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SYNTHETIC STUDIES TOWARDS MARINE NATURAL PRODUCTS

A thesis submitted to the
University of Wales
in candidature for the degree of
Philosophiae Doctor

by

Andrew John Thornhill

October 2000

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CONTENTS

	Page
Abstract	i
Abbreviations	ii
CHAPTER 1	
THE GUANIDINIUM GROUP AND ITS BIOLOGICAL ROLE	1
1.1 Introduction	2
1.2 Basicity and hydrogen bonding	2
1.3 Arginine and the biological role	6
1.3.1 Introduction	6
1.3.2 Arginine and its biological role	6
1.3.3 Alkaline phosphatase	8
1.3.4 Prostaglandin H_2 synthase-1	9
1.3.5 Nitric oxide synthase	12
CHAPTER 2	
GUANIDINE CONTAINING NATURAL PRODUCTS	13
2.1 Introduction	14
2.2 Tetrodotoxin and saxitoxin	14
2.3 Anatoxin-a-(S)	16
2.4 Cylindrospermopsin	17
2.4.1 Isolation and structural elucidation	17
2.4.2 Synthetic approaches	19
2.5 Ptilomycalin A and related compounds	32
2.5.1 Isolation and structural elucidation	32
2.5.2 Synthetic approaches	33
2.5.3 The crambescidins	40
2.5.4 Celeromycalin and fromiamycalin	41

2.5.5 Biological activity 42
2.6 The batzelladine alkaloids 43
2.6.1 Introduction 43
2.6.2 Batzelladines A-E 44
2.6.3 Isolation and structural elucidation 44
2.6.4 Synthetic approaches 45
2.6.5 Batzelladines F-I 56
2.6.6 Biological assessment 57

CHAPTER 3

SYNTHETIC APPROACHES TO BATZELLADINE F 59

3.1 Introduction 60
3.2 Synthesis of the left hand portion 60
3.2.1 NMR correlation 64
3.3 Attempted sythesis of the right hand portion 68
3.3.1 Retrosynthesis 68
3.3.2 Synthetic approaches 69
3.3.3 Biological evaluation 73
3.3.4 Conclusions 74

CHAPTER 4

ATTEMPTED SYNTHESIS OF THE ABC RING SYSTEM OF CYLINDROSPERMOPSIN 75

4.1 Introduction 76
4.2 Retrosynthesis 76
4.3 Synthetic approaches to the AB ring system of cylindrospermopsin 77
4.4 Conclusions 95

CHAPTER 5

GLYCOMIMETICS 97

5.1 Introduction 98
5.2 Preparation of simple five membered cyclic guanidines 99
5.3 Preparation of seven membered cyclic guanidines 103
5.4 Preparation of the substituted five membered cyclic guanidines 110
5.5 Preparation of the substituted six membered cyclic guanidines 113
5.6 Overall conclusions 118

CHAPTER 6

BIOLOGICAL EVALUATION OF THE GLYCOMIMETICS 120

6.1 Introduction 121
6.2 Biological evaluation 121

CHAPTER 7

A SYNTHETIC APPROACH TO THE PLAKORTONE SERIES OF NATURAL PRODUCTS 123

7.1 Introduction 124
7.1.1Introduction to the plakortones 124
7.2 Background work on the synthetic analogues 125
7.3 Preparation of a plakortone analogue 126
7.4 Conclusions 129

CHAPTER 8

EXPERIMENTAL 130

CHAPTER 9

REFERENCES 196

ABSTRACT

This thesis describes the work performed developing synthetic approaches to the guanidine containing marine natural products cylindrospermopsin and batzelladine F, and the bicyclic lactone containing compounds, the plakortones.

(i) A reductive guanidine addition-cyclisation reaction was employed to prepare the left hand portion of batzelladine F from a *bis*-α,β-unsaturated ketone which was prepared from succinaldehyde using Wittig methodology.

(ii) Attempts were made to prepare a 6,6-bicyclic analogue of cylindrospermopsin in four steps from 5-hexen-1-ol utilising as the key step a tandem 1,4-addition/epoxide opening methodology. This was found to lead to a 7,6 product *via* a competitive process.

(iii) Intramolecular epoxide opening reactions involving guanidines were also studied providing access to several glycomimetic analogues. These included a simple five and seven membered ring system, together with a more complex substituted five and six membered ring system as well as a dimeric 7,7 system.

(iv) A bicyclic 5,5-furanofuran was prepared *via* the reaction of *n*-butylmagnesium bromide with an α-hydroxy-γ-ketocarboxylic acid which leads to the formation of a tetrasubstituted γ-butyrolactone which in turn was converted to the required system, an analogue of the bicyclic lactones found in the marine natural products, the plakortones.

ABBREVIATIONS

Å	Angstrom
AcCl	acetyl chloride
Ac_2O	acetic anhydride
AcOH	acetic acid
AIDS	acquired immune deficiency syndrome
AMP	adenosine monophosphate
AP	alkaline phosphatase
Arg	arginine
Asp	aspartic acid
α	alpha
β	beta
Bn	benzyl
BOC	*tert*-butoxycarbonyl
B.pt.	boiling point
br	broad
Bu	butyl
BuLi	butyllithium
°C	degrees Celsius
CBz	benzyloxycarbonyl
CI	chemical ionisation
CIDMS	chemically induced dynamic mass spectroscopy
COLOC	correlated spectroscopy for long range coupling
COSEY	correlated spectroscopy
d	doublet
Δ	heat, reflux
δ	chemical shift
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DEPT	distortionless enhancement by polarisation transfer
DIBAL-H	diisobutylaluminium hydride

DIPEA	diisopropylethylamine
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DMF	N,N-dimethylformamide
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
EDCl	1-ethyl-3-[3-(dimethylamino)-propyl]-carbodiimide hydrochloride
equiv.	mole equivalent(s)
Et	ethyl
EtOAc	ethyl acetate
EtOH	ethanol
Et_3N	triethylamine
FABMS	fast-atom bombardment mass spectroscopy
FT-IR	Fourier transform infra-red spectroscopy
γ	gamma
g	gram(s)
Glu	glutamic acid
gp	glycoprotein
h	hour(s)
HCl	hydrochloric acid
HIS	histidine
HIV	human immunodeficiency virus
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum coherence
HOHAHA	homonuclear Hartmann-Hahn spectroscopy
HRFABMS	high resolution fast-atom bombardment mass spectroscopy
HRMS	high resolution mass spectroscopy
HSV	herpes simplex virus
Hz	Hertz
IC_{50}	concentration at which 50% of target cells are inhibited
^{i}Pr	isopropyl
J	coupling constant

JACS	Journal of the American Chemical Society
JOC	Journal of Organic Chemistry
l	litre
M	molar
m	multiplet or medium intensity
*m*CPBA	*meta*-chloroperoxybenzoic acid
Me	methyl
MeOH	methanol
MeOTf	methyl trifluoromethanesulphonate
MIC	minimum inhibitory concentration
min	minute(s)
ml	millilitre(s)
mmHg	millilitres of mercury
mmol	millimole(s)
mol	mole(s)
M.pt.	melting point
MsCl	mesyl chloride
μg/kg	microgram per kilogram
μM	micromolar
$NaCNBH_3$	sodium cyanoborohydride
NaH	sodium hydride
N	normal
NO	nitric oxide
NOS	nitric oxide synthase
NSAID	nonsteroidal anti-inflammatory drugs
ν	wavenumber(s)
NMR	nuclear magnetic resonance
NOESY	correlated nuclear Overhauser effect spectroscopy
PCC	pyridinium chlorochromate
PGH_2	prostaglandin H_2
PGHS-1	prostagladin H_2 synthase-1
Ph	phenyl

PPh_3	triphenylphosphine
ppm	parts per million
PPTS	pyridinium *p*-toluenesulphonate
Pr	propyl
q	quartet
R_f	retention factor
RT	room temperature
sat.	saturated
s	singlet or strong intensity
sec	second(s)
Ser	serine
SNase	staphylococcal nuclease
t	triplet
TBAF	tetra-n-butylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
^{t}Bu	*tert*-butyl
^{t}BuOH	*tert*-butanol
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMSCl	trimethylsilyl chloride
TMSOTf	trimethylsilyl trifluoromethanesulphonate
TOCSY	total correlated spectroscopy
Ts	*para*-toluenesulphonyl
TsCl	*para*-toluenesulphonyl chloride
Tyr	tyrosine
2D NMR	2-dimensional nuclear magnetic resonance
UV	ultra-violet
w	weak intensity

CHAPTER 1

THE GUANIDINIUM GROUP AND ITS BIOLOGICAL ROLE

THE GUANIDINIUM GROUP AND ITS BIOLOGICAL ROLE

1.1 INTRODUCTION

The guanidinium motif is commonly used by proteins and enzymes to recognise and bind anions.[1] This variety of ion pairing patterns coupled with its strong basicity allows the guanidinium moiety to play a key role in ion recognition and catalysis.

1.2 BASICITY AND HYDROGEN BONDING

Guanidine **[1]** is the imine of urea and is one of the most basic neutral nitrogen containing compounds known with a pKa of 13.5 in water,[2] the strength of which is comparable to that of sodium hydroxide. This strong basicity is a result of the stability of the cation after protonation of the imino nitrogen. The positive charge on the guanidinium ion can spread equally between the three nitrogens by virtue of resonance, producing the three resonance hybrids depicted below in Fig. 1.

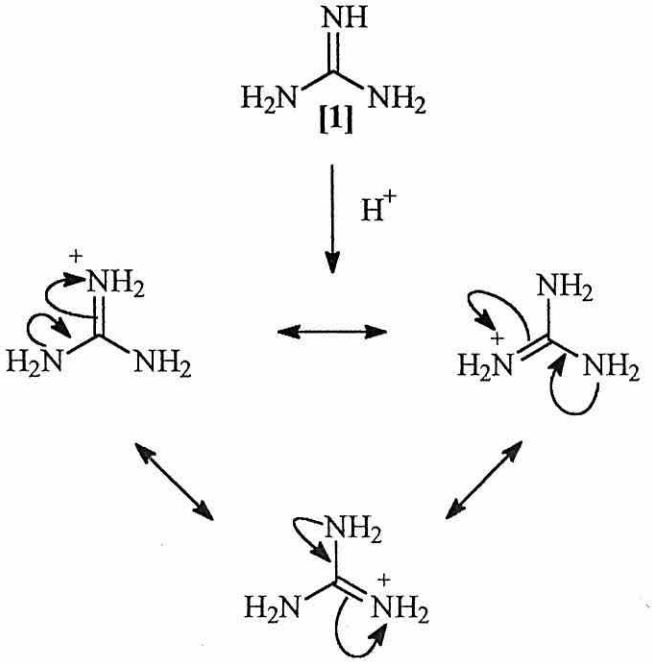


Fig. 1. Guanidine and its protonated resonance hybrids.

In comparison the guanidinium group is a very weak base with a pKa of -11. In aqueous media the guanidinium group is well solvated due to the extensive hydrogen bonding to the solvent. The partial positive charge of the hydrogen bond donors increases their strength for donation to the negative dipole of water[1] and therefore alkyl

substituted guanidines show decreased solvation effects due to the loss of hydrogen bonding sites. The basicity of the substituted guanidines remains high due to the electron donating character of the alkyl groups, this effect is observed in arginine which has a pKa of 12.5 in aqueous media.[3]

In contrast both phenylguanidiniums and acylguanidiniums have lower pKa values than alkylguanidiniums due to the disruption of the canonical forms of the guanidinium ion. Table 1. shows examples of several substituted guanidines and their related pKa values.

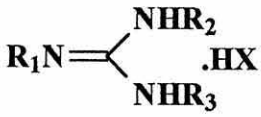
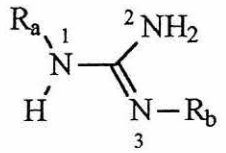
			pKa
R_1	R_2	R_3	
H	H	H	13.6
Me	H	H	13.4
Me	Me	H	13.4
Me	Me	Me	13.9
Ph	H	H	10.77
$CONH_2$	H	H	8.11 ± 0.05
COMe	H	H	8.20 ± 0.05
COPh	H	H	6.98 ± 0.05
CO_2Et	H	H	7.03 ± 0.05
OH	H	H	7.96 ± 0.04

Table 1. pKa values of substituted guanidines.[4]

On comparison of acylguanidiniums and phenylguanidiniums it can be seen that a high degree of amide resonance stabilisation results in acylguanidiniums having lower pKa values than phenylguanidiniums. Moreover substituted phenylguanidines must be considered separately as their pKa values will be dependent upon the nature of the substituent. The presence of an electron donating group will therefore increase the pKa and *vice versa* for electron withdrawing groups.[5]

The dimensions for simple guanidine derivatives[6,7] have been obtained by X-ray crystallographic analysis which has revealed several common features. The C-N single

bond length in alkylguanidines is typically shorter than the usual C-N single bond. The three C-N bonds in the guanidinium group itself are virtually equivalent with an average of 1.33 Å. This is further illustrated in Table 2 with examples of derivatised guanidines, furthermore, the three bond angles (N-C-N) are almost equal at an average of 120°.



Compound		C-N bond lengths (Å)			NCN bond angles (°)		
R_a	R_b	C-N^1	C-N^2	C-N^3	N^1CN^2	N^2CN^3	N^1CN^3
H	NO_2	1.34	1.34	1.35	118	129	112[8]
H	CN	1.333	1.339	1.341	118.7	123.8	117.5[9]
H	$SO_2C_6H_4NH_2$-*p*	1.329	1.334	1.348	118.1	126.0	115.8[10]
H	$OCH_2CH_2CH(NH_3)^+(CO_2)^-$	1.361	1.347	1.302	116.4	125.2	118.4[11]
OH	NO_2	1.391	1.307	1.346	119.3	129.1	111.7[12]

Table 2. Crystallographic dimensions of functionalised guanidines

1.3 ARGININE AND THE BIOLOGICAL ROLE

1.3.1 INTRODUCTION

Many enzymes have incorporated within their active site an arginine residue. It is the guanidine motif within the arginyl residue which plays a vital role in enzymatic activity. In some cases it provides support, in others it aids in the orientation of the guest to the host molecule, the 'Lock and Key' hypothesis. Probably the most common anionic substrates that require arginyl residues for activity are phosphates and carboxylates. This results in the formation of oxoanionic bonds (Fig. 2), which is of great interest to the bio-organic chemist.

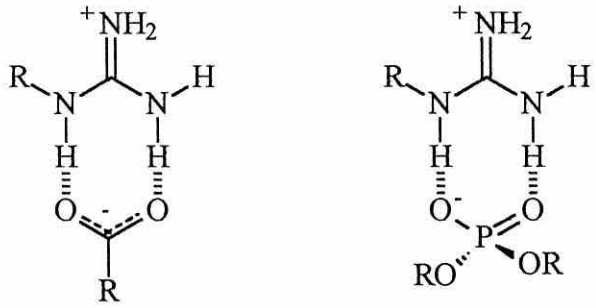


Fig. 2. Oxoanionic bonding with carboxylates and phosphates.

Many examples of arginyl incorporated active sites exist; however, only a few will be discussed within this section. For a more detailed account the reader is advised to read a text by Hannan and Ainsly.[1]

1.3.2 ARGININE AND ITS BIOLOGICAL ROLE

As discussed in section 1.2 the unique structure of the guanidinium ion allows it to form hydrogen bonding patterns which are rare and possibly unique in biological systems. In fact, these properties may account for nature's selection of arginine as one of the twenty naturally occurring amino acids found in proteins.

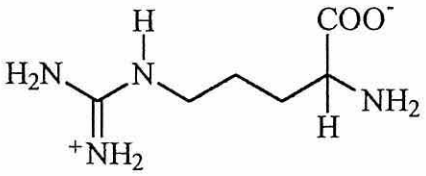


Fig. 3. Arginine in its zwitterionic form.

Arginine (Fig. 3) is one of three amino acids which are positively charged at physiological pH; this ionic charge is provided by protonation of the guanidinium moiety.[3] Nature offers many examples in which arginine is a key component in a biological system, recognition of anionic substrates by enzymes and receptor sites, binding of antibody to antigen[13] and the maintenance of protein conformation.

Enzymes of notable interest containing the arginine residue in the active site include staphylococcal nuclease[14] (Fig. 4), bovine carboxypeptidase A[1] (Fig. 5), transcarbamylases[15] (Fig. 6) (in which in 1980 Marshall and Cohen found Arg 54 and Arg 105 at the carbamyl phosphate binding site of bovine ornithine transcarbamylase and concluded the residues importance for catalysis), alkaline phosphatase[1], prostaglandin H_2 synthase[16] and Nitric oxide synthase oxygenase.[17] The final three are discussed in greater detail.

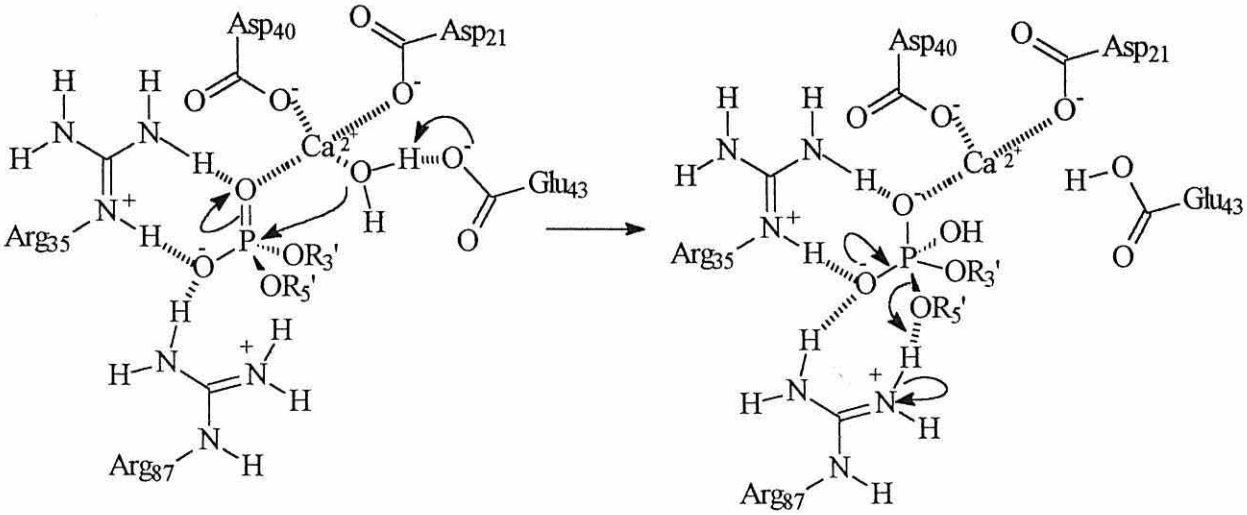


Fig. 4. Current mechanistic view of the initial step in catalysed DNA hydrolysis by SNase.

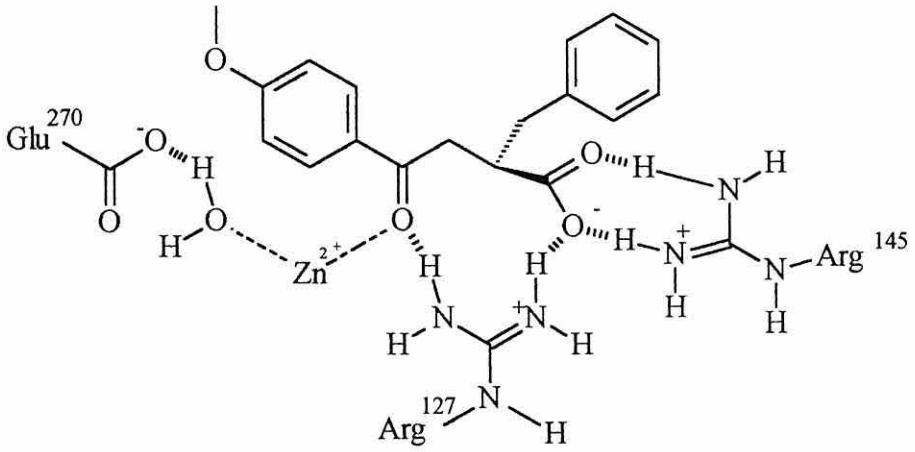


Fig. 5. Structure of bovine carboxypeptidase A bound to (-)-3-(*p*-methoxybenzoyl)-2-benzoylpropanoic acid.

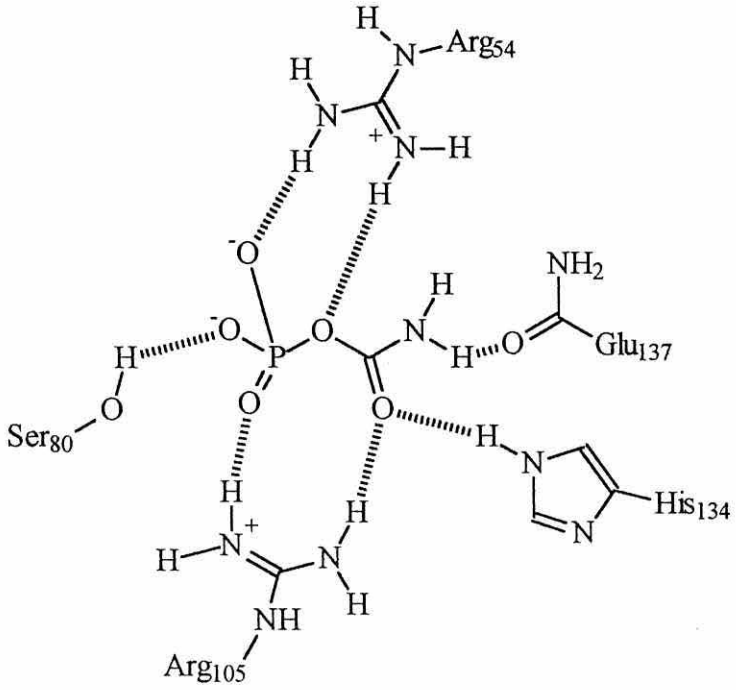


Fig. 6. The active site of carbamyl phosphate in bovine ornithine transcarbamylase.

1.3.3 ALKALINE PHOSPHATASE

Alkaline phosphatase is a dimeric enzyme which contains two Zn^{2+} ions and one Mg^{2+} in each monomer. It is a non-specific phosphomonoesterase that can either produce inorganic phosphate or transfer phosphoryl groups between alcohols.[1] The

important features of the active site (illustrated in Fig. 7) are the amino acids Ser-102 and Arg-166.

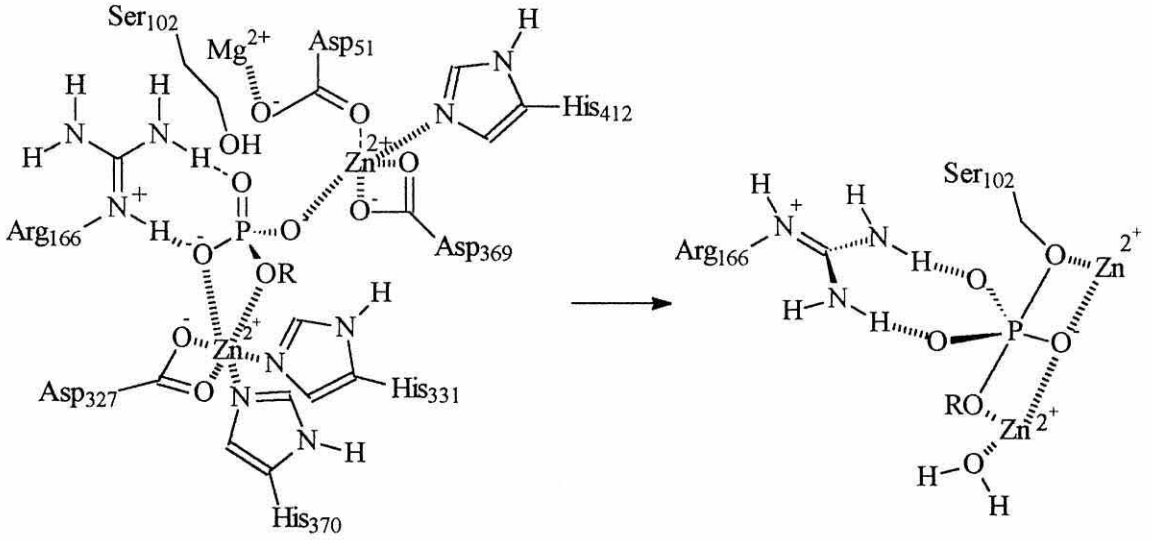


Fig. 7. The coordination sphere of phosphate in alkaline phosphatase.

The current mechanistic interpretation is that the Arg-166 residue aligns the phosphate within the active site correctly, as well as stabilising the charged intermediates and transition state; this coupled with experimental data infers the involvement of a phosphoryl serine intermediate in which the phosphate substrate bridges to both zinc ions, and is linked to the Mg^{2+} ion *via* a water hydrogen bond. This produces a pocket with a 7^{+} charge which provides an extremely electrophilic environment for phosphate binding. Arg-166 is not necessarily involved in catalysis but its presence is beneficial for catalytic activity.[18]

1.3.4 PROSTAGLANDIN H_2 SYNTHASE-1

As prostaglandin biosynthesis has been implicated in the pathophysiology of cardiovascular disease, cancer and inflammatory diseases, the active site of PGHS-1 has been greatly investigated.[19] Non-steroidal anti-inflammatory drugs (NSAID) such as asprin, ibuprofen and indomethacin target PGHS and affect its activity. Prostaglandin belongs to the family of eicosanoids, as do the thromboxanes and leukotrienes which are formed from the essential fatty acid arachidonic acid.[16] PGHS-1 catalyses a key step in the production of prostaglandins and thromboxanes from arachidonic acid. This

enzyme exhibits both cyclooxygenase activity and peroxidase activity. Both sites are functionally and spatially distinct but they do require the presence of a single heme unit.[20]

PGHS has two functions, the initial cyclooxygenase reaction which is the target for NSAID's, and secondly, the conversion of the acid to PGG_2 while the peroxidase active site converts PGG_2 to PGH_2. Examination of the crystal structure of PGHS-1 with S-flurbiprofen (Fig. 8) suggests that Arg-120 has three main functions. It provides a ligand with which the carboxylate of arachidonic acid can bind, forms an important salt bridge with Glu-524, and, as Arg-120 is found near the mouth of the channel opposite the bulky phenyl group of Tyr-355, it may determine the stereochemistry of PGHS-1.

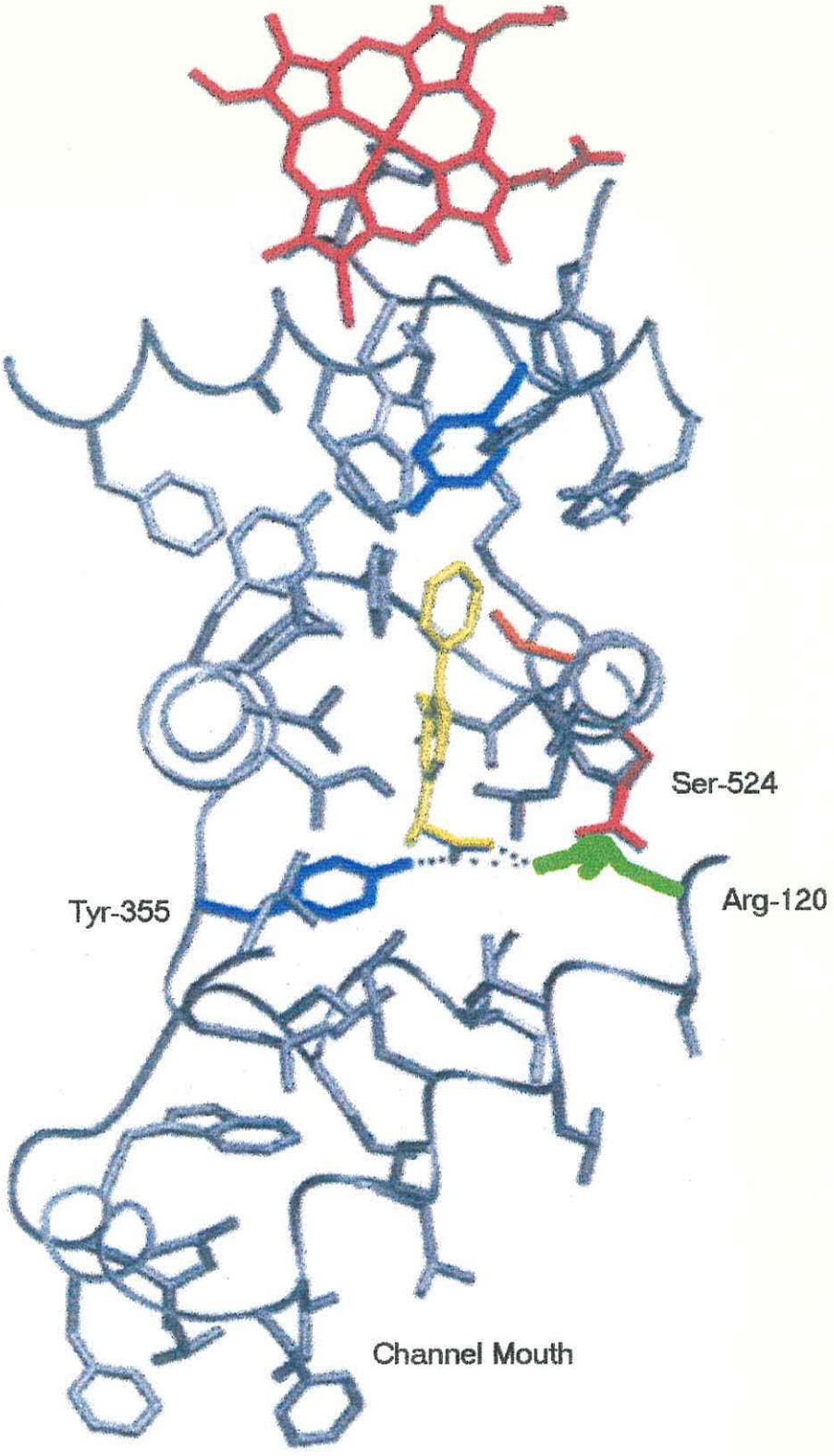


Fig. 8. The active site of cycloxygenase in PGHS-1.

The active site lies between the heme group (Red), at the top and the channel mouth. Bound within the active site is NSAID flurbiprofen (Yellow) which lies near Ser-524 (Orange). Tyr-385 (Blue) lies between the heme and flurbiprofen. The carboxylate of flurbiprofen ligates with Arg-120 (Green) and Tyr-355 (Blue). Glu-524 (Red) may also interact with Arg-120.

1.3.5 NITRIC OXIDE SYNTHASE OXYGENASE

Nitric oxide (NO) is a key intercellular signal and defensive cytotoxin in the nervous, muscular, cardiovascular[17] and immune systems,[21] and is produced by the oxidation of arginine by nitric oxide synthase (NOS). Neuronal NOS and endothelial NOS produce low concentrations of nitric oxide which is subsequently used for neurotransmission, insulin release and penile erection to name a few. The high concentrations of NO which is produced by cytokine-inducible NOS is used to counter pathogens and organise T-Cell response.

Citrulline and nitric oxide are obtained *via* a NOS catalysed two step heme based, five electron oxidation of arginine. The first step of the reaction pathway is a mixed function oxidation in which arginine is hydrolysed to N^{ω}-hydroxy-L-arginine (NOH-L-Arg), and secondly NOH-L-Arg is converted to both NO and L-citrulline. The mechanism by which this occurs (Fig. 9) involves the guanidine moiety of arginine donating a proton to the peroxo-iron intermediate, enabling O-O bond cleavage to occur and subsequent conversion to a proposed peroxoiron (IV) π cation radical species, which then rapidly hydroxylates the neutral guanidinium to NOH-L-Arg, *via* a radical-based mechanism.[22]

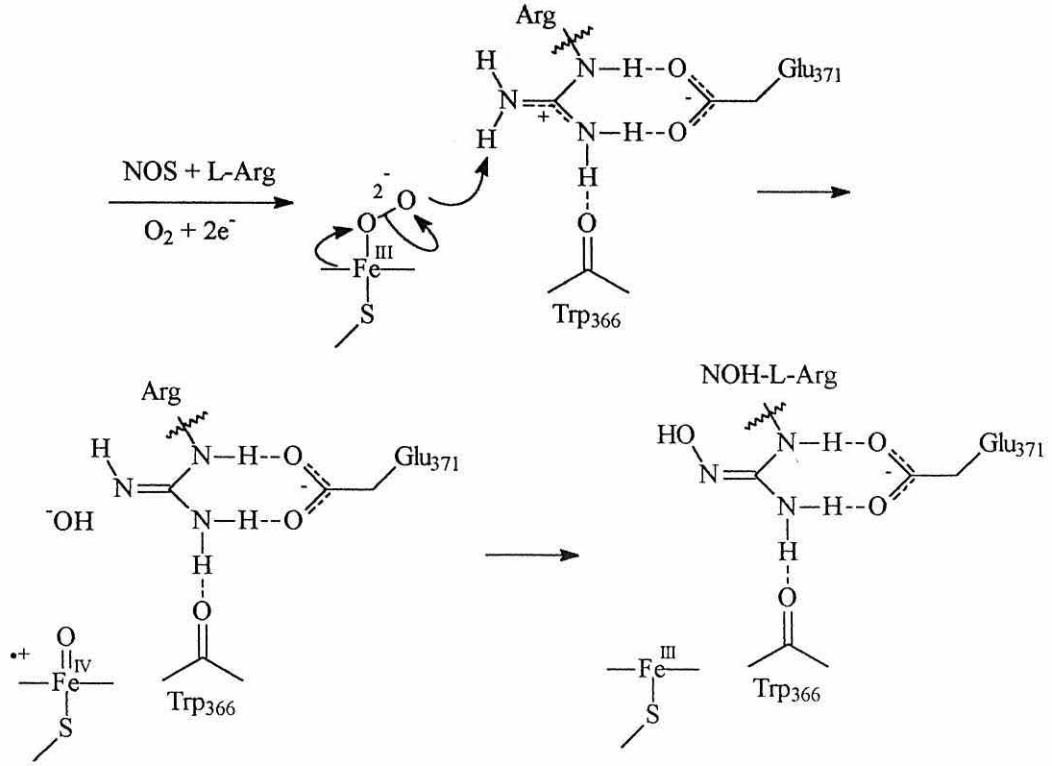


Fig. 9. A mechanistic representation of NOS.

CHAPTER 2

GUANIDINE CONTAINING NATURAL PRODUCTS

2.1 INTRODUCTION

The ocean covers nearly three quarters of the earth's surface, with these wide expansive tracts of water being home to a variety of flora and fauna. This makes the marine environment a huge potential resource for natural products and their associated applications (pharmaceutical science, food additives, cosmetics, novel enzymes and medicinal science). Many of these natural products contain guanidine with the more elaborate products being a great synthetic challenge to the organic chemist.[23]

Improved analytical techniques have led to the isolation and structural elucidation of many natural products, which are more elaborate than their terrestrial counterpart if any exists. Only a selected few will be discussed in this chapter, however, for a more overall account the reader is pointed towards reviews by Mori[24] and more recently Berlink.[25]

2.2 TETRODOTOXIN AND SAXITOXIN

Tetrodotoxin **[2]** is probably the most well known guanidine containing marine natural product. This lethal neurotoxin, found in the ovaries and eggs of the tiger puffer fish *tara fugu* and the closely related puffer fish *ma fugu*, has a lethal dose of 8μg/kg. Structural elucidation was carried out simultaneously in 1964 by American and Japanese groups.[26] However, the first total synthesis was not achieved until 1972 by Kishi and co-workers.[26] Further studies by Mosher and co-workers[27-29] found tetrodotoxin to be present in many organisms, the Californian newt *Taricha torsa,*[27] octopus *Hapalochaena maculosa*[28] and the skin and eggs of the Costa Rican frog *Atelpus chiriaauiensis.*[29] Yasumoto[30] isolated tetrodotoxin from the skin of *Atelopus subornatus, A. peruensis* and *A. oxyrhynchus* Bufonidae toads,[30] and Shimidu and co-workers in 1988 isolated tetrodotoxin from extracts of marine sediment off the coast of Japan.[31]

In 1992 and 1995 several derivatives of tetrodotoxin were found by Yotsu *et al.*[32] in several new species of puffer fish. Compounds **[3]** and **[4]** were isolated from *Arothron nigropunctatus* and *Fugu poecilonotus* respectively, along with a more abundant epimer of **[4]** at C-4 **[5]**. Structural elucidation of both **[4 & 5]** was achieved by analysis of spectroscopic data, along with circular dichroism, which established the configuration at C-9. Yamamoto in 1994 reported an asymmetric synthesis of tetrodotoxin.[33]

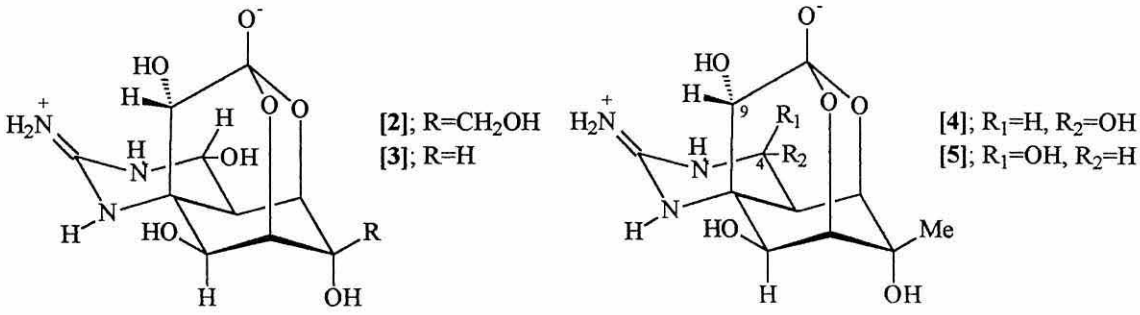


Fig. 10. Tetrodotoxin and its derivatives.

The symptoms of poisoning are well documented and go back to antiquity. The puffer fish *Tetraodon lineatus* has been identified amongst the figures on the walls of the tomb of the Egyptian Pharaoh Ti of the Vth Dynasty. It is believed that the early Egyptians knew the puffer fish to be poisonous and the earliest known reference to fugu poisoning is from the Han Dynasty (202BC-AD220).

Saxitoxin **[6]** was isolated in 1957 by Schantz *et al.*[34] and is found in normally edible bivalves such as clams and mussels *Mytilus californianus*. The toxin accumulates and concentrates within the bivalves when they ingest toxic blooms of dinoflagellates (Plankton) *Gonyaulax catenella*[34] and *Gonyaulax tamarensis*.[35] Consumption of contaminated shellfish by humans results in PSP (Paralytic Shellfish Poisoning) which can be fatal. Saxitoxin is also the paralytic agent of the Alaskan Butterclam *Saxidomad giganteus*.[36]

Structural elucidation of the complex molecule proved to be extremely problematical until Rapport[37] and Schantz[38] in 1975 obtained crystalline derivatives. The first total synthesis of Saxitoxin was reported in 1977 by Kishi *et al.* and *via* a similar route in 1984 by Jacobi.[34b] More recently in 1995 Kishi *et al.*[39] produced a series of bioassays with the natural (+)-saxitoxin **[6]**, (+)-decarbamoylsaxitoxin **[7]** and the unnatural derivatives. This proved that only the natural orientations displayed sodium channel blocking activity.

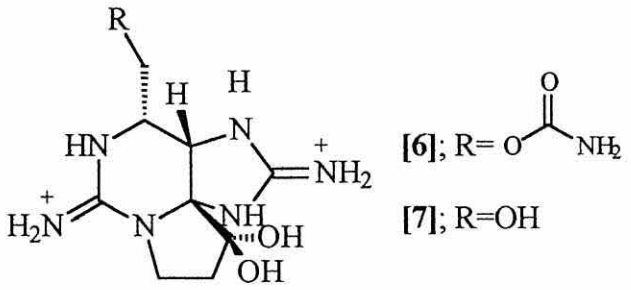


Fig. 11. Saxitoxin and derivative.

Both tetrodotoxin **[2]** and saxitoxin **[6]** display similar activity in terms of their selective affinity towards sodium channel inhibition even though their structures are markedly different.

2.3 ANATOXIN -a-(S)

Anatoxin-a-(S) **[8]** is a neurotoxin which was isolated from the cyanobacteria *Anabaena flos-aquae*[40] in 1989 by Moore and co-workers. It is stable between pH 3 and 5 but under basic conditions decomposes readily. Carmichael *et al* suggested the high level of toxicty anatoxin-a-(s) displayed (LD_{50}-20-40 μg/kg of mice) was due to the exceptional anti-cholinesterase activity.[41]

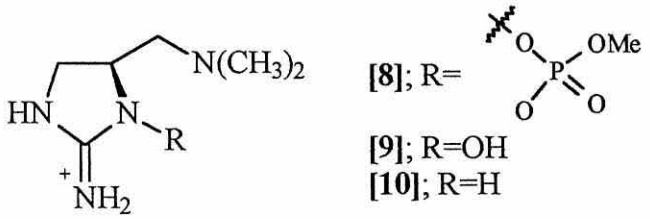
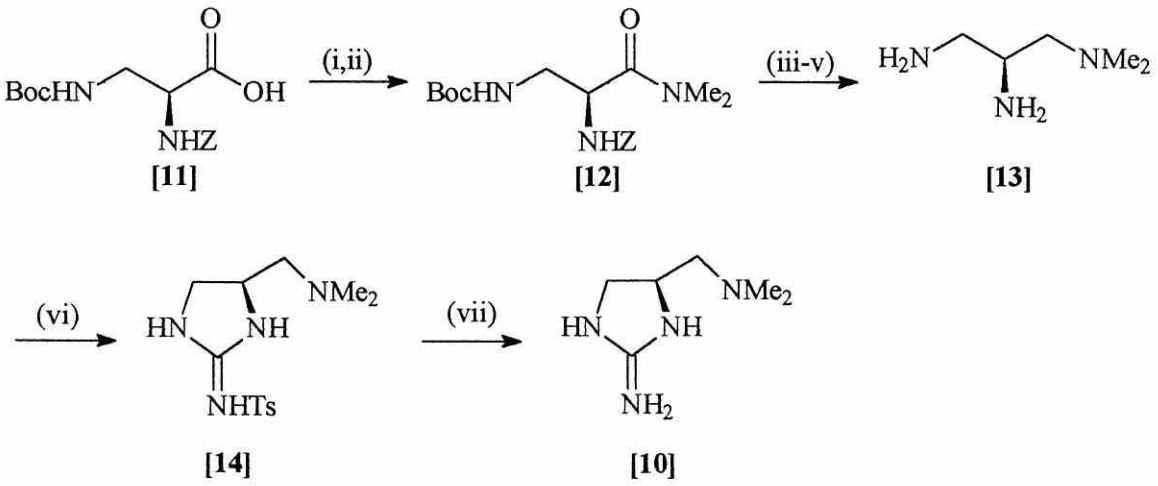


Fig. 12. Anatoxin-a-(S).

Structural elucidation was achieved by spectroscopic analysis ^{1}H, ^{13}C and ^{31}P NMR along with NMR studies on anatoxin-a-(S) that had been uniformly enriched to 50% ^{13}C and 90+ % ^{15}N. Storage of anatoxin-a-(S) led to decomposition products **[9 & 10]** as well as monomethylphosphate. The absolute stereochemistry around C-5 was determined by Moore *et al.* by means of synthesising the R and S geometries of **[10]** from D- and L-asparagine (Scheme 1).



Scheme 1. ***Reagents and Conditions:*** (i) N-hydroxysuccinimide, DCC, dioxane, 0°C 10min to RT 15h; (ii) Me_2NH in Et_2O; (iii) CF_3CO_2H, RT 1h; (iv) 10% Pd-C, H_2, MeOH; (v) excess BH_3/Me_2S, THF, reflux 15h; (vi) S,S-dimethyl-N-tosyliminodithiocarbonimifate, EtOH, reflux 15h; (vii) 48% HBr, reflux 4h.

Acid **[11]** was converted to dimethylamide **[12]**, deprotection with TFA/H_2/Pd-C and subsequent reduction with BH_3-Me_2S complex afforded triamine **[13]** which was treated with S,S-dimethyl-N-tosyliminodithiocarbonimidate to furnish the cyclic guanidine **[14]**. Deprotection of imine **[14]** by heating at reflux with 48%, aq HBr afforded **[10]**. Spectroscopic analysis indicated that synthetic **[10]** derived from L-asparagine had identical NMR characteristics to anatoxin-a-(S).

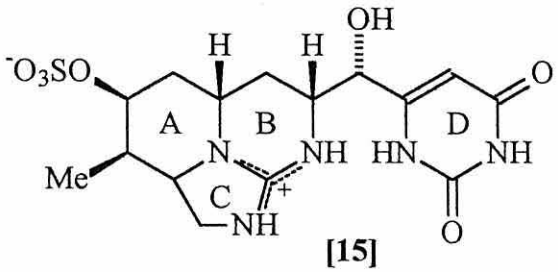
2.4 CYLINDROSPERMOPSIN

2.4.1 ISOLATION AND STRUCTURAL ELUCIDATION

In 1985 Hawkins *et al.* reported on a study concerning an outbreak of hepatoentritis that occurred on Palm Island in Northern Australia in 1979 affecting 148 inhabitants. Within the course of their study three species of cyanobacteria were identified as regular components of phytoplankton. Two varieties of *Anabaena circinalis* were found and were shown to be non-toxic after testing, however *Cylindrospermopsis raciborskii*, a species previously not thought to be toxic was found to be the causative agent in this outbreak.[42] *Cylindrospermopsis raciborskii* was identified by J. Komárek of the Institute of Botany, Třeboň, Czechoslovakia, and isolated using treated agar plates.[43]

Structure elucidation did not occur until 1992 when Moore and co-workers[44] isolated cylindrospermopsin **[15]** from the same cyanobacterium *Cylindrospermopsis raciborskii* as did Hawkins. They utilised 500MHz ^{1}H and 125 MHz ^{13}C NMR spectra in D_2O coupled with 2D COSY, HMQC and HMBC experiments as well as HRFABMS and UV spectral analysis. The intense negative ion FABMS ($M\text{-}H^-$ m/z 414) and UV spectrum in H_2O inferred a substituted uracil. The high chemical shifts of carbons C-10, C-15, C-8 and C-14 (δ45.0, 48.3, 53.6, and 57.9 respectively) suggested nitrogen was attached, whereas oxygen was present on carbons C-7 and C-12 (δ70.7 and 78.2 respectively). Isotope shifts established the positions of the NH's (C-8 and C-15) and the OH (C-7) with CIDMS data supporting the position of the sulphate group (C-12). The *cis*, *trans* and geminal coupling constants associated with the signals for protons on C-8 to C-14 indicated the six membered rings and their attached functional groups. Moore *et al.* established the presence of a tricyclic moiety by HMBC experiments. Protons on C-8 and one proton on C-15 were coupled to a guanidino carbon (δ156.5).

In 1994 Herada *et al.*[45] isolated cylindrospermopsin from a new cyanobacteria *Umezakia natans* found in Lake Mikata, Fukui, Japan and in 1997 Sukenik *et al.*[46] isolated **[15]** from a further cyanophyte *Aphanizomenon ovalisporum* in Lake Kinneret in Israel.



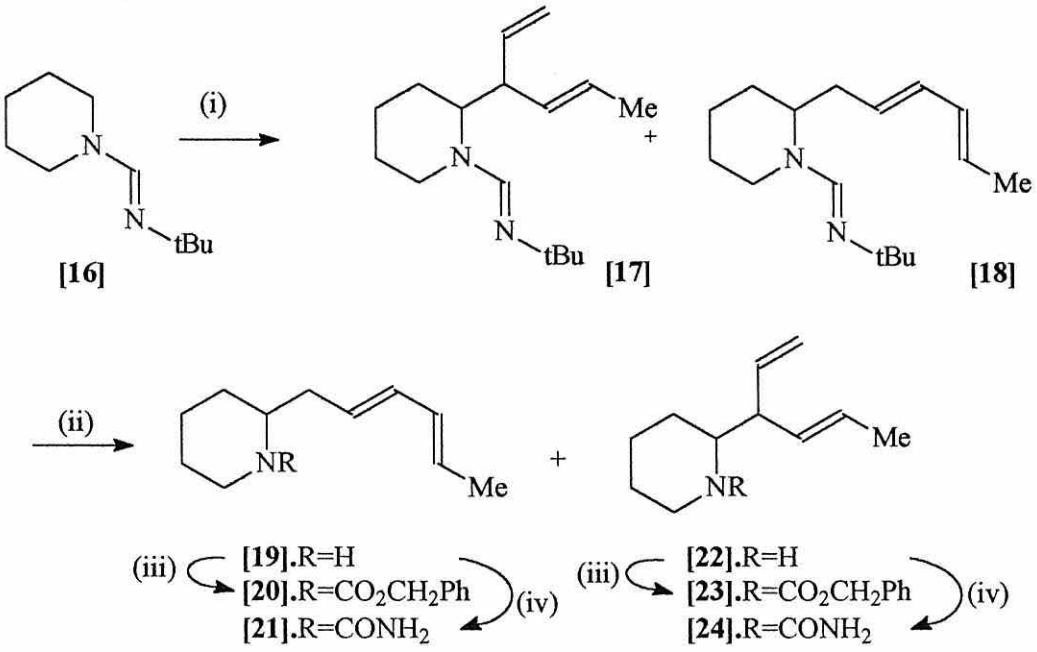
[15]

Fig. 13. Cylindrospermopsin.

Runnegar *et al.*[47] found cylindrospermopsin to be toxic to cultured rat hepatocytes, functioning by inhibition of reduced glutathione synthesis. The preliminary investigations established doses of 3.3-5.0 μM caused 40-67% cell death by LDH release after incubation for 18 hours while nontoxic doses of cylindrospermopsin 1.6-2.5 μM decreased cell glutathione by 50%. They also concluded that toxic doses of 5.0 μM lead to loss of glutathione before the onset of toxicity by six hours.

2.4.2 SYNTHETIC APPROACHES

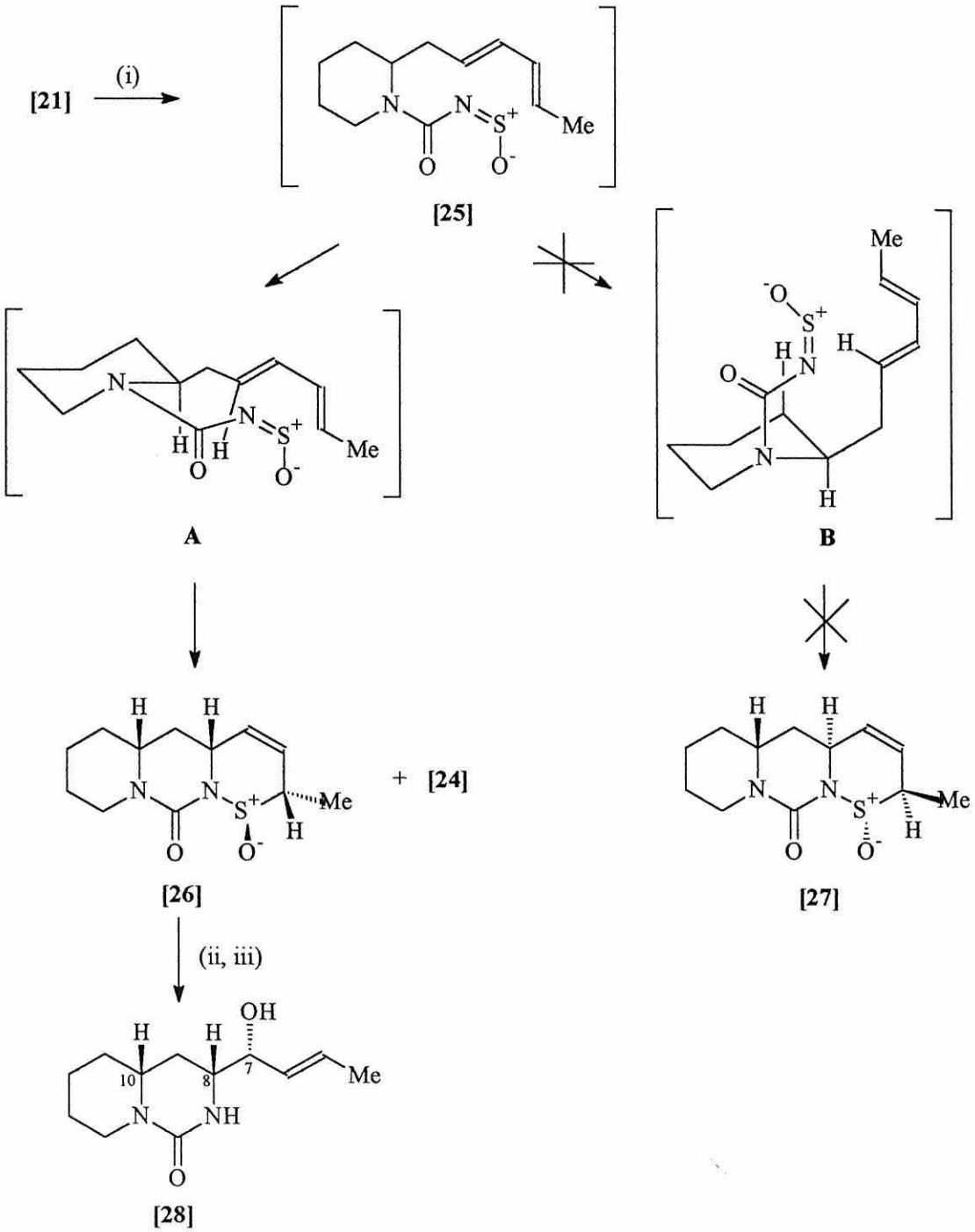
Preliminary efforts towards the synthesis of cylindrospermopsin was attempted by Weinreb and co-workers [48] in 1993. They reported the production of an analogue AB ring system which had the stereochemistry at C-7,8 & 10 comparable to cylindrospermopsin. Amidine **[16]** was lithiated[49] and alkylated producing diene **[18]** and the isomeric unconjugated diene **[17]** as an inseparable mixture in 67% yield. Hydrolysis of **[17 & 18]** afforded an inseparable mixture of isomeric amines **[19 & 22]** which were then further converted to benzyl carbamates **[20 & 23]** in 65% yield. Compounds **[19 & 22]** were secondly converted to a mixture of ureas **[21 & 24]** in 48% yield.



Scheme 2. ***Reagents and Conditions***: (i) ^{t}BuLi, THF, CuC_2H_2Pr, 1-chloro-2,4-hexadiene, 4:1 67% total yield; (ii) KOH, MeOH / H_2O, 94% yield inseparable; (iii) $PhCH_2OCOCl$, Et_3N, CH_2Cl_2, 65%; (iv) NaOCN, HCl, 48%.

Weinreb *et al.* observed that treatment of **[21 & 24]** with thionyl chloride and imidazole at -78°C produced a single Diels Alder adduct **[26]** in 67% yield, based upon **[21]** and urea **[24]** was recovered unchanged. Structural characterisation of **[26]** was established by X-ray crystallography. The diastereomeric adduct **[27]** was not detected and so Weinreb and co-workers performed modelling studies on **[25]** which established two possible reasons for the production of **[26]** and not **[27]**: (a) the presence of better

overlap in an *exo* transition state comparable to intermediate A leading to **[26]** and (b) the presence of a non-bonded interaction which would destabilise B relative to A.

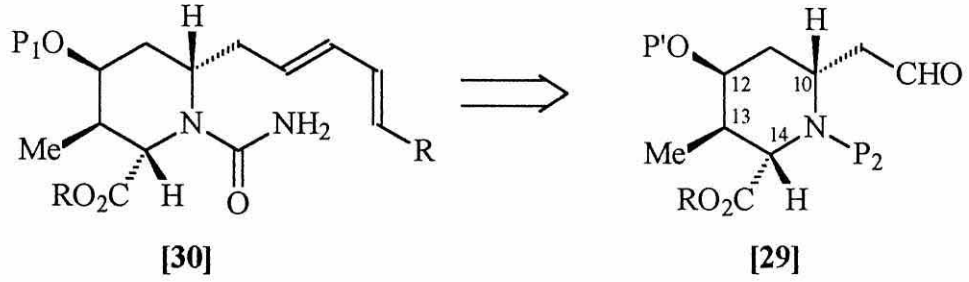


Scheme 3. ***Reagents and Conditions***: (i) $SOCl_2$, $CHCl_2$, imidazole, -78°C; (ii) PhMgBr, THF, -78°C; (iii) $(MeO)_3P$, MeOH, Δ, 65%.

Stereospecific conversion of **[26]** by treatment with phenylmagnesium bromide and a [2,3] sigmatropic shift of the sulfoxide intermediate furnished **[28]** in a 65% yield.

Weinreb *et al.* were pleased to find their AB system had the same stereochemistry at C-7, 8 & 10 as cylindrospermopsin.

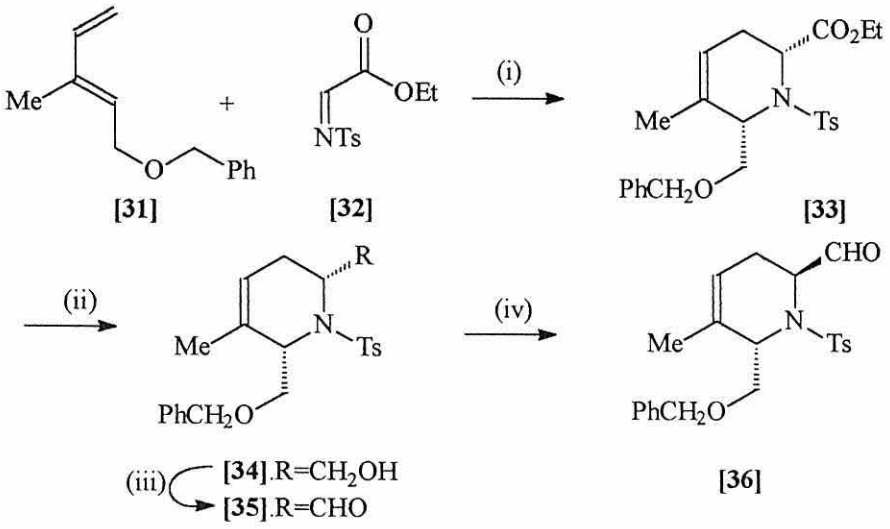
In order to extend their approach to the total synthesis of cylindrospermopsin Weinreb and co-workers[50] needed to access the generalised substituted diene system **[30]** which held the correct functionality and stereochemistry as cylindrospermopsin and envisaged that aldehyde **[29]** would be a suitable precursor to this system.



[30] **[29]**

Fig. 14. Weinreb's retrosynthetic analysis.

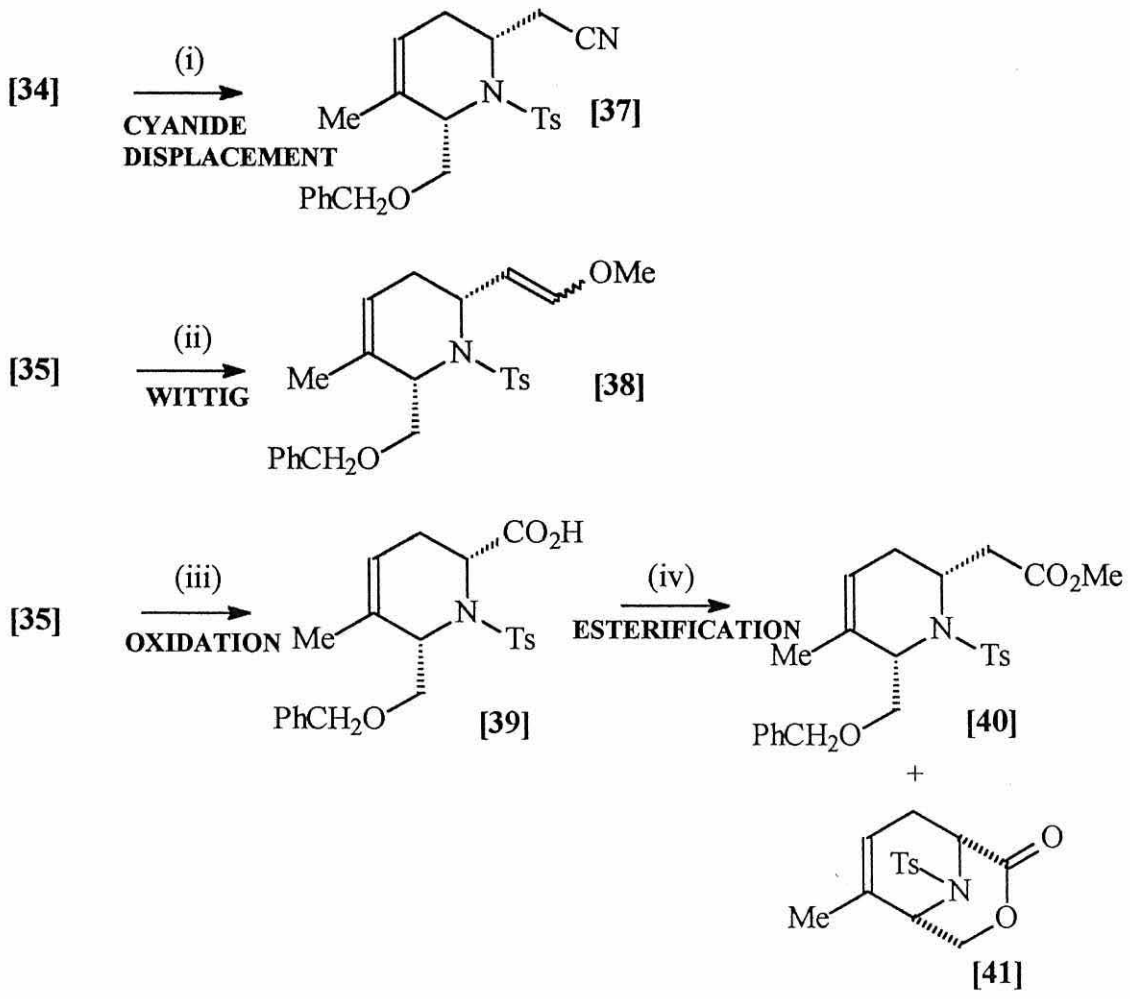
Initial investigations focused on an imino Diels Alder reaction of diene **[31]** with acetate **[32]** forming the cycloadduct **[33]** as a single stereoisomer. However modification of **[33]** to the required intermediate **[29]** proved difficult. Ester **[33]** was first converted to alcohol **[34]** without problem; however, Swern oxidation of alcohol **[34]** to aldehyde **[35]** yielded 89% of the requisite aldehyde but, under basic conditions epimerisation occurred leading to the unwanted *trans* product **[36]**.



[31] **[32]** **[33]** **[36]**

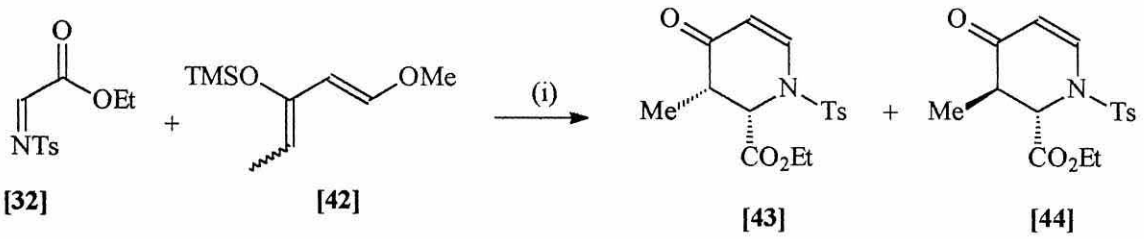
Scheme 4. ***Reagents and Conditions***: (i) $ZnCl_2$, PhMe, RT, 58%; (ii) $LiAlH_4$, Et_2O, 94%; (iii) Swern, 89%; (iv) Base.

As the next stage of the proposed synthesis involved the modification of C-2, several homologation reactions were attempted, including homologation of **[34]** using cyanide, *via* the corresponding mesylate and Wittig homologation of aldehyde **[35]**. Both attempts did produced the desired products **[37]** and **[38]** but in low yields. As these homologation methods proved to be difficult, Weinreb and Heintzelman employed a new strategy in which aldehyde **[35]** was oxidised to the requisite carboxylic acid **[39]** and then treated under Arndt-Eistert homologation conditions. Unfortunately this also led to a complex mixture of products including the required ester **[40]** as well as lactone **[41]**, again in low yields.



Scheme 5. ***Reagents and Conditions***: (i) MsCl, TEA, KCN, DMSO, 14%; (ii) Ph_3P=CHOMe, 5-10%; (iii) Jones oxidation, 83%; (iv) Oxalyl chloride, CH_2N_2, Et_2O, Ag_2O, MeOH, **[40]** 17%, **[41]** 39%.

A new strategy was thus adopted in which N-tosylamine **[32]** was reacted with oxygenated diene **[42]** (4:1 ratio of *E*:*Z*) which, after an *in situ* acid hydrolysis, resulted in formation of *cis* and *trans* enones **[43]** and **[44]** respectively.



[32] **[42]** **[43]** **[44]**

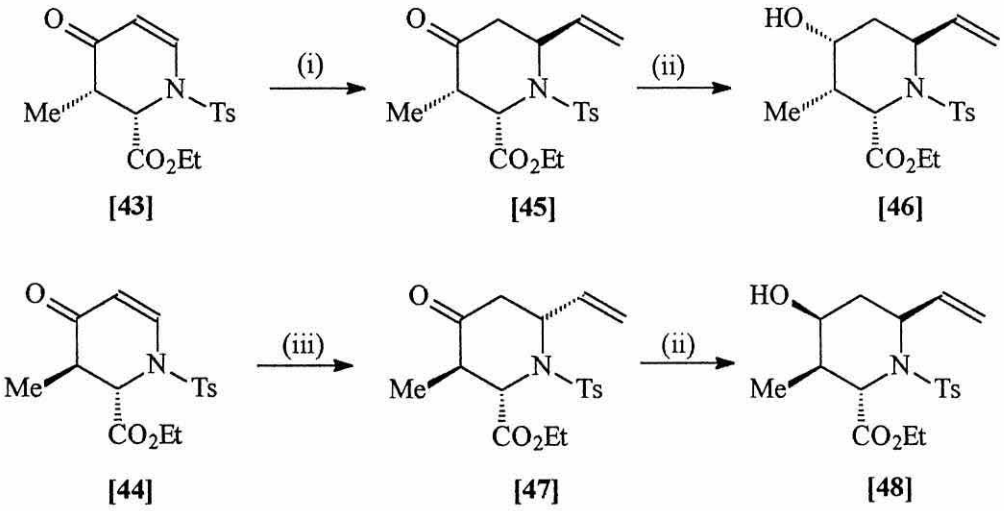
Scheme 6. ***Reagents and Conditions***: (i). PhMe, H_3O^+.

They observed that the ratio yields of **[43]** : **[44]** were dependent upon reaction conditions, (summarised in Table 3) and determined that these differences were a result of *E*/*Z* isomerisation of the diene or epimerisation that occurred during work up and purification.

Catalyst	**Material**	**Ratio [43] : [44]**	**Percentage Yield [43] : [44]**
$AlCl_3$	PhMe, -78°C, 3h.	7:1	53
$ZnCl_3$	PhMe, -78°C, 3h	22:1	60
-	PhMe, RT, 3h	4.7:1	51

Table 3. The various conditions employed for production of **[43]** & **[44]**.

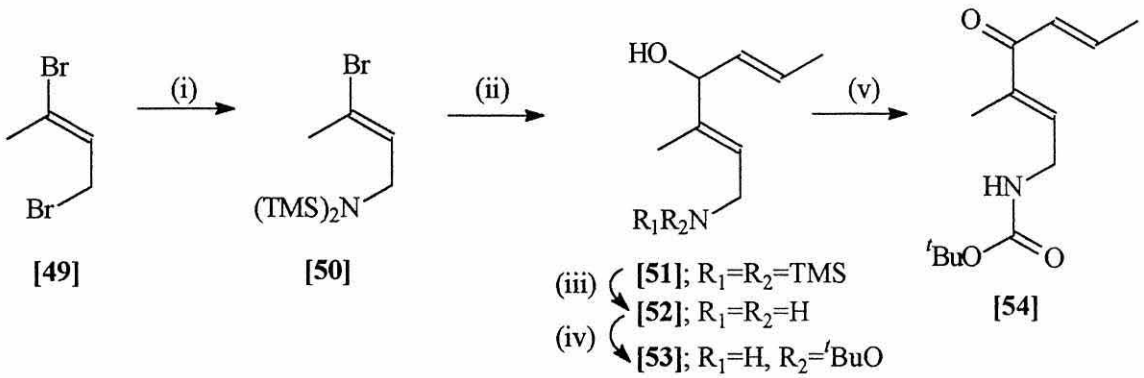
Weinreb *et al.* suggested the major *cis* product **[43]** was derived from a *Z*-diene which ultimately proceeded *via* a transition state which had the carboxylate group of imine **[32]** *endo*. This led to the conclusion that the minor *trans* adduct **[44]** arose from the *E*-diene. Due to the problematic nature of assignment Weinreb *et al.* treated both the major and minor adducts with vinylmagnesium bromide yielding vinyl ketones **[45]** and **[47]** as single stereoisomers. Reduction of both with L-Selectride afforded piperidine alcohols **[46]** and **[48]** as crystalline materials, enabling clarification of the original assignment with **[47]** having the correct stereochemistry for cylindrospermopsin.



Scheme 7. ***Reagents and Conditions***: (i) Vinylmagnesium bromide, CuI, THF, BF_3-Et_2O, 96%; (iii) Vinyl magnesium bromide, CuI, THF, 88%; (ii) L-Selectride, THF, 59%, 49% respectively.

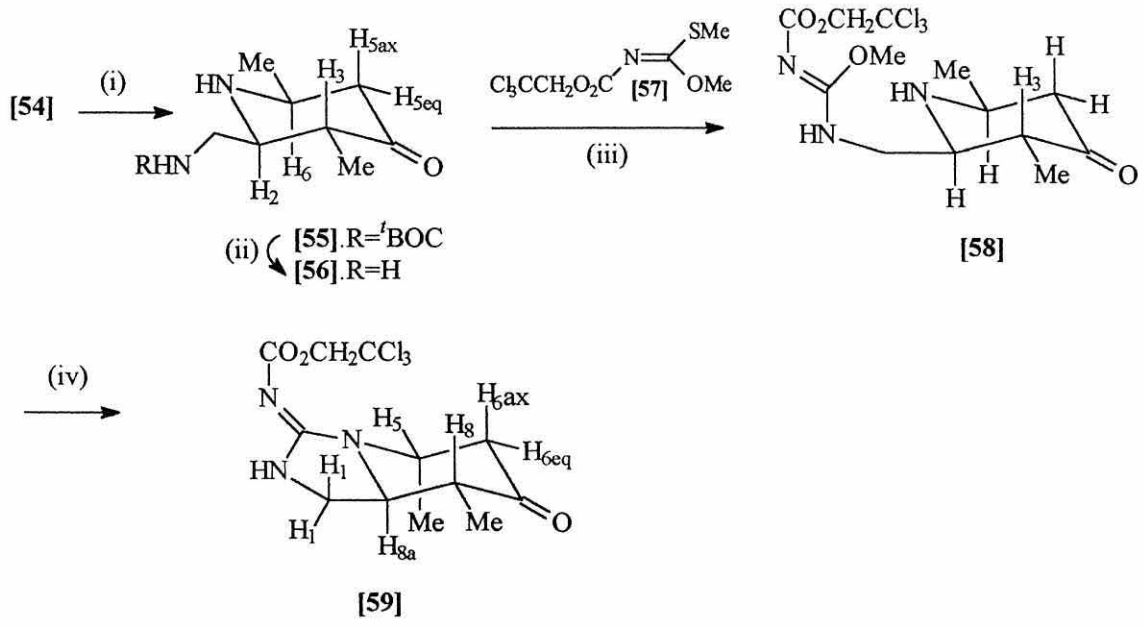
Weinreb and co-workers produced several active intermediates which were further functionalised and predicted that these would be instrumental in developing a total synthesis of cylindrospermopsin.

In 1995 Snider and Harvey[51] repeated their synthesis of an AB model system of cylindrospermopsin *via* a ten step convergent synthesis. The key reaction in this approach being a double Michael addition of ammonia to dienone **[54]**. Dienone **[54]** was prepared from 1,3-dibromo-2*E*-butene **[49]**, firstly by treatment with lithium bis(trimethylsilyl)amide in THF followed by metal halogen exchange, subsequent addition of crotonaldehyde furnished **[51]**. Without any further purification, the TMS protecting group was cleaved, producing **[52]**, and the amine function protected, with Boc_2O producing **[53]** in 44% yield from **[49]**. Further oxidation of **[53]** with MnO_2 gave **[54]** in 86% yield.



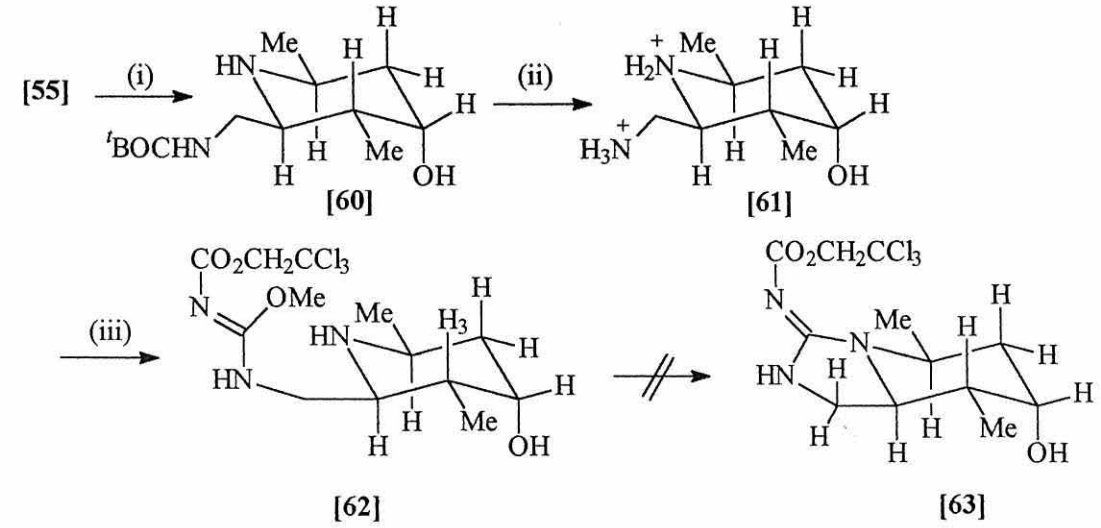
Scheme 8. ***Reagents and Conditions***: (i) $LiN(TMS)_2$, THF 25°C, 2h, 40-50%; (ii) ^{t}BuLi -78°C, crotonaldehyde; (iii) MeOH, overnight 25°C; (iv) BOC_2O, $CHCl_2$, 1M NaOH, 44%; (v) MnO_2, $CHCl_2$, 16h, 25°C, 86%.

Dienone **[54]** was elaborated by a double Michael addition of ammonia producing piperidinone **[55]** as a mixture of diastereomers, the major diastereomer, having all three substituents equatorial (H_2, H_3 and H_6 were axial indicated by large vicinal coupling constants $J_{2,3}$=10.3 and $J_{5ax,6}$=12.0Hz) as required for cylindrospermopsin, in 55% yield after purification. Snider *et al.* observed that equilibration of the mixture of diastereomers under the original reaction conditions afforded further quantities of **[55]**. They envisaged the guanidine moiety could be introduced by deprotection of **[55]** with TFA followed by subsequent treatment under Kay[52] conditions using carbonimidothioate **[57]**. This produced the unforeseen protected O-methylisourea **[58]**; however, cyclisation to produce **[59]** was achieved by heating **[58]** at 60°C in methanol. Preliminary NMR studies of the cyclic intermediate **[59]** indicated a change in stereochemistry of H_5, in terms of an axial to equatorial arrangement. Snider *et al.* suggested that isomerisation could have been a result of a retro-Michael and subsequent Michael reaction being thermodynamically driven by A strain between the equatorial methyl and acyl guanidine.



Scheme 9. ***Reagents and Conditions***: (i) NH_4OH, NH_4Cl, MeOH, 67°C, 16h sealed tube; (ii) TFA, CH_2Cl_2, 25°C, 16h; (iii) **[57]**, DMF, 25°C, 16h; (iv) MeOH, 60°C.

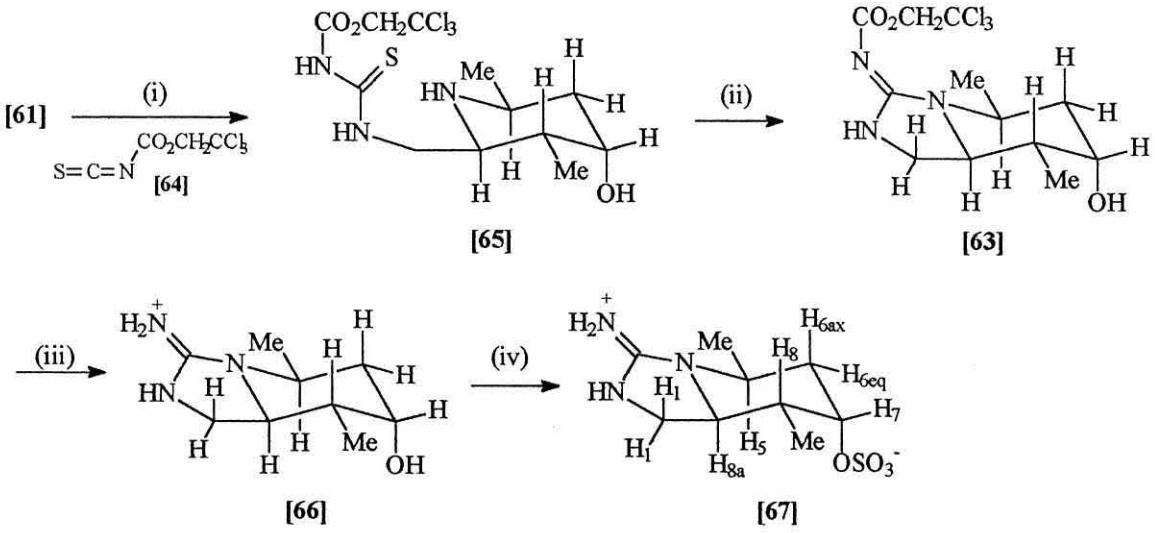
This isomerisation was prevented by reduction of ketone **[55]** with L-Selectride to the desired axial alcohol **[60]** in 88% yield. After deprotection of the amine furnishing **[61]** and treatment with **[57]** to obtain **[62]**, cyclisation to the bicyclic system **[63]** did not occur.



Scheme 10. ***Reagents and Conditions***: (i) L-Selectride; (ii) TFA; (iii) **[56]**, DMF, 25°C, 16h.

As with their first attempt, steric interactions were considered to be the major problem. Therefore, a more reactive precursor was used enabling production of guanidine **[63]**. Snider and Harvey then used isothiocyanate **[64]** which after generation *in situ* was reacted with **[61]** to furnish the protected thiourea **[65]**. Subsequent

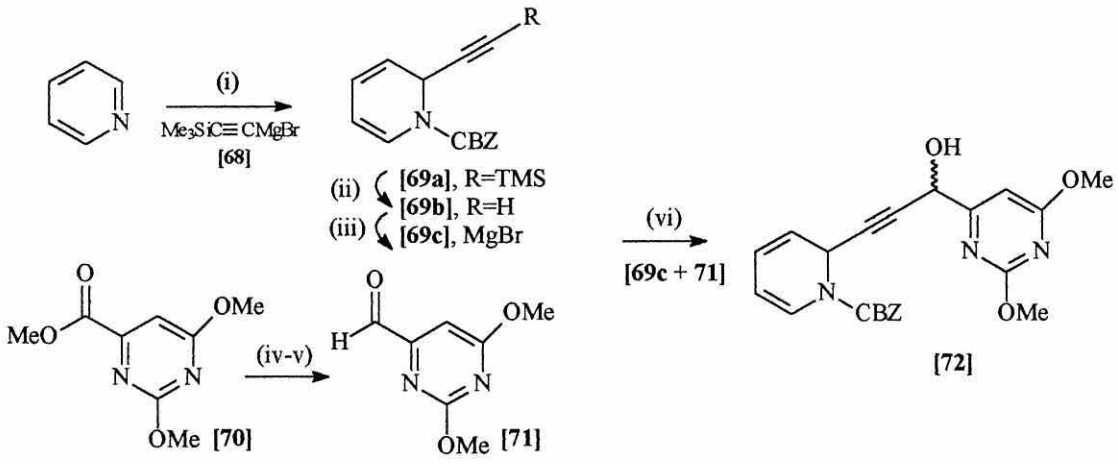
treatment with $HgCl_2$ and Et_3N in DMF produced the bicyclic system **[63]** in 40% yield from **[61]**. Guanidine deprotection and sulfonation gave the cylindrospermopsin model **[67]** which on comparison with the reported data for the natural material, was shown to possess the correct stereochemistry.



Scheme 11. ***Reagents and Conditions***: (i) *in-situ*, **[64]**; (ii) $HgCl_2$, Et_3N, DMF, 40%; (iii) Zn dust, AcOH, H_2S, HCl, 81%; (iv) $DMF.SO_3$, DMF, quantitatively.

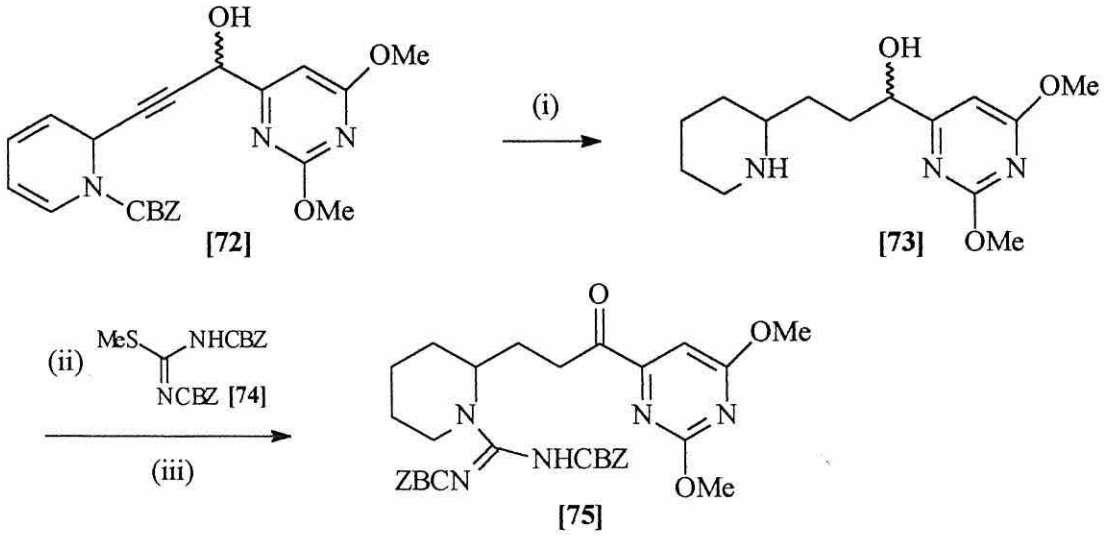
One of the most recent studies towards the total synthesis of cylindrospermopsin was carried out by Snider and Xie in 1998.[53] After reporting the formation of the bicyclic guanidinium sulphate **[67]** they further attempted the synthesis of the third ring of cylindrospermopsin and the hydroxymethyluracil side chain. This was partially achieved by a convergent synthesis based upon the coupling of acetylene **[69b]** with aldehyde **[71]**.

Acetylene **[69b]** was prepared in 97% yield *via* the reaction of pyridine with Grignard **[68]** and BnOCOCl under Yamaguchi conditions,[54] followed by deprotection. Compound **[71]** was obtained from elaboration of orotic acid by the procedure of Gershon[55] forming dimethoxy-4-pyrimidinecarboxylate **[70]**. After treatment with $LiBH_4$ which afforded 93% of the corresponding primary alcohol, this was subsequently oxidised with Dess-Martin reagent to give aldehyde **[71]** in 90% yield. Reaction of **[69b]** with EtMgBr furnished an intermediate acetylenic Grignard which on treatment with aldehyde **[71]** resulted in an 85% yield of **[72]** as a mixture of diastereomers.



Scheme 12. ***Reagents and Conditions***: (i) BnOCOCl, THF, 0°C, 2h, 95%; (ii) K_2CO_3, MeOH, 25°C, 20 min, 97%; (iii) EtMgBr; (iv) $LiBH_4$, THF, 93%; (v) Dess-Martin, 90%; (vi) Direct combination **[69c]** and **[71]**, 85%.

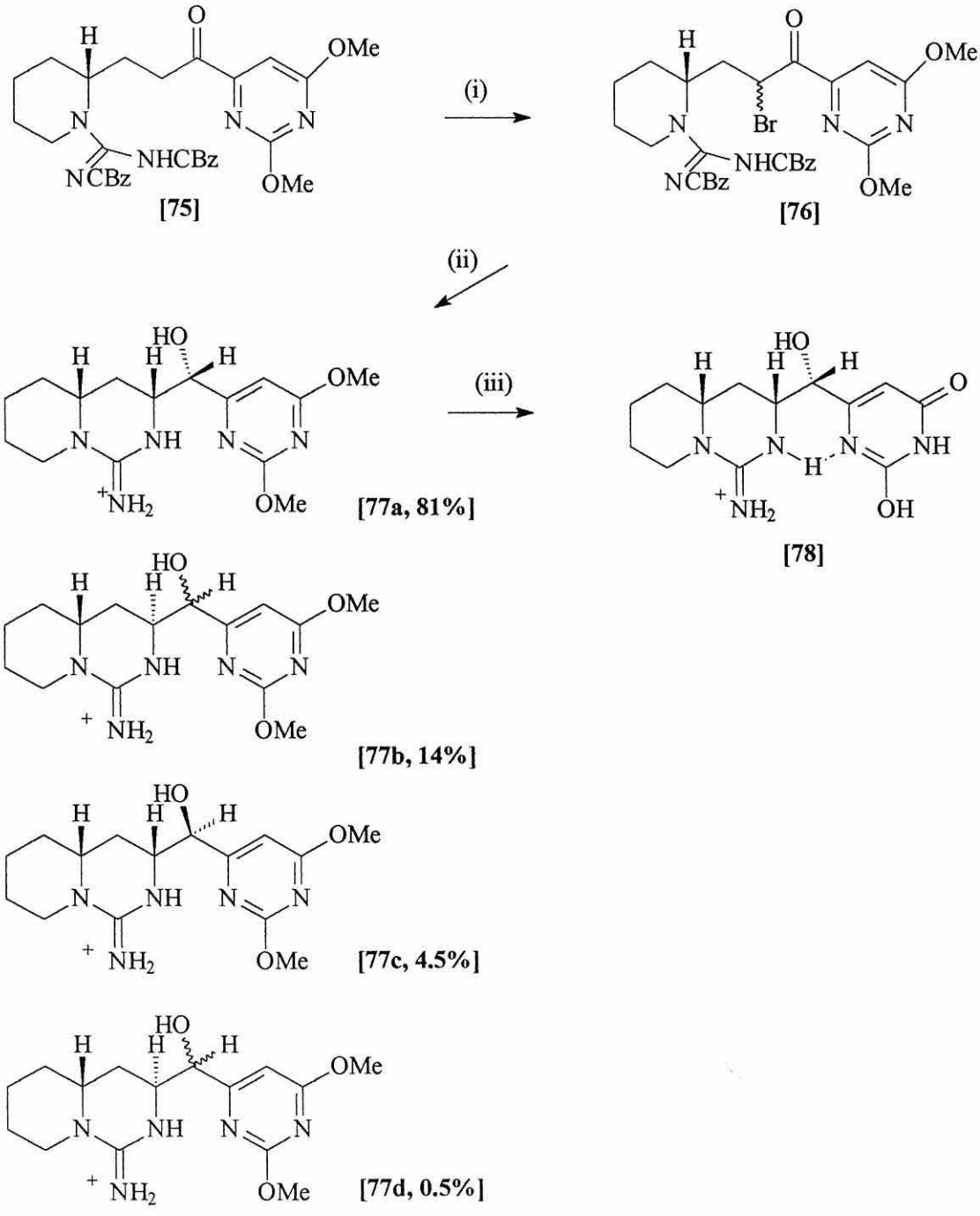
Hydrogenation of **[72]** over 5% Pd/C reduced both the acetylene and pyridine moieties as well as cleaving the CBZ protecting group giving **[73]** in 94% yield as a mixture of diastereomers. Further elaboration of **[73]** by treatment with thiourea **[74]** introduced the guanidine moiety in 74% yield, and following subsequent treatment with $HgCl_2$, Et_3N and oxidation, again with the Dess-Martin reagent, afforded 72% of ketone **[75]**.



Scheme 13. ***Reagents and Conditions***: (i) H_2, 5% Pd/C, MeOH, 94%; (ii) **[74]**, $HgCl_2$, Et_3N, DMF, 74%; (iii) Dess-Martin, 72%.

Ensuing bromination with $CuBr_2$ provided crude bromoketone **[76]** which Snider and Xie found unstable and could not be purified. Hydrogenolysis of **[76]** deprotected the guanidine moiety thus enabling an S_N2 reaction to form the second six membered ring. Under these conditions the ketone was also hydrogenated giving

alcohols **[77a-d]** as a mixture of diastereomers in a ratio of 81:14:4.5:0.5 around C1 and C2. Compound **[77a]** had the correct stereochemistry with relation to cylindrospermopsin even after treatment with conc. HCl thus forming in 95% yield the analogue **[78]** from **[77a]**.

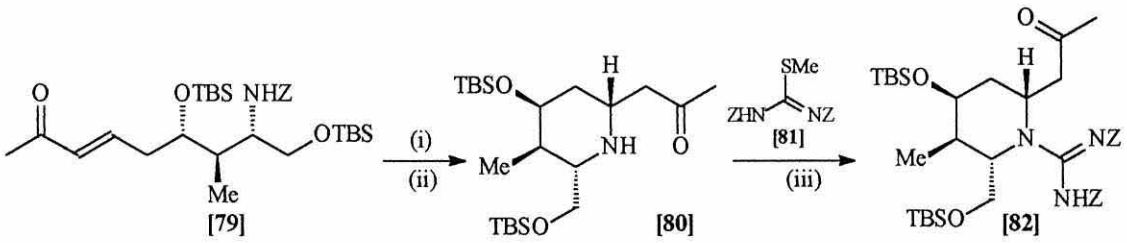


Scheme 14. ***Reagents and Conditions***: (i) $CuBr_2$, EtOAc, 40°C, 15min; (ii) H_2, Pd/C, MeOH, 2h, two steps; (iii) conc. HCl, reflux 6h, 95%.

Snider and Xie developed a short synthetic pathway which enabled them to access the tetrahydropyrimidine ring and hydroxymethyluracil side chain of cylindrospermopsin.

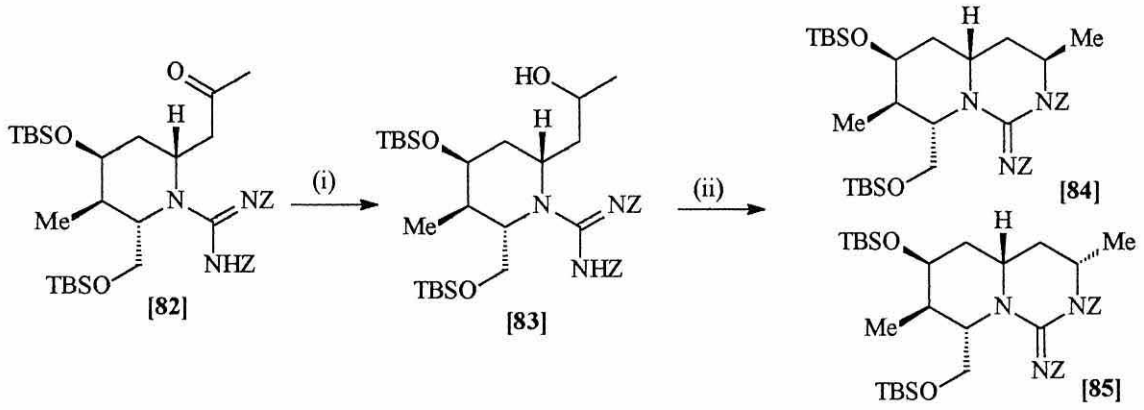
Following the completion of the work described in this thesis, McAlpine and Armstrong[56] reported the synthesis of a tricyclic guanidinium analogue of cylindrospermopsin. In a key step in this synthesis, the advanced intermediate **[79]** was cyclised by treatment with a catalytic amount of p-TsOH in refluxing benzene. This yielded one diastereomer of the corresponding Z-protected piperidine in 74% yield, subsequent hydrogenation giving **[80]** in quantitative yield.

The absolute stereochemistry of **[80]** correlated with that of the A ring of cylindrospermopsin and was assigned by 2D NOESY. Guanylation of **[80]** was achieved by reaction with *bis*-*Z*-methylthiopseudourea **[81]**.



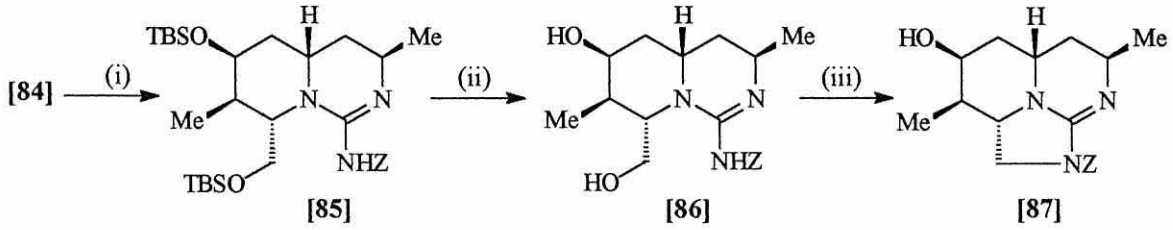
Scheme 15. ***Reagents and Conditions***: (i) *p*TSA, benzene, 74%; (ii) H_2, Pd/C, 100%; (iii) **[81]**, $HgCl_2$, Et_3N, DMF, 85%.

They envisaged the next important step in the synthesis as two S_N2 displacement reactions. The first required the alcohol with appropriate stereochemistry resulting from reduction of the methyl ketone. As this was an initial investigation McAlpine and Armstrong treated **[82]** with $NaBH_4$, which produced an inseparable 5:1 mixture of alcohols **[83]**, which upon Mitsunobu cyclisation generated guanidine bicycles **[84 & 85]** in 75% combined yield. 2D NOESY experiments confirmed that the minor product **[85]** had the equatorial conformation of the methyl group necessary in the B-piperidine ring of cylindrospermopsin.



Scheme 16. ***Reagents and Conditions***: (i) $NaBH_4$, MeOH, 100%; (ii) PPh_3, DIAD, **[84]** 63%, **[85]** 12%.

Despite this, further model studies on **[84]** established that tricycle **[87]** could be formed by initial treatment with NaH in a THF : methanol (1:1) solution to selectively deprotect one Z-group. Subsequent treatment with TBAF in THF removed both TBS protecting groups and cyclisation under Mitsunobu conditions produced the tricycle **[87]** in 27% yield, as the only isolated structure.



Scheme 17. ***Reagents and Conditions***: (i) NaH, THF, MeOH, 67%; (ii) TBAF, THF, 84%; (iii) PPh_3, DIAD, 27%.

McAlpine and Armstrong concluded that the synthesis of tricycle **[87]** (albeit with the wrong stereochemistry) demonstrated the feasability of a double displacement strategy to install the tricyclic guanidinium core.

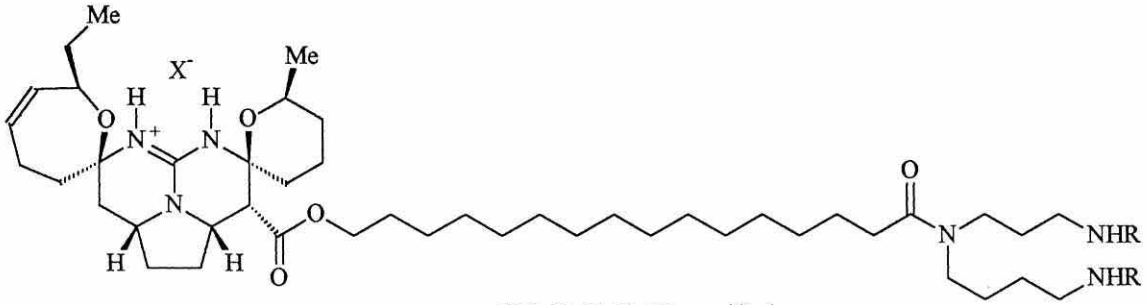
2.5 PTILOMYCALIN A AND RELATED COMPOUNDS

2.5.1 ISOLATION AND STRUCTURAL ELUCIDATION

In 1989 Kashman and Hirsh isolated a new unique polycyclic guanidine containing natural product, ptilomycalin A **[88]**, from the Caribbean sponge *Ptilocaulis spiculifer* and the Red Sea sponge *Hemimycale sp.*.[57] More recently ptilomycalin A has been isolated from the starfish *Fronia manilis* and *Celerina hetfernani*[58] and the Caribbean sponge *Batzella sp.*[59]

Structural elucidation was achieved by a combination of several NMR techniques and mass spectrometry. Ptilomycalin A exhibited cytotoxicity against P388 (IC_{50} 0.1μg/ml), L1210 (IC_{50} 0.4μg/ml) and KB (IC_{50} 1.3μg/ml) as well as antifungal activity against *Candida albicans* (MIC 0.8 μg/ml) and antiviral activity against HSV (0.2μg/ml).

Kashman and co-workers studied the structure and chemical properties of ptilomycalin A extensively; however, most of the structural work was carried out on the *bis*(trifluoroacetyl) derivative **[89]** which gave sharper signals in the ^{1}H and ^{13}C NMR spectra. In a latter publication Kashman related ptilomycalin A to a vessel and anchor,[60] the polycyclic framework being the vessel and the alkyl chain and spermidine moiety the anchor.

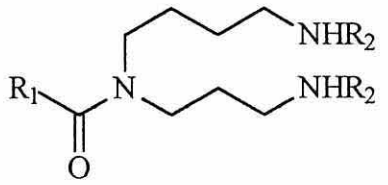


[88]; R=H, X=Unspecified.
[89]; R=$COCF_3$, X=CF_3CO_2.

Fig. 15. Ptilomycalin A and the *bis*(trifluoroacetyl) derivative.

The spermidine unit was assigned using COSY, HOHAHA, COLOC and HMBC experiments as well as degradation studies. Assignment was aided with the isolation of **[90]** from *Ptilocaulis spiculifer* and *Hemimycale sp.*. Kashman and Hirsh noted the accompaniment of small signals with the seven methylene groups, implying the spermidine moiety existed as two rotational isomers. This was confirmed by preparing

[91]. The NMR properties of **[91]** were equivalent to **[89]** with respect to the spermidine moiety.



[90]; R_1=$CH_3(CH_2)_{14}$, R_2=H.
[91]; R_1=CF_3, R_2=$COCF_3$.

Fig. 16.

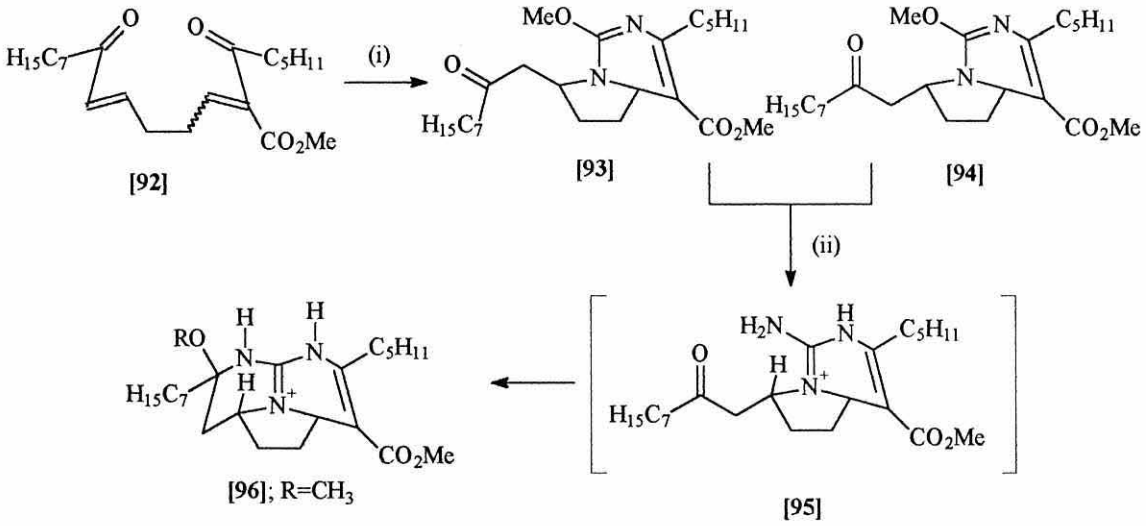
The HOHAHA spectrum also enabled the identification of the central tricyclic unit and both *N,O*-Acetyl units. The downfield NH protons at δ10.22 and δ9.87 suggested an ammonium or guanidinium salt. This was clarified by treatment of a sample in $CDCl_3$ with sodium hydroxide. The peaks in question disappeared but regenerated over a 24hr period strongly suggesting a guanidine. In addition the typical carbon signal of a guanidine δ149.09 remained uncorrelated in any NMR experiments. Final ratification was achieved by treatment of the $CDCl_3$ sample with 3 equivalents of CD_3OD which split the signal at δ149.09 into three new signals δ149.09, δ149.02 and δ148.94 due to the isotopic effect of the deuterium. This suggested the carbon was adjacent to two exchangeable protons indicating a guanidine moiety. The stereochemistry of **[89]** was established by phase sensitive NOESY and ROESY experiments.

2.5.2 SYNTHETIC APPROACHES

Not surprisingly ptilomycalin A became the focus of much synthetic attention. Snider and Shi in 1993[61] developed a biomimetic approach to the tricyclic portion of ptilomycalin A. They found the tricyclic unit could be accessed from a *bis*-α,β–unsaturated ketone **[92]** in two steps.

The required substrate **[92]** was obtained as a 1:1 mixture of stereoisomers in 61% yield from a Knoevenagel condensation reaction. This was then heated in the presence of *O*-methylisourea and $NaHCO_3$ at 50°C, which produced a 3:1 *trans : cis* mixture of the bicyclic adducts **[93]** and **[94]** in 56% yield. The relative stereochemistry was determined by ROSEY experiments, and the authors were pleased to observe both isomers were converted to the tricycle **[96]** in 60% yield by treatment with excess NH_4OAc in methanol, which was saturated with anhydrous NH_3 at 60°C in

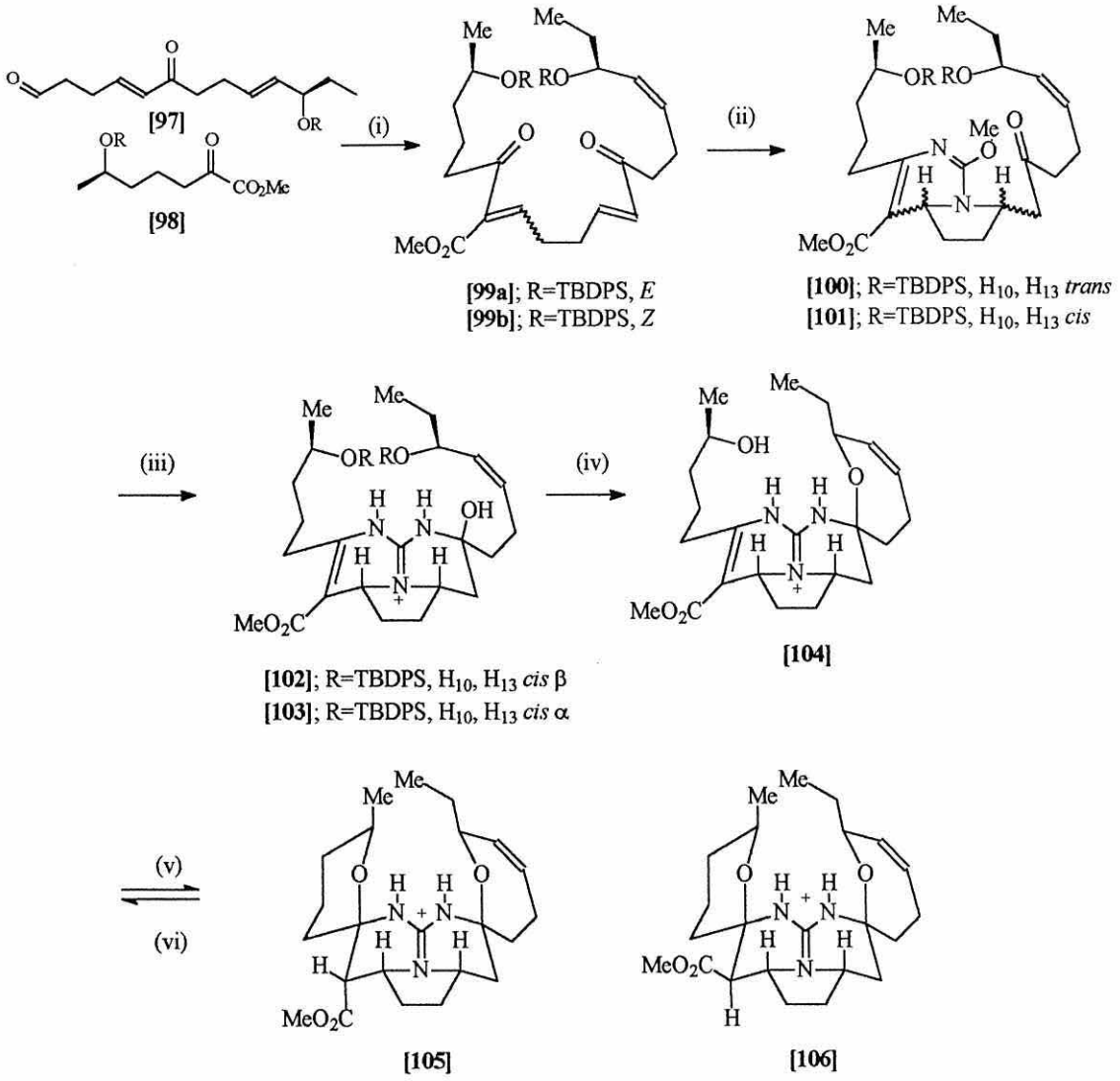
a sealed tube. Again the stereochemistry around C-10 and C-13 was established by ROESY experiments.



Scheme 18. ***Reagents and Conditions***: (i) *O*-methylisourea hydrogen sulphate, $NaHCO_3$, DMF, 2h 3:1 *trans*:*cis*, 56%; (ii) NH_4OAc, MeOH sat. with anhydrous NH_3, 4d, 60°C, 60%.

In 1994 Snider and Shi[62] elaborated on earlier work and synthesised the pentacyclic nucleus of ptilomycalin A, using similar methodology which gave them access to the tricyclic unit. The *bis*-α,b-unsaturated ketone employed in the synthesis was obtained from the Knoevenagel condensation of aldehyde **[97]** and β-ketoester **[98]** (prepared in nine and four steps from commercially available materials).

The Knoevenagel condensation between **[97]** and **[98]** to produce **[99]** as a 1:1 mixture of isomers was achieved using; a catalytic amount of piperidine at -78°C and warming to -20°C in DCM. Double Michael addition and enamine formation was accomplished in DMSO giving the two *trans* and two *cis* diastereomers **[100 & 101]** in 52% yield, both in a 4:1 ratio. Stereochemical assignment was based on NMR correlation with the previously synthesised cyclic systems (δ 4.3-4.5 ppm *cis* isomer and δ 4.1-4.2 *trans* isomer). Deprotection of the silyl ethers **[102 & 103]** with aqueous hydrofluoric acid and acetonitrile followed by cyclisation resulted in a 1.3:1 ratio of the pentacyclic core **[105]** in 34% yield as well as **[106]** in 26% yield where the methyl ester is equitorial.



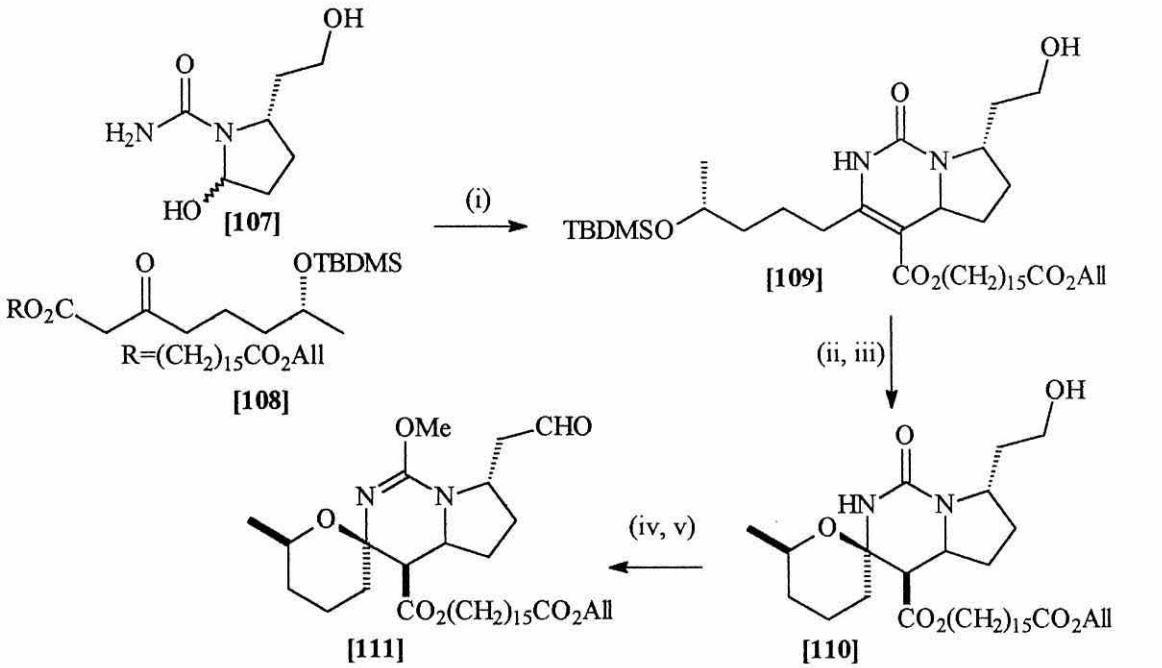
Scheme 19. ***Reagents and Conditions***: (i) Piperidine, DCM, -78°C to -20°C, 20h, 64%; (ii) *O*-methylisourea, *i*-Pr_2EtN, DMSO, 80°C, 1.5h, 52%, (4:1 **[100]**:**[101]**); (iii) NH_3, NH_4OAc, ^tBuOH, 60°C, 40h, 72%, (1:1 **[102]**:**[103]**); (iv) 3:7 HF:CH_3CN, -30°C, 3d; (v) Et_3N, MeOH, 60°C, 20h, 78% from **[102]**, (1.3:1 **[105]**:**[106]**); (vi) Et_3N, 1:1 H_2O:MeOH, 60°C, 16h.

Snider and Shi found that 2D-NMR ROSEY depicted structural similarities between the synthesised pentacyclic core **[105]** and ptilomycalin A.

A latter approach by Overman *et al.* in 1995[63] based on a model study[64] on tethered Biginelli condensation reactions for the preparation of advanced tricyclic intermediates, led to the first total enantioselective synthesis of (-)-ptilomycalin A.

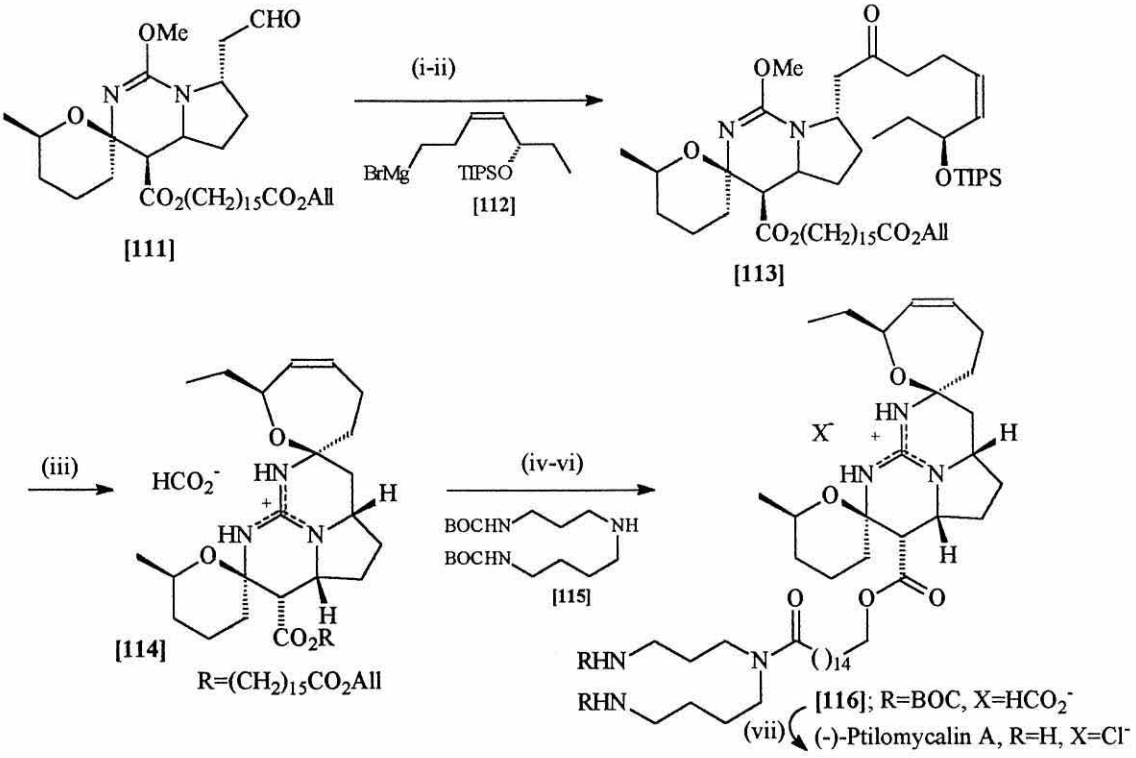
Their approach involved the condensation of β-ketoester **[108]**, urea **[107]**, to give bicyclic urea **[109]** in 44% yield. Condensation occurred with good diastereomer selectivity at 70°C in the presence of morpholinium acetate, a catalytic amount of acetic acid and Na_2SO_4. Cleavage of the TBDMS protecting group enabled subsequent

spirocyclisation with p-TsOH which produced tricycle **[110]**. This was found to be epimeric with ptilomycalin A. Swern oxidation, followed by protection and activation of the urea moiety yielded the intermediate **[111]**.



Scheme 20. ***Reagents and Conditions***: (i) Morpholine, AcOH, EtOH, Na_2SO_4, 70°C, 61%; (ii)PPTS, MeOH, 50°C; (iii) *p*-TsOH, $CHCl_3$, 23°C, 96%; (iv) Swern Oxidation; (v) MeOTf, R_3N, 23°C, 67%.

Condensation of **[111]** with 2 equivalents of the Grignard reagent **[112]** followed by Swern oxidation afforded ketone **[113]** in 58% yield. The pentacyclic core was obtained in 51% yield *via* cleavage of the protecting group and ammonolysis. Further cleavage of the allyl ester and subsequent coupling with **[112]** furnished the amide. Epimerization of the ester with Et_3N in methanol produced the α–ester ptilomycalin A analogue **[113]**. Further deprotection with formic acid furnished (-)-ptilomycalin A in quantitative yield.

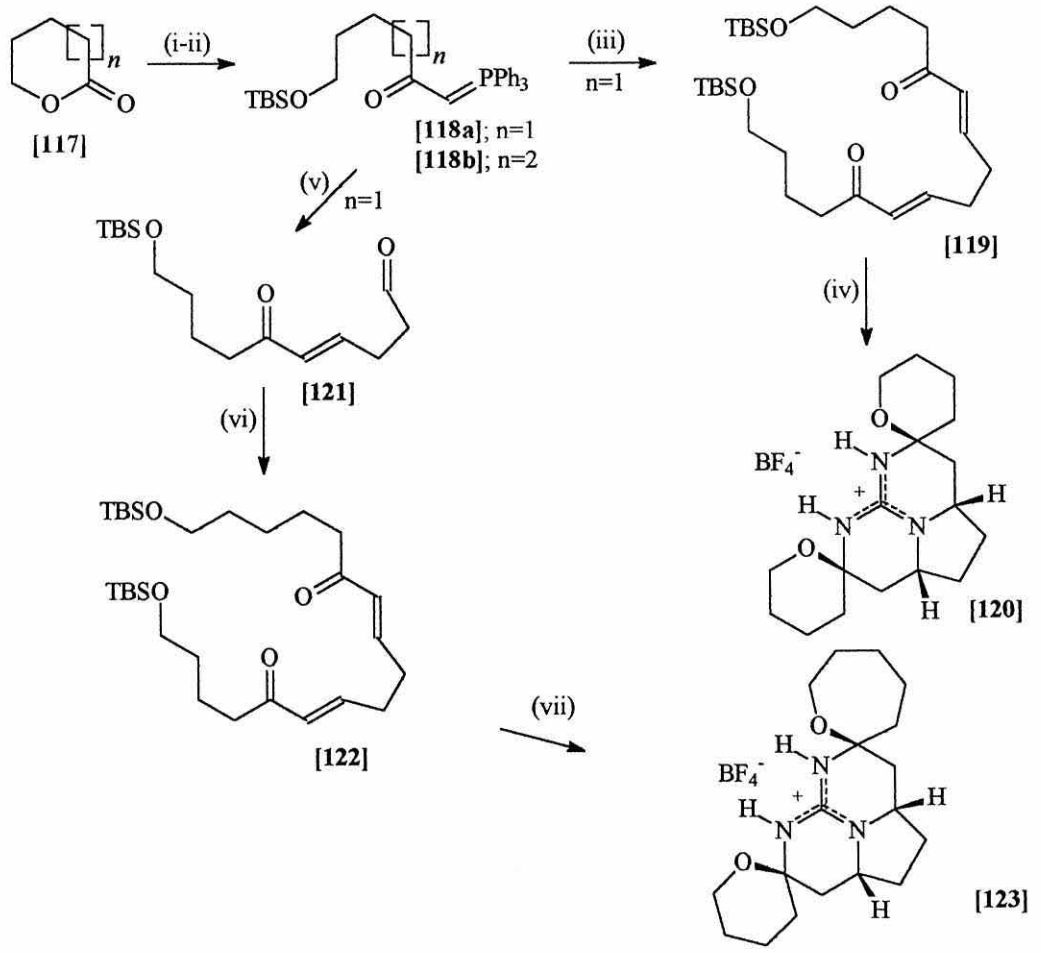


Scheme 21. ***Reagents and Conditions***: (i) 2 equiv. **[112]**, THF, -78°C, morpholinium acetate; (ii) Swern oxidation, 58% overall yield; (iii) TBAF, NH_3, NH_4OAc, ^tBuOH, 60°C, 51%; (iv) $Pd(Ph_3P)_4$, pyrrolidine, MeCN, 23°C, 75%; (v) **[115]**, EDCI, DMAP, DCM, 23°C, 60%; (vi) Et_3N, MeOH, 65°C, 50%; (vii) HCO_2H, 23°C, 100%.

Murphy *et al.*[36, 65] have also been pursuing the total synthesis of ptilomycalin A utilising a strategy based upon the double 1,4-Michael addition of guanidine to a *bis*-α,β-unsaturated ketone. Preliminary studies illustrated that the formation of pentacyclic guanidines **[120]** and **[123]** was possible using this strategy. The substrate utilised in their investigations was prepared by the reaction of lactones **[117]** with two equivalents of methylene triphenylphosphorane, followed by silyl protection of the intermediate phosphonium alkoxide furnishing phosphorane **[118]**. Wittig reaction of **[118a]** with succinaldehyde gave the symmetrical *bis*-α,β-unsaturated ketone **[119]** in 54% yield. Further reaction of **[119]** with one equivalent of guanidine, followed by removal of solvent, deprotection/cyclisation with methanolic HCl and subsequent counter-ion exchange afforded two products identified as the *cis* and *trans* pentacycles **[120]** in a 4:1 ratio. Murphy *et al.* isolated the major *trans* product in 25% yield by recrystallisation.

Furthermore, Murphy *et al.* found reaction of phosphorane **[118a]** with an excess of succinaldehyde led to the formation of aldehyde **[121]** in 43% yield, based upon the lactone starting material. Subsequent reaction with phosphorane **[118b]** gave

the unsymmetrical *bis*-α,β-unsaturated ketone **[122]**. Reaction of **[122]** with guanidine led to the formation of two pentacycles again in a 4:1 ratio, and from which the unsymmetrical pentacycle **[123]** was obtained by recrystallisation in 20% yield.

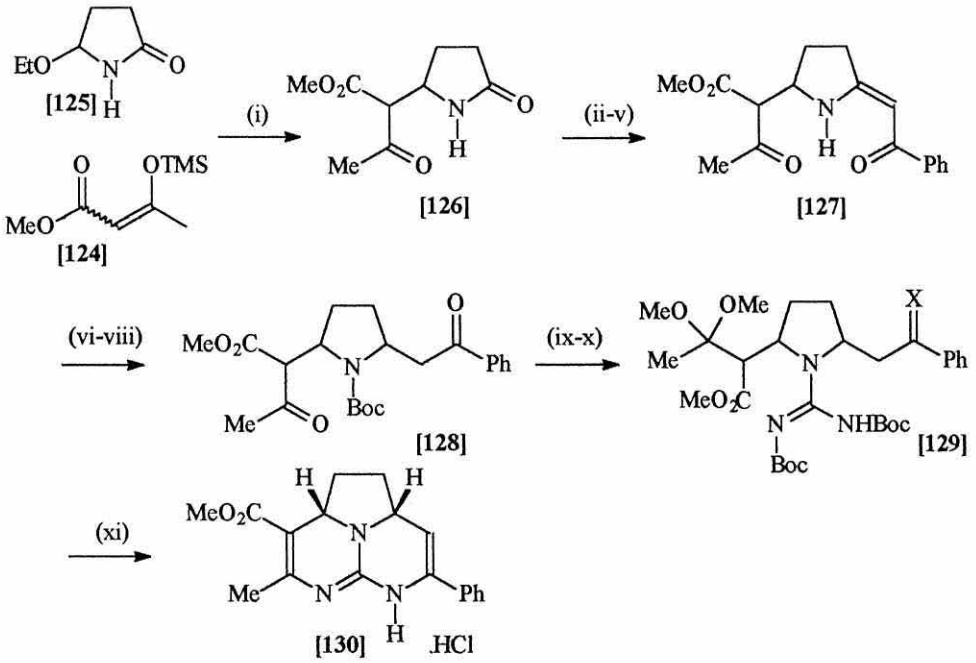


Scheme 22. ***Reagents and Conditions***: (i) 2 Eqv. CH_2=PPh_3, THF, -78°C; (ii) TBDMSCl, Imidazole, DMF; (iii) 0.4 Eqv. succinaldehyde, THF, 48h, 54% overall, (iv)(a) Guanidine, DMF, 3h., (b) MeOH, HCl, 0°C-RT, 24h, (c) aq. $NaBF_4$ (sat), (d) Trituration and crystallisation; 25% overall; (v) steps (i-ii) then 10 Eqv. succinaldehyde, THF, 43%; (vi) **[118b]**, THF, 48 h, 37%; (vii) as (iv) 20%.

An alternative theory to the production of ptilomycalin A was suggested by Hiemstra and co-workers in 1996.[66] In this tricyclic guanidines were prepared from substituted pyrrolidin-2-ones utilising an N-acyliminium ion coupling reaction with silyl enol ethers. Followed by a direct guanylation reaction with *bis*-BOC-thiourea and mercury (II) chloride.

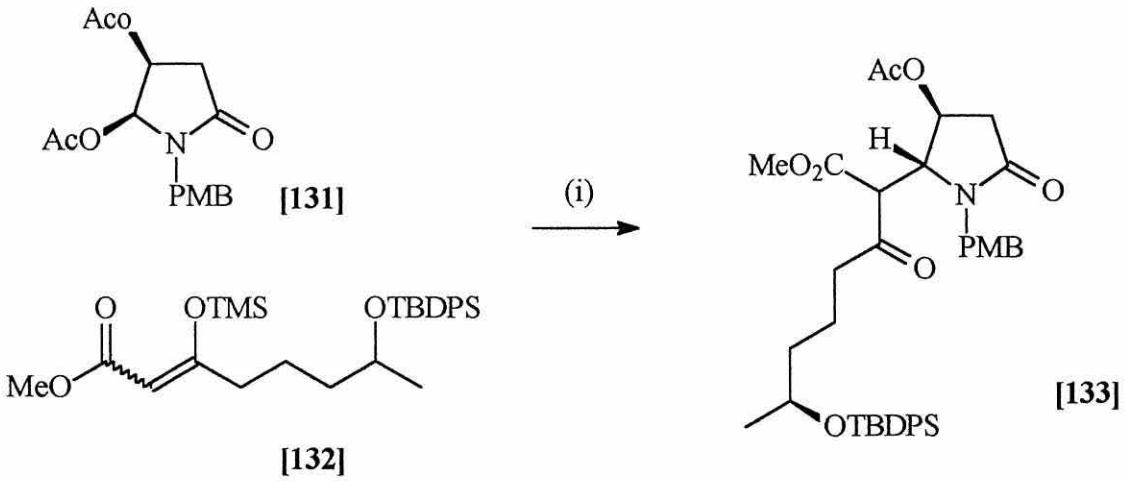
The authors reported that reaction of silyl enol ether **[124]** with lactam **[125]** led to the formation of the substituted lactam **[126]** in a 63% yield. After conversion of **[126]** into the corresponding thiolactam, an Eschenmoser sulfide contraction procedure was utilised leading to the formation of the vinylogous amide **[127]**. Subsequent

reduction and N-BOC protection gave **[128]**. They then subjected **[128]** to a three stage procedure which included the protected guanidine **[129]** as an intermediate. Cyclisation under acidic conditions furnished guanidine **[130]** in 33% overall yield, accompanied by several other *trans*-substituted guanidines which were recycled by treatment with ammonia and ammonium acetate in methanol at 60°C to give further **[130]**.



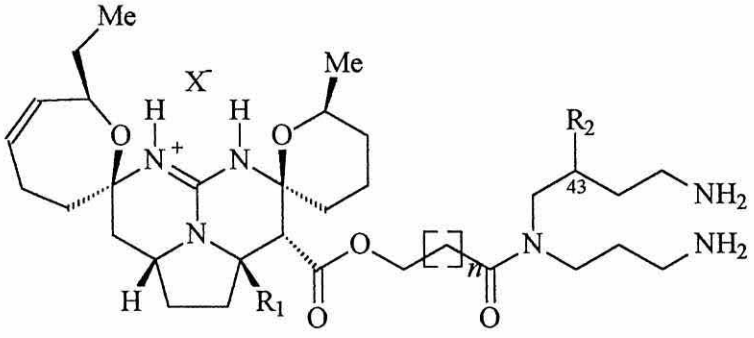
Scheme 23. ***Reagents and Condition:*** (i) TMSOTf, -78°C-RT, DCM, 18h, 63%; (ii) Lawesson's reagent, $PhCH_3$, 80°C, 10 min, 91%; (iii) $PhCOCH_2Br$, Et_2O, RT, 18h; (iv) Et_3N, DCM, RT, 2h, 83%; (v) PPh_3, DCM, 60°C, 18h, 82%; (vi) $NaCNBH_3$, 3:1 AcOH-THF, 0°C, 40 min, 99%; (vii) Boc_2O, DIPEA, THF, RT, 18h, 91%; (viii) PCC, DCM, mol. sieves (4A), RT, 3h, 91%; (ix) $CH(OMe)_3$, H_2SO_4 (cat), MeOH, 50°C, 5h; (x) $SC(NHBoc)_2$, $HgCl_2$, Et_3N, DMF, 0°C-RT, 18h, X=O, $(OMe)_2$; (xi) HCl, MeOH, RT, 3h, 33% for three steps.

In 1996 Hiemstra *et al.*[66] further elaborated on his methodology by reporting the reaction of *bis*-acetoxylactam **[131]** with silyl enol ether **[132]** in a stereocontrolled fashion to furnish **[133]**, which could be an intermediate in the total synthesis of ptilomycalin A.



Scheme 24. ***Reagents and Conditions***: (i) TMSOTf, -78°C-RT, DCM, DIPEA, 1h, 75%.

2.5.3 THE CRAMBESCIDINS



[134]; R_1=R_2=OH, n=14
[135]; R_1=R_2=OH, n=15
[136]; R_1=R_2=OH, n=16
[137]; R_1=H, R_2=OH, n=14

Fig. 17. The Crambescidins 816 **[134]**, 830 **[135]**, 844 **[136]**, 800 **[137]**. X=unspecified.

Crambescidins 816 **[134]**, 830 **[135]**, 844 **[136]** and 800 **[137]** were isolated in 1991 by Rinehart[67] from the red encrusting sponge *Crambe crambe*. More recently **[137]** has been isolated from a Brazilian specimen *Mananchora arbuscula*[68] and *Celerina heffernani*.[58] The structural similarities between ptilomycalin A and the Crambescidins is easily notable, both have the unique pentacyclic core and the linear ω-hydroxy fatty acid which links the core to the spermidine or hydroxy spermidine moiety respectively. These compounds differ only in the length of the ω-hydroxy fatty acid linker and the presence or absence of a hydroxy group at C-43. As in ptilomycalin A the crambescidins are biologically active with **[134]**, **[136]** and **[137]** inhibiting HSV-1 completely, with diffuse cytotoxicity at 1.25μg/ml and are 98% effective against L1210

cell growth at 0.1μg/ml. However, 13,14,15-*iso*crambescidin 800[69] **[138]** (Fig. 18) was substantially less cytotoxic to L1210 and had no observable antiviral activity against HSV-1 and in contrast to the other crambescidins the protons around the pyrrolidine ring were *anti* as were the spirocyclic units present in the pentacycle, depicted in fig. 18.

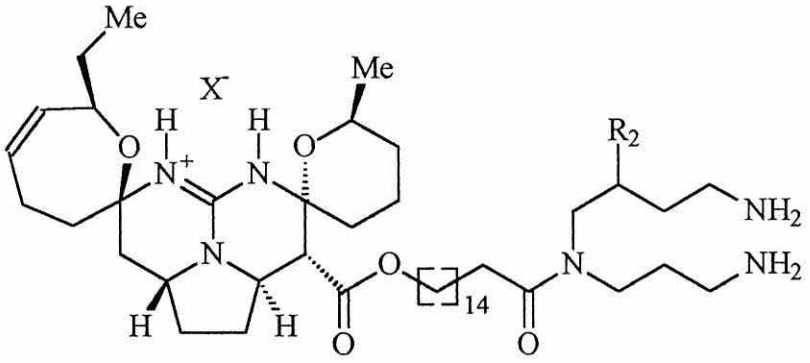
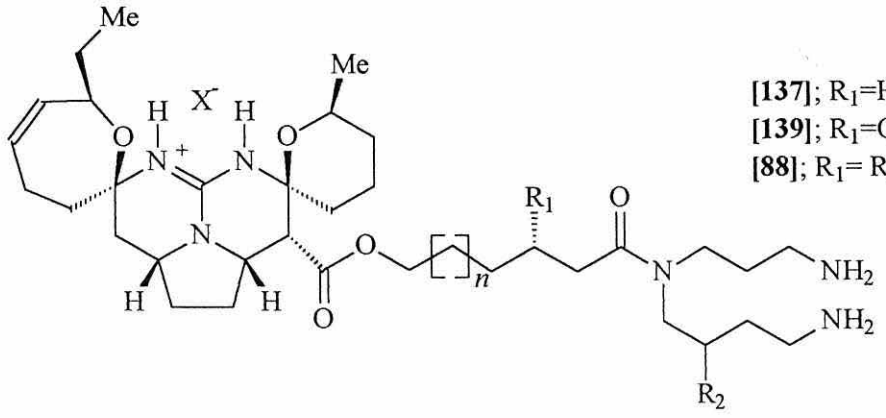


Fig. 18. 13,14,15-*iso*crambescidin 800 **[138]**.

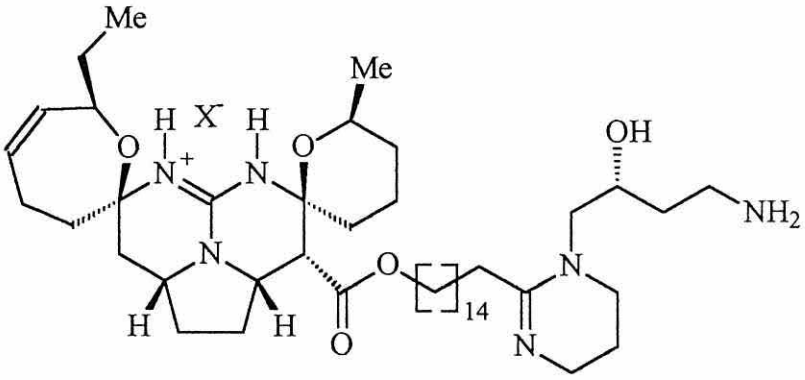
Structural elucidation of the crambescidins was achieved using HRFABMS, ^{1}H and ^{13}C NMR coupled with COSY, COLOC and HMBC experiments.

2.5.4 CELEROMYCALIN AND FROMIAMYCALIN

Furthermore in 1995 Minale and co-workers[58] isolated two new highly cytotoxic guanidine alkaloids from the starfish *Fromia manilis* and *Celerina heffernani* collected off New Calodonia, species which already had been shown to contain: ptilomycalin A and the crambescidins. These metabolites celeromycalin **[139]** and fromiamycalin **[140]** contained the same pentacyclic core as ptilomycalin A and crambescidin 800 and celeromycalin possessed a similar spacer unit and spermidine moiety.



[137]; R_1=H, R_2=OH, n=11
[139]; R_1=OH, R_2=H, n=11
[88]; R_1= R_2=H, n=11



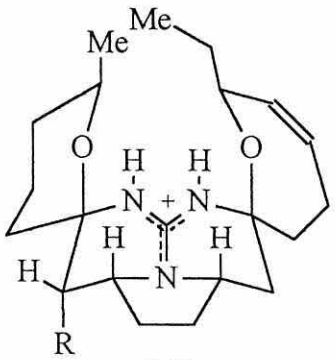
[140]

Fig. 19. Celeromycalin **[139]**, ptilomycalin A **[88]**, crambescidin 800 **[137]**, and fromiamycalin **[140]**.

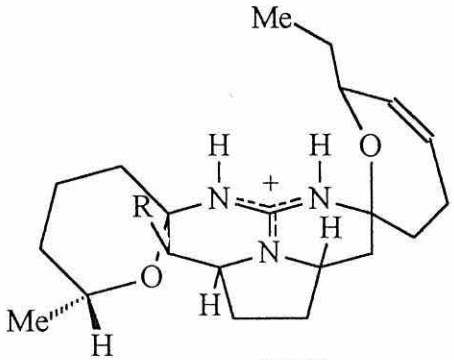
Both celeromycalin and fromiamycalin were shown to be active against cells CEM4 infected by HIV-1 with a IC_{50} of 0.32 μg/ml and 0.11 μg/ml respectively without cytoprotective effects at a dose of <0.1μg/ml. These results relate closely to the other family members of this unique group of alkaloids. This emerging pattern suggests that a relationship exists between the structural features of the alkaloids and its biological activity.

2.5.5 BIOLOGICAL ACTIVITY

There has been much speculation as to the exact biological role of ptilomycalin A which has centred on its similarities to abiotic guanidine based anionic receptor molecules and its involvement in oxoanionic bonding (nucleotides or phosphates). This concept is supported by considering the comparison between the pentacyclic cores of ptilomycalin A **[88]** and the inactive 13,14,15-*iso*crambescidin 800 **[138]**.



[88]

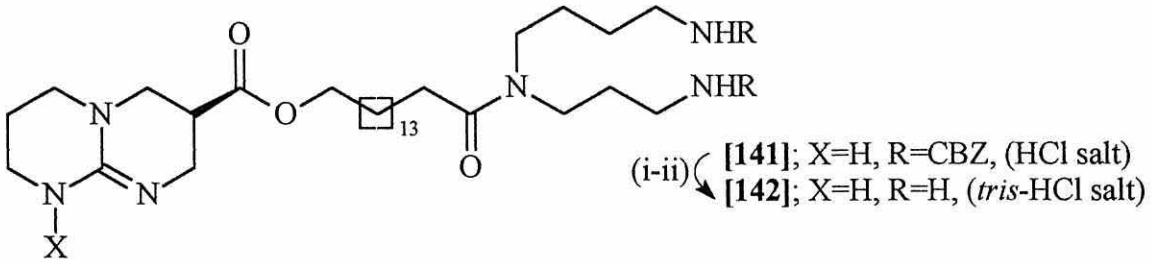


[138]

Fig. 20. Pentacyclic core of **[88]** and **[138]**.

The above diagram (Fig. 20) shows that there exists within the pentacyclic core of ptilomycalin A **[88]** an ionic pocket, which could be acting as a recognition site and conveying much of the biological activity.

In 1995 Hart and Grillot[70] reported the synthesis of a structural analogue which contained the same ω-hydroxy fatty acid spacer and spermidine moiety as ptilomycalin A but replaced the polycyclic core with a bicyclic core. This was prepared in an effort to mimic the biological activity of ptilomycalin A. Analogue **[142]** was prepared in 6% overall yield *via* a 12 step convergent sequence from acyclic precursors. They discovered that although carbamate **[141]** was stable, the amine **[142]** underwent cleavage of the ester linkage *via* an unidentified process, thus precluding the determination of its biological activity. Hart *et al.* did speculate that the role of the spiro-*N*-*O*-acetals linkage in ptilomycalin A could be to protect the ester functionality from hydrolysis or aminolysis.



Scheme 25. ***Reagents and Conditions***: (i) $Pd(OH)_2$, 1,4-cyclohexadiene, EtOH; (ii) HCl, MeOH, 70%, 6% overall yield.

2.6 THE BATZELLADINE ALKALOIDS

2.6.1 INTRODUCTION

Since the emergence of HIV and AIDS a great deal of research has been conducted in an attempt to find a cure and develop effective treatments to alleviate the symptoms. The hallmark of the disease is the progressive decline in the number of $CD4^+$ cells that are present in the body, which ultimately leads to the decline of the immune system and consequently those infected become susceptible to opportunistic diseases such as pneumonia.[71] HIV is known to be the primary cause of AIDS, a fact originally reported by SmithKline and Beecham.[71] The HIV gp120 binds to the $CD4^+$

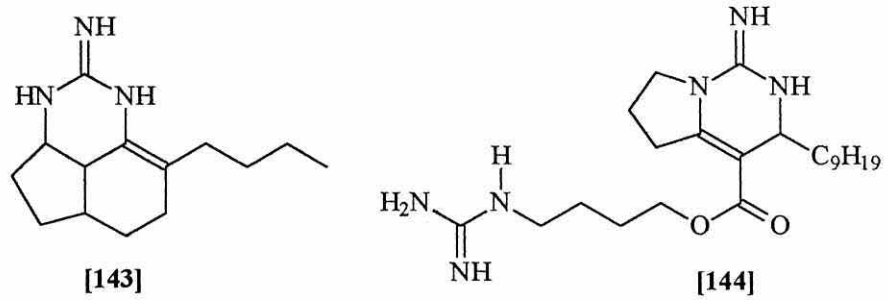
cell surface receptor protein and initiates the decline in the CD4 cells and so, antagonism of HIV replication is a therapeutic approach for the treatment of AIDS.

2.6.2 BATZELLADINES A-E

2.6.3 ISOLATION AND STRUCTURAL ELUCIDATION

The Batzelladines A-E were isolated by Patil *et al.* in 1995[72] from the Caribbean sponge *Batzella sp.* together with ptilomycalin A and several other known metabolites. Originally this sponge was thought to be of a different species to *Ptilocaulis spiculifer*, the sponge from which ptilomycalin A was isolated; however, after re-examination of Kashman's voucher specimen by Van Soest,[72] the correct assignment was achieved and the two were found to be in fact identical.

A total of 21 compounds was obtained during this screening, several known alkaloids: crambsecidin 800 **[137]**, 816 **[134]** and some minor metabolites relating to ptilomycalin A **[88]**, ptilocaulin **[143]** and crambescin A **[144]**.



[143] **[144]**

Fig. 21. Ptilocaulin and crambescin A.

Patil *et al.* used ^{1}H and ^{13}C NMR coupled with *inter alia* DQFCOSY, COSY 45, TOCSY, HMQC, COLOC and HMBC experiments, as well as FABMS and correlated their findings with the data from ptilocaulin and crambescin A.

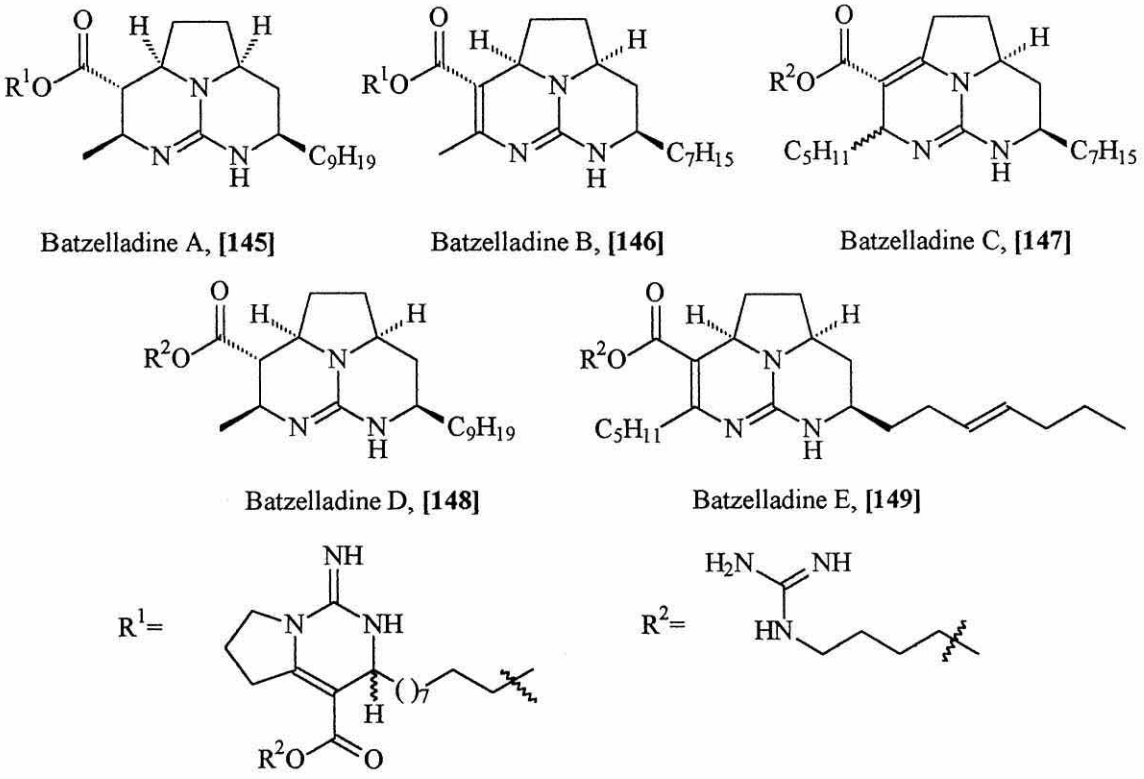


Fig. 22. A representation of the batzelladines A-E with the original stereochemical assignment.

Interestingly batzelladines A **[145]** and B **[146]** were the first low molecular weight natural products to inhibit the binding of HIV gp120 to CD4 cells and thus have potential as anti-AIDS agents.

2.6.4 SYNTHETIC APPROACHES

Several approaches towards the synthesis of the batzelladines have been reported, mainly concentrating on the tricyclic moiety. Initial investigations were pursued by Murphy *et al.* in 1996[73] whereby they reasoned that the tricyclic core could be accessed using a proposed biomimetic approach *via* a sequential double Michael addition of guanidine to a *bis*-α,β-unsaturated ketone, thus producing a hemiaminal structure, which upon selective reduction, would afford the desired tricyclic system fig. 23.

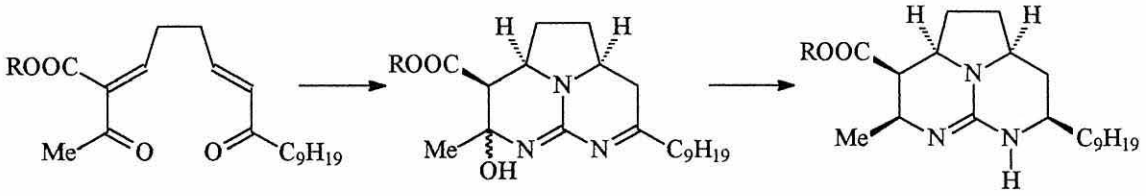
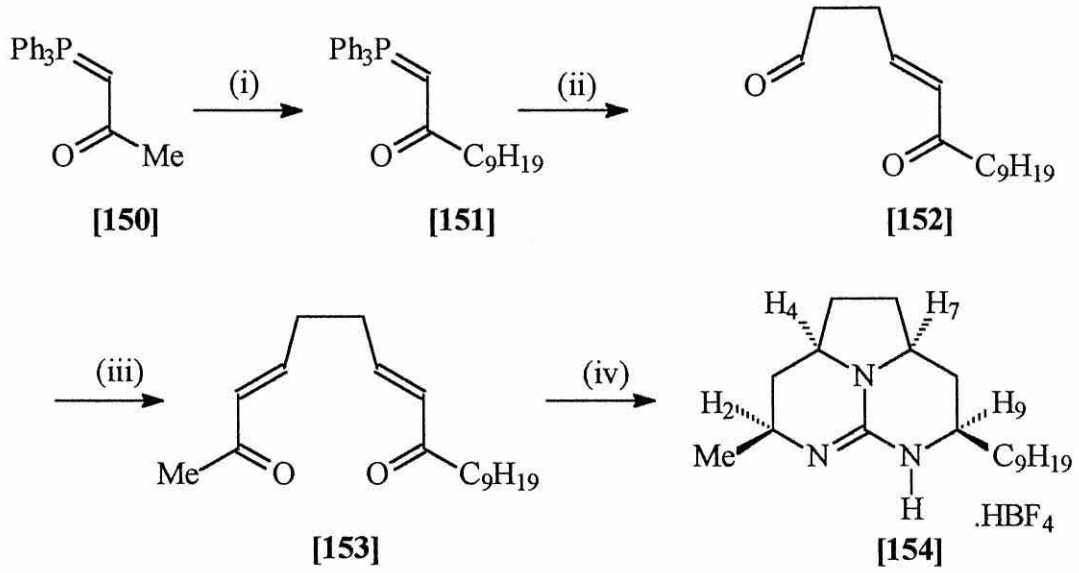


Fig. 23. Murphy *et al.* proposed synthesis.

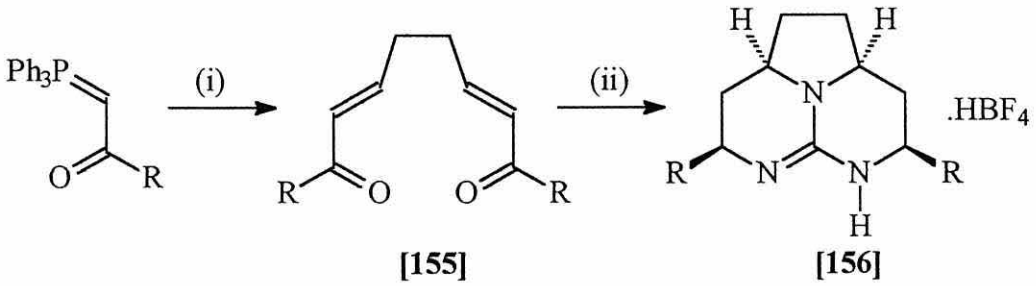
This hypothesis was tested by preparing simple *bis*-α,β-unsaturated ketones in three high yielding steps.



Scheme 24. ***Reagents and Conditions***: (i) *n*-BuLi, -78°C, $C_8H_{17}I$, RT, 16h; (ii) 3.0 eqv. succinaldehyde, THF, 24h; (iii) $MeCOCHPPh_3$, DCM, 24h; (iv)(a) Guanidine, DMF, 0°C-RT, 5h; (b) 3:1:3, DMF / H_2O / MeOH, $NaBH_4$, 16h; (c) HCl; (d) saturated aq. $NaBF_4$.

Deprotonation of the commercially available phosphorane **[150]**, which was achieved with *n*-BuLi, followed by alkylation with octyl iodide produced the desired phosphorane **[151]** in quantitative yield. Subsequent treatment with succinaldehyde yielded α,β-unsaturated ketone **[152]** in 71% overall yield. Further treatment under Wittig conditions with **[150]** produced in 66% yield *bis*-α,β-unsaturated ketone **[153]**. Addition of guanidine *via* subsequent Michael additions, selective reduction with $NaBH_4$ and ion exchange afforded the desired tricyclic analogue **[154]** in 31% yield as a single diastereomer. The relative stereochemistry around the four stereocentres H_2, H_4, H_7 and H_9 was obtained *via* NOE studies and crystallographic data. A series of analogues of **[154]** were produced by varying the phosphorane used (Table 4) and in

line with the previous example **[154]** the relative stereochemistry was identical to that originally reported for the batzelladines.



[155] **[156]**

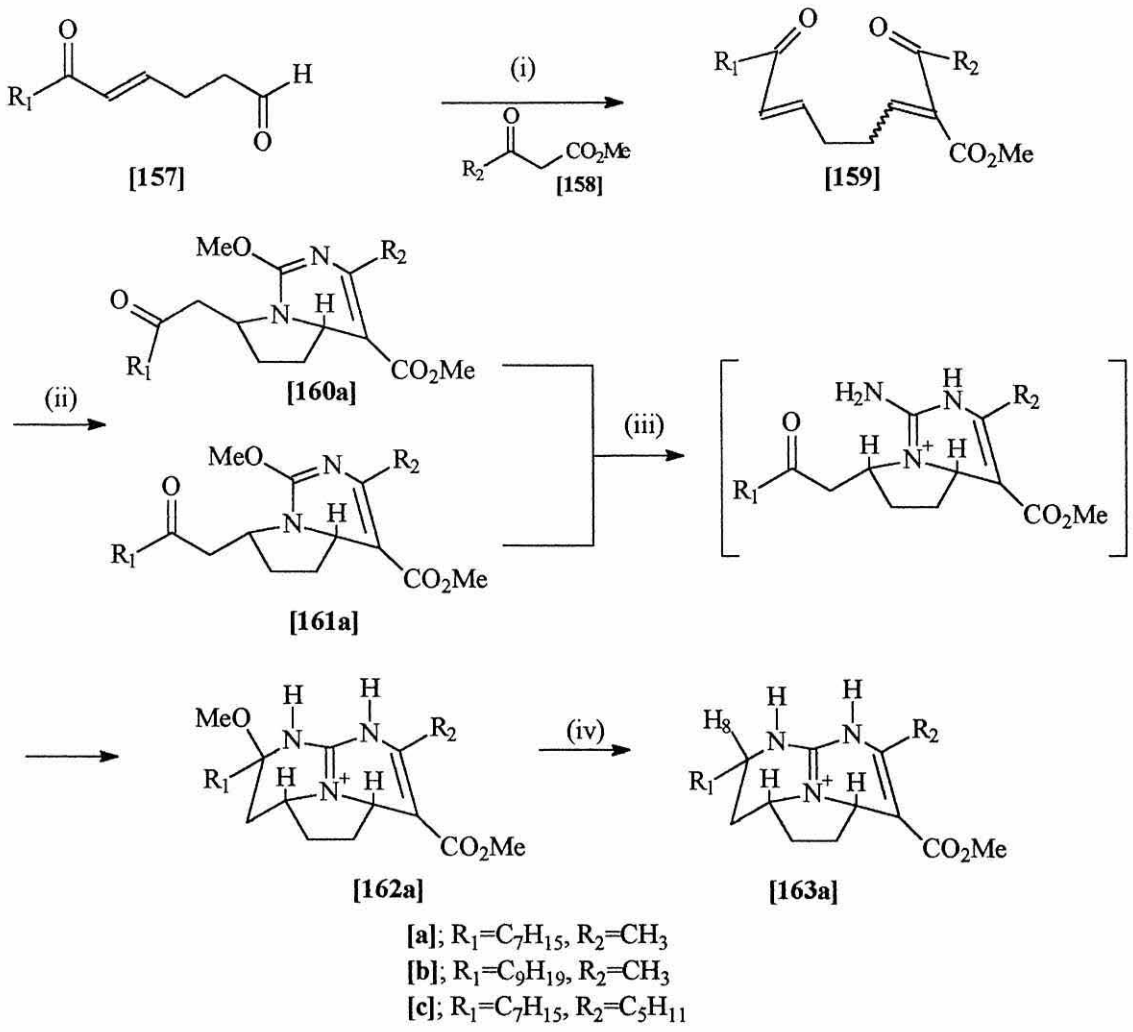
Scheme 27. ***Reagents and Conditions***: (i) 0.4 eqv. succinaldehyde, THF, 24-48h; (ii)(a) Guanidine, DMF, 0°C-RT, 5-8h; (b) 3:1:3, DMF / H_2O / MeOH, $NaBH_4$, 16h; (c) HCl; (d) saturated aq. $NaBF_4$.

R	Percentage Yield of [155]	Percentage Yield of [156]
Me	74	33
Ph	68	32
n-pentyl	54	27
n-nonyl	36	22

Table 4. Yields for tricyclic formation.

A further synthetic approach was reported by Snider and Chen in 1996[74] which initially led to the tricyclic portions of batzelladines A **[145]**, B **[146]**, D **[148]**, E **[149]** and revision of the stereochemistry of the tricyclic moieties of A and D.

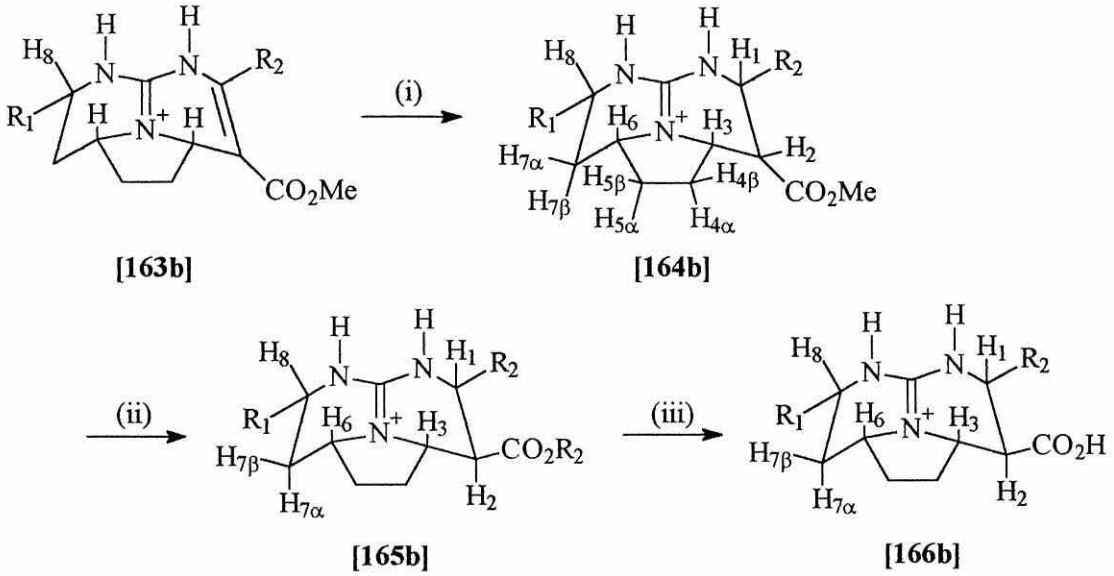
Their approach was based on earlier findings of guanidine systems and involved *trans* **[160a]** being obtained from a Knoevenagel condensation reaction of **[157]** and **[158]**. As with work on ptilomycalin A, Snider *et al.* formed the tricyclic unit with the reaction of *O*-methylisourea under standard conditions leading to a 35% yield of a 6:1 mixture of the dihydropyrimidine **[160a]** and **[161a]**. Ammonolysis of **[160a]** and **[161a]** afforded **[162a]** in 56% yield as the only isolated product. Careful reduction of **[162a]** with $NaCNBH_3$ in NaH_2PO_4-buffered MeOH at 25°C for 16 hours furnished **[163a]** which on comparison with the reported NMR data for the methanolysis product of batzelladine B proved to be identical.



Scheme 28. ***Reagents and Conditions***: (i) **[158]**, DCM, 0.2 eqv. piperidinium acetate, 48h, -20°C; (ii) 1.5 eqv. *O*-methylisourea hydrogen sulphate, *i*-Pr_2EtN, DMSO, 75°C, 5h, 6:1 *trans* **[160]**: *cis* **[161]**, 35% from **[157]**; (iii) NH_4OAc, MeOH sat. with anhydrous NH_3, 48h, 60°C, 56%; (iv) $NaCNBH_3$, NaH_2PO_4-buffered MeOH, 25°C, 16hrs, >90%.

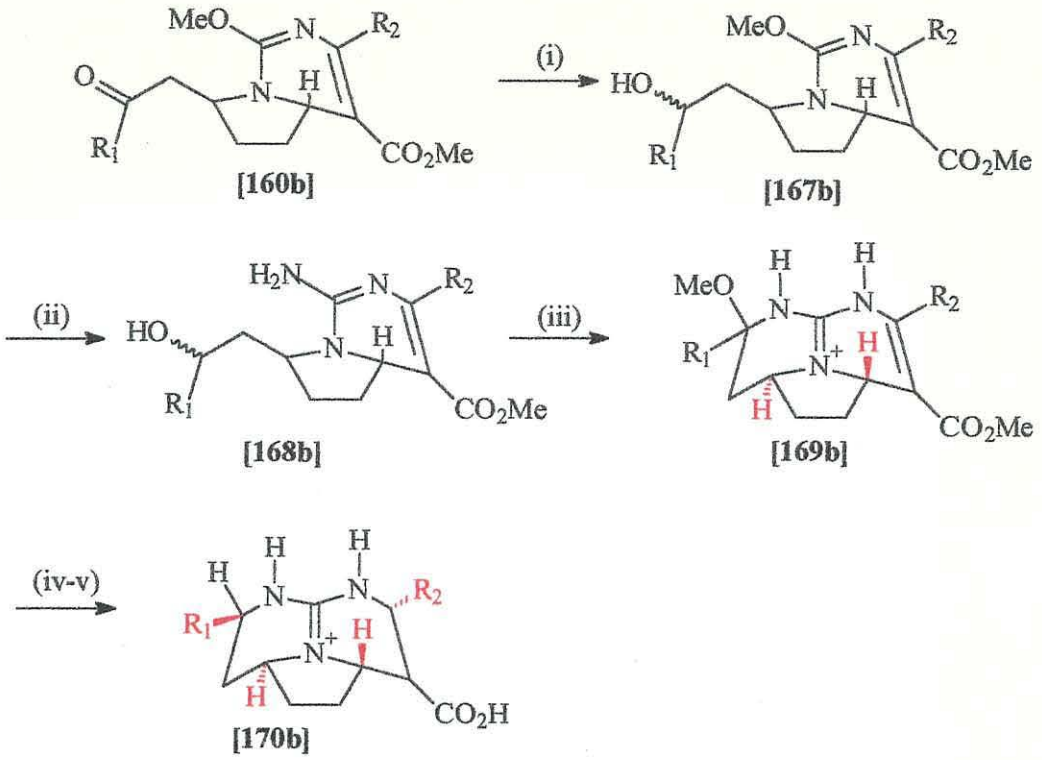
The reduction of **[162a]** forming **[163a]** provided Snider and Chen with an efficient stereoselective route to access the tricyclic core of batzelladine B.

The authors employed the same methodology to access **[163b]** by an analogous series of reactions using decanal, rather than octanal to prepare **[157]**. Hydrogenation of **[163b]** furnished **[164b]** in >90% yield. They observed that the data for **[164b]** was different to the NMR data of the tricyclic portion of batzelladine A, suggesting the tricyclic portions were in fact not the same. As a result of this discrepancy **[164b]** was converted to the hydrolysis product of batzelladine A by epimerisation of **[164b]** with NaOMe, which afforded **[165b]** again in >90% yield followed by subsequent hydrolysis to furnish acid **[166b]** in 85% yield.



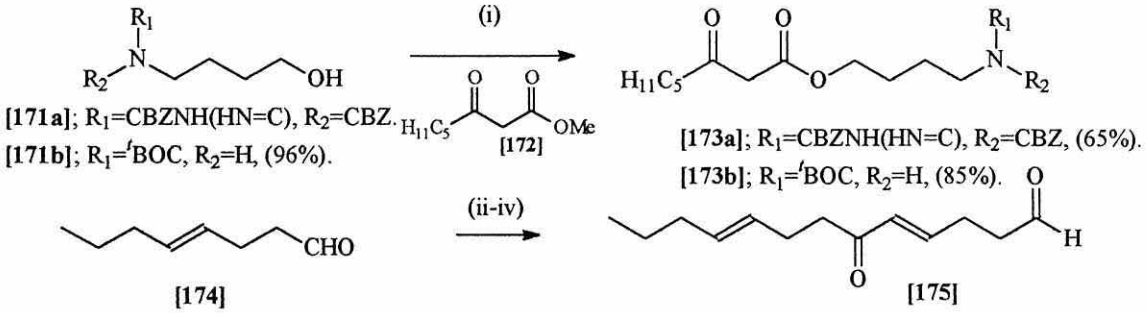
Scheme 29. ***Reagents and Conditions***: (i) $H_2/Rh/Al_2O_3$, 100:1 MeOH-formic acid, >90%; (ii) NaOMe, MeOH, 25°C, 12hrs, >90%; (iii) NaOH, MeOH, 25°C, 12hrs.

Comparison of the NMR data of both the natural and synthetic **[166b]** determined that the protons $H_{1\text{-}3}$ and $H_{6/8}$ were axial in both compounds, inferring the difference to be around the syn/anti arrangement of the two six membered rings. As a result of this Snider and Chen prepared the hydrolysis product from the *trans*-isomer **[160b]**. The ketone function was protected by reduction to alcohol **[167b]**, which was then converted to guanidine **[168b]**. Regeneration of the ketone function led to the formation of the tricyclic guanidine **[169b]** which was reduced and saponified to furnish the hydrolysis product **[170b]**, so establishing the stereochemistry in these two products A and D to be indeed *trans* and not *cis* across the pyrrolidine ring.

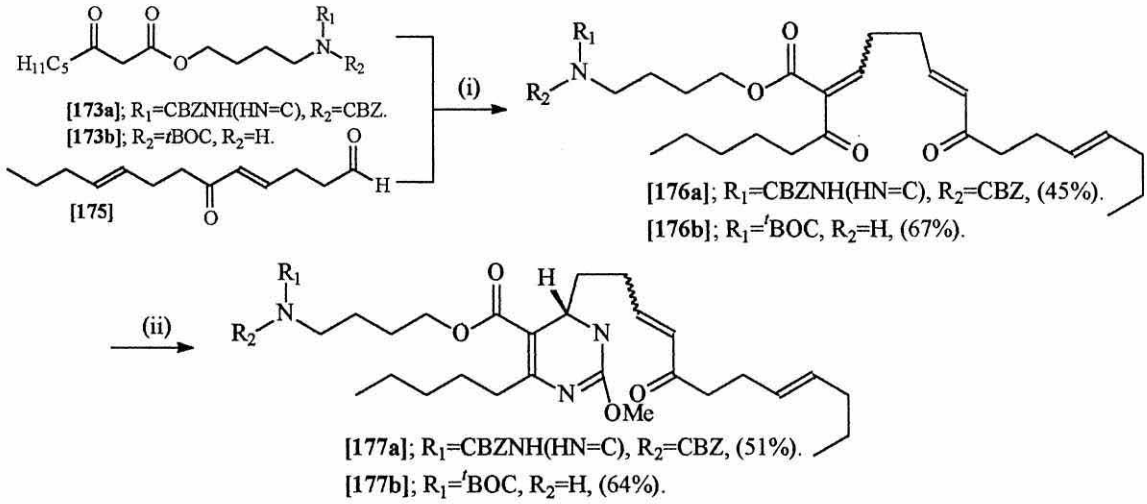


Scheme 30. ***Reagents and Conditions***: (i) $NaBH_4$, *i*-PrOH, 25°C; (ii) NH_3, NH_4OAc, MeOH, 60°C, 2 d, (iii) Dess-Martin reagent, DCM, 25°C, then MeOH, 25°C, 12 h; (iv) $NaCNBH_3$, NaH_2PO_4, MeOH, 25°C, 16 h, then 65°C, 5h; (v) NaOH, MeOH, 25°C, 18h.

In 1998 Snider and Chen[75a] reported the first total synthesis of batzelladine E and its associated isomer using a similar approach to the one reported in 1996 which gave them access to the tricyclic cores of batzelladines A, B and D. Originally **[149E]** was prepared with the guanidino butyl ester present α to the pyrrolidine unit before production of the polycyclic framework. Earlier research had indicated that once the polycyclic framework had been formed the ester functionality could not be introduced.[76] Their initial investigations led to the Knoevenagel condensation of **[173a]** (prepared by DMAP catalysed condensation of **[171a]** with methyl 3-oxooctanoate **[172]**) with **[175]** (prepared from aldehyde **[174]**) producing **[176a]** as a 1:1 mixture of stereoisomers in 45% yield.

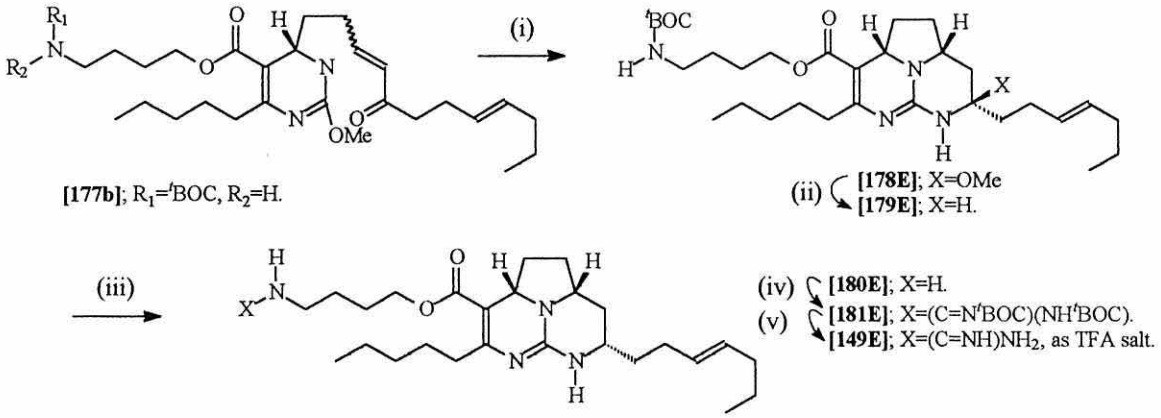


Scheme 31. ***Reagents and Conditions***: (i) **[172]**, DMAP, C_6H_6, 80°C; (ii). $LiCC(CH_2)_3OTBDMS$, THF, -78°C, 86%; (iii) LAH, THF, 65°C, 90%; (iv). Dess-Martin periodinane, 82%.



Scheme 32. ***Reagents and Conditions***: (i) Piperidinium acetate, DCM, -20°C, 72h; (ii). *O*-methylisourea hydrogen sulphate, DMSO, *i*-Pr_2EtN, 55°C, 4h.

Heating **[176a]** with *O*-methylisourea hydrogen sulphate afforded **[177a]** as a ratio of 6:1 *trans:cis* in 67% yield. However, they established that final cyclisation could not occur due to a CBZ protecting group being cleaved during final ring closure, due to this **[173b]** was prepared in two steps from 4-amino-1-butanol in 85%. After a series of analogous reactions **[177a]** was obtained in 64% as a mixture of 6:1 *trans:cis* isomers. On this occasion Snider and Chen found that cyclisation proceeded without any problems forming the tricyclic system **[178E]** which, after reduction with $NaCNBH_3$, formed **[179E]** in 42% yield.

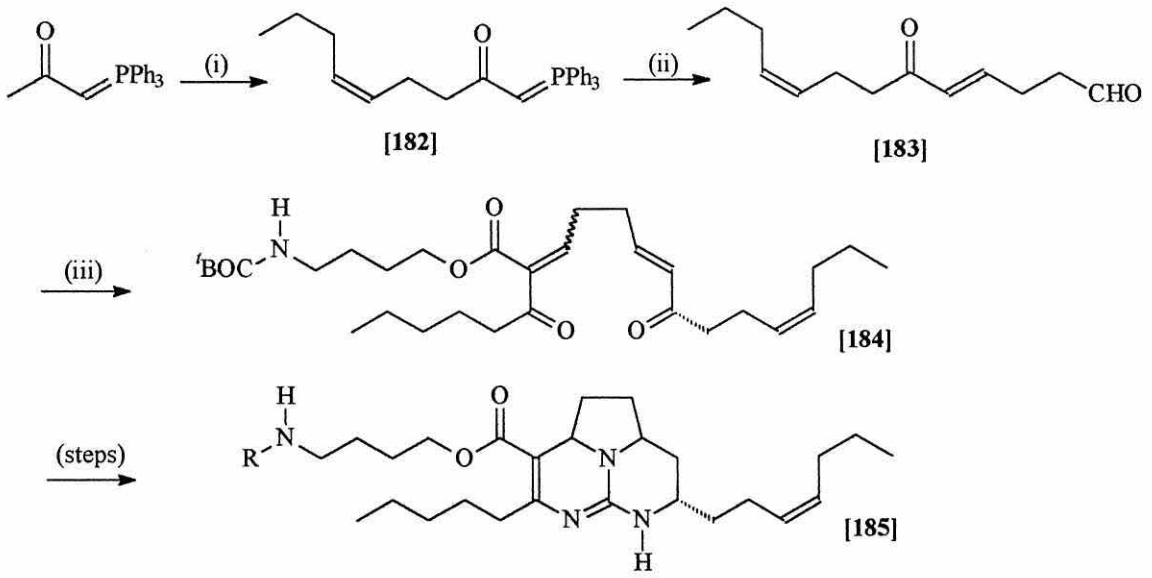


Scheme 33. ***Reagents and Conditions***: (i) NH_4OAc, NH_3, ^tBuOH, 60°C, 24h; (ii) $NaCNBH_3$, NaH_2PO_4, MeOH, 25°C, 42%; (iii) 1:4 TFA / DCM, 90%; (iv) *N,N'-di*-(t-butoxycarbonyl)thiourea, 2-chloro-methylpyridinium iodide, Et3N in DCM, 70%; (v) 1:1 TFA / DCM, 2h, 88%.

Deprotection of **[179E]** with TFA and DCM afforded **[180E]** in 90% yield. The guanidine side chain was introduced by treatment with *N, N'-di*-(t-butoxycarbonyl) thiourea, 2-chloro-methylpyridinium iodide, Et_3N and DCM following Lipton's[75b] procedure. Subsequent deprotection gave **[149E]** in 88%.

NMR correlation of Snider's synthetic analogue **[149E]** and the isolated compound displayed some differences, most notably in the olefinic region. The alkene hydrogens of **[149E]** resonated at δ 5.52 and 5.44 ppm each as a dt J = 15.5 and 6.0 Hz., whereas in the isolated structure the corresponding protons overlapped. Snider and Chen suggested that, because allylic carbons of *cis* alkenes absorb 5-6 ppm upfield of *trans* alkenes in ^{13}C NMR, the data suggested that batzelladine *E* had a *Z* configuration rather than the supposed *E*.

To test their hypothesis Snider *et al.* synthesised (*Z*) batzelladine E in a 9 step convergent synthesis initially utilising methodology developed by Murphy[36, 65] to alkylate the phosphorane and produce **[182]**. Condensation with succinaldehyde produced **[183]** in 65% yield. Subsequent Knoevenagel condensation reaction, with 0.33 equivalents of piperidine and 0.30 equivalents of acetic acid to avoid isomerisation to the unwanted *E* isomer, was conducted producing **[184]**. The original methodology which gave them access to the *E* isomer was employed and **[149Z]** was obtained in a 3% overall yield. The key steps are highlighted in scheme 34.



Scheme 34. ***Reagents and Conditions***: (i) *n*-BuLi, THF, -78°C, 1-bromo-2Z-hexene, 64%; (ii) succinaldehyde, THF, -25°C, 24h, 65%; (iii) 0.33 eqv. piperidine, 0.3 eqv. HOAc, DCM, -20°C, 48h.

Again NMR correlation with **[185]** made apparent some differences but Snider postulated that this was due to concentration factors and the fact that his analogue was a TFA derivative whereas the original species wasn't.

The most recent approach to the batzelladines was reported by Overman and co-workers in 1999.[77,78] They envisaged the use of a tethered Biginelli reaction[77,78] as a strategic key in the construction of the tricyclic core of the batzelladines, the key stage of the reaction centres around guanidine **[186]** and β-ketoester **[187]** combining as illustrated in fig. 24.

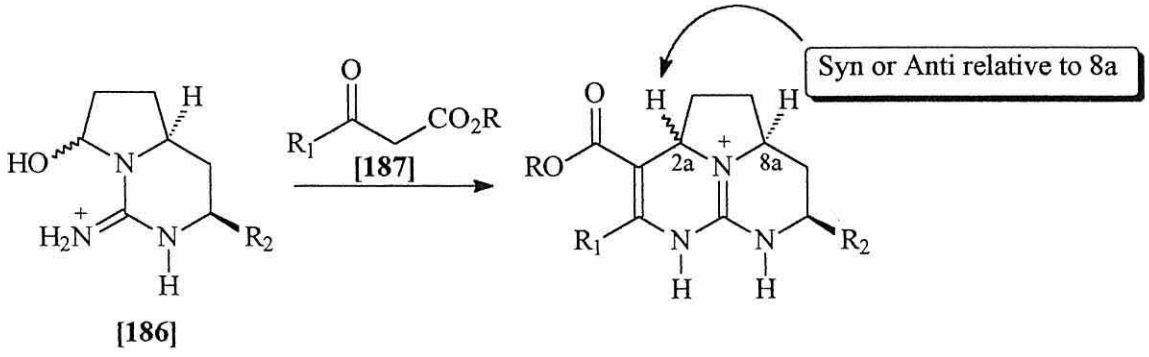
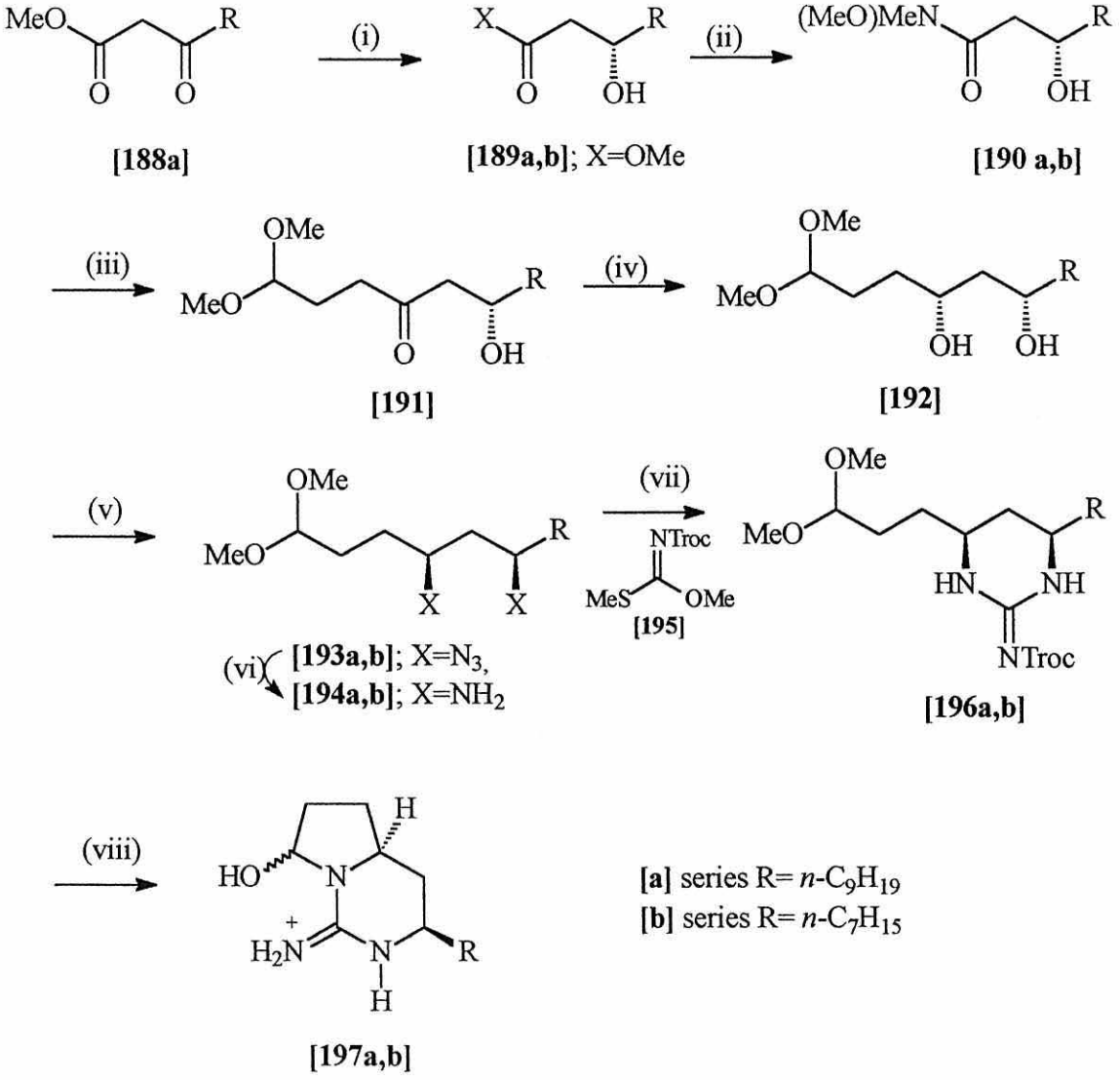


Fig. 24. Key stage in Overman *et al.* synthesis.

Guanidine **[186]** was prepared in two series **[a,b]**. Initial exploratory studies **[a]** utilised a *n*-nonyl side chain intermediate whilst series **[b]** utilised *n*-heptyl side chains. There were several key steps incorporated within the synthetic pathway, firstly, the

enantioselective reduction of **[188a]** using a modification of Noyori's procedure afforded **[189a]** in 93% yield with a 95% ee. Conversion of **[189a]** to Weinreb amide **[190a]** followed by addition of 3,3-dimethoxypropylmagnesium bromide afforded **[191a]** in 71% overall yield. Selective reduction with diethylmethoxy borane and $NaBH_4$ furnished **[192]** in 82% as a single diastereomer.

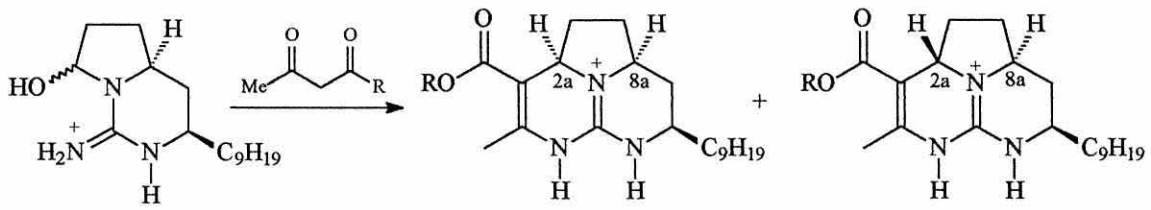


Scheme 35. ***Reagents and Conditions***: (i) Ru, HCl, H_2 (40 psi), **[a]**. 93%, **[b]**. 87%. (ii) $AlMe_3$, HCl.NH(OMe)Me, **[a]**. 84%, **[b]**. 93%; (iii) 3,3-dimethoxypropyl magnesium bromide, THF, **[a]**. 84%, **[b]**. 79%; (iv) Et_2BOMe, $NaBH_4$, **[a]**. 82%, **[b]**. 98%; (v) HN_3, PPh_3, DEAD, **[a]** 98%, **[b]** 94%,(vi) $LiAlH_4$, **[a]** 93%, **[b]** 85%; (vii) **[195]**, DCM, **[a]** 89%, **[b]** 82%; (viii) Zn, AcOH-H_2O, quantitative.

Secondly, introduction of the guanidine moiety by standard Mitsunobu displacement of **[192a]** with hydrazoic acid, followed by reduction of the resulting diazide **[193a]** with $LiAlH_4$ furnished 91% of **[194]**. Further condensation of diamine

[194] with carboniumidothioate **[195]** in DCM formed tetrahydropyrimidine **[196]**. Subsequent deprotection using zinc and aqueous acetic acid produced the desired compound **[197]** as a 1:1 mixture of aminal epimers, with no evidence of monocyclic guanidine-aldehyde tautomers or higher oligomers. This though was only achieved after precipitation of the formed zinc residues with H_2S and acidification with HCl.

Overman *et al.* investigated the formation of the tricyclic unit utilising the aforementioned tethered Biginelli cyclisation on **[197]**. This produced the required products as a 1:1 mixture of stereoisomers, depending on solvents used and reaction temperature. The more efficient conditions for the reaction are summarised in table 5. During this cycle of reactions Overman and co-workers also attempted the cyclisation utilising Knoevenagel conditions (1 equivalent of morpholinium acetate) and found this predominantly formed the *syn* arrangement.



[a]; R=Me, **[b]**; R=$CH_2CH{=}CH_2$

R	Acid[a]	Base[a]	Solvent	Temp. (°C)	Yield	Ratio [198]:[199]
[a]	HOAc	Morpholine	CF_3CH_2OH	90	**b**	9:1
[b]	HOAc	Morpholine[c]	CF_3CH_2OH	90	80	6.5:1
[b]	HOAc	Morpholine	THF	66	80	5.7:1
[a]	HOAc	Morpholine	$Cl(CH_2)_2Cl$	90	95	1.3:1
[b]	HOAc	Morpholine	$Cl(CH_2)_2Cl$	85	64	3.7:1

Table 5. Summary of the results obtained from the tethered Biginelli condensation.
NOTE:- [a]1 equivalent, [b] Not determined, [c] 2 equivalents.

As the batzelladines A, D, F and G (the latter two being discussed in section **2.6.5**) contained the saturated tricyclic core, Overman conducted studies into the selective facial reductions of **[198a]** and **[199a]**. In 1996 Snider *et al.* communicated such hydrogenations using Rh in methanol; because of this and the apparent need to access such structures, Overman used two hydrogenation systems for both *syn* and *anti*

arrangements. They observed that the facial selectivity of hydrogenation, whether α or β face, was dependent on the catalyst used. In the first instance of **[198a]** to **[200]** and **[201]** differing ratios of *syn*:*anti* were observed with Rh in methanol to those for Pd on carbon in methanol.

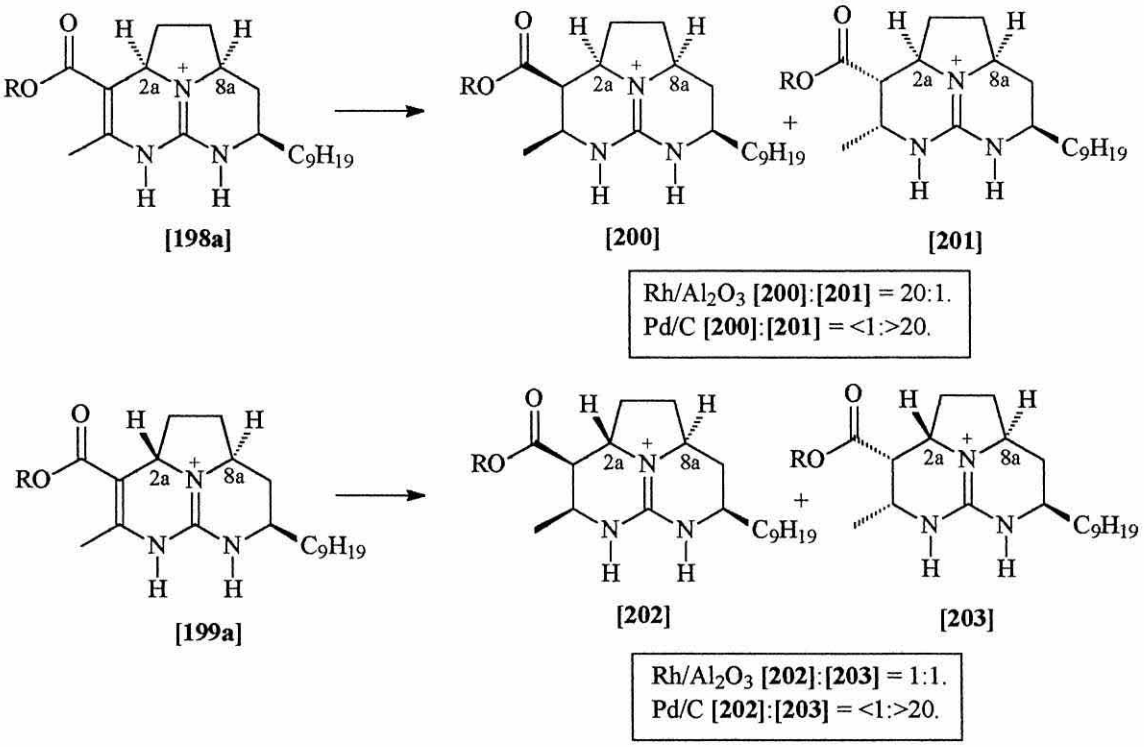


Fig. 25. Hydrogenation of the *syn* and *anti* isomers.

Hydrogenation of **[199a]** was also undertaken under the same conditions, but on this occasion the Rh on alumina in methanol displayed no such selectivity; however, using Pd/C Overman *et al.* observed the single formation of **[203]** in 94% yield.

2.6.5 BATZELLADINES F-I

Patil *et al.* in 1997[79] isolated four new alkaloids from the Jamaican sponge *Batzella sp.* Over 3500 extracts were screened and only two were of interest. Further research unfortunately determined that one of the extracts contained the non-specific halitoxins which demonstrated a broad range of general activity. However, the second methanol extract from *Batzella sp.*, containing the aforementioned alkaloids illustrated remarkable activity in the $p56^{lck}$-CD4 dissociation assay, relevant to immunosuppression and ultimately a valuable treatment of autoimmuno diseases and

allograft rejection. These new natural products were designated batzelladines F-I **[204-207]**.

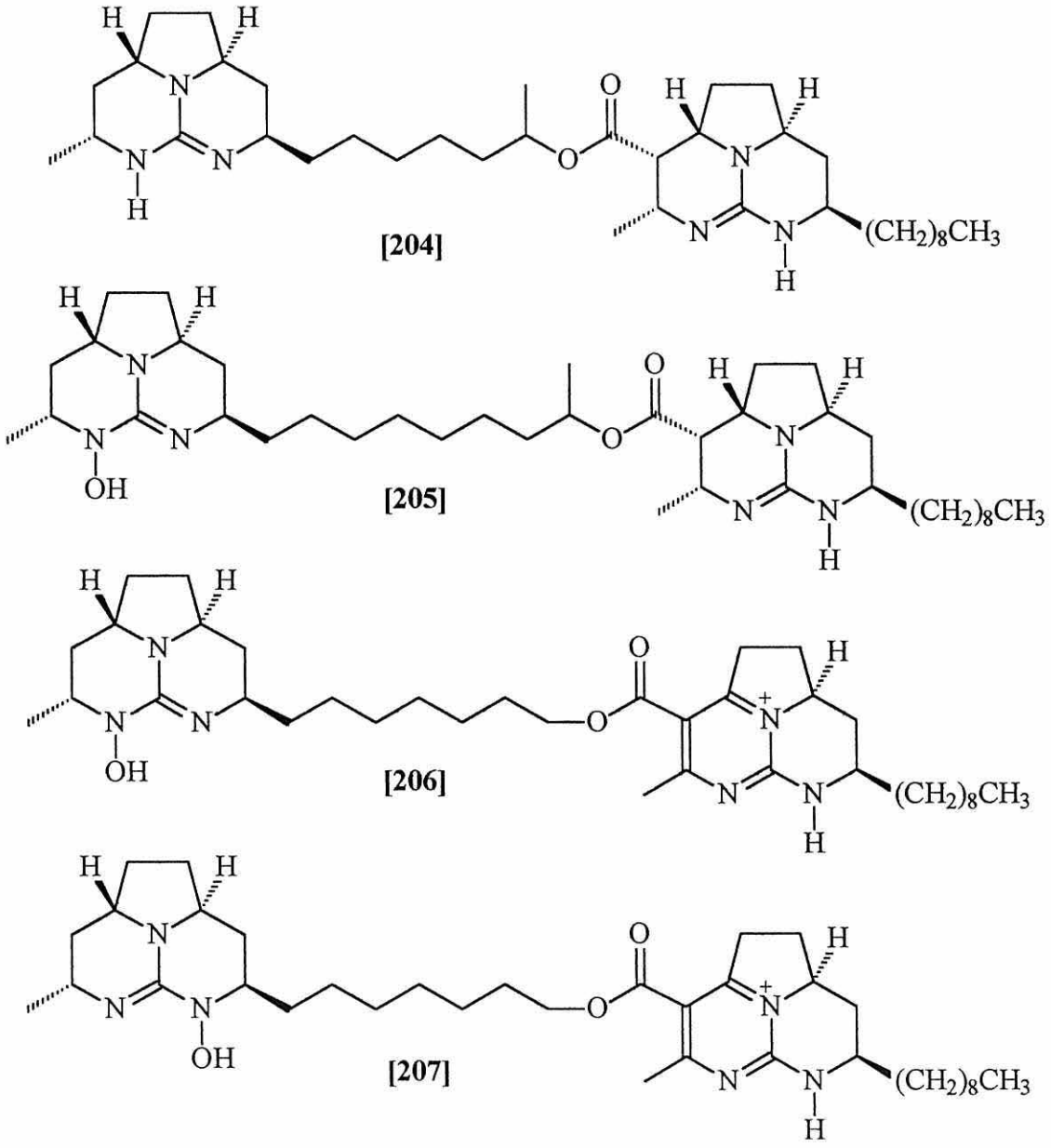


Fig. 26. The batzelladines F-I **[204-207]**.

2.6.6 BIOLOGICAL ASSESSMENT[80]

The biological activity of the batzelladines A-D and crambescin A were tested for comparison of activity in an ELISA-based assay which measures the association of soluble CD4 to immobilised recombinant gp120. This was then followed up with cell

based assays that measured the binding ability of gp120 to $CD4^+$ T-cells. Batzelladines A and B were active in this assay with IC_{50} values of approximately 3μM, suggesting that the tricyclic moiety and crambescin A bicyclic unit must be present for ultimate activity.

Compound	IC_{50}(μM)	
	gp120-CD4 ELISA	**Cell based assay**
Batzelladine A	29 ± 4	10
Batzelladine B	31 ± 12	25
Batzelladine C	>100	>100
Batzelladine D	72 ± 2	>100
Crambescin A	>100	>100

Table 6. Inhibition of HIV-1 gp120 binding to CD4 by the batzelladines A-D and crambescin A.

Batzelladines A and B were tested against HIV-1 infectivity but antiviral activity could not be assessed due to their toxicity. Additional bioassays were carried out on other ligand-receptor interactions to test their diversity and potency and, as previously, batzelladines A and B were generally more active than the remainder. These results are summarised in table 7.

Compound	IC_{50}(μM)				
	PKC[a]	**IL8a[b]**	**IL8b[c]**	**CGRP[d]**	**Cytotoxicity[e]**
Batzelladine A	1.4	4.7	7.8	1.7	1.6
Batzelladine B	1.5	2.6	6.5	1.7	1.8
Batzelladine C	6.8	9.4	9.4	4.3	1.1
Batzelladine D	11	15	14	26	0.5
Crambescin A	9.6	47	41	7.1	0.7

Table.7. The activity of the batzelladines A-D and crambescin A in additional bioassays.

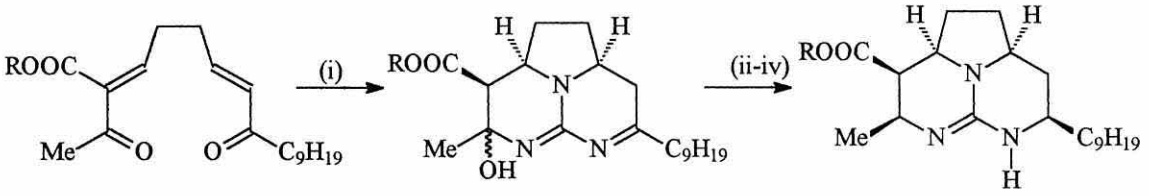
Note:- [a] Protein Kinase C enzyme assay using rat brain enzyme and histone protein as substrate.
[b] Binding of interleukin-8 to the nonpermissive receptor.
[c] Binding of interleukin-8 to the nonpermissive receptor.
[d] Binding of calcitonin gene-related peptide to porcine lung membranes.
[e] Cytotoxicity to proliferating Vero cells, 72 h exposure with an XTT read.
[b,c,d] are all radioligand binding assays.

CHAPTER 3

SYNTHETIC APPROACHES TO BATZELLADINE F

3.1 INTRODUCTION

The methodology for the synthesis of the simple tricyclic and more complex systems has already been established through previous research by members of the Murphy research group.[81] Their synthetic pathway revolved around a biomimetic approach *via* a reductive double Michael addition of guanidine to a suitably functionalised *bis*-α,β-unsaturated ketone.



Scheme 36. ***Reagents and Conditions***: (i) Guanidine, DMF, 0°C-RT, 5h; (ii) 3:1:3, DMF / H_2O / MeOH, $NaBH_4$, 16h; (iii) HCl; (iv) saturated aq. $NaBF_4$.

As stated earlier one of the two main aims of this project was to utilise the methodology already in place to synthesise the left hand portion of batzelladine F. This was of great interest as it lacked the problematic ester functionality α to the nitrogen on the pyrrolidine ring. Secondly, and of similar importance was the continuation of the synthetic pathway to enable access to the right hand portion of batzelladine F.

The synthesis of batzelladine F **[204]** was channelled into two separate approaches: the left hand portion and right hand portion (Fig. 28).

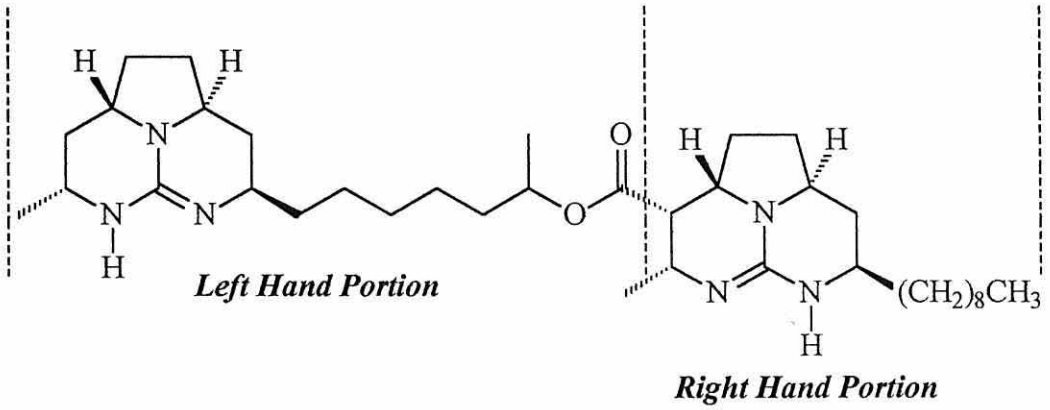
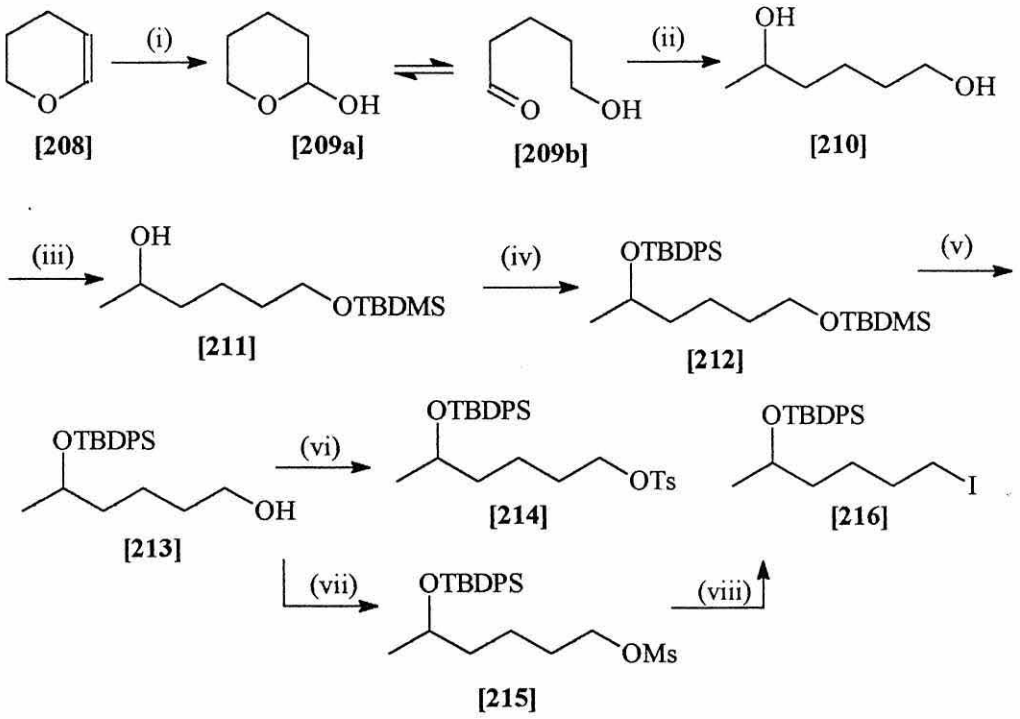


Fig. 28. Synthetic approach

3.2 SYNTHESIS OF THE LEFT HAND PORTION

The *bis*-α,β-unsaturated ketone required for the guanidine addition was prepared in nine steps from tetrahydropyran **[208]**. Acid hydrolysis of **[208]** with dilute HCl[82] under reflux conditions produced hydroxypyran **[209a]**, which existed in

equilibrium with aldehyde **[209b]**.[81] This was most noticeable in the IR spectrum with the occurrence of a C=O stretch at 1723 cm^{-1}. Treatment of the masked aldehyde with methylmagnesium bromide in THF at reflux overnight afforded 1,5-hexandiol **[210]** after chromatography in 95% yield.

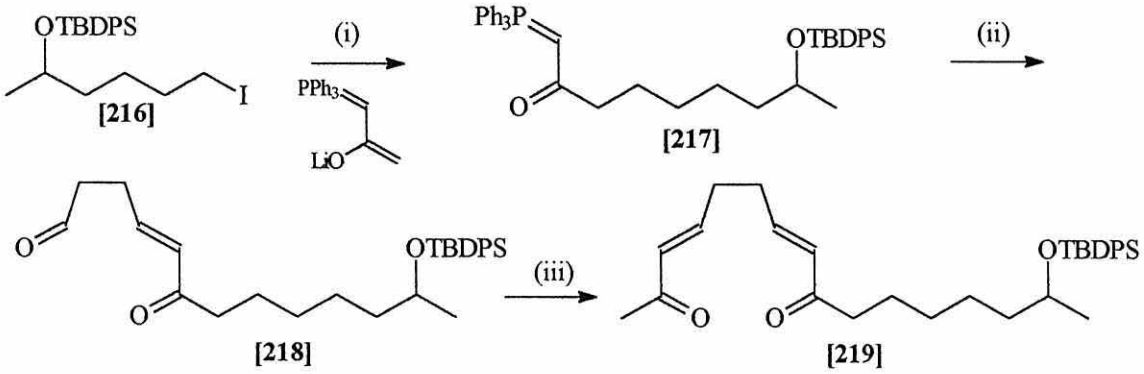


Scheme 37. ***Reagents and Conditions***: (i) 0.2N HCl, reflux overnight, 92%; (ii) MeMgCl, THF, 0°C, THF, warm RT over 30 min, reflux, 95%; (iii) 0°C, DMF, imidazole, TBDMSCl, 92%; (iv) DCM, RT, TBDPSCl, imidazole, DMAP, 89%; (v) absolute EtOH, PPTS, 48h, 91%; (vi) 0°C, pyridine, *p*-toluene sulphonylchloride, 0°C to RT, stir 18h, 30%; (vii) MsCl, Et_3N, DCM, 24h; (viii) Acetone, NaI, reflux 4h, 80% for 2 steps.

Diol **[210]** was then converted into the monoprotected alcohol **[213]** in three high yielding steps. The initial protection of the primary alcohol was achieved with *t*-butyldimethylsilyl chloride in DMF to furnish alcohol **[211]** in 92% yield. Further protection of the secondary alcohol with *t*-butyldiphenylsilyl chloride in DCM proceeded in 84% yield with subsequent deprotection using PPTS, which afforded **[213]** in 91% yield. NMR studies indicated the loss of the singlet at δ 0.01 ppm corresponding to the methyls on the TBDMS group in **[212]**, with other spectral data being consistent with alcohol **[213]**. In order to generate iodide **[216]** monoalcohol **[213]** was originally tosylated with pyridine and tosyl chloride. The presence of a signal at δ 7.7 ppm and increased multiplicity and integration at δ 7.2 ppm indicated the formation of **[214]**. However, this method only produced a 30% yield of **[214]** together

with recovered starting material and further repetition did not improve this. Therefore, alcohol **[213]** was mesylated under standard conditions to form **[215]** with no further purification, the singlet at δ 2.8 ppm indicative of the methyl group present on the mesylate. Iodination by Finkelstein displacement with NaI in acetone afforded **[216]** in 80% crude yield.

Alkylation of phosphorane **[150]** with iodide **[216]** was accomplished by initial treatment of **[150]** at -78°C with 1.1 equivalents of *n*-BuLi.[83] The resulting lithium enolate (red solution) was stirred and allowed to warm to between -50 and -60°C over a period of one hour, when a solution of iodide **[216]** in THF was added at -78°C. The resulting mixture was allowed to warm to RT over 4 hours where a distinct colour change was observed (red to yellow). Removal of the solvent furnished a brown solid which was re-dissolved in ethyl acetate, washed with water and dried to produce phosphorane **[217]** in near quantitative yield.



Scheme 38. ***Reagents and Conditions***: (i) $CH_3COCHPPh_3$, THF, -78°C, *n*-BuLi, warm between -50 & -60°C, 1h, **[216]**, THF, -78°C, 4h warm to RT, quant.; (ii) succinaldehyde, DCM, 24h, 54%; (iii) $CH_3COCHPPh_3$, DCM, 24h, $CH_3COCHPPh_3$, 24h, 91%.

Phosphorane **[217]** was used without any further purification and reacted with excess succinaldehyde[84†] to give the α,β-unsaturated ketone **[218]** in 54%. The *trans* geometry was assigned by the large coupling constant J = 15.8 Hz of the olefinic protons at δ 6.1 ppm as a broad doublet and δ 6.8 ppm as a doublet of triplets.

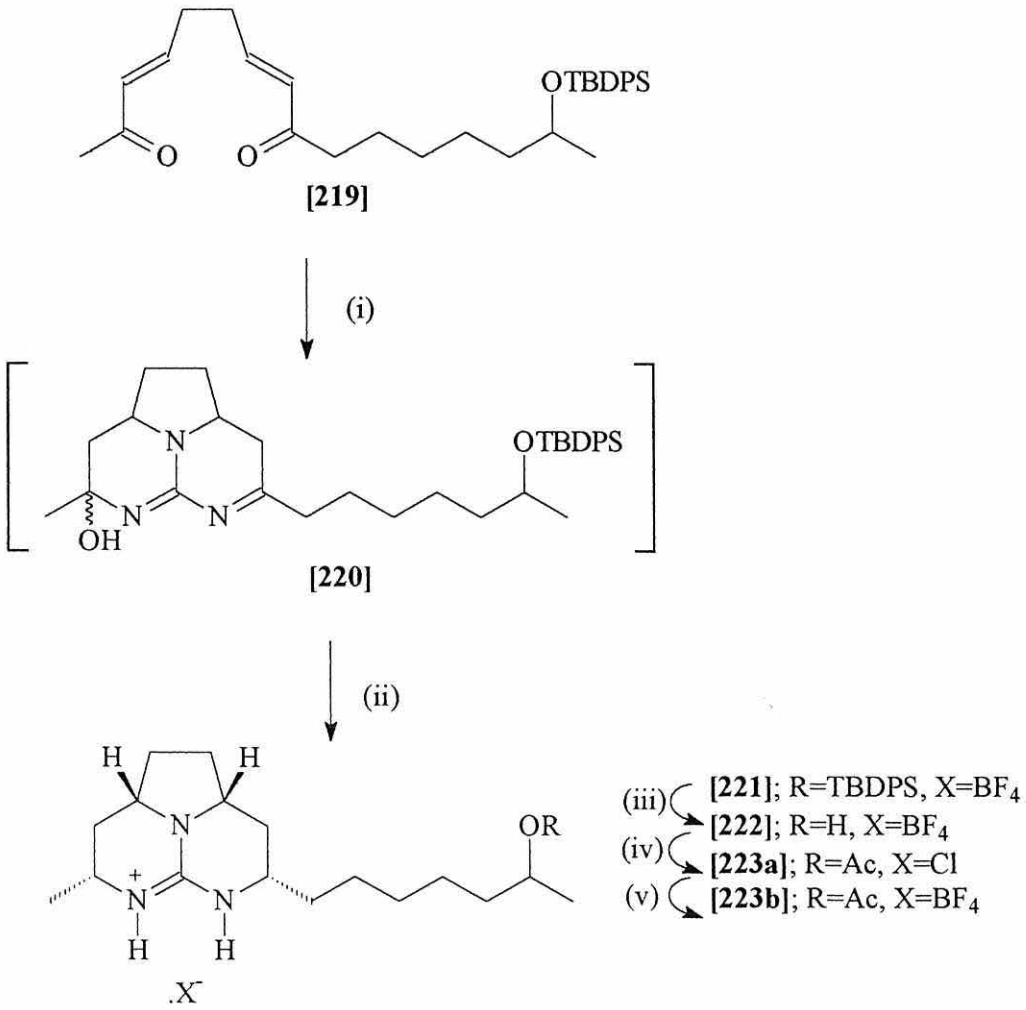
A second Wittig reaction to furnish **[219]** was achieved by treatment of **[218]** with 2 equivalents of phosphorane **[150]**. After 24 hours a further 1.5 equivalents of **[150]** were added and stirring continued for a further 24 hours. This generated the

† Prepared by treatment of 2,5-dimethoxytetrahydrofuran with either 0.1 N HCl or 1% aqueous acetic acid. Both procedures required purification by vacuum distillation.

desired *bis*-α,β-unsaturated ketone **[219]** in 91% yield after purification by flash chromatography.

Thus, the precursor to the tricyclic unit had been prepared in nine high yielding steps, with the exception of the penultimate step ((ii) in scheme 38) which only proceeded in 54% yield.

With the *bis*-enone in hand we effected the formation of the tricycle by addition of a DMF solution of guanidine to **[219]** dissolved in DMF at 0°C. After 4 hours, during which time the reaction was stirred at room temperature, water and methanol were added and the presumed intermediate **[220]** was reduced with sodium borohydride. Work-up and ion exchange with saturated sodium tetrafluoroborate and chromatography led to the formation of the silylated guanidine **[221]** in 29% overall yield.



Scheme 39. ***Reagents and Conditions***: (i) 0°C, DMF, guanidine, DMF, warm RT 4h, 0°C (ii) 3:1:3 DMF/MeOH/H_2O, $NaBH_4$, 16h, DCM, 2N HCl, sat. $NaBF_4$, 29%; (iii) 0°C, MeOH, MeOH/HCl, sat. $NaBF_4$, 91%; (iv) Ac_2O/pyridine, 2N HCl, 41%; (v) sat. $NaBF_4$, quant.

NMR studies of **[221]** indicated the presence of the tricyclic moiety with the key ^{13}C signals at δ 57.97 and 56.05 ppm indicating the presence of two CH carbons on the pyrrolidine ring. Signals at δ 34.45, 35.75, 46.1 and 50.41 ppm confirmed the presence of the six membered rings. Furthermore, the silyl group had remained intact with signals at δ 19.3 and 27.05 ppm. Further correlation was established by the presence of the aryl signals in both ^{1}H and ^{13}C NMR.

The silyl ether **[221]** was deprotected in 91% yield after treatment with methanolic HCl, ion exchange and flash chromatography. The lack of TBDPS signals on both ^{1}H and ^{13}C NMR indicated a complete removal of the protecting group. Further evidence was obtained by the presence of a broad singlet at δ 2.0 ppm which had not been previously correlated in the ^{1}H NMR of **[221]** and the presence of an OH signal at 3370 cm^{-1} in the IR spectrum.

To complete the synthesis of the model for the left hand portion of batzelladine F the ester functionality had to be incorporated at the end of the chain. This was initially attempted using acetic acid in the presence of HOBt and EDCl. However, upon work-up and purification there appeared no indication of the tricyclic compound.

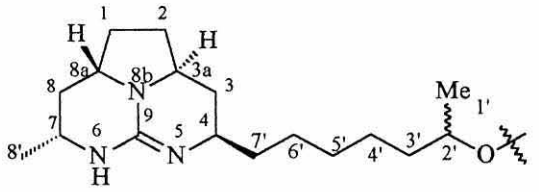
The production of **[223a]** was finally achieved by dissolving alcohol **[222]** in pyridine with subsequent treatment of the solution with acetic anhydride. After stirring at room temperature for 48 hours, work-up and purification by flash chromatography **[223a]** was obtained in 41% yield as the hydrochloride salt. Counter-ion exchange with saturated sodium tetrafluoroborate furnished **[223b]** in quantitative yield. ^{13}C NMR studies indicated a successful conversion by the presence of three methyl groups δ 20.21, 20.72 and 21.23 ppm and the carbonyl signal at δ 172.68 ppm.

3.2.1 NMR CORRELATION

Comparison of the ^{13}C NMR spectra of **[222]** and **[223b]** with that reported for batzelladine F **[204]** by Patil *et al.*[79] indicated a strong correlation. However, the question of stereochemistry around the tricyclic core still exists. In earlier work by Murphy,[36, 65, 73] it was found conclusively that the addition of guanidine to the *bis*-α,β-unsaturated enones leads to the tricyclic products in which H 8, 10, 13 and 15 are syn.

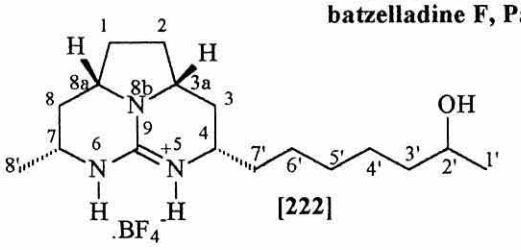
It is apparent from this observation that the assignment of Patil[74] for batzelladine F is incorrect and the real relative stereochemistry for the natural material is as

illustrated in **[222]** and **[223b]**. This contradicted the publication by Patil *et al.* in 1997[79] which indicated the stereochemistry as *anti*.

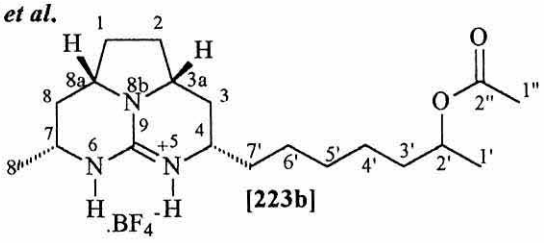


[204]

Proposed structure for batzelladine F, Patil *et al.*



[222]



[223b]

Carbon	Batzelladine F [204] Patil *et al.* (δ ppm)	Analogue [222] (δ ppm)	Analogue [223b] (δ ppm)
1'	20.5	23.5	20.2
2'	73.3	68.4	72.3
3'	36.9	40.0	36.6
4'	**26.5**	**26.6**	26.0
5'	**30.5**	**30.5**	30.2
6'	**26.2**	**26.1**	**26.3**
7'	**35.8**	**35.7**	35.6
8'	**20.7**	**20.7**	**20.7**
1	**31.1**	**31.0**	**31.0**
2	**31.1**	**31.0**	**31.0**
3	**34.8**	**34.6**	**34.7**
3a	**57.4**	**57.4**	**57.4**
4	**51.6**	**51.5**	**51.5**
7	**47.2**	**47.2**	**47.3**
8	**36.9**	36.6	**36.8**
8a	**57.5**	**57.5**	**57.5**
1"	-	-	21.2
2"	-	-	172.7

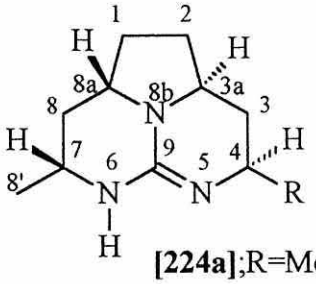
Table 8. ^{13}C NMR correlation between the two analogues and batzelladine F.

†Bold notation equates to better than 0.1 ppm correlation.

‡NMR solvent for **[204]**, **[222]** and **[223b]** was CD_3OD.

Whilst these assignments are not absolute proof of the structure of batzelladine F **[204]** the spectroscopic evidence does suggest the original assignment in terms of stereochemistry around the left hand tricyclic core of batzelladine F is possibly incorrect, and the true assignment is that suggessted above in which the protons at C3a, 4, C7 and C8a exist in a *syn* arrangement. Both analogues **[222]** and **[223b]** show good correlation with the data obtained for the natural product. Furthermore the signals of interest, C2 and C3a, correlate closely to those of batzelladine F **[204]**. This information further supported the stereochemistry assigned.

In 1999 Snider and Busuyek[85] also published a paper on the revision of the stereochemistry of batzelladine F and their unsuccessful synthetic approach to the formation of the hydroxyguanidine moiety of batzelladines G-I. They prepared an *anti* substituted system **[224]** using methodology reported by Murphy *et al.*.[36, 65, 73] They were able to isolate this *anti* isomer in a low 10% (mixed *syn*:*anti*) yield and illustrated its vastly different NMR data to that found in batzelladine F as can be seen in table 9 overleaf.



[224a];R=Me
[224b];R=C_9H_{19}

Fig. 29. Snider *anti* conformer.

Carbon[§]	Batzelladine F [203] Patil *et al* (δ ppm)	Analogue [223b] (δ ppm)	Snider's *anti* analogue [224a]	Snider's *anti* analogue [224b]
1	**31.1**	**31.0**	31.8	31.1
2	**31.1**	**31.0**	31.8	31.1
3	**34.8**	**34.7**	36.4	36.8
3a	**57.4**	**57.4**	56.5	57.5
4	**51.6**	**51.5**	48.0	47.3
7	**47.2**	**47.3**	48.0	51.6
8	**36.9**	**36.8**	36.4	34.8
8a	**57.5**	**57.5**	56.5	57.5
8'	**20.7**	**20.7**	21.7	35.9

Table 9. ^{13}C NMR correlation between batzelladine F analogue **[223b]** and Snider's anti analogues **[224a]** and **[224b]**

[†]Bold notation equates to better than 0.1 ppm correlation.

[‡]NMR solvent for **[204]**, **[223b]**, **[224a]** and **[224b]** was CD_3OD.

[§]Only the important carbons are shown, and the numbering of the tricyclic moiety are kept the same for ease of comparison.

These repeated studies of the Murphy protocol by Snider *et al.* did further confirm that the original assignment of batzelladine F was in fact wrong and, as Murphy *et al.* reported in 1999[86], it should in fact be a *syn* arrangement and not the *anti* arrangement reported by Patil *et al* in 1997.[79]

3.3 ATTEMPTED SYNTHESIS OF THE RIGHT HAND PORTION

3.3.1 RETROSYNTHESIS

The retrosynthetic analysis for the right hand tricyclic core as illustrated in fig. 30 is similar to that previously employed for the left hand side, in that the guanidinium moiety is removed to furnish a *bis*-α,β-unsaturated ketone **[225]**. The only main difference being the presence an ester function as the alkane. Several potential disconnections could be employed to enable the synthesis of **[225]**.

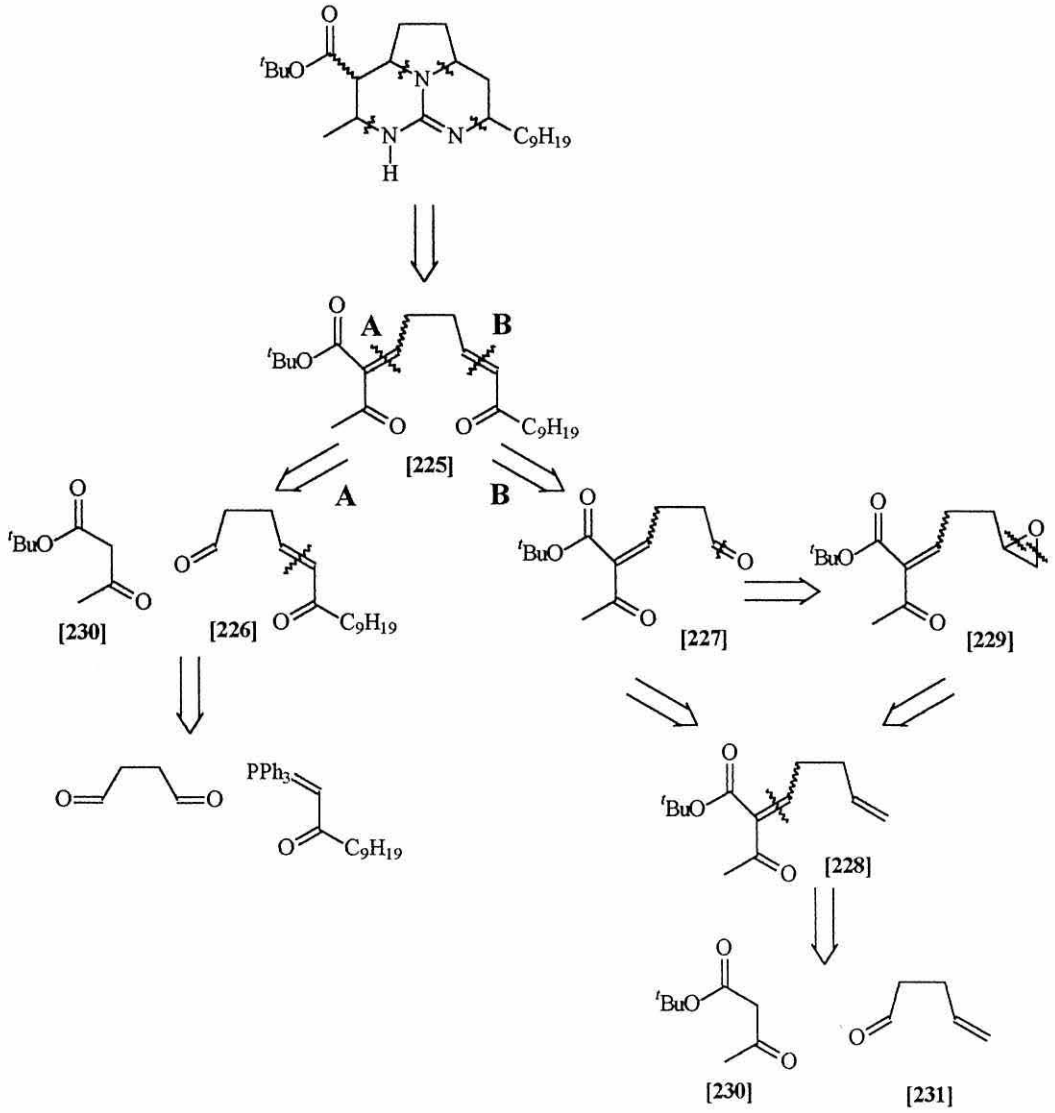
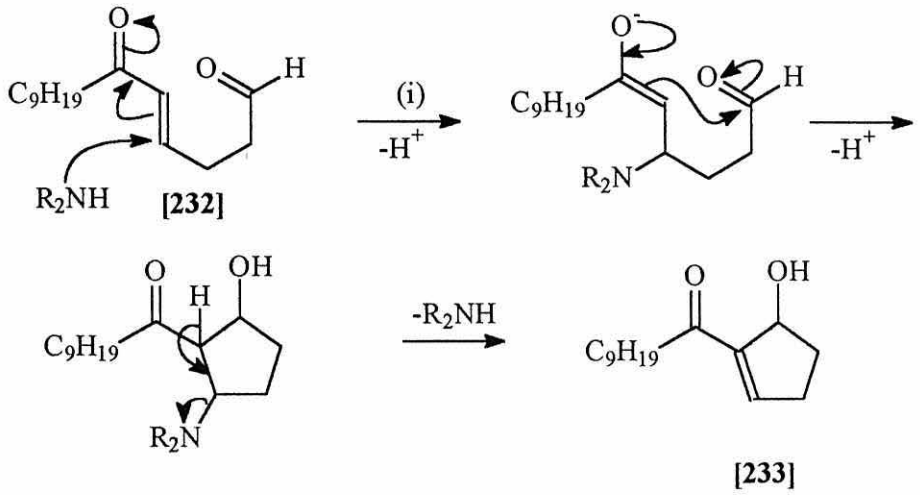


Fig. 30. Retrosynthetic analysis of the right hand core.

One method would be to disconnect bond A to give the β-ketoester **[230]** and aldehyde **[226]** which could then be coupled in a Knoevenagel-like condensation, a protocol employed by Snider *et al.*.[74] However, extensive studies have been carried out within the Murphy research group on this type of reaction, and other similar Knoevenagel condensation reactions in an attempt to prepare **[225]** and related

compounds which have met with little success.[87] Attempts have also been made to repeat the work reported by Snider, again with no success. It is worth noting that in some cases Snider does report difficulty with this type of reaction and also problems with the work-up.[75]

Somewhat surprisingly research has shown the major product of the reaction of **[226]** with piperidine or piperidine acetate (as reported by Snider) is the cyclopenten-ol **[233]** in 50% yield.[81] Mention of this aldol type of reaction is not made by Snider in any of his papers.



Scheme 40. ***Reagents and Conditions***: (i) $CH_3(CO)CH_2CH_2{}^tBu$, piperidine, HOAc.

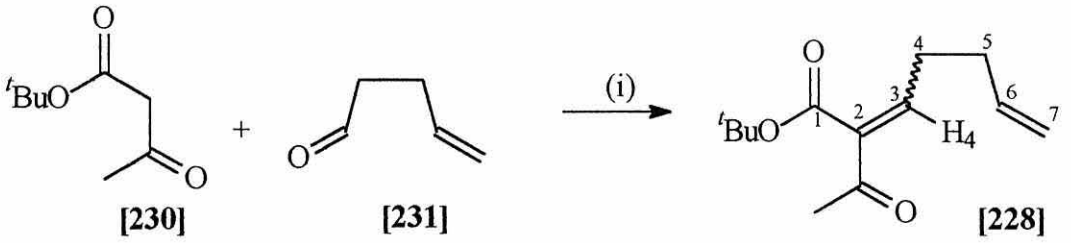
The failure of this methodology enabled two further related synthetic approaches to be investigated, which are outlined in fig. 30. They both centred around disconnecting bond B to give aldehyde **[227]**, which it was hoped could be accessed from alkene **[228]** either by ozonolysis or periodate oxidation of epoxide **[229]**. We hoped that compound **[228]** was accessible from aldehyde **[231]**.

3.3.2 SYNTHETIC APPROACHES

A report by Lehnert[88] detailed the Knoevenagel condensation reaction of simple aliphatic aldehydes using a titanium (IV) chloride-THF complex and pyridine. A protocol which had already been attempted within the Murphy research group,[89] and was repeated with aldehyde **[231]** and β-ketoester **[230]** in an attempt to obtain a greater yield.

$TiCl_4$ was thus dissolved in carbon tetrachloride and added dropwise to a cooled (0°C) solution of THF. The resulting bright yellow suspension was stirred for 15

minutes, whereupon 4-pentenal **[231]** and *tert*-butylacetoacetate **[230]** as a solution in THF were added. This resulted in a colour change to reddish brown. After addition of pyridine in THF over 2 hours at 0°C, the reaction was left to warm to room temperature for 48 hours.

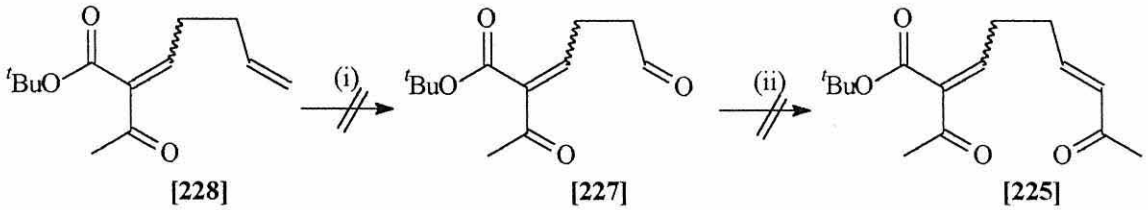


[230] **[231]** **[228]**

Scheme 41. ***Reagents and Conditions***: (i)(a) $TiCl_4$, CCl_4; (b) THF, 0°C, 15 min; (c) **[230 & 231]**, THF, 0°C; (d) Dry Pyridine, THF, 0°C, 43%.

This produced a mixture of unidentified geometric isomers in a 2:1 ratio which was determined by the integration of the olefinic signal for H_4 and, after careful purification, afforded a single unassigned isomer in 19% yield.

With **[228]** in hand, it was dissolved in DCM and cooled to -78°C and one equivalent of ozone was passed into the solution. Triphenylphosphine was then added at -78°C followed by phosphorane **[150]** after the complete dissolution of triphenylphosphine. The reaction was then left to warm to room temperature overnight. Work-up and purification failed to give the required product, all that was observed by ^1H and ^{13}C NMR were decomposition products and nothing resembling *bis*-α-β-unsaturated ketone **[225]**.

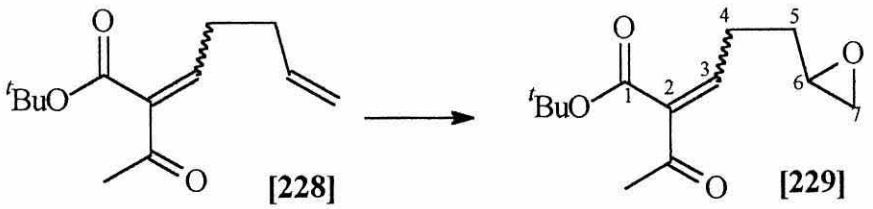


[228] **[227]** **[225]**

Scheme 42. ***Reagents and Conditions***: (i) DCM, O_3, -78°C, 3 mins 30 secs, PPh_3; (ii) -78°C, $Ph_3PCOCHCH_3$, overnight -78°C to RT.

Due to the lack of success with the ozonolysis of **[228]**, the second approach was adopted, which utilised the synthesis of the epoxide intermediate **[229]** and its

treatment with periodic acid to form the aldehyde **[227]** *in situ.* Thus, alkene **[228]** was epoxidised using *m*CPBA which produced **[229]** in 81% yield.



Scheme 43. ***Reagents and Conditions***: (i) *m*CPBA, DCM, 81%.

NMR studies indicate a successful reaction as indicated by the loss of the alkene signals at δ 5.0, 5.8 ppm in the ^{1}H NMR and the signals at δ 115.7 and 136.8 ppm in the ^{13}C NMR. The appearance of an increased integration at δ 2.5 ppm in the ^{1}H NMR and signals at δ 2.7 and 3.0 ppm together with signals at δ 46.7 and 51.3 ppm in the ^{13}C NMR also indicated the presence of an epoxide.

The NMR's below depict the region of most interest, the loss of the olefinic signals of **[228]** and the formation of epoxide signals in **[229]**.

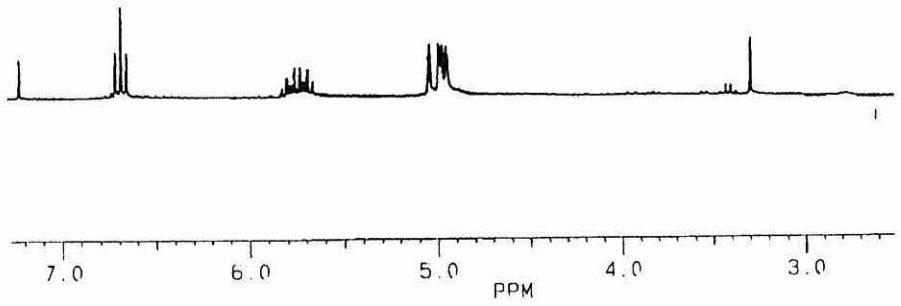


Fig. 31. Olefinic signals **[228]**.

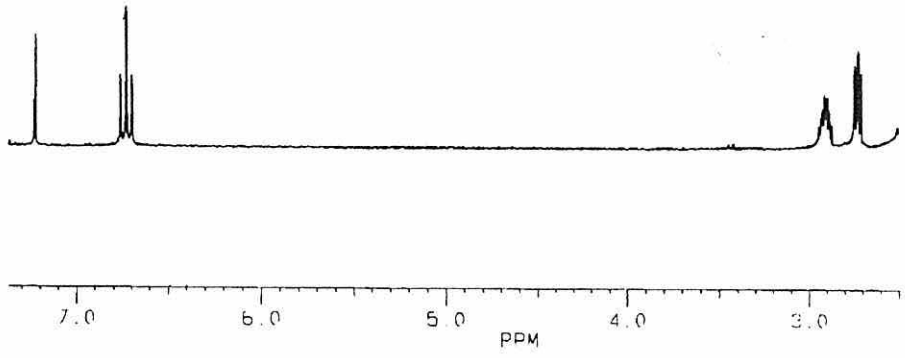
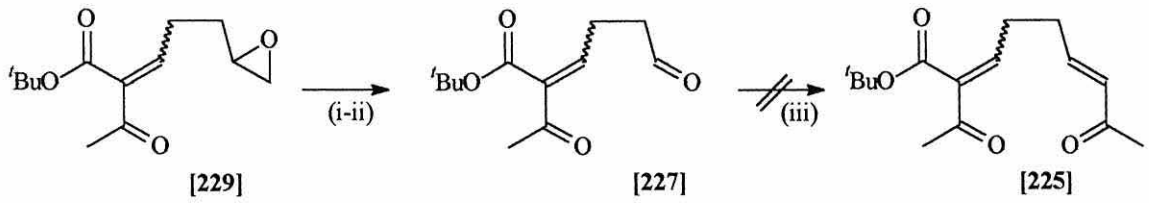


Fig. 32. Epoxide signals **[229]**.

With **[229]** in hand, *in situ* aldehyde formation was attempted by the addition of periodic acid[90] to epoxide **[229]** in THF. This was allowed to stir at room temperature overnight. The reaction mixture was then diluted with DCM, washed and dried over $MgSO_4$. ^1H NMR studies were carried out on a small sample of the product to establish the formation of **[227]** before phosphorane **[150]** was added. ^1H NMR studies established **[227]** had not been produced, nor had starting material been re-isolated as the epoxide signals at δ 2.7 and 3.0 ppm were no longer present.

This procedure was repeated whilst varying the temperature and monitoring the reaction by TLC. ^1H NMR analysis of the product did suggest the formation of **[227]** by the presence of an aldehyde signal at δ 9.7 ppm. However, after addition of phosphorane **[150]** and subsequent work-up, there was no evidence by both ^1H and ^{13}C NMR of the presence of **[225]**.



[229] **[227]** **[225]**

Scheme 44. ***Reagents and Conditions***: (i) THF, 0°C, Periodic Acid, RT, 1h; (ii) DCM, H_2O, $MgSO_4$; (iii) $Ph_3PCOCHCH_3$ **[150]**.

Time limitations prevented further investigation of this synthetic pathway. However, the feasability of an *in situ* aldehyde formation reaction by periodate oxidation, despite not being able to perform the Wittig step has been illustrated. This protocol, although in its infancy, could be a viable methodology to access the *bis*-α,β-unsaturated ketone although more work is required.

3.3.3 BIOLOGICAL EVALUATION.[91]

Several of the synthesised compounds were tested to determine their biological activity against several cancer strains and also tested for activity against HIV-RT1 inhibition.

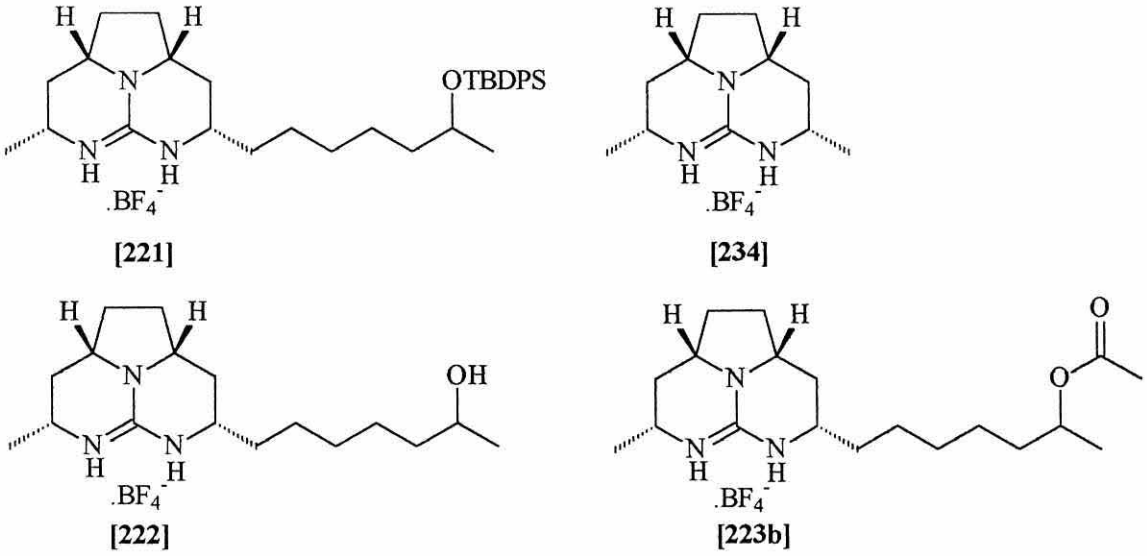


Fig. 33. Structures sent for biological testing.

Structure	K562[†]	A2780[‡]	H-460[§]	P388[ɛ]	HIV[¶]
[221]	1.22	3.87	1.44	1.94	41%
[222]	16.91	46.77	NT	22.18	100%
[234][*]	18.18	41.79	NT	NT	100%
[223b]	6.79	9.94	29.6	17.42	100%

Table 10. IC 50's μg/ml of several synthetic guanidines.
[†]Human chronic myelagenous leukaemia.
[‡]Human ovarian carcinoma.
[§]Human large cell carcinoma lung. High DT-Diaphorase.
[ɛ]Mouse Lymphoid neoplasm.
NT- Non Toxic.
[¶]HIV-RT1 % of initial enzymatic activity at concentration =[10μM] of compound.
[*]Synthesised by Dr G Black 1998.

It can be seen from the above results that the four compounds do show varying activity towards the different cell lines. Of great importance is the fact they do not show toxicity across the whole spectrum and show some signs of selectivity. Compounds **[221]** and **[223b]** show the best activity. This could be related to the lipophilic side chain functionality which is present in both.

In the HIV-RT1 assay the only compound with any activity is the silyl protected tricycle **[221]**, again possibly reflecting that a high degree of lipophilicity is required for significant activity.

3.3.4 CONCLUSIONS

As this section of the work was attempted near the end of the research project, the conversion of epoxide **[229]** to aldehyde **[227]**, and ultimately *bis* α,β-unsaturated ketone **[225]** was not achieved. Further investigative studies could be carried out utilising different oxidising agents as well as varying reaction conditions. This sequence of reactions though in its infancy could, with some further investigation, be a useful route to the required *bis*-α,β-unsaturated ketone and ultimately the right hand side of batzelladine F with installation of the problematic ester functionality.

Current studies by Murphy *et al.* are concentrating on the formation of the required substrate **[225]** by Knoevenagel condensation reactions with protected aldehydes **[235]** and **[236]**.[81] Subsequent deprotection to give **[227]** and standard Wittig methodology should then give access to the *bis*-α,β-unsaturated ketone **[225]**. Coupled with this approach Murphy *et al.* are using an acetal protecting group in a similar fashion. These approaches are summarised below.

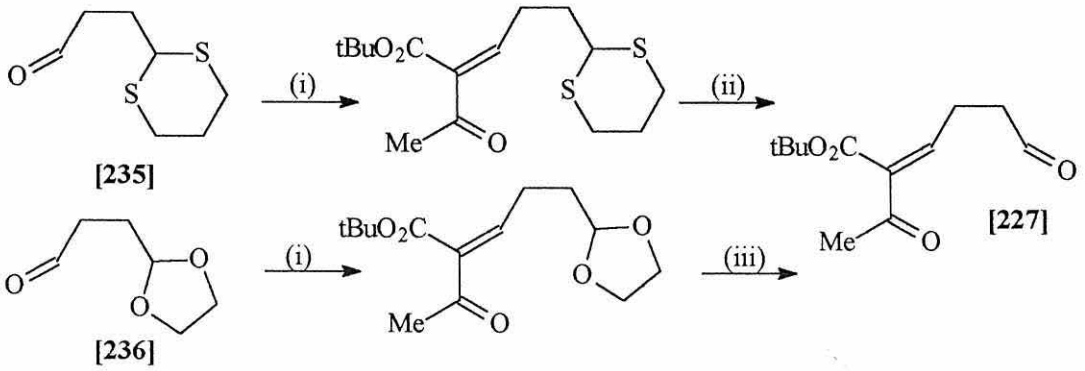


Fig. 34. An outline of the new methodology Murphy *et al.* is utilising to access the right hand portion of batzelladine F. (i) Δ, $^{t}BuO_2CH_2COCH_3$, Knoevenagel condensation; (ii) $HgCl_2$, HgO, H_2O, Δ; (iii) PPh_3, CBr_4, acetone.

CHAPTER 4

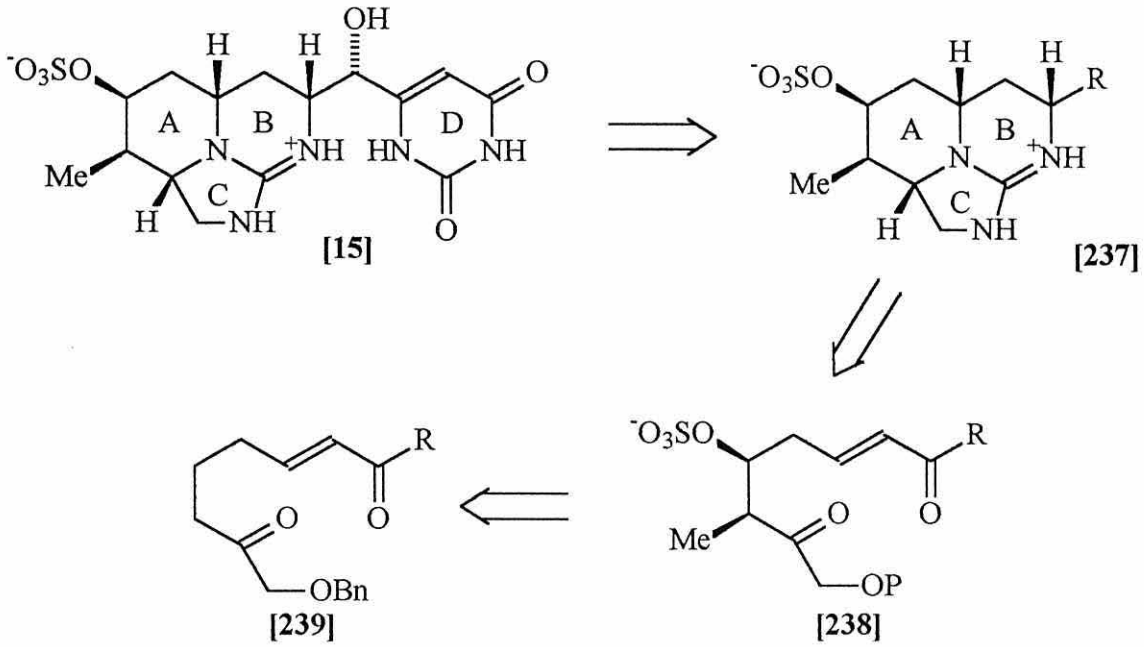
ATTEMPTED SYNTHESIS OF THE ABC RING SYSTEM OF CYLINDROSPERMOPSIN

4.1 INTRODUCTION

Initial investigations were directed towards using the methodology previously discussed in chapter 3, which essentially was to utilise a 1,4-addition of guanidine to a *bis*-α,β-unsaturated ketone. However, on retrosynthetic analysis, slight modifications were required to the synthetic precursors which would ultimately lead to the target compound.

4.2 RETROSYNTHESIS

Upon consideration of cylindrospermopsin the initial target was the tricyclic core containing the guanidine moiety, which, as with other guanidine containing metabolites appears to be the active portion, and of great interest. The remainder, the α-hydroxy uracil chain, was not to be included in the synthetic approach at this early stage and the simplified structure **[237]** was to be considered first.



Scheme 45. Retrosynthetic analysis of the cylindrospermopsin core.

Removal of the guanidine functionality from **[273]** furnished the synthon **[238]** which contained an enone and a ketone function. Continuing retrosynthetic analysis was required leading ultimately to commercially available starting materials. This was initially achieved by simplifying target enone **[238]** by removal of both sulphate and methyl groups to give **[239]**. In essence this is a similar retrosynthesis to the

batzelladine F **[223b]** synthesis as the compound contains four masked electrophilic centres, *viz* the enone (2 centres), ketone (1 centre) and the protected alcohol (1 centre).

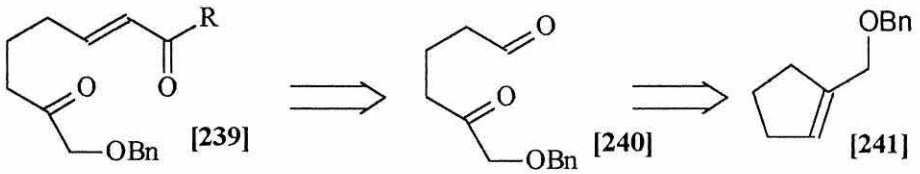
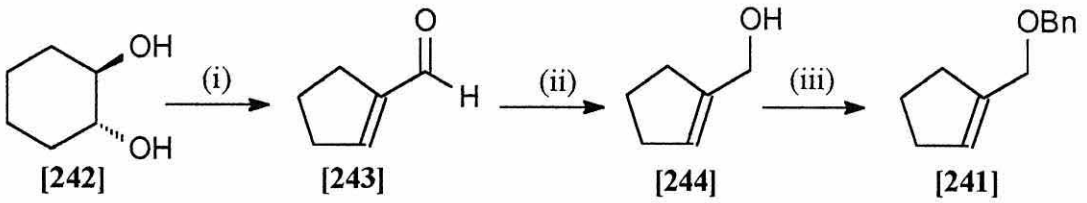


Fig. 35. Retrosynthesis of enone **[239]** to a commercially viable target.

The production of **[239]** was envisaged *via* an *in situ* Wittig reaction from aldehyde **[240]** which could be accessed *via* ozonolysis of alkene **[241]**. This in turn should be accessible from the parent alcohol **[244]**.

4.3 SYNTHETIC APPROACHES TO THE AB RING SYSTEM OF CYLINDROSPERMOPSIN

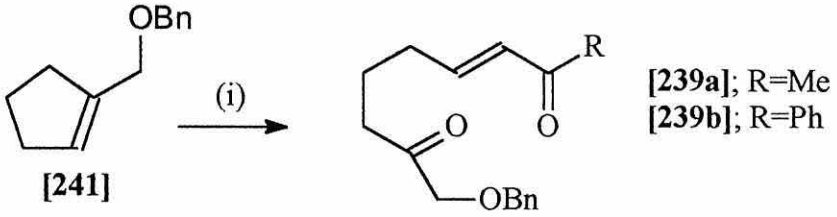
Cyclopent-1-ene-1-methanol **[244]** is a literature compound[92] which is prepared from *trans*-cyclohexane-1,2-diol **[242]** by oxidation with sodium periodate. This yields a dialdehyde intermediate which undergoes an intramolecular aldol condensation on base treatment to give **[243]** in 81% yield. ^{1}H NMR studies clarified the production with a singlet at δ 9.8ppm for the aldehyde functionality and a triplet at δ 6.9 ppm for the olefinic proton. ^{13}C NMR added further confirmation with signals at δ189, 153 and 147 ppm equating to the aldehyde, CH olefin and quaternary carbon respectively. Reduction of the aldehyde with $NaBH_4$ furnished **[244]** in 69% yield, with NMR studies establishing a successful conversion. Infra-red spectroscopy added further evidence for conversion with the appearance of an OH stretch at 3350 cm^{-1} and loss of the carbonyl stretch at 1677cm^{-1}. Subsequent treatment of alcohol **[244]** with sodium hydride followed by benzyl bromide afforded **[241]** in 69%. The presence of aromatic signals at δ 7.4 ppm as a multiplet and a singlet at δ 4.6 ppm for the CH_2Ph respectively, further confirmed the structure of the product.



[242] [243] [244] [241]

Scheme 46. (i)(a) Sodium periodate, water, c.HNO_3, NaOH pH = 4, **[242]**, (b) ether, KOH, 25°C, 81%; (ii) MeOH, 0°C, $NaBH_4$, stir overnight, 69%; (iii) NaH, dry THF, 0°C, **[244]** dry THF, tetrabutylammonium iodide, benzyl bromide THF, 69%.

The principal synthetic step in the preparation of substrates **[239a]** and **[239b]** was ozonolysis of the protected alcohol **[241]** followed by an *in situ* Wittig reaction. Initial attempts with acetylmethylene triphenylphosphorane produced the desired product **[239a]** in a disappointing 21% yield. Further investigation led to the discovery that varying the temperature at which the phosphorane and ylid were added had a drastic effect on the yield of the reaction. These results are summarised in the Table 11 below.



[241]

[239a]; R=Me
[239b]; R=Ph

Scheme 47. (i) Dry DCM, -78°C, O_3, -78°C PPh_3, $PPh_3CHCOCH_3$ (**[239a]**), $PPh_3CHCOPh$ (**[239b]**)

Attempt	R	Temp. PPh_3 added (°C)	Temp. Ylid added (°C)	% Yield
1	Me	-78 to RT	RT	21
2	Me	-78 to -20	-20 to RT	54
3	Me	-78	-78 to RT	95
4	Ph	-78 to RT	RT	71
5	Ph	-78 to -20	-20 to RT	85
6	Ph	-78	-78 to RT	94

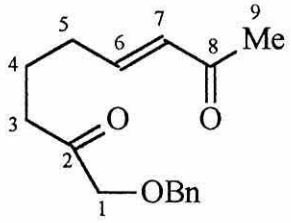
NOTE: Ozonolysis carried out at >-78°C and in DCM.
[244]; R=Me, **[245]**; R=Ph.

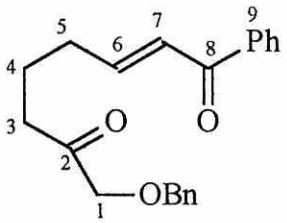
Table 11. Summary of ozonolysis reactions.

Once the blue colouration was observed, it was found warming to RT before the addition of triphenylphosphorane and subsequent addition of the ylid resulted in a low

yield. This could have been due to stability reasons of the intermediate ozonide and warming to RT led to decomposition. Thus, the addition reactions were performed at -20°C and -78°C and found that the best yields were obtained with the latter temperature.

Both ^{1}H and ^{13}C NMR of substrates **[239a]** and **[239b]** indicated the successful production of the enone functionality with olefinic signals at δ 6.7 ppm for **[239a]** as a broad doublet of triplets and a doublet of triplets at δ 6.7 ppm for **[239b]**. The coupling constants J = 15.9 and 1.4 Hz and 15.9 and 6.8 Hz respectively clarifying the *trans* geometry.





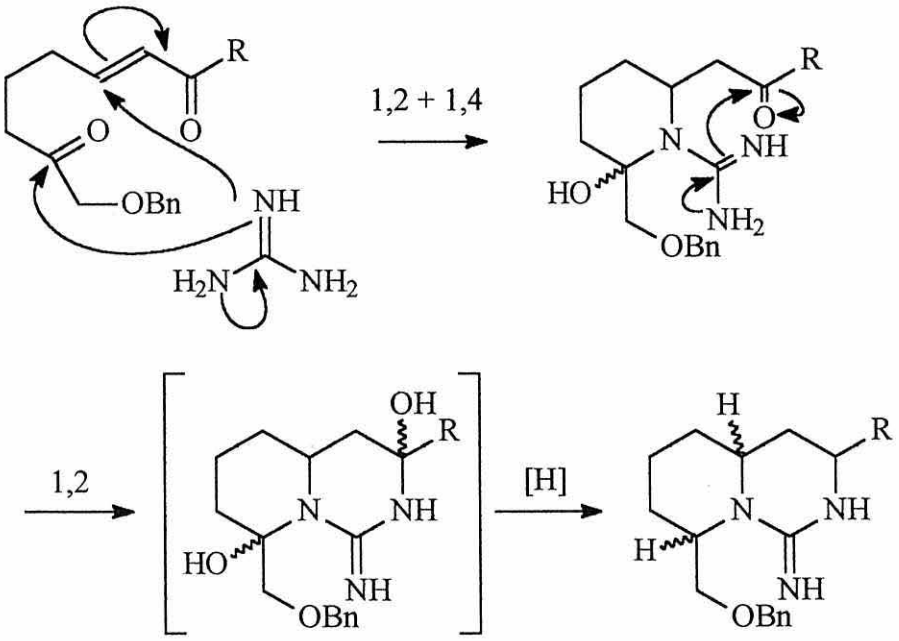
	[239a]		[239b]	
Carbon	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**
1	4.1	75.0	4.1	75.0
2	-	198.5	-	190.6
3	2.5	31.7	2.6	32.0
4	1.8	21.4	1.9	21.5
5	2.25	27.0	2.4	38.0
6	6.7	131.8	7.0	132.7
7	6.1	147.0	6.9	148.4
8	-	208.1	-	208.2
9	2.25	38.0	7.5	133.9-137.8
$PhCH_2$	4.55	73.4	4.6	73.4
$PhCH_2$	7.3	127.9-137.1	7.3	133.6-137.1

Table 12. ^{1}H and ^{13}C NMR details of both enone systems.

The presence of the phenyl ring adjacent to the α,β-unsaturated ketone in **[239b]** and the affect of conjugation resulted in increasing the complexity of the olefinic splitting pattern. Expansion of the region δ 7.0 ppm established a doublet of triplets at δ 6.97-7.1 ppm and a doublet at δ 6.85-6.95 ppm with coupling constants J = 15.4, 6.6

Hz and 15.4 Hz respectively. As with system **[239a]** the large coupling constant inferred the *trans* geometry. Both IR, MS and HRMS further confirmed the production of both enone systems.

With **[239a]** and **[239b]** in hand, both systems were ideally arranged for the reaction of guanidine *via* a 1,4 and 1,2 Michael addition using the methodology described. Thus, guanidine was added as a solution in DMF to a stirred and cooled (0°C) solution of **[239a]** or **[239b]** in DMF. This was allowed to warm to room temperature over 5 hours which was followed by addition of $NaBH_4$, H_2O and MeOH at 0°C and stirring overnight to ambient temperature.



Scheme 48. Proposed route of guanidine addition.

Initial NMR studies were inconclusive, however, signals at δ 3.5-4.0 ppm which in common with other guanidine metabolites (ptilomycalin A and related compounds, chapter 2) were symbolic of the protons α to the nitrogen in the piperidine ring were encouraging. Flash chromotography isolated several fractions which looked encouraging; however, none displayed the characteristic N-H signal expected for a guanidine containing compound in the ^{1}H NMR at 7-9 ppm (N-H) and ^{13}C NMR between 150 and 160 ppm (guanidine C).

As the bicyclic system had not been obtained, the guanidine addition reaction was further investigated using substrates **[239a]** and **[239b]** as the starting materials.

The conditions of the reactions were modified to some extent by changing the concentration of the guanidine in the reaction and by changing the reducing agent to the less harsh $NaCNBH_3$. These changes had no effect, and no guanidine containing compounds were obtained. The results of these reactions are summarised in Table 13.

Attempt	Temp	Substrate	Concentration†	Equivalents‡	Reducing Agent	Yield
1	0	**[244]**	0.21	1	$NaBH_4$	-
2	0	**[244]**	0.21	1	$NaBH_4$	-
3	0	**[244]**	0.25	1.1	$NaCNBH_3$	-
4	0	**[245]**	0.13	1	$NaBH_4$	-
5	0	**[245]**	0.21	1	$NaBH_4$	-
6	0	**[245]**	0.23	1.1	$NaCNBH_3$	-

Note. The concentration and equivalents refers to the addition of guanidine and not the reducing agent.
†Concentration of guanidine in DMF ($x10^{-3}$ g/ml)
‡Equivalents of guanidine with respect to starting substrate

Table 13. A summary of the guanidine addition reactions.

To try and establish what had actually occurred in this reaction, a mass spectrum of one of the major products formed during the addition was obtained. The M^+ obtained was 264 daltons which, compared to the starting material mass of 260 indicated the addition of two equivalents of hydrogen, i.e. the reduction of two double bonds. Thus, as no alkene protons were present in the ^1H NMR spectrum of the product, the likely nature of decomposition is as suggested in fig. 36 in that an intramolecular base catalysed 1,4-addition had occurred, mediated by guanidine, which led to cyclohexanone **[245]**, which is reduced to diol **[246]** on treatment with $NaBH_4$.

Diol **[246]** would be expected to display signals in the ^1H NMR at $\delta = 3.5 - 4.0$ for the CHOH and CHOBn products and has the mass required ($M^+ = 264$) for the material obtained.

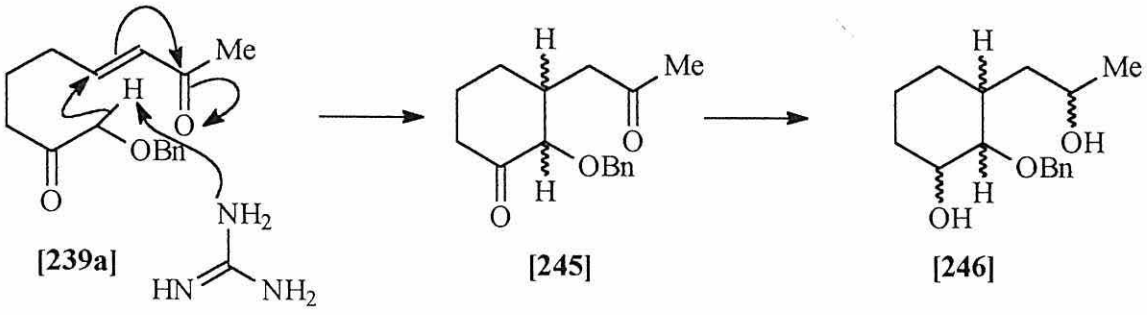
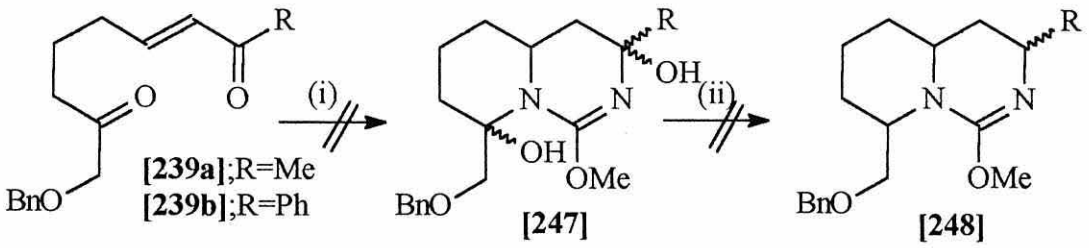


Fig. 36. Possible decomposition mechanism for substrate **[239a]**.

With the failure of this method, we attempted to utilise the methodology described by Snider *et al.*[51] who utilised *O*-methylisourea to introduce the guanidine moiety over two steps. Therefore, the addition of *O*-methylisourea to both enones **[239a]** and **[239b]** was envisaged with which, after reduction, would furnish the bicycle **[248]**. With this in mind both substrates were treated under the conditions reported by Snider,[62] the results obtained are summarised below in Table 14.



Scheme 49. ***Reagents and Conditions***: (i) *O*-methylisourea hydrogen sulphate, $NaHCO_3$, DMF; (ii) $NaCNBH_3$, DMF.

Attempt	Substrate	Temperature	Solvent	Time (hr)	Yield %
1	**[239a]**	50	DMF	2	-
2	**[239a]**	75	DMSO	25	-
3	**[239b]**	50	DMF	2	-
4	**[239b]**	75	DMSO	25	-

Table 14. Summary of addition reactions.

Entries **1** and **3** followed the reported conditions of Snider[62] exactly, but gave no indication of reaction with starting material being recovered from both attempts. With the lack of success both the reaction temperature and time were increased to 75°C and 25 hrs respectively (entries **2** and **4**), again with no success.

It was apparent that the attempts to add guanidine or *O*-methylisoureas hydrogen sulphate to both substrates **[239a]** and **[239b]** were problematic for differing reasons. As a result of this the synthetic approach was changed in order that some progress be made.

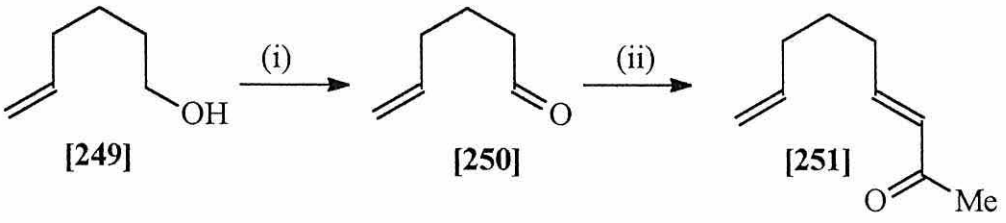
Therefore a more stable substrate was prepared from the commercially avaliable 5-hexen-1-ol **[249]**. 5-Hexen-1-ol was thus oxidised with PCC to afford 5-hexenal **[250]** in somewhat inconsistent yields which seemed to be dependent on the quantity of solvent used to wash the inorganic residues produced by the oxidation. In order to improve this yield the reaction was repeated, but with the addition of Celite as a

dispersant for the PCC by products and filter aid for the work-up. As Table 15 below shows the average yield per reaction was greater with Celite than without.

Entry	Yield (%)	Celite
1	60	-
2	97	-
3	47	-
4	92	Yes
5	77	Yes
6	85	Yes

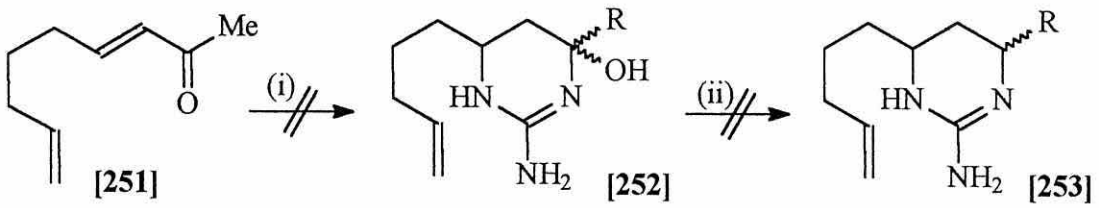
Table 15. Several examples illustrating the difference between Celite / PCC oxidation and just PCC.

NMR analysis of the product formed after oxidation established the successful production of **[250]** with the aldehyde functionality observed at δ 9.7 ppm as a triplet J = 0.95 Hz. The Wittig reaction between **[250]** and **[150]** yielded the required substrate in 88% yield after trituration and column chromatography. NMR analysis indicated the successful production of **[251]** with the olefinic protons appearing at δ 5.0 and 5.6 ppm as two sets of multiplets. ^{13}C NMR further clarified the production with signals at δ 202.3, 137.5 and 115.2 ppm for the ketone and olefinic regions respectively. Again, the stereochemistry around the double bond was found to be *trans* due to the large coupling constant of 15.9 Hz.



Scheme 50. ***Reagents and Conditions***: (i) Dry DCM, 0°C; 2 eqv. PCC / Celite, 90min; 0°C, 0.5 eqv. PCC / Celite, stir overnight, 92%; (ii) acetylmethylene triphenylphosphorane, dry DCM, RT overnight, 88%.

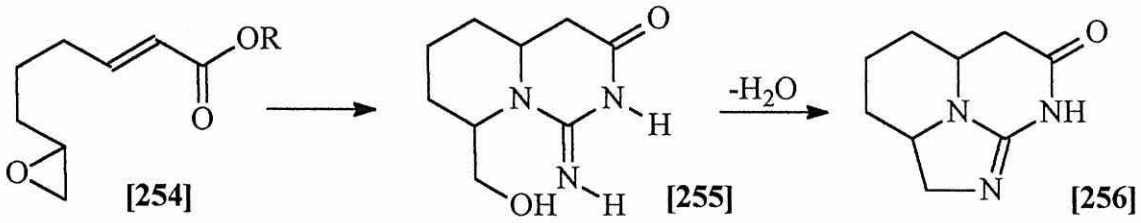
With **[251]** in hand one equivalent of guanidine as a solution in DMF was added to a cooled (0°C) solution of **[251]** in DMF. After 4 hours stirring to room temperature the assumed intermediate was reduced with $NaBH_4$, after addition of methanol and water in a ratio of 3:1:3 (DMF:MeOH:H_2O). On the two occasions this procedure was attempted no guanidine containing products were obtained.



[251] [252] [253]

Scheme 51. ***Reagents and Conditions***: (i) 0°C, DMF, guanidine, DMF, warm RT 4h; (ii) 0°C 3:1:3 DMF/MeOH/H_2O, $NaBH_4$, 16h, HCl/water.

Further investigative studies moved towards a substrate that had an excellent leaving group attached to the enone functionality so the addition reaction would be more favourable. Therefore, a compound with a more stable electrophilic centre that did not possess enolisable protons was prepared. It was apparent that an epoxide function would fit this criteria, hence, the reaction of epoxide **[254]** with guanidine was envisaged which after dehydrative cyclisation would yield tricycle **[256]**.

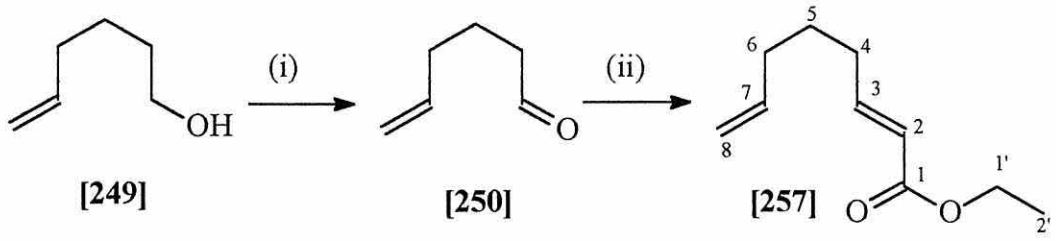


[254] [255] [256]

Fig. 37. Envisaged production of tricycle **[256]**.

The ester functionality was chosen, as the reaction of single acrylides had been proven within the group to lead to pyrimidines in good yields.[93]

This approach utilised the previously prepared aldehyde **[250]**. Treatment of **[250]** with carboethoxymethylene triphenylphosphorane in dry DCM at an ambient temperature furnished ester **[257]** in 88% yield. The ^1H NMR displayed several signals of interest, including the multiplet at δ 5.0 ppm for CH_2-1, a multiplet at δ 5.8 ppm for 2 x CH-2/7 and finally the doublet of triplets at δ 6.9 ppm for CH-6, J = 15.9 and 6.9 Hz.



Scheme 52. ***Reagents and Conditions***: (i) Dry DCM, 0°C; 2 eqv. PCC / Celite, 90min; 0°C, 0.5 eqv. PCC / Celite, stir overnight, 92%; (ii) carboethoxymethylene triphenylphosphorane, dry DCM, RT overnight, 88%.

The next stage in the synthetic approach could have progressed *via* two pathways. Firstly guanidine could have added to substrate **[257]** leading to the monocyclic intermediate **[258]**, after which the olefin at C7 could be epoxidised to give **[259]**, which would then undergo nucleophilic ring closure to the bicyclic system **[260]**. Alternatively these steps could be reversed giving epoxide **[261a]** which could then be cyclised in one step to yield the same product **[260]**.

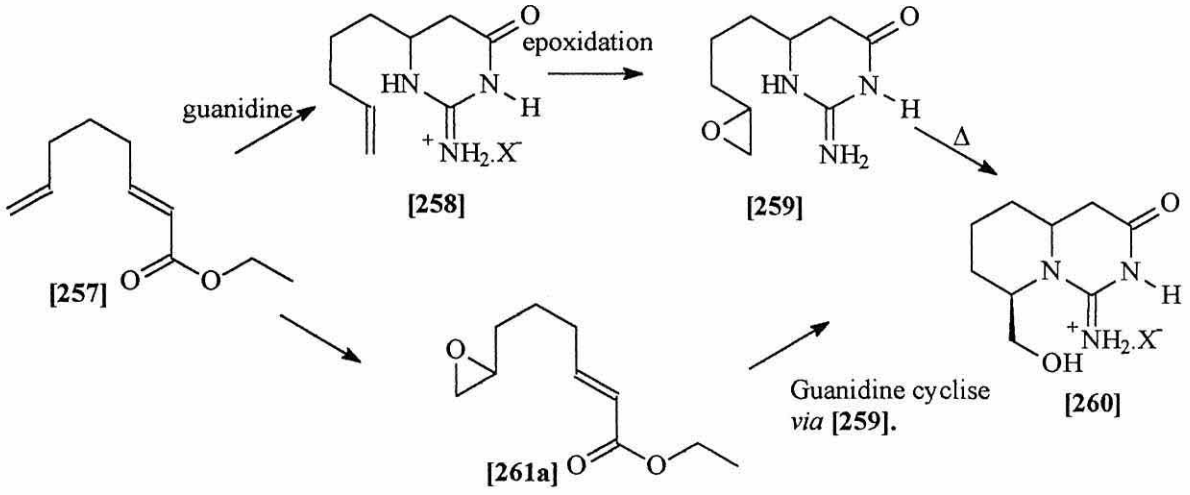
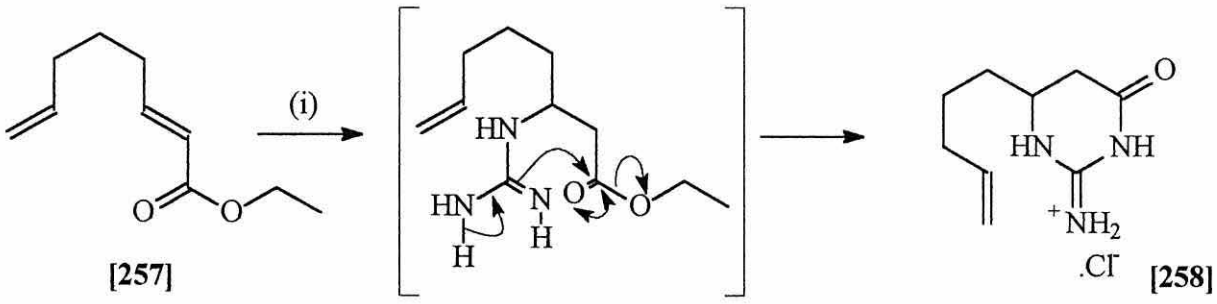


Fig. 38. The possible approaches to obtain the bicyclic system.

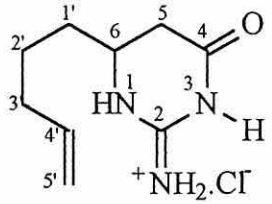
Both these pathways were investigated to establish viable methodology. Thus, ester **[257]** was dissolved in dry DMF and cooled to 0°C, whereupon a solution of guanidine in DMF was added. The reaction was then allowed to warm to room temperature and stir for 72 hours. The reaction mixture was then filtered to give a white solid which was washed with diethyl ether and dried *in vacuo* to furnish **[258]** in 31% yield (Scheme 53).



[257] [258]

Scheme 53. ***Reagents and Conditions***: (i) DMF, 0°C, guanidine, DMF; warm to RT 72 h, methanolic HCl, 27%.

Attempts at producing further **[258]** were made by evaporating the reaction solvent residues and filtrate. However, after trituration and chromatography of the oily residues the only material found was contaminated starting material together with what could have been trace amounts of **[258]**, but were more likely to be by products from the reaction. Mass spectral analysis of **[258]** gave a clear M^+ at 182 daltons with HRMS corresponding exactly to **[258]**. Both ^1H and ^{13}C NMR also aided in structural determination, the data is provided in Table 16.



[258]

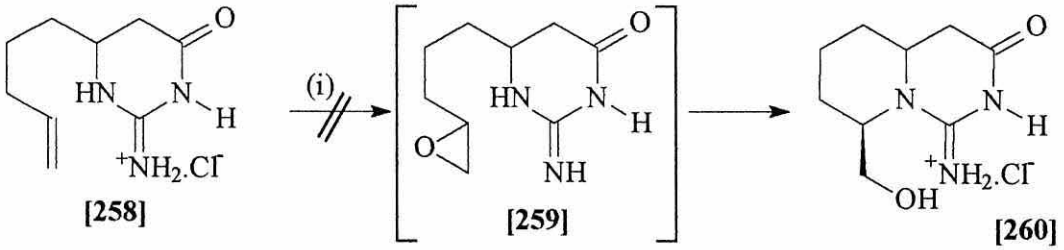
	^{1}H			^{13}C
	δ ppm CD_3OD	**Species**	**J (Hz)**	**δ ppm CD_3OD**
1	-	-	-	-
2	-	-	-	159.00
3	-	-	-	-
4	-	-	-	173.44
5	2.9 2.6	CH CH	J = 5.5 J = 8.2	37.33
6	3.6	CH_2	m	50.88
1'	1.7	CH_2	m	35.16
2'	1.5	CH_2	m	26.11
3'	2.1	CH_2	m	35.05
4'	5.9	CH	m	141.55
5'	5.1	CH_2	m	117.58

Table 16. ^{1}H and ^{13}C NMR for analogue **[258]**.

With **[258]** in hand it remained to introduce the epoxide on C1 which would enable production of the bicyclic system **[260]**. This transformation was envisaged by treatment with *m*CPBA which would then enable nucleophilic attack of the amine group present within the guanidine moiety to open the epoxide and form the bicyclic analogue **[260]**. Which contained the non-functionalised analogue rings A and B, comparable to cylindrospermopsin **[15]**.

Unfortunately treatment with *m*CPBA did not affect the required transformation and all that was isolated was recovered starting material. One possible cause for the lack of success was solubility problems with **[258]**. The product was originally isolated as the free base which would have absorbed atmospheric CO_2 as H_2O and so produce the carbonate, which is highly insoluble. Thus **[258]** was treated with methanolic HCl

to convert the salt to the chloride which would be slightly more reactive however, this failed to improve matters and no epoxide was obtained. Isopropyl alcohol was added to aid solubility, this though could have been interfering with the cyclisation step *via* the epoxide. The attempts to produce **[258]** are shown in Table 17 where varying equivalents of *m*CPBA were utilised.



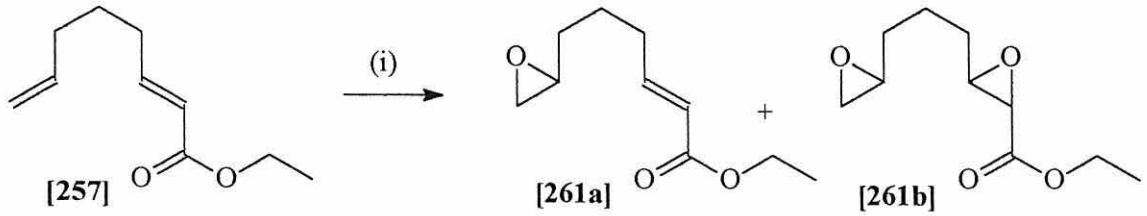
Scheme 54. ***Reagents and Conditions***: (i) DCM, *iso*propyl alchol, *m*CPBA, DCM, RT, 70 h.

Attempt	Equivalents *m*CPBA†	Solvent System	[258].X	% Yield [261]
1	1.5	DCM / IPA	free‡	-
2	1.5	DCM / IPA	free‡	-
3	2.0	DCM / IPA	Cl	-
4	3.0	DCM / IPA	Cl	-

†The equivalents were based on molar concentration of **[258]**
‡Assumed to be $.HCO_3$

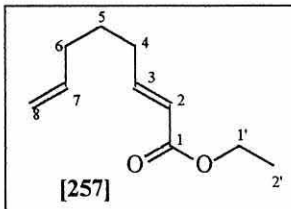
Table 17. Attempted epoxidation reactions of **[258]**.

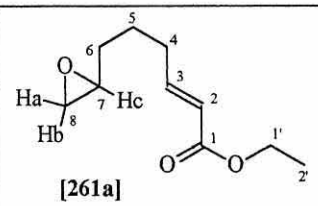
With the failure of route A the next step in the synthesis was to reverse the steps in the reaction, and consider the epoxidation of **[257]**. This involved treatment of **[257]** with *m*CPBA in DCM, which surprisingly produced two epoxides **[261a]** and **[261b]** in a ratio of 80:10 after purification by column chromatography.

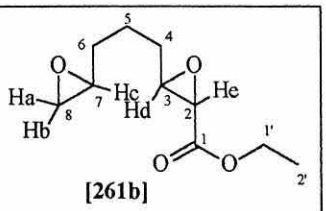


Scheme 55. ***Reagents and Conditions***: (i) *m*CPBA, dry DCM, **[257]**, dryDCM, stir overnight, 80% **[261a]**: 10% **[261b]**.

It was apparent from NMR analysis that **[261a]** was the required *mono*-epoxide and **[261b]** the *bis*-epoxide. The presence of the *bis*-epoxide was confirmed by structural elucidation using ^{1}H and ^{13}C NMR (Table 18).



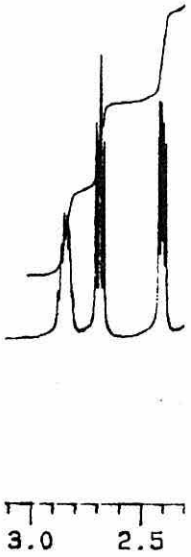




Position	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	-	166.68	-	166.57	-	169.0
2	5.8	148.89	**5.8**	148.32	**H_e3.2**	57.2
3	6.9	137.98	**6.9**	121.79	**H_d3.1**	52.8
4	2.4	33.05	1.6	31.75	1.6	31.8
5	1.5	31.46	1.6	24.44	1.6	22.3
6	2.1	27.13	2.3	46.90	**1.6**	31.9
7	5.8	121.57	**H_c3.0**	51.90	**H_c2.9**	51.8
8	5.0	115.07	**H_a2.5** **H_b2.8**	60.17	**H_a2.5** **H_b2.7**	46.8
1'	4.2	60.12	4.2	60.2	4.2	61.5
2'	1.3	14.25	1.4	14.23	1.3	14.0

Table 18. Comparison of starting substrate to *mono* and *bis*-epoxides.

As can be seen from Table 18 **[261a]** had three epoxide signals corresponding to $H_{a/b/c}$ at δ 2.5, 2.8 and 3.0 ppm respectively (Fig. 39a.) and **[261b]** had five epoxide signals corresponding to $H_{a/b/c/d/e}$ at δ 2.5, 2.7, 2.9, 3.1 and 3.2 ppm respectively (Fig. 39b.).



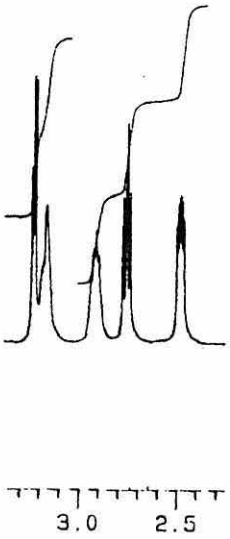


Fig. 39a. ^{1}H NMR of **[261a]**

Fig. 39b. ^{1}H NMR of **[261b]**

With epoxide **[261a]** in hand, guanidine was dissolved in DMF was added to a cooled (0°C) solution of **[261a]** in DMF. This was allowed to warm to room temperature whereupon the reaction was ceased after 96 hours. After filtration of the solid product, washing with diethyl ether and treatment with methanolic HCl a white solid was produced in 16% yield. However, ^{1}H NMR analysis in D_2O established the epoxide signals were still present (δ 2.7, 2.9 and 3.2 ppm). Guanidine had added 1,4 to produce **[259]** but had not then undergone an intramolecular nucleophilic attack to furnish the bicyclic analogue **[260]** (Both ^{1}H and ^{13}C data are shown in Table 19).

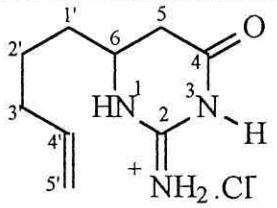
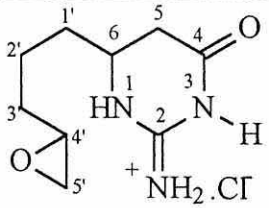
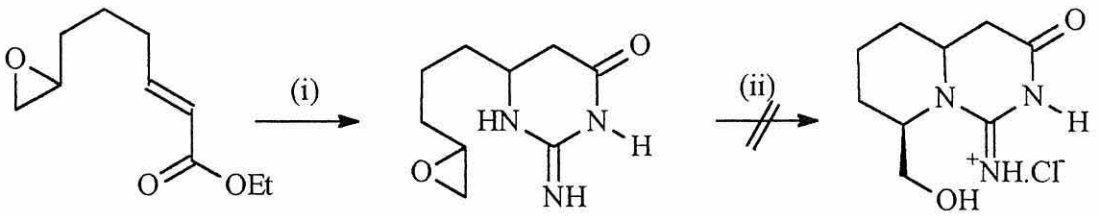
	 [258]		 [259]	
	^{1}H	^{13}C	^{1}H	^{13}C
1	-	-	-	-
2	-	159.00	-	156.00
3	-	-	-	-
4	-	173.44	-	173.40
5	2.9 2.6	37.33	2.3	68.5
6	3.6	50.88	3.7	53.2
1'	1.7	35.16	1.5	33.7
2'	1.5	26.11	1.5	23.1
3'	2.1	35.05	1.5	25.8
4'	5.9	141.55	3.1	53.1
5'	5.1	117.58	2.7 2.9	50.6
-	-	-	-	-

Table 19. NMR data of the two prepared analogues **[258]** and **[259]**.

As a result of the successful production of intermediate **[259]** the next step necessitated the intramolecular nucleophilic opening of the epoxide and ultimately formation of **[260]**. Several attempts were made at facilitating this step, initially utilising the intermediate itself **[259]** by re-dissolving in DMF and heating to 60°C. This though yielded no bicyclic product. The reaction was repeated with the same methodology, guanidine addition in DMF, stir for 96 hours and then warm the stirring reaction mixture for a further 96 hours to 60°C. This though did not produce **[260]**. Several attempts were made to convert **[261a]** to **[260]** these are summarised in Table 20, there was no success.



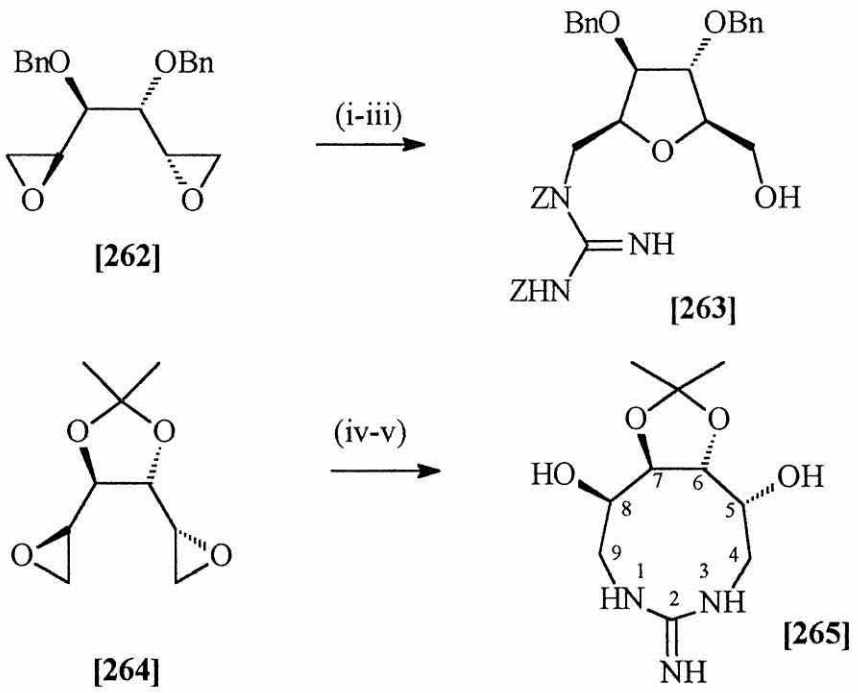
[261a] **[259]** **[260]**

Scheme 56. ***Reagents and Conditions***: (i) DMF, 0°C, guanidine, DMF, RT 96 hrs; D 60°C, 96-168 hrs; (ii) methanoli HCl.

Attempt	Conditions	%Yield
1	(i) dry DMF, 0°C, guanidine, dry DMF, stir RT 96 hrs; (ii) methanolic HCl.	-
2	(i) dry DMF, 0°C, guanidine, dry DMF, stir RT 96 hrs; Δ60°C 96 hrs; (ii) methanolic HCl.	-
3	(i) dry DMF, 0°C, guanidine, dry DMF, stir 96 hrs ;Δ60°C 168 hrs; (ii) methanolic HCl.	-

Table 20. Attempted cyclisation products to form **[260]**.

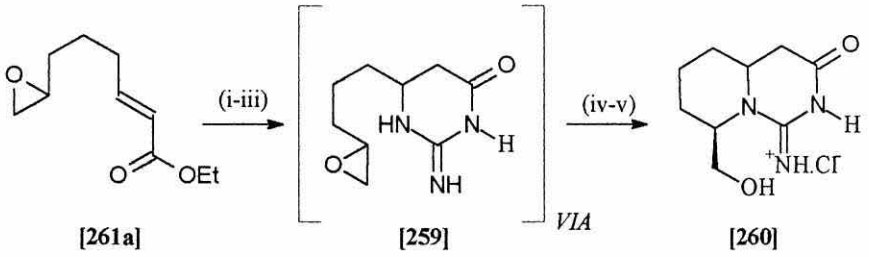
On inspection of the literature, some recent work reported by Le Merrer *et al.* on the nucleophilic attack of epoxides by guanidine, as a route to potential substrates or inhibitors of nitric oxide synthases,[94] enabled an alternative method to be used. They had developed an efficient method for the synthesis of monosubstituted guanidines through the regiospecific nucleophilic opening of an epoxide by free guanidine, followed by an *in situ* selective protection of the guanidine moiety. What was of great importance was both the method of producing the free guanidine and the solvent system used.[95]



Scheme 57. ***Reagents and Conditions***: (i) $(GuanH^{+})_2.SO_4$, NaH, ^{t}BuOH, 60°C, 130 h; (ii) iPr$_2$NEt, TMSCl, $(ClCH_2)_2$, 40°C, 2 h; (iii) iPr$_2$NEt, ZCl, 0°C to 20°C overnight, 51%. (iv) guanidinium hydrochloride, EtOH:H_2O, Amberlite$^{(R)}$; (v) guanidine, EtOH, **[264]**, reflux 1 h, 97%.

As can be seen Le Merrer *et al.* generated the free guanidine initially by treatment of guanidinium sulphate with NaH in ^{t}BuOH followed by *in situ* guanidine cyclisation at 60°C which furnished **[263]** in 51% yield. In a later publication they treated guanidinium hydrochloride with an ethanol : water mix on Amberlite followed by guanidine cyclisation which produced **[265]** in 97% yield.

With this methodology in hand, guanidine hydrochloride was dissolved in ^{t}BuOH and treated with NaH. This mixture was allowed to stir for 30 minutes to enable the free guanidine to be produced. Compound **[261a]** was then added as a solution in ^{t}BuOH at RT. This was stirred for 96 hours before being warmed to 60°C and stirred for a further 96 hours. The reaction mixture was evaporated *in vacuo*, treated with methanolic HCl and then purified using column chromatography which furnished the supposed bicycle **[260]** as a white solid in 41% yield (entry 2 in Table 21).



[261a] [259] [260]

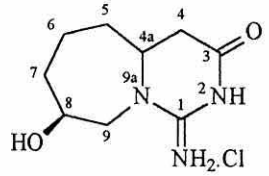
Scheme 58. Attempted synthesis of the AB analogue

Attempt	Conditions	%Yield
1	(i). Guanidine hydrochloride, ^{t}BuOH; (ii). NaH, stir 30mins, RT; (iii). **[261a]**, ^{t}BuOH, RT 96 hrs; (iv), Δ60°C 96 hrs; (v). Methanolic HCl.	21
2	(i). Guanidine hydrochloride, ^{t}BuOH; (ii). NaH, stir 30mins, RT; (iii). **[261a]**, ^{t}BuOH, RT 96 hrs; (iv), Δ60°C 96 hrs; (v). Methanolic HCl.	41
3	(i). Guanidine hydrochloride, ^{t}BuOH; (ii). NaH, stir 30mins, RT; (iii). **[261a]**, ^{t}BuOH, RT 96 hrs; (iv), Δ60°C 96 hrs; (v). Methanolic HCl.	35

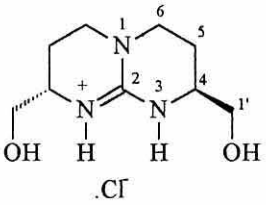
Table 21. Attempts made to cyclise epoxide **[261a]**.

Initial analysis of the product of this reaction was attempted using MS which indicated that the bicyclic compound had been formed, due to the the presence of the required M^+ at 198 Daltons. The ^{1}H NMR of the product was extremely complex but appeared to fit the general structure **[260]**. However, on close inspection of the ^{13}C NMR it was clear that the reaction was not as clean as those previously observed for the formation of the bicycles **[258]** and **[259]**. The second factor was that the ^{13}C NMR of the major component did not appear to correlate with the formation of the 6,6-bicycle **[260]**. Comparison of the ^{1}H NMR with the known compound **[266]**[96] indicated that the expected signals at *ca* δ = 64.0 ($\underline{C}H_2OH$) and δ = 50.3 ($\underline{C}HN$) were not present, instead signals at δ = 71.0 ($\underline{C}HOH$) and 58.4 (CH_2) were indicative of the formation of the 6,7 bicycle **[267]** a more detailed analysis of the major signals in the spectrum (Table 22) seems to support this hypothesis, as very little if no correlation with the known compound **[266]** is apparent.

This result is somewhat disappoiting as this reaction was the cornerstone of our proposed methodology.



[267]



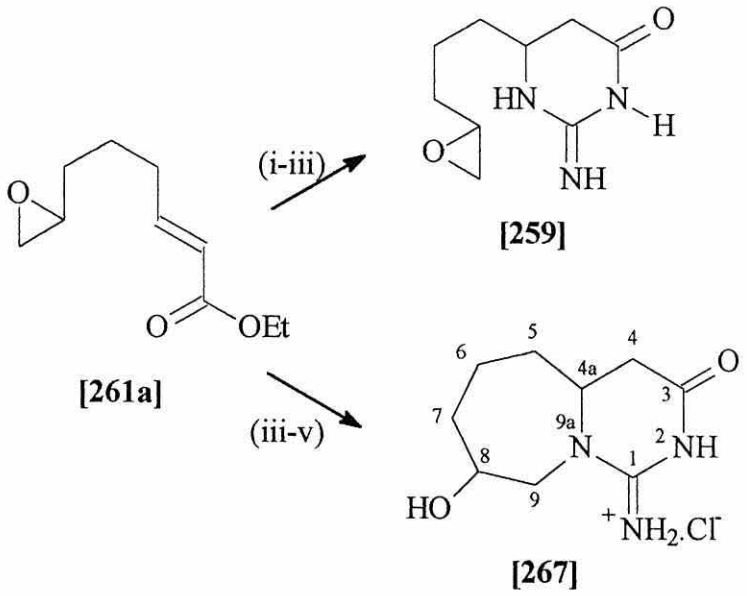
[266]

Position	^{13}C	Position	$^{13}C^{95}$
1	**160.2**	1	-
2	-	2	151.6
3	169.5	3	-
4	37.5	4	50.3
4a	60.8	4a	-
5	**35.3**‡	5	22.7
6	21.1	6	45.5
7	**35.1**‡	7	-
8	71.0	8	-
9	58.4	9	-
1'	-	1'	64.0

Table 22. NMR data of **[267]** with Mendoza's bicycle **[266]**.
†Bold notation indicates simalarity to the natural product **[15]**.
‡Interchangeable assignments.

4.4 CONCLUSIONS

A methodology has been established which has enabled access to a bicyclic intermediate *via* a 1,4-addition of guanidine to **[261a]**. This furnished the epoxide intermediate **[259]** which could be isolated, however subsequent heating of the reaction to 60°C enabled an intramolecular nucleophilic epoxide opening to generate the bicyclic guanidine **[267]** (summarised in scheme 59).



Scheme 59. ***Reagents and Conditions***: (i) guanidine hydrochloride, ^{t}BuOH; (ii) NaH, stir 30min, RT; (iii) **[261a]**, ^{t}BuOH, RT 96 h; (iv) Δ,60°C 96 h; (v) Methanolic HCl..

The reaction methodology gave the 6-7 product **[267]** rather than the desired 6-6 product **[260]**. It was extremely difficult though to obtain a pure sample of **[267]** due to its polar nature and the presence of reaction by-products and polymeric material.

CHAPTER 5

GLYCOMIMETICS

5.1 INTRODUCTION

Owing to the problems which arose during the opening of the epoxide system leading to the AB analogue of cylindrospermopsin, and the inability to obtain pure products or good yields, we decided to investigate the ring opening of epoxies using guanidine in a more fundamental manner, in order that we might ascertain what was actually occurring during the intramolecular nucleophilic epoxide ring opening reactions. The envisaged scheme of work would produce monocyclic guanidines which are related to a class of biologically active compounds called glycosidase inhibitors.

There is an increasing interest in the isolation and synthesis of glycosidase inhibitors[97] due to their potential use as chemotherapeutic agents.[98,99] Furthermore they may constitute useful tools to unravel the catalytic mechanism of the corresponding enzymes.[100,101] Glycosidase inhibitors[102] have been used to treat diabetes and other metabolic disorders,[103, 104, 105] and have been implicated in the blocking of viral infections.[106, 107] Inhibitors for these enzymes are usually designed to mimic the transition-state or transient intermediate present in the active-site during enzyme catalysis.[108, 109]

A key objective in the emerging field of *glycobiology*[99] has been the development of specific glycomimetics, i.e. carbon or heteroanalogues of sugars which mimic the structure and properties of carbohydrates.[110] Such research has produced many linkage and configuration-specific inhibitors of glycosidases such as the polyhydroxylated piperidines[111,104] and amidrazones[112,109] which disrupt the biosynthesis of N-linked glycoproteins and glycolipids that play prominent roles in immune recognition phenomena and cellular adhesion.[108,113] A major challenge for synthetic chemists is to devise non-carbohydrate templates (i.e. saccharide 'look-alikes') with which to assemble bioactive mono- and oligosaccharide analogues. Several examples of these analogues are depicted below in Fig. 41.[102, 110]

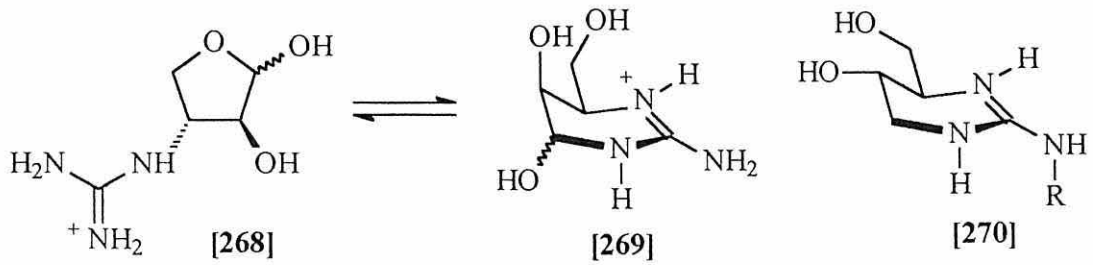


Fig.41. Several examples of glycomimetic analogues Wong *et al.* and Ganem *et al.* respectively.[102,110]

5.2 PREPARATION OF SIMPLE FIVE MEMBERED CYCLIC GUANIDINES

The object of this investigative work was primarily to understand how guanidine was opening the epoxide ring. Thus, similar reaction conditions were used to those utilised in the preparation of the cylindrospermopsin analogue **[260]** (Chapter 4).

To test the feasibility of the methodology a simple epoxide, epibromohydrin, **[271]** was treated with guanidine at RT in ^{t}BuOH to theoretically give the guanidinium hydrobromide **[272]** *in situ.* Deprotonation of this with KO^{t}Bu was followed by heating for 3 days to effect cyclisation to one of the possible products **[273]** and **[274]**. These arise from either a 5-exo-tet or 6-endo-tet reaction illustrated in Fig. 42.

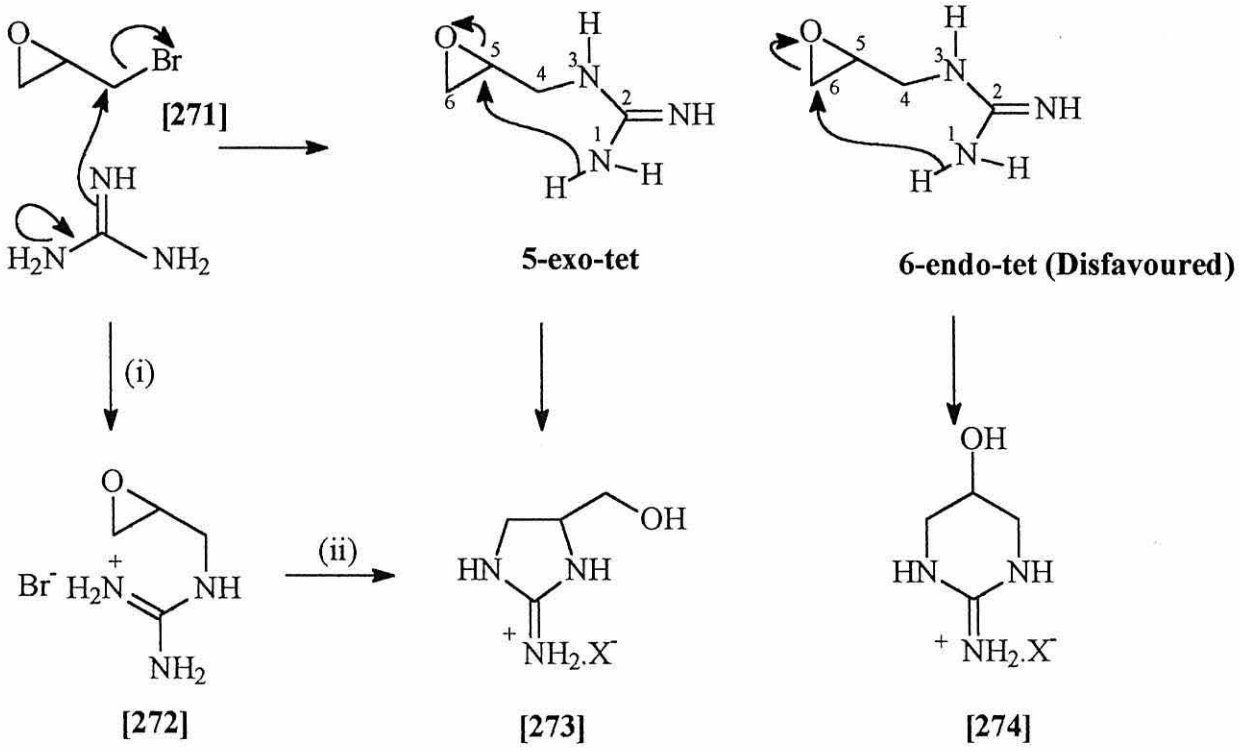
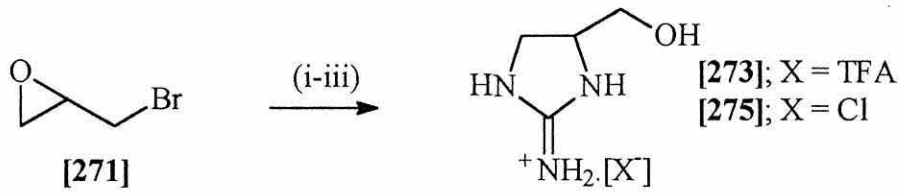


Fig. 42. Determination of which ring would be formed from epibromohydrin. (i) ^{t}BuOH, guanidine,^{t}BuOH, stir 36hrs RT; (ii) K^{t}BuO, Δ 60°C 96hrs.

Analysis of the ^{1}H NMR produced five separate signals at δ 3.73, 3.77, 3.84, 3.93 and 4.3 ppm as four sets of double doublets and a multiplet respectively. This data pointed towards **[273]** as the structure, as this would be expected to show two ABX type coupling patterns.

^{13}C NMR also aided in the structural determination of **[274]** as the plane of symmetry which exists in **[274]** would only produce three signals but four were observed at δ 44.59, 56.46, 62.68 and 158.24 ppm.



[271]

[273]; X = TFA
[275]; X = Cl

Scheme 60. ***Reagents and Conditions***: (i) epibromohydrin, ^{t}BuOH, guanidine, ^{t}BuOH, stir 36h RT; (ii) K^{t}BuO, Δ 60°C 96h; (iii)(a) 0°C, MeOH, TFA stir 5 min, 61%; (b) 0°C, Methanolic HCl, stir 5 min, 75%.

The molarity of the reaction was also investigated to establish whether this would affect the yield of **[273]**. Initially this was not the case, however, after several attempts the yield of the isolated TFA salt increased to 61% at a molarity of 0.27 M with respect to guanidine (Table 23 entries **1-5**). The next step in the optimisation sequence was to repeat the synthesis but alter the acid used in the work-up to establish whether this would make isolation and purification easier. Thus, the reaction was repeated with methanolic HCl replacing TFA. Again the reaction was undertaken at two different molarities 0.27M and 0.8M. The different counterion made no real difference to purification, however, the different concentration increased the yield from 53% at 0.8 M (entry **6**) to 75% at 0.27M (entry **7** and **8**). As a result of this observation all the subsequent reactions were carried out at 0.27 M. These results are summarised in Table 23.

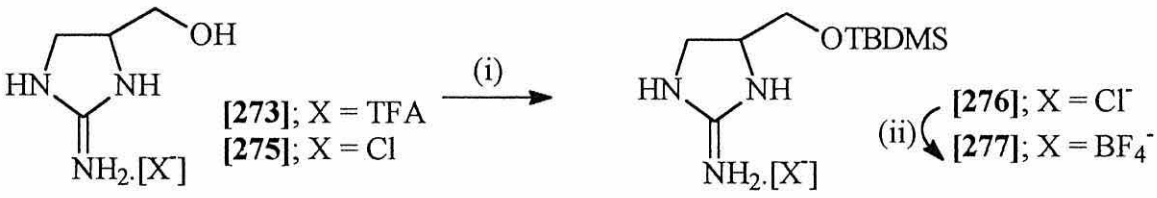
Attempt	%Yield	Molarity[†]
1	12	0.8
2	33	0.8
3	32	0.27
4	61	0.27
5	52	0.27
6	53	0.8
7	75	0.27
8	73	0.27

[†]The molarity was based on the quantity of guanidine used (x10^{-3} g/ml).
[‡]Entries 1-5 **[273]**, entries 6-8 **[275]**.

Table 23. Percentage yields of the different salts of **[273]** at different concentrations.

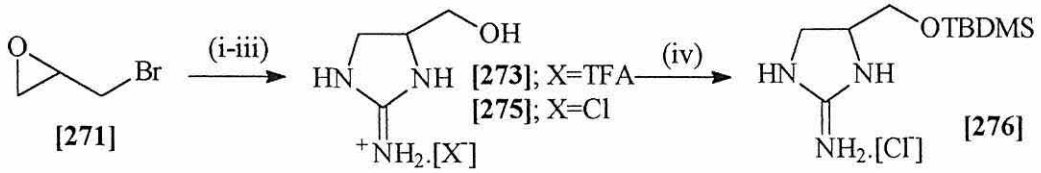
It is worth noting that in all cases several fractions from the purification by column chromatography were obtained which appeared to have polymeric material present, an aspect which has been observed in other guanidine reactions.[81, 86, 87] As NMR studies of these materials showed the presence of trace impurities and exhaustive chromatography was used to produce analytically pure samples, an attempt was made to ease purification by reducing the polarity of the products by derivatisation. Problems with purification were compounded by the presence of mixed counterions as a result of the experimental work-up, this mean't the desired product had differing Rf values which made purification much harder.

Derivatisation was achieved by reacting either **[273]** or **[275]** with *tert*-butyldimethylsilyl chloride in the presence of imidazole. Both salts were used as to ascertain which would undergo protection, purification and isolation the easiest.



Scheme 61. ***Reagents and Conditions***: (i) dry DMF, 0°C, imidazole, TBDMS-Cl, 0°C-RT overnight, 54%(X = TFA); (ii) CH_2Cl_2, sat. $NaBF_4$ solution, RT, overnight, 43% (X = Cl).

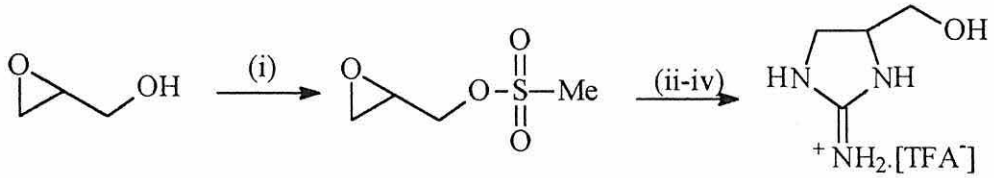
The methodology used to access **[276]** was altered in an attempt to produce a one-pot synthesis. Thus, **[271]** was reacted with guanidine under the standard reaction conditions. The crude product from this reaction was passed through a short silica plug to remove inorganic salts and polymeric material and the product silylated. However, this produced very low yields of **[276]** (10% and 9% respectively from **[273]** and **[275]**), therefore, it was concluded the purification step was essential prior to silylation.



Scheme 62. ***Reagents and Conditions***: (i) epibromohydrin, ^{t}BuOH, guanidine,^{t}BuOH, stir 36h RT; (ii) K^{t}BuO, Δ 60°C 96h; (iii)(a) 0°C, MeOH, TFA stir 5 min; (b) 0°C, Methanolic HCl, stir 5 min; (iv) dry DMF, 0°C, imidazole, TBDMS-Cl, 0°C-RT overnight.

Structural elucidation was achieved by ^{1}H and ^{13}C NMR studies which indicated both salts had undergone protection to initially furnish **[276]** as the hydrochloride. However, MS analysis also indicated the existence of bromide ions. Establishing conversion was achieved initially by IR spectroscopy of the neat compound to prove loss of the broad OH stretch at 3300cm^{-1}. ^{1}H NMR further aided identification with signals at δ 0.0 and 0.8 ppm for the silyl ether with the equivalent ^{13}C signals at δ-5.52, 18.1 and 25.68 ppm. It was seen that conversion to the silylether was more efficient if the original starting material was **[273]**, the TFA based salt yielding 54% of **[276]**, with **[275]** producing only a 20% yield. These materials, as expected, were very easy to purify and analysis of the data was consistent with pure compounds. Ion exchange to the tetrafluoroborate salt **[277]** was achieved by treatment of **[276]** with a saturated solution of sodium tetrafluoroborate in DCM.

The use of mesylate **[279]** was also investigated as a possible starting reagent to access the glycomimetic analogues. Compound **[279]** was obtained by the treatment of glycidol **[278]** with methanesulphonyl chloride and triethylamine to access mesylate **[279]** in 87% yield. Both ^{1}H and ^{13}C NMR established conversion. The ^{1}H NMR signals at δ 2.65, 2.9 and 3.25 ppm equated to the epoxide functionality and δ 3.05 ppm for the methyl group adjacent to the SO_3 group. ^{13}C NMR found signals at δ 37.64, 44.49 ppm for the epoxide carbons and δ 49.09 ppm for the methyl group of the mesylate. Subsequent guanidine addition as with the initial glycomimetics furnished a suspension which was not previously observed with the reactions utilising epibromohydrin **[271]**; however, continuation of the reaction furnished **[273]** in 30% yield.



[278] **[279]** **[273]**

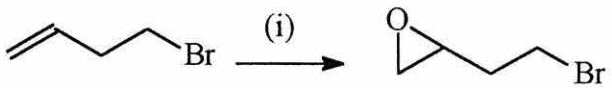
Scheme 63. ***Reagents and Conditions***: (i) 0°C, dry DCM, Et_3N, methanesulphonyl chloride, 0°C to RT overnight, 87%; (ii) ^{t}BuOH, guanidine, ^{t}BuOH, stir 36 h RT, (iii) KO^{t}Bu, Δ, 60°C, 96 h; (iv) 0°C, MeOH, TFA, stir 5 min, 30%

These initial studies proved the best methodology to adopt to form the five membered guanidines **[273]** and **[275]** was treatment of epibromohydrin **[271]** with

guanidine in ^{t}BuOH, with subsequent protection of the free hydroxyl with *tert*-butyldimethylsilyl chloride, and finally ion exchange to the tetrafluroborate salt to remove any other ions such as Br^-, $CH_3SO_3^-$, Cl^- and $CF_3CO_2^-$, which apparently led to purification problems.

5.3 PREPARATION OF SEVEN MEMBERED CYCLIC GUANIDINES

With the previous reactions in hand, the next step was to investigate the preparation of the corresponding six membered guanidine systems. The required starting material for these structures was obtained *via* the epoxidation of 4-bromobut-1-ene **[280]** using *m*CPBA. This furnished the epoxide **[281]** in 92% yield with ^{1}H NMR indicating the loss of the olefinic signals and the appearance of signals at δ 2.4, 2.7 and 3.0 ppm indicating the production of the epoxide. Signals at δ 46.95 and 50.62 ppm in the ^{13}C NMR also confirmed this.

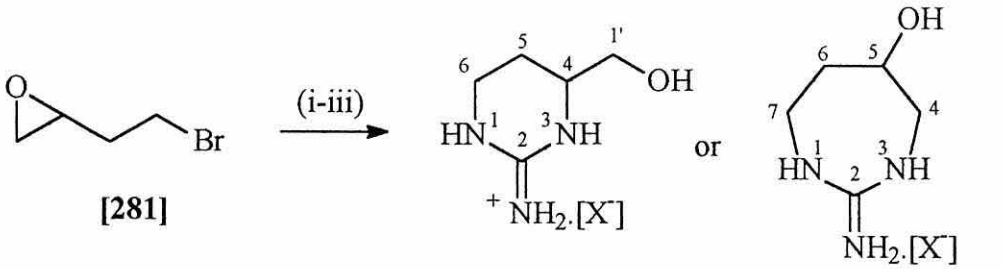


[280] **[281]**

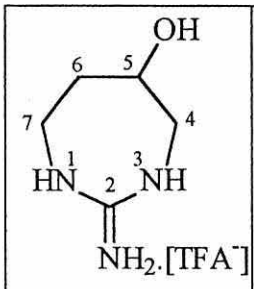
Scheme 64. ***Reagents and Conditions.*** (i) DCM, *m*CPBA, 92%.

With epoxide **[281]** in hand, guanidine was added as with **[271]** to afford what was assumed to be the desired products, **[282a]** with the addition of trifluoroacetic acid and **[282b]** with methanolic HCl in 92% and 84% yield respectively. On inspection of the ^{13}C NMR data for these products it became apparent that this was not actually the case. Structure **[282a/b]** resembled previous isolated compound **[266]**[95] and would be expected to display a CH_2 signal at *ca* 64.0 ppm in the ^{13}C NMR. However, on inspection of the ^{13}C NMR the presence of CH signals at δ = 71.3 and 70.2 ppm were indicative of the formation of the isomeric 7-membered products **[283a/b]**.

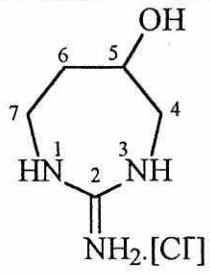
The remainder of the ^{13}C data for these compounds is shown in Table 24 and supports this structure. Unfortunately, no other 7-membered guanidines of this nature are not known in the literature so a direct comparison is not possible, however the 9-membered product **[284]** does show some correlation with **[283a/b]** as do the highlighted signals in **[266]**.



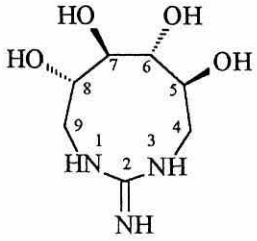
Scheme 65. ***Reagents and Conditions***: (i) ^{t}BuOH, guanidine, ^{t}BuOH, stir 36 h RT, (ii) KO^{t}Bu, Δ, 60°C, 96 h; (iii)(a) 0°C, MeOH, TFA, stir 5 min, 79%; (b) 0°C, Methanolic HCl, stir 5 min, 84%.



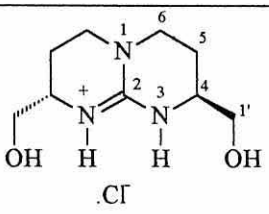
[283a]



[283b]



[284]



[266]

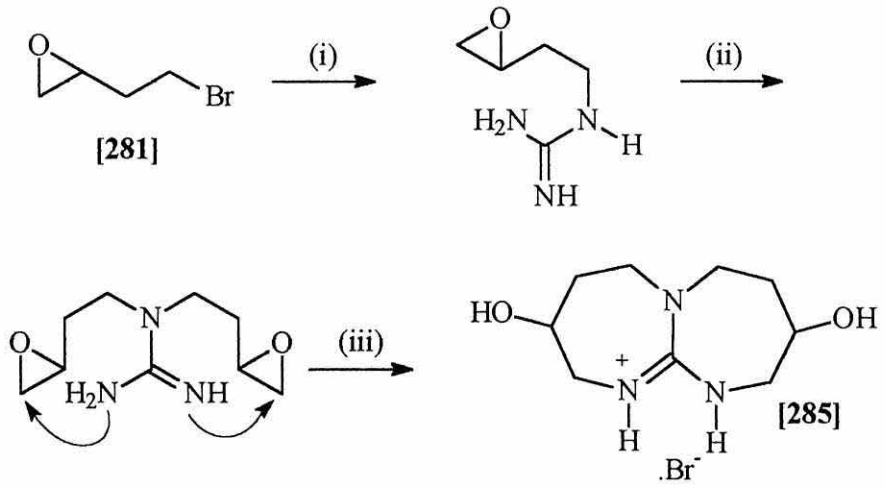
Position	^{13}C δ (ppm)†	^{13}C δ (ppm)†	Position	^{13}C δ (ppm)‡	Position	^{13}C δ (ppm)‡
1	-	-	-	-	-	-
2	158.24	155.02	-	159.6	-	151.6
3	-	-	-	-	-	-
4	**56.7**§	**56.7**	4/9	**54.7**	4	50.3
5	**71.3**	**70.2**	5/6 7/8	**74.2** and **76.1**	-	-
6	34.8	33.7	-	-	5	22.7
7	**46.8**	**45.9**	-	-	6	**45.5**
-	-	-	-	-	1'	64.0

†All NMR's were carried out in CD_3OD.
‡All NMR's in D_2O.
§ bold type shows similar signals.
[284][95], **[266]**[114]

Table 24. ^{1}H and ^{13}C NMR comparison of **[283a]**, **[283b]**, **[284]** and **[266]**.

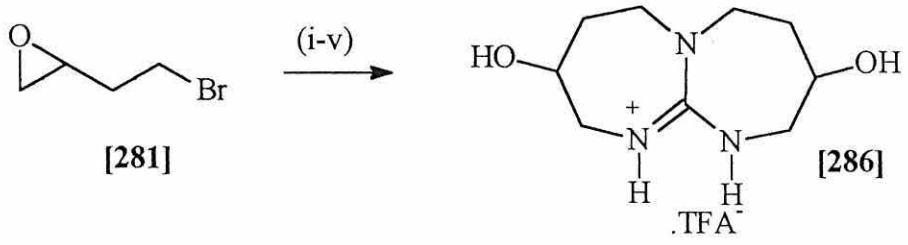
Other spectral data for these materials displayed the presence of a minor impurity which from MS analysis was found to be a dimeric material displaying a mass of 200 Daltons for the protonated guanidine [M^{+}] as opposed to 130 Dalton [M^{+}] for the monomeric adduct **[283]**.

Several dimeric products are possible, however the ^{13}C NMR signals observed seem to indicate an element of symmetry was present in the product and we thus hypothesised that the dimeric structure was **[285]**. This should arise from the double displacement of halide from bromide **[281]** followed by two epoxide opening steps leading to **[285]**.



Scheme 66. ***Reagents and Conditions***: (i) ^{t}BuOH, guanidine, (ii) **[281]**, KO^{t}Bu, (iii) Δ, 60°C.

In order to establish whether this reaction was occurring the initial cyclisation reaction was repeated, but using two equivalents of epoxide **[281]** to enhance production of what was percieved to be the main by-product. A similar method was adopted in which guanidine was added as a solution in ^{t}BuOH to a solution of the epoxide **[281]** in ^{t}BuOH. This was then stirred for 24 hours after KO^{t}Bu was added followed by a further portion of **[281]**, after which the reaction was heated to 60°C for 96 hours.



Scheme 67. ***Reagents and Conditions***: (i) ^{t}BuOH, guanidine, stir 24 h RT, (ii) KO^{t}Bu, stir 24 h RT; (iii) **[281]**, stir 24 h RT; (iv) KO^{t}Bu, stir 24 h RT; (v) Δ, 60°C, 96 h; (vi) 0°C, MeOH, TFA, stir 5 min, 36%.

Two major fractions were isolated after purification by column chromatography with the ^{13}C NMR's of these displaying some interesting signals. Fractions A (Fig. 43) was obviously a mixture composed largely of the previously isolated compound **[283a]**. Fraction B (Fig. 44) however was obviously composed of a mixture of two isomeric compounds with very similar chemical shifts (Fig. 44).

Fig.43. Fraction A.
^{13}C NMR in CD_3OD

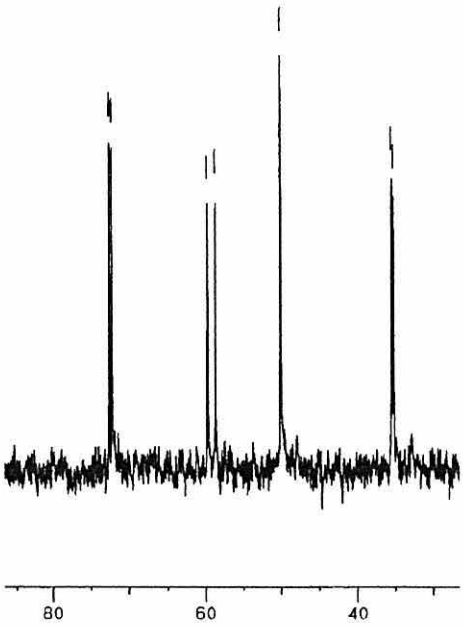
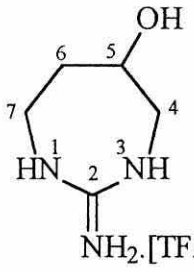
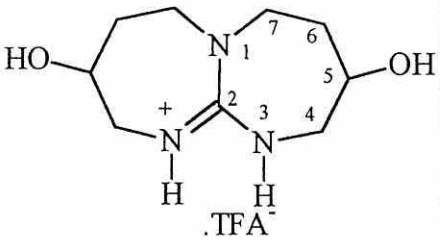


Fig.44. Fraction B.
^{13}C NMR in D_2O

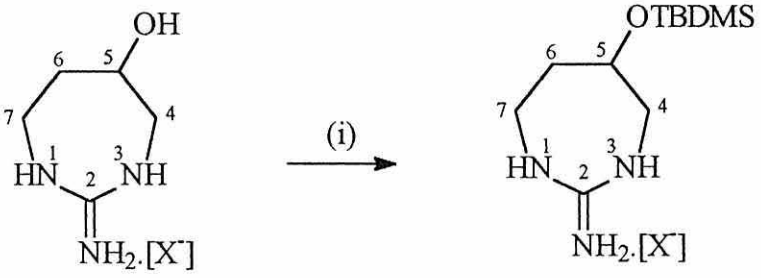
The ^{13}C NMR chemical shifts of both compounds **[283a]** and **[286]** are shown in Table 25 below, and as can be seen there is an excellent correlation of these two structures suggesting that the proposed structure **[286]** is correct..

	 [283a]	 [286]
Position	**^{13}C δ ppm (CD_3OD)**	**^{13}C δ ppm (D_2O)**
1	-	-
2	**158.24**	**158.4**
3	-	-
4	**56.7**[§]	**59.7** and **58.65**
5	**71.3**	**72.56** and **72.22**
6	**34.8**	**35.42** and **35.18**
7	**46.8**	**50.03**

[§]Bold type denotes similar signals

Table 25. ^{13}C NMR comparisons between the monocyclic guanidine **[283a]** and the isolated bicyclic guanidine **[286]**.

As with **[273]** both **[283a]** and **[283b]** were treated with *tert*-butyldimethylsilyl chloride in the presence of imidazole. Both salts were used to ascertain which would undergo protection, purification and isolation to produce the greatest yield.



[283a]; X = TFA
[283b]; X = Cl

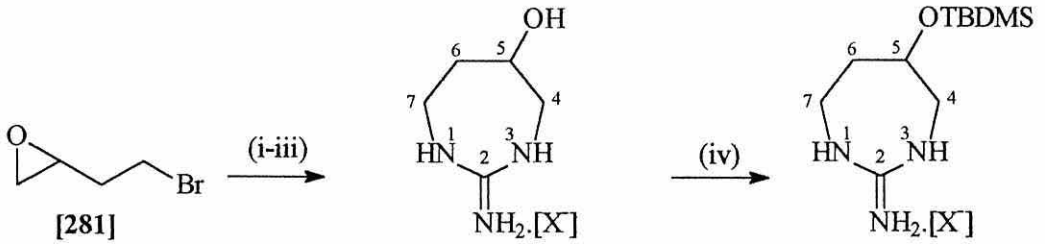
(ii) **[287]**; X = Cl^-
[288]; X = BF_4^-

Scheme 68. ***Reagents and Conditions***: (i) dry DMF, 0°C, imidazole, TBDMS-Cl, 0°C-RT overnight; (ii) $CHCl_3$, sat. $NaBF_4$, RT overnight, 83%.

Structural elucidation was achieved by ^{1}H and ^{13}C NMR studies which indicated both salts had undergone silylation to give **[287]**. Again as seen with **[276]** MS analysis displayed the presence of several counterions. Establishing conversion was achieved initially by IR spectroscopy of the neat compound to prove loss of the broad OH stretch

at 3346cm^{-1} for **[283a]** and 3389cm^{-1} for **[283b]**. The ^{1}H NMR of **[287]** displayed signals at δ 0.0 and 1.3 ppm for the silyl ether with the equivalent ^{13}C signals at δ-4.85, 17.91 and 25.69 ppm. It was seen that conversion to the silyl ether was more efficient if the original starting material was **[283a]** the TFA based salt yielding 64% of **[287]**, with **[283b]** producing only a 14% yield. This step had made purification by column chromatography a great deal easier, due to the reduction in polarity of the species. Ion exchange to the tetrafluoroborate salt **[288]** was achieved in similar fashion to **[277]**. This furnished **[288]** in 83% yield.

As with the five membered analogue conversion to **[287]** was attempted without isolating the intermediate alcohols. This was carried out in exactly the same manner as detailed in Section 5.2 leading to a similar result, in that very low overall yields of 7% and 5% were obtained..



[283a]; X = TFA
[283b]; X = Cl

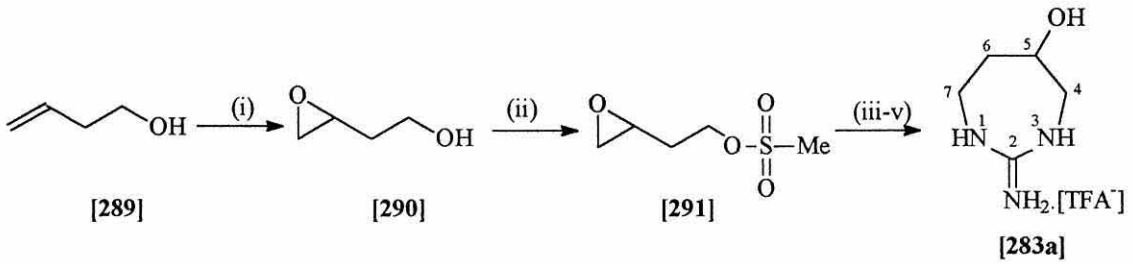
[287]; X = Cl^{-}

Scheme 69. ***Reagents and Conditions***: (i) **[281]**, ^{t}BuOH, guanidine,^{t}BuOH, stir 36h RT; (ii) K^{t}BuO, Δ 60°C 96h; (iii)(a) 0°C, MeOH, TFA stir 5 min; (b) 0°C, Methanolic HCl, stir 5 min; (iv) dry DMF, 0°C, imidazole, TBDMS-Cl, 0°C-RT overnight.

As with the five membered analogue, further investigations were carried out on the starting material used. Originally bromoepoxide **[281]** was utilised to afford addition and subsequent cyclisation, it was however, percieved that a mesylate may allow a more favourable addition and purification, not necessarily seen with **[271]**.

Mesylate **[291]** was prepared by treatment of 1-hydroxy-but-3-ene **[289]** with *m*CPBA to afford epoxide **[290]** in 74% yield. The successful conversion being established by the lack of olefinic signals in both ^{1}H and ^{13}C NMR and the appearance of epoxide signals at δ 2.6, 2.75 and 3.1 ppm and δ 46.71 and 50.43 ppm respectively. Subsequent treatment of **[290]** with methanesulphonyl chloride afforded mesylate **[291]** in 74% yield. Both ^{1}H and ^{13}C NMR studies established successful mesylation with the

appearance of a signal at δ 3.1 ppm in the ^{1}H NMR and a signal at δ 37.31 ppm in the ^{13}C NMR, indicative of the mesylate group.



[289] [290] [291] [283a]

Scheme 70. ***Reagents and Conditions***: (i) 0°C, dry DCM, *m*CPBA, 74%; (ii) 0°C, dry DCM, Et_3N, methanesulphonyl chloride, 0°C to RT overnight, 74%; (iii) ^{t}BuOH, guanidine, ^{t}BuOH, stir 36 h RT, (iv) KO^{t}Bu, Δ, 60°C, 96 h; (v) 0°C, MeOH, TFA, stir 5 min, 30%.

Again as with the five membered analogue guanidine addition was achieved under our standard conditions affording **[283a]** in a 30% yield.

Both studies have proven the feasibility of the guanidine addition to the requisite epoxides and have established a methodology in which to access cyclic guanidine systems. Inspection of the literature of similar amine type cyclisation reactions illustrates that these addition reactions give rise to mainly seven membered ring products.[115- 117] However, six membered systems have been obtained from similar cyclisation reactions but with substantially lower yields.[118,119] It appears the size of the ring system which is formed is extremely dependent on the particular substrate used in the cyclisation. If there exists no large conformational restraint on the system then a mix of six and seven membered rings is formed whereas, if there exists a strong conformational restraint the seven membered ring predominates. It may be that in this system the presence of the planar guanidine group gives sufficient bias to the system to force it to give the seven membered product which attacks the sterically less demanding primary position, as opposed to attack at the more hindered secondary position.

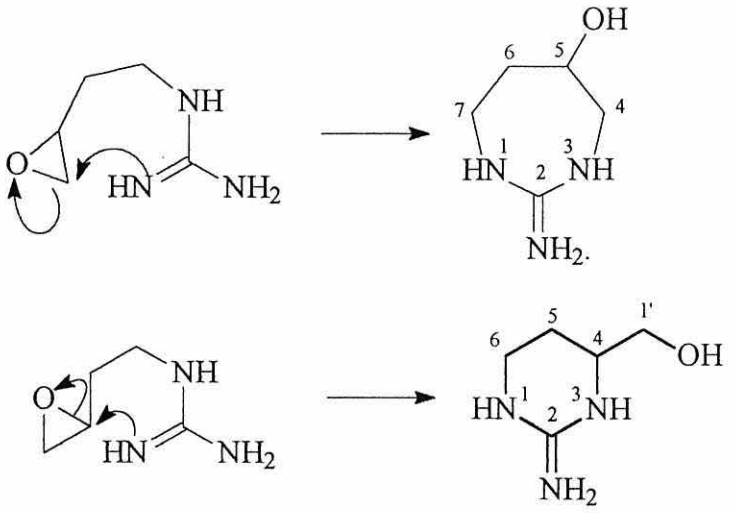
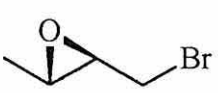


Fig. 45. Representation of the epoxide opening.

Furthermore both studies have established the best methodology for the synthesis of these cyclic guanidines is to prepare the silyl protected system in which the counterions have been exchanged for tetrafluoroborates, thus, removing any problems encountered with mixed ion products which has made isolation problematic.

5.4 PREPARATION OF THE SUBSTITUTED FIVE MEMBERED CYCLIC GUANIDINES

As the previous two experiments of this reaction had been utilising epoxides derived from terminal alkenes and thus, terminal epoxides, synthetic efforts were focused on investigating the use of more substituted epoxides. The two systems **[292]** and **[293]** were chosen for study.



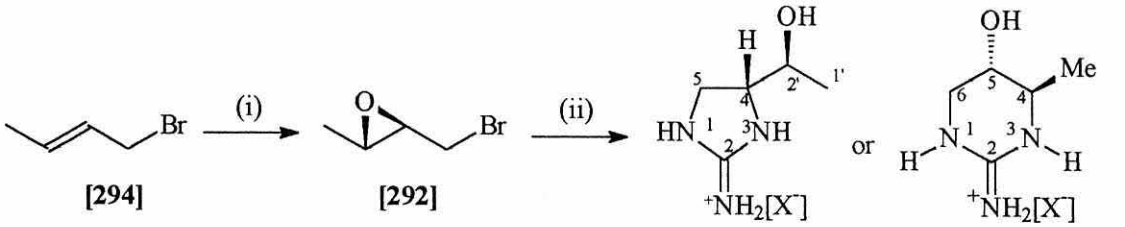
[292]

[293a]; X = Br
[293b]; X = I
[293c]; X = Cl
[293d]; X = Ms

Fig. 45. Substituted epoxides to be usedfor the addition of guanidine.

Initially crotyl bromide **[294]** was treated with *m*CPBA to afford 1-bromo-2,3-epoxybutane **[292]** in 80% yield, with both ^{1}H and ^{13}C NMR indicating a successful reaction with signals at δ 2.9 ppm in the ^{1}H NMR and δ 56.59 and 58.02 ppm in the ^{13}C

NMR for the epoxide. As the previous cyclisation reactions had established that the most successful synthetic route went *via* the TFA work-up method, guanidine was added in ^{t}BuOH to **[292]**, with subsequent addition of KO^{t}Bu after 36 hours, warming to 60°C for 96 hours which produced **[295]** in a moderate 36% yield. The successful addition was established by both ^{1}H and ^{13}C NMR studies. As the yield obtained was disappointing the reaction work-up was repeated with methanolic HCl, which furnished **[296]** in a 52% yield. The data for these two compounds is shown below in Table 26.



[294] **[292]**

[295]; X = TFA
[296]; X = Cl

[295a]; X = TFA
[296a]; X = Cl

Scheme 71. ***Reagents and Conditions***: (i) 0°C, *m*CPBA, dry DCM, 80%; (ii) ^{t}BuOH, guanidine, *t*BuOH, stir 36 h, RT, (iii) KO^{t}Bu, Δ, 60°C, 96 h; (iv)(a) 0°C, MeOH, TFA, stir 5 min, 36%; (b) 0°C, MeOH/HCl, stir 5 min, 52%.

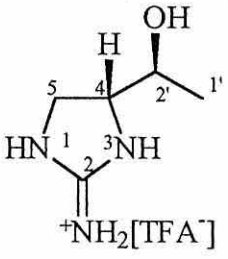
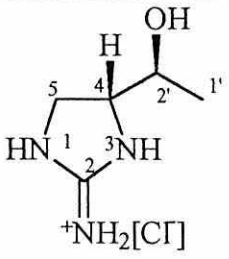
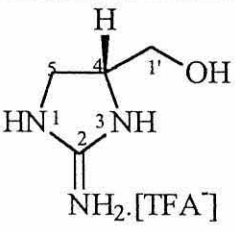
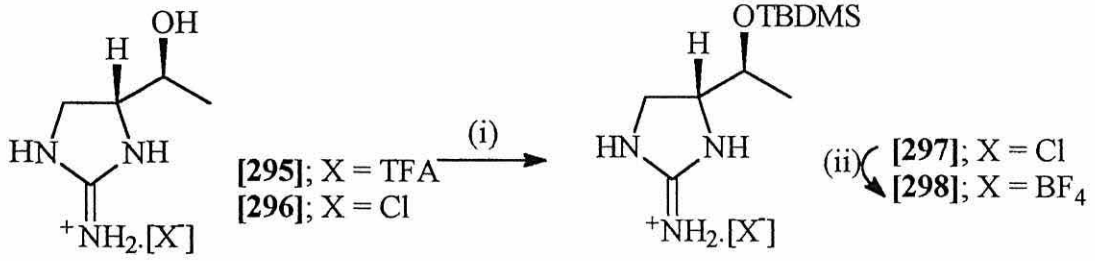
	 **[295]**	 **[296]**	 **[273]**
Carbon	**^{13}C δ ppm (CD_3OD)**	**^{13}C δ ppm (CD_3OD)**	**^{13}C δ ppm (CD_3OD)**
1	-	-	-
2	160.75	160.18	158.24
3	-	-	-
4	62.71	62.29	56.46
5	45.82	45.65	45.59
1'	19.42	19.30	62.68
2'	69.45	69.24	-

Table 27. ^{1}H and ^{13}C NMR correlation of **[295]**, **[296]** and **[273]**.

Table 27 is further evidence of the production of the five membered ring due to the correlation of the signals. If the six membered product **[295/6a]** had been produced the carbon signal for the C-OH would be at approximately δ 75 ppm as seen in compound **[284]**. There does exist some difference between the two salts, especially with the C-NR peaks at 160.75 and 160.18 ppm for **[295]** and **[296]** respectively. Furthermore, the signals relating to C1' and C2' differed in both ^{1}H and ^{13}C NMR with C1' at δ 1.1 ppm as a doublet J = 6.4 Hz, and δ 1.4 ppm as a doublet J = 6.5 Hz and C2' 2.2 ppm and 3.85 ppm for **[295]** and **[296]** respectively. The related ^{13}C NMR signals for C1' were δ 19.42 and 19.30 ppm and C2' were δ 69.45 and 69.24 ppm for **[295]** and **[296]** respectively.

Protection of **[295]** and **[296]** was performed as before, by treatment of each species with *tert*-butyldimethylsilyl chloride and imidazole in DMF to produce the protected salt **[297]** in 59% yield and 32% yield from **[295]** and **[296]** respectively.

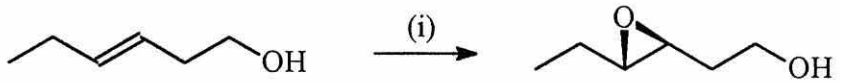


Scheme 72. ***Reagents and Conditions***: (i) dry DMF, 0°C, imidazole, TBDMS-Cl, 0°C-RT overnight; (ii) sat. $NaBF_4$, $CHCl_3$, overnight, 52%.

Structural elucidation was achieved by NMR, MS and HRMS analysis. The ^{1}H NMR had signals which had previously not been assigned at δ 0.0 and 0.7 ppm and δ -4.32, 17.8 and 25.65 ppm. Both MS and HRMS further confirmed the structure with an M^{+} of 244 and 244.1842 daltons respectively. Finally as dictated by the methodology, **[297]** was converted to the fluoroborate **[298]** in 53% yield to remove the mixed counterions seen by negative ion electrospray mass spectrometry.

5.5 PREPARATION OF THE SUBSTITUTED SIX MEMBERED CYCLIC GUANIDINES

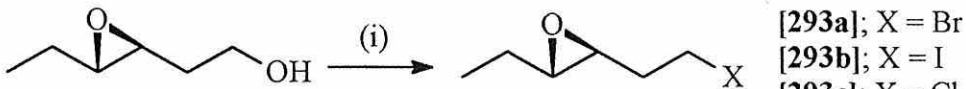
In order to investigate the reaction which led to the seven membered guanidine **[283]**, 1-hydroxy-hex-3-ene **[299]** was treated with *m*CPBA to effect epoxidation. Epoxide **[300]** was isolated in 72% yield with both ^{1}H and ^{13}C NMR showing the characteristic epoxide signals at δ 2.7 and 2.8 ppm as multiplets and δ 56.62 and 59.50 ppm respectively. Again further clarification was obtained from the lack of signals in the olefinic regions in both spectrum.



[299] **[300]**

Scheme 73. ***Reagents and Conditions***: (i) 0°C, dry DCM, mCPBA, 0°C to RT overnight, 72%.

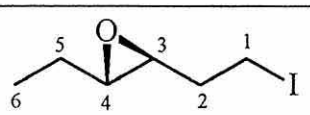
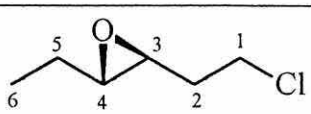
With **[300]** in hand, it was assumed that conversion to the bromide could be effected by treatment of the epoxide with CBr_4 in the presence of triphenylphosphine. This however, proved problematic and the halogenated epoxide **[293a]** was not isolated despite varying the conditions. As a result the iodide **[293b]** was seen as a possible alternative starting material. Therefore, treatment of 1-hydroxy-3,4-epoxyhexane **[300]** with iodine in the presence of triphenylphosphine and imidazole in acetonitrile afforded **[293b]** in 87% yield. The loss of the OH stretch at 3420 cm^{-1} in the IR spectrum indicated successful conversion along with ^{1}H, ^{13}C, MS and HRMS. The chloride epoxide **[293c]** was also prepared in 50% yield by treating **[300]** with CCl_4 in the presence of triphenylphosphine at reflux. The ^{1}H and ^{13}C NMR data is shown in Table 27.



[300]

[293a]; X = Br
[293b]; X = I
[293c]; X = Cl

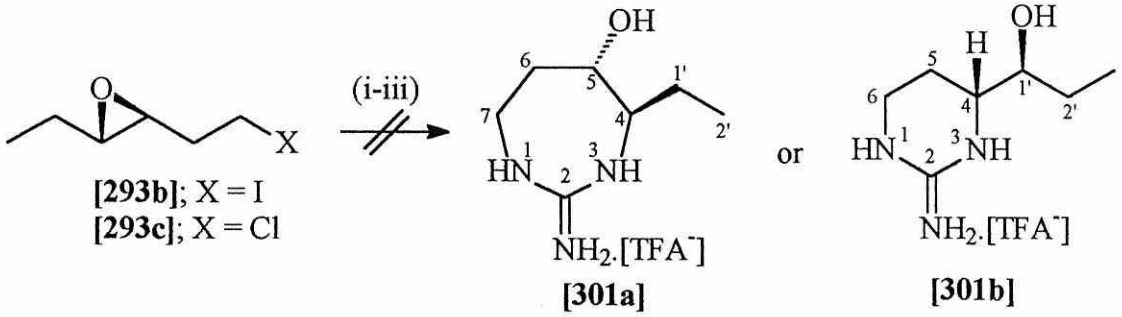
Scheme 74. ***Reagents and Conditions***. (i) CBr_4, PPh_3; (ii) PPh_3, imidazole, CH_3CH / Et_2O, 0°C, I_2 stir 20 min, warm to RT, 0°C, **[300]** over 15 min, stir 1 h warm 0°C-RT, 0°C, pentane, 87%; (iii) CCl_4, 0°C, PPh_3, 0°C-RT overnight, reflux 3h, 50%.

	 [293b]		 [293c]	
Carbon	**^{1}H δ ppm**	**^{13}C δ ppm**	**^{1}H δ ppm**	**^{13}C δ ppm**
1	3.7	0.60	3.6 (J = 7.4 Hz)	41.36
2	2.3	35.86	2.0	35.86
3	2.9	59.75	2.8	59.96
4	2.9	58.22	2.7	55.72
5	1.7	24.93	1.5	24.96
6	1.2 (J = 7.5 Hz)	9.88	0.9 (J = 7.5 Hz)	9.75

†NMR solvent in both instances was $CDCl_3$

Table 27. ^{1}H and ^{13}C NMR correlation of both **[293b]** and **[293c]**.

The addition of guanidine to both **[293b & 293c]** was attempted as previously, however, the predicted outcome was not the same and **[301a** or **b]** were not isolated.



Scheme 75. ***Reagents and Conditions***: (i) ^{t}BuOH, guanidine, ^{t}BuOH, stir 36 h RT, (ii) KO^{t}Bu, Δ, 60°C, 96 h; (iii) 0°C, MeOH, TFA, stir 5 min.

Both ^{1}H and ^{13}C NMR's of both additions indicated olefinic signals present at δ 5.5-6.0 and δ 120-140 ppm respectively. The guanidine C=N signal was present, but at δ 160 ppm in the ^{13}C NMR. This established that guanidine had added to both the epoxides but not in the manner normally observed. It was considered that the basic nature of guanidine and the leaving group tendency of the iodide and chloride had enabled the guanidine to remove a proton at C2 of **[293b & 293c]** and so eliminate HX before attacking the epoxide, as shown below.

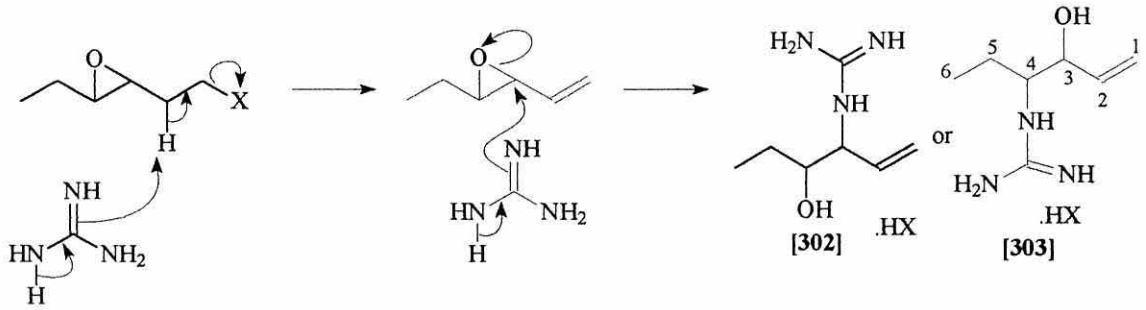
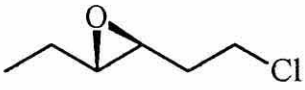
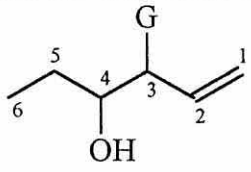
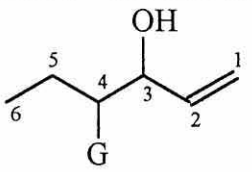


Fig. 46. Possible mechanism for the guanidine addition to **[293b & 293c]** where X = I or Cl.

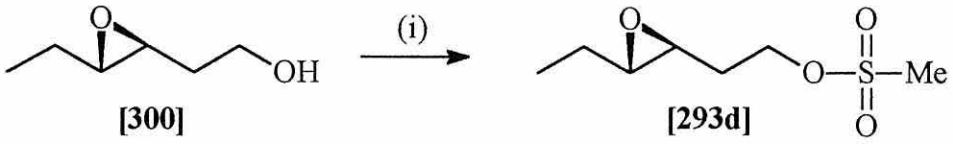
NMR studies established that either **[302]** or **[303]** had been produced due to the presence of characteristic signals for vinylic methylene and methine groups, as well as methine signals indicative of the presence of CHOH and CHON groups.

Starting Material	 **[293b]**	 **[293c]**
Product	 .HX or  .HX X = I or Cl	
Carbon	**[302]**	**[303]**
1	118.98	119.02
2	134.57	134.65
3 / 4	60.11	60.52
4 / 3	75.7	76.1
5	27.5	27.6
6	11.08	11.20

† G is equivalent to guanidine.
Bold notation relates to either structure **[302] / [303]**.
Table 28. ^{13}C NMR correlation.

Due to this failure the mesylate, as with the earlier examples was utilised. Thus, alcohol **[300]** was treated with methanesulphonyl chloride in the presence of Et_3N to give **[293d]** in 73% yield. Both ^{1}H and ^{13}C NMR established protection of the hydroxyl group with the appearance of a methyl signal at δ 3.1 ppm as a singlet in the ^{1}H NMR and δ 37.40 ppm in the ^{13}C NMR respectively equating to the methyl group of the

mesylate. MS and HRMS further clarified production with an [M + NH_4^+] of 212 daltons

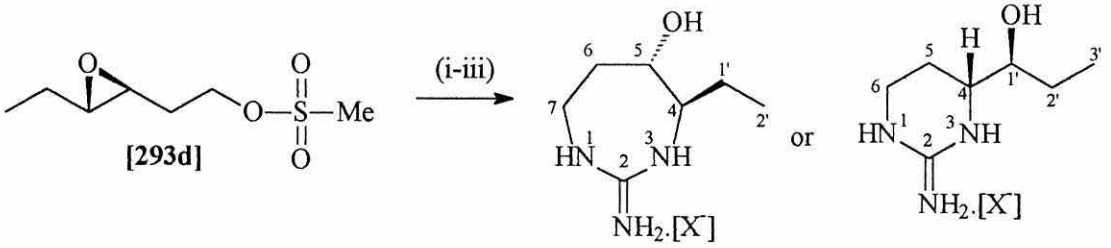


[300] **[293d]**

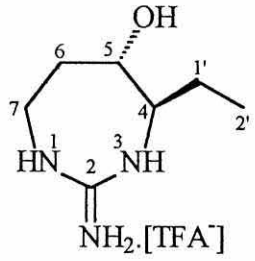
Scheme 76. ***Reagents and Conditions***: (i) 0°C, DCM, Et_3N, methanesulphonyl chloride, 0°C-RT, 1 h, 73%.

With **[293d]** in hand, guanidine was added as a solution in ^{t}BuOH in similar fashion to previously and after the initial 36 hour time period stirring at RT, KO^{t}Bu was added and the reaction warmed to 60°C whilst stirring for a further 96 hours. Both ^{1}H and ^{13}C NMR showed that guanidine had added and formed a cyclic system but initially it was difficult to determine whether the 7-membered product **[310]** or the 6-membered **[310b]** product had been formed. We sought to determine the structure of **[301a/b]** by comparison with known data and we were able to use our previously isolated 7-membered guanidine **[283a]** for our initial comparison, [Table 29, columns 1 and 2]. It was apparent that despite good correlation for the proposed C<u>H</u>OH signal (C-5) adjacent to the OH group and the guanidine carbon (C-2, shown in bold) the two methylene signals correlated very badly (C-6 and C-7 shown in italics). What was most surprising was that the methine signal for C4 in the supposed structure **[301a]** was at δ = 55.0 ppm which when compared with **[283]** at δ = 56.7 ppm, seemed quite similar. However, a down field shift would be expected with the conversion from a methylene (CH_2) to a methine (CH) group, and this suggests that the structure is not **[301a]**.

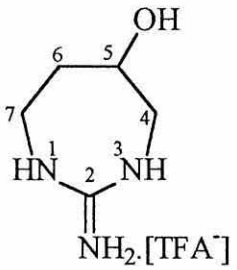
Comparison of the alternate structure **[301b]** with the literature compound **[266]**[95] displayed a better if not totally convincing correlation (Table 29, columns 3 and 4). The methine signal at δ = 55.0 ppm (C-4) shows a reasonable correlation between **[301b]** and **[266]** as do the C-5 and C-6 methylene signals. In addition a value of 74.6 ppm for the C1' methine is also not unreasonable when compared to the literature value of 73.8 ppm for the methine signal of pentan-3-ol. Obviously one must bear in made the obvious structural differences between these compounds when making a comparison.



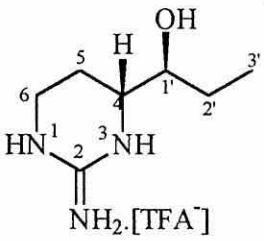
Scheme 77. ***Reagents and Conditions***: (i). ^{t}BuOH, guanidine, ^{t}BuOH, stir 36 h RT, (ii) KO^{t}Bu, Δ, 60°C, 96 h; (iii) 0°C, MeOH, TFA, stir 5 min, 30%.



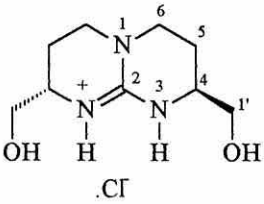
[301a]



[283a]



[301b]



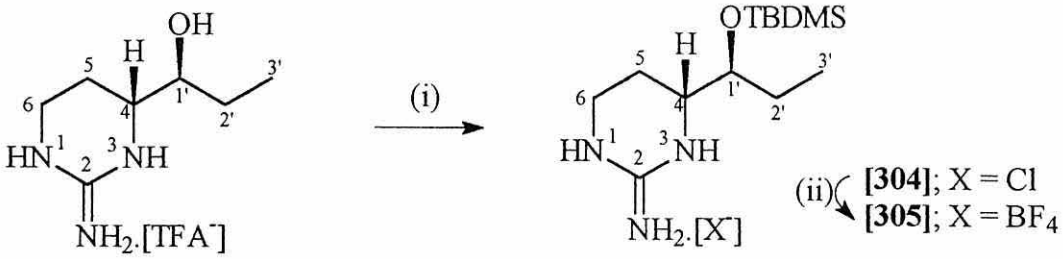
[266]

Carbon	^{13}C δ ppm (CD_3OD)	Carbon	^{13}C δ ppm (CD_3OD)	Carbon	^{13}C δ ppm (CD_3OD)	Carbon	^{13}C δ ppm (CD_3OD)
2	**156.36**§	2	**158.3**	2	**156.36**	2	**151.6**
4	*55.0*¶	4	*56.7*	4	**55.0**	4	**50.3**
5	**74.6**	5	**71.3**	5	**27.0**	5	**22.7**
6	*27.0*	6	*34.8*	6	**38.4**	6	**45.5**
7	*38.4*	7	*46.8*		-		-
1'	21.91	1'	-		74.6		64.0
2'	11.02	2'	-		21.91		-
3'	-	3'	-		11.02		-

§Bold type denotes good correlation, ¶denotes poor correlation.

Table 29. ^{1}H and ^{13}C NMR of **[301a]**, **[283a]**, **[301b]** and **[266]**.

As addition had occurred, continuation of the original methodology produced the silylated compound **[304]** from **[301b]** in 44% yield and subsequent counterion exchange furnished the tetrafluoroborate salt **[305]** in 46% yield which was of high purity.



[301b]

Scheme 78. ***Reagents and Conditions***: (i) dry DMF, 0°C, imidazole, TBDMS-Cl, 0°C-RT overnight; (ii) sat. $NaBF_4$, $CHCl_3$, 46%.

It is now apparent that the intramolecular nucleophilic addition reaction has switched from being under steric control which gave access to the seven membered ring to a position where either species can be obtained and the 6-exo-tet is probably the more favoured reaction pathway where there is equal steric bias.

5.6 OVERALL CONCLUSIONS

The feasibility of the intramolecular nucleophilic reaction with epoxy halides and epoxy mesyl compounds has proven successful This methodology is worth developing into a one-pot type procedure as it gives easy access to a range of glycomimetics. These investigations have led to the following rules to be derived:

(i) 5-exo-tet is favoured

(ii) 6-endo-tet was not observed

(iii) For a terminal epoxide 7-endo-tet is favoured over 6-exo-tet, which may be due to the planar nature of the guanidine nucleophile.

(iv) When 1° and 2° centres are present as with **[282]** 7-endo-tet is favoured as seen in the literature for the amine chemistry, however, when there are only 2° positions the 6-exo-tet reaction occurs.

With this understanding in hand it now appears that the addition of guanidine to substrate **[261a]** in the preparation of cylindrospermopsin will have produced the 7,6

bicyclic system **[267]**; instead of the anticipated **[260]** 6,6 bicyclic system as shown below. This will require further investigative work, however, it does appear a methodology is in place which will give access to pure **[267]**. This would involve silyl protection of the hydroxy group followed by counterion exchange to the tetrafluoroborate and then purification by column chromatography to access **[267]**.

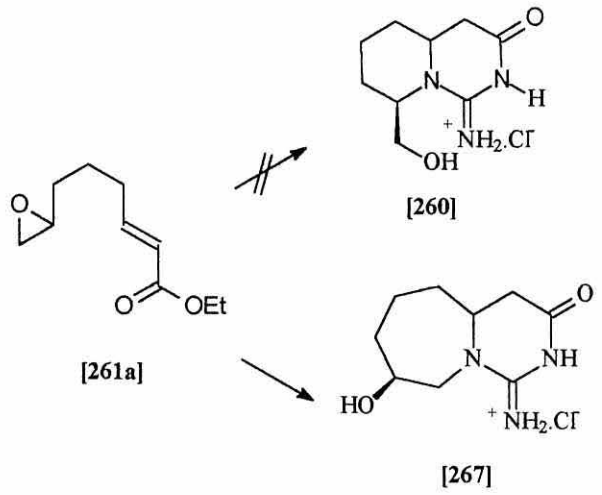


Fig. 46. Attempted synthesis of cylindrospermopsin AB analogue

CHAPTER 6

BIOLOGICAL EVALUATION OF THE GLYCOMIMETICS

6.1 INTRODUCTION

As several glycomimetic analogues had been prepared (Chapter 5) it was important to discover whether any of the cyclic systems **[273]**, **[277]**, **[283a]**, **[288]**, **[295]**, **[298]**, **[301b]** and **[305]** displayed biological activity.

6.2 BIOLOGICAL EVALUATION[120]

The glycosidases assays used in determination included α-glucosidase (yeast), β-glucosidase (almond), α-mannosidase (Jack bean), α-galactosidase (green coffee bean), β-galactosidase (Bovine liver), β-N-acetylglucosaminidase (bovine kidney), naringinase (*Penicillium decumbens*), amyloglucosidase (*Aspergillus niger*) and α-L-fucosidase (human placenta). The substrates were 3.5 mM *p*-nitrophenyl-glycosides and the assays were conducted as described in the literature.[120]

The assays were carried out in microtitre plates with the incubation mixture consisting of 20μl of enzyme solution (approx. 0.1 unit per ml), 20μl of inhibitor solution and 100μl of a 5mM solution of the appropriate *p*-nitrophenyl-glycoside as substrate made up in 50mM phosphate citrate buffer at the optimum pH for the enzyme. Enzymes were tested at 30°C except for β-galactosidase, α-L-fucosidase and β-N-acetylglucosaminidase which were run at 37°C. Below in table 31 is a summary of the biological activity.

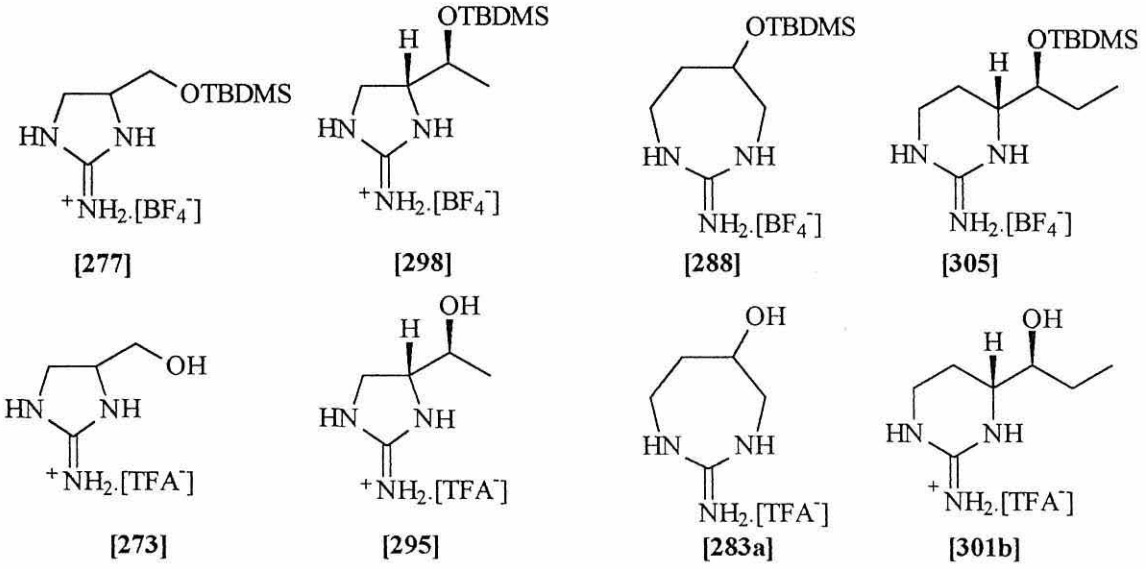


Fig. 47. Samples for biological testing.

Compound	Highest conc. Assays (μg/ml)	1 (%)	2 (%)	3 (%)	4 (%)	5 (IC_{50}) (μg/ml)	6 (%)	7 (%)	8 (%)	9 (%)
[277]	740	×[†]	×	×	×	140	×	×	×	×
[298]	266	×	×	×	×	31	×	×	×	×
[288]	476	×	×	×	×	113	×	×	×	×
[305]	420	×	×	×	×	5.6	×	×	15	×
[273]	1134	×	×	×	×	245	×	×	13	×
[295]	84	×	×	×	×	266	×	×	18	×
[283a]	644	×	×	×	×	49	×	×	20	×
[301b]	1316	×	×	×	×	196	×	×	34	×

[†]Denotes no inhibition, the glycosidase assays used **1**. α-glucosidase (yeast), **2**. β-glucosidase (almond), **3**. α-mannosidase (Jack bean), **4**. α-galactosidase (green coffee bean), **5**. β-galactosidase (Bovine liver), **6**. β-N-acetylglucosaminidase (bovine kidney), **7**. naringinase (*Penicillium decumbens*), **8**. amyloglucosidase (*Aspergillus niger*) and **9**. α-L-fucosidase (human placenta).

Table 30. Biological evaluation of the eight cyclic adducts as the BF_4 salt

The results show that all the compounds appeared to be inhibitors of β-galactosidase, with varying effectiveness most of them being relatively weak, with the exceptions of **[305]**, **[298]** and **[283a]**. Several activities were observed against the glycosidase assay amyloglucosidase. Despite the poor inhibition with the remaining assays, this apparent specificity is of great interest and further testing would be advantageous.

CHAPTER 7

A SYNTHETIC APPROACH TO THE PLAKORTONE SERIES OF NATURAL PRODUCTS

7.1 INTRODUCTION

During the continuing studies towards the synthesis of guanidine containing metabolites work was also carried out in conjunction with, and following on from, Erasmus students on the plakortone series of natural products.

7.1.1 INTRODUCTION TO THE PLAKORTONES

In 1996 Patil *et al.* reported the isolation of four novel metabolites the plakortones A-D **[307-310]** (Fig. 48) from the sponge *Plakortis halichondrioides.*[121] These were identified as a novel class of activators of cardiac sarcoplasmic reticulum (SR) Ca^{2+} pumping ATPase, a property which may be of value in correcting relaxation abnormalities observed in some forms of human heart failure, *ergo.* Hence an interesting synthetic challenge.

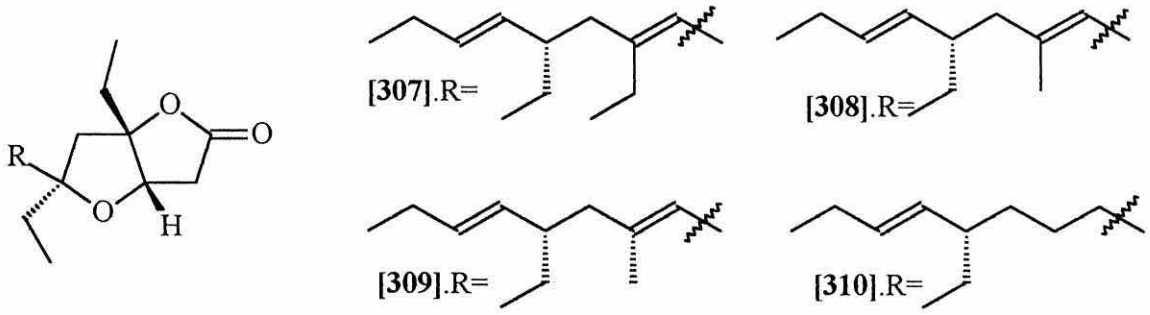


Fig. 48. The family of Plakortones

The metabolites were characterised by the presence of a 2,6-dioxabicyclo[3.3.0] octan-3-one subunit **[313]**. The synthetic strategy to access the bicyclic unit **[313]** was envisaged, and would proceed *via* an intramolecular Wittig cyclisation between a stabilised phosphorane and lactone **[311]**, followed by subsequent hydrogenation of the bicyclic tetronate **[312]** formed (illustrated in Fig. 49).[122]

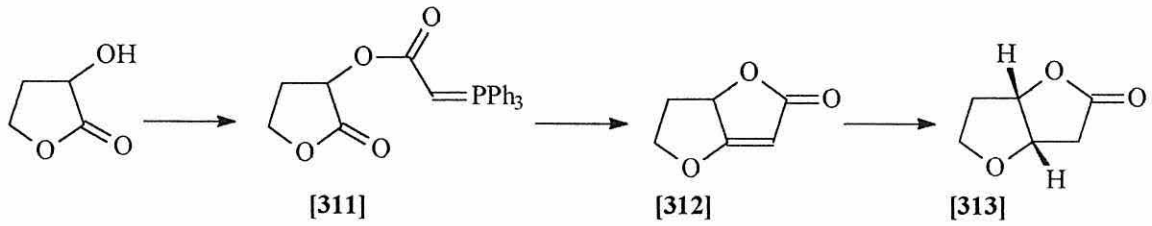


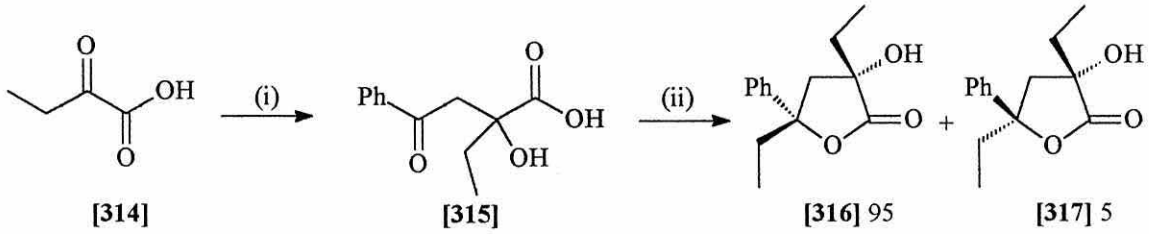
Fig. 49. Envisaged synthetic pathway.

Before the majority of the functionality was introduced into the bicyclic tetronate **[312]** several basic analogues were prepared by other members of the research group to test the methodology.[123]

7.2 BACKGROUND WORK ON THE SYNTHETIC ANALOGUES[124]

Initially several basic analogues were prepared. This work concentrated on the addition of Grignard reagents to an α-hydroxy-γ-ketocarboxylic acid **[315]**, which were prepared by the base catalysed condensation of methyl ketones with 2-oxobutyric acid **[314]** using a modification of a related literature procedure.[125]

Thus, reaction of acetophenone with **[314]** under basic conditions afforded the required ketone **[315]** in 57% yield, which, on treatment with excess ethylmagnesium bromide followed by acid catalysed lactonisation, furnished a 95:5 mixture of **[316]**:**[317]** in a combined 65% yield (Scheme 79). The major lactone was isolated by crystallisation (Fig. 50) with a solvent system of petroleum ether : diethyl ether which enabled the relative stereochemistry to be determined by X-ray crystallography



[314] **[315]** **[316]** 95 **[317]** 5

Scheme 79. ***Reagents and Conditions***: (i) PhCOMe, KOH, MeOH, H_2O, 57%; (ii)(a) EtMgBr, THF, Et_2O; (b) tartaric acid (aq), 65%.

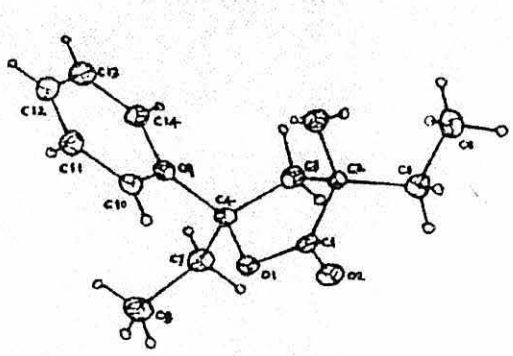
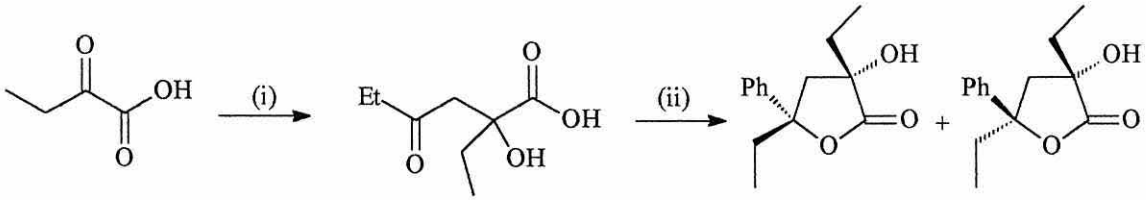


Fig. 50. X-ray crystal structure of **[316]**.

It was observed that **[316]** possessed the opposite stereochemistry to that found in the tetrahydrofuran ring of the plakortones. In order to obtain the correct

stereochemistry the order in which the two γ-substituents were introduced was reversed and so the ethyl ketone **[318]** was prepared in 83% yield from butanone and 2-oxobutyric acid **[314]**. Subsequent treatment of **[318]** with excess phenylmagnesium bromide followed by acidic work-up, afforded an 85:15 ratio of lactone **[317]** with the correct relative stereochemistry, and the previously isolated **[316]** in a 48% combined yield (Scheme 80).



[314] **[318]** **[316]** 15 **[317]** 85

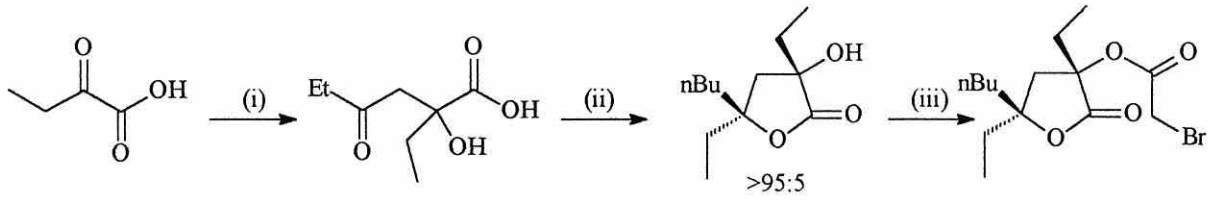
Scheme 78. ***Reagents and Conditions***: (i) EtCOMe, NaOH, MeOH, H_2O, 57%; (ii)(a) PhMgBr, THF, Et_2O; (b) tartaric acid (aq), 48%.

7.3 PREPARATION OF A PLAKORTONE ANALOGUE

As a result of the initial investigative work a methodology had been devised which would enable easy access to analogues of the plakortones with the correct stereochemistry within the ring system.

Thus, the previously synthesised α-hydroxy-γ-ketocarboxylic acid **[318]** was treated with an excess of butylmagnesium bromide which furnished lactone **[319]** in 68% yield, as essentially a single isomer (>95:5) as determined by ^{1}H NMR.

With **[319]** in hand, the bromoacetyl derivative was prepared. Formation of this initially proved problematic with only low yields being obtained after treatment of **[319]** with bromoacetyl bromide, pyridine and DMAP (Table 31). In an attempt to optimise reaction conditions, several combinations of reagents were used, all initially at a concentration of 1.26 x 10^{-3} g/l, and 1.3 equivalents of bromoacetyl bromide (entries **1** to **5** table 31). This though did not have the desired effect with only entry **3** showing any indication of improvement. Due to the inconsistency of the yields obtained, the initial methodology was re-visited (entries **6-8**) but using a concentration of 1.03 x 10^{-3} g/l and 1.1 equivalents of bromoacetyl bromide. This culminated in **[320]** being produced in 63, 66 and 58 % yields (entries **6-8**) respectively.



[314] [318] [319] [320]

Scheme 81. ***Reagents and Conditions***: (i) EtCOMe, NaOH, MeOH, H_2O, 57%; (ii)(a) *n*BuMgBr, THF, 0°C-RT 48 hrs; (b) tartaric acid (aq), 68%; (iii) $BrCOCH_2Br$, pyridine, DMAP, DCM, 0°C-RT 16 hrs, 66%.

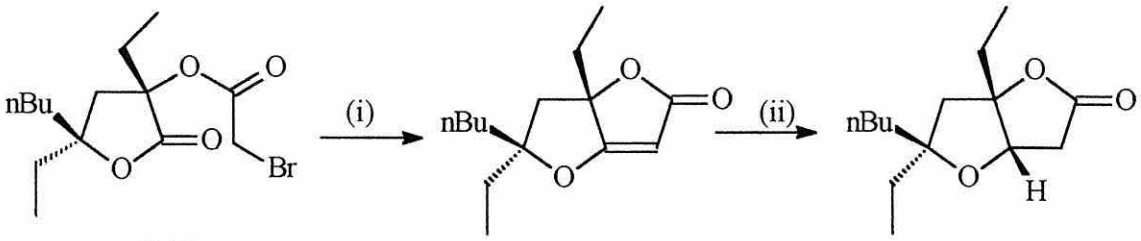
Attempt	Solvent System[†]	Concentration[§]	Equivalents BrAcBr[¶]	Base	% Yield [320]
1	BrAcBr, DCM	1.26	1.3	Pyridine	28
2	BrAcBr, DCM	1.26	1.3	Pyridine	54
3	BrAcBr, DCM	1.26	1.3	Diisopropyl ethylamine	59
4	BrAcBr, Et_2O	1.26	1.3	Pyridine	30
5	BrAcBr, Et_2O	1.26	1.3	Diisopropyl ethylamine	29
6[‡]	**BrAcBr, DCM**	**1.03**	**1.1**	**Pyridine**	**63**
7[‡]	**BrAcBr, DCM**	**1.03**	**1.1**	**Pyridine**	**66**
8[‡]	**BrAcBr, DCM**	**1.03**	**1.1**	**Pyridine**	**58**

[†]A catalytic amount of DMAP was added to all solvent systems
[‡]Solvent system which produced the best results.
[§]Concentration determined with respect to **[319]** ($x10^{-3}$ g/l)
[¶]Based upon mmol of **[319]**.

Table 31. Summary of attempts to produce **[320]**.

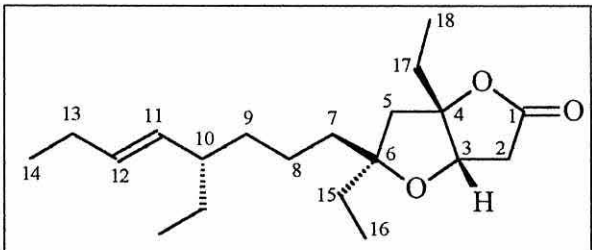
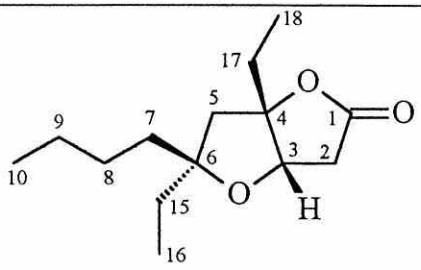
NMR analysis of entries **6** and **7** indicated a successful conversion with the appearance of signals in the ^1H NMR at δ 2.7 ppm and ^{13}C NMR at δ 97.77 ppm equating to the methylene group adjacent to both carbonyl and bromide functionalities. The furan ring was also intact with the existence of an ABX pattern in the ^1H NMR at δ 1.9 ppm as a doublet and at δ 2.25 ppm as a doublet equating to the methylene group within the ring. Compound **[320]** was treated with triphenylphosphine in acetonitrile followed by addition of DBU and refluxing for 90 minutes to furnish **[321]** in 68% yield.

Formation of tetronate **[321]** was essentially determined by the loss of the signal at δ 3.5 ppm in the ^1H NMR spectrum and the appearance of an olefinic methine signal at δ 103.99 ppm in the ^{13}C spectrum. Subsequent hydrogenation with Pd / C gave the analogue **[322]** in 90 % yield. Comparison of both ^1H and ^{13}C NMR of **[322]** with the data from the isolated species shows excellent correlation, as can be seen in Table 32.



[320] [321] [322]

Scheme 82. ***Reagents and Conditions***: (i) PPh_3, MeCN, 40°C 90 min, DBU, MeCN, 0°C-reflux, 90 min, 68%; (ii) H_2, Pd/C, EtOAc, 10 min., 90%.

	 [310]	 [322]
Carbon	**^{13}C (δ, ppm)**	**^{13}C (δ, ppm)**
1	175.6	**175.46**‡
2	37.4	**37.35**
3	80.5	**80.46**
4	97.8	**97.74**
5	45.0	**44.94**
6	87.5	**87.27**
7	38.4	38.10
8	21.5	26.00
9	35.4	23.08
10	44.3	13.99
11	133.2	-
12	132.3	-
13	25.6	-
14	14.2	-
15	31.4	**31.31**
16	8.4	**8.38**
17	30.3	**30.25**
18	8.6	**8.52**

†^{13}C NMR in CDCl

‡Bold notation indicates excellent correlation.

¶The carbon numbers shown do not relate to the numbering used to name **[322]**.

Table 32. ^{13}C NMR comparison of the isolated plakortone and the synthesised analogue **[322]**.

As can be seen from the above table there exists excellent correlation between the similar carbon environments C1-6 and C15-18, in both the isolated product **[310]** and the synthesised analogue **[322]**.

7.4 CONCLUSIONS

This work has shown that by a combination of the stereoselective addition of Grignard reagents to an α-hydroxy-γ-ketocarboxylic acid, an intramolecular Wittig cyclisation and hydrogenation; synthetic analogues to the plakortones can be accessed.

CHAPTER 8

EXPERIMENTAL

SOLVENTS

All solvents used in the reactions were dried using standard methods found in the literature.[126] In particular, diethyl ether and tertrahydrofuran were distilled from sodium wire and benzophenone, chloroform and carbon tertachloride were distilled from phosphorus pentoxide, dichloromethane and dimethylformamide were dried over calcium hydride and freshly distilled.

CHROMATOGRAPHY

All new compounds were homogenous by thin-layer chromatography (TLC) unless otherwise stated. TLC was performed on glass plates coated with Kieselgel 60 F254 (Art. 5554, Merck). Compounds were visualised using ultraviolet light and / or staining with phosphomolybdic acid (PMA) in ethanol or vanillin in ethanol / sulphuric acid and heating to 180°C or staining with iodine. Column chromatography was performed using Merck 7736 silica gel (particle size 40-63μm) under medium pressure with the eluting solvent system specified in each case.

ANALYTICAL METHODS

Melting points were recorded using a Gallenkamp capillary apparatus and are uncorrected. Infra-red spectra were recorded as thin films or solutions where appropriate on a Perkin-Elmer 1600 FT-IR spectrometer. Absorption frequencies are reported in wavenumbers ν, whose unit is the reciprical centimetre (cm^{-1}). Absorption intensities are recorded quantitatively as strong (s), medium (m), weak (w) and broad (br). Microanalyses were obtained using a Carlo-Erba model 1106 CHN analyser. Thermospray (TS) mass spectra were recorded using a Hewlett-Packard engine mass spectrometer. Electron impact (EI) and chemical ionisation (CI) were recorded on a VG Masslab Model 12/253 spectrometer and high resolution mass spectra (HRMS) on a VG Analytical ZAB-E spectrometer at the EPSRC Mass Spectrometry Service Centre at Swansea. Mass measurements are reported in daltons, Br refers to ^{79}Br. Proton NMR spectra were run at 250 MHz on a Bruker AC250 spectrometer unless otherwise stated. Carbon-13 NMR spectra were run at 62.5 MHz on a Bruker AC250 spectrometer unless otherwise stated and were gate decoupled. All spectra were obtained from solutions in deuterated chloroform unless otherwise specified. Chemical shifts are reported as δ

values (ppm) relative to tetramethylsilane as an internal standard. Spin couplings are denoted as J values (Hz), whilst splitting patterns are reported as singlets (s), doublets (d), triplets (t), quartets (q), multiplets (m), broad (br), or any combination of these.

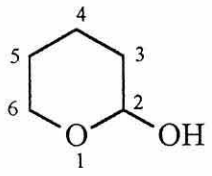
MISCELLANEOUS

All non-aqueous reactions were performed using oven-dried (250°C) glassware which was cooled under a stream of argon or nitogen gas. All experiments were conducted under a positive pressure of inert gas. The solution of *n*-butyllithium in hexanes was titrated[125] against diphenylacetic acid in tetrahydrofuran immediately before use. All yields refer to the pure isolated compound unless otherwise stated. The term *in vacuo* refers to the reduced pressure of a Büchi rotary evaporator at water pump pressure (~15 mm Hg) and or rotary vacuum pump pressure (~1 mm Hg).

METHANOLIC HCl PREPARATION

To cooled (0°C) stirring methanol (9 ml), acetyl chloride (1 ml) is added dropwise. This is then used after several minutes stirring.

2-Hydroxytetrahydropyran[81]



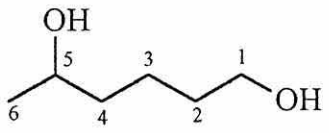
[209]

To a cooled solution (0^0C) of 2,3-dihydropyran (9.22g, 10 ml, 0.1096 mol) was added aqueous hydrochloric acid (0.2M, 30 ml). This was then stirred at 0^0C for 15 minutes then warmed to RT and stirred for a further 19 hours. Extraction with ethylacetate (3 x 50 ml) and diethyl ether (1 x 50 ml), combination of the organic fractions, washing with saturated sodium hydrogen carbonate (50 ml) and subsequent drying over $MgSO_4$ and evaporation *in vacuo* produced the desired product in 79% yield (8.92g) as a clear mobile oil.

FT IR: ν_{max} (cm^{-1}) 3399 (br, s, OH), 2918 (s, CH), 1723 (s, CO)

^{1}H-NMR: δ_{ppm} 1.45-1.60 (4H, m, CH_2-4/5), 1.80 (2H, m, CH_2-3), 3.3 (1H, br, OH), 3.6 (1H, m, CH-6), 4.0 (1H, m, CH-6), 4.9 (1H, m, CH-2).

Hexane-1,5-diol[81]



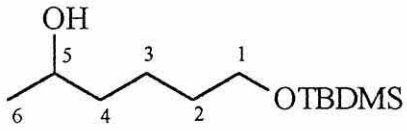
[210]

Methylmagnesium chloride (3.0M, 168 mmol, 56 ml), was added dropwise over 20 minutes to a cooled (0°C), stirred solution of the crude 5-hydroxypentanal (8.50g, 0.083 mol) in dry THF (80 ml). The solution was allowed to warm to RT over 30 minutes and then heated at reflux for 16 hours. The mixture was then cooled (0°C) and quenched by careful addition of saturated ammonium chloride solution (20 ml). The subsequent reaction mixture was dried over $MgSO_4$ and filtered through a sinter packed with $MgSO_4$ and the pad eluted with ethyl acetate (5 x 50 ml). The solvent was removed *in vacuo* yielding the product in 95% yield (9.29g) as a colourless oil which could be purified by eluting through a short plug of silica with diethyl ether. (R_f = 0.1 in diethyl ether).

FT IR: ν_{max} (cm^{-1}) 3374 (br, OH), 2913 (s, CH), 1433 (s, CO).

^{1}H-NMR: δ_{ppm} 1.2 (3H, d, CH_3-6, J = 6.2 Hz), 1.45 (4H, m, CH_2-2/3), 1.6 (2H, m, CH_2-4), 3.6 (1H, t, CH-1, J = 6.1 Hz), 3.8 (1H, br, CH-5).

1-*tert*-Butyldimethylsilyloxyhexan-5-ol[81]



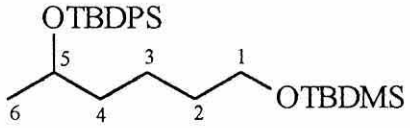
[211]

To a stirred cooled (0°C) solution of hexane-1,5-diol (9.29g, 0.079 mol) in dry DMF (l00 ml), imidazole (10.72g, 0.157 mol) and *tert*-butyldimethylsilyl chloride (1.90g, 0.079 mol) were added and the mixture stirred for 18 hours at RT. The reaction mixture was diluted with diethyl ether (250 ml) and washed with H_2O (5 x 50 ml). The aqueous phase was then extracted with diethyl ether (3 x 50 ml) and the combined organic layers dried over $MgSO_4$ and evaporated *in vacuo* to yield a mobile oil. This was purified using column chromatography eluting with 10% ethyl acetate : petroleum ether) which yielded the product in 83% (15.23g, R_f = 0.17 in 10% ethyl acetate : petroleum ether) as an oil.

FT IR: ν_{max} (cm^{-1}) 3399 (br, OH), 2929 (s, CH)

^{1}H-NMR: δ_{ppm} 0.01 (6H, s, 2 x CH_3), 0.85 (9H, s, ^{t}Bu), 1.15 (3H, d, CH_3-6, J = 6.1 Hz), 1.3-1.5 (6H, m, CH_2-2-4), 3.5 (2H, t, CH_2-1, J = 6.1 Hz), 3.7 (1H, br, CH-5).

1-*tert*-Butyldimethylsilyloxy-5-*tert*-butyldiphenylsilyloxyhexane

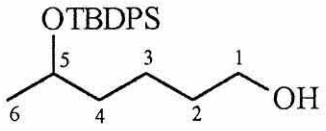


[212]

1-*tert*-Butyldimethylsilyloxyhexan-5-ol (9.80g, 0.042 mol) was dissolved in dry DCM (200 ml) and *tert*-butyldiphenylsilyl chloride (12.73g, 0.046 mol) was added dropwise at room temperature, followed by imidazole (4.3g, 0.063 mol) and a catalytic amount of DMAP (0.1g). A white suspension formed which was stirred overnight at RT. Evaporation and column chromatography eluting with 3% ethyl acetate : petroleum ether gave the desired product in 84% yield (16.61g, R_f = 0.6 in 10% ethyl acetate : petroleum ether).

FT IR: ν_{max} (cm^{-1})	3070 (s, CH), 2929 (s, CH).
^{1}H-NMR: δ_{ppm}	0.0 (6H, s, 2 x CH_3), 0.85 (9H, s, ^{t}Bu of TBDMS), 1.0 (12H, d, ^{t}Bu of TBDPS + CH_3-6, J = 3.8 Hz), 1.2-1.5 (6H, m, CH_2-2-4), 3.5 (2H, t, CH_2-1, J = 5.9 Hz), 3.8 (1H, m, CH-5), 7.3-7.7 (10H, m, 2 x Ph).
^{13}C-NMR: δ_{ppm}	-5.01 (2 x CH_3), 18.37 (Quat. of TBDMS), 19.28 (Quat. of TBDPS), 21.5 (CH_2-3), 23.18 (CH_3-6), 26.00 (^{t}Bu of TBDMS), 27.05 (^{t}Bu of TBDPS), 32.91 (CH_2-4), 39.25 (CH_2-2), 63.20 (CH_2-1), 69.56 (CH-5), 127.38, 127.47, 129.37, 129.44 (8 x CH), 134.58, 134.96 (2 x C), 135.88 (2 x CH).
MS (CI): m/z	488(100% $[M+NH_4]^+$), 357(100% $[M+NH_4\text{-OTBDMS}]^+$) daltons.
HRMS (CI) : m/z	$C_{28}H_{50}O_2Si_2N$ requires $[M+NH_4^+]$ 488.3380; found 488.3380 daltons.

5-*tert*-Butyldiphenylsilyloxyhexan-1-ol

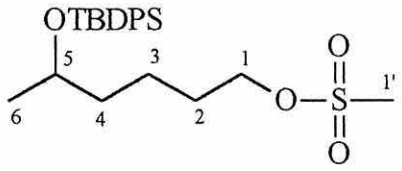


[213]

1-*tert*-Butyldimethylsilyloxy-5-*tert*-butyldiphenylsilyloxyhexane (5.95g,13 mmol) was dissolved in absolute ethanol (55 ml), and PPTS (1.05g, 4.2 mmol) was added. After stirring for 48 hours at RT, the reaction mixture was evaporated and the resulting oil purified by column chromatography eluting with 4% ethyl acetate : hexane to give the product in 91% yield (4.0g, $R_f = 0.14$ in ethyl acetate : hexane).

FT IR: ν_{max} (cm^{-1})	3386 (br, OH) , 2971 (s, CH).
^{1}H-NMR: δ_{ppm}	1.0 (12H, d, *t*Bu of TBDPS + CH_3-6, J = 5.5 Hz), 1.3-1.45 (6H, m, CH_2-2-4), 3.45 (2H, t, CH_2-1, J = 6.3 Hz), 3.8 (1H, m, CH-5), 7.3-7.6 (10H, m, 2 x Ph).
^{13}C-NMR: δ_{ppm}	19.33 (Quat. of TBDPS), 21.42 (CH_2-3), 23.26 (CH_3-6), 27.12 (^{t}Bu of TBDPS), 32.71 (CH_2-2), 39.17 (CH_2-4), 62.76 (CH_2-1), 69.50 (CH-5), 127.48, 127.56, 129.49, 129.55 (8 x CH), 134.60, 134.87 (2 x C), 135.38, 135.93 (2 x CH).
MS (CI): m/z	357 (100% [MH^+]) daltons.
HRMS (CI): m/z	$C_{22}H_{33}O_2Si$ requires [M^+] 357.2250; found 357.2250 daltons.

1-Mesyl-5-*tert*-butyldiphenylsilyloxyhexane



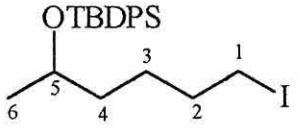
[215]

To a stirred and cooled (0°C) solution of 5-*tert*-butyldiphenylsilyloxyhexan-1-ol (4.51g, 12.67 mmol) in dry DCM (40ml), Et_3N (2.4 ml, 17.2 mmol) and methane sulphonyl chloride (1.33 ml, 17.2 mmol) were added. After stirring for 1 hour at RT H_2O (100 ml) was added, and the reaction was extracted with DCM (2 x 50 ml). The combined organic layers were dried over $MgSO_4$, and evaporated *in vacuo* yielding the crude mesylate in 100% (5.68g) yield.

FT IR: ν_{max} (cm^{-1}) 3070 (s, CH), 2959 (s, CH).

^{1}H-NMR: δ_{ppm} 0.9 (12H, d, TBDPS + CH_3-6, J = 5.4 Hz), 1.2-1.6 (6H, m, CH_2-2-4), 2.8 (3H, s, CH_3-1'), 3.8 (1H, m, CH-5), 4.1 (2H, t, CH_2-1, J = 6.2 Hz), 7.2-7.7 (10H, m, Phenyl).

5-*tert*-Butyldiphenylsilyloxy-1-iodohexane

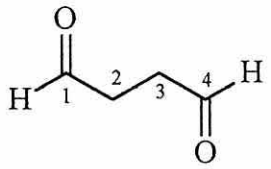


[216]

NaI (9.48g, 0.063 mol) was added to a stirred solution of mesylate **[215]** (5.86g, 22.67 mmol) in acetone (380 ml) and the reaction mixture refluxed for 4 hours. This was then washed with diethyl ether (300 ml), with the filtrate being reduced in *vacuo*. This was then triturated with hexane (6 x 60 ml) and the evaporated triturates further purified using column chromatography (1% ethyl acetate : petroleum ether) to give the title compound in 80% (4.25g) yield as a yellow oil.

FT IR: ν_{max} (cm^{-1})	3069 (s, CH), 2930 (s, CH)
^{1}H-NMR: δ_{ppm}	1.08 (12H, d, *t*Bu, CH_3-6, J = 6.8 Hz), 1.4 (4H, m, CH_2-2/3), 1.7 (2H, m, CH_2-4), 3.1 (2H, t, CH_2-l, J = 7 Hz), 3.9 (1H, m, CH-5), 7.2 (5H, m, Phenyl), 7.7 (5H, m, Phenyl)
^{13}C-NMR: δ_{ppm}	7.02 (CH_2-3), 19.25 (Quat. of ^{t}Bu), 23.2 (CH_2-2), 26.15 (CH-5), 27.05 (^{t}Bu), 33.48 (CH_2-4), 38.15 (CH_3-6), 69.17 (CH_2-1), 127-135 (Phenyl).
MS(CI): m/z	484 (100% [$M+NH_4^+$]), 467 (40% [MH^+]) daltons.
HRMS(CI): m/z	$C_{22}H_{32}OSiI$ requires [M^+] 467.1267; found 467.1267 daltons.

Succinaldehyde [84]



Method A

Aqueous hydrochloric acid (0.1N, 200 ml) was added to 2,5-dimethoxy tetrahydrofuran (50.0g, 0.38 mol) and the solution heated under gentle reflux for 2 hours. On cooling the mixture was basified to pH 8 with saturated aqueous sodium bicarbonate solution (50 ml) and saturated with sodium chloride. The product was extracted with diethyl ether (5 x 100 ml), dried over $MgSO_4$ and evaporated *in vacuo* to give a yellow oil. This oil was distilled under reduced pressure using a short path apparatus, yielding the title compound as a colourless oil. (B.pt. 57-60°C at 15mm Hg, Lit. B.pt 62°C at 14 mm Hg) in 49% yield (31.6g).

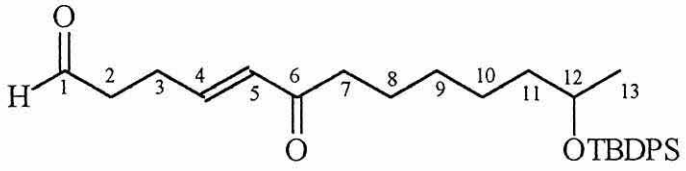
Method B

To 2,5-dimethoxytetrahydrofuran (11.0g, 0.08 mol) was added 1% acetic acid (22 ml) and the solution refluxed for 20 mins. After cooling and neutralisation with saturated sodium bicarbonate the mixture was saturated with sodium chloride, extracted with $CHCl_3$ (3 x 50 ml), dried over $MgSO_4$ and evaporated *in vacuo* to give a yellow oil. This oil was distilled under reduced pressure using a short path apparatus, yielding the title compound as a colourless oil. (B.pt. 38°C at 7.8 mbar, Lit. B.pt 62°C at 14 mm Hg) in 68% yield (4.85g).

FT IR: ν_{max} **(cm^{-1})**, 2958 (s, CH), 1722 (s, C=O), 1634 (w, CO).

^{1}H-NMR: δ_{ppm} 2.8 (4H, s, m, CH_2-2,3), 9.8 (2H, s, CH-1,4).

12-*tert*-Butyldiphenylsilyloxy-6-oxotridec-4-enal



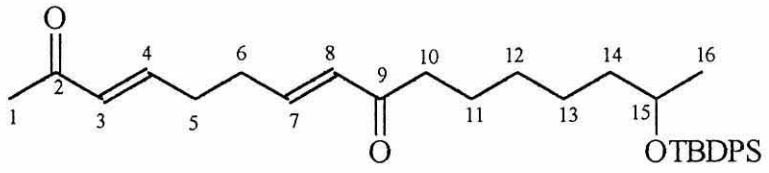
[218]

To a cooled (-78^{0}C) solution of acetylmethylenetriphenylphosphorane (1.19g, 3.73 mmol) in dry THF (20 ml) was added *n*-butyllithium (1.68 ml, 2.44M, 4.09 mmol) dropwise. The resulting red solution was stirred between -50 and -60^{0}C for one hour and then iodide **[216]** (1.8g, 4.01 mmol) was added as a solution in dry THF (10 ml) at -78°C. The mixture was allowed to warm to RT with stirring over 3 hours. Water (100 ml) was added and the reaction extrated with ethyl acetate (2 x 20 ml) which was washed with water (2 x 50 ml), dried over $MgSO_4$ and evaporated

The resulting phosphorane was re-dissolved in dry DCM (10 ml), and succinaldehyde (2.7g, 31 mmol) in dry DCM (10 ml) was added. This was then allowed to stir for 24 hours at RT. The reaction mixture was then washed with water (3 x 250 ml) to remove excess succinaldehyde and the organic phase dried over $MgSO_4$, and evaporated *in vacuo*. The resultant oil was purified by column chromatography with a gradient elution of 1% ethyl acetate : petroleum ether - 20% ethyl acetate : petroleum ether, to give the title compound in 54% yield (0.93g) as a colourless oil. (R_f = 0.21 in 20% ethyl acetate : petroleum ether).

FT IR: ν_{max} (cm^{-1})	3069 (s, CH), 2931 (s, CH)
^{1}H-NMR: δ_{ppm}	1.05 (12H, d, *t*Bu / CH_3-13, J = 3.3 Hz), 1.1-1.5 (8H, m, CH_2-8-11), 2.45 (2H, t, CH_2-2, J = 7.4 Hz), 2.56 (2H, t, CH_2-7, J = 6.4 Hz), 2.6 (2H, m, CH_2-3), 3.8 (1H, m, CH-12), 6.1 (1H, d, CH-5, J = 15.8 Hz), 6.8 (1H, m, CH-4), 9.8 (1H, s, CHO).
^{13}C-NMR: δ_{ppm}	19.27 (Quat. of ^{t}Bu), 23.27 (CH_3-13), 24.03 (CH_2-9), 24.60 (CH_2-10), 25.02 (CH_2-8), 27.05 (^{t}Bu), 29.25 (CH_2-11), 39.24 (CH_2-3), 40.28 (CH_2-7), 41.92 (CH_2-2), 69.49 (CH-12), 127.39, 127.46, 129.38, 129.44 (8 x CH), 130.96 (CH-4), 134.57, 134.89 (2 x C), 135.87 (2 x CH), 144.06 (CH-5), 200.39 (CH-1), 201.73 (CO).
MS(CI):m/z	482 (20% [M+NH_4^+]) daltons.
HRMS(CI): m/z	$C_{29}H_{44}O_3SiN$ [M+NH_4^+] requires 482.3090; found 482.3090 daltons.

15-*tert*-Butyldiphenylsilyloxy-2,9-dioxohexadeca-3,7-diene



[219]

Acetylmethylenetriphenylphosphorane (3.45g, 10.8 mmol) was added to a solution of **[218]** (2.60g, 5.44 mmol) in dry DCM (15 ml) and the reaction mixture stirred at RT. After 24 hours, further acetylmethylenetriphenylphosphorane (2.0g, 6.3 mmol) was added and stirring was continued for a further 24 hours at RT. After evaporation of the solvent, chromatography eluting with a gradient solvent system of 10%-20% ethyl acetate : petroleum ether gave the title compound in 91% (2.46g, R_f = 0.16, 15% ethyl acetate : petroleum ether) yield as an oil.

FT IR: ν_{max} (cm^{-1}) 3070 (s,CH), 2931 (s, CH), 1698 (s, C=O), 1676 (s, C=O), 1628 (s, C=C).

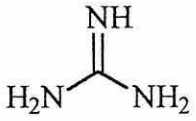
^{1}H-NMR: δ_{ppm} 1.05 (12H, d, ^{t}Bu, CH_3-16, J = 3.3 Hz), 1.15-1.65 (10H, m, CH_2-10-14), 2.26 (3H, s, CH_3-1), 2.4 (4H, m, CH_2-5,6), 3.8 (1H, m, CH-15), 6.1 (2H, d, CH-3,8, J = 17.2 Hz), 6.8 (2H, m, CH-4,7), 7.35 (5H,m, Phenyl), 7.65 (5H,m, Phenyl).

^{13}C-NMR: δ_{ppm} 19.27 (Quat. of ^{t}Bu), 23.26 (CH_3-16), 24.06 (CH_2-12), 25.00 (CH_2-13), 27.04 (^{t}Bu), 29.27 (CH_2-11), 30.68 (CH_2-14), 30.74 (CH_2-6), 39.23 (CH_2-10), 40.35 (CH_2-5), 52.98 (CH_3-1), 69.48 (CH-15), 127.37, 127.45, 129.37, 129.43 (8 x CH), 130.97 (CH-7), 131.91 (CH-4), 134.57, 134.88 (2 x C), 144.41 (CH-8), 145.82 (CH-3), 198.22 (C=O), 200.34 (C=O).

MS(CI): m/z 522 (100% [M+NH_4^+]) daltons.

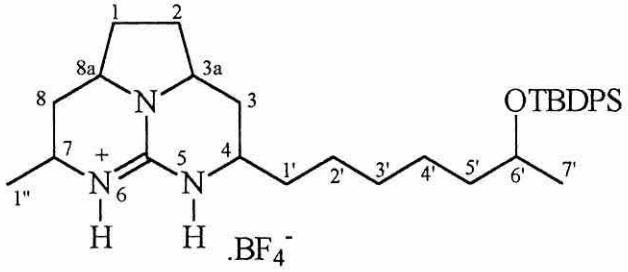
HRMS(CI): m/z $C_{32}H_{48}O_3SiN$ requires [M+NH_4^+] 522.3368; found 522.3368 daltons.

Guanidine [1][81]



Sodium (2.28g, 0.099 mol) was washed with petrol and dissolved in dry methanol (200 ml) at 0°C. Guanidine hydrochloride (10g, 0.104 mol) was added to the solution and the mixture stirred at RT for 18 hours. The resulting residue was evaporated *in vacuo*, triturated with methanol (4 x 70 ml) and filtered several times to remove sodium chloride. Final evaporation of the solvent *in vacuo* gave the title compound as a low melting solid in 90% (5.50g) yield.

***rac*-(*3aS,4S,8aR,7R*)-4-[(*6'R/S*)-6'-(*tert*-Butyldiphenylsilyl)oxy)heptyl]-7-methyl-(1,2,3,4,7,8-hexahydro-5H-5,6,8b-triazaacenapthylene)-6-ium tetrafluoroborate**



[221]

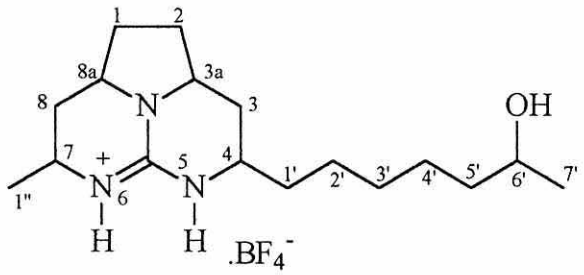
Guanidine (0.12g, 1.99 mmol) as a solution in dry DMF (2.2 ml) was added in a dropwise fashion to a stirred, cooled (0°C) solution of **[219]** (1.0g, 1.99 mmol) in dry DMF (4.3 ml). This was then left to stir and warm to RT over 4 hours at which point the reaction was cooled (0^0C) and methanol (6 ml) and H_2O (2 ml) were added followed by $NaBH_4$ (0.45g, 11.90 mmol). After stirring at RT overnight, the reaction mixture was diluted with DCM (20 ml) and acidified with HCl (2N, 3.5 ml). After extraction with DCM (4 x 40 ml) the combined extracts were washed with water (2 x 40 ml), brine (2 x 40 ml), and LiBr solution (2 x 40 ml) then dried over $MgSO_4$ and the solvent removed *in vacuo* to give a brown oil. This was then dissolved in DCM (10 ml) and treated with a saturated solution of sodium tetrafluoroborate (20 ml). After vigorous stirring overnight at RT the organic layer was separated and the aqueous phase extracted with further DCM (3 x 30 ml). Drying over $MgSO_4$ and evaporation followed by chromatography with a graduated solvent system of, 70 ml DCM, 70 ml 0.25% MeOH / $CHCl_3$, 210 ml 0.5% MeOH / $CHCl_3$, 140 ml 1% MeOH / $CHCl_3$, 140 ml 2% MeOH / $CHCl_3$,70 ml 5% MeOH / $CHCl_3$, 70 ml 10% MeOH / $CHCl_3$ furnished the title compound in 29% (0.35g) yield (TLC in 5% MeOH / $CHCl_3$ run twice up the plate, product off in 2% MeOH / $CHCl_3$) as an oil.

FT IR: ν_{max} (cm^{-1}) 3361 (br, NH), 3025 (s,CH), 2931 (s, CH)

^{1}H-NMR: δ_{ppm} 1.05 (12H, s, ^{t}Bu, CH_3-7'), 1.16-1.45 (13H, m, CH_2-1'-5', CH_3-1"), 1.55-1.7 (4H, m, CH_2-1,2), 2.1 (4H, m, CH_2-3, 8), 3.3 (1H, m, CH-3a), 3.55 (1H, m, CH-8a), 3.7 (2H, m, CH-4, 7), 3.9 (1H, m, CH-6'), 6.7 (1H, brs, NH), 6.9 (1H, brs, NH), 7.4 (5H, m, Phenyl),

	7.7(5H, m, Phenyl).
^{13}C-NMR: δ_{ppm}	19.27 (Quat. of ^{t}Bu), 20.27 (CH_3-7'), 20.7 (CH_3-1''), 25.01 (CH_2-4'), 27.05 (^{t}Bu), 29.29 (CH_2-3'), 29.70 (CH_2-2'), 30.21 (CH_2-5'), 30.26 (CH_2-1'), 33.62 (CH_2-2), 34.40 (CH_2-1), 35.70 (CH_2-3), 39.20 (CH_2-8), 46.09 (CH-4), 50.41 (CH-7), 55.97 (CH-3a), 56.05 (CH-8a), 69.48 (CH-6'), 127.39, 127.47, 129.39, 129.44 (8 x CH), 134.60, 134.90 (2 x C), 135.86 (2 x CH), 149.28 (C=N).
MS(CI): m/z	532 (100% [M^+]), 533 (100% [MH^+]) daltons.
HRMS(CI): m/z	$C_{33}H_{50}OSiN_3$ requires [M^+] 532.3719; found 532.3719 daltons.

***rac*-(*3aS,4S,8aR,7R*)-4-[(*6'R/S*)-6'-Hydroxyheptyl]-7-methyl(1,2,3,4,7,8-hexahydro-5H-5,6,8b-triazaacenapthylene)-6-ium tetrafluoroborate**

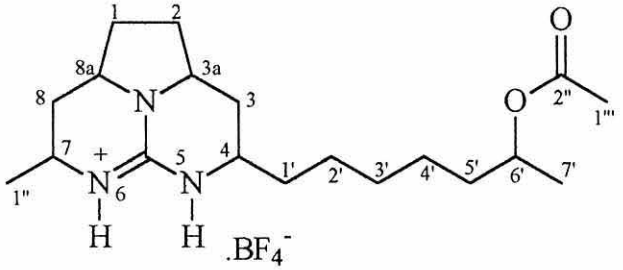


[222]

Compound **[221]** (213mg, 0.34 mmol) was dissolved in methanol (7 ml) and cooled (0°C) and a solution of methanolic HCl (10 ml) was added. After standing at RT overnight the reaction was evaporated, re-dissolved in DCM (10 ml), washed with saturated sodium tetrafluoroborate solution (2 x 20 ml), dried over $MgSO_4$, and the solvent removed *in vacuo*, producing a crude brown oil (224.7mg). This oil was purified by column chromatography utilising a gradient elution of DCM 50 ml, 50 ml 2% MeOH / $CHCl_3$, 50 ml 4% MeOH / $CHCl_3$, 100 ml 6% MeOH / $CHCl_3$, 100 ml 10% MeOH / $CHCl_3$ which produced the title compound in 91% yield (94mg, R_f = 0.03 in 5% MeOH : $CHCl_3$.) as an oil.

FT IR: ν_{max} (cm^{-1})	3370 (OH str), 2929 (s, CH), 1502 (s, CN), 1328 (w, CO).
^{1}H-NMR: δ_{ppm}	1.14 (3H, d, CH_3-7', J = 6.2 Hz), 1.28 (3H, d, CH_3-1", J = 6.4 Hz), 1.3-1,59 (10H, m, CH_2-1'-5'), 1.62 (4H, m, CH_2-1,2), 2.0 (1H, br, OH), 2.2 (4H, m, CH_2-3, 8), 3.3 (1H, m, CH-3a), 3.5 (1H, m, CH-8a), 3.75 (3H, m, CH-6', 4, 7), 6.4 (1H, brs, NH), 6.6 (1H, brs, NH).
^{13}C-NMR: δ_{ppm}	20.7 (CH_3-1"), 23.5 (CH_3-7'), 26.1 (CH_2-2'), 26.6 (CH_2-4'), 30.5 (CH_2-3'), 31.0 (CH_2-2), 31.0 (CH_2-1), 34.60 (CH_2-3), 35.7 (CH_2-1'), 36.6 (CH_2-8), 40.0 (CH_2-5'), 47.2 (CH-7), 51.5 (CH-4), 57.4 (CH-3a), 57.5 (CH-8a), 68.4 (CH-6'), 149.32 (C=N).
MS(CI): m/z	294(30% [M^+]) daltons.
HRMS(CI):m/z	$C_{17}H_{32}0N_3$ requires [M^+] 294.2545; found 294.2545 daltons.

***rac*-(*3aS,4S,8aR,7R*)-4-[(*6'R/S*)-6'-Acetoxyheptyl]-7-methyl-(1,2,3,4,7,8-hexahydro-5H-5,6,8b-triazaacenapthylene)-6-ium chloride and tetrafluoroborate**

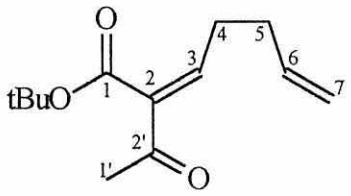


[223b]

Alcohol **[222]** (87mg, 0.29 mmol) was dissolved in dry pyridine (35mg, 0.44 mmol, 0.04 ml) and acetic anhydride (148mg, 1.45 mmol) was added. After stirring for 48 hours at RT the reaction was diluted with $CHCl_3$ (10 ml) and washed with HCl (2N, 2 x 20 ml), dried over $MgSO_4$ and the solvent removed *in vacuo*. Purification by column chromatography with gradient elution $CHCl_3$ to 30% MeOH : $CHCl_3$ gave [Cl^-] (41% R_f = 0.05 in 2% MeOH : $CHCl_3$ eluted twice). Washing of a DCM solution of [Cl^-] with saturated sodium tetrafluoroborate solution (2 x 20 ml) followed by drying over $MgSO_4$ and evaporation, furnished the tetrafluoroborate salt in quantitative yield.

FT IR: ν_{max} (cm^{-1})	3188 (br, NH), 2928 (s, CH), 1726 (s, C=O), 1500 (w, C=N).
^{1}H-NMR: δ_{ppm}	1.17 (3H, d, CH_3-7', J = 6.2 Hz), 1.3 (3H, d, CH_3-1", J = 6.4 Hz), 1.2-1.7 (14H, m, CH_2-1'-5', 1, 2), 2.0 (3H, s, CH_3-1'''), 2.2 (4H, m, CH_2-3, 8), 3.3 (1H, m, CH-3a), 3.5 (1H, m, CH-8a), 3.6 (3H, m, CH-6', 4, 7), 8.5 (1H, brs, NH), 8.7 (1H, brs, NH).
^{13}C-NMR: δ_{ppm}	20.27 (CH_3-7'), 20.7 (CH_3-1"), 26.0 (CH_2-4'), 26.3 (CH_2-2'), 30.2 (CH_2-3'), 31.0 (CH_2-2), 31.0 (CH_2-1), 34.70 (CH_2-3), 35.6 (CH_2-1'), 36.6 (CH_2-5'), 36.8 (CH_2-8), 47.3 (CH-7), 51.5 (CH-4), 57.4 (CH-3a), 57.5 (CH-8a), 72.3 (CH-6'), 149.32 (C=N).
MS(CI): m/z	336 (100% [M^+]) daltons.
HRMS(CI):m/z	$C_{19}H_{34}O_2N_3$ requires [M^+] 336.2651; found 336.2645 daltons.

***E/Z-tert*-Butyl(2-acetyl)hepta-2,6-dieneoate**



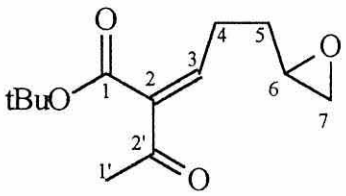
[228]

Titanium (IV) chloride (18.30g, 96.48 mmol, 10.6 ml) was added dropwise to dry carbon tetrachloride (28.8 ml) with subsequent dropwise addition of the solution to cooled (0°C) stirred dry THF (197 ml). The resulting bright yellow suspension was stirred for 15 minutes whereupon 4-pentenal (5.0g, 59 mmol) and *tert*-butyl acetoacetate (8.49g, 53.6 mmol, 8.9 ml) dissolved in dry THF (36.2 ml) were added slowly. The brown solution was then treated dropwise over an hour and a half with dry pyridine (15.7 ml) in dry THF (24 ml) whilst still at 0°C. This mixture was then stirred for 48 hours whilst reaching RT.

Water (70 ml) and diethyl ether (130 ml) were then added, and the organic layer washed with saturated copper sulphate solution (3 x 30 ml). The combined aqueous layers were then back extracted with diethyl ether (3 x 50 ml) and the combined organic phases dried over $MgSO_4$ and the solvent removed *in vacuo* to give the crude product as a yellow oil. Purification by column chromatography with gradient elution 2%, 3%, 4%, 10%, 20% diethyl ether : petroleum ether then 100% diethyl ether gave **[228]** in 19% yield (2.27g, R_f= 0.1, 100% diethyl ether).

FT IR: ν_{max} **(cm^{-1})**	2978 (s, CH), 1723 (s, C=O), 1235(s, CH).
^{1}H-NMR: δ_{ppm}	1.5 (9H, s, *t*Bu), 2.25 (2H, m, CH_2-5), 2.3 (3H, s, CH_3-1'), 2.45 (2H, dt, CH_2-4, J = 7.7, 15.4 Hz), 5.1 (2H, m, CH_2-7), 5.8 (1H, m, CH-6), 6.75 (1H, t, CH-3, J = 7.7 Hz).
^{13}C-NMR: δ_{ppm}	19.78 (Quat. of *t*Bu), 27.89 (CH_3-2''), 28.07 (*t*Bu), 28.83 (CH_2-5), 32.11 (CH_2-4), 115.78 (CH_2-7), 136.72 (CH-6), 138.32 (C-2), 145.79 (CH-3), 201.17 (CO), 201.85 (CO).
MS (CI) : m/z	186 (100% [M^+]) daltons.
HRMS (CI) : m/z	$C_{13}H_{21}O_3$ requires [MH^+] 225.1490; found 225.1488 daltons.

***E/Z-tert*-Butyl(2-acetyl)hepta-2-ene-6-epoxyoate**

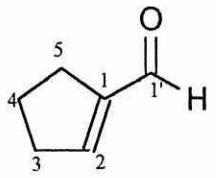


[229]

To a cooled (0°C) solution of **[228]** (1.27g, 5.99 mmol) in dry DCM (15 ml), *m*CPBA (1.24g, 7.2 mmol) was added and the mixture stirred overnight to RT. The reaction mixture was then diluted with DCM (30 ml), washed with $NaHCO_3$ (2 x 30 ml), the organic phase dried with $MgSO_4$ and solvent removed *in vacuo*. Purification was accomplished by column chromatography, eluting with petroleum ether, 25% and 50% diethyl ether : petroleum ether. This furnished the title compound as an oil in 81% yield (1.17g, $R_f = 0.16$ in 50% diethyl ether : petroleum ether).

FT IR: ν_{max} **(cm^{-1})**	3020 (w, CH), 1700 (s, C=O).
^{1}H-NMR: δ_{ppm}	1.5 (9H, s, *t*Bu), 2.25 (2H, m, CH_2-5), 2.3 (3H, s, CH_3-1'), 2.45 (3H, m, CH_2-4, CH-7), 2.75 (1H, m, CH-7), 2.9 (1H, m, CH-6), 6.75 (1H, t, CH-3, J = 7.8 Hz).
^{13}C-NMR: δ_{ppm}	25.6 (C), 26.07 (CH_3-1'), 27.95 (*t*Bu), 31.08 (CH_2-5), 31.3 (CH_2-4), 46.90 (CH_2-7), 51.46 (CH-6), 138.4 (C-2), 144.92 (CH-3), 194.97 (CO), 201.85 (CO).
MS (CI) : m/z	202 (100% [(^{t}BuOOCO)$_2$]) daltons.
HRMS (CI) : m/z	$C_{13}H_{20}O_4$ requires [M^+] 241.1440; found 241.1438 daltons.

Cyclopent-1-enealdehyde[92]

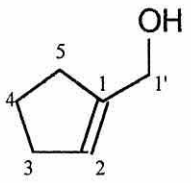


[243]

Sodium periodate (25.0g, 85.1 mmol) was suspended in water (300 ml). Concentrated nitric acid (5.8 ml, 35.6 mmol) was added and the subsequent mixture stirred until the solid dissolved. The solution was then adjusted to pH 4 by the addition of sodium hydroxide solution. *Trans*-cyclohexane-l,2-diol (1.0g, 8.6 mmol) was added with stirring to the periodate solution which was cooled to 25°C over 20 minutes. Diethyl ether (40 ml) and KOH (35 ml, 20%) was added with the subsequent solution being stirred vigorously for 30 minutes. Extraction with diethyl ether (3 x 40 ml), subsequent drying over $MgSO_4$ and evaporation under reduced pressure followed by distillation gave cyclopent-1-enealdeyhde (7.39g) as a yellow oil in 81% yield (Bpt. 50-55°C at 15mm of Hg, Lit Bpt 52°C at 20 mm of Hg).

FT IR: ν_{max} **(cm^{-1})**	3500 (br, OH), 3000 (s, CH), 1700 (s, C=O), 1620 (s, C=C).
^{1}H-NMR: δ_{ppm}	2.0 (2H, m, CH_2-4), 2.6 (4H, m, CH_2-3/5), 6.9 (1H, m, CH-2), 9.8 (1H, s, 1', CHO).
^{13}C-NMR: δ_{ppm}	22.88 (CH_2-4), 28.27 (CH_2-3), 33.65 (CH_2-5), 147.88 (C-1), 153.18 (CH-2), 189.90 (CHO).

Cyclopent-1-ene-1-methanol[127]



[244]

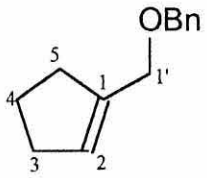
Sodium borohydride (1.90g, 50.8 mmol) was added to a cooled (0°C) stirred solution of cyclopent-1-enealdehyde (7.39g, 76.9 mmol) dissolved in methanol (100 ml) and the solution then stirred overnight to RT. The volume of the resulting liquor was reduced to half its initial volume and the product extracted with diethyl ether (3 x 50 ml). The organic phase was dried over $MgSO_4$ and evaporated *in vacuo* to give cyclopent-1-ene-1-methanol as a yellow oil (5.23g, 69%).

FT IR: ν_{max} **(cm^{-1})**, 3300 (br, OH), 3000 (s, CH), 1650 (s, C=C).

^{1}H-NMR: δ_{ppm} 1.6 (1H, s, OH), 2.0 (2H, m, CH_2-4), 2.6 (4H, m, CH_2-3/5), 4.3 (2H, s, CH_2-1'), 5.6 (1H, m, CH-2).

^{13}C-NMR: δ_{ppm} 22.37 (CH_2-4), 32.26 (CH_2-3), 32.49 (CH_2-5), 62.06 (CH_2-1'), 125.39 (CH-2), 144.20 (C-1).

Benzoylmethylenecyclopent-1-ene



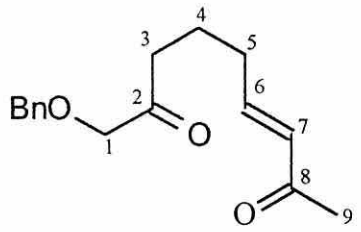
[241]

NaH (3.80g, 0.9612 mol) was washed with dry diethyl ether (3 x 20 ml) and dried under vacuum. Dry THF (50 ml) was then added and the suspension cooled to 0^0C after which cyclopent-1-ene-1-methanol (5.0g, 53 mmol) was added as a solution in dry THF (50 ml) *via* a dropping funnel. After five minutes a catalytic amount of tetrabutylammonium iodide (0.25g, 0.68 mmol) was added followed by benzyl bromide (7.3 ml, 61 mmol) in THF (50 ml) and the resultant reaction mixture stirred to RT over 18 hours.

To the cooled (0°C) reaction mixture methanol (30 ml) was added, and after 2 hours water (100 ml); this was subsequently left to stir for a further 18 hours. The reaction mixture was then extracted with diethyl ether (2 x 20 ml), dried over $MgSO_4$ and the solvent evaporated *in vacuo*. Purification by column chromatography eluting with 5% diethyl ether : petroleum ether gave the title compound in 69% yield (5.76g, R_f = 0.53 in with 5% diethyl ether : petroleum ether).

FT IR: ν_{max} **(cm^{-1})**,	3030 (s, CH), 1453 (s, C=C).
^{1}H-NMR: δ_{ppm}	1.9 (2H, m, CH_2-4), 2.4 (4H, m, CH_2-3/5), 4.1 (2H, s, CH_2-1'), 4.6 (2H, s, CH_2Ph), 5.7 (1H, s, CH-2), 7.3 (5H, m, Ph).
^{13}C-NMR: δ_{ppm}	23.37 (CH_2-4), 32.43 (CH_2-3), 32.99 (CH_2-5), 69.05 (CH_2-1'), 72.03 (CH_2Ph), 127.50, 127.70, 128.35, 128.80 (Ph), 138.58 (C-1), 141.46 (CH-2).

1-Benzyloxy-2,8-dioxonon-6(*E*)-ene

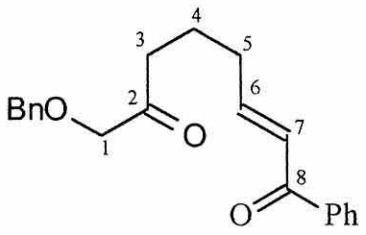


[239a]

Benzyloxymethylcyclopent-1-ene (0.70g, 3.72 mmol) was dissolved in dry DCM (25 ml) in a two necked flask, one neck having a drying tube attached and the second a bubbler. The flask was cooled (-78°C) and flushed with N_2. Ozone was then bubbled through for 15 minutes whereby the solution turned blue. The flask was again flushed with N_2 and triphenylphosphine (1.lg, 4.1 mmol) was added at -78°C. Once all the triphenylphosphine had dissolved, acetylmethylenetriphenylphosphorane (1.42g, 4.46 mmol) was added whilst still maintaining the temperature at -78°C. Once all the phosphorane had dissolved the reaction was allowed to warm to room temperature over 2 hours and the N_2 supply turned off. Once at RT the volume was reduced to half its original volume *in vacuo* and the reaction stirred for a further 18 hours. The remaining solvent was removed *in vacuo* which produced a white solid. The triphenylphosphine oxide was removed by trituration with diethyl ether, before purification by column chromatography eluting with 50% diethyl ether : petroleum ether. This furnished **[239a]** in 97% yield (0.94g, R_f= 0. 18 in 50% diethyl ether : petroleum ether).

FT IR: ν_{max} **(cm^{-1})**,	1727 (s, C=O), 1619 (s, C=C).
^{1}H-NMR: δ_{ppm}	1.6 (2H, m, CH_2-4), 2.2 (3H, s, CH_3-9), and (2H, m, CH_2-5), 2.5 (2H, t, CH_2-3, J = 7.2 Hz), 4.0 (2H, s, CH_2Ph), 4.6 (2H, s, CH_2-1), 6.1 (1H, dt, CH-7, J = 1.4, 15.9 Hz), 6.7 (1H, dt, CH-6, J = 6.8, 15.9 Hz), 7.4 (5H, m, Ph).
^{13}C-NMR: δ_{ppm}	21.38 (CH_2-4), 26.92 (CH_3-9), 31.66 (CH_2-5), 37.97 (CH_2-3), 73.40 CH_2Ph, 75.03 (CH_2-1), 127.90, 128.07, 128.54, 137.07 (Ph), 131.77 (CH-6), 147.05 (CH-7), 198.48 (C-2), 208.14 (C-8).
MS (CI): m/z	278 (100% $[M + NH_4]^+$), 261 (30% $[MH]^+$), 260 (10% [M]) daltons.
HRMS (CI) : m/z	$C_{16}H_{21}O_3$ requires $[MH]^+$ 261.1491; found 261.1491 daltons.

(*E*)-1-Benzoyloxy-8-phenyloct-2-8-dione-6-ene

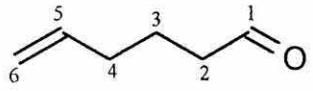


[239b]

Benzyloxymethylcyclopent-1-ene (1.50g, 7.9 mmol) was dissolved in dry DCM (50 ml) in a two necked flask, one neck having a drying tube attached and the second a bubbler. The flask was cooled (-78°C) and flushed with N_2. Ozone was then bubbled through for 25 minutes whereby the solution turned blue. The flask was again flushed with N_2 and triphenylphosphine (2.30g, 8.69 mmol) was added at -78°C. Once all the triphenylphosphine had dissolved, benzoylmethylene-triphenylphosphorane (3.60g, 9.48 mmol) was added whilst still maintaining the -78°C. Once all the phosphorane had dissolved the system was allowed to warm to room temperature over 2 hours and the N_2 supply turned off. Once at RT the solvent volume was reduced to approximately half its original volume *in vacuo* and the reaction stirred for a further 18 hours. The remaining solvent was removed *in vacuo* which produced a white solid. Triphenylphosphine oxide was removed by trituration with diethyl ether, before purification by column chromatography, eluting with 50% diethyl ether : petroleum ether producing the title compound as an oil in 94% yield (2.40g, R_f = 0. 19 in 50% diethyl ether : petroleum ether).

FT IR: ν_{max} **(cm^{-1})**,	1732 (s, C=O), 1620 (s, C=C).
^{1}H-NMR: δ_{ppm}	1.8 (2H, m, CH_2-4), 2.4 (2H, m, CH_2-5), 2.6 (2H, t, CH_2-3, J = 7.3 Hz), 4.1 (2H, s, CH_2Ph), 4.6 (2H, s, CH_2-1), 6.7 (1H, d, CH-7, J = 16.3 Hz), 7.0 (1H, dt, CH-6, J = 6.3, 16.3 Hz), 7.4(10H, m, Ph)
^{13}C-NMR: δ_{ppm}	21.53 (CH_2-4), 31.95 (CH_2-5), 38.03 (CH_2-3), 73.41 (CH_2Ph), 75.04 (CH_2-1), 126.50-128.70 (2 x Ph), 132.71 (CH-6), 133.57, 137.10 (2 x C Ph), 148.41 (CH-7), 190.63 (C-2), 208.20 (C-8).
MS (CI): m/z	340 (100% $[M + NH4]^+$), 323 (50% $[MH]^+$) daltons.
HRMS (CI) : m/z	$C_{21}H_{23}O_3$ requires $[MH]^+$323.1647; found 323.1647 daltons.

5-Hexenal[128]



[250]

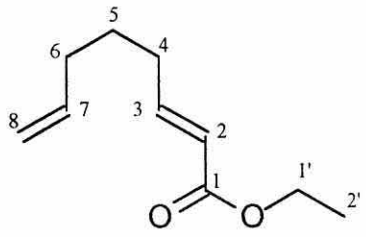
To a cooled (0°C) solution of 5-hexen-1-ol (4.0g, 39.9 mmol) in dry DCM (100 ml) was added Celite (12.9g) and then PCC (12.9g, 59.9 mmol) and this was stirred for 90 minutes. Further portions of Celite (4.30g) and PCC (4.30g, 1.99 mmol) were added and this was left to stir overnight reaching RT. The reaction mixture was then filtered through a sinter funnel with a 6 cm layer of silica, Celite mix (equal portions) and eluted with DCM (3 x 75 ml). This was then evaporated *in vacuo* to give **[250]** as a colourless liquid in 77% yield (3.0g) which was used without futher purification.

FT IR: ν_{max} **(cm^{-1})**, 1730 (s, C=O), 1641 (s, C=C).

^{1}H-NMR: δ_{ppm} 1.6 (2H, m, CH_2-3), 2.1 (2H, m, CH_2-4), 2.4 (2H, m, CH_2-2), 5.0 (2H, m, CH_2-6), 5.7 (1H, m, CH-5), 9.7 (1H, t, CHO, J = 0.94 Hz).

^{13}C-NMR: δ_{ppm} 21.11 (CH_2-3), 32.89 (CH_2-4), 43.03 (CH_2-2), 115.23 (CH_2-6), 137.50 (CH-5), 202.35 (CH-1).

Ethyl (2*E*)-octa-2,7-dieneoate

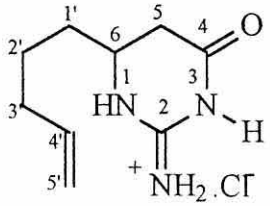


[257]

To a solution of 5-hexenal (3.0g, 30.6 mmol) in dry DCM (80 ml) was added carboethoxymethylenetriphenylphosphorane (21.3g, 61 mmol). This was stirred overnight at RT. The solvent was removed *in vacuo* and purification was achieved initially by trituration with diethyl ether and then by column chromatography, eluting with 3% diethyl ether : petroleum ether. This gave the title compound as an oil in 88% yield (3.40g, $R_f = 0.20$ in 3% diethyl ether : petroleum ether).

FT IR: ν_{max} **(cm^{-1})**,	1735 (s, C=O), 1638 (s, C=C).
^{1}H-NMR: δ_{ppm}	1.3 (3H, t, CH_3-2', J = 7.1 Hz), 1.5 (2H, m, CH_2-5), 2.1 (2H, m, CH_2-6), 2.4 (2H, m, CH_2-4), 4.2 (2H, q, CH_2-1', J = 7.1 Hz), 5.0 (2H, m, CH_2-8), 5.8 (2H, m, CH-2 / 7), 6.9 (1H, dt, CH-3, J = 6.3, 15.9 Hz).
^{13}C-NMR: δ_{ppm}	14.21 (CH_3-2'), 27.10 (CH_2-5), 31.42 (CH_2-6), 33.02 (CH_2-4), 60.06 (CH_2-1'), 115.4 (CH_2-8), 121.50 (CH-7), 137.91 (CH-3), 148.81 (CH-2), 173.57 (C-1).
MS (CI): m/z	186 (100% $[M+NH_4]^+$), 169 (20% $[MH]^+$) daltons.
HRMS (CI) : m/z	$C_{10}H_{20}O_2N$ requires $[M+NH_4]^+$ 186.1494; found 186.1495 daltons.

6-(4'-Pentenyl)2-imino-tetrahydropyrimidin-4-one hydrochloride [258]



Guanidine (0.39g, 6.55 mmol) in dry DMF (1 ml) was added dropwise to a cooled (0°C) solution of **[257]** (1.0g, 5.95 mmol) in dry DMF (1 ml) and stirred for 72 hours to RT. This resulted in a precipitation which was filtered and washed with diethyl ether and the resultant filtrate evaporated *in vacuo*. The solid was then re-dissolved in methanol (10 ml) and treated with methanolic HCl (10 ml). The solvent was removed to produce the title compound as a white solid in 23% yield (0.25g).

Purification of the original filtrate and washings was undertaken after treatment with methanolic HCl (10 ml) initially by trituration with diethyl ether to remove soluble by-products and then by column chromatography, utilising a gradient elution of $CHCl_3$ (50 ml), 1% MeOH : $CHCl_3$ (100 ml), 2% MeOH : $CHCl_3$ (100 ml), 3% MeOH : $CHCl_3$ (100 ml), 4% MeOH : $CHCl_3$ (100 ml), 5% MeOH : $CHCl_3$ (100 ml), 10% MeOH : $CHCl_3$ (400 ml), 20% MeOH : $CHCl_3$ (100 ml), 100% MeOH (100 ml). This yielded further product as a white solid (84mg) in a combined 31% yield (0.33g, R_f = 0.07 in 10% MeOH : $CHCl_3$).

FT IR: ν_{max} **(cm^{-1})**, 1710 (s, C=O), 1550 (s, C=N), 1520 (s, C=C).

^{1}H-NMR: δ_{ppm} **(D_2O)** 1.2 (2H, m, CH_2-2'), 1.4(2H, m, CH_2-1'), 1.9 (2H, m, CH_2-3') 2.4 (1H, dd, CH-3, J = 8.2, 17.1 Hz), 2.7 (1H, dd, CH-3, J = 5.5, 17.1 Hz), 3.6 (1H, m CH-4), 4.8 (2H, m, CH_2-5'), 5.6 (1H, m, CH-4').

^{1}H-NMR: δ_{ppm} **(DMSO)** 1.4 (4H, m, CH_2-2'/1'), 2.0 (2H, m, CH_2-3') 2.3 (1H, dd, CH-3, J = 7.4, 16.1 Hz), 2.6 (1H, dd, CH-3, J = 6.1, 16.1 Hz), 3.6 (1H, m, CH-4), 5.0 (2H, m, CH_2-5'), 5.9 (1H, m, CH-4').

^{1}H-NMR: δ_{ppm} **(CD_3OD)** 1.4 (4H, m, CH_2-2'/1'), 2.0 (3H, m, CH_2-3', CH-3) 2.3 (1H, dd, CH-3, J = 5.4, 15.4 Hz), 5.0 (2H, m, CH_2-5'), 5.9 (1H, m, CH-4'), 6.4 (2H, br, NH_2).

^{13}C-NMR: δ_{ppm} **(D_2O)** 26.11 (CH_2-2'), 35.05 (CH_2-3'), 35. 16 (CH_2-1'), 37.33 (CH_2-3),

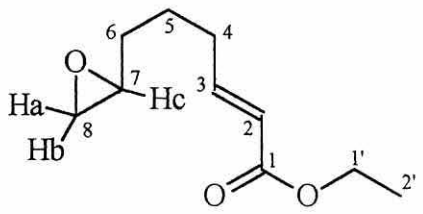
50.88 (CH_2-4), 117.58 (CH_2-5'), 141 .55 (CH-4'), 159 (CN), 173.44 (CO).

^{13}C-NMR: δ_{ppm} **(CD_3OD)** 23.95 (CH_2-2'), 33.02 (CH_2-1'), 34.22 (CH_2-3'), 36.50 (CH_2-3), 48.19 (CH-4), 115.11 (CH-4'), 138.49 (CH_2-5'), 161.30 (CN), 176.84 (CO).

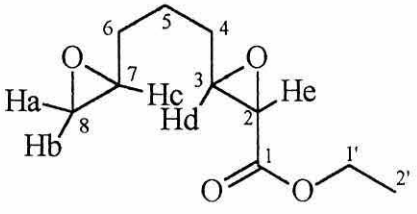
MS (CI) : m/z 182 (100% $[MH]^+$) daltons.

HRMS (CI) : m/z $C_9H_{16}ON_3$ requires $[MH^+]$ 182.1293; found 182.1293 daltons.

Ethyl (*2E*)-epoxy-7,8-oct-2-enoate and ethyl 2,3-7,8-diepoxyoctanoate



[261a]



[261b]

To a cooled (0°C) solution of **[257]** (5.68g, 34.0 mmol) in DCM (20 ml) was added *m*CPBA (8.75 g, 51.0 mmol) in DCM (50 ml) and this was stirred overnight reaching RT. The resulting mixture was diluted with DCM (30 ml), filtered through Celite and subsequently washed with saturated sodium metabisulphite solution (3 x 60 ml) and 5% sodium hydrogen carbonate solution (3 x 60 ml). The organic phase was dried over $MgSO_4$ and the solvent removed *in vacuo* which produced an oil. Purification of the oil by column chromatography eluting with 25% diethyl ether : petroleum ether gave rise to the *mono*epoxide in 80% yield (5.02 g, R_f = 0. 21 in 25% diethyl ether : petroleum ether) and the *bis*epoxide in 10% yield (0.7g).

[261a]

FT IR: ν_{max} **(cm^{-1})** 1710 (s, C=O), 1525 (s, C=C).

^{1}H-NMR: δ_{ppm} 1.4 (3H, t, CH_3-2', J = 7.1 Hz), 1.6 (4H, m, CH_2- 4 / 5), 2.3 (2H, m, CH_2-6), 2.5 (1H, dd, CH^a-8, J = 5.0, 2.7 Hz), 2.8 (1H, dd, CH^b-8, J = 5.0, 3.4 Hz), 3.0 (1H, m, CH^c-7), 4.2 (2H, q, CH_2-1', J = 7.1 Hz), 5.8 (1H, dt, CH^e-2, J = 15.6, 1.0 Hz), 6.9 (1H, dt, CH^d-3, J=15.6, 6.9 Hz).

^{13}C-NMR: δ_{ppm} 14.23 (CH_3-2'), 20.29 (CH_2-1'), 24.44 (CH_2-5), 31.75 (CH_2-4), 46.90 (CH_2-6), 51.90 (CH-7), 60.17 (CH_2-8), 121.79 (CH-3), 148.32 (CH-2), 166.57 (C-1).

MS (CI) : m/z 202 (100% $[M+NH_4]^+$) daltons.

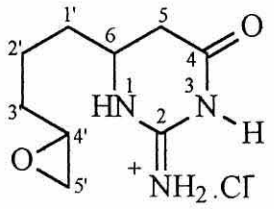
[261b]

FT IR: ν_{max} **(cm^{-1})** 1710 (s, C=O).

^{1}H-NMR: δ_{ppm} 1.3 (3H, t, CH_3-2', J = 7.1 Hz), 1.6 (6H, m, CH_2-4-6), 2.4 (1H, m, CH^a-8), 2.7 (1H, m, CH^b-8), 2.9 (1H, m, CH^c-7), 3.1 (1H, m, CH^d-3), 3.2 (1H, m, CH^e-2), 4.2 (2H, q, CH_2-1', J = 7.1 Hz).

^{13}C-NMR: δ_{ppm} 14.00 (CH_3-2'), 22.3 (CH_2-5), 31.8 (CH_2-4), 31.9 (CH_2-6), 46.8 (CH_2-8), 51.80 (CH-7), 52.8 (CH-3), 57.2 (CH-2), 61.5 (CH_2-1'), 169.0 (CO).

6-(4',5'-Epoxypentyl)-2-iminotetrahydropyrimidin-4-one hydrochloride



[259]

Guanidine (0.141g, 2.39 mmol) as a solution in dry DMF (0.39 ml) was added dropwise to a cooled (0°C) solution of **[261a]** (0.4g, 2.17 mmol) in dry DMF (0.4 ml), and stirred for 96 hours reaching RT.

This resulted in a precipitation which was filtered and washed with diethyl ether and the resultant filtrate evaporated *in vacuo*. The solid was then re-dissolved in methanol (10 ml) and treated with methanolic HCl (10 ml) (prepared from MeOH (10 ml) and CH_3COCl (1 ml)). The solvent was removed to produce the title compound as a white solid in 16% yield (0.07g).

Purification of the original filtrate and washings was undertaken after treatment with methanolic HCl (5 ml), initially by trituration with diethyl ether to remove soluble by-products and then by column chromatography, utilising a gradient elution of $CHCl_3$ (50 ml), 1% MeOH : $CHCl_3$ (100 ml), 2% MeOH : $CHCl_3$ (100 ml), 3% MeOH : $CHCl_3$ (100 ml), 4% MeOH : $CHCl_3$ (100 ml), 5% MeOH : $CHCl_3$ (100 ml), 10% MeOH : $CHCl_3$ (400 ml), 20% MeOH : $CHCl_3$ (100 ml), 100% MeOH (100 ml). This though only yielded recovered starting material **[261a]**.

FT IR: ν_{max} **(cm^{-1})** 1721, (s, C=O), 1575 (s, C=N).

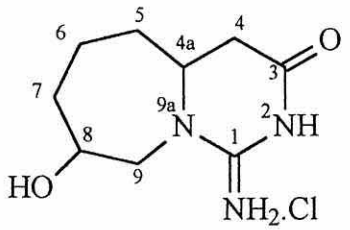
^{1}H-NMR: δ_{ppm} **(D_2O)** 1.5 (6H, m, CH_2-1'-3'), 2.3 (1H, dd, CH-3, J = 7.0, 16.3 Hz), 2.6(1H, dd, CH-3, J = 6.3 , 16.3 Hz), 2.7(1H, m, CH-5'), 2.9 (1H, t, CH-5',J = 4.3 Hz), 3.2 (1H, m, CH-4'), 3.7(1H, m, CH-4).

^{13}C-NMR: δ_{ppm} **(D_2O)** 23.10 (CH_2-2'), 25.81 (CH_2-1'), 35.51 (CH_2-3'), 37.30 (CH_2-3), 50.94 (CH_2-5'), 52.08 (CH-4'), 73.34 (CH-4), 155.91 (CN), 173.39 (CO).

MS (CI) : m/z 198 (100% $[MH]^+$) daltons.

HRMS (CI) : m/z $C_9H_{16}N_3O_2$ requires $[MH]^+$198.1243; found 198.1243 daltons.

8-Hydroxy-1-imino-octahydro-pyrimido[1,6-*a*]azepin-3-one hydrochloride



[267]

NaH (0.24g, 5.94 mmol) was added to a suspension of guanidine hydrochloride (0.82g, 8.64 mmol) in ^{t}BuOH (10 ml) and stirred for 30 minutes. After which **[261a]** (1.0g, 5.4 mmol) was added as a solution in ^{t}BuOH (5 ml). The reaction mixture was stirred for 96 hours at RT. After the formation of a white precipitate **[259]** the reaction mixture was then heated to 60°C and stirred for a further 96 hours. The reaction mixture was then cooled and filtered. The filtrate was washed with diethyl ether (3 x 100 ml) and then redissolved in methanol (5 ml), treated with methanolic HCl (1 ml, 10%), and stirred for 2 hours at 0°C, after which the solvent was removed *in vacuo* which produced a white solid. This was further purified by column chromatography, eluting with a graduated solvent system of 100 % $CHCl_3$, 0.5% MeOH : $CHCl_3$, 1% MeOH : $CHCl_3$, 3% MeOH : $CHCl_3$, 5% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 20% MeOH : $CHCl_3$, 60% MeOH : $CHCl_3$, 100% MeOH, to produce the product in 41% yield. (0.51g, R_f = 0. 10 in 20 % MeOH : $CHCl_3$, fractions 71-90 20% MeOH : $CHCl_3$).

FT IR: ν_{max} **(cm^{-1})** 3345 (br, OH), 2919 (s, CH), 1655 (s, C=O), 1510 (s, C=N).

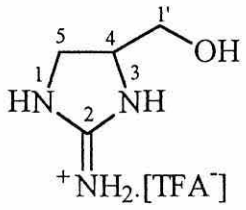
^{1}H-NMR: δ_{ppm}**D_2O** 1.5-2.0 (6H, m, CH_2-5-7), 2.6 (1H, m, CH-4), 3.2 (1H, m, CH-4), 3.4-4.1 (3H, m, CH_2-9, CH-4a), 4.25 (1H, brm, CH-8).

^{13}C-NMR: δ_{ppm}**D_2O** 24.0, 33.7 (2 x CH_2), 36.3 (CH_2), 57.2 (CH_2), 59.9 (CH), 70.7 (CH), 160.21 (CN), 169.48 (CO).

^{13}C-NMR: δ_{ppm}**CD_3OD** 21.1, 35.3, 35.1, 37.5, 58.4 (5 x CH_2), 60.8 (CH), 71.0 (CH), 160.21 (CN), 169.48 (CO).

MS (EI): m/z 198 (100% $[M]^+$) daltons.

(2-Iminoimidazolidin-4-yl)methanoltrifluoroacetate



[273]

A solution of guanidine (0.47g, 8.03 mmol), in ^{t}BuOH (4 ml) was added dropwise to a solution of epibromohydrin (1.0g, 7.3 mmol, 0.62 ml) in ^{t}BuOH (6 ml) and stirred for 36 hours at RT. KO^{t}Bu (0.82g, 7.3 mmol) was added and the reaction heated to 60°C and stirred for a further 96 hours. The resulting reaction mixture was cooled (0°C) and treated with methanol (10 ml) and trifluoroacetic acid (1 ml). This was then allowed to stir for 5 minutes, after which the solvent was removed *in vacuo*, the resultant material was purified using column chromatography and eluting with a graduated solvent system of 100 % $CHCl_3$, 5% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 20% MeOH : $CHCl_3$, 25% MeOH : $CHCl_3$, 30% MeOH : $CHCl_3$, 50% MeOH : $CHCl_3$, 70% MeOH : $CHCl_3$, 100% MeOH. This produced the desired compound in 33% yield (0.56g, 10% / 20% MeOH : $CHCl_3$, R_f = 0.15 in 20% MeOH : $CHCl_3$ run twice) as a semi solid.

FT IR: ν_{max} **(cm^{-1})** 3352 (br, OH), 1550 (s, C=N).

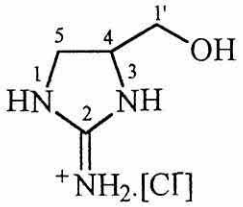
^{1}H-NMR: δ_{ppm} **(CD_3OD)** 3.73 (1H, dd, CH-5, J = 6.1, 10.1 Hz), 3.77 (1H, dd, CH-5, J = 5.2, 12.1 Hz), 3.84 (1H, dd, CH-1', J = 4.3, 12.1 Hz), 3.93 (1H, t, CH-1', J = 10.1 Hz), 4.3 (1H, m, CH-4)

^{13}C-NMR: δ_{ppm} **(CD_3OD)** 44.59 (CH_2-5), 56.46 (CH_2-1'), 62.68 (CH-4), 158.24 (C=N).

MS (CI) : m/z 116 (40% $[MH]^+$) daltons.

HRMS : m/z $[C_4H_{10}N_3O]^+$ requires $[MH]^+$ 116.0824; found 116.0825 daltons.

(2-Iminoimidazolidin-4-yl)methanol hydrochloride



[275]

A solution of guanidine (0.47g, 8.03 mmol), in ^{t}BuOH (10 ml) was added dropwise to a solution of epibromohydrin (1.0g, 7.3 mmol, 0.62 ml) in ^{t}BuOH (20 ml) and stirred for 36 hours at RT. KO^{t}Bu (0.82g, 7.3 mmol) was added and the reaction heated to 60°C and stirred for a further 96 hours. The resulting reaction mixture was then cooled (0°C) and methanolic HCl (2 ml) was added. This was then allowed to stir for 5 minutes, after which the solvent was removed *in vacuo*, and purification was carried out using column chromatogarphy with a graduated solvent system of 100 % $CHCl_3$, 5% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 20% MeOH : $CHCl_3$, 25% MeOH : $CHCl_3$, 30% MeOH : $CHCl_3$, 50% MeOH : $CHCl_3$, 70% MeOH : $CHCl_3$, 100% MeOH. This produced the desired compound in 73% yield (0.8g, 15% / 20% MeOH : $CHCl_3$, R_f = 0.27 in 20% MeOH : $CHCl_3$ run twice).

FT IR: ν_{max} **(cm^{-1})** 3258 (br, OH), 1552 (w, C=N).

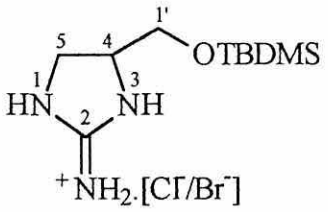
^{1}H-NMR: δ_{ppm} **(CD_3OD)** 3.9-4.1(3H, m, CH-5, 2 x CH-1'), 4.2 (1H, br t, CH-10, J = 10.0 Hz), 4.6 (1H, m, CH-4).

^{13}C-NMR: δ_{ppm} **(CD_3OD)** 45.73 (CH_2-5), 57.50 (CH_2-1'), 63.48 (CH-4), 158.77 (C=N).

MS (CI) : m/z 116 (25% $[MH]^+$) daltons.

HRMS (CI) : m/z See text page 101.

(2-Iminoimidazolidin-4-yl)-4-methyl-1'-(*tert*-butyldimethylsilyloxy) hydrochloride / hydrobromide



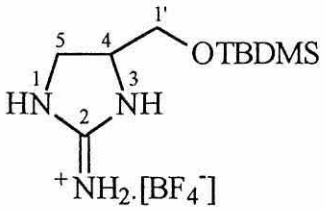
[276]

To a cooled (0°C) stirred solution of alcohol **[275]** (800mg, 5.3 mmol) in dry DMF (10.6 ml) imidazole (0.72g, 10.6 mmol) and TBDMSCl (1.20g, 7.95 mmol) were added. This was stirred overnight reaching RT. The reaction mixture was diluted with ethyl acetate (40 ml), and subsequently washed with H_2O (3 x 50 ml), saturated aqueous lithium bromide (3 x 50 ml), H_2O (3 x 50 ml), brine (3 x 50 ml), and the combined aqueous layers back extracted with $CHCl_3$ (2 x 50 ml). The combined organic layers were again washed with saturated aqueous lithium bromide (2 x 50 ml), dried over $MgSO_4$ and evaporated *in vacuo* giving the crude product as a yellow oil. This was purified by column chromatography eluting with a graduated solvent system of 10% diethyl ether : petroleum ether, 2% MeOH : $CHCl_3$, 2.5% MeOH : $CHCl_3$, 3% MeOH : $CHCl_3$, 3.5% MeOH : $CHCl_3$, 4% MeOH : $CHCl_3$, 4.5% MeOH : $CHCl_3$, 5% MeOH : $CHCl_3$, 5.5% MeOH : $CHCl_3$, 100% MeOH, to produce the title compound in 17% yield (227mg, 3% / 5% MeOH : $CHCl_3$, R_f = 0.44 in 10% MeOH : $CHCl_3$).

FT IR: ν_{max} **(cm^{-1})**	2950 (s, CH), 1685 (w, C=N).
^{1}H-NMR: δ_{ppm}	0.0 (6H, s, 2 x CH_3), 0.8 (9H, s, 3 x CH_3), 3.45 (2H, dd, 2 x CH-5, J = 5.4, 9.8 Hz), 3.55 (1H, d, CH-1', J = 5.4 Hz), 3.65 (1H, t, CH-1', J = 9.8 Hz), 4.0 (1H, m, CH-4).
^{13}C-NMR: δ_{ppm}	-5.52(CH_3), 18.11(C), 25.69 (CH_3), 45.19 (CH_2-5), 56.33 (CH_2-1'), 64.25 (CH-4), 160.415(C=N)
MS (CI) : m/z	230 (100% [M^+]) daltons.
HRMS	$[C_{10}H_{24}ON_3Si]^+$ requires [M^+] 230.1689; found 230.1687[†] daltons.

[†] MS analysis indicated the presence of a bromide ion, it was found that all TBDMS protections of the family of glycomimetics afforded a mixture of ions.

(2-Iminoimidazolidin-4-yl)-4-methyl-1'-(*tert*-butyldimethylsilyloxy) tetrafluoroborate

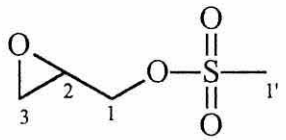


[277]

Saturated sodium tetrafluoroborate solution (10 ml) was added to a solution of **[276]** (227mg, 0.86 mmol) in DCM (10 ml). This was then stirred at RT overnight. The title compound was extracted with DCM (3 x 20 ml) dried over $MgSO_4$ and purified by column chromatography eluting with a graduated solvent system of $CHCl_3$, 2% MeOH : $CHCl_3$, 4% MeOH : $CHCl_3$, 6% MeOH : $CHCl_3$, 8% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 20% MeOH : $CHCl_3$, 100% MeOH. This furnished the title compound in 43% yield (120mg, 8% MeOH : $CHCl_3$, R_f= 0.13 in 10% MeOH : $CHCl_3$).

FT IR: ν_{max} **(cm^{-1})**	3388 (brd, NH_2), 2954 (s, CH), 2929(s, CH), 2857 (s, CH), 1693 (s, CO), 1589 (m, C=N).
^{1}H-NMR: δ_{ppm}	0.0 (6H, s, 2 x CH_3), 0.8 (9H, s, 3 x CH_3), 3.52 (1H, dd, CH-5, J = 5.8, 9.7 Hz), 3.64 (2H, d, CH-1', CH-5, J = 5.2 Hz), 3.80 (1H, t, CH-1', J= 9.7 Hz), 4.13 (1H, m, CH-4), 6.3 (2H, br, NH_2), 6.4 (1H, br, NH), 6.6 (1H, br, NH).
^{13}C-NMR: δ_{ppm}	-5.52 (2 x CH_3), 18.10 (C), 25.68 (3 x CH_3), 45.29 (CH_2-5), 56.83 (CH-4), 64.02 (CH_2-1'), 159.33 (C=N).
MS (CI) : m/z	230 (65% [M^+]) daltons.
HRMS	$[C_{10}H_{24}ON_3Si]^+$ requires [M^+] 230.1689; found 230.1690 daltons.

1-Methanesulphonyl-2,3-epoxypropane

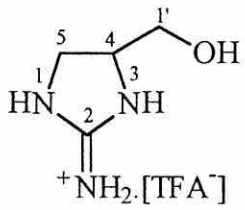


[279]

To a stirred cooled (0°C) solution of glycidol **[278]** (5.0g, 67.5 mmol, 4.5 ml) in dry DCM (169 ml), Et_3N (7.51g, 74.25 mmol) and methane sulphonyl chloride (8.51g, 74.25 mmol, 5.75 ml) were added sequentially, which produced a white suspension. The resultant suspension was then left to stir overnight reaching RT. Water (150 ml) was added and then extracted with DCM (3 x 60 ml), and the extracts dried over $MgSO_4$ with the solvent being removed *in vacuo*. The crude product was then purified using column chromatography eluting with a graduated solvent system of 25% - 50% diethyl ether : petroleum ether. This produced the mesylate in 87% yield (8.95g, R_f = 0.12 in 50 % diethyl ether : petroleum ether).

FT IR: ν_{max} **(cm^{-1})**	3022 (w, CH), 2950 (w, CH).
^{1}H-NMR: δ_{ppm}	2.65 (1H, dd, CH-3, J = 2.5, 4.7 Hz), 2.9 (1H, t, CH-3, J = 4.7 Hz), 3.05 (3H, s, CH_3-1'), 3.25 (1H, m, CH-2), 4.05 (1H, dd, CH-1, J = 6.5, 12 Hz), 4.5 (1H, dd, CH-1, J = 2.7, 12 Hz).
^{13}C-NMR: δ_{ppm}	37.64 (CH-2), 44.49 (CH_2-3), 49.09 (CH_3-1'), 70.39 (CH_2-1).
MS (CI) : m/z	170 (100% $[M+NH_4]^+$) daltons
HRMS	$[C_4H_{12}NO_4S]^+$ requires $[M+NH_4]^+$ 170.0487; found 170.0484 daltons.

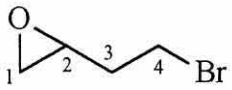
(2-Iminoimidazolidin-4-yl)methanol trifluoroacetate



[273]

A solution of guanidine (0.43g, 7.26 mmol) in ^{t}BuOH (10 ml) was added dropwise to a solution of **[279]** (1.0g, 6.6 mmol) in ^{t}BuOH (17 ml) which was stirred for 36 hours at RT. KO^{t}Bu (0.74g, 6.6 mmol) was added and the reaction heated to 60°C and stirred for 96 hours. The resulting reaction mixture was then cooled (0°C) and treated with methanol (10 ml) and trifluoroacetic acid (1 ml). This was then allowed to stir for 5 minutes, after which the solvent was removed *in vacuo*, and purification was carried out using column chromatogarphy and a graduated solvent system of; 100 % $CHCl_3$, 5% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 20% MeOH : $CHCl_3$, 25% MeOH : $CHCl_3$, 30% MeOH : $CHCl_3$, 50% MeOH : $CHCl_3$, 70% MeOH : $CHCl_3$, 100% MeOH. This produced the desired compound in 30% yield (0.46g, 20% / 30% MeOH : $CHCl_3$, R_f = 0.11 in 20% MeOH : $CHCl_3$). The data was as previously reported.

1,2 Epoxy-4-bromobutane

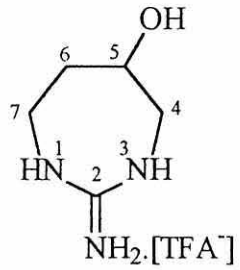


[281]

To a cooled (0°C) solution of 4-bromo-1-butene (2.66g, 19.7 mmol) in dry DCM (70 ml), a solution of *m*CPBA (4.42 g, 25.6 mmol) in dry DCM (25 ml) was added and the reaction stirred overnight reaching RT. The resulting mixture was diluted with DCM (30 ml) then filtered through a pad of $MgSO_4$ and silica eluting with DCM (3 x 50 ml). The organic phase was subsequently washed with sodium metabisulphite solution (3 x 40 ml), 5% sodium hydrogen carbonate solution (3 x 40 ml), dried over $MgSO_4$ and evaporated *in vacuo* producing the title compound in 92 % yield (2.95g), of sufficient purity for further reactions.

FT IR: ν_{max} **(cm^{-1})**	2994 (s, CH), 1265 (s, CO).
^{1}H-NMR: δ_{ppm}	2.0 (2H, m, CH_2-3), 2.4 (1H, m, CH-1), 2.7 (1H, m, CH-1), 3.0 (1H, m, CH-2), 3.5 (2H, t, CH_2-4, J = 7.2 Hz)
^{13}C-NMR: δ_{ppm}	29.00 (CH_2-3), 35.65 (CH_2-4), 46.95 (CH_2-1), 50.62 (CH-2).

2-Imino-[1,3]diazepan-5-ol trifluoroacetate



[283a]

A solution of guanidine (0.43g, 7.33 mmol) in ^{t}BuOH (7.1 ml) was added dropwise to a solution of 1,2-epoxy-4-bromobutane **[281]** (1.0g, 6.67 mmol) in ^{t}BuOH (20 ml) and the reaction stirred for 36 hours at RT. KO^{t}Bu (0.75g, 6.67 mmol) was then added and the reaction heated to 60°C and stirred for a further 96 hours. The resulting reaction mixture was then cooled (0°C) and treated with methanol (10 ml) and trifluoroacetic acid (1 ml). This was then stirred for 5 minutes, after which the solvent was removed *in vacuo*. The crude product was purified by column chromatography eluting with a graduated solvent system of 100 % $CHCl_3$, 5% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 20% MeOH : $CHCl_3$, 25% MeOH : $CHCl_3$, 30% MeOH : $CHCl_3$, 50% MeOH : $CHCl_3$, 70% MeOH : $CHCl_3$, 100% MeOH. This produced the desired compound in 79% yield (1.27g, 10% / 30% MeOH : $CHCl_3$, R_f = 0.11 in 20% MeOH : $CHCl_3$ run twice).

FT IR: ν_{max} **(cm^{-1})** 3346 (br, OH), 1500 (s,C=N).

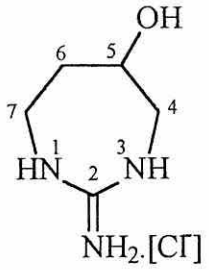
^{1}H-NMR: δ_{ppm} **(CD_3OD)** 2.0 (2H, m, CH_2-5), 3.3 (2H, m, CH_2-6), 3.6 (2H, m, CH_2-1'), 4.5 (1H, m, CH-5).

^{13}C-NMR: δ_{ppm} **(CD_3OD)** 34.8 (CH_2-5), 46.8 (CH_2-6), 56.70 (CH_2-1'), 71.30 (CH-4), 158.24 (C=N).

MS (CI) : m/z 130 (30% $[MH]^+$) daltons.

HRMS See text page 104-107.

2-Imino-[1,3]diazepan-5-ol hydrochloride



[283b]

A solution of guanidine (0.43g, 7.34 mmol) in ^{t}BuOH (7.1 ml) was added dropwise to a solution of 1,2-epoxy-4-bromobutane (1.0g, 6.67 mmol) in ^{t}BuOH (20 ml) which was stirred for 36 hours at RT. KO^{t}Bu (0.75g, 6.67 mmol) was added and the reaction heated to 60°C and stirred for 96 hours. The resulting reaction mixture was then cooled (0°C) and treated with methanolic HCl (10 ml) and stirred for 5 minutes, after which the solvent was removed *in vacuo*. Purification was achieved by column chromatography eluting with a graduated solvent system of 100 % $CHCl_3$, 5% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 20% MeOH : $CHCl_3$, 25% MeOH : $CHCl_3$, 30% MeOH : $CHCl_3$, 50% MeOH : $CHCl_3$, 70% MeOH : $CHCl_3$, 100% MeOH. This produced the desired compound as a mixture of bromide and chloride ions in 84% yield (0.92g, 10 / 30% MeOH : $CHCl_3$, R_f = 0.06 in 20% MeOH : $CHCl_3$ run twice).

FT IR: ν_{max} **(cm^{-1})** 3389 (br, OH), 1510 (s, C=N).

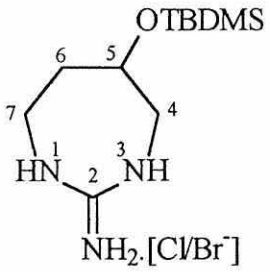
^{1}H-NMR: δ_{ppm} **(CD_3OD)** 2.0 (2H, m, CH_2-5), 3.3 (1H, m, CH-6), 3.6 (3H, m, CH_2-1', CH-6), 4.5 (1H, m, CH-4).

^{13}C-NMR: δ_{ppm} **(CD_3OD)** 33.55 (CH_2-5), 45.52 (CH_2-6), 55.43 (CH_2-1'), 69.98 (CH-4), 155.02 (C=N).

MS (CI) : m/z 130 (30% $[MH]^+$) daltons.

HRMS See text page 104-107.

5-*tert*-Butyldimethylsilyloxy-2-imino-[1,3]diazepan hydrochloride / hydrobromide

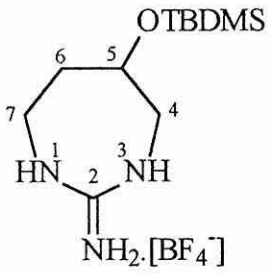


[287]

To a cooled (0°C) stirred solution of alcohol **[282b, 283b]** (244mg, 1.01 mmol) in dry DMF (2 ml), imidazole (138 mg, 2.02 mmol) and TBDMSCl (228mg, 1.51 mmol) were added. This was then stirred overnight reaching RT. The reaction mixture was diluted with ethyl acetate (20 ml), and subsequently washed with H_2O (2 x 20 ml), saturated aqueous lithium bromide (2 x 20 ml), H_2O (2 x 20 ml), brine (2 x 20 ml), with the combined aqueous layers being back extracted with $CHCl_3$ (2 x 20 ml). The combined organic layers were again washed with saturated aqueous lithium bromide (2 x 20 ml), dried over $MgSO_4$ and evaporated *in vacuo* furnishing the crude product as an oil. This was purified by column chromatography eluting with a graduated solvent system of 10% diethyl ether : petroleum ether, 2% MeOH : $CHCl_3$, 2.5% MeOH : $CHCl_3$, 3% MeOH : $CHCl_3$, 3.5% MeOH : $CHCl_3$, 4% MeOH : $CHCl_3$, 4.5% MeOH : $CHCl_3$, 5% MeOH : $CHCl_3$, 5.5% MeOH : $CHCl_3$, 100% MeOH, producing the title compound in 36% yield (102mg, 3% / 4% MeOH : $CHCl_3$, R_f = 0.04 in 10% MeOH : $CHCl_3$ run twice).

FT IR: ν_{max} **(cm^{-1})**	3300 (w, NH_2), 2928 (m, CH), 1620 (w, CO).
^{1}H-NMR: δ_{ppm}	0.0 (6H, s, 2 x CH_3), 1.3 (9H, s, ^{t}Bu), 1.95 (2H, m, CH_2-5), 3.3 (1H, m, CH-6), 3.6 (3H, m, CH_2-1', CH-6), 4.5 (1H, m, CH-4), 7.0 (4H, br, 4 x NH).
^{13}C-NMR: δ_{ppm}	-4.85 (CH_3), 17.91 (Quat. tBu), 25.69 (^{t}Bu), 34.36 (CH_2-5), 45.84 (CH_2-6), 56.46 (CH_2-1'), 70.65 (CH-4), 154.86 (C=N).
MS (EI) : m/z	244 (20% $[MH]^+$) daltons.
MS (CI) : m/z	244 (2% $[MH]^+$) daltons.
HRMS (CI) : m/z	$C_{11}H_{26}ON_3Si$ requires $[MH]^+$ 244.1845; found 244.1843 daltons.

5-*tert*-Butyldimethylsilyloxy-2-imino-[1,3]diazepan tetrafluoroborate

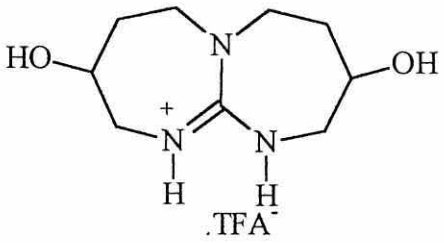


[288]

Saturated sodium tetrafluoroborate solution (5 ml) was added to a solution of **[287]** (102mg, 0.37 mmol) in $CHCl_3$ (5 ml). This was stirred at RT overnight. The reaction was extracted with $CHCl_3$ (3 x 20 ml), dried over $MgSO_4$ and purified by column chromatography eluting with a graduated solvent system of $CHCl_3$, 2% MeOH : $CHCl_3$, 4% MeOH : $CHCl_3$, 6% MeOH : $CHCl_3$, 8% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 20% MeOH : $CHCl_3$, 100% MeOH. This furnished **[288]** in 84% yield (102 mg, 6% / 8% MeOH : $CHCl_3$, R_f = 0.04 in 10% MeOH : $CHCl_3$ run twice).

FT IR: ν_{max} **(cm^{-1})**	3367 (br, NH_2), 1630 (m, C=N).
^{1}H-NMR: δ_{ppm}	0.0 (6H, s, 2 x CH_3), 0.8 (9H, s, ^{t}Bu), 1.9 (2H, m, CH_2-5), 3.2 (1H, br d, CH-6, J = 10.6 Hz), 3.5 (3H, m, CH_2-1', CH-6), 4.5 (1H, m, CH-4).
^{13}C-NMR: δ_{ppm}	-4.91 (2 x CH_3), 17.92 (Quat. ^{t}Bu), 25.69 (^{t}Bu), 34.42 (CH_2-5), 45.03 (CH_2-6), 55.21 (CH_2-1'), 70.73 (CH-4), 154.41 (C=N).
MS (CI) : m/z	244(100% [M^+]) daltons.
HRMS (ES$^+$) : m/z	$[C_{11}H_{26}N_3OSi]^+$ requires [M^+] 244.1845; found 244.1849 daltons.

***rac/meso*-2,3,4,5,6,7,8,9-Octahydro-1H-1,5a,10-triazaheptalene-4,7-diol**



[286]

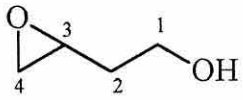
A solution of guanidine (0.26g, 4.40 mmol) in ^{t}BuOH (4ml) was added dropwise to a solution of **[281]** (0.73g, 4.84 mmol) in ^{t}BuOH (8 ml) which was stirred for 24 hours at RT. KO^{t}Bu (0.49g, 4.40 mmol) was added and the reaction stirred for a further 24 hours. A further portion of **[281]** (0.73g, 4.84 mmol) was added and the reaction stirred for 24 hours. A second portion of KO^{t}Bu (0.49g, 4.40 mmol) was added, stirred for 24 hours and then heated to 60°C and stirred for a further 96 hours. The resulting reaction mixture was then cooled (0°C) and treated with methanol (10 ml) and trifluoroacetic acid (1 ml). This was then allowed to stir for 5 minutes, after which the solvent was evaporated *in vacuo*. Purification was carried out using column chromatography eluting with a graduated solvent system of 100 % $CHCl_3$, 5% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 15% MeOH : $CHCl_3$, 20% MeOH : $CHCl_3$, 30% MeOH : $CHCl_3$, 50% MeOH : $CHCl_3$, 70% MeOH : $CHCl_3$, 100% MeOH. This produced 0.72g of the previously isolated structure **[283a]** and 0.36g of the title compound in a 36% combined yield (1.08 g, 70% MeOH : $CHCl_3$, R_f = 0.16 in 20% MeOH : $CHCl_3$ run twice).

FT IR: ν_{max} **(cm^{-1})** 3300 (br, OH), 2952 (s, CH).

^{1}H-NMR: δ_{ppm} **(D_2O)** 1.7-2.25 (4H, m, 2 x CH_2), 3.2-3.9 (8H, m, 4 x CH_2), 4.7 (2H, m, 2 x CH).

^{13}C-NMR: δ_{ppm} **(D_2O)** 35.18, 35.42 (2 x CH_2), 50.3 (2 x CH_2), 58.65, 59.7 (2 x CH_2), 72.22, 72.56 (2 x CH), 158.43 (C=N).

1-Hydroxy-3,4-epoxybutane

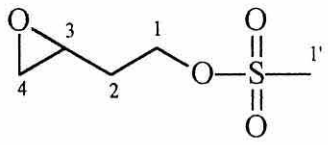


[290]

*m*CPBA (8.60g, 49.92 mmol) was added to a cooled (0°C) solution of but-3-ene-1-ol (3.0g, 41.6 mmol) in dry DCM (104 ml), which was then stirred for 3 hours reaching RT. The solvent was then removed *in vacuo* with purification by column chromatography eluting with a graduated solvent system of 25% diethyl ether : petroleum ether to a 100% diethyl ether in 25% increments. The desired product was obtained in 74% yield (2.70g, 50% diethyl ether : petroleum ether / 100% diethyl ether, R_f= 0.2 in 100% diethyl ether.).

FT IR: ν_{max} **(cm^{-1})**	3500 (s, OH), 1500(w, CO).
^{1}H-NMR: δ_{ppm}	1.7 (1H, m, CH-2), 1.9 (1H, m, CH-2), 2.2 (1H, br, OH), 2.6 (1H, dd, CH-4, J = 2.7, 4.8 Hz), 2.75 (1H, t, CH-4, J = 4.8 Hz), 3.1 (1H, m, CH-3), 3.8 (2H, t, CH_2-1, J = 5.9 Hz)
^{13}C-NMR: δ_{ppm}	34.77 (CH_2-2), 46.71 (CH_2-4), 50.43 (CH-3), 59.64 (CH_2-1).

1-Methanesulphonyoxy-3,4-epoxybutane

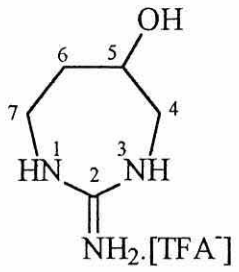


[291]

To a stirred, cooled (0°C) solution of **[290]** (1.5g, 17.04 mmol) in dry DCM (43 ml), Et_3N (1.89g, 18.75 mmol, 2.6 ml), methanesulphonyl chloride (2.15g, 18.75 mmol, 1.45 ml) were added and the reaction stirred overnight to reach RT. Water (70 ml) was added and the product extracted with DCM (2 x 50 ml) with the combined extracts being dried over $MgSO_4$ and the solvent removed *in vacuo*. Purification was achieved by column chromatography eluting with 25%-50% diethyl ether : petroleum ether. This furnished the title compound in 74% yield (2.09g, 50% diethyl ether, R_f= 0.07 in 50 % diethyl ether :petroleum ether).

FT IR: ν_{max} **(cm^{-1})**	3022 (m, CH), 2938 (m, CH), 1470 (w, S=O), 1416 (m, S=O).
^{1}H-NMR: δ_{ppm}	1.6 (1H, m, CH-2), 2.1 (1H, m, CH-2), 2.55 (1H, dd, CH-4, J = 2.6, 4.9 Hz), 2.8 (1H, t, CH-4, J = 4.9 Hz), 3.1 (4H, m, CH_3-1', CH-3), 4.4 (2H, t, CH_2-1, J = 6.1 Hz).
^{13}C-NMR: δ_{ppm}	32.24 (CH_2-2), 37.31 (CH_3-1'), 46.93 (CH_2-4), 48.75 (CH-3), 66.86 (CH_2-1).
MS (CI) : m/z	184 (80% [M+NH_4^+]) daltons.
HRMS (CI) : m/z	$C_5H_{14}NO_4S$ requires [M+NH_4^+] 184.0644; found 184.0645 daltons.

2-imino-[1,3]diazepan-5-ol trifluoroacetate

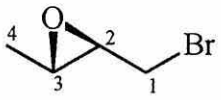


[283a]

A solution of guanidine (391mg, 6.6 mmol) in ^{t}BuOH (10 ml) was added dropwise to a solution of **[291]** (1g, 6.02 mmol) in ^{t}BuOH (14 ml) which stirred for 36 hours at RT. KO^{t}Bu (676mg, 6.02 mmol) was added and the reaction heated to 60°C and stirred for a further 96 hours. The resulting reaction mixture was cooled (0°C) and treated with methanol (10 ml) and trifluoroacetic acid (1 ml). This was then allowed to stir for 5 minutes, after which the solvent was evaporated *in vacuo*. Purification was achieved by column chromatography eluting with a gradient solvent system of 100 % $CHCl_3$, 5% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 20% MeOH : $CHCl_3$, 25% MeOH : $CHCl_3$, 30% MeOH : $CHCl_3$, 50% MeOH : $CHCl_3$, 70% MeOH : $CHCl_3$, 100% MeOH. This produced the desired compound in 30% yield (430mg, 30% MeOH : $CHCl_3$, R_f = 0.1 in 20% MeOH : $CHCl_3$ run twice).

(data as page 170)

(*E*)-1-Bromo-2,3-epoxybutane



[292]

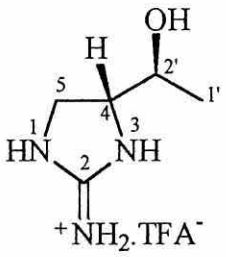
To a cooled (0°C) solution of (*E*)-crotyl bromide (3g, 22.2 mmol) in dry DCM (25 ml) was added *m*-CPBA (7.6g, 44.4 mmol) as a solution in dry DCM (25 ml) and this was left to stir overnight reaching RT. The resulting mixture was diluted with DCM (30 ml) filtered through silica, and subsequently washed with sodium metabisulphite solution (3 x 20 ml), 5% sodium hydrogen carbonate solution (3 x 20 ml) and water (2 x 20 ml). The organic phase was dried over $MgSO_4$ and evaporated *in vacuo* producing the title compound of sufficient purity for further reactions in 60% yield (2g).

FT IR: ν_{max} **(cm^{-1})** 2995 (s, CH), 1451 (s, C=O).

^{1}H-NMR: δ_{ppm} 1.3 (3H, d, CH_3-4, J = 5.2 Hz), 2.9 (2H, m, CH-2/3), 3.3 (1H, dd, CH-1, J = 5.8, 10.6 Hz), 3.4 (1H, dd, CH-1, J = 5.9, 10.5 Hz).

^{13}C-NMR: δ_{ppm} 17.21 (CH_3-4), 32.29 (CH_2-1), 56.59 (CH-3), 58.02 (CH-2).

(2-Iminoimidazolidin-4-yl)-2'-hydroxyethane trifluoroacetate



[295]

A solution of guanidine (0.43g, 7.3 mmol) in ^{t}BuOH (7 ml) was added dropwise to a solution of 2,3 epoxy-1-bromobutane (1g, 6.6 mmol) in ^{t}BuOH (20 ml) which was stirred for 36 hours at RT. KO^{t}Bu (0.74g, 6.6 mmol) was added and the reaction heated to 60°C and stirred for a further 96 hours. The resulting reaction mixture was then cooled (0°C) treated with TFA (1 ml) and stirred for 5 minutes, after which the solvent was evaporated *in vacuo*. Purification was carried out using column chromatography, eluting with a graduated solvent system of 100 % $CHCl_3$, 5% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 20% MeOH : $CHCl_3$, 25% MeOH : $CHCl_3$, 30% MeOH : $CHCl_3$, 50% MeOH : $CHCl_3$, 70% MeOH : $CHCl_3$, 100% MeOH. This gave **[295]** in 52% yield (0.57g, 15% MeOH : $CHCl_3$, R_f = 0.2 in 20% MeOH : $CHCl_3$ run twice).

FT IR: ν_{max} **(cm^{-1})** 3364 (s, OH), 1677 (s, C=N), 1431 (w, CO).

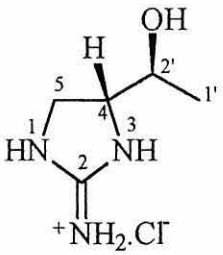
^{1}H-NMR: δ_{ppm} **(CD_3OD)** 1.1 (3H, d, CH_3-7, J = 6.4 Hz), 3.6 (1H, m, CH), 3.7 (2H, m, CH_2), 3.9 (1H, m, CH), 7.0 (2H, br, NH2), 7.5 (1H, br, NH), 7.6, 8.0 (2H, br, 2 x NH)

^{13}C-NMR: δ_{ppm} **(CD_3OD)** 19.42 (CH_3-1'), 45.82 (CH_2-5), 62.71 (CH-4), 69.45 (CH-2'), 160.75 (C=N).

MS (CI) : m/z 130 (75% [M^+]) daltons.

HRMS (CI) : m/z $[C_5H_{12}N_3O]^+$ requires [M^+] 130.0980; found 130.0978 daltons.

(2-Iminoimidazolidin-4-yl)-2'-hydroxyethane hydrochloride



[296]

A solution of guanidine (0.43g, 7.3 mmol) in ^{t}BuOH (7 ml) was added dropwise to a solution of 2,3 epoxy-1-bromobutane (1g, 6.6 mmol) in ^{t}BuOH (20 ml) which was stirred for 36 hours at RT. KO^{t}Bu (0.74g, 6.6 mmol) was added and the reaction heated to 60°C and stirred for a further 96 hours. The resulting reaction mixture was cooled (0°C), treated with methanolic HCl (10 ml) and stirred for 5 minutes, after which the solvent was evaporated *in vacuo*. Purification was carried out using column chromatography eluting with a gradient solvent system of 100 % $CHCl_3$, 5% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 20% MeOH : $CHCl_3$, 25% MeOH : $CHCl_3$, 30% MeOH : $CHCl_3$, 50% MeOH : $CHCl_3$, 70% MeOH : $CHCl_3$, 100% MeOH. This gave **[296]** in 52% yield (0.57g, 15% MeOH : $CHCl_3$, R_f = 0.23 in 20% MeOH : $CHCl_3$ run twice).

FT IR: ν_{max} **(cm^{-1})** 3356 (s), 1660 (s), 1381 (w), 1290 (w).

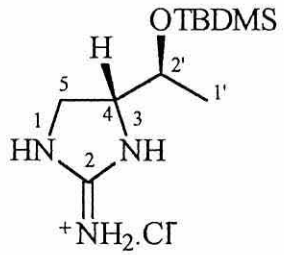
^{1}H-NMR: δ_{ppm} **(CD_3OD)** 1.4 (3H, d, CH_3-1', J = 6.5 Hz), 3.85 (2H, m, CH_2), 3.95 (1H, m, CH), 4.2 (1H, m, CH), 7.15 (2H, s, NH2), 7.7 (1H, s, NH), 7.8, 8.1 (1H, s, 2 x NH)

^{13}C-NMR: δ_{ppm} **(CD_3OD)** 19.30 (CH_3-1'), 45.65 (CH_2-5), 62.29 (CH-4), 69.24 (CH-2'), 160.18 (C=N).

MS (CI) : m/z 130 (100% [M^+]) daltons.

HRMS (CI) : m/z $[C_5H_{12}N_3O]^+$ requires [M^+] 130.0980 found 130.0982 daltons.

(2-Iminoimidazolidin-4-yl)-4-ethyl-2'-(*tert*-butyldimethylsilyloxy) hydrochloride

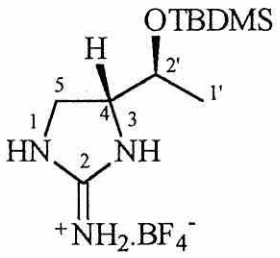


[297]

To a cooled (0°C) stirred solution of alcohol **[296]** (220mg, 0.91 mmol) in dry DMF (2.3 ml), imidazole (496mg, 7.28 mmol) and TBDMSCl (548mg, 3.64 mmol) were added and allowed to stir overnight to reach RT. The reaction mixture was diluted with ethyl acetate (25 ml), and subsequently washed with H_2O (2 x 30 ml), saturated aqueous lithium bromide (2 x 30 ml), H_2O (2 x 20 ml) and brine (2 x 30 ml). The combined aqueous layers were back extracted with $CHCl_3$ (2 x 20 ml). The combined organic layers were again washed with saturated aqueous lithium bromide (2 x 20 ml), dried over $MgSO_4$ and evaporated *in vacuo* giving the crude product. This was purified by column chromatography eluting with a graduated solvent system of 10% diethyl ether : petroleum ether, 2% MeOH : $CHCl_3$, 2.5% MeOH : $CHCl_3$, 3% MeOH : $CHCl_3$, 3.5% MeOH : $CHCl_3$, 4% MeOH : $CHCl_3$, 4.5% MeOH : $CHCl_3$, 5% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 100% MeOH, which produced the title compound in 43% yield (470mg, 5% MeOH : $CHCl_3$, R_f = 0.15 in 10% MeOH : $CHCl_3$).

FT IR: ν_{max} **(cm^{-1})**	3246 (s, NH), 2956 (s, CH), 1591 (w, CO).
^{1}H-NMR: δ_{ppm}	0.0 (6H, s, 2 x CH_3), 0.7 (9H, s, 3 x CH_3), 1.1 (3H, d, CH_2, J = 5.7 Hz), 3.6 (2H, m, 2 x CH), 3.7 (2H, m, 2 x CH).
^{13}C-NMR: δ_{ppm}	-4.32(CH_3), 17.80 (Quat. of ^{t}Bu) 19.98 (CH_3-1'), 25.65 (CH_3), 44.22 (CH_2-5), 61.00 (CH-4), 69.16 (CH-2'), 159.93 (C=N).
MS (ES) : m/z	244 (100% [M^+]) daltons.
HRMS (CI) : m/z	$[C_{11}H_{26}ON_3Si]^+$ requires [M^+] 244.1845; found 244.1842 daltons.

(2-Iminoimidazolidin-4-yl)-4-ethyl-2'-(*tert* butyldimethylsilyloxy) tetrafluoroborate

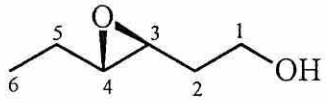


[298]

To a stirred solution of **[297]** (100mg, 0.34 mmol) in $CHCl_3$ (5 ml), saturated sodium tetrafluoroborate solution (5 ml) was added. The title compound was extracted afetr 18 hours with $CHCl_3$ (3 x 20 ml) dried over $MgSO_4$ and purified by column chromatography eluting with a graduated solvent system of $CHCl_3$, 2% MeOH : $CHCl_3$, 4% MeOH : $CHCl_3$, 6% MeOH : $CHCl_3$, 8% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 20% MeOH : $CHCl_3$, 100% MeOH. This furnished **[298]** in 52% yield (61mg, 10% MeOH : $CHCl_3$, R_f= 0.09 in 10% MeOH : $CHCl_3$).

FT IR: ν_{max} **(cm^{-1})**	3385 (s, NH), 2953 (s CH), 2928 (s, CH), 2856 (s, CH).
^{1}H-NMR: δ_{ppm}	0.0 (6H, s, 2 x CH_3), 0.8 (9H, s, 3 x CH_3), 1.1 (3H, d, CH_2, J = 6.0 Hz), 3.6 (2H, m, CH_2), 3.8 (2H, m, 2 x CH), 6.3 (2H, br, NH_2), 6.45 (1H, br, NH), 6.7 (1H, br NH).
^{13}C-NMR: δ_{ppm}	-4.5 (CH_3), 17.75 (C), 19.5 (CH_3-1'), 25.5 (CH_3), 44.13 (CH_2-5), 61.12 (CH-4), 68.84 (CH-2'), 159.21 (C=N).
MS (ES) : m/z	244 (45% [M^+]) daltons.
HRMS (CI) : m/z	$[C_{11}H_{26}ON_3Si]^+$ requires [M^+] 244.1845; found 244.1847 daltons.

***trans*-3,4-Epoxyhexan-1-ol**

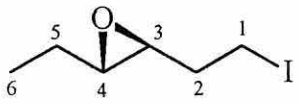


[300]

To a cooled (0°C) solution of (*E*)-hex-3-ene-1-ol (1g, 9.98 mmol) in dry DCM (2.5 ml) was added a solution of *m*CPBA (3.45g, 19.0 mmol) in dry DCM (15 ml) and this was stirred overnight reaching RT. The resulting mixture was diluted with DCM (30 ml) filtered through Celite and subsequently washed with sodium metabisulphite solution (3 x 20 ml), 5% sodium hydrogen carbonate solution (3 x 20 ml), dried over $MgSO_4$ and evaporated *in vacuo*. This produced the title compound **[300]** in 72% yield.

FT IR: ν_{max} **(cm^{-1})**	3420 (br, OH), 1459 (s, CO).
^{1}H-NMR: δ_{ppm}	0.9 (3H, t, CH_3-6, J = 7.6 Hz), 1.5 (2H, m, CH_2-5), 1.9 (2H, m, CH_2-2), 2.7 (1H, m, CH-4), 2.8 (1H, m, CH-3), 3.0 (1H, br, OH), 3.7 (2H, t, CH_2-1, J = 6.3 Hz)
^{13}C-NMR: δ_{ppm}	9.72 (CH_3-6), 24.97 (CH_2-5), 34.23 (CH_2-2), 56.62 (CH-4), 59.50 (CH-3), 59.88 (CH_2-1).
MS (CI) : m/z	115 (100% [M-H$^+$]) daltons.
HRMS (EI) : m/z	$C_6H_{11}O_2$ requires [M-H$^+$] 115.0759; found 115.0757 daltons.

***trans*-1-Iodo-3,4-epoxy-hexane**

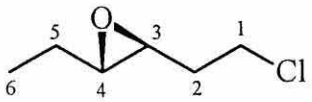


[293b]

Triphenylphosphine (2.06g, 7.84 mmol) and imidazole (0.53g, 7.84 mmol) were dissolved in diethyl ether : acetonitrile (3:1 (20.6 ml, 6.86 ml). The mixture was cooled (0°C) and iodine (1.99g, 7.84 mmol) was added in four portions with vigorous stirring over a 20 minute period. The resulting slurry was warmed to 20°C, re-cooled to 0°C and 3,4-epoxy-hexan-1-ol **[300]** (0.82g, 7.13 mmol) was added over 15 minutes. The resulting mixture was warmed to 20°C, stirred for 1 hour, re-cooled (0°C) and pentane (30 ml) was added. The title compound was extracted with diethyl ether (3 x 50 ml), washed with H_2O (2 x 20 ml) and dried with $MgSO_4$. The excess solvent was removed *in vacuo* and this was purified by column chromatography eluting with 5% diethyl ether : petroleum ether producing the title compound in 45% yield (0.71g, R_f = 0.5 in 30% diethyl ether : petroleum ether).

FT IR: ν_{max} **(cm^{-1})**	2950 (s, CH), 1459 (s, CO).
^{1}H-NMR: δ_{ppm}	1.2 (3H, t, CH_3-6, J = 7.5 Hz), 1.7 (2H, m, CH_2-5), 2.3 (2H, m, CH_2-2), 2.9 (2H, m, CH-3/4), 3.7 (2H, m, CH_2-1)
^{13}C-NMR: δ_{ppm}	0.60 (CH_2-1), 9.88 (CH_3-6), 24.93 (CH_2-5), 35.86 (CH_2-2), 58.22 (CH-4), 59.75 (CH-3).
MS (CI) : m/z	227 (40% $[MH]^+$), 100 (30% $[MH]^+$- I) daltons.
HRMS (CI) : m/z	$C_6H_{12}OI$ requires $[M^+]$ 226.9933; found 226.9932 daltons.

***trans*-1-Chloro-3,4-epoxyhexane**



[293c]

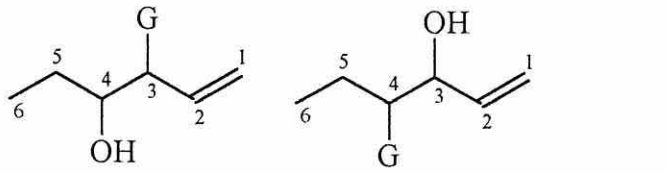
Triphenylphosphine (4.5g, 17.2 mmol) was added to a cooled (0°C) solution of **[300]** (1g, 8.6 mmol) in CCl_4 (24 ml) and this was stirred overnight reaching RT. The reaction mixture was then refluxed until no starting material remained (TLC observation - 50% diethyl ether : petroleum ether), the solvent was removed *in vacuo* and purified by column chromatography with a gradient elution : 40-60% diethyl ether : petroleum ether. This furnished the title compound in 30% yield (0.35g, R_f = 50% diethyl ether : petroleum ether).

FT IR: ν_{max} **(cm^{-1})** 2969 (s, CH), 1461 (s, CO).

^{1}H-NMR: δ_{ppm} 0.9 (3H, t, CH_3-6, J = 7.5 Hz), 1.5 (2H, m, CH_2-5), 2.0 (2H, m, CH_2-2), 2.7 (1H, m, CH-4), 2.8 (1H, m, CH-3), 3.6 (2H, t, CH_2-1, J = 7.4 Hz)

^{13}C-NMR: δ_{ppm} 9.75 (CH_3-6), 24.96 (CH_2-5), 35.86 (CH_2-2), 41.36 (CH_2-1), 55.72 (CH-4), 59.96 (CH-3).

N-[1-(1-Hydroxy-propyl)-allyl]-guanidine hydrochloride/hydroiodide and / or N-(1-Ethyl-2-hydroxy-but-3-enyl)-guanidine hydrochloride/hydroiodide



.HX or .HX X = Cl or I

[302] **[303]**

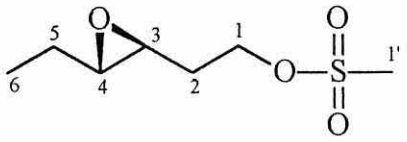
A solution of guanidine (146mg, 2.47 mmol) in ^{t}BuOH (2.3 ml) was added dropwise to a solution of **[293b]** (507mg, 2.24 mmol) in ^{t}BuOH (6 ml) which was stirred for 36 hours at RT. KO^{t}Bu (251mg, 2.24 mmol) was added and the reaction heated to 60°C and stirred for a further 96 hours. The reaction mixture was then cooled (0°C), treated with methanolic HCl (10 ml)) and stirred for 5 minutes, after which the solvent was removed *in vacuo*. Purification was achieved by column chromatography eluting with a graduated solvent system of 100 % $CHCl_3$, 5% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 20% MeOH : $CHCl_3$, 25% MeOH : $CHCl_3$, 30% MeOH : $CHCl_3$, 50% MeOH : $CHCl_3$, 70% MeOH : $CHCl_3$, 100% MeOH. This produced the title compound in 20% yield (129mg, 20 / 25% MeOH : $CHCl_3$, R_f = 0.15 in 20% MeOH : $CHCl_3$ run twice).

FT IR: ν_{max} **(cm^{-1})** 3360 (s, OH), 2494 (s, CH), 1630 (s, CO).

^{1}H-NMR: δ_{ppm} 1.2 (3H, t, CH_3-6, J = 7.7 Hz), 2.2 (2H, dq, CH_2-5, J = 7.7 Hz), 3.7 (1H, m, CH), 4.35 (1H, m, CH), 5.55 (2H, dd, CH-1, J = 11.6,16.6 Hz), 6.1 (1H, ddd, CH-2, J = 7.7, 11.5, 16.5 Hz)

^{13}C-NMR: δ_{ppm} 11.08 (CH_3-6), 27.52 (CH_2-5), 60.11 (CH), 75.73 (CH), 118.98 (CH_2-1), 134.57 (CH-2), 160.37 (C=N).

1-Methansulphonyloxy-3,4-epoxyhexane

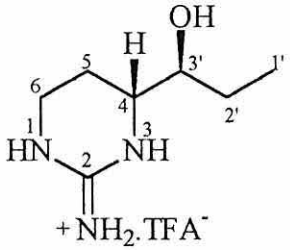


[293d]

To a stirred, cooled (0°C) solution of **[300]** (1.5g, 13 mmol) in dry DCM (33 ml) Et_3N (1.45g, 14.3 mmol, 1.99 ml) and methanesulphonyl chloride (1.64g, 14.3 mmol, 1.1 ml) were added. This was allowed to stir overnight to reach RT. Water (70 ml), DCM (2 x 50 ml) were added, the organic phase was dried over $MgSO_4$ and the solvent removed *in vacuo*. Purification was carried out by column chromatography eluting with 50 % diethyl ether : petroleum ether. This furnished the title compound in 73% yield (1.8g, R_f = 0.1 in 50 % diethyl ether :petroleum ether).

FT IR: ν_{max} **(cm^{-1})**	2976 (m, CH), 1477 (m, S=O), 1355 (s, CO).
^{1}H-NMR: δ_{ppm}	1.0 (3H, t, CH_3-6, J = 7.4 Hz), 1.6 (2H, m, CH_2-5), 1.9 (1H, m, CH-2), 2.15 (1H, m, CH), 2.7 (1H, m, CH-4), 2.9 (1H, m, CH-3), 3.1 (3H, s, CH_3-1'), 4.4 (2H, t, CH_2-1, J = 5.4 Hz).
^{13}C-NMR: δ_{ppm}	9.72 (CH_3-6), 24.89 (CH_2-5), 31.98 (CH_2-2), 37.40 (CH_3-1'), 54.54 (CH-4), 59.91 (CH-1), 66.83 (CH_2-1).
MS (CI) : m/z	212 (100% [M+NH_4]$^+$) daltons.
HRMS (CI) : m/z	$C_7H_{18}O_4SN$ requires [M+NH_4]$^+$ 212.0957; found 212.0955 daltons.

(2-Iminotetrahydropyrimidine-4-yl)-3'-hydroxypropane trifluoroacetate



[301b]

A solution of guanidine (222mg, 3.75 mmol) in ^{t}BuOH (4 ml) was added dropwise to a solution of **[239d]** (1g, 3.41 mmol) in ^{t}BuOH (10 ml) which was stirred for 36 hours at RT. KO^{t}Bu (383mg, 3.41 mmol) was added and the reaction heated to 60°C and stirred for a further 96 hours. The resulting reaction mixture was then cooled (0°C), treated with methanol (10 ml) and trifluoroacetic acid (1 ml). This was then allowed to stir for 5 minutes, after which the solvent was evaporated *in vacuo*. Purification was carried out using column chromatography eluting with a graduated solvent system of 100 % $CHCl_3$, 5% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 15% MeOH : $CHCl_3$, 20% MeOH : $CHCl_3$, 30% MeOH : $CHCl_3$, 50% MeOH : $CHCl_3$, 70% MeOH : $CHCl_3$, 100% MeOH. This produced the desired compound in 30% yield (280mg, 20 / 30% MeOH : $CHCl_3$, R_f = 0.16 in 20% MeOH : $CHCl_3$ run twice).

FT IR: ν_{max} **(cm^{-1})** 3348 (m, NH), 2923 (s, CH), 1678 (w, CO).

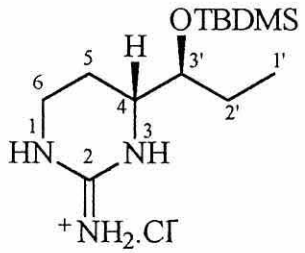
^{1}H-NMR: δ_{ppm} **(CD_3OD)** 1.0 (3H, t, CH_3-1', J = 7.3 Hz), 1.6 (H, m, CH-2'), 2.0 (2H, q, CH_2-5, J = 5.7 Hz), 3.4-3.65 (4H, m, CH-3', CH_2-6, CH-4).

^{13}C-NMR: δ_{ppm} **(CD_3OD)** 11.02 (CH_3-1'), 21.91 (CH_2-2'), 27.02 (CH_2-5), 38.40 (CH_2-6), 55.12 (CH-4), 74.60 (CH-1'), 156.36 (C=N).

MS (CI) : m/z 158 (45% $[M]^+$) daltons.

HRMS (CI) : m/z $C_7H_{16}N_3O$ requires $[M]^+$ 158.1293 found 158.1294 daltons.

(2-Iminotetrahydropyrimidine-4-yl)-3'-*tert*-butyldimethylsilyloxypropane hydrochloride

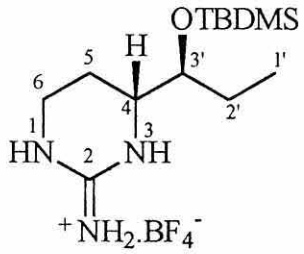


[304]

To a cooled (0°C) stirred solution of alcohol **[301b]** (280mg, 1.03 mmol) in dry DMF (3 ml), imidazole (562mg, 8.26 mmol) and TBDMSCl (623mg, 4.13 mmol) were added, and stirred to RT overnight. The reaction mixture was diluted with ethyl acetate (25 ml), and subsequently washed with H_2O (2 x 30 ml), saturated aqueous lithium bromide (2 x 30 ml), H_2O (2 x 30 ml) and brine (2 x 30 ml), with the combined aqueous layers being back extracted with $CHCl_3$ (2 x 30 ml). The combined organic layers were again washed with saturated aqueous lithium bromide (2 x 20 ml), dried over $MgSO_4$ and evaporated *in vacuo* giving the crude product as an oil. This was purified by column chromatography eluting with a graduated solvent system of 10% diethyl ether : petroleum ether, 2% MeOH : $CHCl_3$, 4% MeOH : $CHCl_3$, 6% MeOH : $CHCl_3$, 8% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 20% MeOH : $CHCl_3$, 100% MeOH, producing the title compound in 53% yield (168mg, 6% / 20% MeOH : $CHCl_3$, R_f = 0.34 in 10% MeOH : $CHCl_3$).

FT IR: ν_{max} **(cm^{-1})**	3300 (m, NH), 2956 (m, CH), 1667 (s, CO).
^{1}H-NMR: δ_{ppm}	0.0 (6H, s, 2 x CH_3), 0.8 (12H, s, ^{t}Bu, t, CH_3-1', J = 7.4 Hz), 1.6 (2H, m, CH_2-2'), 1.8 (2H, brq, CH_2-5, J = 5.9 Hz), 3.2 (1H, m, CH-3'), 3.4 (2H, m, CH_2-6), 3.6 (1H, m, CH-4), 7.0 (2H, br, NH_2), 7.6 (1H, br, NH), 7.9 (1H, br, NH).
^{13}C-NMR: δ_{ppm}	-4.46 (CH_3), 8.58 (CH_3-1'), 17.96 (Quat. of *t*Bu), 20.84 (CH_2-2'), 25.83 (CH_3), 36.82 (CH_2-6), 51.48(CH-4), 74.10 (CH-1'), 154.98 (C=N).
MS (CI) : m/z	272 (40% [M^+]) daltons.
HRMS (ES$^+$) : m/z	$[C_{13}H_{30}N_3OSi]^+$ requires [M^+] 272.2158; found 272.2166 daltons.

(2-Iminotetrahydropyrimidine-4-yl)-3'-*tert*-butyldimethylsilyloxypropane tetrafluoroborate

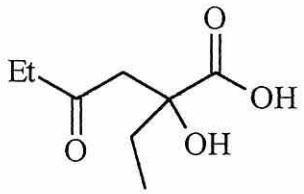


[305]

Saturated sodium tetrafluoroborate solution (5 ml) was added to a solution of **[304]** (100mg, 0.3 mmol) in $CHCl_3$ (5 ml). This was then left to stir at RT overnight. The product was extracted with $CHCl_3$ (3 x 20 ml) dried over $MgSO_4$ and purified by column chromatography eluting with a graduated solvent system of $CHCl_3$, 2% MeOH : $CHCl_3$, 4% MeOH : $CHCl_3$, 6% MeOH : $CHCl_3$, 8% MeOH : $CHCl_3$, 10% MeOH : $CHCl_3$, 20% MeOH : $CHCl_3$, 100% MeOH. This furnished the title compound in 46% yield (50mg, 10% MeOH : $CHCl_3$, $R_f = 0.34$ in 10% MeOH : $CHCl_3$).

FT IR: ν_{max} **(cm^{-1})**	3325 (m, NH), 2975 (m, CH), 1655 (s, CO).
^{1}H-NMR: δ_{ppm}	0.0 (6H, s, 2 x CH_3), 0.8 (12H, s, ^{t}Bu, t, CH_3-1', J = 7.4 Hz), 1.4 (2H, m, CH_2-2'), 1.9 (2H, m, CH_2-5, J = 5.9 Hz), 3.25 (1H, m, CH-3'), 3.4 (2H, m, CH_2-6), 3.6 (1H, m, CH-4), 5.9 (2H, br, NH_2), 6.45 (1H, br, NH), 6.65 (1H, br, NH).
^{13}C-NMR: δ_{ppm}	-4.63 (CH_3), 8.97 (CH_3-1'), 17.93 (Quat. of ^{t}Bu), 20.25 (CH_2-2'), 25.71 (CH_3), 37.33 (CH_2-6), 52.14 (CH-4), 73.99 (CH-1'), 154.98 (C=N).
MS (CI) : m/z	272 (85% [M^+]) daltons.
HRMS (ES^+) : m/z	$[C_{13}H_{30}N_3OSi]^+$ requires [M^+] 272.2158; found 272.2157 daltons.

2-Ethyl-2-hydroxy-4-oxohexanoic acid



[318]

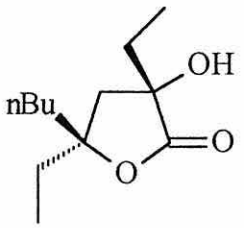
A solution of NaOH (1.96g, 49 mmol) in water (15 ml) was added dropwise to a cooled (0°C) solution of 2-oxobutyric acid (5g, 49 mmol) in water (10 ml). Butan-2-one (10.8g, 150 mmol) and a further portion of NaOH (2.72g, 68 mmol) in methanol (55 ml) were added sequentially and the reaction stirred overnight reaching RT. The reaction mixture was then neutralised with the addition of conc. H_2SO_4 with subsequent additions of H_2O (150 ml), salt (to saturation) and ethyl acetate (50 ml). After stirring for 10 mins, the product was extracted with ethyl acetate (3 x 100 ml) and the solvent removed *in vacuo*. The aqueous phase was then acidified further, more salt was addded to saturation and this was stirred again overnight at RT, after which it was extracted with ethyl acetate (3 x 100 ml). The combined organic phases were dried over $MgSO_4$ filtered and evaporated to give **[318]**. (7.48g, 80%).

FT IR: ν_{max} **(cm^{-1})** 3300 (br, OH), 1710 (s, C=O).

^{1}H-NMR: δ_{ppm} 0.94 (3H, t, CH_3, J = 7.5 Hz), 1.04 (3H, t, CH_3, J = 7.3 Hz), 1.74 (2H, m, CH_2), 2.47 (2H, t, CH_2, J = 7.3 Hz), 2.83 (1H, d, CH, J = 17.5 Hz), 3.10 (1H, d, CH, J = 17.5 Hz), 7.70 (2H, br s, OH, COOH).

^{13}C-NMR: δ_{ppm} 7.27 (CH_3), 7.36 (CH_3), 31.96 (CH_2), 36.70 (CH_2), 49.16 (CH_2), 76.10 (C), 174.20 (C=O), 179.50 (C=O).

(2S,4S)/(2R,4R)-4-Butyl-2,4-diethyl-2-hydroxybutyrolactone



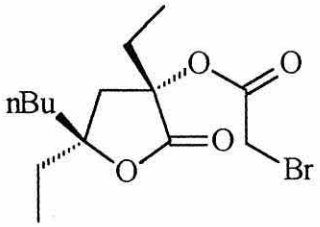
[319]

Half a solution of *n*-butyl bromide (4.45g, 32.48 mmol, 3.5 ml), in dry THF (14.8 ml) was added to magnesium turnings (0.78g, 32.48 mmol) *via* a droping funnel and the reaction refluxed for 5 mins. To this was added the remaining *n*-butyl bromide in THF over a period of 40 mins. After complete addition the reaction was refluxed for a further 40 mins.

The solution was then added dropwise to a cooled (0°C) solution of acid **[318]** (0.77g, 4.06 mmol) and stirred for 48 hours reaching RT. A solution of tartaric acid (9g) in water (50 ml) was then added and this was left to stir for a further one hour after which the product was extracted with diethyl ether (3 x 100 ml). The combined organic phases were washed with sodium carbonate (2 x 20 ml), dried over $MgSO_4$ and the solvent removed *in vacuo* to produce a crude oil. The aqueous layer was then re-acidified and extracted with a further portion of diethyl ether (2 x 50 ml) and dried over $MgSO_4$. The combined extracts were purified by column chromatography eluting with 20% diethyl ether : petroleum ether and 30% diethyl ether : petroleum ether which furnished the title compound in 68% yield (0.59g R_f = 0.17 in 30% diethyl ether : petroleum ether).

FT IR: ν_{max} **(cm^{-1})**	3450 (br, OH), 2943 (s, CH), 1764 (s, C=O)
^{1}H-NMR: δ_{ppm}	0.80-0.96 (9H, m, 3 x CH_3), 1.14-1.32 (5H, m, 2 x CH_2 / OH), 1.46-1.86 (6H, m, 3 x CH_2), 1.97 (1H, d, CH, J = 14.2 Hz), 2.07 (1H, d, CH, J = 14.2 Hz).
^{13}C-NMR: δ_{ppm}	7.58 (CH_3), 8.87 (CH_3), 13.90 (CH_3), 22.89 (CH_2), 25.49 (CH_2), 31.60 (CH_2), 31.68 (CH_2), 38.03 (CH_2), 41.81 (CH_2), 77.55 (C), 87.49 (C), 178.92 (C).
MS (CI) : m/z	214 (25% [M^+]) daltons.
HRMS (CI) : m/z	$C_{12}H_{26}NO_3$ requires [M^+] 232.1913; found 232.1910 daltons.

(2S,4R)/(2R,4S)-4-Butyl-2,4-diethyl-2-bromoacetoxybutyrolactone

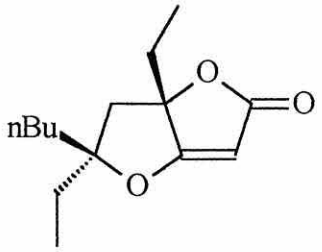


[320]

Dry pyridine (116.3mg, 1.47 mmol, 0.12 ml) was added to a cooled (0°C) solution of **[319]** (315mg, 1.47 mmol) in dry DCM (1.43 ml), followed by a catalytic amount of DMAP and BrAcBr (326mg, 1.62 mmol, 0.14 ml). This was allowed to stir for 48 hours whilst reaching RT. The reaction was then diluted with water (20 ml) and extracted with diethyl ether (3 x 30 ml). The combined organic phases were washed with saturated copper sulphate solution (2 x 25 ml), water (2 x 25 ml) and sodium hydrogen carbonate solution (2 x 30 ml). The organic phase was dried over $MgSO_4$ and the solvent removed *in vacuo*. The crude product was purified by column chromatography with a gradient elution of 5% diethyl ether : petroleum ether and 15% diethyl ether : petroleum ether this gave the title compound in 67% yield (330mg, R_f = 0.22 in 20% diethyl ether : petroleum ether).

FT IR: ν_{max} **(cm^{-1})**	2958 (s, CH), 1777 (s, C=O), 1742 (s, CO).
^{1}H-NMR: δ_{ppm}	0.81 (6H, m, 2 x CH_3), 1.0 (3H, t, CH_3, J = 7.3 Hz), 1.25 (4H, m, 2 x CH_2), 1.5-2.0 (6H, m, 3 x CH_2), 2.2 (1H, d, CH, J = 14.0 Hz), 2.25 (1H, d, CH, J = 14.0 Hz), 3.8 (2H, s, CH_2).
^{13}C-NMR: δ_{ppm}	7.49 (CH_3), 7.87 (CH_3), 13.88 (CH_3), 22.86 (CH_2), 25.51 (CH_2), 30.92 (CH_2), 31.10 (CH_2), 37.80 (CH_2), 40.54 (CH_2), 82.70 (C), 86.30 (C), 165.96 (C=O), 173.24 (C=O).
MS (CI) : m/z	352 (10% [M+NH_4^+]) daltons.
HRMS (CI) : m/z	$C_{14}H_{27}O_4NBr$ requires [M+NH_4^+] 352.1123; found 352.1121 daltons

(6S,8R)/(6R,8S)-6,8-Diethyl-6-butyl-1,5-dioxa-2-oxobicyclo[3.3.0]oct-3-ene

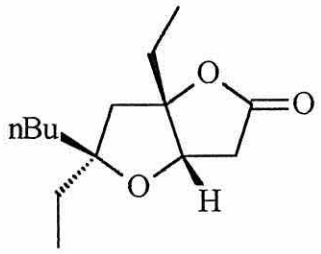


[321]

Triphenylphosphine (348 mg, 1.33 mmol) was added to a solution of **[320]** (340.7mg, 1.02 mmol) in dry acetonitrile (17 ml) and stirred for 80 mins at 40°C. After cooling to 0°C DBU (148mg, 0.97 mmol) was added slowly and the solution refluxed for a further 90 mins. The solvent was removed *in vacuo* and purified by column chromatography eluting with 10%-15% diethyl ether : petroleum ether which furnished the title compound in 68% yield (164.7mg, R_f = 0.23 in 15% diethyl ether : petroleum ether).

FT IR: ν_{max} **(cm^{-1})**	2960 (s, CH), 1752 (s, C=O), 1654 (s, C=C).
^{1}H-NMR: δ_{ppm}	0.79 (3H, t, CH_3, J = 7.5 Hz), 0.88 (6H, t, 2 x CH_3, J = 7.3 Hz), 1.25 (6H, m, 3 x CH_2), 1.7 (4H, dq, 2 x CH_2, J = 6.4 Hz), 1.85 (1H, d, CH, J = 12.8 Hz), 2.10 (1H, d, CH, J = 12.8 Hz), 4.9 (2H, s, CH_2).
^{13}C-NMR: δ_{ppm}	7.56 (CH_3), 7.72 (CH_3), 13.83 (CH_3), 22.87 (CH_2), 26.42 (CH_2), 32.83 (CH_2), 32.97 (CH_2), 37.49 (CH_2), 40.92 (CH_2), 88.08 (CH), 103.96 (C), 186.73 (C=O).
MS (CI) : m/z	276 (100% [M+NH_4^+]) daltons.
HRMS (CI) : m/z	$C_{16}H_{22}NO_3$ requires [M+NH_4^+] 258.1256; found 258.1250 daltons.

(*4R,6S,8R*)/(4S,6R,8S)-6,8-Diethyl-6-butyl-1,5-dioxa-2-oxobicyclo[3.3.0]oct-3-ene



[322]

Pd/C was added to a solution of **[321]** (100mg, 0.42 mmol) in ethyl acetate (4.2 ml). The reaction was flushed with H_2, stirred vigorously whilst under a H_2 environment and monitored by TLC. After 10 mins the reaction had gone to completion. The crude reaction mixture was filtered through a plug of Celite and silica and the solvent removed *in vacuo* to furnish the title compound in 90% yield (89.9mg, R_f = 0.27 in 20% diethyl ether : petroleum ether).

FT IR: ν_{max} **(cm^{-1})**	2975 (s, CH), 1775 (s, C=O).
^{1}H-NMR: δ_{ppm}	0.82 (3H, t, CH_3, J = 7.5 Hz), 0.85 (3H, t, CH_3, J = 7.5 Hz), 0.99 (3H, t, CH3, J = 7.5), 1.1-1.3 (4H, m, 2 x CH_2), 1.5 (4H, m, 2 x CH_2), 1.7 (2H, m, CH_2), 1.85 (1H, d, CH, J = 14.4 Hz), 2.2 (1H, d, CH, J = 14.4 Hz), 2.63(1H, d, CH, J = 14.5 Hz), 2.7 (1H, d, CH, J = 4.7 Hz), 4.3 (1H, dd, CH, J = 1.6, 4.7 Hz).
^{13}C-NMR: δ_{ppm}	8.38 (CH_3), 8.52 (CH_3), 13.99 (CH_3), 23.08 (CH_2), 26.00 (CH_2), 30.25 (CH_2), 31.31 (CH_2), 37.35 (CH_2), 38.10 (CH_2), 44.94 (CH_2), 80.46 (CH), 87.27 (C), 97.74 (C), 175.46 (C=O).
MS (CI) : m/z	258 (100% [M+NH_4^+]) daltons.
HRMS (CI) : m/z	$C_{14}H_{28}NO_3$ requires [M+NH_4^+] 241.1803; found 241.1800 daltons.

CHAPTER.9.

REFERENCES

1. C. L. Hannon, E. V. Anslyn, *Bioorganic Chemistry Frontiers*, 1993, **3**, 193.

2. J. A. Dean, *Lange's Handbook of Chemistry, 13th edn.*, Mc-Graw Hill, New York, 1985.

3. D. Voet and J. G. Voet, *Biochemistry*, Wiley, New York, 1990.

4. Y. Yamamoto, S. Kojima, *The Chemistry of Amidines and Imidates*, Vol. 2, John Wiley and Sons Ltd., 1991.

5. K. T. Leffek, P. Pruszynski, K. Thanapaalasingham, *Can. J. Chem.*, 1989, **67**, 590.

6. (a). W. Sawka-Dobrowolska, T. Glowiak, *Acta. Cryst.*, 1984, **C40**, 174. (b). K. Aoki, K. Nagano, Y. Iitaka, *Acta. Cryst.*, 1971, **B27**, 11. (c). D. M. Salunke, M. Vijayan, 1982, *Acta. Cryst.*, **B38**, 1328. (d). V. Sudhakar, M. Vijayan, 1980, *Acta. Cryst.*, **B36**, 120.

7. (a). P. J. Berthou, A. Laurent, S. Nakajima, 1976, *Acta. Cryst.*, **B32**, 1529. (b). T. Maeda, T. Fujiwara, K. I. Tomita, 1972, *Bull. Chem. Soc. Jpn.*, **45**, 3628. (c). E. G. Steward, D. Warner, G. Clark, 1974, *Acta. Cryst.*, **B30**, 813.

8. J. H. Bryden, L. A. Burkardt, E. W. Hughes, J. Donohue, *Acta. Cryst.*, 1956, **9**, 573.

9. G. R. Sullivan, J. D. Roberts, *J. Org. Chem.*, 1977, **42**, 1095.

10. M. Alleaume, A. Gulko, F. H. Herbstein, M. Kapon, R. E. Marsh, *Acta. Cryst.*, 1976, **B32**, 669.

11. A. Boyar, R. E. Marsh, *J. Am. Chem. Soc.*, 1982, **104**, 1995.

12. S. Rice, M. Y. Cheng, R. E. Cramer, M. Mandel, H. F. Mower and K. Seff, *J. Am. Chem. Soc.*, 1984, **106**, 239.

13. A. Fersht, Enzyme Structure and Mechanism, 2nd Edition, W. H. Freeman, New York.

14. F. A. Cotton, E. E. Hazen, Jr. and M. J. Legg, Proc. *Natl. Acad. Sci.*, 1979, **76**, 2551.

15. M. Marshal and P. P. Cohen, *J. Biol. Chem.*, 1980, **255**, 7301.

16. D. Picot, P. J. Loll and R. M. Garavito, *Nature*, 1994, **367**, 243.

17. B.R. Crane, A. S. Arvai, D. K. Ghosh, C. Wu, E. D. Getzoff, D. J. Stuehr and J. A. Tainer, *Science*, 1998, **279**, 2121.

18. (a). J. E. Butler-Ransohoff, D. A. Kendall, S. Freeman, J. R. Knowles and E. T. Kaiser, *Biochemistry*, 1988, **27**, 4777. (b). A. Chaidaroglou, D. J. Brezinski, S. A. Middleton and E. R. Kantrowitz, *Biochemistry*, 1988, **27**, 8338.

19. Y. Shimizu, M. Alam, Y. Oshima, L. J. Buckley, W. E. Fallon, H. Kasai, I. Miura, V. P. Gullo and K. Nakanishi, *Deleterious Dinoflagellate Toxins*, 261.require book number and code.

20. D. K. Bhattacharyya, M. Lecomte, C. J. Rieke, R. M. Garavito and W. L. Smith, *J. Biol. Chem.*, 1996, **271**, 2179.

21. (a). S. Moncada and A. Higgins, *N. Engl. J. Med.*, 1993, **329**, 2002. (b). H. H. Schmidt and U. Walter, *Cell*, 1994, **78**, 919. (c). C. Nathan, *J. Clin. Invest.*, 1997, **100**, 2417. (d). M. Marletta, *J. Biol. Chem.*, 1993, **268**, 12231. (e). O. W. Griffith and D. J. Stuehr, *Annu. Rev. Physiol.*, 1995, **57**, 707. (f). B. Mayer and E. R. Werner, *Nauyn-Schmiedeberg's Arch. Pharmacol.*, 1995, **351**, 453.

22. M. Sono, M. P. Roach, E. D. Coulter and J. H. Dawson, *Chem. Rev.*, 1996, **96**, 2841.

23. (a). G. M. Cragg, D. J. Newman and K. M. Snader, *J. Nat. Prod.*, 1997, **60**, 52. (b). A. L. Harvey and P. G. Waterman, *Curr. Op. Drug Discovery*, 1998, **1**, 71.

24. A. Mori, *Neuroscience*, 1983, **9**, 143.

25. R. G. S. Berlinck, *Natural Product Reports*, 1996, 377.

26. Y. Kishi, M. Arantani, T. Fukuyama, F. Nakatsubo, T. Goto, S. Inoue, H. Tanino, S. Sugiura and H. Kakoi, *J. Am. Chem. Soc.*, 1972, **94**, 9217.

27. H. A. Elliot, *J. Expt. Zool.*, 1934, **68**, 247.

28. D. Scheumack, M. E. H. Howden, I. Spence, R. J. Quinn, *Science*, 1978, **199**, 188.

29. L. A. Pavelka, Y. H. Kim, H. S. Moscher, *Toxicon*, 1977, **15**, 135.

30. D. Mebs, M. Yatsu-Yamashita, T. Yasumoto, S. Lötters and A. Schlűter, *Toxicon*, 1995, **33**, 246.

31. K. Kogure, H. K. Do, E. V. Thuessen, K. Nanba, K. Ohwada and U. Shimidu, *Mar. Ecol: Prog. Ser.*, 1988, **45**, 303.

32. (a). M. Yotsu, Y. Hayashi, S. S. Khora, S. Sato and T. Yasumoto, *Biosci. Biotech. Biochem.*, 1992, **56**, 370. (b). M. Yotsu-Yamashita, Y. Yamagishi and T. Yasumoto, *Tetrahedron Letters*, 1995, **36**, 9329.

33. N. Yasumoto amd M. Isobe, *Chem. Lett.*, 1994, 2299.

34. E. J. Schantz, J. D. Mold, D. W. Stanger, J. Shavel, F. J. Riel and H. Sommer, *J. Am. Chem. Soc.*, 1957, **79**, 5230.

35. Y. Shimizu, M. Alam, Y. Oshima, L. J. Buckley, W. E. Fallon, H. Kasai, I. Miura, V. P. Gullo and K. Nakanishi, *Deleterious Dinoflagellate Toxins*, 261.

36. (a). P. J. Murphy, H. L. Williams, D. E. Hibbs, M. B. Hursthouse and K. M. Abdul Malik, *Tetrahedron*, 1996, **52**, 8315. (b). P. A. Jacobi, M. J. Martinelli and S. Polanc, *J. Am. Chem. Soc.*, 1984, **106**, 5594.

37. J. L. Wong, R. J. Oesterlin and H. Rapoport, *J. Am. Chem. Soc.*, 1971, **93**, 7344.

38. E. J. Schantz, J. D. Mold, W. C. Howard, J. P. Bowden, D. W. Stanger, J. M. Lynch, O. P. Wintersteiner, J. D. Dutcher, D. R. Walters and B. Riegel, *Can. J. Chem.*, 1961, **39**, 2117.

39. G. R. Strichartz, S. Hall, B. Magnani, C. Y. Hong, Y. Kishi and J. A. Debin, *Toxicon*, 1995, **33**, 723.

40. S. Matsunaga, R. E. Moore and W. P. Niemczura, *J. Am. Chem. Soc.*, 1989, **111**, 8021.

41. (a). N. A. Mahmood and W. W. Carmichael, *Toxicon*, 1987, **25**, 1221. (b). W. O. Cook, V. R. Beasley, A. M. Dahlem, J. A. Dellinger, K. S. Harlin and W. W. Carmichael, *Toxicon*, 1988, **26**, 750.

42. P. R. Hawkins, M. T. C. Runnegar, A. R. B. Jackson and I. R. Falconer, *Applied and Environmental Micobiology*, 1985, **50**, 1292.

43. (a). S. P. Chu, *J. Ecol.*, 1942, **30**, 284. (b). R. Y. Stanier, M. Kunisawa, M. Mandel and G. Cohen-Bazire, *Bacteriol. Rev.*, 1971, **35**, 171.

44. I. Ohtani, R. E. Moore and M. T. C. Runnegar, *J. Am. Chem. Soc.*, 1992, **114**, 7941.

45. K. Harada, I. Ohtani, K. Iwamoto, M. Suzuki, M. F. Watanabe, M. Watanabe and K. Terao, *Toxicon*, 1994, **32**, 73.

46. R. Banker, S. Carmeli, O. Hadas, B. Teltsch, R. Porat and A. Sukenik, *J. Phycol*, 1997, **33**, 613.

47. (a). M. T. Runnegar, S. M. Kong, Y. Z. Zhong, J. L. Ge and S. C. Lu, *Biochem. And Biophys. Res. Comm.*, 1994, **201**, 235. (b). M. T. Runnegar, S. M. Kong, Y. Z. Zhong and S. C. Lu, *Biochem. Pharmacol.*, 1995, **49**, 219.

48. G. R. Heintzelman, M. Parvez and S. M. Weinreb, *Synlett*, 1993, 551.

49. A. I. Meyers, P. D. Edwards, W. F. Rieker and T. R. Bailey, *J. Am. Chem. Soc.*, 1984, **106**, 3270.

50. G. R. Heintzelman, M. Parvez and S. M. Weinreb, *J. Org. Chem.*, 1996, **61**, 4594.

51. B. B. Snider and T. C. Harvey, *Tetrahedron Letters*, 1995, **36**, 4587.

52. P. R. Atkins, S. E. J. Glue and I. T. Kay, *J. Chem. Soc., Perkins Trans. 1.*, 1973, 2644.

53. B. B. Snider and C. Xie, *Tetrahedron Letters*, 1998, **39**, 7021.

54. R. Yamaguchi, Y, Nakazono and M. Kawanisi, *Tetrahedron Letters*, 1983, **24**, 1801.

55. H. Gershon, *J. Org. Chem.*, 1962, **27**, 3507.

56. I. J. McAlpine and R. W. Armstrong, *Tetrahedron Letters*, 2000, **41**, 1849.

57. Y. Kashman, S. Hirsh, O. J. McConnell, I. Ohtani, T. Kusumi and H. Kakisawa, *J. Am. Chem. Soc.*, 1989, **111**, 8926.

58. E. Palagiano, S. DeMarino, L. Minale, R. Riccio, F. Zollo, M. Iorizzi, J. B. Carré, C. Debitus, L. Lucarain and J. Provost, *Tetrahedron Letters*, 1995, **51**, 3675.

59. A. D. Patil, W. C. Kokke, J. Faulkner, J. W. Westley and B. C. M. Potts, *J. Org. Chem.*, 1995, **60**, 1182.

60. I. Ohtani, T. Kusumi, H. Kakisawa, Y. Kashman and S. Hirsh, , *J. Org. Chem.*, 1992, **114**, 8472.

61. B. B. Snider and Z. Shi, *Tetrahedron Letters*, 1993, **34**, 2099.

62. B. B. Snider and Z. Shi, *J. Am. Chem. Soc.*, 1994, **116**, 549.

63. L. E. Overman, M. H. Rabinowitz and P. A. Renhowe, *J. Am. Chem. Soc.*, 1995, **117**, 2657.

64. L. E. Overman and M. H. Rabinowitz, *J. Org. Chem.*, 1993, **58**, 3235.

65. P. J. Murphy and H. L. Williams, *J. Chem. Soc., Chem. Comm.*, 1994, 819.

66. (a). S. Louwrier, M. Ostendorf, A. Tuynman and H. Hiemstra, *Tetrahedron Letters*, 1996, **37**, 905. (b). S. Louwrier, M. Ostendorf, A. Boom, H. Hiemstra and W. N. Speckamp, *Tetrahedron*, 1996, **52**, 2603. (c). S. Louwrier, A. Tuynman and H. Hiemstra, *Tetrahedron Letters*, 1996, **52**, 2629.

67. E. A. Jares-Erijman, R. Sakai and K. L. Rinehart, *J. Org. Chem.*, 1991, **56**, 5712.

68. R. Tavares, D. Daloze, J. C. Braekman, E. Hajdu, G. Muricy and R. W. M. van Soest, *Biochem. Syst. Ecol.*, 1994, **22**, 645.

69. E. A. Jares-Erijman, A. L. Ingrum, J. R. Carney, K. L. Rinehart and R. Sakai, *J. Org. Chem.*, 1993, **58**, 4805.

70. A. L. Grillot and D. J. Hart, *Tetrahedron*, 1995, **51**, 11377.

71. D. J. Capon and R. H. R. Ward, *Annu. Rev. Immunol.*, 1991, **9**, 649.

72. A. D. Patil, N. V. Kumar, W. C. Kokke, M. F. Bean, A. J. Freyer, C. De Brosse, S. Mai, A. Truneh, D. J. Faulkner, B. Carte, A. L. Breen, R. P. Hertzberg, R. K. Johnson, J. W. Westley and B. C. M. Potts, *J. Org. Chem.*, 1995, **60**, 1182.

73. G. P. Black, P. J. Murphy, N. D. A. Walshe, D. E. Hibbs, M. B. Hursthouse and K. M. Abdul Malik, *Tetrahedron Letters*, 1996, **37**, 6943.

74. B. B. Snider, J. Chen, *Tetrahedron Letters*, 1996, **37**, 6977.

75. (a). B. B. Snider and J. Chen, *Tetrahedron Letters*, 1998, **39**, 5697. (b). Y. F. Yong, J. A. Kowalski and M. A. Lipton, *J. Org. Chem.*, 1997, **62**, 1540.

76. (a). B. B. Snider and Z. Shi, *J. Org. Chem.*, 1992, **57**, 2526. (b). B. B. Snider and Z. Shi, *J. Org. Chem.*, 1993, **58**, 3828.

77. A. S. Franklin, S. K. Ly, G. H. Mackin, L. E. Overman and A. J. Shaka, *J. Org. Chem.*, 1999, **64**, 1512.

78. A. I. McDonald and L. E. Overman, *J. Org. Chem.*, 1999, **64**, 1520.

79. A. D. Patil, A. J. Freyer, P. B. Taylor, B. Carté, G. Zuber, R. K. Johnson and D. J. Faulkner, *J. Org. Chem.*, 1997, **62**, 1814.

80. R. L. Jarvest, A. L. Breen, C. M. Edge, M. A. Chaikin, L. J. Jennings, A. Truneh, R. W. Sweet and R. P. Hertzberg, *Bioorg. Med. Chem. Lett.*, 1993, **3**, 2851.

81. H. Lloyd Williams, PhD thesis, UWB, 1996 and G. P. Black, PhD thesis, UWB, 1998.

82. C. K. McClure, K-Y. Jung, *J. Org. Chem.*, 1991, **56**, 2326.

83. D. Taylor and J. F. Wolf, *J. Chem. Soc., Chem. Commun.*, 1972, 876.

84. J. Fakstorp, D. Raleigh and L. E. Schniepp, *J. Am. Chem. Soc.*, 1950, **72**, 869.

85. B. B. Snider and M. V. Busuyek, *J. Nat. Prod.*, 1999, **62**, 1707.

86. G. P. Black, P. J. Murphy, A. J. Thornhill, N. D. A. Walshe and C. Zanetti, *Tetrahedron*, 1999, **55**, 6547.

87. C. G. Moore, PhD thesis, UWB, 1999.

88. W. Lehnert, *Tetrahedron*, 1972, **72**, 663.

89. M. Bella, Erasmus report August 1998, UWB.

90. P. Garner and J. M. Park, *Syn. Comm.*, 1987, 189.

91. (a). A. Hizi and S. Loya, Department of Cell Biology and Histology, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel. (b). A. T. McGown, Christie

CRC Research Centre, Paterson Institute for Cancer Research, Christie NHS Trust, Wilmslow Road, Manchester, M20 4BX.

92. J. B. Brown, H. B. Henbest and E. R. H. Jones, *J. Chem. Soc.*, 1950, 3634.

93. S. Jones, MSc. thesis, UWB, 1999

94. C. Gravier-Pelletier, D. Bourissou, Y. Le Merrer and J-C. Depezay, *Synlett*, 1996, 275.

95. Y. Le Merrer, L. Gauzy, C. Gravier-Pelletier and J-C. Depezay, *Bioorg and Med. Chem.*, 2000, **8**, 307.

96. L. Poitout, Y. Le Merrer and J-C. Depezay, *Tetrahedron Letters*, 1994, **35**, 3293.

97. Y. Bleriot, A. Genre-Grandpierre and C. Tellier, *Tetrahedron Letters*, 1994, **35**, 1867.

98. G. B. Karlsson, T. D. Butters, R. A. Dwek and F. M. Platt, *J. Biol. Chem.*, 1993, **268**, 570.

99. B. Winchester and G. W. Fleet, *Glycobiology*, 1992, **2**, 199.

100. G. Legler, *Adv. Carbohydr. Chem. Biochem.*, 1990, **48**, 319.

101. M. L. Sinnott, *Chem. Rev.*, 1990, **90**, 1171.

102. C. H. Fotsch and C-H. Wong, *Tetrahedron Letters*, 1994, **35**, 3481.

103. C. G. Look, C. H. Fotsch and C-H. Wong, *Acc. Chem. Res.*, 1993, **26**, 182.

104. E. Truscheit, W. Frommer, B. Junge, L. Müller, D. D. Schmidt and W. Wingender, *Angew. Chem. Int. Ed. Engl.*, 1981, **20**, 744.

105. A. D. Elbein, *Ann. Rev. Biochem.*, 1987, **56**, 497.

106. P. M. Schwarz and A. D. Elbein, *J. Biol. Chem.*, 1985, **26**, 14452.

107. (a). R. A. Gruters, J. J. Neefjes, M. Tersmette, R. E. Y. De Goede, A. Tulip, H. G. Huisman, F. Miedena and H. L. Ploegh, *Nature*, 1987, 330, 74. (b). A. Karpas, G. W. J. Fleet, R. A. Dwek, S. Petursson, S. K. Namgoong, N. G. Ramsden, G. S. Jacob and T. W. Rademacher, *Proc Natl. Acad. Sci. USA*, 1988, **85**, 9229.

108. R. C. Bernotas, M. A. Pezzone and B. Ganem, *Carbohydrate Res.*, 1987, **167**, 305.

109. G. Papandreou, M. K. Tong and B. Ganem, *J. Am. Chem. Soc.*, 1993, **115**, 11682.

110. A. W-Y. Chan and B. Ganem, *Tetrahedron Letters*, 1995, **36**, 811.

111. (a). L. E. Fellows, *Chem Brit.*, 1987, **23**, 842. (b). L. E. Fellows, *New Scientist*, 1989, **123**, 45.

112. Y. T. Pan, G. P. Kaushal, G. Papandreou, B. Ganem, A. D. Elbein, *J. Biol. Chem.*, 1992, **267**, 8313.

113. (a). N. Sharon and H. Lis, *Sci. Amer.*, 1993, **268(1)**, 82. (b). T. A. Springer, *Cell*, 1994, **78**, 301. (c). T. A. Springer, *Nature*, 1990, **346**, 425. (d). B. N. Cronstein and G. Weissmann, *Arthritis Rheum.*, 1993, **36**, 147.

114. A. Echavarren, A. Galán, J. De Mendoza, A. Salmerón and J-M. Lehn, *Helv. Chimica Acta*, 1988, **71**, 685.

115. R. C. Bernotas and B. Ganem, *Tetrahedron Letters*, 1984, **25**, 165.

116. B. B. Lohray, Y. Jayamma and M. Chatterjee, *J. Org. Chem.*, 1995, **60**, 5958.

117. X. Qian, F. Morís-Varas, M. C. Fitzgerald and CH. Wong, *Bioorg and Med. Chem.*, 1996, **4**, 2055.

118. J. Piró, M. Rubiralta, E. Giralt and A. Diez, *Tetrahedron Letters*, 1999, **40**, 4865.

119. J. M. McIntosh and L. C. Matassa, *J. Org. Chem.*, 1988, **53**, 4452.

120. R. Nash and A. Winters, IGER/MNL Glycosidase Assays.

121. A. D. Patil, A. J. Freyer, M. F. Bean, B. K. Carte, J. W. Westley and R. K. Johnson, *Tetrahedron*, 1996, **52**, 377.

122. (a). P. J. Murphy and S. T. Dennison, Tetrahedron, 1993, 49, 6695, (b). C. Cagnolini, M. Ferri, P. R. Jones, P. J. Murphy, B. Ayres and B. Cox, *Tetrahedron*, 1997, **53**, 4815.

123. (a). C. Bittner, Erasmus report 1998, (b). A. Burgo, Erasmus report 1998 and (c). C. H. Sung, Bangor UG project 1997.

124. C. Bittner, A. Burgo, C. H. Yund, A. Thornhill and P.J. Murphy, *Tetrahedron Letters*, 1999, **40**, 3455.

125. (a). G. Leclerc, C. G. Wermuth and J. Schreiber, Bull. Chem. Soc. France., 1967, 1302. (b). M. Klaren-De Wit, D. J. frost and J. P. Ward, *Recl. Trav. Chim. Pays-Bas.*, 1971, **90**, 1207.

126. M. Casey, J. Leonard, B. Lygo and G. Procter, *Advanced Practical Organic Chemistry*, Blackie Academic & Professional, Glasgow, London, New York (1990).

127. M. Kawaguchi, O. Hayashi, N. Sakai, M. Hamada, Y. Yamamoto and J. Oda, *Agric. Biol. Chem.*, 1986, **50**, 3107.

128. C. Spino and N.Barriault, *J. Org. Chem.*, 1999, **64**, 5292.