Synthesis of Novel Biologically Active Tropanes

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A thesis submitted for the Degree of Doctor of Philosophy in the Faculty of Science at the University of Leicester

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STATEMENT

The accompanying thesis submitted for the degree of PhD entitled "Synthesis of Novel Biologically Active Tropanes" is based on work conducted by the author in the Department of Chemistry at the University of Leicester mainly during the period between October 1995 and October 1998.

All work recorded in this thesis is original unless otherwise acknowledged in the text or references.

None of the work has been submitted for another degree in this or any other university.

Signed: ..... Date: 6th July 1999.

To Mum and Dad

With love

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Firstly, I would like to express my gratitude to John Malpass for his guidance and support over the past three years.

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To them I dedicate this thesis.

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ABSTRACT

SYNTHESIS OF NOVEL BIOLOGICALLY ACTIVE TROPANES

BY ANNA L. WALLIS

Diels-Alder cycloaddition between 1,3-cycloheptadiene (or its derivatives) and benzyl nitrosoformate produced intermediates from which the N-methyl-8-aza-bicyclo-[3.2.1]octane (tropane) ring system, 8-aza-bicyclo-[3.2.1]octane (nortropane) ring system and a range of oxygenated and 6,7-dehydro- derivatives were constructed.

Homoepibatidine, a homologue of the alkaloid epibatidine, was synthesised using a Heck-type coupling reaction to introduce a chloropyridyl group at the C_6 position of the tropane ring system. ^1H NMR spectroscopy was used to prove the *exo*-stereochemistry of the chloropyridyl moiety. Homoepibatidine is one of the most potent epibatidine analogues to be synthesised to date. Indeed, nicotinic receptor binding assays have shown that the activity of homoepibatidine is comparable to that of epibatidine itself.

The first synthesis of 1-hydroxytropacocaine, an alkaloid recently isolated from *Erythroxylum novogranatense* variants, is described. Adaptation of the synthesis produced 1-hydroxynortropacocaine, which was subsequently found to occur in nature. 1-hydroxytropanes exist as mixtures of tautomers in which the bicyclic hemi-aminal is in equilibrium with the monocyclic amino-ketone. The tautomeric preferences of 1-hydroxytropacocaine were studied using VT NMR. It was established that 1-hydroxytropacocaine exists predominantly as the bicyclic tautomer, although the ratio of the bicyclic hemiaminal and monocyclic amino-ketone could not be measured quantitatively. Similar tautomeric preferences were observed for 1-hydroxynortropacocaine.

Two biosynthetic routes to 1-hydroxtropanes have been proposed which involve tropinone N-oxides as key intermediates. The first proposal involves thermal or base-induced rearrangement of tropinone N-oxides. The second, alternative route, centres on Meisenheimer rearrangement of tropinone N-oxides. Synthetic routes are developed to key doubly-labelled (^{18}O,^2H) intermediates for use in feeding experiments to investigate these proposals.

CONTENTS

ABBREVIATIONS

The following abbreviations are used throughout this thesis:

°C	degrees centigrade
b.p.	boiling point
br	broad
cm^{-1}	wavenumber
CNS	central nervous system
COSY	correlated spectroscopy
d	doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
dd	doublet of doublets
DEAD	diethyl azodicarboxylate
DEPT	distortionless enhancement by polarisation transfer
DHP	dihydropyran
DIBAH	diisobutylaluminium hydride
DMAP	4-dimethylaminopyridine
DMDO	dimethyldioxirane
DME	ethylene glycol dimethyl ether
DMF	dimethylformamide
DMSO	dimethylsulphoxide
dppb	1,4-*bis*(diphenylphosphine)butane
ee	enantiomeric excess
EI	electron impact
FAB	fast atom bombardment
g	grams
HMPA	hexamethylphosphoric triamide
HMPT	hexamethylphosphorous triamide
hr	hour
Hz	hertz
IR	infra-red
lit.	literature
m	multiplet (NMR); medium (IR)
m.p.	melting point
M^+	molecular ion
MCPBA	*meta*-chloroperoxybenzoic acid
MEM	2-methoxyethoxymethyl chloride
MHz	megahertz
min	minutes
ml	millilitres
mmol	millimole
mol	moles
nAChRs	nicotinic acetylcholine receptors
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
PNS	peripheral nervous system
ppm	parts per million

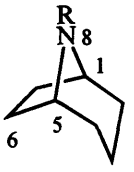
s	singlet (NMR); strong (IR)
t	triplet
TBAF	tetrabutylammonium fluoride
TBDMSCl	*t*-butyldimethylsilylchloride
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THP	tetrahydropyran
TLC	thin layer chromatography
TMS	tetramethylsilane
TMSI	iodotrimethylsilylsilane
v	very
w	weak

Chapter 1

Introduction

1.1 THE TROPANE ALKALOIDS

The tropane alkaloids are a subgroup of the pyrrolidine alkaloids and occur in the plant families Solanaceae, Convolvulaceae, Erythroxylaceae, Euphorbiaceae, Proteaceae and Rhizophoraceae. They are usually hydroxylated derivatives of the tropane (8-methyl-8-azabicyclo[3.2.1]octane) **(1)** or nortropane (8-azabicyclo[3.2.1]-octane) **(2)** skeletons. Many are found as esters derived from relatively simple carboxylic acids (e.g. tropic acid) and many are present in nature as glycosides.

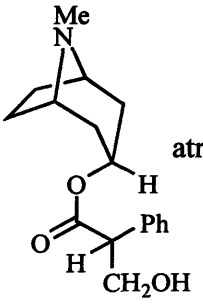


R = Me; tropane **(1)**

R = H; nortropane **(2)**

Figure 1.1

The vast majority of tropane alkaloids were isolated during the late nineteenth and early twentieth centuries, but their pharmacological activity has inspired continuing research focusing mainly on stereochemistry, synthesis and biogenesis.[1] Tropanes can be sub-classified according to the degree of hydroxylation, i.e. from mono- up to penta-hydroxytropanes. In 1833, the first tropane alkaloids, atropine **(3)** and (-)-hyoscyamine, were isolated from *Atropa belladonna* (deadly nightshade), a member of the Solanaceae family.



atropine [(±)-hyoscyamine] **(3)**

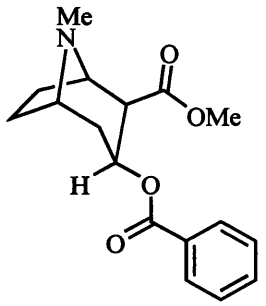
Figure 1.2

Both are tropic acid esters of 3α-hydroxytropane, atropine being the racemic form of (-)-hyoscyamine. The racemisation of (-)-hyoscyamine to atropine is an extremely facile process; heating under vacuum or refluxing in chloroform is sufficient to cause racemisation. It is probable, therefore, that in most instances where atropine has been isolated, it was not present as such in the plant, but that racemisation of hyoscyamine occurred during the isolation process. As a consequence

of its mydriatic properties, atropine was extensively used in ophthalmology, but it has since been replaced by other drugs whose effects are less prolonged. More recently atropine was issued to troops fighting in the Gulf war as an antidote to organophosphate poisoning.

Other species of the Solanaceae family, such as henbane (*Hyoscyamus niger L.*), and the thorn apple (*Datura stramonium L.*), also contain therapeutically important alkaloids. Consequently, these plants have been used for medicinal and ceremonial purposes for centuries. Henbane seeds were used by the Babylonians, three thousand years ago to relieve toothache, and in the Middle Ages the hallucinatory properties of such solanaceous plants were exploited by sorcerers.

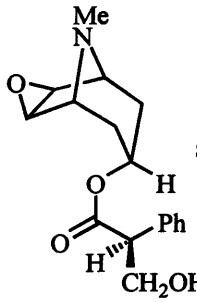
Cocaine **(4)**, a diester of tropan-3β-ol-2β-carboxylic acid, was first isolated in 1862 from the leaves of *Erthroxylum coca lam*, which is indigenous to the higher regions of Peru.[2] In the latter half of the nineteenth century cocaine was used as a local anaesthetic, but because it is highly stimulating to the central nervous system (CNS), its uses today are restricted to surface anaesthesia for the eye, nose and throat.



cocaine (4)

Figure 1.3

Scopolamine **(5)** was first isolated in 1881, under the name (-)-hyoscine, from *Hyoscyamus muticus L.*[3] It also occurs in *Datura metel L.* and, together with hyoscyamine, in various other solanaceous plants.

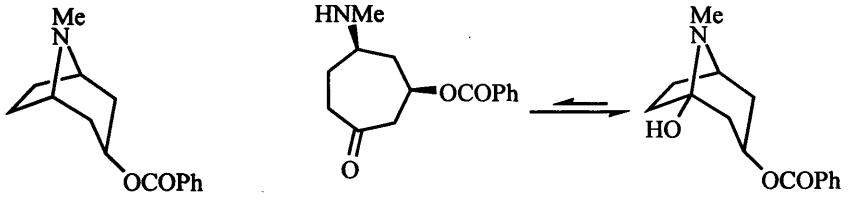


scopolamine (5)

Figure 1.4

Scopolamine is a sedative and is found in numerous over-the-counter sleep aids. It is also a constituent of travel sickness remedies. In conjunction with morphine or meperidine it can be used to induce "twilight sleep" which results in a loss of memory concerning the events during labour.

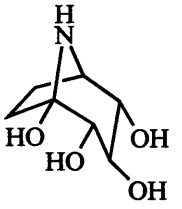
Tropacocaine **(6)** is the benzoyl ester of tropan-3β-ol and occurs in Javanese coca leaves. It is interesting because, like cocaine, it possesses the more unusual tropine skeleton and because it was among the first alkaloids to be synthesised.[4] 1-Hydroxytropacocaine **(7⇌8)** has recently been isolated from the leaves of *Erythroxylum novogranatense* variants and also from *Erythroxylum coca*.[5] The first synthesis of **(7⇌8)** is described in Chapter 3 of this thesis.



(6) **(7)** **(8)**

Figure 1.5

Indeed, significant discoveries have been made in the area of 1-hydroxytropanes over the past decade; namely, the isolation of a family of polyhydroxylated 1-hydroxy-nortropanes, the calystegines, of which calystegine B_2 **(9)** is typical.[6] The calystegines are potent glycosidase inhibitors and have an important role in rhizosphere ecology. Over the past two decades, significant progress has been made in understanding the biosynthetic route to tropane alkaloids such as hyoscyamine and scopolamine.

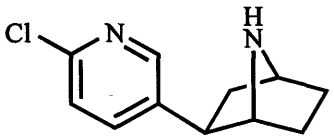


calystegine B_2 **(9)**

Figure 1.6

However, the biosynthetic pathway to 1-hydroxytropanes like the calystegines has yet to be elucidated. Two possible biosynthetic routes to 1-hydroxytropanes are examined in Chapter 4, along with the synthesis of doubly-labelled (^{18}O,^{2}H) intermediates for use in feeding experiments.

Epibatidine **(10)**, one of the most important alkaloids to be discovered in recent years, is not based on tropane ring system.[7] Extracted in 1992, from the skin of the Ecuadorian poison frog *Epipedobates tricolor*, epibatidine has been shown to have analgesic activity 200-500 fold greater than that of morphine. These exciting pharmacological properties, together with the scarcity of natural material, generated unprecedented interest in laboratories around the world. Unfortunately, despite the fact that it is non-addictive and has high potency, epibatidine is unsuitable for clinical use because of its high toxicity. In order to overcome this problem, much synthetic effort has gone into the synthesis of epibatidine analogues. Chapter 2 of this thesis is devoted to discussion of our own efforts in this area, namely, the synthesis of homoepibatidine (the higher homologue of epibatidine) which is one of the most potent epibatidine analogues synthesised to date.



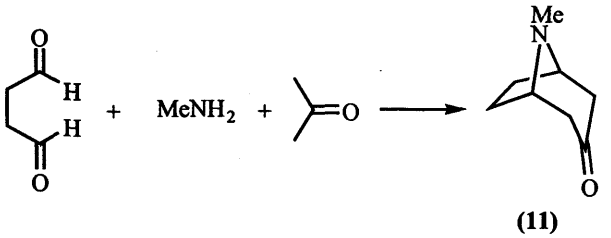
epibatidine **(10)**

Figure 1.7

1.2 ESTABLISHED ROUTES TO THE TROPANE ALKALOIDS

The unique pharmacological properties of the tropane alkaloids, coupled with their varied uses in medicine, have prompted a multiplicity of different syntheses of the tropane skeleton. These can be divided into procedures for constructing the 8-aza-bicyclo[3.2.1]octane ring system and adaptations of these to introduce functionality. A number of different strategies for creating the basic tropane ring system are considered in this section, together with specific examples of natural product syntheses.

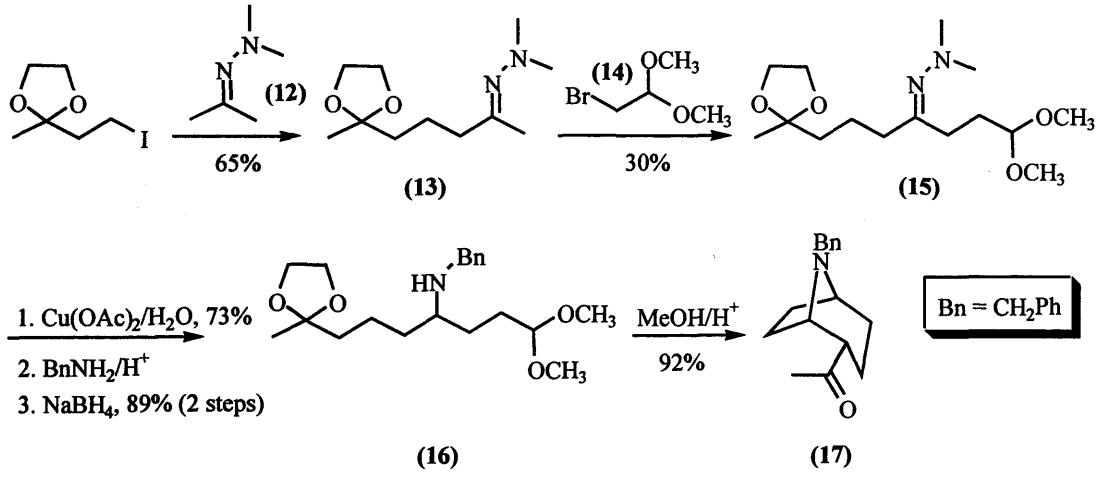
Robinson pioneered the synthesis of the tropane alkaloids in 1917, when he prepared tropinone **(11)** using a Mannich reaction between succinaldehyde, methylamine and acetone (Scheme 1.1).[8]



Scheme 1.1

Schöpf replaced acetone with the calcium salt of acetone dicarboxylic acid and made adjustments to the temperature and pH of the reaction, which led to an improvement in the yield.[9] Although the Robinson synthesis has been successfully adapted to incorporate a wide range of functionality into the tropane ring system,[10] it is limited, in that, it cannot be used for the synthesis of 6,7-dehydro- or 6,7-epoxytropanes.[11]

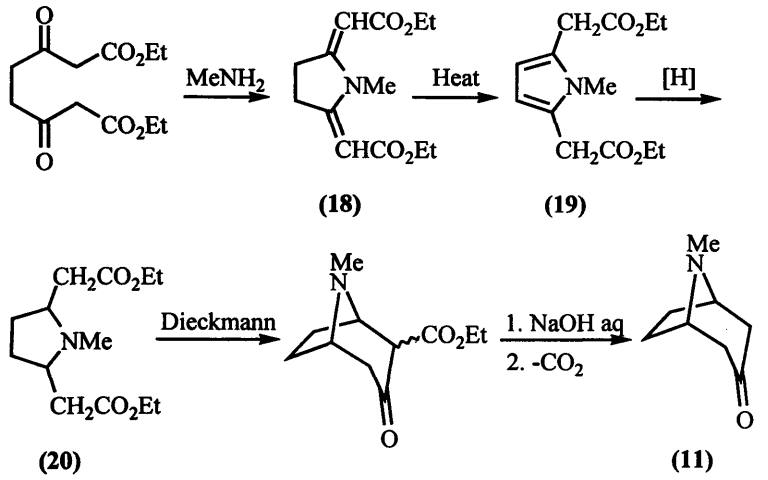
Rapoport has also published a synthesis of the tropane ring system based on the Mannich reaction (Scheme 1.2).[12] Alkylation of acetone dimethylhydrazone **(12)** gave the hydrazone **(13)** in 65% yield. A second alkylation with **(14)** required the addition of hexamethylphosphorus triamide (HMPT) and even then the yield of **(15)** was only 30%.



Scheme 1.2

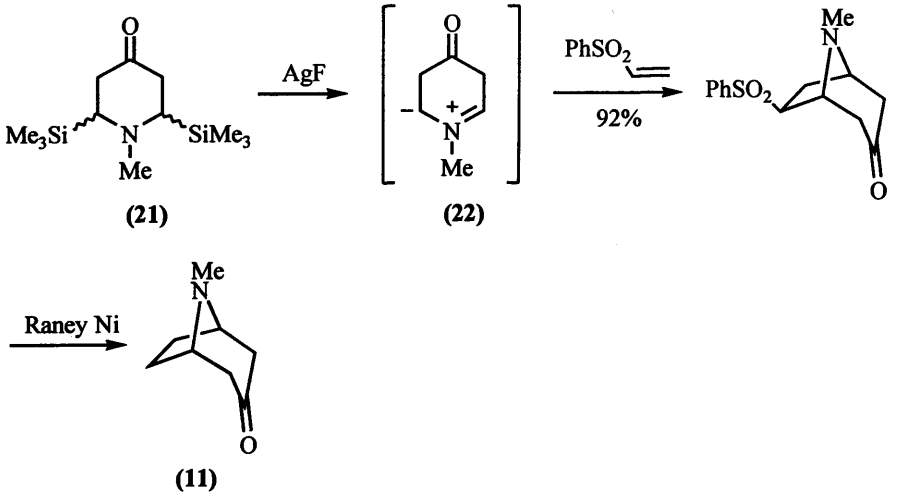
Hydrolysis of the hydrazone with copper(II) acetate gave the corresponding ketone; treatment with benzylamine in the presence of an acid catalyst afforded the imine, which was reduced to the benzyl amine **(16)** with sodium borohydride. Acid catalysed cyclisation of **(16)** gave the acyl tropane **(17)**.

Earlier, Willstätter and Pfannenstiel (Scheme 1.3) developed an alternative strategy for the production of tropinone.[13] Heating the pyrrolidine intermediate **(18)** produced the pyrrole **(19)**, which was hydrogenated to afford **(20)**. In the key step a Dieckmann cyclisation was used to form the tropane skeleton. Saponification followed by decarboxylation yielded **(11)**.



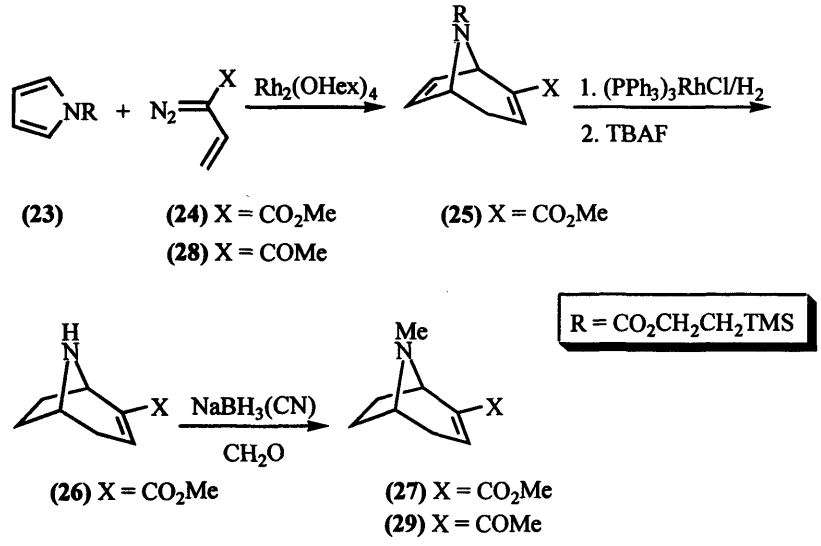
Scheme 1.3

Very recently, Pandey generated tropinone using [3+2] cycloaddition reactions involving cyclic azomethine ylides and vinylsulphones (Scheme 1.4).[14] Treatment of the precursor **(21)** with silver fluoride resulted in two consecutive disilylations to produce the ylide **(22)** as an intermediate. Reaction of **(22)** with phenylvinylsulphone, followed by desulphonylation with Raney nickel gave tropinone **(11)**. This approach has since been adapted to the production of epibatidine.[15]



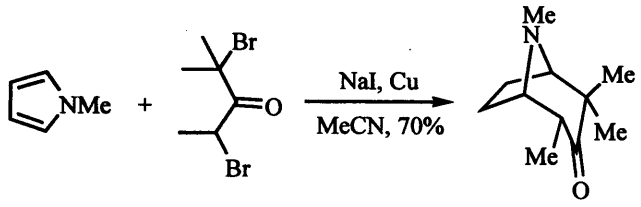
Scheme 1.4

Davies *et al.* have devised a method for the construction of tropane derivatives based on reaction of vinylcarbenoids with N-protected pyrroles. This was then applied to the synthesis of racemic anhydroecgonine methyl ester **(27)** and ferruginine **(29)** (Scheme 1.5).[16] Treatment of **(23)** with the vinyldiazo- compound **(24)** in the presence of a rhodium(II) hexanoate catalyst, yielded **(25)**. Hydrogenation over Wilkinson's catalyst reduced only the less substituted alkene; deprotection then gave **(26)**. Reductive amination of **(26)** with sodium cyanoborohydride and formaldehyde afforded anhydroecgonine methyl ester **(27)**. The synthesis of ferruginine **(29)** was carried out using an identical procedure to that described above, starting with **(28)**. Davies has recently published enantioselective syntheses of both **(27)** and **(29)**.[17]



Scheme 1.5

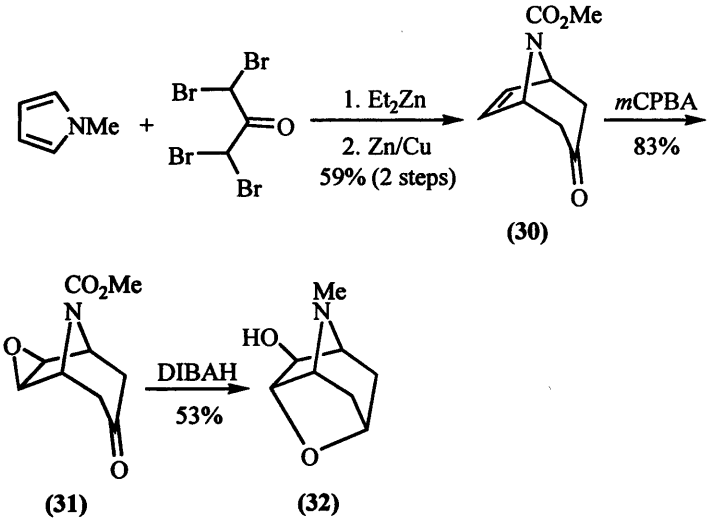
In order to synthesise alkaloids such as scopolamine, it is necessary to introduce functionality into the two-carbon bridge. This can be achieved *via* Diels Alder reaction of N-substituted pyrroles with oxyallyl cations. These have been produced in a variety of different ways. Hoffmann reported that the reductive dehalogenation of αα'-dibromo ketones, using sodium iodide and copper, in the presence of N-protected pyrroles gave substituted 6,7-dehydrotropinones (Scheme 1.6).[18] The disadvantage of this approach is the necessity of using highly substituted αα' dibromoketones in order to produce satisfactory yields.



Scheme 1.6

Noyori reduced the level of substitution required by generating oxyallyls from 1,1,3,3-tetrabromoacetone and nonacarbonyldiiron.[19] However, the toxicity and expense of nonocarbonyldiiron offset this advantage.

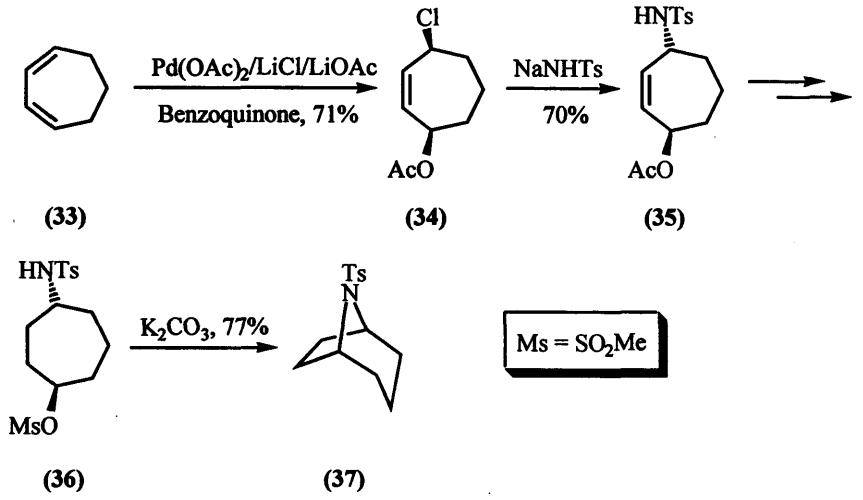
Recent work by Mann, in which oxyallyls are formed by reaction of polybromoketones with diethyl zinc, has led to improved yields of both nitrogen and oxygen-bridged bicycles without the attendant risks of the Noyori synthesis (Scheme 1.7).[20] Treatment of **(30)** with *m*CPBA afforded the *exo*-6,7-epoxytropane derivative **(31)**. However, reduction of the N-protecting group resulted in concomitant opening of the epoxide and formation of scopoline **(32)**.



Scheme 1.7

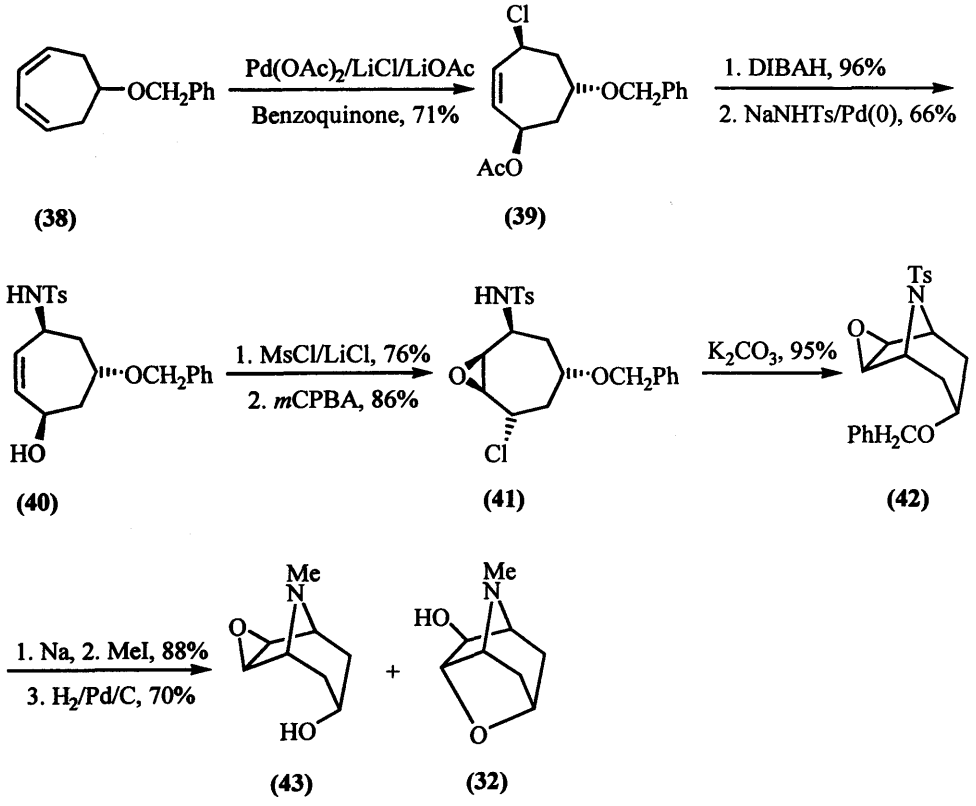
Bäckvall reported a synthesis of simple tropane derivatives based on palladium-catalysed 1,4-chloroacetoxylation of 1,3-cycloheptadiene **(33)**, depicted in Scheme 1.8.[21] Chloroacetoxylation proceeds stereospecifically, yielding only the *cis*-substituted cycloheptene **(34)**. Substitution of the chlorine using sodium *p*-toluenesulphonamide gave **(35)**. Hydrogenation and subsequent saponification of the acetoxy group afforded the corresponding alcohol. Conversion to the mesylate

(36), followed by base-catalysed cyclisation afforded the tropane derivative (37). The deprotection step was not reported.



Scheme 1.8

Bäckvall has since adapted this approach to the synthesis of the natural and non-natural 6,7-epoxytropane derivatives, scopine (43) and pseudoscopine (the 3β-isomer).[22] The route to scopine is depicted in Scheme 1.9. Once again, the synthesis was based on palladium-catalysed 1,4-chloroacetoxylation of a 1,3-cycloheptadiene (38).

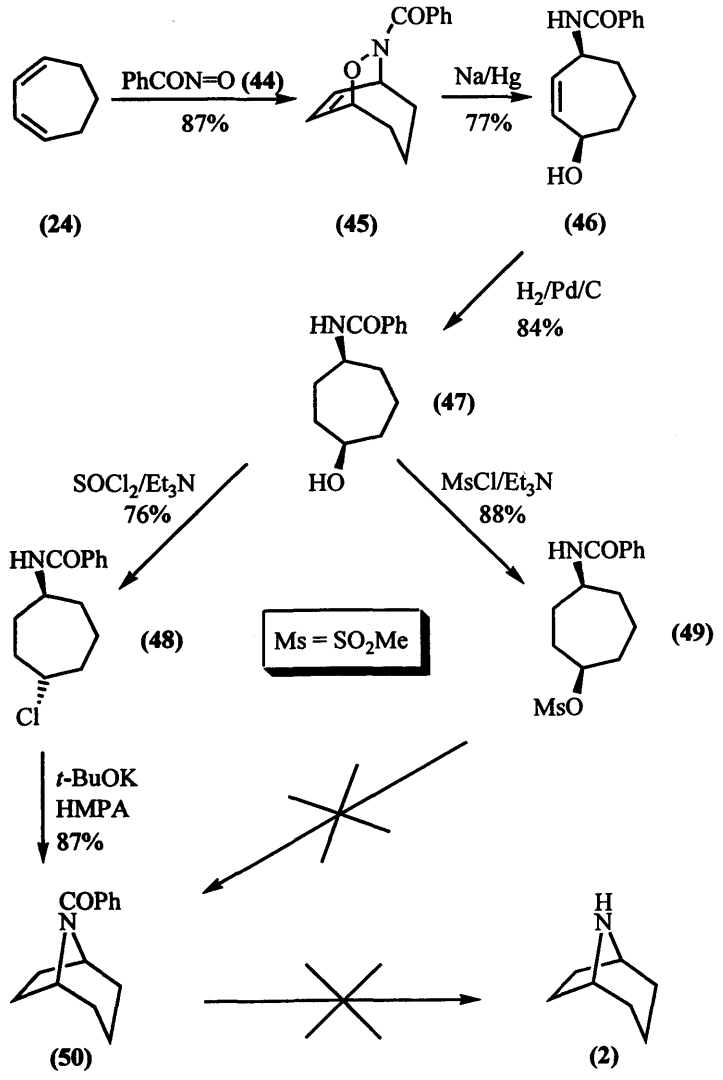


Scheme 1.9

Reduction of the acetoxy group of **(39)**, and subsequent replacement of the chloride using *p*-toluenesulphonamide in the presence of a palladium catalyst, gave the alcohol **(40)**. Following the introduction of the epoxide, the hydroxy group was converted to a chloride, with inversion of configuration, to yield **(41)**. Base-induced cyclisation gave **(42)**, which was converted to the amine and then hydrogenolysed to afford scopine **(43)**. Scopoline **(32)** was formed as a by-product.

1.3 SYNTHESIS OF TROPANES VIA CYCLOADDITION OF CYCLIC DIENES WITH NITROSO COMPOUNDS

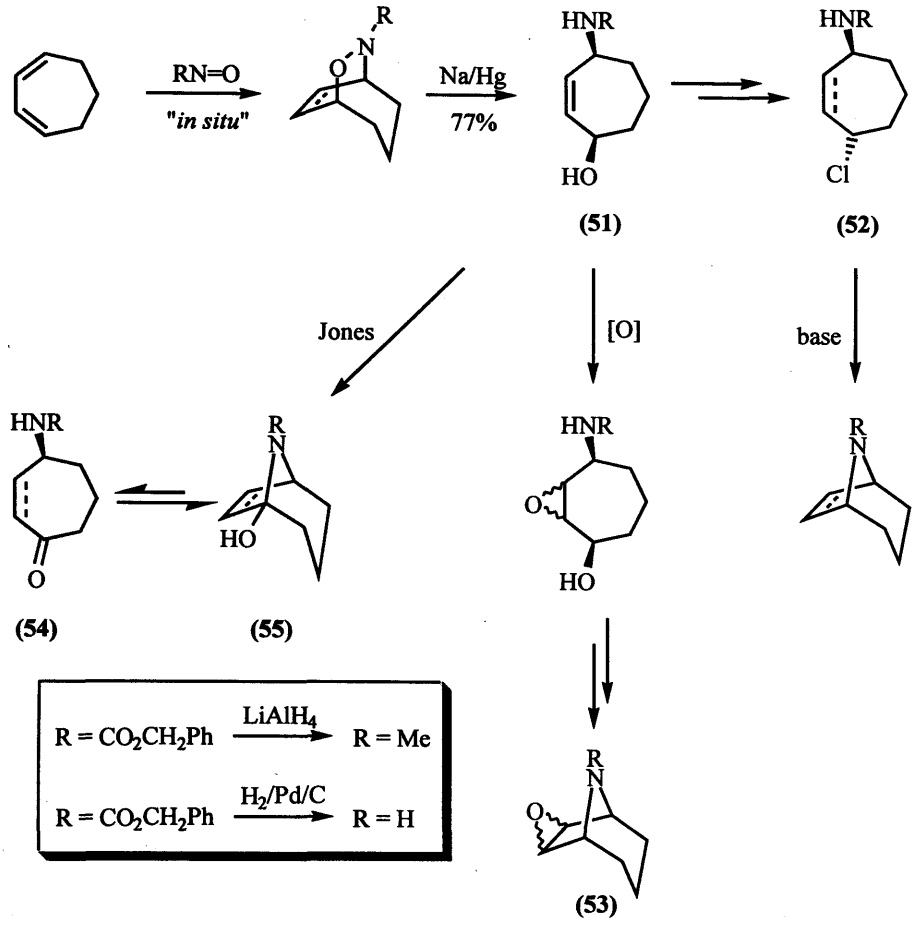
Kibayashi was the first to publish a route to tropane derivatives based on the Diels-Alder cycloaddition of acyl nitroso compounds with a cyclic dienes (Scheme 1.10).[23] N-Benzoylnortropane **(50)** was selected as an initial target.



Scheme 1.10

Treatment of 1,3-cycloheptadiene **(33)** with the nitroso compound **(44)**, produced *in situ* by oxidation of benzohydroxamic acid, gave the cycloadduct **(45)**. Reductive cleavage of the N-O bond afforded the amino alcohol **(46)**, which was subsequently hydrogenated to yield **(47)**. At this point the synthesis diverged and two separate routes to benzoylnortropane were pursued. Treatment of **(47)** with methanesulphonyl chloride gave **(49)**. However, attempts to induce cyclisation of the amido-mesylate **(49)** with a variety of strong bases failed. The corresponding amido-chloride **(48)** was successfully cyclised with potassium *t*-butoxide in hexamethylphosphoric triamide (HMPA) to produce **(50)**. This suggested that an intramolecular S_N2 mechanism was operating, and that a *trans* arrangement of nucleophile and leaving group was required in order for cyclisation to occur. Unfortunately, the amide group could not be hydrolysed to produce nortropane. Kibayashi therefore substituted carbamate protecting groups at nitrogen, but these caused problems in the chlorination step as elimination competed to produce alkene by-products. Despite these difficulties, Kibayashi applied the approach to the synthesis of pseudotropine (tropan-3β-ol) and tropacocaine **(6)**, although the efficiency of these syntheses was low (the overall yield of both products was below ten percent).

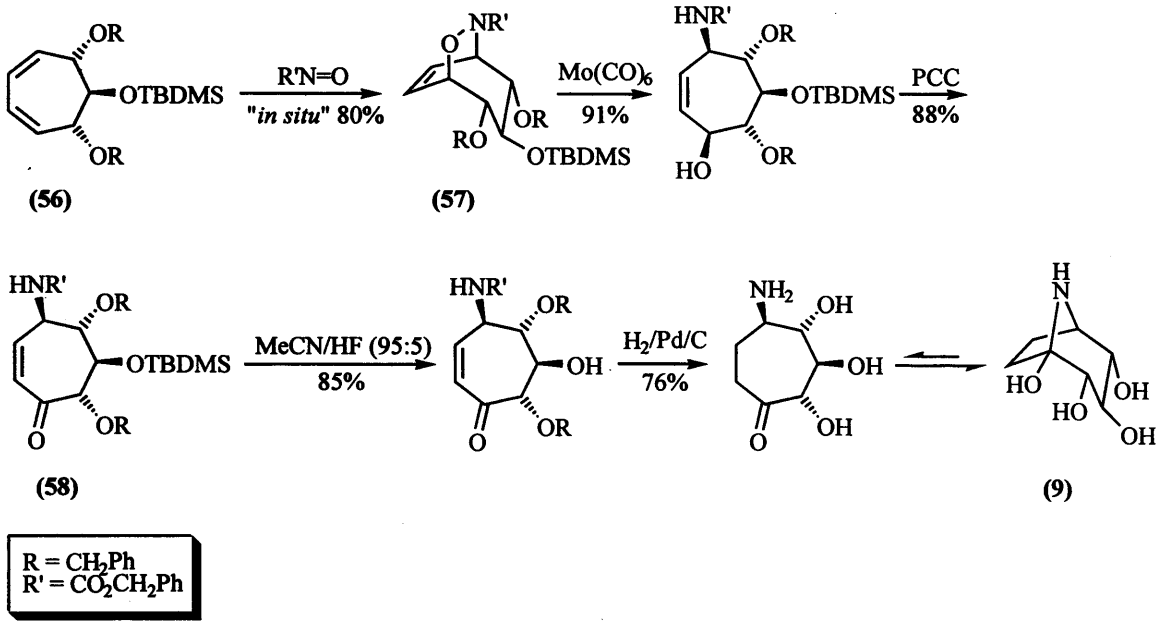
At Leicester cycloaddition between nitroso compounds and cyclic dienes has been developed into a simple but flexible route to the tropane ring system, which has resulted in the successful synthesis of 6,7-dehydro-, 6,7-epoxy- and 1-hydroxy-tropanes as well as simple tropanes[24] and higher homologues.[25] The overall strategy is outlined in Scheme 1.11. Following development work using the N-benzyl protection, the benzyloxycarbonyl group was found to be the most convenient protecting group for nitrogen, since it could be reduced to a methyl group to give tropane derivatives or hydrogenolysed to produce nortropanes. The unsaturated amino-alcohol **(51)** is key to the route, as the approach derives its flexibility from the wide variety of transformations this compound can undergo.



Scheme 1.11

In addition to producing simple tropanes from compounds such as the protected amino-chloride **(52)**, the double bond of **(51)** can be epoxidised to produce both exo- and endo-6,7-epoxytropanes **(53)**.[26] This reaction, which formed the basis of a synthesis of scopine **(43)** and pseudoscopine,[27] is described in more detail in Chapter 2. Jones oxidation of **(51)** affords the amino-ketone **(54)**, which is in tautomeric equilibrium with the bicyclic alcohol **(55)**. This was central to Justice's synthesis of physoperuvine, which is discussed in Chapter 3.[28] Indeed, this approach is the most efficient method of synthesising 1-hydroxytropanes reported to date, and is utilised in the following two syntheses.

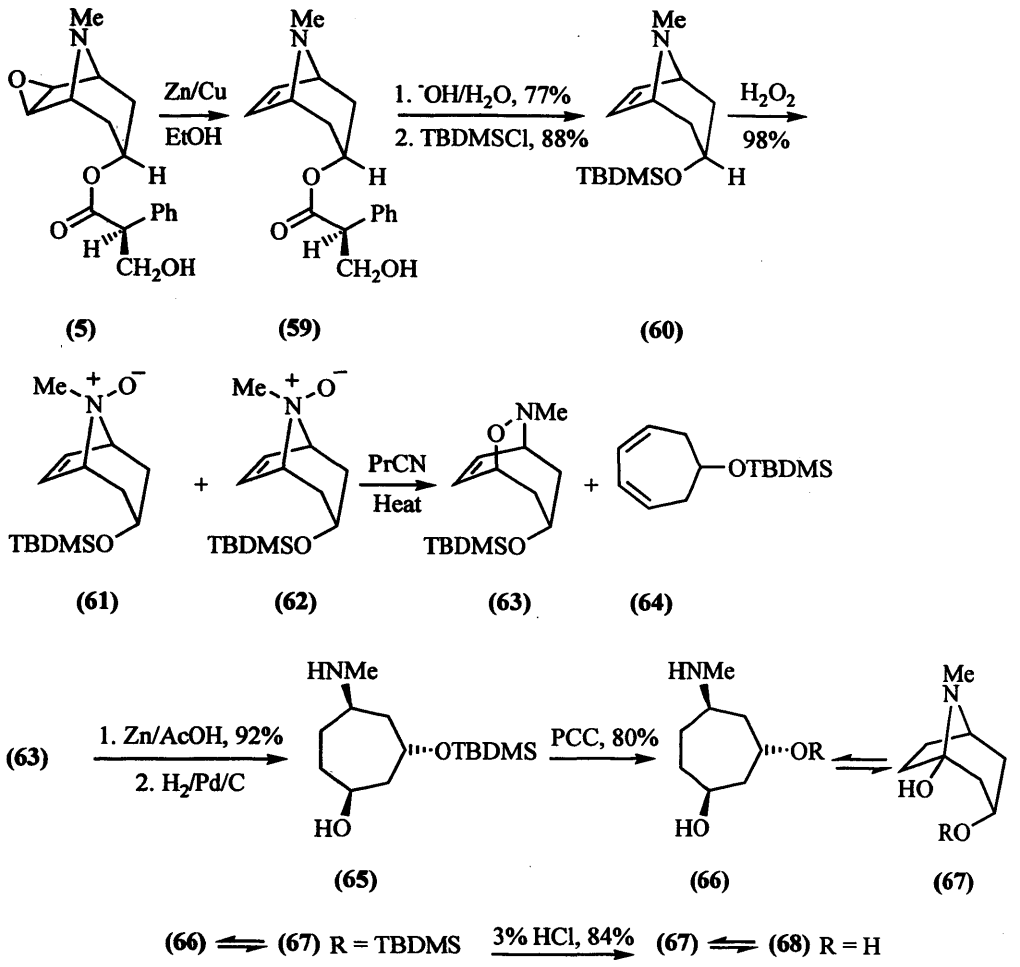
Soulié *et al.* recently reported a synthesis of racemic calystegine B_2, based on cycloaddition of a nitroso compound with a substituted 1,3-cycloheptadiene (Scheme 1.12).[29] In contrast with synthesis of calystegine B_2 from D-glucose,[30] this synthesis involved relatively few steps and proceeded in high overall yield.



Scheme 1.12

The appropriately functionalised 1,3-cycloheptadiene derivative **(56)** was synthesised by catalytic oxidation of protected 2,4,6-cycloheptatrienol and protection of the resulting diol. Reaction of **(56)** under standard nitroso cycloaddition conditions gave the oxazine **(57)**. Molybdenum hexacarbonyl was used to cleave the N-O bond and the resulting amino-alcohol was then oxidised with pyridinium chlorochromate to produce **(58)**. Desilylation followed by hydrogenolysis afforded calystegine B_2 **(9)**.

Bremner has designed an elegant synthesis of the 1,3-dihydroxytropane **(68⇌69)** which uses a Meisenheimer rearrangement to produce an oxazine **(63)** from the tropane N-oxides **(61)** and **(62)** (Scheme 1.13).[31] Saponification of **(59)** and protection of the resulting alcohol gave **(60)**, which was oxidised with hydrogen peroxide to yield a mixture of N-oxides **(61)** and **(62)** (ratio 1.5:1). Heating **(61)** or **(62)** in butyronitrile afforded **(63)**, although rearrangement of **(62)** was significantly slower than **(61)**. The protected 1,3-cycloheptadienol **(64)** was formed as a by-product in both reactions, *via* a retro Diels-Alder reaction.



Scheme 1.13

Reductive cleavage of the N-O bond followed by hydrogenation gave the amino-alcohol **(65)**, which was oxidised with PCC to produce **(66⇌67)**; deprotection with aqueous hydrochloric acid afforded **(68⇌69)**. The ratio of tautomers was not reported. Neither was the 3β-derivative of **(68⇌69)** accessible via this approach. Attempts to extend the synthesis of **(68⇌69)** to produce the 3α-isomer of 1-hydroxytropacocaine were unsuccessful.[32]

This thesis describes work which extends the synthetic route to tropane derivatives developed at Leicester, to encompass the epibatidine analogue, homoepibatidine and novel 1,3-dihydroxytropanes. In addition, Chapter 4 describes investigations into plausible biosynthetic pathways to natural 1-hydroxytropanes.

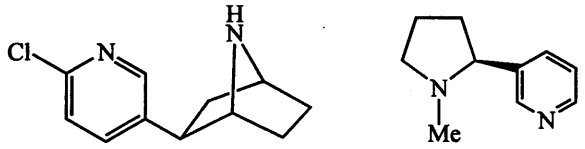
Chapter 2

Total Synthesis of Homoepibatidine

2.1 INTRODUCTION

2.1.1 Biological significance of epibatidine

Epibatidine **(10)** (Figure 2.1) is the first naturally occurring member of a class of alkaloids based on the 7-azabicyclo[2.2.1]heptane skeleton, the lower homologue of the tropane ring system. It was first isolated in 1992 from the skin of the Ecuadorian poison frog *Epipedobates tricolor*.[7] Preliminary biological tests showed epibatidine to be a potent analgesic (200 to 500 times more potent than morphine) with a non-opioid mode of action.[7] Opiate receptors could not be directly involved since naloxone (an opiate antagonist) failed to inhibit epibatidine's analgesic effect.



(10) **(70)**

Figure 2.1

Qian *et al.* highlighted the structural similarities between epibatidine and nicotine **(70)**.[33] Nicotine is a neuronal nicotinic acetylcholine receptor (neuronal nAChR) agonist. nAChRs are located on skeletal muscle at the neuromuscular junction, in the autonomic ganglia of the peripheral nervous system (PNS), on sensory nerves and some peripheral nerve terminals, and numerous sites in the spinal chord and brain. All nAChRs are composed of five protein subunits surrounding a central ion channel. However, there are many different sub-types of neuronal nAChR each made up of different combinations of protein subunits. Their principal functions (Table 2.1) are the release of neurotransmitters and the control of cerebral blood flow.

Qian *et al.* used the mouse tail-flick test (see appendix) to study the antinociceptive effects of both epibatidine and nicotine.[33] In mice pre-treated with mecamylamine (a CNS nicotinic receptor blocker) the analgesia normally produced by epibatidine and nicotine was antagonised. In addition, epibatidine was found to inhibit the binding of [^{3}H]-cysteine (a putative CNS nicotinic receptor ligand). These results imply that the analgesia induced by epibatidine is mediated though the nicotinic acetylcholine receptors. Indeed, epibatidine has a potency in many pharmacological and behavioural assays several hundred times greater than that of

nicotine itself.[34] Since it is probable that epibatidine binds to nAChRs in an analogous manner to nicotine, its high affinity has raised questions about the validity of previous pharamacophores for the central nicotinic receptor.

Table 2.1 Overview of nAChR sub-types (taken from Holladay *et al.*[35])

Receptor Type	Location	Number of Sub-types	Possible Functions
Neuronal Central	CNS	Minimum of four	Cognition Addiction Neurotransmitter release Sensory gating Neuroprotection Dopamine release
Ganglionic	PNS	Minimum of four	Synaptic transmission Neurotransmitter release Cellular function Neurite retraction
Muscle	PNS	One	Contraction of skeletal muscle

A pharmacophore is a model which includes the major structural features necessary for a molecule to bind to a given receptor. These are derived using a combination of structure-activity relationships and computerised molecular modelling. Since there are several sub-types of neuronal nicotinic receptor, each of which is different, each pharmacophore developed is specific to a certain receptor sub-type. However, all nicotinic pharmacophores have the following attributes: a cationic centre (i.e. a quaternary or protonated amine such as the pyrrolidine nitrogen atom of nicotine); an electronegative atom or centre that can act as a hydrogen bond acceptor (e.g. the pyridine nitrogen of nicotine) and lastly, a dummy point in line with the electronegative atom which indicates the direction in which a hydrogen bond may form (e.g. the centroid of the pyridine ring). Molecular modelling is conducted on the protonated forms of epibatidine and nicotine, since amines are protonated at the receptor site.

Early pharmacophores for nicotinic receptors were based on antagonist structures because conformationally restricted ligands were obtainable. The more

flexible agonists were then made to fit these models. However, although agonists and antagonists may bind to the same receptors, it is by no means certain that they bind in precisely the same way. Epibatidine, as a potent and conformationally restricted agonist, is already playing an important role in the development of future pharmacophores for the nAChRs.

Investigations by Glennon *et al.* focused on the relationship between inter-nitrogen distance and activity for a range of pyridine-containing nicotine analogues.[i] Assuming that all the ligands analysed were binding in a similar fashion, to a single population of receptors, these studies suggest that affinity increases with inter-nitrogen distance until an optimal distance of between 5.0 and 5.5 angstroms (Figure 2.2). Epibatidine (shown in green) has a near optimal inter-nitrogen distance of 5.51 angstroms and an affinity which exceeds that of all other known nicotinic receptor ligands (nicotine has an inter-nitrogen distance of 4.87 angstroms).

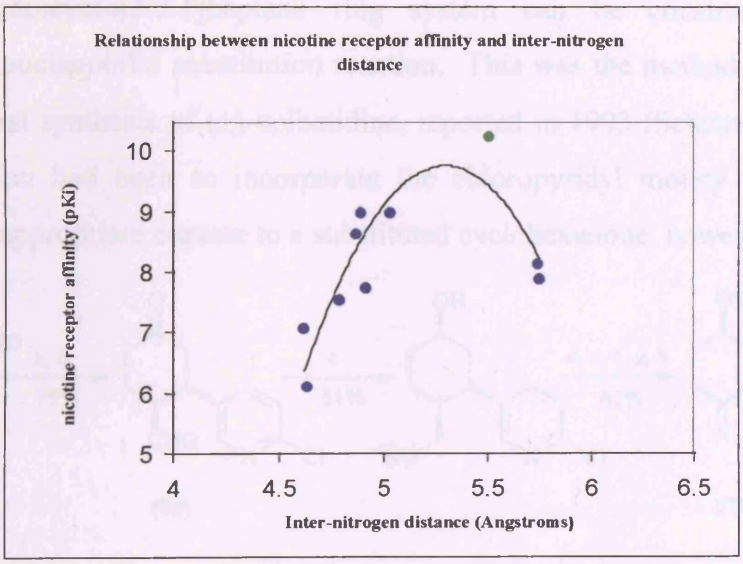


Figure 2.2 (taken from Glennon *et al.*[36])

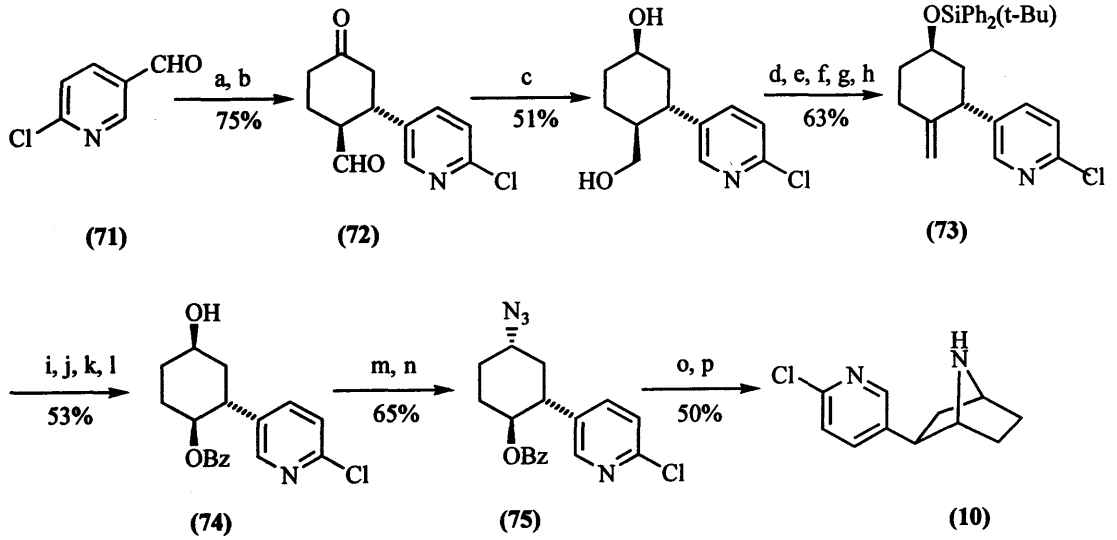
Although epibatidine remains the most potent naturally occurring nAChR ligand reported to date, it is unlikely to be developed as a drug since analgesic effects are only observed at doses slightly below those causing severe hypertension, convulsions and respiratory depression. However, nAChRs are thought to be involved in several human diseases including Parkinson's disease and Alzheimer's disease; the high affinity and selectivity of epibatidine for the nAChRs may prove useful in determining the role of these receptors in such diseases.

2.1.2 Synthetic approaches to epibatidine

The unusually high potency of **(10)**, its novel structure and the dearth of natural sources of material (750 frog skins yielded only 1 mg of the alkaloid) has inspired tremendous synthetic interest (to date, some 27 syntheses have been described in the literature). A wide range of synthetic strategies for generating the 7-azabicyclo[2.2.1]heptane skeleton have been reported. These fall predominantly into one of two classes: intramolecular nucleophilic substitution reactions and Diels Alder cycloaddition reactions. However, several syntheses of epibatidine fall outside these two main categories; these include, for example, Bai's novel route which utilises a Favorskii rearrangement to construct the azabicycle.[37] Selected syntheses are discussed below.

2.1.2.1 Intramolecular nucleophilic substitution reactions

The 7-azabicyclo[2.2.1]heptane ring system can be constructed *via* an intramolecular nucleophilic substitution reaction. This was the method employed by Broka in the first synthesis of (±)-epibatidine, reported in 1993 (Scheme 2.1).[38] The original intention had been to incorporate the chloropyridyl moiety by conjugate addition of the appropriate cuprate to a substituted cyclohexenone; however, this



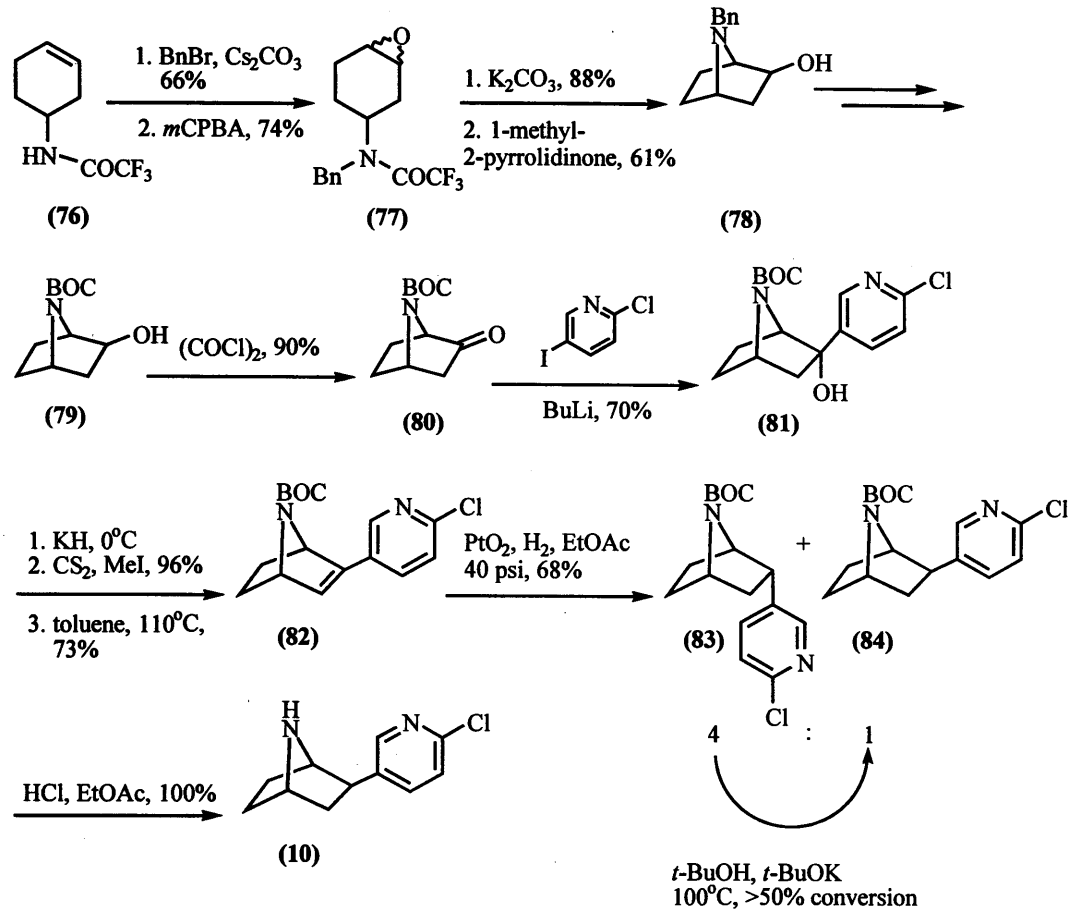
a) Ph_3P=CHCHO, toluene; b) 2-(trimethylsilyloxy)-1,3-butadiene (6eq, neat, 150°C) then H^+/H_2O; c) L-Selectride, -78°C; d) TsCl, pyridine; e) PhSK, THF/DME; f) silylation; g) *m*CPBA; h) 200°C, xylene; i) OsO_4 (cat), NMO then $Pb(OAc)_4$; j) $NaBH_4$, MeOH; k) BzCl, pyridine; l) TBAF; m) MsCl, NEt_3; n) LiN_3, DMF, 55°C; o) hydrolysis then m); p) $SnCl_2$, THF/MeOH, then $CHCl_3$, 55°C, 1 day.

Scheme 2.1

methodology was unsuccessful. Broka therefore turned his attention to a strategy that would allow formation of the cyclohexane ring and concomitant introduction of the chloropyridine group.

6-Chloronicotin-3-aldehyde **(71)** was chosen as the starting material. This was reacted with (triphenylphosphoranylidiene)acetaldehyde to give the corresponding enal by means of a Wittig reaction. Diels-Alder reaction of the product with 2-(trimethylsilyloxy)-1,3-butadiene, followed by treatment with dilute acid gave **(72)** as a single stereoisomer. The superfluous carbon atom of **(72)** was removed by conversion to the alkene **(73)** and subsequent cleavage of the methylene unit. Immediate reduction with borohydride yielded mainly the equatorial isomer. Acylation followed by desilylation then afforded **(74)**. Hydrolysis of the benzyl group and subsequent mesylation was followed by reduction of the azido group with tin(II)chloride followed by heating at 55°C in chloroform for a day gave epibatidine **(10)**.

This synthesis was quickly followed by one from Fletcher.[39] Fletcher's approach, shown in Scheme 2.2, hinged on the condensation reaction of the protected 7-azabicyclo[2.2.1]heptan-2-one **(80)** with 5-litho-2-chloropyridine. The bicyclic precursor was prepared from n-trifluoroacetylaminocyclohex-3-ene **(76)**. Benzylation followed by treatment with *m*CPBA gave a mixture of epoxides **(77)**. Hydrolysis of this mixture and subsequent cyclisation in N-methyl-pyrrolidinone, yielded only the azabicycle **(78)**. The N-benzyl protecting group was exchanged for N-BOC protection at this stage, since it was considered easier to remove without damaging the chloropyridyl group. Swern oxidation of **(79)** afforded the ketone **(80)**, which was subsequently coupled with 5-litho-2-chloropyridine (generated from reaction of *n*-butyllithium with 2-chloro-5-iodopyridine). The resulting alcohol **(81)** was dehydrated, *via* conversion to the corresponding S-methyl xanthate, to yield **(82)**. Hydrogenation using Adams' catalyst produced a 4:1 mixture of the *exo* and *endo* isomers **(83)** and **(84)**. Fortuitously, the endo isomer **(83)** could be epimerised to the desired product **(84)** by heating with potassium *t*-butoxide. Deprotection afforded (±)-**(10)** in quantitative yield.



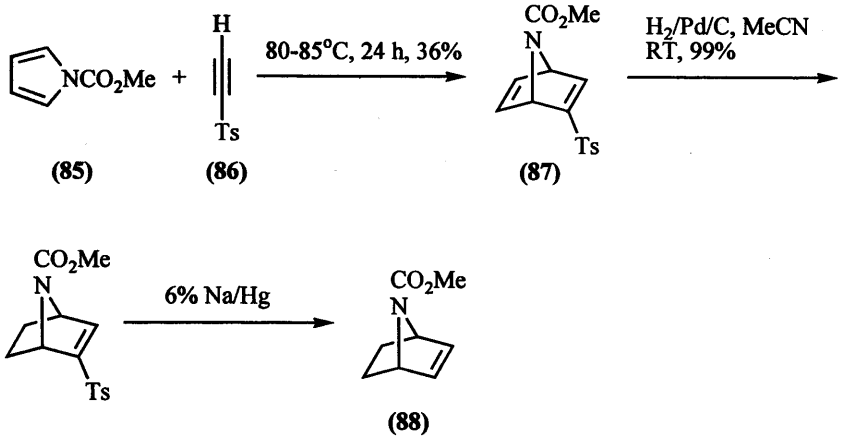
Scheme 2.2

Fletcher *et al.* proceeded to resolve racemic epibatidine by separating the enantiomers of the alcohol **(81)**. Treatment of **(81)** with (R)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (R)-(-)-Mosher's acid chloride) gave the corresponding Mosher esters, which were separated by recystallisation. X-ray crystallography of the Mosher esters was used to determined the absolute configuration of each of the enantiomers of **(81)**. Deprotection of the esters with potassium hydroxide in ethanol gave the enantiopure alcohols which were converted to (+) and (-)-epibatidine as using an identical procedure to that used for racemic **(81)**.

2.1.2.2 Diels-Alder cycloaddition reactions

A concise synthesis of (±)-epibatidine was published by Clayton and Regan, the key step of which was a reductive Heck-type coupling between 2-chloro-5-iodopyridine **(91)**, and the azabicycle **(88)**.[40] The bicyclic framework was constructed using the method of Altenbach *et al.*[41] (Scheme 2.3) which relies on a Diels-Alder cycloaddition reaction between N-methoxycarbonylpyrrole **(85)** and *p*-toluenesulphonylacetylene **(86)** to yield **(87)**. Selective catalytic hydrogenation, and

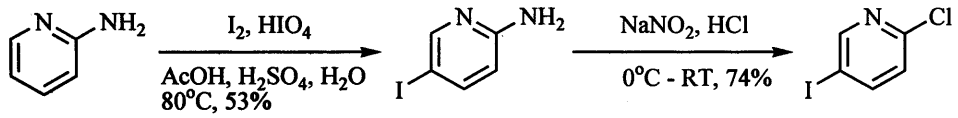
subsequent reductive cleavage of the *p*-toluenesulphonyl group gave the alkene **(88)** necessary for the key coupling reaction.



(85) **(86)** **(87)** **(88)**

Scheme 2.3

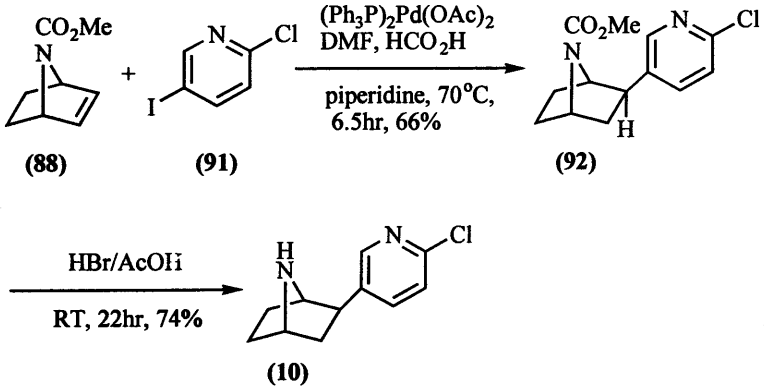
2-Chloro-5-iodopyridine **(91)** was synthesised from 2-aminopyridine **(89)** *via* iodination to give **(90)**, followed by diazotisation in concentrated hydrochloric acid (Scheme 2.4).



(89) **(90)** **(91)**

Scheme 2.4

This then underwent a reductive Heck coupling with the alkene **(88)** to produce **(92)** (Scheme 2.5). Completely stereoselective coupling was observed, with only the *exo*-isomer formed, in accordance with literature precedent.[42,43] Deprotection with hydrogen bromide in acetic acid gave **(10)**.

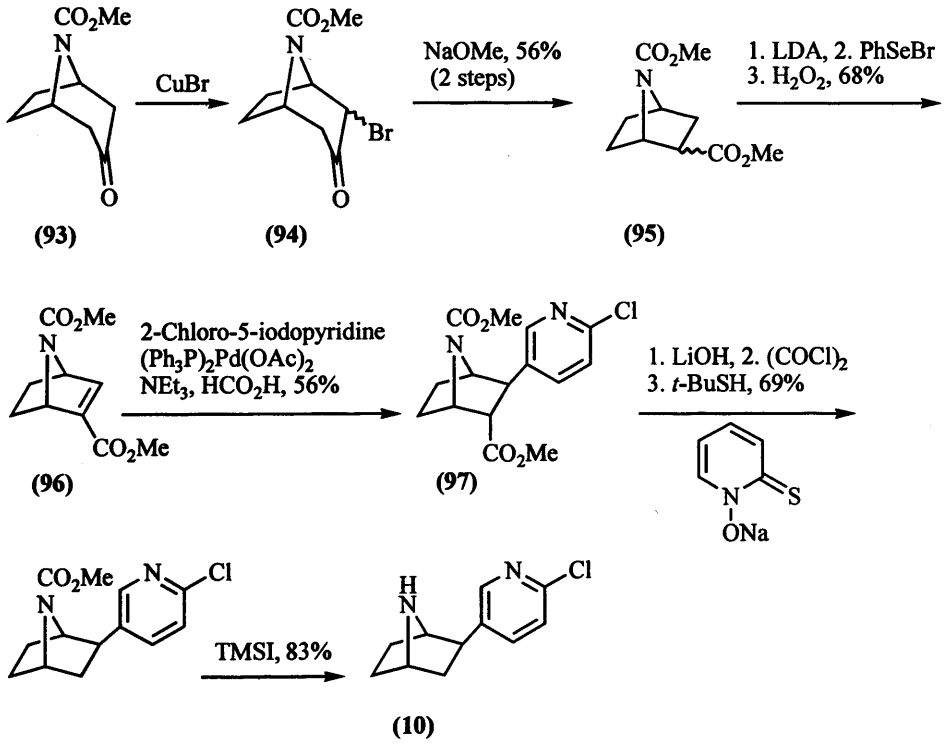


(88) **(91)** **(92)** **(10)**

Scheme 2.5

2.1.2.3 An alternative strategy: the Favorskii Rearangement

Bai published a synthesis of epibatidine starting from N-methoxy-carbonyltropinone **(93)**, which can easily be prepared from tropinone (Scheme 2.6).[37] Treatment of **(93)** with cupric bromide formed an isomeric mixture of the monobromides **(94)**. In the presence of base, both isomers were converted to the ester **(95)**, by means of a Favorskii rearrangement; α-selenation and subsequent selenoxide elimination yielded the key intermediate **(96)**. Various methods for attaching the pyridyl ring were investigated. Conjugate addition of a 5-pyridyl cuprate to **(96)** was unsuccessful, and consequently a palladium-catalysed reductive coupling reaction was used. Initially, this also proved troublesome, but lower reaction temperatures prevented the occurrence of a retro-Diels Alder reaction, and yielded the desired product **(97)**. Hydrolysis of **(97)**, decarboxylation, and deprotection yielded racemic epibatidine.

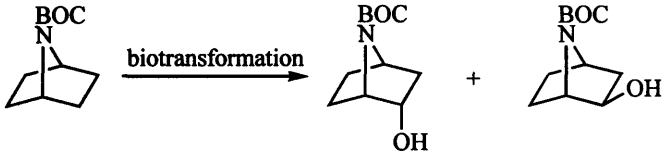


Scheme 2.6

2.1.2.4 Methods of incorporating the chloropyridyl group

The intense synthetic interest in epibatidine has also produced several different methods of incorporating the chloropyridyl moiety. Two of the most popular strategies have already been mentioned, i.e. a palladium-catalysed reductive Heck-type coupling reaction between an appropriately substituted pyridine and a 7-

azabicyclo[2.2.1]heptene derivative, or the condensation of a lithiated pyridine derivative with the protected 7-azabicyclo[2.2.1]heptan-2-one **(80)**. The latter method has led to several formal syntheses of epibatidine which have focused on finding a short and efficient route to **(80)**, rather than a total synthesis of **(10)**. The most interesting of these are two independent publications reporting microbial hydroxylation of N-substituted-7-azabicyclo[2.2.1]heptanes.[44,45] Bio-transformations provide a convenient way of oxygenating unactivated hydrocarbon sites which are unreactive in conventional oxidations.



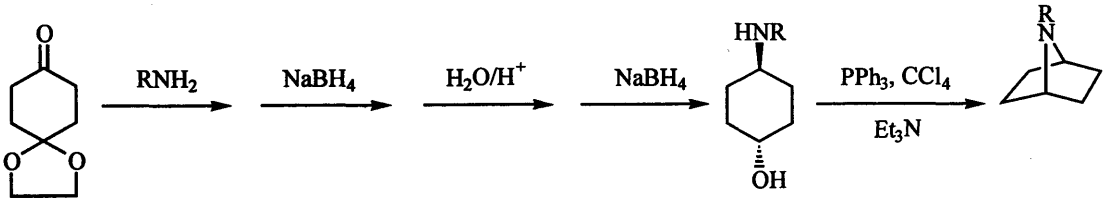
(98) **(99)** **(100)**

62% 27%

(+) or (-) (+) or (-)

Scheme 2.7

Davies *et al.* oxidised **(98)** using *Rhizopus nigricans* to a mixture of *exo-* and *endo-* alcohols **(99)** and **(100)** (Scheme 2.7).[45] Since further oxidation of either alcohol will lead to the key intermediate **(80)**, this work provides a link between the synthesis of 7-alkyl-7-azabicyclo[2.2.1]heptane described by Hassner[46] (Scheme 2.8) and the synthesis of epibatidine reported by Fletcher.[39]

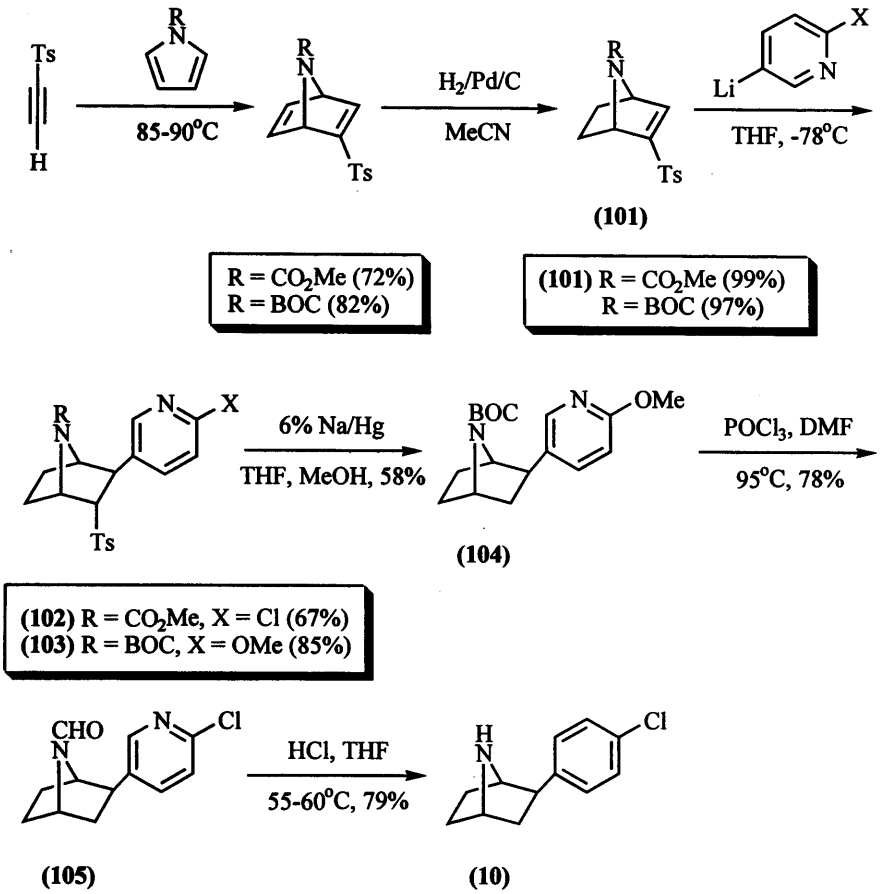


R = Bn, *i*-Pr, *i*-Bu, *t*-Bu

Scheme 2.8

Simpkins *et al.* have developed a synthesis of racemic epibatidine in which the chlororopyridine group was attached *via* Michael addition to an activated *alkene* (an alkenyl sulphone) (Scheme 2.9).[47] The method used to construct the bicyclic framework was identical to that described by Regan.[40] Nucleophilic attack by

2-chloro-5-lithiopyridine derivative on the bicycle **(101)** led exclusively to exo substitution.

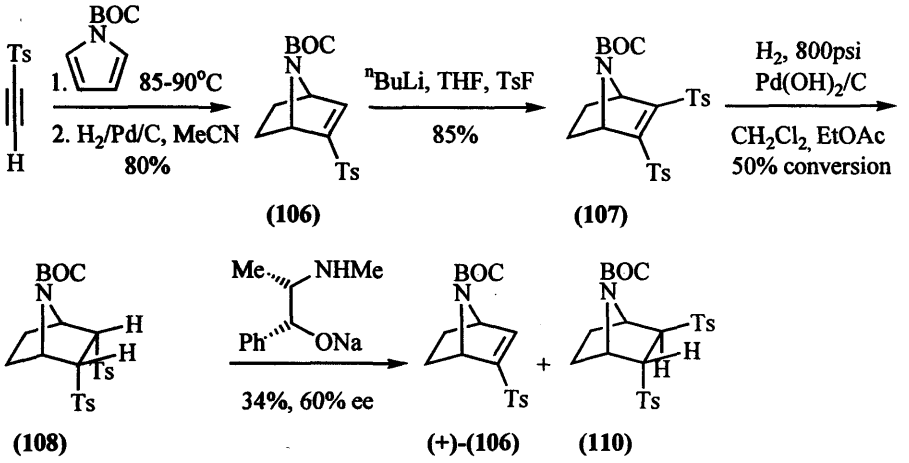


Scheme 2.9

However, attempts to remove the sulphone group from **(102)** resulted in concomitant removal of the chloride from the chloropyridine ring. The analogous reaction using the corresponding methoxypyridine derivative **(103)** proceeded smoothly to give **(104)**. Subsequent treatment with phosphorus oxychloride in DMF converted the methoxy group into the desired chlorine substituent as well as unexpectedly deprotecting the nitrogen to afford **(105)**. Treatment with acid followed by basic work up gave **(10)**.

An asymmetric variation of this synthesis has recently been published (Scheme 2.10).[48] In this route the sulphone (+)-**(106)** was generated by asymmetric elimination from a *bis*-sulphone **(108)**. Regioselective metallation of racemic **(106)** produced **(107)**; subsequent hydrogenation gave the key intermediate **(108)**. Treatment with the chiral base **(109)** produced (+)-**(106)** in 34% yield and 60% ee, together with the trans-*bis*-sulphone **(110)**. The absolute configuration of (+)-**(106)** was assigned by

conversion into natural epibatidine using the route shown in Scheme 2.10. Several other asymmetric syntheses of epibatidine have been reported.[49] However, such syntheses are often protracted. Since both enantiomers of epibatidine show equal biological activity, and access to either enantiomer may be gained by including a resolution step at an appropriate stage of a synthesis, the rewards of following an asymmetric route to epibatidine seem minimal.



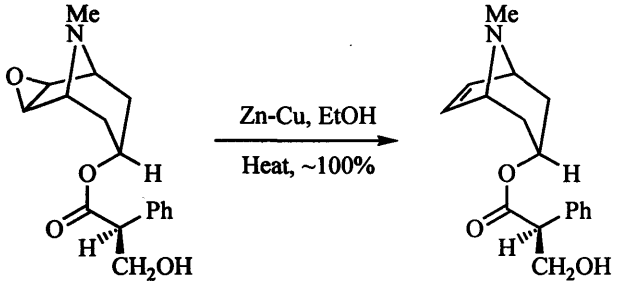
Scheme 2.10

2.2 SYNTHESIS OF HOMOEPIBATIDINE

Following the race to prepare epibatidine in the laboratory, synthetic interest in its analogues was confined to those based on the 7-azabicyclo[2.2.1]heptane skeleton. In previous research at Leicester, the unusual character of the bridging nitrogen in the azanorbornane (7-azabicyclo[2.2.1]heptane) ring system has been studied. This is reflected by the considerable deshielding seen in the ^{15}N NMR spectra of such systems, when compared to simple amines,[50] and in the abnormally high nitrogen-inversion barrier observed for these compounds.[51,52] Preliminary ^{15}N NMR spectra of epibatidine showed unexpected temperature-dependent behaviour, but were not fully analysed.[53] Earlier work suggested that the unusual properties exhibited by the bridging nitrogen in the azanorbornane ring system may not be shared by its higher homologues (i.e. tropanes and homotropanes). Therefore, if the pharmacological activity of epibatidine is related to the unusual nature of the bridging nitrogen, a significant difference in activity should be observed for higher homologues. Our aim was to prepare higher homologues of epibatidine in order to investigate their

biological activity and spectroscopic properties; homoepibatidine was selected as the initial target.

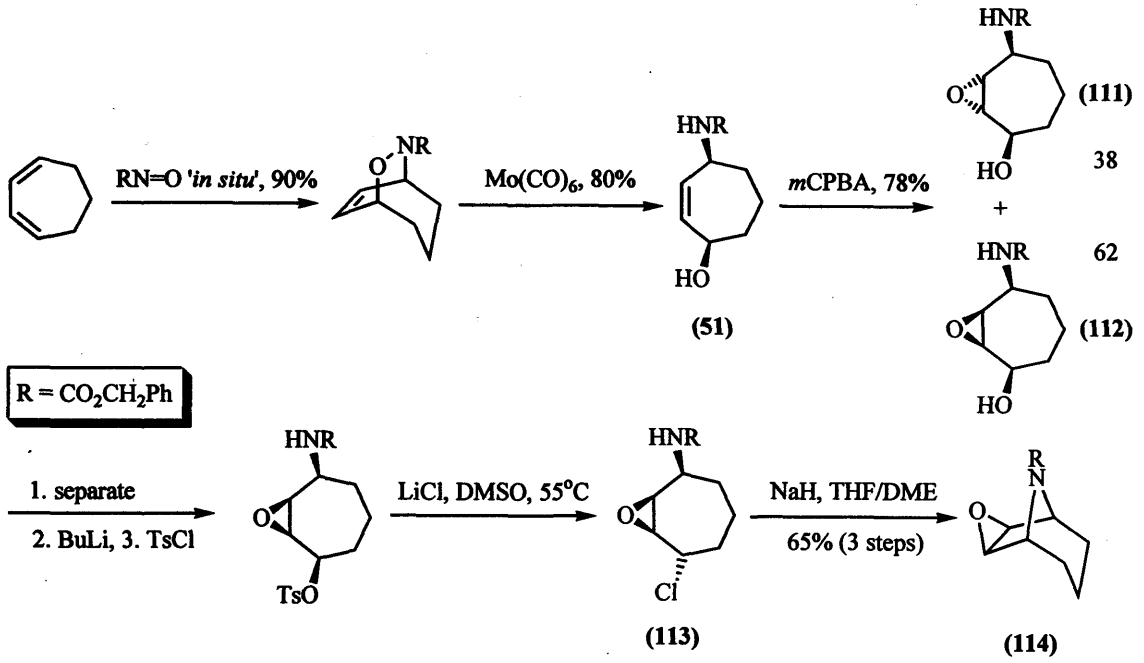
The Heck-type coupling reaction, developed by Regan,[40] provided the most convenient method for attaching the chloropyridyl ring to the tropane skeleton. This required the production of a 6,7-dehydrotropane but simple derivatives are not easily prepared. Howarth attempted to synthesise nortrop-6-ene *via* the nitroso cyclo-addition/intramolecular displacement strategy; however, poor yields were obtained.[24] These problems were overcome following a report by Bremner of the deoxygenation of scopolamine **(5)** using a zinc-copper couple which was discussed in Chapter 1 (Figure 2.3).[31]



(5) **(59)**

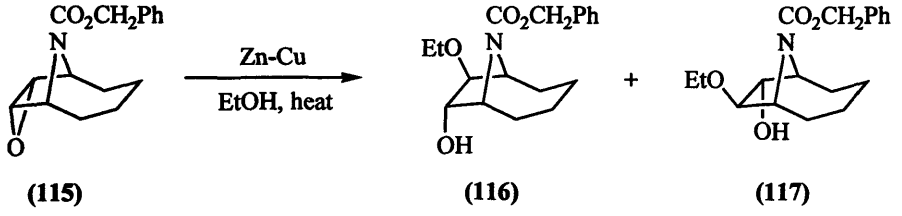
Figure 2.3

Homoepibatidine was therefore synthesised by extension of the route to 6,7-epoxy-tropanes developed by Justice (Scheme 2.11).[26] The key step was epoxidation of the amino-alcohol **(51)**. This afforded a mixture of *cis*- and *trans*- epoxides **(112)** and **(111)**; the ratio could be controlled to some extent and the epoxides were separable by flash chromatography. The *cis* epoxide **(112)** was carried through to give the *exo*-6,7-epoxytropane derivative **(114)**, firstly, because it was the major isomer and, secondly, because the route to **(114)** was easier than that to the corresponding *endo*-6,7-epoxytropane derivative. Conversion of the alcohol **(112)** into the chloride **(113)** proceeded smoothly, providing the trans- 1,4 relationship between the nucleophile and the leaving group necessary for cyclisation. Treatment of **(113)** with sodium hydride gave the epoxide **(114)**.



Scheme 2.11

Bremner reported that the deoxygenation of scopolamine, could be achieved in quantitative yield by refluxing in ethanol over a zinc-copper couple at atmospheric pressure. The epoxide **(114)** proved significantly more stable than scopolamine, and heating in a Young's tube at 150°C for 48 hours, with ethanol as solvent, was found to give the best yield of the alkene **(118)**, although this was still modest at 54% (with 20% recovery of starting material) (Scheme 2.13). In the case of the higher homologue **(115)**, nucleophilic attack of ethanol on the *endo*-epoxide led to the isolation of significant quantities of the ring-opened products **(116)** and **(117)** (Scheme 2.12).[54]



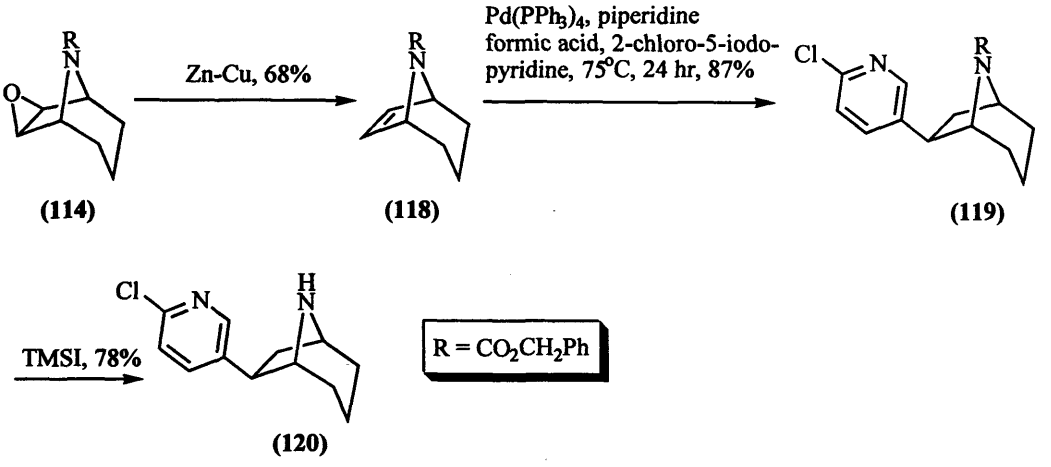
(115) **(116)** **(117)**

Scheme 2.12

No such by-products were observed in the deoxygenation of the *exo*-epoxide **(114)**. Attempts to increase the yield by changing the solvent and/or reducing the reaction time were unsuccessful. As a result of slow rotation about the N-CO bond, the ^{1}H NMR spectrum of the alkene **(118)** (Scheme 2.13) showed two broad singlets at δ 6.02 and δ6.05 for the alkene protons. Double irradiation of the bridgehead protons

produced a sharpening of the alkene protons, but the spectrum was second order and no further analysis was undertaken.

2-Chloro-5-iodopyridine was synthesised as described by Regan.[40] Basic conditions were essential in order to obtain a good yield from the palladium-catalysed coupling reaction, which afforded **(119)** in 87% yield. The ^{1}H NMR spectrum of **(119)** was complicated by the presence of a pair of rotamers (in the ratio 40:60 as measured by ^{1}H NMR signal integrations).



(114) **(118)** **(119)** **(120)**

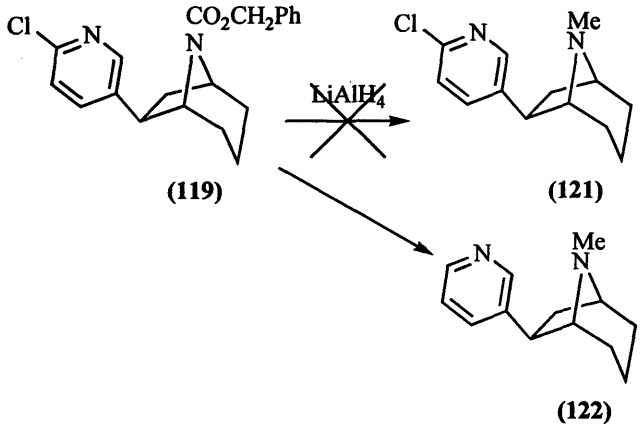
Scheme 2.13

The H_5-bridgehead proton appeared as two broad singlets at δ 4.12 (major rotamer) and δ 4.22 (minor rotamer); lack of coupling with $H_{6\text{-}endo}$ confirmed the *exo* incorporation of the chloropyridyl group. The other bridgehead proton (H_1) was observed as two broad doublets at δ 4.47 (minor) and δ 4.53 (major) as a result of vicinal coupling to $H_{7\text{-}exo}$. Irradiation of H_1 led to the simplification of a multiplet at δ 1.95 ($H_{7\text{-}exo}$), leaving $H_{7\text{-}endo}$ at δ 2.28 unchanged. A general downfield shift in the position of signals for the bridgehead protons is observed in moving up the homologous series from epibatidine to bis-homoepibatidine. The diastereotopic protons of the benzyloxy CH_2 group appeared as two sets of AB quartets at δ 5.16 (major) and δ 5.20 (minor) with a geminal coupling of 12.4 Hz. The ^{13}C NMR spectrum also showed duplication of signals as a result of slow N-CO rotation. Deprotection of **(119)** with iodotrimethylsilane gave (±) homoepibatidine **(120)** in 78% yield. Removal of the protecting group simplified the NMR spectra of **(120)** compared with those for **(119)** since slow rotation was no longer a factor. Otherwise, the spectra displayed similar characteristics to those seen for **(119)**.

2.3 SYNTHESIS OF HOMOEPIBATIDINE DERIVATIVES

The successful synthesis of homoepibatidine, together with encouraging biological data (discussed in Section 2.5), provided the incentive to produce more derivatives of the parent compound. The obvious compound to make was the N-methyl derivative **(121)**, since it should have been easily accessible by reduction of the N-protected compound **(119)**. From a biological perspective, N-methylepibatidine has been shown to have similar pharmacological activity to epibatidine itself [33] and so there was a good chance, given the activity of homoepibatidine, that **(121)** would also be active.

At low temperature, lithium aluminium hydride failed to reduce **(119)** (Scheme 2.14) and so the reaction was monitored as it warmed to room temperature. However, the product was not **(121)** as desired, but the deschloro compound **(122)**. Attempts to reduce **(119)** with DIBAH also failed.

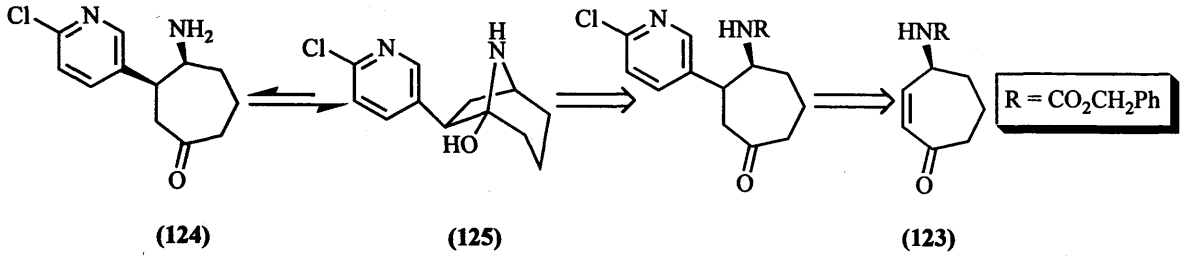


Scheme 2.14

It was decided to attempt the synthesis of an unsymmetrical homoepibatidine analogue in the hope that this would produce enantioselective binding at the nicotinic receptor. The research group in Leicester has developed a simple and efficient route to 1-hydroxytropanes, which is discussed in detail in Chapter 3. This was to be the basis of a synthesis of 1-hydroxyhomoepibatidine **(124⇌125)**.

Retro-synthetic analysis for the synthesis of **(124⇌125)** is shown in Scheme 2.15. The αβ-unsaturated ketone **(123)** is easily accessible *via* oxidation of the corresponding allylic alcohol.[28] It was hoped that conjugate addition to **(123)** could be

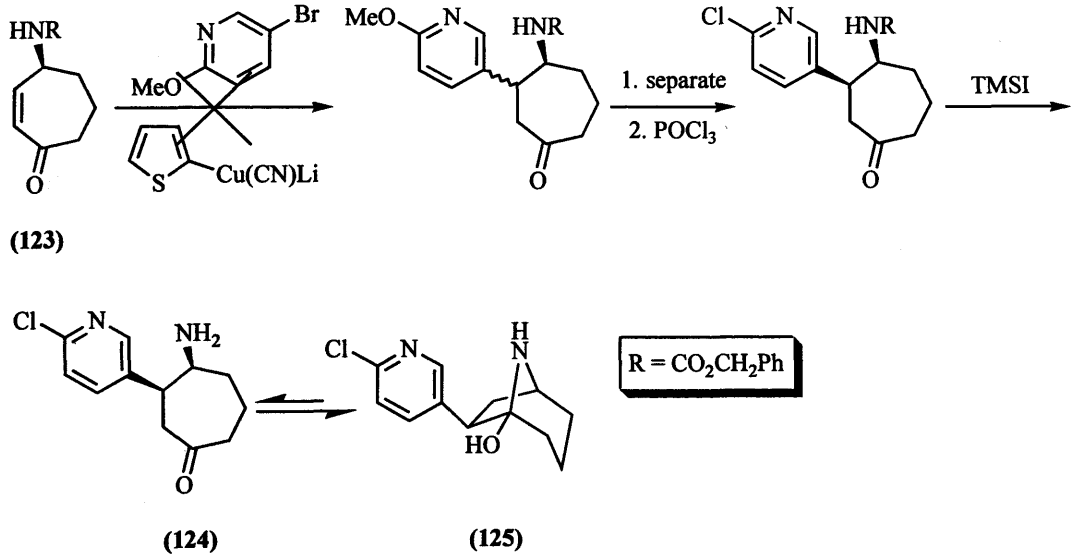
used to insert the chloropyridyl moiety. Deprotection of the nitrogen would conclude the synthesis.



(124) (125) (123)

Scheme 2.15

There were several potential problems with this strategy. Firstly, the cuprate would deprotonate the amine before it added to the αβ-unsaturated ketone. Secondly, dimerisation of the cuprate may occur. Thirdly, Broka had tried to react Gilman reagents with a substituted cyclohexenone in his synthesis of epibatidine, but without success (Section 2.1.2.1). However, in their synthesis of epibatidine, Sestanj *et al.* converted 2-methoxy-5-lithiopyridine into a "higher-order" cuprate.[55] This was then successfully used to effect conjugate addition to a cyclohexenone derivative. Applying this methodology in our own synthesis should give the best possible chance of a successful reaction, and should avoid problems with dimerisation by replacing the chlorine with the more stable methoxy group. Finally, using two equivalents of cuprate should allow for deprotonation of the amine group.



(123)

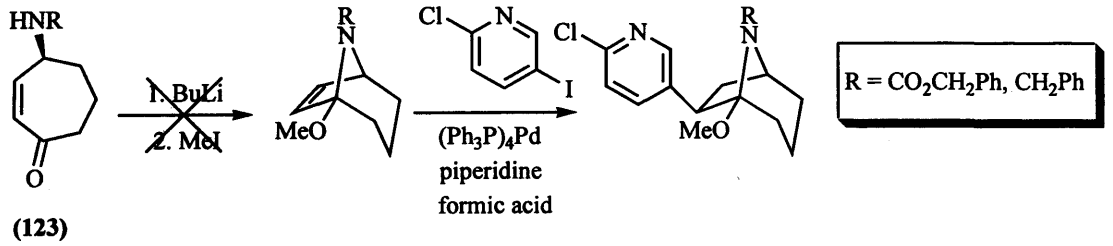
(124) (125)

Scheme 2.16

5-Bromo-2-methoxypyridine was lithiated using *n*-butyl lithium (Scheme 2.16). Sestanj noted the importance of using dry diethyl ether as solvent as all attempts to

generate the lithiopyridine in THF failed. Lithium 2-thienylcyanocuprate (commercially available as a solution in THF) was then added. Decomposition of the lithiopyridine does not occur under these conditions.[55] Finally, the ketone **(123)** was added, as a solution in THF. However, despite several attempts, the reaction was unsuccessful. There are several possible reasons for this. Firstly, it is not certain that the αβ-unsaturated ketone **(123)** is fully conjugated, since molecular modelling suggests that there are several possible low-energy conformations. However, conjugate addition to cycloheptenone itself has been reported by House *et al.*[56] Secondly, although, when protonated, the ketone exists exclusively in the monocyclic form, it is possible that deprotonation causes the equilibrium to shift in favour of the bicylic form. In this case reaction *via* the αβ-unsaturated ketone **(123)** would be slow.

If the second hypothesis is correct, it should be possible to alkylate the bridgehead oxygen and therefore fix the molecule in the bicyclic configuration. A precedent for this is found in the homotropane series, where the removable MEM protecting group was used.[57] With the conformation fixed as bicyclic, the chloropyridyl moiety could be attached using the reductive Heck reaction described above. Deprotection at both oxygen and nitrogen would then give 1-hydroxyhomoepibatidine **(124⇌125)**.



Scheme 2.17

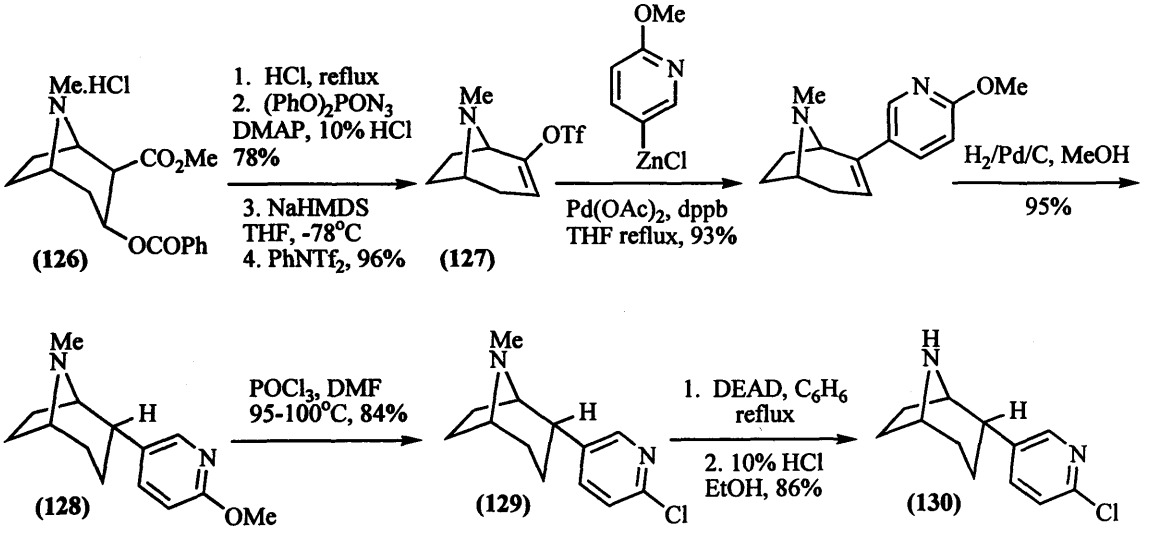
Initial investigations involved alkylation of **(123)** with methyl iodide, as shown in Scheme 2.17. Although alkylation would be irreversible in this case, the reaction was a quick and convenient way to test the viability of the route. When the nitrogen was protected with a benzyloxycarbonyl group, only starting material was recovered from the reaction. It was hoped that changing the hybridisation of the nitrogen from sp^2 to sp^3 would shift the equilibrium towards the bicycle and allow some O-alkylated product to be obtained. Unfortunately, this was not the case. Therefore, it seems that the equilibrium is heavily weighed in favour of the monocyclic tautomer in both the

deprotonated carbamate and N-alkyl derivatives. Hence, the second possible explanation for the failure of the conjugate addition is invalid.

2.4 SYNTHESIS OF EPIBATIDINE ANALOGUES

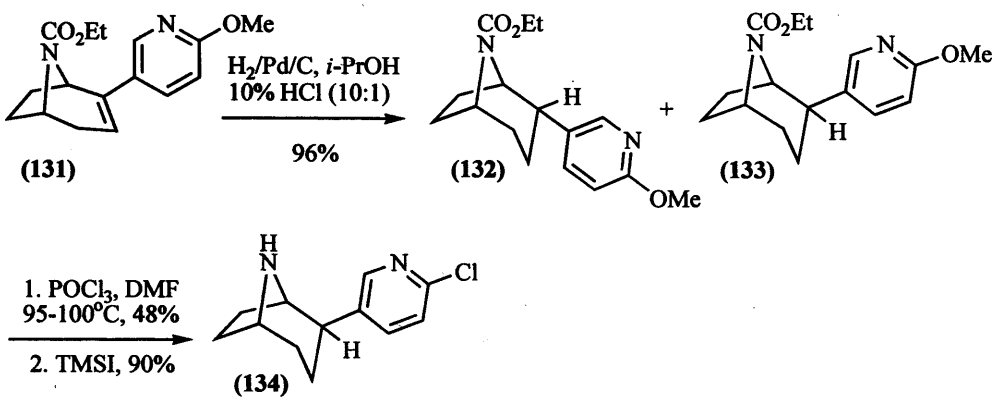
Many different epibatidine analogues, based a variety of ring systems, have been synthesised and some of these have been evaluated biologically. Selected syntheses are discussed below.

Trudell *et al.* synthesised the epibatidine analogues **(130)** and **(134)** from cocaine (Scheme 2.18).[58] The enol triflate **(127)** was prepared in four steps from (-)-cocaine hydrochloride **(126)**. The pyridine moiety was then attached using a palladium catalysed coupling reaction. Catalytic hydrogenation gave only the endo-isomer **(128)**, and was followed by a Vilsmeier reaction to afford **(129)**. Demethylation and subsequent hydrolysis yielded **(130)**.



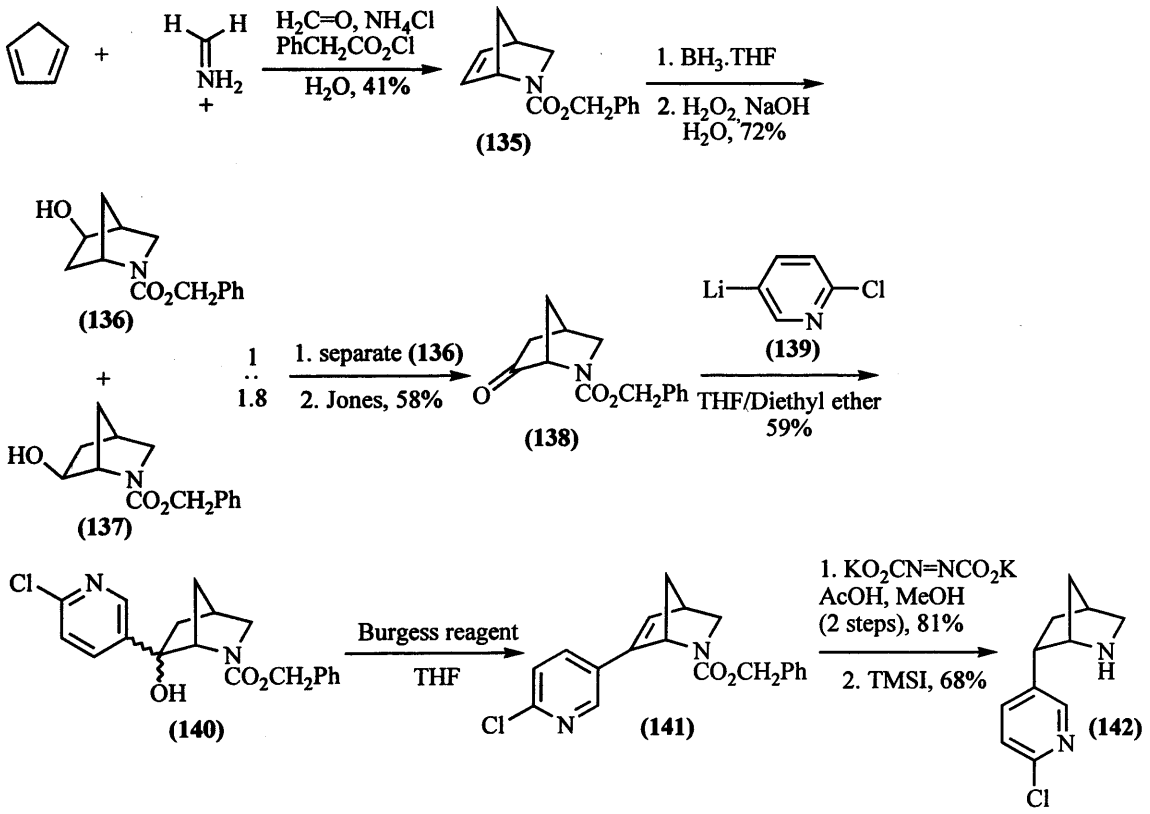
Scheme 2.18

To produce the *exo*-isomer **(134)**, the nitrogen was protected by treatment with ethyl chloroformate in the presence of a catalytic amount of potassium carbonate, yielding **(131)** (Scheme 2.19). Catalytic hydrogenation in *iso*-propanol-10% HCl afforded a mixture the *exo*- and *endo*-isomers **(132)** and **(133)** in a 4:1 ratio. Treatment with phosphorus oxychloride followed by deprotection gave **(134)**.



Scheme 2.19

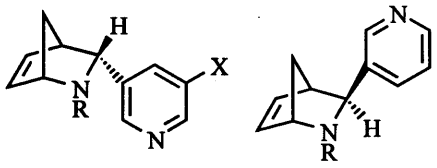
Two novel epibatidine analogues have recently been synthesised at Leicester (Scheme 2.20).[59] N-(Benzyloxycarbonyl)-2-azabicyclo[2.2.1]hept-5-ene **(135)** was prepared using the method of Carroll.[60] Hydroboration of the alkene resulted in a mixture of *exo*-alcohols **(136)** and **(137)**. The alcohols were separated and each taken through the steps described below, but for simplicity only the intermediates involved in the route to the C_6-isomer are depicted.



Scheme 2.20

Oxidation with Jones reagent gave the ketone **(138)**, and subsequent reaction with the lithiated chloropyridine **(139)** yielded **(140)** as a mixture of stereoisomers.

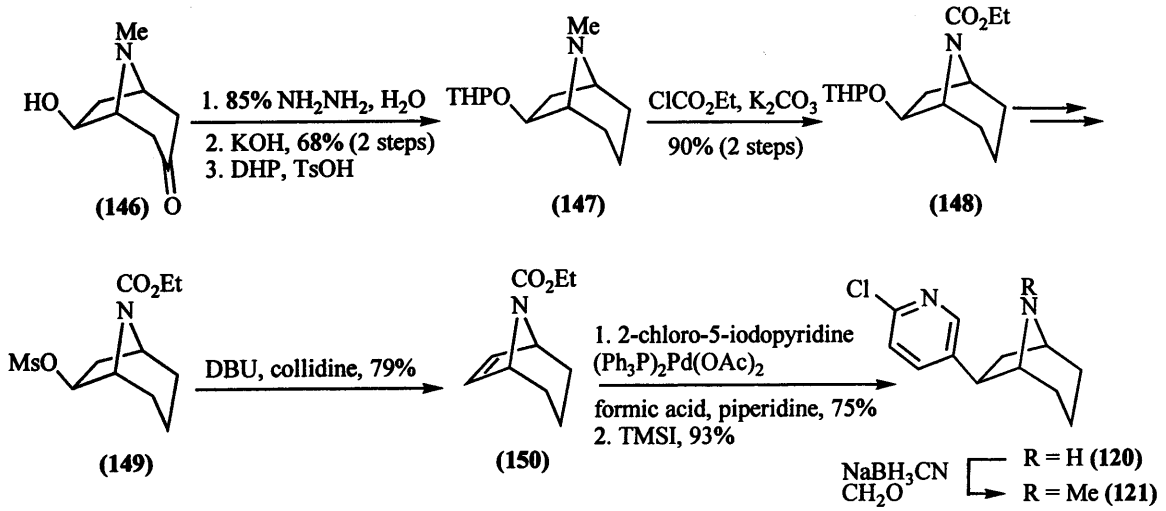
Dehydration of **(140)** proceeded smoothly; however, catalytic hydrogenation of the resulting alkene **(141)** led to concomitant dechlorination of the pyridine ring. An alternative reduction with diimide was successful, and this was followed by N-deprotection with iodotrimethylsilane to afford **(142)**. Analogous treatment of **(136)** produced the C_5-isomer. These compounds have yet to be tested for biological activity. However, the 2-azabicyclo[2.2.1]hept-5-ene derivatives **(143)** **(144)** and **(145)** (Figure 2.4) have all been shown to bind to nicotinic receptors.[35] The molecules **(144)** and **(145)** have an affinity for rat nAChRs (α4β2 subtype) similar to that of nicotine. The 5-bromopyridine derivative **(143)** was considerably weaker than either **(144)** or **(145)**.



(143) R = Me, X = Br **(145)** R = Me
(144) R = Me, X = H

Figure 2.4

Bai has recently reported a synthesis homoepibatidine **(120)** in the first work outside Leicester to target a higher homologue of epibatidine (Scheme 2.21).[37] Wolff Kischner reduction of 6β-hydroxytropinone **(146)** yielded 6β-tropinol.



Scheme 2.21

The hydroxy group was protected as the THP ether **(147)** before treatment with ethyl chloroformate to give the carbamate **(148)**. Following cleavage of the THP ether, the hydroxy group was converted into a mesylate **(149)**; this was then eliminated by refluxing with one equivalent of DBU in collidine to afford **(150)**. Stereoselective introduction of the chloropyridyl group was achieved using a Heck-type coupling reaction. Deprotection of the nitrogen with iodotrimethylsilane yielded **(120)**. Bai also suceesfully produced N-methylhomoepibatidine **(121)** by reductive amination of homoepibatidine with sodium cyanoboro-hydride and formaldehyde.

2.5 BIOLOGICAL ACTIVITY OF HOMOEPIBATIDINE

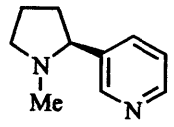
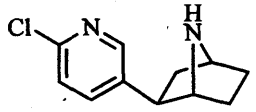
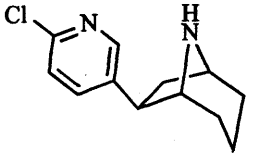
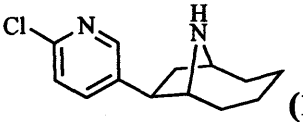
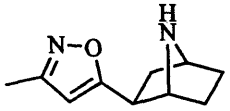
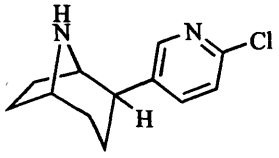
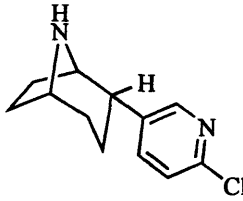
2.5.1 Nicotinic receptor binding assays

An analytically pure sample of (±)-**(119)** was resolved using chiral HPLC on a Chiralpak AD column, eluting with 30% ethanol in hexane. Having established the correct solvent system in work at Leicester, the enantiomers were separated on a preparative scale by Merck Sharp and Dohme Research Laboratories. Each enantiomer of **(119)** was then deprotected using iodotrimethylsilane to give samples of (+)- and (-)-homoepibatidine.

Nicotinic receptor binding assays were carried out (see appendix for procedure); the results are shown in Table 2.2 together with data for epibatidine itself and selected analogues. The data revealed that the enantiomers of homoepibatidine have the same level of activity as epibatidine, but differ slightly in activity; (-)-homoepibatidine was 2.6-fold more active than (+)-homoepibatidine. This parallels results for epibatidine, which show (-)-epibatidine to have 2.4-fold greater activity than (+)-epibatidine. Both enantiomers of homoepibatidine are over ten times more potent than nicotine. Tests for receptor sub-type selectivity are now required to more fully characterise their pharamacological properties.

The results of investigations by Bai, into the analgesic activity of homoepibatidine, compliment our own data. Analgesic activity was evaluated using hot-plate assays (see appendix). Homoepibatidine elicited significant antinociceptive activity at a dose of 40 μg/kg, which was similar to that produced by 10 μg/kg of racemic epibatidine.

Table 2.2 Inhibition of binding at nicotinic receptors

	IC50 (nM)	Number of experiments	Ki (nM)	nH	Analgesic activity
 **(70)**	7.8_c	5_c	1.01_a	-	
 **(10)**	(+) 0.24_c (-) 0.10_c (±) $2.9 \times 10^2{}_d$	5_c 5_c 3	0.058_a 0.045_a -	- - -	(±) $10\mu g/kg_e$
 **(120)**	(+) 0.8_c (-) 0.3_c	2 2	0.35_c 0.13_c	0.72 0.55	(±) $40\mu g/kg_e$
 **(151)**	2.85_c	2	1.25_c	0.6	
 **(152)**	-	-	0.6_b	>0.9	$100\mu g/kg_b$
 **(134)**	$7.19 \times 10^3{}_d$	2	-	-	
 **(130)**	$6.74 \times 10^5{}_d$	2	-	-	

IC50 = 50% inhibition of binding of [^{3}H]-nicotine.
Ki = apparent affinity (IC50 corrected for ligand occupancy).
nH = Hill slope.
a. Taken from Daly *et al.*[61] (assays performed using rat brain).
b. Taken from Daly *et al*[62] (assays performed using rat brain).
c. Assays performed using rat brain (courtesy of Dr S.R. Fletcher, Merck, Sharp and Dohme).
d. Taken from Trudell *et al.*[58] (assays performed using electronic organ membranes of the *Torpedo californica* eel.
e Taken from Bai *et al.*[37] (evaluated using hotplate analgesia in mice).

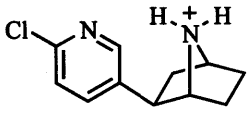
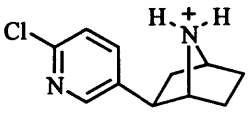
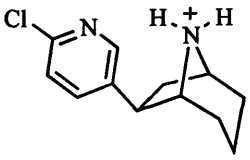
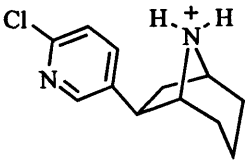
The higher homologue, *bis*-homoepibatidine **(151)**, synthesised by Hemmings,[63] was considerably less active than either epibatidine or homoepibatidine, and so the racemate was not resolved.

(±)-Epiboxidine **(152)**, synthesised by Daly *et al.*, is approximately 10-fold less potent as a nAChR agonist than (+) or (-)-epibatidine, but has been shown to be about 20-fold less toxic.[62] Compounds **(130)** and **(134)** were compared with (±)-epibatidine in their ability to displace [^{3}H](±)-epibatidine from nAChRs. **(134)** was ca. 25-fold less potent than (±)-epibatidine, while **(130)** was found to be 2500-fold less active.[58]

2.5.2 Molecular modelling

The protonated forms of epibatidine and homoepibatidine were modelled using Desk Top Molecular Modeller version 3.0. The results are shown in Table 2.3.

Table 2.3 Molecular modelling results for epibatidine and homoepibatidine

Compound/ Conformation	Energy (kcal mol^{-1})	Internitrogen distance (Angstroms)
 **(10a)**	-14.5	4.6
 **(10b)**	-14.4	5.3
 **(120a)**	-33.4	4.6
 **(120b)**	-33.3	5.5

Looking at the two possible conformations of epibatidine **(10a)** and **(10b)**, it is clear that the inter-nitrogen distance of **(10b)** (5.3 Å) matches the values of between 5.0 - 5.5 Å quoted by Glennon and other authors.[36,64] The analogous conformation for

homoepibatidine **(120b)** has a similar inter-nitrogen distance of 5.5 Å. The alternative conformation **(120a)** has an inter-nitrogen distance which is much shorter at only 4.6 Å.

2.6 CONCLUSION

This chapter has described a successful synthetic strategy to homoepibatidine. This has been possible due to the use of an epoxide as a convenient and effective protecting group for the alkene of **(118)**. Homoepibatidine is one of the most potent epibatidine analogues to be synthesised to date, having an affinity for the nicotinic receptors equal to that of epibatidine itself. Like epibatidine, the higher homologue shows little enantioselectivity in binding to nAChRs. If 1-substituted homoepibatidine derivatives can be synthesised they may bind enantioselectively. Finally, molecular modelling has shown that there is a good match between the inter-nitrogen distances of epibatidine and homoepibatidine.

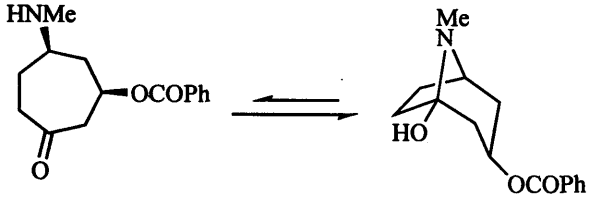
Chapter 3

Total Synthesis of 1-Hydroxytropacocaine and Analogues

3.1 INTRODUCTION

3.1.1 Biological significance of 1-hydroxytropanes

1-Hydroxytropacocaine **(153⇌154)**, a new alkaloid possessing the 1-hydroxy-tropane skeleton, was first isolated from the leaves of *Erythroxylum novogranatense* variants in 1994.[5] In addition to the significant quantities isolated from these plants, 1-hydroxytropacocaine was also found to be present, in small amounts, in *Erythroxylum coca*. 1-Hydroxytropacocaine joins a family of polyhydroxylated 1-hydroxynortropanes, the calystegines (Table 3.1) which have been isolated from well-known (and well-studied) plants only during the past decade.



(153) **(154)**

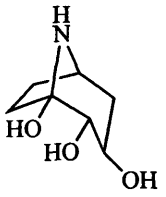
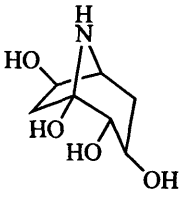
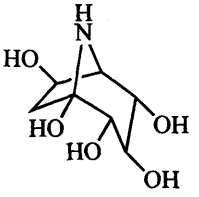
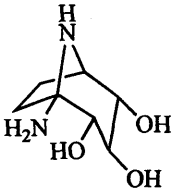
Figure 3.1

Calystegines were originally isolated from the bindweed *Calystegia sepium* (morning glory), from which they derive their name.[6] They have now been identified in three different plant families: the Convolvulaceae, the Moraceae and the Solanaceae. The calystegines are sub-divided according to their degree of hydroxylation. Calystegine N_1, the latest member of the family to be discovered, is unique in possessing a 1-amino group in place of the usual 1-hydroxy substituent. The large number of hydroxy groups makes calystegines extremely hydrophilic, hence, during the usual alkaloid extraction procedures, involving a lipophilic extraction step, they remain in the aqueous layer. This may explain why they have only recently been discovered.

Calystegines are glycosidase inhibitors and as such, may have an important role in plant defence. Indeed they have been found in certain types of lepidoptera (e.g. the Death's Head Hawk Moth) and are presumed to be sequested by these insects to deter predators.[65] In addition, they may provide a carbon and nitrogen source for soil bacteria which are beneficial to the rhizosphere of calystegine-producing plants. It is thought that calystegines are responsible for several instances of livestock poisoning, including the bovine neurological disorders, "Maldronksiekte" and "Crazy Cow

Syndrome". Calystegines also pose a potential risk to human health, as they occur in vegetables such as aubergines and potatoes and are reported to be potent inhibitors of human liver β-glycosidase.

Table 3.1 The Calystegines

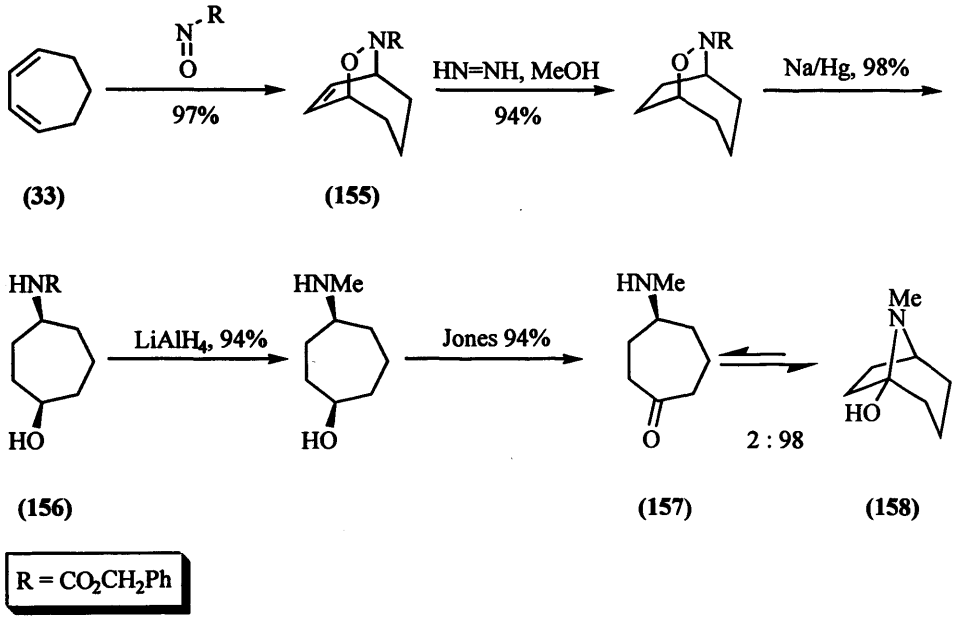
Sub-class	Degree of hydroxylation	Representative structure
calystegine A group	trihydroxy derivatives	 Calystegine A_3
calystegine B group	tetrahydroxy derivatives	 Calystegine B_1
calystegine C group	pentahydroxy derivatives	 Calystegine C_1
calystegine N group	1-amino-trihydroxy derivative	 Calystegine N_1

In addition to their biological activity, 1-hydroxytropanes are also interesting because they can exhibit tautomerism between the bicyclic hemi-aminal and monocyclic amino-ketone forms. This was first shown in the case of physoperuvine **(157⇌158)**, the first naturally-occurring 1-hydroxytropane to be discovered.[66,28,67] In contrast, calystegine A_3, B_1 and B_2 are reported to be exclusively bicyclic.[68] However, Lydon *et al.* characterised 1-hydroxytropacocaine as the O-heptafluorobutanoyl (HFB)

derivative, because of its apparent tendency to decompose in methanol.[5] Obviously, such derivatisation prevented tautomerism occurring in this case.

3.1.2 Synthesis of 1-hydroxytropanes

1-Hydroxytropanes are not directly accessible using routes based on cycloaddition chemistry. However, the novel approach developed at Leicester can be adapted to the production of both 1-hydroxytropanes and their higher homologues. This is illustrated by the synthesis of physoperuvine **(157 ⇋ 158)** (Scheme 3.1).[28]



Scheme 3.1

The oxazine **(155)** was synthesised in 97% yield from cyclohepta-1,3-diene **(33)** by addition of benzylnitrosoformate, which was formed *in situ* by the reaction of benzyl N-hydroxycarbamate and tetramethylammonium periodate. Reduction with diimide and subsequent cleavage of the NO bond gave the key 4-aminocycloheptanol **(156)** in an overall yield of 92% from **(155)**. Reduction with lithium aluminium hydride followed by Jones oxidation afforded physoperuvine **(157⇋158)** in 79% overall yield from cycloheptadiene.

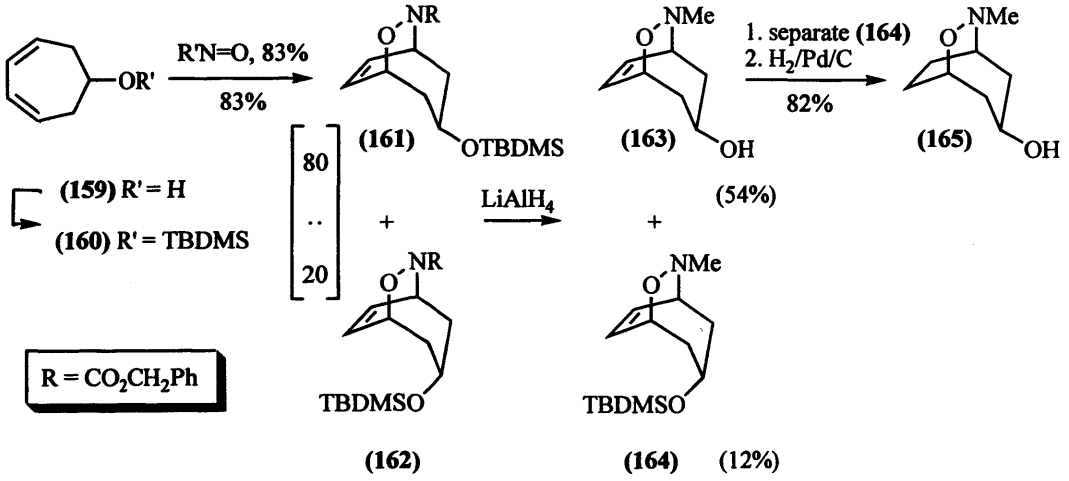
3.2 SYNTHESIS OF 1-HYDROXYTROPACOCAINE

The successful synthesis of physoperuvine within the group inspired us to adapt our route to tropanes to encompass 1,3-dihydroxytropane derivatives, focusing particularly on **(153⇋154)**. Since all the calystegines discovered thus far are based on

the 1-hydroxy-*nor*-tropane skeleton, it was envisaged that nor-derivatives of 1-hydroxytropacocaine or similar compounds might well be isolated from plants in the future. With this in mind, 1-hydroxynortropacocaine **(182⇌183)** was also selected as a target. In addition, it was our intention to investigate the tautomeric preferences of these compounds by means of VT NMR.

The method of choice for preparing **(153⇌154)** is shown in Scheme 3.2 and involved the cycloaddition of benzyl nitrosoformate to the protected cyclohepta-3,5-dienol **(160)** [instead of the parent compound **(159)**] since the presence of the bulky TBDMS group confers facial selectivity, leading to the preferential formation of the 3β-isomer **(161)**.[27] The mixture of **(161)** and **(162)** could not be separated by column chromatography.

The stereostructures of **(161)** and **(162)** could be assigned on the basis on the relative chemical shifts on the of the α-silyl ether protons. The 3α-proton of the major cycloadduct **(161)** appeared up-field (δ 3.68) as it was shielded by the double bond. The 3β-proton of **(162)** was further down-field at δ 4.35.

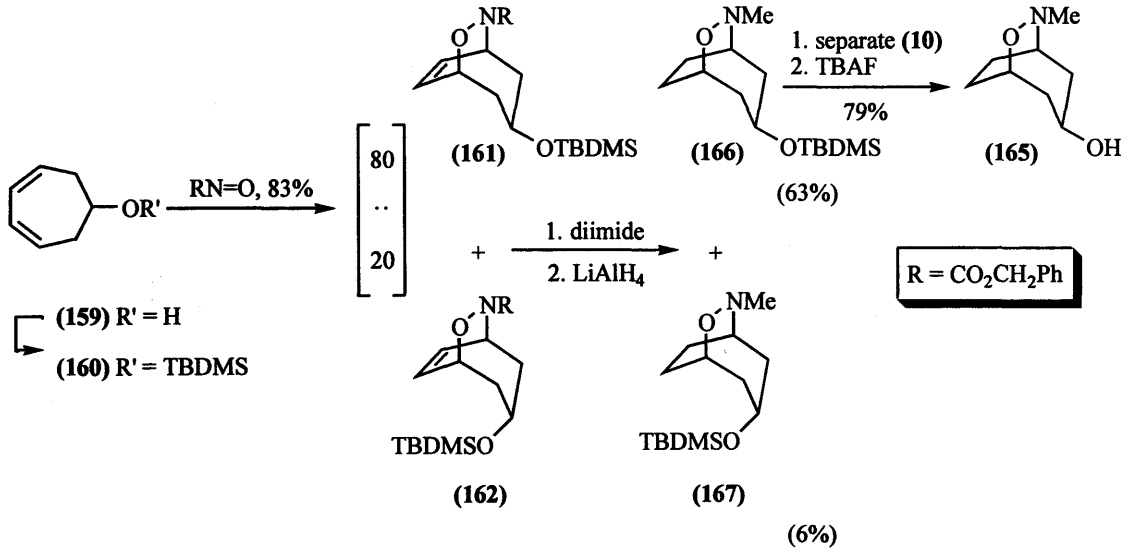


Scheme 3.2

Initially, direct reduction of **(161)** and **(162)** to the corresponding N-methyl derivatives was attempted. Treatment of a mixture of **(161)** and **(162)** with lithium aluminium hydride in refluxing THF resulted, not only in reduction of the N-protecting group, but also in concomitant desilylation of **(161)**. Surprisingly, the 3α-isomer retained the TBDMS protecting group under these conditions and hence **(163)** and **(164)** were easily separable by chromatography. Catalytic hydrogenation of **(163)** then afforded **(165)**. Unfortunately, the conditions leading to selective

desilylation of the 3β-isomer were not easily reproducible; mixtures containing differing amounts of the TBDMS derivative of **(163)** were obtained in later experiments and it was decided that a more reliable route to **(165)** had to be developed.

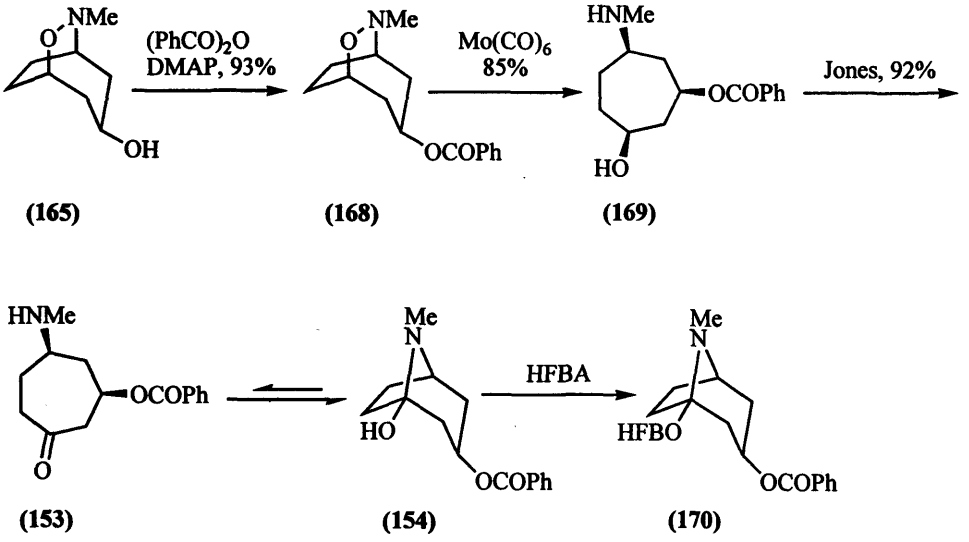
An alternative strategy for the production of **(165)** is outlined in Scheme 3.3. Reduction of **(161)** and **(162)** with diimide and subsequent treatment with lithium aluminium hydride yielded a mixture of **(166)** and **(167)**; these isomers were separated chromatographically to provide a pure sample of **(166)** in 63% overall yield from **(161)**. The α-silyl ether proton of **(166)** appeared in the ^{1}H NMR spectrum as a multiplet at δ 4.12. Its chemical shift was significantly down-field of the corresponding signal for **(161)** since it was no longer shielded by the double bond. A singlet, observed at δ 2.60 in the ^{1}H NMR spectrum of **(166)** and a signal at δ 44.7 in the ^{13}C NMR spectrum were assigned to the N-methyl group. Desilylation of **(166)** with TBAF afforded **(165)** in 79% yield; an exchangeable proton at δ 3.45 in the ^{1}H NMR spectrum confirmed the deprotection of the 3-hydroxyl.



Scheme 3.3

Esterification of **(165)** with benzoic anhydride and DMAP gave **(168)** in excellent yield (Scheme 3.4). The α-ester proton of **(168)** was visible in the ^{1}H NMR spectrum as an approximate triplet of triplets at δ 5.53. This arises from the axial nature of the proton, which shows a large vicinal coupling (J = 10.6 Hz) to each of the neighbouring axial protons. The axial-equatorial coupling was smaller (J = 6.6 Hz). These observations are precedented by the literature assignments for scopine and

pseudoscopine, where the axial 3α-proton of pseudoscopine appeared as a triplet of triplets, while the equatorial 3α-proton of scopine is seen as a triplet because the vicinal equatorial-equatorial coupling is virtually zero.[27] Signals for C_2 and C_4 in the ^{13}C NMR spectrum of **(168)** were broadened due to VT effects and could not be assigned with confidence. Cleavage of the NO bond with molybdenum hexacarbonyl afforded the *cis*-hydroxy amine **(169)**. Treatment of **(169)** with Jones reagent under standard conditions then provided the ketone **(153)**, which existed almost exclusively as the bicyclic tautomer **(154)**, in an overall yield of 33% from **(160)**.



Scheme 3.4

The oxidation of **(169)** was confirmed by a reduction in relative molecular mass of 2 as shown by mass spectrometry. The ^1H and ^{13}C NMR spectra for **(153⇌154)** were in good agreement with the data published by Lydon (Tables 3.2 and 3.3).[5]

Careful inspection of the ^{13}C NMR spectrum of **(153⇌154)** recorded at ambient temperature led to the assignment of all the carbon signals for **(154)** except that for C_1, which was missing. However, some signals, particularly those for C_2 and C_4, were broadened at this temperature. Lowering the temperature resulted in a sharpening of the spectrum and the appearance of a characteristic quaternary carbon signal at δ 88.8 which was assigned to C_1. These data compare well with those recorded by Justice for physoperuvine [28] and confirm that tautomerism is occurring despite the fact that the proportion of monocyclic tautomer is insufficient to allow the signals due to **(153)** to be assigned with any degree of certainty.

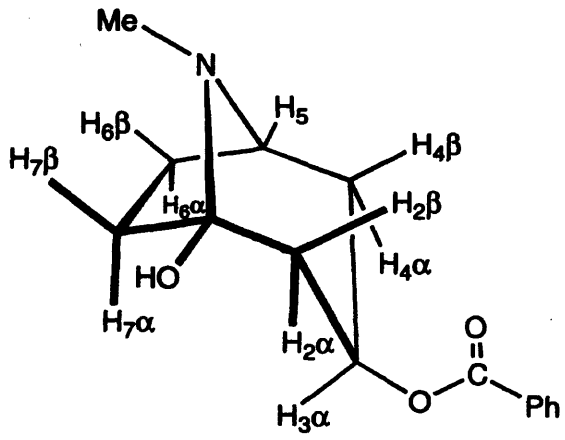
Table 3.2 NMR data for 1-hydroxytropacocaine (153⇋154), 1-OHFB derivative (170), and 1-hydroxynortropacocaine (182⇋183).

proton/ carbon	(153) ⇋ (154) $^1H^b$ 223K	$(170)^5$ ^1H	(182) ⇋ (183) $^1H^b$ 223K	(153) ⇋ (154) ^{13}C 223K	(153) ⇋ (154) ^{13}C 300K	$(170)^5$ ^{13}C	(182) ⇋ (183) ^{13}C 223K	(182) ⇋ (183) ^{13}C 223K
1				88.8	*c*	99.8	90.6	*c*
2β (axial)	~1.93 m	2.09 d^3	1.92 brd^3	33.9	35.2*d*	31.7	43.8	44.8
2α (equatorial)	~1.93 m	2.71 d^3	2.53 d^3					68.4
3α (axial)	5.30 d^4	5.32 d^4	5.38 d^4	68.4	68.2	67.2	68.1	68.4
4β (axial)	2.02 m	2.00 d^4	1.65 d^4	27.4	29.0*d*	28.8	36.8	38.0
4α (equatorial)	1.75 d^3	1.85 d^{3+}	2.15 m					
5	3.43 d^4	3.52 d^4	3.68 brd^3	56.3	57.2	56.5	52.0	52.3
6β (exo-)	2.08 m	2.18 d^4	~2.10 m	25.2	25.0	25.0	27.1	27.8
6α (endo-)	1.69 brd^3	1.67 d^4	1.75 brd^3					
7β (exo-)	1.82 brd^3	2.03 d^4	~1.90 m	36.0	36.1	33.5	34.5	35.4
7α (endo-)	2.05 m	2.61 d^3	2.05 d^3					
Me	2.42 s	2.53 s		29.2	29.7	29.9		
1'				130.4	130.4	130.0	130.3	130.8
2', 6'	8.05 d^2	8.01 d^2	8.04 d^2	129.8	129.6	129.6	129.9	129.8
3', 5'	7.48 d^2	7.44 d^2	7.48 brt	128.8	128.4	128.4	129.0	128.7
4'	7.62 t^2	7.56 t^2	7.61 t^2	133.5	133.0	133.2	133.7	133.3
PhC=O				166.0	166.0	165.7	166.1	166.1
NH/OH	*b*		5.04 brs					

Spectra in CD_2Cl_2 at 223 and 300K; d^3 = ddd; t^2 = tt etc.
Aryl signals were assigned by analogy with the data reported for **(170)**[5] and using a CH COSY spectrum

* Lydon did not mention which NMR solvent was used to obtain spectra for **(170)**.
b: Some signals in the ^1H NMR spectra of **(153⇋154)** and **(182⇋183)** were second-order. These were simplified using homonuclear spin-decoupling experiments and interpreted (assuming pseudo first-order behaviour) with the aid of ^1H-^1H and ^1H-^{13}C COSY spectra. The OH and NH signals were broad and their positions varied.
c: Signal not visible at this temperature as a result of monocyclic/bicyclic tautomerism.
d: Broad signal as a result of tautomerism.

Table 3.3 **J values for 1-hydroxytropacocaine (153⇋154), 1-OHFB derivative (170) and 1-hydroxynortropacocaine (182⇋183) (values in Hz)**



$J_{H,H}$	(153) ⇋ (154)	(170)[5]	(182) ⇋ (183)
$J_{2,2}$	*	12.4	11.9
$J_{2\alpha,3\alpha}$	6.5	6.1	6.5
$J_{2\beta,3\alpha}$	10.5	10.5	10.7
$J_{2\alpha,4\alpha}$	< 1	< 1	ca. 0.6
$J_{2\beta,7\beta}$	*	ca. 2	2.4
$J_{3\alpha,4\alpha}$	6.5	6.5	6.5
$J_{3\alpha,4\beta}$	10.5	10.7	10.7
$J_{4,4}$	12.5	13.3	13.3
$J_{4\alpha,5}$	2.5	2.5	2.2
$J_{4\beta,5}$	3.0	3.6	3.4
$J_{4\beta,6\beta}$	*	ca. 1	1.3
$J_{5,6\beta}$	7.0	7.3	7.2
$J_{5,6\alpha}$	< 1	< 1	ca. 0.6
$J_{6,6}$	13	13.3	12.7
$J_{6\alpha,7\alpha}$	10	9.0	9.8
$J_{6\alpha,7\beta}$	4.0	ca. 5	4.3
$J_{6\beta,7\alpha}$	*	ca. 5	4.8
$J_{6\beta,7\beta}$	13.5	*	12.7
$J_{7,7}$	13.5	13.3	13.3

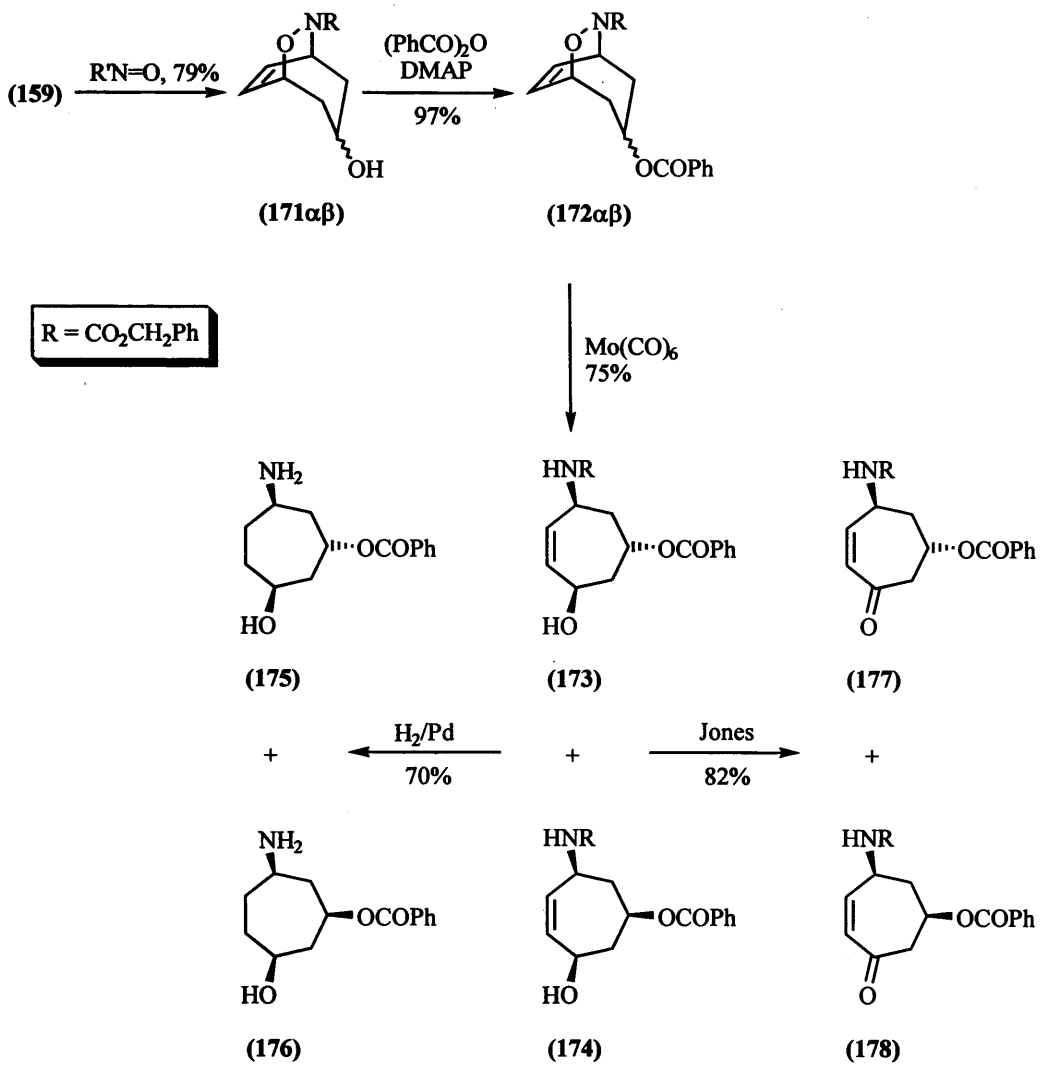
The 3α proton appeared in the ^{1}H NMR spectrum of **(154)** as a doublet of doublet of doublet of doublets (an approximate triplet of triplets) at δ 5.30 and hence, verified that the ester group was equatorial.

Lydon *et al.* characterised 1-hydroxytropacocaine as 1-OHFB derivative **(170)** because of fears about its instability in methanol.[5] In contrast, we found 1-hydroxytropacocaine to be stable during purification by flash chromatography (eluting with ethyl ethanoate/methanol/ammonia). It was, therefore, necessary to convert a sample of **(153⇋154)** into **(170)** in order to be able to make a direct comparison with the spectral data published by Lydon.[5] However, this proved more difficult than anticipated. Lydon did not make any reference to the NMR solvent used in the characterisation of **(170)**. Different solvents were found to elicit small changes

in chemical shift. Drying and basifying a sample of **(170)** (in $CDCl_3$) with anhydrous potassium carbonate, finally produced a spectrum consistent with the literature data.[5]

3.3 SYNTHESIS OF 1-HYDROXYNORTROPACOCAINE

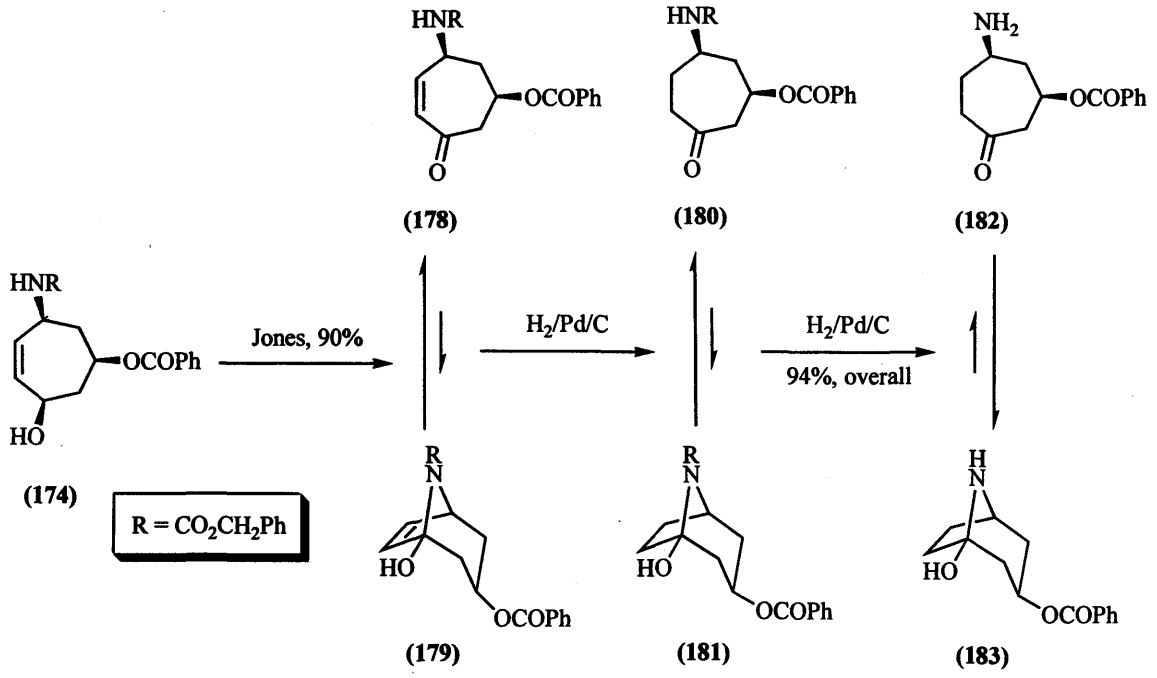
1-Hydroxynortropacocaine **(182⇌183)** was synthesised by a route based on that described above. The mixture of cycloadducts **(171αβ)** (ratio 35:65) was produced by reaction of cyclohepta-3,5-dienol **(159)** under standard nitroso cycloaddition conditions (Scheme 3.5).[27] The compounds could not be separated chromatographically, but it was hoped that the lack of stereoselectivity in this reaction could still be turned to advantage by later separation of derivatives, thus providing easy access to both the 3α- and 3β-derivatives of **(182⇌183)**. Hence, the mixture **(171αβ)** was esterified using an identical procedure to that described for the esterification of **(165)**, to produce **(172αβ)** in unchanged ratio after chromatography on silica (eluting with 2:3 diethyl ether:petroleum ether (b.p. 40-60°C)). The 3α-proton of **(172β)** appeared as an approximate triplet of triplets (J = 11.0, 6.4 Hz) at δ 5.03, up-field of the 3β-proton of **(172α)** which was seen at δ 5.51 (~tt, J = 5.3, 3.6 Hz). This observation is in good agreement with the relative chemical shifts of **(161)** and **(162)**. A pure sample of the 3β-ester **(172β)** was obtained by recystallisation from 1:1 diethyl ether:petroleum ether (b.p. 60-80°C) (m.p. 105°C). Subsequent treatment of the mixture **(172αβ)** with molybdenum hexacarbonyl cleaved the NO bond to yield **(173)** and **(174)**. Separation of **(173)** and **(174)** was difficult, and it was hoped that either oxidation to **(177)** and **(178)** or reduction to **(175)** and **(176)** would provide an easier alternative. Removal of the N-protecting group and concomitant hydrogenation of **(173)** and **(174)** afforded a mixture of **(175)** and **(176)**. However, the high polarity of these compounds made them difficult to separate effectively. Attention was turned to the production of the ketones **(177)** and **(178)**. Although formed in excellent yield, it transpired that **(177)** and **(178)** were inseparable. With care, a pure sample of **(174)** was separated chromatographically and was recystallised from 1:1 diethyl ether:petroleum ether (b.p. 60-80°C) to give a white cystalline solid (m.p. 145-147°C); however, the minor isomer **(173)** co-eluted with an equal amount of **(174)**.



Scheme 3.5

Jones oxidation of pure **(174)** proceeded smoothly to give **(178)** (Scheme 3.6) which existed only in the monocyclic form with no spectroscopic evidence for the hemiaminal **(179)**. Simultaneous deprotection and hydrogenation over 3 hours afforded 1-hydroxy-nortropacocaine **(182⇌183)**. Hydrogenation over a shorter period produced the saturated derivative **(180⇌181)**, however, this could not be purified. Key features of the ^{1}H NMR spectrum of **(182⇌183)** were: firstly, the appearance of the 3α-proton as a doublet of doublet of doublet of doublets (for J values see Table 3.3) which again provided evidence for the equatorial nature of the ester group; secondly, a broad singlet at δ 5.04 which was assigned to the NH proton. On cooling to -50°C only one tautomer was observed, with a new signal appearing in the ^{13}C NMR spectrum at δ 90.6 which was assigned to the quaternary carbon of the

bicyclic tautomer **(183)**. The signals for C_2 and C_4 were significantly down-field of those observed for the corresponding carbons of **(154)**.



Scheme 3.6

Owing to time constraints, work was confined to the conversion of **(174)** into **(182⇌183)**. Nevertheless, in principle, it should be possible to synthesise the 3α - isomer of **(182⇌183)** from **(173)** using the route outlined in Scheme 3.6.

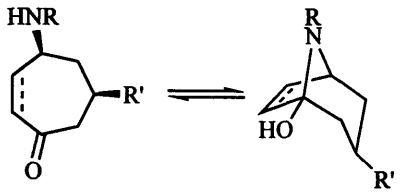
3.4 CONCLUSION

The ratios of monocyclic:bicyclic tautomers observed for compounds **(153⇌154)** and **(182⇌183)** together with those for the N-benzyloxycarbonyl derivatives **(178⇌179)** and **(180⇌181)** are summarised in Table 3.4. Physoperuvine **(157⇌158)** and its analogues, synthesised by Justice,[28] are included for comparison. Both physoperuvine and norphysoperuvine exist almost exclusively as the bicyclic hemi-aminals.[28] 1-Hydroxytropacocaine **(153⇌154)** and 1-hydroxynortropacocaine **(182⇌183)** also show a marked preference for the bicyclic tautomer.

The opposite is true for both the unsaturated **(178⇌179)** and saturated **(180⇌181)** carbamates, which existed only in the monocyclic form. This dichotomy

can be rationalised in several ways. It can be argued that for the unsaturated derivative **(178⇌179)**, the resonance stabilisation of the α,β-unsaturated ketone contributes to the preference for the monocyclic tautomer **(178)**.

Table 3.4 Tautomeric ratios for 1,3-dihydroxytropane/3-hydroxy-5-aminocycloheptanone derivatives

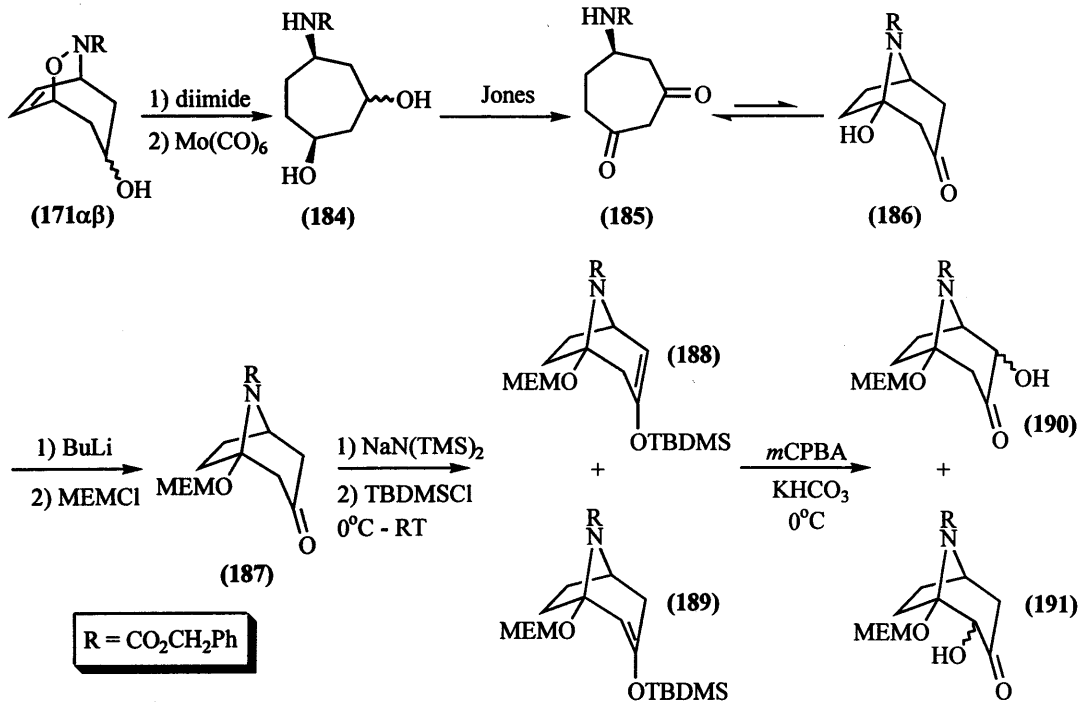
Compound			Ratio (MC:BC)
			
1-hydroxytropacocaine	R = Me R' = OCOPh	**(153⇌154)**	ca. 0 : 100
1-hydroxynortropacocaine	R = H R' = OCOPh	**(182⇌183)**	ca. 0 : 100
Physoperuvine	R = Me, R' = H	**(157⇌158)**	2 : 98
norphysoperuvine	R = H, R' = H		ca. 0 : 100
N-benzyloxycarbonyl-1-hydroxynortropacocaine	R = CO_2CH_2Ph R' = OCOPh	**(180⇌181)**	ca. 100 : 0
N-benzyloxycarbonyl-norphysoperuvine	R = CO_2CH_2Ph R' = H		ca. 100 : 0
N-benzyloxycarbonyl-6,7-dehydro-1-hydroxy-nortropacocaine	R = CO_2CH_2Ph R' = OCOPh	**(178⇌179)**	ca. 100 : 0
6,7-dehydrophysoperuvine	R = Me, R' = H		major : minor

However, the greater influence is likely to be the hybridisation of the bridging nitrogen. In the bicyclic form **(179)** the sp^2-nitrogen is forced to adopt a bond angle much smaller than the optimal value of 120°, whereas the sp^3-nitrogens of **(154)** and **(183)** are less distorted from their ideal angle of 109.5°. This is borne out by the shift from monocyclic to bicyclic tautomer in going from **(180⇌181)** to **(182⇌183)**. These findings corroborate previous results in which the equilibrium is heavily weighted towards the monocyclic form where the bridging nitrogen is in conjugation with a carbamate protecting group, but reverses to favour the bicyclic tautomer in both physoperuvine and norphysoperuvine.

3.5 FURTHER FUNCTIONALISATION OF 1-HYDROXYTROPANES

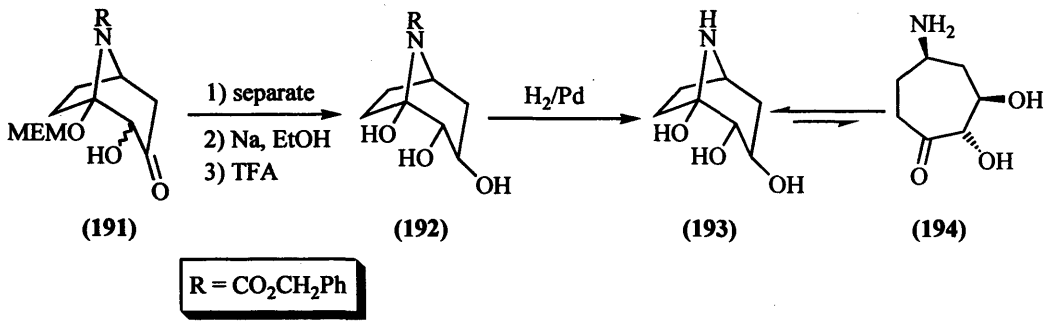
This chapter has described a successful synthetic strategy to 1-hydroxy-tropacocaine **(153⇌154)** and the novel nor-derivative **(182⇌183)**. A method of introducing further oxygen functionality into the tropane ring was now sought. This would provide the opportunity of extending the present synthesis of 1,3-dihydroxytropanes to include the calystegines and/or calystegine analogues.

A possible route to calystegine A_3 is outlined in Schemes 3.7 and 3.8. The bicyclic oxazines **(171α)** and **(171β)** were intermediates in the synthesis of 1-hydroxynortropacocaine. Reduction of the double bond, followed by treatment with molybdenum hexacarbonyl should give the amino alcohol **(184)**. Oxidation will produce the key diketone **(185)** which will be in equilibrium with the bicyclic form **(186)**. This can be trapped out as the ether **(187)**.[57] Conversion of **(187)** into the corresponding silyl enol ethers **(188)** and **(189)**, followed by oxidation with *m*CPBA should result in α-hydroxylation of the ketone to give **(190)** and **(191)**.[69]



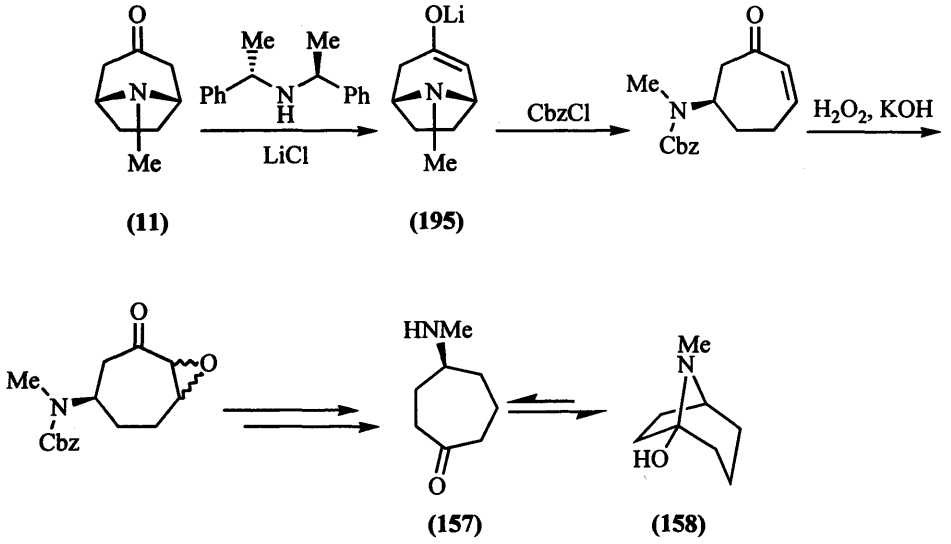
Scheme 3.7

Separation of the regio- and stereoisomers, reduction of the 3-keto group and dealkylation of the 1-hydroxy position should give the protected trihydroxy derivative **(192)**. Hydrogenolysis would then afford calystegine A_3 **(193⇌194)**.



Scheme 3.8

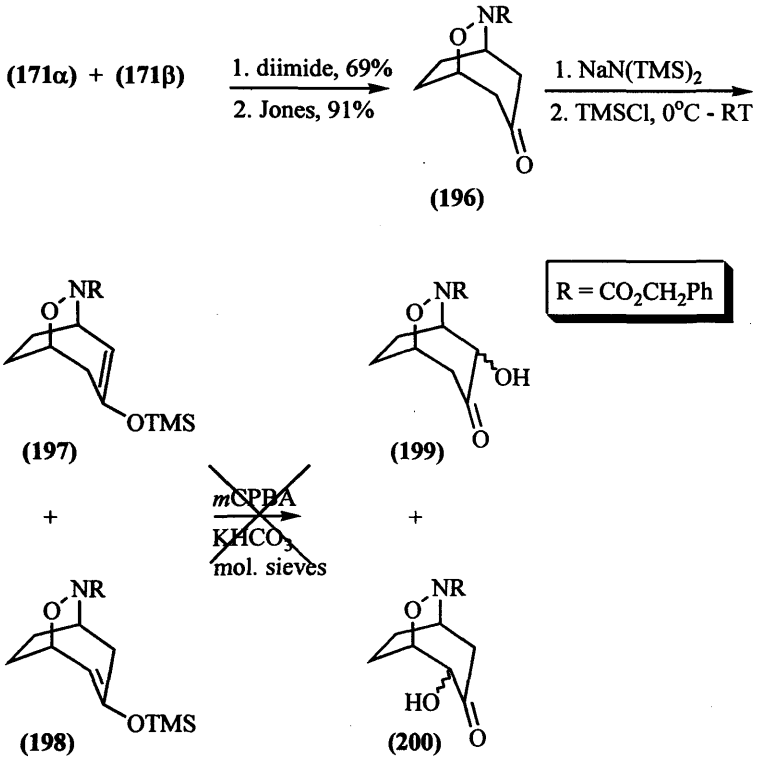
A precedent for this approach is found in the work of Majewski who has investigated enolate formation in tropinone **(11)**.[70] Chiral lithium amide bases were used to deprotonate tropinone enantioselectively (with enantiomeric excesses of up to 96%). Enolates such as **(195)** undergo a ring opening reaction on treatment with a chloroformate to give αβ-unsaturated ketones, a reaction exploited by Majewski in a synthesis of physoperuvine **(157⇌158)** (Scheme 3.9).[70]



Scheme 3.9

Preliminary investigations focused on α-hydroxylation of the 3-keto group of **(196)**, which was produced by reaction of **(171αβ)** with diimide and subsequent oxidation with Jones reagent (Scheme 3.10). Treatment of **(196)** with sodium bis(trimethyl-silyl)amide, followed by the addition of a solution of trimethyl-silylchloride and triethylamine in THF, gave a crude mixture of **(197)** and **(198)**. The ^{1}H NMR spectrum displayed characteristic α-N bridgehead protons of similar shift to those of cycloadducts prepared previously. Two broad singlets at δ 6.00 and δ 6.09 were also visible and were assigned to the alkene protons of **(199)** and **(200)**

respectively. The relative integration of these signals suggested a 1:1 ratio of regioisomers. Attempts to purify this mixture by column chromatography resulted in isolation of the starting ketone **(196)** as the only product. Therefore, the oxidation step was attempted on a crude mixture of silyl enol ethers. A solution of **(199)** and **(200)** in dry dichloromethane was stirred over pre-dried potassium hydrogen carbonate and powdered molecular sieves.[71] To this mixture a solution of *m*CPBA, in dichloromethane, was added, over a period of 4 hours, at 0°C. Only the ketone **(196)** was isolated from this reaction.



Scheme 3.10

With hindsight, it would have been better to use a more stable silyl group such as the TBDMS group. This would have allowed purification of the intermediate silyl enol ethers and made them less susceptible to hydrolysis before the oxidation took place.

An alternative oxidation, which may avoid these problems altogether, could be performed with dimethyldioxirane (DMDO).[72] This is a very simple reaction which can be carried out in dichloromethane at room temperature. With the advent of simpler methods for the preparation of DMDO[73] this route would appear to be a feasible alternative to that described above.

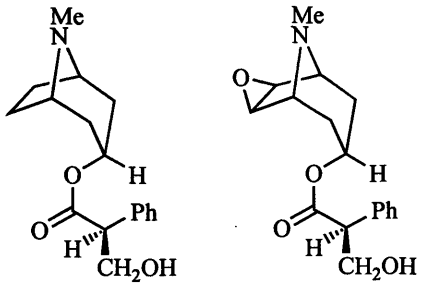
Chapter 4

Investigation of the Biosynthetic Route to 1-Hydroxytropanes

4.1 INTRODUCTION

4.1.1 Biosynthesis of the tropane alkaloids

The pharmacological importance of compounds such as (-)-hyoscyamine **(201)** and scopolamine **(5)** has led to considerable efforts to elucidate their biosynthetic pathways. However, it is only during the past two decades that significant progress in determining the biosynthetic routes to these alkaloids has been made.[74]

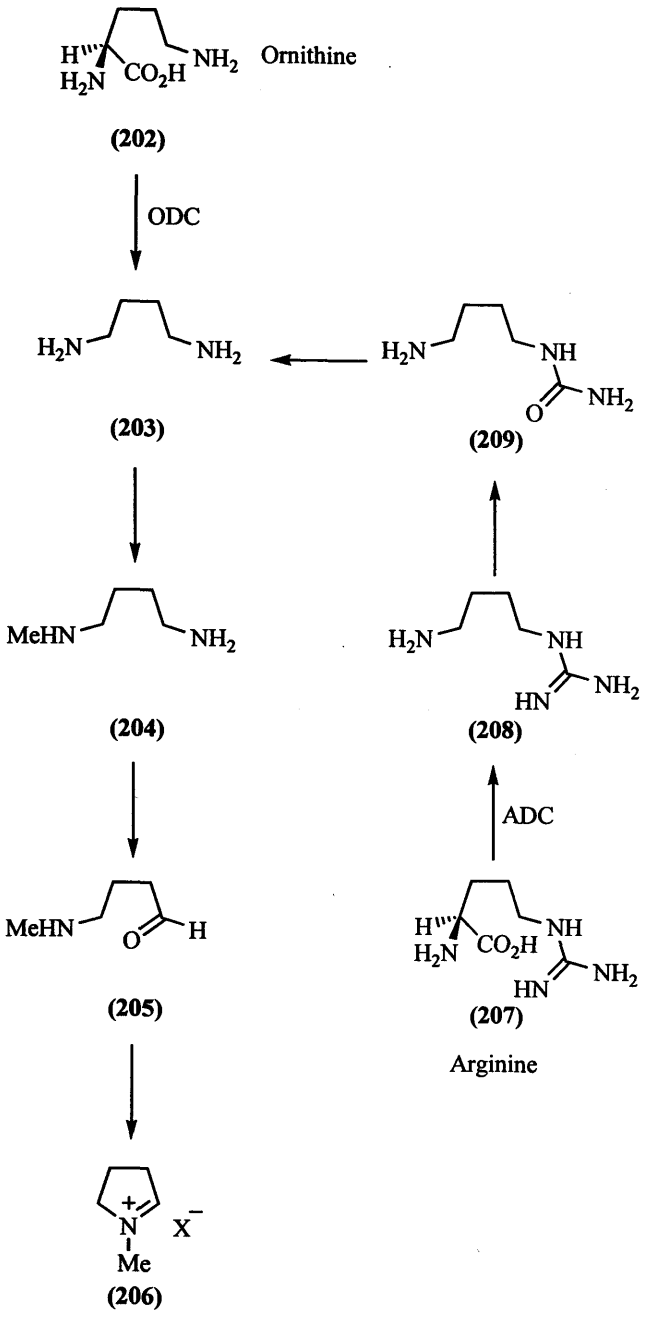


(201) **(5)**

Figure 4.1

4.1.1.1 Biosynthesis of the N-methylpyrrolinium salt (206)

It has been shown by radio- and stable-isotope labelling experiments that the pyrrolidine ring of the tropane alkaloids is derived from either ornithine **(202)** or arginine **(207)** (Scheme 4.1). Decarboxylation of ornithine, catalysed by ornithine decarboxylase (ODC) leads directly to the diamine putrescine **(203)**. Arginine is also decarboxylated, by arginine decarboxylase (ADC) to produce agmatine **(208)** which is rapidly converted to **(203)** *via* N-carbamyl-putrescine **(209)**. Since putrescine is involved in other biological processes within cells, N-methylputrescine **(204)** is often said to be the first true metabolite in the tropane alkaloid pathway. N-methyl-putrescine is converted into 4-methylaminobutanal **(205)** which then undergoes intramolecular condensation to yield the imininium salt **(206)**. It is probable that the exact mechanism by which these transformations occur differs slightly between plant species and that unbound putrescine is not a true intermediate in every case.

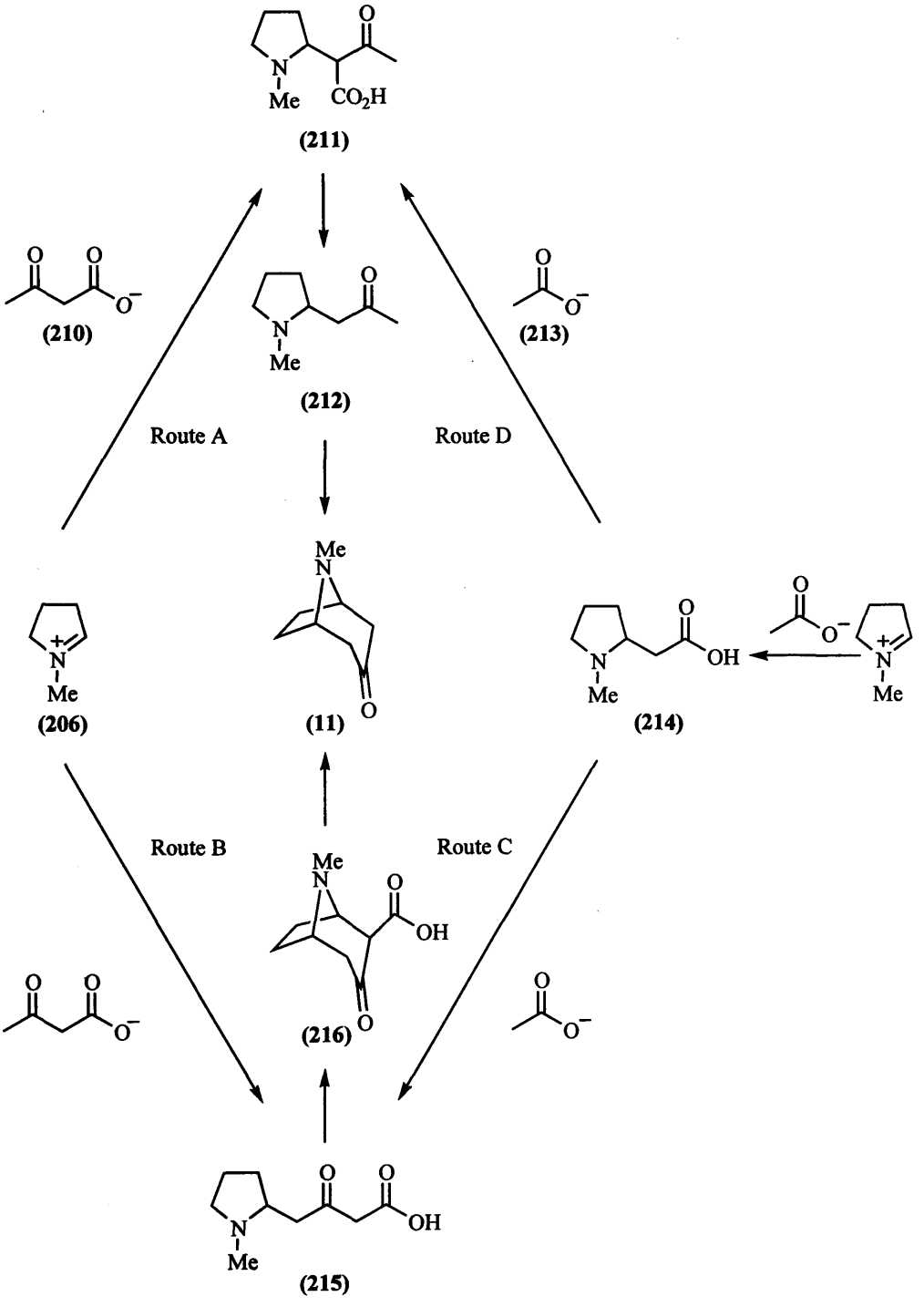


Scheme 4.1

4.1.1.2 The identity of the intermediates between the N-methylpyrrolinium salt (206) and tropinone (11)

The identity of the intermediates between the N-methylpyrrolinium salt **(206)** and tropinone **(11)** has been a source of controversy over recent years. It is generally believed that the 3-carbon bridge of the tropane skeleton is derived from acetate, *via* acetyl co-enzyme A. However, four possible routes have been proposed for the addition of acetate **(213)** to **(206)** (Scheme 4.2). It was originally postulated that all tropane alkaloids were biosynthesised by addition of acetoacetate **(210)** to **(206)** to

give **(211)** (Route A). Decarboxylation then produced hygrine **(212)**. A further Mannich reaction was thought to produce tropinone **(11)**. However, it has recently been shown that, while **(212)** occurs in the roots of many tropane-producing species, it is not a direct precursor to cocaine, (-)-hyoscyamine **(201)** or scopolamine **(5)**.[75]

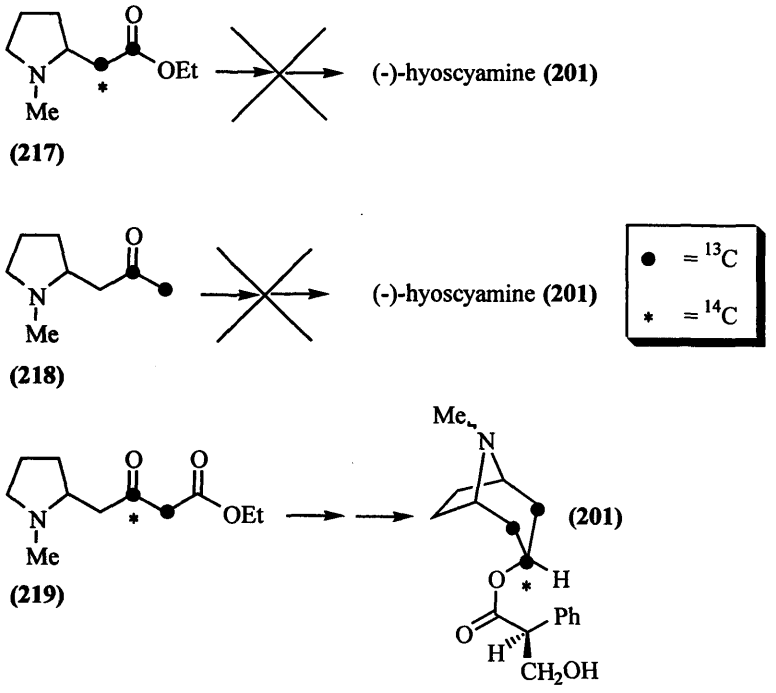


Scheme 4.2

It was later suggested that cocaine was biosynthesised by the consecutive addition of two units of acetate **(213)** to **(206)** to give firstly **(214)** and then **(215)** as an

intermediate (Route C). As a result, Route C was also proposed for the biosynthesis of (-)-hyoscyamine **(201)** and scopolamine **(5)**. Ring closure of **(215)** could then yield 2-carboxytropinone **(216)** (or the ester thereof); decarboxylation would give tropinone **(11)**.

Recent work by Robins *et al.*[75] has tested these proposals using the labelled precursors shown in Scheme 4.3 and has led to substantial clarification of tropane biosynthesis.



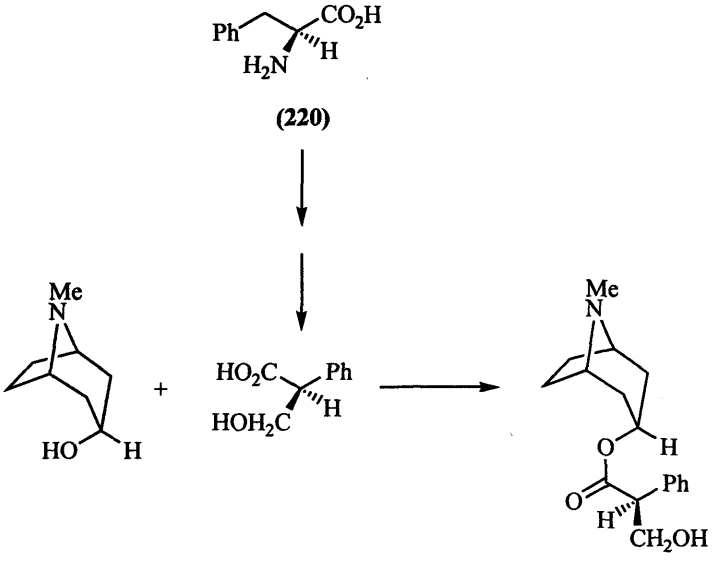
Scheme 4.3

Ethyl (R,S)-[1,2-$^{13}C_2$,2-^{14}C]-2-(1-methyl-2-pyrrolidinyl)-acetate **(217)** was not incorporated by root cultures into (-)-hyoscyamine **(201)**; this casts doubt on the intermediacy of **(214)** and hence makes it improbable that **(201)** is formed from **(206)** *via* Routes C or D. The failure to obtain any incorporation of (R,S)-[2',3'-$^{13}C_2$]-hygrine **(218)** confirms that **(212)** is not an intermediate for the biosynthesis of **(201)** and therefore, that Route A does not operate. In contrast, ethyl (R,S)-[2,3-$^{13}C_2$,3-^{14}C]-4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate **(219)**, found to be incorporated into scopolamine in whole plants (*Datura innoxia*), was also shown to be incorporated into **(201)** in root cultures of *Datura stramonium*. This result suggests that either Route B or C (with **(215)** as an intermediate) is involved in the biosynthesis of hyoscyamine, while Routes A and D are not.

Taken as a whole, this evidence suggests, that all three remaining carbons are added to the pyrrolidine ring of the tropane skeleton in a single step. It is currently thought that the azabicyclo[3.2.1]octane ring is formed by condensation of **(206)** with acetoacetate **(210)**, followed by a further Mannich reaction (Route B). Decarboxylation of **(216)** then gives tropinone **(11)**. The stereochemistry of the reaction has yet to be determined, as has the intermediacy of **(210)**, which is not a satisfactory precursor in *Datura stramonium* owing to rapid hydrolysis.

4.1.1.3 Conversion of tropinone (11) to (-)-hyoscyamine (201)

Tropinone **(11)** is reduced by tropinone reductase I to tropine **(221)**, which is then esterified with tropic acid **(222)** (activated as a co-enzyme A thioester) to give (-)-hyoscyamine **(201)** (Scheme 4.4). It has been established the tropic acid is derived from phenylalanine **(220)**,[76] however, there has been little research to date into the enzyme that esterifies **(221)** with activated tropic acid.

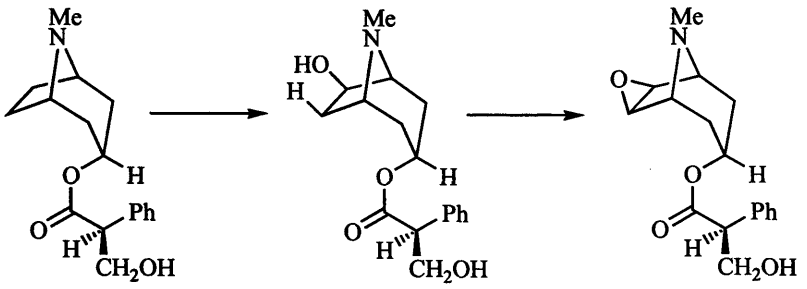


Scheme 4.4

4.1.1.4 Conversion of hyoscyamine (201) into scopolamine (5)

Many species of plants synthesise scopolamine **(5)** in addition to (-)-hyoscyamine **(201)**. The enzyme responsible for the conversion of **(201)** into **(5)** has now been isolated from root cultures of *H. niger*.[77] The epoxidation must occur at a late stage in the biosynthetic pathway, as the enzyme does not epoxidise the hydroxytropane **(221)**. It has been shown by ^{18}O-labelling experiments that

epoxidation of **(201)** is a two step process involving the intermediate **(223)**, with the same enzyme catalysing both steps (Scheme 4.5).[78]

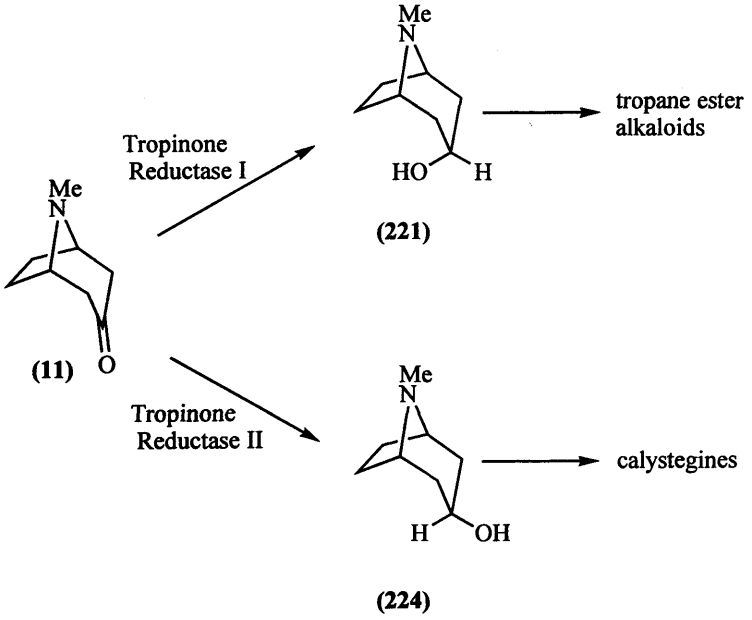


(201) **(223)** **(5)**

Scheme 4.5

4.1.2 Biosynthesis of the calystegines

Despite intensive investigations into the biological role of the calystegines, the biosynthetic pathway to 1-hydroxytropanes has yet to be elucidated. Dräger has proposed that calystegines are synthesised by an adaptation of the tropane alkaloid pathway. She has argued, that pseudotropine **(224)** is a plausible intermediate for the biosynthesis of calystegines, since all calystegines possess a 3β-hydroxy group, with the exception of calystegine A_6, which is not hydroxylated at the 3-position.[79] The proposed route is summarised in Scheme 4.6 and involves reduction of **(11)** to **(224)** by the enzyme tropinone reductase II.



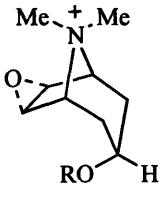
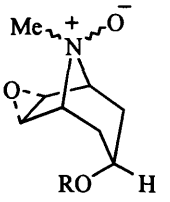
Scheme 4.6

There are several flaws in this proposal. Pseudotropine has been isolated in significant amounts from the leaves of field bindweed (*Convolvulus arvensis*); however, no calystegines were detected in the study.[80] As stated in the previous chapter, all known calystegines are based on the 1-hydroxy*nor*tropane skeleton. This poses a fundamental problem, as N-methylation occurs at a very early stage in the tropane alkaloid pathway (*vide supra*) and seems to be necessary for subsequent transformations. Hence, the biosynthetic pathway to calystegines remains unclear.

4.2 N-OXIDATION OF TROPANE DERIVATIVES

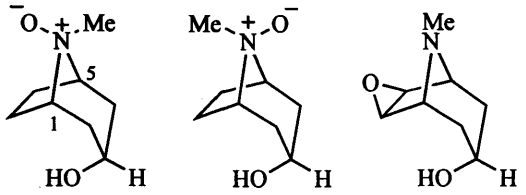
Tropane derivatives have played an important role in investigations of the stereochemistry of amine quaternisation reactions. This is because they have a semi-rigid structure, which results in the products of quaternisations having a similar structure to the transition state; in addition, unusually high stereoselectivity is observed for these reactions. It has been established that in the quaternisation reactions of tropanes, equatorial attack of the electrophile predominates.[81] As an extension of this work the configurations of various tropane N-oxides were investigated. Fodor *et al.* studied the stereochemistry of tropine, atropine and scopolamine N-oxides by comparing their ^{1}H NMR spectra with the corresponding methiodides (Table 4.1).[82]

Table 4.1 Comparison of N-methyl signals in N-oxides and methiodides

Parent skeleton	 **Methiodide** **δ NCH$_3$**		 **N-oxide** **δ NCH$_3$**	
	Equatorial	**Axial**	**Equatorial**	**Axial**
Tropine	3.16	3.10	3.20	-
Atropine	3.19	3.08	3.17	-
Scopolamine	3.30	3.12	3.32	-

Using this analogy, Fodor concluded that these N-oxides possessed an equatorial N-methyl group. Hence, oxidation occurred *via axial* attack of the oxidising agent, in contrast to the situation in quaternisation reactions. However, the analogy proved to be invalid, as a later X-ray study of scopolamine N-oxide, conducted by the same author, showed that the major isomer was, in fact, the equatorial N-oxide.[83] This was corroborated by a review of the ^{1}H NMR data. Based on this result, Fodor also reversed his assignments of the major isomers of tropine and atropine N-oxides.

However, it was not until the following year, that von Philipsborn proved that the equatorial tropine N-oxide **(225)** was, indeed, the major product in the N-oxidation of tropine.[84]



(225) **(226)** **(43)**

Figure 4.2

Tropine was treated with aqueous hydrogen peroxide solution.[85] In order to ascertain the structure of the major product and hence, the stereochemical course of the reaction, von Philipsborn *et al.* undertook a detailed analysis of the ^{1}H NMR spectra. Their assignments for each N-oxide are shown in Table 4.2.

Table 4.2 ^{1}H NMR data for tropine N-oxides (225) and (226)

Proton	Tropine N-oxide (225) (major)	Tropine N-oxide (226) (minor)
NCH_3	3.18 s	3.17 s
$H_{1,5}$	3.51 brm	2.42 m
$H_{2,4eq}$	1.98 d	1.69 d
$H_{2,4ax}$	2.40 m	2.84 dt
$H_{6,7\alpha}$	2.27 d	2.52 d
$H_{6,7\beta}$	2.45 m	2.11 m
$H_{3\beta}$	3.96 t	3.97 t

Spectra in CD_3OD at 220 MHz.

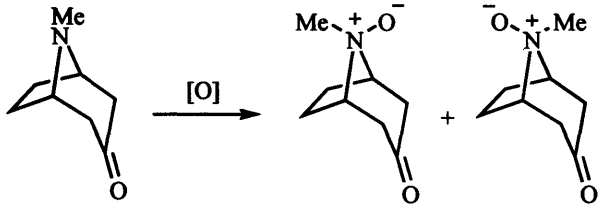
A pure sample of the major isomer **(225)** was obtained by recrystallisation from ethanol-diethyl ether and its spectrum compared to that of scopine **(43)**. This led von Philipsborn *et al.* to assign the up-field doublet at δ 1.98 to the equatorial protons on carbons two and four. The corresponding axial protons appeared as a multipet at δ 2.40. This was in contrast to earlier work where the up-field doublet had been erroneously assigned to $H_{2,4ax}$.[85] Signals due to the minor isomer **(226)** were assigned using a mixture of N-oxides enriched in the minor isomer. The equatorial protons at the two and four positions again appeared as a high field doublet (δ 1.69) while the axial protons (δ 2.84) gave rise to an approximate doublet of triplets as the result of coupling to the bridgehead protons and to $H_{3\beta}$. The chemical shifts of all protons in the N-oxides **(225)** and **(226)** were then expressed relative to the shifts of the corresponding protons in tropine ($\Delta\nu^{N}_{NO} = \nu_{N} - \nu_{NO}$). These values are shown in Table 4.3.

Table 4.3 Chemical shifts of tropine N-oxides relative to those of tropine $\Delta\nu^{N}_{NO}$ (Hz at 220 MHz)

	$H_{1,5}$	$H_{2,4eq}$	$H_{2,4ax}$	$H_{6,7\alpha}$	$H_{6,7\beta}$	$H_{3\beta}$	NCH_3
N-oxide (225) (major)	-94	-61	-74	-28.5	-101	-6	-205
N-oxide (226) (minor)	-74	+3	-171	-83.5	-26	-10	-202

Working on the premise that these changes in the chemical shift were caused mainly by the electric dipole in the NO bond, von Philipsborn *et al.* deduced that for N-oxide **(225)** the change in chemical shift of $H_{6,7\beta}$ would be greater than that for $H_{2,4ax}$. This is because the C-H and N-O bonds are almost coplanar in this structure. In N-oxide **(226)** $H_{2,4ax}$ should show an even bigger shift due to their closer proximity to the centre of the N-O dipole. From the data in Table 4.3 it is clear that the major isomer must be tropine N-oxide **(225)** ($H_{6,7\beta}$: $\Delta\nu^{N}_{NO}$ = -101 Hz; $H_{2,4ax}$: $\Delta\nu^{N}_{NO}$ = -74 Hz) and the minor isomer structure **(226)** ($H_{2,4ax}$: $\Delta\nu^{N}_{NO}$ = -171 Hz; $H_{6,7\beta}$: $\Delta\nu^{N}_{NO}$ = -26 Hz). Therefore, the oxidising agent approaches equatorially, not axially, as reported previously.[85]

The N-oxidation of tropinone (Scheme 4.7) was investigated by Shvo *et al.*[86] The tropinone N-oxides **(227)** and **(228)** were prepared by three different methods: a) oxidation with hydrogen peroxide solution; b) cycloaddition of cyclohepta-2,6-dienone with N-methylhydroxylamine; [87] c) oxidation with *m*CPBA.



(11) **(227)** **(228)**

Scheme 4.7

^{1}H NMR spectra showed that each reaction produced mixtures of **(227)** and **(228)** and that the composition of the mixture depended on the method of preparation. The N-oxides were separated and converted to picrate salts. The infra-red spectrum of the picrate of **(227)** shows no carbonyl absorption due to nucleophilic attack by the oxygen of the N-oxide (Figure 4.3). No such attack is possible in **(228)**.

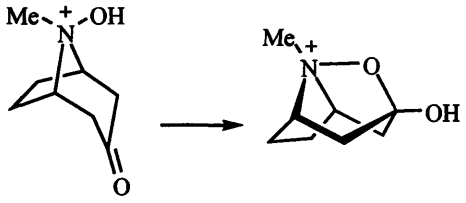


Figure 4.3

From this information the stereostructure of the major isomer in each reaction was determined and related to the chemical shift of the corresponding N-methyl group in the ^{1}H NMR spectrum. The results are summarised in Table 4.4.

Table 4.4 Composition of mixtures of tropinone N-oxides

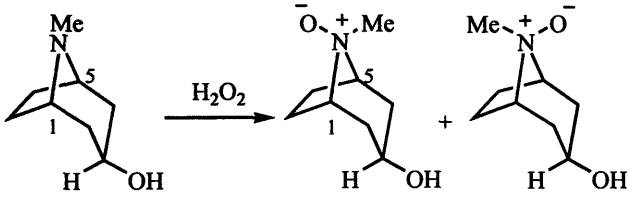
Reaction method	**% composition**	
	(227)	**(228)**
H_2O_2 oxidation	90	10
cycloaddition	60	40
***m*CPBA oxidation**	40	60

Surprisingly, the results implied preferential *axial* attack of hydrogen peroxide, the direct opposite of the situation in the N-oxidation of tropine. In contrast oxidation

with *m*CPBA results in equatorial attack being favoured. Shvo rationalised these results by showing that **(227)** and **(228)** were in equilibrium. Heating mixtures of the N-oxides in organic solvents, or placing picrates of either the pure isomers or a mixture of isomers in contact with basic aluminia, was found to interconvert the two stereoisomers. The thermodynamic ratio of **(227)**:**(228)** was observed to be 9:1.

Hence, it can be concluded that N-oxidation of tropinone with *m*CPBA is kinetically controlled, while N-oxidation with hydrogen peroxide is thermodynamically controlled and that the kinetically favoured isomer is the thermodynamically less stable one. This in turn means that equatorial attack of the oxidising agent is preferred, in line with other tropane quaternisations.

The N-oxidation reactions of tropine, pseudotropine and tropinone (Section 4.4) have been investigated at Leicester with the intention of preparing ^{18}O-labelled N-oxides for biosynthetic studies.[88] The starting point was the synthesis of tropine N-oxide using the method of Werner and Schickfluss.[85] ^1H NMR data for **(225)** and **(226)** agreed with the literature data and confirmed that the major isomer was the equatorial N-oxide **(225)**.



(229) **(230)** **(231)**

Scheme 4.8

As far as we are aware, the N-oxidation of pseudotropine **(229)** has not been reported previously. Oxidation of pseudotropine with aqueous hydrogen peroxide solution produced a mixture of N-oxides **(230)** and **(231)** in the ratio 3:1 (Scheme 4.8).

Stereochemical assignment of the pseudotropine N-oxides was made by comparision with the data reported by von Philipsborn and that reported for scopine and pseudoscopine.[27] The ^1H NMR data for pseudotropine and the N-oxides **(230)** and **(231)** are shown in Table 4.5.

Table 4.5 **^{1}H NMR data for pseudotropine (229) and pseudotropine N-oxides (230) and (231)**

Proton	Pseudotropine (229)[a,b]	Pseudotropine N-oxide (230) (major)[a,c]	Pseudotropine N-oxide (231) (minor)[a,c]
NCH_3	2.30 s	3.36 s	3.19 s
$H_{1,5}$	3.20 brt, J = ~ 3.3	3.63 brs	3.96 brs
$H_{2,4eq}$	1.85 ddd, J = ~13, 12, 2.5	~1.90 – 2.10 m	~1.78 m
$H_{2,4ax}$	1.70 ddd, J = ~13, 6.0, 3.2	~1.90 – 2.10 m	~2.60 m
$H_{6,7\alpha}$	1.57 m	1.85 m	2.13 m
$H_{6,7\beta}$	2.02 brm	2.52 m	2.23 m
$H_{3\beta}$	3.90 ~tt, J = 12, 6.0	4.17 tt, J = 10.5, 7.0	4.05 tt, J = ~11.2, 5.0

a: J values in Hz.
b: Spectra in $CDCl_3$ at 300 MHz.
c: Spectra in CD_3OD at 300 MHz.

As already discussed, the chemical shifts of the $H_{6,7}$ and $H_{2,4}$ protons are of particular value in the stereochemical assignment of tropane N-oxides. A comparison of the shifts of the $H_{6,7}$ protons in tropine N-oxides **(226)** and **(227)** with the corresponding values for the pseudotropine N-oxides **(231)** and **(232)** (Table 4.6) shows that in both major isomers the $H_{6,7\beta}$ protons are deshielded with respect to those of the minor isomer. This is indicative that the equatorial N-oxide is the major isomer.

Table 4.6 **Summary of ^{1}H NMR data for $H_{6.7}$ of tropine N-oxides (225) and (226) and pseudotropine N-oxides (230) and (231)**

	Major Isomer		Minor Isomer	
	$H_{6,7\beta}$	$H_{6,7\alpha}$	$H_{6,7\beta}$	$H_{6,7\alpha}$
Tropine N-oxides (225) &(226)[84]	2.45 m	2.27 d	2.11 m	2.52 d
Pseudotropine N-oxides (230) & (231)	2.52 m	1.85 m	2.23 m	2.13 m

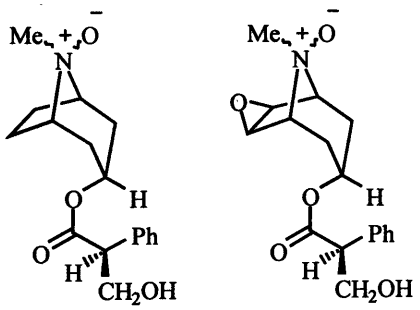
Interestingly, the $H_{6,7\alpha}$ protons in the minor isomer of tropine N-oxide appear downfield of the $H_{6,7\beta}$ protons, this is probably due to the deshielding effect of the 3α-hydroxy group. Unfortunately, in the ^{1}H NMR spectrum of **(230)** signals for $H_{2,4ax}$ and $H_{2,4eq}$ overlapped, making the assignment of exact chemical shifts difficult.

However, the difference in chemical shift between $H_{2,4ax}$ and $H_{2,4eq}$ cannot be greater that ca. 0.2 ppm. In the minor isomer **(231)** $H_{2,4ax}$ protons are deshielded by ca. 0.8 ppm, with respect to $H_{2,4eq}$, suggesting that this is an axial N-oxide. Therefore, all the evidence suggests that **(230)** is the major isomer and **(231)** the minor one i.e. N-oxidation of pseudotropine occurs *via* preferential equatorial attack.

4.3 PROPOSED BIOSYNTHETIC ROUTES TO 1-HYDROXYTROPANES

4.3.1 Background

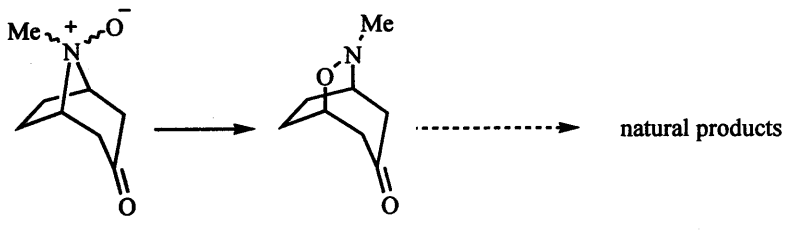
This chapter outlines two plausible routes for the biosynthesis of 1-hydroxy-tropanes, both of which feature tropinone N-oxides as key intermediates. N-oxide derivatives of tropane esters are known to occur in nature, for example hyoscyamine N-oxide **(232)** and scopolamine N-oxide **(233)**.



(232) **(233)**

Figure 4.4

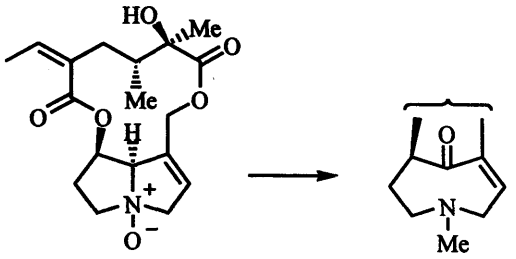
In each of the following proposals, the amine-oxides **(227)** and **(228)** rearrange to the oxazine **(234)** (Scheme 4.9). This could then undergo further transformations, similar to those used in the synthesis of 1-hydroxytropacocaine (Chapter 3), to yield a variety of natural products based on the 1-hydroxytropane skeleton (Section 4.6).



(227)/(228) **(234)**

Scheme 4.9

Indeed, an apparently similar conversion has already been reported in the pyrrolizidine alkaloids. Senecionine N-oxide **(235)** is converted directly into senkirkine **(236)** in root cultures of *Senecio. veralis*.[89]

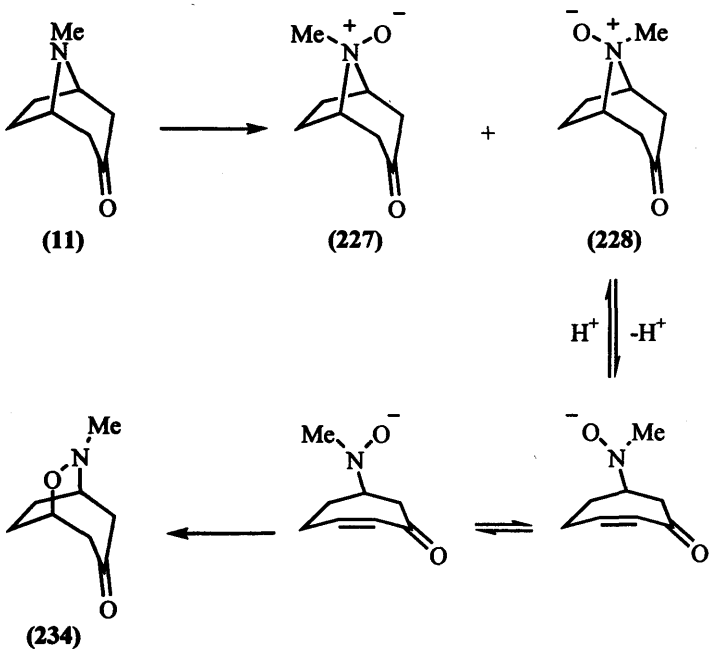


(235) **(236)**

Figure 4.5

4.3.2 Thermal or base-induced rearrangement of tropinone N-oxide

The first proposal involves a thermal or base-induced rearrangement of tropinone N-oxide proposed *in vitro* by Shvo and is shown in Scheme 4.10.[86]



(11) **(227)** **(228)** **(234)**

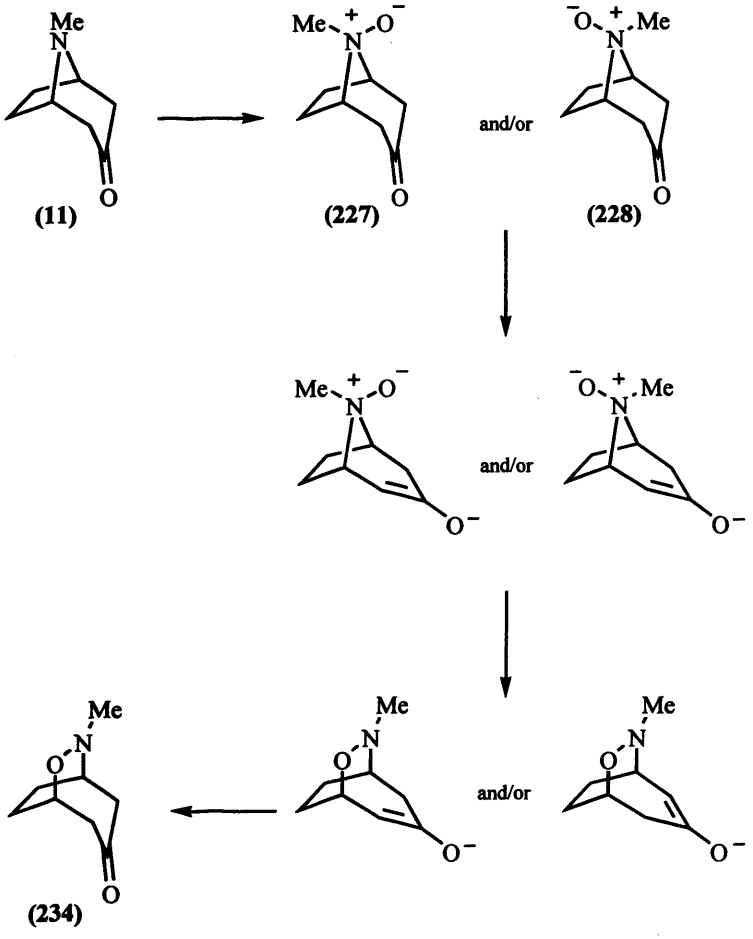
Scheme 4.10

The oxazine **(234)** is isomeric with the N-oxides **(227)** and **(228)** and may be formed in the equilibration process. However, it was not detected as a stable product *in vitro*. If the reaction occurs *in vivo* under enzyme control, the outcome may be different from that observed in the laboratory.

4.3.3 Meisenheimer rearrangement of tropinone N-oxide

The second pathway centres on the Meisenheimer rearrangement of tropinone N-oxides, a reaction exploited by Bremner in his synthesis of the 1,3-dihydroxytropane **(68⇌69)** (see Chapter 1).[31] A possible route is outlined in Scheme 4.11. The Meisenheimer rearrangement is well known *in vitro*, but does not appear to have been proposed *in vivo* before. There is a need for unsaturation in order to produce an intermediate allylic radical. Corresponding stabilisation in the proposed route could come from enolisation of **(227)** and **(228)** which would provide the unsaturation necessary for the Meisenheimer rearrangement. This is likely to be enzyme controlled *in vivo*.

In order to test the validity of these proposals, it was decided to synthesise isotopically labelled tropinone N-oxide, which could then be used in feeding experiments with calystegine-producing root cultures.[90] ^{18}O-labelling would be essential if the origin of the 1-hydroxy group was to be determined.



Scheme 4.11

It was also deemed necessary to incorporate some form of deuterium labelling to give the best chance of seeing any incorporation of the N-oxides using mass spectrometry. Since the doubly labelled N-oxide would show M+4 peaks in addition to those for M+1 and M+2.

4.4 N-OXIDATION OF TROPINONE

Before the synthesis of labelled tropinone N-oxide was undertaken, a thorough investigation into the N-oxidation of tropinone was made. It was necessary to ascertain the ratio of tropinone N-oxides which would be used in the feeding experiments, since it was not known whether both isomers would be metabolised. N-Oxidation of tropinone with aqueous hydrogen (30%), using the method of Werner and Schichfluss,[85] resulted in a mixture of **(227)** and **(228)** (ratio 9:1) in accordance with the literature.[86] Recrystallisation from ethyl acetate produced a pure sample of the major isomer **(227)**, however, obtaining a sufficiently pure sample of **(228)** for full ^{1}H NMR assignments to be made was difficult. Shvo obtained a pure sample of **(228)** using chromatography over neutral alumina (eluting with chloroform:methanol 9:1). However, column chromatography using these conditions failed to separate **(228)**. An alternative strategy was adopted in which tropinone was oxidised with *m*CPBA, according to the method of Shvo,[86] to produce **(228)** as the major isomer. Interestingly, the ratio of **(227)**:**(228)** in the crude reaction mixture (as calculated from ^{1}H NMR signal integrations) was 1:4, which was even more "kinetic" than the 2:3 ratio reported by Shvo. The removal of residual *m*CPBA from the reaction was a problem, since washing with aqueous sodium bicarbonate solution appeared to result in decomposition of the product. However, stirring a solution of the crude product over anhydrous potassium carbonate provided a clean sample of **(227)** and **(228)** (ratio 1:1) and, from this, definitive ^{1}H HMR assignments for **(228)** could be made. Higher resolution ^{1}H NMR data were obtained than those recorded by Shvo. This allowed all the signals for both **(227)** and **(228)** to be assigned (Table 4.7). The ^{13}C NMR data for **(227)** and **(228)** have not been reported to date and are recorded in the experimental section.

Table 4.7 ^{1}H NMR data for tropinone (11) and tropinone N-oxides (227) and (228)

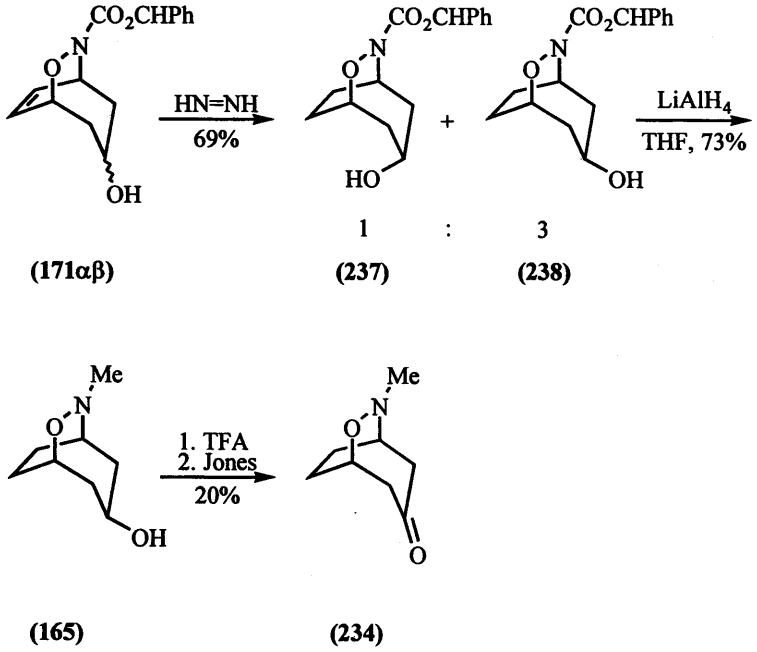
Proton	Tropinone (11)[a,b]	Tropinone N-oxide (227) (major)[a,c]	Tropinone N-oxide (228) (minor)[a,c]
NCH_3	2.49 s	3.42 s	3.56 s
$H_{1,5}$	3.45 brm	3.75 brm	3.88 brm
$H_{2,4eq}$	2.20 dd, J =16.1, 1.5	2.23 d, J = 16.1	2.58 brd, J ≈ 18
$H_{2,4ax}$	2.69 brdd, J = 16.1, 4.3	3.86 dd, J = 16.1, 4.4	~2.96 brdd, J ≈ ~18, 4.1
$H_{6,7\alpha}$	1.61 m	2.09 m	1.77 m
$H_{6,7\beta}$	2.12m	2.32 m	2.96 m

a: J values in Hz.
b: Spectra in $CDCl_3$ at 300 MHz.
c: Spectra in $CDCl_3$ at 250 MHz over anhydrous potassium carbonate.

Chemical shifts for **(227)** and **(228)** were found to be extremely pH-dependent e.g. values for the bridgehead protons of **(227)** ranged from δ 4.50 when protonated with TFA, to δ 3.75 when in solution over anhydrous potassium carbonate. To combat this problem, all ^{1}H NMR spectra were recorded as solutions in $CDCl_3$ over anhydrous potassium carbonate. The major product **(227)** showed an N-methyl signal at δ 3.42, this was in agreement with the value reported by Shvo. The doublet at δ 2.23, which showed a geminal coupling of 16.1 Hz was assigned to the equatorial protons on carbons two and four. The corresponding axial protons, which were deshielded by the proximity of the NO bond, appeared as a doublet of doublets at δ 3.86 (J= 16.1, 4.4 Hz, 2H) as the result of additional coupling to the bridgehead protons ($H_{1,5}$). The methylene groups on the ethano bridge gave rise to multiplets at δ 2.09 ($H_{6,7\alpha}$) and 2.32 ($H_{6,7\beta}$). The minor product **(228)** displayed an N-methyl signal at δ 3.56; the $H_{2,4eq}$ protons were visible as a doublet at δ 2.58 (2H), while the $H_{2,4ax}$ appeared as a doublet of doublets at δ 2.96 (2H). These signals overlapped with those for $H_{6,7\beta}$, as a result of deshielding of the latter by the NO bond. The corresponding α-protons appeared up-field at δ 1.77.

The oxazine **(234)** was proposed by Shvo as a possible intermediate in the equilibration of **(227)** and **(228)** although there was no evidence for its formation. However, the presence of small amounts of **(234)** in mixture of **(227)** and **(228)** could easily have been missed at low resolution. Compound **(234)** was synthesised, using

the route shown in Scheme 4.12, and its ^{1}H NMR spectrum compared with those of mixtures of **(227)** and **(228)**.



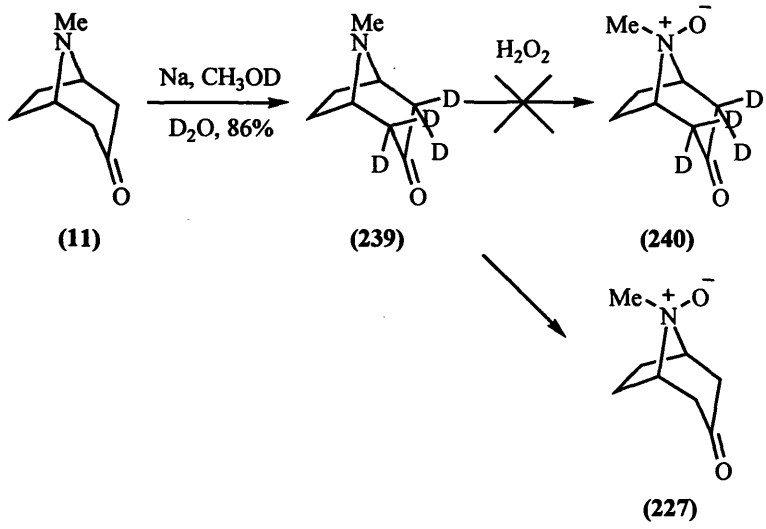
Scheme 4.12

A mixture of **(171αβ)** was reduced with diimide to give **(237)** and **(238)** which were separable by column chromatography. Treatment of **(238)** with lithium aluminium hydride under standard conditions gave **(165)** in good yield. Oxidation with Jones reagent afforded **(234)**. The N-methyl of **(234)** appeared at δ 2.72, while the bridgehead protons gave rise to a broad multiplet at δ 4.34. No evidence of either of these signals was seen in the spectra of **(227)** and **(228)**. The other signals arising from **(234)** were of little diagnostic value as they overlapped with signals from the N-oxides.

Subsequent experiments showed that N-oxidation of tropinone could be carried out with concentrations of hydrogen peroxide as low as 0.5%. This proved that a reaction with dilute ^{18}O-labelled peroxide would be viable. All biosynthetic work would be carried out in aqueous solution at pH 5.8. It was necessary, therefore, to determine the equilibrium distribution of **(227)** and **(228)** under these conditions. A sample of pure **(227)** was stirred in aqueous solution at pH 5.8 for 2 days after which the ratio of **(227)** to **(228)** was approximately 9:1 (as calculated from ^{1}H NMR integrations) in agreement with the data reported by Shvo.

4.5 SYNTHESIS OF ISOTOPICALLY LABELLED TROPINONE N-OXIDES

It was thought that 2,2,4,4-d_4-tropinone **(239)** would be worth investigating, since it could be easily synthesised in a single step from tropinone **(11)**,[91] which is commercially available. Oxidation with ^{18}O-labelled hydrogen peroxide would then give an appropriately labelled tropinone N-oxide **(240)** (Scheme 4.13). However, there was a serious risk of "washout" of deuterium *via* enolisation. Compound **(239)** was successfully prepared and showed 100% incorporation of deuterium by ^1H NMR, but treatment with aqueous hydrogen peroxide solution did, indeed, result in complete wash-out of deuterium, to give **(227)**, as the major product.

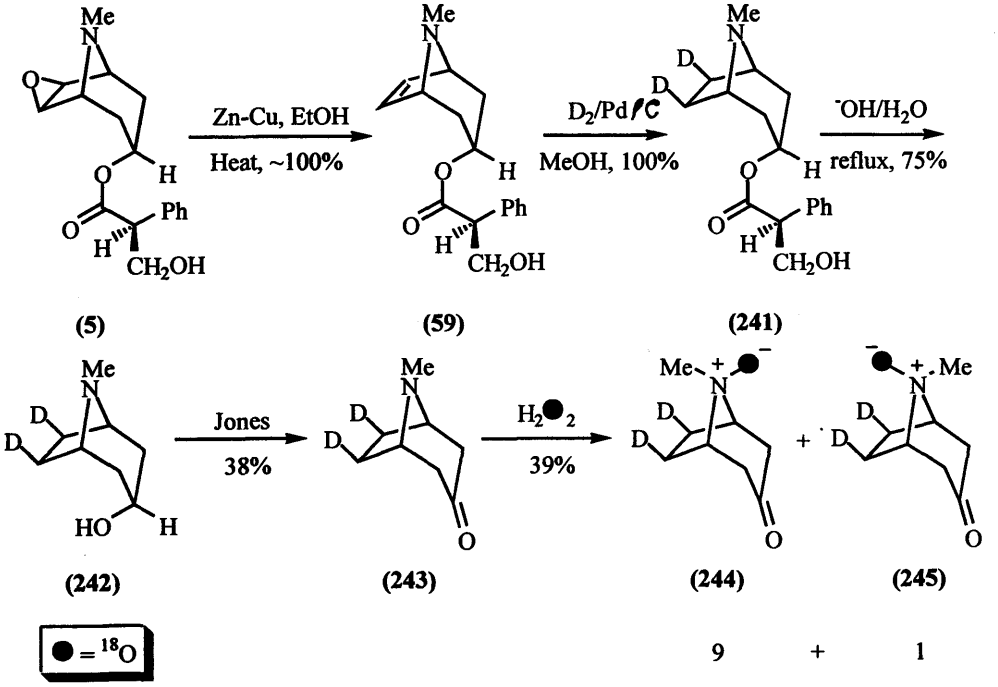


Scheme 4.13

Further, under the conditions of the feeding experiments (pH 5.8), the deuterium in 2,2,4,4-d_4-tropinone **(239)** was completely lost after six days stirring in aqueous solution at this pH. Hence, it became necessary to incorporate deuterium into the ethano bridge of tropinone N-oxide.

An efficient synthesis of 6β,7β-d_2-^{18}O-tropinone N-oxide **(244)** and **(245)** was devised, starting from scopolamine **(5)**, which is commercially available (Scheme 4.14). Deoxygenation of scopolamine proceeded smoothly to give **(59)**.[31] Reduction of **(59)** under a deuterium atmosphere with a catalytic amount of palladium-on-carbon, produced **(241)**. The ^1H NMR data for **(241)** were in agreement with those reported by Hashimoto *et al.*[78] The ^{13}C NMR data, which has not been reported to date, showed two 1:1:1 triplets for carbons six and seven, which confirmed the presence of deuterium in these positions. Saponification of **(241)** yielded **(242)**. Jones oxidation

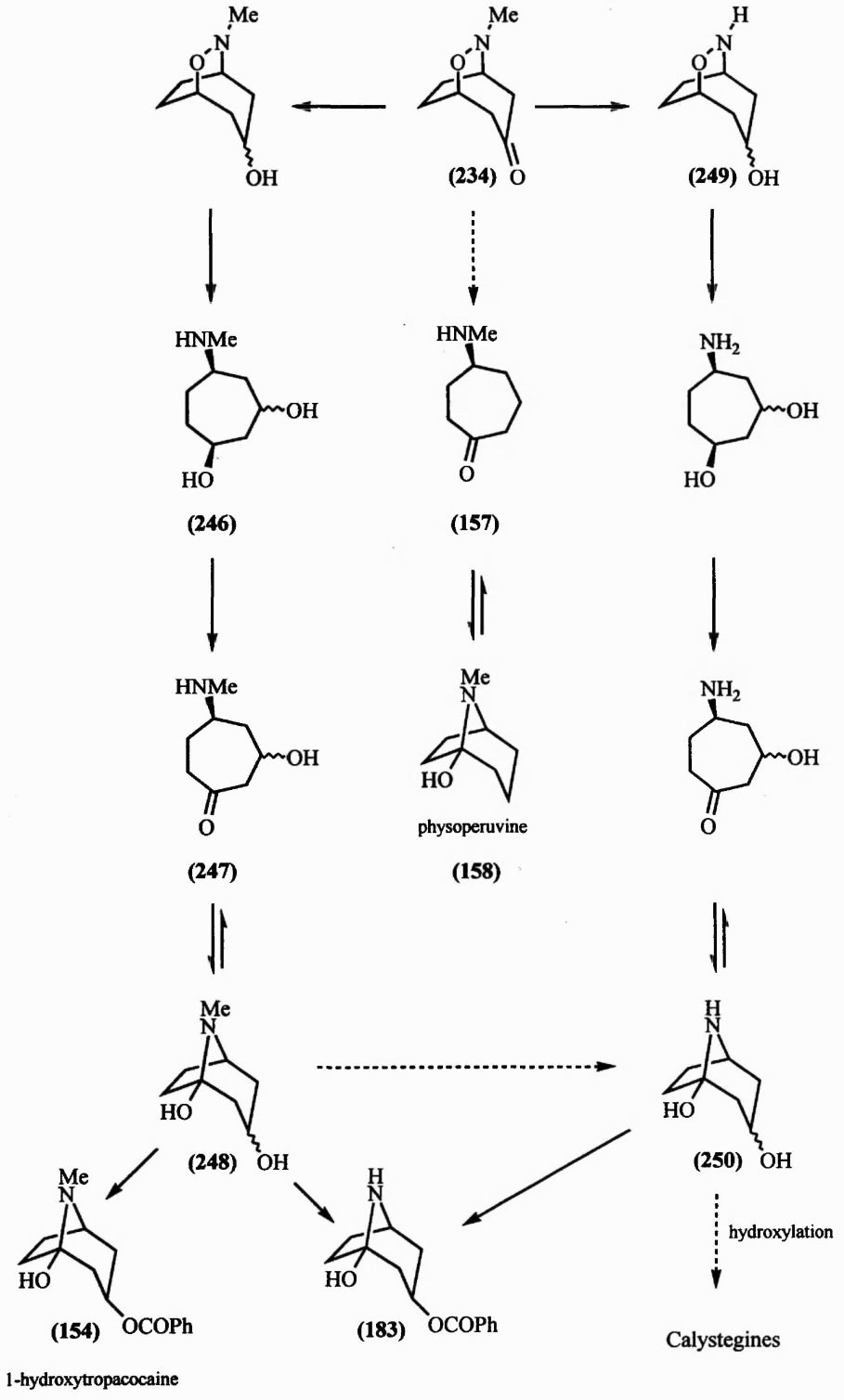
afforded 6,7-d_2-tropinone **(243)**; However, the yield, though comparable to that in the literature, was disappointingly low.[91] The group has subsequently found oxidation with tetrapropylammonium perruthenate (TPAP) to be an effective and simple alternative to Jones oxidation and it is possible that the use of TPAP would improve the yield in this case.[92] Oxidation with 0.5% ^{18}O-labelled hydrogen peroxide[93] gave the desired product as a mixture of isomers **(244)** and **(245)** (ratio 9:1) in 39% yield after trituration with petroleum ether (b.p. 60 - 80°C). Mass spectrometry showed a peak at M^++4, as compared with unlabelled tropinone N-oxide and indicated an 87% incorporation of ^{18}O. The N-methyl signal of **(244)** was visible at δ 3.50. The α-protons of the ethano-bridge appeared as a singlet at δ 2.09, confirming the *exo*-incorporation of deuterium. Protons $H_{2,4eq}$ gave rise to a doublet at δ 2.26. As expected, the $H_{2,4ax}$ protons appeared as a doublet of doublets down-field of the corresponding equatorial protons at δ 3.78. Wash out experiments using **(243)** in aqueous solution at pH 5.8 showed no significant loss of deuterium over a period of months.



Scheme 4.14

4.6 CONCLUSION

This chapter has described work leading to the synthesis of the doubly labelled tropinone N-oxides **(244)** and **(245)**, which forms the basis for investigations into the biosynthetic route to 1-hydroxytropanes.



Scheme 4.15

The simple and efficient route to **(244)** and **(245)** is easily adaptable to the synthesis of variants of these compounds, which may be necessary as the investigation of the pathway progresses.

Feeding experiments are currently underway to see if **(244)** and **(245)** are incorporated into root cultures of *Atropa belladonna.* The timing of subsequent oxidations and reductions remains to be established, subject to proof that one of the two proposed pathways is operating. However, the synthesis of 1-hydroxy-tropacocaine illustrates that it is possible, in the laboratory at least, to produce 1-hydroxytropanes from compounds such as the bicyclic oxazine **(234)**. Scheme 4.15 outlines some possible routes for converting **(234)** into 1-hydroxytropanes.

Reduction of **(234)** followed by reductive cleavage of the NO bond (achieved *in vitro* using reagents such as molybdenum hexacarbonyl or sodium or aluminium amalgams) would give the monocyclic intermediate **(246)**. Selective oxidation of **(246)** (*via* an enzyme-bound intermediate) would produce the amino-ketone **(247)** which is a tautomer of the 1,3-dihydroxytropane **(248)**. It is possible that these transformations may occur *via* a series of enzyme-bond intermediates, starting with the enolate of **(234)**. Esterification of **(247⇌248)** would give 1-hydroxytropacocaine **(154)**; demethylation of **(247⇌248)** prior to esterification would produce the nor-derivative **(183)** which has also been isolated from plant sources recently (see Chapter 3). Alternatively, demethylation of **(234)** may occur (either as shown, or at a later stage) to produce **(249)**. An analogous series of oxidations and reductions to that described above would provide the 1,3-dihydroxytropane **(250)**. Further hydroxylation would then give calystegines. Finally, conversion of **(234)** into physoperuvine **(157⇌158)** can be envisaged, *via* a similar route to that taken by Justice in his laboratory synthesis.[28]

Chapter 5

Experimental

INSTRUMENTATION

Routine ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX 250 spectrometer (250 and 63 MHz). Higher field and variable temperature ^{1}H and ^{13}C NMR spectra were obtained on a Bruker DRX 400 spectrometer (400 and 101 MHz). Chemical shifts were recorded in ppm (δ) downfield from the internal reference, tetramethylsilane (TMS). Signal characteristics are described using standard abbreviations: s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets) etc., t (triplet), q (quartet), quin (quintet), m (multiplet), br (broad) and v (very); protons identified as NH or OH were shown to be exchangeable with D_2O. In some circumstances, signals that appear in a more simplified form than the molecule allows are given the prefix ~. For example, a dddd which appears as a quintet is quoted as ~quin. Where data are quoted for two isomers or rotamers, overlapping signals are shown in italics but may be quoted separately for reasons of clarity even though they are not fully resolved or assigned. Where the abbreviation ABq (AB quartet) is used, chemical shifts are taken at the centre of gravity of each doublet and J valves are approximate and are estimated on a pseudo first order basis. In the ^{13}C spectra, C, CH, CH_2, CH_3 are used to indicate quaternary, methine, methylene and methyl carbons respectively, as shown by off-resonance decoupling or DEPT experiments.

IR spectra were recorded on PE 1604 FT or PE 298 IR spectrometers as solutions in CH_2Cl_2 unless indicated otherwise. Band intensities are described using standard abbreviations: s (strong), m (medium), w (weak), br (broad), v (very).

Mass spectra were measured on a Kratos Concept spectrometer using ionisation by electron impact (EI) except where fast atom bombardment (FAB) was used; intensities are given as percentages of the base peak.

Melting point measurements were made using a Kofler hot stage apparatus and are uncorrected.

Combustion Analyses were performed by Butterworth Laboratories Ltd., Teddington, Middlesex.

TECHNICAL

Reactions were performed under dry nitrogen using solvents dried by standard methods. Diethyl ether was distilled from lithium aluminium hydride. Dichloromethane was distilled from calcium hydride. Petroleum ether was distilled prior to use. Tetrahydrofuran was distilled from sodium- benzophenone. All other solvents were dried and purified as described by Perrin.[94]

Flash chromatography was carried out according to the method of Still[95] using Silica gel 60 (35 – 70 μm) supplied by Fluka. Analytical thin-layer chromatography was conducted on standard commercial aluminium sheets pre-coated with a 0.2 mm layer of silica gel 60.

Tetramethylammonium periodate[96]

A solution of paraperiodic acid (50.97 g, 0.224 mol), in water (120 ml), was added in portions to a stirred 25% solution of tetramethylammonium hydroxide (81.65 g, 0.224 mol) at 0°C. The resultant white precipitate was filtered, washed with methanol (100 ml) and dried to give tetramethylammonium periodate (43.24 g, 73%) as a crystalline white solid.

Benzyl-N-hydroxycarbamate[97]

Benzyl chloroformate (50 ml, 0.35 mol) was dripped into a stirred solution of hydroxylamine hydrochloride (26.76 g, 0.39 mol) and sodium hydroxide (40.3 g, 1.06 mol) in water (300 ml) at 0°C. After complete addition the solution was warmed to room temperature and stirred for a further 22 hr. Hydrochloric acid solution (6 M) was then added until a pH of 2 was obtained. The liberated oil was then extracted with diethyl ether (3 x 90 ml); the combined organic layers were washed with water (50 ml) and dried over anhydrous magnesium sulphate. After filtration and rotary evaporation of the solvent, the yellow solid was recrystallised twice from toluene and petroleum ether (b.p. 60 - 80°C) to afford benzyl-N-hydroxycarbamate (25.95 g, 44%) as an off-white crystalline solid, m.p. 69 - 70°C (lit.[97] m.p. 71°C).

Potassium azodicarboxylate [98]

Azodicarbonamide (20 g, 0.17 mol) was stirred with degassed aqueous potassium hydroxide solution (50 ml; 1:1 solution by weight) at 0°C. After complete evolution

of ammonia, the solution was filtered (taking care to exclude carbon dioxide) leaving a yellow solid. This was dissolved in the minium quantity of cold water (75 ml) at 0°C and poured into 4 volumes of ethanol (300 ml). The resulting bright yellow precipitate was filtered and washed with methanol (3 x 25 ml) to afford potassium azadicarboxylate (23.7 g, 72%), which was dried over P_2O_5.

Tetrakis[triphenylphosphine]palladium(0)[99]

A mixture of palladium (II) chloride (5.00 g, 28.2 mmol) and triphenylphosphine (36.98 g, 141.0 mmol) in DMSO (375 ml) was heated at 150°C until all the solid was in solution. The heat was removed and hydrazine hydrate (5.5 ml, 112.97 mmol) was carefully added. The product crystallised out, as a yellow solid, on cooling to room temperature. The reaction mixture was filtered under a nitrogen atmosphere, and the solid washed with ethanol (4 x 100 ml), followed by diethyl ether (4 x 100 ml). The product was dried under vacuum to afford tetrakis[triphenylphosphine]palladium(0) (30 g, 92%).

δ_P (101MHz, CD_2Cl_2): 12.0 (brs). lit[100] δ 15.5 (CD_2Cl_2).

5-bromo-2-methoxypyridine[101]

A cooled solution of bromine (9.9 ml, 0.192 mol) in glacial acetic acid (34 ml) was dripped, with vigorous stirring, into a suspension of 2-methoxypyridine (20 g, 0.183 mol) and sodium acetate (15.6 g, 0.192 mol) in glacial acetic acid (115 ml) at 0°C. The reaction was stirred for 24 hr at room temperature. It was then poured into crushed ice (170 ml). An aqueous solution of sodium hydroxide (6M) was added at 0°C until the mixture was alkaline (pH 12). The aqueous phase was extracted with diethyl ether (3 x 100 ml) and the combined organic layers were dried over anhydrous magnesium sulphate. Filtration and evaporation left a crude brown oil. Vacuum distillation of the oil at 7 mbar, 30-35°C removed impurities. Further distillation at 7 mbar, 56-58°C afforded 5-bromo-2-methoxypyridine (14.84 g, 43%).

δ_H (250MHz, $CDCl_3$): 3.90 (s, 3H, OMe), 6.66 (dd, J = 8.7, 0.5 Hz, 1H, H_3), 7.63 (dd, J = 8.7, 2.5 Hz, 1H, H_4), 8.20 (d, J = 2.5Hz), 1H, H_6).

N-(Benzyloxycarbonyl)-6-oxo-7-azabicyclo[3.2.2]non-8-ene (155)[102]

Cyclohepta-1,3-diene **(33)** (1.90 g, 0.020 mol) and tetramethylammonium periodate (6.38 g, 0.024 mol) in dichloromethane (20 ml) were stirred at 0°C. A solution of benzyl-N-hydroxycarbamate (4.0 g, 0.024 mol) in dichloromethane (8 ml) was dripped in over 15 min. On complete addition the mixture was allowed to warm to ambient temperature and stirred for a further 4 hr. The solution was filtered, washed with sodium thiosulphate solution (2 x 30 ml) and then water (30 ml). The organic layer was separated, dried over anhydrous magnesium sulphate, filtered and the solvent removed using a rotary evaporator. The residual yellow oil was purified by flash chromatography using 1:4 diethyl ether:petroleum ether (b.p. 40 - 60°C) to afford **(155)** (4.68 g, 90%) as a colourless oil which crystallised on refrigeration.

δ_H (250MHz, $CDCl_3$): 1.28 - 1.60 (series of m, 2H), 1.82 (brm, 4H), 4.74 (brm, 1H, α-N), 4.87 (brm, 1H, α-O), 5.18 (s, 2H, CH_2Ph), 6.19 (ddd, J = 9.2, 6.1, 1.3 Hz, 1H, =CH), 6.31 (ddd, J = 9.0, 6.8, 1.1 Hz, 1H, =CH), 7.34 (m, 5H, Ph).

Cis-4-([Benzyloxycarbonyl]amino)cyclohept-2-enol (51)

The oxazine **(155)** (5 g, 0.019 mol) was dissolved in acetonitrile (150 ml) and water (50 ml) Molybdenum hexacarbonyl (3.5 g, 0.013 mol) was added and the mixture was heated at reflux for 24 hr under a nitrogen atmosphere. After cooling, the suspension was filtered through a plug of silica gel which was then washed thoroughly with dichloromethane. Further filtration through celite followed by removal of solvent using a rotary evaporator yielded the crude product as a brown solid. This was chromatographed on silica using diethyl ether petroleum ether (b.p. 40 - 60°C) (7:3) to yield **(51)** as a yellow oil (3.18 g, 64%). The ^1H NMR spectrum was identical to that of a sample prepared by Justice.[102]

δ_H (250MHz, $CDCl_3$): 1.40 - 2.10 (brm, 6H), 4.30 (brm, 2H, α-N & α-O), 4.80 (brm, 1H, NH), 5.15 (s, 2H, CH_2Ph), 5.55 (brd, J = 12 Hz, 1H, HC=), 5.80 (brd J = 12 Hz, 1H, HC=), 7.35 (m, 5H, Ph).

1β-Hydroxy-2α,3α-epoxy-4β-([benzyloxycarbonyl]amino)cycloheptane (111) and 1β-Hydroxy-2β,3β-epoxy-4β-([benzyloxycarbonyl]amino)cycloheptane (112)[102]

To a stirred solution of **(51)** (4.34 g, 16.63 mmol) in dichloromethane (250 ml) was added *m*CPBA (57 - 86% purity, 4.85 g, 28.12 mmol) and stirring was continued at ambient temperature for 24 hr. The solution was transferred to a separating funnel and washed with sodium hydrogen carbonate solution (3 x 50 ml) and water (2 x 50 ml). The combined organic layers were dried over anhydrous magnesium sulphate, filtered and the solvent removed using a rotary evaporator to afford a mixture of the *cis*- and *trans* epoxides in the ratio 38:62. Purification of the crude solid by flash chromatography eluting with diethyl ether:petroleum ether (b.p. 40 - 60°C) (in ratios ranging from 7:3 - 10:0) yielded firstly **(111)** (938 mg, 21%) as a crystalline white solid, and secondly **(112)**, again as a crystalline white solid (2.6 g, 57%).

(111): δ_H (250MHz, CD_3COCD_3): 1.42 (m, 1H), 1.50 - 1.81 (series of m, 5H), 3.02, (m, 2H, 2 x HCO), 3.64 (m, 1H, α-N), 3.79 (m, 1H, α-O), 4.47 (brs, 1H, exch), 5.07 (s, 2H, CH_2Ph), 6.54 (brm, 1H, NH), 7.34 (m, 5H, Ph).

(112): δ_H (250MHz, $CDCl_3$): 1.01 (m, 1H), 1.36 - 1.89 (series of m, 5H), 1.98 (brs, 1H, exch), 3.24 (d, J = 5.0 Hz, 1H, HCO), 3.29 (d, J = 5.0 Hz, 1H, HCO), 4.01 (m, 2H, α-N & α-O), 5.10 (m, 3H, CH_2Ph & NH), 7.34 (m, 5H, Ph).

1β-[(*p*-Toluenesulphonyl)oxy]-2β,3β-epoxy-4β-[(benzyloxycarbonyl)amino]-cycloheptane [102]

A solution of **(112)** (930 mg, 3.36 mmol) in dry THF (20 ml) was stirred at 0°C under a nitrogen atmosphere. *n*-Butyllithium (1.6 ml, 2.5 M in hexane) was injected and the solution stirred for 5 min before the addition of *p*-toluenesulphonylchloride (829.3 mg, 4.37 mmol) in THF (4 ml). The solution was warmed to room temperature and stirred for a further 1.5 hr before being quenched with the minimum of water-saturated diethyl ether at 0°C. The solution was transferred to a separating funnel and washed with water (2 x 10 ml) and brine (10 ml). After separation the ethereal layer was dried over anhydrous magnesium sulphate, filtered, and the solvent removed on a rotory evaporator to afford the tosylate as a dark yellow oil (889 mg), which was coverted to the chloride **(113)** without further purification.

δ_H (250 MHz, $CDCl_3$): 0.87 (m, 1H), 1.38 (m, 1H), 1.72 (m, 4H), 2.42 (s, 3H), 3.18 (m, 2H, 2 x CHO), 3.96 (m, 1H), 4.74 (m, 1H), 5.07 (s inc m, 3H, CH_2Ph & NH), 7.31 - 7.39 (m, 7H), 7.82 (d, J = 8.5 Hz, 2H).

1α-Chloro-2β,3β-epoxy-4β-([benzyloxycarbonyl]amino)cycloheptane (113)[102]

Lithium chloride (1.11 g, 26.4 mmol) and 1β-[(*p*-Toluenesulphonyl)oxy]-2β,3β-epoxy-4β-[(benzyloxycarbonyl)amino]-cycloheptane (1.90 g, 4.40 mmol) were added to DMSO (20 ml) and heated to 55°C with stirring for 4 hr. On cooling, the solution poured into an equal volume of water and repeatedly extracted with diethyl ether (3 x 20 ml). The combined organic layers were washed with water (2 x 5 ml) and dried over anhydrous magnesium sulphate. Filtration and removal of the solvent using a rotary evaporator gave the crude product as a pale yellow solid (1.15 g) which was cyclised to **(113)** without further purification.

δ_H (250MHz, $CDCl_3$): 1.55 (m, 3H), 1.74 (m, 1H), 1.91 (m, 2H), 3.30 (m, 2H, CHO), 4.21 (m, 1H), 4.74 (m, 1H), 5.11 (s, 2H, CH_2Ph), 5.29 (brd, J = 7.8 Hz, NH), 7.34 (m, 5H, Ph).

N- (Benzyloxycarbonyl)-6β,7β-epoxy-8-azabicyclo[3.2.1]octane (114)[102]

To a stirred slurry of sodium hydride (60% dispersion in mineral oil, 338 mg, 8.4 mmol) in dry THF:DME (8:1, 2 ml) was injected a solution of **(113)** (1.24 g, 4.2 mmol) in THF:DME (8:1, 25 ml) at 0°C. The solution was stirred at ambient temperature for 1 hr and then at 50°C for a further 1.5 hr. Excess hydride was destroyed by addition of water at -78°C and diethyl ether (25 ml) was added. The ethereal layer was washed with water (2 x 10 ml), brine (10 ml), separated and dried over magnesium sulphate. After filtration and rotary evaporation of the solvent, the residual oil was purified by flash chromatography, eluting with 2:3 diethyl ether:petroluem ether (b.p. 40 - 60°C), to give **(114)** (717 mg, 65%, 3 steps) as a yellow oil. Signals quoted in italics are common to both rotamers.

δ_H (250MHz, $CDCl_3$): *1.46 - 1.89* (series of m, 6H), *3.44* (m, 2H, CHO), 4.33 (brs, 1H, α-N), 4.41 (brs,1H, α-N), *5.12* (s, 2H, CH_2Ph), *7.34* (m, 5H, Ph).

N-(Benzyloxycarbonyl)-nortrop-6-ene (118)

Zinc/copper couple (2.41 g) was added to a solution of **(114)** (134 mg, 0.52 mmol) in absolute ethanol (5 ml) and heated in a Young's tube at 150°C under pressure for 48 hr. On cooling, the solution was filtered through celite and the bulk of the solvent removed using a rotary evaporator. The residual solution was partitioned between dichloromethane (30 ml) and water (10 ml). The organic layer was separated and repeatedly washed with water (2 x 10 ml). The combined organic layers were dried over anhydrous magnesium sulphate. Filtration followed by rotary evaporation of the solvent yielded a crude oil (125 mg, 99%) which (from ^{1}H NMR integration) was found to contain **(118)** (77%) and the starting material. Separation by column chromatography over silica (eluting with diethyl ether:petroleum ether in ratios ranging from 2:3 to 3:2) afforded **(118)** as a pale yellow oil (68 mg, 54%) and recovered starting material (20%). Signals shown in italics are common to both rotamers (1:1 ratio).

δ_H 250MHz, $CDCl_3$): *1.30 - 1.85* (series of m, 6H), *4.58* (brm, 2H, α-N), *5.16* (s, 2H, CH_2Ph), 6.02 (brs, 1H, HC=), 6.05 (brs, 1H, HC=), *7.35* (m, 5H, Ph).

δ_C (63MHz, $CDCl_3$): *16.7* (CH_2), 23.9 (2 x CH_2), 24.8 (2 x CH_2), *58.9* (2 x NCH), *66.8* (CH_2PH), *128.2*, 128.*3* & *128.8* (3 x aryl CH), 130.5 (HC=), 130.9 (HC=), *137.5* (aryl C), *152.8* (C=O).

ν_{max} (CH_2Cl_2; film): 3070w, 3030w, 2940brs, 2860m, 1700brs, 1595w, 1500w, 1440brs, 1420brs, 1365m, 1340m, 1305m, 1260m, 1225s, 1215m, 1165w, 1095 brs, 1060s, 1035w, 1030w, 1010s, 955s, 920w, 825m, 760brm, 750brm, 715s, 695s cm^{-1}.

$^m/z$ (FAB): 244 (MH^+). $C_{15}H_{18}NO_2$ [MH^+] requires $^m/z$ 244.1337; observed $^m/z$ 244.1337.

N-(benzyloxycarbonyl)-6β-(2'-chloro-5'-pyridyl)-8-azabicyclo[3.2.1]octane (119)

To a solution of **(118)** (86 mg, 0.35 mmol) in dry DMF (570 µl) was added tetrakis(triphenyl(phosphine)) palladium (0) (60.7 mg, 0.053 mmol), 2-chloro-5-iodopyridine (251 mg, 1.05 mmol), piperidine (121 µl, 1.23 mmol) and formic acid (39.6 µl, 1.05 mmol). The mixture was heated at 75°C with stirring for 24 hr and afterwards diluted with dichloromethane (15 ml), transferred to a separating funnel

and washed with water (3 x 5 ml). The organic layer was dried over anhydrous magnesium sulphate, filtered and the solvent evaporated using a rotary evaporator. The crude oil was purified by flash chromatography eluting with diethyl ether:petroleum ether (b.p. 40 - 60°C) (in ratios ranging from 1:9 to 1:1) to afford **(119)** as a colourless oil (108.6 mg, 87%). NMR spectra of **(119)** showed duplication of signals due to the presence of rotamers. Signals shown in italics are common to both rotamers.

δ_H (250MHz, $CDCl_3$): *1.2 - 1.9* (series of m, 6H), *1.95* (m, 1H, H_7-exo), *2.28* (m, 1H, H_7-endo), *3.21* (dd, J = 9.4, 4.7 Hz, 1H, H_6-endo), 4.12 (brs, 1H, H_5), 4.22 (brs, 1H, H_5), 4.47 (brd, 1H, H_1), 4.53 (brd, 1H, H_1), 5.16 (ABq, J = 12.4, 2H, CH_2Ph), 5.20 (ABq, J = 12.4, 2H, CH_2Ph), 7.13, (d, J = 8.2 Hz, 1H, H_3'), 7.20 (d, J = 8.2 Hz, 1H, H_3'), 7.40, (dd, J = 8.2, 2.5 Hz, 1H, H_4'), 7.47 (dd, J = 8.2, 2.5 Hz, 1H, H_4'), *8.20* (br, 1H, H_6'), *7.28 - 7.40* (series of m, 5H, Ph).

δ_C (63MHz, $CDCl_3$): *17.3* (CH_2, C_3), 30.1, 30.5, 30.9 & 31.3 (4 x CH_2, C_2, C_4), 38.7 & 39.8 (2 x CH_2, C_7), 43.7 & 44.6 (2 x CH_2, C_6), 55.4 & 55.5 (2 x NCH, C_1), 61.9 & 62.3 (2 x NCH, C_5), 67.2 (CH_2Ph), 124.9 (CH, C_3'), 128.3, 128.4 & 128.9 (3 x aryl CH; benzyl), 136.9 (CH, C_4') 137.2 (aryl C; benzyl), 141.9 (CH, C_5'), 148.5 & 148.6 (CH, C_6'), 149.8, (C, C_2'), 153.8 (C=O).

ν_{max} (CH_2Cl_2; film): 3062m, 2930s, 2863m, 1694s, 1587w, 1554w, 1454s, 1432s, 1339m, 1319s, 1267s, 1220m, 1185w, 1143m, 1102s, 1036s, 974w, 955w, 912w, 872w, 837w, 802w, 738brs, 696s cm^{-1}.

$^m/z$: (%): 356 (M^+, 8), 277 (2), 221 (17), 194 (1), 173 (3), 151 (2), 126 (4), 104 (3), 91 (100), 82 (27), 55 (15). $C_{20}H_{21}N_2O_2Cl$ [M^+] requires $^m/z$ 356.1292; observed $^m/z$ 356.1291.

Homoepibatidine (120)

Trimethylsilyliodide (63 µl, 0.44 mmol) was injected into a solution of **(119)** (35.3 mg, 0.099 mmol) in $CHCl_3$ (20 ml). Acidified methanol (5 ml) was then added and the solvent removed using a rotary evaporator, this was followed by the addition of basified methanol (5 ml) after which the solvent was again evaporated. The residue was taken up in chloroform and the precipitate removed by filtration to yield a yellow oil. Purification by flash column chromatography, eluting with diethyl ether:

petroleum ether (b.p. 40 - 60) (in ratios ranging from 1:9 to 3:2) afforded **(120)** (17.2mg, 78%).

δ_H (250MHz, $CDCl_3$): 1.5 - 1.9 (series of m, 7H), 2.24 (dd, J = 13.2, 9.4, Hz, 1H, H_7-endo, 3.16 (dd, J = 9.1, 5.0, Hz, H_6-endo), 3.34 (brs, 1H, H_5), 3.70 (m, 1H, H_1), 7.23 (d, J = 8.2 Hz, 1H, H_3'), 7.75 (dd, J = 8.2, 2.5 Hz, 1H, H_4'), 8.28, (d, J = 2.5 Hz, 1H, H_6').

δ_C (63MHz, $CDCl_3$): 18.1 (CH_2, C_3), 33.2 & 33.8 (2 x CH_2, C_2, C_4), 39.8 (CH_2, C_7), 44.8 (CH_2, C_6), 56.1 (NCH, C_1), 63.2 (NCH, C_5), 124.5 (aryl CH, C_3'), 137.6 (aryl CH, C_4'), 143.1 (aryl C, C_5'), 148.7 (aryl CH, C_6'), 149.3 (aryl C, C_2').

ν_{max} (CH_2Cl_2): 3045w, 2920s, 2870w, 2850m, 1584w, 1560m, 1454s, 1405m, 1390m, 1290w, 1265s, 1140m,1100s, 1084w, 862m, 840w, 825m, 805w, 790w, 735brs, 700s cm^{-1}.

$^m/z$ (%): 222 (M^+, 12), 193 (2), 179 (9), 155 (5), 127 (4), 107 (4), 91 (10), 83 (100), 68 (18), 57 (9). $C_{12}H_{15}N_2Cl$ [M^+] requires $^m/z$ 222.0924; observed $^m/z$ 222.0924.

N-methyl-6β-(5'-pyridyl)-8-azabicyclo[3.2.1]octane (122)

To a stirred solution of **(120)** (54 mg, 0.151 mmol) in THF (5 ml) was added lithium aluminium hydride (23 mg, 0.604 mmol) at -78°C. The reaction was allowed to warm slowly to ambient temperature. Analysis by TLC after 1.5 hr showed only starting material. A further 23 mg of lithium aluminium hydride was added at 0°C and the reaction allowed to warm to room temperature. TLC analysis after a further 2.5 hr still indicated that starting material was present. Stirring was continued overnight, after which time no starting material remained. The reaction was quenched by the addition of the minimum quantity water-saturated diethyl ether at 0°C. The suspension was dried with anhydrous sodium sulphate and filtered through celite. The inorganic residues were washed with ethyl acetate and the solvent evaporated using a rotary evaporator to yield a crude yellow oil. Purification by column chromatography yielded **(122)** (18 mg, 58%) as pale yellow oil.

δ_H (250MHz, $CDCl_3$): 1.17 (m, 1H), 1.26 (m, 1H), 1.66 (m, 1H), 1.80 (m, 1H) 1.96 - 2.11 (series of m, 3H), 2.24 (dd, J = 13.1, 9.3 Hz, 1H, H_7-endo), 2.52 (s, 3H, NCH_3), 3.16 (brs, 1H, H_5), 3.19 (dd, J = 9.3, 5.0 Hz, 1H, H_6-endo), 3.34 (m, 1H, H_1), 7.21 (dd,

J = 8.0, 4.6 Hz, 1H, H_5'), 7.80 (dt, J = 8.0, 2.1 Hz, 1H, H_4'), 8.43 (d, J = 4.6 Hz, 1H, H_6'), 8.58 (s, 1H, H_2').

δ_C (63MHz, $CDCl_3$): 17.7 (CH_2, C_3), 23.8 & 24.4 (2 x CH_2, C_2, C_4), 35.2 (CH_3), 39.2 (CH_2, C_7), 45.4 (CH_2, C_6), 60.1 (NCH, C_1), 66.5 (NCH, C_5), 123.8 (aryl CH, C_5'), 134.6 (aryl CH, C_4'), 144.4 (aryl C, C_3'), 147.6 (aryl CH), 149.3 (aryl CH).

ν_{max} (CH_2Cl_2): 2930s, 2870w, 2860m, 1575w, 1460brm, 1425m, 1325brw, 1015brw, 850brs cm^{-1}.

$^m/z$ (FAB): 203 (MH^+). $C_{13}H_{19}N_2$ [MH^+] requires $^m/z$ 203.1548; observed $^m/z$ 203.1548.

4-[(Benzyloxycarbonyl)amino]cyclohept-2-enone (123)

A solution of **(51)** (2 g, 7.66 mmol) in dichloromethane (100 ml) was stirred at room temperature. Barium manganate (15.6 g, 61 mmol) was added and the mixture was stirred for a further 36 hr. The slurry was filtered through celite and the inorganic residues were washed with dichloromethane (95 ml) and ethanol (155 ml). The solutions were combined and the solvent removed using a rotary evaporator. The residual oil was purified by flash chromatography eluting with diethyl ether to afford **(123)** (1.59 g, 80%) as a pale yellow oil.

δ_H (250MHz, $CDCl_3$): 1.78 (m, 3H), 2.12 (m, 1H), 2.55 (m, 2H), 4.53 (brm, 1H, α-N), 5.08 (s, 2H, CH_2Ph), 5.22 (brd, J ≈ 7.6 Hz, 1H, NH), 5.92 (dd, J = 12.6, 2.2 Hz, 1H, =CH), 6.34 (dd, J = 12.6, 3.3 Hz), 7.31 (m, 5H, Ph).

Cyclohepta-3,5-dienol (159)[102]

Peroxyacetic acid (36 - 40 %, by weight, 125 g, 0.63 mol) was dripped into a stirred solution of cycloheptatriene (40 g, 0.43 mol) in dichloromethane (300 ml) containing anhydrous sodium carbonate (100 g, 0.94 mol) over 30 min at 0°C. Stirring was continued for a further 3 hr at 0°C after which the solution was filtered though celite. The solution was transferred to separating funnel and washed with saturated sodium bicarbonate solution (2 x 60 ml) and brine (50 ml). The organic layer was separated, dried over anhydrous magnesium sulphate, filtered and the solvent distilled at atmospheric pressure. The crude epoxide was then dissolved in diethyl ether (100 ml) and dripped into a slurry of $LiAlH_4$ (7.60 g, 0.2 mol) in diethyl ether (250 ml) at 0°C.

On complete addition the mixture was stirred overnight. An aliquot was removed for NMR analysis which indicated complete reaction. Excess hydride was destroyed by the careful addition of sodium hydroxide solution (2 M) at 0°C and the mixture was dried over anhydrous sodium sulphate. Filtration through celite and removal of the solvent using a rotary evaporator yielded a crude yellow oil. Vacuum distillation of the oil at 20 mbar, 110°C removed impurities. Futher distillation at 5 mbar, 110°C afforded **(159)** (3.66 g, 90% pure) and a further 3.64 g (70% pure (by ^{1}H NMR)) as a pale yellow oil.

δ_H (250MHz, $CDCl_3$): 2.54 (~t, J = 4.7 Hz, 4H), 4.21 (quin, J = 4.7 Hz, 1H, α-OH), 5.61 – 5.72 (series of m, 2H), 5.86 (m, 1H), 5.91 (m, 1H).

6-[(*t*-Butyldimethylsilyl)oxy]cyclohepta-1,3-diene (160)[102]

A solution of **(159)** (1.98 g, 0.018 mol) in dry DMF (15 ml) was stirred at 0°C under a nitrogen atmosphere. Imidazole (1.97 g, 0.028 mol) and *t*-butyldimethylsilyl chloride (3.63 g, 0.023 mol) were added and the solution gradually warmed to ambient temperature and stirred overnight. The reaction mixture was poured into water (20 ml) and repeatedly extracted with diethyl ether (2 x 75 ml, 1 x 50 ml). The combined ethereal layers were washed with water (10 ml), brine (10 ml) and then dried over magnesium sulphate. Filtration and removal of the solvent using a rotary evaporator yielded a crude oil which was purified by flash chromatography, eluting with 1:9 diethyl ether:petroleum ether (b.p. 40 - 60°C) to afford **(160)** (3.03 g, 75%) as a pale yellow oil.

δ_H (250MHz, $CDCl_3$): 0.12 [s, 6H, $(CH_3)_2Si$], 0.95 [s, 9H, $(CH_3)_3CSi$], 2.51 (m, 4H), 4.11 (tt, J = 8.0, 4.8 Hz, 1H, α-OSi), 5.71 (m, 2H), 5.82 (m, 2H).

N-Benzyloxycarbonyl-3β-([*t*-butyldimethylsilyl)oxy]-6-oxa-7-azabicyclo[3.2.2] non-8-ene (161) and N-Benzyloxycarbonyl-3α-([*t*-butyldimethylsilyl)oxy]-6-oxa-7-azabicyclo[3.2.2]non-8-ene (162)[102]

Tetramethylammonium periodate (4.13 g, 15.6 mmol) and **(160)** (3.0 g, 13.0 mmol) in dichloromethane (65 ml) were stirred at -78°C. A solution of benzyl-N-hydroxycarbamate (2.61 g, 15.6 mmol) in dichloromethane (10 ml) was dripped in over 10 min and the solution was then warmed to ambient temperature and stirred for 1.5 hr. An identical work up procedure to that described for the preparation of the

unsubstituted cycloadduct **(155)** was used. The residual dark yellow oil was purified by flash chromatography using 1:3 diethyl ether:petroleum ether (b.p. 40-60°C) to afford **(161)** (containing *ca.* 20% of the 3α-isomer **(162)**, as calculated from ^{1}H NMR signal integrations) as a yellow oil (4.22 g, 83%). The NMR spectra were identical to those of a sample prepared by Justice (in 91% yield).

δ_H (250 MHz, $CDCl_3$): 0.01 [s, 6H, $(CH_3)_2Si$], 0.85 [s, 9H, $(CH_3)_3CSi$], 1.88 - 2.14 (series of m, 4H), 3.68 (~tt, J = 10.3, 6.3 Hz, 1H, α-OSi), 4.70 (brt, J ≈ 5 Hz, 1H, α-N), 4.84 (brt, J ≈ 7 Hz, 1H, α-O), 5.15 (s, 2H, CH_2Ph), 6.18 (ddd, J = 9.1, 6.2, 1.3 Hz, 1H), 6.31 (ddd, J = 9.1, 6.8, 0.8 Hz, 1H), 7.32 (m, 5H). Small signals from the 3α-isomer **(162)** were visible.

N-Methyl-3β-hydroxy-6-oxa-7azabicyclo[3.2.2]non-8-ene (163) and N-Methyl-3α-([*t*-butyldimethylsilyl)oxy]-6-oxa-7-azabicyclo[3.2.2]non-8-ene (164)

A flame-dried 2-necked flask, fitted with a septum cap and reflux condenser, was charged with $LiAlH_4$ (1 g, 26.5 mmol). Dry THF (10 ml) was injected and the system was alternately evacuated and purged with nitrogen gas. The slurry was cooled with stirring to 0°C and a solution of **(161)** and **(162)** (2.58 g, 6.63 mmol) in dry THF (40 ml) was introduced. The mixture was heated under reflux for 3 hr, after which time no starting material remained. The solution was cooled to 0°C and the minimum amount of water-saturated diethyl ether was added carefully to destroy the excess hydride. The suspension was dried with anhydrous sodium sulphate, filtered though celite and the inorganic residues washed with ethyl acetate (3 x 20 ml). The combined organic extracts were evaporated, using a rotary evaporator, to leave a yellow oil (1.4 g). Further washing with methanol yielded a yellow solid (524 mg). These extracts were combined and purified by flash chromatography, eluting with 1:4 diethyl ether:petroleum ether (b.p. 40-60°C), to yield **(164)** (225mg, 12%) (the sample still contained a small amount of benzyl alcohol which was difficult to separate chromatographically).

δ_H (250 MHz, $CDCl_3$): 0.12 [s, 6H, $(CH_3)_2Si$], 0.91 [s, 9H, $(CH_3)_3CSi$], 1.46 (m, 2H), 2.36 (m, 2H), 2.61 (s, 3H, NCH_3), 3.46 (brt, J ≈ 6 Hz, 1H, α-N), 4.36 (brm, 1H, α-O), 4.56 (m, 1H, α-OSi), 6.26 (brdd, J ≈ 9.1, 6.4 Hz, 1H, =CH), 6.46 (brdd, J ≈ 9.1, 6.6 Hz, 1H, =CH).

Further elution with methanol:diethyl ether (1:9) afforded the desired compound **(163)** as a single stereoisomer (486 mg, 54%).

δ_H (250 MHz, $CDCl_3$): 1.46 (brdd, J = 13.2, 10.5 Hz, 1H), 1.68 (m, 1H), 1.88 (m, 1H), 2.02 (m, 1H), 2.24 (s, 3H, NCH_3), 3.20 (brt, J ≈ 7 Hz, 1H, α-N), 3.35 (~tt, J = 10.2, 4.8 Hz, 1H, α-O), 4.18 (m, 2H, α-O and OH), 5.89 (brdd, J = 9.1, 6.1 Hz, 1H), 6.05 (brdd, J = 9.1, 5.9 Hz, 1H).

δ_c (63MHz, $CDCl_3$): 38.7 (br, CH_2), 41.1 (CH_2), 45.5 (CH_3), 58.0 (NCH), 65.5 (COH), 69.5 (OCH), 127.6 HC=), 128.8 (HC=).

ν_{max} (CH_2Cl_2): 3600m, 3400br, 3040m, 2940s, 2880m, 2855m, 1600m, 1145w, 1075w, 935m, 910m, 830w, 800w cm^{-1}.

$^m/z$ (%): 155 (M^+, 69), 149 (8), 138 (8), 126 (10), 120 (16), 110 (63), 95 (36), 82 (100), 67 (72), 59 (34), 55 (80). $C_8H_{13}NO_2$ [MH^+] requires $^m/z$ 155.0946; observed $^m/z$ 155.0946.

N-Methyl-3β-hydroxy-6-oxa-7-azabicyclo[3.2.2]nonane (165) from (163)

A solution of **(163)** (486 mg, 3.14 mmol) in absolute ethanol (25 ml) was hydrogenated using a catalytic amount of 10% palladium on charcoal at 1 atmosphere pressure. After 20 hr the solution was basified with gaseous ammonia, filtered through celite, dried over anhydrous magnesium sulphate, and the solvent removed using a rotary evaporator to yield **(165)** as a yellow oil (408 mg, 82%) which showed identical spectroscopic properties to the sample prepared from **(166)**.

N-Benzyloxycarbonyl-3β-([*t*-butyldimethylsilyl)oxy]-6-oxa-7-azabicyclo[3.2.2] nonane and N-Benzyloxycarbonyl -3α-([*t*-butyldimethylsilyl)oxy]-6-oxa-7-aza-bicyclo[3.2.2]nonane

To a stirred solution of potassium azodicarboxylate (6.67 g, 34.4 mmol) and a mixture of **(161)** and **(162)** (ratio 80:20, 1.34 g, 3.44 mmol) in methanol (30 ml) was added glacial ethanoic acid (3.9 ml, 68.2 mmol) over 10 min. The mixture was warmed to ambient temperature and stirred for a further 17 hr. The mixture was quenched with water (3 ml), filtered, and the bulk of the solvent removed usig a rotary evaporator. The residual oil was taken up in dichloromethane (60 ml), washed with saturated sodium bicarbonate solution (2 x 15 ml), and with water (15 ml). The organic layer

was separated, dried over anhydrous magnesium sulphate, filtered, and the solvent removed using a rotary evaporator to leave the mixture of N-benzyloxycarbonyl-3-([*t*-butyldimethylsilyl)oxy]-6-oxa-7-azabicyclo[3.2.2]nonanes as an oil (1.25 g). This mixture was partially purified by column chromatography using 1:4 diethyl ether:petroleum ether (b.p. 40-60°C) to yield a yellow oil (993 mg).

δ_H (250 MHz, $CDCl_3$): 0.06 [s, 6H, $(CH_3)_2Si$], 0.88 [s, 9H, $(CH_3)_3CSi$], 1.6 - 2.25 (series of m, 8H), 4.20 (m, 1H, α-OSi), 4.36 (brm, 1H, α-N), 4.40 (brm, 1H, α-O), 5.25 (s, 2H, CH_2Ph), 7.35 (m, 5H).

δ_c (63 MHz, $CDCl_3$): -4.7 [$(CH_3)_2Si$], 18.1 [$(CH_3)_3CSi$], 21.4 & 22.1 (2 x CH_2), 25.8 [$(CH_3)_3CSi$], 42.4 (br, CH_2), 48.6 (br, NCH), 66.3 (CHOSi), 67.3 (CH_2Ph), 73.3 (OCH), 128.1 (2 x aryl CH), 128.5 (aryl CH), 136.5 (aryl C), 154.3 (C=O). Some signals were broadened and not all were visible at this temperature owing to slow rotation about the N-CO bond.

ν_{max} (CH_2Cl_2): 2960s, 2930s, 2890m, 2860m, 1720s, 1690s, 1450brm, 1365m, 1345m, 1330m, 1310m, 1090brs, 1005m, 905m, 855m, 840s, 740bm cm^{-1}.

$^m/z$ (FAB): 414 (MNa^+), 392 (MH^+). $C_{21}H_{34}NO_4Si$ [MH^+] requires $^m/z$ 392.2258; observed $^m/z$ 392.2258.

N-Methyl-3β-([*t*-butyldimethylsilyl)oxy]-6-oxa-7-azabicyclo[3.2.2]nonane (166) and N-Methyl-3α-([*t*-butyldimethylsilyl)oxy]-6-oxa-7-azabicyclo[3.2.2]nonane (167)

A mixture of N-Benzyloxycarbonyl-3β-([*t*-butyldimethylsilyl)oxy]-6-oxa-7-azabicyclo[3.2.2]nonane and N-Benzyloxycarbonyl -3α-([*t*-butyldimethylsilyl)oxy]-6-oxa-7-azabicyclo[3.2.2]nonane was dissolved in diethyl ether (70 ml), dried over anhydrous magnesium sulphate, filtered and the solvent evaporated using a rotary evaporator. A sample of this mixture (544 mg, 1.39 mmol) in dry diethyl ether (25 ml) was added dropwise to $LiAlH_4$ (211 mg, 5.5 mmol) with stirring at 0°C and allowed to warm to ambient temperature over 1 hr. The reaction was quenched by dropwise addition of water-saturated diethyl ether and the resulting suspension was dried with anhydrous magnesium sulphate. After filtration through celite, the filter cake was washed thoroughly with ethyl acetate and the combined extracts were evaporated using a rotary evaporator to give an oil which was chromatographed over silica using diethyl

ether:petroleum ether in ratios ranging from 2:3 up to 3:2. A sample of the minor N-methyl compound **(167)** was eluted first (22 mg, 6% overall yield). This sample contained a small amount of the major isomer; a pure sample of the major isomer **(166)** was then eluted (237 mg, 63% overall).

(166): δ_H (250 MHz, $CDCl_3$): 0.06 [s, 6H, $(CH_3)_2Si$], 0.88 [s, 9H, $(CH_3)_3CSi$], 1.58 (m, 2H), 1.80 - 2.20 (series of m, 6H), 2.60 (s, 3H, NCH_3), 2.90 (brt, J ≈ 6 Hz, 1H, α-N), 4.00 (brm, 1H, α-O), 4.12 (m, 1H, α-OSi).

δ_c (63MHz, $CDCl_3$): -4.9 [$(CH_3)_2Si$], 18.1 [$(CH_3)_3CSi$], 21.2 (br, CH_2), 22.3 (CH_2), 25.9 [$(CH_3)_3CSi$], 38.0 (br, CH_2), 44.7 (CH_3), 46.2 (CH_2), 55.6 (NCH), 66.7 (CHOSi), 69.9 (OCH).

ν_{max} ($CDCl_3$): 2960s, 2948s, 2890m, 2860s, 1470m, 1462m, 1445w, 1435w, 1410w, 1390w, 1372w, 1362w, 1350w, 1325w, 1280w, 1260s, 1205w, 1165w, 1090s, 1005w, 995w, 965w, 855s, 840s, 815w, 805w cm^{-1}.

$^m/z$ (FAB): 272 (MH^+). $C_{14}H_{30}NO_2Si$ [MH^+] requires $^m/z$ 272.2046; observed $^m/z$ 272.2046.

(167): δ_H (400 MHz, $CDCl_3$): 0.08 [s, 6H, $(CH_3)_2Si$], 0.91 [s, 9H, $(CH_3)_3CSi$], 1.60 - 2.20 (series of m, 6H), 2.30 (m, 1H), 2.48 (m, 1H), 2.70 (s, 3H, NCH_3), 3.05 (brm, 1H, α-N), 4.15 (brm, 2H, 2 x α-O).

δ_c (101MHz, $CDCl_3$): -4.5 [$(CH_3)_2Si$], 18.4 [$(CH_3)_3CSi$], 22.7 & 23.8 (2 x CH_2), 26.2 [$(CH_3)_3CSi$], 44.3 & 46.6 (2 x CH_2), 53.2 (CH_3), 57.1 (NCH), 67.3 (CHOSi), 70.2 (OCH).

ν_{max} ($CDCl_3$): 2960s, 2945s, 2890m, 2860s, 1470m, 1260s, 855s, 840s cm^{-1}.

N-Methyl-3β-hydroxy-6-oxa-7-azabicyclo[3.2.2]nonane (165) from (166)

Tetrabutyl ammonium fluoride (1 M in THF, 2.2 ml, 2.2 mmol) was injected into a solution of **(166)** (200 mg, 0.738 mmol) in THF (10 ml) under a nitrogen atmosphere at 0°C. The solution was stirred as it was allowed to warm to ambient temperature. After a further 22 hr, the bulk of the solvent was distilled off using a rotary evaporator. The residual oil was dissolved in chloroform (20 ml) and washed with potassium carbonate solution (5 ml, 10% by weight) and brine (5 ml). The organic layer was dried over anhydrous magnesium sulphate, filtered, and the solvent removed

using a rotary evaporator. The oily product was chromatographed on silica using ethyl acetate/ammonia and then 5% methanol in ethyl acetate/ammonia to yield **(165)** as a pale yellow oil (92 mg, 79%).

δ_H (250MHz, $CDCl_3$): 1.58 (d, J = 9 Hz, 2H), 1.93 - 2.27 (series of m, 6H), 2.63 (s, 3H, NCH_3), 3.02 (m, 1H, α-N), 3.45 (brs, 1H, OH, exch.), 4.13 (m, 2H, 2 x α-O).

δ_c (63MHz, $CDCl_3$: *19.8*, 23.4 & *39.0* (3 x CH_2), 44.5 (CH_3), 45.7 (CH_2), 56.6 (NCH), 66.6 (COH), 70.7 (OCH). Signals in italics were broadened owing to VT effects.

ν_{max} ($CDCl_3$): 3610w, 3340vbrw, 2995w, 2960s, 2955s, 2950s, 2935s, 2920s, 2890m, 2870m, 2850w, 1110m, 1058s, 1055s, 1048s, 1040s, 1030s, 1025m, 960m, 910brm, 900m, 890m cm^{-1}.

$^m/z$ (FAB): 158 (MH^+). $C_8H_{16}NO_2$ [MH^+] requires $^m/z$ 158.1181; observed $^m/z$ 158.1181.

N-Methyl-3β-[(benzoyl)oxy]-6-oxa-7-azabicyclo[3.2.2]nonane (168)

To a solution of **(165)** (170 mg, 1.08 mmol) in dry pyridine (5 ml) was added benzoic anhydride (741 mg, 3.28 mmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP). The reaction mixture was stirred for 4 hr. A ^1H NMR spectrum of a small sample showed that no starting material remained and the bulk of the solvent was removed using a rotary evaporator. The off-white solid residue was purified by flash chromatography eluting with diethyl ether : petroleum ether (4:1) to yield **(168)** as a pale yellow oil (261 mg, 93%).

δ_H (250MHz, $CDCl_3$): 1.81 (m, 2H), 2.15 - 2.42 (series of m, 6H), 2.72 (s, 3H, NCH_3), 3.11 (brt, J ≈ 6 Hz, 1H, α-N), 4.22 (brm, 1H, α-O), 5.53 (tt, J = 10.6, 6.6 Hz, 1H, α-OCOPh), 7.44 (m, 2H, $H_{3',5'}$), 7.56 (brtt, J = 7.3, 1.3 Hz, 1H, $H_{4'}$), 8.05, (brm, 2H, $H_{2',6'}$).

δ_C (63MHz, $CDCl_3$): 22.4 (CH_2), 42.1 (CH_2), 44.3 (CH_3), 55.8 (NCH), 69.7 (COCOPh), 70.1 (OCH), 128.7 ($C_{3',5'}$), 130.2 ($C_{2',6'}$), 130.8 ($C_{1'}$), 133.3 ($C_{4'}$), 166.6 (COPh). Some signals were too broad at this temperature to be assigned with confidence.

ν_{max} (CH_2Cl_2): 3060w, 3050w, 3040w, 2960m, 2940m, 2910m, 2885w, 2860w, 2850w, 2835w, 1715brs, 1605w, 1585w, 1285s, 1275s, 1262s, 1255s, 1120s, 1115s, 1110s, 978m, 974m, 970m cm^{-1}.

$^m/z$ (FAB): 262 (MH^+). $C_{15}H_{20}NO_3$ [MH^+] requires $^m/z$ 262.1443; observed $^m/z$ 262.1443.

All-cis-1-Hydroxy-3-benzoyloxy-5-[methylamino]-cycloheptane (169)

The oxazine **(168)** (46 mg, 0.176 mmol) was dissolved in acetonitrile (10 ml) and water (2.5 ml). Molybdenum hexacarbonyl (50.6 mg, 0.192 mmol) was then added. The mixture was heated at reflux for 7 hr under a nitrogen atmosphere. An identical work up procedure to that described for the preparation of the unsubstituted amino alcohol **(51)** was used. A crude brown solid was obtained which was chromatographed on silica using 2% methanol in ethyl acetate to yield **(169)** as a yellow oil (39.1 mg, 85%).

δ_H (250MHz, $CDCl_3$): 1.68 - 1.86 (series of m, 7H), 2.25 (m, 1H), 2.35 (brs, 1H, OH exch), 2.39 (s, 3H, NMe), 2.68 (m, 1H, α-N), 3.98 (m, 1H, α-O), 5.04 (tt, J = 10.4, 2.8 Hz), 1H, α-OCOPh), 7.42 (brt, J = 7.5 Hz, 2H, $H_{3',5'}$), 7.55 (brtt, J = 7.5, 1.3 Hz, 1H, $H_{4'}$), 8.01 (brdd, J = 7.5, 1.3 Hz, $H_{2',6'}$).

δ_c (63MHz, $CDCl_3$): 28.4 & 32.7 (2 x CH_2), 34.3 (CH_3), 41.5, 44.2 (2 x CH_2), 56.8 (NCH), 68.0 (HCOCOPh), 70.5 (OCH), 128.7 ($C_{3',5'}$), 129.9 ($C_{2',6'}$), 130.8 ($C_{1'}$), 133.3 ($C_{4'}$), 166.2 (COPh).

ν_{max} (CH_2Cl_2): 3610w, 3415w, 3050w, 2920m, 2860w, 2795w, 1715brs, 1290s, 1280s, 1268s, 1258s, 1115m, 1025m, 910m cm^{-1}.

$^m/z$ (%): 263 (M^+, 3), 243 (1), 206 (4), 190 (6), 175 (1), 158 (20), 142 (100), 124 (22), 105 (72), 96 (15), 84 (45), 77 (51), 70 (72), 57 (39). $C_{15}H_{21}NO_3$ [M^+] requires $^m/z$: 263.1521; $^m/z$: observed 263.1521.

8-Methyl-3β-benzoyloxy-8-azabicyclo[3.2.1]octan-1-ol (1-hydroxytropacocaine) (153⇌154)

A stirred solution of **(169)** (8.2 mg, 0.03 mmol) in propanone (3 ml) was cooled to 0°C and titrated with Jones reagent[102] until the green solution had a permanent orange tinge. After 1 minute, the remaining oxidant was reduced by the dropwise addition of

isopropanol. The green solution was basified to pH 9 with a solution of sodium bicarbonate and the bulk of the solvent removed using a rotary evaporator. The residual aqueous layer was extracted with dichloromethane (3 x 10 ml). The organic extracts were combined and dried over anhydrous sodium sulphate. Filtration and evaporation afforded the crude product as an off-white solid. Purification by flash chromatography eluting with ethyl acetate:methanol (95:5) saturated with ammonia yielded **(153⇌154)** as a white crystalline solid (7.2 mg, 92%). A sample was recrystallised from petroleum ether (b.p. 60 - 80°C) and had m.p. 116 - 118°C.

δ_H (400MHz, 223 K, CD_2Cl_2): 1.69 (brddd, J = 13, 10, 4.0, 1H, $H_{6\alpha}$), 1.75 (ddd, J = 12.5, 6.5, 2.5, 1H, $H_{4\alpha}$), 1.82 (brddd, J = 13.5, 13.5, 4.0, 1H, $H_{7\beta}$), ~1.93 (m, 2H, $H_{2\alpha, 2\beta}$), 2.02 (m, 1H, $H_{4\beta}$), 2.05 (m, 1H, $H_{7\alpha}$), 2.08 (m, 1H, $H_{6\beta}$), 2.42 (s, 3H, NMe), 3.43 (dddd, J = 7.0, 3.0, 2.5, <1, 1H, H_5), 5.30 (dddd, J = 10.5, 10.5, 6.5, 6.5 Hz, 1H, $H_{3\alpha}$), 7.48 (dd, J = 7.5, 1.2 Hz, 2H, $H_{3',5'}$), 7.62 (tt, J = 7.5, 1.2 Hz, 1H, $H_{4'}$), 8.05 (dd, J = ~7.5,1.2 Hz, 2H, $H_{2',6'}$).

Parts of the ^1H NMR spectrum of **(153⇌154)** were second order at 400MHz but were analysed as far as possible on a 'pseudo-first- order' basis with the help of ^1H-^1H and ^1H-^{13}C COSY spectra and selective spin-decoupling experiments. Some signals in the 1.6 - 2.1δ region which overlapped at 300 K were separated at 223 K. The OH signal was broad and varied in position according to temperature, concentration and moisture content.

δ_c (100MHz, 300 K, CD_2Cl_2) (signals in italics were broadened due to VT effects): 25.0 (CH_2, C_6), *29.0* (CH_2, C_4), *35.2* (CH_2, C_2), 29.7 (CH_3), 36.1 (CH_2, C_7), 68.2 (CH, C_3), 57.2 (NCH), 128.4 (2 x aryl CH, $C_{3',5'}$), 129.6 (2 x aryl CH, $C_{2',6'}$), 130.4 (aryl C, $C_{1'}$), 133.0 (aryl CH, $C_{4'}$), 166.0 (C=O).

δ_c (100MHz, 223 K, CD_2Cl_2): 25.2 (CH_2, C_6) 27.4 (CH_2, C_4), 33.9 (CH_2, C_2), 29.2 (CH_3), 36.0 (CH_2, C_7), 68.4 (CH, C_3), 56.3 (NCH), 88.8 (COH), 128.8 (2 x aryl CH, $C_{3',5'}$), 129.8 (2 x aryl CH, $C_{2',6'}$), 130.4 (aryl C, $C_{1'}$), 133.5 (aryl CH, $C_{4'}$), 166.0 (C=O).

ν_{max} (CH_2Cl_2): 3580brw, 2950m, 2930m, 2920m, 2910m, 2895w, 2875w, 2850w, 1715s, 1605w, 1450m, 1315m, 1295m, 1280s, 1270s, 1255s, 1250s, 1120m, 1095m, 1070m, 1025m, 1010m, 970m cm^{-1}.

^{m}/z (%): 261 (M^+, 10), 156 (19), 140 (100), 122 (30), 110 (38), 105 (66), 98 (43), 84 (16), 77 (61), 70 (38), 57 (40), 51 (20). $C_{15}H_{19}NO_3$ [M^+] requires ^{m}/z 261.1365; observed ^{m}/z 261.1365.

Figures from combustion analysis determinations were variable, probably as a result of hydrate formation: e.g. found: C, 64.31; H, 7.64; N, 4.80%. $C_{15}H_{19}NO_3$:H_2O requires C, 64.50; H, 7.58; N, 5.01%. However, a sample of **(153⇌154)** which had been dried over P_2O_5 under vacuum for 24 hr at 30°C analysed correctly: found: C, 68.66; H, 7.07; N, 5.26%. $C_{15}H_{19}NO_3$ requires C, 68.94; H, 7.33; N, 5.36%.

Heptafluorobutanoyl ester of 1-hydroxytropacocaine (170)

To a stirred solution of **(153⇌154)** (7 mg, 0.027 mmol) in dry acetonitrile (2 ml) at 0°C was injected heptafluorobutyric anhydride (20 µl, 0.027 mmol) using a micro-syringe. The solution was allowed to warm to ambient temperature and stirred for 2 hr. After dilution with diethyl ether (15 ml), washing with saturated $NaHCO_3$ (2 x 1 ml), water (1 ml), drying with anhydrous magnesium sulphate, and evaporation, the crude product was purified by chromatography on silica using 5% methanol in ethyl acetate. **(170)** was isolated as a colourless oil (3.6 mg, 30%). ^{1}H NMR data were in agreement with literature data.[5]

N-Benzyloxycarbonyl-3α-hydroxy-6-aza-7oxabicyclo[3.2.2]non-8-ene (171α) and N-Benzyloxycarbonyl-3β-hydroxy-6-aza-7oxabicyclo[3.2.2]non-8-ene (171β) [102]

Tetramethylammonium periodate (5.4 g, 20.4 mmol) and **(159)** (3.81 g, 17.0 mmol) in dichloromethane (70 ml) were stirred at -78°C under nitrogen. A solution of benzyl-N-hydroxycarbamate (3.41 g, 20.4 mmol) in dichloromethane (20 ml) was dripped in over 10 min and the solution was then warmed to ambient temperature and stirred overnight. The solution was filtered, washed with sodium thiosulphate solution (2 x 30 ml) and water (30 ml). The organic layer was separated, dried over anhydrous magnesium sulphate, filtered and the solvent removed using a rotary evaporator. The residual dark yellow oil was purified by flash chromatography using diethyl ether, to afford an inseparable mixture of stereoisomers **(171α)** and **(171β)** in a 30:70 ratio (from ^{1}H NMR signal integrations), as a yellow oil (3.69 g, 79%). The 250 MHz NMR spectrum was identical to that of a sample prepared by Justice;[27] partial analysis was possible. Signals shown in italics were common to both isomers.

δ_H (250MHz, $CDCl_3$): *1.78 - 2.07 (series of m, 5H, inc OH), 2.20 - 2.67 (series of m, 5H inc OH), 3.67* (~tt, J = 6.2, 4.4 Hz, 1H, α-OSi, **(171β)**), 4.24 (~ quin, J = 5.5 Hz, 1H, α-OSi, **(171α)**), *4.74 (m, 1H, α-N), 4.90 (m, 1H, α-O),* 5.15 (s, 2H, CH_2Ph, **(171β)**), 5.17 (s, 2H, CH_2Ph, **(171α)**), 6.17 (ddd, J = 9.1, 6.2, 1.2 Hz, 1H, HC=, **(171β)**, 6.32, (ddd, J = 9.1, 6.8, 0.6 Hz, 1H, HC=, **(171β)**), 6.39 (ddd, J = 9.1, 6.4, 1.3 Hz, 1H, HC=, **(171α)**), 6.50 (ddd, J = 9.1, 6.4, 1.1 Hz, 1H, HC=, **(171α)**), *7.33 (m, 5H).*

N-Benzyloxycarbonyl-3α-[(benzoyl)oxy]-6-aza-7oxabicyclo[3.2.2]non-8-ene (172α) and N-Benzyloxycarbonyl-3β-[(benzoyl)oxy]-6-aza-7oxabicyclo[3.2.2]non-8-ene (172β)

To a 30:70 mixture of **(171α)** and **(171β)** (131 mg, 0.48 mmol) in dry pyridine (2 ml) was added benzoic anhydride (163 mg, 0.72 mmol) and a catalytic amount of DMAP. The reaction mixture was stirred for 18 hr, then diluted with diethyl ether (20 ml). The organic layer was washed with saturated copper sulphate solution (3 x 25 ml), dried over anhydrous magnesium sulphate, filtered and the solvent removed using a rotary evaporator to afford **(172α)** and **(172β)** as a pale yellow oil (177 mg, 97%), in unchanged ratio after chromatography on silica using diethyl ether:petroleum ether (b.p. 40 - 60°C) in a 2:3 ratio. Spectroscopic data for the 3α-ester **(172α)** are derived from the mixture (signals common to both isomers are shown in italics):

(172α): δ_H (250MHz, $CDCl_3$): *2.2 - 2.4 (m, 2H), 2.4 - 2.6 (m, 2H), 4.8 - 5.2 (m, 2H, α-N, α-O), 5.20 (s, 2H, CH_2Ph),* 5.51 (~tt, J ≈ 5.3, 3.6 Hz, 1H, α-OCOPh), *6.42 (m, 1H, HC=),* 6.58 (ddd, J = 9.2, 6.9, 1.0 Hz, 1H, HC=), *7.3 - 7.5 (series of m, 8H, aryl H), 8.0 (m, 2H, aryl H).*

δ_C (63MHz, $CDCl_3$): 35.4 & 38.2 (2 x CH_2), *51.0 (NCH), 67.8 (CH_2Ph), 68.7 (HCOCOPh),* 73.0 (OCH), 128.2 (aryl CH; benzyl), 128.6 (aryl CH, $C_{3'}$,$C_{5'}$), 129.4 (aryl CH; benzyl), 129.5 (aryl CH, $C_{2'}$,$C_{6'}$), 130.0 (aryl CH; benzyl), 130.3 (aryl CH, $C_{1'}$), 131.6 (2 x HC=), 132.9 (aryl CH, $C_{4'}$), 135.9 (aryl C; benzyl), 156.4, (CO_2CH_2Ph), 165.3 (COPh).

ν_{max} (CH_2Cl_2) (mixture of **(172α)** and **(172β)**): 3050w, 3040w, 2960w, 2940w, 2925w, 1715s, 1705s, 1605w, 1265brs, 1115m, 1085brm, 1070m, 1025m cm^{-1}.

^{m}/z (%) (mixture of **(172α)** and **(172β)**): 379 (M^+, 3), 335 (11), 213 (2), 183 (1), 122 (2), 105 (26), 91 (100), 77 (13), 65 (4). $C_{22}H_{21}NO_5$ [M^+] requires ^{m}/z 379.1420; observed ^{m}/z 379.1420.

A pure sample of the 3β-ester **(172β)**, m.p. 105-106°C, was obtained by recrystallisation from 1:1 diethyl ether:petroleum ether (b.p. 60 - 80°C).

(172β): δ_H (250MHz, $CDCl_3$; assignments made with the help of an HH COSY spectrum): 2.10 (ddd, J ≈ 13, 11, 1.0 Hz, 1H, $H_{2\beta}$), 2.15 (ddd, J ≈ 13, 11, 1.7 Hz, 1H, $H_{4\beta}$), 2.48 (m, 2H, $H_{2\alpha}$, $H_{2\beta}$), 4.84 [brdd (~brt) J ≈ 6.3, 4.5 Hz, 1H, α-N], 4.97 [brdd (~brt) J ≈ 6.6, 5.0 Hz, 1H, α-O], 5.03 (~tt, J = 11.0, 6.4 Hz, 1H, $H_{3\alpha}$), 5.20 (s, 2H, CH_2Ph), 6.31 (ddd, J = 9.2, 6.3, 1.2 Hz, 1H, H_6), 6.45 (ddd, J = 9.2, 6.4, 1.3 Hz, 1H, H_5), 7.3 - 7.5 (series of m, 7H, aryl H), 7.56 (tt, J = 7.6, 1.5 Hz), 8.01 (brd, J = 8.4 Hz, 2H, aryl H).

δ_C (63MHz, $CDCl_3$): 33.9 & 36.7 (2 x CH_2), 51.4 (NCH), 68.3 (CH_2Ph), 69.2 (HCOCOPh), 72.4 (OCH), 128.5, 128.7, 128.8, 128.9, 129.1, 130.0, 130.4, 130.5, 133.5, 136.4, (aryl and alkenyl C), 156.8, (CO_2CH_2Ph), 166.2 (COPh).

(172β): found: C, 69.50; H, 5.29; N, 3.71%. $C_{22}H_{21}NO_5$ requires C, 69.64; H, 5.58; N, 3.69%.

1β-Hydroxy-4β[(benzyloxycarbonyl)amino]-6α-benzoyloxy-cyclohept-2-ene (173)
1β-Hydroxy-4β[(benzyloxycarbonyl)amino]-6β-benzoyloxy-cyclohept-2-ene (174)

To a solution of **(172α)** and **(172β)** (1:3, 4.6 g, 0.012 mol) in acetonitrile:water (4:1, 125 ml) was added molybdenum hexacarbonyl (3.5 g, 0.013 mol). The mixture was heated under reflux for 24 hr and then filtered through a silica plug which was washed thoroughly with ether:methanol (95:5). The solvent was removed using a rotary evaporator and the dark brown residue dissolved in dichloromethane. This solution was filtered again through celite to yield a crude brown solid which was partially purified by flash chromatography eluting with diethyl ether:petroleum ether (b.p. 40 - 60°C) in a ratio ranging from 3:2 to 4:1. Some remaining material was washed off the column using ethyl acetate and the combined fractions were evaporated to give **(173)** and **(174)** (3.46 g, 75%) as a white solid. A sample of the mixture was recrystallised from 1:1 diethyl ether : petroleum ether (b.p. 60 - 80°C) to give a mixed sample (m.p. 132 - 145°C).

δ_H (250MHz, $CDCl_3$) (signals are quoted in italics where they overlap or where they are common to both isomers): **(173)**: *1.80 - 2.45 (series of m, 5H)*, 4.77 (brm, 2H, α-O and α-N), *5.08 (brs, CH_2Ph and NH, 3H)*, 5.56 (m, 1H, α-OCOPh), 5.69 (dd J = 12.9, 3.0 Hz, 1H, HC=), *5.85 (m, 1H, HC=)*, 7.2 - 7.5 (m, 8H), 8.1 (brdd, J ≈ 7.5 , 1.2 Hz, $H_{2',6'}$).

(174) *1.80 - 2.45 (series of m, 5H)*, 4.31 (brm, 1H, α-N), 4.47 (brm, 1H, α-O) *5.08 (brs, CH_2Ph and NH, 3H,)*, 5.53 (m, 1H, α-OCOPh), 5.56 (m, 1H, HC=), *5.85 (m, 1H, HC=)*, 7.2 - 7.5 (m, 8H), 8.01 (brdd, J ≈ 7.5 , 1.2 Hz, $H_{2',6'}$).

ν_{max} (CH_2Cl_2 (mixture of **(173)** and **(174)**): 3440w, 3055m, 3035m, 2950m, 2920m, 1720s, 1715s, 1510m, 1505m, 1500m, 1450m, 1425m, 1420m, 1415m, 1315w, 1277s, 1270s, 1260s, 1252s, 1248s, 1210m, 1200m, 1175w, 1115w, 1070w, 1035m, 1025s cm^{-1}.

$^m/z$ (FAB %) (mixture of **(173)** and **(174)**): 404 (MNa^+, 25), 382 (MH^+, 49), 364 (($MH^+ - H_2O$, 100).

Found: C, 69.13; H, 6.20; N, 3.70%. $C_{22}H_{23}NO_5$ requires C, 69.28; H, 6.08. N, 3.67%.

A pure sample of **(174)** (1.0 g) was separated on silica (chromatotron) eluting with diethyl ether:petroleum ether (b.p. 40 - 60°C) (ratio ranging from 1:1 to 3:2). Early fractions contained pure **(174)** (0.46 g), a sample of which was recrystallised from 1:1 diethyl ether:petroleum ether (b.p. 60 - 80°C) to give a white crystalline solid, m.p. 145 - 147°C.

δ_H (250MHz, $CDCl_3$): 1.8 - 2.0 (brm, 3H, inc. OH), 2.2 - 2.4 (m, 2H), 4.34 (brm, 1H, α-N), 4.50 (brd, J = 10 Hz, 1H, α-OH), 5.05 (brm, 1H, NH), 5.10 (brs, 2H, CH_2Ph), 5.33 (tt, J = 10.7, 3.7 Hz, 1H, α-OCOPh), 5.59 (ddd, J ≈ 11.5, 3.4, 2.2 Hz, 1H, HC=), 5.84 (brd, J = 11.5 Hz, 1H, HC=), 7.35 (m, 5H), 7.41 (brt, J ≈ 7.5 Hz, 2H, $H_{3',5'}$), 7.56 (brtt, J = 7.5, 1.2 Hz, 1H, $H_{4'}$), 8.01 (brdd, J ≈ 7.5, 1.2 Hz, $H_{2',6'}$).

δ_c (63MHz, $CDCl_3$): 39.6 & 42.4 (2 x CH_2), 48.0 (NCH), 66.9 (HCOCOPh), 67.3 (CH_2Ph), 71.4 (HCOH), 128.5, 128.6, 128.7 (3 x aryl CH; benzyl), 129.0 (aryl CH, $C_{3',5'}$), 130.0 (aryl CH, $C_{2',6'}$), 130.6 (aryl C, $C_{1'}$), 132.3 (HC=), 133.5 (aryl CH, $C_{4'}$), 136.7 (aryl C; benzyl), 137.5 (HC=), 155.9 (NCO), 165.8 (COPh).

Found: C, 69.15; H, 6.05. N, 3.71%. $C_{22}H_{23}NO_5$ requires C, 69.28; H, 6.08; N, 3.67%.

Later fractions, together with ethyl acetate washings, contained a mixture of **(173)** and **(174)** (0.395 g).

β-1-Hydroxy-3α-benzoyloxy-5β-aminocycloheptane (175) and all-cis-(all-β-)-1-hydroxy-3-benzoyloxy-5-aminocycloheptane (176)

A 30:70 mixture of **(173)** and **(174)** (45 mg, 0.118 mmol) in dry methanol (7 ml) was hydrogenolysed using a catalytic amount of 10% palladium on charcoal at atmospheric pressure. After 1.5 hr, gaseous ammonia was bubbled through the mixture which was filtered though celite and the solvent evaporated to yield the mixture of **(175)** and **(176)** in quantitative yield (30 mg).

(175): δ_H (250MHz, $CDCl_3$): *1.6 - 2.4 (series of m. 9H)*, 3.45 (brm, 1H, α-N), 4.15 (brm, 1H, α-OH), 5.58 (m, 1H, $H_{3\beta}$), *7.43 (m, 2H, $H_{3',5'}$), 7.56 (m, 1H, $H_{4'}$), 8.02 (m, 2H, $H_{2',6'}$)*

δ_c (63MHz, $CDCl_3$): 46.7 (NCH), 66.7 (HCOCOPh), 69.4 (HCOH), *128.8 (2 x aryl CH, $C_{3',5'}$)*, 129.91 (2 x aryl CH, $C_{2',6'}$), 130.8 (aryl C, $C_{1'}$), 133.27 (aryl CH, $C_{4'}$), 166.20 (C=O); additional minor peaks were observed in the ^{13}C NMR spectrum of the mixture but these could not be assigned with confidence to the ring CH_2 signals of the minor isomer.

(176) δ_H (250MHz, $CDCl_3$): *1.6 - 2.4 (series of m. 9H)*, 3.10 (brm, 1H, α-N), 4.05 (brm, 1H, α-OH), 5.04 (tt, J = 10.7, 2.2 Hz, 1H, $H_{3\alpha}$), *7.43 (m, 2H, $H_{3',5'}$), 7.56 (m, 1H, $H_{4'}$), 8.02 (m, 2H, $H_{2',6'}$).*

δ_c (63MHz, $CDCl_3$): 31.9, 32.6, 42.0 & 43.9 (4 x CH_2), 49.0 (NCH), 68.1 (HCOCOPh), 70.0 (HCOH), *128.8 (2 x aryl CH, $C_{3',5'}$)*, 129.94 (2 x aryl CH, $C_{2',6'}$), 131.0 (aryl C, $C_{1'}$), 133.35 (aryl CH, $C_{4'}$), 166.26 (C=O).

ν_{max} (CH_2Cl_2) (mixture of **(175)** and **(176)**): 3600m, 2860m, 1710s, 1605w, 1585w, 1560w, 1545w, 1465w, 1450w, 1317m, 1270brs, 1260brs, 1178w, 1115s, 1070w, 1025m cm^{-1}.

The compounds could not be separated completely. Partial separation of the two stereoisomers was achieved using preparative thin layer chromatography (5% ethanol

in diethyl ether). This yielded, firstly, a sample containing ca. 80% of the 3α-ester **(175)** as a pale yellow oil (1.2 mg, 5%).

δ_H (250MHz, $CDCl_3$): 1.7 - 2.4 (series of m. 9H), 3.40 (m, 1H, α-N), 4.18 (brm, 1H, α-O), 5.70 (m, 1H, $H_{3\beta}$), 7.48 (m, 2H, $H_{3',5'}$), 7.58 (m, 1H, $H_{4'}$), 8.02 (m, 2H, $H_{2',6'}$).

Mixed fractions were then eluted and finally the 3β-ester **(176)** followed, also as a pale yellow oil (2.8 mg, 11%).

δ_H (250MHz, $CDCl_3$): 1.70 - 2.45 (series of m. 9H), 2.90 (m, 1H, α-N), 4.05 (brm, 1H, α-O), 5.10 (tt, J = 10.7, 2.2 Hz, 1H, $H_{3\alpha}$), 7.45 (m, 2H, $H_{3',5'}$), 7.55 (m, 1H, $H_{4'}$), 8.04 (m, 2H, $H_{2',6'}$).

$^m/z$ (FAB): 272 (MNa^+), 250 (MH^+). $C_{14}H_{20}NO_3$ [M^+] requires $^m/z$ 250.1444; observed $^m/z$ 250.1443.

4β[(Benzyloxycarbonyl)amino]-6α-benzoyloxycyclohept-2-enone (177) and 4β[(benzyloxycarbonyl)amino]-6β-benzoyloxycyclohept-2-enone (178)

To a stirred solution of **(173)** and **(174)** (50 mg, 0.13 mmol) in acetone was added chromic acid following the procedure used for compound **(153⇌154)** to afford a mixture of **(177)** and **(178)** as a pale yellow oil (40mg, 82%).

δ_H (250MHz, $CDCl_3$): 2.0 - 2.2 (brm, 2H), 2.5 & 2.65 (2 x m, 2H), 3.0 (m, 4H, α-CO), 4.78 (brm, 2H, 2 x α-N), 5.10 (s, 4H, CH_2Ph), 5.3 - 5.6 (brm, 4H, 2 x α-OCOPh + 2 x NH), 6.0 (m, 2H), 6.6 (m, 2H), 7.2 - 7.6 (series of m, 16 H), 7.9 & 8.0 (2 x d, J = 7.8 Hz, 2H).

δ_c (63 MHz, $CDCl_3$): 39.2, 40.0 & 47.9 (3 x CH_2), 48.3 (NCH), 48.8 (CH_2), 48.9 (NCH), 66.3 and 66.9 (2 x CHOCOPh), 67.1 (2 x CH_2Ph), [aryl and alkene CH signals were observed at 128.2, 128.29, 128.33, 128.45, 128.5, 128.6, 129.6, 129.7, 131.3, 132.0, 133.3, 133.4 together with aryl C signals at 136.0 and 136.1 but there was signal overlap and these were not assigned], 155.5 (2 x NCO), 165.5 and 165.6 (2 x COPh), 197.4 and 197.6 (2 x C=O).

ν_{max} (CH_2Cl_2, film): 1715s, 1665m, 1605w, 1585w, 1510m cm^{-1}.

$^m/z$ (FAB): 380 (MH^+). $C_{22}H_{22}NO_5$ [MH^+] requires $^m/z$ 380.1498; observed $^m/z$ 380.1498.

Conversion of (174) into (178⇌179)

Conversion of **(174)** (95 mg, 0.249 mmol) into **(178⇌179)** followed the general procedure used for the preparation of **(177)** and **(178)** above. The oily product was chromatographed using diethyl ether:petroleum ether (b.p. 40 - 60°C) (7:3) to give **(178⇌179)** (85 mg, 90%) as a yellow oil.

δ_H (400MHz, CD_2Cl_2, 300K): 2.13 (m, 1H) & 2.64 (m, 1H), 3.0 (m, 2H, α-CO), 4.71 (brm, 1H, α-N), 5.10 (s, 2H, CH_2Ph), 5.41 (brd, J ≈ 6 Hz, NH), 5.54 (~quintet, J ≈ 5.7 ± 1Hz, 1H, α-OCOPh), 6.08 (dd, J = 12.3, 2.4 Hz, 1H), 6.58 (dd, J = 12.3, 2.9 Hz, 1H), 7.32 - 7.37 (m, 5H), 7.41 (brt, J = 7.3 Hz, 2H, $H_{3'5'}$), 7.56 (tt, J = 7.3, 1.2 Hz, 1H, $H_{4'}$), 7.94 (m, 2H, $H_{2',6'}$).

δ_c (101MHz, CD_2Cl_2, 300K): 40.3 & 48.2 (2 x CH_2), 49.3 (NCH), 67.3 (CH_2Ph), 67.5 (HCOCOPh), [aryl and alkene CH signals were observed at 128.4, 128.6, 128.85, 128.9, 129.9, 132.3, 133.6 together with aryl C signals at 130.3 and 136.9 but there was overlap and these were not assigned], 155.8 (NCO), 165.7 (COPh), 197.7 (C=O). Measurements at 223 K showed no evidence of the bicyclic tautomer.

ν_{max} (CH_2Cl_2): 3430m, 3050w, 2960w, 2870w, 1725s, 1680m, 1620w, 1605w, 1510w, 1450m, 1400w, 1315w, 1265brs, 1175m, 1110s, 1100m, 1070m, 1040m, 1025s cm^{-1}

$^m/z$ (FAB) 402 (MNa^+), 380 (MH^+). $C_{22}H_{22}NO_5$ [MH^+] requires $^m/z$ 380.1498; observed: $^m/z$ 380.1498.

Hydrogenation of (178⇌179) to 3β-benzoyloxy-5β [(benzyloxycarbonyl)amino]-cycloheptanone (180⇌181)

The hydrogenation of the double bond in **(178⇌179)** was performed at atmospheric pressure in methanol using standard conditions. The product **(180⇌181)** was shown by ^1H NMR spectroscopy to have lost the double bond but to have retained the N-benzyloxycarbonyl group; it was not purified.

Direct hydrogenolysis/hydrogenation of (178⇌179) to 1-hydroxynortropacocaine (182⇌183)

A solution of the ketone **(178⇌179)** in dry methanol was hydrogenolysed over a catalytic amount of 10% palladium on charcoal at atmospheric pressure. The progress

of the reaction was monitored by TLC and after 3 hours, there remained no trace of either **(178⇌179)** or the intermediate **(180⇌181)**. The reaction mixture was filtered through a pad of celite which was subsequently washed thoroughly with ethanol. The combined solutions were evaporated to yield **(182⇌183)** as a yellow oil, (ca. 85 mg). Chromatography on a small silica column using ethyl acetate:ammonia:5-10% methanol gave pure **(180⇌181)** as a crystalline solid (82 mg; 94%). A sample was recrystallised from ethyl acetate to give a sample which melted with decomposition at 103-105°C.

δ_H (400MHz, CD_2Cl_2, 300 K): 1.65 (dddd, J = 13.3, 10.7, 3.6, ca. 1 Hz, 1H, $H_{4\beta}$), 1.75 (brddd, J = 13.3, 9.0, ca. 5 Hz, 1H, $H_{6\alpha}$), ~1.90 (m, 1H, $H_{7\beta}$), 1.92 (brddd, J = 12.4, 10.5, ca. 2 Hz, 1H, $H_{2\beta}$), 2.05 (ddd, J = 13.3, 9.0, ca. 5 Hz, 1H, $H_{7\alpha}$), ~2.10 (m, 1H, $H_{6\beta}$), 2.15 (m, 1H, $H_{4\alpha}$), 2.53 (ddd, J = 12.4, 6.1, <1 Hz, 1H, $H_{2\alpha}$), 3.68 (brddd, J = 7.3, 3.6, 2.5 Hz, 1H, H_5), 5.38 (dddd, J = 10.7, 10.5, 6.5, 6.1 Hz, 1H, $H_{3\alpha}$), 7.48 (brt, J ≈ 7.5 Hz, 2H, $H_{3',5'}$), 7.61 (tt, J = 7.5, 1.2 Hz, 1H, $H_{4'}$), 8.04 (dd, J = 8.2, 1.2 Hz, 2H, $H_{2',6'}$).

δ_c (101MHz, CD_2Cl_2, 300 K): 27.8 (CH_2, C_6), 35.4 (CH_2, C_7), 38.0 (CH_2, C_4), 44.8 (CH_2, C_2), 52.3 (NCH), 68.4 (CH, C_3), 128.7 (2 x aryl CH, $C_{3',5'}$), 129.8 (2 x aryl CH, $C_{2',6'}$), 130.8 (aryl C, $C_{1'}$), 133.3 (aryl CH, $C_{4'}$), 166.1 (C=O).

δ_c (101MHz, CD_2Cl_2, 223 K): 27.1 (CH_2, C_6), 34.5 (CH_2, C_7), 36.8 (CH_2, C_4), 43.8 (CH_2, C_2), 52.0 (NCH), 68.1 (CH, C_3), 90.6 (COH), 129.0 (2 x aryl CH, $C_{3',5'}$), 129.9 (2 x aryl CH, $C_{2',6'}$), 130.3 (aryl C, $C_{1'}$), 133.7 (aryl CH, $C_{4'}$), 166.1 (C=O).

ν_{max} (CH_2Cl_2): 3045w, 2945w, 1720s, 1605w, 1540w, 1455w, 1385s, 1318w, 1265s, 1180w, 1150w, 1115m, 1070w, 1030w cm^{-1}.

$^m/z$ (%): 247 (M^+, 4), 229 (4), 203 (1), 188 (2), 158 (2), 143 (12), 126 (64), 105 (100), 96 (35), 84 (28), 77 (67), 69 (16), 56 (32). $C_{14}H_{17}NO_3$ [M^+] requires $^m/z$ 247.1208; observed $^m/z$ 247.1208.

N-Benzyloxycarbonyl-3-oxo-6-oxa-7-azabicyclo[3.2.2]nonane (196)

Oxidation of **(238)** and **(239)** (389 mg, 1.40 mmol) with Jones reagent followed the general procedure used for the preparation of **(153⇌154)** above to yield **(196)** as a yellow oil (353 mg, 91%).

δ_H (250MHz, $CDCl_3$): 1.60 – 1.87 (series of m, 2H), 2.25 (m, 2H), 2.67 (m, 2H), 3.04 (m, 2H), 4.50 (m, 1H, αN), 4.60 (m, 1H, αO), 5.20 (s, 2H, CH_2Ph), 7.35 (m, 5H, Ph).

δ_c (63MHz, $CDCl_3$): 22.9, 23.1 & 48.1 (3 x CH_2), 49.2 (CNH), 49.9 (CH_2), 68.1 (CH_2Ph), 72.7 (CHO), 128.7, 128.9, 129.0 (3 x aryl CH), 136.3 (aryl C), 155.4 (NCO), 208.8 (C=O).

ν_{max} (CH_2Cl_2): 3020m, 2960m, 1710s, 1500w, 1455m, 1435m, 1405m, 1360m, 1350m, 1335m, 1325w, 1305m, 1280m, 1245m, 1235m, 1220m, 1100s, 1030w, 1000m, 890m, 860w, 805w, 770brm, 675brm cm^{-1}.

$^m/z$ (FAB): 276 (MH^+). $C_{15}H_{18}NO_4$ [MH^+] requires $^m/z$ 276.1235; observed: $^m/z$ 276.1236.

N-Benzyloxycarbonyl-3-trimethylsilyloxy-6-oxa-7-azabicyclo[3.2.2]non-2-ene (197) and N-Benzyloxycarbonyl-3-trimethylsilyloxy-6-oxa-7-azabicyclo[3.2.2]-non-3-ene (198)

To a stirred solution of **(196)** (186 mg, 0.68 mmol) in THF (1.5 ml) was added sodium *bis*(trimethylsilyl)amide (0.95 ml, 0.95 mmol) (1 M solution in THF) and the mixture stirred at -78°C for 1 hr. To this mixture was added a solution of trimethylsilylchloride (388 μl, 3.07 mmol) and triethylamine (100 μl) in THF (2 ml). A white solid precipitated out of the solution. The mixture was then allowed to reach ambient temperature and stirred for a further 30 min. Analysis by TLC indicated the absence of starting material. The resulting solution was diluted with dichloromethane (10 ml), washed with sodium hydrogen carbonate (2 x 1 ml) and water (1 ml), and dried with anhydrous magnesium sulphate. Removal of the solvent using a rotary evaporator yielded **(197)** and **(198)** as a crude brown oil (227 mg). The product was taken on to the next step without further purification, since chromatography on silica resulted in hydrolysis, to give only the starting ketone **(196)**.

Some signals in the crude spectrum of **(197)** and **(198)** could not be assigned with confidence owning the presence of impuries.

δ_H (250 MHz, $CDCl_3$) [mixture of **(197)** and **(198)**]: 0.19 (s, 18H, 2 x $Si(CH_3)_3$), 2.17 (m, 4H), 2.32 – 2.50 (series of m, 2H), 2.58 – 2.87 series of m, 4H), 3.09 (m, 2H), 4.39 (m, 1H), 4.55 (m, 1H), 5.15 (s, 2H, CH_2Ph), 6.00 (brs, 1H, =CH), 6.09 (brs, 1H, =CH), 6.66 (m, 2H), 7.30 (m, 5H, Ph).

Tropinone-N-oxides (227) and (228) (oxidation with hydrogen peroxide)

A solution of tropinone **(11)** (1 g, 7.19 mmol), in ethanol (13 ml) was oxidised with aqueous hydrogen peroxide solution (30%) (1.48 ml, 14.38 mmol) according to the procedure of Werner and Schickfluss.[85] The solution was stirred at 75°C for 14 hr. On cooling, an aliquot was removed for NMR analysis, which showed the reaction to be complete. Stirring over palladium-on-carbon destroyed excess hydrogen peroxide. The solution was filtered and the filtrate evaporated. The residual oil was taken up in chloroform (30 ml), dried over anhydrous magnesium sulphate, filtered and the solvent evaporated to afford a yellow solid (1 g, 89%). This was shown by ^{1}H NMR to be composed of a mixture of **(227)** and **(228)** in the ratio 90:10. A small sample was recystallised from ethyl acetate to give pure **(227)** as a white solid m.p. 97 - 99°C (lit[86] 100°C).

(227) [from a mixture of **(227)** and **(228)**]: δ_H (250 MHz, $CDCl_3$): 2.09 (m, 2H, $H_{6,7\alpha}$), 2.23 (d, J = 16.1 Hz, 2H, $H_{2,4eq}$), 2.32 (m, 2H, $H_{6,7\beta}$), 3.42 (s, 3H, NCH_3), 3.86 (dd, J = 16.1, 4.4 Hz, 2H, $H_{2,4ax}$), 3.75 (brm, 2H, $H_{1,5}$).

δ_c (63MHz, $CDCl_3$): 25.7 (2 x CH_2, $C_{6,7}$), 44.6 (2 x CH_2, $C_{2,4}$), 55.3 (2 x NCH, $C_{1,5}$), 72.1 (CH_3), 205.3 (C=O).

Signals due to the minor isomer **(228)** were visible in the ^{1}H NMR spectrum of **(227)** however, these could not be assigned with confidence. In the ^{13}C NMR spectrum of **(227)** and **(228)**, the carbonyl signal of the minor isomer **(228)** was not visible.

(228): δ_c (63MHz, $CDCl_3$): 27.9 (2 x CH_2, $C_{6,7}$), 46.2 (2 x CH_2, $C_{2,4}$) 57.7 (2 x NCH, $C_{1,5}$), 73.0 (CH_3).

ν_{max} (CH_2Cl_2) [mixture of **(227)** and **(228)**]: 3660m, 3040m, 2980m, 1725s, 1480w, 1440m, 1420m, 1260brs, 980w, 895w, 720vbrm cm^{-1}.

$^m/z$ (FAB) [mixture of **(227)** and **(228)**]: 156 (MH^+). $C_8H_{14}NO_2$ [MH^+] requires $^m/z$ 156.1025; observed $^m/z$ 156.1025.

Tropinone-N-oxides (227) and (228) (oxidation with MCPBA)

A solution of tropinone **(11)** (1.4 g, 10 mmol) in dichloromethane (50 ml), was oxidised with MCPBA (3.72 g, 15 mmol), according to the method of Shvo.[86] After stirring at ambient temperature for 72 hr, TFA was added (0.92 ml). The resulting

solid was found to contain a mixture of protonated N-oxides **(227)** and **(228)** in the ratio 80:20 together with a large amount of *m*CPBA. A small portion of the crude solid (ca. 100 mg) was dissolved in dichloromethane (15 ml) and stirred overnight with anhydrous potassium carbonate. The solution was filtered and evaporated to leave a mixture of neutral N-oxides **(227)** and **(228)** in the ratio 50:50.

(228) δ_H (250MHz, $CDCl_3$): 1.77 (m, 2H, $H_{6,7\alpha}$), 2.58 (brd, J ≈ 18 Hz, 2H, $H_{2,4eq}$), 2.96 (brdd, J ≈ 18, 4.1 Hz, 2H, $H_{2,4ax}$), 2.96 (m, 2H, $H_{6,7\beta}$), 3.56 (s, 3H, NCH_3), 3.88 (brm, 2H, $H_{1,5}$).

N-Benzyloxycarbonyl-3α-hydroxy-6-oxa-7-azabicyclo[3.2.2]nonane (237) and N-Benzyloxycarbonyl-3β-hydroxy-6-oxa-7-azabicyclo[3.2.2]nonane (238)

To a stirred slurry of potassium azadicarboxylate (7 g, 0.35 mol) and **(171αβ)** (2 g, 7 mmol) in dry methanol (20 ml) was added glacial ethanoic acid (42 ml) over 10 min at 0°C. The mixture was allowed to warm to ambient temperature and stirred for a further 20 hr, after which the reaction was quenched by addition of the minimum quantity of water at 0°C. Filtration through celite and evaporation of the bulk of the solvent left a residual oil, which was taken up in dichloromethane (30 ml) and washed with sodium bicarbonate solution (10 ml) and water (10 ml). The organic layer was dried over anhydrous magnesium sulphate, filtered and the solvent removed using a rotary evaporator to give a crude oil. Purification by flash chromatography [diethyl ether – diethyl ether:methanol (5%)] afforded firstly **(237)** as a pale yellow oil (340 mg, 18%). Further elution gave **(238)** (1 g, 52%) also as a yellow oil.

(237): δ_H (250 MHz, $CDCl_3$): 190 (m, 1H), 2.00 – 2.19 (series of m, 5H), 2.42 (m, 2H), 4.32 (~quin, J = 5.3 Hz, α-OH), 4.51 (brs, 2H, α-N & α-O), 5.20 (s, 2H, CH_2Ph), 7.35 (m, 5H, Ph).

δ_c (63MHz, $CDCl_3$): 22.4 & 23.0 (2 x CH_2), 41.6 (2 x CH_2), 50.5 (NCH), 66.2 (COH), 67.8 (CH_2Ph), 74.8 (OCH), 128.5, 128.6, 128.9 (3 x aryl CH), 136.7 (aryl C), 155.1 (CO).

ν_{max} (CH_2Cl_2): 3600m, 3460brm, 3030m, 2940s, 1685s, 1420brs, 1355m, 1330s, 1305s, 1255brs, 1090s, 1050s, 1000m, 935w, 885w, 860w, 690vbrm cm^{-1}.

^{m}/z (FAB): 278 (MH^+). $C_{15}H_{20}NO_4$ [MH^+] requires ^{m}/z 278.1392; observed ^{m}/z 278.1392.

(238): δ_H (250 MHz, $CDCl_3$): 1.72 (m, 2H), 1.90 – 2.19 (series of m, 4H), 2.29 (m, 2H), 4.22 (~tt, J = 10.5, 6.4 Hz, 1H, α-OH), 4.40 (brs, 1H, α-N), 4.49 (brt, J ≈ 5.7 Hz, 1H, α-O), 5.17 (s, 2H, CH_2Ph), 7.35 (m, 5H, Ph).

δ_c (63MHz, $CDCl_3$): 21.6 & 22.5 (2 x CH_2), 42.3 (2 x CH_2), 49.1 (NCH), 66.0 (COH), 67.8 (CH_2Ph), 73.8 (OCH), 128.5, 128.6, 128.9 (3 x aryl CH), 136.7 (aryl C), 154.9 (CO).

ν_{max} (CH_2Cl_2): 3600m, 3440brm, 3020m, 2940s, 1690s, 1415brs, 1350m, 1300brs, 1260brs, 1210m, 1105s, 1085s, 1055s, 1000w, 890w, 840w, 690vbrm cm^{-1}.

^{m}/z (FAB): 278 (MH^+). $C_{15}H_{20}NO_4$ [MH^+] requires ^{m}/z 278.1392; observed ^{m}/z 278.1392.

N-Methyl-3β-hydroxy-6-oxa-7-azabicyclo[3.2.2]nonane (165) from (238)

To a stirred slurry of lithium aluminium hydride (239 mg, 6.28 mmol) in dry THF (2 ml) under a nitrogen atmosphere, was added a solution of **(238)** (434 mg, 1.57 mmol) in THF (10 ml) at 0°C. The reaction mixture was allowed to warm to ambient temperature and then stirred for a further 3 hr, before addition of the minimum amount of water-saturated diethyl ether. The reaction mixture was diluted with diethyl ether (10 ml) and then dried over anhydrous magnesium sulphate. The mixture was filtered and the inorganic solids washed with ethyl acetate (2 x 7 ml). The filtrate was then evaporated using a rotary evaporator to yield a crude oil, which was purified by flash chromatography, eluting with chloroform:methanol (9:1) to give **(165)** as a pale yellow oil (180 mg, 73%) which showed identical spectroscopic properties to the sample prepared from **(166)**.

N-methyl-3-oxo-6-aza-7-oxobicyclo[3.2.2]nonane (234)

A stirred solution of **(165)** (50 mg, 0.318 mmol) in propanone (7 ml) was acidified with TFA (49 μl, 0.636 mmol) and then titrated with Jones reagent, at ambient temperature, until the green solution had a permanent orange tinge. Stirring was continued for a further 20 min before the addition of excess isopropyl alcohol. The solution was filtered though celite and the inorganic solid washed with methanol (2 x

5 ml). The bulk of the solvent was evaporated and the residue taken up in water (5 ml). The pH of the solution was adjusted to 12 by the dropwise addition of 2 M sodium hydroxide solution. The solution was transferred to a separating funnel and the aqueous layer repeatedly extracted with ethyl acetate (3 x 10 ml). The combined organic layers were dried over anhydrous magnesium sulphate, filtered and evaporated using a rotary evaporator to yield a pale yellow oil. Purification by column chromatography eluting with diethyl ether/ammonia yielded **(234)** as a pale yellow oil (10 mg, 20%).

δ_H (250MHz, $CDCl_3$): 1.70 (m, 2H), 2.36 (m, 2H), 2.55 (m, 2H), 2.72 (s, 3H, NCH_3), 3.10 (m, inc brm, 2H & α-N), 4.34 (brm, 1H, α-O).

δ_c (63MHz, $CDCl_3$): 23.7 (2 x CH_2), 44.6 (CH_3), 51.2 (2 x CH_2), 56.1 (CHN), 69.6 (CHO), 210.9 (C=O).

ν_{max} (CH_2Cl_2): 2950m, 2930m, 1705s, 1470w, 1405w, 1100w, 1070w, 1035w cm^{-1}.

$^m/z$ (%):155 (M^+, 87), 149 (12), 143 (50), 126 (9), 112 (58), 100 (100), 84 (84), 72 (70), 55 (59). $C_8H_{13}NO_2$ (M^+) requires $^m/z$ 155.0946; observed $^m/z$ 155.0946.

2,2',4,4'-d_4-tropinone (239)

Tropinone **(11)** (130 mg, 0.935 mmol) was dissolved in methyl alcohol-d (26 ml) under an nitrogen atmosphere. Sodium (129 mg, 5.61 mmol) was added at 0°C, followed by D_2O (6.5 ml). The reaction was then heated at reflux for 1 hr. The solution was cooled and the solvent evaporated using a rotary evaporator. The residual yellow oil was taken up in diethyl ether (20 ml) and washed with water (2 ml). The ethereal layer was dried over anhydrous magnesium sulphate, filtered and the solvent evaporated to yield **(239)** as a white crystalline solid (81 mg, 61%) m.p. 38°C (lit.[91] m.p. 38°C).

δ_H (250MHz, $CDCl_3$): 1.52 (m, 2H, $H_{6,7\alpha}$), 2.04 (m, 2H, $H_{6,7\beta}$), 2.41 (s, 3H, NCH_3), 3.36 (dd, J = 4.0, 2.6 Hz, 2H, 2 x α-N).

δ_c (63 MHz, $CDCl_3$): 27.9 (2 x CH_2), 38.6 (CH_3), 46.6, 46.9, 47.2, 47.5, 47.9 (quin, 2 x CD_2), 60.9 (2 x NCH), 210 (C=O).

ν_{max} (CH_2Cl_2): 3030w, 2950s, 2880w,2850w, 2800m, 1705s, 1470m, 1450m, 1350m, 1340m, 1330m, 1310w, 1260brm, 1235m, 1215m, 1155m, 1140m, 1120s, 1100s, 1080brm, 1030s, 980w, 960m, 920w, 910w, 860m, 700vbrm cm^{-1}.

^{m}/z (%): 143 (M^+, 26), 136 (4), 123 (2), 107 (10), 98 (8), 82 (100), 77 (20), 69 (12), 63 (3), 55 (20), 51 (7). $C_8H_9D_4NO$ requires ^{m}/z 143.1248; observed ^{m}/z 143.1248.

(-)-6,7-Dehydrohysocyamine (59)

(-)-Dehydrohysocyamine **(59)** was prepared by deoxygenation of scopolamine in accordance with the method of Bremner.[31] Scopolamine hydrobromide trihydrate (4.80 g, 10.96 mmol) was partially dissolved in ethanol and basified with ammonia gas until all the solid was in solution. To this solution was added zinc/copper couple (10 g) and the mixture heated at reflux, with stirring, for 16 hr. An aliquot was removed for NMR analysis and showed complete reaction. On cooling, the solution was filtered through celite and the filter cake washed thoroughly with ethanol. Evaporation of the filtrate yielded **(59)** as yellow oil (3.14 g, 100%), which was converted to **(242)** without further purification.

^{1}H NMR data was in agreement with that of Noyori.[19] The mass spectrum was identical to that reported by Blossey.[91] The ^{13}C NMR data and full IR data are so far unreported.

δ_H (250MHz, $CDCl_3$): 1.52 (brd, J ≈ 15.5 Hz, 1H), 1.67 (brd, J ≈ 15.5 Hz, 1H), 2.1 -2.3 (brm, 2H, $H_{2,4ax}$), 2.24 (s, 3H, NCH_3), 3.27 (m, 1H, α-N), 3.38 (m, 1H, α-N), 3.74 (ABX type m, 1H, CHC**H**$_2$OH), 3.75 (ABX type m, 1H, C**H**CH$_2$OH), 4.0 (s, 1H, OH) 4.12 (ABX type m, 1H, CHC**H**$_2$OH), 4.99 (t, J = 5.2 Hz, 1H, $H_{3\beta}$), 5.44 (dd, J = 5.3, 1.0 Hz, 1H, =CH), 5.82 (dd, J = 5.3, 1.0 Hz, 1H, =CH), 7.29 (m, 5H, Ph).

δ_c (63 MHz, $CDCl_3$): 33.2 & 33.4 (2 x CH_2), 41.5 (CH_3), 54.9 (**C**HCH$_2$OH), 64.1 (CHCH$_2$OH), 65.6 & 66.7 (2 x NCH), 67.7 (C_3), 127.9, 128.6 & 129.0 (3 x aryl CH), 136.4 (aryl C), 172.2 (C=O).

ν_{max} (CH_2Cl_2): 3620 brw, 3050brw, 2945s, 3880w, 1720s, 1595w, 1495w, 1455w, 1420w, 1370brw, 1360w, 1345w, 1340w, 1300w, 1265brm, 1225s, 1175s, 1100m, 1060w, 1040s, 710vbrm cm^{-1}.

^{m}/z (%): 287 (M^+, 100), 271 (3), 222 (1), 164 (4), 138 (57), 121 (86), 94 (73), 81 (26), 53 (1). $C_{17}H_{21}NO_3$ [M^+] requires ^{m}/z 287.1522; observed ^{m}/z 287.1521.

(-)-6,7-d_2-hysocyamine (241)

The alkene **(59)** (1.60 g, 5.57 mmol) was dissolved in methanol (50 ml), and stirred with over a catalytic amount of palladium on charcoal (5%), under a deuterium atmosphere for 5 hr. An aliquot was removed for NMR analysis and showed complete reduction of the double bond. The reaction mixture was then filtered though celite, and the solvent evaporated using a rotary evaporator to yield **(241)** as a pale yellow solid (1.62 g, 100%).

The ^{1}H NMR data was in agreement with that of Hashimoto *et al.*[103] The ^{13}C NMR, IR and mass spectrometric data are so far unreported.

δ_H (250MHz, $CDCl_3$): 1.19 & 1.76 (brd, J = 9.4 Hz, 1H, $H_{6,7\alpha}$), 1.50 & 1.70 (brd, J = 14.8 Hz, 1H, $H_{2,4ax}$) 2.07 & 2.14 (m, 1H, $H_{2,4eq}$), 2.23 (s, 3H, NCH_3), 2.95 & 3.07 (brs, 1H, α-N),) 3.80 (ABX type m, 2H, CHC**H$_2$**OH & C**H**CH$_2$OH), 4.17 (ABX type m, 1H, CHC**H$_2$**OH), 5.03 (t, J = 5.3 Hz, 1H, $H_{3\beta}$), 7.30 (m, 5H, Ph).

δ_c (63 MHz, $CDCl_3$): 24.4 (1:1:1 t, 2 x CHD, 34.9 & 35.1 (2 x CH_2, $C_{2,4}$), 39.5 (CH_3), 54.8 (**C**HCH$_2$OH), 61.2 & 61.3 (2 x NCH), 64.0 (CH**C**H$_2$OH), 66.3 (C_3), 127.7, 128.4 & 129.3 (3 x aryl CH), 136.0 (aryl C), 172.2 (C=O).

ν_{max} (CH_2Cl_2): 3600w, 3360brw, 3020m, 2860s, 2825s, 1730s, 1490w, 1455w, 1420w, 1230m, 1170s, 1090m, 1075m, 1045s, 1040s, 1015w, 1000w, 910w, 705vbrm cm^{-1}.

^{m}/z (%): 291 (M^+, 27), 275 (3), 164 (7), 142 (11), 126 (100), 94 (16), 85 (26), 68 (7), 51 (37). $C_{17}H_{21}D_2NO_3$ [M^+] requires ^{m}/z 291.1803; observed ^{m}/z 291.1804.

(-)-6,7-d_2-tropine (242)

Sodium hydroxide (100 mg, 2.5 mmol) and **(241)** (338 mg, 1.16 mmol) in water (15 ml) was heated at reflux, with stirring, for 2 hr. On cooling the bulk of the solvent was removed using a rotary evaporator. The residue was partitioned between chloroform (10 ml) and sodium bicarbonate solution (15 ml). The aqueous layer was separated, basified with ammonia gas, and repeatedly extracted with chloroform (3 x 10 ml). The combined organic layers were dried over anhydrous magnesium

sulphate, filtered, and the solvent removed using a rotary evaporator, to afford **(242)** as a white crystalline solid (122 mg, 74%) m.p. 47°C (lit.[104] m.p. 45°C).

^{1}H NMR data was in agreement with that of Bishop *et al.*[104] The mass spectrum was identical to that reported by Blossey *et al.*[91] The ^{13} C NMR data and full IR data are so far unreported.

δ_H (250MHz, $CDCl_3$): 1.58 (d, J = 14.2 Hz, 2H, $H_{2,4eq}$), 2.00 (m, 2H, $H_{2,4ax}$), 2.03 (s, 2H, $H_{6,7\alpha}$), 2.17 (s, 3H, NCH_3), 2.98 (brs, 2H, $H_{1,5}$), 3.62 (brs, 1H, exch), 3.91 (t, J = 4.9 Hz, 1H, $H_{3\beta}$).

δ_c (63 MHz, $CDCl_3$): 24.4 (1:1:1 t, 2 x CHD, $C_{6,7}$), 39.3 (2 x CH_2, $C_{2,4}$), 40.5 (CH_3), 60.6 (2 x NCH), 63.9 (COH).

ν_{max} (CH_2Cl_2): 3680brw, 3610m, 2970m, 2940s, 1460w, 1420m, 1340w, 1320w, 1260brm, 1120w, 1085m, 1050m, 1015m, 950w, 920w, 815w, 785w, 700vbrm cm^{-1}.

^{m}/z (%): 143 (M^+, 52), 126 (48), 113 (33), 98 (63), 84 (100), 77 (3), 68 (17), 57 (30). $C_8H_{13}D_2NO$ [M^+] requires ^{m}/z 143.1279; observed ^{m}/z 143.1279.

(-)-6,7-d_2-tropinone (243)

A solution of **(242)** (140 mg, 0.98 mmol) in acetone (15 ml) was stirred with TFA (90 µl, 1.18 mmol) at room temperature for 15 min. This solution was then titrated with Jones reagent until a permanent orange tinge was obtained. After 40 min the excess oxidant was destroyed by the dropwise addition of isopropyl alcohol at 0°C. The solution was basified with 1 M sodium hydroxide, and filtered though celite and the inorganic solids washed with chloroform (2 x 10 ml). The bulk of the solvent was evaporated and the residue partitioned between chloroform (15 ml) and water (2 ml). The aqueous layer was further extracted with chloroform (2 x 10 ml). The combined organic layers were dried over anhydrous magnesium sulphate, filtered and the solvent removed using a rotary evaporator, to yield **(243)** as pale yellow oil (52 mg, 36%). This was converted into a mixture of **(244)** and **(245)** without further purification.

δ_H (250MHz, $CDCl_3$): 1.58 (s, 2H, $H_{6,7\alpha}$), 2.20 (d, 16.0 Hz, 2H, $H_{2,4eq}$), 2.48 (s, 3H, NCH_3), 2.69 (dd, J = 16.0, 2.6 Hz, 2H, $H_{2,4ax}$), 3.43 (brs, 2H, $H_{1,5}$).

δ_c (63 MHz, $CDCl_3$): 27.6 (1:1:1 t, CHD), 27.9 (1:1:1 t, CHD), 38.7 (CH_3), 47.9 (2 x CH_2, $C_{2,4}$), 61.1 (2 x NCH), 210.0 (C=O).

ν_{max} (CH_2Cl_2): 2940s, 2860m, 1710s, 1455m, 1410brm, 1355m, 1225brm, 1205m, 1140w, 1110m, 1000brm, 890w, 700vbrm cm^{-1}.

$^m/z$ (%): 141 (M^+, 32), 125 (7), 110 (13), 98 (31), 91 (1), 83 (100), 77(3), 69 (12), 59 (62), 56 (22), 51 (8). $C_8H_{12}D_2NO$ [M^+] requires $^m/z$ 141.1279; observed $^m/z$ 141.1279.

(-)-6,7-d_2-tropinone [N-^{18}O]-N-oxides (244) and (245)

To a solution of **(243)** in ethanol (28 µl) was added ^{18}O-labelled hydrogen peroxide solution (0.5%, 100% ^{18}O). The reaction was stirred at 75°C for 3 hr. On cooling, the solution was stirred with Pd/C (5%) for 1 hr. The reaction mixture was then filtered though celite, which was washed thoroughly with ethanol. Evaporation of the solvent using a rotary evaporator yielded a crude yellow oil, which was triturated with petroleum ether (b.p. 60 - 80°C) to yield **(244)** and **(245)** (ratio 9:1, as calculated from ^{1}H NMR signal integrations) as a white solid (12 mg, 39%).

δ_H (250MHz, $CDCl_3$): 2.09 (s, 2H, $H_{6,7\alpha}$), 2.26 (d, J = 16.9 Hz, 2H, $H_{2,4eq}$), 3.50 (s, 3H, NCH_3), 3.78 (brdd, J = 16.9, 4.4 Hz, 2H, $H_{2,4ax}$), 3.99 (brd, J = 4.4 Hz, 2H, $H_{1,5}$).

$^m/z$ (%): 159 (M^+, 7), 141 (7), 126 (9), 111 (100), 98 (10), 83 (44), 69 (10), 56 (13). $C_8H_{11}D_2N^{18}O^{16}O$ (M^+) requires $^m/z$ 159.1114; observed $^m/z$ 159.1114.

Appendix

APPENDIX

Nicotinic receptor binding assay (courtesy of Dr S.R. Fletcher, Merck, Sharp and Dohme)

Displacement of [^{3}H](-)-nicotine binding was determined using a modification of the method described by Romano and Goldstein.[105] Whole rat brains were homogenised in 1:10 (wet w/v) of 20 mM HEPES buffer, pH 7.4, and centrifuged at 17,500 g for 30 mins. The pellet was resuspended in ice-cold distilled water 1:20 (wet w/v) and incubated on ice for 1 hr. This suspension was centrifuged at 17,500 g for 30 min, and the pellet resuspended in ten volumes of buffer. After a final centrifugation (17,500 g for 30 mins) the pellet was resuspended in buffer at a concentration of 15 mg ml^{-1}.

Binding assays were conducted in polypropylene tubes containing: 100 ml of [^{3}H](-)-nicotine (final concentration 10 nM), 10 ml of the displacing compound and 390 ml of buffer. Non specific binding was defined by incorporation of 10 ml of carachol (final concentration 1 mM). The reaction was initiated by adding 500 ml of the membrane suspension with vortex mixing. Samples were incubated for 60 min at 30°C. The reaction was terminated by filtration over filters pre-soaked in 0.05% polyethylamine followed by washing with 10 ml of ice-cold saline. The radioactivity of filters was estimated by liquid scintillation spectroscopy.

Data from binding assays were subjected to non-linear least squares regression analysis using RSI (BNN Research Systems, Cambridge, Ma, USA) and a computerised iterative procedure written by Dr A. Richardson, Neuroscience Research Centre, Terlings Park.

Example of hot-plate assay for antinociceptive activity (mouse tail-flick test) (taken from Daly *et al.*[62])

Adult male NIH Swiss strain mice, weighing 25-30 g were used. All drugs were dissolved in a 20 : 80 v/v mixture of Emulphor EL-620 (Rhône-Poulenc, Cranbury, NJ, USA) and 0.9% saline solution and administered intraperitonaeally in a volume corresponding to 5ml/kg body weight. In the hot-plate antinociceptive assay mice were placed on a metal plate heated to 55 - 56°C, enclosed by a glass cylinder. Time

to the appearance of the first sign of pain (flicking of the tail, licking or shaking of the hind paw, jumping or climbing the sides of the cylinder) was measured. The reaction time for each mouse without drug was determined twice. Each mouse was then injected intraperitoneally with the test agent and the reaction time of each mouse was again determined. Mice were used only once for each test agent.

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