Studies Towards the Total Synthesis of a Natural Product Alkaloid

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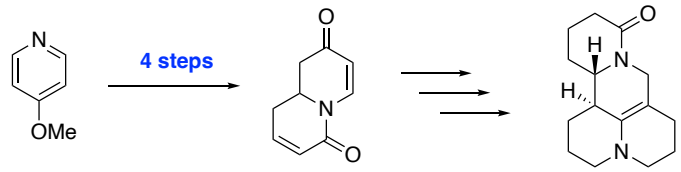
Abbreviations

Aq.	Aqueous
Bu	Butyl
br	broad
DCM	Dichloromethane
DIBAL	Di-isobutylaluminium hydride
DIPEA	*N,N*-Di-isopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	*N,N*-Dimethylformamide
d	doublet
dd	doublet of doublets
dt	doublet of triplets
ddt	doublet of doublet of triplets
ddd	doublet of doublet of doublets
ESI	Electrospray Ionisation
Et	Ethyl
Hz	Hertz
h	Hour(s)
HPLC-MS/MS	High Performance Liquid Chromatography-Mass Spectrometry/Mass Spectrometry
HMPA	Hexamethylphosphoramide
HRMS	High-resolution Mass Spectrometry
*i*Bu	Isobutyl
IR	Infrared
J	Coupling Constant
LDA	Lithium diisopropylamide
LiHMDS	Lithium *bis*(trimethylsilyl)amide
Me	Methyl
min	Minute(s)
M	Molar
m	Multiplet
*n*Bu	*Normal*-butyl
ppm	parts per million
Ph	Phenyl
PPTS	Pyridinium *p*-toluenesulfonate
q	quartet
quin	quintet
RCM	Ring Closing Metathesis
R_f	Retention factor
RNA	Ribonucleic Acid
r.t.	Room temperature
s	singlet

S_N2	Bimolecular Nucleophilic Substitution Reaction
sext	sextet
TBSCl	*tert*-Butyldimethylsilyl chloride
TBAF	*Tetra*-N-butylammonium fluoride
*t*Bu	*Tertiary*-butyl
THF	Tetrahydrofuran
TMEDA	Tetramethylethylenediamine
TLC	Thin Layer Chromatography
TMS	Trimethylsilane
t	triplet
tt	triplet of triplets
UV	Ultraviolet

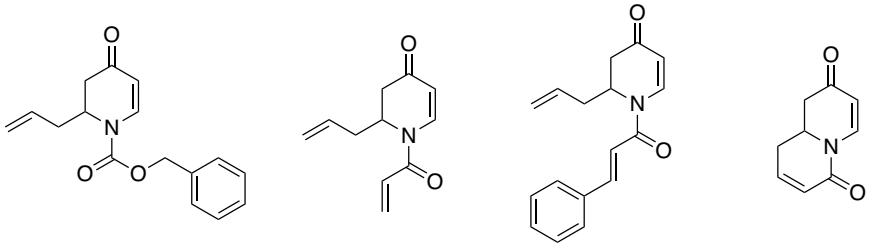
Abstract

This thesis outlines work towards the optimisation and total synthesis of dehydromatrine **[C]**, a quinolizidine alkaloid found in the root of the *Sophora flavescens Aiton* plant. Four steps **[A-B]** in the proposed total synthesis were successfully undertaken and each intermediate that was made was isolated and comprehensively analysed. Each individual step was optimised and enhanced to increase yield by changing solvents, reagents, or equivalents. This was pivotal as due to the total synthetic route having nine hypothesised steps at the start it was crucial that yield was preserved after each reaction. Aligning to this, different proposed synthetic routes had to be updated and changed depending on the feedback gained from each result as sometimes reactions did not work, and yield could not be improved greatly.



[A] [B] [C]

When undertaking this research, the main aims were to ensure that the natural product was created from commercially available starting reagents and once made was analytically identical to the quinolizidine alkaloid **[C]**, that had been extracted from the natural source.



[D] [E] [F] [G]

Several novel molecules **[D-G]** were successfully synthesised using a range of different strategies from the literature and conditions were changed accordingly. These reactions were developed and optimised so that the best possible yields could be attained.

1. Introduction

1.1. Natural Product Synthesis

The first reference of natural product synthesis as a concept occurred in 1828 when Friedrich Wöhler first synthesised urea, **2**, from evaporating a solution of ammonium cyanate, **1,** in water as seen in Figure 1.[1,2] This revelation changed the attitudes of scientists all around the world as they finally realised that natural products could be made from chemicals and did not just have to be produced inside the body.[1] The synthetic industry thrived from this discovery as complex molecular structures could now be formed from simpler starting reagents through the use of well-constructed chemical synthesis routes.[2] Natural product chemistry has a range of different uses varying from structure-elucidation studies used to examine different natural products made in plants and the body to chemical biology, which includes drug discovery or the synthesis of agrochemical products like fertilisers.[1,2]

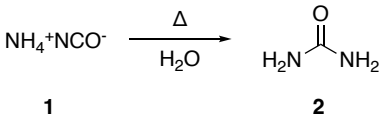


Figure 1. Synthesis of Urea by evaporating a solution ammonium cyanate in water invented by Friedrich Wöhler in 1828.

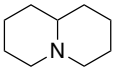
Natural products are important as they offer a range of structurally diverse scaffolds with complex interactions between their atoms and functional groups that can be modified by industry to serve a certain purpose.[3] These molecules have been optimised by Nature for years to help regulate certain biological activities happening throughout the body.[3] Therefore, by studying these molecules, scientists can gain insights into what interactions can be

favourable when creating drugs or fertilisers to be used against certain diseases or pests.[3] For example, knowledge of protein-protein interactions between drugs and their active sites came from the greater rigidity found in natural products.[3] This rigidity can come from the addition of extra cyclic rings or bigger functional groups and can be employed when making more diverse drugs for different therapeutic areas like cancer or infectious diseases.[3]

1.2. Quinolizidine Alkaloids

Quinolizidine alkaloids are categorized as containing at least one quinolizidine ring system in their structure.[4] The quinolizidine ring system, **3**, is composed of two fixed piperidine [5/5] rings that both share the same nitrogen atom as seen in Figure 2.[5] There are seven distinct groups that quinolizidine alkaloids can be named under, for example shown in Figure 3: the lupinine type (**4**), the leontidine type (**5**), the sparteine/lupanine/multiflorine type (**6a, 6b** and **6c**), the α-pyridone type (**7**), the matrine type (**8**), the ormosia type (**9**), and piperidine and dipiperidines (**10**).[4]

The expansive range of biological activities that quinolizidine alkaloids show is due to the accessible lone pair on the tertiary cycloaliphatic amine in the piperidine ring system.[6,7] This nitrogen atom lone pair is free to move into either a *trans* or *cis* arrangement with the angular hydrogen atom as seen in Figure 4.[7]



3

Figure 2. The quinolizidine ring structure.

This can be seen with the example of the quinolizidine alkaloid myrtine.[7] Theoretical calculations of this alkaloid have shown that the energies of the three conformers, **11**, **12** and **13**, are very much alike leading to it existing in all three forms equally.[7] However epimyrtine, exists solely as one conformer, which in this case is the *cis* isomer, **15.**[7] The varying addition of different substituents on the ring may have a pivotal effect on the conformation of the quinolizidine system, which in turn may be the reason for the observed different biological activities of these compounds.[7] Furthermore, due to this stereochemistry, the basicity of these alkaloids varies widely with the freeness of this lone pair. Hence, the more accessible the lone pair is, the more basic the alkaloid.[8]

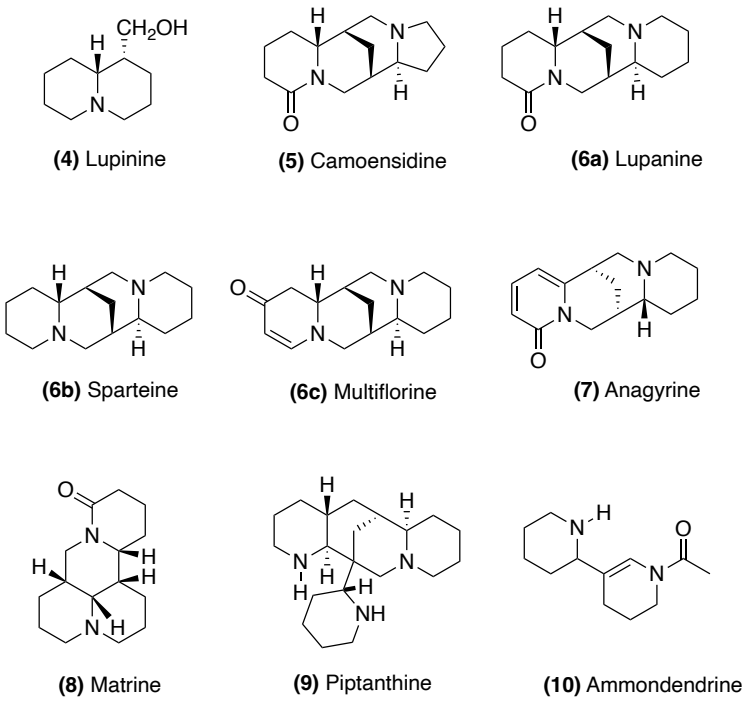


Figure 3. The seven distinct groups of quinolizidine alkaloids; the lupinine type (**4**), the leontidine type (**5**), the sparteine/lupanine/multilorine type (**6a, 6b** and **6c**), the α-pyridone type (**7**), the matrine type (**8**), the ormosia type (**9**), and piperidine and dipiperidines (**10**).

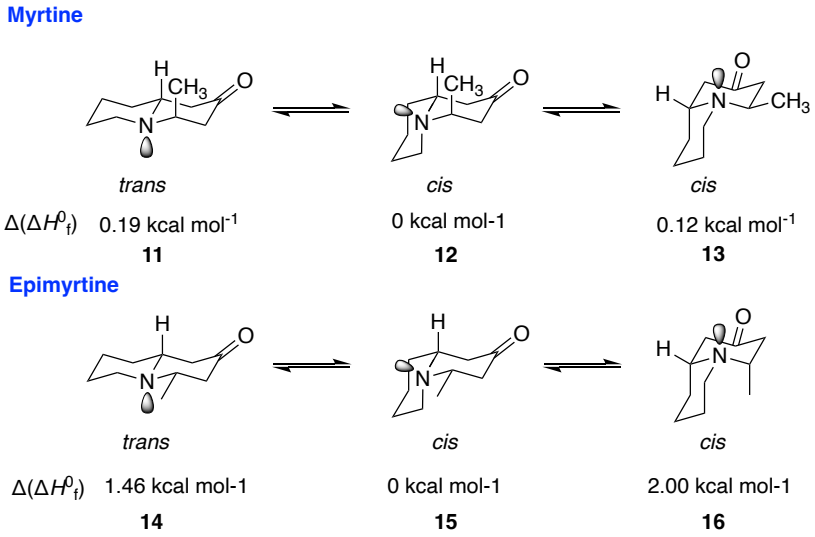


Figure 4. Quinolizidine alkaloids, myrtrine and epimyrtine, in their *cis* and *trans* arrangements between the nitrogen lone pair and angular hydrogen. The corresponding theoretical energies $\Delta(\Delta H^0_f)$ of each conformer are also shown.

Quinolizidine alkaloids are mainly found within the Leguminosae plant family and are especially common in the Genisteae, Sophoreae and Thermopsideae tribes.[1,9] The presence of these alkaloids in plants prevents the feeding of herbivores such as mammals, insects, and pests.[10] These alkaloids are found widely in Australia, China, Japan and North and South America.[11,12]

1.2.1. Biosynthesis of Quinolizidine Alkaloids

In plants, the quinolizidine backbone structure is formed by the oxidative cyclisation of cadaverine, **18**, which is the decarboxylated intermediate made from the essential amino acid lysine, **17** as seen in Figure 5.[13] This reaction takes place because of the enzyme, Lys decarboxylase, which forms a coenzyme (PLP)-dependent decarboxylase intermediate with lysine to initiate the biosynthesis.[14] Amino acids are organic compounds synthesised in the

chloroplast of the plant, which are then transported to other areas to help with different chemical reactions that need to occur or to make new molecules.[15]

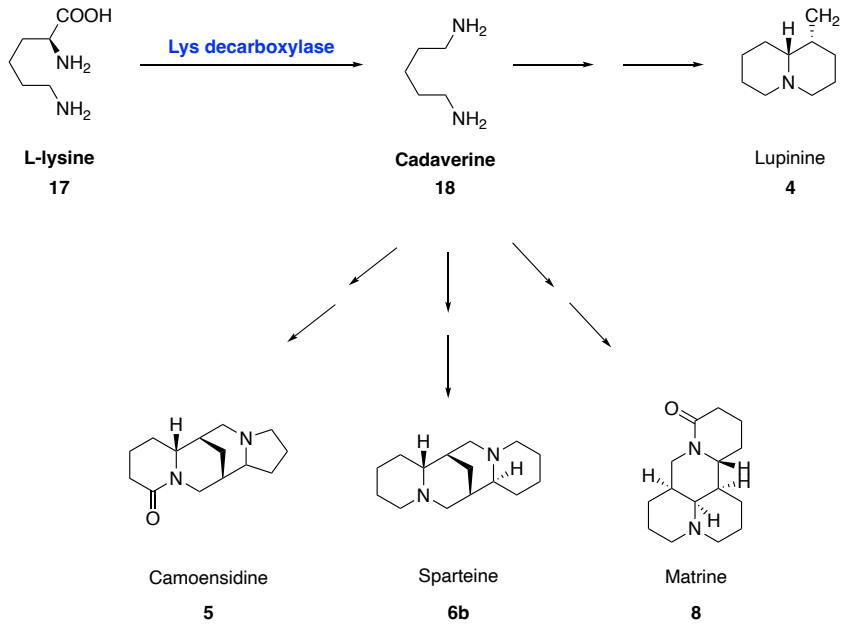
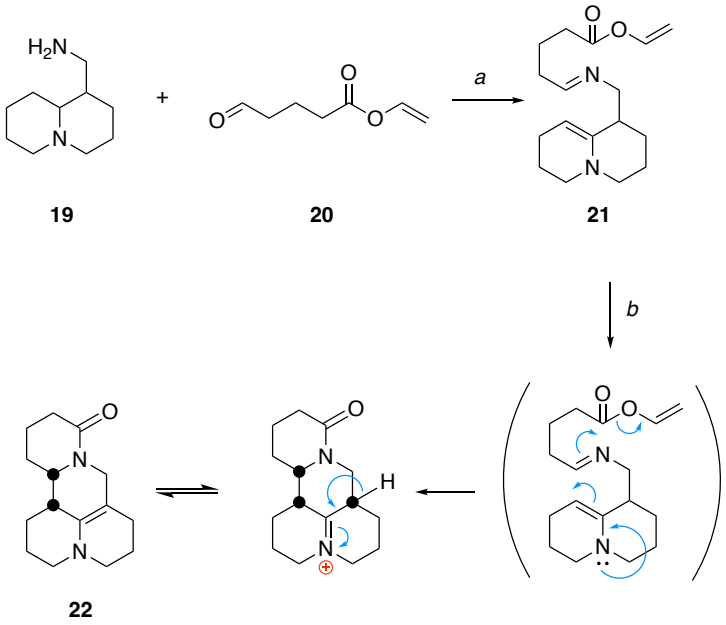


Figure 5. The biosynthesis of different quinolizidine alkaloids starting from L-lysine and catalysed by the enzyme Lys decarboxylase.

After the cyclisation of cadaverine, different quinolizidine alkaloids can be made such as the tetracylic alkaloid (+)-matrine, **8**, or the bicyclic alkaloid (+)-lupinine, **4**, using different modification reactions like esterification or oxygenation.[14] Unfortunately, unlike other alkaloids not much is known about the molecular mechanism for the biosynthesis of quinolizidine alkaloids, especially matrine.[14]

Back in 1966, Bohlmann and colleagues tried to recreate the reaction they proposed happened in plants to make dehydromatrine to gain some insight into how nature may initiate this reaction itself.[16] As little was known at this time Bohlmann and co-workers tried to prove that part of the biogenetic pathway happens through the intermediate **21.**[16] This

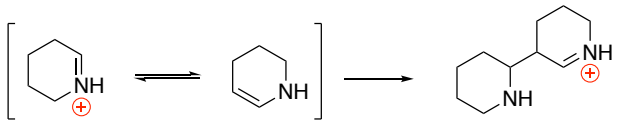
intermediate would have been made, in the plant, by the process mentioned in the above paragraph followed by an esterification reaction and condensation to make the imine group that forms the Schiff base.[14,16] Therefore, this compound was made by reacting **19** with **20** to give the Schiff base, **21**.[16] Compound **21** then undergoes dehydration with mercury(II) acetate in acetonitrile to give **22** in a yield of 5%. The cyclisation reaction mechanism takes place as shown in Scheme 1.[16]



Scheme 1. Biogenetic pathway for the synthesis of matrine-type alkaloids proposed to take place by Bohlmann *et al* in 1966. Reaction conditions *a* = 30% MeOH ammonia solution for 4 h at 150 °C, *b* = mercury(II) acetate in CH_3CN for 20 - 48 h.

In the late 20th century further work was undertaken to investigate and identify the biogenetic route used to form matrine in plants.[17] This involved measuring the distribution of radioactively labelled carbons within the product by using chemical degradation sequences.[14] Leeper *et al* labelled Δ^1-piperideine, **23**, and suggested that this molecule was formed via the intermediate cadaverine after lysine had been decarboxylated.[17] It was thought that three of

these Δ^1-piperidine are then used to make the alkaloid, however two Δ^1-piperideine molecules react first to form tetrahydroanabasine, **24,** as seen in Scheme 2.[17] From here the C_{10} unit of **24** and the C_5 unit of **23** undergo a series of oxidation-reduction and cyclisation steps.[17] Two models were hypothesised to explain how this reaction takes place as shown in Figure 6.[17] Interestingly, from the labelled carbons it was found that some carbons in matrine have different specific activity.[17] This confirms that the red carbon in each model is supplied from **23** whereas the blue carbons, which both have the same specific activity, are supplied from the same C_{10} unit, **24**.[17]



Δ1-piperidine **23**

Tetrahydroanabasine **24**

Scheme 2. Hypothesised formation of tetrahydrobasine from Δ^1-piperidine by Leeper *et al.*

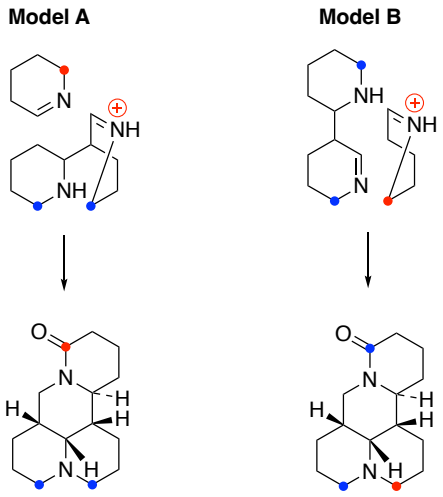
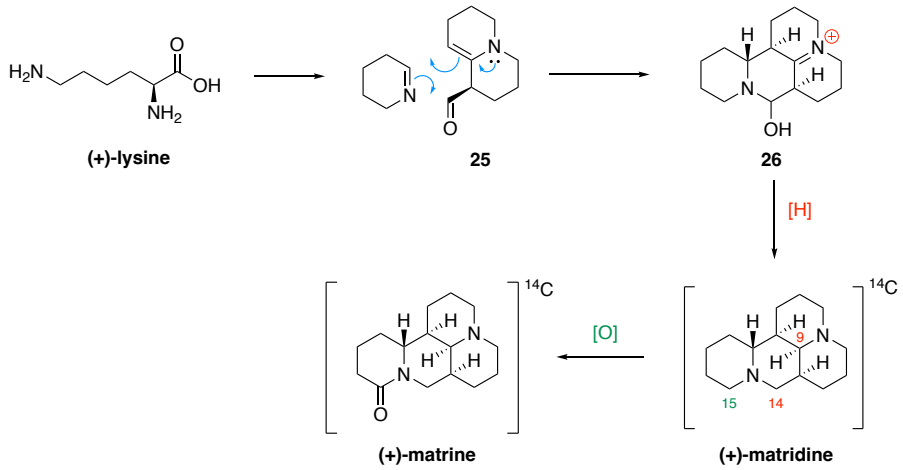


Figure 6. Hypothesised model for the formation of matrine by Leeper *et al.*

This theory was half correct, with Leeper realising that three molecules of Δ^1-piperidine were involved in the biogenetic mechanism and that (-)-lysine is converted into Δ^1-piperidine by

Lys decarboxylase shown in Scheme 3.[17,18] However, with further research conducted in the 21st century it was found that Δ^1-piperidine is dimerized followed by an oxidation and isomerization reaction to give **25**.[18] Compound **25** then undergoes a Mannich addition to a third molecule of Δ^1-piperidine.[17,18] The terminal aldehyde in this molecule cyclizes to generate the fourth fused nitrogen heterocycle backbone with a hydroxy functional group, **26**.[18] When this alcohol is reduced, (+)-matridine, **27**, is formed.[18] Abdusalamov and co-workers investigated the last step of this proposed mechanism by feeding ^{14}C-labelled (+)-matridine to *Goebelia pachycarpa,* where they proved selective oxidation takes place on carbon 15 to form (+)-matrine.[18]



Scheme 3. The modern proposed biosynthesis of matrine with the last step being investigated by Abdusalamov *et al.*

1.3. Matrine-type Alkaloids

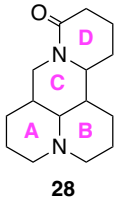
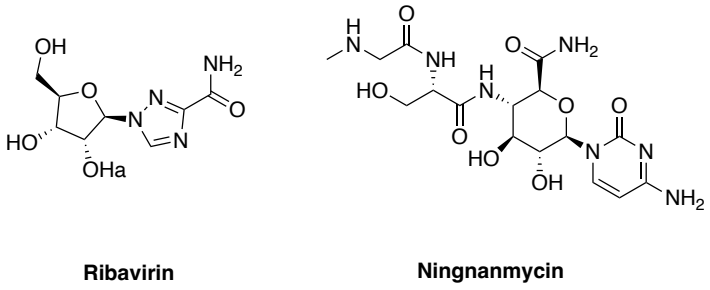


Figure 7. The structure of matrine.

Matrine-type alkaloids have a tetracyclo-quinolizidine structure in which there are two quinolizidine rings fused together with a tertiary amide-like functional group on the D ring as seen in Figure 7.[19] The compound matrine, **28**, was first isolated in 1895 in Japan from the *Sophora flavescens* plant by Professor Nagai's group.[20,21] It was found to have many different biological activities such as antiviral, antitumour, anti-inflammatory, antibacterial and insecticidal.[21] Furthermore, it was also seen to have strong anti-viral activity against Hepatitis B Virus (HBV) which is a serious liver infection that can be lethal.[21,22] Matrine has also been investigated to see whether it has a significant anticancer effect.[19] It has been found to impede cancer cell growth, seize the cell cycle, reduce anticancer drug resistance, encourage the process of cell death and lessen the overall toxicity of anticancer drugs. [19]



Ribavirin **Ningnanmycin**

Figure 8. Structures of the well-known pesticides, ribavirin and ningnanmycin.

As well as having a wide range of medicinal activities, matrine is also used in environmentally-safe pesticides and insecticides.[12] It has especially shown promising activity against the tobacco mosaic virus (TMV).[12] TMV is defined as a single-stranded RNA virus that infects a broad-spectrum of plant species including tobacco, tomato and more with mosaic disease.[23,24] Once infected with the mosaic virus, there is no cure for plants therefore leading to reduced quality of crops and yield.[23,25] To help prevent this, natural products could

potentially be used as treatment to protect plants from TMV whilst at the same time also ideally curing plants that have already been infected.[23] However no such natural product has yet been found that does both.[23] Ribavirin and ningnanmycin, in Figure 8, are examples of pesticides that show only average activity towards inhibiting TMV, therefore proving that there is a need to find better antiviral natural products with a greater effective inhibitory effect against TMV.[23]

Matrine and its derivatives have been used in traditional Chinese medicine for a long time.[19] It is specifically found in Kushen which is the root of the *Sophora flavescens Aiton* plant from the Leguminosae plant family.[19] It is used as an injection to treat lung cancer[26,19], breast cancer[19,27], esophageal cancer[19,28], gastric cancer[19.29], colon cancer[19,30,31], liver cancer[19,32] and pancreatic cancer[19,33].

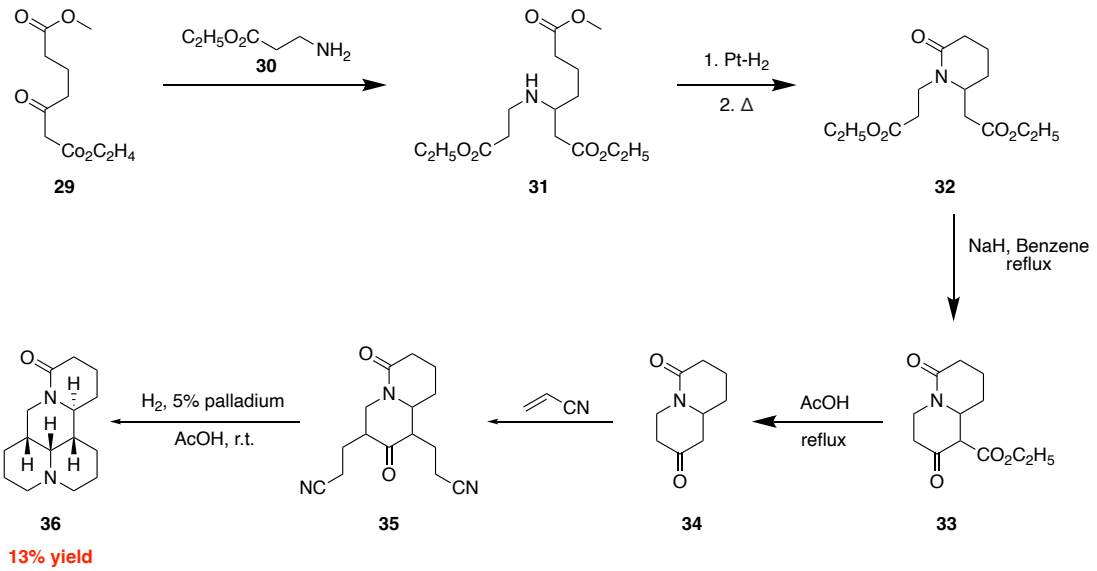
1.3.1 Extraction of Matrine-type Alkaloids

It is now common practice to extract matrine from *Sophora flavescens* Ait. by heating it in aqueous ethanol.[34,35] After decocting, matrine can be purified by dissolving it in 0.5% hydrochloric acid, filtering, performing alkalization using pH 12 ammonia water and then finally using ion exchange resin adsorption.[34,35] When these techniques are fully optimised, a final high purity for extraction of 82% can be achieved.[34] Matrine can be extracted from Kushen in a yield of more than 1g for 10kg of Kushen.[36]

In the literature other extraction methods have been discussed, such as solid phase extraction which is a very rapid technique, however due to its reduced selectivity it is not normally used.[37,38] Counter current chromatography has been tried, which could positively separate a substantial number of products in one sample with a high purity and no column needs to be used.[37,39] As no column is used, there are no problems with column adsorption, degradation, or infinite retention but this technique can require a lot of harmful solvent, which is not favourable from a sustainable point of view.[37,39] Recently, a new technique has been developed using double-templated molecularly imprinted polymers with HPLC-MS/MS, which means the polymers can recognise the desired molecule in the sample and specifically bind to it.[37] This technique can therefore stop similar analytes from eluting at the same time during the separation which is very advantageous. It has also been found that these polymers are easy, cheap, and very stable to make, although complete template removal and loading levels are limitations.[37]

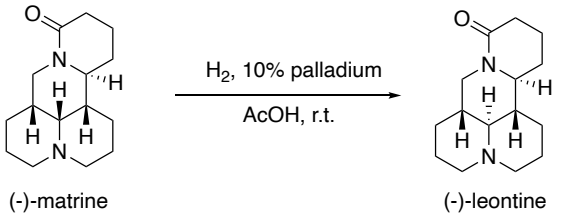
1.4 Total Synthesis of Matrine Through Different Synthetic Methods

1.4.1. First Reported Total Synthesis of Matrine



Scheme 4. The total synthesis of (-)-matrine by Mandell and co-workers in 1965.

The first total synthesis of (±)-matrine, seen in Scheme 4, was reported in 1965 by Mandell *et al*, and took six steps in total and gave the desired product in a low yield of 13%.[40] In this reaction scheme, Mandell and colleagues created the first two heterocycles in the form **34.**[40] This scheme started by condensing **29** with **30**.[40] **31** was reduced over Adams catalyst to give **32** then subjected to a Dieckmann cyclisation to give **33**.[40] The decarboxylation of the Dieckmann product using glacial acetic acid under reflux conditions gave **34** in a moderate 55% yield.[40] This product underwent Stork enamine alkylation with acrylonitrile then cyanoethylation twice to form the dinitrile product, **35**.[40] The final step included the reductive cyclisation of the dinitrile functional groups giving the pure matrine product, **36**.[40]



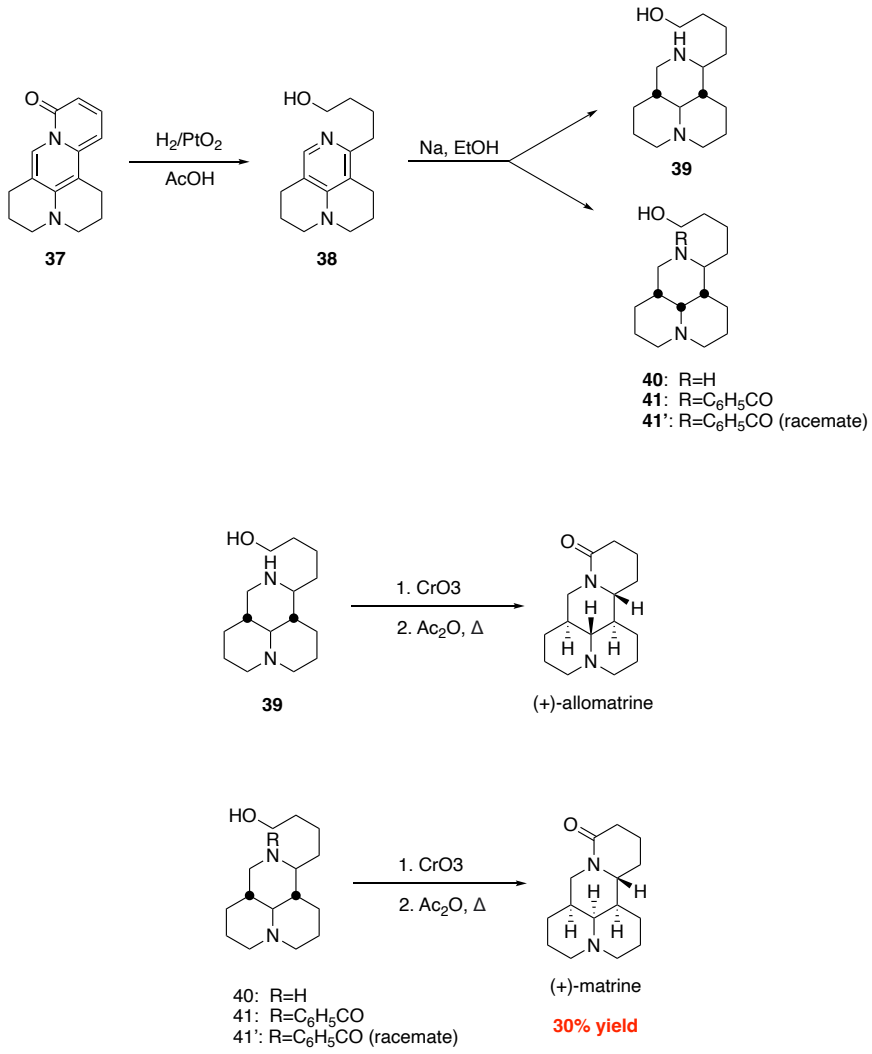
Scheme 5. Formation of (-)-leontine using hydrogenation on palladium performed by Mandell and co-workers.

In this paper, it is also reported that this reductive cyclisation step can be used to form the optical antipode of allomatrine, an isomer of matrine, called *d,l*-leontine.[40] This can be completed by using a higher catalyst loading of 10% palladium on charcoal as seen in Scheme 5.[40]

1.4.2. Synthesis of Optically Active (+)-Matrine

Even though Mandel and co-workers constructed the impressive, first ever total syntheses for matrine, they were not able to accomplish the optical resolution of this alkaloid.[41] Okuda *et al* constructed the second total synthesis of matrine in 1966 as shown in Scheme 6, and were able to find a way to make this optically active compound.[41] First, compound **37** was hydrogenated at room temperature using platinum oxide to form **38.**[41] When **38** is reacted under reflux conditions with a surplus of sodium in ethanol compounds **39** and **40** are made.[41] A large amount of **39** was removed from the reaction mixture by recrystallisation using an acetone-ether solution.[41] After this removal, the remaining reaction mixture was chromatographed many times to give pure **40**.[41] A crystallised (±)-matrinol could not be formed, however the crystallised dibenzoyl-(+)-tartaric acid salt of *N*-benzoyl-(±)-matrinol,

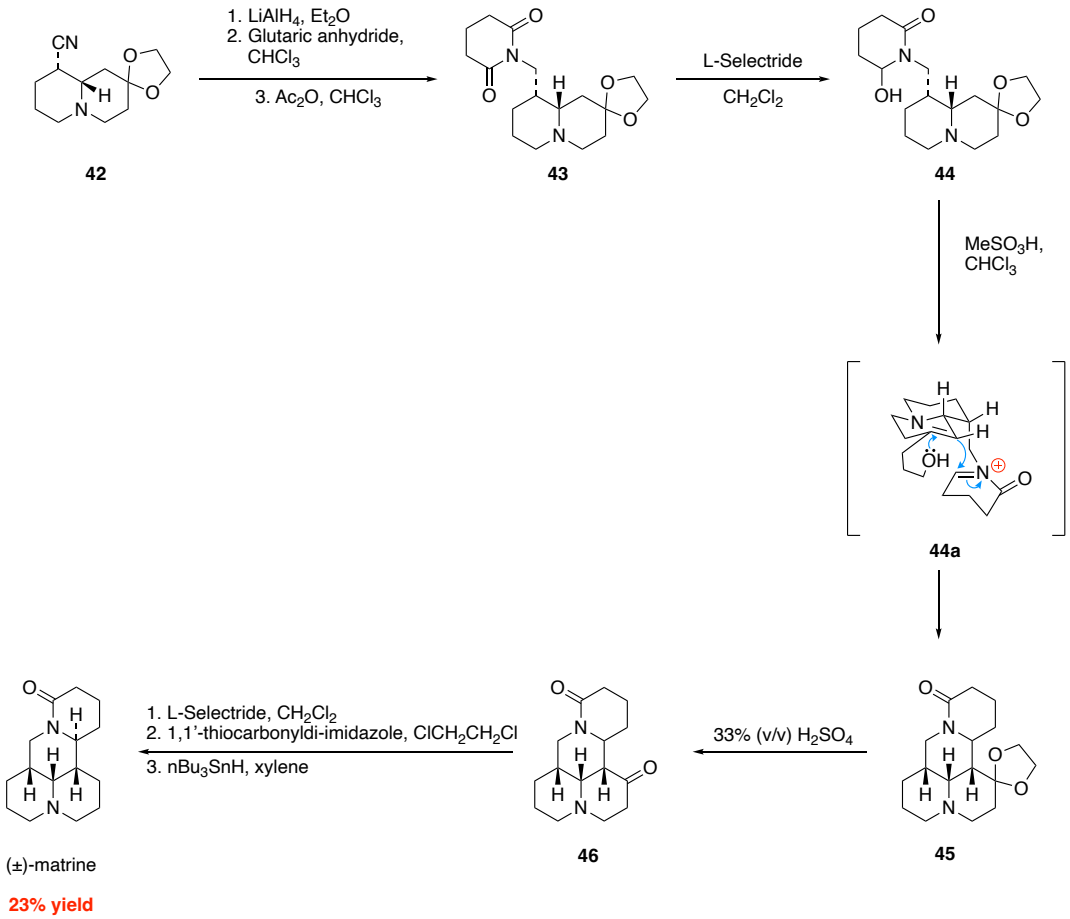
41', was isolated.[41] The optical resolution of this salt was carried out through nine repeated recrystallisations of this compound from acetone to give pure *N*-benzoyl-(+)-matrinol, **41.**[41]



Scheme 6. The synthesis of (+)-matrine by Okuda and co-workers in 1966.

Compound **41** was reduced using lithium aluminium hydride followed by catalytic hydrogenation to give **40**.[41] This now optically pure compound was oxidized using chromium trioxide in 20% sulfuric acid to form the analogous amino acid, which could then be cyclized with the addition of acetic acid to form (+)-matrine in 30% yield.[41]

1.4.3. The Third Total Synthesis Using An *N*-acyliminium Ion Cyclisation Step



Scheme 7. The total synthesis of (±)-matrine by Chen and co-workers in 1986.

In 1986, Chen *et al* demonstrated the total synthesis of matrine in 9 steps, as seen in Scheme 7, whilst also employing an *N*-acyliminium ion cyclisation step to give moderate yield and good stereoselectivity.[42] This step took place using methanesulfonic acid in chloroform for 20 hours at room temperature, which converted **44** to **45**.[42] Cyclisation reactions are defined as the construction of one or more rings in a molecule or chemical compound.[43] This type of cyclisation utilises the Mannich reaction so the molecule is first reduced to an alkenic carbon nucleophile bearing an enol ether functional group.[44,45] This enol ether shows aldol-type

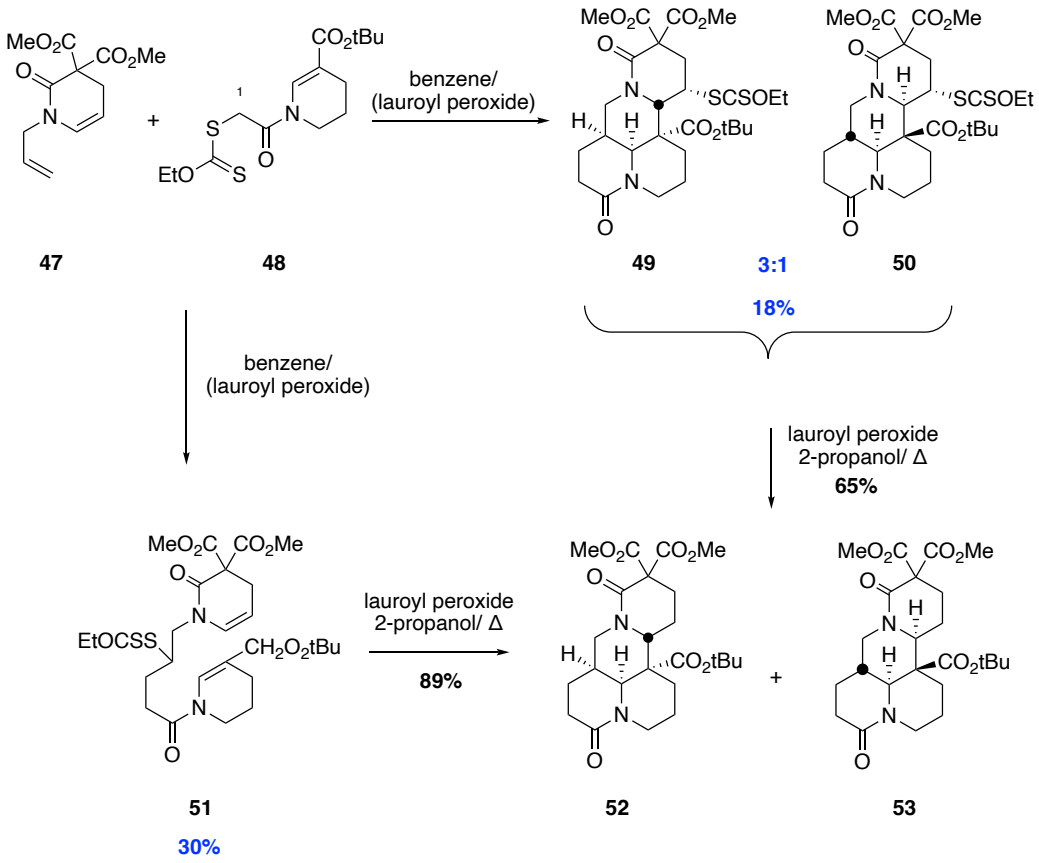
reactivity, when formed by the addition of acid, as it initiates the reaction through the transference of the lone pair of electrons on the alcohol to the double bond in molecule **44a**.[42] As this enol ether is an electron-rich nucleophile it attacks the electron deficient *N*-acyliminium ion, which acts as the electrophilic component to form the 6 membered nitrogen heterocycle.[46] After this, deacetalization and deoxygenation takes place to allow matrine to be made in 23% yield.[42]

1.4.4. Synthesis of Matrine Using Two Consecutive Radical Cyclisations

In the late 20th century, Boiteau and co-workers utilised various radical reactions, including a key cascade radical reaction using a xanthate group (RSSCOEt) to make (±)-matrine.[47] Radicals are atoms, molecules, or ions that have an unpaired valence electron in their molecular orbital.[48] This single valence electron means that radicals have a greater reactivity towards other reagents. In this radical cyclisation, the radical forms when lauroyl peroxide breaks down under heating to form a lauroyl radical, which can then react with the xanthate group on **48** via a Free radical addition-transfer reaction.[49] From here the radical forms on carbon 1, next to the carbonyl in product **48**, and can react with the terminal alkene on **47** initiating the cascade reaction.[47]

The important matrine backbone structure, **49,** was formed in a ratio of 3:1 with the latter being that of the allomatrine backbone, **50**, in a combined 18% yield as seen in Scheme 8.[47] This novel cascade reaction allowed for the creation of four new bonds and five adjacent chiral centres with decent efficiency and adequate stereoselectivity in one step.[47] The

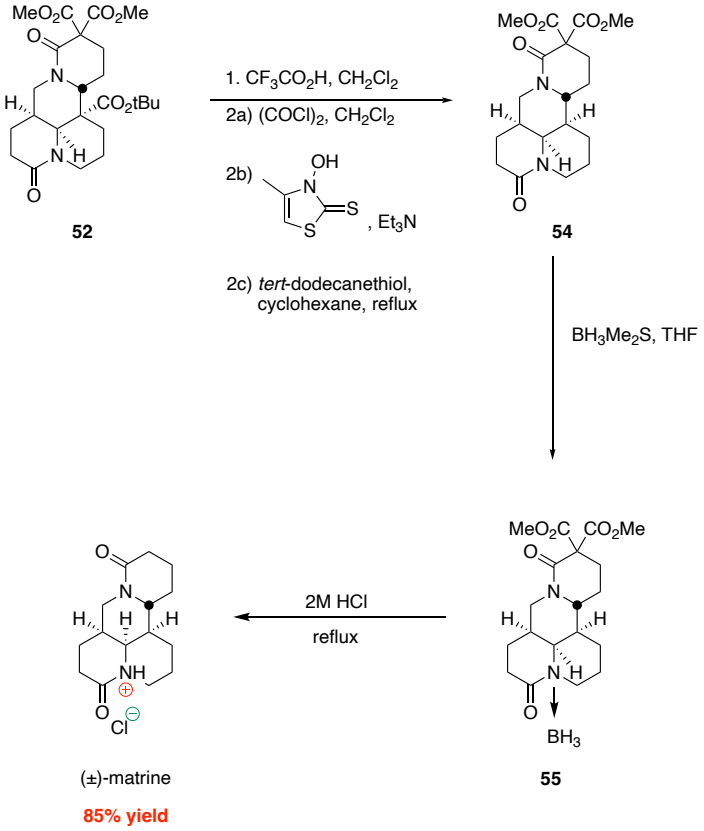
conditions used for this radical cascade involved the heating of products, **47** and **48**, in benzene with the use of a small quantity of lauroyl peroxide which initiated the reaction.[47]



Scheme 8. A Scheme showing the consecutive radical cyclisation reactions performed by Boiteau and co-workers to form **51, 52** and **53.**

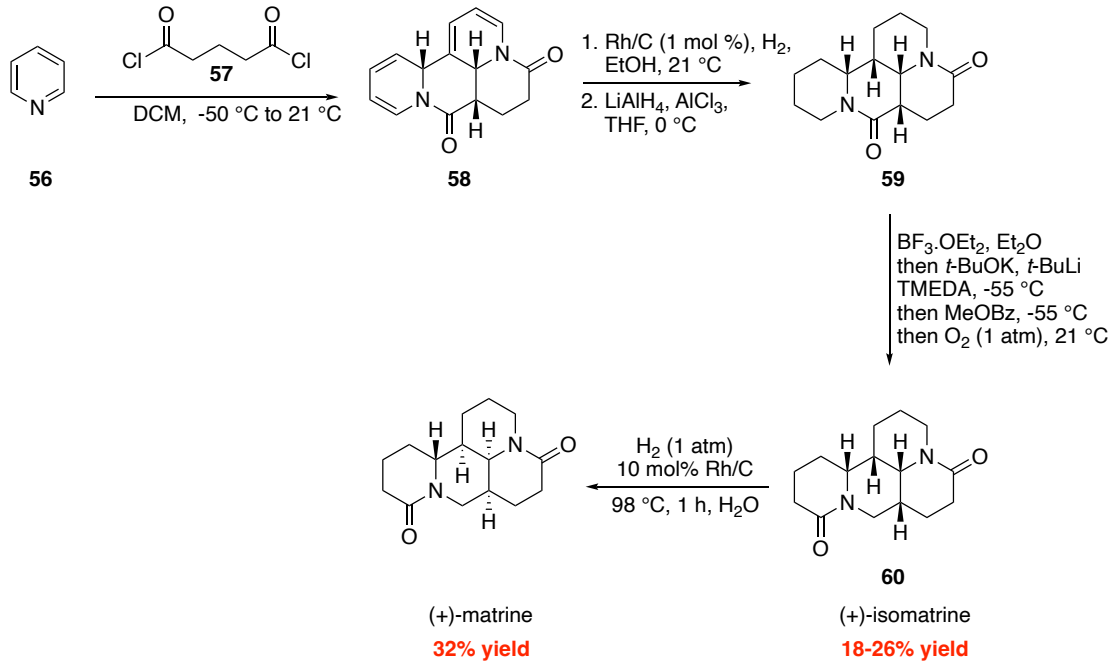
The reductive cleavage of the xanthate group took place under reflux conditions with lauroyl peroxide and 2-propanol.[47] Overall, after this removal products **52** and **53** were made in a yield of 65%.[47] The major product of the first reaction in benzene was the bicyclic intermediate, **51**, due to the slow transfer of the hydrogen from 2-propanol to the radical.[47] This means that this product can directly undergo the same conditions for the reductive cleavage affording the desired tetracyclic products, **52** and **53**, in 89% yield.[47] From here

another four steps, forming product **54** and **55** in the process in Scheme 9, were undertaken to remove other functional groups on the matrine skeleton and in the end afforded (±)-matrine in 85% yield as the hydrochloride salt.[47]



Scheme 9. The final steps of the total synthesis by Boiteau *et al* to make the (+)-matrine hydrochloride salt.

1.4.5. The Most Recent Total Synthesis of Matrine Using Dearomatization of Pyridine



Scheme 10. The total synthesis of (+)-matrine by Kerkovius and co-workers in 2022.

Kerkovius *et al* published a paper very recently, after the practical work for my Master's degree had finished, showing the four-step synthesis of matrine.[18] This synthetic route was based upon the hypothesised biosynthetic route proposed by scientists to explain how this alkaloid is made naturally in plants.[18] This route, shown in Scheme 10, started by reacting **56** with **57** in dichloromethane to form the full tetra-cyclised nitrogen heterocyclic backbone structure, **58**.[18] This elegant reaction formed three of the main carbon chiral centres of (+)-isomatrine in one step, all with the correct stereochemistry.[18] The selectivity determining step to give **58** as the only enantiomer was due to the final deprotonation within the reaction mechanism in Scheme 11 showing syn-syn (±)-**58** being favoured as it follows the lowest energy pathway. Compound **58** was then subjected to hydrogenation using a Rh/C catalyst followed by reduction with alane to fully dearomatize the pyridine and form the last chiral

carbon centre in compound **59**.[18] From here selective deprotonation took place followed by oxidation to form (+)-isomatrine, **60**, in yields of 18-26%.[18] This selective deprotonation was inspired by work done by Kessar *et al* that showed amine-BF_3 adducts could be deprotonated using *tert*-butyl lithium (*t*-BuLi) and potassium *tert*-butoxide.[18] It was possible to trap the deprotonated product with methyl benzoate, which could then be aerobically oxidised to the carbonyl group on (+)-isomatrine, **60**.[18] Compound **60** could then be isomerized with a Rh/C catalyst to form (+)-matrine in 32% yield.[18]

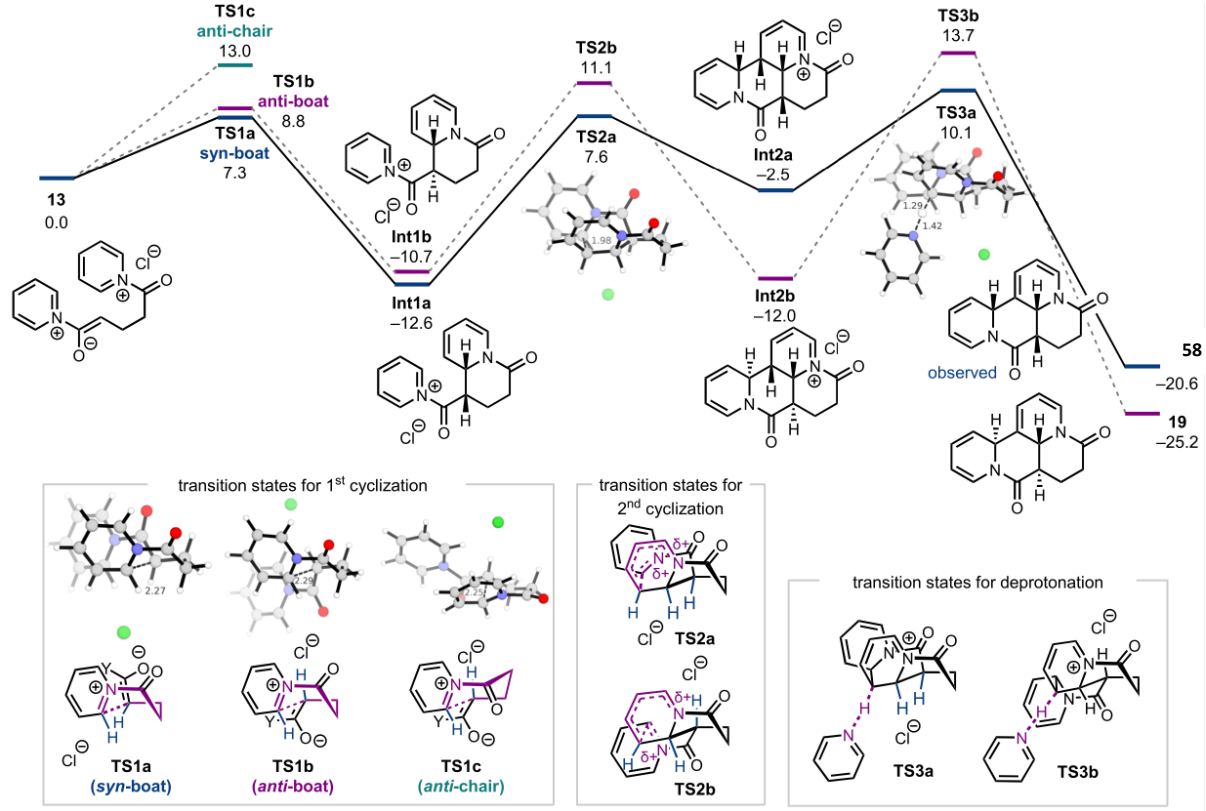


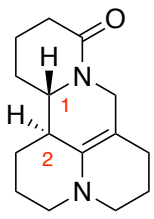
Figure 9. Reaction coordinate diagram performed with Gaussian, using ωB97XD/def2-TZVP/SMD(DCM) by Kerkovius *et al* showing the selectivity for the formation of compound **58** from compound **56** and **57**.[18]

1.5. Project Aims

The aim of the project was to develop a synthetic route for the synthesis of product **67** in Figure 10, using a variety of reactions with good stereoselectivity, optimised conditions and good yield. With matrine only having five reported total syntheses since it was first synthetically created in 1963 and dehydromatrine having none, there was an obvious area to be investigated to try and discover an easier synthetic route to make this product, particularly due to its noted pharmacological activities and its use in agrochemicals.

The main area of challenge when making dehydromatrine is the formation of the correct stereochemistry when synthesising each of the four fused nitrogen heterocycles. In this molecule there are two chiral centres, therefore four different diastereoisomers can be created. Dehydromatrine has an (R,R) configuration with one hydrogen atom facing forwards on one carbon and one hydrogen atom facing backwards on another. We aim to investigate a range of reactions that will create the desired final quinolizidine alkaloid with the correct stereochemistry at carbon 1 and 2 in compound **67** in Figure 10.

The concept of sustainability will be applied along each step of the synthetic route to ensure the target molecule is made from basic, economically attainable starting materials and is analytically indistinguishable from the naturally occurring quinolizidine alkaloid.



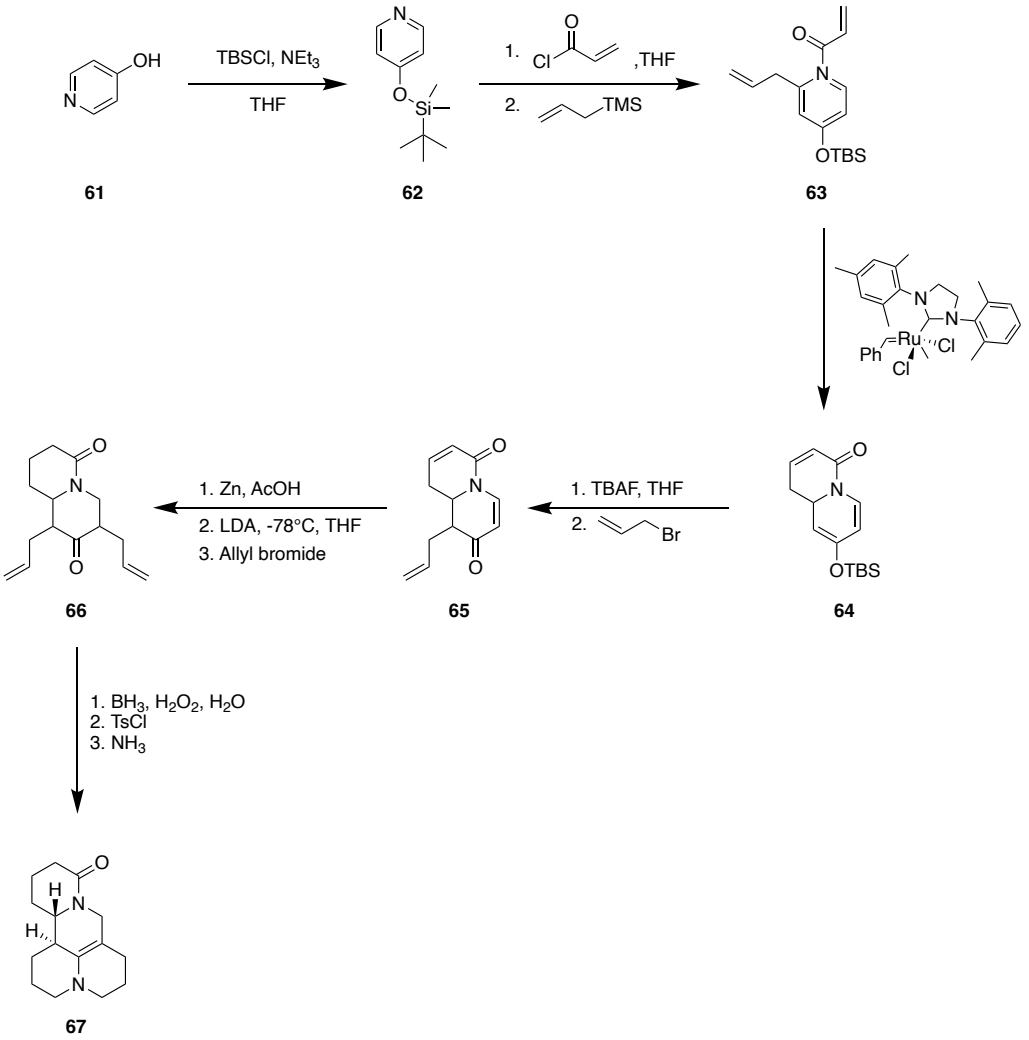
67

Figure 10. Structure of dehydromatrine, **67**.

2. Results and Discussion

2.1 Original Proposed Total Synthesis

The planned synthesis involves starting with the commercially and readily available reagent, 4-hydroxypyridine, which will first be protected as a silyl ether using TBSCl as shown in Scheme 11. After this protection, the addition of the acyl chloride to the nitrogen of compound **63** will be undertaken followed by the *ortho* addition of allyl trimethyl silane. The closing of the first nitrogen heterocycle will then be attempted using the concept of ring closing metathesis, with Grubbs 2nd generation catalyst initiating this reaction.



Scheme 11. Original proposed total synthetic route to make the desired quinolizidine alkaloid, **67**.

This then allows for the deprotection of the silyl ether, leaving an enolate functional group to nucleophilically attack the allyl bromide to form **65**. From here a reduction of the enone components can take place using zinc and acetic acid followed by a deprotonated alkylation sequence with allyl bromide leaving **66**. The last step will utilize a one pot three-step reaction with the initial step being a hydroboration-oxidation reaction using borane, hydrogen peroxide and water. The resulting alcohol groups will then be converted into a tosyl groups followed by the addition of ammonia to create the final two fused nitrogen heterocycles in **67**.

2.1.1. Synthesis of Protected Silyl Ether Intermediate, 62

The route originally started with the protection of the alcohol group on 4-hydroxypyridine using *tert*-butyldimethylsilyl chloride. No reaction was observed when triethylamine was used as the base in DMF so the solvent system was changed to toluene and the reaction was performed under reflux.[50,51] The ^{1}H NMR spectrum of the crude product looked promising as all the correct peaks for the silylated compound were observed with the correct integrations. However, when purifying the crude product using silica gel chromatography, none of the fractions obtained compound **62**. One possible reason for this could be considering the stability of the silyl group on the column as due to the slight acidity of the silica gel this may have led to the occurrence of its hydrolysis.

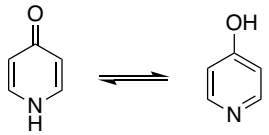


Figure 11. Figure showing the tautomerisation of 4-pyridone to 4-

Under equilibrium conditions, 4-hydroxypyridine can tautomerise into 4-pyridone with it favouring the latter when intermolecular hydrogen bonding is present in solution as shown in Figure 11.[52] Due to the changing forms of the starting material and the weakly acidic nature of silica gel, this could in theory cause the silyl group to potentially fall off providing an explanation as to why **62** wasn't observed after the column. The nitrogen atom on the pyridine ring becomes protonated by the weakly acidic silica which in turn makes the silyl group a good leaving group when water attacks the silicon atom. This water could come from the solvent being used in the column to separate the products as it was not distilled prior to use. As seen in the mechanism in Figure 12, once the silyl group leaves, **62** tautomerises through the 4-pyridone intermediate back into the original 4-hydroxypyridine starting material.

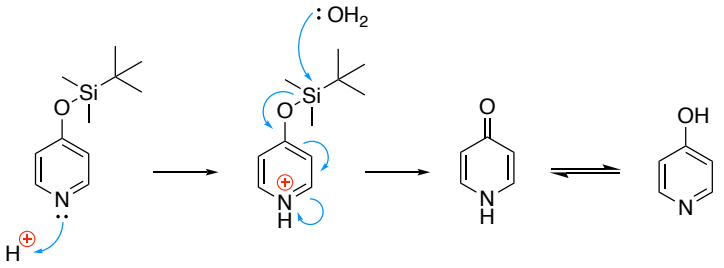
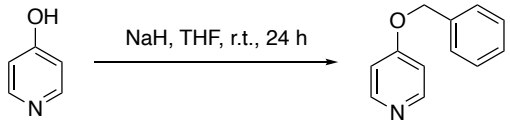


Figure 12. Mechanism for the hydrolysis of the silyl group from compound **62.**

2.1.2. Synthesis of Protected Benzyloxy Intermediate, 68

Due to the problems that had arisen from the use of TBSCl as the protecting group, it was decided that the alcohol group would be protected using benzyl bromide instead. This reaction, as seen in Scheme 12, took place with NaH as a strong base in THF for twenty-four

hours at room temperature.[53] At first, this reaction looked very promising, however after purification only a 2% yield of **68** was achieved. An explanation for this low yield could be because **68** did not elute off the column properly so had to be flushed off with 100% methanol. This elution problem could be a result of the chemisorption of the weakly basic pyridinyl nitrogen with the weakly acidic silanol groups on the surface of the silica gel, leading to the product sticking to the column.[53,55] This sticking would lead to the higher possibility of the product degrading on the column hence lower yield.[56]

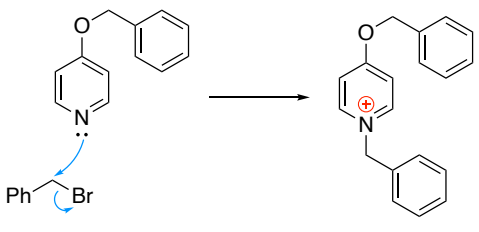


68

2% yield

Scheme 12. Scheme showing the formation of **68**, from 4-hydroxypyridine.

When repeating the reaction another two times, **68** was not made, instead over-benzylation occurred on the nitrogen creating product **69**, which exists in the salt form, even though the same method was followed. The mechanism of this reaction is seen in Figure 13. To try to combat this, the reaction time was reduced to 4 hours instead of overnight, however this did not work and still led to over-benzylation. As NaH has a pK_a of around 35 and the pK_a of 4-hydroxypyridine is only 3.2, a much weaker base needs to be used to stop this over reaction.[57] In the future, an amine base with a pK_a of around 9-11 could be tried like triethylamine.[57]

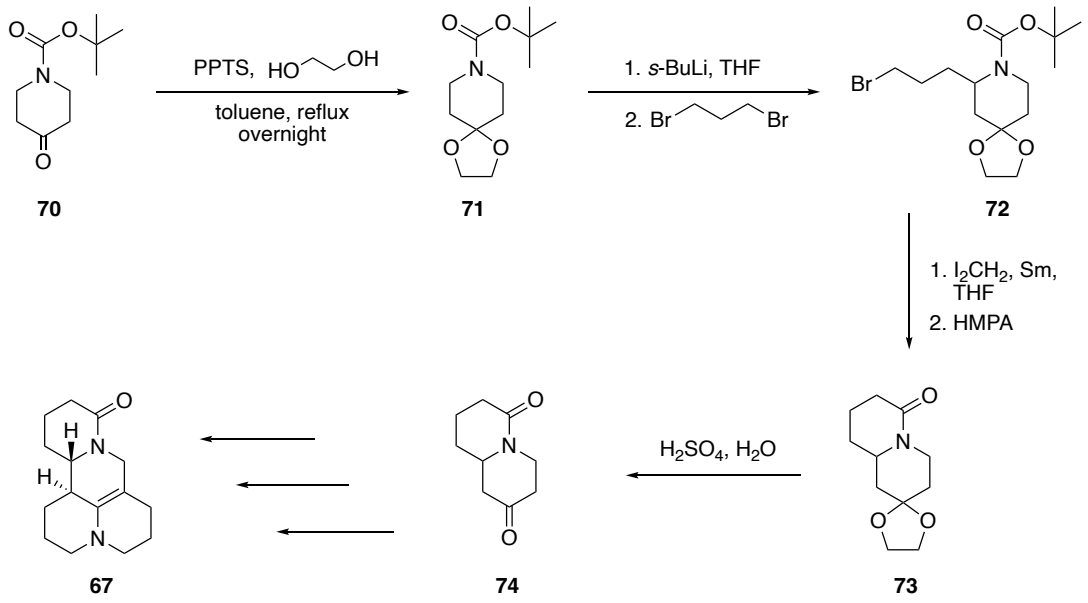


69

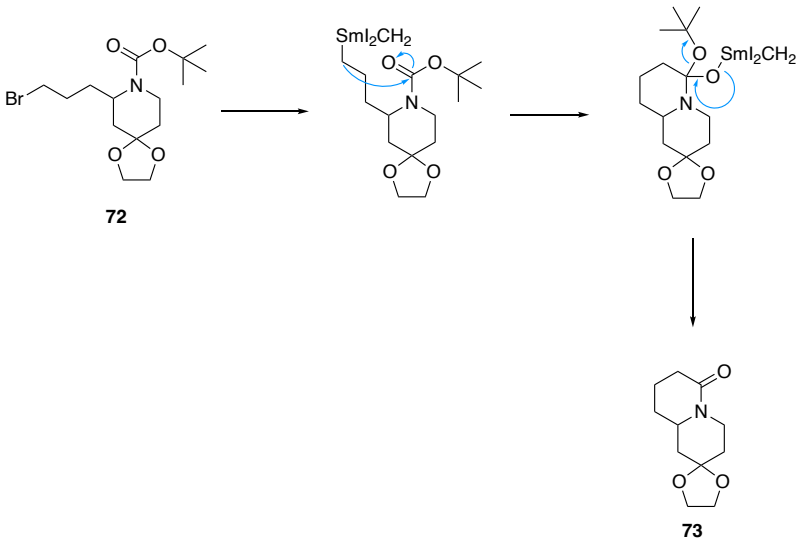
Figure 13. Mechanism for the formation of the overbenzylated product, **69**.

2.2. Proposed Second Synthesis for Tetracyclic Final Product, 67

The second attempted synthetic route followed Scheme 13 whereby the ketone functional group is first protected with an acetal. After this an α-lithiation reaction will take place to allow **71** to act as the nucleophile and attack the 1,3-dibromopropane to form **72**. The second fused nitrogen heterocycle would then be formed from a Barbier-type cyclisation reaction developed by Molander and co-workers.[58] This reaction takes place using samarium diiodide, which forms an organosamarium species in place of the bromine atom is, followed by nucleophilic acyl substitution on the carbonyl of the carboxylated nitrogen atom.[58] This nucleophilic attack leads to a tetrahedral intermediate forming briefly before collapsing to form the amide functional group in the newly made nitrogen heterocycle as shown in the mechanism in Figure 14.[58] Acetal hydrolysis would then take place using sulphuric acid and water as the solvent to form the ketone functionality. A multitude of other reactions would then be tested to form the final alkaloid natural product, **67**.



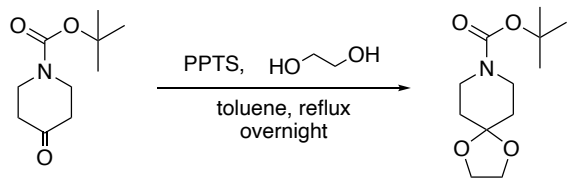
Scheme 13. Second proposed total synthetic route to make the desired alkaloid, **67**.



72 73

Figure 14. Mechanism highlighting the formation of compound **73** from compound **72**.

2.2.1. Synthesis of Carboxylated Intermediate, 71



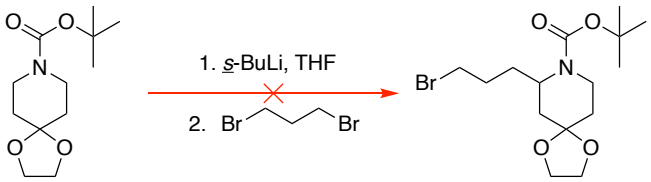
70 **71**

34% yield

Scheme 14. Scheme showing the conditions using for the protection of the carbonyl functional group in **70** to form **71**.

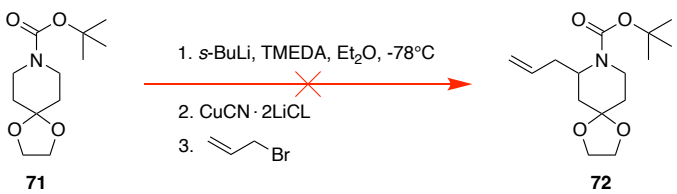
Following on from these results a new route, in Scheme 13, was attempted using a different starting material, **70**. First the ketone was protected by converting it into an acetal group. This reaction, seen in Scheme 14, used ethylene glycol and PPTS as the catalyst in toluene under reflux with Dean-Stark apparatus for eight hours and gave relatively low yields with the highest being 34%.[59] From here an S_N2 reaction was employed to try and allow the addition of 1,3-dibromopropane at the α-position to the protected nitrogen to form product, **72**.[60] *sec*-BuLi was used as a powerful base to deprotonate the alkyl α-carbon due to its high pK_a of

51.[60,61] However, this reaction did not work as only starting material was recovered from the reaction. A reason for this could be because of steric factors due to the bulky tert-butyloxycarbonyl (Boc) protecting group on the nitrogen atom of compound **71.** This extra steric bulk could mean that the alkyl-lithium base may not be able to ensure the selective deprotonation of the correct alkyl carbon *ortho* to the nitrogen.



Scheme 15. Scheme showing the hypothesised formation of compound **72**, however this reaction did not work.

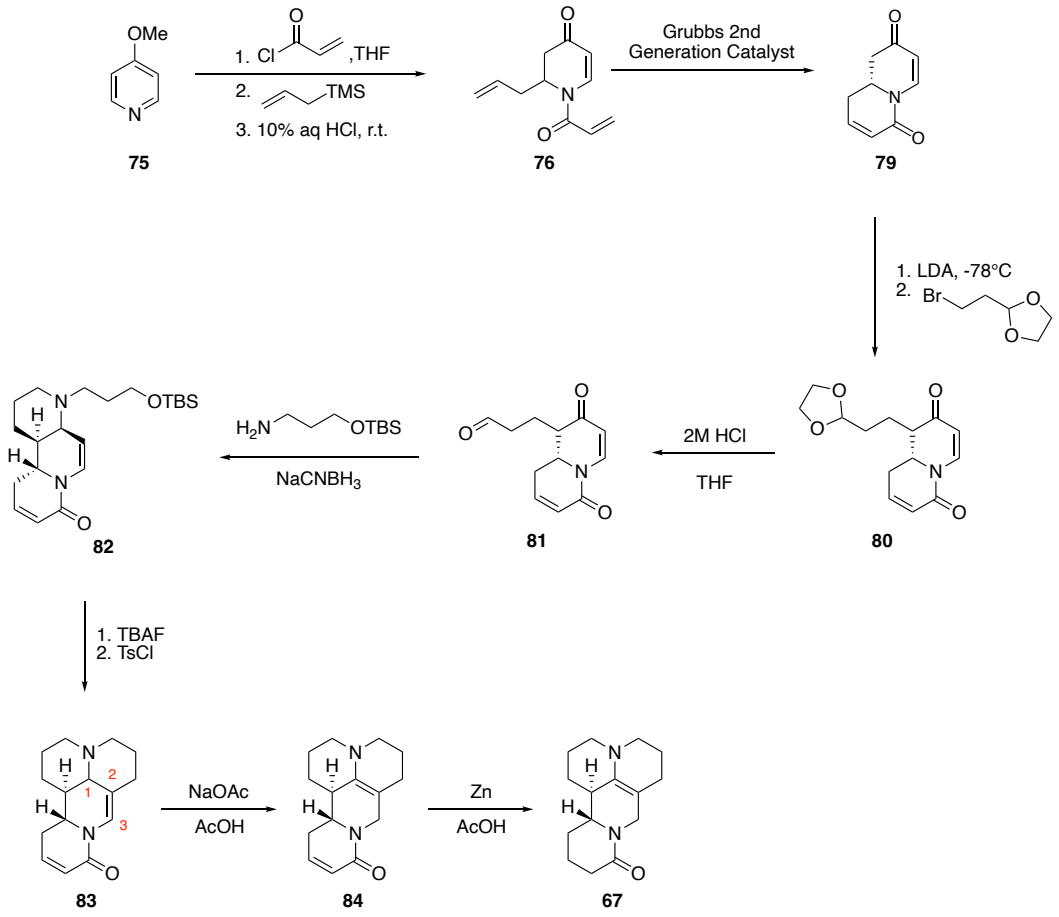
A different reaction for lithiation was attempted, still using *sec*-BuLi as the base but with allyl bromide as the reagent instead alongside TMEDA in diethylether as seen in Scheme 15.[62] A key difference in this reaction was the employment of TMEDA which is used as a ligand to reduce the formation of alkyl lithium aggregates in solution.[63] If these aggregates form, this can lead to reduced activity due to the reduced basicity found when there is less free *sec*-BuLi floating in solution ready to deprotonate.[63] Even though these changes were made, this reaction still did not work which once again may be due to the use of the wrong organolithium reagent.



Scheme 16. Scheme showing the hypothesised formation of compound **72**, however this reaction did not work.

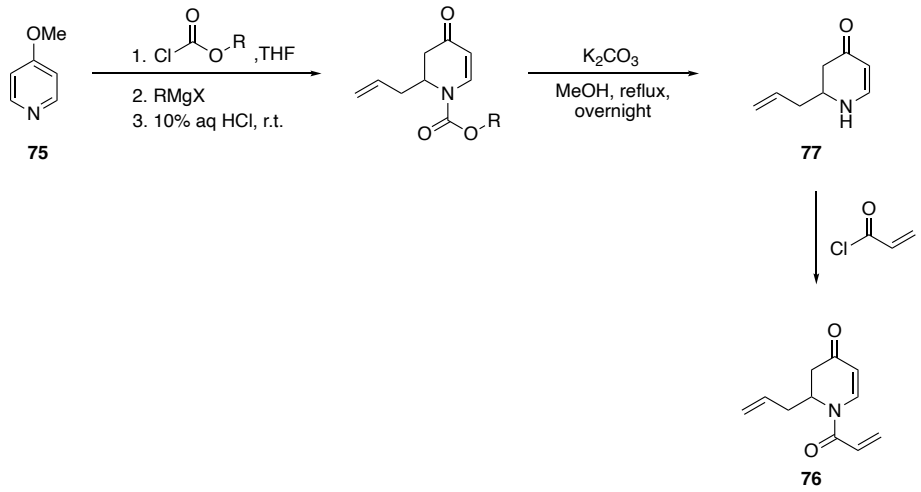
To try and make this step work a reaction demonstrated by Coldham *et al* using an organocuprate compound, otherwise known as Gilman's Reagent, was attempted as these reagents are more effective nucleophiles for S_N2 reactions.[62,64,65] In this reaction, seen in Scheme 16, transmetallation takes place whereby *sec*-BuLi with TMEDA is added first followed by CuCN.2LiCl in diethyl ether at -78°C to make the organocuprate.[62,64] Allyl bromide is then added which reacts with the organocuprate to create the carbon-carbon bond.[62,64,65] The ^1H NMR spectrum of the crude product looked promising as the correct hypothesised peaks for the compound were seen. However, when trying to purify the reaction mixture it appeared that there was an inseparable mixture of five different compounds with very similar retention factors. A gradient column was performed using the solvent systems stated in the literature however the compounds did not separate successfully thereby leading to the next route idea.[62]

2.3. Final Proposed Synthetic Route to Desired Tetracyclic Molecule, 67



Scheme 17. Final proposed total synthetic route for the formation of the desired quinolizidine alkaloid, **67**.

The first reaction, shown in Scheme 17 involves a one pot three-step synthesis in which the tertiary amine on the pyridine ring of **75** nucleophilic attacks acryloyl chloride to form an acylated nitrogen cation. From here allyl TMS reacts like a nucleophile to attach at the *ortho* position next to the nitrogen. Dilute hydrochloric acid will be used to hydrolyse the methyl enol ether to form the carbonyl to form compound **76**. This all happens via the mechanism stated in Figure 15.



Scheme 18. Revised final route for the synthesis of compound **76.**

Grubbs 2nd generation catalyst will be used in a ring closing metathesis reaction to form the second fused nitrogen heterocycle, leaving compound, **79**. After this, it is hoped that LDA deprotonates α to the carbonyl group allowing for the alkylation of the bromo dioxolane compound to give **80**. The acetal group will be hydrolysed to an aldehyde followed by a reductive amination reaction using sodium cyanoborohydride to form the third nitrogen heterocycle.

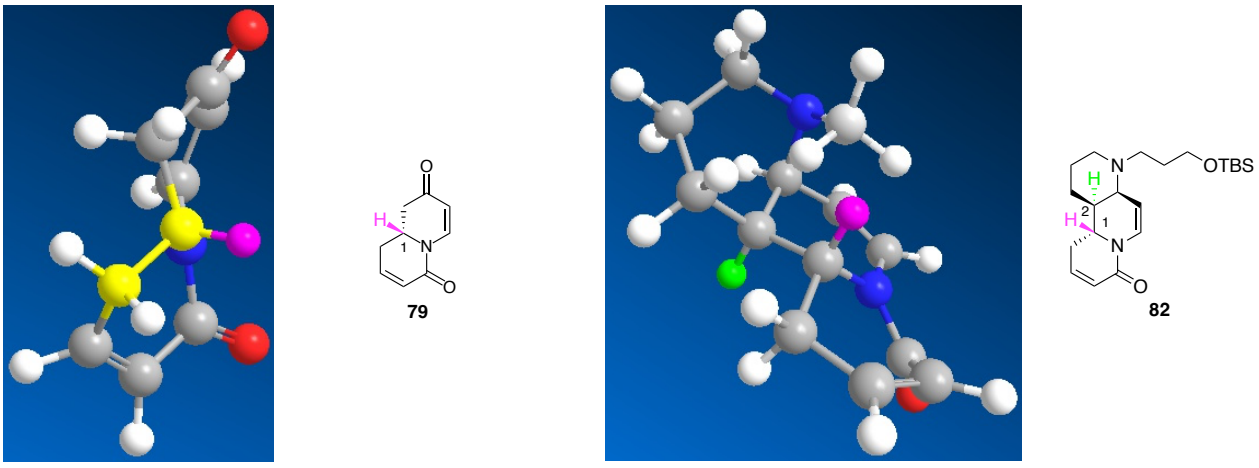


Figure 15. Chem3D images of compounds **79** and **82**. The red atoms correspond to oxygen and the blue atoms correspond to nitrogen. The pink atom corresponds to the forward-facing hydrogen and the green atom corresponds to the backward-facing hydrogen thereby creating the (R,R) stereochemistry of dehydromatrine.

The silyl ether on compound, **82**, is removed using TBAF to form a hydroxy group which will be converted into a tosyl group as this is a better leaving group. This will hopefully allow for the intramolecular cyclisation of the fourth and final nitrogen heterocycle.

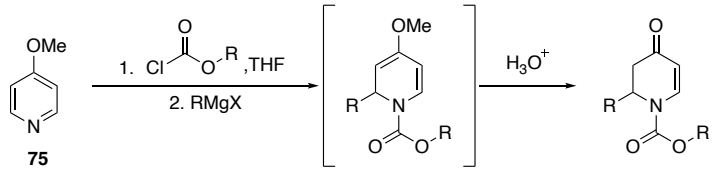
In Figure 15, the Chem3D images for the formation of compounds **79** and **82** can be seen which highlights the installation of the two stereocenter's of dehydromatrine to create the (R,R) configuration. In compound **79**, after ring closing metathesis, the first stereocenter is installed on carbon 1. Following on from this, the formation of the third nitrogen heterocycle installs the second stereocenter on carbon 2 of compound **82** leading to a *trans* relationship between the pink hydrogen in Figure 15 and the green hydrogen.

The position of the vinyl component will hopefully form in the correct place with the correct stereochemistry when NaOAc is used first as a weak base to deprotonate at chiral carbon centre, 1, in **83** to form the new alkene between carbons 1 and 2. Acetic acid will then isomerise the old carbon-carbon double bond between carbons 2 and 3 to an alkane forming the new compound, **84.** The enone component will be able to be reduced in a radical reaction designed by Comins, using zinc and acetic acid. This reaction and mechanism are further described in the Further Work section shown in Figure 32. In Scheme 18, the revised final route for the synthesis of compound **76** is highlighted due to the first reaction in Scheme 17 not reacting leaving only the original starting material. In the revised route, a one pot three-step synthesis with compound **75** can be used however different chloroformate compounds had to be used instead of the original acryloyl chloride starting reagent. The secondary amine of, **77**, can then be formed using a deprotection reaction to allow for the removal of the acyl

group. A nucleophilic addition and elimination reaction between acryloyl chloride and **77** can then take place to form **76**. This is further explained in chapter 2.3.1.

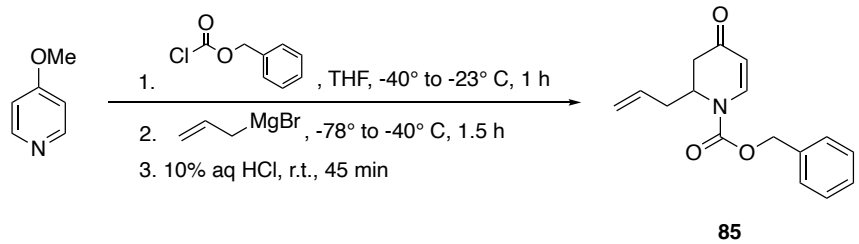
2.3.1. Synthesis of Dihydropyridine Intermediate, 85

When reviewing the literature, it became apparent that a large amount of similar chemistry had been completed by Comins in the late 20th century.[66] Therefore, utilising the findings from Professor Comins research, a new type of one-pot two-step reaction was used as seen in Scheme 19, and it took a several attempts to gain a positive result. In the first attempt, acryloyl chloride was added to **75** in THF at -23°C to form the 1-acryloyl-4-methoxypyridin-1-ium salt. This seemed viable because **75** has an imine-like nitrogen with its lone pair of electrons in an sp^2 orbital.[67] This means that this lone pair is not a part of the aromatic π-system in the pyridine ring therefore making it available to bond with the electrophilic carbon present in the carbonyl bond of acryloyl chloride.[67,68] Once this protonated nitrogen cation was formed, allyl TMS was added at -23°C to attach at the *ortho* position next to it and was left stirring for one hour and then at room temperature for thirty minutes.[66] This reaction did not work so instead allyl magnesium bromide was tried, which is a much more nucleophilic reagent, however again a complex mixture was made.



Scheme 19. The proposed reaction route, produced from research done by Comins *et al*, for the formation of compounds **85** and **86**.[69]

Some problems with this initial reaction could have arisen from the use of acryloyl chloride. It is known that acid chlorides react violently with water to form unsaturated carboxylic acids, for example acrylic acid from acryloyl chloride.[70] As there is moisture present in the air too, this reaction needed to take place in oven-dried glassware, under an inert atmosphere using nitrogen or argon and all solvents needed to be freshly distilled so there was no water content.[71] Even the tiniest amount of water could hinder the reaction significantly due to the acid chloride's highly reactive nature. This would lead to the breakdown of the original acryloyl chloride reagent before it has much chance to react.[48] This could explain why no reaction was found because if a low yield of the original starting salt formed and with this this being a one-pot reaction then there could possibly not have been enough material for the additional allyl reagents to attack.

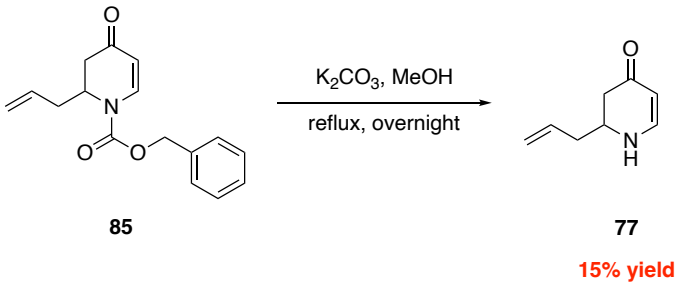


85

Scheme 20. Reaction route used for the formation of compound, **85**.

Another key problem could have come from the use of allyl TMS which reacts like a weak nucleophile. Possibly this is not reactive enough to react with the acyl pyridinium electrophile. Further, the acyl group has a further site of potential reaction as a conjugate acceptor. Therefore, a more nucleophilic reagent such as allyl magnesium bromide should be used instead.

From this conclusion, benzyl chloroformate was used instead of acryloyl chloride and the conditions of the reaction were also changed slightly. For this, as seen in Scheme 20, benzyl chloroformate was added at -40°C to 4-methoxypyridine in THF and left for one hour, followed by allyl magnesium bromide at -78°C which was left stirring for one hour and then thirty minutes at room temperature giving **85** in a low yield of 18%.[66] Once made, the benzyl chloroformate group was removed using K_2CO_3 in MeOH as seen in Scheme 21.[72] This was left overnight under reflux conditions and gave **77** in a low yield of 15%.[72]



85 **77**

15% yield

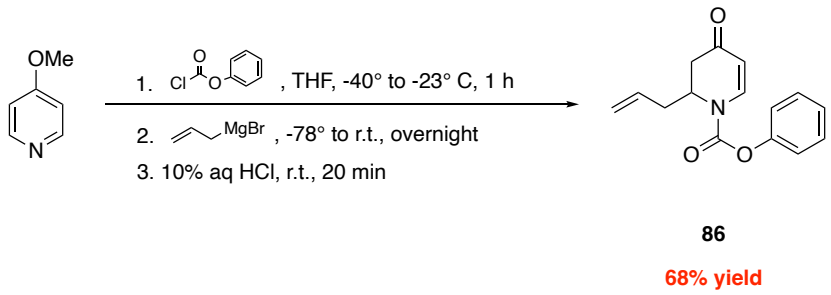
Scheme 21. Reaction route used for the deprotection of the tertiary amine in compound **85** forming compound **77**.

2.3.2 Synthesis of Dihydropyridine Intermediate, 86

As both reactions gave significantly low yields, phenyl chloroformate was used instead as this group would be easier to remove in the second reaction and had been used by Comins. Benzyloxycarbonyl groups often require harsh conditions for removal as they are very stable under either acidic or basic conditions.[73] These harsh conditions are normally undesirable as they can sometimes change the structure of other parts of the desired molecule too.[74] Therefore, in the first trial reaction phenyl chloroformate was added to **75** under the same conditions as previously described for benzyl chloroformate giving a low yield again, but better than the previous reaction with the benzyloxycarbonyl group, of 29% for **86.**

The conditions of this reaction were optimised by changing solvents, equivalents of reagents and reaction time as seen in Scheme 22. Instead of using a polar, aprotic solvent like THF, the non-polar solvent toluene was used instead.[75] This is because the oxygen atom in the THF ring can coordinate the allyl Grignard when used which reduces its nucleophilic activity. This coordination happens due to ligation therefore leading to aggregates forming in solution. However, when using toluene there are no polar atoms in the solvent to do this, therefore leading to an enhancement in reactivity and yield.

Phenyloxycarbonyl and benzyloxycarbonyl groups are both electron-withdrawing functional groups caused by the inductive effects formed by the δ^+carbon and δ^- oxygen double bond.[48] This means these functional groups destabilise the nitrogen carbocation that is formed when the salt is made making it more reactive for attack from the allyl Grignard.[48] Thereby explaining why these groups are good choice for this reaction.



86

68% yield

Scheme 22. Fully optimised reaction route for the formation of compound **86.**

Phenyl chloroformate was also added in a slight excess to ensure the reaction could proceed with maximum efficiency and that there were no limiting factors.[76] The reaction was

left overnight instead of three and a half hours to ensure the reaction reached full completion and the allyl Grignard was titrated to ensure the correct molarity was known and being used.[77] After all these different conditions were implemented a fair yield of 68% was achieved for the first reaction. The mechanism of this one-pot reaction is shown in Figure 16.

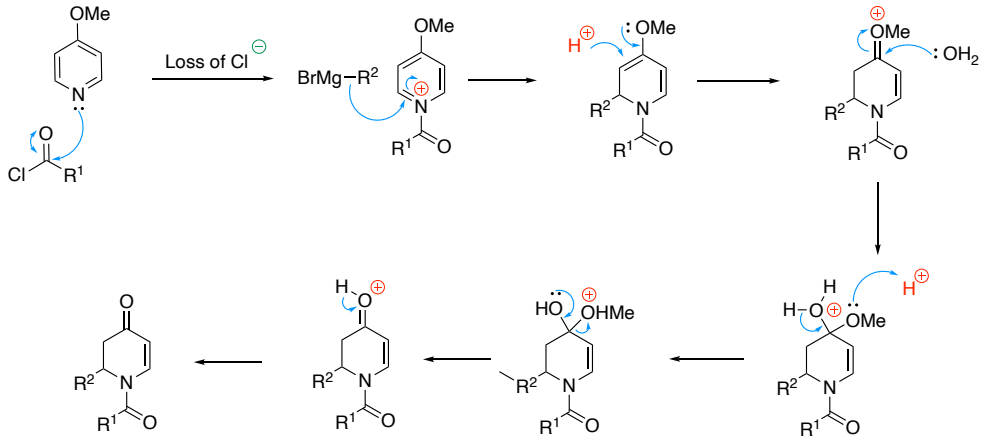
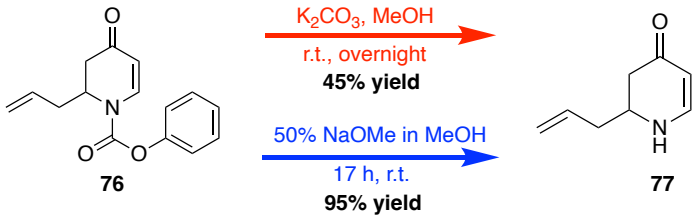


Figure 16. Mechanism for the one pot reaction, created by Comins et al and used in the formation of **85** and **86.**[48]

2.3.3. Synthesis of Deprotected Pyridine Intermediate, 77



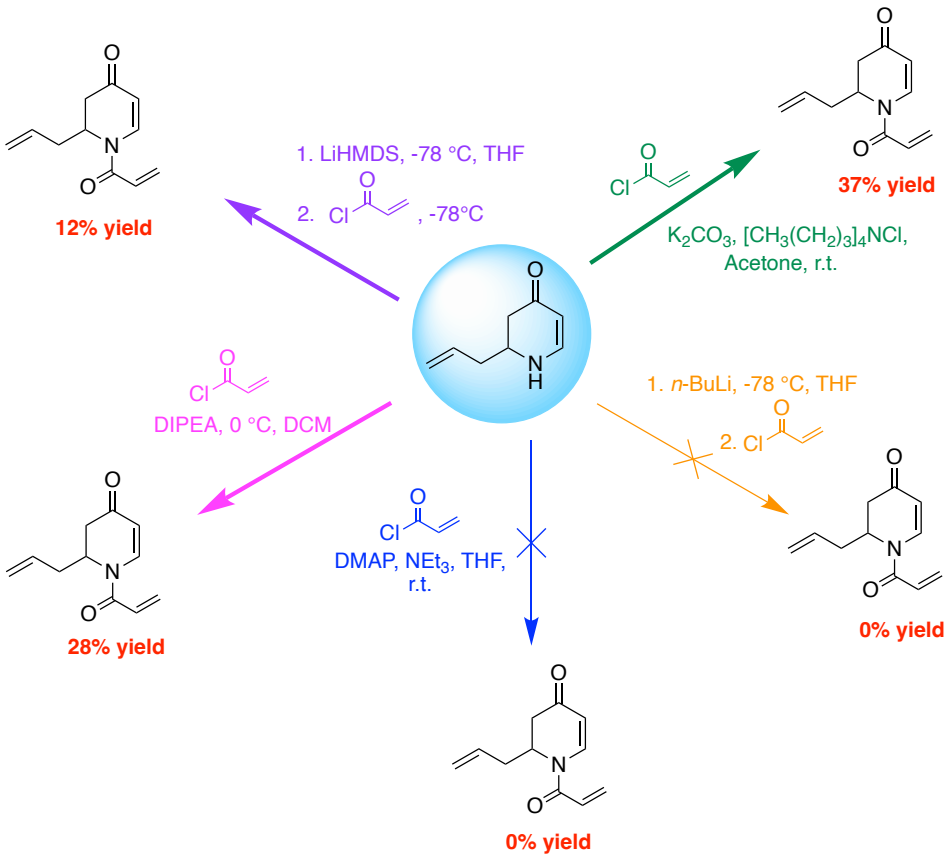
Scheme 23. Reaction routes tested for the formation of compound, **77**.

The first reaction investigated for the removal of the phenyl chloroformate group was the same procedure employed for the removal of the benzyl chloroformate group using K_2CO_3 in MeOH.[72] However this time, less harsh conditions were needed so the reaction was left stirring at room temperature overnight giving a moderate yield of 45% for **77**.[72] To try and improve this yield a slightly stronger base like NaOMe in MeOH, with a higher pK_a value of 16

compared to K_2CO_3 with a pK_a of 10, was used to allow for full removal.[69] Contrasting to the original procedure 8.7M NaOMe in MeOH was used instead of 4.4M.[69] This is because in the procedure the reaction was left for two hours, however after monitoring the reaction via TLC analysis for four hours, the reaction mixture still had a lot of starting material in it. Therefore, extra base was added to increase the rate of the reaction. This second reaction involved adding this base to **76** in MeOH and leaving it to stir for seventeen hours at room temperature to give a high yield of 95%.[69] Both of these reactions are seen in Scheme 23.

2.3.4. Synthesis of Acryloyl Substituted Dihydropyridine Intermediate, 78

Now that the free secondary amine had been formed in **77**, acryloyl chloride could be added via a nucleophilic addition and elimination reaction to allow for closing of the second fused nitrogen heterocycle in the next step.[48] Various methods were tried, as seen in Scheme 24 and Table 1, due to only achieving with poor yield. The first one involved stirring the starting material, acryloyl chloride, K_2CO_3 and tetrabutylammonium bromide in acetone at room temperature overnight giving **78** in a poor yield of 37%.[77] The conditions used in methods two and three in Table 1 did not lead to a reaction and only gave the original starting material and reagent.[78,79] When using DIPEA as the sterically hindered base and acryloyl chloride in DCM at 0°C for eighteen hours, a poor yield of 28% was also obtained.[80] An even poorer yield of 12% was also obtained when LiHMDS, a strong base, was used at -78°C in THF for two hours.[80] These poor yields could also be attributed to the instability of acryloyl chloride due to the reasons previously mentioned above.



Scheme 24. Reaction routes tested for the formation of compound, **78.**

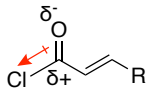
As **77** has a pK_a of around 20-25 as estimated from the Bordwell pK_a table, this means that LDA or an amine base like ammonia with a pK_a of around 35 should be considered as an alternative.[57] DIPEA, K_2CO_3 and DMAP all have too low pK_a values so are too weak a base to fully deprotonate the secondary amine of **77.**[57] Whereas *n*-BuLi and LiHMDS are too strong a base to be used and may have a competing reaction of deprotonation at another carbon in **77**, hence why no yield or a low yield is seen.[57]

Synthesis of Compound,	Reagents and Conditions	Yield (%)
Method 1	Acyloyl chloride, K_2CO_3, tetrabutyl ammonium chloride, acetone, r.t.	37
Method 2	4. *n*-BuLi, -78 °C, THF 5. Acryloyl chloride	0
Method 3	DMAP, NEt_3, acryloyl chloride, THF, r.t.	0
Method 4	DIPEA, acryloyl chloride, 0 °C, DCM	28
Method 5	1. LiHMDS, -78 °C, THF 2. Acryloyl chloride, -78 °C	12

Table 1. Methods used for the synthesis of **78**, with the corresponding reagents, conditions and yields listed.

2.3.5. Synthesis of Cinnamoyl Chloride, 87

As seen from the extended search and trying of a range of different experiments to improve the poor yields found, it was obvious that a different reagent was needed. A relatively more stable acyl chloride was chosen, cinnamoyl chloride, due to the enhanced stability from the extra phenyl substituent.[83,84] Within an acyl chloride functional group, the chlorine atom displays a strong electron withdrawing inductive effect that destabilises the carbonyl group hence making it a more reactive electrophile and more unstable like in Figure 17, as seen with acryloyl chloride.[85] However, when a vinyl group is added to the molecule, this alkene group displays more electron donating effects which stabilises the strong effect seen from the chlorine atom on the carbonyl group slightly.[83,85] The π-electrons from the phenyl and alkene group also further stabilise the acyl chloride substituent through resonance effects shown in Figure 18.[48]



R = H or Phenyl

Figure 17. Figure showing the electron withdrawing inductive effect of the chloride on the carbonyl group in an acyl chloride.

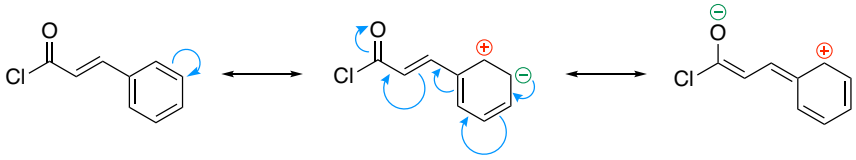


Figure 18. The resonance structures predicted for cinnamoyl chloride to explain the higher stabilisation seen in this acyl chloride.

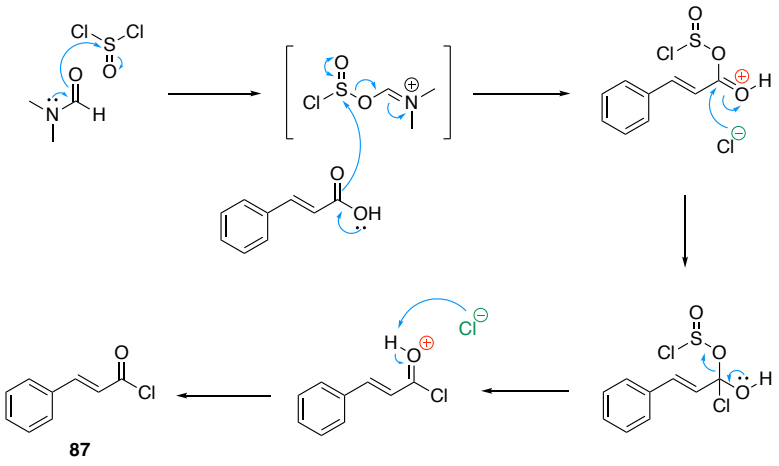
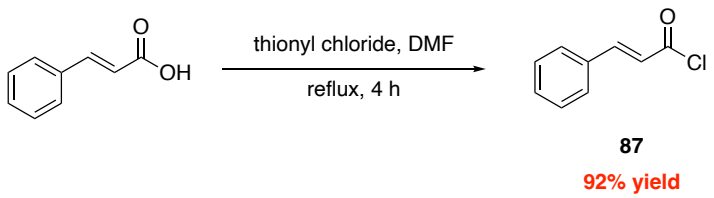


Figure 19. Mechanism that describes the formation of compound **87**.

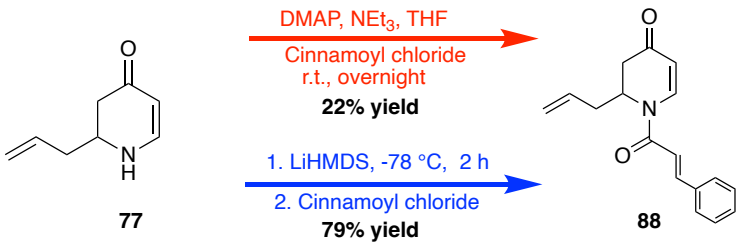
To make cinnamoyl chloride, cinnamic acid was heated at 75°C in thionyl chloride and a catalytic amount of DMF for four hours giving a high yield of 92% as seen in Scheme 25.[81] Mechanistically, as seen in Figure 19, the hydroxy group in the carboxylic acid functional



Scheme 25. Reaction route for the formation of compound **87**.

group is not a good leaving group so to improve this it is converted into a chlorosulfite group first.[82] The chloride anion formed after leaving then nucleophilic attacks the carbonyl to give cinnamoyl chloride.[82]

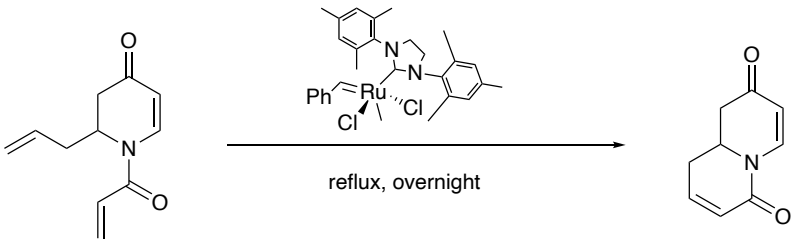
2.3.6. Synthesis of Cinnamoyl Substituted Dihydropyridine Intermediate, 88



Scheme 26. Methods that were used to make compound **88**.

After making cinnamoyl chloride, it was then used in a nucleophilic attack and elimination reaction with **77**.[48] This was first done using DMAP and NEt_3 in THF at room temperature overnight giving a poor yield of 22%.[80] In this reaction DMAP acts catalytically by reacting with the acid chloride to form an *N*-acylpyridinium, salt which is a better nucleophile to transfer the acyl group onto the amine.[86] As this reaction did not seem to work very well a stronger base, LiHMDS, was used at -78°C in THF for two hours to deprotonate the secondary amine in **77** and allow it to readily nucleophilic attack the acyl chloride.[80] This method gave a significantly improved higher yield of 79%.[80] Both reactions are seen in Scheme 26.

2.3.7. Synthesis of Bicyclic Intermediate, 79 from Compound 78



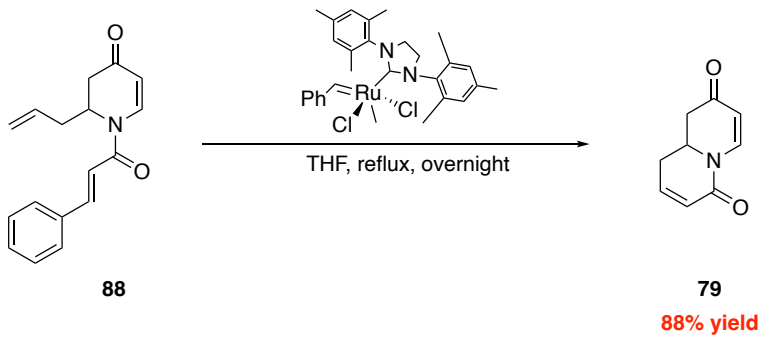
78 **79**

22% yield

Scheme 27. Ring closing metathesis conditions used to create the second nitrogen heterocycle in compound **79** from **78**.

To form the second fused nitrogen heterocycle, Grubbs 2nd generation catalyst was used.[48] This ruthenium catalyst first undergoes initiation with the starting material so that the original ruthenium precursor can add to one of the terminal carbon-carbon double bonds of the starting material forming a metal carbene complex via a [2+2] cycloaddition.[48,87] Once this complex is generated, this compound enters a catalytic cycle where it forms another key metallacyclobutane intermediate which undergoes a cycloreversion step to form the final ring closed product.[48,87] This mechanism is seen in Figure 20. This was first attempted with **78** in DCM under reflux conditions for sixteen hours giving a low yield of 22% for **79** as seen in Scheme 27.[88]

2.3.8. Synthesis of Bicyclic Intermediate, 79 from Compound 88



88 **79**

88% yield

Scheme 28. Ring closing metathesis conditions used to create the second nitrogen heterocycle in compound **79** from compound **88.**

When this ring closing step was tried with **88** using the same reaction conditions as with **78** but with THF as the solvent as shown in Scheme 28, a high yield of 88% was obtained.[88] In this reaction, the metal carbene forms on the monosubstituted terminal alkene as this reaction is more entropically favourable compared to the metal carbene forming on the disubstituted terminal alkene with a phenyl substituent at the vinyl position.[89,90]

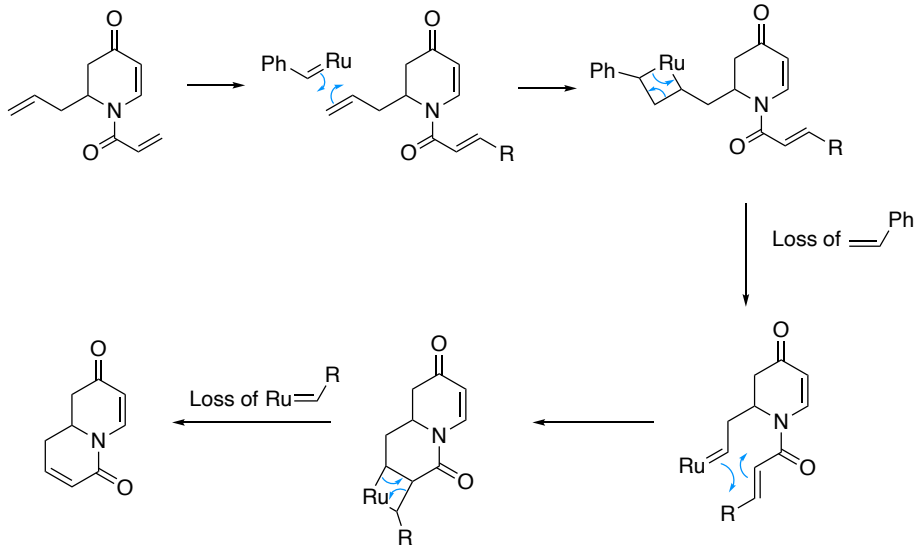


Figure 20. Mechanism for the ring closing metathesis reaction with Grubbs 2nd Generation catalyst.

This is proven by the reaction with the monosubstituted terminal alkene theoretically having a higher equilibrium constant (K_{eq}) and more negative free energy value, as assumed by work done by Lane *et al* which is shown in Table 2, due to there being less substitution therefore making this reaction more favourable.[89,90] These values are also seen because having a bulkier phenyl group is sterically unfavourable for the ruthenium species when it is trying to form metal carbene complex as there would not be optimal space available for the initiation reaction to take place. From here, the [2+2] cycloreversion step can happen as normal to form the second 6-membered nitrogen heterocycle.

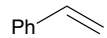
Substrate	K_{eq}	ΔG (mean) (kcal/mol)
	8.66	-1.251
	0.304	0.695

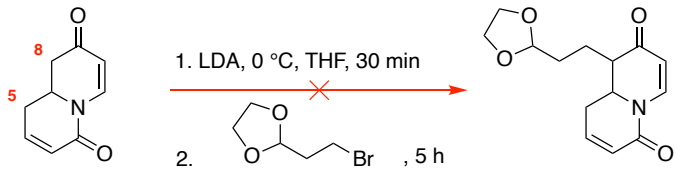
Table 2. Table showing the equilibrium constant values (K_{eq}) and free energy values (kcal/mol) of a terminal unsubstituted alkene versus a phenyl substituted alkene to explain why the metal carbene forms on the monosubstituted terminal alkene compared with the disubstituted terminal alkene bearing the phenyl group in compound **88**. Having a higher K_{eq} and more negative ΔG leads to a more favourable reaction.

A reason for the low yield seen when **78** is used could be because of the competing side reaction that happens alongside RCM called Acyclic Diene Metathesis polymerization.[91] This cross-metathesis step is very unfavourable in this instance because of the loss of yield of the desired product, **79**.[91] Due to there being two monosubstituted terminal alkenes in this compound, this could in theory lead to enhanced reactivity towards polymerization.[91] The fact that there are no substituents means that the diene is unhindered and therefore

kinetically favours oligomerization.[91] Therefore, attaching the phenyl group in **88** could potentially reduce the rate of Acyclic Diene Metathesis polymerization and increase the rate of RCM due to this compound being more sterically hindered.

In the original paper by Grossmith *et al*, the solvent used for the RCM reaction was DCM.[88] However, it was found that THF produced a higher yield for this reaction with an improvement of 15% yield. This can be explained using research conducted by Lane et al where it was seen that THF had a better initiation rate of 0.032 s^{-1} compared to DCM, with an initiation rate of 0.021 s^{-1} when using Grubbs catalysts in solution.[90] A higher initiation rate means that more material is reacting in solution at a faster rate therefore resulting in a higher yield when the same time frame is used.

2.3.9. Attempted Synthesis of Substituted Bicyclic Intermediate, 80

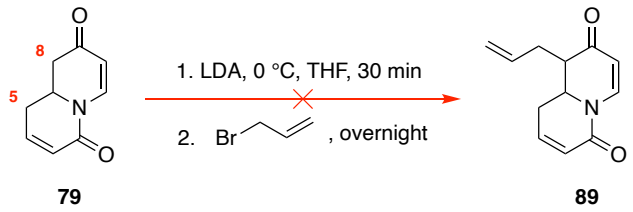


79 **80**

Scheme 29. Scheme showing the hypothesised formation of compound **80**, however this reaction did not work.

After these two-fused nitrogen heterocycles had been formed, the next step was to react **79** with 2-(2-bromoethyl)-1,3-dioxolane using LDA in THF for five hours as seen in Scheme 29.[92] It was hoped that this would allow for an S_N2 reaction to take place with the dioxolane compound alkylating at the carbon 8 position in **79**.[93] With carbon 8 in molecule **79** being less substituted, this should allow the sterically hindered LDA base to selectively

deprotonate this carbon forming a reactive nucleophilic enolate ion intermediate.[93] However, this reaction did not work, leaving the original starting material and dioxolane unreacted in the mixture. The reason for this happening was thought to be because 2-(2-bromoethyl)-1,3-dioxolane was not electrophilic enough to react with **79**.



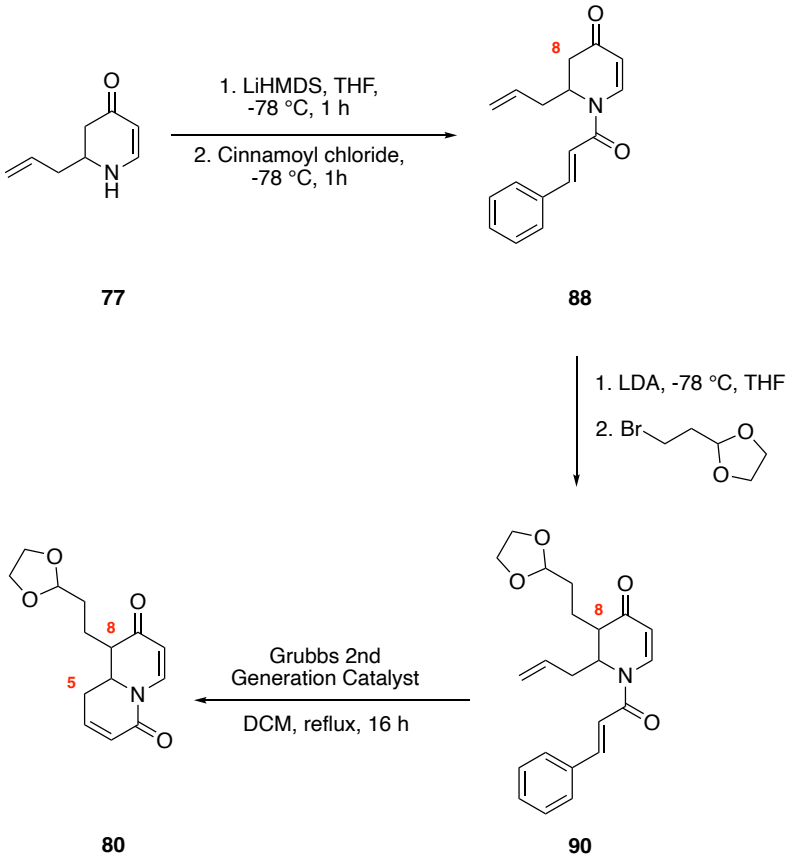
Scheme 30. Scheme showing the hypothesised formation of compound **89**, however this reaction did not work.

Therefore, a more reactive electrophile was employed, allyl bromide as shown in Scheme 30, using the same reaction conditions, however this time the reaction was left to react overnight to try and make compound **89**. This was because, after monitoring with TLC analysis it was found that a lot of starting material was still left in the reaction by the end of the day. This reaction did not work either and led to a complex formation of 4 different products with very similar retention factors. Nonetheless, since this reaction was done with a more reactive nucleophile, it probably didn't need to be left reacting at such a long timescale. This could have led towards problems with over-reaction hence why the desired product was not seen, and four different products were.

Three different bases were tried to initiate this reaction, including LiHMDS, LDA and *n*-BuLi due to their varying differences in pK_a values and therefore deprotonating activities.[57] However, none of these bases worked so a different route was taken. Instead of commencing

alpha alkylation on **79**, it was decided to redo this step with **88** instead before the original ring closing metathesis step commenced as seen in Scheme 31.[92] This is because when using **88**, it only allows for the deprotonation of carbon centre 8, whereas in compound **79** LDA can also deprotonate at carbon 5 on the bottom ring. Therefore using **88** removes this extra possible area for deprotonation and increases the chance of the bromo-dioxolane adding at the correct carbon centre to form **90**.

The reaction was attempted on a small scale, with 16 mg of starting material, using LDA as the base at -78°C for three hours. This reaction looked more promising as the exact molecular weight of the desired product, **90**, was seen when the product was run on the mass spectrometer. If there had been more time, a scale-up reaction would have been done so that more spectroscopic data could have been collected to prove this step works.



77 **88** **80** **90**

Scheme 31. New reaction scheme for the improved addition of the bromo dioxolane compound.

From here the RCM step would take place using the same conditions as already tested to afford compound **80** with the dioxolane compound already *ortho* alkylated as seen in Scheme 31. This would then allow for the original total synthetic route to be followed as initially expected.

3. Conclusions and Further Work

In conclusion, work towards synthesising the desired natural product has been achieved. This has been done by implementing, changing, and optimising a range of different reactions, conditions, and synthetic routes. The research completed was highly dependent on the results and feedback gained from each step in the synthesis as sometimes certain ideas had to be changed if they did not work or yield was low. Whilst conducting this research, four novel compounds were synthesised and characterised which were molecules **76**, **79**, **85** and **88** in Figure 21.

More research needs to be undertaken to close the final two fused nitrogen heterocyclic rings. If, when completed on a larger scale, the proposed revised synthetic route, in Scheme 31, for the addition of the bromodioxolane compound works then the final total synthetic route in Scheme 32 can be followed as hypothesised.

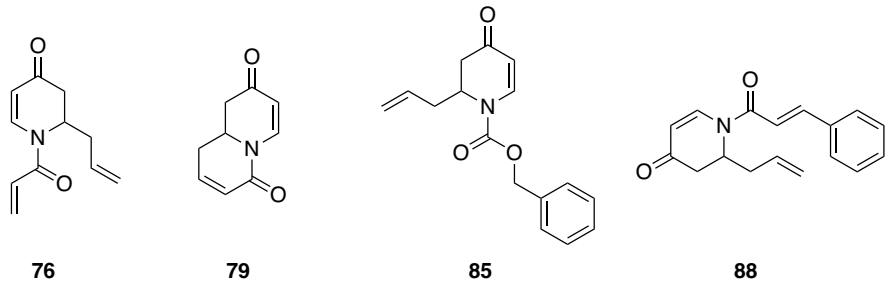
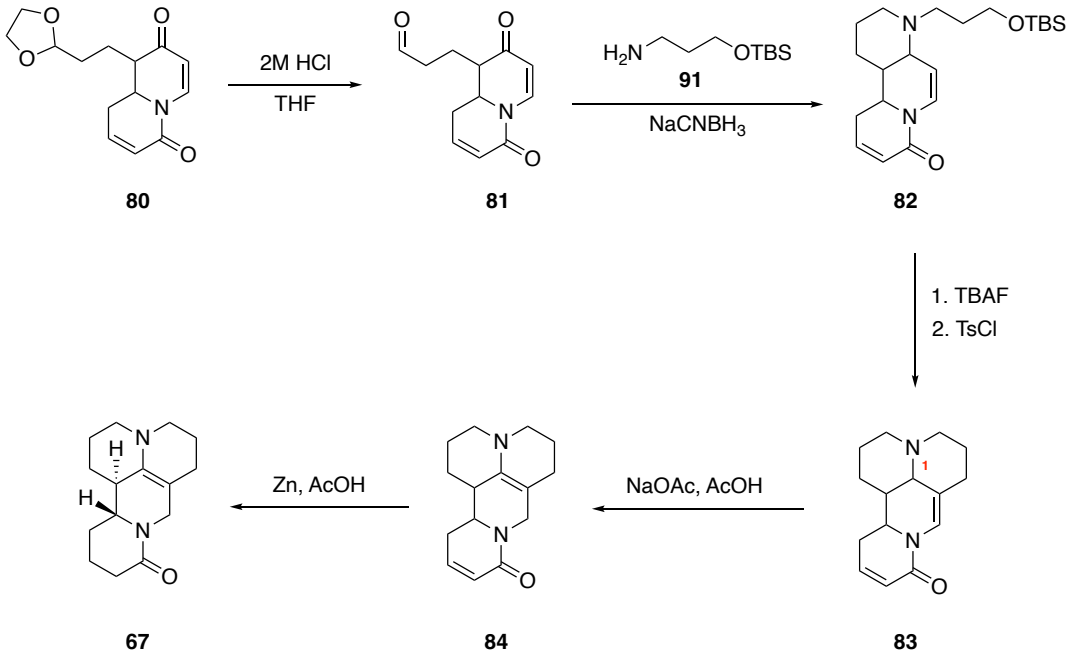


Figure 21. The structures of the four novel compounds that were made.



Scheme 32. The remaining steps of the third proposed total synthetic route.

This would allow for the removal of the acetal group by reducing the dioxolane in **80** to an aldehyde in **81** using 2M hydrochloric acid in tetrahydrofuran. The intramolecular reductive amination can then take place via the mechanism in Figure 22 where the primary amine **91** first reacts with the carbonyl in **81** to form an imine functional group. This imine functional group can be reduced by $NaCNBH_3$ to form a secondary amine that can react with the terminal aldehyde through the same kind of mechanism. In the next one-pot reaction the silyl ether functionality in **82** can be deprotected using TBAF to form the hydroxy group. This alcohol can be turned into a tosylate so that it is a better leaving group when forming the fourth nitrogen heterocycle. This will happen via the mechanism in Figure 23. NaOAc can be used to deprotonate at carbon 1 in **83** so that the alkene functionality can form in the correct position with the correct stereoselectivity. Acetic acid will be used to reduce the enone by protonating the double carbon-carbon bond.

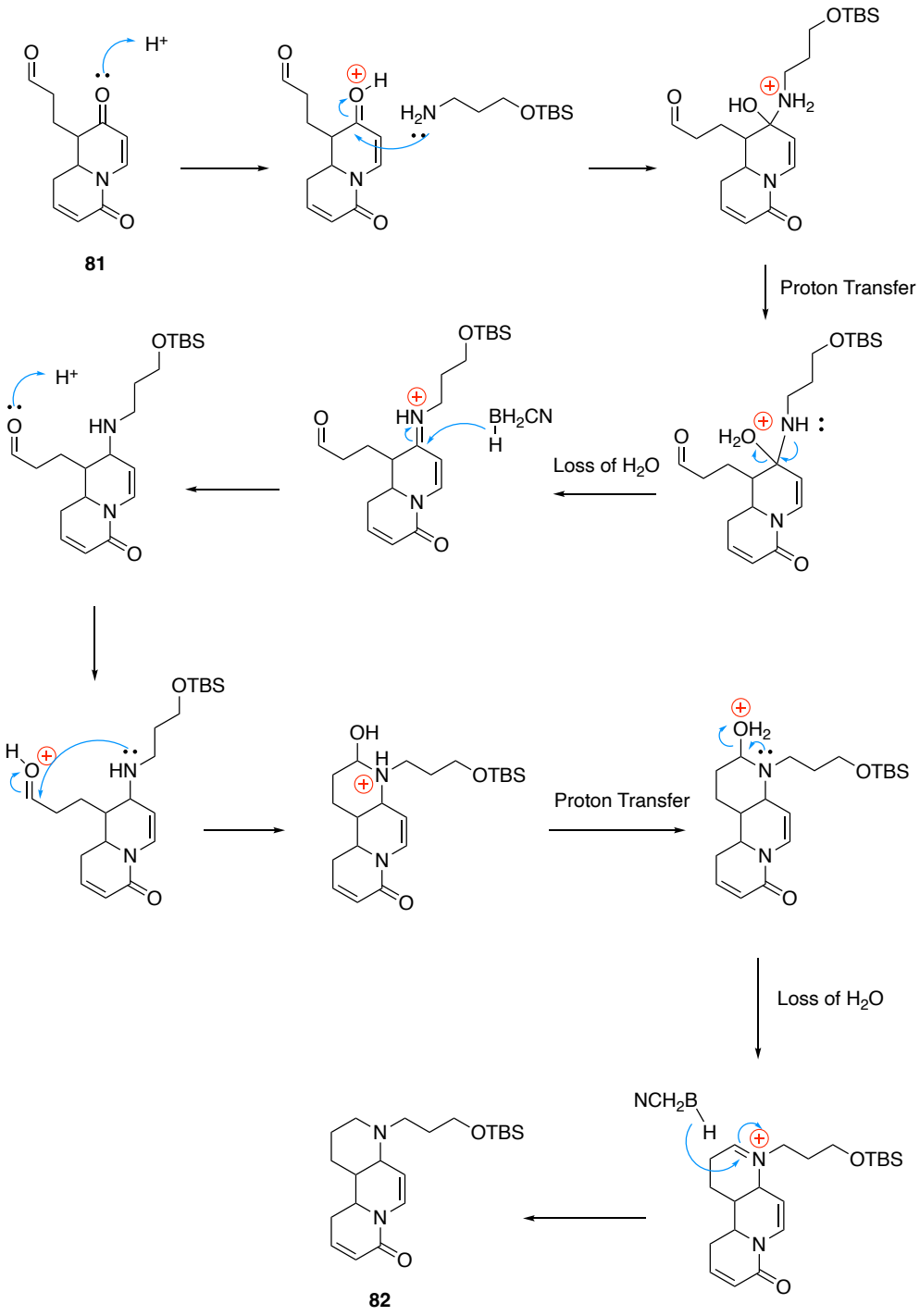


Figure 22. Mechanism for the reductive amination reaction from compound **81** to compound **82.**

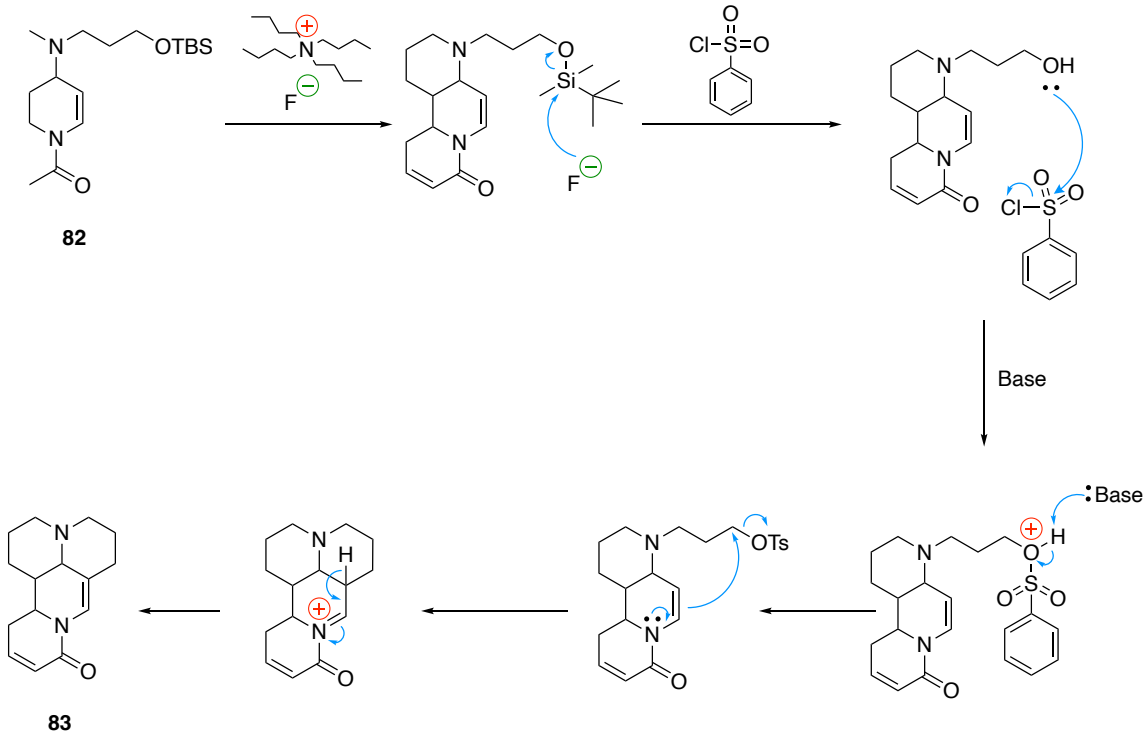


Figure 23. Mechanism for the deprotection reaction using TBAF followed by the formation of the tosylate functional groups from compound **82** to compound **83.**

The enone can be reduced using zinc and acetic acid, which is a radical reaction formulated by Comins et al.[94] These reduction conditions are preferable to that of others due to the low-cost of zinc dust, the mild conditions needed as this reaction can be done at room temperature and the high chemoselectivity of the reaction.[94] The mechanism for this last radical step is seen in Figure 24.

Another way of making dehydromatrine, seen in Scheme 33, was found when searching through the literature as it was apparent that the vinyl groups in **79** could be reduced to form **94** using standard reduction conditions with $NaBH_4$, $NiCl_2$ and MeOH. The enone functionalities could also be reduced by the same procedure produced by Comins et al using Zn and acetic acid too as described above.[94] A similar route used by Mandell et al in the first

total synthesis of matrine could then be optimised to make dehydromatrine.[41] This involved the use of the Stork-enamine dialkylation with acrylonitrile and pyrrolidine as the base to deprotonate the hydrogens at carbons 8 and 2 in **92** to make **93**. From here a one-pot four-step reaction could be tried to close the final two fused nitrogen heterocycles. First the carbonyl functional groups would have to be protected using an acid-catalysed reaction with ethylene glycol.

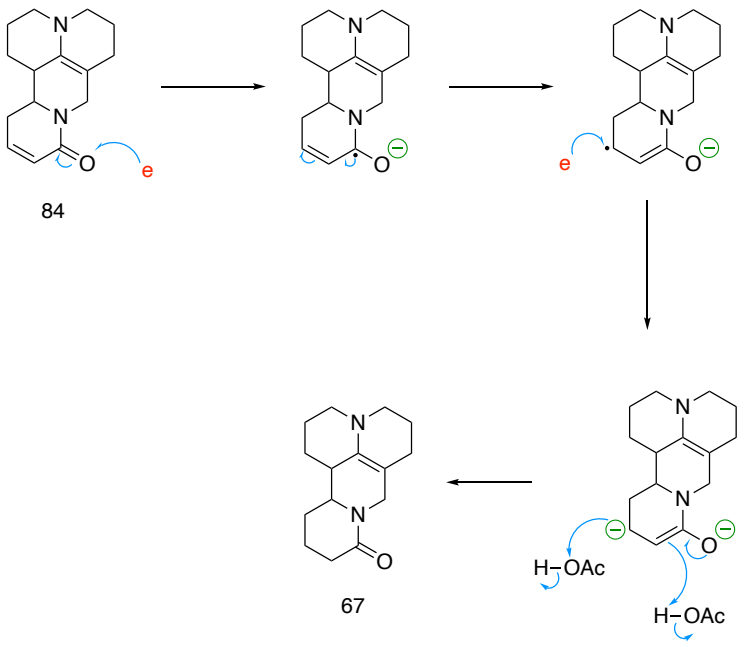
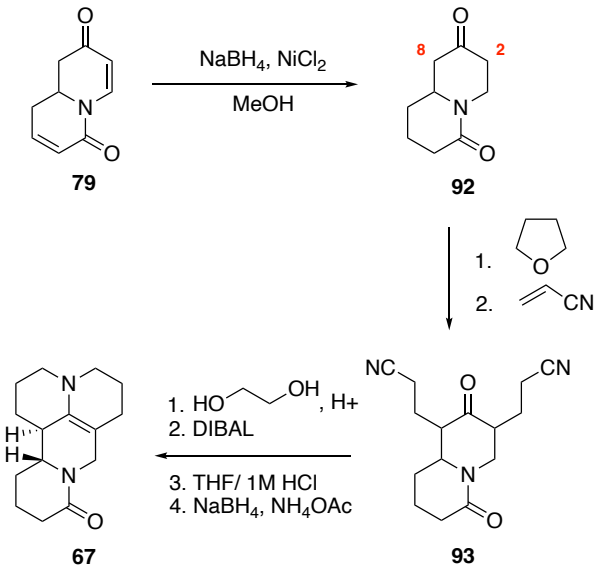


Figure 24. Mechanism for the radical reaction for enone reduction using Zn and AcOH in compound **84** forming compound **67**. *e* is for the electron from the Zn which catalyses the radical reaction.

This means a nitrile reduction can take place using the bulky reducing agent, DIBAL, to form two aldehyde functional groups. The aldehydes can then take part in a reductive amination reaction with NH_4OAc to create the final overall dehydromatrine backbone. The final reductive amination takes place via the mechanism in Figure 25. Due to NH_4OAc existing in solution as $^{+}NH_4$ and $HCOO^{-}$, this means the ammonium cation is in equilibrium with NH_3

which reacts with the aldehyde in **94** to form a protonated imine which catalyses the formation of the third and fourth nitrogen heterocycles.



Scheme 33. An alternative proposed route to make the final desired natural product **67** from **79**.

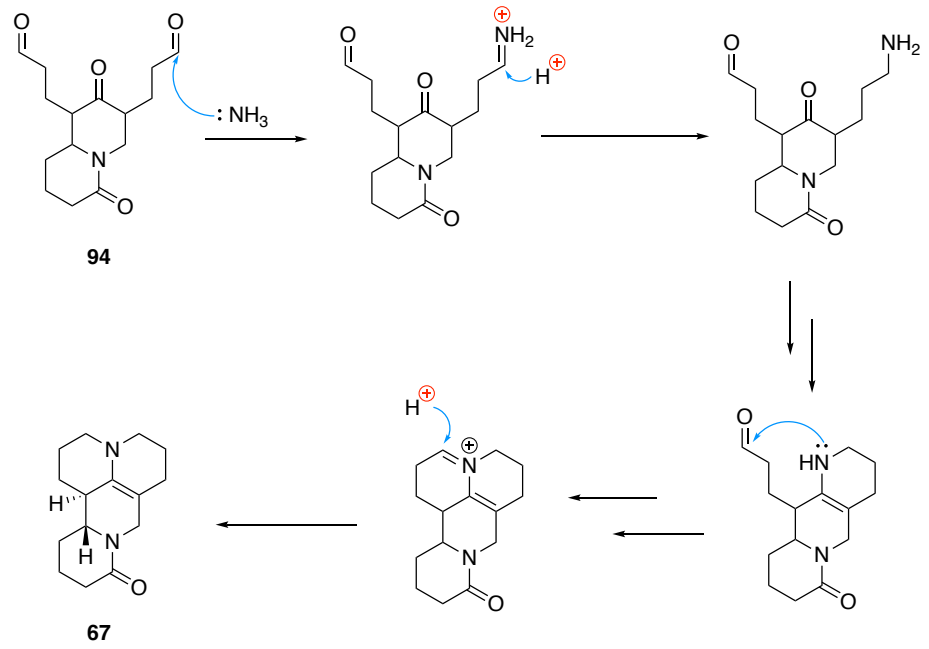


Figure 25. Mechanism for the final reductive amination in the alternative proposed route, in Figure 12, from compound **94** to **67**.

4. Experimental

4.1 General Experimental

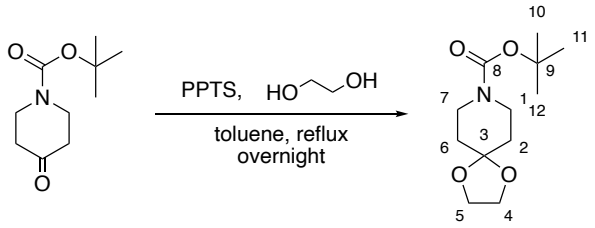
Starting reagents were either purchased from commercial suppliers and used without further purification or were made according to procedures found in the literature. Reactions took place in round bottomed flasks and were sealed with a glass stopper or a rubber septum. All reactions were conducted in oven-dried glassware. When an inert atmosphere was needed flasks were flushed three times with argon using a Schlenk line and an Ar balloon would be added to the flask through a rubber septum. Anhydrous solvents, toluene and THF, were obtained from a solvent purification system. Anhydrous DCM was distilled over calcium hydride under lab conditions. The concentration of allyl MgBr was determined by titration, under argon, against a 0.5 M solution of LiCl in THF (4 mL) and iodine (254 mg). Reactions were monitored with thin layer chromatography using Merck silica gel 60 F_{254} aluminium backed sheets and were visualized under UV light before staining with either potassium permanganate or an acidic solution of vanillin in ethanol. Column chromatography was performed manually using Merck silica gel 60, 35-70 μm particles with different specified solvents.

All Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker AV (III) 400HD, Bruker AV400 or Bruker AV(III)400 NMR spectrometer in $CDCl_3$ at 298 K. For ^{1}H NMR, samples were prepared using ca. 5-10 mg of compound dissolved in 0.5 mL of $CDCl_3$ and for ^{13}C NMR, ca. 20-30 mg of compound was used dissolved in 0.5 mL of $CDCl_3$. All spectra were referenced with respect to $CDCl_3$, with the residual hydrogen solvent peaks for ^{1}H NMR at δ_H 7.26 ppm

and the solvent peak for ^{13}C NMR at δ_C 77.0 ppm. NMR chemical shifts (δ) are reported in ppm, coupling constants (*J*) are reported in Hertz (Hz); multiplicities of each individual signals are assigned as follows s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, sext = sextet, m = multiplet, br = broad signal, dd = doublet of doublets, ddt = doublet of doublet of triplets, dt = doublet of triplets, tt = triplet of triplets and ddd = doublet of doublet of doublets. High resolution mass spectrometry (HRMS) was recorded on a Bruker micrOTOF II mass spectrometer using electrospray ionization (ESI) in the positive and negative mode. Infrared spectra were performed on a Bruker ALPHA FT-IR spectrometer and signals were recorded as wavenumbers in cm^{-1}. The compound nomenclature was generated using Chemdraw 20.0 software with numbering of structures corresponding to ^1H and ^1C NMR assignments. The atom numbering used was aimed to give an easier understanding of peak assignments so does not always align with the systemic numbering from the IUPAC names for each compound. References to the literature are indicated where applicable when common procedures were followed, although some have been modified and changed.

4.2 Experimental Procedures

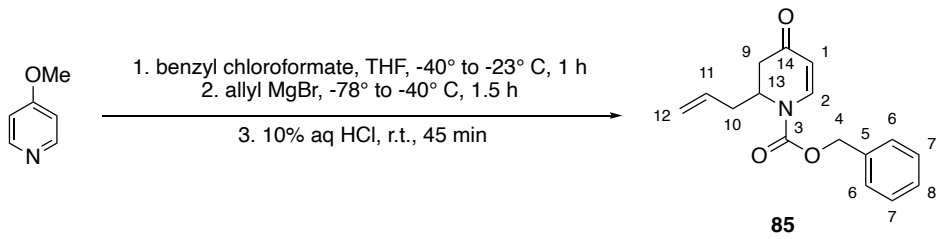
Tert-butyl 1,4-dioxa-8-azaspiro[4.5]decane-8-carboxylate



71

Tert-butyl 4-oxopiperidine-1-carboxylate (943 mg, 4.74 mmol), ethylene glycol (350 mg, 0.315 mL, 5.68 mmol) and pyridinium p-toluenesulfonate (PPTS) (119 mg, 0.474 mmol) were stirred in toluene (50 mL) and refluxed under Dean-Stark conditions overnight. In the morning an orange solution was obtained, and the solvent was removed *in vacuo*. The mixture was redissolved in ethyl acetate (50 mL), washed with water (50 mL), brine (50 mL) and dried with $MgSO_4$. After filtering, the filtrate was concentrated *in vacuo* to give the crude carboxylate as an orange viscous oil (0.622 g, 2.56 mmol). Purification by silica gel chromatography (2.5:1.5 hexane:EtOAc) gave the title compound **71** (329 mg, 29%) as a colourless crystalline solid: m.p. 39-42 °C; R_f 0.74 (1:1 petroleum ether 40-60:ethyl acetate); IR ν_{max} (thin film)/cm^{-1} 1733 (ester); δ_H (400 MHz, $CDCl_3$) 3.96 (4H, s, 5-H_2, 4-H_2), 3.50 (4H, t, J 5.8 Hz, 1-H_2, 7-H_2), 1.65 (4H, t, J 5.8 Hz, 2-H_2, 6-H_2), 1.46 (9H, s, 10-H_3, 11-H_3, 12-H_3); δ_C (101 MHz, $CDCl_3$) 154.7 (8-C), 107.2 (3-C), 79.6 (9-C), 64.4 (4-C, 5-C), 41.9 (1-C, 7-C), 34.9 (2-C, 6-C), 28.4 (10-C, 11-C, 12-C); HRMS: Found 266.1360. $C_{12}H_{21}NO_4Na$ ($M + Na^+$) requires 266.1368.

Benzyl 2-allyl-4-oxo-3,4-dihydropyridine-1(2*H*)-carboxylate



85

Formation of Grignard

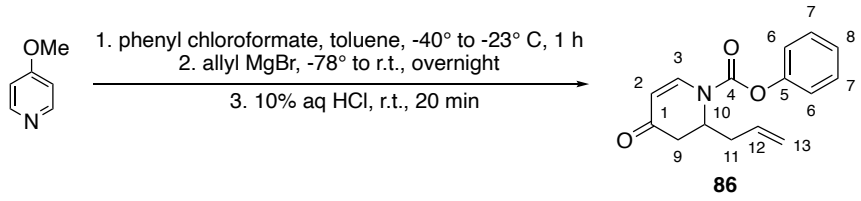
Magnesium turnings (1.07 g, 44 mmol) were stirred in dry THF (16 mL) under argon. Iodine (490 mg, 1.92 mmol) was added at 35 °C-45 °C over 2 h to give a purple solution. The mixture was cooled to -25 °C and a solution of allyl bromide (5.16 g, 3.69 mL, 42.6 mmol) was added dropwise over 2 h. The reaction was stirred for a further 1 h at -25 °C then warmed up to r.t. and stirred for another 2 h to give a grey solution with white precipitate. The resulting mixture was allowed to settle then the allylmagnesium bromide was obtained by decanting the upper layer.

Method

Benzyl chloroformate (1.56 g, 1.30 mL, 9.163 mmol) was added dropwise to a stirred solution of 4-methoxypyridine (1 g, 1 mL, 9.163 mmol) in dry THF (60 mL) under an argon atmosphere at -40 °C to give a cloudy white solution. After 1 h of stirring at -23 °C, the reaction mixture was cooled down to -78 °C and allylmagnesium bromide was added dropwise through a double tipped needle. The reaction was stirred for 1 h at -78 °C to -40 °C then at r.t for 30 min. After this 10% aqueous HCl (25 mL) was added and stirred at r.t. for 45 min to give a clear pale-yellow solution. The layers were then separated, and the aqueous layer was extracted with ether (5 x 15 mL). The combined organic layers were washed with brine (60 mL), dried

over $MgSO_4$, filtered, and concentrated *in vacuo* to give the crude dihydropyridine as a pale-yellow oil. Purification by silica gel chromatography (8.5:1.5 petroleum ether 40-60:EtOAc) gave the title compound **85** (440 mg, 18%) as a colourless oil: R_f 0.22 (8.5:1.5 petroleum ether 40-60:ethyl acetate); IR ν_{max} (thin film)/cm^{-1} 1721 (ester), 1667 (Ketone), 1599 (alkene); δ_H (400 MHz, $CDCl_3$) 7.80 (1H, d, *J* 6.2 Hz, 2-H), 7.47-7.35 (5H, m, 6-H_2, 7-H_2, 8-H), 5.71 (1H, sext, *J* 9.1 Hz, 11-H), 5.36 (1H, d, *J* 6.2 Hz, 1-H), 5.28 (2H, s, 4-H_2), 5.13-5.01 (2H, m, 12-H_2), 4.72-4.62 (1H, m, 13-H), 2.80 (1H, dd, *J* 16.6, 6.7 Hz, 9-H_a), 2.57-2.30 (3H, m, 13-H_b, 14-H_a, 14-H_b); δ_C (101 MHz, $CDCl_3$) 192.8 (14-C), 173.8 (3-C), 141.6 (2-C), 135.0 (5-C), 132.8 (11-C), 128.9 (7-C), 128.8 (8-C), 128.6 (6-C), 119.3 (12-C), 107.4 (1-C), 69.1 (4-C), 52.8 (13-C), 39.3 (9-C), 35.3 (10-C) ; HRMS: Found 272.1277. $C_{16}H_{18}NO_3$ ($M + H^+$) requires 272.1287. Found 294.1100. $C_{16}H_{17}NO_3Na$ ($M + Na^+$) requires 294.1106.

Phenyl 2-allyl-4-oxo-3,4-dihydropyridine-1(2*H*)-carboxylate



86

Titrating allylmagnesium chloride procedure – 0.5 M solution of LiCl

Anhydrous LiCl (4.239g, 100 mmol) was placed in a 250 mL round bottomed flask under argon and dried under vacuum using a Schlenk line for 4 h at 120°C. After cooling to r.t. dry THF (200 mL) was added and stirred for 24 h until the LiCl dissolved.

Titration using Iodine

Three 10 mL round bottomed flasks equipped with a stirrer bar were dried in the oven for 1 h. Iodine (254 mg, 1 mmol) was added, the flask was flushed with nitrogen and a septum was added. A saturated solution of LiCl in THF (4 mL) was added and stirring was started. After iodine had dissolved, the brown solution was cooled to 0 °C using an ice bath and allylmagnesium bromide was added dropwise using a 1 mL syringe using 0.01 mL graduations. This was done for each round bottomed flask until the brown colouration turned colourless.

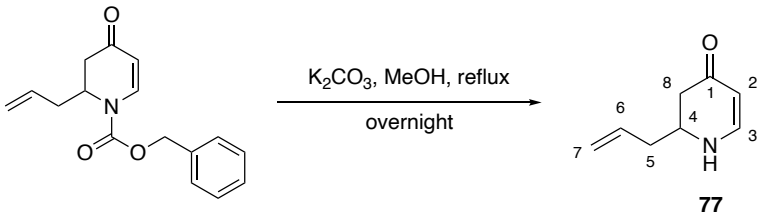
Method

Phenyl chloroformate (2.87 g, 18.33 mmol) was added dropwise to a stirred solution of 4-methoxypyridine (2.00 g, 18.33 mmol) in dry toluene (120 mL) at -40 °C under argon. The mixture was stirred at -40 °C for 1 h to give a white cloudy solution. After this, allylmagnesium chloride was added dropwise at -78 °C and left to warm up to r.t. overnight to give a dark

orange clear solution. 10% aqueous HCl (120 mL) was added and stirred for 20 min to give an orange solution. The layers were separated, and the aqueous layer was extracted with ether (3 x 100 mL). The combined organic layers were washed with brine (100 mL), dried with $MgSO_4$, and concentrated *in vacuo* to give the crude dihydropyridine as an orange oil. Purification by gradient silica gel chromatography (9:1 petroleum ether 40-60:EtOAc, 8:2 petroleum ether 40-60:EtOAc, 100% EtOAc) gave the title compound **86** (3.20 g, 68%) as an orange/yellow oil: R_f 0.13 (ethyl acetate); IR ν_{max} (thin film)/cm^{-1} 1733 (ester), 1667 (Ketone), 1599 (alkene); δ_H (400 MHz, $CDCl_3$) 7.90 (1H, dd, *J* 8.3, 1.6 Hz, 3-H), 7.46-7.37 (2H, m, 7-H_2), 7.32-7.24 (1H, m, 8-H), 7.19-7.11 (2H, m, 6-H_2), 5.81 (1H, ddt, *J* 16.8, 10.2, 7.4 Hz, 12-H), 5.45 (1H, d, *J* 8.3 Hz, 2-H), 5.21-5.08 (2H, m, 13-H_2), 4.80 (1H, d, *J* 7.6 Hz, 10-H), 2.90 (1H, dd, *J* 16.8, 6.6 Hz, 9-H_a), 2.58 (1H, t, *J* 1.5 Hz, 9-H_b), 2.54 (1H, t, *J* 1.5 Hz, 11-H_a), 2.42 (1H, quin, *J* 7.7 Hz, 11-H_b); δ_C (101 MHz, $CDCl_3$) 192.7 (1-C), 150.6 (4-C), 141.1 (3-C), 133.0 (12-C), 129.8 (7-C), 126.6 (8-C), 121.4 (6-C), 119.6 (13-C), 108.4 (2-C), 53.2 (10-C), 39.6 (9-C), 35.6 (11-C); HRMS: Found 267.1954. $C_{15}H_{16}NO_3$ (M + H^+) requires 267.1955. Found 289.1777. $C_{15}H_{15}NO_3Na$ (M + Na^+) requires 289.1774.

2-allyl-2,3-dihydropyridin-4(1*H*)-one

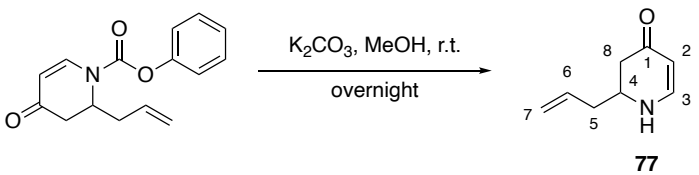
Method 1



77

K_2CO_3 (450 mg, 3.25 mmol) was added to a stirred solution of benzyl 2-allyl-4-oxo-3,4-dihydropyridine-1(2*H*)-carboxylate (440 mg, 1.62 mmol) in methanol (13 mL) to give a yellow solution. The reaction was stirred under reflux overnight to give a dark brown/orange solution. The solvent was removed *in vacuo* to give a very viscous dark brown/orange oil. EtOAc (15 mL) was added to the oil and the sides of round bottomed flask were scraped with a spatula to give a solid precipitate. Following this the precipitate was removed via Buchner Filtration and deionised water (15 mL) was added. The layers were separated, and the aqueous layer extracted with EtOAc (15 mL). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated *in vacuo* to give the crude dihydro compound as an orange oil. Purification by silica gel chromatography (7:3 petroleum ether 40-60:EtOAc) gave the title compound **77** (66.8 mg, 15%) as a yellow oil.

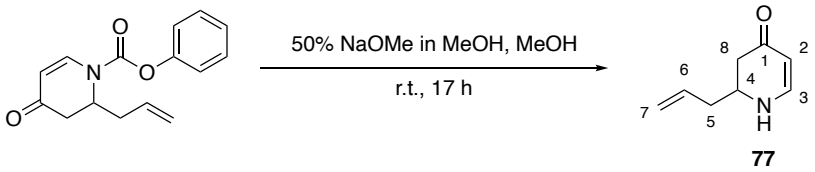
Method 2



77

K_2CO_3 (720 mg, 5.24 mmol) was added to a stirred solution of Phenyl 2-allyl-4-oxo-3,4-dihydropyridine-1(2*H*)-carboxylate (674 mg, 2.62 mmol) in methanol (25 mL) to give a yellow cloudy solution. The reaction was stirred at r.t. overnight to give a yellow clear solution. The solvent was removed *in vacuo* to give a very viscous orange oil. DCM (50 mL) was added to the oil and the sides of round bottomed flask were scraped with a spatula to give a solid precipitate. Following this the mixture was filtered through a celite pad with a DCM (20 mL) wash and concentrated *in vacuo* to give the crude dihydro compound as a yellow oil. Purification by silica gel chromatography (8:2 petroleum ether 40-60:EtOAc) gave the title compound **77** (0.165 g, 45%) as a yellow oil.

Method 3



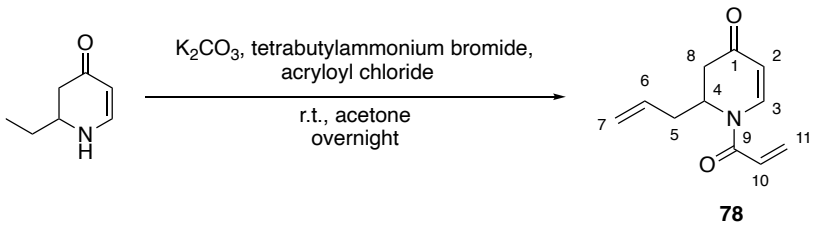
77

Phenyl 2-allyl-4-oxo-3,4-dihydropyridine-1(2*H*)-carboxylate (2.95 g, 11.5 mmol) was dissolved in methanol (18 mL) for 10 min. A 50% solution of NaOMe in methanol (0.88 g in 1.77 mL) was added and stirred for 17 h at r.t. to give an orange then purple solution. After this, the reaction was neutralized by the dropwise addition of 12 M HCl. The solvent was removed *in vacuo* to give the crude dihydropyridine as a viscous orange oil. Purification by silica gel chromatography (1:1 petroleum ether 40-60:EtOAc) gave the title compound **77** (1.50 g, 95%) as an orange oil: R_f 0.32 (100% ethyl acetate: 1% methanol); IR ν_{max} (thin film)/cm^{-1} 3233 (amine), 1619 (Ketone), 1563 (alkene); δ_H (400 MHz, $CDCl_3$) 7.15 (1H, d, *J* 7.0 Hz, 3-H), 5.88-

5.61 (1H, m, 6-H), 5.25-5.13 (2H, m, 7-H_a, 2-H), 5.00 (1H, t, *J* 6.9 Hz, 7-H_b), 3.67 (1H, sext, *J* 5.7 Hz, 4-H), 2.49-2.25 (4H, m, 8-H_2, 5-H_2), 1.24 (1H, br s, NH) ; δ_C (101 MHz, $CDCl_3$) 192.7 (1-C), 150.67(3-C), 133.0 (6-C), 119.3 (7-C), 99.3 (2-C), 52.0 (4-C), 42.1 (8-C), 38.5 (5-C); HRMS: Found 138.0922. $C_8H_{12}NO$ ($M + H^+$) requires 138.0919. Found 160.0742. $C_8H_{11}NONa$ ($M + Na^+$) requires 160.0738.

1-acryloyl-2-allyl-2,3-dihydropyridin-4(1*H*)-one

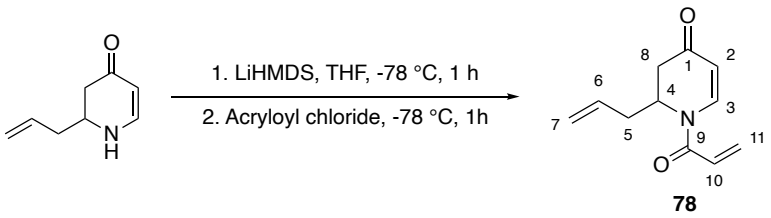
Method 1



78

2-Allyl-2,3-dihydropyridin-4(1*H*)-one (165 mg, 1.20 mmol) was stirred for 10 min at r.t. in acetone (10 mL). K_2CO_3 (332 mg, 2.40 mmol) and tetrabutyl ammonium bromide (33 mg, 0.12 mmol) was added and stirred for 20 minutes to give an orange solution. Acryloyl chloride (130 mg, 1.44 mmol) was added and left to stir overnight to give a red solution. The reaction mixture was filtered by gravity filtration and the solvent removed *in vacuo*. The red oil was dissolved in chloroform (10 mL), washed with deionised water (50 mL), dried with $MgSO_4$, and filtered. The reaction mixture was concentrated *in vacuo* to give the crude dihydropyridine as a dark orange oil. Purification by silica gel chromatography (1:1 petroleum ether 40-60:EtOAc) gave the title compound **78** (858 mg, 37%) as an orange oil.

Method 2

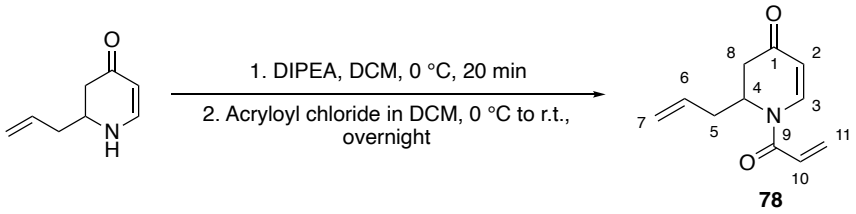


78

2-Allyl-2,3-dihydropyridin-4(1*H*)-one (235 mg, 1.71 mmol) was dissolved in dry THF (50 mL) under argon at -78 °C. LiHMDS (1.06 M in THF, 2.57 mmol) was added dropwise and stirred

for 1 h. Acryloyl chloride (233 mg, 2.57 mmol) was added dropwise at -78 °C and stirred for 1 h. After this the reaction mixture was quenched with NH_4Cl (70 mL) and the layers separated. The aqueous layer was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with brine (100 mL). The combined organic layers were dried with $MgSO_4$, filtered and the solvent was concentrated *in vacuo* to give the crude dihydropyridine as an orange oil. Purification by gradient silica gel chromatography (8:2, 7:3, 6:4, 1:1 petroleum ether 40-60:EtOAc) gave the title compound **78** (40.4 mg, 12 %) as an orange oil.

Method 3



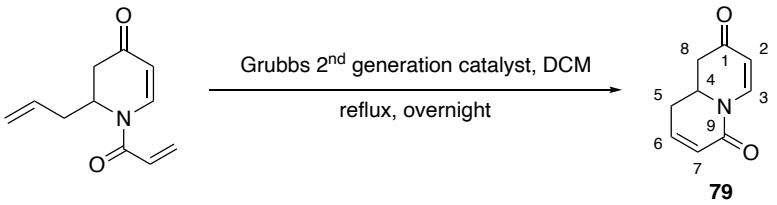
78

2-Allyl-2,3-dihydropyridin-4(1*H*)-one (191 mg, 1.39 mmol) was dissolved in DCM (2 mL) at 0 °C in an ice/water bath. DIPEA (170 mg, 1.39 mmol) was added and left to stir for 20 min. After this acryloyl chloride (132 mg, 1.46 mmol) in DCM (0.5 mL) was added dropwise at 0 °C and left stirring to warm up to r.t. overnight. After this the reaction mixture was washed with 5% aqueous HCl (3 mL), saturated aqueous $NaHCO_3$ (3 mL), dried with $MgSO_4$ and filtered. The solvent was removed *in vacuo* to give the crude dihydropyridine as an orange oil. Purification by silica gel chromatography (100% EtOAc) gave the title compound **78** (75.5 mg, 28%) as an orange oil: R_f 0 (ethyl acetate); IR ν_{max} (thin film)/cm^{-1} 1660 (ketone), 1588 (alkene); δ_H (400 MHz, $CDCl_3$) 7.75 (1H, s, 3-H), 6.76-6.63 (1H, m, 10-H), 6.51 (1H, d, *J* 16.7 Hz, 11-H_a), 5.98 (1H, dd, *J* 10.4, 1.6 Hz, 2-H), 5.76 (1H, ddt, *J* 17.3, 10.2, 7.2 Hz, 6-H), 5.44 (1H, d, *J* 8.3 Hz, 11-H_b),

5.18-5.06 (2H, m, 7-H_a, 7-H_b), 3.97-3.72 (1H, m 4-H), 2.80 (1H, dd, *J* 16.6, 6.4 Hz, 8-H_a), 2.61 (1H, dt, *J* 16.6, 1.6 Hz, 8-H_b), 2.21-1.97 (1H, m, 5-H_a), 1.50 (1H, m, 5-H_b); δ_C (101 MHz, $CDCl_3$) 192.8 (1-C), 164.87 (9-C), 140.6 (3-C), 132.7 (6-C), 132.1 (10-C), 125.8 (11-C), 119.5 (7-C), 108.3 (2-C), 53.0 (4-C), 39.3 (8-C), 35.1 (5-C); HRMS: Found 192.1028. $C_{11}H_{14}NO_2$ ($M + H^+$) requires 192.1025. Found 214.0852. $C_{11}H_{13}NO_2Na$ ($M + Na^+$) requires 214.0844.

9,9a-dihydro-2*H*-quinolizine-2,6(1*H*)-dione

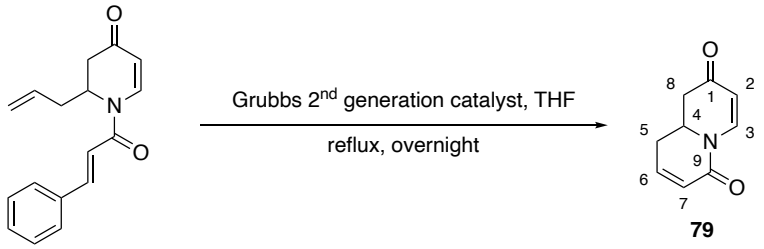
Method 1



79

2-Allyl-2,3-dihydropyridin-4(1*H*)-one (49.2 mg, 0.257 mmol) was dissolved in dry DCM (5 mL) under Argon. Grubbs second generation catalyst (22.1 mg, 0.026 mmol) was then added and left stirring overnight under reflux. The solvent was removed *in vacuo* to give the crude dione as a black solid. Purification by silica gel chromatography (1:1 petroleum ether 40-60:EtOAc) gave the title compound **79** (9 mg, 22%) as a black/silver solid.

Method 2

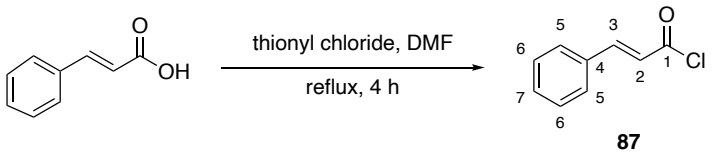


79

2-allyl-1-cinnamoyl-2,3-dihydropyridin-4(1*H*)-one (288 mg, 1.08 mmol) was dissolved in dry THF (40 mL) under Argon. Grubbs second generation catalyst (91.8 mg, 0.108 mmol) was added and left stirring overnight under reflux. The solvent was removed *in vacuo* to give the crude dione as a black solid. Purification by silica gel chromatography (1:1 petroleum ether

40-60:EtOAc) gave the title compound **79** (156 mg, 88%) as a black/brown crystalline solid: R_f 0.1 (1:1 petroleum ether 40-60:ethyl acetate); IR ν_{max} (thin film)/cm^{-1} 1687 (ketone), 1640 (ketone), 1590 (alkene); δ_H (400 MHz, $CDCl_3$) 8.29 (1H, d, *J* 8.4 Hz, 3-H), 6.88 (1H, ddd, *J* 10.1, 6.4, 2.2 Hz, 6-H), 6.16 (1H, dd, *J* 10.1, 3.0 Hz, 7-H), 5.56 (1H, dd, *J* 8.4, 0.9 Hz, 2-H), 4.13 (1H, tt, *J* 12.9, 5.5 Hz, 4-H), 2.73-2.58 (3H, m, 8-H_a, 8-H_b, 5-H_a), 2.50 (1H, ddt, *J* 18.2, 12.7, 2.6 Hz, 5-H_b); δ_C (101 MHz, $CDCl_3$) 192.0 (1-C), 161.6 (9-C), 142.2 (6-C), 141.4 (3-C), 124.2 (7-C), 108.8 (2-C), 52.0 (4-C), 43.0 (8-C), 30.1 (5-C); HRMS: Found 186.0527. C_9HgNO_2Na ($M + Na^+$) requires 186.0531.

Cinnamoyl chloride

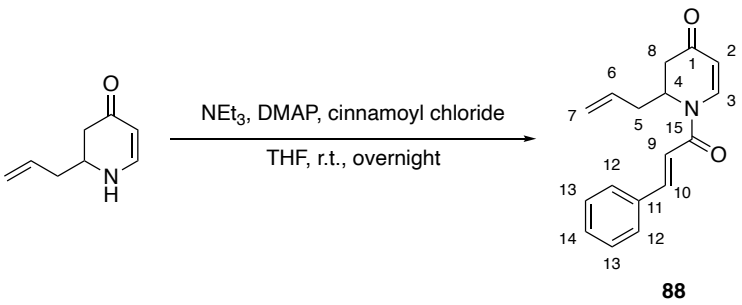


87

Cinnamic acid (3.00 g, 20.2 mmol) was dissolved in thionyl chloride (10.12 g, 85.0 mmol) and left stirring for 10 min. A catalytic amount of DMF (0.15 mL, 2.03 mmol) was added and effervescence was seen. The reaction was performed under reflux for 4 h to give a yellow solution. The solvent was removed *in vacuo* to give the pure title compound **88** (3.11 g, 92%) as a yellow gum: R_f 0.69 (1:1 petroleum ether 40-60:ethyl acetate); IR ν_{max} (thin film)/cm^{-1} 1677 (ketone), 1611 (alkene); δ_H (400 MHz, $CDCl_3$) 7.85 (1H, d, *J* 15.6 Hz, 3-H), 7.58 (2H, d, *J* 6.5 Hz, 5-H_2), 7.46 (3H, ddd, *J* 7.7, 5.8, 4.1 Hz, 6-H_2, 7-H), 6.66 (1H, d, *J* 15.6 Hz, 2-H); δ_C (101 MHz, $CDCl_3$) 166.2 (1-C), 150.7 (3-C), 133.1 (4-C), 132.0 (6-C), 129.3 (5-C), 129.1 (7-C), 122.4 (2-C); HRMS: Found 131.0503. C_9H_7O ($M - Cl^-$) requires 131.0497.

2-allyl-1-cinnamoyl-2,3-dihydropyridin-4(1*H*)-one

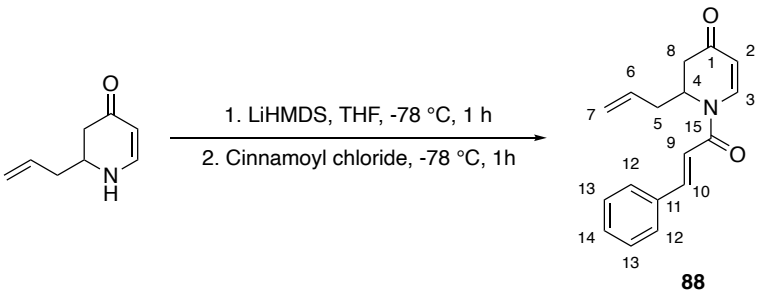
Method 1



88

2-allyl-2,3-dihydropyridin-4(1*H*)-one (100 mg, 0.729 mmol) was dissolved in THF (1 mL) and left stirring for 10 min. Triethylamine (296 mg, 29.3 mmol), DMAP (196 mg, 1.60 mmol) and cinnamoyl chloride (304 mg, 1.82 mmol) was added consecutively and left stirring at r.t. overnight. The solvent was removed *in vacuo* to give the crude dihydropyridine as orange/brown oil. Purification by gradient silica get chromatography (7:3, 6:4 petroleum ether 40-60 to EtOAc) gave the title compound **89** (43 mg, 22%) as a yellow oil.

Method 2



88

2-allyl-2,3-dihydropyridin-4(1*H*)-one (188 mg, 1.36 mmol) was dissolved in dry THF (25 mL) under Argon at -78 °C. LiHMDS (1.06 M in THF, 2.04 mmol) was added dropwise to give a dark

yellow solution and left stirring for 1 h. Cinnamoyl chloride (350 mg, 2.04 mmol) was added at -78 °C and left to stir for 1 h to give a bright orange solution. The reaction was quenched with brine (25 mL) and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic layers were washed with brine (100 mL), dried with $MgSO_4$ and the solvent was removed *in vacuo* to give the crude dihydropyridine as an orange viscous oil. Purification by gradient silica gel chromatography (100% petroleum ether, 9:1, 1:1 petroleum ether 40-40:EtOAc) gave the title compound **89** (288 mg, 79%) as an orange/yellow oil: R_f 0.62 (7.3 petroleum ether 40-60:ethyl acetate); IR ν_{max} (thin film)/cm^{-1} 1733 (ketone), 1657 (ketone), 1616 (alkene) 1589 (alkene); δ_H (400 MHz, $CDCl_3$) 7.85 (1H, d, *J* 15.3 Hz, 3-H), 7.63-7.51 (2H, m, 12-H_2), 7.47-7.30 (4H, m, 13-H_2, 14-H, 10-H), 6.98 (1H, d, *J* 15.4 Hz, 9-H), 5.79 (1H, ddt, *J* 17.3, 10.3, 7.3 Hz, 6-H), 5.46 (1H, d, *J* 8.3 Hz, 2-H), 5.21-5.03 (2H, m, 7-H_2), 2.82 (1H, dd, *J* 16.7 6.5 Hz, 4-H), 2.61 (2H, d, *J* 16.7 Hz, 8-H_2), 2.54-2.32 (2H, m, 5-H_2); δ_C (101 MHz, $CDCl_3$) 193.1 (1-C), 165.0 (15-C), 147.0 (3-C), 140.1 (10-C), 134.3 (11-C), 132.8 (6-C), 130.9 (13-C), 129.1 (12-C), 128.3 (14-C), 119.5 (9-C), 114.8 (7-C), 108.0 (2-C), 39.4 (4-C), 35.2 (8-C), 24.0 (5-C). HRMS: Found 268.1313. $C_{17}H_{18}NO_2$ (M + H^+) requires 268.1338. Found 290.1133. $C_{17}H_{17}NO_2Na$ (M + Na^+) requires 290.1157.

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