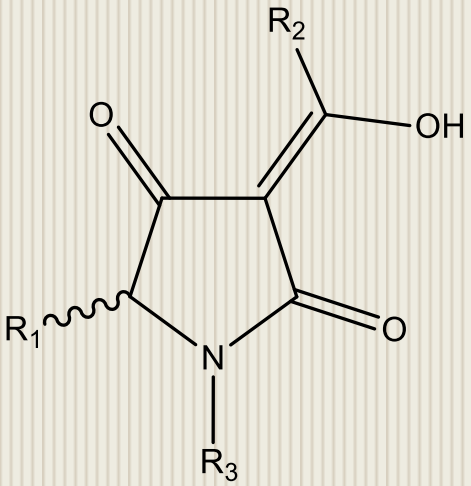
New Routes to Acyltetramic Acids and Analogues

A Doctoral Thesis



3-Acyltetramic Acid Motif

DEPARTMENT OF CHEMISTRY, LOUGHBOROUGH UNIVERISTY

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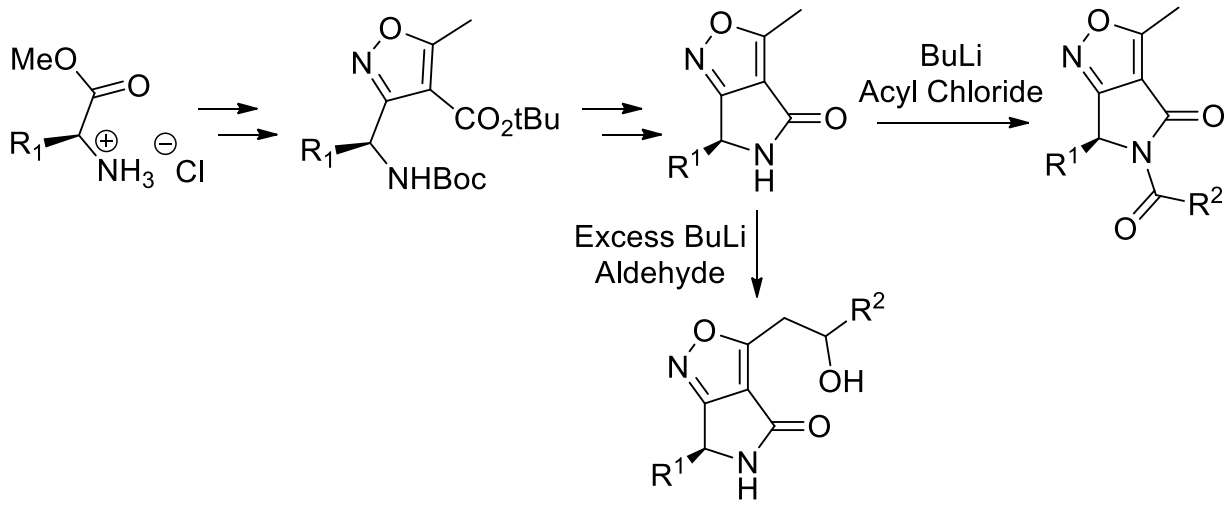
Research completed under the supervision of Prof. Ray C. F. Jones

Abstract

3-Acyltetramic acids, such as reutericyclin, belong to a group of natural products which contain a 5-membered pyrrolidine-2,4-dione heterocycle with an acyl group at the 3-position. Molecules containing this motif have been shown to contain a wealth of desirable bio-activity such as antibiotic, antitumor, antiviral, antiulcerative, fungicidal and cytotoxic properties.[1,2] The motivation for synthetic efforts towards reutericyclin and analogues is that it has been shown to have potential as an antibiotic treatment against superbug *C. difficile*.[3]

Our synthetic approach used a pyrroloisoxazole bicyclic system as a 'masked' form of the acyltetramic acid core structure, which enables us to make selective modifications towards these bio-active products and produce more analogues suitable for biological testing. We report the synthesis of several novel compounds closely related to a masked reutericyclin as well as elaborations at the C-3 methyl group through aldol chemistry.

The route began with a naturally occurring amino acid that underwent N-protection, carboxyl reduction and conversion to an oxime. This oxime is precursor to a nitrile oxide used in a 1,3-dipolar cycloaddition to achieve a substituted isoxazole that was deprotected and, through an intramolecular peptide coupling reaction, provided the pyrroloisoxazole core as the masked acyltetramic acid. Acylation reactions were completed upon this pyrroloisoxazole using butyllithium as a base and a range of acyl chlorides (Scheme 1).



Scheme 1. Synthesis overview

Other developments made in this synthesis were the isolation of the chlorinated oxime and its use in a solvent study and significant improvements in the peptide coupling reaction. Also, synthetic efforts were made to produce an analogue of the natural product laccarin A using this methodology.

Acknowledgements

I would like to thank my supervisor, Prof. Ray Jones, for choosing me to progress with this research and his help and guidance throughout the research conducted.

I would most like to thank my wife, Vikki, and our son, Sean, for putting up with me for the last 10 years, but especially the last 3 years. Their emotional support helped me through the ups and downs of research, without which I am not sure I would be writing this thesis today. Having my son at a young age really gave me the focus I needed to achieve the goals I have and, with his mother standing at my side, I am confident I will continue achieve my goals.

Obviously, I would not be able to be here without the support of my parents, Julie and Paul, my grandparents, Joan, Allan, Sylvia and Norman, as well as the support of my in-laws, Pauline, Trevor, Sam and Tom. These special people, as well as both mine and my wife's extended family, have provided child, financial and more emotional support which was been invaluable to me and my family.

During my research, I have had the honour of working with some great young scientists, some of whom I am sure will go on to do some fantastic research. This peer support, not only from fellow Ph. D students but also lecturers, has provided priceless research ideas, laugh-a-minute morale boosts and general good times, thanks to Rossi, Alex, Jimmy, Capel, Sam, Nat, Tash, Tom, Marc, Ben and Gaz. I can only hope I have been able to help them half as much as they have helped me.

Lastly, thanks to everyone who made this possible, no matter how unlikely this may have seemed 10 years ago!!!!

Abbreviations

Ar	Aryl
Boc	*tert*-Butoxycarbonyl
br.	Broad
Brine	Saturated aqueous Sodium Chloride solution
n-BuLi	*n*-Butyllithium
DCC	1,3-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIBAH	Diisobutylaluminium hydride
DMF	*N,N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
DIPA	Diisopropylamine
EDCI	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
Eq	Equivalent
FMO	Frontier Molecular Orbital
g	Gram
h	Hour
HOMO	Highest Occupied Molecular Orbital
HOSu	*N*-Hydroxysuccinimide
IR	Infrared
LDA	Lithium Diisopropylamide
LUMO	Lowest Unoccupied Molecular Orbital
M	Molar or mol dm^{-3}
mp	Melting point
mL	Millilitre
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MS	Mass Spectrometry

NCS	*N*-Chlorosuccinimide
NMR	Nuclear Magnetic Resonance
NRPS	Non-ribosomal Peptide Synthase
Phth	Phthaloyl
PKS	Polyketide Synthase
PPA	Polyphosphoric acid
PS	Polystyrene
PS-CDI	Polystyrene carbodiimide
PTSA	*p*-Toluenesulfonic acid
rt	Room Temperature
Et_3N	Triethylamine
T3P	Propylphosphonic anhydride
TBAF	Tetra-n-butylammonium fluoride
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMSCl	Chlorotrimethylsilane
UV	Ultraviolet

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1.0 – Introduction

1.1 - 3-Acyltetramic Acids

Tetramic acids are a family of naturally occurring metabolites consisting of a 5-membered heterocycle with the general structure of a pyrrolidine-2,4-dione. Discovered in the early twentieth century, it was not until the mid-1960's that their true advantages became known.[4,1,2] The beneficial properties of the class were investigated upon the realisation that several naturally occurring compounds contain this heterocyclic moiety, which is most common with the addition of an acyl group at the 3-position in the ring (**1**), giving rise to the popularity of 3-acyltetramic acids.

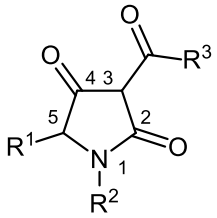

Figure 1. The 3-acyltetramic acid motif

There is a wealth of desirable activities that these compounds have been shown to possess which include antibiotic, antitumor, antiviral, antiulcerative, fungicidal, mycotoxic and cytotoxic properties[1,2]. What is most compelling is that all these potential health benefits can arise from relatively simple modifications to the R^3, C-5 and N-substituted groups, providing the assumption that most biological activity originates from the tricarbonyl structure at the centre. This ability to continue to produce slightly different compounds still containing the biological activities, as seen in penicillin, is massively desirable in drug development due to risks associated with resistance. This high variety of biological activity and challenging structural complexity makes the acyltetramic acids ideal targets for synthetic organic chemists.

Although this chemical moiety was found to be beneficial in the mid-1960's, the first synthetic example was produced in 1914[5], synthesised due to its occurrence in some in natural products and its (at the time) synthetically challenging structure. The 3-acyltetramic acids have been shown to possess a p*Ka* in the range of 3.0-3.5[6], which is thought to be due

to the methine carbon at C-3 which is at the centre of the three carbonyl groups and, therefore, holds a very acidic proton. The charge left behind on removal of this proton can be delocalised by three separate oxyanion forms seen in Figure 2, which leads to this highly acidic proton.

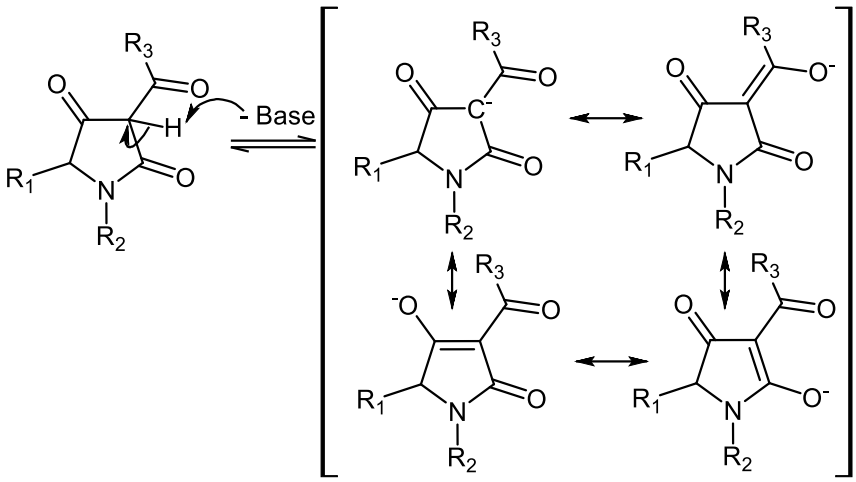

Figure 2. An explanation of the acidity of the methine proton

Also related to the tricarbonyl structure is, as with most enolisable ketones, keto-enol tautomerism. Along with this, the addition of the nitrogen in the heterocycle also allows the projection of over nine possible tautomers, see Figure 3.

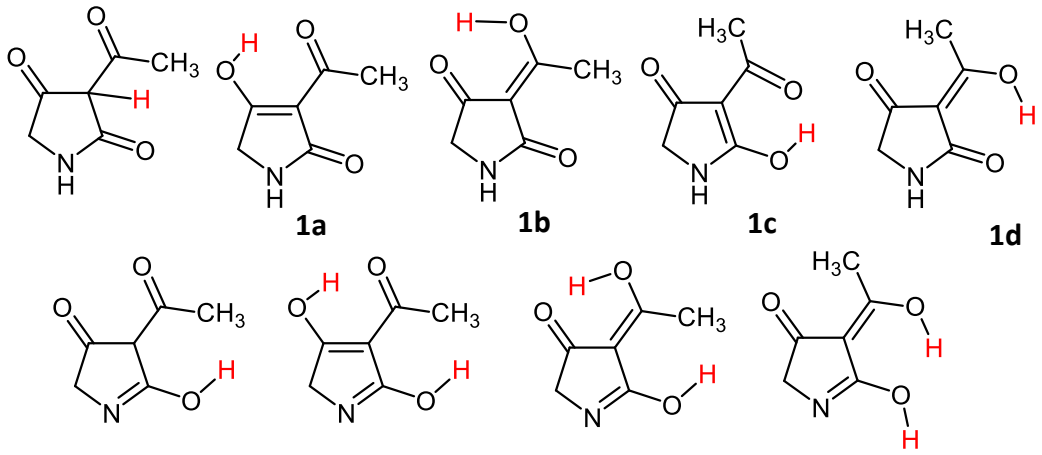

Figure 3. Some theoretical tautomers of the 3-acyltetramic acid motif

Research has shown that only four of these nine are observed in solution (**2a**-**d**). As with most compounds, behaviour is determined by the structure of these tautomers as well as any subsequent intramolecular forces that may arise. The conversion between the tautomers shown has been observed and it was identified that the exchange between the internal tautomers (**2a** ⇌ **2b**, **2c** ⇌ **2d**) is too rapid to detect on an NMR spectroscopy timescale because it relies simply upon a proton transfer along the shown intramolecular hydrogen bond, see Figure 4 for the 5-sec-butyl compounds.

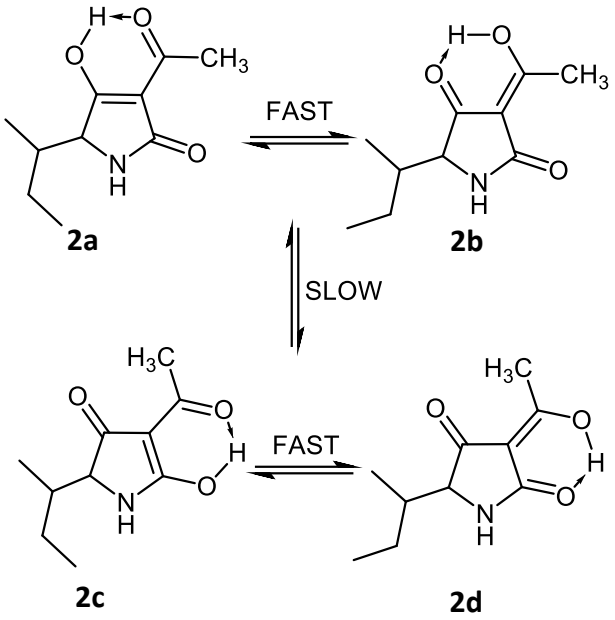

Figure 4. The most common tautomers and their relative rate of interchange

The interconversion between the internal and external tautomers of the 5-sec-butyl compounds (**3a**,**3b** ⇌ **3c**,**3d**) has been shown to occur at a much slower rate, which can be understood as it relies upon the rotation of the 3-acyl chain[7]. The major tautomer at room temperature in the solid state has been identified as the 2,4-diketo external-enol (**3d**)[8]. This was extensively researched and led to conflicting views in the contradictory paper by Yamaguchi[9], who concluded the major tautomers to be both internal (**3a** and **3b**) from his studies involving electron density and CNDO/2 calculations. The true major tautomer (**3d**) was confirmed by the production of more ^{13}C and ^{1}H NMR experiments[10], in which it was shown that the ratio between **3a:b:c:d** is 5:15:0:80 respectively in deuterated chloroform, as

well as crystal structures[11] proving **3d** as the major tautomer in the solid state.

1.2 - 3-Acyltetramic Acids Present in Natural Products

As previously discussed the most common form of tetramic acids in natural products is the 3-acyltetramic acid derivative. These arise in the form of simple lactam compounds, with only very minor variations to the general structure **1,** and can include large aliphatic or aromatic side chains and compounds that integrate the 3-acyltetramic acid into a larger macrocycle, but several of these minor variations give rise to a large library of compounds. Examples of these will now be discussed as reasons for the on-going research into the synthesis of 3-acyltetramic acids.

The first, and simplest in terms of structural complexity, naturally occurring 3-acyltetramic acid derivative to be discussed is tenuazonic acid (**3**), Figure 5.

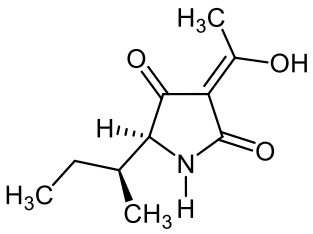

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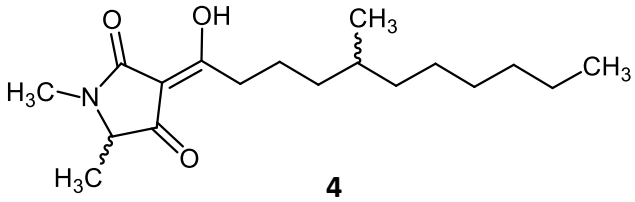
Figure 5. Tenuazonic acid

This compound was isolated in 1957 from a culture of *Alternaria tenuis*, which is a fungus that causes brown spots in leaves, although this particular strain was grown in a laboratory on a glucose medium[12,13]. Tenuazonic acid is a secondary metabolite and inhibits protein synthesis machinery, which is seen in its mycotoxic properties, the ability to inhibit viruses such as herpes and poliovirus[12]. It is important to note that it can be toxic to mammals and this high toxicity limits its potential in human patients. More recently the compound was shown to inhibit tumour growth in mice[14], which could lead to more attempts at the synthesis of non-toxic derivatives of this 3-acyltetramic acid.

The next naturally occurring 3-acyltetramic acid derivative is a group known as melophlins (Figure 6, Table 1). Melophlins C-O, differing mainly in the length of the 3-acyl group, were isolated in 2003 by Wang *et al.* from a marine sponge, *Melophlus sarassinoru*. It is

interesting to note that one-third of all natural products are isolated from sponges, leading them to be the most abundant providers of natural products in the sea[15].



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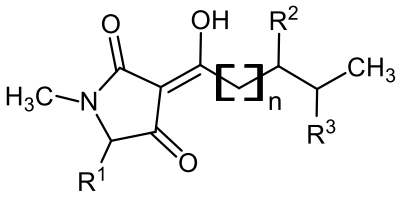

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Figure 6. Melophlins C-O

Table 1. Different possible substituents of Melophlins C-O

Melophlin	R^1	R^2	R^3	n
C (4)	-	-	-	-
D (5)	H	H	H	11
E (6)	H	H	CH_3	11
F (7)	H	CH_3	H	11
G (8)	H	H	H	10
H (9)	H	H	CH_3	10
I (10)	H	CH_3	H	10
J (11)	CH_3	H	H	10
K (12)	H	H	CH_3	9
L (13)	CH_3	H	H	9
M (14)	CH_3	H	H	8
N (15)	CH_3	H	CH_3	8
O (16)	CH_3	CH_3	H	8

Melophlins (**4**-**16**) have all been shown to demonstrate advanced antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*, a strain of which is methicillin-resistant *Staphylococcus aureus* (MRSA). Melophlin C (**4**) has also revealed antifungal activity in that it can inhibit *Candida albicans*[15]. These applications and small chain differences around a single central structure make another intriguing target for synthetic chemists based on the tetramic acid motif.

Another medicinal compound containing this versatile acyltetramic acid is a molecule known as equisetin (**17**), Figure 7, discovered in the mid-1970's upon isolation from the white mould *Fusarium equiseti*[16]. Equisetin is an *N*-methyl 3-enolised-acyltetramic acid that contains a bicyclic system as the acyl group. It has also been shown to display a range of biological activity such as antibiotic and HIV integrase inhibition, cytotoxicity and ability to bind to mammalian DNA[17].

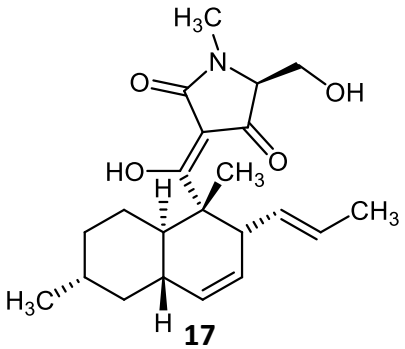

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Figure 7. Equisetin

The desirable biological activity and structural complexity of equisetin make it a suitable target for multiple synthetic attempts; more details will be given later[18].

An additional biological property seen in compounds containing this pyrrolidine-2,4-dione is commercially desirable nematocidal activity. Nematodes are organisms that have been described as hard to distinguish, yet 28,000 different examples have been found, with 16,000 being parasitic[19]. A compound possessing this desirable feature is geodin A (**18**, Figure 8) (characterised as a magnesium salt) that was able to be isolated by Capon *et al.* in 1999. Along with this potent nematocidal activity, this example was also chosen as it contains a macrocyclic polyketide ring, linking the 3-acyl and C-5 chains. The structure was

assigned on the basis of detailed spectroscopic analysis[20].

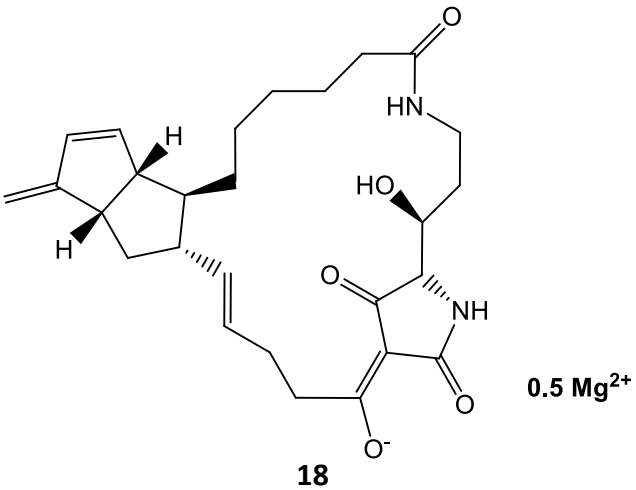

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Figure 8. Geodin A Magnesium Salt

These natural products are only some of the over 150 examples found since 1960[1,2] and the possibilities of thousands more can be easily imagined by simple changes to this small 3-acyltetramic acid centrepiece. The huge range of diversity in these biological activities is also fascinating and can be assumed to have a strong basis in this intriguing pyrrolidine-2,4-dione core.

1.3 – Specific Naturally Occurring Examples Relevant to the Research Undertaken and their Previous Synthesis

1.3.1 – Reutericyclin and Analogues

When discussing naturally occurring 3-acyltetramic acid targets, reutericyclin (**19**, Figure 9) was a clear candidate. Reutericyclin and analogues possess a wealth of biological activity, including potential for use in an antibiotic treatment against hospital superbug *Clostridium difficile* [21,3,22,23,2], which is currently very critical as antibiotic resistance by this bacterium is causing it to be spread "fast and easy" leading to fatalities in older patients and people with a compromised immune system[24]. The structure of the acyltetramic was also deemed an appropriate target for this research due to its relative lack of chiral complexity, as compared with equisetin (**17**), coupled with this highly desirable biological activity.

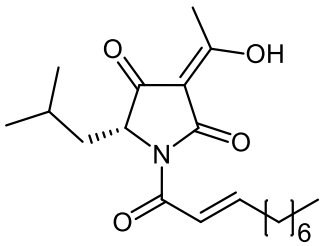

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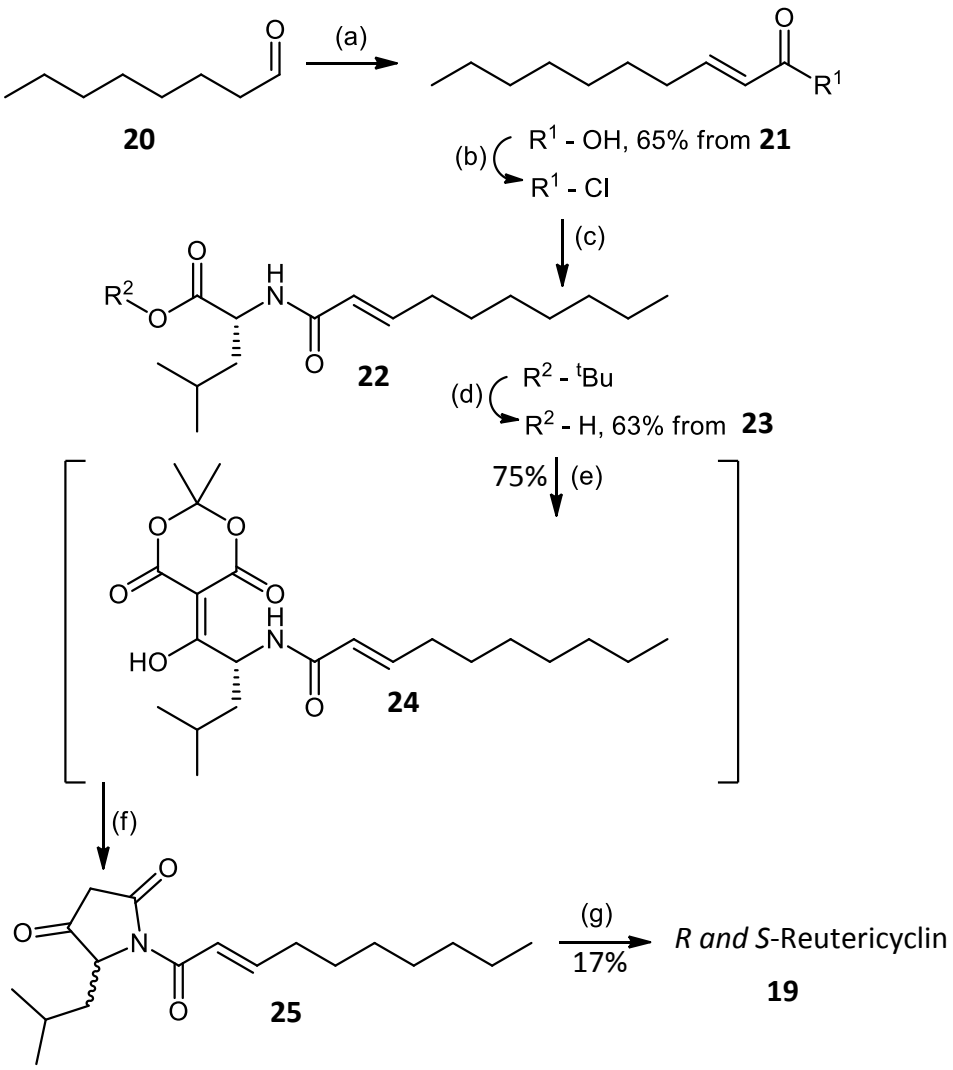
Figure 9. Reutericyclin

Reutericyclin was discovered in 2000 by Jung and co-workers as the first low molecular weight antibiotic from a bacterium found in cereals fermented in lactic acid. In this particular case the lead compound was identified from *Lactobacillius reuteri* LTH2584[21], found in sourdough which is used as a baking aid. This initial discovery was of importance as it was able to inhibit Gram-positive bacteria, the category of bacteria in which *Clostridium difficile* resides.

The first published synthesis of reutericyclin was very quickly provided by its discoverers, Jung and co-workers, in the same year as the publication of its discovery, 2000[25]. This was a racemic synthesis providing the only chiral centre as both enantiomers in 7 steps with an overall yield of 5%. The synthetic product was confirmed by direct comparison of the spectral analysis with the data obtained from the natural product, which was found to occur only in one enantiomer, *R*. The synthesis began with the formation of 2-decenoic acid in

65% yield by the condensation of octanal with malonic acid, followed by chlorination of this acid using thionyl chloride in a quantitative yield. The acyl chloride was then used to acylate *tert*-butyl *O*-protected L-leucine and, after acidic ester cleavage with TFA the *N*-acylated amino acid was isolated in a 63% yield from the acyl chloride. This amino acid was then converted to the *N*-acylated tetramic acid in a 75% yield by coupling with Meldrum's acid and subsequent thermal cyclisation with racemisation, losing the introduced chirality. Racemic reutericyclin was produced and isolated in a 17% yield after HPLC purification from the non-acylated tetramic acid derivative by C-acylation using excess acetyl chloride and titanium tetrachloride (Scheme 2).



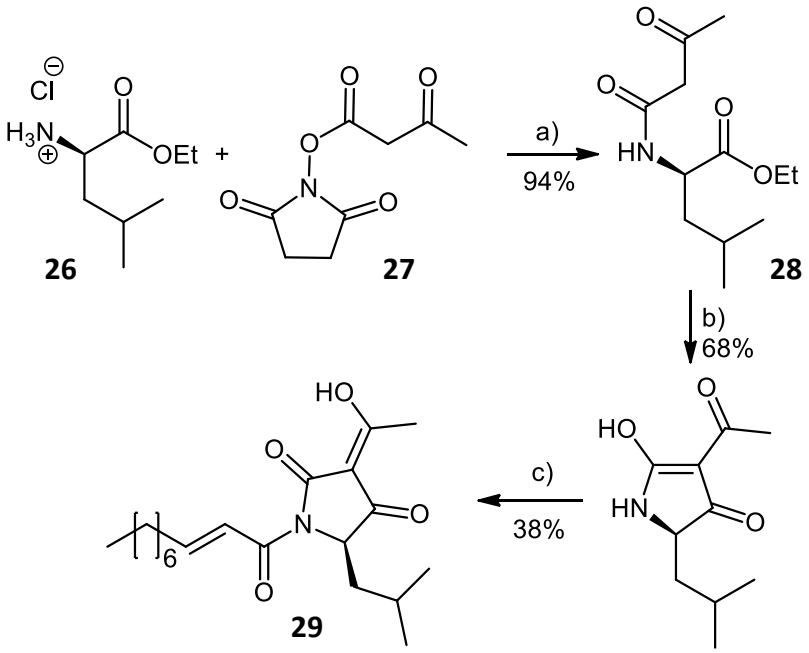
Scheme 2. Racemic synthesis of reutericyclin

Reagents and conditions: (a) $CH_2(COOH)_2$, $-CO_2$, (b) $SOCl_2$, (c) L-Leu-O^{t}Bu, (d) 50% TFA in CH_2Cl_2, -isobutene, (e) Meldrum's acid, DCC, DMAP, (f) Heat, -acetone, $-CO_2$, (g) AcCl, $TiCl_4$.

The synthesis requires a strong Lewis acid, $TiCl_4$, and also requires a highly controlled step to thermolyse the Meldrum's acid, which the authors state needs a specific temperature requirement to be reproducible at the yields they report.

This was a successful first synthesis but biological studies showed the racemic mixture exhibited slightly lower antibiotic activity than the naturally occurring enantiomerically pure reutericyclin[25]. Therefore an enantiospecific total synthesis was required, and was provided by Jung and his team again in 2005[26].

This new synthesis produces *R*-reutericyclin in 3 steps with an overall yield of 24% using chiral pool chemistry. Starting by coupling the *R*-leucine ethyl ester with N-hydroxysuccinimidoyl acetoacetate in dichloromethane, Jung *et al.* were able to add an activated ketoester to the amine. This modified amino acid could then be cyclised to the acyltetramic acid using an amended Dieckmann cyclisation with sodium ethoxide in ethanol, which was developed as standard conditions were unsuccessful. This acyltetramic structure then needed to be N-acylated to add the crucial dec-2-enoyl group, which was finally completed using *n*-butyllithium in a 38% yield after the lower effectiveness of other methods attempted, Scheme 3.



Scheme 3. Total synthesis of R-reutericyclin

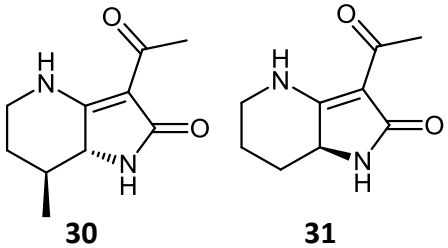
a)CH_2Cl_2, r.t., 3 h. b) EtONa/EtOH, 3 h. c) 1. THF, BuLi (2.2. equiv.), -70^{o}C; 2. (E)-dec-2-enoyl chloride; 3. H_3O^+

This synthesis was successful in producing enantiomerically pure *R*-reutericyclin from *R*-leucine in 3 steps, without requirement for chiral separation. This chiral pool chemistry retained the chirality from the starting amino acid, which was lost in the previous synthesis.

It is suggested by the authors that the NMR data for reutericyclin failed to identify a major tautomer as a crystal could not be produced and an oil was repeatedly produced after slow evaporation. Jung and co-workers also suggested his method might be suitable for mass production of the target.

1.3.2 –Laccarin A

In the search for possible targets and applications for the synthetic route being developed it became apparent that, although not specifically an acyltetramic acid, an analogue (**18**) of laccarin A (**30**) could be targeted, see Figure 10. The synthetic plan towards this interesting molecule is described later in this report.



30 **31**

Figure 10. Laccarin (30) and a synthetically feasible analogue (31)

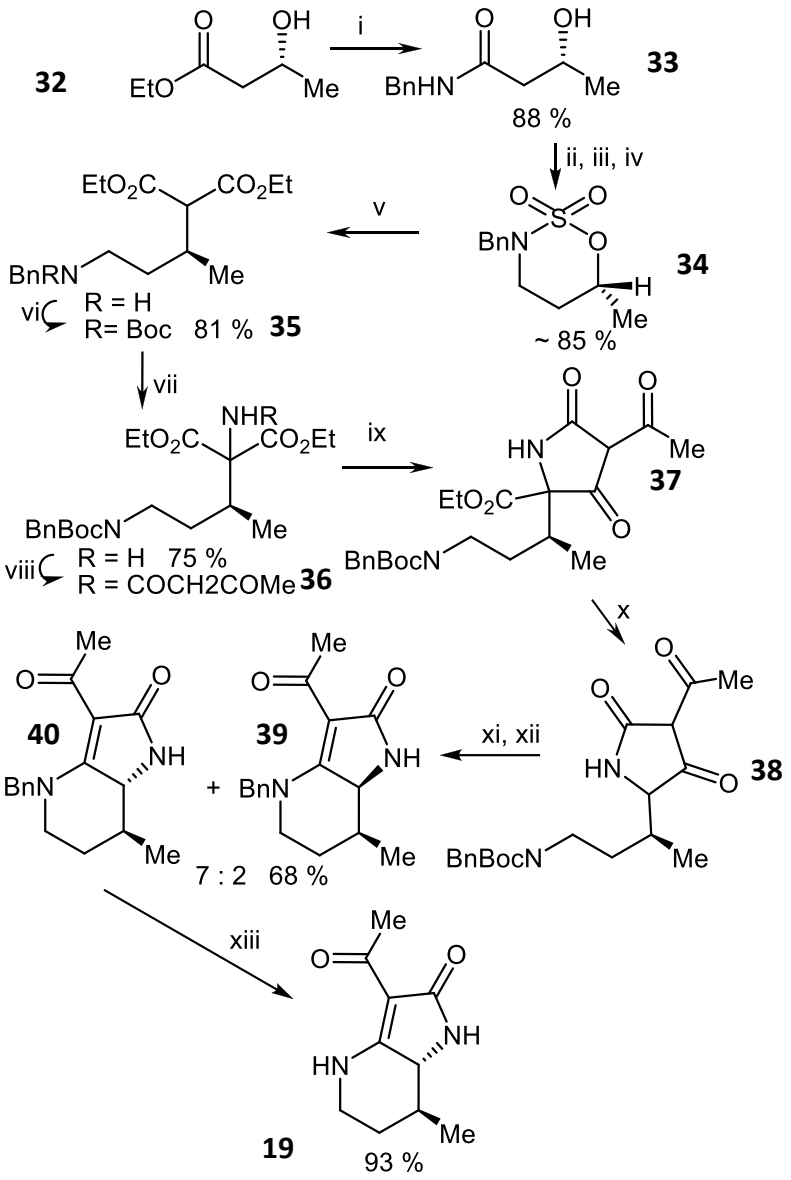
The widely growing but inedible mushroom fungus *Lactarius subplinthogalus*, found in China, has been extensively researched for its production of biologically active alkaloids[27,28]. In 1996, Matsuda *et al.* discovered laccarin and determined its structure based on spectroscopic evidence[29]. Further evidence was found confirming this structure and several similar compounds in 2003 by Wang *et al.* using more accurate spectroscopic methods[30].

Upon its first discovery, laccarin was shown to exhibit potent biological activity which allowed interest to grow in its synthesis and potential medical uses. The activity displayed was similar to that of tenuazonic acid (**3**) in its ability to moderately inhibit phosphodiesterase activity. It was also shown to contain phytotoxic and antineoplastic characteristics[29].

This biological activity displayed by this molecule and its small molecular weight caused it to be of interest to synthetic chemists, with a total synthesis of laccarin being reported in 2007 by Gallagher[31], see Scheme 4.

This first total synthesis, which is also an asymmetric synthesis, was developed by Gallaher *et al.* and remains the only published synthesis to date, obtaining the correct enantiomeric product in 13 steps with an overall yield of 18.4%. This is an impressive yield over 13 steps and the chemistry used is notable. Firstly, (3*R*)-hydroxybutyrate (**32**) had its ethyl ester converted into *N*-benzyl amide (**33**) which was subsequently reduced and the amino alcohol

formed protected as a cyclic sulfamidate (**34**). Ring opening was then achieved by addition of the sodium enolate of diethyl malonate and subsequent protection of the now free amine was completed (**35**) to ensure there would be no early formation of a lactam ring. An amino group was added using monochloroamine under basic conditions and this free amine was then acylated using diketene. Cyclisation of the pyrrolo unit by base was followed by decarboxylation using aqueous acid. TFA removal of the remaining Boc group led to the cyclisation of the second ring system which was shown to form the target (*trans*) and also its *cis* diastereoisomer in a 7:2 ratio (**40**:**39**). The two were separated and the *trans* diastereoisomer identified based on a detailed spectroscopic analysis and X-ray crystallography. Debenzylation of the product was then achieved to yield the target compound laccarin, whose structure was confirmed by detailed spectroscopic analysis, X-ray crystallography and direct comparison with the natural product.



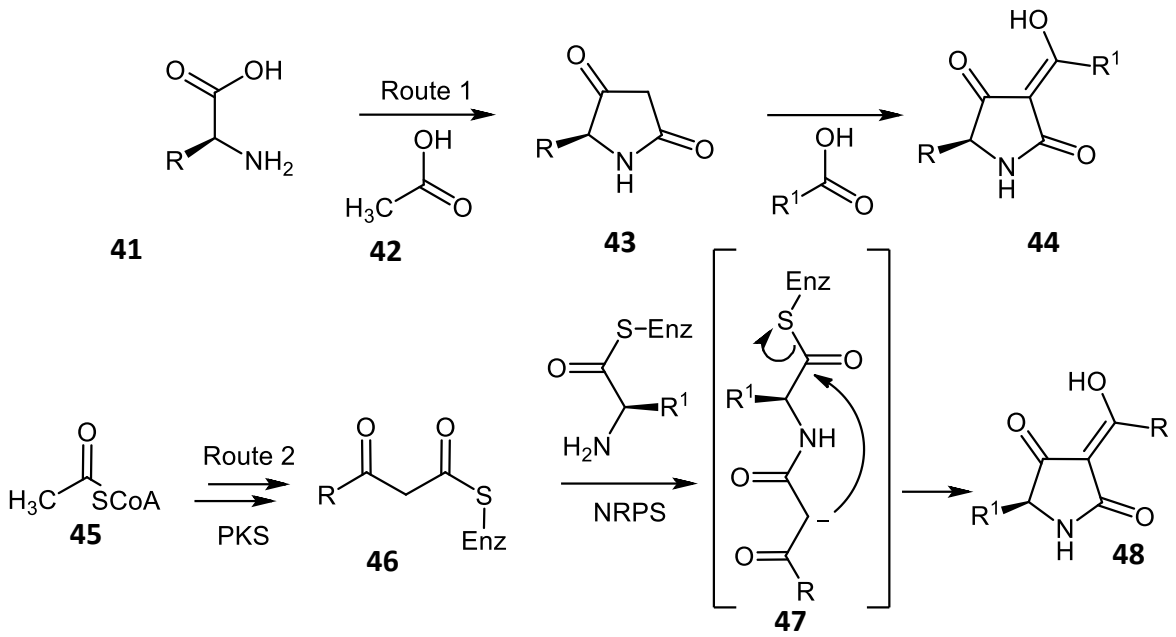
Scheme 4. Synthesis of Laccarin

Reagents and conditions: i, $AlMe_3$, $BnNH_2$, PhMe, 0 ^{0}C; ii, $LiAlH_4$, THF, reflux; iii, $SOCl_2$, Et_3N, imidazole, CH_2Cl_2, -20 ^{0}C to 0 ^{0}C; iv, $RuCl_3$ (0.25 mol%), $NaIO_4$, MeCN–H_2O, 0 ^{0}C; v, diethyl malonate, NaH, DMF, 110 ^{0}C; then 5 M HCl; vi, Boc_2O, $NaHCO_3$, MeCN; vii, NH_2Cl (Et_2O), t-BuOK, THF, 0 ^{0}C; viii, diketene, cat. DMAP, THF, 60 ^{0}C; ix, NaOEt, EtOH, 60 ^{0}C; x, H_2O, then 5 M HCl; xi, TFA, CH_2Cl_2 then Et_3N; xii, PhMe, 80 ^{0}C; xiii, 10% Pd/C (65 wt%), H_2 (5.5 bar), TFA.

1.4 – Suggested Biosynthesis of 3-Acyltetramic Acids in Natural Products

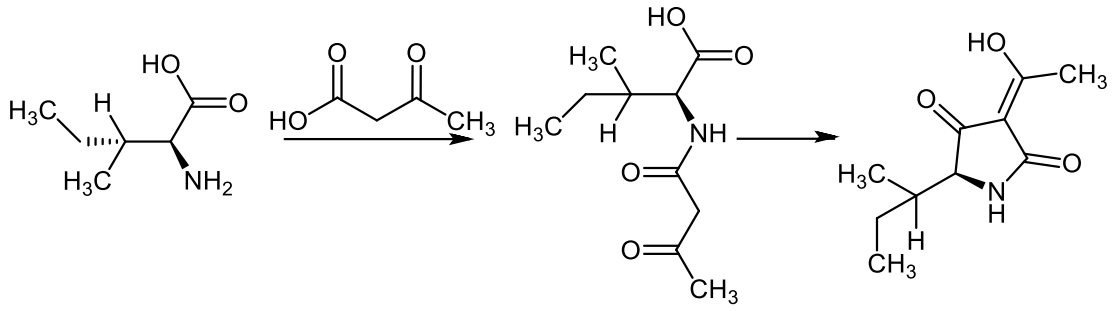
Some consideration must be given to the natural production of this diverse and interesting group of metabolites. The biosynthesis of such molecules has been investigated thoroughly due to their production in a multitude of different species including sponges, cyanobacteria, bacteria and fungi as well as their discussed biological activity, although it is still unknown what the genetic determinants for the biosynthesis of these compounds are in fungi and bacteria[32].

It has been proposed that 3-acyltetramic acids can be formed in biological systems via two main reactions, one involves the combination of an amino acid and an acetyl group followed by subsequent acylation at C-3 (route 1). The other notion comprises the cyclisation of an amidated polyketide chain, fashioned by polyketide synthases (PKS), forming the lactam ring in the presence of non-ribosomal peptide synthase (NRPS) in the cytoplasm spontaneously or enzymatically (route 2)[33–35], Scheme 5. It is worth noting that any N-acylation, as seen in reutericyclin and other examples, is assumed to occur before the lactam ring closure by the enzymatic addition of the relevant acyl substituent.



Scheme 5. Biosynthetic routes to the 3-acyltetramic acids

It was concluded by ^{14}C labelling of the substrates provided to a fungal broth in a feeding study that the intermediate **43** (route 1) was not observed, yet all intermediate substrates involved in route 2 (**45**-**48**), were seen in an individual example which was the biosynthesis of tenuazonic acid[1,36,37], Scheme 6.

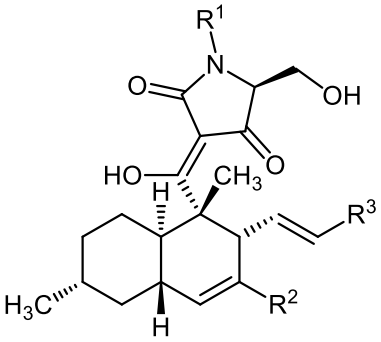


Scheme 6. Biosynthetic route to 3-acyltetramic acids

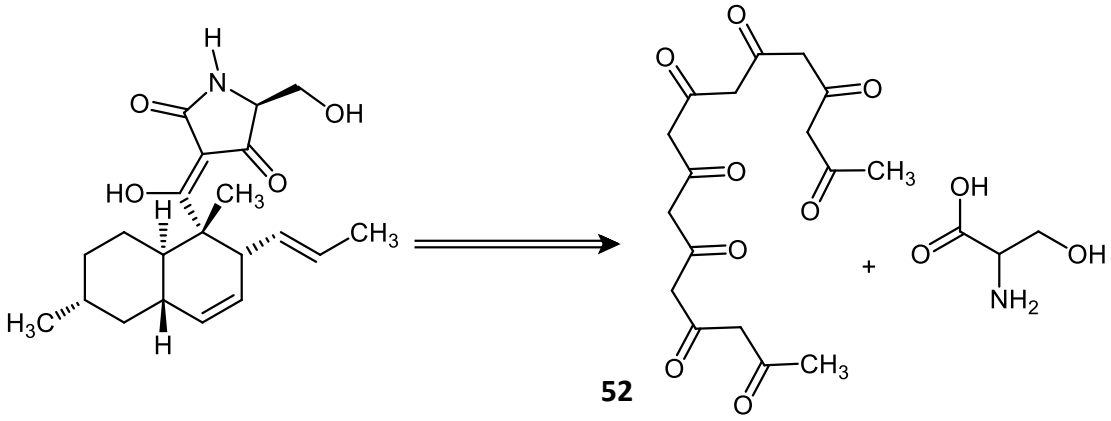
Numerous further studies have been conducted on this biosynthetic process, including that of Cox[38], in which he recently isolated a fungus, *Beauveria bassiana*, grown on spiders, that contain new tetramic acid derivatives. These genetic studies support route 2 of Scheme 5.

Other known biosynthetic pathways to popular tetramic acids include the assembly of trichosetin (**49**), equisetin (**17**) and phomasetin (**50**)[39]. It was found that these compounds, due to the similarity of large portions of the structure, are all made correspondingly from two separate reactants which can be abundantly produced by the organism. These units include an amino acid (in the case of trichosetin it was found to be L-serine (**51**) which can also be assumed to be used in the biosynthesis of the other two) and a series of eight acetate groups, known as an octaketide (**52**), joined in a linear manner, Scheme 7. It is assumed that upon the addition of these two units the (reduced) polyacetate tail undergoes an intramolecular Diels–Alder reaction to produce the modified bicyclic ring system.

Table 2. Different substituents in suggested Equisetin Biosynthesis



Number	R^1	R^2	R^3
49	H	H	CH_3
17	CH_3	H	CH_3
50	CH_3	CH_3	$CHCHCH_3$



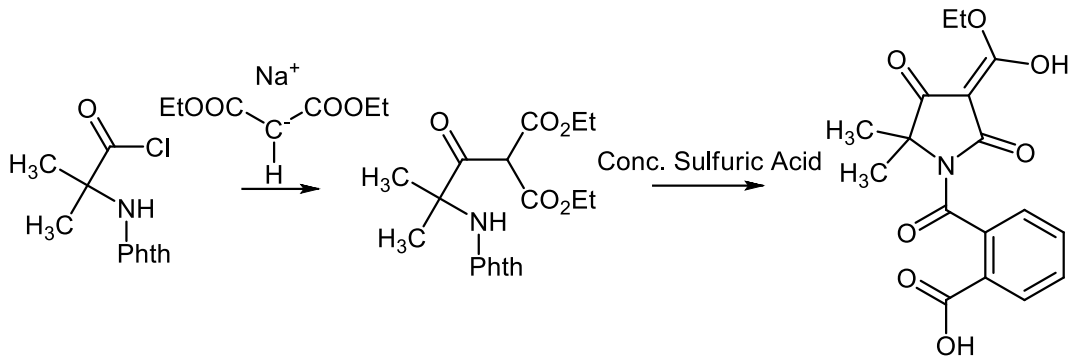
Scheme 7. A proposed biosynthetic origin of Trichosetin (49), Equisetin (17) and Phomasetin (50)

The intramolecular Diels-Alder reaction proposed in this suggested biosynthesis is common to natural products containing this ring structure[40].

1.5 – Selected General Synthetic Routes to 3-Acyltetramic Acids

Due to the large applications and challenging structural features of these tetramic acid analogues there has been substantial research into their synthesis. Both racemic and (later) enantioselective syntheses have been developed, with stereoselective syntheses being sought because only some enantiomers and diastereoisomers are biologically active. These routes will now be discussed.

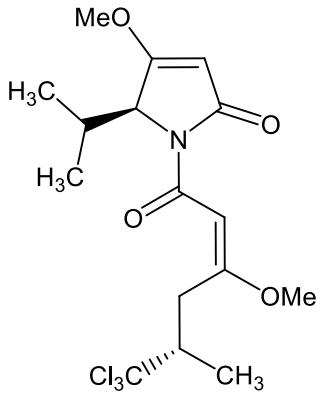
The first synthesis of a 3-acyltetramic acid was achieved by Gabriel in 1914[41,5] causing the ring closure by forming a new C(2)-N carbon-nitrogen bond. This was accomplished by reaction of phthalimidoisobutyryl chloride (**53**) with diethyl sodiomalonate to yield the addition-elimination product **54**. This, in turn, was subjected to strong sulfuric acid conditions which produced the novel tetramic acid **55,** Scheme 8.



53 **54** **55**

Scheme 8. The first published synthesis of a 3-acyltetramic acid derivative

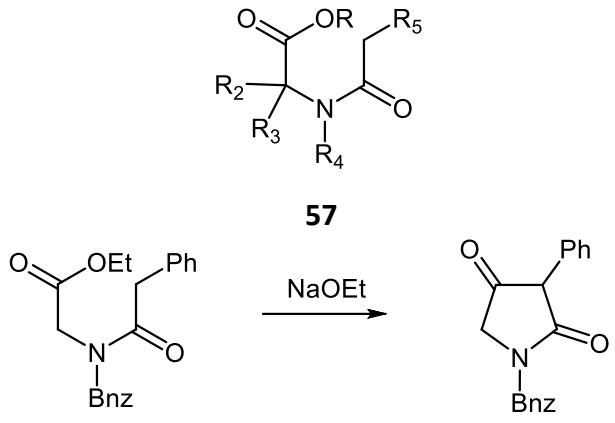
This method was successfully implemented in the 1984 racemic synthesis of natural product dysidin (**56**) by Willard and Laszlo[42]. Note that dysidin is not a 3-acyltetramic acid but does show some structural similarities, demonstrating the diversity in the uses of these synthetic strategies.



56

Figure 11. Dysidin, which was successfully produced using this early synthetic technique

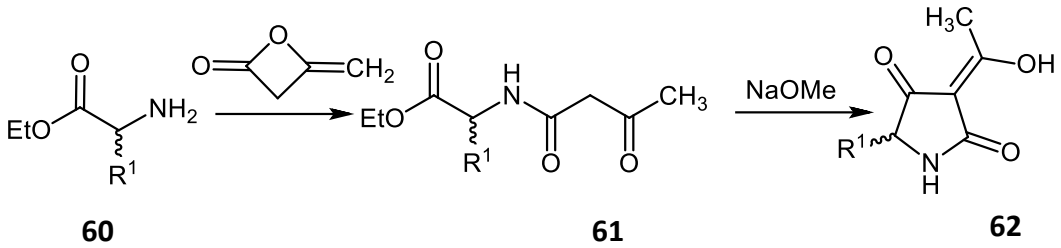
A second racemic synthesis, derived after the original 1914 work by Gabriel[5], was developed by means of a Dieckmann cyclisation which in effect is an intramolecular Claisen condensation, by King *et al.*[43,44] in 1950. This involved the use of an *N*-acyl-α-amino ester (**57**) in the basic conditions caused by sodium ethoxide and it was shown that a tetramic acid could be afforded but this method lacked the ability to add a 3-acyl group, although manipulation at C-5 was possible, Scheme 9.



Scheme 9. An example of the ring closure reaction to produce tetramic acids

This method was advanced by Lacey in 1954[45] to produce a 3-acetyltetramic acid from readily available amino acids. This was a major advance in the synthesis of racemic tetramic acids and is still the most prominent method used today. This widely popular method

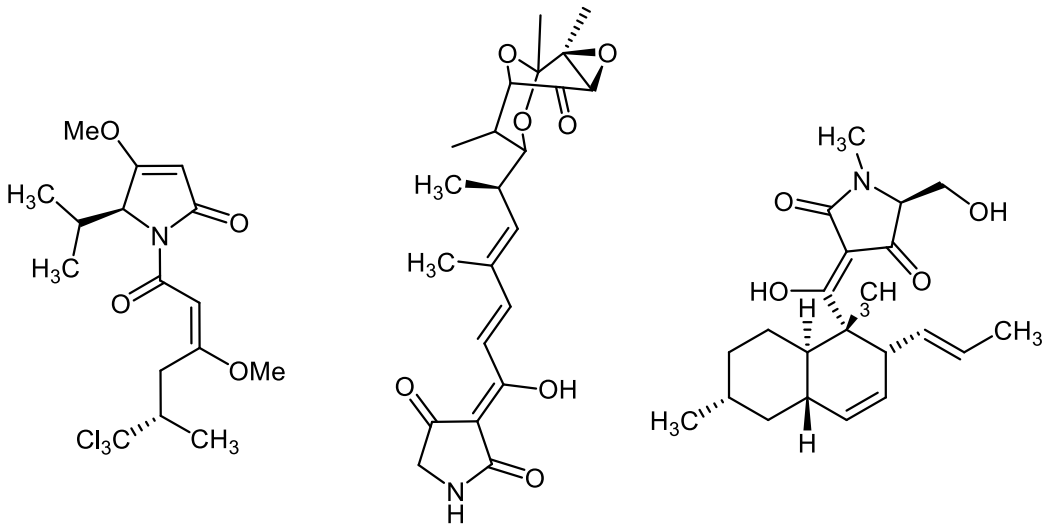
involves the addition of diketene to the *N*-terminus of any readily available amino acid (**60**) followed by Dieckmann cyclisation to give the corresponding 3-acetyltetramic acid (**62**). The diketene restricts variation at C-3 but the amino acid used provides a variety of possibilities at the C-5 substituent, Scheme 10.



60 **61** **62**

Scheme 10. 3-Acetyltetramic acid synthesis by diketene addition and ring closure

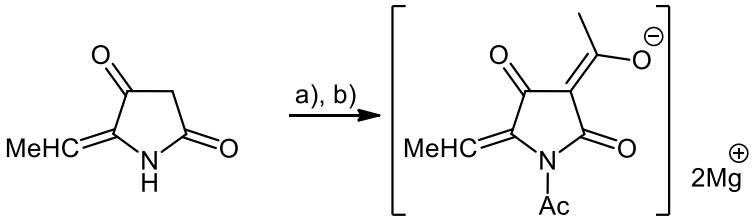
This method has been developed further to integrate new asymmetric synthetic discoveries, such as using chiral naturally occurring compounds to produce single diastereoisomers[46,47] that have been shown to exhibit desirable biological activity. These prepared compounds include (-)-dysidin (**56**) and (-)-tirandamycin A (**62**). In the early 1990's, this synthetic method was improved upon by Ley and others in order to introduce more enantiocontrol at the C-5 position, in Scheme 10 where R^1 is not hydrogen.[48,49] This control was assessed by using a variety of different bases in the base mediated ring closure of the final lactam ring, concluding that Lacey's original method of refluxing in methanol with sodium methoxide overnight was too harsh and led to further racemisation. The studies identified two conditions that were found to be more efficient in controlling the enantioselectivity, these were potassium *t*-butoxide in *t*-butanol for 5 minutes at room temperature and tetra-n-butylammonium fluoride (TBAF) in THF for 5 minutes at room temperature. Both provided excellent yields for the cyclisation product and retained the chirality in the starting material. This base mediated technique to close the ring, along with an intramolecular Diels-Alder reaction, was also built upon recently in a synthesis by Ley[50] in which a long conjugated chain closed thermally to form equisetin (**17**) from the thioester.



56 **63** **17**

Figure 12. (-)-Dysidin (56), (-)-Tirandamycin A (63) and Equisetin (17)

Another milestone in the synthesis of 3-acyltetramic acids was a technique first exploited by Kohl in 1974.[51] This involved forming the lactam ring through the Lacey–Dieckmann cyclisation seen above with an alkene in the R^1 position, without the 3-acyl substituent. This group was added later via a C-3 acylation using an acyl chloride and boron trifluoride-etherate, Scheme 14.

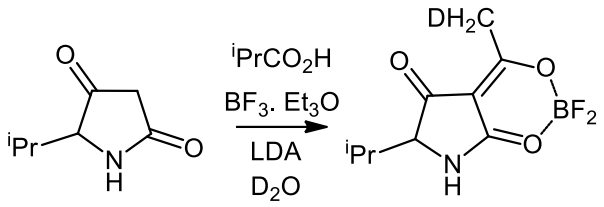


64 **65**

a) RCOCl, $BF_3.OEt_2$; b) Ac_2O, NaOAc, $MgCl_2$

Scheme 11. Kohl method of 3-Acylation on pyrrolidine-2,4-dione

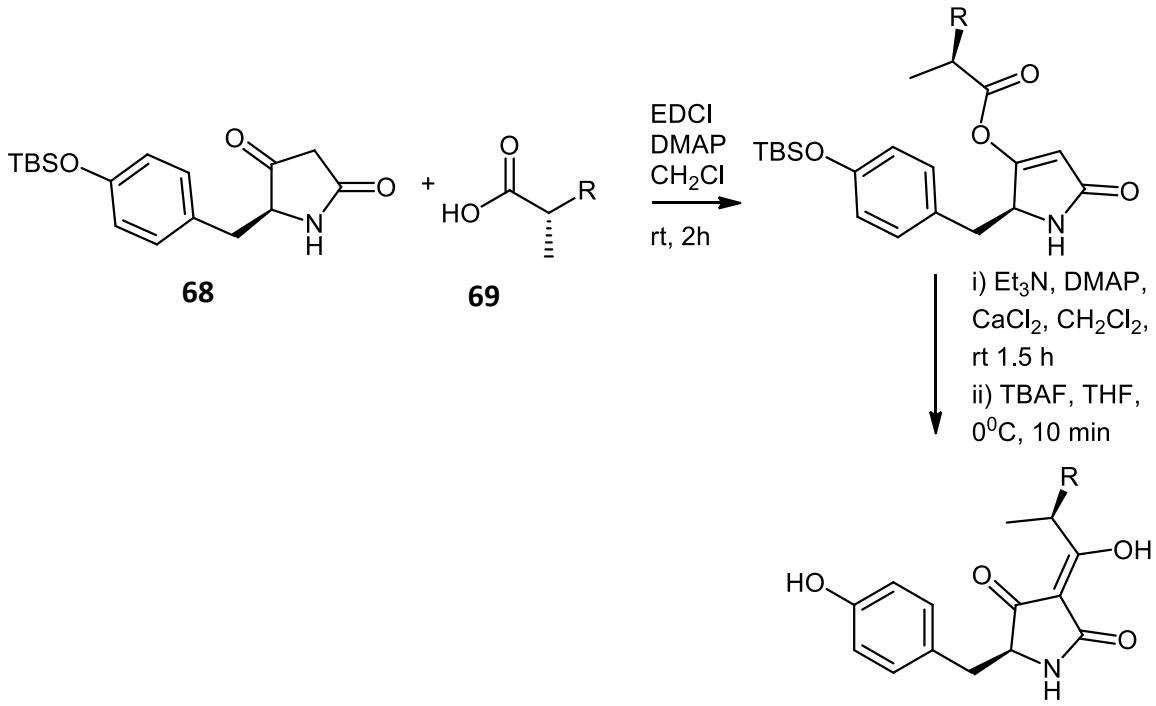
This strategy was later developed by Jones and colleagues in an attempt to produce complex naturally occurring products.[52] The preliminary results were not encouraging when using $TiCl_4$ as the Lewis acid, but using boron trifluoride with alterations to the work up the 3-acyltetramic acid products were able to be identified as their boron difluoride complexes and then isolated pure after liberation with methanol, Scheme 12[53,54]



66 **67**

Scheme 12. Jones method of 3-Acylation on pyrrolidine-2,4-dione

More recently, Yoda and colleagues have used a procedure involving the synthesis of the pyrrolidine-2,4-dione species and a 3-acylation was accomplished by an O- to C-acyl rearrangement.[55] This was developed in the synthesis of the antibacterial agent epicoccarine A and the key rearrangement step involved firstly, the coupling of the C-4 oxygen with an acid to form an ester which was then rearranged using 4-dimethylaminopyridine (DMAP) and calcium chloride, Scheme 13.

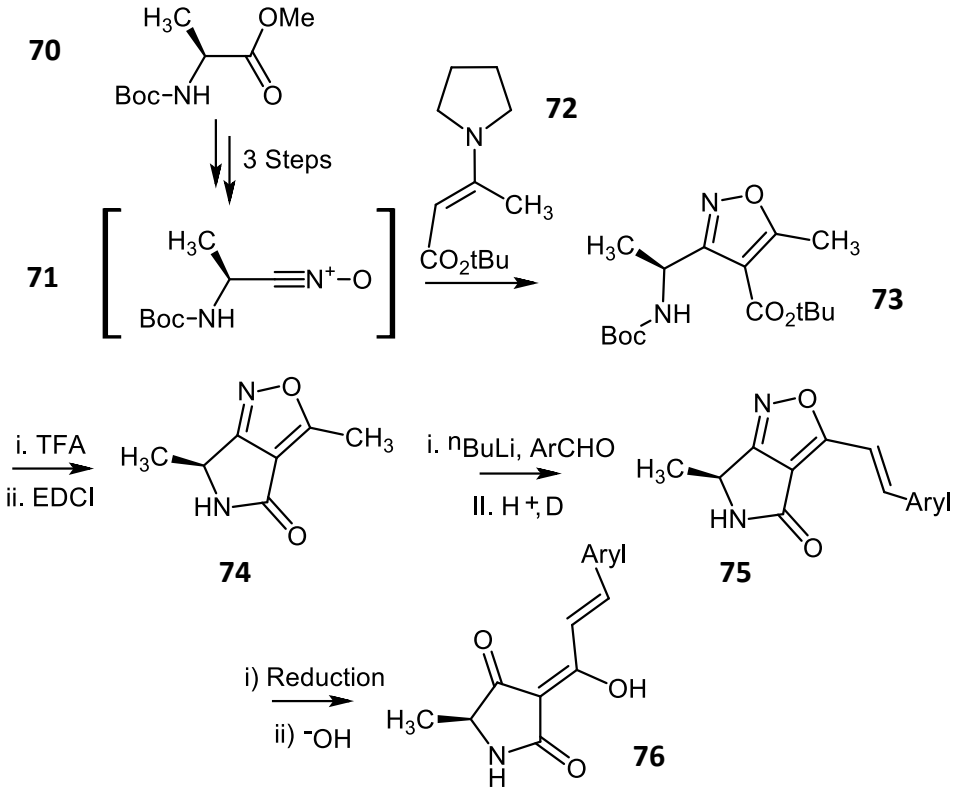


68 **69**

Scheme 13. Yoda method for O- to C-acyl rearrangement, where R is a long alkene with multiple double bonds and branched substitution

Due to the wide use of these previously discussed methods and their variants there has been a lack of new developments until Jones *et al.* devised a method where the tricarbonyl tetramic acid structure is masked as a pyrroloisoxazole to allow elaboration at the C-3

position[56,57]. This method has moved into its second generation and involves the 1,3-dipolar cycloaddition of a nitrile oxide, that originates from an amino acid ester hydrochloride, and an enamine. This produces an isoxazole that can then be deprotected via the use of an acid and subsequently, by using a peptide coupling reaction, the pyrrolo-ring can be closed. This now provides an acidic proton on the methyl group that is directly bound to the pyrroloisoxazole core and will become, after nitrogen-oxygen bond reduction to the 3-acyltetramic acid, the 3-acyl group. This method therefore allows flexible manipulation of the C-3 acyl groups, Scheme 14.

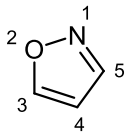


Scheme 14. A synthesis of 3-acyltetramic acids involving masking the core as a pyrroloisoxazole

The research work undertaken in this thesis follows on directly from this methodology.

1.6 – Isoxazoles

An isoxazole (or 1,2-oxazole) is an azole, or 5-membered ring including an integrated nitrogen, with an oxygen atom directly bonded to the nitrogen atom and formally containing one double carbon-carbon bond and one double nitrogen-carbon bond (**77**), see Figure 13.

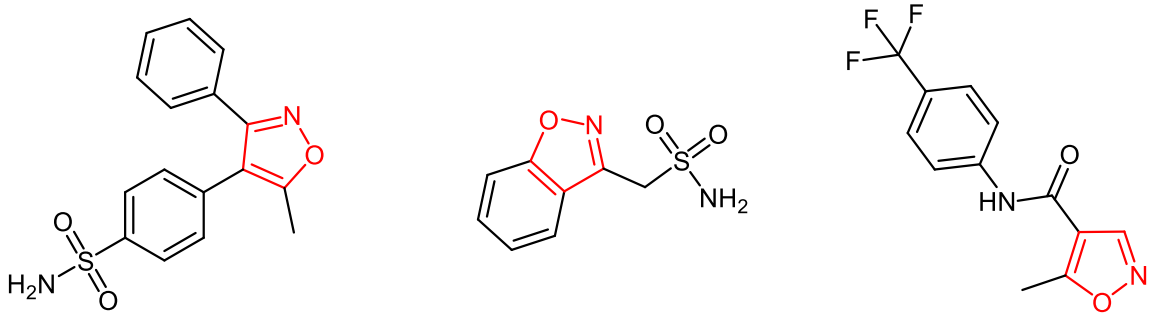


77

Figure 13. Isoxazole

Isoxazoles are important due to their aromaticity, their presence in biologically active products and simple synthesis. Although the targets chosen in this research do not include the isoxazole unit, as it is used as an intermediate in the route, it is important to understand the uses of such compounds and their possible syntheses.

The occurrence of isoxazoles in medicinal drugs is very widely known as they have been demonstrated as being used to treat psychological conditions, obesity, migraines, general anti-inflammatory and specific illness or disease states. Some examples of these commonly used drugs are Valdecoxib[58] (**58**), Zonisamide[59] (**59**) and Leflunomide (**60**)[60], see Figure 14.



78 **79** **80**

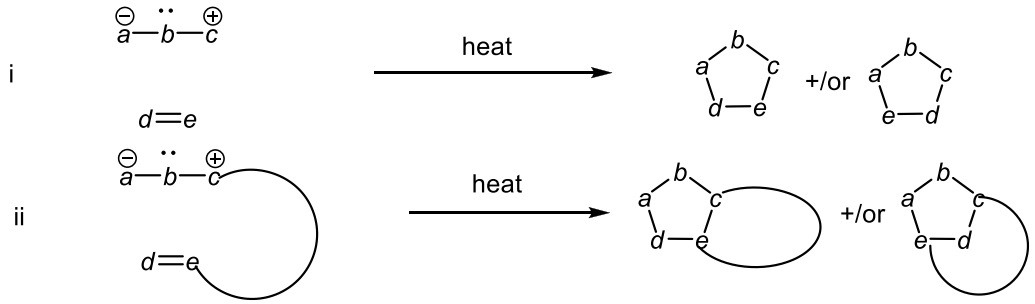
Figure 14. Examples of isoxazoles in medicinal drugs: Valdecoxib (78), Zonisamide (79) and Leflunomide (80).

Due to these biological applications, the general interest in isoxazoles and their physical aromatic properties, there are traditionally two routes known towards isoxazole systems.

The first isoxazole was developed in 1888 by Claisen using hydroxylamine to cyclise β-keto esters and yield 3-hydroxyisoxazoles[61]. This route can be used with various 1,3-dicarbonyl compounds. The more common method of making isoxazoles is now to implement a 1,3-dipolar cycloaddition, which was to be the selected method for the production of the isoxazole required in this research. The 1,3-dipolar cycloaddition will now be discussed in more detail.

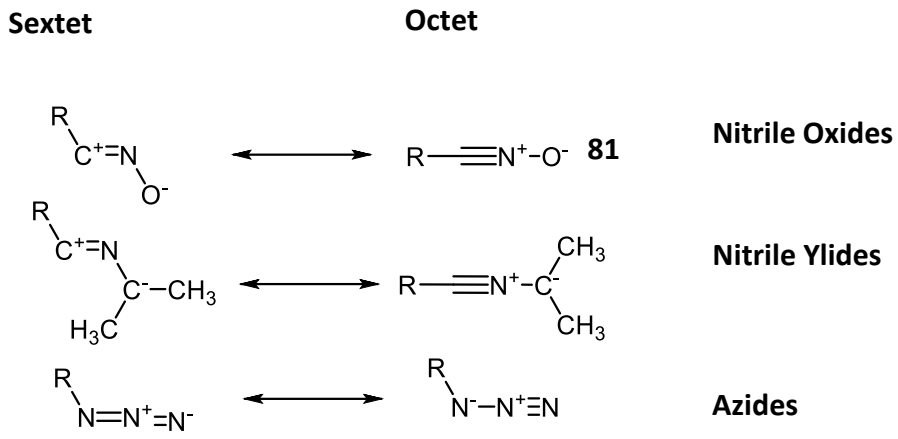
1.7 - 1,3-Dipolar Cycloaddition

As previously discussed, isoxazoles can be formed by a 1,3-dipolar cycloaddition, but the scope of a 1,3-dipolar cycloaddition goes much further than just the synthesis of these useful molecules. The 1,3-dipolar cycloaddition (or [3+2]-cycloaddition) can be used to accomplish a large variety of reactions in which 5-membered heterocycles are formed. The general scheme is that 1,3-dipolar compounds are added across double bonds causing the formation of two new σ bonds and a new ring, which has been demonstrated to be very efficient in general alkaloid and asymmetric synthetic pathways[44,45]. The next chapter has in depth discussion of the uses of just one of these possible 1,3-dipoles, a nitrile oxide (**81**). Another interesting feature is that the 1,3-dipolar cycloaddition can occur intermolecularly and intramolecularly, with the only requirements being a 1,3-dipole (typically represented *a*-*b*-*c*) being sterically able to approach a multipule bond (typically represented *d*=*e*), see Scheme 15.

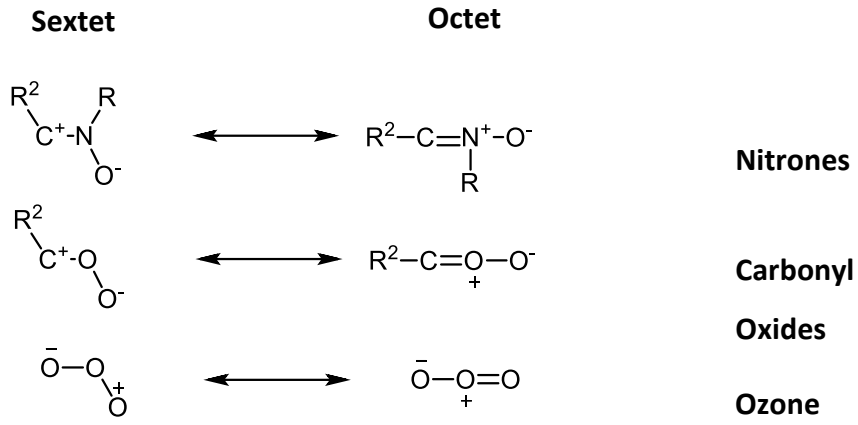


Scheme 15. 1,3-dipolar cycloaddition, where i) intermolecular and ii) intramolecular

A 1,3-dipole is an extremely polarised system, also called a zwitterion, that contains charge separated across three bonded atoms with four π electrons distributed between them, shown above as *a*-*b*-*c*. Some commonly used 1,3-dipoles are shown in Scheme 16 and Scheme 17; note that there are type 1 and 2 dipoles. Both dipoles have a sequence of *a*-*b*-*c*, where *c* has a sextet of electrons available in its outer-shell and *a* has an octet of electrons but vitally, *b* must contain at least one unshared pair of electrons to stabilise the sextet terminus. Type 1 dipoles possess a double bond in their sextet canonical forms and a triple bond in other canonical forms, see Scheme 16. Type 2 dipoles possess a single bond in their sextet canonical forms and a double bond in other canonical forms, see Scheme 17[64].



Scheme 16. Some examples of Type 1 1,3-dipoles in both the sextet and octet forms



Scheme 17. Some examples of Type 2 1,3-dipoles in both the sextet and octet forms

Dipolarophile is the name given to the other species required for this kind of cycloaddition to occur. Dipolarophiles contain two π electrons and are commonly carbon-carbon π-systems, such as alkenes and alkynes. Examples of heteronuclear diatomic molecules in a π bond are carbonyls, nitriles and thials.

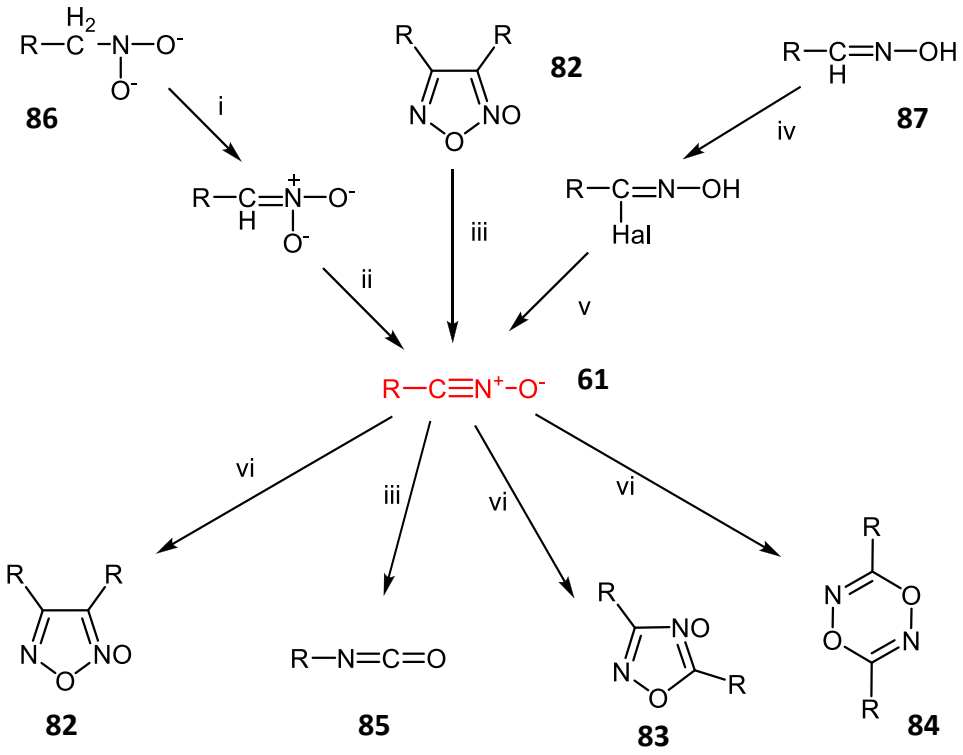
It should be noted that some systems shown require formation *in situ* due to their instability; reactions are still possible with the above examples yet, not all with carbon-carbon dipolarophiles[65]. It is also known that not all dipolarophiles, or even alkenes, are successful in a 1,3-cycloaddition. For a successful cycloaddition it is usual that the dipolarophile must be considered a good dienophile in the Diels-Alder reaction. The 1,3-cycloaddition has been found to be stereospecific and *syn* or suprafacial, meaning both

bonds of the new ring form on the same face of the double bond[66]. The orientation of the dipolarophile and the 1,3-dipole is deduced by considering the Frontier Molecular Orbitals[67], which will be discussed later in more detail.

In order to produce the 1,3-dipolar cycloaddition required to obtain the desired isoxazole product, it was decided the 1,3-dipole needed was a nitrile oxide, which had to be formed *in situ*. Nitrile oxides will now be discussed in more depth.

1.8 – Nitrile Oxides

The chemistry of the nitrile oxide species (**81**, Scheme 16) has been extremely well documented and is well appreciated in the synthetic chemistry community with reactions dated back to 1896 and through to the present[68,69]. The nitrile oxide species is traditionally used to prepare several different other functionalities including its ability to dimerise to furoxans (**82**), 1,2,4-oxadiazole-4-oxides (**83)**, 1,4,2,5-dioxadiazines (**84**) and isomerise to isocyanates (**85**)[70], see Scheme 18.



Scheme 18. Reaction of and forming nitrile oxides, where i) Et_3N, ii) PhNCO, iii) heat, iv) NCS, v) base, vi) dimerisation

The synthesis of nitrile oxides has therefore been extensively researched and there are a number of different commonly used techniques for forming them *in situ*. For example, they can be formed from aliphatic nitro compounds (**86**) in a process known as the Mukaiyama procedure[71], formed by cycloreversion from (**82**)[72], or from an aldoxime (**87**) in fundamentally an overall dehydrogenation (oxidation) process, which was the chosen method for this research, see Scheme 18[70].

1.9 – Frontier Molecular Orbital Theory

As previously stated, the orientation and stereoselectivity of 1,3-dipolar cycloadditions can be explained using Frontier Molecular Orbital (FMO) theory. FMO theory, first suggested by Fukui in 1954[73], takes into consideration the state of the electrons in the orbitals that wil undergo bonding, which for a dipole and a dipolarophile is sigma (σ) and pi (π) bonding, see Figure 15.

Figure 15. Sp^3 and p Orbitals

Using the shading shown it is possible to see different phases, where the shaded area can be considered as + and the lighter area as -. The coming together of these can differ depending on their orientation, for example if a + and a – area align (where + and – represent the sign of the wave function), the energy requirement is higher and it is known as anti-bonding. If, oppositely, a + and +, or – and -, align the energy requirement is lower and it is known as bonding[74]. This information can be used to predict or explain regiochemistry in 1,3-dipolar cycloaddition products, see Figure 16 and Figure 17.

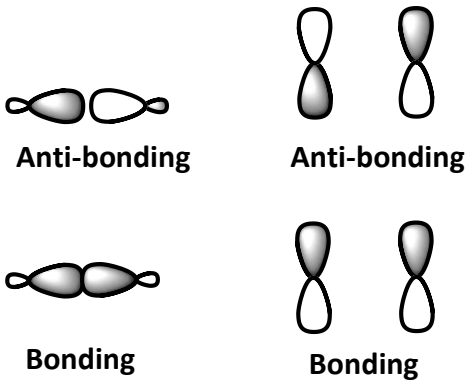


Figure 16. Bonding and anti-bonding in σ and π orbitals

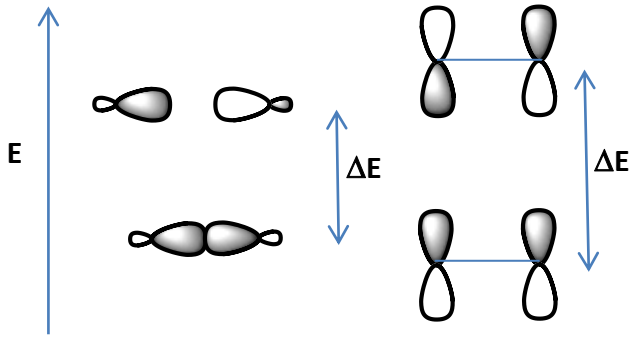


Figure 17. Energy difference in σ and π Orbitals

In terms of electrons in these orbitals, there are two energy levels to be considered. The highest occupied molecular orbital (HOMO) contains the electrons to be donated in the formation of new covalent bonds. The orbital into which these electrons are to go would be the lowest unoccupied molecular orbital (LUMO) of the other component. The interactions of these orbitals are dominant in determining the orientations observed in standard 1,3-dipolar cycloadditions, but all filled and empty combinations contribute to the bonding. It has been deduced that there are three different types of FMO combination for cycloadditions, each describing which orbital determines the regiochemistry in the product based on the energy differences. It is known that electrophilic dipolarophiles have a lower energy LUMO whilst nucleophilic dipolarophiles have a higher energy HOMO[74]. This relationship has been shown in Figure 18, where type 1 is HOMO-determined with HOMO of dipole and LUMO of dienophile as dominant interaction, type 2 is either HOMO- or LUMO-determined with both interactions significant, and type 3 is LUMO-determined with LUMO of dipole and HOMO of dienophile as dominant interaction.

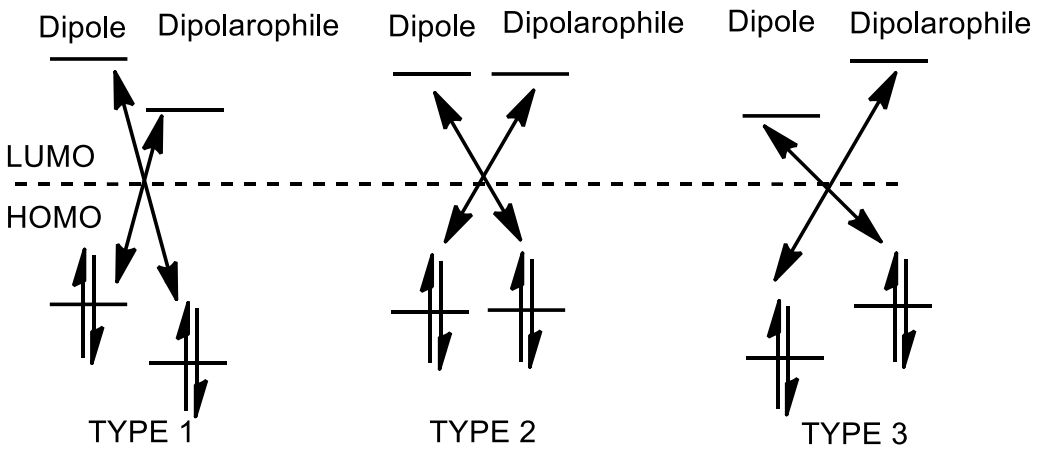


Figure 18. Illustration of different energy levels required depicting which orbital determines stereoselectivity[75]

Using the methodology we had selected, which involved using a nitrile oxide, an electron rich dipole, coupled with an enaminoester, an electron deficient dipolarophile, we could propose that we have a high energy HOMO and a low energy LUMO[74], lending itself to a type 1 reaction.

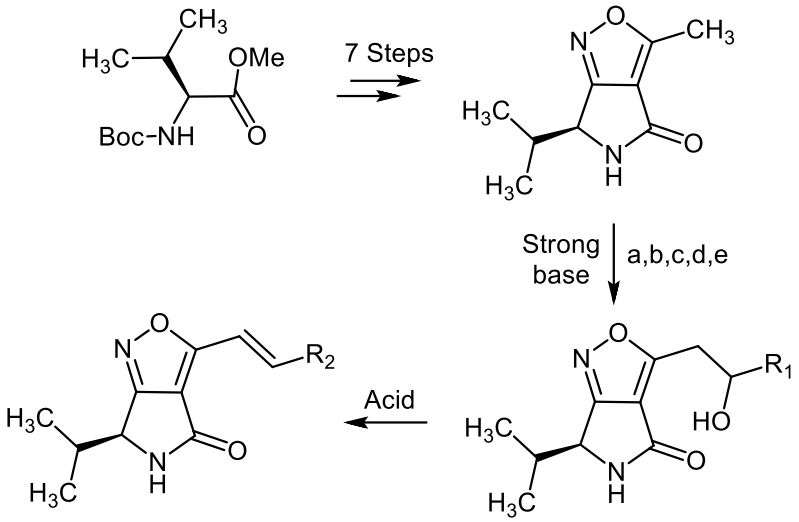
However, it is important to note that FMO theory can predict incorrect regiochemistry[76]. This can arise when electrostatic or steric effects occur. For example in an intramolecular system the hindrance caused could lead to another regiochemistry being adopted. This is often planned into the experimental methodologies for these reactions. In respect of what is planned for this research, it is intended to use an enamine with a substituted carboxyl group (electron withdrawing) and pyrrolidine group (electron donating). This way the FMO theory and electrostatic effects can combine to give a better reaction yield. This information was used in the synthetic plan for the research undertaken, which will now be discussed in more detail.

1.10 - Synthetic Aim

1.10.1 – Pyrroloisoxazole Formation and Elaboration

The research undertaken in this project was prompted by the second generation of the scheme developed by Jones *et al.*[57] which included the formation of an isoxazole via 1,3-cycloaddition[77] of a nitrile oxide and an enamine. This is then subsequently converted to the pyrroloisoxazole and thus, allowing manipulation of the potential C-3 tetramic acyl unit as well as the nitrogen in the lactam ring. A general aim was to construct natural and unnatural examples of acyltetramic acids using a methodology revolving around a 1,3-dipolar cycloaddition and have them tested for biological activity.

An initial aim of this research was to successfully construct the pyrroloisoxazole ring system from a selection of readily available amino acids and prepared enamines. Primarily, these pyrroloisoxazoles were then to be treated with strong base in excess, deprotonating at N-1 and at the C-3 methyl position, then utilising aldol reactions at C-3 to add a range of enolisable aliphatic aldehydes with a range of structural features including long and short chain lengths, α-substitution and unsaturation. The alcohol adducts would then be subjected to acid leading to the dehydrated unsaturated product, (Scheme 19). This project leads on from earlier work that successfully utilised non-enolisable aldehydes, such as benzaldehydes to these same pyrroloisoxazole systems under thermodynamic enolate generation[57]. These reactions are more well-known[56] and therefore an investigation into the less known reactions of enolisable aldehydes was required.



93 – R_2 – $CH_2CH_2CH_2CH_3$

94 – R_2 – $CH_2(CH_2)_7CH_3$

95 – R_2 – $CH_2[CH_3]_2$

96 – R_2 – C_6H_{11}

97 – R_2 – $CHCHCH_3$

88 – R_1 – $CH_2CH_2CH_2CH_3$

89 – R_1 – $CH_2(CH_2)_7CH_3$

90 – R_1 – $CH_2[CH_3]_2$

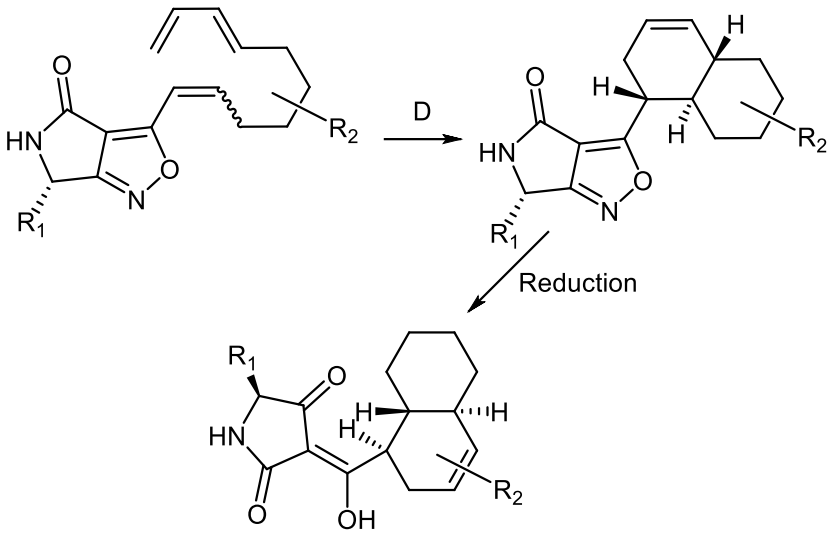
91 – R_1 – C_6H_{11}

92 – R_1 – $CHCHCH_3$

(Reagents: (a) Pentanal, (b) Decanal, (c) Isobutyraldehyde, (d) Cyclohexanecarboxylaldehyde, (e) Crotonaldehyde)

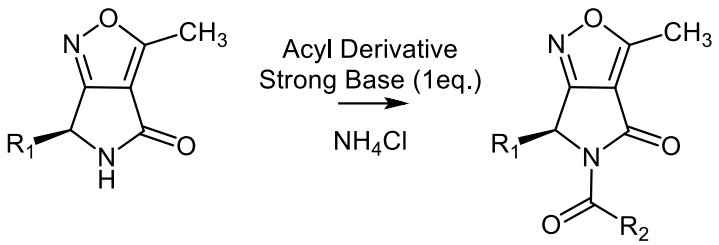
Scheme 19. The initial aims of this research

These elaborations at the C-3 position are investigations towards the possibility of adding long chain unsaturated aldehydes that could later undergo intramolecular Diels-Alder cyclisation and produce tetramic acids that contain a bicyclic system in the 3-acyl group such as seen in trichosetin (**49**), equisetin (**17**) and phomasetin (**50**)[39], Scheme 20.



Scheme 20. The proposed synthesis of a compound structurally similar to equisetin (17)

The second form of elaboration to be attempted on these pyrroloisoxazole systems was to use one equivalent of strong base, which would deprotonate at the amide of the lactam ring. This could then be used in *N*-acylation reactions to add functionality here as seen in tetramic acids such as the previously discussed reutericyclin, among other examples. After the acylation using a range of substrates (including long and short chained aliphatic, aromatic and sterically hindered acyl derivatives), see Scheme 21, the later discussed pyrroloisoxazole reduction will be implemented to achieve the *N*-substituted 3-acyltetramic acids.



a – R_1 – $CH(CH_3)_2$

b – R_1 – $CH_2CH(CH_3)_2$

c – R_1 – C_6H_5

98 – R_2 – $CH_2CH_2CH_3$

99 – R_2 – $CH_2(CH_2)_7CH_3$

100 – R_2 – $C(CH_3)_3$

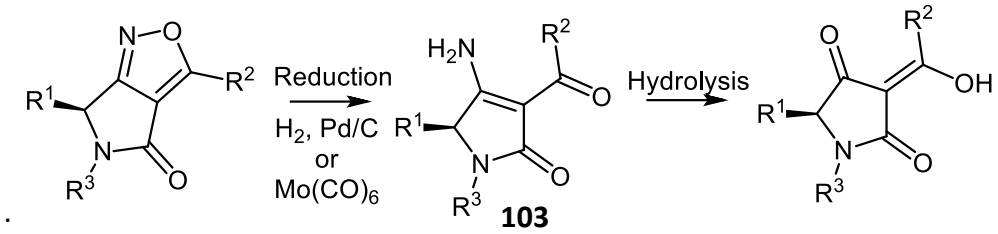
101 – R_2 – $CHCHCH_3$

102 – R_2 – Ph

Scheme 21. *N*-Acylation of the pyrroloisoxazole

1.10.2 – Pyrroloisoxazole Reduction to 3-Acyltetramic Acid

The reduction of the isoxazole could present some issues as the unmasking of the 1,3-dicarbonyl moiety occurs in two stages, (i) the reduction of the nitrogen-oxygen bond yielding the enaminoketone, (ii) hydrolysis leading to the substitution of the amine group to give an enolised 1,3-diketone, Scheme 22. Two reductive methods have been developed for the formation of the enaminoketone (**103**) involving hydrogenation, for use in the case of no unsaturation in the added side chains, or using molybdenum hexacarbonyl where there are other reducible groups.[78,57,75] A potential issue in the hydrolysis step is deacylation where *N*-acyl derivatives are concerned.

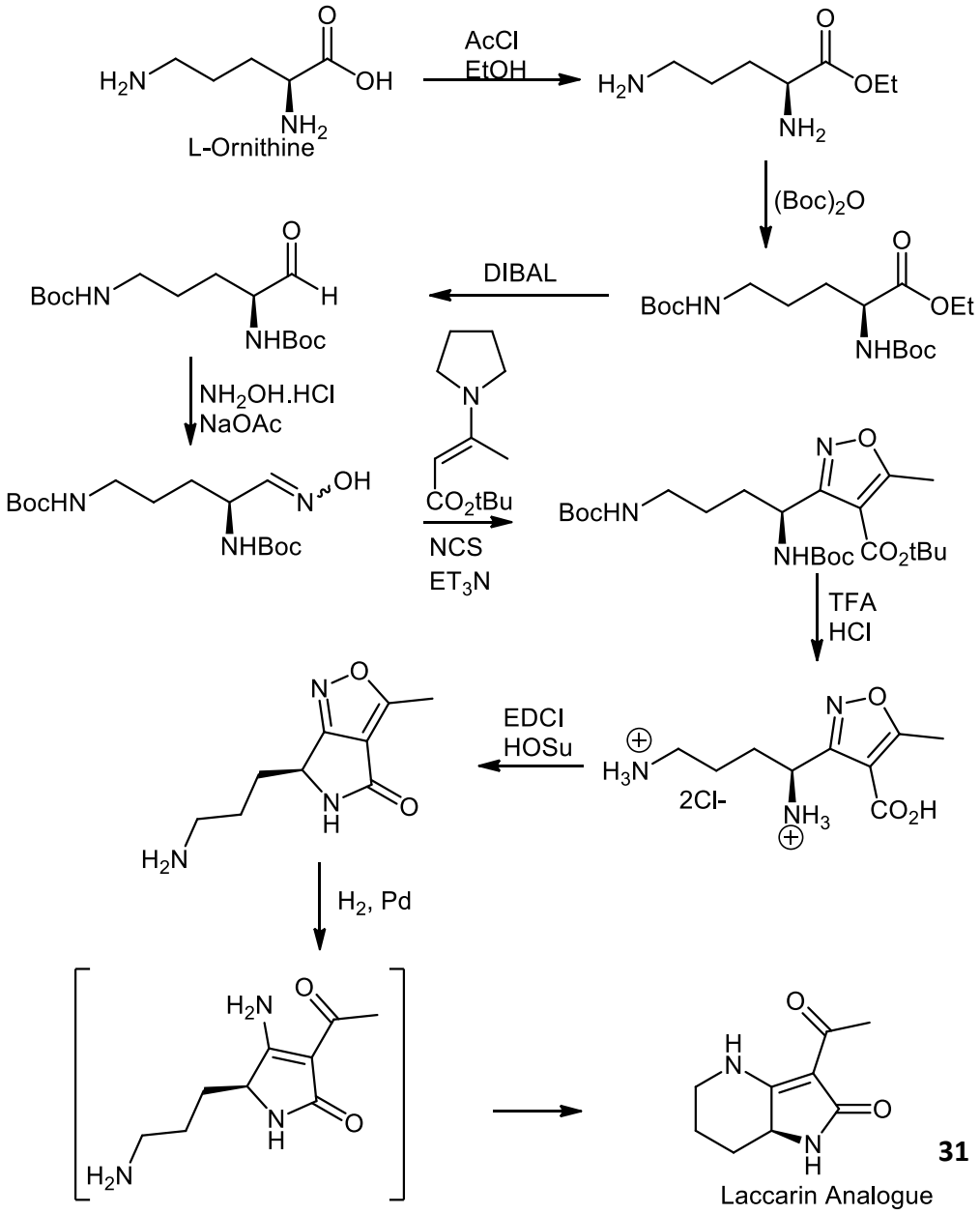


R^1 = H, CH_3, $CH(CH_3)_2$
R^2 = $CH_2CH_2C_6H_5$, $CHCHC_6H_5$
R^3 = H, $C(O)CH_2CH_2CH_3$

Scheme 22. Unmasking the pyrroloisoxazole

1.10.4 – Laccarin Synthesis by Means of Dipolar Cycloaddition Route

As previously stated, laccarin and possible analogues became of interest as research targets when it was confirmed the pyrroloisoxazole could be synthesised effectively. The proposal was to start with commercially available L-ornithine, bis-Boc protect and react through to the pyrroloisoxazole with the above described methodology. The idea was then to reduce the isoxazole to the corresponding enaminoketone and allow the terminal amine to perform an intramolecular nucleophilic substitution reaction to form the second ring in the bicyclic product (**31**), see Scheme 23.



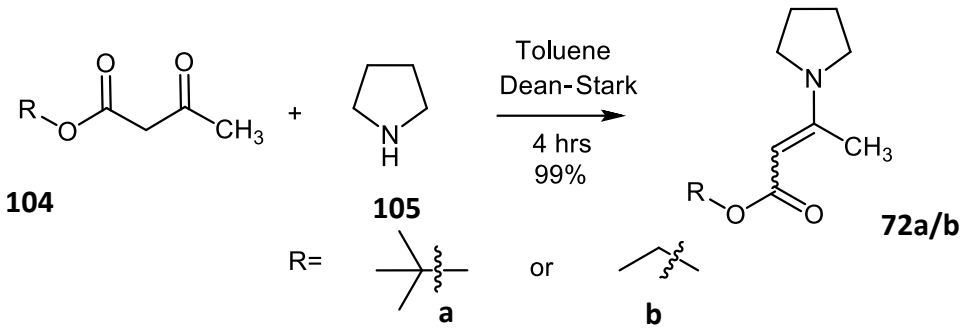
Scheme 23. Scheme for potential synthesis of a laccarin analogue

2.0 – Results and Discussion

2.1 – Pyrroloisoxazole Formation using *tert*-Butyl 3-(pyrrolidin-1-yl)but-2-enoate and L-Valine or L-Leucine

2.1.1 – Preparation of a Cycloaddition Reactant, the Dipolarophile

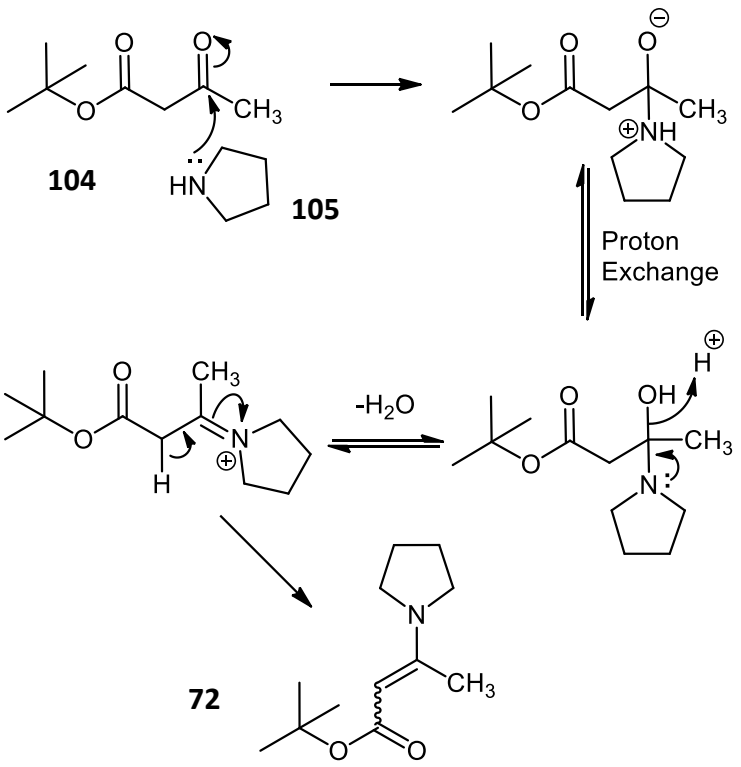
In order for the planned 1,3-dipolar cycloaddition reactions, we needed to produce a dipolarophile with a suitable leaving group substituted on the double bond as it would need to be readily eliminated after the cycloaddition driven by the aromaticity gained in the isoxazole. It was deduced that the best group to use as a dipolarophile was an enamine containing a pyrrolidine group that would self-eliminate after cycloaddition. The other characteristic that this enamine required was a protected acid group that could, eventually, close the pyrrolidine ring in a peptide bonding fashion with an amine group derived from an amino acid. It was therefore reasoned that a reactant containing these characteristics could be easily made by the addition of pyrrolidine to *tert*-butyl acetoacetate or ethyl acetoacetate under Dean-Stark conditions, Scheme 24.



Scheme 24. The production of an enamine for the 1,3-dipolar cycloaddition

The reaction involved heating the reactants with toluene in a Dean-Stark apparatus and a condenser. The solution was heated to reflux for 4 hours and confirmation of completion of the reaction was determined by the condensation of the calculated amount of water. This solution then required no washings and was simply evaporated under reduced pressure to yield the product, either *tert*-butyl 3-(pyrrolidin-1-yl)but-2-enoate (**72a**) or ethyl 3-(pyrrolidin-1-yl)but-2-enoate (**72b**), both as yellow powders in excellent yields, 97-99%.

The *tert*-butyl 3-(pyrrolidin-1-yl)but-2-enoate (**72a**) and ethyl 3-(pyrrolidin-1-yl)but-2-enoate (**72b)** were identified by clear corresponding signals in the IR spectra, the appearance of a singlet at δ = 4.5 ppm for the alkene proton and 85.0 ppm for the methine carbon in the ^{1}H and ^{13}C NMR spectra, respectively, and mass spectrometric identification of the molecular ions. These reactants were produced as a stock intermediate, under the assumption that they would not degrade if stored away from moisture and therefore would be available on demand as and when the oxime (and hence dipole) was produced. A suggested mechanism for production of the enamine via dehydration is shown in Scheme 25 using producing *tert*-butyl 3-(pyrrolidin-1-yl)but-2-enoate.



Scheme 25. A proposed mechanism for the enamine production.

Also important to note is that the scale of the reaction did not affect the yield produced as is commonly seen in some synthetic procedures. 99% Yield was achieved when reacting 10 g of pyrrolidine and 20 g of *tert*-butyl acetoacetate to give 26.4 g of enamine product as well as when using 1.0 g of pyrrolidine and 2.0 g of *tert*-butyl acetoacetate producing 2.60 g of product.

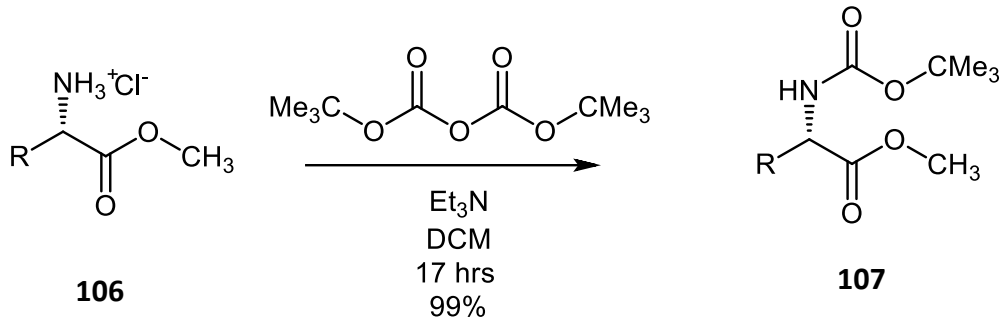
2.1.2 – Preparation of a Cycloaddition Reactant, the Dipole via an Oxime

The assembly of this oxime, to be used in the 1,3-dipolar cycloaddition as precursor to a 1,3-dipole, requires 3 individual steps. The oxime was chosen as a suitable precursor as it could be oxidised to a nitrile oxide by chlorination – dehydrochlorination, in Scheme 18.

The oxime synthesis began with a commercially available amino ester, initially L-valine methyl ester hydrochloride but later L-leucine methyl ester hydrochloride.[79,75] The aim of this project was not only to develop the cycloaddition step but to utilise it as a means of providing a masked tetramic acid permitting manipulation at C-3 and other elaborations including *N*-acylations.

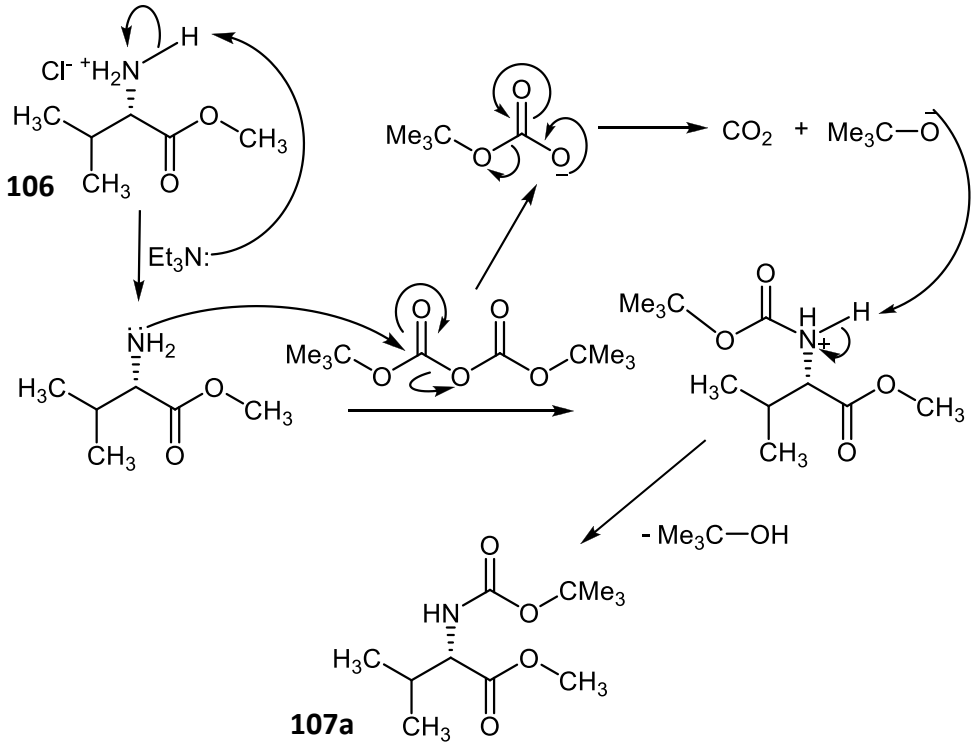
The S-valine and S-leucine amino esters were obtained from a commercial provider (Sigma-Aldrich) as the methyl ester hydrochloride salts (**106a/b**, respectively**)**, which enabled us to directly protect the amino group. The protecting group used was tert-butyloxycarbonyl (Boc) because its removal is relatively simple under acidic conditions, a characteristic that will be exploited later in this synthesis when removed in tandem with another *t*-butyl based protecting group. This protection was initiated by the addition of triethylamine, which was required to deprotonate the hydrochloride salts, at 0 ^{0}C over 30 minutes. To this chilled solution was added di-tert-butyl dicarbonate [$(Boc)_2O$] suspended in dichloromethane (DCM) dropwise, to avoid localised heating, after which the reaction was stirred at 0 ^{0}C for an hour and then at room temperature for a further 17 hours. It was found by repeat experiments that the addition could be done either dropwise or in one portion with no effect on the yield, also that a longer reaction time (17 or 48 hours) similarly did not affect the yield, showing the reaction was complete after 17 hours. However, when up to 20.0 g of amino ester hydrochloride salt was used a reaction time of up to 48 hours was required for completion otherwise unreacted di-tert-butyl dicarbonate required separation from the product by column purification, laborious due to large sample size.

Excess basic contaminants (triethylamine) were removed by an acidic wash with 1 M citric acid solution, followed by a saturated brine wash. The solution was then dried and concentrated under reduced pressure to yield both tert-butyl (2-methyl-4-oxohexan-3-yl)carbamate (**107a**) and tert-butyl (2-methyl-4-oxoheptan-3-yl)carbamate (**107b**) as colourless oil in 99% yields, Scheme 26.



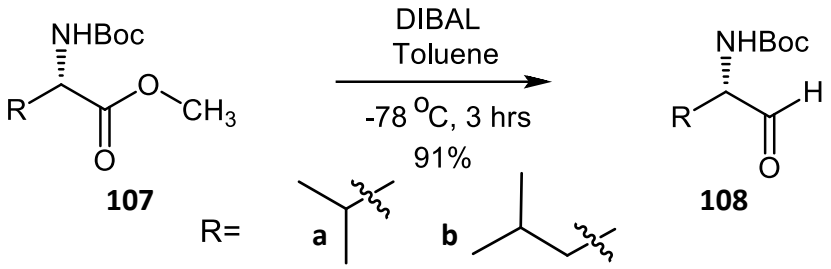
Scheme 26. Boc protection of an amino acid methyl ester hydrochloride

This reaction yield was also unaffected by a large scale, achieving the best yield of 99% in the production of 27.0 g of the valine based product **107a**. Another encouraging aspect of this reaction is that no purification was necessary; the pure product was confirmed by IR, ^{1}H and ^{13}C NMR spectroscopy, and mass spectrometric identification of molecular ion. A suggested mechanism for this reaction is shown below, Scheme 27.



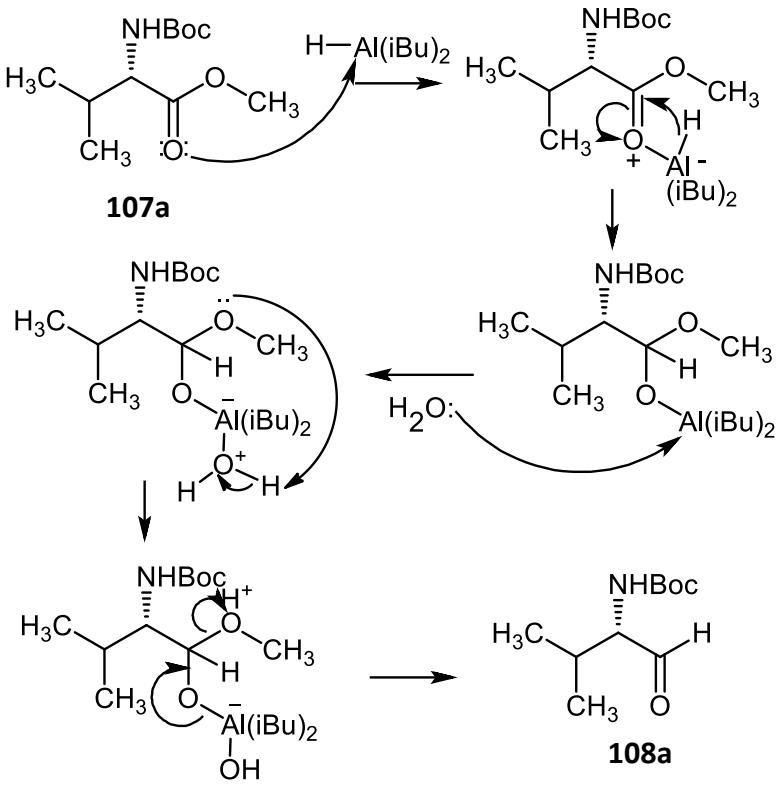
Scheme 27. A suggested mechanism for a Boc protection of an amino acid methyl ester hydrochloride

The next step was to reduce the methyl ester to an aldehyde, ready for the ensuing transformation to an oxime. This was achieved by cooling a suspension of the tert-butyl (2-methyl-4-oxohexan-3-yl)carbamate (**107a**) or tert-butyl (2-methyl-4-oxoheptan-3-yl)carbamate (**107b**) in dry toluene to -78 ^{0}C followed by the addition of a 1.0 M solution of diisobutylaluminium hydride (DIBAL) in toluene, completed dropwise with the aid of a syringe pump over 4 hours. Again, the addition was dropwise to avoid any localised heating in the flask as temperatures above -70 ^{0}C could lead to either the further reduction of the aldehyde to an alcohol, or to racemise. The reaction was quenched whilst at -78 ^{0}C by slowly adding methanol, which led to the production of large amounts of hydrogen gas as the reactions were done on a large scale. Once satisfactorily quenched, the reaction mixture was added to a solution of sodium potassium tartrate (Rochelle salt) in water and stirred vigorously for 2 hours. The Rochelle salt is a separation agent to enable emulsions to successfully disperse; in this case it causes the organic reaction mixture, containing aluminium residues, to interact with the water and enable successful separation, which is notably more difficult when no Rochelle salt is used. After extraction with ethyl acetate the product solution was dried and concentrated under a reduced pressure to yield tert-butyl (3-methyl-1-oxobutan-2-yl)carbamate (**108a**) and tert-butyl (4-methyl-1-oxopentan-2-yl)carbamate (**108b**) as clear and colourless oils in a highest yield of 91%, Scheme 28.



Scheme 28. Reduction of a methyl ester to an aldehyde

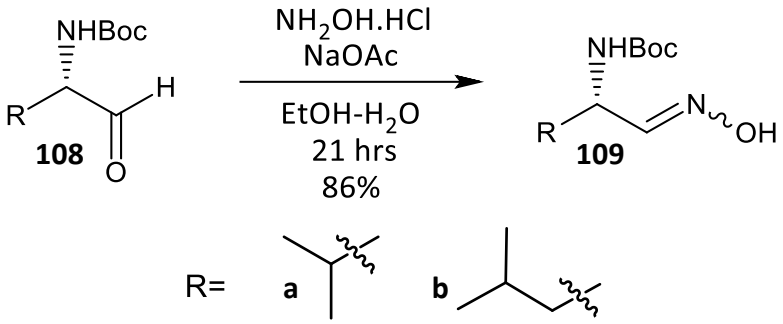
A mechanism for this reaction has been suggested, Scheme 29. It Is important to note that these aldehydes were then immediately used in the synthesis of the oxime to avoid any racemisation at the chiral centre as this chirality needs to be retained to the final product.



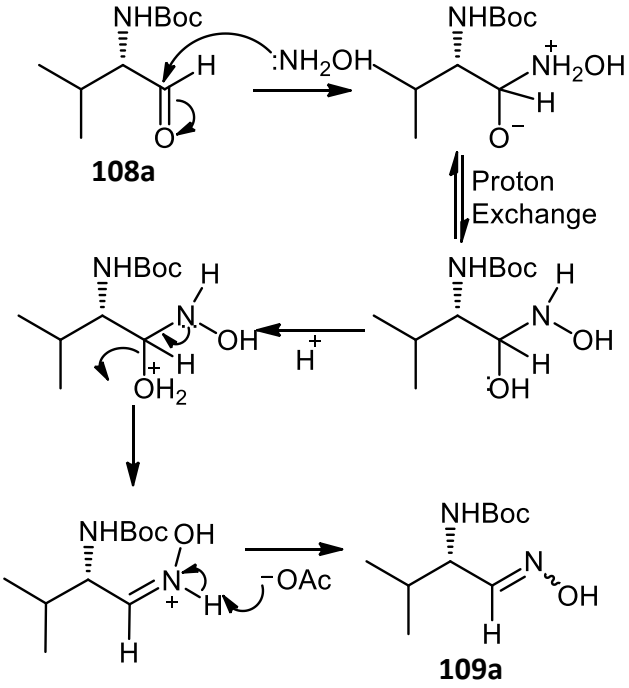
Scheme 29. A suggested mechanism for the interaction of DIBAL and an ester to give an aldehyde

The crude aldehydes (**108a/b**) were dissolved in the least amount of ethanol and added to an aqueous solution containing hydroxylamine hydrochloride and sodium acetate. This method of oxime formation from aldoximes by condensation of the aldehyde with hydroxylamine hydrochloride and sodium acetate has been previously used in our labs and shown to be reliable[80]. Immediately it was noted that a white precipitate formed, which had to be redissolved with the minimum amount of ethanol. The solution was heated to 70 ^{0}C on a heating mantle for 30 minutes, during which time a watchglass was placed over the neck of the round bottomed reaction flask to restrict solvent loss, and after this time the flask was cooled to room temperature and placed in the refrigerator (2-5 ^{0}C) for 18 hours. After this time a white precipitate had formed which was removed by filtration and was identified as the product oximes, *tert*-butyl [1-(hydroxyimino)-3-methylbutan-2-yl]carbamate (**109a**) and *tert*-butyl [1-(hydroxyimino)-4-methylpentan-2-yl]carbamate (**109b**), by NMR spectroscopy in d_6-DMSO and by mass spectrometric identification of the molecular ion. In some cases it was noted that no precipitate formed and therefore the reaction mixture was extracted with ethyl acetate followed by a single wash with a sodium bicarbonate solution to remove the remaining acetic acid. Successive drying and

concentration under vacuum led to the desired product, *tert*-butyl [1-(hydroxyimino)-3-methylbutan-2-yl]carbamate (**109a**) and *tert*-butyl [1-(hydroxyimino)-4-methylpentan-2-yl]carbamate (**109b**) with a yield maximum of 86% sometimes as a clear oil or a sticky white solid or a dry white powder, all of which were confirmed as the oxime by IR and NMR spectroscopy and mass spectrometry of the molecular ion, Scheme 30. Although this reaction is common practice the mechanism has been included for completeness, Scheme 31.



Scheme 30. The conversion of an aldehyde to an oxime



Scheme 31. Mechanism for the conversion of an aldehyde to an oxime

This provided the oxime in good yields on all small-scale reactions, larger scales were not attempted. The highest yield recorded from both series was 86% and this was achieved

when producing 2.2g of product but it is essential to note the yield fluctuated significantly when following the exact same reaction procedure with the range of yield achieved being 15%-86% with all reactions producing between 1g and 4g.

The oximes could be stored and they were recorded as being stable for at least 4 weeks when kept at 2-5 ^{0}C under a nitrogen atmosphere. Usually, however, the oximes were reacted in the 1,3-dipolar cycloaddition sequence within 24 hours of their preparation.

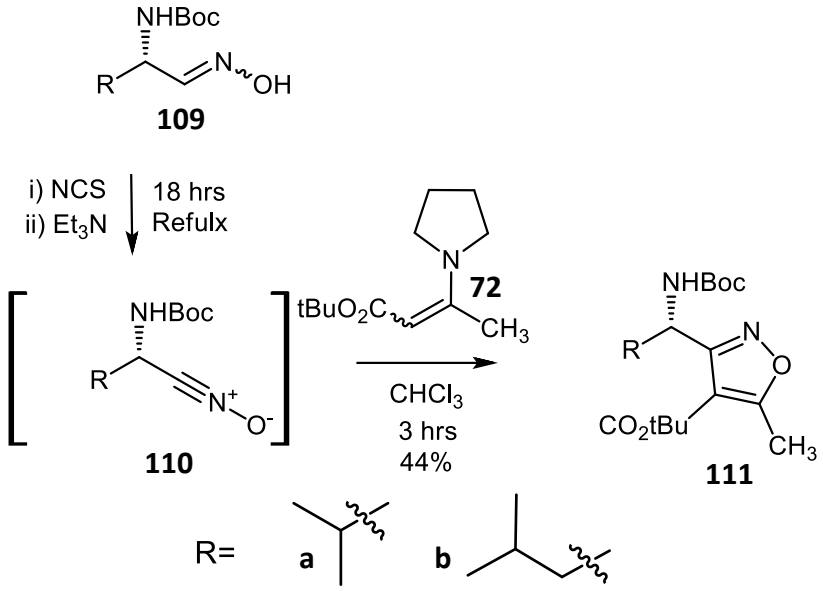
2.1.3 – 1,3-Dipolar Cycloaddition Reactions

Following the original synthesis, this work utilised initial C-chlorination using *N*-chlorosuccinimide (NCS) followed by the addition of a base to form the nitrile oxide *in situ* by eliminating the chloro-substituent. This would then allow the 1,3-dipolar cycloaddition to occur upon the addition of the dipolarophile (the enamine *tert*-butyl (2Z)-3-(pyrrolidin-1-yl)but-2-enoate, **72**).

The first protocol involved the oximes, *tert*-butyl [1-(hydroxyimino)-3-methylbutan-2-yl]carbamate (**109a**) and *tert*-butyl [1-(hydroxyimino)-4-methylpentan-2-yl]carbamate (**109b**), being suspended in chloroform at 0 ^{0}C. To them was added N-chlorosuccinimide dropwise over 30 minutes at this constant temperature to ensure a controlled reaction, although introducing the NCS in one portion was later found not to affect the results. This mixture was then returned to room temperature and heated to reflux with stirring for a further 18 hours. When quantities of less than 2.0g of the oximes *tert*-butyl [1-(hydroxyimino)-3-methylbutan-2-yl]carbamate (**109a**) and *tert*-butyl [1-(hydroxyimino)-4-methylpentan-2-yl]carbamate (**109b**) were used it was noted that after 18 hours at reflux the mixture had turned a blue green, but when using more than 2.0g of oxime **85a** and **85b** the solution became dark orange/brown. Through thin layer chromatographic (TLC) analysis it was found that both different coloured samples were the same chlorinated product.

These chlorinated oximes were then immediately dehydrochlorinated using triethylamine, added directly through the reflux condenser, to form the unstable nitrile oxide intermediate **110** *in situ*. To this intermediate was immediately added 2.2 equivalents of the enamine *tert*-butyl 3-(pyrrolidin-1-yl)but-2-enoate (**72**) in one portion and this reaction mixture was

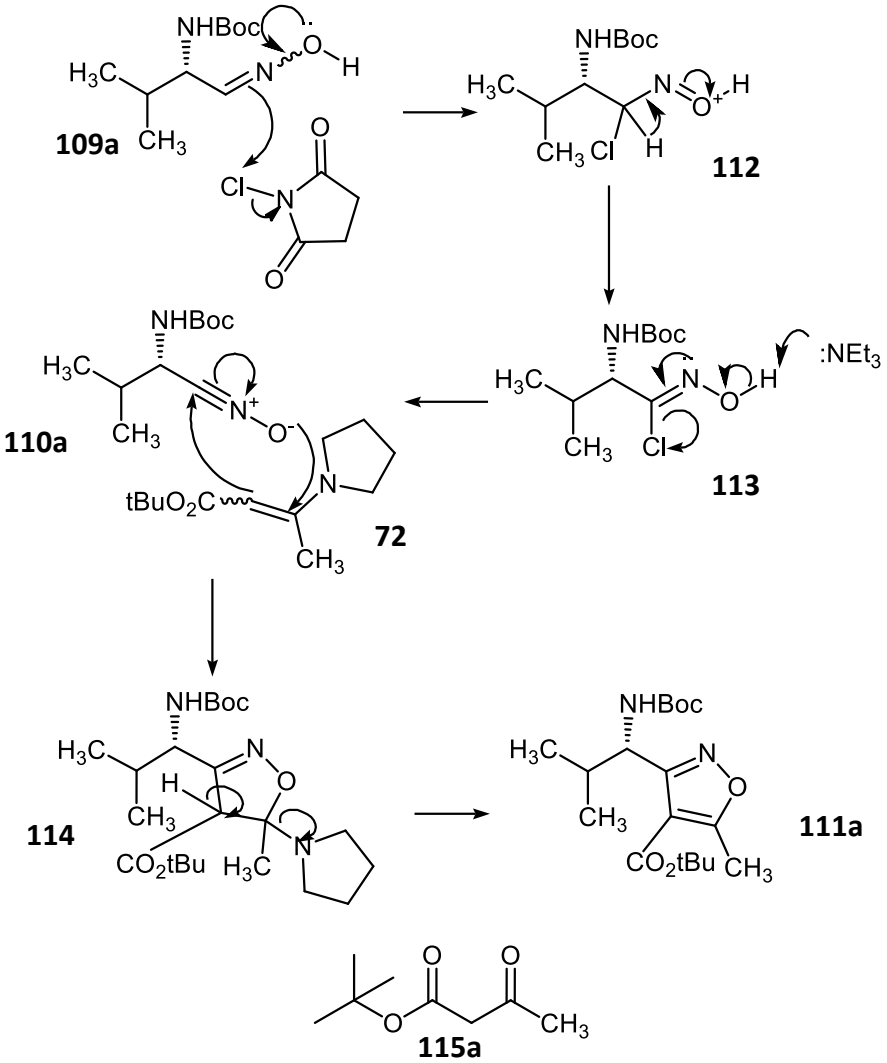
heated at reflux for a further 3 hours, after which time it was added to water and the organic layer separated and removed, Scheme 32. This was followed by multiple washings with 1 M citric acid, to remove any excess basic amines, 5 % w/v sodium hydroxide, to remove any acid remaining from chloro-elimination, and finally with saturated brine solution. This yielded the organic fraction that, after drying and concentrating under reduced pressure, contained the crude product isoxazole as a thick brown oil. This was then subjected to separation via column chromatography eluting with light petroleum and ethyl acetate (3:1 v/v) which provided the isoxazoles, *tert*-butyl 3-[(1-*tert*-butoxycarbonylamino-2-methylpropyl)]-5-methylisoxazole-4-carboxylate (**111a**) and (*S*)-*tert*-butyl [(1-chloro-1-(hydroxyimino)-4-methylpentan-2-yl)]carbamate (**111b**), as mobile yellow oils in 44% and 50% maximum yields, respectively.



Scheme 32. The formation of the 1,3-dipole in solution and subsequent production of the isoxazole

The dipole formation is presumably initiated by the electrophilic addition of a chlorine atom to the carbon adjacent to the oxime nitrogen and simultaneous injection of the oxygen lone pair into nitrogen-oxygen bond to give a protonated α-chloronitroso intermediate, as illustrated in the valine series, **112**. This oxygen charge is then quenched by the elimination of a proton and formation of a carbon-nitrogen double bond, producing the C-chlorinated oxime (**113**) (Scheme 33).

The suggested mechanism for the production of the isoxazole via the 1,3-dipolar cycloaddition is shown in Scheme 33. Deprotonation of the hydroxyl group of the hydroximoyl choride, **113**, by triethylamine is accompanied by chloride expulsion to provide the reactive intermediate dipole **110,** to which is immediately added the dipolarophile (enamine **72**) to partake in the 1,3-dipolar cycloaddition, the initial product of which is presumed to be the isoxazoline **114**. Due to the potential leaving ability of the pyrrolidine and driving force to achieve aromaticity, the pyrrolidine is directly eliminated from the ring forming the isoxazole **111**.

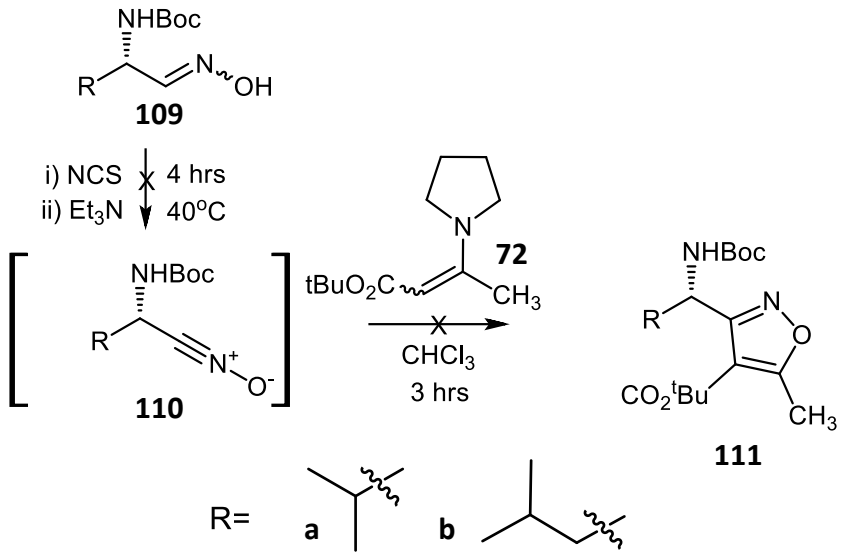


Scheme 33. The proposed mechanism for the 1,3-dipolar cycloaddition

The yield obtained with this reaction was acceptable, 44% and 50% for the valine and leucine based series respectively, at its highest over the two steps. The main by-product is the β-ketoester **115a**, which it is assumed is formed by the hydrolysis of the excess enamine

post-reaction. This has been observed before and, with optimisation, it had been determined that 2.0 equivalents of the enamine relative to the oxime was the most effective.[75] This issue is complicated by the fact that the β-ketoester by-product is very difficult to separate from the isoxazole product due to very similar R_F values in all solvent systems explored. This led occasionally to inability to isolate the pure isoxazole, therefore a study was launched to investigate if this yield could be improved.

The first adaption made to the original procedure for the attempted synthesis of the isoxazole was made on the assumption that extended high temperature (18 hours at 60 ^oC) could be causing decomposition of the chloro-oxime and limiting the yield of the reaction. We attempted to avoid this by adding NCS to the oxime *tert*-butyl [1-(hydroxyimino)-3-methylbutan-2-yl]carbamate (**109a**), suspended in chloroform as before, at 0 ^0C in one portion, but then heating at 40 ^0C for 4 hours. During this process the reaction solution did not change colour as seen before but it was still reacted further with triethylamine and the enamine. This reaction, unfortunately, gave none of the desired product and only starting materials were collected, showing these milder conditions did not lead to a successful chlorination, Scheme 34.

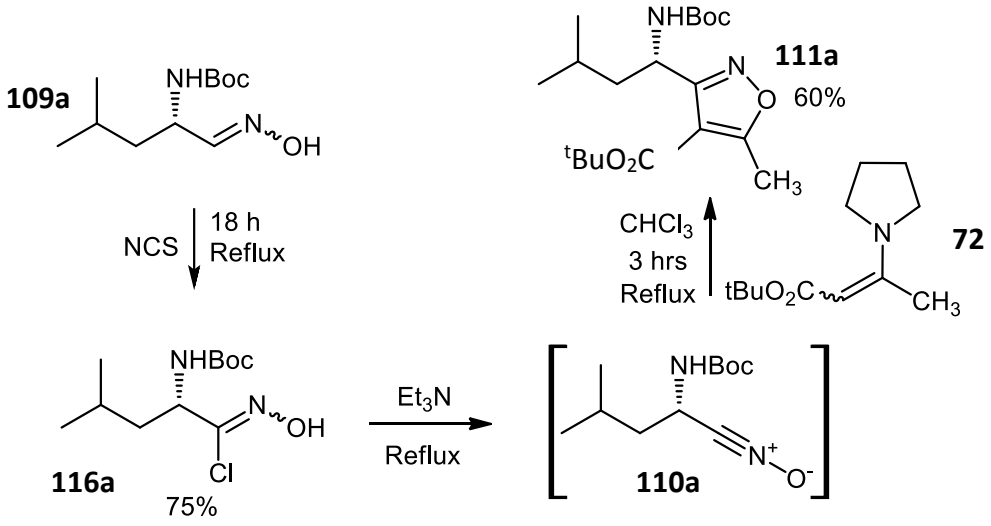


Scheme 34. An attempted alternative isoxazole synthesis

Our next investigation was to isolate the chloro-oxime to determine the yields for each individual step. The chlorinated products that we were able to isolate were the (*S*)-*tert*-

butyl [1-chloro-1-(hydroxyimino)-3-methylbutan-2-yl]carbamate from the (S)-valine series and (S)-*tert*-butyl [1-chloro-1-(hydroxyimino)-4-methylpentan-2-yl]carbamate, which is from the (S)-leucine series. After following the protocol as before, addition of NCS at 0^0C and heating at reflux for 18 hours, the work up included washing the cooled solution with water and saturated brine, to remove excess NCS, before drying the organic layers with $MgSO_4$, filtration and concentration under reduced pressure to yield the chlorinated oxime as an orange oil in a good 75% and 82% yield, respectively. This was surprising as it had been assumed that this simple step was yielding close to a quantitative yield, but did provide an anticipation that the subsequent 1,3-dipolar cycloaddition reaction was higher yielding than previously assumed.

The cycloaddition reaction was completed by directly following the procedure described before, with the chloro-oxime being suspended in chloroform and heated at reflux before the addition of the triethylamine and dipolarophile enamine. This cycloaddition reaction, following work up and purification by column chromatography, yielded the isoxazole in 60% yield from the chloro-oxime, which agrees with the average 44% overall achieved in the 'one pot' methodology. This developed reaction scheme is shown in Scheme 35.



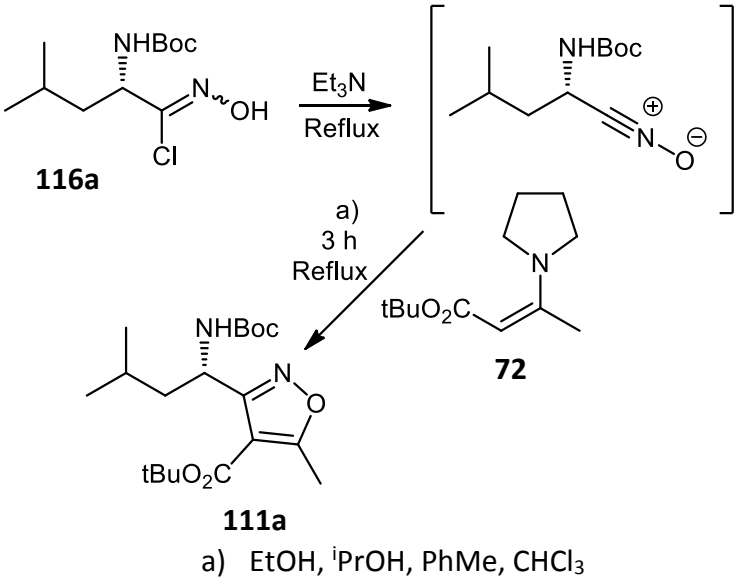
Scheme 35. Isolation of chloro-oxime followed by isoxazole synthesis

Searching for new methods for a 1,3-dipolar cycloaddition reaction yielded a procedure utilising microwave radiation as a source of energy for the reaction. It was shown by Touaux

et al. in 1998 that aldoximes reacted in a one-pot reaction with NCS and alkenes to form isoxazoles under microwave radiation in yields of up to 75% over both stages.[81] Therefore, the conditions attempted following these new protocols were to absorb (*S*)-*tert*-butyl [1-(hydroxyimino)-3-methylbutan-2-yl]carbamate (2.5 mmol), *tert*-butyl 3-(pyrrolidin-1-yl)but-2-enoate (5.0 mmol), and NCS (2.5 mmol) on Al_2O_3 (5 g). These reactants were sealed in a closed Teflon tube and irradiated to 30 W for 4 minutes in a microwave (Biotage Eight). This reaction was unsuccessful and, after work up by extraction with CH_2Cl_2, drying with $MgSO_4$, filtration and concentration under a reduced pressure, no isoxazole product was identified with only starting materials being recovered.

The final variation to be explored was the solvent in which the cycloaddition reaction is performed, which can be polar, non-polar or a median between the two. Isolation of the chloro-oxime provided this opportunity as due to insolubility of the oxime, only $CHCl_3$ was used for the chlorination, whereas the chloro-oxime was soluble in a range of solvents.

The solvents attempted in the study were ethanol, 2-propanol, toluene and chloroform as a standard to compare the results of the reaction. The specific reaction studied was (*S*)-*tert*-butyl [1-chloro-1-(hydroxyimino)-3-methylbutan-2-yl]carbamate (2.0 mmol) with *tert*-butyl 3-(pyrrolidin-1-yl)but-2-enoate (4.0 mmol) and triethylamine (2.2 mmol), which was repeated four times identically, following the initial protocol above (Scheme 36). The relative solvent polarities are shown in Table 3 below as well as the yields achieved which were calculated after separate work-ups and isolation of the pure isoxazole by preparative TLC, confirmed by comparison with previously synthesised samples.



a) EtOH, ^{i}PrOH, PhMe, $CHCl_3$

Scheme 36. Reaction of the chloro-oxime in the isoxazole synthesis

Table 3. Table showing the results and relative polarities[82] of solvent used

Solvent	***Polarity**[82]*	***Yield***
Ethanol	0.654	41%
2-Propanol	0.546	36%
Chloroform	0.259	54%
Toluene	0.099	9%

**The values for relative polarity are normalized from measurements of solvent shifts of absorption spectra*

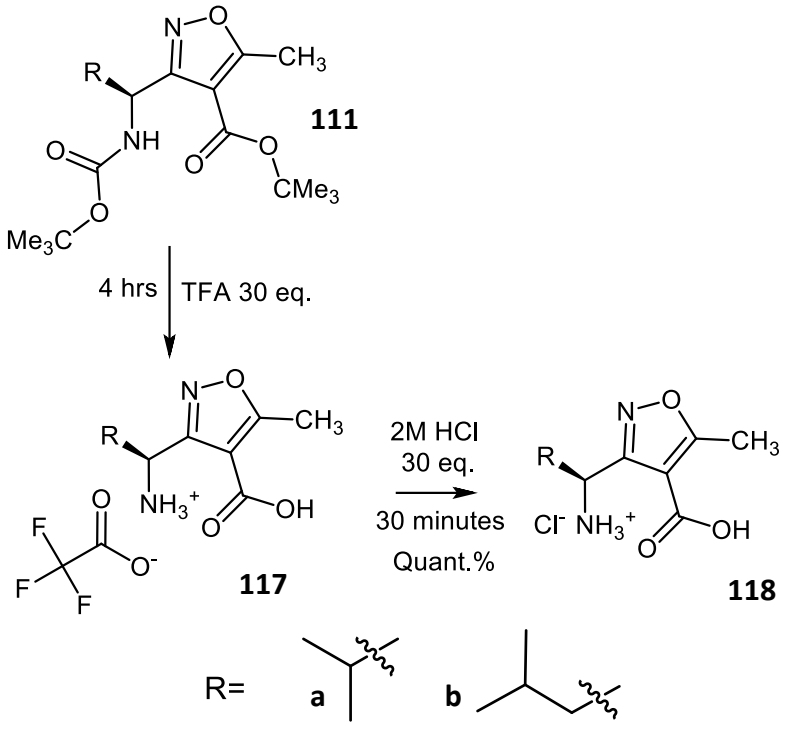
The results showed that the reaction completed in $CHCl_3$ achieved the highest yield after isolation and therefore it was deduced that this is after all the best solvent to use under these reaction conditions.

The next stage was to form the bicyclic pyrroloisoxazole, which was intended to be achieved by the deprotection of the isoxazoles and subsequent ring closure by a peptide coupling reaction forming the lactam ring.

2.1.4 – Formation of the Masked Acyltetramic acid, a Pyrroloisoxazole

In the formation of the pyrroloisoxazole moiety, there needs to first be a removal of the *tert*-butyloxycarbonyl (Boc) protection group on the amine as well as cleavage of the *tert*-butyl ester group to give an acid. This free acid and amine could then be reacted in a peptide-like coupling reaction.

Careful selection of the protecting groups used, meant that both protecting groups could be removed in a single step using an acid-mediated cleavage (and decarboxylation in the Boc removal). The chosen acid, trifluoroacetic acid (TFA), successfully cleaved the groups but left them extremely sensitive to moisture due to the trifluoroacetate salts formed. This was overcome by concentration of the salt under reduced pressure to remove any excess TFA and subsequent addition of hydrochloric acid producing the hydrochloride salt, which is less moisture sensitive and therefore allows for storage, Scheme 37. This protocol was found to give better results than direct hydrochloric acid cleavage.

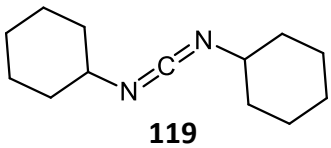


Scheme 37. Deprotection of the isoxazole with acid

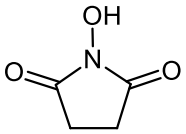
TFA was added to the isoxazoles (**111a** and **111b**) and stirred at room temperature for 4.5 hours; note that 30 equivalents were used to ensure the TFA in large excess, to guarantee a successful deprotection. Excess TFA was then removed under reduced pressure and 2M

hydrochloric acid added, followed by 30 minutes stirring and successive washings with ethyl acetate where the aqueous layer was retained, because the product was a salt and therefore water soluble. This was then evaporated under reduced pressure and any remaining water was removed under a high vacuum, yielding 1-(4-carboxy-5-methyl-1,2-oxazol-3-yl)-2-methylpropan-1-aminium hydrochloride (**118a**) and 1-(4-carboxy-5-methyl-1,2-oxazol-3-yl)-3-methylbutan-1-aminium hydrochloride (**118b**) as dark brown sticky oils in quantitative yields. The product was identified by ^{1}H NMR spectroscopy, with loss of two *tert*-butyl groups, and mass spectrometric identification of molecular ion. The deprotected isoxazole amine hydrochloride salts were ready for the peptide coupling reaction.

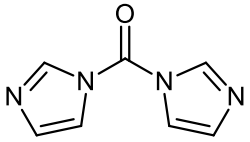
Peptide coupling reactions are a method of efficiently combining an acid and an amine to form a new amide bond and are immensely useful to synthetic organic chemists and thus have been the subject of a number of reviews and much interest[83]. There are a large number of potential coupling agents available, each with their own applications, advantages and disadvantages, and include compounds possessing carbodiimides, phosphonium and imidazolium salt functionalities. The most commonly used peptide coupling reagents are carbodiimides, as they are readily available and easily handled, but disadvantages include the potential for side reactions to occur including the formation of *N*-acylureas which are notoriously difficult to remove from the product. This issue has been shown to be overcome by adding an activator which promotes a successful reaction. A selection of the commercially available coupling reagents and activators is provided in Figure 19.



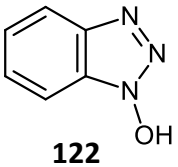
119
DCC[84]
1,3-dicyclohexylcarbodiimide



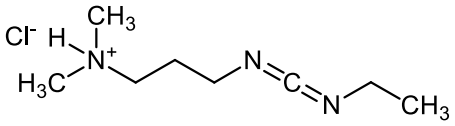
120
HOSu
Hydroxysuccinimide



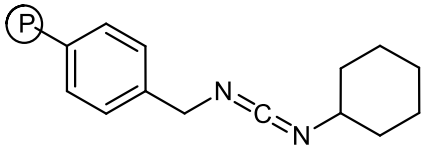
121
CDI
Carbonyldiimidazole



122
HOBt
1-hydroxybenzotriazole



123
EDCI
1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride



124
PS-CDI
Polystyrene-carbodiimide

Figure 19. Common peptide coupling reagents and activators

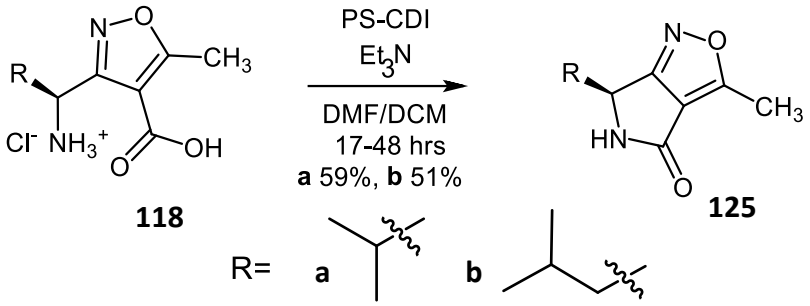
The most popular of the carbodiimides used in peptide bond synthesis is 1,3-dicyclohexylcarbodiimide (DCC, **119**), first used in 1955[84], making it the obvious choice. However, disadvantages associated with DCC include the fact that the by-product of peptide coupling reactions (dicyclohexylurea) is hard to separate from the reaction mixture which causes problems in obtaining pure material.

Therefore, it was decided to use another, less common, peptide coupling reagent, polystyrene-bound carbodiimide (PS-CDI, **124**) which possesses the DCC functionality whilst being permanently bound to a polystyrene bead. Although less frequently used, possibly due to the high price (approx. £60 per gram), this carbodiimide source has excellent

potential as the urea by-product remains bound to the polystyrene after the reaction has occurred and therefore is very easily removed by filtration. Polystyrene-bound carbodiimide was also shown to be effective by an earlier group member[75].

In this reaction it was necessary to ensure all glassware was thoroughly dry and this was confirmed by heating glassware in an oven overnight and cooling under a nitrogen flow before the reaction and also by leaving reactants in a sealed desiccator containing phosphorus pentoxide.

The deprotected isoxazoles, 1-(4-carboxy-5-methyl-1,2-oxazol-3-yl)-2-methylpropan-1-aminium hydrochloride (**118a**) and 1-(4-carboxy-5-methyl-1,2-oxazol-3-yl)-3-methylbutan-1-aminium hydrochloride (**118b**), were suspended in a mixture of dry dichloromethane (DCM) and dry *N*,*N*-dimethylformamide (DMF) (9:1 v/v). To this solution was charged triethylamine, required to remove the extra proton at the ammonium group and allow it to undergo the peptide coupling reaction upon the subsequent addition of the polystyrene-bound carbodiimide (**124**). After all reactants were added, the reaction was stirred at room temperature for between 17 and 48 hours, although no difference was observed in the yield over longer reaction times, before removing the polystyrene-bound by-products by filtration. The filtrate was washed with saturated potassium carbonate solution, to remove the triethylamine hydrochloride, and also with saturated brine. The crude amide product was confirmed by ^{1}H NMR spectroscopy, seen by the removal of an amine proton and the acidic proton, and was separated from the impurities and unreacted starting materials by flash column chromatography eluting with ethyl acetate and light petroleum (5:1 v/v). This process yielded 3-methyl-6-(propan-2-yl)-5,6-dihydro-4H-pyrrolo[3,4-c][1,2]oxazol-4-one (**125a**) and 3-methyl-6-(2-methylpropan1-yl)-5,6-dihydro-4H-pyrrolo[3,4-c][1,2]oxazol-4-one (**125b**), both confirmed by IR, ^{1}H and ^{13}C NMR spectroscopic, and mass spectrometric identification of the molecular ion, as white solids in a range of yields, 30-59%, Scheme 38.



Scheme 38. The formation of a pyrroloisoxazole from a deprotected isoxazole using PS-CDI

To add to the array of evidence spectroscopic methods, it was decided to define the structure completely with X–ray crystallography, which confirmed the correct structure for the valine-derived pyrroloisoxazole (**125a**), Figure 20.

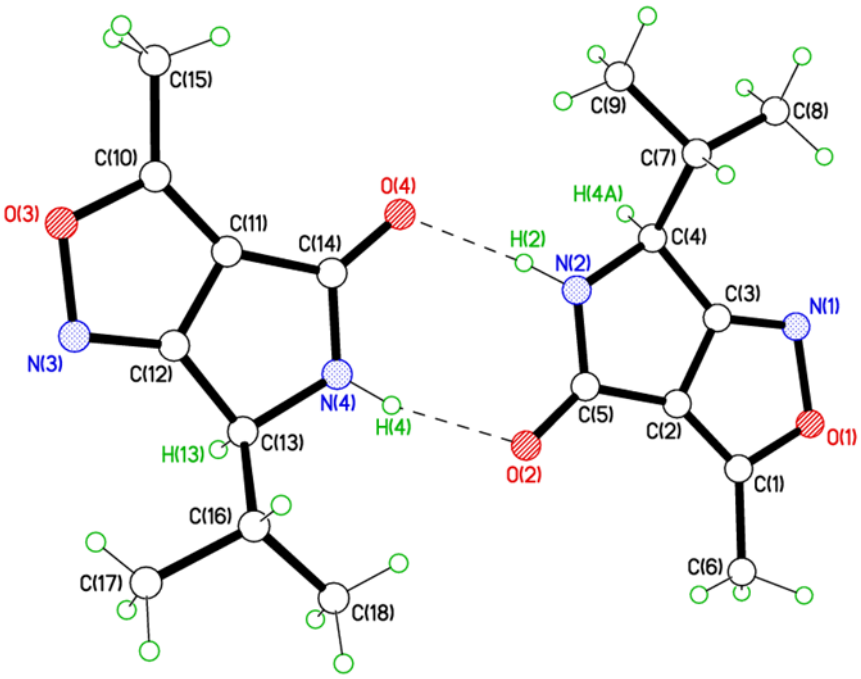
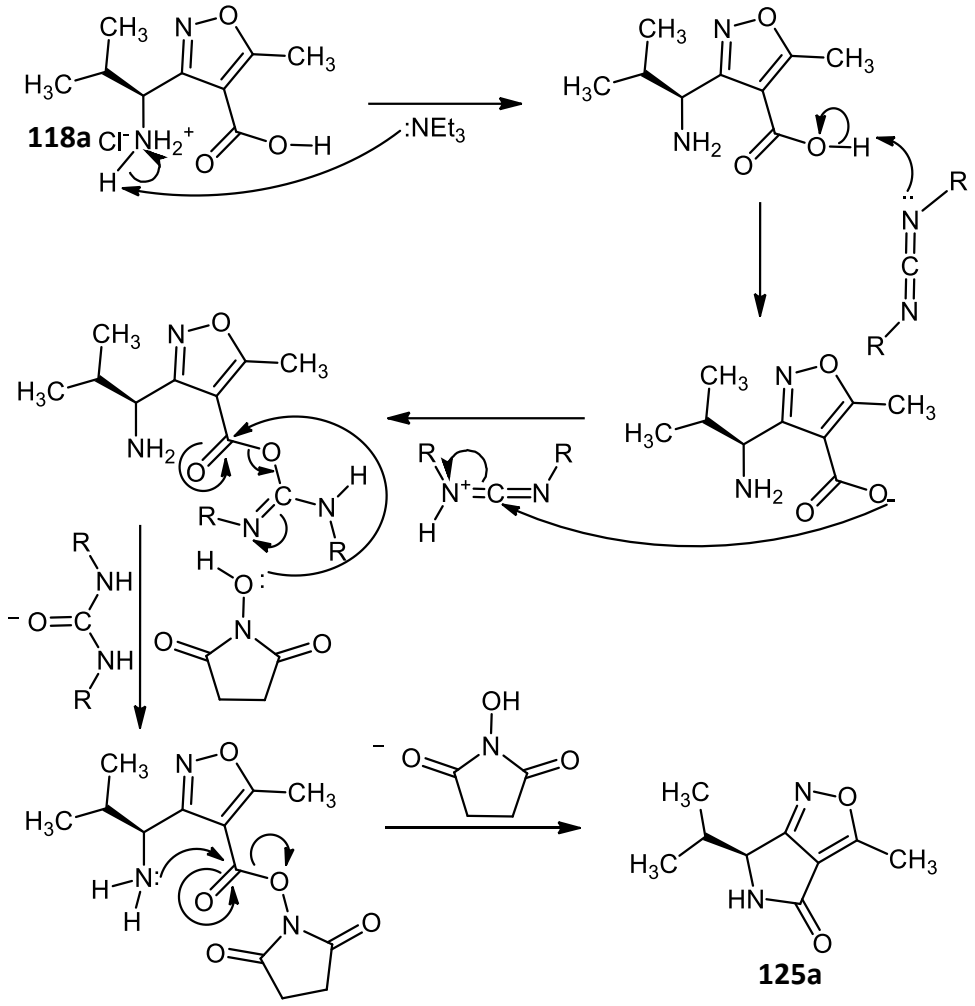


Figure 20. X-ray structure of 3-methyl-6-(propan-2-yl)-5,6-dihydro-4*H*-pyrrolo[3,4-*c*][1,2]oxazol-4-one (125a)

Due to some moderate yields achieved (between 30% and 59%) it was decided that another method would be attempted in parallel to deduce if it was more advantageous. The other selected method involved the use of 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (EDCI, **123**) in the presence of a hydroxysuccinimide (HOSu, 1**20**) activator. The main advantage of EDCI is that the urea products remain water soluble while the

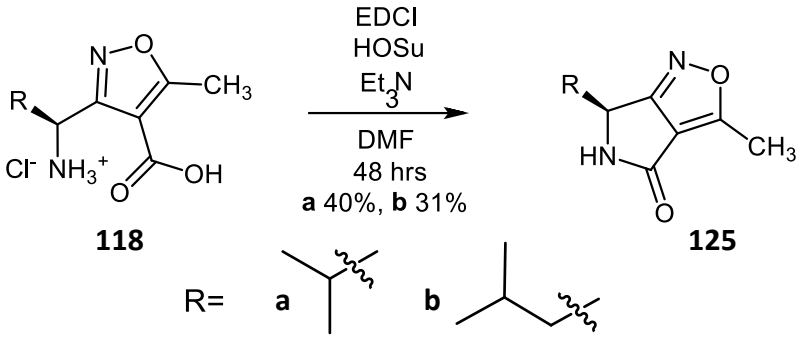
product is kept in the organic layer, allowing for an easy separation. A suggested mechanism for the carbodiimide reaction is shown in Scheme 39.



Scheme 39. Mechanism for the reaction of the isoxazole with a general carbodiimide giving the pyrroloisoxazole

In this reaction the glassware was dried as before and the reactants also left to dry in a desiccator. EDCI (**98**) was added to the suspension of deprotected isoxazoles and HOSu (**120**) in DMF dropwise over 30 minutes at 0 ^{0}C and the mixture was then stirred at room temperature for a further 17 hours before the addition of triethylamine, followed by 17 hours of stirring at room temperature, Scheme 40. After an acidic work-up, drying and evaporation under reduced pressure, the beige solid was subjected to separation via flash column chromatography eluting with an ethyl acetate and light petroleum (5:1 v/v). This yielded the product pyrroloisoxazoles 3-methyl-6-(propan-2-yl)-5,6-dihydro-4H-pyrrolo[3,4-

c][1,2]oxazol-4-one (**125a**) and 3-methyl-6-(2-methylpropan-1-yl)-5,6-dihydro-4H-pyrrolo[3,4-c][1,2]oxazol-4-one (**125b**), as a white solids in a maximum 40% yield.



Scheme 40. The formation of a pyrroloisoxazole from a deprotected isoxazole using EDCI/HOSu

The yields achieved were less than hoped for and as this was stage 7; the amount of material was limited with a maximum of 600mg being produced in this step.

The yields routinely achieved ranged from moderate to good but it was felt these could be improved upon. When cost of starting materials is considered, the higher yielding PS-CDI reaction was deemed less practical. This left the 40% yielding EDCI reaction as the choice reaction, for which the yield was undesirable this late in the synthesis. This issue was resolved by the discovery of a new peptide coupling and water scavenging reagent, propylphosphonic anhydride, marketed as T3P by Archimica in Germany[85], Figure 21

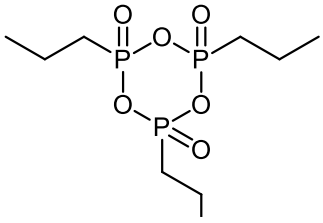


Figure 21. Propylphosphonic anhydride or T3P

Not only did using T3P provide the products in a shorter reaction time but also as pure white solids with no requirement for any further purification. Figure 22 and Figure 23 show NMR spectra for the S-valine and S-leucine pyrroloisoxazoles after washings and removal of solvent provided them in 59% and 68% yields, respectively, Scheme 41. This was the highest yield achieved for this reaction, showing a successful development of the methodology.

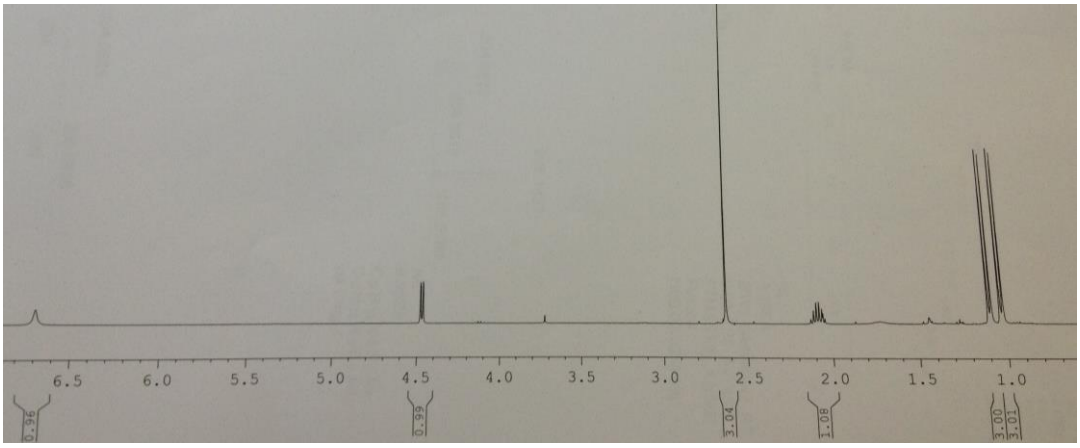


Figure 22. NMR of S-valine pyrroloisoxazole (125a) straight after washings and reduction of excess solvent

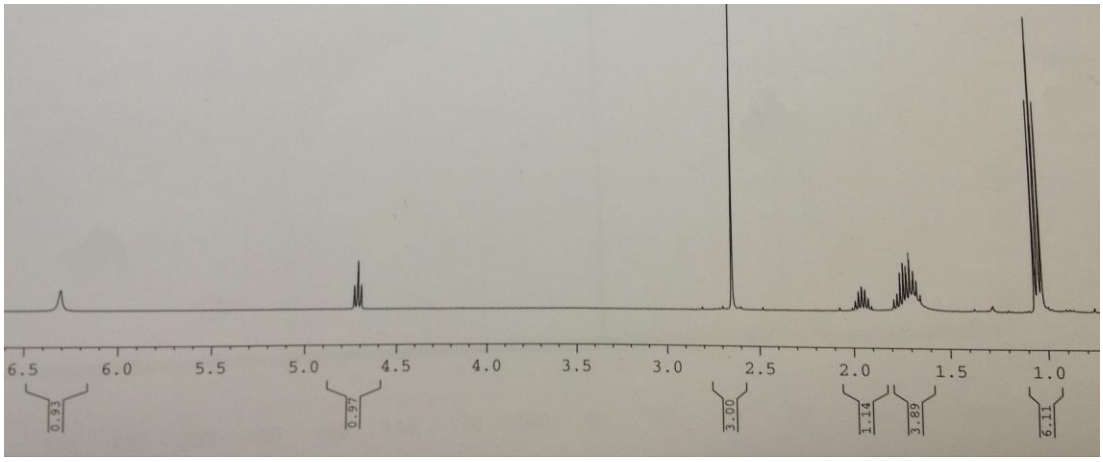
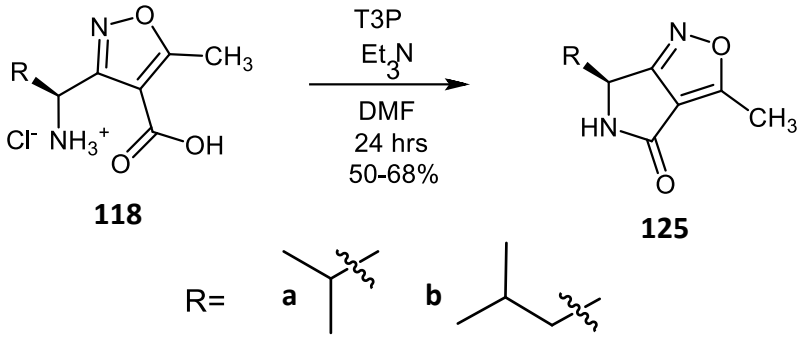
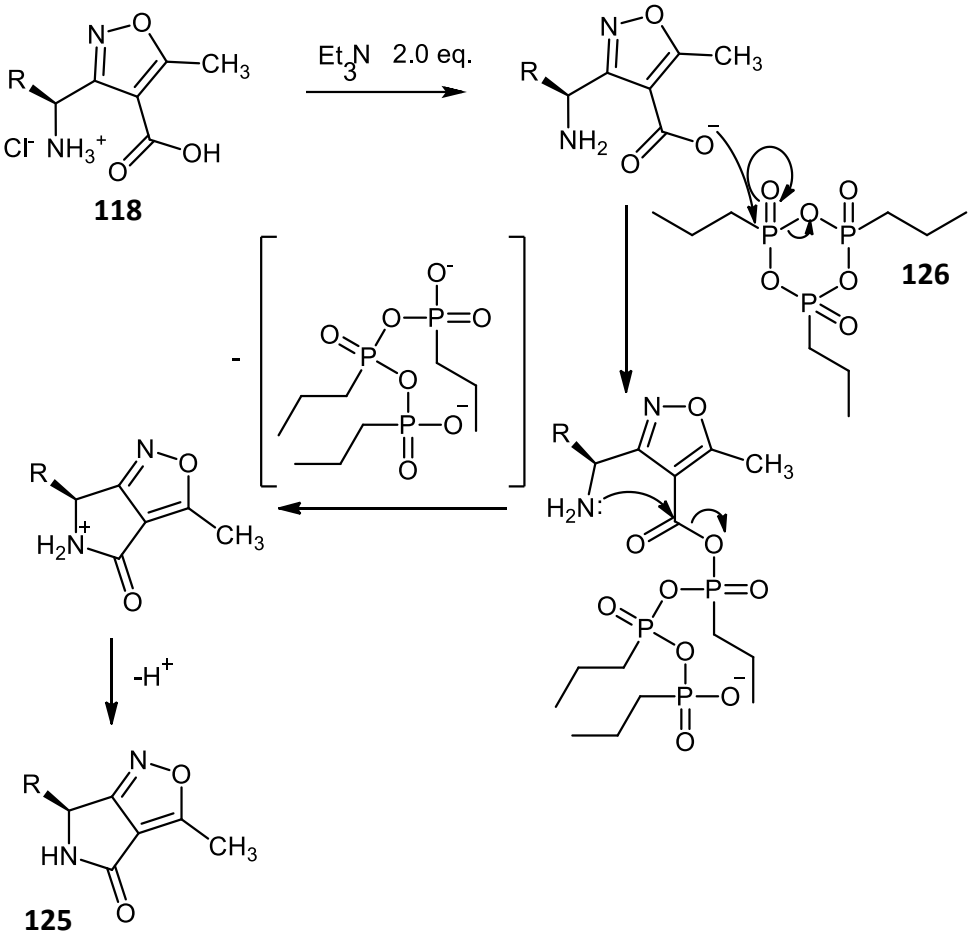


Figure 23. NMR spectrum of crude S-leucine pyrroloisoxazole (125b) (straight after washings and removal of excess solvent)



Scheme 41. The formation of a pyrroloisoxazole from a deprotected isoxazole using T3P

A mechanism for this reaction has been suggested below, Scheme 42, and involves the opening of the phosphonic heterocycle by a nucleophilic attack of the carboxylate oxygen on a phosphorus atom due to its partial positive charge and the affinity of oxygen for phosphorus. This then allows the nitrogen to attack the acid and provide the phosphorus-containing by-product as a dianion, soluble in the aqueous layer.



Scheme 42. Peptide coupling mechanism using T3P

This method using T3P provided the S-valine and S-leucine pyrroloisoxazole; from the latter a crystal was grown suitable for X-ray crystallography which provided the structure seen in Figure 24.

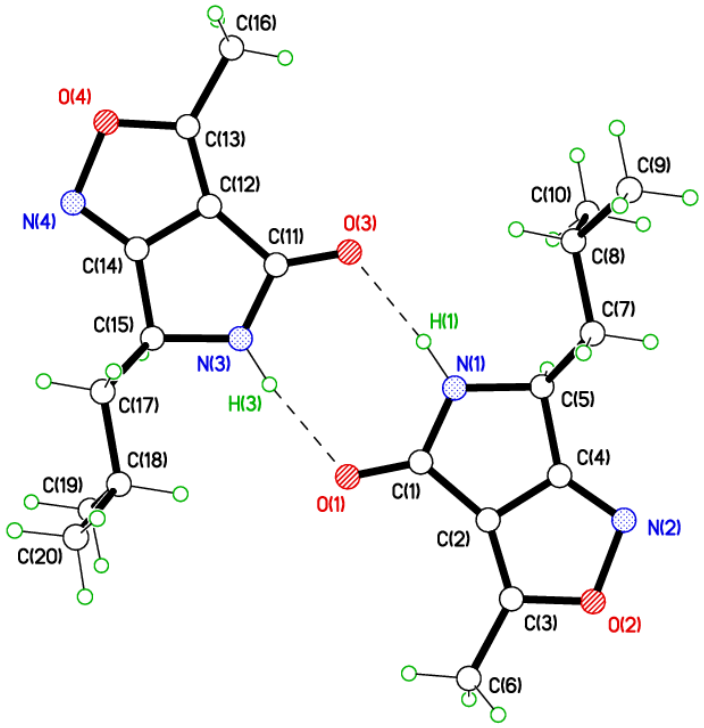


Figure 24. X-ray structure of S-leucine pyrroloisoxazole (125b)

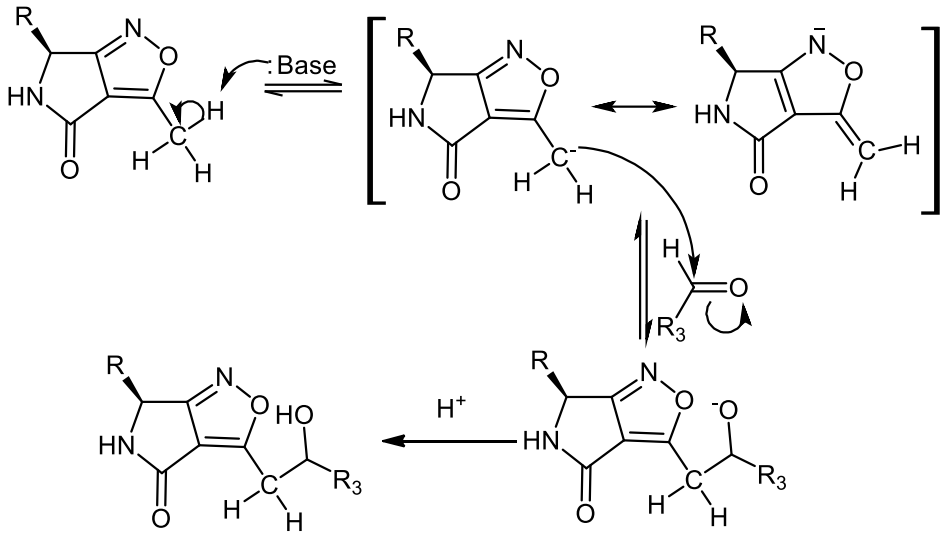
Now the pyrroloisoxazoles had been successfully produced, elaborations on the methyl group could be undertaken, trialled on the L-valine series only, as well as other interesting extensions. For example, both pyrroloisoxazoles were used in the synthesis of reutericyclin and analogues, which will be discussed later.

The ability of these pyrroloisoxazole units to undergo hydrogenation and hydrolysis to form the 3-acyltetramic acids has previously been shown by the group so it was deemed unnecessary to repeat and discard valuable material.[75,57]

2.2 - Elaboration at C-3 Position of the L-Valine Pyrroloisoxazole

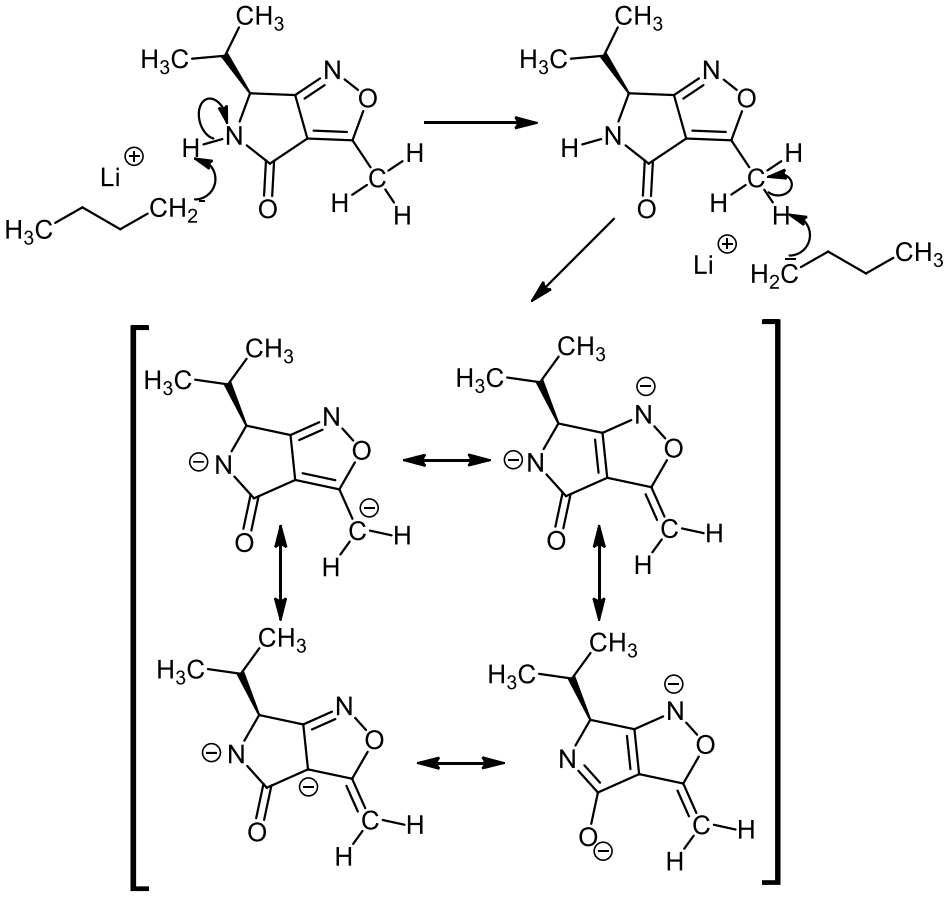
2.2.1 – Aldol Reaction at C-3 Position of the L-Valine Pyrroloisoxazole

This stage was key to one of the initial aims of the project and involved an anion condensation on to an aldehyde to provide a new carbon-carbon bond and allowing chain extension. This can typically be done by an aldol-type reaction, using the C-3 position methyl group via its anion, which is accessed via basic conditions and subsequent addition to an aldehyde, followed by acidification to yield the alcohol adduct, Scheme 43.



Scheme 43. A general mechanism for an aldol-type reaction under basic conditions

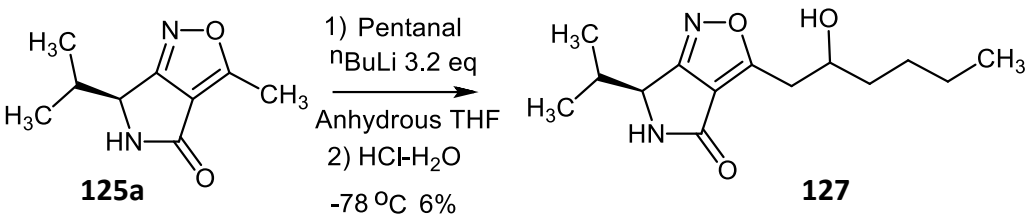
It has also been previously demonstrated, both by our group and very recently by Wells *et al.*, that the C-3 methyl position can be selectively deprotonated with lithium diisopropylamide (LDA) or *n*-butyllithium (n-BuLi), and subsequently added to an aldehyde to allow a chain extension using non-enolisable aldehydes in low yields (typically up to 30%).[79,75,86] The basic condition selected was deprotonation via ^{n}BuLi based on previous work finding this to be more effective than LDA. It should be noticed that the acidic proton that was removed was the second most acidic, with the most acidic being on the lactam nitrogen. This meant a minimum of 2.2 equivalents of base was required, which was modified to 3.2 equivalents and even 5 equivalents in some cases providing the best yields, Scheme 44. Although it is thought the first deprotonation will occur at the amide, it is assumed that the product from this attack is reversible under the acidic conditions of work up.



Scheme 44. Some possible resonance structures for the pyrroloisoxazole dianion

The first attempt made on the chain extension was the addition of pentanal. *n*-Butyllithium was added dropwise to a solution of the pyrroloisoxazole 3-methyl-6-(propan-2-yl)-5,6-dihydro-4*H*-pyrrolo[3,4-*c*][1,2]oxazol-4-one (**125a**) suspended in anhydrous tetrahydrofuran (THF) at -78 ^{0}C and kept under a nitrogen atmosphere to allow a controlled and selective deprotonation. The reaction mixture was stirred at this temperature for 2 hours, after which a large excess of freshly distilled (bp 103 ^{0}C) pentanal was added in one portion, typically 1 mL. After a further 2 hours of stirring the reaction mixture was quenched by the addition of water and acidified with hydrochloric acid, Scheme 45. The reaction mixture was then concentrated under vacuum and the resulting yellow oil was suspended in water and ethyl acetate. The aqueous phase was extracted by using ethyl acetate which was dried under vacuum to yield a yellow solid. This material was then purified using flash column chromatography eluting with ethyl acetate and light petroleum (5:1 v/v) to yield the product 3-(2-hydroxyhexyl)-6-(propan-2-yl)-5,6-dihydro-4*H*-pyrrolo[3,4-*c*][1,2]oxazol-4-one (**127**) as

a white solid (≤1 mg, 6 %). The product was confirmed only by ^{1}H NMR spectroscopy with the disappearance of the methyl group at 2.5-2.6 ppm, ^{13}C NMR spectroscopy and identification of the molecular ion by mass spectrometry due to a lack of available sample.



Scheme 45. Aldol addition of pentanal to pyrroloisoxazole

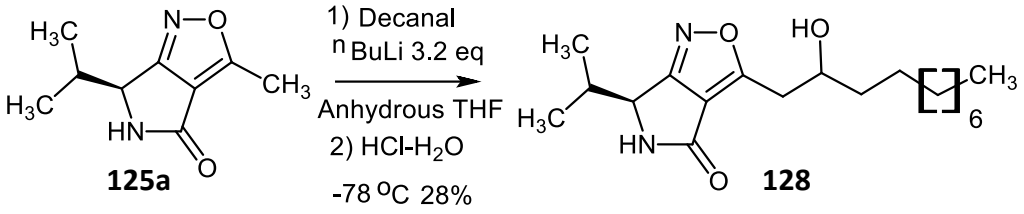
This yield was poor when compared with the non-enolisable aldehydes and surprising considering the aldol reaction is well established and typically high yielding[87]. This low yield required analysis to identify the issue, which will be discussed later. The equivalence of base used in the attempt was 3.2, which was established by previous research showing it to be the most productive.[75]

It was also identified that there would be diastereomers formed because of the introduction of a new chiral centre at the hydroxyl group carbon. This stereochemistry was not investigated since this chirality was intended to be immediately removed in the next stage via dehydration. It can be suggested that a roughly equal mixture of diastereomers would be obtained as the existing chiral centre is too remote to have any significant effect.

The next attempted chain extension was to add decanal, which was chosen as an alternative saturated analogue due to its longer chain length. The boiling point of this aldehyde was considerably higher than pentanal (207-209 ^{0}C[88]) and therefore required another form of purification. The aldehyde was suspended in diethyl ether and the solution washed several times with saturated potassium carbonate solution to remove any acidic impurities. The organic fraction was then dried and concentrated under a reduced pressure to achieve the pure aldehyde, confirmed by a clean NMR spectrum with the expected signals.

In this preparation, 3.2 equivalents of $^{n}BuLi$ was added dropwise to a solution of the pyrroloisoxazole 3-methyl-6-(propan-2-yl)-5,6-dihydro-4*H*-pyrrolo[3,4-*c*][1,2]oxazol-4-one (**125a**) in THF at -78 ^{0}C, under a nitrogen atmosphere. The solution was stirred at this

temperature for 2 hours, after which the excess purified decanal was added in one portion and stirring continued for at -78 ^{0}C for 2 hours. The reaction solution was then quenched by the addition of water and acidified with hydrochloric acid. The reaction mixture was concentrated under vacuum and the resulting yellow oil was partitioned between water and ethyl acetate. The aqueous phase was then extracted using ethyl acetate, which was dried and concentrated under vacuum to yield a crude yellow solid (900 mg, >100%). This was purified by flash chromatography eluting with ethyl acetate and light petroleum (5:1 v/v) to yield a mixture of products, which was chromatographed again under the same conditions to give the desired product 3-(2-hydroxyundecyl)-6-(propan-2-yl)-5,6-dihydro-4*H*-pyrrolo[3,4-*c*][1,2]oxazol-4-one (**128**) as a white solid (27 mg, 28 %), Scheme 46. The product was confirmed only by ^{1}H NMR spectroscopy with the disappearance of the methyl group at 2.5-2.6 ppm and identification of the molecular ion by mass spectrometry.



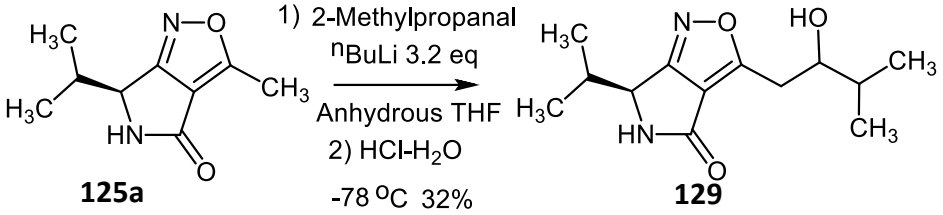
Scheme 46. Aldol addition of decanal to pyrroloisoxazole

This yield of 28% was more promising, but it is notable that this reaction was attempted more than 15 times and only yielded the desired compound once. This unreliability and recurring low yields led to the conclusion that the anion formation is not as dependable as first assumed.

The next aldehyde reaction was to add an α-substituted aldehyde, the simplest being 2-methylpropanal. This aldehyde was also purified by washing with saturated potassium carbonate solution to remove any acid by-product that could interfere with the reaction conditions. These precautions for purifying the reactants were taken even in cases where the commercial aldehyde was supplied freshly and were all confirmed by a clean ^{1}H NMR spectrum.

In the addition of 2-methylpropanal, ^{n}BuLi was added dropwise to a solution of the

pyrroloisoxazole 3-methyl-6-(propan-2-yl)-5,6-dihydro-4*H*-pyrrolo[3,4-*c*][1,2]oxazol-4-one (**125a**) in THF at -78 ^{0}C under a nitrogen atmosphere. The solution was stirred at this temperature for 2 hours, after such time the purified 2-methylpropanal was added in one portion and stirring continued for at -78 ^{0}C for a further 2 hours. The reaction was quenched as before, the mixture evaporated under a reduced pressure and suspended in water and ethyl acetate. Extraction with ethyl acetate and washings as before yielded crude yellow oil (845 mg, >100%), which was purified by flash chromatography eluting with ethyl acetate and light petroleum (5:1 v/v) to yield 3-(2-hydroxy-3-methylbutyl)-6-(propan-2-yl)-5,6-dihydro-4*H*-pyrrolo[3,4-*c*][1,2]oxazol-4-one (**129**) as a light yellow oil (30 mg, 32%), Scheme 47. The product was again confirmed only by ^{1}H NMR spectroscopy with the disappearance of the methyl group at 2.5-2.6 ppm and identification of the molecular ion by mass spectrometry.



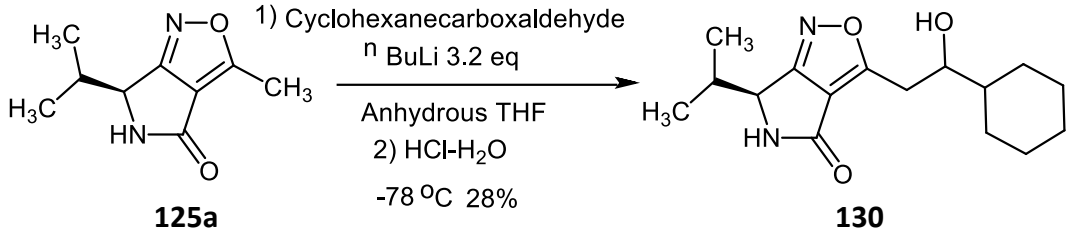
Scheme 47. Aldol addition of 2-methylpropanal to pyrroloisoxazole

The yield of 32% was the highest observed yield to date of this type of reaction in the group and provided promise, but this reaction was, also, only successful once in multiple attempts.

Progressing through our library of aldehydes the next to be tried was cyclohexanecarboxylaldehyde, another α-disubstituted aldehyde, also containing a ring. Again, due to a high boiling point, this aldehyde was purified by washing with saturated potassium carbonate solution.

The reaction was completed as seen before and was shown to yield the crude 3-(2-cyclohexyl-2-hydroxyethyl)-6-(propan-2-yl)-5,6-dihydro-4*H*-pyrrolo[3,4-*c*][1,2]oxazol-4-one (**130**) as a yellow oil (800 mg, >100%),%), which was purified by flash chromatography eluting with ethyl acetate and light petroleum (5:1 v/v) to yield 3-(2-cyclohexyl-2-hydroxyethyl)-6-(propan-2-yl)-5,6-dihydro-4*H*-pyrrolo[3,4-*c*][1,2]oxazol-4-one (**130**) as a

light yellow oil (45 mg, 28%), Scheme 48. Products were confirmed only by ^{1}H NMR spectroscopy, with the disappearance of the methyl group at 2.5-2.6 ppm, and identification of the molecular ion by mass spectrometry.

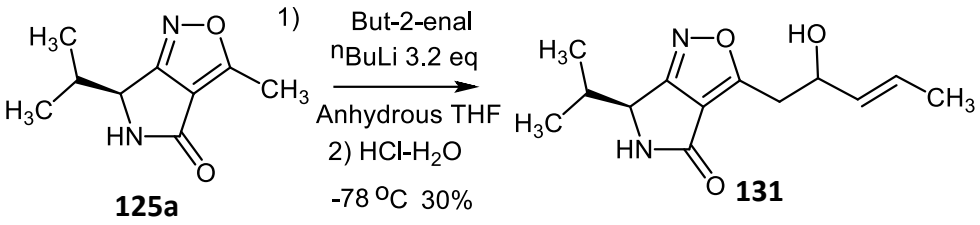


Scheme 48. Aldol addition of cyclohexanecarboxaldehyde to pyrroloisoxazole

This yield of 28% was another successful result, but unfortunately this result could not be reproduced under similar conditions in several attempts.

The last aldehyde analogue utilised in this stage of the project was unsaturated at the 2,3-position, namely but-2-enal, Scheme 49. This was chosen as there may be the need to add unsaturated chain extensions en route to naturally occurring 3-acyltetramic acids in the future, and also for completeness of the study. Again, this aldehyde was purified by washings which saturated potassium carbonate solution.

The reaction was completed as above and was shown to yield the crude 3-[(3*E*)-2-hydroxypent-3-en-1-yl]-6-(propan-2-yl)-5,6-dihydro-4*H*-pyrrolo[3,4-*c*][1,2]oxazol-4-one (**131**) as a yellow oil (500 mg, >100%). This was purified by flash chromatography eluting with ethyl acetate and light petroleum (5:1 v/v) to yield 3-[(3*E*)-2-hydroxypent-3-en-1-yl]-6-(propan-2-yl)-5,6-dihydro-4*H*-pyrrolo[3,4-*c*][1,2]oxazol-4-one (**131**) as a light yellow oil (10 mg, 30%). This compound was confirmed by ^{1}H NMR spectroscopy, with the disappearance of the methyl group at 2.5-2.6 ppm, ^{13}C NMR spectroscopy, identification of the molecular ion by mass spectrometry and IR analysis. The full analysis was able to be acquired on this molecule when the reaction was reproduced a further one time. This was a breakthrough in that this specific aldol addition was reproduced, although it was only twice in over 15 attempts. A 30% yield was a relatively good yield for this reaction based on previous attempts.



Scheme 49. Aldol addition of crotonaldehyde to pyrroloisoxazole

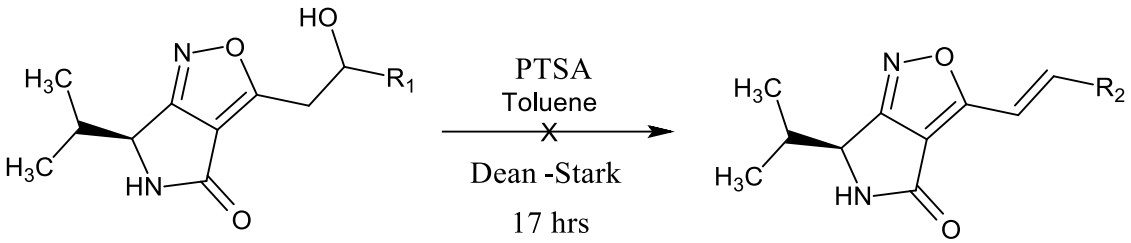
These five aldehydes were all successfully added to the C-3 methyl group at least once and the adduct structures were suggested by mass spectrometry, IR analysis in one case and NMR analysis. These aldol adduct pyrroloisoxazoles were then required to undergo a condensation elimination of the hydroxyl group to form the double carbon-carbon bond in the product.

These studies and further investigations seen later in this thesis have however shown that the anion formation is unreliable and its ability to reproducibly attack any appropriate carbonyl moiety is questionable. This issue will be discussed in further detail later.

2.2.2 – Extended Pyrroloisoxazole Dehydration

A dehydration step was required to show the enolisable aldehyde addition product was able to be converted into an unsaturated compound. This was desirable as many 3-acyltetramic acid natural products exhibit this unsaturated functionality at the C-3 acyl substituent.

The dehydration appears simple and involves using an organic soluble acid, such as *p*-toluenesulfonic acid (PTSA) in toluene, to protonate the hydroxyl group and make a good leaving group. The potential conjugation in the system will then drive elimination of water forming a new carbon-carbon double bond. Due to the possible reverse reaction, the water produced must be removed via a Dean-Stark apparatus set-up. All of the attempted dehydrations of alcohols **127-131** failed to afford any isolated alkene products, Scheme 50.



127 – R_1 –$CH_2CH_2CH_2CH_3$

128 – R_1 –$CH_2(CH_2)_7CH_3$

129 – R_1 –$CH_2[CH_3]_2$

130 – R_1 –C_6H_{11}

131 – R_1 –$CHCHCH_3$

132 – R_2 –$CH_2CH_2CH_2CH_3$

133 – R_2 –$CH_2(CH_2)_7CH_3$

134 – R_2 –$CH_2[CH_3]_2$

135 – R_2 –C_6H_{11}

136 – R_2 –$CHCHCH_3$

Scheme 50. General synthetic attempts at dehydrating the aldol adducts

It was unknown why they should fail based on successful, high yielding, reactions being performed on the non-enolisable aldehyde derivatives in the past. Possible explanations include the fact the reactions were attempted on small scale, around 20mg-30mg, which could have led to detection errors. Also, a separation was required and although crude NMR spectra were obtained, separations were not completed successfully as R_F values were too similar and the products contained too many impurities. It is also thought that a condensation under acidic conditions could be destructive to the molecule, so therefore further work on this reaction would be needed to investigate the substitution of other leaving groups (such as mesylates) into this position.

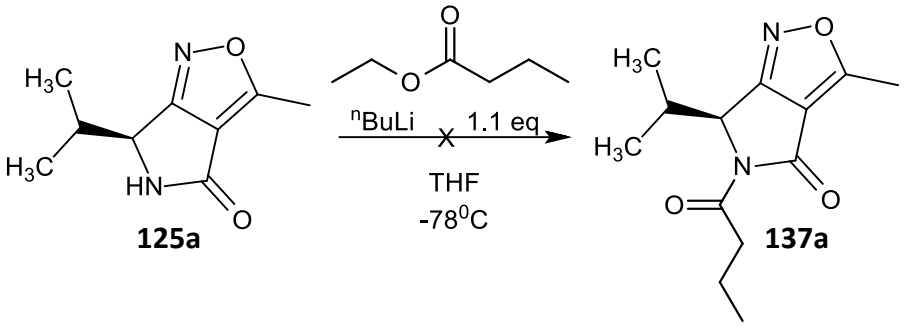
An attempt was made to produce the aldol adducts as acetyl adducts by quenching with freshly distilled acetyl chloride. Due to the unreliability of the aldol reaction, this did not prove possible and therefore it was not determined if this leaving group would have eliminated to yield the unsaturated target.

2.3 – Reutericyclin and Analogue Synthesis from a Variety of Pyrroloisoxazoles, Elaboration via *N*-Acylation and Alkylation.

2.3.1 – *N*-Acylation Reactions of Pyrroloisoxazoles

As previously discussed, reutericyclin and analogues have been shown to have potent biological activity against Gram-positive bacteria, such as hospital superbug *C. difficile*, so it was decided to synthesise a library of structurally similar compounds, all with different N-substitutions. N-Acylpyrroloisoxazoles could be regarded as masked reutericyclin analogues.

Once a method had been investigated for the aldol reactions at the C-3 methyl group of our pyrroloisoxazoles, our attention was diverted to the potential of *N*-acyl and -alkyl derivatisation. As we recognised from the aldol studies, n-butyllithium was a sufficiently strong base to remove the amide proton and so was chosen as the base in these acylation reactions. The concept was an *N*-acylation reaction coupling an amide anion and an ester (as esters are cheap and easy to store), but preliminary results showed the ester was not reactive enough to undergo the acylation, only yielding quantitative amounts of the starting materials indicating no reaction occurred. The valine-derived pyrroloisoxazole (**125a**) was suspended in dry THF and cooled to -78^{0}C before adding ethyl butyrate followed by stirring the reaction mixture for 1 hour at this temperature. This solution was quenched with saturated ammonium chloride solution to pH 7, extracted with ethyl acetate, dried over magnesium sulfate and filtered. The filtrate was evaporated under reduced pressure and only starting material was identified using ^{1}H NMR spectroscopy.

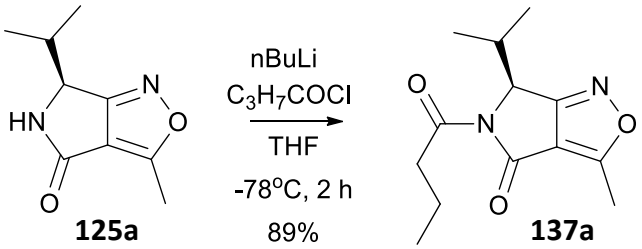


Scheme 51. Attempted N-acylation using an ester, ethyl butyrate

The next attempts were made using a selection of acyl chlorides to provide variety in the library of compounds produced. This diversity was also accomplished by using both of the pyrroloisoxazoles synthesised, the leucine- and valine-derived pyrroloisoxazoles, and also one prepared by another member of the group from phenylglycine.[75]

We opted to work with pyrroloisoxazoles derived from the (S)-amino acids, although this is enantiomeric to reutericyclin, based on availability and cost. We reasoned that our studies would be transferable to the other enantiomeric series.

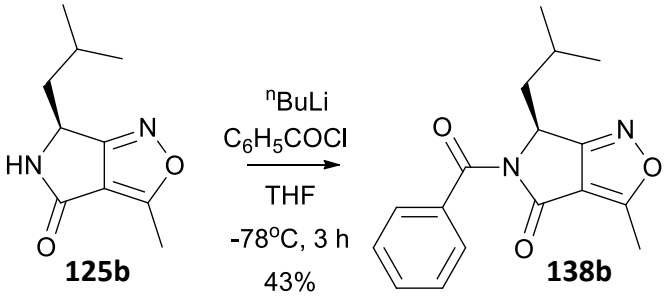
The prototype reaction was completed using the valine-based pyrroloisoxazole (**125a**) with readily available butanoyl chloride. This reaction was undertaken following a developed procedure using standard acylation conditions and a base validated in the aldol reactions, but is similar to the methodology reported by Schobert *et al.* in 2006[35]. The valine-derived pyrroloisoxazole was suspended in dry THF and cooled to -78 ^{o}C before the addition of 1.1 equiv of n-butyllithium to deprotonate the amide. This was allowed to stir at -78^{o}C for 10 minutes, to form the anion, followed by the addition of 1.1 equiv of butyrl chloride, after which the reaction was left to stir for a further 2 hours at -78 ^{o}C and subsequently quenched with saturated ammonium chloride solution. The aqueous layer was extracted with ethyl acetate and the organic fraction dried over magnesium sulfate before being concentrated under a reduced pressure to yield pure (S)-5-butyryl-6-(propan-2-yl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**137a**) in 89% yield (Scheme 52).



Scheme 52. *N*-Acylation of valine pyrroloisoxazole using butyryl chloride

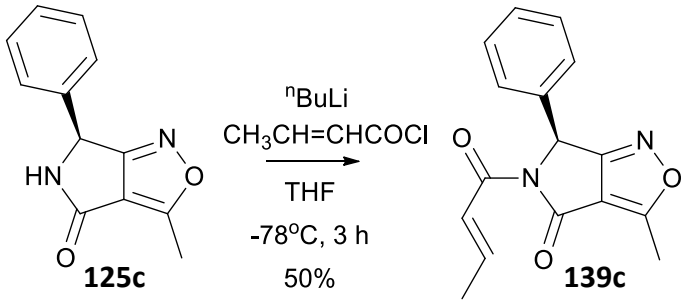
The next example attempted was to couple the leucine-derived pyrroloisoxazole (**125b**) with a sterically bulky acyl chloride, benzoyl chloride. In this reaction the leucine-derived pyrroloisoxazole was suspended in dry THF and cooled to -78^{o}C before the addition of 1.1 equiv of n-butyl-lithium to deprotonate the amide, as seen above. This was allowed to stir at -78^{o}C for 10 minutes, to form the anion, followed by the addition of 1.1 equiv of benzoyl

chloride, after which the reaction was left to stir for a further 2 hours at -78°C and subsequently quenched with saturated ammonium chloride solution. The aqueous layer was extracted with ethyl acetate and the organic fraction dried over magnesium sulfate before being concentrated under a reduced pressure to yield crude (S)-5-benzoyl-6-(2-methylpropyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**137b**), which was could not be isolated pure in a quantifiable yield after prep-TLC. This lead to an increase in reaction time from 2 hours to 3 hours and, when the reaction was completed under these new time conditions pure benzoyl N-substituted product (**137b**) was isolated after purification by prep-TLC eluting with light petroleum:ethyl acetate (3:1 v/v) in a yield of 43%, Scheme 53.



Scheme 53. *N*-Acylation of leucine pyrroloisoxazole using benzoyl chloride

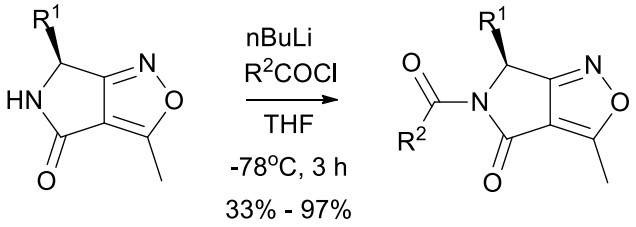
Another illustration of this chemistry was using the phenylglycine-derived pyrroloisoxazole (**125c**) to couple to a conjugated acyl chloride, 2-butenoyl chloride, which was produced by an earlier student[75] and used in this synthesis after ^{1}H NMR confirmation. In this reaction the phenylglycine-derived pyrroloisoxazole was suspended in dry THF and cooled to -78 °C before the addition of 1.1 equiv of n-butyllithium to deprotonate the amide, as seen above. This was allowed to stir at -78 °C for 10 minutes, to form the anion, followed by the addition of 1.1 equiv of benzoyl chloride, after which the reaction was left to stir for a further 3 hours at -78 °C and subsequently quenched with saturated ammonium chloride solution. The aqueous layer was extracted with ethyl acetate and the organic fraction dried over magnesium sulfate before being concentrated under a reduced pressure to yield crude (S,E)-5-(but-2-enoyl)-6-(propan-2-yl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**138c**), which could be isolated pure in a 50 % yield after prep-TLC, Scheme 54.



Scheme 54. *N*-Acylation of phenylglycine pyrroloisoxazole using crotonyl chloride

Based on the success of these reactions, it was decided to create a collection of compounds that would all be 'masked' analogues of reutericyclin, all with potential for biological activity.

The library of compounds that was devised had to show structural diversity and this was accomplished by using a selection of acyl chlorides including long and short aliphatic chains, an unsaturated substituent, a bulky sterically hindered group and an aromatic moiety. This was achieved by successfully coupling 2-butanoyl chloride, decanoyl chloride, 2-butenyl chloride, trimethylacetyl chloride and benzoyl chloride. The diversity, as previously mentioned, was also built upon by not only using one example of a pyrroloisoxazole but three (Scheme 55), all accomplished in yields of 33% to 97% producing 12 novel compounds. A full illustration of the compounds synthesised in this research is shown in **Table 4** and Scheme 58 with individual yields.



a – R^1 –$(CH_3)_2CH$,

b – R^1 – $(CH_3)_2CHCH_2$

c – R^1 – $CH(C_6H_5)$

137 – R^2– C_3H_7 **139** – R^2– $CHCHCH_3$

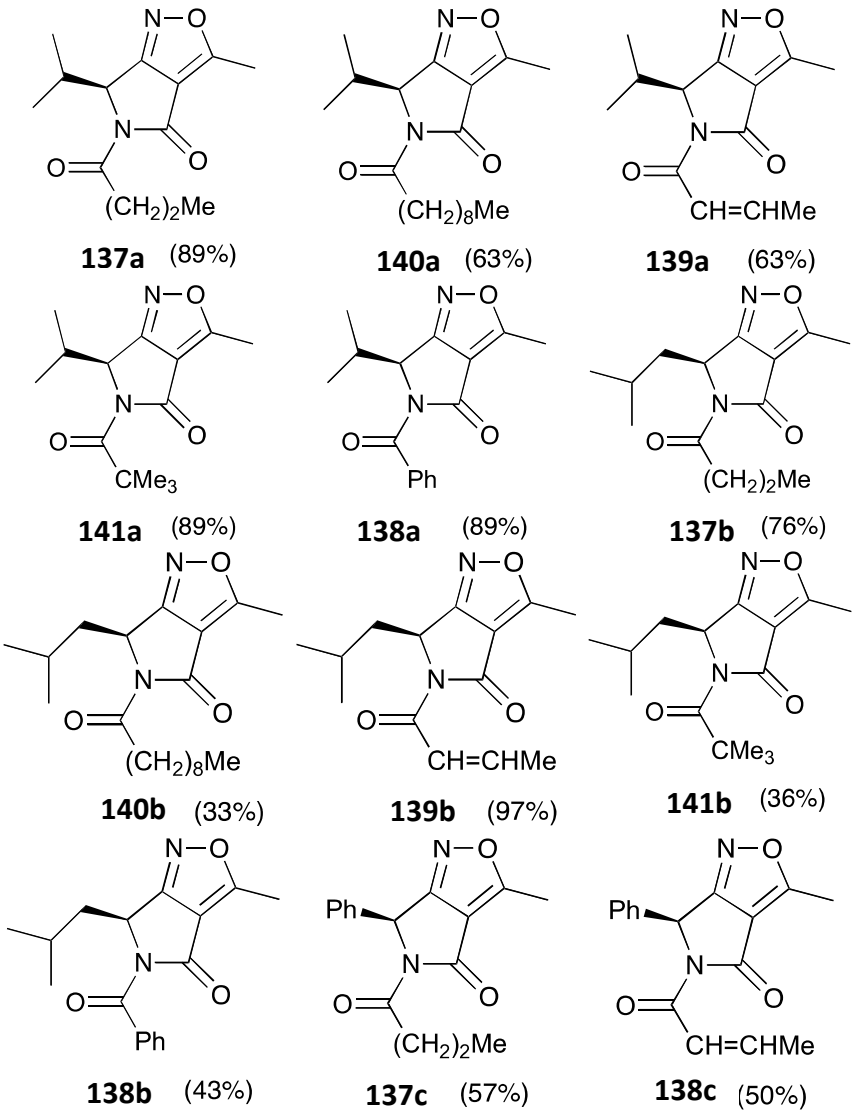
140 – R^2– C_9H_{17} **141** – R^2– $C(CH_3)_3$

138 – R^2– C_6H_5

Scheme 55. *N*-Acylation reactions summary

There were some important alternations made to the synthesis protocol when using different acyl chlorides, with the initial alteration required being a purification step. But-2-enoyl chloride was able to be removed by evaporation under a reduced pressure, as was crotonyl chloride, but the other three acyl chloride starting materials required removal via preparative thin layer chromatography (Prep-TLC) eluting with light petroleum:ethyl acetate (3:1 v/v). It was deduced that the increase in residual starting material, as these reactions were lower yielding, meant they were unable to be removed completely simply under a reduced pressure. This led to the adoption of an increased reaction time from the initial 2 hours to an improved 3 hours, which ultimately increased yields of all the acylations, especially the more sterically bulky examples. Even after this extended reaction time, prep-TLC was required to provide the pure *N*-acyl products.

Table 4. Library of *N*-acyl masked reutericyclin analogues (with respective yields)



Confirmation of all the products was obtained by accurate mass spectrometric identification of the molecular ion, infra-red analysis, ^{13}C and ^{1}H NMR spectroscopy. The latter often showed a characteristic splitting in the protons alpha to the carbonyl group on the added substituent as well as disappearance of the amide proton on the pyrroloisoxazole. In addition, a crystal was able to be produced suitable for X-ray crystallography from the valine based pyrroloisoxazole and crotonyl chloride, with the product characterised as (S,E)-5-(but-2-enoyl)-6-isopropyl-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**139a**, Figure 25). This structure also confirmed that the N-acylation had occurred rather than the possible lactam O-acylation.

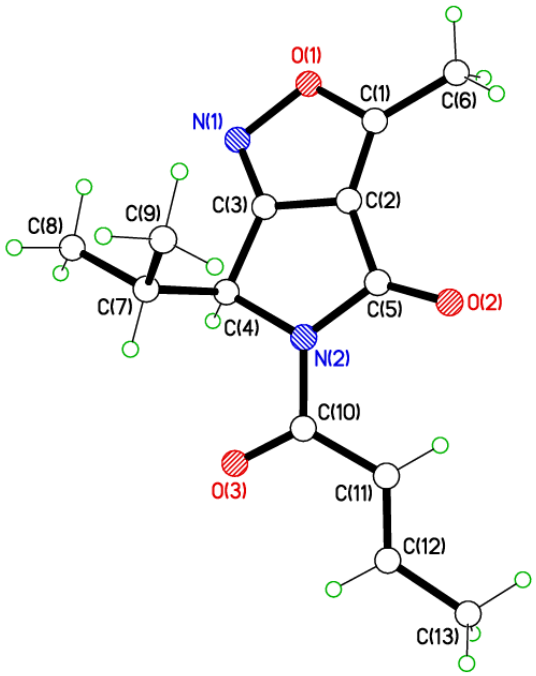
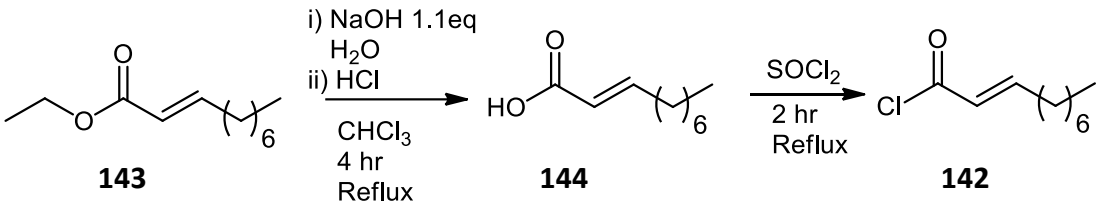


Figure 25. X-ray structure of (S,E)-5-(But-2-enoyl)-6-isopropyl-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (139a)

In an attempt to add the N-acyl substituent found in reutericyclin, we tried to prepare the appropriate acyl chloride, (E)-dec-2-enoyl chloride (**142**), was produced from the commercially available ethyl ester, ethyl (E*)*-2-decenoate (**143**), Scheme 56.

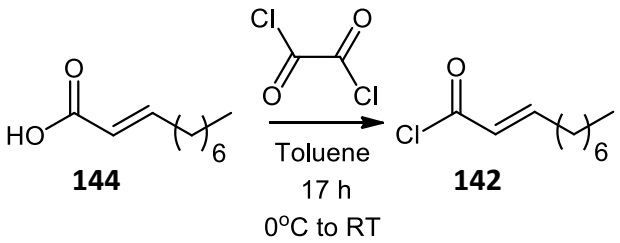
To ethyl (E)-2-decanoate (**143**) was added aqueous sodium hydroxide and the mixture was heated to reflux for 4 hours. After cooling, the solution was washed with chloroform to remove any starting material and the aqueous layer was acidified to pH 2-3 using concentrated hydrochloric acid. The resultant white precipitate was collected by filtration, dissolved in chloroform, which was then dried over magnesium sulfate, filtered and reduced under a reduced pressure to yield (E)-2-decenoic acid (**144**) as a white solid. After characterisation this carboxylic acid was chlorinated by being suspended solventless in thionyl chloride and stirred for 2 h to reflux. Removal of the excess thionyl chloride was attempted under a reduced pressure to yield (E)-dec-2-enoyl chloride (**142**).



Scheme 56. Formation of acyl chloride precursor for reutericyclin analogue synthesis

This reaction was successful, with characterisation of the desired product but TLC analysis continually showed remaining thionyl chloride, which was evident by repeated failed attempts at the *N*-acylation reaction. Several different attempts were made to remove this excess thionyl chloride. Standard methods involving column chromatography would lead to the degradation of the desired acyl chloride so alternatives were required. These included extended periods of heating under a reduced pressure, 7 hours at 60^0C, as well as adding formic acid in an attempt to decompose the thionyl chloride to carbon dioxide and sulfur dioxide, but all were unsuccessful.

Another attempt was made to access the acyl chloride using oxalyl chloride.[89] This was completed by adding oxalyl chloride to dropwise to the (E)-2-decanoic acid in toluene at 0^oC which, after concentration under a reduced pressure yielded the desired product, yet still with impurities which were identified by ^{13}C NMR spectroscopy as the oxalyl chloride, Scheme 57. This was disappointing but due to the collection of 12 compounds already synthesised as part of the studies towards reutericyclin analogues, it was decided to leave the synthesis at this point.



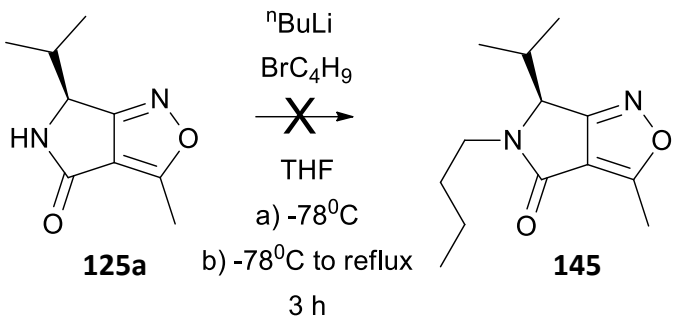
Scheme 57. Formation of acyl chloride precursor for reutericyclin analogue synthesis with oxalyl chloride

2.3.2 – *N*-Alkylation Reactions of Pyrroloisoxazoles

After the success of the acylations, and some examples of *N*-alkyl reutericyclin derivative having being reported to have biological activity[3], it was decided to attempt alkylations of pyrroloisoxazoles under the same conditions as the successful acylations.

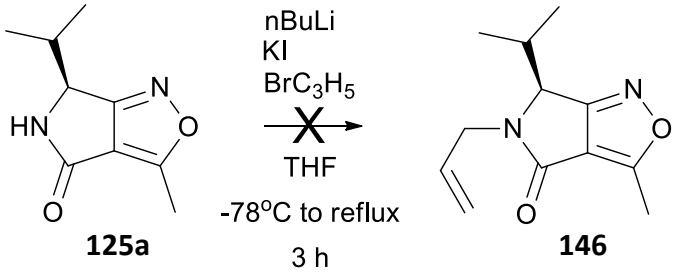
The first to be attempted was to use an alkyl halide to be incorporated by a nucleophilic substitution reaction. 1-Bromobutane was selected as the haloalkane and the pyrroloisoxazole used in all cases attempted was from the valine-based series, **125a**. The reaction was methodologically the same as the acylations, with the pyrroloisoxazole being stirred to -78 ^{o}C in dry THF and the base (1.1 equiv n-butyllithium) added. After 10 minutes the 1-bromobutane was charged to the reaction mixture which was left to stir for 2 hours followed by quenching with saturated ammonium chloride. After extraction of the aqueous layer with ethyl acetate, drying over magnesium sulfate and concentration under a reduced pressure, quantitative recovery of the valine pyrroloisoxazole starting material was obtained, with no sign of any alkylation product, Scheme 58.

This result was disappointing, so modifications were made to the reaction conditions in that the temperature was increased; instead of quenching with saturated ammonium chloride solution at -78 ^{o}C the reaction was allowed to warm to room temperature over the 2 hours and quenched at 23 ^{o}C. This also, after washings, drying and concentration, returned all the pyrroloisoxazole starting material. The assumption was made that the alkyl halide was not reactive enough and therefore it was decided to use 3-bromo-1-propene as it is more reactive. Again allowing the reaction to reach room temperature before quenching also led to the isolation of starting material, with no indication of any desired reaction occurring. The next alteration was to again increase reaction temperature, with the idea that if any observed reaction occurred, of any yield, the reaction time could be increased later if required. The reaction was now heated to reflux after initial addition of the n-butyl-lithium at -78^{o}C, and addition of the 3-bromo-1-propene, but yet again only pyrroloisoxazole starting material was apparent after work up, Scheme 58.



Scheme 58. Attempts at *N*-alkylation of valine pyrroloisoxazole using 1-bromobutane

The last attempt at *N*-alkylation was to increase the reactivity of the allyl bromide by adding potassium iodide as a nucleophilic catalyst. The reaction was performed as before, heated to reflux, but with 1 equiv potassium iodide with the intention that there would be ion exchange forming 1-iodo-prop-3-ene *in situ* and therefore increasing the reactivity of the allyl halide. This did not lead to a successful reaction and instead, as seen in all other attempts, only returned the pyrroloisoxazole starting material after work up, Scheme 59. A summary of all the conditions tried is shown in Table 5.



Scheme 59. Attempts at *N*-alkylation of valine pyrroloisoxazole using 3-bromo-prop-3-ene

Table 5. *N*-Alkylation conditions attempted

Ref.	*1*	*2*	*3*	*4*	*5*
Substrate	1-Bromobutane	1-Bromobutane	Allyl Bromide	Allyl Bromide	Allyl Bromide
Time	2 Hr	2 Hr	2 Hr	2 Hr	2 Hr
Temperature	-78°C	-78°C to rt	-78°C to rt	-78°C to reflux	-78°C to reflux
Ion Catalyst	None	None	None	None	Potassium Iodide

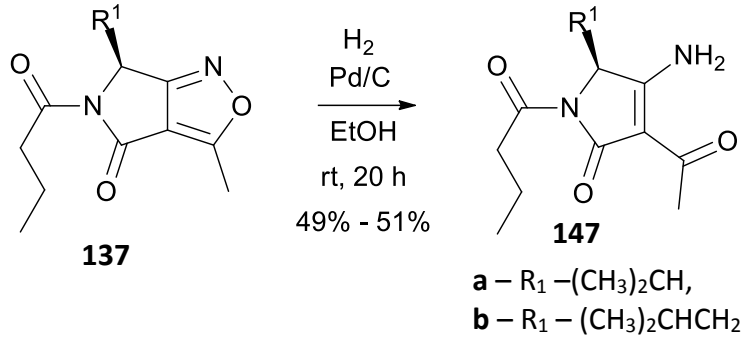
These results were disappointing, but it was decided that the *N*-acyl compounds contained sufficient diversity to continue the synthesis with them alone and not to spend more time developing the alkylation reaction. This also indicates that the nucleophilicity of the nitrogen anion formed by the deprotonation is not sufficient enough to attack the alkyl and allyl halides.

2.3.3 – Reduction of the *N*-Acyl Pyrroloisoxazoles

In the synthesis of acyltetramic acids, the produced *N*-acyl pyrroloisoxazoles would next require the isoxazole to be ring opened to give an enaminoketone which could subsequently be hydrolysed to give the *N*,3-diacyltetramic acid. The conditions for this reduction in the case of NH-pyrroloisoxazoles have already been established by Jones in previous publications[56,57] and consisted of two different reaction protocols, one for saturated and one for unsaturated C-3 acyl substituents.

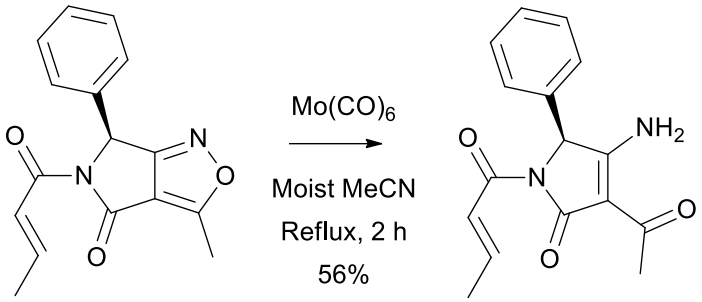
The reaction conditions for the opening of the isoxazole ring developed for pyrroloisoxazoles with saturated C-3 substituents were a hydrogenation using hydrogen gas with a palladium on carbon catalyst. This was successfully completed on the (S)-5-butyryl-6-(2-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**137a**) and (S)-5-butyryl-6-(2-methylpropanyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**137b**) systems and involved suspending the pyrroloisoxazoles in ethanol followed by addition of this suspension to a dry, nitrogen-filled reaction flask already containing the palladium on carbon in ethanol. The reaction vessel was then evacuated using a vacuum pump and subsequently filled with nitrogen gas, repeated three times before repeating this purge technique with hydrogen gas. The reaction solution was then left under an atmosphere of hydrogen gas and stirred vigorously for 17 hours at room temperature before being purged a further three times with nitrogen gas before allowing in air. The reaction mixture was then filtered through a celite pad and concentrated under a reduced pressure to yield the crude enaminoketone (**147**), which was purified using Prep-TLC eluting with ethyl acetate:light petroleum (3:1 v/v). Confirmation of the desired product was obtained by accurate mass spectrometry, IR analysis, ^{13}C and ^{1}H NMR spectroscopy (Scheme 60). The ^{1}H NMR spectra were distinctive in that a peak pattern identical to the pyrroloisoxazole was observed but with the addition of two broad singlets for the new amine group, one at 6.1

ppm and another at 9.4 ppm. This large splitting between the two peaks also identified that the product was an enaminoketone, with strong hydrogen bonds between one of the amine hydrogen atoms and the adjacent ketone, and not the alternative tautomeric imino-enol.



Scheme 60. Hydrogenation ring opening of isoxazole ring

The other reaction conditions utilised were to produce the same enaminoketone but using an *N*-unsaturated acyl pyrroloisoxazole, namely (S,*E*)-5-(But-2-enoyl)-3-methyl-6-phenyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**148**). The double carbon-carbon bond in the acyl group can be reduced by H_2 and palladium on carbon, so an alternative method had to be used, specifically using molybdenum hexacarbonyl in moist acetonitrile. This ability of molybdenum hexacarbonyl was first observed and explained by Nitta in 1985[90] and following his work it was utilised by our group in earlier syntheses.[57] The reaction required (S,*E*)-5-(But-2-enoyl)-3-methyl-6-phenyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**139c**) to be dissolved in acetonitrile:water (4:1 v/v) and added to molybdenum hexacarbonyl, upon which the reaction mixture was heated to reflux for 1 hour before the addition of 2M aqueous hydrochloric acid, followed by a further 1 hour stirring at reflux. The reaction mixture was then allowed to cool before filtering through celite. The aqueous filtrate was extracted with dichloromethane, washed with water and saturated brine, dried over magnesium sulfate and concentrated under a reduced pressure to yield the impure enaminoketone. This was isolated pure using Prep-TLC eluting with ethyl acetate:light petroleum (3:1 v/v) with confirmation of the desired product obtained by accurate mass spectrometry, IR analysis, ^{13}C and ^{1}H NMR spectroscopy, Scheme 61. The ^{1}H NMR spectrum again contained the distinguishing broad singlets signifying the formation of an enaminoketone whilst also containing the relevant hydrogen resonances for the unsaturated carbon side chain.



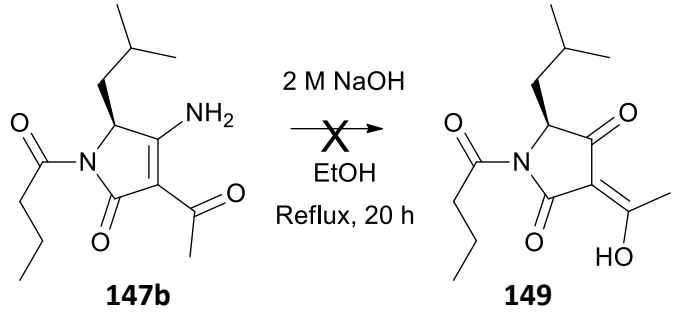
139c **148**

Scheme 61. Reduction using molybdenum hexacarbonyl ring opening of isoxazole ring

This process provided us with three novel enaminoketone compounds containing *N*-acylation that were also suitable for biological testing as the structure of the enaminoketone pyrrolidine is electronically similar to the final acyltetramic acid.

2.3.4 – Hydrolysis of the *N*-Acyl Pyrrolidine compounds

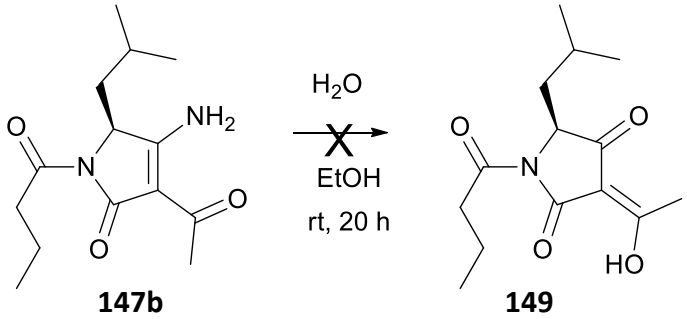
From the outset of this synthesis, this stage was a concern that required research. The issue was that this step required a hydrolysis reaction, which in earlier examples of its use had not presented a problem, but we now had *N*-acylation to give an imide which can easily be hydrolysed back to the amide. The first attempted hydrolysis used the previously established sodium hydroxide hydrolysis in THF:water[91,57] which involved adding the (S)-3-acetyl-4-amino-1-butanoyl-5-isobutyl-1H-pyrrol-2(5H)-one (**147b**) to a solution of THF and 2M sodium hydroxide and stirring at reflux for 20 hours, Scheme 62. The work up required an acidification to remove excess hydroxide and an organic extraction, drying and concentration under a reduced pressure. This inevitably led to the hydrolysis of the imide and yielded a known acyltetramic acid, which was characterised by the reappearance of the amide proton at 6.5 ppm in a ^{1}H NMR analysis and an upfield shift in the alpha proton on the adjacent carbon, 2.5 ppm in imide and 2.0 ppm in amide. Although this compound was not fully characterised extensive separations led to the decomposition of this compound. It was decided to attempt very mild conditions and work toward more vigorous conditions, with the idea to preserve material.



147b **149**

Scheme 62. Attempted leucine enaminoketone hydrolysis using 2 M sodium hydroxide

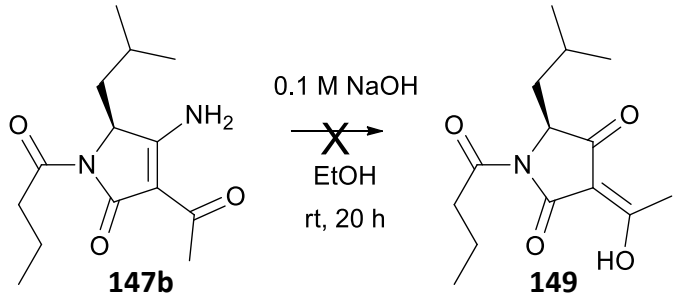
The next effort was made using just water as a source of hydrolysis, which after 24 hours at room temperature yielded only unchanged (S)-3-acetyl-4-amino-1-butyryl-5-isobutyl-1H-pyrrol-2(5H)-one (**147b**) starting material based on ^{1}H NMR spectroscopy data, obtained after work up and concentration under a reduced pressure, Scheme 63.



147b **149**

Scheme 63. Attempted leucine enaminoketone hydrolysis using water

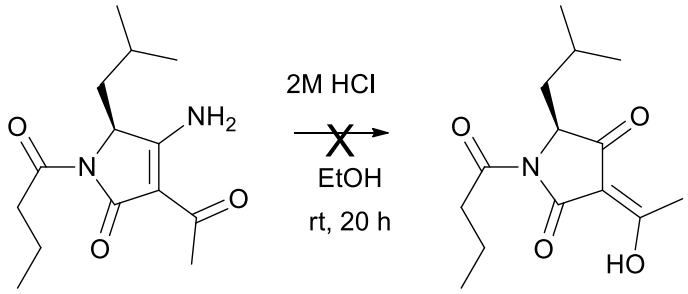
A subsequent reaction, using a 0.1 M solution of sodium hydroxide at room temperature overnight, again lead to the hydrolysis of the imide, as identified by NMR spectroscopy, Scheme **64**, so it was decided that other methods for the amine removal would be investigated.



147b **149**

Scheme 64. Attempted leucine enaminoketone hydrolysis using 0.1 M sodium hydroxide

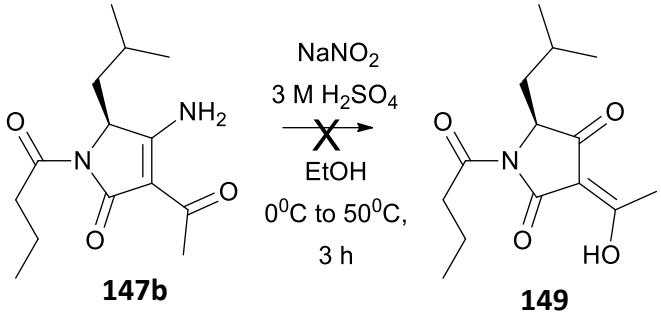
After the attempts made at a basic hydrolysis, the next logical step was an acid hydrolysis. This was attempted by dissolving (S)-3-acetyl-4-amino-1-butyryl-5-isobutyl-1H-pyrrol-2(5H)-one (**147b**) in a minimum amount of ethanol and stirring with 2M hydrochloric acid for 24 hours at room temperature. After an organic extraction using dichloromethane, the potential product was concentrated under a reduced pressure and analysis by NMR spectroscopy showed there had been no reaction and enaminoketone starting material was re-isolated, Scheme 65.



147b **149**

Scheme 65. Attempted leucine enaminoketone acid hydrolysis using 2M hydrochloric acid

This was disappointing as all attempts at hydrolysis had failed, therefore another methodology was required. In 2009, a previous group member had used a method of diazotisation to remove an amine in a related pyridone series[92]. In this technique the (S)-3-acetyl-4-amino-1-butyryl-5-isobutyl-1H-pyrrol-2(5H)-one (**147b**) was dissolved in ethanol and added to 3M sulfuric acid, before adding sodium nitrite in water at 0 ^{o}C. This solution was then stirred at 0 ^{o}C for 1 hour before being heated to 50 ^{o}C and further stirred for another hour. The aqueous layer was extracted with ethyl acetate, dried over magnesium sulfate and concentrated under a reduced pressure to yield a yellow oil. Upon analysis by ^{1}H NMR spectroscopy the isolated compound was shown to be a mixture of residual (S)-3-acetyl-4-amino-1-butyryl-5-isobutyl-1H-pyrrol-2(5H)-one (**147b**) and degraded material, presumable due to the strong acid conditions. Separation by prep-TLC yielded none of the desired acyltetramic acid, Scheme 66.



147b **149**

Scheme 66. Attempted leucine enaminoketone diazotisation using sodium nitrite

All available known routes to obtain this elusive acyltetramic acid structure have ended in either isolation of (S)-3-acetyl-4-amino-1-butyryl-5-isobutyl-1H-pyrrol-2(5H)-one (**147b**) starting material or the removal of the *N*-acyl elaboration. A summary of the condition attempted is shown in Table 6. This result is disappointing in the synthesis of acyltetramic acids but along the way, this synthetic approach has yielded a large library of novel compounds as masked reutericyclin analogues (pyrroloisoxazoles and enaminoketones), all suitable for biological testing.

Table 6. Enaminoketone hydrolysis or diazotisation methods

REF.	*1*	*2*	*3*	*4*	*5*
TYPE OF REACTION	Base Hydrolysis	Base Hydrolysis	Base Hydrolysis	Acid Hydrolysis	Diazotisation
REACTANT (CONCENTRATION)	H_2O	NaOH (0.1 M)	NaOH (2 M)	HCl (2 M)	$NaNO_2$
TIME	20 Hr	20 Hr	20 Hr	20 Hr	3 Hr
TEMPERATURE	reflux	rt	-rt	rt	0°C to 50°C
RESULT	no reaction	N-acyl hydrolysis	N-acyl hydrolysis	no reaction	no reaction

This work was accepted for publication in Chemical Communications[93] and can be found in the appendix of this thesis.

2.4 – Model Isoxazole Synthesis and Elaboration

Due to the expense of synthesising the pyrroloisoxazole units, both in time and money, it was decided that a model of the (S)-5-butyryl-6-isopropyl-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) would be produced which would be used to assess the success of elaboration techniques and determine which should transferred to the full pyrroloisoxazole.

The model would need to contain the isoxazole ring with a methyl group at C-5 (corresponding to C-3 of the pyrroloisoxazole) as well as a carbonyl group substituted at the C-4 position. It was initially proposed that an ester carbonyl (**150**) would be suitable to mimic the amide we see in the pyrroloisoxazole but, as discussed later, this proved unsuitable and an amide (**151**) was used (Figure 26).

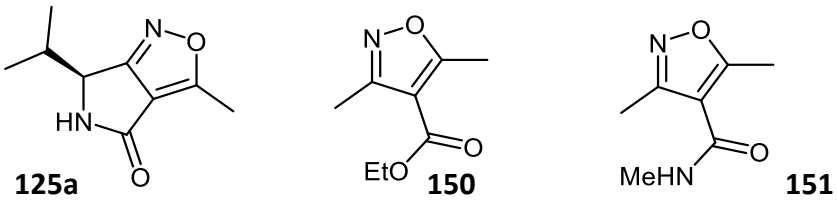
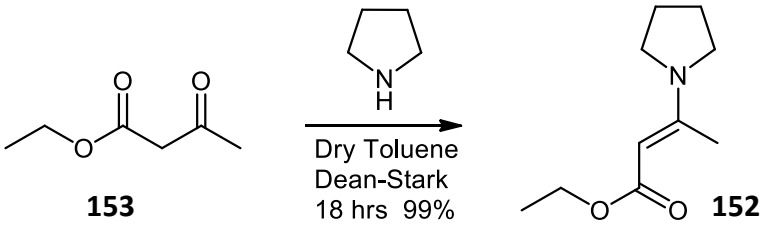


Figure 26. A pyrroloisoxazole example (125a), ester model (150) and amide model (151)

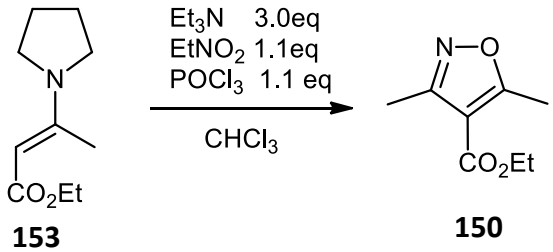
The synthesis of these isoxazoles was developed from a synthesis used previously in our research group[94] which involved a two stage one-pot reaction to provide the ester containing isoxazole.

The first of these reactions is an enamine (**152**) formation, as seen before by the addition of pyrrolidine to ethyl acetoacetate (**153**) dissolved in toluene with heating to reflux under Dean-Stark conditions to remove the water produced, Scheme 67. The amount of water formed can be measured and from this it was determined the reaction was complete after 18 h and, after removing excess solvent under a reduced pressure, a sample was taken for spectroscopic analysis. ^{1}H NMR spectroscopy showed the formation of the new double bond and the addition of the pyrrolidine group to the molecule, with IR spectroscopy and mass spectrometry confirming this in a 99% yield.

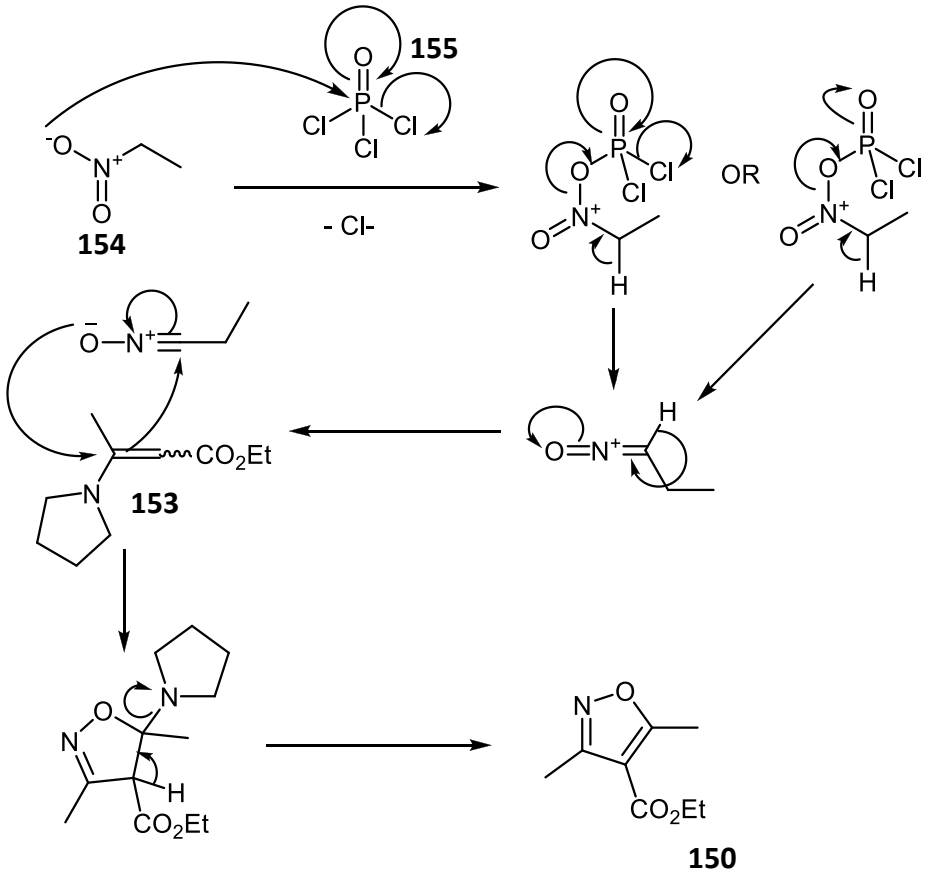


Scheme 67. Enamine formation from ethyl acetoacetate

The second of the two stages was the cycloaddition to form an isoxazole ring and subsequent elimination of the pyrrolidine to produce the aromaticity seen in the final isoxazole. The reactive 1,3-dipole nitrile oxide (**81**) required had to be formed *in situ* due to its instability. To produce this, firstly, triethylamine was added to the newly formed enamine, followed by nitroethane (**154**) in chloroform and the reaction mixture was cooled to 0 ^{0}C. When the mixture had reached 0 ^{0}C phosphorus oxychloride (**155**) was added dropwise over 1.5 h to ensure a controlled reaction, after which time the reaction mixture was allowed to warm to room temperature and stirred overnight, Scheme 68. The organic layer was then washed with aqueous acid, base and brine to remove excess triethylamine and other water soluble impurities. After drying and concentration under reduced pressure, ethyl 3,5-dimethylisoxazole-4-carboxylate (**150**), as a yellow thick oil, was confirmed by NMR spectroscopy showing the new ring system, supported by IR spectroscopy and mass spectrometric data in a 47% yield. A suggested mechanism for the formation of the nitrile oxide and subsequent 1,3-dipolar cycloaddition can be seen in Scheme 69.

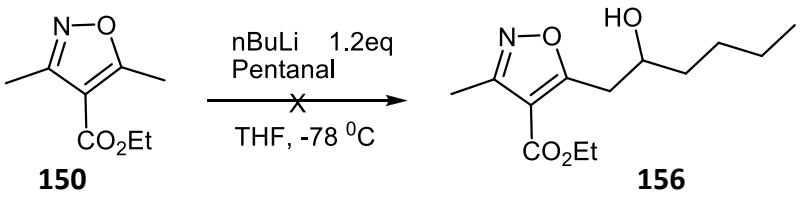


Scheme 68. 1,3-Dipolar cycloaddition producing model isoxazole



Scheme 69. Proposed mechanism for the 1,3-dipolar cycloaddition producing ethyl 3,5-dimethylisoxazole-4-carboxylate (150)

Once the ethyl ester containing isoxazole (**150**) had been synthesised a series of reactions were investigated. The first was a repeat of the anion formation and aldol reaction with pentanal using nBuLi as a base, previously successfully completed on the valine-based pyrroloisoxazole, (S)-5-Butyryl-6-isopropyl-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) but not reproducibly. This required adding nBuLi dropwise to the isoxazole in dry THF at -78^{0}C over 1 h. The freshly distilled pentanal was added in excess and the reaction mixture stirred for a further hour before the reaction was quenched by the addition of water and 2M HCl, and evaporated to yield a yellow oil, Scheme 70. Preliminary NMR spectroscopy showed a mixture of starting materials and impurities, with no peaks suggesting desired product. The IR also showed very minimal amounts of hydroxyl group, which would be expected from aldol addition.

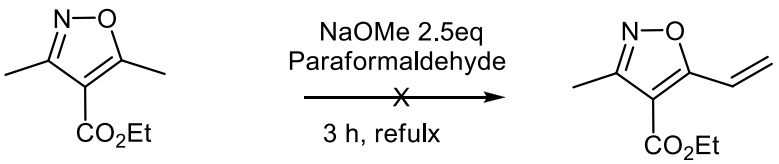


150 **156**

Scheme 70. Attempted aldol reaction of isoxazole 156 using nBuLi

After this unsuccessful reaction it was decided to attempt to a new reaction where an aldol style nucleophilic addition and elimination would occur on a formaldehyde unit. This would provide a vinyl group for cross metathesis or conjugate addition investigations. As formaldehyde is a gas and only available commercially as a gas or as an aqueous solution, which would be detrimental to this dry reaction; it was decided to use paraformaldehyde, a polymerised version of the aldehyde that requires heat to depolymerise and produce formaldehyde.

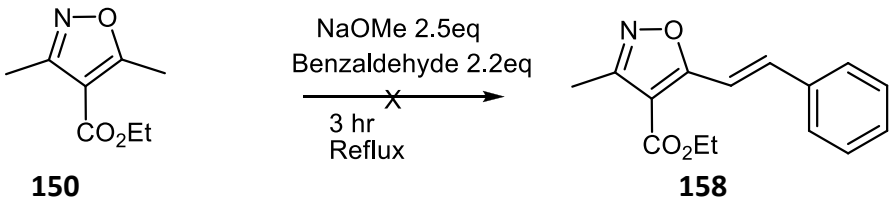
Based on past success on the pyrroloisoxazole with non-enolisable aldehydes, sodium methoxide was used as a base, bought in commercially as it was assumed that the n-BuLi could have reacted as a nucleophile in the previous reaction. Sodium methoxide was dissolved in methanol and added to a suspension of ethyl 3,5-dimethylisoxazole-4-carboxylate (**150**) in dry methanol and stirred at room temperature for 2 minutes. The paraformaldehyde was then suspended in dry methanol and heated to reflux under a nitrogen atmosphere for 3 hours. After separation in water and extraction using chloroform, drying and evaporation under vacuum afforded a yellow oil, Scheme 71. Again, preliminary NMR spectroscopy showed a mixture of starting materials and impurities, with no desired peaks such as that of the new alkene (**157**).



150 **157**

Scheme 71. Attempted aldol reaction of isoxazole using sodium methoxide and formaldehyde

With this unsuccessful reaction, it was decided to repeat a previously successful experiment performed on the pyrroloisoxazole in earlier published work[75,94] to test the reactivity of the isoxazole model. The reaction was to perform the aldol condensation with an aromatic aldehyde, benzaldehyde, which was attempted using the same methodology as seen for the paraformaldehyde reaction where sodium methoxide was dissolved in methanol and added to a suspension of the ethyl 3,5-dimethylisoxazole-4-carboxylate (**150**) in dry methanol and the mixture stirred at room temperature for 2 minutes. The benzaldehyde was then added in dry methanol and the mixture heated to reflux under a nitrogen atmosphere for 3 hours. After separation in water and extraction using chloroform, drying and reduction under vacuum, a yellow oil was obtained, Scheme 72. Again, preliminary NMR spectroscopy showed a mixture of starting materials and impurities, with none of the expected peaks relating to the new alkene bond (**158**).



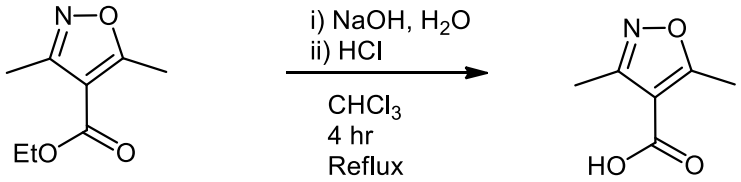
150 **158**

Scheme 72. Attempted aldol reaction of isoxazole using sodium methoxide on to benzaldehyde

This led to the assumption that ethyl 3,5-dimethylisoxazole-4-carboxylatewas not a valid model and was not proving to accurately mimic the behaviour of the pyrroloisoxazole. The task was then to synthesis a new model with a secondary amide in the place of the ethyl ester. This functional group conversion is a reliable and commonly accepted process which could be completed in three stages.

The first stage was to hydrolyse the ester to produce an acid. Base hydrolysis was initiated by addition of sodium hydroxide in water to the ethyl 3,5-dimethylisoxazole-4-carboxylate (**150**) and stirring to reflux for 4 h, after which the solution was left to cool and washed with chloroform to remove any unreacted organic starting material, retaining the aqueous layer. This fraction was then acidified with concentrated hydrochloric acid to pH 3, at which time a thick white precipitate had formed. The white precipitate was collected by Buchner

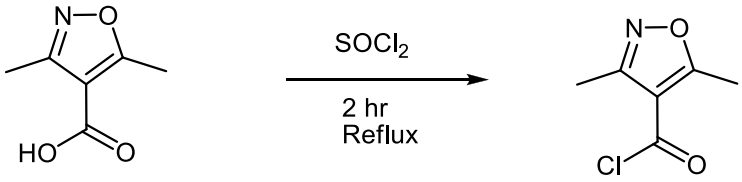
filtration and dissolved in chloroform, dried over magnesium sulfate and concentrated under a reduced pressure to yield 3,5-dimethylisoxazole-4-carboxylic acid (**159**) as a white crystalline solid in an 81% yield, Scheme 73. This was confirmed by NMR spectroscopy with the absence of the ethyl group signals and the appearance in the IR spectrum of a large hydroxyl peak.



150 **159**

Scheme 73. Functional group conversion of an ethyl ester to carboxylic acid on isoxazole

The carboxylic acid was now to be converted in one-pot to the amide over two stages. The first stage was to halogenate the carboxylic acid to yield an acid chloride which would provide a good leaving group. The 3,5-dimethylisoxazole-4-carboxylic acid (**159**) was stirred in neat thionyl chloride for 2 h and heated to reflux; during the heating (before reaching reflux) effervescence was observed which was assumed to be the formation of sulfur dioxide, Scheme 74. The excess thionyl chloride was removed under a reduced pressure and the brown residue (**160**) was then taken up in dichloromethane. Due to the instability of the acid chloride in air it was decided to continue directly through to the next stage of the reaction with no further analysis or purification.

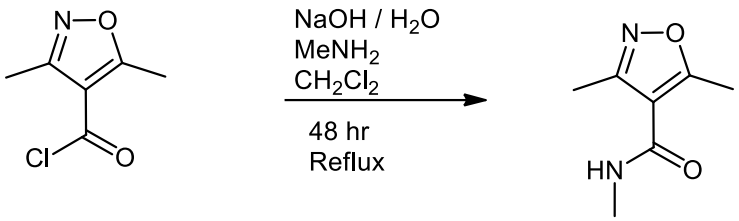


159 **160**

Scheme 74. Conversion of a carboxylic acid to an acid chloride

In order to mimic the pyrroloisoxazole, the most accurate amide to form was a secondary amide with a methyl group on the nitrogen atom. It was therefore decided to use methylamine to form the amide, which is only available commercially in aqueous solution. Therefore, to 3,5-dimethylisoxazole-4-carbonyl chloride (**160**) in dichloromethane was

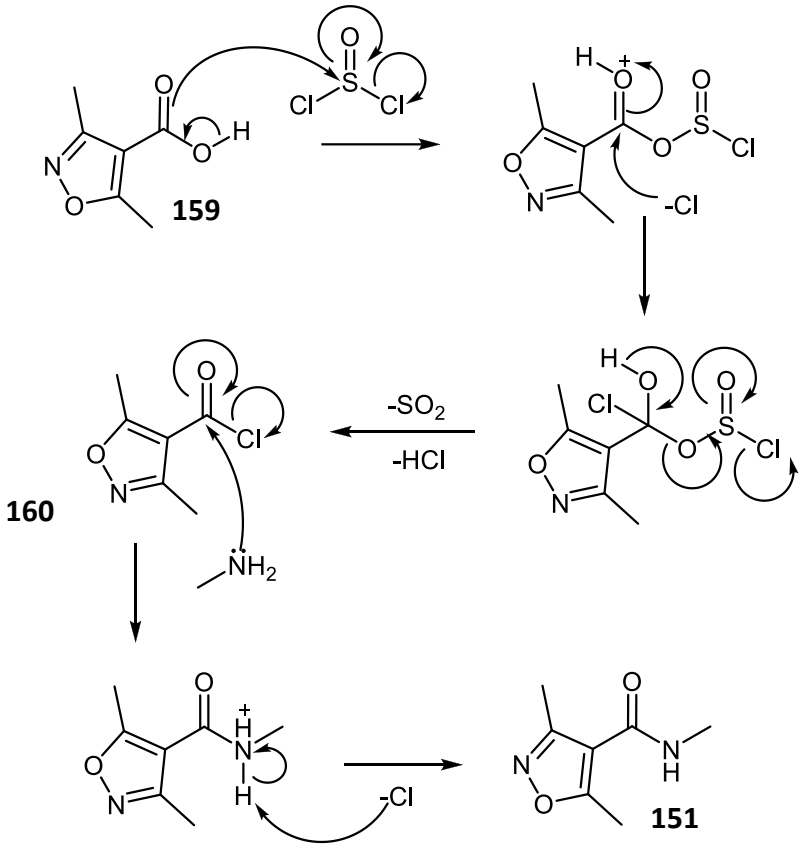
added methylamine in 1M sodium hydroxide solution to ensure the aqueous layer remained basic and guarantee the amine would continue to be reactive and not over-protonated. The reaction occurred at the interface between the layers, which therefore required the reaction to be stirred vigorously for 48 h, Scheme 75. The product was isolated after separation with water, extraction with dichloromethane, drying of the organic layer and evaporation under a reduced pressure to afford the *N*,3,5-trimethylisoxazole-4-carboxamide (**151**) in 84% yield, which was confirmed by ^{1}H NMR spectroscopy by the addition of amide signals to the isoxazole.



160 **151**

Scheme 75. Conversion of an acid chloride to an amide

A mechanism for this one-pot synthesis is illustrated in Scheme 76.



Scheme 76. Mechanism for the conversion of carboxylic acid to an amide

Once *N*,3,5-trimethylisoxazole-4-carboxamide (**151**) had been produced, it was now possible to assess the reactivity of this model to provide a comparison with the pyrroloisoxazole, which was done with repeats of the two experiments conducted on the ethyl 3,5-dimethylisoxazole-4-carboxylate (**150**). These methodologies can be seen above but the reactions are also shown in Scheme 77 and Scheme 78. The results obtained, however, were the same as seen in the earlier model with both reactions resulting in unidentifiable products. The NMR spectroscopic signature that was required to confirm product formation was the new alkene proton signals shown to be around 6-7 ppm with a specific splitting pattern, which was not observed in any fraction.

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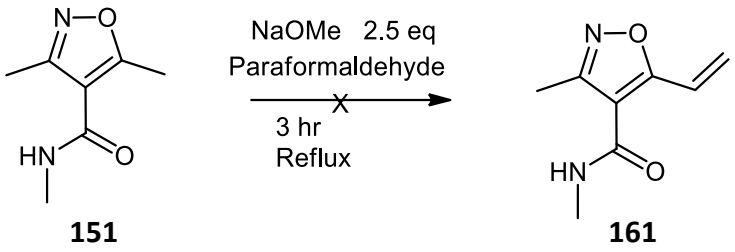
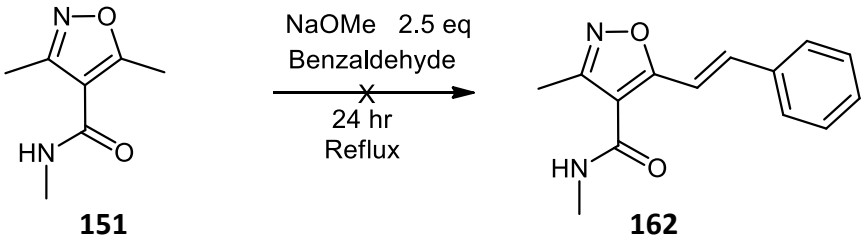


151 **161**

Scheme 77. Attempted aldol reaction on paraformaldehyde on *N*,3,5-trimethylisoxazole-4-carboxamide



151 **162**

Scheme 78. Attempted aldol reaction on benzaldehyde on *N*,3,5-trimethylisoxazole-4-carboxamide

These experiments were repeated several times with different reaction times, solvents and bases until successful conditions were identified, which is summarised in Table 7.

Table 7. Table showing range of results from a range of model isoxazole reactions

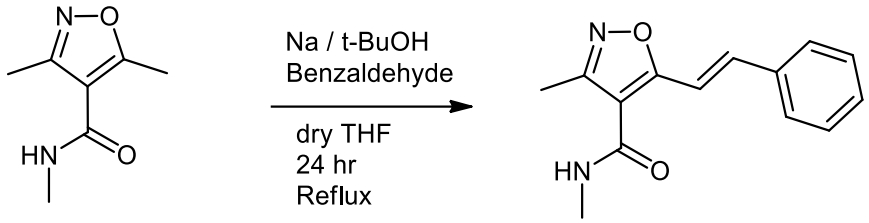
*Ref.**	*Isoxazole*	*Base (2.5 eq.)*	*Time/ Temperature*	*Aldehyde*	*Solvent*	*Yield*
156	Ethyl Ester	nBuLi	2 h / -78^{0}C	Pentanal	THF	-
157	Ethyl Ester	NaOMe	3 h / reflux	Paraformaldehyde	Methanol	-
158	Ethyl Ester	NaOMe	3 h / reflux	Benzaldehyde	Methanol	-
162	Amide	**NaOMe	3 h / reflux	Benzaldehyde	Methanol	-
162	Amide	**NaOMe	24 h/ reflux	Benzaldehyde	Methanol	-
161	Amide	**NaOMe	3 h / reflux	Paraformaldehyde	Methanol	-
162	Amide	nBuLi	3 h / reflux	Benzaldehyde	THF	-
162	Amide	**NaO^{t}Bu	24 h/ reflux	Benzaldehyde	THF/^{t}BuOH	24%
161	Amide	**NaO^{t}Bu	24 h/ reflux	Paraformaldehyde	THF/^{t}BuOH	-

Note that all solvents used were dry, either freshly distilled or bought dry. Also that ethyl ester isoxazole is ethyl 3,5-dimethylisoxazole-4-carboxylate *(**150**) and amide isoxazole is N*,3,5-trimethylisoxazole-4-carboxamide *(**151**). *Denotes the number reference for the product, ** Denotes where the base was freshly prepared*

An advance came when it was decided to use a slightly stronger base, sodium *tert*-butoxide, due to concerns the deprotonation was not occurring. The sodium *tert*-butoxide was freshly prepared by adding sodium to *tert*-butanol at room temperature under a nitrogen atmosphere and heated to reflux until the sodium had fully dissolved to give a cloudy, colourless solution, typically around taking 45 minutes. This solution was then transferred directly to a vessel containing *N*,3,5-trimethylisoxazole-4-carboxamide (**151**) and benzaldehyde suspended in freshly distilled dry THF via a cannula. The solution immediately began to darken and turn red as the deprotonation occurred; this became a signal that the deprotonation was successful.

This red colour remained in the solution throughout the 3 hours of stirring at reflux, until separation by water and ethyl acetate, with the aqueous layer being extracted with ethyl acetate. The organic phase, now a yellow colour, was then dried and concentrated under a reduced pressure to yield the crude mixture containing alkene product (**162)** as a yellow oil.

The ^{1}H NMR spectroscopic analysis showed two new doublets with couplings of 16.3 Hz. This was used to identify the product fraction isolated by column chromatography, eluting with light petroleum: ethyl acetate (2:1 v/v), which enabled full characterisation of the desired aldol product, (E)-*N*,3-dimethyl-5-(2-phenylethenyl)isoxazole-4-carboxamide (**162**). Although the experiment was successful, the yield was low (26%). Scheme 79 is shown below which depicts the successful reaction conditions.



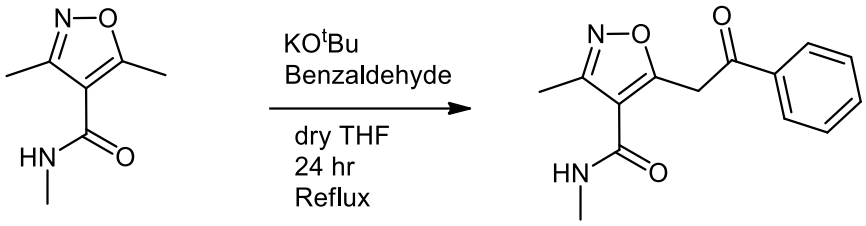
151 **162**

Scheme 79. Successful conditions for the aldol reaction on to benzaldehyde

The paraformaldehyde reaction has not yet been successful and the conclusion was drawn that paraformaldehyde may not be a good source of formaldehyde in this reaction. Due to this lack of success of this reaction, cross metathesis investigations could not be completed.

However, once it was identified that the *tert*-butoxide anion was able to remove the proton on the C-5 methyl group, the results then needed to be transferred from the model to the pyrroloisoxazole. The aim was now to complete the reaction without the use of a protic solvent, as the pyrroloisoxazole had previously undergone aldol reactions under these conditions.[57] The next reaction to attempt on the model was therefore to use *tert*-butoxide without the excess protic *tert*-butanol solvent, which was completed by using commercially available potassium *tert*-butoxide dissolved in dry THF. The reaction was attempted by dissolving *N*,3,5-trimethylisoxazole-4-carboxamide in dry THF followed by addition of the potassium *tert*-butoxide in dry THF at room temperature, which led to no obvious colour change. This clear and colourless solution was then refluxed for 1 hour before adding the benzaldehyde, after which the reaction mixture turned a vibrant yellow and, after a further 3 hours at reflux, darkened to a deep red. The presumed aldol reaction was then heated to reflux overnight with stirring and after workup, involving a water wash, drying over $MgSO_4$ and concentration under a reduced pressure, yielded *N*,3-dimethyl-5-(2-oxo-2-

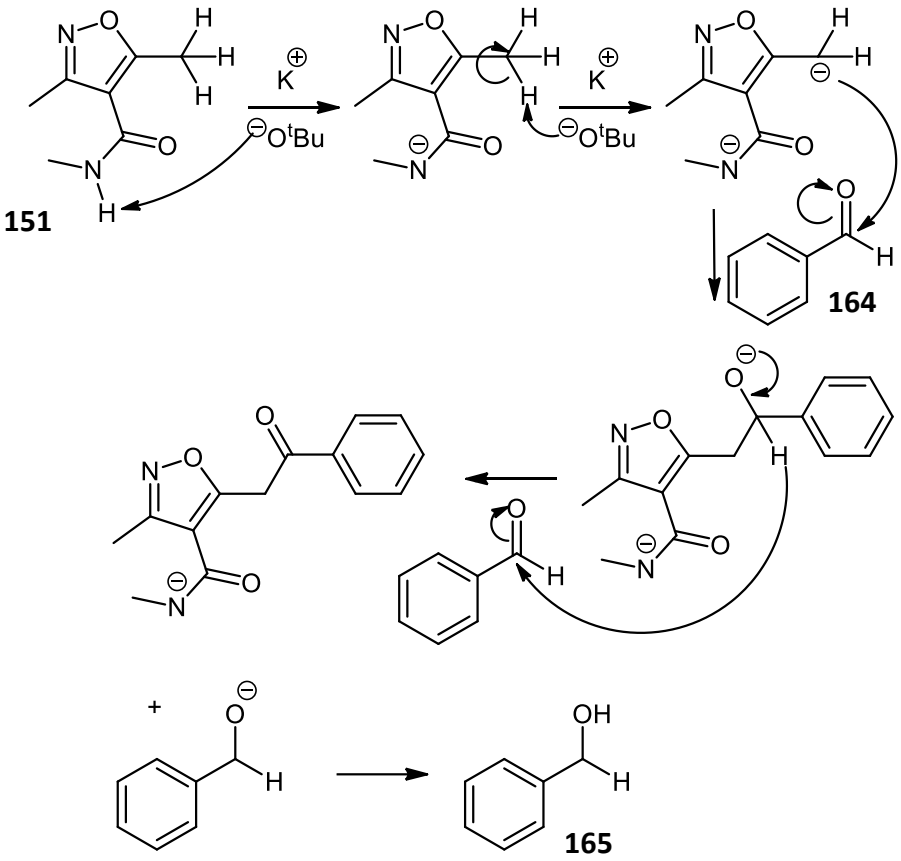
phenylethyl)isoxazole-4-carboxamide (**163**) as a major product at 33%, with no other recognisable minor products, Scheme 80.



151 **163**

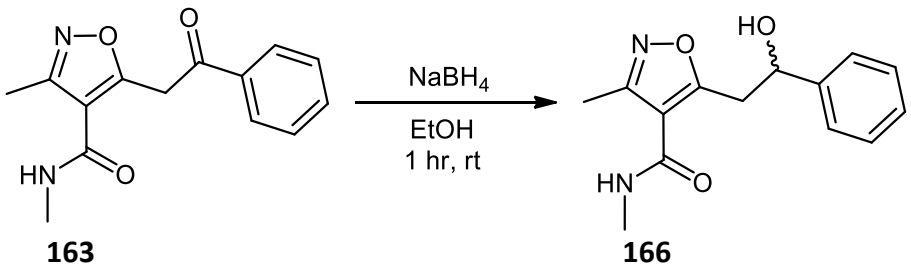
Scheme 80. Conditions for the aldol reaction to form the ketone with benzaldehyde

The reaction was unexpected as the oxidised form of the aldol adduct alcohol was achieved. The presence of the ketone was confirmed initially by the occurrence of a singlet integrating for 2 protons at 4.63 ppm in the NMR spectrum (which was assigned as the 2 hydrogens on the alpha methylene group to the ketone) and the absence of the previous methyl group at 2.55 ppm and of signals seen in the aldol condensation product alkene. The product was also confirmed by high resolution mass spectrometry by identification of the molecular ion. This mode of reaction can be identified as a possible disproportionation and in 1853 was specifically described for aldehydes with no alpha hydrogens such as benzaldehyde by Cannizzaro.[95] One possible pathway is hydride transfer from the initial aldol hydroxy adduct to reduce the excess benzaldehyde (Scheme 81).



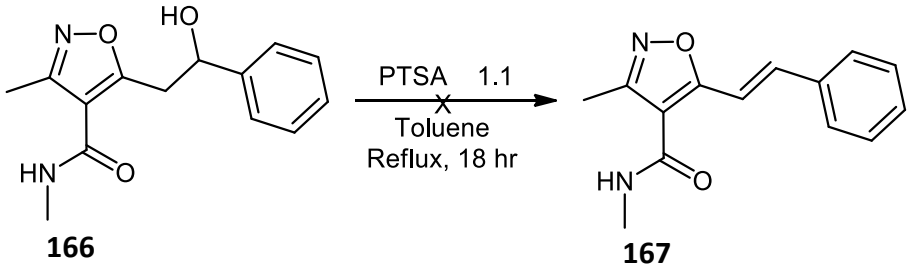
Scheme 81. A suggested mechanism for the disproportionation reaction described

Upon further investigation this ketone could be successfully reduced to the alcohol by stirring with sodium borohydride in ethanol for 1 hour to yield the alcohol 5-(2-hydroxy-2-phenylethyl)-*N*,3-dimethylisoxazole-4-carboxamide (**166**) in 98%, Scheme 82. This was again confirmed by NMR analysis which showed the complete removal of the peak at 4.63 ppm and the addition of a new splitting pattern for 2 protons at 3-3.5 ppm (assigned as the methylene group) and a further new peak showing doublet of doublets coupling and integrating for 1 proton at 5.10 ppm (assigned as the new methine hydrogen on the alcoholic carbon).



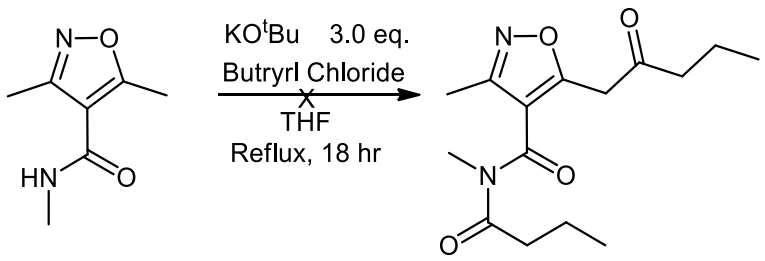
Scheme 82. Conditions for the reduction to form the alcohol from the ketone

Once the alcohol was confirmed an attempt was made to dehydrate it using para-toluenesulfonic acid (PTSA) as seen before under Dean-Stark conditions,[75] but this reaction failed and no alkene was identified. The reaction was done on a very small scale which could have contributed to the failure to identify desire product as the analysis showed no major product, instead suggesting degradation, Scheme 83.



Scheme 83. Conditions for the attempted dehydration of the alcohol to form an alkene

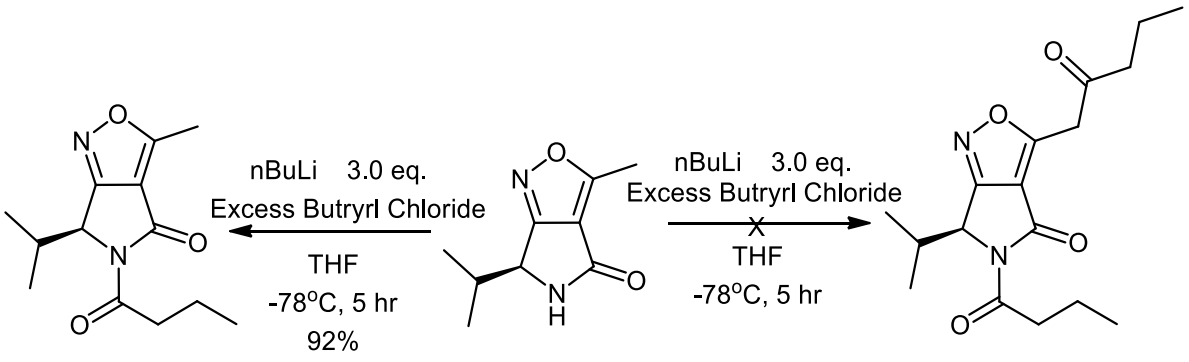
Given the success of the N-acylation reactions on the pyrroloisoxazole series, it was decided to attempt a diacylation on the model isoxazole amide. This reaction therefore required a base, commercial potassium *tert*-butoxide added in 3 equivalence, to be added to *N*,3,5-trimethylisoxazole-4-carboxamide (**151**) suspended in dry THF at room temperature before the addition of an excess amount of butyryl chloride and heating to reflux overnight, **Scheme 84**. This reaction yielded no desired product of either the mono- or diacylated isoxazole, which confirms concerns that the anion formation or reactivity of this methyl group is unreliable. This provides an explanation of the non-reproducibility and relatively low yields for the aldol reactions attempted.



151 **168**

Scheme 84. Conditions for the attempted diacylation of amide model isoxazole

At this point it was found that we had some (S)-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**151**) remaining and therefore this diacylation was attempted on it. As seen before the base, n-butyllithium in this case, was added (3 equiv) to the pyrroloisoxazole at -78^{0}C and the mixture stirred for 3 hours at this temperature under a nitrogen atmosphere to form the appropriate anions. To this reaction was charged an excess amount of butyryl chloride and stirred for a further 2 hours at -78^{0}C under a nitrogen atmosphere. After work-up and purification, the major product isolated was the mono-N-acylated compound, (S)-5-Butyryl-6-isopropyl-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**137a**), in a 92% yield. This was confirmed by comparing analysis data with the previous data collected for this compound, Scheme 85 and it gave further evidence as to the lack of formation of any methyl anion.



137a **125a** **169**

Scheme 85. Conditions for the attempted diacylation of valine pyrroloisoxazole

2.5 – Laccarin Analogue Synthesis

The next, and final, strand of research completed is the attempted synthesis of an analogue (**18**) of the natural alkaloid laccarin (**30**, Figure 27), which began with the commercially available and cheap L-ornithine (**170**). There are protected versions of L-ornithine available commercially but they are more expensive and only offer protection to the two free amines and not the acid function. Therefore, the first stage in the synthesis of the laccarin analogue (**18**) was to protect the acid by converting it to an ethyl ester in an esterification. Note that the analogue contains the opposite enantiomeric configuration to that seen in the natural product; this would require starting with D-ornithine, which is more expensive, so was not used in this work.

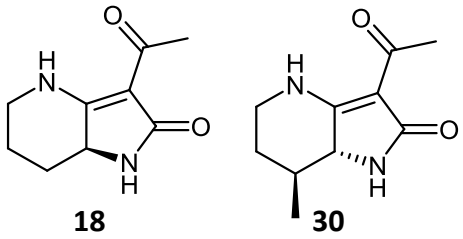
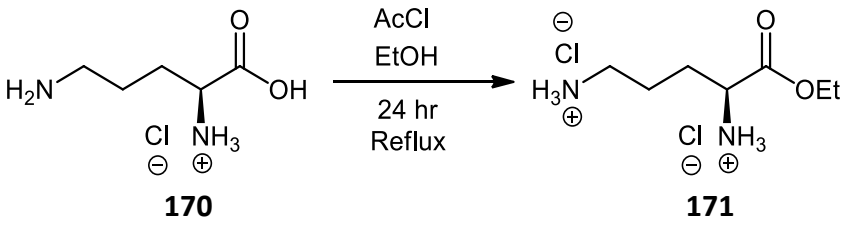


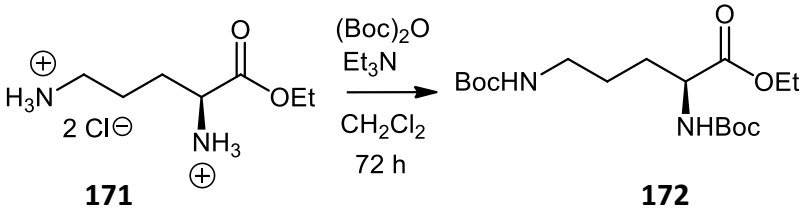
Figure 27. Laccarin (30) and the synthetically fessable anaolouge (18)

To the L-ornithine hydrochloride salt or (S)-2,5-diaminopentanoic acid hydrochloride (**170**) in dry ethanol was added freshly distilled acetyl chloride, stirred and heated to reflux for 18 hours overnight. This process produced the desired ethyl ester, as salts of both amine groups due to the hydrogen chloride produced during the reaction. The product required extensive heating, over 7 hours, in a vacuum oven at 80 ^{0}C to drive off all the water and solvent to yield (S)-5-ethoxy-5-oxopentane-1,4-diaminium chloride (**171**) as a dry white powder in 99% yield, Scheme 86. The product structure was confirmed by NMR spectroscopy, with the appearance of the ethyl group and disappearance of the acidic proton, mass spectrometric identification of a molecular ion and IR analysis showing appropriate bond vibrations.



Scheme 86. Esterification of L-ornithine

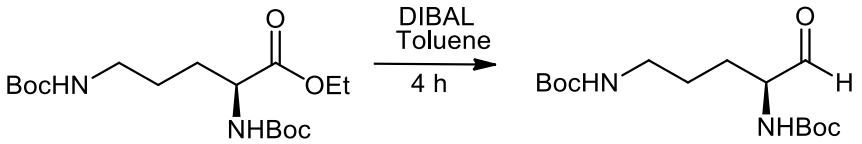
The next reaction was another protection step to protect the free amine groups as Boc derivatives, as seen in earlier syntheses but now with two to protect. This was completed as seen before, differing only in the requirement to add 2 equiv di-*tert*-butyl dicarbonate dropwise to a solution in dichloromethane containing L-ornithine ethyl ester bis-hydrochloride salt (**171**), which was followed by 2 equivs of triethylamine and stirring the mixture at room temperature overnight. Subsequent washing with citric acid, to remove unreacted starting material, and a saturated brine wash was followed by drying over magnesium sulfate and concentrating under a reduced pressure to yield the desired ethyl (S)-2,5-bis((tert-butoxycarbonyl)amino)pentanoate (**172**) as a colourless oil in good yield (64%), Scheme 87. The product was confirmed by the appearance of two tert-butyl groups in the NMR analysis along with the L-ornithine ethyl ester. IR spectroscopy and mass spectrometry identifying the molecular ion also confirmed the structure.



171 **172**

Scheme 87. Di-protection of L-ornithine ethyl ester

As seen earlier in the synthesis of the valine- and leucine-based pyrroloisoxazoles, the next stage required is to reduce the ethyl ester in (S)-ethyl 2,5-bis((*tert*-butoxycarbonyl)amino)pentanoate (**172**) to an aldehyde in (S)-2,5-bis (*tert*-butyloxycarbonylamino)pentanal (**173**). This was performed using 2.5 equivalence of diisobutylaluminium hydride in toluene over 4 hours at -78 ^{0}C to prevent epimerization of the central chiral unit. This is usually a very successful method and provided good results yielding the aldehyde (64%) as a clear colourless oil, Scheme 88, which required no further purification and was added directly to the next reaction. Characterisation was achieved on a sample removed before commencing with the next reaction and confirmed the structure as (S)-tert-butyl 5-oxopentane-1,4-diyldicarbamate (**173**) by ^{1}H NMR spectroscopy, acknowledged by the removal of the ethyl group and addition of an aldehyde proton signal. This was supported by mass spectrometric identification of the molecular ion and IR analysis.

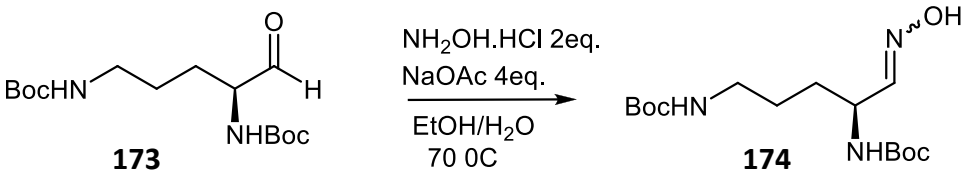


172 **173**

Scheme 88. Reduction of L-ornithine ethyl ester to aldehyde

The formation of the oxime was the next stage: the crude (S)-di-*tert*-butyl (5-oxopentane-1,4-diyl)dicarbamate (**173**) was dissolved in the least amount of ethanol and added to an aqueous solution containing 2 equivs of hydroxylamine hydrochloride and 4 equivs of sodium acetate. This method of oxime formation from aldoximes by condensation of the aldehyde with hydroxylamine hydrochloride and sodium acetate has been previously used and is shown to be reliable both in our work and that of others[80].

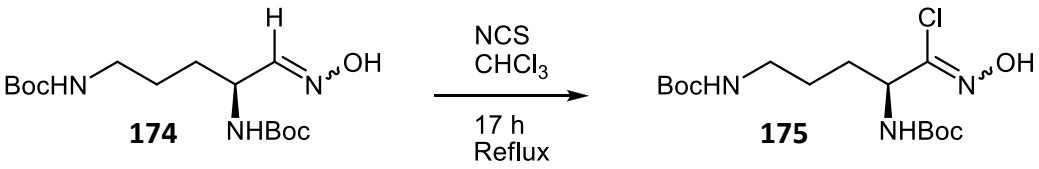
As seen before in the L-valine and L-leucine series, an initial white precipitate was observed, which had to be re-dissolved with the minimum amount of ethanol. The solution was heated to 70 ^{0}C for 30 mins, during which time a watchglass was placed over the neck of the round bottomed reaction flask to restrict solvent loss, and after this time the flask was cooled to room temperature and placed in the refrigerator (2-5 ^{0}C) overnight. After this time a white precipitate had formed which was removed by filtration and identified as the product oxime (S)-*tert*-butyl 5-(hydroxyimino)pentane-1,4-diyldicarbamate (**174**). A white solid, remaining after extraction with ethyl acetate from the filtrate and drying, filtering and concentrating the combined organic under a reduced pressure, was also identified as the desired product (**174**), Scheme 89. The total yield collected from this reaction was an excellent 96% and, in both cases the NMR spectrum in d_6-DMSO, showed shifting of the sp^2 CH signal, and mass spectrometric identification of the molecular ion and IR analysis supported the oxime assignment.



173 **174**

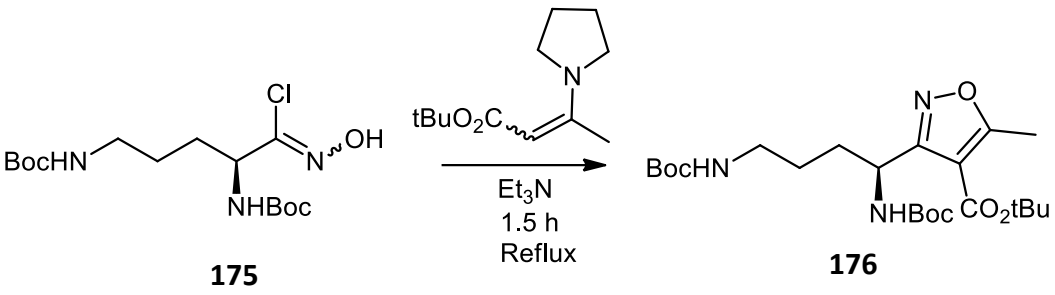
Scheme 89. Oxime formation from aldehyde of Boc-protected L-ornithine

The next stage was the 1,3-dipolar cycloaddition, the first part of which was the chlorination of the oxime (**174)**. This chlorination was achieved by adding *N*-chlorosuccinimide portionwise to the oxime (**174**) at 0 ^{0}C in chloroform, followed by heating to reflux overnight, during which time a colour change from pale blue to green to brown was observed, Scheme 90. The structure of (S)-*tert*-butyl 5-chloro-5-(hydroxyimino)pentane-1,4-diyldicarbamate (**175**) was confirmed, after work-up, drying and concentration, by ^{1}H NMR spectroscopy, with the disappearance appearance of oxime alpha-proton, mass spectrometric identification of the molecular ion and IR analysis.



Scheme 90. Chlorination of the L-ornithine oxime

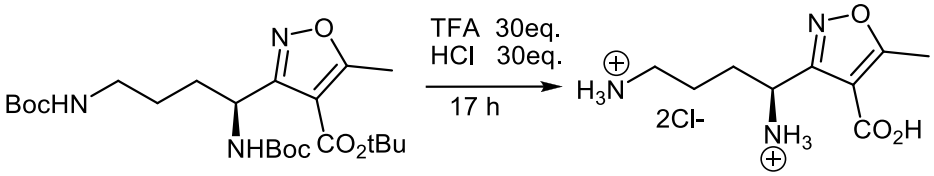
To a solution of (S)-*tert*-butyl 5-chloro-5-(hydroxyimino)pentane-1,4-diyldicarbamate (**175**) in chloroform heated to reflux was added 2 equivalence *tert*-butyl 3-(pyrrolidin-1-yl)but-2-enoate (**72**) followed by an equivalent amount of triethylamine dropwise over 1 h, required to initiate the reaction by forming the nitrile oxide *in situ*. The mixture was heated to reflux for a further 3 h, then separated between water and chloroform and the organic layer washed with citric acid solution, to remove excess base, and sodium hydroxide solution, to remove acidic impurities, and finally with saturated brine solution. The organic layer was dried over magnesium sulfate, filtered and concentrated under reduced pressure to yield a crude dark brown thick oil. The oil was subjected to purification by column chromatography eluting with light petroleum:ethyl acetate (3:1 v/v) to finally provide the new isoxazole (**176**) as a yellow oil in a disappointing 5% yield, Scheme 91. This reaction was shown to have produced some isoxazole with a full characterisation by NMR spectroscopy, the ^{13}C spectrum confirming the formation of 3 new quaternary carbons and the incorporation of the tert-butyl group, mass spectrometric identification of the molecular ion and IR analysis. We do not know the reason for the low yield; it may be due to side chain interference, but we have no evidence for this.



175 **176**

Scheme 91. 1,3-Dipolar cycloaddition of L-Ornithine

The next stage was to remove all the protecting groups to prepare the molecule for a peptide coupling reaction. This was done using the same methodology as described earlier, using TFA in 30 fold excess and stirring for 4 hours, followed by concentration to yield a brown residue and subsequent addition of 2M HCl and stirring overnight, Scheme 92. The product bis-hydrochloride salt (S)-1-(4-carboxy-5-methylisoxazol-3-yl)butane-1,4-diammonium dichloride (**177**) was identified in quantitative recovery and confirmed by ^{13}C NMR spectroscopy and mass spectrometry but due to the complexity of the ^{1}H spectrum it could not be confirmed using this technique. The complexity observed was possibly due to the mixture of different possible salts present and their interaction with deuterated protic solvent, D_2O.

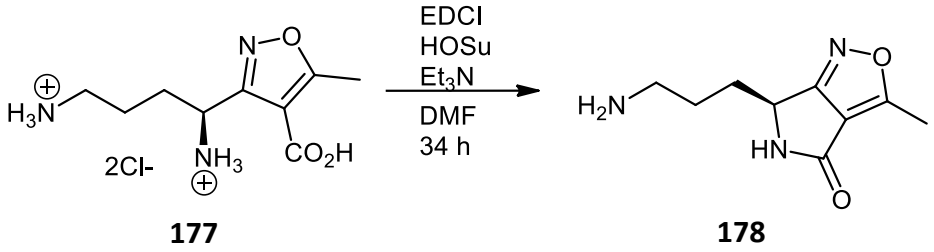


176 **177**

Scheme 92. Deprotection of L-ornithine isoxazole

The formation of the new amide, bond closing the pyrrolidone ring, was the next stage in the synthesis of a laccarin analogue. This was attempted, as seen before in the L-valine series, using the EDCI (**123**) method, which was chosen as the amount of PS-CDI (**124**) available to us was low and this material is very expensive so it was determined to use the less expensive method and the T3P reaction had not yet been established at this point in our research. The EDCI (**123**) process required the use of N-hydroxysuccinimide (**120**) as an activator and dimethylformamide as a solvent. All these were combined with the 3-(1-

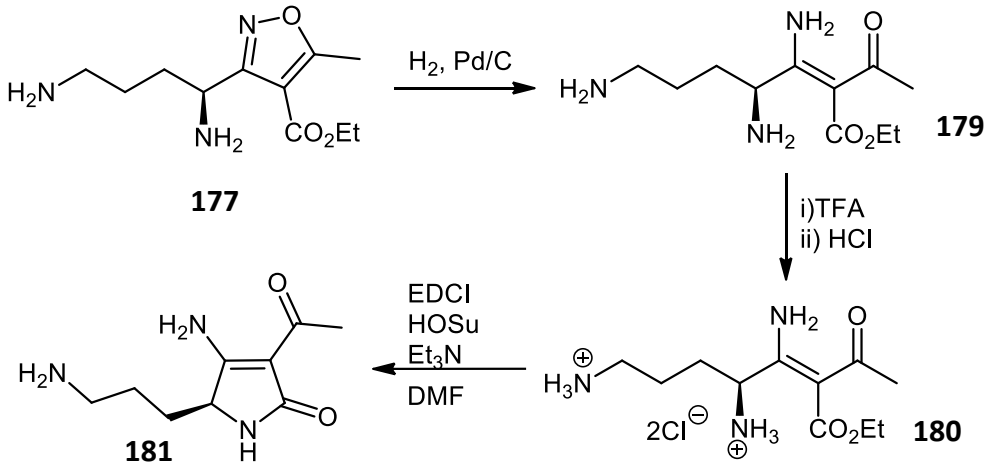
amino-2-methylpropyl)-5-methylisoxazole-4-carboxylic acid hydrochloride salt (**177**) at 0^0C and stirred at room temperature overnight, 17 hours. Triethylamine was then added to neutralise salts and the reaction left again overnight stirring. After extracting the aqueous layer with ethyl acetate and washing with acid and base to remove the impurities, the solution was concentrated under a reduced pressure, Scheme 93. (S)-6-(3-aminopropyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**178**) could not be identified in a crude NMR spectrum or by mass spectrometry. The acid wash was analysed to see if any material had been transferred into it due to protonation making it more soluble in an aqueous layer. Unfortunately, it was not found to be present in any washings and the reaction was deemed unsuccessful. Repeat attempts also failed and consequently a new strategy was required.



177 **178**

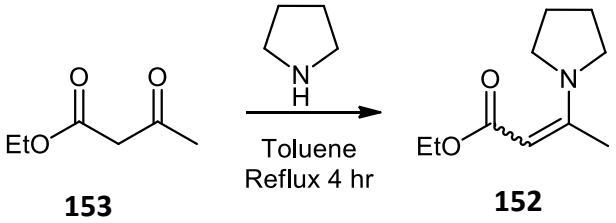
Scheme 93. Attempted peptide coupling reaction using L-ornithine series

Acting on the assumption that the additional long carbon chain at C-5 of the new ring was interfering with lactam formation, it was decided to reduce the isoxazole ring before the lactam formation. This method required the synthesis of a protected isoxazole species which could then undergo a hydrogenation to form an enaminoketone, and as seen before in the *N*-acylation reactions, we also know these enaminoketones are stable under acidic conditions. This would help to add flexibility for the new ring formation by removing the N-O bond. It then followed that by changing the enamine to contain an ethyl group may enable a potential spontaneous lactam ring closure, Scheme 94.



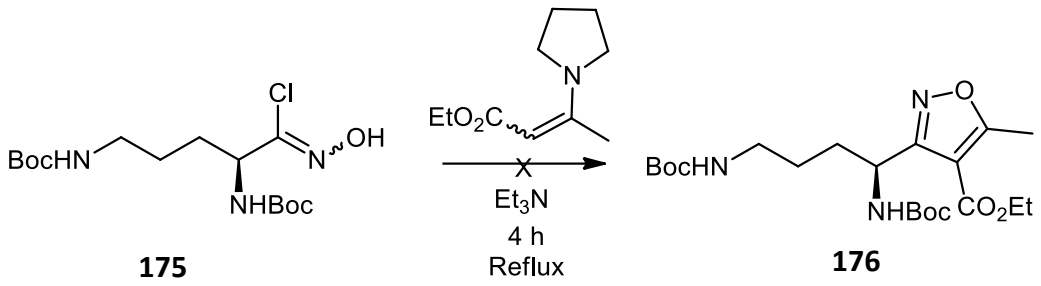
Scheme 94. Suggested route towards L-ornithine-derived lactam enaminoketone

The next reaction required was therefore to form the ethyl ester enamine, which was successfully synthesised from ethyl acetoacetate (**152**) and pyrrolidine under Dean-Stark conditions in a maximum of 99% yield, Scheme 95.



Scheme 95. Ethyl ester enamine formation

The next stage was again to form the isoxazole, except now with the ethyl ester enamine. This was attempted following the earlier discussed procedure by adding ethyl 3-(pyrrolidin-1-yl)but-2-enoate (**153**) to (S)-tert-butyl 5-chloro-5-(hydroxyimino)pentane-1,4-diyldicarbamate (**175**) in chloroform after which the solution was heated to reflux followed by the addition of triethylamine dropwise over 1 h. The mixture was heated to reflux for a further 3 h, then worked up as previously described, dried over magnesium sulfate, filtered and concentrated under a reduced pressure to yield a crude dark brown thick oil. The oil was subjected to purification by column chromatography eluting with light petroleum:ethyl acetate but none of the fractions isolated were identifiable by NMR spectroscopy, Scheme 96. A marker for the isoxazole is the presence of the methyl group giving a singlet integrating for 3 protons at 2.5-2.6 ppm in the ^{1}H NMR spectrum, but no fractions displayed this signal.



175 **176**

Scheme 96. Attempted 1,3-dipolar cycloadddition using L-ornithine series and ethyl enamine

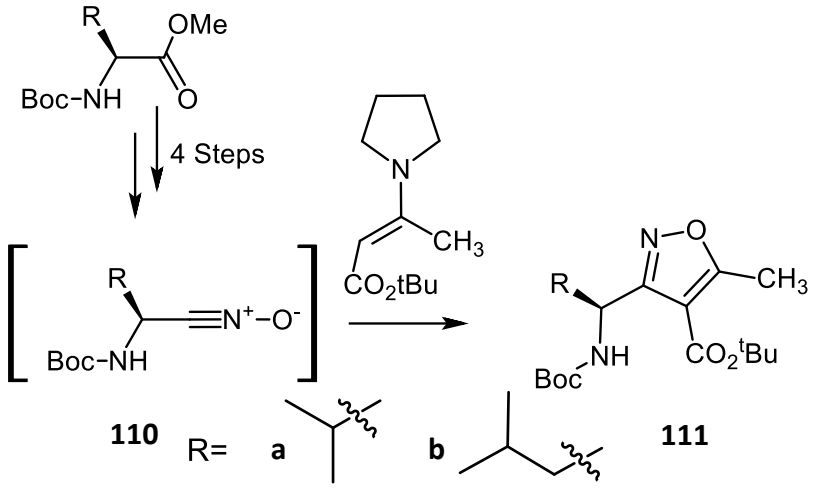
This was disappointing and the research ended due to a lack of time and other research priorities. This provides a strong basis for other projects to build upon with potential further work to be discussed later.

3.0 – Conclusion

3.1 – L-Valine and L-Leucine Pyrroloisoxazole Formation and Elaboration

This report has shown the research conducted towards pyrroloisoxazoles in their role as masked forms of acyltetramic acids.

Across both amino ester series investigated the desired 1,3-dipolar cycloaddition reactants were successfully completed to yield the protected isoxazoles *tert*-butyl 3-(1-*tert*-butoxycarbonylamino-2-methylpropyl)-5-methylisoxazole-4-carboxylate (**111a**) and *tert*-butyl 3-(1-*tert*-butoxycarbonylamino-2-methylbutyl)-5-methylisoxazole-4-carboxylate (**111b**). The production of these isoxazoles proceeded in good overall yields with a maximum of 35% being achieved over the 5 steps, Scheme 97.

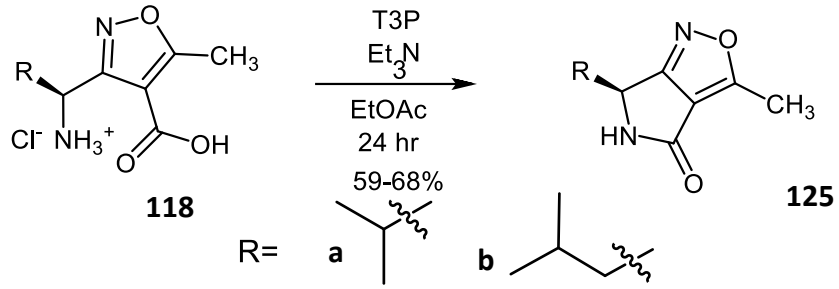


Scheme 97. Synthesis conducted from starting material to isoxazole

The isolation of the chlorination product of the oxime products was developed in this research and was significant as it provided evidence the cycloaddition reaction itself was higher yielding than first perceived. The solubility of the isolated chloro-oximes in a range of solvents also enabled a solvent study to be completed, which confirmed that the most efficient solvent in a selection of three with varied polarity was chloroform.

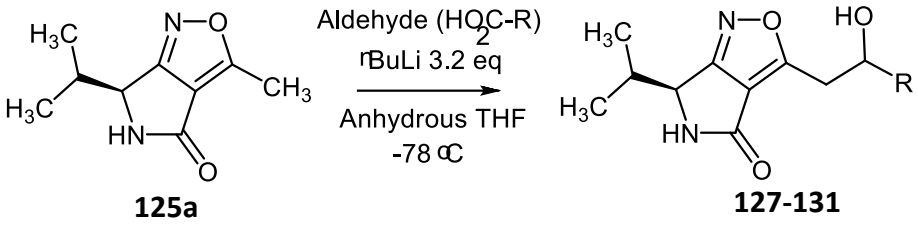
This isoxazoles were subsequently deprotected leaving the molecules as the hydrochloride salts 1-(4-carboxy-5-methyl-1,2-oxazol-3-yl)-2-methylpropan-1-ammonium hydrochloride (**118a**) and 1-(4-carboxy-5-methyl-1,2-oxazol-3-yl)-2-methylbutan-1-ammonium

hydrochloride (**118b**). These salts were then cyclised to form the bicyclic pyrroloisoxazole systems using different coupling agents (EDCI (**123**), along with an activator (HOSu, **120**), and PS-CDI (**124**). No significant difference was observed in the yield with a high of 59% being reported when using PS-CDI after purification by column chromatography, which was required in all cases. This was until a method was developed utilising the recently commercialised peptide coupling agent T3P (**126**), which enabled the L-valine- and L-leucine-derived pyrroloisoxazoles to be synthesized in maximum of 68% yield with no requirement for purification after a simple work up, Scheme 98.



Scheme 98. Successful peptide coupling reaction producing the pyrroloisoxazole

Upon successful synthesis, 3-methyl-6-(propan-2-yl)-5,6-dihydro-4*H*-pyrrolo[3,4-*c*][1,2]oxazol-4-one (**125**), was subjected to an anion condensation on to an aldehyde in an aldol fashion, Scheme 99. Table 8 shows the selection of aldehydes added to the C-3 methyl position, albeit in low yields. Limited material and non-reproducibility meant that full characterisation was not obtained except in the of crotonaldehyde addition.

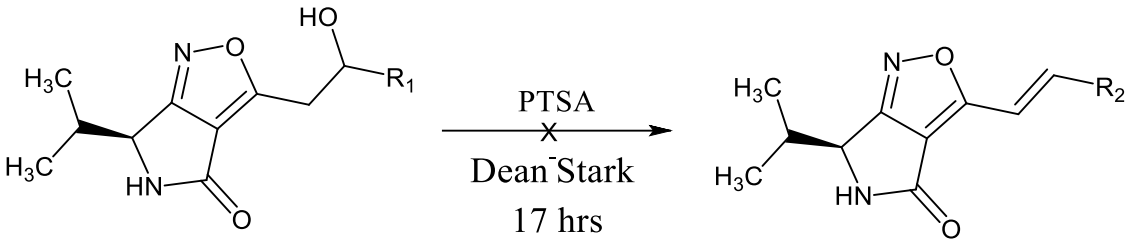


Scheme 99. General synthesis for the aldol reaction

The dehydration of these alcohol products by PTSA acidification was attempted and, in all cases, yielded no desired products, Scheme 100. Product decomposition could have occurred as the TLC showed a large number of compounds with similar Rf values in all solvent systems attempted.

Table 8. Products formed in the aldol reaction

Ref.	Aldehyde	Yield
127	Pentanal	6%
128	Decanal	28%
129	Isobutyraldehyde	32%
130	Cyclohexanecarboxylaldehyde	28%
131	Crotonaldehyde	30%



127 – R_1 –$CH_2CH_2CH_2CH_3$

128 – R_1 –$CH_2(CH_2)_7CH_3$

129 – R_1 –$CH_2[CH_3]_2$

130 – R_1 –C_6H_{11}

131 – R_1 –$CHCHCH_3$

132 – R_2 –$CH_2CH_2CH_2CH_3$

133 – R_2 –$CH_2(CH_2)_7CH_3$

134 – R_2 –$CH_2[CH_3]_2$

135 – R_2 –C_6H_{11}

136 – R_2 –$CHCHCH_3$

Scheme 100. Summary of failed dehydration reactions

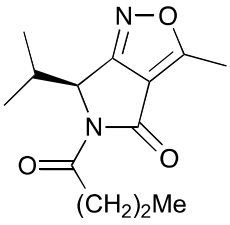
Possible explanations include small scales necessary, around 20mg-30mg, which could have led to detection errors. Also, a separation was required and, although crude NMR spectra were obtained, separations were not completed successfully as R_f values were too similar and the products contained too many impurities. It is also thought that a condensation under acidic conditions could be destructive to the molecule, so therefore further work on this reaction could investigate the substitution of other leaving groups at the alcohol position.

In a modification to add a different leaving group to the alcohol, preparation of the acetyl adduct, was attempted by quenching the aldol reaction with freshly distilled acetyl chloride. Results here are to date inconclusive.

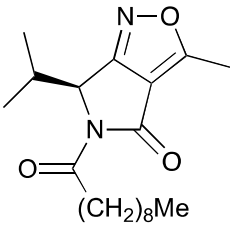
In conclusion of the further elaborations on the pyrroloisoxazole moieties, acylations at the lactam nitrogen were successfully completed. The conditions required for this were 1.1 equiv of n-butyl-lithium stirred at -78°C for 10 minutes followed by addition of the acyl chloride (1.1 equiv) and stirring for 3 hours, which after work up provided a library of *N*-acylpyrroloisoxazoles in a range of moderate to excellent yields. The library of analogues produced is shown in **Table 9** with individual yields.

N-Alkylation reactions were unsuccessful after several attempts in a range of conditions, summarised in Table 10. These results were disappointing, but it was determined that the *N*-acyl compounds contained sufficient diversity to continue the synthesis with them alone and further effort was not expanded in developing the alkylation reaction. This indicates that the nucleophilicity of the nitrogen anion formed by the deprotonation is not sufficient to attack the alkyl and prop-1-enyl halides

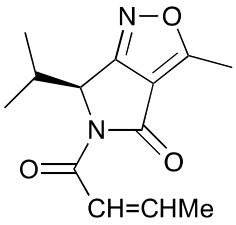
Table 9. Library of *N*-Acyl Compounds formed with respective yields



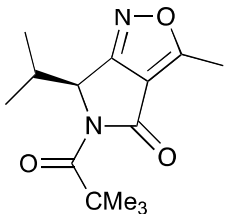
137a 89%)



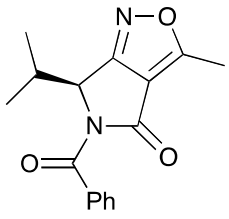
140a (63%)



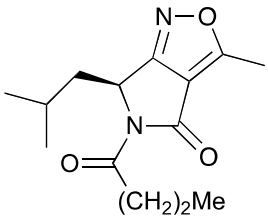
***E*-139a** (63%)



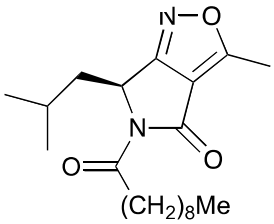
141a 89%)



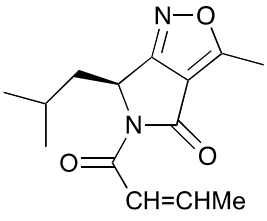
138a (89%)



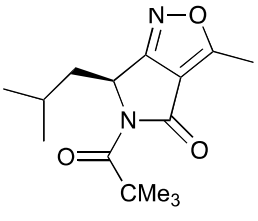
137b f (76%)



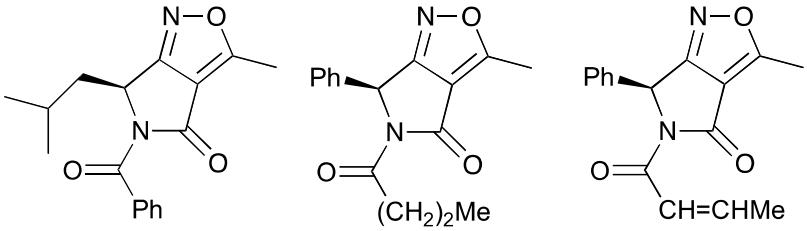
140b (33%)



***E*-139b** (97%)



141b (36%)

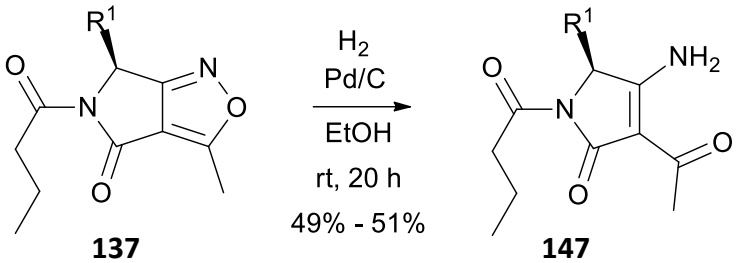


138b (43%) **137c** (57%) ***E*-138c** (50%)

Table 10. *N*-Alkylation conditions attempted

Ref.	*1*	*2*	*3*	*4*	*5*
Substrate	1-Bromobutane	1-Bromobutane	Allyl Bromide	Allyl Bromide	Allyl Bromide
Time	2 Hr	2 Hr	2 Hr	2 Hr	2 Hr
Temperature	-78°C	-78°C to rt	-78°C to rt	-78°C to reflux	-78°C to reflux
Ion Catalyst	None	None	None	None	Potassium Iodide

The acylations were successful in providing a library of 12 novel 'masked' analogues of the biologically active natural product reutericyclin, which are available for biological testing. They are of interest as mimics of the natural products. The next requirement in the pursuit of the 3-acyltetramic acids was to cleave the isoxazole ring, for which two successful method were described. The first of these techniques involved a hydrogenation: subjecting two saturated *N*-acylpyrroloisoxazoles to a hydrogen atmosphere over palladium on carbon provided the enaminoketones in an average 50% yield after purification, Scheme 101.

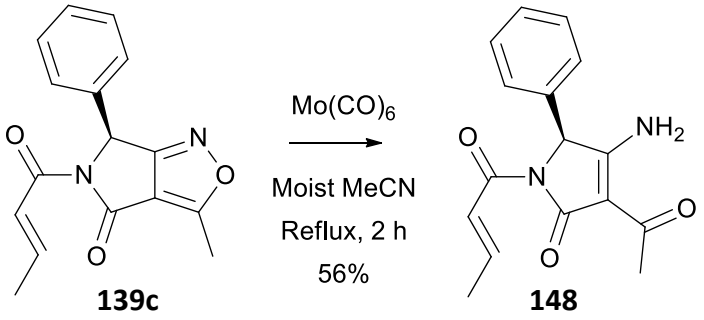


a – R_1 –$(CH_3)_2CH$,

b – R_1 – $(CH_3)_2CHCH_2$

Scheme 101. Hydrogenation ring opening of isoxazole ring

The second reduction conditions utilised also produced the enaminoketone functionality but were unsuitable for an *N*-unsaturated acylpyrroloisoxazole, namely the *N*-crotonyl phenylglycine-derived pyrroloisoxazole which contained a double carbon-carbon bond in the acyl group. This alkene can be reduced by H_2 and palladium on carbon, so the alternative method had to be used. This was accomplished using molybdenum hexacarbonyl in moist acetonitrile in an acceptable 50% yield, **Scheme 102**.



Scheme 102. Reduction using molybdenum hexacarbonyl ring opening of isoxazole ring

This process provided us with a further 3 novel enaminoketone compounds containing *N*-acylation that were also suitable for biological testing as the structure of the enaminoketone pyrrolidine is electronically similar to the final acyltetramic acid.

The last stage identified a problem in which the hydrolysis of the enaminoketone to form a 1,3-dicarbonyl function was difficult to achieve. This occurred as the *N*-acyl groups either were hydrolysed, leading to a previously synthesised 3-acyltetramic acid, or the conditions were not strong enough to complete the required hydrolysis. A summary of the conditions

and their results is shown in Table 11.

Table 11. Enaminoketone hydrolysis or diazotisation methods

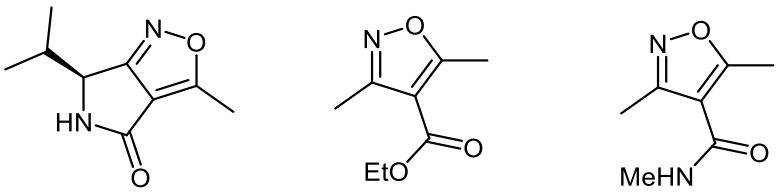
REF.	*1*	*2*	*3*	*4*	*5*
TYPE OF REACTION	Base Hydrolysis	Base Hydrolysis	Base Hydrolysis	Acid Hydrolysis	Diazotisation
REACTANT (CONCENTRATION)	H_2O	NaOH (0.1 M)	NaOH (2 M)	HCl (2 M)	$NaNO_2$
TIME	20 Hr	20 Hr	20 Hr	20 Hr	3 Hr
TEMPERATURE	reflux	rt	-rt	rt	0°C to 50°C
RESULT	no reaction	N-acyl hydrolysis	N-acyl hydrolysis	no reaction	no reaction

These results mean further study is necessary, but the main stream of the research did produce a range of compounds that would be suitable for biological testing and showed a new style of reactivity to which the useful pyrroloisoxazole moieties can be utilised.

This work was accepted for publication in Chemical Communications[93] and can be found in the appendix of this thesis.

3.2 –Model Formation and Reactivity

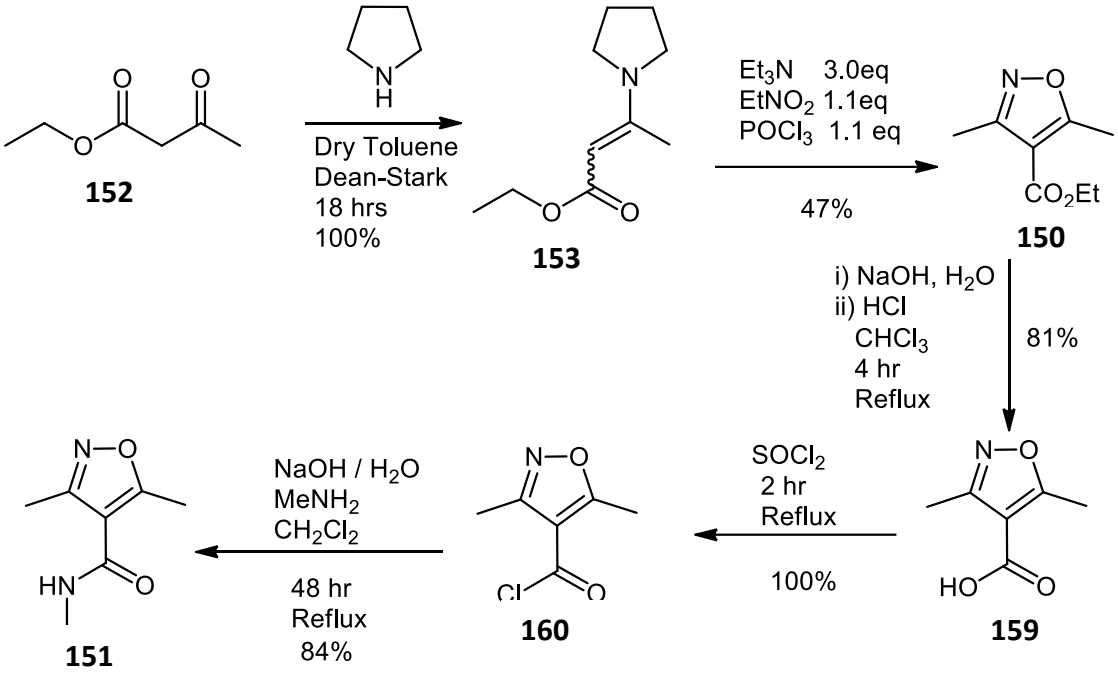
The model chemistry was planned as a route to towards quickly and inexpensively determining which reactions and condition may lead to a successful reaction on the pyrroloisoxazole and, therefore, maximising time spent on following encouraging leads. The model was made in two versions after several failed reactions with ethyl 3,5-dimethylisoxazole-4-carboxylate (ethyl ester, **150**), where *N*,3,5-trimethylisoxazole-4-carboxamide (amide, **151**) (Scheme 103) is a closer model and yielded a successful result.



125a **150** **151**

Scheme 103. Models and pyrroloisoxazole.

The *N*,3,5-trimethylisoxazole-4-carboxamide model (**151**), was produced in 5 steps from ethyl acetoacetate (**152**) and is shown in Scheme 104.



Scheme 104. Summary of model product

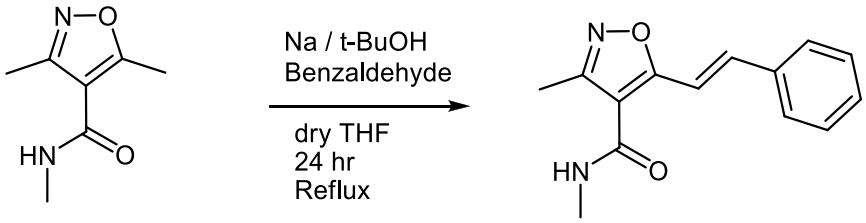
Once the model was formed a number of reactions were completed attempting to find appropriate reaction conditions, these are summarised in Table 12.

Table 12. Summary showing results from model isoxazole reaction

*Ref.**	*Isoxazole*	*Base*	*Time/ Temperature*	*Aldehyde*	*Solvent*	*Yield*
156	Ethyl Ester	nBuLi	2 h / -78^{0}C	Pentanal	THF	-
157	Ethyl Ester	NaOMe	3 h / reflux	Paraformaldehyde	Methanol	-
158	Ethyl Ester	NaOMe	3 h / reflux	Benzaldehyde	Methanol	-
162	Amide	**NaOMe	3 h / reflux	Benzaldehyde	Methanol	-
162	Amide	**NaOMe	24 h/ reflux	Benzaldehyde	Methanol	-
161	Amide	**NaOMe	3 h / reflux	Paraformaldehyde	Methanol	-
162	Amide	nBuLi	3 h / reflux	Benzaldehyde	THF	-
162	Amide	**NaO^{t}Bu	24 h/ reflux	Benzaldehyde	THF/^{t}BuOH	24%
161	Amide	**NaO^{t}Bu	24 h/ reflux	Paraformaldehyde	THF/^{t}BuOH	-
163	Amide	KOtBu	24 h/ reflux	Benzaldehyde	THF	33%[a]
166	Amide Ketone	-	24 h/ reflux	PTSA	Toluene	99%
168	Amide	KOtBu	24 h/ reflux	Butryrl Chloride	THF	-

Note that all solvents used were dry, either freshly distilled or brought dry. Also that ethyl ester isoxazole is ethyl 3,5-dimethylisoxazole-4-carboxylate *(**150**) and amide isoxazole is N*,3,5-trimethylisoxazole-4-carboxamide *(**151**).*Denotes were the base was freshly prepared.** Denotes the number reference for the product, [a] Represents the synthesis of the ketone*

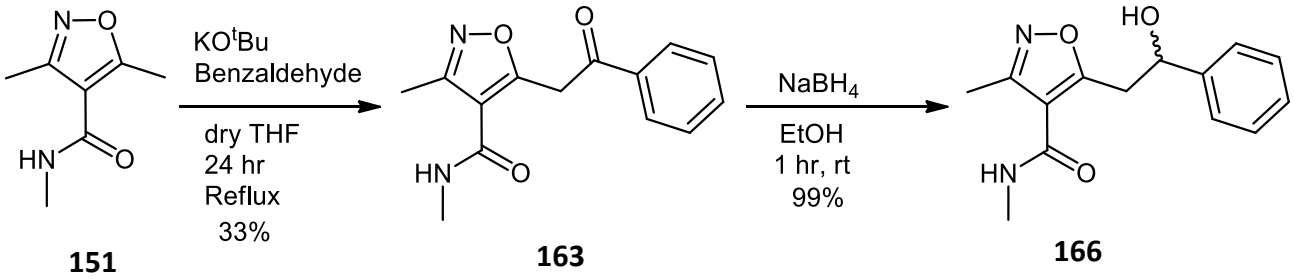
The table shows that only one condition tested was successful in accomplishing the aldol condensation reaction, producing a new alkene bond. This required *tert*-butoxide as a base in dry THF with stirring overnight for 24 hours, and the specific reaction is shown in Scheme 105.



151 **167**

Scheme 105. Successful aldol condensation reaction

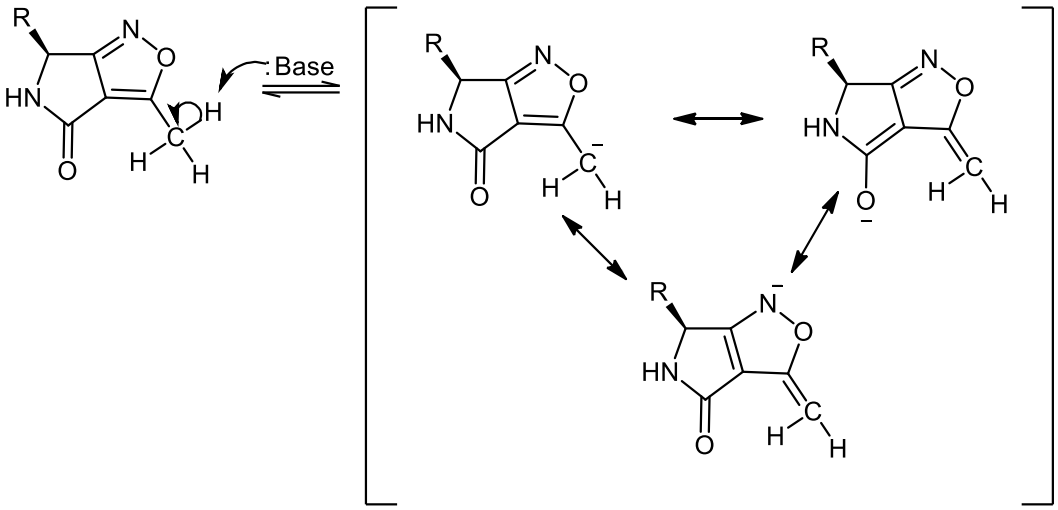
The table also shows the assumed disproportionation reaction in which the attempted aldol reaction resulted in the synthesis of the ketone, an oxidised version of the desired alcohol. This was confirmed by NMR spectroscopy. This ketone could be reduced with sodium borohydride to give an alcohol, Scheme 106. This alcohol could not be dehydrated to the previously synthesised alkene through addition of PSTA and under Dean-Stark conditions, after which only unidentified decomposed material was recovered.



151 **163** **166**

Scheme 106. Conditions for the aldol reaction to form the ketone with benzaldehyde and subsequent reduction by $NaBH_4$.

An N,C-diacylation attempt was made on the amide model and the L-valine-based pyrroloisoxazole using previously successful bases and an excess of butyryl chloride. In the case of the model amide, no identifiable products were isolated after several attempts. The pyrroloisoxazole did not afford the diacylated product but did return the mono-*N*-acylated compound in a 92% yield. This confirmed that the anion formed at the methyl group was only weakly nucleophilic, being extensively stabilised by delocalisation, Scheme 107.

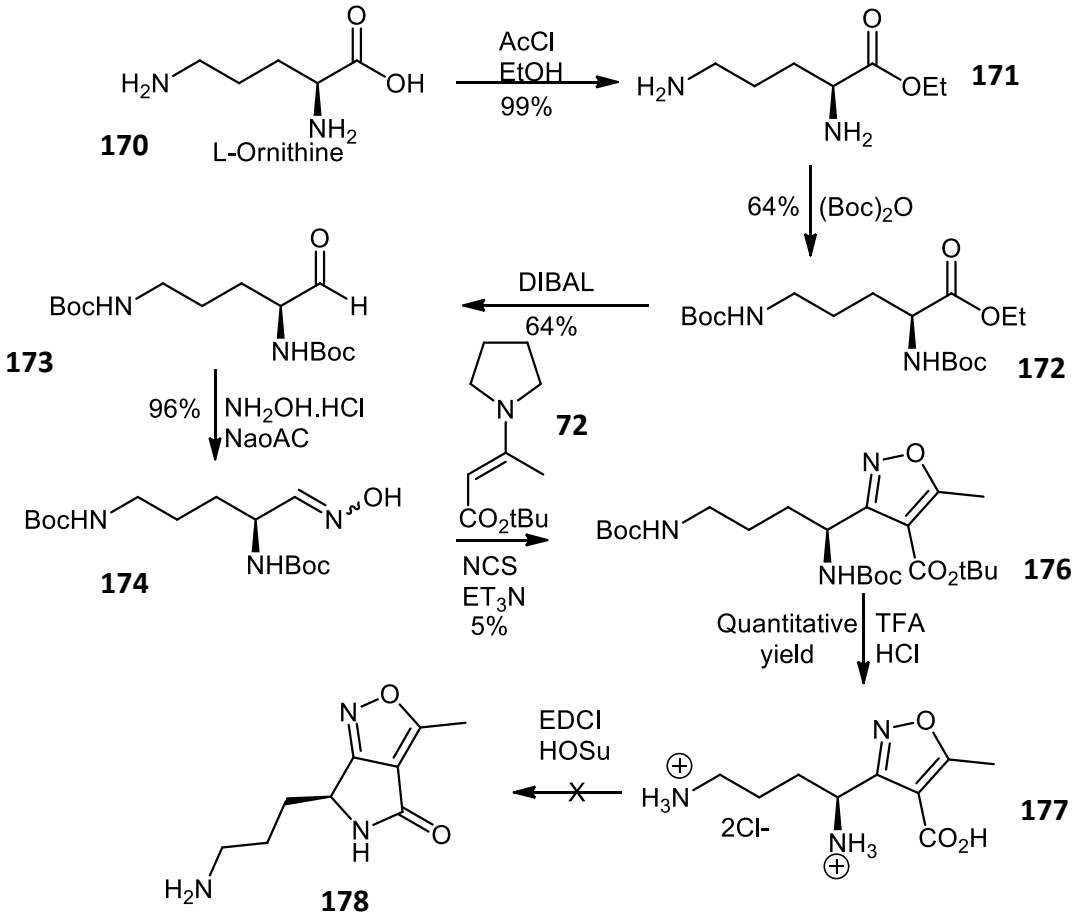


Scheme 107. Possible resonance structures seen in pyrroloisoxazole

Further investigation of C-deprotonation and reaction is required.

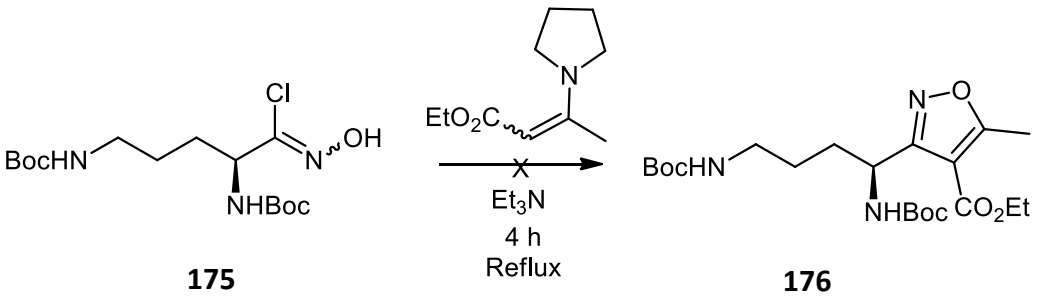
3.3 –Laccarin Analogue Synthesis

The synthesis of a laccarin analogue appeared to be an ideal extension of the synthesis to more than the directly targeted 3-acyltetramic acids. The Boc protected oxime, (S)-ethyl 2,5-bis((tert-butoxycarbonyl)amino)pentanoate (**172**) was produced in four stages from the commercially available and cheap L-ornithine (**170**) on an overall yield of 40%, which was good over the four stages as an additional esterification was required. The cycloaddition was then completed successfully with enamine *tert*-butyl 3-(pyrrolidin-1-yl)but-2-enoate (**72**) in a low 5% yield over two stages directly from the oxime. The next deprotection reaction was confirmed based on mass spectrometry and ^{13}C NMR. This hydrochloride salt was then tried in a cyclisation to form the new pyrrolidine-ring, which proved unsuccessful in several attempts. The research undertaken towards synthesis of the laccarin analogue has been summarised in Scheme 108.



Scheme 108. Summary towards synthesis of a laccarin analogue

We attempted to reverse the order of steps, by the synthesis of an ethyl ester substituted isoxazole which would be ring opened by hydrogenation then treated with a peptide coupling agent. This synthesis was unsuccessful in that although the chloro-oxime was isolated, the 1,3-dipolar cycloaddition was unsuccessful. The characteristic methyl group seen at 2.5-2.6 ppm in the NMR spectra of the 5-methyl isoxazoles was not observed (Scheme 109).



175 **176**

Scheme 109. Attempted 1,3-dipolar cycloadddition using L-ornithine series and ethyl enamine

This was disappointing but the project could be built upon by either completing the synthesis with the tert-butyl ester followed immediately by hydrogenation as well as potentially using the recent modification of T3P as a coupling agent.

3.4 – Potential Further Work

Further investigations are needed to find a reliable route to C-3 (Me) deprotonation of the pyrroloisoxazoles. Enaminoketone hydrolysis of N-acylated tetramides also requires additional investigation.

The laccarin synthesis has several potential routes that could be attempted, for example following the developed procedure to form the tert-butyl ester isoxazole then hydrogenation. The other main alteration is to try using T3P as an effective coupling agent for the lactam ring closing reaction, which could result in the successful formation of the pyrroloisoxazole which could under the enaminoketone formation and subsequent hydrolysis as previously planned.

4.0 – Experimental

4.1 – General Procedure

All reactions, unless otherwise stated, were completed under a nitrogen atmosphere and room temperature, which refers to an ambient temperature of 19-25 ^{0}C. All glassware, needles and syringes used were either new, therefore used straight from the sealed original packaging, or were washed prior to use using acetone and dried in an oven (150 ^{0}C), with the exception of plastic syringes, which were dried on the bench top. Temperatures of 0 ^{0}C were achieved by using an ice bath, -78 ^{0}C was reached by using a dry ice-acetone mixture in a Dewar vessel. Experiments requiring heat were completed by using heating mantles, with a thermometer, and a hot plate fitted around a round bottomed flask. Dry experiments were ensured by placing a round bottomed flask under a nitrogen flow and then heating it strongly with a flame gun to remove the moisture.

TLC plates used to analyse products and reaction progress were aluminium backed silica gel F_{245} plates (produced by Merck) and were visualised either under UV light (at 254 nm) or by using a phosphomolybdic acid, ninhydrin or vanillin dip. When chromatography was used to separate products, columns were loaded with silica gel (60 Å) which was purchased from Fluorochem.

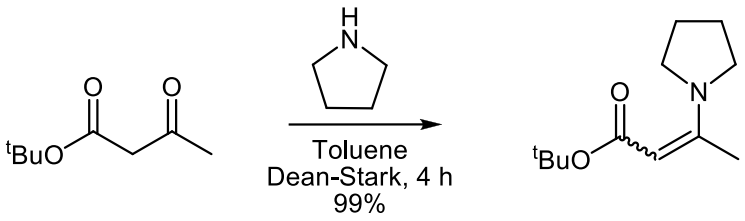
Anhydrous THF was freshly distilled from sodium-benzophenone. Where light petroleum is stated, this refers to the 40 ^{0}C – 60 ^{0}C boiling range and most other reactants used were not further purified after purchase, with the exception of the aldehydes used which were purified by distillation or by dissolving in diethyl ether and washing with sodium bicarbonate, and all products were acquired from Sigma-Aldrich or Merck. Deuterated-chloroform ($CDCl_3$), deuterated-water (D_2O) and deuterated-dimethyl sulfoxide (d_6-DMSO) were used in all cases as nuclear magnetic resonance spectroscopy (NMR) solvents and kept dry using molecular sieves, which were oven heated and dried on the high vacuum for 1 h prior to adding them to the solvent, and stored in a desiccator. Tetramethylsilane (TMS) was added to the NMR solvents as a standard.

The NMR spectra, both carbon (^{13}C) and hydrogen (^{1}H), were acquired using a Bruker DPX-400MHz spectrometer which provided frequencies of 100MHz for ^{13}C and 400MHz for ^{1}H analysis. All chemical shifts are reported in parts per million (ppm) where the TMS peak was an internal standard (at 0 ppm) and coupling constants (*J*) are all reported in Hz. The infra-red spectroscopic analysis was completed using a Perkin Elmer Paragon 1000 spectrometer and sodium chloride plates, where the sample was dried onto the plates by dissolving it in DCM and then loading it to the plate. Mass spectrometry results were obtained on a Thermo Fisher Exactive Accurate Mass Spectrometer with Advion Triversa Nanomate automation. Melting points were identified by using a Stewart Scientific SMP3 Melting Point Apparatus.

References have been given if the methodology used was similar and if the characterisation data were comparable to the source.

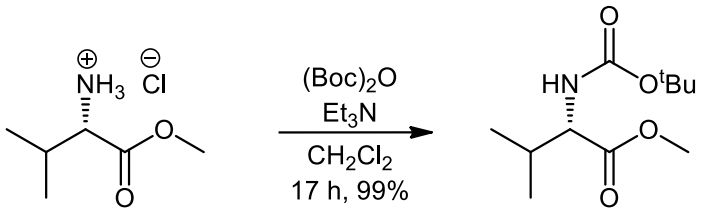
I would like to thank Dr. Mark Elsegood of Loughborough University for his help and support in obtaining the X-ray crystallography data shown in this report. Also I would like to thank Dr. Mark Edgar for his expertise and advice in NMR spectroscopy analysis and techniques.

4.2 – Preparation of Pyrroloisoxazole from L-Valine



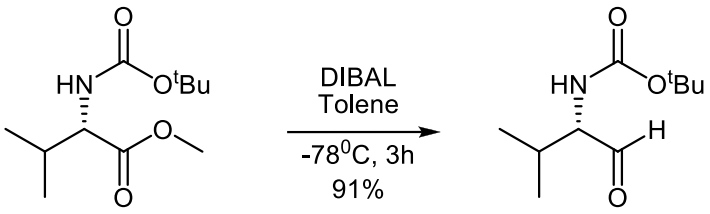
tert-Butyl 3-(pyrrolidin-1-yl)but-2-enoate (72)[75]

Pyrrolidine (10 g, 11.74 mL, 141 mmol) was added to a 500 mL round bottomed flask containing *tert*-butyl 3-oxobutanoate (20 g, 20.81 mL, 126 mmol) in toluene (250 mL). The reaction mixture was heated to reflux under Dean-Stark conditions for 4 h. The reaction mixture was then left to cool to room temperature before removing the excess solvent under reduced pressure. This yielded the product as a cream solid (26.4 g, 99%); mp 97.1-100.2 °C, lit.[79] mp 96-98 °C; MS: *m/z* calculated for $C_{12}H_{21}NO_2$ (MNa^+): 234.1465. Found 234.1461; IR ν_{max} ($CHCl_3$)/cm^{-1} 2959 (CCH), 2800 (CH_2, CH_3), 1634 (CO), 1583 (CC), 1130 (CN); ^1H NMR δ_H (400 MHz; $CDCl_3$) 1.39 (9H, s, $C(CH_3)_3$), 1.83 (4H, t, *J* 7.6, 2xsymmetrical (CH_2)), 2.35 (3H, s, $CHCCH_3$), 3.16-3.20 (4H, br. m, 2xsymmetrical (CH_2)N), 4.33 (1H, s, $CHCCH_3$); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 16.5 (CH_3), 25.2 (CH_2), 28.7 ($C(CH_3)_3$), 47.8 (CH_2), 85.0 ($CHCCH_3$), 158.9 (*C*N), 169.2 (*C*OO).



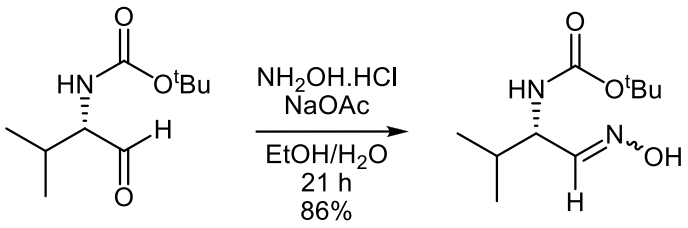
(S)-methyl 2-((tert-butoxycarbonyl)amino)-3-methylbutanoate (106a)[75]

Triethylamine (2 eq, 24.2 g, 33.3 mL, 239 mmol) was added dropwise to *S*-valine methyl ester hydrochloride salt (20.0 g, 119 mmol) in dichloromethane (400 mL) over 60 minutes at 0 ^{o}C. Di-*tert*-butyl dicarbonate (1.1 eq, 27.2 g, 125 mmol) in DCM (22 mL) was added dropwise over 60 minutes at 0 ^{o}C, after which the reaction mixture was stirred for a further 60 minutes at 0 ^{o}C. After this time the clear colourless reaction mixture was cooled to room temperature and stirred continuously for 18 h, under a nitrogen atmosphere. The reaction mixture, now a colourless clear solution, was washed with citric acid solution (1 M, 250 mL) and saturated brine (2 x 200 mL), then dried using magnesium sulfate which was removed by filtration and the product was achieved removing the excess solvent under a reduced pressure as a clear colourless oil (27.0 g, 99%); $[\alpha]_D^{rt}$ +21.5 (c 1, $CHCl_3$), lit.[96] $[\alpha]_D^{rt}$ +22 (c 1, MeOH); MS: *m/z* calculated for $C_{11}H_{21}NO_4$ (MNa^+): 254.1363. Found 254.1358; IR ν_{max} ($CHCl_3$)/cm^{-1} 3206 (NH), 2962 (CH_2, CH_3), 1726 (C=O), 1556 (NH), 1309 (CN), 1254 (C-O); ^{1}H NMR δ_H (400 MHz; ($CDCl_3$)) 0.82 (3H, d, *J* 6.8 Hz, CH(C*H*$_3$)), 0.88 (3H, d, *J* 7.2 Hz, CH(C*H*$_3$)), 1.38 (9H, s, C(C*H*$_3$)$_3$), 1.99-2.11 (1H, m, C*H*(CH_3)$_2$), 3.66 (3H, s, CO_2CH_3), 4.15 (1H, dd, J_a 9.0 Hz, J_b 5.0 Hz, C*H*NH), 5.01 (1H, broad s, CHN*H*); ^{13}C NMR δ_C (100 MHz; ($CDCl_3$)) 17.4 ((*C*H$_3$)$_2$), 18.9 ((*C*H$_3$)$_2$), 28.3 (*C*H(CH$_3$)$_2$), 31.2 (C(*C*H$_3$)$_3$), 51.9 (CO_2CH_3), 58.5 (*C*HNH), , 79.7 (*C*(CH$_3$), 155.6 (N*C*OO), 172.9 (*C*OO).



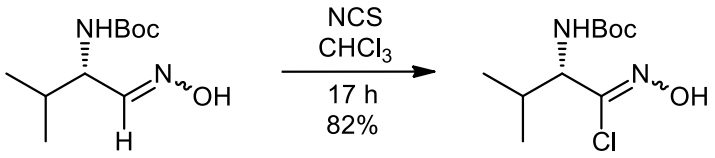
(S)-tert-Butyl (3-methyl-1-oxobutan-2-yl)carbamate (107a)[75]

Diisobutylaluminium hydride (1.0 M in toluene, 41 mL, 41 mmol) was added dropwise to (S)-tert-butyl (2-methyl-4-oxohexan-3-yl)carbamate (83) (3.3 g, 14.3 mmol) suspended in dry toluene (100 mL) under nitrogen at -78 ^{o}C over 4 h. The reaction mixture was stirred under these conditions for a further 1 h. Methanol (70 mL) and water (100 mL) were added and the mixture was then stirred vigorously for 2 h. The aqueous phase was separated and extracted with ethyl acetate (3 x 50 mL) and the combined organic layers were washed with brine (2 x 50 mL). The organic fraction was dried using magnesium sulfate, filtered and concentrated under reduced pressure to yield the aldehyde as a clear, colourless oil (2.60 g, 91%); $[\alpha]_D^{rt}$ +36.2 (c 1, $CHCl_3$); MS: *m/z* calculated for $C_{10}H_{19}NO_3$ (MNa^+): 224.1257. Found 224.1255; IR ν_{max} ($CHCl_3$)/cm^{-1} 3299 (NH), 2942 (CH_2, CH_3), 1709 (C=O), 1367 (CN), 1269 (C-O); ^{1}H NMR δ_H (400 MHz; ($CDCl_3$)) 0.87 (3H, d, *J* 5.6 Hz, CH(C*H*$_3$)), 0.95 (3H, d, *J* 6.0 Hz, CH(C*H*$_3$)), 1.38 (9H, s, C(C*H*$_3$)$_3$), 2.12-2.28 (1H, m, C*H*(CH_3)$_2$), 4.15 (1H, dd, J_a 7.9 Hz, J_b 5.1 Hz, C*H*NH), 5.21 (1H, d, *J* 7.9 Hz, CHN*H*), 9.57 (1H, broad, C*H*O); ^{13}C NMR δ_C (100 MHz; ($CDCl_3$)) 17.5 ((*C*H$_3$)$_2$), 18.7 ((*C*H$_3$)$_2$), 29.0 (C(*C*H$_3$)$_3$), 29.6 (*C*H(*C*H$_3$)$_2$), 64.5 (*C*HNH), 151.9 (N*C*OO), 199.8 (*C*HO).



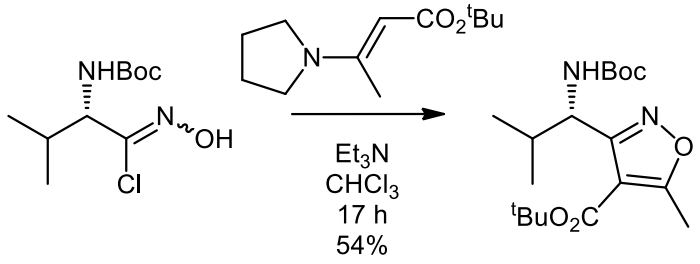
(S)-tert-Butyl 1-(hydroxyimino)-3-methylbutan-2-yl]carbamate (109a)[75]

(S)-*tert*-Butyl (3-methyl-1-oxobutan-2-yl)carbamate (84) (2.4 g, 11.9 mmol) in ethanol (50 mL) was added to hydroxylamine hydrochloride (1.65 g, 23.8 mmol) and sodium acetate (3.91 g, 47.7 mmol) suspended in water (50 mL). A white precipitate formed and was re-dissolved with ethanol (15 mL), the solution was then heated to 70 ^{0}C and stirred for 0.5 h, during which a watchglass was placed over the neck of the round bottomed flask to avoid solvent evaporation. The reaction mixture was then allowed to cool to ambient temperature and was placed in the refrigerator (2-5 ^{0}C) to remove impurities via crystallisation for 20 h. After this a white precipitate had formed which was removed via filtration. The filtrate was then separated and extracted with ethyl acetate (3 x 70 mL). The organic phases were dried over magnesium sulfate. The excess solvent was removed under a reduced pressure to leave the product *tert*-butyl 1-(hydroxyimino)-3-methylbutan-2-yl]carbamate as a white solid (2.20 g, 86 %); mp 111-115 °C; MS: *m/z* calculated for $C_{10}H_{20}N_2O_3$ (MNa^+): 239.1366. Found 239.1365; IR ν_{max} (nujol)/cm^{-1} 3415 (OH) 3379 (NH), 2976 (CH_2, CH_3), 1709 (C=O), 1678 (CN), 1399 (NH), 1349 (CN), 1249 (C-O); ^{1}H NMR δ_H (400 MHz; $(CD_3)_2SO$)) 0.88 (6H, 2 x d, *J* 6.4 Hz, $CH(CH_3)_2$), 1.38 (9H, s, $C(CH_3)_3$), 1.72-1.78 (1H, m, $CH(CH_3)_2$), 4.55 (1H, dd, J_a 7.6 Hz, J_b 10.6 Hz, CHNH), 6.51 (1H, d, *J* 7.6 Hz, CHNOH), 6.99 (1H, d, *J* 10.6 Hz, CHNH), 10.9 (1H, s, OH); ^{13}C NMR δ_C (100 MHz; $((CD_3)_2SO)$) 18.6 ($(CH_3)_2$), 28.2 ($C(CH_3)_3$), 39.4 ($CH(CH_3)_2$), 50.2 (CHNH), 149.9 (CHN), 155.2 (NCOO).



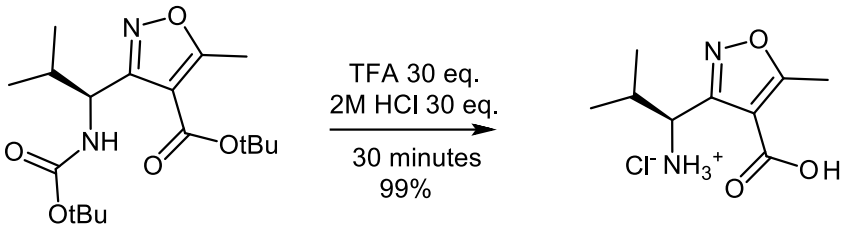
(S)-tert-Butyl 1-chloro-1-(hydroxyimino)-3-methylbutan-2-yl]carbamate (116a)[75]

N-Chlorosuccinimide (1.1 eq, 1.47 g, 11.0 mmol) was added dropwise to (S)-tert-butyl 1-(hydroxyimino)-3-methylbutan-2-yl]carbamate (85) (2.16 g, 9.98 mmol) suspended in chloroform (50 mL) at 0 ^{o}C over 60 minutes. The reaction mixture was then heated at reflux for 18 h to produce the chlorinated light brown solution which was added to directly without purification; $[\alpha]_D^{rt}$ +19.6 (c 1, $CHCl_3$); MS: *m/z* calculated for $C_{10}H_{21}N_2O_3{}^{35}Cl$ (MNa^+-HCl): 238.1293. Found 238.1294; IR ν_{max} ($CHCl_3$)/cm^{-1} 3350 (OH), 2985 (CH_2, CH_3), 1711 (C=O), 1525 (NH), 1298 (CN), 1259 (C-O) 1095 (N-O); ^{1}H NMR δ_H (400 MHz; ($CDCl_3$)) 0.89 (3H, d, *J* 4.0 Hz, CH(C*H*$_3$)), 0.92 (3H, d, *J* 4.0 Hz, CH(C*H*$_3$)), 1.46 (9H, s, C(C*H*$_3$)$_3$), 1.96-2.03 (1H, m, C*H*(CH_3)$_2$), 4.25 (1H, br. s, O*H*), 4.39 (1H, t, *J* 9.6 Hz, C*H*NH), 4.97 (1H, d, *J* 9.6 Hz, CHN*H*); ^{13}C NMR δ_C (100 MHz; ($CDCl_3$)) 18.5 ((*C*H$_3$)$_2$), 19.3 ((*C*H$_3$)$_2$), 27.6 (C(*C*H$_3$)$_3$), 30.4 (*C*H(CH$_3$)$_2$), 59.2 (*C*HNH), 79.8 (*C*(CH$_3$)$_3$, 138.2 (*C*HN), 167.2 (N*C*OO).



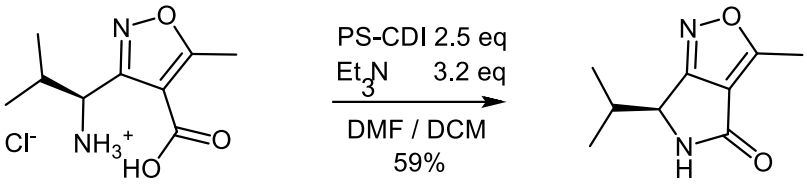
(S)-tert-Butyl 3-(1-tert-butoxycarbonylamino-2-methylpropyl)-5-methylisoxazole-4-carboxylate (111a)[75]

To freshly prepared (S)-*tert*-butyl 1-chloro-1-(hydroxyimino)-3-methylbutan-2-yl]carbamate (86) (1.3 g, 5.80 mmol) was added directly *tert*-butyl 3-(pyrrolidin-1-yl)butyl-2-enoate (81) (2 eq, 2.28 g, 10.52 mmol) in one portion, followed by triethylamine (1.1 eq, 0.81 mL, 5.78 mmol) over 60 minutes at room temperature. The reaction mixture was heated at reflux for a further 3 h under a nitrogen atmosphere, cooled and subsequently poured into water (100 mL). The organic layer was separated and washed with citric acid solution (2 M, 200 mL), sodium acetate solution (5%, 100 mL) and brine (2 x 100 mL). The brown oil was then dried over magnesium sulfate, filtered and concentrated under a reduced pressure to yield a light brown/yellow oil (1.33 g, crude 75 %). This crude material was purified by flash chromatography eluting with light petroleum:ethyl acetate (3:1 v/v) to yield the product isoxazole as a yellow oil (0.88 g, 45% over two steps); $[\alpha]_D^{rt}$ -6.2 (c 1, $CHCl_3$), lit.[75] $[\alpha]_D^{rt}$ -8.0 (c 10, $CHCl_3$); MS: *m/z* calculated for $C_{18}H_{30}N_2O_5$ (MNa^+): 377.2047. Found 377.2039; IR ν_{max} ($CHCl_3$)/cm^{-1} 3203 (NH), 2986 (CH_2, CH_3), 1716 (C-O), 1691 (CN), 1651 (CC), 1487 (NH), 1296 (C-O); ^1H NMR δ_H (400 MHz; ($CDCl_3$)) 0.86 (3H, d, *J* 7.2 Hz, $CH(CH_3)_2$), 0.90 (3H, d, *J* 7.2 Hz, $CH(CH_3)_2$), 1.33 (9H, s, $C(CH_3)_3$), 1.45 (9H, s, $C(CH_3)_3$), 1.99-2.09 (1H, m, $CH(CH_3)_2$), 2.58 (3H, s, $COCH_3$), 4.91 (1H, dd, J_a 7.9 Hz, J_b 15.2 Hz CHNH), 5.69 (1H, d, *J* 7.9 Hz, CHNH); ^{13}C NMR δ_C (100 MHz; ($CDCl_3$)) 13.2 (5-CH_3), 17.7 ((CH_3)$_2$), 19.8 ((CH_3)$_2$), 28.0 ($C(CH_3)_3$), 28.2 ($C(CH_3)_3$), 31.0 ($CH(CH_3)_2$), 81.5(CHNH), 107.8 ($C(CH_3)_3$), 155.8 ($C(CH_3)_3$), 161.2 (CCO), 165.4 (CN), 166.0 (CCO), 174.4 (NCOO), 200.0 (COO).



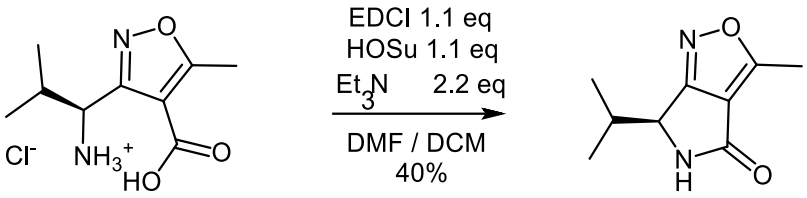
(S)-3-(1-Amino-2-methylpropyl)-5-methylisoxazole-4-carboxylic acid hydrochloride (118a)[75]

Trifluoroacetic acid (30 eq, 34.6 mL, 448.6 mmol) was added to *tert*-butyl (*S*)-3-(1-*tert*-butyloxycarbonylamine-2-methylpropyl)-5-methylisoxazole-4-carboxylate (87) (5.30 g, 14.95 mmol). The mixture was stirred at room temperature for 4.5 h to produce a brown paste, which was concentrated under a reduced pressure. The dry residue was then resuspended in hydrochloric acid (30 eq, 2 M, 224 mL, 448.6 mmol) and the mixture was stirred at room temperature for a further 1 h. The excess acid was removed *under a reduced pressure* producing a pink/brown residue which was dissolved in water (100 mL) and washed with ethyl acetate (3 x 70 mL), retaining the aqueous phase. The aqueous phase was evaporated to dryness under reduced pressure, yielding 3-(1-amino-2-methylpropyl)-5-methylisoxazole-4-carboxylic acid hydrochloride as a yellow/brown crystalline solid (3.5 g, 99%); $[\alpha]_D^{rt}$ -5.6 (c 1, MeOH), lit.[75] $[\alpha]_D^{rt}$ -9.8 (c 13, MeOH); mp 183-187 ^oC; MS: *m/z* calculated for $C_9H_{15}N_2O_3$ (MH^+): 199.1076. Found 199.1076; IR ν_{max} ($CHCl_3$)/cm^{-1} 3010 (OH), 2923 (CH_3), 1709 (CO), 1689 (CN), 1646 (CC), 1429 (NH), 1396 (OH), 1352 (CN); ^1H NMR δ_H (400 MHz; ($(CD_3)_2SO$)) 0.90 (3H, d, *J* 6.8 Hz, CH(CH_3)$_2$), 1.05 (3H, d, *J* 6.8 Hz, CH(CH_3)$_2$), 2.21-2.33 (1H, m, C*H*(CH_3)$_2$), 2.67 (3H, s, CH_3), 4.64-4.65 (1H, m, C*H*NH), 8.78 (3H, s, NH_3); ^{13}C NMR δ_C (100 MHz; ($(CD_3)_2SO$)) 13.1 (5-*C*H_3), 18.3 ((CH_3)$_2$), 17.9 ((CH_3)$_2$), 30.6 (*C*H(CH_3)$_2$), 51.2 (*C*HNH), 108.7 (*C*C), 160.3 (*C*N), 162.7 (N*C*OO), 175.7 (*C*OO).



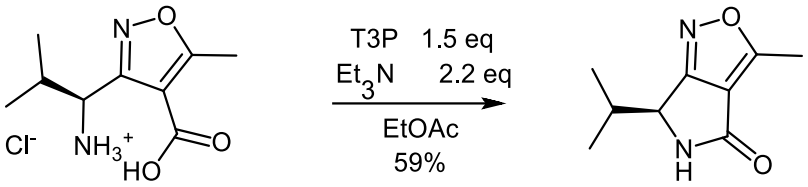
(S)-5,6-Dihydro-6-(1-methylethyl)-3-methyl-4H-pyrrolo[3,4-c]isoxazol-4-one (125a)[75]

(S)-3-(1-Amino-2-methylpropyl)-5-methylisoxazole-4-carboxylic acid hydrochloride (93) (1.5 g, 6.39 mmol) was dissolved in dry DMF/DCM (1:9, 20 mL) and stirred under a nitrogen atmosphere for 15 minutes. To this mixture was then added the polystyrene-carbodiimide (2.5 eq, 13.32 g, 15.98 mmol) and triethylamine (1.97 mL, 14.06 mmol) and stirring was continued at room temperature and under a nitrogen atmosphere for a further 48 h. The remaining polystyrene-carbodiimide was removed by filtration, washing with dry DMF/DCM (1:9, 5 mL). The filtrate was then washed with saturated potassium carbonate solution (2 x 30 mL) and brine (2 x 30 mL) and dried using magnesium sulfate. The solution was concentrated under reduced pressure to obtain an orange/brown solid (1.2 g, 80%). This solid was separated using flash chromatography with ethyl acetate and light petroleum (5:1 v/v) to give a pale yellow solid identified as (S)-5,6-dihydro-6-(1-methylethyl)-3-methyl-4H-pyrrolo[3,4-c]isoxazol-4-one (600 mg, 59%); $[\alpha]_D^{rt}$ +7.9 (c 1, $CHCl_3$), lit.[75] $[\alpha]_D^{rt}$ +7.9 (c 1, $CHCl_3$), lit.[75] $[\alpha]_D^{rt}$ +6.8 (c 12.3, $CHCl_3$); mp 167-170 °C; MS: m/z calculated for $C_9H_{12}N_2O_2$ (MH^+): 181.0970. Found 181.0970; IR ν_{max} ($CHCl_3$)/cm^{-1} 3220 (NH), 3017 (CH_3), 1700 (CO), 1653 (CC), 1465 (NH), 1390 (CN), 1133 (COC); ^1H NMR δ_H (400 MHz; ($CDCl_3$)) 1.04 (3H, d, *J* 4.0 Hz, $CH(CH_3)_2$), 1.10 (3H, d, *J* 6.2 Hz, $CH(CH_3)_2$), 2.05-2.14 (1H, m, $CH(CH_3)_2$), 2.65 (3H, s, CH_3), 4.50 (1H, d, *J* 5.3 Hz, CHNH), 6.32 (1H, broad, CHNH); ^{13}C NMR δ_C (400 MHz; ($CDCl_3$)) 12.1 (CH_3), 17.6 (($CH_3)_2$), 18.3 (($CH_3)_2$), 31.5 ($CH(CH_3)_2$), 58.6 (CHNH), 113.8 (CCO), 163.1 (CN), 166.2 (CO), 171.3 (NCO). Crystals were able to be grown from DCM and the data can be found later in this report.



(S)-5,6-Dihydro-6-(1-methylethyl)-3-methyl -4H-pyrrolo[3,4-c]isoxazol-4-one (125a)

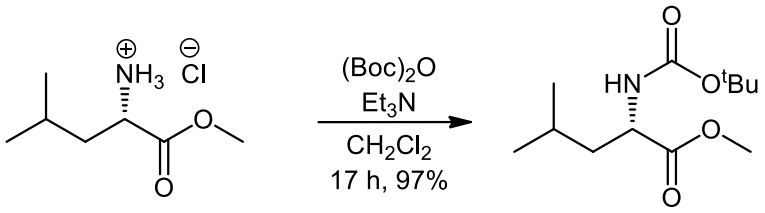
1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) (1.09 g, 7.03 mmol, 1.1 eq) was added to a suspension of (S)-3-(1-amino-2-methylpropyl)-5-methylisoxazole-4-carboxylic acid hydrochloride (93) (0.50 g, 2.13 mmol) and *N*-hydroxysuccinimide (0.81 g, 7.03 mmol, 1.1 eq) in dry dimethylformamide (50 mL) at 0 ^{0}C. The reaction mixture was stirred at room temperature for 17 h. Triethylamine (1.96 mL, 15.46 mmol, 2.2 eq) was added and the reaction mixture left to stir for a further 17 h and then concentrated under a reduced pressure. The residue was dissolved in ethyl acetate (60 mL) and washed with water (60 mL), hydrochloric acid (2M, 60 mL), saturated sodium bicarbonate solution (60 mL) and saturated brine (60 mL). The organic layer was dried over magnesium sulfate, filtered and concentrated under a reduced pressure to yield a beige solid (0.23 g, 60 %). The solid was purified by column chromatography using silica gel as absorbent and ethyl acetate:light petroleum (5:1 v/v) as eluent to yield (100) as a white solid (600 mg, 40%); Characterisation data were identical to those reported above.



(S)-5,6-Dihydro-6-(1-methylethyl)-3-methyl-4H-pyrrolo[3,4-c]isoxazol-4-one (125a)

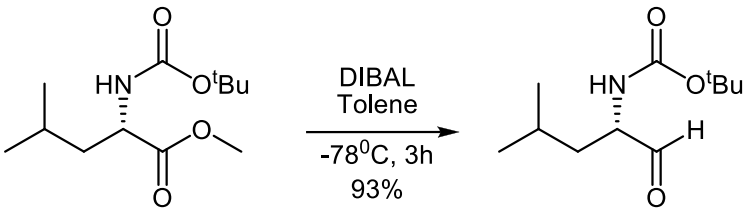
(*S*)-3-(1-Amino-2-methylpropyl)-5-methylisoxazole-4-carboxylic acid hydrochloride (93) (2.70 g, 11.5 mmol) in ethyl acetate (20.0 mL) was stirred under a nitrogen atmosphere for 15 minutes at 0 ^{0}C. To this was then added triethylamine (4.00 mL, 28.8 mmol) and the reaction mixture stirred for a further 10 minutes before the addition of propylphosphonic anhydride solution (T3P) (50% (w/w) in ethyl acetate, 8.78 g, 6.59 mL, 13.8 mmol) at 0^{0}C. Stirring was continued and the mixture allowed to warm to room temperature and under a nitrogen atmosphere for a further 17 h. The reaction mixture was then washed with citric acid solution (1M, 50.0 mL) saturated potassium carbonate solution (2 x 30.0 mL) and brine (2 x 30.0 mL) and dried using magnesium sulfate. The solution was then concentrated under a reduced pressure to obtain pure (S)-5,6-dihydro-6-(1-methylethyl)-3-methyl-4H-pyrrolo[3,4-c]isoxazol-4-one as a white solid (1.15 g, 59%); Characterisation data were identical to those reported above.

4.3 – Preparation of the Pyrroloisoxazole from L-Leucine



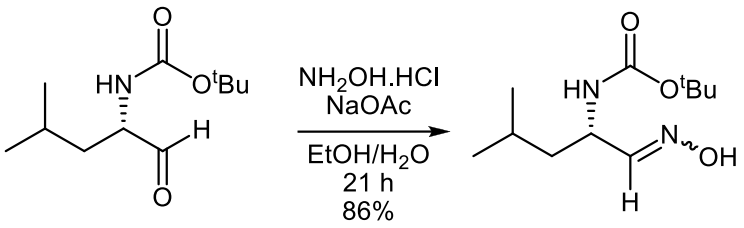
(S)-Methyl 2-((tert-butoxycarbonyl)amino)-4-methylpentanoate (106b)

Triethylamine (2 eq, 22.26 g, 30.68 mL, 220.0 mmol) was added dropwise to L-leucine methyl ester (20.0 g, 110.0 mmol) in dichloromethane (400 mL) over 60 minutes at 0 °C. Di-*tert*-butyl dicarbonate (1.1 eq, 25.11 g, 115.5 mmol) in DCM (60 mL) was added dropwise over 60 minutes at 0 °C, after which the reaction mixture was stirred for a further 60 minutes at 0 °C. After this time the clear, colourless reaction mixture was cooled to room temperature and stirred continually for 18 h, under a nitrogen atmosphere. The reaction mixture, now a colourless clear solution, was washed with citric acid solution (1 M, 250 mL) and saturated brine (2 x 200 mL), then dried using magnesium sulfate which was removed by filtration and the product was achieved removing the excess solvent under a reduced pressure as a clear colourless oil (26.2 g, 97%); $[\alpha]_D^{rt}$ -13.2 (c 1, $CHCl_3$); MS: *m/z* calculated for $C_{12}H_{23}NO_4$ (MNa^+): 268.1519. Found 268.1515; IR ν_{max} ($CHCl_3$)/cm^{-1} 3436 (NH), 2984 (CH_2, CH_3), 1713 (C=O), 1502 (NH), 1368 (CN), 1265 (C-O); ^1H NMR δ_H (400 MHz; ($CDCl_3$)) 0.90 (3H, d, *J* 2.4 Hz, CH(C*H*$_3$)), 0.95 (3H, d, *J* 2.4 Hz, CH(C*H*$_3$)), 1.42 (9H, s, C(C*H*$_3$)$_3$), 1.49-1.51 (1H, m, C*H*(CH_3)$_2$), 1.52-1.59 (1H, m, C*H*$_2$CH(CH_3)$_2$), 1.65-1.69 (1H, m, C*H*$_2$CH(CH_3)$_2$), 3.61 (3H, s, CO_2CH_3), 4.39 (1H, dd, J_a 8.1 Hz, J_b 4.2 Hz, C*H*NH), 4.98 (1H, broad s, CHN*H*); ^{13}C NMR δ_C (100 MHz; ($CDCl_3$)) 21.8 ((*C*H$_3$)$_2$), 22.8 ((*C*H$_3$)$_2$), 24.7 (*C*H(CH$_3$)$_2$), 28.7 (C(*C*H$_3$)$_3$), 41.7 (*C*H$_2$), 51.4 (CO_2CH_3), 56.0 (*C*HNH), 79.7 (*C*(CH$_3$)$_3$, 155.4 (N*C*OO), 174.0 (*C*OO).



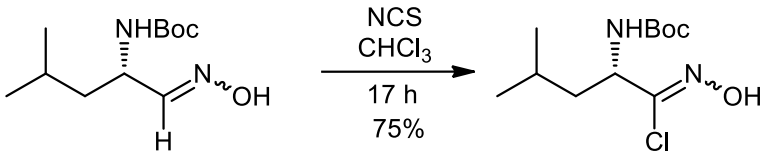
(S)-tert-Butyl (4-methyl-1-oxopentan-2-yl)carbamate (108b)

Diisobutylaluminium hydride (1.0 M in toluene, 133 mL, 133 mmol) was added dropwise to (S)-methyl 2-((*tert*-butoxycarbonyl)amino)-4-methylpentanoate (**106b**) (13.1 g, 53.3 mmol) suspended in dry toluene (100 mL) under nitrogen at -78 ^{o}C over 4 h. The reaction mixture was stirred under these conditions for a further 1 h. Methanol (70 mL) was added to quench excess DIBAL and the solution was added to Rochelle salt (50 g) in water (100 mL) and the mixture was then stirred vigorously for 2 h. The aqueous phase was separated and extracted with ethyl acetate (3 x 50 mL) and the combined organic layers were washed with brine (2 x 50 mL). The organic fraction was dried using magnesium sulfate, filtered and concentrated under a reduced pressure to yield the aldehyde as a clear, colourless oil (10.63 g, 93%); $[\alpha]_D^{rt}$ +21.5 (c 1, $CHCl_3$); MS: *m/z* calculated for $C_{11}H_{22}NO_3$ (MNa^+): 239.1492. Found 239.1487; IR ν_{max} ($CHCl_3$)/cm^{-1} 3429 (NH), 2986 (CH_2, CH_3), 2820 (OC-H), 2730 ((OC-H), 1709 (C=O), 1502 (NH), 1368 (CN), 1265 (C-O); ^{1}H NMR δ_H (400 MHz; ($CDCl_3$)) 0.87 (3H, d, *J* 2.4 Hz, CH(C*H*$_3$)), 0.87 (3H, d, *J* 2.4 Hz, CH(C*H*$_3$)), 1.28-1.35 (1H, m, C*H*(CH_3)$_2$), 1.40 (9H, s, C(C*H*$_3$)$_3$), 1.57-1.65 (1H, m, C*H*$_2$CH(CH_3)$_2$), 1.68-1.77 (1H, m, C*H*$_2$CH(CH_3)$_2$), 4.15-4.21 (1H, br. m, C*H*NH), 5.13 (1H, br. d, *J* 4.0 Hz, CHN*H*), 9.50 (1H, s, C*H*O); ^{13}C NMR δ_C (100 MHz; ($CDCl_3$)) 22.3 ((*C*H$_3$)$_2$), 23.7 ((*C*H$_3$)$_2$), 25.3 (*C*H(CH_3)$_2$), 28.3 (C(*C*H$_3$)$_3$), 37.9 (*C*H$_2$), 57.9 (*C*HNH), 80.8 (*C*(CH_3)$_3$, 156.1 (N*C*OO), 200.6 (*C*HO).



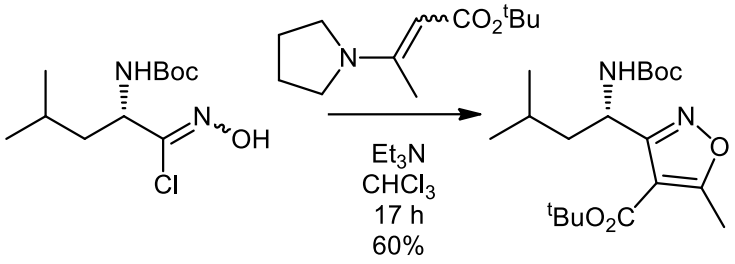
(S)-tert-Butyl (1-(hydroxyimino)-4-methylpentan-2-yl)carbamate (109b)

(S)-*tert*-Butyl (4-methyl-1-oxopentan-2-yl)carbamate (**108b**) (10.62 g, 49.4 mmol) in ethanol (50 mL) was added to hydroxylamine hydrochloride (6.86 g, 98.7 mmol) and sodium acetate (16.15 g, 197.0 mmol) suspended in water (100 mL). A white precipitate formed and was re-dissolved with ethanol (18 mL), the solution was then heated to 70 ^{0}C and stirred for 0.5 h, during which a watchglass was placed over the neck of the round bottomed flask to avoid solvent evaporation. The reaction mixture was then allowed to cool to ambient temperature and was placed in the refrigerator (2-5 ^{0}C) to remove impurities via crystallisation for 20 h. After this a white precipitate had formed which was removed via filtration. The filtrate was then separated and extracted with ethyl acetate (3 x 70 mL) and then washed with saturated potassium carbonate solution (100 mL). The combined organic phases were dried over magnesium sulfate. The excess solvent was removed under reduced pressure to leave the product (S)-*tert*-butyl (1-(hydroxyimino)-4-methylpentan-2-yl)carbamate as a white solid (2.20 g, 86 %); mp 182-184^{o}C; $[\alpha]_D^{rt}$ -18.87 (c 3.9, $CHCl_3$); MS: *m/z* calculated for $C_{11}H_{22}N_2O_3$ (MNa^+): 253.1523. Found 253.1519; IR ν_{max} ($CHCl_3$)/cm^{-1} 3427 (OH), 3389 (NH), 2982 (CH_2, CH_3), 1701 (C=O), 1501 (NH), 1367 (CN), 1266 (C-O) 1028 (N-O); ^{1}H NMR δ_H (400 MHz; ($(CD_3)_2SO$)) 0.85 (3H, d, *J* 2.4 Hz, CH(C*H*$_3$)), 0.87 (3H, d, *J* 2.4 Hz, CH(C*H*$_3$)), 1.19-1.25 (1H, m, C*H*$(CH_3)_2$), 1.37 (9H, s, C(C*H*$_3)_3$), 1.37-1.40 (1H, m, C*H*$_2$CH$(CH_3)_2$), 1.51-1.57 (1H, m, C*H*$_2$CH$(CH_3)_2$), 2.50 (1H, s, O*H*), 4.69-4.75 (1H, br. m, C*H*NH), 6.50 (1H, d, *J* 3.6 Hz, CHN*H*), 10.83 (1H, s, C*H*N); ^{13}C NMR δ_C (100 MHz; ($(CD_3)_2SO$)) 21.9 (($CH_3)_2$), 23.0 (($CH_3)_2$), 24.2 (*C*H$(CH_3)_2$), 28.2 (C(*C*H$_3)_3$), 39.8 (*C*H$_2$), 44.1 (*C*HNH), 77.2 (*C*$(CH_3)_3$, 151.9 (*C*HN), 155.3 (N*C*OO).



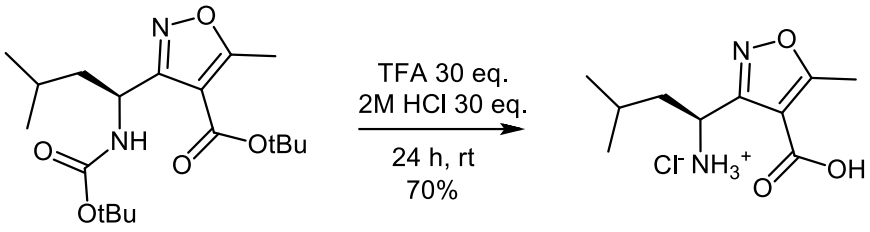
***(S)-tert-Butyl (1-chloro-1-(hydroxyimino)-4-methylpentan-2-yl)carbamate* (116b)**

N-Chlorosuccinimide (1 eq, 0.772g, 5.78 mmol) was added dropwise to (S)-*tert*-butyl (1-(hydroxyimino)-4-methylpentan-2-yl)carbamate (**109b**) (1.14 g, 5.26 mmol) suspended in chloroform (50 mL) at 0 ^{o}C over 60 minutes. The reaction mixture was then heated at reflux for 18 h to produce the chlorinated light brown solution. The chlorinated product was able to be isolated by washing the cooled solution with water (200 mL) and saturated brine (200 mL) before drying the organic layers with $MgSO_4$, filtration and concentration under reduced pressure to yield the chlorinated oxime as an orange oil (5.85 g, 75 %); $[\alpha]_D^{rt}$ +21.5 (c 1, $CHCl_3$); MS: *m/z* calculated for $C_{11}H_{21}N_2O_3Cl$ (MNa^+- HCl): 251.1362. Found 251.1366; IR ν_{max} ($CHCl_3$)/cm^{-1} 3321 (OH), 2962 (CH_2, CH_3), 1701 (C=O), 1503 (NH), 1368 (CN), 1265 (C-O) 1166 (N-O); ^{1}H NMR δ_H (400 MHz; ($CDCl_3$)) 0.90 (3H, d, *J* 2.4 Hz, CH(C*H*$_3$)), 0.93 (3H, d, *J* 2.4 Hz, CH(C*H*$_3$)), 1.20-1.30 (1H, m, C*H*(CH_3)$_2$), 1.45 (9H, s, C(C*H*$_3$)$_3$), 1.45-1.49 (1H, m, C*H*$_2$CH(CH_3)$_2$), 1.54-1.58 (1H, m, C*H*$_2$CH(CH_3)$_2$), 3.69 (1H, s, O*H*), 4.61-4.67 (1H, br. m, C*H*NH), 4.95 (1H, d, *J* 3.9 Hz, CHN*H*); ^{13}C NMR δ_C (100 MHz; ($CDCl_3$)) 21.9 ((*C*H$_3$)$_2$), 23.1 ((*C*H$_3$)$_2$), 24.8 (*C*H(CH_3)$_2$), 28.4 (C(*C*H$_3$)$_3$), 41.8 (*C*H$_2$), 43.1 (*C*HNH), 79.9 (*C*(CH_3)$_3$, 140.5 (*C*HN), 156.9 (N*C*OO).



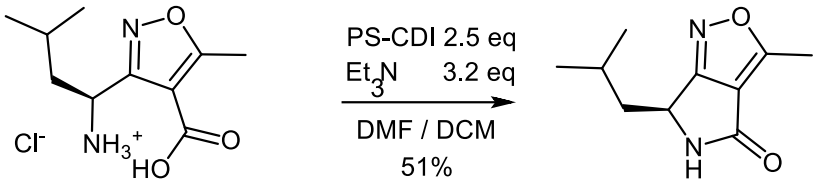
(S)-tert-Butyl 3-(1-((tert-butoxycarbonyl)amino)-3-methylbutyl)-5-methylisoxazole-4-carboxylate (111b)

To freshly prepared (*S*)-*tert*-butyl (1-chloro-1-(hydroxyimino)-4-methylpentan-2-yl)carbamate (**116b**) (3.26 g, 12.30 mmol) was added *tert*-butyl 3-(pyrrolidin-1-yl)butyl-2-enoate (81) (2 eq, 5.32 g, 24.60 mmol) in one portion, followed directly by triethylamine (1.1 eq, 1.89 mL, 13.53 mmol) over 60 minutes at room temperature. The reaction mixture was heated at reflux for a further 3 h under a nitrogen atmosphere, cooled and subsequently poured into water (200 mL). The organic layer was separated and washed with citric acid solution (2 M, 200 mL), sodium acetate solution (5%, 100 mL) and brine (2 x 100 mL). The brown oil was then dried over magnesium sulfate, filtered and concentrated under a reduced pressure to yield a light brown/yellow oil (4.4 g, crude 97 %). This crude material was purified by flash chromatography eluting with light petroleum:ethyl acetate (7:1 v/v) to yield the product isoxazole as a yellow oil (1.60 g, 60%); $[\alpha]_D^{rt}$ -6.0 (c 2.5, $CHCl_3$); MS: *m/z* calculated for $C_{19}H_{32}N_2O_5$ (MNa^+): 391.2203. Found 391.2200; IR ν_{max} ($CHCl_3$)/cm^{-1} 3365 (NH), 3055, 2982 (CH_2, CH_3), 1708 (C=O), 1643 (C=C), 1502 (NH), 1393 (CN), 1318 (C-O), 1168 (C-O-C); ^{1}H NMR δ_H (400 MHz; ($CDCl_3$)) 0.97 (3H, d, *J* 3.6 Hz, CH(C*H*$_3$)), 0.99 (3H, d, *J* 3.6 Hz, CH(C*H*$_3$)), 1.45 (9H, s, C(C*H*$_3$)$_3$), 1.51-1.54 (2H, m, C*H*$_2$CH(CH$_3$)$_2$), 1.62(9H, s, C(C*H*$_3$)$_3$), 1.65-1.71 (1H, m, C*H*(CH$_3$)$_2$), 2.54 (3H, s, C*H*$_3$), 4.80-4.89 (1H, br. m, C*H*NH), 5.50 (1H, d, *J* 4.2 Hz, CHN*H*); ^{13}C NMR δ_C (100 MHz; ($CDCl_3$)) 13.5 (5-*C*H$_3$) 22.6 ((*C*H$_3$)$_2$), 24.8 ((*C*H$_3$)$_2$), 27.1 (*C*H(CH$_3$)$_2$), 27.8 (C(*C*H$_3$)$_3$), 43.6 (*C*H$_2$), 47.0 (*C*HNH), 79.6 (*C*(CH$_3$)$_3$, 81.5 (*C*(CH$_3$)$_3$, 154.9 (O*C*NH), 161.5 (*C*NO), 163.7 (CH$_3$*C*O), 175.1 (*C*O$_2$^{t}Bu).



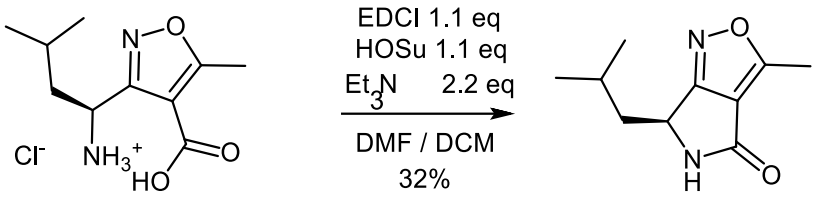
(S)-3-(1-Amino-3-methylbutyl)-5-methylisoxazole-4-carboxylic acid hydrochloride (118b)[75]

Trifluoroacetic acid (30 eq, 8.0 mL, 105 mmol) was added to *(S)-tert*-butyl 3-(1-((tert-butoxycarbonyl)amino)-3-methylbutyl)-5-methylisoxazole-4-carboxylate (**111b**). The mixture was stirred at room temperature for 4.5 h to produce a brown paste, which was concentrated under a reduced pressure. The dry residue was then resuspended in hydrochloric acid (30 eq, 2 M, 52.5 mL, 105 mmol) and the mixture was stirred at room temperature for a further 1 h. The excess acid was removed under a reduced pressure producing a pink/brown residue which was dissolved in water (100 mL) and washed with ethyl acetate (3 x 70 mL), retaining the aqueous phase. The aqueous phase was evaporated to dryness under reduced pressure, yielding (S)-3-(1-amino-3-methylbutyl)-5-methylisoxazole-4-carboxylic acid hydrochloride as a yellow/brown crystalline solid (610 mg g, 70 %); $[\alpha]_D^{rt}$ -3.0 (c 2.0, H_2O); mp 189-190 ^oC; MS: *m/z* calculated for $C_{10}H_{16}N_2O_3Cl$ (MNa^+-HCl): 235.1053. Found 235.1053; IR ν_{max} ($CHCl_3$)/cm^{-1} 3365 (NH), 3055, 2982 (CH_2, CH_3), 1708 (C=O), 1643 (C=C), 1502 (NH), 1393 (CN), 1318 (C-O), 1168 (C-O-C); ^1H NMR δ_H (400 MHz; (D_2O)) 0.75 (3H, d, *J* 2.8 Hz, CH(C*H*$_3$)), 0.79 (3H, d, *J* 2.8 Hz, CH(C*H*$_3$)), 1.35-1.42 (1H, m, C*H*(CH_3)$_2$), 1.66-1.73 (1H, m, C*H*$_2$CH(CH_3)$_2$), 1.78-1.85 (1H, m, C*H*$_2$CH(CH_3)$_2$), 2.58 (3H, s, C*H*$_3$), 4.80 (1H, t, *J* 7.6 Hz, C*H*NH); ^{13}C NMR δ_C (100 MHz; (D_2O)) 13.2 (5-*C*H_3) 21.1 ((*C*H_3)$_2$), 23.6 ((*C*H_3)$_2$), 23.9 (*C*H(CH_3)$_2$), 39.9 (*C*H_2), 45.7 (*C*HNH), 108.0 (*C*CO), 160.0 (*C*NO), 168.9 (C*C*O), 178.3 (*C*O_2H).



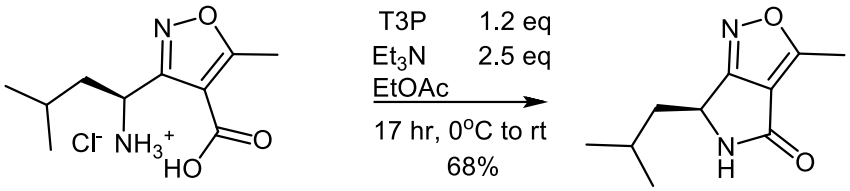
(S)-5,6-Dihydro-2-methylpropyl-3-methyl-4H-pyrrolo[3,4-c]isoxazol-4-one (125b)[75]

(*S*)-3-(1-Amino-3-methylbutyl)-5-methylisoxazole-4-carboxylic acid hydrochloride (**116b**) (1.3 g, 5.54 mmol) was dissolved in dry DMF/DCM (1:9, 20 mL) and stirred under a nitrogen atmosphere for 15 minutes. To this mixture was then added the polystyrene-carbodiimide (2.5 eq, 11.55 g, 13.85 mmol) and triethylamine (1.71 mL, 12.2 mmol) and stirring was continued at room temperature and under a nitrogen atmosphere for a further 48 h. The remaining polystyrene-carbodiimide was removed by filtration, washing with dry DMF/DCM (1:9, 5 mL). The filtrate was then washed with saturated potassium carbonate solution (2 x 30 mL) and brine (2 x 30 mL) and dried using magnesium sulfate. The solution was concentrated under a reduced pressure to obtain an orange/brown solid (1.0 g, 70%). This solid was separated using flash chromatography with ethyl acetate and light petroleum (5:1 v/v) to give a pale yellow solid identified as (*S*)-5,6-dihydro-2-methylpropyl-3-methyl-4H-pyrrolo[3,4-c]isoxazol-4-one (520 mg, 51%); $[\alpha]_D^{rt}$ +1.2 (c 2.0, $CHCl_3$), mp 180-182 °C; m/z calculated for $C_{10}H_{14}N_2O_2$ (MNa^+): 217.0947. Found 217.0948; IR ν_{max} ($CHCl_3$)/cm^{-1} 3372 (N-H), 3054 (C-H), 1701 (C=O), 1650 (C=C), 1603 (C-C), 1421 (NH), 1265 (CN), 1130 (COC); ^{1}H NMR δ_H (400 MHz; ($CDCl_3$)) 0.98 (3H, d, *J* 7.2 Hz, $CH(CH_3)_2$), 1.00 (3H, d, *J* 7.2 Hz, $CH(CH_3)_2$), 1.65-1.76 (2H, m, CH_2), 1.92-1.97 (1H, m, $CH(CH_3)_2$), 2.65 (3H, s, CH_3), 4.72 (1H, t, J 7.2 Hz, CHNH), 6.32 (1H, br. s, CHNH); ^{13}C NMR δ_C (400 MHz; ($CDCl_3$)) 13.9 (*C*H_3), 21.0 ((*C*H_3)$_2$), 22.1 ((*C*H_3)$_2$), 29.7 (*C*H(CH_3)$_2$), 51.3 (*C*H_2), 60.4 (*C*HNH), 113.4 (*C*CO), 163.2 (*C*N), 166.1 (*C*O), 171.1 (N*C*O).



(S)-5,6-Dihydro-2-methylpropyl-3-methyl-4H-pyrrolo[3,4-c]isoxazol-4-one (125b)[75]

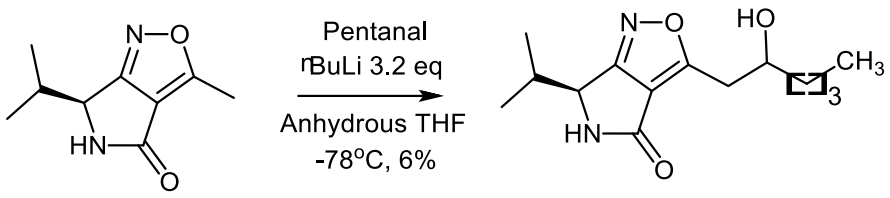
1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) (1.1 eq., 0.872 g, 5.62 mmol) was added to a suspension of (*S*)-3-(1-amino-3-methylbutyl)-5-methylisoxazole-4-carboxylic acid hydrochloride (**116b**) (0.400 g, 1.70 mmol) and *N*-hydroxysuccinimide (1.1 eq., 0.65 g, 5.62 mmol) in dry dimethylformamide (50 mL) at 0 ^{0}C. The reaction mixture was stirred at room temperature for 17 h. Triethylamine (2.2 eq., 1.57 mL, 12.4 mmol) was added and the reaction mixture was left to stir for a further 17 h and then concentrated under a reduced pressure. The residue was dissolved in ethyl acetate (60 mL) and washed with water (60 mL), hydrochloric acid (2M, 60 mL), saturated sodium bicarbonate solution (60 mL) and saturated brine (60 mL). The organic layer was dried over magnesium sulfate, filtered and concentrated in *vacuo* to yield a beige solid (0.184 g, 48%). The solid was purified by column chromatography using silica gel as absorbent and ethyl acetate:light petroleum (5:1 v/v) as eluent to yield a white solid (480 mg, 32%); Characterisation data were identical to those reported above.



(S)-5,6-Dihydro-2-methylpropyl-3-methyl-4H-pyrrolo[3,4-c]isoxazol-4-one (125b)[75]

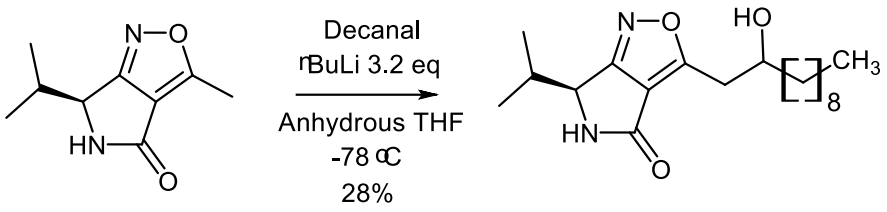
(*S*)-3-(1-Amino-3-methylbutyl)-5-methylisoxazole-4-carboxylic acid hydrochloride (**116b**) (250 mg, 1.00 mmol) in ethyl acetate (20 mL) was stirred under a nitrogen atmosphere for 15 minutes at 0^0C. To this was then added triethylamine (2.5 eq, 0.35 mL, 2.50 mmol) and the reaction mixture stirred for a further 10 minutes before the addition of propylphosphonic anhydride solution (T3P) (50% w/w in ethyl acetate, 0.76 g, 0.57 mL, 1.2 mmol) at 0^0C. Stirring was continued and the mixture allowed to warm to room temperature and under a nitrogen atmosphere for a further 17 h. The reaction mixture was then washed with citric acid solution (1M, 50 mL), saturated potassium carbonate solution (2 x 30 mL) and brine (2 x 30 mL) and dried using magnesium sulfate. The solution was then concentrated under a reduced pressure to obtain the pure pyrroloisoxazole product with no need for further purification (131.3 mg, 68%); Characterisation data were identical to those reported above.

4.4 – Aldol Reactions at C-3 Position of L-Valine Pyrroloisoxazole (125a)



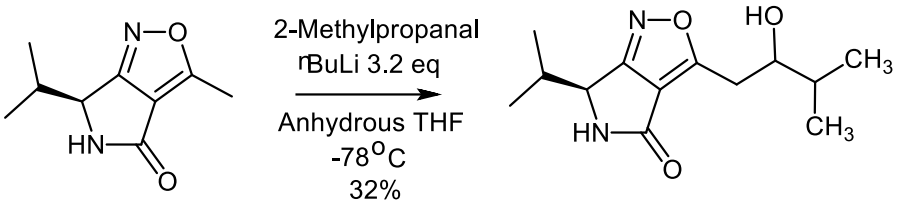
(S)-3-(2-Hydroxyhexyl)-6-(-1-methylethyl)-5,6-dihydro-4H-pyrrolo[3,4-c][1,2]oxazol-4-one (127)[75]

n-Butyllithium (3.2 eq, 2.5 M solution in hexanes, 0.08 mL, 0.2 mmol) was added dropwise to a solution of the pyrroloisoxazole (S)-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) (10 mg, 0.063 mmol) in THF (5 mL) at - 78 ^{0}C and kept under a nitrogen atmosphere. The solution was stirred at this temperature for 2 h, after which freshly distilled pentanal (1 mL, excess) was added in one portion and stirring continued for at - 78 ^{0}C for 2 h, after which the solution turned yellow. The reaction was quenched by the addition of water (5 mL) and the mixture acidified with hydrochloric acid (5 mL, 2 M). The mixture was concentrated under vacuum and the resulting yellow oil was suspended in water (5 mL) and ethyl acetate (5 mL). The aqueous phase was then extracted using ethyl acetate (2 x 5 mL), before the combined organic phases were washed with brine (10 mL) and dried over $MgSO_4$, filtered and concentrated under a vacuum to yield a yellow solid. This material was then purified using flash chromatography eluting with ethyl acteate and petroleum ether (5:1 v/v) to yield the product as a white solid (1 mg, 6 %); mp 115-120 ^{o}C; *m/z* calculated for $C_{14}H_{21}N_2O_3$ (MH^+): 267.1625. Found 267.1629; ^{1}H NMR δ_H (400 MHz; ($CDCl_3$)) 0.82 (3H, d, *J* 8.7 Hz, $CH(CH_3)_2$), 0.88 (3H, d, *J* 6.8 Hz, $CH(CH_3)_2$), 1.38 (9H, br.s, $CH_2CH_2CH_2CH_3$), 2.01-2.09 (1H, m, $CH(CH_3)_2$), 3.67 (2H, s, CH_2), 3.90 (1H, br. s, CH(O*H*), 4.12-4.19 (1H, m, C*H*NH), 4.93-5.01 (1H, m, CHN*H*); ^{13}C NMR δ_C (100 MHz; ($CDCl_3$)) 14.1 (CH_3CH_2), 17.7 ((CH_3)$_2$), 18.2 ((CH_3)$_2$), 22.5 (CH_3CH_2) 22.6 ($CH_3CH_2CH_2$), 28.0 (CH_2CHOH), 31.5 ($CH(CH_3)_2$), 36.7 (CH_2), 59.1 (*C*HNH), 67.5 (*C*HOH), 112.8 (*C*CO), 169.7 (*C*O), 170.9 (*C*N), 171.0 (N*C*O).



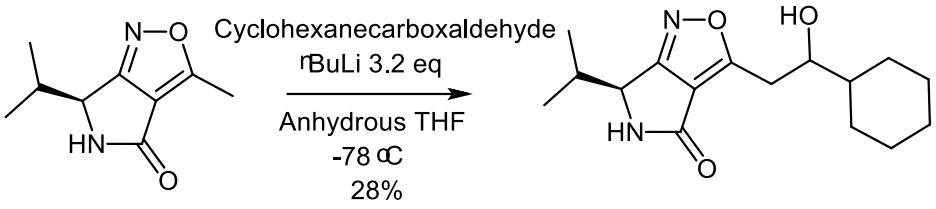
(S)-3-(2-Hydroxyundecyl)-6-(1-methylethyl)-5,6-dihydro-4H-pyrrolo[3,4-c][1,2]oxazol-4-one (128)[75]

Decanal was suspended in diethyl ether and washed with saturated potassium carbonate solution to remove any acidic impurities. The organic fraction was dried ($MgSO_4$), filtered and concentrated under a reduced pressure to achieve the pure aldehyde, confirmed by NMR spectroscopy. *n*-Butyllithium (3.2 eq, 2.5 M solution in hexanes, 0.08 mL, 0.2 mmol) was added dropwise to a solution of the pyrroloisoxazole (*S*)-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) (100 mg, 0.55 mmol) in THF (5 mL) at - 78 ^{0}C and kept under a nitrogen atmosphere. The solution was stirred at this temperature for 2 h. Purified decanal (1 mL, excess) was then added in one portion and stirring continued for at - 78 ^{0}C for 2 h, at t. The reaction was quenched by the addition of water (5 mL) and the mixture acidified with hydrochloric acid (5 mL, 2 M). The mixture dried over $MgSO_4$, filtered and concentrated under a reduced pressure and the resulting yellow oil was suspended in water (5 mL) and ethyl acetate (5 mL). The aqueous phase was then extracted by using ethyl acetate (2 x 5 mL), before the combined organic phases were washed with brine (10 mL) and dried under vacuum to yield a yellow oil (900 mg, 100%). This was purified by flash chromatography eluting with ethyl acetate and light petroleum (5:1 v/v) to yield a mixture of products, which was separated again under the same conditions to give the desired product as an pale yellow oil (27 mg, 28 %); *m/z* calculated for $C_{19}H_{32}N_2O_3$ (MH^+): 337.2486. Found 337.2488; ^{1}H NMR δ_H (400 MHz; ($CDCl_3$)) 0.77 (3H, t, *J* 8.1Hz, C*H*$_3$), 0.92 (3H, d, *J* 5.8 Hz, CH(C*H*$_3$)$_2$), 1.01 (3H, d, *J* 5.3 Hz, CH(C*H*$_3$)$_2$), 1.12-1.29 (14H, m, 7 x C*H*$_2$), 1.41-1.50 (2H, m, C*H*$_2$CHOH), 1.96-2.05 (1H, m, C*H*(CH$_3$)$_2$), 2.55 (2H, s, C*H*$_2$CHOH), 4.10 (1H, s, C*H*OH), 4.50-4.52 (1H, m, C*H*NH), 6.55 (1H, d, *J* 8.0 Hz, CHO*H*), 8.22 (1H, broad, CHN*H*).



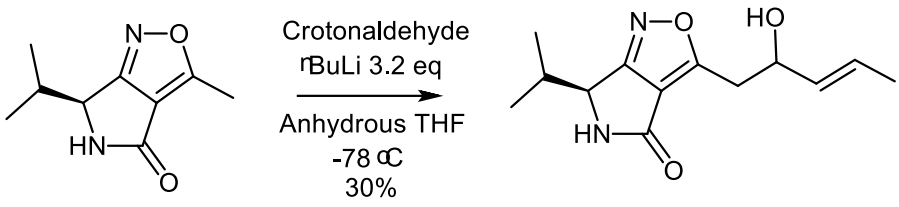
(S)-3-(2-Hydroxy-3-methylbutyl)-6-(1-methylethyl)-5,6-dihydro-4H-pyrrolo[3,4-c][1,2]oxazol-4-one (129)[75]

2-Methylpropanal was suspended in diethyl ether and washed with saturated potassium carbonate solution to remove any acidic impurities. The organic fraction was then dried ($MgSO_4$), filtered and concentrated under a reduced pressure to achieve the pure aldehyde, confirmed by NMR spectroscopy. *n*-Butyllithium (3.2 eq, 2.5 M solution in hexanes, 0.08 mL, 0.2 mmol) was added dropwise to a solution of the pyrroloisoxazole (S)-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) (100 mg, 0.55 mmol) in THF (5 mL) at - 78 ^{0}C and kept under a nitrogen atmosphere. The solution was stirred at this temperature for 2 h. Purified 2-methylpropanal (1 mL, excess) was then added in one portion and stirring continued for at - 78 ^{0}C for 2 h. The reaction was quenched by the addition of water (5 mL) and the mixture acidified with hydrochloric acid (5 mL, 2 M). The mixture was dried over $MgSO_4$, filtered and concentrated under a reduced pressure and the resulting yellow oil was suspended in water (5 mL) and ethyl acetate (5 mL). The aqueous phase was then extracted by using ethyl acetate (2 x 5 mL), before the combined organic phases were washed with brine (10 mL) and dried under vacuum to yield a yellow oil (845 mg, 100%). This was purified by flash chromatography eluting with ethyl acetate and light petroleum (5:1 v/v) to yield the desired product as a light yellow oil (30 mg, 32 %); *m/z* calculated for $C_{13}H_{20}N_2O_3$ (MNa^+): 275.1366. Found 275.1370; ^{1}H NMR δ_H (400 MHz; ($CDCl_3$)) 0.92 (3H, d, *J* 2.8 Hz, CH(CH_3)$_2$), 0.96 (3H, d, *J* 3.6 Hz, C(CH_3)$_2$) , 0.99 (3H, d, *J* 3.6 Hz, C(CH_3)$_2$), 1.02 (3H, d, J_a 2.8 Hz, CH(CH_3)$_2$), 2.01-2.11 (1H, m, C*H*(CH_3)$_2$), 2.95-3.01 (2H, m, CH_2CHOH), 3.82 (1H, s, CHO*H*), 4.43 (1H, dd, J_a 2.8 Hz, J_b 6.0 Hz, C*H*NH), 6.25 (1H, broad, CHN*H*), 7.20 (1H, s, C*H*OH).



(S)-3-(2-Cyclohexyl-2-hydroxyethyl)-6-(1-methylethyl)-5,6-dihydro-4H-pyrrolo[3,4-c][1,2]oxazol-4-one (130)[75]

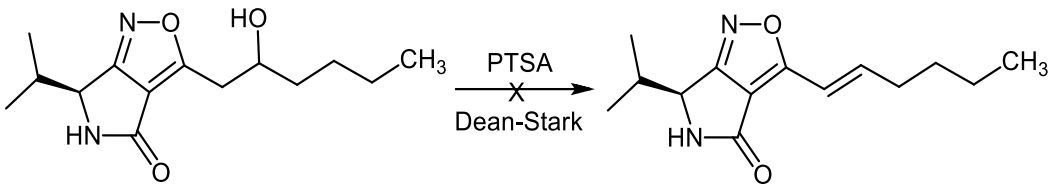
Cyclohexanecarboxaldehyde was suspended in diethyl ether and washed with saturated potassium carbonate solution to remove any acidic impurities. The organic fraction was then dried ($MgSO_4$), filtered and concentrated under a reduced pressure to achieve the pure aldehyde, confirmed by NMR spectroscopy. *n*-Butyllithium (3.2 eq, 2.5 M solution in hexanes, 0.08 mL, 0.2 mmol) was added dropwise to a solution of the pyrroloisoxazole (S)-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) (100 mg, 0.55 mmol) in THF (5 mL) at - 78 ^{0}C and kept under a nitrogen atmosphere. The solution was stirred at this temperature for 2 h. Purified cyclohexanecarboxaldehyde (1 mL, excess) was then added in one portion and stirring continued for at - 78 ^{0}C for 2 h. The reaction was quenched by the addition of water (5 mL) and the mixture acidified with hydrochloric acid (5 mL, 2 M). The mixture was dired over $MgSO_4$, filtered and concentrated under a reduced pressure and the resulting yellow oil as suspended in water (5 mL) and ethyl acetate (5 mL). The aqueous phase was then extracted by using ethyl acetate (2 x 5 mL), before the organic phases were washed with brine (10 mL) and dried under vacuum to yield a crude yellow oil (800 mg, 100%). This was purified by flash chromatography eluting with ethyl acetate and light petroleum (5:1 v/v) to yield the desired product as a light yellow oil (45 mg, 28%); *m/z* calculated for $C_{16}H_{24}N_2O_3$ (MH^+): 293.1860. Found 293.1856; ^{1}H NMR δ_H (400 MHz; ($CDCl_3$)) 1.04 (3H, d, J_a 3.6 Hz, CH(CH_3)$_2$), 1.09 (3H, d, J_a 4.0 Hz, CH(CH_3)$_2$), 1.29 (10H, s, C_5H_{10}), 2.03-2.09 (1H, m, C*H*(CH_3)$_2$), 3.04-3.08 (2H, m, CH_2CHOH), 3.82 (1H, s, CHO*H*), 4.90 (1H, dd, J_a 2.0 Hz, J_b 5.2 Hz, C*H*NH), 6.65 (1H, broad, CHN*H*), 7.19 (1H, s, C*H*OH).



(S)-3-[(2-Hydroxypent-3-en-1-yl]-6-(1-methylethyl)-5,6-dihydro-4H-pyrrolo[3,4-c][1,2]oxazol-4-one (131)[75]

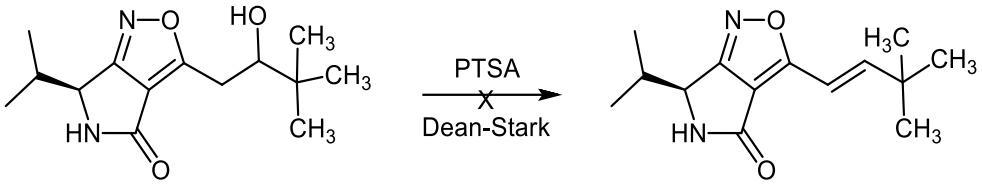
n-Butyllithium (10.0 eq, 0.975 M solution in hexanes, 1.20 mL, 1.10 mmol) was added dropwise to a solution of the pyrroloisoxazole (S)-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**130**) (20.0 mg, 0.110 mmol) in THF (10 mL) at - 78 ^{0}C and kept under a nitrogen atmosphere. The solution was stirred at this temperature for 2 h. But-2-enal (cis:trans mixture, 1 mL, excess) was then added in one portion and stirring continued for at - 78 ^{0}C for 2 h. The reaction was quenched by the addition of water (5 mL) and the mixture acidified with hydrochloric acid (5 mL, 2 M) and separated by adding ethyl acetate (30 mL). The aqueous phase was then extracted (2 x 30 mL), before the combined organic phases were washed with brine (30 mL) and dried over $MgSO_4$, filtered and concentrated under a reduced pressure to yield a crude yellow oil which was purified by prep-TLC eluting with ethyl acetate:petrol (1:1 v/v) to yield the desired product (S)-3-[(2-hydroxypent-3-en-1-yl]-6-(1-methylethyl)-5,6-dihydro-4H-pyrrolo[3,4-c][1,2]oxazol-4-one as a clear colourless oil (8.70 mg, 30 %); *m/z* calculated for $C_{13}H_{18}N_2O_3$ (MH^+): 251.1351. Found 251.1351; IR ν_{max} (nujol)/cm^{-1} 3262 (OH), 2896 (CH_3), 1709 (CO), 1659 (CN); ^{1}H NMR δ_H (400 MHz; ($CDCl_3$)) 1.12 (3H, d, *J* 4.2 Hz, CH(CH_3)$_2$), 1.20 (3H, d, *J* 4.2 Hz, CH(CH_3)$_2$), 1.72 (3H, d, *J* 7.2 Hz, CHCH_3), 2.11-2.19 (1H, m, C*H*(CH_3)$_2$), 3.21 (2H, d, *J* 7.2 Hz, CH_2CHOH), 4.51 (1H, dd, J_a 2.8 Hz, J_b 6.0 Hz, C*H*NH), 4.61 (1H, br. s, O*H*), 5.60-5.70 (1H, m, C*H*CH_3), 5.81-5.89 (1H, m, C(OH)C*H*), 6.12 (1H, broad, CHN*H*), ^{13}C NMR δ_c (100 MHz; ($CDCl_3$)) 15.6 (CH_2), 10.2 (CH_3), 21.0 ((CH_3)$_2$), 22.1 ((CH_3)$_2$), 29.7 (*C*H(CH_3)$_2$), 51.3 (CH_2), 57.1 (*C*HOH) 60.4 (*C*HNH), 113.4 (CCO), 125.6 (H*C*=CH), 130.1 (HC=*C*H), 163.2 (*C*N), 166.1 (*C*O), 171.1 (N*C*O).

4.5 – Attempted Dehydration of the Aldol Adducts



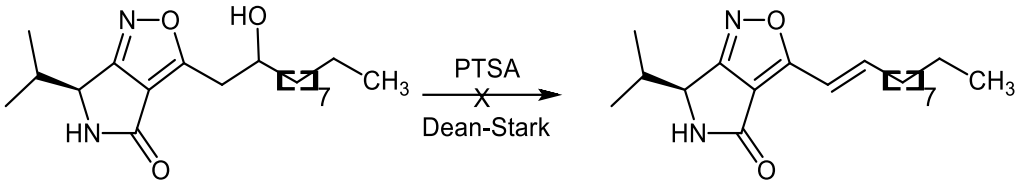
(S,E)-3-(Hex-1-en-1-yl)-6-isopropyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (132)[75]

p-Toluenesulfonic acid (26 mg, 0.138 mmol) was suspended in toluene (10 mL) and added dropwise to (S)-3-(2-Hydroxyhexyl)-6-(-1-methylethyl)-5,6-dihydro-4H-pyrrolo[3,4-c][1,2]oxazol-4-one (**127**) (22.8 mg, 0.120 mmol) in toluene (20 mL) and heated under Dean-Stark conditions for 17 h with stirring. The reaction mixture was then partitioned between ethyl acetate (30 mL) and saturated sodium carbonate solution (30 mL), followed by a washing with saturated brine (30 mL). The organic fraction was collected, dried over magnesium sulfate and concentrated under reduced pressure. Upon thin layer chromatographic analysis it was shown to contain multiple products but, after separation via flash column chromatography eluting with ethyl acetate and light petroleum (5:1 v/v), no dehydrated product could be identified.



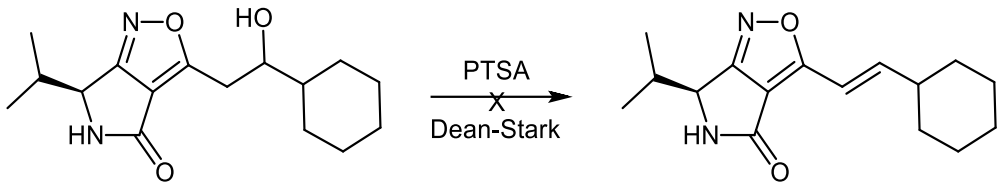
(S,E)-3-(3,3-Dimethylbut-1-en-1-yl)-6-isopropyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (134)[75]

p-Toluenesulfonic acid (20.8 mg, 0.110 mmol) was suspended in toluene (10 mL) and added dropwise to (S)-3-(2-hydroxy-3-methylbutyl)-6-(1-methylethyl)-5,6-dihydro-4H-pyrrolo[3,4-c][1,2]oxazol-4-one (**129**) (34.2mg, 0.096 mmol) in toluene (20 mL) and heated under Dean-Stark conditions for 17 h with stirring. The reaction mixture was then partitioned between ethyl acetate (30 mL) and saturated sodium carbonate solution (30 mL), followed by a washing with saturated brine (30 mL). The organic fraction was collected, dried over magnesium sulfate and concentrated under reduced pressure. Upon thin layer chromatographic analysis it was shown to contain multiple products but, after separation via flash column chromatography eluting with ethyl acetate and light petroleum (5:1 v/v), no dehydrated product could be identified.



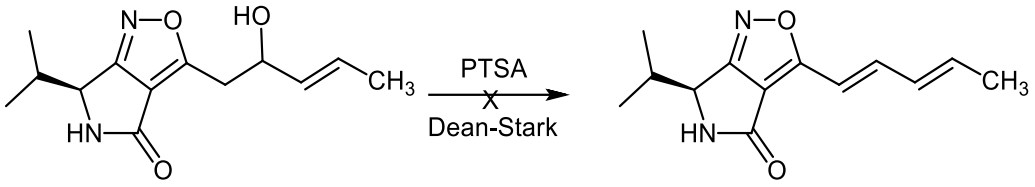
3-[Undecyl-1-en-1-yl]-6-(1-methylethyl)-5,6-dihydro-4H-pyrrolo[3,4-c][1,2]oxazol-4-one (133)[75]

p-Toluenesulfonic acid (18.2 mg, 0.097 mmol) was suspended in toluene (10 mL) and added dropwise to (S)-3-(2-hydroxyundecyl)-6-(1-methylethyl)-5,6-dihydro-4H-pyrrolo[3,4-c][1,2]oxazol-4-one (**128**) (30.0 mg, 0.084 mmol) in toluene (20 mL) and heated under Dean-Stark conditions for 17 h with stirring. The reaction mixture was then partitioned between ethyl acetate (30 mL) and saturated sodium carbonate solution (30 mL), followed by a washing with saturated brine (30 mL). The organic fraction was collected, dried over magnesium sulfate and concentrated under reduced pressure. Upon thin layer chromatographic analysis it was shown to contain multiple products but, after separation via flash column chromatography eluting with ethyl acetate and light petroleum (5:1 v/v), no dehydrated product could be identified.



3-[2-Cyclohexylethenyl]-6-(1-methylethyl)-5,6-dihydro-4H-pyrrolo[3,4-c][1,2]oxazol-4-one (135)[75]

p-Toluenesulfonic acid (15.6 mg, 0.083 mmol) was suspended in toluene (10 mL) and added dropwise to (S)-3-(2-Cyclohexyl-2-hydroxyethyl)-6-(1-methylethyl)-5,6-dihydro-4H-pyrrolo[3,4-c][1,2]oxazol-4-one (**130**) (25.7 mg, 0.072 mmol) in toluene (20 mL) and heated under Dean-Stark conditions for 17 h with stirring. The reaction mixture was then partitioned between ethyl acetate (30 mL) and saturated sodium carbonate solution (30 mL), followed by a washing with saturated brine (30 mL). The organic fraction was collected, dried over magnesium sulfate and concentrated under reduced pressure. Upon thin layer chromatographic analysis it was shown to contain multiple products but, after separation via flash column chromatography eluting with ethyl acetate and light petroleum (5:1 v/v), no dehydrated product could be identified.

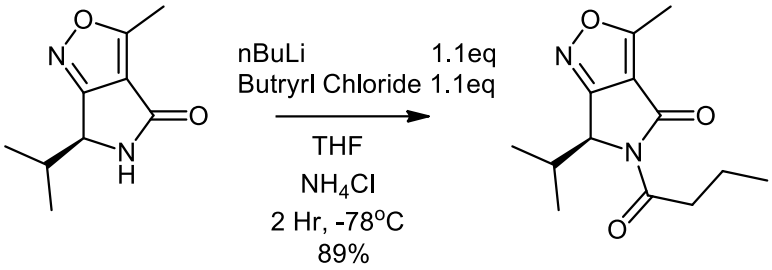


3-[Penta-1,3-dien-1-yl]-6-(1-methylethyl)-5,6-dihydro-4H-pyrrolo[3,4-c][1,2]oxazol-4-one (136)[75]

p-Toluenesulfonic acid (13 mg, 0.069 mmol) was suspended in toluene (10 mL) and added dropwise to (S)-3-[(2-Hydroxypent-3-en-1-yl]-6-(1-methylethyl)-5,6-dihydro-4H-pyrrolo[3,4-c][1,2]oxazol-4-one (**131**) (21.4 mg, 0.06 mmol) in toluene (20 mL) and heated under Dean-Stark conditions for 17 h with stirring. The reaction mixture was then partitioned between ethyl acetate (30 mL) and saturated sodium carbonate solution(30 mL), between by a washing with saturated brine (30 mL). The organic fraction was collected, dried over magnesium sulfate and concentrated under reduced pressure. Upon thin layer chromatographic analysis it was shown to contain multiple products but, after separation via flash column chromatography eluting with ethyl acetate and light petroleum (5:1 v/v), no dehydrated product could be identified.

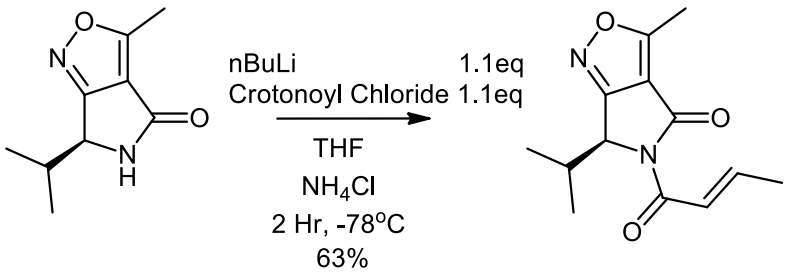
4.6 – *N*-Acylation and Alkylation on Pyrroloisoxazole Moiety

4.6.1 – *N*-Acylation Reactions



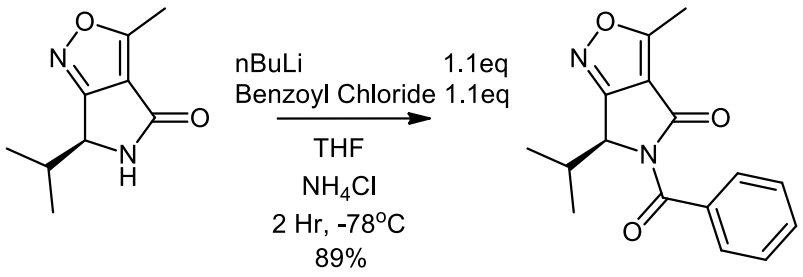
(S)-5-Butanoyl-5,6-dihydro-6-(1-methylethyl)-3-methyl-4H-pyrrolo[3,4-c]isoxazol-4-one (137a)

(S)-6-(1-Methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) (72.8 mg, 0.404 mmol) was suspended in dry THF (25 mL), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was then added n-butyl-lithium (1.1 eq., 2.0 M in hexanes, 0.22 mL, 0.444 mmol) in one portion and the mixture stirred for 15 minutes at this temperature, during which time the solution turned yellow. Butanoyl chloride (1.1 eq., 50.0 μL, 0.44 mmol) was then added in two equal portions over 10 minutes and the reaction mixture stirred at -78 ^{0}C for a further hour before being quenched by saturated ammonium chloride solution. The reaction solution was then tested for pH to ensure neutrality had been achieved, and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, dried over $MgSO_4$, filtered and concentrated under reduced pressure to produce the product as a yellow oil (90 mg, 89%); $[\alpha]_D^{rt}$ +36.0 (c 5.0x10^{-3}, $CHCl_3$); MS: *m/z* calculated for $C_{13}H_{18}N_2O_3$ (MNa^+): 273.1210. Found 273.1211; IR ν_{max} ($CHCl_3$)/cm^{-1} 3025 (CH_3), 1725 (C=O), 1689 (C=C), 1650 (NC=O), 1389 (CN), 1250 (C-C), 1131 (COC); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 0.50 (3H, d, *J* 6.8 Hz, CH(CH_3)$_2$) 0.91 (3H, t, *J* 7.2 Hz, CH_2CH_3), 1.18 (3H, d, *J* 6.8 Hz, CH(CH_3)$_2$), 1.55-1.61 (2H, m, CH_2CH_3), 2.62 (3H, s, 5-CH_3), 2.68-2.72 (1H, m, $CH(CH_3)_2$), 2.80 (1H, dt, J_a 7.6 Hz, J_b 14.8 Hz, C(O)CH_2), 2.90 (1H, dt, J_a 7.6 Hz, J_b 14.8 Hz, C(O)CH_2), 5.13 (1H, d, *J* 4 Hz, $CHN(CO)_2$); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 11.8 (5-CH_3), 12.8 (CH_2CH_3), 13.1 (CH_3), 17.0 (CH_2CH_3), 17.9 (CH_3), 27.0 ($CH(CH_3)_2$), 38.2 (C(O)CH_2), 59.9 ($CHN(CO)_2$), 113.0 ($CCCH_3$), 159.5 (CHCN), 165.0 ($CCCH_3$), 168.6 (NC(O)C), 173.2 (NC(O)CH_2).



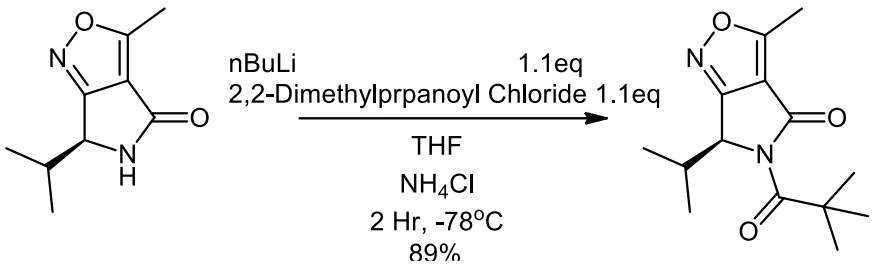
(S,E)-5-(But-2-enoyl)-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (139a)

(S)-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) (56.0 mg, 0.311 mmol) was suspended in dry THF (25 mL), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was then added n-butyllithium (1.1 eq., 2.0 M in hexanes, 0.171 mL, 0.342 mmol) in one portion and the mixture stirred for 15 minutes at this temperature, during which time the solution turned yellow. But-2-enoyl chloride (1.1 eq., 33.0 μL, 0.342 mmol) was then added in two equal portions over 10 minutes and the reaction mixture stirred at -78 ^{0}C for a further hour before being quenched by saturated ammonium chloride solution. The reaction solution was then tested for pH to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, washed with saturated potassium carbonate solution (50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to produce the product as a white crystalline solid (37.5 mg, 63%); mp. 109-111^{o}C; $[\alpha]_D^{rt}$ +65.3 (c 15.0 x10^{-3}, $CHCl_3$); MS: *m/z* calculated for $C_{13}H_{16}N_2O_3$ (MNa^+): 271.1053. Found 271.1056; IR ν_{max} ($CHCl_3$)/cm^{-1} 3054 (CH_3), 1741 (C=O), 1682 (C=C), 1642 (NC=O), 1337 (CN), 1292 (C-C), 1138 (COC); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 0.53 (3H, d, *J* 6.8 Hz, CH(C*H*$_3$)$_2$), 1.16 (3H, d, *J* 6.8 Hz, CH(C*H*$_3$)$_2$), 1.58 (3H, dd, J_a 1.6 Hz, J_b 4.2 Hz, CHC*H*$_3$), 2.62 (3H, s, 5-C*H*$_3$), 2.68-2.71 (1H, m, C*H*(CH$_3$)$_2$), 5.18 (1H, d, *J* 3.6 Hz, C*H*N(CO)$_2$), 7.10 (1H, dq, J_a 6.8 Hz, J_b 11.6 Hz, C*H*CH$_3$), 7.22 (1H, d, J_a 1.6 Hz, C(O)C*H*); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 12.4 (5-*C*H$_3$), 14.6 (*C*H$_3$), 18.5 (*C*H$_3$), 18.6 (CH*C*H$_3$), 28.1 (*C*H(CH$_3$)$_2$), 61.0 (*C*HN(CO)$_2$), 114.0 (*CC*CH$_3$), 122.1 (*C*HCH$_3$), 146.5 (C(O)*C*H), 160.7 (CH*C*N), 166.0 (C*C*CH$_3$), 167.3 (N*C*(O)C), 169.6 (N*C*(O)CH$_2$). X-ray data for this compound are available at the end of this report.



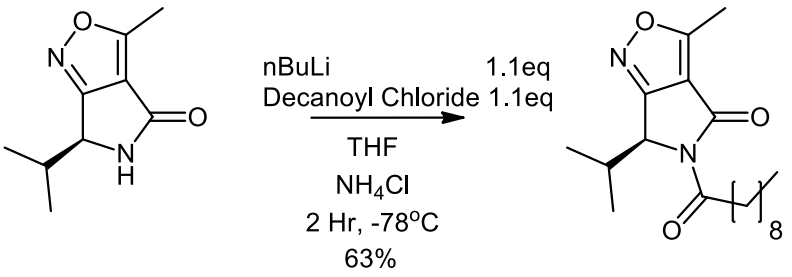
(S)-5-Benzoyl-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (138a)

(S)-6-(1-Methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) (59.0 mg, 0.327 mmol) was suspended in dry THF (25 mL) and cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was then added n-butyllithium (1.1 eq., 2.0 M in hexanes, 0.180 mL, 0.360 mmol) in one portion and the mixture stirred for 15 minutes at this temperature, during which time the solution turned yellow. Benzoyl chloride (1.1 eq., 42.0 μL, 0.360 mmol) was then added in two equal portions over 10 minutes and the reaction mixture stirred at -78 ^{0}C for a further hour before being quenched by saturated ammonium chloride solution. The reaction solution was then tested for pH to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, washed with saturated potassium carbonate solution (50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to produce the product as a yellow oil (82.0 mg, 89%); $[\alpha]_D^{rt}$ +52.0 (c 5.0 x10^{-3}, $CHCl_3$); MS: *m/z* calculated for $C_{16}H_{11}N_2O_3$ (MH^+): 279.0764. Found 279.0742; IR ν_{max} ($CHCl_3$)/cm^{-1} 3608 (Ar-H), 3054 (CH_3), 1796 (C=O), 1688 (C=C), 1421 (NC=O), 1389 (CN), 1265 (C-C), 1155 (COC); ^{1}H NMR δ_H (400 MHz; $CDCl_3$), 0.85 (3H, d, *J* 6.8, CH(C*H*$_3$)$_2$), 1.28 (3H, d, *J* 6.8, CH(C*H*$_3$)$_2$), 1.55-1.58 (1H, m, C*H*(CH$_3$)$_2$), 2.65 (3H, s, 5-C*H*$_3$), 5.54 (1H, d, *J* 4 Hz, C*H*N(CO)$_2$), 7.20-7.52 (2H, m, Ar-H), 7.54-7.59 (1H, m, Ar-H), 7.65-7.71 (2H, m, Ar-H); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 12.3 (5-*C*H$_3$), 15.3 (*C*H$_3$), 18.7 (*C*H$_3$), 28.4 (*C*H(CH$_3$)$_2$), 60.9 (*C*HN(CO)$_2$), 113.6 (*C*CCH$_3$), 128.0 (Ar-*C*), 129.0 (2Ar-*C*), 132.3 (2Ar-*C*), 134.6 (Ar-*C*), 159.7 (CH*C*N), 167.5 (C*C*CH$_3$), 169.9 (N*C*(O)C), 170.4 (N*C*(O)CH$_2$).



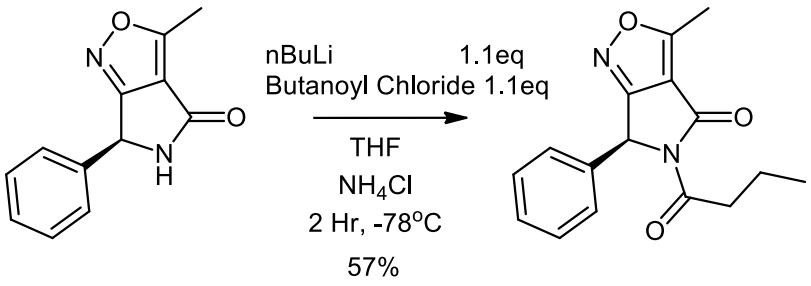
(S)-(1-Methylethyl)-3-methyl-5-(2,2-dimethylpropanoyl)-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (141a)

(S)-6-(1-Methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) (57.0 mg, 0.316 mmol) was suspended in dry THF (25 mL), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was then added n-butyllithium (1.1 eq., 2.0 M in hexanes, 0.174 mL, 0.348 mmol) in one portion and the mixture stirred for 15 minutes at this temperature, during which time the solution turned yellow. 2,2-Dimethylpropanoyl chloride (1.1 eq., 42.8 μL, 0.348 mmol) was then added in two equal portions over 10 minutes and the reaction mixture stirred at -78 ^{0}C for a further hour before being quenched by saturated ammonium chloride solution. The reaction solution was then tested for pH using indicator paper to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, washed with saturated potassium carbonate solution (50 mL), dried over $MgSO_4$, filtered and concentrated under a reduced pressure to produce the product as a yellow oil (74.1 mg, 89%); $[\alpha]_D^{rt}$ +42.1 (c 4.75 x10^{-3}, $CHCl_3$); MS: *m/z* calculated for $C_{14}H_{20}N_2O_3$ (MNa^+): 287.1366. Found 287.1368; IR ν_{max} ($CHCl_3$)/cm^{-1} 3029 (CH_3), 1717 (C=O), 1656 (C=C), 1621 (NC=O), 1398 (CN), 1261 (C-C), 1139 (COC); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 0.61 (3H, d, *J* 6.8, CH(C*H*$_3$)$_2$), 1.20 (3H, d, *J* 6.8, CH(C*H*$_3$)$_2$), 1.40 (9H, s, C(C*H*$_3$)$_3$), 2.45-2.51 (1H, m, C*H*(CH$_3$)$_2$), 2.66 (3H, s, 5-C*H*$_3$), 5.13 (1H, d, *J* 4 Hz, C*H*N(CO)$_2$); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 12.3 (5-*C*H$_3$), 14.7 (*C*H$_3$), 18.5 (*C*H$_3$), 26.3 (C(*C*H$_3$)$_3$), 29.4 (*C*H(CH$_3$)$_2$), 42.2 (*C*(CH$_3$)$_3$), 62.0 (*C*HN(CO)$_2$), 114.1 (*C*CCH$_3$), 159.3 (CH*C*N), 167.3 (C*C*CH$_3$), 169.4 (N*C*(O)C), 180.5 (N*C*(O)CH$_2$).



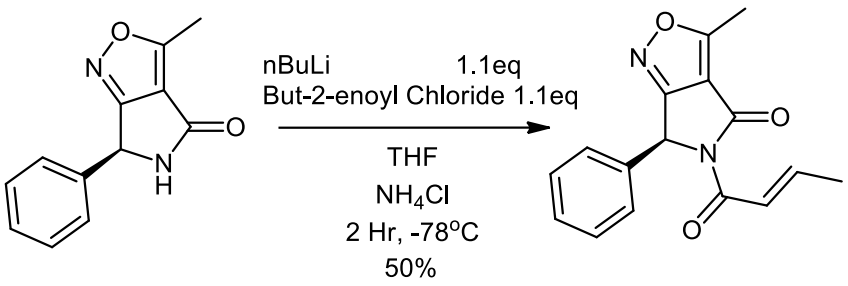
(S)-5-Decanoyl-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (140a)

(S)-6-(1-Methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) (50.0 mg, 0.277 mmol) was suspended in dry THF (25 mL), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was then added n-butyllithium (1.1 eq., 1.65 M in hexanes, 0.185 mL, 0.305 mmol) in one portion and the mixture stirred for 15 minutes at this temperature, during which time the solution turned yellow. Decanoyl chloride (1.1 eq., 63.0 μL, 0.305 mmol) was then added in two equal portions over 10 minutes and the reaction mixture stirred at -78 ^{0}C for a further hour before being quenched by saturated ammonium chloride solution. The reaction solution was then tested for pH to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, washed with saturated potassium carbonate solution (50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to produce the product as a yellow oil (57.4 mg, 63%); $[\alpha]_D^{rt}$ -40.0 (c 5.0 x10^{-3}, $CHCl_3$); MS: *m/z* calculated for $C_{19}H_{30}N_2O_3$ (MNa^+): 357.2149. Found 357.2149; IR ν_{max} ($CHCl_3$)/cm^{-1} 2965 (CH_3), 1749 (C=O), 1675 (C=C), 1599 (NC=O), 1398 (CN), 1265 (C-C), 1131 (COC); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 0.57 (3H, d, *J* 6.8, $CH(CH_3)_2$) 0.87 (3H, t, *J* 7.2, CH_2CH_3), 1.23 (3H, d, *J* 6.8, $CH(CH_3)_2$), 1.27-1.45 (12H , br. m, $6CH_2$), 1.64-1.72 (1H, m, $C(O)CH_2CH_2$), 2.68 (3H, s, $5\text{-}CH_3)_2$), 2.71-2.77 (1H, m, $CH(CH_3)_2$), 2.91 (1H, dt, J_a 7.6 Hz, J_b 14.8 Hz, $C(O)CH_2$), 3.01 (1H, dt, J_a 7.6 Hz, J_b 14.8 Hz, $C(O)CH_2$), 5.15 (1H, d, *J* 3.6 Hz, $CHN(CO)_2$); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 12.3 ($5\text{-}CH_3$), 14.1 (CH_2CH_3), 14.6 ($CH(CH_3)_2$), 18.5 ($CH(CH_3)_2$), 22.7 (CH_2), 24.6 (CH_2), 28.1 ($CH(CH_3)_2$), 29.2 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 31.9 ($C(O)CH_2CH_2$), 37.4 ($C(O)CH_2$), 60.9 ($CHN(CO)_2$), 114.0 ($CCCH_3$), 160.5 ($CHCN$), 167.3 ($CCCH_3$), 169.6 ($NC(O)C$), 174.5 ($NC(O)CH_2$).



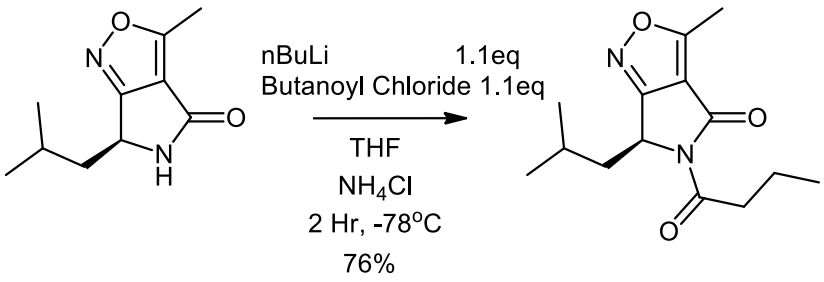
(S)-5-Butyryl-3-methyl-6-phenyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (137c)

(S)-3-Methyl-6-phenyl-5,6-dihydropyrrolo[3,4-c]isoxazol-4-one (**125c**) (54.0 mg, 0.252 mmol) was suspended in dry THF (25 mL), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was then added n-butyllithium (1.1 eq., 2.0 M in THF, 0.139 mL, 0.278 mmol) in one portion and the reaction was then stirred for 15 minutes at this temperature, during which time the solution turned yellow. Butanoyl chloride (1.1 eq., 30 mg, 30 μL, 0.278 mmol) was then added in two equal portions over 10 minutes and the reaction mixture stirred at -78 ^{0}C for a further hour before being quenched by saturated ammonium chloride solution. The reaction solution was then tested for pH to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, dried over $MgSO_4$, filtered and concentrated under reduced pressure to produce the product as a white crystalline solid (31.0 mg, 57%); mp 120-121^{o}C; $[\alpha]_D^{rt}$ +4.0 (c 12.5 x10^{-3}, $CHCl_3$); MS: *m/z* calculated for $C_{16}H_{16}N_2O_3$ (MNa^+): 307.1053. Found 307.1054; IR ν_{max} ($CHCl_3$)/cm^{-1} 3054 (CH_3), 1745 (C=O), 1701 (C=C), 1644 (NC=O), 1381 (CN), 1227 (C-C), 1111 (COC); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 0.85 (3H, t, *J* 7.2 Hz, CH_2CH_3), 1.53-1.61 (2H, q, *J* 7.6 Hz, CH_2CH_3), 2.62 (3H, s, CH_3), 2.85-2.90 (2H, m, $CH_2CH_2CH_3$), 6.17 (1H, s, CHNH), 7.17-7.23 (5H, m, Ar(*H*)); ^{13}C NMR δ_C (100 MHz; ($CDCl_3$)) 11.5 (CH_2CH_3), 12.6 ($2CH_3$), 16.6 (CH_2CH_3), 38.1 ($CH_2CH_2CH_3$), 57.2 (*C*HNH), 111.5 (*C*CO), 125.3, 127.7, 128.0 (Ar(*C*)), 134.9 (*C*-Ar), 158.9 (*C*N), 168.2 (C*C*O), 169.9 (N*C*OC), 172.2 (N*C*OCH_2).



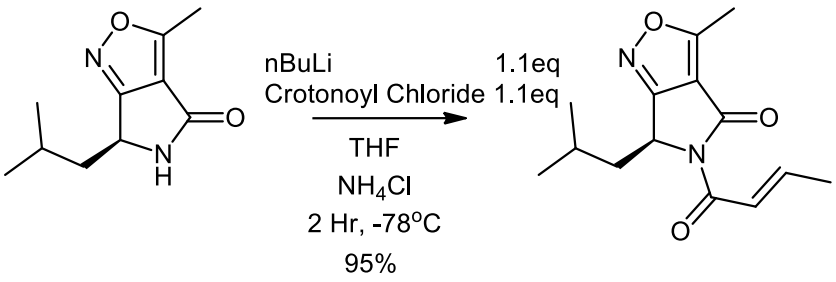
(S,E)-5-(But-2-enoyl)-3-methyl-6-phenyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (139c)

(S)-3-Methyl-6-phenyl-5,6-dihydropyrrolo[3,4-c]isoxazol-4-one (**125c**) (33.0 mg, 0.154 mmol) was suspended in dry THF (25 mL), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was then added n-butyllithium (1.1 eq., 1.408 M in THF, 0.120 mL, 0.170 mmol) in one portion and the mixture stirred for 15 minutes at this temperature, during which time the solution turned yellow. But-2-enoyl chloride (1.1 eq., 16.0 μL, 0.170 mmol) was then added in two portions over 10 minutes and the reaction mixture stirred at -78 ^{0}C for a further hour before being quenched by saturated ammonium chloride solution. The reaction solution was then tested for pH to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, dried over $MgSO_4$, filtered and concentrated under reduced pressure to produce the product as a white crystalline solid (22.0 mg, 50%); mp 122-125^{o}C; $[\alpha]_D^{rt}$ -4.6 (c 11.0 x10^{-3}, $CHCl_3$); MS: *m/z* calculated for $C_{16}H_{14}N_2O_3$ (MNa^+): 305.0900. Found 305.0900; IR ν_{max} ($CHCl_3$)/cm^{-1} 3054, 2986 (CH_3), 1744 (C=O), 1718 (C=C), 1641 (NC=O), 1421 (CN), 1217 (C-C); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 1.98 (3H, dd, J_a 1.6 Hz, J_b 6.8 Hz, CHC*H*$_3$), 2.73 (3H, s, C*H*$_3$), 6.53 (1H, s, C*H*NH), 7.14 (1H, qd, J_a 6.8 Hz, J_b 13.6, (C(O)C*H*), 7.27 (1H, dq, J_a 6.8 Hz, J_b 7.2 Hz, C*H*CH$_3$), 7.30-7.38 (5H, m, Ar(*H*)); ^{13}C NMR δ_C (100 MHz; ($CDCl_3$)) 11.2 (5-*C*H$_3$), 13.2 (CHC*H*$_3$), 57.4 (*C*HN(CO)$_2$), 111.6 (*C*CCH$_3$), 122.4 (*C*HCH$_3$), 125.4 (Ar-*C*), 127.8 (2Ar-*C*), 134.7 (2Ar-*C*), 146.2 (C(O)*C*H), 159.2 (CH*C*N), 165.8 (C*C*CH$_3$), 169.2 (N*C*(O)C), 171.2 (N*C*(O)CH$_2$).



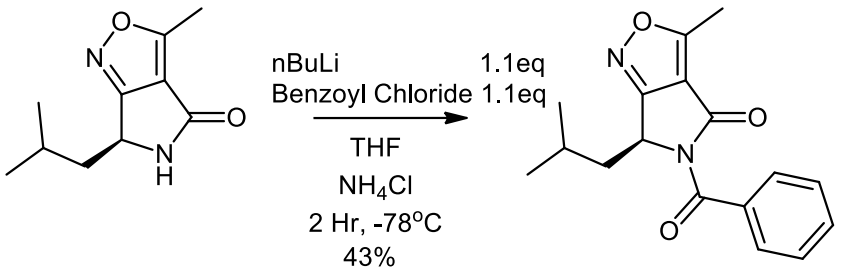
(S)-5-Butanoyl-6-(3-methylbutyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (137b)

(S)-6-(1-Methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125b**) (33.0 mg, 0.170 mmol) was suspended in dry THF (25 mL), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was added n-butyllithium (1.1 eq., 1.408 M in THF, 0.133 mL, 0.187 mmol) in one portion and the mixture stirred for 15 minutes at this temperature, during which time the solution turned yellow. Butanoyl chloride (1.1 eq., 19.3 μL, 0.187 mmol) was then added in two equal portions over 10 minutes and the reaction mixture stirred at -78 ^{0}C for a further hour before being quenched by saturated ammonium chloride solution. The reaction solution was then tested for to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, washed with saturated potassium carbonate solution (50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to produce the product as a yellow oil (33.8 mg, 76%); $[\alpha]_D^{rt}$ +86.2 (c 14.15 x10^{-3}, $CHCl_3$); MS: *m/z* calculated for $C_{14}H_{20}N_2O_3$ (MNa^+): 287.1366. Found 287.1367; IR ν_{max} ($CHCl_3$)/cm^{-1} 3054 (CH_3), 1738 (C=O), 1696 (C=C), 1644 (NC=O), 1382 (CN), 1215 (C-C), 1143 (COC); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 0.84 (3H, d, *J* 6.8, CH(CH_3)$_2$), 0.95 (3H, t, *J* 7.2, CH_2CH_3), 1.01 (3H, d, *J* 6.8, CH(CH_3)$_2$), 1.40 (1H, t, *J* 9.6 Hz, $CHCH_2CH$), 1.60-1.69 (1H, m, CH_2CH_3), 1.92-1.95 (1H, m, $CH(CH_3)_2$), 2.01 (1H, t, *J* 9.6 Hz, $CHCH_2CH$), 2.61 (3H, s, 5-CH_3)$_2$), 2.82-2.92 (2H, m, C(O)CH_2), 5.19 (1H, d, *J* 3.6 Hz, $CHN(CO)_2$); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 11.3 (5-CH_3), 12.7 (CH_2CH_3), 16.7 (CH_3), 22.7 (CH_2CH_3), 23.5 (CH_3), 26.9 ($CH(CH_3)_2$), 38.2 ($CHCH_2CH$), 39.6 (C(O)CH_2), 53.7 ($CHN(CO)_2$), 111.9 ($CCCH_3$), 159.0 (CHCN), 168.4 ($CCCH_3$), 170.0 (NC(O)C), 173.1 (NC(O)CH_2).



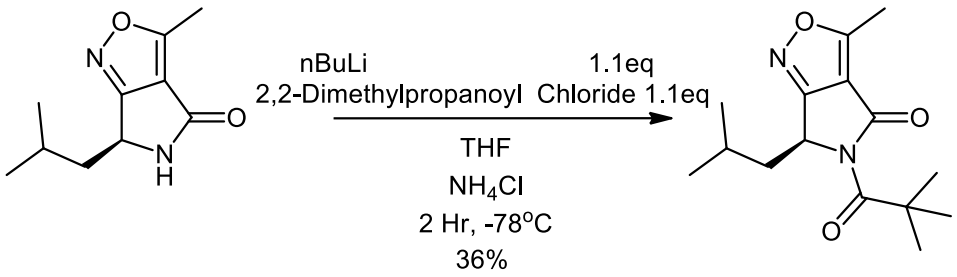
(S,E)-5-(But-2-enoyl)-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (139b)

(S)-6-(1-Methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125b**) (50.0 mg, 0.275 mmol) was suspended in dry THF (25 mL), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was added n-butyllithium (1.1 eq., 1.408 M in THF, 0.201 mL, 0.283 mmol) in one portion and the mixture stirred for 15 minutes at this temperature, during which time the solution turned yellow. But-2-enoyl chloride (1.1 eq., 27.0 μL, 0.283 mmol) was then added in two equal portions over 10 minutes and the reaction mixture stirred at -78 ^{0}C for a further hour before being quenched by saturated ammonium chloride solution. The reaction solution was then tested fro pH to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, washed with saturated potassium carbonate solution (50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to produce the product as a yellow oil (65.0 mg, 95%); $[\alpha]_D^{rt}$ +67.1 (c 32.5 x10^{-3} $CHCl_3$); MS: *m/z* calculated for $C_{14}H_{18}N_2O_3$ (MNa^+): 285.1210. Found 285.1212; IR ν_{max} ($CHCl_3$)/cm^{-1} 3054, 2986 (CH_3), 1739 (C=O), 1679 (C=C), 1642 (NC=O), 1335 (CN), 1216 (C-C), 1141 (COC); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 0.93 (3H, d, *J* 6.8, CH(C*H*$_3$)$_2$), 1.08 (3H, d, *J* 6.8, CH(C*H*$_3$)$_2$), 1.50 (1H, td, J_a 3.6 Hz, J_b 10.4 Hz, CHC*H*$_2$CH), 1.98 (3H, dd, J_a 3.6 Hz, J_b 7.2 Hz, CHC*H*$_3$), 2.01-2.04 (1H, m, C*H*(CH$_3$)$_2$), 2.15 (1H, td, J_a 3.6 Hz, J_b 10.4 Hz, CHC*H*$_2$CH), 2.67 (3H, s, 5-C*H*$_3$)$_2$), 5.32 (1H, d, *J* 3.6 Hz, C*H*N(CO)$_2$), 7.16 (1H, qd, J_a 6.8 Hz, J_b 13.6 Hz, (C(O)C*H*), 7.22 (1H, dq, J_a 6.8 Hz, J_b 7.2 Hz, C*H*CH$_3$); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 12.4 (5-*C*H$_3$), 18.1 (CHC*H*$_3$), 18.6 (*C*H$_3$), 21.2 (*C*H$_3$), 23.6 (*C*H(CH$_3$)$_2$), 40.6 (CH*C*H$_2$CH), 54.2 (*C*HN(CO)$_2$), 113.0 (*C*CCH$_3$), 123.8 (*C*HCH$_3$), 146.5 (C(O)*C*H), 160.2 (CH*C*N), 166.0 (C*C*CH$_3$), 169.8 (N*C*(O)C), 171.2 (N*C*(O)CH$_2$).



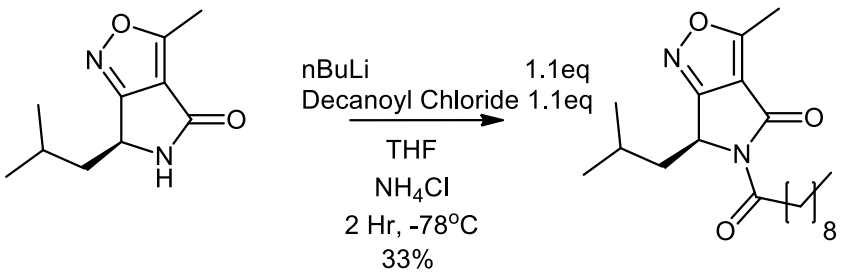
(S)-5-Benzoyl-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (138b)

(S)-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125b**) (43.0 mg, 0.237 mmol) was suspended in dry THF (25 mL), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was added n-butyllithium (1.1 eq., 1.408 M in THF, 0.185 mL, 0.261 mmol) in one portion and the mixture stirred for 15 minutes at this temperature, during which time the solution turned yellow. Benzoyl chloride (1.1 eq., 30.3 μL, 0.261 mmol) was then added in two portions over 10 minutes and the reaction mixture stirred at -78 ^{0}C for a further hour before being quenched by saturated ammonium chloride solution. The reaction solution was then tested for pH to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, washed with saturated potassium carbonate solution (50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to produce the product as a pale orange oil (30.0 mg, 43%); $[\alpha]_D^{rt}$ +158.7 (c 15.0 x10^{-3}, $CHCl_3$); MS: *m/z* calculated for $C_{17}H_{18}N_2O_3$ (MNa^+): 321.1236. Found 321.1237; IR ν_{max} ($CHCl_3$)/cm^{-1} 3054, 2986 (CH_3), 1750 (C=O), 1678 (C=C), 1643 (NC=O), 1421 (CN), 1265 (C-C), 1156 (COC); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 1.03 (3H, d, *J* 6.8, CH(C*H*$_3$)$_2$), 1.13 (3H, d, *J* 6.8, CH(C*H*$_3$)$_2$), 1.75 (1H, t, *J* 9.2 Hz, CH(C*H*$_3$)$_2$), 2.05-2.13 (2H, m, CHC*H*$_2$CH), 2.65 (3H, s, 5-C*H*$_3$)$_2$), 5.55 (1H, d, *J* 3.6 Hz, C*H*N(CO)$_2$), 7.44-7.52 (2H, m, Ar-H), 7.53-7.60 (3H, m, Ar-H); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 12.3 (5-*C*H$_3$), 21.4 (*C*H$_3$), 23.7 (*C*H$_3$), 24.7 (*C*H(CH$_3$)$_2$), 40.5 (CH*C*H$_2$CH), 52.8 (*C*HN(CO)$_2$), 112.7 (*C*CCH$_3$), 128.0 (2Ar-*C*H), 128.5 (2Ar-*C*H), 132.3 (Ar-*C*H), 134.5 (Ar-*C*), 159.2 (CH*C*N), 169.4 (C*C*CH$_3$), 169.9 (N*C*(O)C), 170.3 (N*C*(O)CH$_2$).



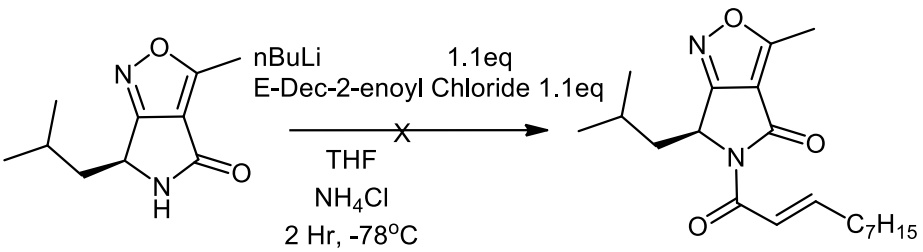
(S)-6-(1-Methylethyl)-3-methyl-5-(2,2-dimethylpropanoyl)-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (141b)

(S)-6-(1-Methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125b**) (33.0 mg, 0.170 mmol) was suspended in dry THF (25 mL), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was added n-butyllithium (1.1 eq., 1.408 M in THF, 0.133 mL, 0.187mmol) in one portion and the mixture stirred for 15 minutes at this temperature, during which time the solution turned yellow. 2,2-dimethylpropanoyl chloride (1.1 eq., 23.0 μL, 0.187 mmol) was then added in two equal portions over 10 minutes and the reaction mixture stirred at -78 ^{0}C for a further hour before being quenched by saturated ammonium chloride solution. The reaction solution was then tested for pH to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, washed with saturated potassium carbonate solution (50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to produce the product as a yellow oil (20.0 mg, 36%); $[\alpha]_D^{rt}$ +47.5 (c 8.0 x10^{-3}, $CHCl_3$); MS: *m/z* calculated for $C_{15}H_{22}N_2O_3$ (MNa^+): 301.1526. Found 301.1524; IR ν_{max} ($CHCl_3$)/cm^{-1} 3054, 2986 (CH_3), 1794 (C=O), 1741 (C=C), 1643 (NC=O), 1421 (CN), 1265 (C-C), 1167 (COC); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 0.98 (3H, d, *J* 6.8, CH(C*H*$_3$)$_2$), 1.07 (3H, d, *J* 6.8, CH(C*H*$_3$)$_2$), 1.37 (9H, s, C(C*H*$_3$)$_3$), 1.69 (2H, t, *J* 9.6 Hz, CHC*H*$_2$CH), 1.91-1.94 (1H, m, C*H*(CH$_3$)$_2$), 2.62 (3H, s, 5-C*H*$_3$)$_2$), 5.38 (1H, d, *J* 3.6 Hz, C*H*N(CO)$_2$); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 12.0 (5-*C*H$_3$), 21.1 (*C*H$_3$), 22.5 (*C*H$_3$), 25.3 (*C*H(CH$_3$)$_2$), 26.2 ((C*H*$_3$)$_3$), 40.7 (CH*C*H$_2$CH), 51.2 (*C*HN(CO)$_2$), 113.0 (*C*CCH$_3$), 158.9 (CH*C*N), 169.4 (C*C*CH$_3$), 169.5 (N*C*(O)C), 180.2 (N*C*(O)CH$_2$).



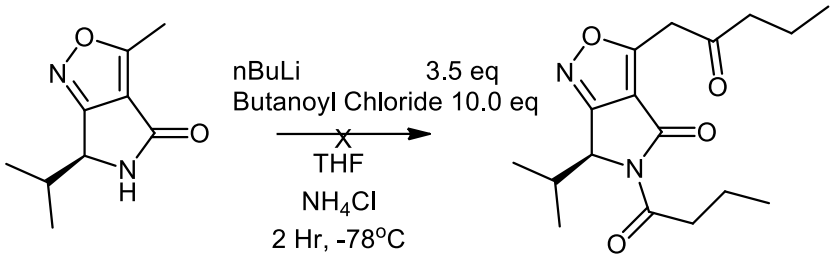
(S)-5-Decanoyl-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (140b)

(S)-6-(1-Methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125b**) (33.0 mg, 0.170 mmol) was suspended in dry THF (25 mL), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was added n-butyllithium (1.1 eq., 1.408 M in THF, 0.133 mL, 0.187 mmol) in one portion and the reaction was then stirred for 15 minutes at this temperature, during which time the solution turned yellow. Decanoyl chloride (1.1 eq., 38.8 μL, 0.187 mmol) was then added in two equal portions over 10 minutes and the reaction mixture stirred at -78 ^{0}C for a further hour before being quenched by saturated ammonium chloride solution. The reaction solution was then tested for pH to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, washed with saturated potassium carbonate solution (50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to produce the product as a pale yellow oil (16.0 mg, 33%); $[\alpha]_D^{rt}$ +94.0 (c 10.0 x10^{-3}, $CHCl_3$); MS: *m/z* calculated for $C_{20}H_{32}N_2O_3$ (MNa^+): 371.2305. Found 371.2305; IR ν_{max} ($CHCl_3$)/cm^{-1} 3054, 2987 (CH_3), 1739 (C=O), 1643 (C=C), 1605 (NC=O), 1421 (CN), 1265 (C-C), 1156 (COC); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 0.88 (3H, t, J 6.4 Hz, CH_2CH_3), 0.94 (3H, d, *J* 6.8, $CH(CH_3)_2$), 1.10 (3H, d, *J* 6.8, $CH(CH_3)_2$), 1.50 (1H, td, J_a 2.0 Hz, J_b 3.6 Hz, $CHCH_2CH$), 1.61-1.71 (1H, m, $C(O)CH_2CH_2$), 1.98-2.04 (1H, m, $CH(CH_3)_2$), 2.12 (1H, td, J_a 2.0 Hz, J_b 3.6 Hz, $CHCH_2CH$), 2.68 (3H, s, 5-$CH_3)_2$), 2.94-2.98 (2H, m, $C(O)CH_2$), 5.26 (1H, d, *J* 3.6 Hz, $CHN(CO)_2$); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 12.3 (5-CH_3), 14.1 (CH_3), 21.2 (CH_2CH_3), 22.7 (CH_2CH_3), 23.7 (CH_3), 24.3 (CH_2), 24.6 ($CH(CH_3)_2$), 29.2 (CH_2), 29.3 (CH_2), 29.3 (CH_2), 29.7 (CH_2), 35.2 ($C(O)CH_2CH_2$), 39.8 ($C(O)CH_2$), 61.5 ($CHN(CO)_2$), 115.1 ($CCCH_3$), 161.0 (CHCN), 167.4 ($CCCH_3$), 169.8 (NC(O)C), 175.1 ($NC(O)CH_2$).



(S,E)-5-(dec-2-enoyl)-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (19)

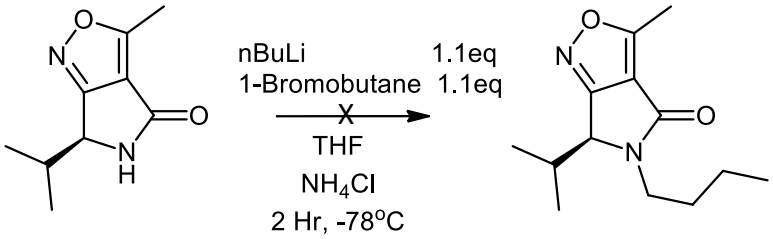
(S)-6-(1-Methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125b**) (8.0 mg, 0.040 mmol) was suspended in dry THF (10 mL), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was added n-butyllithium (1.5 eq., 1.65 M in THF, 38 μL, 0.062 mmol) in one portion and the mixture stirred for 15 minutes at this temperature, during which time the solution turned yellow. (*E*)-Dec-2-enoyl chloride (1.5 eq., 11 mg, 12 μL, 0.062 mmol) was then added in two equal portions over 10 minutes and the reaction mixture stirred at -78 ^{0}C for a further hour before being quenched by saturated ammonium chloride solution. The reaction solution was then tested for pH to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, washed with saturated potassium carbonate solution (50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to produce none of the desired product. It is thought that some thionyl chloride remained in the reaction mixture from a previous step, which could have reacted with the anion, eventually returning the pyrroloisoxazole after work-up.



(S)-5-Butanoyl-6-(1-methylethyl)-3-(2-oxopentyl)-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (168)

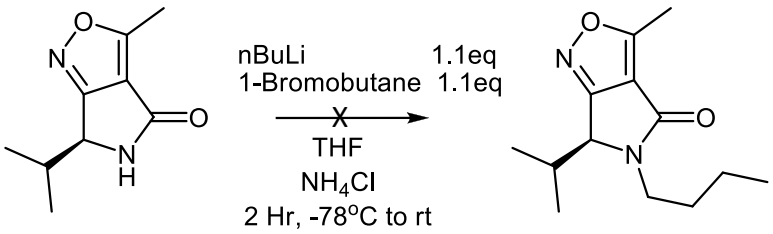
(S)-6-(1-Methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) (20.0 mg, 0.110mmol) was suspended in dry THF (25 mL), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was then added n-butyllithium (3.5 eq., 0.960 M in hexanes, 0.401 mL, 0.385 mmol) in one portion and the mixture stirred for 15 minutes at this temperature, during which time the solution turned yellow. Butanoyl chloride (10.0 eq., 113.0 μL, 1.10 mmol) was then added in two equal portions over 10 minutes and the reaction mixture stirred at -78 ^{0}C for a further hour before being quenched by saturated ammonium chloride solution. The reaction solution was then tested for pH to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, dried over $MgSO_4$, filtered and concentrated under reduced pressure to produce a crude mixture, in which the desired product could not be identified.

4.6.2 – *N*-Alkylation Attempts



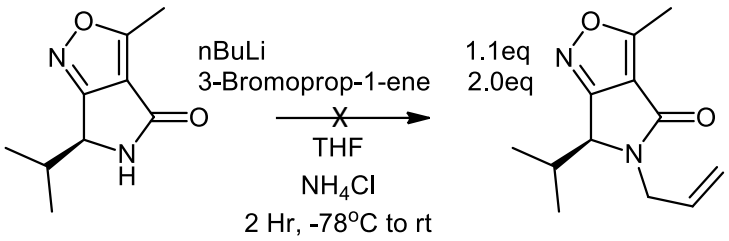
(S)-5-Butyl-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (145)

(S)-6-(1-Methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) (46.8 mg, 0.280 mmol) was suspended in dry THF (25 mL), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was added n-butyllithium (1.1 eq., 2.0 M in THF, 0.143 mL, 0.286 mmol) in one portion and the mixture stirred for 15 minutes at this temperature, during which time the solution turned yellow. 1-Bromobutane (1.1 eq., 40.0 mg, 0.037 mL, 0.286 mmol) was then added in two equal portions over 10 minutes and the reaction mixture stirred at -78 ^{0}C for a further hour before being quenched by saturated ammonium chloride solution. The reaction solution was then tested for pH to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, dried over $MgSO_4$, filtered and concentrated under a reduced pressure to produce a white solid identified as returned pyrroloisoxazole starting material.



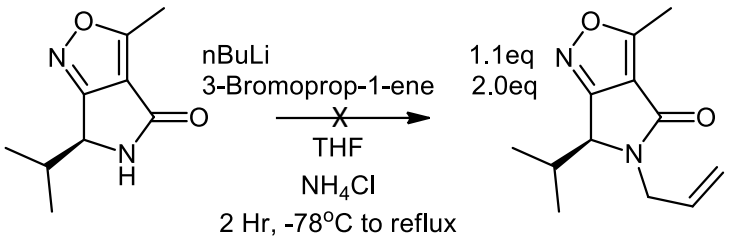
(S)-5-Butyl-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (145)

(S)-6-(1-Methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) (46.8 mg, 0.280 mmol) was suspended in dry THF (25 mL), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was added n-butyllithium (1.1 eq., 2.0 M in THF, 0.143 mL, 0.286 mmol) in one portion and the mixture stirred for 15 minutes at this temperature, during which time the solution turned yellow. 1-Bromobutane (1.1 eq., 40.0 mg, 0.037 mL, 0.286 mmol) was then added in two equal portions over 10 minutes and the reaction mixture stirred and allowed to warm to room temperature over 2 h before being quenched by saturated ammonium chloride solution. The reaction solution was tested for pH to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, dried over $MgSO_4$, filtered and concentrated under a reduced pressure to produce a white solid identified as returned pyrroloisoxazole starting material.



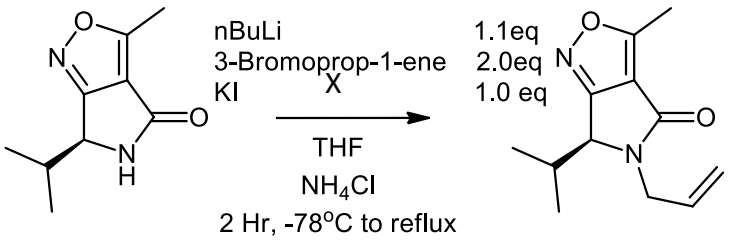
(S)-5-Prop-2-enyl-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (146)

(S)-6-(1-Methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) (57.5 mg, 0.319 mmol) was suspended in dry THF (25 mL), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was added n-butyllithium (1.1 eq., 2.0 M in THF, 0.175 mL, 0.351 mmol) in one portion and the mixture stirred for 15 minutes at this temperature, during which time the solution turned yellow. 3-Bromoprop1-ene (2 eq., 77.2 mg, 0.055 mL, 0.638 mmol) was then added in two equal portions over 10 minutes and the reaction mixture stirred and allowed to warm to room temperature over 2 h before being quenched by saturated ammonium chloride solution. The reaction solution was tested for pH to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, dried over $MgSO_4$, filtered and concentrated under a reduced pressure to produce a white solid identified as returned pyrroloisoxazole starting material.



(S)-5-Prop-2-enyl-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (146)

(S)-6-(1-Methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) (43.5 mg, 0.241 mmol) was suspended in dry THF (25 mL), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was added n-butyllithium (1.1 eq., 2.0 M in THF, 0.133 mL, 0.266 mmol) in one portion and mixture stirred for 15 minutes at this temperature, during which time the solution turned yellow. 3-Bromoprop-1-ene (excess, 140 mg, 0.10 mL, 1.165 mmol) was then added in two equal portions over 10 minutes and the reaction mixture stirred at reflux over 1 h before being quenched by saturated ammonium chloride solution. The reaction solution was then tested for pH to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, dried over $MgSO_4$, filtered and concentrated under a reduced pressure to produce a white solid identified as returned pyrroloisoxazole starting material.

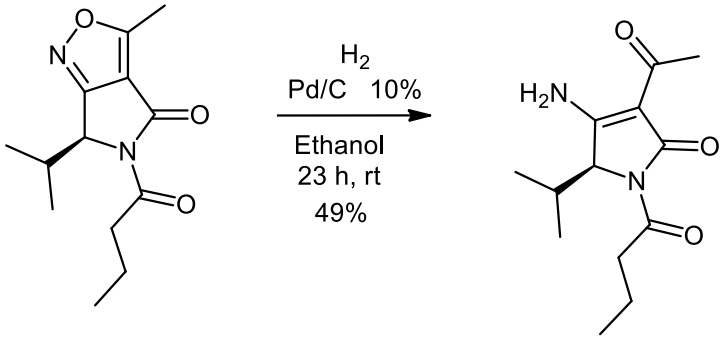


(S)-5-Prop-2-enyl-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (146)

(S)-6-(1-Methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) (43.5 mg, 0.241 mmol) was suspended in dry THF (25 mL) with potassium iodide (40.0 mg, 0.241 mmol), cooled to -78 ^{0}C and stirred under a nitrogen atmosphere. To this solution was added n-butyl-lithium (1.1 eq., 2.0 M in THF, 0.133 mL, 0.266 mmol) in one portion and the mixture stirred for 15 minutes at this temperature, during which time the solution turned yellow. 3-Bromoprop-1-ene (excess, 140 mg, 0.10 mL, 1.165 mmol) was then added in two equal portions over 10 minutes and the reaction mixture stirred at reflux over 1 h before being quenched by saturated ammonium chloride solution. The reaction solution was then tested for pH to ensure neutrality had been achieved and separated between water (20 mL) and ethyl acetate (25 mL). The organic layer was retained, dried over $MgSO_4$, filtered and concentrated under a reduced pressure to produce a white solid identified as returned pyrroloisoxazole starting material.

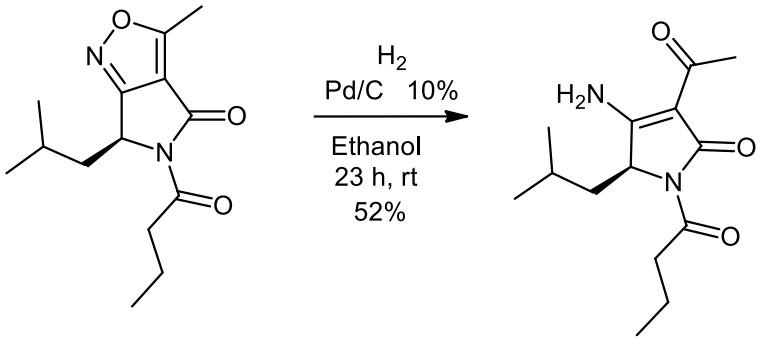
4.7 – Isoxazole Cleavage in Pyrroloisoxazoles and Attempted Hydrolysis

4.7.1 – Isoxazole Cleavage by Hydrogenation



(S)-3-Acetyl-4-amino-1-butanoyl-5-(1-methylethyl)-1H-pyrrol-2(5H)-one (147a)

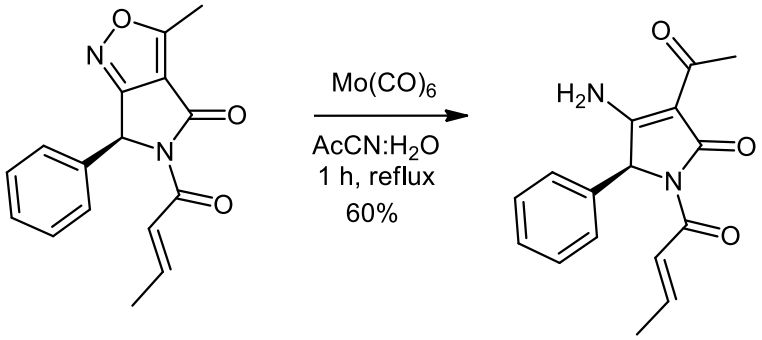
To a flask flushed with nitrogen was added palladium on carbon (10% by mass, 12.0 mg) followed dropwise by ethanol (20 mL). This produced a slurry, to which was added (S)-5-butanoyl-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**) (120 mg, 0.506 mmol) in ethanol (20 mL) with vigorous stirring. The reaction vessel was then purged and subsequently evacuated 3 times with nitrogen and then a further 3 times with hydrogen gas, eventually being left under a hydrogen atmosphere for 25 hours with vigorous stirring. The reaction vessel was then evacuated and purged 3 times with nitrogen and the slurry filtered through a Celite® pad to remove palladium on carbon. The filtrate was concentrated under reduced pressure to yield product as a yellow oil (58.0 mg, 49%); $[\alpha]_D^{rt}$ +26.1 (c 8.0 x10^{-3}, $CHCl_3$); MS: *m/z* calculated for $C_{13}H_{20}N_2O_3$ (MNa^+): 275.2987. Found 275.2986; IR ν_{max} ($CHCl_3$)/cm^{-1} 3567 (NH), 2981 (CH_3), 1769 (C=O), 1659 (C=C), 1568 (NC=O), 1378 (CN), 1219 (C-C), 1142 (COC);); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 0.80 (3H, d, *J* 6.8, $CH(CH_3)_2$), 0.91 (3H, t, *J* 7.2, CH_2CH_3), 1.14 (3H, d, *J* 6.8, $CH(CH_3)_2$), 1.61-1.69 (2H, m, CH_2CH_3), 2.50 (3H, s, 5-CH_3), 2.53-2.65 (1H, m, $CH(CH_3)_2$), 2.85 (1H, dt, J_a 7.6 Hz, J_b 14.8 Hz, $C(O)CH_2$), 2.98 (1H, dt, J_a 7.6 Hz, J_b 14.8 Hz, $C(O)CH_2$), 4.62 (1H, d, *J* 7.2 Hz, $CHN(CO)_2$), 5.80 (1H, br. s, HN*H*), 9.41 (1H, br. s, *H*NH); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 12.8 (5-CH_3), 13.2 (CH_2CH_3), 14.4 (CH_3), 17.1 (CH_2CH_3), 17.6 (CH_3), 27.5 ($CH(CH_3)_2$), 38.0 ($C(O)CH_2$), 59.4 ($CHN(CO)_2$), 101.4 ($CCCH_3$), 166.9 (CHCN), 170.9 ($CCCH_3$), 172.3 (NC(O)C), 196.2 ($NC(O)CH_2$).



(S)-3-Acetyl-4-amino-1-butanoyl-5-(1-methylethyl)-1H-pyrrol-2(5H)-one (147b)

To a flask flushed with nitrogen was added palladium on carbon (10% by mass, 3.00 mg) followed dropwise by ethanol (20 mL). This produced a slurry, to which was added (S)-5-butanoyl-6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125b**) (28 mg, 0.106 mmol) in ethanol (20 mL) with vigorous stirring. The reaction vessel was then purged and subsequently evacuated 3 times with nitrogen and then with a further 3 times with hydrogen gas, eventually being left under a hydrogen atmosphere for 25 hours with vigorous stirring. The reaction vessel was then evacuated and purged 3 times with nitrogen and the slurry filtered through a Celite® pad to remove palladium on carbon. The filtrate was concentrated under reduced pressure to yield product as a yellow oil (15.0 mg, 52%); $[\alpha]_D^{rt}$ +31.5 (c 12.0 x10^{-3}, $CHCl_3$); MS: *m/z* calculated for $C_{14}H_{22}N_2O_3$ (MNa^+): 289.1523. Found 289.1523; IR ν_{max} ($CHCl_3$)/cm^{-1} 3550 (NH), 2985 (CH_3), 1740 (C=O), 1650 (C=C), 1521 (NC=O), 1374 (CN), 1246 (C-C), 1168 (COC); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 0.91 (3H, d, *J* 6.4 Hz, CH(C*H*$_3$)$_2$), 0.94 (3H, d, *J* 2.4 Hz, CH(C*H*$_3$)$_2$), 1.01 (3H, t, *J* 7.2, CH_2C*H*$_3$), 1.25 (1H, t, *J* 9.6 Hz, CHC*H*$_2$CH), 1.65-1.74 (2H, m, C*H*$_2$CH$_3$), 2.01-2.06 (1H, m, C*H*(CH$_3$)$_2$), 2.07 (1H, t, *J* 9.6 Hz, CHC*H*$_2$CH), 2.52 (3H, s, 5-C*H*$_3$)$_2$), 2.92-3.02 (2H, m, C(O)C*H*$_2$), 4.75 (1H, d, *J* 7.2 Hz, C*H*N(CO)$_2$), 6.01 (1H, br. s, HN*H*), 9.30 (1H, br. s, *H*NH); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 12.2 (CH_2*C*H$_3$), 17.4 (*C*H$_2$CH$_3$), 22.4 (*C*H$_3$), 22.6 (CH$_3$), 25.4 (*C*H(CH$_3$)$_2$), 28.7 (5-*C*H$_3$), 37.9 (C(O)*C*H$_2$), 39.0 (CH*C*H$_2$CH), 55.1 (*C*HN(CO)$_2$), 100.6 (*C*CCH$_3$), 166.8 (CH*C*N), 172.3 (C*C*CH$_3$), 172.4 (N*C*(O)C), 196.16 (N*C*(O)CH$_2$).

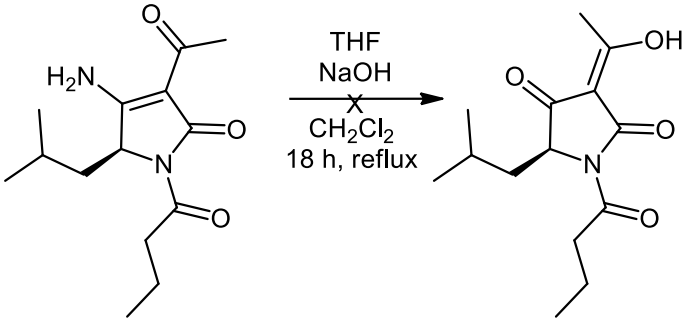
4.7.2 – Isoxazole Cleavage by Molybdenum Hexacarbonyl



(S,E)-3-Acetyl-4-amino-1-(but-2-enoyl)-5-phenyl-1H-pyrrol-2(5H)-one (148)

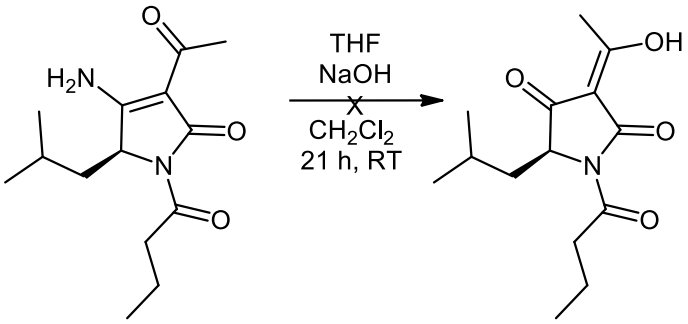
(S,E)-5-(But-2-enoyl)-3-methyl-6-phenyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125c**) (10.0 mg, 35.5 μmol) was added to molybdenum hexacarbonyl (1.0 eq., 9.40 mg, 35.5 μmol) in a acetonitrile:water (4:1 v/v, 5.00 mL) and stirred under reflux for 1 hour. To this refluxing mixture was then added 2.0 M aqueous hydrochloric acid (2.00 mL) and the reaction solution stirred under reflux for a further hour. The solution was then filtered through Celite® and the filtrate extracted with DCM before being washed with water and brine. The organic fraction was dried over magnesium sulfate and concentrated under reduced pressure to yield the crude enaminoketone, which was able to be purified by prep-TLC eluting with ethyl acetate:light petrol (3:1 v/v). The product was isolated as a clear and colourless oil (6.0 mg. 60%); $[\alpha]_D^{rt}$ +8.9 (c 9.0 x10^{-3}, $CHCl_3$); MS: *m/z* calculated for $C_{16}H_{16}N_2O_3$ (MNa^+): 307.1053. Found 307.1052; IR ν_{max} ($CHCl_3$)/cm^{-1} 3250 (NH), 3059, 2916 (CH_3), 1779 (C=O), 1710 (C=C), 1649 (NC=O), 1455 (CN), 1097 (C-C); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 1.25 (3H, dd, J_a 1.6 Hz, J_b 6.8 Hz, CHC*H*$_3$), 2.59 (3H, s, C*H*$_3$), 5.56 (1H, s, C*H*N), 5.62 (1H, br. s, HN*H*), 7.05 (1H, qd, J_a 6.8 Hz, J_b 13.6 Hz, (C(O)C*H*), 7.35 (1H, dq, J_a 3.6 Hz, J_b 7.2 Hz, C*H*CH_3), 7.30-7.48 (5H, m, Ar(*H*)), 9.01 (1H, br. s, *H*NH); ^{13}C NMR δ_C (100 MHz; ($CDCl_3$)) 17.8 (5-*C*H_3), 24.4 (CHC*H*$_3$), 60.2 (*C*HN(CO)$_2$), 123.1 (*C*CCH_3), 126.0 (*C*HCH_3), 128.3 (Ar-*C*), 128.5 (2Ar-*C*), 144.3 (2Ar-*C*), 154.0 (C(O)*C*H), 160.1 (CH*C*N), 163.8 (*C*CCH_3), 167.2 (N*C*(O)C), 170.4 (N*C*(O)CH_2).

4.7.3 – Attempted Hydrolysis of Enaminoketone



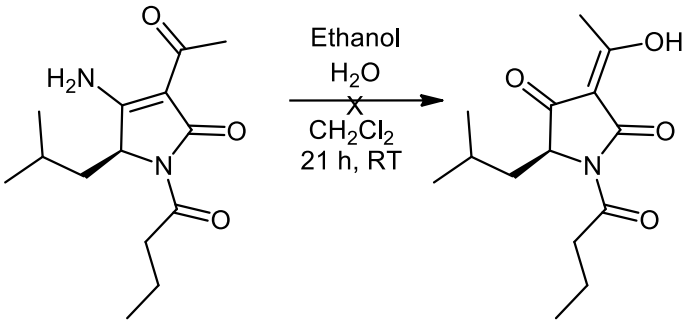
(S,Z)-1-Butanoyl-3-(1-hydroxyethylidene)-5-(1-methylethyl)pyrrolidine-2,4-dione (149)

To the crude enaminone (90.0 mg, 0.360 mmol) was added tetrahydrofuran (20 mL) and sodium hydroxide solution (2M, 60 mL) and the reaction mixture heated to reflux and stirred for 18 h. After allowing the reaction mixture to return to room temperature, the solution was acidified using hydrochloric acid solution (1M) and the aqueous layer was extracted with dichloromethane (3 x 30 mL). The combined organic layers were dried over magnesium sulfate, filtered and the solvent removed under reduced pressure to yield the crude material as a yellow/orange oil. Further purification by column chromatography eluting with light petroleum and ethyl acetate (1:3 v/v) afforded none of the desire 3-acyltetramic acid. Hydrolysis of the imide bond was inferred by identification of butanoic acid from the column.



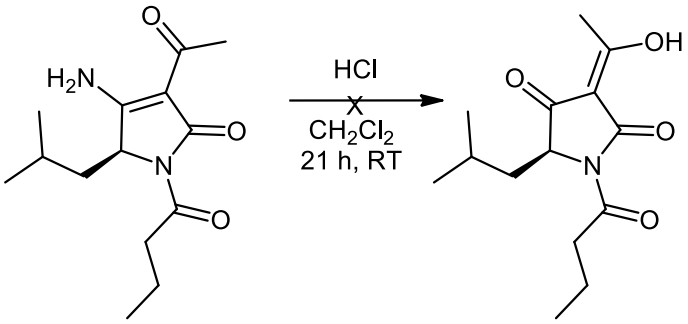
(S,Z)-1-Butanoyl-3-(1-hydroxyethylidene)-5-(1-methylethyl)pyrrolidine-2,4-dione (149)

To the pure enaminone (10.0 mg, 0.039 mmol) was added tetrahydrofuran (20 mL) and sodium hydroxide solution (1.1 eq., 0.1M, 0.44 mL, 0.044 mmol) and the reaction mixture was left at room temperature and stirred for 18 h. The solution was acidified using hydrochloric acid solution (1M) and the aqueous layer was extracted with dichloromethane (3 x 30 mL). The combined organic layers were dried over magnesium sulfate, filtered and the solvent removed under reduced pressure to yield the crude material as a yellow/orange oil. Further purification was required by column chromatography eluting with light petroleum and ethyl acetate (1:3 v/v) to produce none of the desire 3-acyltetramic acid. Hydrolysis of the imide bond was inferred by identification of butanoic acid from the column.



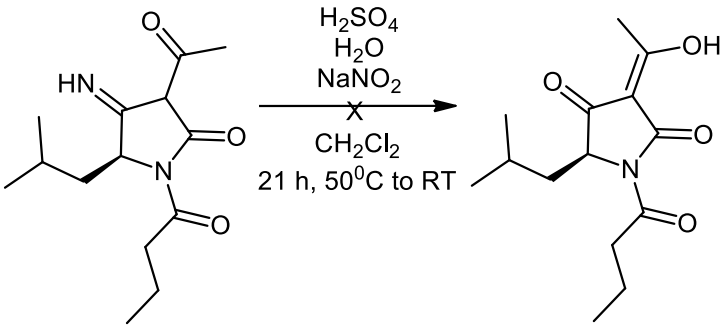
(S,Z)-1-Butanoyl-3-(1-hydroxyethylidene)-5-(1-methylethyl)pyrrolidine-2,4-dione (149)

To the pure enaminone (1050 mg, 0.059 mmol) was added ethanol (5 mL) followed by H_2O (5 mL) and the reaction mixture was left at room temperature and stirred for 18 h. The solution was acidified using hydrochloric acid solution (1M) and the aqueous layer was extracted with dichloromethane (3 x 30 mL). The combined organic layers were dried over magnesium sulfate, filtered and the solvent removed under a reduced pressure to yield the crude material as a white powder which was identified as *N*-acyl enaminoketone starting material. Further purification by column chromatography eluting with light petroleum and ethyl acetate (1:3 v/v) revealed none of the desired 3-acyltetramic acid, and no hydrolysis.



(S,Z)-1-Butanoyl-3-(1-hydroxyethylidene)-5-(1-methylethyl)pyrrolidine-2,4-dione (149)

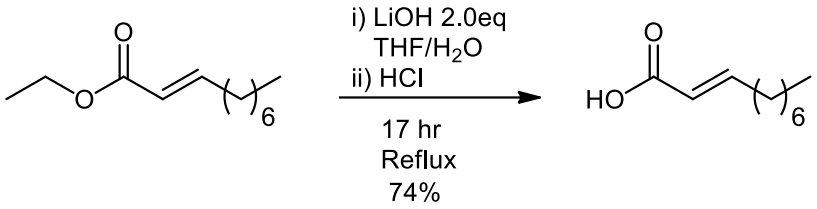
To the pure enaminone (30.0 mg, 0.113 mmol) was added ethanol (5 mL) and hydrochloric acid (2 M, 5 mL) and the reaction mixture was left at room temperature and stirred for 18 h. The aqueous layer was then extracted with dichloromethane (3 x 30 mL). The combined organic layers were dried over magnesium sulfate, filtered and the solvent removed under a reduced pressure to yield the crude material as a white powder which was identified as *N*-acyl enaminoketone starting material. Further purification by column chromatography eluting with light petroleum and ethyl acetate (1:3 v/v) revealed none of the desire 3-acyltetramic acid and no hydrolysis.



(S,Z)-1-Butanoyl-3-(1-hydroxyethylidene)-5-(1-methylethyl)pyrrolidine-2,4-dione (149)

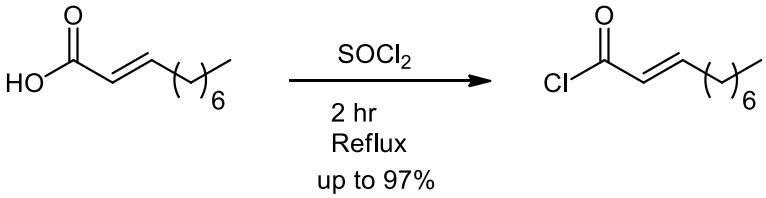
The pure enaminone (30.0 mg, 0.113 mmol) was suspended in sulfuric acid (3.0 M, 5 mL) and H_2O (5 mL) to which was added sodium nitrite (1.5 eq., 20.0 mg, 0.170 mmol) and the reaction mixture was left at room temperature and stirred for 1 h. The reaction mixture was then heated to 50^0C for 40 minutes and, after allowing the reaction mixture to return to room temperature, the aqueous layer was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were dried over magnesium sulfate, filtered and the solvent removed under reduced pressure to yield the crude material as a white powder which was identified as *N*-acyl enaminoketone starting material. Further purification by column chromatography eluting with light petroleum and ethyl acetate (1:3 v/v) revealed none of the desired 3-acyltetramic acid and no hydrolysis.

4.8 – Production of the Acyl Chloride Required to Synthesise Reutericyclin Analogues



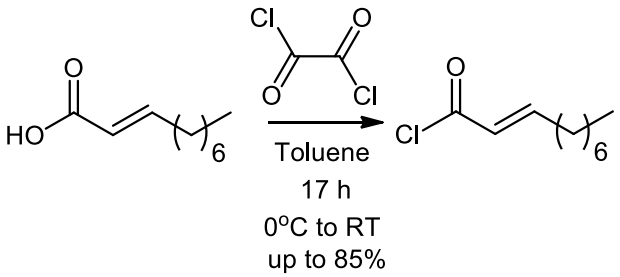
E-Dec-2-enoic acid (144)

To ethyl *E*-dec-2-enoate (0.88g, 1 mL, 4.44 mmol) was added lithium hydroxide (2.0 eq., 0.22g, 8.89 mmol) in water and THF (10 mL, 1:1 v/v) and the mixture was heated to reflux for 17 h. After cooling, the solution was washed with diethyl ether (50 mL) and the aqueous layer was acidified to pH 2-3 using concentrated hydrochloric acid. The resultant white precipitate was collected by filtration and dissolved in chloroform (30 mL) which was then dried over magnesium sulfate, filtered and evaporated under a reduced pressure to yield the title compound as an oil (0.554 g, 74%); MS: *m/z* calculated for $C_{10}H_{18}O_2$ (MH^+): 171.1380. Found 171.1379; IR ν_{max} ($CHCl_3$)/cm^{-1} 3404 (OH), 2935 (CH_2, CH_3), 1685 (CO), 1659 (CC), 1423 (CN); ^1H NMR δ_H (400 MHz; $CDCl_3$) 0.89 (3H, t, *J* 6.8 Hz, C*H*$_3$), 1.13-1.53 (8H, m, 4xC*H*$_2$), 1.45-1.57 (2H, m, C*H*$_2$), 2.21 (2H, dt, J_a 7.2 Hz, J_b 14.4 Hz, CHC*H*$_2$), 6.07 (1H, d, *J* 15.2 Hz, CH=C*H*COOH), 7.20-7.28 (1H, m, CH$_2$C*H*=CHCOOH), 11.50 (1H, br. s, O*H*); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 14.0 (*C*H$_3$), 22.8 (*C*H$_2$), 24.7 (*C*H$_2$), 27.4 (*C*H$_2$), 29.0 (*C*H$_2$), 30.8 (*C*H$_2$), 31.7 (*C*H$_2$), 32.5 (*C*H$_2$), 149.6 (*C*H), 153.4 (*C*H). This was a literature compound and the data agreed with that seen earlier.



E-Dec-2-anoyl chloride (142)

E-Dec-2-enoic acid (0.225 g, 1.33 mmol) was suspended in thionyl chloride (10 eq., 1.57 g, 0.96 mL, 13.3 mmol and stirred for 2 h at reflux. The excess thionyl chloride was removed under reduced pressure to yield the chlorinated product (241 mg, up to 97%, note it was not isolated); MS: *m/z* calculated for $C_{10}H_{18}O_2$ (MH^+): 171.1380. Found 171.1379; IR ν_{max} ($CHCl_3$)/cm^{-1} 2930 (CH_2, CH_3), 1695 (CO), 1650 (CC), 1420 (CN); ^1H NMR δ_H (400 MHz; $CDCl_3$) 0.88 (3H, t, *J* 6.8 Hz, C*H*$_3$), 1.13-1.51 (8H, m, 4xC*H*$_2$), 1.45-1.59 (2H, m, C*H*$_2$), 2.29 (2H, dt, J_a 7.2 Hz, J_b 14.4 Hz, CHC*H*$_2$), 6.07 (1H, d, *J* 15.2 Hz, CH=C*H*COOH), 7.20-7.29 (1H, m, CH_2C*H*=CHCOOH); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 14.0 (*C*H_3), 22.8 (*C*H_2), 24.7 (*C*H_2), 27.4 (*C*H_2), 29.0 (*C*H_2), 30.8 (*C*H_2), 31.7 (*C*H_2), 32.5 (*C*H_2), 152.6 (*C*H), 157.4 (*C*H).

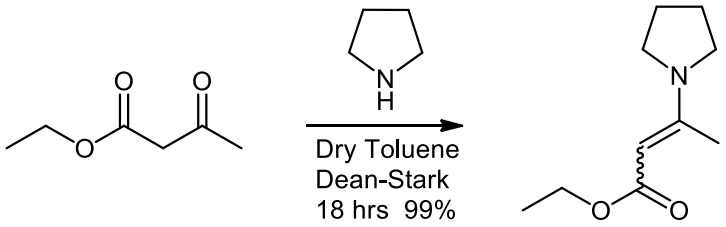


E-Dec-2-enoyl chloride (142)

E-Dec-2-enoic acid (298 mg, 1.76 mmol) was suspended in toluene (20 mL) and cooled to 0°C before adding oxalyl chloride (2.5 eq., 0.378 mL, 560 mg, 4.41 mmol) and stirred for 17 h to room temperature. The excess oxalyl chloride was removed under reduced pressure to yield the chlorinated product (305 mg, up to 85%, note it was not fully characterised); the analysis was identical to that seen above.

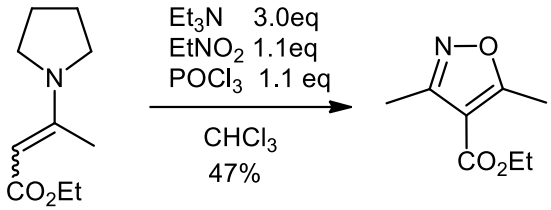
4.9 – Methods using a Model Isoxazole

4.9.1 – Model Isoxazole Synthesis



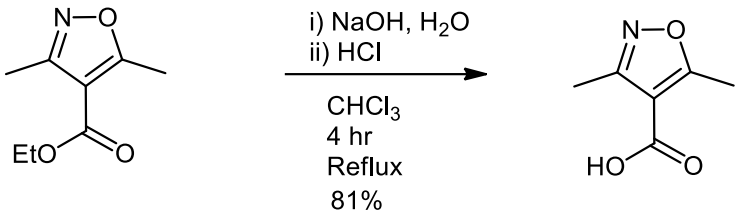
Ethyl 3-(pyrrolidine-1-yl)but-2-enoate (152)[94]

Ethyl 3-oxobutanoate (26.00g, 25.5 mL, 0.20 mol) and pyrrolidine (14.2g, 16.4 mL, 0.20 mol) were heated to reflux in try toluene (50 mL) under Dean-Stark conditions for 18 hrs, producing the theoretical amount of water (3.30 mL, 0.20 mol). The solvent was then removed under a reduced pressure to yield ethyl 3-(pyrrolidine-1-yl)but-2-enoate as a dark brown mobile oil (34.6g, 99%) MS: *m/z* calculated for $C_{10}H_{17}NO_2$ (MH^+): 184.1301. Found 184.1499; IR ν_{max} ($CHCl_3$)/cm^{-1} 2970 (CH), 2796 (CH_2, CH_3), 1652 (CO), 1569 (CC), 1196 (CN); ^1H NMR δ_H (400 MHz; $CDCl_3$) 1.13-1.19 (3H, t, *J* 7.0 Hz, C*H*$_3$CH$_2$), 1.78-1.87 (4H, m, RNCH$_2$(C*H*$_2$)$_2$CH$_2$), 2.35 (3H, s, C*H*$_3$C(N)=CH), 3.06-3.29 (4H, br.m, RNC*H*$_2$(CH$_2$)$_2$C*H*$_2$), 4.35 (1H, s, C*H*CCH$_3$); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 16.5 (*C*H$_3$), 25.2 (*C*H$_3$), 47.8 (*C*H$_2$), 86.0 (*C*HCCH$_3$), 160.5 (*C*N), 171.9 (*C*OO). This was a literature compound and the data agreed with that seen earlier.



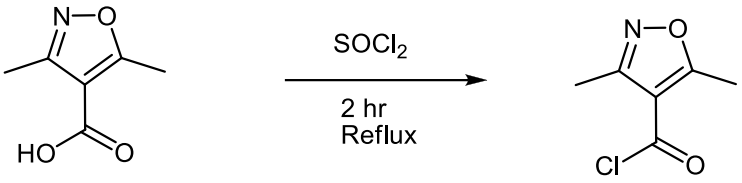
Ethyl 3,5-dimethylisoxazole-4-carboxylate (150)[94]

Triethylamine (3 eq., 80 mL, 0.60 mol) was added directly to ethyl 3-(pyrrolidine-1-yl)but-2-enoate (**152**) (34.6g, 0.20 mol) followed by nitroethane (1.1 eq., 15.80 mL, 0.22 mol) in chloroform (100 mL) and the solution was cooled to 0 ^{0}C. Phosphorus (V) oxychloride (1.1 eq., 34.0g, 0.22 mol) in chloroform (40 mL) was added dropwise over 1.5 h, the mixture stirred and allowed to warm to room temperature and left for 18 h. The solution was then added to water (200 mL), separated, and the organic fraction washed successively with hydrochloric acid (6M, 70 mL), sodium hydroxide solution (5% w/v, 100 mL) and saturated brine (100 mL). The organic phase was dried over magnesium sulfate and concentrated under reduced pressure to yield ethyl 3,5-dimethylisoxazole-4-carboxylate (15.8g, 47%,). bp 59-61^{0}C at 0.5 mmHg, lit.[94] bp 60-62^{0}C at 0.5 mmHg; MS: *m/z* calculated for $C_8H_{11}NO_3$ (MNa^+): 170.0856. Found 170.0849; IR ν_{max} ($CHCl_3$)/cm^{-1} 2980 (CH_3), 2951 (CH_2, CH_3), 1719 (CO), 1612(CC), 1303 (CN); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 1.30 (3H, t, *J* 6.8 CH_2CH_3), 2.36 (3H, s, $OCCH_3$), 2.58 (3H, s, $NCCH_3$), 4.24 (2H, q, J_a 6.8, CH_2CH_3); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 11.6 (CH_2CH_3), 13.2 ($NCCH_3$), 13.9 ($OCCH_3$), 60.4 (CH_3CH_2), 108.7 ($C(C)_4$), 159.8 (*C*N), 162.3 (CH_3CO) 175.0 (CH_2COC).



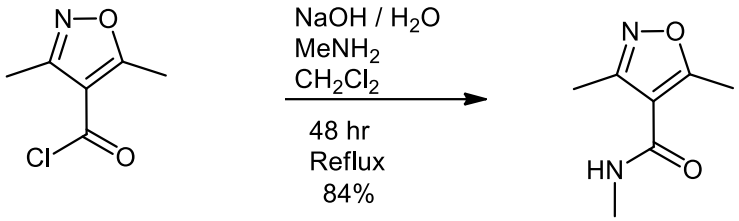
3,5-Dimethylisoxazole-4-carboxylic acid (159)

To ethyl 3,5-dimethylisoxazole-4-carboxylate (**150**) (9.40g, 55.5 mmol) was added sodium hydroxide (2.70g, 6.66 mmol) in water (100 mL) and the mixture was heated at reflux for 4 h. After cooling, the solution was washed with chloroform (2 x 100 mL) and the aqueous layer was acidified to pH 2-3 using concentrated hydrochloric acid. The resultant white precipitate was collected by filtration, dissolved in chloroform (100 mL) which was then dried over magnesium sulfate, filtered and evaporated under a reduced pressure to yield the title compound as a white solid (6.32g, 81%). mp 145-147^{0}C, lit.[94] mp 146-147^{0}C; MS: *m/z* calculated for $C_6H_7NO_3$ (MH^+): 142.0568. Found 142.0562; IR ν_{max} ($CHCl_3$)/cm^{-1} 3110-3390 (OH), 2950 (CH_2, CH_3), 1726 (CO), 1609 (CC), 1506 (CN); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 2.40 (3H, s, NCC*H*$_3$), 2.65 (3H, s, OCC*H*$_3$), 10.18 (1H, s, CO_2*H*)); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 11.7 (NC*C*H$_3$), 13.5 (OC*C*H$_3$), 108.0 (C(*C*)$_4$), 160.3 (N*C*CH$_3$), 167.9 (O*C*CH$_3$), 176.9 (*C*O$_2$H).



3,5-dimethylisoxazole-4-carbonyl chloride (160)[87]

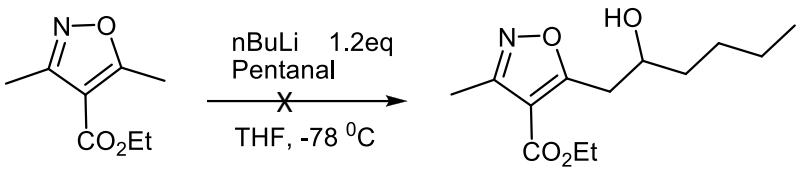
4-Carboxy-3,5-dimethylisoxazole (**159**) (6.33g, 44.9 mmol) was suspended in thionyl chloride (45 mL, 72.0g, 605 mmol) and the mixture stirred for 2h at reflux. The excess thionyl chloride was removed under reduced pressure and the black oily chlorinated product used directly in the next reaction without purification. No spectroscopic analysis was taken at his point.



N,3,5-trimethylisoxazole-4-carboxamide (151)[97]

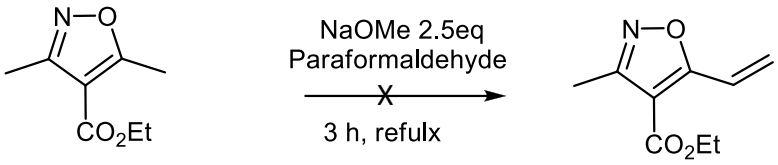
To a solution of crude 3,5-dimethylisoxazole-4-carbonyl chloride (**160**) (7.16g, 44.9 mmol) in DCM (80 mL) was added methylamine (40% w/w in aqueous solution, 4.17g, 53.8 mmol) in a sodium hydroxide solution (1M, 80 mL) and the mixture stirred at reflux for 20 h. The reaction mixture was cooled to room temperature and the aqueous layer extracted with DCM (2 x 100 mL). The organic fractions were combined and dried using magnesium sulfate, filtered and concentrated under reduced pressure to yield N,3,5-trimethylisoxazole-4-carboxamide as a white solid (5.81g, 84%). MS: *m/z* calculated for $C_7H_{10}N_2O_2$ (MH^+): 155.0895. Found 155.0901; ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 2.36 (3H, s, NCC*H*$_3$), 2.55 (3H, s, OCC*H*$_3$), 2.90 (3H, d, *J* 4.8, NHC*H*$_3$) 5.61 (1H, br. s, N*H*CH_3); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 13.9 (NC*C*H_3), 15.7 (OC*C*H_3), 26.5 (CONH*C*H_3), 110.0 (C(*C*)$_4$), 160.8 (N*C*CH_3), 168.6 (O*C*CH_3), 176.9 (*C*O_2NHCH_3). No melting point test or IR analysis was done on this compound.

4.9.2 – Model Aldol Reactions



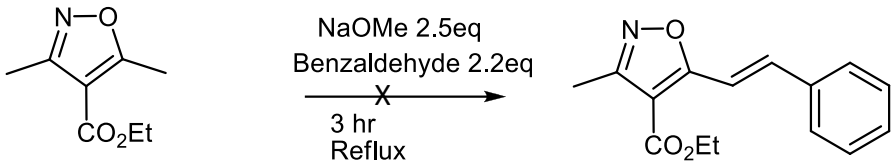
Ethyl 5-(2-hydroxylhexyl)-3-methyliosoxazole-4-carboxylate (156)[75]

n-Butyllithium (1.30 M in hexanes, 54.6 mL, 70.9 mmol, 1.2 eq.) was added dropwise to ethyl 3,5-dimethylisoxazole-4-carboxylate (**150**) (10.0g, 59.0 mmol) in THF (50 mL) at -78 ^{0}C and under nitrogen atmosphere. The mixture was stirred for 1 h at -78 ^{0}C and freshly distilled pentanal (10 mL, excess) was added followed by a further 1 h of stirring. The reaction mixture was then added to water (50 mL) and hydrochloric acid (2 M, 50 mL) which was subsequently removed under reduced pressure to leave a thick yellow/brown oil. The oil was then separated by the addition of ethyl acetate (50 mL) and water (50 mL) and the organic phase further extracted with ethyl acetate (50 mL). The combined organic layers were washed with saturated brine (50 mL) and dried over magnesium sulfate before being concentrated under a reduced pressure to yield a brown oil which was shown by ^{1}H NMR spectroscopy to contain no desired product.



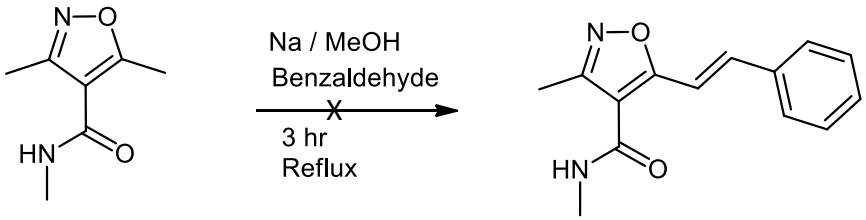
Ethyl 3-methyl-5-ethenylisoxazole-4-carboxylate (157)[79]

Sodium methoxide (2.5 eq, 0.37g, 16.23 mmol) was dissolved in methanol (100 mL) and was then added to a suspension of ethyl 3,5-dimethylisoxazole-4-carboxylate (**150**) (1.0 g, 6.49 mmol) in dry methanol (20 mL) and the mixture stirred at room temperature for 2 minutes. To this mixture was added paraformaldehyde (0.42g, 7.16 mmol) in dry methanol (50 mL). The reaction mixture was then heated at reflux under a nitrogen atmosphere for 3 h. The cooled mixture was then separated between water (50 mL) and chloroform (50 mL) and the aqueous layer further extracted with chloroform (2 x 50 mL), the organic layers were combined, dried over magnesium sulfate and concentrated under a reduced pressure to give a yellow oil that was shown to contain a number of compounds by TLC (3:1 petrol:ethyl acetate v/v), none of which was identified by ^{1}H NMR spectroscopy as the desired product.



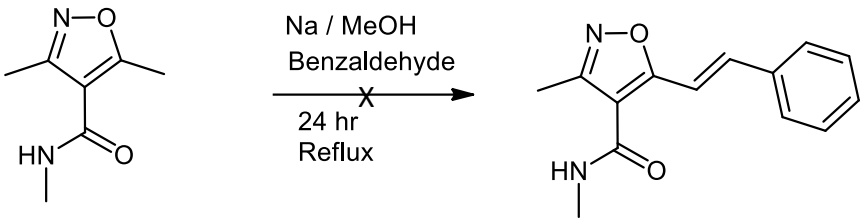
Ethyl 3-methyl-5-(2-phenylethenyl)isoxazole-4-carboxylate (158)[79]

Sodium methoxide (2.5 eq, 2.24g, 41.55 mmol) was dissolved in methanol (100 mL) and was then added to a suspension of ethyl 3,5-dimethylisoxazole-4-carboxylate (**150**) (3.0 g, 16.62 mmol) in dry methanol (20 mL) and the mixture stirred at room temperature for 2 minutes. To this mixture was added benzaldehyde (3.90g, 3.72 mL, 36.6 mmol) in dry methanol (50 mL). The reaction mixture was then heated at reflux under a nitrogen atmosphere for 3 h. The cooled solution was separated between water (50 mL) and chloroform (50 mL) and the aqueous layer extracted with chloroform (2 x 50 mL), the organic layers were combined, dried over magnesium sulfate and concentrated under reduced pressure to give a yellow oil that was shown to contain a number of compounds by TLC (3:1 light petrol:ethyl acetate v/v), none of which were identified by ^{1}H NMR spectroscopy as the desired product.



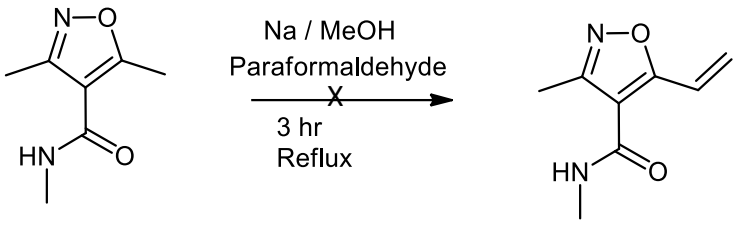
(E)-N,3-Dimethyl-5-(2-phenylethenyl)isoxazole-4-carboxamide (162)[79]

Sodium (2.2 eq, 0.329, 14.29 mmol) was dissolved in methanol (25 mL) and the mixture stirred at 0 ^{0}C under nitrogen atmosphere for 30 minutes until all the sodium had dissolved. This cloudy mixture was then added to a suspension of *N*,3,5-trimethylisoxazole-4-carboxamide (**151**) (1.0 g, 6.49 mmol) in dry methanol (20 mL) and stirred at room temperature for 2 minutes and to this reaction mixture was added benzaldehyde (1.51g, 14.29 mmol) and the mixture heated at reflux under a nitrogen atmosphere for 3 h. The solution was then separated between water (50 mL) and chloroform (50 mL) and the aqueous layer further extracted with chloroform (2 x 50 mL), the organic layers were combined, dried over magnesium sulfate and concentrated under reduced pressure to give a yellow oil that was shown to contain a number of compounds by TLC (3:1 light petrol:ethyl acetate v/v), none of which were identified by ^{1}H NMR spectroscopy as the desired product.



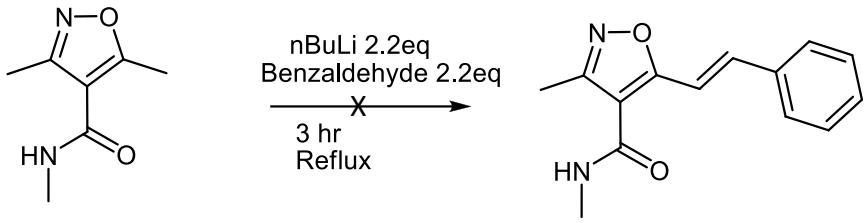
(E)-N,3-Dimethyl-5-(2-phenylethenyl)isoxazole-4-carboxamide (162)[79]

Sodium (2.2 eq, 0.329, 14.29 mmol) was dissolved in methanol (25 mL) and the mixture stirred at 0 ^{0}C under nitrogen atmosphere for 30 minutes until all the sodium had dissolved. This cloudy mixture was then added to a suspension of *N*-3,5-trimethylisoxazole-4-carboxamide (**151**) (1.0 g, 6.49 mmol) in dry methanol (20 mL) and the mixture stirred at room temperature for 2 minutes and to this reaction mixture was added benzaldehyde (1.51g, 14.29 mmol) and the mixture then heated at reflux under a nitrogen atmosphere overnight, 17 h. The cooled solution was then separated between water (50 mL) and chloroform (50 mL) and the aqueous layer extracted with chloroform (2 x 50 mL), the organic layers were combined, dried over magnesium sulfate and concentrated under reduced pressure to give a yellow oil that was shown to contain a number of compounds by TLC (3:1 light petrol:ethyl acetate v/v), none of which were identified by ^{1}H NMR spectroscopy as the desired product.



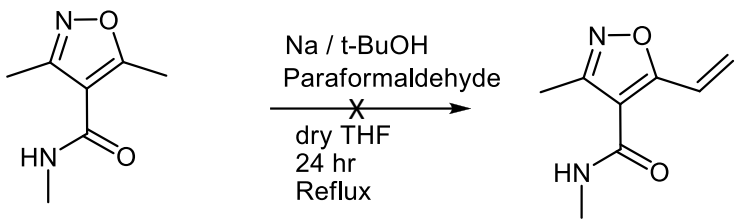
N,3-Dimethyl-5-(2-phenylethenyl)isoxazole-4-carboxamide (161)[79]

Sodium (2.2 eq, 0.329, 14.29 mmol) was dissolved in methanol (25 mL) and the mixture stirred at 0 ^{0}C under nitrogen atmosphere for 30 minutes until all the sodium had dissolved. This cloudy mixture was then added to a suspension of *N*,3,5-trimethylisoxazole-4-carboxamide (**151**) (1.0 g, 6.49 mmol) in dry methanol (20 mL) and the mixture stirred at room temperature for 2 minutes and to this reaction mixture was added paraformaldehyde (0.84g, 14.29 mmol) and the mixture was then heated at reflux under a nitrogen atmosphere for 3 h. The solution was then separated between water (50 mL) and chloroform (50 mL) and the aqueous layer further extracted with chloroform (2 x 50 mL), the organic layers were combined, dried over magnesium sulfate and concentrated under a reduced pressure to give a yellow oil that was shown to contain a number of compounds by TLC (3:1 light petrol:ethyl acetate v/v), none of which were identified by ^{1}H NMR spectroscopy as the desired product.



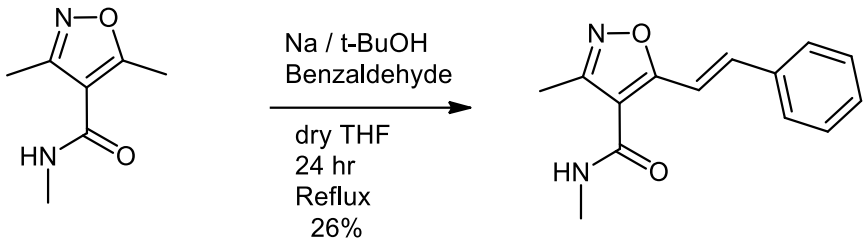
E-N,3-Dimethyl-5-(2-phenylethenyl)isoxazole-4-carboxamide (162)[75]

n-Butyl-lithium (2.29M in toluene, 5.68 mL, 13.0 mmol) was added dropwise to a suspension of *N*,3,5-trimethylisoxazole-4-carboxamide (**151**) (0.91 g, 5.91 mmol) in dry THF (20 mL) and the mixture stirred at room temperature for 2 minutes and to this reaction mixture was added benzaldehyde (1.38g, 13.0 mmol) the mixture was then heated to reflux under a nitrogen atmosphere overnight, 17 h. The solution was then separated between water (50 mL) and chloroform (50 mL) and the aqueous layer further extracted with chloroform (2 x 50 mL), the organic layers were combined, dried over magnesium sulfate and concentrated under reduced pressure to give a yellow oil that was shown to contain a large amount of compounds by TLC (3:1 light petrol:ethyl acetate v/v), none of which were identified by ^{1}H NMR spectroscopy to have characteristics of a new alkene bond being formed.



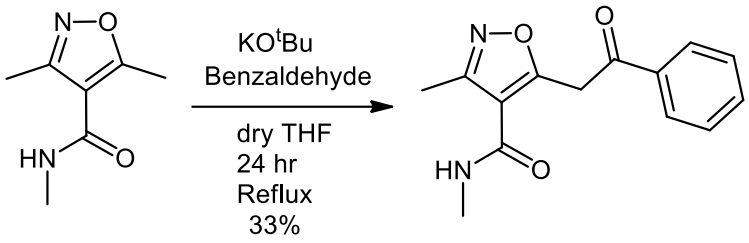
N,3-Dimethyl-5-vinylisoxazole-4-carboxamide (161)[79]

Sodium (2.5 eq, 0.185g, 8.11 mmol) was added directly to *tert*-butanol (25 mL) and the mixture heated at reflux with stirring under nitrogen atmosphere for 45 minutes until all the sodium had dissolved. This cloudy mixture was then added to a suspension of *N,*3,5-trimethylisoxazole-4-carboxamide (**151**) (0.5 g, 3.25 mmol) in dry THF (25 mL) and the mixture stirred at room temperature for 2 minutes, after which the reaction mixture turned red. To this reaction mixture was added paraformaldehyde (0.21g, 7.14 mmol) the mixture was then heated to reflux under a nitrogen atmosphere overnight, 18 h. The red solution was then separated between water (50 mL) and ethyl acetate (50 mL) and the aqueous layer further extracted with ethyl acetate (2 x 50 mL), the organic layers were combined, dried over magnesium sulfate and concentrated under reduced pressure to give a yellow oil that was identified as a mixture of starting materials my ^{1}H NMR spectroscopy and TLC (3:1 light petrol:ethyl acetate v/v).



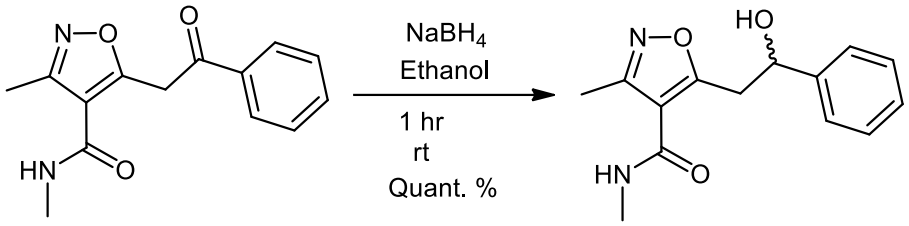
(E)-N,3-Dimethyl-5-(2-phenylethenyl)isoxazole-4-carboxamide (162)[79]

Sodium (2.5 eq, 0.185g, 8.11 mmol) was added in small pieces to *tert*-butanol (25 mL) and the mixture heated at reflux with stirring under a nitrogen atmosphere for 45 minutes until all the sodium had dissolved. This cloudy mixture was then added to a suspension of *N*,3,5-trimethylisoxazole-4-carboxamide (**151**) (0.5 g, 3.25 mmol) in dry THF (20 mL) and stirred at room temperature for 2 minutes, after which the reaction mixture turned red. To this reaction mixture was added benzaldehyde (0.75g, 7.14 mmol) and the mixture was then heated at reflux under a nitrogen atmosphere overnight, 18 h. The red solution was cooled then separated between water (50 mL) and ethyl acetate (50 mL) and the aqueous layer extracted with ethyl acetate (2 x 50 mL). The organic layers were combined, dried over magnesium sulfate and concentrated under a reduced pressure to give the crude product as a yellow oil that was identified by characteristic peaks in the ^{1}H NMR spectrum corresponding to the *trans* double bond hydrogens. The product was purified by column chromatography using ethyl acetate and light petrol (2:1 v/v) to give the desired product as a pale yellow solid (220 mg/ 26%). mp 210-211^{0}C; MS: *m/z* calculated for $C_{14}H_{14}N_2O_2$ (MH^+): 243.1106. Found 246.1101; IR ν_{max} ($CHCl_3$)/cm^{-1} 3426 (NH), 3032, 3011 (CH_2, CH_3), 1716 (CO), 1695 (CC), 1598 (CN); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 2.33 (3H, s, NCC*H*$_3$), 2.91 (3H, d, *J* 4.8, NHC*H*$_3$) 6.59 (1H, br. s, N*H*CH_3), 7.19 (1H, d, *J* 16.3, OCC*H*CH), 7.30-7.35 (3H, m, Ar-H), 7.35 (1H, d, *J* 16.3, PhC*H*CH), 7.44-7.48 (2H, m, Ar-H) ; ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 11.1 (NC*C*H_3), 26.5 (NHC*C*H_3), 112.1 (OC*C*H), 128.3 (2Ar*C*), 128.6 (2Ar*C*), 129.1 (Ar*C*), 135.6 (Ar*C*), 136.9 (Ph*C*H), 110.0 (C(*C*)$_4$), 158.8 (N*C*CH_3), 162.6 (O*C*CH_3), 167.9 (*C*O_2NHCH_3).



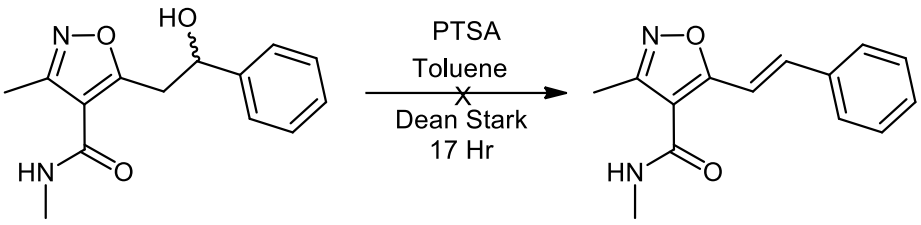
N,3-Dimethyl-5-(2-oxo-2-phenylethyl)isoxazole-4-carboxamide (163)[79]

To a suspension of *N*,3,5-trimethylisoxazole-4-carboxamide (**151**) (100 mg, 0.649 mmol) in dry THF (10 mL) was added potassium *tert*-butoxide (3.0 eq., 218 mg, 1.95 mmol) and the mixture stirred at room temperature for 2 minutes. To this was added benzaldehyde (10 eq., 0.671 mL, 691 mg, 6.49 mmol) which was then heated at reflux under a nitrogen atmosphere overnight, 18 h. The red solution was then separated between water (50 mL) and ethyl acetate (50 mL) and the aqueous layer extracted with ethyl acetate (2 x 50 mL). The organic layers were combined, dried over magnesium sulfate and concentrated under a reduced pressure to give the crude product as a yellow oil that was determined to be the ketone. The product was purified by column chromatography using ethyl acetate and light petrol (1:3 v/v) to give the product as a white solid (55.0 mg/ 33%). mp 198-202^{0}C; MS: *m/z* calculated for $C_{14}H_{14}N_2O_2$ (MH^+): 258.1004. Found 258.1005; IR ν_{max} ($CHCl_3$)/cm^{-1} 3256 (NH), 3012, 2985 (CH_2, CH_3), 1712 (CO), 1679 (CC), 1601 (CN); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 2.50 (3H, s, NCC*H*$_3$), 2.97 (3H, d, *J* 4.8, NHC*H*$_3$), 4.64 (2H, s, C*H*$_2$), 7.29 (1H, br. s, N*H*), 7.50-7.55 (3H, m, Ar-H), 7.65 (1H, d, *J* 16.3, PhC*H*CH), 8.14-8.18 (2H, m, Ar-H); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 11.3 (NC*C*H$_3$), 26.5 (NHC*C*H$_3$), 36.3 (*C*H$_2$), 115.2 (*C*(C)$_3$), 129.0 (2Ar*C*), 129.1 (2Ar*C*), 134.7 (Ar*C*), 135.4 (Ar*C*), 158.8 (N*C*CH$_3$), 162.6 (O*C*CH$_3$), 167.9 (*C*O$_2$NHCH$_3$), 195.1 (*C*(O)).



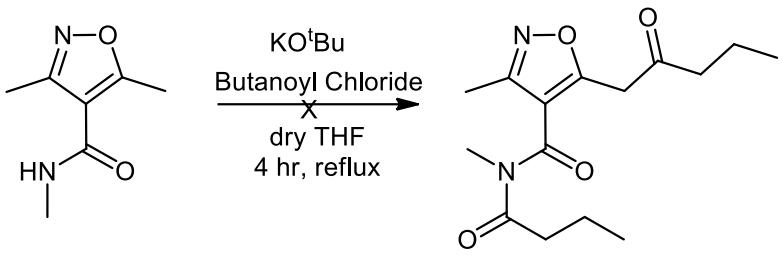
N,3-Dimethylisoxazole-5-(2-hydroxy-2-phenylethyl)-4-carboxamide (166)[79]

Sodium borohydride (1.0 eq., 6.0 mg, 0.161 mmol) was added portionwise to *N*,3-dimethyl-5-(2-oxo-2-phenylethyl)isoxazole-4-carboxamide (**163**) (40.0 mg, 0.161 mmol) suspended in ethanol (5 mL). This reaction was stirred at room temperature and monitored by TLC to observe the disappearance of the ketone starting material, which took 1 hour, after which the reaction was quenched with the addition of saturated ammonium chloride solution (5 mL). The mixture was then concentrated to remove ethanol under reduced pressure and separated between water (5 mL) and ethyl acetate (5 mL). The aqueous layer was extracted further with ethyl acetate (5 mL) and the organic fraction washed with saturated brine, dried over magnesium sulfate and concentrated under reduced pressure to yield the title compound as a white solid (41 mg/ quantitative yield). mp 195-198^{0}C; MS: *m/z* calculated for $C_{14}H_{14}N_2O_2$ (MH^+): 261.1194. Found 261.1195; IR ν_{max} ($CHCl_3$)/cm^{-1} 3402 (OH), 3298 (NH), 3010, 2996 (CH_2, CH_3), 1713 (CO), 1689 (CC), 1581 (CN); ^{1}H NMR δ_H (400 MHz; $CDCl_3$) 2.45 (3H, s, NCC*H*$_3$), 2.96 (3H, d, *J* 4.8, NHC*H*$_3$), 3.19 (1H, dd, J_a 3.6 Hz, J_b 14.4 Hz, C*H*H), 3.31 (1H, dd, J_a 10.0 Hz, J_b 14.4 Hz, CH*H*), 3.55 (1H, br. s, O*H*), 5.13 (1H, dd J_a 3.6 Hz, J_b 10.0 Hz, C*H*), 7.28-7.36 (1H, m, Ar-H), 7.38-7.41 (4H, m, 4Ar-H), 7.42 (1H, br. s, N*H*); ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 11.2 (NC*C*H$_3$), 26.4 (NHC*C*H$_3$), 36.3 (*C*H$_2$), 73.2 (*C*H(OH)), 114.8 (*C*(C)$_3$), 125.4 (2Ar*C*), 128.5 (Ar*C*), 128.9 (2Ar*C*), 142.6 (Ar*C*), 159.3 (N*C*CH$_3$), 163.2 (O*C*CH$_3$), 168.8 (*C*O$_2$NHCH$_3$).



(E)-N,3-Dimethyl-5-(2-phenylethenyl)isoxazole-4-carboxamide (167)[79]

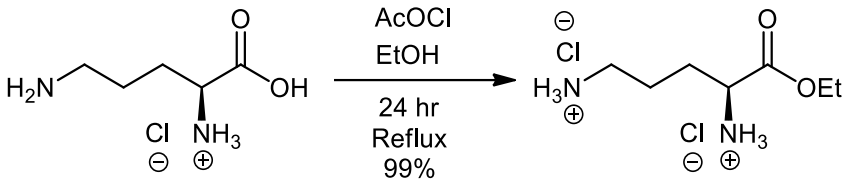
p-Toluenesulfonic acid (1.15 ea., 31.9 mg, 0.185 mmol) was suspended in toluene (10 mL) and added dropwise to 5-(2-hydroxy-2-phenylethyl)-N,3-dimethylisoxazole-4-carboxamide (**166**) (40.0 mg, 0.161 mmol) in toluene (30 mL) and the mixture heated under Dean-Stark conditions for 17 h with stirring. The cooled reaction mixture was then partitioned between ethyl acetate (30 mL) and saturated sodium carbonate solution (30 mL), followed by a washing with saturated brine (30 mL). The organic fraction was collected, dried over magnesium sulfate and concentrated under a reduced pressure. Upon TLC analysis it was shown to contain several products but, after separation via flash column chromatography eluting with ethyl acetate and light petroleum (5:1 ratio), no dehydrated product could be identified.



N-Butanoyl-N,3-dimethyl-5-(2-oxopentyl)isoxazole-4-carboxamide (167)

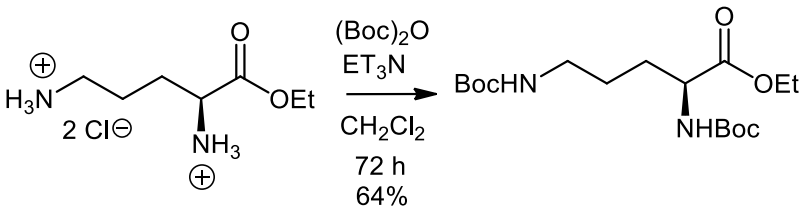
To a suspension of *N*,3,5-trimethylisoxazole-4-carboxamide (**151**) (100 mg, 0.649 mmol) in dry THF (10 mL) was added potassium tert-butoxide (3.2 eq., 233 mg, 2.08 mmol) and the mixture stirred at room temperature for 2 minutes. To this was added benzaldehyde (10 eq., 0.671 mL, 691 mg, 6.49 mmol) and the mixture was then heated at reflux under a nitrogen atmosphere overnight, 18 h. The cooled red solution was then separated between water (50 mL) and ethyl acetate (50 mL) and the aqueous layer extracted with ethyl acetate (2 x 50 mL). The organic layers were combined, dried over magnesium sulfate and concentrated under a reduced pressure to give the crude product as a yellow oil that could not be confirmed as the containing the diacylated product. After purification it was found that no diacylated product was formed.

4.10 – Laccarin Analogue Synthesis



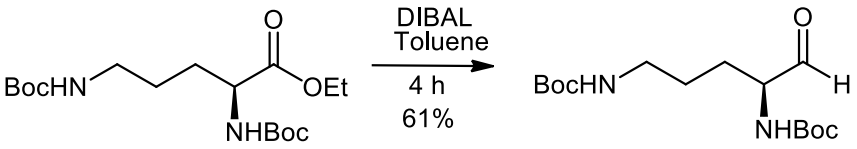
Ethyl 2,5-diaminopentanoate dihydrochloride (171)[75]

Acetyl chloride (4.2 eq, 9.40 g, 9.49 mL, 124.5 mmol) was added dropwise to a suspension of *S*-ornithine monohydrochloride (5.00 g, 29.7 mmol) in dry ethanol (50 mL, 1.13 mol) at 0°C over 4 h. The reaction mixture was then heated at reflux for 20 h; it was cooled to room temperature and then concentrated under reduced pressure to yield a viscous white gel which was evaporated further in a high vacuum oven at 100 ^{0}C to give a white powdered solid that was identified as the title compound (6.51 g, 99%). mp 60-63 ^{o}C; MS: *m/z* calculated for $C_7H_{17}N_2O_2Cl_2$ (MH^+): 162.1465 Found 162.1461; IR ν_{max} ($CHCl_3$)/cm^{-1} 3299 (NH), 2987, 2942 (CH_2, CH_3), 1706 (CO), 1398 (CN), 1186 (COC); ^{1}H NMR δ_H (400 MHz; (D_2O)) 1.29 (3H, t, *J* 7.2 Hz, CH_2CH_3), 1.61-1.75 (2H, m, $NCHCH_2CH_2$), 1.86-1.91 (2H, m, $NCHCH_2CH_2$), 2.78 (2H, br. s, CH_2NH_3), 3.99 (1H, t, *J* 6.0 Hz, $CHNH_3$), 4.20 (1H, d, *J* 7.2 Hz, CH_2CH_3), 4.24 (1H, d, J_a 7.2 Hz, CH_2CH_3), 8.28 (1H, br. s, CH_2NH_3), 8.78 (1H, br, s, $CHNH_3$); ^{13}C NMR δ_C (100 MHz; ($CDCl_3$)) 13.9 (CH_3), 22.7 ($NCHCH_2CH_2$), 27.0 ($NCHCH_2CH_2$), 37.9 (NH_3CH_2), 51.3 (NH_3CH), 61.8 (OCH_2), 169.2 (*C*OO). This was a literature compound and the data agreed with that seen earlier.



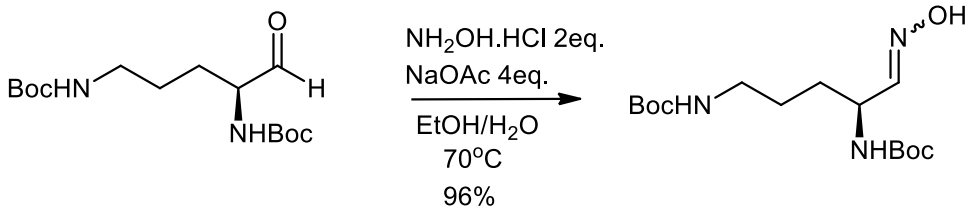
(S)-Ethyl 2,5-bis(tert-butoxycarbonylamino)pentanoate (172)[75,980]

Triethylamine (4 eq, 12.0 g, 16.57 mL, 118.8 mmol) was added dropwise to ethyl 2,5-diaminopentanoate dihydrochloride (**171**) (5.87 g, 29.7 mmol) in dichloromethane (100 mL) over 1 h at 0^0C. Di-*tert*-butyl dicarbonate (2.1 eq, 13.56 g, 67.4 mmol) in dichloromethane (50 mL) was added dropwise over 1 h at 0 ^{o}C, after which the reaction mixture was stirred for a further h at 0 ^{o}C. After this time the clear and colourless reaction mixture was allowed to warm to room temperature and continually stirred for 18 h, under a nitrogen atmosphere. The reaction mixture, now a colourless clear solution, was washed with citric acid solution (1 M, 200 mL) and saturated brine (2 x 200 mL), then dried using magnesium sulfate which was removed by filtration and the excess solvent evaporated under reduced pressure to afford the product as a clear colourless oil (7.09 g, 64%); MS: *m/z* calculated for $C_{17}H_{32}N_2O_6$ (MH^+): 361.2354 Found 361.2359; IR ν_{max} ($CHCl_3$)/cm^{-1} 3301 (NH), 2994, 2936 (CH_2, CH_3), 1729 (CO), 1406 (CN), 1197 (COC); ^{1}H NMR δ_H (400 MHz; ($CDCl_3$)) 1.28 (3H, t, *J* 7.2 Hz, CH_2CH_3), 1.45 (18H, br. s, 2x^{t}Bu), 1.58-1.62 (2H, m, $NCHCH_2CH_2$), 1.62-1.65 (2H, m, $NCHCH_2CH_2$), 1.81-1.88 (1H, m, CHNHBoc), 3.12 (2H, br. s, CH_2NHBoc), 4.10 (1H, d, *J* 7.2 Hz, CH_2CH_3), 4.15 (1H, d, *J* 7.2 Hz, CH_2CH_3), 4.67 (1H, br. s, CH_2NHBoc), 5.21 (1H, br, s, CHNHBoc). ^{13}C NMR δ_C (100 MHz; ($(CD_3)_2SO$)) 25.6 (CH_2), 26.9 (2x($CH_3)_3$), 38.5 (CH_2), 51.6 (CHNH), 68.9 (CH_2NH), 72.3 ($C(CH_3)_3$), 125.6 (C(O)), 165.8 (NCOO), 191.6 (NCOO). This was a literature compound and the data agreed with that seen earlier.



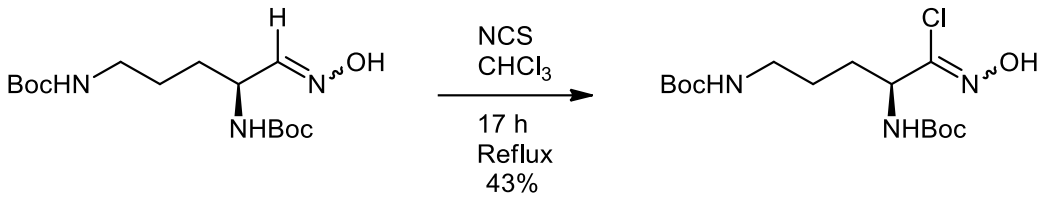
(S)-tert-Butyl 5-oxopentane-1,4-dicarbamate (173)[75]

Diisobutylaluminium hydride (2.5 eq., 1.0M in toluene, 47.87 mL, 47.87 mmol) was added to a suspension of (S)-ethyl 2,5-bis(tert-butoxycarbonylamino)pentanoate (**172**) (7.09 g, 19.15 mmol) in dry toluene (150 mL) under nitrogen with stirring at -78 °C for 1 h. The reaction mixture was left stirring for a further 30 min at -78 °C. Methanol (25 mL) was added to the mixture which was then poured into a solution of Rochelle salt (60 g) in water (200 mL). The reaction mixture was stirred vigorously for 2 h. The aqueous phase was separated and extracted with ethyl acetate (3 x 50 mL). The combined organic layers were washed with saturated brine (100 mL), then dried over magnesium sulfate, filtered and concentrated under reduced pressure to yield the aldehyde as a colourless oil (3.8 g, 61 %). This was then used directly for oxime formation without further purification. MS: *m/z* calculated for $C_{15}H_{27}N_2O_5$ (MH^+): 316.1998. Found 316.1998; IR ν_{max} ($CHCl_3$)/cm^{-1} 3265 (NH), 2994, 2923 (CH_2), 1759 (CO), 1621 (Ar), 1421 (CN), 1121 (COC); ^{1}H NMR δ_H (400 MHz; ($CDCl_3$)) 1.45 (18H, br. s, 2x^{t}Bu), 1.59-1.66 (2H, m, NCHC*H*$_2$CH$_2$), 1.61-1.69 (2H, m, NCHCH$_2$C*H*$_2$), 1.80-1.86 (1H, m, C*H*NHBoc), 3.22 (2H, br. s, C*H*$_2$NHBoc), 4.68 (1H, br. s, CH$_2$N*H*Boc), 5.23 (1H, br, s, CHN*H*Boc), 9.48 (1H, br. s, C*H*O). ^{13}C NMR δ_C (100 MHz; ($(CD_3)_2SO$)) 26.8 (*C*H$_2$), 27.9 (2x(*C*H$_3$)$_3$), 41.2 (*C*H$_2$), 54.6 (*C*HNH), 69.3 (*C*H$_2$NH), 71.2 (*C*(CH$_3$)$_3$), 129.9 (*C*H(O)), 167.8 (N*C*OO), 195.6 (N*C*OO).



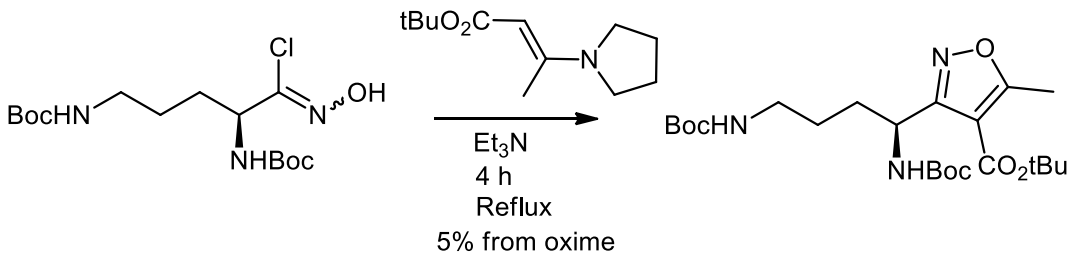
(S)-tert-Butyl 5-(hydroxyimino)pentane-1,4-dicarbamate (174)[75]

The crude aldehyde, (S)-*tert*-butyl 5-oxopentane-1,4-diyldicarbamate (**173**) (3.8 g, 11.66 mmol) in ethanol (50 mL) was added to hydroxylamine hydrochloride (2 eq., 1.62 g, 23.3 mmol) and sodium acetate (4 eq., 3.8 g, 46.65 mmol) in water (50 mL). A few drops of ethanol were added to dissolve the precipitate and the solution was warmed to 70 °C for 30 min. The reaction mixture was then allowed to cool to room temperature and stored in a refrigerator (2-5^{0}C) for 24 h. A white precipitate was seen and filtered off. The remaining mixture was extracted with ethyl acetate (3 x 50 mL) and the combined organic layers dried over magnesium sulfate, filtered and concentrated under reduced pressure to yield the title compound as a white solid (3.7 g, 96%); mp 159-160°C; MS: *m/z* calculated for $C_{15}H_{28}N_3O_5$ (MH^+): 332.2130. Found 332.2128; IR ν_{max} ($CHCl_3$)/cm^{-1} 3402 (OH), 2995, 2940 (CH_2), 1726 (CO), 1601 (Ar), 1395 (CN), 1251 (COC); ^{1}H NMR δ_H (400 MHz; ($(CD_3)_2SO$)) 1.45 (18H, br. s, 2x^{t}Bu), 1.49-1.51 (2H, m, NCHC*H*$_2$CH$_2$), 1.55-1.61 (2H, m, NCHCH$_2$C*H*$_2$). 3.22 (2H, br. s, C*H*$_2$NHBoc), 4.38 (1H, br. s, O*H*), 4.69 (1H, br. s, CH$_2$N*H*Boc), 5.25 (1H, br, s, CHN*H*Boc), 7.43 (1H, s, C*H*NOH), 9.46 (1H, br. s, C*H*O); ^{13}C NMR δ_C (100 MHz; ($(CD_3)_2SO$)) 26.9 (*C*H$_2$), 27.3 (2x(*C*H$_3$)$_3$), 40.8 (*C*H$_2$), 53.9 (*C*HNH), 64.2 (*C*H$_2$NH), 72.2 (*C*(CH$_3$)$_3$), 129.1 (*C*N(OH)), 167.2 (N*C*OO), 201.6 (N*C*OO).



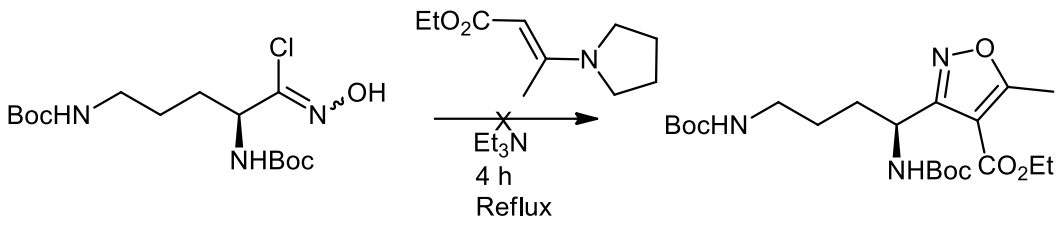
(S)-tert-Butyl 5-chloro-5-(hydroxyimino)pentane-1,4-dicarbamate (175)[75]

N-Chlorosuccinimide (1.1 eq., 4.44 g, 33.2 mmol) was added portionwise to (S)-*tert*-butyl 5-(hydroxyimino)pentane-1,4-diyldicarbamate (**174**) (10.0 g, 30.2 mmol) in chloroform (100 mL) at 0°C over 30 minutes. The reaction mixture was then heated at reflux for 16 h. A gradual colour change from pale yellow to dark orange/brown was noted during this time. This solution was then washed with water (3x100 mL) and saturated brine (2 x 100 mL) before being dried over $MgSO_4$, filtered and concentrated under a reduced pressure to yield the chlorinated oxime (S)-tert-butyl 5-chloro-5-(hydroxyimino)pentane-1,4-diyldicarbamate as a dark orange thick oil (4.63g, 43%); MS: *m/z* calculated for $C_{15}H_{28}N_3O_5{}^{35}Cl$ (MH^+): 365.1717. Found 365.1717; IR ν_{max} ($CHCl_3$)/cm^{-1} 3456 (OH), 2998 (CH_2), 1726 (CO), 1624 (Ar), 1401 (CN), 1112 (COC); ^1H NMR δ_H (400 MHz; ($(CD_3)_2SO$)) 1.48 (18H, br. s, 2x^{t}Bu), 1.59-1.62 (2H, m, NCHC*H*$_2$CH$_2$), 1.55-1.61 (2H, m, NCHCH$_2$C*H*$_2$), 3.15 (2H, br. s, C*H*$_2$NHBoc), 4.38 (1H, br. s, O*H*), 4.19-4.23 (1H, m, C*H*NH), 4.68 (1H, br. s, CH$_2$N*H*Boc), 5.25 (1H, br, s, CHN*H*Boc); ^{13}C NMR δ_C (100 MHz; ($(CD_3)_2SO$)) 26.4 (*C*H$_2$), 28.3 (2x(*C*H$_3$)$_3$), 40.0 (*C*H$_2$), 53.2 (*C*HNH), 61.4 (*C*H$_2$NH), 72.2 (*C*(CH$_3$)$_3$), 119.1 (*C*N(OH)), 165.8 (N*C*OO), 226.1 (N*C*OO).



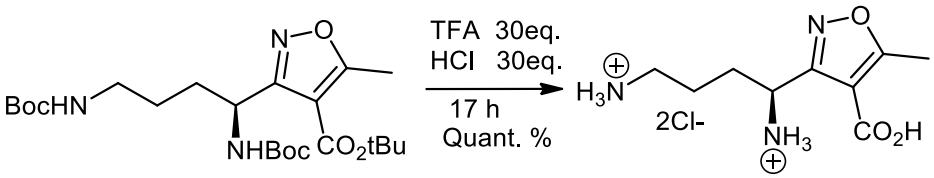
(S)-tert-Butyl 5-methyl-3-[1,4-bis(tert-butoxycarbonylamino)butyl]isoxazole-4-carboxylate (176)[75]

To the refluxing suspension of (S)-*tert*-butyl 5-chloro-5-(hydroxyimino)pentane-1,4-diyldicarbamate (**175**) (4.63 g, 12.65 mmol) in chloroform (150 mL) was added *tert*-butyl 3-(pyrrolidin-1-yl)but-2-enoate (**72**) (2 eq., 5.33 g, 25.22 mmol) in one portion followed by triethylamine (1.1 eq., 1.40 g,1.93 mL, 13.87 mmol) dropwise over 1 h. The mixture was heated at reflux for a further 2 h and allowed to cool to room temperature. The mixture was poured into water (200 mL) and the organic layer was separated and washed with citric acid solution (2M, 100 mL), sodium hydroxide solution (5 % w/v, 100 mL) and saturated brine (100 mL). The organic layer was dried over magnesium sulfate, filtered and concentrated under reduced pressure to provide a dark brown thick oil. The oil was purified by column chromatography eluting with light petroleum:ethyl acetate (3:1 v/v) to yield the title compound as a yellow oil (271 mg, 5%); MS: *m/z* calculated for $C_{29}H_{39}N_3O_7$ (MNa^+): 493.7891 Found 493.7886; IR ν_{max} ($CHCl_3$)/cm^{-1} 3402 (NH), 2981, 2939 (CH_2), 1726 (CO), 1601 (Ar), 1395 (CN), 1251, 1167, 1121 (COC); ^{1}H NMR δ_H (400 MHz; ($CDCl_3$)) 1.31 (27H, s, 3xC(CH_3)$_3$), 1.49-1.51 (2H, m, NCHCH_2CH$_2$), 1.55-1.61 (2H, m, NCHCH$_2$CH_2),1.65-1.75 (1H, m, CH_2), 1.75-1.85 (2H, m, NHC*H*), 1.89 (3H, s, CH_3), 2.95-3.06 (2H, m, CH_2), 4.97-5.06 (1H, m, CH$_2$N*H*), 5.29-5.36 (1H, m, C*H*NH); ^{13}C NMR δ_C (100 MHz; ($CDCl_3$)) 13.9 (5-*C*H$_3$), 21.6 (*C*H$_2$), 27.6 (C(*C*H$_3$)$_3$), 27.7 (C(*C*H$_3$)$_3$), 31.5 (*C*H$_2$), 42.6 (*C*H$_2$), 50.1 (*C*HNH), 60.1 (*C*(CH$_3$)$_3$), 66.5 (*C*(CH$_3$)$_3$), 69.5 (*C*(CH$_3$)$_3$), 155.1 (*C*O), 156.2 (*C*N), 170.2 (N*C*OO), 172.5 (N*C*OO), 173.6(*C*OO).



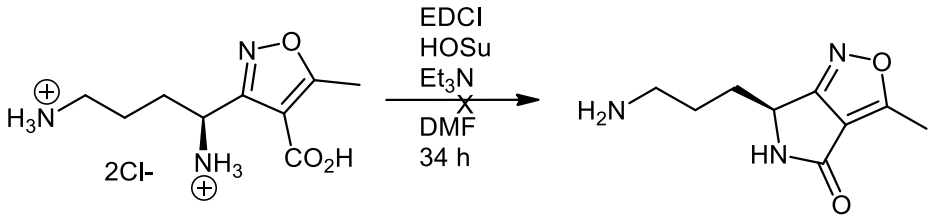
(S)-Ethyl 5-methyl-3-[1,4-bis(tert-butoxycarbonylamino)butyl]isoxazole-4-carboxylate (176)[75]

To the refluxing suspension of (S)-*tert*-butyl 5-chloro-5-(hydroxyimino)pentane-1,4-diyldicarbamate (**175**) (2.00 g, 5.47 mmol) in chloroform (100 mL) was added ethyl 3-(pyrrolidin-1-yl)but-2-enoate (**72**) (2.0 eq., 1.853 g, 10.9 mmol) in one portion followed by triethylamine (1.1 eq., 0.60 g, 0.840 mL, 6.02 mmol) dropwise over 1 h. The mixture was heated to reflux for a further 2 h and allowed to cool to room temperature. The mixture was poured into water (200 mL) and the organic layer was washed with citric acid solution (2M, 100 mL), sodium hydroxide solution (5 % w/v, 100 mL) and saturated brine (100 mL). The organic layer was dried over magnesium sulfate, filtered and concentrated under reduced pressure to provide a dark brown thick oil. The oil was subjected to purification by column chromatography eluting with light petroleum:ethyl acetate (3:1 v/v) to yield a yellow oil which was shown to contain a mixture of compounds, from which none could be identified by ^{1}H NMR spectroscopy.



(S)-tert-Butyl 5-methyl-3-[1,4-bis(tert-butoxycarbonylamino)butyl]isoxazole-4-carboxylic acid dihydrochloride (177)[75,57]

To *S-tert*-Butyl 5-methyl-3-[1,4-bis(tert-butoxycarbonylamino)butyl]isoxazole-4-carboxylate (**176**) (271 mg, 0.577 mmol) was added trifluoroacetic acid (30 eq., 1.33 mL, 17.3 mmol). The reaction mixture was stirred at room temperature for 4 h, and then concentrated under a reduced pressure, after which hydrochloric acid (30 eq., 2M, 8.65 mL, 17.3 mmol) was added to the residue and the reaction mixture was stirred at room temperature for a further 17 h. After this time the reaction mixture was again concentrated under reduced pressure. The residue was dissolved in water (50 mL) and washed with ethyl acetate (3 x 50 mL). The aqueous phase was evaporated to dryness in a vacuum oven set to 80^0C to yield a light brown solid (200 mg, quantitative %). MS: *m/z* calculated for $C_9H_{17}N_3O_3Cl_2$ (MH^+) 215.1367: Found 215.1369; ^{13}C NMR δ_C (100 MHz; $CDCl_3$) 13.9 (5-*C*H_3), 22.9 (*C*H_2), 28.1 (C(*C*H_3)$_3$), 28.6 (C(*C*H_3)$_3$), 30.5 (*C*H_2), 45.1 (*C*H_2), 51.3 (*C*HNH), 156.9 (*C*O), 158.6 (*C*N), 175.1 (*C*OOH).



(S)-6-(3-Aminopropyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (178)[75,57]

1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) (**123**) (1.1 eq., 119.4 g, 0.77 mmol) was added to a suspension of 3-(1,4-diaminobutyl)-5-methylisoxazole-4-carboxylic acid dihydrochloride (**177**) (200 mg, 0.70 mmol) and *N*-hydroxysuccinimide (1.1 eq., 88.5 mg, 0.77 mmol) in dry dimethylformamide (30 mL) at 0 ^{0}C. The reaction mixture was stirred at room temperature for 17 h. Triethylamine (3.2 eq., 0.226 g, 0.31 mL, 2.24 mmol, 2.2 eq) was added to the mixture and it was left to stir for a further 17 h and then concentrated under reduced pressure. The residue was dissolved in ethyl acetate (60 mL) and washed with water (60 mL), hydrochloric acid (2M, 60 mL), saturated sodium bicarbonate solution (60 mL) and saturated brine (60 mL). The organic layer was dried over magnesium sulfate, filtered and concentrated under reduced pressure to yield a beige solid (0.125 g, 99%). The solid was analysed by NMR spectroscopy, which showed no peaks that could be associated with the product. The initial aqueous layer was then basified with sodium bicarbonate (7.0 g) to pH 12 and the aqueous phase now extracted with ethyl acetate (60 mL) and washed with water (60 mL) and saturated brine (60 mL). The organic layer was dried over magnesium sulfate, filtered and concentrated under a reduced pressure to yield a yellow solid (99.6 mg) which was shown by ^{1}H NMR spectroscopy to contain none of the relevant peaks.

4.11 – X-Ray Crystal Data

4.11.1 – 6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (125a) X-Ray Data

X-Ray data has been identified for 6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125a**), Figure 28, as prepared by the PS-CDI route as described above.

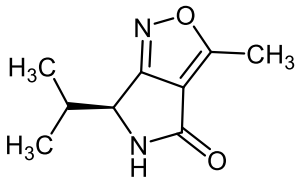
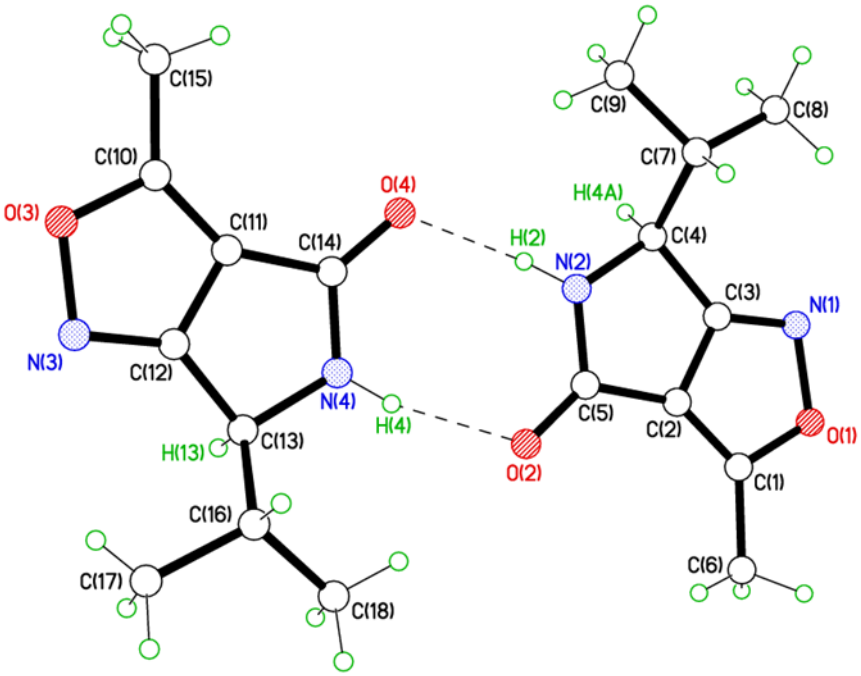
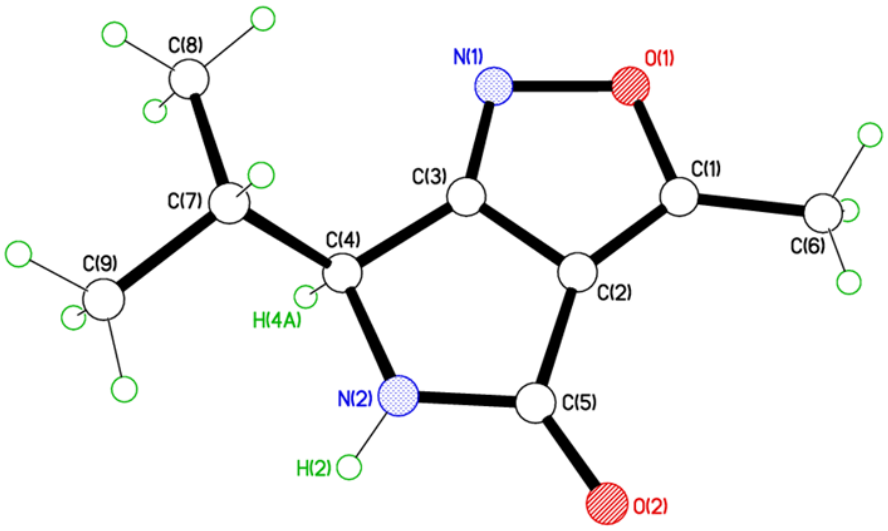


Figure 28. Structural illustration of 6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (125a)

rcfj40.figs




C(8)
N(1)
O(1)
C(3)
C(1)
C(7)
C(4)
C(6)
C(9)
C(2)
H(4A)
N(2)
C(5)
H(2)
O(2)

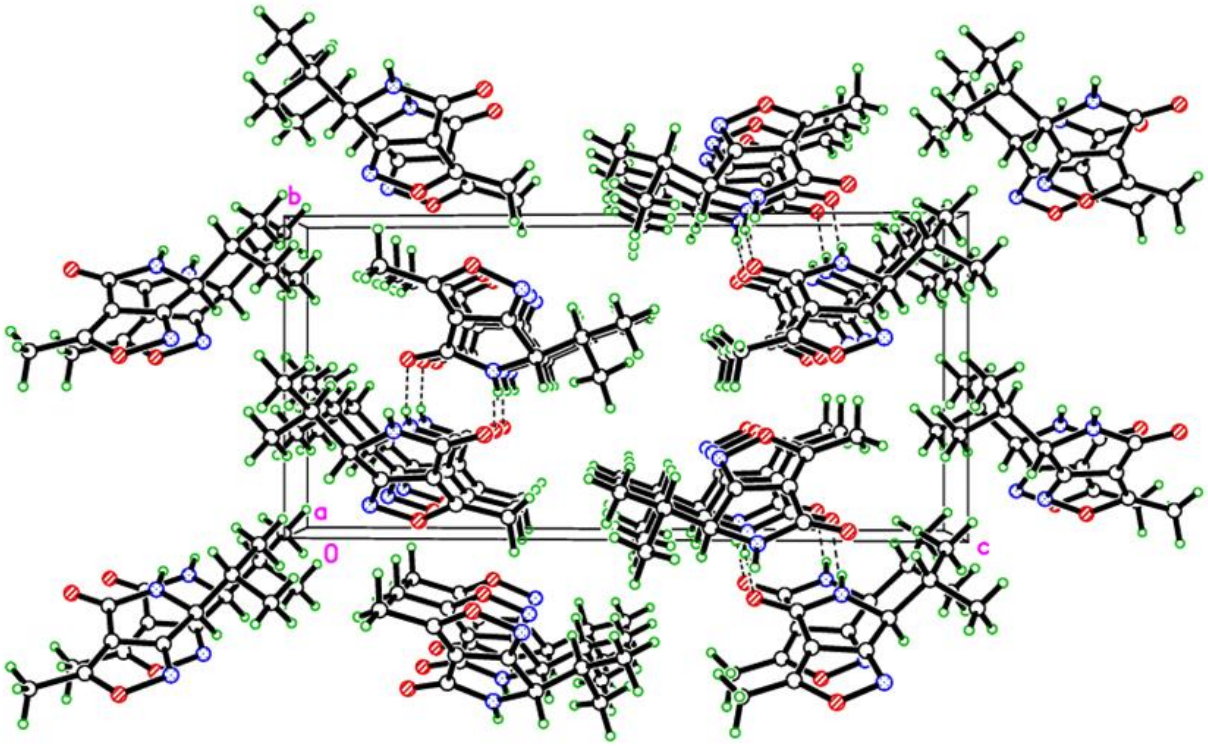

b
a
0
c

Table 13. Crystal data and structure refinement for 6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (125a).

Identification code	**rcfj40**	
Chemical formula	$C_9H_{12}N_2O_2$	
Formula weight	180.21	
Temperature	150(2) K	
Radiation, wavelength	MoK, 0.71073 Å	
Crystal system, space group	Monoclinic, $P2_1$	
Unit cell parameters	a = 5.5512(4) Å	□ = 90°
	b = 8.7805(7) Å	□ = 95.4252(12)°
	c = 18.5613(15) Å	□ = 90°
Cell volume	900.67(12) $Å^3$	
Z	4	
Calculated density	1.329 g/cm^3	
Absorption coefficient □	0.096 mm^{-1}	
F(000)	384	
Crystal colour and size	colourless, 0.72 × 0.17 × 0.14 mm^3	
Reflections for cell refinement	4955 (□ range 2.20 to 30.55°)	

Data collection method	Bruker APEX 2 CCD diffractometer
	□ rotation with narrow frames
□ range for data collection	1.10 to 30.56°
Index ranges	h –7 to 7, k –12 to 12, l –25 to 26
Completeness to □ = 30.56°	99.3 %
Intensity decay	0%
Reflections collected	10700
Independent reflections	5424 (R_{int} = 0.0189)
Reflections with F^2>2□	4950
Absorption correction	semi-empirical from equivalents
Min. and max. transmission	0.934 and 0.987
Structure solution	direct methods
Refinement method	Full-matrix least-squares on F^2
Weighting parameters a, b	0.0707, 0.0133
Data / restraints / parameters	5424 / 1 / 331
Final R indices [F^2>2□]	R1 = 0.0385, wR2 = 0.0990

R indices (all data)	R1 = 0.0429, wR2 = 0.1029
Goodness-of-fit on F^2	1.025
Absolute structure parameter	−0.7(7)
Largest and mean shift/su	0.000 and 0.000
Largest diff. peak and hole	0.386 and −0.164 e $Å^{-3}$

Table 14. Atomic coordinates and equivalent isotropic displacement parameters ($Å^2$) for 6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (125a). U_{eq} is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U_{eq}
N(1)	1.6350(2)	0.11416(13)	0.13835(6)	0.0279(2)
O(1)	1.75696(18)	0.06367(12)	0.20609(5)	0.0287(2)
C(1)	1.6411(2)	0.11762(14)	0.26202(6)	0.0233(2)
C(2)	1.4491(2)	0.20206(13)	0.23411(6)	0.0207(2)
C(3)	1.4557(2)	0.19480(13)	0.15846(6)	0.0214(2)
C(4)	1.2430(2)	0.27965(14)	0.12084(6)	0.0212(2)
N(2)	1.14016(19)	0.34402(13)	0.18484(5)	0.0230(2)
C(5)	1.2442(2)	0.29806(14)	0.25040(6)	0.0204(2)
O(2)	1.17821(16)	0.33258(11)	0.30988(5)	0.02568(19)
C(6)	1.7425(3)	0.07619(18)	0.33616(7)	0.0303(3)
C(7)	1.3039(2)	0.39857(15)	0.06492(6)	0.0239(2)

C(8)	1.4203(3)	0.32018(19)	0.00329(7)	0.0322(3)
C(9)	1.0752(3)	0.48259(18)	0.03473(7)	0.0324(3)
N(3)	0.2631(2)	0.75585(14)	0.36071(6)	0.0290(2)
O(3)	0.16064(17)	0.82211(12)	0.29363(5)	0.0302(2)
C(10)	0.2802(2)	0.77101(14)	0.23783(7)	0.0238(2)
C(11)	0.4588(2)	0.67594(13)	0.26543(6)	0.0217(2)
C(12)	0.4364(2)	0.66976(14)	0.34033(6)	0.0225(2)
C(13)	0.6165(2)	0.55956(14)	0.37699(6)	0.0214(2)
N(4)	0.74898(19)	0.51830(13)	0.31417(5)	0.0227(2)
C(14)	0.6623(2)	0.57864(14)	0.24934(6)	0.0215(2)
O(4)	0.74328(16)	0.55436(12)	0.19058(5)	0.0269(2)
C(15)	0.1888(3)	0.82215(17)	0.16413(8)	0.0300(3)
C(16)	0.7785(2)	0.62062(16)	0.44194(6)	0.0243(2)
C(17)	0.6228(3)	0.6617(2)	0.50271(8)	0.0336(3)
C(18)	0.9673(3)	0.5019(2)	0.46789(7)	0.0323(3)

Table 15. Bond lengths [Å] and angles [°] for 6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (125a).

N(1)–C(3)	1.3047(16)	N(1)–O(1)	1.4402(14)
O(1)–C(1)	1.3574(14)	C(1)–C(2)	1.3600(17)
C(1)–C(6)	1.4821(18)	C(2)–C(3)	1.4097(15)

C(2)–C(5)	1.4700(16)	C(3)–C(4)	1.5103(16)
C(4)–N(2)	1.4781(15)	C(4)–C(7)	1.5324(16)
N(2)–C(5)	1.3579(14)	C(5)–O(2)	1.2335(14)
C(7)–C(9)	1.5282(18)	C(7)–C(8)	1.5298(18)
N(3)–C(12)	1.3071(16)	N(3)–O(3)	1.4416(15)
O(3)–C(10)	1.3582(14)	C(10)–C(11)	1.3589(17)
C(10)–C(15)	1.4829(18)	C(11)–C(12)	1.4086(16)
C(11)–C(14)	1.4693(17)	C(12)–C(13)	1.5068(17)
C(13)–N(4)	1.4811(15)	C(13)–C(16)	1.5312(17)
N(4)–C(14)	1.3604(15)	C(14)–O(4)	1.2368(14)
C(16)–C(18)	1.524(2)	C(16)–C(17)	1.5278(17)

C(3)–N(1)–O(1)	103.04(9)	C(1)–O(1)–N(1)	110.04(9)
O(1)–C(1)–C(2)	108.05(10)	O(1)–C(1)–C(6)	117.30(11)
C(2)–C(1)–C(6)	134.65(12)	C(1)–C(2)–C(3)	105.16(10)
C(1)–C(2)–C(5)	145.90(11)	C(3)–C(2)–C(5)	108.92(10)
N(1)–C(3)–C(2)	113.69(11)	N(1)–C(3)–C(4)	136.05(10)
C(2)–C(3)–C(4)	110.21(10)	N(2)–C(4)–C(3)	99.29(9)
N(2)–C(4)–C(7)	114.24(10)	C(3)–C(4)–C(7)	115.87(10)
C(5)–N(2)–C(4)	116.28(10)	O(2)–C(5)–N(2)	126.30(11)
O(2)–C(5)–C(2)	128.75(11)	N(2)–C(5)–C(2)	104.95(9)

C(9)–C(7)–C(8)	109.76(10)	C(9)–C(7)–C(4)	110.39(10)
C(8)–C(7)–C(4)	109.62(11)	C(12)–N(3)–O(3)	102.97(9)
C(10)–O(3)–N(3)	110.12(9)	O(3)–C(10)–C(11)	107.89(11)
O(3)–C(10)–C(15)	117.02(11)	C(11)–C(10)–C(15)	135.04(12)
C(10)–C(11)–C(12)	105.44(10)	C(10)–C(11)–C(14)	145.91(12)
C(12)–C(11)–C(14)	108.65(10)	N(3)–C(12)–C(11)	113.56(11)
N(3)–C(12)–C(13)	135.71(11)	C(11)–C(12)–C(13)	110.70(10)
N(4)–C(13)–C(12)	99.17(9)	N(4)–C(13)–C(16)	114.03(9)
C(12)–C(13)–C(16)	116.48(10)	C(14)–N(4)–C(13)	116.15(10)
O(4)–C(14)–N(4)	125.83(11)	O(4)–C(14)–C(11)	129.04(11)
N(4)–C(14)–C(11)	105.13(10)	C(18)–C(16)–C(17)	110.40(12)
C(18)–C(16)–C(13)	110.16(11)	C(17)–C(16)–C(13)	109.45(10)

Table 16. Hydrogen coordinates and isotropic displacement parameters (Å^2) for 6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (125a).

	x	y	z	U
H(4A)	1.120(3)	0.2048(18)	0.0959(8)	0.020(4)
H(2)	1.007(3)	0.395(2)	0.1814(9)	0.033(4)
H(6A)	1.676(5)	0.136(3)	0.3670(14)	0.069(7)
H(6B)	1.915(5)	0.088(3)	0.3408(12)	0.067(7)
H(6C)	1.742(6)	−0.028(5)	0.3447(16)	0.099(10)

H(7)	1.418(3)	0.473(2)	0.0879(8)	0.023(4)
H(8A)	1.298(4)	0.253(3)	−0.0222(11)	0.047(5)
H(8B)	1.568(4)	0.264(2)	0.0201(10)	0.038(5)
H(8C)	1.453(3)	0.396(2)	−0.0306(11)	0.041(5)
H(9A)	0.963(3)	0.412(2)	0.0084(10)	0.036(5)
H(9B)	1.113(4)	0.562(3)	−0.0016(11)	0.045(5)
H(9C)	0.992(3)	0.531(2)	0.0723(10)	0.033(4)
H(13)	0.523(3)	0.470(2)	0.3887(8)	0.026(4)
H(4)	0.875(4)	0.455(3)	0.3195(11)	0.047(5)
H(15A)	0.290(4)	0.795(3)	0.1310(12)	0.054(6)
H(15B)	0.165(4)	0.929(3)	0.1624(10)	0.041(5)
H(15C)	0.031(4)	0.784(3)	0.1493(13)	0.065(7)
H(16)	0.869(4)	0.712(2)	0.4276(10)	0.038(5)
H(17A)	0.548(3)	0.568(3)	0.5222(10)	0.039(5)
H(17B)	0.516(4)	0.732(3)	0.4893(12)	0.048(6)
H(17C)	0.726(4)	0.703(3)	0.5458(11)	0.048(6)
H(18A)	0.900(3)	0.406(2)	0.4828(10)	0.036(5)
H(18B)	1.075(4)	0.546(3)	0.5101(11)	0.046(5)
H(18C)	1.070(3)	0.485(3)	0.4299(10)	0.041(5)

Table 17. Torsion angles [°] for 6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (125a).

C(3)–N(1)–O(1)–C(1)	0.14(14)	N(1)–O(1)–C(1)–C(2)	−0.33(15)
N(1)–O(1)–C(1)–C(6)	179.91(11)	O(1)–C(1)–C(2)–C(3)	0.37(14)
C(6)–C(1)–C(2)–C(3)	−179.92(15)	O(1)–C(1)–C(2)–C(5)	−178.02(16)
C(6)–C(1)–C(2)–C(5)	1.7(3)	O(1)–N(1)–C(3)–C(2)	0.10(14)
O(1)–N(1)–C(3)–C(4)	−177.05(13)	C(1)–C(2)–C(3)–N(1)	−0.30(14)
C(5)–C(2)–C(3)–N(1)	178.74(11)	C(1)–C(2)–C(3)–C(4)	177.59(10)
C(5)–C(2)–C(3)–C(4)	−3.37(13)	N(1)–C(3)–C(4)–N(2)	−177.45(15)
C(2)–C(3)–C(4)–N(2)	5.33(12)	N(1)–C(3)–C(4)–C(7)	−54.65(19)
C(2)–C(3)–C(4)–C(7)	128.13(11)	C(3)–C(4)–N(2)–C(5)	−6.02(13)
C(7)–C(4)–N(2)–C(5)	−129.98(11)	C(4)–N(2)–C(5)–O(2)	−176.34(11)
C(4)–N(2)–C(5)–C(2)	4.31(14)	C(1)–C(2)–C(5)–O(2)	−1.4(3)
C(3)–C(2)–C(5)–O(2)	−179.74(12)	C(1)–C(2)–C(5)–N(2)	177.94(18)
C(3)–C(2)–C(5)–N(2)	−0.41(13)	N(2)–C(4)–C(7)–C(9)	−61.83(13)
C(3)–C(4)–C(7)–C(9)	−176.36(11)	N(2)–C(4)–C(7)–C(8)	177.14(11)
C(3)–C(4)–C(7)–C(8)	62.61(13)	C(12)–N(3)–O(3)–C(10)	−0.06(14)
N(3)–O(3)–C(10)–C(11)	−0.89(14)	N(3)–O(3)–C(10)–C(15)	176.88(11)

O(3)–C(10)–C(11)–C(12)	1.41(14)	C(15)–C(10)–C(11)–C(12)	−175.77(15)
O(3)–C(10)–C(11)–C(14)	−178.59(17)	C(15)–C(10)–C(11)–C(14)	4.2(3)
O(3)–N(3)–C(12)–C(11)	0.99(14)	O(3)–N(3)–C(12)–C(13)	−176.53(13)
C(10)–C(11)–C(12)–N(3)	−1.56(14)	C(14)–C(11)–C(12)–N(3)	178.44(11)
C(10)–C(11)–C(12)–C(13)	176.58(10)	C(14)–C(11)–C(12)–C(13)	−3.41(13)
N(3)–C(12)–C(13)–N(4)	−178.03(14)	C(11)–C(12)–C(13)–N(4)	4.40(12)
N(3)–C(12)–C(13)–C(16)	−55.27(19)	C(11)–C(12)–C(13)–C(16)	127.16(11)
C(12)–C(13)–N(4)–C(14)	−4.22(13)	C(16)–C(13)–N(4)–C(14)	−128.72(12)
C(13)–N(4)–C(14)–O(4)	−177.81(12)	C(13)–N(4)–C(14)–C(11)	2.43(14)
C(10)–C(11)–C(14)–O(4)	0.9(3)	C(12)–C(11)–C(14)–O(4)	−179.06(12)
C(10)–C(11)–C(14)–N(4)	−179.31(18)	C(12)–C(11)–C(14)–N(4)	0.69(13)
N(4)–C(13)–C(16)–C(18)	−59.38(14)	C(12)–C(13)–C(16)–C(18)	−174.02(10)

N(4)–C(13)–C(16)–C(17)	179.06(12)	C(12)–C(13)–C(16)–C(17)	64.41(15)

Table 18. Hydrogen bonds for 6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (125a). [Å and °].

D–H...A	d(D–H)	d(H...A)	d(D...A)	<(DHA)
N(2)–H(2)...O(4)	0.862(19)	2.04(2)	2.8846(14)	165.1(17)
N(4)–H(4)...O(2)	0.89(2)	2.02(2)	2.8946(14)	167.0(19)

4.11.2 – 6-(2-methylpropyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (125b) X-Ray Data

X-Ray data has also been identified for 6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**125b**), Figure 29, as prepared by the T3P route described above.

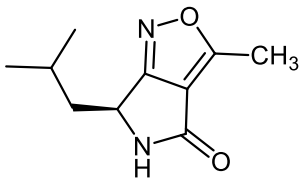
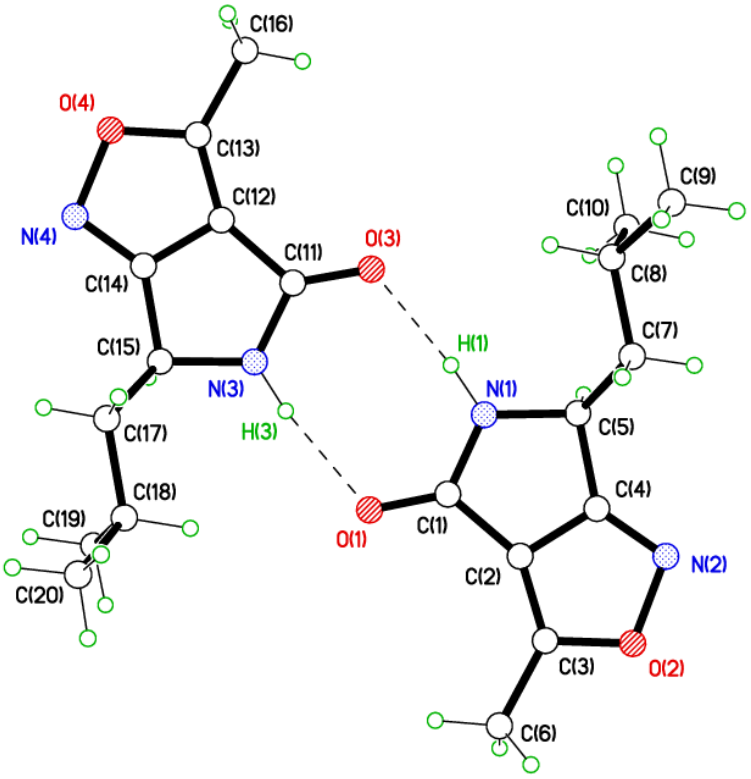
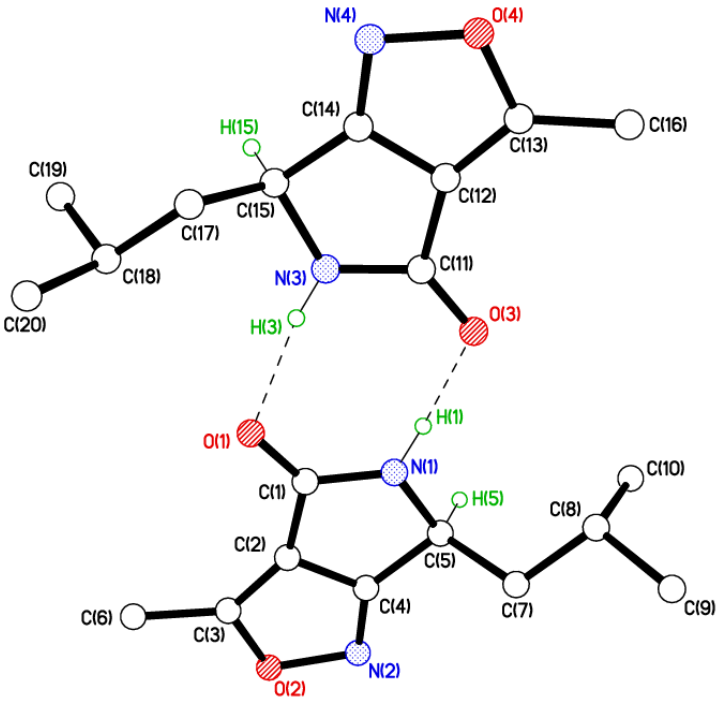


Figure 29. Structural illustration of 6-(2-methylpropyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (125b)

rcfj49.figs


C(16)
O(4)
C(13)
C(12)
N(4)
C(11)
O(3)
C(14)
C(15)
H(1)
N(3)
N(1)
C(17)
H(3)
C(5)
C(18)
C(4)
C(19)
O(1)
C(1)
N(2)
C(20)
C(2)
C(3)
O(2)
C(6)
C(9)
C(10)
C(8)
C(7)


N(4)
O(4)
C(14)
H(15)
C(16)
C(13)
C(19)
C(12)
C(15)
C(17)
C(18)
N(3)
C(11)
C(20)
O(3)
H(3)
H(1)
O(1)
N(1)
C(10)
C(1)
H(5)
C(8)
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C(4)
C(7)
C(9)
C(6)
C(3)
N(2)
O(2)

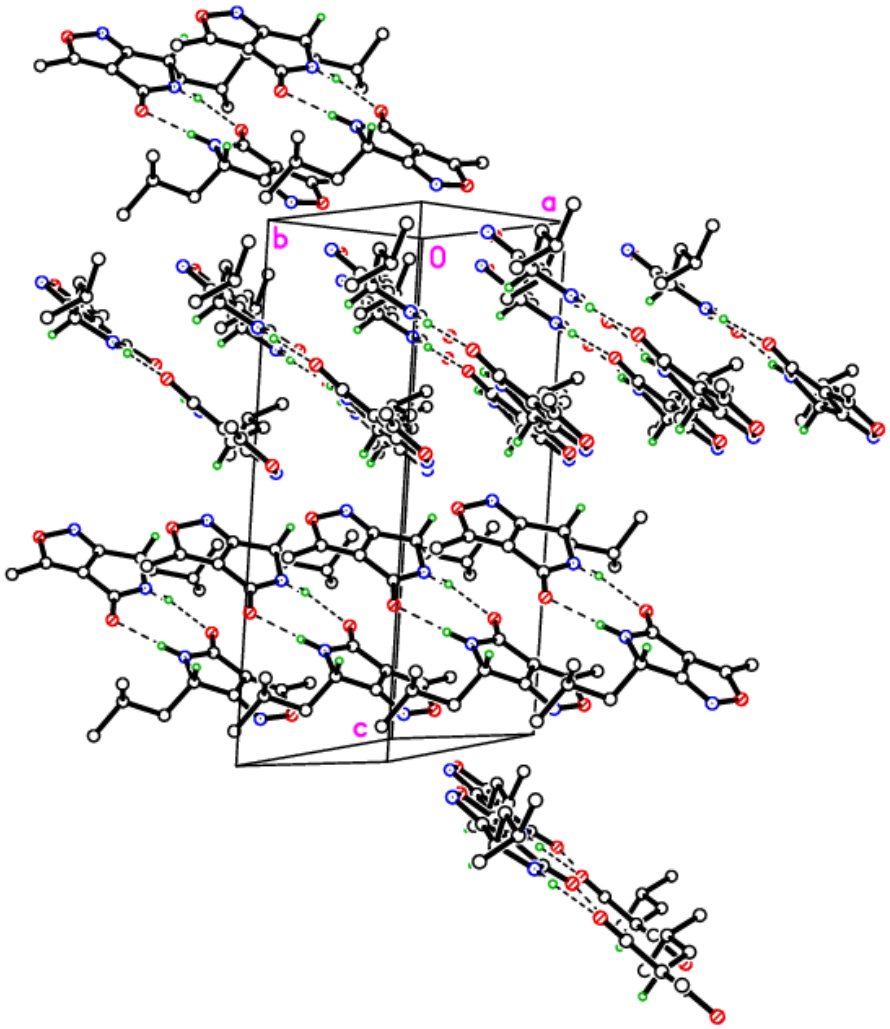

a
b
0
c

Table 19. Crystal data for 6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (125b)

C10H14N2O2	**F(000) = 416**
Mr = 194.23	Dx = 1.253 Mg m-3
Monoclinic, P21	Mo K radiation, = 0.71073 Å
a = 5.558 (1) Å	Cell parameters from 6151 reflections
b = 10.6051 (19) Å	= 2.3–28.3°
c = 17.647 (3) Å	= 0.09 mm-1
= 98.036 (3)°	T = 150 K
V = 1030.0 (3) Å3	Rod, colourless
Z = 4	1.10 × 0.23 × 0.10 mm

Table 20. Data collection for **125b**

Bruker APEX 2 CCD diffractometer	**4526 reflections with I > 2(I)**
Radiation source: fine-focus sealed tube	**Rint = 0.040**
rotation with narrow frames scans	**max = 28.4°, min = 2.3°**
Absorption correction: multi-scan	
TWINABS v20012/1, Sheldrick, G.M., (2012)	**h = -7 7**
Tmin = 0.909, Tmax = 0.991	**k = -14 14**
18525 measured reflections	**l = -23 23**

5115 independent reflections

Table 21. Refinement for **125b**

Refinement on F2 **Secondary atom site location: All non-H atoms found by direct methods**

Least-squares matrix: full **Hydrogen site location: difference Fourier map**

R[F2 > 2□(F2)] = 0.034 **All H-atom parameters refined**

wR(F2) = 0.089 **w = 1/[□2(Fo2) + (0.0563P)2]**

where P = (Fo2 + 2Fc2)/3

S = 1.04 **(□/□)max < 0.001**

5115 reflections **□□max = 0.23 e Å-3**

365 parameters **□□min = -0.17 e Å-3**

16 restraints **Absolute structure: Flack x determined using 1971 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons and Flack (2004), Acta Cryst. A60, s61).**

Primary atom site location: structure-invariant direct methods **Flack parameter: -0.3 (6)**

Table 22. Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å2) for 6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (125b)

	x	y	z	Uiso*/Ueq
N1	0.5354 (3)	0.37681 (16)	0.66552 (9)	0.0258 (3)
H1	0.682 (5)	0.349 (3)	0.6918 (15)	0.041 (7)*
C1	0.4480 (3)	0.49305 (18)	0.67757 (10)	0.0229 (4)
O1	0.5438 (2)	0.57230 (13)	0.72354 (7)	0.0268 (3)
C2	0.2171 (3)	0.50300 (18)	0.62533 (10)	0.0241 (4)
O2	-0.1300 (3)	0.50612 (14)	0.54890 (8)	0.0315 (3)
N2	-0.0221 (3)	0.38518 (17)	0.53771 (9)	0.0316 (4)
C3	0.0178 (3)	0.57315 (19)	0.60207 (10)	0.0267 (4)
N3	1.0190 (3)	0.50850 (15)	0.80497 (9)	0.0233 (3)
H3	0.876 (4)	0.537 (2)	0.7802 (12)	0.029 (6)*
O3	0.9641 (3)	0.30199 (13)	0.76370 (8)	0.0302 (3)
C4	0.1813 (3)	0.38949 (18)	0.58396 (10)	0.0258 (4)
O4	1.6529 (3)	0.36342 (13)	0.93243 (8)	0.0280 (3)
N4	1.5748 (3)	0.49293 (15)	0.93334 (9)	0.0262 (3)
C5	0.3938 (3)	0.30169 (19)	0.60419 (10)	0.0254 (4)
H5	0.489 (4)	0.297 (2)	0.5620 (12)	0.023 (5)*
C6	-0.0653 (4)	0.7008 (2)	0.62054 (14)	0.0349 (5)
H6A	-0.054 (6)	0.755 (3)	0.5799 (18)	0.067 (10)*

H6B	-0.224 (7)	0.696 (4)	0.6250 (19)	0.074 (10)*
H6C	0.036 (6)	0.739 (3)	0.6677 (17)	0.060 (9)*
C7	0.3248 (4)	0.17108 (19)	0.63002 (12)	0.0274 (4)
H7A	0.218 (4)	0.134 (2)	0.5864 (11)	0.018 (5)*
H7B	0.236 (4)	0.183 (2)	0.6737 (13)	0.025 (5)*
C8	0.5389 (4)	0.08160 (19)	0.65233 (12)	0.0310 (4)
H8	0.651 (4)	0.115 (2)	0.6949 (13)	0.027 (6)*
C9	0.4441 (5)	-0.0434 (2)	0.67883 (16)	0.0465 (6)
H9A	0.337 (5)	-0.084 (3)	0.6359 (13)	0.053 (8)*
H9B	0.356 (6)	-0.032 (4)	0.7243 (15)	0.088 (12)*
H9C	0.581 (5)	-0.103 (3)	0.6947 (19)	0.067 (10)*
C10	0.6833 (4)	0.0587 (3)	0.58620 (16)	0.0423 (5)
H10A	0.572 (5)	0.025 (3)	0.5408 (17)	0.060 (9)*
H10B	0.813 (5)	-0.002 (3)	0.6030 (15)	0.050 (8)*
H10C	0.760 (5)	0.137 (3)	0.5682 (16)	0.055 (8)*
C11	1.0814 (3)	0.38468 (17)	0.80155 (10)	0.0225 (4)
C12	1.3142 (3)	0.37371 (17)	0.85237 (10)	0.0218 (3)
C13	1.4936 (3)	0.29570 (18)	0.88356 (10)	0.0245 (4)
C14	1.3761 (3)	0.49291 (17)	0.88452 (9)	0.0211 (3)
C15	1.1931 (3)	0.59071 (16)	0.85327 (10)	0.0213 (4)
H15	1.272 (4)	0.648 (2)	0.8232 (12)	0.021 (5)*

C16	1.5480 (5)	0.1600 (2)	0.87611 (14)	0.0358 (5)
H16A	1.715 (8)	0.154 (4)	0.860 (2)	0.099 (13)*
H16B	1.546 (6)	0.117 (3)	0.9242 (18)	0.069 (10)*
H16C	1.441 (5)	0.126 (3)	0.8392 (16)	0.051 (8)*
C17	1.0788 (3)	0.66370 (17)	0.91327 (10)	0.0216 (4)
H17A	1.009 (4)	0.600 (2)	0.9439 (12)	0.024 (5)*
H17B	1.207 (4)	0.704 (2)	0.9485 (12)	0.027 (6)*
C18	0.8962 (4)	0.76490 (18)	0.88012 (11)	0.0274 (4)
H18	0.771 (5)	0.724 (2)	0.8404 (14)	0.041 (7)*
C19	1.0196 (5)	0.8719 (2)	0.84346 (15)	0.0454 (6)
H19A	0.909 (5)	0.937 (2)	0.8189 (16)	0.055 (8)*
H19B	1.112 (5)	0.848 (4)	0.8027 (16)	0.078 (11)*
H19C	1.144 (5)	0.915 (3)	0.8815 (16)	0.061 (9)*
C20	0.7611 (4)	0.8155 (2)	0.94284 (15)	0.0389 (5)
H20A	0.687 (6)	0.746 (3)	0.9632 (16)	0.052 (8)*
H20B	0.648 (6)	0.880 (3)	0.9233 (17)	0.060 (9)*
H20C	0.879 (5)	0.860 (3)	0.9791 (14)	0.042 (7)*

Table 23. Geometric parameters (Å, º) for 6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (125b)

N1—C1	**1.353 (3)**	**C8—H8**	**0.97 (2)**
N1—C5	1.479 (2)	C9—H9A	0.993 (19)
N1—H1	0.93 (3)	C9—H9B	1.00 (2)
C1—O1	1.236 (2)	C9—H9C	0.99 (2)
C1—C2	1.475 (3)	C10—H10A	1.01 (3)
C2—C3	1.350 (3)	C10—H10B	0.98 (3)
C2—C4	1.407 (3)	C10—H10C	1.00 (3)
O2—C3	1.359 (2)	C11—C12	1.472 (2)
O2—N2	1.441 (2)	C12—C13	1.352 (3)
N2—C4	1.299 (3)	C12—C14	1.408 (2)
C3—C6	1.481 (3)	C13—C16	1.481 (3)
N3—C11	1.362 (2)	C14—C15	1.502 (2)
N3—C15	1.481 (2)	C15—C17	1.520 (2)
N3—H3	0.90 (2)	C15—H15	0.95 (2)
O3—C11	1.232 (2)	C16—H16A	1.01 (4)
C4—C5	1.507 (3)	C16—H16B	0.96 (3)
O4—C13	1.353 (2)	C16—H16C	0.89 (3)
O4—N4	1.441 (2)	C17—C18	1.535 (3)
N4—C14	1.302 (2)	C17—H17A	0.98 (2)

C5—C7	1.524 (3)	C17—H17B	0.98 (2)
C5—H5	0.97 (2)	C18—C19	1.517 (3)
C6—H6A	0.93 (3)	C18—C20	1.520 (3)
C6—H6B	0.90 (4)	C18—H18	1.01 (3)
C6—H6C	1.02 (3)	C19—H19A	0.99 (2)
C7—C8	1.530 (3)	C19—H19B	0.97 (2)
C7—H7A	0.99 (2)	C19—H19C	1.00 (2)
C7—H7B	0.98 (2)	C20—H20A	0.94 (3)
C8—C9	1.524 (3)	C20—H20B	0.96 (3)
C8—C10	1.525 (3)	C20—H20C	0.97 (3)
C1—N1—C5	116.08 (16)	C8—C10—H10B	108.5 (16)
C1—N1—H1	121.2 (17)	H10A—C10—H10B	111 (2)
C5—N1—H1	122.5 (17)	C8—C10—H10C	113.8 (18)
O1—C1—N1	126.27 (17)	H10A—C10—H10C	106 (2)
O1—C1—C2	128.23 (18)	H10B—C10—H10C	108 (2)
N1—C1—C2	105.49 (16)	O3—C11—N3	126.27 (17)
C3—C2—C4	105.25 (17)	O3—C11—C12	128.85 (17)
C3—C2—C1	146.56 (19)	N3—C11—C12	104.88 (16)
C4—C2—C1	108.10 (17)	C13—C12—C14	105.11 (16)
C3—O2—N2	109.70 (14)	C13—C12—C11	146.26 (18)
C4—N2—O2	102.99 (16)	C14—C12—C11	108.59 (16)

C2—C3—O2	108.25 (18)	C12—C13—O4	108.33 (17)
C2—C3—C6	134.82 (19)	C12—C13—C16	135.00 (19)
O2—C3—C6	116.92 (18)	O4—C13—C16	116.67 (17)
C11—N3—C15	116.31 (15)	N4—C14—C12	113.79 (16)
C11—N3—H3	120.7 (15)	N4—C14—C15	135.29 (17)
C15—N3—H3	123.0 (15)	C12—C14—C15	110.93 (15)
N2—C4—C2	113.80 (18)	N3—C15—C14	99.16 (14)
N2—C4—C5	135.33 (18)	N3—C15—C17	113.42 (14)
C2—C4—C5	110.86 (16)	C14—C15—C17	115.03 (14)
C13—O4—N4	109.97 (14)	N3—C15—H15	111.7 (12)
C14—N4—O4	102.80 (14)	C14—C15—H15	107.7 (13)
N1—C5—C4	99.20 (15)	C17—C15—H15	109.4 (13)
N1—C5—C7	113.65 (16)	C13—C16—H16A	107 (3)
C4—C5—C7	114.25 (16)	C13—C16—H16B	110 (2)
N1—C5—H5	107.3 (12)	H16A—C16—H16B	110 (3)
C4—C5—H5	110.1 (13)	C13—C16—H16C	109.4 (18)
C7—C5—H5	111.5 (14)	H16A—C16—H16C	109 (3)
C3—C6—H6A	110 (2)	H16B—C16—H16C	111 (3)
C3—C6—H6B	108 (3)	C15—C17—C18	114.13 (14)
H6A—C6—H6B	106 (3)	C15—C17—H17A	105.8 (13)
C3—C6—H6C	112.9 (17)	C18—C17—H17A	113.5 (12)

H6A—C6—H6C	107 (3)	C15—C17—H17B	109.1 (13)
H6B—C6—H6C	113 (3)	C18—C17—H17B	108.6 (13)
C5—C7—C8	114.93 (17)	H17A—C17—H17B	105.3 (17)
C5—C7—H7A	106.2 (12)	C19—C18—C20	110.19 (18)
C8—C7—H7A	108.2 (12)	C19—C18—C17	111.81 (17)
C5—C7—H7B	107.3 (14)	C20—C18—C17	109.69 (17)
C8—C7—H7B	109.6 (13)	C19—C18—H18	109.5 (15)
H7A—C7—H7B	110.5 (17)	C20—C18—H18	107.0 (15)
C9—C8—C10	109.8 (2)	C17—C18—H18	108.5 (15)
C9—C8—C7	109.17 (18)	C18—C19—H19A	114.9 (18)
C10—C8—C7	112.04 (19)	C18—C19—H19B	116 (2)
C9—C8—H8	107.0 (13)	H19A—C19—H19B	103 (3)
C10—C8—H8	107.6 (13)	C18—C19—H19C	111.4 (18)
C7—C8—H8	111.1 (13)	H19A—C19—H19C	108 (3)
C8—C9—H9A	109.7 (17)	H19B—C19—H19C	103 (3)
C8—C9—H9B	111 (2)	C18—C20—H20A	106.6 (18)
H9A—C9—H9B	111 (3)	C18—C20—H20B	110.9 (19)
C8—C9—H9C	110.6 (19)	H20A—C20—H20B	113 (3)
H9A—C9—H9C	107 (3)	C18—C20—H20C	107.2 (16)
H9B—C9—H9C	107 (3)	H20A—C20—H20C	115 (2)
C8—C10—H10A	109.7 (17)	H20B—C20—H20C	104 (3)

C5—N1—C1—O1	-176.78 (17)	C5—C7—C8—C10	-59.7 (2)
C5—N1—C1—C2	3.8 (2)	C15—N3—C11—O3	-178.58 (17)
O1—C1—C2—C3	-4.2 (4)	C15—N3—C11—C12	1.6 (2)
N1—C1—C2—C3	175.3 (3)	O3—C11—C12—C13	-1.9 (4)
O1—C1—C2—C4	-179.85 (18)	N3—C11—C12—C13	177.9 (3)
N1—C1—C2—C4	-0.40 (19)	O3—C11—C12—C14	-178.91 (18)
C3—O2—N2—C4	-0.65 (19)	N3—C11—C12—C14	0.91 (19)
C4—C2—C3—O2	-0.5 (2)	C14—C12—C13—O4	0.7 (2)
C1—C2—C3—O2	-176.2 (3)	C11—C12—C13—O4	-176.3 (2)
C4—C2—C3—C6	-179.4 (2)	C14—C12—C13—C16	-179.6 (2)
C1—C2—C3—C6	4.9 (5)	C11—C12—C13—C16	3.4 (5)
N2—O2—C3—C2	0.7 (2)	N4—O4—C13—C12	-0.2 (2)
N2—O2—C3—C6	179.83 (16)	N4—O4—C13—C16	-179.92 (17)
O2—N2—C4—C2	0.3 (2)	O4—N4—C14—C12	1.00 (19)
O2—N2—C4—C5	-179.0 (2)	O4—N4—C14—C15	-178.84 (18)
C3—C2—C4—N2	0.1 (2)	C13—C12—C14—N4	-1.1 (2)
C1—C2—C4—N2	177.61 (16)	C11—C12—C14—N4	177.12 (15)
C3—C2—C4—C5	179.57 (15)	C13—C12—C14—C15	178.75 (15)

C1—C2—C4—C5	-2.9 (2)	C11—C12—C14—C15	-3.00 (19)
C13—O4—N4—C14	-0.52 (19)	C11—N3—C15—C14	-3.19 (19)
C1—N1—C5—C4	-5.2 (2)	C11—N3—C15—C17	-125.67 (17)
C1—N1—C5—C7	-126.89 (17)	N4—C14—C15—N3	-176.6 (2)
N2—C4—C5—N1	-176.0 (2)	C12—C14—C15—N3	3.59 (18)
C2—C4—C5—N1	4.63 (19)	N4—C14—C15—C17	-55.3 (3)
N2—C4—C5—C7	-54.8 (3)	C12—C14—C15—C17	124.89 (16)
C2—C4—C5—C7	125.87 (17)	N3—C15—C17—C18	-68.0 (2)
N1—C5—C7—C8	-65.9 (2)	C14—C15—C17—C18	178.84 (16)
C4—C5—C7—C8	-178.82 (16)	C15—C17—C18—C19	-67.4 (2)
C5—C7—C8—C9	178.48 (18)	C15—C17—C18—C20	170.04 (16)

Table 24. Hydrogen-bond geometry (Å, º)for 6-(1-methylethyl)-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (125b)

D—H•••A	D—H	H•••A	D•••A	D—H•••A
N1—H1•••O3	0.93 (3)	1.94 (3)	2.855 (2)	169 (2)
N3—H3•••O1	0.90 (2)	2.01 (2)	2.905 (2)	171 (2)

Computing details

Data collection: Bruker APEX 2; cell refinement: Bruker SAINT; data reduction: Bruker SAINT; program(s) used to solve structure: SHELXS97 (Sheldrick, 2008); program(s) used to refine structure: SHELXL2012 (Sheldrick, 2012); molecular graphics: Bruker SHELXTL; software used to prepare material for publication: Bruker SHELXTL.

Special details

Geometry. All esds (except the esd in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell esds are taken into account individually in the estimation of esds in distances, angles and torsion angles; correlations between esds in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell esds is used for estimating esds involving l.s. planes.

4.11.3 – (S,E)-5-(But-2-enoyl)-6-isopropyl-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**139a**) X-Ray Data

X-Ray data has also been identified for (S,E)-5-(But-2-enoyl)-6-isopropyl-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**139a**), Figure 29, as prepared by the T3P route described above.

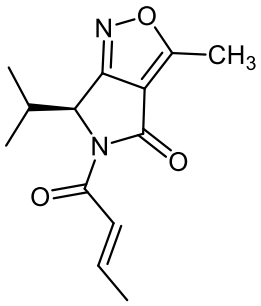
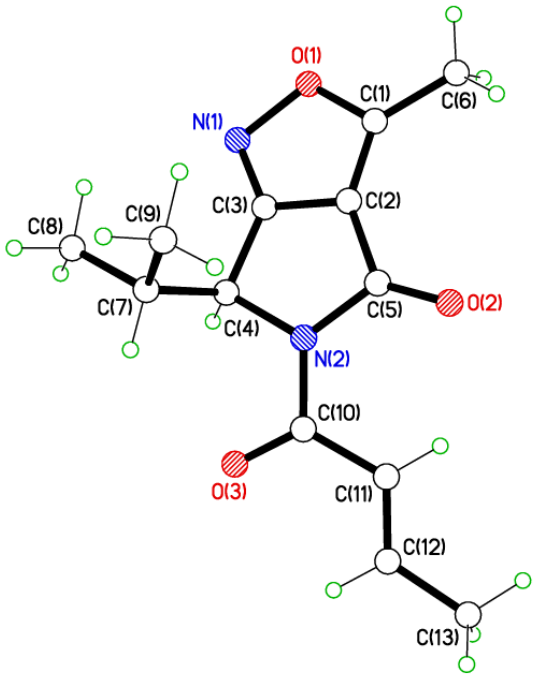


Figure 30. Structural illustration of (S,E)-5-(But-2-enoyl)-6-isopropyl-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (139a)

Rcfj51.figs



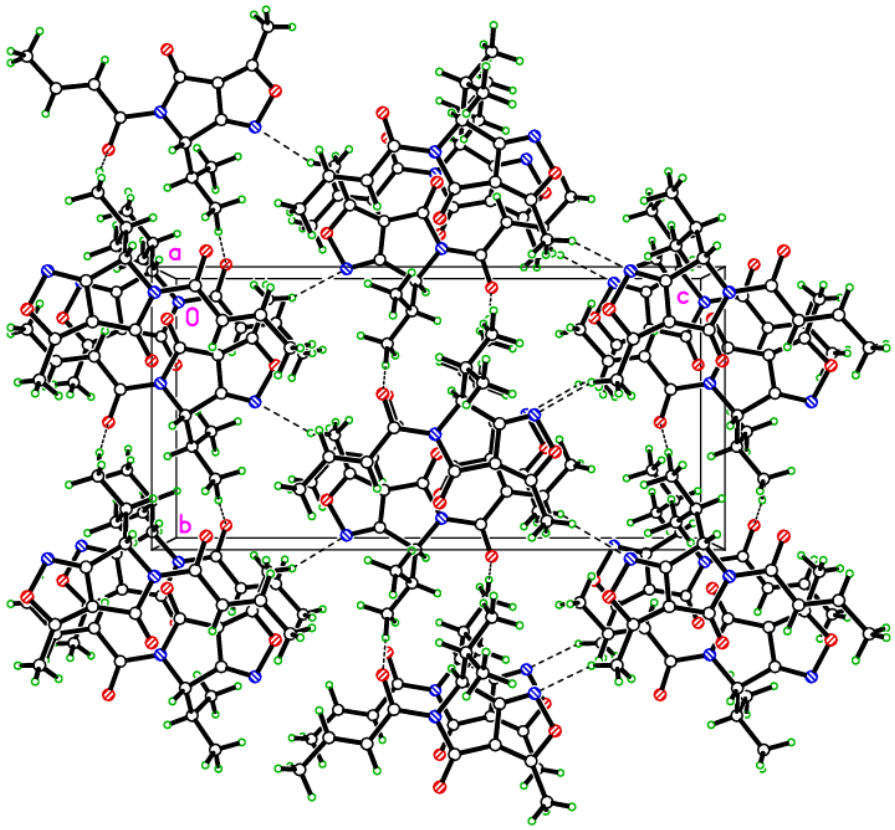


Table 25. Crystal Data for (S,E)-5-(But-2-enoyl)-6-isopropyl-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (139a)

$C_{13}H_{16}N_2O_3$	**D_x = 1.294 Mg m^{-3}**
M_r = 248.28	Mo *K*a radiation, l = 0.71073 Å
Orthorhombic, $P2_12_12_1$	Cell parameters from 3130 reflections
a = 6.9698 (12) Å	q = 2.4–23.3°
b = 9.5108 (16) Å	m = 0.09 mm^{-1}
c = 19.233 (3) Å	T = 150 K
V = 1274.9 (4) Å^3	Lath, colourless
Z = 4	1.70 × 0.28 × 0.07 mm
F(000) = 528	

Table 26. Data collection for (S,E)-5-(But-2-enoyl)-6-isopropyl-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**139a**)

Bruker APEX 2 CCD diffractometer	**2390 reflections with *I* > 2s(*I*)**
Radiation source: fine-focus sealed tube	R_{int} = 0.034
w rotation with narrow frames scans	q_{max} = 26.4°, q_{min} = 2.1°
Absorption correction: multi-scan *SADABS* v2012/1, Sheldrick, G.M., (2012)	*h* = -8®8
T_{min} = 0.858, T_{max} = 0.994	*k* = -11®11
11350 measured reflections	*l* = -24®23
2618 independent reflections	

Table 27. Refinement for (S,E)-5-(But-2-enoyl)-6-isopropyl-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**139a**)

Refinement on F^2	**Secondary atom site location: all non-H atoms found by direct methods**
Least-squares matrix: full	Hydrogen site location: difference Fourier map
$R[F^2 > 2s(F^2)]$ = 0.032	All H-atom parameters refined
$wR(F^2)$ = 0.078	$w = 1/[s^2(F_o^2) + (0.0451P)^2 + 0.0725P]$ where $P = (F_o^2 + 2F_c^2)/3$
S = 1.03	$(D/s)_{max} < 0.001$
2618 reflections	$D\tilde{n}_{max}$ = 0.16 e Å^{-3}

227 parameters	$D\tilde{n}_{min}$ = -0.15 e Å^{-3}
0 restraints	Absolute structure: Flack x determined using 939 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons and Flack (2004), Acta Cryst. A60, s61).
Primary atom site location: structure-invariant direct methods	Flack parameter: 0.0 (6)

Table 28. Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å^2) for (S,E)-5-(But-2-enoyl)-6-isopropyl-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**139a**)

	x	***y***	***z***	U_{iso}*/U_{eq}
N1	0.6760 (3)	0.47667 (18)	0.17030 (8)	0.0289 (4)
O1	0.6596 (2)	0.34386 (15)	0.20662 (7)	0.0294 (4)
C1	0.5953 (3)	0.2431 (2)	0.16305 (10)	0.0251 (4)
C2	0.5658 (3)	0.3043 (2)	0.09976 (10)	0.0227 (4)
C3	0.6192 (3)	0.4450 (2)	0.10789 (10)	0.0231 (4)
C4	0.5887 (3)	0.5260 (2)	0.04159 (9)	0.0225 (4)
H4	0.704 (3)	0.563 (2)	0.0234 (11)	0.020 (5)*
N2	0.5322 (2)	0.40813 (16)	-0.00600 (8)	0.0219 (4)
C5	0.5092 (3)	0.2767 (2)	0.02828 (9)	0.0215 (4)
O2	0.4512 (2)	0.16826 (13)	0.00244 (7)	0.0276 (3)
C6	0.5773 (4)	0.0993 (2)	0.19076 (12)	0.0341 (5)

H6A	0.491 (5)	0.044 (3)	0.1630 (16)	0.068 (9)*
H6B	0.690 (7)	0.052 (4)	0.191 (2)	0.100 (13)*
H6C	0.506 (4)	0.092 (3)	0.2330 (14)	0.055 (8)*
C7	0.4362 (3)	0.6423 (2)	0.04790 (11)	0.0273 (5)
H7	0.416 (3)	0.679 (2)	0.0018 (11)	0.025 (5)*
C8	0.5136 (4)	0.7610 (2)	0.09322 (13)	0.0359 (5)
H8A	0.644 (4)	0.802 (2)	0.0798 (14)	0.039 (7)*
H8B	0.419 (4)	0.842 (3)	0.0956 (14)	0.058 (8)*
H8C	0.536 (4)	0.731 (2)	0.1422 (14)	0.041 (7)*
C9	0.2457 (3)	0.5881 (3)	0.07569 (14)	0.0353 (5)
H9A	0.196 (4)	0.512 (3)	0.0474 (13)	0.037 (6)*
H9B	0.257 (4)	0.549 (3)	0.1259 (14)	0.046 (7)*
H9C	0.147 (4)	0.667 (3)	0.0803 (15)	0.052 (8)*
C10	0.5543 (3)	0.4308 (2)	-0.07795 (10)	0.0245 (4)
O3	0.5795 (3)	0.55123 (15)	-0.09796 (7)	0.0363 (4)
C11	0.5554 (3)	0.3089 (2)	-0.12532 (10)	0.0253 (4)
H11	0.532 (3)	0.216 (2)	-0.1071 (11)	0.029 (6)*
C12	0.5902 (3)	0.3271 (2)	-0.19257 (10)	0.0284 (5)
H12	0.610 (4)	0.422 (3)	-0.2100 (12)	0.036 (6)*
C13	0.6051 (4)	0.2113 (3)	-0.24419 (13)	0.0388 (6)
H13A	0.517 (4)	0.226 (3)	-0.2797 (14)	0.047 (8)*

H13B	0.736 (5)	0.208 (3)	-0.2643 (14)	0.054 (8)*
H13C	0.582 (4)	0.116 (3)	-0.2224 (14)	0.056 (8)*

Table 29. Geometric parameters (Å, º) for (S,E)-5-(But-2-enoyl)-6-isopropyl-3-methyl-5,6-dihydro-4H-pyrrolo[3,4-c]isoxazol-4-one (**139a**)

N1—C3	**1.299 (3)**	**C7—C8**	**1.525 (3)**
N1—O1	1.448 (2)	C7—H7	0.96 (2)
O1—C1	1.350 (2)	C8—H8A	1.02 (3)
C1—C2	1.365 (3)	C8—H8B	1.02 (3)
C1—C6	1.473 (3)	C8—H8C	1.00 (3)
C2—C3	1.397 (3)	C9—H9A	0.97 (3)
C2—C5	1.454 (3)	C9—H9B	1.04 (3)
C3—C4	1.505 (3)	C9—H9C	1.02 (3)
C4—N2	1.500 (2)	C10—O3	1.221 (2)
C4—C7	1.539 (3)	C10—C11	1.475 (3)
C4—H4	0.95 (2)	C11—C12	1.327 (3)
N2—C10	1.409 (2)	C11—H11	0.97 (2)
N2—C5	1.422 (2)	C12—C13	1.486 (3)
C5—O2	1.214 (2)	C12—H12	0.97 (2)
C6—H6A	0.96 (3)	C13—H13A	0.93 (3)
C6—H6B	0.91 (4)	C13—H13B	0.99 (3)

C6—H6C	0.95 (3)	C13—H13C	1.01 (3)
C7—C9	1.521 (3)		

C3—N1—O1	102.66 (15)	C8—C7—C4	109.44 (19)
C1—O1—N1	110.25 (14)	C9—C7—H7	108.7 (13)
O1—C1—C2	107.52 (17)	C8—C7—H7	108.1 (12)
O1—C1—C6	117.57 (17)	C4—C7—H7	106.6 (12)
C2—C1—C6	134.88 (19)	C7—C8—H8A	117.0 (14)
C1—C2—C3	105.56 (17)	C7—C8—H8B	110.9 (16)
C1—C2—C5	143.81 (19)	H8A—C8—H8B	107 (2)
C3—C2—C5	110.54 (16)	C7—C8—H8C	112.3 (14)
N1—C3—C2	114.00 (18)	H8A—C8—H8C	102 (2)
N1—C3—C4	134.98 (18)	H8B—C8—H8C	106 (2)
C2—C3—C4	110.96 (16)	C7—C9—H9A	111.5 (15)
N2—C4—C3	99.88 (14)	C7—C9—H9B	112.6 (15)
N2—C4—C7	113.83 (16)	H9A—C9—H9B	106.4 (19)
C3—C4—C7	113.49 (16)	C7—C9—H9C	111.5 (15)
N2—C4—H4	106.1 (13)	H9A—C9—H9C	111 (2)
C3—C4—H4	112.7 (13)	H9B—C9—H9C	103 (2)
C7—C4—H4	110.3 (13)	O3—C10—N2	117.98 (17)
C10—N2—C5	127.03 (15)	O3—C10—C11	122.82 (17)

C10—N2—C4	117.15 (15)	N2—C10—C11	119.13 (16)
C5—N2—C4	113.81 (14)	C12—C11—C10	120.02 (19)
O2—C5—N2	126.48 (17)	C12—C11—H11	120.3 (12)
O2—C5—C2	129.10 (18)	C10—C11—H11	119.7 (13)
N2—C5—C2	104.41 (15)	C11—C12—C13	124.5 (2)
C1—C6—H6A	111.2 (17)	C11—C12—H12	118.9 (14)
C1—C6—H6B	113 (3)	C13—C12—H12	116.6 (14)
H6A—C6—H6B	106 (3)	C12—C13—H13A	109.7 (16)
C1—C6—H6C	115.0 (17)	C12—C13—H13B	110.3 (16)
H6A—C6—H6C	96 (2)	H13A—C13—H13B	109 (2)
H6B—C6—H6C	114 (3)	C12—C13—H13C	112.1 (15)
C9—C7—C8	111.03 (19)	H13A—C13—H13C	109 (2)
C9—C7—C4	112.79 (17)	H13B—C13—H13C	106 (2)

C3—N1—O1—C1	0.3 (2)	C7—C4—N2—C5	-115.02 (18)
N1—O1—C1—C2	-0.9 (2)	C10—N2—C5—O2	-22.4 (3)
N1—O1—C1—C6	177.61 (19)	C4—N2—C5—O2	174.39 (19)
O1—C1—C2—C3	1.1 (2)	C10—N2—C5—C2	158.92 (19)
C6—C1—C2—C3	-177.0 (2)	C4—N2—C5—C2	-4.3 (2)
O1—C1—C2—C5	177.2 (3)	C1—C2—C5—O2	5.6 (5)
C6—C1—C2—C5	-1.0 (5)	C3—C2—C5—O2	-178.4 (2)

O1—N1—C3—C2	0.4 (2)	C1—C2—C5—N2	-175.7 (3)
O1—N1—C3—C4	177.3 (2)	C3—C2—C5—N2	0.2 (2)
C1—C2—C3—N1	-0.9 (2)	N2—C4—C7—C9	59.8 (2)
C5—C2—C3—N1	-178.48 (18)	C3—C4—C7—C9	-53.6 (2)
C1—C2—C3—C4	-178.61 (17)	N2—C4—C7—C8	-176.08 (16)
C5—C2—C3—C4	3.9 (2)	C3—C4—C7—C8	70.6 (2)
N1—C3—C4—N2	177.1 (2)	C5—N2—C10—O3	-177.35 (19)
C2—C3—C4—N2	-5.9 (2)	C4—N2—C10—O3	-14.6 (3)
N1—C3—C4—C7	-61.4 (3)	C5—N2—C10—C11	-0.3 (3)
C2—C3—C4—C7	115.6 (2)	C4—N2—C10—C11	162.47 (17)
C3—C4—N2—C10	-158.75 (16)	O3—C10—C11—C12	1.9 (3)
C7—C4—N2—C10	80.0 (2)	N2—C10—C11—C12	-175.02 (19)
C3—C4—N2—C5	6.3 (2)	C10—C11—C12—C13	176.6 (2)

Computing details

Data collection: Bruker *APEX* 2; cell refinement: Bruker *SAINT*; data reduction: Bruker *SAINT*; program(s) used to solve structure: *SHELXS97* (Sheldrick, 2008); program(s) used to refine structure: *SHELXL2013* (Sheldrick, 2013); molecular graphics: Bruker *SHELXTL*; software used to prepare material for publication: Bruker *SHELXTL*.

Special details

Geometry. All esds (except the esd in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell esds are taken into account individually

in the estimation of esds in distances, angles and torsion angles; correlations between esds in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell esds is used for estimating esds involving l.s. planes.

5.0 – References

1. B. J. L. Royles, *Chem. Rev.*, 1995, **95**, 1981–2001.

2. R. Schobert and A. Schlenk, *Bioorg. Med. Chem.*, 2008, **16**, 4203–21.

3. J. G. Hurdle, A. E. Heathcott, L. Yang, B. Yan, and R. E. Lee, *J. Antimicrob. Chemother.*, 2011, **6**, 1773.

4. C. O. Gitterman, *J. Med. Chem.*, 1965, **8**, 483–6.

5. S. Gabriel, *Ber. Dtsch. Chem. Ges*, 1914, 3033–3037.

6. S. Ito and Y. Hirata, *Tetrahedron Lett.*, 1972, 1181–1185.

7. C. Skylaris, *Chem. Phys.*, 2003, **293**, 355–363.

8. P. S. Steyn and P. L. Wessels, *Tetrahedron Lett.*, 1978, **19**, 4707–4710.

9. K. Saito, T. Yamaguchi, and C. Centre, *J. Chem. Soc. Perkin Transcr. 2*, 1979, 1605–1609.

10. P. L. Steyn, P. S., Wessels, *J. Chem. Soc. Perkin Transcr. 1*, 1980, 1057–1065.

11. P. H. Van Rooyen, *Acta. Cyrstallogr. Sect. C*, 1992, **48**, 551–555.

12. T. Rosett, R. Sankhala, C. Stickings, M. Taylor, and R. Thomas, *Biochem. J.*, 1957, **67**, 390–401.

13. C. Stickings, *Biochem. J.*, 1958, **72**, 332–341.

14. M. Antony, K. Gupta, K. Janardaanan, and N. Mehrota, *Cancer Lett.*, 1991, **61**, 21.

15. C.-Y. Wang, B.-G. Wang, S. Wiryowidagdo, V. Wray, R. van Soest, K. G. Steube, H.-S. Guan, P. Proksch, and R. Ebel, *J. Nat. Prod.*, 2003, **66**, 51–6.

16. H. Burmeister, G. Bennet, R. Vesonder, and C. Hesseltine, *J. Antimicrob. Agents Chemother.*, 1974, 634–637.

17. L. T. Burke, D. J. Dixon, S. V. Ley, and F. Rodríguez, *Org. Lett.*, 2000, **2**, 3611–3613.

18. N. a Butt and C. J. Moody, *Org. Lett.*, 2011, **13**, 2224–7.

19. J. P. Hugot, P. Baujard, and S. Morand, *Nematology*, 2001, **3**, 199–208.

20. R. Capon, C. Skene, E. Lacey, J. Gill, D. Wadsworth, and T. Friedel, *J. Nat. Prod.*, 1999,

62, 1256–9.

21. a Höltzel, M. Gänzle, G. Nicholson, W. Hammes, and G. Jung, *Angew. Chem. Int. Ed. Engl.*, 2000, **39**, 2766–2768.

22. R. Yendapally, J. G. Hurdle, E. I. Carson, R. B. Lee, and R. E. Lee, *J. Med. Chem.*, 2008, **51**, 1487–91.

23. T. Wakimoto, T. Mori, H. Morita, and I. Abe, 2011, 4746–4749.

24. BBC, www.bbc.co.uk [accessed 12.06.2012].

25. U. Marquardt, D. Schmind, and G. Jung, *Synlett*, 2000, **8**, 1131–1132.

26. R. Böhme, G. Jung, and E. Breitmaier, *Helv. Chim. Acta*, 2005, **88**, 2837–2841.

27. T. Suortti, V. A. Wright, and A. Koskinen, *Phytochemistry*, 1983, **22**, 2873–2874.

28. D. J. Klamann, B. Fugmann, and W. Steglich, *Phytochemistry*, 1989, **28**, 3519–3522.

29. M. Matsuda, T. Kobayashi, S. Nagao, T. Ohta, and S. Nozoe, *Heterocycles*, 1996, **43**, 685–690.

30. Y. Wang, S.-P. Yang, Y. Wu, and J.-M. Yue, *Nat. Prod. Res.*, 2004, **18**, 159–62.

31. J. F. Bower, T. Riis-Johannessen, P. Szeto, A. J. Whitehead, and T. Gallagher, *Chem. Commun. (Camb).*, 2007, **2**, 728–30.

32. M. G. Gänzle, *Appl. Microbiol. Biotechnol.*, 2004, **64**, 326–32.

33. S. Gatenbeck and J. Sierankiewicz, *J. Acta. Chem. Scand.*, 1973, 1825–1830.

34. C. Stickings and R. Townsend, *Biochem. J.*, 1961, **78**, 412–420.

35. R. Schobert, M. Dietrich, G. Mullen, and J.-M. Urbina-Gonzalez, *Synthesis (Stuttg).*, 2006, **2006**, 3902–3914.

36. M. Nukina and T. Saito, *Biosci. Biotech. Biochem.*, 1992, 1314–1318.

37. S. Gatenbeck and J. Sierankiewicz, *J. Antimicrob. Agents Chemother.*, 1973, 308–311.

38. M. Isaka, P. Chinthanom, S. Supothina, P. Tobwor, and N. L. Hywel-Jones, *J. Nat. Prod.*, 2010, **73**, 2057–60.

39. E. Marfori, *Tetrahedron*, 2002, **58**, 6655–6658.

40. A. . Fieseler, L.; Hentschel, U.; Grozdanov, L.; Schirmer, D. . Z. Wen, G.; Platzer, M.;

Hrvatin, S.; Butzke, and J. K.; Piel, *Appl. Environ. Microbiol.*, 2007, 2144–2155.

41. S. Gabriel, *Ber. Dtsch. Chem.Ges.*, 1913, 1319–1322.

42. P. G. Williard and S. E. de Laszlo, *J. Org. Chem.*, 1984, **49**, 3489.

43. J. A. King and F. H. McMillan, *J . Am. Chem. Soc*, 1950, 1236–1240.

44. S. Larsen and J. Bernstein, *J. Am. Chem. Soc.*, 1950, 4447–4450.

45. R. N. Lacey, *J. Chem. Soc.*, 1954, 850–853.

46. H. Kohler and H. Gerlach, *Helu. Chim. Acta*, 1984, 1783–1786.

47. S. R. H. B. G. R., P. Lin, and A. J. Poss, *J. Am. Chem. Soc.*, 1985, 1777–1780.

48. J. Poncet, P. Jouin, B. Castro, L. Nicolas, M. Boutar, and A. Gaudemer, *J. Chem. Soc. Perkin Trans. 1*, 1990, 611.

49. S. V Ley, S. C. Smith, and P. R. Woodward, *Tetrahedron*, 1992, **48**, 1145.

50. L. T. Burke, D. J. Dixon, S. V Ley, and F. Rodríguez, *Org. Biomol. Chem.*, 2005, **3**, 274–80.

51. H. Kohl, S. V. Bhat, J. R. Patell, N. M. Ghandi, J. Nazareth, P. V. Divekar, N. J. de Souza, H. G. Bergscheid, and H.-W. Fehlhaber, *Tetrahedron Lett.*, 1974, 983.

52. R. C. F. Jones and S. Sumaria, *Tetrahedron Lett.*, 1978, 3173.

53. R. C. F. Jones and G. E. Peterson, *Tetrahedron Lett.*, 1983, **24**, 475.

54. R. C. F. Jones, M. J. Begley, G. E. Peterson, and S. J. Sumaria, *Chem. Soc., Perkin Trans. 1*, 1990, 1959.

55. Y. Ujihara, K. Nakayama, T. Sengoku, and M. Takahashi, 2012, 1–4.

56. R. Jones, E. Vultoa, K. Duller, P. Carter, and G. Bhalay, *J. Chem. Soc. Perkin Transcr. 1*, 1994, 2513–2515.

57. R. C. Jones and T. a. Pillainayagam, *Synlett*, 2004, 2815–2817.

58. J. J. Talley, D. L. Brown, J. S. Carter, M. J. Graneto, C. M. Koboldt, J. L. Masferrer, W. E. Perkins, and R. S. Rogers, *J. Med. Chem.*, 200AD, **43**, 775.

59. Y. Ueda, T. Doi, J. Tokumaru, and J. L. Willmore, *Mol. Brain Res.*, 2003, **116**, 1–6.

60. M. Dougados, P. Emery, E. M. Lemmel, C. A. Zerbini, S. Brin, and P. Van Riel, *Ann.*

Rheum. Dis., 2005, **64**, 44–51.

61. L. Claisen and O. Lowwan, *Chem. Ber.*, 1888, **21**, 1149.

62. G. Broggini and G. Zecchi, *Synthesis (Stuttg).*, 1999, 905.

63. S. Karlsson and H. E. Hogberg, *Org. Prep. Proceed. Int.*, 2001, **33**, 103.

64. J. March, *Advanced Organic Chemistry*, Wiley, 2007.

65. G. Bianchi, C. De Micheli, R. Gandolfi, and S. Patai, *Supplement A: The Chemistry of Double-Bonded Functional Groups, pt. 1*, 1977.

66. C. Di Valentin, M. Freccero, R. Gandolfi, and A. Rastelli, *J. Org. Chem.*, 2000, **65**, 6112.

67. P. Caramella, R. W. Gandour, J. A. Hall, C. G. Deville, and K. N. Houk, *J. Am. Chem. Soc.*, 1977, **99**, 385.

68. A. Hantzsch and O. W. Schultze, *Chem. Ber.*, 1896, **29**, 699.

69. H. Takayama, *Chem. Pharm. Bull.*, 1978, **26**, 2575.

70. K. B. G. Torssell, *Nitrile Oxides, Nitrones, and Nitronates in Organic Synthesis*, 1988.

71. T. Mukaiyama and T. Hoshino, *J. Am. Chem. Soc.*, 1960, **82**, 5339.

72. U. Schollkopf and P. Tome, *Liebigs Ann.*, 1971, **753**, 135.

73. K. Fukui, T. Yonezawa, T. Nagata, and H. Shingu, *J. Chem. Phys.*, 1554, **22**, 1433.

74. M. B. Smith, *Organic Synthesis 2nd Ed*, 2002.

75. C. Law, Ph. D Thesis, Loughborough University, 2008.

76. F. Carey and R. Sundberg, *Advanced Organic Chemistry 4th Ed.*, Plenum, 2000.

77. R. Huisgen, *Angew. Chem. Int. Ed.*, 1963, **2**, 633–696.

78. G. Stork, S. Danishefsky, and M. Ohashi, *J. Am. Chem. Soc.*, 1967, **89**, 5459.

79. T. A. Pillainayagam, Ph. D Thesis, Loughborough University, 2005.

80. R. Huisgen and W. Mack, *Tetrahedron Lett.*, 1961, 583–586.

81. J. Hamelin, J. Org. Chem., 1998, **9**, 351–354.

82. C. Reichardt, *Solvents and Solvent Effects in Organic Chemistry*, Wiley-VCH Publishers,

3 rd. Edit., 2003.

83. S. Y. Han and Y. A. Kim, *Tetrahedron*, 2004, 2447–22480.

84. J. Sheehan and G. Hess, *J . Am. Chem. Soc*, 1955, **74**, 1067–1068.

85. Archimica, *http://www.euticals.com./attachments/075_EUTICALS_T3P-Coupling-Agents_2012_final_web.pdf*.

86. R. Wells, M. Moccia, and M. F. a. Adamo, *Tetrahedron Lett.*, 2013.

87. J. Clayden, N. Greeves, S. Warren, and P. Wothers, *Organic Chemistry*, 2006.

88. Sigma Aldrich, *www.sigmasldrich.com*, [accessed 1.12.2010].

89. K. Omura and D. Swern, *Tetrahedron*, 1978, **34**, 1651.

90. M. Nitta and T. Kobayashi, *J. Chem. Soc. Perkin. Trans. 1.*, 1985, 1401–1406.

91. R. C. F. Jones, C. E. Dawson, and M. J. O'Mahony, *Synlett*, 1999, **S1**, 873.

92. A. K. Choudhury, Ph. D Thesis, Loughborough University, 2010.

93. R. C. F. Jones, J. P. Bullous, C. C. M. Law, and M. J. R. Elsegood, *Chem. Commun.*, 2013.

94. G. Bhlay, Ph. D Thesis, Loughborough University, 2005.

95. S. Cannizzaro, *Justus Liebigs Ann. Chem.*, 1853, **88**, 129.

96. V. Jager and M. Muller, *Tetrahedron*, 1985, **41**, 3519.

97. K. Phukan, M. Ganguly, and N. Devi, *Synth.Comm.*, 2008, **39**, 2649–2701.

98. T. Greene and P. Wuts, *Protective Groups in Organic Synthesis*, Wiley Publications 2007.

6.0 – Appendix