Synthesis of analogues of the norditerpenoid alkaloid methyllycaconitine: a selective nicotinic acetylcholine receptor antagonist

Trigg, William John

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SYNTHESIS OF ANALOGUES OF THE NORDITERPENOID ALKALOID METHYLLYCACONITINE: A SELECTIVE NICOTINIC ACETYLCHOLINE RECEPTOR ANTAGONIST

submitted by William John Trigg B.Sc.

for the degree of Ph.D.

of the University of Bath

1997

Signed: .....

ProQuest LLC
789 East Eisenhower Parkway
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Abstract

In this thesis, an investigation into the synthesis of novel analogues of the norditerpenoid alkaloid methyllycaconitine (MLA) is described. Analogues were designed and synthesised which contain parts of the carbon skeleton, along with some of the oxygenation pattern present in the norditerpenoid skeleton. Structure-activity relationship studies, using the prepared analogues, were undertaken.

Chapter 1 gives an introduction to the norditerpenoid alkaloids covering their biological activity, isolation and nomenclature. Chapter 1 also includes a review of recent synthetic routes to the hexacyclic ring system found within these norditerpenoids. Chapter 2 describes a dianion alkylation approach to the synthesis of C10-C11 substituted AE-bicyclic analogues of lycoctonine, inuline and MLA. γ-Substituted β-ketoesters were synthesised and used in a double Mannich reaction to yield 3-azabicyclo[3.3.1]nonanones which mimic the AE bicycle of the norditerpenoids. In chapter 3, a study of the application of an ester migration to the synthesis of novel γ-substituted β-ketoesters for use in a double Mannich reaction is discussed. Investigation into the Michael reaction of 1-carbethoxycyclohexan-2-one and subsequent ester migration is also described. Chapter 4 describes an acetylide addition strategy to the synthesis of C6-C5 substituted AE-bicyclic analogues of MLA. A bicyclic ketoester was linked to substituted cyclohexanone using acetylene as a two carbon fragment. Chapter 5 is a study of the attempted dehydroxylation of a bicyclic propargylic alcohol which led, *via* an unusual rearrangement, to the formation of a substituted dihydropyridinone. Chapter 6 explains the design and synthesis of small molecule analogues of lycoctonine which contain the C1-methoxy present in the norditerpenoid hexacycle. This employed a tandem Michael addition-aldol reaction to form an hydroxy-substituted [3.3.1]azabicycle.

To My Family

ACKNOWLEDGEMENTS

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And finally I would like to thank all of the friends that I have made at the University of Bath. Within the Department of Pharmacy and Pharmacology I would especially like to thank the "great mates"; Gareth Adlam, Graham Kay, Martyn "Sparky" Clarke, Stuart Carter and Chris Ward, and all of the people with whom I have enjoyed working and socialising. I would also like to thank all of the friends that I made within the University of Bath Hockey Club, especially Alexi, Zorba and Litch, with whom I enjoyed three years of great hockey and friendship.

Abbreviations

The following abbreviations have been used in this thesis:

Ac	acetyl
AcOH	acetic acid
bp	boiling point
n-BuLi	*n*-butyl lithium
CI	chemical ionisation
CMR	^{13}C nuclear magnetic resonance
COSY	correlated spectroscopy
d	doublet
DBU	1,8-diazobicylo[5.4.0]undec-7-ene
dd	doublet of doublets
DCM	dichloromethane
DMAP	4-dimethylaminopyridine
DMSO	dimethyl sulphoxide
dt	doublet of triplets
DMF	*N*,*N*-dimethylformamide
DMPU	1,3-dimethyl-3,4,5,6-tetrahydo-2(1H)-pyrimidinone
EI	electron impact
Et	ethyl
FAB	fast atom bombardment
GC	gas chromatography
h	hours
HMPA	hexamethylphosphoric triamide
IR	infrared

IC_{50}	median inhibitory concentration
J	coupling constant
K_i	inhibition constant
LAH	lithium aluminium hydride
LDA	lithium diisopropylamide
m	multiplet
$[M]^+$	molecular ion
Me	methyl
$[MH]^+$	protonated molecular ion
min	minutes
ml	millilitres
mmol	millimoles (10^{-3} moles)
MS	mass spectrometry
MW	relative molecular weight
m/z	mass by charge ratio
nAChR	nicotinic acetylcholine receptor
NMR	nuclear magnetic resonance
Ph	phenyl
PMR	^1H nuclear magnetic resonance
ppm	parts per million
*p*TSA	*para*-toluenesulphonic acid
q	quartet
RT	room temperature
t	triplet
THF	tetrahydofuran
tlc	thin layer chromatography

TMS	tetramethylsilane
UV	ultraviolet
$^{w}/_{w}$	weight by weight
$^{v}/_{v}$	volume by volume
+ve	positive
-ve	negative
°C	degrees Celsius

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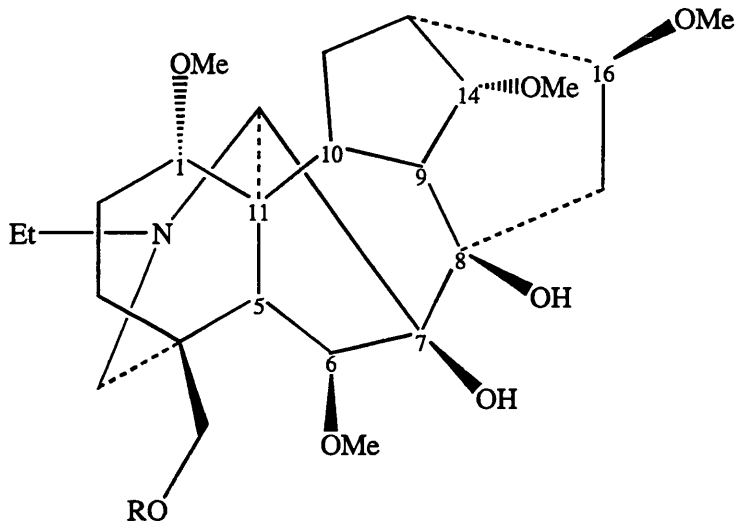
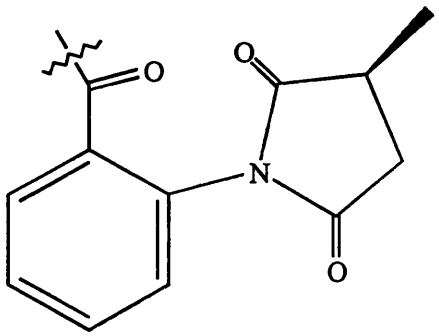
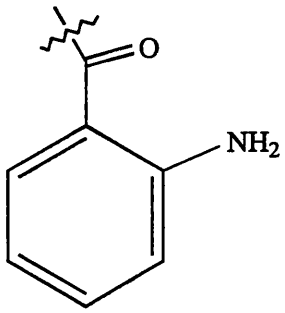
Chapter 1

INTRODUCTION

1.1 Aims

The aims of these studies were to synthesise novel analogues of the norditerpenoid alkaloid methyllycaconitine (MLA) **1** which is a highly potent selective nicotinic acetylcholine receptor (nAChR) antagonist, in order to perform structure-activity relationship (SAR) studies.



R =  R =  R = H

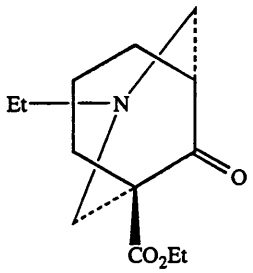
MLA **1** inuline **2** lycoctonine **3**

There is literature precedent for the rapid and efficient conversion of the neopentyl-like alcohol lycoctonine **3** firstly into inuline **2** and then into MLA **1** (Coates *et al.* 1994b). By preparing small molecule analogues of lycoctonine **3**, we have been able to use this literature protocol to synthesise novel analogues of inuline **2** and MLA **1**.

We aimed to synthesise C10-C11 substituted AE-bicyclic analogues of lycoctonine **3** *via* a dianion alkylation of a cyclic β-ketoester followed by a double

Mannich reaction to form a [3.3.1]azabicyclononane which mimics the AE-bicyclic unit of the hexacyclic norditerpenoid ring system.

We envisaged synthesising C5-C6 substituted AE-bicyclic analogues of MLA **1** using a variety of carbon nucleophile strategies to attack regioselectively the ketone functional group of bicyclic ketoester **4**.



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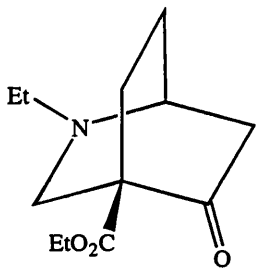
Using this strategy, we aimed to synthesise analogues of MLA **1** containing the D-ring of the carbon skeleton and the oxygen functions at C16/C14 and C8 of MLA **1**.

We wanted to synthesise small molecule analogues of lycoctonine **3** which contained the methoxy group at C1 in the norditerpenoid skeleton. We intended to do this by using a double Mannich reaction on a suitably substituted β-ketoester, but this strategy led to the formation of a [2.2.2]azabicyclooctane **5** rather than the desired [3.3.1]bicycle **6**. As an alternative strategy we aimed to employ a tandem Michael addition-aldol condensation strategy to synthesise the desired [3.3.1] compound **6**.



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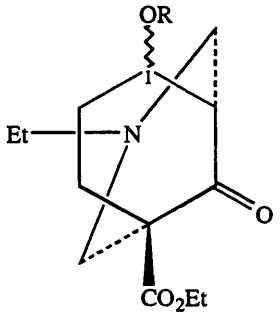

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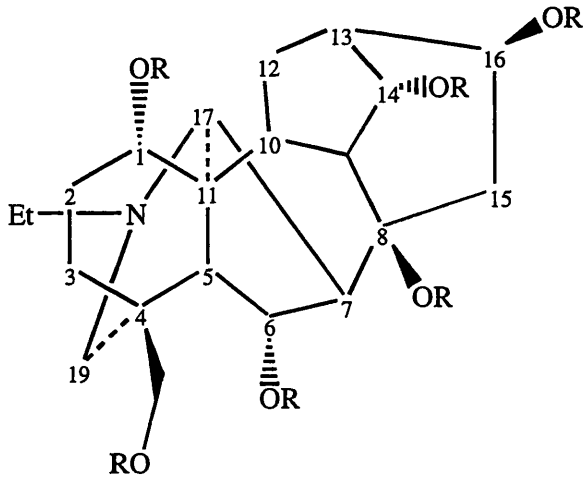
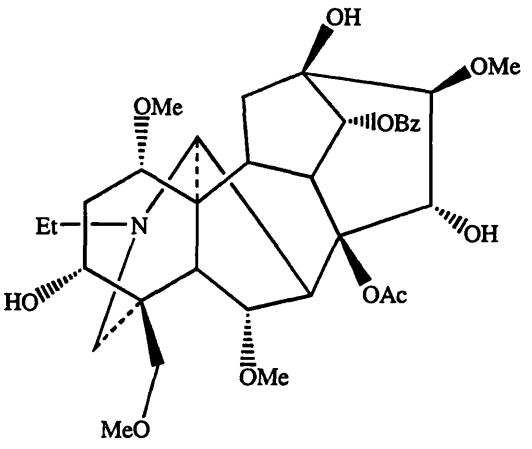
1.2 NORDITERPENOID ALKALOIDS

There are many norditerpenoid alkaloids isolated from *Delphinium, Consolida, Aconitum and Inula* species (Benn and Jacyno 1983; Pelletier *et al.* 1984; Pelletier and Joshi 1991; Yunusov 1991; Yunusov 1993). These alkaloids exhibit a range of interesting biological effects mainly involved with either nAChR or voltage gated sodium channels. Norditerpenoid alkaloids are C_{19} hexacyclic alkaloids (as opposed to the significantly less toxic C_{20} diterpenoid alkaloids) with a highly elaborate system of oxygen functionalisation. Norditerpenoid alkaloids have trivial names which are generally derived from the name of the plant from which the alkaloid was first isolated. There are literally hundreds of alkaloids which have been isolated (some of which may be artefacts of their extraction process) and so some form of classification system is appropriate. The recent work of Hanuman and Katz (1994) on the spectroscopy of the norditerpenoid alkaloids classifies the norditerpenoid alkaloids into 6 types depending on the oxygenation pattern around the hexacycle. The basic norditerpenoid alkaloid is considered to be oxygenated at C-1, C-6, C-8, C-14 and C-16 and any additional oxygenation is used to provide the classification system.



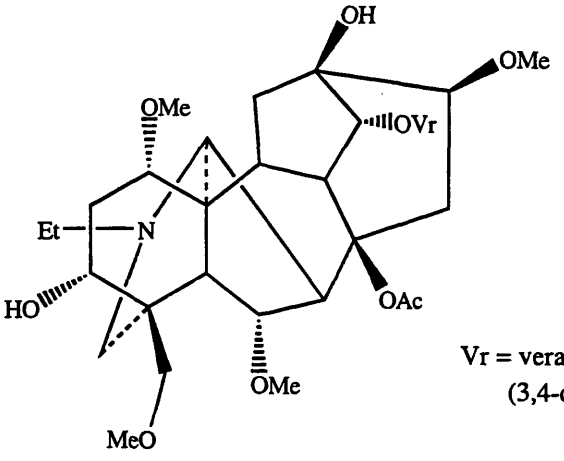
Basic norditerpenoid

I *Aconitine type:* additional hydroxyls at C-3, C-13 and C-15



aconitine **7**

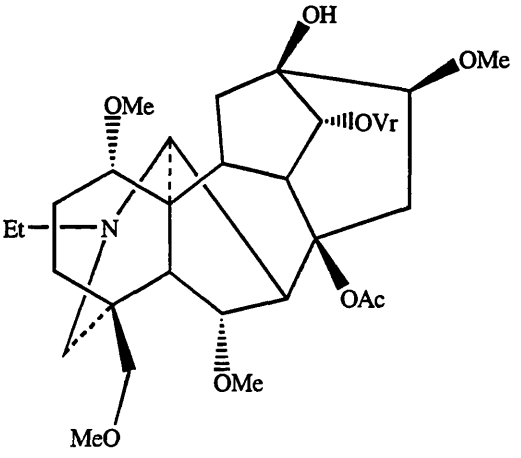
II *Pseudaconitine type:* additional hydroxyls at C-3 and C-13



Vr = veratroyl
(3,4-dimethoxybenzoyl)

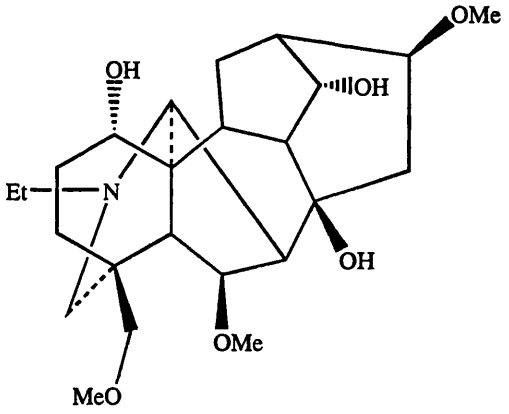
pseudaconitine **8**

III *Bikhaconitine type:* additional hydroxyl C-13



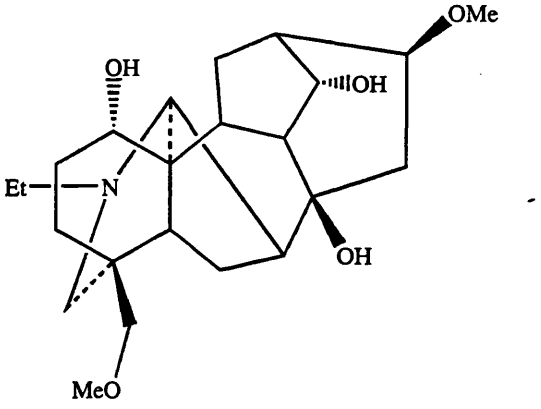
Bikhaconitine **9**

IV *Neoline type:* generally no additional oxygenation at C-3, C-13 and C-15



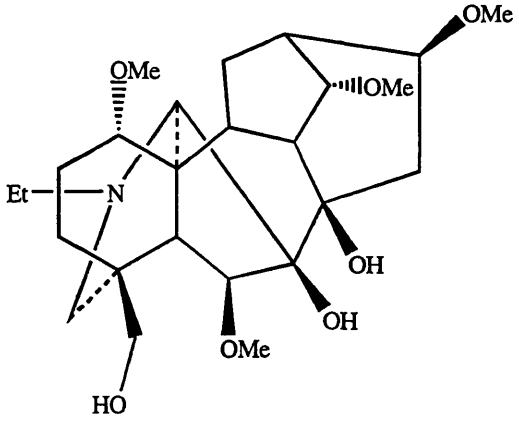
neoline **10**

V *Isotalatizidine type:* no oxygenation at C-3, C-6, C-13 or C-15



isotalatizidine **11**

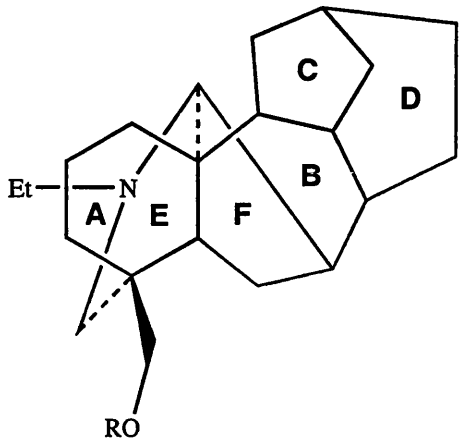
VI *Lycoctonine type:* oxygenation at C-6 and C-7 is common, but no oxygenation at C-3, C-13 or C-15



Lycoctonine **3**

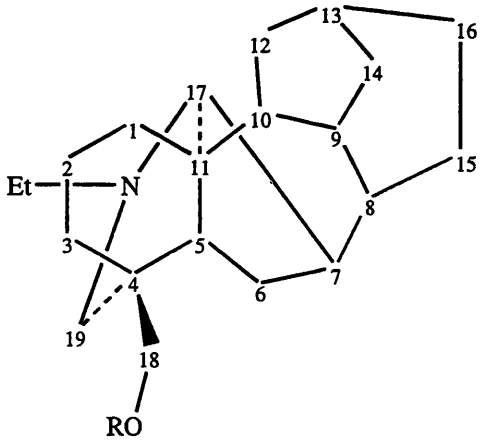
1.2a Numbering of atoms and rings

The hexacyclic ring system of the norditerpenoid alkaloids is labelled alphabetically from left to right and from front to back as indicated below:



Ring labelled part structure **12**

The carbon atoms of the norditerpenoid alkaloids are numbered as follows:

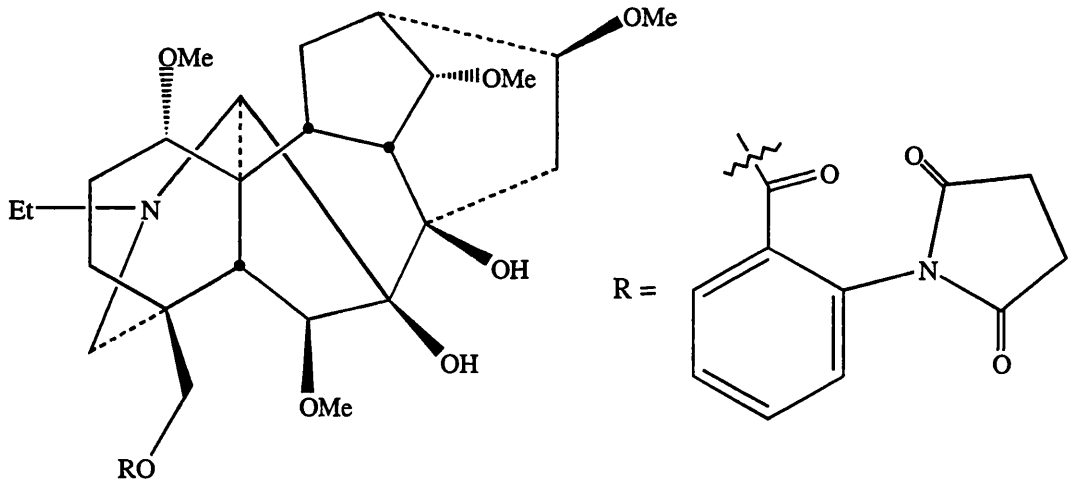


Atom labelled part structure **13**

Not all of the alkaloids have an *N*-ethyl group and there are some which have methyl or methoxyethyl substitution at the alkaloid amine. The 2-(methyl-succinimido)benzoate ester moiety found in MLA **1** is a relatively unusual substituent.

1.2b Biological activity of certain norditerpenoids

The norditerpenoid alkaloids display a range of biological activities. MLA **1** is a selective nicotinic acetylcholine receptor (nAChR) antagonist. The high affinity binding of MLA **1** to nAChRs depends upon the 2-(methylsuccinimido)benzoate ester function as the parent alcohol lycoctonine **3** has a considerably lower affinity for the receptor (Hardick *et al.* 1996). The 2-(methylsuccinimido)benzoate ester containing alkaloids have a significantly higher affinity and thus a higher toxicity (MLA **1** having an LD_{50} in mice of 7.5mg/kg compared to lycoctonine **3** which has an LD_{50} of 443.5mg/kg) (Manners *et al.* 1993; Manners *et al.* 1995). Not having the methyl group attached to the succinimide (as in lycaconitine **14**) reduces the affinity for certain nAChR by half as recently shown by Jacyno *et al.* (1996).

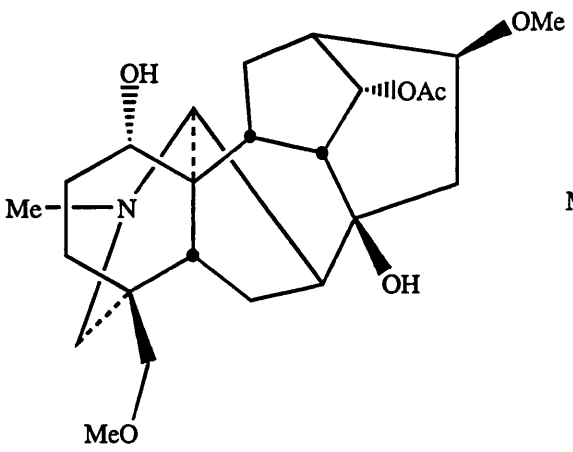


lycaconitine **14**

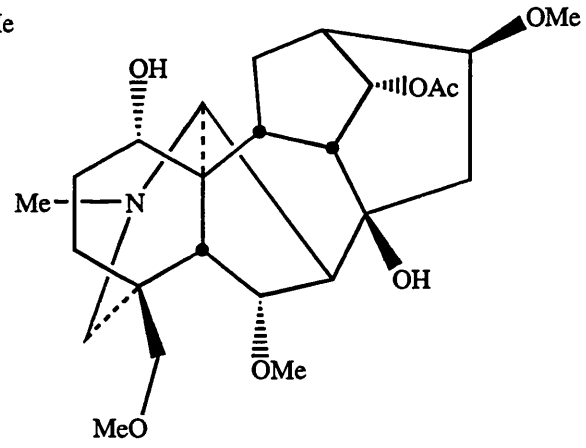
Simple anthranilic esters do not possess the same affinity with a reduction of around 3 orders of magnitude in nAChR affinity and subsequently the toxicity is also lower (inuline **2** having an LD_{50} of 20.8mg/kg) (Manners *et al.* 1995). In a study by Kukel and Jennings (Kukel and Jennings 1994) it was shown that by changing the nature of the ester (or by the lack of the ester) of the C-18 alcohol that selectivity for the insect receptor over the mammalian receptor can be increased to as much as 50,000:1 (insect :

rat neuronal). However, not all of the norditerpenoid alkaloids which have a high affinity for the nAChR do possess this moiety. Compounds such as condelphine **15**, neoline **10** and isotalatizidine **11** display activity at insect nAChRs in the nanomolar range without having the 2-(methylsuccinimido)benzoate ester function present (Wonnacott *et al.* 1994).

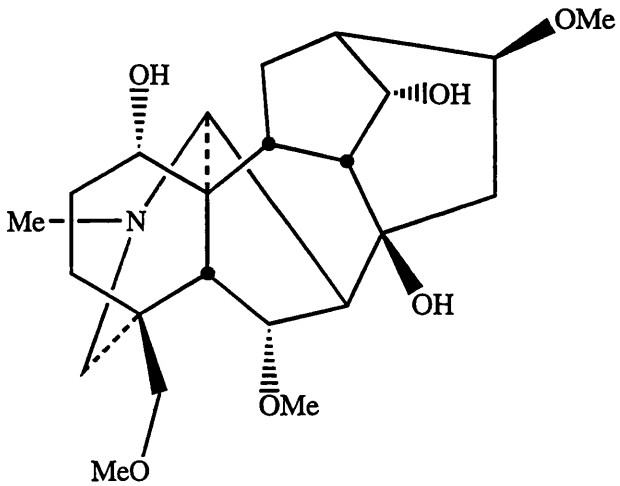
Changing the oxygenation pattern of the hexacycle also leads to a different spectrum of affinities for mammalian or insect nAChR. Compounds possessing a methylenedioxy bridge between C-7 and C-8 showed a higher affinity for the insect receptor than for the mammalian receptor (Kukel and Jennings 1994; Hardick *et al.* 1996).



condelphine **15**

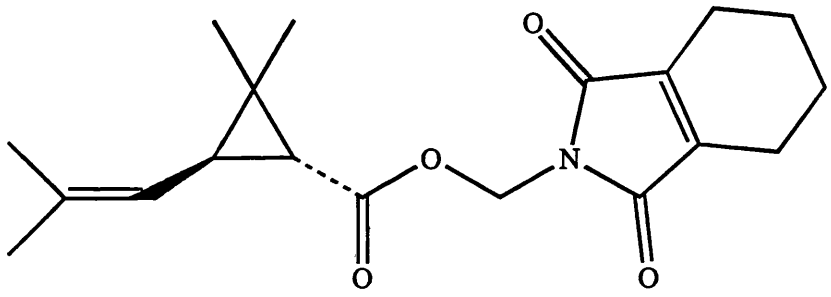


neoline **10**

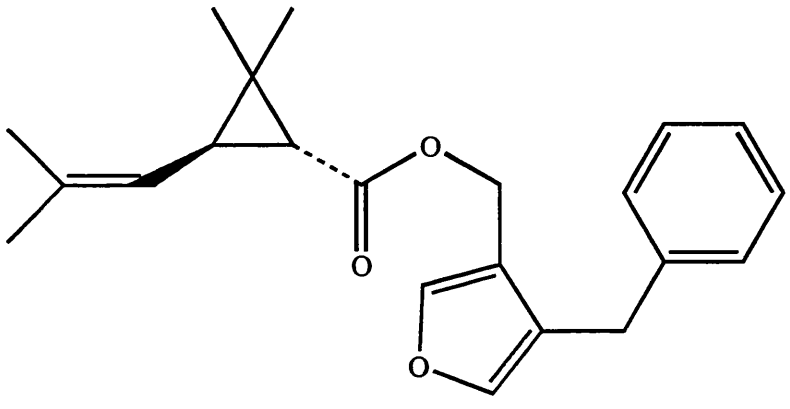


isotalatizidine **11**

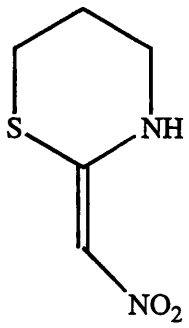
Aconitine **7** acts at voltage-gated sodium channels as do other toxins such as tetrodotoxin (isolated from puffer fish), saxitoxin (isolated from "red tide" plankton) and the steroidal alkaloid batrachotoxin (isolated from the Colombian arrow-poison frog *Phyllobates aurotaenia*) (Voet and Voet 1990; Cestele *et al.* 1996; Qu *et al.* 1995). When aconitine **7** is bound to the protein, the pore (channel) is locked in an open conformation once it has been opened by the passage of an action potential. This is also the mechanism of action of pyrethroid insecticides such as tetramethrin **16a** and bioresmethrin **16b** which are semi-synthetic compounds based upon the pyrethrins isolated from the *Chrysanthemum* species (George and Kalyanasundaram 1994). The nitromethylene insecticides (Rozen and Margulies 1991) also work by this mechanism (e.g. tetrahydro-2-(nitromethylene)-2*H*-1,3-thiazine (NMH) **17a** and imidacloprid **17b**).



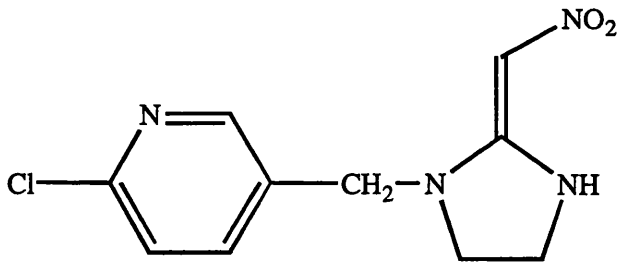
Tetramethrin **16a**



Bioresmethrin **16b**



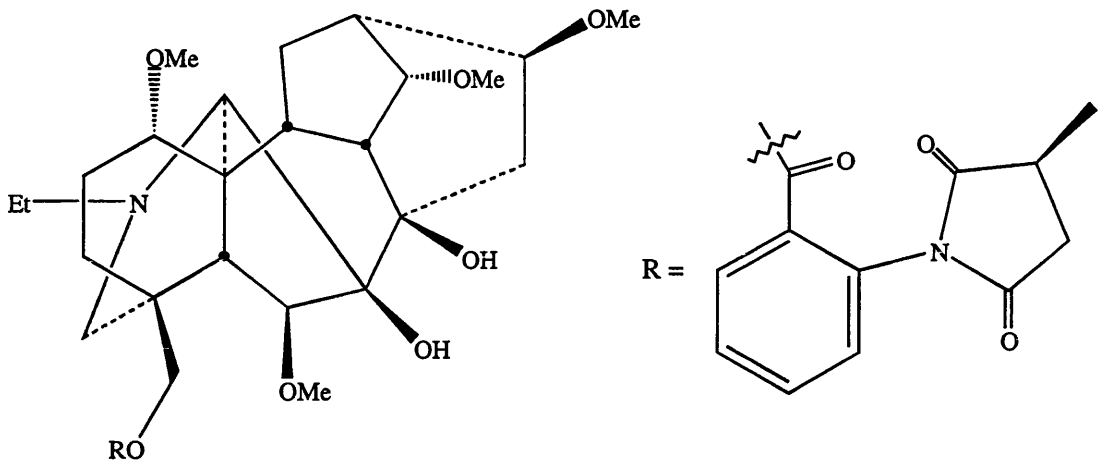
NMH **17a**



Imidacloprid **17b**

The voltage-gated sodium channel is present in all nerve axons and opening facilitates the entry of sodium ions into the intracellular space and subsequent depolarization of the nerve. By preventing closure of the voltage dependent sodium channel, aconitine **7** causes a blockade of the nerve signal and hence paralysis. Important structural differences between nAChR active alkaloids and aconitine **7** include the addition of C3 hydroxyl, acetate and benzoate esters, and these probably play important roles in aconitine binding. That the different oxygen substitution pattern does not prevent binding to nAChR was shown by the synthesis of an MLA-aconitine hybrid by Blagbrough and co-workers (Hardick *et al.* 1994).

1.3 Methyllycaconitine: a general introduction



MLA **1**

MLA **1** is a norditerpenoid alkaloid of the lycoctonine type which occurs in many *Delphinium* species as well as *Consolida ambigua* and *Inula royaleana* (Pelletier *et al.* 1984; Pelletier and Joshi 1991; Yunusov 1991 and 1993). MLA **1** is one of hundreds of norditerpenoid alkaloids which can be isolated from the *Delphinium, Inula, Consolida* and *Aconitum* species. These plants, especially the *Delphinium* species, are known to possess nervous system toxicity to mammals and a wide variety of insect species. The insecticidal property of *D. staphisagria* extracts (which contain neoline **10** and related norditerpenoids, but possibly not MLA) was exploited as a herbal treatment for lice infestations of the hair and body as reported by Pliny the Elder (Plinius AD77) and a preparation of *Delphinium* seeds was standard issue in the British army until the 19th century. *Delphinium* ingestion (and hence MLA **1**) causes the death of more grazing cattle than any other poisonous plant in north-west American ranges (as there are significant numbers of wild larkspur species on the grasslands of North America) (Keeler *et al.* 1975; Nambi Aiyar *et al.* 1979). The insecticidal and mammalian toxic effects have been shown to be a result of MLA **1** acting as a selective, competitive nAChR antagonist (Jennings and Brown 1986; Ward *et al.* 1990; Manners *et al.* 1995).

MLA **1** was first isolated and named in 1938 by Manske (1938) from the aerial parts of *Delphinium brownii.* MLA **1** was shown by Manske, and also by Goodson, to be the 2-(methylsuccinimido)benzoate ester of lycoctonine **3** (Manske 1938; Goodson 1943). Jennings and Brown first reported the insecticidal properties of MLA **1** (Jennings *et al.* 1986; Jennings *et al.* 1987). Benn and colleagues have worked on the isolation of MLA **1** and many other alkaloids from *Delphinium* and other species (Majak *et al.* 1987). The norditerpenoid alkaloids have been isolated from a wide range of plants which occur throughout the northern hemisphere. MLA **1** can be isolated from many *Delphinium* and *Consolida* species as well as from *Inula royaleana.* There have been several reported protocols for the rapid and efficient isolation and purification of MLA **1** from the seeds and aerial parts of a variety of plant species. Blagbrough and co-workers (Coates *et al.* 1994a) have reported an efficient extraction of MLA **1** from garden hybrid *Delphinium* seeds and the subsequent purification of the crude alkaloid mixture by liquid chromatography (yielding 439mg of pure MLA **1** from 600g of seeds). Benn and co-workers (Majak *et al.* 1987) have taken a wild low larkspur (*Delphinium nuttallianum*) from the plains of British Columbia and extracted a freeze-dried sample of whole plant which yielded a significant amount of MLA **1** (70% of the total alkaloid extracted (0.15% of the dry weight of plant matter)). This work shows that MLA **1** is present in a significant amount in wild species and is present in large enough quantities to present a significant risk to grazing cattle.

Insecticidal modes of action which are based upon nAChR as a target involve either over-stimulation (agonism) e.g. nicotine, or antagonism (competitive or non-competitive, allosteric or channel blocking). An alternative mode of insecticidal action is to increase the constitutive agonist acetylcholine by inhibiting the enzyme acetylcholine esterase (which deactivates ACh at the synapse, by cleaving the ester) with a compound such as an organophosphate insecticide. The nAChR neurotransmission pathway is a

well established target for insecticidal compounds and research into affecting this pathway is ongoing in the agrochemicals industry. MLA **1** exhibits a high level of insecticidal activity, acting by blocking insect nAChR. Making small molecule analogues will allow SAR studies to be carried out to ascertain which parts of the diterpenoid skeleton and its oxygenation pattern are required to give high affinity for particular nAChR subtypes and high selectivity for insect over mammalian nAChR. The benefits of this would be that the norditerpenoid pharmacophore would be better understood and this may lead to the design and discovery of more selective compounds which could be used as insecticides with significantly reduced mammalian toxicity.

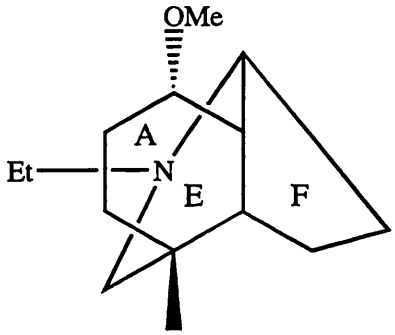
The nicotinic pathway has been the target of few therapeutic compounds other than in the use of agonists and cholinesterase inhibitors in the treatment of Myasthenia gravis (Katzung 1989) and the use of antagonists as neuromuscular blocking drugs as muscle relaxants in surgery (Katzung 1989). This is because nAChR is so important and widespread within mammalian physiology. Only highly selective drugs could be used as interference with autonomic and muscular function would occur using compounds which were not selective. The need for selective compounds which distinguish between the different types of receptor is an obvious requirement for further investigation of the mammalian nervous system. More recently, nAChRs have been identified as playing a pivotal role in some neurodegenerative disease states and so pharmaceutical interest into nAChR has also increased (Wonnacott *et al.* 1993). As MLA **1** has been shown to be a highly selective compound for neuronal nAChR, analogues of MLA **1** which display slightly different pharmacological profiles would be useful tools in the research into neurodegeneration (Wonnacott *et al.* 1993).

1.4 Literature survey

1.4a Synthesis of the norditerpenoid skeleton

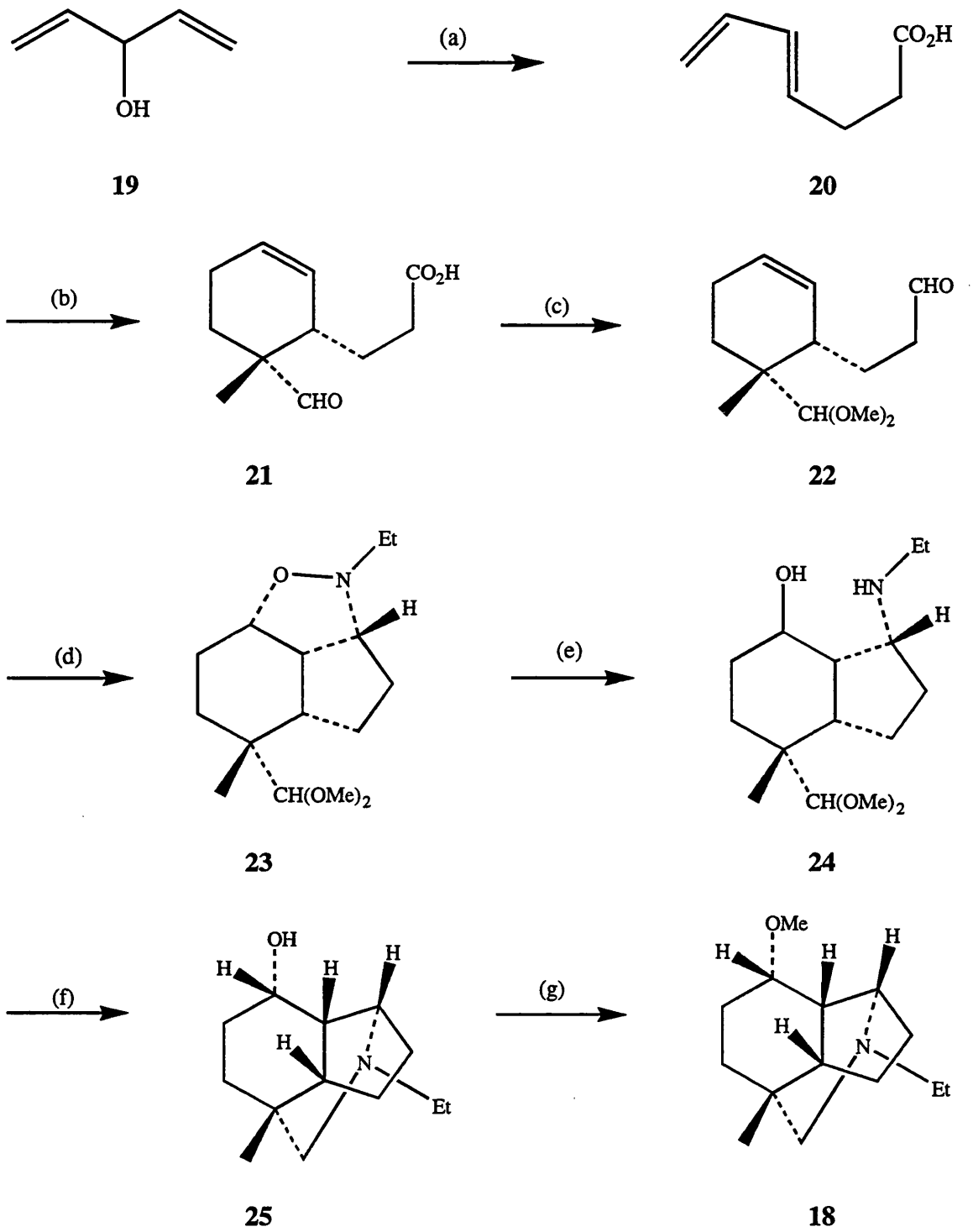
There have been remarkably few attempts at the total synthesis of norditerpenoid alkaloids. The main body of synthetic work has concerned the synthesis of parts of the norditerpenoid ring system whilst others have completed semi-synthetic studies to produce novel hybrid alkaloids as well as a variety of different esters and ether conjugates of the norditerpenoid alkaloids.

The elegant work of Whiting and co-workers (Baillie *et al.* 1994) on the synthesis of an AEF tricyclic section of the norditerpenoid skeleton involves a multi-step asymmetric synthesis of the nitrogen containing tricycle **18**.



18

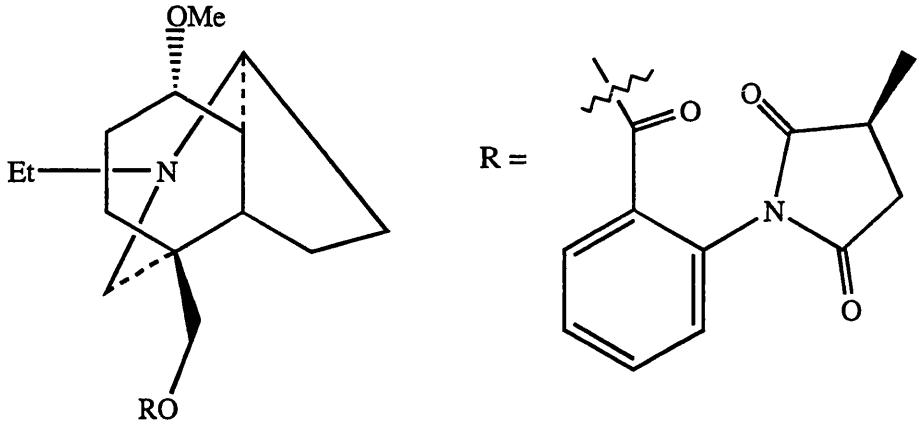
The key step in this synthesis is a nitrone 1,3-dipolar cycloaddition to form the A- and F-rings of the norditerpenoid skeleton. This work has the advantage of being stereospecific and also introduces the oxygenation at C-1 of MLA **1**, but does not possess the C-18 oxygen which is required for the addition of the 2-(methylsuccinimido)benzoate ester function of MLA **1**. Without this moiety, which is apparently important for high affinity for the nAChR and hence antagonism of the receptor, the AEF tricycle **18** would probably not be active.



(a) i. $MeC(OEt)_3$, $EtCO_2H$, 142°C ii. KOH, MeOH (b) CH_2=C(Me)CHO, H_2O, $NaHCO_3$, RT (c) i. $HC(OMe)_3$, MeOH, pTSA ii. DIBAL, toluene, -80°C (d) EtNHOH•TFA, Et_3N, benzene, reflux (e) $NiCl_2$•$6H_2O$, $NaBH_4$, MeOH (f) i. 5M HCl ii. pH 5.5 buffer iii. $NaCNBH_3$ (g) NaH, MeI, THF

In a subsequent publication (Baillie *et al.* 1997) this synthesis was modified to allow the incorporation of the 2-(methylsuccinimido)benzoate ester yielding compound

26. The modification was made by simply replacing the methyl group present with an acetyl protected hydroxyl which when deprotected yields the desired C18 hydroxyl (norditerpenoid numbering). This was converted into the homochiral 2-(methylsuccinimido)benzoate ester.



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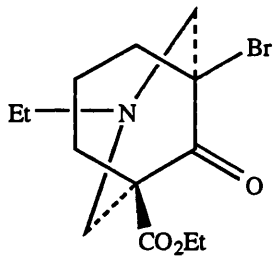
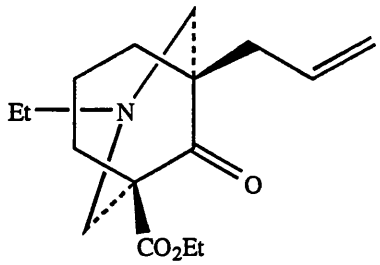
The work of Kraus *et al.* (1993) on the synthesis of the norditerpenoid alkaloid carbon skeleton involves, as the key step, the alkylation of a bridgehead radical (Kraus and Shi 1990; Kraus and Shi 1991). Using this radical strategy, Kraus and co-workers have been able to synthesise an ABDE tetracyclic system which includes some oxygenation (not necessarily in the correct regiochemistry as MLA **1**), notably the functionality at a position equivalent to C-18 of MLA **1** which would allow the introduction of the 2-(methylsuccinimido)benzoate ester. Using a radical alkylation of γ-bromo bicyclic β-ketoester **27** to give **28** is the key step. Ozonolysis of **28** yielded an aldehyde which was subjected to immediate Wittig reaction to give enone **30** (after acid catalysed elimination of the secondary alcohol from intermediate ketal **29**). Enone **30** then acted as the dieneophile in a Diels-Alder reaction which yields **31** (introducing the 6 membered D-ring). The final step of the synthesis was the closure of the seven membered B-ring by an intramolecular aldol reaction with potassium bis(trimethylsilyl)amide as the base to give **32**. Compound **32** could have been reduced

to give the neopentyl type alcohol instead of the ethyl ester and this would allow the incorporation of the 2-(methylsuccinimido)benzoate ester.



27

(a)



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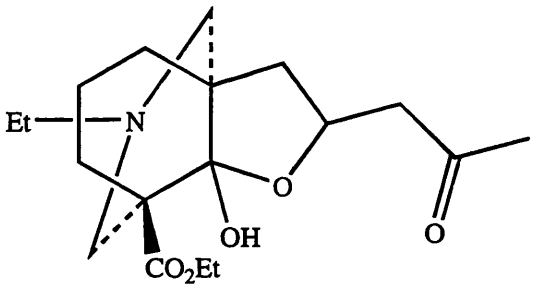
(b)



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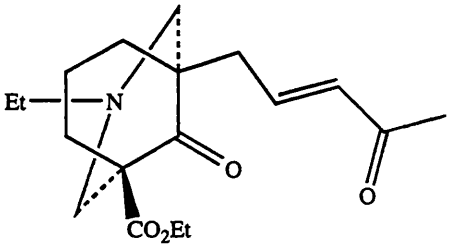
(c)



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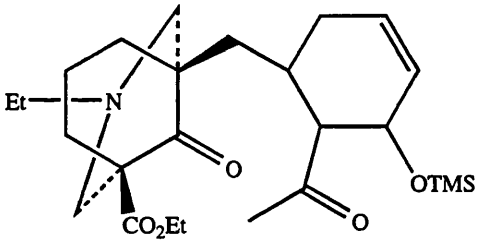
(d)



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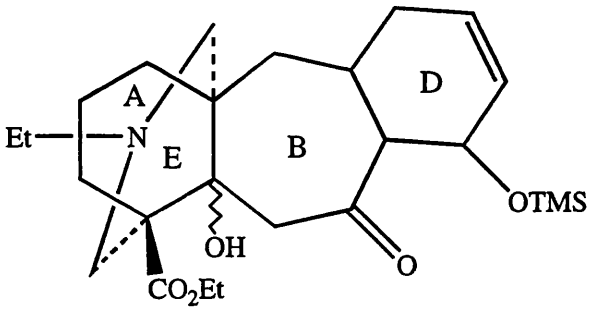
(e)

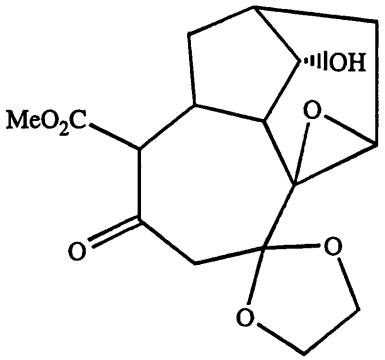


32

(a) allytributyltin, AIBN (b) i. O_3, ii. $Ph_3P{=}CHCOMe$ (c) H_2SO_4

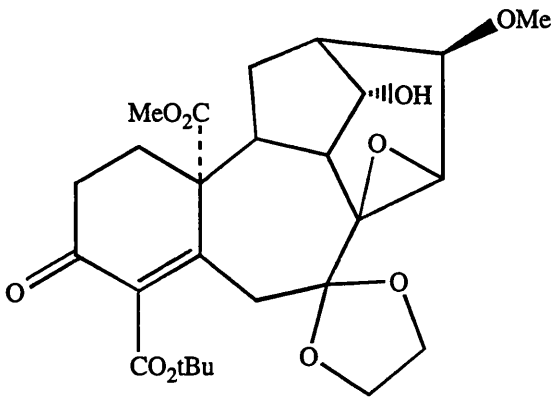
(d) $CH_2{=}CHCH{=}CHOTMS$ (e) $(Me_3Si)_2NK$

The work of van der Baan and van Beek (van der Baan *et al.* 1986; van Beek *et al.* 1992) on the synthesis of the norditerpenoid skeleton provided an elegant synthesis. They have concentrated on the synthesis of the front four rings of MLA **1** and have synthesised the BCD and ABCD ring systems. Initially, in their 1986 publication, the tricarbocyclic system **33** was produced by a ring expansion reaction of a five membered enamino-ester with an acetylenic ester.



33

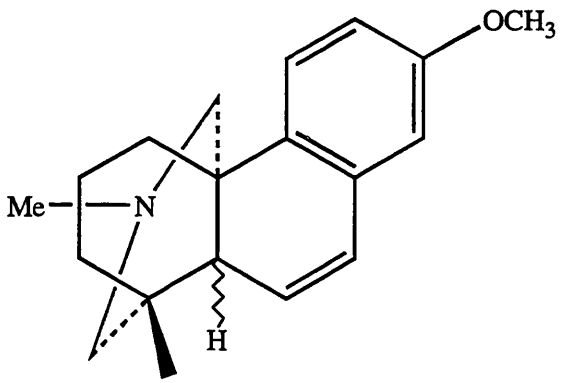
Then in 1992, conversion of this compound into an epoxide substituted tetracyclic ABCD ring system **34** was achieved with a Michael addition of the β-ketoester followed by intramolecular aldol condensation to form the A-ring. Incorporation of the C-16 methoxy was achieved by an allylic oxidation followed by reduction of the ketone formed and subsequent *O*-methylation (methyl iodide/silver oxide/calcium sulphate).



34

This work is an elegant and stereoselective synthesis which is in contrast to most other attempts at the synthesis of the norditerpenoid skeleton in that the direction of building the ring system starts from the eastern region of the molecule.

Shimizu and co-workers (Shimizu *et al.* 1963a and b; Ogiso *et al.* 1963a and b) have made a significant contribution to the area of norditerpenoid alkaloid synthesis, in that, as well as synthesising some interesting intermediates towards the synthesis of the diterpenoid skeleton, they were the first group to recognise the importance of a double Mannich reaction in the synthesis of the piperidine E-ring within the norditerpenoid skeleton. This has been an integral part to the approaches of both our synthetic studies on the norditerpenoid skeleton and those of Kraus and co-workers. In their approaches to the alkaloid synthesis, an acetylenic addition to the ketone of the azabicyclo[3.3.1]nonane formed by a double Mannich reaction is a key part of the strategy. Using this in combination with an acid catalysed electrophillic substitution gave tetracyclic compound **35**.



35

Fukumoto and co-workers performed a total synthesis of the diterpenoid *Aconitum* alkaloid (±)atisine **36** (Ihara *et al.* 1988) and also later produced a stereoselective synthesis based upon tandem Michael reactions and enzyme (porcine liver esterase, porcine pancreas lipase and *Candida cylindracea* lipase) mediated resolutions (Ihara *et al.* 1990a; Ihara *et al.* 1990b; Ihara *et al.* 1995). This is a hexacyclic ring

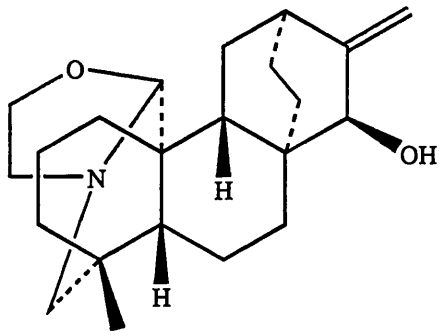
system, but it does not contain the exact ring system of the norditerpenoids of the lycoctonine class. Instead, it has a 5-membered oxazolidine ring connecting the alkaloid nitrogen to C-17 of the carbon skeleton. Their strategy has four main components:

a) double Mannich reaction to form the AE-bicycle,

b) enatioselective acylation catalyzed with lipase,

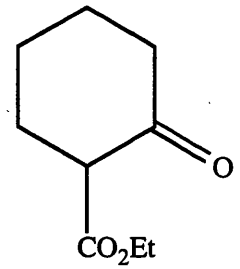
c) stereoselective hydroboration,

d) construction of the DE rings by intramolecular double Michael reactions closing 2 of the rings of atisine.



atisine **36**

In work by Blagbrough and co-workers (Coates *et al.* 1994b; Coates *et al.* 1996) the A and E rings of MLA **1** are mimicked by 3-azabicyclo[3.3.1]nonanes synthesised via a double Mannich reaction on ethyl 2-oxocyclohexane-1-carboxylate **37** to yield **4**. The ketone moiety of the azabicyclononanone produced by the double Mannich reaction was further reacted by firstly reducing to the secondary alcohol and then *O*-methylation to give **38**. This ketone was also used in a Wittig reaction to yield a mixture of enol ethers **39** which were then hydrogenated to yield the *O*-methyl ethers **40** (with the methoxy representing the functionality of C-6 in the norditerpenoid skeleton). LAH reduction of the ester yielded a series of neopentyl-like alcohols which can undergo regioselective anthranoylation with isatoic anhydride. Conversion of the aniline into the methylsuccinimide with methylsuccinic anhydride is a practical step.



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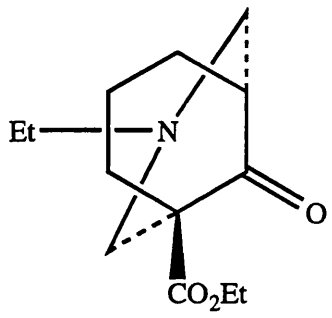

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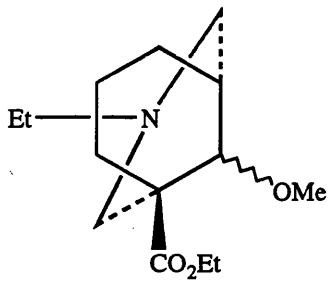

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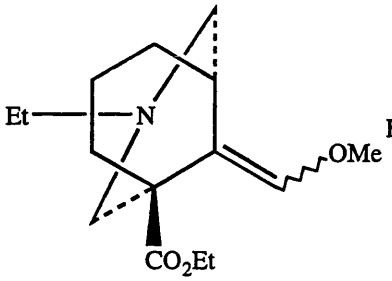

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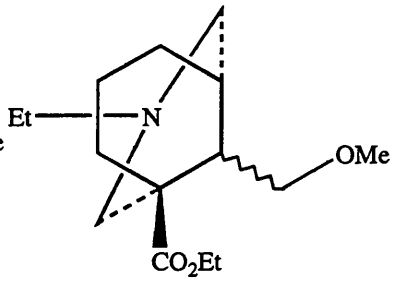
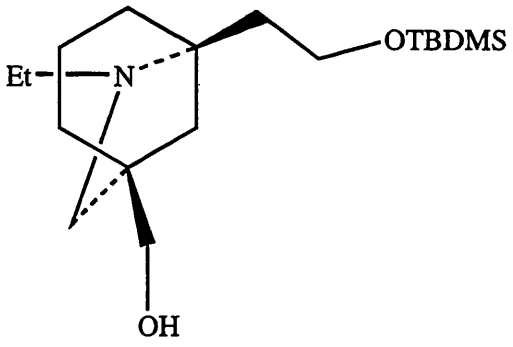


40

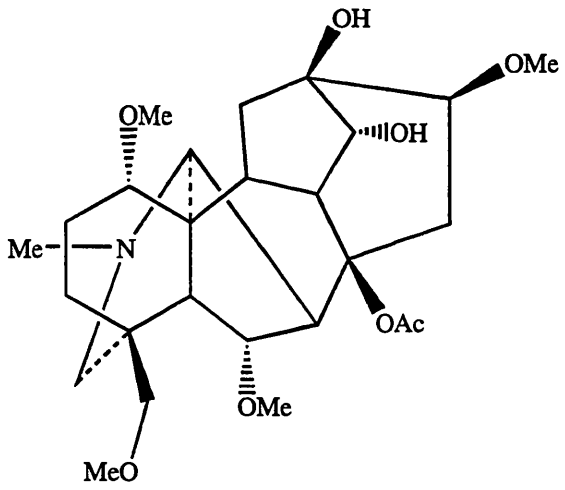
In their work, Potter and co-workers used a tandem Horner-Emmons olefination-conjugate addition to synthesise the 1,5-disubstituted-6-azabicyclo[3.2.1]octane **41** to investigate the effect of ring contraction on the antagonism of nAChRs (Callis *et al.* 1996).



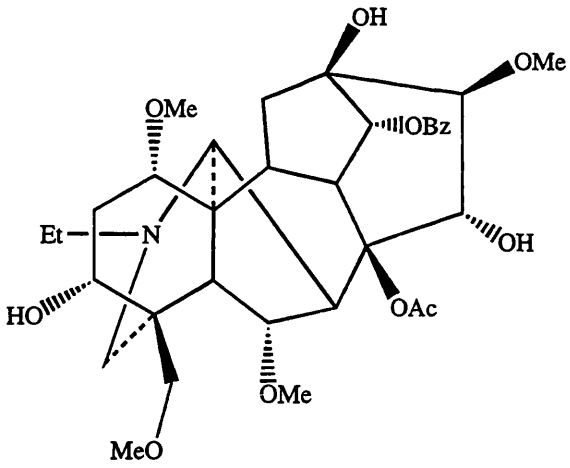
41

1.4b Semi-synthesis using norditerpenoid alkaloids

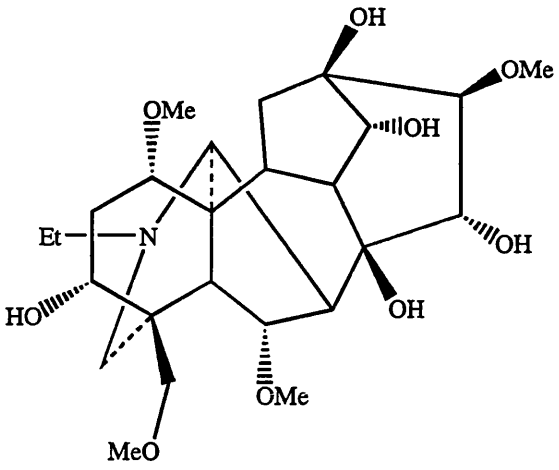
Pelletier and Ross (Pelletier and Ross 1990) have prepared some novel semi-synthetic ethers and esters from the alkaloids lycoctonine **3** and delphinine **42**. In the case of lycoctonine **3** they have synthesised primarily C-18 substituted esters in which various C18-methoxybenzoate and C18-nitrobenzoate esters were prepared rather than the 2-(methylsuccinimido)benzoate moiety (present in MLA **1**). In addition, long chain hydrocarbon esters (both branched and unsaturated) and simple alkyl ethers were synthesised. Delphinine C-13 and C-14 esters as well as C-13 ethers were synthesised. Ross and Pelletier (1991) also reported the synthesis of a series of 15 different poly-acetyl derivatives of aconitine **7** and aconine **43** as well as the synthesis of *N*-substituted derivatives of *N*-deacetyllappaconitine **44**. A quaternised variant of (*N*-methylated rather than *N*-ethylated)delphionine **45** was also prepared.



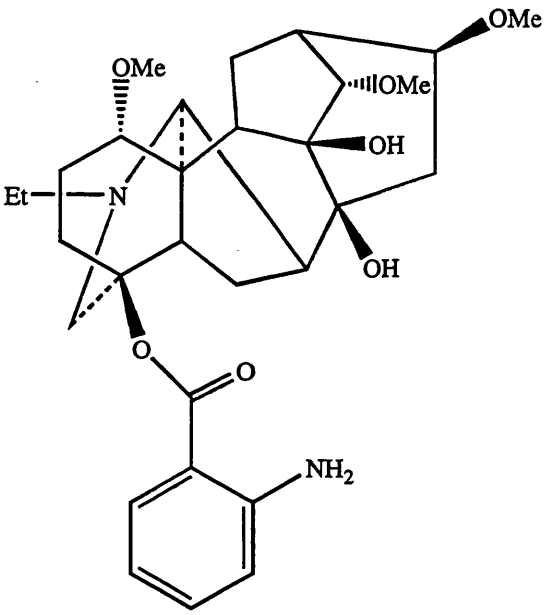
delphinine **42**



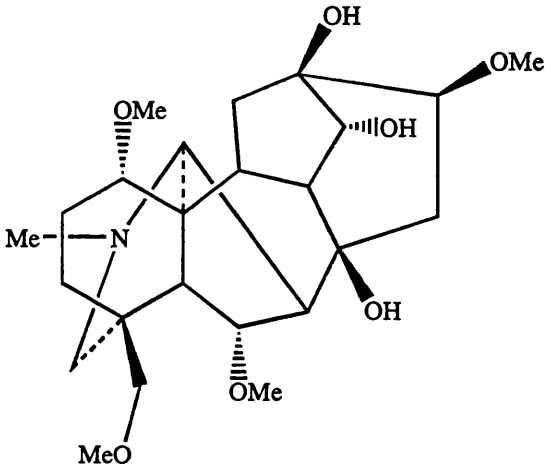
aconitine **7**



aconine **43**



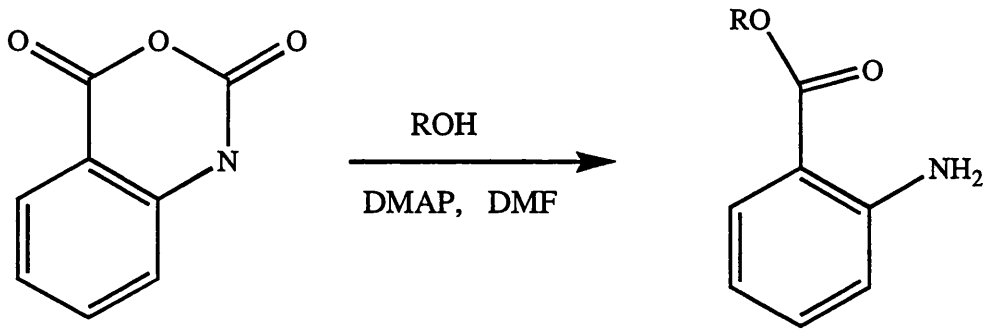
N-deacetyllappaconitine **44**



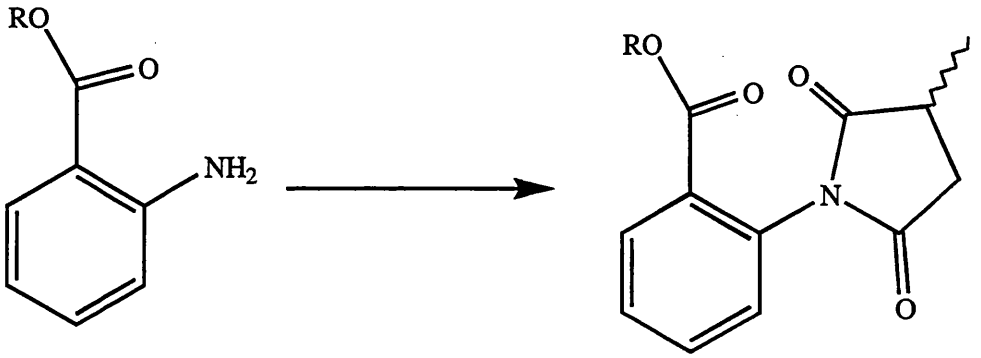
delphionine **45**

The work of Blagbrough and co-workers. (Blagbrough *et al.* 1994b; Hardick *et al.* 1994) on the conversion of neopentyl-like alcohols, (e.g. lycoctonine **3** or C3-dehydroxy C18-demethylated aconitine **7** or in the case of later work (Coates *et al.* 1994b) small molecule analogues of lycoctonine **3**), into benzoate succinimide esters, analogous to that found in MLA **1**, has shown that the small molecule compounds synthesised can be readily converted into analogues of MLA **1**. Reacting neopentyl-like alcohols with isatoic anhydride **46** under base catalysis, 2-aminobenzoate esters **47** can

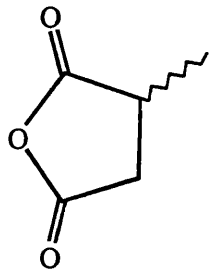
be formed. Under other conditions, esters of this type are difficult to prepare (primarily due to the difficulty with regiochemistry and/or the difficulty in orthogonal protection of the free acid and amine functional groups) and this reaction is of major importance to our synthetic strategy. Another problem which is overcome by this strategy is the problem of regiochemistry, as isatoic anhydride **46** reacts at the neopentyl-like centre selectively over the free secondary alcohols present in lycoctonine **3**. The subsequent conversion of the amino function into a methylsuccinimide **48** by the reaction with methylsuccinic anhydride **49** (initially yielding a mixture of half acid amides which can be dehydrated *in situ* with 1,1'-carbonyldiimidazole) is facile and high yielding. In the work by Blagbrough and co-workers (Blagbrough *et al.* 1994a; Hardick *et al.* 1994; Hardick *et al.* 1995), on the regioselective demethylation of aconitine **7** and the subsequent conversion to the MLA-aconitine hybrid, methods of producing homochiral methylsuccinic anhydride are reported which allow formation of compounds with the same stereochemistry as in the natural product. Related work (Coates *et al.* 1994a; Coates *et al.* 1996) has also demonstrated an efficient protocol for isolation of MLA **1** and subsequent absolute assignment of the natural product stereochemistry at the methylsuccinimide as *S*.



isatoic anhydride **46** anthranilate ester **47**

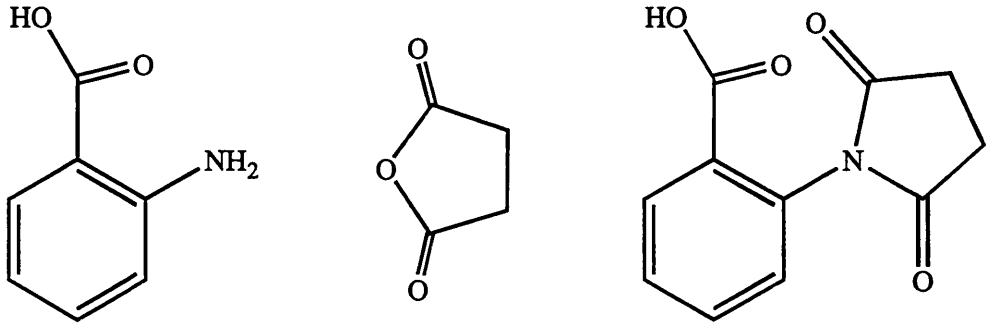


47 **48**



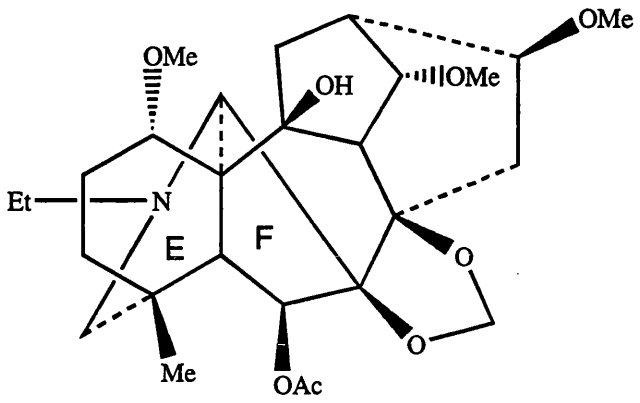
methylsuccinic anhydride **49**

The work of Jacyno *et al.* (1996) on the conversion of lycoctonine **3** into lycaconitine **14** uses an ester formation with the succinimide already in place on the anthranoyl unit **52.** Ester formation is base catalysed, but the reactivity is increased by the formation of the mixed anhydride with tosyl chloride. Formation of the succinimide is by reaction of anthranilic acid **50** with succinic anhydride **51,** but in this case cyclisation of the initially formed half-acid amide is by a base catalysed dehydration under Dean-Stark (azeotropic) conditions.



50 **51** **52**

Pelletier and co-workers (Srivastava *et al.* 1995) reported an unexpected fragmentation of deltaline **53**.



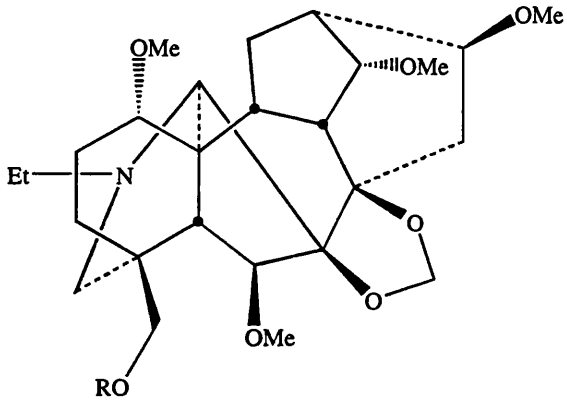
deltaline **53**

Pelletier showed that, during the attempted synthesis of 10-chloro-10-deoxydeltaline, deltaline **53** is fragmented with cleavage of the E- and F-rings under deoxygenation reaction conditions to give acyclic ethyl amines. They reported that the E- and F-rings of the alkaloid structure appear to be vulnerable to fragmentation. This is an important publication as it shows some of the conditions under which the small molecule analogues we hoped to make may be degraded.

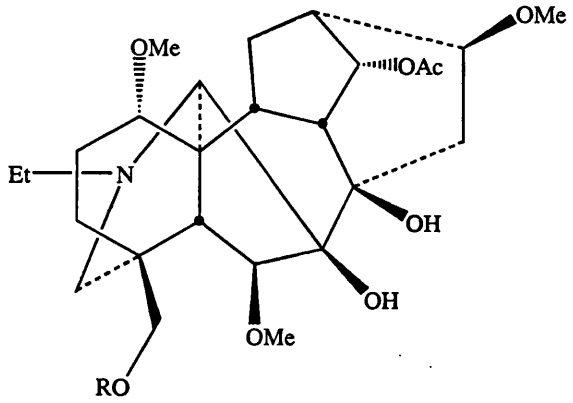
As outlined above, Hanuman and Katz (1994) have recently published a comprehensive review of the spectroscopy of norditerpenoid alkaloids which includes the use of a wide range of spectroscopic techniques to give detailed information on bond angles in the structures. They categorise 52 alkaloids into 6 different classes (**I.** Aconitine type **II.** Pseudaconitine type **III.** Bikhaconotine type **IV.** Neoline type **V.** Isotalatizidine type **VI.** Lycoctonine type) depending on oxygenation patterns.

1.4c Recent work on biological activity

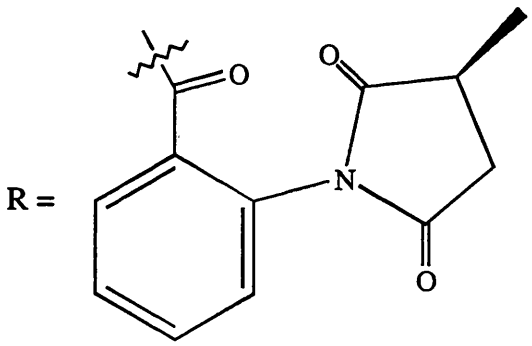
The work of Hardick *et al.* (1995) on the binding of MLA **1**, and the related alkaloids elatine **54** and nudicauline **55**, to mammalian neuronal nAChR has demonstrated firstly the high potency and selectivity of the alkaloids for the α-bungarotoxin (α7/α8 type) receptor and secondly the importance of the 2-(methyl-succinimido)benzoate moiety for binding to the receptor.



nudicauline **54**



elatine **55**



The work shows that without the 2-(methylsuccinimido)benzoate moiety present (e.g. lycoctonine **3**) activity is reduced by 4-orders of magnitude and that even with a 2-aminobenzoate ester (e.g. inuline **2**) the activity is still 3-orders of magnitude less than MLA **1**. The reliance on the 2-(methylsuccinimido)benzoate is further illustrated by the semi-synthetic work discussed earlier in which the sodium channel activating compound

aconitine **7** is converted into a potent nicotinic antagonist by the incorporation of the methylsuccinimidobenzoate moiety (Hardick *et al.* 1994; Hardick *et al.* 1995).

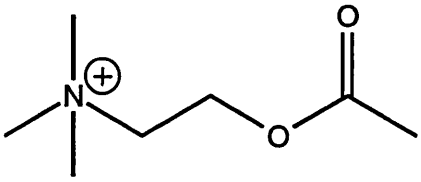
Pelletier and co-workers (Manners *et al.* 1995) have also recently published on the SAR of a group of *Delphinium* alkaloids. They report the LD_{50} values, in mice, of a series of alkaloids. These data enable them to say that there are two structural features essential for high toxicity. These are firstly, an *N*-ethyl bicyclo substituted tertiary alkaloid nitrogen atom and a C-18 anthranilic acid ester. They report that nudicauline **54** is the most toxic of the *Delphinium* alkaloids investigated, closely followed by MLA **1**.

Kukel and Jennings (Kukel and Jennings 1994) have recently published a study on the inhibition of α-bungarotoxin binding to rat and insect membrane receptors. They tested a series of alkaloids for inhibition using radio-labelled α-bungarotoxin in rat and housefly neural tissue. The results show the significance of the 2-(methyl-succinimido)benzoate moiety for high activity and the selectivity for insect over mammalian receptors. This selectivity makes these alkaloids lead compounds for insecticidal agents.

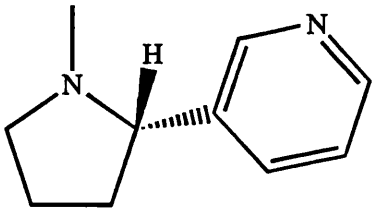
1.5 Nicotinic acetylcholine receptors

1.5a Structure and Function

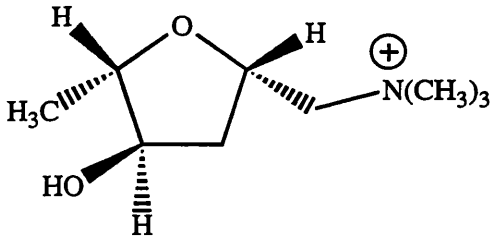
Acetylcholine **56** (an endogenous ligand) acts at two structurally and physiologically different receptor sites in the mammalian nervous system (Dale 1914). These receptors were defined by the ligands that act selectively at each AChR subtype and are the nicotinic (defined by nicotine **57**) and muscarinic (defined by muscarine **58** from the poisonous mushroom *Amanita muscaria*) acetylcholine receptors, nAChR and mAChR respectively.



acetylcholine **56**



nicotine **57**



muscarine **58**

Both of these receptor subtypes are heterogeneous and have been subdivided into more specific receptor classes. MLA **1** acts as an antagonist at nAChRs which are members of the superfamily of ligand-gated ion channel (LGIC) receptors. nAChRs constitute a well studied group within this superfamily of receptors which includes GABA receptors and inhibitory glycine receptors, though not necessarily the ionotropic glutamate receptors (e.g. NMDA receptor) which may be more closely allied with certain potassium channels.

The nAChR allows the passage of cations such as sodium or calcium into the cell, and the egress of intracellular potassium ions, when the channel opens upon activation. The passage of ions is dependent upon their relative concentration gradients. The receptor is a pentameric protein structure (of stoichiometry e.g. $\alpha_2\beta\gamma\delta$) with each of the five subunits contributing to the pore through the centre of the receptor. There are many different subtypes of the receptor due to the heterogeneity of the subunits (e.g. $\alpha1$-$\alpha10$). The most well studied receptor in terms of the structure and physiology is the receptor found in the electric organ of certain fish (e.g. *Torpedo*). This receptor is of high abundance and is therefore amenable to isolation for patch clamp analysis and protein sequencing studies. This receptor is analogous in subunit makeup to the receptor found at the mammalian neuromuscular junction and has the subunit stoichiometry $\alpha_2\beta\gamma\delta$. Receptors do not require all of the different classes of subunit to be functional and as long as there are at least two alpha subunits within the five, then the receptor will be functional (e.g. receptors at autonomic ganglia are composed generally of two alpha subunits and three beta subunits). Recombinant techniques (Yu *et al.* 1996; Eisele *et al.* 1993) have shown that even receptors made up entirely of alpha subunits (e.g. five $\alpha7$ subunits) can function. This enables a potentially huge number of possible receptor subtypes which can explain how nicotinic transmission can have many varied roles in both the peripheral and central nervous systems.

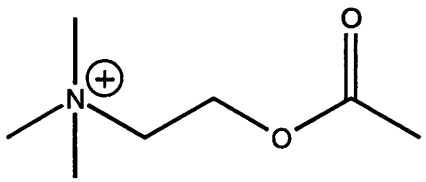
The binding site for acetylcholine is on the alpha subunit and two molecules of acetylcholine are required for channel opening (one at each of the alpha subunits in the receptor). MLA **1** blocks the acetylcholine site on the α-subunit of the nAChR and is therefore a competitive antagonist of nAChR. The nAChR occurs at many physiologically important sites in mammalian systems (e.g. neuromuscular junction, autonomic ganglia and at many CNS sites). The nAChR is vital in mammalian

physiology and plays a vital role in muscle control and autonomic nervous transmission and has a more subtle and complex role in the function of the CNS. Malfunction of the activity of the nicotinic receptor is involved in many disease states with an important role in neurodegeneration (Wonnacott 1993) and stroke within the CNS, and with Myasthenia gravis in the periphery.

1.5b Ligands

Many ligands of the nAChR are known and they all have certain similar structural properties which have been collated into the Beers-Reich (1970) model for the nicotinic pharmacophore. The vital part of this model is the distance between the quaternary ammonium ion (at physiological pH) and the lone pair on the heteroatom approximately 5 atoms away (generally oxygen as with acetylcholine and MLA **1** or pyridine nitrogen as in nicotine). As there are many different variants of the subunits of nAChR, ligands which are selective for a particular subtype are valuable and sought after. As with all receptors there are agonists, partial agonists and antagonists of the receptor acting at the same site as well as compounds which can act in an allosteric fashion (affecting the function of channel without competing for the binding site of the endogenous ligand) and compounds which can block the channel itself.

Acetylcholine:

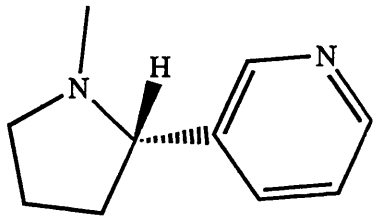


56

This small molecule choline ester is an agonist and a vitally important neurotransmitter which is the endogenous ligand at both nicotinic and muscarinic

receptors. Acetylcholine **56** is stored in vesicles and is released from the synapse when an action potential arrives at the nerve terminus. It is readily metabolised at the synapse by acetylcholinesterase which cleaves the ester function. The choline released by the esterase is taken up by the surrounding cells and re-used.

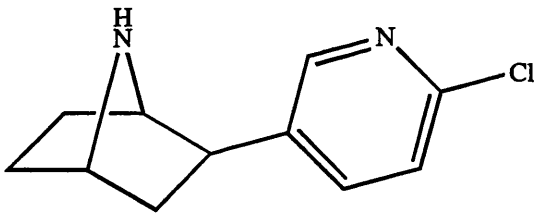
S-(-)-Nicotine:



57

This is a naturally occurring ligand found in the tobacco plant. It is a partial agonist which acts selectively at the nicotinic sites. This agonist was used to define the two main subtypes of acetylcholine receptor along with muscarine which defines mAChR. Nicotine **57** is an addictive substance and causes dependence in smokers. Nicotine **57** has insecticidal properties. Nicotine **57** exhibits selectivity for α4 neuronal nAChR at a high affinity binding site (Wonnacott *et al.* 1993).

Epibatidine:

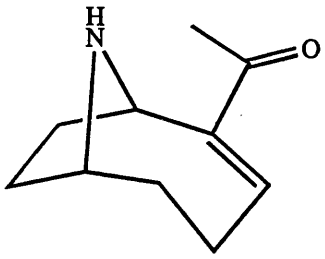


59

This is an agonist isolated from the skin of an Ecuadoran tree frog *Epipedobates tricolor* which is of great interest because it has an analgesic effect. Epibatidine **59** is 1000x more potent than morphine, without acting at opioid receptors. The amount of

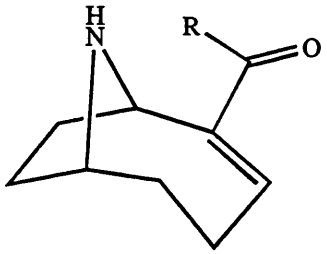
epibatidine **59** isolated from each frog is only micrograms. Therefore hundreds of frogs are required to produce milligrams of the compound and hence, epibatidine **59** has been an important synthetic target (Corey *et al.* 1993).

Anatoxin-a:



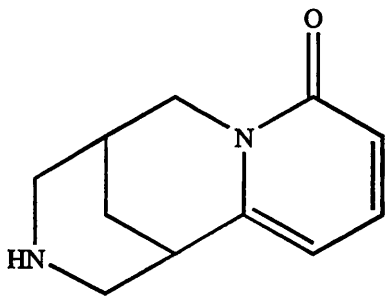
60

This is a naturally occurring agonist found in toxic the blue-green alga *Anabaena flos-aquae* (Devlin *et al.* 1977) which grows in lakes and ponds and in warmer weather can be seen as large blooms which makes the lake poisonous (several reported deaths of pets in the newspapers). This compound has been a target of many synthetic attempts and is a lead compound in the search for new compounds which act at nAChR (such as insecticides). Gallagher and co-workers have synthesised anatoxin analogues (**61a-c**; **a**) R = ethyl, **b**) R = propyl, **c**) R = isopropyl) for SAR studies on nAChR (Magnus *et al.* 1997).



61a-c

Cytisine



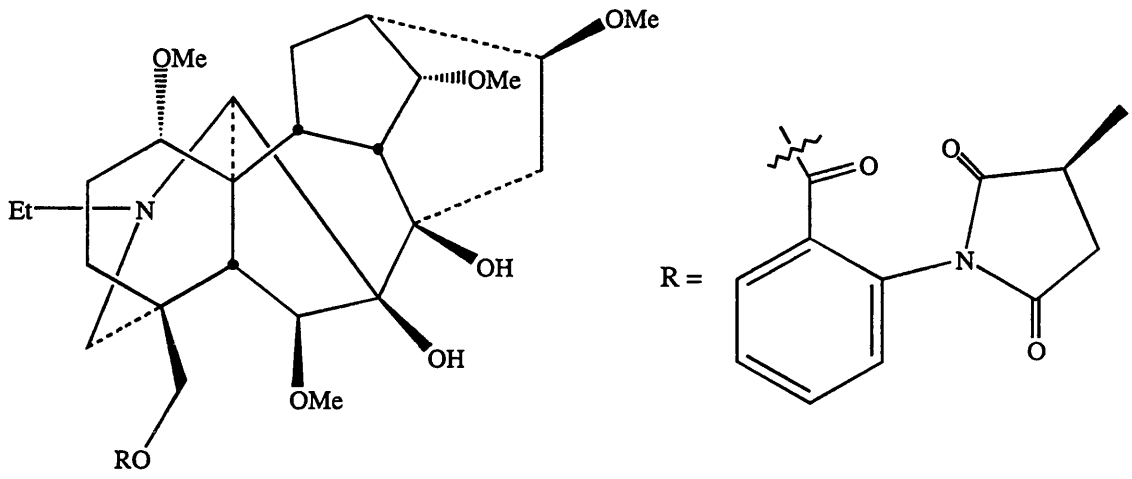
62

The quinolizidine alkaloid cytisine **62** is isolated from *Laburnum* species. Cytisine **62** is a potent agonist of nAChR (Barlow and Johnson 1989) and is a powerful insecticide as well as a mammalian toxin. Cytisine is a lead compound for the synthesis of novel nAChR agonists (Blackall *et al.* 1995) and contains a aza[3.3.1]bicyclic system.

α-Bungarotoxin

This is a peptide antagonist that is isolated from the venom of the Banded Krait (*Bungarus fasciatus*), a venomous snake from Asia (Marks *et al.* 1986). α-Bungarotoxin is a member of an homologous family of 7-8 kDa proteins which includes erabutoxin from sea snakes and cobratoxin from cobras. This peptide antagonist has a high selectivity for the α1 (neuromuscular junction type) and the α7/α8 type neuronal receptor (which are defined as being α-bungarotoxin sensitive) as opposed to the nicotine sensitive α4 receptor. α-Bungarotoxin binds (tightly) to the α-subunit of nAChR in an almost irreversible way to prevent channel opening. This compound is used labelled with ^{125}I in a binding assay in which the level of binding to the nAChR is measured by the displacement of the labelled α-bungarotoxin from the receptor (Wonnacott *et al.* 1993).

MLA:



1

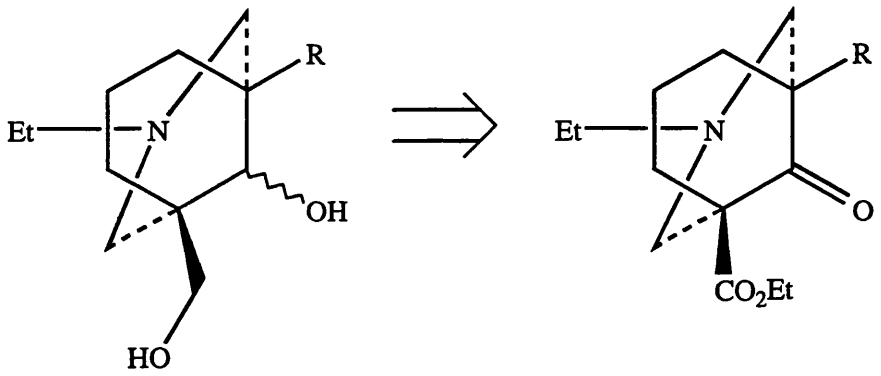
MLA **1** is a nAChR antagonist with high affinity and significant selectivity for certain subtypes of nAChR. MLA **1** antagonises ligand binding to nAChR in flyheads, locust ganglia and cockroach motorneurones with K_i values in the nanomolar range (at α7 and/or α8), whereas there is a significantly lower affinity/antagonism at rat neuromuscular junction nAChR (α1 subtype of stoichiometry $\alpha_2\beta\gamma\delta$) and also at the mammalian CNS α4 containing nicotinic binding site which shows high affinity for nicotine **57** (Macallan *et al.* 1988). This shows the potential for MLA **1** as both an insecticide (selective for insect over mammalian sites) and as a pharmacological tool as it binds selectively to α7/8 over α4 containing receptors. In comparison with the snake venom toxin α-bungarotoxin, which displays nM affinity for the α1 and α7/8 nAChR, MLA **1** is as potent, but more selective. At the start of these studies MLA **1** was the most potent and selective nAChR antagonist known.

Chapter 2

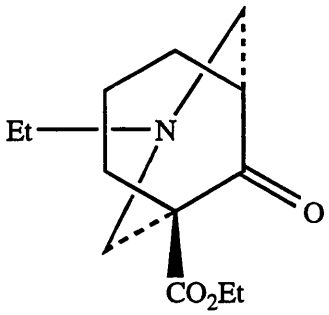
A DIANION ALKYLATION APPROACH TO C10-C11 SUBSTITUTED AE-BICYCLIC ANALOGUES OF MLA

2.1 Design of synthetic targets

In order to investigate the role of the BCD rings (of MLA **1**) in binding to nAChR, a series of substituted AE-bicyclic analogues of MLA **1** was required. Initially, compounds were designed which contained substitution that occupied the space taken up by the rings of the norditerpenoid skeleton, and then intact C- and D-rings were to be incorporated into the analogues. Substituted analogues which contain functionality that could be further elaborated were also desired. To synthesise MLA **1** analogues it is necessary initially to synthesise analogues of lycoctonine **3** and then using the method of Blagbrough and co-workers conversion into the 2-(methylsuccinimido)benzoate ester is facile and efficient (Coates *et al.* 1994b). Given that a double Mannich reaction on a β-ketoester was to be used to synthesise the piperidine ring, the target molecules were of type **63**:

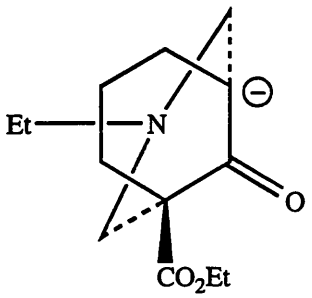


63 **64**

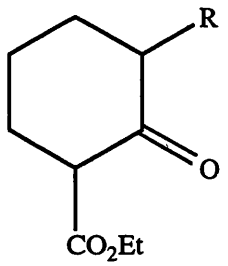


4

Retrosynthetically, compound **63** can be derived from the corresponding β-ketoester **64** by reduction of the carbonyl functionalities with LAH. Bicyclic β-ketoester **4** can be synthesised by a double Mannich reaction on β-ketoester ethyl 2-oxocyclohexane-1-carboxylate **37** in reasonable yield, but to synthesise a substituted β-ketoester **64** (where R= alkyl, aryl or allyl) a method of substituting at this position (C5) is required. It is not possible to deprotonate bicyclic β-ketoester **4** (forming carbanion **65**) as this would form an anti-Bredt bridgehead carbanion/enolate. Hence, the direct alkylation of such an anion is not a viable strategy. However, the work of Kraus *et al.* (1990, 1991 and 1993) has shown that a bridgehead radical can be formed (from the bridgehead bromo compound) and that alkylation of this radical with allyltributyl tin is efficient (see Chapter 1).



65



66

The double Mannich reaction works well on γ-substituted β-ketoesters **66** (Shimizu *et al.* 1963a; Blackall *et al.* 1995) and so it is possible to use 3-substituted β-ketoesters in this reaction to form the desired substituted bicyclic β-ketoesters.

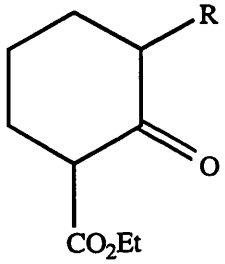
2.2 Alkylation of the dianion of ethyl 2-oxocyclohexane-1-carboxylate

In a β-ketoester, the most acidic proton is not at the γ-position, but at the α-position which is between the two carbonyls and the reactivity at this centre has been widely exploited in synthetic chemistry. The anion produced by deprotonation at the α-position can be alkylated (Sakai *et al.* 1980), perform aldol reactions (Kociolek and Leplawy 1977) and add to electron-deficient unsaturated systems in 1,4-conjugate addition Michael reactions (Bergmann *et al.* 1959; Barbee *et al.* 1991).

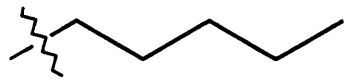
However, this enolate can be further deprotonated to form a dianion/di-enolate which can be regioselectively alkylated at the γ-position. The dianion of a β-ketoester was first reported by Hauser and Harris in the 1950's using two equivalents of potassium amide in liquid ammonia to effect the deprotonations (Hauser and Harris 1958). The regioselective γ-alkylation of ethyl acetoacetate and other β-ketoesters has literature precedent and was studied extensively by Weiler and co-workers in the 1970's. The method developed by Weiler uses one equivalent of sodium hydride followed by one equivalent of *n*-BuLi generally with THF as the solvent (Weiler 1970). Weiler showed that the dianion of acyclic β-ketoesters (primarily with ethyl acetoacetate) could be alkylated by simple alkyl halides (Weiler 1970; Huckin and Weiler 1974) and other electrophiles such as α-chloroethers (Sum and Weiler 1977) and halo ketals (Flannery and Hampton 1972), as well as undergo aldol condensations (Huckin and Weiler 1971). Under the conditions used by Weiler and co-workers, yields were modest to good, but there were some cases when the use of a co-solvent (HMPA) was required to obtain good yields. It also seems likely that the dianion of a β-ketoester would perform Claisen type condensations onto other esters, but there is no report in the literature that the

dianion of a β-ketoester would perform a conjugate 1,4-Michael-type addition to an unsaturated system.

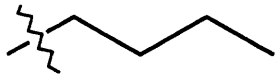
We envisaged that we could use the conditions described by Weiler to form the dianion of the cyclic β-ketoester, ethyl 2-oxocyclohexane-1-carboxylate **37**, and react this dianion with a series of electrophiles to yield a series of γ-substituted β-ketoesters **66a-f** which could be used in a double Mannich reaction to form the AE-bicyclic portion of MLA.



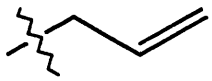
R =



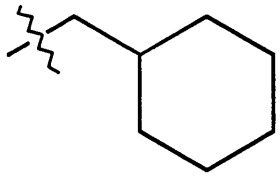
66a



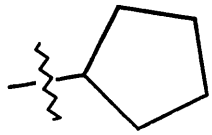
66b



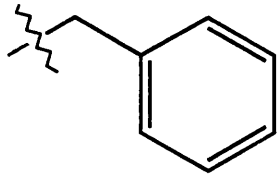
66c



66d



66e

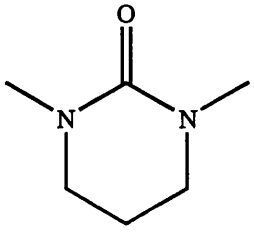


66f

In the first instance, we were concerned with occupying the space taken up by the remaining 4 rings of the norditerpenoid carbon skeleton. This we hoped to achieve by inserting an alkyl chain which could access all the carbon atoms of the remaining rings.

This can be achieved with a 5 carbon unit and so 1-iodopentane was used as the electrophile to yield **66a** (and 1-iodobutane to yield **66b**). Secondly, it was hoped that we could insert one or more of the rings into our compounds in an intact form, with the correct regiochemistry required for the norditerpenoid skeleton. For this we used cyclohexylmethyl bromide (yielding **66d**) and cyclopentyl bromide (yielding **66e**) as the electrophiles to represent the D and C rings respectively. We also wanted to create some unnatural analogues and so a planar ring D analogue was formed with the use of benzyl bromide as the electrophile to yield **66f**. Alkylation with allyl bromide (to give **66c**) would give a route to the type of compound synthesised by Kraus and co-workers (Kraus *et al.* 1993) and also allow the insertion of a useful synthetic handle into our small molecule analogues.

Initial attempts at alkylating the dianion proved to be low yielding or did not yield the desired product at all. These initial attempts used the conditions described by Weiler (1970) to form the dianion, that is 1.2 equivalent of NaH and 1.05 equivalent of nBuLi in THF at -78°C, followed by addition of the electrophile. The reaction temperature was allowed to rise to RT, and then stirred for a minimum of 4h. It was thought that the low yields could be due to a solvent effect, as in some of the experiments reported by Weiler the co-solvent HMPA was used to increase the reactivity and yields. We therefore added a co-solvent, replacing the HMPA used by Huckin and Weiler with DMPU **67** (which is considerably less toxic than HMPA) as first described by Seebach (Mukhopadhyay and Seebach 1982).



67

The co-solvent was used as a solvent for the electrophile and added after the dianion had been formed in THF. A 3:7 mixture of DMPU to THF was found to increase yields and reaction rates considerably. However, the reactions still produced a complex mixture of by-products and the reason for this was probably due to an interaction between the highly basic dianion (or residual *n*-BuLi) and the solvent. Careful control of the reaction temperature was investigated as at lower temperatures the solvent is less likely to be attacked by the dianion. At temperatures below -20°C there appeared to be no reaction between the dianion and the electrophile, but as the temperature was raised the reaction proceeded and the yields increased until 5°C when the yields fell and the complexity of the product mixture (by tlc analysis) increased, possibly due to unwanted interaction between the basic dianion and THF yielding acetaldehyde enolate.

The reaction conditions which gave the best yields are formation of the mono-anion with 1.2 equivalents of NaH at 0°C followed by cooling to -78°C and addition of 1.05 equivalents of *n*-BuLi to form the dianion, all carried out in rigorously dried THF. To this dianion was added the electrophile dissolved in DMPU (enough to give the optimum total ratio of solvents 3:7 DMPU-THF) at -78°C and then the reaction temperature was allowed to rise to 4°C (by putting the reaction into a refrigerator). The reactions were left for 4-12h depending upon the electrophile used. The best yields were obtained using the primary alkyl halides 1-iodobutane (58% over two steps) and 1-iodopentane (57% over two steps) followed by benzyl bromide (47% over two steps) and allyl bromide (27% over two steps) and modest yields were obtained with the more hindered cyclohexylmethyl bromide and cyclopentyl bromide. That the secondary enolate reacted with the secondary alkyl halide, cyclopentyl bromide (32% over two

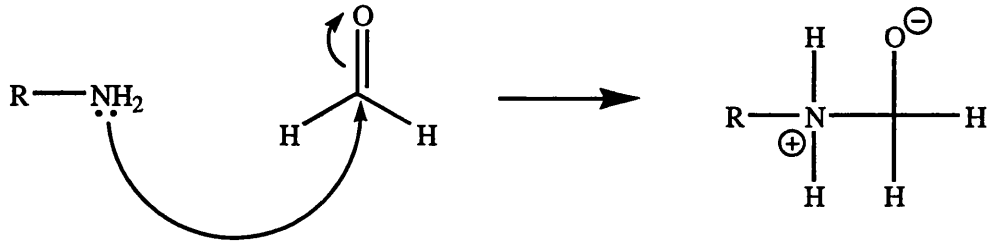
steps) and the hindered primary halide cyclohexylmethyl bromide (39% over two steps), shows that this dianion route is an efficient route to 3-substituted cyclic β-ketoesters.

The product formed by alkylation of the dianion is a β-ketoester which still contains a readily enolisable α-proton and hence exists in an equilibrium between the keto and enol tautomers. This, in conjunction with the presence of unreacted starting material (which has a similar polarity and boiling point to the products), meant that the purification of the γ-alkylated β-ketoesters (i.e. separation of **66a-f** from unreacted starting material **37**) was difficult both by flash silica gel chromatography and distillation. We discovered that it was both easier and higher yielding to use the crude product in the double Mannich reaction rather than to attempt to purification at this stage. Proton NMR of the crude alkylated product showed that the enolisable (and acidic) methine proton α- to 2 carbonyl groups is still present (δ ~3.5ppm). Sharp singlets are seen at 12-13ppm (which are assigned to the OH of the enol tautomer) in the spectrum in $CDCl_3$ with an integral corresponding to the decrease in the integral of the methine proton at ~3.5ppm indicating that some enolisation is occurring.

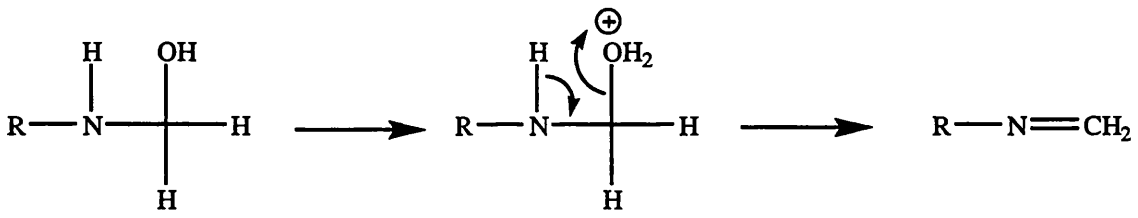
2.3 The double Mannich reaction

The double Mannich reaction was first used to synthesize the piperidine ring of norditerpenoid alkaloid structures by Shimizu *et al.* (1963a) during the attempted synthesis of atisine, a diterpenoid alkaloid. The double Mannich reaction requires a ketone with at least 1 acidic α-proton on either side of the ketone. The reaction does not proceed with cyclohexanone and Shimizu and co-workers (1963a) showed that it was necessary for at least one of the α-protons to be activated by another electron

withdrawing group such as an electron deficient aromatic ring or another carbonyl functional group. The double Mannich reaction is an acid catalyzed reaction and the first step is the formation of the enol form of the β-ketoester. The reaction requires a primary amine **68** and two equivalents of an aldehyde (usually formaldehyde **69**). The first equivalent of the aldehyde reacts with the primary amine to give the corresponding imine and the reaction protocol requires the amine and aldehyde to be mixed before addition of the of the ketone. Imine formation is exothermic and so addition of the reagents must be carefully controlled with the aldehyde solution being added slowly to the amine.

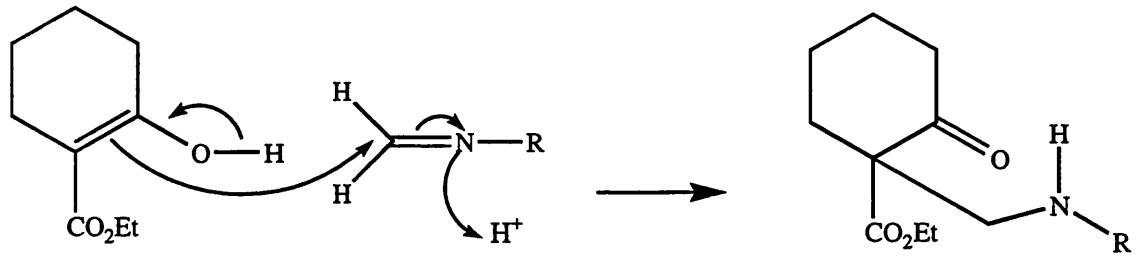


68 **69**



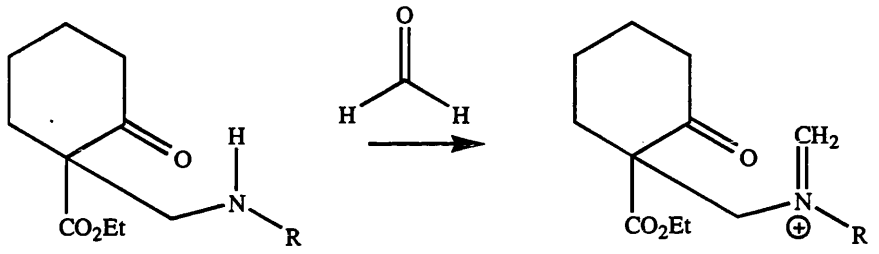
70

The imine **70** (or the iminium ion) then reacts with the activated ketone (in enol form) to give the methylamino substituted β-ketoester **71** which was not isolated.



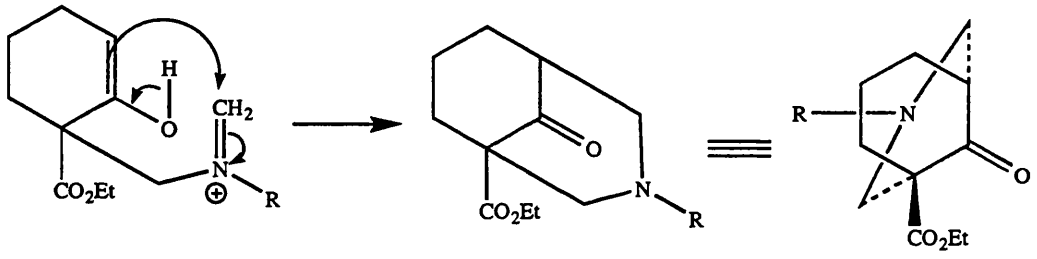
37 **71**

The enol tautomer of β-ketoester **34** can react with the imine from either face yielding both axial and equatorial aminomethyl species. The newly formed secondary amine **71** then reacts with another molecule of formaldehyde **69** to give the iminium species **72.**

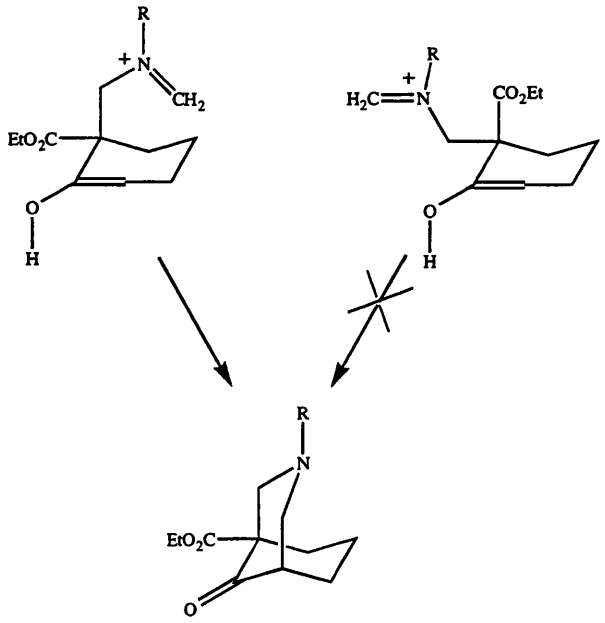


71 **72**

In order for the cyclisation to occur affording [3.3.1]azabicycle **73**, the iminium substituent must be in an axial orientation or the reacting enol species will not be able to reach (intramolecularly) the C-terminus of the iminium ion and react.

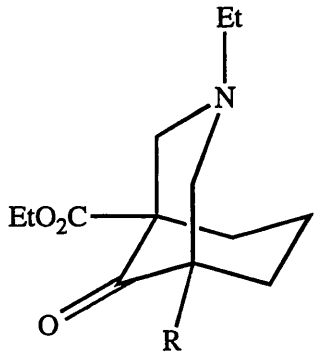


72 **73** **73**



73

The γ-alkylated β-ketoesters **66a-f** contain two chiral centres, including the readily enolisable α-carbon of the β-ketoester, resulting in a mixture of diastereomeric compounds in the crude product (this partially accounts for the purification difficulties). However, in order to cyclise and yield the piperidinone, the iminium ion must be in an axial position relative to the carbocycle. The fact that only a di-axial conformation of the new C-C bonds is possible ensures that the carboxylate ester function and the alkyl group must occupy equatorial positions. This restriction means that only one of the possible diastereoisomers is formed (together with it's enantiomer). This allows for easier purification by silica gel chromatography and also simplifies the spectroscopy of the products. The double Mannich reaction is a relatively poor yielding reaction (40-60%), but it gives higher yields of [3.3.1]bicycles when there is substitution at the γ-position of the β-ketoester than when there is no substitution (i.e. to give **4**). Using a double Mannich reaction of formaldehyde, ethylamine and the crude products of the dianion alkylation of ethyl 2-oxocyclohexane-1-carboxylate and 1-carbethoxy-cyclohexan-2-one itself yielded a series of 5-substituted 3-ethyl-3-aza-bicyclo[3.3.1]nonan-9-ones **64a-f** in yields ranging from 30-60% over 2 steps (and **4** in 37%).

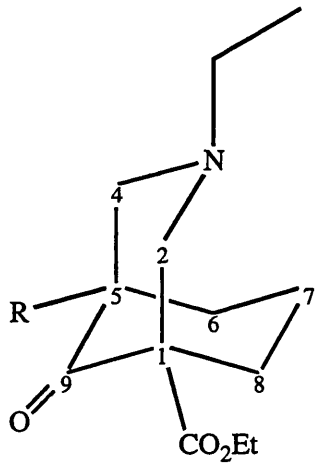


64a-f

a) R= *n*-pentyl, **b)** R= *n*-butyl, **c)** R= allyl, **d)** R= cyclohexylmethyl, **e)** R= cyclopentyl, **f)** R= benzyl **4)** R= H

The polarities of the 5-substituted bicyclic β-ketoesters (**64a-f**) are sufficiently different from both the starting monocyclic β-ketoesters (**66a-f**), and the unsubstituted bicyclic β-ketoester **4** (which is also present in the reaction mixture), to allow efficient separation by silica gel chromatography. The purified bicyclic β-ketoesters are viscous colourless oils which exhibit characteristic proton and carbon NMR spectra. The methylenes of the piperidinone ring have characteristic down field chemical shifts (2.5-3.2ppm as opposed to 1.4-2.5 for a carbocyclic methylene) and large geminal (10-11Hz) coupling constants. In the CMR, there are patterns of three methylenes (next to nitrogen) with two of them (the piperidinone pair) at 55-60ppm and the third (ethyl amine) at 50-52ppm characteristic of a methylene α- to an amine.

2.4 The conformations of 3-aza[3.3.1]bicyclononanones

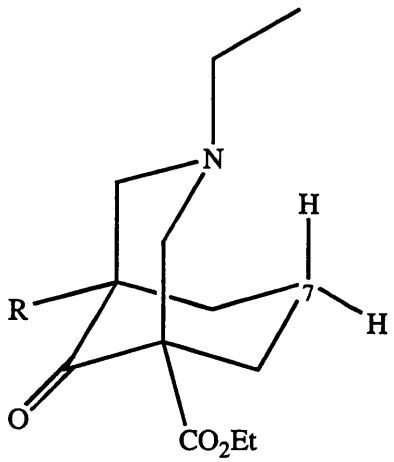


64

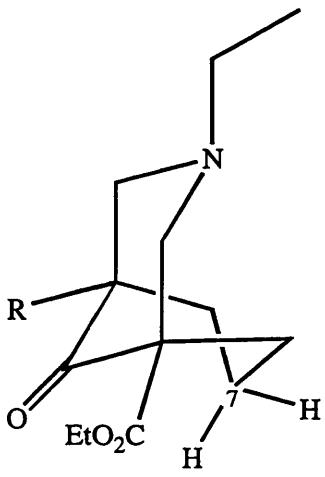
3-Ethyl-3-aza[3.3.1]bicyclononan-9-ones (**64a-f** and **4**) can possibly exist in a number of conformations. They can exist as a chair-chair, chair-boat or a boat-boat conformation or a distortion of any of these. It is unlikely that it would be a true match to any of these conformations because of the sp^2 hybridization of the carbonyl which will

distort the ring(s). However, after reduction to the secondary alcohol, the bicyclic structure can adopt one of the true conformations. There are two conflicting opinions in the literature about the conformations with Speckamp and co-workers (1970) proposing a chair-boat conformation and Shimizu and co-workers (1963a) proposing a chair-chair conformation.

Work within our research group has shown that there is ^{1}H NMR evidence for a chair-chair conformation. This evidence is based upon the chemical shift of the carbocyclic methylenes (Coates 1996). The methylene at position 7 appears as two separate signals at significantly different chemical shifts (H7ax m 2.90-2.71ppm and H7eq m 1.57-1.49ppm). These data show that the axial proton is de-shielded (i.e. lower electron density around the proton) by the lone-pair of the nitrogen, moving the δ value downfield (Kemp 1986), which confirms the chair-chair conformation **64x**.



64x

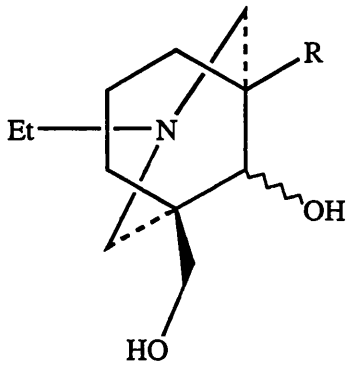


64y

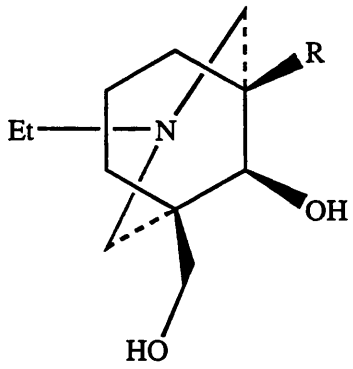
In the chair-boat conformation **64y**, the nitrogen lone-pair is too far away from the H7 proton to have a de-shielding effect. The chair-chair conformation is adopted by the A and E rings of MLA **1** and hence the 3-ethyl-3-azabicyclo[3.3.1]nonan-9-one core is an accurate template upon which to build analogues of MLA **1**.

2.5 LAH reduction of bicyclic ketoesters: synthesis of lycoctonine analogues

In order to introduce the 2-(methylsuccinimido)benzoate ester moiety into our compounds, reduction of the ethyl ester to a primary (neopentyl-like) alcohol must be performed (to yield compounds **74a-g**). This was achieved with LAH and proceeds with concurrent reduction of the ketone to a secondary alcohol. This yielded a diastereoisomeric mixture of bicyclic diols in a ratio of ~9:1. The least prevalent diastereoisomer was not converted into 2-(methylsuccinimido)benzoate esters as there was not generally enough material to proceed on a practical scale. The preferential angle of attack was presumably with the hydride attacking in an equatorial position yielding primarily the axial alcohol relative to the carbocyclic ring as was found with the sodium borohydride reduction of this ketone (Coates 1996). This is presumably due to nitrogen-metal co-ordination guiding the incoming hydride attack.



74a-g



Major diastereoisomer

Following the reaction by tlc, it was obvious when all the bicyclic β-ketoester starting material had reacted as the product (**74a-g**) has a considerably lower Rf than the starting material (0.05 as opposed to 0.5 for the n-pentyl compound **64a→74a** eluting with 1:9 EtOAc-hexane).

Compound	R	Yield (%)
74a	*n*-pentyl	66
74b	*n*-butyl	46
74c	allyl	60
74d	cyclohexylmethyl	72
74e	cyclopentyl	39
74f	benzyl	47
74g	H	46

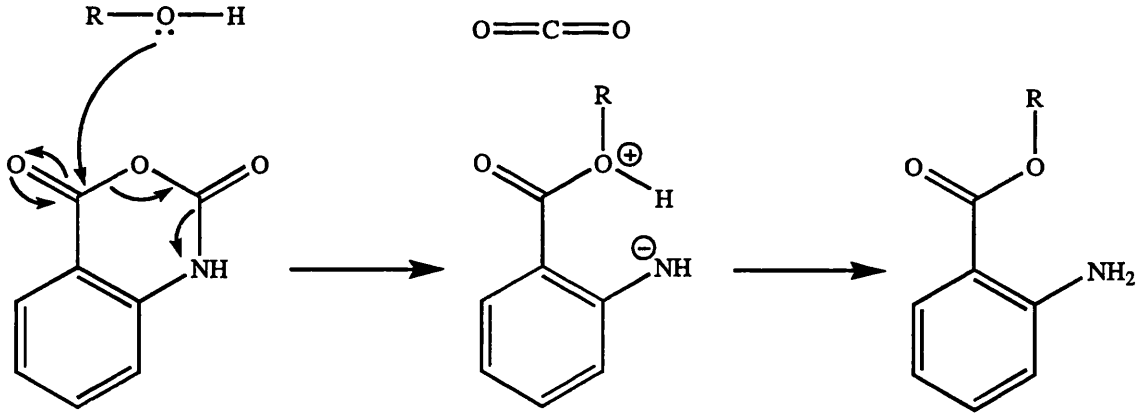
Significant changes in both the proton and CMR spectra also show that the reaction has proceeded as expected. In the PMR spectra, the quartet (~4.2ppm) and the triplet ~1.1ppm) of the ethyl ester are lost and a doublet (~3.4ppm) with a geminal coupling (~11Hz) appears which is the newly formed hydroxy-methylene analogous to C18 of the norditerpenoid skeleton. In the more easily assigned spectra (e.g. **74g**), a doublet at around 3.5-4.0ppm with a fine coupling (3.1Hz) representing the methine of the secondary alcohol is observed. In the proton decoupled CMR spectra, loss of the ethyl ester is also observed and the two new hydroxyl substituted carbons appear at ~75 and ~71ppm (secondary and primary alcohols respectively as identified by the 135 DEPT experiment).

The conformation of the aza[3.3.1]bicyclononanone ring system matches that of the AE-rings MLA, but the secondary alcohol which remains after reduction of the ketone with LAH does not appear in MLA. This is not as much of a problem as it may at first appear because NMR studies (Coates 1996) have shown that this hydroxyl is most likely to adopt an axial position (relative to the carbocyclic ring) and hence not occupy the space filled by C6 in MLA **1** and related alkaloids of the lycoctonine class.

2.6 Selective esterification of lycoctonine analogues: synthesis of inuline analogues

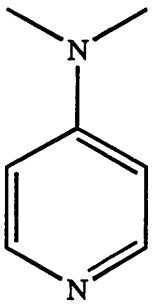
Conversion of the bicyclic diols **74a-g** into the 2-aminobenzoate esters is an efficient reaction (Coates *et al.* 1994b). Reacting the bicyclic diol with 1.1 equivalents of isatoic anhydride **46** with base catalysis (DMAP **75**) in anhydrous DMF at 70°C gives regioselective acylation at the primary (neopentyl-like) alcohol over the secondary alcohol (hence more hindered).

There are two possible mechanisms for this reaction which involve DMAP **75** either as a base or as a nucleophile/leaving group. The favoured mechanism is the one in which DMAP **75** acts as a base and as it has been shown that the reaction works under catalysis with other, non-nucleophilic bases (e.g. potassium carbonate) (Staiger and Miller 1959).

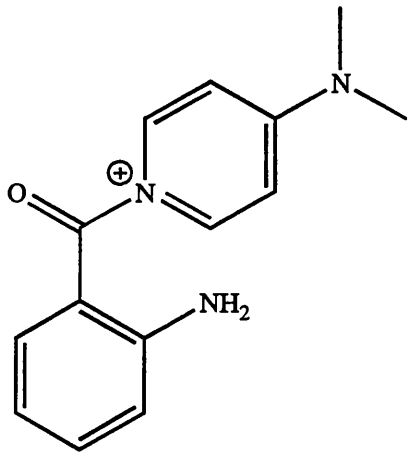


Isatoic anhydride **46** **47**

In the other proposed mechanism (which is the more usual mechanism when DMAP is involved), DMAP adds to the isatoic anhydride, via the pyridine nitrogen, in an analogous manner causing the loss of CO_2 and the formation of an activated amido species **76**, which can easily undergo nucleophilic displacement by the primary alcohol.

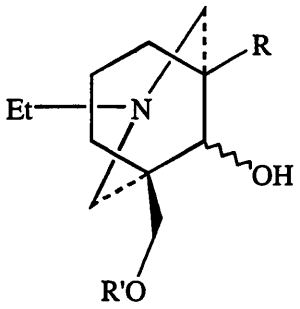


DMAP **75**

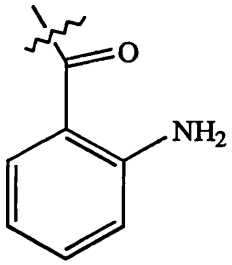


76

The conversion of **74a-g** into 2-aminobenzoate esters is a moderate-good yielding reaction (60-90%) and allows the formation of the series of inuline **2** analogues **77a-g.**



R' =

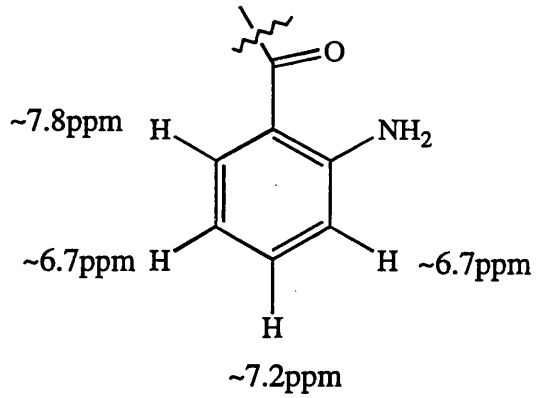


77a-g

a) R= *n*-pentyl, **b)** R= *n*-butyl, **c)** R= allyl, **d)** R= cyclohexylmethyl, **e)** R= cyclopentyl, **f)** R= benzyl **g)** R= H

The formation of the 2-aminobenzoate is monitored by tlc and a marked increase in Rf between the starting diols (**74a-g**) and the esters (**77a-g** which are characteristically UV active) with a typical increase being from 0.3 for the diol to 0.8 for the ester (1:9 MeOH-DCM). Evidence in the PMR and CMR spectra of the conversion are the appearance of aromatic signals and, in the proton NMR, the new broad singlet at ~5.2ppm (exchangeable) which is the NH_2 group.

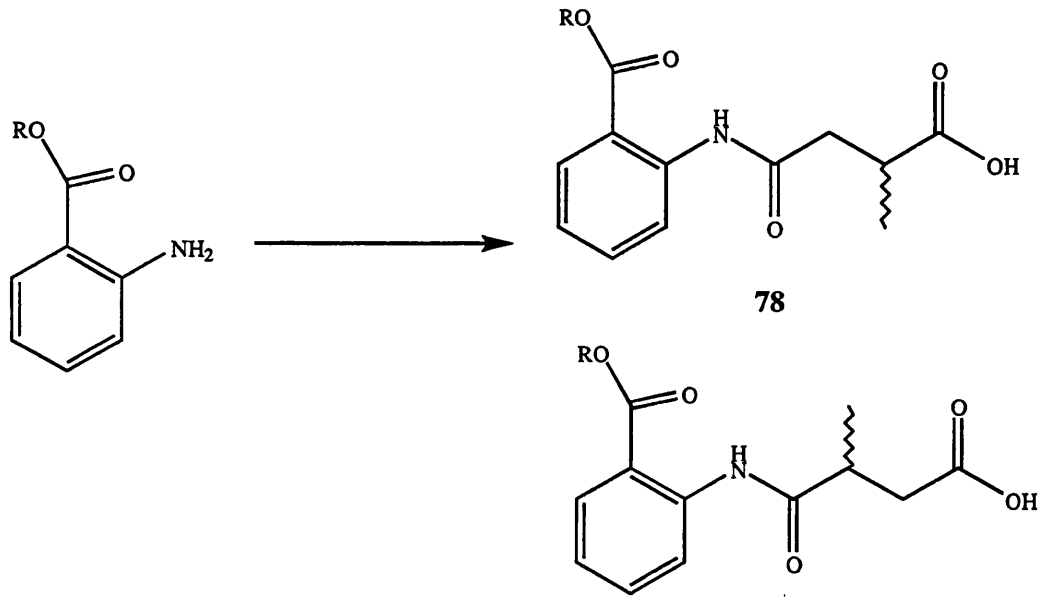
The pattern of the proton NMR aromatic signals is well defined and is a useful diagnostic tool in later experiments.



The 2 protons *meta* to the ester group appear as an overlapping multiplet and the *para* (multiplet) and *ortho* (doublet of doublets) protons appear singly giving a 1:1:2 integration pattern (as opposed to the 1:1:1:1 pattern observed later in the methylsuccinimido compounds, see below).

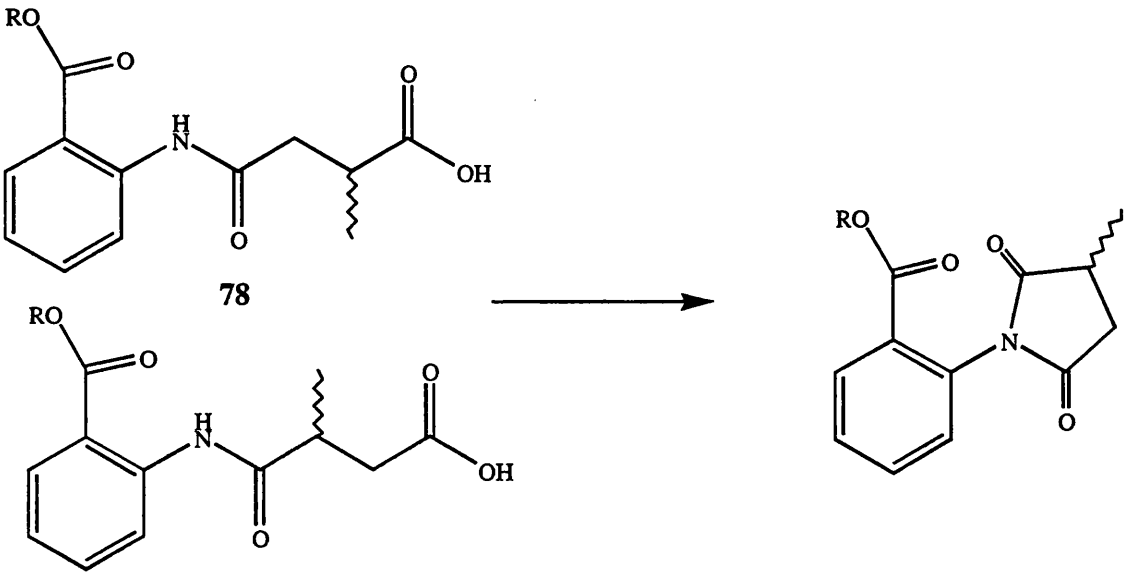
2.7 Conversion of inuline analogues into MLA analogues

The final step in the conversion into MLA **1** analogues is the transformation of the aniline amine into a methylsuccinimide. This is achieved using the protocol of Blagbrough and co-workers (Coates *et al.* 1994b). The 2-aminobenzoates **77a-g** were reacted with 3 equivalents of (±)methylsuccinic anhydride **49** (in DCM) which initially yielded the half-acid amides **78** and **79** by nucleophilic ring opening of the methylsuccinic anhydride **49**. This takes 16-24h for complete conversion and the reaction was monitored by tlc (the half-acid amide has a low Rf ~0.05 compared to ~0.5 for the anthranilate ester 2:3 EtOAc-hexane).

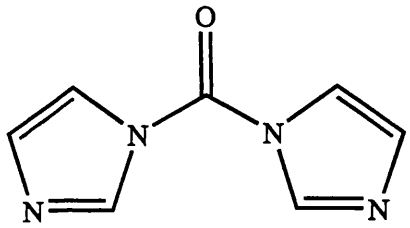


77a-g **79**

This mixture of half-acid amides **78** and **79** must be dehydrated (and cyclised) to yield the methylsuccinimide and there are a number of ways of achieving this conversion (Coates *et al.* 1994b; Jacyno *et al.* 1996). The method of Blagbrough and co-workers (Coates *et al.* 1994b) is to dehydrate *in situ* with 1,1'-carbonyldiimidazole (CDI) **81.** This method works well and was the method of choice in all cases attempted. The CDI is added to the reaction mixture after 16-24h and the reaction was stirred for a further 24h.

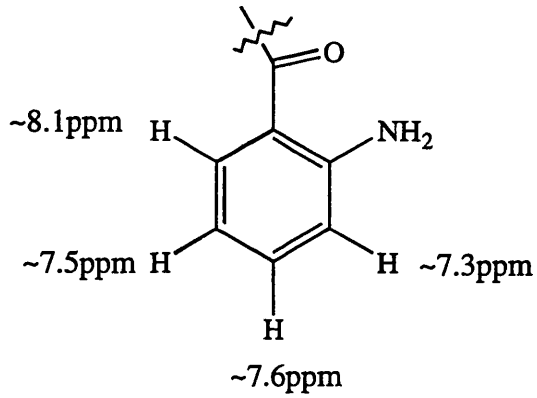


79 **80a-f**



1,1'-carbonyldiimidazole **81**

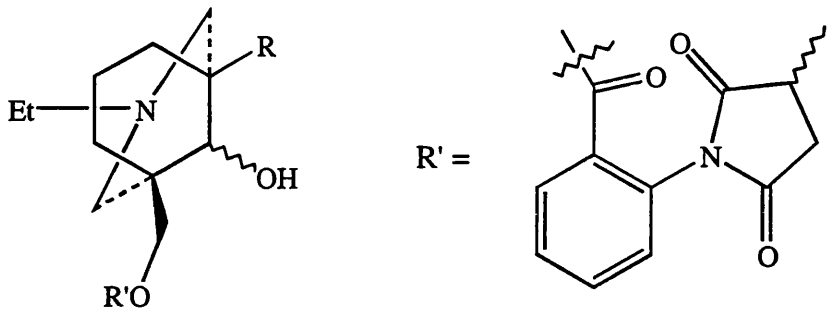
After addition of CDI **81**, tlc monitoring showed the appearance of a new compound with Rf ~0.3 (2:3 EtOAc-hexane). The conversion of the anthranilic esters into 2-(methylsuccinimido)benzoate esters has a moderate yield (50-60%). The 2-methylsuccinimido compounds **80a-f** exhibit some interesting spectroscopic data. The pattern of aromatic signals in the proton NMR has changed so that each individual proton is now evident (integrating 1:1:1:1).



Reaction between 2-aminobenzoate ester **77g** (R = H) and methylsuccinic anhydride did not however yield the desired product, but instead yielded a compound of higher mass than expected (543.4 as opposed to 414 which is required for $C_{23}H_{30}N_2O_5$) which could not be identified. This is probably due to a side reaction involving the methylsuccinic anhydride (or the half acid amide) and the secondary alcohol which is less hindered than in the other compounds due to the lack of substitution at the γ-position.

Using this reaction, the series of MLA **1** analogues **80a-f** has been synthesised in a good yield over 5 steps. These compounds have been tested by biologists at Zeneca

Agrochemicals for both insecticidal activity and for nAChR affinity in a radiolabeled iodo-α-bungarotoxin displacement assay (using neuronal nAChR from blowfly).



80a-f

Compound	R	% displacement of α-BgTx at 10ppm
80a	*n*-pentyl	53
80b	*n*-butyl	20
80c	allyl	37
80d	cyclohexylmethyl	34
80e	cyclopentyl	47
80f	benzyl	39

Compounds **80a-80f** did not show significant insecticidal activity against 6 different insect species. The insect species in which the screens were performed are:

Tetranychus urticae 2 spotted spider mite

Musca domestica housefly

Myzu persicae peach potato aphid

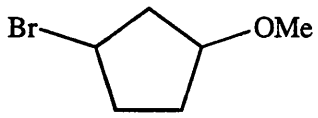
Spodoptera exiqua lesser armyworm

Heliothis virescens tobacco budworm

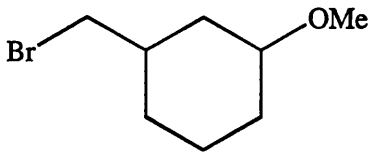
Meloidogyne incognita rootknot nematode

2.8 Attempted Michael additions using a dianion

As one aim of this project was to create small molecule analogues of MLA **1**, some consideration of the oxygenation pattern of MLA is essential. One method of introducing oxygenation into our analogues would have been to alkylate with an electrophile which has an oxygen functionality in place (e.g. 1-bromo-3-methoxycyclopentane **82** or (3-methoxy)cyclohexylmethyl bromide **83**).

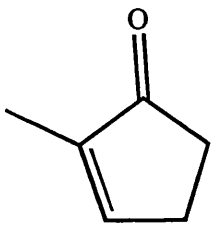


82

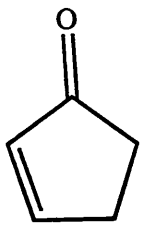


83

However, neither of these alkyl halide compounds (**82** or **83**) was commercially available and their synthesis may have been problematic. This led to the idea of performing a Michael addition on an enone with the dianion as the Michael donor. This reaction has no obvious literature precedent and so the conditions required were not known. Using this direct approach, we tried a number of reaction conditions and the results are summarised below. All reactions were attempted with the dianion prepared in the usual way (1.2 equivalents of NaH, then 1.05 equivalents of *n*-BuLi, THF, -78°C).



84



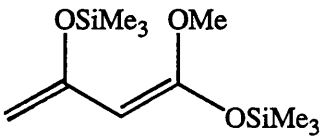
85

Enone	Conditions	Result
2-methylcyclopent-2-enone **84**	30% DMPU/THF, -78°C rising to 0°C	No Michael addition Mainly polymerisation
2-methylcyclopent-2-enone **84**	30% DMPU/THF, -78°C rising to 0°C 0.5 eq. CuI	No Michael addition Mainly polymerisation
2-methylcyclopent-2-enone **84**	THF, -78°C rising to 0°C	No Michael addition Mainly polymerisation
cyclopent-2-enone **85**	30% DMPU, -78°C rising to 0°C	No Michael addition Mainly polymerisation

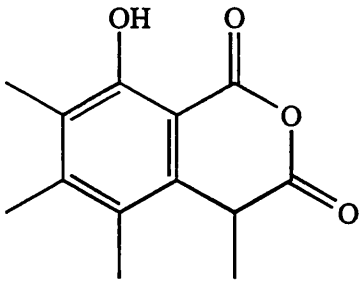
In all cases, there was a small amount (generally 5% or less) of 1,2-addition in an aldol fashion. Weiler showed that it is possible to perform aldol reactions with dianions of ethyl acetoacetate (Huckin and Weiler 1971) showing that the dianion reacts well in a 1,2-addition to a carbonyl. Therefore, the Michael addition of a dianion is difficult to perform as the dianion is either too basic and causes a polymerisation reaction to occur with the enone reacting with itself, or it is too "hard" a nucleophile and attacks the harder centre in the enone i.e. 1,2-addition across the carbonyl double bond. 2-Methylcyclopent-2-enone **84** was replaced by cyclopent-2-enone **85** as it was thought the methyl group may be hindering the reactivity due to the electron donating (inductive) effect of the methyl group. The solvent system also appears to be an important consideration as a large number of Michael additions are performed under protic conditions (e.g. ethanol or methanol). The use of DMPU in this reaction seemed to have no beneficial effects and may even hamper the process. The use of copper(II) in Michael reactions has literature precedent (Desimoni *et al.* 1995). The copper (II) forms higher

order cuprates which are softer nucleophiles and preferentially attack in a 1,4-Michael-type fashion. However, the addition of a copper salt was not effective in this case. This could be due to non-formation of the cuprate or perhaps the formation of more tightly bonded higher order cuprate complexes which are too stable to react.

There is literature precedent (Chan and Brownbridge 1981; Brownbridge *et al.* 1983; Miyashita *et al.* 1984) for the formation of a di-silyl enol ether of a β-ketoester which acts as a dianion equivalent. The silyl enol ethers react under acid conditions ($TiCl_4$ as a Lewis acid) and can add in a 1,2 aldol fashion as well as in nucleophillic displacement reactions (Brownbridge *et al.* 1983). There is some evidence, mainly in self condensation reactions, that these enol ethers can react in conjugate 1,4-Michael type reactions (Brownbridge *et al.* 1983). Using the disilyl enol ether of methyl acetoacetate **86**, Chan and Brownbridge have been able to synthesise biomimetically the naturally occurring aromatic anhydride sclerin **87** (Chan and Brownbridge 1981; Brownbridge *et al.* 1983).

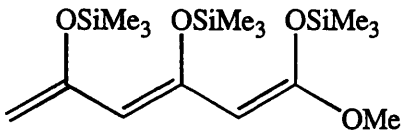


86

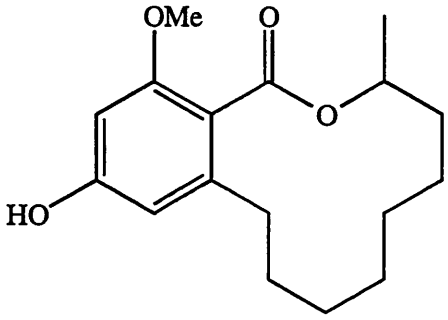


87

In a later publication, Chan and Stössel reported the use of the trisilyl enol ether **88** as a trianion equivalent of a β-ketoester in the synthesis of the natural product lasiodiplodin **89** (Chan and Stössel 1986).

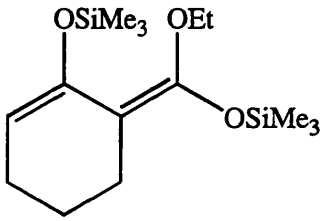


88



89

It may be possible in future work to investigate the use of the methods developed Chan and Brownbridge to react, via the γ-position, ethyl 2-oxocyclohexane-1-carboxylate **34** in a conjugate 1,4-Michael addition reaction using the disilyl enol ether **90**.



90

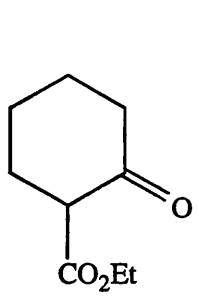
In 1987, Mukaiyama reported that "the Michael reaction of a metal enolate, especially with an αβ-unsaturated ketone still remains a formidable problem" (Mukaiyama *et al.* 1987). These results show that it is not yet possible to make the bond analogous to C10-C11 in MLA via a direct Michael addition of the dianion. However, Mukaiyama (Mukaiyama *et al.* 1994) has also shown that the use of tin (II) enolates can facilitate Michael additions to αβ-unsaturated ketones. Therefore, it may be possible in future work to undertake an investigation into tin (II) enolate techniques with the mono-enolate of a protected cyclic β-ketoaldehyde. After deprotection, this aldehyde could still undergo a double Mannich reaction and hence allow the synthesis of some AE-bicyclic MLA mimics.

Chapter 3

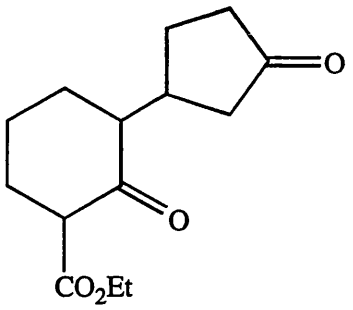
AN 1,3-ESTER MIGRATION APPROACH TO γ-SUBSTITUTED β-KETOESTERS

3.1 Design of synthetic targets

As the synthesis of the γ-substituted β-ketoester **91** was not possible by direct Michael addition of the dianion of ethyl 2-oxocyclohexane-1-carboxylate **37** to cyclopent-2-en-1-one **85** (see Chapter 2) alternative routes to the synthesis of **91** were sought.

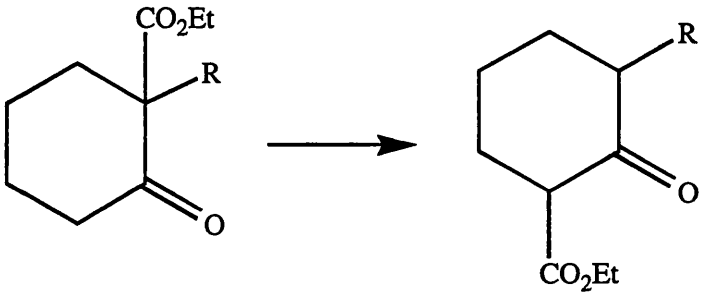


37



91

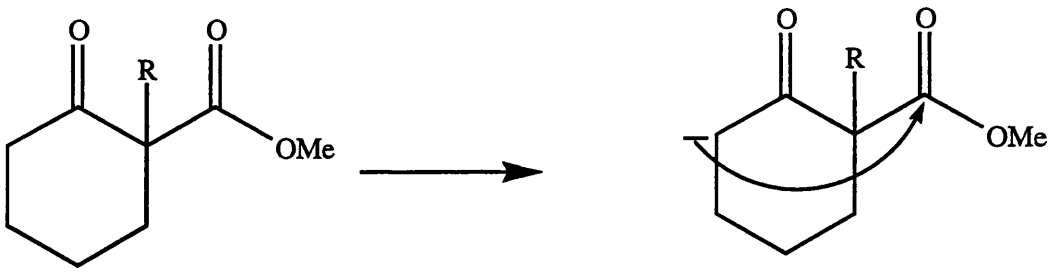
Habi and Gravel (1994) reported an efficient synthesis of γ-substituted β-ketoesters starting from the corresponding α-substituted β-ketoesters by base induced 1,3-ester shift. Several examples were presented and the reaction appeared to be general. Openshaw and Robinson (1937) also reported a similar 1,3-ester shift which they used in the synthesis of the carbon skeleton of strychnine. In this chapter, attempts at using these literature methodologies to synthesise γ-substituted β-ketoesters **66** from the corresponding α-substituted β-ketoesters **92** are discussed.



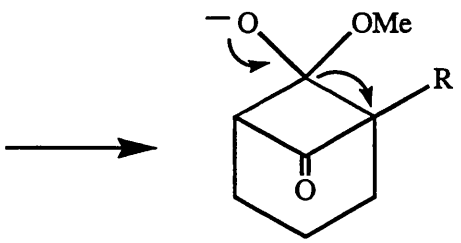
92 **66**

3.2 Rearrangement of α-substituted β-ketoesters to γ-substituted β-ketoesters

Habi and Gravel (1994) reported their findings studying Cope rearrangements of α-allyl β-ketoester silyl enol ethers. Their method uses potassium hydride as the base, with 18-crown-6 (to give a naked hydride ion) in THF. The reaction is rapid and takes around 15min to reach completion. The mechanism they proposed involves the formation of ketone enolate **93** (although drawn as the carbanion below) which reacts intramolecularly with the ester to form the cyclobutanone intermediate **94**.



92 **93**

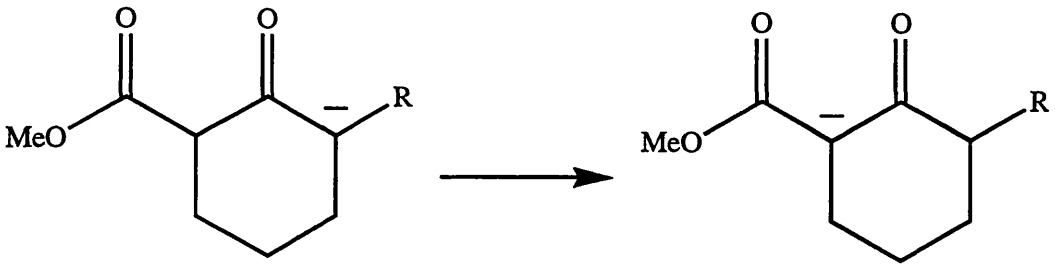


94

R = allyl

The highly strained cyclobutanone intermediate **94** collapses to give anion **95** (with the carbanion as a leaving group). In a Claisen (or Dieckmann) ester condensation (to which the first step of this reaction is analogous) the leaving group would be the methoxide anion yielding a 1,3-dicarbonyl compound, but in this case the product would be a four membered ring with two sp^2 hybridised ketone carbonyl carbon atoms and the resultant ring strain makes the β-ketoester product more favoured. Carbanion **95** then rearranges

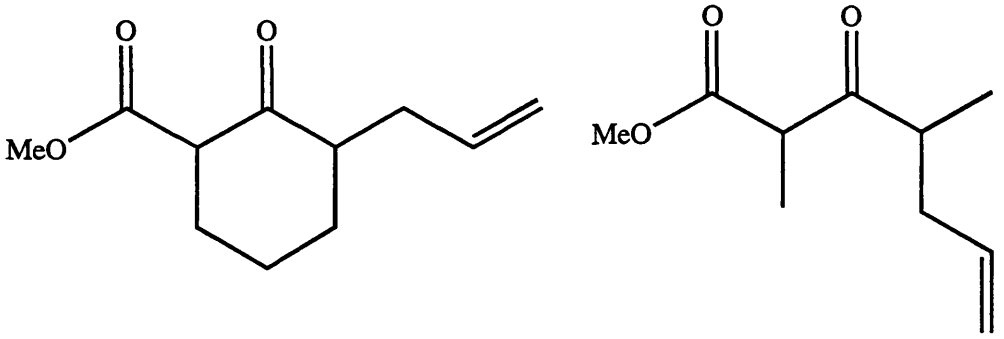
to the more stable carbanion **96** (almost certainly as its enolate) as the final step. Acidic aqueous work-up yields the desired γ-substituted β-ketoester.



95 **96**

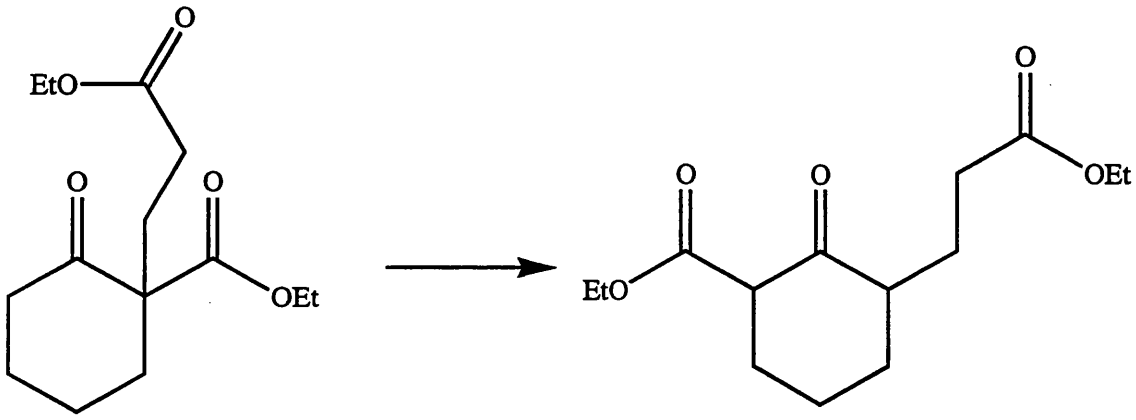
R = allyl

Habi and Gravel reported the synthesis of 5 different γ-substituted β-ketoesters in good to excellent yields. Both cyclic and acyclic rearrangements are reported including the synthesis of **97** and **98** (no mention of stereoselectivity was made and it would appear that this reaction produces a mixture of diastereoisomers). Also discussed is the importance of the metal ion used ($K^+ > Na^+ > Li^+$) in increasing both the yield and the reaction rate.



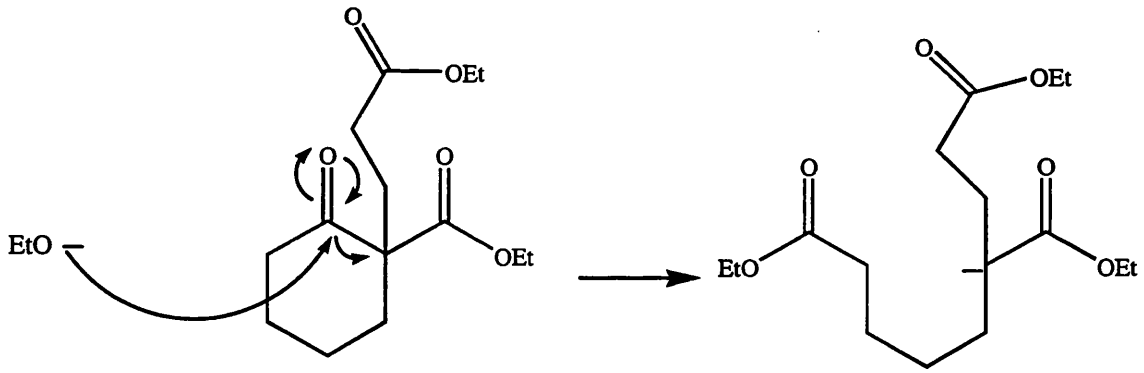
97 **98**

Openshaw and Robinson (1937) reported, in preliminary synthetic studies on the toxic alkaloids strychnine and brucine, the rearrangement of one particular α-substituted β-ketoester **99** (synthesised by alkylation of ethyl 2-oxocyclohexane-1-carboxylate **37** with ethyl β-chloropropionate) to the γ-substituted β-ketoester **100**.

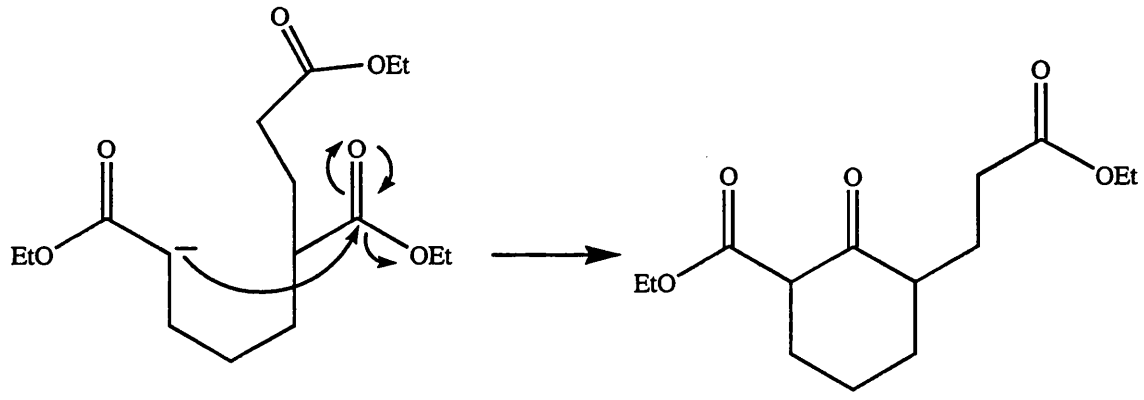


99 **100**

This transformation was afforded by refluxing in ethanolic sodium ethoxide (rather than using potassium hydride) and Openshaw and Robinson proposed a different mechanism to that proposed by Habi and Gravel. They postulated that the mechanism was "obviously alcoholysis and ring closure in a new position". This mechanism involves the formation of the triester carbanion **101** as the key intermediate followed by rearrangement to carbanion **102** which self-condenses to give keto-diester **100**.



99 **101**

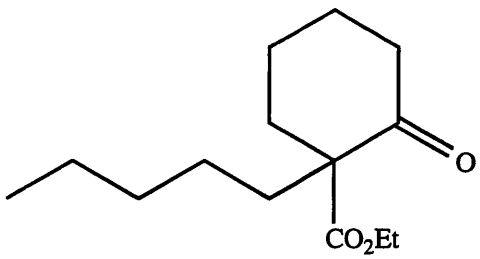


102 **100**

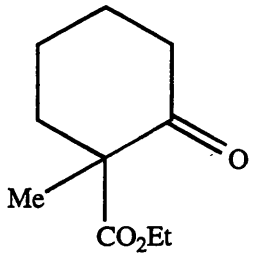
The reaction is complete when ethoxide deprotonates the product **100** (removing the most acidic α-proton) trapping the product as the corresponding sodium enolate. This reaction was not shown to be general by Openshaw and Robinson, but as the product (**100**) was similar to those we require, it was thought that this may be a feasible route to γ-substituted cyclic β-ketoesters such as **91**.

3.3 Synthesis of α-substituted β-ketoesters

Using these two literature methods we intended to synthesise novel γ-substituted β-ketoesters from the corresponding α-substituted β-ketoesters. We prepared some oxygenated α-substituted β-ketoesters (such as **92**) to be used in the migration reactions. In the first instance, *n*-pentyl and methyl substituted ketoesters **103** and **104** were synthesised by alkylation of ethyl 2-oxocyclohexane-1-carboxylate with the corresponding alkyl iodides (using DMPU **67** as a co-solvent in anhydrous THF).



103

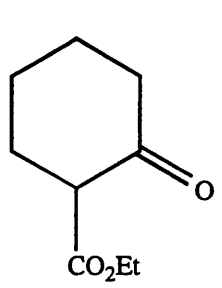


104

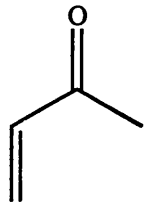
Compounds **103** and **104** were difficult to purify by distillation and silica gel chromatography and, in order to obtain pure samples for use in the migration reactions, the crude product was used in a double Mannich reaction. All of the unalkylated starting material reacted in the double Mannich, but the desired product could not. This allowed the purification of the α-substituted β-ketoesters **103** and **104**. Analysis of the PMR

spectra of **103** and **104** show that the enolisable α-methine proton (between two carbonyls) at ~3.5ppm was not present, illustrating that α-substitution had occurred.

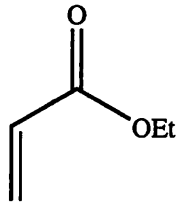
Synthesis of the α-substituted β-ketoesters with oxygenation in the substituent was achieved by Michael addition of ethyl 2-oxocyclohexane-1-carboxylate **37** to α,β-unsaturated carbonyl compounds. Michael addition to methylvinyl ketone **105** (but-1-en-3-one) gave **107** and addition to ethyl acrylate (ethyl propenoate) **106** gave **99**. Compound **99** was also synthesised by alkylation of the sodium enolate **108** with ethyl 3-chloropropanoate as previously described by Openshaw and Robinson (1937).



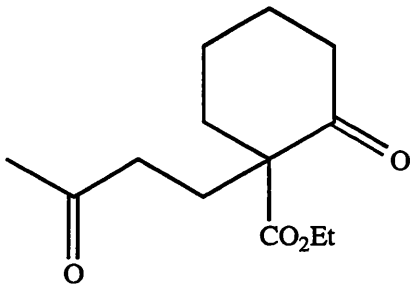
37



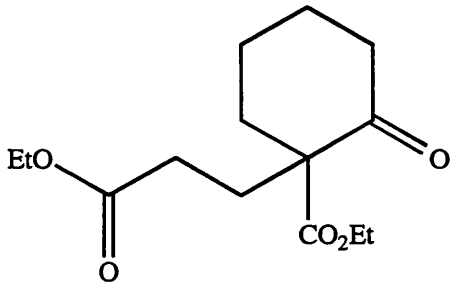
105



106

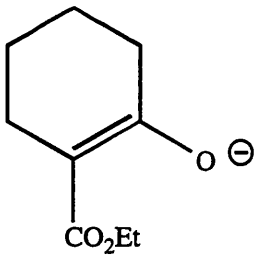


107

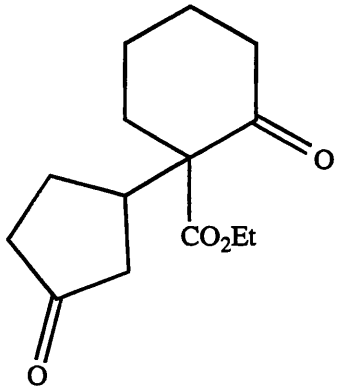


99

Addition of ethyl 2-oxocyclohexane-1-carboxylate **37** to cyclopent-2-en-1-one **85** gave the analogous α-substituted β-ketoester **109**, reacting with enolate **108** of ketoester **37**.

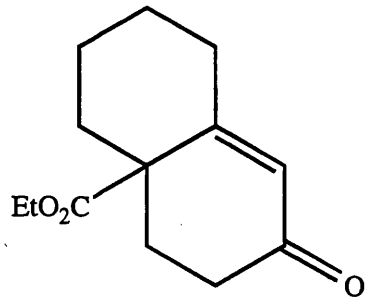


108



109

Several attempts at performing these Michael additions were made (varying the solvent and base) in order to determine the optimum conditions. The use of stoichiometric sodium ethoxide as base did not yield the adducts of a 1,4-conjugate addition in good yield. Thus, attempted Michael additions to cyclopent-2-en-1-one **85** gave only starting β-ketoester and a polymeric product assigned to cyclopent-2-en-1-one **85** self-condensation. In the case where addition to methylvinyl ketone **105** was attempted, the main product formed was decalone **110** (Dreiding and Tomasewki 1955) which is the Robinson ring-annelation product (i.e. the required 1,4-conjugate addition followed by an intramolecular aldol condensation and dehydration afforded the α,β-enone).



110

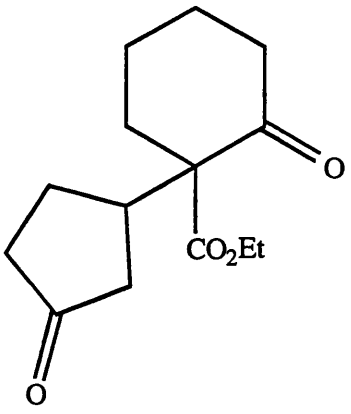
That decalone **110** was formed showed that Michael addition had occurred, but that sodium ethoxide is a strong enough base to enolise the resulting ketone facilitating aldol condensation onto the cyclohexanone ketone. Dehydration of the β-hydroxy ketone (aldol) gave enone **110**. This leads to the conclusion that a milder base was

required to afford the Michael addition adducts as the resulting ketone from the addition is reactive under basic conditions. Changing to stoichiometric quantities of Triton B™ (benzyl trimethyl ammonium hydroxide) did not yield the desired Michael adducts, but using a catalytic amount (<1%) of this mild base (with methanol as the solvent) allowed us to prepare the desired adducts **107**, **99** and **109** albeit in modest yields (Bergmann *et al.* 1959; Ginsburg and Pappo 1951).

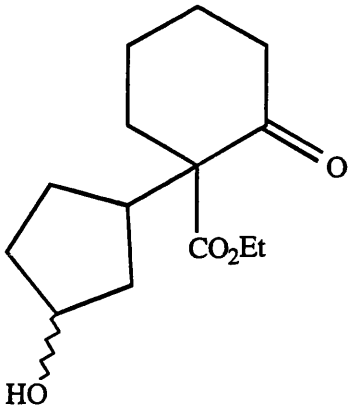
Compound	Yield (%)
107	30
99	52
109	59

The relatively poor yield of **107** can be explained by the ready formation of the Robinson annelation product **110**. Adduct **99** could only form a 4-membered ring as the product of a Robinson annelation reaction (which is not favoured) and **109** could form a relatively strained tricycle (either a 6-6-5 or a 6-5-5 tricycle) which is also not favoured. Analysis of the PMR spectra of Michael adducts **107**, **99** and **109** show that the addition has occurred regioselectively at the α-position (as the enolisable α-methine proton is not present). Analysis of the CMR spectra shows the presence of new carbonyl signals either another ketone (~210ppm) in **107** and **109**, or another ester (~170ppm) signal in **99**. As in the natural product, the oxygen at C14 (norditerpenoid numbering) is at a lower oxidation level than in compound **109** (an alcohol as opposed to a ketone) an attempt was made to reduce regioselectively the newly introduced ketone before ester migration was performed. As the newly added ketone functionality appears to be less sterically hindered than the starting ketone in the molecule, we predicted that using 1 equivalent of

hydride (from sodium borohydride) would regioselectively reduce the less hindered ketone to the corresponding secondary alcohol.



109

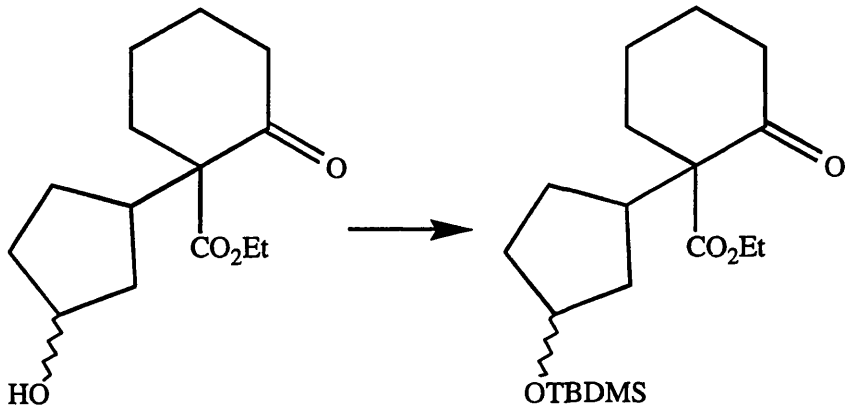


111

This reaction proceeded as predicted using 0.3 molar equivalents of sodium borohydride in ethanol giving diastereoisomeric alcohol **111** in an excellent yield (92%). Analysis of the PMR spectra was not conclusive, but the CMR spectra showed the loss of the cyclopentanone ketone (217.98/217.76ppm) signals and the introduction of the secondary alcohol signal (73.43/73.28ppm). Mass spectroscopy confirmed the transformation had increased the mass by 2 Daltons (CI gives 255; $C_{14}H_{22}O_4$ requires 254).

Strong bases are required in the ester migration and an alcohol would be readily deprotonated and may interfere with the reaction, so the secondary alcohol **111** was protected as its *t*-butyldimethylsilyl ether. Synthesis of the required methyl ether would have been preferable, but selective *O*-methylation would have been difficult given the presence of the ketone (with acidic α-protons) within the molecule. Protection of the alcohol was afforded with *t*-butyldimethylsilyl chloride, using imidazole as a base which gave silyl ether **112** in good yield (72%). Purification of this significantly less polar product was achieved by passing through a short column of silica gel (eluting with 1:9 EtOAc-hexane). Analysis of the PMR spectra showed the presence of the *t*-butyl protons at 0.84ppm and the dimethylsilyl protons appeared at 0.00ppm. Analysis of the

mass spectrum showed the significant increase in mass expected (CI gives 369; $C_{20}H_{36}O_4Si$ requires 368).

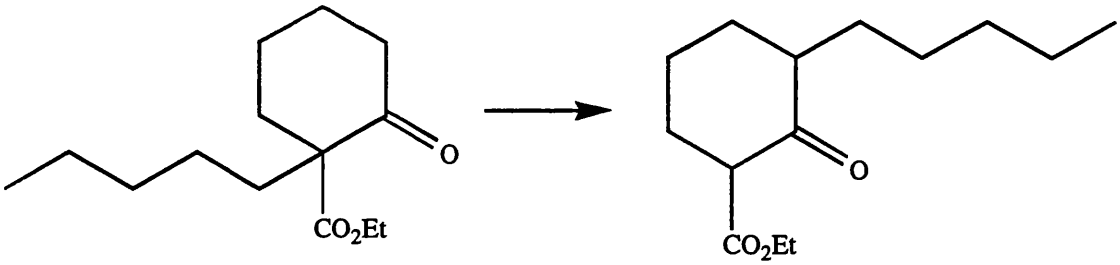


111 **112**

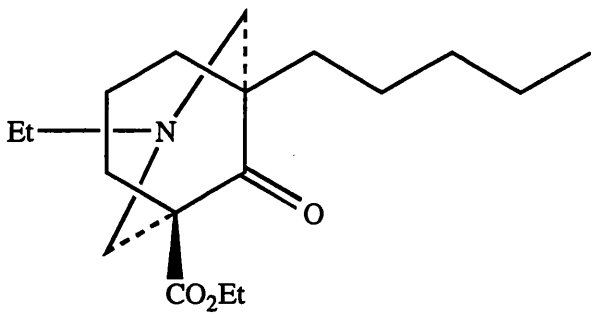
This series of reactions gave 7 different compounds with which to attempt the ester migration reactions using the conditions described by separately by Habi and Gravel (1994) and Openshaw and Robinson (1937).

3.4 Attempted ester migrations

The first migration attempted was the conversion of the *n*-pentyl substituted compound **103** into **66a** (which had been previously synthesised by the dianion route) using the conditions of Habi and Gravel (1.4 equivalents of KH and 3 equivalents of 18-crown-6 in anhydrous THF for ~30min). A relatively poor recovery of crude product was obtained (53%) and this was not purified. Crude **66a** was reacted in a double Mannich reaction to give bicyclic β-ketoester **64a** in good yield (75%). This gave an overall yield of 42% starting from the α-substituted β-ketoester.

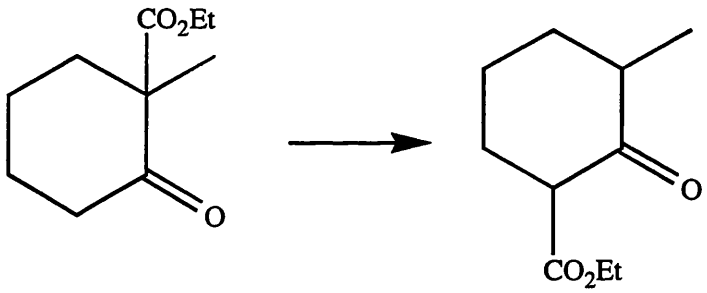


103 **66a**



64a

Ethyl 1-methyl-2-oxocyclohexane-1-carboxylate **104** was then reacted under the same conditions giving a 41% crude yield of the γ-substituted β-ketoester **113.** Analysis of the PMR spectra of **113** shows that the enolisable α-methine proton signal at ~3.5ppm (between the two carbonyls) is present which was not there in the starting material.

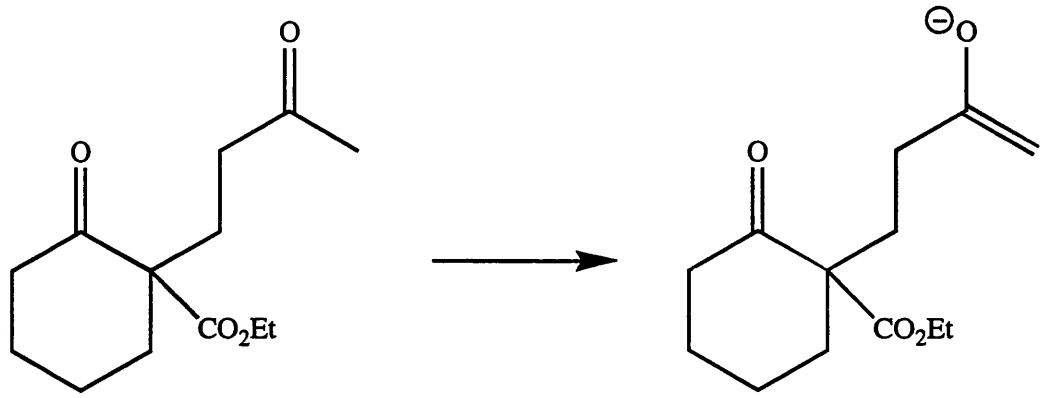


104 **113**

Attempts at converting the α-alkyl substituted β-ketoesters into the corresponding γ-substituted compounds using the method described by Openshaw and Robinson (refluxing in an ethanolic solution of sodium ethoxide) did not yield the desired compounds and a quantitative recovery of the starting material was possible.

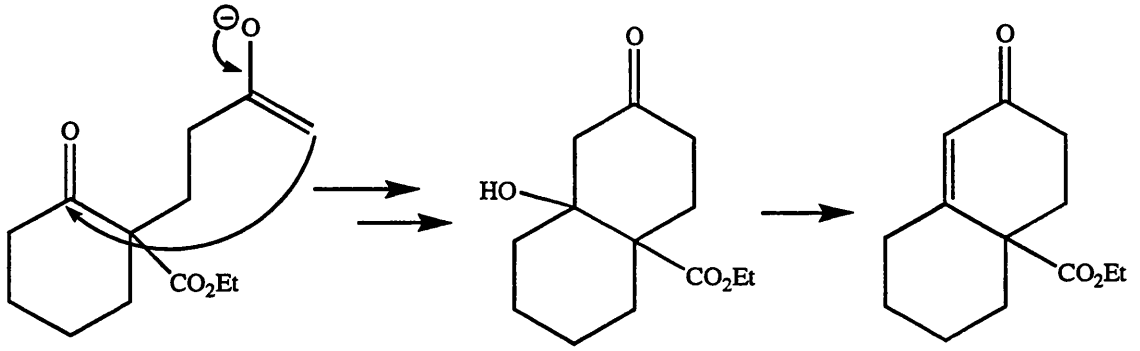
Attempts at migrating the 1-(butan-3-one) substituted adduct **107** using Openshaw and Robinson's method gave the annelation product **110** (observed by tlc

analysis and comparison with the authentic material) and this reaction was not worked up. This was expected given that bicyclic enone **110** was obtained whilst attempting to synthesise **107** with sodium ethoxide as the base.



107 **114**

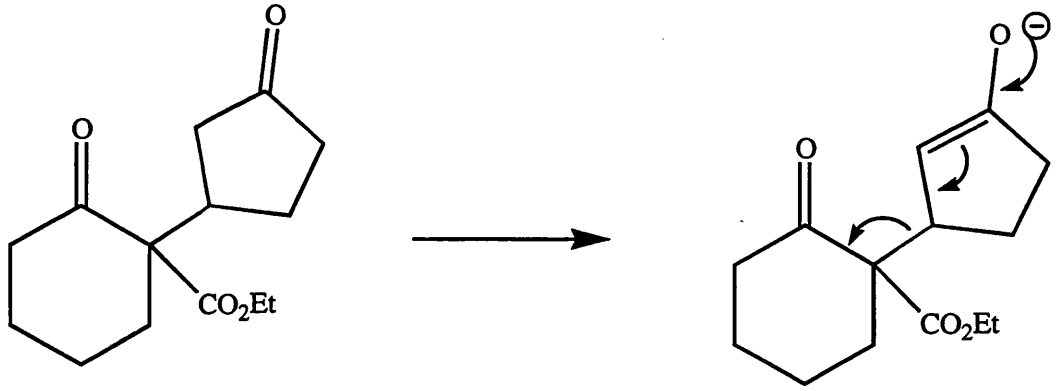
Deprotonation of **107** gave enolate **114** which then self-condensed to give (after a proton transfer) bicyclic aldol **115**. Dehydration of bicyclic aldol **115** yielded the α,β-enone **110**.



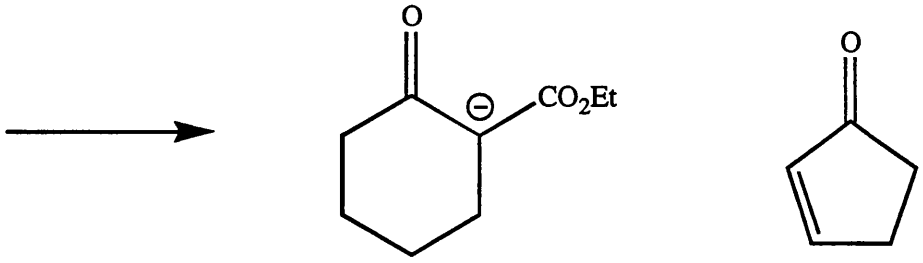
114 **115** **110**

The more rigorous conditions of the Habi and Gravel methodology were not tried on the reactive di-ketone **106** as we thought that the potassium hydride used would cause the formation of **110** more readily than sodium ethoxide.

Using the cyclopent-2-en-1-one Michael adduct **109** under both sets of conditions (potassium hydride and sodium ethoxide) gave ethyl 2-oxocyclohexane-1-carboxylate as the main product presumably by a reverse Michael addition mechanism or by a simple β-elimination.

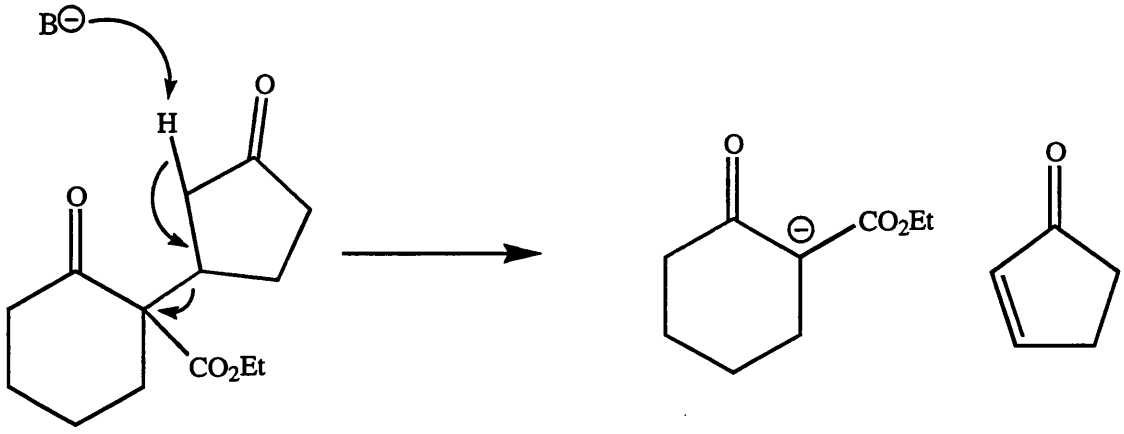


109 **116**



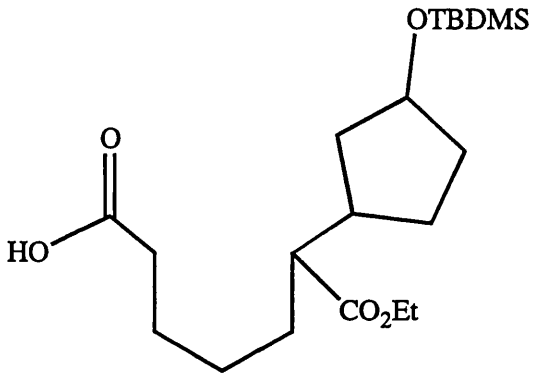
108 **85**

In the reverse Michael addition mechanism, the first step is the formation of enolate **116**, which then collapses eliminating enolate **108** (represented as the carbanion) of ethyl 2-oxocyclohexane-1-carboxylate **37** which is a stable anion and hence a good leaving group. The β-elimination mechanism does not involve enolate **116**, but the base (hydride or ethoxide) deprotonates α- to the cyclopentanone carbonyl and this deprotonation is accompanied by elimination of the ethyl 2-oxocyclohexane-1-carboxylate **37** anion as a leaving group.



109 **108** **85**

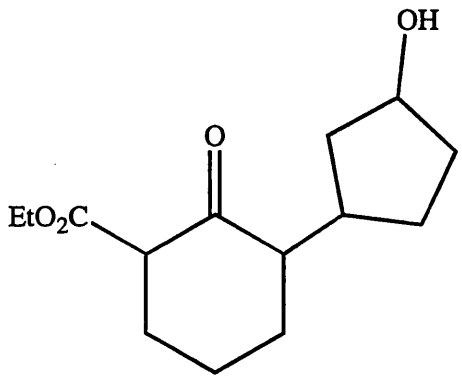
Attempts at performing ester migrations with the 1-(cyclopentan-3-ol) substituted compound **111** and the silyl protected compound **112** using the Openshaw and Robinson conditions failed and quantitative recovery of starting materials was possible. Using the Habi and Gravel conditions, with the silyl protected compound **112** did not yield the desired γ-substituted β-ketoester and recovery of unreacted **112** was also possible. However, there was some degradation of **112** to give a more polar by-product which was not conclusively identified, but was probably acid **117** (an hydrolysis product from the work-up).



117

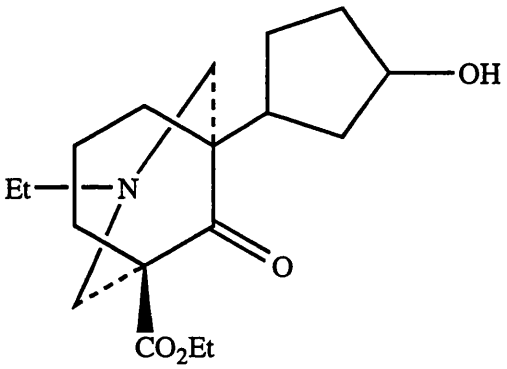
Evidence for this structure is the loss of the ketone carbonyl signal (207ppm) in the CMR spectra and the presence of signals at ~175ppm which may be the acid carbonyl. The mass spectrum provides the most conclusive evidence for the structure **117** as the molecular ion in the CI (isobutane) has the correct mass (386.9 $C_{20}H_{38}O_5Si$ requires 386) and the fragmentation of this molecular ion (the loss of 46 (EtOH), the loss of 58 (*t*-butyl) and the loss of 132 (OSiTBDM)) represent reasonable breakdown of this structure.

Rearrangement of **111** (using 2.2 equivalents of KH and 4 equivalents of 18-crown-6 to compensate for the presence of the alcohol) to yield γ-substituted β-ketoester **118** proceeded, but only in a poor yield (4%) with some recovery of starting material possible (21%).



118

The PMR spectra of **118** show the presence of the α-methine proton (a multiplet at ~3.4ppm) and also the presence of the methine next to the hydroxyl group (a multiplet at ~4.3ppm). The CMR spectra are poorly resolved due to the small amount of material available (~4mg), but the ester and ketone carbonyl signals are present and signals representing the three methines can be seen by using DEPT experiments (although the diastereoisomeric nature of **118** means that multiple signals are observed). Mass spectroscopy (CI; isobutane) gives a molecular ion of 254.9 ($C_{14}H_{22}O_4$ requires 254) confirming the structure of **118**. Repetition of this experiment followed by use of the crude product in a double Mannich reaction yielded the bicyclic ketoester **119** in a poor yield (2.6% over 2 steps).

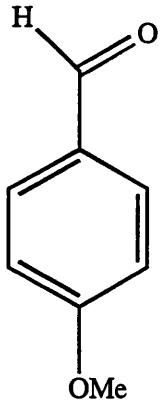


119

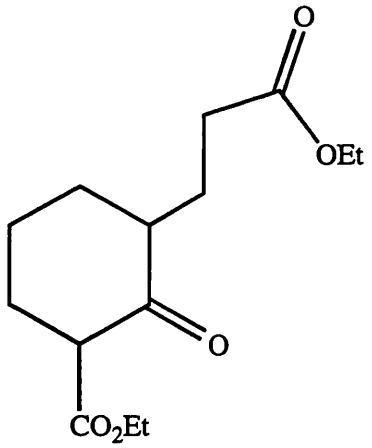
Analysis of the PMR spectra of **119** shows the presence of two ethyl groups (OCH_2CH_3 quartet at ~4.2ppm and triplet at ~1.2ppm; NCH_2CH_3 quartet at ~2.5ppm and triplet at ~1.1ppm) as well as the usual pattern of methylenes from the bicyclic

system. Examination of the CMR spectra show the presence of both an ester and a ketone carbonyl signal (171.52 and 214.23ppm respectively) as well as the methine adjacent to the hydroxyl functional group. Many of the signals are "doubled up" due to the presence of diastereoisomers within the product mixture as expected. Analysis of the mass spectrum shows a molecular ion of 324.1 ($C_{18}H_{27}NO_4$ requires 323) confirming the structure.

Finally, attempts to repeat the transformation achieved by Openshaw and Robinson were made. Using the conditions of Habi and Gravel, some rearrangement occurred to give **100**, but the reverse Michael reaction (or β-elimination) to give ethyl 2-oxocyclohexane-1-carboxylate **37** also occurred. This reaction was monitored by tlc with anisaldehyde **120** visualisation (as well as uv activity) clearly showing the formation of cyclic β-ketoester **37** as the reaction progressed (observed as a bright red spot with Rf 0.7, 1:9 EtOAc-hexane).



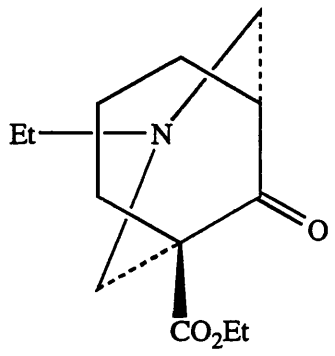
120



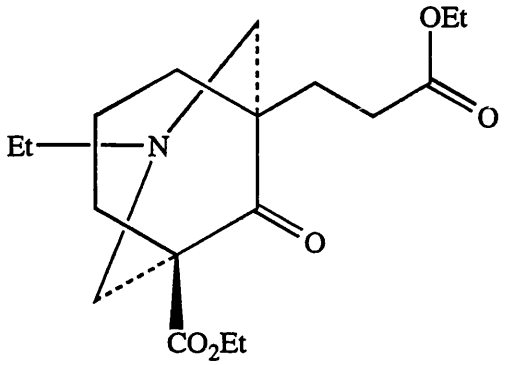
100

Using the crude mixture of β-ketoesters in a double Mannich reaction yielded a mixture of **121** and the unsubstituted bicyclic β-ketoester **4**, formed in a ~1:1 ratio. Thus, an overall yield of bicyclic β-ketoester **121** of 15% over two steps (rearrangement and double Mannich) has been achieved. Analysis of the PMR spectra of **121** shows that there are two different ethyl esters present (only one in **4**) with two quartets at 4.21 and

4.12ppm (2H each, the methylenes) and a multiplet (6H, two overlaid triplets representing the two methyl groups) at ~1.3ppm. The characteristic signals for the [3.3.1]azabicycle are present (a double triplet at ~3.2ppm and the down-field shifted axial proton ~3.0ppm from the C2 carbocyclic methylene).

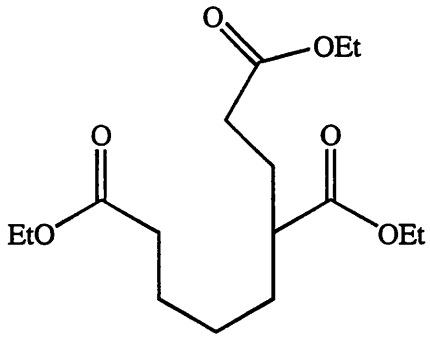


4

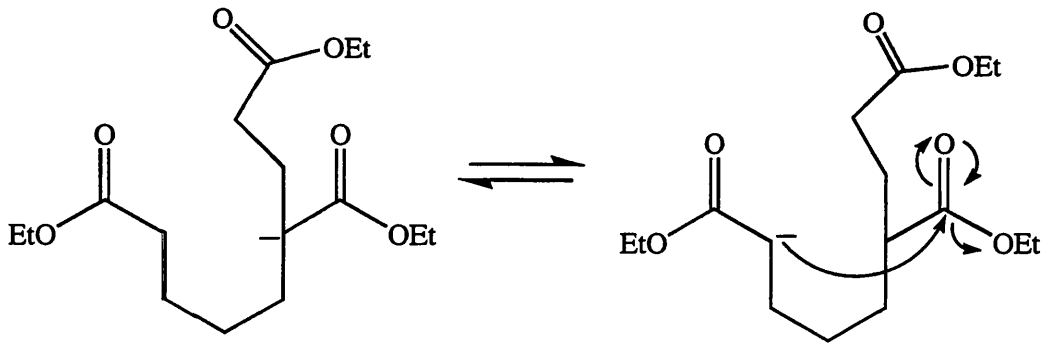


121

Having succeeded in gaining poor-moderate ester migration using the conditions described by Habi and Gravel, an attempt was made to repeat the work of Openshaw and Robinson (converting **99** into **100**) using their conditions (that is sodium ethoxide in refluxing ethanol). However, several attempts (varying the concentration of ethoxide) failed to yield **100**, affording acyclic triester **122** instead. This can only be explained by the failure of the conversion of intermediate enolate **101** into the required enolate **102** or failure of enolate **102** (shown as carbanions for clarity) to add in a Claisen (Dieckmann) ester condensation at the correct centre (as shown below). Product **122** is the conjugate acid of enolates **101** and **102.**



122



101 **102**

Examination of the PMR spectra of triester **122** showed that a simplification of the coupling patterns of the system had occurred (as the acyclic methylene protons were now equivalent) and that, due to the complexity of the ester methylene signals, there were at least two, if not more, ester signals present. Examination of the CMR spectra show that three ester signals are indeed present (175.47, 173.55 and 173.02ppm) and that a new methine signal (44.64ppm) has appeared. This methine is α- to an ester and β- to one of the others. Mass spectroscopy allowed confirmation of the structure, with FAB+ve MS giving a molecular ion of 317.1 Daltons ($C_{16}H_{28}O_6$ requires 316).

Compound **122** cannot undergo a double Mannich reaction as none of the esters is sufficiently activating to facilitate attack on the imine (formed by the addition of ethylamine to formaldehyde). Attempts to force the ring closure of **122** under thermal conditions failed and hence we thought that an impurity from the previous step of the Openshaw and Robinson synthesis (alkylation of 1-carbethoxycylohexan-2-one with ethyl β-chloropropionate) may facilitate the desired conversion. However, exact repetition of the Openshaw and Robinson methods (albeit on a smaller scale) did not yield the desired cyclic β-ketoester. The only product we could obtain was triester **122**.

3.5 Conclusions

The ester migrations that were attempted are summarised in the table below.

	Openshaw and Robinson	Habi and Gravel
α-substituted β-ketoester	**1.2 eq. NaOEt in EtOH at reflux**	**1.4 eq. KH, 3 eq. 18-crown-6, in THF**
pentyl **103**	no reaction, SM recovered	migration 53%
methyl **104**	no reaction, SM recovered	migration 40%
butan-3-one **107**	annelation reaction	not tried
ethyl propionate **99**	formation of triester	some rearrangement and some reverse Michael reaction
cyclopentanone **109**	reverse Michael reaction with 1-carbethoxy-cyclohexan-2-one recovered	reverse Michael reaction with 1-carbethoxy-cyclohexan-2-one recovered
cyclopentanol **111**	no reaction, SM recovered	4% rearrangement with 2.2 equivalents of base and 4 equivalents of 18-crown-6
O-silyl ether **112**	no reaction, SM recovered	no rearrangement, possibly hydrolysis of compound to acid

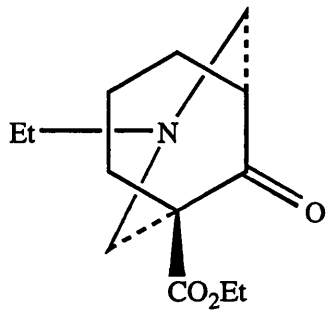
In conclusion, it appears that the ester migration approach to the synthesis of γ-substituted β-ketoesters is not a viable route as in most cases the yields are either nil or poor. In the cases where the yields were moderate (the synthesis of γ-alkyl β-ketoesters **113** and **66a**) another more efficient route was already available (i.e. dianion alkylation).

Chapter 4

AN ACETYLIDE ADDITION STRATEGY TO C5-C6 SUBSTITUTED AE-BICYCLIC ANALOGUES OF MLA

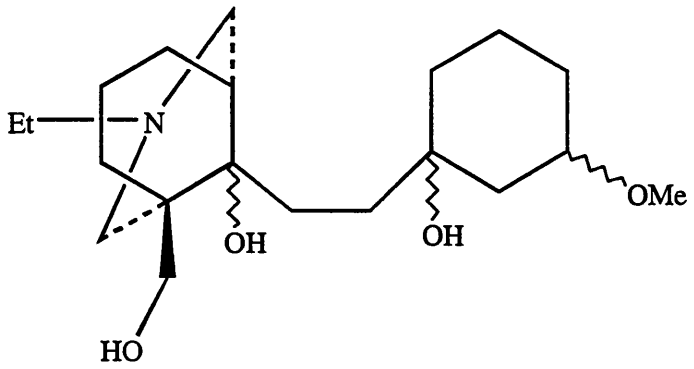
4.1 Design of synthetic targets

In order to advance our SAR studies on the AE-bicyclic analogues of MLA **1**, a series of C5-C6 substituted compounds was needed to investigate the role of the D-ring and the C5-C8 carbons of MLA. We envisaged that the C5-C6 analogues could be synthesised starting from the bicyclic β-ketoester **4** with the substitution being introduced by nucleophilic attack of the ketone functional group (representing C5 of the norditerpenoid skeleton).



4

Targets were designed which are AED analogues of lycoctonine which could be converted into 2-(methylsuccinimido)benzoate esters (analogues of MLA **1**).



123

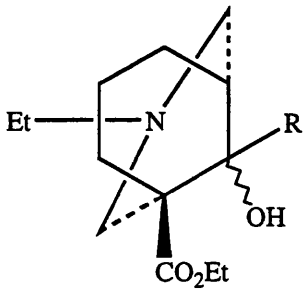
The main target compound was **123**, and this chapter deals with synthetic approaches to this compound and related compounds containing the AED six membered rings of MLA **1**.

4.2 Nucleophilic attack on 3-ethyl-3-azabicyclo[3.3.1]nonan-9-one

The AE-bicycle mimicking piperidinone **4** is easily prepared using the double Mannich reaction of 1-carbethoxycyclohexan-2-one **37** (as described in Chapter 2). The ketone moiety of **4** is a relatively unreactive centre due to steric crowding. The nature of [3.3.1]bicycles means that nucleophilic attack is likely to occur from either face of the ketone yielding an epimeric mixture of the tertiary alcohols formed by nucleophilic addition.

4.2a Grignard and alkyl lithium nucleophiles

In unpublished work by Blagbrough and co-workers (reactions performed by M.S. Othman), treatment of piperidinone **4** with Grignard reagents (such as ethyl or methyl magnesium bromide) or alkyl lithium salts (such as *n*-BuLi) gave tertiary alcohols **124a-c**.



124

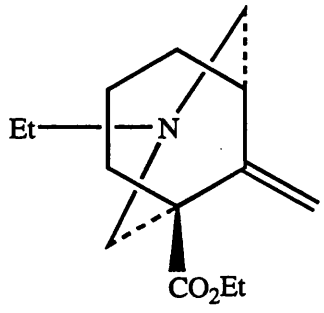
124a) R = Me, **124b**) R = Et, **124c**) R = *n*-Bu

These organometallic reagents attack regioselectively at the ketone over the ester (Buhler 1973; Marshall *et al.* 1979). A mixture of diastereoisomeric alcohols was formed which could be separated (with varying degrees of success) by silica gel chromatography. In order to introduce the D-ring of MLA, however, Grignard reagents or alkyllithium salts with relatively complex structures would be required and the

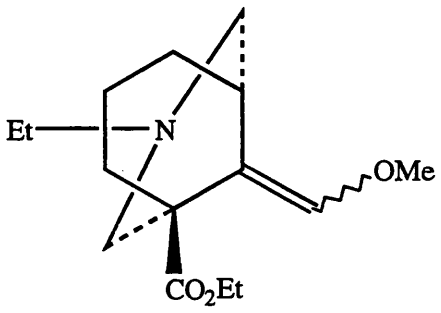
synthesis of such compounds was not considered an efficient way to introduce the D-ring of MLA into the desired analogues.

4.2b Wittig and other phosphorus-containing carbon nucleophiles

Wittig reactions at the ketone of the bicyclic keto-ester, along with Horner-Emmons/Wadsworth Emmons/Wittig-Horner variants, were extensively studied by Dr Géraldine Grangier in unpublished work within our research group, and only small and hence reactive ylids afforded a reaction. A one-carbon homologation of the ketone to yield the exocyclic methylene (**125**) has been performed by Fukumoto and co-workers (Ihara *et al.* 1988) and formation of the methyl enol ether (**126**) has been reported by Blagbrough and co-workers (Coates *et al.* 1994b).



125

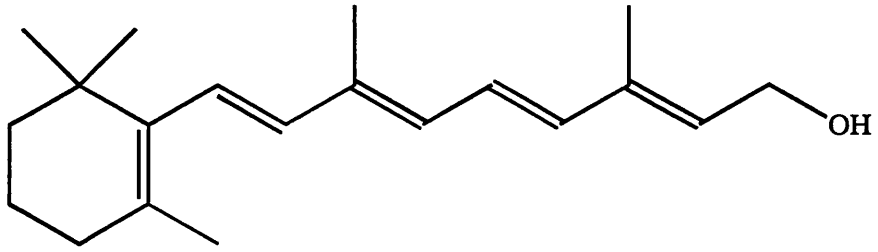


126

The use of more complex ylids (i.e. larger and more hindered) did not yield the desired alkenes. Attempted homologation with different phosphorus stabilised carbon nucleophiles (the more reactive Horner-Emmons type with phosphonates rather than a phosphine) also proved unsuccessful and did not yield the desired alkenes. The failure of these reactions is primarily due to steric hindrance around the ketone and the un-reactivity of ketones in comparison with aldehydes in Wittig-type reactions. This showed that the use of a Wittig reagent had limited synthetic utility in the synthesis of more complex C5-C6 substituted analogues of MLA **1**.

4.3 Acetylide additions to cyclic ketones

The uses of acetylide anions as nucleophiles to attack carbonyls (Weyerstahl *et al.* 1988; Weyerstahl *et al.* 1994) and perform alkylations (Sagar and Scheinmann 1976) have literature precedent and as the acetylide is a linear structure it is less affected by steric constraints than more bulky carbanion nucleophiles. A significant use of acetylide addition to substituted (hindered) cyclic ketones has been in a variety of syntheses of vitamin A **127** (Milas *et al.* 1948; Attenburrow *et al.* 1952; Olson *et al.* 1976; Hollinshead *et al.* 1983). There is also a recent example of stereoselective additions of acetylide anions to ketones using a chiral ligand to promote enantioselectivity (Scharpwinkel *et al.* 1996).



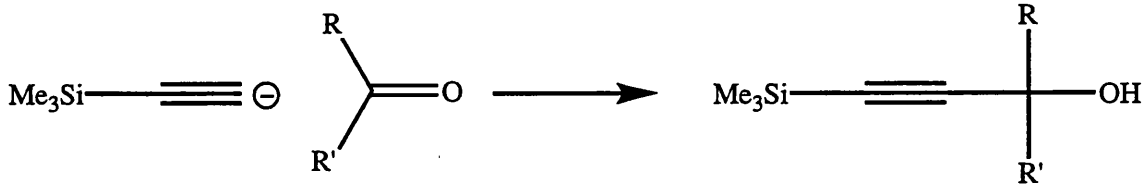
Vitamin A **127**

This improved reactivity over phosphorus ylids, and the increased structural variability over readily available organometallic reagents, allows for the introduction of a two carbon unit representing C6 and C7 of the norditerpenoid skeleton. It is possible to substitute an alkyne at both termini and this allows the flexibility to synthesise more complex analogues of lycoctonine **3** starting from bicyclic β-ketoester **4**. The mono-anion of acetylene is easily prepared using a strong base (e.g. *n*-BuLi, EtMgBr or sodium amide). This anion can then be reacted in a nucleophilic manner. However, using acetylene itself can be problematic as it is possible to form the anion of the newly formed propargylic alcohol (or terminal alkyne) and then this anion can react with another molecule of the electrophile used (ketone or alkyl halide) leading to a mixture of mono-

and di-substituted products. A more selective and ordered synthetic strategy is to used a protected acetylide anion (either formed *in situ* or purchased) which can only react at one terminus, and then deprotect the other terminus and react this via a propargylic alkoxide dianion.

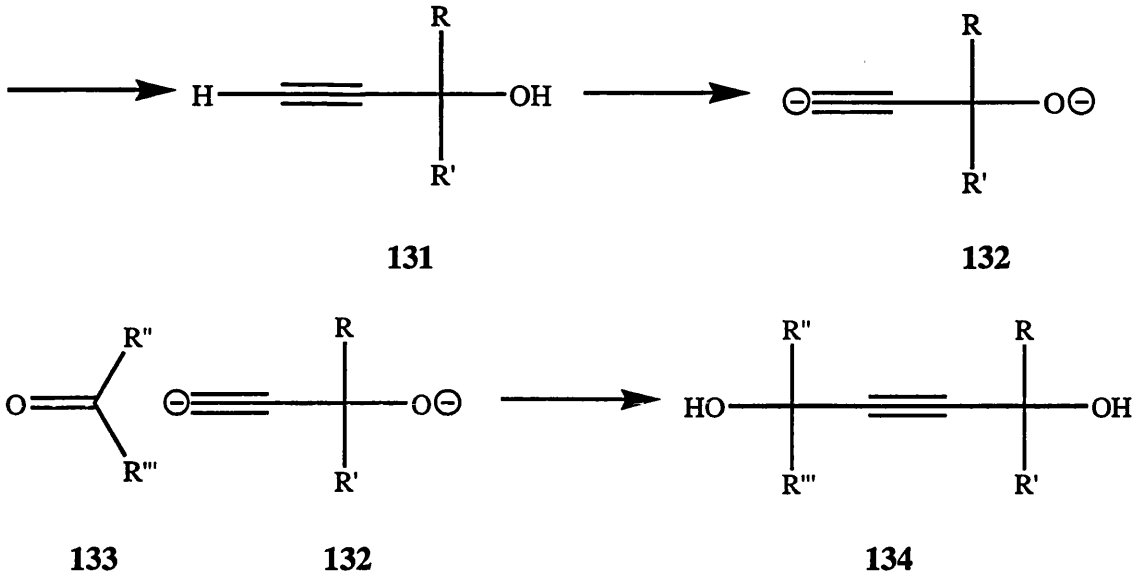
Babler and co-workers recently reported that it is possible to form acetylide anions using a tertiary alkoxide as the base if DMSO is used as the solvent (Babler *et al.* 1996). The ability of a base to deprotonate a terminal alkyne relies upon the difference of the p*K*a of the conjugate acid (the tertiary alcohol in this case) and the alkyne. Babler and co-workers report that the p*K*a varies depending on the solvent allowing the use of an alkoxide as base. Normal values for the p*K*a of a tertiary alcohol is ~17 whereas an alkyne has a p*K*a of ~25. This implies that a strong base (e.g. alkyl lithium, ethane has a p*K*a of 50) is needed to deprotonate a terminal alkyne. In DMSO, the relative acidities change with phenylacetylene having a p*K*a of 28.7 and *t*-butyl alcohol 32.2 showing that a tertiary alkoxide is capable of deprotonating a terminal alkyne.

In order to produce the disubstituted alkyne required to synthesise **123** two ketones must be linked. The first step is to form the acetylide anion **128** with a strong base such as *n*-BuLi or a Grignard reagent (e.g. EtMgBr Weyerstahl *et al.* 1988). Reacting this acetylide with ketone **129** yields the propargylic alcohol **130**.



128 **129** **130**

Deprotection of this alkynyl silane is facile with NaOH in methanol or with tetrabutyl ammonium fluoride (Montalbetti *et al.* 1995). By forming the dianion **132** of the newly formed propargylic alcohol **131** with two equivalents of a strong base (e.g. *n*-BuLi) and then reacting this with another ketone **133** a disubstituted alkyne **134** is formed.



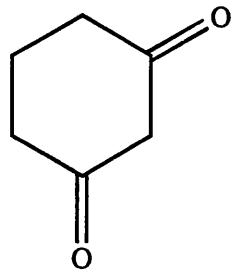
4.4 Synthesis of AED tricycle

Having decided upon the strategy with which to proceed towards the AED analogue **123** of lycoctonine **3**, the two ketones to be linked by the alkyne had to be synthesised. As described in Chapter 2, 3-ethyl-3-azabicyclo[3.3.1]nonan-9-one **4** can be synthesised in a moderate yield in multi-gram quantities by a double Mannich reaction on 1-carbethoxycyclohexan-2-one **37**.

In lycoctonine **3**, the D-ring is embellished with two methoxy substituents at C14 and C16 and a hydroxyl at C8. The hydroxyl at C8 will be introduced into the analogues as the tertiary alcohol produced by acetylide addition to a cyclohexanone (representing the D-ring). By having an appropriately substituted cyclohexanone (with a 3-methoxy) the methoxy at C14 or C16 can be mimicked. Hence, a synthesis of 3-methoxycyclohexan-1-one **135** is required.

This relatively simple compound is not commercially available presumably due to stability problems. We have made this compound by two distinct routes. Firstly, we synthesised 3-methoxycyclohexanone **135** in two steps from cyclohexan-1,3-dione **136**.

Cyclohexan-1,3-dione **136** was converted into the methyl enol ether **137** by reaction with trimethylorthoformate under acid catalysis (pTSA) with methanol as the solvent (82% yield) using a method described by Kraus for the conversion of 1,3-diones into vinylogous acid esters (Kraus *et al.* 1989). Enol ether **137** was then converted into 3-methoxycyclohexan-1-one **135** by catalytic hydrogenation (10% Pd/C, 1 atm, methanol, 43%). This gives a combined yield of only 35% over two steps.



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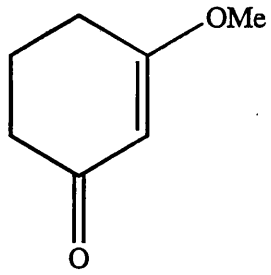
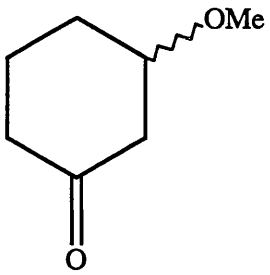
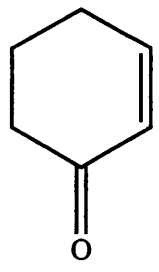


137

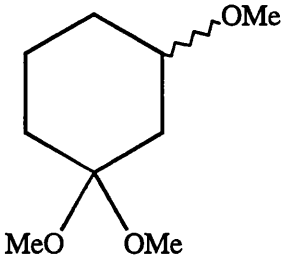


135

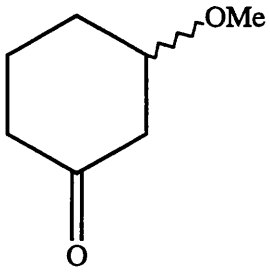
This method of synthesising 3-methoxycyclohexan-1-one **135** is relatively poor yielding and an alternative route was sought. A conjugate 1,4-addition of methanol to cyclohex-2-enone **138** under acid catalysis (conc. H_2SO_4) yields initially the dimethyl ketal **139** which when hydrolysed *in situ* yields 3-methoxycyclohexan-1-one **135** in 72% yield (Lambert *et al.* 1988). This reaction occurs at a relatively slow rate (24-48h) and heating the reaction mixture causes the equilibrium to shift towards the starting enone. Hydrolysis of the ketal is completed, in the work-up procedure, by pouring the reaction mixture into water which affords complete hydrolysis of the dimethyl ketal **139** to the ketone 3-methoxycyclohexan-1-one **135**.



138

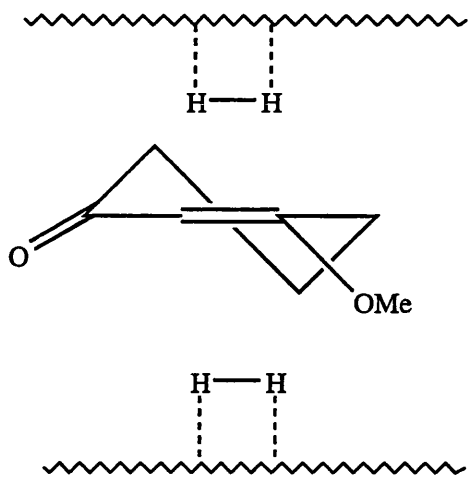


139

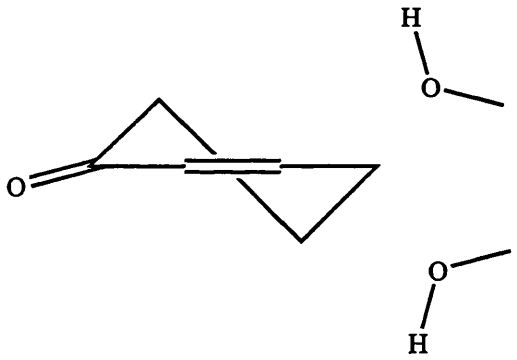


135

Both of these synthetic strategies yield a racemic mixture of 3-methoxycyclohexan-1-one **135** as would be expected with an achiral starting material. This is because the hydrogenation reaction can occur from either face of the molecule and hence give both enantiomers.

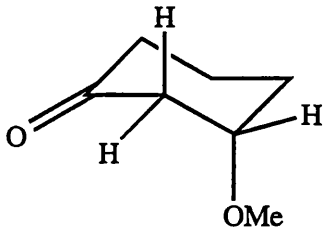


Addition to the enone can also give either enantiomer as methanol can approach from either face of the double bond.

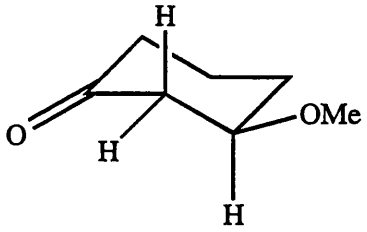


The work of Djerassi and co-workers (Gorthey *et al.* 1985) has shown that β-heteroatom substituted cyclohexanones have a higher than expected proportion of axial heteroatom (when the heteroatom is highly electronegative O or F). 3-Methoxycyclohexan-1-one has 51% axial methoxy whereas 3-methylcyclohexanones have ~17% axial methyl (however this is higher then the corresponding cyclohexane (Bowen and Allinger 1987). They have proposed that the axial preference is due to what is known as the "3-alkyl ketone effect" which shows some relationship to the van der Waals radius of the heteroatom (and hence the electronegativity). Electrostatic or

dipole-dipole interactions are also considered to play a role as are orbital-orbital interactions. The C2 methylene protons couple geminally to each other (J = 14.1 Hz) and have vicinal couplings to the methine at C3 of 4.0 and 7.0 Hz respectively which may represent an equatorial-equatorial coupling and an axial-equatorial coupling respectively but more likely represents an average signal from both axial and equaitorial C3 methoxy conformations as predicted by Djerassi and co-workers (Gorthey *et al.* 1985). Coupling constants are generally reduced when an electronegative substituent is present (Williams and Fleming 1989; Jackmann and Sternhill 1969) and it is feasible that 7.0Hz may represent an axial-axial coupling and that these signals overlap those for the axial methoxy conformer.



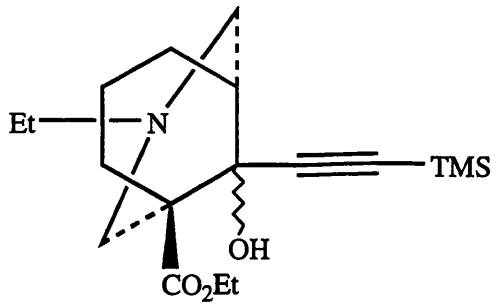
135a



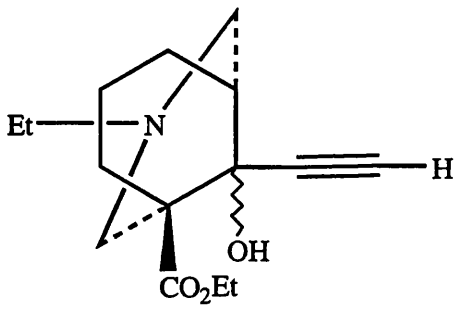
135b

As a mixture of enantiomers is formed the compounds are inseparable by silica gel chromatography and appear homogenous by tlc (as silica gel is not enantiospecific). This means that when 3-methoxycyclohexan-1-one **135** is reacted further at the 1-position diastereoisomeric mixtures will be formed.

Now that both the bicyclic ketone **4** and 3-methoxycyclohexan-1-one **135** have been synthesised, the next step was to link the two together using the acetylide anion addition strategy. Initial attempts began with the synthesis of ethyl 9-(trimethylsilylethynyl)-9-hydroxy-3-ethyl-3-azabicyclo[3.3.1]nonane-1-carboxylate **140** by reacting the bicyclic ketone **4** with lithium trimethylsilylacetylide formed *in situ* from trimethylsilylacetylene and *n*-BuLi.



140

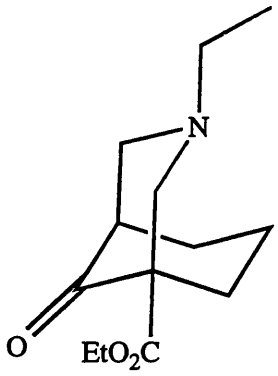


141

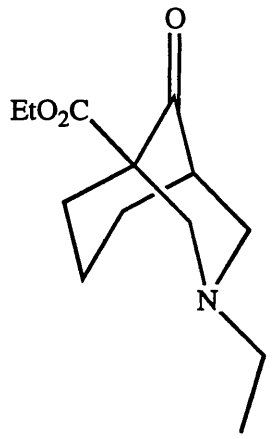
The reaction between lithium trimethylsilylacetylene and bicyclic ketone **4** afforded **140** in moderate yield (40-50%). The reaction was followed by tlc with the diastereoisomeric mixture of products formed having a higher Rf than the starting material (0.6-0.55 as opposed 0.4 for the starting material 1:4 EtOAc-hexane). At this stage, it was not possible to separate the diastereoisomeric products completely by silica gel chromatography. The appearance of singlets at 4.69ppm (1H) and 0.15ppm (9H) in the PMR are diagnostic of a successful reaction. The singlet at 4.69ppm represents the tertiary hydroxyl at C5 (norditerpenoid numbering) and the large singlet at 0.15ppm is the 9 protons of the trimethylsilyl group. In the CMR spectra new signals at 107.87 and 91.69ppm are alkyne carbons (quaternary by DEPT experiments) and the carbonyl signal at 212.79 in the starting material is replaced by the tertiary alcohol signal at 70.77ppm. The methyl groups of the trimethylsilyl group appear at 0.05ppm.

Removal of the trimethylsilyl protecting group by stirring **140** with TBAF in THF was facile, but relatively poor yielding for a deprotection step (51% isolated yield). The reason for this moderate yield was probably due to acid sensitivity of **140** or **141** as there is often residual HF present in THF solutions of TBAF. Fortunately, however, it was now possible to separate the diastereoisomers **141a** and **141b** by silica gel chromatography. Interestingly, there was an unequal mixture of the 2 diastereoisomeric pairs with a 6.3:1 bias of one isomer over the other (we do not know which is which). This mixture is surprising, as although bicyclic ketoester **4** is itself enantiomeric, it has no

obvious functionality to direct the addition of the acetylide to the ketone. When **4** is in the chair-chair conformation both of the pendant 3 atom sections are sufficiently far away from the reacting centre for them to have no effect on the path of attack. There is a possibility that the amine nitrogen may in some way coordinate to the lithium and hence cause the acetylide to attack from that face. The 2D representation below shows that there are no obvious steric reasons for there to be diastereoselectivity.



4



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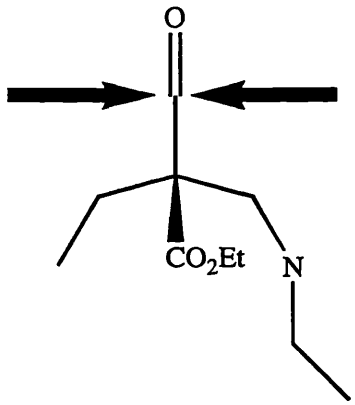
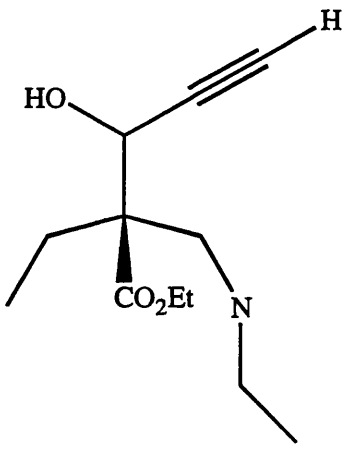
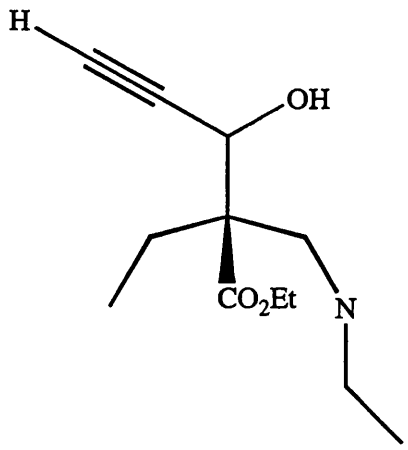


4

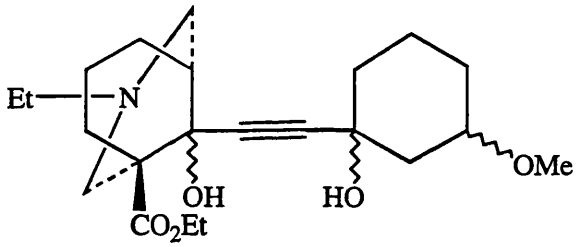
The resulting mixture of diastereoisomers is most likely therefore caused by a difference in stability of the products.





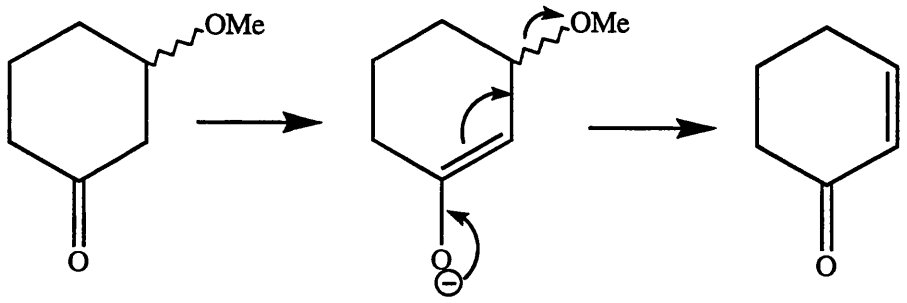
141a **141b**

The dianion of the more predominant isomer was then prepared with 2.4 equivalents of *n*-BuLi, in THF, which it was hoped would react efficiently with 3-methoxycyclohexan-1-one **135** to yield disubstituted alkyne **142.**

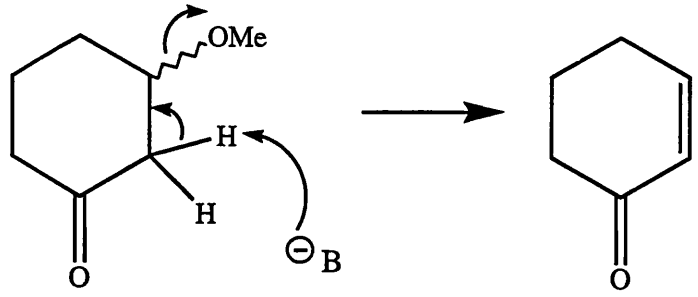


142

However, when 3-methoxycyclohexan-1-one **135** was added, the dianion rather than acting as a nucleophile and attacking the ketone, reacted as a base and facilitated the elimination of methanol from 3-methoxycyclohexan-1-one **135** to give back the starting α,β-enone (cyclohex-2-en-1-one **138**). The elimination of methanol can occur in either a reverse Michael-type reaction (of the enolate **143** formed by deprotonation of 3-methoxycyclohexan-1-one **135**) or via a β-elimination.



135 **143** **138**



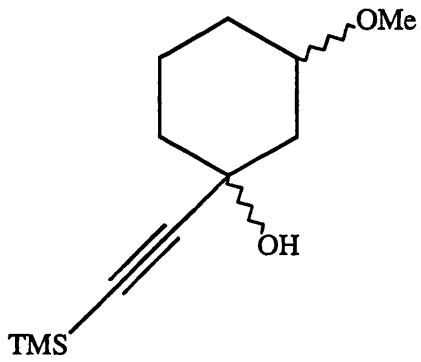
135 **138**

The reaction was followed by both tlc and GC. The loss of 3-methoxycyclohexan-1-one **135** was observed by tlc (Rf 0.1 1:4 EtOAc-hexane) and by GC it was possible to observe the formation of cyclohex-2-en-1-one **138** (a characteristic peak with retention time 2.52 min). The starting bicyclic propargylic alcohol was recovered quantitatively. The reason that this reaction did not work was probably due to the bicyclic propargylic alcohol being too bulky to react as a nucleophile despite the fact that the acetylide anion is linear. The dianion is also highly basic and it may be that the destruction of the electrophile (by the dianion reacting as a base) is more significant in preventing reaction.

As it is possible to substitute acetylene at both termini then there is no reason for the order of the substitution to be fixed. Hence, if the more reactive acetylide (lithium trimethylsilylacetylide as opposed to the bicyclic propargylic dianion) is used to attack 3-methoxycyclohexan-1-one it could be possible to form the dianion of the newly formed propargylic alcohol which could then be used to attack bicyclic β-ketoester **4**. Adding lithium trimethylsilylacetylide to 3-methoxycyclohexan-1-one might however cause

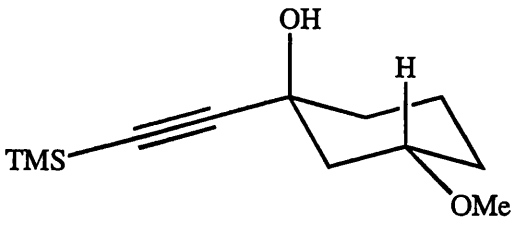
elimination of methanol from 3-methoxycyclohexan-1-one in the same way as the dianion of the bicyclic propargylic alcohol **141**.

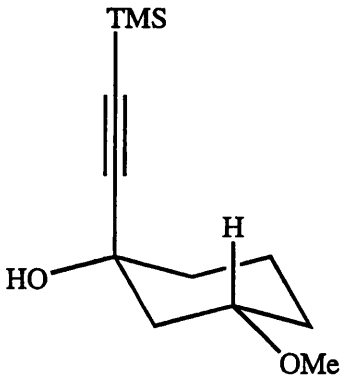
Addition of the acetylide did yield the desired propargylic alcohol **144** in 78% overall yield and the diastereoisomers could be separated by silica gel chromatography. The reaction was followed by tlc and the product had a significantly higher Rf than the starting material (0.6 as opposed to 0.4, 1:1 EtOAc-hexane). Diagnostic changes in the PMR spectra include the introduction of a large singlet at 0.00ppm which represent the 9 protons of the trimethylsilyl group. In the CMR spectra, signals at 110.28 and 86.47 representing the alkyne carbons (quaternary by DEPT experiments) and a quaternary signal at 67.76ppm for the tertiary alcohol produced by the addition to the ketone (209.37ppm no longer present) appeared.



144

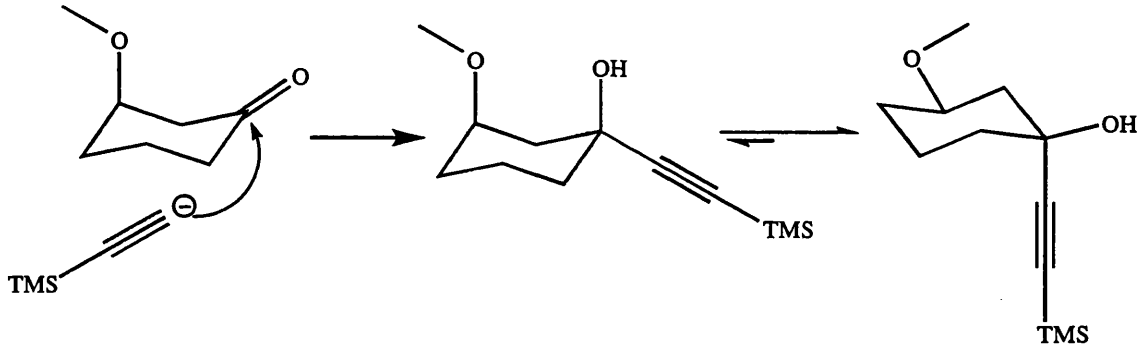
The ratio of diastereoisomers produced was 1:11.6 of the axial alkyne **144a** to the equatorial alkyne **144b** with the methoxy group always in an equatorial position (confirmed by NMR studies using NOE difference and NOESY experiments). This contrasts to 3-methoxycyclohexan-1-one **135** which appears to have a significant proportion of axial methoxy (by PMR studies), but has been reported to have 51:49 axial-equatorial conformation by Djerassi and co-workers (Gorthey *et al.* 1985). We infer that the methoxyl substituent takes priority over the alkyne in terms of defining conformation.





144a **144b**

The fact the methoxy group is always equatorial shows that, at this centre, the preferred conformation is the equatorial position in a fully saturated cyclohexane as reported by Djerassi and co-workers (Gorthey *et al.* 1985). The conformation of 3-methoxycyclohexan-1-one **135** may also play a role in this selectivity as the axial methoxy will induce addition from the other face which would give an axial hydroxyl and, as this is not favoured, the chair would flip to the other chair conformation giving the equatorial methoxy and axial alkyne **144b**.

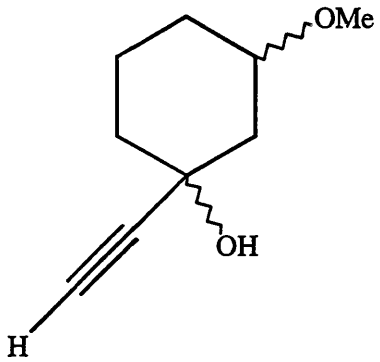


135 **144** **144b**

The ratio of diastereoisomers produced is such that practically useful quantities of the equatorial alkyne containing isomer **144a** are not available (0.154g from 1.5g of 3-methoxycyclohexan-1-one, 7% yield) and only the more favoured isomer **144b** is used further in our synthesis.

Removal of the trimethylsilyl group by stirring in methanolic sodium hydroxide solution gave an excellent yield (90%) of terminal alkyne **145**. Diagnostic spectroscopic

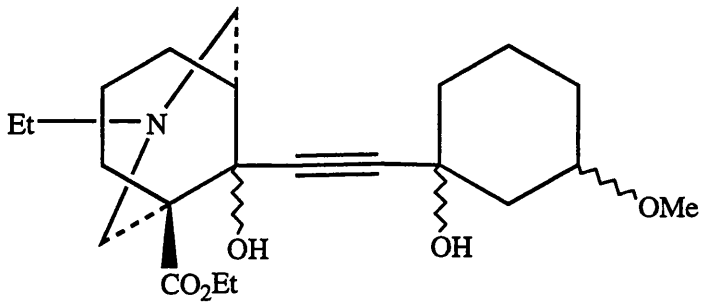
features include the loss of the large singlet at 0.00ppm (the trimethylsilyl protons) in the PMR spectra and the appearance of a sharp singlet at 2.46ppm representing the terminal alkynyl proton. In the CMR spectra, it is now possible to distinguish between the alkyne signals (by DEPT experiments) and the terminal alkyne signal appears at 71.42ppm and the internal alkyne at 86.91ppm.



145

An attempt was made using the methods of Babler and co-workers with potassium *t*-butoxide as the base (Babler *et al.* 1996) to react propargylic alcohol **145** with bicyclic β-ketoester **4**. However, though unsurprisingly, this reaction failed as the double deprotonation of propargylic alcohol **145** is more complex than the simple deprotonation of a terminal alkyne. Quantitative recovery of both starting materials was achieved.

Formation of the dianion of propargylic alcohol **145** (a single pair of enantiomers) was achieved using 2.4 equivalents of *n*-BuLi, in anhydrous THF at 0°C, and to this was added bicyclic β-ketoester **4** and the reaction temperature allowed to rise to room temperature. After stirring at 20°C for 2.5h, the reaction had gone to completion, monitored by tlc (Rf 0.2 and 0.25 1:4 EtOAc-hexane) giving an 83% isolated yield of diol **142** as a colourless viscous oil.



142

Diagnostic spectroscopic features include the appearance of a new broad singlet at 4.7ppm in the PMR (representing the new tertiary alcohol), the loss of the sharp singlet at 2.46ppm (representing the terminal alkyne) and the appearance of a new tertiary alcohol signal (70.60 or 68.44) in the CMR (coupled with the loss of the ketone signal at 212.79 from the starting bicyclic ketoester **4**). Mass spectroscopy confirmed the formation of the desired product by the appearance of a strong peak at 394.1 (the molecular ion $C_{22}H_{35}O_5N$ requires 393) in the CI (isobutane) spectrum.

As was expected, the reaction produced a mixture of diastereoisomeric compounds due to the formation of both possible sterochemistries of the newly formed tertiary alcohol (with both the axial and equatorial hydroxyls formed relative to the carbocyclic ring), along with all of the other sterochemical variables. By tlc, the product of the addition reaction appears to yield only two distinct compounds, whereas the number of compounds which might have been expected was four. Separation of the compounds was difficult by silica gel chromatography and only the less polar of the two could be separated and purified from the other with any real success. The fact that in the original addition the bicyclic structure is racemic means there are now 5 chiral centres in the molecule including the newly formed tertiary alcohol. That some of the chiral centres are fixed (in pairs) due to the previous reactions allow for the following combination of isomers:

Listed in the following order of chiral centres **4 11 5 8 14/16** (norditerpenoid numbering)

RRSRS *SSRSR*

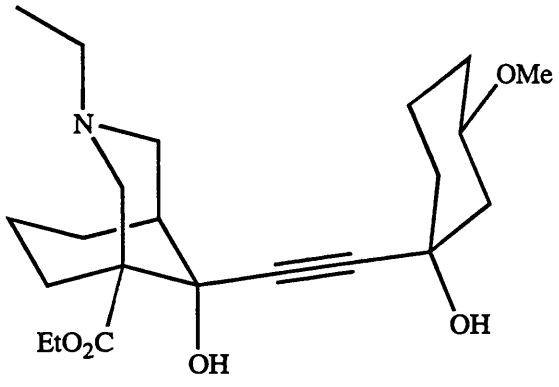
RRSSR *SSRRS*

SSSRS *RRRSR*

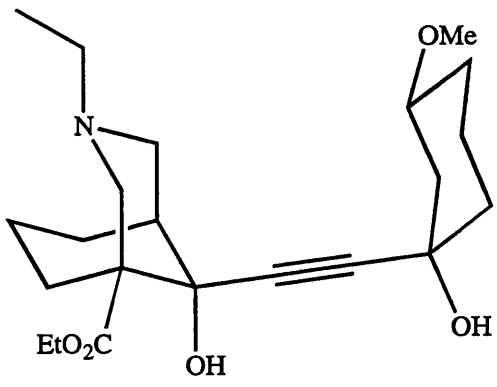
SSSSR *RRRRS*

This means that there are certainly 4 diastereoisomers and their enantiomers within the products of the initial addition which can only be partially separated by silica gel chromatography. Each of the two separate "compounds" which were observed by tlc almost certainly represents two diastereoisomeric compounds and the corresponding enantiomer of each diastereoisomer (making 4 isomers in total per tlc spot).

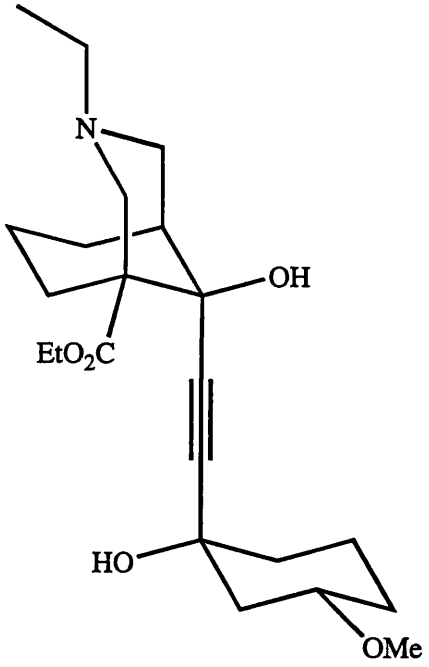
Extrapolating the data from NMR experiments performed on the *cis*-alkenes **148a** and **148b** by Dr. Martin Kipps (Zeneca Agrochemicals) it was possible to assign the relative stereochemistry of each fraction obtained (or those which would have been obtained given better separation). The less polar fraction contains a mixture of diastereoisomers in which the addition of acetylide anion has occurred in an equatorial manner relative to the carbocycle (giving an axial hydroxyl). In the more polar fraction, acetylide addition has occurred from the opposite face giving an axial alkyne and an equatorial hydroxyl relative to the carbocycle.



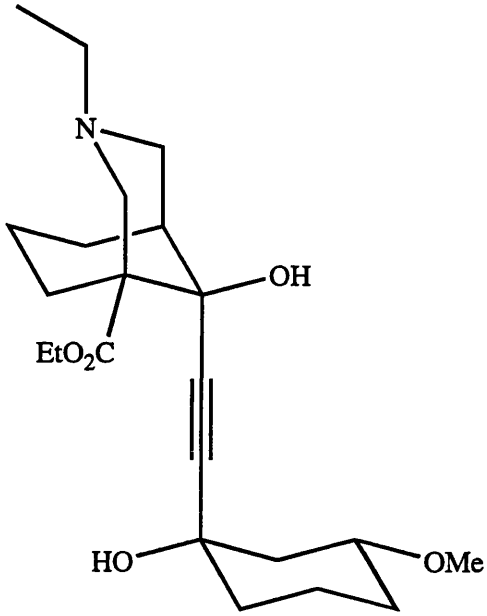
142a(1)



142a(2)



142b(1)



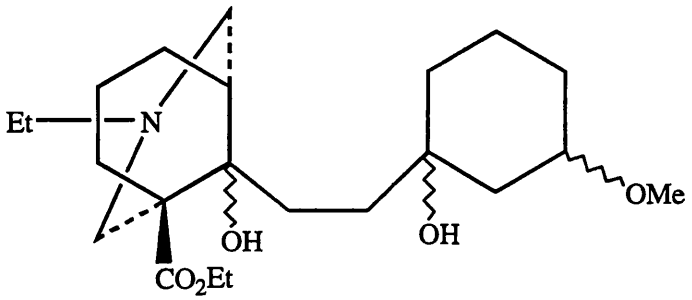
142b(2)

The only difference between the two diastereoisomers within each fraction is the stereochemistry of the monocyclic ring. However, in some ways, this is desirable because within each fraction, there is a compound containing a methoxy functional group which represents the C16 methoxy and one which represents the C14 methoxy. Having now synthesised the AED carbon skeleton (compound **142**) the triple bond can be manipulated to synthesise a series of analogues of lycoctonine **3**, and, by using the previously described methods, analogues of inuline **2** and MLA **1**.

4.5 Synthesis of saturated AED lycoctonine analogue 123

The initial target molecule diol **123** requires that the triple bond in compound **142** is reduced completely to the alkane and this was achieved by catalytic hydrogenation of **142** with molecular hydrogen and palladium catalysis (10% Pd/C, 1 atm H_2, 48h, 84% combined yield). Although it has been shown in the literature that alkynes can be reduced to alkanes using pyridine poisoned palladium (Montalbetti *et al.* 1995) using this

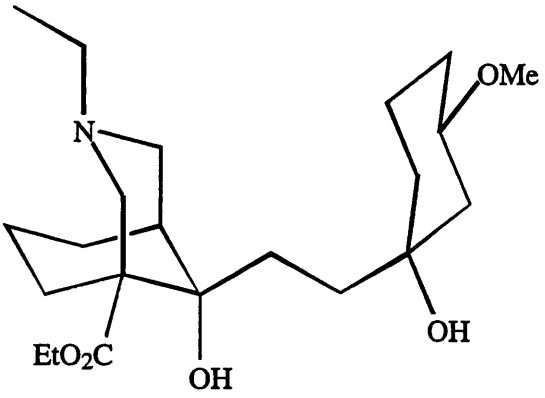
reaction protocol on alkyne **142** only afforded reduction to the (Z)-alkene. Disubstituted propargylic alcohol **142** was reacted as a mixture of diastereoisomers which could not be separated by silica gel chromatography, but the product (still a mixture of compounds) could be efficiently separated to give a 1.5:1 (less polar (Rf 0.4)-more polar (Rf 0.3) 1:19 MeOH-DCM) ratio of two viscous oils which exhibited spectroscopic properties consistent with those of the desired product **146.**



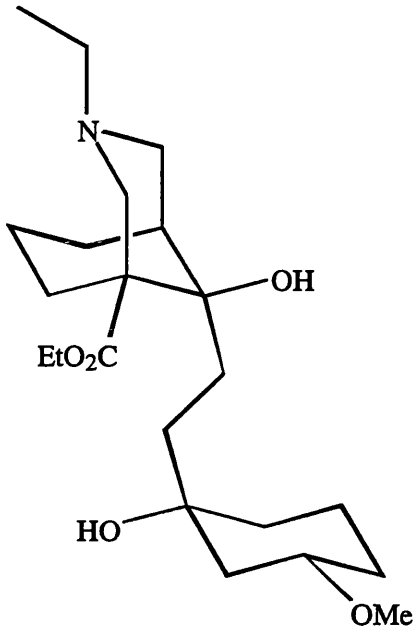
146

The PMR spectra of **146** are not diagnostic, but the loss of the quaternary alkyne carbon signals (90.46 and 88.55) in the CMR spectra indicate that reduction has occurred. The newly formed C6 and C7 (norditerpenoid numbering) methylenes appear in the CMR spectra as a complex pattern of 4 signals within the region 25-40ppm which is due to the presence of diastereoisomers within each isolated fraction. Mass spectroscopy indicates the correct increase of 4 Daltons (CI gives 398.3 $C_{22}H_{39}O_5N$ requires 397) from the starting material **142** to the product **146.** The reduction yields a pair of isolable "compounds" (homogenous by tlc) which presumably have the conformations **146a** and **146b** with both compounds having equatorial methoxy groups in the D-ring (within each fraction both orientations of the methoxy group are present) and with **146a** having axial hydroxyl in the A-ring and **146b** having equatorial hydroxyl in the A-ring if the conformation of the cyclohexyl D-ring is retained throughout. Now that the bond C6-C7 is fully saturated there will be free rotation about the bonds and the D-ring will take up the lowest energy conformation and not necessarily take up the

conformation seen in the norditerpenoid skeleton, (but these structures will be flexible and could fit into many different conformations).

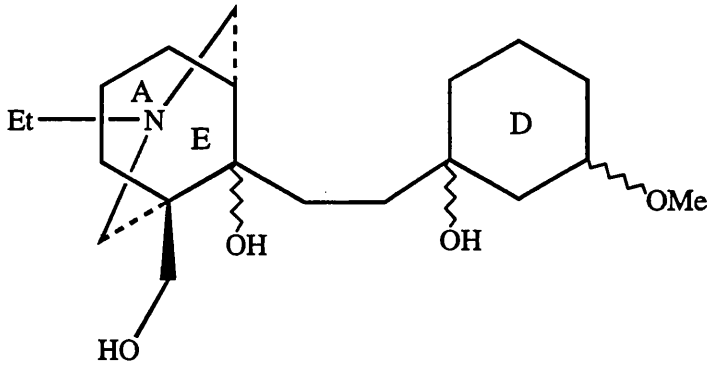


146a



146b

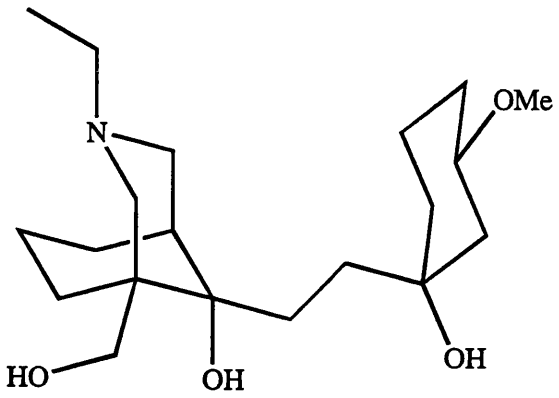
Compounds **146a** and **146b** were then reduced with LAH (Et_2O, 20h) in excellent yields (88 and 97% respectively) to give **147a** and **147b** which are separated diastereoisomers of the desired saturated AED lycoctonine analogue **123**.



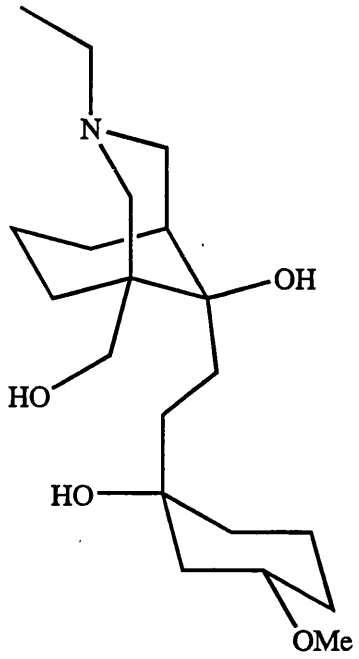
123

The reaction was monitored by tlc with the triol product being considerably more polar than the starting ester (Rf 0.2 1:9 MeOH-DCM as opposed to 0.5 for SM). Diagnostic features in the PMR spectra include the loss of the ethyl ester signals (quartet at 4.2ppm and triplet at 1.1ppm) and the introduction of a new doublet (3.6-4.0ppm) representing the newly formed hydroxymethyl at C18 (norditerpenoid numbering). In

the CMR spectra, the ester carbonyl signal at ~170ppm and the ethyl group signals (CH_2 at ~60ppm and CH_3 at ~14ppm) had disappeared and there is a new signal at ~70ppm representing the new primary (neopentyl-like) alcohol.



147a



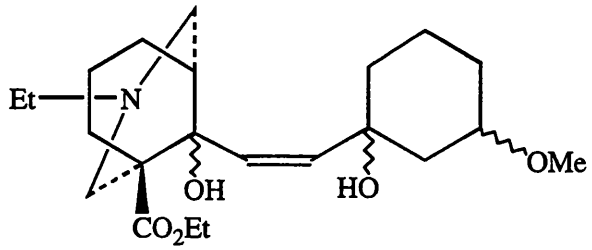
147b

As the hydrogenation reaction and the LAH reduction do not introduce any new chiral centres, the ratio of diastereoisomers does not change and unfortunately the increased polarity of the triol (produced by LAH reduction) did not aid in the resolution of the diastereoisomers.

4.6 Synthesis of unsaturated AED-analogues of lycoctonine

Using Lindlar's catalyst (palladium on calcium carbonate, poisoned with lead) it was possible to reduce the co-eluting mixture of diastereoisomeric alkynes **142** to the (*Z*)-alkenes **148a** and **148b** (10% catalyst, 72h, 1 atm H_2, EtOH), but this reaction only proceeded in poor yield (27%). We found that using the method described by Montalbetti *et al.* (1995), to reduce an alkyne to an alkane, which employs pyridine

poisoned palladium (10% Pd/C, 1 atm H_2, 72h) on charcoal, actually yields the (*Z*)-alkene (not alkane) in a better yield (40%). After this reduction step, it was possible to separate the previously inseparable diastereoisomeric pair of compounds yielding **148a** and **148b** in a 1.5:1 ratio of the less polar **148a** to the more polar compound **148b**. This ratio of compounds is the same as the one encountered for the saturated AED analogues **146a** and **146b** as expected.



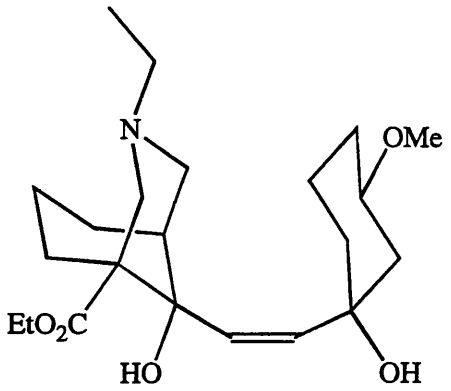
148

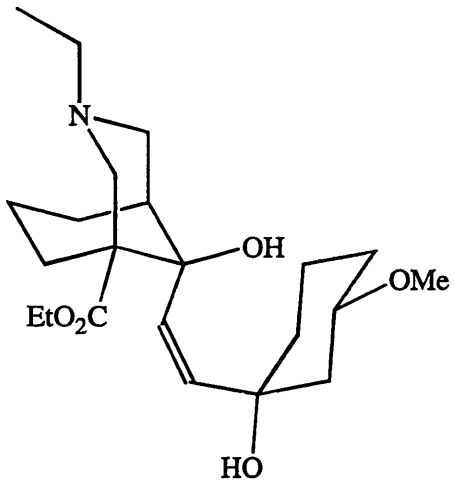
This reaction was monitored by tlc (1:3 EtOAc-hexane) and the products were slightly more polar then the starting material (0.4 as opposed to 0.3). The PMR of the products showed that conversion into the alkene had occurred by the appearance of two new sets of signals at ~6ppm and ~5.5ppm and CMR showed that the alkyne signals (90.46 and 88.55ppm) had disappeared and two new signals at ~134ppm and ~133ppm were present which were the alkene carbons.

Initial examination of the spectra led us to believe, however, that we had synthesised the (*E*)-alkene as the vicinal coupling constants between the alkene protons was ~13.5Hz which is above the range which is commonly accepted for (*Z*)-alkenes of 7-12Hz (Williams and Fleming 1989; Kemp 1986; Jackman and Sternhill 1969) and was more typical of an (*E*)-alkene (14-18Hz). An (*E*)-alkene would not be the expected product of a catalytic hydrogenation, but using Lindlar's catalyst has been shown to yield occasionally the (*E*)-alkene (Heublin and Stadermann 1972) and the time taken for the hydrogenation, and hence the contact time between the alkene and the catalyst, was long (allowing for possible isomerisation of the alkene). By using NOE difference and

NOESY NMR techniques it was possible to show that the compounds **148a** and **148b** were in fact of (*Z*)- and not (*E*)-geometry.

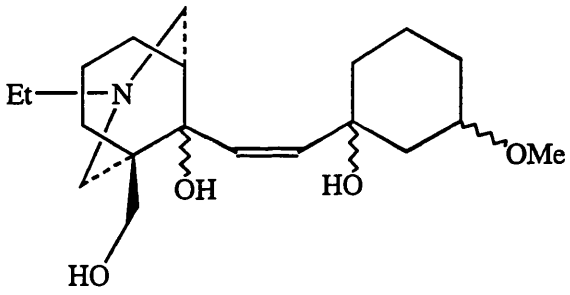
There will only be an NOE between the alkene protons if the double bond has *cis*-geometry, as in the *trans*-geometry the protons are too far away from each other. However, not all *cis*-double bonds show an NOE and hence the absence of an NOE would not have confirmed the *trans*- geometry (i.e. the presence of a NOE confirms the *cis*-geometry, but the absence of an NOE would not have ruled it out). Subsequent analysis of the spectra also show an unusual doubling on the alkene signals which can only be attributed to longer range (4 bond) coupling to the tertiary alcohol adjacent to the alkene if the isolated compound is truly a single diastereoisomer (together with its enantiomer), but it is more likely that the doubling observed is in fact the evidence that the product is in fact a mixture of diastereoisomers which are co-eluting, as expected. Evidence reinforcing this possibility is the C14/C16 methoxy signal and its accompanying methine which appear as double signals with the combined integral being for 3 and 1 protons respectively. As with the alkane synthesis, the reduction to the (*Z*)-alkene does not introduce any new chirality into the molecule (although conformational isomers may be present) and so the ratio of diastereoisomers is unchanged. Catalytic hydrogenation did not increase the separation of the diastereoisomers and hence only two fractions were separated (rather than the 4 expected).





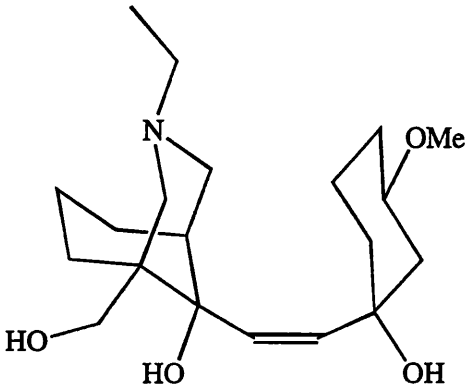
148a **148b**

The pair of separated (Z)-alkenes **148a** and **148b** was then reduced with LAH (THF, 22h, 20°C, 84 and 78% respectively) to give the triols **149a** and **149b**. These reactions were monitored by tlc (1:19 MeOH-DCM) and the products had a significantly lower Rf than the starting material (0.2 for **149** as opposed to 0.5 for **148**).

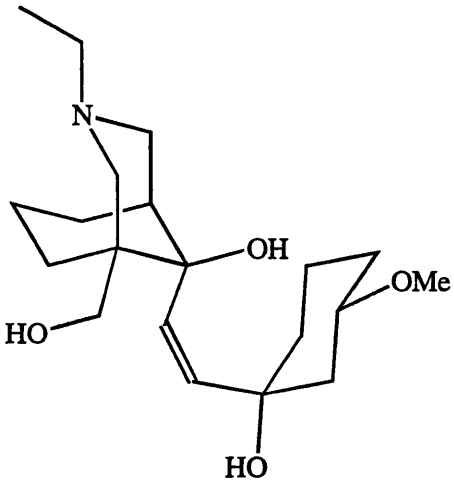


149

Diagnostic features in the PMR spectra include the loss of the ethyl group signals (triplet at ~1.1ppm and quartet at ~4.2ppm) and the introduction of the hydroxymethyl group signals (in these compounds a multiplet at ~3.6ppm). In the CMR spectra, the ester signal (~170ppm) has been lost and a new primary (neopentyl-like) alcohol signal appears at ~70ppm. The mass spectrum also confirms the reduction of the ester to the primary alcohol (low resolution FAB +ve gives 354.2 $C_{20}H_{35}NO_4$ requires 353).



149a

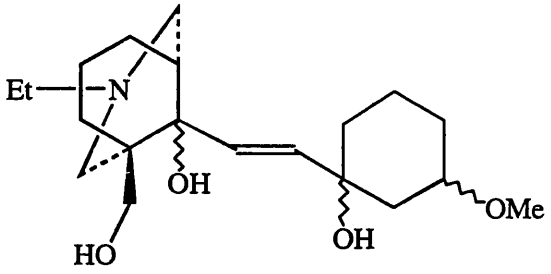


149b

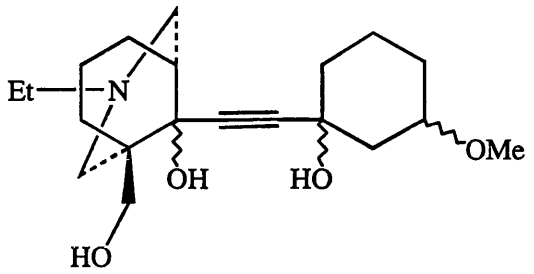
Now (*Z*)-alkenes **149a** and **149b** which are analogues of lycoctonine **3** can be converted firstly into inuline **2** analogues and finally into MLA **1** analogues using the literature synthetic methods described earlier (Coates *et al.* 1994b). These unsaturated analogues (**149a** and **149b**) of lycoctonine are conformationally restrained and some of the conformations (especially from **149a**) will be close to those of the norditerpenoid skeleton (although some will be necessarily different).

Having synthesised the (*Z*)-alkene, it was appropriate that the (*E*)-alkene, which can also be synthesised from the alkyne **142**, should be synthesised firstly in order to advance the SAR study, but also as it will help to confirm the geometry of the (*Z*)-alkene by analogy. The reduction of propargylic alcohols to (*E*)-alkenes using LAH has literature precedent (Walborsky and Wüst 1982) and, using an excess of LAH, will also reduce the ester functionality present in **142** to the required primary alcohol. Reduction of alkynic ester **142** with LAH (Et_2O, 48h, 20°C) proceeded in good overall yield (82%), but afforded a more complex mixture of products than had been expected. Rather than the two separate (*E*)-alkene fractions expected, three apparently homogeneous fractions were isolated. Initially, it was thought that more of the diastereoisomers had become separable, but analysis of the PMR and CMR showed that this was not the case. In the least polar fraction (Rf 0.4 1:9 MeOH-DCM), a mixture of an (*E*)-alkene **150a**

(presumably a pair of diastereoisomers by analogy) and an alkynic triol **151a** was present. In the second fraction (Rf 0.3), an alkynic triol **151b** was present, and in the final fraction (Rf 0.2) the other (*E*)-alkene **150b** was present.



150 (a +b)



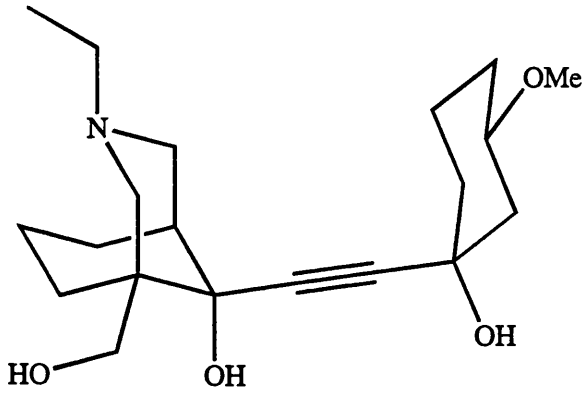
151

This can be explained by the reduction of the propargylic alcohol not going to completion whilst the faster and more facile ester reduction did go to completion leaving a mixture of compounds. Attempts could have been made to force the reaction to completion by adding more LAH or by heating the reaction mixture, but as this reaction had been performed on a relatively large scale and separation of most of the products was possible, it was decided that an attempt would be made to isolate the alkynic triols **151a** and **151b** to allow the synthesis of some other novel unsaturated analogues of MLA **1**.

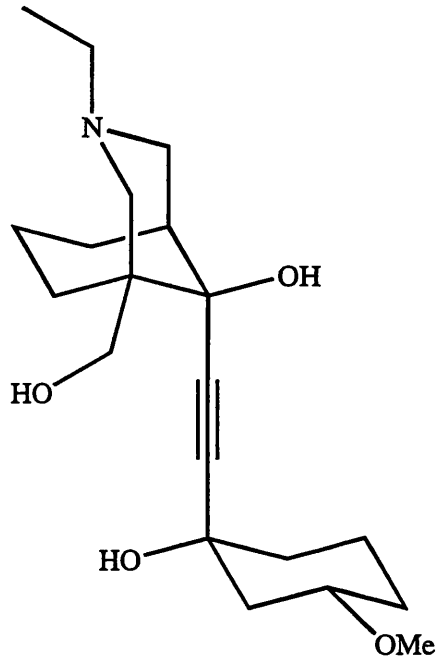
Examination of the PMR spectra of both the fractions containing the (*E*)-alkene showed that the vicinal coupling between the two alkene protons was ~15-16Hz and by comparing these data to that produced by the (Z)-alkenes **149** confirms the geometry of both alkenes as those which would have been predicted. PMR spectra also show that the ethyl ester (~1.1ppm and ~4.2ppm) had been reduced completely to the primary alcohol required (doublet at ~3.5ppm *J*=9.2Hz and broad singlet at ~4.5ppm). In the CMR spectra, the ester signals are no longer present (carbonyl ~170ppm, OCH_2 at ~60ppm CH_3 at ~14ppm) and a new signal (the primary alcohol) is observed at ~70ppm. The alkene carbons resonate at ~137 and ~128 ppm (but we do not know which is which).

At this stage, it was not possible to separate the less polar alkynic triol **151a** from the (*E*)-alkenic triol **150a** and so these compounds were reacted further as a mixture with a view to separating them at a later stage.

The PMR of the alkynic triols **151a** and **151b** showed that the ester functionality was no longer present and that the newly formed primary alcohol had been introduced. In the CMR spectra, it was possible to detect the quaternary alkyne carbons (~77 and 70ppm) and that the ester had been reduced to a primary alcohol.



151a

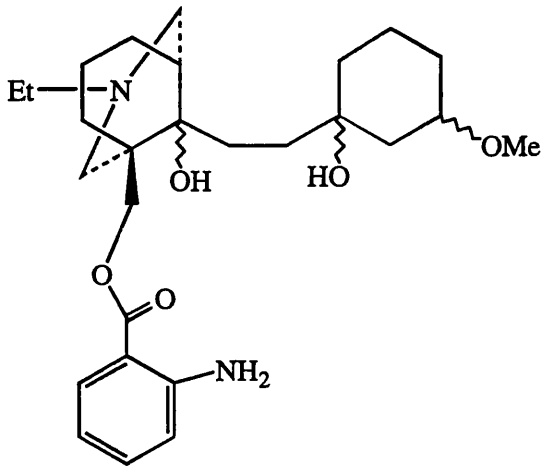


151b

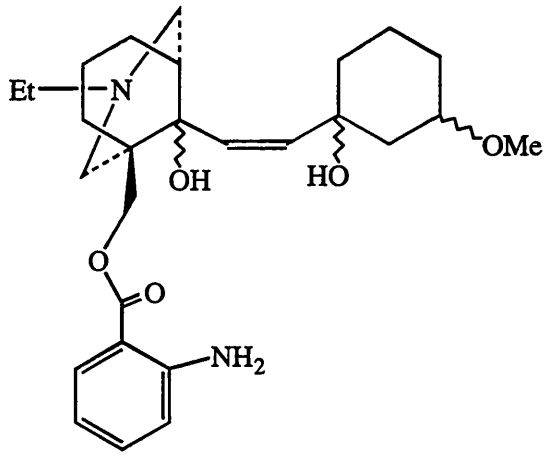
4.7 Conversion of AED lycoctonine analogues into inuline analogues

Using the methods described in Chapter 2 developed by Blagbrough an co-workers (Coates *et al.* 1994b; Hardick *et al.* 1994) the series of AED analogues of lycoctonine **147**, **149**, **150**, and **151** (each having 2 fractions **a** and **b**) were reacted with isatoic anhydride **46** under base catalysis (DMAP **75**) in DMF at 70°C to yield the 2-aminobenzoate esters **152, 153, 154** and **155** respectively. The only difference between

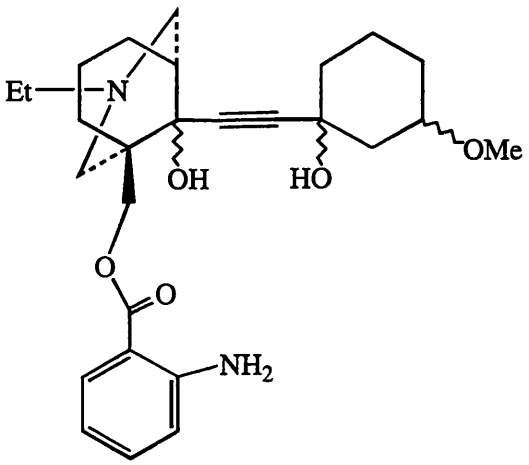
this procedure and the procedure described in Chapter 2 is that the filtration step to remove any excess of isatoic anhydride was omitted and silica gel chromatography performed directly after removal of the DMF by bulb-to-bulb distillation. In all cases, both of the fractions (which contain a mixture of diastereoisomers) were reacted with isatoic anhydride and hence eight inuline analogues were made, which are a mixture of diastereoisomers (and their corresponding enantiomers). Despite this reaction being generally high yielding, some of the yields in this series of reactions are disappointingly low, but there was always enough material to proceed with the conversion into the 2-methylsuccinimido derivatives.



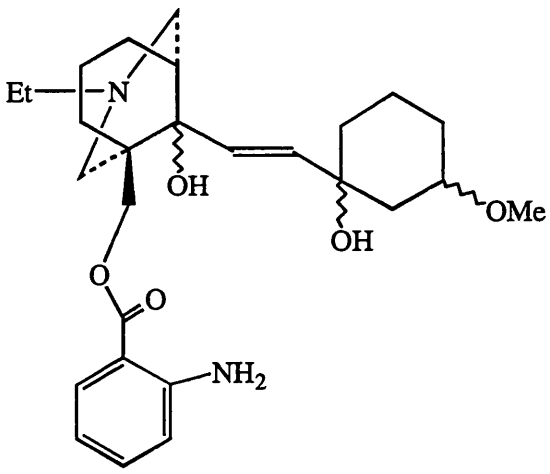
152



153



154



155

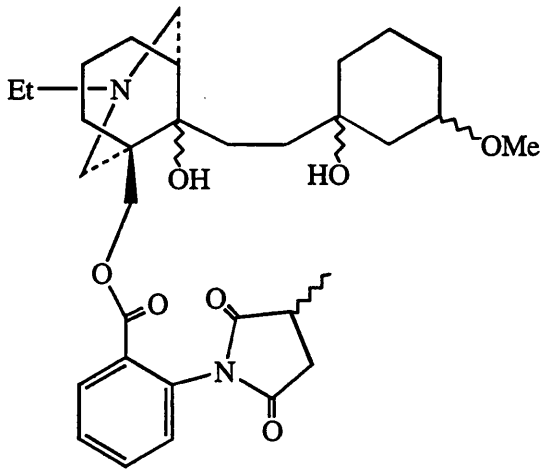
This reaction was monitored by tlc and an increase in the Rf (from ~0.3 to 0.4 1:19 MeOH-DCM) was observed on completion of the esterification. In the PMR spectra, a new set of aromatic signals is observed belonging to the 2-aminobenzoate ester and these have characteristic coupling constants (as described in Chapter 2) and a broad singlet at ~5.5 appeared which represents the aniline protons. In the CMR spectra, the new ester carbonyl (~170ppm) was observed as were the 6 aromatic carbon signals (2 quaternaries at ~110 and ~150ppm and 4 CHs at ~133, 131, 116 and 115ppm). Mass spectroscopy confirmed that the esterification had taken place by the addition of 121 Daltons.

Compound	Reaction time h	Isolated Yield %
152a	48	83
152b	48	68
153a	20	82
153b	24	82
154a	20	15 (combined 28)
154b	20	93
155a	20	13 (combined 28)
155b	17	36

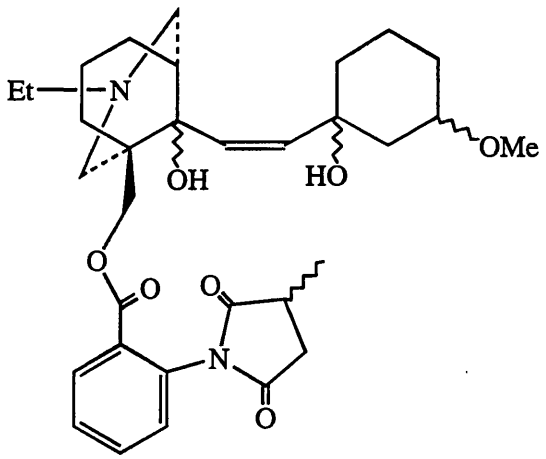
4.8 Conversion of AED inuline analogues into MLA analogues

Each of the 2-aminobenzoate esters **152-155** was reacted with (±)-methylsuccinic anhydride to form the 2-(methylsuccinimido)benzoate esters **156-159** analogous to MLA **1** using the methods described by Blagbrough and co-workers (Coates *et al.* 1994b; Hardick *et al.* 1994). The 2-aminobezoate esters were stirred with methylsuccinic

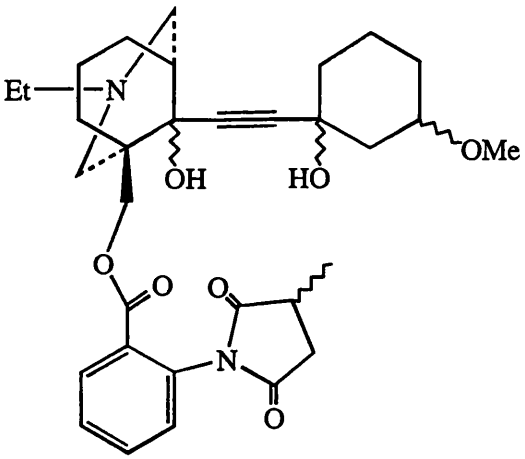
anhydride for 20h until the half-acid-amides were formed. Dehydration and thus cyclisation was afforded by the addition of CDI and a further period of stirring (24h).



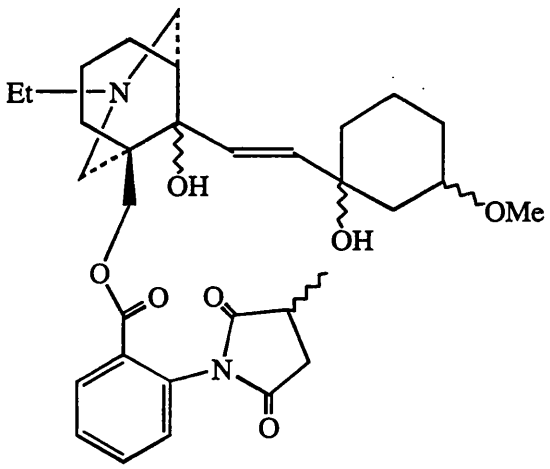
156



157



158



159

This conversion into the half-acid amide could be monitored by tlc as the half acid amide did not run on neutral silica plates, but the starting material does. When all of the starting material had reacted, CDI **81** was added and the final product does chromatograph showing that cyclisation was occurring. All of these reactions worked except for the conversion of the more polar **158b** which failed to convert into the corresponding methylsuccinimide.

Diagnostic features in the PMR spectra, confirming that the reactions had worked were the change in pattern of the aromatic protons (as described in Chapter 2) and the

disappearance of the broad singlet at ~5.5ppm which shows the aniline nitrogen has reacted. Close inspection of the PMR spectra, along with ^{1}H-^{13}C COSY data, show the emergence of new signals attributable to the methylsuccinimide (namely the CH_3, CH_2 and CH of the methylsuccinimide appearing between 1 and 2.5ppm). In the CMR spectra, the pattern within the aromatic signals is again different to the starting compound with the signals being more equivalent than in the 2-aminobenzoate ester. The succinimide carbonyl signals also appear in the region 178-180ppm, but are often of low intensity. All of the new carbons can be identified in the CMR spectra (CH_3, CH_2 and CH appearing at ~16, 34 and 35ppm respectively). Interestingly, but perhaps not unsurprisingly, the succinimide carbons display some doubling (even in the proton de-coupled spectrum) due primarily to the diastereoisomeric nature of the compounds.

Compound	Isolated Yield (%)	% Displacement of α-BGTx at 10ppm
156a	47	24
156b	100	47
157a	28	22
157b	46	71
158a	50	-
158b	-	-
159a	46	-
159b	57	19

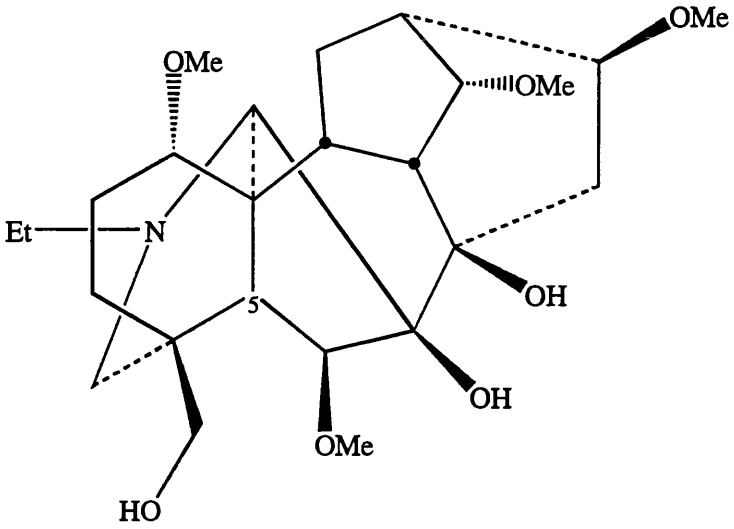
None of the compounds showed significant insecticidal activity in preliminary screens on a variety of insect species (in experiments performed by Zeneca Agrochemicals). The insect species used are the same as those described in Chapter 2.

Chapter 5

STUDIES IN C5 DEHYDROXYLATION

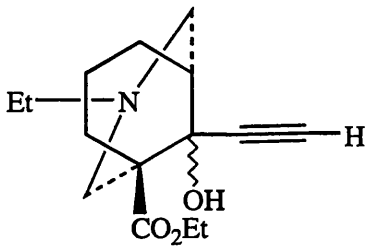
5.1 Design of synthetic targets

In the lycoctonine class of norditerpenoids (e.g. lycoctonine **3**) there is no hydroxyl group at C5 which has been a feature of many of the substituted AE-bicyclic analogues of MLA synthesised so far, due primarily to the manner in which the azabicyclo[3.3.1]nonane system has been prepared.



3

Therefore, it was desirable to synthesise analogues of MLA **1** that did not contain this unnatural moiety. This, coupled with the failure of the dianion of compound **141** to react with 3-methoxycyclohexan-1-one **135**, led us to attempt the synthesis of compound **160** which could be deprotonated to give an acetylide mono-anion which may be more reactive (more nucleophilic) than the basic dianion derived from propargylic alcohol **141**.



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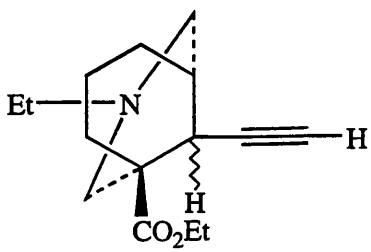
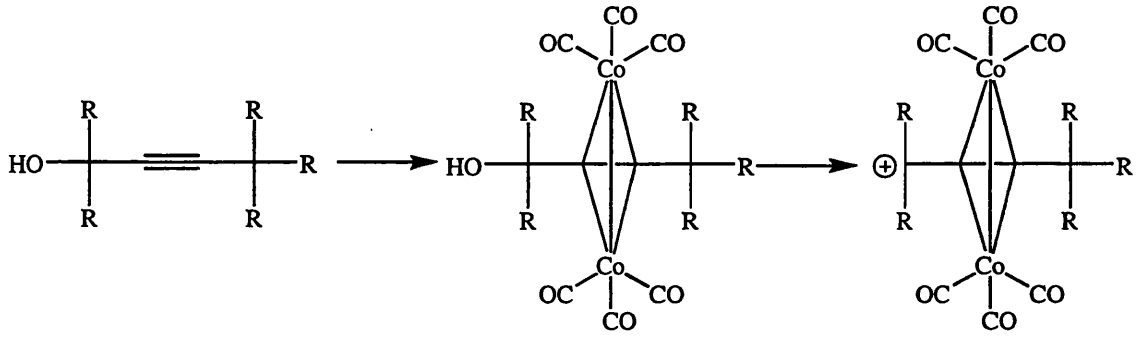


160

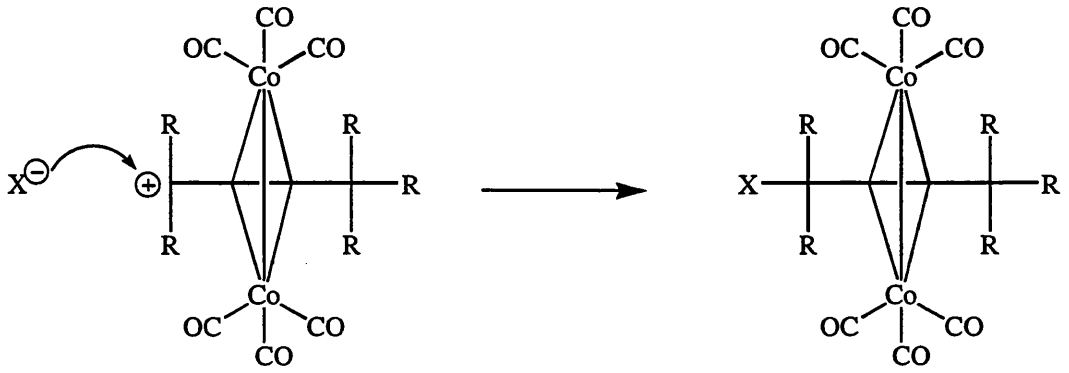
5.2 Dehydroxylation of propargylic alcohols

Nicholas and co-workers have shown that the synthetic utility of propargylic alcohols can be greatly increased by the use of a dicobalt hexacarbonyl protecting/activating strategy (for a review, see; Nicholas 1987). By forming the dicobalt hexacarbonyl complex **162** of acetylene **161** (by reacting the alkyne with 1 equivalent of dicobalt octacarbonyl), carbocation **163** can be formed by acid catalysed elimination of the hydroxyl functionality of the propargylic alcohol.



161 **162** **163**

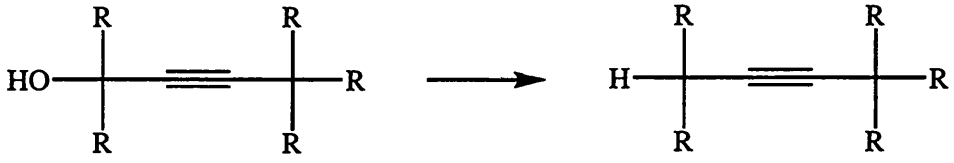
This carbocation **163** is stabilised by the transition metal complex, and salts of the carbocation have been studied by IR and NMR spectroscopic techniques (Nicholas 1987). However, despite this inherent stability, this carbocation can react with a number of nucleophiles in what is described as the Nicholas reaction (Schreiber *et al.* 1986).



163 **164**

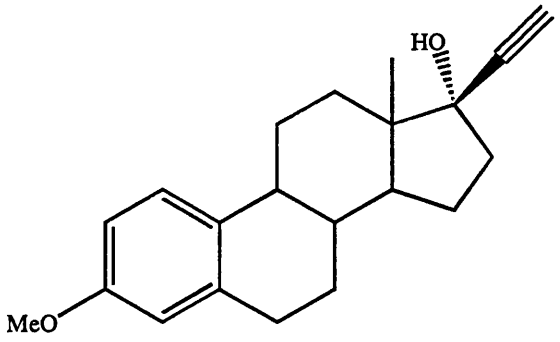
After quenching the carbocation with a nucleophile to give **164**, the dicobalt complex can be removed by oxidative cleavage using solid ferric nitrate (Nicholas 1987)

in the reaction solvent (or with solid ferric nitrate in an ethanolic solution of the complex). There are many examples of nucleophiles (electron rich aromatics (e.g. anisole), allylsilanes, organometallic reagents (e.g. trialkyl aluminiums), enolisable ketones (e.g. acetone) and β-dicarbonyl nucleophiles) which react with the carbocation (Nicholas 1987; Schreiber *et al.* 1986; Melikyan *et al.* 1991), but of most significance to us is the hydride variant of this reaction (Nicholas and Siegel 1985; McComsey *et al.* 1986). By reacting the carbocation (formed by trifluoroacetic acid induced elimination of water) with a hydride source such as sodium borohydride (Nicholas and Siegel 1985) or borane (McComsey *et al.* 1986) it is possible to synthesise the corresponding *sec*-acetylene **165** from the propargylic alcohol **161** of this complex (after demetallation).



161 **165**

This dehydroxylation reaction appears to be general and it was hoped that our target compound **160** could be synthesised via this route. An excellent example of the synthetic utility of this strategy is the conversion of steroid derivative **166** into the *sec*-alkyne **167**, which is an intermediate in the synthesis of 17-β-substituted steroids which are otherwise inaccessible (Nicholas and Siegel 1985). Interestingly, both the α- and β-epimers of the propargylic alcohol yield only the β-*sec*-alkyne, indicating the formation of a common carbocation which is attacked by BH_4 solely from a favoured (less hindered) face of the molecule. Also interestingly, the reaction conditions, as illustrated by several examples in the literature from Nicholas and co-workers, require first an excess of hydride and then the addition of trifluoroacetic acid (TFA) to form the stabilised carbocation for hydride reaction *in situ*.



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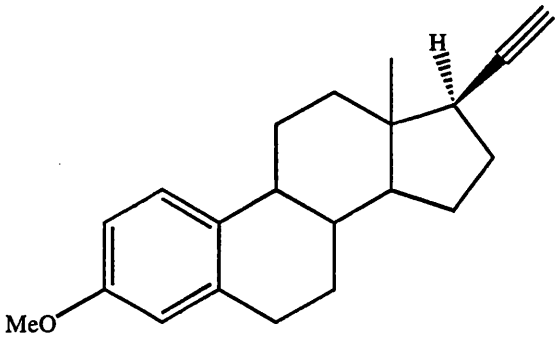
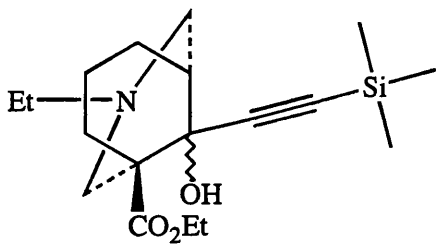


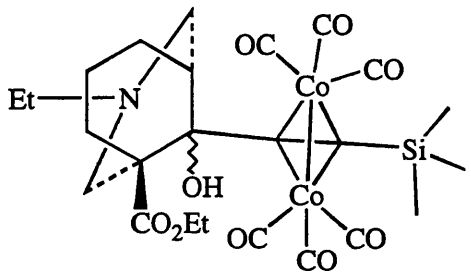
167

5.3 Attempted dehydroxylation of 9-(trimethylsilylethynyl)-9-hydroxy-3-azabicyclo[3.3.1]nonane 140

9-(Trimethylsilylethynyl)-9-hydroxy-3-azabicyclo[3.3.1]nonane **140** was synthesised in good yield (74%) by addition of lithium trimethylsilylacetylide to bicyclic β-ketoester **4** (as detailed in Chapter 4). The dicobalt hexacarbonyl complex was synthesised in the manner described by Nicholas (Nicholas 1987) by the addition of dicobalt octacarbonyl to a solution of **140** in DCM. The complexation reaction was monitored by tlc with the dicobalt complex being clearly visible as a purple-brown spot (Rf 0.6, 1:4 EtOAc-hexane). Visualisation with $KMnO_4$ showed both the complex **166** and the starting alkyne **140**.



140

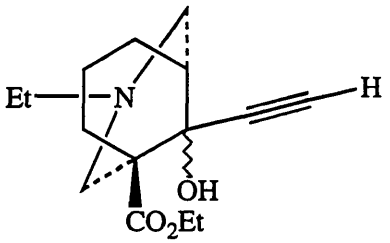


166

After stirring for 28h (complexation was slower than expected) the reaction mixture was cooled to -5°C with a salt water-ice bath and sodium borohydride (3 molar

equivalents relative to the β-ketoester i.e. 12 equivalents of hydride) was added. To this was then added trifluoroacetic acid (vigorous evolution of hydrogen gas was observed) and the temperature of the reaction mixture was allowed to rise to room temperature and then stirred for 20h. Monitoring by tlc showed that no reaction had occurred and that the complex was unaffected by the reaction conditions. Demetallation with an ethanolic slurry of ferric nitrate yielded an almost quantitative recovery of starting material.

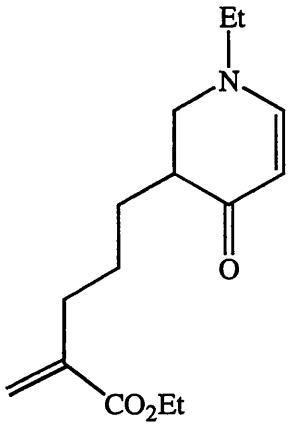
The reason for the failure of this reaction could be firstly that the carbocation did not form which could be explained by the nitrogen atom being protonated and this ammonium ion decreasing the likelihood of the hydroxyl becoming protonated. Secondly, it could be that the carbocation did form, but that it was too hindered to allow the hydride anion to reach the positive carbon. Molecular models show that the tertiary alcohol is hindered and that the trimethylsilyl protecting group increases the steric crowding. In order to test whether steric crowding was the primary reason for the reaction not working, the trimethylsilyl group was removed to leave a less hindered propargylic alcohol **141**. This deprotection was afforded by stirring with TBAF (in THF) as described in Chapter 4.



141

Formation of the cobalt complex of **141** proceeded as expected (1 equivalent of Co_2CO_8) as observed by the appearance of a purple-brown spot (Rf 0.5, 1:4 EtOAc-hexane) in the tlc. However, as the reaction was monitored a new spot on the tlc appeared (Rf 0.05) which was considerably more polar than both the starting propargylic alcohol and the complex. As this new compound **167** was also not coloured, we inferred

that the cobalt complex had degraded (neither borohydride or TFA was added). Tlc analysis showed that all of the starting material had reacted and that furthermore all of the complex had degraded. Purification of the reaction mixture yielded a pale yellow viscous oil (45% isolated yield) which displayed entirely different NMR spectroscopic properties to the starting bicyclic propargylic alcohol **141**. Mass spectroscopy showed that the product had the same molecular weight as the starting material **141**. From detailed analysis of the PMR, CMR, 1H-1H COSY and FLOC spectra, we determined that the compound was dihydropyridinone **167**. Important features in the PMR that indicated that the new compound was dihydopyridinone **167** are the pair of coupled (J = 7Hz) doublets at 6.99 and 4.89 which are the enone protons at C3 and C2 respectively, the pair of singlets at 6.15 and 5.54 which are the alkene protons at C12. The PMR also indicates that both ethyl groups are intact (4.19: 2H, q, 7 and 1.30: 3H, t, 7 representing OCH_2CH_3 and 3.28: 2H,q, 7 and 1.22: 3H, t, 7 representing NCH_2CH_3). The CMR spectra shows the presence of a ketone (194.2ppm) and an ester (167.1ppm) carbonyls and 3 distinct alkene carbons (1 CH_2 and 2 CHs) and proton-carbon correlation helped us to assign these carbon resonances. Long range correlation experiments confirm the connectivity of these groups. Analysis of the mass spectrum also confirms the structure with the detection of a fragment of mass 124 which is ethyl dihydropyridinone.



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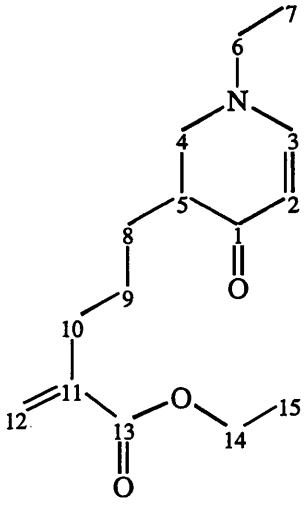
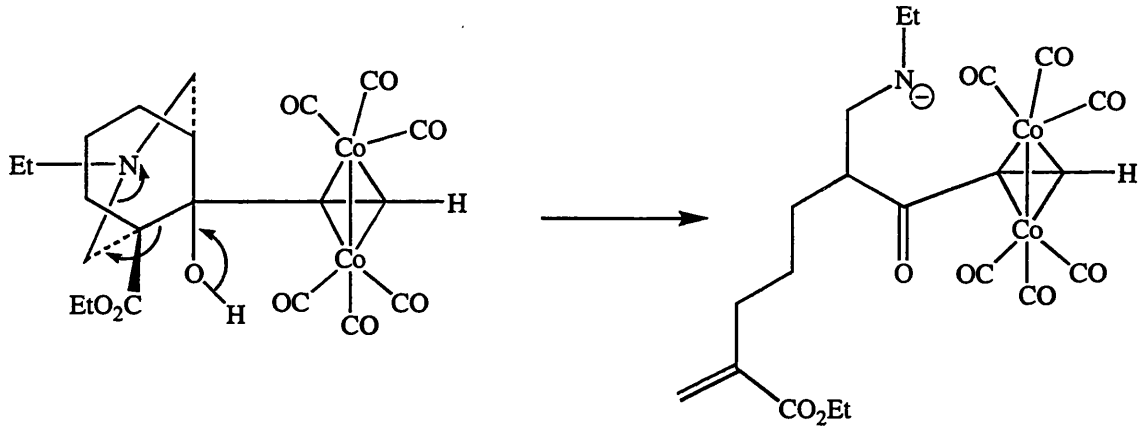


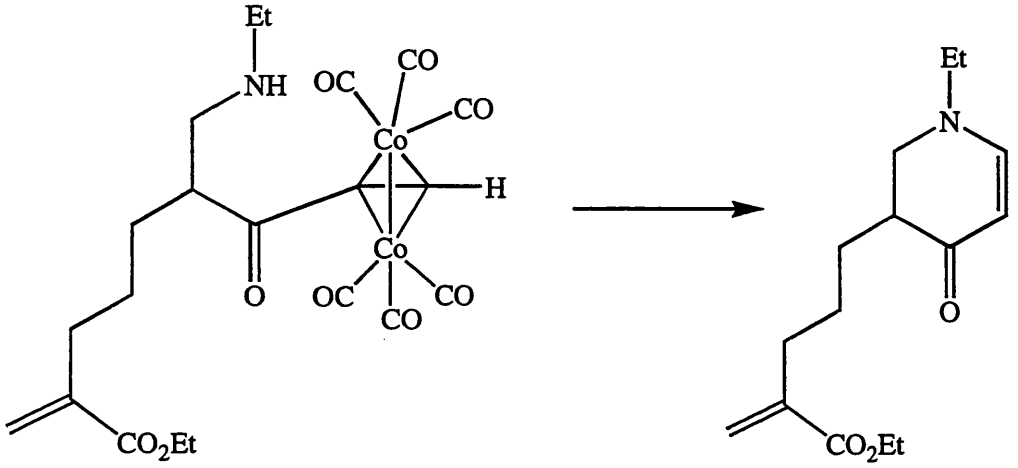
167

Presumably, a Grob-type fragmentation (a retro-aldol-type reaction) has occurred (cleaving C4-C5 bond, norditerpenoid numbering) with the amine (ammonium ion if the tertiary amine is protonated) acting as a leaving group to afford acyclic ketone **169** containing a 2-substituted ethyl acrylate moiety.



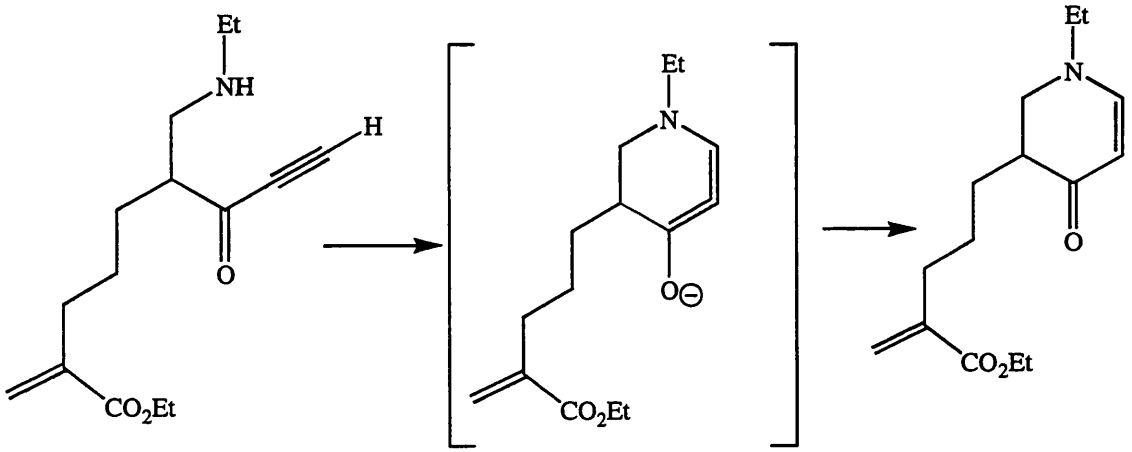
168 **169**

This reaction is probably auto-catalytic, in that the tertiary nitrogen of one molecule of **168** can facilitate the deprotonation of the tertiary alcohol initiating the reaction and then donate this proton back to intermediate **169** giving the secondary amine **170** which then attacks intramolecularly the terminus of the dicobalt protected alkyne. However, it may be that the formation of the quaternary ammonium species facilitates the fragmentation as this makes a more efficient leaving group.



170 **167**

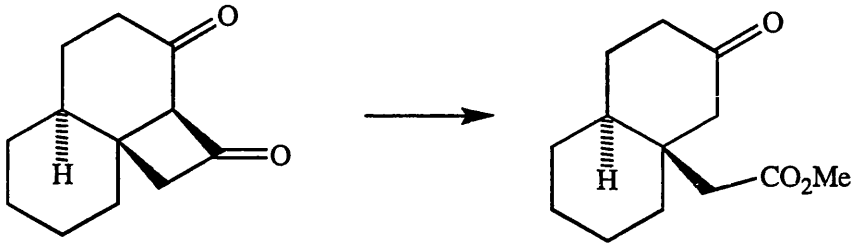
Dihydropyridinone ring closure accompanies loss of the dicobalt hexacarbonyl protecting group in a reaction analogous to a conjugate 1,4-addition to alkynone **171**. That the dicobalt hexacarbonyl group is essential for this reaction is shown by looking at intermediate **172** which would be formed if the complex was not in place. Cyclic allenyl intermediate **172** would probably not form within a six-membered ring (due to its linearity) and hence the cobalt complex must still be intact until the reaction is complete. The cobalt may take part in the reaction, acting as a leaving group or more probably enabling the reaction to take place by allowing the conformationally strained cyclic allenyl species (or an equivalent thereof) to be part of the six-membered ring. The cobalt presumably forms a transient complex with this allenyl species which is cleaved when cyclisation is complete and the enone is formed. This addition of an amine to an ethynyl ketone has been used in the synthesis of β-amino α,β-unsaturated ketones (Bowden *et al.* 1946; Tripathi *et al.* 1979), but we have not found any examples of the formation of a cyclic ketone by an intramolecular reaction. If this reaction were general, which we have not investigated, it could represent a new route to substituted dihydropyridinones which previously have been synthesised by Birch-type reduction of pyridinone (Guerry and Neier 1984).



171 **172** **167**

Grob fragmentation reactions are not uncommon and have been used in the synthesis of medium to large cyclic ketones (Zhang and Dowd 1996), taxane ring

derivatives (Kerkar *et al.* 1997) and in the synthesis of 3-substituted cyclohexanones such as **174** from diketone **173** (DeGiacomo *et al.* 1997). The heterolytic fragmentation as a class of reactions has been comprehensively reviewed (Grob and Schiess 1967; Grob 1969; March 1992).



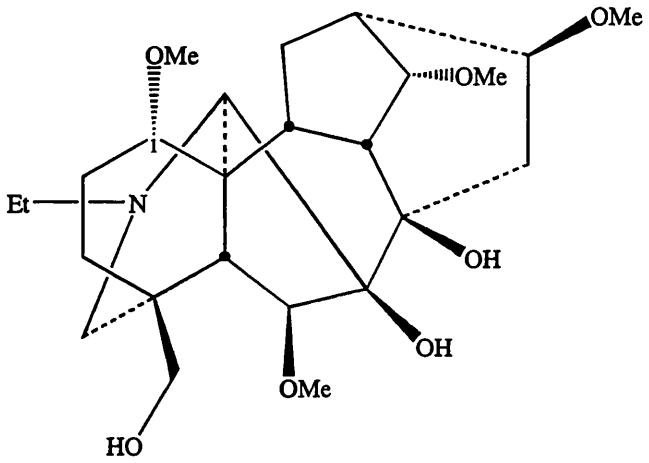
173 **174**

Chapter 6

SYNTHESIS OF 1-METHOXY SUBSTITUTED ANALOGUES OF LYCOCTONINE

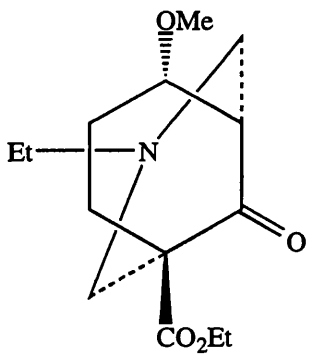
6.1 Design of synthetic targets

The C1 methoxy functional group of the lycoctonine class of alkaloids may play an important role in the natural alkaloid (and analogues) binding to nAChR. Furthermore, it has been shown, by molecular modelling, that the C1 methoxy is isolated from the other oxygen functionality in lycoctonine **3** being located on the opposite face of the molecule to the majority of the oxygen atoms.



lycoctonine **3**

In order to synthesise analogues of lycoctonine **3** containing this C1 methoxy functional group, we decided to synthesise the aza[3.3.1]bicyclic compound **175** as our initial target which would then be converted into lycoctonine analogue **176** on reduction with LAH.



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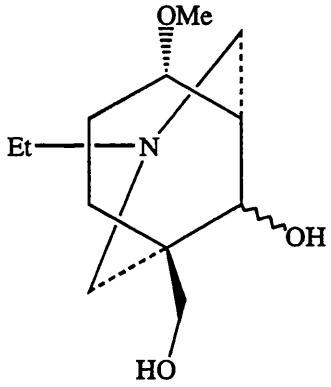
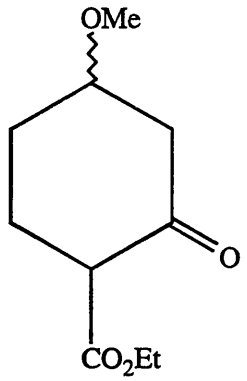


176

6.2 Synthesis of 1-carbethoxy-4-methoxycyclohexan-2-one

In order to synthesise the desired bicyclic β-ketoester **175** via a double Mannich reaction, it was necessary to synthesise the 4-methoxy (C1 using norditerpenoid numbering) substituted monocyclic β-ketoester **177**.



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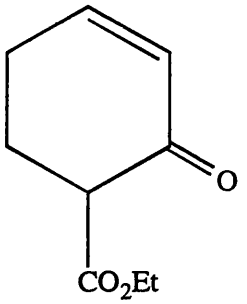
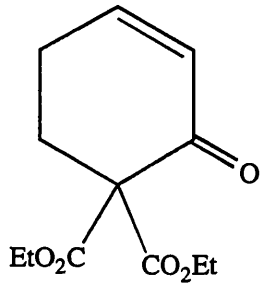


178

We envisaged that this compound could be prepared by a 1,4-conjugate addition of methanol to the corresponding enone **178** (cf conversion of cyclohex-2-en-1-one **138** into 3-methoxycyclohexan-1-one **135** in Chapter 4).

Enone **178** is not commercially available and was therefore synthesised from (46% isolated yield) by reacting the enolate of cyclohex-2-en-1-one **138**, (deprotonated with 2 equivalents of LDA made *in situ*) at -78°C, with ethyl cyanoformate (1 equivalent) (Mander and Sethi 1983). This reaction is hampered by the formation of the diacylated product **179** (25%) and carbamate **180** (the result of attack of LDA or diisopropylamine on the acylating agent) which were both identified by GC/MS analysis (VG Triol-1, 100-250°C, 15min) of the reaction mixture (diacylated product **179**: retention time 9.6min, molecular ion 240 $[M]^+$ $C_{12}H_{16}O_5$ requires 240, carbamate **180**: retention time 4.7min, molecular ion 173 $[M]^+$ $C_9H_{19}NO_2$ requires 173). Mander and Sethi (1983) reported that the use of cyanoformates enables regioselectivity and high yields, but we found that in our hands the yields obtained were only modest.



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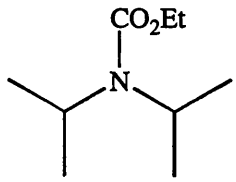
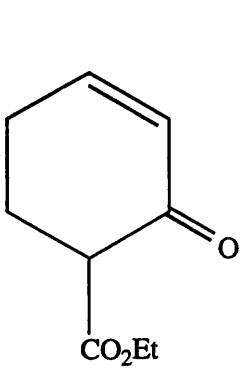


180

This reaction was monitored by tlc (with $KMnO_4$ visualisation) and the product had a slightly higher Rf (0.5, 2:3 EtOAc-hexane) than the starting material (0.4). Purification by silica gel chromatography gave a colourless oil. Evidence in the PMR spectra that the reaction had worked was the appearance of ethyl ester signals (a quartet at 4.22ppm (OCH_2) and a triplet at 1.29ppm (CH_3)) and the doublet of doublets at 3.41ppm (J = 9.9 and 5.7Hz) representing the methine between the two carbonyls.

Ethyl cyanoformate (the Mander protocol) was found to be the best acylating agent after attempting to use ethyl chloroformate which was too reactive and gave mainly diacylated product **179** and carbamate **180**. The use of diethyl carbonate also failed to give a practical yield (no reaction) of the desired enone **178**. Use of an alternative less nucleophilic base (LiHMDS) did not give a practically useful yield of the desired enone **178**.

Conversion of enone **178** into *O*-methyl ether **177** by conjugate addition of methanol (slight excess) was performed under acid catalysis (concentrated H_2SO_4) and yielded the desired methoxy β-ketoester in a reasonable yield (40%), a modification of the procedure of Lambert *et al.* (1988). Unsurprisingly, given the reaction conditions, this addition was accompanied by trans-esterification giving methyl ester **181** as a significant by-product. Using methyl cyanoformate as the acylating agent, would have yielded the methyl ester rather than the ethyl ester eliminating this problem and as the nature of the ester is not critical to the synthesis, this would not have affected the final product, the desired neopentyl-like alcohol **176**.



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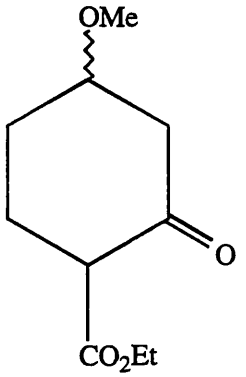

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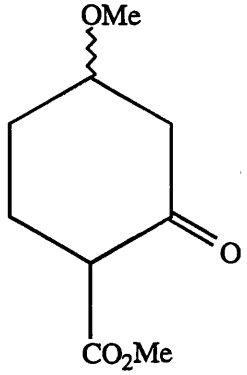
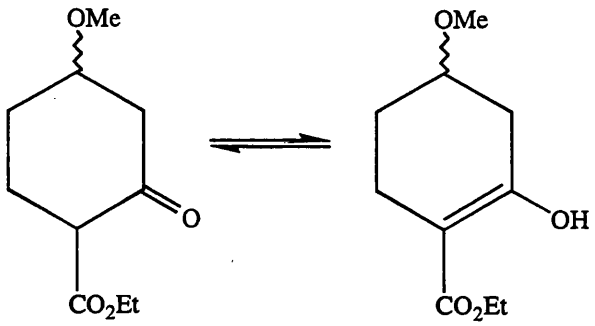


181

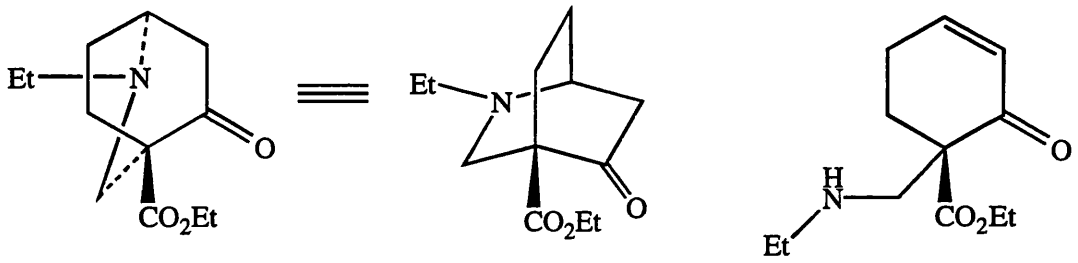
The conversion of enone **178** to methoxy β-ketoester **177** is a slow reaction (48-72h) and the reaction rate could not be increased by heating. The reaction was followed by tlc analysis (visualised with $KMnO_4$) and the product had a slightly higher Rf than the starting material even though an oxygen atom was added (0.6 as opposed to 0.5, 2:3 EtOAc-hexane). Significant diagnostic features in the PMR spectra include the appearance of the methoxy group protons (singlet at 3.39ppm) and the methine accompanying the methoxy group (a mulitiplet at 3.62-3.51ppm). Interestingly, there are signals in both the PMR and CMR spectra (singlet at 12.20ppm in the PMR and quaternary signals at 97.55 and 78.03ppm in the CMR) that indicate significant enolisation of the β-ketoester. In enone **178**, enolisation does not occur as having two double bonds in the six-membered ring leads to a structure of higher energy than that with only one. The change from enone to a fully saturated structure allows enolisation to occur as the cyclic enol tautomer of **177** has only one double bond in the ring.



177 **177**

6.3 Attempted double Mannich reaction of 1-carbethoxy-4-methoxycyclohexan-2-one

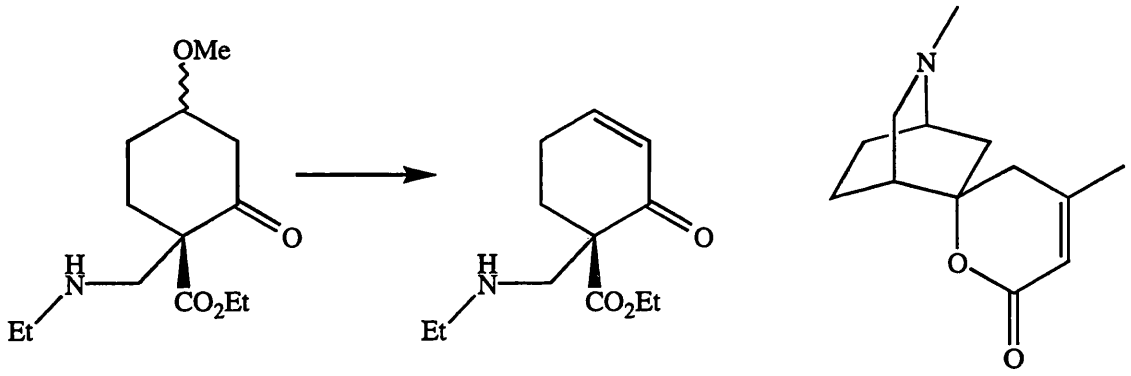
Having synthesised the suitably substituted β-ketoester **177**, an attempt was made to synthesise the aza[3.3.1]bicycle by performing a double Mannich reaction. Using the conditions described in Chapter 2, (1 equivalent of ethylamine and 2 equivalents of formaldehyde) except without acid catalysis, the double Mannich reaction did not proceed as expected, and did not yield 3-ethyl-3-azabicyclo[3.3.1]nonanone **175**. Silica gel chromatography gave a colourless, viscous oil (51% isolated yield) which did not display the expected spectroscopic properties of a [3.3.1]bicyclic system. Inspection of the mass spectrum (EI gave molecular ion 225, compound **175**: $C_{14}H_{23}NO_4$ requires 269) showed that the product was 44 Daltons less than the expected product (representing 2 carbon atoms, 1 oxygen atom and 4 hydrogens). Analysis of the PMR spectra showed that the two ethyl groups were still intact, but that the expected pattern of methylene signals (from the piperidine and carbocyclic six membered rings) had not appeared. The PMR spectra also show that the methoxy group and the corresponding methine are not present. Analysis of the CMR spectra show that the methoxy group has gone, but that both the ester and ketone carbonyl signals are still present. The CMR spectra (DEPT experiments) also show that there are not enough methylene groups for the expected [3.3.1]bicyclic system to have formed. From these data, combined with the knowledge that the methoxy group can readily eliminate from the starting material (especially when heated), we infer that the compound produced is in fact azabicyclo[2.2.2]octane **182**.



182 **182** **183**

Presumably this compound is formed via intermediate **183**, with ring closure afforded by an intramolecular 1,4-conjugate addition to the enone, i.e. a tandem Mannich-Michael reaction.

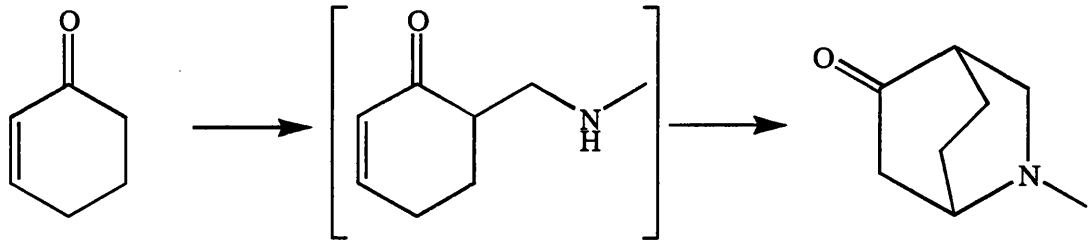
Intermediate **183** would be formed by a Mannich reaction (yielding **184**) involving the enol of starting methoxy substituted β-ketoester **177** followed by elimination of methanol to give the enone. Alternatively the elimination of methanol may have occurred before the Mannich reaction took place.



184 **183** **185**

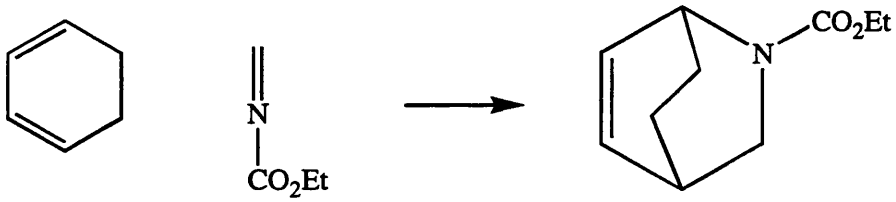
The 2-azabicyclo[2.2.2]octane system (an isoquinuclidine) occurs in the natural product dioscorone **185**, and in the iboga alkaloids. The isoquinuclidine nucleus **186** has been synthesised in a tandem Mannich-Michael reaction (Page and Pinder 1964) from cyclohex-2-en-1-one. Although the double Mannich reaction does not occur on simple ketones (such as cyclohexanone) this tandem Mannich-Michael reaction is possible because the product of the mono-Mannich (which is a minor by-product of the reaction)

reaction is immediately trapped by reacting in a Michael reaction which is presumably more facile than the second Mannich reaction.



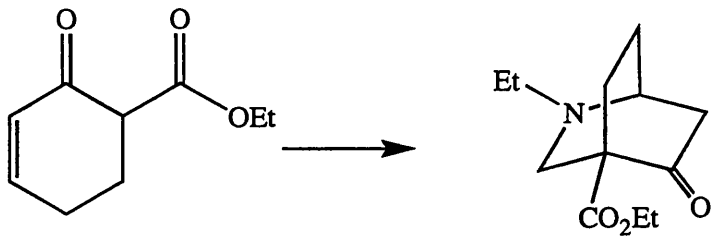
138 **186**

Another synthetic approach to the isoquinuclidine bicyclic system is a cyclo-addition strategy in which cyclohexa-1,3-diene **187** adds to the transient intermediate methyleneurethan **188** (Cava *et al.* 1965) yielding the unsaturated isoquinuclidine derivative **189**.



187 **188** **189**

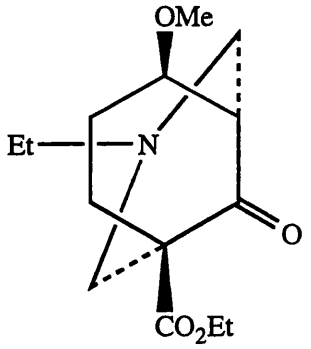
In order to confirm that formation of **182** can proceed via intermediate **183**, unsaturated β-ketoester **178** was used in a Mannich reaction (1 equivalent of ethylamine and 2 equivalents of formaldehyde) and as expected the [2.2.2]bicyclic compound was formed in good yield (79%).



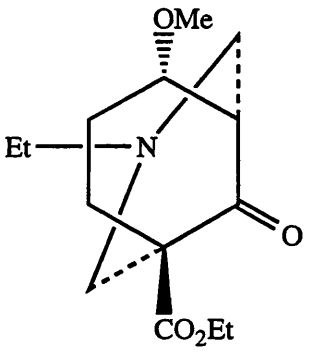
178 **182**

The Mannich reaction is normally performed under acid catalysis, but in this case the acid was omitted and it may have been that acid catalysis would have allowed

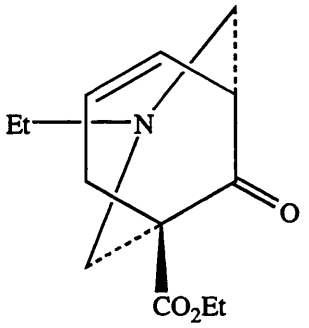
formation of the [3.3.1]bicycle. To test this hypothesis, an attempt to perform a one-pot synthesis of the [3.3.1]bicyclic compound **175** was made. This was done by adding the pre-formed imine **70** directly to the reaction mixture in which the addition of methanol to enone **178** had taken place. This reaction mixture contained acid which could potentially catalyse the double Mannich reaction. However, despite the presence of an acid source, this reaction still produced the [2.2.2]bicycle **182** as the major product. Analysis by GC/MS (VG Triol-1, 100-250°C, 15min) showed that within the product mixture was some of the desired product **175** (with both diastereoisomers **175a** and **175b**, present retention times 7.63 and 7.93min with $[M]^+$ 269 ($C_{14}H_{23}NO_4$ requires 269) although we do not know which diastereoisomer was which). There is also evidence of an unsaturated [3.3.1]bicyclic compound **190** or an isomer of it (retention time 6.57min $[M]^+$ 237 ($C_{13}H_{19}NO_3$ requires 237).



175a



175b



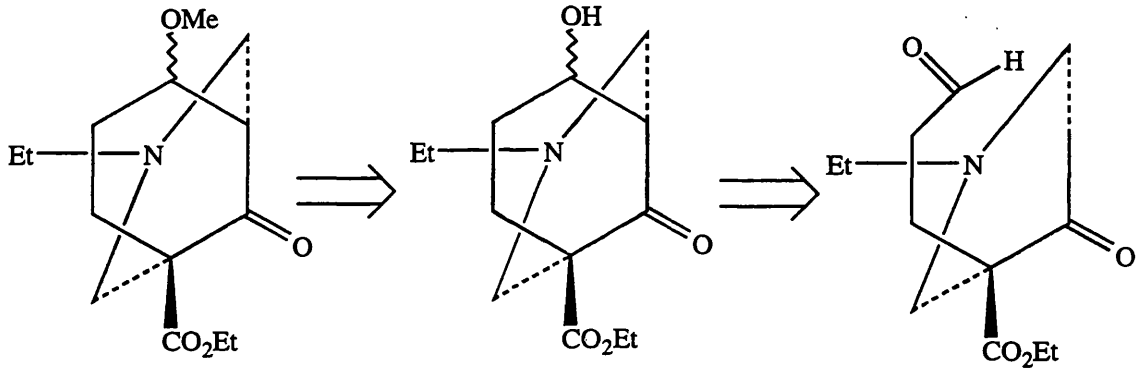
190

This route, despite being more successful than using no acid in the double Mannich reaction, still does not allow the synthesis of practical quantities of the desired

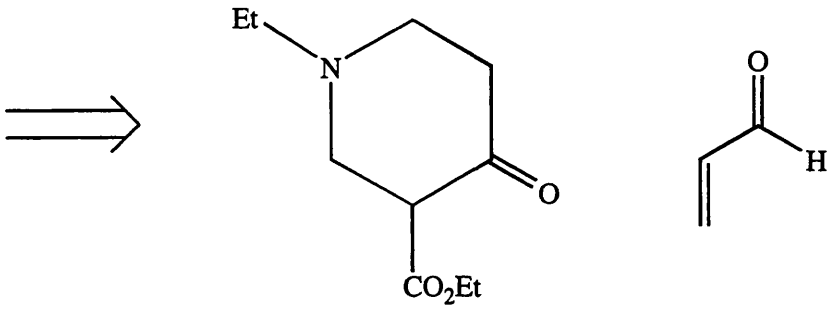
[3.3.1]bicyclic β-ketoester **175**. However, it does present a potentially valuable route to 2-azabicyclo[2.2.2]octanes (e.g. **182**) which are useful synthetic building blocks.

6.4 A tandem Michael addition-aldol condensation approach to oxygenated 3-azabicyclo[3.3.1]nonanes

Using the double Mannich reaction to synthesise the azabicyclo[3.3.1]nonane skeleton involves the addition of a piperidine ring to a pre-formed carbocycle, but it is possible, making a different disconnection, to envisage the synthesis of the bicyclic system in an alternative order.



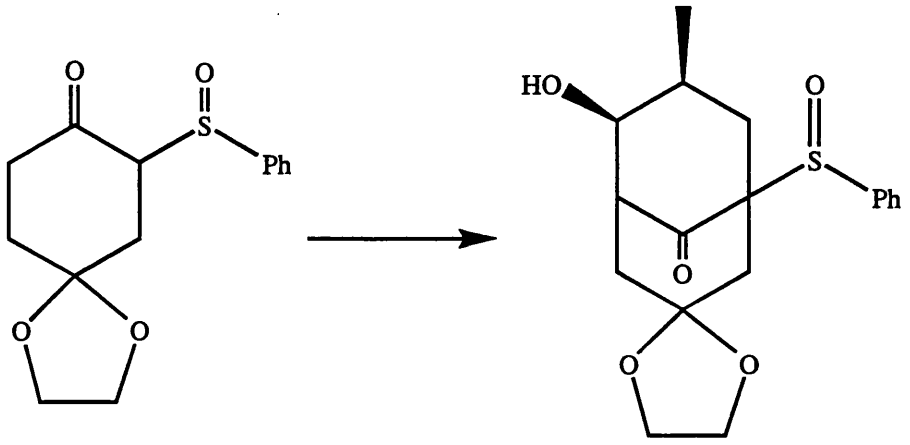
175 **191** **192**



193 **194**

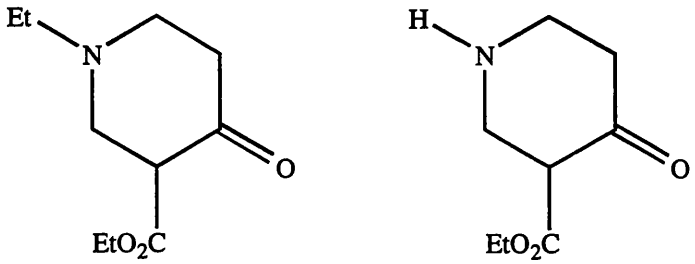
Thus, taking a pre-formed 4-piperidinone β-ketoester **193** and reacting with acrolein **194** under base catalysis should yield hydroxybicyclic compound **191** (via intermediate aldehyde **192**). This type of strategy has been used by Kraus and co-workers to

synthesise a bridgehead portion of the naturally occurring nootropic (i.e. memory enhancing) agent huperzine A (Kraus *et al.* 1992) as well as by Hesse and co-workers in their synthesis of bicyclo[3.3.0]octene systems (Lorenzi-Riatsch *et al.* 1984). As the key step in the synthesis, Kraus and co-workers used a β-ketosulphoxide **195** rather than a carboxylic acid ester as the Michael donor, and methacrolein as the Michael acceptor to synthesise their key intermediate **196**. This reaction was performed in anhydrous acetonitrile using DBU as base.



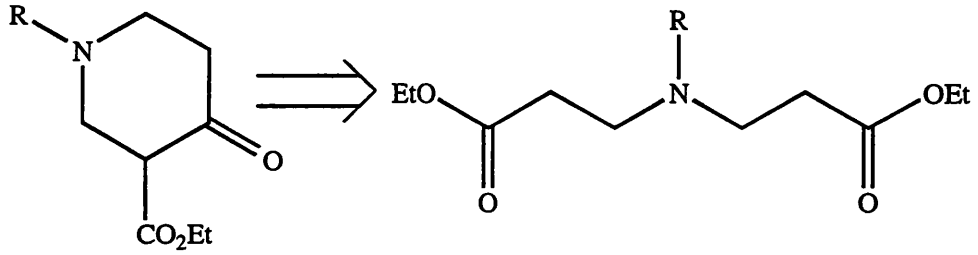
195 **196**

In order to apply this strategy to the synthesis of lycoctonine analogues, the appropriate 4-piperidinone β-ketoester **193** was required with an *N*-ethyl substituted piperidinone and preferably an ethyl ester. This compound is not commercially available, but the unsubstituted secondary amino compound **197** is available. Attempts at direct *N*-alkylation (NaH, EtI in THF) and reductive alkylation (MeCHO, $NaCNBH_3$, EtOH) of **197** did not yield the desired tertiary amine.



193 **197**

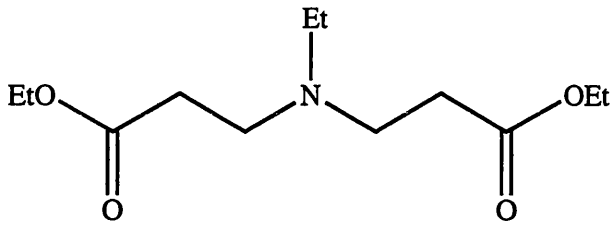
1,3-Dicarbonyl compounds (e.g. β-ketoesters) can be synthesised by Claisen ester condensation reactions and cyclic 1,3-dicarbonyl compounds by intramolecular condensation (acylation), the Dieckmann cyclisation reaction. Extensive literature searches uncovered the synthesis of piperidinone β-ketoester **193** by McElvain and co-workers in the 1920's and 30's (McElvain 1926; Prill and McElvain 1933). In investigations into the synthesis of simple piperidine derivatives (in SAR studies on cocaine), McElvain reported a general synthesis of *N*-alkylpiperidine β-ketoesters **198** from the corresponding diesters **199.**



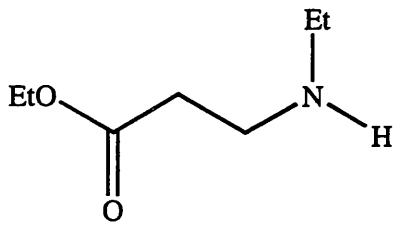
198 **199**

The diesters were synthesised from 2 equivalents of ethyl acrylate **106** (or by using the β-halopropionate ester) and 1 equivalent of the appropriate amine (e.g. ethylamine, *iso*-propylamine or *iso*-butylamine). Cyclisation was achieved with either sodium metal or with sodium ethoxide. Interestingly, the cyclisation reaction is not trivial and requires careful control of the reaction conditions. For a good yield to be obtained, any excess of ethanol (formed when deprotonation occurs using ethoxide as the base and by elimination during cyclisation) must be removed by distillation. The best way to achieve this was either to use no solvent, and to distil any ethanol produced out of the reaction vessels, or to use an inert solvent of higher boiling point than ethanol (e.g. toluene) and to azeotrope the ethanol out of the reaction mixture (Prill and McElvain 1933). This is presumably because the reaction is an equilibrium which can be reversed by reaction with a molecule of ethoxide. Thus, by removing the ethanol, the reaction is forced to the product side of the equilibrium.

In order to synthesise diester **200**, 2 equivalents of ethyl acrylate **107** were reacted with 1 equivalent of ethylamine (2M in THF) in refluxing ethanol for 6h. Without significant heating of the reaction mixture, only one addition occurs and **201** is formed, which can be converted into **200** by the addition of another equivalent of ethyl acrylate **107** and heating under reflux.

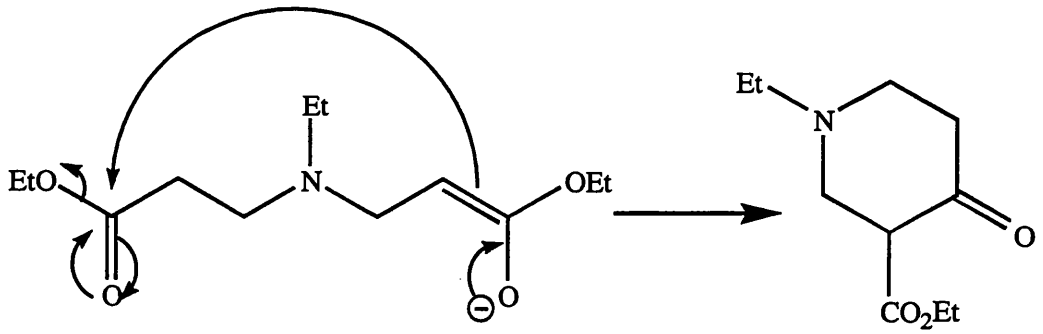


200



201

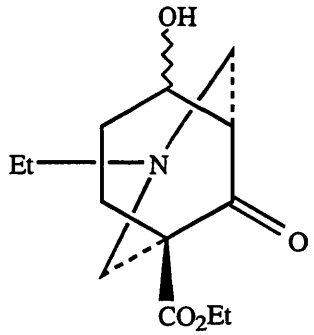
Purification by silica gel chromatography gave **200** as a pale yellow oil in moderate yield (49%). The main diagnostic feature in the PMR spectra was the ratio of integrals of the ethyl group signals with the ethylamine portion having an integral half that of the ethyl ester signals. Dieckmann cyclisation to yield the cyclic β-ketoester was achieved with sodium ethoxide in anhydrous toluene. Sodium ethoxide was prepared *in situ* with a slight excess of ethanol and then diester **200** was added dissolved in toluene. The reaction mixture was heated to 90°C with a water condenser set for downward distillation to remove ethanol (the excess and the eliminated ethanol). After 3h, no more ethanol was collected and the sodium salt of the product (as its enolate) separated as a crystalline white precipitate. Quenching this salt with acetic acid, and removal by filtration of the resulting sodium acetate yielded a solution of the product **193** which, after concentration under reduced pressure, was purified by silica gel chromatography to give a pale yellow oil in 55% yield. Tlc analysis showed that the product had a similar Rf to the diester (~0.5, 1:9 MeOH-DCM), but the product was observed by UV visualisation whereas the starting diester was not (the enolisable β-ketoester is detectable, but the starting material is not).



200 **193**

Inspection of the PMR spectra showed that the ratio of integrals of the two ethyl groups (ethyl amine and ethyl ester) was now 1:1, and that there was a multiplet at 3.48-3.44 for the α-methine between the two carbonyls. The CMR spectra showed the presence of the α-methine, but also that some enolisation of the β-ketoester had occurred by the presence of a quaternary alkenyl signal at 96.61ppm (assigned to the enol tautomer of the β-ketoester).

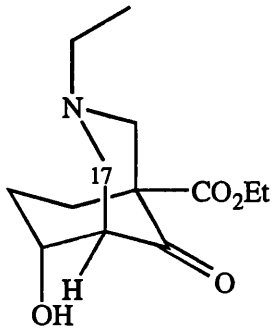
Having now synthesised the correct piperidinone β-ketoester **193**, the tandem Michael-aldol reaction was performed. To a solution of **193** in anhydrous acetonitrile (distilled from calcium hydride), and a stoichiometric amount of DBU as base, was added acrolein (propenal) and the reaction mixture stirred at 20°C for 16h. The resulting pale orange solution was concentrated under reduced pressure and silica gel chromatography (2:3 EtOAc-hexane) yielded **191** as a colourless oil in an overall yield of 85%. Unlike the reaction performed by Kraus and co-workers (Kraus *et al.* 1992), the product was a mixture of diastereoisomers which could be partially separated into three fractions (2 minor pure fractions (4*S* 11*S* 1*R* and 4*S* 11*S* 1*S* and their enantiomers) and a major mixed fraction). In the synthesis of huperzine A, reported by Kraus *et al.* (1992), methacrolein was used and the methyl group directed the stereochemistry of the product yielding one diastereoisomer. In the further stages of our synthesis, reactions were performed on the mixture of diastereoisomers, with a view to a more efficient separation of the products at a later stage.



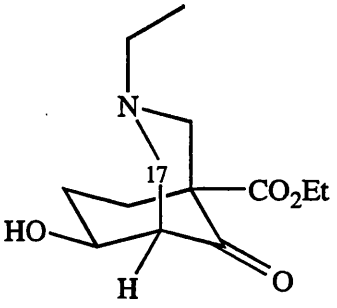
191

Analysis of the pure fractions of **191** by PMR spectroscopy indicated that the reaction had proceeded as expected. The enolisable α-proton (methine at ~3.5ppm) was no longer present and a new methine proton (a multiplet at~4.2-4.4ppm) was present which represents the methine next to the hydroxyl formed by the aldol condensation. Confirmation of the expected [3.3.1]azabicyclic structure (and its conformation) was shown by the splitting of the methylene proton signals adjacent to the newly formed hydroxyl with the axial proton being deshielded by the lone-pair on the nitrogen (appearing in the diastereoisomers at 2.85-2.75 and 3.24-3.02ppm respectively) and the equatorial proton not being affected (appearing at 1.96-1.88 and 1.70-1.61 respectively).

Analysis of the CMR spectra showed the methine substituted with a hydroxyl (72.15 and 76.53ppm) and the expected two new methylene signals at ~30ppm. Interestingly, in the less polar isomer, one of the amino methylenes (C17 norditerpenoid numbering) has a slightly lower chemical shift than expected (53.71ppm as opposed to the usual ~56ppm) which possibly indicates an interaction between the hydroxyl group and the C17 carbon atom. When the hydroxyl is in an axial orientation it is too far away from this methylene to affect it, but in an equatorial orientation the hydroxyl is in close proximity and may shield the carbon atom. From this we infer that the more polar isomer has an axial hydroxyl **191a** and the less polar has an equatorial hydroxyl **191b**.

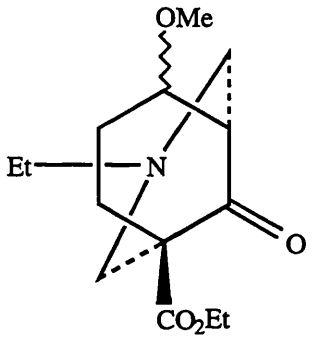


191a



191b

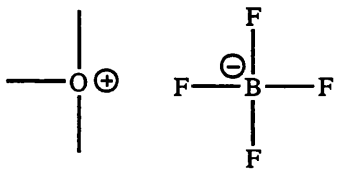
Thus, an efficient route to C1 oxygenated 3-ethyl-3-azabicyclo[3.3.1]nonan-9-ones has been established. In order to synthesise the desired lycoctonine analogue **175,** the newly formed hydroxyl must be *O*-methylated.



175

O-Methylation was attempted in two ways. Initially, the hydroxy-bicycle **191** was deprotonated with sodium hydride and reacted with methyl iodide, but this gave only a poor yield (4%) of the desired *O*-methyl ether **175.** We believe this could possibly be due to the formation of a quaternary ammonium species, with the more nucleophilic nitrogen (tertiary amine) reacting with methyl iodide in preference to the secondary alkoxide as the starting material was not recovered.

The second method of *O*-methylation attempted was with trimethyloxonium tetrafluoroborate **202** as the methylating agent (Evans *et al.* 1994; Ishii *et al.* 1996). This procedure used potassium carbonate as the base, but still gave a poor yield (6%) of the desired *O*-methyl ether **175.**

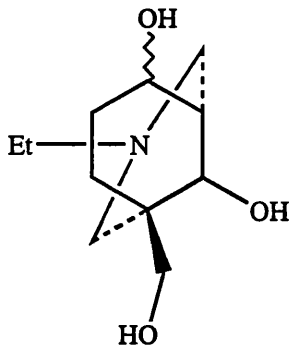


202

The failure of this reaction was possibly due (in part) to the formation of the tetramethylammonium species as the recovery of products from the reaction mixture after aqueous work-up was poor (indicating a polar, in this case ionic, species remaining in the aqueous fraction). Despite the poor yield, it was possible to separate the pair of diastereoisomeric *O*-methyl ethers and analyse them by PMR and CMR spectroscopy. Analysis of the PMR spectra showed that the required methoxy group is present (a singlet at 3.34 in the less polar isomer and a singlet at 3.32 in the more polar isomer). Analysis of the CMR spectra also showed new CH_3 signals at 56.40 and 55.91ppm respectively (with the multiplicity shown by DEPT experiments). Another possible explanation of the difficulties with methylation is that the alkoxide may not form due to the presence of a strong hydrogen bond between the 1-hydroxy and the tertiary amine. In order for this hydrogen bond to be present, the A-ring must be in a boat conformation and there is some precedent for this in natural products containing a C1-hydroxy functional group (Pelletier *et al.* 1974a and 1974b; Pelletier *et al.* 1976).

Having not been able to synthesise practical quantities of the desired *O*-methyl ether, the hydroxy-bicycle **191a** was reduced with LAH to give the *O*-demethylated analogue of lycoctonine **203** (there are many *Delphinium* and *Aconitum* alkaloids which possess a hydroxy at C1 rather than a methoxy e.g. neoline **10**, isotalatizidine **11**). The single diastereoisomer was used, as a mixture of diastereoisomers would have led to the formation of a complex mixture of four diastereoisomers which would have been difficult to separate. Reducing **191a** yielded two distinct diastereoisomers, in a ~5:1 ratio, which were separated by silica gel chromatography. Distinctive features of the PMR spectra

include the loss of the ethyl ester signals and the introduction of a new signal at 3.72ppm (doublet with J = 3.0 Hz) representing the methine of the secondary alcohol formed by ketone reduction. In the CMR spectra, the ester and ketone carbonyls are lost and the presence of a new methine at 75.61ppm which is the secondary alcohol at C5 (using norditerpenoid numbering). Comparatively, the 2 methylenes of the carbocyclic ring have a slightly higher chemical shift then in the non-oxygenated system as would be expected. Hence, a facile route to 1-hydroxy AE-bicyclic analogues of lycoctonine has been designed and carried out.



203

Chapter 7

EXPERIMENTAL

General Experimental Procedures

Chemicals

All chemicals were purchased from the Aldrich Chemical company (Gillingham, Dorset), with the exception of dicobalt octacarbonyl which was purchased from Fluka (Gillingham, Dorset). The purchased chemicals were used without further purification unless specified.

Experimental procedures

"Concentrated under reduced pressure" refers to removal of solvent with a Buchi rotary evaporator. When sodium or potassium hydride was used the mineral oil was removed by washing with anhydrous hexane (dried over shavings of sodium). When *n*-BuLi was used it was initially titrated against diphenylacetic acid to determine its molarity. Drying over magnesium sulphate refers to shaking over powdered $MgSO_4$ monohydrate followed by removal of the solid by filtration. Water refers to distilled water. All solvents were purchased from Fisons/Fisher (Loughborough) and were of HPLC grade unless otherwise specified. Anhydrous solvents were dried by distillation in the manner specified by Purification of Laboratory Chemicals (Perrin, Armarego and Perrin 1980) or Practical Organic Chemistry (Vogel 1989).

THF: After pre-drying by passing through a 10cm column of activated grade I alumina (which also removes peroxides), or by drying over KOH pellets for 16h, THF was distilled from sodium (5g) and benzophenone (5g). Refluxing under nitrogen was continued until a deep blue colour (the sodium benzophenone ketyl radical) was observed indicating that the THF was ready to be collected. Freshly distilled THF was used on all occasions.

Et_2O: (to dry 500ml) After first washing the diethyl ether with 5-10ml of acidified iron (II) sulphate solution (60g $FeSO_4$, 6ml of conc. H_2SO_4 and 110ml of water), the diethyl ether was passed down a 15cm column of grade I alumina. This diethyl ether was then refluxed over sodium (5g) and benzophenone (5g). Refluxing under nitrogen was continued until a deep blue colour (the sodium benzophenone ketyl radical) was observed indicating that the diethyl ether was ready to be collected. Freshly distilled diethyl ether was used on all occasions.

DMF: Anhydrous DMF was purchased from the Aldrich chemical company or reagent grade DMF was distilled from CaH_2 (5-10g/500ml).

DCM, EtOAc, DMPU and acetonitrile: 500ml of HPLC grade solvent was distilled from CaH_2 (5-10g/500ml).

Ethanol and methanol: Approximately 100ml of absolute ethanol or HPLC grade methanol was added to magnesium turnings (2.5g) and iodine (0.25g) and then warmed until all of the iodine and magnesium had disappeared. The remaining solvent was then added and heated under reflux for 30min-1, and finally collected and stored over 4Å molecular sieves.

Chromatography

Thin layer chromatography: (tlc) was performed on aluminium backed silica gel plates (Merck 60 F_{254}) using HPLC grade solvents unless otherwise specified. Visualisation methods were UV activity (254/366nm) and iodine staining, or with anisaldehyde, or $KMnO_4$, or ninhydrin dips as described in Practical Organic Chemistry (Vogel 1989).

Silica gel chromatography: refers to flash silica gel chromatography using the method of Still, Khan and Mitra (1978). All solvents were of HPLC grade unless otherwise

specified. Columns were dry packed with Sorbsil C60-H silica gel purchased from Merck (Eccles, Manchester). Pressure was applied to the columns via hand bellows.

NMR

All NMR experiments were carried out using either Jeol GX 270 or Jeol EX 400 spectrometers. All samples were in $CDCl_3$ using tetramethylsilane as internal standard unless otherwise specified. Assignments were made using a combination of NMR experiments including 135- and 90-DEPT carbon pulse experiments, standard 2D-correlation experiments (^1H-^1H and ^1H-^{13}C) as well as more complex semi-interpreted techniques such as FLOC long range correlation experiments.

Assignments are listed as:

PMR: δ chemical shift in ppm (number of protons, multiplicity, coupling constant *J* in Hz, assignment/interpretation)

CMR: δ chemical shift (assignment/interpretation)

Italicised numbers refer to norditerpenoid numbering unless otherwise specified.

Mass Spectrometry

All mass spectrometry was performed on a VG autospec spectrometer. Electron impact ionisation was carried out at 70eV and <70eV. Chemical ionisation was performed using isobutane and +ve and -ve FAB spectroscopy used 3-nitrobenzyl alcohol as the matrix.

Microanalysis

All microanalyses were performed using a Carla Erba 1106 elemental analyser.

Unless otherwise stated the compounds synthesised have not been previously reported.

General procedure 1: 3-Alkylation of ethyl 2-oxocylohexane-1-carboxylate with an alkyl iodide or bromide

Example: synthesis of ethyl 2-oxo-3-pentylcyclohexane-1-carboxylate **66a**

To a slurry of sodium hydride (0.546g of 60% $^{w}/_{w}$ in mineral oil, 12.94mmol) in anhydrous THF (25ml) at 0°C, was added ethyl 2-oxocylohexane-1-carboxylate **37** (2.000g, 11.76mmol) dissolved in anhydrous THF (10ml). Vigorous evolution of hydrogen gas was observed. The reaction mixture was stirred for 10min under an atmosphere of nitrogen and then cooled to -78°C with an acetone/dry ice bath. To this was added dropwise *n*-BuLi (5.56ml of 2.2M solution in hexanes, 12.35mmol) over 2 minutes. A solution of 1-iodopentane (2.329g, 11.76mmol) dissolved in DMPU (15ml, 30% of the total solvent volume) was then added dropwise (1 minute). The reaction temperature was then allowed to rise to 5°C. The reaction mixture was stirred for 16h (at 5°C) under nitrogen before being poured into saturated aqueous ammonium chloride solution. The organic (upper) layer was removed and concentrated under reduced pressure before re-dissolving in hexane (20ml). This hexane fraction was then washed sequentially with water (10ml) and saturated aqueous sodium chloride solution (10ml) and finally dried over magnesium sulphate. Filtration and concentration under reduced pressure gave a yellow oil (2.789g, 98% crude yield).

PMR: δ 12.43 (0.3H, s, $\underline{H}OC{=}CCO_2CH_2CH_3$), 12.25 (0.1H, s, $\underline{H}OC{=}CCO_2CH_2CH_3$), 4.27-4.13 (2H, m, $OC\underline{H}_2CH_3$), 3.42-3.34 (0.6H, m, $OCC\underline{H}CO_2CH_2CH_3$), 2.69-1.23 (18H, m), 0.88 (3H, t, 7.0, $CH_2CH_2C\underline{H}_3$).

Synthesis of ethyl 3-butyl-2-oxocyclohexane-1-carboxylate **66b**

Using general procedure 1. ethyl 2-oxocylohexane-1-carboxylate **37** (3.000g, 17.6mmol), NaH (0.847g of 60% $^{w}/_{w}$ in mineral oil, 21.10mmol), *n*-BuLi (7.7ml of 2.4M in hexanes, 18.48mmol), 1-iodobutane (3.560g, 19.36mmol), DMPU:THF (15:25ml).

PMR: δ 12.42 (0.1H, s, HOC=CCO$_2$CH$_2$CH$_3$), 12.23 (0.6H, s, HOC=CCO$_2$CH$_2$CH$_3$), 4.19 (2H, q, 7.0, OCH$_2$CH$_3$), 3.35 (0.3H, m, O=CCHCO$_2$CH$_2$CH$_3$), 2.47-1.56 (12H, m), 1.28 (3H, t, 7.0, OCH$_2$CH$_3$), 0.89 (3H, t, 7.0, CH$_2$CH$_2$CH$_3$).

Synthesis of ethyl 2-oxo-3-(prop-2-enyl)cyclohexane-1-carboxylate **66c** (previously synthesised by Gravel and Labelle (1985))

Using general procedure 1. ethyl 2-oxocylohexane-1-carboxylate **37** (5.000g, 29.4mmol), NaH (1.140g of 60% $^{w}/_{w}$ in mineral oil, 21.10mmol), *n*-BuLi (10.25ml of 2.4M in hexanes, 30.87mmol), allyl bromide (3.550g, 29.40mmol), DMPU:THF (30:70ml).

PMR: δ 12.44 (0.25H, s, HOC=CCO$_2$CH$_2$CH$_3$), 12.26 (0.25H, s, HOC=CCO$_2$CH$_2$CH$_3$), 5.84-5.69 (1H, m, CH=CH$_2$), 5.09-4.99 (2H, m, CH=CH$_2$), 4.27-4.17 (2H, m, OCH$_2$CH$_3$), 3.43-3.34 (0.5H, m, O=CCHCO$_2$CH$_2$CH$_3$), 2.65-1.21 (13H, m).

Synthesis of ethyl 3-cyclohexylmethyl-2-oxocyclohexane-1-carboxylate **66d**

Using general procedure 1. ethyl 2-oxocylohexane-1-carboxylate **37** (2.000g, 11.60mmol), NaH (0.547g of 60% $^{w}/_{w}$ in mineral oil, 14.12mmol), *n*-BuLi (5.56ml of 2.4M in hexanes, 12.35mmol), cyclohexylmethyl bromide (2.080g, 11.76mmol), DMPU:THF (15:30ml). Used without purification.

Synthesis of ethyl 3-cyclopentyl-2-oxocyclohexane-1-carboxylate **66e**

Using general procedure 1. ethyl 2-oxocylohexane-1-carboxylate **37** (3.000g, 17.60mmol), NaH (0.847g of 60% $^{w}/_{w}$ in mineral oil, 21.10mmol), *n*-BuLi (8.2ml of

2.17M in hexanes, 18.48mmol), cyclopentyl bromide (2.880g, 19.36mmol), DMPU:THF (10:20ml).

PMR: δ 12.50 (0.25H, s, HOC=CCO$_2$CH$_2$CH$_3$), 12.26 (0.25H, s, HOC=CCO$_2$CH$_2$CH$_3$), 4.25-4.16 (2H, m, OCH$_2$CH$_3$), 3.45-3.35 (0.5H, m, O=CCHCO$_2$CH$_2$CH$_3$), 2.49-1.07 (19H, m).

Synthesis of ethyl 3-benzyl-2-oxocyclohexane-1-carboxylate **66f** (previously synthesised by Godfrey and Ganem (1992))

Using general procedure 1. ethyl 2-oxocylohexane-1-carboxylate **37** (3.000g, 17.6mmol), NaH (0.847g of 60% $^{w}/_{w}$ in mineral oil, 21.10mmol), *n*-BuLi (8.2ml of 2.17M in hexanes, 18.48mmol), benzyl bromide (3.310g, 19.36mmol), DMPU:THF (10:20ml).

PMR: δ 12.52 (0.25, s, HOC=CCO$_2$CH$_2$CH$_3$), 12.26 (0.25H, s, HOC=CCO$_2$CH$_2$CH$_3$), 7.30-7.13 (5H, m, 5 x aromatic CH), 4.27-4.12 (2H, m, OCH$_2$CH$_3$), 3.44-3.21 (0.5H, m, O=CCHCO$_2$CH$_2$CH$_3$), 2.59-1.12 (11H, m).

General procedure 2: Double Mannich reaction to form azabicyclo[3.3.1]nonanes

Example: synthesis of ethyl 3-ethyl-5-pentyl-3-azabicyclo[3.3.1]nonan-9-one-1-carboxylate **64a**

Modified from the procedure of Shimizu *et al.* (1963a).

To formaldehyde (1.910g, 37% $^{w}/_{w}$ in water, 23.52mmol) was added ethylamine (0.756g 70% $^{w}/_{w}$ in water, 11.76mmol) dissolved in absolute ethanol (10ml). To this was then added crude 3-pentyl-ethyl 2-oxocylohexane-1-carboxylate **66a** (2.789g, 11.76mmol, assuming 100% alkylation) dissolved in absolute ethanol (20ml). To this was then added glacial acetic acid (0.5ml) and the reaction mixture was heated under reflux for 16h. The

reaction mixture was cooled and quenched with solid sodium hydrogen carbonate (until pH 8.0). The reaction mixture was then filtered and concentrated under reduced pressure. The residual orange yellow oil was dissolved in DCM (30ml) and then sequentially washed with saturated aqueous sodium hydrogen carbonate solution (10ml), water (10ml) and saturated aqueous sodium chloride solution (10ml) and finally dried over sodium sulphate filtered and concentrated under reduced pressure. Purification of the residual yellow oil by silica gel chromatography (1:49 EtOAc-hexane) gave a clear viscous oil (2.080g, 57% over 2 steps).

PMR: δ 4.20 (2H, q, 7.2, $OC\underline{H}_2CH_3$), 3.19 (1H, dd, 11.4, 2.2, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 3.05 (1H, dd, 11.0, 2.4, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 3.02-2.83 (2H, m, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19* and 1 of $C\underline{H}_2$ *2* ax), 2.58-2.46 (2H, m), 2.39 (2H, q, 8.8, $NC\underline{H}_2CH_3$), 2.34-2.08 (3H, m), 1.55-1.45 (1H, m, $C\underline{H}_2$ *2* eq), 1.43-1.33 (8H, m), 1.29 (3H, t, 7.0, $OCH_2C\underline{H}_3$), 1.10 (3H, t, 7.1, $NCH_2C\underline{H}_3$), 0.87 (3H, t, 6.8, $CH_2CH_2C\underline{H}_3$).

CMR: δ 213.46 (cyclohexanone carbonyl), 171.49 (ester carbonyl), 64.69, 61.77 $\underline{C}H_2N\underline{C}H_2$), 60.97 ($O\underline{C}H_2CH_3$), 58.98 (quaternary $EtO_2C\underline{C}C{=}O$ *4*), 51.20 ($N\underline{C}H_2CH_3$), 49.18 (quaternary $NCH_2\underline{C}C{=}O$), 39.23, 36.87 (2 x $C\underline{H}_2$ *1* and *3*), 34.69, 32.66, 22.91, 22.49 (4 x $C\underline{H}_2$ *7, 8, 9, 10*), 20.45, ($\underline{C}H_2$ *2*), 14.12 ($OCH_2\underline{C}H_3$), 13.99 ($CH_2CH_2\underline{C}H_3$), 12.65 ($NCH_2\underline{C}H_3$).

MS: low eV ionisation gave m/z 309 $[M]^+$ ($C_{18}H_{31}O_3N$ requires 309).

Synthesis of ethyl 3-ethyl-5-butyl-3-azabicyclo[3.3.1]nonan-9-one-1-carboxylate **64b**

Using general procedure 2. 3-Butyl-ethyl 2-oxocylohexane-1-carboxylate **66b** (4.012g crude, assume 17.60mmol), formaldehyde (2.850g of 37% $^w/_w$ H_2O, 35.20mmol), ethylamine (1.130g of 70% $^w/_w$ H_2O, 17.60mmol), glacial acetic acid (0.5ml), absolute ethanol (40ml). Silica gel chromatography (1:19 EtOAc-hexane) gave a colourless oil (3.016g, 58% over 2 steps).

PMR: δ 4.21 (2H, q, 7.2, $OC\underline{H}_2CH_3$), 3.18 (1H, dd, 11.4, 2.4, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 3.05 (1H, dd, 11.2, 2.4, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 2.93-2.89 (2H, m), 2.59-2.45 (1H, m), 2.39 (2H, q, 7.1, $NC\underline{H}_2CH_3$), 2.26-2.07 (4H, m), 1.83-1.69 (1H, m), 1.57-1.43 (1H, m, $C\underline{H}_2$ *2* eq), 1.42-1.23 (8H, m), 1.10 (3H, t, 7.4, $NCH_2C\underline{H}_3$), 0.89 (3H, t, 6.7, $CH_2CH_2C\underline{H}_3$).

CMR: δ 213.48 (cyclohexanone carbonyl), 171.46 (ester carbonyl), 64.63, 61.73 ($\underline{C}H_2N\underline{C}H_2$ *17* and *19*), 60.96 ($CH_3\underline{C}H_2O$), 58.95 (quaternary $EtO_2C\underline{C}C=O$), 51.17 ($CH_3\underline{C}H_2N$), 49.11 (quaternary $NCH_2\underline{C}C=O$), 39.20, 36.84 (2 x CH_2 *1* and *3*), 34.43, 25.43, 23.51 (3 x CH_2 *10, 9, 8*), 20.42 ($\underline{C}H_2$ *2*), 14.05 ($OCH_2\underline{C}H_3$), 13.94 ($CH_2CH_2\underline{C}H_3$), 12.62 ($NCH_2\underline{C}H_3$).

MS: CI gave m/z 296 $[M+H]^+$ ($C_{17}H_{29}O_3N$ requires 295).

Synthesis of ethyl 3-ethyl-5-(3-prop-1-enyl)-3-azabicyclo[3.3.1]nonan-9-one-1-carboxylate **64c** (previously synthesised by Kraus *et al.* (1993))

Using general procedure 2. 3-(3-Prop-1-enyl)-ethyl 2-oxocylohexane-1-carboxylate **66c** (6.150g crude, assume 29.4mmol), formaldehyde (4.770g of 37% $^w/_w$ H_2O, 58.80mmol), ethylamine (1.89g of 70% $^w/_w$ H_2O, 29.40mmol), glacial acetic acid (1.0ml), absolute ethanol (75ml). Silica gel chromatography (1:9 EtOAc-hexane) gave a colourless oil (2.252g, 27% over 2 steps).

PMR: δ 5.86-5.70 (1H, m, $C\underline{H}=CH_2$), 5.06-4.99 (2H, m, $CH=C\underline{H}_2$), 4.22 (2H, q, 7.2, $OC\underline{H}_2CH_3$), 3.19 (1H, dd, 11.3, 2.2, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 3.02 (1H, dd, 11.1, 2.4, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 2.97-2.86 (2H, m), 2.58-2.47 (1H, m), 2.39 (2H, q, 7.1, $NC\underline{H}_2CH_3$), 2.32-2.06 (5H, m), 1.84-1.73 (1H, m), 1.54-1.47 (1H, m, $C\underline{H}_2$ *2* eq), 1.29 (3H, t, 7.1, $OCH_2C\underline{H}_3$), 1.09 (3H, t, 7.1, $NCH_2C\underline{H}_3$).

CMR: δ 212.89 (cyclohexanone carbonyl), 171.38 (ester carbonyl), 133.83 ($\underline{C}H=CH_2$), 118.03 ($CH=\underline{C}H_2$), 64.56, 61.74 ($\underline{C}H_2N\underline{C}H_2$), 61.12 ($O\underline{C}H_2CH_3$), 59.02 (quaternary

$EtO_2C\underline{C}C{=}O$), 51.19 ($N\underline{C}H_2CH_3$), 48.90 (quaternary $NCH_2\underline{C}C{=}O$), 39.39, 39.26, 36.91(3 x CH_2 *1, 3, 10*), 20.39 ($\underline{C}H_2$ *2*), 14.17($OCH_2\underline{C}H_3$), 12.69 ($NCH_2\underline{C}H_3$).

MS: CI gave m/z 280 $[M+H]^+$ ($C_{16}H_{25}O_3N$ requires 279).

Synthesis of ethyl 3-ethyl-5-cyclohexylmethyl-3-azabicyclo[3.3.1]nonan-9-one-1-carboxylate **64d**

Using general procedure 2. 3-Cyclohexylmethyl-ethyl 2-oxocylohexane-1-carboxylate **66d** (3.460g crude, assume 11.76mmol), formaldehyde (1.907g of 37% $^w/_w$ H_2O, 23.52mmol), ethylamine (0.756g of 70% $^w/_w$ H_2O, 11.76mmol), glacial acetic acid (0.5ml), absolute ethanol (30ml). Silica gel chromatography (1:49 EtOAc-hexane) gave a colourless oil (1.500g, 39% over 2 steps).

PMR: δ 4.21 (2H, q, 7.1, $OC\underline{H}_2CH_3$), 3.18 (1H, dd, 11.3, 2.1, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 3.07 (1H, dd, 11.0, 2.1, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 3.03-2.88 (2H, m), 2.58-2.08 (10H, m), 1.89-1.43 (11H, m), 1.32-1.22 (3H, m, $OCH_2C\underline{H}_3$), 1.10 (3H, t, 7.1, $NCH_2C\underline{H}_3$).

CMR: δ 213.23 (cyclohexanone carbonyl), 171.47 (ester carbonyl), 64.95, 61.64 ($\underline{C}H_2N\underline{C}H_2$ *17* and *19*), 60.98 ($O\underline{C}H_2CH_3$), 58.98 (quaternary, $EtO_2C\underline{C}C{=}O$), 51.25 ($N\underline{C}H_2CH_3$), 50.16 (quaternary, $NCH_2\underline{C}C{=}O$), 41.84, 39.71, 36.89, 25.69, 35.64 (5 x $\underline{C}H_2$), 33.38 ($\underline{C}H$), 26.50, 26.16 (2 x $\underline{C}H_2$), 20.48 ($\underline{C}H_2$ *2*), 14.11 ($OCH_2\underline{C}H_3$), 12.70 ($NCH_2\underline{C}H_3$)

MS: low eV EI gave m/z 335 $[M]^+$ ($C_{20}H_{33}O_3N$ requires 335).

Synthesis of ethyl 3-ethyl-5-cyclopentyl-3-azabicyclo[3.3.1]nonan-9-one-1-carboxylate **64e**

Using general procedure 2. 3-Cyclopentyl-ethyl 2-oxocylohexane-1-carboxylate **66e** (0.576g crude, assume 2.94mmol), formaldehyde (0.477g of 37% $^w/_w$ H_2O, 5.88mmol),

ethylamine (0.189g of 70% $^{w}/_{w}$ H_2O, 2.94mmol), glacial acetic acid (0.5ml), absolute ethanol (10ml). Silica gel chromatography (1:49 EtOAc-hexane) gave a colourless oil (0.286g, 32% over 2 steps).

PMR: δ 4.24-4.17 (2H, m, $OC\underline{H}_2CH_3$), 3.10 (1H, dd, 11.2, 2.4, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 2.96 1H, dd, 10.7, 2.0, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 2.92 (1H, d, 11.7, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 2.70-2.59 (1H, m, CH_2 *2* ax), 2.55 -2.35 (3H, m), 2.31 (1H, d, 11.7, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 2.23-2.14 (1H, m), 2.10-1.95 (2H, m), 1.86-1.45 (10H, m), 1.28 (3H, t, 6.8, $OCH_2C\underline{H}_3$), 1.09 (3H, t, 7.3, $NCH_2C\underline{H}_3$).

CMR: δ 213.55 (cyclohexanone carbonyl), 171.87 (ester carbonyl), 63.88, 61.70 ($\underline{C}H_2N\underline{C}H_2$), 61.04 ($CH_3\underline{C}H_2O$), 59.14 (quaternary $EtO_2C\underline{C}C{=}O$), 52.05 (quaternary $NCH_2\underline{C}C{=}O$), 51.19 ($CH_3\underline{C}H_2N$), 42.23 ($\underline{C}H$), 36.32, 36.15, 27.27, 26.98, 26.01, 25.88, 20.79 (7 x $\underline{C}H_2$), 14.17 ($OCH_2\underline{C}H_3$), 12.67 ($NCH_2\underline{C}H_3$).

MS: low eV EI gave m/z 307 $[M]^+$ ($C_{18}H_{29}O_3N$ requires 307).

Synthesis of ethyl 3-ethyl-5-benzyl-3-azabicyclo[3.3.1]nonan-9-one-1-carboxylate **64f**

Using general procedure 2. 3-Benzyl-ethyl 2-oxocylohexane-1-carboxylate **66f** (0.500g crude, assume 2.94mmol), formaldehyde (0.477g of 37% $^{w}/_{w}$ H_2O, 5.88mmol), ethylamine (0.189g of 70% $^{w}/_{w}$ H_2O, 2.94mmol), glacial acetic acid (0.5ml), absolute ethanol (10ml). Silica gel chromatography (1:9 Et_2O-hexane) gave a colourless oil (0.328g, 34% over 2 steps).

PMR: δ 7.28-7.09 (5H, m, 5 x aromatic $C\underline{H}$), 4.21 (2H, q, 7.3, $OC\underline{H}_2CH_3$), 3.52-3.48 (1H, m, 1 of $C\underline{H}_2NC\underline{H}_2$), 3.22 (1H, dd, 13.5, 2.4, 1 of $C\underline{H}_2NC\underline{H}_2$), 2.97-2.77 (2H, m, $C\underline{H}_2Ar$), 2.62-2.37 (3H, m), 2.29-2.01 (5H, m), 1.58-1.45 (2H, m), 1.29 (3H, t, 7.3, $OCH_2C\underline{H}_3$), 1.10 (3H, t, 7.1, $NCH_2C\underline{H}_3$).

MS: CI gave m/z 330 $[M+H]^+$ ($C_{20}H_{27}O_3N$ requires 329).

Synthesis of ethyl 3-ethyl-3-azabicyclo[3.3.1]nonan-9-one-1-carboxylate **4**

Using general procedure 2. ethyl 2-oxocylohexane-1-carboxylate **37** (5.000g, 29.40mmol), formaldehyde (5.720g of 37% $^{w}/_{w}$ H_2O, 70.56mmol), ethylamine (2.260g of 70% $^{w}/_{w}$ H_2O, 35.3mmol), glacial acetic acid (1.0ml), absolute ethanol (30ml). Silica gel chromatography (1:9 EtOAc-hexane) gave a colourless oil (2.604g, 37%).

PMR: δ 4.21 (2H, q, 7.2, $OC\underline{H}_2CH_3$), 3.22 (1H, dd, 11.2, 2.4 $C\underline{H}_2NCH_2CH$ *19*), 3.15 (1H, dt, 11.2, 2.0, 1 of $CH_2NC\underline{H}_2CH$ *17*), 2.93 (1H, dd, 11.2, 2.0, 1 of $C\underline{H}_2NCH_2CH$ *19*), 2.90-2.81 (1H, m, $C\underline{H}_2$ *2* ax), 2.59-2.35 (5H, m), 2.27-2.02 (3H, m), 1.57-1.49 (1H, m, $C\underline{H}_2$ *2* eq), 1.29 (3H, t, 7.3, $OCH_2C\underline{H}_3$), 1.10 (3H, t, 7.3, $NCH_2C\underline{H}_3$).

CMR: δ 212.70 (ketone carbonyl), 171.18 (ester carbonyl), 61.60 ($\underline{C}H_2NCH_2CH$ *19*), 61.04 ($CH_3\underline{C}H_2O$), 59.86 ($CH_2N\underline{C}H_2CH$ *17*), 58.76 (quaternary), 51.05 ($CH_3\underline{C}H_2N$), 38.13 (<u>C</u>H), 36.77, 34.13 (2 x $\underline{C}H_2$ *1* and *3*), 20.49 ($\underline{C}H_2$ *2*), 14.09 ($OCH_2\underline{C}H_3$), 12.68 ($NCH_2\underline{C}H_3$).

General procedure 3: Reduction of bicyclic β-ketoester compounds to diols

Example: synthesis of 3-ethyl-5-pentyl-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-methanol **74a**

To a slurry of lithium aluminium hydride (0.096g, 2.54mmol) in anhydrous diethyl ether (15ml) was added a solution of **64a** (0.784g, 2.54mmol) in anhydrous diethyl ether (5ml). This was stirred for 3h at 20°C under an atmosphere of nitrogen. The reaction was quenched with 5 drops of 0.5M aqueous sodium hydroxide solution. The resulting suspension was filtered through celite and concentrated under reduced pressure to give a white solid (0.774g). Purification by silica gel chromatography (3:7→67:33 EtOAc-hexane) gave a major product (0.365g, 55%) and a minor product (0.070g, 10%) which were both white foams.

Spectroscopic data for major isomer:

PMR: δ 3.46 (1H, d, 10.8, 1 of C<u>H</u>OH), 3.38 (2H, d, 10.8, 1 of C<u>H</u>$_2$OH), 2.77 (1H, dd, 11.2, 1.0, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.73-2.65 (1H, m, C<u>H</u>$_2$ *2* ax), 2.61 (1H, dd, 10.7, 1.0, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.46 (2H, s, 2 x OH), 2.23-2.13 (2H, m), 2.05-1.94 (1H, m), 1.82 (1H, dd, 11.2, 2.0, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 1.75 (1H, dd, 10.8, 2.0, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 1.58-1.12 (12H, m), 1.02 (3H, t, 6.8, NCH$_2$C<u>H</u>$_3$), 0.88 (3H, t, 7.3, alkyl C<u>H</u>$_3$).

CMR: δ 78.47 (<u>C</u>HOH), 71.03 (<u>C</u>H$_2$OH), 62.32, 60.70 (<u>C</u>H$_2$N<u>C</u>H$_2$) 52.47 (CH$_3$<u>C</u>H$_2$N), 39.01 (quaternary HOCH$_2$<u>C</u>CHOH), 38.24 (<u>C</u>H$_2$ *1* or *3*), 37.82 (quaternary HO<u>C</u>CH$_2$N), 32.86, 30.01, 26.39, 22.67, 22.48 (5 x <u>C</u>H$_2$ *1* or *3, 10, 9, 8, 7*), 20.48 (CH$_2$ *2*) 14.10 (NCH$_2$<u>C</u>H$_3$), 12.66 (<u>C</u>H$_3$).

MS: EI gave m/z 269.3 $[M]^+$ ($C_{16}H_{31}O_2N$ requires 269).

Synthesis of 3-ethyl-5-butyl-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-methanol **74b**

Using general procedure 3. **64a** (1.000g, 3.38mmol), LAH (0.128g, 3.38mmol), Et_2O (10ml). Silica gel chromatography (1:19 MeOH-DCM) gave a white foam (major isomer 0.393g, 46%).

PMR: δ 3.48-3.35 (3H, m, C<u>H</u>OH and 2 x C<u>H</u>$_2$OH), 2.85-2.51 (3H, m), 2.20 (1H, s), 2.01-1.71 (2H, m), 1.61-1.40 (2H, m), 1.37-1.15 (2H, m), 1.10-0.98 (13H, m), 0.90 (3H, t, 6.4).

Synthesis of 3-ethyl-5-(3-prop-1-enyl)-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-methanol **74c**

Using general procedure 3. **64c** (0.630g, 2.60mmol), LAH (0.100g, 2.60mmol), Et_2O (15ml). Silica gel chromatography (2:3 EtOAc-hexane) gave a homogenous white foam (0.323g, 60%).

PMR: δ 5.93-5.80 (1H, m, C<u>H</u>=CH_2), 5.08-5.01 (2H, m, CH=C<u>H_2</u>), 3.47-3.37 (3H, m, C<u>H</u>OH and 2 x C<u>H_2</u>OH), 2.75-2.60 (4H, m), 2.5 (2H, bs, 2 x OH), 2.23-2.11 (2H, m), 2.08-1.90 (1H, m), 1.85 (1H, dd, 11.3, 2.4, 1 of C<u>H_2</u>NC<u>H_2</u> *17* or *19*), 1.76 (1H, dd, 11.0, 2.5, 1 of C<u>H_2</u>NC<u>H_2</u> *17* or *19*), 1.70-1.61 (2H, m), 1.52-1.41 (2H, m), 1.30-1.19 (2H, m), 1.01 (3H, t, 7.0, $NCH_2C\underline{H}_3$).

CMR: δ 134.77 (<u>C</u>H=), 117.40 (<u>C</u>H_2=), 78.34 (<u>C</u>HOH), 71.02 (<u>C</u>H_2OH), 62.64, 60.74 (<u>C</u>H_2N<u>C</u>H_2), 52.29 ($CH_3\underline{C}H_2N$), 42.88 (CH_2=CH<u>C</u>H_2), 39.09, 38.49 (2 x quaternary), 29.93, 26.31 (2 x <u>C</u>H_2 *1* and *3*), 20.51 (<u>C</u>H_2 *2*), 12.70 ($NCH_2C\underline{H}_3$).

MS: CI gave m/z 240 $[M+H]^+$ ($C_{14}H_{25}O_2N$ requires 239).

<u>Synthesis of 3-ethyl-5-cyclohexylmethyl-1-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-methanol **74d**</u>

Using general procedure 3. **64d** (0.950g, 2.84mmol), LAH (0.108g, 2.84mmol), Et_2O (20ml). Silica gel chromatography (3:7 EtOAc-hexane) gave a homogenous white foam (0.323g, 39%).

PMR: δ 3.48-3.35 (3H, m, C<u>H_2</u>OH and C<u>H</u>OH), 2.82 (1H, d, 11.0, 1 of C<u>H_2</u>NC<u>H_2</u> *17* or *19*), 2.79-2.59 (2H, m), 2.41 (1H, s), 2.26-2.11 (2H, m), 1.92-0.92 (24H, m).

CMR: δ 78.98 (<u>C</u>HOH), 71.19 (<u>C</u>H_2OH), 62.75, 60.72 (<u>C</u>H_2N<u>C</u>H_2), 52.43 ($CH_3\underline{C}H_2N$), 45.78 (<u>C</u>H_2 *10*), 39.04, 38.94 (2 x quaternary), 36.25, 36.13 (2 x <u>C</u>H_2 *1* and *3*), 32.45 (<u>C</u>H), 30.21, 26.63, 26.56, 26.29, 26.17 (5 x <u>C</u>H_2), 20.60 (<u>C</u>H_2 *2*), 12.75 ($NCH_2C\underline{H}_3$).

MS: CI gave m/z 296 $[M+H]^+$ EI gave m/z 295 $[M]^+$ ($C_{18}H_{33}O_2N$ requires 295).

Synthesis of 3-ethyl-5-cyclopentyl-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-methanol **74e**

Using general procedure 3. **64e** (0.400g, 1.30mmol), LAH (0.050g, 1.30mmol), Et_2O (10ml). Silica gel chromatography (3:7 EtOAc-hexane) gave a homogenous white foam (0.228g, 66%).

PMR: δ 3.89-3.77 (2H, m, $C\underline{H}_2OH$), 3.52-3.36 (3H, m), 2.77-2.58 (2H, m), 2.22-1.23 (19H, m), 1.02 (3H, t, 7.2, $NCH_2C\underline{H}_3$).

CMR: 77.84/77.10 (<u>C</u>HOH), 71.26/69.18 ($\underline{C}H_2OH$), 60.59, 58.06 ($\underline{C}H_2N\underline{C}H_2$), 52.58 ($CH_3\underline{C}H_2N$), 46.04/40.20 (<u>C</u>H), 31.57, 30.15, 30.10, 28.56, 27.34, 26.22, 25.97, 25.83, 25.46, 25.35, 25.02, 24.20, 23.45 (6 x $\underline{C}H_2$), 20.64 ($\underline{C}H_2$ *2*), 12.75 ($NCH_2\underline{C}H_3$).

MS: CI gave m/z 268 $[M+H]^+$ ($C_{16}H_{29}O_2N$ requires 269).

Synthesis of 3-ethyl-5-benzyl-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-methanol **74f**

Using general procedure 3. **64f** (1.000g, 3.03mmol), LAH (0.115g, 3.03mmol), Et_2O (20ml). Silica gel chromatography (3:7 EtOAc-hexane) gave a homogenous white foam (0.415g, 47%).

PMR: δ 7.30-7.15 (5H, m, 5 x aromatic C<u>H</u>), 3.47-3.38 (3H, m, $C\underline{H}_2OH$ and C<u>H</u>OH), 2.97 (1H, bs, OH), 2.73-2.55 (6H, m), 2.19-1.91 (3H, m), 1.85-1.65 (3H, m), 1.54-1.36 (2H, m), 1.30-1.22 (1H, m, CH_2 *2 eq*), 0.95 (3H, t, 7.2, $NCH_2C\underline{H}_3$).

CMR: δ 137.88 (quaternary aromatic), 130.62 (2 x aromatic <u>C</u>H), 127.81 (2 aromatic <u>C</u>H), 125.96 aromatic (<u>C</u>H), 77.71 (<u>C</u>HOH), 71.08 ($\underline{C}H_2OH$) 62.53, 60.49 ($\underline{C}H_2N\underline{C}H_2$), 52,28 ($CH_3\underline{C}H_2N$), 44.29 (benzylic $\underline{C}H_2$), 39.20, 39.08 (2 x quaternary), 30.02, 26.34 (2 x $\underline{C}H_2$), 20.55 (CH_2 *2*), 12.67 ($NCH_2\underline{C}H_3$).

MS: CI gave m/z 290.2 $[M+H]^+$($C_{18}H_{27}O_2N$ requires 289).

Synthesis of 3-ethyl-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-methanol **74g**

Using general procedure 3. **4** (0.500g, 2.10mmol), LAH (0.080g, 2.10mmol), Et_2O (20ml). Silica gel chromatography (1:9 EtOAc-hexane) gave a homogenous colourless oil (0.238g, 57%).

PMR: δ 3.70 (1H, d, 3.1, C<u>H</u>OH), 3.39 (2H, dd, 33.0, 10.7, C<u>H</u>$_2$OH), 2.96 (1H, dt, 21.0, 2.6, 1 of $CH_2NC\underline{H}_2CH$ *17*), 2.90 (1H, bs, OH), 2.68-2.55 (2H, m, CH_2 *2* ax and 1 of $C\underline{H}_2NCH_2CH$ *19*), 2.25-2.13 (3H, m, $NC\underline{H}_2CH_3$ and 1 of $CH_2NC\underline{H}_2CH$ *17*), 2.06-1.76 (4H, m, 2 of $C\underline{H}_2CH_2C\underline{H}_2$, CH *11* and 1 of $C\underline{H}_2NCH_2CH$ *19*), 1.54-1.43 (2H, m, CH_2 *2* eq and 1 of $C\underline{H}_2CH_2C\underline{H}_2$), 1.37 (1H, dd, 13.2, 3.0, 1 of $C\underline{H}_2CH_2C\underline{H}_2$), 1.02 (3H, t, 7.3, $NCH_2C\underline{H}_3$).

CMR: δ 75.67 (<u>C</u>HOH), 71.26 (<u>C</u>H_2OH), 60.52, 58.38 ($\underline{C}H_2N\underline{C}H_2$), 52.24 ($CH_3\underline{C}H_2N$), 38.07 (quaternary) 36.24 (<u>C</u>H) 26.51, 23.93 (2 x <u>C</u>H_2), 20.56 ($CH_2\underline{C}H_2CH_2$), 12.75 ($NCH_2\underline{C}H_3$).

MS: EI gave m/z 199 $[M]^+$ ($C_{11}H_{21}O_2N$ requires 199).

General Procedure 4: The synthesis of anthranilate esters of bicyclic diols

Example: synthesis of 3-ethyl-9-hydroxy -5-pentyl-3-azabicyclo[3.3.1]nonane-1-methyl 2-aminobenzoate **77a**

Modified from a procedure by Coates *et al.* (1994b).

A solution of **74a** (0.300g, 1.14mmol), isatoic anhydride (0.205g, 1.25mmol) and DMAP (trace) in anhydrous DMF (1.5ml) was stirred at 70°C for 6h. The solvent was removed by bulb-to-bulb distillation. The products were then dissolved in DCM (20ml) and filtered through celite to remove the excess of isatoic anhydride. This solution was then concentrated under reduced pressure and purification by silica gel chromatography (1:4 EtOAc-hexane) gave a white foam (0.261g, 59%).

PMR: δ 7.85 (1H, dd, 8.5, 1.6, aromatic C<u>H</u> *o*-ester), 7.32-7.26 (1H, m, aromatic C<u>H</u> *p*-ester), 6.69-6.64 (2H, m, 2 x aromatic C<u>H</u> *m*-ester), 5.74 (2H, s, N<u>H</u>$_2$), 4.44 (1H, d, 11.4, 1 of OC<u>H</u>$_2$ *18*), 3.74 (1H, d, 11.1, 1 of OC<u>H</u>$_2$ *18*), 3.18 (1H, d, 4.2, C<u>H</u>OH), 2.87 (1H, d, 11.0, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.80 (1H, d, 7.0, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.73-2.64 (1H, m, C<u>H</u>$_2$ *2* ax), 2.56 (1H, d, 4.6, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.20 (2H, q, 7.2, NC<u>H</u>$_2$CH$_3$), 2.09 (1H, dd, 11.0, 1.0, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 1.79 (1H, dd, 9.0, 1.0, 1 of C<u>H</u>$_2$CH$_2$C<u>H</u>$_2$ *1* or *3*), 1.71-1.21 (13H, m), 1.04 (3H, t, 7.0, NCH$_2$C<u>H</u>$_3$), 0.88 (3H, t, 6.2, alkyl C<u>H</u>$_3$).

CMR: δ 168.83 (ester carbonyl), 150.73 (quaternary aromatic <u>C</u>NH$_2$), 134.33 (<u>C</u>H aromatic *o*-ester), 131.20 (<u>C</u>H aromatic *p*-ester), 116.77, 116.31 (2 x <u>C</u>H aromatic *m*-ester) 110.42 (quaternary CH=<u>C</u>(CO$_2$)CNH$_2$) 74.54 (<u>C</u>HOH), 69.20 (<u>C</u>H$_2$O) 62.78, 61.03 (<u>C</u>H$_2$N<u>C</u>H$_2$) 52.43 (CH$_3$<u>C</u>H$_2$N), 39.78 (quaternary *4* or *11*), 38.71 (<u>C</u>H$_2$ *1* or *3*), 37.29 (quaternary *4* or *11*), 32.86 (<u>C</u>H$_2$ *1* or *3*), 29.96, 27.29, 22.64, 22.49 (4 x <u>C</u>H$_2$ *10, 9, 8, 7*), 20.65 (CH$_2$ *2*), 14.10 (<u>C</u>H$_3$CH$_2$N), 12.82 (<u>C</u>H$_3$).

Mass Spec: EI gave m/z 388 $[M]^+$ ($C_{23}H_{36}O_3N_2$ requires 388).

<u>Synthesis of 5-butyl-3-ethyl-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-methyl 2-aminobenzoate **77b**</u>

Using general procedure 4. **74b** (0.350g, 1.37mmol), isatoic anhydride (0.246g, 1.51mmol), DMAP (trace), DMF (1.5ml). Silica gel chromatography (1:4 EtOAc-hexane) gave a white foam (0.368g 72%).

PMR: δ 7.85 (1H, dd, 8.4, 1.7, aromatic C<u>H</u> *o*-ester), 7.32-7.26 (1H, m, aromatic C<u>H</u> *p*-ester), 6.69-6.61 (2H, m, 2 x aromatic C<u>H</u> *m*-ester), 5.74 (2H, s, NH$_2$), 4.45 (1H, d, 11.2, 1 of OC<u>H</u>$_2$ *18*), 3.74 (1H, d, 11.3, 1 of OC<u>H</u>$_2$ *18*), 3.18 (1H, d, 4.0, C<u>H</u>OH), 2.87 (1H, d, 11.0, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.80 (1H, d, 11.2, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.74-2.65 (1H, m, C<u>H</u>$_2$ *2* ax), 2.55 (1H, d, 14.6, 1 of C<u>H</u>$_2$CH$_2$C<u>H</u>$_2$ *1* or *3*), 2.20 (2H, q,

7.2, $NC\underline{H}_2CH_3$), 2.10 (1H, dd, 11.0, 2.4, 1 of $C\underline{H}_2NCH_2$ *17* or *19*), 1.81-1.10 (12H,m), 1.04 (3H, t, 7.2, $NCH_2C\underline{H}_3$), 0.88 (3H, t, 7.0, alkyl $C\underline{H}_3$).

CMR: δ 168.52 (ester carbonyl), 150.73 (quaternary aromatic $\underline{C}NH_2$), 134.44 ($\underline{C}H$ aromatic *o*-ester), 131.20 ($\underline{C}H$ aromatic *p*-ester), 116.77, 116.31 (2 x aromatic $\underline{C}H$ *m*-ester), 110.42 (quaternary aromatic $CH=\underline{C}(CO_2)CNH_2$), 74.52 ($\underline{C}HOH$), 69.18 ($\underline{C}H_2O$), 62.80, 61.03 ($\underline{C}H_2N\underline{C}H_2$), 52.45 ($CH_3\underline{C}H_2N$), 39.80 (quaternary *4* or *11*), 38.50 ($\underline{C}H_2$ *1* or *3*), 37.25 (quaternary *4* or *11*), 29.96 ($\underline{C}H_2$ *1* or *3*), 27.29, 25.09, 23.72 (3 x $\underline{C}H_2$ *10, 9, 8*), 20.65 ($\underline{C}H_2$ *2*), 14.13 ($\underline{C}H_3CH_2N$), 12.82 (alkyl $\underline{C}H_3$).

MS: CI gave m/z 375 $[M+H]^+$ ($C_{22}H_{34}O_3N_2$ requires 374).

Synthesis of 3-ethyl-9-hydroxy-5-(3-prop-1-enyl)-3-azabicyclo[3.3.1]nonane-1-methyl 2-aminobenzoate **77c**

Using general procedure 4. **74c** (0.300g, 1.25mmol), isatoic anhydride (0.410g, 2.51mmol), DMAP (trace), DMF (1.5ml). Silica gel chromatography (1:3 EtOAc-hexane) gave a white foam (0.369g 83%).

PMR: δ 7.85 (1H, dd, 8.4, 1.4, aromatic $C\underline{H}$ *o*-ester), 7.31-7.25 (1H, m, aromatic $C\underline{H}$ *p*-ester), 6.69-6.64 (2H, m, 2 x aromatic $C\underline{H}$ *m*-ester), 5.90-5.75 (3H, m, $\underline{H}C=CH_2$ and $N\underline{H}_2$), 5.01 (2H, d, 12.5, $HC=C\underline{H}_2$), 4.44 (1H, d, 11.2, 1 of $OC\underline{H}_2$ *18*), 3.74 (1H, d, 11.2, 1 of $OC\underline{H}_2$ *18*), 3.21 (1H, bs, $C\underline{H}OH$), 2.87 (1H, d, 11.0, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 2.78-2.65 (3H, m), 2.24-1.69 (8H, m), 1.50-1.26 (3H, m), 1.03 (3H, t, 7.2, $NCH_2C\underline{H}_3$).

CMR: δ 168.62 (ester carbonyl), 150.70 (quaternary aromatic $\underline{C}NH_2$), 134.64 ($\underline{C}H$ allyl), 134.33 ($\underline{C}H$ aromatic *o*-ester), 131.15 ($\underline{C}H$ aromatic *p*-ester), 117.22 ($\underline{C}H_2$ allyl), 116.76, 116.26 (2 x $\underline{C}H$ aromatic *m*-ester), 110.32 (quaternary aromatic $CH=\underline{C}(CO_2)CNH_2$), 74.07 ($\underline{C}HOH$), 69.03 ($\underline{C}H_2O$), 62.86, 60.93 ($\underline{C}H_2N\underline{C}H_2$), 52.31 ($CH_3\underline{C}H_2N$), 43.13 ($\underline{C}H_2CH=CH_2$), 39.84, 37.79 (2 x quaternary *4* or *11*), 29.87, 27.13 (2 x $\underline{C}H_2$ *1* and *3*), 20.65 ($\underline{C}H_2$ *2*), 12.79 ($\underline{C}H_3$).

MS: FAB +ve gave m/z 359 $[M+H]^+$ ($C_{21}H_{30}O_3N_2$ requires 358).

Synthesis of 5-cyclohexylmethyl-3-ethyl-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-methyl 2-aminobenzoate **77d**

Using general procedure 4. **74d** (0.300g, 1.02mmol), isatoic anhydride (0.333g, 2.04mmol), DMAP (trace), DMF (1.5ml). Silica gel chromatography (1:3 EtOAc-hexane) gave a white foam (0.304g 72%).

PMR: δ 7.85 (1H, dd, 8.4, 1.4, aromatic C<u>H</u> *o*-ester), 7.32-7.26 (1H, m, aromatic C<u>H</u> *p*-ester), 6.69-6.64 (2H, m, 2 x aromatic C<u>H</u> *m*-ester), 5.74 (2H, s, N<u>H</u>$_2$), 4.43 (1H, d, 11.2, 1 of OC<u>H</u>$_2$ *18*), 3.74 (1H, d, 11.3, 1 of OC<u>H</u>$_2$ *18*), 3.16 (1H, d, 4.0, C<u>H</u>OH), 2.86 (2H, d, 11.0, C<u>H</u>$_2$CH *10*), 2.69-2.64 (1H, m, C<u>H</u>$_2$ *1* ax), 2.54 (1H, d, 4.4, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.23-2.05 (3H, m, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19* and NC<u>H</u>$_2$CH$_3$), 1.81 (1H, d, 11.4, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 1.66-1.15 (18H, m), 1.04 (3H, t, 7.2, NCH$_2$C<u>H</u>$_3$).

CMR: δ 169.00 (ester carbonyl), 150.73 (quaternary aromatic <u>C</u>NH$_2$), 134.30 (<u>C</u>H aromatic *o*-ester), 131.18 (<u>C</u>H aromatic *p*-ester), 116.77, 116.31 (2 x <u>C</u>H aromatic *m*-ester), 110.00 (quaternary aromatic CH=<u>C</u>(CO$_2$)CNH$_2$), 74.81 (<u>C</u>HOH), 69.22 (<u>C</u>H$_2$O), 63.13, 60.94 (<u>C</u>H$_2$N<u>C</u>H$_2$), 52.46 (CH$_3$<u>C</u>H$_2$N), 46.24 (<u>C</u>H$_2$ *1* or *3*), 39.78, 38.40 (2 x quaternary *4* and *11*), 36.25, 36.08, (2 x <u>C</u>H$_2$ *1* or *3* and *10*), 32.61 (<u>C</u>H *9*), 30.02, 29.69, 27.16, 26.60, 26.21 (5 x <u>C</u>H$_2$), 20.71 (<u>C</u>H$_2$ *2*), 12.84 (<u>C</u>H$_3$CH$_2$N).

MS: CI gave m/z 415 $[M+H]^+$ ($C_{25}H_{38}O_3N_2$ requires 414).

Synthesis of 5-cyclopentyl-3-ethyl-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-methyl 2-aminobenzoate **77e**

Using general procedure 4. **74e** (0.200g, 0.77mmol), isatoic anhydride (0.140g, 8.43mmol), DMAP (trace), DMF (1ml). Silica gel chromatography (1:3 EtOAc-hexane) gave a white foam (0.267g 90%).

PMR: δ 7.86-7.81 (1H, m, aromatic C<u>H</u> *o*-ester), 7.30-7.24 (1H, m, aromatic C<u>H</u> *p*-ester), 6.68-6.63 (2H, m, 2 x aromatic C<u>H</u> *m*-ester), 5.75 (2H, s, N<u>H</u>$_2$), 4.64 (1H, d, 11.5, 1 of OC<u>H</u>$_2$ *18*), 4.40 (1H, d, 11.6, 1 of OC<u>H</u>$_2$ *18*), 4.15-4.10 (1H, m, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 3.78 (1H, d, 11.4, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 3.33 (1H, bs, C<u>H</u>OH), 2.86 (1H, d, 11.0, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.76 (1H, d, 11.0, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.71-2.56 (1H, m, C<u>H</u>$_2$ *2* ax), 2.20 (2H, q, 7.3, NC<u>H</u>$_2$CH$_3$), 2.08-1.22 (15H, m), 1.04 (3H, t, 7.2, NCH$_2$C<u>H</u>$_3$).

MS: CI gave m/z 387 $[M+H]^+$ ($C_{23}H_{34}O_3N_2$ requires 386).

<u>Synthesis of 5-benzyl-3-ethyl-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-methyl 2-aminobenzoate **77f**</u>

Using general procedure 4. **74f** (0.400g, 1.38mmol), isatoic anhydride (0.271g, 1.66mmol), DMAP (trace), DMF (1.5ml). Silica gel chromatography (1:4 EtOAc-hexane) gave a white foam (0.513g 91%).

PMR: δ 7.80 (1H, dd, 7.9, 1.2, aromatic C<u>H</u> *o*-ester), 7.31-7.12 (6H, m, 5x CH benzyl and aromatic C<u>H</u> *p*-ester), 6.69-6.63 (2H, m, 2 x aromatic C<u>H</u> *m*-ester), 5.75 (2H, s, N<u>H</u>$_2$), 4.48 (1H, d, 11.6, 1 of OC<u>H</u>$_2$ *18*), 3.71 (1H, d, 11.3, 1 of OC<u>H</u>$_2$ *18*), 3.20 (1H, d, 4.3, C<u>H</u>OH), 2.87 (1H, d, 1.0, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.82 (1H, d, 11.0, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.70-2.56 (4H, m, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*, C<u>H</u>$_2$ *2* ax and benzylic C<u>H</u>$_2$), 2.20-2.07 (2H, m, 2 of C<u>H</u>$_2$ *1* and *3*), 2.03 (1H, dd, 11.0, 2.1, 1 of C<u>H</u>$_2$ *1* and *3*), 1.84-1.72 (3H, m, 1 of C<u>H</u>$_2$ *1* and *3* and NC<u>H</u>$_2$CH$_3$), 1.61 (1H, s, O<u>H</u>), 1.52-1.25 (2H, m), 0.96 (3H, t, 7.2, NCH$_2$C<u>H</u>$_3$).

CMR: δ 168.73 (ester carbonyl), 150.74 (quaternary aromatic <u>C</u>NH$_2$), 138.00 (quaternary aromatic CH$_2$<u>C</u>), 134.41 (<u>C</u>H aromatic *o*-ester), 131.19 (<u>C</u>H aromatic *p*-ester), 130.57, 127.68, 125.87 (5 x <u>C</u>H aromatic benzyl), 116.76, 116.31 (2 x <u>C</u>H aromatic *m*-ester), 110.23 (quaternary aromatic CH=<u>C</u>(CO$_2$)CNH$_2$), 73.51 (<u>C</u>HOH),

69.03 ($\underline{C}H_2O$), 62.77, 60.71 ($\underline{C}H_2N\underline{C}H_2$), 52.29 ($CH_3\underline{C}H_2N$) , 44.61 ($\underline{C}H_2$ benzyl), 40.04, 38.43 (quaternary *4* and *11*), 29.98, 27.14 (2 x $\underline{C}H_2$ *1* and *3*), 20.69 ($\underline{C}H_2$ *2*), 12.77 ($NCH_2\underline{C}H_3$).

Mass Spec: EI gave m/z 408 $[M]^+$ ($C_{25}H_{32}O_3N_2$ requires 408).

Synthesis of 3-ethyl-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-methyl 2-aminobenzoate

77g

Using general procedure 4. **74g** (0.300g, 1.02mmol), isatoic anhydride (0.333g, 2.04mmol), DMAP (trace), DMF (1.5ml). Silica gel chromatography (1:3 EtOAc-hexane) gave a white foam (0.304g 72%).

PMR: δ 7.85 (1H, d, 7.3, aromatic C$\underline{H}$ *o*-ester), 7.31-7.16 (1H, m, aromatic C$\underline{H}$ *p*-ester), 6.68-6.64 (2H, m, 2 x aromatic C$\underline{H}$ *m*-ester), 5.74 (2H, s, $N\underline{H}_2$), 4.47 (1H, d, 9.2, 1 of $OC\underline{H}_2$ *18*), 3.65 (1H, d, 11.7, 1 of $OC\underline{H}_2$ *18*), 3.50 (1H, s, C$\underline{H}$OH), 2.96 (1H, d, 10.8, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 2.87 (2H, d, 10.8, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 2.67-2.57 (1H, m, 1 of $C\underline{H}_2$ *2* ax), 2.23 (2H, q, 6.9, $NC\underline{H}_2CH_3$), 2.13 (2H, m, 2 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 2.04-1.24 (7H, m), 1.05 (3H, t, 7.3, $NCH_2C\underline{H}_3$).

CMR: δ 168.73 (ester), 150.76 (quaternary aromatic$\underline{C}NH_2$), 134.39 ($\underline{C}H$ aromatic *o*-ester), 131.20 ($\underline{C}H$ aromatic *p*-ester), 116.78, 116.33 (2 x $\underline{C}H$ aromatic *m*-ester), 110.31 (quaternary aromatic CH=$\underline{C}(CO_2)CNH_2$), 71.06 ($\underline{C}HOH$), 68.97 ($\underline{C}H_2O$), 60.83, 58.77 ($\underline{C}H_2N\underline{C}H_2$), 52.28 ($CH_3\underline{C}H_2N$) 38.94 (quaternary *4*), 35.43 ($\underline{C}H$ *11*) 27.33, 24.18 (2 x $\underline{C}H_2$), 20.69 ($CH_2\underline{C}H_2CH_2$), 12.86 ($NCH_2\underline{C}H_3$).

MS: EI gave m/z 318 $[M]^+$ ($C_{18}H_{26}O_3N_2$ requires 318).

General Procedure 5: The conversion of anthranilate esters into methylsuccinimido compounds

Example: synthesis of 3-ethyl-9-hydroxy-5-pentyl-3-azabicyclo[3.3.1]nonane-1-methyl [2-(*RS*)-methylsuccinimido]benzoate **80a**

Modified from a procedure by Coates *et al.* (1994b).

A solution of **77a** (0.200g, 0.51mmol) and (±) methylsuccinic anhydride (0.222g, 1.55mmol) in DCM (3ml) was stirred at room temperature for 16h. To this was then added 1,1'-carbonyldiimidazole (0.210g, 1.29mmol). After stirring for a further 24h, the reaction mixture was concentrated under reduced pressure and purification by silica gel chromatography (2:3 EtOAc-hexane) gave a white foam (0.168g, 67.5%).

PMR: δ 8.09-8.06 (1H, m, aromatic C<u>H</u> *o*-ester), 7.69-7.65 (1H, m, aromatic C<u>H</u> *p*-ester), 7.56-7.52 (1H, m, aromatic C<u>H</u> *m*-ester), 7.29-7.26 (1H, m, aromatic C<u>H</u> *m*-ester), 4.47-4.39 (1H, m, 1 of O<u>CH</u>$_2$ *18*), 3.73-3.66 (1H, m, 1 of O<u>CH</u>$_2$ *18*), 3.19 (1H, d, 3.3 ,C<u>H</u>OH), 3.16-3.01 (2H, m), 2.83 (1H, d, 11.0, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$), 2.79 (1H, d, 11.3, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.74-2.63 (2H, m), 2.62-2.32 (2H, m), 2.19 (2H, q, 7.3, NC<u>H</u>$_2$CH$_3$), 2.10-2.05 (1H, m, 1 of C<u>H</u>$_2$CH$_2$C<u>H</u>$_2$), 1.81 (1H, dd, 11.0, 1.8, 1 of C<u>H</u>$_2$CH$_2$C<u>H</u>$_2$), 1.71-1.16 (16H, m), 1.03 (3H, t, 7.0, NCH$_2$C<u>H</u>$_3$), 0.87 (3H, t, 7.3, alkyl C<u>H</u>$_3$).

CMR: δ 180.00 (O=<u>C</u>CH$_2$), 176.00 (O=<u>C</u>CH$_2$) 165.00 (ester carbonyl), 133.44 (aromatic <u>C</u>H aromatic *o*-ester), 132.67 (aromatic quaternary <u>C</u>N), 131.28 (<u>C</u>H aromatic *p*-ester), 129.71, 129.32 (2 x aromatic <u>C</u>H aromatic *m*-ester), 127.35 (aromatic quaternary CH=<u>C</u>(CO$_2$)CN), 74.06 (<u>C</u>HOH), 70.01 (<u>C</u>H$_2$O), 62.72, 60.93 (<u>C</u>H$_2$N<u>C</u>H$_2$), 52.38 (N<u>C</u>H$_2$CH$_3$), 39.71, 38.61 (2 x <u>C</u>H$_2$ *1* and *3*), 37.26 , 36.97 (2 x quaternary *4* and *11*), 35.40/35.23 (<u>C</u>H methylsuccinimide), 32.89, 29.95, 27.26, 22.61, 22.43 (5 x <u>C</u>H$_2$), 20.64 (<u>C</u>H$_2$ *2*), 16.41 (<u>C</u>H$_3$ methylsuccinimide), 14.11 (<u>C</u>H$_3$ amine), 12.83 (<u>C</u>H$_3$ alkyl).

MS CI gave m/z 485 $[M+H]^+$ ($C_{28}H_{40}O_5N_2$ requires 484).

Microanalysis: Observed C 69.3 H 8.4 N 5.6 ($C_{28}H_{40}O_5N_2$ requires C 69.4 H 8.3 N 5.8).

Synthesis of 5-butyl-3-ethyl-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-methyl [2-(*RS*)-methylsuccinimido]benzoate **80b**

Using general procedure 5. **77b** (0.450g, 0.73mmol), methylsuccinic anhydride (0.316g, 2.20mmol), CDI (0.296g, 1.83mmol), DCM (3ml). Silica gel chromatography (3:7 EtOAc-hexane) gave a white foam (0.175g 51%).

PMR: δ 8.02-7.96 (1H, m, aromatic C<u>H</u> *o*-ester), 7.62-7.56 (1H, m, aromatic C<u>H</u> *p*-ester), 7.48-7.43 (1H, m, aromatic C<u>H</u> *m*-ester), 7.21 7.19 (1H, m, aromatic C<u>H</u> *m*-ester), 4.32 (1H, d, 11.2, 1 of OC<u>H</u>$_2$ *18*), 3.65 (1H, d, 11.2, 1 of OC<u>H</u>$_2$ *18*), 3.13 (1H, s, C<u>H</u>OH), 3.10-2.94 (2H, m, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19* and O<u>H</u>), 2.78 (1H, d, 12.1, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.74 (1H, d, 12.1, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.70-2.40 (3H, m, C<u>H</u>$_2$ *2* ax and C<u>H</u>$_2$CHCH$_3$), 2.17-1.96 (4H, m, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19* 3 of C<u>H</u>$_2$ *1* and *3*), 1.76 (1H, d, 11.0, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 1.59-1.12 (13H, m), 0.97 (3H, t, 7.1, NCH$_2$C<u>H</u>$_3$), 0.81 (3H, t, 6.8 alkyl C<u>H</u>$_3$).

CMR: δ 175.85 (O=<u>C</u>CH$_2$), 174.39 (O=<u>C</u>CH), 165.16 (ester carbonyl), 133.34 (aromatic <u>C</u>H *o*-ester), 132.59 (aromatic quaternary <u>C</u>N), 131.18, 129.64, 129.24 (3 x aromatic <u>C</u>H), 127.31 (aromatic quaternary CH=<u>C</u>(CO$_2$)CN), 73.80 (<u>C</u>HOH), 69.86 (<u>C</u>H$_2$O), 62.53, 60.73 (<u>C</u>H$_2$N<u>C</u>H$_2$), 52.48 (N<u>C</u>H$_2$CH$_3$), 39.64 (quaternary *4* or *11*), 38.31 (<u>C</u>H$_2$ methylsuccinimide), 37.19 (quaternary*4* or *11*), 36.91 (<u>C</u>H$_2$ *1* or *3*), 35.27 (<u>C</u>H methylsuccinimide), 29.81, 27.12, 24.91, 23.64 (4 x <u>C</u>H$_2$ *1* or *3, 8, 9, 10*), 20.50 (<u>C</u>H$_2$ *2*), 16.33 (<u>C</u>H$_3$ methylsuccinimide), 14.04 (NCH$_2$<u>C</u>H$_3$ amine), 12.63 (alkyl <u>C</u>H$_3$).

MS: FAB +ve gives 470.9($C_{27}H_{38}O_5N_2$ requires 470).

High resolution FAB +ve gave 471.2855 ($C_{27}H_{38}O_5N_2$ +H requires 471.2858).

Synthesis of 3-ethyl-9-hydroxy-5-(3-prop-1-enyl)-3-azabicyclo[3.3.1]nonane-1-methyl [2-(*RS*)-methylsuccinimido]benzoate **80c**

Using general procedure 5. **77c** (0.300g, 0.83mmol), methylsuccinic anhydride (0.362g, 2.51mmol), CDI (0.339g, 2.09mmol), DCM (5ml). Silica gel chromatography (2:3 EtOAc-hexane) gave a white foam (0.154g 40%).

PMR: δ 8.08-8.05 (1H, m, aromatic C<u>H</u> *o*-ester), 7.70-7.66 (1H, dt, 7.6, 1.6, aromatic C<u>H</u> *p*-ester), 7.56-7.52 (1H, dt, 7.3, 0.6, aromatic C<u>H</u> *m*-ester), 7.29-7.27 (1H, bd, 7.9 aromatic C<u>H</u> *m*-ester), 5.90-5.79 (1H, m, C<u>H</u>=CH_2), 5.03-4.99 (2H, m, CH=C<u>H_2</u>), 4.49-4.41 (1H, m, 1 of OC<u>H_2</u> *18*), 3.71-3.64 (1H, m, 1 of OC<u>H_2</u> *18*), 3.22 (1H, d, 4.6, C<u>H</u>OH), 3.11-3.07 (2H, m, C<u>H_2</u>CH=CH_2), 2.83 (1H, d, 10.9, 1 of C<u>H_2</u>NC<u>H_2</u> *17* or *19*), 2.75 (1H, d, 11.3, 1 of C<u>H_2</u>NC<u>H_2</u> *17* or *19*), 2.72-2.62 (1H, m, C<u>H_2</u> *2* ax), 2.57-2.51 (2H, m), 2.19 (2H, q, 7.3, NC<u>H_2</u>CH_3), 2.15-1.95 (2H, m), 1.82 (1H, d, 11.3, 1 of C<u>H_2</u>NC<u>H_2</u> *17* or *19*), 1.72-1,62 (3H, m), 1.48-1.24 (4H, m), 1.02 (3H, t, 7.3, NCH_2C<u>H_3</u>).

CMR: δ 182.00 (O=<u>C</u>CH_2), 180.00 (O=<u>C</u>CH), 165.00 (ester carbonyl), 134.73, 133.47 (2 x <u>C</u>H aromatic), 132.66 (quaternary aromatic <u>C</u>N), 131.29, 129.72, 129.35 (2 x <u>C</u>H aromatic and <u>C</u>H=CH_2 allyl), 127.32 (quaternary aromatic CH=<u>C</u>(CO_2)CN), 117.23 (CH=<u>C</u>H_2), 73.66 (<u>C</u>HOH), 69.84 (<u>C</u>H_2O), 62.86, 60.88 (<u>C</u>H_2N<u>C</u>H_2), 52.29 (CH_3<u>C</u>H_2N), 42.12 (<u>C</u>H_2CH=CH_2), 39.83, 37.80 (2 x quaternary *4* and *11*), 36.98 (<u>C</u>H_2 methylsuccinimide), 35.22 (<u>C</u>H methylsuccinimide), 29.86, 27.16 (2 x <u>C</u>H_2 *1* and *3*), 20.62 (<u>C</u>H_2 *2*), 16.44 (<u>C</u>H_3 methylsuccinimide), 12.82 (<u>C</u>$H_3$$CH_2$N).

MS: FAB +ve gave m/z 454.9 $[M+H]^+$ ($C_{26}H_{34}O_5N_2$ requires 454).

High resolution FAB +ve gave 455.2540 $[M+H]^+$ ($C_{26}H_{34}O_5N_2$ +H requires 455.2545).

Synthesis of 5-cyclohexylmethyl-3-ethyl-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-methyl [2-(*RS*)-methylsuccinimido]benzoate **80d**

Using general procedure 5. **77d** (0.275g, 0.67mmol), methylsuccinic anhydride (0.288g, 2.00mmol), CDI (0.270g, 1.67mmol), DCM (5ml). Silica gel chromatography (2:3 EtOAc-hexane) gave a white foam (0.206g 61%).

PMR: δ 8.08-8.05 (1H, m, aromatic C<u>H</u> *o*-ester), 7.69-7.65 (1H, m, aromatic C<u>H</u> *p*-ester), 7.55-7.52 (1H, m, aromatic C<u>H</u> *m*-ester), 7.29-7.26 (1H, m, aromatic C<u>H</u> *m*-ester), 4.43-4.36 (1H, m, 1 of O<u>CH</u>$_2$ *18*), 3.74-3.68 (1H, m, 1 of O<u>CH</u>$_2$ *18*), 3.17-3.06 (3H, m, C<u>H</u>OH and CH_2 methylsuccinimide), 2.86 (1H, d, 10.7, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.83 (1H, d, 9.2, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.75-2.14 (6H, m), 2.08 (1H, d, 9.1, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 1.81 (1H, d, 10.7, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 1.65-0.89 (21H, m).

CMR: δ 180.00 (O=<u>C</u>CH$_2$), 176.00 (O=<u>C</u>CH), 166.00 (ester carbonyl), 133.38 (aromatic <u>C</u>H, *o*-ester), 132.00 (aromatic quaternary <u>C</u>N), 131.22, 129.69, 129.28 (3 x aromatic <u>C</u>H), 127.38 (aromatic quaternary CH=<u>C</u>(CO$_2$)CN), 74.51 (<u>C</u>HOH), 70.07 (<u>C</u>H$_2$O), 63.08, 60.85 (<u>C</u>H$_2$N<u>C</u>H$_2$), 52.38 (N<u>C</u>H$_2$CH$_3$), 46.13 (<u>C</u>H$_2$), 39.67 , 38.39 (2 x quaternary *4* and *11*), 36.97, 36.22, 36.11 (3 x <u>C</u>H$_2$ *1, 3* and *10*), 35.23 (<u>C</u>H methylsuccinimide), 32.49 (CH cyclohexylmethyl *9*), 29.85, 27.13, 26.58, 26.52, 26.18 (5 x <u>C</u>H$_2$ *8, 13, 14, 15, 16*), 20.65 (<u>C</u>H$_2$ *2*), 16.39 (<u>C</u>H$_3$ methylsuccinimide), 12.89 (NCH$_2$<u>C</u>H$_3$).

MS: FAB +ve gave 511 $[M+H]^+$ ($C_{30}H_{42}O_5N_2$ requires 510).

Microanalysis: Observed C 70.7 H 8.4 N 5.4 ($C_{30}H_{40}O_5N_2$ requires C 70.3 H 8.2 N 5.5).

Synthesis of 5-cyclopentyl-3-ethyl-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-methyl [2-(*RS*)-methylsuccinimido]benzoate **80e**

Using general procedure 5. **77e** (0.250g, 0.65mmol), methylsuccinic anhydride (0.279g, 1.94mmol), CDI (0.262g, 1.62mmol), DCM (3ml). Silica gel chromatography (2:3 EtOAc-hexane) gave a white foam (0.131g 42%).

PMR: δ 8.11-8.06 (1H, m, aromatic C<u>H</u> *o*-ester), 7.68-7.63 (1H, m, aromatic C<u>H</u> *p*-ester), 7.56-7.48 (1H, m, aromatic C<u>H</u> *m*-ester), 7.28-7.23 (1H, m, aromatic C<u>H</u> *m*-ester), 4.44-4.36 (1H, m, 1 of O<u>CH</u>$_2$ *18*), 3.74 (1H, d, 9.3, 1 of O<u>CH</u>$_2$ *18*), 3.65 (1H, d, 6.4, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 3.32 (1H, d, 5.2, C<u>H</u>OH), 3.09-3.04 (2H, m), 2.96-2.52 (4H, m), 2.44-2.33 (2H, m), 2.23-2.16 (2H, m), 2.05 (1H, d, 12.2, 1 of C<u>H</u>$_2$ *1* or *3*), 2.00-1.83 (2H, m), 1.74-1.19 (13H, m), 1.03 (3H, t, 7.0, NCH$_2$C<u>H</u>$_3$).

CMR: δ 179.94/179.80 (O=<u>C</u>CH$_2$), 175.87/174.40 (O=<u>C</u>CH), 165.00 (ester carbonyl), 133.31 (aromatic <u>C</u>H *o*-ester), 132.72/132.63 (aromatic quaternary <u>C</u>N), 131.19, 129.62, 129.23 (3 x aromatic <u>C</u>H), 127.26 (aromatic quaternary CH=<u>C</u>(CO$_2$)CN), 73.60 (<u>C</u>HOH), 70.05 (<u>C</u>H$_2$O), 60.76, 58.47 (<u>C</u>H$_2$N<u>C</u>H$_2$), 52.48 (N<u>C</u>H$_2$CH$_3$), 46.31 (<u>C</u>H cyclopentyl *10*), 39.51 , 38.39 (2 quaternary *4* and *11*), 36.88 (<u>C</u>H$_2$ methylsuccinimide), 35.30/35.14 (<u>C</u>H methylsuccinimide), 30.18, 27.02, 25.98/25.94, 25.72, 25.41, 24.93 (6 x <u>C</u>H$_2$ *1, 3, 9, 12, 13, 14*), 20.71 (<u>C</u>H$_2$ *2*), 16.32 (<u>C</u>H$_3$ methylsuccinimide), 12.71 (NCH$_2$<u>C</u>H$_3$).

MS: CI gave m/z 483 $[M+H]^+$ ($C_{28}H_{38}O_5N_2$ requires 482).

Microanalysis: Observed C 67.0 H 7.6 N 4.8 ($C_{20}H_{40}O_5N_2$ requires C 69.7 H 7.8 N 5.8).

Synthesis of 5-benzyl-3-ethyl-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-methyl [2-(*RS*)methylsuccinimido]benzoate **80f**

Using general procedure 5. **77f** (0.480g, 1.17mmol), methylsuccinic anhydride (0.509g, 3.54mmol), CDI (0.386g, 2.93mmol), DCM (5ml). Silica gel chromatography (2:3 EtOAc-hexane) gave a white foam (0.365g 60%).

PMR: δ 8.04-8.00 (1H, m, aromatic C<u>H</u> *o*-ester), 7.68 (1H, dt, 8.0, 1.5, aromatic C<u>H</u> *p*-ester), 7.52 (1H, dt, 7.7, 1.2, aromatic C<u>H</u> *o*-ester), 7.30-7.10 (6H, m, aromatic C<u>H</u> *m*-ester and 5 x aromatic C<u>H</u> benzyl), 4.50-4.43 (1H, m, 1 of O<u>CH</u>$_2$ *18*), 3.70-3.63 (1H, m, 1 of O<u>CH</u>$_2$ *18*), 3.24 (1H, d, 4.5, C<u>H</u>OH), 3.09 (2H, d, 10.7, <u>CH</u>$_2$Ar), 2.81-2.52 (6H, m), 2.19-2.03 (3H, m), 1.85-1.64 (4H, m), 1.48-1.24 (6H, m), 0.95 (3H, t, 7.0, NCH$_2$<u>CH</u>$_3$).

CMR: δ 180.00 (O=<u>C</u>CH$_2$), 176.00 (O=<u>C</u>CH), 165.34 (ester carbonyl), 138.04 (quaternary aromaticCH$_2$<u>C</u>), 133.48 (aromatic <u>C</u>H), 132.66 (quaternary aromatic <u>C</u>N), 131.27, 131.22, 130.60, 129.72, 129.35, 2 x [127.74] (6 x aromatic <u>C</u>H), 127.26 (quaternary aromatic CH=<u>C</u>(CO$_2$)CN), 125,88 (aromatic <u>C</u>H), 73.54 (<u>C</u>HOH), 69.91 (<u>C</u>H$_2$O), 62.84, 60.72, (<u>C</u>H$_2$N<u>C</u>H$_2$), 52.29 (CH$_3$<u>C</u>H$_2$N), 44.62 (benzylic <u>C</u>H$_2$), 39.38, 38.50 (2 x quaternary *4* and *11*), 36.91 (<u>C</u>H$_2$ methylsuccinimide), 35.40/35.22 (<u>C</u>H methylsuccinimide), 29.65, 27.16 (2 x <u>C</u>H$_2$ *1* and *3*), 20.65 (CH$_2$ *2*), 16.48 (<u>C</u>H$_3$ methylsuccinimide), 12.78 (<u>C</u>H$_3$CH$_2$N).

MS: CI gave m/z 505 $[M+H]^+$ ($C_{30}H_{36}O_5N_2$ requires 504).

Microanalysis: Observed C 70.5 H 7.2 N 5.3 ($C_{30}H_{34}O_5N_2$ requires C 71.7 H 6.8 N 5.3).

General Procedure 6: Preparation of dianion for attempted Michael additions

To a slurry of sodium hydride (0.071g of 60% in mineral oil, 1.76mmol) in anhydrous THF (10ml) was added ethyl 2-oxocylohexane-1-carboxylate (0.250g, 1.47mmol) at 0°C under an atmosphere of nitrogen. The reaction mixture was then cooled to-78°C and *n*-BuLi (0.7ml of 2.2M in hexanes, 1.54mmol) was added. After 1h of stirring at , the enone was added (together with any co-solvent or other additive) as described below.

Enone	Additive	Result
2-methylcyclopent-2-enone **84** (0.280g, 2.94mmol)	30%DMPU	mainly polymerisation of enone
2-methylcyclopent-2-enone **84** (0.141g, 1.47mmol)	30%DMPU 0.5eq. CuI	mainly polymerisation of enone
2-methylcyclopent-2-enone **84** (0.141g, 1.47mmol)	none	mainly polymerisation of enone
cyclopent-2-enone **85** (0.121g, 1.47mmol)	30% DMPU	mainly polymerisation of enone

Synthesis of ethyl 2-oxo-1-pentylcyclohexane-1-carboxylate 103 (previously synthesised by Westermann *et al.* 1993)

To a suspension of sodium hydride (0.547g of 60% in mineral oil, 14.12mmol, washed with hexane) at 0°C in anhydrous THF (15ml) was added a solution of ethyl 2-oxocylohexane-1-carboxylate **37** (2.000g, 11.76mmol) in anhydrous THF (5ml) under an atmosphere of nitrogen. After stirring for 30min a solution of 1-iodopentane (2.329g, 11.76mmol) in DMPU (10ml) was added. After a further 48h the reaction mixture was poured into saturated aqueous ammonium chloride solution (20ml) and then extracted

into hexane (5 x 20ml). The combined hexane extracts were washed with water (2 x 20ml), saturated aqueous sodium chloride solution (20ml) and then dried over magnesium sulphate, filtered and concentrated under reduced pressure to yield a pale yellow oil (2.861g, crude yield > 100%). Purification of this compound was achieved by firstly, using the crude reaction mixture in a double Mannich reaction (see general procedure 2) using 1 equivalent of ethylamine and 2 equivalents of formaldehyde and then removing any cyclic β-ketoester formed by silica gel chromatography (1:9 EtOAc-hexane) to give a colourless oil (1.575g, 56%).

PMR: δ 4.19 (2H, q, 7.2, $OC\underline{H}_2CH_3$), 2.55-2.41 (3H, m), 2.05-1.37 (13H, m), 1.26 (3H, t, 7.2, $OCH_2C\underline{H}_3$), 0.87 (3H, t, 6.9, $CH_2CH_2CH_2C\underline{H}_3$).

Synthesis of ethyl 1-methyl-2-oxocyclohexane-1-carboxylate 104 (previously synthesised by Linstead and Millidge (1936))

To a suspension of sodium hydride (0.565g of 60% in mineral oil, 14.12mmol, washed with hexane) at 0°C in anhydrous THF (20ml) was added a solution of ethyl 2-oxocylohexane-1-carboxylate **37** (2.000g, 11.76mmol) in anhydrous THF (5ml) under an atmosphere of nitrogen. After stirring for 30min a solution of iodomethane (1.836g, 12.94mmol) in DMPU (10ml) was added. After a further 48h the reaction mixture was poured into saturated aqueous ammonium chloride solution (20ml) and then extracted into hexane (5 x 20ml). The combined hexane extracts were washed with water (2 x 20ml), saturated aqueous sodium chloride solution (20ml) and then dried over magnesium sulphate, filtered and concentrated under reduced pressure to yield a pale yellow oil (2.139g, crude yield). Purification of this compound was achieved by firstly, using the crude reaction mixture in a double Mannich reaction (see general procedure 2) using 1 equivalent of ethylamine and 2 equivalents of formaldehyde and then removing

any cyclic β-ketoester formed by silica gel chromatography (1:9 EtOAc-hexane) to give a colourless oil (0.500g, 25%).

PMR: δ 4.26-4.14 (2H, m, $OC\underline{H}_2CH_3$), 2.54-2.40 (2H, m, $O{=}CC\underline{H}_2$), 2.10-1.38 (9H, m), 1.26 (3H, t, 7.0, $OCH_2C\underline{H}_3$).

General Procedure 7: Michael additions of ethyl 2-oxocylohexane-1-carboxylate

Example: synthesis of ethyl 2-oxo-1-(3-oxocylopentyl)cyclohexane-1-carboxylate **109**

To a stirred solution of ethyl 2-oxocylohexane-1-carboxylate **37** (0.500g, 2.94mmol) in methanol was added a few drops (approximately 0.015g) of a 40% solution of Triton B in methanol (2ml). To this was then added cyclopent-2-enone **85** (0.264g, 3.23mmol) dissolved in methanol (3ml). After stirring for 4h at 20°C, the reaction mixture was diluted with diethyl ether (30ml) and then washed with dilute aqueous hydrochloric acid (10ml of 2M), saturated aqueous sodium hydrogen carbonate solution (10ml) and brine (10ml). The organic fraction was dried over magnesium sulphate and then filtered and concentrated. Purification by silica gel chromatography (3:17 EtOAc-hexane) gave a colourless oil (0.430g, 59%).

PMR: δ 4.29-4.19 (2H, m, $OC\underline{H}_2CH_3$), 2.74-1.51 (13H, m), 1.29 (3H, dt, 7.0, 2.8, $OCH_2C\underline{H}_3$).

CMR: δ 217.98/217.76 (cyclopentanone carbonyl), 207.10 (cyclohexanone), 170.85/170.72 (ester carbonyl), 62.53/62.27 (quaternary), 61.43 ($O\underline{C}H_2$), 40.85 ($\underline{C}H$), 41.37, 40.79, 40.10, 38.54, 38.38, 34.24, 34.10, 27.29, 27.22, 24.97, 24.04, 22.58 (6 $\underline{C}H_2$), 14.10 ($OCH\underline{C}H_3$).

MS: CI gave m/z 253 $[M+H]^+$ ($C_{14}H_{20}O_4$ requires 252).

Synthesis of ethyl 1-ethoxycarbonyl-2-oxocyclohexanepropanoate **99** (previously synthesised by Openshaw and Robinson (1933))

Using general procedure 7. ethyl 2-oxocylohexane-1-carboxylate **37** (2.000g, 11.76mmol), Triton B (approximately 0.015g) and ethyl acrylate **106** (1.293g, 12.93mmol). Silica gel chromatography (3:17 EtOAC-hexane) gave a colourless oil (1.665g, 52%).

PMR: δ 4.21 (2H, dq, 7.2, 2.8, O=CCCO$_2$C$\underline{H}_2$CH$_3$), 4.12 (2H, q, 7.1, CH$_2$CH$_2$CO$_2$C$\underline{H}_2$CH$_3$), 2.51-1.47 (12H, m), 1.28 (3H, t, 7.3, OCH$_2$C$\underline{H}_3$), 1.28 (3H, t, 7.2, OCH$_2$C$\underline{H}_3$).

CMR: δ 207.52 (ketone carbonyl), 173.04, 171.68 (2 x ester carbonyl), 61.38, 60.39 (2 x O$\underline{C}$H$_2$CH$_3$), 59.97 (quaternary), 40.98 ($\underline{C}$H$_2$C=O), 36.28 ($\underline{C}$H$_2$CO$_2$CH$_2$CH$_3$), 29.61, 29.55, 27.47 (3 x $\underline{C}$H$_2$), 22.49 (CH$_2\underline{C}$H$_2$CH$_2$), 14.16, 14.08 (2 x OCH$_2\underline{C}$H$_3$).

MS: CI gave m/z 271.1 $[M+H]^+$ ($C_{14}H_{22}O_5$ requires 270).

Synthesis of ethyl 2-oxo-1-(3-oxobutyl)cyclohexane-1-carboxylate **107** (previously synthesised by McQuillan and Robinson (1941))

Using general procedure 7. ethyl 2-oxocylohexane-1-carboxylate **37** (0.500g, 2.94mmol), Triton B (approximately 0.015g) and but-1-en-3-one **105** (0.226g, 3.23mmol). Silica gel chromatography (3:17 EtOAC-hexane) gave a colourless oil (0.205g, 30%).

PMR: δ 3.29-4.15 (2H, m, OC$\underline{H}_2$CH$_3$), 2.65-1.43 (15H, m), 1.28 (3H, t, 7.0, OCH$_2$C$\underline{H}_3$)

Synthesis of ethyl 1-ethoxycarbonyl-2-oxocyclohexanepropanoate 99 (previously synthesised by Openshaw and Robinson (1933))

To a freshly prepared solution of sodium ethoxide (0.676g, 29.4mmol of sodium in 25ml of anhydrous EtOH) was added ethyl 2-oxocylohexane-1-carboxylate **37** (5.000g, 29.4mmol), sodium iodide (0.050g, 0.33mmol) and ethyl β-chloropropionate (4.020g, 29.4mmol). The reaction mixture was heated to reflux for 2h under an atmosphere of nitrogen and then stirred at 20°C for a further 16h. The reaction mixture was then poured into saturated aqueous ammonium chloride solution and extracted into ether (3 x 20ml). The ether fractions were combined and then washed with water (20ml), saturated aqueous sodium chloride solution (20ml) and then dried over magnesium sulphate, filtered and concentrated under reduced pressure. The residual oil was purified by silica gel chromatography (1:10 EtOAc-hexane) to give a colourless oil (4.964g, 63%).

PMR: δ 4.21 (2H, dq, 7.2, 2.8, O=CCCO$_2$C$\underline{H}_2$CH$_3$), 4.12 (2H, q, 7.1, CH$_2$CH$_2$CO$_2$C$\underline{H}_2$CH$_3$), 2.51-1.47 (12H, m), 1.28 (3H, t, 7.3, OCH$_2$C$\underline{H}_3$), 1.28 (3H, t, 7.2, OCH$_2$C$\underline{H}_3$).

CMR: δ 207.52 (ketone carbonyl), 173.04, 171.68 (2 x ester carbonyl), 61.38, 60.39 (2 x O$\underline{C}$H$_2$CH$_3$), 59.97 (quaternary), 40.98 ($\underline{C}$H$_2$C=O), 36.28 ($\underline{C}$H$_2$CO$_2$CH$_2$CH$_3$), 29.61, 29.55, 27.47 (3 x $\underline{C}$H$_2$), 22.49 (CH$_2\underline{C}$H$_2$CH$_2$), 14.16, 14.08 (2 x OCH$_2\underline{C}$H$_3$).

MS: CI gave m/z 271.1 $[M+H]^+$ ($C_{14}H_{22}O_5$ requires 270).

Synthesis of ethyl 1-(3-hydroxycyclopentyl)-2-oxocyclohexane-1-carboxylate 111

To a stirred solution of sodium borohydride (0.004g, 0.99mmol) in absolute ethanol (5ml) was added ethyl 2-oxo-1-(3-oxocyclopentyl)cyclohexane-1-carboxylate **109** (0.100g, 4.00mmol) dissolved in absolute ethanol (5ml). After stirring at 20°C for 30min the reaction was quenched with glacial acetic acid (2ml) and then concentrated under

reduced pressure. Purification by silica gel chromatography (3:7 EtOAc-hexane) gave a colourless oil (0.052g, 51%).

PMR: δ 4.23 (2H, q, 7.1, $OC\underline{H}_2CH_3$), 2.47-1.43 (16H, m), 1.31-1.24 (3H, m, $OCH_2C\underline{H}_3$).

CMR: δ 209.05/207.80/207.75 (cyclohexanone carbonyl), 172.05/171.89/171.65 (ester carbonyl), 73.43, 73.28 (<u>C</u>HOH), 63.04/63.00/62.87 (quaternary), 61.37/61.17 ($O\underline{C}H_2CH_3$), 42.74/42.61 (<u>C</u>H), 41.75, 41.60, 41.31, 41.17, 37.34, 37.12, 36.74, 35.84, 35.69, 35.51, 34.98, 34.94, 34.82, 34.38, 33.98, 27.41, 27.37, 27.28, 25.43, 25.22, 25.22, 22.73 (7 $\underline{C}H_2$), 14.34, 14.17 ($OCH_2\underline{C}H_3$)

Mass spec.: CI m/z 255 $[M+H]^+$ ($C_{14}H_{22}O_4$ requires 254).

IR: broad 3700-3000 peak.

Synthesis of ethyl 2-oxo-1-(3-trimethylsilyloxycyclopentyl)cyclohexane-1-carboxylate 112

To a stirred solution of *t*-butyldimethylsilyl chloride (0.187g, 0.944mmol) and imidazole (0.177g, 1.96mmol) dissolved in anhydrous DMF (5ml) was added ethyl 1-(3-hydroxycyclopentyl)-2-oxocyclohexane-1-carboxylate **111** (0.200g, 0.787mmol) dissolved in DMF (5ml). After 90min the reaction was poured into water (10ml) and extracted into ethyl acetate (20ml). The combined organic layers were dried over magnesium sulphate filtered and concentrated under reduced pressure. The colourless oil obtained was passed through a short column of silica gel (1:9 EtOAc-hexane) yielding a colourless oil (0.210g, 72%).

PMR: δ 4.20 (2H, q, 7.1, $OC\underline{H}_2CH_3$), 2.66-2.33 (2H, m), 2.17-1.30 (14H, m), 1.24 (3H, t, 2.2), 0.84 (9H, s, 3 x $CC\underline{H}_3$), 0.00 (6H, s, 2 x $SiC\underline{H}_3$).

CMR: δ 207.87/207.78/207.74 (cyclohexanone carbonyl), 171.75/171.29/171.24 (ester carbonyl), 77.21 (*t*-butyl carbon), 73.69/73.59/73.28, (COSi), 63.16/63.10 (quaternary), 41.52/41.32/41.06 (CH), 41.61, 37.62, 37.41, 37.30,37.22, 35.50, 35.07, 34.99, 34.50, 32.69, 31.93, 29.69, 27.37, 27.28, 25.63, 25.46, 24.65, 24.59, 22.65, 22.59, 22.54 (7 CH_2),25.85 (*t*butyl CH_3), 14.14 (OCH_2CH_3),-4.71 ($Si(CH_3)_2$).

Mass spec.: CI m/z 369 $[M+H]^+$ ($C_{20}H_{36}O_4Si$ requires 368).

Synthesis of 1-carbethoxy-3-pentylcyclohexan-2-one 66a by ester migration

Modified from a procedure by Habi and Gravel (Habi and Gravel 1994).

To a slurry of potassium hydride (0.150g of 35% in mineral oil, 3.75mmol, washed with hexane) in anhydrous THF (20ml), at room temperature and under an atmosphere of nitrogen, was added ethyl 2-oxo-1-pentylcyclohexane-1-carboxylate **103** (0.640g, 2.67mmol) and 18-crown-6 (2.100g, 8.01mmol). After stirring for 45min, the reaction mixture was poured into saturated aqueous ammonium chloride solution (20ml) and the THF layer removed and concentrated under reduced pressure. The resulting yellow oil was dissolved in hexane and washed with water (2 x 20ml), saturated aqueous sodium chloride solution (20ml) and then dried over magnesium sulphate, filtered and concentrated under reduced pressure to yield a pale yellow oil (0.497g, crude yield 55%).

PMR: δ 12.43 (0.3H, s, $HOC=CCO_2CH_2CH_3$), 12.25 (0.1H, s, $HOC=CCO_2CH_2CH_3$), 4.27-4.13 (2H, m, OCH_2CH_3), 3.42-3.34 (0.6H, m, $OCCHCO_2CH_2CH_3$), 2.69-1.23 (18H, m), 0.88 (3H, t, 7.0, $CH_2CH_2CH_3$).

Synthesis of 1-carbethoxy-3-methylcyclohexan-2-one 113 by ester migration (previously synthesised by Sengupta (1953))

Modified from a procedure by Habi and Gravel (Habi and Gravel 1994).

To a slurry of potassium hydride (0.150g of 35% in mineral oil, 3.75mmol, washed with hexane) in anhydrous THF (20ml), at room temperature and under an atmosphere of nitrogen, was added ethyl 3-methyl-2-oxocyclohexane-1-carboxylate **104** (0.640g, 2.67mmol) and 18-crown-6 (2.100g, 8.01mmol). After stirring for 45min, the reaction mixture was poured into saturated aqueous ammonium chloride solution (20ml) and the THF layer removed and concentrated under reduced pressure. The resulting yellow oil was dissolved in hexane and washed with water (2 x 20ml), saturated aqueous sodium chloride solution (20ml) and then dried over magnesium sulphate, filtered and concentrated under reduced pressure to yield a pale yellow oil (0.041g, crude yield 41%).

PMR: δ 4.28-4.15 (2H, m, $OC\underline{H}_2CH_3$), 3.43-3.35 (1H, m, $OCC\underline{H}CO_2CH_2CH_3$), 2.48-1.03 (13H, m)

Double Mannich reaction with rearranged ester 66a to give 64a (cf. General procedure 2)

Modified from a procedure by Shimizu *et al.* (Shimizu *et al.* 1963a).

To formaldehyde (0.610g, 37% $^{w}/_{w}$ in water, 7.50mmol) was added ethylamine (0.240g 70% $^{w}/_{w}$ in water, 3.75mmol) dissolved in absolute ethanol (10ml). To this was then added the crude 1-carbethoxy-3-pentylcyclohexan-2-one **66a** (0.497g crude from rearrangement) dissolved in absolute ethanol (20ml) and glacial acetic acid (0.5ml). After refluxing for 6h, the reaction mixture was cooled and then quenched with solid sodium hydrogen carbonate (until pH 8.0). The reaction mixture was then filtered and

concentrated under reduced pressure. The orange yellow oil obtained was dissolved in DCM (30ml) and then sequentially washed with saturated aqueous sodium hydrogen carbonate solution (10ml), water (10ml) and saturated aqueous sodium chloride solution (10ml) and finally dried over sodium sulphate filtered and concentrated under reduced pressure. Purification by silica gel chromatography (1:49 EtOAc-hexane) gave two clear viscous oils (0.183g, of the desired product and 0.050g of the unalkylated equivalent).

PMR: δ 4.20 (2H, q, 7.2, $OC\underline{H}_2CH_3$), 3.19 (1H, dd, 11.4, 2.2, 1 of $C\underline{H}_2NC\underline{H}_2$), 3.05 (1H, dd, 11.0, 2.4, 1 of $C\underline{H}_2NC\underline{H}_2$), 3.02-2.83 (2H, m, 1 of $C\underline{H}_2NC\underline{H}_2$ and 1 of $C\underline{H}_2$ *2* ax), 2.58-2.46 (2H, m), 2.39 (2H, q, 8.8, $NC\underline{H}_2CH_3$), 2.34-2.08 (3H, m), 1.55-1.45 (1H, m, $C\underline{H}_2$ *2* eq), 1.43-1.33 (8H, m), 1.29 (3H, t, 7.0, $OCH_2C\underline{H}_3$), 1.10 (3H, t, 7.1, $NCH_2C\underline{H}_3$), 0.87 (3H, t, 6.81 $CH_2CH_2C\underline{H}_3$).

CMR: δ 213.46 (cyclohexanone carbonyl), 171.49 (ester carbonyl), 64.69, 61.77 $\underline{C}H_2N\underline{C}H_2$), 60.97 ($O\underline{C}H_2$), 58.98 (quaternary $EtO_2C\underline{C}C$=O), 51.20 ($N\underline{C}H_2CH_3$), 49.18 (quaternary $NCH_2\underline{C}C$=O), 39.23, 36.87 (2 x $C\underline{H}_2$ *1* and *3*), 34.69, 32.66, 22.91, 22.49 (4 x $C\underline{H}_2$ *10, 9, 8, 7*), 20.45, ($\underline{C}H_2$ *2*), 14.12 ($OCH_2\underline{C}H_3$), 13.99($NCH_2\underline{C}H_3$), 12.65 ($CH_2CH_2\underline{C}H_3$).

MS: low eV Ionisation gave m/z 309 $[M]^+$ ($C_{18}H_{31}O_3N$ requires 309).

Attempted rearrangement of 112

Modified from procedures by Habi and Gravel (1994).

To a slurry of potassium hydride (0.083g of 35% in mineral oil, 0.59mmol, washed with hexane) in anhydrous THF (10ml), at room temperature and under an atmosphere of nitrogen, was added of ethyl 2-oxo-1-(3-trimethylsilyloxycyclopentyl)cyclohexane-1-carboxylate **112** (0.190g, 0.52mmol) and 18-crown-6 (0.412g, 1.56mmol). After stirring for 1h the reaction mixture was poured into saturated aqueous ammonium chloride

solution (10ml) and the THF layer removed and concentrated under reduced pressure. The resulting yellow oil was dissolved in hexane and washed with water (2 x 10ml), saturated aqueous sodium chloride solution (10ml) and then dried over magnesium sulphate, filtered and concentrated under reduced pressure to yield a pale yellow oil. Purification by silica gel chromatography (3:7 EtOAc-hexane) gave two fractions (0.049g (26%) of starting material **112** and 0.053g (28%) of **117**).

PMR: δ 4.15-4.07 (2H, m, $OC\underline{H}_2CH_3$), 2.33-1.20 (21H, m), 0.85-0.83 (9H, bs, 3 x $C\underline{H}_3$ *t*-Bu), 0.00 (6H, s, 2 x $SiC\underline{H}_3$)

MS: CI (isobutane) gives $[M+H]^+$ 386.9 ($C_{20}H_{38}O_5Si$ requires 386)

Synthesis of ethyl 3-(3-hydroxycyclopentyl)-2-oxocyclohexane-1-carboxylate 118

To a slurry of potassium hydride (0.100g of 35% in mineral oil, 0.87mmol, washed with hexane) in anhydrous THF (10ml), at room temperature and under an atmosphere of nitrogen, was added of ethyl 1-(3-hydroxycyclopentyl)-2-oxocyclohexane-1-carboxylate **111** (0.100g, 0.87mmol) and 18-crown-6 (0.416g, 1.56mmol). After stirring for 1h the reaction mixture was poured into saturated aqueous ammonium chloride solution (10ml) and the THF layer removed and concentrated under reduced pressure. The resulting yellow oil was dissolved in hexane and washed with water (2 x 10ml), saturated aqueous sodium chloride solution (10ml) and then dried over magnesium sulphate, filtered and concentrated under reduced pressure to yield a pale yellow oil. Purification by silica gel chromatography (3:7 EtOAc-hexane) gave two fractions (0.021g (21%) of starting material **111** and 0.004g (4%) of **118**).

PMR: δ 4.29-4.17 (3H, m, $OC\underline{H}_2CH_3$ and $C\underline{H}OH$), 3.42-3.35 (1H, m, O=CC$\underline{H}$C=O), 2.52-1.25 (15H, m), 0.88 (3H, t, 6.6, $OCH_2C\underline{H}_3$)

MS: CI (isobutane) gives $[M+H]^+$ 254.9 ($C_{14}H_{22}O_4$ requires 254)

Synthesis of ethyl 5-(3-hydroxycyclopentyl)-3-ethyl-3-aza-bicyclo[3.3.1]nonan-9-one-1-carboxylate 119

Modified from procedures by Habi and Gravel (1994) and Shimizu *et al.* (1963a).

To a slurry of potassium hydride (0.745g of 35% in mineral oil, 6.49mmol, washed with hexane) in anhydrous THF (20ml), at room temperature and under an atmosphere of nitrogen, was added ethyl 1-(3-hydroxycyclopentyl)-2-oxocyclohexane-1-carboxylate **111** (0.750g, 2.95mmol) and 18-crown-6 (3.120g, 11.80mmol). After stirring for 1h, the reaction mixture was poured into saturated aqueous ammonium chloride solution (20ml) and the THF layer removed and concentrated under reduced pressure. The resulting yellow oil was dissolved in hexane and washed with water (2 x 20ml), saturated aqueous sodium chloride solution (20ml) and then dried over magnesium sulphate, filtered and concentrated under reduced pressure to yield a pale yellow oil (0.364g, crude yield of **118** 49%). To formaldehyde (0.478g, 37% $^{w}/_{w}$ in water, 5.90mmol) was added ethylamine (0.190g 70% $^{w}/_{w}$ in water, 2.95mmol) dissolved in absolute ethanol (10ml). To this was then added the crude 3-alkylated product **118** (0.364g crude from rearrangement) dissolved in absolute ethanol (10ml) and glacial acetic acid (0.5ml). After refluxing for 2h the reaction mixture was cooled and then quenched with solid sodium hydrogen carbonate (until pH 8.0). The reaction mixture was then filtered and concentrated under reduced pressure. The orange yellow oil obtained was re-dissolved in DCM (30ml) and then sequentially washed with saturated aqueous sodium hydrogen carbonate solution (10ml), water (10ml) and saturated aqueous sodium chloride solution (10ml) and finally dried over sodium sulphate, filtered and concentrated under reduced pressure. Silica gel chromatography (1:19 EtOAc-hexane) gave two colourless viscous oils (0.025g, (2.6%) of the desired product and 0.046g of the unalkylated equivalent).

PMR: δ 4.2 (2H, q, 7.1, $OC\underline{H}_2CH_3$), 3.12 (1H, dt, 11.5, 2.5, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 2.98-1.46 (19H, m), 1.28 (3H, t, 7.2, $OCH_2C\underline{H}_3$), 1.10 (3H, t, 7.1, $NCH_2C\underline{H}_3$).

CMR: δ 214.23 (ketone carbonyl), 171.52 (ester carbonyl), 73.37/73.19 (CHOH), 64.53, 64.01, 61.69, 61.16 (2 x CH_2N, CH_2O), 59.25 (quaternary), 51.87 (quaternary), 51.12 (CH_3CH_2N), 42.72/42.61 (CH), 38.26, 37.53, 37.03, 36.76, 36.59, 36.54, 36.05, 35.79, 29.70, 25.09, 24.84 (5 CH_2), 20.65/20.56 (CH_2 *2*) 14.14 (OCH_2CH_3), 12.67 (NCH_2CH_3)

MS: CI gave m/z 324 $[M+H]^+$ ($C_{18}H_{29}O_4N$ requires 323).

Synthesis of ethyl 3-ethyl-5-(3-ethoxy-3-oxopropyl)-3-aza-bicyclo[3.3.1]nonan-9-one-1-carboxylate 121

Modified from procedures by Habi and Gravel (1994) and Shimizu *et al.* (1963a).

To a slurry of potassium hydride (0.120g of 35% in mineral oil, 1.03mmol, washed with hexane) in anhydrous THF (10ml), at room temperature and under an atmosphere of nitrogen, was added 1-carbethoxy-1-(ethyl 3-proprionyl)cyclohexan-2-one **108** (0.200g, 0.74mmol) and 18-crown-6 (0.587g, 2.22mmol). After stirring for 15min, the reaction mixture was poured into saturated aqueous ammonium chloride solution (20ml) and the THF layer removed and concentrated under reduced pressure. The resulting yellow oil was dissolved in hexane and washed with water (2 x 20ml), saturated aqueous sodium chloride solution (20ml) and then dried over magnesium sulphate, filtered and concentrated under reduced pressure to yield a pale yellow oil (0.129g, crude yield 65% of **120**). To formaldehyde (0.152g, 37% $^w/_w$ in water, 1.77mmol) was added ethylamine (0.058g 70% $^w/_w$ in water, 0.88mmol) dissolved in absolute ethanol (5ml). To this was then added the crude 3-alkylated product (0.120g crude from rearrangement) dissolved in absolute ethanol (5ml) and glacial acetic acid (0.5ml). After refluxing for 2h the reaction mixture was cooled and then quenched with solid sodium hydrogen carbonate (until pH 8.0). The reaction mixture was then filtered and concentrated under reduced

pressure. The orange yellow oil obtained was re-dissolved in DCM (30ml) and then sequentially washed with saturated aqueous sodium hydrogen carbonate solution (10ml), water (10ml) and saturated aqueous sodium chloride solution (10ml) and finally dried over sodium sulphate, filtered and concentrated under reduced pressure. Silica gel chromatography (1:9 EtOAc-hexane) gave two clear viscous oils (0.037g, of the desired product and 0.022g of the unalkylated equivalent).

PMR: δ 4.21 (2H, q, 7.1, $OC\underline{H}_2CH_3$), 4.12 (2H, q, 7.1, $OC\underline{H}_2CH_3$), 3.19 (1H, dt, 13.5, 2.5, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 3.04-2.84 (3H, m, 2 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*, and CH_2 *2* ax), 2.58-1.63 (11H, m), 1.58-1.48 (1H, m, CH_2 *2* eq), 1.31-1.22 (6H, m, 2 x $OCH_2C\underline{H}_3$), 1.10 (3H, t, 7.0, $NCH_2C\underline{H}_3$).

Synthesis of diethyl 4-ethoxycarbonylnonanedioate 122

Following a procedure from Openshaw and Robinson (1933).

To a solution of freshly prepared sodium ethoxide (0.020g of Na in 10ml of anhydrous ethanol, 0.88mmol) was added 1-carbethoxy-1-(ethyl 3-proprionyl)cyclohexan-2-one **108** (wt2/17) (0.200g, 0.741mmol). The reaction mixture was heated under reflux under an atmosphere of nitrogen. After 18h, the reaction mixture was cooled and concentrated under reduced pressure and the residual oil dissolved in DCM. This solution was then washed with saturated aqueous ammonium chloride solution (20ml), water (2 x 20ml), saturated aqueous sodium chloride solution (20ml) and then dried over magnesium sulphate, filtered and concentrated under reduced pressure to yield a pale yellow oil which was homogeneous by tlc.

PMR: δ 4.19-4.08 (6H, m, 3 x $OC\underline{H}_2CH_3$), 2.44-2.24 (5H, m, 2 x $C\underline{H}_2C{=}O$ and $C\underline{H}C{=}O$), 1.93-1.31 (8H, m, 4 x CH2), 1.29-1.23 (9H, m, 3 x $OCH_2C\underline{H}_3$).

CMR: δ 175.47, 173.55, 173.02 (3 x ester carbonyl), 60.42, 60.33, 60.26 (3 x $O\underline{C}H_2CH_3$), 44.64 ($\underline{C}HC=O$), 34.13, 32.00, 31.93, 27.16, 26.74, 24.80 (6 x $\underline{C}H_2$), 14.30, 14.23, 14.10 (3 x $OCH_2\underline{C}H_3$).

MS: FAB +ve and CI gave m/z 317.0 $[M+H]^+$ ($C_{16}H_{28}O_6$ requires 316).

Synthesis of 3-methoxycyclohex-2-en-1-one 137 (previously synthesised by Agosta and Lowrance Jr. (1970))

A stirred solution of cyclohexane-1,3-dione **136** (5.000g, 44.59mmol), trimethylorthoformate (4.720g, 44.59mmol) and pTSA (trace) in anhydrous methanol (10ml) was heated to reflux for 16h. The reaction mixture was cooled and then concentrated under reduced pressure. The residue was passed through a short column of silica gel (7:3 EtOAc-hexane) to give a yellow crystalline solid (5.500g, 98%).

PMR: δ 5.38 (1H, s, alkenyl $C\underline{H}$), 3.71 (3H, s, $OC\underline{H}_3$), 2.44-2.33 (4H, m, 2 x $C\underline{H}_2$), 2.03-1.94 (2H, m, $CH_2C\underline{H}_2CH_2$).

CMR: δ 199.56 ($\underline{C}=O$), 178.56 ($C=\underline{C}OCH_3$), 132.66 ($=\underline{C}H$), 102.17 ($O\underline{C}H_3$), 36.65 ($O=C\underline{C}H_2$), 28.72 ($\underline{C}H_2COCH_3$, 21.13 ($CH_2\underline{C}H_2CH_2$).

Synthesis of 3-methoxycyclohexan-1-one 135 (previously synthesised by Lambert et al. (1988))

Method A

A solution of 3-methoxycyclohex-2-en-1-one **137** (3.668g, 29.10mmol) in methanol (40ml) was stirred under an atmosphere of hydrogen with a catalytic amount of palladium on charcoal (10% Pd on C) for 22h at room temperature. The reaction mixture was then filtered through celite, concentrated under reduced pressure. Final

purification by silica gel chromatography (3:7 EtOAc-hexane) gave a colourless oil (1.611g, 43%).

Method B

From a procedure by Lambert *et al.* (1988)

A solution of cyclohex-2-en-1-one **138** (2g, 20.83mmol) and concentrated sulphuric acid (catalytic) in methanol (3ml) was stirred for 48h at 20°C. The reaction mixture was poured into water (20ml) and extracted into DCM (3 x 20ml). The combined organic layers were washed with saturated sodium chloride solution (20ml) and dried over magnesium sulphate, filtered and concentrated under reduced pressure. Silica gel chromatography (3:7 EtOAc-hexane) gave a pale yellow oil (1.919g, 72%).

PMR: δ 3.72-3.65 (1H, m, C<u>H</u>OCH$_3$), 3.33 (3H, s, OC<u>H</u>$_3$), 2.62 (1H, dd, 14.1, 4.0, C<u>H</u>$_2$ eq), 2.47 (1H, dd, 14.1, 6.8, C<u>H</u>$_2$ ax), 2.33 (2H, t, 6.4, O=CC<u>H</u>$_2$CH2), 2.10-1.64 (4H, m, 2 x C<u>H</u>$_2$).

CMR: δ 209.37 (<u>C</u>=O), 78.04 (<u>C</u>HOCH$_3$), 55.80 (O<u>C</u>H$_3$), 46.98 (CO<u>C</u>H$_2$CHOCH$_3$), 40.98 (O=C<u>C</u>H$_2$), 29.36 (O=CH$_2$<u>C</u>H$_2$), 20.47 (CH(OCH$_3$)<u>C</u>H$_2$).

Synthesis of ethyl 3-ethyl-9-hydroxy-9-(trimethylsilylethynyl)-3-aza-bicyclo[3.3.1]nonane-1-carboxylate 140

To a stirred solution of (trimethylsilyl)acetylene (1.812g, 18.48mmol) in anhydrous THF (50ml) at 0°C was added *n*-BuLi solution (7.39ml of 2.5M in hexanes, 18.48mmol) under an atmosphere of nitrogen. After 45mins a solution of bicyclic β-ketoester **4** (4.000g, 16.8mmol) was added in anhydrous THF (10ml). After 20h, the reaction mixture was poured into saturated aqueous ammonium chloride solution (20ml) and the organic layer removed and concentrated under reduced pressure. The residue was

dissolved in diethyl ether and washed with water (40ml) and saturated aqueous sodium chloride solution (40ml) before being dried over magnesium sulphate, filtered and concentrated under reduced pressure. Silica gel chromatography gave a colourless oil (3.717g, 67%) which is a mixture of diastereoisomers.

PMR: δ 4.69 (1H, s, O<u>H</u>), 4.27-4.13 (2H, m, OC<u>H</u>$_2$CH$_3$), 3.15 (1H, d, 10.7, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.85 (1H, d, 11.0, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.70-2.51 (3H, m, 2 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19* and CH$_2$ *2* ax), 2.39-2.19 (4H, m, NC<u>H</u>$_2$CH$_3$ and 2 of C<u>H</u>$_2$ *1* and *3*), 1.99-1.91 (1H, m, C<u>H</u> 11), 1.77-1.36 (3H, m, 2 of C<u>H</u>$_2$ *1* and *3* and C<u>H</u>$_2$ *2* eq), 1.29 (3H, t, 7.1, OCH$_2$C<u>H</u>$_3$), 1.07 (3H, t, 7.0, NCH$_2$C<u>H</u>$_3$), 0.15 (9H, s, Si(C<u>H</u>$_3$)$_3$).

CMR: δ 176.62 (ester carbonyl), 107.87, 91.69 2 (2 x quaternary C≡C *6* and *7*), 70.77 (quaternary <u>C</u>OH *5*), 61.12 (O<u>C</u>H$_2$CH$_3$), 57.39, 56.50 (<u>C</u>H$_2$N<u>C</u>H$_2$ *17* and *19*), 52.18 (N<u>C</u>H$_2$CH$_3$), 49.67 (quaternary *4*), 38.87 (<u>C</u>H *11*), 30.10, 24.72 (2 x <u>C</u>H$_2$ *1* and *3*) 20.12 (<u>C</u>H$_2$ *2*), 14.38 (OCH$_2$<u>C</u>H$_3$), 12.63 (NCH$_2$<u>C</u>H$_3$), 0.05 (3 x <u>C</u>H$_3$ TMS).

Synthesis of ethyl 3-ethyl-9-hydroxy-9-ethynyl-3-azabicyclo[3.3.1]nonane-1-carboxylate 141

To a stirred solution of propargylic alcohol **140** (0.980g, 2.91mmol) was added TBAF (2.9ml of 1.1M in THF, 3.19mmol) at 0°C. After 2h the reaction mixture was poured into water and extracted into DCM (3 x 20ml). The combined DCM layers were washed with water (20ml) and saturated aqueous sodium chloride solution (20ml) and dried with magnesium sulphate before being filtered and concentrated under reduced pressure. Silica gel chromatography gave two pale yellow oils which are diastereoisomers (A 0.336g 44% and A+B 0.058g, 7.5%).

Compound **141a**

PMR: δ 4.76 (1H, s, O<u>H</u>), 4.21 (2H, q, 7.1, OC<u>H</u>$_2$CH$_3$), 3.19 (1H, d, 10.7, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.87 (1H, d, 11.0, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.72-2.56 (2H, m, 2 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.53 (1H, s, C≡C<u>H</u>), 2.38-2.12 (4H, m, NC<u>H</u>$_2$CH$_3$ and C<u>H</u>$_2$ *2* ax and 1 of C<u>H</u>$_2$ *1* or *3*), 2.03-1.98 (1H, m, C<u>H</u> *11*), 1.79-1.38 (4H, m, 3 of C<u>H</u>$_2$ *1* and *3* and C<u>H</u>$_2$ *2* eq), 1.29 (3H, t, 6.4, OCH$_2$C<u>H</u>$_3$), 1.06 (3H, t, 7.1, NCH$_2$C<u>H</u>$_3$).

CMR: δ 177.00 (ester carbonyl), 85.80 (quaternary acetylenic), 75.17 (<u>C</u>H acetylene), 71.50 (quaternary <u>C</u>OH *5*), 61.28 (O<u>C</u>H$_2$CH$_3$), 57.19, 56.38 (<u>C</u>H$_2$N<u>C</u>H$_2$ *17* and *19*), 52.04 (N<u>C</u>H$_2$CH$_3$), 49.80 (quaternary *4*), 39.02 (<u>C</u>H *4*), 30.15, 24.69 (2 x <u>C</u>H$_2$ *1* and *3*), 20.17 (<u>C</u>H$_2$ *2*), 14.30 (OCH$_2$<u>C</u>H$_3$), 12.87 (NCH$_2$<u>C</u>H$_3$).

MS: CI gave m/z 266 $[M+H]^+$ ($C_{15}H_{23}O_3N$ requires 265).

Compound **141b**

PMR: δ ~4.7 (1H, bs, O<u>H</u>), 4.24-4.12 (2H, m, OC<u>H</u>$_2$CH$_3$), 2.88-2.60 (4H, m, 4 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* and *19*), 2.55 (1H, s, C≡C<u>H</u>), 2.35-2.08 (4H, m, NC<u>H</u>$_2$CH$_3$, C<u>H</u>$_2$ *2* ax and 1 of C<u>H</u>$_2$ *1* or *3*), 2.02-1.97 (1H, m, C<u>H</u> *11*), 1.81-1.71 (2H, m, 2 of C<u>H</u>$_2$ *1* and *3*), 1.53-1.41 (2H, m, C<u>H</u>$_2$ *2* eq and 1 of C<u>H</u>$_2$ *1* or *3*), 1.28 (3H, t, 6.4, OCH$_2$C<u>H</u>$_3$), 1.04 (3H, t, 7.1, NCH$_2$C<u>H</u>$_3$).

CMR: δ 176.50 (ester carbonyl), 86.20 (quaternary acetylenic), 74.64 (<u>C</u>H acetylene), 70.08 (quaternary <u>C</u>OH *5*) 61.35 (O<u>C</u>H$_2$CH$_3$), 55.56, 52.18 (<u>C</u>H$_2$N<u>C</u>H$_2$ 17 and 19), 52.18 (N<u>C</u>H$_2$CH$_3$), 50.88 (quaternary), 39.78 (<u>C</u>H *11*), 31.83, 29.37 (2 x <u>C</u>H$_2$ *1* and *3*) 20.98 (<u>C</u>H$_2$ *2*), 14.26 (OCH$_2$<u>C</u>H$_3$), 12.70 (NCH$_2$<u>C</u>H$_3$).

Attempted synthesis of ethyl 3-ethyl-9-hydroxy-9-(1-hydroxy-3-methoxycyclohexylethynyl)-3-azabicyclo[3.3.1]nonane-1-carboxylate 142

To a stirred solution of propargylic alcohols **141a** and **b** (0.650g, 2.45mmol) in anhydrous THF (20ml) was added *n*-BuLi (2.35ml of 2.5M in hexanes, 5.86mmol) at 0°C. After stirring for 45min, 3-methoxycyclohexan-1-one **135** (0.376g, 2.94mmol) was added. After stirring at 20°C for 48h the reaction mixture was poured into saturated ammonium chloride solution (10ml) and the organic fraction removed. The THF layer was concentrated under reduced pressure and then dissolved in DCM (20ml) and washed with water (2 x 20ml), saturated aqueous sodium chloride solution (10ml) and then dried over magnesium sulphate, filtered and concentrated under reduced pressure to give a yellow oil (0.785g). Purification by silica gel chromatography (1:4 EtOAc-hexane) allowed a quantitative recovery of **141a** and **b** but did not yield any 3-methoxycyclohexan-1-one **137**.

Synthesis of 3-methoxy-1-(2-trimethylsilylethynyl)cyclohexanol 144

Modified from a procedure by Weyerstahl *et al.* (1994).

To a stirred solution of (trimethylsilyl)acetylene (1.091g, 11.13mmol) in anhydrous THF (30ml) at 0°C was added *n*-BuLi solution (4.45ml of 2.5M in hexanes, 11.13mmol) under an atmosphere of nitrogen. After 45mins, 3-methoxycyclohexan-1-one (1.500g, 11.72mmol) was added in anhydrous THF (10ml). After 6h, the reaction mixture was poured into saturated aqueous ammonium chloride solution. The upper (THF) layer was removed and concentrated under reduced pressure. The residue was dissolved in DCM (20ml) and washed sequentially with water (2 x 20ml) and saturated aqueous sodium chloride solution (20ml) before being dried with magnesium sulphate, filtered and concentrated under reduced pressure. Silica gel chromatography (1:3 EtOAc-hexane

gave a 2 clear viscous oils A and B in an 1:11.6 ratio (0.154 and 1.794g respectively, 78% combined yield).

Compound **144a:**

PMR: δ 3.37-3.26 (1H, m, C<u>H</u>OCH$_3$), 3.20 (3H, s, OC<u>H</u>$_3$), 2.19-2.12 (2H, m, HOCC<u>H</u>$_2$CHOCH$_3$), 1.89-1.84 (1H, m, COHC<u>H</u>$_2$CHOCH$_3$ ax), 1.73-1.63 (1H, m, 1H, m, COHC<u>H</u>$_2$CHOCH$_3$ eq), 1.53-1.36 (3H, m,3 of C<u>H</u>$_2$), 1.12-0.96 (1H, m, C<u>H</u>$_2$), 0.00 (9H, s, 3 x SiC<u>H</u>$_3$).

CMR: δ 110.28 (C≡<u>C</u>TMS), 86.47 (<u>C</u>≡CTMS), 75.33 (<u>C</u>HOCH$_3$), 67.76 (quaternary), 55.81 (O<u>C</u>H$_3$), 43.61 (HOC<u>C</u>H$_2$CHOCH$_3$), 38.55 (HOC<u>C</u>H$_2$CH$_2$CH$_2$), 31.25 (HOCCH$_2$CH$_2$<u>C</u>H$_2$), 19.41 (HOCCH$_2$<u>C</u>H$_2$CH$_2$), 0.00 (3 x Si<u>C</u>H$_3$).

MS: CI gave m/z 227.1 $[M+H]^+$ ($C_{12}H_{22}O_2Si$ requires 226).

Compound **144b**

PMR: δ 3.35-3.25 (1H, m, C<u>H</u>OCH$_3$), 3.18 (3H, s, OC<u>H</u>$_3$), 1.94 (1H, dd, 10.8, 3.4, COHC<u>H</u>$_2$CHOCH$_3$ eq), 1.68-1.46 (5H, m, 2 x C<u>H</u>$_2$ and COHC<u>H</u>$_2$CHOCH$_3$ ax), 1.40-1.25 (2H, m, CH$_2$C<u>H</u>$_2$CH$_2$), 0.00 (9H, m, 3 x SiC<u>H</u>$_3$).

CMR: δ 109.03 (C≡<u>C</u>TMS), 88.13 (<u>C</u>≡CTMS), 77.08 (<u>C</u>HOCH$_3$), 68.42 (quaternary), 56.15 (O<u>C</u>H$_3$), 43.17 (HOC<u>C</u>H$_2$CHOCH$_3$), 39.31 (HOC<u>C</u>H$_2$CH$_2$CH$_2$), 29.61 (HOCCH$_2$CH$_2$<u>C</u>H$_2$), 18.49 (HOCCH$_2$<u>C</u>H$_2$CH$_2$) 0.00 (3 x Si<u>C</u>H$_3$).

MS: EI gave m/z 226.1 $[M]^+$ ($C_{12}H_{22}O_2Si$ requires 226).

Synthesis of 1-ethynyl-3-methoxycyclohexanol 145

To a stirred solution of 3-methoxy-1-(2-trimethylsilylethynyl)cyclohexanol **144** (1.300g, 2.2mmol) in methanol (20ml) was added aqueous sodium hydroxide (11ml of 1M). After 4h, the reaction mixture was poured into dilute hydrochloric acid solution (22ml of

0.5M) and extracted into DCM (3 x 20ml). The combined DCM layers were washed with saturated aqueous sodium chloride solution (20ml), dried with magnesium sulphate and then filtered and then concentrated under reduced pressure. The crude product was then passed through a short column of silica (2:3 EtOAc-hexane) gave a colourless oil (0.809g, 91%)

PMR: δ 3.53-3.48 (1H, m, C<u>H</u>OCH$_3$), 3.34 (3H, s, OC<u>H</u>$_3$), 2.46 (1H, s, C≡C<u>H</u>), 2.09 (1H, dd, 13.1, 2.9, CHOCH$_3$C<u>H</u>$_2$COH eq), 1.85-1.72 (2H, m, CHOCH$_3$C<u>H</u>$_2$COH ax and C<u>H</u>$_2$), 1.56-1.46 (5H, m, C<u>H</u>$_2$).

CMR: δ 86.91 (HC≡<u>C</u>), 76.42 (<u>C</u>HOCH$_3$), 71.42 (H<u>C</u>≡C), 67.58 (quaternary), 55.79 (O<u>C</u>H$_3$), 42.53 (HOC<u>C</u>H$_2$CHOCH$_3$), 38.78 (HOC<u>C</u>H$_2$CH$_2$CH$_2$), 28.92 (HOCCH$_2$CH$_2$<u>C</u>H$_2$), 17.71 (HOCHCH$_2$<u>C</u>H$_2$CH$_2$).

Attempted synthesis of ethyl 3-ethyl-9-hydroxy-9-(1-hydroxy-3-methoxycyclohexylethynyl)-3-aza-bicyclo[3.3.1]nonane-1-carboxylate 142

To a solution of propargylic alcohol **145** (0.270g, 1.75mmol) in anhydrous DMSO (5ml) was added potassium *t*-butoxide (0.040g, 0.36mmol). After stirring for 1h, bicyclic β-ketoester 4 (0.400g, 1.68mmol) was added. After stirring for 120h, the reaction mixture was poured into 10% aqueous sodium chloride solution (10ml) and then extracted into ether (3 x 10ml) and then dried over magnesium sulphate, filtered and concentrated under reduced pressure to give a pale yellow oil (0.521g). Silica gel chromatography (1:4 EtOAc-hexane) gave a quantitative recovery of both starting materials.

Synthesis of ethyl 3-ethyl-9-hydroxy-9-(1-hydroxy-3-methoxycyclohexylethynyl)-3-azabicyclo[3.3.1]nonane-1-carboxylate 142

To a solution of 1-ethynyl-3-methoxycyclohexanol **145** (0.924g, 6.0mmol) in anhydrous THF (30ml) was added *n*-BuLi (6.85ml of 2.1 M in hexanes, 14.4mmol) at 0 °C under an atmosphere of nitrogen. After 1h, ethyl 3-ethyl-3-azabicyclo[3.3.1]nonan-9-one-1-carboxylate **4** was added (1.434g, 6.0mmol) as a solution in anhydrous THF (10ml). After stirring for 16h, the reaction mixture was poured into saturated aqueous ammonium chloride solution (30ml) and the organic layer removed and concentrated under reduced pressure. The resulting yellow oil was dissolved in DCM (25ml) and washed with water (2 x 20ml), washed with saturated aqueous sodium chloride solution (20ml), dried over magnesium sulphate, filtered and then concentrated under reduced pressure to yield a viscous yellow oil. Silica gel chromatography (3:7 EtOAc-hexane) gave a colourless viscous oil (2.060g 83%) which is a mixture of diastereoisomers. Subsequent silica gel chromatography allowed limited separation of the less polar diastereoisomer from the mixture.

Compound A:

PMR: δ 4.71 (1H, s, O<u>H</u>), 4.25-4.12 (2H, m, $CH_3C\underline{H}_2O$), 3.43-3.25 (1H, $C\underline{H}OCH_3$), 3.35 (3H, s, $OC\underline{H}_3$), 3.24 (1H, bs, O<u>H</u>), 3.19 (1H, dd, 12.2, 1.5, $C\underline{H}_2NCH_2$, *19*), 2.87 (1H, d, 11.2, $C\underline{H}_2NCH_2$, *18*), 2.68-1.40 (19H, m), 1.29 (3H, dt, 12.2, 2.5, $C\underline{H}_3CH_2O$), 1.07 (3H, dt, 8.3, 1.4, $C\underline{H}_3CH_2N$).

CMR: δ 176.72 ($O{=}\underline{C}CH_2CH_3$), 90.46, 88.55 (2 x $\underline{C}{\equiv}\underline{C}$), 77.42 ($H\underline{C}OCH_3$), 70.60, 68.44 (HO<u>C</u> quaternary *8*), 61.29 ($CH_3\underline{C}H_2O$), 57.52, 56.65 ($\underline{C}H_2N\underline{C}H_2$), 56.28 ($\underline{C}H_3O$), 52.26 ($CH_3\underline{C}H_2N$), 49.92 (quaternary *4*), 44.54 ($CH_3OCH\underline{C}H_2COH$ *15*), 39.66 (CH_2 *1* or *3*), 39.60 ($NCH_2\underline{C}HCOH$), 30.51, 30.49, 30.24, 24.78 (4 x CH_2 *1* or *3*, *9*, *13*, *14*), 20.18 ($NCH_2CHCH_2\underline{C}H_2CH_2$ *2*), 19.86 ($CH_3OCHCH_2\underline{C}H_2CH_2$ *14*), 14.36 ($OCCH_2\underline{C}H_3$), 12.85 ($NCH_2\underline{C}H_3$).

MS: CI gave m/z 394.1 $[M+H]^+$ ($C_{22}H_{35}O_5N$ requires 393).

Synthesis of ethyl 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)ethyl)-3-azabicyclo[3.3.1]nonane-1-carboxylate 146

To a slurry of palladium on charcoal (~100mg 10% Pd/C) in ethanol (20ml) was added **142** (1.115g, 2.8mmol) as a solution in ethanol (10ml). This was stirred under an atmosphere of hydrogen gas (atmospheric pressure) at 20°C for 72h. The reaction mixture was then filtered through a bed of celite and the filter cake washed with ethanol (5 x 20ml). The combined extractions were concentrated under reduced pressure and then purified by silica gel chromatography (3:7 EtOAc-hexane) to yield the 2 separate diastereoisomers A (Rf 0.3, 0.474g) and B (Rf 0.2, 0.310g) in a combined yield of 84%.

Compound **146a:**

PMR: δ 4.24-4.11 (2H, m, OCH_2CH_3), 4.04 (1H, s, OH), 4.01 (1H, s, OH), 3.67 (1H, d, 11.8, 1 of CH_2NCH_2 *17* or *19*), 3.63-3.60 (1H, m, $CHOCH_3$), 3.34 (3H, s, OCH_3), 3.09 (1H, d, 11.7, 1 of CH_2NCH_2 *17* or *19*), 2.79 (1H, d, 11.2, 1 of CH_2NCH_2 *17* or *19*), (2.72-2.60 (2H, m, 1 of CH_2NCH_2 *17* or *19* and CH_2 *2* ax), 2.43-1.32 (19H, m), 1.28 (3H, dt, 7.3, 1.6 OCH_2CH_3), 1.06 (3H, t, 6.8, NCH_2CH_3).

CMR: δ 177.20/177.11 (ester carbonyl), 76.89 ($CHOCH_3$), 72.43/72.37, 71.20/71.17 (2 x quaternary *5* and *8*), 60.58 (OCH_2CH_3), 56.37/56.33 (CH_2NCH_2 *17* or *19*), 56.13 (OCH_3), 55.16/55.09 (CH_2NCH_2 *17* or *19*), 51.91 (NCH_2CH_3), 49.99 (quaternary *4*), 39.88/39.02, 37.79/36.75 (2 x CH_2 *6* and *7*), 34.42/34.35 (CH *11*), 32.43, 28.46/28.39, 26.87, 26.81, 26.07 (5 x CH_2 *1, 3, 9, 13, 15*), 20.23 (CH_2 *2*), 16.19 (CH_2 *14*), 12.66/12.63 (NCH_2CH_3).

MS: CI gave m/z 398.3 $[M+H]^+$ ($C_{22}H_{39}O_5N$ requires 397).

Compound **146b:**

PMR: δ 4.08 (2H, q, 7.3, $OC\underline{H}_2CH_3$), 3.99 (1H, d, 23.4, O<u>H</u>), 3.90 (1H, bs, O<u>H</u>), 3.56-3.53 (1H, m, $C\underline{H}OCH_3$), 3.26 ($OC\underline{H}_3$), 2.83 (1H, d, 9.8, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 2.71 (1H, d, 10.2, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 2.62-2.54 (2H, m 2 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 2.21 (2H, q, 7.3, $NC\underline{H}_2CH_3$), 2.04-1.23 (19H, m), 1.20 (3H, dt, 7.3, 1.5, $OCH_2C\underline{H}_3$), 0.96 (3H, t, 7.3, $NCH_2C\underline{H}_3$).

CMR: δ 177.19/177.12 (ester carbonyl), 77.25 ($\underline{C}HOCH_3$), 72.81/72.75, 71.58/71.54 (2 x quaternary $\underline{C}OH$ *5* and *8*), 61.03 ($O\underline{C}H_2CH_3$), 58.16, 56.48 ($C\underline{H}_2N\underline{C}H_2$ *17* and *19*), 54.01 ($O\underline{C}H_3$), 52.25 ($N\underline{C}H_2CH_3$), 51.19 (quaternary *4*), 40.22/39.30, 38.15/37.00 (2 x $\underline{C}H_2$ *6* and *7*), 35.74/35.65 ($\underline{C}H$ *11*), 34.95/34.86, 30.73, 28.81/28.73, 28.11/28.07, 27.34/27.29 (5 x $\underline{C}H_2$ *1, 3, 9, 13, 15*), 20.63 ($\underline{C}H_2$ *2*), 16.51 ($\underline{C}H_2$ *14*), 14.36, ($OCH_2\underline{C}H_3$), 12.84 ($NCH_2\underline{C}H_3$).

MS: CI gave m/z 398.3 $[M+H]^+$ ($C_{22}H_{39}O_5N$ requires 397).

Synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)ethyl)-3-azabicyclo[3.3.1]nonane-1-methanol 147a

To a slurry of lithium aluminium hydride (0.090g, 2.4mmol) in anhydrous THF (15ml) was added **146a** (0.474g, 1.2mmol), as a solution in anhydrous THF (5ml), under an atmosphere of nitrogen at 20°C. After 20h, saturated aqueous sodium potassium tartrate solution (~2ml) was added. The resulting emulsion was poured into saturated aqueous sodium potassium tartrate solution (25ml) and after settling the diethyl ether layer was removed. The aqueous layer was extracted with diethyl ether (3 x 10ml). The combined ether layers were washed with saturated aqueous sodium chloride solution (25ml), dried over magnesium sulphate and concentrated. Silica gel chromatography (1:19 MeOH-DCM) gave a colourless viscous oil (0.372g, 88%).

PMR: δ 4.74 (1H, bs, 1 of $C\underline{H}_2OH$ *18*), 4.63 (1H, bs, 1 of $C\underline{H}_2OH$ *18*), ~4.2 (1H, bs, O<u>H</u>), 3.80 (1H, dd, 10.4, 3.4, 1 of $\underline{C}H_2N\underline{C}H_2$ *17* or *19*), 3.62 (1H, bs, C<u>H</u> *16*), 3.07 (1H, dd, 10.4, 2.4, 1 of $\underline{C}H_2N\underline{C}H_2$ *17* or *19*), 2.78 (1H, d, 10.6, 1 of $\underline{C}H_2N\underline{C}H_2$ *17* or *19*), 2.72-1.21 (22H, m), 1.01 (3H, t, 7.4, $NCH_2C\underline{H}_3$).

CMR: δ 77.56/77.05 ($\underline{C}HOCH_3$), 74.78, 72.79, 72.13 (3 x quaternary *4, 5* and *8*), 68.89 ($\underline{C}H_2OH$), 58.87 ($\underline{C}H_2N\underline{C}H_2$ *17* or *19*), 56.46 ($O\underline{C}H_3$), 55.84 ($\underline{C}H_2N\underline{C}H_2$ *17* or *19*), 40.75/40.71, 38.77/38.39 (2 x $\underline{C}H_2$ *6* and *7*), 36.59 ($\underline{C}H_2$ *1* or *3*), 36.19/36.03 (<u>C</u>H *11*) 34.71/34.55 ($\underline{C}H_2$ *15*), 30.76 ($\underline{C}H_2$ *1* or *3*), 28.73/28.68, 26.96/25.73 (2 x $\underline{C}H_2$ *15* and *17*), 20.61 ($\underline{C}H_2$ *2*), 16.63/16.50 ($\underline{C}H_2$ *14*), 12.93 ($NCH_2\underline{C}H_3$).

MS: FAB +ve $[M+H]^+$ 356.3 ($C_{20}H_{37}O_4N$ requires 355).

Synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)ethyl)-3-azabicyclo[3.3.1]nonane-1-methanol 147b

To a slurry of lithium aluminium hydride (0.060g, 1.6mmol) in anhydrous THF (15ml) was added **146b** (0.310g, 1.2mmol), as a solution in anhydrous THF (5ml), under an atmosphere of nitrogen at 20 °C. After 20h, saturated aqueous sodium potassium tartrate solution (~2ml) was added. The resulting emulsion was poured into saturated aqueous sodium potassium tartrate solution (25ml) and after settling the diethyl ether layer was removed. The aqueous layer was extracted with diethyl ether (3 x 10ml). The combined ether layers were washed with saturated aqueous sodium chloride solution (25ml), dried over magnesium sulphate and concentrated. Silica gel chromatography (1:19 MeOH-DCM) gave a colourless viscous oil (0.271g, 98%).

PMR: δ 3.67 (1H, dd, 10.0, 2.4, 1 of $C\underline{H}_2OH$ *18*), 3.38-3.31 (1H, m, $C\underline{H}OCH_3$), 3.26 (3H, s, $OC\underline{H}_3$), 3.16 (1H, d, 11.0, 1 of $C\underline{H}_2OH$ *18*), 2.92-2.36 (6H, m), 2.02-1.36 (19H, m), 1.03 (3H, t, 7.1, $NCH_2C\underline{H}_3$).

Synthesis of ethyl 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)-(*Z*)-ethenyl)-3-azabicyclo[3.3.1]nonane-1-carboxylate 148

To a slurry of palladium on charcoal (~200mg 10% Pd/C) in pyridine-ethanol (3:17ml) was added **142** (2.500g, 6.4mmol) as a solution in ethanol (10ml). This was stirred under an atmosphere of hydrogen gas (atmospheric pressure) at 20°C for 72h. The reaction mixture was then filtered through a bed of celite and the filter cake washed with ethanol (5 x 25ml). The combined extractions were concentrated under reduced pressure and then purified by silica gel chromatography (1:19 MeOH-DCM) to yield the 2 separate diastereoisomers A (0.600g) and B (0.387g) in a combined yield of 40%.

Compound **148a**:

PMR: δ 5.91 (1H, bs, O<u>H</u>), 5.86 (1H, dd, 13.4, 6.2, 1 of C<u>H</u>=C<u>H</u>), 5.59 (1H, d, 6.8, O<u>H</u>), 5.49 (1H, dd, 13.5, 2.3, 1 of C<u>H</u>=C<u>H</u>), 4.16-4.11 (2H, m, OC<u>H</u>$_2$CH$_3$), 3.53-3.44/3.44-3.34 (1H, m, C<u>H</u>OCH$_3$), 3.34/3.33(3H, s, OC<u>H</u>$_3$), 3.11 (1H, d, 12.2, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.80 (1H, d, 11.2, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.69-1.38 (19H, m), 1.28-1.22 (3H, m, OCH$_2$C<u>H</u>$_3$), 1.05 (3H, t, 7.1, NCH$_2$C<u>H</u>$_3$).

Compound **148b**:

PMR: δ 6.09 (1H, dd, 13.5, 8.8, C<u>H</u>=C<u>H</u>), 5.75 (1H, s, O<u>H</u>), 5.61 (1H, s, O<u>H</u>), 5.36 (1H, dd, 13.7, 5.3, C<u>H</u>=C<u>H</u>), 4.19-4.04 (2H, m, OC<u>H</u>$_2$CH$_3$), 3.61-3.54/3.54-3.47 (1H, m, C<u>H</u>OCH$_3$), 3.34/3.31 (3H, s, OC<u>H</u>$_3$), 3.01 (1H, d, 10.4, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.87-1.38 (20H, m), 1.23 (3H, dt, 7.2, 3.7, OCH$_2$C<u>H</u>$_3$), 1.04 (3H, t, 7.2, NCH$_2$C<u>H</u>$_3$).

CMR: δ 175.51 (ester carbonyl), 134.44/14.22, 133.05 (<u>C</u>H=<u>C</u>H *6* and *7*), 76.77 (<u>C</u>HOCH$_3$), 73.25/73.12, 72.91 (quaternary <u>C</u>OH *5* and *8*), 60.38 (O<u>C</u>H$_2$CH$_3$), 56.36 (1 of <u>C</u>H$_2$N<u>C</u>H$_2$ *17* or *19*), 56.10 (O<u>C</u>H$_3$), 53.42 (1 of <u>C</u>H$_2$N<u>C</u>H$_2$ *17* or *19*), 52.14 (N<u>C</u>H$_2$CH$_3$), 50.29/50.13 (quaternary *4*), 41.98/41.84 (CH *11*), 38.94, 38.01 (2 x <u>C</u>H$_2$ *1*

and *3*), 32.05 ($\underline{C}H_2$ *15*), 28.87/ 28.64, 28.51 (2 x $\underline{C}H_2$ *9* and *14*), 20.21 ($\underline{C}H_2$ *2*), 17.00 ($\underline{C}H_2$ *13*), 14.02 ($OCH_2\underline{C}H_3$), 12.55 ($NCH_2\underline{C}H_3$).

Synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)(*Z*)-ethenyl)-3-azabicyclo[3.3.1]nonane-1-methanol 149a

To a slurry of lithium aluminium hydride (0.096g, 2.5mmol) in anhydrous THF (15ml) was added **148a** (0.500g, 1.3mmol), as a solution in anhydrous THF (5ml), under an atmosphere of nitrogen at 20 °C. After 20h, saturated aqueous sodium potassium tartrate solution (~2ml) was added. The resulting emulsion was poured into saturated aqueous sodium potassium tartrate solution (25ml) and after settling the diethyl ether layer was removed. The aqueous layer was extracted with diethyl ether (3 x 10ml). The combined ether layers were washed with saturated aqueous sodium chloride solution (25ml), dried over magnesium sulphate and concentrated. Silica gel chromatography (1:19 MeOH/DCM) gave a colourless viscous oil (0.378g, 84%).

PMR: δ 6.90/6.58 (1H, 2 x bs, O$\underline{H}$), 5.95 (1H, dd, 13.8, 8.4, C$\underline{H}$=CH), 5.66/5.51 (1H, 2 x bs, O$\underline{H}$), 5.24 (1H, ddd, 13.5, 4.8, 2.2, C$\underline{H}$=CH), ~4.2 (1H, bs, O$\underline{H}$), 3.71-3.64 (2H, m, C$\underline{H}OCH_3$ and 1 of $C\underline{H}_2OH$ *18*), 3.31/3.30 (3H, s, $OC\underline{H}_3$), 2.99 (1H, dd, 8.2, 1.0, 1 of $C\underline{H}_2OH$ *18*), 2.76 (1H, d, 11.4, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 2.71-2.59 (1H, m, $C\underline{H}_2$ *2* ax), 2.57-1.19 (19H, m), 0.99 (3H, t, 7.1 $NCH_2C\underline{H}_3$).

CMR: δ 135.76/135.40, 132.25/132.05 (2 x CH=$\underline{C}$H), 76.47 ($\underline{C}HOCH_3$), 73.89, 73.58 (2 x quaternary *5* and *8*), 68.99/68.93 ($\underline{C}H_2OH$), 58.16/58.13 ($\underline{C}H_2N\underline{C}H_2$ *17* or *19*), 56.15 ($O\underline{C}H_3$), 58.15 ($\underline{C}H_2N\underline{C}H_2$ *17* or *19*), 52.38 ($N\underline{C}H_2CH_3$), 42.97/42.78 ($\underline{C}H$ *11*), 40.00 (quaternary *4*), 39.88/39.84, 37.84/37.81 (2 x $\underline{C}H_2$ *1* and *3*), 29.18, 27.34/27.20, 26.32 (3 x $\underline{C}H_2$ *9,13, 15*), 20.25 ($\underline{C}H_2$ *2*), 15.53/15.42 ($\underline{C}H_2$ *14*), 12.35 ($NCH_2\underline{C}H_3$).

MS: FAB +ve gave m/z 354.2 $[M+H]^+$ ($C_{20}H_{35}O_4N$ requires 353).

Synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)(*Z*)-ethenyl)-3-azabicyclo[3.3.1]nonane-1-methanol 149b

To a slurry of lithium aluminium hydride (0.060g, 1.5mmol) in anhydrous THF (15ml) was added **148b** (0.300g, 0.8mmol), as a solution in anhydrous THF (5ml), under an atmosphere of nitrogen at 20 °C. After 22h, saturated aqueous sodium potassium tartrate solution (~2ml) was added. The resulting emulsion was poured into saturated aqueous sodium potassium tartrate solution (25ml) and after settling the diethyl ether layer was removed. The aqueous layer was extracted with diethyl ether (3 x 10ml). The combined ether layers were washed with saturated aqueous sodium chloride solution (25ml), dried over magnesium sulphate and concentrated. Silica gel chromatography (1:19 MeOH-DCM) gave a colourless viscous oil (0.208g, 78%).

PMR: δ 7.03/6.75 (1H, 2 x bs, O<u>H</u>), 5.81 (1H, dd, 13.5, 8.3, C<u>H</u>=CH), 5.65/5.55 (1H, 2 x bs, O<u>H</u>), 5.24 (1H, dd, 13.5, 5.1, C<u>H</u>=CH), 4.25 (1H, bs, O<u>H</u>), 3.65-3.56 (2H, m, C<u>H</u>OCH$_3$ and 1 of C<u>H$_2$</u>OH *18*), 3.23 (3H, s, OC<u>H$_3$</u>), 2.99 (1H, dd, 14.0, 2.0, 1 of C<u>H$_2$</u>OH *18*), 2.59-1.29 (21H, m), 0.99 (3H, t, 6.7, NCH$_2$C<u>H$_3$</u>).

CMR: δ 136.47/136.19, 131.73/131.50 (2 x CH=<u>C</u>H), 76.53 (<u>C</u>HOCH$_3$), 73.89, 73.63 (2 x quaternary *5* and *8*), 69.76 (<u>C</u>H$_2$OH), 56.42 (<u>C</u>H$_2$N<u>C</u>H$_2$ *17* or *19*), 56.18 (O<u>C</u>H$_3$), 53.71 (<u>C</u>H$_2$N<u>C</u>H$_2$ *17* or *19*), 52.88 (N<u>C</u>H$_2$CH$_3$), 42.64/42.46 (<u>C</u>H *11*), 40.48/40.35 (quaternary *4*), 40.02, 37.88 (2 x <u>C</u>H$_2$ *1* and *3*), 31.49, 28.53, 27.44/27.30 (3 x <u>C</u>H$_2$ *9,13, 15*), 19.82 (<u>C</u>H$_2$ *2*), 15.68 (<u>C</u>H$_2$ *14*), 12.24 (NCH$_2$<u>C</u>H$_3$).

MS: FAB +ve gave m/z 354.2 $[M+H]^+$ ($C_{20}H_{35}O_4N$ requires 353).

Synthesis of 3-ethyl-9-hydroxy-9-(2-(-1-hydroxy-3-methoxycyclohexyl)(*E*)-ethenyl)-3-azabicyclo[3.3.1]nonane-1-methanol 150 and 3-ethyl-9-hydroxy-9-(1-hydroxy-3-methoxycyclohexylethynyl)-3-azabicyclo[3.3.1]nonane-1-methanol 151

Modified from a procedure by Walborsky and Wust (1982).

To a slurry of lithium aluminium hydride (0.390g, 10.2mmol) in anhydrous diethyl ether (20ml) was added **142** (2.000g 5.1mmol) under an atmosphere of nitrogen at 20 °C. After stirring for 20h, saturated aqueous sodium potassium tartrate solution (~2ml) was added. The resulting emulsion was poured into saturated aqueous sodium potassium tartrate solution (30ml) and after settling the diethyl ether layer was removed. The aqueous layer was extracted with diethyl ether (3 x 10ml). The combined ether layers were washed with saturated aqueous sodium chloride solution (20ml), dried over magnesium sulphate and concentrated to yield a white foam (~1.9g). Silica gel chromatography gave 4 compounds:

Fraction 1: (*E*)-alkenic triol **150a** + alkynic triol **151a**

Fraction 2: alkynic triol **151b**

Fraction 3: (*E*)-alkenic triol **150a**

Compound **151b** (fraction 2):

PMR: δ ~4.7 (3H, bs, 3 x OH), 3.90 (1H, dd, 10.8, 5.1, 1 of OCH_2 *18*), 3.42-3.33 (1H, m, $CHOH_3$), 3.30 (3H, s, OCH_3), 3.22 (1H, dd, 10.9, 4.1, 1 of OCH_2 *18*), 2.88 (1H, d, 11.0, 1 of CH_2NCH_2 *17* or *19*), 2.78-1.13 (20H, m), 1.00 (3H, t, 7.1 NCH_2CH_3).

CMR: δ 90.20, 85.74 (2 x C≡C *6* and *7*), 77.31 ($CHOCH_3$), 73.51, 69.74 (2 x quaternary COH), 68.26 (CH_2OH), 55.91/55.88 (OCH_3), 54.83, 52.49 (CH_2NCH_2), 52.28 (NCH_2CH_3), 44.57/44.41 (CH_2 *1* or *3*), 40.71/40.68 (quaternary *4*), 40.38/40.33 (CH *11*), 39.24/39.05, 32.40, 32.36, 30.24, 29.12, 19.52 (6 x CH2 *1/3, 2, 9, 13, 14,15*), 12.26 (NCH_2CH_3).

Compound **150b** (fraction 3):

PMR: δ 6.23 (1H, d, 15.4, 1 of CH=CH *6* or *7*), 5.87 (1H, d, 15.4, 1 of CH=CH *6* or *7*), ~4.5 (3H, bs, 3 x OH), 3.53 (2H, d, 9.2, OCH_2 *18*), 3.27 (3H, s, OCH_3), 3.10 (1H, d, 9.2, 1 of CH_2NCH_2 *17* or *19*), 3.03-2.94 (2H, m, 1 of CH_2NCH_2 *17* or *19* and CHOCH$_3$), 2.60-1.16 (20H, m), 1.06 (NCH_2CH_3).

CMR: δ 137.51, 128.88 (CH=CH *6* and *7*), 77.07 (CHOCH$_3$), 75.55, 72.40/72.35 (2 x quaternary COH *5* and *8*), 69.50 (CH_2OH), 56.49 (1 of CH_2NCH_2 *17* or *19*), 56.24 (OCH_3), 54.01 (1 of CH_2NCH_2 *17* or *19*), 52.71 (NCH_2CH_3), 41.24 (CH *11*), 37.55, 38.35 (2 x CH_2 *1* and *3*), 30.95, 28.43, 28.22 (3 x CH_2 *9, 13* and *15*), 18.95 (CH_2 *2*), 16.74 (CH_2 *14*), 12.21 (NCH_2CH_3).

MS: FAB +ve gave m/z 354.0 $[M+H]^+$ ($C_{20}H_{35}O_4N$ requires 353).

General Procedure 8: Conversion of neopentyl-like alcohols into anthranilate esters

Example: synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)ethyl)-3-azabicyclo[3.3.1]nonane-1-methyl 2-aminobenzoate **152a**

From a procedure by Coates *et al.* (1994b).

A solution of **147a** (0.365g, 1.03mmol), isatoic anhydride (0.251g, 1.54mmol) and DMAP (trace) in anhydrous DMF (1.5ml) was stirred at 70°C for 20h under an atmosphere of nitrogen. The DMF was removed by bulb-to-bulb distillation. Silica gel chromatography (1:19 MeOH-DCM) of the residual gum gave a colourless viscous oil (0.402g 83%).

PMR: δ 7.85-7.82 (1H, m, aromatic CH *o*-ester), 7.25 (1H, dt, 7.7, 1.0, aromatic CH *p*-ester), 6.68-6.61 (2H, m, 2 x aromatic CH *m*-ester), ~5.8 (2H, bs, NH_2), 4.44 (1H, d, 19.6, 1 of OCH_2 *18*), 4.19 (1H, d, 5.8, 1 of OCH_2 *18*), 3.63-3.59 (1H, m, CHOCH$_3$),

2.95 (3H, s, OCH_3), 2.77-2.62 (1H, m, $C\underline{H}_2$ *2* ax), 2.40-1.25 (27H, m), 1.03 (3H, t, 7.0, $NCH_2C\underline{H}_3$).

CMR: δ 168.04/167.98 (ester carbonyl), 148.90 (quaternary aromatic $\underline{C}NH_2$), 133.89 (aromatic $\underline{C}H$ *p*-ester), 130.90 (aromatic $\underline{C}H$ *o*-ester), 116.61, 116.08 (2 x aromatic $\underline{C}H$ *m*-ester), 110.71 (quaternary aromatic $CH=\underline{C}(CO_2)CNH_2$), 76.80/76.69 ($C\underline{H}OCH_3$), 72.81/72.67, 71.62/71.44 (2 x quaternary $\underline{C}OH$ *5* and *8*), 68.28 ($\underline{C}H_2O$ *18*), 58.82/58.79 ($\underline{C}H_2N\underline{C}H_2$ *17* or *19*), 56.13 ($O\underline{C}H_3$), 55.75/55.71 ($\underline{C}H_2N\underline{C}H_2$ *17* or *19*), 52.15 ($N\underline{C}H_2CH_3$), 41.28/41.25 (quaternary *4*), 39.84, 38.14/38.05 (2 x $\underline{C}H_2$ *6* and *7*), 36.46 ($\underline{C}H_2$ *1* or *3*), 36.05/35.89 ($\underline{C}H$ *11*), 34.60/34.46 ($\underline{C}H_2$ *1* or *3*), 30.05, 28.24, 26.40, 25.74/25.65 (4 x $\underline{C}H_2$ *9, 13, 15*), 20.22 ($\underline{C}H_2$ *2*), 16.06/15.97 ($\underline{C}H_2$ *14*), 12.61 ($NCH_2\underline{C}H_3$)

MS: FAB +ve gave m/z 475 $[M+H]^+$ ($C_{27}H_{42}O_5N_2$ requires 474).

Synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)ethyl)-3-azabicyclo[3.3.1]nonane-1-methyl 2-aminobenzoate **152b**

Using general procedure 8. **147b** (0.200g, 0.56mmol), isatoic anhydride (0.138g, 0.85mmol), DMAP (trace) in anhydrous DMF (1ml). Silica gel chromatography (1:19 MeOH-DCM) gave a yellow oil (0.180g, 67%).

PMR: δ 7.85-7.82 (1H, d, 7.9, aromatic $C\underline{H}$ *o*-ester), 7.25 (1H, dt, 6.9, 1.5, aromatic $C\underline{H}$ *p*-ester), 6.67-6.61 (2H, m, 2 x aromatic $C\underline{H}$ *m*-ester), ~5.8 (2H, bs, $N\underline{H}_2$), ~4.4 (2H, bs, 2 x $O\underline{H}$), 4.28 (2H, m, $OC\underline{H}_2$ *18*), 3.61 (1H, bs, $C\underline{H}OCH_3$), 3.31 (3H, s, OCH_3), 2.87-2.36 (25H, m), 1.07 (3H, t, 7.0, $NCH_2C\underline{H}_3$).

CMR: δ 167.98 (ester carbonyl), 150.43 (quaternary aromatic $\underline{C}NH_2$), 133.89 (aromatic $\underline{C}H$ *p*-ester), 130.85 (aromatic $\underline{C}H$ *o*-ester), 116.59, 116.06 (2 x aromatic $\underline{C}H$ *m*-ester), 110.63 (quaternary aromatic $CH=\underline{C}(CO_2)CNH_2$), 77.00 ($C\underline{H}OCH_3$), 71.53, 71.38 (2 x quaternary $\underline{C}OH$ *5* and *8*), 68.41 ($\underline{C}H_2O$ *18*), 57.12 ($\underline{C}H_2N\underline{C}H_2$ *17* or *19*),

56.12 (O$\underline{C}H_3$), 53.56 ($\underline{C}H_2N\underline{C}H_2$ *17* or *19*), 52.02 ($N\underline{C}H_2CH_3$), 41.58 (quaternary *4*), 39.53/38.45, 37.73/36.76 (2 x $\underline{C}H_2$ *6* and *7*), 36.09/35.85 ($\underline{C}H$ *11*), 34.75/34.65, 31.63 ($\underline{C}H_2$ *1* and *3*), 28.32/28.24, 28.08, 25.54/25.43 (3 x $\underline{C}H_2$ *9, 13, 15*), 18.97/18.78 ($\underline{C}H_2$ *2*), 16.08/16.03 ($\underline{C}H_2$ *14*), 12.39 ($NCH_2\underline{C}H_3$).

Synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)(*Z*)-ethenyl)3-azabicyclo[3.3.1]nonane-1-methyl 2-aminobenzoate **153a**

Using general procedure 8. **149a** (0.300g, 0.85mmol), isatoic anhydride (0.206g, 1.27mmol), DMAP (trace) in anhydrous DMF (1ml). Silica gel chromatography (3:97 MeOH-DCM) gave a yellow oil (0.329g, 82%).

PMR: δ 7.78-7.76 (1H, m, aromatic C$\underline{H}$ *o*-ester), 7.18-7.15 (1H, m, aromatic C$\underline{H}$ *p*-ester), 6.59-6.56 (2H, m, 2 x aromatic C$\underline{H}$ *m*-ester), 6.39/6.27 (1H, 2 x bs, O$\underline{H}$), 5.98 (1H, dd, 13.6, 11.2, C$\underline{H}$=C$\underline{H}$ *6* or *7*), 5.69 (2H, bs, $N\underline{H}_2$), 5.44/5.29 (1H, 2 x bs O$\underline{H}$), 5.15 (1H, dd, 13.6, 11.2, C$\underline{H}$=C$\underline{H}$ *6* or *7*), 4.21-4.11 (2H, m, $C\underline{H}_2O$ *18*), 3.61-3.55 (1H, m, C$\underline{H}$OCH$_3$), 3.26/3.25 (3H, s, OC$\underline{H}_3$), 2.93 (1H, d, 11.7, 1 of $C\underline{H}_2N\underline{C}H_2$ *17* or *19*), 2.79 (1H, d, 13.6, 1 of $C\underline{H}_2N\underline{C}H_2$ *17* or *19*), 2.63-2.43 (1H, m, $C\underline{H}_2$ *2* ax), 2.40 (1H, d, 15.0, 1 of $C\underline{H}_2N\underline{C}H_2$ *17* or *19*), 2.25-1.18 (17H, m), 0.97 (3H, t, 6.9, $NCH_2C\underline{H}_3$).

CMR: δ 168.24/168.20 (ester carbonyl), 150.32 (quaternary aromatic $\underline{C}NH_2$), 135.08/134.85, 133.75, 133.13/132.85, 131.10 (2 x aromatic $\underline{C}H$ *o*- and *p*- ester and 2 x alkene $\underline{C}H$), 116.56, 116.06/116.01 (2 x aromatic $\underline{C}H$ *m*-ester), 111.09 (quaternary aromatic CH=$\underline{C}(CO_2)CNH_2$), 77.00 ($\underline{C}HOCH_3$), 73.59/73.27, 72.67/72.63 (2 x quaternary *5* and *8*), 68.99 ($\underline{C}H_2O$ *18*), 58.58 (1 of $\underline{C}H_2N\underline{C}H_2$ *17* or *19*), 56.28 (O$\underline{C}H_3$), 56.21 (1 of $\underline{C}H_2N\underline{C}H_2$ *17* or *19*), 52.20 ($N\underline{C}H_2CH_3$), 43.08/42.77 ($\underline{C}H$ *11*), 41.14/40.94 (quaternary *4*), 39.84/39.13, 38.19/37.70 (2 x $\underline{C}H_2$ *1* and *3*), 29.76/29.58 ($\underline{C}H_2$ *15*), 27.51/27.29, 26.60 (2 x $\underline{C}H_2$ *9* and *13*), 20.32 ($\underline{C}H_2$ *2*), 15.50/15.46 ($\underline{C}H_2$ *14*), 12.61 ($NCH_2\underline{C}H_3$).

MS: FAB +ve gave m/z 473.2 $[M+H]^+$ ($C_{27}H_{40}O_5N_2$ requires 472).

Synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)(Z)-ethenyl)-3-azabicyclo[3.3.1]nonane-1-methyl 2-aminobenzoate **153b**

Using general procedure 8. **149b** (0.150g, 0.43mmol), isatoic anhydride (0.103g, 0.64mmol), DMAP (trace) in anhydrous DMF (1ml). Silica gel chromatography (3:97 MeOH-DCM) gave a yellow oil (0.165g, 82%).

PMR: δ 7.78-7.75 (1H, m, aromatic C<u>H</u> *o*-ester), 7.20-7.14 (1H, m, aromatic C<u>H</u> *p*-ester), 6.57-6.54 (2H, m, 2 x aromatic C<u>H</u> *m*-ester), 5.92 (1H, dd, 13.2, 10.0, C<u>H</u>=C<u>H</u> *6* or *7*), 5.70 (2H, bs, N<u>H</u>$_2$), 5.25-5.20 (1H, m, C<u>H</u>=C<u>H</u> *6* or *7*), 4.21-4.14 (2H, m, C<u>H</u>$_2$O *18*), 3.57-3.53 (1H, m, C<u>H</u>OCH$_3$), 3.24/3.23 (3H, s, OC<u>H</u>$_3$), 2.92 (1H, d, 8.3, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.85-2.73 (3H, m, 3 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* and *19*), 2.61-2.54 (C<u>H</u>$_2$ *2* ax), 2.49-2.35 (2H, m, NC<u>H</u>$_2$CH$_3$), 2.12-1.18 (17H, m), 0.97 (3H, t, 6.9, NCH$_2$C<u>H</u>$_3$).

CMR: δ 168.11 (ester carbonyl), 150.35 (quaternary aromatic <u>C</u>NH$_2$), 136.00/135.76 (alkene <u>C</u>H *6* or *7*), 133.8 (aromatic <u>C</u>H *p*-ester), 132.14/131.88 (alkene <u>C</u>H *6* or *7*), 131.04 (aromatic <u>C</u>H *o*-ester), 116.69, 116.04 (2 x aromatic <u>C</u>H *m*-ester), 110.87 (quaternary aromatic CH=<u>C</u>(CO$_2$)CNH$_2$), 76.60 (<u>C</u>HOCH$_3$), 73.49/73.25, 72.39/72.30 (2 x quaternary *5* and *8*), 68.79 (<u>C</u>H$_2$O *18*), 56.66 (1 of <u>C</u>H$_2$N<u>C</u>H$_2$ *17* or *19*), 56.19/56.15 (O<u>C</u>H$_3$), 53.28 (1 of <u>C</u>H$_2$N<u>C</u>H$_2$ *17* or *19*), 52.92/52.84 (N<u>C</u>H$_2$CH$_3$), 42.47/42.24 (<u>C</u>H *11*), 41.56/41.42 (quaternary *4*), 40.00/39.53, 38.08/37.70 (2 x <u>C</u>H$_2$ *1* and *3*), 31.00/30.97 (<u>C</u>H$_2$ *15*), 28.63, 27.57/27.35 (2 x <u>C</u>H$_2$ *9* and *13*), 19.47 (<u>C</u>H$_2$ *2*), 15.64 (<u>C</u>H$_2$ *14*), 11.93/11.86 (NCH$_2$<u>C</u>H$_3$).

MS: FAB +ve gave m/z 473.2 $[M+H]^+$ ($C_{27}H_{40}O_5N_2$ requires 472).

Synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)ethynyl)-3-azabicyclo[3.3.1]nonane-1-methyl 2-aminobenzoate **154a**

Using general procedure 8. **151a** (0.500g, 1.41mmol), isatoic anhydride (0.346g, 2.12mmol), DMAP (trace) in anhydrous DMF (1ml). Silica gel chromatography (1:19 MeOH-DCM) gave a yellow oil (0.150g, 22%).

PMR: δ 7.74 (1H, d, 8.2, aromatic C<u>H</u> *o*-ester), 7.21-7.16 (1H, m aromatic C<u>H</u> *p*-ester), 6.58 (2H, t, 8.2, 2 x aromatic C<u>H</u> *m*-ester), 5.70 (2H, bs, N<u>H</u>$_2$), 4.30-4.19 (2H, m, OC<u>H</u>$_2$ *18*), 3.44-3.36 (1H, m, C<u>H</u>OCH$_3$), 3.26/3.25 (3H, s, OC<u>H</u>$_3$), 2.87-2.80 (2H, m, 2 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *18*), 2.62-2.55 (2H, m 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *18* and C<u>H</u>$_2$ *2* ax), 2.35-1.21 (19H, m), 0.97 (3H, t, 7.1, NCH$_2$C<u>H</u>$_3$).

CMR: δ 168.09 (ester carbonyl), 150.45 (quaternary aromatic <u>C</u>NH$_2$), 132.61, 130.91 (2 x aromatic <u>C</u>H *o*- and *p*-ester), 116.81, 116.37 (2 x aromatic <u>C</u>H *m*-ester), 110.68 (quaternary aromatic CH=<u>C</u>(CO$_2$)CNH$_2$), 91.23, 85.69 (2 x quaternary C≡C *6* and *7*), 77.16 (<u>C</u>HOCH$_3$), 71.62 (1 of quaternary *5* or *8*), 69.54 (O<u>C</u>H$_2$ *18*), 68.28/68.24 (1 of quaternary *5* or *8*), 59.52, 56.79 (2 x <u>C</u>H$_2$N<u>C</u>H$_2$ *17* and *19*), 56.03 (O<u>C</u>H$_3$), 52.14 (N<u>C</u>H$_2$CH$_3$), 44.01 (quaternary *4*), 43.93 (<u>C</u>H$_2$ *1* or *3*), 41.21 (<u>C</u>H *11*), 39.26/39.07 (<u>C</u>H$_2$ *1* or *3*), 30.07 (<u>C</u>H$_2$ *15*), 28.09, 25.04 (2 x <u>C</u>H$_2$ *9* and *13*), 20.19 (<u>C</u>H$_2$ *2*), 19.33 (<u>C</u>H$_2$ *14*), 12.52 (NCH$_2$<u>C</u>H$_3$).

Synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)ethynyl)-3-azabicyclo[3.3.1]nonane-1-methyl 2-aminobenzoate **154b**

Using general procedure 8. 3-Ethyl-3-aza-9-hydroxy-9-(2-(1-ethynyl-1-hydroxy-3-methoxycyclohexane))-bicyclo[3.3.1]nonane-1-methanol **151b** (0.356g, 1.01mmol), isatoic anhydride (0.246g, 1.52mmol), DMAP (trace) in anhydrous DMF (1ml). Silica gel chromatography (1:19 MeOH-DCM) gave a yellow oil (0.173g, 36%).

PMR: δ 7.81 (1H, d, 7.8, aromatic C<u>H</u> *o*-ester), 7.28-7.25 (1H, m aromatic C<u>H</u> *p*-ester), 6.68-6.63 (2H, m, 2 x aromatic C<u>H</u> *m*-ester), 5.29 (2H, bs, N<u>H</u>$_2$), 4.44-4.39 (1H, m, 1 of OC<u>H</u>$_2$ *18*), 4.29 (1H, d, 11.2, 1 of OC<u>H</u>$_2$ *18*), 3.60 (2H, bs 2 x O<u>H</u>), 3.44-3.40 (1H, m, C<u>H</u>OCH$_3$), 3.33/3.32 (3H, s, OC<u>H</u>$_3$), 2.74-1.26 (21H, m), 0.97 (3H, t, 7.1, NCH$_2$C<u>H</u>$_3$).

CMR: δ 168.15 (ester carbonyl), 150.46 (quaternary aromatic <u>C</u>NH$_2$), 134.19, 130.91 (2 x aromatic <u>C</u>H *o*- and *p*-ester), 116.80, 116.34 (2 x aromatic <u>C</u>H *m*-ester), 110.63 (quaternary aromatic CH=<u>C</u>(CO$_2$)CNH$_2$), 90.61, 84.45 (2 x quaternary C≡C *6* and *7*), 77.00 (<u>C</u>HOCH$_3$), 71.53 (1 of quaternary *5* or *8*), 69.52 (O<u>C</u>H$_2$ *18*), 68.20 (1 of quaternary *5* or *8*), 56.00 (O<u>C</u>H$_3$), 55.97/55.53 (1 of <u>C</u>H$_2$N<u>C</u>H$_2$ *17* or *19*), 52.35, 52.07 (1 of <u>C</u>H$_2$N<u>C</u>H$_2$ *17* or *19* and N<u>C</u>H$_2$CH$_3$), 43.97/43.90 (<u>C</u>H$_2$ *1* or *3*), 41.84 (quaternary *4*), 41.16/41.12 (<u>C</u>H *11*), 39.19, 39.03 (<u>C</u>H$_2$ *1* or *3* and *15*), 30.07 (<u>C</u>H$_2$ *15*), 32.71, 30.07/30.01/29.85 (2 x <u>C</u>H$_2$ *9* and *13*), 19.70, 19.32 (2 x <u>C</u>H$_2$ *2* and *14*), 12.46 (NCH$_2$<u>C</u>H$_3$).

MS: FAB +ve gave m/z 471.2 $[M+H]^+$ ($C_{27}H_{38}O_5N_2$ requires 470).

<u>Synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)(*E*)-ethenyl)-3-azabicyclo[3.3.1]nonane-1-methyl 2-aminobenzoate **155a**</u>

Using general procedure 8. **150a** (0.500g, 1.41mmol), isatoic anhydride (0.346g, 2.12mmol), DMAP (trace) in anhydrous DMF (1.5ml). Silica gel chromatography (1:19 MeOH-DCM) gave a yellow oil (0.162g, 24%).

PMR: δ 7.82-7.78 (1H, m, aromatic C<u>H</u> *o*-ester), 7.30-7.23 (1H, m, aromatic C<u>H</u> *p*-ester), 6.69-6.64 (2H, m, aromatic 2 x C<u>H</u> *m*-ester), 6.53 (1H, dd, 15.5, 10.1, C<u>H</u> alkene 6 or 7), 5.83 (1H, dd, 15.6, 5.3, C<u>H</u> alkene 6 or 7), 5.75 (2H, bs, N<u>H</u>$_2$), 4.29-4.15 (2H, m, 2 x O<u>H</u>), 4.11-4.00 (2H, m C<u>H</u>$_2$O *18*), 3.60-3.55 (1H, m, C<u>H</u>OCH$_3$), 3.34/3.32/3.31 (3H, s, OCH$_3$), 2.88-1.52 (24H, m), 1.06 (3H, t, 7.1, NCH$_2$C<u>H</u>$_3$).

Synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)(*E*)-ethenyl)3-azabicyclo[3.3.1]nonane-1-methyl 2-aminobenzoate **155b**

Using general procedure 8. 3-Ethyl-3-aza-9-hydroxy-9-(2-(1-(*E*)-ethenyl-1-hydroxy-3-methoxycyclohexane))-bicyclo[3.3.1]nonane-1-methanol **150b** (0.400g, 1.13mmol), isatoic anhydride (0.275g, 1.70mmol), DMAP (trace) in anhydrous DMF (1ml). Silica gel chromatography (1:19 MeOH-DCM) gave a yellow oil (0.507g, 93%).

PMR: δ 7.74-7.71 (1H, m, aromatic C<u>H</u> *o*-ester), 7.20-7.16 (1H, m, aromatic C<u>H</u> *p*-ester), 6.59-6.56 (2H, m, 2 x aromatic C<u>H</u> *m*-ester), 6.35 (1H, dd, 15.3, 11.0, C<u>H</u>=C<u>H</u> *6* or *7*), 5.80 (1H, dd, 15.3, 7.0, C<u>H</u>=C<u>H</u> *6* or *7*), 5.63 (2H, bs, N<u>H</u>$_2$), 4.12-4.02 (2H, m, C<u>H</u>$_2$O *18*), 3.50 (1H, bs, C<u>H</u>OCH$_3$), 3.26/3.24 (3H, s, OC<u>H</u>$_3$), 2.87-2.81 (1H, m, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.74 (1H, d, 10.7, 1 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* or *19*), 2.59-1.18 (19H, m), 1.00 (3H, t, 7.0, NCH$_2$C<u>H</u>$_3$).

CMR: δ 168.20/168.13 (ester carbonyl), δ 150.77 (quaternary aromatic <u>C</u>NH$_2$), 137.48 (1 of <u>C</u>H=<u>C</u>H *6* or *7*), 134.37, 131.24 (2 x aromatic <u>C</u>H *o*- and *p*- ester), 129.27 (1 of <u>C</u>H=<u>C</u>H *6* or *7*), 117.00, 116.59 (2 x aromatic <u>C</u>H *m*-ester), 110.98 (quaternary aromatic CH=<u>C</u>(CO$_2$)CNH$_2$), 77.00 (<u>C</u>HOCH$_3$), 74.06, 72.23/72.17 (2 x quaternary *5* and *8*), 69.47 (O<u>C</u>H$_2$ *18*), 57.25 (1 of <u>C</u>H$_2$N<u>C</u>H$_2$ *17* or *19*), 56.61/56.57 (O<u>C</u>H$_3$), 54.16 (1 of <u>C</u>H$_2$N<u>C</u>H$_2$ *17* or *19*), 52.37 (CH$_3$<u>C</u>H$_2$N), 42.11 (CH *11*), 41.72 (quaternary *4*), 40.42/40.31, 38.16/37.97 (2 x <u>C</u>H$_2$ *1* and *3*), 31.87 (<u>C</u>H$_2$ *15*), 28.79, 28.37/28.32 (2 x <u>C</u>H$_2$ *9* and *13*), 19.25/19.19 (<u>C</u>H$_2$ *2*), 16.52/16.39 (<u>C</u>H$_2$ *14*), 12.73 (NCH$_2$<u>C</u>H$_3$).

MS: FAB +ve gave m/z 473.2 $[M+H]^+$ ($C_{27}H_{40}O_5N_2$ requires 472).

General Procedure 9: Conversion of anthranilate esters to methylsuccinimides

Example: synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)ethyl)3-azabicyclo[3.3.1]nonane-1-methyl [2-(*RS*)-methylsuccinimido]benzoate **156a**

Modified from a procedure by Coates *et al.* (1994).

A solution of **152a** WT4/57 (0.200g, 0.42mmol) and (±)-methylsuccinic anhydride (0.182g, 1.26mmol) in DCM (5ml) was stirred at room temperature for 16h. To this was then added 1,1-carbonyldiimidazole (0.171g, 1.06mmol). After stirring for a further 24h, the reaction mixture was concentrated under reduced pressure and then purified by silica gel chromatography to give a viscous colourless oil (0.115g, 47%).

PMR: δ 8.11 (1H, d, 7.3, aromatic C<u>H</u> *o*-ester), 7.65 (1H, dt, 7.6, 1.5, aromatic C<u>H</u> *p*-ester), 7.52 (1H, tt, 8.2, 1.2, aromatic C<u>H</u> *m*-ester), 7.25 (1H, d, 7.9, aromatic C<u>H</u> *o*-ester), 4.57 (2H, bs, 2 x O<u>H</u>), 4.17 (2H, d, 3.7, OC<u>H</u>$_2$ *18*), 3.62 (1H, bs, C<u>H</u>OCH$_3$), 3.31 (3H, s, OC<u>H</u>$_3$), 3.17-2.86 (5H, m, 4 of C<u>H</u>$_2$NC<u>H</u>$_2$ *17* and *19*, and 1 of C<u>H</u>$_2$ *15*), 2.74 (1H, dd, 16.5, 8.0 1 of C<u>H</u>$_2$ *15*), 2.66-1.21 (25H, m), 1.05 (3H, t, 7.0, NCH$_2$C<u>H</u>$_3$).

CMR: δ 179.89 (O=<u>C</u>CH$_2$), 176.01 (O=<u>C</u>CH), 162.72 (ester carbonyl), 132.65 (quaternary aromatic <u>C</u>N), 133.25 (aromatic <u>C</u>H *p*-ester), 131.32 (aromatic <u>C</u>H *o*-ester), 129.72, 129.32 (2 x aromatic <u>C</u>H *m*-ester), 127.27 (quaternary aromatic CH=<u>C</u>(CO$_2$)CN), 76.80/76.69 (<u>C</u>HOCH$_3$), 72.46/72.37, 71.73/71.51 (2 x quaternary <u>C</u>OH *5* and *8*), 69.08 (O<u>C</u>H$_2$ *18*), 58.42/58.34, 55.53/55.49 (<u>C</u>H$_2$N<u>C</u>H$_2$ *17* and *19*), 56.17 (O<u>C</u>H$_3$), 52.13 (N<u>C</u>H$_2$CH$_3$), 41.36/41.32 (quaternary 4), 38.12/37.53/36.88/36.49/36.42/36.09 (2 x <u>C</u>H$_2$ *6* and *7*), 35.93/35.74 (<u>C</u>H *11*), 35.36/35.14 (<u>C</u>H methylsuccinimide), 34.66/34.48 (<u>C</u>H$_2$ methylsuccinimide), 29.76/29.58, 28.19 (2 x <u>C</u>H$_2$ *1* and *3*), 26.14/26.10, 25.78, 25.67 (3 x <u>C</u>H$_2$ *9, 13* and *15*), 19.89/19.85 (<u>C</u>H$_2$ *2*), 16.43/16.19/16.06 (<u>C</u>H$_3$ methylsuccinimide), 15.93 (<u>C</u>H$_2$ *14*), 12.28/12.24 (NCH$_2$<u>C</u>H$_3$).

MS: Low resolution FAB +ve gave 571.1 $[M+H]^+$ and 553.1 $[M+H]^+$-H_20 ($C_{32}H_{46}N_2O_7$ requires 570).

High resolution FAB +ve gave 571.3383 $[M+H]^+$ ($C_{32}H_{46}N_2O_7$ +H requires 571.3383).

Synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)ethyl)-3-azabicyclo[3.3.1]nonane-1-methyl [2-(*RS*)-methylsuccinimido]benzoate **156b**

Using general procedure 9: **152b** (0.100g, 0.21mmol), (±)-methylsuccinic anhydride (0.091g, 0.63mmol), CDI (0.085g, 0.53mmol), in DCM (5ml). Silica gel chromatography (1:19 MeOH-DCM) gave a pale yellow foam (0.120g, 100%).

PMR: δ 8.03 (1H, d, 7.5, aromatic C<u>H</u> *o*-ester), 7.59 (1H, dt, 7.9, 1.1, aromatic C<u>H</u> *p*-ester), 7.46 (1H, t, 7.5, aromatic C<u>H</u> *m*-ester), 7.18 (1H, d, 7.9, aromatic C<u>H</u> *m*-ester), 4.89 (2H, bs, 2 x O<u>H</u>), 4.23 (1H, d, 11.2, 1 of O<u>CH</u>$_2$ 18), 4.13 (1H, d, 11.2, 1 of O<u>CH</u>$_2$ 18), 3.57 (1H, bs, C<u>H</u>OCH$_3$), 3.25/3.24 (3H, s, OC<u>H</u>$_3$), 3.04-2.25 (10H, m), 1.99-1.13 (21H, m), 1.09 (3H, t, 7.2, NCH$_2$C<u>H</u>$_3$).

CMR: δ 179.90 (O=<u>C</u>CH$_2$), 175.80 (O=<u>C</u>CH), 164.30 (ester carbonyl), 133.38 (aromatic <u>C</u>H *p*-ester), 133.72 (quaternary aromatic <u>C</u>N), 131.35 (aromatic <u>C</u>H *o*-ester), 129.80, 129.43 (2 x aromatic <u>C</u>H *m*-ester), 127.15 (quaternary aromatic CH=<u>C</u>(CO$_2$)CN), 77.20/76.80 (<u>C</u>HOCH$_3$), 71.90/71.66, 71.48 (2 x quaternary <u>C</u>OH *5* and *8*), 69.14 (O<u>C</u>H$_2$ *18*), 56.13 (O<u>C</u>H$_3$), 56.12, 53.39/53.19 (<u>C</u>H$_2$N<u>C</u>H$_2$ *17* and *19*), 53.00 (N<u>C</u>H$_2$CH$_3$), 41.58 (quaternary 4), 39.99 (<u>C</u>H$_2$ methylsuccinimide), 39.98/38.12, 37.15/36.91 (2 x <u>C</u>H$_2$ *6* and *7*), 36.35/36.05 (<u>C</u>H *11*), 35.78/35.30/35.18 (<u>C</u>H methylsuccinimide), 34.94/34.72, 31.08 (2 x <u>C</u>H$_2$ *1* and *3*), 28.17/28.12, 27.51/27.46, 25.28/25.23 (3 x <u>C</u>H$_2$ *9, 13* and *15*), 18.42/18.28 (<u>C</u>H$_2$ *2*), 16.45/16.28 (<u>C</u>H$_3$ methylsuccinimide), 16.01/15.88 (<u>C</u>H$_2$ *14*), 11.38 (NCH$_2$<u>C</u>H$_3$).

MS: Low resolution FAB +ve gave 571.2 $[M+H]^+$ ($C_{32}H_{46}N_2O_7$ requires 570).

High resolution FAB +ve gave 571.3433 $[M+H]^+$ ($C_{32}H_{46}N_2O_7$ +H requires 571.3383).

Synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)(*Z*)-ethenyl)-3-azabicyclo[3.3.1]nonane-1-methyl [2-(*RS*)-methylsuccinimido]benzoate **157a**

Using general procedure 9: **153a** (0.150g, 0.32mmol), (±)-methylsuccinic anhydride (0.137g, 0.95mmol), CDI (0.128g, 0.79mmol), in DCM (5ml). Silica gel chromatography (1:19 MeOH-DCM) gave a pale yellow foam (0.050g, 28%).

PMR: δ 8.10-8.06 (1H, m, aromatic C<u>H</u>, *o*-ester), 7.73-7.69 (1H, m, aromatic C<u>H</u>, *p*-ester), 7.61-7.56 (1H, m, aromatic C<u>H</u>, *m*-ester), 7.33 (1H, d, 7.8, aromatic C<u>H</u>, *m*-ester), 6.11 (1H, t, 13.2, alkene C<u>H</u> *6* or *7*), 5.54 (1H, dd, 13.2, 5.4, alkene C<u>H</u> *6* or *7*), 4.20-4.14 (2H, m, OC<u>H</u>$_2$ *18*), 3.63-3.34 (1H, m, C<u>H</u>OCH$_3$), 3.33/3.30 (3H, s, OC<u>H</u>$_3$), 3.18-1.39 (27H, m), 1.06 (3H, t, 6.9, NCH$_2$C<u>H</u>$_3$).

CMR: δ 182.06 (O=<u>C</u>CH$_2$), 178.21 (O=<u>C</u>CH), 165.92 (ester carbonyl), 136.38/135.90 (alkene <u>C</u>H *6* or *7*), 134.49 (aromatic <u>C</u>H *p*-ester), 134.19 (quaternary aromatic <u>C</u>N), 133.25/133.03 (alkene <u>C</u>H *6* or *7*), 132.10 (aromatic <u>C</u>H *o*-ester), 131.17, 130.51 (2 x aromatic m-ester), 128.90 (quaternary aromatic CH=<u>C</u>(CO$_2$)CN), 78.24 (<u>C</u>HOCH$_3$), 74.26/74.20, 73.95/73.81 (2 x quaternary <u>C</u>OH *5* and *8*), 70.52 (O<u>C</u>H$_2$ *18*), 59.22, 57.32 (<u>C</u>H$_2$N<u>C</u>H$_2$ *17* and *19*), 56.21(O<u>C</u>H$_3$), 53.94 (N<u>C</u>H$_2$CH$_3$), 43.88/43.80 (<u>C</u>H *11*), 42.47/42.36 (quaternary *4*), 39.83, 39.71 (2 x <u>C</u>H$_2$ *1*, *3*), 37.90/37.63 (<u>C</u>H$_2$ methylsuccinimide), 36.95/36.77/36.46 (<u>C</u>H methylsuccinimide), 30.62/30.40, 30.16, 27.12 (<u>C</u>H$_2$ *9*, *13* and *15*), 21.06 (<u>C</u>H$_2$ *2*), 19.21/18.92 (<u>C</u>H$_2$ *14*), 16.44/16.28 (<u>C</u>H$_3$ methylsuccinimide), 12.55 (NCH$_2$<u>C</u>H$_3$).

MS: Low resolution FAB +ve gave 569.1 $[M+H]^+$ ($C_{32}H_{44}N_2O_7$ requires 568).

High resolution FAB +ve gave 569.3152 $[M+H]^+$ ($C_{32}H_{44}N_2O_7$ +H requires 569.3226).

Synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)(*Z*)-ethenyl)3-azabicyclo[3.3.1]nonane-1-methyl [2-(*RS*)-methylsuccinimido]benzoate **157b**

Using general procedure 9: **153b** (0.100g, 0.21mmol), (±)-methylsuccinic anhydride (0.095g, 0.64mmol), CDI (0.085g, 0.53mmol), in DCM (5ml). Silica gel chromatography (1:19 MeOH-DCM) gave a pale yellow foam (0.060g, 46%).

PMR: δ δ 8.06 (1H, d, 6.3, aromatic CH, *o*-ester), 7.60 (1H, t, 7.3, aromatic CH, *p*-ester), 7.48 (1H, t, 6.8, aromatic CH, *m*-ester), 7.19 (1H, d, 6.8, aromatic CH, *m*-ester), 6.45 (1H, bs, 1 x OH), 5.95 (1H, dd, 13.0, 10.0, alkene CH *6* or *7*), 5.50 and 5.38 (1H, 2 x bs 1 x OH), 5.27-5.21 (1H, m, alkene CH *6* or *7*), 4.18-4.16 (2H, m, OCH_2 *18*), 3.63-3.57 (1H, m, $CHOCH_3$), 3.29/3.27 (3H, s, OCH_3), 3.10-1.28 (27H, m), 1.03 (3H, t, 6.9, NCH_2CH_3).

CMR: δ 178.85 ($O=CCH_2$), 176.05/175.95/175.81 (O=CCH), 164.38/164.16 (ester carbonyl), 135.87/135.65 (alkene CH *6* or *7*), 133.13 (aromatic CH *p*-ester), 132.72/132.43 (alkene CH *6* or *7*), 131.54 (quaternary aromatic CN), 131.43 (aromatic CH *o*-ester), 129.70, 129.25 (2 x aromatic m-ester), 127.51 (quaternary aromatic $CH=C(CO_2)CN$), 76.60 ($CHOCH_3$), 73.51/73.23, 72.55 (2 x quaternary COH *5* and *8*), 69.90/69.77 (OCH_2 *18*), 56.98 (1 of CH_2NCH_2 *17* or *19*), 56.24/56.18 (OCH_3), 53.57/53.37 (1 of CH_2NCH_2 *17* or *19*), 52.38 (NCH_2CH_3), 42.65/42.45 (CH *11*), 41.65/41.50 (quaternary *4*), 40.05/39.49, 38.10/37.74 (2 x CH_2 *1*, *3*), 36.88 (CH_2 *15*), 35.27/35.10 (CH methylsuccinimide), 31.08, 28.97 (CH_2 *9* and *13*), 27.62/27.40 (CH_2 methylsuccinimide), 19.89 (CH_2 *2*), 16.43/16.17(CH_3 methylsuccinimide), 15.68 (CH_2 *14*), 12.46/12.16 (NCH_2CH_3).

MS: Low resolution FAB +ve gave 569.1 $[M+H]^+$ ($C_{32}H_{44}N_2O_7$ requires 568).

High resolution FAB +ve gave 569.3224 $[M+H]^+$ ($C_{32}H_{44}N_2O_7$ +H requires 569.3226).

Synthesis of 3-ethyl-9-hydroxy-9-(1-hydroxy-3-methoxycyclohexylethynyl)-3-azabicyclo[3.3.1]nonane-1-methyl [2-(*RS*)-methylsuccinimido]benzoate **158a**

Using general procedure 9: **154a** (0.100g, 0.21mmol), (±)-methylsuccinic anhydride (0.095g, 0.64mmol), CDI (0.090g, 0.53mmol), in DCM (5ml). Silica gel chromatography (1:19 MeOH-DCM) gave a pale yellow foam (0.060g, 50%).

PMR: δ δ 8.09 (1H, d, 7.7, aromatic CH, *o*-ester), 7.68 (1H, dt, 6.8, 1.8, aromatic CH, *p*-ester), 7.48 (1H, t, 7.7, aromatic CH, *m*-ester), 7.27 (1H, d, 6.6, aromatic CH, *m*-ester), 4.33-4.24 (1H, d, 1 of OCH_2 *18*), 3.73-3.65 (1H, d, 1 of OCH_2 *18*), 3.50-3.40 (1H, m, $CHOCH_3$), 3.34/3.33 (3H, s, OCH_3), 3.18-1.21 (27H, m), 1.05 (3H, t, 7.1, NCH_2CH_3).

CMR: δ 180.12 ($O=CCH_2$), 176.21 (O=CCH), 164.54 (ester carbonyl), 133.56 (aromatic CH *p*-ester), 132.68 (quaternary aromatic CN), 131.38 (aromatic CH *o*-ester), 129.86/129.75, 129.68/129.50 (2 x aromatic CH *m*-ester), 90.94, 85.02 (2 x quaternary C≡C *6* and *7*), 77.23/77.18 ($CHOCH_3$), 71.73 (quaternary COH *5* or *8*), 71.73 (OCH_2 *18*), 68.12/68.08 (quaternary COH *5* or *8*), 56.18/56.13 (OCH_3), 55.47 (1 of CH_2NCH_2 *17* or *19*), 52.39, 52.14 (1 of CH_2NCH_2 *17* or *19* and CH_2NCH_3), 43.75 (CH_2 *1* or *3*), 41.83 (quaternary *4*), 41.85 (CH *11*), 39.28/39.22 (CH_2 *1* or *3*), 37.00 (CH_2 *15*), 35.38/35.22 (CH methylsuccinimide), 32.79, 29.79 (CH_2 *9* and *13*), 29.77/29.70/29.61 (CH_2 methylsuccinimide), 19.71, 19.53/19.22 (CH_2 *2*and *14*), 16.57/16.39 (CH_3 methylsuccinimide), 12.55 (NCH_2CH_3).

MS: Low resolution FAB +ve gave 567.1 $[M+H]^+$ ($C_{32}H_{44}N_2O_7$ requires 566).

High resolution FAB +ve gave 567.3070 $[M+H]^+$ ($C_{32}H_{44}N_2O_7$ +H requires 567.3070).

Synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)(*E*)-ethenyl)-3-bicyclo[3.3.1]nonane-1-methyl [2-(*RS*)-methylsuccinimido]benzoate **159a**

Using general procedure 9: **155a** (0.090g, 0.19mmol), (±)-methylsuccinic anhydride (0.082g, 0.57mmol), CDI (0.077g, 0.47mmol), in DCM (5ml). Silica gel chromatography (1:19 MeOH-DCM) gave a pale yellow foam (0.050g, 46%).

PMR: δ 8.15-8.07 (1H, m, aromatic CH, *o*-ester), 7.67 (1H, t, 9.0, aromatic CH *p*-ester), 7.54 (1H, t, 8.6, aromatic CH *m*-ester), 7.26 (1H, d, 8.0, aromatic CH *m*-ester), 6.52 (1H, dd, 15.4, 8.6, alkene CH *6* or *7*), 5.82 (1H, dd, 15.6, 6.8 alkene CH *6* or *7*), 4.36-3.89 (4H, m, 2 x OH, and OCH_2 *18*), 3.71-3.58 (1H, m, $CHOCH_3$), 3.34/3.31 (3H, s, OCH_3), 3.15-2.17 (27H, m), 1.06 (3H, t, 7.1, NCH_2CH_3).

CMR: δ 179.91 ($O=CCH_2$), 176.07 (O=CCH), 164.29 (ester carbonyl), 136.78 (alkene CH *6* or *7*), 133.38 (aromatic CH *p*-ester), 132.86 (quaternary aromatic CN), 131.37 (aromatic CH *o*-ester) 2 x (129.80), 129.43 (2 x aromatic m-ester and alkene CH *6* or *7*), 127.24 (quaternary aromatic $CH=C(CO_2)CN$), 76.76 ($CHOCH_3$), 73.60, 71.86/71.79 (2 x quaternary COH *5* and *8*), 69.85/69.78 (OCH_2 *18*), 58.20 (1 of CH_2NCH_2 *17* or *19*), 56.30 (OCH_3), 55.80 (1 of CH_2NCH_2 *17* or *19*), 52.29 (NCH_2CH_3), 42.18 (CH *11*), 40.91 (quaternary *4*), 40.19, 37.77, 36.94 (3 x CH_2 *1*, *3* and *15*), 35.35/35.17 (CH methylsuccinimide), 29.62 (CH_2 *9* or *13*), 28.09/27.93 (CH_2 methylsuccinimide), 26.50 (CH_2 *9* or *13*), 20.11 (CH_2 *2*), 16.48/16.32 (CH_3 methylsuccinimide), 16.12 (CH_2 *14*), 12.53 (NCH_2CH_3).

MS: Low resolution FAB +ve gave 569.1 $[M+H]^+$ ($C_{32}H_{44}N_2O_7$ requires 568).

High resolution FAB +ve gave 569.3222 $[M+H]^+$ ($C_{32}H_{44}N_2O_7$ +H requires 569.3226).

Synthesis of 3-ethyl-9-hydroxy-9-(2-(1-hydroxy-3-methoxycyclohexyl)(*E*)-ethenyl)-3-azabicyclo[3.3.1]nonane-1-methyl [2-(*RS*)-methylsuccinimido]benzoate **159b**

Using general procedure 9: **155b** (0.200g, 0.42mmol), (±)-methylsuccinic anhydride (0.183g, 1.27mmol), CDI (0.171g, 1.06mmol), in DCM (5ml). Silica gel chromatography (1:19 MeOH-DCM) gave a pale yellow foam (0.137g, 57%).

PMR: δ 8.09 (1H, d, 7.2, aromatic CH, *o*-ester), 7.65 (1H, t, 7.8, aromatic CH, *p*-ester), 7.52 (1H, t, 7.8, aromatic CH, *m*-ester), 7.24 (1H, d, 7.8, aromatic CH, *m*-ester), 6.41 (1H, dd, 15.6, 9.8, alkene CH *6* or *7*), 5.86 (1H, dd, 15.1, 8.3, alkene CH *6* or *7*), 5.25 (2H, bs, 2 x OH), 4.22 (1H, dd, 11.2, 4.6, 1 of OCH_2 *18*), 4.00 (1H, dd, 21.0, 9.3, 1 of OCH_2 *18*), 3.59 (1H, bs, $CHOCH_3$), 3.34/3.31 (3H, s, OCH_3), 3.10-2.90 (2H, m, CH methylsuccinimide and 1 of CH_2 *1, 13* or *15*), 2.93 (1H, dd, 10.7, 5.4, 1 of CH_2NCH_2 *17* or *19*), 2.75 (1H, d, 11.2, 1 of CH_2NCH_2 *17* or *19*), 2.60-2.40 (3H, m, 2 of CH_2NCH_2 *17* or *19* and CH_2 *2* ax), 2.35 (2H, q, 7.3, NCH_2CH_3), 1.98-1.38 (19H, m), 1.06 (3H, t, 6.8, NCH_2CH_3).

CMR: δ 179.93 ($O=CCH_2$), 176.07/175.83 (O=CCH), 164.22/164.01 (ester carbonyl), 137.15/137.11 (alkene CH *6* or *7*), 134.99 (aromatic CH *p*-ester), 133.27 (quaternary aromatic CN), 132.65 (aromatic CH *o*-ester), 131.32, 129.70/129.38 (2 x aromatic m-ester), 128.86/128.82 (alkene CH *6* or *7*), 127.24 (quaternary aromatic $CH=C(CO_2)CN$), 77.20/76.69 ($CHOCH_3$), 73.67, 71.93/71.86 (2 x quaternary COH *5* and *8*), 70.09 (OCH_2 *18*), 56.88 (1 of CH_2NCH_2 *17* or *19*), 56.21(OCH_3), 53.81 (1 of CH_2NCH_2 *17* or *19*), 51.76 (NCH_2CH_3), 41.72 (quaternary *4*), 41.36 (CH *11*), 40.19, 37.75/37.72, 36.86 (3 x CH_2 *1, 3* and *15*), 35.26/35.08 (CH methylsuccinimide), 31.35, 29.56 (CH_2 *9* and *13*), 28.48/28.44/28.01 (CH_2 methylsuccinimide), 18.70, 18.66 (CH_2 *2* and CH_2 *14*), 16.37/16.23/16.10 (CH_3 methylsuccinimide), 12.46 (NCH_2CH_3).

MS: Low resolution FAB +ve gave 569.1 $[M+H]^+$ ($C_{32}H_{44}N_2O_7$ requires 568).

High resolution FAB +ve gave 569.3226 $[M+H]^+$ ($C_{32}H_{44}N_2O_7$ +H requires 569.3226).

Attempted dehydroxylation of ethyl 3-ethyl-9-hydroxy-9-(2-trimethylsilyl)-1-ethynyl-3-azabicyclo[3.3.1]nonane-1-carboxylate 140

To a solution of **140** (1.500g, 4.45mmol) in DCM was added dicobalt octacarbonyl (1.856g, 5.34mmol) under an atmosphere of nitrogen. After stirring at room temperature for 23h the reaction mixture was cooled to -5°C and sodium borohydride (0.504g, 13.35mmol) was added. After a further 10min trifluoroacetic acid was added (5.0ml, 64.73mmol) and a vigorous evolution of gas was observed. After 20h the reaction mixture was poured into ice water (30ml) and the DCM layer was washed with water (3 x 20ml) and then concentrated under reduced pressure. The residual purple-brown oil was re-dissolved in EtOH (50ml) and to this was added an ethanolic solution of ferric nitrate (6.675g $Fe(NO_3)_3$ in 20ml EtOH). After 24h the reaction mixture was concentrated under reduced pressure and re-dissolved in EtOAc (50ml) and washed with water (3 x 20ml), saturated aqueous sodium chloride solution (20ml) and dried over magnesium sulphate. Concentration under reduced pressure yielded a viscous colourless oil (0.980g 65%) which had the same GC retention time, Rf by tlc and the same proton and carbon NMR as the starting propargylic alcohol.

Attempted synthesis of ethyl 3-ethyl-9-ethynyl-3-azabicyclo[3.3.1]nonane-1-carboxylate 160

To a solution of **141** (0.330g, 1.25mmol) in DCM was added dicobalt octacarbonyl (0.511, 1.49mmol) under an atmosphere of nitrogen. After 6h, tlc indicated (1:9 EtOAc-hexane) that a new compound of lower Rf was appearing, but not all of the starting material had been converted to the dicobalt hexacarbonyl complex. After 20h, tlc indicated that all of the starting material had been consumed, but there was none of the dicobalt hexacarbonyl complex present. The reaction mixture was concentrated under

reduced pressure and purified by silica gel chromatograhy (1:9 EtOAc-hexane) to yield a pale yellow gum (0.150g, 45%).

PMR: δ 6.95 (1H, d, 7.1, O=CC<u>H</u>=CHN), 6.14 (1H, s, 1 of C<u>H</u>$_2$=C), 5.56 (1H, s, 1 of C<u>H</u>$_2$=C), 4.89 (1H, d, 7.1, O=CCH=C<u>H</u>N), 4.20 (2H, q, 7.0, OC<u>H</u>$_2$CH$_3$), 3.50 (1H, dd, 12.8, 5.7, CHC<u>H</u>$_2$N ax), 3.31-3.18 (3H, m, NC<u>H</u>$_2$CH$_3$ and CHC<u>H</u>$_2$N eq), 2.43-2.21 (3H, m, 3 of C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$), 1.88-1.35 (3H, m, 3 of C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$), 1.31 (3H, t, 6.4, OCH$_2$C<u>H</u>$_3$), 1.23 (3H, t, 7.1, NCH$_2$C<u>H</u>$_3$).

CMR: δ 194.38 (ketone carbonyl), 167.36 (ester carbonyl), 152.71 (O=C<u>C</u>H=CHN), 140.58 (quaternary CH$_2$=<u>C</u>), 124.97 (<u>C</u>H$_2$=C), 97.10 (O=CCH=<u>C</u>HN), 60.72 (O<u>C</u>H$_2$CH$_3$), 50.77 (N<u>C</u>H$_2$CH), 50.512 (N<u>C</u>H$_2$CH$_3$), 44.00 (O=CC<u>H</u>CH$_2$N), 32.09 (CH$_2$=C<u>C</u>H$_2$CH$_2$), 28.41, 26.11 (CH$_2$=CCH$_2$<u>C</u>H$_2$<u>C</u>H$_2$), 14.37 (OCH$_2$<u>C</u>H$_3$), 14.13 (NCH$_2$<u>C</u>H$_3$).

MS: CI gave m/z 266 $[M+H]^+$ ($C_{15}H_{23}NO_3$ requires 265). High resolution FAB +ve gave 266.1763 $[M+H]^+$ ($C_{15}H_{23}NO_3$ +H requires 266.1756).

Synthesis of ethyl 3-oxocyclohexene-4-carboxylate 178 (previously synthesised by Brenner (1961))

To a solution of diisopropylamine (1.052g, 10.42mmol) in anhydrous THF (15ml) at 0°C was added *n*-BuLi (4.38ml of 2.5M in hexanes, 10.94mmol) under an atmosphere of nitrogen. After stirring for 45min the reaction mixture was cooled to -78°C and cyclohex-2-en-1-one **138** (0.500g, 5.21mmol) was added. Following another 45min stirring ethyl cyanoformate (0.516g, 5.21mmol) was added (some white precipitate formed). The reaction mixture was allowed to rise slowly (6h) to room temperature and stirred for a further12h. The reaction mixture was then poured into saturated aqueous ammonium chloride solution (20ml) and the THF layer and 2 further THF extracts (2 x

20ml) were combined and concentrated. The residual oil was then dissolved in EtOAc and washed with water (2 x 20ml), brine (20ml) and then dried over magnesium sulphate, filtered and concentrated under reduced pressure. Silica gel chromatography (3:7 EtOAc-hexane) gave a colourless oil (0.403g, 46%).

PMR: δ 7.06-6.97 (1H, m, O=CH=C<u>H</u>), 6.09 (1H, d, 10.6, O=C<u>H</u>=CH), 4.22 (2H, q, 7.1, OC<u>H</u>$_2$CH$_3$), 3.41 (1H, dd, 9.9, 5.7 (O=CC<u>H</u>CO$_2$Et), 2.59-2.16 (4H, m, C<u>H</u>$_2$C<u>H</u>$_2$), 1.29 (3H, t, 7.1, OCH$_2$C<u>H</u>$_3$).

Synthesis of ethyl 4-methoxy-2-oxocyclohexanecarboxylate 177

To a solution of ethyl 3-oxocyclohexene-4-carboxylate **178** (0.500g, 2.97mmol) in methanol (0.25ml) was added concentrated sulphuric acid (0.05ml). After 72h, the reaction mixture was poured into water and the products extracted into DCM (5 x 10ml). The combined DCM layers were washed with saturated aqueous sodium hydrogen carbonate solution (10ml), water (2 x 10ml), saturated aqueous sodium chloride (10ml) and then dried over magnesium sulphate filtered and concentrated. Silica gel chromatography (1:4 EtOAc-hexane) yielded a colourless oil (0.240g, 40%).

PMR: δ 12.20 (1H, s, C=CO<u>H</u>), 4.22 (2H, 2, 7.1, OC<u>H</u>$_2$CH$_3$), 3.62-3.51 (1H, m, C<u>H</u>OCH$_3$), 3.39 (3H, s, OC<u>H</u>$_3$), 2.69-1.65 (6H, m, 3 x <u>C</u>H$_2$), 1.30 (3H, t, 7.1, OCH$_2$C<u>H</u>$_3$).

CMR: δ 203.90 (ketone carbonyl), 169.26 (ester carbonyl), 97.55 (C=<u>C</u>OH), 78.03 (<u>C</u>=COH), 74.53 (C<u>H</u>OCH$_3$), 60.46 (O<u>C</u>H$_2$CH$_3$), 56.15 (O<u>C</u>H$_3$), 51.80 (O=C<u>C</u>HCO$_2$Et), 35.02, O=C<u>C</u>H$_2$CHOCH$_3$), 26.87 (O=CCH<u>C</u>H$_2$CH$_2$), 19.34 (O=CCHCH$_2$<u>C</u>H$_2$), 14.45 (OCH$_2$<u>C</u>H$_3$).

Attempted synthesis of ethyl 3-ethyl-6-methoxy-3-azabicyclo[3.3.1]nonan-9-one-1-carboxylate 175

To a solution of formaldehyde (0.196g of 37% $^{w}/_{w}$ in water, 2.42mmol) in absolute ethanol (5ml) was added a solution of ethylamine (0.078g of 70% $^{w}/_{w}$ in water, 1.21mmol) in absolute ethanol (5ml). After stirring for 15min, ethyl 4-methoxy-2-oxocyclohexanecarboxylate **177** (0.220g, 1.10mmol) was added and the reaction mixture heated to reflux. After 5h, the reaction mixture was cooled and concentrated under reduced pressure. The residual oil was dissolved in DCM and washed with water (2 x 10ml), brine (10ml), dried with magnesium sulphate, filtered and then concentrated under reduced pressure. Silica gel chromatography (1:9 MeOH-DCM) yielded a colourless oil (0.128g, 51%) as the major product **182**.

PMR: δ 4.21 (2H, q, 7.0, $OC\underline{H}_2CH_3$), 3.39 (1H, d, 11.4), 3.16-3.12 (1H, m), 2.84 (1H, d, 10.7), 2.71 (1H, dt, 17.8, 2.0), 2.60 (2H, q, 7.1, $NC\underline{H}_2CH_3$), 2.32-1.98 (4H, m), 1.75-1.60 (1H, m), 1.28 (3H, t, 7.1, $OCH_2C\underline{H}_3$), 1.10 (3H, t, 7.1, $NCH_2C\underline{H}_3$).

CMR: δ 209.11 (ketone carbonyl), 170.36 (ester carbonyl), 61.26 ($O\underline{C}H_2CH_3$), 55.34 (quaternary $\underline{C}CO_2Et$), 54.29 ($N\underline{C}H_2CCO_2Et$), 52.94 ($\underline{C}HCH_2C{=}O$), 50.07 ($N\underline{C}H_2CH_3$), 43.46 ($O{=}C\underline{C}H_2$), 25.14, 22.48 (2 x $\underline{C}H_2\underline{C}H_2$), 14.25 ($OCH_2\underline{C}H_3$), 13.51 ($NCH_2\underline{C}H_3$).

MS: EI gave m/z 225 $[M]^+$ ($C_{12}H_{19}O_3N$ requires 225).

Synthesis of 4-carbethoxy-2-ethyl-2-azabicyclo[2.2.2]octan-5-one 182

To a solution of formaldehyde (0.193g of 37% $^{w}/_{w}$ in water, 2.38mmol) in absolute ethanol (5ml) was added a solution of ethylamine (0.076g of 70% $^{w}/_{w}$ in water, 1.19mmol) in absolute ethanol (5ml). After stirring for 15min, ethyl 3-oxocyclohexene-4-carboxylate **178** (0.200g, 1.19mmol) was added and the reaction mixture heated to reflux. After 5h, the reaction mixture was cooled and concentrated under reduced

pressure. The residual oil was dissolved in DCM and washed with water (2 x 10ml), brine (10ml), dried over magnesium sulphate, filtered and then concentrated under reduced pressure. Silica gel chromatography (1:19 MeOH-DCM) yielded a colourless oil (0.212g, 79%).

PMR: δ 4.21 (2H, q, 7.0, $OC\underline{H}_2CH_3$), 3.39 (1H, d, 11.4), 3.16-3.12 (1H, m), 2.84 (1H, d, 10.7), 2.71 (1H, dt, 17.8, 2.0), 2.60 (2H, q, 7.1, $NC\underline{H}_2CH_3$), 2.32-1.98 (4H, m), 1.75-1.60 (1H, m), 1.28 (3H, t, 7.1, $OCH_2C\underline{H}_3$), 1.10 (3H, t, 7.1, $NCH_2C\underline{H}_3$).

CMR: δ 209.11 (ketone carbonyl), 170.36 (ester carbonyl), 61.26 ($O\underline{C}H_2CH_3$), 55.34 (quaternary $\underline{C}CO_2Et$), 54.29 ($N\underline{C}H_2CCO_2Et$), 52.94 ($\underline{C}HCH_2C{=}O$), 50.07 ($N\underline{C}H_2CH_3$), 43.46 ($O{=}C\underline{C}H_2$), 25.14, 22.48 (2 x $\underline{C}H_2\underline{C}H_2$), 14.25 ($OCH_2\underline{C}H_3$), 13.51 ($NCH_2\underline{C}H_3$).

MS: EI gave m/z 225 $[M]^+$ ($C_{12}H_{19}O_3N$ requires 225).

Synthesis of diethyl 4-aza-4-ethylheptanedioate 200 (previously synthesised by McElvain (1926))

To a solution of ethyl acrylate **106** (10.000g, 100mmol) in absolute ethanol (30ml) was added ethylamine solution (25ml of 2M in THF, 50mmol). After refluxing for 6h, the reaction mixture was cooled and then concentrated under reduced pressure. Purification by silica gel chromatography (1:9 EtOAc-hexane) gave a colourless oil (6.013g, 49%).

PMR: δ 4.11 (4H, q, 7.1, $OC\underline{H}_2CH_3$), 2.76 (4H, t, 7.0, $CH_2C\underline{H}_2N$), 2.50 (2H, q, 7.1, $NC\underline{H}_2CH_3$), 2.42 (4H, t, 7.0, $C\underline{H}_2CH_2N$), 1.24 (6H, t, 7.1, $OCH_2C\underline{H}_3$), 1.00 (3H, t, 7.1, $NCH_2C\underline{H}_3$).

CMR: δ 172.67 (ester carbonyl), 60.30 (2 x $OCH_2\underline{C}H_3$), 48.65, 47.26 (2 x $N\underline{C}H_2CH_2$ and $N\underline{C}H_2CH_3$), 32.68 ($NCH_2C\underline{H}_2$), 14.19 ($OCH_2\underline{C}H_3$), 11.95 ($NCH_2\underline{C}H_3$).

Synthesis of 1-carbethoxy-5-ethyl-5-azacyclohexan-2-one 193 (previously synthesised by McElvain (1926))

To a freshly prepared solution of sodium ethoxide (0.413g of Na in anhydrous ethanol, 10ml) was added a solution of **200** (4.000g, 16.32mmol) in anhydrous toluene (50ml). The reaction flask was attached to a water condenser set for downward distillation and heated to 100°C under an atmosphere of nitrogen. The excess ethanol, along with any formed by the reaction was collected, and a white precipitate formed. Heating was continued until no further solvent distilled over (3h). The reaction mixture was neutralised with glacial acetic acid and the resulting precipitate (NaOAc) was removed by filtration. The remaining toluene was removed under reduced pressure. Purification by silica gel chromatography (1:1 EtOAc-hexane) yielded a colourless oil (1.789g, 55%).

PMR: δ 4.22 (2H, q, 7.3, $OC\underline{H}_2CH_3$), 3.48-3.44 (1H, m, $O{=}CC\underline{H}C{=}O$), 3.16-3.14 (2H, m, $CHC\underline{H}_2N$), 2.62 (2H, t, 6.2, $C\underline{H}_2C{=}O$), 2.54 (2H, q, 7.0, $NC\underline{H}_2CH_3$), 2.45-2.42 (2H, m, $O{=}CCH_2C\underline{H}_2$), 1.29 (3H, t, 7.0, $OCH_2C\underline{H}_3$), 1.14 (3H, t, 7.3, $NCH_2C\underline{H}_3$).

CMR: δ 204.08 (ketone carbonyl), 170.93/170.10 (ester carbonyl), 96.61 ($HOC{=}\underline{C}$), 61.11/60.14 ($O\underline{C}H_2CH_3$), 56.30 ($\underline{C}HCH_2N$).

Synthesis of ethyl 3-ethyl-6-hydroxy-3-azabicyclo[3.3.1]nonan-9-one-1-carboxylate 191

To a solution of **193** (1.000g, 5.03mmol) in anhydrous MeCN (30ml) was added DBU (0.841g, 5.52mmol) followed by acrolein (0.309g, 5.52mmol). After stirring for 16hrs the reaction mixture was concentrated under reduced pressure. Silica gel chromatography gave three fractions (A 0.108g, A+B 1.092g, B 0.048g, combined yield 84%) which were colourless viscous oils.

Compound A

PMR: δ 4.21-4.08 (3H, m, C<u>H</u>OH and OC<u>H</u>$_2$CH$_3$), 3.54 (1H, dt, 11.2, 2.4, 1 of CH$_2$NC<u>H</u>$_2$CH *17*), 3.20 (1H, dd, 11.2, 2.4, 1 of CH$_2$NC<u>H</u>$_2$CC=O *19*), 2.95 (1H, dd, 11.2, 2.0, 1 of CH$_2$NC<u>H</u>$_2$CH *17*), 2.85-2.75 (1H, m, C<u>H</u>$_2$ *2* ax), 2.68-2.61 (1H, m, C<u>H</u> *11*), 2.51-2.33 (4H, m, 1 of CH$_2$NC<u>H</u>$_2$CC=O *19* NC<u>H</u>$_2$CH$_3$ and C<u>H</u>$_2$ *3* ax), 2.19-2.13 (1H, m, C<u>H</u>$_2$ *3* eq), 1.96-1.88 (1H, m, C<u>H</u>$_2$ *2* eq), 1.30 (3H, t, 7.3, OCH$_2$C<u>H</u>$_3$), 1.12 (3H, t, 6.8, NCH$_2$C<u>H</u>$_3$).

CMR: δ 209.81 (ketone carbonyl), 170.79 (ester carbonyl), 72.15 (<u>C</u>HOH), 61.30, 61.25 (O<u>C</u>H$_2$CH$_3$, and CH$_2$NCH$_2$CH *19*), 57.94 (quaternary *4*), 53.74 (CH *11*), 53.71 (CH$_2$NCH$_2$CC=O *17*), 50.78 (N<u>C</u>H$_2$CH$_3$), 30.32 (<u>C</u>H$_2$ *3*), 29.90 (<u>C</u>H$_2$ *2*), 14.01 (OCH$_2$<u>C</u>H$_3$), 12.50 (NCH$_2$<u>C</u>H$_3$).

MS: FAB +ve gives m/z 255.9 $[M+H]^+$ ($C_{13}H_{21}O_4N$ +H requires 255).

High resolution FAB +ve gave 256.1559 $[M+H]^+$ ($C_{13}H_{21}O_4N$ +H requires 256.1548).

<u>Compound B</u>

PMR: δ 4.41-4.40 (1H, m, C<u>H</u>OH), 4.18 (2H, q, 7.0, OC<u>H</u>$_2$CH$_3$), 3.24-3.02 (3H, m, 1 of CH$_2$NC<u>H</u>$_2$CC=O *19*, 1 of CH$_2$NC<u>H</u>$_2$CH *17* and C<u>H</u>$_2$ *2* ax), 2.93-2.53 (2H, m , 1 of CH$_2$NC<u>H</u>$_2$CC=O *19*, C<u>H</u>$_2$ *3* ax), 2.49-2.35 (4H, m, 1 of CH$_2$NC<u>H</u>$_2$CH *17*, NC<u>H</u>$_2$CH$_3$ and C<u>H</u> *11*), 2.16-2.03 (1H, m, C<u>H</u>$_2$ *3* eq), 1.70-1.61 (1H, m, C<u>H</u>$_2$ *2* eq), 1.26 (3H, t, 7.1, OCH$_2$C<u>H</u>$_3$), 1.06 (3H, t, 7.2, NCH$_2$C<u>H</u>$_3$).

CMR: δ 210.52 (ketone carbonyl), 170.80 (ester carbonyl), 76.53 (<u>C</u>HOH), 61.72 (O<u>C</u>H$_2$CH$_3$), 61.16 (CH$_2$NCH$_2$CH *19*), 58.49 (quaternary *4*), 56.16 (CH$_2$NCH$_2$CC=O *17*), 55.01 (CH *11*), 50.74 (N<u>C</u>H$_2$CH$_3$), 31.77 (<u>C</u>H$_2$ *3*), 29.05 (<u>C</u>H$_2$ *2*), 14.08 (OCH$_2$<u>C</u>H$_3$), 12.52 (NCH$_2$<u>C</u>H$_3$).

MS: FAB +ve gives m/z 256.1 $[M+H]^+$ ($C_{13}H_{21}O_4N$ +H requires 255).

High resolution FAB +ve gave 256.1549 $[M+H]^+$ ($C_{13}H_{21}O_4N$ +H requires 256.1548).

Synthesis of ethyl 3-ethyl-6-methoxy-3-azabicyclo[3.3.1]nonan-9-one-1-carboxylate 175

Method A:

To a slurry of sodium hydride (0.043g of 60% in mineral oil, 1.08mmol) in anhydrous DMF (10ml) was added bicyclic alcohol **191** (0.250g, 0.98mmol). After 30min of stirring, iodomethane (0.140g, 0.98mmol) was added. After a further 2h, the reaction mixture was neutralised with dilute aqueous hydrochloric acid (~1ml). DMF was removed by bulb-to-bulb distillation and the residual brown oil dissolved in EtOAc (10ml). This solution was washed with water (2 x 10ml), saturated aqueous sodium hydrogen carbonate solution (10ml), brine (10ml), dried over magnesium sulphate, filtered and then concentrated under reduced pressure. Purification by silica gel chromatography (1:99 MeOH-DCM) gave a colourless viscous oil (0.009g, 3%).

Method B:

A solution of **191** (0.250g, 0.98mmol), trimethyloxonium tetrafluoroborate (0.292g, 1.96mmol) and potassium carbonate (0.548g, 3.92mmol) in anhydrous DCM was stirred for 20h. The reaction mixture was filtered (to remove the potassium carbonate) and then washed with water (10ml), brine (10ml) and then dried over magnesium sulphate and concentrated under reduced pressure. Silica gel chromatography (1:3 EtOAc-hexane) of the residual oil gave a two colourless viscous oils (compound **175a** (0.005g) and **175b** (0.007g) in a combined yield of 5%).

Compound **175a**:

PMR: δ 4.22 (2H, q, 7.1, $OC\underline{H}_2CH_3$), 3.61-3.55 (1H, m, $C\underline{H}OCH_3$), 3.42 (1H, dt, 12.4, 3.4, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 3.34 (3H, s, $OC\underline{H}_3$), 3.21 (1H, dd, 11.5, 2.4, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 3.06-2.88 (2H, m, CH_2 *2* ax and 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 2.84-

2.80 (1H, m, C<u>H</u> *11*), 2.48-1.95 (6H, m), 1.29 (3H, t, 7.1 $OCH_2C\underline{H}_3$), 1.09 (3H, t, 7.1, $NCH_2C\underline{H}_3$)

CMR: δ 209.25 (ketone carbonyl), 170.77 (ester carbonyl), 77.23 ($\underline{C}HOCH_3$), 61.28, 61.19 ($O\underline{C}H_2CH_3$ and 1 of $\underline{C}H_2N\underline{C}H_2$ *17* or *19*), 58.26 (quaternary *4*), 56.40 ($O\underline{C}H_3$), 53.36 (1 of $\underline{C}H_2N\underline{C}H_2$ *17* or *19*), 50.95 ($N\underline{C}H_2CH_3$), 50.87 (<u>C</u>H *11*), 30.54, 27.30 ($\underline{C}H_2$ *1* and *3*), 14.13 ($OCH_2\underline{C}H_3$), 12.58 ($NCH_2\underline{C}H_3$)

MS: FAB +ve gives m/z 270.2 $[M+H]^+$ ($C_{14}H_{23}O_4N$ +H requires 269).

High resolution FAB +ve gave 270.170532 $[M+H]^+$ ($C_{14}H_{23}O_4N$ +H requires 270.170534 error = -0.0ppm).

<u>Compound **175b**:</u>

PMR: δ 4.26-4.17 (2H, m, $OC\underline{H}_2CH_3$), 3.87-3.84 (1H, m, $C\underline{H}OCH_3$), 3.32 (3H, s, $OC\underline{H}_3$), 3.22 (1H, dd, 11.5, 2.2, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 3.12 (1H, dt, 11.3, 2.5, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19*), 3.07-2.94 (2H, m, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19* and $C\underline{H}_2$ *2* ax), 2.75-2.63 (2H, m, 1 of $C\underline{H}_2NC\underline{H}_2$ *17* or *19* and 1 of $C\underline{H}_2C\underline{H}_2$ *2* or *3*), 2.46-2.38 (2H, m, $NC\underline{H}_2CH_3$), 2.10-1.74 (2H, m, 2 of $C\underline{H}_2C\underline{H}_2$ *2* or *3*), 1.28 (3H, t, 7.1, $OCH_2C\underline{H}_3$), 1.09 (3H, t, 7.1, $NCH_2C\underline{H}_3$)

CMR: δ 209.26 (ketone carbonyl), 170.80 (ester carbonyl), 77.95 ($\underline{C}HOCH_3$), 61.93, 61.13 (OCH2CH3 and 1 of $\underline{C}H_2N\underline{C}H_2$ *17* or *19*), 58.48 (quaternary *4*), 56.53 ($O\underline{C}H_3$), 56.53 (1 of $\underline{C}H_2N\underline{C}H_2$ *17* or *19*), 50.81 ($N\underline{C}H_2CH_3$), 51.63 (<u>C</u>H *11*), 31.67, 25.88 ($\underline{C}H_2$ *1* and *3*), 14.12 ($OCH_2\underline{C}H_3$), 12.58 ($NCH_2\underline{C}H_3$)

MS: FAB +ve gives m/z 270.2 $[M+H]^+$ ($C_{14}H_{23}O_4N$ +H requires 269).

High resolution FAB +ve gave 270.1704 ($C_{14}H_{23}O_4N$ +H requires 270.1705).

Synthesis of 3-ethyl-6,9-dihydroxy-3-azabicyclo[3.3.1]nonane-1-methanol 203

To a slurry of LAH (0.040g, 1.06mmol) in anhydrous THF (15ml) was added bicyclic β-ketoester **191a** (0.090g, 3.53mmol). After stirring for 20h, the reaction mixture was quenched with saturated aqueous sodium potassium tartrate solution (10ml). The organic layer was removed and dried over magnesium sulphate, filtered and concentrated to yield a colourless oil. Purification by silica gel chromatography (1:4 MeOH-DCM) gave two colourless oils (**203a** 0.040g and **203b** 0.010g, 66% combined yield).

Compound **203a**:

PMR: δ 4.25 (1H, m, $CH_2C\underline{H}OH$ *1*), 3.72 (1H, d, 3.0, $CHC\underline{H}OH$ *11*), 3.37-3.27 ($C\underline{H_2}OH$ *18* and 1 of $C\underline{H_2}NC\underline{H_2}$ *17* or *19*), 2.84 (1H, d, 11.3, 1 of $C\underline{H_2}NC\underline{H_2}$ *17* or *19*), 2.68-2.56 (1H, m, $C\underline{H_2}$ *2* ax), 2.35-2.15 (2H, m, 2 of $C\underline{H_2}NC\underline{H_2}$ *17* or *19*), 1.98-1.96 (3H, m, $NC\underline{H_2}CH_3$ and 1 of $C\underline{H}$ 11), 1.81-1.68 (2H, m, $C\underline{H_2}$ 3), 1.47-1.45 (1H, m, $C\underline{H_2}$ *2* eq), 1.05 (3H, t, 7.0, $NCH_2C\underline{H_3}$)

CMR: δ 75.61 ($\underline{C}HOH$ *5*), 69.32 ($\underline{C}H_2OH$ *18*), 68.75 ($\underline{C}HOH$ *5*), 62.00 (1 of $\underline{C}H_2N\underline{C}H_2$ *17* or *19*), 53.68, 53.59 (1 of $\underline{C}H_2N\underline{C}H_2$ *17* or *19* and $CH_3\underline{C}H_2N$), 44.30 ($\underline{C}H$ *11*), 39.80 (quaternary *4*), 30.95, 27.76 (2 x $\underline{C}H_2$ *2* and *3*), 13.21 ($NCH_2\underline{C}H_3$)

MS: FAB +ve gives m/z 216.2 $[M+H]^+$ ($C_{11}H_{21}O_3N$ requires 215).

High resolution FAB +ve gave 216.1599 ($C_{11}H_{21}O_3N$ +H requires 216.1599).

Chapter 8

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Appendix 1

PUBLICATIONS TO WHICH THIS WORK HAS CONTRIBUTED

Selective Probes for Nicotinic Acetylcholine Receptors from Substituted AE-Bicyclic Analogs of Methyllycaconitine

William J. Trigg[1], David J. Hardick[1], Géraldine Grangier[1], Susan Wonnacott[2], Terence Lewis[3], Michael G. Rowan[1], Barry V. L. Potter[1], and Ian S. Blagbrough[1*]

[1]Department of Medicinal Chemistry, School of Pharmacy and Pharmacology, and [2]School of Biology and Biochemistry, University of Bath, Bath BA2 7AY, [3]Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell RG12 6EY, U.K.

A concise, practical approach to substituted [3.3.1]-AE-bicyclic analogs of methyllycaconitine (MLA) has been developed employing dianion alkylation and double Mannich reactions. Acetylide anion addition to substituted cyclohexanones and manipulation of the acetylide alkoxide dianion generates useful bis-propargyl tertiary alcohols. The design and synthesis of probes for nicotinic acetylcholine receptors (nAChR) affords possible leads for pesticides. MLA also has potential in probing neuronal nAChR which are implicated in some mechanisms of neurodegeneration.

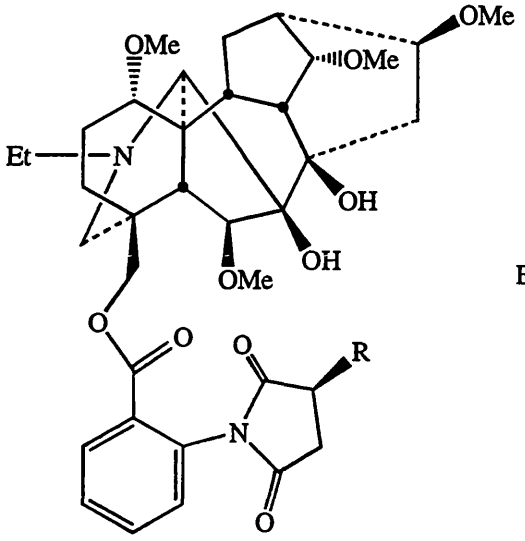
Methyllycaconitine (MLA) **1** is a hexacyclic norditerpenoid alkaloid which occurs in many *Delphinium* species as well as in *Consolida ambigua* and *Inula royaleana* (*1-4*). These plants, especially the *Delphinium* species, are known to be toxic to mammals and to a wide variety of insect species (*5-9*). The insecticidal property of *D. staphisagria* which contains related norditerpenoids, but possibly not MLA, has long been exploited as a herbal treatment for head lice infestations, first reported by Pliny the Elder (*5,9,10*). Each year, ingestion of various wild *Delphinium* spp. is one of the major causes of death for significant numbers of grazing cattle across North America (*7,8*). The toxicity of *Delphinium* plants is in part due to neuromuscular blockade, but a contribution also comes from MLA which acts as a competitive antagonist at insect and mammalian neuronal nicotinic acetylcholine receptors (nAChR) (*11-13*). Thus, MLA has use as a potent, selective ligand for molecular studies of neuronal nAChR implicated in neurodegeneration, but MLA is more potent at neuronal insect nAChR than at the mammalian neuromuscular junction and has potential as a lead compound for the rational design of insecticides acting at nicotine binding sites (*9,14*). We are continuing our structure-activity relationship (SAR) studies of diverse insect and mammalian nAChR (*15*). In this *Chapter*, we report our concise synthetic routes to substituted [3.3.1]bicyclic analogs of MLA, templates for the design of selective molecular probes, in order to investigate the structure and function of nAChR.

MLA Isolation and Characterization

MLA was first isolated and identified in 1938 by Manske (*16*) from the aerial parts of *D. brownii.* Goodson determined (*17*) the structure of MLA **1** in the 1940s, and we unambiguously confirmed that the 2-methylsuccinimidobenzoate ester contains an *S*-stereocenter (*18*). Benn (*19,20*), Pelletier (*1,2,21,22*) and their independent research groups have worked extensively on the isolation of alkaloids from *Delphinium* and other plant species. Recent research work in this area of norditerpenoid alkaloid phytochemistry has been comprehensively reviewed by Yunusov (*3,4*). In addition to our studies (*15,18,23-30*), other research groups who have recently reported efficient syntheses of MLA analogs include those of Kraus (*31-33*) and Whiting (*34*).

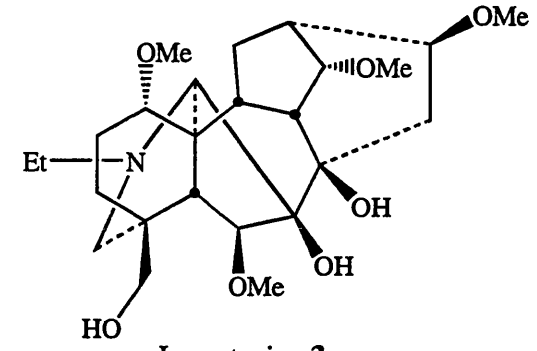
Nicotinic Receptors and MLA Neurotoxicity

Jennings, Brown and co-workers (*5,6*) first investigated the insecticidal properties of MLA. The insect and mammalian toxicity are a result of MLA **1** acting as a competitive nAChR antagonist. Substituted hexacyclic norditerpenoid alkaloids are the most potent, non-proteinaceous antagonists at nAChR and MLA **1** binds to rat α7-type (α-bungarotoxin sensitive nAChR) with a K_i in the nanomolar region. The closely related norditerpenoid alkaloid lacking the angular methyl group on the succinimide, lycaconitine (LA) **2**, has also been isolated from *Delphinium.* Compared to MLA **1**, LA **2** displays 5-fold reduced binding at nAChR (*35*). Invertebrate and mammalian nAChR have a multisubunit protein structure and are pentameric. There are many different subtypes of nAChR due to the structural heterogeneity of the subunits (e.g. vertebrate α1-9, β1-4, and also insects subunits) (*36*). MLA competes with ligand binding to nAChR in flyheads, locust ganglia, Manduca (tobacco hornworm) (*37*) and cockroach motorneurone with K_i values in the nanomolar range (and at mammalian receptors containing α7 and chick α8 subunits), whereas there is a significantly lower affinity at vertebrate neuromuscular junction nAChR (α1 subtype with nAChR stoichiometry $\alpha 1_2\beta 1\gamma\delta$) (*13*). MLA is therefore an attractive lead compound for the design of insecticides. Furthermore, MLA shows a significantly greater affinity (10,000-fold) for nAChR than that displayed by the parent alcohol lycoctonine **3**. Following SAR comparisons between natural products MLA **1**, LA **2**, and lycoctonine **3**, we conclude that the 2-methylsuccinimidobenzoate ester function is significant for efficient nAChR antagonism within this series of small molecules. Our related studies with the *Aconitum* hexacyclic norditerpenoid alkaloid aconitine **4** confirm this conclusion (*28*). Aconitine **4** is a potent, well-characterized neurotoxin which has essentially no activity at nAChR and acts by maintaining voltage-sensitive sodium channels in an open conformation. In order to test the above hypothesis, we have designed and prepared an MLA-aconitine hybrid, the 2-*S*-methylsuccinimido-benzoate ester of 3-deoxy-18-*O*-demethylaconitine **5**. This semi-synthetic alkaloid was essentially equipotent with MLA **1** and lacked biological activity at voltage-sensitive sodium channels (*28*). Therefore, such an ester functional group appears to be important for nAChR antagonism (*15,25,28*).

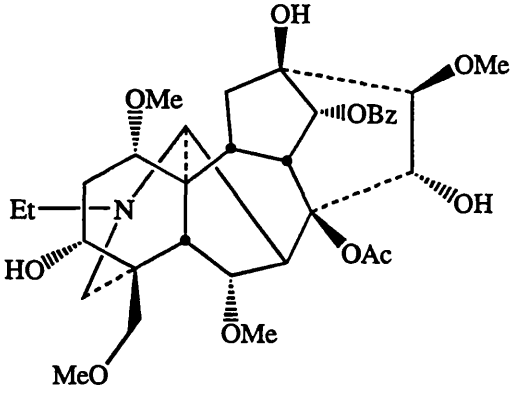


R = Me Methyllycaconitine (MLA) **1**

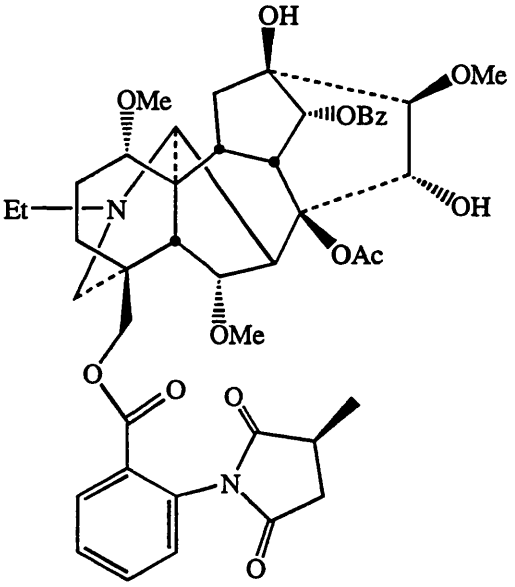
R = H Lycaconitine (LA) **2**



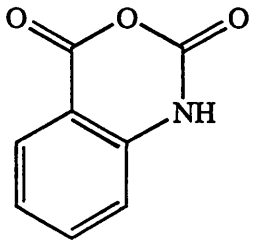
Lycoctonine **3**



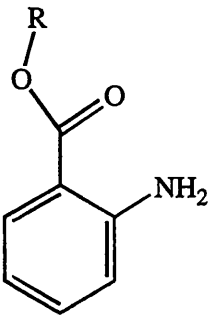
Aconitine **4**



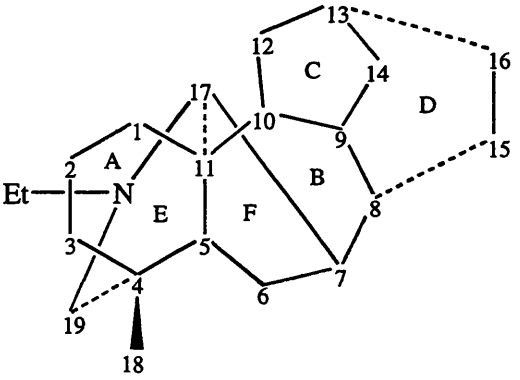
3-Deoxy-18-*O*-demethylaconitine-MLA-hybrid **5**



Isatoic anhydride **6**



Anthranilate ester **7**



Norditerpenoid labelling **8**

Design of [3.3.1]Bicyclic Analogs of MLA

Practical quantities of alcohols that mimic the AE-bicycle of MLA are therefore required for the next phase of our SAR program. These sterically hindered, neopentyl-like alcohols will be esterified in order to introduce the important 2-methylsuccinimidobenzoate moiety. We can achieve this esterification by reaction of an alcohol (ROH) with isatoic anhydride **6**, under basic catalysis, to afford the desired anthranilate ester **7** together with the liberation of an equivalent of carbon dioxide and no detectable formation of the corresponding isatoate (carbamate functional group). In the penultimate step in the preparation of our MLA analogs, this neopentyl alcohol esterification will need to be performed in the presence of secondary and sometimes tertiary alcohols with regiochemical control. Based upon literature precedent (*38*), we have established a practical protocol for this esterification (*25*). Neopentyl alcohols were esterified in good yield (~60 %) in this reaction, and in significantly better yields than secondary alcohols; not unexpectedly, tertiary alcohols did not react.

We decided to prepare the required AE-bicycles (see: **8**) by a double Mannich reaction between a cyclic β-ketoester, formaldehyde (two equivalents), and ethyl-amine. In order to add the desired carbon-chain substituents (incorporating C11-C10 of MLA) to these small molecule MLA analogs, a way to substitute efficiently at the γ-position of ethyl cyclohexan-2-onecarboxylate was required. The alkylation of a β-ketoester is relatively easy to achieve at the α-position as has been shown by the extensive use of substituted ethyl acetoacetates in synthesis. In the early 1970s, Huckin and Weiler (*39,40*) showed that ethyl acetoacetate could be alkylated at the γ-position by making a dianion of the β-ketoester using NaH followed by n-butyl lithium as the bases. This is an extension and an improvement to the work of Hauser and Harris (*41*) in the 1950s who employed two equivalents of sodium amide as the base in liquid ammonia. Dianion formation in effect prevents any alkylation at the α-carbon atom making the process regioselective, due to preferential alkylation of the more reactive, least stabilized enolate under kinetic control. If this strategy is successful, dianion alkylation followed by double Mannich reaction will allow access to AE-bicycles further substituted with rings C or D **8**. Appropriately chosen carbon chains will allow subsequent transformations in order to introduce oxygenation at patterns which mimic those found in MLA. Thus, we can incorporate oxygen substituents at C6, C7, C8, C14, and C16 (see: **8**) individually or in selected patterns. Such a flexible approach is highly desirable for SAR studies at selected nAChR.

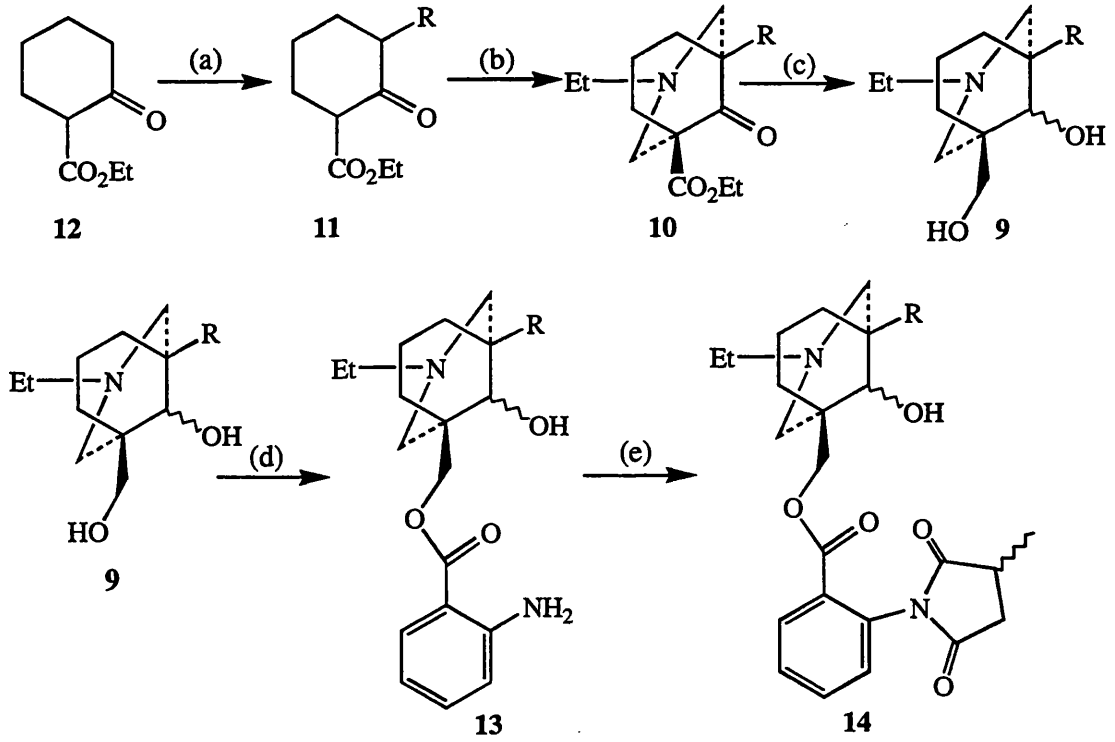
Preparation of C11-C10 Substituted Analogs

Therefore, we wanted to prepare bicyclic diols represented as **9**. Disconnection to piperidinone **10** and from thence to substituted β-ketoester **11**, gave the requirement for regioselective alkylation at the γ-position of cyclic β-ketoester **12**. The forward synthesis is in Scheme 1. Ethyl cyclohexan-2-onecarboxylate (Aldrich) **12** was alkylated with a range of alkyl halides (allyl, benzyl, cyclohexylmethyl, and cyclo-pentyl bromides and n-butyl and n-pentyl iodides). Whilst it may be assumed that both the ethyl ester and the newly introduced carbon chain prefer to occupy equatorial

conformations (around a cyclohexanone chair), nevertheless, there was always a significant proportion of the corresponding enol tautomer present. This cyclohexenol was detected by ^{1}H NMR spectroscopy in $CDCl_3$, the integrals of the acidic α-proton (methine) and the enol signal combining for one proton. Even with the addition of a carbon chain (or ring) at γ-position in the starting material, this enolization contributes to the similarity in polarity of the starting material and the alkylated product. Although less likely, the ethyl ester could also be in an axial orientation after protonation of the enol tautomer from the less favored face. This tautomerization process will give rise to mixtures of diastereoisomers, rather than to an enantiomeric pair. The dianions were not reactive under the conditions initially investigated (THF as the solvent with the anions formed at -78 °C and then the reaction mixture warmed to room temperature after the electrophile had been added). We thought that this lack of nucleophilicity might be due to a solvent effect (THF solvating the dianion). Therefore, we investigated the use of a co-solvent to increase the reactivity. Following literature precedent (*42*), we used a dimethylpyrimidone urea (1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone, DMPU) as co-solvent and we established that a 30 % mixture of DMPU in THF (v/v) both increased the reaction rate and improved the alkylation yields. Another way to increase the reactivity and rate of reaction is to use a concentrated reaction mixture, but with simple alkyl halides the use of DMPU as a co-solvent is an efficient protocol. Yields were further improved by carefully controlling the reaction conditions, the temperature was only allowed to rise to 0 °C rather than to room temperature.

Although the dianion alkylation can be performed in good yield (typically 50-80 %), it was neither practical nor essential to separate product **11** from unreacted starting materials at this stage, in part due to enolization modulating the polarity. An immediate double Mannich reaction on the alkylated β-ketoester afforded the desired piperidinone **10**. We were able to effect an efficient separation of this product from the unsubstituted [3.3.1]bicycle by flash silica gel chromatography. Therefore, it was expedient to take the ethereal extracts from the dianion alkylation directly on to the double Mannich reaction, utilizing (in effect) an excess of reagents based upon an equivalent of aqueous ethylamine pretreated with two equivalents of formalin (37 % aqueous formaldehyde) relative to the starting β-ketoester. When R = H (Scheme 1), the unsubstituted case, the double Mannich reaction proceeded in ~40 % yield. When R = alkyl, the yields were always equal to or greater than the unsubstituted case (40-60 % over two steps). The possibility of diastereoisomeric pairs (previously alluded to) was not a problem, as the double Mannich reaction is under equilibrium conditions (acetic acid catalysis) (*43*). The newly formed carbon-carbon bonds, by alkylation of the iminium salt formed *in situ*, firstly intermolecular and then intramolecular alkylations, must both be axial relative to the cyclohexanone, if the piperidinone ring is to be closed. Thus, although the intermolecular Mannich reaction will proceed initially from either face of the cyclic β-ketoester, only those conformers with a newly formed axial aminomethyl substituent can form the piperidinone, the most stable product. Therefore, given that (in the desired product) these aminomethyl substituents must both be axial, the quaternary carbon atoms in **10** must have *R,S*- or *S,R*-chirality. Reduction of the ketone and ester functional groups ($LiAlH_4$, Et_2O, typically 65 %) afforded the corresponding diols **9**.

As piperidinones **10** have been formed as an *R,S/S,R*-enantiomeric pair, the $LiAlH_4$ reduction gives the opportunity to form diastereoisomers with the generation of a new chiral center. The newly formed secondary alcohol **9** was typically isolated (R = n-pentyl) as a 9:1 mixture of diastereoisomers (relative to the *R,R/S,S* pair of enantiomers whose priority follows from ester reduction). That the hydride ion has probably adopted an equatorial position is possibly due to $LiAlH_4$ coordination to the basic nitrogen atom (tertiary amine) with corresponding directing effects. Possibly, there is a less hindered path for attack generating an axial hydroxyl functional group (axial relative to the cyclohexane, but of course equatorial relative to the piperidine in the [3.3.1]bicycle). Diol **9** was then esterified with isatoic anhydride **6**, using *N,N*-dimethylaminopyridine as the nucleophilic base in hot (70 °C) DMF to afford anthranilate ester **13** (~60 %). Treatment of this series of aniline esters **13** with methylsuccinic anhydride afforded the corresponding half-acid amides which were cyclized *in situ* with 1,1'-carbonyldiimidazole as the dehydrating agent to give the desired 2-methylsuccinimidobenzoates **14**.

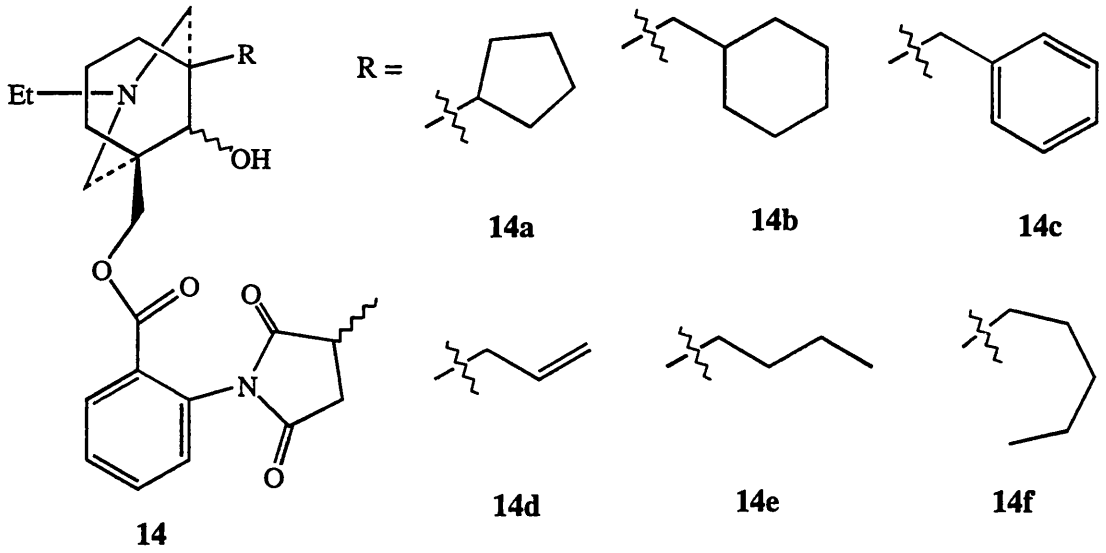


12 11 10 9 9 13 14

SCHEME 1

(a) NaH, nBuLi, RX, 30 % DMPU/THF (b) 2 CH_2O, $EtNH_2$, EtOH, AcOH
(c) $LiAlH_4$, Et_2O (d) isatoic anhydride **6**, DMAP, DMF
(e) methylsuccinic anhydride, CDI, DCM

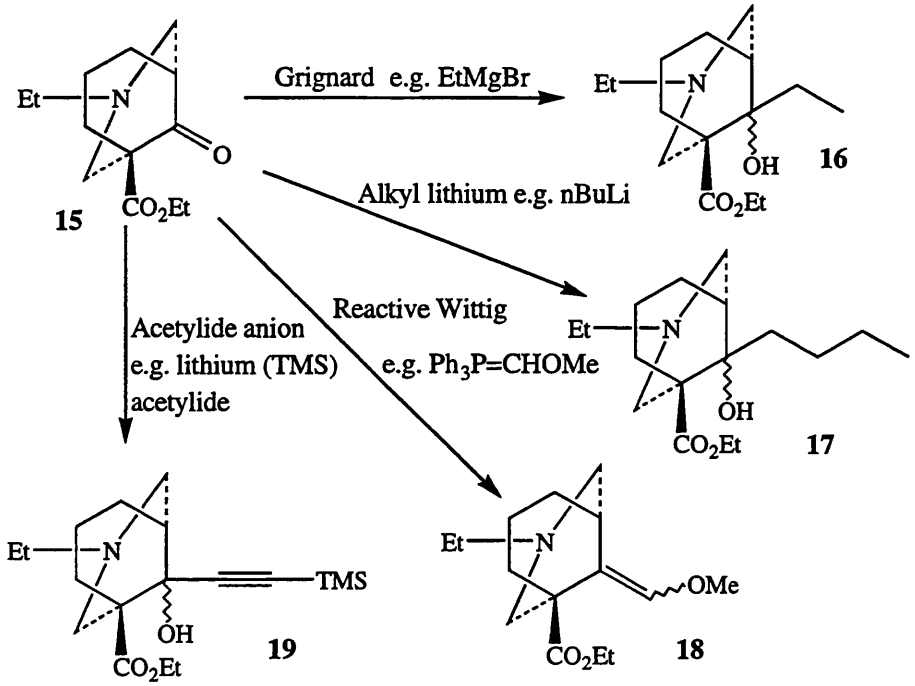
This reaction proceeded in good yield (~65 %) and the AE-bicyclic analogs containing a five-membered ring **14a**, mimicking ring C of MLA **1**, a six-membered ring D mimic **14b**, the corresponding planar aromatic analog **14c**, allyl **14d**, n-butyl **14e**, and n-pentyl **14f** analogs were prepared by this concise route.



Substituted AE-bicyclic analogs **14a-14f** showed no significant insecticidal activity when tested on a range of pest species. They also displayed low affinity for insect brain nAChR in a competition assay, displacing iodo-α-bungarotoxin (*15*). In this assay, where MLA **1** achieved 100 % displacement of [^{125}I]α-bungarotoxin at 0.1 ppm, analogs **14a-14f** reached only 1-20 % at 1 ppm and 20-53 % at 10 ppm.

Preparation of C5-C6 Substituted Analogs

[3.3.1]AE-Bicyclic piperidinone **15** is easily prepared by the double Mannich strategy outlined above. Using this hindered ketone **15**, we have designed routes to take our SAR studies further by preparing C5-C6 containing MLA analogs.

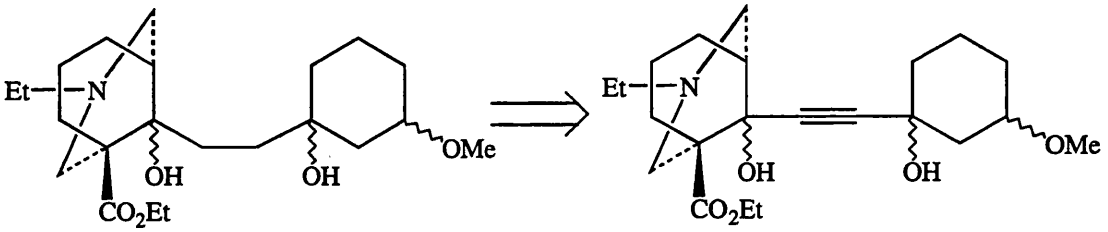


With this synthetic strategy, we can investigate the significance of carbon substituents around ring B and of linking from the AE-bicycle to ring D through C6 and C7. We can now selectively introduce each of the carbon atoms in MLA and regioselectively vary the substitution pattern. The contribution of these substituents

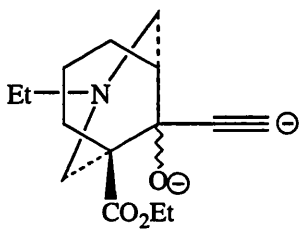
to nAChR affinity, within a defined conformational space, can therefore be assessed. Treatment of piperidinone **15** with a Grignard reagent (*44,45*) regioselectively gave tertiary alcohol **16**. Reaction of **15** with an alkyl lithium salt (*46*) afforded tertiary alcohol **17**. Wittig reaction of **15** was only practical with reactive (and small) ylids, yielding enol ether **18**. Stabilized ylids did not react under typical Horner-Emmons conditions. A one-carbon homologation of ketone **15** to enol ether **18** (*47*) is interesting, but of somewhat limited utility. The nucleophilic addition of an acetylide anion (e.g. lithium TMS-acetylide) to ketone **15** afforded propargyl alcohol **19** in good yield (*48-51*). The functionality present in this C5-C6 substituted analog allows us to design practical entries to highly oxygenated, functionalized probes for SAR studies.

Acetylide Alkoxide Dianion Strategy

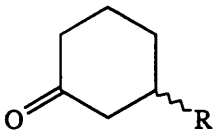
One such potentially important entry for the design of molecular probes is the highly oxygenated carbon framework containing the three 6-membered MLA rings A, D, and E **20**. We rationalized that such a molecule could be prepared from the corresponding alkyne **21** by reduction (*52,53*). Using acetylenic nucleophiles (*48-51*), we can incorporate C6 and C7 (norditerpenoid numbering) introducing appropriately positioned functional groups. One illustration of the versatility of this strategy is that partial reduction of this alkyne functional group **21** will allow (enantioselective) epoxidation of the resultant allylic alcohol. Vicinal-diol synthesis and potentially regiochemically controlled methyl ether formation can also be designed. Such changes in oxygenation pattern around C6-C7-C8 closely mimic some of the important differences between *Delphinium* (e.g. LA **2** and lycoctonine **3**) and *Aconitium* (e.g. aconitine **4**) alkaloids. Therefore, a practical route to alkyne **21** was devised.



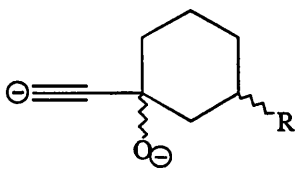
20 **21**



22



23a R = H
23b R = OMe

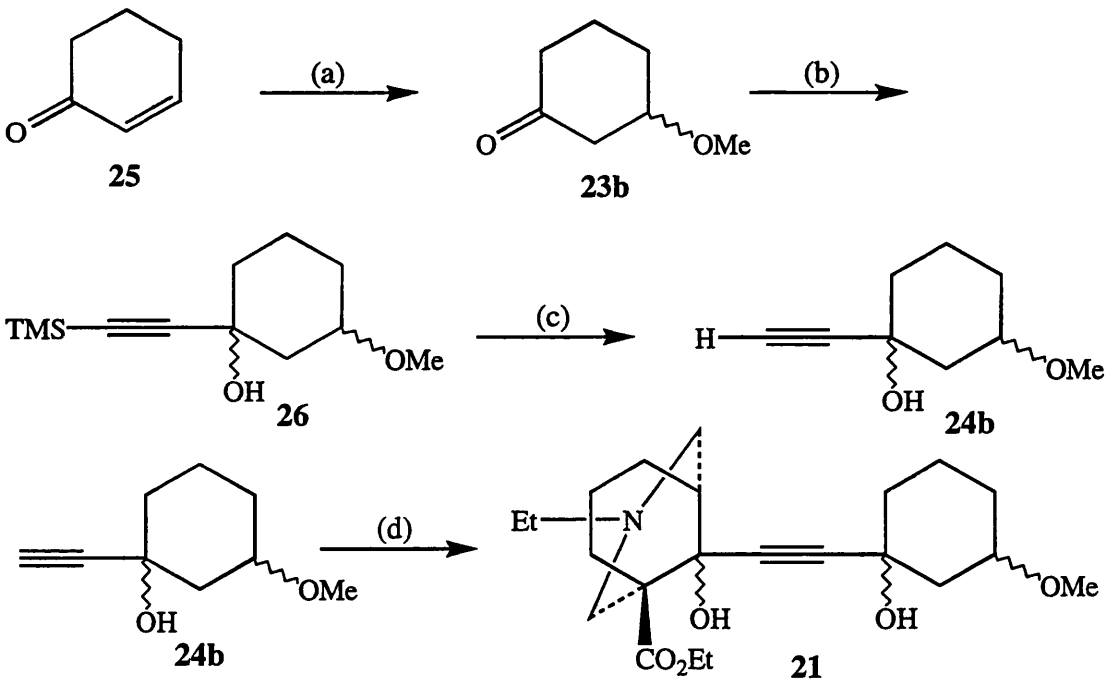


24a R = H
24b R = OMe

Acetylide alkoxide dianion **22** was prepared from TMS-acetylene, but reaction with cyclohexanone **23a** gave only a poor yield (~5 %) of the desired product. Reaction of basic dianion **22** with 3-methoxycyclohexanone **23b** gave only cyclohex-2-enone by elimination of methanol. The alternative order of C-C bond formation, forming C5-C6 after C7-C8, was more rewarding. Acetylide alkoxide dianions prepared by reaction of TMS-acetylene with cyclohexanone **24a**, and from 3-methoxycyclohexanone **24b**, did react in the expected manner with bicyclic β-ketoester **15**.

Preparation of C14/C16-Methoxy-Substituted Analogs

Cyclohex-2-enone (Aldrich) **25** was reacted with methanol under acid catalysis to afford an enantiomeric pair of methyl ethers **23b** (Scheme 2) where the 3-methoxy functional group was presumed to prefer an axial orientation (following the literature precedent of axial cyanide addition to a cyclohex-2-enone). The acetylide anion was prepared from TMS-acetylene, using n-BuLi in THF at 0 °C, and this reacted smoothly with ketone **23b** (with no detectable elimination of methanol to give cyclohex-2-enone in this case, *vide supra*). The diastereoisomeric mixture of propargyl alcohols **26** formed was not purified, but was efficiently deprotected under basic conditions using aqueous sodium hydroxide in methanol (95 %) to afford key acetylenic alcohol **24b** which incorporates the (C14/C16)-methoxy-substituted cyclohexane ring D of MLA **1**.

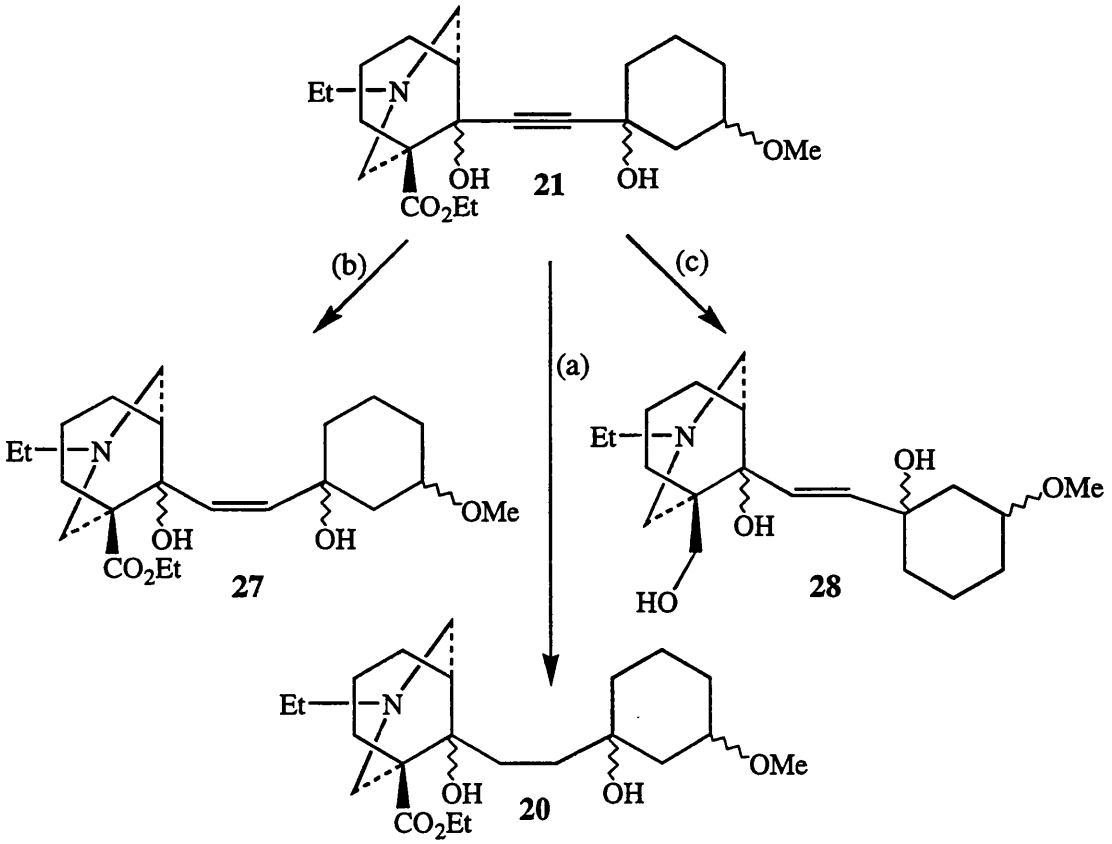


SCHEME 2

(a) MeOH, H^+ (b) TMS-acetylene, nBuLi, THF (c) NaOH, MeOH

(d) 2.4 nBuLi, bicyclic ketoester **15**, THF

Treatment of propargyl alcohol **24b** with 2.4 equivalents of n-BuLi gave the corresponding dianion which reacted (THF, 0 °C) with bicyclic β-ketoester **15** at the more electrophilic cyclic ketone functional group, in preference to the ethyl ester. After silica gel chromatography, bis-tertiary alcohol **21** was isolated as a colorless oil (~60 %). Bis-tertiary alcohol **21** is an advanced synthetic intermediate for the design and preparation of small molecules which will be useful as nAChR probes. This compound contains all of the carbon framework of MLA norditerpenoid rings A, D, and E, an appropriately located *N*-ethylated tertiary amine, tertiary alcohols at C5 (unrequired for the natural product series) and C8 (norditerpenoid numbering), and a C14/C16-methoxy substituent. Complete reduction (Scheme 3) of the C6-C7 alkyne functional group **21** (hydrogenation, 10 % Pd/C, EtOH, 15 °C, 60 %) gave alkane diol **20** as a colorless oil. Partial reduction of this alkyne **21**, in the presence of Lindlar's catalyst (*53*) or better with Pd/C poisoned with 10 % pyridine in ethanol (*52*), gave *Z*-alkene **27** as a colorless oil. These compounds now require functional group interconversion (ethyl ester into neopentyl-like alcohol) for final conversion into anthranilate esters. We were also able to take advantage of the propargylic alcohol functionality in **21** which was reduced with $LiAlH_4$ (*53*), with useful concomitant reduction of the ethyl ester, to afford *E*-alkene triol **28** (40 %). Thus, concise routes to synthetic AE-bicyclic analogs of MLA have been designed and developed.



SCHEME 3

(a) H_2, Pd/C, EtOH (b) H_2, Lindlar's catalyst, EtOH (c) $LiAlH_4$, Et_2O

Acknowledgments

We wish to acknowledge financial support from Zeneca Agrochemicals and EPSRC through the CASE award scheme (WJT), Fondation pour la Recherche Médicale (GG), and The Wellcome Trust (Project Grants 036214 and 045023).

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Synthesis of C5-Substituted AE-Bicyclic Analogues of Lycoctonine, Inuline and Methyllycaconitine

Géraldine Grangier[1], William J. Trigg[1], Terence Lewis[2], Michael G. Rowan[1], Barry V. L. Potter[1] and Ian S. Blagbrough[1*]

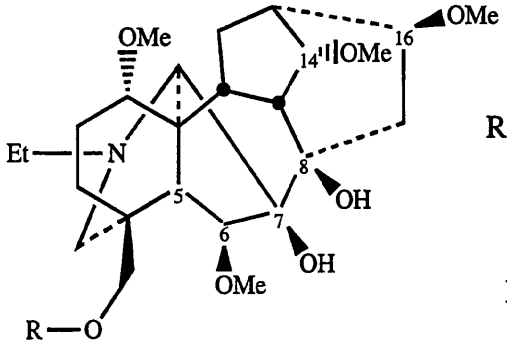
[1]Department of Medicinal Chemistry, School of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY,

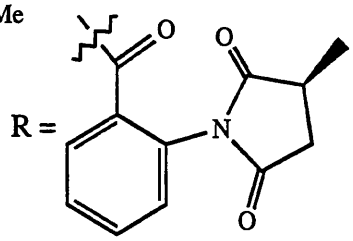
[2]Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell RG12 6EY, U.K.

Abstract: We have prepared C5-substituted AE-bicyclic analogues of norditerpenoid alkaloids lycoctonine, inuline and methyllycaconitine via an acetylide anion addition strategy. Using two acetylide anions, we have regioselectively linked two cyclic ketones to acetylene.

Methyllycaconitine (MLA) **1** is a hexacyclic norditerpenoid alkaloid which occurs in many *Delphinium* species as well as in *Consolida ambigua* and *Inula royaleana.*[1] MLA **1** is the 2-(*S*)-methylsuccinimido-benzoate ester of neopentyl-like alcohol lycoctonine **2**.[2] These plants, especially the *Delphinium* species, are known to be toxic to mammals[3,4] and to a wide variety of insect species.[5,6] The insecticidal property of MLA (and of *D. staphisagria* extracts which contain related norditerpenoids, but possibly not MLA) has long been exploited as a herbal treatment for head lice infestations, first reported by Pliny the Elder.[7,8] MLA has use as a potent, selective ligand for molecular studies of neuronal nAChR implicated in neurodegeneration,[9] and potential as a lead compound for the rational design of insecticides acting at nicotine binding sites.[6,10] Previous work on the synthesis of the carbon skeleton of these hexacyclic alkaloids of the lycoctonine family includes the synthesis of an AEF-tricycle by Whiting[11,12] and co-workers, an AEBD-tetracycle by Kraus *et al.*[13] and the synthesis of the BCD- and ABCD-carbocycles of the C_{19} norditerpenoid alkaloid skeleton by van der Baan and co-workers.[14,15]

In this *Letter,* we report the syntheses of substituted AE-bicyclic analogues of lycoctonine **2**, inuline **3** and MLA **1** involving a double acetylide addition[16,17] linking a monocyclic ketone (**13** or **14**) to bicyclic ketone **6**.[18] We chose substituted bicyclic triol **4** as our target. Ketone **6** is formed via a double Mannich[19] reaction which yields an enantiomeric mixture of products as both new chiral centres must have new C-C bonds generated with axial stereochemistry (relative to the cyclohexanone) in order to close the piperidine ring. On addition of a nucleophile to ketone **6**, a new chiral centre is generated yielding a mixture of diastereoisomers. β-Ketoester **6** reacts regioselectively with reactive Wittig reagents to produce compounds such as enol ether **7**.[18,20] However, attempts to react this ketone with larger and less reactive phosphorus ylids failed, due either to steric hinderance around the ketone or lack of sufficient nucleophilicity in the ylid.

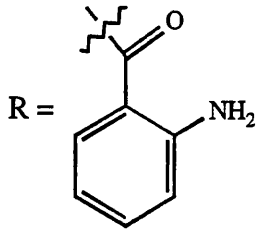




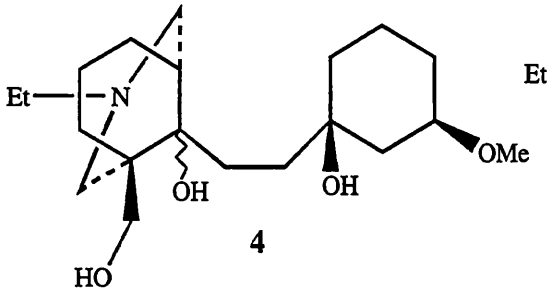
Methyllycaconitine **1**

R = H

Lycoctonine **2**



Inuline **3**



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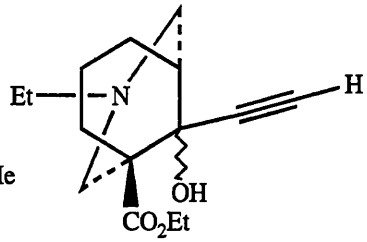

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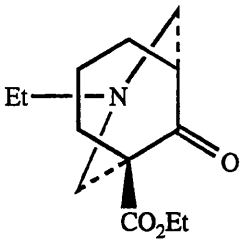
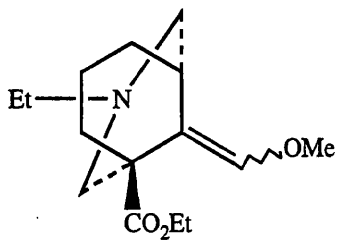


6

β-Ketoester **6** could also be regioselectively attacked with a variety of organometallic nucleophiles. We investigated a series of Grignard[21] and alkyllithium[22] reagents which readily added across this ketone to form tertiary alcohols **8, 9** and **10** in good yields. The use of alkyllithium and to a lesser extent Grignard reagents, however, did not allow the flexibility of substitution required to synthesise our target molecule **4** mimicking three of the six rings found in MLA **1**. By contrast, the use of lithium acetylide[23-29] as the nucleophile allows us to incorporate C6 and C7 (norditerpenoid numbering) as in propargylic alcohols **5**, **11** and **12** (~60 %). However, a dianion derived from **5** (via **12**) reacted with cyclic ketones **13** and **14** to give the desired products **17** and **18** respectively, only in poor yields (<5 %). This strategy allows us to incorporate a methoxy group which is in the correct regiochemical orientation to represent the methoxy at either C14 or C16 of MLA **1** and the tertiary alcohol at C8 is also incorporated in **4**. Hence, an alternative route to alkyne **17** was devised, forming C5-C6 after C7-C8, which allowed efficient synthesis of practical quantities of **17**. Cyclohex-2-enone was reacted with methanol under acid catalysis to afford an enantiomeric pair of methyl ethers **14** where the 3-methoxy functional group was presumed to add with an axial orientation and then equilibrate to an axial-equatorial mixture which Djerassi and co-workers have measured (NMR) as 51:49 respectively.[30,31]



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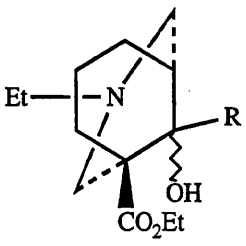
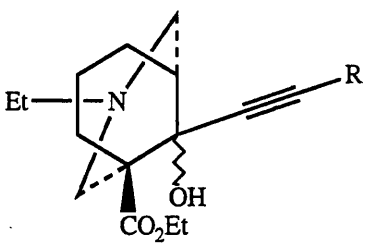
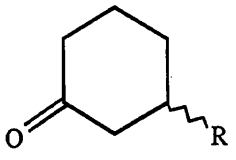


8 R = Me
9 R = Et
10 R = *n*Bu

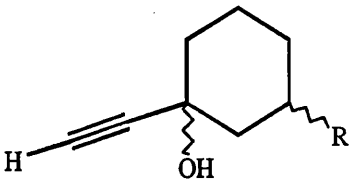


11 R = *n*Pr
12 R = $SiMe_3$

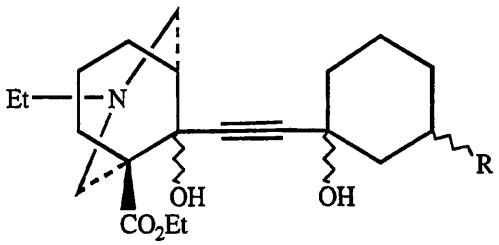
The acetylide anion was prepared from TMS-acetylene, using n-BuLi in THF at -78 °C, and this reacted smoothly with cyclohexanones **13** and **14**. The diastereoisomeric mixture of propargylic alcohols formed was not purified, but was efficiently deprotected under basic conditions (aq. methanolic NaOH) to afford key acetylenic tertiary alcohols **15** and **16** respectively (95 % overall). Purification of **16** gave a 12:1 ratio of diastereoisomers with the favoured isomer having an equatorial 3-methoxy and an equatorial 1-hydroxy substitution pattern (shown by nOe experiments and analysis of coupling constants to be *RS/SR* stereochemistry). The major diastereoisomer **16** (with di-equatorial C-O bonds and axial C-alkyne as required in MLA **1**) was then used for the rest of the synthesis.



13 R = H
14 R = OMe

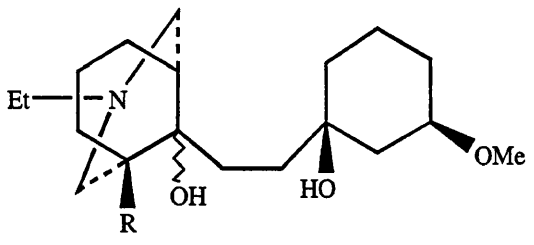


15 R = H
16 R = OMe

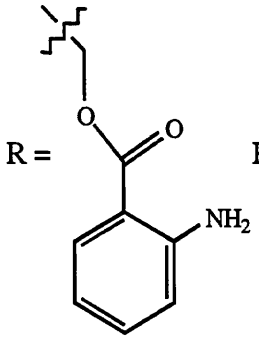


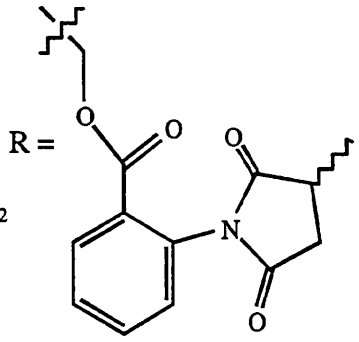
17 R = H
18 R = OMe

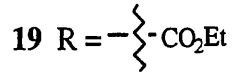
Treatment of propargylic alcohols **15** and **16** with 2.4 equivalents of n-BuLi gave the corresponding dianions which were reacted (THF, 0 °C) with bicyclic β-ketoester **6** at the more electrophilic cyclic ketone functional group, in preference to the ethyl ester. After silica gel chromatography (30% EtOAc-hexane), bis-tertiary alcohols **17** and **18** were isolated as colourless oils (~60 %), co-eluting mixtures of diastereoisomers. Bishydroxy acetylene **18** was then reduced to afford alkane **19** (Pd/C, EtOH, 72 h, 80 %) where the diastereo-isomers were separated into two fractions.[16,17] Reduction of ester **19** with LAH yielded triol **4** (Et_2O, 20 °C, 16 h, 70 %), the desired target, as a colourless viscous oil. Triol **4** was converted into inuline analogue **20** by reaction with isatoic anhydride with base catalysis (DMF, DMAP, 70 °C, 16 h, 65 %). The resulting anthranilate ester **20** was converted into MLA analogue **21** by reaction with methylsuccinic anhydride. Initially this reaction afforded a mixture of half acid amides which was cyclised *in situ* to yield the desired methylsuccinimide **21** by the addition of 1,1'-carbonyldiimidazole as a dehydrating agent.[32]



19, 20, 21





19 R =  **20** **21**

Acknowledgements: We thank EPSRC and Zeneca Agrochemicals (CASE award to WJT) and Fondation pour Recherche Médicale (GG) for financial support. We thank Dr. Martin R. Kipps (Zeneca Agrochemicals) for the detailed NMR spectroscopy and for his interest in these studies.

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Synthesis of Novel Unsaturated AE-Bicyclic Analogues of Lycoctonine, Inuline and Methyllycaconitine: with olefinic J = 13.5 Hz, but still *cis*

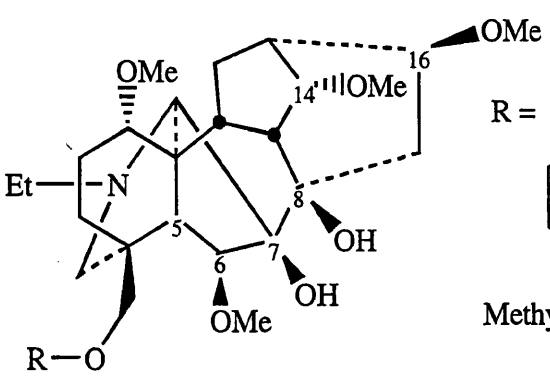
William J. Trigg[1], Géraldine Grangier[1], Terence Lewis[2], Michael G. Rowan[1], Barry V. L. Potter[1] and Ian S. Blagbrough[1*]

[1]Department of Medicinal Chemistry, School of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY,

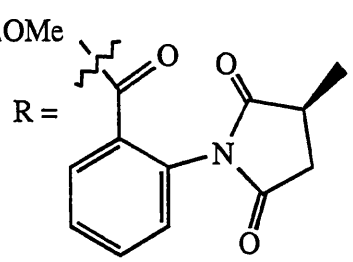
[2]Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell RG12 6EY, U.K.

Abstract: We have synthesised unsaturated AE-bicyclic analogues of lycoctonine class norditerpenoid alkaloids by acetylide addition and regiochemically controlled reductions. Reduction of a substituted propargylic alcohol with hydrogen gas (poisoned Pd catalyst) gave an alkene with vicinal J = 13.5 Hz. This was shown to be of *Z*-geometry by unambiguously preparing the corresponding *E*-alkene (J = 15.4 Hz).

Methyllycaconitine (MLA) **1** is a hexacyclic norditerpenoid alkaloid of the lycoctonine class.[1-3] MLA **1** is the 2-(*S*)-methylsuccinimidobenzoate ester of neopentyl-like alcohol lycoctonine **2**.[4-6] MLA **1** and closely related alkaloids occur in many *Delphinium* species as well as in *Consolida ambigua* and *Inula royaleana*.[6,7] *Delphinium* species are toxic to mammals causing many (economically significant) cattle deaths each year across North American ranges.[8] Furthermore, these norditerpenoid alkaloids are toxic to a wide variety of insect species, and this potentially important insecticidal property of MLA **1** has been investigated by Jennings and co-workers.[9,10] We have used MLA **1** as a selective ligand for molecular studies of neuronal nAChR and for the rational design of insecticides acting at nicotine binding sites.[1-3] We are continuing our structure-activity relationship (SAR) studies of these norditerpenoid alkaloids by synthesising novel C5-substituted unsaturated AE-bicyclic analogues of MLA **1**.[3] Other workers have recently contributed significantly to this research area, achieving controlled syntheses of several of the carbocycles found in the alkaloid skeleton.[11-15]

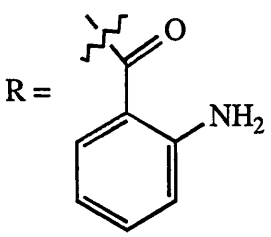


Methyllycaconitine **1**



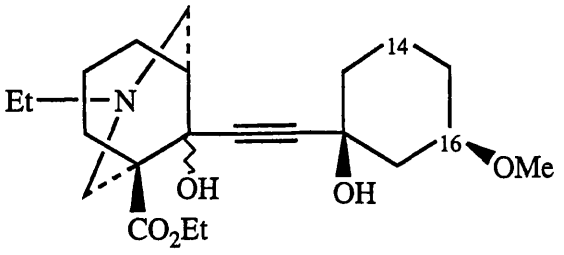
R = H

Lycoctonine **2**

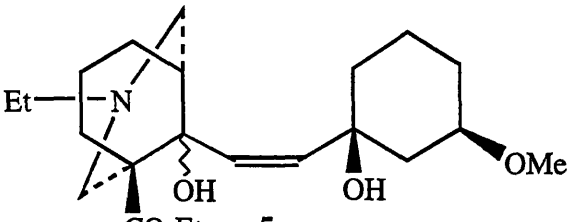


Inuline **3**

In the preceding *Letter*,[3] we reported a route to C5-substituted AE-bicyclic analogues of lycoctonine **2**, inuline **3**, and MLA **1**. Herein, we describe regio- and stereochemically selective reductions of alkyne **4**[3] to unsaturated analogues of lycoctonine **2**, and their subsequent conversion into the corresponding analogues of inuline **3** and MLA **1**. To synthesise *Z*-alkene **5**, alkyne **4** (as a co-eluting mixture of four diastereoisomers with methoxy at C14*R* or C16*R*, both are required in MLA **1**, but only one pair has been shown for clarity)[3] was reduced with hydrogen gas in the presence of a poisoned palladium catalyst. Initially, we employed Lindlar's catalyst (Pd on $CaCO_3$ poisoned with lead, EtOH), but this gave a relatively poor yield (25 %).[16,17] When we used 10 % Pd/C poisoned with 10 % pyridine in EtOH, a higher yield was obtained (50 %).[18] Both reactions were stirred at 20 °C for 72 h (little reaction was detected after 24 h) under an atmosphere of hydrogen. The resulting colourless viscous oil was purified over flash silica gel (1:19 MeOH-DCM) and yielded two homogeneous fractions **5a** and **5b** (each a mixture of two co-eluting diastereoisomers).



4

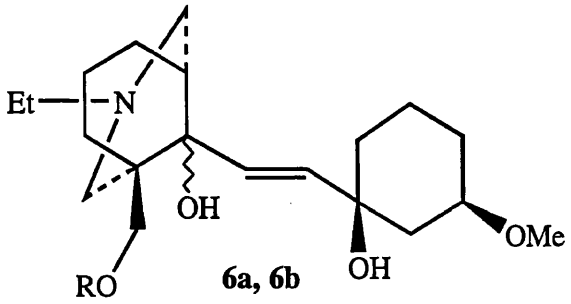


5a

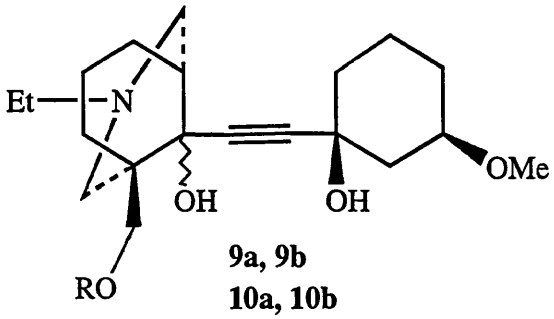
5b

Spectroscopic analysis (^{1}H NMR) of each fraction showed that alkene formation had occurred, but the vicinal (alkenyl) coupling constant was higher (J = 13.5 and 13.6 Hz) than is normally predicted for a *Z*-alkene (0-12 Hz, typically 6-8 Hz).[19-22] As 13.5 Hz is above the typical higher limit for a *Z*-alkene and within the limits (12-18 Hz, typically 14-16 Hz) for an *E*-alkene, there was some doubt about the geometry of this carbon-carbon double bond. Furthermore, the long reaction time (with the alkene in contact with the catalyst) could have allowed isomerism to the *E*-alkene, and there is literature precedent for Lindlar's catalyst mediating the formation of an *E*-alkene.[23] Thus, there was some ambiguity with respect to our assignment of the geometry of this double bond. Therefore, in order to assign this geometry and to prepare (unambiguously) *E*-alkenes for our SAR programme, we took advantage of the ready reduction of propargylic alcohols with $LiAlH_4$ (Et_2O, 18 h, 20 °C) to afford the desired *E*-alkenes,[17,24] allylic alcohols **6a** and **6b** which were purified over flash silica gel (3:17 MeOH-DCM). The key vicinal coupling constants (J = 15.4 and 15.6 Hz) are indicative of *trans*-geometry. That the ester functional group was reduced before the alkyne was also confirmed by the isolation of alkyntriols **12a** and **12b** (combined yield 82 %, ~1:1:1:1). We then converted separated diastereoisomeric esters **5a** and **5b** into the corresponding neopentyl-like alcohols, triols **12a** and **12b** ($LiAlH_4$, THF, 16 h, 20 °C) which were purified over flash silica gel (1:19 MeOH-DCM, ~80 % each diastereoisomeric pair).

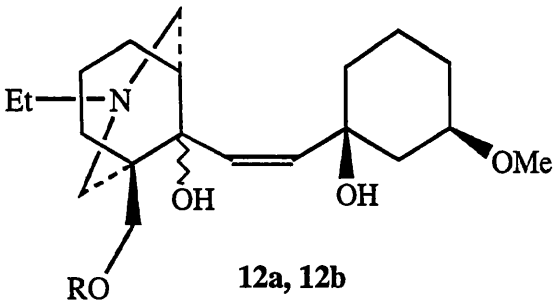
Triols **12a** and **12b** are directly comparable with triols **6a** and **6b**. For the former, vicinal coupling constants, although high (J = 13.5 and 13.6 Hz, comparable with **5a** and **5b**), are indicative of *cis*-geometry with respect to those displayed by the latter (J = 15.4 and 15.6 Hz) and indicative of *trans*-geometry when taken together with the respective synthetic routes. As J depends strongly upon the electronegativity of substituents (decreasing with increasing electronegativity)[19-22] and upon the C.C.H angles of coupled nuclei, and given that J_{trans} is always greater than J_{cis},[19-22] then there must be significant angle strain in **5a** and **5b** and in **12a** and **12b** in comparison with typical *cis*-alkenes. We conclude that J = 13.0-13.6 Hz is within the acceptable range for vicinal coupling across a *cis*-alkene when substituted with two quaternary carbons (tertiary alcohol functional groups). Weyerstahl and co-workers have reported J = 12.5 Hz for certain *cis*-alkenes derived from C_{13}-degraded carotenoids (alkynol-isophorones) in their studies on fragrances.[25,26]



6a, 6b
7a, 7b
8a, 8b



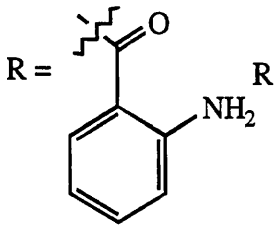
9a, 9b
10a, 10b
11a, 11b



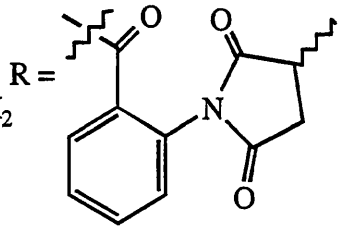
12a, 12b
13a, 13b
14a, 14b

R = H

6, 9, 12



7, 10, 13



8, 11, 14

All six of the above purified neopentyl-like alcohols (**6a**, **6b**, **9a**, **9b**, **12a** and **12b**), analogues of lycoctonine **2**, were converted into anthranilate esters (**7a**, **7b**, **10a**, **10b**, **13a** and **13b** respectively), analogues of inuline **3**, by reaction with isatoic anhydride using 4-dimethylaminopyridine as catalyst (DMF, 16 h, 70 °C, ~50-85 %).[27] Reaction of anthranilate esters (**7a**, **7b**, **10a**, **10b**, **13a** and **13b**) with methylsuccinic anhydride initially yielded half acid amides which were then cyclised to form 2-methylsuccinimidobenzoate esters (**8a**, **8b**, **11a**, **11b**, **14a** and **14b** respectively) *in situ* with 1,1'-carbonyldiimidazole as a dehydrating agent (DCM, 48 h, 20 °C), analogues of MLA **1**.[27] These compounds were purified by flash chromatography over silica gel (1:19 MeOH-DCM) and isolated as colourless viscous oils (~80 %).

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