Synthesis and Biological Activity of Pyrrolizidine Alkaloids and Analogues

A thesis presented in part fulfilment of the requirement for the Degree of Doctor of Philosophy

by

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to Marion

"Then a ploughman said, Speak to us of work.

And he answered, saying:

You work that you may keep pace with the earth and the soul of the earth.

For to be idle is to become a stranger unto the seasons, and to step out of life's procession that marches in majesty and proud submission towards the infinite.

When you work you are a flute through whose heart the whispering of the hours turns to music.

Which of you would be a reed, dumb and silent when all else sings together in unison?"

"The Prophet"

Kahlil Gibran

"If I answered these questions, it would kill the suspense. It would resolve the conflict and turn intriguing possibilities into boring old facts."

Calvin in "The Days are Just Packed" by Bill Watterson

"This is the essence of science Scully - ask an impertinent question, you're on your way to a pertinent answer."

Special Agent Fox Mulder

Acknowledgements

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Finally I would like to thank my wife Valerie, without whom. . . , my Dad, Brian, my mum Marion, without whom I wouldn't be here, or anywhere else for that matter, Tom Waits for the musical accompaniment to the writing of this thesis, and my friends Scott, Campbell and Gary for all the other important stuff.

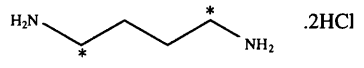
Summary

The work presented in this thesis is concerned with the synthesis and biological activity of pyrrolizidine alkaloids and analogues and has been divided into seven main areas: (a) the isolation and preparation of simple derivatives of pyrrolizidine alkaloids isolated from plants and root cultures available within the University; (b) the synthesis of synthanecine A, and derivatives of this compound for investigation of their anti-tumour activity; (c) approaches to the synthesis of one enantiomer of synthanecine A; (d) approaches to the synthesis of a novel synthanecine; (e) attempted synthesis of novel necic acids for esterification with synthanecine A; (f) the synthesis of novel pyrrolizidine alkaloid analogues via a 1,3-dipolar cycloaddition; (g) investigation of the biological activity of some of the compounds isolated and synthesised during the project. Those topics of greatest significance in the thesis are summarised more fully below.

Isolation and Derivatisation of Pyrrolizidine Alkaloids

A number of pyrrolizidine alkaloids are available in the Chemistry Department, either from plant sources or from root cultures. Samples of these compounds were requested by Dr. Bryan Hanley, MAFF, Norwich, for metabolic studies. These compounds were also required to be radiolabelled with ^{14}C. This was achieved by feeding [1,4-^{14}C]putrescine (**A**) to the plants and root cultures. The *N*-oxides of the pyrrolizidine alkaloids isolated were synthesised. *N*-Oxides of the radiolabelled alkaloids were also synthesised. The quaternary methiodide derivatives were also prepared for biological testing.

Rosmarinine (**B**) can be readily isolated in gram quantities from *Senecio pleistocephalus*. This compound can be converted into the toxic alkaloid senecionine (**C**) by elimination of the hydroxyl group to give a double bond in the 1,2-position. This elimination was carried out with limited success.



* = position of label

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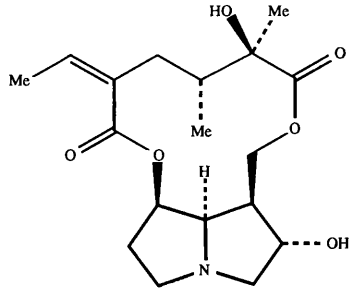

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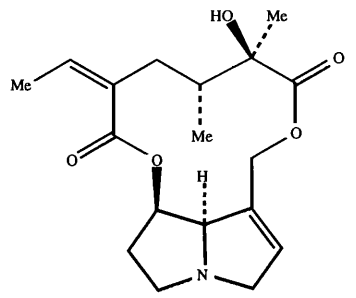
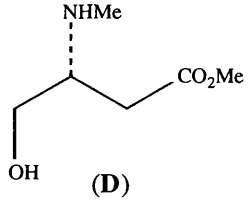


(C)

Approaches to the Synthesis of One Enantiomer of Synthanecine A

In order to prepare a closer pyrrolizidine alkaloid analogue, the synthesis of one enantiomer of synthanecine A (**H**, R=H) was undertaken. Several approaches were tried in this synthesis. In all approaches the key intermediate methyl (*R*)-3-(*N*-methylamino)-4-hydroxybutanoate was identified (**D**). Syntheses starting from (*S*)-malic acid (**E**) and from D-aspartic acid (**F**) were attempted. L-Aspartic acid was also used because it is cheaper and more readily available. The synthesis from L-aspartic acid proved most successful, and this route was advanced beyond the key intermediate to the diester (**G**).



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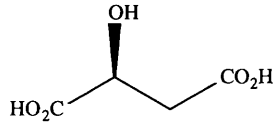

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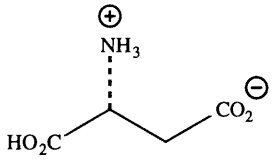

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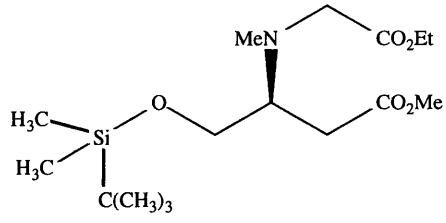
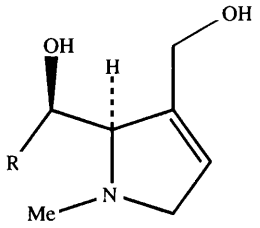


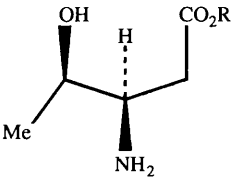
(G)

Approaches to the Synthesis of a Novel Optically Active Synthanecine

Synthanecine A differs from a pyrrolizidine alkaloid in the number of carbon atoms in the molecule. Thus it was decided to attempt a synthesis of the novel synthanecine (**H**, R=Me). The substituted pentanoic acid derivative (**I**) was identified as the key intermediate. This compound could be derived from L-threonine. (11)



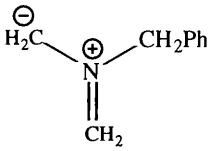
(**H**)



(**I**)

Synthesis of Novel Pyrrolizidine Alkaloids Analogues via a 1,3-Dipolar Cycloaddition

The 1,3-dipolar cycloaddition reaction of the azomethine ylide (**J**) derived from *N*-benzyl-*N*-(trimethylsilylmethyl)aminomethyl methyl ether (**K**) with a variety of dipolarophiles was used to prepare a range of pyrrolidines of the general structure (**L**) and the 3-pyrroline (**M**). Simple derivatives of the pyrrolidines were prepared by removal of the benzyl groups and by reduction and further reaction of the ester functions.



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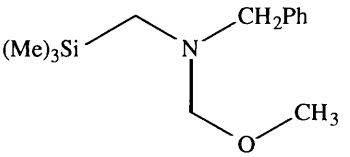

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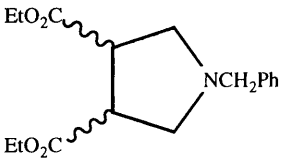
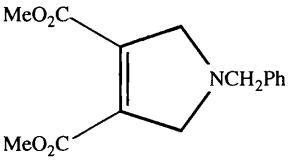


(**L**)



(**M**)

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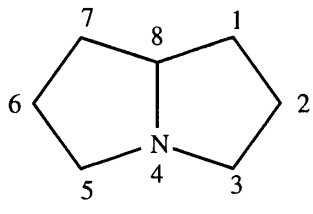
Chapter 1

Introduction

1.1 Pyrrolizidine Alkaloids

Pyrrolizidine alkaloids are secondary metabolites produced by certain higher plants.[1] They have become the subject of considerable interest over recent years since the recognition that the ingestion of plants containing pyrrolizidine alkaloids is responsible for serious livestock loss and many incidents of human poisoning principally by hepatotoxicity.[2-6]

Pyrrolizidine alkaloids contain the 1-azabicyclo[3.3.0]octane system (**1**). A number of alkaloids are derivatives of 1-aza-1-methylcyclo-octan-5-one (**2**). The numbering system generally used for these compounds is shown below. The base portions of pyrrolizidine alkaloids are termed necines. Most necines possess a hydroxymethyl group at C-1 as in (-)-trachelanthamidine (**3**), and many have a double bond in the 1,2-position as in retronecine (**4**). Although these features are typical of necines, many examples exist which have other structural features in addition to or instead of the above, for example the diol platynecine (**5**), the triol crotanecine (**6**) and the non-basic pyrrole derivative (**7**). These factors and the stereochemistry of the substituents account for the large number of necine structures.



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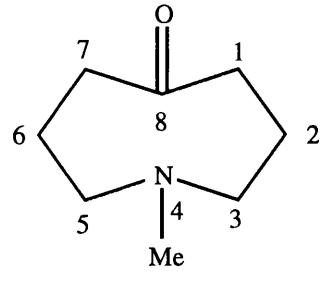

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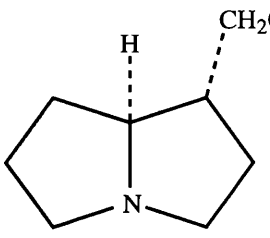

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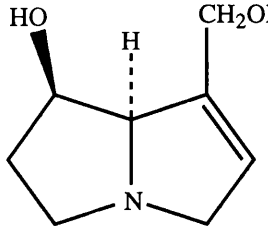

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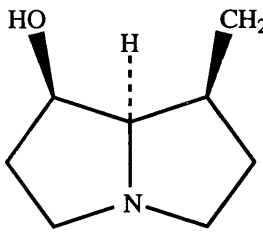

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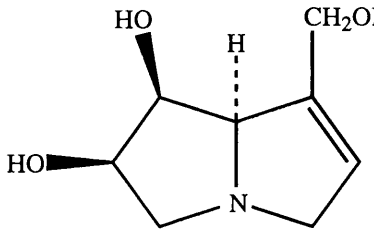

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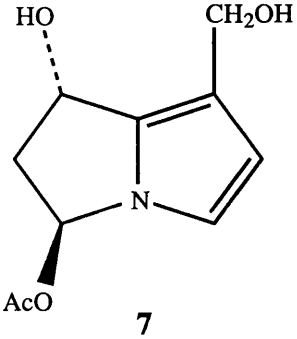

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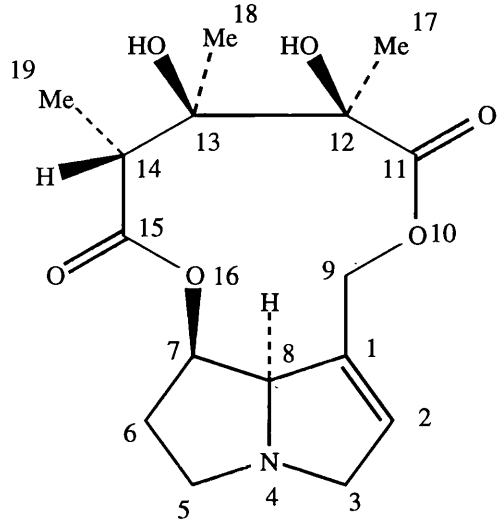
Pyrrolizidine alkaloids are usually found as ester derivatives. These can be monoesters (usually esterified at the 7- or 9- positions), diesters or macrocyclic diesters, for example monocrotaline (**8**) (the conventional numbering system for pyrrolizidine alkaloids is given for this compound). These macrocyclic diesters can have varying ring sizes from 11 in monocrotaline up to 13 in bulgarsenine (**9**). The esterifying acids are known as necic acids and are often highly substituted and oxygenated as shown by the C_7 acid (-)-trachelanthic acid (**10**) and the C_{10} acid seneciphyllic acid (**11**). Pyrrolizidine alkaloids also exist as *N*-oxides within the plant, for example rosmarinine *N*-oxide (**12**). These are normally chemically reduced to the free base after extraction from the plant.

1.2 Occurrence of Pyrrolizidine Alkaloids

Pyrrolizidine alkaloids occur widely both geographically and botanically. They have been found in 14 unrelated plant families[7] and over 300 plant species[8] and occur in plant species on every continent. It has been estimated that approximately 3% of the world's flowering plants contain pyrrolizidine alkaloids.[9] A

certain degree of species specificity has been observed, for example in the Boraginaceae family most of the pyrrolizidine alkaloids found are monoesters and diesters whereas in the Asteraceae (Compositae) family, macrocyclic pyrrolizidine alkaloids are more frequent.[10]



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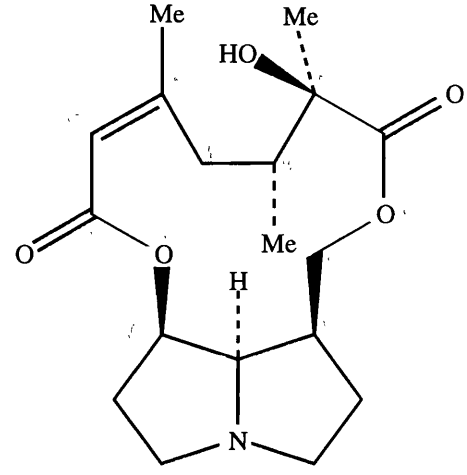

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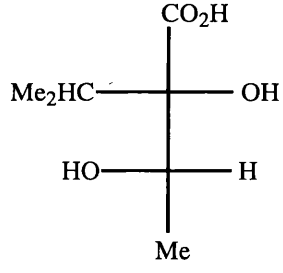

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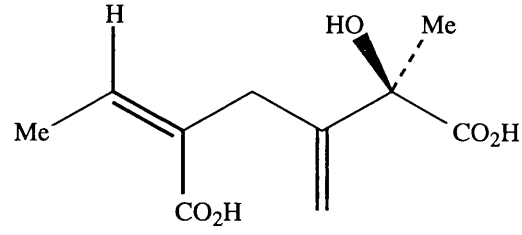

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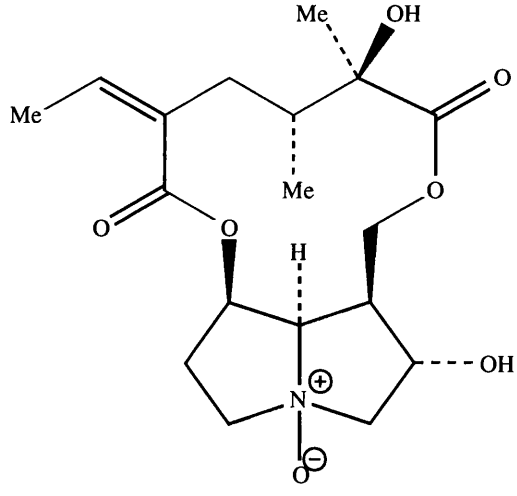

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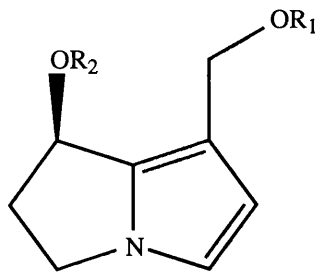
The particular alkaloid found within a plant and the quantity isolated is dependent upon the part of the plants harvested (e.g. roots, leaves, flowers, etc.), the climate where the plant grew, the soil conditions and the time of harvesting.[11] For example *Senecio hygrophyllus* contains differing proportions of rosmarinine, platyphylline and hygrophylline depending upon its time of harvest, the season and the location of the plant.[12,13] Ratios of free base to *N*-oxides can vary between different parts of the plant; for example in *Crotalaria retusa*, basic alkaloids accumulate in the seeds whereas *N*-oxides often predominate in the green parts of the plant.[14,15]

The yields of pyrrolizidine alkaloids isolated from a particular plant can vary considerably. For example, Johnson *et al.*[16] observed pyrrolizidine alkaloid levels which varied form 0.18 % to 17.99 % in *Senecio ridellii* from different locations.

1.3 Analogues of Pyrrolizidine Alkaloids

The amounts of pyrrolizidine alkaloids which can be isolated from plants are usually small, and the syntheses of such compounds are often lengthy and may result in a poor overall yield or are impractical to scale up. Many or all of the toxic effects of pyrrolizidine alkaloids can be attributed to the formation of the pyrrole ring (**13**).[17] When considering the synthesis of model compounds the saturated ring in the didehydropyrrolizidine nucleus is not involved in metabolism to a toxic intermediate and could thus be omitted.[18] This has led to the preparation of a series of monocyclic analogues of the necine bases, which have been called synthanecines.[18] The most commonly prepared of these are synthanecine A (**14**), synthanecine B (**15**) and synthanecine C (**16**). Preparation of monoesters, diesters and macrocyclic diesters of these compounds has served to give representative analogues of pyrrolizidine alkaloids. A wide variety of such compounds has been prepared.[19-22]



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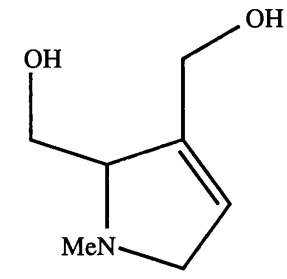

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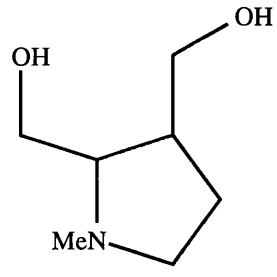

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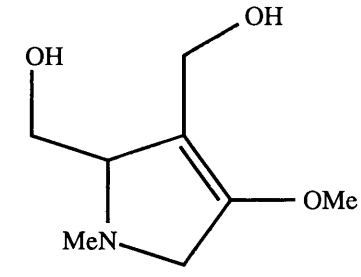

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1.4 Metabolism of Pyrrolizidine Alkaloids and Analogues

In considering the toxic actions of pyrrolizidine alkaloids it is important to consider whether it is the alkaloid itself or a metabolite of the alkaloid that is responsible for the toxic action. There is considerable weight of evidence suggesting that it is the metabolites of the alkaloids that are responsible for their toxic action. This evidence is summarised below:

- Pyrrolizidine alkaloids are not locally toxic at the site of administration or injection.[17]
- Some organisms e.g. cinnabar moth larvae are uninjured by the alkaloids, despite storing large quantities within their tissues.[23]
- The main organ damaged by pyrrolizidine alkaloids is the liver which is the site of their metabolism.[17]
- Inhibition of liver drug metabolism enzymes results in decreased toxicity.[17]
- Promotion of liver drug metabolism enzymes results in increased toxicity.[24]

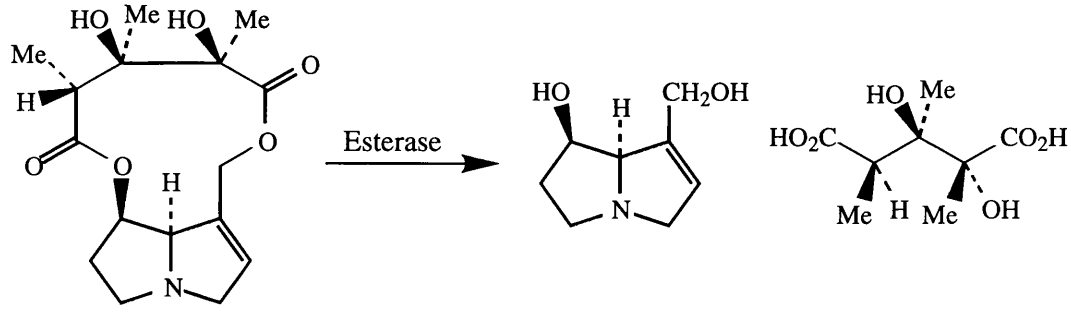
It has been established that only pyrrolizidine alkaloids containing a double bond in the 1,2-position are hepatotoxic. Indeed the metabolite responsible for this hepatotoxicity is also implicated in the other toxic actions that pyrrolizidine alkaloids can exert.[17]

1.4.1 Metabolic Pathways of Hepatotoxic Pyrrolizidine Alkaloids and Analogues.

Hepatotoxic pyrrolizidine alkaloids are able to undergo a variety of metabolic fates within the body.

i. Hydrolysis

Hydrolysis of a hepatotoxic pyrrolizidine alkaloid produces the non-hepatotoxic necine and necic acid. For example the enzymatic or chemical hydrolysis of hepatotoxic monocrotaline (**8**) results in the liberation of non-hepatotoxic retronecine (**4**) and monocrotalic acid (**17**) (**scheme 1**).



8 **4** **17**

Scheme 1 : Products of PA Hydrolysis

The rates of enzymatic hydrolysis of pyrrolizidine alkaloids are important in estimating the level of toxicity of a particular alkaloid. A fast turnover of

pyrrolizidine alkaloid to necine and necic acid results in less pyrrolizidine alkaloid reaching the liver intact. Dehydrogenation of the necine does not result in an active alkylating species (see **section 1.5.1iii** below). Steric hindrance around the ester groups inhibits enzymatic hydrolysis as shown in **table 1**.[25] This results in pyrrolizidine alkaloids bearing hindered ester groups tending to exhibit higher liver toxicity.

Acid[a]	Alkyl Group of Acid	Enzymatic Hydrolysis Rate μmol/min/g liver[b]
Valerate	Me $(CH_2)_3$-	20
Isovalerate	Me_2CHCH_2-	4.3
Pivalate	Me_3C-	0.5
Senecioate	Me_2C=CH-	0.35

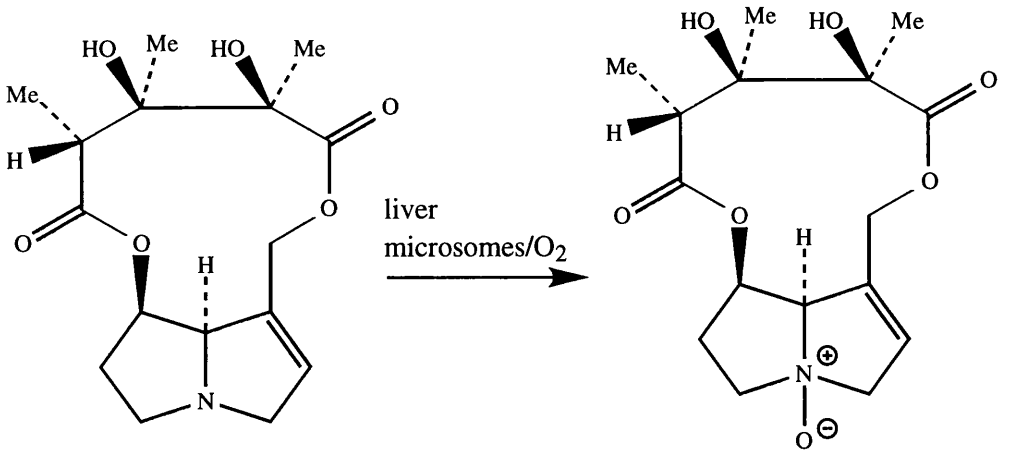
[a] Retronecine was the base in all cases

[b] Anaerobic rates in rat liver homogenate at pH 7.5, 37 °C

Table 1 : Comparison of Rates of Enzyme Catalysed Hydrolysis of Retronecine Diesters

ii. *N*-Oxidation

Enzymatic oxidation produces two major metabolites in pyrrolizidine alkaloid metabolism, and the product of *N*-oxidation is considered here. The product of dehydrogenation is considered below (**section 1.4.1iii**). The formation of the *N*-oxide results in excretion of this compound due to its low lipophilicity. This reaction is brought about by the liver microsomal system.[26] The *N*-oxide is non-hepatotoxic. the mechanism of *N*-oxidation has yet to be elucidated but probably involves a mixed function oxidase requiring both oxygen and $NADP^+$. The *N*-oxidation of monocrotaline (**8**) to monocrotaline *N*-oxide (**18**) is shown in **scheme 2**.



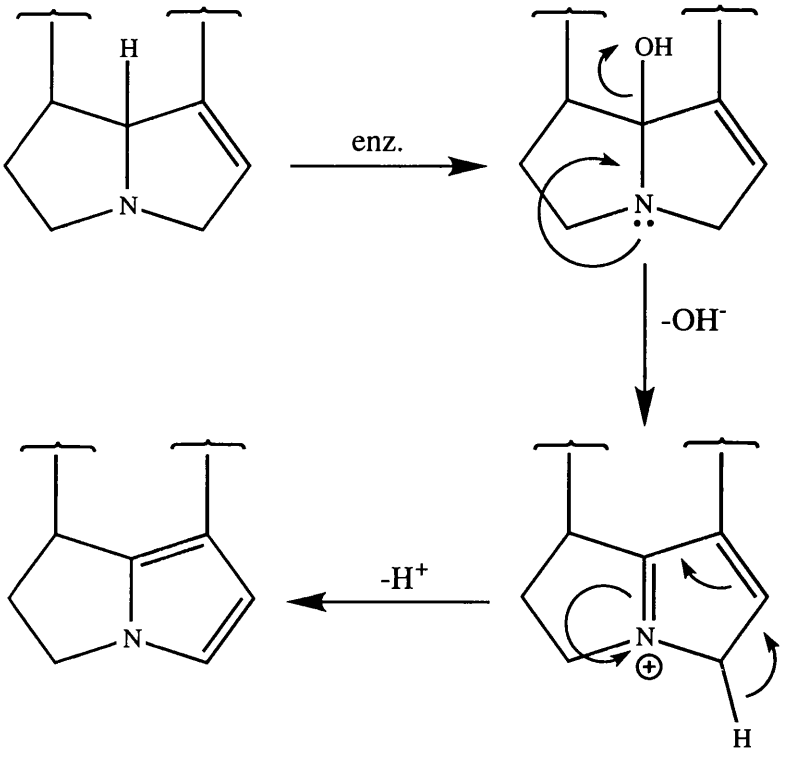
8 **18**

Scheme 2 : Enzymatic Conversion of Monocrotaline (8) into Monocrotaline *N*-Oxide (18)

iii. Dehydrogenation

Hepatotoxic pyrrolizidine alkaloids are metabolised to pyrrole derivatives. It is these derivatives that are responsible for the hepatotoxic action of these compounds. Pyrrolic metabolites are formed by the action of microsomal enzymes in the liver which formally dehydrogenate the unsaturated ring of the pyrrolizidine alkaloid. The proposed mechanism of pyrrole formation via hydroxylation is presented in **scheme 3**.[26, 27, 28]

Although both *N*-oxidation and dehydrogenation are carried out by liver microsomal enzymes, the relative proportions of these compounds that are formed is dependent upon the structure and physical properties of the alkaloid.[26, 29] The acid moieties can exert steric hindrance over C-8, the putative site of hydroxylation in the formation of the pyrrole derivative. As a result of this, diesters who are able to hinder the C-8 position the most, often show the greatest proportion of *N*-oxide formation, whereas monoesters and macrocyclic diesters often show greater amounts of pyrrole formation since the steric hindrance at C-8 is less in these compounds.[26]



Scheme 3 : Mechanism of Pyrrolic Metabolite Formation

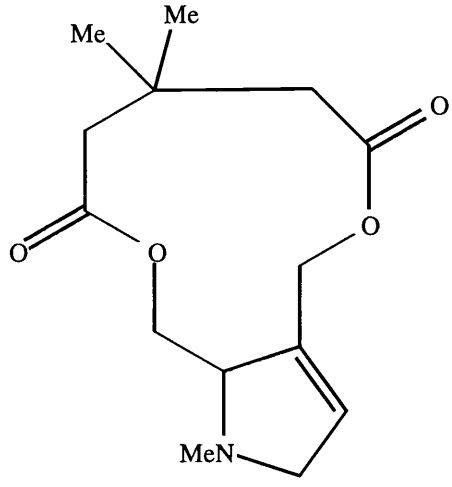
iv. Other Metabolic Products

Alternative metabolic fates of pyrrolizidine alkaloids have been poorly studied. Hydroxylation of the necic acid portion of senecionine has been observed,[30] although this was a very minor metabolite. Other metabolic products include demethylated species[31] and epoxides.[1,32]

v. Metabolism of Pyrrolizidine Alkaloid Analogues

Macrocyclic diesters of synthanecine A (**14**) have been prepared and their toxicity in rats has been tested. 3,3-Dimethylglutaryl synthanecine A (**19**) proved to be the most toxic. Moreover, the toxicity of this compound was increased when esterases were inhibited by pretreatment with the esterase inhibitor tri-orthocresyl phosphate (TOCP) (see **section 1.5.1iii**). This is consistent with pyrrolizidine

alkaloid toxicity. The metabolic behaviour and toxicity of this compound are comparable to those of a hepatotoxic pyrrolizidine alkaloid.[33]



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1.5 Toxicity of Pyrrolizidine Alkaloids, Metabolites and Analogues

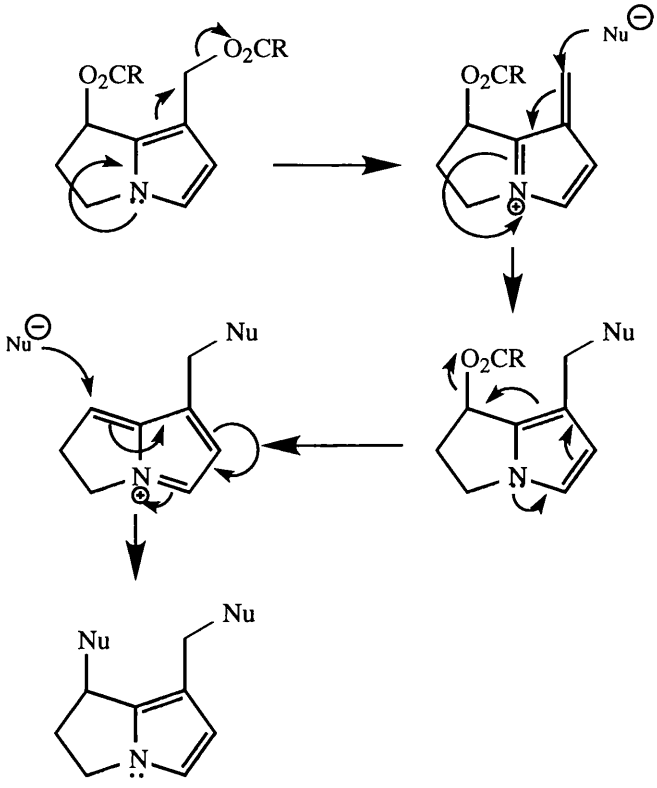
The wide variety of pyrrolizidine alkaloids can have an equally wide spectrum of toxic effects. These effects can be classified into two categories. Pharmacological effects (e.g. effect of a pyrrolizidine alkaloid on certain types of smooth muscle) can result in rapid death. Cytotoxic effects (e.g. the irreversible binding of a pyrrolizidine alkaloid metabolite to a nucleophile in the cytoplasm) can lead to later death as a result of tissue damage.

This thesis is concerned with the cytotoxic effects of pyrrolizidine alkaloids. Cytotoxicity may be divided into two categories. Acute cytotoxicity may result in the death of an animal up to a week after administration of the alkaloid. Chronic cytotoxicity can occur when an animal survives a dose of pyrrolizidine alkaloids. Such effects can occur when the diet is contaminated by pyrrolizidine alkaloids (see **section 1.6**).

The conversion of pyrrolizidine alkaloids with a 1,2-double bond into the hepatotoxic pyrroles occurs almost exclusively in the liver and this is the primary site of damage. Pyrrolizidine alkaloid metabolites are also found in the lungs and the kidneys. Transport of the active metabolites to these organs is a reflection of their stability or half life. The more labile a metabolite, the less likely it is to be found in organs other than the liver where it is produced.[34]

Lipophilicity and base strength of the alkaloid are two factors which affect toxicity.[17] Higher lipophilicity means that the alkaloids are more susceptible to oxidation by hepatic microsomal enzymes, and hence are more toxic. Alkaloids of higher base strength are usually less lipophilic and therefore less toxic. This reduced toxicity is due to the fact that proportionally more of the alkaloid is protonated at physiological pH and thus can be excreted.

The pyrrole metabolites formed by hepatotoxic pyrrolizidine alkaloids can react with cellular nucleophiles as shown in **scheme 4** below.



Scheme 4 : Reaction of Nucleophiles with a Pyrrolic Metabolite

1.5.1 Pretreatments Which Affect Metabolism

i. Hepatic Microsomal Enzyme Inducers

The susceptibility of an animal to pyrrolizidine alkaloid toxicity is increased by treatment with a substance such as phenobarbitone which increases the activity of hepatic microsomal enzymes. This enhances the rate of conversion of the pyrrolizidine alkaloid into the toxic pyrrolic metabolite.[29, 35]

ii. Inhibitors of Microsomal Enzyme Activity

Compounds which inhibit the activity of liver microsomal enzymes such as carbon monoxide reduce the susceptibility of animals to pyrrolizidine alkaloid toxicity by decreasing the rate of conversion of pyrrolizidine alkaloids into pyrrolic metabolites.[36] Treatment with zinc lowers the ability of rat liver microsomes to convert pyrrolizidine alkaloids into pyrrolic metabolites.[37]

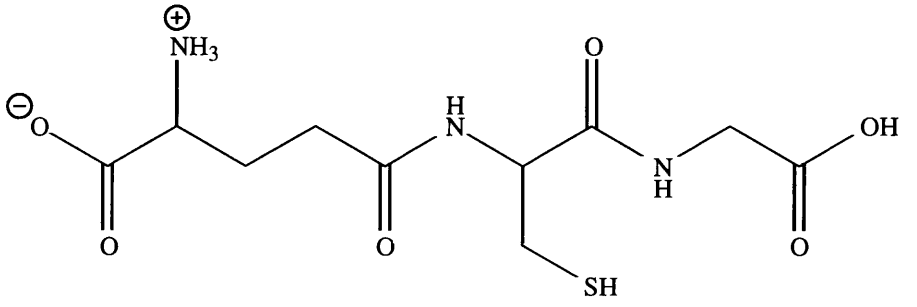
iii. Esterase Inhibitors

Hydrolysis of the ester groups of a pyrrolizidine alkaloid is a detoxification pathway because the hydroxyl groups left after hydrolysis are poor leaving groups compared to the ester groups. Pretreatment of animals with esterase inhibitors such as TOCP results in a larger proportion of the pyrrolizidine alkaloid reaching the liver intact where it may form a toxic pyrrolic metabolite.

1.5.2 Liver Thiol Levels

The pyrrolic metabolites formed by hepatotoxic pyrrolizidine alkaloids are potent alkylating agents and will alkylate a wide variety of cellular

nucleophiles.[39,40] Some protection can be afforded against this by pretreating the animal with a thiol such as mercaptoethylamine or cysteine[41] thus providing a ready source of nucleophile which may be alkylated instead of vital cellular constituents. Pretreatment with cysteine increases the levels of the tripeptide glutathione (GSH) (**20**) found in the liver.[42] It is this increase in levels of glutathione that decreases the pyrrolizidine alkaloid's toxicity.



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1.5.3 Other Biological Effects of Pyrrolizidine Alkaloids

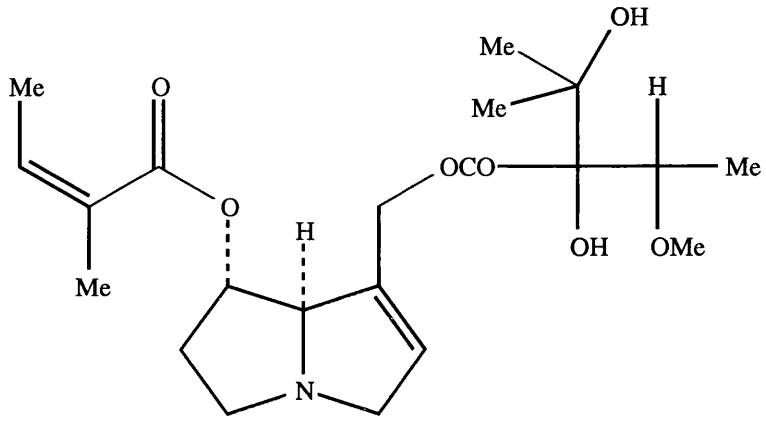
Although liver toxicity is the major component of the toxicological profile of pyrrolizidine alkaloids, these compounds have a broad spectrum of minor actions.

i. Mitochondria

Mitochondria are the sites of oxidative phosphorylation, the process in which the energy supplying molecule ATP is formed. Pyrrolizidine alkaloids have a pKa of about 7 and are partly protonated at physiological pH. The protonated form of the alkaloid competes for sites on mitochondria normally occupied by NAD^+, a key molecule in oxidative phosphorylation, and thus disrupts the function of the mitochondria.[43]

ii. Protein and RNA Synthesis

Lasiocarpine (**21**) has been shown to inhibit protein synthesis in rat liver.[44] This inhibition is proposed to be due to alkylation of messenger RNA. Lasiocarpine has also been shown to inhibit RNA polymerase.[45]



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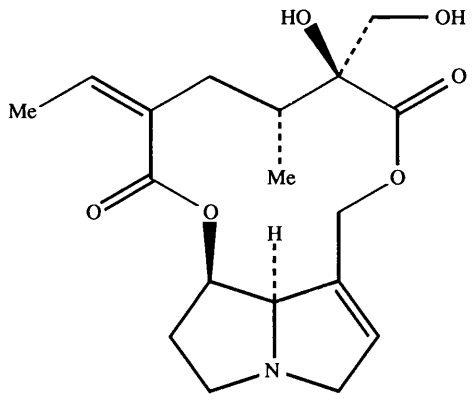
iii. Hepatic Microsomal Enzymes

Treatment of rats with a non-lethal dose of retrorsine (**22**) reduces the ability of their liver microsomal enzymes to metabolise subsequent administrations of the alkaloid to its pyrrolic metabolite.[29] This implies that metabolites formed in the initial administration are able to inhibit the action of the microsomal enzymes.

iv. Mineral Metabolism

The levels of minerals in rabbits were altered when they were fed on a diet containing *Senecio jacobaea*. The liver levels of zinc and copper and the levels of plasma iron were affected.[46]



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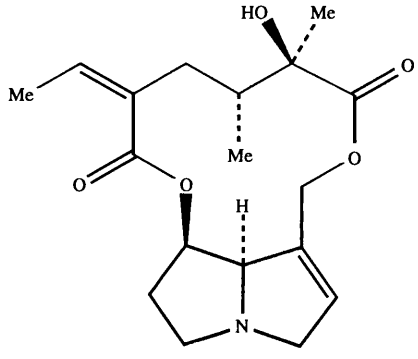
v. Embryotoxicity

It has been shown that senecionine (**23**) can cross the placenta[47] and disrupt the pregnancy of rats. Some litters were born prematurely and others were born dead or died shortly after birth. The precise reason for this is unclear. Unborn and new-born rats have a poor capacity for metabolising pyrrolizidine alkaloids to the toxic pyrrolic metabolites.[48] It is likely that any damage caused in the foetus is due to metabolism in the maternal liver and transport of the toxic metabolites to the foetus.



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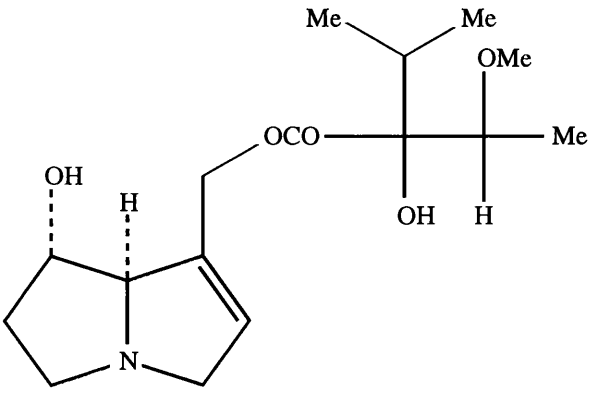
vi. Teratogenicity

Injection of heliotrine (**24**) into rats in the second week of pregnancy caused a variety of birth defects including growth retardation, and skeleto-muscular defects.[49]



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vii. Carcinogenicity

The carcinogenicity of many pyrrolizidine alkaloids has now been established.[50, 51] This carcinogenic action almost always gives rise to liver tumours suggesting that it is a metabolite of the alkaloid that is causing the tumour.

If one considers the alkylating activity of pyrrolizidine alkaloids (**scheme 4**) then it is clear that DNA may supply both the nucleophilic centres. This could result in cross-linked DNA. This cross-linking, although ultimately causing cell death, because of its antimitotic activity may provide some protection against carcinogenicity.[1]

Studies on the carcinogenic activity of pyrrolic metabolites of pyrrolizidine alkaloids have shown that pyrrolic diesters are not carcinogenic,[52] but the hydrolysed product, dehydroretronecine (**13**) (R_1=R_2=H), known to be a secondary metabolite of pyrrolizidine alkaloids,[53] can cause tumour growth.[54, 55]

1.5.4 Pyrrolizidine Alkaloid Toxicity in Livestock

Poisoning of livestock by the pyrrolizidine alkaloid-containing plant, ragwort (*Senecio jacobaea*) is said to cause more livestock losses in the U.K. than all other poisonous plants put together.[9] Although ragwort does not contain the

highest concentration of pyrrolizidine alkaloids, it is a very common weed and is difficult to eradicate. In the north-western United States poisoning of cattle is said to be a major economic problem.[56]

Ragwort is not the only plant responsible for livestock poisoning. **Table 2**[57] summarises a number of serious outbreaks of pyrrolizidine alkaloid poisoning. Such poisoning was often attributed to diseases until the toxic actions of pyrrolizidine alkaloids was better understood.

Country	Disease	Animal	Plant	Reference
New Zealand	Winton	Horse	*Senecio jacobaea*	58
Canada	Pictou	Cattle	*Senecio jacobaea*	59
South Africa	Molteno	Cattle	*Senecio colifolius*	60
U.S.A.	Dunskierte	Horse	*Senecio vernalis*	61
U.S.S.R	Zd'or	Horse	*Senecio erraticus*	62
Central Asia	Suilfuk	Horse	*Trichodesma incanum*	63

Table 2 : Pyrrolizidine Alkaloid Poisoning of Livestock

Pyrrolizidine alkaloid toxicity in livestock is seen primarily in the liver, but other organs are also often affected.[9]

1.5.5 Pyrrolizidine Alkaloid Toxicity in Humans

Pyrrolizidine alkaloid poisoning in humans is characterised by an acute liver disease known as veno-occlusive disease (VOD).[64, 65] Many cases of VOD prove to be fatal. Outbreaks of pyrrolizidine alkaloid poisoning in humans often derive from contaminated sources of grain, although some poisoning is due to other sources (see **section 1.6**). In South Africa in the 1920s seeds of *Senecio ilicifolius* and *S. burchellii* plants growing in wheat fields were harvested with the wheat and were responsible for over 80 cases of poisoning.[17] The largest case of pyrrolizidine

alkaloid poisoning in humans was in Afghanistan in 1974 and affected a population of 35,000. The source of the poisoning was bread contaminated with a *Heliotropium* species.[66, 67]

1.6 Sources of Exposure to Pyrrolizidine Alkaloids

Livestock are exposed to pyrrolizidine alkaloids through either contaminated food stock or by grazing upon pyrrolizidine alkaloid-containing plants.[17]

In humans, exposure to pyrrolizidine alkaloids can come from a variety of different sources. Pyrrolizidine alkaloid-containing plants are employed all over the world as foods or as medicines and can also be consumed accidentally when contamination occurs (see **section 1.5.5**).

i. Food Sources

No records of poisoning by pyrrolizidine alkaloid-containing plants used as foods have been found although a direct correlation between diet and cause of death is often difficult to establish. It seems likely however that some liver damage must accrue from consuming these plants. For example *Crotalaria retusa* is known to contain the alkaloid monocrotaline (**8**), which causes liver damage in rats, and is also carcinogenic.[50] This plant is used as a vegetable in India and parts of East Africa.[67]

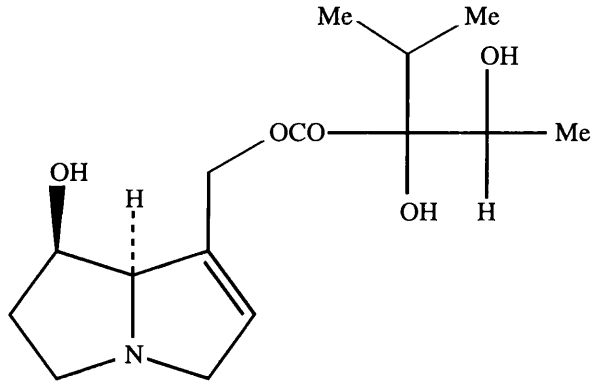
If milk is derived from animals which graze on pyrrolizidine alkaloid-containing plants then there is a possibility of the animal's milk being contaminated with the alkaloids. The quantity of alkaloid would be extremely low and is unlikely to cause any harm to anyone consuming such contaminated milk. There is no evidence to suggest that humans have been harmed by pyrrolizidine alkaloids in milk.[17]

ii Medicinal Sources

Herbal medicines have been employed throughout the world and are still used widely today, particularly in areas where modern medicines are unavailable. There has also been an increase in their use in the developed world as people seek alternative remedies to illnesses.

Herbal remedies can be split into two categories: those that involve consumption of dried plant matter and those that are prepared as teas.

Heliotropum indicum is used in dried form to treat a wide variety of ailments in southern Africa including sores, snakebites, prevention of abortion and strangely enough inducement of abortion.[68] *H. indicum* contains the alkaloid indicine (**25**).



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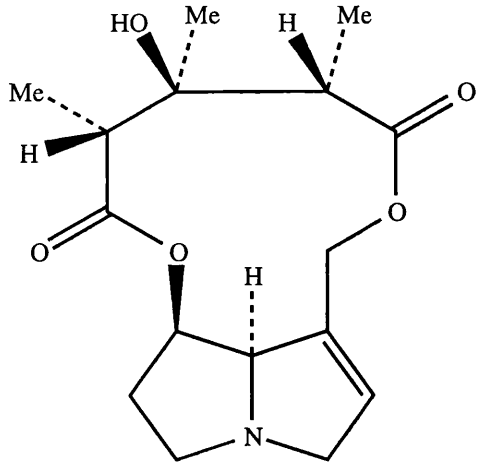
Teas are a popular remedy in parts of the West Indies. Their use is prescribed for treating fevers, coughs, colds and in pregnancy. Teas made with pyrrolizidine alkaloid-containing plants, for example *Crotalaria fulva* have been known to cause liver damage and death.[69] *C. fulva* contains fulvine (**26**).



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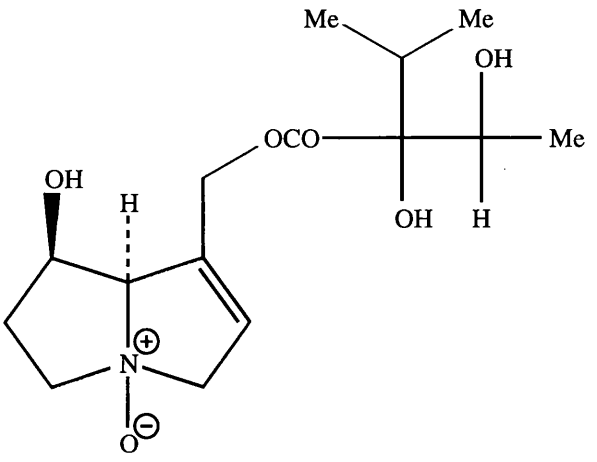
1.7 Antitumour Activity of Pyrrolizidine Alkaloids and Analogues

A variety of pyrrolizidine alkaloids has been tested for antitumour activity. These have included both alkaloids which can be metabolised to pyrrolic derivatives[70] and those which cannot.[71] The results of these studies showed that an unsaturated necine was not a prerequisite for anti-tumour activity.

One compound which has been tested and has shown some promise is indicine *N*-oxide (**27**) (INO).

INO is the only pyrrolizidine alkaloid to have entered clinical trials, where it was tested against both advanced solid tumours and advanced leukaemia. Against the solid tumours there was no therapeutic response. The major toxic action was bone marrow suppression.[72] Against leukaemia, INO prompted complete remission in two of the ten patients who underwent the treatment. Again the major toxic action was bone marrow suppression, although in the treatment of leukaemia, liver failure was seen in two patients.[73]



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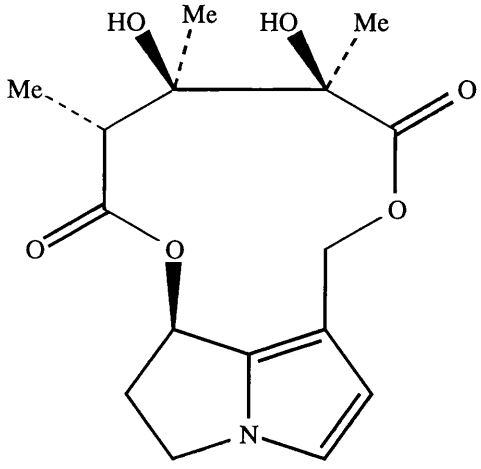
The mode of action of INO is unclear. Only a small amount of INO is converted into indicine base and so it seems unlikely that it is the base which exerts the antitumour action.[74] The pyrrole is also a poor candidate since with only a small amount of base present there would be a slow turnover to pyrrole, and indicine base itself has a low lipophilicity and thus is fairly resistant to pyrrole formation.[36, 75] From this evidence it would seem that it is INO itself that exerts the antitumour action, or some unknown metabolite.

Given the cytotoxic action of pyrrolic metabolites of pyrrolizidine alkaloids, it might be expected that these derivatives would be effective alkylating agents in treating tumours. The results however are inconclusive with some dehydro-alkaloids having similar activity to the parent alkaloid whilst others have a greater activity. For example dehydromonocrotaline (**28**) is more active than monocrotaline (**8**) itself, whilst dehydroheliotrine (**29**) is less active than heliotrine (**24**). Pyrrolic alcohols, for example dehydroheliotridine (**30**) are also active.[76] The main drawback with such compounds is their lack of stability and some of the results may have been affected by their rapid decomposition. Pyrrole carbamates such as (**31**) were synthesised as potential antitumour agents and exhibited some antitumour action.[77]



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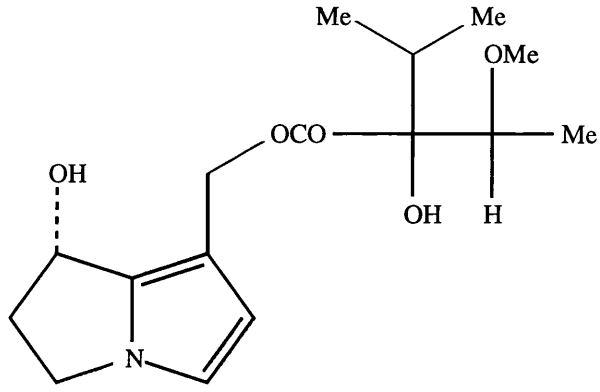

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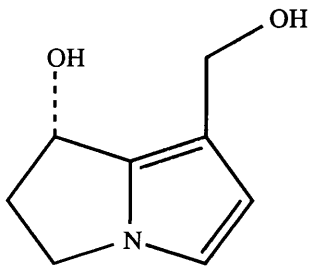

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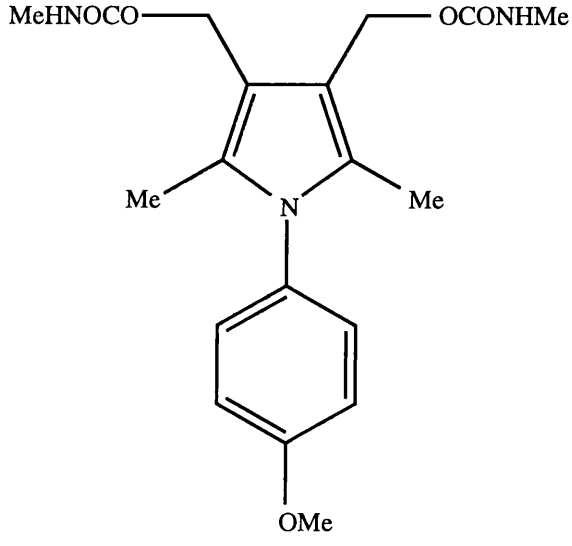

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1.8 Comfrey

Comfrey is a fast-growing leafy perennial. It has been widely consumed as a medicinal herb, as a salad plant and as a herbal remedy.[78]

1.8.1 Uses of Comfrey

The use of herbal remedies has increased sharply over recent years. This is based on the mistaken belief that a natural remedy must be safer and healthier than a

synthetic alternative. The use of comfrey is an example of one such herbal remedy which has seen a resurgence in its use.

Medicinal comfrey contains either the roots or leaves of *Symphytum officinale* or *Symphytum* x *uplandicum*. The latter is a hybrid of *S. officinale* and *S. asperum*. Reported applications of comfrey include the treatment of colds, arthritis, gall and kidney stones, headaches, cancer and many other illnesses.[78,79] Indeed comfrey was described as "being good for almost every ill of mankind".[80]

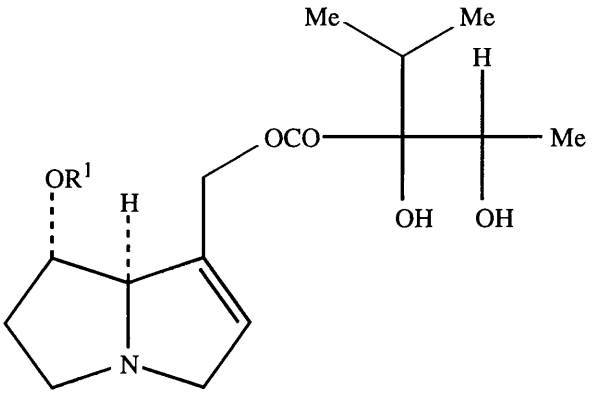
1.8.2 Toxic Pyrrolizidine Alkaloids in Comfrey

Several pyrrolizidine alkaloids have been isolated from comfrey. The amount of pyrrolizidine alkaloid isolated depends upon the part of the plant used, its age and its condition,[17] as shown in **table 3**.

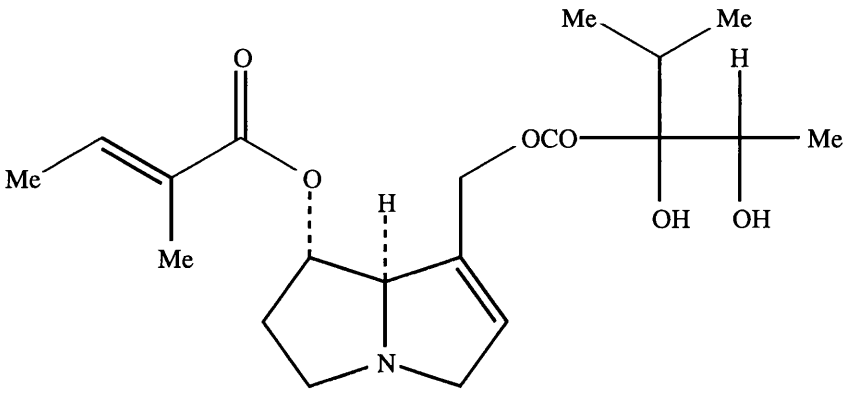
	Fresh Leaves	Dried Leaves	Roots
% Dry Weight of Alkaloids + *N*-Oxides	0.006 - 0.15	0.05 - 0.22	0.07 - 0.37

Table 3 : Percentage weight of alkaloids in comfrey

The most common alkaloids found in comfrey are lycopsamine (**32**), 7-acetyllycopsamine (**33**) and symphytine (**34**). More comprehensive listings of the pyrrolizidine alkaloids found in comfrey can be found in Culvenor *et al.*[81, 82]



32 R^1=H, **33** R^1=Ac



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1.8.3 Risks Associated with Comfrey Use

Comfrey contains several hepatotoxic pyrrolizidine alkaloids and as such could cause VOD.

The carcinogenicity of comfrey has been tested.[83] Both ground leaves and ground roots were fed to rats. The comfrey caused tumours to form in both the liver and the bladder. The ground comfrey root caused the higher incidence of tumours.

1.9 Risks to Humans from Pyrrolizidine Alkaloid Exposure

The risks to humans from pyrrolizidine alkaloid poisoning can be classified into two categories: the risk due to acute poisoning and the risks due to chronic intoxication.

Acute poisoning by pyrrolizidine alkaloids can be due to ingestion of herbal medicines containing unsaturated pyrrolizidine alkaloids or through ingestion of contaminated cereal products (see **section 1.5.5**).

A dose of retronecine-based macrocyclic pyrrolizidine alkaloid exceeding 10 mg/kg or a dose of a monoesterified pyrrolizidine alkaloid in excess of 50 mg/kg could be sufficient to cause acute liver damage.[17]

The incidence of acute pyrrolizidine alkaloid poisoning appears to on the decline. This is due to modern farming techniques decreasing the incidence of crop contamination and of an increasing awareness in developing countries of the dangers of herbal medicines. However, in industrialised countries, herbal medicines are currently in vogue and although the overall trend may be down, the incidence in industrialised countries like the U. K. may be on the increase.[17]

Some sources of pyrrolizidine alkaloids (e.g. herbal teas) provide very low levels of the alkaloids and as such are extremely unlikely to cause any acute liver damage. The danger from continued ingestion of such products is that chronic toxicity may occur. The risk of this seems low. Although many pyrrolizidine alkaloids found in herbal remedies have been shown to be carcinogenic, the levels required to induce a tumour are extremely high compared to the levels that would normally be ingested, indeed a person weighing 60 kg would need to drink over 700 cups of comfrey tea at one time to approach the LD_{50} value.[17,84] Topical application of pyrrolizidine alkaloid-containing herbal medicines represents the lowest risk since under 5 % of the alkaloid that could be ingested orally is actually absorbed through the skin.[85] Liver cancer is rare in countries where comfrey is widely used,[17] although other dietary factors should be considered in these cases. The connection between herbal remedies and liver cancer in regions where liver cancer is common is unproven.[86] A more insidious risk may be through an additive effect when pyrrolizidine alkaloids are combined with other carcinogens in the diet.

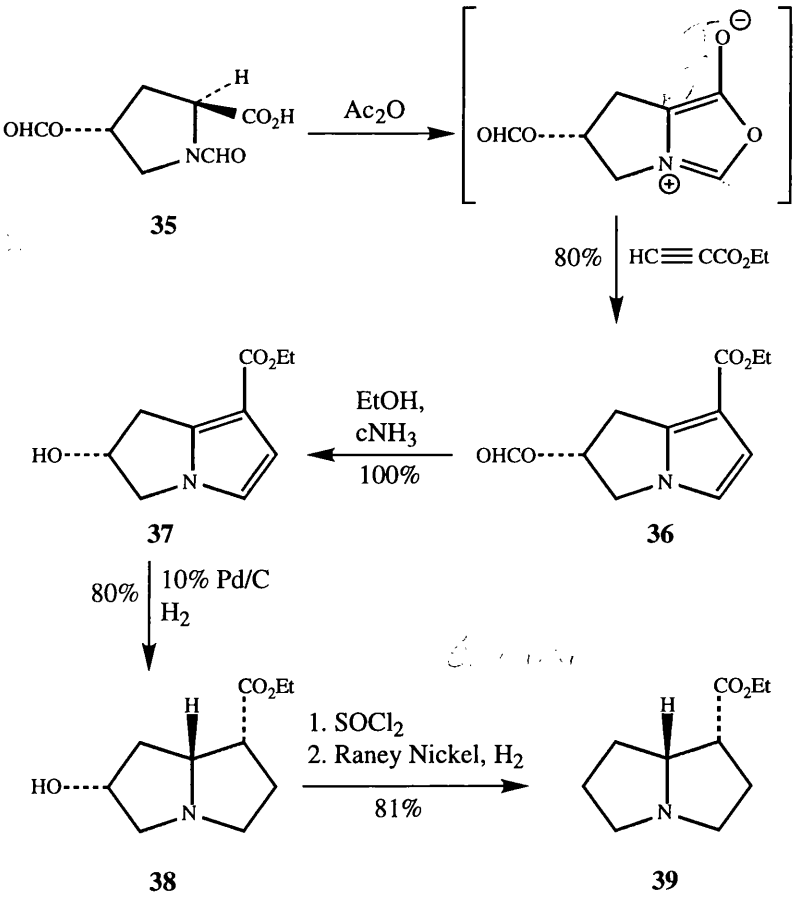
1.10 Synthesis of Pyrrolizidine Alkaloids

Natural products often provide challenging goals for synthetic chemists and pyrrolizidine alkaloids have been no exception. Such syntheses have been of value both in providing novel compounds for biological testing, and in establishing the stereochemistry of the isolated natural products.

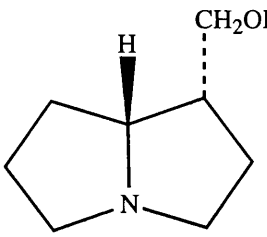
1.10.1 Synthesis of Necines

A large number of necine bases has been prepared, both in racemic and in enantiomerically pure form. Early literature on this subject was reviewed by Kotchetkov and Likhosherstov[87] and by Warren.[88] More recent literature was comprehensively reviewed by Robins.[89, 90]

The first total synthesis of an optically active pyrrolizidine base was by Robins and Sakdarat[91] utilising a 1,3-dipolar cycloaddition of ethyl propiolate to a derivative of (-)-4-hydroxy-L-proline (**35**) as shown in **scheme 5** to give the pyrrolizidine ester (**39**) which can be transformed into (+)-isoretronecanol (**40**), (+)-trachelanthamidine (**41**) or (+)-supinidine (**42**) depending upon the final steps in the synthesis.



Scheme 5 : Synthesis of an optically active pyrrolizidine base.



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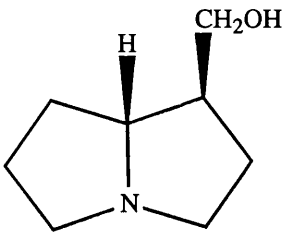

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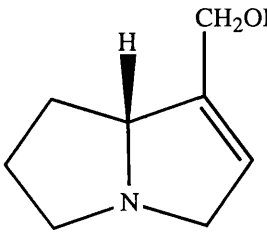

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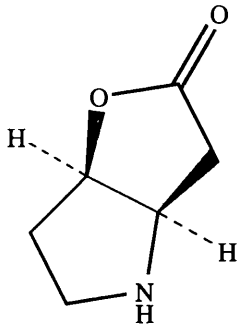
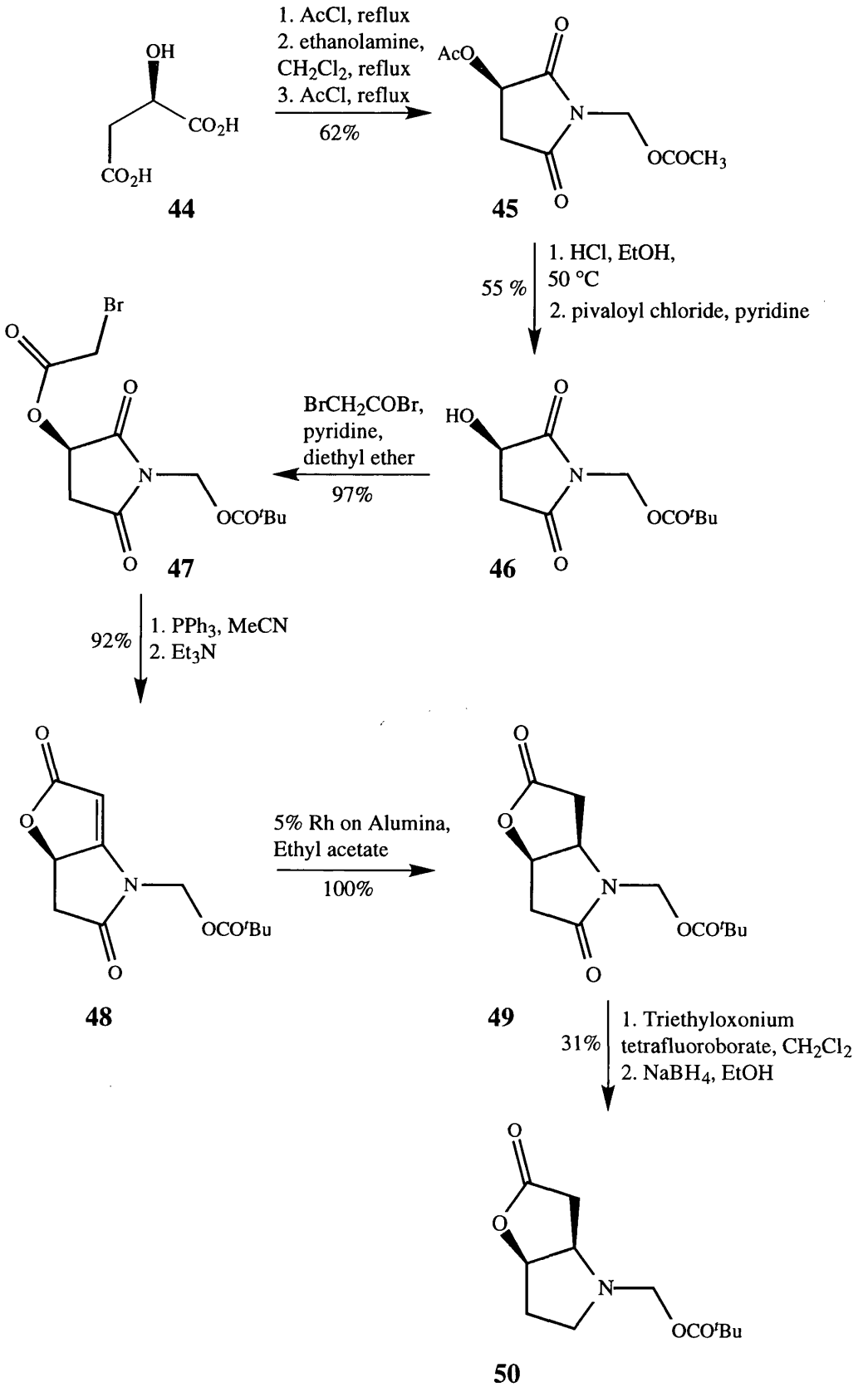


43

One compound which has been popular as a synthon in the synthesis of necine bases is the Geismann Waiss lactone (**43**) which was used in the first synthesis of (+)-retronecine (**4**) in 1962.[92] A recent synthesis of (+)-retronecine used a derivative of this lactone (**50**), which was prepared according to **scheme 6**,[93] starting from (*R*)-(+)-malic acid (**44**). Formation of a necine base from such a compound involves cleavage of the ester to give the primary alcohol and subsequent conversion into a good leaving group, followed by formation of the anion α to the lactone carbonyl and substitution of the alcohol derivative. Alcoholic cleavage of the lactone followed by reduction of the ester gives the necine base.



Scheme 6 : Synthesis of a derivative of the Geismann Waiss lactone

Many other recent publications have made use of this lactone.[90]

1.10.2 Synthesis of Necic Acids

Syntheses of necic acids have been comprehensively reviewed by Warren[88] and by Robins.[90]

1.10.3 Synthesis of Pyrrolizidine Alkaloids

The total synthesis of many pyrrolizidine alkaloids has been carried out over the years and has utilised much diverse and interesting synthetic methodology. The synthesis of macrocyclic pyrrolizidine alkaloids presents the greatest problems due to closure of the large ring system. Some solutions to this problem are discussed in **chapter 7**.

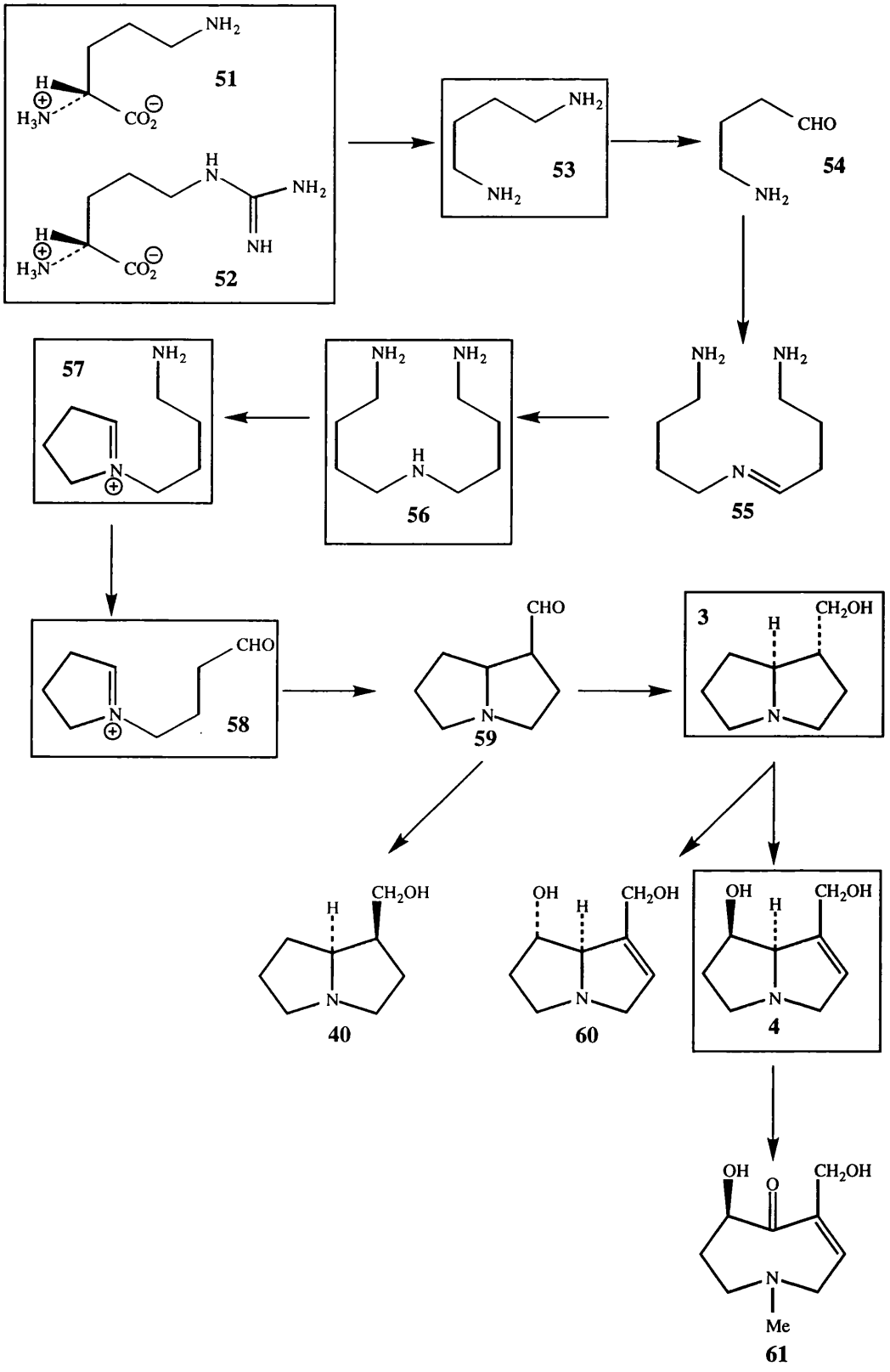
1.11 Biosynthesis of Pyrrolizidine Alkaloids

1.11.1 Biosynthesis of Necine Bases

The biosynthesis of pyrrolizidine alkaloids has been a topic of interest for over 30 years since Nowacki and Byerrum[94] used *Crotalaria spectabilis* to examine the pathway to monocrotaline (**8**). These initial experiments demonstrated the incorporation of ornithine (**51**) into the base portion retronecine (**4**) using ^{14}C-labelled ornithine.

Progress in elucidating the biosynthetic pathway to necines was hindered by the lack of good degradations of retronecine (**4**) required to determine the positions of all the radiolabels. The incorporation of stable isotopes such as deuterium and ^{13}C into pyrrolizidine alkaloids allowed complete labelling patterns to be determined by NMR spectroscopy.[95]

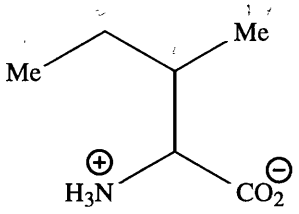
The biosynthetic pathway to the necine bases has now been established and is shown in **scheme 7**. The known intermediates are boxed. L-Ornithine (**51**) undergoes decarboxylation to give putrescine (**53**).[96, 97] Putrescine is derived from L-arginine (**52**) in some plant species.[98, 99] One of the amine groups of putrescine is then oxidised to give the aminoaldehyde (**54**). This aldehyde then condenses with another molecule of putrescine to afford the imine (**55**).[95] This imine is then reduced to give the symmetrical intermediate homospermidine (**56**).[100, 101] Oxidation of this intermediate and condensation gives the iminium ion (**57**).[102, 103] Further oxidation of the remaining amino group to give the aldehyde (**58**) is followed by cyclisation to give the pyrrolizidine aldehyde (**59**).[95] After this stage, a series of reductions, hydroxylations and eliminations can give rise to heliotridine (**60**), retronecine (**4**), isoretronecanol (**40**) or trachelanthamidine (**3**).[104, 105] Otonecine (**61**) can be formed from retronecine (**4**), possibly after hydroxylation at C-8 and methylation of the nitrogen, allowing cleavage of the bicyclic ring.[106]



Scheme 7 : Biosynthesis of necine bases

1.11.2 Biosynthesis of Necic Acids

Necic acids appear at first glance to be terpenoid in origin, but feeding experiments with mevalonic acid derivatives showed no incorporation into these acids.[107] It was then shown by Crout *et al.*[108] that isoleucine (**62**) and threonine (**63**) were incorporated into seneciphyllic acid (**11**) produced by *Senecio douglasii.*



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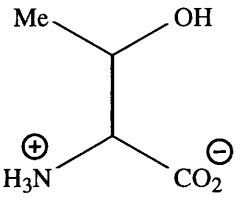
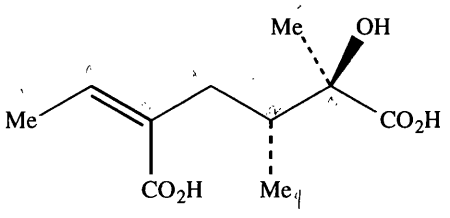


63

In the alkaloid senecionine (**23**) two units of isoleucine are incorporated into senecic acid (**64**) with loss of both carboxyl carbons of the isoleucine units. This raised the interesting question of how these molecules are coupled together. Crout *et al.*[109] suggested that the intermediate could be β-methylenenorvaline (**65**) and indeed a radiolabelled form of this molecule was incorporated into senecic acid. Unfortunately degradations could not be carried out to determine the positions of the radiolabels.



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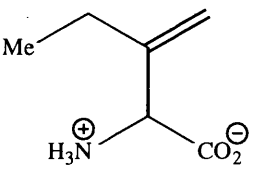
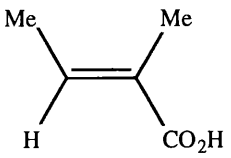
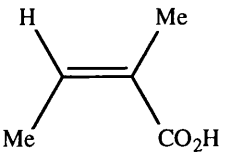


65

The C_5 compound tiglic acid (**66**) appears to undergo isomerisation to give angelic acid (**67**).[110]



66



67

Clearly a lot less is known about the biosynthesis of necic acids than about the necines and this is an area worthy of further investigation.

The topic of pyrrolizidine alkaloid biosynthesis has been comprehensively reviewed by Robins.[95]

1.12 Aims of this Project

The aim of this project was to assess the toxicity, and anti-tumour activity of a wide number of pyrrolizidine alkaloids and analogues to establish further the potential risk to humans. In particular the toxicity of comfrey was studied.

To this end a number of pyrrolizidine alkaloids were isolated and simple derivatives were prepared of them. This work is discussed in **chapter 3**. In order to increase the number of compounds available for testing, novel pyrrolizidine alkaloid analogues were prepared. These analogues included compounds derived from synthanecine A and pyrrolines and pyrrolidines prepared via a 1,3-dipolar cycloaddition. In addition to this work several approaches were tried in an attempt to synthesise a single enantiomer of synthanecine A. Considerable progress was made in this route. The synthesis of analogues of pyrrolizidine alkaloids is discussed in **chapters 3-7**

Radiolabelled alkaloids were prepared for metabolic studies; however at the time of writing this thesis no data were available on this testing.

In order to evaluate their anti-tumour activity a number of compounds were tested for their ability to inhibit the growth of the PLC/PRF/5 hepatoma cell line. The biological data obtained are presented in **chapter 8**.

Chapter 2

Isolation and Derivatisation of Pyrrolizidine Alkaloids

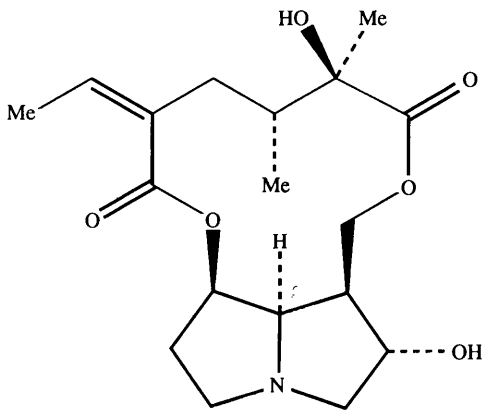
2.1 Introduction

Although the focus of this thesis is on the synthesis of analogues of pyrrolizidine alkaloids, a number of the alkaloids were isolated from the available plant and root culture sources and characterised and derivatised to provide standards for comparison in biological studies.

2.2 Isolation and Characterisation of Pyrrolizidine Alkaloids

Available within the Chemistry Department are a number of alkaloid-producing plants and root cultures. The plant *Senecio pleistocephalus* produces the non-hepatotoxic alkaloid rosmarinine (**68**). Transformed root cultures of *Senecio vulgaris* produce the hepatotoxic alkaloid senecionine (**23**). *Symphytum officinale* (comfrey) was both gathered locally and cultivated to provide a crude mixture of alkaloids.

The alkaloids were isolated from the plants using the method of Robins and Sweeney[111] and were purified by recrystallisation.



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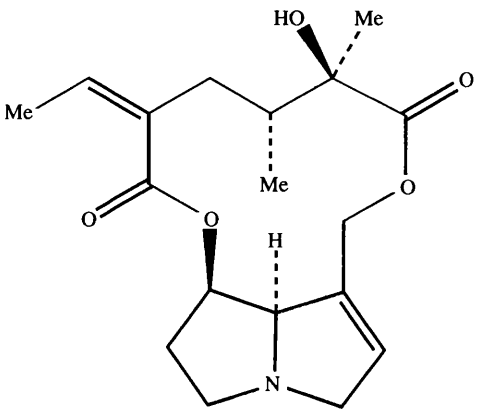


23

Isolation of rosmarinine from the leaves of *Senecio pleistocephalus* gave a yield of 0.069 % based on the weight of the leaves extracted. The analytical data obtained were identical to those obtained by Roitman.[112]

Isolation of senecionine was carried out as described for rosmarinine and gave a yield of 0.088 % based on the weight of roots extracted. The analytical data concurred with those given by Culvenor and Smith.[113] Additional data are given in **Chapter 9**.

As previously discussed (**Chapter 1.8**) comfrey is a common herbal remedy prescribed for a wide variety of ailments. Volmer *et al.*[79] described the isolation of the alkaloids from comfrey and a quantitative analysis of their toxic alkaloidal components. We analysed a locally gathered batch of comfrey in a similar experiment. The roots and leaves were analysed separately and the alkaloids were isolated by the method of Robins and Sweeney.[111] A known quantity of dibromobenzene (DBB) was then added to each sample and the samples were analysed by ^{1}H NMR spectroscopy. The integration of the dibromobenzene signal allowed calculation of the amount of toxic alkaloid present in each sample based upon the size of the alkenic signals due to the hydrogen attached to C-2 in each pyrrolizidine alkaloid. The weight of pyrrolizidine alkaloid was calculated according to **equation 1**.[114]

$$\text{wt. of PA} = (\text{wt of DBB}) \left(\frac{\text{mol. wt. of PA}}{\text{mol. wt. of DBB}}\right) \left(\frac{\text{A(2-H)}}{0.25\ \text{A(DBB)}}\right)$$

Where A is the magnitude of the integral.

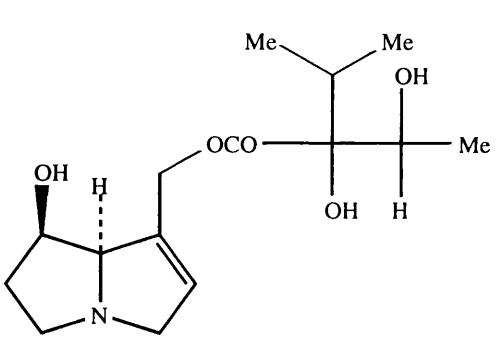
Equation 1 : Calculation of weight of PAs

The results of this experiment are given in **table 4.** The alkaloids were identified by comparison with previously published ^{1}H NMR spectra.[17,79]

Source	Alkaloid	δ_H for 2-H	Quantity of Alkaloid based on DBB integral	% of Total Alkaloidal Mass Isolated
Root	Lycopsamine (**32**) / intermedine (**69**)	5.86	53.8 mg	55.6 %
Root	Echimidine (**70**)	5.77	3.0 mg	3.1 %
Leaf	Echimidine (**70**)	5.76	60.6 mg	87.9 %

Table 4 : Quantity of Hepatotoxic PAs in Comfrey

A portion of the ^1H NMR spectrum of the comfrey leaf extract after the addition of 5.9 mg of dibromobenzene is shown in **figure 1**.



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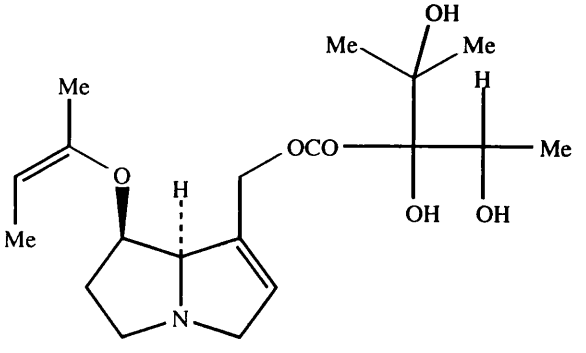

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2.3 Synthesis of *N*-Oxides of Pyrrolizidine Alkaloids

Pyrrolizidine alkaloids are often accompanied in the plants by varying quantities of the corresponding *N*-oxides. These compounds are reduced to the basic alkaloid during the extraction procedure using Zn/H^+. *N*-Oxides were synthesised to study their metabolism and to determine their cytotoxicty. The ability of *N*-oxides to kill cells is worthy of study because of the anti-tumour activity of indicine *N*-oxide (**27**).[74, 75]

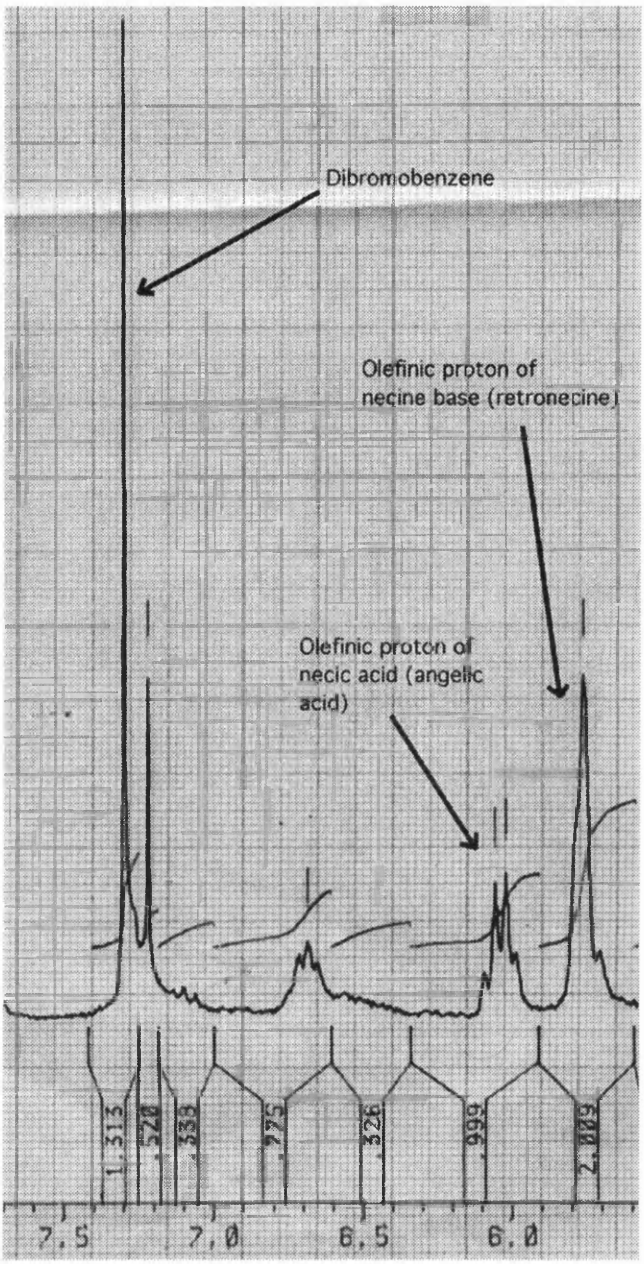
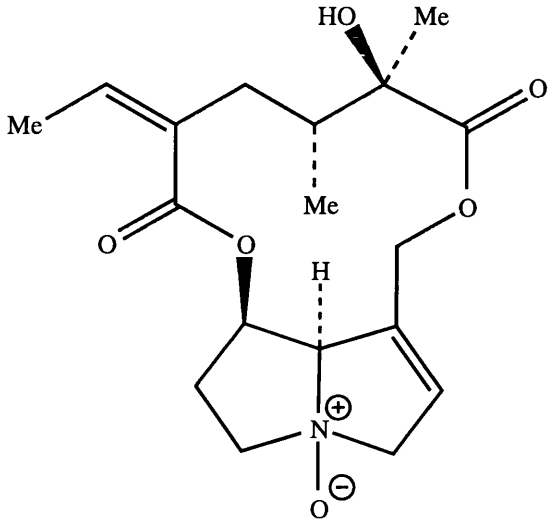


Figure 1: ^{1}H NMR spectrum of comfrey leaf extract + 5.9 mg of dibromobenzene

The *N*-oxide of rosmarinine (**12**) was prepared by treatment of rosmarinine with *m*-chloroperbenzoic acid in chloroform to give the *N*-oxide in 78 % yield. The IR spectrum of this compound showed a strong absorption at 975 cm^{-1} corresponding to the stretching of the N^{+}-O^{-} bond. NMR spectroscopic data are discussed below. This compound had been previously prepared by Koekemoer and

Warren.[115] The analytical data provided for this compound in **chapter 9** are in addition to those given in the original literature.

Treatment of senecionine (**23**) with *m*-chloroperbenzoic acid failed to give any senecionine *N*-oxide (**71**). For this compound the method of Culvenor and Smith[14] employing H_2O_2 proved successful if a 1:1 mixture of methanol and chloroform was employed as the solvent, yielding the *N*-oxide in 38 % yield. The IR spectrum of this compound had the predicted strong absorption at 968 cm^{-1} due to the stretching of the N^+-O^- bond. NMR data are examined below. Additional analytical data for this compound are presented in **chapter 9**.



71

Monocrotaline *N*-oxide (**18**) was prepared using *m*-chloroperbenzoic acid in 27 % yield; however the method of Culvenor and Smith[14] using hydrogen peroxide solution proved more successful. Careful monitoring of the reaction mixture by TLC (chloroform/methanol/conc. NH_3; 85:14:1) and an increased reaction time gave a yield of 92 %, improving the literature yield[14] by 10 %. The IR spectrum of this compound showed a strong absorption at 955 cm^{-1} correlating to the stretching of the N^+-O^- bond. NMR data are discussed below. The analytical data provided for this compound in **chapter 9** are in addition to those given in the original synthesis.[14]

Formation of the *N*-oxides of rosmarinine, senecionine, and monocrotaline gave characteristic downfield shifts of the NMR signals attributable to the hydrogens and carbons at positions 3, 5 and 8 due to the introduction of a positive charge at position 4. This information is summarised in **table 5**.

Alkaloid	**δ 3-H** (multiplicity)	**δ 5-H** (multiplicity)	**δ 8-H** (multiplicity)	**δ 3-C**	**δ 5-C**	**δ 8-C**
Rosmarinine (**68**)[112]	2.90 (3β-H, dd), 3.06 (3α-H, dd)	2.59 (5β-H, m), 3.24 (5α-H, ddd)	3.55 (dd)	61.3	53.3	69.5
Rosmarinine *N*-oxide (**12**)	3.31 (3β-H, bd), 5.05 (3α-H, m)	4.16 (m)	4.16 (m)	73.1	68.1	84.5
Senecionine (**23**)	3.28 (3α-H, dd), 3.90 (3β-H, bd)	2.42 (5β-H, m), 3.28 (5α-H, m)	4.24 (d)	60.4	53.0	77.5
Senecionine *N*-oxide (**69**)	3.59 (3α-H, m), 4.26 (3β-H, m)	3.59 (5α-H, m), 4.26 (5β-H, m)	5.24 (m)	78.6	69.1	96.3
Monocrotaline (**8**)[117]	*a*	*a*	*a*	61.3	53.6	76.9
Monocrotaline *N*-oxide (**18**)	3.57 (m)	4.60 (m)	4.60 (m)	77.8	67.8	95.3

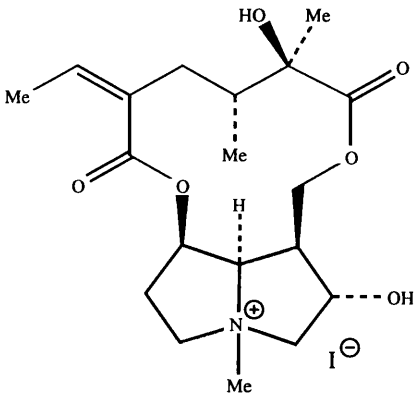
a no accurate data available

Table 5 : NMR spectroscopic data for PA free bases and *N*-oxides

2.4 Synthesis of Methiodides of Pyrrolizidine Alkaloids

Previous workers have prepared a number of semisynthetic quaternary pyrrolizidine derivatives by reaction of pyrrolizidine alkaloids with a variety of alkyl halides.[118] These compounds have been investigated pharmacologically and exhibited varying degrees of ganglion blocking activity resulting in hypertension.[119] In order to investigate any anti-tumour activity that the adducts of pyrrolizidine alkaloids and methyl iodide may exhibit, a range of these compounds were synthesised. A comprehensive review of many semisynthetic derivatives of pyrrolizidine alkaloids has been published.[120]

Rosmarinine methiodide (**72**), senecionine methiodide (**73**) and monocrotaline methiodide (**74**) were prepared by reaction of the alkaloids with iodomethane in ethanol in 56 %, 22 %, and 47 % yields, respectively.



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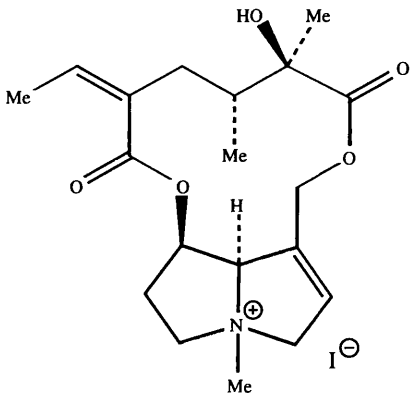

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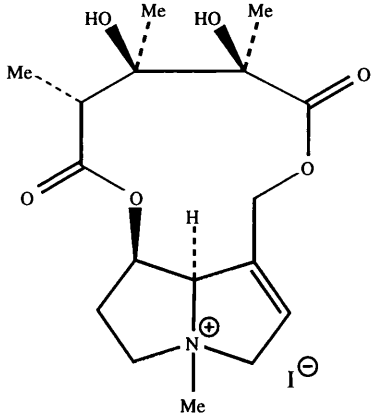


74

In common with the *N*-oxides of rosmarinine, senecionine, and monocrotaline, the methiodides of these compounds gave the same characteristic downfield shift of the NMR signals due to the hydrogens and carbons at the positions adjacent to the nitrogen atom due to the introduction of the positive charge at this position. This information is summarised in **table 6**.

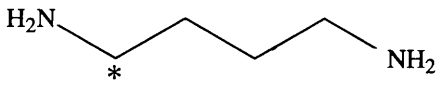
Alkaloid	**δ 3-H (multiplicity)**	**δ 5-H (multiplicity)**	**δ 8-H (multiplicity)**	**δ 3-C**	**δ 5-C**	**δ 8-C**
Rosmarinine (**68**)[112]	2.90 (3_β-H, dd), 3.06 (3_α-H, dd)	2.59 (5_β-H, m), 3.24 (5_α-H, ddd)	3.55 (dd)	61.3	53.3	69.5
Rosmarinine methiodide (**72**)	3.42 (3-H_β, dd), 4.86 (3-H_α, m)	2.88 (5_β-H, m), 3.86 (5_α-H, m)	3.86 (m)	73.4	66.2	80.9
Senecionine (**23**)	3.28 (3_α-H, dd), 3.90 (3_α-H, bd)	2.42 (5_β-H, m), 3.28 (5_α-H, m)	4.24 (d)	60.4	53.0	77.5
Senecionine methiodide (**71**)	4.42 (m)	4.42 (m)	5.35 (m)	74.5	65.1	90.7
Monocrotaline (**8**)[117]	*a*	*a*	*a*	61.3	53.6	76.9
Monocrotaline methiodide (**72**)	4.49 (m)	3.66 (m)	4.95 (bd)	72.7	63.3	88.5

[a] no accurate data available

Table 6 : NMR spectroscopic data for PA free bases and methiodides

2.5 Synthesis of Labelled Compounds

In order to investigate the metabolism of pyrrolizidine alkaloids it was necessary to prepare radiolabelled samples of these compounds. Previous workers[96, 97] have shown that putrescine (**53**) is a biosynthetic precursor of pyrrolizidine alkaloids, thus [1,4-^{14}C]putrescine (**75**) was fed to *Senecio pleistocephalus*, *S. vulgaris* and *Symphytum officinale.* This results in labelling of the base portion as shown in (**76**).



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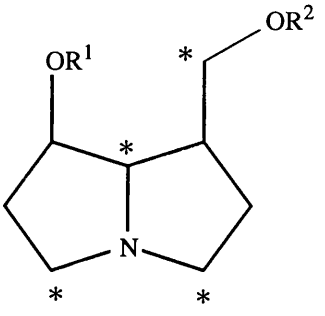


* position of label

76

Feeding of the radiolabelled putrescine was carried out by the wick method of Robins and Sweeney,[111] when whole plants were used. Direct addition to the growth media of seven day old roots was employed with root cultures. Whole plants were allowed to grow for 7 days after feeding was complete before extraction. Root cultures were allowed to grow for 16 days after feeding before extraction. *N*-Oxides of rosmarinine and senecionine were prepared as previously discussed from the radiolabelled alkaloids.

The results of these experiments are shown in **table 7**.

The results obtained for feeding to the whole plant were considerably poorer than those gained when root cultures were used. This is because in the whole plant the labelled putrescine has to be transported to the roots of the plant to be incorporated into the pyrrolizidine alkaloid. During this process some putrescine may be lost to other biosynthetic pathways. Feeding to root cultures is a more direct method where the labelled putrescine is incorporated directly into the root.

These compounds were sent to Dr Hanley, MAFF, Norwich for use in metabolic studies. At the time of writing this thesis no results had been received.

Alkaloid	Source	Activity Fed	Weight	Activity of isolated alkaloid	Incorporation
Rosmarinine (**68**)	*S. pleistocephalus*	225 μCi	42 mg	0.037 μCi / mg	0.69 %
Rosmarinine *N*-oxide (**12**)	Semisynthetic	-	7.4 mg	0.035 μCi / mg	-
Senecionine (**23**)	*S. vulgaris*	275 μCi	37 mg	0.45μCi / mg	7.4 %
Senecionine *N*-oxide (**71**)	Semisynthetic	-	9.2 mg	0.43μCi / mg	-
Comfrey Root Alkaloids	*Sym. officinale*	250 μCi	96 mg	0.37nCi / mg	0.014 %
Comfrey Leaf Alkaloids	*Sym. officinale*	250 μCi	45 mg	1.3nCi / mg	0.023 %

Table 7 : Results from feeding of [1,4-^{14}C]putrescine

2.6 Synthesis of Senecionine from Rosmarinine

Senecionine (**23**) has 1,2-unsaturation and thus is a toxic pyrrolizidine alkaloid. This compound would be interesting in both metabolic and toxicity testing but only a small amount is available through isolation from root cultures. Rosmarinine (**68**) is however easily isolated in gram quantities from *S. pleistocephalus* and conversion into senecionine would be possible if elimination across the 1,2-position could be achieved. A number of methods have been tried to carry out this conversion.

Koekemoer and Warren[121] had previously shown that senecionine (**23**) could be prepared from 2-*O*-tosyl rosmarinine (**77**) in moderate yield (**scheme 8**). This route was repeated with a number of modifications to see if the yield of senecionine could be improved.

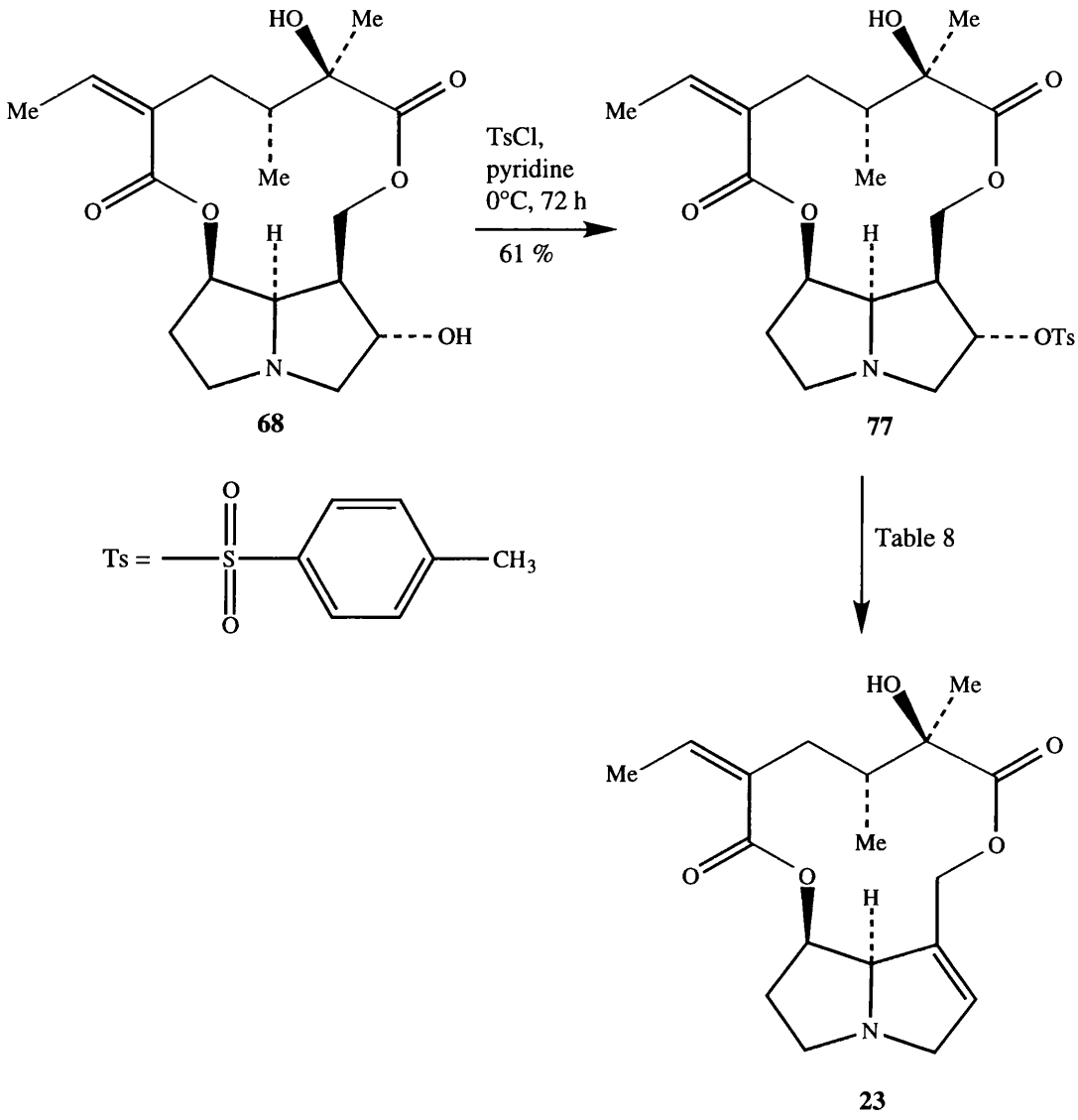
2-*O*-Tosyl rosmarinine was prepared from rosmarinine (**66**) in 61 % yield by treatment of rosmarinine with *p*-toluenesulfonyl chloride at 0 °C.[121] Additional IR, NMR and MS data were gathered to prove the identity of the compound. The IR spectrum showed two strong bands at 1365 and 1190 cm^{-1} corresponding to the $-SO_2O-$ group as well as peaks indicative of the presence of an aromatic ring. The ^1H NMR spectrum showed a singlet for the $C\underline{H}_3$-Ar group at δ 2.46 and the aromatic protons were observed as an AA'BB' system at δ 7.35 and 7.82. High resolution MS gave the expected elemental composition for the M^+, whilst a low resolution MS of the compound showed a fragment at *m/z* 155 corresponding to the $-SO_2$-Ar group.

According to the procedure of Koekomoer and Warren,[121] 3 hours at reflux temperature in pyridine should convert the 2-*O*-tosyl rosmarinine into senecionine in moderate yield. In our hands this reaction produced no senecionine. TLC analysis indicated that the major component of the mixture was starting material. A variety of conditions were tried to affect this transformation. These are summarised in **table 8**.

CONDITIONS	YIELD
Pyridine, reflux, 22h	4%
Pyridine, DMAP, room temp, 96h	no reaction
Pyridine, DMAP, 65°C, 96h	9%
Pyridine, DMAP, reflux, 22h	11%
DMF, DMAP, reflux, 4.5h	20%, mixture of rosmarinine and senecionine

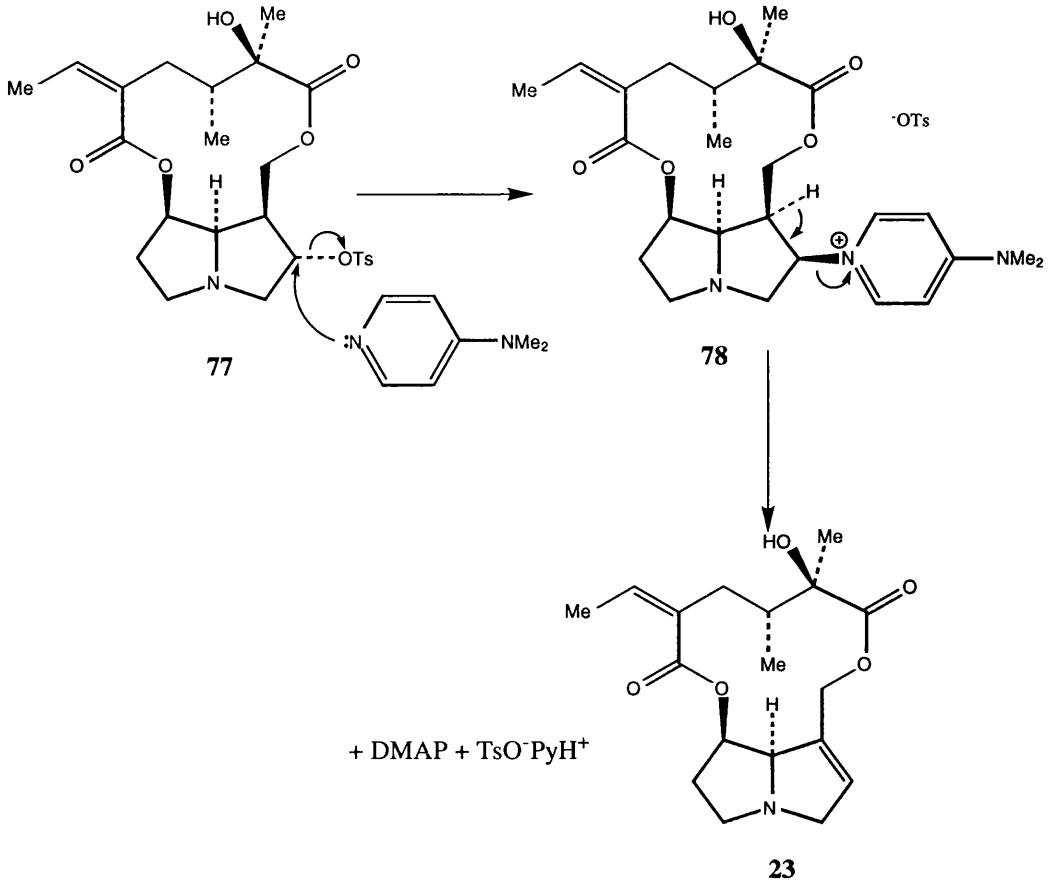
Table 8 : Formation of Senecionine (23) from 2-*O*-Tosyl Rosmarinine (77)

At reflux temperature considerable decomposition of the 2-*O*-tosyl rosmarinine occurred. Addition of a catalytic amount of DMAP resulted in a slightly improved yield. This is perhaps due to the DMAP acting as a nucleophile at the 2-position (**scheme 9**) although the approach of a nucleophile at this position would be sterically hindered.



Scheme 8 : Synthesis of Senecionine from Rosmarinine via 2-*O*-tosyl rosmarinine

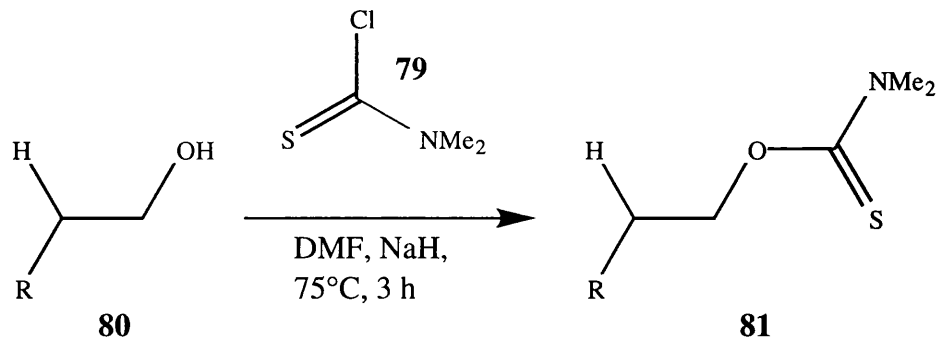
The dihedral angle between the OTs group and the methine hydrogen in (**77**) is approximately 45°. The conformation is fixed and so a *syn*-elimination must take place (a dihedral angle of 180° is required for an *anti*-elimination).[122] Without isotope studies it is impossible to determine the mechanism of this reaction. The rate of this reaction compared to the decomposition of the starting material was very low, and hence a small yield of senecionine was obtained.



Scheme 9 : Possible course of reaction involving DMAP

The addition of DMAP to the reaction mixture increased the rate of formation of senecionine. Such effects were observed by Parker and co-workers[123] who classified such a reaction as an E_2C reaction. If displacement of the tosyl group does occur then a dihedral angle of approximately 180° is possible between the two leaving groups and could account for the increase in rate. The yield is still very low however and an alternative approach to the elimination was formulated.

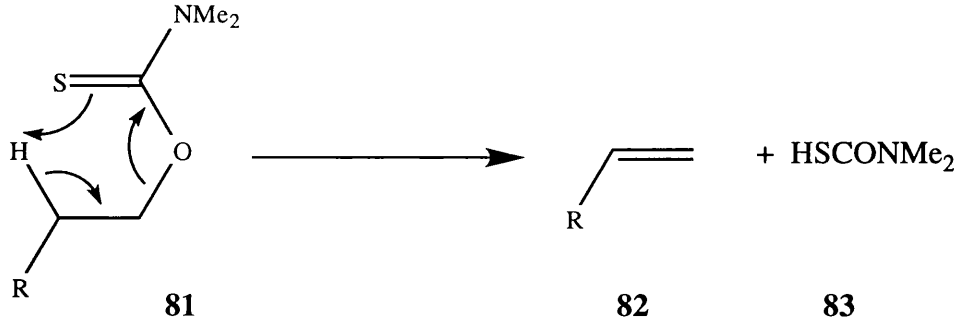
Given the closeness in space of any 2-substituent and the hydrogen atom at the 1-position, a reaction which exclusively favoured a *syn*-elimination seemed appropriate. Newman and Hetzel[124] reported a variation of the Chugaev reaction where pyrolysis of O-alkyl dimethylthiocarbamates proceeded in good yields to gave a wide range of alkenes. The dimethylthiocarbamates could be formed from dimethylthiocarbamoyl chloride (DMTCC) (**scheme 10**) (**79**).



80 81

Scheme 10: Synthesis of a dimethylthiocarbamate

This reaction has several advantages over the Chugaev reaction, namely, the thiocarbamate could be prepared in a single step, and the reaction avoided use of the highly toxic carbon disulfide. Elimination was proposed to take place by the mechanism shown in **scheme 11**.[124]



81 **82** **83**

Scheme 11 : Mechanism of elimination

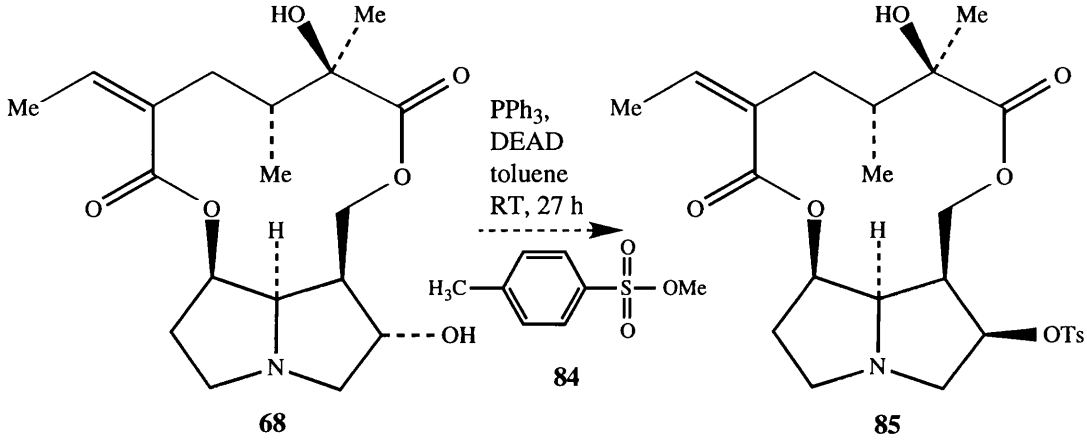
Rosmarinine proved to be too unstable to form the dimethylthiocarbamate under the wide variety of conditions given in **table 9**. In all cases the reactions produced a complex mixture of inseparable products.

Again the instability of alkaloids in reactions requiring long reaction times and/or high temperatures played a role in the failure of this approach.

Conditions	Yield
DMF, 2 equiv. NaH, 1.2 equiv. DMTCC, 80°C, 48h	mixture
DMF, 1.2 equiv. DMTCC, 80°C, 48h	mixture
DMF, 0.5 equiv. NaH, 1.2 equiv. DMTCC, 80°C, 48h	mixture
DMF, 4 equiv. DMTCC, 80°C, 48h	mixture
DMF, 4 equiv. DMTCC, DMAP, 80°C, 48h	mixture

Table 9 : attempted preparation of dimethylthiocarbamate

The final approach taken to the conversion of rosmarinine into senecionine involved a Mitsunobu inversion of the secondary alcohol centre at the 2-position **(scheme 12)** with simultaneous formation of the tosylate (**85**) using methyl *p*-toluenesulfonate (**84**). Mitsunobu methodology has proved reliable in giving both good yields of products and clean inversion at the reaction centre.[125] The advantage of this reaction was that it involved a short reaction time and would remove the necessity of carrying out a *syn*-elimination. Unfortunately no identifiable material could be isolated from the reaction mixture.



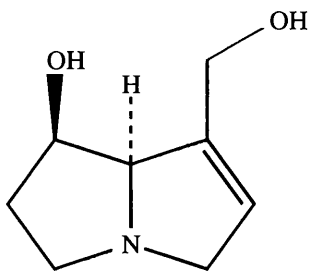
Scheme 12 : attempted synthesis of a 2-*O*-tosyl rosmarinine epimer

Chapter 3

Synthesis of Synthanecine A

3.1 Introduction

Synthetic analogues of pyrrolizidine alkaloids were required for metabolic and toxicological studies. The first synthesis of the most common necine retronecine (**4**) was reported by Geissman and Waiss in 1962.[92] The route to this compound was lengthy and the overall yield was low (<1 %). Although many syntheses of necine bases have been carried out since then,[90] they are still lengthy and often low yielding. This has led to the preparation of a range of monocyclic analogues of necine bases, called synthanecines.[18, 126] These have included synthanecine A (**14**) which is 2,3-bishydroxymethyl-1-methylpyrroline and synthanecine B (**15**) which is the corresponding saturated derivative. The structural similarity of synthanecine A to retronecine (**4**) is shown in **figure 2**.



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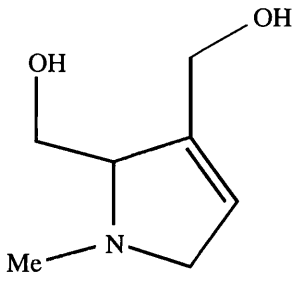


14

Figure 2 : structural similarity of synthanecine A (14) to retronecine (4)

The synthesis of synthanecine A and B has been well documented.[18,127,128] Mattocks originally prepared a range of analogues for toxicology studies, while possible anti-tumour activity of macrocyclic diesters of synthanecine A was investigated by Barbour[127] and by Baxter.[128] It was intended that synthanecine A

and B would be prepared, using known procedures, and esterified with novel necic acid derivatives. This work is further discussed in **chapter 6**.

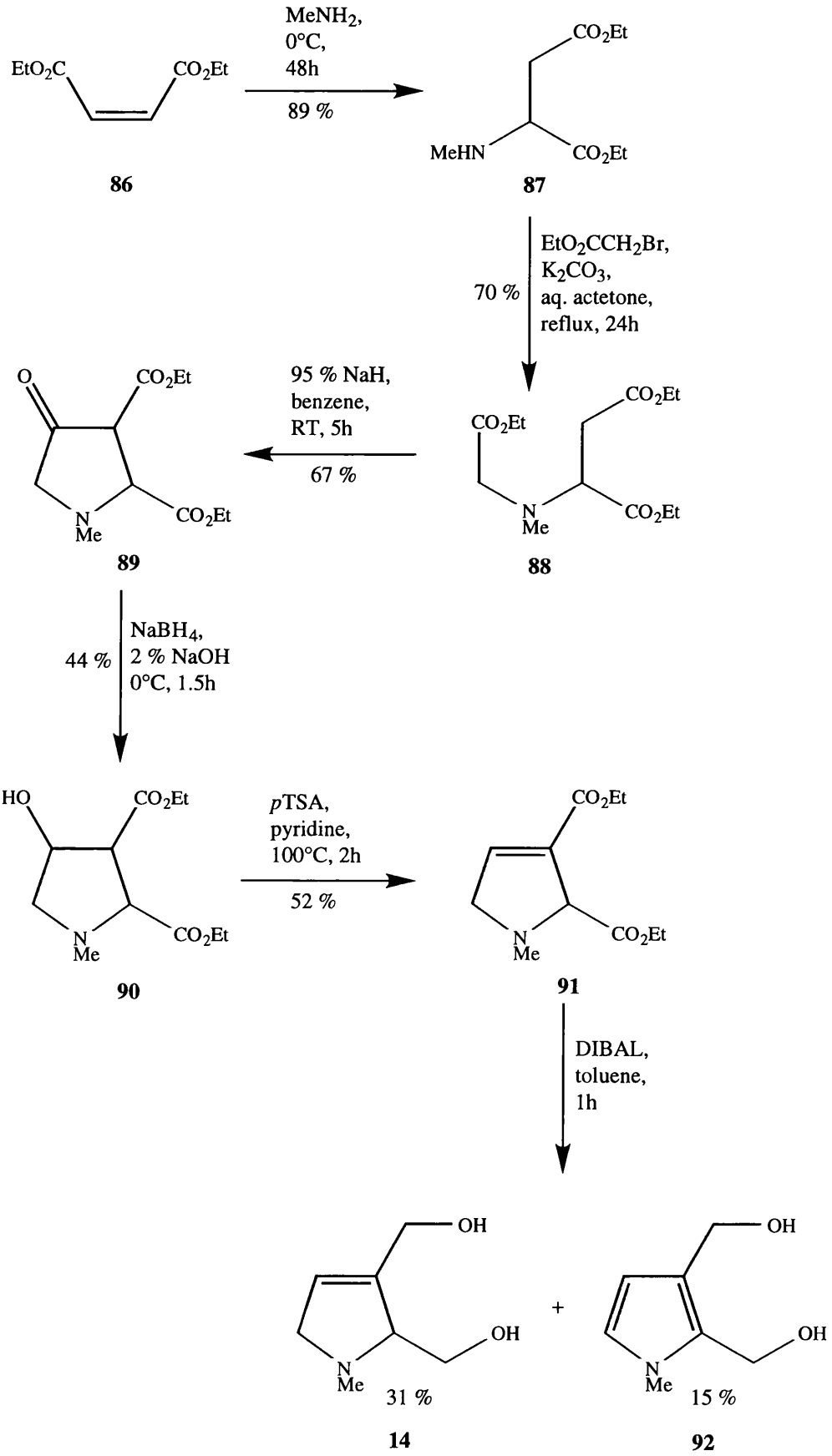
3.2 Synthesis of Synthanecine A

A modified version[127] of the procedure initially developed by Mattocks[18] was used to form synthanecine A (**scheme 13**).

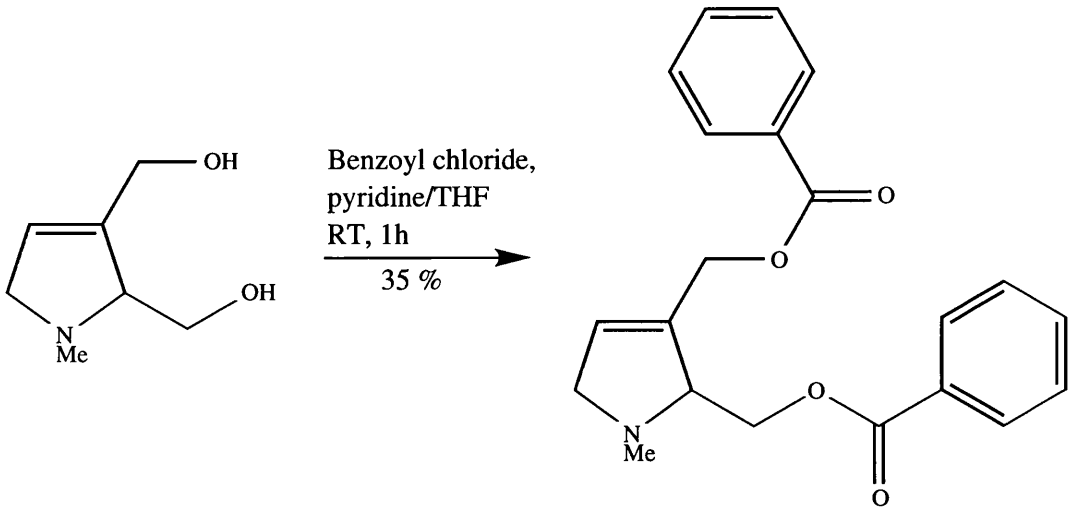
The overall yield of synthanecine A was 3 % over 6 steps.

3.3 Synthesis of Derivatives of Synthanecine A

The dibenzoyl ester of synthanecine A (**93**) was prepared to provide material for biological testing. Treatment of synthanecine A (**14**) with benzoyl chloride in pyridine/THF gave the dibenzoyl ester in 35 % yield (**scheme 14**). The IR spectrum showed absorptions at 1601, 1584 and 1491 cm^{-1} corresponding to an aromatic ring and at 1719 cm^{-1} corresponding to the ester carbonyl groups. The ^1H NMR spectrum had resonances due to the aromatic protons at δ 7.19-7.47 and 7.88-7.93.



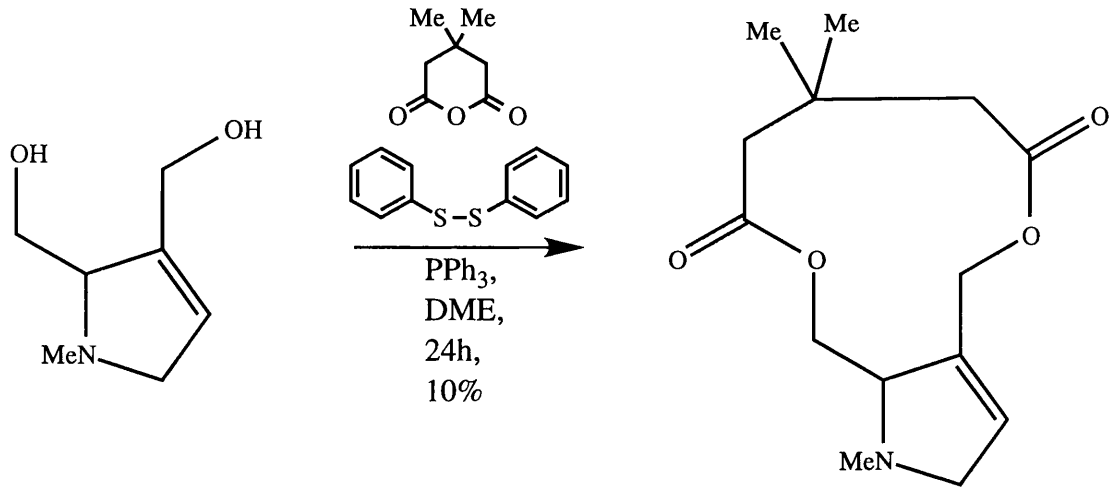
Scheme 13 : Synthesis of Synthanecine A (14)



14 **93**

Scheme 14 : Synthesis of (±)-6,7-*O,O*-Dibenzoyl Synthanecine A

In **chapter 8** the attempted synthesis of a macrocyclic diester of a synthanecine analogue is discussed. One of the methods attempted to prepare this compound was the Corey Nicolaou method.[129] (±)-6,7-*O,O*-(3,3-Dimethylglutaryl)synthanecine A (**19**) was synthesised to test the validity of this approach (**scheme 15**). This compound had previously been synthesised by Barbour and Robins.[19]



14 **19**

Scheme 15 : Synthesis of (±)-6,7-*O,O*-(3,3-Dimethylglutaryl)synthanecine A (19)[19]

Treatment of synthanecine A with 3,3-dimethylglutaric anhydride, di-2-pyridyl disulfide and triphenylphosphine afforded the macrocyclic diester in 10 % yield. All analytical data were identical to those previously published.[19]

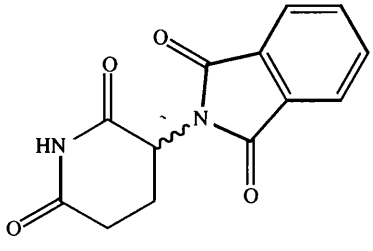
A further discussion of the formation of macrocyclic diesters can be found in **chapter 8**.

Chapter 4

Approaches to the Synthesis of a Single Enantiomer of Synthanecine A

4.1 Introduction

Synthanecine A (**14**) was first synthesised by Mattocks[18] in 1974 and has proved to be a useful analogue in the study of pyrrolizidine alkaloid toxicity.[33] Synthanecine A contains a single chiral centre at the 2-position. In a necine base the equivalent position is the 8-position, which is also a chiral centre. In the common necine base retronecine (**4**) the absolute configuration at this center is *R*. It is likely that if synthanecine A could be synthesised with the same configuration at the 2-position, then the biological activity of its derivatives would be enhanced. This enhancement might be evident in anti-tumour activity of derivatives of synthanecine A. Any enhancement could be attributed to the fact that receptor sites in biological systems are optically active and have the ability to distinguish between two enantiomers of a racemate. This property of receptor sites in the body cannot only cause a particular enantiomer to be active whilst its mirror-image is inactive but can cause both enantiomers to have different properties. The most infamous case of this involves the use of the drug thalidomide (**94**). When administered in its racemic form to pregnant women there was a high incidence of birth defects.[130] The teratogenic properties of thalidomide were attributed to the (*S*)-(-)-enantiomer.[131]



94

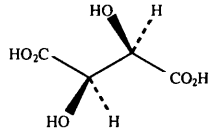
4.2 The Synthesis of Enantiomerically Pure Compounds

If a single enantiomer of a molecule is required then there are a number of approaches to achieving this.

4.2.1 Resolution

Traditionally optically pure compounds were obtained by resolution of racemic material at some stage in the synthesis. This resolution should ideally be at the earliest point in the synthesis to avoid carrying any unwanted material through a large number of synthetic stages. Although often wasteful, this method is worthy of consideration if it is possible to recycle the unwanted isomer. Resolution is often carried out using naturally occurring optically active acids or bases such as L-tartaric acid (**95**) or quinine (**96**) respectively, to form diastereoisomeric salts of sufficient crystallinity which can then be readily separated by crystallisation.

A covalent bond can also be formed between a racemic substrate and an optically pure compound thus forming a pair of diastereoisomers which can then be separated. The desired enantiomer can then be regenerated from the appropriate diastereoisomer. This method requires that the racemic material has an appropriate "handle" for the attachment of the optically pure compound as well as there being high yielding reactions available for the necessary transformations.



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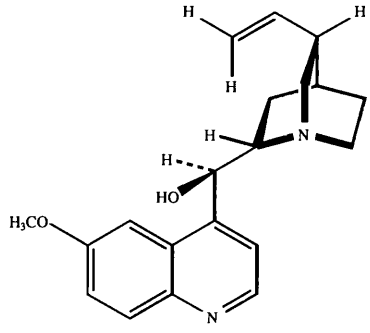
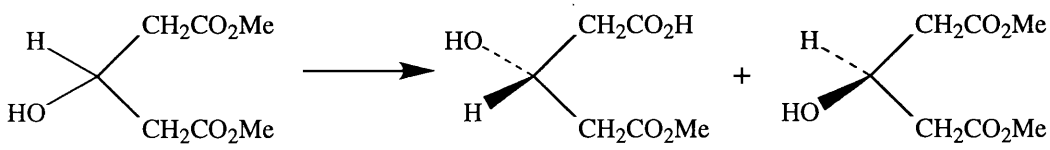


96

A related and much more expensive method is to use a chiral stationary phase and column or high pressure liquid chromatography.

Resolution can also be carried out enzymatically. For example treatment of dimethyl β-hydroxyglutarate (**97**) with α-chymotrypsin gave selective hydrolysis of the pro-(S) ester group to give the mono-acid (**98**) (**scheme 16**).[132]



97 **98** **99**

Scheme 16: Separation of β-hydroxyglutarate enantiomers

Unfortunately resolution can quickly become impractical as the number of chiral centres within the molecule begins to rise, since the number of possible enantiomers then becomes 2^n where n is the number of chiral centres.

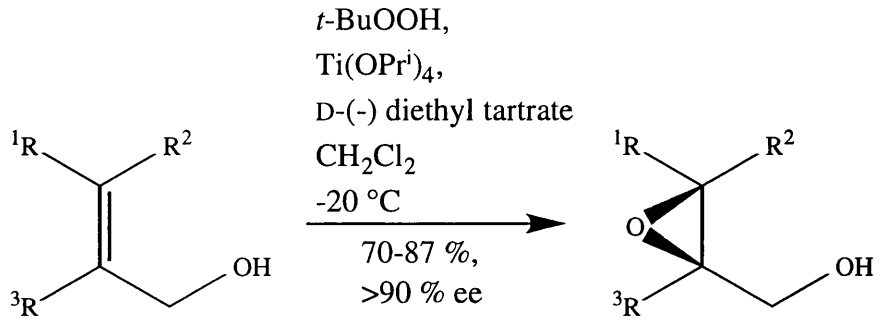
4.2.2 Optically Active Starting Materials

The strategy that is utilised in the synthetic approaches discussed later in this chapter is that of disconnecting the target molecule into a readily available optically active starting material. Compounds that are particularly useful in this respect are amino acids[133] and sugars.[134] Such compounds are known as chirons.[134]

4.2.3 Stereospecific Reactions

Asymmetry in a target molecule can be induced by using a chiral auxiliary. Such auxiliaries may form electrostatic or hydrogen bonds with the substrate or the chiral auxiliary may be attached covalently to the substrate and removed later in the synthesis. Such reactions tend to control the absolute stereochemistry. An example

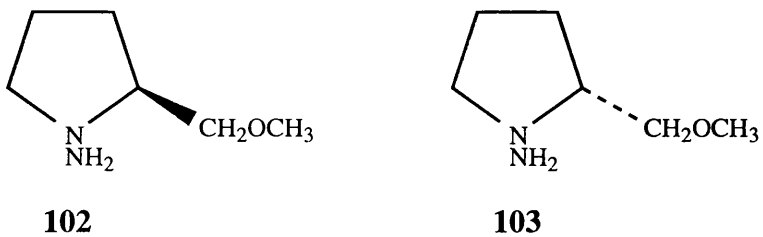
of the former is the enantioselective epoxidation of allylic alcohols using diethyl tartrate as a catalyst (**scheme 17**).[135]



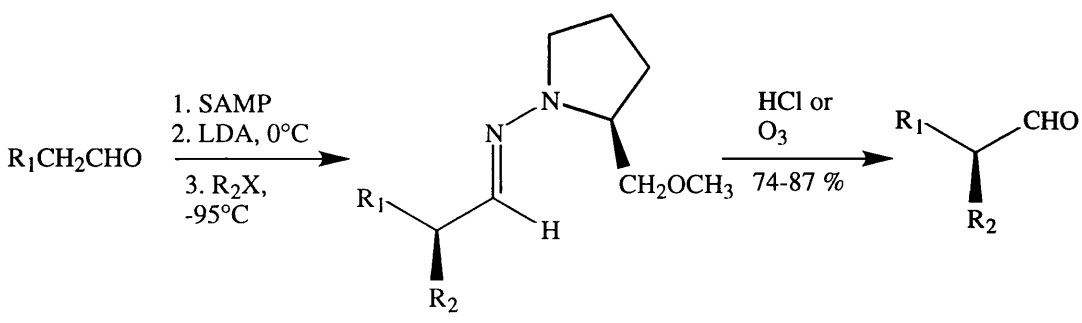
100 **101**

Scheme 17 : Epoxidation of an allylic alcohol

An example of the latter is the use of the chiral auxiliaries (*S*)- and (*R*)-1-amino-2-methoxymethylpyrrolidine, (**102**) and (**103**). These compounds are also known as SAMP and RAMP respectively.[136, 137] They can be used to prepare aldehydes via alkylation of SAMP-hydrazones (**105**) (**scheme 18**).[138] Typically the enantiomeric excess for such compounds is >90 %.[138]



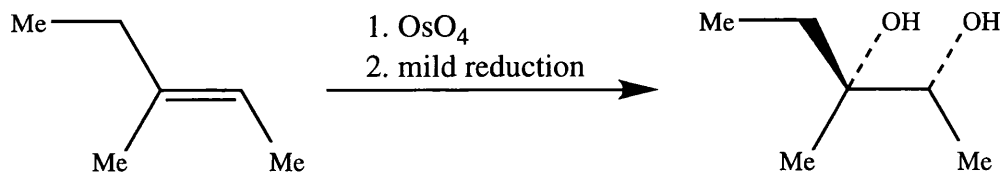
102 **103**



104 **105** **106**

Scheme 18 : Synthesis of optically active aldehydes

When only the relative stereochemistry needs to be controlled then many reagents by their mechanism control the relative stereochemistry in a reaction, for example the synthesis of a *cis*-diol using osmium tetroxide (**scheme 19**).



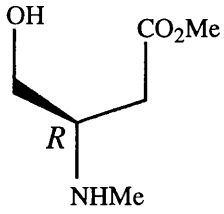
107 **108**

Scheme 19 : Synthesis of a *cis*-diol

There are a large number of reviews available on these subjects.[139]

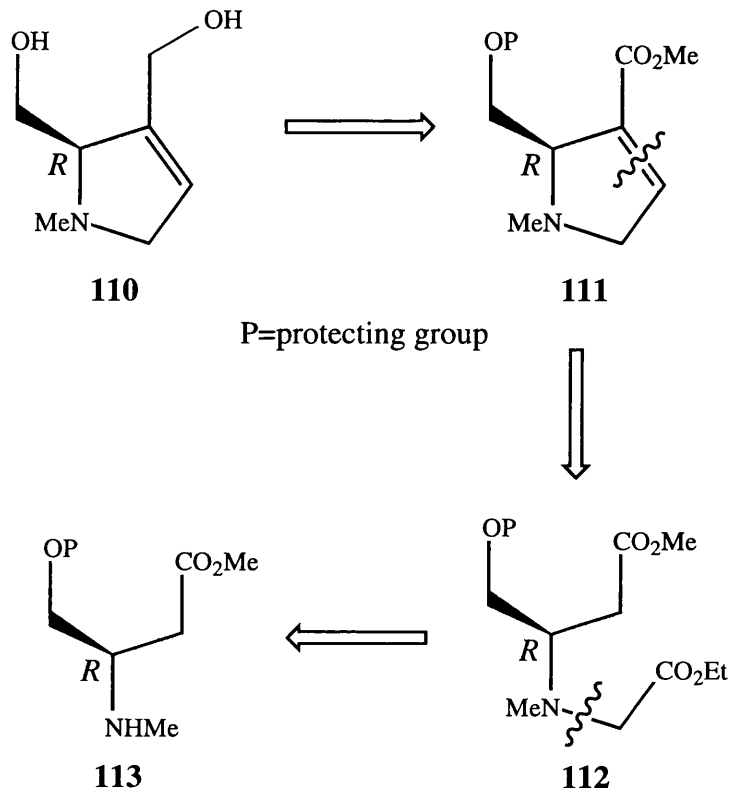
4.3 Retrosynthetic Analysis of the Synthesis of a Single Enantiomer of Synthanecine A

Synthanecine A (**14**) contains a single chiral centre at the 2-position. For much of Mattocks' original synthesis of synthanecine A (**scheme 13**), this position is α to an ester group and has an approximate pKa of 16.[140] This position is subject to racemisation particularly under some of the harsh conditions employed in the original synthesis. It was thus decided to reduce the ester to the hydroxymethyl group at the earliest possible stage hopefully protecting the compound from racemisation. The retro-synthetic analysis shown in **scheme 20** was proposed. This retained as many of Mattocks' original procedures as possible and identified methyl (*R*)-3-(*N*-methylamino)-4-hydroxybutanoate (**109**) as the key intermediate. The methyl ester was selected arbitrarily and is shown below for clarity.

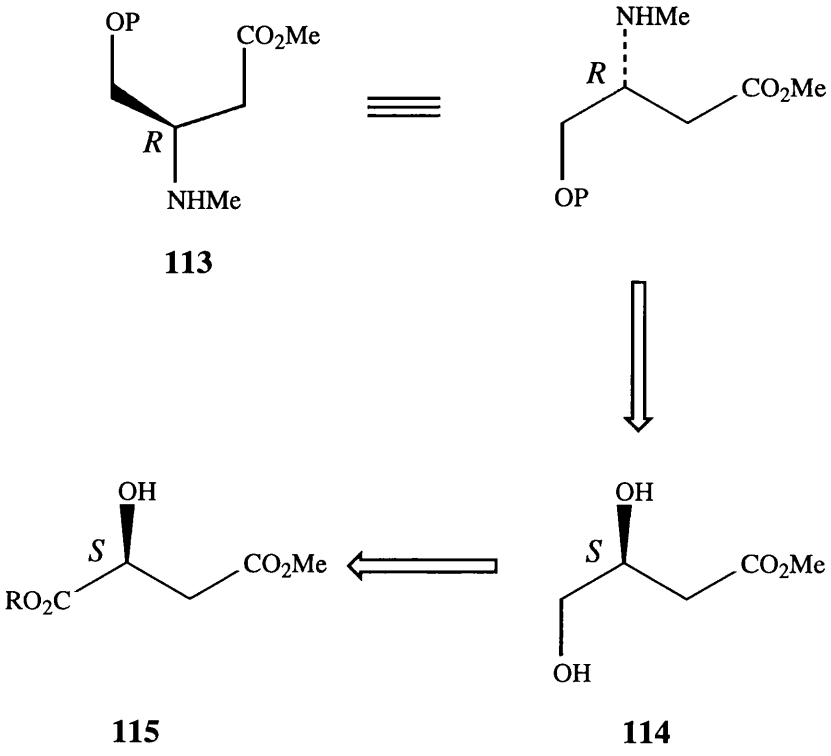


109

Approaches were then considered to the synthesis of methyl (*R*)-3-(*N*-methylamino)-4-hydroxybutanoate (**109**), preferably with the alcohol group protected. Two possible disconnections suggested suitable routes to the desired compound. One route gave a derivative of (*S*)-malic acid (**115**) as the starting material (**scheme 21**), whereas the other gave a derivative of D-aspartic acid (**116**) (**scheme 22**).

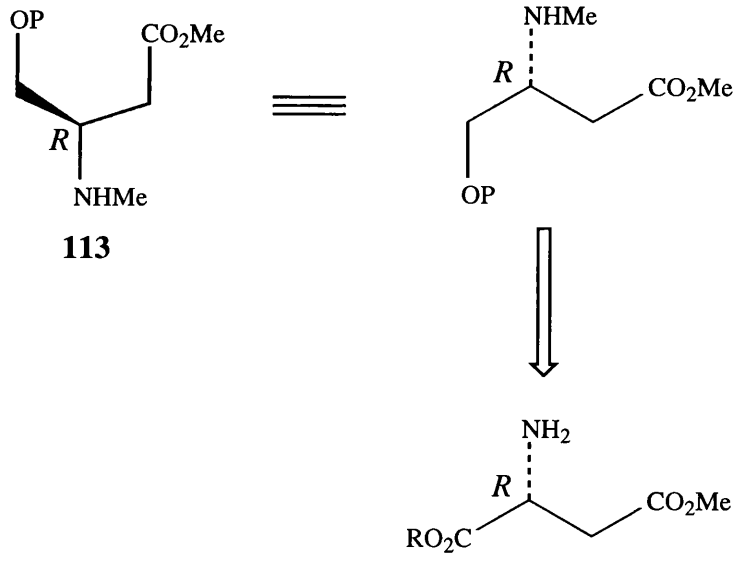


Scheme 20 : Disconnection of (*R*)-Synthanecine A (110) to methyl (*R*)-3-(*N*-methylamino)-4-hydroxybutanoate (109)



113 **115** **114**

Scheme 21 : Disconnections to (*S*)-Malic Acid

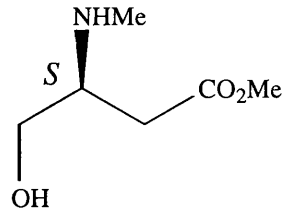


113 **116**

Scheme 22 : Disconnections to D-Aspartic Acid

The availability and cost of starting materials dictated that much of the development of this route would be done via the synthesis of methyl (*S*)-3-(*N*-

methylamino)-4-hydroxybutanoate (**117**), allowing the use of the readily available and cheaper L-aspartic acid (**118**).



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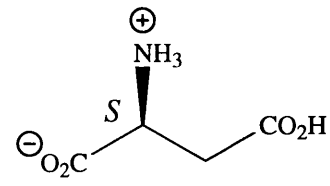

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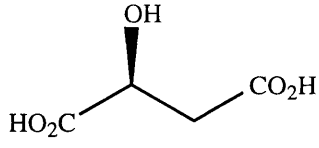
4.4 Approaches to the Synthesis of a Single Enantiomer of Synthanecine A Using Dimethyl (*S*)-Malate (121)

4.4.1 Introduction

Hydroxyacids such as (*S*)-malic acid (**119**) are useful and inexpensive chiral synthons. Saito *et al.*[141] recently reported a site selective reduction of diethyl malate (**120**), to give a diol of structure similar to (**114**) required in the retrosynthetic analysis given in **scheme 21**. The ease of preparation of a key intermediate in the synthesis based on malic acid prompted selection of this route over the route based upon L-aspartic acid. The development of this route is discussed below.



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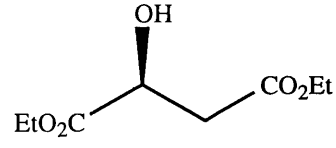
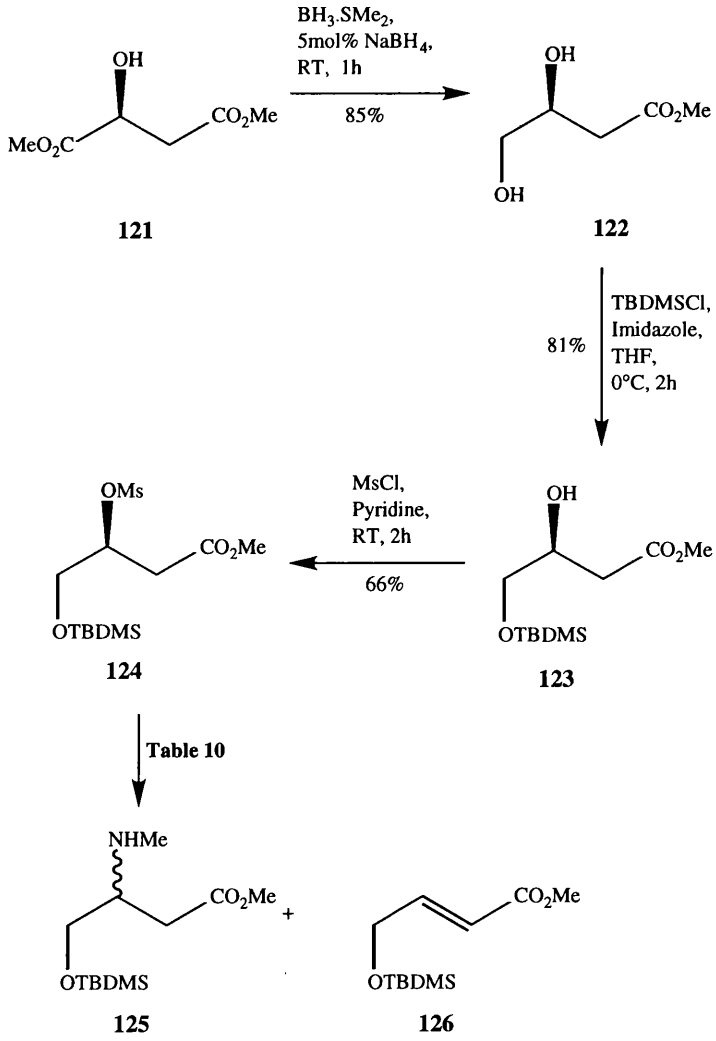


120

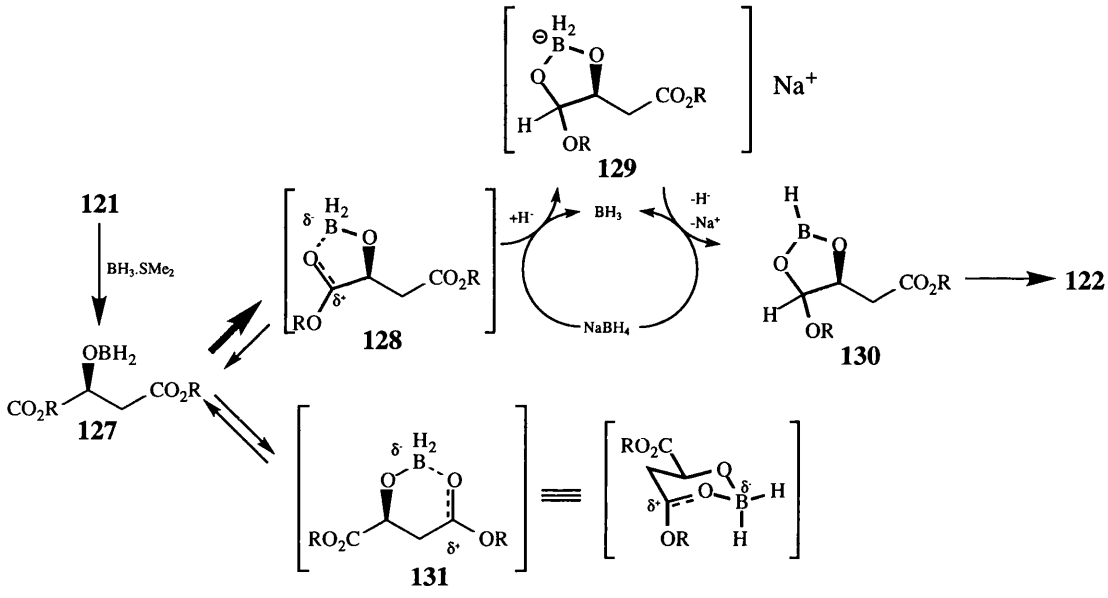
4.4.2 Attempted Synthesis of a Single Enantiomer of Synthanecine A Using Dimethyl Malate

Treatment of dimethyl malate (**121**) with one mole equivalent of boron methyl sulfide complex caused evolution of hydrogen which continued for 30 minutes during which time it is postulated[141] that the oxyborane intermediate (**128**) is formed. Addition of 5 mol% $NaBH_4$ gave the diol in 85 % yield. The appearance of an ABX system at δ 3.45 and 4.00 due to the methylene hydrogens next to the oxygen and the methine hydrogen, and the absence of a singlet due to one of the methyl esters in the ^1H NMR spectrum of the product confirmed its structure to be (**122**).



Scheme 23 : Attempted synthesis of methyl (*R*)-3-(*N*-methylamino)-4-hydroxybutanoate (109)

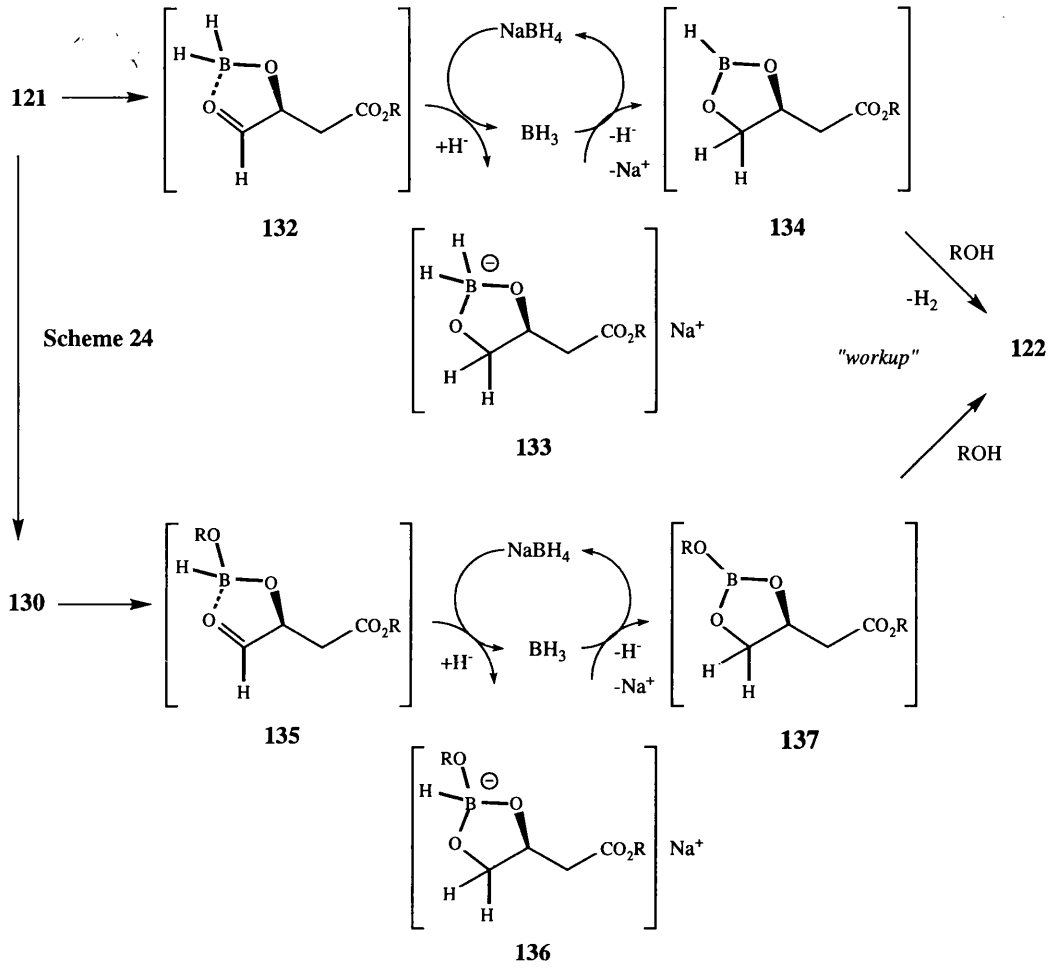
Saito *et al.*[142] originally proposed the mechanism given in **scheme 24** to account for the site selectivity of the reduction.



Scheme 24 : Originally proposed mechanism for selective reduction of (121)

This mechanism was then revised[141] to incorporate the observation that only a small amount of hydrogen is evolved on work-up. The revised mechanism is presented in **scheme 25**. The revised mechanism accounts for this by reasoning that two possible intermediates may give rise to the product (**122**). Only one of these intermediates (**134**) has an intact B-H bond which would be capable of liberating hydrogen gas on work-up.

The five-membered transition state (**128**) is favoured over the six-membered transition state (**131**) because neighbouring group participation is favoured in a five-membered cyclic array[143] and in the six-membered transition state there is a severe 1,3-diaxial interaction between the ester alkoxy group and one of the hydrogens attached to the boron.



Scheme 25 : Revised mechanism for selective reduction of (121)

The *tert*-butyldimethylsilyl ether was then prepared, reaction taking place selectively at the primary alcohol to give the monoprotected diol (**123**) in 81 % yield after column chromatography. The IR spectrum of this compound showed absorptions at 1074 and 838 cm^{-1} corresponding to the stretching of the Si-O bond. The ^1H NMR spectrum showed a singlet at δ 0.01 due to the geminal dimethyl groups attached to the silicon and a singlet at δ 0.83 due to the methyl groups of the *tert*-butyl group.

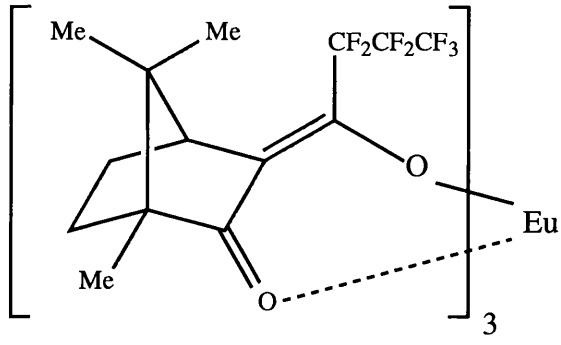
In order to transform the secondary alcohol of (**123**) into a better leaving group it was converted into the mesylate (**124**) by treatment with methanesulfonyl chloride in pyridine. The product was isolated by column chromatography in 66 % yield. The expected absorptions due to the stretching of the SO_2O- group were

found at 1360 and 1176 cm^{-1}. The ^1H NMR spectrum showed a singlet at δ 2.99 correlating with the methyl group of the mesylate.

Upon treatment of the mesylate (**124**) with methylamine it was hoped that the mesylate would undergo an S_N2 reaction to give the protected form of methyl (*R*)-3-(*N*-methylamino)-4-hydroxybutanoate (**109**). Unfortunately this proved not to be the case. Treatment of the mesylate with methylamine in ethanol at reflux temperature gave the racemised amine (**125**) as the major product.

The IR spectrum of the amine showed a broad absorption at 3344 cm^{-1} corresponding to an N-H stretch, and the loss of the absorptions representing the $-SO_2O$ group. The ^1H NMR spectrum showed the absence of the methyl singlet due to the methyl group of the mesylate and the appearance of a singlet at δ 2.37 due to the methyl group attached to the nitrogen. At δ 1.97 there was a D_2O exchangeable signal due to the N-H hydrogen.

The racemisation of the compound (**125**) was proven by measurement of the compound's optical rotation which was found to be 0°, and by examination of the ^1H NMR spectrum of the compound after addition of 3 % (by mole) of the chiral shift reagent europium (III) tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato] (**138**).



138

A portion of ^1H NMR spectrum after treatment with the shift reagent is shown in **figure 3**. The doubling of the signal due to the methoxy group can be clearly seen. The same experiment was carried out on the mesylate (**124**) to ensure that the starting material was optically pure. No doubling of the signals was noted.

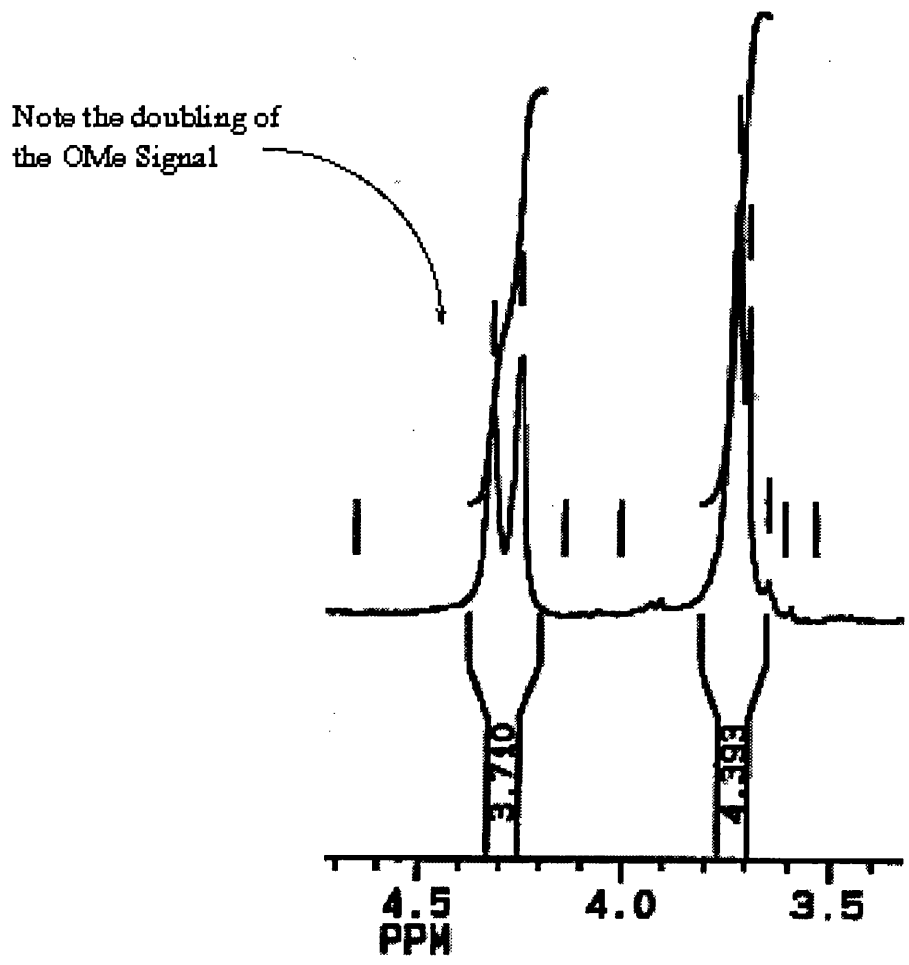
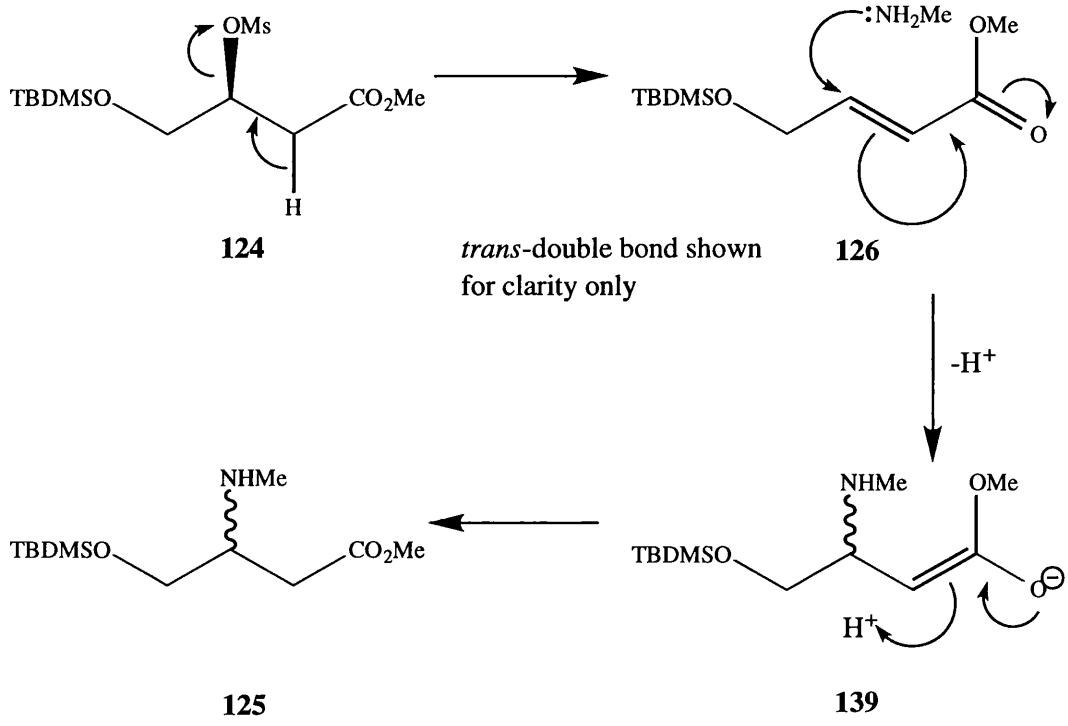


Figure 3 : A portion of the ^{1}H NMR spectrum of 125 after addition of 3 mol % of chiral shift reagent (135)

Clearly visible in the ^{1}H NMR spectrum of the racemised amine were signals corresponding to a trace amount of the alkene (**126**)[144] formed by elimination of the mesyl group to give the α, β-unsaturated ester. It is assumed that the racemisation of the amine is due to this elimination being the first stage of the reaction, followed by a conjugate addition to the double bond (**scheme 26**).



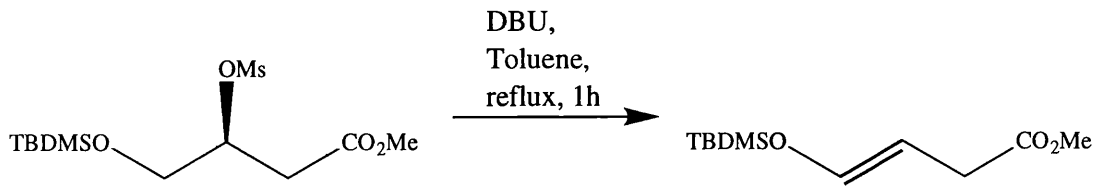
124 *trans*-double bond shown for clarity only **126**

125 **139**

Scheme 26 : Proposed mechanism of amination of 124

McElvain *et al.*[145,146] have shown that treatment of β-bromoesters with piperidine gave a mixture of the α,β-unsaturated ester and the product of conjugate addition with the unsaturated ester. The reaction mechanism proposed above is analogous to the observations of McElvain *et al.*

The amount of α,β-unsaturated ester (**126**) remaining at the end of the reaction was too small for isolation and characterisation and so it was decided to synthesise this ester to show that its ^{1}H NMR spectrum was identical to that observed as an impurity in the ^{1}H NMR spectrum of the amine (**125**). This reaction was carried out by treatment of the mesylate (**124**) with the non-nucleophilic nitrogen base DBU in toluene at reflux temperature (**scheme 27**).



124 **140**

Scheme 27 : Reaction of mesylate (124) with DBU in toluene

This reaction gave an unexpected product: the silyl enol ether (**140**) was formed in preference to the predicted α,β-unsaturated ester. The IR spectrum of the ether showed absorptions at 1744 cm^{-1} corresponding to the ester carbonyl and 1660 cm^{-1} due to the stretching of the C=C bond. It should be noted that these absorptions are at too high a frequency to be due to the α,β-unsaturated compound. The ^1H NMR spectrum showed a doublet of triplets at δ 4.66 with coupling constants of 5.8 and 7.0 Hz and another doublet of triplets at δ 6.30 with coupling constants of J_{vic} 5.8 Hz and $J_{allylic}$ 1.6 Hz corresponding to the alkenic hydrogens. The fact that the downfield alkenic hydrogen shows the allylic coupling also suggests that the compound formed is the silyl enol ether (**140**). The coupling constant between the alkenic protons is 5.8 Hz suggesting a *cis* double bond. Selected ^1H NMR data are shown in **figure 4**.

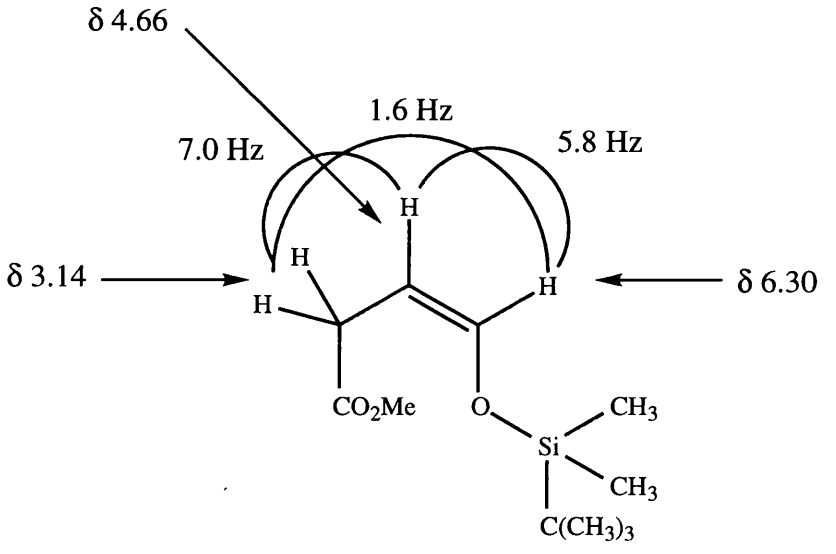


Figure 4 : Selected chemical shifts and coupling constants for the silyl enol ether (140)

No previous reference can be found in the literature to this method of formation of silyl enol ethers. However it assumed that the reaction goes via the α,β-unsaturated ester, which then undergoes isomerisation to the silyl enol ether. The

implication is that the silyl enol ether is more thermodynamically stable than the α,β-unsaturated ester.

A variety of conditions was tried to affect the substitution of the mesylate with methylamine without racemisation. These methods are summarised in **table 10**. None of the methods proved successful and this synthetic approach was abandoned.

Conditions	Racemised ?	Yield
reflux, EtOH, $MeNH_2$, 1h	Yes	47% of **125** & trace **126**
rt, EtOH, $MeNH_2$	Yes	82% of **125** & trace**126**
Et_2O, -78→0°C, $MeNH_2$ via cold finger	Yes	49% of **125** & trace**126**
hexane, -78→0°C, $MeNH_2$ via cold finger	Yes	37% of **125** & trace**126**
MeCN, 0°C, $MeNH_2$ via bubbler	Yes	31% of **125** & trace **126**
hexane, -78°C, $MeNH_2$ via bubbler	Yes	42% of **125** & trace**126**

Table 10 : Attempted formation of methylamine without racemisation

4.5 Approaches to the Synthesis of a Single Enantiomer of Synthanecine A Using L-Aspartic Acid (118)

4.5.1 Introduction

L-Aspartic acid (**118**) [(*S*)-2-aminosuccinic acid] is an essential amino acid in the diet of mammals, and plays an important role in a wide variety of biochemical processes. The relative pKa values of the amino group and the two acid groups are shown in **figure 5**. The use of aspartic acid in organic synthesis has been comprehensively reviewed.[133]

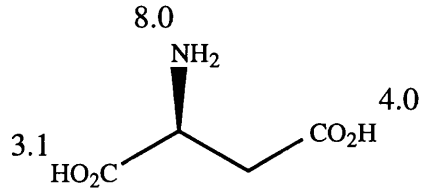


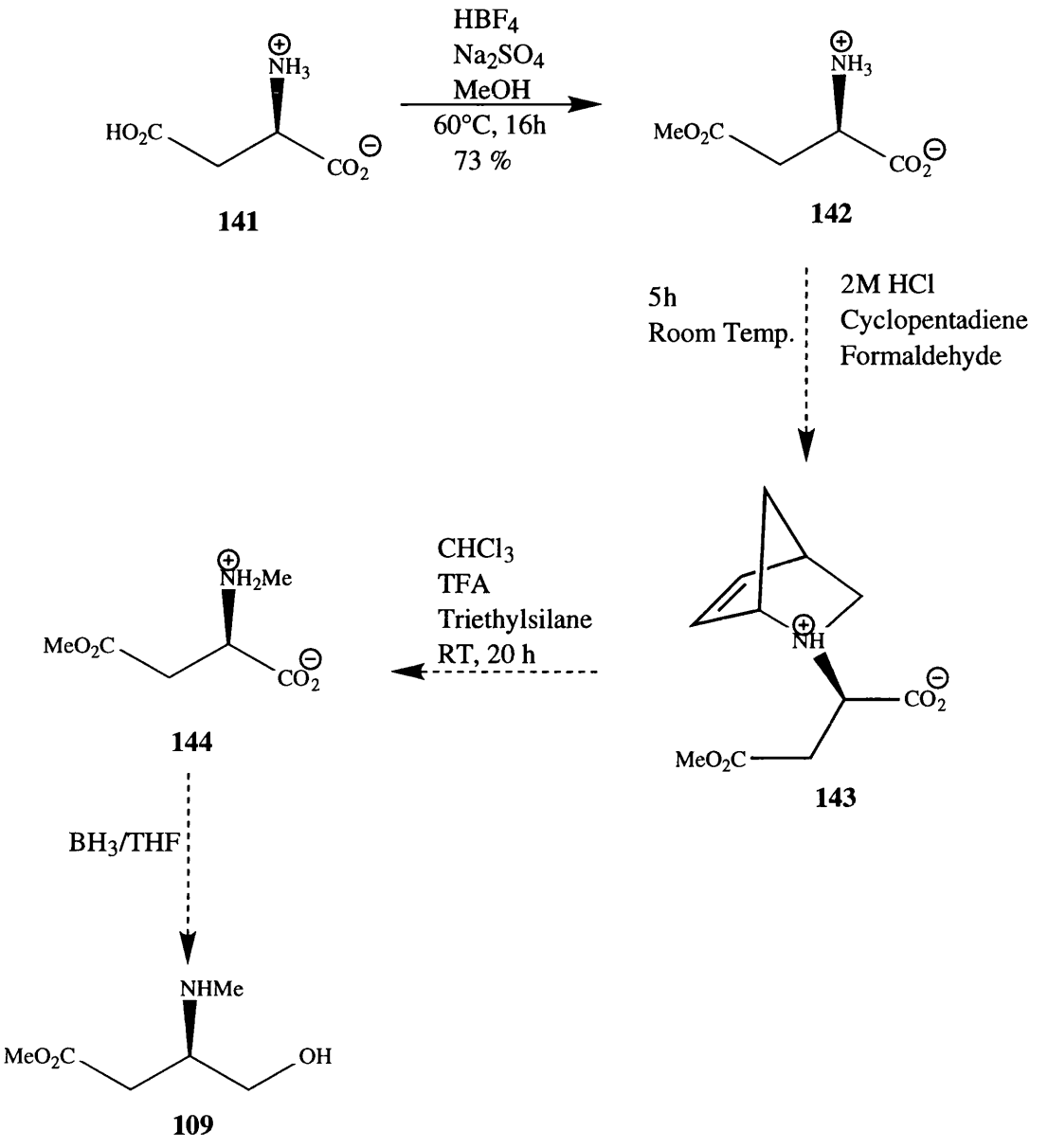
Figure 5 : pKa values of L-aspartic acid

The first route which was attempted to synthesise a single enantiomer of synthanecine A utilised D-aspartic acid (**141**) (**scheme 28**). Later attempts used L-aspartic acid.

4.5.2 Attempted Synthesis of a Single Enantiomer of Synthanecine A Using Aspartic Acid

The initial attempt to synthesise the key intermediate methyl (*R*)-3-(*N*-methylamino)-4-hydroxybutanoate (**109**) is detailed in **scheme 28**.

β-Methyl D-aspartic acid (**142**) was required in the first stage of this synthesis. Acid catalysts used to effect this esterification include sulfuric acid,[147] hydrochloric acid,[148] boron trifluoride etherate[149] or chlorotrimethylsilane.[150] In all of these reactions diester formation was reported as a significant side reaction.[148,151] Albert *et al.* reported that tetrafluoroboric acid is an excellent catalyst for this reaction in terms of yield, selectivity, and ease of work-up.[152] This reaction was carried out in 73 % yield with no diester formation. Additional spectroscopic data are provided in **chapter 9**. The IR spectrum of this compound showed a strong absorption at 1728 cm^{-1} corresponding to the ester carbonyl stretch. The antisymmetrical and symmetrical stretches of the acid carbonyl were found at 1644 and 1376 cm^{-1} respectively. The ^1H NMR spectrum showed the methyl singlet at δ 3.60 due to the methyl ester.



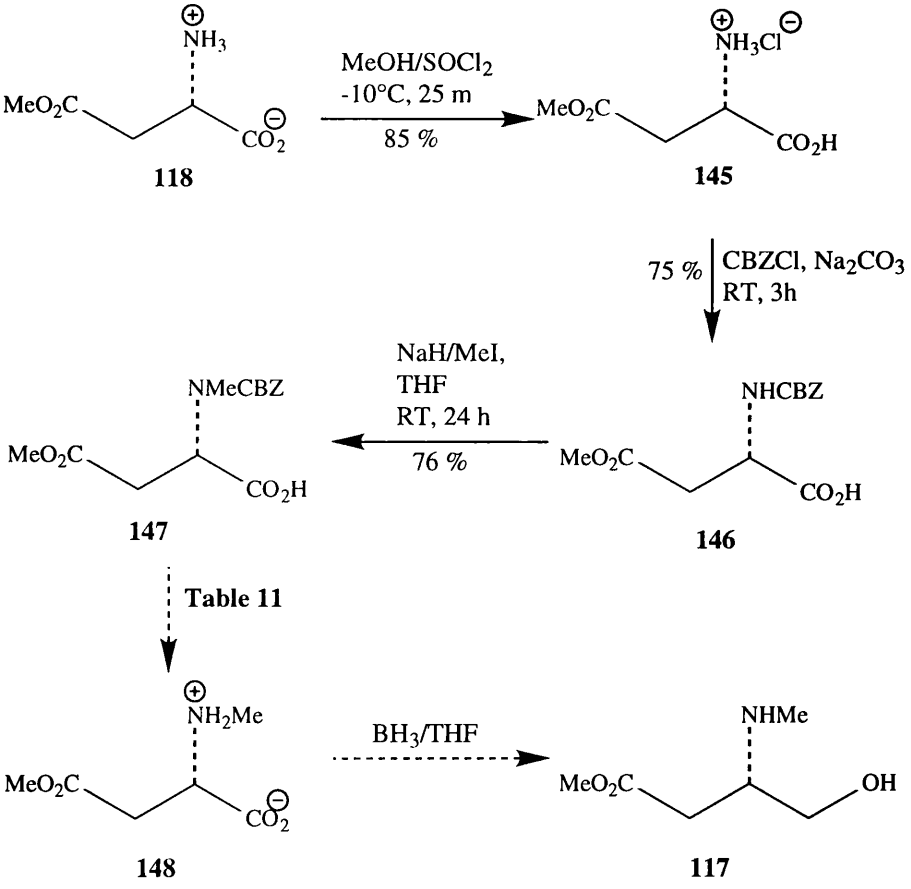
Scheme 28 : Attempted synthesis of methyl (*R*)-3-(*N*-methylamino)-4-hydroxybutanoate

Grieco and Bahsas[153] reported a novel method of *N*-methylation via a retro aza Diels-Alder reaction. They used a wide variety of substrates (no aspartic acid derivatives were reported) and reported that no protection of the α-carbonyl was necessary and that no racemisation took place. The reaction was reported to proceed via the acid-catalysed formation of the imine adduct of the amino acid and formaldehyde, and subsequent trapping of this adduct via the aza Diels-Alder reaction. The retro aza Diels-Alder reaction then proceeded smoothly in the

presence of trifluoroacetic acid. Triethylsilane reduced the liberated iminium ion to the *N*-methyl compound. Treatment of β-methyl D-aspartic acid (**142**) with formaldehyde, HCl and cyclopentadiene failed to give the aza Diels-Alder product (**143**). This route was abandoned.

The final stage of this route would have been BH_3/THF reduction of the *N*-methyl compound (**144**) to give methyl (*R*)-3-(*N*-methylamino)-4-hydroxybutanoate (**109**). Yoon *et al.*[154] had shown that BH_3/THF could be used to reduce carboxylic acids in the presence of esters citing the slow rate of reduction of esters by BH_3/THF as the reason for the selectivity.

L-Aspartic acid (**118**) was then adopted as the starting material for the new approach to the synthesis of the key intermediate methyl (*S*)-3-(*N*-methylamino)-4-hydroxybutanoate (**117**). This synthesis is detailed in **scheme 29**.



Scheme 29 : Attempted synthesis of methyl (*S*)-3-(*N*-methylamino)-4-hydroxybutanoate (114)

Despite reports to the contrary by Albert *et al.*[152] thionyl chloride and methanol were used to synthesise the hydrochloride salt of β-methyl L-aspartic acid (**145**) using the method of Schwarz *et al.*[155] with no diester formation noted. This compound was obtained in 85 % yield. Additional spectroscopic data are presented in **chapter 9**. The IR spectrum of this compound showed an absorption at 1732 cm^{-1} corresponding to a carbonyl stretch. The ^1H NMR spectrum shows a singlet at δ 3.53 corresponding to the methoxy group.

In order for the *N*-methylation to take place the nitrogen in **145** had to be protected as its benzyloxycarbonyl derivative.[156] This protection was carried out using benzyl chloroformate, with sodium carbonate acting as the base. The product (**146**) was isolated in 75 % yield after recrystallisation. The IR spectrum of this compound showed strong absorptions at 1694 and 1588 cm^{-1} corresponding to the carbamate carbonyl and the aromatic ring respectively. The CBZ group gave characteristic resonances in the ^1H NMR spectrum: a broad singlet at δ 7.33 due to the aromatic ring, and a singlet at δ 5.10 due to the methylene group next to the aromatic ring.

McDermott and Benoiton[157] had prepared *N*-methyl derivatives of a variety of amino acids including β-*tert*-butyl L-aspartic acid using sodium hydride and methyl iodide in THF. The *N*-methylation of the protected β-methyl ester (**146**) proceeded in 76 % crude yield to give a thick yellow oil. This oil could not be induced to crystallise, nor could it be successfully purified by column chromatography. McDermott and Benoiton had described the purification of their aspartic acid derivative as its dicyclohexylamine salt. This also proved unsuccessful for the *N*-methyl derivative (**146**). The compound was thus used crude in the next stage of the reaction. The ^1H NMR spectrum of the crude material showed a singlet at δ 2.9 due to the *N*-methyl group.

In order to facilitate purification it was decided to remove the protecting group from the nitrogen which should result in an easily purified crystalline

derivative. A variety of methods are available for the removal of such benzyloxycarbonyl protecting groups.[158] It was elected to remove the protecting group by hydrogenolysis since this method should leave the labile methyl ester untouched and also presented a wide variety of possible methods for carrying out the hydrogenolysis. The deprotection proceeds by decarboxylation of the free carbamic acid resulting from hydrogenolysis of the benzyl residue.[159] A variety of methods was attempted to remove the protecting group. These are summarised in **table 11**.

Method	Yield	Reference
10 % Pd/C catalyst, H_2, 80 % acetic acid	-	160
10 % Pd/C catalyst, cyclohexene, ethanol	-	161
10 % Pd/C catalyst, ammonium formate, methanol	26 %	162

Table 11 : Deprotection of β-methyl (*N*-benzyloxycarbonyl-*N*-methyl)-L-aspartic acid (147)

The standard method of hydrogenolysis[159] using 10 % Pd/C catalyst and hydrogen gas, where both the hydrogen gas and the organic substrate are absorbed onto the catalyst facilitating their contact, failed to give any product and only starting material could be isolated.

Jackson and Johnstone[161] reported that using cyclohexene as a hydrogen source, removal of benzyloxycarbonyl protecting groups would be complete in 2 h at 25 °C. This reaction failed to give any product and only starting material could be isolated.

The method of Makowski *et al.*[162] using ammonium formate as a hydrogen source proved successful in removing the protecting group but only in a poor yield of 26 %. The IR spectrum of this compound showed an absorption at 1740 cm^{-1} corresponding to the ester carbonyl and further peaks at 1591 and 1377 cm^{-1}

corresponding to antisymmetrical and symmetrical stretching of the carboxylate ion respectively. No peaks due to the CBZ protecting group were seen.

The *N*-methyl derivative proved particularly resistant to hydrogenolysis. This lack of success in the deprotection step was probably due to the lack of purity of the *N*-methyl derivative causing catalyst poisoning. A likely constituent of this catalyst poison may be residual iodine liberated in the previous stage of the reaction.[159] In view of the poor yield obtained in the deprotection step this route was abandoned.

A protected form of the key intermediate methyl (*S*)-3-(*N*-methylamino)-4-hydroxybutanoate (**154**) was finally synthesised as shown in **scheme 30**. This route was continued beyond the synthesis of **154** and toward a single enantiomer of synthanecine A.

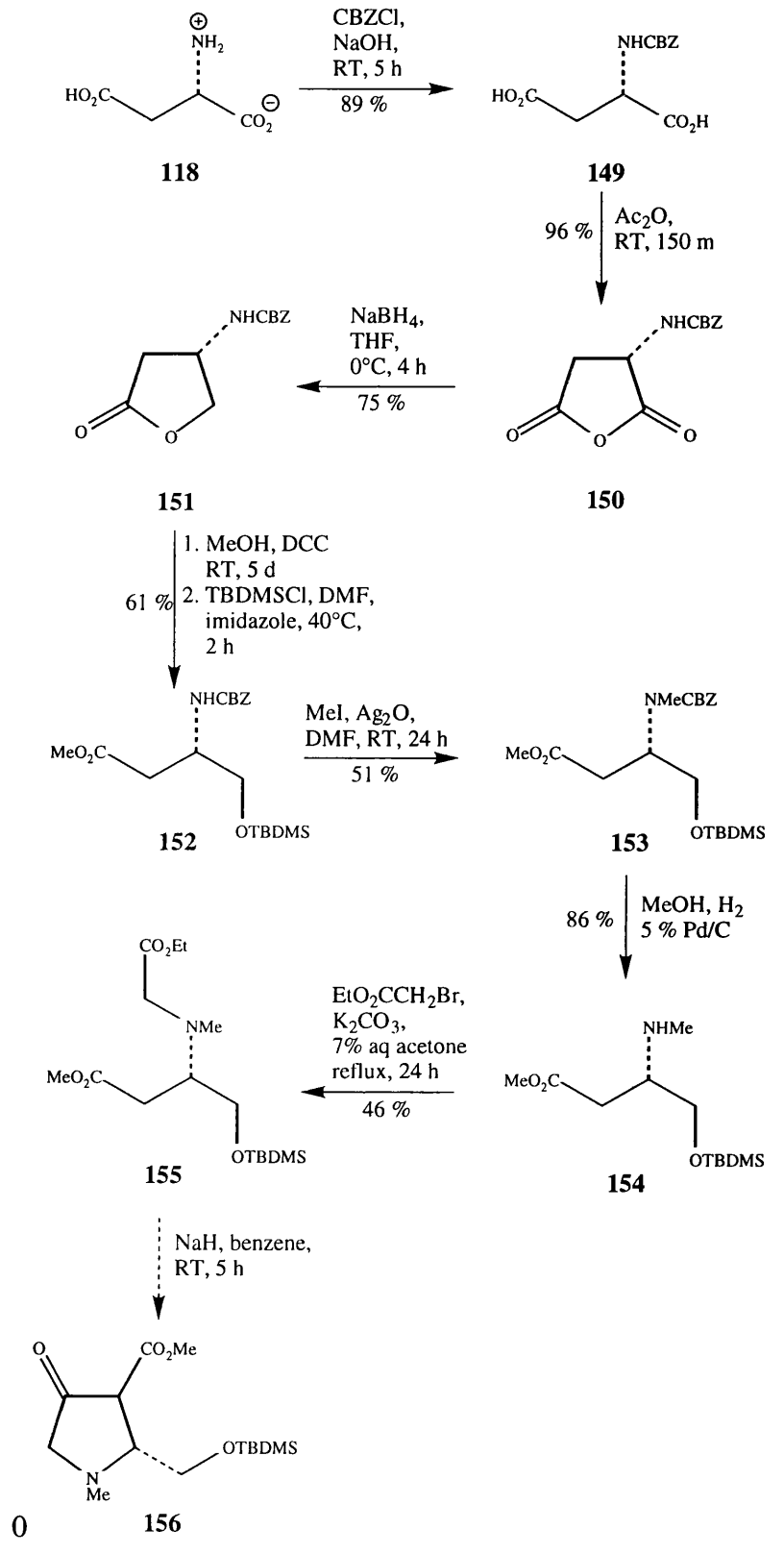
In order to achieve the correct oxidation states in the synthesis as early as possible, the method of McGarvey *et al.*[163] was used to reduce the α-carbonyl of L-aspartic acid. Additional spectroscopic data are provided in **chapter 9**.

Treatment of L-aspartic acid (**118**) with benzyl chloroformate and NaOH afforded the protected amino acid (**149**) in 89 % yield after recrystallisation. The IR spectrum of this compound showed absorptions at 1705 cm^{-1} due to stretching of the carbamate carbonyl and at 1585 and 1533 cm^{-1} due to the aromatic ring. The ^1H NMR spectrum of this compound showed a broad singlet at δ 7.34 due to the aromatic hydrogens and at δ 5.05 corresponding to the methylene group next to the aromatic ring.

N-Benzyloxycarbonyl-L-aspartic anhydride (**150**) was prepared in 96 % yield by the method of Lutz *et al.*[164] This compound gave an elemental composition and melting point consistent with product formation. The IR spectrum showed a strong absorption at 1306 cm^{-1} due to C-O stretching.

Reduction of the anhydride (**150**) was effected using $NaBH_4$ in THF and gave the lactone (**151**) in 75 % yield. The IR spectrum of this compound was significantly altered from that of the starting material and showed an absorption at

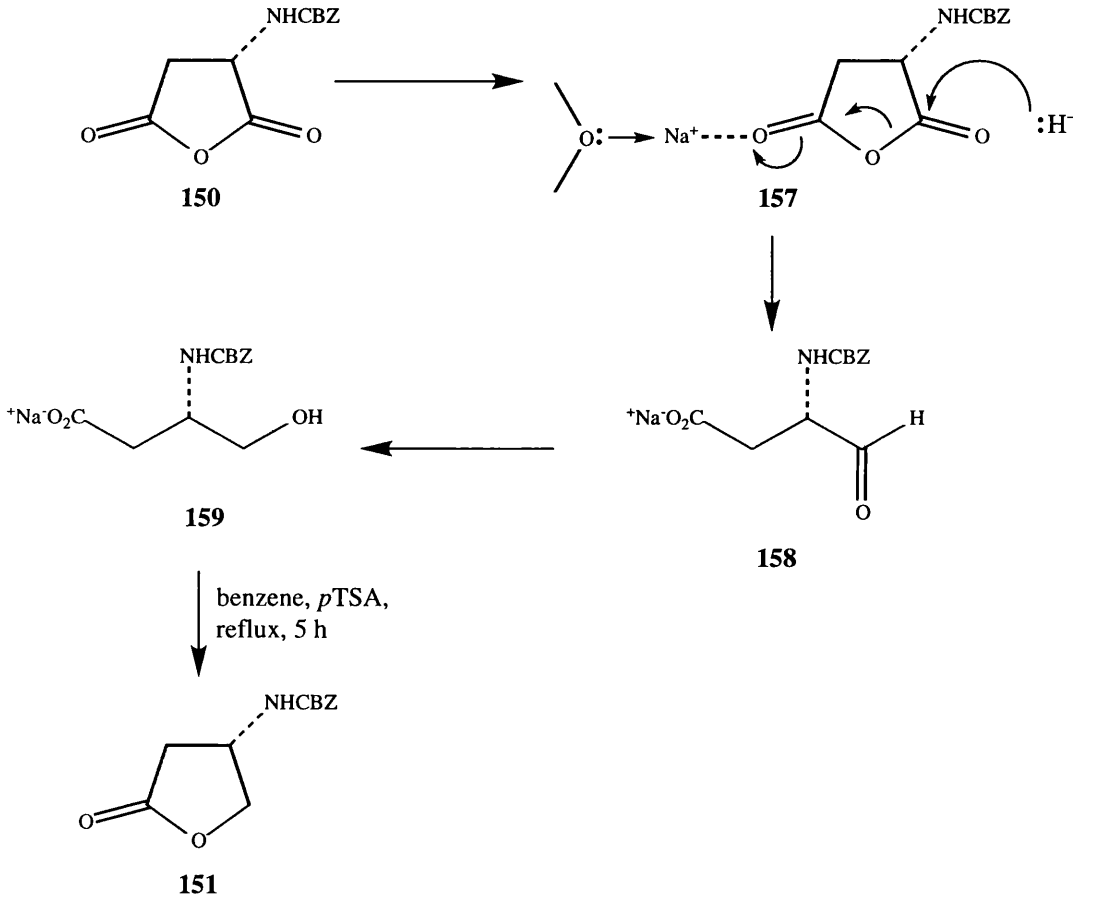
1780 cm^{-1} due to a 5-membered lactone. The ^{1}H NMR spectrum showed a new multiplet a δ 4.14-4.17 assigned to the newly formed methylene group. The ^{13}C NMR spectrum showed only two quaternary carbons corresponding to carbonyl groups at δ 155.9 and 175.8 due to the carbamate carbonyl and the lactone carbonyl respectively.



Scheme 30 : Attempted synthesis of a single enantiomer of synthanecine A

Reduction to give the lactone took place at the more hindered position α to the protected amino group. This is the normal steric course of reductions using

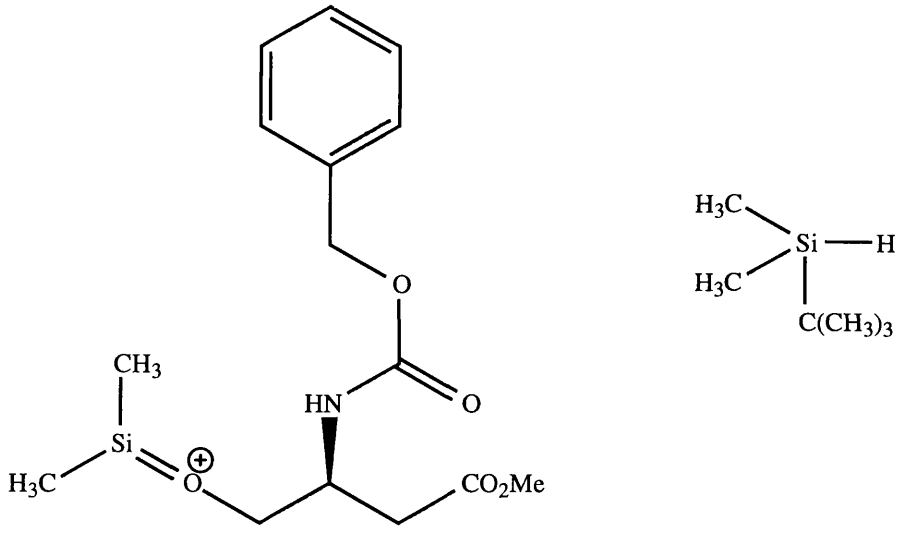
$NaBH_4$.[165, 166] This selectivity is due to the less hindered carbonyl complexing with a solvated cation, in this case Na^+, to give the species (**157**) shown in **scheme 31**. This makes this site the more hindered. Attack of a hydride ion at the position α to the amino group can then occur producing an aldehyde (**158**) which is then rapidly reduced by borane liberated in the reaction to give the alcohol (**159**). Heating at reflux in benzene in the presence of *p*-toluenesulfonic acid then closed the ring to form the lactone (**151**).



Scheme 31 : Reduction of anhydride (150) with $NaBH_4$

In their synthesis of the anthracycline antibiotic L-daunosamine, Jurzal *et al.*[167] detailed a method for the ring opening and subsequent protection of the lactone (**151**) to give the TBDMS protected methyl ester (**152**). Following this method and treating the lactone first with methanol and DCC for 5 days at room temperature followed by treatment with TBDMSCl and imidazole in THF at 40 °C

gave the protected ester (**152**) in 61 % yield after purification by column chromatography. Additional spectroscopic data to that originally published[167] are presented in **chapter 9**. The IR spectrum of this compound showed a strong absorption at 1728 cm^{-1} assigned to stretching of the ester carbonyl group. Absorptions at 1256 and 1088 cm^{-1} corresponded to stretching of the $SiMe_2$ group and stretching of the Si-O bond respectively. The silyl protecting group was distinctive in the ^1H NMR spectrum showing singlets at δ 0.00 and δ 0.85 due to the geminal methyl groups and the methyl groups of the *tert*-butyl group respectively. A singlet at δ 3.59 corresponded to the methyl ester. The MS of this compound showed fragments at *m/z* 324 and 116. These fragments are indicative of the presence of TBDMS protecting group (**figure 6**).



160 *m/z* 324

161 *m/z* 116

Figure 6 : Identity of fragments from MS of 152

Previously, when an *N*-methyl compound was required, the method of McDermott and Benoiton[157] had proved successful (**scheme 29**) using sodium hydride and iodomethane. However when the amine (**152**) was the substrate for this reaction it proved unsuccessful giving only starting material. The conditions for this

reaction were varied as shown in **Table 12**. None of these conditions succeeded in forming the product (**153**).

Reagents	Conditions	Product
iodomethane (8 equiv.), sodium hydride (1.5 equiv.), THF	room temperature, 24 h	starting material, 85 %
iodomethane (8 equiv.), sodium hydride (3 equiv.), THF	room temperature, 24 h	starting material, 14 %
iodomethane (8 equiv.), sodium hydride (3 equiv.), THF	reflux, 3 h	starting material, 6 %
iodomethane (8 equiv.), sodium hydride (3 equiv.), THF	reflux, 24h	complex mixture of products.

Table 12 : Attempted synthesis of *N*-methyl amine (153)

A wide variety of methods is available for the *N*-methylation of amines including the use of organocopper reagents,[168] alkylation of Schiff bases,[169] and via a retro aza Diels-Alder reaction.[153] The method selected to affect the formation of the *N*-methylamine (**153**) was that of Olsen[170] using silver (I) oxide and iodomethane in DMF. Treatment of the amine (**152**) with these reagents at room temperature for 24 hours gave the product (**153**) in 51 % yield after purification by column chromatography. Although apparently pure by TLC, both the ^{1}H and ^{13}C NMR spectra show a doubling of some of the signals (**figure 7**).

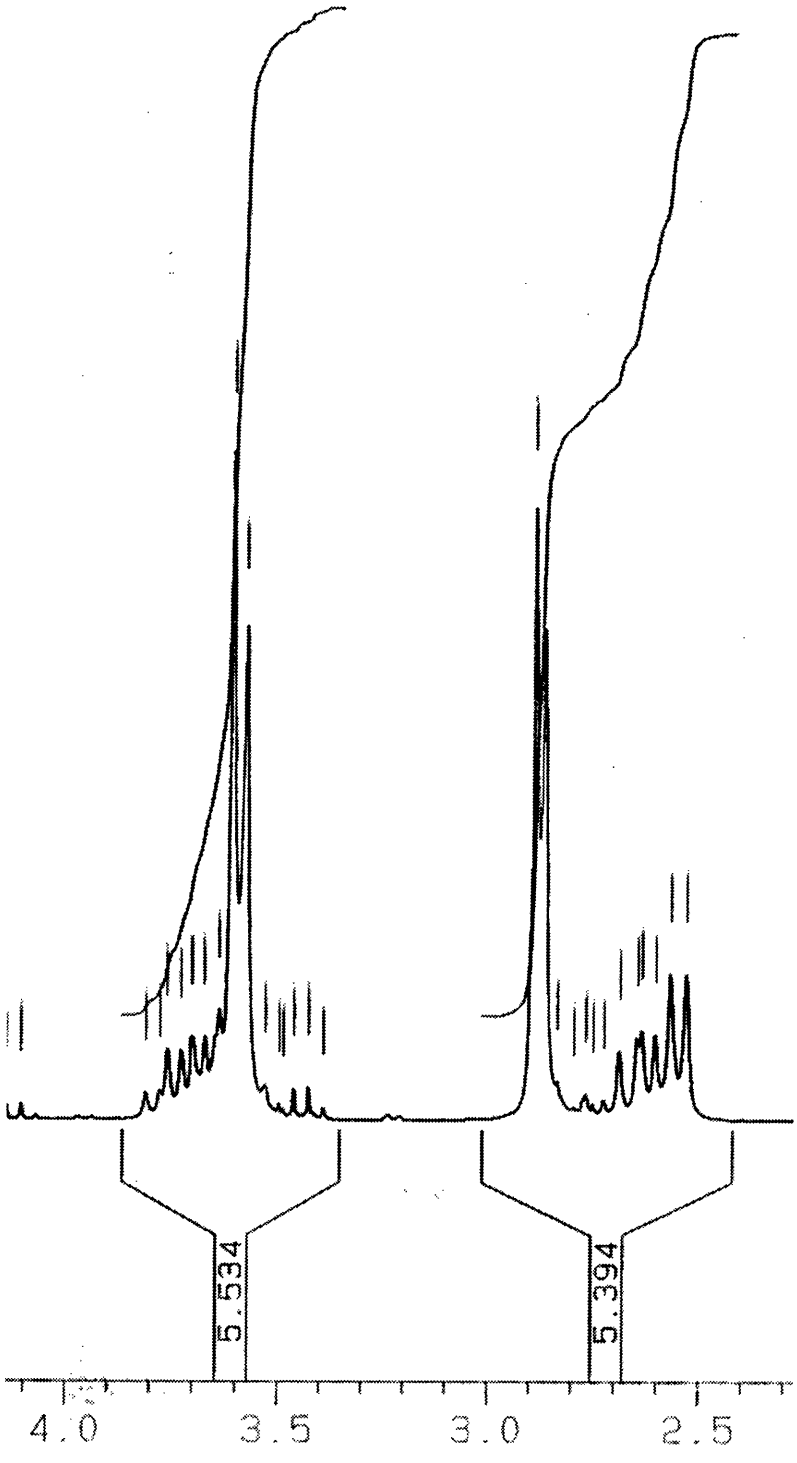
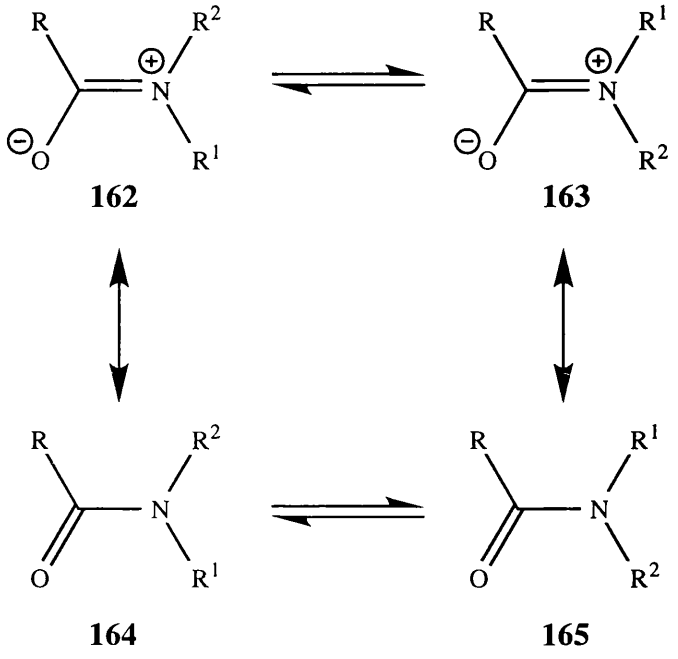


Figure 7 : ^{1}H NMR spectrum of the *N*-methyl amine (150)

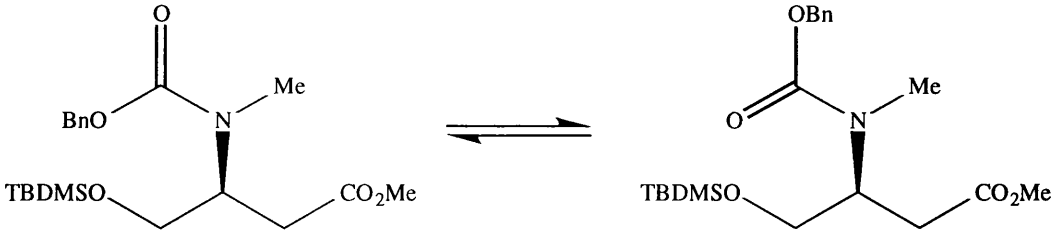
Such doubling of the signals in the ^{1}H and ^{13}C NMR spectra suggest that the *N*-methylamine can adopt two distinct conformational isomers. This doubling of signals is not observed in either the starting material (**152**) or in the next stage of the

synthesis (after removal of the CBZ protecting group) (**117**). The presence of distinct conformational isomers can be observed in some amides and thioamides where resonance gives the molecule some double bond character and thus slows rotation about the C-N bond[171] (**Scheme 32**).



Scheme 32 : Conformational isomers of an amide

Such isomerisation is possible (**scheme 33**) in the *N*-methyl carbamate (**153**).



Scheme 33 : Possible isomerisation of the *N*-methyl carbamate (153)

The IR spectrum of this compound shows a strong absorption at 2856 cm^{-1} due to the stretching of the N-Me bond.

The CBZ- protecting group was removed by treatment with 5 % Pd/C in dry methanol to give the free amine (**154**) in 86 % yield.

The IR spectrum of this compound showed no signals attributable to either carbonyl stretching in a carbamate or the presence of an aromatic ring. A moderate absorption was observed at 3442 cm^{-1} attributable to the stretching of the N-H bond. The ^1H NMR spectrum of this compound had no signals due to the CBZ- protecting group. A singlet at δ 2.37 was correlated with the hydrogens attached to the *N*-methyl group.

The *O*-protected form of the key intermediate methyl (*S*)-3-(*N*-methylamino)-4-*tert*-butyldimethylsilyloxybutanoate (**154**) has now been synthesised in 6 steps from L-aspartic acid in 17 % overall yield.

It is hoped that the remainder of the synthesis of a single enantiomer of synthanecine A will proceed using the same synthetic procedures originally used by Mattocks[18] (**Scheme 13**).

To this end methyl (*S*)-3-(*N*-methylamino)-4-*tert*-butyldimethylsilyloxybutanoate (**154**) was treated with ethyl bromoacetate and hydrated potassium carbonate in aqueous acetone to give the tertiary amine (**155**) in 46 % yield after purification by column chromatography.

The ^1H NMR spectrum of this compound exhibited a doublet at δ 3.37 with a coupling constant of 5.1 Hz corresponding to the new methylene group. This is an example of four bond coupling to the methine hydrogen. The high coupling constant could be accounted for if this were an example of *W* coupling which is mediated by the overlap of the σ bonds. The ethyl ester gives a characteristic quartet at δ 4.12 and triplet at δ 1.22.

Ring closure of this tertiary amine via a Dieckmann ring closure to give the pyrrolidine (**156**) was attempted using sodium hydride in dry benzene. Only starting material was recovered from the reaction.

It is likely that the failure of this Dieckmann ring closure was a result of the extremely small scale that the reaction was carried out on. Only 5 mg of sodium hydride were required for this reaction and such a small amount of an air sensitive reagent is likely to be deactivated quickly by exposure to even very small quantities

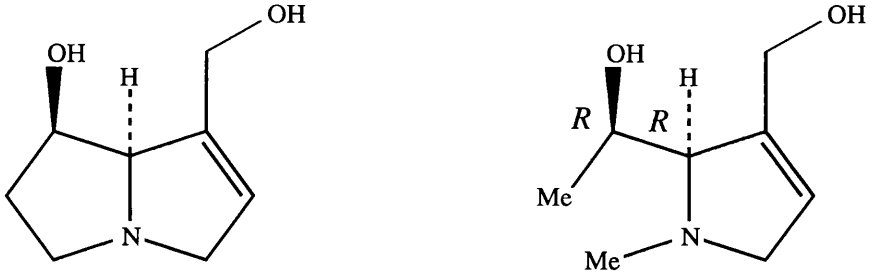
of moisture. Time constraints prevented this reaction from being repeated on a larger scale.

Chapter 5

Approaches to the Synthesis of a Novel Optically Active Synthanecine

5.1 Introduction

Synthanecine A (**14**) has been shown to be a good analogue of pyrrolizidine alkaloids in both structure and biological behaviour.[33] It does however differ from a pyrrolizidine base such as retronecine (**4**) in the number of carbon atoms in the molecule and in containing two primary alcohols as opposed to a primary and a secondary alcohol. Thus it was decided to attempt a synthesis of the novel synthanecine (2*R*,6*R*)-2-(1-hydroxyethyl)-3-hydroxymethyl-1-methyl-3-pyrroline (**167**). The structural similarity of this compound to the common necine base retronecine (**4**) is shown below (**figure 9**).



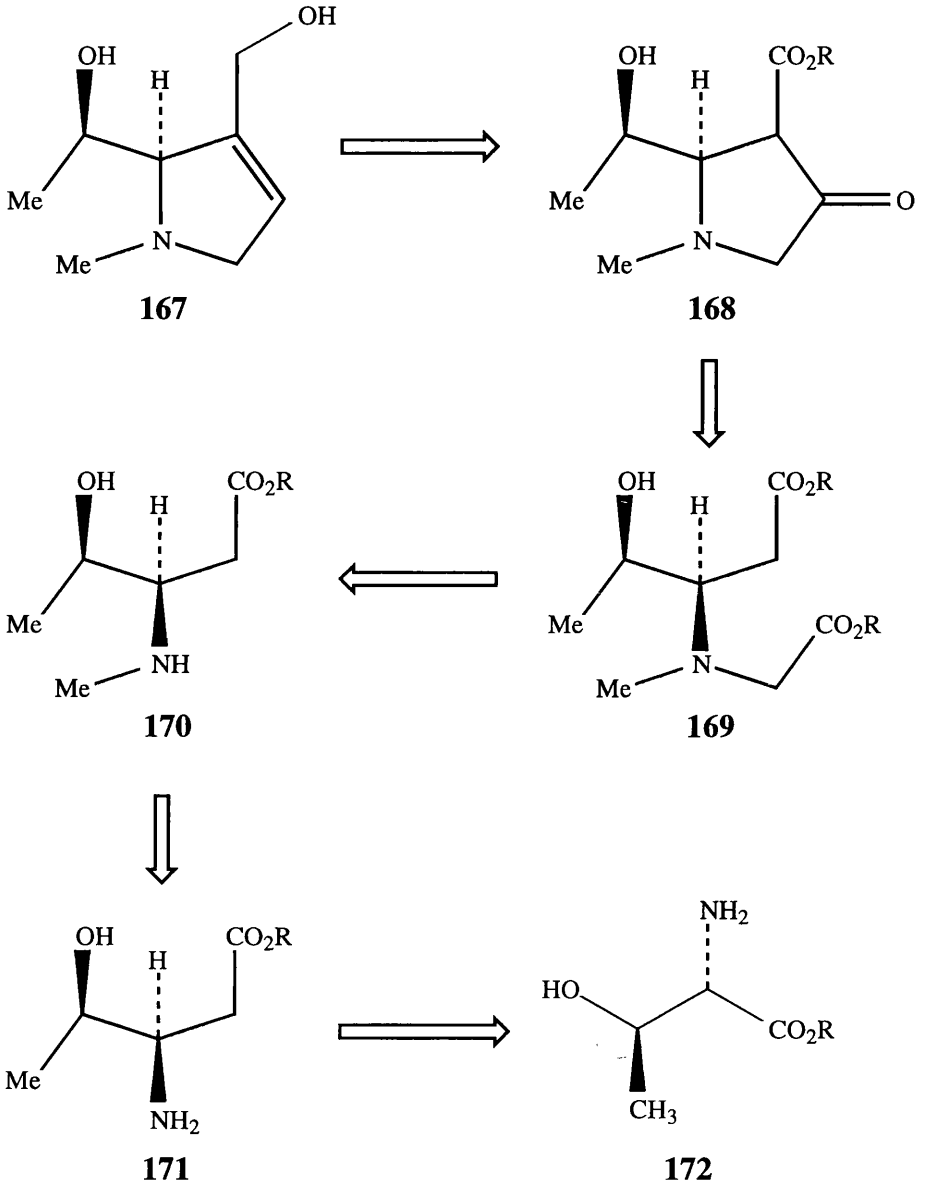
4 **167**

Figure 9 : Comparison of retronecine (4) and a novel synthanecine (167)

5.2 Retrosynthetic Analysis of (2*R*,6*R*)-2-(1-hydroxyethyl)-3-hydroxymethyl-1-methyl-3-pyrroline (167)

The retrosynthetic analysis of (2*R*,6*R*)-2-(1-hydroxyethyl)-3-hydroxymethyl-1-methyl-3-pyrroline (**167**) is given in **scheme 34** below. As was the case with the

synthesis of a single enantiomer of synthanecine A (**chapter 4**) as many of Mattocks' original procedures as possible were incorporated.



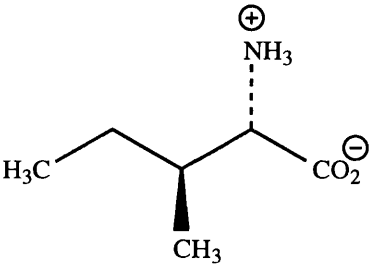
Scheme 34 : Retrosynthetic analysis of (2*R*,6*R*)-2-(1-hydroxyethyl)-3-hydroxymethyl-1-methyl-3-pyrroline (167)

(3*R*,4*R*)-3-Amino-4-hydroxypentanoic acid (**171**) (R=H) was identified as the key intermediate. This compound could be derived from L-threonine (**172**) (R=H).

5.3 Approaches to the Synthesis of (3*R*,4*R*)-3-amino-4-hydroxypentanoic acid (165)

5.3.1 Introduction

L-Threonine is an essential amino acid. Besides L-isoleucine (**173**), it is the only other member of the common amino acids to contain two asymmetric carbon atoms. This makes L-threonine and its derivatives extremely useful as chiral starting materials in syntheses (see **chapter 4.2.2**). The use of threonine in organic synthesis has been extensively reviewed.[133]

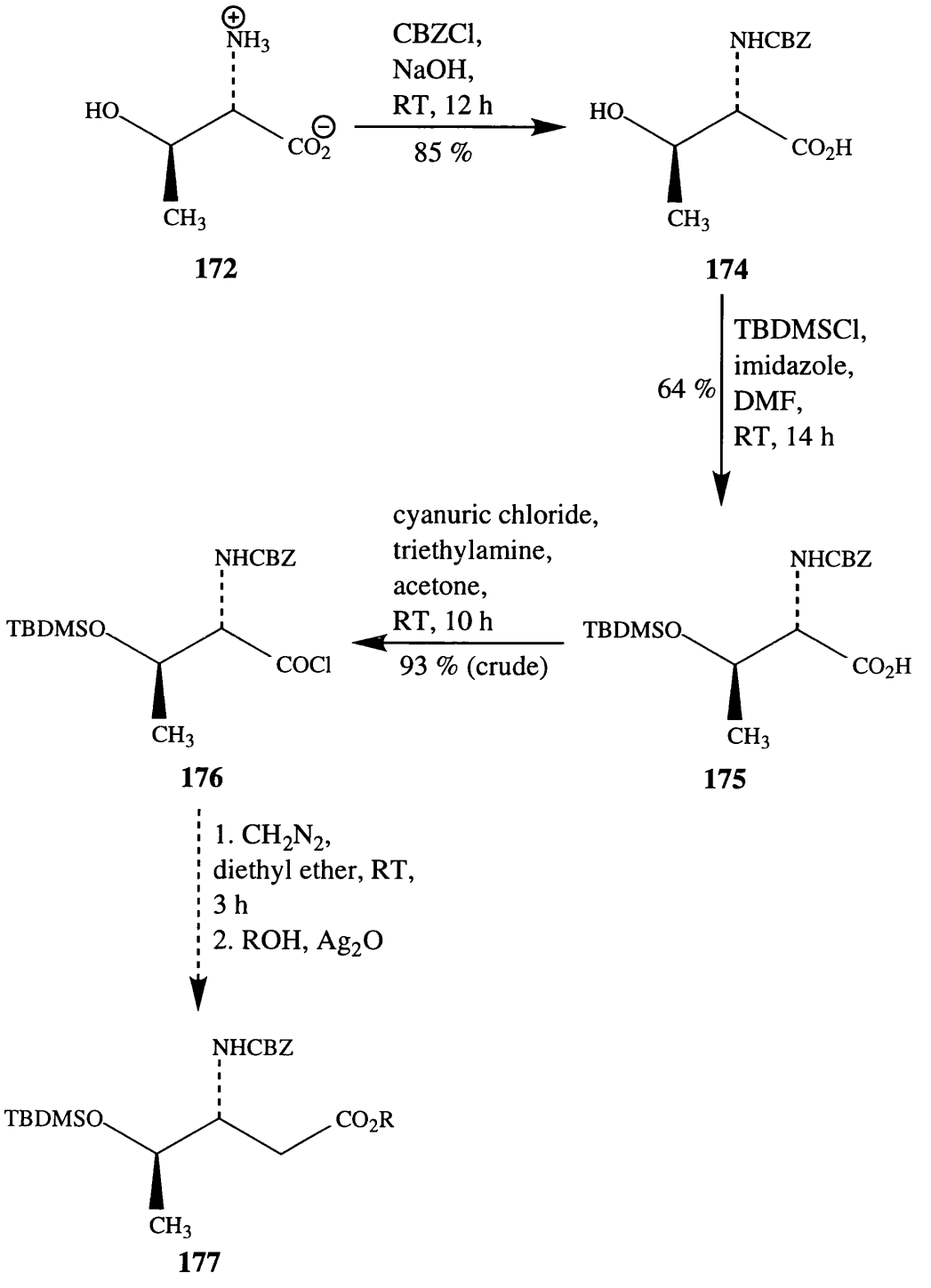


173

5.3.2 The Attempted Synthesis of (3*R*,4*R*)-3-amino-4-hydroxypentanoic acid (171) from L-threonine (172)

The attempt to synthesise (3*R*,4*R*)-3-amino-4-hydroxypentanoic acid from L-threonine is shown in **scheme 35**.

L-Threonine was first converted into *N*-CBZ-L-threonine in 85 % yield using a variation of the standard methodology.[172] Additional spectroscopic data are presented in **chapter 9**. The IR spectrum of the protected threonine (**174**) showed an absorption at 1717 cm^{-1} assigned to the carbamate carbonyl. Absorptions at 1612, 1565 and 1463 cm^{-1} corresponded to the aromatic ring. The ^1H NMR spectrum showed the expected broad singlet at δ 7.26 due to the aromatic hydrogens and a singlet at δ 4.99 corresponding to the methylene group next to the aromatic ring.



Scheme 35 : Attempted synthesis of *N*-protected (3*R*, 4*R*)-3-amino-4-hydroxypentanoic acid from L-threonine

In Kurokawa and Ohfune's synthesis of the antibiotic echinocandin D,[173] they gave a method for the preparation of *N*-(benzyloxycarbonyl)-*O*-(*tert*-butyldimethylsilyl)-L-threonine (**175**). Additional spectroscopic data are presented

in **chapter 9**. Treatment of the *N*-protected L-threonine (**174**) with TBDMS chloride and imidazole in DMF gave the diprotected L-threonine (**175**) in 64 % yield. The IR spectrum of this compound showed an absorption at 1251 cm^{-1} corresponding to stretching of the Si-O bond. The ^{1}H NMR spectrum gave singlets at δ -0.03, 0.00 and 0.78 corresponding to the methyl groups of the TBDMS protecting group. The two diastereotopic geminal methyl groups attached to the silicon come into resonance at slightly different frequencies. The ^{13}C NMR spectrum also shows the diastereotopic nature of the methyl groups as the carbons of the geminal methyl groups resonate at δ -6.6 and -6.1.

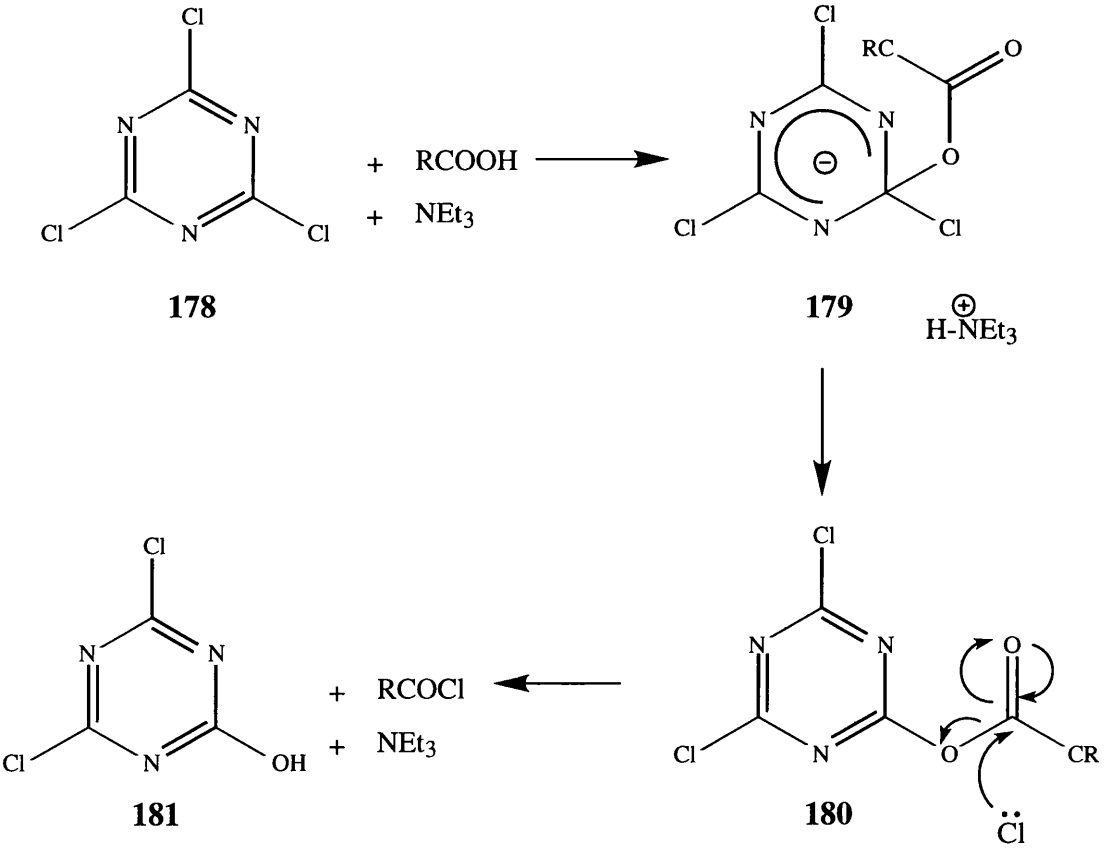
The initial attempt to form the acid chloride of the L-threonine derivative (**176**) using oxalyl chloride in THF proved unsuccessful. However, the method of Venkataraman and Wagle[174] using cyanuric chloride (**178**) gave the acid chloride (**176**) in 93 % crude yield. The proposed mechanism[174] for this reaction is presented below in **scheme 36**. Cyanuric chloride can supply a single chorine forming dichlorohydroxy-*s*-triazine (**181**) or twice to form chlorodihydroxy-*s*-triazine.

Both the ^{1}H and ^{13}C NMR spectra of this compound (**176**) showed a doubling of signals attributable to a 2:1 mixture of diastereoisomers. The mechanism illustrated (**scheme 36**) suggests that a catalytic amount of triethylamine would be sufficient to catalyse the reaction. The reaction was carried out using one molar equivalent of triethylamine as suggested by Venkataraman and Wagle.

Racemisation of amino acids can occur in basic[175] and acidic media[176] and at neutral pH.[177] Conversion of the amino group into an amide can cause a dramatic increase in the rate of racemisation.[178] Hydroxyl groups located on the carbon atom next to the methine carbon bearing the α-hydrogen are the most effective groups for increasing the rate of racemisation.[179] Ethers are still effective but the rate of racemisation is reduced by approximately 55%.[179] Although the free hydroxyl group causes a number of effects which lead to an increased rate of racemisation, including solvation of the base and hydrogen bonding to the carboxylate anion, in the ether the only possible effect is that of induction stabilising the α-anion through

the σ-system. The effect of ether formation on the racemisation of hydroxyamino acids has been studied by Smith *et al.*[179] Such an inductive effect could contribute to the racemisation of the threonine derivatives **175** and **176**. No silyl ethers were studied by Smith *et al.* Silicon is more electropositive than carbon and the O-Si bond is more polarised than the O-H bond, and the O-C bond. However the inductive effect of an atom so far removed from the methine carbon is unlikely to have any effect on the rate of racemisation.[179]

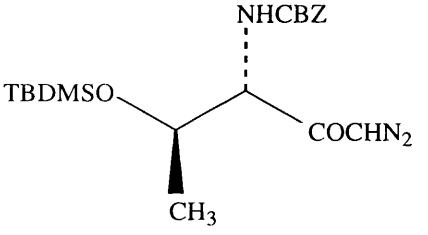
It cannot be said whether the racemisation takes place before or after the formation of the acid chloride. However the formation of a carboxylate anion adjacent to the α-hydrogen makes it less likely to be removed and so it would seem that racemisation is more likely to occur after formation of the acid chloride.



Scheme 36 : Proposed mechanism for acid chloride formation using cyanuric chloride

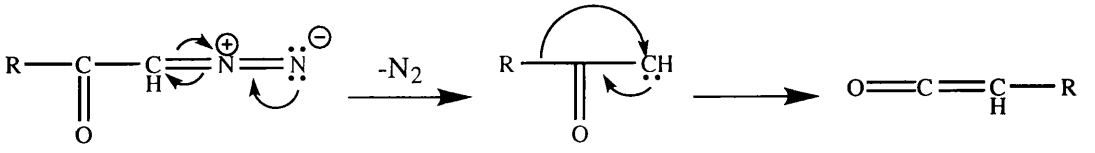
The ^{1}H NMR spectrum of the proposed acid chloride showed no signal attributable to the acid proton of the starting material (**175**) which had previously been observed at δ 10.74. The ^{13}C NMR spectrum showed the acid chloride carbonyl carbon at δ 164.3 and 167.8 as opposed to δ 174.2 for the carboxylic acid carbonyl carbon in the starting material. This upfield shift of the carbonyl signal in the acid chloride is always observed in acid chloride formation.

The mixture of diastereoisomers prepared in the previous step was then treated with diazomethane in diethyl ether to give the diazoketone (**182**). The ^{1}H NMR spectrum of this compound showed a singlet corresponding to one hydrogen at δ 5.09 which suggests that the diazoketone had formed. Unfortunately attempted purification of this compound by column chromatography on neutral alumina resulted in its decomposition. Time constraints prevented a further investigation of this reaction.



182

The next stage of this synthesis was to have been a Wolff rearrangement[180] involving treatment with an alcohol and silver (I) oxide as a catalyst, to give the carboxylic ester with the carbon chain increased by one. The mechanism of this reaction is thought to involve a ketene (**scheme 37**).[180]



183 **184** **185**

Scheme 37 : Formation of ketene in Wolff rearrangment

The ketene (**185**) then reacts with the alcohol to give the carboxylic ester. Replacing the alcohol with water or an amine will give the carboxylic acid or amide respectively. Because the R group migrates with its bonding electrons the optical purity is maintained. Overall the homologation of the carboxylic acid is known as the Arndt Eistert synthesis.

Chapter 6

Attempted Synthesis of Necic Acid Analogues

6.1 Introduction

Necic acids are generally highly substituted and oxygenated compounds. A wide variety of necic acids form the acid moiety of pyrrolizidine alkaloids. These compounds are often closely related, differing only in their stereochemistry. Examples of necic acid structures are given in **chapter 1**. The necic acid portion of a pyrrolizidine alkaloid has a considerable influence over its biological activity. **Table 13** compares the biological properties of several pyrrolizidine alkaloids differing only in the nature of their necic acid: the macrocyclic diester monocrotaline (**8**); and the "open" diesters heliotrine (**24**) and indicine (**25**).

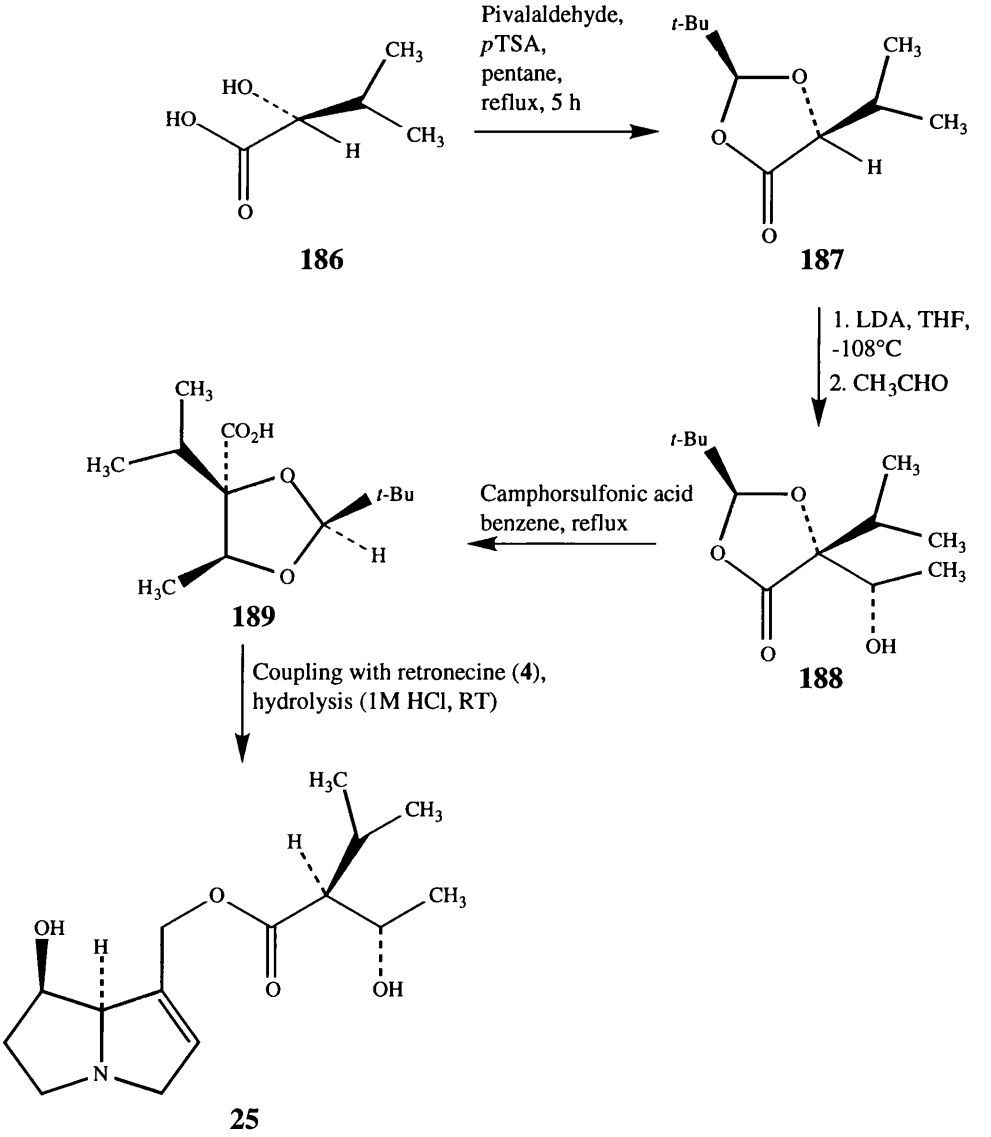
Alkaloid	**Approximate acute LD_{50} (mg/kg)**[36, 176]	**Enzymic activity for conversion into pyrroles and N-oxides (nmol/min/mg microsomal protein)**[26]	**Pyrrolic metabolite levels per dose of 100mg/kg**[a, 36, 181]
Monocrotaline (**8**)	109	2.62	0.64
Heliotrine (**24**)	280	1.70	0.27
Indicine (**25**)	>1000	0.325	0.09

a arbitrary units representing absorbance of Ehrlich colour from 0.5 g of liver tissue

Table 13 : Comparison of the biological activities of several alkaloids

The choice of necic acid also affects the lipophilicity of the pyrrolizidine alkaloid. A high lipophilicity makes the pyrrolizidine alkaloid more susceptible to activation by hepatic microsomal enzymes and thus potentially more toxic.

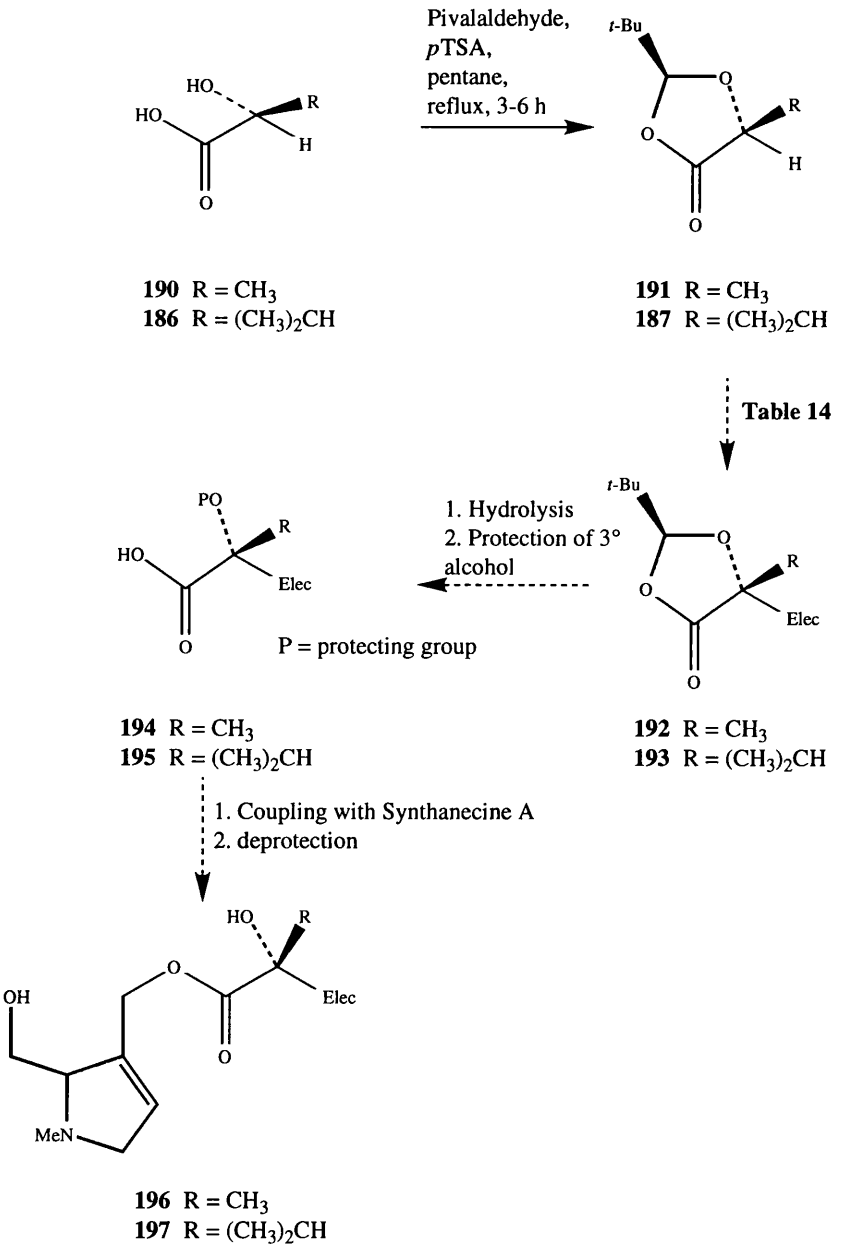
In **chapter 3** the synthesis of synthanecine A was briefly discussed. The purpose of this synthesis was to provide material which would be used to esterify a range of novel necic acids. Ogawa *et al.*[182] had used methodology first designed by Seebach *et al.*[183] to synthesise a (-)-trachelanthic acid derivative (**189**) (**scheme 38**) as part of their synthesis of indicine *N*-oxide. It was decided to use this approach to synthesise a range of necic acid analogues.



Scheme 38 : Synthesis of indicine (25)[182]

6.2 Attempted Synthesis of Necic Acids Via Alkylation of Dioxolanones

Scheme 39 shows the synthetic route which was planned to give novel necic acid analogues and thus novel pyrrolizidine alkaloid analogues after esterification with synthanecine A (**14**).



Scheme 39 : Attempted synthesis of novel pyrrolizidine alkaloid analogues

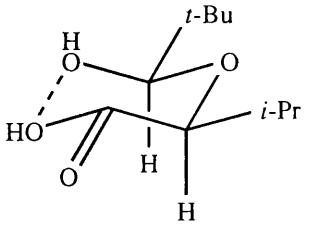
Two dioxolanones were prepared using L-lactic acid (**190**) and (*S*)-(+)-2-hydroxy-3-methylbutanoic acid (**186**) using the method of Seebach *et al.*[183]

(2*S*,5*S*)-2-(*t*-Butyl)-5-methyl-1,3-dioxolan-4-one (**191**) was synthesised in 54 % yield and 92 % de. It was found that any purification other than an aqueous wash reduced the diastereoisomeric excess and repeated attempts at recrystallisation could not improve the de, thus the compound was used without any further purification. The spectroscopic data for this compound are identical to those given by Seebach *et al.*[183]

(2*S*,5*S*)-2-(*t*-Butyl)-5-(isopropyl)-1,3-dioxolan-4-one (**187**) was synthesised in 85 % yield and 99 % de. As above the diastereoisomeric excess was reduced by any attempts at purification. Spectroscopic data in addition to that given by Seebach *et al.*[183] are given in **chapter 9**. A strong absorption at 1090 cm^{-1} in the IR spectrum corresponded to the stretching of the C-O bond. The ^1H NMR spectrum showed a doublet, *J* 7.0 Hz, at δ 1.12 due to the methyl groups of the isopropyl group. The hydrogen α to the carbonyl group was found as a double doublet at δ 4.10 (*J* 4.0 & 1.3 Hz). The smaller coupling constant is due to coupling through oxygen to the methine hydrogen next to the *tert*-butyl group.

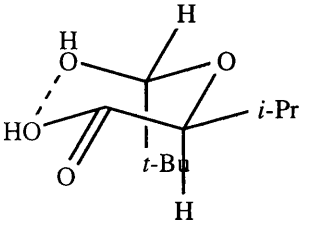
Diastereoisomeric excesses for these compounds were determined by ^1H NMR spectroscopy.

The diastereoselectivity found in the synthesis of the dioxolanones is due to the formation of the chair-like transition state shown in **198** leading to the *cis*-isomer. The transition state leading to the *trans*-isomer (**199**) has a strong 1,3-diaxial interaction between the *t*-butyl group and the hydrogen α to the carbonyl. The configurations were originally assigned using NOE measurements.[183] After irradiation at the frequency of the *tert*-butyl protons, the *trans*-isomer showed an enhancement of the signal due to the hydrogen α to the carbonyl. The *cis*-isomer showed no such enhancement.



198

cis-isomer



199

trans-isomer

Despite the literature precedent for the alkylation of such dioxolanones,[182, 183] only a complex mixture of products was formed and no products of alkylation could be isolated when the anions of the dioxolanones were treated with a range of simple electrophiles (**table 14**). As a result of this, this route had to be abandoned. The electrophiles should have approached the anion on the opposite face from the *tert*-butyl group because of steric hindrance, giving rise to the diastereoisomers shown in **scheme 39**.

Dioxolanone	Electrophile	Result
187	methyl iodide	Complex mixture of products
187	benzyl bromide	Complex mixture of products
191	methyl iodide	Complex mixture of products
191	benzyl bromide	Complex mixture of products

Table 14 : Attempted alkylation of the dioxolanones 187 & 191

The remaining stages of this synthesis would have involved hydrolysis of the dioxolanone to liberate the hydroxy acid, protection of the hydroxyl group of the acid and subsequent coupling with synthanecine A and deprotection to give the novel pyrrolizidine alkaloid analogue (**196** or **197**).

Chapter 7

Synthesis of Novel Pyrrolizidine Alkaloids Analogues via a 1,3-Dipolar Cycloaddition

7.1 Introduction

In order to prepare more compounds for anti-tumour testing that have structures related to pyrrolizidine alkaloids, the work of Hosomi *et al.*[184] was utilised to prepare a range of novel necine base analogues based upon pyrroline and pyrrolidine structures via 1,3-dipolar cycloadditions. The comparison between the pyrroline (**200**) and (+)-retronecine (**4**), and the general pyrrolidine structure (**202**) and (-)-rosmarinecine (**201**) are shown below (**figure 10**).

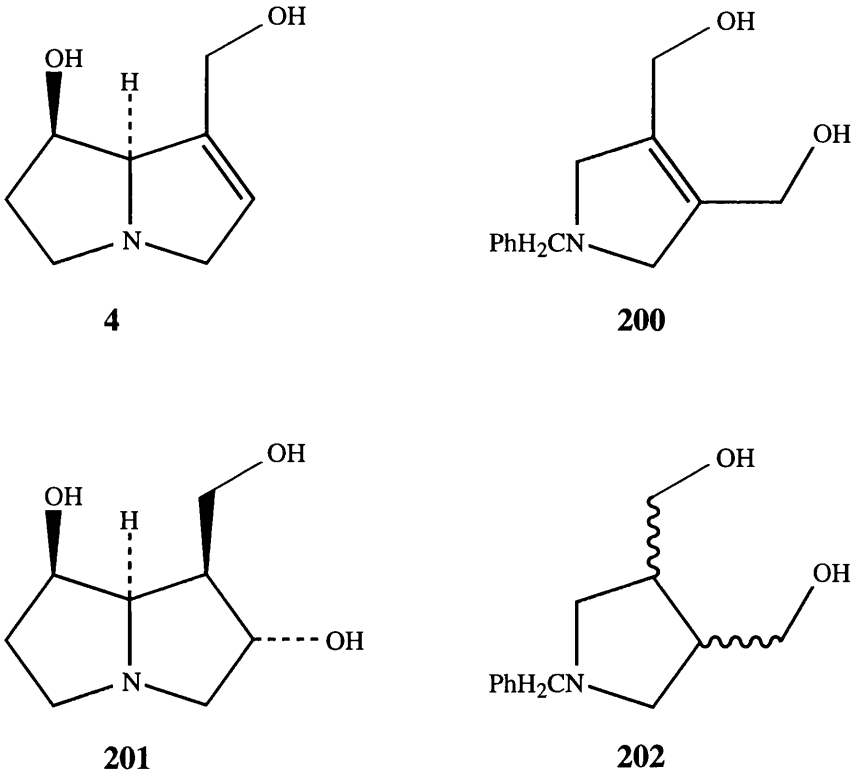


Figure 10 : Comparison of pyrroline (200) and pyrrolidine (202) with (+)-retronecine (4) and (-)-rosmarinecine (201)

7.2 The 1,3-Dipolar Cycloaddition

7.2.1 Introduction

The 1,3-dipolar cycloaddition reaction proceeds through a 6π electron "aromatic" transition state. The name of the reaction is derived from the 4π electron component of the reaction (**203**) which contains a 1,3-dipole. This component also contains at least one hetero-atom. The 2π electron component is known as the dipolarophile (**204**). A generic 1,3-dipolar cycloaddition reaction is shown in **scheme 40**. Such cycloadditions lead to five-membered heterocycles. It is generally agreed that 1,3-dipolar cycloadditions are concerted.[185]

203 **204** **205**

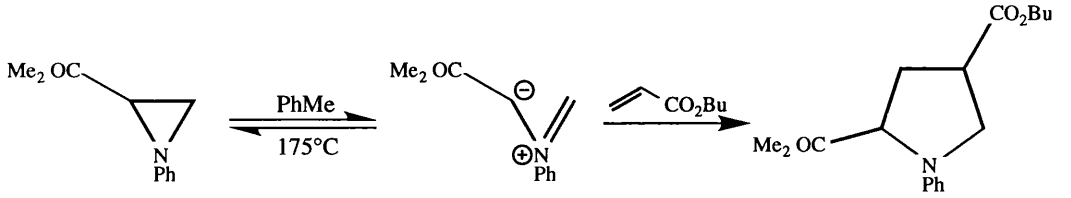
Scheme 40 : Generic 1,3-dipolar cycloaddition reaction

1,3-Dipoles have been classified into two groups: propargyl allenyl types, e.g the nitrile ylide (**206**), and allyl types, e.g. the azomethine ylide (**207**).[185, 186]

206 **207**

The work which is discussed below is concerned with syntheses involving azomethine ylides. Reactions with azomethine ylides take place most easily with dipolarophiles bearing electron withdrawing substituents and occur via a HOMO

(dipole) - LUMO (dipolarophile) interaction.[187] With dipolarophiles containing double bonds, the stereochemistry of the double bond is maintained in the product of cycloaddition. With mono-substituted alkenes the reactions are regioselective, giving preferentially 2, 4-disubstituted pyrrolidines for example (**scheme 41**).

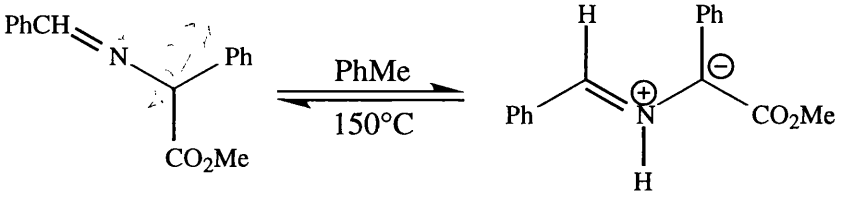


208 **209** **210**

Scheme 41 : Formation of a 2,4-disubstituted pyrrolidine via a 1,3-dipolar cycloaddition

The example given in **scheme 41** is an example of the thermolysis of an aziridine (**208**): a common method of azomethine ylide formation. The azomethine ylide (**209**) shown above is a stabilised ylide. This stabilisation is due to the electron withdrawing group next to the carbon bearing the negative charge.

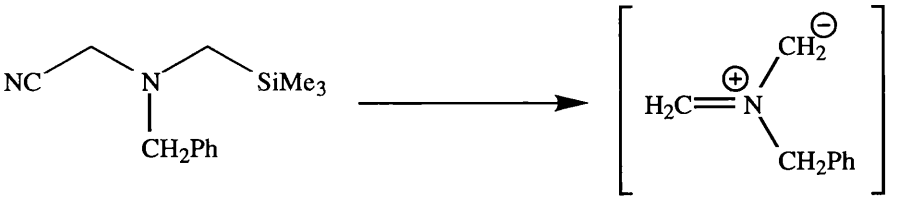
Azomethine ylides can also be generated from imines of α-amino acid esters (**scheme 42).** This is an example of a 1,2-prototropic shift.



211 **212**

Scheme 42 : Generation of an azomethine ylide from an imine

Desilylation of trimethylsilylmethylamines has provided a route to non-stabilised azomethine ylides (**scheme 43**). It is this method of forming an azomethine ylid which is utilised in the reactions discussed below.

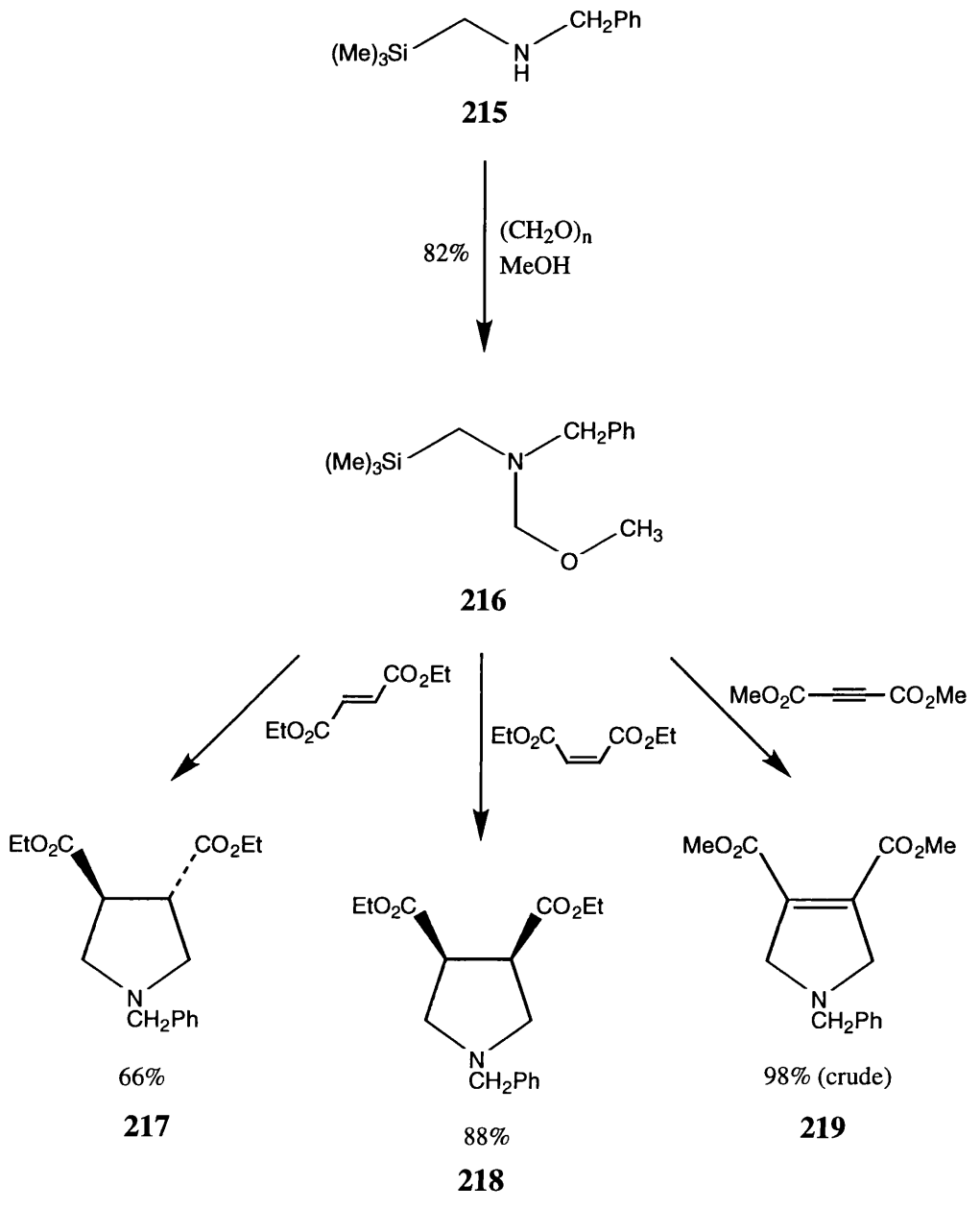


213 **214**

Scheme 43 : Formation of a non-stabilised azo-methine ylide

7.2.2 Synthesis of Pyrrolidines and Pyrrolines Using a 1,3-Dipolar Cycloaddition

A variation of the method of Hosomi *et al.*[184] was used to prepare the precurser to the azomethine ylide. Treatment of *N*-(trimethylsilylmethyl)benzylamine (**215**) with paraformaldehyde in methanol gave *N*-benzyl-*N*-(trimethylsilylmethyl)aminomethyl methyl ether (**216**). The use of a suspension of paraformaldehyde in methanol as opposed to the formalin solution used by Hosomi *et al.* resulted in an improved yield of 82 % compared to 51 % in the original work. Additional spectroscopic data are presented in **chapter 9**. No purification was necessary. The IR spectrum of this compound showed a strong absorption at 2841 cm^{-1} corresponding to stretching of the O-CH_3 bond. The ^1H NMR spectrum of this compound showed singlets at δ 3.10 and 3.95 attributed to the methoxy hydrogens and the hydrogens on the methylene group attached to the oxygen, respectively.

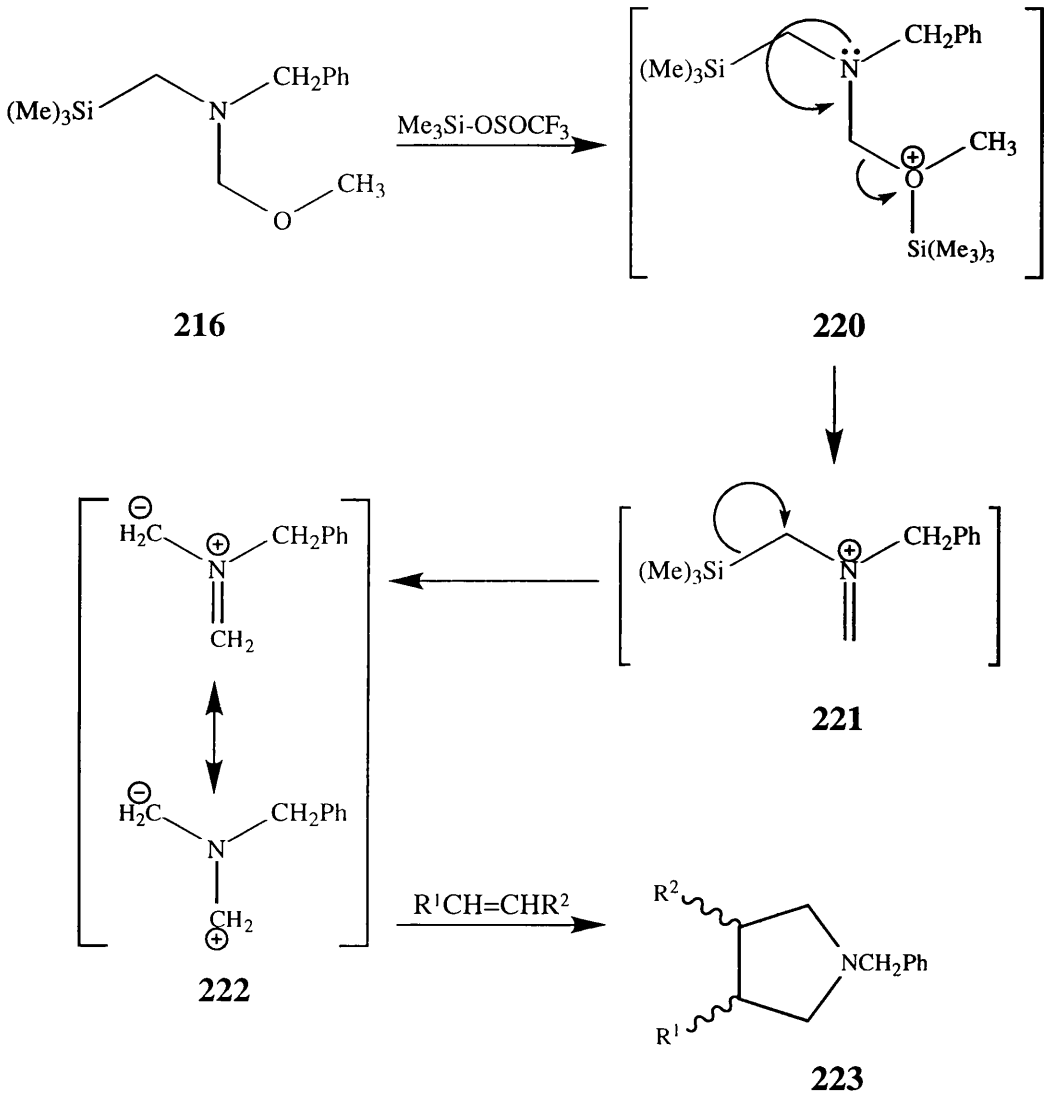


Conditions: CsF, THF, 60°C, 18h

Scheme 44 : Preparation of pyrrolidines and pyrrolines via a 1,3-dipolar cycloaddition

The azomethine ylid (**222**) was generated by treating the trimethylsilylmethylamine (**216**) with trimethylsilyl triflate and caesium fluoride. The proposed mechanism for this reaction is shown in **scheme 45**.[188] The trimethylsilylmethylamine undergoes 1,3-elimination of methoxytrimethylsilane catalysed by the trimethylsilyl triflate. Addition of a small amount of caesium

fluoride improves the yield of the cycloaddition reaction presumably aiding desilylation of **221**. The reaction proceeds in poorer yield if the addition of caesium fluoride is omitted.[184]



216 **220** **221** **222** **223**

Scheme 45 : Mechanism of generation of azomethine ylide (222) from *N*-benzyl ether (216)[188]

Three compounds were prepared by this cycloaddition reaction. The pyrrolidines (**217 and 218**) were first prepared by Hosomi *et al.*[184] although no analytical data were given. Complete analytical data for these pyrrolidines and for the pyrroline (**219**) are presented in **chapter 9**.

The azomethine ylide (**222**) was generated *in situ* and treated with diethyl fumarate to give diethyl (±)-1-benzylpyrrolidine-3,4-dicarboxylate (**217**) in 66 % yield after purification by column chromatography. An absorption at 1732 cm^{-1} in the IR spectrum corresponded to the stretching of the ester carbonyl group. The ^1H NMR spectrum of this compound showed a characteristic triplet and quartet at δ 1.24 and 4.15 respectively with a coupling constant of 7.1 Hz corresponding to the two ethyl esters. The signals corresponding to the hydrogens on the pyrrolidine ring are complex. The signal due to the hydrogens α to the ester groups appear as a multiplet at δ 3.39-3.50. The hydrogens of the methylene groups adjacent to the nitrogen are seen at δ 2.77-2.94.

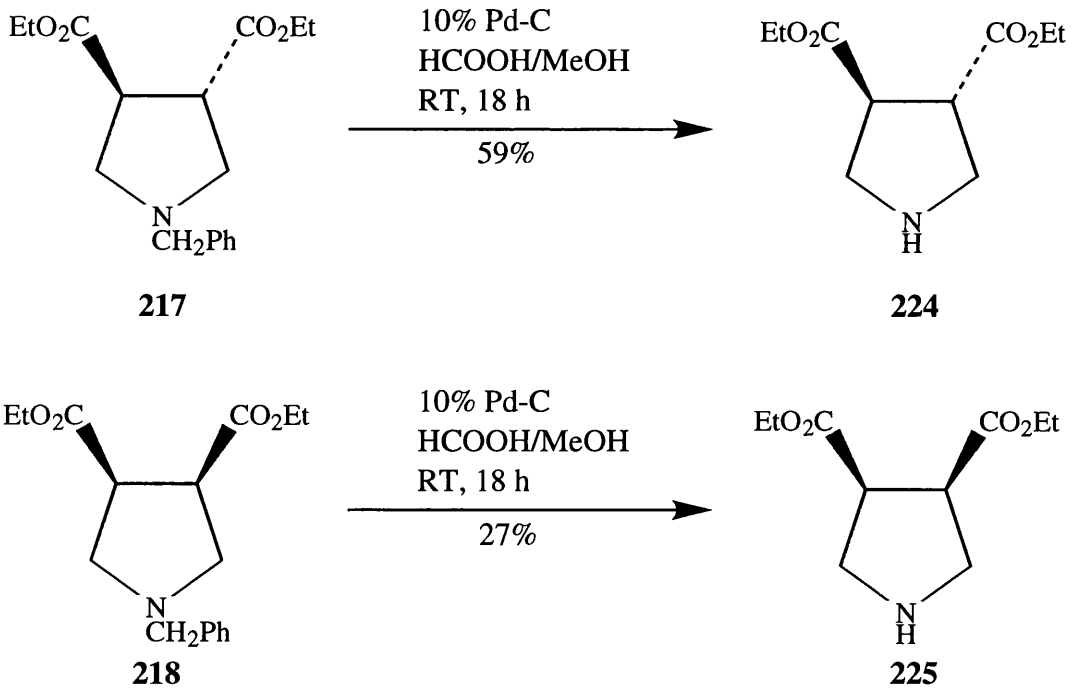
Treatment of the azomethine ylide (**222**) with diethyl maleate gave diethyl *meso*-1-benzylpyrrolidine-3,4-dicarboxylate (**218**) in 88 % yield after purification by column chromatography. An absorption at 1732 cm^{-1} in the IR spectrum was attributed to the stretching of the ester carbonyl group. No peaks due to the trimethylsilyl group were seen. The ^1H NMR spectrum of this compound also showed a characteristic triplet and doublet pattern at δ 1.16 and 4.04. Once again the signals corresponding to the hydrogens of the pyrrolidine ring are complex. The signal due to the hydrogens α to the ester groups is seen as a multiplet at δ 3.18-3.26. The methylene groups next to the nitrogen are found as a pair of multiplets at δ 2.61-2.69 and δ 3.03-3.07 each integrating to two hydrogens.

The azomethine ylid (**222**) reacted with diethyl acetylenedicarboxylate to give dimethyl 1-benzyl-3-pyrroline-3,4-dicarboxylate (**219**) in 98 % crude yield. Attempts at the purification of this compound by column chromatography on both silica and neutral alumina both resulted in extremely low yields of impure product. 3-Pyrrolines such as **219** are easily converted into pyrroles,[189] and this is perhaps the reason for the difficulty in successfully purifying this material. Because of this lack of stability and purity this compound was not used in any of the transformations discussed below. The spectroscopic data were consistent with product formation. A strong absorption at 1738 cm^{-1} was seen in the IR spectrum corresponding to

stretching of the ester carbonyl. An absorption at 1622 cm^{-1} corresponded to the αα',ββ' unsaturated alkene. The ^1H NMR spectrum of this compound showed a singlet at δ 3.77 due to the methoxy groups and a singlet at δ 3.82 correlating to the methylene groups next to the nitrogen. The ^{13}C NMR spectrum showed the alkenic carbons at δ 137.1.

7.3 Synthesis of Compounds Prepared from diethyl (±)-1-benzylpyrrolidine-3,4-dicarboxylate (217) and diethyl *meso*-1-benzylpyrrolidine-3,4-dicarboxylate (218)

Simple compounds were prepared from the pyrrolidines **217** and **218**.



217 **224**

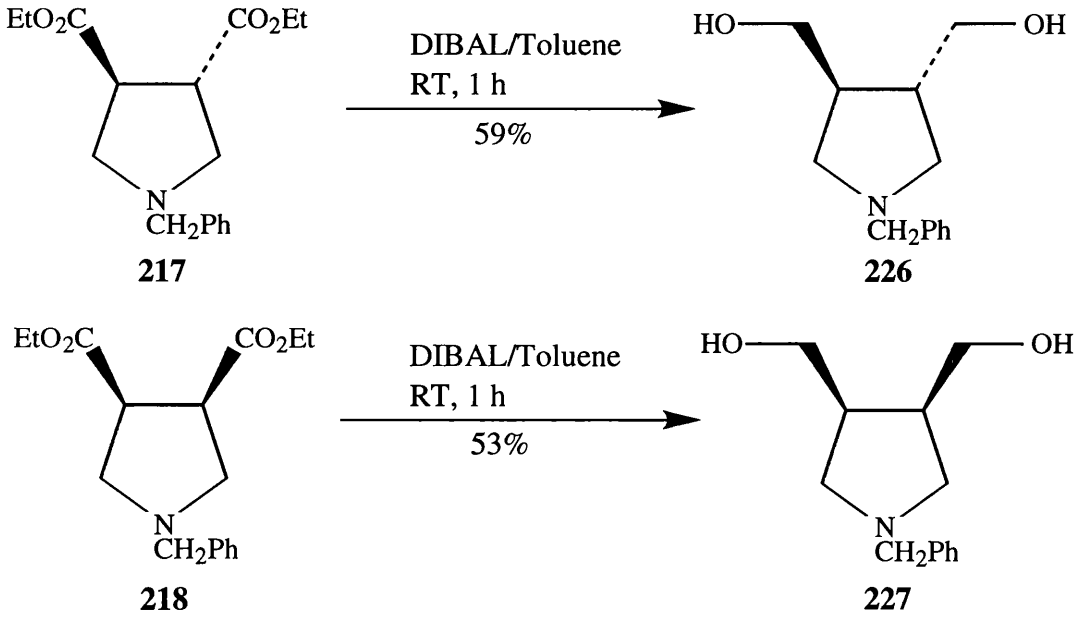
218 **225**

Scheme 46 : Hydrogenolysis of pyrrolidines

Treatment of the pyrrolidines **217** and **218** with 10 % Pd/C in a 5 % solution of formic acid in methanol using the methodology of El Amin *et al.*[190] resulted in hydrogenolysis to give the free amines in moderate yield (**scheme 46**).

Diethyl (±)-1-benzylpyrrolidine-3,4-dicarboxylate (**217**) was hydrogenolysed to give diethyl (±)-pyrrolidine-3,4-dicarboxylate (**224**) in 59 % yield after purification by column chromatography. A broad absorption at 3318 cm^{-1} in the IR spectrum was correlated with the stretching of the N-H bond. The ^1H NMR spectrum of this compound showed a deuterium exchangeable signal at δ 2.51 due to the N-H hydrogen. Signals attributable to the benzyl group were absent in the ^1H NMR spectrum.

Hydrogenolysis of diethyl *meso*-1-benzylpyrrolidine-3,4-dicarboxylate (**218**) gave diethyl *meso*-pyrrolidine-3,4-dicarboxylate (**225**) in 29 % yield after column chromatography. The ^1H NMR spectrum of this compound showed no signals attributable to the benzyl group. A deuterium exchangeable signal at δ 3.68 corresponded to the N-H hydrogen.



217 **226**

218 **227**

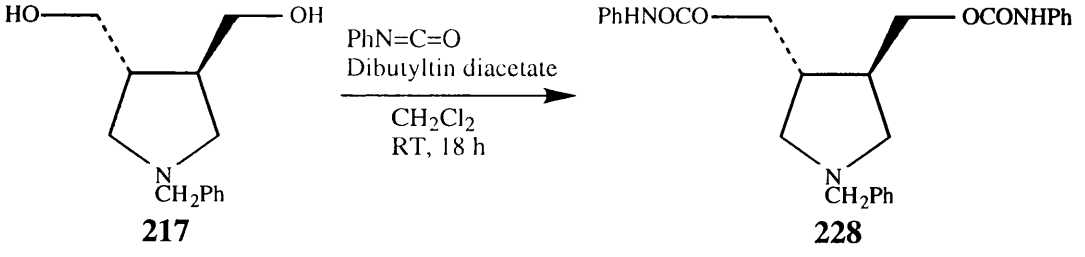
Scheme 47 : DIBAL reduction of pyrrolidines

Treatment of the pyrrolidines **217** and **218** with DIBAL in toluene gave the bishydroxymethyl compounds in moderate yield (**scheme 47**).

Diethyl (±)-1-benzylpyrrolidine-3,4-dicarboxylate (**217**) gave (±)-3,4-bishydroxymethyl-1-benzylpyrrolidine (**226**) in 53 % yield after purification by

column chromatography. The strong absorption at 3366 cm^{-1} in the IR spectrum was correlated with the stretching of the O-H bond. No absorptions attributable to carbonyl stretching were present. The ^1H NMR spectrum of this compound exhibited a broad multiplet integrating to two hydrogens at δ 2.16 corresponding to the methine hydrogens in the pyrrolidine ring. The methylene hydrogens of the hydroxymethyl groups were found as a multiplet at δ 3.47-3.58.

Diethyl *meso*-1-benzylpyrrolidine-3,4-dicarboxylate (**218**) gave *meso*-3,4-bishydroxymethyl-1-benzylpyrrolidine (**227**) in 59 % yield after purification by column chromatography. The IR spectrum of this compound showed a strong absorption at 3342 cm^{-1} due to the stretching of the O-H bond and no absorption due to a carbonyl stretch. A multiplet at δ 2.48 in the ^1H NMR spectrum corresponded to the methine hydrogens of the pyrrolidine ring. The methylene hydrogens of the hydroxymethyl groups were found as a complex multiplet at δ 3.55-3.70.



217 **228**

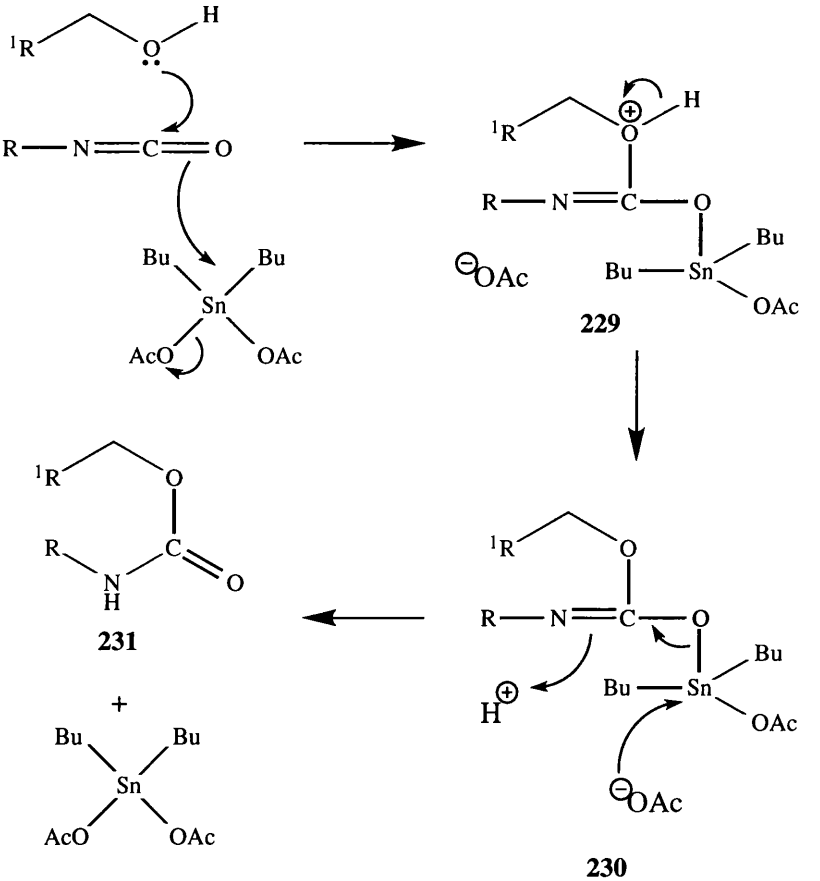
Scheme 48 : Synthesis of (±)-bis(phenylaminocarbonyloxymethyl)-1-benzyl pyrrolidine (228)

Mattocks had previously studied the metabolism and toxicity of carbamates of both synthanecine A (**14**) and synthanecine B (**15**) and found them to be good models for pyrrolizidine alkaloid toxicity, with the unsaturated synthanecine A carbamates proving extremely toxic.[34,181] Based upon this earlier work, it was decided to synthesise the bisphenylcarbamates of the pyrrolidines **220** and **227**. These derivatives would be saturated and thus should be unable to form pyrrolic derivatives. It was hoped that these compounds would show a degree of anti-tumour

activity without exhibiting liver toxicity due to pyrrole formation and should be more stable than pyrrole derivatives previously tested.[77]

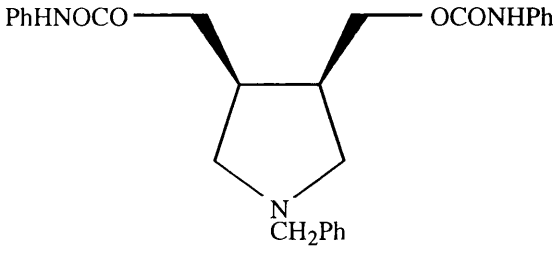
Treatment of diethyl (±)-1-benzylpyrrolidine-3,4-dicarboxylate (**226**) with phenyl isocyanate using dibutyltin diacetate as a catalyst gave the (±)-biscarbamate (**228**) in 11 % yield after purification by column chromatography. A strong absorption at 1702 cm^{-1} in the IR spectrum corresponded to the stretching of the carbonyl group in the carbamate. The ^1H NMR spectrum showed a complex multiplet at δ 6.86-7.34 integrating to 15 hydrogens and corresponding to the three phenyl rings now present in the molecule. The methylene hydrogens of the hydroxymethyl groups showed an acylation shift of ~0.5 ppm downfield to δ 3.95-4.14 from δ 3.47-3.58 in the starting material.

The dibutyltin diacetate in this reaction acts as a catalyst by activating the carbonyl group of the isocyanate (**scheme 49**).[191]



Scheme 49 : Mechanism of catalysis by dibutyltin diacetate

The attempted synthesis of the *meso*-biscarbamate (**232**) failed to give any isolable or characterisable material probably due to the steric hindrance of having two bulky phenyl carbamates *cis* to each other. This steric hindrance may also account for the low yield obtained in the synthesis of the (±)-biscarbamate.



232

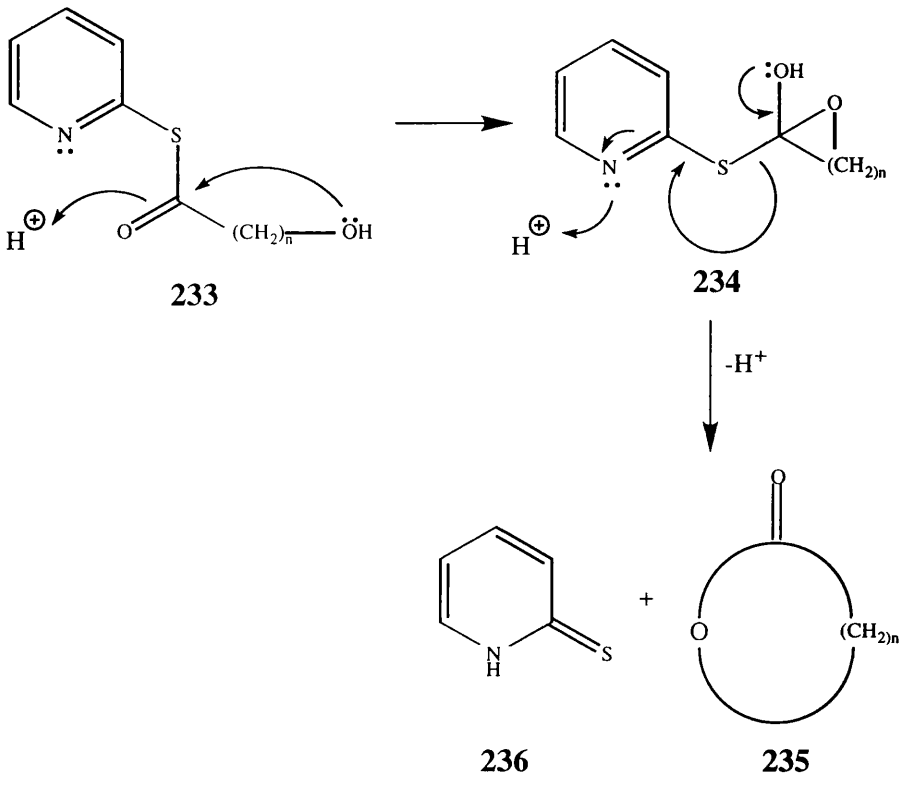
7.4 Attempted Synthesis of Macrocycles of (±)-3,4-bishydroxymethyl-1-benzylpyrrolidine (226)

Pyrrolizidine alkaloids often exist as macrocyclic diesters, thus it was decided to attempt to synthesise macrocyclic diesters of the pyrrolidine **226** to form a novel pyrrolizidine alkaloid analogue for anti-tumour testing.

7.4.1 Introduction

Synthesis of macrocyclic diesters of pyrrolizidine alkaloids is one of the most challenging goals of organic synthesis and a great number of reagents have been used to affect macrocycle formation. These processes rely upon carboxyl and/or hydroxyl activation techniques to overcome the unfavourable entropic factors and polymerisation processes which may occur. Some of the most frequently utilised procedures for the synthesis of macrocyclic diesters of pyrrolizidine alkaloids are presented below.

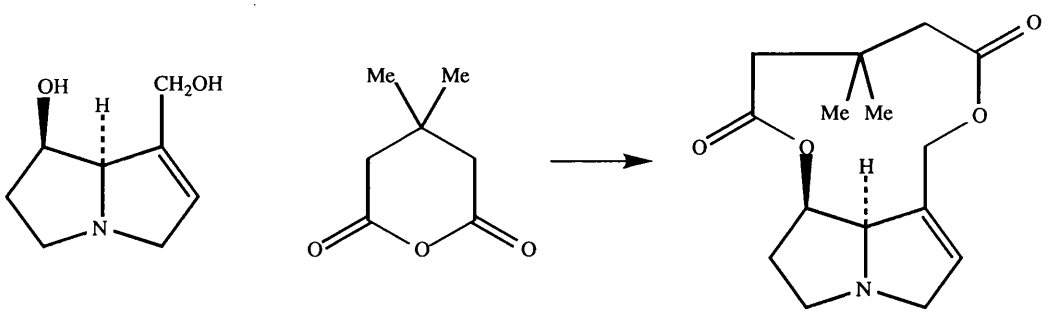
The first synthesis of a macrocyclic diester pyrrolizidine alkaloid was by Robins and Sakdarat[192] using the Corey-Nicolaou method.[129] The Corey-Nicolaou method employs a 2-pyridylthiol ester generated *in situ* from aldrithiol-2 and relies upon a double activation sequence as shown in **scheme 50**.



233 **234** **236** **235**

Scheme 50 : Corey-Nicolaou macrocyclisation process

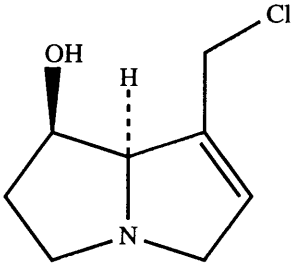
Treatment of (+)-retronecine (**4**) with 3,3-dimethylglutaric anhydride (**237**) resulted in formation of the two possible monoesters. Once the monoesters had formed, addition of aldrithiol-2 and heating at reflux in dimethyl formamide gave 13,13-dimethyl-1,2-didehydrocrotalanine in 50 % yield (**238**) (**scheme 51**).[192]



4 **237** **238**

Scheme 51 : Synthesis of 13,13-dimethyl-1,2-didehydrocrotalanine (238)[192]

Robins and Burton extended the scope of this methodology by using the allylic chloride derivative of (+)-retronecine (**239**), significantly improving the yields in some macrolactonisations.[20]

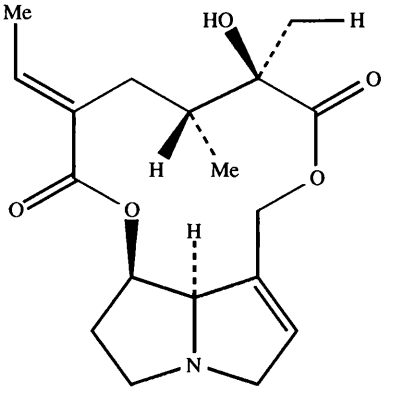


239

This methodology involving the allylic chloride was also used to synthesise a range of macrocyclic diesters of synthanecine A.[21, 22]

An alternative cyclisation method was used by White *et al.*[193] in their synthesis of (-)-integerrimine (**240**) and (+)-usaramine (**241**). Treatment of the lithium alkoxide, prepared by treatment of the necine base (**242**) with BuLi, with the protected necic acid (**243**), DMAP and the carboxylate activating reagent diethyl phosphoryl chloride gave the protected monoester (**244**). Macrolactonisation was then affected by deprotection of the primary alcohol to give **245** and subsequent formation of the mesylate, which resulted in macrocycle formation (**246**). A series

of deprotections then afforded (+)-usaramine (**241**) (**scheme 52**). A similar sequence of reactions afforded (-)-integerrimine (**240**).



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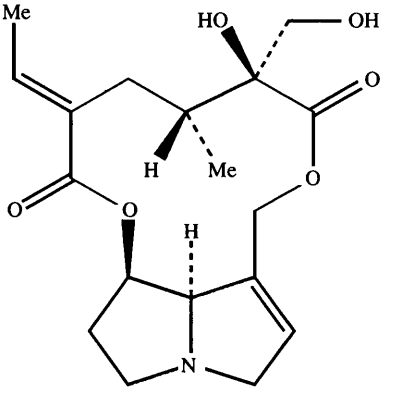

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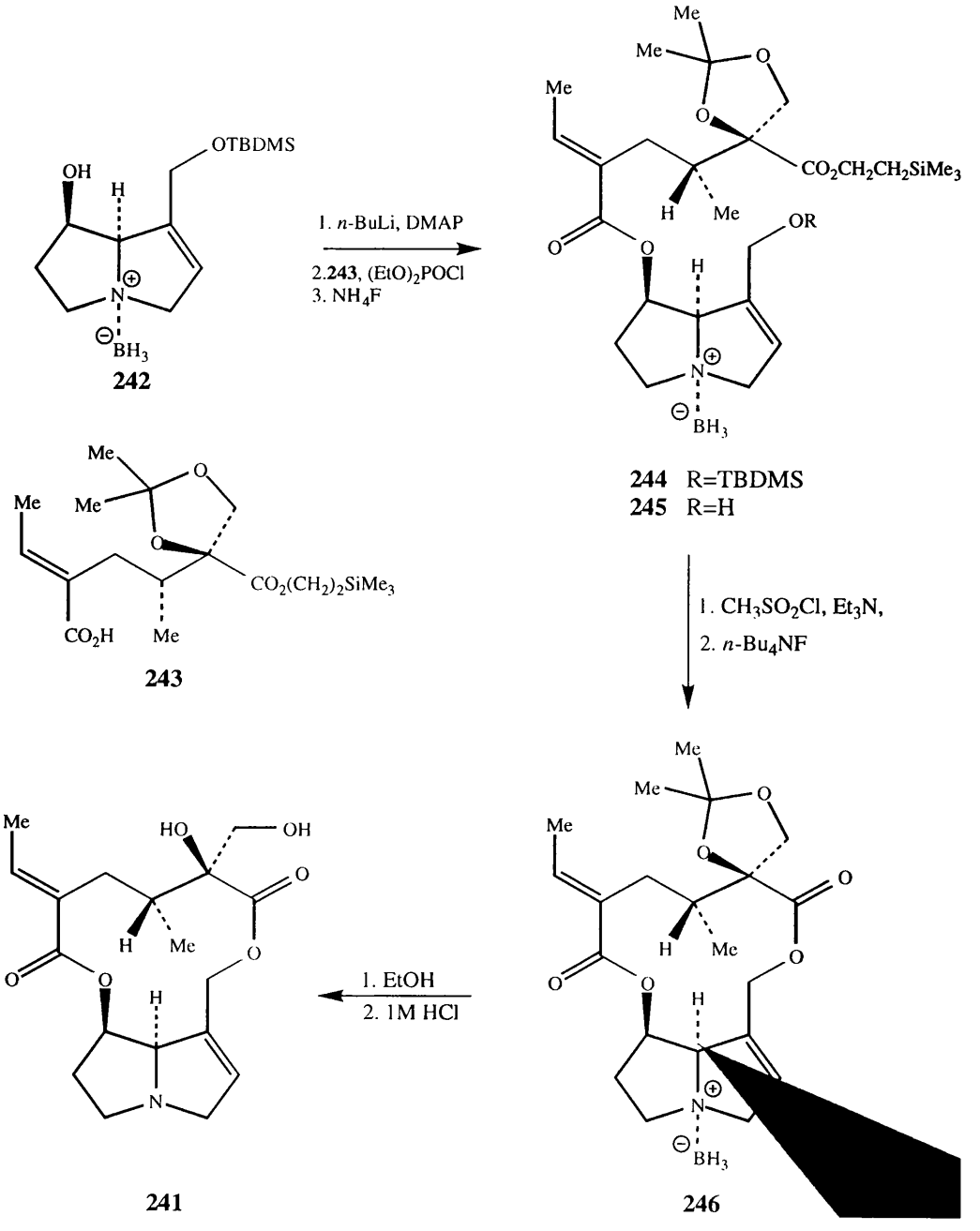
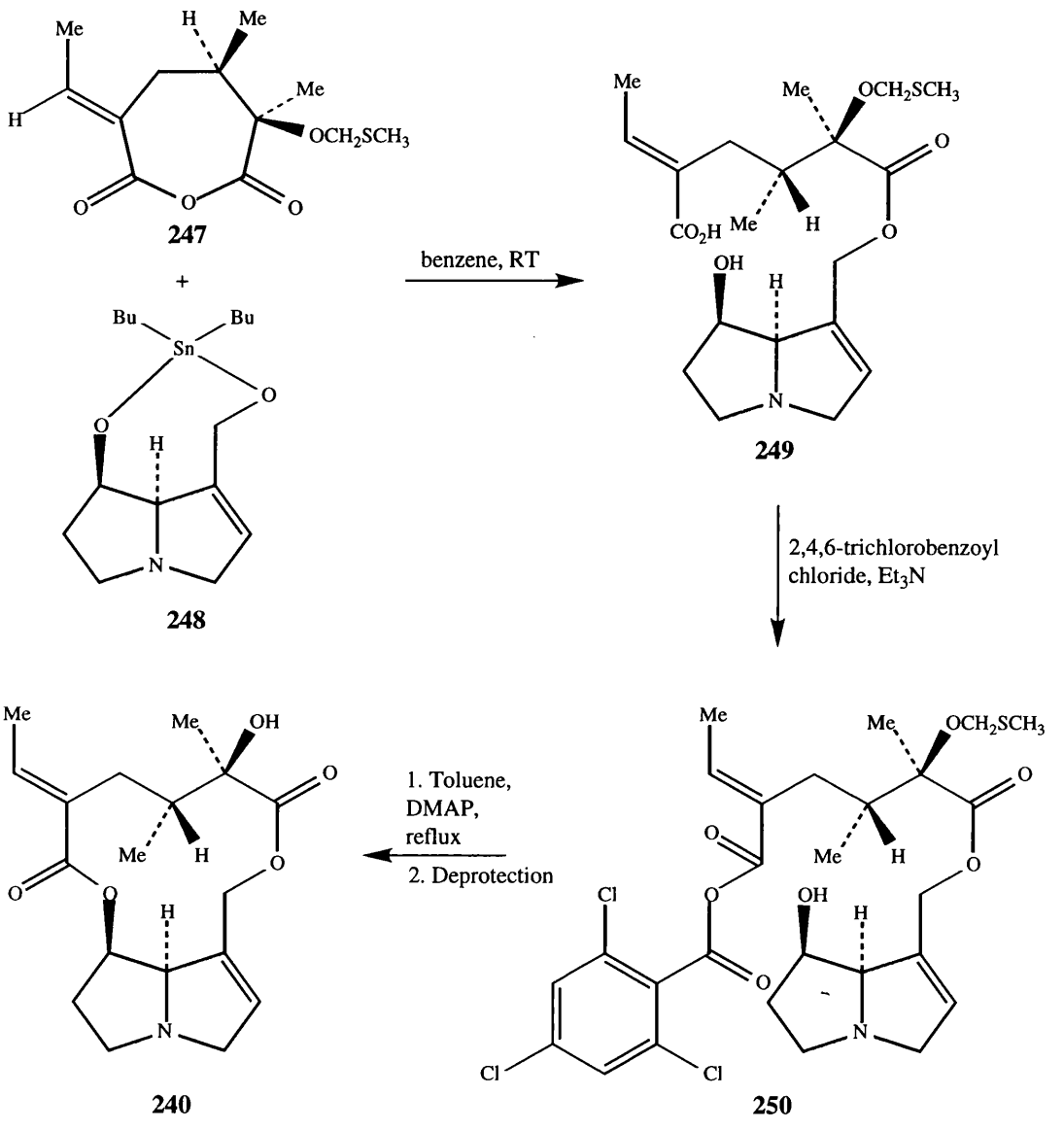


Scheme 52 : Synthesis of (+)-usaramine[193]

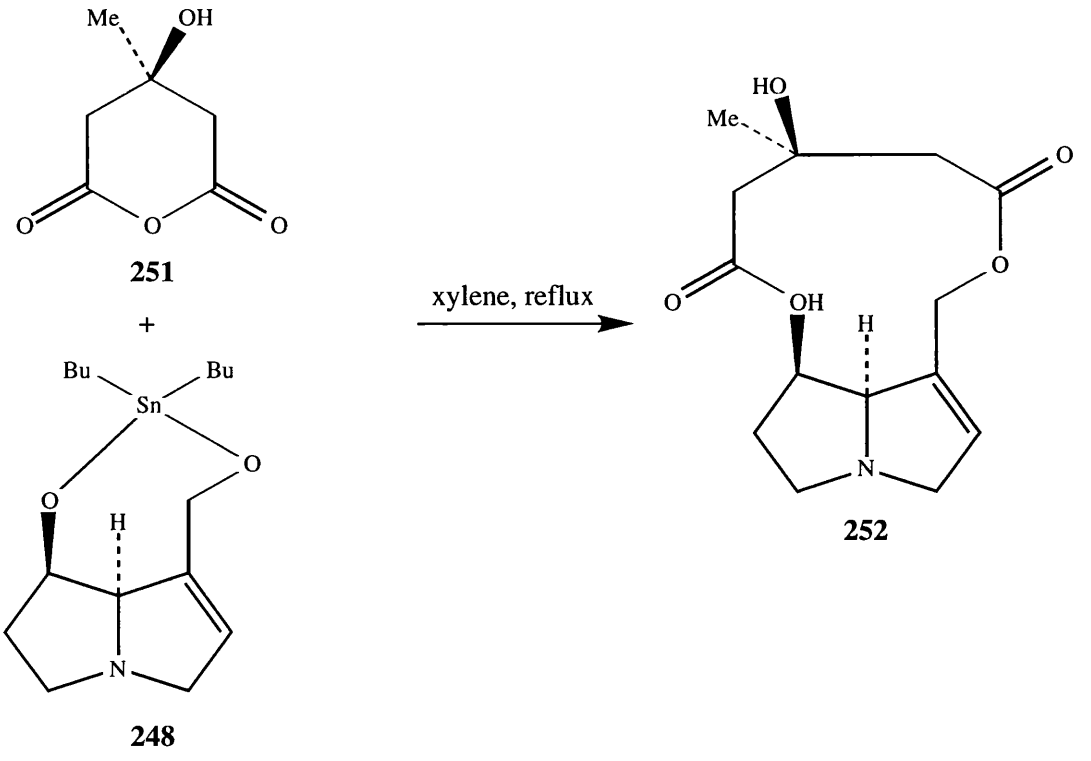
The reactions of stannoxanes derived from diols and dibutyltin oxide have been used in the synthesis of macrocyclic pyrrolizidine alkaloid diesters. Niwa *et al.*[194] used the cyclic stannoxane **248** (this is the proposed structure only: it hasn't been isolated) to form the monoester **249** in their synthesis of integerrimine (**240**). The final ester linkage was formed using the procedure reported by Yamaguchi *et al.*[195] This method involves formation of the mixed anhydride (**250**) using 2,4,6-

trichlorobenzoyl chloride and triethylamine followed by heating at reflux with DMAP and toluene. Subsequent deprotection afforded integerrimine (**240**) (**scheme 53**).



Scheme 53 : Synthesis of integerrimine (240) via a cyclic stannoxane[194]

This method was also utilised in the synthesis of (+)-dicrotaline (**252**) where reaction of the cyclic stannoxane (**248**) with the anhydride (**257**) afforded the pyrrolizidine alkaloid in a single step (**scheme 54**).[196]

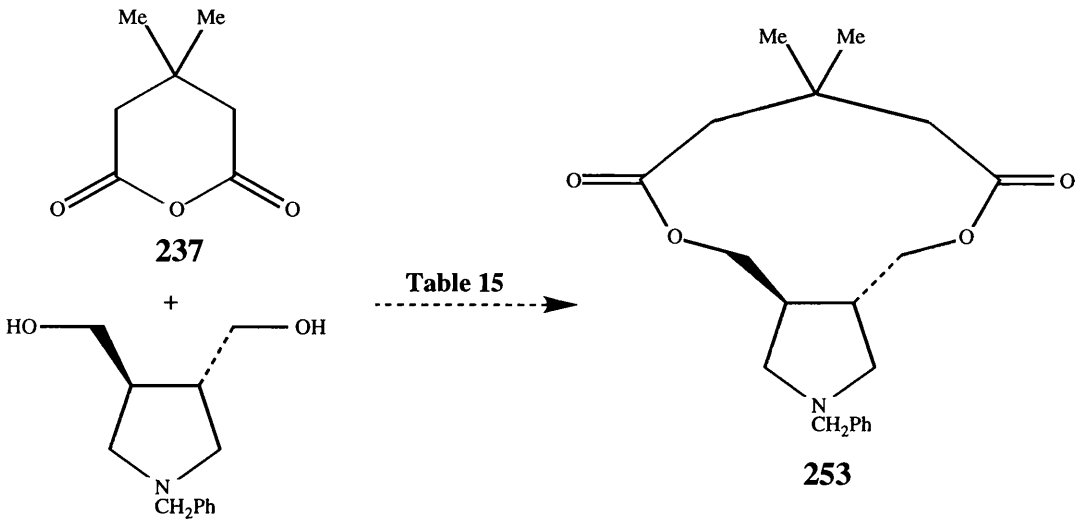


Scheme 54 : Synthesis of (+)-dicrotaline (252) via a cyclic stannoxane

A number of variations on these methods have been used. The total synthesis of pyrrolizidine alkaloids, including methods of macrocyclic diester formation has been comprehensively reviewed.[90]

7.4.2 Attempted Synthesis of the Macrocyclic Adduct of 3,3-dimethylglutaric anhydride (237) and (±)-3,4-bishydroxymethyl-1-benzylpyrrolidine (226)

A variety of methods was used in the attempted synthesis of (±)-6,7-*O*,*O*-(3,3-dimethylglutaryl)-3,4-bishydroxymethyl-1-benzylpyrrolidine (**253**) (**scheme 55**). These methods are summarised in **table 15**.



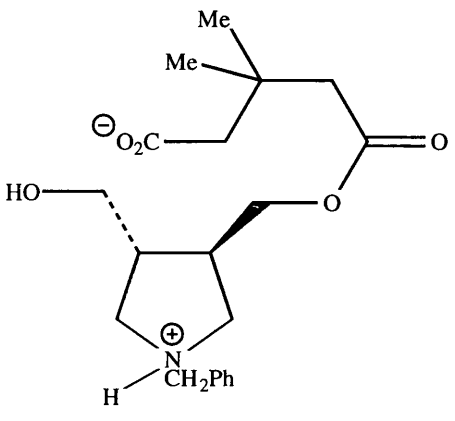
Scheme 55: Attempted Synthesis of the Macrocyclic Adduct of 3,3-dimethylglutaric anhydride (237) and (±)-3,4-bishydroxymethyl-1-benzylpyrrolidine (226)

Conditions	Result
1. **226** + **237,** DME, 40°C, 24 h 2. Aldrithiol-2, PPh_3, DME, RT 24 h	Complex mixture
1. **226**, BuLi, THF, 0°C, 2h 2. **237**, THF, RT, 16 h 3. $(EtO)_2POCl$, DMAP,	Complex mixture
226, dibutyltin oxide, benzene, reflux, 24h	insoluble product

Table 15: Attempted Synthesis of the Macrocyclic Adduct of 3,3-dimethylglutaric anhydride (237) and (±)-3,4-bishydroxymethyl-1-benzylpyrrolidine (226)

Following the Corey-Nicoloau method used by Robins and Kelly,[197] 3,3-dimethylglutaric anhydride (**237**) was added to a solution of the pyrrolidine in DME and the reaction was carefully monitored by TLC (chloroform/methanol/conc. ammonia; 85:14:1) until the spot for the pyrrolidine had been replaced by a base-

line spot assumed to be the zwitterionic monoester (**254**). Aldrithiol-2 and triphenylphosphine were then added and the mixture was stirred for a further 24 h. After this time a new spot had appeared in the TLC assumed to represent the thioester (**255**). The mixture was then heated at reflux for 24 h. This gave a complex mixture of products. ^{1}H NMR spectroscopy of this complex mixture suggested that it contained mainly pyridine derivatives.



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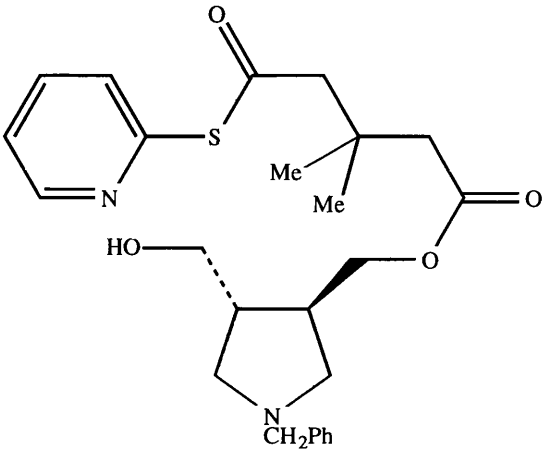
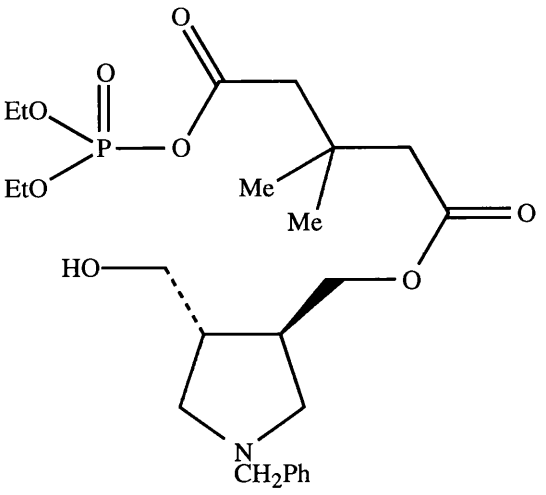


255

The next method attempted was based upon the methodology of White *et al.*[193] and involved the formation of the lithium alkoxide of the pyrrolidine (**226**) and treatment of this with 3,3-dimethylglutaric anhydride. Monitoring of the reaction by TLC showed when the 3,3-dimethylglutaric anhydride had reacted (R_f=0.0). Diethylphosphoryl chloride and DMAP were then added. After stirring for 24 h TLC showed the formation of a new species, assumed to be the mixed anhydride (**256**). The reaction was then heated at reflux for a further 24 h. No products could be isolated from the reaction mixture.

The final attempt to form the macrocyclic diester was via the cyclic stannoxane (**257**) and was based on the work of Niwa *et al.*[194] Treatment of the pyrrolidine (**226**) with dibutyltin oxide in benzene at reflux temperature gave a insoluble residue from which no product could be isolated.

Time limitations prevented a further investigation of this synthetic route.



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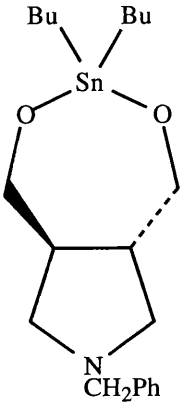

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Chapter 8

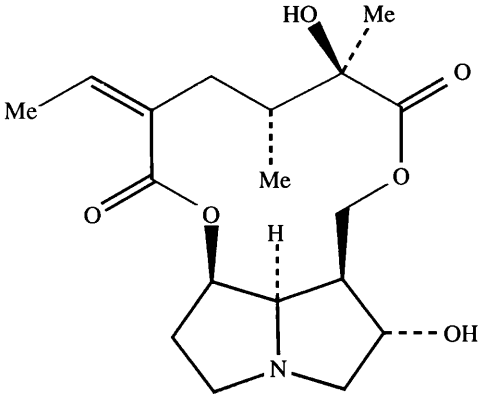
Biological Activity of Pyrrolizidine Alkaloids and Derivatives

8.1 Anti-tumour Activity of Pyrrolizidine Alkaloids and Derivatives

Certain pyrrolizidine alkaloids, particularly indicine *N*-oxide (**27**) and some analogues have shown anti-tumour activity (see **chapter 1.7**). One of the goals of this project was to evaluate the anti-tumour activity of a range of pyrrolizidine alkaloids and analogues. To this end a number of samples were sent to Dr Alan McGown at the Paterson Institute in Manchester for evaluation as anti-tumour agents. The compounds were tested for their inhibition of the growth of the PLC/PRF/5 hepatoma cell line. This cell line is a liver cancer cell line and was chosen because of its high levels of microsomal enzymes which should be able to transform alkaloids with 1,2-unsaturation into pyrrolic derivatives. Such derivatives would be expected to be toxic to the cells.

8.2 Results of Anti-tumour Testing

The compounds that were submitted for testing are shown below. The results of their anti-tumour testing are given in **Table 16**.



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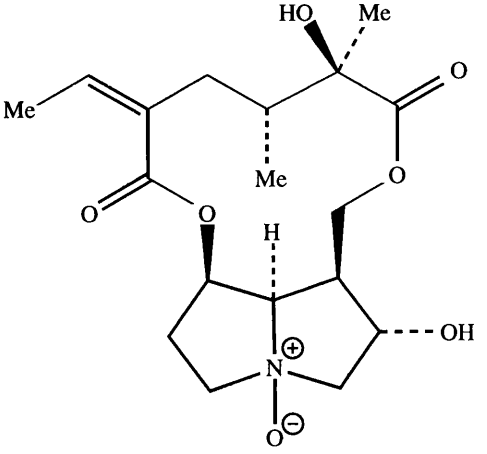

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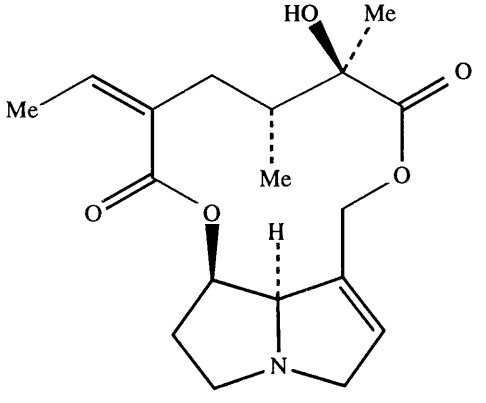

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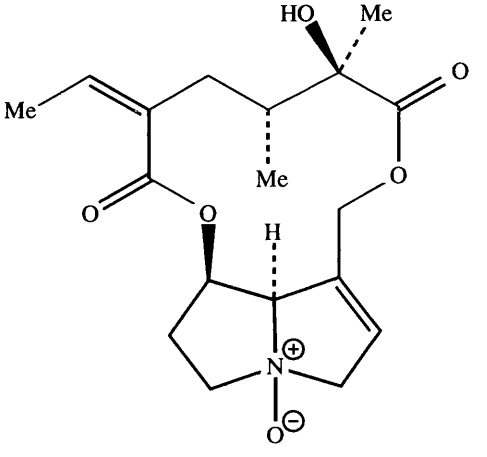

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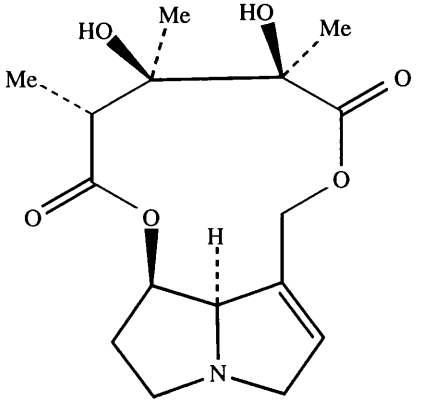

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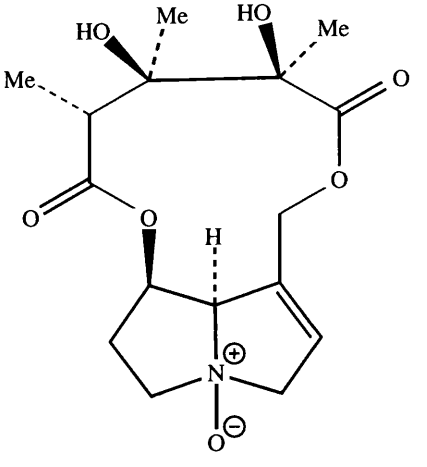

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Compound	Result[a]
Rosmarinine (**68**)	39 μg/ml
Rosmarinine N-oxide (**12**)	No toxicity[b]
Senecionine (**23**)	No toxicity[b]
Senecionine N-Oxide (**69**)	No toxicity[b]
Monocrotaline (**8**)	No toxicity[b]
Monocrotaline N-oxide (**18**)	No toxicity[b]

[a] conc (μg/ml) which inhibits 50 % of cell growth

[b] no toxicity up to 50 μg/ml against PLC/PRF/5 cell line

Table 16 : Results of anti-tumour testing

As can be seen from the results only rosmarinine (**68**) exhibited any inhibition of cell growth. A graph of this inhibition is given in **figure 12**. Rosmarinine does not contain a double bond at the 1,2-position and as such is unlikely to form a hepatotoxic pyrrole derivative. This suggests that the inhibition of cell growth is by some other unknown mechanism.

Rosmarinine *N*-oxide (**12**) was non-toxic to the cells. This implies that the free nitrogen in rosmarinine is important for its toxicity and that the cell line does not possess the reducing power to liberate the free nitrogen from the *N*-oxide.

Those alkaloids which contained 1,2-unsaturation proved non-toxic. This is the opposite of the predicted result based upon previous work,[17] and suggests that this cell line does not have the oxidising capability to convert the alkaloid into the pyrrole derivative. However it is possible that the pyrroles may have been formed but in this cell system they are non-toxic. Different cell lines may give different results for these experiments. It should also be noted that the work cited above[17] was carried out predominantly *in vivo*, whereas the testing carried out in this project was *in vitro*. This may have had an effect on the results.

Additional compounds have been submitted for anti-tumour testing, but at the time of writing no results were available.

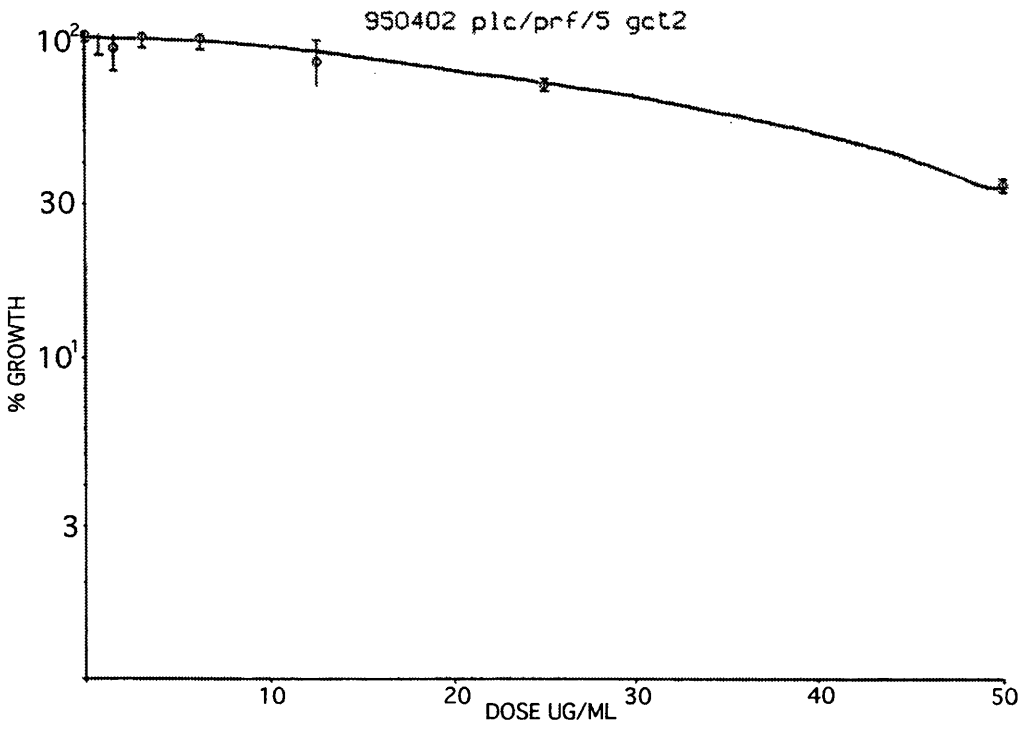


Figure 12 : Graph of cell growth inhibition by rosmarinine (68)

8.3 Metabolism of Pyrrolizidine Alkaloids and Derivatives

^{14}C-Labelled pyrrolizidine alkaloids were isolated and their *N*-oxides were synthesised (see **chapter 2.5**) as requested by Dr. Bryan Hanley, MAFF, Norwich. At the time of writing, no results were available on the metabolic studies on these compounds.

Chapter 9

Experimental

9.1 General Experimental

Melting points were measured on a Kofler hot-stage apparatus and are uncorrected. Mass spectra were obtained with A.E.I. MS 12 or 902 spectrometers. Nuclear magnetic resonance (NMR) spectra were recorded with a Perkin Elmer R32 spectrophotometer operating at 90 MHz (δ_H), a Varian EM 390 spectrophotometer operating at 90 MHz (δ_H), a Bruker AM200 or WP200-SY spectrophotometer operating at 200 MHz (δ_H) or 50 MHz (δ_C). Coupling constants are quoted in hertz. The multiplicities of the ^{13}C NMR resonances were determined using DEPT spectra with pulse angles of $\theta = 90°$ and $\theta = 135°$. Tetramethylsilane (TMS) was used as an internal standard in organic NMR solvents. Infrared spectra (IR) were obtained on either a Perkin Elmer 983 spectrophotometer or a Philips PU 9800 FTIR spectrophotometer. Elemental analyses were performed using a Carlo-Erba 1106 elemental analyser. Optical rotations were determined with an Optical Activity Limited AA10 polarimeter operating at 589 nm.

The diastereomeric compositions of products were determined by ^1H NMR spectroscopy. Enantiomeric compositions were determined by the use of an appropriate chiral shift reagent as discussed in **chapter 4**.

Thin layer chromatography (TLC) was carried out on Merck Kieselgel G (silica) plates of 0.25 mm thickness. Column chromatography was carried out using Merck silica gel 60 (230-400 mesh) or Brackmann standard grade neutral alumina (150 mesh).

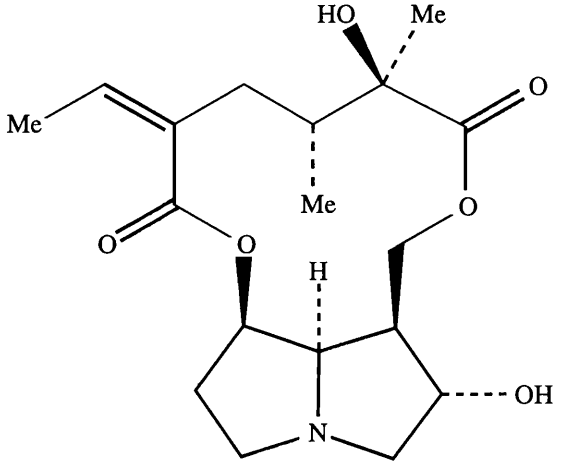
Organic reagents and solvents requiring purification were purified according to the methods given in "Purification of Laboratory Chemicals".[198]

Organic solvents were dried with anhydrous magnesium sulfate unless otherwise stated and solvents were evaporated under reduced pressure below 50°C.

9.2 Experimental For Chapter 2

Extraction of Rosmarinine (68) from *Senecio pleistocephalus*[111]

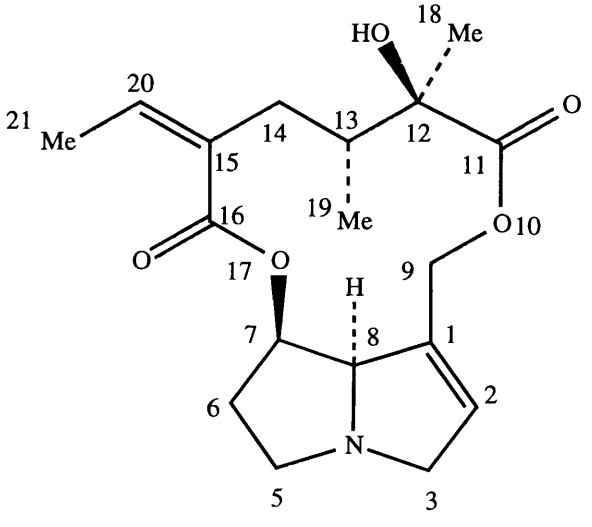
Fresh leaves of *Senecio pleistocephalus* (*ca.* 1 kg) were chopped and blended with methanol (*ca.* 1.5 l). Extracts were filtered and concentrated under reduced pressure. The green residues were then dissolved in dichloromethane (100 ml) and extracted with 1M sulfuric acid (3 x 50 ml). The acidic extracts were combined and washed with dichloromethane (6 x 75 ml). Powdered zinc metal (*ca.* 5 g) was added to the acidic solution which was stirred at room temperature for 1 h. The solution was filtered through a pad of Celite which was then washed with water (25 ml). The filtrate was basified with concentrated ammonia solution to pH 9 and the alkaline solution was extracted with dichloromethane (4 x 100 ml). Combined organic extracts were dried, filtered and concentrated to give the crude alkaloid as a brown residue (1.61 g). This crude extract was recrystallised from acetone-dichloromethane (1:1) to give the pure alkaloid as fine white crystals (0.69 g, 0.069 % based on weight of leaves).



68

Extraction of Senecionine (23) from *Senecio vulgaris*[111]

Transformed root cultures of *Senecio vulgaris* were grown for 21 days in Gamborg's B5 media. They were then filtered and the media discarded to leave the roots. The roots thus prepared (*ca.* 225 g) were chopped and blended with methanol (*ca.* 1 l). Extracts were filtered and concentrated under reduced pressure. The brown residues were then dissolved in dichloromethane (100 ml) and extracted as for rosmarinine (above) to give the crude alkaloid as a brown solid (0.258 g). Recrystallisation from acetone-dichloromethane (1:1) gave the pure alkaloid as fine white crystals (0.199 g, 0.088 % based on weight of roots).



23

m.p. 235 °C (dec.) (acetone - dichloromethane; 1: 1), (lit.,[113] 232 °C (dec.)); $[\alpha]^{22}_{D}$ -54.2 ° (*c* 1.02 in $CHCl_3$), (lit., [113] -56 ° (*c* 1.0 in $CHCl_3$)); Rf 0.30 ($CHCl_3$ / MeOH / conc. NH_3; 85:14:1); (Found: M^+, 335.1744. $C_{18}H_{25}NO_5$ requires M^+ 335.1733); ν_{max} (KBr disc) / cm^{-1} 3400 (br, OH stretch), 1740 (s, ester C=O), 1712 (s, α,β unsaturated ester), 1654 (w, alkene), 1640 (w, α,β unsaturated alkene); δ_H (200 MHz, $CDCl_3$) 0.84 (3 H, d, *J* 6.3, 19 - Me), 1.26 (3 H, s, 18 - Me), 1.78 (3 H, dd, *J* 7.1, 1.4, 21 - Me), 1.54 - 1.69 (2 H, m, 6 - H_α & 13 - H), 1.95 - 2.15 (2 H, m, 14 - H), 2.27 - 2.56 (2 H, m, 5 - H_β, 6 - H_β), 3.18 - 3.38 (2 H, m, 3 - H_α & 5 - H_α), 3.90

(1 H, bd, *J* 16.0, 3 - H_{α}), 3.98 (1 H, d, J 11.7, *pro-R* 9 - H), 4.24 (1 H, d, *J* 2.1, 8 - H), 4.95 - 4.98 (1 H, m, 7 - H), 5.44, (1 H, d, *J* 11.7, *pro-S* 9 - H), 5.66 (1 H, dq, *J* 7.1, 0.9, 20 - H), 6.13 (1 H, bs, 2 - H); δ_C (50 MHz, $CDCl_3$) 11.0 (19 - C), 14.0 (18 - C), 24.9 (21 - C), 29.5 (6 - C), 34.7 (6 - C), 38.2 (14 - C), 38.3 (13 - C), 53.0 (5 - C), 60.4 (3 - C), 62.7 (9 - C), 74.8 (7 - C), 77.0(12 - C), 77.5 (8 - C), 131.3 (1 - C), 132.9 (15 - C), 134.2 (2 - C), 136.4 (20 - C), 167.4 (16 - C), 178.0 (11 - C); *m/z* 335 (M^+, 4.8%), 138 (30.4), 111 (4.1), 106 (19.6), 94 (85.8), 82 (12.4), 80 (544.2), 43 (100).

Extraction of Alkaloids From Comfrey (*Symphytum officinale*) Leaves

The leaves (472 g) of flowering *Symphytum officinale* collected locally were extracted as detailed above for *Senecio pleistocephalus* to give a mixture of alkaloids (9 mg, 0.0019 % based on weight of leaves).

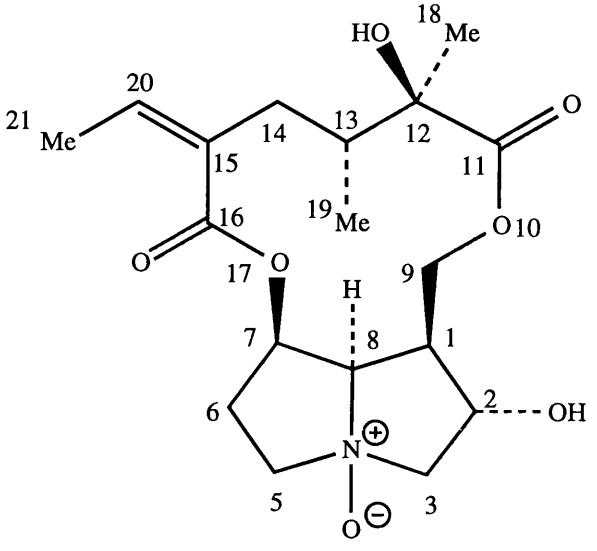
Extraction of Alkaloids From Comfrey (*Symphytum officinale*) Roots

The roots (338 g) of flowering *Symphytum officinale* were extracted as detailed above for *Senecio pleistocephalus* to give a mixture of alkaloids (97 mg, 0.029 % based on weight of roots).

Rosmarinine *N*-Oxide (12)

Rosmarinine (**68**) (0.500 g, 1.33 mmol) was dissolved in $CHCl_3$ (50 ml). 3-Chloroperoxybenzoic acid (0.330 g, 1.65 mmol) was dissolved in $CHCl_3$ (15 ml) and added dropwise to the rosmarinine solution. The mixture was stirred at room temperature for 45 min. The $CHCl_3$ was removed under reduced pressure and the

residue was dissolved in water (25 ml) and washed with diethyl ether (6 x 50 ml). The aqueous layer was concentrated to give the title compound as pale brown crystals (0.383 g, 78 %).

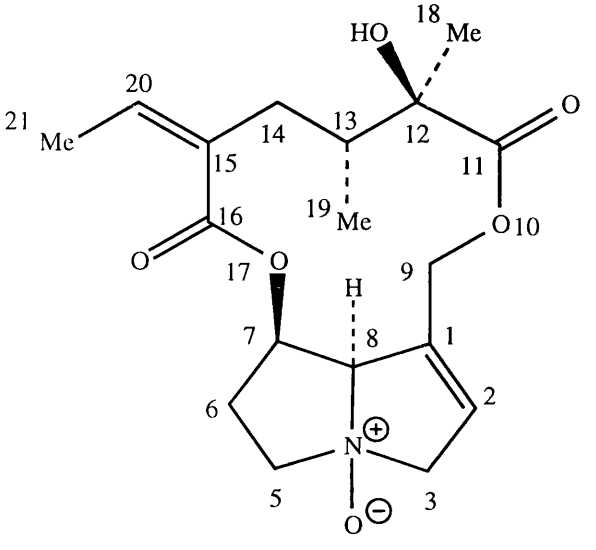


12

m.p. 141-142 °C (dec.), (lit.,[115] 164 °C); Rf 0.08 ($CHCl_3$ / MeOH / conc. NH_3; 85:14:1); (Found: M^+ - 16, 353.1836. $C_{18}H_{27}NO_6$ requires M^+-16, 353.1838); ν_{max} (nujol) / cm^{-1} 3400 (br, OH stretch), 1720 (br, ester C=O & α, β unsaturated ester), 1630 (w, α,β unsaturated alkene), 975 (N^+- O^-); δ_H (200 MHz, $CDCl_3$ / D_6 - DMSO) 0.94 (3 H, d, *J* 6, 19 - Me), 1.32 (3 H, s, 18 - Me), 1.86 (3 H, d, *J* 6, 21 - Me), 1.97 - 2.23 (4 H, m, 6_α - H, 13 - H & 14 - H); 2.88 (1 H, m, 6_β - H); 3.31 (1 H, m, 3_β - H); 3.79 - 4.53 (7 H, m, 1 - H, 2 - H, 5 - H, 8 - H & 9 - H), 5.05 (1 H, m, 3_α - H); 5.51 (1 H, bd, 7 - H); 5.93 (1 H, q, *J* 6, 20 - H); δ_C (50 MHz, $CDCl_3$ / D_6 DMSO) 12.1 (19 - C), 15.6 (21 - C), 25.8 (18 - C), 31.4 (6 - C), 37.8 (13 - C), 39.2 (14 - C), 47.0 (1 - C), 61.1 (9 - C), 69.8 (2 - C), 68.1 (5 - C), 73.1 (3 - C), 75.6 (12 - C), 76.5 (7 - C), 84.5 (8 - C), 131.3 (15 - C), 137.6 (20 - C), 166.7 (16 - C), 178.6 (11 - C); *m/z* 353 (1.0), 153 (29.8), 135 (57.8), 81 (38.9), 43 (100).

Senecionine *N* - Oxide (71)[14]

Senecionine (**23**) (0.429 g, 1.28 mmol) was dissolved in methanol / chloroform (1:1) (20 ml). Hydrogen peroxide solution (30% aqueous solution, 0.44 g, 0.44 ml, 3.84 mmol) was added dropwise to the senecionine solution. The mixture was then stirred at room temperature for 5 d. The solvent was removed under reduced pressure to give a white solid. Recrystallisation from methanol / diethyl ether gave the title compound as white crystals (0.170 g, 38 %).



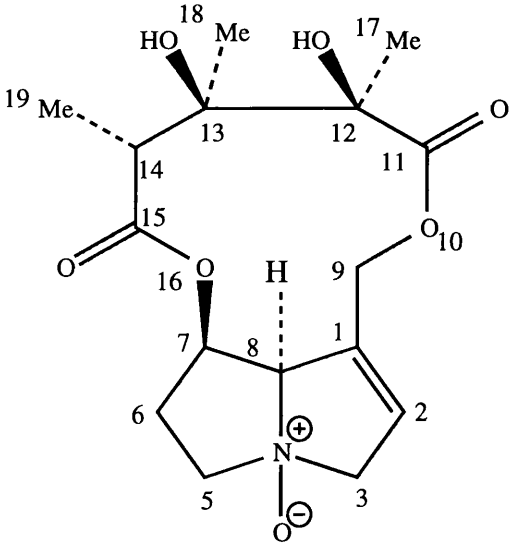
71

m.p. 132 - 144 °C (dec.), (lit.,[116] 141 - 142 °C (dec.)); $[\alpha]^{22}_D$ -21.3 ° (*c* 1 in methanol); Rf 0.09 ($CHCl_3$ / MeOH / conc. NH_3; 85:14:1); (Found: M^+ - 16, 335.1731. $C_{18}H_{25}NO_6$ requires M^+-16, 335.1681); ν_{max} (nujol) / cm^{-1} 3400 (br, OH stretch), 1716 (s, ester C=O & α,β unsaturated ester), 1651 (s, α,β unsaturated alkene), 968 (N^+- O^-); δ_H (200 MHz, D_2O) 0.60 (3 H, d, J 6.1, 19 - Me), 1.00 (3 H, s, 18 - Me), 1.44 - 1.61 (2H, m, 13 - H & 14 - H_α), 1.54 (3 H, d, *J* 7.2, 21 - Me), 1.92 (1H, bd, 14 - H_β), 2.30 (1 H, bd, 6 - H_α), 2.53 (1 H, bm, 6 - H_β), 3.59 (2 H, bm, 3 - H_α & 5 - H_α), 4.01 - 4.61 (4 H, m, 3 - H_β. 5 - H_β, & 9 - H), 5.19 - 5.29 (2 H, m, 7 - H & 8 - H), 5.75 (1 H, q, *J* 7.2, 20 - H), 6.05 (1 H, bs, 2 - H); δ_C (50 MHz, D_2O)

10.0 (18 - C), 15.4 (19 - C), 24.6 (21 - C), 33.29 (6 - C), 38.3 (14 - C), 39.2 (13 - C), 60.1 (9 - C), 69.1(5 - C), 74.8 (7 - C), 78.6 (3 - C), 96.3 (8 - C), 129.2 (1 - C), 131.8 (15 - C), 132.3 (2 - C), 138.7 (20 - C), 169.9 (16 - C), 178.4 (11 - C); *m/z* 335 (0.7), 198 (1.7), 127 (14.1), 119 (100), 94 (9.3), 81 (31.9).

Monocrotaline *N*-Oxide (18); Procedure 1

Monocrotaline (0.175 g, 0.5 mmol) was dissolved in $CHCl_3$ (20 ml). 3-Chloroperoxybenzoic acid (0.126 g, 0.63 mmol) was dissolved in $CHCl_3$ (6 ml) and added dropwise to the monocrotaline solution. The mixture was stirred at room temperature for 45 m. The $CHCl_3$ was then removed under reduced pressure and the residue dissolved in water (10 ml) and washed with diethyl ether (6 x 20 ml). The aqueous layer was evaporated to approximately a quarter of its volume. On standing the product crystallised as clear needles (0.064 g, 27 %).



18

m.p. 188 - 194 °C (dec.), (lit.,[14] 192 - 196 °C (dec.)); $[\alpha]^{22}_D$ -11.5 ° (*c* 0.73 in methanol), Rf 0.05 ($CHCl_3$ / MeOH / conc. NH_3; 85:14:1); ν_{max} (nujol) / cm^{-1} 3500 (br, OH stretch), 1732 (s, ester C=O), 1720 (s, ester C=O), 1680 (w, alkene), 955

(N^+- O^-); δ_H (200 MHz, D_2O) 1.02 (3 H, d, *J* 7.2, 19 - Me), 1.15 (3 H, s, 18 - Me), 1.30 (3 H, s, 17 - Me), 2.07 (1 H, m, 6 - H_α), 2.54 (1 H, m, 6 - H_β), 2.94 (1 H, q, *J* 7.2, 14 -H), 4.18 (1 H, *J* 17, pro *R* 9 - H), 3.57 (2 H, m, 5 - H), 4.49 (1 H, *J* 17, pro *S* 9 - H), 4.51 - 4.70 (3 H, m, 3 - H & 8 - H), 5.28 (1 H, bdd, *J* 12.4, 5.6, 7 - H), 6.13 (1 H, bs, 2 - H) δ_C (50 MHz, D_2O) 14.1 (19 - C), 18.1 (18 - C), 22.1 (17 - C), 32.3 (6 - C), 43.5 (14 - C), 60.5 (9 - C), 67.8 (5 - H), 73.5 (7 - H), 77.4 (12 - C), 77.8 (3 - C), 80.4 (13 - C), 95.3 (8 -C), 130.3 (1 - C), 132.8 (2 - C), 175.9 (15 - C), 177.1 (11 - C); *m/z* 117 (13.5), 111 (1.2), 99 (21.4), 94 (2.2), 83 (9.3), 80 (2.0), 43 (100).

Monocrotaline *N*- Oxide (18); Procedure 2[14]

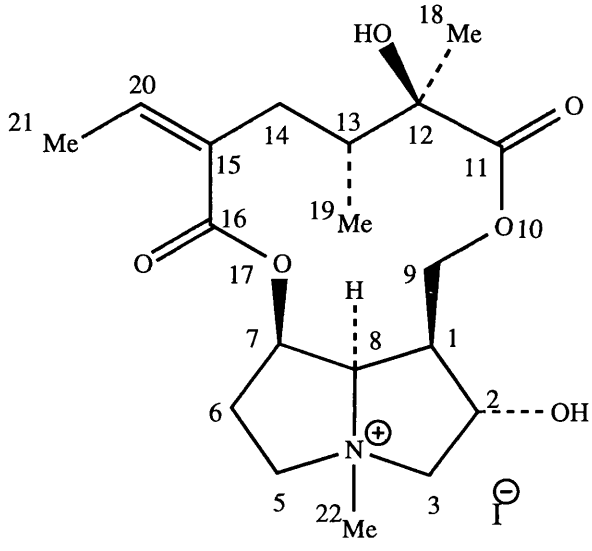
Monocrotaline (0.5 g, 1.54 mmol) was dissolved in ethanol (5 ml). Hydrogen peroxide solution (30% aqueous solution, 0.34 g, 0.36 ml, 2.32 mmol) was added dropwise to the monocrotaline solution. The mixture was stirred at room temperature for 5 d. The ethanol was removed under reduced pressure to give the title compound as white crystals (0.485 g, 92%). The sample was identical to that shown above.

Quaternisation Procedure 2.2.1

A mixture of the alkaloid and iodomethane (quantities given under individual compounds) was stirred at room temperature in methanol (5 ml) for 5 h. The methanol and excess iodomethane were removed under reduced pressure to give a thick oil. Upon trituration with diethyl ether this oil crystallised. Recrystallisation from ethanol / hexane gave the pure material.

Rosmarinine Methiodide (72)

Rosmarinine (**68**) (0.2 g, 0.57 mmol) and iodomethane (0.113 g, 0.05 ml, 0.8 mmol) were treated according to procedure **2.2.1** to afford the title compound as plates (0.156 g, 56 %).



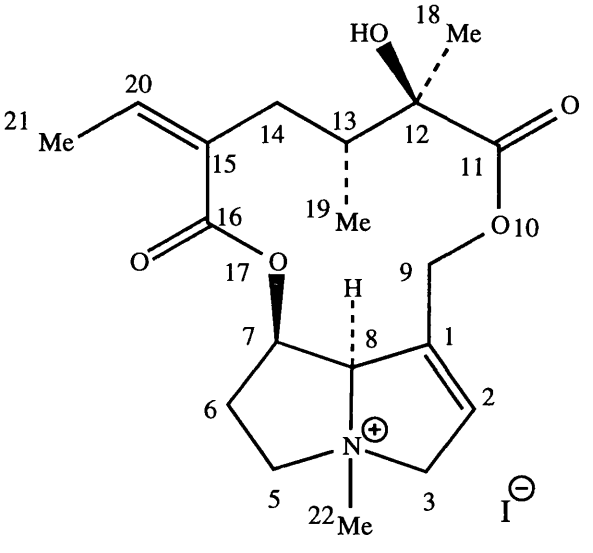
72

m.p. 243 - 245 °C; $[\alpha]^{22}_D$ -62.1 ° (c 2.31 in methanol), Rf 0.05 ($CHCl_3$ / MeOH / conc. NH_3; 85:14:1); (Found: C, 46.1; H, 6.2; N, 2.8; M^+-15, 353.1844. $C_{19}H_{28}NO_5I$ requires C, 46.1; H, 6.1; N, 2.8 %; M^+-15, 353.1838); ν_{max} (nujol) / cm^{-1} 3418 & 3272 (s, OH stretch), 1736 (s, ester C=O) 1707 (α, β unsaturated ester), 1640 (w, α, β unsaturated alkene); δ_H (200 MHz, D_2O) 0.74 (3 H, d, *J* 6.6, 19 - Me), 1.20 (3 H, s, 18 - Me), 1.65 (3 H, d, *J* 7.2, 21 - Me), 1.71 - 2.11 (3 - H, m, 13 - H & 14 - H), 2.43 - 2.54 (2 H, m, 6 - H), 2.88 (1 H, m, 5β - H), 3.24 (3 H, s, 22 - Me), 3.42 & 4.86 (2 H, ABX, J_{AB} 10, J_{AX} 6, 3β & 3α - H), 3.68 - 4.05 (4 H, m, 1 - H, 2 - H, 5α - H, 8 - H), 4.33 - 4.43 (2 H, m, 9 - H), 5.40 (1H, bd, 7 - H), 5.9 (1 H, q, *J* 7.2, 20 - H); δ_C (50 MHz, D_2O) 12.0 (19 - C), 15.7 (18 - C), 25.4 (21 - C), 31.8 (6 - C), 38.3 (13 - C), 39.5 (14 - C), 46.8 (1 - C), 55.9 (22 - C), 60.4 (9 - C), 66.2 (5 - C), 69.8 (7 - C), 73.4 (3 - C), 75.3 (2 - C), 78.9 (12 - C), 80.9 (8 - C), 131.3 (15 - C),

139.2 (20 - C), 169.8 (16 - C), 179.4 (11 - C); *m/z* 353 (M^+ - 15, 3.1), 142 (31.4), 127 (21.4), 112 (24.2), 98 (21.6), 82 (73.5), 43 (100).

Senecionine Methiodide (73)

Senecionine (**23**) (0.2 g, 0.60 mmol) and iodomethane (0.113 g, 0.05 ml, 0.8 mmol) were treated according to procedure **2.2.1** to afford the title compound as pale brown cubes (0.062 g, 22 %).



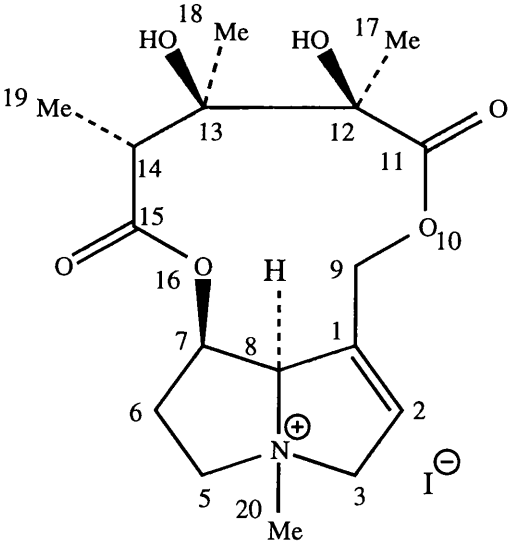
73

m.p. 248 - 249 °C (dec.), (lit.,[17] 249 °C (dec.)); $[\alpha]^{22}_D$ -16.2 ° (*c* 0.53 in methanol), Rf 0.07 ($CHCl_3$ / MeOH / conc. NH_3; 85:14:1); (Found: C, 47.8; H, 6.0; N, 2.8; M^+-15, 335.1708. $C_{19}H_{28}NO_5I$ requires C, 47.8; H, 5.9; N, 3.00 %; M^+-15, 335.1733); ν_{max} (nujol) / cm^{-1} 3500 (br, OH stretch), 1730 (s, ester C=O) 1710 (w, α,β unsaturated ester), 1640 (w, α,β unsaturated alkene); δ_H (200 MHz, D_2O) 0.74 (3 H, d, *J* 6.4, 19 - Me), 1.24 (3 H, s, 18 - Me), 1.68 (3H, d, *J* 7.3, 21 - Me), 1.59 - 1.71 (2H, m, 13 - H & 14 - H_α), 2.07 (1H, bd, 14 - H_β), 2.58 (2 H, bs, 6 - H), 3.19 (3 H, s, 22 - Me), 3.58 - 3.88 (2 H, m, 5 - H), 4.12 - 4.35 (3 H, m, 3 - H & *pro R* 9 - H), 4.90 (1 H, bs, pro *S* 9 - H), 5.32 - 5.38 (2 H, m, 7 - H & 8 - H), 5.88 (1 H, q, *J* 7.2,

20 - H), 6.20 (1 H, bs, 2 - H); δ_C (50 MHz, D_2O) 11.0 (19 - C), 15.5 (18 - C), 24.6 (21 - C), 33.4 (6 - C), 38.4 (14 - C), 39.3 (13 - C), 55.2 (22 - C), 59.3 (9 - C), 65.1 (5 - C), 74.5 (3 - C), 75.1 (7 - C), 78.7 (12 - C), 90.7 (8 - C), 129. 4 (1 - C), 131.7 (15 - C), 132.5 (2 - C), 138.7 (20 - C), 169.8 (16 - C), 178.5 (11 - C); *m/z* 351 (M^+ - 15, 0.2%), 335 (1.6), 198 (1.2), 127 (21.3), 120 (37.5), 111 (3.2), 94 (39.8), 80 (21.8), 43 (100).

Monocrotaline Methiodide (74)

Monocrotaline (0.2 g, 0.62 mmol) and iodomethane (0.113 g, 0.05 ml, 0.8 mmol) were treated according to procedure **2.2.1** to afford the title compound as white crystals (0.136 g, 47 %).



74

m.p. 204 - 205 °C (dec.), (lit.,[17] 205 - 206 °C (dec.)); $[\alpha]^{22}_D$ +22.6 ° (*c* 3.1 in methanol) (lit.,[17] +23.4° (*c* 3.1 in methanol)); Rf 0.06 ($CHCl_3$ / MeOH / conc. NH_3; 85:14:1); (Found: C, 44.4; H, 5.6; N, 3.1. $C_{17}H_{26}NO_6I$ requires C, 44.7; H, 5.6; N, 3.0 %); ν_{max} (nujol) / cm^{-1} 3500 (br, OH stretch), 1728 (s, ester C=O), 1711 (s, ester C=O), 1613 (w, alkene); δ_H (200 MHz, D_2O) 1.08 (3 H, d, *J* 7.2, 19 - Me), 1.19 (3

H, s, 18 - Me), 1.34 (3 H, s, 17 - Me), 2.20 - 2.35 (1 H, m, 6 - H_α), 2.46 - 2.60 (1 H, m, 6 - H_β), 3.00 (1 H, q, *J* 7.2, 14 - H), 3.19 (3H, s, 20 - Me), 3.53 - 3.80 (2 H, m, 5 - H), 4.26 - 4.72 (4 H, m, 3 - H & 9 - H), 4.95 (1 H, bd, *J* 7.2, 8 - H), 5.26 (1H, dt, J_d 6.2, J_t 6.6, 7 - H), 6.21 (1H, bs, 2 - H); δ_C (50 MHz, D_2O) 13.9 (19 - C), 17.9 (18 - C), 21.9 (17 - C), 31.6 (6 - C), 43.2 (14 - C), 54.0 (20 - C), 59.7 (9 - C), 63.3 (5 - C), 72.7 (3 - C), 73.9 (7 - C), 77.2 (13 - C), 80.2 (12 - C), 88.5 (8 - C), 130.5 (1 - C), 132.8 (2 - C), 175.5 (15 - C), 176.8 (11 - C); *m/z* 142 (7.2), 126 (7.4), 111 (2.8), 99 (16.4), 94 (3.5), 80 (2.2), 43 (100)

Preparation of Labelled Compounds

[1,4-^{14}C]Putrescine dihydrochloride was fed to the following plants and root cultures and the alkaloids thus produced were isolated by the method given above.[111]

1. *Senecio pleistocephalus*

[1,4-^{14}C]Putrescine dihydrochloride (3.6 mg, 225μCi) was dissolved in distilled water (4 ml) and fed to six six month old plants at the rate of 1 ml/day by the wick method. After a further seven days the plants were harvested and the labelled rosmarinine extracted by the procedure shown above[111] to give 42 mg of labelled rosmarinine with an activity of 0.037 μCi/mg.

2. *Senecio vulgaris*

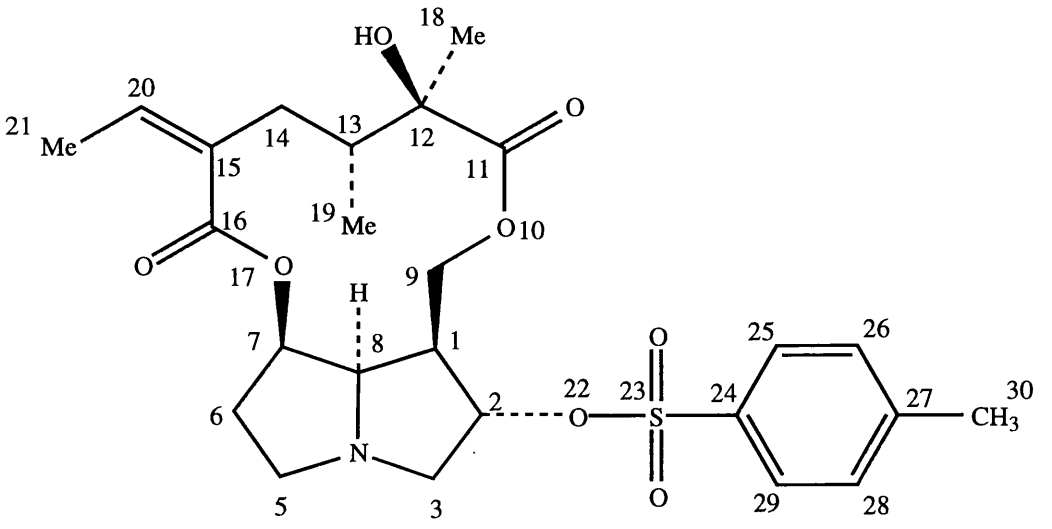
[1,4-^{14}C]Putrescine dihydrochloride (4.4 mg, 275μCi) was dissolved in sterile water and added to twenty flasks of seven day old *S. vulgaris* root cultures. After a further sixteen days of growth, the cultures were harvested and the labelled senecionine extracted by the procedure shown above[111] to give 37 mg of labelled senecionine with an activity of 0.45 μCi/mg.

3. *Symphytum officinale*

[1,4-^{14}C]Putrescine dihydrochloride (3.6 mg, 225 μCi) was dissolved in distilled water (4 ml) and fed to two eighteen month old plants in a single batch by the wick method. After a further twelve days the plants were harvested and the labelled alkaloids extracted from the roots and leaves separately by the procedure shown above[111] to give 96 mg of root alkaloids with an activity of 0.37 nCi/mg, and 45 mg of leaf alkaloids with an activity of 1.3 nCi

2-*O*-Tosyl Rosmarinine (77)[121]

Rosmarinine (**68**) (3.0 g, 8.4 mmol), in dry pyridine (30 ml) was cooled to 0 °C and added to a solution of *p*-toluenesulfonyl chloride (3.1 g, 16.8 mmol) in dry pyridine (10 ml) with cooling such that the temperature of the reaction mixture did not rise above 0 °C. The reaction mixture was then flushed with nitrogen, stoppered and refrigerated for 72 h. After this time the red reaction mixture was poured into ice-cooled 5 M ammonia solution (75 ml) and extracted with diethyl ether (3 x 100 ml). The combined organic extracts were dried, filtered and concentrated to give a brown oil which was induced to crystallise upon drying *in vacuo*. The brown solid was recrystallised from acetone / dichloromethane (1:1) to afford the title compound as orange crystals (2.61 g, 61 %).



77

m.p. 121 - 122 °C (dec.), (lit.,[122] 120 °C (dec.)); $[\alpha]^{22}_D$ -43.6 ° (c 1.02 in $CHCl_3$), Rf 0.62 ($CHCl_3$/MeOH/conc. NH_3; 85:14:1); (Found: M^+, 507.1919. $C_{25}H_{33}NO_8S$ requires M^+, 507.1927); ν_{max} ($CHCl_3$ soln.) / cm^{-1} 3532 (br, OH stretch), 1719 (s, ester C=O), 1624 (w, alkene), 1537 - 1576 (v, benzene ring), 1365 (s, SO_2O-), 1190 (m, SO_2O-), 849 (s, *p*-disubstituted benzene ring); δ_H (200 MHz, $CDCl_3$) 0.96 (3 H, d, *J* 6.8, 19 - Me), 1.27 (3 H, s, 18 - Me), 1.82 (3 H, d, *J* 7.1, 21 - Me), 1.86 - 1.96 (1H, m, H - 13), 2.10 - 2.28 (4 H, m, H - 6 & H - 14), 2.46 (3 H, s, 30 - Me), 2.60 - 2.78 (2 H, m, H - 1 & H - 5β), 2.92 - 3.10 (3 H, m, H - 3 & H - 5α), 3.56 (1H, dd, *J* 7.2, 4.5, 8 - H), 3.88 (1 H, dd, *J* 11.8, 1.9, pro *R* 9 - H), 4.58 - 4.68 (1H, m, pro *S* 9 - H), 4.77 - 4.87 (1H, bq, *J* 6.3, H - 2), 5.25 (1H, q, *J* 4.2 H - 7), 5.85 (1 H, q, *J* 7.1, H - 20), 7.35 (2 H, dd, *J* 8.3, 26- H & 28 - H), 7.82 (2 H, dd, *J* 8.3, 25- H & 29 - H); δ_C (50 MHz, $CDCl_3$) 13.2 (19 - C), 15.5 (18 - C), 21.7 (21 - C), 26.0 (30 - C), 35.0 (14 - C), 37.2 (13 - C), 39.2 (6 - C), 45.5 (1 - C), 51.9 (3 - C), 59.1 (5 - C), 62.6 (9 - C), 67.4 (8 - C), 74.1 (2 - C), 75.9 (12 - C), 81.6 (7 - C), 128.0 (26 - C & 28 - C), 129.9 (25 - C & 29 - C), 131.8 (15 - C), 133.4 (27 - C), 135.6 (20 - C), 145.2 (24 - C), 167.5 (16 - C), 178.1 (11 - C); *m/z* 507 (M^+, 0.4%), 353 (0.5), 335 (1.4), 155 (6.4), 138 (39), 120 (55.9), 94 (45.1), 91 (61.8), 82 (44.4), 80 (49.0), 43 (100).

Senecionine (23) From 2-*O*-Tosyl Rosmarinine (77) in Refluxing Pyridine

A solution of 2-*O*-tosyl rosmarinine (**77**) (0.15 g, 0.30 mmol) in dry pyridine (3 ml) was heated at reflux under nitrogen for 22 h, after which time TLC ($CHCl_3$ / MeOH / conc. NH_3; 85:14:1) showed no remaining starting material. The solution was cooled and poured into 5M ammonia solution (25 ml). The aqueous layer was extracted with chloroform (3 x 15 ml). The combined organic layers were dried, filtered and concentrated. Remaining pyridine was removed by azeotroping the residue with water (3 x 1 ml) and toluene (3 x 1 ml) to give a brown solid. This was purified by preparative TLC ($CHCl_3$ / MeOH / conc. NH_3; 85:14:1; Rf 0.30) to give the title compound as a white powder (0.004 g, 4 %).

Attempted Synthesis of Senecionine (23) From 2-*O*-Tosyl Rosmarinine (77) in Pyridine / DMAP

A solution of 2-*O*-tosyl rosmarinine (**77**) (0.3 g, 0.59 mmol), DMAP (0.029 g, 0.24 mmol), and triethylamine (0.1 ml, 0.64 mmol) in dry pyridine (6 ml) was stirred under dry nitrogen for 24 h. TLC analysis ($CHCl_3$ / MeOH / conc. NH_3; 85:14:1) showed that no product had formed.

Senecionine (23) From 2-*O*-Tosyl Rosmarinine (77) in Refluxing Pyridine / DMAP

A solution of 2-*O*-tosyl rosmarinine (**77**) (0.15 g, 0.30 mmol) and DMAP (0.015 g, 0.12 mmol) in dry pyridine (6 ml) was heated at reflux under dry nitrogen for 48 h, after which time TLC ($CHCl_3$ / MeOH / conc. NH_3; 85:14:1) showed no remaining starting material. The solution was cooled and poured into 5 M ammonia solution. The aqueous layer was extracted with chloroform (3 x 50 ml). The

combined organic layers were dried, filtered and concentrated. Excess pyridine was removed by azeotroping the residue with water (3 x 5 ml) and toluene (3 x 5 ml) to give a brown solid. This was purified by preparative TLC ($CHCl_3$ / MeOH / conc. NH_3; 85:14:1; Rf 0.30) to give the title compound as a white powder (0.009 g, 9 %).

Senecionine (23) From 2-*O*-Tosyl Rosmarinine (77) in DMF

A solution of 2-*O*-tosyl rosmarinine (**77**) (0.20 g, 0.39 mmol) in dry DMF (10 ml) was heated at reflux under dry nitrogen for 5 h, after which time TLC ($CHCl_3$ / MeOH / conc. NH_3; 85:14:1) showed no remaining starting material. The solution was cooled and poured into 5 M ammonia solution. The aqueous layer was extracted with diethyl ether (3 x 25 ml). The combined organic layers were dried, filtered and concentrated to give a brown solid. This was purified by preparative TLC ($CHCl_3$ / MeOH / conc. NH_3; 85:14:1) to give the title compound as a white powder (0.015 g, 12 %) and rosmarinine (0.009 mg, 6 %).

Attempted Synthesis of 2-*O*-Dimethylthiocarbamoyl Rosmarinine (79)

To a solution of rosmarinine (**68**) (0.4 g, 1.1 mmol) in dry DMF (5 ml) under nitrogen was added NaH (60 % in mineral oil, 0.088 g, 2.2 mmol). The resulting solution was stirred at room temperature until TLC ($CHCl_3$ / MeOH / conc. NH_3; 85:14:1) suggested formation of the sodium salt (Rf 0.0) (*ca.* 1 h). Dimethylthiocarbamoyl chloride (0.16 g, 1.3 mmol) was then added and the reaction mixture was heated at 80 °C for a further 3 h. The reaction mixture was cooled and poured into brine (10 ml) and extracted with chloroform (3 x 25 ml). The combined organic extracts were washed with brine (3 x 25 ml), and extracted with 5 M HCl (3 x 25 ml). The combined acidic extracts were washed with chloroform (2 x 25 ml),

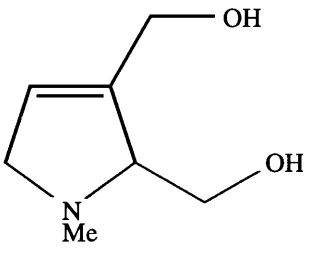
basified with conc. NH_3 solution and extracted with chloroform (3 x 25 ml). The combined organic extracts were dried, filtered and concentrated to give a brown oil. In this complex mixture of products no alkaloidal product could be detected by TLC ($CHCl_3$/MeOH/conc. NH_3; 85:14:1) and visualisation using Dragendorff's reagent. A variety of other conditions were tried to affect this reaction. These are detailed in **table 9** in **chapter 2**.

Attempted Synthesis of a 2-*O*-Tosyl Rosmarinine Epimer (85) Via a Mitsunobu Reaction

Diethyl azodicarboxylate (0.192 g, 0.17 ml, 1.1 mmol) in dry toluene (2 ml) was added to a solution of rosmarinine (**68**) (0.353 g, 1 mmol) and triphenylphosphine (0.288 g, 1.1 mmol) in dry toluene (5 ml). This mixture was stirred until complete dissolution had occurred (*ca.* 15 min). Methyl *p*-toluenesulfonate (0.208 g, 1.1 mmol) was then added dropwise and stirring was continued for a further 27 h. A complex, inseparable mixture of products was obtained. The main component of this mixture was shown by TLC ($CHCl_3$ / MeOH / conc. NH_3; 85:14:1; Rf 0.21 - 0.22) to have the same Rf value as rosmarinine.

9.3 Experimental For Chapter 3

Preparation of Synthanecine A (14)[127]

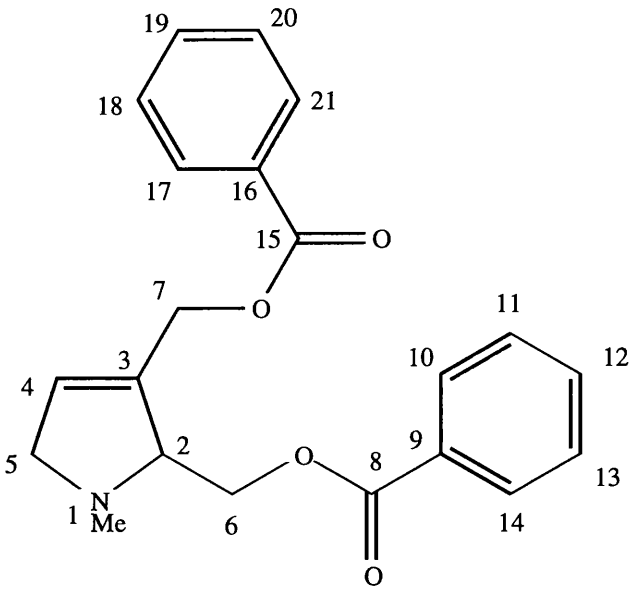


14

Synthanecine A (**14**) was prepared according to the method of Mattocks[18] as modified by Barbour and Robins.[127] All analytical data agreed with literature values.

(±)-6,7-*O*,*O*-Dibenzoyl synthanecine A (93)

Distilled benzoyl chloride (0.37 g, 0.31 ml, 2.64 mmol) was added dropwise with ice cooling to a solution of synthanecine A (0.12 g, 0.88 mmol) in a mixture of dry THF (20 ml), and dry pyridine (5 ml) under nitrogen. The mixture was stirred at room temperature for 1 h then poured into ice water (25 ml). The solution was acidified with 2 M HCl and washed with diethyl ether (3 x 50 ml). The aqueous solution was basified with conc. NH_3 solution and extracted with chloroform (3 x 50 ml). The combined organic layers were dried, filtered and concentrated to give the crude product as a thick oil. This was purified by column chromatography (silica; $CHCl_3$/methanol/triethylamine; 85:14:1) to give the title compound as an oil (0.108 g, 35 %).

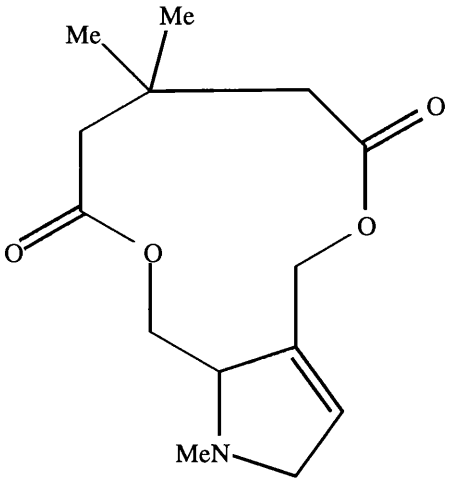


93

(Found: M^+-135, 216.1034 $C_{13}H_{14}NO_2$ requires M^+-135, 216.1024); ν_{max} (KBr disc) / cm^{-1} 2943 (m, CH_2 stretch), 2785 (m, N-Me stretch), 1719 (s, C=O stretch), 1664 (m, C=C stretch), 1601 (m, aromatic ring), 1584 (m, aromatic ring), 1491 (m, aromatic ring); δ_H (200 MHz, $CDCl_3$) 2.53 (3 H, s, 1 - Me), 3.18 - 3.28 (1 H, m, 5 - H_a), 3.45 - 3.47 (2 H, m, 2 - H), 3.57 (1 H, dd, J_{ab} 9.7, 6 - H_a), 3.62 (1 H, dd, J_{ab} 9.7, 6 - H_b), 3.74 - 3.84 (1 H, m, 5 - H_b), 4.89 (2 H, bs, 7 - H), 5.89 (1 H, bs, 4 - H), 7.19 - 7.47 (6 H, m, aromatic H), 7.88 - 7.93 (4 H, m, aromatic H); *m/z* 216 (9.0), 112 (9.0), 94 (100), 77 (25.6), 42 (12.8).

(±)-6,7-*O*,*O*-(3,3-Dimethylglutaryl)synthanecine A (19)

Treatment of synthanecine A (0.142 g, 1 mmol) and 3,3-dimethylglutaric anhydride (0.142 g, 1 mmol) according to the method of Barbour and Robins gave the title compound in 10 % yield. All analytical data were identical to those previously published.[19]

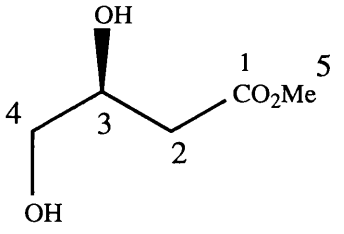


19

9.4 Experimental For Chapter 4

Methyl (3*S*)-3,4-dihydroxybutanoate (122)

To a solution of dimethyl (*S*)-malate (1 g, 6.17 mmol) in dry THF (10 ml) under nitrogen was added boron methyl sulfide complex (10 M, 0.64 ml, 6.35 mmol) dropwise over 20 min, maintaining the temperature at 20 °C. The solution was then stirred at room temperature for 30 min until the evolution of hydrogen had ceased. After this time the flask was cooled to 0 °C and $NaBH_4$ (12 mg, 5 mol %) was added with vigorous stirring in one portion. When the exothermic reaction had subsided the cooling bath was removed and stirring was continued for a further 1 h. The reaction was quenched by addition of methanol (6 ml), stirred for 30 min and concentrated to give a colourless oil. This was purified by column chromatography (silica, ethyl acetate, Rf = 0.34) to give the product (**122**) as an oil (0.701g, 85%).

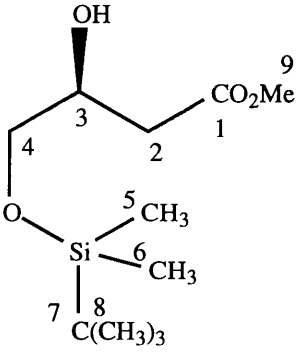


122

$[\alpha]_D^{22}$ -4.7 ° (*c* 1.7 in methanol); (M^+-31, 103.0394. $C_4H_7O_3$ requires M^+-31, 103.0395); ν_{max} (thin film) / cm^{-1} 3362 (br, OH stretch), 2954 (s, C-H stretch), and 1738 (s, ester C=O stretch); δ_H (200 MHz, $CDCl_3$) 2.39 (2 H, d, *J* 6.6, 2 - H), 3.45 & 4.00 (3 H, ABX system, J_{AB} 11.5, 4 - H_a, 4 - H_b & 3 - H), 3.58 (3 H, s, 5 - Me); δ_C (50 MHz, $CDCl_3$) 37.9 (2 - C), 51.9 (5 - Me), 65.7 (4 - C), 68.8 (3 - C), 172.8 (1 - C); *m/z* 103 (13.2), 71 (19.7), 61 (19.1), 43 (100).

Methyl (3*S*)-3-hydroxy-4-(*tert*-butyldimethylsilyl)butanoate (123)

A solution of the diol (**122**) (0.5 g, 3.73 mmol) in dry THF (5 ml) under nitrogen was cooled to 0 °C. Imidazole (0.33 g, 4.85 mmol) and TBDMSCl (0.59 g, 3.92 mmole) were then added with stirring. Stirring was continued for 2 h at 0 °C before the solution was partitioned between diethyl ether (30 ml) and water (20 ml). The aqueous layer was extracted with ether (2 x 30 ml) and the organic extracts combined, washed with brine (3 x 30 ml), dried and concentrated to give the crude product (0.79 g). This was purified by column chromatography (silica; EtOAc / hexane; 75:25) to give the pure monoprotected diol (0.75 g, 81 %);

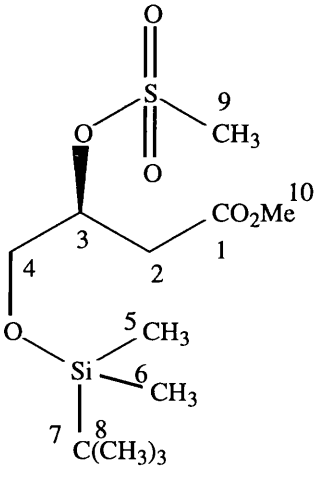


123

$[\alpha]_D^{22}$ -8.9 ° (*c* 1.50 in $CHCl_3$); (M^+-31, 217.1251. $C_{10}H_{21}O_3Si$ requires M^+-31, 217.1260); ν_{max} (thin film) / cm^{-1} 2954 - 2856 (s, C-H stretch), 1740 (s, ester C=O stretch), and 1122 (m, C-O stretch), 1074 (s, Si-O), 838 (s, Si-O); δ_H (200 MHz, $CDCl_3$) 0.01 (6 H, s, 5 & 6 - H), 0.83 (9 H, s, 8 - H), 2.45 (2 H, m, 2 - H), 2.93 (1 H, d, *J* 5, O-H), 3.53 (2 H, m, 4 - H), 3.75 (3 H, s, 9 - Me), 4.00 (1 H, m, 3 - H); δ_C (50 MHz, $CDCl_3$) -5.6 (5 - C, 6 - C), 18.2 (7 - C), 25.8 (8 - C), 37.8 (2 - C), 51.6 (9 - Me), 66.1 (4 - C), 68.4 (3 - C), 172.5 (1 - C); *m/z* 217 (4.9), 191 (18.8), 117 (73.2), 75 (100).

Methyl (3*R*)-3-methanesulfonyl-4-(*tert*-butyldimethylsilyl)butanoate (124)

To a solution of the monoprotectected diol (**123**) (2 g, 8.06 mmol) in dry pyridine (25 ml) under nitrogen was added methanesulfonyl chloride (0.95 g, 0.64 ml, 1.65 mmol) with stirring. Stirring was continued for 2 h at room temperature. After this time diethyl ether (100 ml) was added to precipitate pyridine hydrochloride. The solution was filtered and evaporated to give the crude product. This was purified by column chromatography (silica; EtOAc) to give the product as a yellow oil (1.74 g, 66 %);

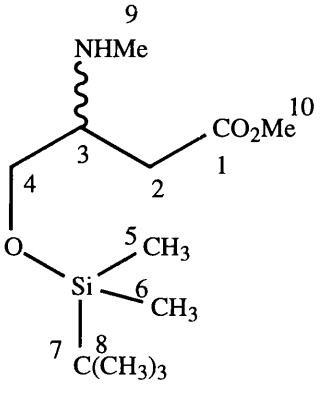


124

$[\alpha_D]^{22}$ -30.6 ° (*c* 1.56 in $CHCl_3$); (Found: C, 44.3; H, 8.0; M^+-31, 295.1037. $C_{12}H_{26}SO_6Si$ requires C, 44.2; H, 8.0 %; M^+-31 295.1035); ν_{max} (thin film) / cm^{-1} 2954 - 2858 (s, C-H stretch), 1740 (s, ester C=O stretch), 1360 & 1176 (s, SO_2O-) and 1126 (s, C-O stretch), 1035 (s, Si-O), 840 (s, Si-O); δ_H (200 MHz, $CDCl_3$) 0.00 & 0.00 (2 x 3 H, 2 x s, 5 & 6 - H), 0.81 (9 H, s, 8 - H), 2.70 (2 H, m, 2 - H), 2.99 (3 H, s, 9 - H), 3.63 (3 H, s, 10 - Me), 3.74 (2 H, m, 4 - H), 4.90 (1 H, m, 3 - H); δ_C (50 MHz, $CDCl_3$) -5.6 (5 - C, 6 - C), 18.2 (7 - C), 25.7 (8 - C), 36.0 (2 - C), 38.1 (9 - C), 52.0 (10 - Me), 64.2 (4 - C), 78.8 (3 - C), 170.5 (1 - C); *m/z* 295 (2.3), 269 (5.8), 173 (70.4), 153 (100), 89 (46.2), 75 (24.4).

Attempted Synthesis of Methyl (3*R*)-3-(*N*-methylamino)-4-(*tert*-butyldimethylsilyl)butanoate (125) 1

The mesylate (**124**) (0.4 g, 1.23 mmol), was stirred with methylamine (33% solution in ethanol, 1.1g, 1.16 ml, 1.23 mmol) at room temperature for 18 h. 5 M ammonia solution (10 ml) was then added and the aqueous layer was extracted with chloroform (3 x 15 ml). The combined organic extracts were dried, filtered and concentrated to give a pale yellow oil. Purification by column chromatography (silica, $CHCl_3$ / MeOH / Et_3N; 85:14:1) gave the racemised amine (**125**) (0.161 g, 50 %) and the olefin (**126**) (0.014 g, 5 %).



125

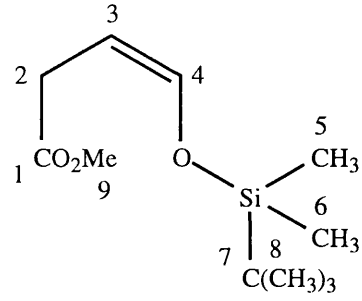
$[\alpha_D]^{22}$ 0 ° (*c* 1.51 in $CHCl_3$); (Found: M^+-31, 230.1569. $C_{11}H_{24}NO_2Si$ requires M^+-31 230.1576); ν_{max} (thin film) / cm^{-1} 3344 (w, N-H stretch), 2954 - 2856 (s, C-H stretch), 1736 (s, ester C=O stretch), 1534 (w, N-H bend), 1041 (s, Si-O), 830 (s, Si-O); δ_H (200 MHz, $CDCl_3$) 0.00 (6 H, s, 5 - Me & 6 - Me), 0.84 (9 H, s, 8 - Me), 1.60 (1 H, bs, N-H), 2.37 (3 H, s, 9 - Me), 2.40 (2 H, d, *J* 6.4, 2 - H), 2.85 - 2.97 (1 H, m, 3 - H), 3.47 - 3.60 (2 H, m, 4 - H), 3.63 (3 H, s, 10 - Me); δ_C (50 MHz, $CDCl_3$) -5.6 (5 - C & 6 - C), 18.1 (7 - C), 25.7 (8 - C), 33.7 (9 - Me), 35.9 (2 - C), 51.4 (10 - C), 57.8 (3 - C), 63.7 (4 - C), 172.7 (1 - C); *m/z* 261 (M^+, 0.1 %), 188 (5.4), 173 (7.2), 130 (41.5), 116 (100), 89 (25.5), 75 (22.7).

Attempted Synthesis of Methyl (3*R*)-3-(*N*-methylamino)-4-(*tert*-butyldimethylsilyl)butanoate (113) 2

The mesylate (**124**) (0.4 g, 1.23 mmol), was dissolved in dry acetonitrile (5 ml). The reaction mixture was cooled to 0 °C and methylamine gas was bubbled through the solution for 1 min. The solution was then stirred for 15 min at 0 °C after which time 5 M ammonia solution (10 ml) was added and the aqueous layer washed with chloroform (3 x 15 ml). The combined organic layers were dried, filtered and concentrated to give a pale yellow oil. Purification by column chromatography (silica, $CHCl_3$ / MeOH / Et_3N; 85:14:1) gave the racemised amine (**125**) (0.100 g, 31 %) and trace amounts of the alkene (**126**). Further attempts to synthesise the amine (**113**) were carried out (see **Table 10**). None of these gave enantiomerically pure material.

Methyl 4-(*tert*-butyldimethylsilyl)but-3-enoate (140)

To a solution of the mesylate (**124**) (0.5 g, 1.53 mmol) in toluene (10 ml) was added DBU (1.16 g, 1.14 ml, 7.67 mmol) and the mixture heated at reflux for 1 h. The solution was then allowed to cool and poured into 0.1 M HCl (50 ml) and extracted with dichloromethane (3 x 75 ml). The combined organic layers were dried, filtered and evaporated to give the crude product. This was purified by column chromatography (silica; hexane / ethyl acetate; 9:1) to give the title compound as a clear oil (0.066 g, 19 %).

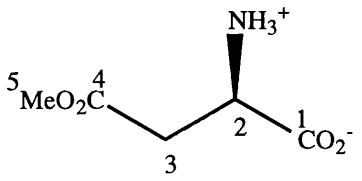


140

(Found: C, 57.2; H, 9.8; M^+-31, 199.1157. $C_{10}H_{19}O_2Si$ requires C, 57.4; H 9.6 %; M^+-31, 199.1154); ν_{max} (thin film) / cm^{-1} 2954 - 2858 (s, C-H stretch), 1744 (s, ester C=O stretch), 1660 (s, olefin), 1046 (s, Si-O), 840 (s, Si-O); δ_H (200 MHz, $CDCl_3$) 0.12 (6 H, s, 5 - Me & 6 - Me), 0.90 (9 - H, s, 8 - Me), 3.14 (2 H, dd, J_{vic} 7.0, $J_{allylic}$ 1.6, 2 - H), 3.66 (3H, s, 9 - Me), 4.66 (1 H, dt, J 5.8, 7.0, 3 - H), 6.30 (1 H, dt, J_{vic} 5.8, $J_{allylic}$ 1.6, 4 - H); δ_C (50 MHz, $CDCl_3$) -5.5 (5 - C & 6 - C), 18.1 (7 - C), 25.5 (8 - C), 29.3 (2 - C), 51.6 (9 - C), 101.4 (3 - C), 140.9 (2 - C), 172.8 (1 - C); *m/z* 200 (0.4), 173 (51.3), 145 (16.8), 115 (5.8), 89 (100), 73 (39.9), 59 (28.3).

β-Methyl D-Aspartic Acid (142)[152]

D-Aspartic Acid (2.0 g, 15 mmol) and sodium sulfate (2.0 g, 14 mmol) were suspended in dry methanol (25 ml) under nitrogen. To this suspension was added tetrafluoroboric acid diethyl etherate (4.7 ml, 30 mmol) dropwise via a syringe. The mixture was heated to 60 °C and stirred for 15 h. After this time the sodium sulfate was removed by filtration, washed with methanol (2 x 10 ml) and the filtrate was neutralised with triethylamine. The excess methanol was removed under reduced pressure and the residue was triturated with ethyl acetate / acetone (4:1). The solid was filtered off and recrystallised from water/acetone to give the title compound as white platelets (1.60 g, 73 %).



142

m.p. 190 - 193 °C , (lit.,[152] 180 - 181 °C); $[\alpha]_D^{22}$ -20.1 ° (*c* 2 in 1 M HCl) (lit.,[152] -20.6 ° (*c* 2 in 1 M HCl)), Rf 0.27 (MeOH/ether/water; 50:35:15); (Found: MH^+, 148.0583. $C_5H_{10}NO_4$ requires MH^+, 148.0610.); ν_{max} (nujol) / cm^{-1} 3100 (w, NH_3^+ stretch), 2922 & 2852 (s, C-H stretch), 1728 (s, ester C=O stretch), 1644 (s, CO_2^- antisymmetrical stretch), 1376 (s, CO_2^- symmetrical stretch); δ_H (200 MHz, D_2O) 2.93 (2 H, d, *J* 5.6, 3 - H), 3.60 (3 H, s, 5 - Me), 4.02 (1 H, t, *J* 5.6, 2 - H); δ_C (50 MHz, D_2O) 35.1 (3 - C), 51.1 (2 - C), 53.7 (5 - Me), 173.3 (1 - C & 4 - C); *m/z* 148 (MH^+ 1.0%), 102 (100), 88 (10.2), 74 (65.9), 60 (46.7), 43 (98.1).

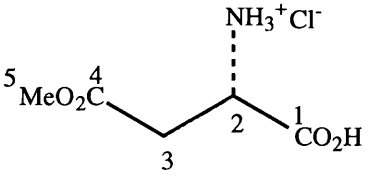
Attempted Synthesis of β-Methyl (N-[2-norborn-5-ene])-D-aspartic Acid (143)

To a homogeneous solution of β-methyl D-aspartic acid (**142**) (0.5 g, 3.4 mmol) in 2 M HCl were added with vigorous stirring, cyclopentadiene (1.78 g, 2.2 ml, 27.2 mmol), and formaldehyde (37 % aqueous solution, 0.81 g, 9.96 mmol). Stirring was continued for 22 h. The reaction mixture was then poured into water (25 ml) and extracted with dichloromethane (3 x 25 ml). The combined organic layers were dried, filtered and concentrated to give a crude brown oil from which no identifiable products could be isolated.

β-Methyl L-Aspartic Acid Hydrochloride (145)[155]

A solution of L-aspartic acid (17 g, 0.13 mol) in dry methanol (80 ml), was cooled to -10 °C. To this cooled flask was added thionyl chloride (20.2 g, 12.4 ml,

0.17 mol) dropwise with stirring. The flask was allowed to warm slowly to room temperature. Stirring was then stopped and the flask was allowed to stand for a further 25 min. Diethyl ether (250 ml) was added and the flask was vigorously shaken and cooled to 0 °C. A white precipitate formed and the flask was refrigerated for 1 h to induce further precipitation. The precipitate was collected by filtration and placed in a desiccator for 24 h at *ca.* 20 mmHg to remove any residual SO_2. Recrystallisation from methanol/ether (with a few drops of dilute HCl added) gave the product (**145**) (23.4 g, 85 %).



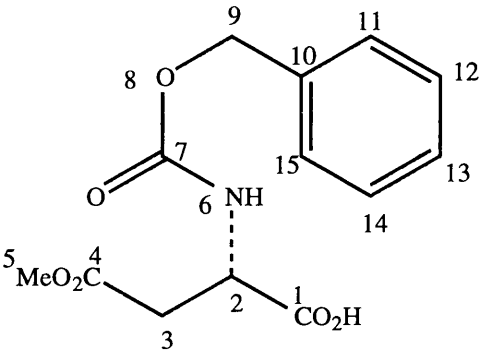
145

m.p. 201 - 204 °C , (lit.,[155] 190 °C); $[\alpha]_D^{22}$ +17.4° (*c* 1 in EtOH / water [3:1]) (lit.,[155] +21.4 ° [*c* 1 in EtOH / water (3:1)]), (Found: MH^+-HCl, 148.0593. $C_5H_{10}NO_4$ requires MH^+-HCl, 148.0610.); ν_{max} (nujol) / cm^{-1} 2923 (m, amino salt), 2695 - 2458 (m, acid O-H stretch), 1732 (s, C=O stretch), 1500 (s, N-H bend); δ_H (200 MHz, D_2O) 2.94 (2 H, d, *J* 5.3, 3 - H), 3.53 (3 H, s, 5 - Me), 4.22 (1 H, t, *J* 5.2, 2 - H); δ_C (50 MHz, D_2O) 34.5 (3 - C), 50.0 (2 - C), 53.8 (5 - Me), 171.4 (4 - C), 172.6 (1 - C); *m/z* 148 (MH^+ 1.1%), 102 (99.8), 88 (12.7), 74 (65.2), 60 (48.5), 43 (100).

β-Methyl (*N*-Benzyloxycarbonyl)-L-aspartic Acid (146)[156]

The aspartic acid derivative (**145**) (5 g, 27 mmol) and sodium carbonate (2.97 g, 28 mmol) were dissolved in water (30 ml) at 0 °C. When evolution of CO_2 had ceased benzyl chloroformate (5.12 g, 4.28 ml, 30 mmol) was added dropwise via syringe simultaneously with sodium carbonate (4 g, 38 mmol in 30 ml water) via

a dropping funnel to the vigorously stirred reaction mixture. The reaction was allowed to warm to room temperature and stirring was continued for 3 h. The reaction mixture was then washed with diethyl ether (3 x 50 ml), acidified to pH 1 with conc HCl and extracted with ethyl acetate (3 x 75 ml). The combined organic extracts were dried, filtered and concentrated to leave a clear oil. Cooling and vigorous scratching induced crystallisation. Recrystallisation from ethyl acetate/petroleum ether (40 - 60 °C) gave the product (**146**) as white crystals (5.66 g, 75 %).

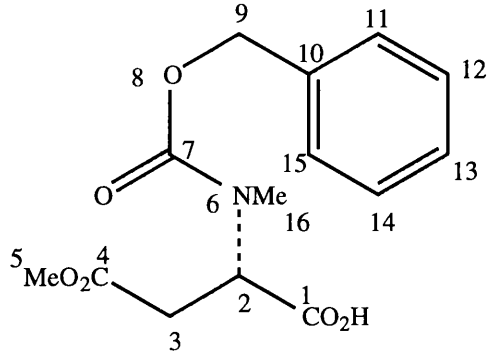


146

m.p. 89 - 90 °C, (lit.,[156] 97 - 98 °C); $[\alpha]_D^{22}$ +33.4 ° (*c* 1 in chloroform), (Found: C, 55.3; H, 5.5; N, 5.1; M^+, 281.0903. $C_{13}H_{15}NO_6$ requires C, 55.5; H, 5.3; N, 5.0 %; M^+, 281.0899); ν_{max} (nujol) / cm^{-1} 3318 (s, acid O-H stretch), 1745 (s, C=O stretch), 1694 (s, carbamate), 1588 (s, aromatic ring); δ_H (200 MHz, $CDCl_3$) 2.95 & 4.66 (3 H, ABX system, J_{AB} 17.4, 3 - H & 2 - H), 3.65 (3 H, s, 5 - Me), 5.10 (2 H, s, 9 - H), 6.00 (1 H, d, *J* 8.6, 6 - H), 7.33 (5 - H, bs, aromatic H), 10.35 (1 H, s, 1 - $CO_2\underline{H}$); δ_C (50 MHz, $CDCl_3$) 36.2 (3 - C), 50.1 (2 - C), 52.2 (5 - Me), 67.3 (9 - C), 128.1 - 128.5 (aromatic C-H), 135.9 (10 - C), 156.3 (7 - C), 171.5 (4 - C), 175.0 (C - 1); *m/z* 281 (M^+, 1.9 %), 146 (4.7), 108 (36.4), 91 (100), 79 (12.2), 43 (16.6).

β-Methyl (*N*-Benzyloxycarbonyl-*N*-methyl)-L-aspartic Acid (147)

The amino acid derivative (**146**) (0.7 g, 2.5 mmol) and iodomethane (1.25 ml, 20 mmol) were dissolved in dry THF (8 ml) under nitrogen. The mixture was cooled to 0 °C and sodium hydride (60 % dispersion, 0.3 g, 7.5 mmol) was added cautiously with gentle stirring. After the addition was complete the suspension was stirred at room temperature for 24 h after which time the reaction mixture was homogeneous. Ethyl acetate (15 ml) was added followed by a dropwise addition of water (10 ml) to destroy any remaining sodium hydride. The solution was then concentrated and the oily residue was partitioned between diethyl ether (25 ml) and water (40 ml). The organic layer was extracted with 5 % sodium bicarbonate (3 x 25 ml) and the combined aqueous extracts were acidified to pH 2 with 5 M HCl. The aqueous layer was extracted with ethyl acetate (3 x 30 ml), and the organic extracts were further washed with water (2 x 25 ml), 5 % sodium thiosulfate (25 ml) and brine (15 ml). The combined organic extracts were dried, filtered and concentrated to give a thick yellow oil (0.557 g, 76 %). This oil could not be induced to crystallise, nor could it be successfully purified by column chromatography and thus it was used crude in the next stage of the synthesis.



147

δ_H (90 MHz, $CDCl_3$) 2.7 - 3.0 (2 H, m, 3 - H), 2.9 (3 H, s, 16 - Me), 3.6 (3 H, s, 5 - Me), 4.6 - 4.9 (1 H, m , 2 - H), 5.05 (2 H, s, 9 - H), 7.2 (5 H, bs, aromatic H), 8.55 (1 H, bs, 1 - $CO_2\underline{H}$); *m/z* 295 (M^+, 0.1 %), 160 (4.1), 116 (7.8), 91 (100), 43 (4.0).

Attempted Synthesis of β-Methyl (*N*-methyl)-L-aspartic Acid (148)

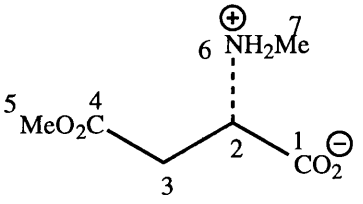
(**i**) A solution of the amino acid derivative (**147**) (0.25 g, 0.85 mmol) in 80 % acetic acid solution (5 ml) was mixed with 10 % Pd - C catalyst (0.2 g). The mixture was vigorously stirred in a H_2 atmosphere for 5 h. The mixture was filtered through Celite to remove the catalyst. The catalyst was extracted with ethanol (2 x 20 ml) and the combined filtrates were concentrated to give only starting material (0.24 g, 98 %).

(**ii**) A solution of the amino acid derivative (**147**) (0.25 g, 0.85 mmol) in dry ethanol (5 ml) was mixed with cyclohexene (0.1 ml) and 10 % Pd - C catalyst (0.2 g). The mixture was heated at reflux for 2 h, before being filtered through Celite to remove the catalyst. The catalyst was extracted with ethanol (2 x 20 ml) and the combined filtrates were concentrated to give only starting material (0.18 g, 72 %).

β-Methyl (*N*-methyl)-L-aspartic Acid (148)

Ammonium formate (5 g, 17.8 mmol) was heated at 40 °C at 2 mmHg for 1 h. Dry methanol (60 ml), the amino acid derivative (**147**) (5 g, 17.8 mmol) and 10 % Pd - C catalyst (5 g) were then added. The mixture was vigorously stirred under a nitrogen atmosphere for 5 h before being filtered through Celite to remove the catalyst. The catalyst was then extracted with methanol (2 x 50 ml) and the combined filtrates were concentrated to give a brown oil. Crystallisation was

induced by dissolving the residue in a small amount of methanol and carefully adding diethyl ether dropwise to give the title compound as white crystals (0.74 g, 26 %).

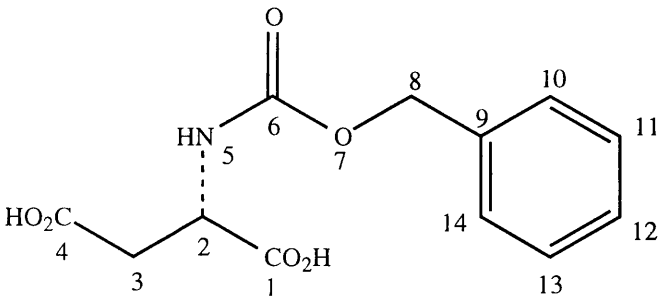


148

m.p. 176 - 180 °C (dec.); $[\alpha]_D^{22}$ +19.1 ° (*c* 0.65 in methanol); ν_{max} (nujol) / cm^{-1} 1740 (s, C=O stretch), 1591 (s, antisymmetrical CO_2^- stretch), 1377 (s, symmetrical CO_2^- stretch); δ_H (200 MHz, D_2O) 2.57 (3 H, s, 7 - Me), 2.77 - 2.85 (2 H, m, 3 - H), 3.54 (3 H, s, 5 - Me), 3.63 - 3.73 (1 H, m, 2 - H); δ_C (50 MHz, D_2O) 32 .9 (6 - C), 33.9 (3 - C), 53.5 (5 - C), 59.9 (2 - C), 173.1 (4 - C), 175.5 (1 - C); *m/z* 161 (M^+, 0.1 %), 130 (4.7), 116 (38.4), 102 (3.2), 88 (31.8), 74 (12.7), 42 (100).

N-Benzyloxycarbonyl-L-aspartic Acid (149)

L-Aspartic acid (2.9 g, 22 mmol) was dissolved in 2 M NaOH (17 ml) and diethyl ether (5 ml). The solution was cooled to 0 °C and benzyl chloroformate (8.2 g, 6.9 ml, 48 mmol) was added dropwise with vigorous stirring simultaneously with a 4 M NaOH solution (12 ml) over 45 m. The solution was stirred for a further 5 h after which time the reaction mixture was washed with diethyl ether (2 x 25 ml), acidified to pH 1 with 5 M HCl and extracted with ethyl acetate (3 x 50 ml). The combined ethyl acetate extracts were dried, filtered and concentrated to give a clear oil. Careful dropwise addition of chloroform afforded the title compound as white crystals (5.23 g, 89 %).

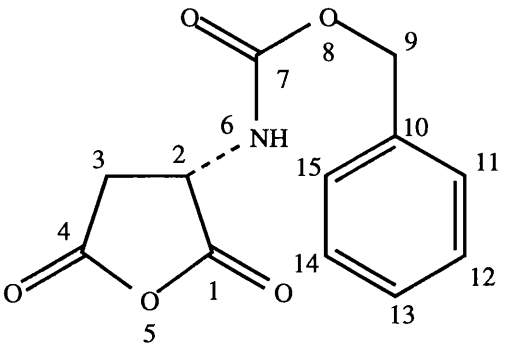


149

m.p. 116 - 117 °C (lit., [199] 117 - 119 °C); $[\alpha]_D^{22}$ +8.7 ° (*c* 7 in acetic acid), (lit., [199] +8.6 (*c* 7 in acetic acid)); (Found: C, 53.9; H, 4.9; N, 5.2. $C_{12}H_{13}NO_6$ requires C, 53.9; H, 4.9; N, 5.2 %); ν_{max} (nujol) / cm^{-1} 2855 - 2924 (s, O-H stretch), 1705 (s br, acid C=O stretch, O-CO-N stretch), 1585 (m, aromatic ring), 1533 (s, aromatic ring), 775 - 736 (aromatic C-H bending); δ_H (200 MHz, $CDCl_3$ / D_6 DMSO) 2.55 - 2.82 (2 H, m, 3 - H), 4.45 (1 H, q, 2 - H), 4.52 (1 H, s, N-H), 5.05 (2 H, s, 8 - H), 7.34 (5 H, bs, aromatic H), 9.68 (2 H, bs, 2 x $CO_2\underline{H}$); δ_C (50 MHz, $CDCl_3$ / D_6 DMSO) 36.0 (3 - C), 50.5 (2 - C), 65.6 (8 - C), 127.6 - 128.3 (aromatic C), 136.8 (9 - C), 155.9 (6 - C), 171.7 (1 - C), 172.7 (4 - C); *m/z* 107 (27.3), 91 (100), 79 (25.7), 77 (19.2).

N-Benzyloxycarbonyl-L-aspartic Anhydride (150)

A mixture of CBZ-L-aspartic acid (**149**) (5 g, 18.7 mmol) and acetic anhydride (11.9 g, 11 ml, 0.11 mol) were gently stirred for 1 h. After this time the mixture was allowed to stand for a further 2 h. The acetic anhydride and acetic acid formed were removed under reduced pressure to afford a thick oil. Remaining acetic anhydride and acetic acid were removed by azeotroping the residue with dioxane (6 ml), and xylene (6 ml) to leave a colourless solid. This solid was stored in a desiccator at *ca* 20 mmHg for 72 h. Recrystallisation from ethyl acetate / hexane afforded the title compound as an colourless solid (4.47 g, 96 %).

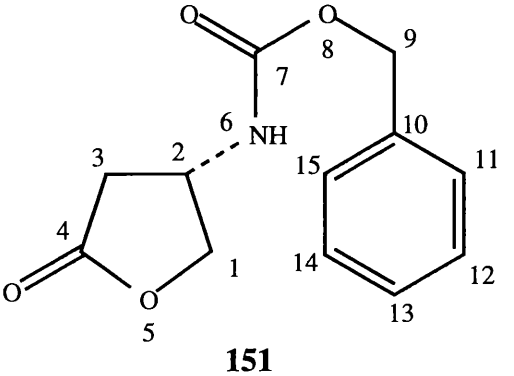


150

m.p. 82 - 84 °C (lit., [164] 79.5 - 81.5 °C); $[\alpha]_D^{22}$ -5.3 ° (*c* 1.8 in methanol); (Found: M^+, 249.0634. $C_{12}H_{11}NO_5$ requires M^+, 249.0637); ν_{max} (nujol) / cm^{-1} 1702 (s, br, anhydride C=O stretch, O-CO-N stretch), 1586 (m, aromatic ring), 1534 (s, aromatic ring), 1306 (s, C-O stretch), 1274 (s, C-O stretch), 1192 (s, C-O stretch), 776 - 736 (aromatic C-H bending); δ_H (200 MHz, D_6-DMSO) 2.71 & 4.42 (3 H, ABX system, J_{ax} 7.8, J_{bx} 7.9, 3 - H & 2 - H); 5.09 (2 H, s, 9 - H), 7.39 (5 H, bs, aromatic H); δ_C (50 MHz, D_6-DMSO) 36.1 (3 - C), 50.6 (2 - C), 65.6 (9 - C), 127.8 - 128.5 (aromatic C), 137.0 (10 - C), 156.0 (7 - C), 171.8 (1 - C), 172.8 (4 - C); *m/z* 249 (M^+, 4.1 %), 107 (27.3), 91 (100), 79 (25.7), 77 (19.2).

3(*S*)-[(Benzyloxycarbonyl)amino]-γ-butyrolactone (151)[163]

Treatment of the anhydride (**150**) (11 g, 44.2 mmol) in dry THF (50 ml) with sodium borohydride (1.68 g, 44.2 mmol) in dry THF (50 ml) according to the method of McGarvey *et al.*[163] gave the title compound in 75 % yield.

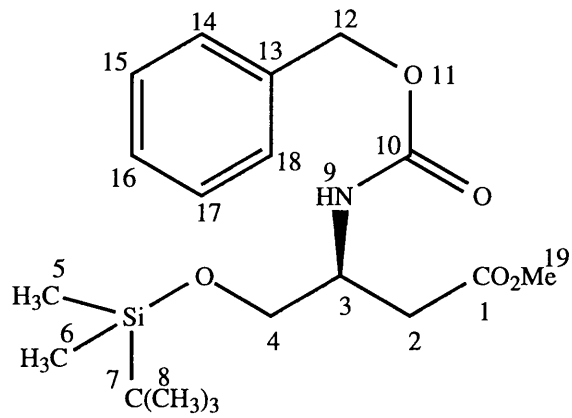


151

m.p. 103 - 104 °C (lit., [163] 103 - 104 °C); Rf = 0.63 (silica, ethyl acetate); $[\alpha]_D^{22}$ -51.6 ° (*c* 2.25 in chloroform), (lit., [163] -54.9 ° (*c* 2.27 in chloroform)); (Found: M^+, 235.0840. $C_{12}H_{13}NO_4$ requires M^+, 235.0844); ν_{max} (nujol) / cm^{-1} 1780 (s, lactone C=O stretch), 1674 (s, O-CO-N stretch), 1550 (m, aromatic ring), 1462 (s, aromatic ring), 786 - 722 (aromatic C-H bending); δ_H (200 MHz, $CDCl_3$) 2.35 - 2.77 (2 H, m, 3 - H), 4.14 - 4.17 (1 H, m, 2 - H), 4.34 - 4.40 (2 H, m, 1 - H), 5.05 (2 H, s, 9 - H), 6.00 (1 H, bs, N-H), 7.30 (5 H, bs, aromatic H); δ_C (50 MHz, $CDCl_3$) 34.6 (3 - C), 47.9 (2 - C), 67.0 (1 - C), 73.7 (9 - C), 128.1 - 129.6 (aromatic C), 135.9 (10 - C), 155.9 (7 - C), 175.8 (4 - C); *m/z* 235 (M^+, 20.0 %), 108 (42.7), 107 (22.4), 91 (100), 79 (15.8), 77 (9.9).

Methyl (*S*)-3-[(benzyloxycarbonyl)amino]-4-*tert*-butyldimethylsilyloxybutanoate (152)[167]

Treatment of the lactone (**151**) (3 g, 12.8 mmol) in dry methanol (60 ml) with DCC (2.91 g, 14.1 mmol) followed by imidazole (1.74 g, 25.6 mmol) and *tert*-butyldimethylsilyl chloride (2.89 g, 19.2 mmol) in dry DMF (20 ml) according to the method of Jurzal *et al.*[167] gave the title compound in 75 % yield.



152

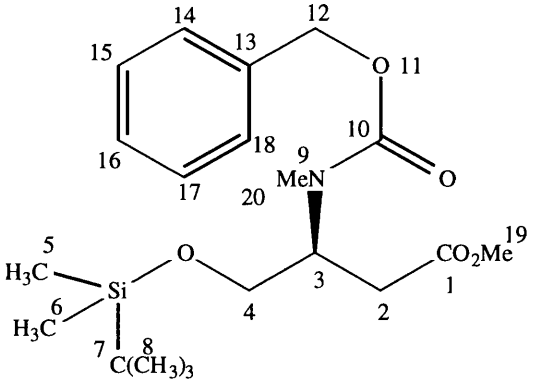
Rf = 0.52 (silica, diethyl ether /hexane; 50:50); $[\alpha]_D^{22}$ -4.1 ° (*c* 1.0 in chloroform), (lit.,[167] -4.1 (*c* 1.0 in chloroform)); (Found: M^+-57, 324.1267. $C_{15}H_{22}NO_5Si$ requires M^+-57, 324.1267); ν_{max} (thin film) / cm^{-1} 2953 & 2932 (s, C-H stretch), 1728 (bs, ester C=O stretch & O-CO-N stretch), 1612 (m, aromatic ring), 1508 (s, aromatic ring), 1256 ($SiMe_2$), 1088 (Si-O), 777 - 698 (aromatic C-H bending); δ_H (200 MHz, $CDCl_3$) 0.00 (6 H, s, 5 - Me & 6 - Me), 0.85 (9 H, s, 8 - Me), 2.56 (2 H, d, *J* 6.1, 2 - H), 3.59 (3 H, s, 19 - Me), 3.62 - 3.70 (2 H, m, 4 - H), 4.02 - 4.11 (1 H, m, 3 - H), 5.04 (2 H, s, 12 - H), 5.51 (1 H, d, J 8.8, N-H), 7.28 (5 H, bs, aromatic H); δ_C (50 MHz, $CDCl_3$) -5.6 (5 - C & 6 - C), 18.2 (7 - C), 25.8 (8 - C), 35.3 (2 - C), 49.3 (3 - C), 51.6 (19 - C), 63.9 (4 - C), 66.7 (12 - C), 128.1 - 128.5 (aromatic C), 136.5 (13 - C), 156.0 (10 - C), 172.0 (1 - C); *m/z* 381 (M^+, 0.1 %), 350 (0.6), 324 (10.1),, 116 (8.7), 91 (100), 79 (5.7), 77 (4.5), 73 (18.1).

Attempted Synthesis of methyl (*S*)-3-[(benzyloxycarbonylamino)-*N*-methyl]-4-*tert*-butyldimethylsilyloxybutanoate (153)

The amine (**152**) (0.381 g, 1 mmol) and iodomethane (1.13 g, 0.5 ml, 8 mmol) were dissolved in dry THF (5 ml) under nitrogen. The flask was cooled to 0 °C and sodium hydride (0.036 g, 1.5 mmol) was added cautiously with gentle stirring. Stirring was continued at room temperature for 24 h. Ethyl acetate (5 ml) was added followed by water (5 ml). The solution was concentrated and the residue that remained was partitioned between diethyl ether (25 ml) and water (25 ml). The aqueous layer was washed with extracted with diethyl ether (3 x 25 ml) and the combined organic extracts were dried, filtered and concentrated to give a yellow oil. TLC (silica; diethyl ether /hexane; 50:50) and NMR spectroscopic analysis showed this to be a mixture of products of which the major component was the starting amine (**152**). The conditions of this reaction were varied with no further success. These conditions are summarised in **table 12**.

Methyl (*S*)-3-[(benzyloxycarbonylamino)-*N*-methyl]-4-*tert*-butyldimethylsilyloxybutanoate (153)

The amine (**152**) (0.381 g, 1 mmol) was dissolved in dry DMF (5 ml) and iodomethane (1.13 g, 0.5 ml, 8 mmol) and silver (I) oxide (0.972 g, 4 mmol) were added. The reaction was stirred at room temperature for 24 h. The mixture was filtered through Celite to remove the silver (I) oxide. The Celite was washed with diethyl ether (2 x 10 ml). A white solid was precipitated. Filtration removed this solid and the filtrate was diluted with diethyl ether (20 ml) and washed with 2 M HCl (2 x 25 ml), water (2 x 25 ml) and brine (30 ml). The organic solution was dried, filtered and concentrated to give an oil. This was purified by column chromatography (silica; diethyl ether / hexane; 60:40) to give the title compound as a clear oil (195 mg, 51 %).



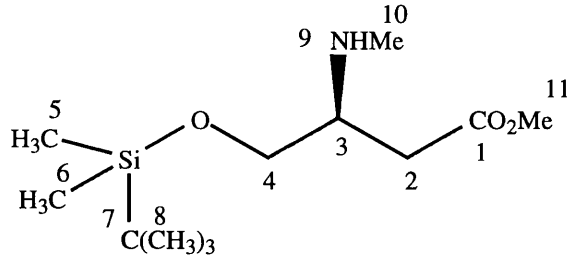
153

Rf = 0.47 (silica, diethyl ether /hexane; 60:40); $[\alpha]^{22}_{D}$ -5.2 ° (*c* 0.81 in chloroform); (Found: M^{+}-15, 380.1886. $C_{19}H_{30}NO_5Si$ requires M^{+}-15, 380.1893); ν_{max} (thin film) / cm^{-1} 2954 - 2886 (s, C-H stretch), 2856 (s, N-Me), 1741 (s, ester C=O stretch), 1703 (O-CO-N stretch), 1538 (m, aromatic ring), 1471 (s, aromatic ring), 1257 ($SiMe_2$), 1007 (Si-O), 778 - 698 (aromatic C-H bending); δ_H (200 MHz, $CDCl_3$) -0.03 / 0.00 (6 H, 2 x s, 5 - Me & 6 - Me), 0.84 (9 H, s, 8 - Me), 2.53 - 2.68 (2 H, m, 2 - H), 2.86 / 2.89 (3 H, 2 x s, 20 - Me), 3.60 / 3.63 (3 H, 2 x s, 19 - Me),

3.67 - 3.81 (2 H, m, 4 - H), 4.31 - 4.51 (1 H, m, 3 - H), 5.09 (2 H, bs, 12 - H), 7.31 (5 H, bs, aromatic H); δ_C (50 MHz, $CDCl_3$) -5.7 / -5.6 (5 / 6 - C), 18.0 (7 - C), 30.8 / 32.0 (20 - C), 33.7 / 34.2 (2 - C), 51.7 (19 - C), 63.3 / 63.4 (4 - C), 66.8 / 67.2 (12 - C), 127.7 - 128.4 (aromatic C), 137.0 (13 - C), 156.0 (10 - C), 171.6 / 171.8 (1 - C); *m/z* 380 (0.3), 338 (21.2), 206 (5.4), 130 (9.9), 116 (1.3), 91 (100), 73 (9.8).

Methyl (*S*)-3-(*N*-methylamino)-4-*tert*-butyldimethylsilyloxybutanoate (154)

A solution of the protected amine (**153**) (0.131 g, 0.34 mmol) was dissolved in dry methanol (5 ml). To this solution was added 5 % Pd - C (100 mg) and the mixture was stirred at room temperature in a hydrogen atmosphere for 18 h. The mixture was filtered through Celite to remove the catalyst, washed with methanol (2 x 15 ml) and the organic solution was concentrated to leave the title compound as an oil (0.076 g, 86 %).



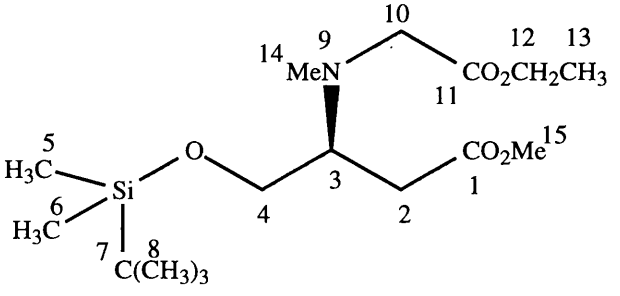
154

Rf = 0.24 (silica, diethyl ether /hexane; 60:40); $[\alpha]_D^{22}$ -6.7 ° (*c* 0.73 in chloroform); (Found: M^+, 261.1744. $C_{12}H_{27}NO_3Si$ requires M^+, 261.1760); ν_{max} (thin film) / cm^{-1} 3442 (m, N-H stretch), 2954 - 2887 (s, C-H stretch), 2798 (m, N-Me), 1739 (s, ester C=O stretch), 1255 ($SiMe_2$), 1106 (Si-O); δ_H (200 MHz, $CDCl_3$) 0.00 (6 H, s, 5 & 6 Me), 0.84 (9 H, s, 8 - Me), 2.37 (3 H, s, 10 - Me), 2.38 - 2.42 (2 H, m, 2 - H), 2.85 - 2.97 (1 H, m, 3 - H), 3.46 - 3.60 (2 H, m, 4 - H), 3.63 (3 H, s, 11 - Me); δ_C (50 MHz, $CDCl_3$) -7.2 (5 & 6 - C), 16.6 (7 - C), 24.2 (8 - C), 32.2 (10 -Me), 34.4 (2 -

C), 49.9 (11 - C), 56.2 (3 - C), 62.1 (4 - C), 171.2 (1 - C); *m/z* 261 (M^+, 0.1 %), 188 (5.0), 130 (34.8), 116 (100), 75 (22.5).

Methyl (*S*)-3-(*N*-methylamino-*N*-ethoxycarbonylmethyl)-4-*tert*-butyldimethylsilyloxybutanoate (155)

A solution of the secondary amine (**154**) (0.105 g, 0.4 mmol), ethyl bromoacetate (0.084 g, 0.06 ml, 0.5 mmol), and hydrated potassium carbonate (0.083 g, 0.6 mmol) were heated at reflux in 7 % aqueous acetone (5 ml) for 24 h. The potassium carbonate was removed by filtration and the organic solution was concentrated to give a yellow oil. This was purified by column chromatography (silica; diethyl ether / hexane; 60:40) to give the title compound as a clear oil (0.064 g, 46 %).



155

Rf=0.56 (silica, diethyl ether /hexane; 60:40); (Found: M^+ 347.2126. $C_{16}H_{33}NO_5Si$ requires 347.2128); ν_{max} (thin film) / cm^{-1} 2954 - 2887 (s, C-H stretch), 1741 (s, ester C=O stretch), 1255 ($SiMe_2$), 1112 (Si-O); δ_H (200 MHz, $CDCl_3$) 0.00 (6 H, s, 5 & 6 - Me), 0.84 (9 H, s, 8 - Me), 1.22 (3 H, t, *J* 7.1, 13 - Me), 2.38 (3 H, s, 14 - Me), 2.49 (2 H, d, *J* 6.9, 2 - H), 3.15 - 3.27 (1 H, m, 3 - H), 3.37 (2 H, d, *J* 5.1, 10 - H), 3.63 (3 H, s, 15 - Me), 3.60 - 3.76 (2 H, m, H - 4), 4.12 (2 H, q, *J* 7.1, 12 - H); δ_C (50 MHz, $CDCl_3$) -7.3 (5 & 6 - C), 12.6 (13 - C), 16.5 (7 - C), 24.2 (8 - C), 31.6 (2 - C), 37.3 (14 - C), 49.9 (15 - C), 54.6 (10 - C), 58.8 (4 - C), 59.7 (3 - C), 61.6 (12

- C), 170.0 (1 - C), 171.29 (11 - C); *m/z* 347 (M^+, 0.9 %), 274 (16.5), 202 (100), 170 (53.5), 143 (6.8), 89 (11.0), 73 (25.6).

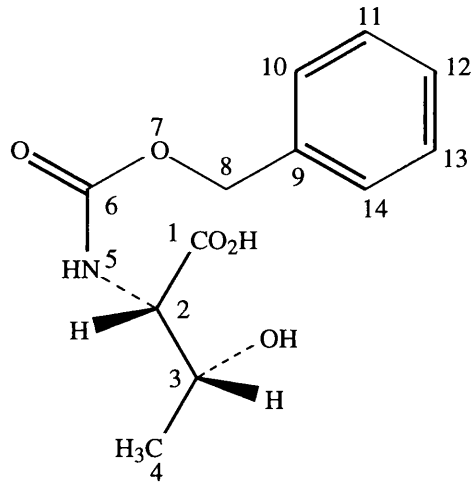
Attempted Synthesis of Methyl 1-methyl-4-oxopyrrolidine-(*S*)-2-*tert*-butyldimethylsilyloxymethyl-3-carboxylate (156)

To a solution of the tertiary amine (**155**) (0.052 g, 0.16 mmol) in dry benzene under nitrogen was added sodium hydride (5 mg, 0.21 mmol). The mixture was stirred at room temperature for 5 h. Saturated ammonium chloride solution was added (10 ml) and the resulting solution was extracted with diethyl ether (3 x 10 ml). The combined organic extracts were dried, filtered and concentrated to give starting material (0.039 g, 75 %).

9.5 Experimental For Chapter 5

N-Benzyloxycarbonyl-L-threonine (174)[172]

L-Threonine (10 g, 0.084 mol) and sodium hydroxide pellets (8.4 g, 0.21 mol) were dissolved in water (50 ml). To this solution was added benzyl chloroformate (17.6 g, 14.3 ml, 0.1 mol) at 0 °C in three equal portions 5 m apart. The solution was then allowed to warm to room temperature and left stirring for 12 h. After this time the reaction mixture was washed with diethyl ether (3 x 30 ml), acidified to pH 1 with 6 M HCl and extracted with ethyl acetate (4 x 50 ml). The combined organic extracts were dried, filtered and evaporated to give a thick oil. Crystallisation was induced by careful addition of a 1:1 mixture of ethyl acetate and petroleum ether (40 - 60 °C) to give the product as a white solid (18.1 g, 85 %).



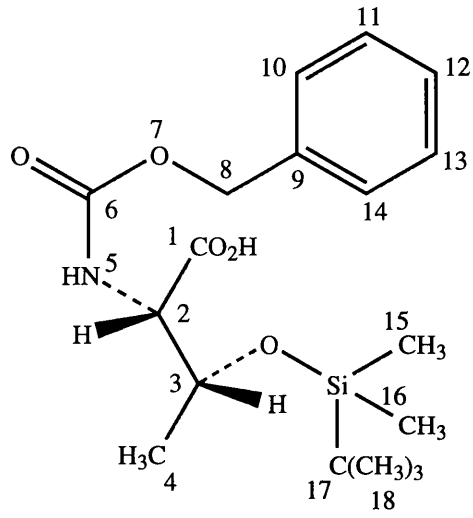
174

m.p. 105 - 106 °C (lit.[172] 101 - 102 °C); $[\alpha]_D^{22}$ -5.7 ° (*c* 2 in ethanol), (lit.,[172] -5.9 ° (*c* 2 in ethanol)); (Found: C, 56.8; H, 6.0; N, 5.5; M^+, 253.0962. $C_{12}H_{15}NO_5$ requires C, 56.9; H 5.9; N 5.5 %; M^+, 253.0950); ν_{max} (nujol) / cm^{-1} 3404 (br, O-H stretch), 2923 & 2854 (s, C-H stretch), 1717 (s, acid C=O stretch & O-CO-N stretch), 1660 (s, N-H bending), 1612 (m, aromatic ring), 1565 (s, aromatic ring),

1463 (s, aromatic ring), 760 (aromatic C-H bending), 694 (aromatic C-H bending); δ_H (200 MHz, D_6 DMSO) 1.04 (3 H, d, *J* 6.3, 4 - Me), 3.92 (1 H, dd, *J* 8.9, 3.3, 2 - H), 3.99 - 4.06 (1 H, m, 3 - H), 4.99 (2 H, s, 8 - H), 6.90 (1 H, d, *J* 8.9, 5 - H), 7.26 (5 H, m, aromatic H); δ_C (50 MHz, D_6 DMSO) 20.5 (4 - C), 60.1 (2 - C), 65.7 (8 - C), 66.6 (3 - C), 127.3, 127.8, 127.9, 128.5 (all aromatic C), 137.1 (9 - C), 156.6 (6 - C), 172.5 (1 - C); *m/z* 253 (M^+, 0.2 %), 209 (3.8), 148 (9.6), 108 (16.7), 91 (100), 79 (22.8), 65 (12.6).

***N*-Benzyloxycarbonyl-*O*-(*tert*-butyldimethylsilyl)-L-threonine (175)**[173]

To a solution of imidazole (2.42 g, 36 mmol) and the L-threonine derivative (3.0 g, 12 mmol) in dry DMF (10 ml) was added *tert* - butyldimethylsilyl chloride (5.38 g, 36 mmol) in dry DMF (10 ml) at 0 °C under nitrogen. The mixture was warmed to room temperature and stirred for 14 h. The reaction mixture was poured into ice water (50 ml) and extracted with diethyl ether (3 x 50 ml). The organic extract was dried, filtered and concentrated. The residue was dissolved in THF (10 ml) and 0.5 M KOH (10 ml), stirred for 4 h and washed with diethyl ether (3 x 40 ml) to remove organic impurities. The aqueous layer was acidified to pH 3 with 1 M HCl and extracted with diethyl ether (3 x 50 ml). The combined organic layers were washed with brine (50 ml), dried, filtered and concentrated to give a crude solid. Careful trituration with hexane gave the title compound as white crystals (2.83 g, 64 %).



175

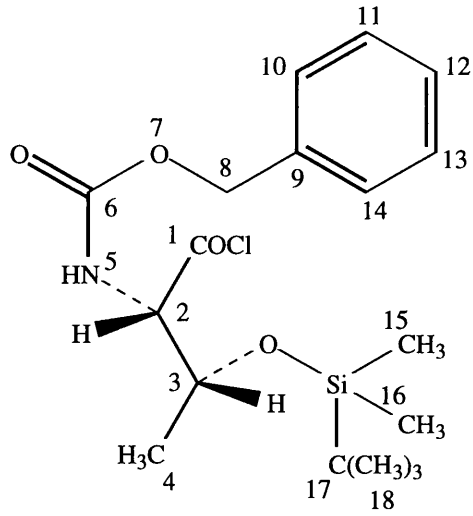
m.p. 146 - 148 °C (lit.[173] 150.5 - 152.5 °C); $[\alpha]_D^{22}$ +12.1 ° (*c* 1 in chloroform), (lit., [173] +13.2 ° (*c* 1 in chloroform)); (Found: C, 58.6; H, 7.9; N, 3.9; M^+, 367.1801. $C_{18}H_{29}NO_5Si$ requires C, 58.9; H 7.9; N 3.8 %; M^+, 367.1815); ν_{max} (nujol) / cm^{-1} 2925 & 2854 (s, C-H stretch), 2725 (w, acid O-H stretch), 1755 (s, acid C=O stretch) 1737 (s, O-CO-N stretch), 1688 (s, N-H bending), 1526 (s, aromatic ring), 1458 (s, aromatic ring), 1251 (m, Si-O), 745 (aromatic C-H bending), 696 (aromatic C-H bending); δ_H (200 MHz, $CDCl_3$) -0.03 (3 H, s, 15 - Me / 16 - Me), 0.00 (3 H, s, 15 - Me / 16 - Me), 0.78 (9 H, s, 18 - Me), 1.14 (3 H, d, *J* 6.2, 4 - Me), 4.27 (1 H, dd, *J* 9.2, 1.9, 2 - H), 4.42 (1 H, m, 3 - H), 5.08 (2 H, s, 8 - H), 5.42 (1 H, d, *J* 9.1, 5 - H), 7.30 (5 H, m, aromatic H), 10.74 (1 H, bs, -$CO_2\underline{H}$); δ_C (50 MHz, $CDCl_3$) -6.6 (C - 15 / C - 16), -6.1 (C - 15 / C - 16), 16.2 (17 - C), 18.8 (18 - C), 24.0 (4 - C), 58.0 (2 - C), 65.9 (8 - C), 67.0 (3 - C), 126.6 (aromatic C), 126.8 (aromatic C), 129.0 (aromatic C), 134.5 (9 - C), 155.1 (6 - C), 174.2 (1 - C); *m/z* 367 (M^+, 0.1 %), 202 (5.1), 159 (22.7), 130 (24.3), 91 (100), 73 (43.7).

Attempted Synthesis of *N*-Benzyloxycarbonyl-*O*-(*tert*-butyldimethylsilyl)-L-threonine chloride (176)

The amino acid derivative (**175**) (0.5 g, 1.36 mmol) was dissolved in dry THF (20 ml). Oxalyl chloride (0.173 g, 0.12 ml, 1.36 mmol) was added and the mixture was stirred at room temperature for 16 h. After this time the solvent was removed under reduced pressure to give starting material (0.46 g, 92 %).

N-Benzyloxycarbonyl-*O*-(*tert*-butyldimethylsilyl)-L-threonine acid chloride (176)

The amino acid derivative (**165**) (1.14 g, 3 mmol) and cyanuric chloride (0.369 g, 2 mmol) were dissolved in dry acetone (20 ml) under nitrogen. Triethylamine (0.304 g, 0.42 ml, 3 mmol) was added and the solution was stirred for 10 h at room temperature. The solvent was removed under reduced pressure to give a 2:1 mixture of diastereoisomers (1.08 g, 93 %). This mixture was used without further purification in the next stage of the synthesis.



176

δ_H (200 MHz, $CDCl_3$) -0.02 (3 H, s, 15 - Me / 16 - Me), 0.00 (3 H, s, 15 - Me / 16 - Me), 0.78 & 0.80 (9 H, s, 18 - Me), 1.16 - 1.28 (3 H, m, 4 - Me), 4.20 (1 H, bd, *J* 6.6, 2 - H), 4.39 (1 H, m, 3 - H), 4.48 & 5.06 (2 H, s, 8 - H), 5.59 (1H, d, *J* 9.6, 5 - H), 7.27 (5 H, m, aromatic H), δ_C (50 MHz, $CDCl_3$) -7.2 & -6.9 (C - 15 / C - 16), -6.0 & -5.8 (C - 15 / C - 16), 16.0 & 16.1 (17 - C), 18.7 & 19.2 (18 - C), 23.9 & 24.0 (4 - C), 62.7 (2 - C), 65.6 (8 - C), 65.8 & 66.3 (3 - C), 125.3 - 127.0 (aromatic C), 134.4 & 135.8 (9 - C), 151.8 & 154.9 (6 - C), 164.3 & 167.8 (1 - C).

Preparation of Diazomethane[200]

Diazomethane was prepared according to the procedure given in "Advanced Practical Organic Chemistry".[200]

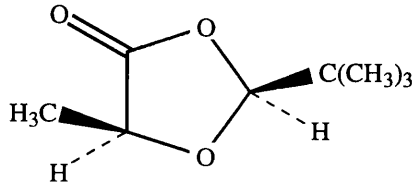
Attempted Synthesis of *N*-Benzyloxycarbonyl-1-diazo-*O* -(*tert*-butyldimethylsilyl)-L-threonine chloride (182)

The acid chloride (**176**) (1.06 g, 2.75 mmol) was dissolved in dry diethyl ether (5 ml) under nitrogen. A solution of diazomethane was added at 0 °C dropwise until a yellow colour persisted. The mixture was allowed to warm to room temperature and stirred for a further 3 h. After this time dilute acetic acid was added to remove the yellow colour, and the solution was dried, filtered and concentrated to give a thick yellow oil. Attempted purification of this oil (neutral alumina; diethyl ether) resulted in decomposition.

9.6 Experimental For Chapter 6

(2*S*,5*S*)-2-(*tert* - Butyl)-5-(methyl)-1,3-dioxolan-4-one (191)[183]

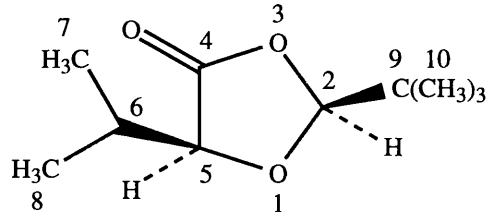
Lactic acid (**190**) (10 g, 0.11 mol) trimethylacetaldehyde (19.1 g, 24 ml, 0.22 mol) and *p*-toluenesulfonic acid (0.2 g) were dissolved in pentane (150 ml). Two drops of conc. H_2SO_4 were added and the mixture was heated at reflux in a Dean Stark apparatus for 6 h. After this time the reaction mixture was washed with water (3 x 100 ml) and brine (75 ml) and the organic layer was dried, filtered and concentrated to give the product (9.5 g, 54 %, 92 % de). The analytical data for this compound are identical to those given by Seebach *et al.*[183]



191

(2*S*,5*S*)-2-(*tert*-Butyl)-5-(isopropyl)-1,3-dioxolan-4-one (187)[183]

(*S*)-(+)-2-Hydroxy-3-methylbutanoic acid (**186**) (4 g, 33.9 mmol) trimethylacetaldehyde (5.83 g, 7.36 ml, 67.8 mmol) and *p*-toluenesulfonic acid (0.20 g) were dissolved in pentane (80 ml). One drop of conc. H_2SO_4 was added and the mixture was then heated at reflux in a Dean Stark apparatus for 3 h. After this time the reaction mixture was washed with water (3 x 40 ml) and brine (40 ml) and the organic layer was dried, filtered and concentrated to give the product (5.39 g, 85 %, 99 % de).



187

$[\alpha]_D^{22}$ -7.1 ° (*c* 3 in chloroform), (lit.,[183] -6.9 ° (*c* 2.9 in chloroform)); (Found: M^+, 186.1257. $C_{10}H_{18}O_3$ requires M^+, 186.1256); ν_{max} (thin film) / cm^{-1} 2938, 2909 & 2878 (s, C-H stretch), 1798 (s, C=O stretch), 1366 (s, -OCOC-H stretch), 1090 (C-O stretch); δ_H (200 MHz, $CDCl_3$) 0.99 (9 H, s, 10 - Me), 1.12 (6 H, d, *J* 7.0, 7 & 8 - Me), 2.12 - 2.26 (1 H, m, 6 - H), 4.10 (1 H, dd, *J* 4.0, 1.3, 5 - H), 5.10 (1 H, d, *J* 1.3, 2 - H); δ_C (50 MHz, $CDCl_3$) 17.1 (7 / 8 - C), 18.5 (7 / 8 - C), 23.6 (10 - C), 29.6 (6 - C), 34.4 (9 - C), 79.5 (5 - C), 108.9 (2 - C), 172.9 (4 - C); *m/z* 186 (M^+, 0.1 %), 129 (21.7), 101 (92.1), 85 (10.8), 73 (100), 57 (91.8), 43 (47.1).

Attempted Synthesis of (2*S*,5*R*)-2-(*tert*-Butyl)-5-(methyl)-5-(isopropyl)-1,3-dioxolan-4-one

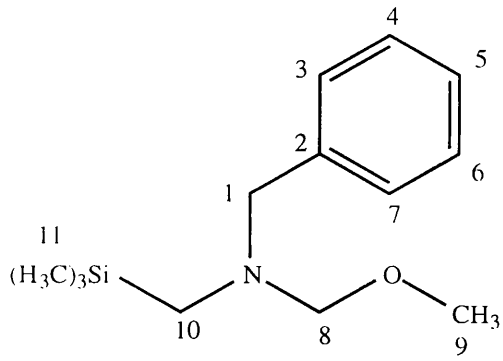
To a solution of diisopropylamine (0.23 ml, 1.6 mmol) in dry THF (7 ml) under nitrogen at -78 °C was added *n*-butyl lithium (1.6 M in hexanes, 0.98 ml, 1.5 mmol) dropwise via syringe. The solution was stirred for 30 min with no further cooling. The resulting solution of LDA was cooled to -78 °C and a solution of the dioxolanone (**187**) (0.19 g, 1.62 mmol) in THF (1 ml) was added dropwise via syringe. After stirring for 1 h at -78 °C, methyl iodide (0.33 g, 0.14 ml, 2.3 mmol) was added dropwise. The mixture was allowed to warm to room temperature with continuous stirring for 1 h before being quenched by the addition of saturated NH_4Cl solution (2 ml). The reaction mixture was extracted with diethyl ether (4 x 20 ml) and the combined organic extracts were dried, filtered and concentrated to give a brown oil. No characterisable products could be isolated from this oil. This

reaction was repeated using the dioxolanone **191** and other nucleophiles as shown in **table 14**. None gave any characterisable products.

9.7 Experimental For Chapter 7

N-Benzyl-*N*-(trimethylsilylmethyl)aminomethyl methyl ether (216)

To a suspension of paraformaldehyde (5 g) in methanol (50 ml) was added *N*-(trimethylsilylmethyl)benzylamine (6.16 g, 31.8 mmol) dropwise at 0 °C. The mixture was stirred for 3 h after which time potassium carbonate was added and the oily layer was separated. The residue was extracted with diethyl ether (3 x 50 ml) and the combined organic extracts were dried over anhydrous potassium carbonate. Filtration and solvent evaporation gave the title compound as a colourless oil (6.17 g, 82 %). Analytical data was identical to that previously published.[184]



216

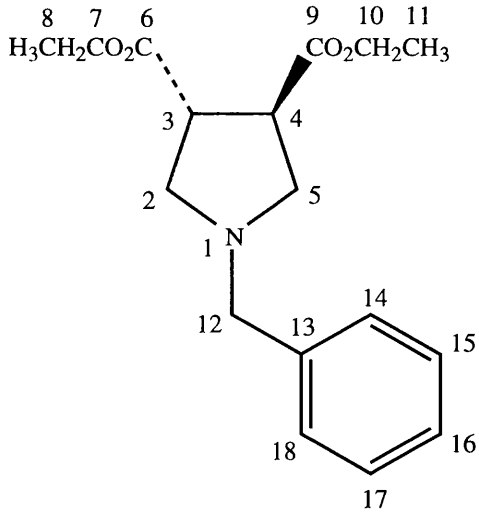
Cycloaddition Procedure 9.7.1[184]

Caesium fluoride was heated under vacuum at 40°C for 2 h. Dry THF, the aminomethyl methyl ether (**216**) and the dienophile were then added under a nitrogen atmosphere. To the resulting solution, trimethylsilyl triflate was added and the reaction mixture was stirred at 60 °C for 18 h. The flask was cooled to 0 °C and quenched with 15% NaOH. The organic layer was separated and the aqueous phase

was extracted with diethyl ether. The combined organic extracts were dried, filtered and concentrated to give the crude product.

Diethyl (±)-1-benzylpyrrolidine-3,4-dicarboxylate (217)[184]

Caesium fluoride (0.749 g, 4.9 mmol), the aminomethyl ether (**216**) (5.80 g, 24.5 mmol), diethyl fumarate (4.21 g, 4 ml, 24.5 mmol) and trimethylsilyl triflate (1.09 g, 0.89 ml, 4.9 mmol) were treated in THF (90 ml) according to **procedure 9.7.1** to give the crude pyrrolidine (**217**). This was purified by column chromatography (silica; diethyl ether / hexane; 25:75) to give the title compound as a colourless oil (4.89 g, 66 %).



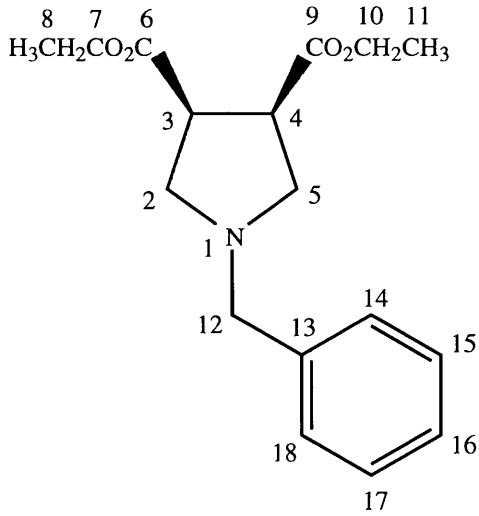
217

(Found: M^+, 305.1627. $C_{17}H_{23}NO_4$ requires M^+, 305.1627); Rf 0.31 (diethyl ether / hexane; 25:75); ν_{max} (thin film) / cm^{-1} 2981 & 2936 (s, C-H stretch), 2800 (s, N-CH_2-, stretch), 1732 (s, C=O stretch), 1604 (w, aromatic ring), 1495 (m, aromatic ring), 741 (m, monosubstituted benzene ring, C-H bend), 700 (m, monosubstituted benzene ring, C-H bend); δ_H (200 MHz, $CDCl_3$) 1.24 (6 H, t, *J* 7.1, 8 - Me & 11 - Me), 2.77 - 2.94 (4 H, m, 2 - H & 5 - H), 3.39 - 3.50 (2 H, m, 3 - H & 4 - H), 3.60 (2 H, s, 12 - H), 4.15 (4 H, q, *J* 7.1, 7 - H & 10 - H), 7.29 (5 H, m, aromatic H); δ_C (50

MHz, $CDCl_3$) 14.2 (8 - C & 11 - C), 45.5 (3 - C & 4 - C), 56.6 (2 - C & 5 - C), 61.0 (7 - C & 10 - C / 12 - C), 61.3 (7 - C & 10 - C / 12 - C), 127.1 - 128.6 (aromatic C), 138.4 (13 - C), 175.5 (6 - C & 9 - C); *m/z* 305 (M^+, 4.9), 276 (3.6), 260 (13.9), 230 (7.8), 214 (9.9), 168 (38.6), 140 (18.6), 91 (100).

Diethyl *meso*-1-benzylpyrrolidine-3,4-dicarboxylate (218)

Caesium fluoride (0.602 g, 3.97 mmol), the aminomethyl ether (**216**) (4.7 g, 19.8 mmol), diethyl maleate (3.41 g, 3.2 ml, 19.8 mmol) and trimethylsilyl triflate (0.88 g, 0.72 ml, 3.97 mmol) were treated in THF (100 ml) according to **procedure 9.7.1** to give the crude pyrrolidine (**218**). This was purified by column chromatography (silica; diethyl ether / hexane; 25:75) to give the title compound as a colourless oil (5.32 g, 88 %).



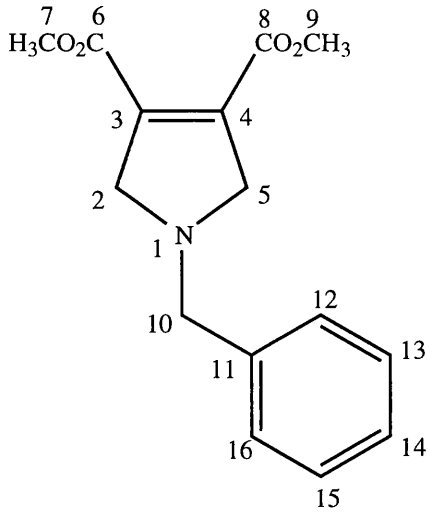
218

(Found: M^+, 305.1622. $C_{17}H_{23}NO_4$ requires M^+, 305.1627); Rf 0.31 (diethyl ether / hexane; 25:75); ν_{max} (thin film) / cm^{-1} 2980 & 2906 (s, C-H stretch), 2810 (s, N-CH_2-, stretch), 1732 (s, C=O stretch), 1604 (w, aromatic ring), 1494 (m, aromatic ring), 742 (m, monosubstituted benzene ring, C-H bend), 700 (m, monosubstituted benzene ring, C-H bend); δ_H (200 MHz, $CDCl_3$) 1.21 (6 H, t, *J* 7.1, 8 - Me & 11 -

Me), 2.61 - 2.69 (2 H, m, 2 - H_a & 5 - H_a), 3.03 - 3.07 (2 H, m, 2 - H_b & 5 - H_b), 3.18-3.26 (2H, m, 3 - H & 4 - H), 3.63 (2 H, s, 12 - H), 4.09 (2 H, q, *J* 7.1, 7 - H & 10 - H), 7.29 (5 H, m, aromatic H); δ_C (50 MHz, $CDCl_3$) 14.0 (8 / 11 - C), 14.1 (8 / 11 - C), 45.3 (3 - C & 4 - C), 56.1 (2 - C & 5 - C), 59.9 (7 - C / 10 - C / 12 - C), 60.6 (7 - C / 10 - C / 12 - C), 61.2 (7 - C / 10 - C / 12 - C), 127.1 - 129.8 (aromatic C), 138.5 (13 - C), 165.2 (6 - C / 9 - C), 172.5 (6 - C / 9 - C); *m/z* 305 (M^+, 1.3), 276 (2.7), 260 (14.5), 230 (5.1), 214 (19.5), 168 (13.0), 140 (8.6), 91 (100).

Dimethyl 1-benzyl-3-pyrroline-3,4-dicarboxylate (219)

Caesium fluoride (0.573 g, 3.77 mmol), aminomethyl ether (**216**) (4.47 g, 18.9 mmol), dimethyl acetylenedicarboxylate (2.68 g, 2.32 ml, 18.9 mmol) and trimethylsilyl triflate (0.837 g, 0.68 ml, 3.77 mmol) were treated in THF (100 ml) according to **procedure 9.7.1** to give the crude pyrroline (5.06 g, 98 %). No further purification was carried out.



219

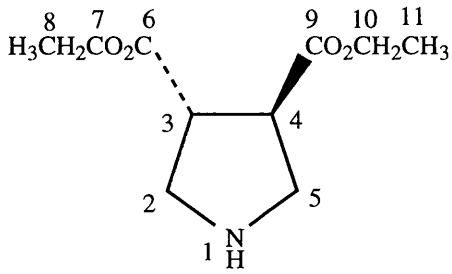
(Found: M^+, 275.1146. $C_{15}H_{17}NO_4$ requires M^+, 275.1157); Rf 0.42 (diethyl ether / hexane; 25:75); ν_{max} (thin film) / cm^{-1} 2952 & 2898 (s, C-H stretch), 2818 (s, N-CH_2-, stretch), 1738 (s, C=O stretch), 1622 ($\alpha\alpha'\beta\beta'$ unsaturated alkene), 1568 (w, aromatic ring), 1496 (m, aromatic ring), 746 (m, monosubstituted benzene ring, C-H bend), 700 (m, monosubstituted benzene ring, C-H bend); δ_H (200 MHz, $CDCl_3$) 3.77 (6 H, s, 7 & H & 9 - H), 3.79 (2 H, s, 10 - H), 3.82 (4 H, s, 2 - H & 5 - H), 7.33 (5 H, s, aromatic H); δ_C (50 MHz, $CDCl_3$) 52.2 (7 - C & 9 - C), 59.7 (10 - C), 60.6 (2 - C & 5 - C), 127.3 - 128.6 (aromatic C), 137.1 (3 - C & 4 - C), 138.4 (11 - C), 164.0 (6 - C & 8 - C); *m/z* 275 (M^+, 1.8), 242 (5.0), 184 (6.1), 91 (100), 59 (7.7).

Debenzylation Procedure 9.7.2

The benzylated amine was dissolved in a solution of formic acid in dry methanol (5 %) under nitrogen. To this solution was added 10 % Pd / C. The mixture was stirred at room temperature for 18 h. After this time the reaction mixture was filtered through Celite to remove the catalyst and the Celite was washed with methanol and water. The solvent was then removed under reduced pressure to give the crude product. Conc. ammonia solution was then added dropwise to the residue and the filtrate extracted with ethyl acetate. The combined organic extracts were dried, filtered and evaporated. The remaining residue was purified by column chromatography (silica; chloroform / methanol / triethylamine; 85:14:1) to give the pure amine.

Diethyl (±)-pyrrolidine-3,4-dicarboxylate (224)

The amine (**217**) (0.40 g, 1.31 mmol), in 5% formic acid in methanol (10 ml) and 10 % Pd / C (0.40 g) were treated according to **procedure 9.7.2** to give the title compound (0.166 g, 59 %).

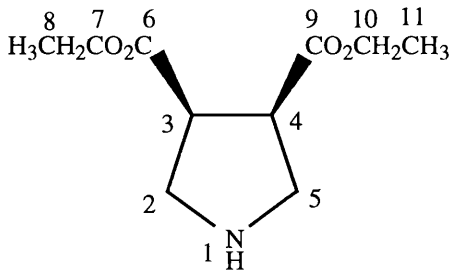


224

(Found: M^+, 215.1154. $C_{10}H_{17}NO_4$ requires M^+, 215.1157); ν_{max} (thin film) / cm^{-1} 3318 (w, N-H stretch), 2942 & 2908 (s, C-H stretch), 1728 (s, C=O stretch), 1304 & 1232 (s, C-O stretch); δ_H (200 MHz, $CDCl_3$) 1.27 (6 H, t, *J* 7.1, 8 - Me & 11 - Me), 2.51 (1 H, s, N-H), 3.04 - 3.33 (6 H, m, 2 - H, 3 - H, 4 - H & 5 - H), 4.17 (4 H, q, *J* 7.1, 7 - H & 10 - H); δ_C (50 MHz, $CDCl_3$) 14.2 (8 - C & 11 - C), 48.3 (3 - C & 4 - C), 52.1 (2 - C & 5 - C), 61.0 (7 - C & 10 - C). 173.9 (6 - C & 9 - C); *m/z* 215 (M^+, 2.9), 170 (32.6), 142 (20.5), 96 (25.2), 68 (100), 43 (44.1).

Diethyl *meso*-pyrrolidine-3,4-dicarboxylate (225)

The amine (**218**) (0.43 g, 1.42 mmol), in 5% formic acid in methanol (10 ml) and 10 % Pd / C (0.43 g) were treated according to **procedure 9.7.2** to give the title compound (0.166 g, 67 %).



225

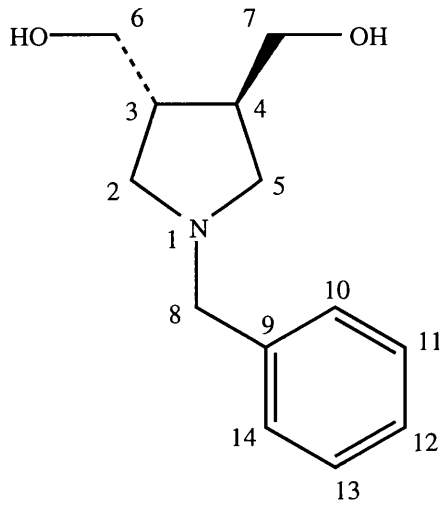
(Found: M^+, 215.1160. $C_{10}H_{17}NO_4$ requires M^+, 215.1157); ν_{max} (thin film) / cm^{-1} 294 & 2907 (s, C-H stretch), 1737 (s, C=O stretch); δ_H (200 MHz, $CDCl_3$) 1.18 (6 H, t, *J* 7.1, 6 - Me & 11 - Me), 3.08 - 3.32 (6 H, m, 2 - H, 3 - H, 4 - H & 5 - H), 4.06 (4 H, q, *J* 7.1, 7 - H & 10 - H); δ_C (50 MHz, $CDCl_3$) 14.0 (8 - C & 11 - C), 47.2 (3 - C & 4 - C), 50.0 (2 - C & 5 - C), 60.7 (7 - C & 10 - C). 172.4 (6 - C & 9 - C); *m/z* 215 (M^+, 2.5), 170 (13.0), 141 (12.3), 96 (8.2), 87 (8.9), 68 (100), 43 (40.3).

Reduction Procedure 9.7.3

A solution of DIBAL in toluene was added, with stirring and ice cooling via syringe over 30 min to a solution of the diester in dry toluene under nitrogen. The reaction mixture was stirred at room temperature for 1 h. Ethyl acetate was then added to consume the excess DIBAL. After a further 5 min acetone and Celite were added. Methanol was then added dropwise with ice cooling. The mixture was shaken vigorously until gelling occurred (5 min), then water was added. The mixture was shaken vigorously again to break up the gel, then stirred at room temperature for 1.5 h. The resulting suspension was filtered, and the solid residue was washed, firstly with hot water then with hot methanol. The combined filtrates were concentrated under reduced pressure to give the crude product which was purified by column chromatography (silica; chloroform / methanol / triethylamine; 85:14:1) to give the pure aminodiol.

(±)-3,4-Bishydroxymethyl-1-benzylpyrrolidine (226)

The amine (**217**) (2.0 g, 6.55 mmol), DIBAL (1.5 M in toluene, 39 ml, 58.5 mmol) and dry toluene (5 ml) were treated according to **procedure 9.7.3**. Work-up using ethyl acetate (7 ml), acetone (35 ml), Celite (7.1 g) and methanol (7 ml) gave the title compound after purification (0.772 g, 53 %).

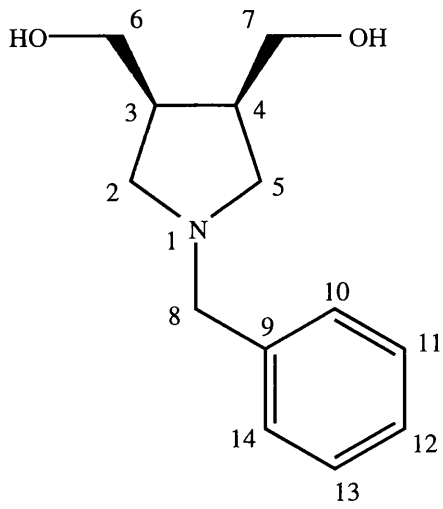


226

m.p. 74 - 77 °C; (Found: M^+, 221.1408. $C_{13}H_{19}NO_2$ requires M^+, 221.1416); ν_{max} (nujol) / cm^{-1} 3366 (s, O-H stretch), 2902 (s, C-H stretch), 2848 (s, N-CH_2-, stretch), 1602 (s, aromatic ring), 1584 (s, aromatic ring), 1496 (m, aromatic ring), 1044 (s, C-O stretch), 756 (m, monosubstituted benzene ring, C-H bend), 700 (m, monosubstituted benzene ring, C-H bend); δ_H (200 MHz, $CDCl_3$) 2.16 (2 H, m, H - 3 & H - 4), 2.36 - 2.43 (2 H, m, 2 - H_a & 5 - H_a), 2.74 - 2.82 (2 H, m, 2 - H_b & 5 - H_b), 3.47 - 3.58 (4 H, m, 6 - H & 7 - H), 4.61 (2 H, s, 8 - H), 4.75 (2 H, bs, 2 x O-H), 7.29 (5 H, bs, aromatic H); δ_C (50 MHz, $CDCl_3$) 44.3 (C - 3 & C - 4), 56.9 (2 - C & 5 - C), 60.1 (8 - C), 64.9 (6 - C & 7 - C), 127.4 - 130.5 (aromatic C), 137.2 (9 - C); *m/z* 221 (M^+, 8.0 %), 132 (6.4), 112 (12.3), 91 (100), 55 (8.2).

meso-3,4-Bishydroxymethyl-1-benzylpyrrolidine (227)

The amine (**218**) (0.603 g, 2.18 mmol), DIBAL (1.5 M in toluene, 13.1 ml, 19.6 mmol) and dry toluene (5 ml) were treated according to **procedure 9.7.3**. Work-up using ethyl acetate (2 ml), acetone (10 ml), Celite (1.8 g) and methanol (2 ml) gave the title compound as an oil after purification (0.286 g, 59 %).



227

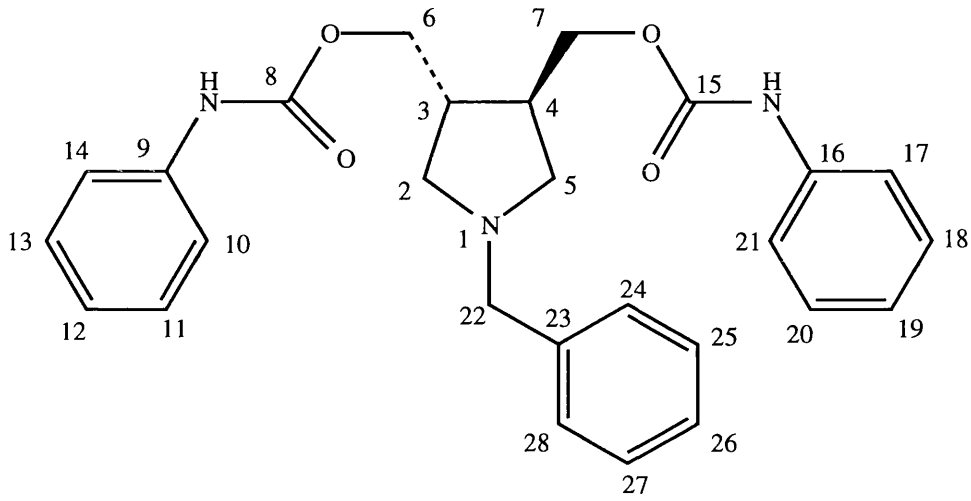
(Found: M^+, 221.1410. $C_{13}H_{19}NO_2$ requires M^+, 221.1416); ν_{max} (thin film) / cm^{-1} 3342 (s, O-H stretch), 2902 (s, C-H stretch), 2854 (s, N-CH_2-, stretch), 1600 (s, aromatic ring), 1586 (s, aromatic ring), 1495 (m, aromatic ring), 1036 (s, C-O stretch), 756 (m, monosubstituted benzene ring, C-H bend), 702 (m, monosubstituted benzene ring, C-H bend); δ_H (200 MHz, $CDCl_3$) 2.19 - 2.27 (2 H, m, H - 3 & H - 4), 2.48 (2 H, m, 2 - H_a & 5 - H_a), 2.69 - 2.77 (2 H, m, 2 - H_b & 5 - H_b), 3.52 (2 H, s, 8 - H), 3.55 - 3.70 (2 H, bs, 2 x O-H), 7.25 (5 H, bs, aromatic H), δ_C (50 MHz, $CDCl_3$) 39.7 (C - 3 & C - 4), 55.1 (2 - C & 5 - C), 58.7 (8 - C), 60.2 (6 - C & 7 - C), 125.7 - 127.3 (aromatic C), 136.4 (9 - C); *m/z* 221 (M^+, 4.5 %), 132 (12.5), 112 (16.9), 91 (100), 55 (5.6).

Carbamate Formation Procedure 9.7.4

A mixture of the aminodiol, phenyl isocyanate and 2 drops of dibutyltin diacetate were stirred at room temperature for 18 h in dichloromethane. The reaction was then concentrated and the oily solid thus obtained was subjected to column chromatography (silica; diethyl ether / hexane; 1:1).

(±)-3,4-Bis(phenylaminocarbonyloxymethyl)-1-benzylpyyrolidine (228)

The aminodiol (**226**) (0.05 g, 0.23 mmol), phenyl isocyanate (0.068 g, 0.06 ml, 0.58 mmol) and dibutyltin diacetate were treated according to **procedure 9.7.4** in dichloromethane (5 ml) to give the product as a white solid after purification (0.012 g, 11 %).



228

Rf 0.20 (diethyl ether / hexane; 1:1); ν_{max} (nujol) / cm^{-1} 2922 (s, C-H stretch), 2852 (s, N-CH_2-, stretch), 1702 (s, R-O-CO-N stretch), 1600 (s, aromatic ring), 1594 (s, aromatic ring), 1522 (m, aromatic ring), 752 (m, monosubstituted benzene ring, C-H bend), 696 (m, monosubstituted benzene ring, C-H bend); δ_H (200 MHz, $CDCl_3$) 2.17 - 2.26 (2 H, m, H - 3 & H - 4), 2.34 - 2.42 (2 H, m, 2 - H_a & 5 - H_a), 2.65 - 2.73 (2 H, m, 2 - H_b & 5 - H_b), 3.62 (2 H, s, 22 - H), 3.95 - 4.14 (4 H, m, 6 - H & 7 - H), 6.86 - 7.34 (15 H, m, aromatic H), δ_C (50 MHz, $CDCl_3$) 47.1 (C - 3 & C - 4), 58.0 (2 - C & 5 - C), 61.2 (22 - C), 67.9 (6 - C & 7 - C), 119.9, 120.4, 123.8, 124.1, 128.4, 129.4, 129.8 & 130.2 (all aromatic C), 139.3 & 140.1 (aromatic quaternary carbons), 155.9 (C - 8 & C - 15); *m/z* 212 (13.0), 151 (7.3), 119 (11.6), 93 (100), 65 (22.2).

Attempted Synthesis of *meso* -3,4-bis(phenylaminocarbonyloxymethyl)-1-benzylpyrrolidine (232)

Procedure 9.7.4 failed to produce any isolable or characterisable product.

Attempted Synthesis of the Macrocyclic Adduct of 3,3-dimethylglutaric anhydride (237) and (±)-3,4-bishydroxymethyl-1-benzylpyrrolidine (226)

(**i**) 3,3-Dimethylglutaric anhydride (0.053 mg, 0.37 mmol) was added to a solution of the aminodiol (**226**) (0.075 mg, 0.34 mmol) in dry DME (10 ml) under nitrogen. The solution was heated at 40 °C for 1 h and then stirred at room temperature for a further 48 h. After this time TLC (chloroform / methanol / conc. NH_3; 85:14:1) indicated the formation of a new species (Rf 0.0) assumed to be the zwitterion. Di-2-pyridyl disulfide (0.123 g, 0.56 mmol) and triphenylphosphine (147 mg, 0.56 mmol) were added and the solution was stirred at room temperature for 24 h when TLC (chloroform / methanol / conc. NH_3; 85:14:1) suggested formation of the thioester (Rf 0.33). The solution was diluted with dry DME (50 ml) and heated at reflux temperature for 24 h. No products could be isolated from the reaction mixture.

(**ii**) To a solution of the aminodiol (**226**) (0.075 g, 0.34 mmol) in dry THF (5 ml) was added *n*-butyl lithium (0.22 ml, 0.34 mmol) at 0 °C. The mixture was stirred for 2 h. TLC (chloroform / methanol / conc. NH_3; 85:14:1) suggested formation of the lithium alkoxide (Rf 0.0). This solution was then added dropwise to a solution of 3, 3-dimethylglutaric anhydride (0.053 mg, 0.37 mmol) in dry THF (5

ml) under nitrogen. The resulting solution was stirred at room temperature for 16 h, after which time diethylphosphoryl chloride (0.059 g, 0.05 ml, 0.34 mmol) and DMAP (4 mg, 0.03 mmol) were added. After stirring for 24 h, the reaction mixture was heated at reflux for a further 24 h. No products could be isolated from the reaction mixture.

(**iii**) A mixture of the aminodiol (**226**) (0.075 g, 0.34 mmol) and dibutyltin oxide (0.101 g, 0.41 mmol) in dry benzene (8 ml) under nitrogen was heated at reflux temperature for 24 h with continuous removal of water by the use of 4 Å molecular sieves (*ca.* 2 g). After cooling, an insoluble and unidentifiable solid was obtained. No product could be detected by TLC (chloroform/methanol/conc. NH_3; 85:14:1).

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