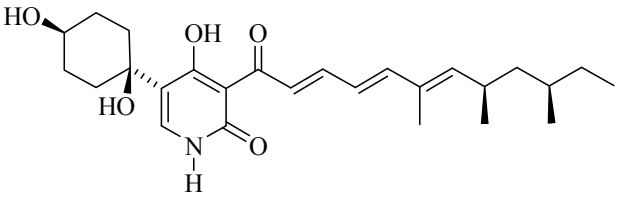
From the mycelium extract of the insect pathogenic fungus *Paecilomyces militaris*, was isolated the novel bioactive natural product militarinone A **48**.[18] This pyridone alkaloid contains similar structural features to tenellin **3**, consisting of the polyene side chain, hydroxamic acid functionality, and in addition the *cis*-(1,4-substituted cyclohexyl) moiety.



Militarinone A **48**

The discovery was made by Schmidt and co-workers after pursuing their interest in secondary metabolites of imperfect fungi.[18] Their findings demonstrated that a mycelium extract of *Paecilomyces militaris* strain RCEF 0095 exhibited neuritogenic activity on PC-12 cells and no cytotoxicity in PC-12 cells, and the bioassay revealed fractions to contain the natural product. PC-12 cells are isolated from a rat medulla, which has the ability to respond to nerve growth factors and therefore it is commonly used as a model for neuronal differentiation.

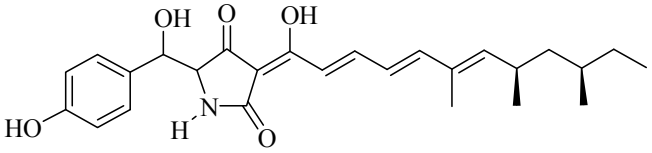
Further investigation into *Paecilomyces militaris* strain RCEF 0097, led to the discovery of the novel pyridone alkaloid (+)-*N*-deoxymilitarinone A **49**.[19] This compound is almost structurally identical to militarinone A **48**, differing by a single oxygen atom. This compound also exhibited neuritogenic activity on PC-12 cells.



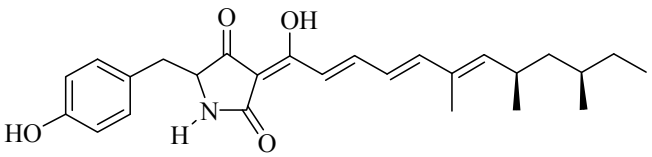
(+)-*N*-deoxymilitarinone A **49**

Further novel alkaloids were isolated in 2002 by Schmidt and co-workers from the mycelium extract of the pathogenic fungus *Paecilomyces militaris*, three yellow pigments

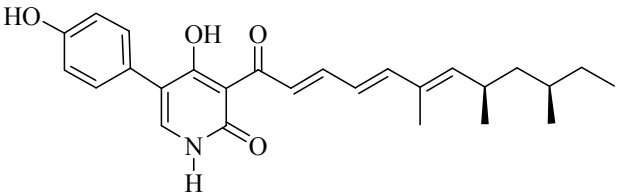
identified as militarinone B **50**, C **51** and D **52**.[20] The structure of militarinone D **52** is almost identical to tenellin **3** and other pyridone fungal metabolites; it consists of the 5-(4-hydroxyphenyl)pyridine-2-one ring system, and the extended acylated moiety at C-3. Militarinone B **50** and C **51** are structurally almost identical, both containing the acyltetramic acid functionality, the 4-hydroxyphenyl moiety, the polyene side chain and the stereochemistry is unknown at the centres. The two differ by the presence of the extra benzylic hydroxyl group in militarinone B **50**. Militarinones **50** and **51** both contain the pyrrolidine-2,4-dione (tetramic acid) ring, which has been reported in other microbial natural products as antibiotics and antiviral agents.[21] Militarinones B-D were tested for neuritogenic properties in PC-12 cells and test results were negligible, however militarinones D **52** confirmed evidence of cytotoxicity in PC-12 cells.[20]



Militarinone B **50**



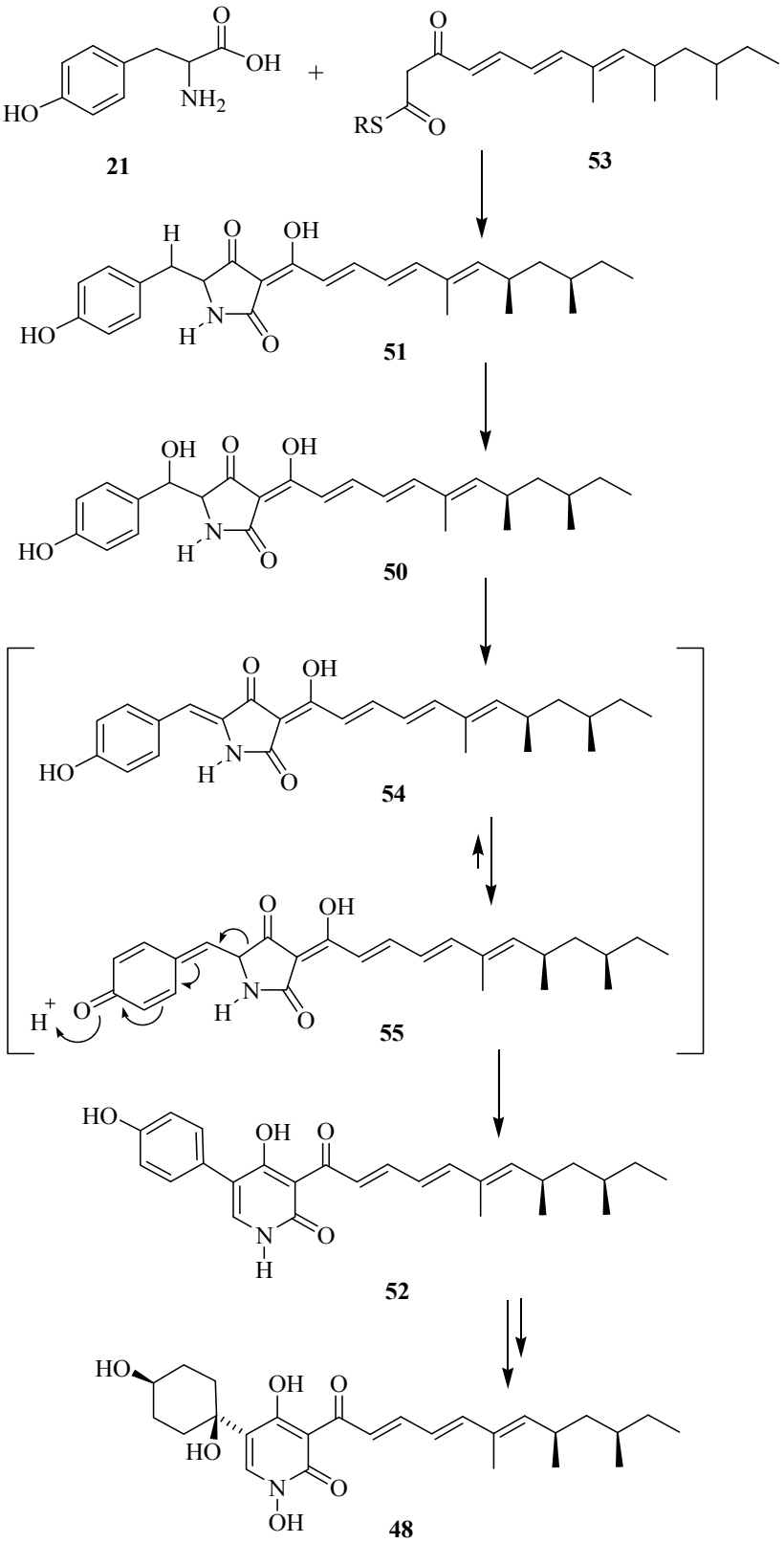
Militarinone C **51**



Militarinone D **52**

The biosynthetic pathway proposed for militarinones A-D is similar to the tenellin **3** biosynthetic pathway, and is suggested to proceed *via* an acyltetramic acid intermediate (Scheme 11).[20] The hypothesis illustrates tyrosine **21** fused with a polyketide derived chain **53** to form the acyltetramic acid intermediate militarinone C **51**, followed by

oxidation to provide the corresponding 6-hydroxy derivative militarinone B **50**. This is suggested to undergo dehydration and tautomerisation to construct a possible quinoid intermediate **55**. Rearrangement of the intermediate directs the formation of the pyridone ring in militarinone D **52**. The interesting conversion of the aromatic substituent to produce the 1,4-disubstituted cyclohexane is accompanied by further reactions, such as N-oxidation, as well as oxidative and reductive steps to complete the biosynthetic pathway to furnish militarinone A **48**. It may be that the radical ring expansion sequence proposed for tenellin (Scheme 10, Path C)[15] also operates here, as the militarinone proposal is based not on experimental evidence but on metabolite co-occurrence.[20]

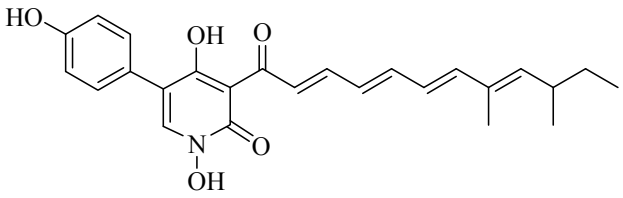


Scheme 11. Proposed biosynthesis for the militarinone alkaloids.

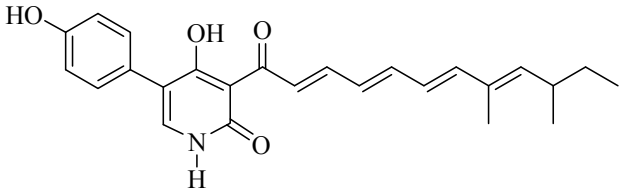
Two new pyridone alkaloids have been reported by Hamburger and co-workers, farinosones A **56** and B **57**.[22] A third compound farinosone C **58** was also isolated, which is possibly involved in the pyridone biosynthesis (Scheme 12).[22] These were isolated from the mycelium extracts of the fungus *Paecilomyces farinosus* as yellow pigments (A and B) and their structures were confirmed through extensive NMR and MS studies. The structures of **56** and **57** are almost identical to that of tenellin, the difference being that these have an extended polyene side chain. Previously tenellin **3** and pretenellin B **17**, were reported to have the hydroxylamine group and simple NH respectively. Here we have the same case with farinosones A **56** and B **57**. The only difference in structure is the hydroxylamine group in **56** and simple NH in **57**.

The presence of the tyrosine-like moiety in structure C **58** supports a biosynthetic pathway that could be similar to other members of the related pyridone family. The presence of tyrosine in the biosynthetic pathway of the tenellin and militarinone series was discussed previously. Again it is possible the sequence proposed for tenellin also operates here, for instance tyrosine fused with a poly-β-keto acid could lead to such metabolites.

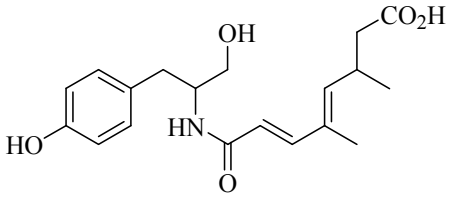
Interests in these metabolites arise due to their bioactivity, such as in cancer treatment and as antibiotics. These compounds are of great interest to the medicinal field, in this case towards neurodegenerative disorders.[22] These disorders are often caused by misfolding of proteins and more commonly known as Alzheimer's disease (AD). Cells of the brain are lost and eventually lead to CNS related dysfunction. Clinical studies of AD are aimed at neurotrophic factors such as nerve growth factors, however success to date has been limited.[22] Natural products are often screened for neurotrophic properties and entomopathogenic imperfect fungi are known to have shown CNS bioactivity, and often produce metabolites which are responsible for the interaction with their host insect, causing behavioural changes and even death. The farinosone family was tested for neurotrophic activity towards PC-12 cells, and compound A **56** and C **58** showed some activity, whilst compound **57** was inactive.



Farinosone A **56**



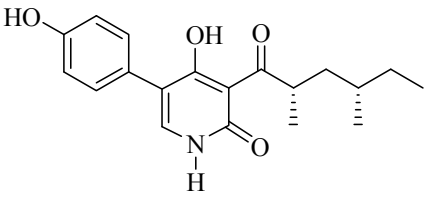
Farinosone B **57**



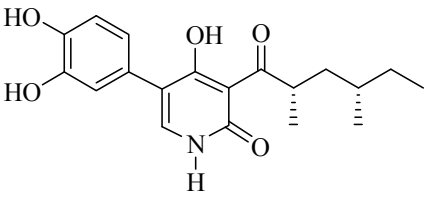
Farinosone C **58**

Scheme 12

From extracts of the fungus *Aspergillus nidulans* were isolated the natural products aspyridones A **59** and B **60**.[17] The structures of these compounds are similar to that of other 2-pyridones such as tenellin **3** but not identical. Similarities arise through the 4-hydroxyphenyl group and the 3-acyl aliphatic side chain with notable differences: the hydroxylamine group is absent, as was also observed in pretenellin B **17**, and the 3-acyl side chain is saturated. The structures of these compounds were confirmed through extensive NMR and MS studies. This demonstrated aspyridone B **60** to have an extra quaternary carbon, absence of an aromatic H and increase in mass which confirmed its structure to be the 3,4-dihydroxyphenyl analogue of aspyridone A **59**. These compounds were screened for bioactivity and results showed moderate cytotoxicity.[17]

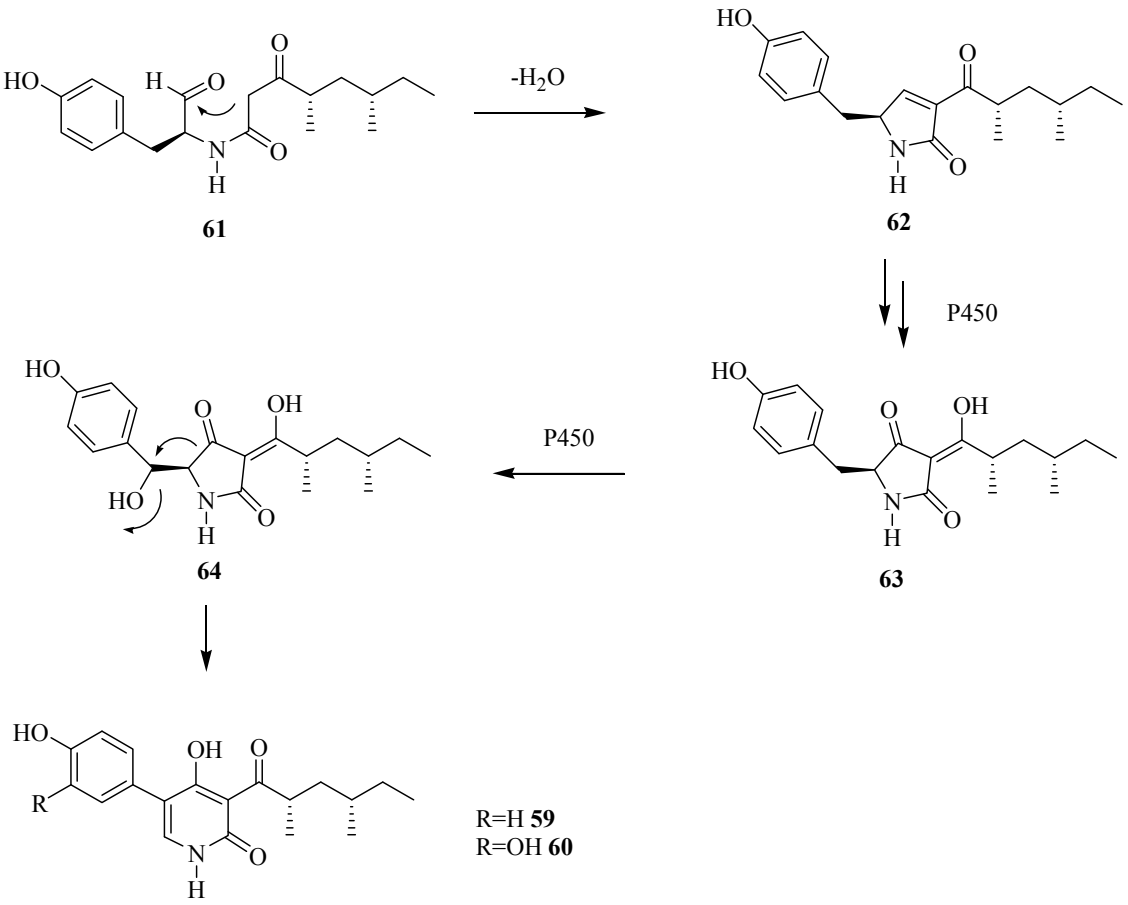


Aspyridone A **59**



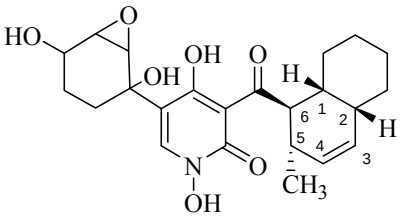
Aspyridone B **60**

The proposed biosynthetic pathway for the aspyridones (Scheme 13)[17] suggests the key backbone is assembled from a polyketide pathway from the PKS–NRPS hybrid to afford an aldehyde **61**, which would readily undergo an intramolecular Knoevenagel condensation reaction, with the loss of water to the pyrrolinone intermediate **62**. This must be followed by several oxidation reactions, catalysed by cytochrome P450 to the tetramic acid intermediate **63**. Further oxidation of this intermediate leads to the 6-hydroxy tetramic acid **64**. In the next step of this pathway, rearrangement follows with the loss of the hydroxyl group to the aspyridones. Comparing this biosynthetic proposal to that of related 2-pyridones the difference arises in this ring expansion step. Here the acyltetramic acid intermediate is proposed to undergo dehydration and tautomerisation to an intermediate, which then proceeds *via* rearrangement to the natural product. In contrast, the evidence for tenellin **3** suggested a radical route (Scheme 10).[15] To date, the exact mechanisms and the pathways to the 2-pyridones have been studied extensively but still remain uncertain. Once again it seems that the sequence proposed for tenellin may also operate here since these are only rationalised deductions of the hypothetical biosynthetic pathway.



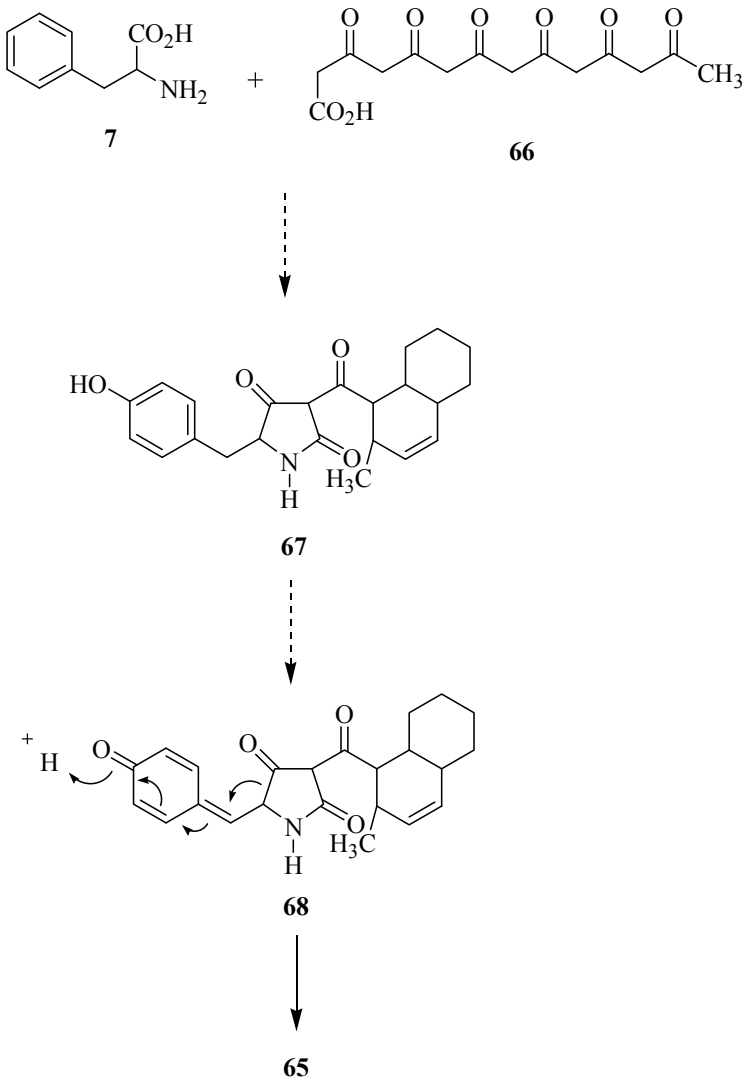
Scheme 13. Hypothetical biosynthetic pathway for aspyridones A and B

From the acetone extracts of the fungus *Aspergillus fumigatus* was isolated the toxic metabolite fischerin **65**, which is known to cause severe peritonitis in mice followed by death.[23] Its structure was solved by extensive ^{1}H and ^{13}C NMR analysis to consist of a hydroxamic acid group, 1,4-dihydroxycyclohexane derivative (stereochemistry not known at the centres) and a decalin ring system. The signals observed in the ^{13}C NMR spectrum of fischerin at the C1 and C2 positions suggests a *cis*-decalin, and the proton configurations between C6 and C5, C6 and C1 are trans diaxial. A similar decalin ring system has been observed in other natural products such as ilicicolin **5**.



Fischerin **65**

The proposed biosynthesis (Scheme 14)[23] is imagined to proceed similarly to that of other fungal metabolites such as tenellin **3**. Again the sequence for the biosynthetic pathway follows a polyketide pathway with phenylalanine fused with the poly-β-keto acid and significant modification to form the acyltetramic acid intermediate **67**, followed by oxidation reactions and ring rearrangement to afford the natural product **65**.



Scheme 14

Summary

The isolation of natural products with the core 3-acyl-4-hydroxypyridone moiety and its acyltetramic acid analogues has been discussed in this section and the key natural products are summarised in Table 1. These structurally diverse groups of natural products display a range of biological activity, including antibiotic and antiviral. For instance they have been screened for CNS-related bioactivities and some have shown neuritogenic activity and cytotoxicity in PC-12 cells.

The fungus *Beauveria bassiana* was first to undergo biosynthetic study due to its insect pathogenic properties which cause white muscardine disease in domestic silkworms.[2] This fungus has been shown to produce yellow pigments such as the 2-pyridone tenellin **3**,[1] and surprisingly a number of other natural products similar in structure have been isolated from other fungi such as the farinosones A **56** and B **57**, isolated from the mycelium extracts of the fungus *Paecilomyces farinosus* as yellow pigments.[24]

Structural similarities arise though the core 3-acyl-4-hydroxypyridone and differences show in the side chains, for instance bassianin **4** has an extended polyene side chain compared to tenellin **3**. In the case of the natural product ilicicolin **5**,[7] the polyene side chain is replaced by a *trans*-decalin ring system while the 4-hydroxyphenyl functionality remains intact. In the case of the 2-pyridone fischerin **65** the 4-hydroxyphenyl functionality is absent and replaced by a 1,4-dihydroxycyclohexane moiety.

Early biosynthetic studies suggested the condensation of a possible intermediate α-formylphenylacetyl CoA with a poly-β-keto acid to form the key carbon skeleton for tenellin **3** (Scheme 1). Over the years, as the interest in the biosynthetic pathway of 2-pyridones developed, much of the research was focused on isotope-labeling methods in polyketides. Through a number of feeding experiments the first hypothesis for tenellin **3** was developed by Wright *et al.* to show that they are derived from amino acids and polyketide precursors, and the amino acid undergoes a late stage rearrangement (Scheme 2).[6] The hypothesis initially derived from tenellin **3** biosynthetic studies illustrated the use of an aromatic amino acid, phenylalanine fused with a polyketide chain to form a 5-

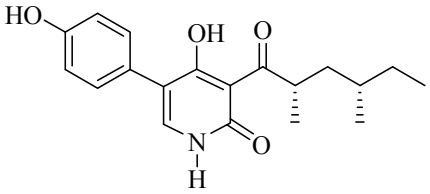
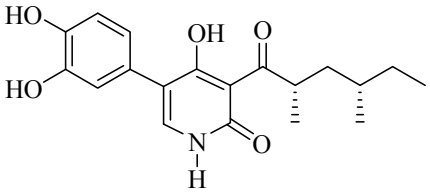
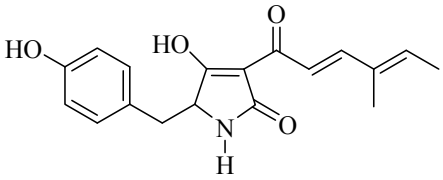
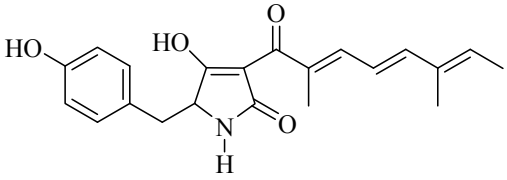
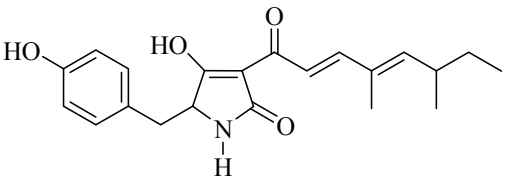
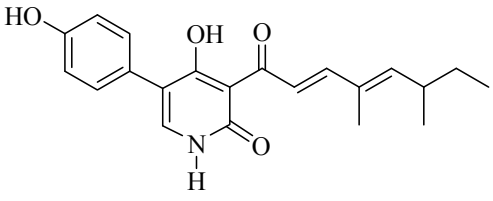
membered acyltetramic acid ring, which then undergoes oxidative ring expansion followed by rearrangement to tenellin **3**. A similar hypothesis was proposed for the biosynthesis of ilicicolin by Wright *et al.* [6] Again, the biosynthetic proposal starts with the interaction of a ploketide and phenylalanine precursors to follow the tenellin **3** sequence. It seems the proposed biosynthetic pathway for tenellin and related compounds have in common a polyketide pathway consisting of an aromatic amino acid such as phenylalanine or tyrosine and polyketides derived from acetates and malonates. The proposed biosynthetic pathway for other 2-pyridones such as the militarinone family is based around acyltetramic acid intermediates and oxidation and reduction reactions. These hypothesis are based on co-occurrences and not on actual experimental data, therefore it may be that the sequence proposed for tenellin also operates in the biosynthesis of other fungal 2-pyridones.

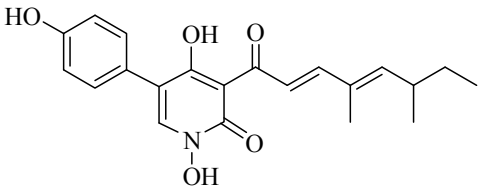
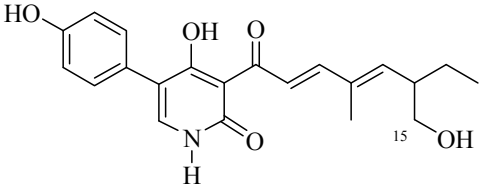
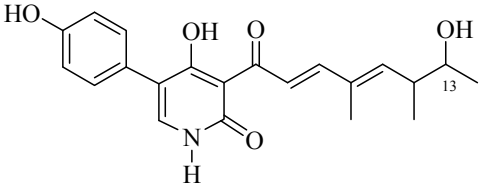
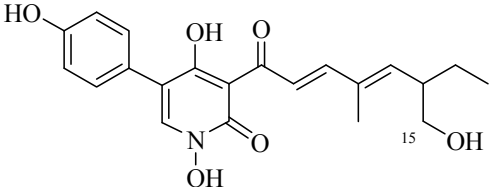
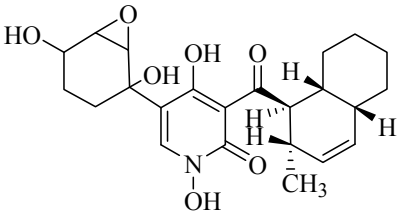
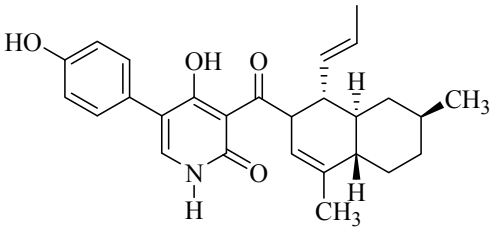
Recent advances in the biosynthetic studies of 2-pyridones use the more modern technology of gene encoding, which has led to the identification of gene clusters in fungi that encode for the proteins governing the biosynthesis of 2-pyridones. Cox *et al.* have shown for the first time that PKS-NRPS are involved in the biosynthesis of the 2-pyridone natural products.[8] These large multifunctional proteins assemble acyl units in a programmed method to build natural products. The functionality of the natural products is controlled by each individual functional domain. They prepared PKS-NRPS genes and conducted several experiments to suggest possible precursors in tenellin biosynthesis. The studies have reported both the pretenellin A **27** and pretenellin B **17** to be possible intermediates in the biosynthesis, and programmed a number of domains to develop specific functionality (Scheme 9). The mechanistic aspect of the ring expansion from the acyltetramic acid pretenellin A to pretenellin B is yet to be fully understood, and currently a radical route *via* the formation of a benzylic radical intermediate in path C of Scheme 10[15] seems to be in favour. However, a major challenge remains in understanding the key aspects of the programming of the PKS proteins. Controlling the domains to determine the chain length and the starter/extender selection, degree of methylation and reduction, all remain uncertain. The existing studies have revealed the

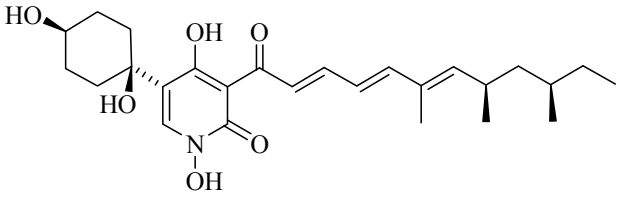
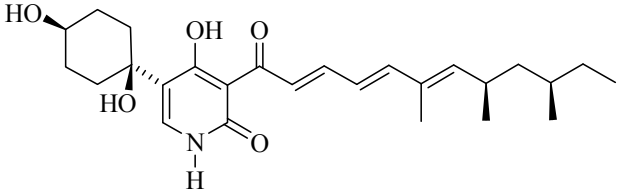
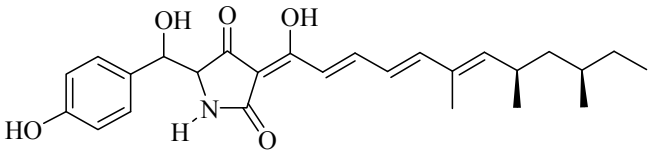
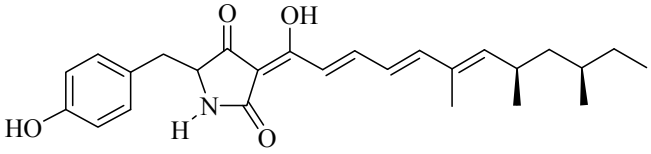
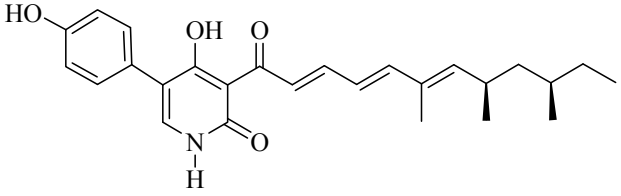
functions of each domain, which it is believed are the key to understanding the fundamental aspects of programming.

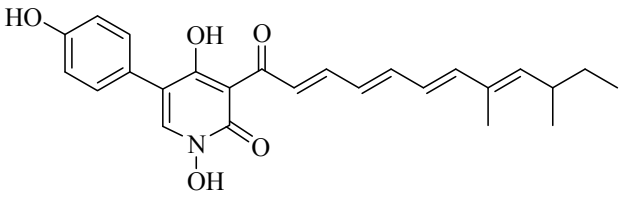
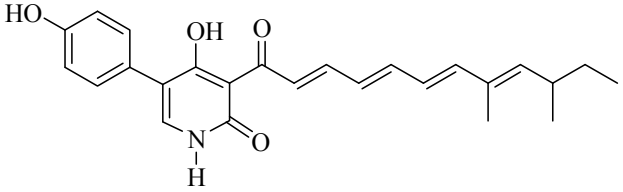
In this section we have discussed the biosynthetic origin of the natural products and in the next part of the thesis will concentrate on the synthesis of some of these natural products and of other 2-pyridones.

Table 1. Summary of natural products

Natural Product	Structure	Isolation
Aspyridone A **59**		*Aspergillus nidulans*
Aspyridone B **60**		*Aspergillus nidulans*
Prototenellin B **33**		*Beauveria bassiana*
Prototenellin A **32**		*Beauveria bassiana*
Pretenellin A **27**		*Beauveria bassiana*
Pretenellin B **17**		*Beauveria bassiana*

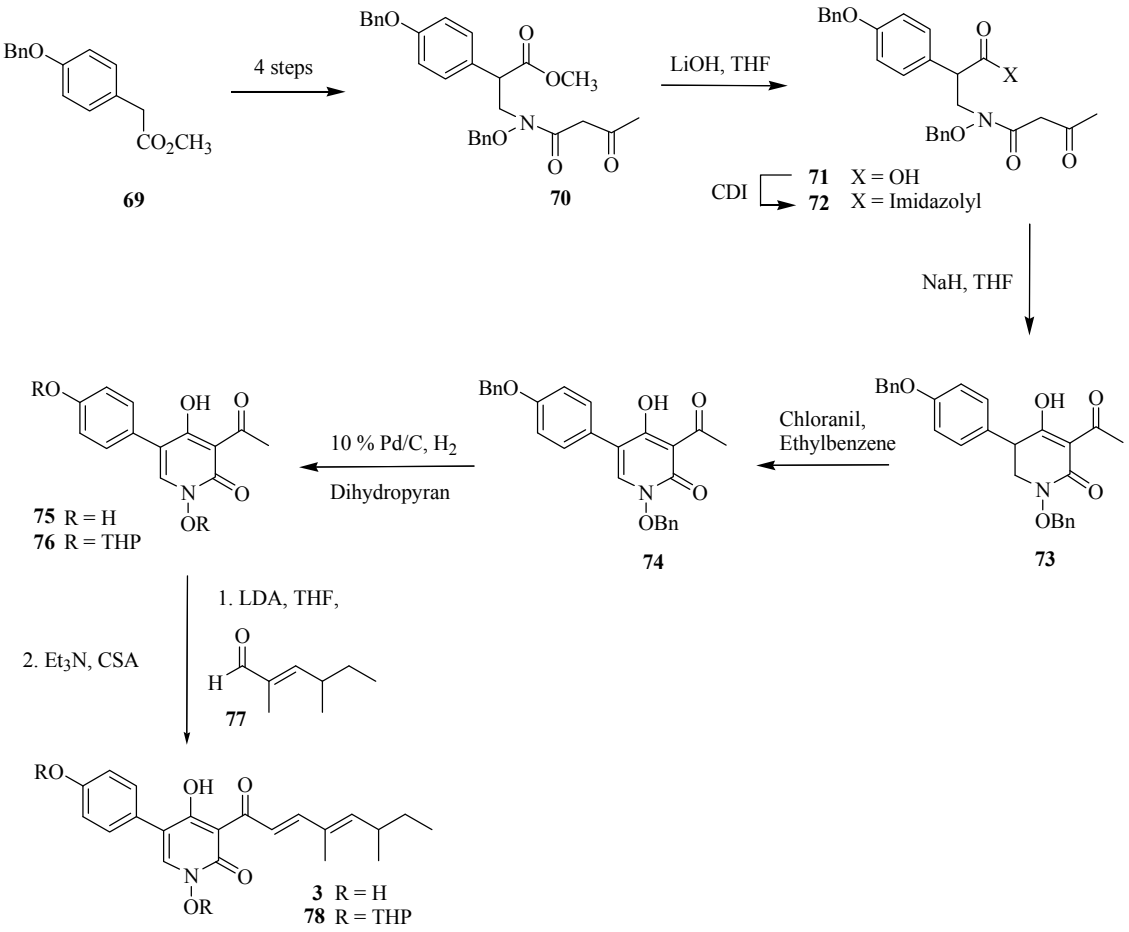
Natural Product	Structure	Isolation
Tenellin **3**		*Beauveria bassiana*
Pyridovercin **36**		*Beauveria bassiana*
13-Hydroxy-tenellin **37**		*Beauveria bassiana*
15-Hydroxy-tenellin **38**		*Beauveria bassiana*
Fischerin **65**		*Aspergillus fumigatus*
Ilicicolin **5**		*Cylindrocladium ilicicola*

Natural Product	Structure	Isolation
Militarinone A **48**		*Paecilomyces militaris*
(+)-*N*-Deoxy militarinone A **49**		*Paecilomyces militaris*
Militarinone B **50**		*Paecilomyces militaris*
Militarinone C **51**		*Paecilomyces militaris*
Militarinone D **52**		*Paecilomyces militaris*

Natural Product	Structure	Isolation
Farinosone A **56**		*Paecilomyces farinosus*
Farinosone B **57**		*Paecilomyces farinosus*

1.3 Synthesis of the 3-acyl-4-hydroxypyridone natural products

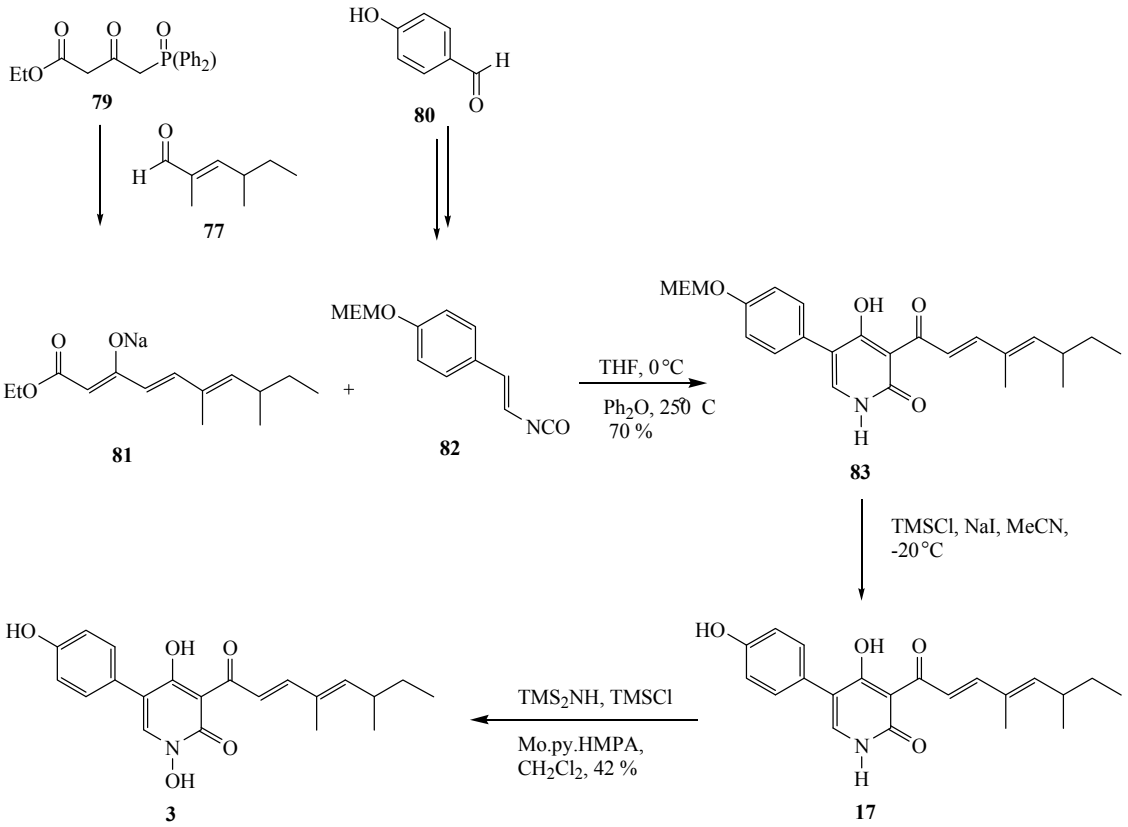
The total synthesis of tenellin **3** was reported by Williams and co-workers during 1982.[25] They prepared the hydroxamic acid derivate **70** in four steps from methyl 2-[4-(benzyloxy)phenyl]acetate **69** (Scheme **15**), which was then subjected to saponification of the methyl ester by treatment with lithium hydroxide in THF, followed by treatment with 1,1'-carbonyldiimidazole in a separate step to yield the imidazolide **72**. Addition of sodium hydride triggered cyclisation to afford **73**. Oxidation with chloranil yielded **74**, and removal of benzyl protection yielded **75**, which was reported as the bis-THP derivative **76**. Tenellin **3** was finally furnished by the use of an aldol condensation reaction, which was achieved upon treating **76** with lithium diisopropylamide (LDA) in THF with corresponding aldehyde **77** followed by dehydration to leave **78** in 56% yield. Finally deprotection followed under acidic conditions to furnish racemic tenellin in 86 % yield.



Scheme 15. Total synthesis of tenellin **3** reported by Williams and co-workers

Rigby and coworkers considered an alternative approach to achieve the total synthesis of tenellin.[26] They had much interest in the cyclocondensation of vinyl isocyanates to form pyridone derivatives. The strategy involved treating the vinyl isocyanate **82** with cyclocondensation partner β-keto ester salt **81** (Scheme 16). The vinyl isocyanate **82** was prepared in two steps from the commercially available 4-hydroxybenzaldehyde **80** in 90% yield. The β-keto ester was obtained upon treating (*E*)-2,4-dimethyl-2-hexanal **77**[25] with a β-keto ester phosphine oxide **79** in the presence of the base *n*-BuLi. Treating the sodium salt of the ester **81** with the vinyl isocyanate **82** afforded the pyridone **83** in 70% yield as a yellow solid. The next step of the synthesis was to introduce the hydroxamic acid functionality. It was necessary to remove the MEM protection group before the *N*-oxidation step, as this step was found to cleave the N-OH bond. The *N*-oxidation was accomplished by *O*-silylating the amide using hexamethydisilazane (HMDS) and a

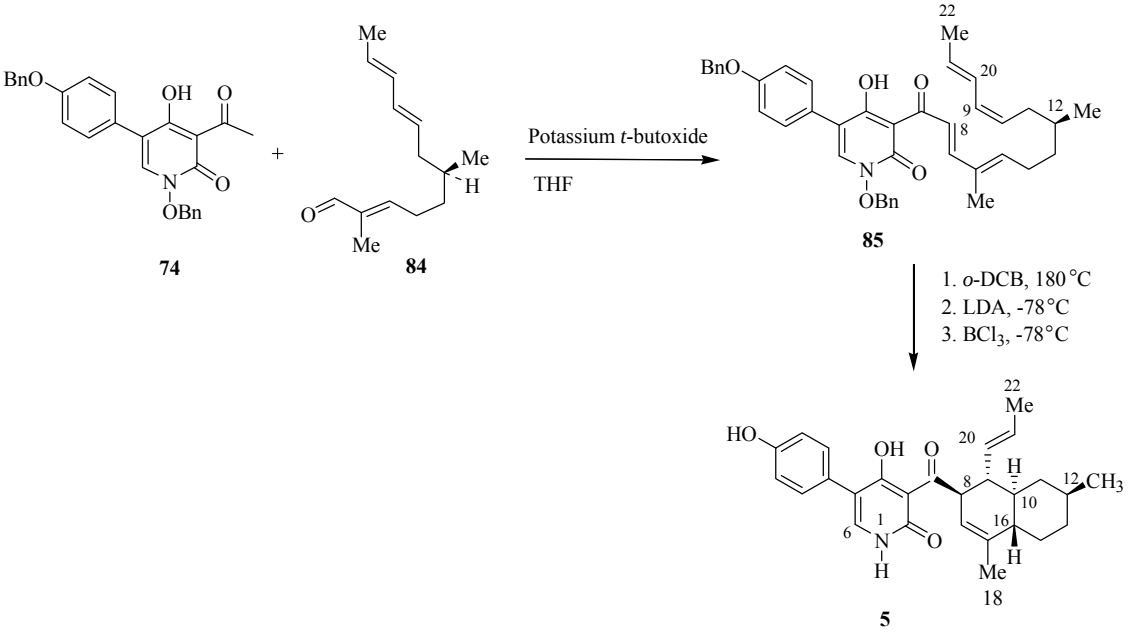
catalytic amount of trimethylsilyl chloride (TMSCl). The extremely moisture sensitive silyl intermediate was not isolated or characterized. This was immediately treated with oxodiperoxymolybdenum(pyridine)(HMPA) complex[27] to afford tenellin **3** in 42% yield.



Scheme 16. Synthesis of tenellin **3** reported by Rigby and Qabar

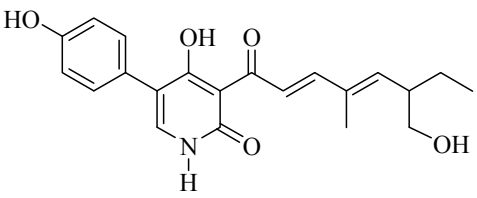
Williams and coworkers were the first to report the total synthesis of the natural product ilicicolin **5** with a unique intramolecular Diels-Alder step in a late stage of the synthesis (Scheme 17).[28] Ilicicolin with antifungal and antibiotic activity is isolated from the imperfect fungus *Cylindrocladium ilicicola*, and contains interesting structural features. It has the 5-(4-hydroxyphenyl)-2-pyridone as in tenellin **3**, and it has a distinctive *trans*-decalin system. Having previously synthesised the benzyl protected pyridine **74,**[25] which is a good starting point for this synthesis, the pyridone was treated with the α,β-unsaturated aldehyde **84** to afford the tetraene intermediate **85**. This reaction preceded *via*

an aldol condensation between the aldehyde **84** and the dianion of the pyridone **74** using potassium *tert*-butoxide. An intramolecular Diels-Alder reaction followed upon applying heat to a solution of tetraene **85** in *o*-dichlorobenzene to construct the *trans*-decalin system. Removal of the N-1 benzyloxy function was carried out by elimination of benzaldehyde using strong a base, and the remaining phenolic benzyl ether was cleaved using boron trichloride to afford the racemic natural product **5**

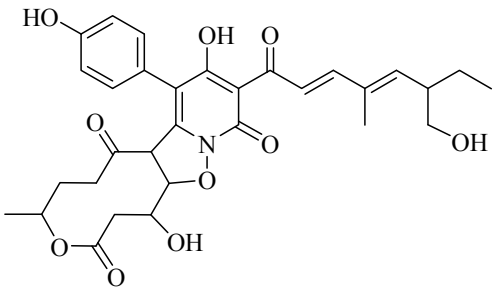


Scheme 17. Total synthesis of ilicicolin **7**

During 1998 Nakagawa and co-workers reported the isolation of two novel natural products, pyridovericin **36** and pyridomacrolidin **86** from the entomopathogenic fungus *Beauveria bassiana*.[29] These compounds have been shown to inhibit protein tyrosine kinase (PTK), and therefore are useful as therapeutic agents against inflammatory diseases. A number of compounds which inhibit protein tyrosine kinase (PTK) have in common the *p*-hydroxyphenyl functionality, which is assumed to mimic tyrosine.

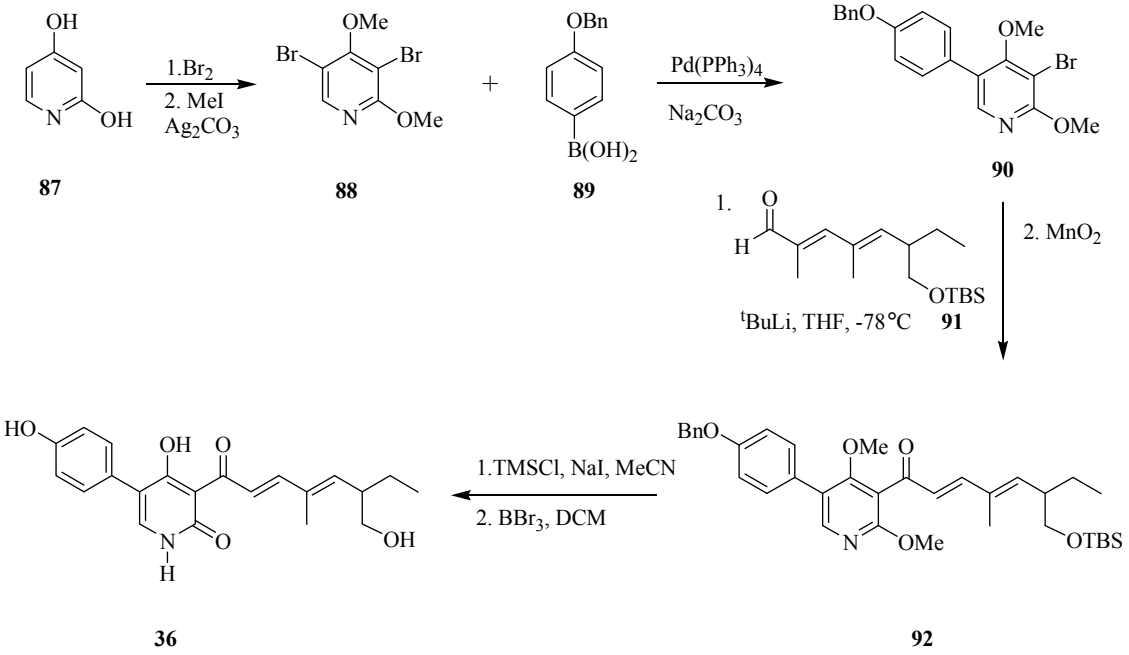


36



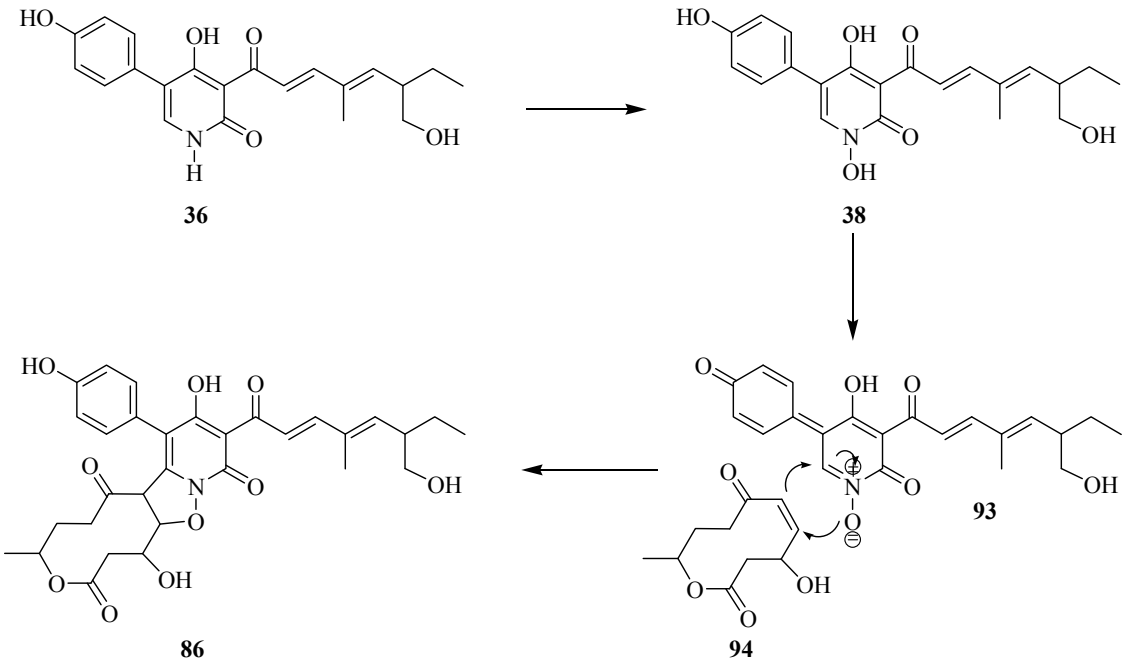
86

With the obvious biological interest, the biosynthetic pathway of pyridomacrolidin **86** has yet to be studied, while the biosynthesis of pyridovericin **36** is assumed to follow the pathway of previously reported pyridone alkaloids such as tenellin **3**. The total synthesis of pyridovericin **36** has been reported by Baldwin and co-workers (Scheme 18).[30] They prepared pyridine **88** from the commercially available 2,4-dihydroxypyridine **87** in two steps, which was then treated with the boronic acid **89** under Suzuki conditions to afford 4-benzyloxypyridone **90** as the major product, from a mixture of mono- and bis-coupled adducts. To construct the polyene side chain, treatment with *t*-BuLi in a metal-halogen exchange of the bromopyridine, and then with the aldehyde **91** afforded an alcohol adduct. Subsequent oxidation gave **92** and deprotection furnished the target natural product **36** in total of thirteen steps.



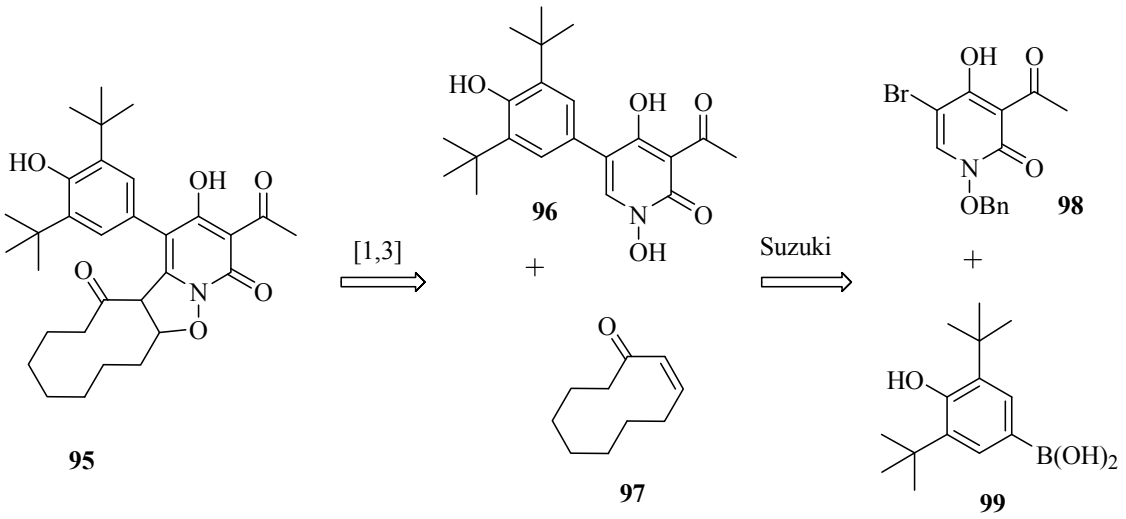
Scheme 18. Synthesis of pyridovericin **36**

Nageswara and co-workers have proposed a possible biosynthetic formation of pyridomacrolidin **86** from pyridovericin **36** (Scheme 19).[31-32] The first step of the pathway involves the *N*-oxidation of pyridovericin **36** to yield the hydroxamic acid functionality as in **38**. This is followed by further oxidation to the acyl nitrone intermediate **93**, which undergoes a 1,3-dipolar cycloaddition reaction with cephalosporolide B **94**, and finally rearomatisation takes place to furnish pyridomacrolidin **86**.



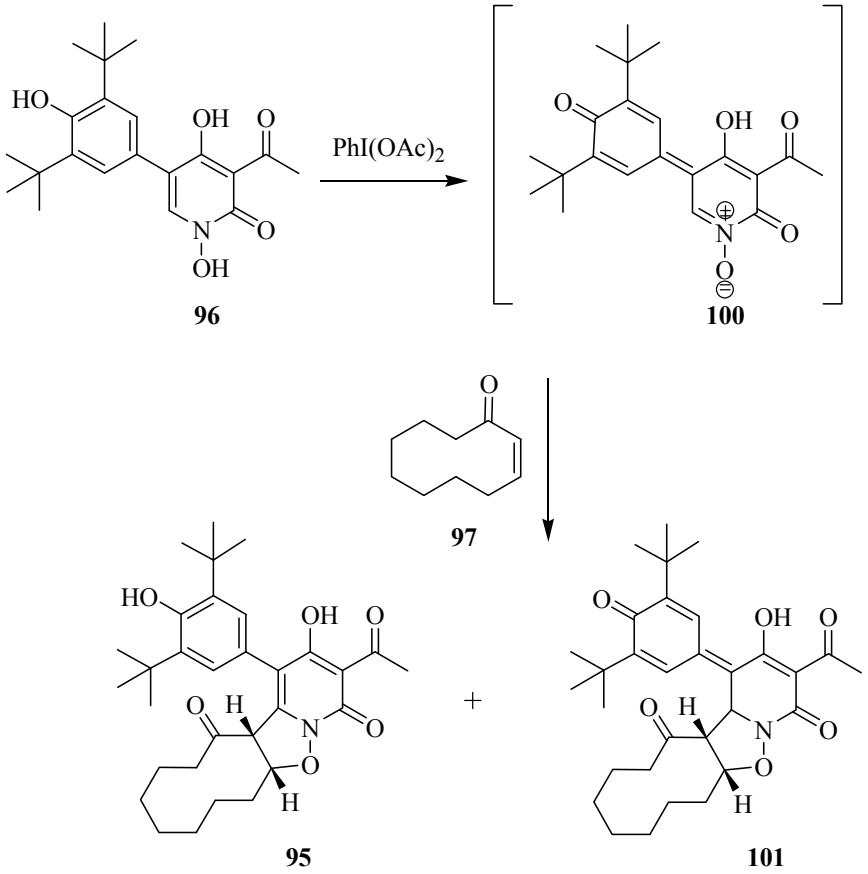
Scheme 19. Proposed biomimetic pathway of pyridomacrolidin **86**

A biomimetic retrosynthesis, based on the proposal of scheme 19, was devised for the synthesis of pyridomacrolidin analogue **95**. The pyridone **98** and boronic acid **99** in a Suzuki-cross coupling reaction would provide the 5-(4-hydroxyphenyl)pyridone **96** (Scheme 20). The reactivity of the phenolic group in **96** is blocked by using sterically hindered *tert*-butyl groups, as previous attempts failed to trap the quinonoid intermediate **93** shown in the biosynthetic proposal (Scheme 19), due to competing additions to this highly electron deficient system. The polyene side chain as in pyridovericin **36**, was omitted in this synthesis, and replaced with an acetyl group to allow the synthesis to proceed without further complexity. The target compound **96** would oxidise to the nitrone/quinonoid intermediate, and proceed in a 1,3-dipolar cycloaddition reaction with a simplified cycloaddition partner **97** to furnish **95**.



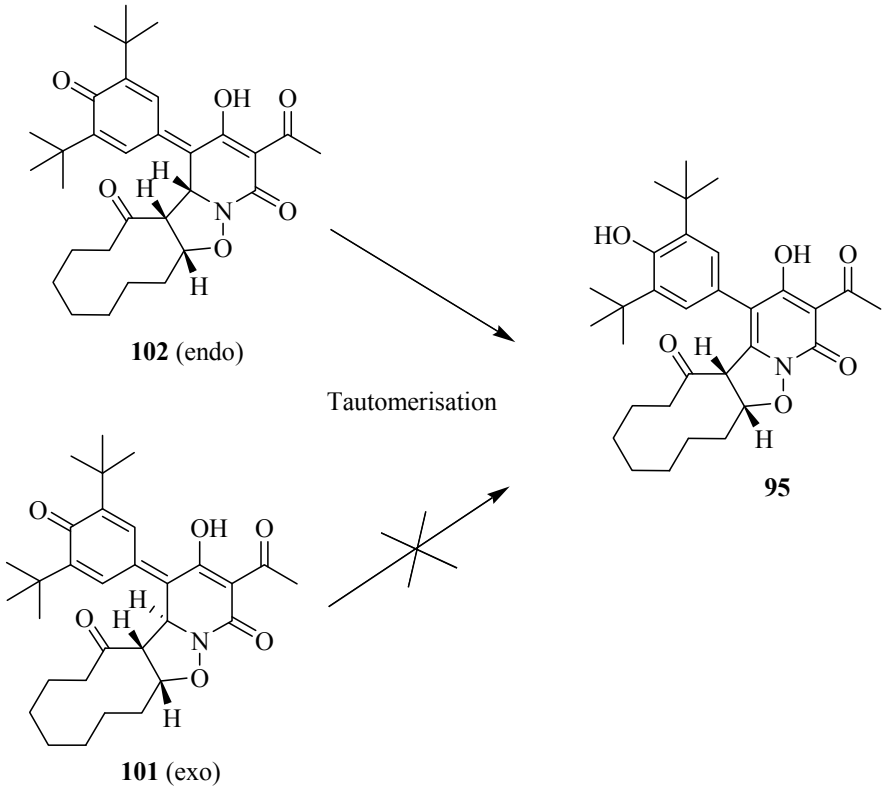
Scheme 20. Retrosynthesis of pyridomacrolidin analogue **95**

Bromide **98** was prepared using similar methods to those developed previously by Williams and co-workers.[33] The boronic acid **99** was prepared from the commercially available 4-bromo-2,6-di-*tert*-butylphenol in one step. Treating bromide **98** and boronic acid **99**, under Suzuki conditions, followed by deprotection of the benzyl group with hydrogen and 10% palladium on carbon presented the pyridone **96** in good yield (Scheme 21). Oxidation of pyridone **96** in the presence of the cycloaddition partner **97** led to [3+2] cycloaddition *via* nitrone **100** to give a mixture of two products, phenol **95** and quinone methide **101** in 60% combined yield with a cis ring junction, in a 1:1.4 ratio respectively. The structures of these two tautomers were confirmed by single-crystal X-ray crystallography,



Scheme 21

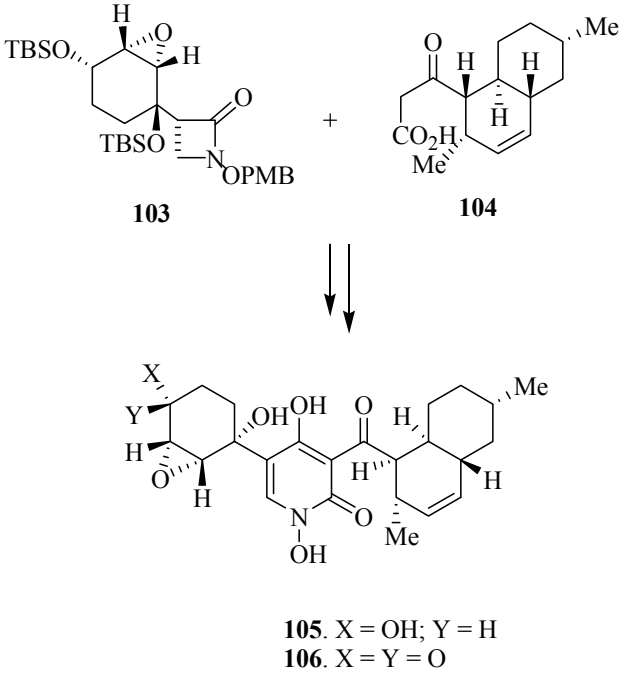
Many attempts were made to equilibrate the two products **95** and **101**, however this proved to be unsuccessful. The formation of both these compounds was rationalised by the formation of the *exo* **101** and the *endo* **102** quinone methide adducts: under the cycloaddition conditions a mixture of *endo* and *exo* adducts was obtained (Scheme 22). The *endo* adduct **102** can undergo facile tautomerisation to yield phenol **95**, whereas tautomerisation was not observed in the case of the *exo* adduct **101**. In conclusion a [3+2] cycloaddition *via* a nitrone intermediate has been investigated to form analogues of pyridomacrolidin and supports the proposed biosynthetic route.



Scheme 22

Apiosporamide **105**, an anti fungal agent, was isolated in 1994 by Gloer and co-workers from the fungus *Apiospora montagnei*.[34] Like the other natural products mentioned this also contains the core 3-acyl-4-hydroxy-2-pyridone unit, in this particular case displaying a range of activity including; antifungal activity against the coprophilous fungus *Ascobolus furfuraceus*, antibacterial characteristics against the Gram-positive bacteria *Bacillus subtilis* and the human pathogen *Staphylococcus aureus*.

Williams and co-workers have achieved the total synthesis of (+)-apiosporamide **105** and the corresponding ketone **106**.[35] They prepared two precursors in a stereocontrolled manner, the left side of **105** as an epoxydiol **103** and the right side as a *trans*-decalin system **104** (Scheme 23). Preparation of the epoxydiol **103** involved nucleophilic attack of a β-lactam hydroxamic acid derivative on a cyclohexanone derivative. The *trans*-decalin system **104** was achieved *via* intramolecular Diels-Alder reaction of an α,β-unsaturated aldehyde similar to **84**. Fusing the two precursors together afforded apiosporamide **105**.

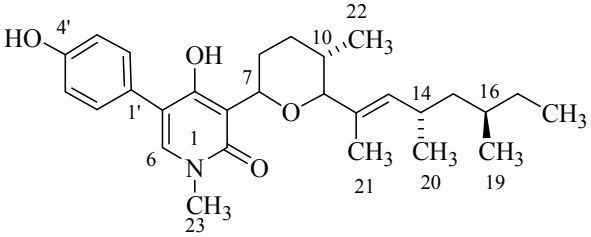


105. X = OH; Y = H
106. X = Y = O

Scheme 23. Total synthesis of (+)-apiosporamide

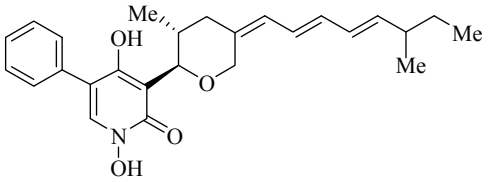
1.4 Other natural products with the core 4-hydroxypyridone unit

The isolation of the natural product (+)-sambutoxin **107** was first reported by Lee and co-workers from wheat cultures of *Fusarium sambucinum* PZF-4. [36] (+)-Sambutoxin **107** in feed sources has been found to cause hemorrhagic lesions of the gastrointestinal tract of livestock. Structural features of **107** include the pyridone core, 4-hydroxyphenyl substituent and the tetrahydropyran ring. Structural determination was able to identify the absolute stereochemistry of the tetrahydropyran ring, however at this stage the absolute stereochemistry remained uncertain. Further studies by Williams and Turske led to the total synthesis of (+)-sambutoxin and definition of the absolute stereochemistry.[37]

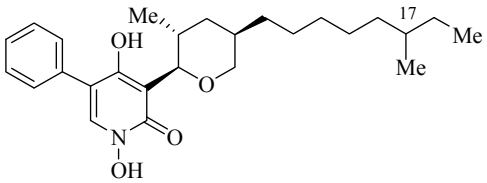


107

Other natural products belonging to the family of funiculosin **6** include TMC-69(6H) **108** and its saturated analogue TMC-69 **109** and these have been isolated from the fungus *Chrysosporium*.[38] These compounds are known to show antitumour activity, cytotoxic activity and **109** has shown significant activity towards P388 murine leukaemia *in vivo*.



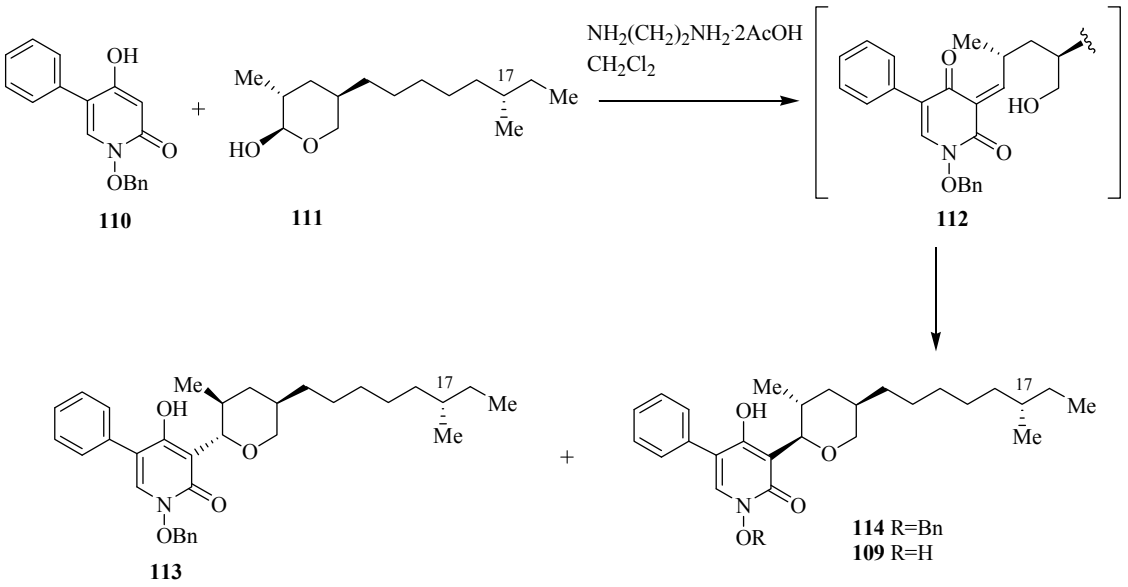
108



109

Like tenellin **3**, these compounds have the core pyridone nucleus, but the 4-hydroxyphenyl substituent is replaced with just a phenyl group on the left hand side of the natural products. The right hand side of the compound consists of a tetrahydropyran system with saturated or unsaturated side chains.

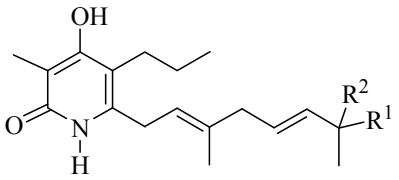
The structural interest combined with the potential biological activity led to the total synthesis of TMC-69 **109**. Previously Furstner and co-workers reported the Pd-C catalysed reaction of **108** to the saturated analogue compound **109**,[39] however the absolute configuration at C-17 was not confirmed. Sugawara and co-workers have prepared compound **109** along with its stereoisomer **113**.[38] They treated the protected pyridone **110** with the tetrahydropyran moiety **111**, which underwent a condensation reaction and cyclisation to afford (17*S*)-TMC-69 **109** and its stereoisomer **113** (Scheme 24).



Scheme 24. Synthesis of TMC-69

During 2007 Surup and coworkers proposed the biosynthesis of a new family of natural products, the iromycins **115**, isolated from a soil sample of *Streptomyces bottropensis* and having the core *N*-hydroxy-2-pyridone nucleus.[40] The structures of these natural products are of interest due to the presence of the two distinctive alkyl chains. The feeding experiments in biosynthetic studies suggested a possible polyketide pathway, which includes acetate, propionate, pentanoate and isobutyrate, however the source of nitrogen is still unknown.

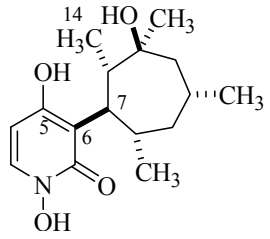
This group of natural products shows useful activity: they can selectively inhibit the production of endothelial nitric oxide synthase (NOS) over neuronal NOS. The NOS can be split into three isoforms, neuronal NOS, endothelial NOS and inducible NOS, all of which trigger the production of nitric oxide (NO). Too much NO build up in cells can be harmful and can lead to conditions such as chronic inflammatory disease. These studies can be useful in the medicinal field to aid therapeutic advances.



R^1	R^2
1 CH_3	H
2 CH_3	OH
3 H	H
4 H	OH

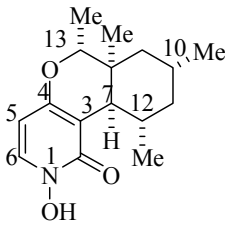
115

From the entomopathogenic fungus *Akanthomyces gracilic* the natural product akanthomycin **116** was isolated.[41] The structure was confirmed by X-ray crystallographic study, to contain the *N*-hydroxypyridone moiety linked *via* a single bond to an unusual cycloheptane ring. The X-ray studies revealed the two rings to be approximately perpendicular to each other in the solid state, with the C-5 hydroxyl group on the same side as the C-14 methyl group in the cycloheptane ring. Like other 2-pyridones, this compound was also tested for bioactivity and assay studies of akanthomycin **116** revealed antibiotic activity against *Staphylococcus aureus*.

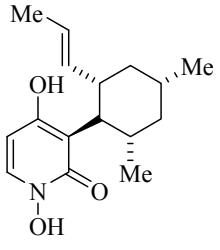


116

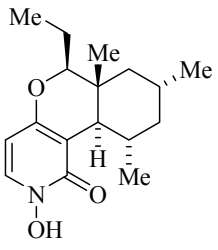
A number of fungi produce 4-hydroxypyridone antibiotics such as tenellin **3** and ilicicolin **5**.[1] Other related naturally occurring compounds which demonstrate this include PF1140 **117**, pyridoxatin **118**, fusaricide **119** and leporin **120**.[42]



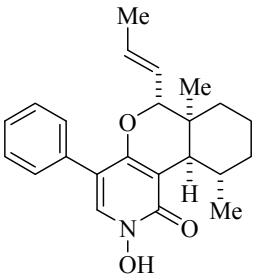
PF1140 **117**



pyridoxatin **118**



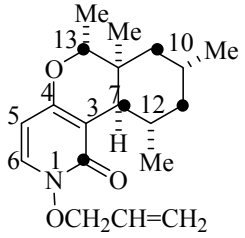
fusaricide **119**



leporin B **120**

The structure of **117** has been reported but the absolute configuration was uncertain. Fujita and co-workers have clarified the absolute configuration *via* numerous feeding experiments and have proposed the biosynthesis of PF1140 **117**.[42]

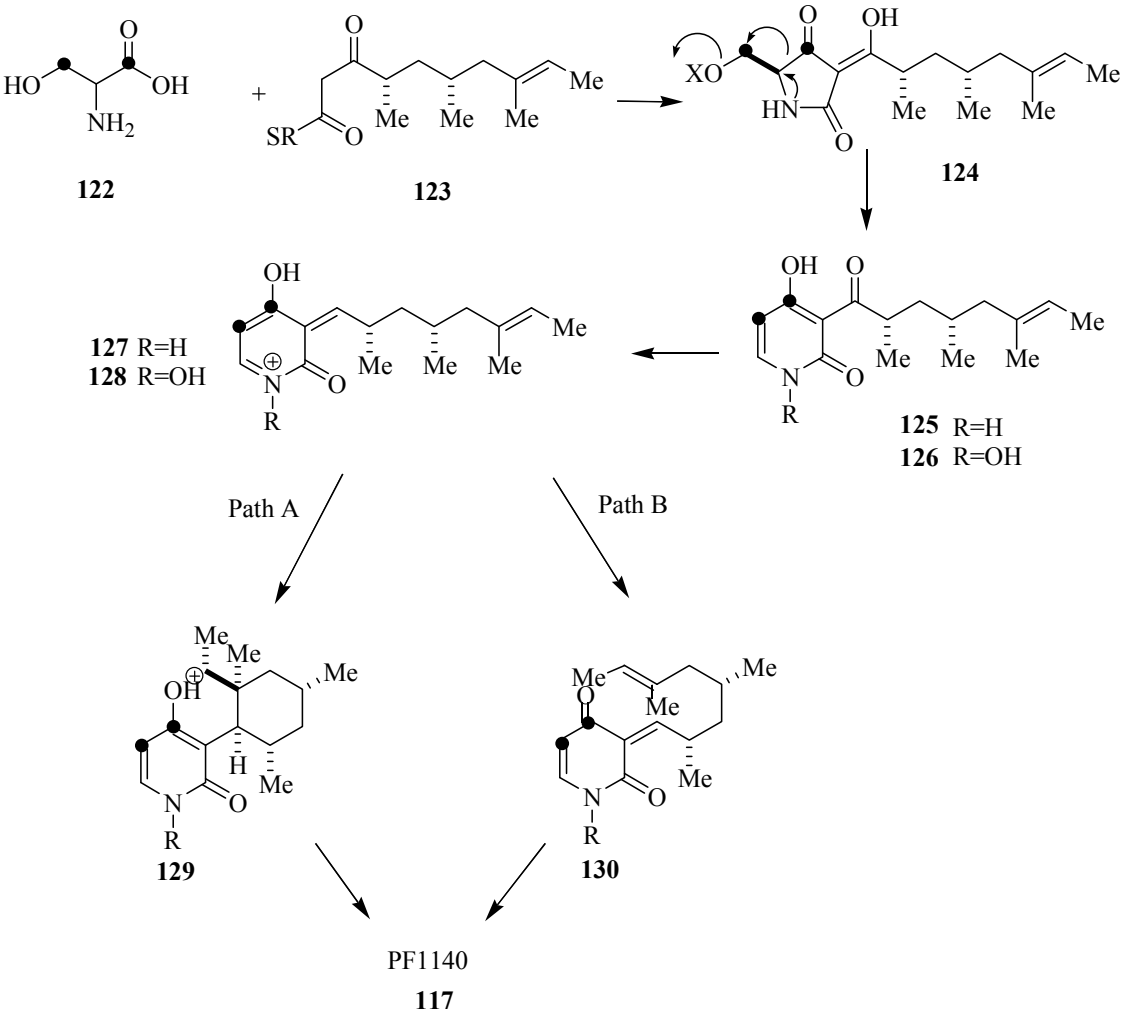
In the initial feeding experiment sodium [1-^{13}C]acetate was incorporated into cultures of *Eupenicillium* sp. PF1140, which were extracted with acetone to isolate **117** and also uncovered a new compound **121**. After extensive analysis, the ^{13}C NMR spectrum clearly illustrated sites of incorporation at C-13, C-11, C-9, C-7 and C-2 compared to the unlabeled sample. Results from a further feeding experiment with sodium [1,2-$^{13}C_2$]acetate provided evidence to suggest that five intact acetate units were involved in a head to tail fashion to form a pentaketide building block. Experiments with L-[Me-^{13}C]methionine confirmed the three branching methyl groups, at carbon positions 12, 10 and 8 originate from the *S*-methyl group of L-methionine. To verify the role of amino acids in the biosynthesis of PF1140 **117**, L-[1-^{13}C]serine was introduced to the feeding experiments and an enhanced signal in the ^{13}C NMR spectrum was confirmed to occur at C4. Based on these positive results, L-[1,3-$^{13}C_2$]serine was introduced into the feeding experiments and ^{13}C NMR spectroscopy revealed coupling between the adjacent C4 and C5, suggesting a possible intramolecular rearrangement of the carbon skeleton. A possible rearrangement has been discussed earlier in this thesis for biosynthesis of tenellin **3**.[4]



121

Following the results of the feeding experiments, a biosynthetic pathway was proposed for PF1140 **117** (Scheme 25). With verification from the feeding experiments, L-serine **122** was integral to the pyridone framework, and therefore the proposal began with this the amino acid. The amino acid comes together with a polyketide chain **123** to form the acyltetramic acid intermediate **124**, which rearranges to give the 4-hydroxy-2-pyridinone

125 framework by the loss of the hydroxyl group and aromatisation. After a reduction, there are two possible routes suggested for the last step to compose the PF1140 **117** unit, either path A, which is a stepwise cyclisation or path B, which is a hetero-Diels-Alder reaction.

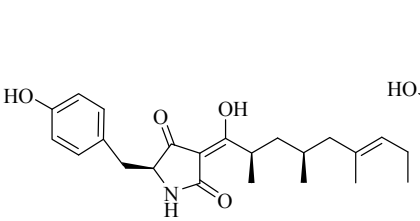


Scheme 25

The acyltetramic acids as intermediates in tenellin **3** biosynthetic pathway have been the subject of biological studies for several years and such intermediates are also considered to appear in other 2-pyridone biosynthesis as discussed earlier. There are several naturally occurring tetramic acids which have been found to be of medicinal importance and isolated from marine sponges and fungi.[43] Two novel tetramic acid derivatives,

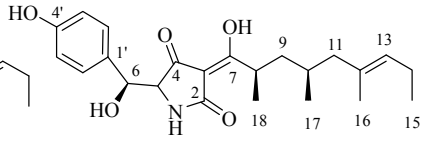
epicoccarine A **131** and B **132**, and a new pyridone alkaloid epipyridone **133**, were reported by Hilaire and co-workers, isolated from the fungus *Epicoccum* sp.[44]

Epipyridone **133** was isolated as a red oil, and the structure was determined by HR-EIMS and extensive NMR analysis. Structural similarities with other natural products are evident: like tenellin this natural product contains the 4-hydroxyphenyl substituent, and the atypical tricyclic ring system similar to leporin B **120**, with additional methyl groups. Epicoccarine A **131** and B **132** are almost structurally identical; both embrace the tetramic acid functionality, unsaturated aliphatic side chain and the *p*-hydroxyphenyl ring. The only difference arises as epicoccarine B **132** contains the hydroxymethylene group in the C-6 position.



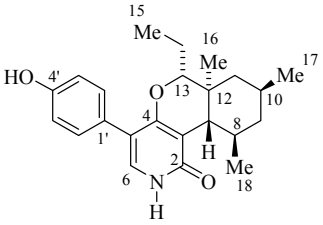
Epicoccarine A

131



Epicoccarine B

132

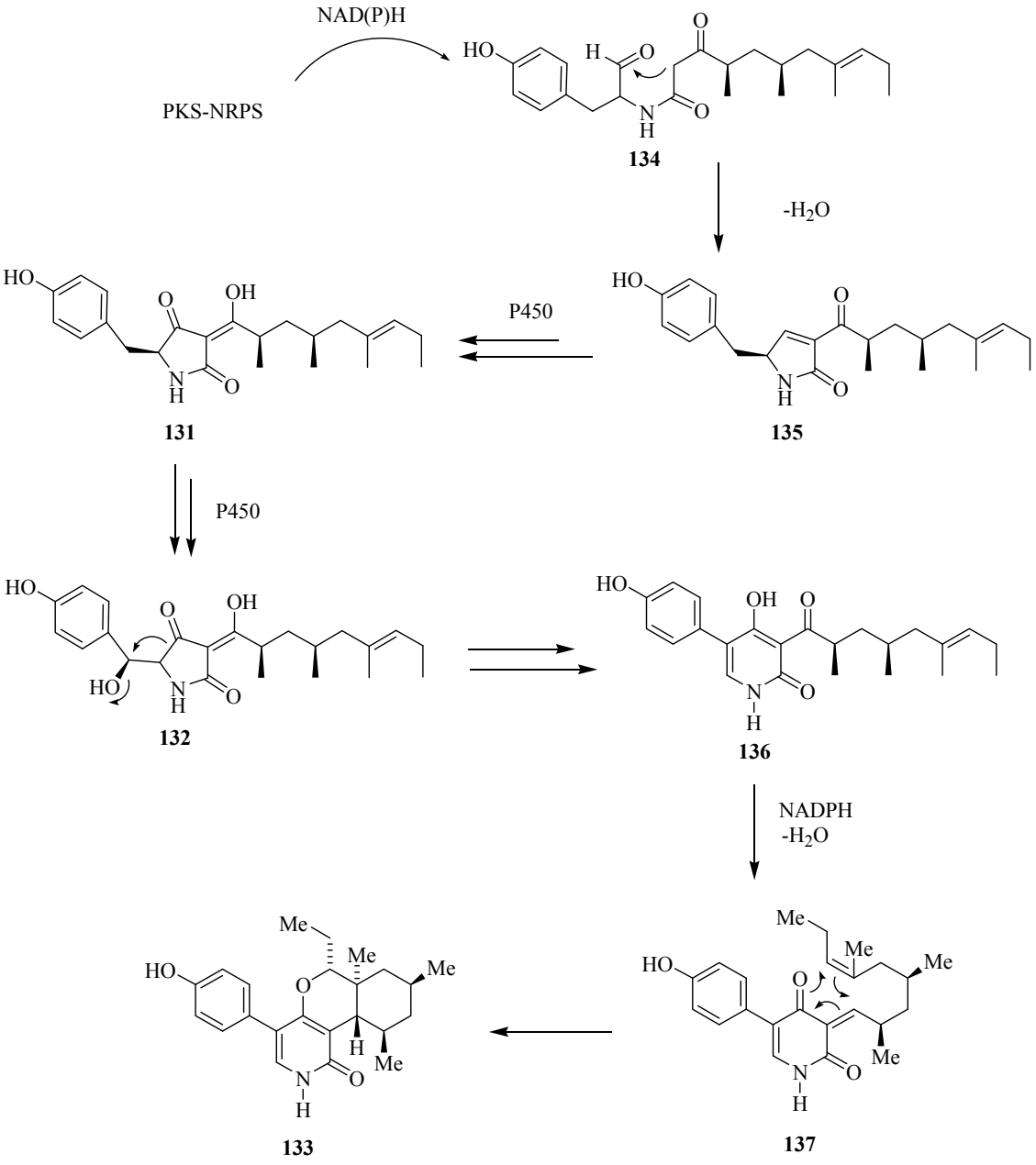


Epipyridone

133

The structural similarities of these compounds suggest the possibility of their being derived from the same biosynthetic pathway. Since as previously discussed, biosynthetic pathways normally involve an aromatic amino acid and a polyketide chain through condensation reactions, a similar concept is also adopted here to follow the tenellin **3** sequence. The polyketide synthase non-ribosomal peptide synthetase (PKS–NRPS) hybrid assembles the polyketide-amino acid backbone **134**. Ring closure followed by further oxidation reactions catalysed by the enzyme P450 could lead to the tetramic acid with the tricarbonyl moiety **132**. This undergoes rearrangement as seen in other 2-pyridone biosyntheses to form the tenellin like pyridone alkaloid **136**. Finally it is thought the natural product **133** is formed *via* an intramolecular cyclisation in a hetero Diels-Alder reaction (Scheme 26).

Epipyridone **133** and epicoccarine B **132** were found to show only moderate activity against Gram positive bacteria. Epicoccarine A **131** on the other hand exhibited antibacterial activity against the Gram positive bacterium *Mycobacterium vaccae*.



Scheme 26. Proposed biosynthetic pathway for epipyridone

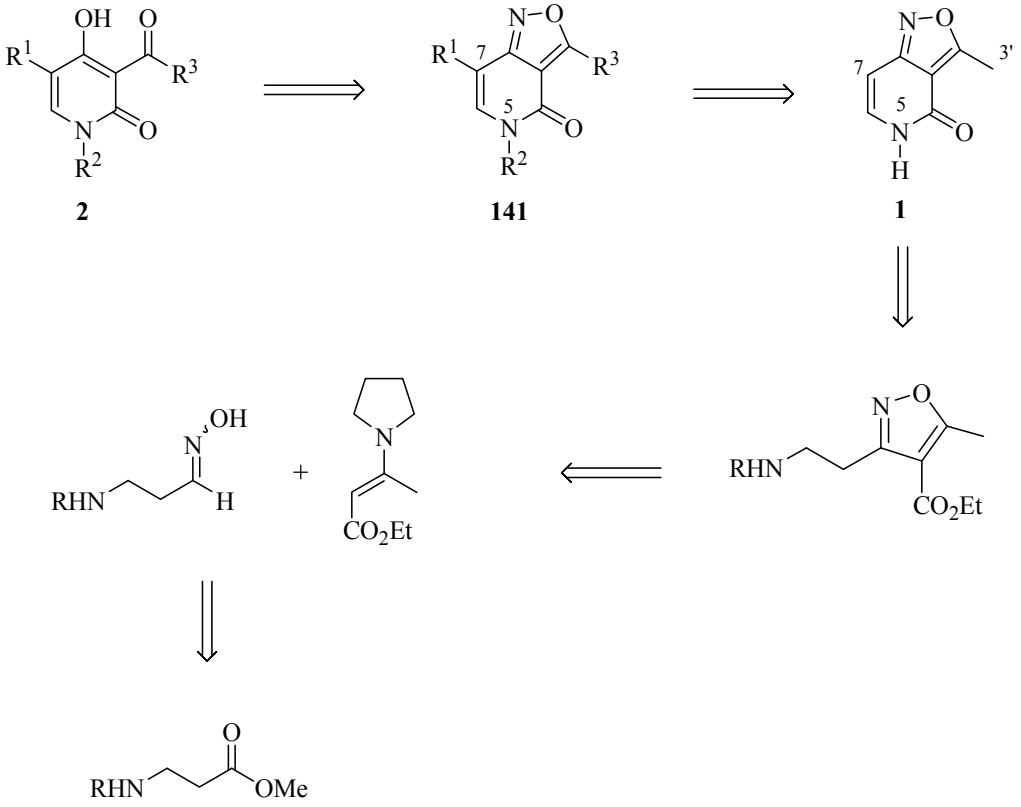
Results and Discussion

2. 1 The 3-acyl-4-hydroxypyridone

2.1.1 Synthetic strategy

After the review presented in chapter one of the natural products containing the acylpyridone motif **141**, we sought original and flexible ways to access and elaborate the 3-acyl-4-hydroxypyridone unit *via* the isoxazolopyridone **1** as the masked form (Scheme 27). In a strategy to develop a building block with the 3-acyl-4-hydroxypyridone core, we planned to disguise the highly polar functionality of the heterocyclic triones **2**, by masking them in the form of an isoxazolopyridone **1**, as a more amenable building block. The polar nature makes them challenging to work with, particularly with regards to handling and purification. The isoxazolopyridone **1** would then be elaborated at the sites, C-3' (Me), N-5 and C-7 to allow the development of natural products and non-natural analogues with potential biological activity. The unmasking of the elaborated isoxazolopyridone **141** to reveal the heterocyclic trione would follow in the final step of the synthesis, by cleavage of the N-O bond.

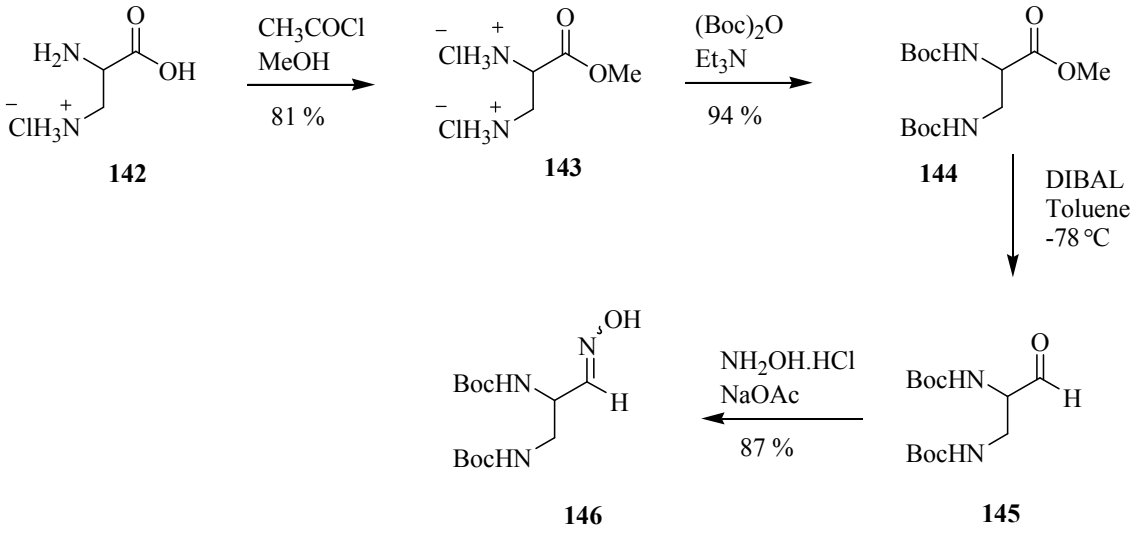
The proposed synthetic strategy to develop the isoxazolopyridone **1** commences with an amino acid (Scheme 27). The protected amino acid would be converted to the stable oxime *via* the corresponding aldehyde. The oxime would undergo a 1,3-dipolar cycloaddition of the nitrile oxide formed *in situ* with a cycloaddition partner to form the isoxazole. A spontaneous cyclisation of the isoxazole would yield the isoxazolopyridone. Other amino acids, such as 2,3-diaminopropionic acid could also be employed in this strategy to afford the isoxazolopyridone building block **1**.



Scheme 27

2.1.2 Synthesis of the isoxazolopyridone building block

The preparation of the isoxazolopyridone **1** building block was achieved in seven steps from the commercially available 2,3-diaminopropionic acid monohydrochloride **142** (Scheme 28). Esterification of 2,3-diaminopropionic acid monohydrochloride was followed by *N*-protection to the ester **144**, and reduction of the ester to an aldehyde **145**, which was transformed directly to diamino-oxime **146**. The nitrile oxide of the oxime **146** took part in a 1,3-diopolar cycloaddition reaction (Scheme 29), followed by spontaneous cyclisation at the β-amine to yield a bicyclic ring **151**. The unsaturated bond was introduced by diazotization of the α-amine to yield the isoxazolopyridone **1** (Scheme 30).



Scheme 28

a) Esterification

The commercially available 2,3-diaminopropionic acid monohydrochloride **142** was treated with freshly distilled methanol and acetyl chloride, which was heated at reflux under nitrogen for four days. The resulting white precipitate was filtered to leave a mixture of the starting material and the methyl ester. The alpha proton signal of the amino acid was present in the ^{1}H NMR spectrum suggesting the presence of the starting material, therefore a second treatment with acetyl chloride and methanol was required to ensure complete conversion to the methyl ester **143**. At that time it was found that the filtrate contained starting material, and as a result, a third treatment was required. The ^{1}H NMR spectrum of the methyl ester revealed double doublets for the beta protons, as they are diastereotopic, with a chiral centre in the molecule.

b) Amine Protection

The amine protection was readily achieve by addition of di-*tert*-butyl dicarbonate ($(Boc)_2O$) to a suspension of the ester dihydrochloride **143** in dichloromethane (DCM) was followed by dropwise addition of triethylamine at 0°C to afford the Boc protected methyl ester **144** in yield of 94%. The excess ($(Boc)_2O$) could be removed by

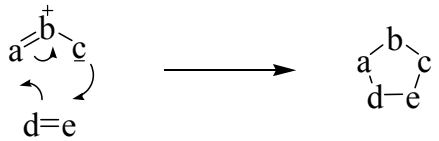
purification using silica column chromatography, however this was not required since we later discovered that this did not cause any complications in the following steps of the synthesis.

c) Oxime Formation

The Boc-protected methyl ester **144** was treated with diisobutylaluminium hydride (DIBAL-H) in toluene at -78 °C, to afford the aldehyde **145**, which was used immediately without further purification to prevent any decomposition from occurring. The presence of the aldehyde proton was observed in the ^{1}H NMR spectrum by a signal at 9.7 ppm as a singlet. Treating the aldehyde with hydroxylamine hydrochloride and sodium acetate, afforded the desired oxime **146** as a colourless oil (70-87 %). Often the oxime precipitated out as a solid if it was dissolved in the minimum amount of ethanol and left in the fridge overnight, otherwise, the oxime was extracted with ethyl acetate. The oxime **146** was isolated as diastereoisomers, both syn and anti oximes being observed, which is consistent with the literature report.[45]

d) 1,3-Dipolar cycloaddition and nitrile oxides

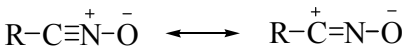
1,3-Dipolar cycloaddition reactions are used to make five-membered rings, with one or more heteroatom. These are considered to be concerted reactions, whereby two new bonds are formed and one is broken. They require a 1,3-dipole, which consists of three atoms with a four π electron system, and a dipolarophile which can be a simple alkene consisting of a two π electron system. Two new σ-bonds are made at the expense of two π-bonds.



The electrons in the dipole are delocalised over the three atoms. An important feature of

the 1,3-dipole is that it has both nucleophilic and electrophilic ends, as shown by the example resonance structures below.

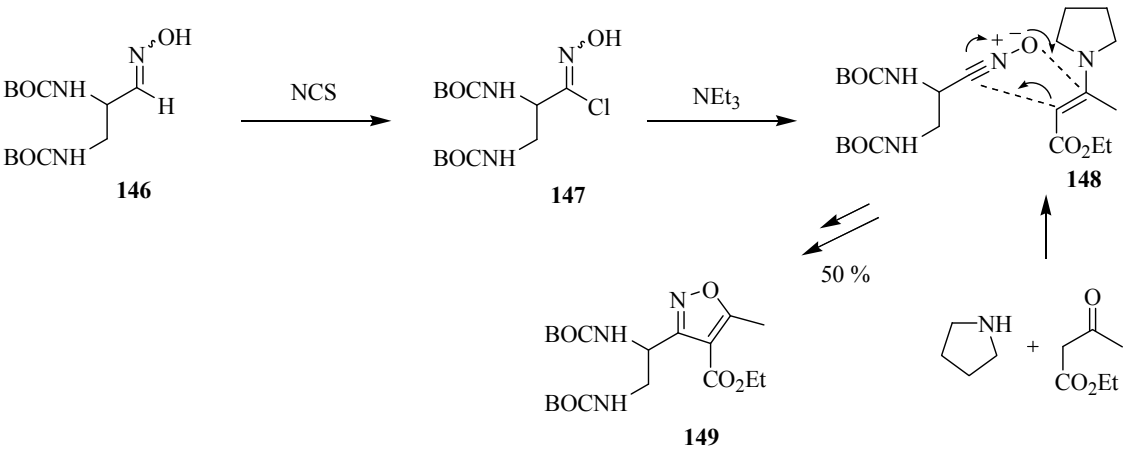
Nitrile oxide



This thesis focuses on nitrile oxide dipoles. Nitrile oxide species are very reactive and often are short-lived. In the absence of reactants, or if the concentration of the nitrile oxides at any one time is high, they will react with themselves. We employed the cycloaddition of a nitrile oxide with an olefin to develop an isoxazole as a key step in our synthetic strategy.

Formation of the isoxazole **149** proved to be difficult at first, followed by a second challenge, which was to improve the yield of the isoxazole **149** (Scheme 29). In order to obtain a reasonable yield of the cycloaddition product, it was necessary for the oxime **146** to be dry prior to use. This was achieved by drying the oxime **146** in a vacuum desiccator over phosphorus pentoxide, followed by drying in a vacuum oven. The dry oxime **146** was then treated with freshly distilled DCM under a nitrogen atmosphere, followed by addition of N-chlorosuccinimide (NCS), and the mixture was heated at reflux for 2 h (Scheme 29). The progress of the reaction was monitored by TLC analysis and as a result, a further portion of NCS was added, and the reaction mixture was heated at reflux overnight. The formation of a blue/green solution indicated the formation of the chloro-oxime **147**. The isolation of the chloro-oxime **147** proved optional, it could either be isolated by simple extraction with ethyl acetate to remove any impurities, or it was possible to carry out the next reaction *in situ.* The cycloaddition partner, the enamine **148**, previously prepared from ethyl acetoacetate and pyrrolidine in toluene under Dean-Stark conditions, was added in excess to the chloro-oxime **147**. Triethylamine was then added dropwise to the reaction mixture to form the reactive nitrile oxide species. The nitrile oxides are very reactive so it could react with various species present, including itself; therefore the concentration of the nitrile oxide was kept at a minimum, which should increase the likelihood of reacting with the cycloaddition partner **148** *via* a 1,3-dipolar

cycloaddition reaction. Finally a spontaneous elimination of pyrrolidine followed to form the isoxazole **149**, the yield varying between 40-50 % after purification by column chromatography.



Scheme 29

e) Deprotection of the amines

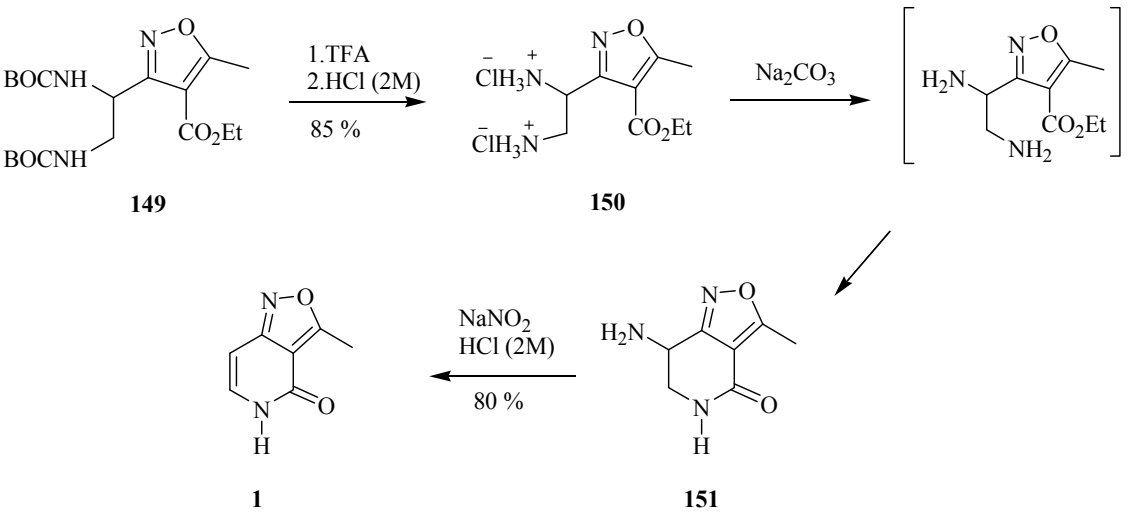
The deprotection was necessary in order to free the amine to allow the formation of the bicyclic pyridone **151** (Scheme 30). Removal of the amine protection was achieved readily by the addition of trifluoroacetic acid to the isoxazole **149**, followed by treatment with hydrochloric acid (HCl, 2M) to afford the less hygroscopic dihydrochloride salt **150** in excess of 85 % yield.

f) Formation of the isoxazolopyridone building block

The dihydrochloride salt **150** was treated with aqueous sodium carbonate solution, and stirred overnight to yield the bicyclic pyridone **151** (Scheme 30). Sodium carbonate removes the hydrochloride salt releasing the amines, which activates spontaneous ring cyclisation by condensation with the ester group to form a six-membered ring. The formation of the five-membered ring is not observed, these are difficult to synthesise due to the ring strain. It has been previously shown in the tetramic acid series, the formation of the five-membered ring does not readily occur in a simple ring closure between the α-amino substituent and the C-4 ester of the isoxazole.[71,46] In a more complex procedure,

peptide coupling was employed to form an active ester, which then proceeded on to form a five-membered ring. Thus, in our case the α-amine does not take part in the cyclisation step to form a five-membered ring, whereas the β-amine is more readily involved to afford the six-membered ring, with the reduced ring strain provided by the extra sp^3 carbon, when compared to the alternative five-membered ring.

Finally to introduce the C6-C-7 unsaturated bond in the isoxazolopyridone, the free amine **151** was treated with sodium nitrite and 2M HCl in water for 1 h at 0 ºC and then at 50 °C for another hour. Upon treating the free amine **151** with a solution of sodium nitrite in HCl (2M), nitrous acid forms, which then undergoes dehydration forming a nitrosonium cation. The nitrosonium cation interacts with the amine forming a diazonium salt at 0 °C and as heat is applied, the stable dinitrogen molecule is lost readily leaving a cation intermediate, which undergoes spontaneous elimination of a proton to provide extra conjugation to furnish the desired isoxazolopyridone **1**. This diazotisation reaction has proven to be very successful and yield of the isoxazolopyridone has reached 80 %.



149 **150**

1 **151**

Scheme 30

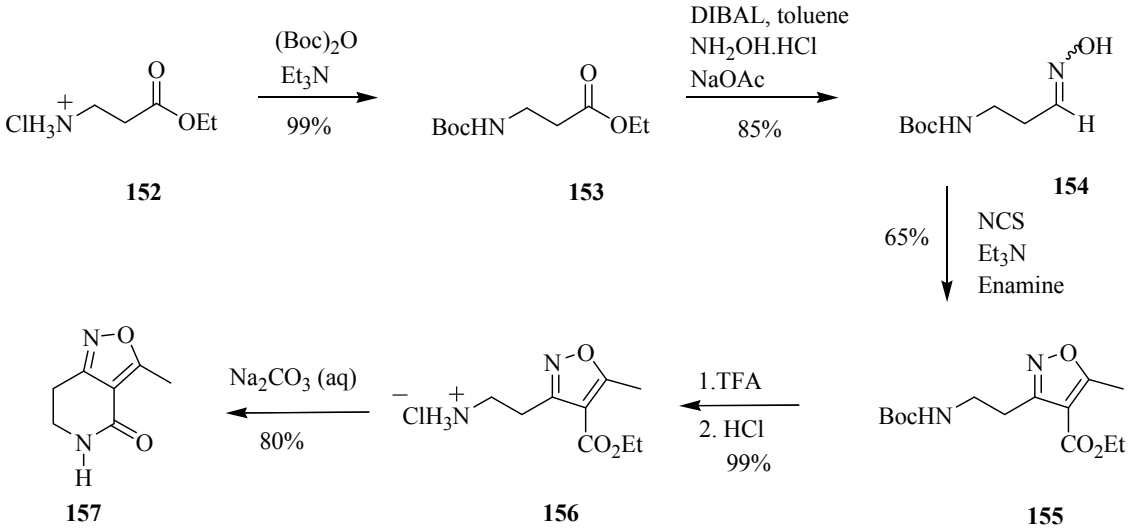
Following the synthetic strategy, the isoxazolopyridone **1** has been synthesised in seven steps from the commercially available 2,3-diaminopropionic acid monohydrochloride. However due to the high cost of the 2,3-diaminopropionic acid monohydrochloride, we

investigated the use of other amino acids, such as β-alanine as a cheaper alternative. In addition, the inexpensive β-alanine ethyl ester hydrochloride is commercially available; therefore the esterification step of the acid is avoided, which can take up to a week to ensure full conversion to the corresponding acid.

2.2 β-Alanine route

2.2.1 Synthesis of the bicyclic lactam

An alternative route to the isoxazolopyridone **1** was the use of other amino acids, in this particular case a cheaper alternative to the 2,3-diaminopropionic acid monohydrochloride was the β-alanine ethyl ester **152**. A similar synthetic route was employed in the β-alanine series to develop the bicyclic lactam **157** (Scheme 31). The bicyclic lactam was synthesised in five steps following the same sequence employed previously with the 2,3-diaminopropionic acid monohydrochloride **142**.



Scheme 31

a) Amine Protection

The β-alanine ethyl ester **152** is commercially available, therefore the esterification step is avoided. We were able to employ the *N*-protection of the amine immediately. The *N*-protection of the β-alanine ethyl ester **152** was achieved readily, by adding triethylamine to a solution of β-alanine ethyl ester **152** in DCM at 0 °C, followed by the addition of di-*tert*-butyl dicarbonate. The reaction mixture was stirred at 23 ºC for 24 h and after an

aqueous workup the *N*-Boc-β-alanine ethyl ester **153** was formed in quantitative yield (Scheme 31).

b) Oxime Formation

The next step involved the preparation of the oxime **154** *via* the corresponding aldehyde. The *N*-Boc-β-alanine ethyl ester **153** was treated with diisobutylaluminium hydride (DIBAL-H) in toluene at -78 °C over a period of 1 h, to afford an aldehyde, which was used without further purification. We were concerned about the stability of the aldehyde to a possible β-elimination, we therefore treated this immediately with an aqueous solution of hydroxylamine hydrochloride and sodium acetate, followed by a workup, which afforded the desired oxime **154** as a colourless oil in 85 % yield. The oxime was isolated as diastereoisomers, both syn and anti oximes being observed.

c) Formation of the isoxazolopyridone *via* 1,3 dipolar cycloaddition

The 1,3-dipolar cycloaddition reaction was pursued to develop the isoxazole **155**. The oxime **154** prepared previously was dried in a vacuum desiccator over phosphorus pentoxide, followed by drying in a vacuum oven. The oxime **154** was then dissolved in dry DCM and NCS was added under a nitrogen atmosphere. The reaction was monitored by TLC analysis and as a result a second batch of NCS was required. The resulting mixture was heated at reflux for 24 h. The reaction mixture was cooled to 23 °C and the cycloaddition partner, the enamine **148** was added in excess to the chloro-oxime, followed by the dropwise addition of triethylamine over a period of 1 h to form the reactive nitrile oxide species. The mixture was heated at reflux for a further 24 h, to follow a spontaneous elimination of pyrrolidine to form the isoxazole **155** in 65 % yield. This is an improved yield compared to the isoxazole **149** (50 % yield) from the 2,3-diaminopropionic acid monohydrochloride route.

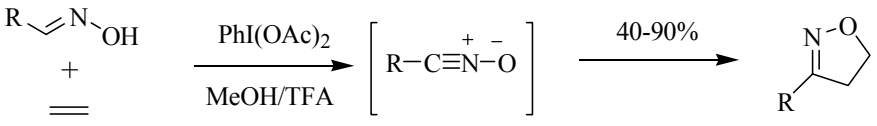
d) Deprotection of the amine

Removal of the amine protection was readily achieved by the addition of excess trifluoroacetic acid to the isoxazole **155**, followed by treatment with hydrochloric acid (2M), to afford the less hygroscopic hydrochloride salt **156** in 99 % yield.

e) Lactam formation

The bicyclic lactam **157** was formed from a base-promoted cyclisation of the hydrochloride salt **156**. The hydrochloride salt **156** was dissolved in water and treated with sodium carbonate. The resulting mixture was stirred for 24 h at 23 ºC. The reaction mixture was extracted with ethyl acetate, dried and the solvent was removed under reduced pressure to leave an off white solid in 80 % yield, which did not require further purification. As mentioned previously, the cyclisation favours the formation of the six-membered ring compared to the analogous five-membered ring due to the reduced ring strain provided by the extra sp^3 carbon in the six-membered ring.

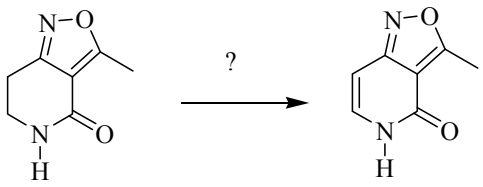
Further investigation could be conducted to improve the yield of the isoxazole **149** and in particular isoxazole **155**. A recent publication has reported the oxidation of oximes to nitrile oxides with hypervalent iodine (Scheme 32).[47] Methanol and a catalytic amount of TFA were used as the solvent, which provided the best yields. These reaction conditions are to be considered in the future for further investigation to increase the yield of the isoxazoles, but due to time constraints this was not pursued at present.



Scheme 32. Formation of reactive nitrile oxides

2.2.2 Development of the C6-C7 unsaturation

Following the β-alanine route has its consequence: the α-amine is no longer available as seen in the 2,3-diaminopropionic acid route, and therefore diazotisation is no longer an option to introduce the C6-C7 unsaturation.



157 **1**

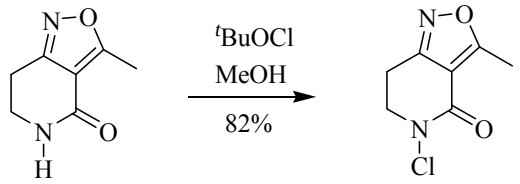
There have been a number of strategies previously attempted to develop the C6-C7 unsaturation, including dehydrogenation using Pd/C and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).[48, 49] But these were unsuccessful, instead we planned to generate an N-chlorolactam and investigate dehydrochlorination.

a) *N*-chlorination

To introduce the unsaturated bond, we pursued the *N*-chlorination strategy. This involved chlorination at the N-5(H) of the bicyclic lactam **157** to yield the *N*-chlorolactam **158** (Scheme 33), which would take part in a photolytic dehydrochlorination to yield the desired isoxazolopyridone **1** (Scheme 36). Simple base mediated dehydrochlorination had proved unsuccessful for a previous worker.[99]

The *N*-chlorination was achieved using *tert*-butyl hypochlorite (Scheme 33), a reagent described by Mintz and Walling.[50] This was prepared by treating *tert*-butanol and glacial acetic acid in commercial bleach (250 ml). Although best used freshly prepared, it was found to be possible to store this reagent at 0 °C in the absence of light to avoid decomposition. Without any significant decomposition the *tert*-butyl hypochlorite was added to a solution of the bicyclic lactam **157** in methanol to yield the *N*-chlorolactam **158** as a white solid in 82% yield. The ^{1}H NMR spectrum of this compound **158**

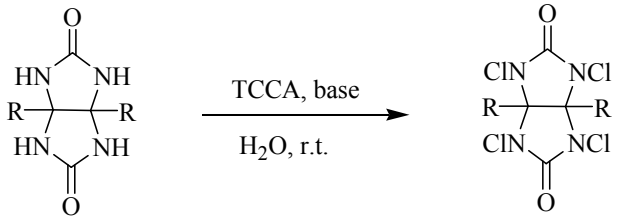
demonstrated a downfield shift of the C-6/7 protons due to the presence of the electronegative chlorine, and the absence of a broad N-H signal verified that the *N*-chlorination had taken place.



157 **158**

Scheme 33

N-chloro compounds have received attention in organic synthesis, in industry and the medicinal field, and more recent studies have described simple methods of preparing *N*-chloro compounds.[51] Here the authors have reported a clean and efficient method of synthesis under mild conditions using trichloroisocyanuric acid (TCCA) **(**Scheme 34). The use of this reagent is growing rapidly and is finding its way into commercial products, such as detergents and disinfectants. It is hoped that this method might be employed successfully in our sequesnce to enhance our current yield.

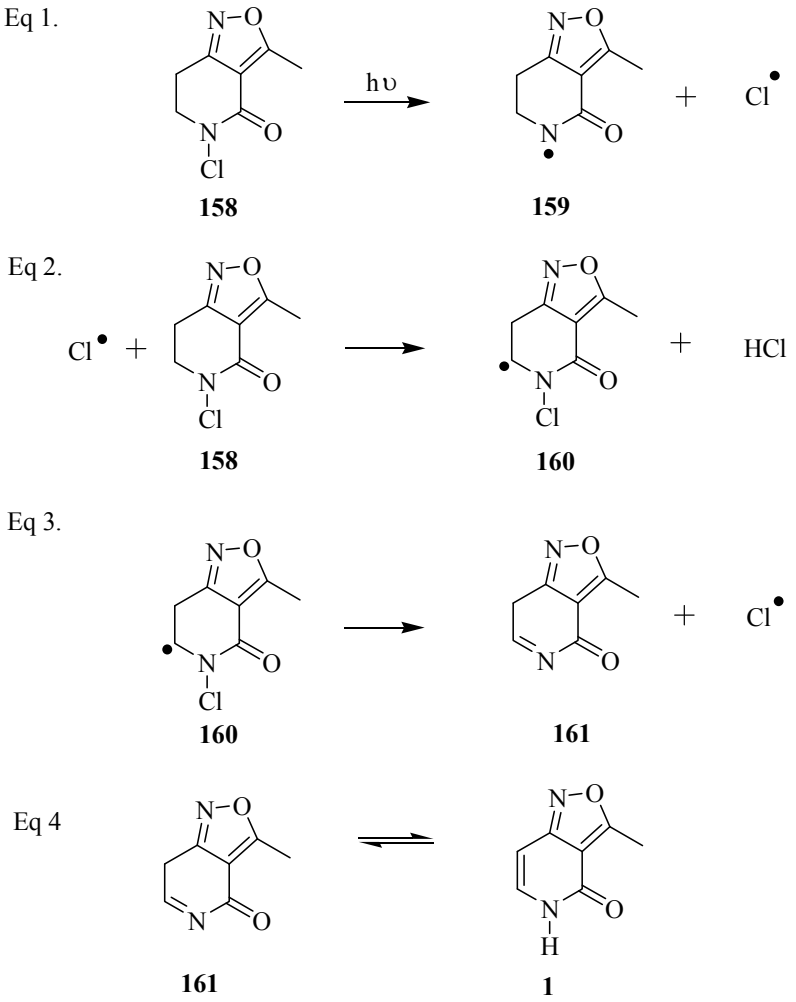


Scheme 34

b) Photolytic dehydrochlorination

Next step in the preparation of the unsaturated pyridone **1** was by using a UV light source in a photolytic dehydrochlorination reaction, which would initiate radical cascade reactions to eventually form the unsaturated pyridone precursor **1**. A reasonable pathway

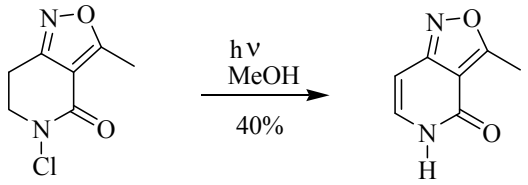
(Scheme 35)[52] would involve the homolysis of the N-Cl bond (Eq 1), followed by hydrogen abstraction from C-6 of the N-chlorolactam **158** to leave the radical **160** (Eq 2). This radical **160** would undergo β-scission to afford the acyl imine **161** and regenerate the chlorine atom (Eq 3), and then tautomerise to yield the favourable acyl enamine tautomer, i.e. the isoxazolopyridone **1** (Eq 4).



Scheme 35

The *N*-chlorolactam **158** in freshly distilled methanol was degassed for 10 minutes under a constant nitrogen flow in the presence of two home Solaria UV lamps. The reaction was monitored using TLC analysis: no change was observed for the first 2 h, but after 4 h a yellow solution developed, and the TLC analysis revealed a second spot. The reaction

was allowed to proceed for an additional 1 h, after which point the solvent was concentrated and the residue purified by column chromatography to yield the isoxazolopyridone **1** in 40 % yield, or 60% yield based on the recovery of the starting material. However, the results were not consistent when repeat reactions were carried out with this light source.

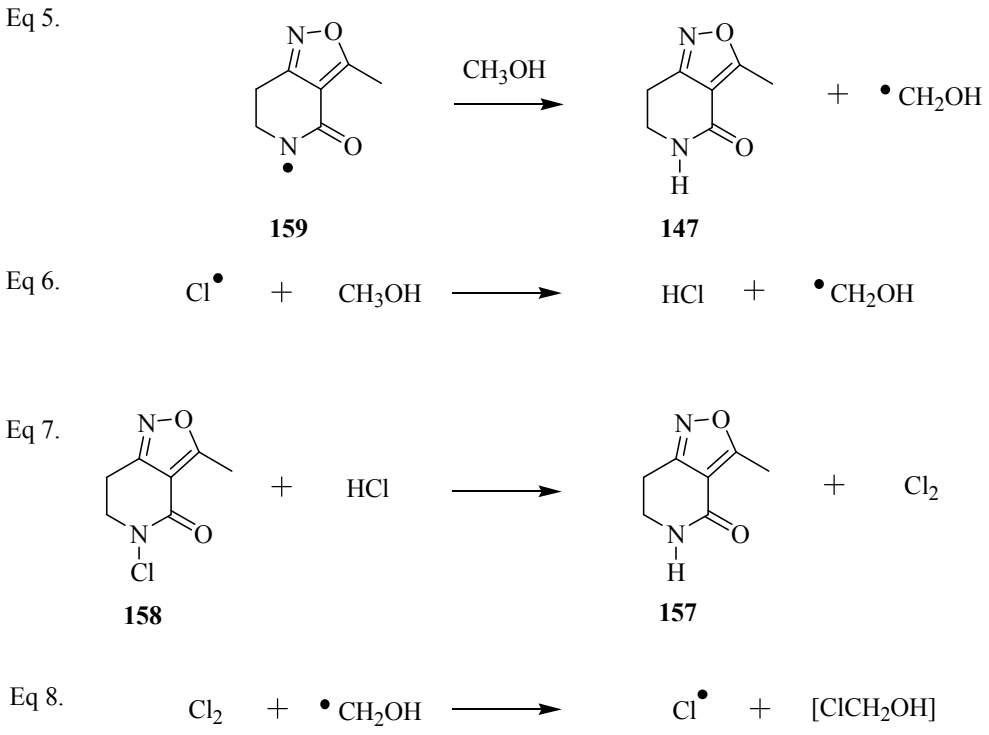


158 **1**

Scheme 36

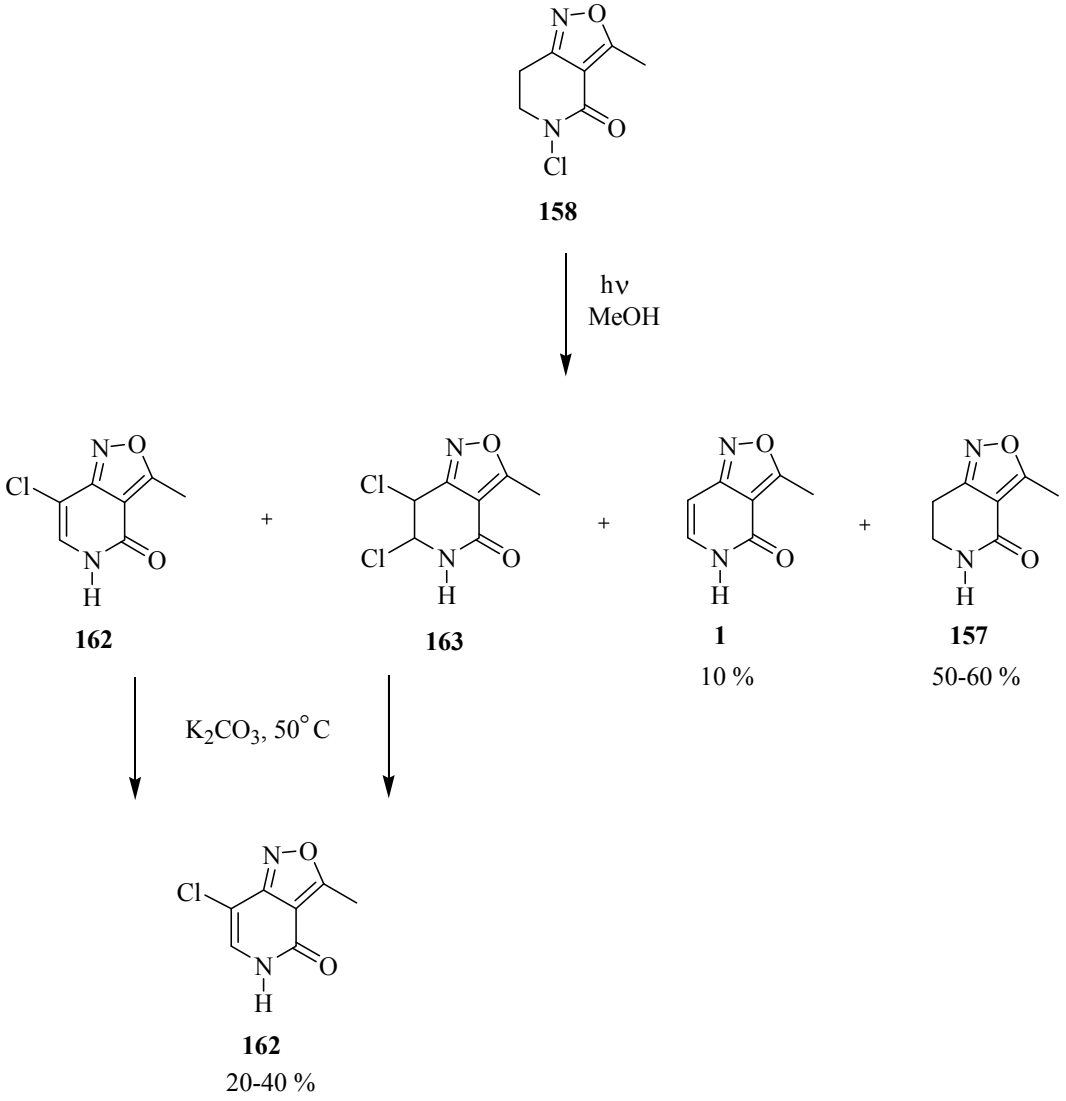
Further investigation followed using a medium-pressure Hg Hanovia lamp in a water-cooled quartz vessel. The *N*-chlorolactam **158** in methanol was purged with nitrogen for 15 minutes before irradiation with the lamp for 1 h, to yield the isoxazolopyridone **1** in 80 % yield as our best procedure. The starting bicyclic lactam **157** was also recovered, which can be re-used.

The regeneration of the bicyclic lactam **157** is proposed *via* two possible mechanisms in competing processes by chlorination of the solvent (Scheme 37, Eq 5).[52] The chlorine atom is terminated as HCl is formed (Eq 6). Under acidic conditions, the N-chlorolactam **158** is converted to the bicyclic lactam **157** (Eq 7) as the by-product chlorine forms.



Scheme 37

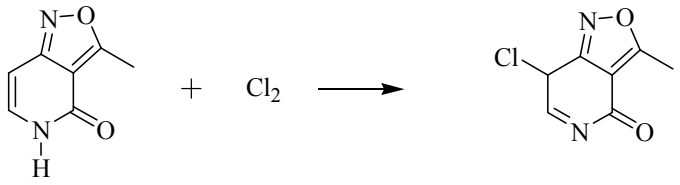
During a placement period at Syngenta, an alternative light source was used as the medium-pressure Hg Hanovia lamp was unavailable, and as a substitute a tungsten UV lamp was utilised. The *N*-chlorolactam **158** in methanol was degassed for 10 minutes under a constant nitrogen flow, before being irradiated with the light source. The reaction was constantly monitored by TLC analysis, which showed all the starting material had been consumed after 5 h. Following work up and purification, the desired isoxazolopyridone **1** was obtained in a low yield and often in repeat reactions this was only observed in trace amounts. The unexpected 7-chloroisoxazolopyridone **162** was isolated as the major product up to 40 % yield (Scheme 38), which was obtained in a mixture with the 6,7-dichlorodihydroisoxazolopyridone **163**. It was possible to heat this mixture at 50 ºC under basic conditions, to ensure complete conversion to the 7-chloroisoxazolopyridone **162**. This elimination reaction confirms the presence of the 6,7-dichloroisoxazolopyridone **163**. The reactions were repeated several times, the reaction conditions were altered, and even the use of different glassware was also investigated to give only the same result.



Scheme 38

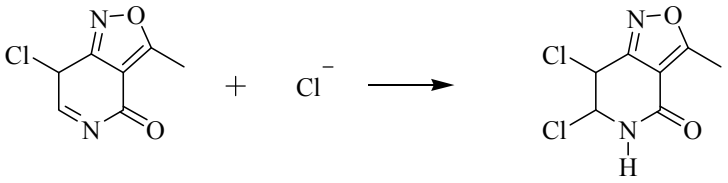
To account for the formation of the chlorinated species, a possible mechanistic proposal *via* an electrophilic addition reaction is shown below (Scheme 39), which would follow on from formation of the unsaturated compound **1** as shown previously. The reaction between the unsaturated pyridone **1** and a chlorine molecule results in the imine **164** (Eq 9), which undergoes addition with a chloride ion to give the di-chloro adduct **163**. Finally elimination of hydrogen chloride follows, to leave the 7-chloroisoxazolopyridone **162**.

Eq 9.



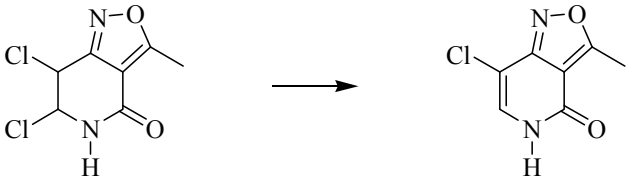
1 **164**

Eq 10.



164 **163**

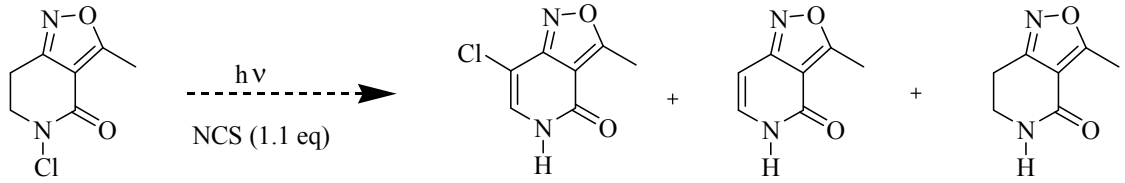
Eq 11.



163 **162**

Scheme 39

The 7-chloroisoxazolopyridone **162** seems to be the major product other than the recovery of the saturated bicyclic lactam **157**; the new light source could be advantageous if the reaction conditions could be optimised to afford a higher yield of this compound. Our interest lies in such halides, since we wish to pursue Suzuki cross coupling reactions between such alkenyl halides and boronic acids, to develop new C-C bonds at C-7. From a mechanistic aspect, we are chlorinating the C-6 and C-7 of the isoxazolopyridone, therefore we thought we might be able to increase the yield of the 7-chloroisoxazolopyridone **162** by introducing a chlorine source. We introduced NCS to the reaction mixture during the photolytic dehydrochlorination step, and monitored the reaction progress by TLC analysis, but upon reaction completion and purification of the crude material, the same results were obtained as in the previous case and no change in the yield was observed (Scheme 40).



158 **162** **1** **157**

Scheme 40

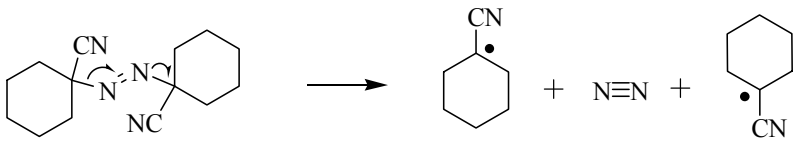
c) Alternative radical approach

An alternative route to introduce the double bond in the C-6 and C-**7** position of the bicyclic lactam **157**, was to investigate the use of radical H-atom abstraction methods. Radical reactions are very useful, since radicals can be used as intermediates in reactions, which may be difficult to achieve *via* other routes. They are formed by homolysis, by either applying light or heat, or radical initiators are used to form reactive radical species, examples including AIBN and ACCN.

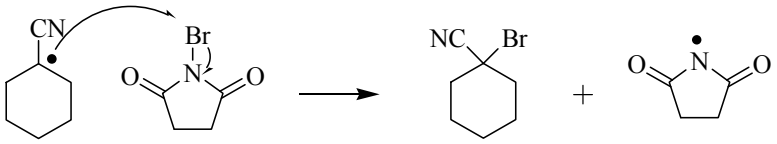
To pursue our interest in this new strategy, the bicyclic lactam **157** was treated with the initiator 1,1-azobis(cyclohexanecarbonitrile) (ACCN) in the presence of N-bromosuccinimide (NBS) under anhydrous conditions (Scheme 41). NBS is a good source of bromine atoms, "$Br^{\bullet}$", and releases molecular bromine slowly during the reaction and as a result, keeps its concentration low. This is important to prevent any side reactions from occurring. The reaction was carried out in cyclohexane, which was degassed for 10-15 minutes prior to use. In the early stages of the reaction, cyclohexane was selected as a suitable solvent due to its high boiling point, as the initiator ACCN undergoes thermal homolysis at 60-70°C to generate the reactive radical species. A possible mechanism for this reaction would proceed through a series of radical chain reactions. Initially, the homolysis of the initiator forms the stabilised cyclohexane nitrile radicals (Eq 12), followed by the formation of the *N*-succinimide radical (Eq 13), which could abstract a hydrogen atom from the bicyclic lactam **157** (Eq 14). In the next step (eq 15), the radical formed **165** should be able to abstract the bromine atoms from NBS, which is known to be a good source of bromine. Finally re-aromatization of the ring

would take place with the loss of hydrogen bromide to yield the unsaturated pyridone **1** (Eq 1). Similar mechanisms have been reported by Wohl-Zeigler.[53]

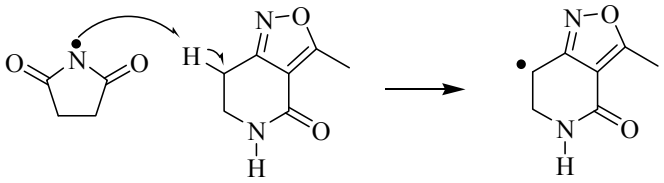
Eq 12.



Eq 13.

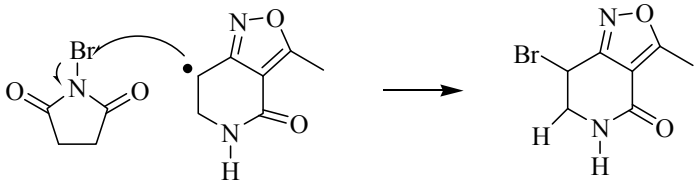


Eq 14.



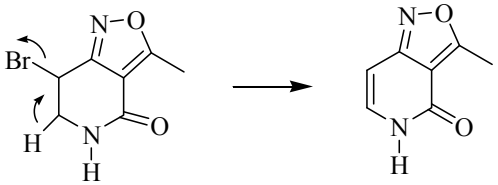
157 **165**

Eq 15.



165 **166**

Eq 16.

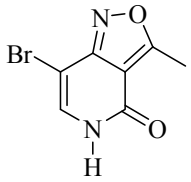


166 **1**

Scheme 41

In the first attempt, 8% of the initiator ACCN and 1.2 equivalents of NBS were used. The initiator ACCN, the bicyclic lactam **157** and NBS freshly recrystallised (from water) in cyclohexane were heated at refluxed overnight. Following analysis, the ^{1}H NMR

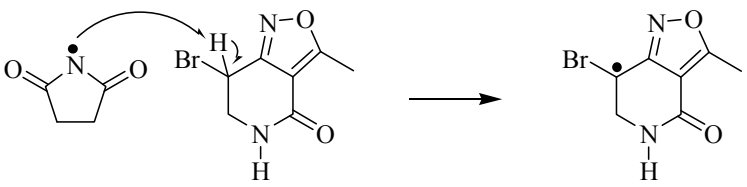
spectrum revealed signals corresponding to the starting material, however TLC analysis revealed 3 spots with distinguishable R_f values. Purification by column chromatography resulted in recovery of the bicyclic lactam **157**, and a trace amount of the 7-bromo-3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **167**.



167

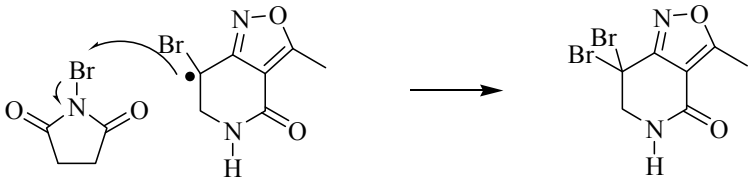
The 7-unsubstituted isoxazolopyridone **1** was not observed, and to account for the formation of the 7-bromoisoxazolopyridone **167**, it is possible that after a first bromination, a second bromination *via* hydrogen abstraction took place at the same position, C-7 (Scheme 42, Eq 17/18). A radical formation at this position **168** is favored due to the extra stability offered by the electron withdrawing bromine substituent. The reaction proceeds with the elimination of hydrogen bromide from **169**, to yield the 7-bromoisoxazolopyridone **167** (Eq 19).

Eq 17.



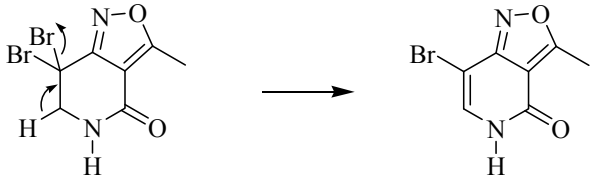
166 **168**

Eq 18



168 **169**

Eq 19.



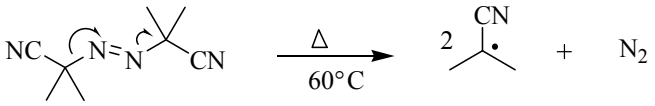
169 **167**

Scheme 42

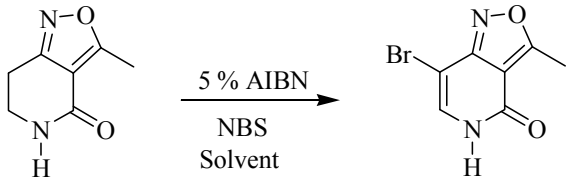
Despite no trace of the 7-unsubstituted isoxazolopyridone **1**, this route provided access the 7-bromoisoxazolopyridone **167** in one step from the bicyclic lactam **157**, therefore it might be possible to carry out a Suzuki cross-coupling reaction with this compound and thus avoid the halogenation reaction of the isoxazolopyridone **1** (see later). However, it was necessary to obtain a good yield of 7-bromoisoxazolopyridone **167** in order to continue with the Suzuki cross-coupling reactions.

Many attempts were made to increase the yield of the 7-bromo compound **167** by altering the reaction conditions. The ACCN initiator was substituted by azobisisobutylonitrile (AIBN), since there have been many reports of successful radical brominations using this initiator.[54] The thermal decomposition of AIBN begins at 60 °C to form the radical species as the stabilised nitrile intermediate (Scheme 43).

Eq 20.



Eq 21.



157 **167**

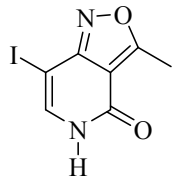
Scheme 43

A range of solvents were investigated in these reactions and it was discovered that the bicyclic lactam **157** had limited solubility in cyclohexane and toluene. Carbon tetrachloride is widely used in radical reactions, and after exploiting this solvent under the current reaction conditions, it provided a 30 % yield of the 7-bromo isoxazolopyridone **167**, and also there was no observation of the non-halogenated isoxazolopyridone. The use of other solvents such as cyclohexane and toluene provided poor results and the majority of the starting material was recovered. The experimental procedure was then slightly adjusted due to the reactive nature of radicals: the initiator, AIBN was added in small portions every hour for 5 h under nitrogen, and the mixture was heated at reflux overnight. Conducting the reaction in *tert*-butanol, provided an improved yield of the 7-bromoisoxazolopyridone **167** of 30-35 % based on the recovery of the starting material.

Further investigation into the 7-bromoisoxazolopyridone **167** *via* radical reactions led to pursuing similar reactions in the presence of tributyltin. The reaction did not prove successful and only a trace of 7-bromoisoxazolopyridone **167** was observed.

The radical reactions were investigated further using *N*-iodosuccinimide (NIS) as an alternative source of halogen. The Suzuki cross coupling reactions between a halide and organoboron compounds often proceeds more efficiently as the reactivity increases with

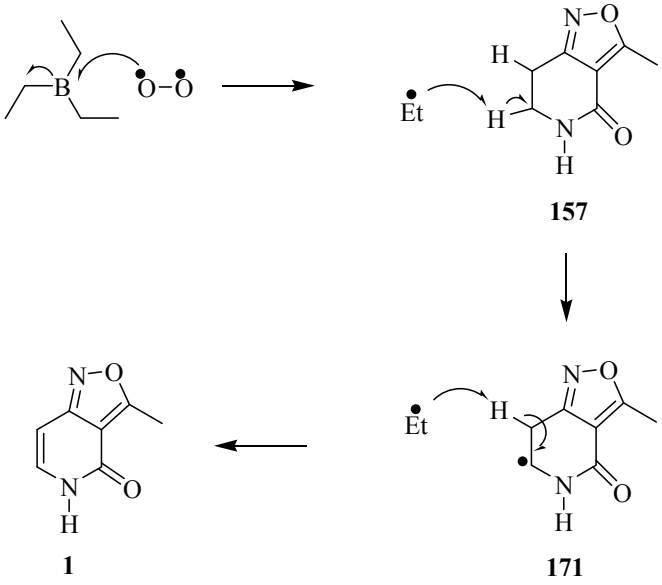
R-X, X = I > Br > Cl. NIS is a good source of iodine atoms and iodine, and a well known iodination reagent. The electrophilic iodine allows good stereoselective reactions on a range of functional groups. The radical reaction described previously was employed once again, but using the electropositive iodine source. Unfortunately, only trace amounts of 7-iodoisoxazolopyridone **170** was observed and the starting material **157** was recovered.



170

Another approach to the unsaturated isoxazolopyridone **1** was investigated using an alternative radical approach with the highly reactive triethylborane reagent (Scheme 44). The proposed reaction mechanism requires dioxygen, itself a radical, to attack the borane, thereby producing the ethyl radical. The ethyl radical abstracts a labile hydrogen atom from C-6 of the isoxazolopyridone **157**, producing ethane as the by-product, which would be followed by further H-atom loss from the ring to yield the desired isoxazolopyridone **1**.

Triethylborane ignites spontaneously in air therefore care needs to be taken while handling this reagent. The procedure applied was as follows; to a round bottomed flask was added the bicyclic lactam **157** and freshly distilled dry DCM. The flask was purged with nitrogen to ensure it was oxygen free, and fitted with a rubber septum. Triethylborane in excess was added via a syringe directly into the solution avoiding the walls of the flask. Whilst the solution was being constantly stirred, a small needle was inserted into the rubber septum to allow a slow flow of oxygen. The mixture was stirred overnight, followed by TLC analysis, which confirmed only one spot corresponding to the starting material. A second treatment with triethylborane in the same manner was applied, however following another day of stirring and TLC analysis, only the starting material was recovered.



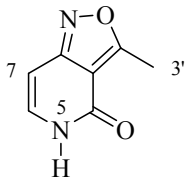
Scheme 44

Thus the best yield obtained for the 7-bromoisoxazolopyridone **167** from the saturated bicyclic lactam **157** was 35 %. This compares to the preparation of the unsubstituted isoxazolopyridone **1** by N-chlorination (82 %) and photolytic dehydrochlorination (80 %). Subsequent halogenation of **1** is discussed in the next section.

2.3 Elaboration at the C-7 of the isoxazolopyridone

2.3.1 Methodology

Having constructed the isoxazolopyridone building block, we require to elaborate at the sites C-3' (Me), C-7, and N-5 (Figure 1). By accessing these sites we will be able to work towards the family of natural products containing the 3-acyl-4-hydroxypyridone core. A number of strategies are proposed to achieve elaboration at these sites, which will allow the development of natural and unnatural analogues.



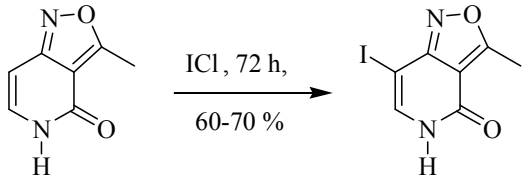
1

Figure 1

We investigated anion-type chemistry to construct a polyene side chain at C-3', Suzuki cross-coupling reactions, with a palladium-based catalyst for C-C coupling to develop aryl substituents such as the 4-hydroxyphenyl group at C-7, and *N*-oxidation to develop hydroxamic acid functionality at N-5.

After the successful synthesis of the isoxazolopyridone precursor **1**, we required to elaborate and functionalise at the C-7 position. The methodologies developed are based around a 7-iodo compound, and its participation in a Suzuki cross coupling reaction with an organoboron compound to develop aryl substituents. The iodine substituent at the C-7 position would lead to an electrophilic isoxazolopyridone, which can be used in Suzuki cross-coupling reactions in the presence of a Pd(0) catalyst to introduce aryl groups. The methodology to develop such a 7-iodo compound utilises iodine monochloride (ICl). This reagent serves as good iodinating agent, since chlorine is more electronegative than iodine, and this ICl acts as a good source of "I^+".

The isoxazolopyridone **1** was treated with ICl and stirred at 23 °C for 72 h, when the resulting precipitate was filtered to yield the 7-iodo-3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **170** in a 60-70 % yield (Scheme 45).



1 **170**

Scheme 45

2.3.2 Suzuki cross-coupling reactions

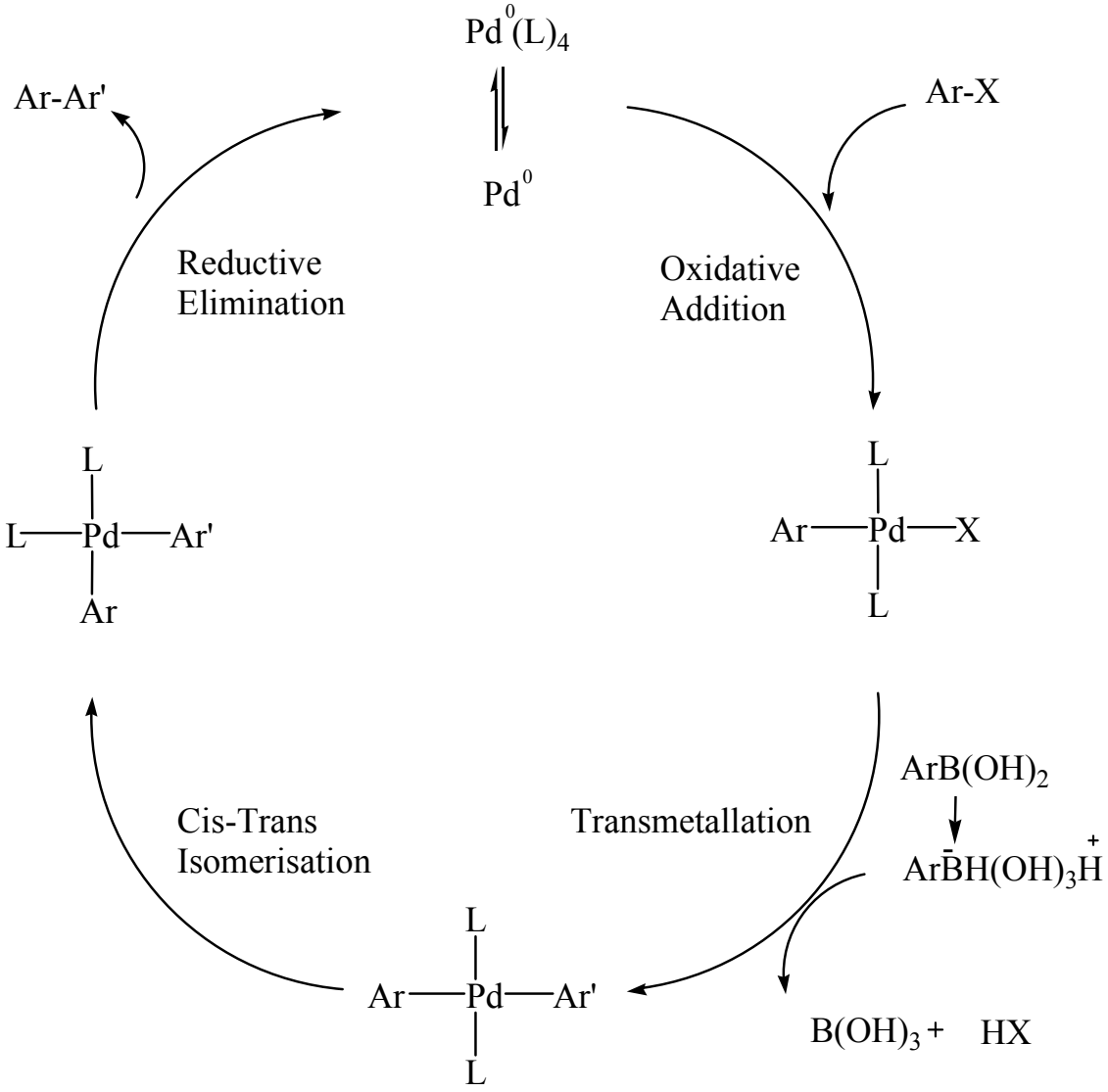
With the 7-iodo-3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **170** in hand, we continued the synthesis towards the natural product tenellin **3**. The natural product has a phenolic aryl ring at the C-7 position. To achieve this, we investigated the Suzuki cross-coupling reaction, with a palladium-based catalyst for C-C coupling. There have been many advances in palladium-catalysed C-C coupling. The Suzuki palladium-catalysed cross-coupling reactions of organoboron[55] compounds with organohalides have been found to be extremely efficient, and an important tool in the synthesis of biaryls.[56, 57] This method of synthesising biaryls has many advantages;

- Relatively experimental conditions
- Tolerant to many functional groups, weak nucleophilic characteristic of boronic acids tolerates reactants containing groups such as aldehydes, ketones and nitriles
- Stability of boron reagents in air and moisture
- Boron reagents usually non toxic
- Easy removal of side products from mixture
- Accessibility of a wide range of commercially available boronic acids

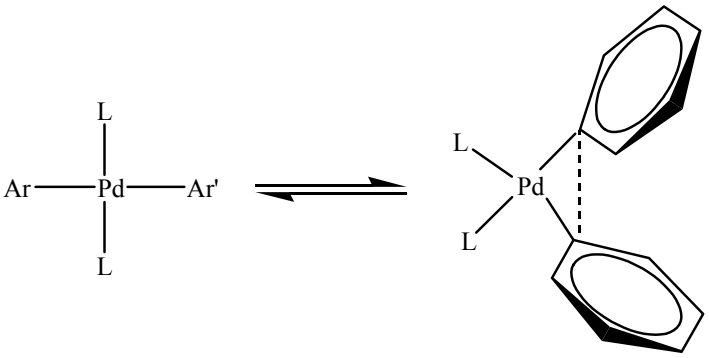
A range of C-C coupling reactions have been reported previously; the cross coupling reactions are accessible using a variety of organometallic reagents, as reported by

Tamaoin 1972.[58] A number of palladium-catalysed reactions for Grignard reagents were first reported by Murahashi.[59] The use of organoboron compounds has been extensively studied by Miyaura and Suzuki. Organoboron compounds are extremely electrophilic, and the organic groups attached to the boron are weakly nucleophilic, therefore limiting the use of these compounds. However, with the addition of a base to generate a boronate, the nucleophilicity is enhanced.

The mechanistic cycle for the palladium-catalysed cross-coupling reaction, between aryl halide and an organoboron reagent involves 3 stages, oxidative addition, transmetallation and reductive elimination (Scheme 46). The oxidative addition step to the aryl halide is the rate determining step, and the reactivity decreases with R-X, X = I > Br > Cl. In the first step, oxidative addition proceeds smoothly between the Pd(0) and Ar-X to form a stable *trans*-σ-Pd(II) complex. For the transmetallation step to proceed with an organoboron compound, it is crucial to use precise reaction conditions and reagents. Organoboron compounds have been previously reported to be inert to organopalladium halides, and do not take part in the transmetallation step under neutral conditions, as the organoboron compounds are electrophilic and the attached organic groups are weakly nucleophilic. Under neutral conditions, the cross-coupling reaction between Ar-I and Ar'$B(OH)_2$ at pH = 7.5-8.5 is hindered, however the reaction proceeds smoothly in aqueous solution with a negatively charged base, such as sodium carbonate at pH = 9.5-11.[60, 61] The pKa values of phenylboronic acid and carbonate are 8.8 and 10.3 respectively, therefore it is assumed the transmetallation step proceeds *via* the boronate anion $RB(OH)_3^-$, which is formed upon addition of the base to enhance the nucleophilicity as pH > pKa. The formation of the boronate anion $RB(OH)_3^-$ at pH = 11-12 has been previously reported.[62] After transmetallation the reductive elimination step regenerates the palladium(0) catalyst and releases the Ar-Ar' complex. The reaction proceeds as the *cis*-complex is formed *via* a *cis-trans* isomerisation. The order of reactivity for the reductive elimination step decreases with; diaryl- > (alkyl)aryl- > dipropyl- > diethyl- > dimethylpalladium(2). This is due to the possible interaction of the π-orbital of the aryl groups as the *cis* complex is formed (Scheme 47).

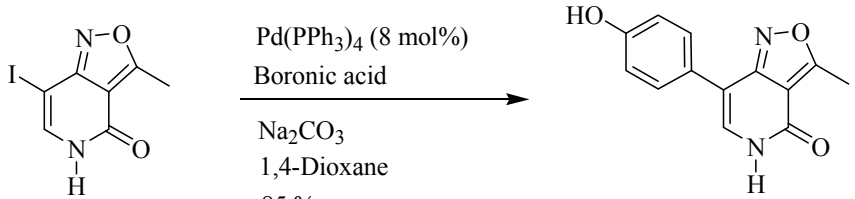

Pd0(L)4
Pd0
Ar-Ar'
Ar-X
Reductive
Elimination
Oxidative
Addition
L
L—Pd—Ar'
Ar
L
Ar—Pd—X
L
ArB(OH)2
ArB̄H(OH)3H+
Cis-Trans
Isomerisation
Transmetallation
L
Ar—Pd—Ar'
L
B(OH)3 + HX

Scheme 46



Scheme 47

There are various Pd(0)-based catalysts used in Suzuki-type reactions, common ones include tetrakis(triphenylphosphine)palladium $Pd(PPh_3)_4$, or the Pd(II) species, palladium acetate $Pd(OAc)_2$ and palladium chloride $PdCl_2(PPh_3)_2$ that are reduced to Pd(0) *in situ*. In our first attempt, the Suzuki reaction was followed using the catalyst $Pd(PPh_3)_4$ (Scheme 48). The procedure applied was as follows: to a degassed solution of 1,4-dioxane were added 7-iodo-3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **170** and $Pd(PPh_3)_4$ (8 mol%). The resulting solution was degassed again for another 10 minutes. To this solution, 4-hydroxyphenylboronic acid in degassed ethanol and aqueous sodium carbonate (2M), in degassed water were added sequentially. The resulting solution was heated at reflux for 18 h. Following work up, the crude residue was purified by column chromatography to leave the 7-(4-hydroxyphenyl)isoxazolopyridone **172** in 80-85 % yield.



170 **172**

Scheme 48

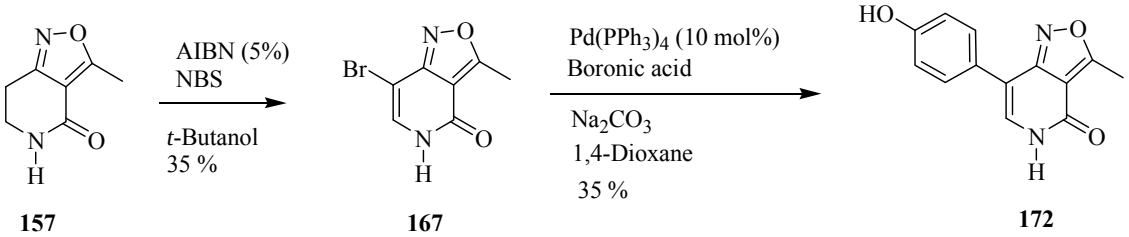
In order to further optimise the reaction, we explored the use of alternative solvents and catalyst (Table 2). The reactions were repeated with $Pd(OAc)_2$ (20%) and

triphenylphosphine to create Pd(0) *in situ*, however only the starting material was recovered. Reactions with $Pd(OAc)_2$ were monitored by TLC, and there were only two spots throughout the reaction corresponding to the starting materials, and no product was isolated in these reactions. Altering the reaction conditions, by varying the solvent and the base also resulted in recovery of the starting material. We therefore utilised the method described in Scheme 48 to develop the aryl substituents.

Table 2

Solvent	**Base**	**Catalyst**	**Result**
1,4-dioxane	Sodium carbonate	$Pd(PPh3)_4$	80-85 % product
1,4-dioxane	Potassium carbonate	$Pd(OAc)_2$	SM
1,4-dioxane	Sodium carbonate	$Pd(OAc)_2$	SM
Dimethoxy ethane	Potassium carbonate	$Pd(OAc)_2$	SM

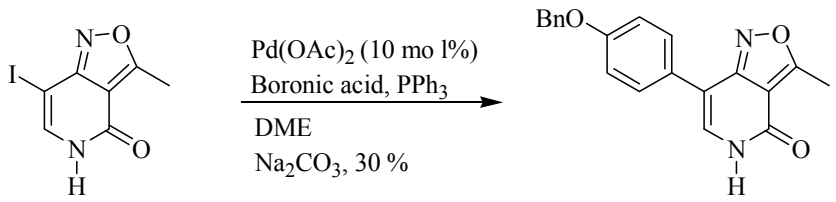
Earlier the unexpected 7-bromoisoxazolopyridone **167** was reported, and our radical route allowed us to acquire the 7-bromoisoxazolopyridone **167** directly from the bicyclic lactam **157**. Therefore, we felt it might be possible to carry out a Suzuki cross-coupling reaction with this compound, and thus avoid the halogenation reaction of the isoxazolopyridone **1**. However, it was necessary to obtain a good yield of the 7-bromoisoxazolopyridone **167**, and our investigation achieved a 35 % yield (Scheme 49). We investigated a Suzuki cross-coupling reaction of the 7-bromoisoxazolopyridone **167** with 4-hydroxyphenylboronic acid using the procedure developed previously. The reaction was indeed successful but only 35 % of the desired 7-(4-hydroxyphenyl)isoxazolopyridone adduct **172** was isolated. Thus overall the approach *via* iodination of the isoxazolopyridone **1** was felt to be more practical.



Scheme 49

After establishing a suitable method of synthesis of biaryls by means of a Suzuki cross-coupling reaction with the catalyst $Pd(PPh_3)_4$, we explored the use of other organoboron reagents as a substitute for the 4-hydroxyphenylboronic acid. It is possible the 4-hydroxyphenyl group might interfere with the aldol chemistry at the isoxazolopyridone C-3'(Me) during the construction of the C-3 side chains, therefore 4-benzyloxyphenylboronic acid was explored as an alternative. In this case, with the benzyl protection we could rule out the possibility of the anion formation at this position in the presence of a strong base such as *n*-BuLi.

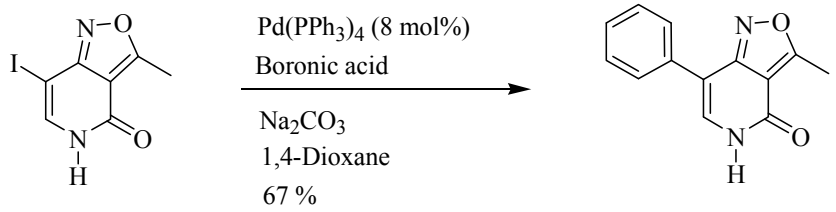
The same procedure was applied using 1,4-dioxane, $Pd(PPh_3)_4$, 4-benzyloxyphenylboronic acid and sodium carbonate solution (Scheme 50). However, this particular coupling product was difficult to isolate due to several spots on the TLC plate with similar R_f values. Following further purification, only a trace amount of the coupled product **173** was isolated. Changing the reaction conditions by varying the solvent and the catalyst to dimethoxyethane (DME) and $Pd(OAc)_2$, afforded the desired coupled compound **173** in just 30% yield, which also proved to be difficult to purify.



170 **173**

Scheme 50

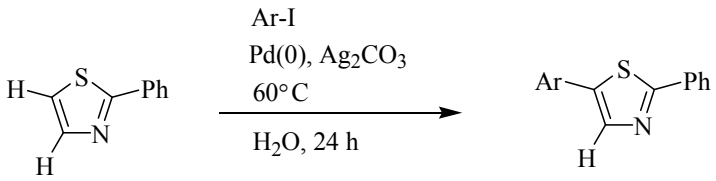
We continued the Suzuki cross-coupling reaction with the commercially available phenylboronic acid. The original procedure was utilised using phenylboronic acid, 1,4-dioxane, $Pd(PPh_3)_4$ and sodium carbonate solution. Following work up, the crude material was purified by column chromatography to leave the 7-(phenyl)isoxazolopyridone **174** in 67 % yield (Scheme 51).



170 **174**

Scheme 51

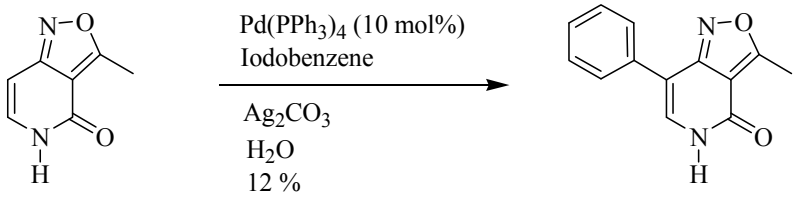
It has recently been demonstrated that an aryl halide with Pd(0) catalyst can take part in direct arylation *via* an electrophilic aromatic substitution mechanism of a nucleophilic partner, for example the 2-substituted thiazoles in water (Scheme 52).[63] The reactions also work in a similar manner in acetonitrile and silver carbonate, which could act as a silver source or a base but the exact role of this remains unclear.



175 **176**

Scheme 52

The reaction conditions were then exploited in the next reaction with the isoxazolopyridone **1** in which C-7 can be regarded as a nucleophilic 'enamine' centre. The palladium catalyst, $Pd(PPh_3)_4$, isoxazolopyridone, iodobenzene and silver carbonate in water were heated 60 ºC to afford the 7-(phenyl)isoxazolopyridone **174** in 12 % yield (Scheme 53). Although the yield is low, we have demonstrated the cross coupling reaction can be carried out in water as a green solvent. To optimise the reaction conditions, acetonitrile may well serve as a better solvent, and both the catalyst loading and the reaction temperature could be increased. However no further experimentation was conducted and this approach remains to be optimised.



1 **174**

Scheme 53

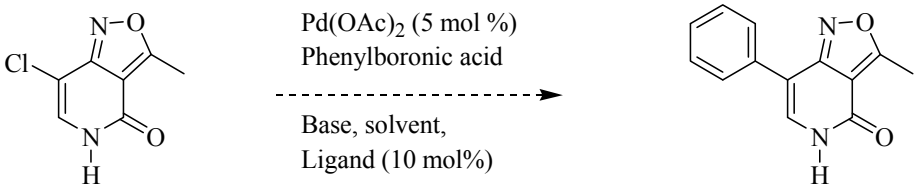
We have successfully developed the aryl ring at the C-7 position of the isoxazolopyridone. The Suzuki cross-coupling reaction of an organoboron compound and the 7-iodoisoxazolopyridone **170** led to the development of the 7-substituted phenyl **174**, 4-hydroxyphenyl **172**, and 4-benzyloxyphenyl **173** isoxazolopyridones. The 7-(phenyl)isoxazolopyridone **174** has also been synthesised *via* the electrophilic aromatic substitution of the isoxazolopyridone by an organopalladium halide, however our preferred method to date of synthesising these compounds follows the Suzuki cross-coupling reaction between the organoboron compound and the halopyridone.

The Suzuki cross coupling reactions using the 7-iodo and 7-bromo compounds were successful in the development of the 7-(phenyl)isoxazolopyridones and analogues. Having developed a suitable procedure, we continued this by attempting to use the 7-chloroisoxazolopyridone **162** to construct the 7-(phenyl)isoxazolopyridone according to the procedure developed previously. The 7-chloroisoxazolopyridone **162** was treated with its coupling partner, phenylboronic acid in the presence of $Pd(PPh_3)_4$, to only afford the starting material. It was therefore concluded that the conditions developed previously are not suitable for the cross-coupling reaction with aryl chlorides. The cross coupling reactions with chlorides are generally known to be difficult. The mechanism for the palladium catalysed cross-coupling reaction suggests the oxidative addition step is the rate determining step, and the reactivity decreases with R-X, X = I > Br > Cl. The chlorides are generally inactive and due to their high bond dissociation energy, for instance Ph-X: Cl 96, Br 81 and I 65 kcal mol^{-1}, they are slow during the oxidative step to add to the Pd(0) centre.[64]

We nevertheless found several reports of successful cross-couplings of inactivated aryl chlorides and bromides by Buchwald and co-workers.[65] They reported the use of general catalysts in Suzuki-Miyaura coupling reactions of aryl chlorides in water. Reactions conducted in water are environmentally friendly since water is non-toxic, non-flammable and its low cost. Much of the report focused on electron poor aryl chlorides, which are activated towards the oxidative addition step.

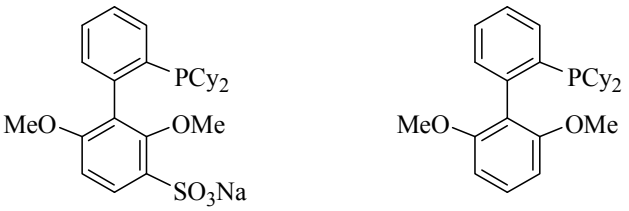
Using the catalysts reported, we investigated the coupling reaction between the 7-chloroisoxazolopyridone **162** and phenylboronic acid (Scheme 54). In the first instance, the alkenyl chloride was not protected as several reports have debated the need to protect functional groups, such as amines and alcohols prior to the coupling reactions.[66] These reactions often depend on the basicity of the heteroaryl chlorides, and strong basicity could increase the chance to coordinate to the palladium, and as a result terminate the catalytic cycle. The selection of ligands is also crucial, especially with inactivated aryl chlorides, and the correct choice of ligand would aid the oxidative addition and reductive elimination steps. The use of a bulky ligand would increase the electron density of palladium when complexed to an electron rich alkyl phosphine, such as the trialkylphosphane (PCy_3) compared to an aryl phosphine, which would accelerate this step by facilitating the oxidative addition of the Ar-Cl to the Pd(0).

The reactions were investigated using ligands **1** and **2**, both containing the bulky PCy group, where ligand **1** contains a water solubilising sulfonate group. Initially the 7-chloro compound **162** was treated with ligand **1** in water, under microwave conditions (Scheme 54), to only afford the starting material and a trace amount of the 7-(phenyl)isoxazolopyridone **174**. In a second reaction, using the same reactants with ligand **2** and heating at reflux in toluene gave the same result.



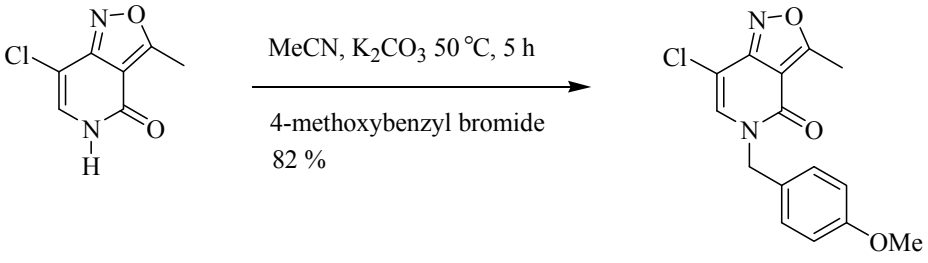
162 **174**

Scheme 54



ligand 1 **ligand 2**

With these poor findings, further reactions were explored using the protected 7-chloroisoxazolopyridone **177**, which was achieved by treating the 7-chloroisoxazolopyridone **162** with 4-methoxybenzyl bromide (Scheme 55). Further reactions made use of both ligands **1** and **2** under microwave and reflux conditions (Table 3), to afford mainly starting material and only trace amounts of the biaryl product **174.**



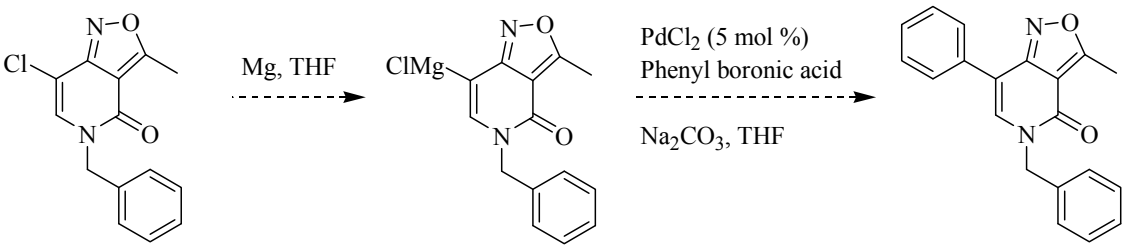
162 **177**

Scheme 55

Table 3

R Group	Solvent	Reaction conditions	Ligand	Base
R=H	Water	MW at 150 ºC 10 mins	1	K_2CO_3
R=H	Toluene	Reflux 24 h	2	K_3PO_4
R=Bn	Water	MW at 150 ºC 10 mins	1	K_2CO_3
R=Bn	Toluene	Reflux 24 h	2	K_3PO_4
R=Bn	Toluene	MW at 150 ºC 10 mins	2	K_3PO_4

Further investigation into coupling reactions of the aryl chloride was followed using Grignard reagents, commonly seen in the Kumada reaction.[67] This type of reaction is still young in the synthetic field compared to the reactions described previously. The use of Grignard reagents is often limited due to their incompatibility with functional groups. The following reactions were conducted based on the literature method; the benzyl protected 7-chloroisoxazolopyridone **178** (prepared from benzyl bromide using the method in Scheme 55) was treated with magnesium turnings in THF and heated at reflux for 5 h (Scheme 56). Addition of the palladium catalyst and the phenylboronic acid followed, and the mixture was heated at reflux for 24 h, to afford only the starting material. There was no observation of the homo-coupled product, dehalogenated material or the desired product **180**. It is possible the chloromagnesium intermediate had decomposed.

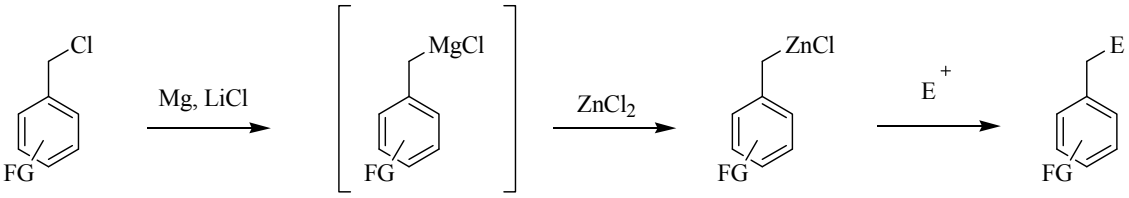


178 **179** **180**

Scheme 56

Knochel and co-workers have reported the use of benzylic zinc reagents to aid coupling reactions of aryl chlorides (Scheme 57).[68] The zinc reagents are compatible with

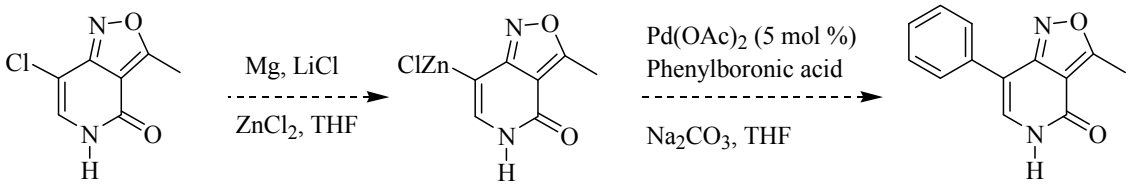
numerous functional groups, and combined with their highly reactive nature they are becoming attractive synthetic intermediates. The authors have reported the use of LiCl to accelerate the insertion of metals such as zinc and magnesium into the halide to give the reactive zinc intermediate, followed by subsequent reaction with an electrophile.



Scheme 57

We followed the synthesis by treating the 7-chloroisoxazolopyridone **162** with LiCl, Mg turnings and $ZnCl_2$ in THF for 2 h at 23 ºC. The unstable chloromagnesium intermediate readily decomposes in the absence of $ZnCl_2$, therefore it was transmetalated *in situ* to the reactive chlorozinc intermediate **181** (Scheme 58). This intermediate was neither isolated nor purified. The LiCl solution was prepared by drying in a Schlenk-flask under vacuum at 140 ºC for 5 h, cooled and THF was added. The $ZnCl_2$ solution was prepared in the same manner. For the coupling step, a palladium catalyst and the phenylboronic acid were added to the presumed chlorozinc intermediate **181**. The reaction mixture was stirred at 23 ºC and monitored by TLC analysis. There was no change in the reaction after several hours, so the reaction was heated at 50 ºC for several hours, to afford the starting material and a trace amount of the 7-(phenyl)isoxazolopyridone **174**. The reaction was repeated with an alternative procedure to form the chlorozinc intermediate. This involved the addition of the reagents, LiCl, $ZnCl_2$ and the 7-chloroisoxazolopyrdione **162** in THF, and the mixture was stirred for 24 h at 23 ºC. After this point, the addition of a palladium catalyst and the phenylboronic acid followed and the mixture was heated at reflux for 24 h, to provide only recovery of the starting material. It is possible the current conditions for the cross coupling reaction with the aryl chloride is not compatible in this case, and the formation of the pyridone chlorozinc intermediate **181** remains uncertain. With very little success, we decided not to explore this further, since we had access to the 7-

(phenyl)isoxazolopyridone **174** *via* the 7-iodo compound and a Sukuki coupling (Scheme 51).



162 **181** **174**

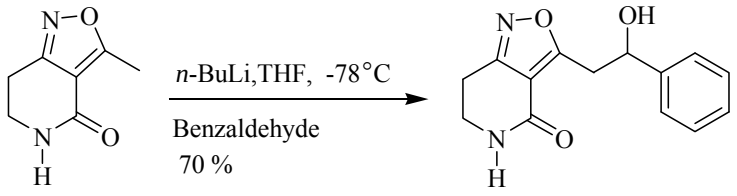
Scheme 58

2.4 Elaboration at the C-3' of the isoxazolopyridone

2.4.1 Aldol Reactions

The natural product tenellin **3** and others belonging to the same family, all possess an unsaturated acyl side chain at C-3 (corresponding to an unsaturated substituent at isoxazolopyridone C-3). The current strategy applied towards the construction of such side chains and analogues, is the use of aldol-type chemistry. A typical reaction would entail the use of a base, such as LDA or *n*-BuLi, to deprotonate at the C-3' methyl position of the isoxazolopyridone, forming an anion. It is also possible to form dianion or trianion species by deprotonating at the N-H proton and/or the O-H in the case of the 7-(4-hydroxyphenyl)isoxazolopyridone. Once the anion is formed by addition of the base, it can be trapped with an electrophile, such as an aldehyde. The aldehydes are suitable electrophiles as they would form an alcohol adduct, which can undergo dehydration to the unsaturated side chains as observed in the natural products. Also, should a reaction take place at the nitrogen, it would be reversible upon work up back to the 2-pyridone.

In a test reaction, *n*-BuLi (2.2 eq) was added dropwise to the bicyclic lactam **157** in THF at -78 ºC. The resulting solution was stirred for 1 h at -78 ºC, followed by the addition of excess benzaldehyde (20 eq), and the mixture was stirred for a further 5 minutes. After work up and column chromatography, the benzaldehyde adduct **182** was isolated in 70% yield (Scheme 59).



157 **182**

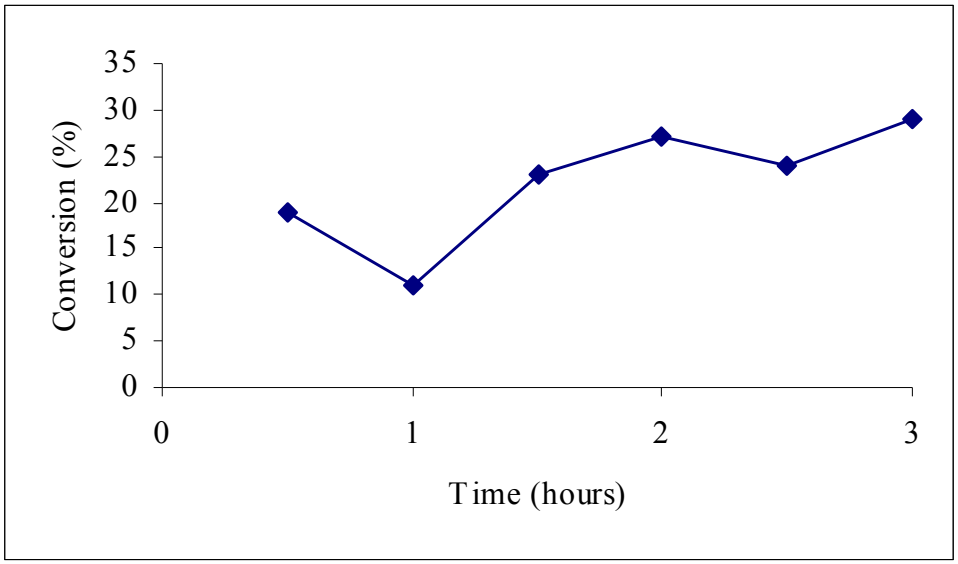
Scheme 59

Following this we repeated the reaction using the unsaturated isoxazolopyridone **1** under the same reaction conditions described for the bicyclic lactam **157**. The resulting benzaldehyde adduct was isolated in poor yield, and the starting material was recovered. Repeating the reaction a second time gave a similar result, and after several repeat reactions variable results were obtained. The methodology developed here is clearly not a reliable method for developing side chains since we are not observing reproducible results. In order to develop a reliable methodology, the reaction conditions were investigated further. It is possible the reactions are proceeding *via* a dianion intermediate as there are two possible sites of anion formation, and the stability of these anion species remains uncertain. The current experimental conditions were carried out at -78 ºC with stirring for an hour; it is uncertain if the anions are formed at this temperature and their stability is unknown. There have also been several reports of aldol type reactions carried out at -78 ºC with warming to -20 °C for 30 minutes, to allow formation of the anion and then cooled back to -78 °C, before trapping the anion with an electrophile. There are also issues based around the solubility of these anion species, whether they remain in solution or precipitate. Before we started experimenting with different temperatures to carry out the reactions, we conducted several experiments by varying the time to allow the formation of the anion species. This was pursued by carrying out a series of six reactions by varying the time at which *n*-BuLi was allowed to stir at -78 ºC, from 0.5 h to 3 h before the addition of the benzaldehyde. The procedure was as follows; to a solution of *n*-BuLi (10 eq) in THF at -78 ºC was added isoxazolopyridone **1** dropwise. The resulting solution was stirred at -78 ºC for the required time, followed by the addition of excess benzaldehyde (20 eq), and the resulting reaction mixture stirred for a further 2 h. The crude mixtures were worked up, and then were subjected to GC-MS to allow the investigation of the optimum reaction conditions. From the results obtained (Table 4, Graph 1), the conversions to the benzaldehyde adduct **183** are at best using 2 h and 3 h reaction time, however the results show a decrease in conversion from 2 h to 2.5 h. This could be an anomaly and also the reactions need to be repeated in order to obtain reproducible data. In the meantime the following reactions (Scheme 60) were carried out with 2 h for anion formation, as the stability of the anion for a longer period of time remains uncertain. These reactions were repeated using excess base (10 eq) and

benzaldehyde (20 eq), which always ensured reproducible results. It was later determined that using less base (5 eq) and benzaldehyde (10 eq) also gave similar results.

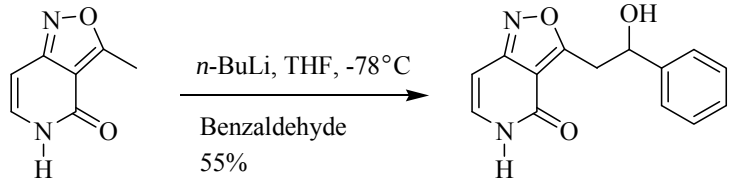
Table 4

Time (hours)	Conversion (%)
0.5	19
1	11
1.5	23
2	27
2.5	24
3	29



Graph 1. Conversion to the benzaldehyde adduct by varying the reaction time.

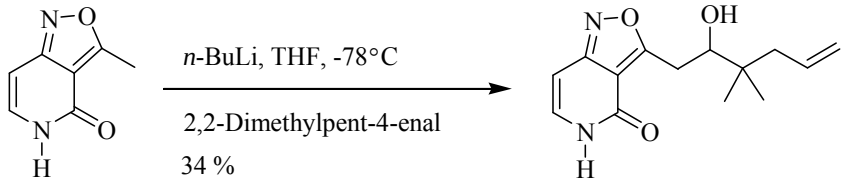
From the results obtained, the following reaction was confirmed using the procedure developed. The isoxazolopyridone **1** was treated with *n*-BuLi (10 eq) for 2 h at -78 ºC, before the addition of benzaldehyde (20 eq), and then a work up and column chromatography afforded the benzaldehyde adduct **183** in 55 % yield (Scheme 60)



1 **183**

Scheme 60

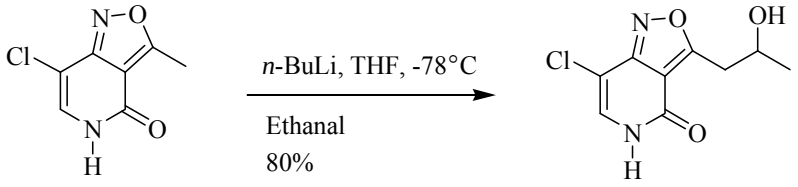
Thus we have developed a reliable method for the aldol chemistry, and by this method we have so far developed aromatic side chains. The next step of the synthesis was to develop aliphatic side chains, such as the ones observed in the natural products. In a first attempt, we used a non-enolisable aldehyde, 2,2-dimethylpent-4-enal, to construct the side chain. This was achieved readily in THF and excess *n*-BuLi (10 eq) at -78 °C, followed by addition of 2,2-dimethylpent-4-enal (20 eq), to leave the desired alcohol adduct **184** in 34 % yield along with the recovery of starting material (Scheme 61).



1 **184**

Scheme 61

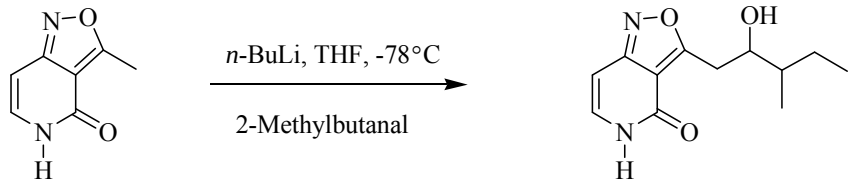
Treating the 7-chloroisoxazolopyridone with ethanal and *n*-BuLi in the same manner as the above reaction furnished the alcohol adduct **185** in 80 % yield (Scheme 62).



162 **185**

Scheme 62

The same procedure was applied using 2-methylbutanal (Scheme 63). This reaction proved to be unsuccessful and majority of the starting material was recovered. However, a trace amount of the alcohol adduct **186** was isolated from the crude mixture. A possible explanation for the lack of success of this reaction would be the enolisable aldehyde interfering with the reaction, and therefore only a trace amount of this compound was observed.

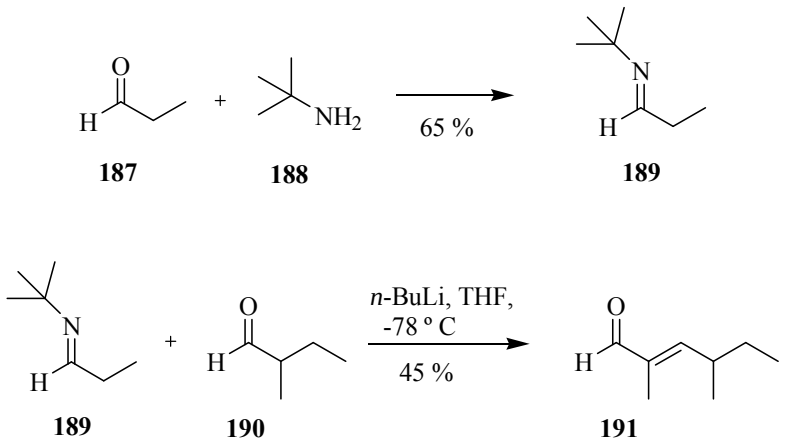


Scheme 63

Having successfully synthesised isoxazolopyridones with aromatic and aliphatic side chains, we pursued our research towards the synthesis of tenellin aliphatic side chain. The aldehyde required to construct the tenellin side chain is not commercially available, therefore we synthesised this in two steps (Scheme 64).[69]

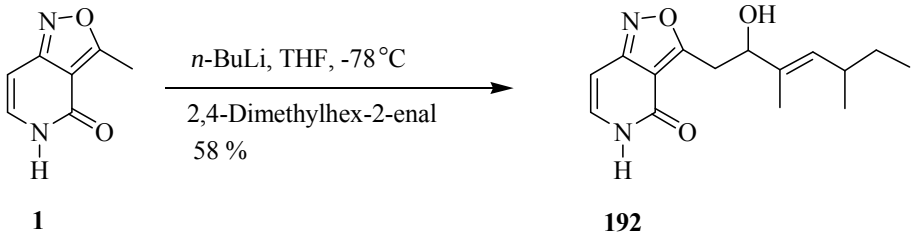
The first step involved the formation of the imine of propanal, by dropwise addition of propanal **187** to *tert*-butylamine **188** at 0 °C. After the addition was complete, potassium hydroxide pellets were added. The resulting solution was allowed to separate at 0 °C over a period of 12 h. Once separation of the layers were visible, the upper yellow layer was decanted and distilled from potassium hydroxide at atmospheric pressure to yield the *tert*-butylpropylideneamine **189** as a colourless oil. It is then possible to store the imine **189** over molecular sieves at 0 °C, however distillation is required prior to further use.

In the second step of the synthesis, the previously prepared *tert*-butylpropylideneamine **189** was freshly distilled. To a solution in THF, at -78 °C, was added *n*-BuLi, the resulting solution was stirred for 30 minutes and then 2-methylbutanal **190** was introduced. An aqueous acidic work up followed, and purification of the crude material by column chromatography afforded the desired aldehyde **191** as a colourless oil (Scheme 64).



Scheme 64

With the tenellin aldehyde **191** (2,4-dimethylhex-2-enal) available, we continued the anion chemistry using the procedure described previously, to furnish the alcohol adduct **192** in 58 % yield (Scheme 65).

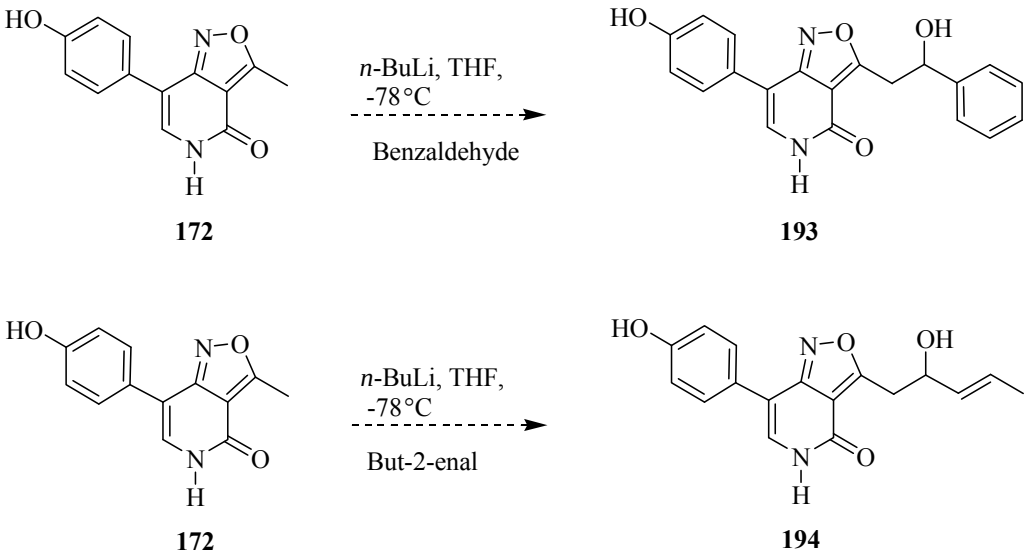


Scheme 65

The natural product tenellin **3** contains the 4-hydroxyphenyl group on C-5 corresponding to the C-7 of the isoxazolopyridone, and also the unsaturated polyene chain system on the C-3' site of the isoxazolopyridone. We have been able to prepare the aromatic ring on the C-7 position of the isoxazolopyridone and also developed the tenellin side chain in separate reactions. To further functionalise these compounds, it was necessary to consider the order of the reactions, whether to functionalise the C-7 before or after C-3' of the isoxazolopyridone. The 7-iodoiosxazolopyridone **170** used in the cross coupling reactions would interfere with the metallation in the aldol reactions, and the unsaturated C-3' side chain would be reactive during the C-7 iodination step. It was therefore decided to pursue the construction of the C-3' side chain after the cross coupling reaction to form the 7-(4-

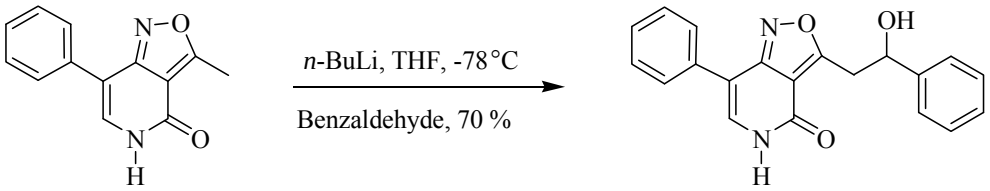
hydroxyphenyl)isoxazolopyridone **172**.

In the early stage of the synthesis, we attempted a number of reactions with the 7-(4-hydroxyphenyl)isoxazolopyridone **172** to develop the C-3' side chains (Scheme 66). We anticipated the reaction to proceed *via* a possible trianion intermediate, since the *n*-BuLi could possibly deprotonate at three sites in **172**. The pKa values of the core 2-pyridone unit and phenol are 24 and 18 respectively in DMSO,[70] therefore the phenol is likely to deprotonate first. In these reactions excess *n*-BuLi and the freshly distilled aldehydes were used. The aromatic benzaldehyde was used as this has proven to be successful as noted previously and but-2-enal, since it has a similar functionality to the side chain of tenellin **3**. Neither of the products **193**/**194** were isolated in pure form, and after several attempts to purify them using column chromatography and preparative TLC, only trace amounts of the products and starting material were recovered. In the case of but-2-enal the ^{1}H NMR spectrum revealed signals corresponding to the alkene in the side chain. However, TLC analysis revealed five spots very close together, which proved to be very difficult to separate. A sample was submitted for mass spectrometry and a signal at 313.11883 was observed, corresponding to the mass of the but-2-enal adduct **194**. With some evidence the reaction had taken place, even though no pure sample was isolated, we turned our attention to an alternative weaker base, LDA, which also proved to be unsuccessful. With no obvious explanation to why the reaction was unsuccessful, our immediate suspicions were either the possible trianion intermediate was no longer in solution upon its formation, and/or this intermediate failed to form due to stability issues.



Scheme 66

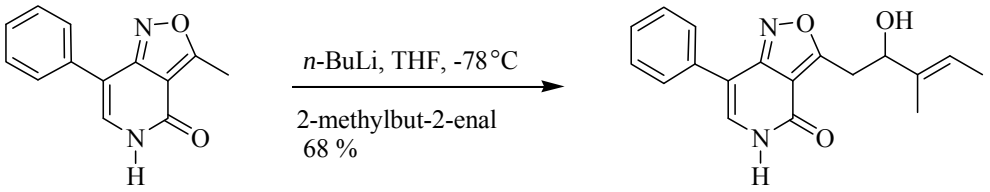
With the possibility the 4-hydroxyphenyl group might be interfering with the anion chemistry, we investigated similar reactions in the absence of the hydroxyl group. We anticipated the reaction to proceed *via* a dianion intermediate of the 7-(phenyl)isoxazolopyridone **174**, the two obvious reactive sites being the nitrogen and the methyl positions (Scheme 67); therefore the initial treatment with excess *n*-BuLi (10 eq) would aid the formation of such an intermediate, and the addition of excess benzaldehyde would ensure maximum interaction between the dianion intermediate and the aldehyde. In an initial reaction, once the *n*-BuLi (10 eq) was added to the reaction mixture, the reaction was allowed to stir for only 1 h before the aldehyde was added. We were reluctant to allow for a longer reaction time for the anion formation due to stability issues, however the product was isolated in poor yield. Following this, the resulting basic mixture was allowed to stir at -78 °C for 2 h and then the addition of benzaldehyde (20 eq) followed; the resulting reaction mixture was stirred at -78 °C for a further 2 h before allowing the reaction to reach room temperature. After which point, work up and purification was conducted to leave the benzaldehyde adduct **195** as a white solid in 70 % yield (Scheme 67). Analysis of this compound revealed ^{1}H NMR signals at 3.56 and 5.19 ppm corresponding to signals produced by the CH*CH*$_2$ and CH$_2$*CH* protons, and the methyl signal was absent.



174 **195**

Scheme 67

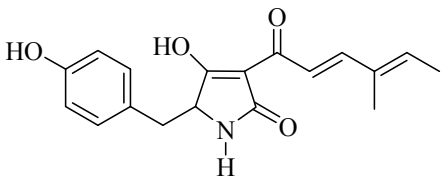
Applying the same experimental procedure with 2-methylbut-2-enal, the alcohol adduct **196** was isolated in 68 % yield, which provides a similar backbone to that of tenellin **3** (Scheme 68).



174 **196**

Scheme 68

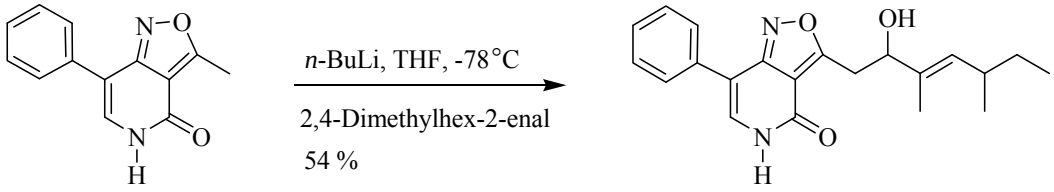
The corresponding dehydrated side chain has not been isolated in the biosynthesis of 2-pyridones, however the acyltetramic acid containing a side chain that would be derived from 2-methylbut-2-enal has previously been observed in prototenellin B **33**, and was discussed in the introduction part of this thesis.



33

We continued the synthesis to develop the tenellin side chain using the 7-(phenyl)isoxazolopyridone **174** with the previously prepared 2,4-dimethylhex-2-enal. Applying the same procedure used previously, the 7-(phenyl)isoxazolopyridone **174** was treated with *n*-BuLi (10 eq) and 2,4-dimethylhex-2-enal (20 eq) in THF, to yield the

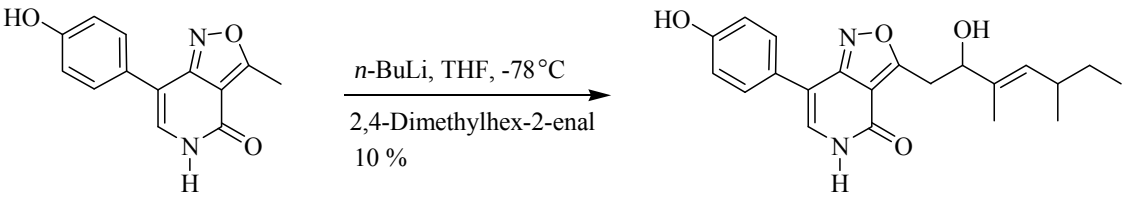
desired compound **197** in 54 % yield and recovery of some starting material (Scheme 69). It is worth noting the R_f values of the starting material and the aldehyde adduct are similar causing difficulty during purification. The mixtures were separated using column chromatography and eluted with light petroleum and a steady increase of polarity to light petroleum and ethyl acetate (1:1 v/v). Although, there were fractions from the column containing both starting material and the product, this could be followed through to the dehydration step (see next section) where good separation was observed during purification to isolate any unreacted starting material.



174 **197**

Scheme 69

Having synthesised the tenellin side chain using the 7-(phenyl)isoxazolopyridone **174**, we exploited the same reaction conditions with the 7-(4-hydroxyphenyl)isoxazolopyridone **172**. In this case with the presence of the extra 4-hydroxy group, it is possible this reaction would proceed *via* a trianion intermediate. Following the reaction through resulted in a complex mixture and after further purification, the desired tenellin-like adduct **198** was obtained in a low yield of 10 % along with recovery of the starting material (Scheme 70)



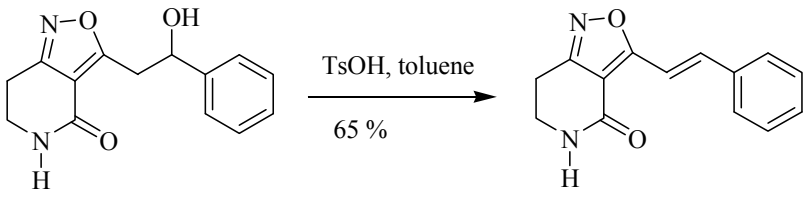
172 **198**

Scheme 70

2.4.2 Dehydration Reactions

The alcohol adducts previously prepared would require a dehydration to the corresponding condensation product, to leave the unsaturated side chains as seen in the natural products.

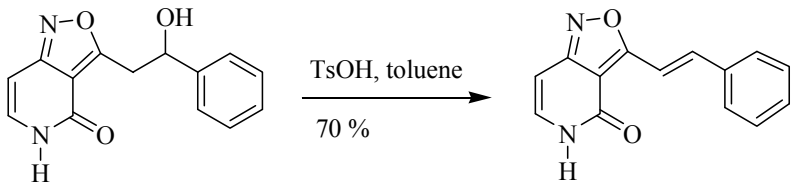
Dehydration of the benzaldehyde adduct **182** was achieved by reaction in toluene, under acidic conditions using *para*-toluenesulfonic acid, and heating at reflux overnight under Dean-Stark conditions (Scheme 71). The reaction was monitored by TLC analysis, and after 24 h of reflux all the starting material had been consumed, and the unsaturated benzaldehyde adduct **199** was obtained in 65 % yield. It seems that some of the starting material may have decomposed during the reaction, which may account for recovery of only 65 %.



182 **199**

Scheme 71

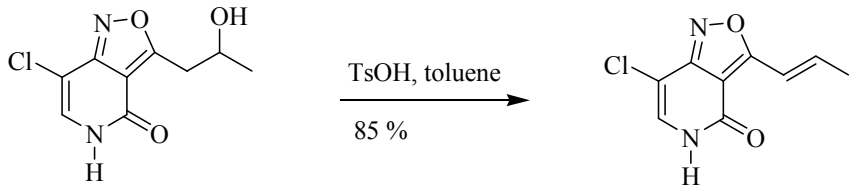
The same procedure was followed through using the benzaldehyde adduct of the unsaturated pyridone **183** to leave the condensation product **200** in 70 % yield (Scheme 72).



183 **200**

Scheme 72

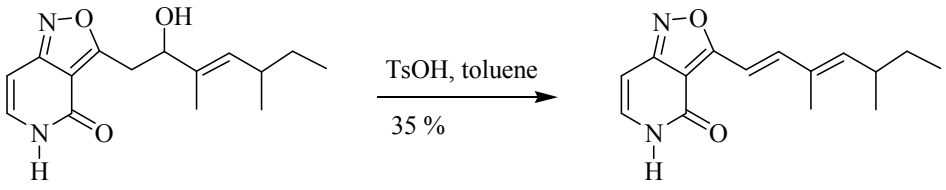
Dehydration of the alcohol adduct **185** was also successful to leave the unsaturated adduct **201** in 85 % yield (Scheme 73).



185 **201**

Scheme 73

Dehydration of the alcohol adduct **192** followed to leave the unsaturated side chain **202** as seen in tenellin, which was observed in 35 % yield. (Scheme 74); it is possible that some starting material had decomposed. An alternative to the current method of dehydration might be to substitute the reaction solvent, toluene, for polar protic solvent such as methanol, which would aid the reaction and may avoid possible loss of material.

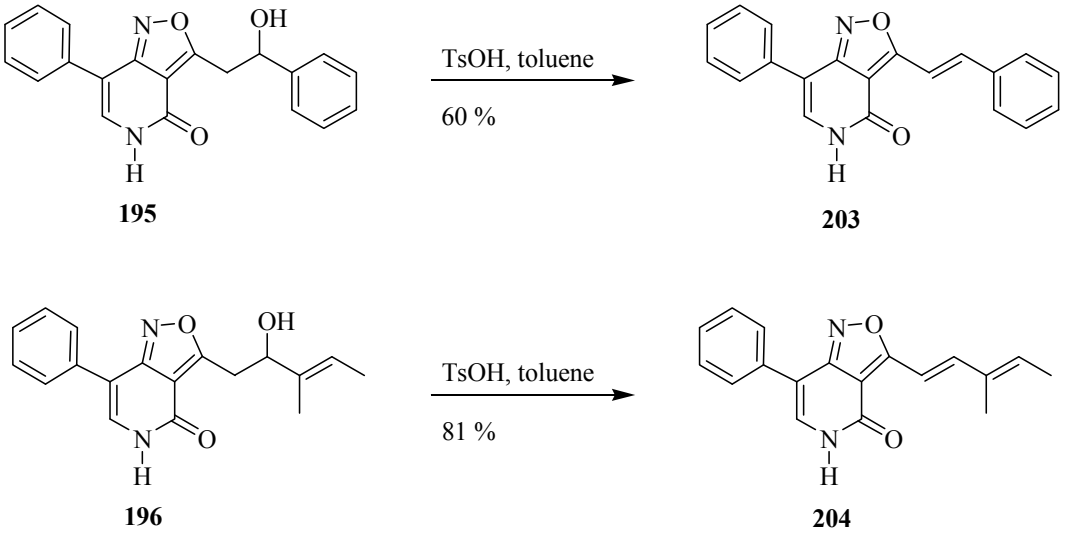


192 **202**

Scheme 74

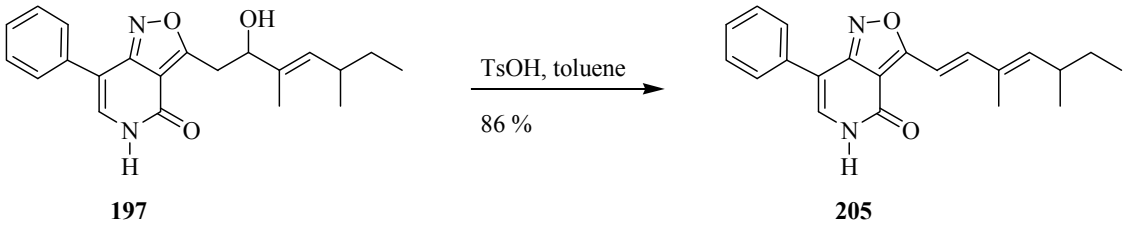
Dehydration of the 7-substituted compounds, such as the 7-(phenyl)isoxazolopyridone benzaldehyde adduct **195** was achieved in 60 % yield, whilst the dehydration of the (2-hydroxy-3-methylpent-3-enyl)-7-(4-hydroxyphenyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one

196 was observed in 81 % yield (Scheme 75). In the case of the condensation product **204** of 2-methylbut-2-enal, the unsaturated side chain was observed in an acyltetramic acid intermediate during studies of the biosynthesis of 2-pyridones; however this has yet to be discovered in 2-pyridones.



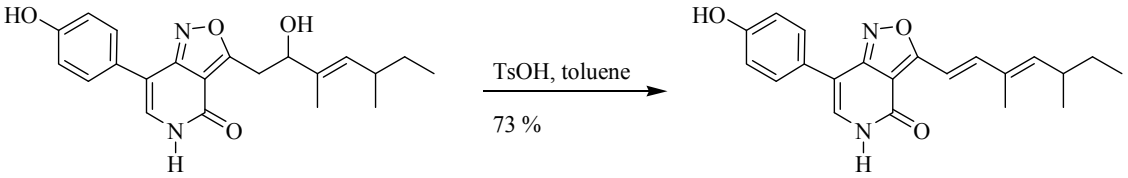
Scheme 75

To complete the synthesis of the tenellin side chain in the isoxazolopyridone with a 7-substituted phenyl substituent, the corresponding alcohol adduct **197** was subjected to a dehydration reaction to furnish the unsaturated tenellin-like side chain **205** in 86 % yield. This isoxazolopyridone is a near analogue of pretenellin B **17** (Scheme 76).



Scheme 76

Dehydration of the corresponding 7-(4-hydroxyphenyl) substituent **198** proved to be difficult during purification and for this reason we were unable to obtain full structural analysis. After several attempts to purify the compound **206**, it was observed in approximately 73 % yield with some unidentified impurities (Scheme 77), to give another close analogue of pretenellin B **17**.

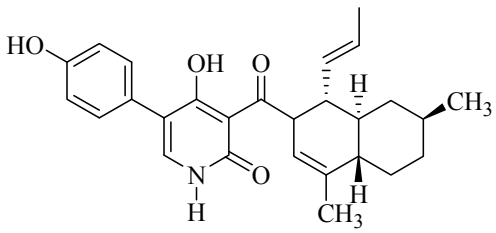


198 **206**

Scheme 77

2.4.3 Towards the synthesis of tricyclic side chains

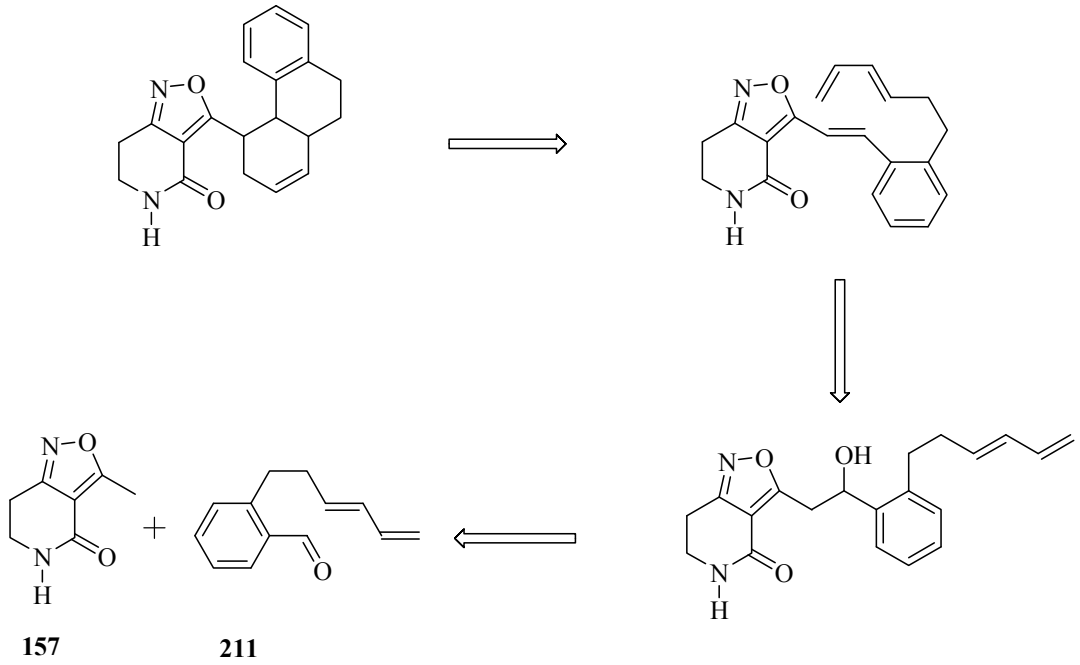
To date we have synthesised the tenellin side chain and other aliphatic C-3' side chains of the isoxazolopyridone core. The aldol reaction with benzaldehyde afforded an aromatic ring, which is the only example of an aromatic substituent at C3'. We were interested in developing other alicyclic side chains; such systems have been observed in natural products like ilicicolin **3** to show a *trans*-decalin ring system. We pursued our interests to develop a simple model of this ring system.



3

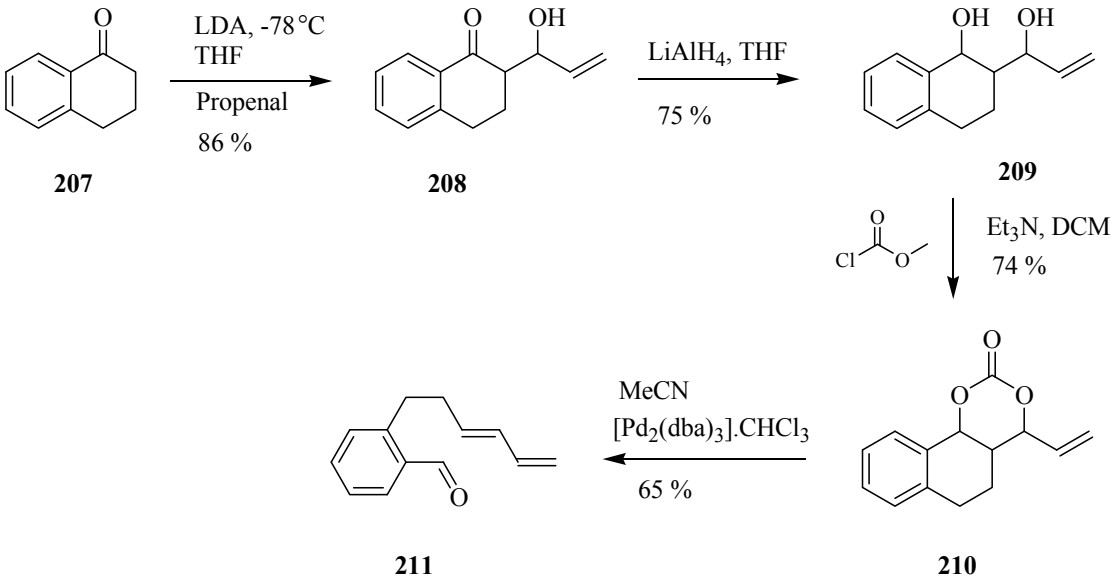
Our proposed method for developing such a ring system incorporates the aldol synthesis method in the development of side chains using an aldehyde reported in the literature

(Scheme 78).[71, 72] We suggest the model tricyclic side chain, which indicates a retrosynthetic analysis *via* an intramolecular Diels-Alder reaction. The cycloaddition precursor would be available *via* the aldol chemistry.



Scheme 78

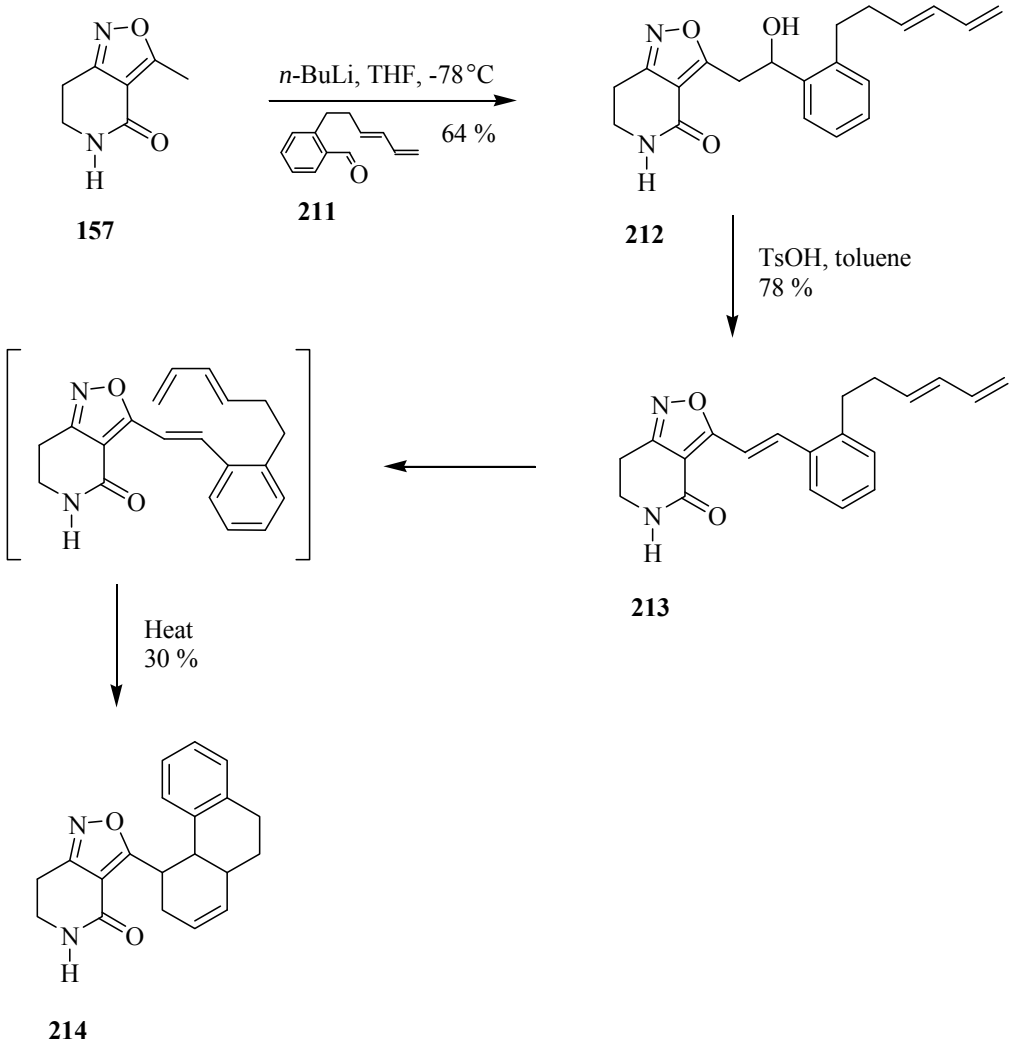
The required aldehyde **211** was prepared in 4 steps, *via* a novel palladium-catalysed decarboxylative ring opening of cyclic carbonates (Scheme 79). The methodology involved the aldol addition of α-tetralone **207** with propenal in the presence of the base lithium diisopropylamide. The second step involved the reduction of the β-hydroxyketone **208**, which was achieved using lithium aluminium hydride in ether to give the 1,3-diol **209** in 75 % yield. The next step was the synthesis of the cyclic carbonate **210** by treating the 1,3-diol **209** with methyl chloroformate in 74 % yield. In a final step, compound **210** was treated with palladium dibenzylideneacetone [$Pd_2(dba)_3$] in dry acetonitrile to afford the aldehyde **211** in 65 % yield.[72,72]



Scheme 79

After the synthesis of the aldehyde **211**, we pursued our interest in the development of polycyclic side chains. The synthesis commenced with an aldol condensation reaction with the bicyclic lactam **157** using the methodology previously developed, to afford the alcohol adduct **212** in 64 % yield as the major product (Scheme 80). A minor product, the dehydrated analogue **213** was also observed. For complete conversion, the dehydration of the alcohol adduct was readily achieved in toluene, under acidic conditions using *para*-toluenesulfonic acid, and heating at reflux overnight under Dean-Stark conditions. The unsaturated **213** analogue was isolated in 78 % yield. In order to develop the tricyclic ring system, compound **213** in toluene, in an NMR tube was heated at 100 ºC. The reaction was monitored initially for 5 h and ^{1}H NMR analysis appeared to only show the starting material. After 24 h, the disappearance of the terminal alkene in the ^{1}H NMR spectrum and a shift in the other alkene signals were observed. TLC analysis also showed that the entire unsaturated compound **213** had been consumed and new spots in the TLC plate appeared. Purification of the crude material by chromatography followed to afford a white solid. Extensive ^{1}H NMR and ^{13}C NMR analysis of the new material proved to be a challenge, as a complex NMR spectrum was obtained to show the presence of a possible diastereomeric mixture. Further analysis revealed the accurate mass to be 321.1602 (H^{+}) of the desired tricyclic ring system **214**. We were unable to obtain any

crystals of the sample, as X-ray crystal analysis would solve the structure. With all the material consumed in the structural analysis our conclusion, using evidence from the NMR spectra, the accurate mass and absence of the starting material (dehydrated adduct), is to suggest the tricyclic cycloadduct as a possible result in this synthesis.



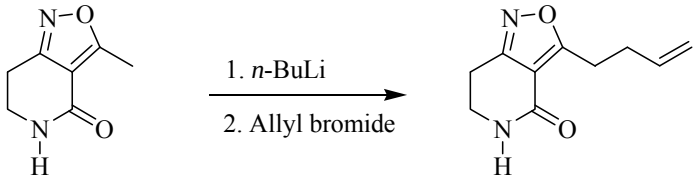
Scheme 80

2.5 Pyridone *N*-protection

2.5.1 Allyl protection of the pyridone

Difficulty arising with the anion chemistry previously with the 7-(4-hydroxyphenyl)isoxazolopyridone could be due to a number of reasons; it may be difficult to form a trianion intermediate, although we do not have any evidence suggesting the formation of such intermediate and if this is formed at all, it may be unstable. A possible strategy to tackle this problem would be to protect the functional groups. Protecting groups, such as the allyl group, could be employed to protect the amide and the phenol group simultaneously. With these functional groups protected, the anion chemistry could then proceed *via* a possible mono-anion intermediate.

On a small scale the allyl group protection was tested on the bicyclic lactam **157** (Scheme 81). The bicyclic lactam was treated with excess *n*-BuLi in THF at -78 °C and stirred for 2 h, followed by the addition of allyl bromide (1 eq). The reaction mixture was stirred at -78 °C for a further 2 h before being allowed to reach room temperature. Work up and purification was then conducted, which showed the presence of the starting material and a C-3'-alkylated product. It seems the allyl group preferred to react at the C-3' methyl position of the isoxazolopyridone. The ^{1}H NMR spectrum revealed the presence of the broad NH signal at 5.8 ppm, the alkene signals were observed, and absence of the methyl signal confirmed the formation of the compound **215**.

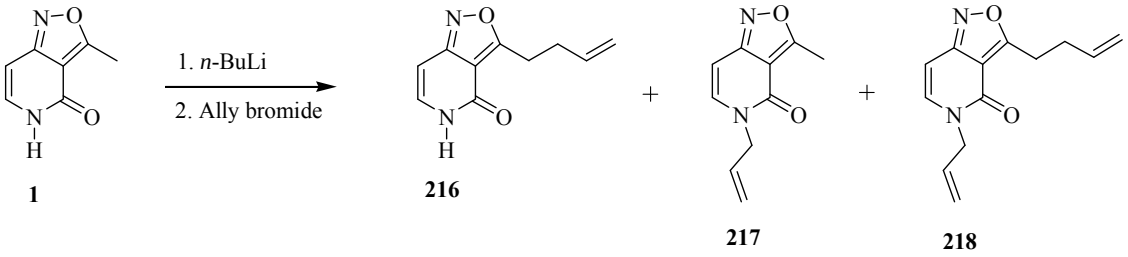


157 **215**

Scheme 81

Further investigation was undertaken using the isoxazolopyridone **1**, which was treated with an excess of *n*-BuLi and excess allyl bromide in the same manner as the previous

reaction. It seems, as expected, that the allyl group reacted at both the methyl position and at the amide to leave a mixture of three compounds (Scheme 82). Investigation of the crude mixture in GC-MS resulted in two major mass signals at 190 and 230 corresponding to compounds **216/217** and **218**. The three compounds were all isolated in ratio 3 (**216**) : 3 (**217**) : 1 (**218**)



1 **216** **217** **218**

Scheme 82

The strategy to protect the amide using allyl protection may not be best suited, as using only one equivalent of allyl bromide results in the C-3'-alkylated compound **215**, where the allyl group reacts on the methyl position of the isoxazole. It is in our interest to avoid this and leave this site vacant for constructing side chains. The amide protection was achieved using excess allyl bromide, however this also yields unwanted compounds. For this reason we did not pursue allyl protection or other alkyl, and further investigation is required to protect the pyridone prior to the anion chemistry with the 4-hydroxyphenyl pyridone, in order to develop further side chains.

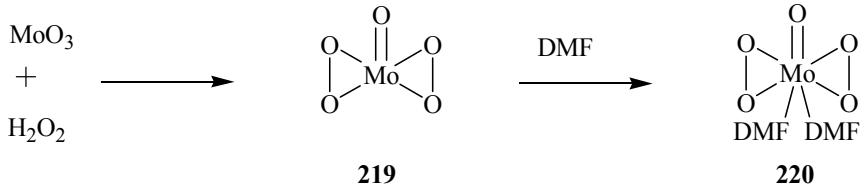
2.6 *N*-Oxidation

Having investigated the anion chemistry at the C-3' position of the isoxazolopyridone **1**, and demonstrated successful Suzuki cross-coupling reactions to introduce C-C bonds at the C-7 of the isoxazolopyridone, we pursued our research to develop the hydroxamic acid functionality *via N*-oxidation of the pyridone **1**. The natural product tenellin contains the hydroxamic acid as do other natural products such as bassianin **4** and leporine **124**. The strategy proposed for the *N*-oxidation was *O*-silylation of the amide, followed by oxidation using a molybdenum complex.

2.6.1 *O*-Silylation and Oxidation

Rigby and co-workers have achieved the *N*-oxidation by *O*-silylation of the amide in related pyridones, followed by a treatment with oxodiperoxymolybdenum(pyridine)(HMPA) complex to form the hydroxamic acid functionality as in tenellin **3**.[73, 74] With hexamethylphosphoric triamide (HMPA) being carcinogenic, we looked at an alternative options. Sammes and co-workers reported the use of an alternative molybdenum complex **220** for the *N*-oxidation step.[75]

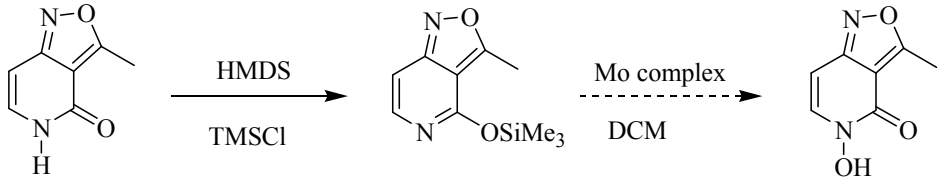
The synthesis of the complex **220** was attempted using the procedure described by Sammes and co-workers (Scheme 83),[75] which involved treatment of molybdic acid with 30% hydrogen peroxide at 35 °C, resulting in a yellow solution. This was cooled to 15 °C and two equivalents of dimethylformamide (DMF) were added to yield the desired molybdenum complex **220** as a yellow solid. The procedure was repeated and a yellow solid was obtained, however mass spectrometric data were unable to confirm the presence of the molybdenum complex due to solubility issues. In the meantime, assuming the complex had formed with support from IR data, we progressed through to the next step of the synthesis.



219 **220**

Scheme 83

Before treatment with the molybdenum complex **220**, we pursued *O*-silylation of the amide (Scheme 84). The isoxazolopyridone **1** was treated with an excess of hexamethydisilazane (HMDS), and a few drops of trimethylsilyl chloride, and heated at reflux for 24 h. The HMDS was removed under reduced pressure and the resulting precipitate was analysed by ^{1}H NMR spectroscopy. Assuming that the *O*-silylation had taken place due to the presence of the TMS signal in the ^{1}H NMR spectrum at 0.09 ppm, and since intermediate **221** is extremely moisture sensitive, the precipitate was treated with the molybdenum complex **220** in DCM at 20 °C and stirring for 12 h followed immediately. After this time, a work up with 1M - Na_3(ethylenediaminetetraacetic acid) (EDTA) was applied to release the molybdenum ion from the expected hydroxamic acid. Extraction with DCM and purification of the crude material by column chromatography resulted in the recovery of the starting material **1**.

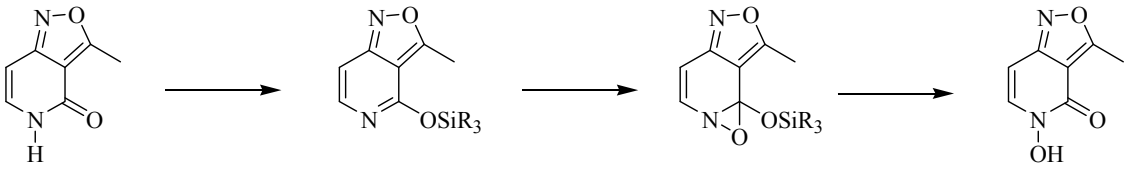


1 **221** **222**

Scheme 84

Further conditions were explored for the *N*-oxidation as there are uncertainties regarding the formation of the silyl enol ether intermediate **221** and the oxidation step with molybdenum complex. Since trimethylsilylation of organic compounds is well known in preparative organic chemistry.[76] The isoxazolopyridone **1** was treated with an excess of

hexamethydisilazane (HMDS), and a few drops of trimethylsilyl chloride, and heated at reflux for 24 h in an attempt to form the silyl enol ether **223** (Scheme 85). The HMDS was removed under reduced pressure and the resulting precipitate was analysed by ^{1}H NMR spectroscopy, which indicated some evidence that the silylation had taken place due to the presence of a signal at 0.09 ppm. Therefore assuming the *O*-silylation had taken place we pursued the oxidation step. Treatment of the double bond with a peracid such as *m*CPBA as in an epoxidation reaction should form the strained three membered oxaziridine ring **224**. Relief of the ring strain would encourage rearrangement with the loss of the silyl group to present the hydroxamic acid functionality **222**.



1 **223** **224** **222**

Scheme 85

For the oxidation step, DCM was used as a suitable solvent since previous reports of similar successful expoxidation reactions support this.[77] *m*CPBA is a strong oxidising agent, it is highly reactive and more selective than other peracids such as hydrogen peroxide. We therefore utilised this as a suitable oxidising agent. The crude material containing the possible intermeidate **223** in DCM was treated with *m*CPBA and was stirred at room temperature. The progress of the reaction was monitored for several days and no change was observed. The reaction was then heated at 35 ºC followed by heating at reflux, which also resulted in no change in the reaction progress. All these attempts failed and we only observed the starting material **1**.

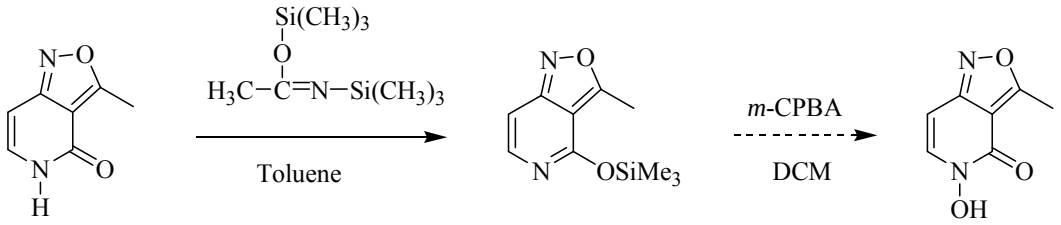
We revised the approach by focusing on trialkylsilyl intermediates,[78] in hope that bulky trialkylsilyl intermediates would be stable and therefore isolable. However, with bulky groups on the silicon, the formation of silyl enol ether intermediates may prove to be difficult due to steric hindrance. To aid this type of reaction a catalytic amount of, for instance a weak base such as imidazole could be used to increase the rate of reaction. Stronger conditions are required for the removal of bulky silicon-based protecting groups, such as the use of tetrabutylammonium fluoride (TBAF).

Further attempts were made to trap the bulky trialkylsilyl intermediate by treating the isoxazolopyridone **1** with excess *n*BuLi at -78 °C to form a possible enolate intermediate followed by addition of triisopropylsilyl chloride (TIPSCl) or and tert-butyl(diphenyl)silyl chloride in separate reactions. Although there was little evidence from the ^{1}H NMR spectrum to suggest the formation of these intermediates, we pursued further investigation by treating the crude material with *m*CPBA to result in the isolation of the starting material. It is possible that the bulky silyl enol ether intermediates are not forming due to steric hindrance.

Since we were unable to isolate the silyl enol ether intermediates, and unsure if the intermediates had formed, further investigation was undertaken. We continued by exploring the use of more potent silylating reagents.[79, 80] N,O-bis(Trimethylsilyl)acetamide (BSA) is a common silylating reagent, it is reactive and is used to silylate a wide range of functional groups, such as alcohols and amides. The reactivity of BSA can be enhanced by addition of a catalyst and the use of polar solvents as they are thought to accelerate the reaction.

BSA was added to a mixture of isoxazolopyridone **1** in anhydrous toluene (Scheme 86),[79, 80] and the reaction was stirred for 1 h before the progress was analysed by TLC. With only the starting material present the resulting mixture was stirred overnight, and following further TLC analysis, an unidentified spot and starting material were present. Since the silyl enol ether intermediate **225** is susceptible to hydrolysis, the oxidation step immediately followed in freshly distilled DCM using excess *m*-CPBA. Extraction with DCM and purification of the crude material by column chromatography resulted in the

recovery of the starting material **1**. These conditions were repeated using more potent silylating agents, for example TMSCl was introduced to increase the reactivity of BSA (BSA ; TMSCl 5:1 ratio) and in another attempt BSA, TMSCl, and N-trimethylsilylimidazole were all used in one pot.[79, 80] N-trimethylsilylimidazole is known to react quickly with unhindered hydroxyls and carbonyls, therefore this was utilised. The three reagents together are highly potent silylating reagents, but applying the reagents in a similar manner as previous reactions proved to be unsuccessful.



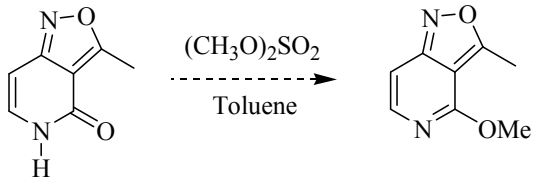
1 **225** **222**

Scheme 86

2.6.2 *O*-Methylation

The silyl enol ethers are highly susceptible to hydrolysis, and therefore isolation of such intermediates remains difficult. The ease of derivatisation of various functional groups using silylating agents follows this order; alcohol > phenol > carboxylic acid > amine > amide. We therefore devised a new strategy, avoiding the use of silylating agents, to form imino ethers, by *O*-methylation. Ideally the imino ethers formed should be stable intermediates and therefore isolable. It has been previously shown that both *N*-methylation and *O*-methylation have been achieved using dimethyl sulfate in benzene.[81-82] We therefore incorporated these methods using the isoxazolopyridone **1** in order to determine the site at which methylation would take place. The isoxazolopyridone **1** was treated with dimethyl sulfate in toluene, and heated at reflux overnight (Scheme 87). At this point TLC analysis only presented the starting material, and as a result the reaction was heated at reflux for a further 24 h, until an unidentified spot was observed on the TLC plate along with the starting material. Further reflux for 24 h resulted in no change since the previous TLC analysis. Following work up, the crude mixture was analysed

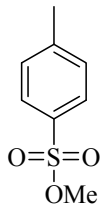
using column chromatography and the fractions were isolated. The starting material was obtained along with the unidentified spot, which was analysed initially by ^{1}H NMR spectroscopy. The signals observed seemed to correspond to the imino ether **226**. It is possible *O*-methylation had taken place, as a signal appeared at 3.74 ppm which would be a typical signal expected for OMe, whereas *N*-methyl signals in the ^{1}H NMR spectrum would be upfield of this.



1 **226**

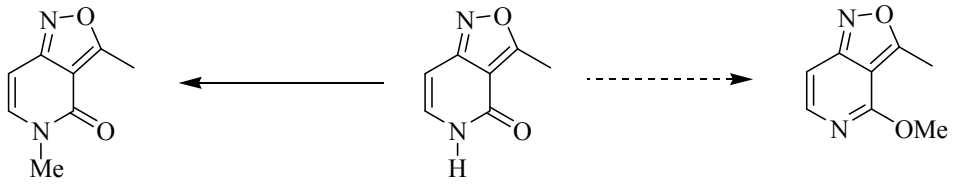
Scheme 87

Mass spectrometry revealed a signal at *m/z* 186, which does not correspond to the mass of the desired imino ether **226**. A mass at 186 coincides with that of toluenesulfonic acid methyl ester **227** and this structure is consistent with the ^{1}H NMR signals. A possible explanation for the formation of the toluenesulfonic acid methyl ester **227** is the sulfonation of toluene. The sulfur atom is acting as an electrophile, and the reaction proceeds *via* an electrophilic substitution mechanism between toluene and dimethyl sulfate, with methanol as the by-product. The electrophilic substitution reaction is fairly slow, no change in reaction was observed in the first 24 h, and after refluxing for 48 h the toluenesulfonic acid methyl ester was isolated in poor yield, this suggests that the sulfur in this case is a poor electrophile.



227

Similar reactions were reported by Hutchinson and co-workers,[83] who carried out reactions in neat dimethyl sulfate, and applied heat up to 55 °C to present good yields of such imino ethers. Implementing these conditions using the isoxazolopyridone **1** only afforded the recovery of the starting material. The conditions were then adjusted to heat these at reflux in neat dimethyl sulfate, however, these reaction proved to be unsuccessful and starting material was again recovered (Scheme 88, Table 5, entry 1 and 2)



228 **1** **226**

Scheme 88

Table 5

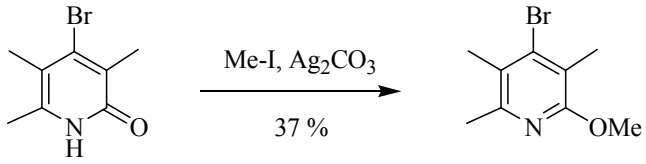
Entry	Solvent	Reagent(s)	Temperature (ºC)	Time (h)	Result
1	-	$(CH_3O)_2SO_2$	55	24	SM
2	-	$(CH_3O)_2SO_2$	Reflux	24	SM
3	THF	*n*BuLi, $(CH_3O)_2SO_2$	-78	2	**228** (65 %)
4	MeCN	Ag_2CO_3, MeI (MW)	90	10 mins	SM
5	$CHCl_3$	Ag_2CO_3, MeI	55	24	SM/**228**

In another reaction the isoxazolopyridone **1** was treated with *n*BuLi in THF at -78 °C and the mixture was stirred for 1 hour. Dimethyl sulfate was then added to the reaction mixture, which was stirred for 1 hour at -78 °C. Following work up, purification and analysis, the imino ether was not present, but the *N*-methyl pyridone **228** was obtained as indicated by the ^{1}H NMR signal at 3.44 ppm to correspond to that of an N-Me signal (Scheme 88, Table 5, entry 3).

Further investigation into the imino ether of the isoxazolopyridone led us in the direction of microwave (MW) reactions. Early studies of the use of MW in organic synthesis were reported by Gedye and co-workers during 1986.[84] The use of MW technology has recently been very popular and a useful technology in organic synthesis, and is described as a general tool for improving the overall efficiency of reactions and rate enhancement. High temperature and pressure are often readily obtained, and as a result increase in yields and reduction in reaction time are observed. For instance, it has been demonstrated that the esterification of benzoic acid was 100 times faster in a MW oven compared to under reflux.[84]

The isoxazolopyridone **1** was dissolved in acetonitrile and treated with silver carbonate and iodomethane. The resulting mixture was irradiated in a microwave system (Biotage Initiator Eight EXP) for 10 minutes at 90 °C, followed by work up to only give the starting material (Scheme 88, Table 5, entry 4).

Failure of the microwave reactions was disappointing, however a final attempt using silver carbonate and iodomethane was considered for *O*-methylation. In a previous report a bromopyridone **230** was heated with silver carbonate and iodomethane in the dark at 55 °C for a period of 24 h to yield the *O*-methylated pyridine **231** (Scheme 89).[85]



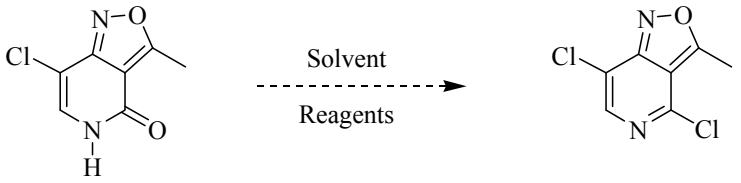
230 **231**

Scheme 89

Thus, to a solution of isoxazolopyridone **1** in chloroform were added silver carbonate and iodomethane, and the mixture stirred in the dark for 24 h at 55 °C (Scheme 88, Table 5, entry 5). Purification of the crude material by column chromatography afforded two fractions, which were subjected to analysis to identify one fraction as the starting material **1** and the other as the unexpected *N*-methylpyridone **228**. The ^{1}H NMR and the ^{13}C NMR

spectra for compound **228** presented signals at 3.44 and 34 ppm respectively to correspond to that of a N-Me signal. With the bulk of the starting material recovered, only a small percentage of the *N*-methylpyridone **228** was isolated. We initially anticipated reactions with silver carbonate would take place at the oxygen which was demonstrated by previous reports,[85] however, in this case it seems silver carbonate may be acting as a weak base to allow methylation to take place at the nitrogen.

The strategy was reviewed and we explored alternative ways to synthesise the enol ethers. There are several examples of synthesis of imino-chlorides from 2-pyridones, which can subsequently be treated with sodium methoxide to give the enol ether *via* an addition-elimination (overall substitution) reaction.[86,87,88] A number of chlorinating reagents were explored in attempts to synthesis the imino-chlorides and the details are discussed below (Scheme 90 and Table 6).



162 **232**

Scheme 90

The 7-chloroisoxazolopyridone **162** in dichloroethane (DCE), phosphorus oxychloride ($POCl_3$) and catalytic amount of dimethylformamide (DMF) was stirred at room temperature. The reaction was monitored by LC-MS, and no change was observed in the reaction. The reaction mixture was then heated at reflux for 24 h to leave a trace amount of the imino-chloride **232**, and also the starting material was recovered (Table 6, entry 1).

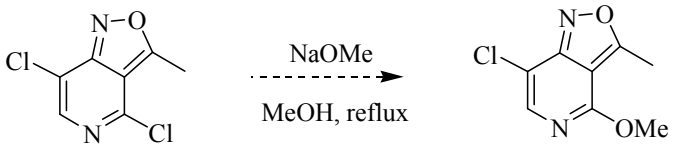
Table 6

Entry	Solvent	Reagent(s)	Temperature (ºC)	Time (h)	Result
1	DCE	$POCl_3$ (excess) DMF	23/Reflux	24	SM
2	MeCN	$POCl_3$ (1.5 eq)	Reflux	24	SM
3	MeCN	$POCl_3$ (10 eq)	Reflux	24	SM
4	-	$POCl_3$ (excess)	80	24	SM
5	-	$POCl_3$ (excess)	Reflux	24	SM
6	-	$SOCl_2$ (7 eq), DMF (7 eq)	Reflux	24	SM
7	MeCN	PCl_5 (10 eq)	23	24	Complex mixture

The reaction was repeated in acetonitrile using $POCl_3$, and the reaction was heated at reflux overnight (Table 6, entry 2). The reaction was monitored by TLC analysis until all the starting material had been consumed. On cooling, the acetonitrile solution was decanted from the insoluble sludge, and evaporated *in vacuo*. The residue was extracted with hot ethyl acetate and then concentrated under reduced pressure to leave a red solid, which was stirred with diethyl ether overnight at 23 °C. The ethereal solution was decanted from the red solid and concentrated under reduced pressure to an oil. After purification, the 7-chloroisoxazolopyridone **162** was isolated along with a second compound, which proved difficult to characterise and provided a complex ^{1}H NMR spectrum, which indicated the presence of a possible broad NH signal. A second attempt at this reaction using excess $POCl_3$ (10 eq) in acetonitrile again provided only the recovery of the starting material (Table 6, entry 3). The reaction was repeated using only the $POCl_3$ reagent in the absence of any solvent and heated at 80 °C overnight. An aqueous workup and extraction with ethyl acetate afforded only starting material (Table 6, entry 4). The reaction was repeated using excess $POCl_3$ reagent and the reaction mixture was heated at reflux overnight, followed by a non-aqueous workup to recover only the

starting material (Table 6, entry 5). Similar results were observed using thionyl chloride and DMF (Table 6, entry 6). In another attempt the 7-chloroisoxazolopyridone was treated with phosphorus pentachloride in acetonitrile and after stirring for 24 h only the starting material was recovered (Table 6, entry 7). The failed attempts to form the imino-chloride **232** were rather disappointing, in our case only the starting 7-chloroisoxazolopyridone **162** was isolated. It appears that the isoxazolopyridone does not behave like traditional 2-pyridones.

It is possible the previous attempts to synthesis the imino-chlorides **232** were susceptible to hydrolysis therefore in numerous experiments we pursued the synthesis of the enol ether without isolating the imino-chloride **232**. There have been several reports of the nucleophilic displacement of a chloro substituent by treatment with sodium methoxide to give a methoxy compound.[89] The crude material from Table 6, entry 1 was treated with sodium methoxide and methanol (Scheme 91) and stirred at 23 °C, but no change was observed in the reaction, and thereafter the reaction was heated at reflux for 24 h. Following workup and purification, very little success was observed, there was no indication of the enol ether **233** and the ^{1}H NMR spectrum indicated the presence of the 7-chloroisoxazolopyridone **162**.

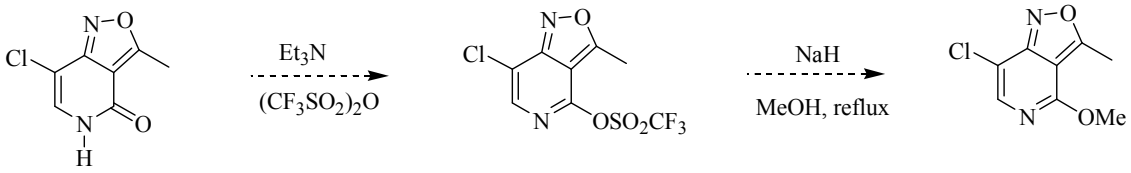


232 **233**

Scheme 91

With these disappointing results, we looked at introducing alternative functional groups at C-4 of the isoxazolopyridone. Triflates and mesylates are very good leaving groups in nucleophilic substitution reactions. By introducing such functionality at C-4 of the iosxazolopyridone, a nucleophilic displacement by, for instance, sodium methoxide would introduce the enol ether.

In the first instance we pursued the synthesis of the highly reactive triflate (Scheme 92). To the 7-chloroisoxazolopyridone **162** in DCM, was added triethylamine and trifluoromethanesulfonic anhydride at 0 °C and the reaction was stirred at 5 ºC and monitored by TLC analysis. After no indication of any product, the reaction was stirred at 23 ºC for 3 h before subjected to a workup. The TLC analysis indicated the possible presence of the unstable trifluoromethanesulfonate intermediate **234**, which was not characterised and used without further purification in a reaction with sodium methoxide in an attempt to form the enol ether compound **233**. The sodium methoxide was prepared *in situ* from sodium hydride and methanol at 0 °C and stirred for 30 minutes before the addition of the possible trifluoromethanesulfonate intermediate **234**. The reaction mixture was stirred for 4 h at 23 ºC and the reaction was monitored by LC-MS but no change was observed. The reaction was then heated at reflux for 24 h, followed by workup and purification to return the 7-chloroisoxazolopyridone **162** and no enol ether **233** was observed.

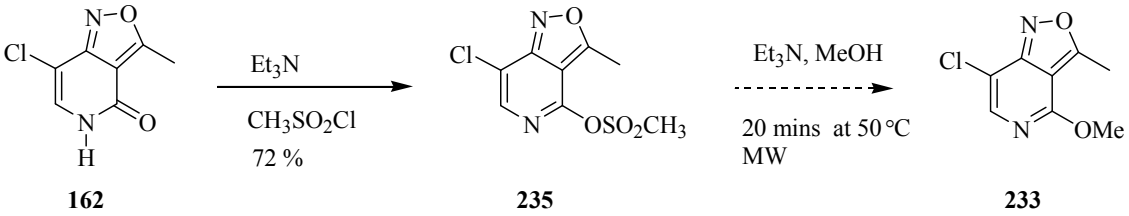


162 **234** **233**

Scheme 92

Since we had little success with the triflate intermediate **234** and the formation of the enol ether **233**, the mesylate compound **235** was our next interest (Scheme 93). To a stirred solution of the 7-chloroisoxazolopyridone **162** was added triethylamine and methanesulfonyl chloride at 0 °C, and the mixture was stirred for 3 h at 23 °C, by which point the reaction had gone to completion by TLC analysis and LC-MS. After workup and purification by column chromatography the methanesulfonyl intermediate **235** was isolated in 72 % yield. The next step of this sequence was to prepare the enol ether **233**, the mesylate was treated with methanol and triethylamine under microwave conditions for 20 minutes at 50 °C, to only afford the 7-chloroisoxazolopyridone **162**. The mesylate intermediate **235** was not recovered. In this case, it appears the nucleophile is attacking

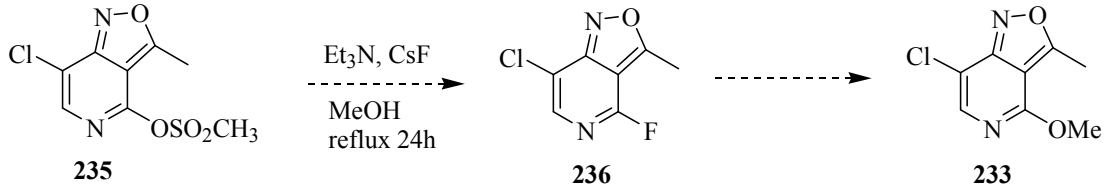
the sulfur atom of the mesylate to liberate the 7-chloroisoxazolopyridone **162** instead of attacking the C-4 centre to provide the enol ether **233**.



162 **235** **233**

Scheme 93

Finally for the first time we had successfully functionalised the C-4 position of the isoxazolopyridone, however the challenge remained to form the enol ether. An alternative synthesis for the enol ether was investigated by treating the mesylate **235** with cesium fluoride to afford an imino-fluoride intermediate **236** (Scheme 94). Cesium fluoride being a good fluoride source should displace the mesylate to a possible unstable imino-fluoride intermediate **236**, and treatment with methanol would afford the enol ether **233**. The mesylate **235** in methanol was treated with triethylamine and cesium fluoride, and the reaction mixture was heated at reflux overnight. The reaction progress was monitored by LC-MS, which indicated no sign of the fluoropyridine **236** or the enol ether **233**. Following preliminary purification, a complex mixture was obtained making characterisation difficult. Again some of the starting 7-chloroisoxazolopyridone **162** was isolated indicating nucleophilic attack on the sulphur atom of the mesylate **235**.

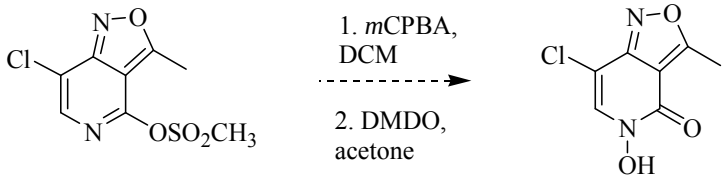


235 **236** **233**

Scheme 94

Earlier in our studies, the oxidising agent *m*-CPBA was used in several attempts to oxidise the silyl compounds to introduce the hydroxamic acid functionality. The same

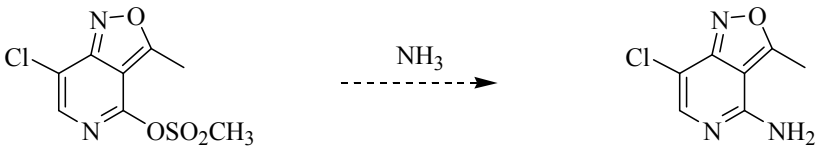
procedure was applied with the mesylate **235** but again the reaction was not successful (Scheme 95). A similar reaction was investigated using an alternative oxidising agent dimethyldioxirane (DMDO) in acetone. Again the mesylate **235** was recovered with no indication of the hydroxamic acid derivative **237**.



235 **237**

Scheme 95

We were not sure if the previous attempts to form the enol ether from the mesylate **235** were either a difficult substitution or we were just using the wrong conditions, so we attempted another reaction using a saturated solution of ammonia with the mesylate **235** (Scheme 96). In a nucleophilic substitution reaction, the ammonia should attack at the C-4 position of compound **235** and displace the mesylate. However, the nucleophilic displacement to give the amine **238** was not observed; only the 7-chloroisoxazolopyridone **162** was isolated. From the results it was concluded that since the nucleophilic ammonia appears unable to attack at the C-4 position to give the amine, it would be difficult to find other suitable reagents to afford the enol ether. Also it is possible that the corresponding *N*-mesylate is formed rather than compound **235**. With time constraints we did not pursue further experiments but it may be worth revisiting the triflate chemistry in future, as it should be more reactive than the mesylate intermediate.



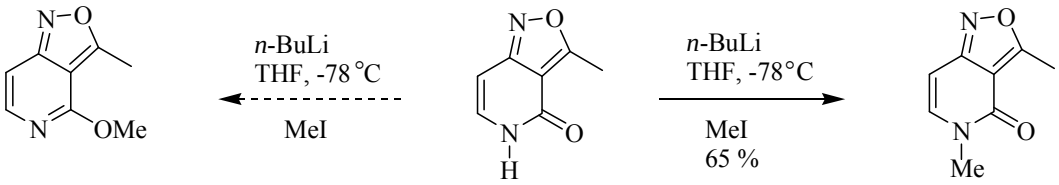
235 **238**

Scheme 96

2.6.3 *N*-Methylation

Many naturally occurring compounds with the 3-acyl-4-hydroxypyridone core contain the hydroxamic acid functionality, and some also contain the *N*-methyl group as in funiculosin **6**. There are many literature reports for *N*-methylation, and a methylating reagent widely used in such reactions is iodomethane.[81]

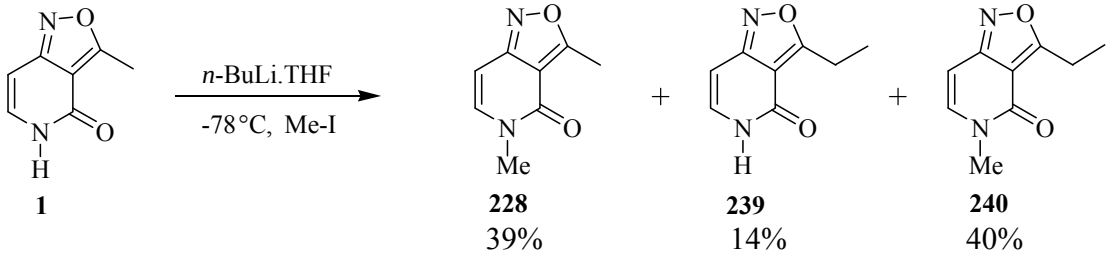
Applying the literature procedure to the isoxazolopyridone **1**, using *n*BuLi (1.5 eq) and iodomethane (1.5 eq) resulted in the *N*-methylpyridone **228** in 65 % yield and *O*-methylation to **226** was not observed. The ^{1}H NMR spectrum revealed a signal at 3.44 ppm, which corresponds to an N-Me signal and the broad NH signal was absent. Iodomethane is a good substrate for an S_N2 substitution reaction, the anion formed upon addition of the base, *n*BuLi, is able to attack the sterically unhindered iodomethane to leave compound **228** (Scheme 97). The *N*-methylation had also been observed previously using similar conditions with dimethyl sulfate as methylating agent (Table 5).



226 **1** **228**

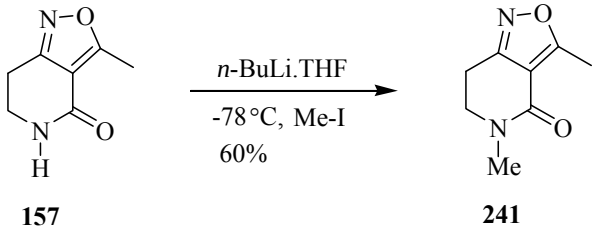
Scheme 97

Applying similar conditions in a second reaction, in which only excess *n*BuLi and excess iodomethane were used, afforded a mixture of compounds **228**, **239**, and **240** in ratio 3: 1: 3 respectively (Scheme 98), which again indicates the acidity at the C-3'(Me) along with the N-5(H).



Scheme 98

Applying similar conditions to the bicyclic lactam **157** using *n*BuLi (1.5 eq) and iodomethane (1.5 eq) afforded the *N*-methylpyridone **241** and *O*-methylation was also not observed in this reaction. The ^{1}H NMR and ^{13}C NMR spectra revealed a signal at 3.0 and 33 ppm repectively, which corresponds to the N-Me signal, and the broad NH signal was absent (Scheme 99).

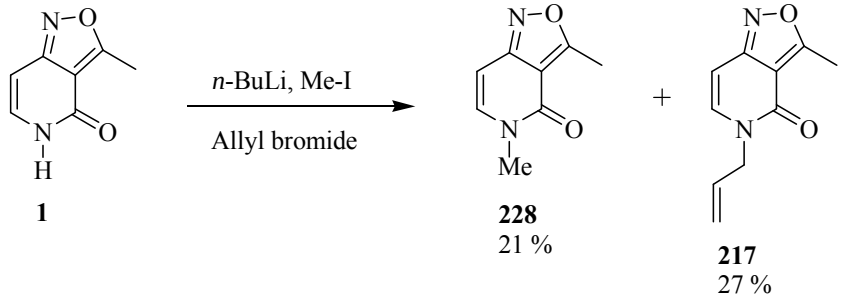


Scheme 99

In the examples shown above, the reactions with iodomethane prefer to form predominantly the *N*-methylpyridone. Also as demonstrated earlier in the report, the reaction of the unsaturated pyridone with *n*-BuLi and excess allyl bromide also prefer to react at the N-5(H) as well as the C3' (Me). From these reactions it is obvious the reaction is proceeding *via* a dianion intermediate.

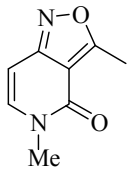
To confirm the reactivity at these possible sites, we continued the enolate type chemistry with the isoxazolopyridone **1** using both iodomethane and allyl bromide in one pot. To the isoxazolopyridone **1** in THF was added excess *n*-BuLi at -78 °C, followed by addition of iodomethane (1.1 eq), and the resulting mixture was stirred at this temperature for 30 minutes. Allyl bromide (1.1 eq) was then added and the reaction stirred for a further 1 h. Following work up, both *N*-substituted compounds **228** and **217** were isolated in ratio

1:1.3 respectively (Scheme 100). This reaction confirms the reactivity of the nitrogen anion over the methyl anion, since only *N*-substituted compounds were observed and no other compounds were isolated from the reaction.



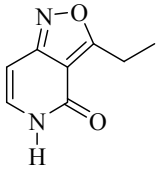
Scheme 100

However, after analysing a small portion of the crude material in the GC-MS, a range of *m/z* values were observed corresponding to analogues of **228** and **217**. The chromatogram revealed *m/z* values at 164, 178, 190 and 204 (Scheme 101). These represent monomethyl, dimethyl, monoallyl and methyl/allyl dialkylated derivatives, respectively. The ratio of the individual substituted pyridones remains uncertain as some of the products are isomeric. The *N*-methyl or the *N*-allyl pyridones seem to be present in most cases. Clearly examples of isoxazolopyridones are also observed, where reaction has taken place at the C-3'(Me) of the isoxazolopyridone. However, these compounds are only seen as trace amounts.



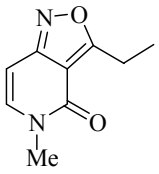
228

m/z 164



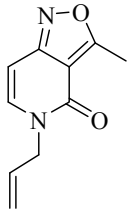
239

164



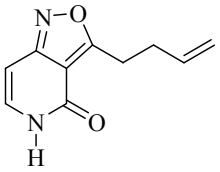
240

178



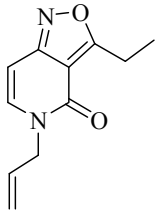
217

m/z 190



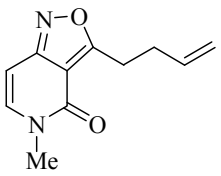
242

190



243

204



244

204

Scheme 101

2.7 Isoxazole unmasking to reveal the tricarbonyl moiety

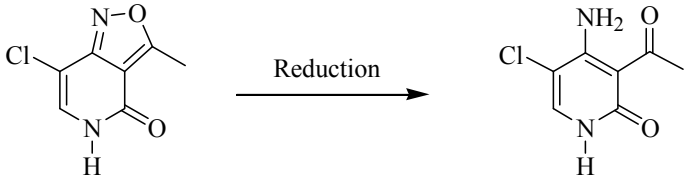
Having functionalised the isoxazolopyridone to develop the aromatic rings *via* coupling reactions and construction of the polyene side chains, we need to cleave the N-O bond of the isoxazolopyridone to prove its validity in the synthetic strategy.

There are several reports of N-O bond cleavage of isoxazoles using molybdenum hexacarbonyl $Mo(CO)_6$ in moist acetonitrile.[90, 91] Other reports include the use of samarium iodide, which is also known as Kagan's reagent, and titanium(III) chloride.[92, 93]

The use of hydrogen and a palladium catalyst to cleave the weak N-O bond has been of great interest in synthetic studies.[94] These reactions are simple to carry out and the work up requires only filtering of the catalyst, and often further purification is not necessary. We pursued our interest in the isoxazole cleavage *via* a number of methods (Scheme 102, Table 7). In the first instance the 7-chloroisoxazolopyridone **162** was subjected to hydrogenolysis. The 7-chloroisoxazolopyridone **162** was treated with palladium on carbon (5 % w/w) in methanol and treated with hydrogen with a Buchi pressflow gas controller at 2 bar pressure for 3 h to yield the enaminone **245** in 84 %. The reaction was also repeated in the H-cube in order to optimise the reaction conditions. Thus, treatment of the 7-chloroisoxazolopyridone **162** in a mixture of methanol/acetone for 10 minutes at 80 bar pressure and 100 ºC over palladium on carbon gave the enaminone **245** in 30 % yield. The reaction was repeated a third time using palladium on carbon (10 % ww) in methanol, and the reaction was stirred for 5 h at 23 ºC at atmospheric pressure to leave the enaminone **245** in 80 % yield. The use of hydrogen and palladium/carbon has thus been proven to be successful in the cleavage of the weak N-O bond of the 7-chloroisoxazolopyridone (Scheme 102).

The reduction of the N-O bond for the more complex isoxazolopyridones prepared, such as the tenellin analogues, using the hydrogen and palladium/carbon method may cause complications. These compounds contain an unsaturated side chain, therefore this method would not be suitable since it would cause over-reduction of the side chain. The proposed

method for cleaving the N-O bond would require the use of the reagent, $Mo(CO)_6$, which should selectively reduce the N-O bond and leave the unsaturated side chain intact.[95,46] The 7-chloroisoxazolopyridone **162** was treated with $Mo(CO)_6$ in moist acetonitrile, and heated at reflux for 3 h to leave the enaminone **245** in 54 % yield.



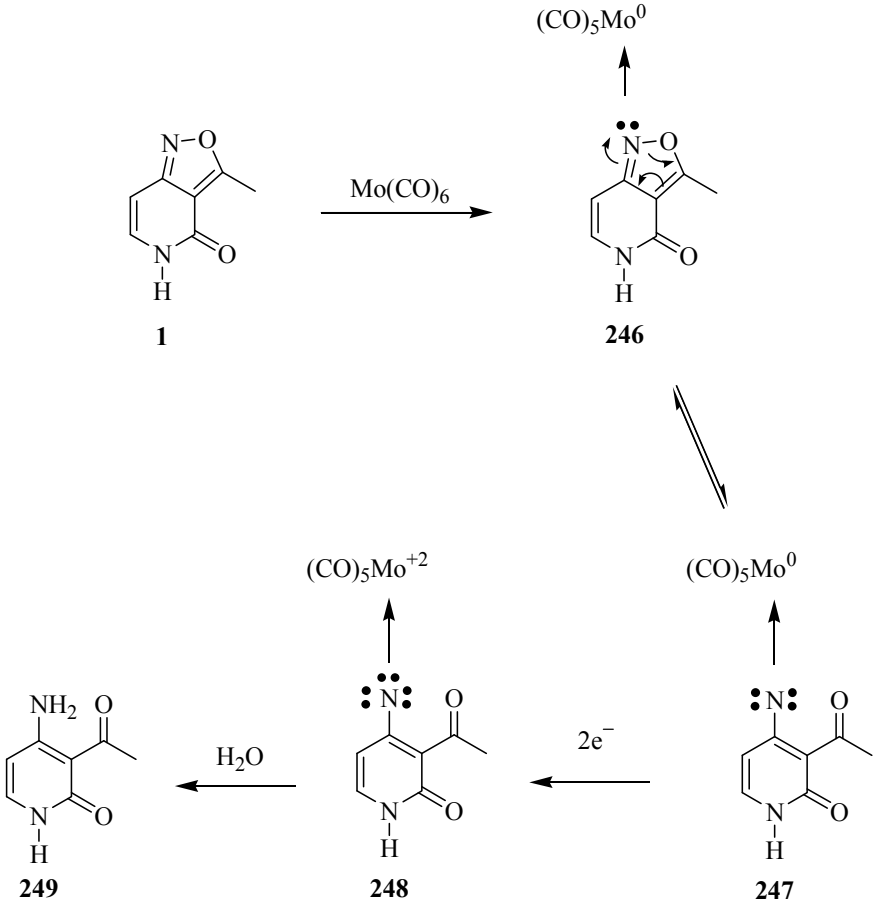
162 **245**

Scheme 102

Table 7

Condition/Reagent	**Yield (%)**
Pd/C, H_2 (Buchi pressflow)	84
Pd/C, H (H Cube)	30
Pd/C, H_2	80
$Mo(CO)_6/H_2O$	54

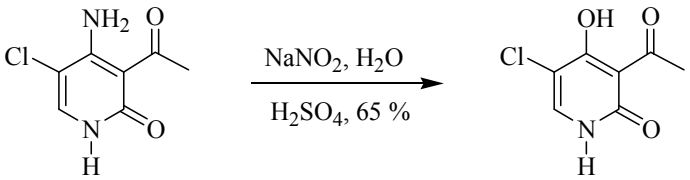
The proposed mechanism for the reductive cleavage of the N-O bond to the enaminone **249** requires the molybdenum to complex with the nitrogen atom of the isoxazolopyridone **1**. As the N-O bond breaks, a possible intermediate **246** will form a complex with the molybdenum, and finally hydrolysis will follow to the leave the enaminone **249** (Scheme 103).[95] However, we propose an additional dianion intermediate **248** as a result of reduction, which would then follow hydrolysis to the enaminone **249**.



Scheme 103

With the successful reduction of the N-O bond, we pursued the development of the 4-hydroxypyridone to reveal the tricarbonyl moiety. It has previously been shown in our research group, the reduction of pyrroloisoxazoles to the corresponding enaminone was achieved under hydrogenation conditions (H_2, Pd/C, MeOH), followed by subsequent hydrolysis (1M aq NaOH, THF, reflux) to reveal the tricarbonyl moiety in acyl tetramic acids.[46] We incorporated this base-catalysed hydrolysis of the amine to the alcohol; the 4-amino-5-chloropyridone **245** was treated with excess sodium hydroxide in THF and heated at reflux for 8 h. The reaction was monitored every hour, and only the starting material was recovered. Clearly C-4 was not sufficiently electrophilic. In a second reaction we investigated a diazotisation procedure to reveal the tricarbonyl moiety (Scheme 104). The 4-amino-5-chloropyridone **245** in water was stirred at 23 ºC to ensure

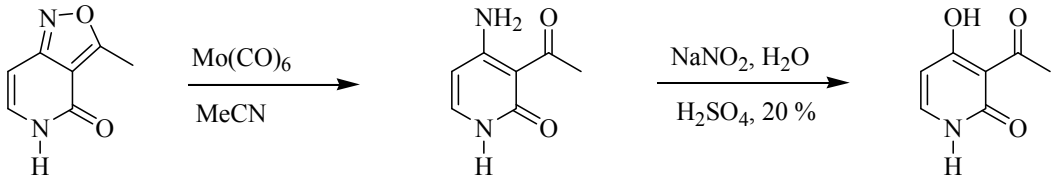
it was in suspension, the reaction mixture was then cooled to 0 ºC and a solution of sodium nitrite in hydrochloric acid (2M) was added, ensuring the temperature did not exceed 5 ºC. The resulting mixture was stirred for 1 h at this temperature, and then another 1 h at 50 ºC. Following work up and purification, the 4-hydroxypyridone **250** was revealed in 65 % yield.



245 **250**

Scheme 104

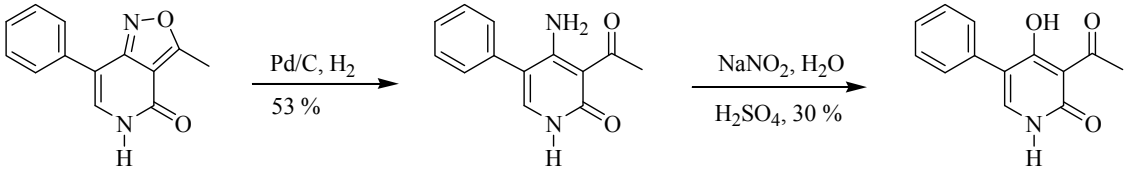
The isoxazolopyridone **1** was treated with $Mo(CO)_6$ to leave the enaminone **252**, which was used without further purification in a diazotisation step to yield the 4-hydroxypyridone **252** in 20 % yield.



1 **251** **252**

Scheme 105

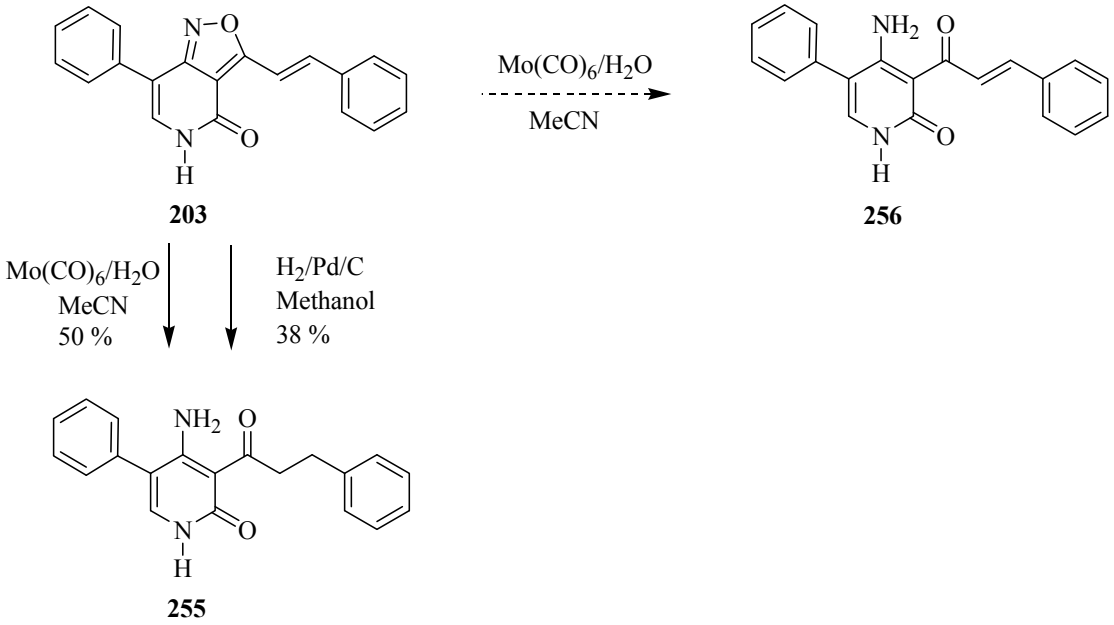
Further reactions were investigated using the 7-phenylisoxazolopyridone **174**, under the hydrogenation conditions. The phenyl compound **174** was treated with palladium on carbon (5 %, w/w) in methanol, with the Buchi pressflow gas controller at 2 bar pressure for 3 h to yield the enaminone **253** in 53 %. To reveal the tricarbonyl moiety, a diazotisation reaction followed to yield the 4-hydroxy adduct **254** in 30 % (Scheme 106).



174 **253** **254**

Scheme 106

To cleave the N-O bond of the C-3 benzaldehyde condensation product **203**, the molybdenum hexacarbonyl method was pursued to avoid side chain reduction. The benzaldehyde adduct **203** was treated with $Mo(CO)_6$ in moist acetonitrile and heated at reflux for 3 h. The result of this reaction was very surprising; we did not see the desired enaminone **256**, but the reduced side chain analogue **255** was observed (Scheme 107). The ^1H NMR spectrum clearly indicated the presence of the two CH_2 triplets at 2.89 and 3.47 ppm confirming the saturated bond and the alkene signals were absent. An accurate mass of the compound **255** was obtained; a molecular ion of *m/z* 319.1445 supports the side chain reduction. This reaction was repeated several times to confirm the results. A thorough literature search of $Mo(CO)_6$ reactivity to support such side chain reduction resulted in no hits.[91, 96, 97] We are unsure of the mechanism by which this reaction proceeds, and the hydrogen source remains unknown. A speculation for the exceptional result, which contrasts with literature reports and with our research group's experience with pyrroloisoxazoles leading to the acyltetramic acids,[46] would relate to the extended conjugation in the 3-(2-phenylethenyl) bicycle possibly *via* electron transfer reactions. We pursued our investigation by conducting the N-O bond cleavage of the benzaldehyde adduct **203** using palladium on carbon (10 % w/w) in methanol. We would expect the side chain reduction in this case and it would allow the direct comparison of the two results, therefore confirming the structure. The reaction was stirred for 5 h at 23 ºC at atmospheric pressure to leave the enaminone **255** with the expected side chain reduction in 38 % yield. The ^1H NMR spectra of the two samples were identical, the presence of the two CH_2 triplets at 2.89 and 3.47 ppm confirming the saturated bond. Finally we obtained an X-ray crystal structure of the reduced product from the $Mo(CO)_6$ method, which proves the N–O reduction accompanied by the side chain reduction (Figure 2).



203 **256** **255**

Scheme 107

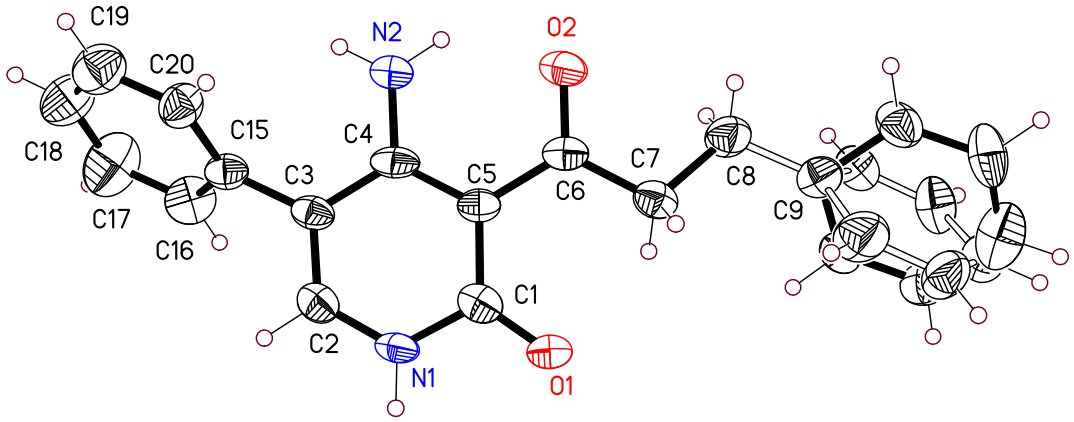
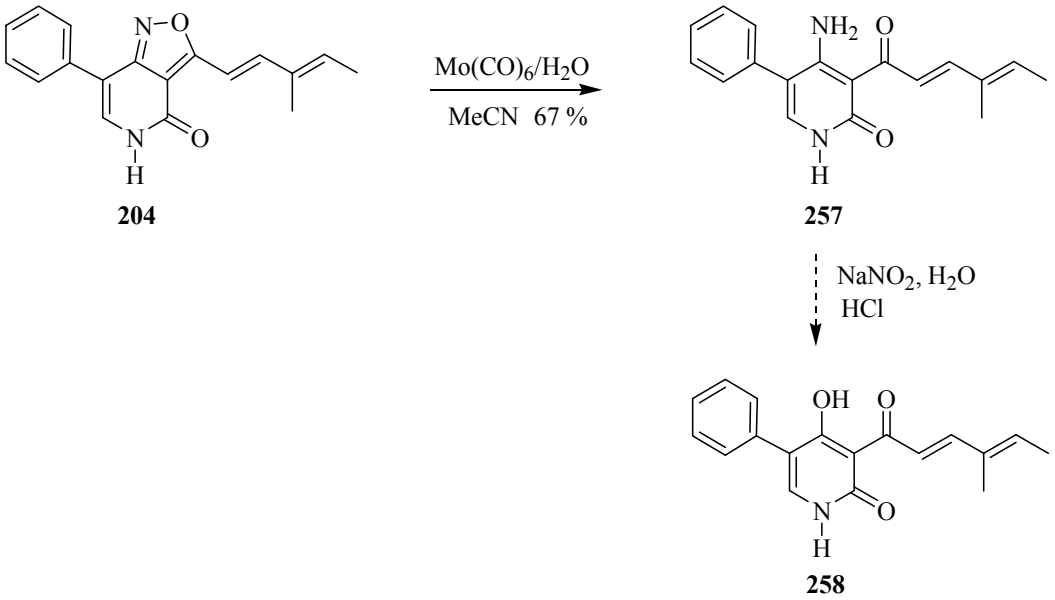


Figure 2. X-Ray crystal structure of reduction product **255** (disorder in phenyl ring; crystallographic numbering)

As we felt the alkene reduction using $Mo(CO)_6$ in the above example was exceptional, and due to the extended conjugation in the phenyl ethenyl system, we decided to proceed with the $Mo(CO)_6$ protocol. The 3-(3-methylpenta-1,3-dienyl)-7-phenyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **204** was treated with $Mo(CO)_6$ in moist acetonitrile and heated at reflux for 3 h, to leave the enaminone **257** in 67 % yield (Scheme 108), with no side chain reduction. Diazotisation of this compound using the previously described procedure was attempted to leave trace amounts of the starting material **257**, and the tricarbonyl adduct **258** was not observed. It appears the compound may have decomposed during the diazotisation step or due to the smale scale nature of the reaction the compound **258** was not observed.

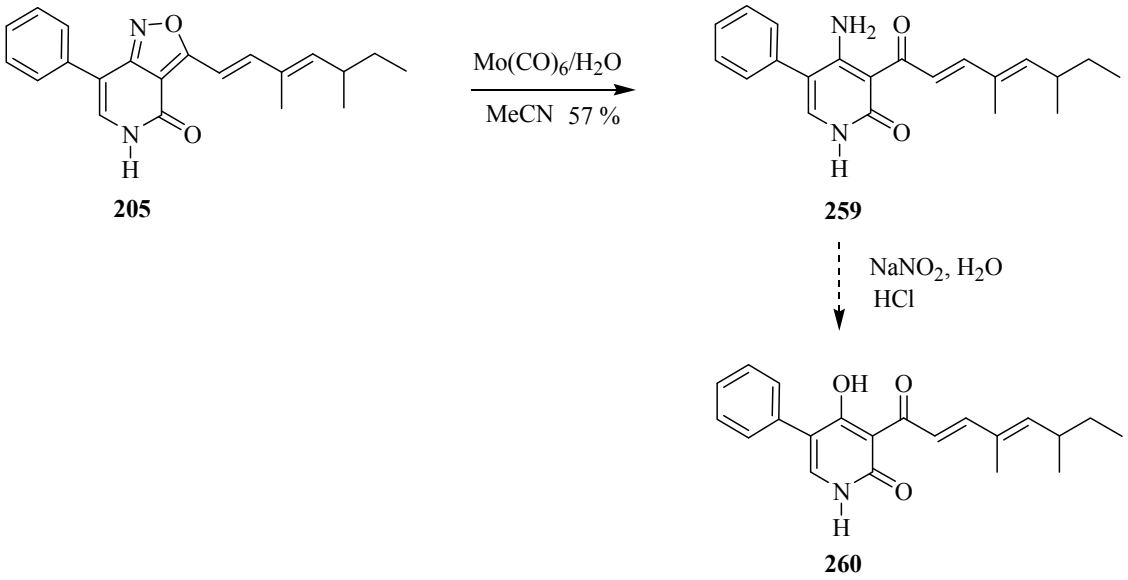


204 **257** **258**

Scheme 108

The 3-(3,5-dimethylhepta-1,3-dienyl)-7-phenyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **205** was similarly treated with $Mo(CO)_6$ in moist acetonitrile and heated at reflux for 3 h to leave the enaminone **259** in 57 % yield (Scheme 109). Diazotisation of this compound was attempted to leave trace amounts of the starting material **259**, and the tricarbonyl adduct **260** was not observed. It again appears again that the compound may have decomposed during the diazotisation step or due to the smale scale nature of the reaction the compound **260** was not observed. In the earlier examples of the 7-

chloroisoxazolopyridone **162**, diazotisation of the enaminone **245** (Scheme 104) was successful although the yields were low. Further investigation is required to reveal the tricarbonyl moiety in the more complex structures containing the aliphatic side chain, to avoid possible decomposition of the material.



Scheme 109

2.8 Conclusion

This work has demonstrated the development of the building block containing the 3-acyl-4-hydroxypyridone core, by masking it in the form of an isoxazolopyridone building block, and has proven this to be a valid synthetic strategy. The isoxazolopyridone has been synthesised in 7 steps, from the commercially available β-alanine. The keys steps in the synthesis of the building block involved a 1,3-dipolar cycloaddition and photolytic dehydrochlorination reactions. The nitrile oxide from the oxime underwent a 1,3-dipolar cycloaddition reaction to construct an isoxazole. The development of the C6-C7 isoxazolopyridone unsaturated bond was achieved using a medium-pressure Hg Hanovia lamp in a photolytic dehydrochlorination reaction.

The construction of substituents at the C-3' of the isoxazolopyridone was successful with both aliphatic and aromatic aldehydes. The tenellin side chain was successfully constructed in the isoxazolopyridone. The Suzuki palladium-catalysed cross-coupling reactions were employed to synthesise biaryls. We have incorporated both the phenyl and the 4-hydroxyphenyl substituent in the C-7 position of the isoxazolopyridone. To reveal the 3-acyl-4-hydroxypyridone, we successfully cleaved the N-O bond of the isoxazolopyridone to prove its validity in the synthetic strategy. The cleavage of the N-O bond afforded the enaminone, which was followed by a diazotisation reaction, to reveal the tricarbonyl moiety. Although, the diazotisation reaction employed previously on the less complex isoxazolopyridones proved to reveal the tricarbonyl moiety, this was not the case with the more complex analogues. This step requires further investigation.

Many attempts to develop the hydroxamic functionality failed. Standard pyridone chemistry to develop the imino-chlorides from 2-pyridones was unsuccessful. We therefore concluded the isoxazolopyridone does not behave like a typical 2-pyridone.

This thesis has contributed towards the development of tenellin and the 3-acyl-4-hydroxy-pyridone analogues.[98] A final step would reveal the tricarbonyl moiety from the enaminone to afford the natural and unnatural analogues of tenellin.

Experimental

General Details

^{1}H NMR spectra were measured using a Bruker DPX 400 MHz spectrometer or a Varian 400 MHz spectrometer. Chemical shift, δ, values are given in ppm (parts per million) and coupling constants in hertz.

Multiplets are denoted by the following symbols: s, singlet; d, doublet; t, triplet; q, quartet and m, multiplet.

^{13}C NMR were recorded on a Bruker DPX 400 MHz instrument operating at 100.62 MHz. All spectra were recorded using tetramethylsilane (TMS) as the internal reference in $CDCl_3$ or d_6-DMSO as solvent.

IR spectra were determined using a Perkin Elmer FT-IR Paragon 1000 spectrometer or a Perkin Elmer Spectrum One spectrometer.

All the infrared spectra were recorded in the range 4000-600 cm^{-1}.

Distillation of reagents was carried out 1 atmospheric pressure unless otherwise stated.

Mass spectra were recorded on a Joel SX-102 spectrometer (FAB and EI) and the Thermo Exactive (Orbi) accurate mass spectrometer (ESI), fitted with a Triversa Advion Nanomate sample delivery system using nano-ESI of methanol or methanol(1% w/w acetic acid). In the description of mass spectra $[M+H^+]$ and M^+ refer to the molecular ion peak obtained by ionisation. The intensity of principle peaks (*m/z*) are given as a percentage relative to the base peak. GC-MS used was Fisons GC 800 with autosampler, Fisons mass lab MD 800 EI^+ and DB5-MS 30 m column.

Melting points were determined using an Electrothermal-IA 9100 and are uncorrected. They are given in degree Celsius (°C).

Thin Layer Chromatography using silica gel as the absorbent was carried out with aluminium backed plates with silica gel.

Column Chromatography using silica gel was carried out with Zeoprep 60 HYD 40-63 Micron silica.

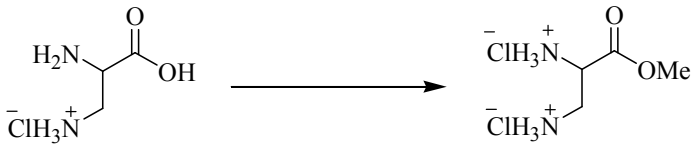
HPLC analysis was conducted in a Waters Fraction Lynx system comprising a 2767 injector/collector with a 2525 gradient pump, CFO, 2996 photodiode array, 2420 ELSD and Micromass ZQ2000 equipped with a Waters XBridge dC18 column (column length 20 mm, internal diameter of column 3 mm, particle size 3.5 micron). The analysis was conducted using a three minute run time using H_2O with 10mM Ammonium Acetate and CH_3CN.

Solvents were distilled before use. Petroleum ether (b.p. 40-60 °C) and ethyl acetate were distilled from calcium chloride. Dichloromethane (DCM) was distilled from calcium hydride. Tetrahydrofuran (THF) was freshly distilled from sodium and benzophenone under an atmosphere of nitrogen. Methanol was freshly distilled from magnesium methoxide.

Hydrogenation reactions were carried out in a Buchi Pressflow gas controller pbc 1202 or the H-cube (where stated).

Microwave reactions were carried out in a Biotage Initiator Eight EXP microwave system.

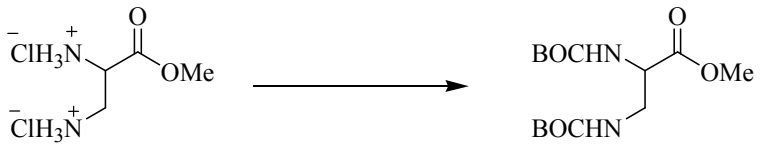
Methyl 2,3-diaminopropanoate dihydrochloride 143



142 **143**

To a stirred suspension of 2,3-diaminopropionic acid monohydrochloride **142** (6.20 g, 41.1 mmol) in dry methanol (200 mL) was added freshly distilled acetyl chloride (70.6 g, 900 mmol) dropwise over 2 h at 0 °C. The mixture was warmed to room temperature and heated at reflux for 4 days. The resulting white precipitate was filtered and treated with methanol and acetyl chloride in the same manner for a further 2 days. The resulting white precipitate was filtered, washed with methanol (20 mL) and dried to give the title compound **143** (6.8 g, 81%) as a white solid; δ_H (400 MHz; D_2O) 3.47 (1H, dd, *J* 13.8 & 8.4 CHC*H*$_2$, H^A), 3.47 (1H, dd, *J* 13.8 & 5.6, CHC*H*$_2$, H^B), 3.79 (3H, s, C*H*$_3$) & 4.42 (1H, dd, *J* 8.4 & 5.6, CH_2C*H*); δ_C (100 MHz; D_2O) 37.9 (CH_2), 49.6 (CH), 54.3 (CH_3) & 167.3 (C).

Methyl 2,3-bis(*tert*-butyloxycarbonylamino)propanoate 144

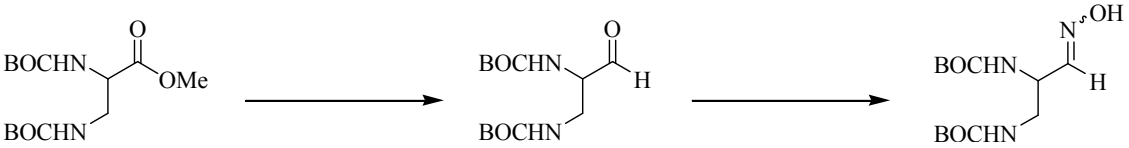


143 **144**

To a stirred suspension of methyl 2,3-diaminopropanoate dihydrochloride **143** (4.01 g, 20.9 mmol) in dichloromethane (100 mL) was added di-*tert*-butyl dicarbonate (9.60 g, 43.9 mmol) in one portion followed by triethylamine (8.46 g, 83.8 mmol) dropwise over 1 h at 0 °C, and the mixture stirred for 18 h. The resulting mixture was washed with aqueous citric acid (1M, 100 mL), saturated aqueous sodium hydrogen carbonate (2 × 50

mL), brine (50 mL), dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure to give the title compound **144** (6.32 g, 94%) as a colourless oil; ν_{max} (DCM)/cm^{-1} 3366, 2976, 1786, 1716, 1515, 1392, 1366, 1250, 1165 & 1077; δ_H (400 MHz; $CDCl_3$) 1.27 (18H, s, 2 × C[CH_3]$_3$), 3.28-3.35 (2H, m, NC*H*$_2$CH), 3.54 (3H, s, OC*H*$_3$), 4.13 (1H, br, NHC*H*CH_2), 5.36 (1H, br, $CHCH_2$N*H*) & 5.64 (1H, br, CH_2CHN*H*); δ_C (100 MHz; $CDCl_3$) 27.6 (CH_3), 28.0 (CH_3), 41.9 (CH_2), 52.1 (CH_3), 54.2 (CH), 84.9 (C), 146.5 (C), 155.3 (C), 156.2 (C) & 171.2 (C); *m/z* (ESI) 319.1862 ([M + H^+], $C_{14}H_{27}N_2O_6$ requires 319.1864).

2,3-Bis(*tert*-butyloxycarbonylamino)propanal oxime 146



144 **145** **146**

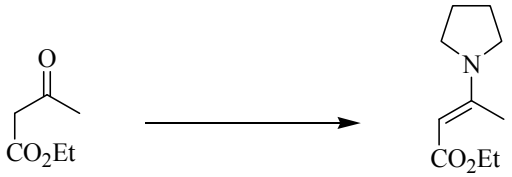
Preparation of the aldehyde **145**

To a solution of methyl 2,3-bis(*tert*-butyloxycarbonylamino)propanoate **144** (6.32 g, 19.8 mmol) in toluene (100 mL) was added DIBAL-H (1M in toluene, 79 mL, 79 mmol) dropwise over 1 h at -78 °C. After stirring the mixture at 23 °C for a 1 h, methanol (20 mL) was added slowly followed by potassium sodium tartrate solution (100 g in 200 mL water) and the mixture stirred vigorously at 23 °C for 1.5 h. The organic phase was separated, and the aqueous phase extracted with ethyl acetate (3 × 50 mL), the organic phases were combined, washed with brine (2 × 50 mL), dried ($MgSO_4$), filtered and evaporated under reduced pressure to yield the crude aldehyde **145** as a viscous oil (5.5 g). The aldehyde prepared was used immediately and without further purification.

Preparation of the Oxime **146**

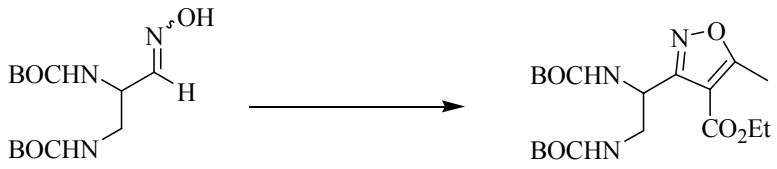
To hydroxylamine hydrochloride (2.75 g, 39.6 mmol) and sodium acetate (9.73 g, 118 mmol) in water (50 mL), was added the crude aldehyde **145** (5.5 g) in ethanol (10 mL). The reaction mixture was warmed to 70 °C for 15 minutes, cooled and the organic phase extracted with ethyl acetate (3 × 100 mL), the organic fractions were combined, washed with brine (50 mL), dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure to give the title compound **146** (5.21 g, 87%) as a colourless oil; ν_{max} (DCM)/cm^{-1} 3342, 2977, 2932, 2358, 1693, 1519, 1455, 1393, 1367 & 1251; δ_H (400 MHz; $[CD_3]_2SO$) (isomer one) (partially assigned by COSY and HMQC) 1.36 (18H, s, 2 × C[C*H*$_3$]$_3$) [or 1.36 and 1.37 (2 × 9H, C[C*H*$_3$]$_3$)], 3.14 (2H, m, CHC*H*$_2$), 4.15 (1H, m, CH_2C*H*), & 7.11 (1H, d *J* 6.4, CHC*H*N), (isomer two) 1.37 (18H, s, 2 × C[C*H*$_3$]$_3$), 3.38 (2H, m, CHC*H*$_2$), 4.62-4.75 (1H, m, CH_2C*H*), & 6.50 (1H, d *J* 6.2, CHC*H*N); δ_C (100 MHz; $CDCl_3$) 28.1 (CH_3), 40.4 (CH_2), 50.0 (CH), 148.2 (CH), 155.6 (C) & 175.7 (C); *m/z* (EI) 303.1786 (M^+, $C_{13}H_{25}N_3O_5$ requires 303.1794), 57 (100 %), 60 (100), 73(16), 85 (23), 101(22), 103 (32), 104 (42), 117 (60), 118 (70), 130 (14), 156 (2), 161 (27), 191 (19) & 301 (1) which is in accordance with data published in the literature.[45]

Ethyl 3-(pyrrolidin-1-yl)but-2-enoate 148



Ethyl acetoacetate (21.75 g, 167.1 mmol) and pyrrolidine (13.55 g, 190.5 mmol) were heated together in toluene (100 mL) at reflux under Dean-Stark conditions. After 24 h the mixture was cooled and the solvent was evaporated under reduced pressure to yield the title compound **148** (31.12 g) as a brown oil; δ_H (400 MHz; $CDCl_3$) 1.24 (3H, t, *J* 7.1, CH_2C*H*$_3$), 1.91-1.92 (4H, m, NCH_2C*H*$_2$C*H*$_2$), 2.45 (3H, s, C*H*$_3$), 3.21-3.27 (4H, m, C*H*$_2$NC*H*$_2$), 4.06 (2H, q, *J* 7.1, CH_3C*H*$_2$) & 4.44 (1H, s, COC*H*).

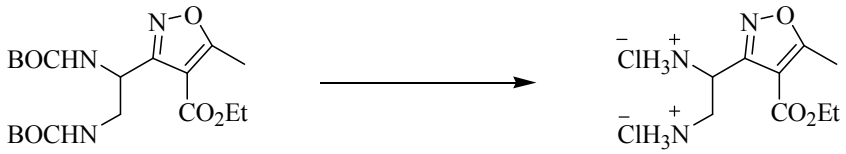
Ethyl 3-[1,2-bis(*tert*-butoxycarbonylamino)ethyl]-5-methylisoxazole-4-carboxylate 149



146 **149**

To a stirred solution of 2,3-bis(*tert*-butyloxycarbonylamino)propanaldehyde oxime **146** (5.66 g, 18.7 mmol) in freshly distilled DCM (150 mL) was added *N*-chlorosuccinimide (NCS) (2.70 g, 20.5 mmol) and the mixture was heated at reflux for 2 h. A second addition of NCS (2.70 g, 20.5 mmol) followed and the resulting mixture was heated at reflux overnight. The mixture (blue/green solution) was cooled to 23 ºC and a solution of ethyl 3-(pyrrolidin-1-yl)but-2-enoate **148** (11.91 g, 66.48 mmol) in DCM (10 mL) was added in one portion, followed by dropwise addition of triethylamine (3.20 g, 31.7 mmol) over 2 h. The mixture was heated at reflux overnight, cooled, washed with aqueous citric acid (100 mL), saturated aqueous sodium hydrogen carbonate (100 mL), and brine (50 mL), dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The crude oil was purified by column chromatography eluted with light petroleum : ethyl acetate (4:1, v/v) to give the title compound **149** (3.39 g, 44%) as a yellow oil; ν_{max} (DCM)/cm^{-1} 3372, 2976, 2359, 1715, 1606, 1507, 1455, 1391, 1366, 1304 and 1250; δ_H (400 MHz; $CDCl_3$) 1.30 (9H, s, C[C*H*$_3$]$_3$), 1.32 (3H, t, *J* 7.2, CH_2CH_3), 1.36 (9H, s, C[C*H*$_3$]$_3$), 2.59 (3H, s, 5-C*H*$_3$), 3.48 (2H, m, CHC*H*$_2$), 4.29 (2H, q, *J* 7.2, CH_3CH_2), 4.87 (1H, br, N*H*), 5.23 (1H, br, NHCH$_2$C*H*) & 5.85 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 13.5 (CH_3), 14.2 (CH_3), 28.2 (CH_3), 28.3 (CH_3), 43.4 (CH_2), 48.6 (CH), 60.8 (CH_2), 77.3 (C), 80.0 (C), 107.9 (C), 155.4 (C), 156.6 (C), 161.4 (C), 162.0 (C) & 175.9 (C); *m/z* (EI) 413.2173 (M^+, $C_{19}H_{31}N_3O_7$ requires 413.2162), 51 (100), 137 (39), 155 (50), 183 (79) & 228 (97).

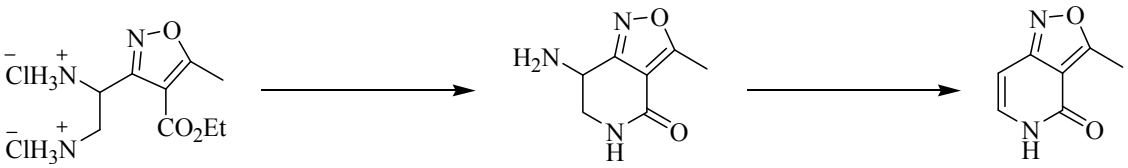
Ethyl 3-(1,2-diaminoethyl)-5-methylisoxazole-4-carboxylate dihydrochloride 150



149 **150**

Trifluoroacetic acid (10.5 mL, 136 mmol) was added to ethyl 3-[1,2-bis(*tert*-butoxycarbonylamino)ethyl]-5-methylisoxazole-4-carboxylate **149** (2.80 g, 6.78 mmol) and the mixture stirred for 4.5 h at 23 °C. The solution was then concentrated under reduced pressure. Aqueous hydrochloric acid (2M, 50 mL) was added and the mixture stirred for 30 minutes, after which time the solution was concentrated under reduced pressure, water added (40 mL) and washed with ethyl acetate (3 × 50 mL). The aqueous layer was concentrated under reduced pressure to give the title compound **150** (1.6 g, 85%) as a pale brown solid, which was used without further purification.

3-Methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 1



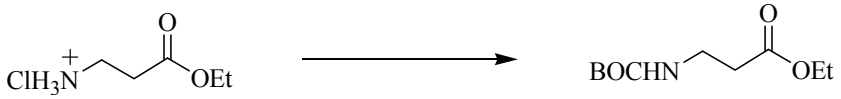
150 **151** **1**

Sodium carbonate was added to ethyl 3-(1,2-diaminoethyl)-5-methylisoxazole-4-carboxylate dihydrochloride **150** (1.20 g, 4.19 mmol) in water (50 mL) at 23 °C and stirred overnight. The aqueous layer was then extracted with ethyl acetate (3 × 100 mL). The organic fractions were combined, dried over ($MgSO_4$), filtered and the solvent removed under reduced pressure to yield amine **151** (0.53 g, 75%) as a white solid, which was used without further purification; δ_H(400 MHz; $CDCl_3$) 2.63 (3H, s, 3-C*H*$_3$), 3.36 (1H, ddd, *J* 12.4, 7.6 & 2.4 ,CHC*H*H), 3.71 (1H, ddd, *J* 12.4, 5.2 & 3.2, CHCH*H*), 4.27

(1H, dd, *J* 7.6 & 5.2, C*H*CH_2) & 5.82 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 12.3 (CH_3), 44.2 (CH_2), 48.8 (CH), 106.4 (C), 154.2 (C) ,164.3 (C) & 172.8 (C).

A solution of sodium nitrite (0.33 g, 4.8 mmol) in hydrochloric acid (2M, 30 mL) was added to the amine **151** (0.53 g) in water (10 mL) at 0 °C and stirred for 1 h, and at 50 °C for 1 h. The organic layers were then extracted with ethyl acetate (3 × 50 mL), combined, dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure to yield the title compound **1** (0.39 g, 80%) as a white solid; m.p. 205-207°C (lit.mp.,[45] 206 °C); ν_{max} (DCM)/cm^{-1} 3425, 3224, 2987, 1696, 1643, 1382 & 1324; δ_H (400 MHz; $CDCl_3$) 2.81 (3H, s, 3-C*H*$_3$), 6.32 (1H, d, *J* 7.6, NHCHC*H*), 6.89 (1H, dd, *J* 7.6 & 5.6, NH*CH*) & 8.92 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 13.1 (CH_3), 95.1 (CH), 108.2 (C), 132.8 (CH), 158.7 (C), 162.5 (C) & 171.2 (C); *m/z* (EI) 150.0427 (M^+, $C_7H_6N_2O_2$ requires 150.0429), 53 (9),1 80 (21), 108 (12) & 150 (100).

Ethyl 3-*tert*-butoxycarbonylaminopropanoate 153

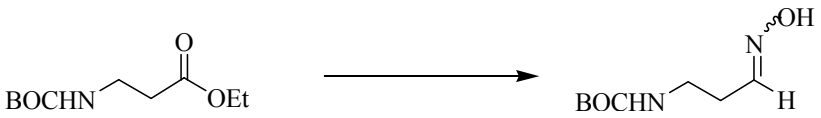


152 **153**

To a stirred suspension of β-alanine ethyl ester hydrochloride **152** (2.90 g, 18.9 mmol) in DCM (100 mL) was added di-*tert*-butyl dicarbonate (8.68 g, 39.7 mmol) in one portion, followed by triethylamine (7.66 g, 75.5 mmol) dropwise over 1 h at 0 °C, and the mixture was stirred for 18 h. The mixture was washed with aqueous citric acid (1M, 100 mL), saturated aqueous sodium hydrogen carbonate (2 × 50 mL), and brine (50 mL), dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give the title compound **153** (4.02 g, 99%) as a colourless oil; ν_{max} (DCM)/cm^{-1} 3385, 2979, 2936, 1808, 1716, 1510, 1459, 1392, 1370 & 1251; δ_H (400 MHz; $CDCl_3$) 1.32 (3H, t, *J* 7.1, CH_2CH_3), 1.37 (9H, s, C[CH_3]$_3$), 2.41 (2H, t, *J* 6.2, $NHCH_2CH_2$), 3.27 (2H, t, *J* 6.2,

NHCH_2CH$_2$), 4.01 (2H, q, *J* 7.1, CH$_3$CH_2) & 4.87 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 14.0 (CH_3), 27.0 (CH_3), 34.6 (CH_2), 36.0 (CH_2), 60.4 (CH_2), 146.6 (C), 155.7 (C) & 172.3 (C); *m/z* (FAB) 218.1389 ([M + H^+], $C_{10}H_{20}NO_4$ requires 218.1392), 57 (36), 116 (28), 118 (66), 162 (100), 218 & (36).

tert-Butyl (3-hydroxyiminopropyl)carbamate 154



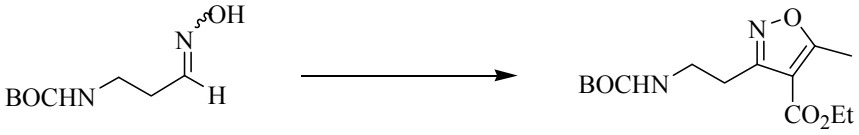
153 **154**

To a solution of ethyl 3-*tert*-butoxycarbonylaminopropanoate **153** (4.60 g, 21.2 mmol) in toluene (100 mL) was added DIBAL-H (1M in toluene, 54 mL, 47 mmol) dropwise over 1 h at -78 °C. After stirring the mixture at 23 °C for 1 h, methanol (20 mL) was added slowly, followed by addition of potassium sodium tartrate solution (100 g in 200 mL water) and the mixture stirred vigorously at 23 °C for 1.5 h. The organic phase was separated, and the aqueous phase extracted with ethyl acetate (3 × 100 mL). The organic phases were combined, washed with brine (100 mL), dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure to yield the corresponding aldehyde, which was used immediately and without further purification.

To hydroxylamine hydrochloride (2.90 g, 41.7 mmol) and sodium acetate (6.95 g, 84.7 mmol) in water (30 mL) was added the crude aldehyde in ethanol (5 mL). The reaction mixture was warmed to 70 °C for 15 minutes, cooled and the organic phase extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure to give the title compound **154** (3.30 g, 85%) as a colourless oil; ν_{max} (DCM)/cm^{-1} 3362, 2978, 2935, 2360, 1689, 1529, 1479 1392, 1278, & 1170; δ_H (400 MHz; $CDCl_3$) (isomer one) 1.38 (9H, s, C[CH_3]$_3$), 2.33-2.30 (2H, m, NHCH$_2$CH_2), 3.18-3.26 (2H, m,

NHCH_2CH$_2$) & 7.37 (1H, br, CH$_2$CHN), (isomer two) 1.38 (9H, s, C[CH_3]$_3$), 2.45-2.50 (2H, m, NHCH$_2$CH_2), 3.18-3.26 (2H, m, NHCH_2CH$_2$) & 7.72 (1H, br, CH$_2$CHN); δ_C (100 MHz; CDCl$_3$) 26.0 (CH$_2$), 28.3 (CH$_3$), 36.9 (CH$_2$), 149.3 (CH), 157.2 (C) & 175.9 (C); *m/z* (ESI) 189.1239 ([M + H$^+$], $C_8H_{16}N_2O_3$ requires 189.1239).

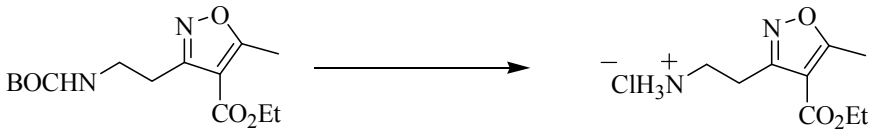
Ethyl 3-(2-*tert*-butoxycarbonylaminoethy)-5-methylisoxazole-4-carboxylate 155



154 **155**

To a stirred solution of *tert*-butyl(3-hydroxyiminopropyl)carbamate **154** (3.30 g, 17.6 mmol) in freshly distilled DCM (100 mL) was added NCS (2.61g, 19.5 mmol) and the mixture was heated at reflux for 2 h, followed by a second addition of NCS (2.61g, 19.5 mmol) and heated at reflux overnight. The reaction (blue/green solution) was allowed to cool 23 °C and ethyl 3-(pyrrolidin-1-yl)but-2-enoate **148** (6.28 g, 35.1 mmol) in DCM (10 mL) was added in one portion, followed by addition of triethylamine (1.95 g, 19.3 mmol) over 2 h. The mixture was heated at reflux overnight, cooled, washed with aqueous citric acid (100 mL), saturated aqueous sodium hydrogen carbonate (100 mL), brine (100 mL) and dried (MgSO$_4$). After filtration and removal of the solvent under reduced pressure, the crude oil was purified by column chromatography eluting with light petroleum : ethyl acetate (4:1, v/v) to give the title compound (3.45 g, 65%) as a colourless oil **155**; ν_{max} (DCM)/cm^{-1} 3414, 2978, 2347, 1709, 1685, 1511, 1451,1391, 1366 & 1250; δ_H (400 MHz; CDCl$_3$) 1.34 (3H, t, *J* 7.2, CH$_2$CH_3), 1.42 (9H, s, C[CH_3]$_3$), 2.66 (3H, s, 5-CH_3), 3.06 (2H, t, *J* 6.4, NHCH$_2$CH_2), 3.54 (2H, m, NHCH_2), 4.32 (2H, q, *J* 7.2, CH$_3$CH_2) & 5.00 (1H, br, NH); δ_C (100 MHz; CDCl$_3$) 13.3 (CH$_3$), 14.2 (CH$_3$), 27.1 (CH$_2$), 27.9 (CH$_3$), 37.9 (CH$_2$), 60.7 (CH$_2$) , 79.1 (C), 155.8 (C), 161.2 (C), 162.2 (C) & 171.1 (C) & 175.4 (C); *m/z* (ESI) 321.1419 ([M + Na$^+$], $C_{14}H_{22}N_2O_5Na$ requires 321.1421).

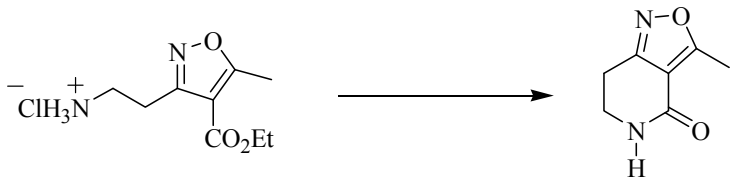
2-(4-Ethoxycarbonyl-5-methylisoxazole-3-yl)ethylammonium chloride 156



155 **156**

Trifluoroacetic acid (12.9 mL, 168 mmol) was added to ethyl 3-(2-*tert*-butoxycarbonylaminoethy)-5-methylisoxazole-4-carboxylate **155** (2.5 g, 8.4 mmol) and the solution was stirred for 4.5 h at 23 °C. The solution was then concentrated under reduced pressure. Hydrochloric acid (2M, 50 mL) was added and the mixture stirred for 30 minutes. After this time the solution was concentrated under reduced pressure, water was added (10 mL) and the solution washed with ethyl acetate (3 × 50 mL). The aqueous layer was concentrated under reduced pressure to give the title compound **156** (1.63 g, 99%) as a pale brown solid, which was used without further purification.

3-Methyl-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 157

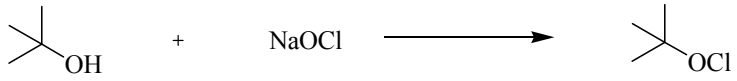


156 **157**

Anhydrous sodium carbonate (1.07 g, 10.2 mmol) was added to a solution of 2-(4-ethoxycarbonyl-5-methylisoxazole-3-yl)ethylammonium chloride **156** (1.61 g, 6.88 mmol) in water (50 mL) at 23°C and stirred overnight. The aqueous layer was then extracted with ethyl acetate (3 × 100 mL). The organic fractions were combined, dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give the title compound **157** as a white solid (0.82 g, 80%); m.p. 195-197 °C (lit.mp.,[99] 190 °C); ν_{max} (DCM)/cm^{-1} 3279, 3071, 1689, 1629, 1513, 1425, 1382, 1300, 1339, 1262 & 1142; δ_H

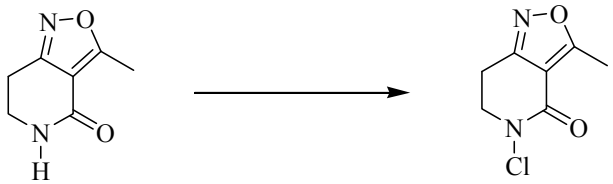
(400 MHz; $CDCl_3$) 2.62 (3H, s, *CH*$_3$), 2.94 (2H, t, *J* 6.4, NCH_2CH_2), 3.55 (2H, dt, *J* 2.8 & 6.4, NCH_2CH_2) & 6.29 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 12.3 (CH_3), 21.4 (CH_2), 40.7 (CH_2), 107.8 (C), 160.6 (C), 163.4 (C) & 172.1 (C); *m/z* (EI) 152.0584 (M^+, $C_7H_8N_2O_2$ requires 152.0586), 81 (28), 123 (30) & 152 (100).

tert-Butyl hypochlorite[50]



tert-Butanol (18 ml) and glacial acetic acid (12 ml) were added to commercial bleach (250 ml) at 5 °C in the absence of light. After stirring for 10 minutes, the organic phase was separated, washed with 10% aqueous sodium carbonate solution and water (50 ml) to leave the title compound as a yellow oil (9.6 g).

5-Chloro-3-methyl-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 158

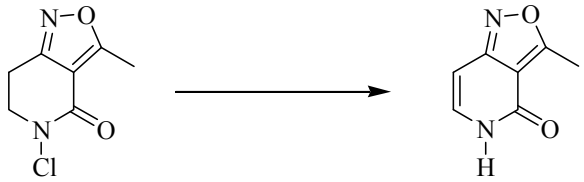


157 **158**

To a solution of 3-methyl-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **157** (1.22 g, 8.03 mmol) in freshly distilled methanol (50 mL) was added *tert*-butyl hypochlorite (1.30 g, 12.0 mmol) dropwise at 0 °C in the absence of light. The resulting mixture was stirred at 0 °C for 2 h and then at 23 °C for 2 h. The solvent was then removed under reduced pressure, water (30 mL) added and the organic phase extracted with ethyl acetate (3 × 150 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure to give the title

compound **158** as a yellow solid (1.21 g, 82%); m.p. 107-109 °C (lit.mp.,[99] 108 °C); ν_{max} (DCM)/cm^{-1} 2220, 1689, 1629, 1513, 1426, 1382, 1331, 1300, 1262, 1142 & 1088; δ_H (400 MHz; $CDCl_3$) 2.62 (3H, s, C*H*$_3$), 3.14 (2H, t, *J* 6.5, NCH_2CH_2) & 3.95 (2H, t, *J* 6.5, NCH_2CH_2); δ_C (100 MHz; $CDCl_3$) 12.4 (CH_3), 22.6 (CH_2), 55.4 (CH_2), 109.6 (C), 159.4 (C), 160.4 (C) & 173.4 (C); *m/z* (FAB) 187.0279 ([M + H^+], $C_7H_8N_2O_2{}^{35}Cl$ requires 187.0274).

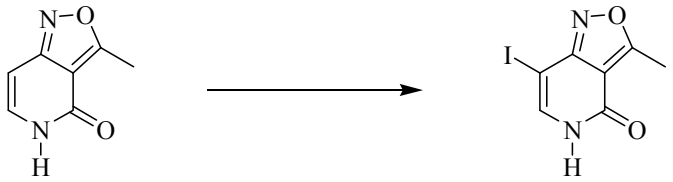
3-Methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 1



158 **1**

5-Chloro-3-methyl-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **158** (150 mg, 0.802 mmol) in freshly distilled methanol (200 mL) was degassed for 10 minutes. Under a constant flow of nitrogen the solution was stirred for 1 h under irradiation medium pressure mercury Hanovia lamp. Concentrating the solvent under reduced pressure and purification of the crude residue using column chromatography, eluting with light petroleum : ethyl acetate (1:1, v/v) yielded the title compound **1** as a white solid (96 mg, 80%), identical to a sample prepared from aminopyridone, see above.

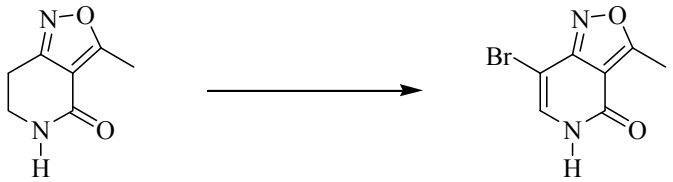
7-Iodo-3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 170



1 **170**

Iodine monochloride (1.0M in DCM, 5.0 mL, 5.0 mmol) was added to a solution of 3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **1** (0.50 g, 3.3 mmol) in DCM (10 mL) and the mixture was stirred for 72 h at 23 °C. The resulting purple precipitate was filtered and washed with DCM (10 mL) to yield the title compound **170** as a red/brown solid (554 mg, 60 %); m.p. 223-225 °C (lit.mp.,[45] 218 °C); ν_{max} (DCM)/cm^{-1} 2360, 1665, 1620, 1519 & 1330; δ_H (400 MHz; $[CD_3]_2SO$) 2.82 (3H, s, 3-C*H*$_3$), 7.50 (1H, d, *J* 5.0, NHC*H*) & 11.12 (1H, br, s, N*H)*; δ_C (100 MHz; $[CD_3]_2SO$) 12.9 (CH_3), 51.6 (C), 107.4 (C), 140.4 (CH), 158.9 (C), 159.0 (C) & 175.2 (C); *m/z* (EI) 275.9394 (M^+, $C_7H_5N_2O_2{}^{127}I$ requires 275.9398), 91 (17), 108 (12), 234 (11), 276 (100)& 277 (15).

7-Bromo-3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 167

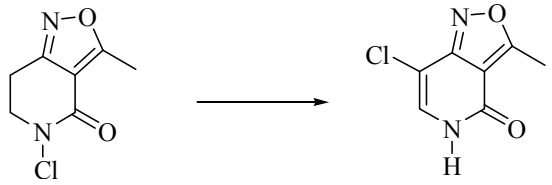


157 **167**

To a solution of degassed *tert*-butanol (10 mL) were added 3-methyl-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **157** (100 mg, 0.658 mmol) and recrystallised *N*-bromosuccinimide (0.14 g, 0.789 mmol) under a nitrogen atmosphere. AIBN (9 mg, 0.05 mmol) was added portionwise every hour for 5 h, and heated at reflux overnight. The solvent was removed under reduced pressure, and to the residue water (20 mL) was

added, and the mixture was extracted with DCM (4 × 40 mL). The combined organic extracts were dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. Purification of the crude residue using column chromatography, eluting with light petroleum : ethyl acetate (1:1, v/v) yielded the title compound **167** as a off white solid (35 mg, 35%); m.p. 268-269 °C; ν_{max} (DCM)/cm^{-1} 3436, 1774, 1665, 1427, 1370, 1296 & 1188 ; δ_H (400 MHz; $[CD_3]_2SO$) 2.78 (3H, s, 3-C*H*$_3$), 7.48 (1H, s, NHC*H*) & 11.32 (1H, br, s, N*H)*; δ_C (100 MHz; $[CD_3]_2SO$) 12.7 (CH_3), 104.5 (C), 107.4 (C), 136.7 (CH), 156.9 (C), 159.6 (C) & 174.2 (C); *m/z* (EI) 227.9534 (M^+, $C_7H_5N_2O_2{}^{79}Br$ requires 227.9534), 57(68), 73 (100), 147 (40) & 252 (70).

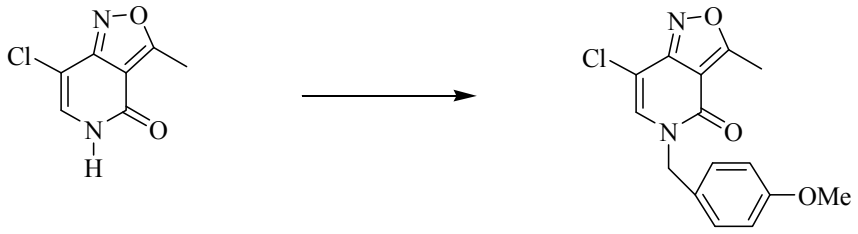
7-Chloro-3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 162



158 **162**

A solution of 5-chloro-3-methyl-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **158** (2.21, 11.9 mmol) in freshly distilled methanol (50 mL), under a constant flow of nitrogen, the reaction was stirred at 23 °C for 5 h while irradiated by a tungsten halogen UV lamp. The solvent was concentrated under reduced pressure and the residue was purified by column chromatography, eluting with light petroleum : ethyl acetate (1:1, v/v) to yield the title compound **162** as a white solid (0.88 g, 40%); m.p. 230-232 °C; ν_{max} (DCM)/cm^{-1} 2898, 1672, 1632, 1519 & 1342; δ_H (400 MHz; $[CD_3]_2SO$) 2.71 (3H, s, *CH*$_3$) & 7.38 (1H, s, *CH*); δ_C (100 MHz; $[CD_3]_2SO$) 12.6 (CH_3), 98.1 (C), 107.9 (C), 133.8 (CH), 156.4 (C), 158.8 (C) & 175.2 (C); *m/z* (FAB) 185.0114 ([M + H^+], $C_7H_6N_2O_2{}^{35}Cl$ requires 185.0117).

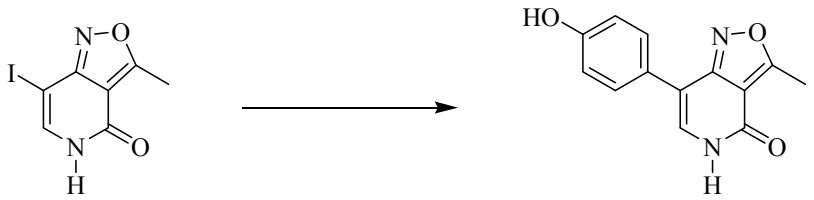
7-Chloro-5-(4-methoxybenzyl)-3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 177



162 **177**

To a solution of 7-chloro-3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **162** (200 mg, 1.09 mmol) in acetonitrile (20 mL) was added 4-methoxybenzyl bromide (260 mg, 1.30 mmol) and potassium carbonate (225 mg, 1.63 mmol) and the mixture stirred for 5 h at 50 °C. Water (10 mL) was added to the reaction mixture and extracted with ethyl acetate (4 × 30 mL), the organic layers were combined, washed with brine (50 mL), dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (1:1, v/v) to leave the title compound **177** as a white solid (271 mg, 82 %); m.p. 155-157 °C; ν_{max} (DCM)/cm^{-1} 3053, 1669, 1633, 1588, 1513, 1432 & 1362; δ_H (400 MHz; $CDCl_3$) 2.88 (3H, s, C*H*$_3$), 3.79 (3H, s, OC*H*$_3$), 4.94 (2H, s, NC*H*$_2$), 6.89 (2H, d, *J* 8.4, Ar-C*H*), 7.06 (1H, s, NC*H*) & 7.31 (2H, d, *J* 8.4, Ar-C*H*); δ_C (100 MHz; $CDCl_3$) 12.9 (CH_3), 49.7 (CH_2), 55.2 (CH_3), 101.3 (C), 108.2 (C), 114.3 (CH), 129.3 (C), 129.7 (CH), 134.8 (CH), 156.2 (C), 158.3 (C), 159.5 (C) & 175.6 (C); *m/z* (FAB) 305.0688 ([M + H^+] $C_{15}H_{14}N_2O_3{}^{35}Cl$ requires 305.0690).

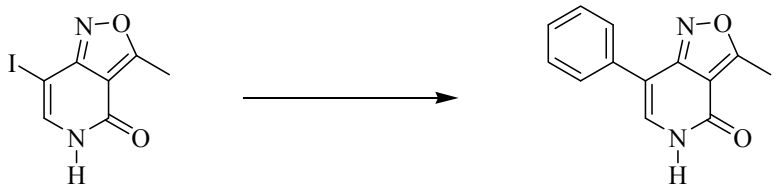
7-(4-Hydroxyphenyl)-3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 172



170 **172**

Tetrakis(triphenylphosphine)palladium (12 mg, 0.014 mmol) was added to degassed 1,4-dioxane (10 mL), followed by addition of 7-iodo-3-methyl-5*H*-isoxozalo[4,3-*c*]pyridin-4-one **170** (100 mg, 0.36 mmol) and the solution was purged with nitrogen for ten minutes at 23 °C. Two separate solutions of 4-hydroxyphenylboronic acid (75 mg, 0.54 mmol) in ethanol (10 mL) and aqueous sodium carbonate (2M, 0.36 mL, 0.72 mmol)) were degassed with nitrogen, and added to the reaction sequentially. The resulting mixture was heated at reflux for 18 h under a nitrogen atmosphere, cooled, water (50 mL) added and the mixture extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure to give a brown oil. This was purified using column chromatography with ethyl acetate as eluent to leave the title compound **172** as a white solid (70 mg, 80%); m.p. 268-269°C (lit.mp.,[45] decomposes 250 °C); ν_{max} (DCM)/cm^{-1} 3425, 2359, 1642, 1436, 1177 & 1118; δ_H (400 MHz; $[CD_3]_2SO$) 2.87 (3H, s, 3-C*H*$_3$), 6.86 (2H, d, *J* 8.4, Ar-C*H*), 7.29 (1H, d, *J* 6.0, NHC*H*), 7.60 (2H, d, *J* 8.4, Ar-C*H*) & 11.05 (1H, br, N*H*); δ_C (100 MHz; $[CD_3]_2SO$) 12.4 (CH_3), 107.1 (C), 108.1 (C), 115.3 (CH), 124.2 (C), 128.7 (CH), 131.5 (CH), 156.7 (C), 157.6 (C), 159.0 (C) & 173.7 (C); *m/z* (EI) 242.0694 (M^+, $C_{13}H_{10}N_2O_3$ requires 242.0691), 51 (16), 77 (10), 118 (20). 133 (18), 171 (33), 227 (46), 242 (100) & 243 (32).

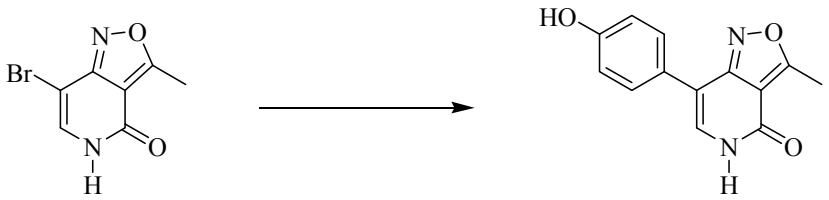
3-Methyl-7-phenyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 174



170 **174**

The same procedure was applied as for 7-(4-hydroxyphenyl)-3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **172**, using the following quantities: tetrakis(triphenylphosphine)palladium (4.2 mg, 3.6 $\times 10^{-3}$ mmol), 1.4-dioxane (10 mL), 7-iodo-3-methyl-5*H*-isoxozolo[4,3-*c*]pyridin-4-one **170** (20 mg, 0.072 mmol), phenylboronic acid (13 mg, 0.11 mmol) in ethanol (10 mL) and aqueous sodium carbonate (2M, 0.02 mL, 0.14 mmol). The residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (1:1, v/v) to leave the title compound **174** as a white solid (10 mg, 67 %); m.p. 253-254 °C; ν_{max} (DCM)/cm^{-1} 3245, 2359, 1676, 1629 & 1404; δ_H (400 MHz; $CDCl_3$) 2.86 (3H, s, 3-C*H*$_3$), 7.33 (1H, d, *J* 6.0, NHC*H*), 7.36 (1H, t, *J* 7.2, Ar-C*H*), 7.43 (2H, m, Ar-C*H*), 7.75 (2H, d, *J* 7.2, Ar-C*H*) & 8.72 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 13.0 (CH_3), 108.6 (C), 112.5 (C), 127.3 (CH), 128.0 (CH), 128.8 (CH), 129.1 (CH), 133.2 (C), 158.8 (C), 161.7 (C) & 174.2 (C); *m/z* (FAB) 227.0825 ([M + H^+], $C_{13}H_{11}N_2O_2$ requires 227.0822).

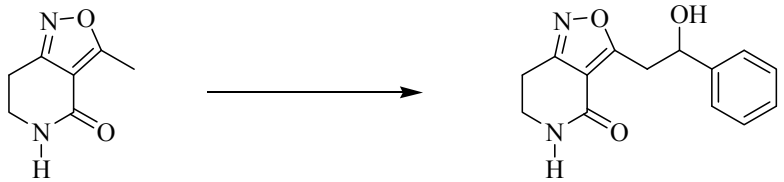
7-(4-Hydroxyphenyl)-3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 172



167 **172**

The same procedure was applied as for 7-(4-hydroxyphenyl)-3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **172**, using the following quantities: tetrakis(triphenylphosphine)palladium (11 mg, 0.001 mmol), 1,4-dioxane (10 mL), 7-bromo-3-methyl-5*H*-isoxozolo[4,3-*c*]pyridin-4-one **167** (73 mg, 0.32 mmol), 4-hydroxyphenylboronic acid (65 mg, 0.48 mmol) in ethanol and aqueous sodium carbonate (2M, 0.32 mL, 0.64 mmol) to afford a brown oil. This was purified using column chromatography, eluting with ethyl acetate : light petroleum (1:1, v/v) to leave the title compound **172** as a white solid (25 mg, 36%); m.p. 268-269 °C; identical to a sample prepared from the 7-iodoisoxazolopyrdone (see above).

3-(2-Hydroxy-2-phenylethyl)-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 182

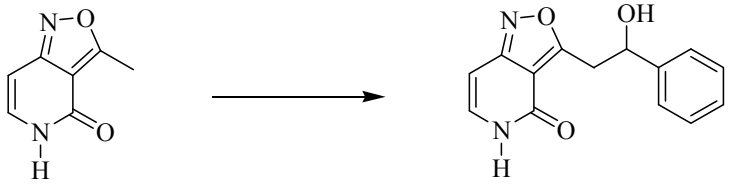


157 **182**

To a solution of *n*-BuLi (0.6 mL of a 2.5M solution in hexanes, 1.5 mmol) in THF at -78 ºC was added 3-methyl-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **157** (100 mg, 0.65 mmol). The resulting solution was stirred for 1 h at -78 ºC followed by addition of benzaldehyde (1.0 mL, 1.0 mmol), and stirred for a further 10 minutes. The reaction was quenched with water (20 mL) and acidified with 2M hydrochloric acid (20 mL) at -78 ºC.

The product was extracted with ethyl acetate (3 × 30 mL), the organic layers were combined, washed with brine (50 mL), dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (1:1, v/v) to leave the title compound **182** (0.12 g, 70%) as a white solid; m.p. 174-175 °C; ν_{max} (DCM)/cm^{-1} 3475, 3215, 3050, 1672, 1620 1479, 1451, 1341, 1134 & 1052; δ_H (400 MHz; $CDCl_3$) 3.05 (2H, t, *J* 6.4, $NHCH_2CH_2$), 3.50 (2H, m, $CHCH_2$), 3.62 (2H, td, *J* 2.8 & 6.4 $NHCH_2CH_2$), 5.14-5.18 (1H, m, CH_2CH), 6.05 (1H, br, NH), 7.29 (1H, t, *J* 6.4, Ar-CH), 7.36 (2H, t, *J* 6.4, Ar-CH) & 7.41 (2H, d, *J* 6.4, Ar-CH); δ_C (100 MHz; $CDCl_3$) 21.2 (CH_2), 37.3 (CH_2), 40.6 (CH_2), 72.2 (CH), 110.0 (C), 125.5 (CH), 127.8 (CH), 128.5 (CH), 143.7 (C), 160.4 (C), 163.8 (C) & 172.5 (C). *m/z* (EI) 258.1001 (M^+, $C_{14}H_{14}N_2O_3$ requires 258.0999).

3-(2-Hydroxy-2-phenylethyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 183

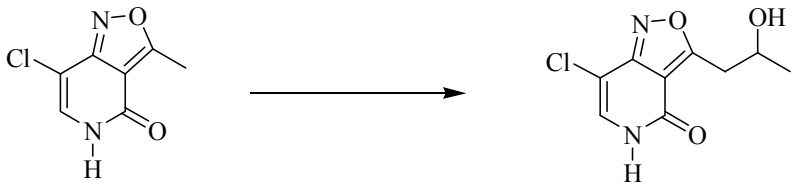


1 **183**

To a solution of 3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **1** (50 mg, 0.33 mmol) in THF (20 mL) at -78 ºC was added *n*-BuLi (0.67 mL of a 2.5M solution in hexanes, 1.7 mmol) The resulting solution was stirred for 2 h before benzaldehyde (0.68 mL, 6.67 mmol) was added, and stirred for 2 h at -78 ºC. The mixture was quenched with water (20 mL) and acidified with 2M hydrochloric acid (20 mL) at -78 °C. The mixture was extracted with ethyl acetate (3 × 40 mL), the organic layers were combined, washed with brine (50 mL), dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure to a yellow oil. The residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (1:1, v/v) to leave the title compound **183** (43 mg, 50%) as a white solid; m.p. 182-184 °C (lit.m.p.,[45] 181 °C); ν_{max} (DCM)/cm^{-1} 3395, 3131,

2962, 1675, 1643, 1457, 1217 & 1049; δ_H (400 MHz; $[CD_3]_2SO$) 3.55 (2H, m, CHC*H*$_2$), 5.19 (1H, q, *J* 7.7, CH_2C*H*), 5.74 (1H, d, *J* 4.9, CH*OH*), 6.38 (1H, d, *J* 7.6, NHCHC*H*), 7.15 (1H, dd, *J* 7.6 & 5.7, NHC*H*), 7.39-7.46 (5H, m, Ar-C*H*) & 10.15 (1H, br, N*H*); δ_C (100 MHz; $[CD_3]_2SO$) 36.9 (CH_2), 70.4 (CH), 92.6 (CH), 108.5 (C), 125.6 (CH), 127.3 (CH), 128.2 (CH), 134.1 (CH), 144.4 (C), 157.3 (C), 158.9 (C) & 174.8 (C); *m/z* (ESI) 257.0924 ([M + H^+], $C_{14}H_{13}N_2O_3$ requires 257.0921), 150 (100), 77 (94), 51 (70) & 43 (62)

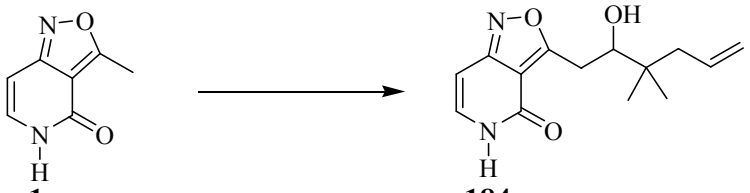
7-Chloro-3-(2-hydroxypropyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 185



162 **185**

The same procedure was applied as for 3-(2-hydroxy-2-phenylethyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **183** using the following quantities: 7-chloro-3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **162** (100 mg, 0.54 mmol), *n*-BuLi (2.1 mL of a 2.5M solution in hexanes, 5.4 mmol) and ethanal (0.61 mL, 10.9 mmol) to yield the title compound **185** as a white solid (92 mg, 80 %); ν_{max} (DCM)/cm^{-1} 3420, 2875 & 1653; δ_H (400 MHz; $CDCl_3$) 1.32 (3H, d, *J* 6.0, C*H*$_3$), 3.41 (2H, m, CHC*H*$_2$), 4.35 (1H, m, CH_2C*H*), 7.03 (1H, d, *J* 4.8, NHC*H*) & 8.60 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 23.3 (CH_3), 37.0 (CH_2), 66.3 (CH), 102.6 (C), 108.9 (C), 130.3 (CH), 156.6 (C), 159.0 (C) & 176.6 (C); *m/z* (ESI) 229.0376 ([M+H^+], $C_9H_{10}N_2O_3{}^{35}Cl$ requires 229.0374)

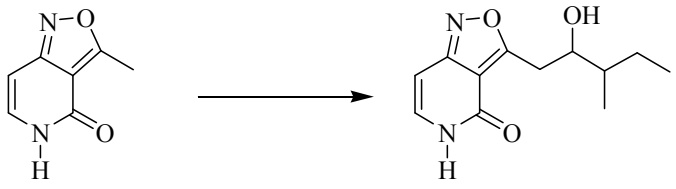
3-(2-Hydroxy-3,3-dimethylhex-5-enyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 184



1 **184**

The same procedure was applied as for 3-(2-hydroxy-2-phenylethyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **183** using the following quantities: 3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **1** (44 mg, 0.29 mmol), *n*-BuLi (1.2 mL of a 2.5M solution in hexanes, 2.9 mmol) and 2,2-dimethylpent-4-enal (0.8 mL, 6 mmol) to yield the title compound **184** as a white solid (48 mg, 63 %); m.p. 99-101 °C; ν_{max} (DCM)/cm^{-1} 3415, 3201, 2959, 1677, 1643, 1467, 1219 & 1059; δ_H (400 MHz; $CDCl_3$) 1.10 (6H, s, 2×C*H*$_3$), 2.09 and 2.25 (each 1H, dd, *J* 13.6 & 7.6, CH_2=CHC*H*$_2$), 3.41 (2H, m, CH(OH)C*H*$_2$), 3.81 (1H, dd, *J* 10 & 2.4, CH_2C*H*(OH)), 5.08-5.13 (2H, m, CH=C*H*$_2$), 5.89 (1H, m, CH_2=C*H*), 6.54 (1H, d, *J* 7.6, NHCHC*H*), 6.97 (1H, dd, *J* 7.6 & 5.6, NHC*H*) & 8.94 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 22.2 (CH_3), 22.9 (CH_3), 30.8 (CH_2), 38.4 (C), 43.5 (CH_2), 77.3 (CH), 95.7 (CH), 109.3 (C), 117.8 (CH_2), 132.5 (CH), 134.8 (CH), 159.2 (C), 161.5 (C) & 176.7 (C); *m/z* (FAB) 263.1388 ([M + H$^+$], $C_{14}H_{19}N_2O_3$ requires 263.1396) 57 (100), 219 (24), 245 (12.3) & 285 (10).

3-(2-Hydroxy-3-methylpentyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 186

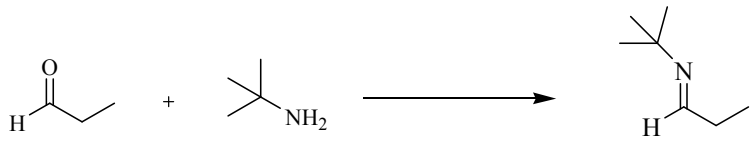


1 **186**

The same procedure was applied as for 3-(2-hydroxy-2-phenylethyl)-5*H*-isoxazolo[4,3-

c]pyridin-4-one **183** using the following quantities: 3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **1** (50 mg, 0.33 mmol) , *n*-BuLi (3.1 mL of a 1.6M solution in hexanes, 5.0 mmol) and 2-methylbutanal (0.54 mL, 5.0 mmol) to leave the title compound **186** in trace amounts. δ_H (400 MHz; $CDCl_3$) 0.86-0.98 (6H, m, 2 × (CH_3)), 1.51 (2H, m, CH_3CH_2), 2.33-2.41 (1H, m, CH_3CH), 3.19-3.28 (2H, m, $CH(OH)CH_2$), 3.92 (1H, br, *OH*), 4.56 (1H, m, $CH_2C(OH)H$), 5.91 (1H, d, *J* 7.6, NHCHC*H*), 6.98 (1H, m, NHC*H*) & 10.52 (1H, br, *NH*). No further analysis to date.

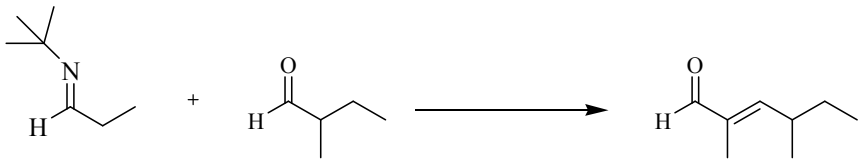
tert-Butylpropylideneamine 189[25]



187 **188** **189**

Propanal **187** (16.0 mL, 125 mmol) was added dropwise over 2 h to *tert*-butylamine **188** (13.1 mL, 125 mmol) and stirred at 0 °C. After the addition was complete, potassium hydroxide pellets (5 g) were added and the resulting solution was allowed to separate over 12 h at 0 °C. The yellow upper layer was decanted and distilled over potassium hydroxide pellets to yield the *tert*-butylpropylideneamine **189** (9.3 g, 65%) as a colourless oil, which was stored over molecular sieves at 0 °C; bp 103-105 °C (lit.bp.,[25] 101-103 °C); ν_{max} (DCM)/cm^{-1} 3393, 2965, 2873, 1725, 1644, 1627, 1460, 1359, 1306, 1210 & 1048**;** δ_H (400 MHz; $CDCl_3$) 1.05 (3H, t, *J* 7.6, CH_2CH_3), 1.15 (9H, s, $C[CH_3]_3$), 2.22 (2H, qd, *J* 7.6 & 5.2 CH_3CH_2) & 7.57 (1H, t, *J* 5.2, N=C*H*); δ_C (100 MHz; $CDCl_3$) 10.7 (CH_3), 29.7 (CH_3), 29.7 (CH_2), 56.3 (C) & 160.1 (CH).

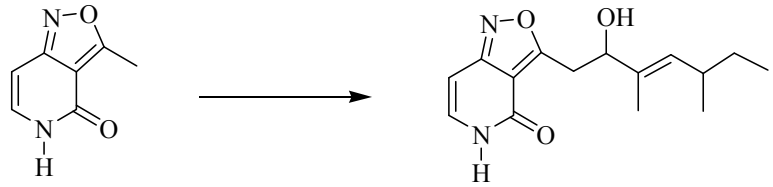
_E_-2,4-Dimethylhex-2-enal 191[25]



189 **190** **191**

To freshly distilled *tert*-butylpropylideneamine **189** (9.3 g, 82 mmol) was added *n*-BuLi (36.2 mL of 2.5M solution in hexanes, 905 mmol) dropwise over 10 minutes in THF (25 mL) at -78 °C. The solution was stirred for 30 minutes before 2-methylbutanal **190** (9.8 mL, 905 mmol) was added dropwise over 5 minutes. The solution was allowed to warm to 0 °C and stirred for a further 2 h. After this point aqueous citric acid (2M, 100 mL) was added and the resulting biphasic mixture was stirred vigorously for a period of 24 h at 23 °C. The mixture was extracted with DCM (3 × 75 mL), and the organic layers were combined, dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure to leave a colourless oil, which was purified by column chromatography using DCM as eluent to yield the title compound **191** as a colourless oil (4.5 g, 45 %); lit.bp.,[25] 63-65 °C (17 mmHg); ν_{max} (DCM)/cm^{-1} 2961, 2928, 2865, 1717, 1638, 1462, 1379, 1215 & 1148; δ_H (400 MHz; $CDCl_3$) 0.79 (3H, t, *J* 7.6, CH_2CH_3), 0.99 (3H, d, *J* 6.7, $CHCH_3$), 1.17-1.19 (2H, m, CH_3CH_2), 1.69 (3H, s, CH_3), 2.49-2.51 (1H, m, CH_2CH), 6.18 (1H, d, *J* 10.0, $CH_3C=CH$) & 9.32 (1H, s, *CHO*). No further characterization as the aldehyde does not store well at -20 ºC and was chromatographed prior to use.

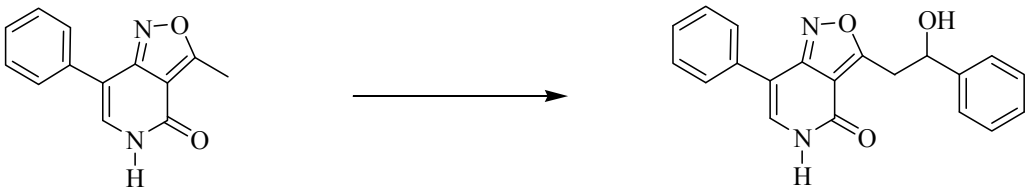
3-(2-Hydroxy-3,5-dimethylhept-3-enyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 192



1 **192**

The same procedure was applied as for 3-(2-hydroxy-2-phenylethyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **183** using the following quantities: 3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **1** (30 mg, 0.20 mmol), THF (20 mL), *n*-BuLi (0.80 mL of a 2.5M solution in hexanes, 2.0 mmol) and *E*-2,4-dimethylhex-2-enal **191** (0.50 g, 4.0 mmol) to leave the title compound **192** as a yellow solid (35 mg, 64 %); m.p. 111-113 °C (lit.m.p.,[45] 110-115 °C); ν_{max} (DCM)/cm^{-1} 3332, 3037, 2958, 2872, 1675, 1653, 1587, 1457, 1217 & 1059; δ_H (400 MHz; $CDCl_3$) 0.69-0.76 (3H, m, CH_2CH_3), 0.82-0.85 (3H, m, $CHCH_3$), 1.02-1.21 (2H, m, CH_3CH_2), 1.71 (3H, s, CH_3), 2.18-2.26 (1H, m, CH_3CH), 3.48-3.60 (2H, m, $CH(OH)CH_2$), 3.82 (1H, br, *OH*), 4.56 (1H, dd, *J* 5.7 & 11.9, CH_2CH), 5.16 (1H, m, C=C*H*), 6.39 (1H, d, *J* 7.6, NHCHC*H*), 6.98 (1H, m, NHC*H*) & 10.13 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 11.4 (CH_3), 12.1 (CH_3), 20.4 (CH_3), 30.0 (CH_2), 33.3 (CH), 34.3 (CH_2), 75.2 (CH), 95.5 (CH), 109.3 (C), 132.9 (CH), 133.3 (CH), 135.3 (C), 158.4 (C), 161.6 (C) & 174.9 (C); *m/z* (ESI) 294.1815 ([M + NH_4^+] $C_{15}H_{24}N_3O_3$ requires 294.1812) 165 (100).

3-(2-Hydroxy-2-phenyl-ethyl)-7-phenyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 195

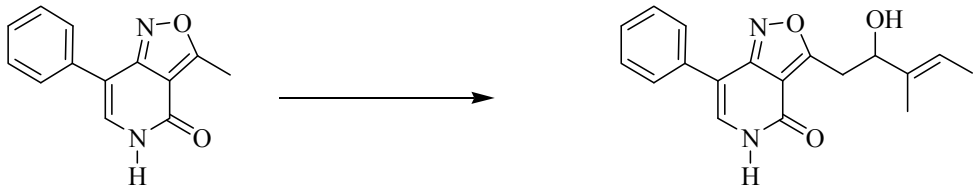


174 **195**

The same procedure was applied as for 3-(2-hydroxy-2-phenylethyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **183** using the following quantities: 3-methyl-7-phenyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **174** (75 mg, 0.28 mmol), *n*-BuLi (0.56 mL of a 2.5M solution in hexanes, 1.4 mmol) and benzaldehyde (0.61 g, 3.3 mmol) to yield the title compound **195** as a white solid (76 mg, 81 %); m.p. 229-233 °C; ν_{max} (DCM)/cm^{-1} 3424, 2358, 1681, 1641, 1579 & 1445; δ_H (400 MHz; $[CD_3]_2SO$) 3.56 (2H, d, *J* 7.7, $CH(OH)CH_2$), 5.19 (1H, m, CH_2CH), 5.85 (1H, m, CH*OH*), 7.26-7.80 (8H, m, Ar-C*H*), 7.26-7.80 (1H, m,

NHC*H*) & 7.82 (2H, m, Ar-C*H*) ; δ_C (100 MHz; $[CD_3]_2SO$) 36.9 (CH_2), 70.5 (CH), 107.9 (C), 108.5 (C), 125.6 (Ar-CH), 126.8 (Ar-CH), 127.2 (Ar-CH), 127.3 (Ar-CH), 128.2 (Ar-CH), 128.6 (Ar-CH), 132.2 (CH), 133.6 (C), 144.4 (C), 161.0 (C), 161.8 (C) & 170.6 (C); *m/z* (ESI) 333.1238 ([M + H^+], $C_{20}H_{17}N_2O_3$ requires 333.1234).

(2-Hydroxy-3-methylpent-3-enyl)-7-(4-hydroxyphenyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 196

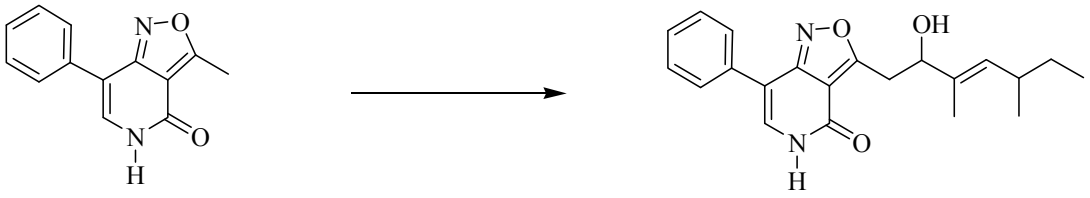


174 **196**

The same procedure was applied as for 3-(2-hydroxy-2-phenylethyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **183** using the following quantities: 3-methyl-7-phenyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **174** (50 mg, 0.19 mmol), *n*-BuLi (0.75 ml of a 2.5M solution in hexanes, 1.9 mmol) and 2-methylbut-2-enal (0.36 mL, 3.8 mmol) to yield the title compound **196** as a white solid (40 mg, 68 %); m.p. 125-128 °C; ν_{max} (DCM)/cm^{-1} 3480, 2962, 1673, 1470 & 1364; δ_H (400 MHz; $CDCl_3$) 1.62 (3H, d, *J* 6.8, CHC*H*$_3$), 1.75 (3H, s, C*H*$_3$), 3.58 (2H, m, CH(OH)C*H*$_2$), 4.59 (1H, m, CH_2(OH)C*H*), 5.61 (1H, q, *J* 6.8, C=C*H*), 7.16 (1H, d, *J* 5.6, NHC*H*), 7.39 (1H, t, *J* 7.2, Ar-C*H*), 7.47 (2H, t, *J* 7.2, Ar-C*H*), 7.76 (2H, d, *J* 7.2, Ar-C*H*) & 8.65 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 11.5 (CH_3), 12.2 (CH_3), 34.2 (CH_2), 75.5 (CH), 111.0 (C), 121.3 (CH), 124.0 (C) 127.4 (Ar-CH), 128.2 (Ar-CH), 128.9 (Ar-CH), 129.2 (CH), 133.5 (C), 136.0 (C), 156.0 (C), 161.1 (C) & 176.3 (C); *m/z* (ESI) 311.1394 ([M+H^+], $C_{18}H_{19}N_2O_3$ requires 311.1390)

3-(2-Hydroxy-3,5-dimethylhept-3-enyl)-7-phenyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one

197

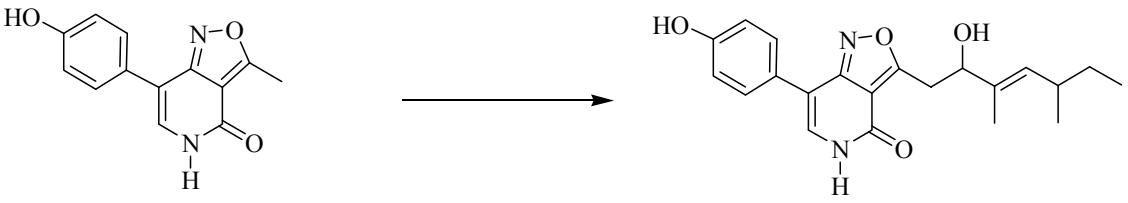


174 **197**

The same procedure was applied as for 3-(2-hydroxy-2-phenylethyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **183** using the following quantities: 3-methyl-7-phenyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **174** (100 mg, 0.44 mmol), *n*-BuLi (1.5 ml of a 2.5M solution in hexanes, 3.8 mmol) and *E*-2,4-dimethylhex-2-enal **191** (0.95 g, 7.5 mmol) to yield the title compound **197** as a white solid (70 mg, 54 %); m.p. 143-145 °C; ν_{max} (DCM)/cm^{-1} 3432, 3230, 2955, 2358, 1665, 1650 & 1371; δ_H (400 MHz; $CDCl_3$) 0.63-0.70 (3H, m, CH_2CH_3), 0.74-0.79 (3H, m, $CHCH_3$), 1.05-1.25 (2H, m, CH_3CH_2), 1.66 (3H, s, CH_3), 2.11-2.20 (1H, m, CH_3CH), 3.47-3.57 (2H, m, $CH(OH)CH_2$), 4.56 (1H, m, $CH_2(OH)CH$), 5.14 (1H, m, C=C*H*), 7.10 (1H, d, *J* 5.6, NHC*H*), 7.30 (1H, t, *J* 7.2, Ar-C*H*), 7.38 (2H, t, *J* 7.2, Ar-C*H*). 7.66 (2H, t, *J* 7.2, Ar-C*H*) & 9.67 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 10.8 (CH_3), 11.2 (CH_3), 19.4 (CH_3), 29.0 (CH_2), 32.5 (CH), 33.2 (CH_2), 74.5 (CH), 108.2 (C), 109.7 (C), 126.3 (Ar-CH), 126.9 (Ar-CH), 127.8 (Ar-CH), 128.4 (CH), 132.6 (CH), 132.9 (C), 133.0 (C), 156.6 (C), 160.1 (C) & 174.3 (C); *m/z* (ESI) 353.1861 ([M+H$^+$], $C_{21}H_{25}N_2O_3$ requires 353.1860)

3-(2-Hydroxy-3,5-dimethylhept-3-enyl)-7-phenyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one

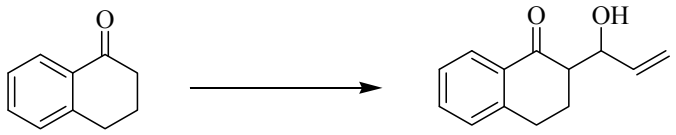
198



172 **198**

The same procedure was applied as for 3-(2-hydroxy-2-phenylethyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **183** using the following quantities: 7-(4-hydroxyphenyl)-3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **172** (50 mg, 0.21 mmol), *n*-BuLi (0.83 ml of a 2.5M solution in hexanes, 2.1 mmol) and *E*-2,4-dimethylhex-2-enal **191** (0.52 g, 4.1 mmol) to yield the title compound **198** as a white solid (7.6 mg, 10 %); m.p. not determined; ν_{max} (DCM)/cm^{-1} 3411, 28522, 1679, 1650 & 1467; δ_H (400 MHz; $CDCl_3$) 0.74 (3H, m, CH_2CH_3), 0.86 (3H, m, $CHCH_3$), 1.26 (2H, m, CH_3CH_2), 1.72 (3H, s, CH_3), 2.22 (1H, m, CH_3CH), 3.50 (2H, m, $CH(OH)CH_2$), 4.58 (1H, m, $CH_2(OH)CH$), 5.24 (1H, m, C=CH), 6.91 (2H, d, *J* 8.4, Ar-CH), 7.04 (1H, d, *J* 5.6, NHCH), 7.58 (2H, d, *J* 8.4, Ar-CH) & 8.59 (1H, br, NH); δ_C (100 MHz; $CDCl_3$) 11.8 (CH_3), 11.9 (CH_3), 29.5 (CH_3), 29.7 (CH_2), 30.1 (CH), 34.3 (CH_2), 76.7 (CH), 109.1 (C), 109.9 (C), 125.8 (Ar-CH), 128.2 (CH), 128.7 (Ar-CH) 133.0 (CH), 134.1 (C), 134.9 (C), 156.1 (C), 161.5 (C), 176.4 (C) & 207.1 (C); *m/z* (ESI) 369.1809 ([M+H^+], $C_{21}H_{25}N_2O_4$ requires 369.1811)

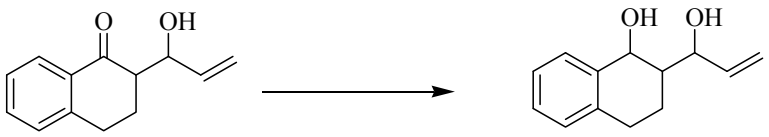
2-(1-Hydroxyprop-2-enyl)-3,4-dihydro-2*H*-naphthalen-1-one 208[71, 72]



207 **208**

To a solution of diisopropylamine (4.6 mL, 33 mmol) in THF (50 mL) at -78 °C was added *n*-BuLi (13.2 mL of a 2.5M solution in hexanes, 33.1 mmol). The resulting solution was stirred for 1 h at this temperature before α-tetralone **207** (3.9 mL, 30 mmol) was added dropwise and stirring continued for a further 1 h. Freshly distilled propenal (2.2 mL, 33 mmol) was added to the resulting solution dropwise over 15 mins at -78 °C and stirred for a further 30 mins. The mixture was then quenched with water (10 mL) at -78 °C and neutralised using 5M hydrochloric acid. Colour change was observed at this point, from a yellow-green to a blue-green solution. The mixture was extracted with ethyl acetate (3 × 30 mL), the organic layers were combined, washed with aqueous hydrochloric acid (2M, 30 mL), saturated sodium hydrogen carbonate (50 mL), brine (50 mL), dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure to a green oil. The residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (1:7, v/v) to leave the title compound **208** as a colourless oil (obtained as a single compound) (5.2 g, 86 %); ν_{max} (DCM)/cm^{-1} 3447, 2930, 1673, 1598 & 1454; δ_H (400 MHz; $CDCl_3$) 2.18 (2H, m, COCHC*H*$_2$), 2.58 (1H, m, COC*H*), 3.00 (2H, m, COCHCH$_2$C*H*$_2$), 4.27 (1H, s, OH), 4.50 (1H, m, C(OH)C*H*), 5.23 (1H, d, *J* 10.4 CH=C*H*H), 5.34 (1H, d, *J* 17.2 CH=CH*H*), 5.90 (1H, m, CH$_2$=C*H*), 7.25 (1H, d, *J* 7.6, Ar-C*H*), 7.31 (1H, t, *J* 7.6, Ar-C*H*), 7.49 (1H, t, *J* 7.6, Ar-C*H*) & 8.03 (1H, d, *J* 7.6, Ar-C*H*); δ_C (100 MHz; $CDCl_3$) 25.7 (CH_2), 28.8 (CH_2), 52.4 (CH), 73.9 (CH), 117.3 (CH_2), 126.7 (Ar-CH), 127.4 (Ar-CH), 128.7 (Ar-CH),132.4 (C), 133.8 (Ar-CH), 137.8 (CH), 144.3 (C) & 201.6 (C); *m/z* (ESI) 203.1065 ([M+H^+], $C_{13}H_{15}O_2$ requires 203.1067).

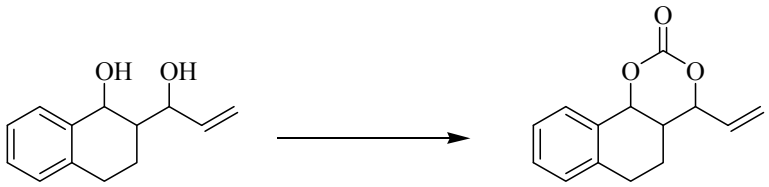
2-(1-Hydroxyprop-2-enyl)-1,2,3,4-tetrahydronaphthalen-1-ol 209[71, 72]



208 **209**

To a stirred suspension of lithium aluminium hydride (0.61 g, 18 mmol) in THF (40 mL) was added the 2-(1-hydroxyprop-2-enyl)-3,4-dihydro-2*H*-naphthalen-1-one **208** (2.0 g, 9.9 mmol) in THF (5 ml) over 5 mins at 0 °C and stirred for 30 mins. The resulting mixture was quenched with THF : water solution (1:1 v/v, 20 mL). Sodium hydroxide solution (15 % w/v, 20 mL) was added and then the mixture was extracted with ethyl acetate (3 × 30 mL), the organic layers were combined, dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure to a yellow oil. The residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (1:4, v/v) to leave the title compound **209** as a yellow oil (1.5 g, 75 %); Data for major isomer; ν_{max} (DCM)/cm^{-1} 3352, 2925, 1490 & 1431; δ_H (400 MHz; $CDCl_3$) 1.71 (2H, m, C(OH)CHC*H*$_2$), 1.85 (1H, m, C(OH)C*H*CH_2), 2.75 (2H, m, C(OH)CHCH_2C*H*$_2$), 4.01 (1H, m, C*H*(OH)), 4.78 (1H, m, ArC*H*(OH)), 5.24 (2H, m, CH=C*H*$_2$), 5.86 (1H, m, CH_2=C*H*) & 7.01-753 (4H, m, Ar-C*H*); δ_C (100 MHz; $CDCl_3$) 23.6 (CH_2), 28.6 (CH_2), 46.7 (CH), 68.5 (CH), 70.9 (CH), 116.2 (CH_2), 126.9 (Ar-CH), 127.2 (Ar-CH), 129.2 (Ar-CH), 129.8 (Ar-CH), 136.4 (C), 137.9 (CH) & 138.6 (C); *m/z* (ESI) 227.1042 ([M+Na^+], $C_{13}H_{16}O_2Na$ requires 227.1043)

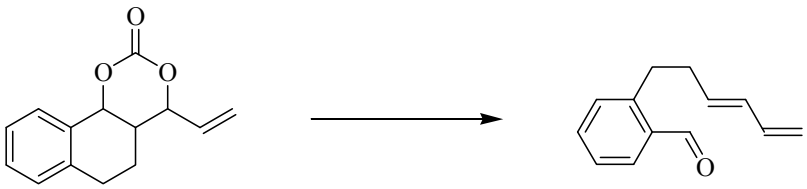
4-Ethenyl-4a,5,6,10b-tetrahydro-4*H*-naphtho[1,2-*d*][1,3]dioxin-2-one 210[71, 72]



209 **210**

To a solution of 2-(1-hydroxyprop-2-enyl)-1,2,3,4-tetrahydronaphthalen-1-ol **209** (0.54 g, 2.7 mmol) and triethylamine (3.10 mL, 22.0 mmol) in dry DCM (20 mL) at 0 °C was added dropwise methyl chloroformate (1.35 mL, 17.5 mmol). The resulting mixture was stirred for 24 h at 23 °C and then the solvent concentrated under reduced pressure. To the resulting mixture, aqueous hydrochloric acid (2M, 50 ml) was added and the mixture was extracted with ethyl acetate (3 × 30 ml), the organic layers were combined, washed with brine (50 mL), dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure to an off-white solid. The residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (1:5, v/v) to yield a white solid (0.44 g, 74 %) of the title compound **210** as a diastereomeric mixture; m.p. 150-152°C (lit.mp.,[72] 152-154 °C); Data for major isomer; ν_{max} (DCM)/cm^{-1} 2879, 1684, 1660, 1532, 1490 & 1362; δ_H (400 MHz; $CDCl_3$) 2.06 (2H, m, $CH_2CH_2CH_2CH$), 2.95 (3H, m, CH_2CH_2CH), 4.71 (1H, m, CH_2=CHCH), 5.31 (1H, d, *J* 10.0, COCH), 5.48 (2H, m, CH=CH_2), 5.93 (1H, m, CH_2=CH) & 7.14-7.61 (4H, m, Ar-CH); *m/z* (ESI) 248.1283 ([$M+NH_4^+$], $C_{14}H_{18}NO_3$ requires 248.1281).

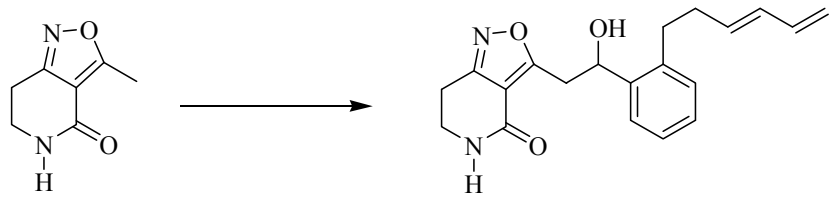
2-(Hexa-3,5-dienyl)-benzaldehyde 211[71, 72]



210 **211**

To solution of 4-ethenyl-4a,5,6,10b-tetrahydro-4*H*-naphtho[1,2-*d*][1,3]dioxin-2-one **210** (2.40 g, 10.4 mmol) in dry acetonitrile (20 mL) was added $[Pd_2(dba)_3].CHCl_3$ (0.54 g, 0.52 mmol) and stirred for 48 h at 23 °C. The resulting reaction mixture was diluted with diethyl ether (10 mL) and filtered through a Celite® pad. The filtrate was washed with saturated sodium bicarbonate solution (30 mL), dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure to a yellow oil. The residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (1:10, v/v) to leave the title compound **211** as yellow oil (1.24 g, 65 %); ν_{max} (DCM)/cm^{-1} 3442, 2358, 1696, 1598 & 1451; δ_H (400 MHz; $CDCl_3$) 2.42 (2H, m, $ArCH_2CH_2$), 3.14 (2H, t, *J* 7.6, $ArCH_2CH_2$), 4.99 (1H, d, *J* 10.0, CH=C*H*H), 5.12 (1H, d, *J* 16.8, CH=CH*H*), 5.75 (1H, m, CH_2CH=CH), 6.01 (1H, m, CH_2CH=C*H*), 6.30 (1H, m, CH_2=C*H*), 7.26 (1H, d, *J* 7.6, Ar-C*H*), 7.38 (1H, t, *J* 7.6, Ar-C*H*), 7.49 (1H, t, *J* 7.6, Ar-C*H*), 7.84 (1H, d, *J* 7.6, Ar-C*H*) & 10.25 (1H, s, C*H*O); δ_C (100 MHz; $CDCl_3$) 32.4 (CH_2), 32.8 (CH_2), 115.6 (CH_2), 126.7 (Ar-CH), 131.1 (Ar-CH), 131.9 (Ar-CH), 132.2 (Ar-CH), 133.5 (CH), 133.7 (C), 133.8 (CH), 136.9 (CH), 144.4 (C) & 192.4 (CH); *m/z* (ESI) 204.1380 ($[M+NH_4^+]$, $C_{13}H_{18}NO$ requires 204.1383)

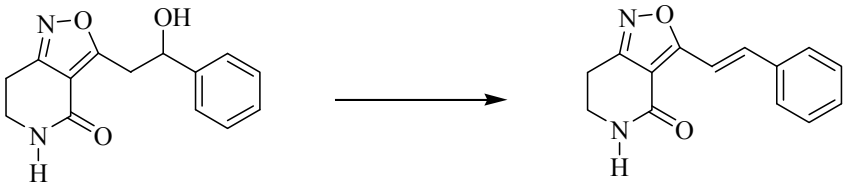
3-[2-(2-Hexa-3,5-dienylphenyl)-2-hydroxyethyl]-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 212



157 **212**

The same procedure was applied as for 3-(2-hydroxy-2-phenylethyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **183** using the following quantities: 3-methyl-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **157** (50 mg, 0.33 mmol), *n*-BuLi (0.66 ml of a 2.5M solution in hexanes, 1.6 mmol) and 2-(hexa-3,5-dienyl)benzaldehyde **211** (0.61 g, 3.3 mmol) to yield the title compound **212** as a white solid (58 mg, 53 %); m.p. 123-125 °C; ν_{max} (DCM)/cm^{-1} 3314, 2951, 1666, 1478 & 1334; δ_H (400 MHz; $CDCl_3$) 2.49 (2H, m, $ArCH_2CH_2$), 2.89 (2H, t, *J* 7.6, $ArCH_2CH_2$), 3.01 (2H, t, *J* 6.4, $NHCH_2CH_2$), 3.38-3.51 (2H, m, $CH(OH)CH_2$), 3.62 (2H, t, *J* 6.4, $NHCH_2CH_2$), 4.58 (1H, br, CHO*H*), 4.99 (1H, d, *J* 10.0 $CH=CH_2$), 5.12 (1H, d, *J* 16.8 $CH=CH_2$), 5.39 (1H, m, $CH_2CH(OH)$, 5.78 (1H, m, $CH_2CH=CH$), 6.08 (1H, m, $CH_2CH=CH$), 6.35 (1H, m, $CH_2=CH$), 7.18-7.29 (3H, m, Ar-C*H*) & 7.56 (1H, m, Ar-C*H*); δ_C (100 MHz; $CDCl_3$) 21.3 (CH_2), 31.8 (CH_2), 34.3 (CH_2), 36.7 (CH_2), 40.6 (CH_2), 68.5 (CH), 109.1 (C), 115.5 (CH_2), 125.5 (Ar-CH), 126.7 (Ar-CH), 127.8 (Ar-CH), 129.5 (Ar-CH), 131.7 (CH), 133.9 (CH), 137.0 (CH), 137.9 (C), 140.9 (C), 160.5 (C), 163.8 (C) & 172.7 (C); *m/z* (ESI) 339.1707 ([M+H$^+$], $C_{20}H_{23}N_2O_3$ requires 339.1703)

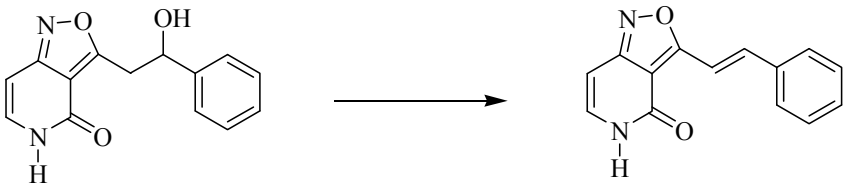
3-(2-Phenylethenyl)-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 199



182 **199**

To a solution of 3-(2-hydroxy-2-phenylethyl)-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **182** (50 mg, 0.19 mmol) in toluene (30 mL) was added *p*-toluenesulfonic acid (48 mg, 0.25 mmol) and the mixture was heated overnight under Dean-Stark conditions. The reaction was cooled and water (20 mL) added. The mixture was extracted with ethyl acetate (3 × 30 mL), the organic layers were combined, washed with brine (50 mL), dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure to leave the title compound **199** as a white solid (29 mg, 63 %); m.p. 219-217 °C; ν_{max} (DCM)/cm^{-1} 3078, 2931, 1674, 1653, 1597, 1473, 1404 & 1334; δ_H (400 MHz; $CDCl_3$) 3.05 (2H, t, *J* 6.8, $NHCH_2CH_2$), 3.64 (2H, dt, *J* 6.8 & 2.8, $NHCH_2$), 6.04 (1H, br, N*H*) 7.36-7.43 (3H, m, Ar-C*H*), 7.48 (1H, d, *J* 16.4, Ph-CH=C*H*), 7.62-7.64 (2H, m, Ar-C*H*) & 8.21 (1H, d, *J* 16.4, Ph-C*H*=CH); δ_C (100 MHz; $CDCl_3$) 21.4 (CH_2), 40.6 (CH_2), 107.0 (C), 112.1 (CH), 128.0 (CH), 128.9 (CH), 130.0 (CH), 135.2 (C), 139.3 (CH), 160.9 (C), 163.0 (C) & 168.6 (C); *m/z* (EI) 240.0894 (M^+, $C_{14}H_{12}N_2O_2$ requires 240.0893).

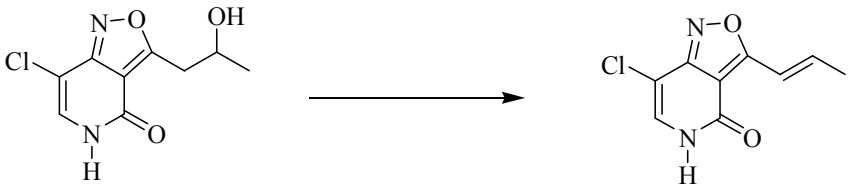
3-(2-Phenylethenyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 200



183 **200**

The same procedure was applied as for 3-(2-phenylethenyl)-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **199** using the following quantities: 3-(2-hydroxy-2-phenylethyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **183** (30 mg, 0.12 mmol) and *p*-toluenesulfonic acid (29 mg, 0.15 mmol) to yield the title compound **200** as a yellow solid (18 mg, 65 %); m.p. 217-219 °C (lit.m.p.,[45] 215 °C); ν_{max} (DCM)/cm^{-1} 3131, 2922, 1672, 1643, 1569, 1457 & 1217; δ_H (400 MHz; $[CD_3]_2SO$) 6.38 (1H, d, *J* 7.5, NHCHC*H*), 7.15 (1H, d, *J* 7.5, NHC*H*), 7.42-7.51 (3H, m, Ar-C*H*), 7.56 (1H, d, *J* 16.4, Ph-CH=C*H*), 7.75 (2H, m, Ar-C*H*), 8.21 (1H, d, *J* 16.4, Ph-C*H*=CH) & 10.95 (1H, br, N*H*); δ_C (100 MHz; $[CD_3]_2SO$) 95.1 (CH), 107.8 (C), 112.6 (CH), 128.2 (CH), 128.9 (CH), 130.3 (CH), 135.2 (C), 135.9 (CH), 139.9 (CH), 158.3 (C), 159.9 (C) & 171.8 (C); *m/z* (EI) 238.0741 (M^+, $C_{14}H_{10}N_2O_2$ requires 238.0741), 238 (100), 209 (12), 77 (26) & 43 (19).

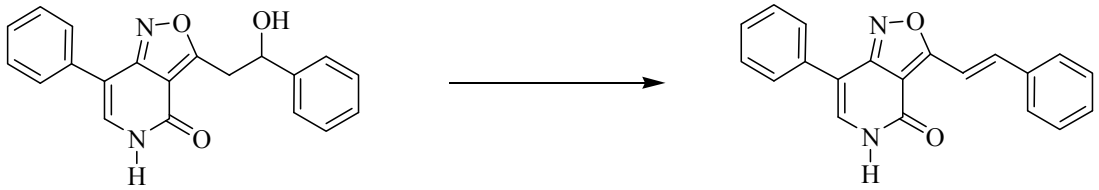
7-Chloro-3-propenyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 201



185 **201**

The same procedure was applied as for 3-(2-phenylethenyl)-6,7-dihydro-5H-isoxazolo[4,3-*c*]pyridin-4-one **199** using the following quantities: 7-chloro-3-(2-hydroxy-propyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **185** (50 mg, 0.23 mmol) and *p*-toluenesulfonic acid (67 mg, 0.53 mmol) to yield the title compound **201** as a white solid (39 mg, 85 %); m.p. 224-227 °C; ν_{max} (DCM)/cm^{-1} 2875, 1677 & 1310; δ_H (400 MHz; $CDCl_3$) 2.06 (3H, d, *J* 6.8, C*H*$_3$), 6.95 (1H, d, *J* 16.0, C*H*=CH), 7.03 (1H, d, *J* 6.0, NHC*H*), 7.34 (1H, m, CH=C*H*) & 8.72 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 19.2 (CH_3), 102.1 (C), 105.8 (C), 116.5 (CH), 130.2 (CH), 142.2 (CH), 156.8 (C), 158.9 (C) & 171.9 (C); *m/z* (ESI) 211.0270 ([M+H$^+$], $C_9H_8N_2O_2Cl$ requires 211.0269)

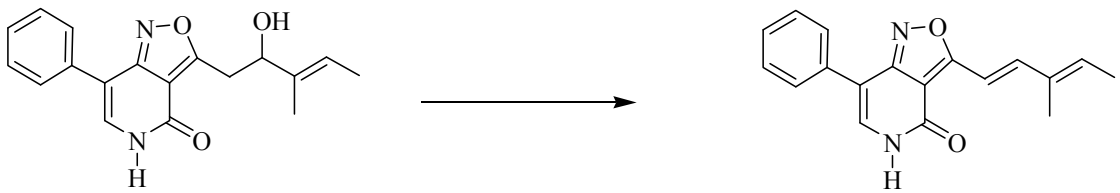
7-Phenyl-3-(2-phenylethenyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 203



195 **203**

The same procedure was applied as for 3-(2-phenylethenyl)-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **199** using the following quantities: 3-(2-hydroxy-2-phenyl-ethyl)-7-phenyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **195** (50 mg, 0.15 mmol) and *p*-toluenesulfonic acid (42 mg, 0.23 mmol) to yield the title compound **203** as a yellow solid (39 mg, 83 %); m.p. 264-266 °C; ν_{max} (DCM)/cm^{-1} 3190, 3054, 2368, 1672, 1643, 1488 & 1375; δ_H (400 MHz; $[CD_3]_2SO$), 7.34 (1H, m, NHC*H*), 7.44-7.50 (6H, m, Ar-C*H*), 7.66 (1H, d, *J* 16.8, Ph-CH=C*H*) 7.77-7.79 (2H, m, Ar-C*H*), 7.83-7.85 (2H, m, Ar-C*H*), 8.14 (1H, d, *J* 16.8, Ph-C*H*=CH) & 9.05 (1H, br, N*H*); δ_C (100 MHz; $[CD_3]_2SO$) 107.5 (C), 108.0 (C), 112.4 (CH), 127.4 (Ar-CH), 127.7 (Ar-CH), 128.4 (Ar-CH), 129.0 (Ar-CH), 129.6 (Ar-CH), 130.8 (Ar-CH), 132.9 (CH), 134.0 (C), 135.3 (C), 140.3 (CH), 158.6 (C), 159.4 (C) & 170.1 (C); *m/z* (ESI) 315.1132 ([M + H$^+$], $C_{20}H_{15}N_2O_2$ requires 333.1128).

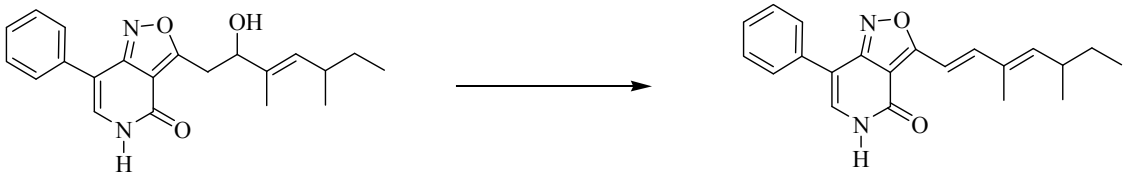
3-(3-Methylpenta-1,3-dienyl)-7-phenyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 204



196 **204**

The same procedure was applied as for 3-(2-phenylethenyl)-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **199** using the following quantities: 3-(2-hydroxy-3-methylpent-3-enyl)-7-(4-hydroxyphenyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **196** (40 mg, 0.13 mmol) and *p*-toluenesulfonic acid (36 mg, 0.19 mmol) to yield the title compound **204** as a yellow solid (30g, 81%); m.p. 197-198 °C; ν_{max} (DCM)/cm^{-1} 2923, 1681, 1640, 1420 & 1350; δ_H (400 MHz; $CDCl_3$) 1.62 (3H, d, *J* 7.2, CHC*H*$_3$), 1.87 (3H, s, C*H*$_3$), 6.05 (1H, q, *J* 7.2, CH$_3$C*H*), 6.93 (1H, d, *J* 16.0, C*H*=CHC(CH$_3$)), 7.09 (1H, d, *J* 6.0, NHC*H*), 7.29 (1H, t, *J* 7.2, Ar-C*H*), 7.38 (2H, t, *J* 7.2, Ar-C*H*), 7.55 (1H, d, *J* 16.0, CH=C*H*C(CH$_3$)), 7.68 (2H, d, *J* 7.2, Ar-C*H*) & 9.04 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 11.7 (CH$_3$), 14.8 (CH$_3$), 109.5 (C), 109.5 (C), 110.4 (CH), 127.4 (Ar-CH), 127.9 (Ar-CH), 128.8 (Ar-CH), 129.6 (CH), 133.4 (C), 134.8 (C), 137.2 (CH), 145.8 (CH), 158.0 (C), 160.2 (C) & 171.6 (C); *m/z* (ESI) 293.1289 ([M+H$^+$], $C_{18}H_{17}N_2O_2$ requires 293.1285)

3-(3,5-Dimethylhepta-1,3-dienyl)-7-phenyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 205

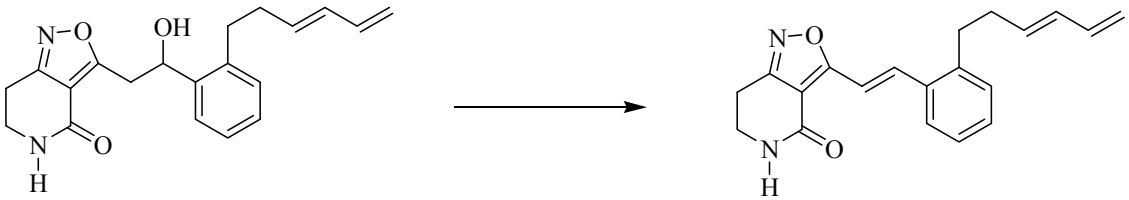


197 **205**

The same procedure was applied as for 3-(2-phenylethenyl)-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **199** using the following quantities: 3-(2-hydroxy-3,5-dimethylhept-3-enyl)-7-phenyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **197** (60 mg, 0.17 mmol) and *p*-toluenesulfonic acid (49 mg, 0.26 mmol) to yield the title compound **205** as a yellow solid (48 mg, 86 %); m.p. 216-219 ºC; ν_{max} (DCM)/cm^{-1} 3061, 2959, 2922, 1673, 1640, 1562 & 1359; δ_H (400 MHz; $CDCl_3$) 0.88 (3H, t, *J* 7.6, CH$_2$C*H*$_3$), 1.01-1.06 (3H, m, CHC*H*$_3$), 1.31-1.46 (2H, m, CH$_3$C*H*$_2$), 1.95 (3H, s, C*H*$_3$), 2.48-2.56 (1H, m, CH$_3$C*H*), 5.81 (1H, d, *J* 9.6, C=C*H*), 7.03 (1H, d, *J* 16.0, C*H*=CHC(CH$_3$)), 7.17 (1H, d, *J* 6.0,

NHC*H*), 7.34-7.38 (1H, m, Ar-C*H*), 7.43-7.47 (2H, m, Ar-C*H*), 7.65 (1H, d, *J* 16.0, CH=C*H*C(CH_3)), 7.75-7.79 (2H, m, Ar-C*H*) & 9.41 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 12.0 (CH_3), 12.3 (CH_3), 20.2 (CH_3), 30.0 (CH_2), 35.2 (CH), 106.5 (C), 109.7 (CH), 110.4 (C), 127.4 (Ar-CH), 127.9 (Ar-CH), 129.6 (Ar-C), 130.7 (CH), 132.6 (C), 133.4 (C), 146.3 (CH), 149.3 (CH), 158.0 (C), 160.4 (C) & 171.6 (C); *m/z* (FAB) 335.1766 ([M + H^+], $C_{21}H_{23}N_2O_2$ requires 335.1760).

3-[2-(2-Hexa-3,5-dienylphenyl)-ethenyl]-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 213

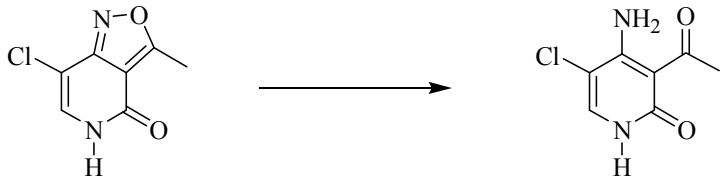


212 **213**

To a solution of 3-[2-(2-hexa-3,5-dienylphenyl)-2-hydroxyethyl]-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **212** (50 mg, 0.15 mmol) in toluene (5 mL) was added *p*-toluenesulfonic acid (42 mg, 0.22 mmol) and the mixture was heated for 4 h under Dean-Stark conditions. The reaction was cooled and water (10 mL) added. The mixture was extracted with ethyl acetate (4 × 30 mL), the organic layers were combined, washed with brine (50 mL), dried ($MgSO_4$), filtered, the solvent was removed under reduced pressure and the residue was purified by column chromatography, eluting with light petroleum : ethyl acetate (1:1, v/v) to yield the title compound **213** as a white solid (37 mg, 78 %); m.p. 160-164 ºC; ν_{max} (DCM)/cm^{-1} 3019, 2956, 2385, 1671, 1597, 1482 & 1336; δ_H (400 MHz; $CDCl_3$) 2.49 (2H, q, *J* 7.2, $ArCH_2CH_2$), 2.90 (2H, t, *J* 7.2, $ArCH_2CH_2$), 3.04 (2H, t, *J* 6.8, $NHCH_2CH_2$), 3.64 (2H, t, *J* 6.8, $NHCH_2CH_2$), 4.98 (1H, d, *J* 10.0, CH=C*H*H), 5.12 (1H, d, *J* 16.8, CH=CH*H*), 5.76 (1H, m, CH_2CH=CH), 6.08 (1H, m, CH_2CH=C*H*), 6.29 (1H, m, CH_2=C*H*), 7.17-7.32 (3H, m, Ar-C*H*), 7.38 (1H, d, *J* 16.4, ArCH=C*H*), 7.74 (1H, m, Ar-C*H*) & 8.19 (1H, d, *J* 16.4, ArC*H*=CH); δ_C (100 MHz; $CDCl_3$) 21.4 (CH_2), 33.2

(CH_2), 34.4 (CH_2), 40.6 (CH_2), 106.9 (C), 113.2 (CH), 115.4 (CH_2), 126.3 (Ar-CH), 126.7 (Ar-CH), 129.9 (Ar-CH), 130.1 (Ar-CH), 131.9 (CH), 133.7 (CH), 137.0 (CH), 137.04 (CH), 141.3 (C), 161.0 (C), 163.3 (C), 168.9 (C) & 175.5 (C); *m/z* (ESI) 321.1607 ([$M+H^+$], $C_{20}H_{21}N_2O_2$ requires 321.1598)

3-Acetyl-4-amino-5-chloro-1*H*-pyridin-2-one 245



162 **245**

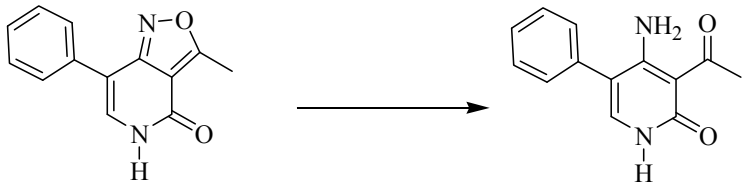
Method A

A stirred suspension of 5-chloro-3-methyl-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **162** (50 mg, 0.27 mmol) in methanol (2 mL) and palladium on carbon (5 % w/w, 14 mg, 0.27 mmol) was treated in the hydrogenation Buchi pressflow gas controller at 2 bar pressure for 3 h. The reaction mixture was filtered, the solvent concentrated under reduced pressure and the residue was purified by column chromatography, eluting with ethyl acetate : methanol (10:1, v/v) to leave the title compound **245** as a white solid (42 mg, 84 %); m.p. 267-269 °C; ν_{max} (DCM)/cm^{-1} 3346.7, 2888, 1669, 1632, 1508, 1455 & 1362; δ_H (400 MHz; $[CD_3]_2SO$) 2.50 (3H, s, C*H*$_3$) & 7.41 (1H, s, NHC*H*); δ_C (100 MHz; $[CD_3]_2SO$) 33.1 (CH_3), 104.1 (C), 105.3 (C), 137.3 (CH), 159.1 (C), 164.9 (C) & 202.6 (C); *m/z* (FAB) 187.0271 ([M + H$^+$], $C_7H_8N_2O_2{}^{35}Cl$ requires 187.0273)

Method B

Chloro-3-methyl-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **162** (50 mg, 0.27 mmol) and molybdenum hexacarbonyl (107 mg, 0.40 mmol) in moist acetonitrile (3 mL) and water (0.01 mL) were heated at reflux for 3 h. The reaction mixture was cooled and solvent removed under reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (2:1, v/v) to leave the title compound **245** as a white solid (55 mg, 54 %);

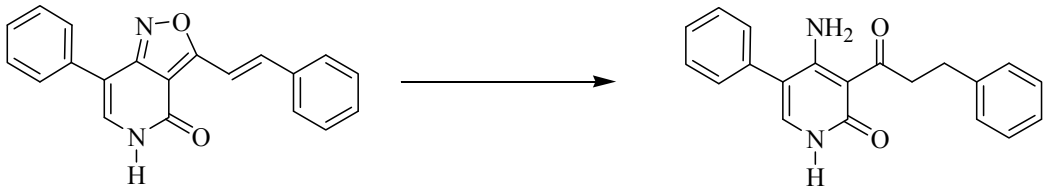
3-Acetyl-4-amino-5-phenyl-1*H*-pyridin-2-one 253



174 **253**

The same was procedure applied as for 3-acetyl-4-amino-5-chloro-1H-pyridin-2-one **245** using the following quantities: 3-methyl-7-phenyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **174** (150 mg, 0.56 mmol), methanol (2 mL) and palladium on carbon (5 % w/w, 30 mg, 0.56 mmol) to yield the title compound **253** as a white solid (80 mg, 53 %); m.p. 250-252 °C; ν_{max} (DCM)/cm^{-1} 2924, 2812, 1637, 1620, 1518, 1445 & 1261; δ_H (400 MHz; $CDCl_3$) 2.69 (3H, s, C*H*$_3$), 7.31 (1H, d, *J* 6.0, NHC*H*); 7.42 (2H, m, Ar-C*H*), 7.55 (1H, m, Ar-C*H*) & 7.69 (2H, m, Ar-C*H*); δ_C (100 MHz; $CDCl_3$) 33.4 (CH_3), 104.1 (C), 112.7 (C), 128.5 (Ar-CH), 129.8 (Ar-CH), 131.9 (Ar-CH), 136.2 (CH), 157.1 (C), 159.5 (C), 165.1 (C) & 201.9 (C); *m/z* (ESI) 229.0971 ([M + H^+], $C_{13}H_{13}N_2O_2$ requires 229.0972)

4-Amino-5-phenyl-3-(3-phenylpropionyl)-1*H*-pyridin-2-one 255



203 **255**

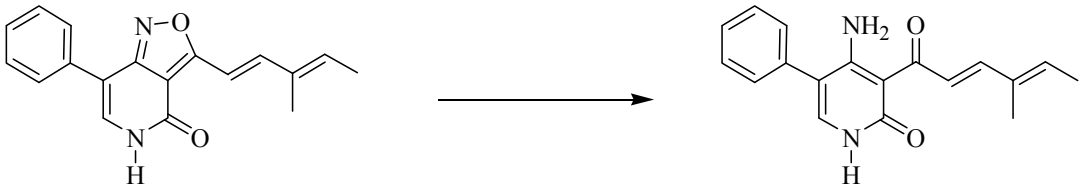
Method A

7-Phenyl-3-(2-phenylethenyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **203** (100 mg, 0.32 mmol) and molybdenum hexacarbonyl (92 mg, 0.35 mmol) in moist acetonitrile (5 mL) and water (0.1 mL) was heated at reflux for 3 h. The reaction mixture was cooled and solvent removed under reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (2:1, v/v) to leave the title compound **255** as a yellow solid (50 mg, 50 %); m.p. 241-243 ºC; ν_{max} (DCM)/cm^{-1} 3445, 1644, 1558, 1510, 1445 & 1226; δ_H (400 MHz; $[CD_3]_2CO$), 2.98 (2H, t, *J* 8.0, $ArCH_2CH_2$), 3.47 (2H, t, *J* 8.0, $ArCH_2CH_2$), 7.14 (2H, m, Ar-C*H*), 7.31 (1H, m, NHC*H*) & 7.41-7.52 (8H, m, ArC*H*); δ_C (100 MHz; $[CD_3]_2CO$) 30.9 (CH_2), 45.6 (CH_2) 102.6 (C), 111.7 (C), 125.4 (Ar-CH), 128.0 (Ar-CH), 128.1 (Ar-CH) 128.4 (Ar-CH), 129.1 (Ar-CH), 129.9 (Ar-CH), 134.0 (C), 136.6 (CH), 142.8 (C), 160.0 (C), 162.6 (C) & 202.2 (C); *m/z* (ESI) 319.1445 ([M+H$^+$], $C_{20}H_{19}N_2O_2$ requires 319.1441)

Method B

A stirred suspension of 7-phenyl-3-(2-phenylethenyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **203** (8 mg, 0.3 mmol) in methanol (2 mL) and palladium on carbon (10 % w/w, 14 mg, 1.3 mmol) was stirred at 23 °C at atmospheric pressure for 5 h. The reaction mixture was filtered, the solvent concentrated under reduced pressure to leave the title compound **255** as a white solid (3 mg, 28 %). Identical to a sample prepared in method A.

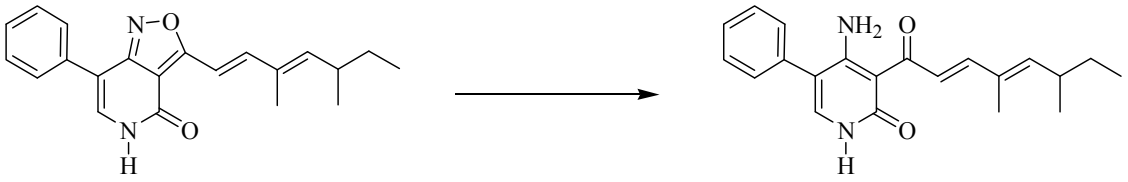
4-Amino-3-(4-methylhexa-2,4-dienoyl)-5-phenyl-1*H*-pyridin-2-one 257



204 **257**

3-(3-Methylpenta-1,3-dienyl)-7-phenyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **204** (30 mg, 0.10 mmol) and molybdenum hexacarbonyl (40 mg, 0.15 mmol) in moist acetonitrile (3 mL) and water (0.1 mL) was heated at reflux for 3 h. The reaction mixture was cooled and solvent removed under reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (2:1, v/v) to leave the title compound **257** as a yellow solid (20 mg, 67 %); m. p. 177-180 °C; ν_{max} (DCM)/cm^{-1} 3437, 1648, 1510 & 1357; δ_H (400 MHz; $CDCl_3$) 1.74 (3H, d, *J* 7.2, CHC*H*$_3$), 1.79 (3H, s, C*H*$_3$), 5.95 (1H, q, *J* 7.2, CH_3C*H*), 7.24-7.28 (2H, m, Ar-C*H*), 7.28-7.31 (1H, m, COCH=C*H*), 7.34-7.43 (1H, m, NHC*H*), 7.38-7.44 (3H, m, Ar-C*H*), 7.63 (1H, d, *J* 15.2, COC*H*=CH), 10.34 (1H, br, N*H*) & 10.89 (1H, br, N*H*);); m.p. 177-180 °C; ν_{max} (DCM)/cm^{-1} 3437, 1648 & 1357; δ_C (100 MHz; $CDCl_3$) 11.3 (CH_3), 13.1 (CH_3), 109.5.0 (C), 109.5 (C), 110.4 (CH), 127.4 (Ar-CH), 127.9 (Ar-CH), 128.8 (Ar-CH), 129.6 (CH), 133.4 (C), 134.8 (C), 137.2 (CH), 145.8 (CH), 158.0 (C), 160.2 (C) & 171.6 (C); *m/z* (ESI) 295.1442 ([M + H$^+$], $C_{18}H_{19}N_2O_2$ requires 295.1441).

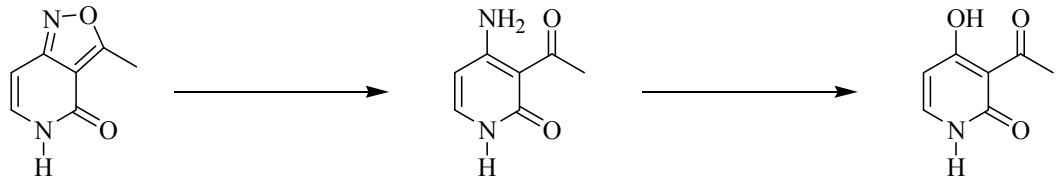
4-Amino-3-(4,6-dimethylocta-2,4-dienoyl)-5-phenyl-1*H*-pyridin-2-one 259



205 **259**

3-(3,5-Dimethylhepta-1,3-dienyl)-7-phenyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **205** (35 mg, 0.10 mmol) and molybdenum hexacarbonyl (30 mg, 0.12 mmol) in moist acetonitrile (3 mL) and water (0.1 mL) was heated at reflux for 3 h. The reaction mixture was cooled and solvent removed under reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (2:1, v/v) to leave the title compound **259** as a yellow solid (20 mg, 57 %); m. p. 211-213 °C; ν_{max} (DCM)/cm^{-1} 3340, 2960, 2920, 1624, 1590 & 1510; δ_H (400 MHz; $CDCl_3$) 0.77 (3H, t, *J* 7.2, CH_2CH_3), 0.92 (3H, d, *J* 6.4 $CHCH_3$), 1.31 (2H, m, CH_3CH_2), 1.83 (3H, s, CH_3), 2.40 (1H, m, CH_3CH), 5.81 (1H, d, *J* 9.6, C=C*H*), 7.27 (2H, m, Ar-C*H*), 7.31 (1H, m, COC*H*=CH), 7.38-7.45 (1H, m, NHC*H*), 7.38-7.45 (3H, m, Ar-C*H*), 7.63 (1H, d, *J* 16, COCH=C*H*), 10.14 (1H, br, N*H*) & 10.37 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 11.9 (CH_3), 12.9 (CH_3), 20.2 (CH_3), 30.3 (CH_2), 34.9 (CH), 106.7 (C), 113.4 (C), 126.1 (CH), 128.8 (CH), 129.6 (Ar-CH), 129.9 (Ar-CH), 132.9 (C), 133.1 (C), 146.6 (CH), 147.9 (CH), 159.7 (C), 164.0 (C) & 192.6 (C); *m/z* (ESI) 337.1912 ([M + H$^+$], $C_{21}H_{25}N_2O_2$ requires 337.1911).

3-Acetyl-4-hydroxy-1*H*-pyridin-2-one 252

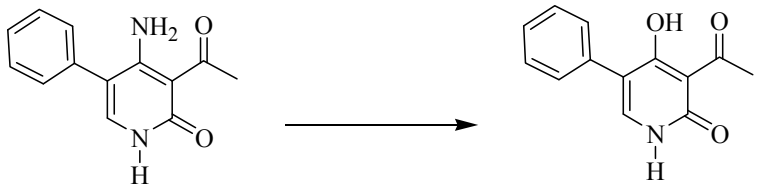


1 **251** **252**

The same procedure was applied as for 3-acetyl-4-amino-5-chloro-1*H*-pyridin-2-one **245** using the following quantities: 3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **1** (10 mg, 0.07 mmol), methanol (2 mL) and palladium on carbon (5 % w/w, 4 mg, 0.07 mmol) to yield the amine which was used in the next step without further purification.

To the amine in water (5 mL) at 0 °C was added sodium nitrite (45 mg, 6.6 mmol) in hydrochloric acid (2M, 5 mL) and the mixture stirred for 1 h, and then at 50 °C for 1 h. The reaction mixture was then extracted with ethyl acetate (3 × 30 mL), the organic layers were combined, washed with brine (50 mL), dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate : methanol (10:1, v/v) to leave the title compound **252** as a waxy solid (2 mg, 20 %); ν_{max} (DCM)/cm^{-1} 3406, 166.4, 1602 & 1468 ; δ_H (400 MHz; $CDCl_3$) 2.74 (3H, s, C*H*$_3$), 6.03 (1H, d, *J* 7.2, C*H*), 7.31 (1H, d *J* 7.2, NHC*H*) & 10.40 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 31.4 (CH_3), 101.6 (CH), 139.8 (CH) & 203.5 (C); *m/z* (ESI) 176.0323 ([M + Na^+], $C_7H_7NO_3Na$ requires 176.0318). Data obtained to date.

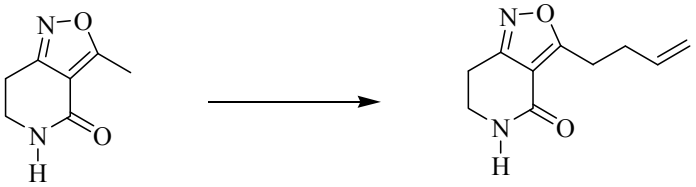
3-Acetyl-4-hydroxy-5-phenyl-1*H*-pyridin-2-one 254



174 **254**

To a stirred suspension of 3-acetyl-4-amino-5-phenyl-1*H*-pyridin-2-one **174** (70 mg, 0.26 mmol) in water (5 mL) at 0 °C was added sodium nitrite (20 mg, 0.29 mmol) in hydrochloric acid (2M, 10 mL) and the mixture stirred for 1 h, and then at 50 °C for 1 h. The reaction mixture was then extracted with ethyl acetate (3 × 30 mL), the organic layers were combined, washed with brine (50 mL), dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate : methanol (10:1, v/v) to leave the title compound **254** as a white solid (21 mg, 30 %); m.p. 271-273 °C; ν_{max} (DCM)/cm^{-1} 3005, 2809, 1630, 1602, 1429 & 1243; δ_H (400 MHz; $[CD_3]_2SO$) 2.42 (3H, s, C*H*$_3$), 7.28 (1H, t, *J* 7.2, Ar-C*H*), 7.35 (2H, m, Ar-C*H*), 7.42 (2H, m, Ar-C*H*) & 7.62 (1H, s, NHC*H*); δ_C (100 MHz; $[CD_3]_2SO$) 30.9 (CH_3), 104.2 (C), 113.7 (C), 127.3 (Ar-CH), 128.0 (Ar-CH), 130.0 (Ar-CH), 141.8 (CH), 135.5 (C), 159.5 (C), 174.2 (C) & 195.6 (C); *m/z* (ESI) 230.0810 ([M + H^+], $C_{13}H_{12}NO_3$ requires 230.0814).

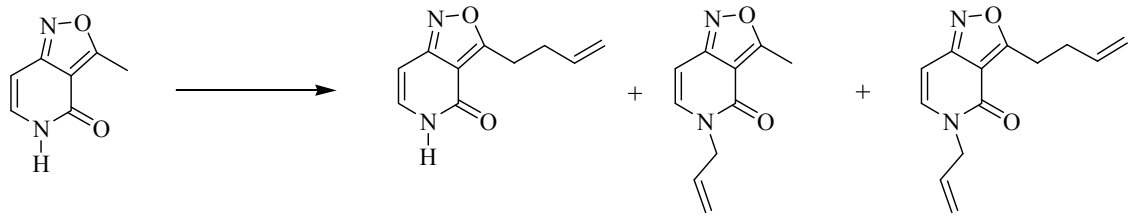
3-(But-3-enyl)-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 215



157 **215**

The same procedure was applied as for 3-(2-hydroxy-2-phenylethyl)-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **183** using the following quantities: 3-methyl-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **157** (20 mg, 0.13 mmol), *n*-BuLi (0.13 mL of a 2.5M solution in hexanes, 0.33 mmol) and prop-2-enyl bromide (0.02 mL, 0.2 mmol) to yield the title compound **215** as a white solid (5 mg, 20 %); m.p. 107 -109 °C; ν_{max} (DCM)/cm^{-1} 3128, 3017, 1628, 1616, 1461 & 1419; δ_H (400 MHz; $CDCl_3$) 2.47 (2H, m, CHC*H*$_2$), 2.95 (2H, t, *J* 6.4 NCH$_2$C*H*$_2$), 3.13 (2H, t, *J* 7.2, CHCH$_2$C*H*$_2$), 3.53 (2H, dt, *J* 2.8 & 6.4, NC*H*$_2$CH$_2$), 4.96 (1H, dd, *J* 1.6 & 10, CH=C*H*$_2$), 5.03 (1H, dd, *J* 1.6 & 17.2, CH=C*H*$_2$), 5.78 (1H, m, CH$_2$=C*H*) & 5.82 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 21.4 (CH_2), 25.9 (CH_2), 31.0 (CH_2), 40.7 (CH_2), 107.6 (C), 116.2 (CH_2), 136.1 (CH), 160.5 (C), 163.0 (C) & 175.1 (C); *m/z* (ESI) 193.0971 ([M + H$^+$], $C_{10}H_{13}N_2O_2$ requires 193.0972).

5-(prop-2-enyl)-3-but-3-enyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one



216 **217** **218**

The same procedure was applied as for 3-(2-hydroxy-2-phenylethyl)-5*H*-isoxazolo[4,3-

c]pyridin-4-one **183** using the following quantities: 3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one (20 mg, 0.13 mmol), *n*-BuLi (0.32 mL of a 2.5M solution in hexanes, 0.80 mmol) and allyl bromide (0.04 mL, 0.5 mmol). The residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (1:1, v/v) to leave the following compounds **216**, **217** and title compound **218**.

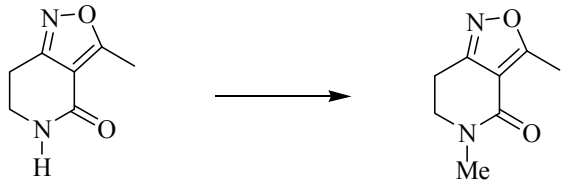
216 was obtained as a brown solid (5 mg, 20 %); m.p. 141-143 °C; ν_{max} (DCM)/cm^{-1} 3181, 3044, 2919, 1682, 1644, 1455 & 1373; δ_H (400 MHz; $CDCl_3$) 2.52 (2H, m, CHC*H*$_2$), 3.30 (2H, t, *J* 7.2, CHCH$_2$C*H*$_2$), 4.96 (1H, dd, *J* 1.2 & 10, CH=C*H*H), 5.03 (1H, dd, *J* 1.2 & 16.8, CH=CH*H*), 5.78 (1H, m, CH$_2$=C*H*), 6.33 (1H, d, *J* 7.6, NHCHC*H*) & 6.89 (1H, dd, *J* 7.6 & 5.6, NHC*H*) & 8.38 (1H, br, N*H*); δ_C (100 MHz; $CDCl_3$) 26.7 (CH_2), 31.1 (CH_2), 95.0 (CH), 108.9 (C), 116.5 (CH_2), 132.6 (CH), 135.8 (CH), 158.2 (C), 160.3 (C) & 177.1 (C); *m/z* (ESI) 213.0634 ([M + Na$^+$], $C_{10}H_{10}N_2O_2Na$ requires 213.0634).

217 was obtained as a brown solid (10 mg, 40 %); m.p. 76-78 °C; ν_{max} (DCM)/cm^{-1} 2927, 2851, 1671, 1643, 1512 & 1419; δ_H (400 MHz; $CDCl_3$) 2.79 (3H, s, 3-C*H*$_3$), 4.41 (2H, d, *J* 6.8, NC*H*$_2$), 5.16 (1H, dd, *J* 1.2 & 17.2, CH=C*H*$_2$), 5.19 (1H, dd, *J* 1.2 & 10, CH=C*H*$_2$), 5.84 (1H, m, CH$_2$=C*H*), 6.30 (1H, d, *J* 7.6, NCHC*H*) & 6.87 (1H, d, *J* 7.6, NC*H*CH); δ_C (100 MHz; $CDCl_3$) 12.9 (CH_3), 49.0 (CH_2), 94.9 (CH), 108.7 (C), 118.3 (CH_2), 132.5 (CH), 137.2 (CH), 158.1 (C), 159.3 (C) & 174.2 (C); *m/z* (ESI) 213.0634 ([M + Na$^+$], $C_{10}H_{10}N_2O_2Na$ requires 213.0634).

218 was obtained as brown oil (8 mg, 27 %); ν_{max} (DCM)/cm^{-1} 3079, 2925, 2851, 1675, 1638, 1452 & 1374; δ_H (400 MHz; $CDCl_3$) 2.52 (2H, m, CH$_2$C*H*$_2$CH), 3.31 (2H, t, *J* 7.2, CHCH$_2$C*H*$_2$), 4.41 (2H, d, *J* 6.8, NC*H*$_2$), 4.96 (1H, dd, *J* 1.2 & 10, CH$_2$CH$_2$CH=CH*H*), 5.03 (1H, dd, *J* 1.6 & 16.8, CH$_2$CH$_2$CH=C*H*H), 5.16 (1H, dd, *J* 1.2 & 17.2, NCH$_2$CH=C*H*$_2$), 5.19 (1H, dd, *J* 1.2 & 10, NCH$_2$CH=C*H*$_2$), 5.74-5.87 (1H, m, CH$_2$=C*H*), 5.74-5.87 (1H, m, CH$_2$=C*H*), 6.32 (1H, d, *J* 7.6, NCHC*H*) & 6.89 (1H, d, *J* 7.6, NC*H*); δ_C (100 MHz; $CDCl_3$) 26.6 (CH_2), 31.1 (CH_2), 49.1 (CH_2), 94.9 (CH), 108.4 (C), 116.3

(CH_2), 118.3 (CH_2), 132.5 (CH), 135.9 (CH), 137.1 (CH), 158.0 (C), 159.1 (C) & 177.1 (C).

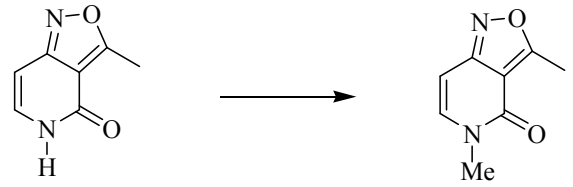
3,5-Dimethyl-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 241



157 **241**

To a solution of 3-methyl-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **157** (50 mg, 0.33 mmol) in THF at -78 ºC was added *n*-BuLi (0.18 mL of a 2.5M solution in hexanes, 0.46 mmol). The resulting solution was stirred for 1 h at -78 ºC followed by addition of iodomethane (0.03 mL, 0.5 mmol), and the reaction mixture was stirred for 2 h at -78 ºC. The reaction was quenched with water (20 mL) and the mixture was extracted with ethyl acetate (4 × 30 mL), the organic layers were combined, washed with brine (50 mL), dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure to leave the title compound **241** as a yellow oil (33 mg, 60%); ν_{max} (DCM)/cm^{-1}; 3071, 1695, 1629, 1526, 1425, 1329 & 1312. δ_H (400 MHz; $CDCl_3$) 2.60 (3H, s, 3-C*H*$_3$), 2.96 (2H, t, *J* 6.8, NCH_2CH_2), 3.00 (3H, s, NC*H*$_3$) & 3.55 (2H, t, *J* 6.8, NCH_2CH_2); δ_C (100 MHz; $CDCl_3$) 12.0 (CH_3), 21.2 (CH_2), 33.8 (CH_3), 48.6 (CH_2), 108.2 (C), 160.3 (C), 161.5 (C) & 171.7 (C); *m/z* (ESI) 167.0823 ([M + H^+], $C_8H_{11}N_2O_2$ requires 167.0821), 137 (60) & 154 (100).

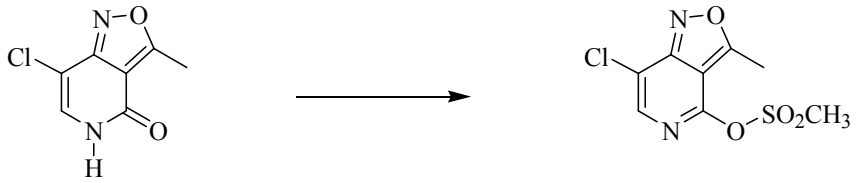
3,5-Dimethyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one 228



1 **228**

The same procedure was applied as for 3,5-dimethyl-6,7-dihydro-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **241** using the following quantities: 3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one (50 mg, 0.33 mmol) **1**, *n*-BuLi (0.19 mL of a 2.5M solution in hexanes, 0.47 mmol) and iodomethane (0.03 mL, 0.5 mmol) to leave the title compound **228** as a white solid (36 mg, 65 %); m.p. 108-109 °C; ν_{max} (DCM)/cm^{-1} 3061, 2357, 1661, 1447, 1365, 1329, 1198 & 1039; δ_H (400 MHz; $CDCl_3$) 2.86 (3H, s, 3-C*H*$_3$), 3.44 (3H, s, NC*H*$_3$), 6.34 (1H, d, *J* 8.0, NHCHC*H*) & 6.95 (1H, d, *J* 8.0, NHC*H*); δ_C (100 MHz; $CDCl_3$) 12.9 (CH_3), 35.3 (CH_3), 94.5 (CH), 108.7 (C), 138.4 (CH), 158.2 (C) 159.7 (C) & 173.8 (C); *m/z* (ESI) 165.0663 ([M + H$^+$], $C_8H_9N_2O_2$ requires 165.0664) 165 (100).

7-Chloro-4-methanesulfonyloxy-3-methylisoxazolo[4,3-*c*]pyridine 235



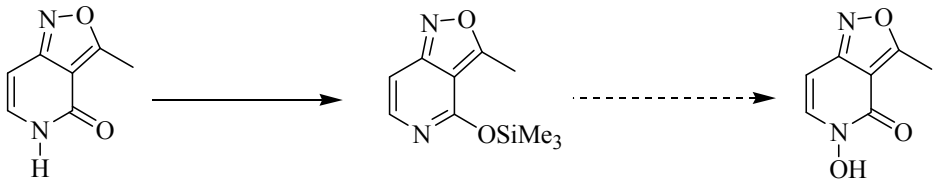
162 **235**

To a solution of 7-chloro-3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **162** (100 mg, 0.54 mmol) in DCM (50 mL) at 0 °C was added triethylamine (66 mg, 0.65 mmol) and methanesulfonyl chloride (68 mg, 0.59 mmol) and the reaction mixture was stirred for 2 h at 23 °C. Water (20 mL) was then added to the reaction mixture, which was and extracted with ethyl acetate (3 × 30 mL), the organic layers were combined, washed with brine (50

mL), dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (1:1, v/v) to leave the title compound **235** as a white solid (102 mg, 72 %); m.p. 214-216 °C; ν_{max} (DCM)/cm^{-1} 2923, 2853, 1699, 1637, 1463 & 1356; δ_H (400 MHz; $[CD_3]_2SO$) 2.89 (3H, s, C*H*$_3$), 3.62 (3H, s, SC*H*$_3$) & 7.78 (1H, s, C*H*); δ_C (100 MHz; $CDCl_3$) 13.2 (CH_3), 42.6 (CH_3), 104.2 (C), 107.9 (C), 128.7 (CH), 156.1 (C) 157.3 (C) & 178.0 (C); *m/z* (ESI) 262.9887 ([M + H^+], $C_8H_8N_2O_4S^{35}Cl$ requires 262.9892).

Attempted preparation of 5-hydroxy-3-methyl-5H-isoxazolo[4,3-c]pyridin-4-one 222

Method A



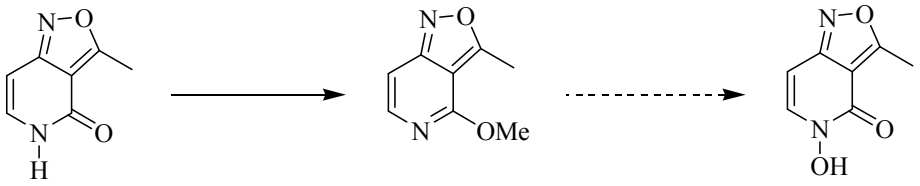
1 **221** **222**

Trimethylsilyl chloride (0.01 mL, 0.4 mmol) was added to a solution of 3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **1** (50 mg, 0.33 mmol) in hexamethyldisilazane (1.39 mL, 6.67 mmol). The resulting mixture was heated at reflux for 24 h. The solvent was removed under reduced pressure, and the resulting crude material was used immediately in the next step.

m-CPBA (149 mg, 0.67 mmol) was added to a solution of the crude material in freshly distilled DCM (50 mL) at 0 °C and stirred for 1 h, after which point the resulting mixture was stirred for a further 18 h at 23 °C, water added (20 mL), and the mixture extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The

residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (1:1, v/v) to only recover the 3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **1**.

Method B

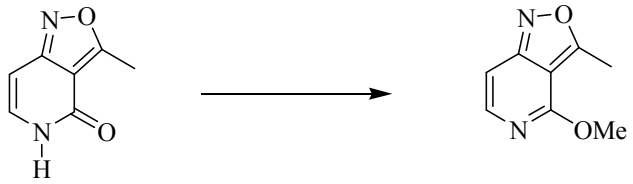


1 **226** **222**

A solution of 3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **1** (50 mg, 0.33 mmol) in anhydrous toluene (50 mL) was heated at reflux under Dean-Stark conditions for 1 h. The reaction was cooled and dimethyl sulfate (0.07 mL, 0.7 mmol) was added, and the resulting reaction mixture was heated at reflux under Dean-Stark conditions for 24 h. The mixture was then cooled, water (20 mL) added, and the mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with aqueous sodium hydrogen carbonate (2 × 50 mL), brine (50 mL), dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure, and remaining crude material was used immediately in the next step.

m-CPBA (149 mg, 0.67 mmol) was added to a solution of the crude material in freshly distilled DCM (50 mL) at 0 °C and stirred for 1 h. The mixture was stirred for a further 18 h at 23 °C, water added (20 mL), and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure to leave a solid. The residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (1:1, v/v) to only recover the 3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **1**.

Attempted preparation of 4-methoxy-3-methylisoxazolo[4,3-*c*]pyridine 226



1 **226**

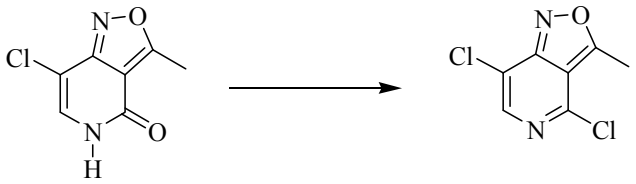
Method A

To a solution of 3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **1** (42 mg, 0.28 mmol) in acetonitrile (2 mL) was added iodomethane (0.026 mL, 0.42 mmol) and silver carbonate (85 mg, 0.31 mmol). The resulting solution was stirred in a microwave (Biotage Initiator Eight EXP microwave system) for 10 minutes at 90 °C. The reaction mixture was cooled, filtered and the solvent removed under reduced pressure to leave a brown oil. The residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (1:1, v/v) to only recover the 3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **1**.

Method B

To a solution of 3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **1** (42 mg, 0.28 mmol) in acetonitrile (2 mL) was added iodomethane (0.026 mL, 0.42 mmol) and silver carbonate (85 mg, 0.31 mmol). The resulting solution was stirred at reflux for 24 h in the dark. The resulting reaction mixture was cooled, filtered and the solvent removed under reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate : light petroleum (1:1, v/v) to yield the *N*-methylpyridone **228** and the recovery of the 3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **1**.

Attempted preparation of 4,7-dichloro-3-methylisoxazolo[4,3-*c*]pyridine 232



162 **232**

To a solution of 7-chloro-3-methyl-5*H*-isoxazolo[4,3-*c*]pyridin-4-one **162** (200 mg, 1.09 mmol) in dichloroethane (5 mL) at 23 °C was added dimethylformamide (0.5 mL) and phosphorus oxychloride (2 mL) and the reaction mixture was heated at reflux for 12 h. To the resulting mixture was added saturated sodium hydrogen carbonate solution (20 mL) and it was then extracted with ethyl acetate (3 × 30 mL), the organic layers were combined, washed with brine (50 mL), dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure to only recover the starting material **162**.

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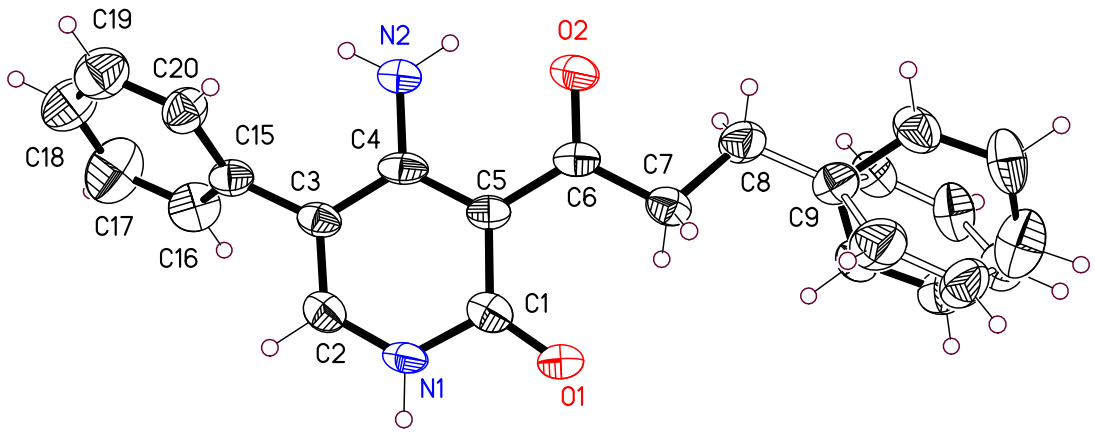
RCFJ32



The data were collected at 150(2)K on a Bruker Apex II CCD diffractometer using MoK_α radiation (λ = 0.71073Å). The structure was solved by direct methods and refined on F^2 using all the reflections*. All the non-hydrogen atoms were refined using anisotropic atomic displacement parameters and hydrogen atoms were inserted at calculated positions using a riding model. Parameters for data collection and refinement are summarised in Table 1.

* G.M. Sheldrick, Acta Cryst. 2008, A64, 112-122.

Notes:

This is a weak data set (and CIFCHECK will complain about this a bit) but it's the best I could do given the size of the crystals. This results in lower bond length precision than normal.

In spite of this, and the fact that there is only one electron difference between O and N, the refinement for $-NH_2$ is significantly better than that with –OH. This is clear from the ellipsoid plots.

One of the phenyl groups is disordered, it was modelled with equal occupancy of two sites, related by rotation and almost perpendicular to each other.

H-bonding links the structure into 2-dimensional sheets, one molecule thick and perpendicular to the a axis.

Table 1. Crystal data and structure refinement for rcfj32.

Identification code	rcfj32	
Empirical formula	C20 H18 N2 O2	
Formula weight	318.36	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	C2/c	
Unit cell dimensions	a = 35.47(2) Å	α= 90°.
	b = 4.896(3) Å	β= 111.575(7)°.
	c = 19.665(12) Å	γ = 90°.
Volume	3176(3) $Å^3$	
Z	8	
Density (calculated)	1.332 Mg/m^3	
Absorption coefficient	0.087 mm^{-1}	
F(000)	1344	
Crystal size	0.56 x 0.07 x 0.03 mm^3	
Crystal description	colourless needle	
Theta range for data collection	2.11 to 25.00°.	
Index ranges	-42<=h<=42, -5<=k<=5, -23<=l<=23	
Reflections collected	11441	
Independent reflections	2788 [R(int) = 0.1813]	
Completeness to theta = 25.00°	100.0 %	
Absorption correction	None	
Max. and min. transmission	0.9974 and 0.9529	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2788 / 36 / 263	
Goodness-of-fit on F^2	0.808	
Final R indices [I>2sigma(I)]	R1 = 0.0490, wR2 = 0.0871	
R indices (all data)	R1 = 0.1652, wR2 = 0.1122	
Extinction coefficient	0.0019(3)	
Largest diff. peak and hole	0.218 and -0.200 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for rcfj32. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	7395(1)	9563(7)	5664(2)	35(1)
O(1)	7104(1)	10876(5)	5232(1)	41(1)
N(1)	7778(1)	10205(6)	5684(1)	35(1)
C(2)	8122(1)	8998(7)	6151(2)	38(1)
C(3)	8122(1)	7057(7)	6634(2)	32(1)
C(4)	7736(1)	6249(7)	6638(2)	34(1)
N(2)	7728(1)	4236(6)	7108(1)	38(1)
C(5)	7373(1)	7416(7)	6151(2)	32(1)
C(6)	6974(1)	6450(7)	6125(2)	31(1)
O(2)	6954(1)	4960(5)	6620(1)	45(1)
C(7)	6595(1)	7157(7)	5511(2)	39(1)
C(8)	6251(1)	5189(8)	5450(2)	48(1)
C(9)	5854(1)	5762(8)	4842(2)	41(1)
C(10)	5777(2)	6280(16)	4139(4)	41(2)
C(11)	5399(3)	6790(20)	3625(5)	52(3)
C(12)	5061(4)	6760(40)	3808(9)	66(7)
C(13)	5115(2)	6274(17)	4526(5)	58(3)
C(14)	5495(2)	5744(16)	5036(4)	51(2)
C(9')	5854(1)	5762(8)	4842(2)	41(1)
C(10')	5709(3)	8380(17)	4662(5)	66(3)
C(11')	5349(2)	8843(18)	4092(5)	59(3)
C(12')	5136(5)	6750(30)	3654(10)	55(6)
C(13')	5301(2)	4168(18)	3805(5)	56(2)
C(14')	5656(2)	3733(16)	4384(4)	45(2)
C(15)	8508(1)	5802(7)	7108(2)	35(1)
C(16)	8752(1)	4536(8)	6790(2)	54(1)
C(17)	9124(1)	3422(10)	7242(3)	77(2)
C(18)	9242(1)	3495(9)	7978(2)	63(1)
C(19)	9003(1)	4778(8)	8287(2)	59(1)
C(20)	8637(1)	5909(8)	7849(2)	44(1)

Table 3. Bond lengths [Å] and angles [°] for rcfj32.

(1)-O(1)	1.248(4)
C(1)-N(1)	1.380(4)
C(1)-C(5)	1.444(5)
N(1)-C(2)	1.362(4)
C(2)-C(3)	1.343(4)
C(3)-C(4)	1.427(5)
C(3)-C(15)	1.479(5)
C(4)-N(2)	1.358(4)
C(4)-C(5)	1.412(5)
C(5)-C(6)	1.473(5)
C(6)-O(2)	1.240(4)
C(6)-C(7)	1.481(4)
C(7)-C(8)	1.526(4)
C(8)-C(9)	1.500(4)
C(9)-C(10)	1.332(7)
C(9)-C(14)	1.458(7)
C(10)-C(11)	1.372(10)
C(11)-C(12)	1.373(16)
C(12)-C(13)	1.375(15)
C(13)-C(14)	1.377(9)
C(10')-C(11')	1.373(9)
C(11')-C(12')	1.372(16)
C(12')-C(13')	1.380(16)
C(13')-C(14')	1.368(9)
C(15)-C(20)	1.360(4)
C(15)-C(16)	1.386(4)
C(16)-C(17)	1.399(5)
C(17)-C(18)	1.352(5)
C(18)-C(19)	1.366(5)
C(19)-C(20)	1.382(5)

O(1)-C(1)-N(1)	117.5(3)
O(1)-C(1)-C(5)	126.3(4)
N(1)-C(1)-C(5)	116.2(3)
C(2)-N(1)-C(1)	123.1(3)
C(3)-C(2)-N(1)	123.5(4)
C(2)-C(3)-C(4)	116.7(3)
C(2)-C(3)-C(15)	119.9(3)
C(4)-C(3)-C(15)	123.4(3)
N(2)-C(4)-C(5)	120.8(3)
N(2)-C(4)-C(3)	117.7(3)
C(5)-C(4)-C(3)	121.4(3)
C(4)-C(5)-C(1)	119.0(4)
C(4)-C(5)-C(6)	121.1(3)
C(1)-C(5)-C(6)	119.8(4)
O(2)-C(6)-C(5)	119.7(3)
O(2)-C(6)-C(7)	118.7(3)
C(5)-C(6)-C(7)	121.6(3)
C(6)-C(7)-C(8)	112.1(3)
C(9)-C(8)-C(7)	115.7(3)
C(10)-C(9)-C(14)	114.0(5)
C(10)-C(9)-C(8)	129.6(5)
C(14)-C(9)-C(8)	116.4(4)
C(9)-C(10)-C(11)	124.9(7)
C(10)-C(11)-C(12)	121.1(10)
C(13)-C(12)-C(11)	117.7(11)
C(12)-C(13)-C(14)	120.7(9)
C(13)-C(14)-C(9)	121.6(7)
C(10')-C(11')-C(12')	121.4(9)
C(11')-C(12')-C(13')	117.2(11)
C(14')-C(13')-C(12')	120.7(9)
C(20)-C(15)-C(16)	118.7(4)
C(20)-C(15)-C(3)	121.8(3)
C(16)-C(15)-C(3)	119.4(3)
C(15)-C(16)-C(17)	119.1(4)
C(18)-C(17)-C(16)	121.2(4)

C(17)-C(18)-C(19)	119.3(4)
C(18)-C(19)-C(20)	120.1(4)
C(15)-C(20)-C(19)	121.4(4)

Table 4. Anisotropic displacement parameters ($Å^2$x 10^3)for rcfj32. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U^{11} + ... + 2 h k a^* b^* U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	45(3)	34(2)	31(2)	-9(2)	19(2)	-8(2)
O(1)	44(2)	37(2)	41(2)	13(1)	16(1)	-1(1)
N(1)	44(2)	30(2)	34(2)	7(2)	19(2)	-7(2)
C(2)	39(3)	36(2)	40(3)	-3(2)	18(2)	-7(2)
C(3)	43(3)	29(2)	29(2)	-4(2)	19(2)	-7(2)
C(4)	56(3)	28(2)	25(2)	-3(2)	23(2)	-5(2)
N(2)	42(2)	39(2)	36(2)	9(2)	18(2)	-2(2)
C(5)	41(3)	30(2)	27(2)	1(2)	17(2)	0(2)
C(6)	48(3)	24(2)	26(2)	-4(2)	18(2)	-3(2)
O(2)	50(2)	46(2)	45(2)	12(2)	25(1)	-2(1)
C(7)	44(3)	33(2)	44(3)	2(2)	21(2)	-2(2)
C(8)	50(3)	36(2)	52(3)	11(2)	13(2)	-2(2)
C(9)	47(3)	32(3)	38(3)	-2(2)	10(2)	-6(2)
C(10)	41(6)	39(5)	46(6)	-2(5)	20(5)	-4(4)
C(11)	60(8)	53(7)	35(6)	-3(5)	9(6)	-8(7)
C(12)	58(10)	45(11)	75(12)	-27(9)	-1(10)	-1(7)
C(13)	36(6)	70(7)	69(7)	-25(6)	22(5)	1(5)
C(14)	47(6)	70(6)	40(5)	-6(5)	22(5)	-9(5)
C(9')	47(3)	32(3)	38(3)	-2(2)	10(2)	-6(2)
C(10')	68(7)	47(6)	72(7)	9(6)	12(6)	-11(5)
C(11')	50(6)	45(6)	66(7)	14(5)	2(5)	-5(5)
C(12')	47(11)	40(11)	70(11)	2(9)	10(10)	-7(10)
C(13')	40(6)	54(7)	73(7)	-18(6)	20(5)	-2(5)
C(14')	47(6)	34(5)	59(6)	-7(5)	28(5)	-2(4)
C(15)	45(3)	28(2)	34(2)	1(2)	17(2)	0(2)
C(16)	64(3)	47(3)	54(3)	-5(2)	25(2)	6(2)
C(17)	72(4)	75(4)	81(4)	-7(3)	27(3)	36(3)
C(18)	69(3)	54(3)	61(3)	8(3)	18(3)	20(3)
C(19)	67(3)	52(3)	56(3)	1(3)	19(3)	2(3)
C(20)	42(2)	46(3)	43(3)	3(2)	15(2)	8(2)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for rcfj32.

	x	y	z	U(eq)
H(1)	7801	11453	5379	42
H(2)	8375	9560	6135	45
H(2N)	7494	3644	7111	57
H(1N)	7956	3524	7408	57
H(7A)	6646	7124	5048	46
H(7B)	6513	9036	5581	46
H(8A)	6338	3319	5383	57
H(8B)	6206	5217	5918	57
H(10)	6000	6300	3981	49
H(11)	5371	7159	3135	62
H(12)	4798	7071	3450	80
H(13)	4887	6303	4672	69
H(14)	5524	5358	5526	61
H(10')	5860	9880	4936	80
H(11')	5246	10651	3998	71
H(12')	4885	7069	3264	66
H(13')	5167	2676	3504	67
H(14')	5767	1943	4467	54
H(16)	8669	4425	6273	64
H(17)	9295	2598	7026	92
H(18)	9490	2663	8278	76
H(19)	9088	4893	8804	71
H(20)	8472	6780	8071	52

Table 6. Hydrogen bonds for rcfj32 [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(1)-H(1)...O(1)#1	0.88	1.89	2.767(4)	175.4
N(2)-H(2N)...O(2)	0.88	1.91	2.578(4)	131.0
N(2)-H(1N)...O(2)#2	0.88	2.52	3.137(4)	127.8

Symmetry transformations used to generate equivalent atoms:

#1 -x+3/2,-y+5/2,-z+1 #2 -x+3/2,y-1/2,-z+3/2