DOCTOR OF PHILOSOPHY

Synthetic studies towards guanidine alkaloids

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SYNTHETIC STUDIES TOWARDS GUANIDINE ALKALOIDS

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by

Gregory Philip Black

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To Alice Marie

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CONTENTS

ABSTRACT

This thesis describes work performed towards developing a synthetic approach to the marine alkaloid Batzelladine A and also towards a template system for another marine alkaloid, Ptilomycalin A.

a) A reductive guanidine addition-cyclisation process is employed to prepare a tricyclic model compound for the tricyclic core of Batzelladine A. The *bis*-α,β-unsaturated precursor, (3*E*,7*E*)-2,9-dioxooctadeca-3,7-diene is prepared from succinaldehyde by a Wittig approach. The tricyclic guanidine compound is obtained as a single diastereoisomer and the relative stereochemistry is assigned by nOe experiments and X-ray diffraction analysis of a symmetrical model compound.

b) An ester-substituted precursor to guanidine addition is prepared *via* a somewhat problematic Knoevenagel condensation. The attempted preparation of a *tert*-butyl ester derivative of the tricyclic guanidine core of Batzelladine A is also described.

c) This methodology is modified to enable the preparation of the left-hand tricyclic portion of the marine alkaloid Batzelladine F.

d) The guanidine addition-cyclisation process is employed in the diastereospecific synthesis of a hexacyclic guanidine compound to assess the viability of this route for the preparation of an advanced intermediate for a Ptilomycalin A mimic. The compound incorporates two tetrahydropyranyl spiro *N*,*O*-acetal units and possesses the same relative stereochemistry as Ptilomycalin A, determined by X-ray crystallography. Studies towards a pyrrole-fused system are also described.

ABBREVIATIONS

Å	angstrom(s)
AcCl	acetyl chloride
Ac_2O	acetic anhydride
AcOH	acetic acid
AIDS	acquired immune deficiency syndrome
All	allyl
AMP	adenosine monophosphate
AP	alkaline phosphatase
Arg	arginine
Asp	aspartic acid
AZT	3'-azido-3'-deoxythymidine
Bn	benzyl
BOC	*tert*-butoxycarbonyl
B.pt.	boiling point
br	broad
Bu	butyl
cat.	catalytic
^{o}C	degrees Celsius
CBZ	benzyloxycarbonyl
CI	chemical ionisation
COLOC	correlated spectroscopy for long-range coupling
COSY	correlated spectroscopy
d	doublet or days
Δ	reflux
δ	chemical shift
dAA	deoxyadenosyladenosine
DCC	dicyclohexylcarbodiimide
d.e.	diastereomeric excess

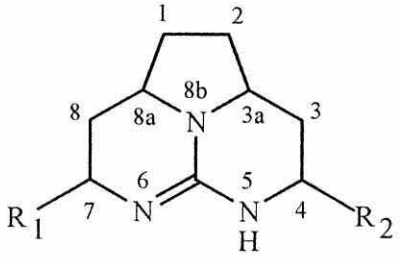
DEPT	distortionless enhancement by polarisation transfer
DIBAL-H	diisobutylaluminium hydride
DIPEA	diisopropylethylamine
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DMB	3,5-dimethoxybenzyl
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
E	entgegen
EDCI	1-ethyl-3-[3-(dimethylamino)-propyl]-carbodiimide hydrochloride
ELISA	enzyme-linked immunosorbent assay
equiv.	mole equivalent(s)
Et	ethyl
EtOAc	ethyl acetate
FABMS	fast-atom bombardment mass spectrometry
FGI	functional group interconversion
FT-IR	Fourier transform infra-red spectroscopy
g	gram(s)
Glu	glutamic acid
gp	glycoprotein
h	hour(s)
HIS	histidine
HIV	human immunodeficiency virus
HMBC	heteronuclear multiple bond correlation
HOHAHA	homonuclear Hartmann-Hahn spectroscopy
HRMS	high resolution mass spectroscopy
HSV	herpes simplex virus
Hz	hertz
^{i}Bu	isobutyl
IC_{50}	concentration at which 50% of

	target cells are inhibited
Im	imidazole
^{i}Pr	isopropyl
J	coupling constant
JACS	Journal of The American Chemical Society
JOC	Journal of Organic Chemistry
l	litre
LDA	lithium diisopropylamide
M	molar
m	multiplet or medium intensity
Me	methyl
MeOTf	methyl trifluoromethanesulphonate
MHz	megahertz
MIC	minimum inhibitory concentration
min	minute(s)
ml	millilitre(s)
mm Hg	millimetres of mercury
mmol	millimole(s)
mol	mole(s)
M.pt.	melting point
N	normal
ν	wavenumber(s)
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
NOESY	correlated nuclear Overhauser effect spectroscopy
PCC	pyridinium chlorochromate
Ph	phenyl
Phe	phenylalanine
ppm	parts per million
PMB	4-methoxybenzyl
Pr	propyl

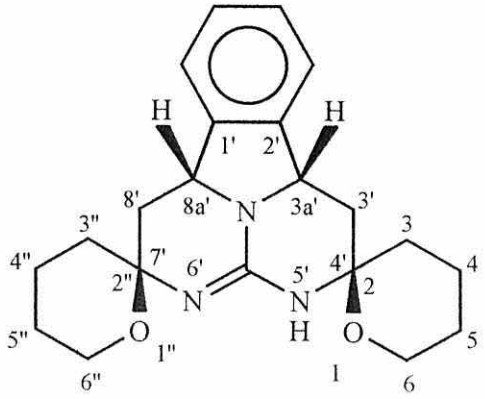
q	quartet
quant.	quantitative
R_f	retention factor
RNA	ribonucleic acid
ROESY	through-space correlated nuclear Overhauser effect spectroscopy
RT	room temperature
s	singlet or strong intensity
Ser	serine
SES	(trimethylsilyl)ethyl sulphone
SNase	staphylococcal nuclease
t	triplet
TBAB	tetra-*n*-butylammonium bromide
TBAF	tetra-*n*-butylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
^{t}Bu	*tert*-butyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TL	Tetrahedron Letters
TMS	trimethylsilyl
TMSOTf	trimethylsilyl trifluoromethanesulphonate
TOCSY	total correlated spectroscopy
TOSMIC	*para*-toluenesulphonylmethyl isocyanide
Trp	tryptophan
Ts	*para*-toluenesulphonyl
TsCl	*para*-toluenesulphonyl chloride
TsOH	*para*-toluenesulphonic acid
2D NMR	2-dimensional nuclear magnetic resonance
Val	valine
w	weak intensity
Z	zusammen

NOMENCLATURE

The numbering system used to name the guanidine compounds described in this thesis is based on the acenaphthylene ring system and is as follows:



The nomenclature of the guanidine compound incorporating spiro *N,O*-acetal rings is based on the acenaphthylene ring system, but is numbered in the following way:



All NMR assignments are made on the basis of the numbering scheme illustrated above, unless otherwise stated.

CHAPTER 1

THE BIOLOGICAL ROLE OF GUANIDINE

1.1 OVERVIEW

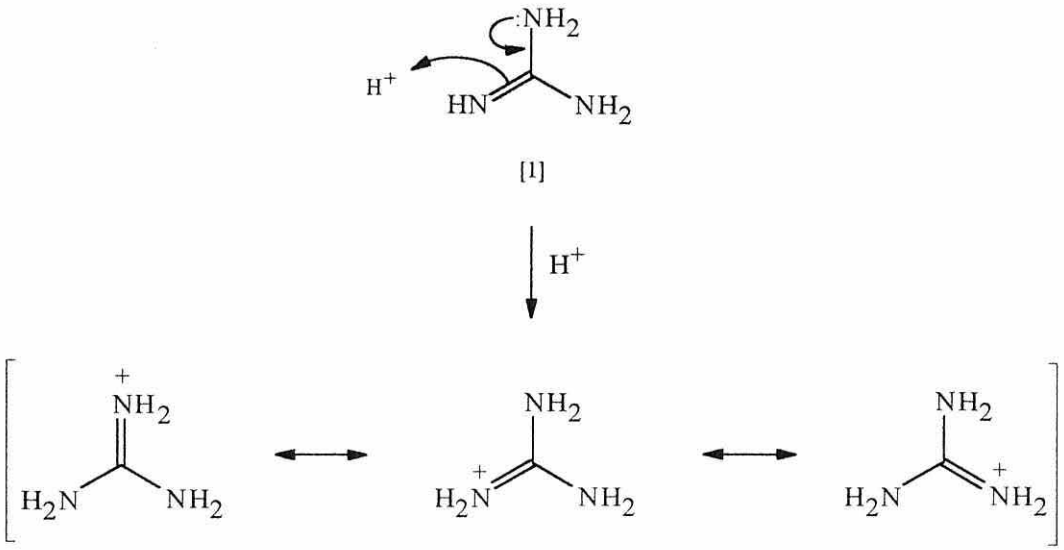
The guanidinium ion has played a key role in the development of anion recognition studies due to its ability to hydrogen-bond and mediate oxoanionic interactions with a variety of guest molecules. During the past decade in particular, an increasing number of novel guanidine natural products have been isolated from a variety of organisms. This has encouraged the development of synthetic receptors which mimic the role of the guanidinium moiety in the natural products.

The analogy which is relied upon greatly in the field of molecular recognition is the "lock-and-key" principle. Molecular recognition, especially in a biological environment, requires a very high degree of complementarity. For example, in the binding of proteins to DNA, or a specific chemical reaction at the active site of an enzyme. Thus, only one specific molecule (the key or guest) can fit the large molecule (the lock or host). However, a rigid lock-and-key analogy is a rather simplified approach since most biological systems (*e.g.* enzymes) have a high degree of flexibility in the lock. Enzymes act as catalysts in complex multi-step reactions and this flexibility allows the active site of the enzyme to conform to and stabilise each of the transition states involved in such a process.

This relatively new but rapidly expanding field involves the study of small molecules which mimic the activity of larger biomolecules. Unlike complex natural systems, the model compounds can easily be studied using familiar techniques such as spectroscopy. An excellent review article on the biological role of guanidine was published recently by Hannon and Anslyn.[1]

1.2 STRUCTURE OF THE GUANIDINIUM ION

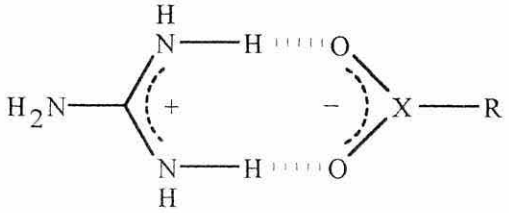
Guanidine itself is one of the strongest organic bases known, the pK_a of the conjugate acid (guanidinium ion) being 13.5 in water.[2] The positive charge on the guanidinium ion is delocalised over the three nitrogen atoms, thus increasing the stability. The protonation of guanidine [1] and the three resonance forms of the guanidinium ion are shown in Fig.1.



[1]

Figure 1. The resonance forms of the guanidinium ion

Using valence bond theory, Pauling[3] has estimated that the guanidinium ion is 6-8 kcal/mol more stable than the free base itself. The basicity of substituted guanidines has been extensively studied. Simple alkyl derivatives are strong bases,[4] and the ubiquitous amino acid arginine has pK_a=13.5 in water.[5] When the guanidine is conjugated with a phenyl or acyl group, pK_a values can drop by several orders of magnitude as a result of resonance into the aromatic ring and amide bond respectively.[6] The guanidinium ion is quite hydrophilic, making it suitable for aqueous biological media and the resonance stabilisation leads to the strong hydrogen-bonding interaction of guanidinium ion with dioxygen containing anions. It is this key interaction (Fig. 2) that is the basis of anionic recognition observed in naturally occurring host molecules (enzymes) and synthetic receptor systems.



X = C or P, R = $(-O)_2$ or -R

Figure 2. Interaction of guanidinium ion with dioxygen anions

1.3 BIOLOGICAL FUNCTIONS

The role of the guanidine motif and some applications in host-guest chemistry are discussed below.

1.3.1 STAPHYLOCOCCAL NUCLEASE

The enzyme Staphylococcal nuclease (SN) has been shown to catalyse the hydrolysis of the 5'-phosphodiester linkage in RNA and DNA (Fig. 3). The enzyme increases the rate of the reaction by a remarkable factor of 10^{16}. SN is a Ca^{2+}-dependent enzyme containing 149 amino acids as a linear polypeptide.

The mechanism of action was elucidated by crystallographic studies in 1979 by Cotton, *et al.*,[7] and the same model is in use today. The structure revealed that two water molecules are hydrogen-bonded to the Ca^{2+} ion, although the current belief is that only one of these water molecules is involved in the mechanism. The mechanism proposed by Cotton involves Glu-43 assisting the nucleophilic attack of water on the 5' phosphate, forming a trigonal bipyramidal transition state and phosphorane intermediate. The line of attack puts the 5'-OR group in an apical position and makes it a good leaving group. The guanidinium moieties of Arg-35 and Arg-87 both form hydrogen-bonds to the 5' phosphate, with additional hydrogen-bonding to the SN polypeptide. The ionic bond which forms between Ca^{2+} ion and the phosphate, as well as the guanidinium hydrogen-bonds are thought to stabilise the negative charge on phosphate as the nucleophile approaches. The charge neutralisation and bond polarisation both contribute to a lowering in energy of the transition state. The displacement of 5'-OR requires a proton whose most likely source is Arg-87; this enables the leaving group to be alcohol rather than a less favourable alkoxide ion.

Gorenstein[8] has postulated that the cleavage of the 5' O-P bond is governed by stereoelectronic factors. In his mechanism, the 5' and 3' phosphate ester bonds are held in different conformations by the guanidiniums of Arg-35 and Arg-87. Fitting this in with the crystal structure evidence, it is found that the 5' ester bond is *trans* and the 3' ester bond is *gauche*. Thus only the 5' bond has the antiperiplanar lone pair of electrons necessary for the elimination.

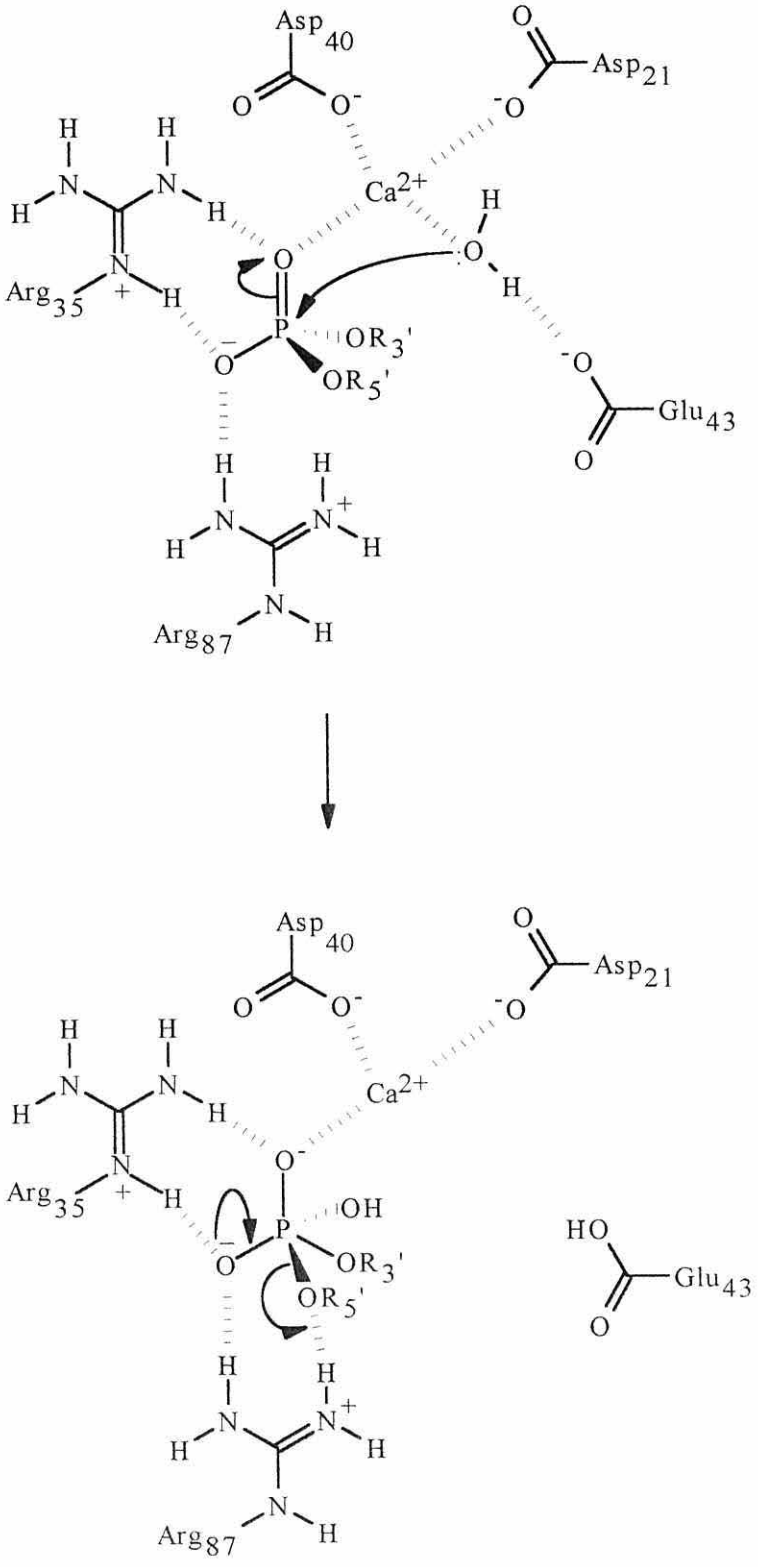


Figure 3. DNA hydrolysis at the active site of Staphylococcal Nuclease

1.3.2 ALKALINE PHOSPHATASE

Alkaline phosphatase (AP) is a non-specific phosphomonoesterase which contains two Zn^{2+} and one Mg^{2+} ion in each monomer (Fig. 4). The enzyme is dimeric, and produces inorganic phosphate or transfers phosphoryl groups between alcohols. The key amino acids at the active site are serine (Ser-102), and arginine (Arg-166). Recent X-ray diffraction studies by Kim and Wyckoff[9] have given some insight into the mode of action of this enzyme. The three metal ions mentioned above are in close proximity, and both zinc centres coordinate to two different oxygen atoms of the phosphate. The other two phosphate oxygens are tightly hydrogen-bonded to the guanidinium sub-unit of Arg-166, which provides orientation of the substrate and stabilisation of the intermediates. It has been shown that it is not essential for catalysis, but does impart a considerable advantage.[10]

The current trend is towards a mechanism which involves a phosphoranyl serine intermediate. The phosphate bridges both zinc ions and is linked to the magnesium ion *via* a water hydrogen-bond. The total charge in the pocket above the phosphate is 7+, creating a very electrophilic environment for anionic binding to the phosphate. The subsequent hydrolysis of the phosphate occurs with retention of configuration, indicating a probable double inversion of stereochemistry in the two-step process.[11]

Remembering the structure of the previous phosphodiesterase (SN), it is worth noting the absence of a general acid to protonate the leaving group, although Zn^{2+} ion is thought to assist this. Also absent is the base to deprotonate the zinc-bound serine. However, a water molecule near the Mg^{2+} coordination sphere could effect the proton transfer.[9]

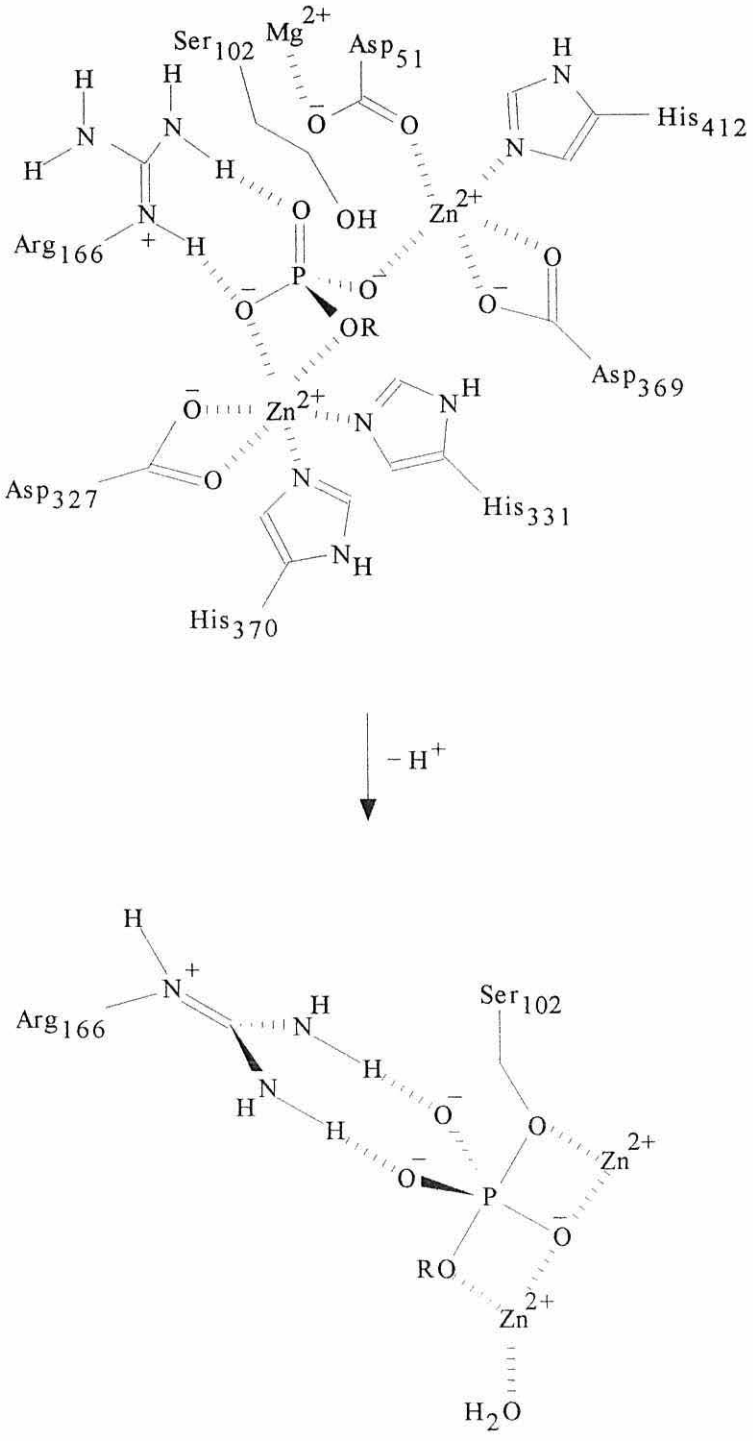


Figure 4. The phosphate coordination environment in alkaline phosphatase

1.3.3 ARGININE-DEPENDENT ENZYMES

As discussed above, arginine plays an important role in most of the enzymes that bind anionic substrates. These enzymes are not discussed individually here but include bovine carboxypeptidase, glycolyte enzymes and kinases among many others. The discovery that 1,2-dicarbonyl compounds are specific to arginine to the exclusion of other amino acids led to a variety of experiments designed to determine the role of the arginine. Yankeelov[12] used 2,3-butanedione to block the activity of the guanidinium moiety of arginine. This process was mediated by a phosphate buffer at pH 8.2. Later work by Riordan[13] showed that a borate buffer increased the rate of reaction as well as stabilising the product. Takahashi[14] has described the use of ^{14}C-labelled phenylglyoxal to bind with arginine for quantitation purposes. Phenylglyoxal normally forms a 2:1 adduct with arginine, but depending on steric and concentration factors, this ratio can be reduced.

Incubation of an enzyme with its substrate, substrate analog or inhibitor prior to treatment with the 1,2-dicarbonyl compound can give information about the binding site. If the 1,2-dicarbonyl compund reacts in the absence of the substrate but not in the presence of the substrate, then it is clear that the substrate is bound to the arginine, thereby blocking the binding to arginine. If the substrate does not inhibit the binding, this suggests a second active site, or that arginine is not essential for binding.

Site-directed mutagenesis can also provide useful information about the binding and the active site in an enzyme. This often involves substituting lysine or alanine residues for the arginine. Modification to lysine maintains a positive charge at the active site, whereas modification to alanine results in complete loss of positive charge. These results can be used to determine the extent of electrostatic involvement and structural requirement for binding and therefore information about the mode of action of the enzyme.

CHAPTER 2

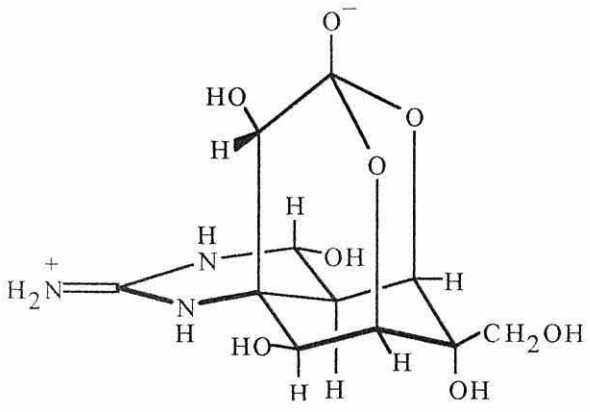
GUANIDINE NATURAL PRODUCTS

2.1 OVERVIEW

The emergence of numerous unique molecular architectures from marine sources has undoubtedly sparked the current interest in these fascinating products of nature. The majority of the more elaborate natural products that contain guanidine are of marine origin and they are worthy synthetic targets due to their extensive range of biological properties. Although simple linear guanidine derivatives have been known for several decades, the development of advanced analytical techniques has led to the isolation and elucidation of more complex structures. Many of the linear compounds are derived from the amino acid arginine, the importance of which was discussed in the previous chapter. It is not practical to discuss every natural product containing guanidine, but a representative selection of more recent discoveries is given. Tetrodotoxin [2] and saxitoxin [3] are included for their historical significance. For an account of the early guanidine derivatives, the reader is referred to a review by Chevelot.[15]

2.2 TETRODOTOXIN AND SAXITOXIN

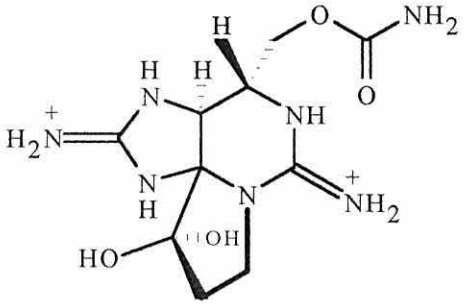
Tetrodotoxin [2] was first obtained from the California newt, *Taricha torosa* in 1934.[16] It was also found to be present in the liver and ovaries of the Japanese Tiger Puffer fish *Sphoeroides rubripes*[17] and in later years was isolated from three other sources.[18-20] The elaborate structure of [2] was simultaneously elucidated in 1964 by three research groups.[21-23] It has been shown to be a potent neurotoxin which acts as a highly specific sodium-channel blocker.[24] It is one of the most toxic non-protein based substances known with a lethal dose of 8-20μg/kg in mice.[25]



[2]

The first total synthesis was accomplished in 1972 by Kishi and co-workers.[26] The long history of research into tetrodotoxin has established that this molecule and its derivatives have bacterial origins.[16b]

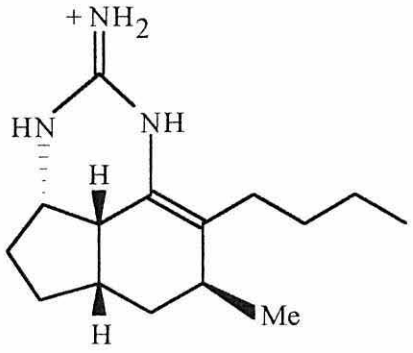
Saxitoxin [3], the paralytic agent of the Alaska butter clam *Saxidomas giganteus*, is also an extremely toxic non-protein alkaloid. The physiological activity of saxitoxin arises from an increase in the sodium ion permeability normally associated with excitation. It was isolated in 1957 by Schantz *et al.*,[27] and extensive structural studies by Rapoport[28] and Schantz[29] were finally concluded in 1975 when both groups obtained crystalline derivatives of saxitoxin. The structure was deduced by X-ray diffraction[30-31] to be that shown below. The total synthesis of saxitoxin was reported first by Kishi in 1977[32] and *via* a similar route by Jacobi in 1984.[33]



[3]

2.3 PTILOCAULIN

Ptilocaulin [4], obtained from the Caribbean marine sponge *Ptilocaulis spiculifer*, was elucidated in 1981 by Rinehart, *et al.*[34] The structure was assigned on the basis of NMR data and X-ray crystallography. It was found that ptilocaulin exhibited a range of bioactivities, including $IC_{50} = 0.39\mu g/ml$ against L1210 leukaemia cells and the following antimicrobial MICs (μg/ml): *Streptococcus pyogenes*, 3.9; *S. pneumoniae*, 15.6; *S. faecalis*, *Staphylococcus aureus* and *Escherichia coli*, 62.5. An isomer of [4] (isoptilocaulin) was also isolated, but found to be slightly less biologically active. The biogenetic origin of [4] would appear to be addition of guanidine to a polyketide. A synthesis of (±)-[4] was reported in 1983[35] and shortly afterwards, the enantioselective synthesis of the unnatural (-)-isomer was achieved.[35, 36]



[4]

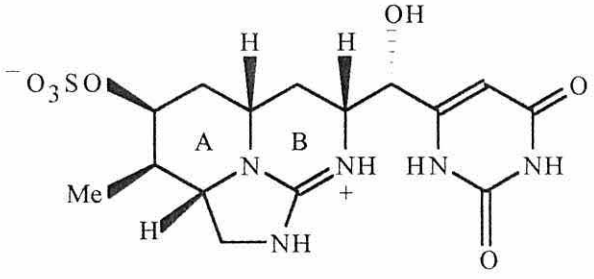
2.4 CYLINDROSPERMOPSIN

2.4.1 ISOLATION AND STRUCTURE

In 1992, Moore[37] reported the isolation and structure of the marine hepatotoxin cylindrospermopsin [5] isolated from the blue-green alga *Cylindrospermopsis raciborskii.* It has also been isolated from the cyanobacterium *Umesakia natans.*[38] Cylindrospermopsin was proved to be the cause of an outbreak of hepatoenteritis in Australia in 1979.[37]

2.4.2 SYNTHETIC APPROACHES TOWARDS CYLINDROSPERMOPSIN

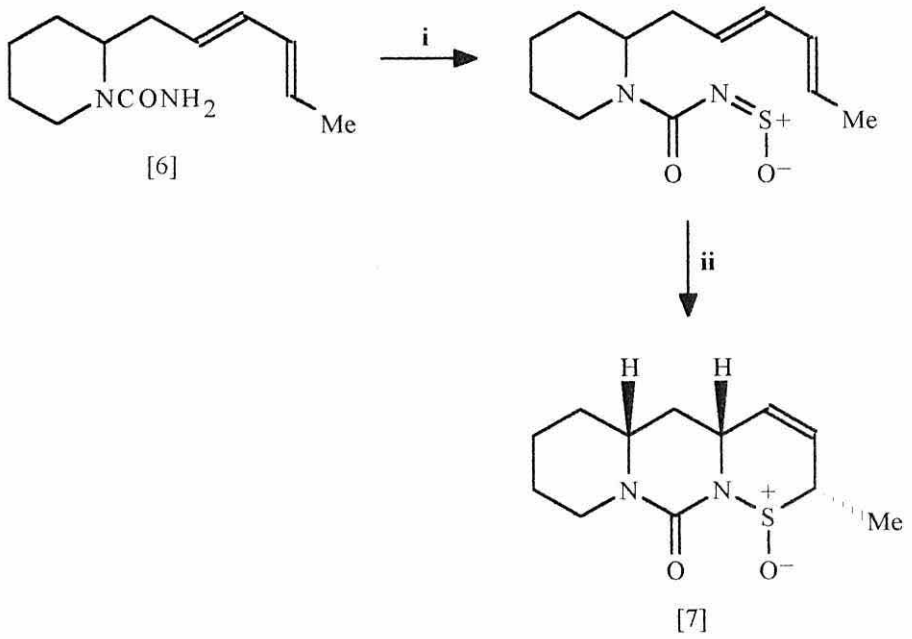
The complex array of functionality and stereochemistry in [5] offers an interesting synthetic challenge.



[5]

In a model study published in 1993 by Weinreb,[39] *hetero*-Diels-Alder rationale using an *N*-sulphinyl dienophile derived from urea derivative [6] was applied, giving the

AB ring system of the natural product as the major isomer [7] (Scheme 1). A second bicyclic model for cylindrospermopsin was developed by Snider[40] using the addition of ammonia to a *bis*-α,β-unsaturated ketone.

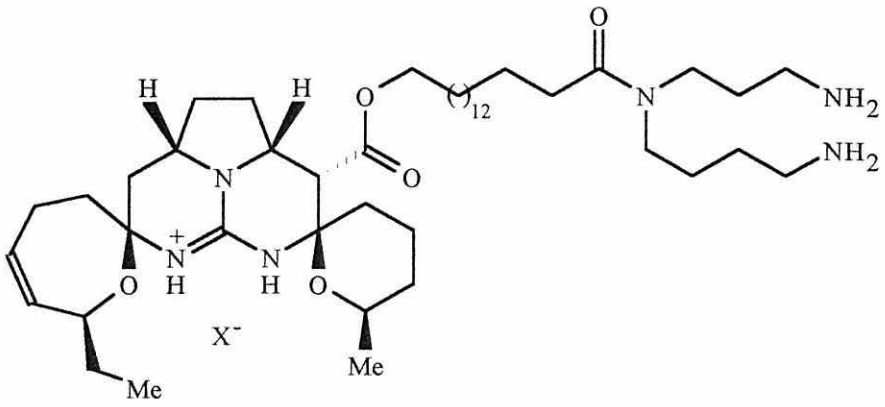


Scheme 1 *Reagents and conditions*: i) $SOCl_2$, CH_2Cl_2, imidazole, -78°C; ii) spontaneous *hetero*-Diels-Alder cyclisation, 67% overall.

2.5 PTILOMYCALIN A

2.5.1 ISOLATION AND STRUCTURE

(-)-Ptilomycalin A [8] was first isolated in 1989 by Kashman and Kakisawa[41] from the Caribbean sponge *Ptilocaulis spiculifer* and the Red Sea sponge *Hemimycale* sp. In later screens, the same metabolite was isolated from three other sources, the starfishes *Fromia monilis*, *Celerina heffernani*[42] and the Caribbean sponge *Batzella* sp.[63] The structure was elucidated by a combination of mass spectrometry and correlated NMR techniques to reveal a unique pentacyclic arrangement as shown. Ptilomycalin A exhibited cytotoxicity against P388 (IC_{50} = 0.1μg/ml), L1210 (IC_{50} = 0.4μg/ml and KB (IC_{50} = 1.3μg/ml) and antifungal and antimicrobial activity against *Candida albicans* (MIC = 0.8μg/ml) in addition to antiviral activity (HSV) at a concentration of 0.2μg/ml.



[8]

Kashman and co-workers[43] extensively investigated the structure and chemical properties of this new alkaloid. Most of the structure elucidation work was carried out on the *bis*(trifluoroacetyl) derivative of [8] as this gave sharper resonances in the ^{1}H and ^{13}C NMR spectra.

The spermidine side-chain was assigned on the basis of COSY, HOHAHA, COLOC and HMBC experiments. Interestingly, the seven methylene resonances were accompanied by a minor set of signals, indicating a degree of rotational isomerism about the amide linkage; this confirmed that the secondary amine of the spermidine was bonded to the carbonyl group.

The HOHAHA spectrum of the pentacyclic portion allowed the two spiro *N,O*-acetal rings and the central tricycle to be identified. The downfield chemical shifts of the two N-H protons (δ 10.22 and 9.87) suggested an ammonium or guanidinium salt.[44] On addition of sodium hydroxide to the NMR sample, these signals disappeared then slowly regenerated over 24-48 hours; this inferred the presence of a guanidinium moiety. In addition, the typical guanidine-type carbon signal at δ 149.09 remained uncorrelated to any proton in the system. The stereochemistry around the pentacycle was deduced from phase-sensitive NOESY and ROESY experiments.[45] For example, nOe was observed between the two pyrrolidine bridge hydrogens, confirming the *cis*-arrangement. Analysis of a three-dimensional model of [8] shows that the spiro *N,O*-acetal and pyrrolidine bridge hydrogens form a well-defined pocket above the guanidinium unit, sequestering the positive charge (Fig. 5).

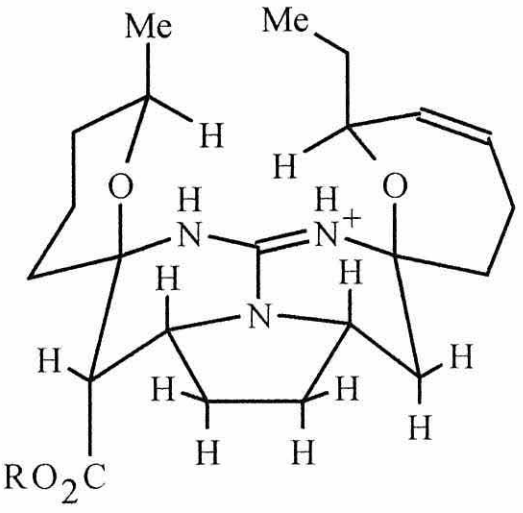


Figure 5. Three-Dimensional Representation of the Ptilomycalin A Core

It was proposed in this study that the guanidine portion could act as a 'vessel' encapsulating a host molecule and that the spermidine could be acting as an 'anchor' portion (possibly to a cell wall) and that the molecule could be involved in cellular transport. However, ptilomycalin A is a relatively non-polar substance, despite the presence of the guanidinium residue. It was therefore suggested that the molecule adopts a conformation such that the spermidine moiety is encapsulating the counter-ion above the guanidinium core, in which the positive charge is itself sequestered by the shape of the pentacycle.

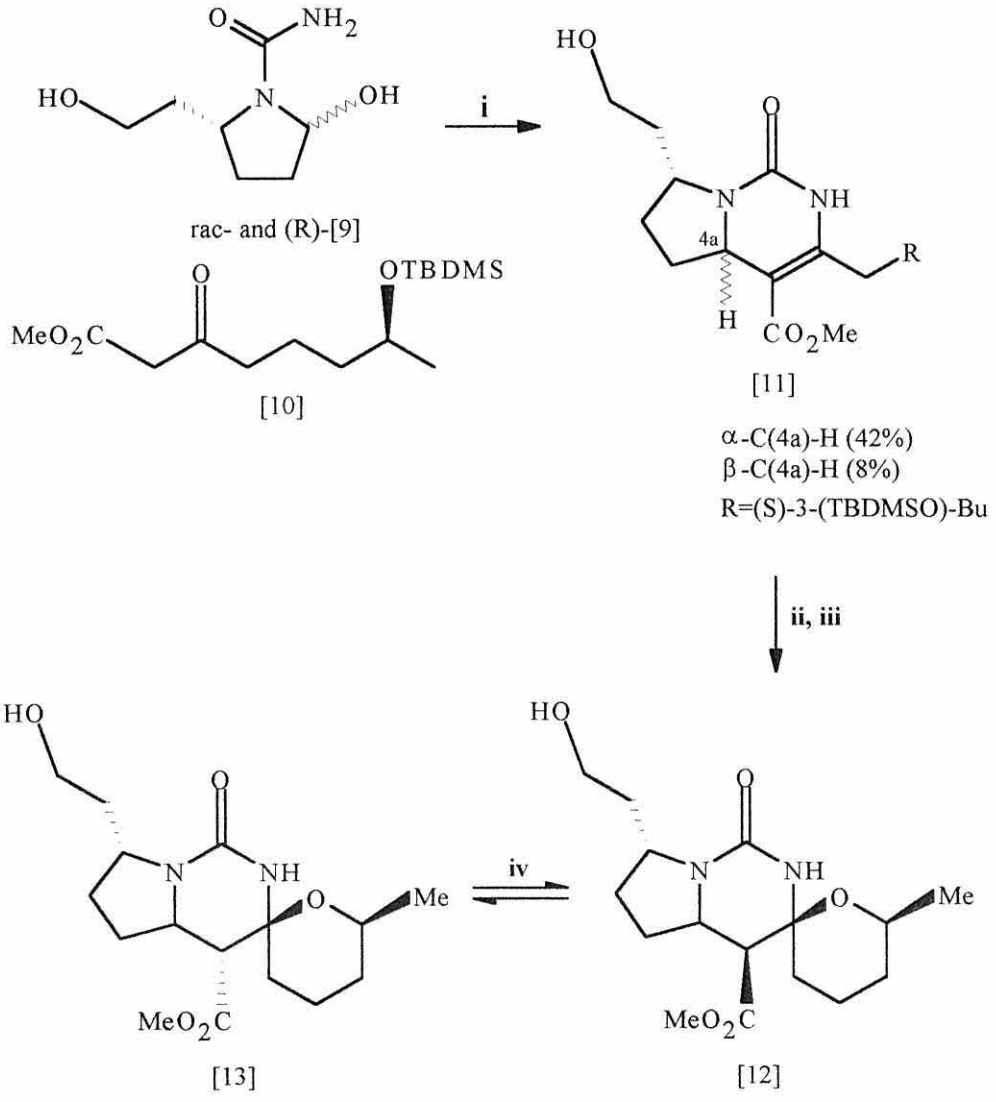
Recently, two similar guanidine alkaloids, fromiamycalin and celeromycalin, have been isolated from the starfishes, *Fromia monillis* and *Celerina heffernani* respectively.[42] They both possess the same pentacyclic core as ptilomycalin A and crambescidin 800, and celeromycalin has a similar spermidine side-chain. These compounds were found to be highly cytotoxic towards cells infected with the human immunodeficiency virus-type 1 (HIV-1).

2.5.2 SYNTHETIC APPROACHES TOWARDS PTILOMYCALIN A

Not surprisingly, ptilomycalin A became the focus of much attention from synthetic organic chemists. In 1993, Snider[46] published a model study towards the synthesis of the tricyclic guanidine portion of ptilomycalin A, utilising the addition of *O*-methylisourea to a *bis*-α,β-unsaturated ketone system. A later approach from

Overman[47] employed an intramolecular Biginelli condensation in the preparation of an advanced tricyclic intermediate (Scheme 2).

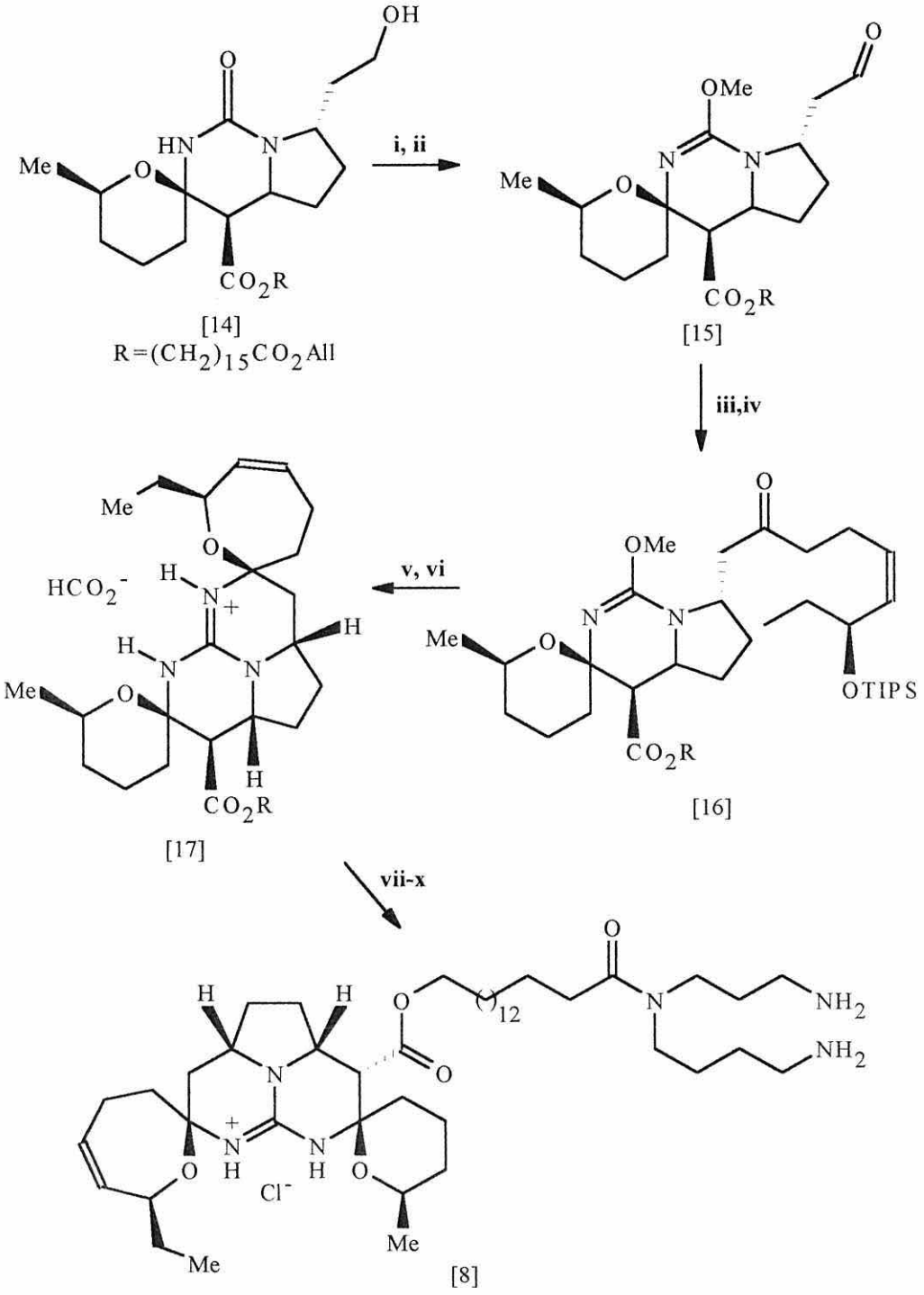
Thus, urea derivative [9] was found to react with optically pure acetoacetate [10] to give a 5:1 mixture of α/β-[11]. TBAF deprotection followed by treatment with *p*-toluenesulphonic acid in chloroform gave the undesired epimer [12] in quantitative yield.



Scheme 2 *Reagents and conditions*: i) Piperidine, AcOH, CH_2Cl_2, 70°C, 50%; ii) TBAF, 23°C, 20 h, 95%; iii) *p*-TsOH, $CHCl_3$, 20°C, quant.; iv) *p*-TsOH, MeOH, 60°C, 2:1 mixture of epimers.

However, it was found that the epimerisation could be effected by *p*-toluenesulphonic acid in methanol to give the desired epimer [13] in a 2:1 ratio.

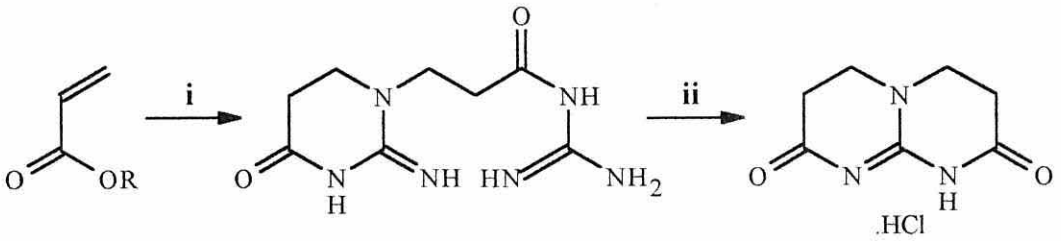
At this time, the absolute stereochemistry of ptilomycalin A was unknown. In 1995, however, the enantioselective total synthesis of (-)-ptilomycalin A by Overman and co-workers[48] established the absolute configuration. Using a slightly modified version of the strategy illustrated above (Scheme 2), an analogue of the enantiomer of tricycle [13] was converted in essentially a four-step sequence to (-)-ptilomycalin A (Scheme 3). Exhaustive comparison with an authentic sample of ptilomycalin A and its derivatives showed that synthetic (-)-[8] had identical stereochemistry to that of the natural product.



Scheme 3 *Reagents and conditions*: i) $(COCl)_2$, Et_3N, DMSO; ii) MeOTf, 2,6-di-*tert*-butylpyridine, CH_2Cl_2, 23°C, 67% overall; iii) $BrMg(CH_2)_2CH{=}CHCH(OTIPS)CH_2CH_3$, THF, -78°C; iv) Swern, 58% overall; v) TBAF; vi) NH_3/NH_4OAc, *t*-BuOH, 60°C, 51%; vii) $Pd(PPh_3)_4$, pyrrolidine, MeCN, 23°C, 75%; viii) *Bis*-BOC-spermidine, EDCI, DMAP, CH_2Cl_2, 23°C, 60%; ix) Et_3N, MeOH, 65°C, 50%; x) HCO_2H, 23°C, 100%.

During the course of model studies towards ptilomycalin A by Murphy and Williams,[49-51] it was found that polycyclic guanidine compounds incorporating the pyrrolidine ring and spiro *N,O*-acetal moieties could be readily accessed *via* a proposed biomimetic route. Following model studies on simple bicyclic and tricyclic systems, pentacyclic guanidines analogous to ptilomycalin A were prepared.

The methodology was based on the findings of Sugino and Tanaka[52] who found that addition of guanidine to alkyl acrylates *via* double Michael addition gave pyrimidinediones of the type shown in Scheme 4.

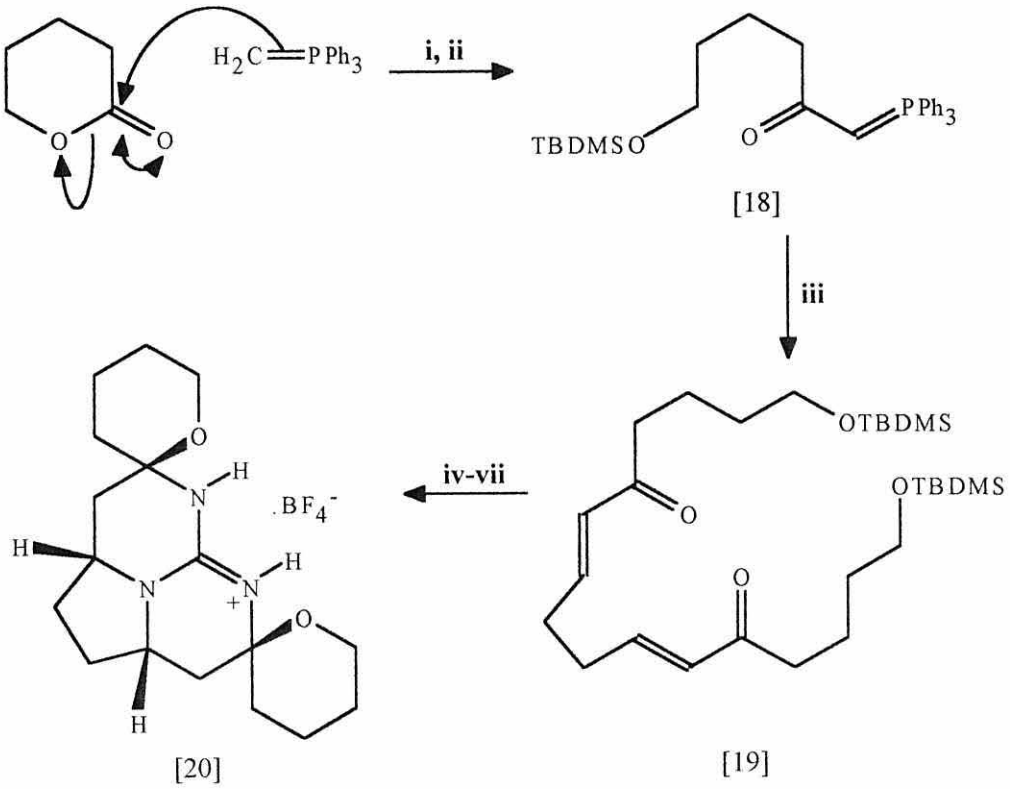


[18]

Scheme 4 *Reagents and conditions*: i) Guanidine, DMF; ii) c. HCl.

It was proposed that polycyclic frameworks containing guanidine could be constructed by the addition of guanidine to a suitable *bis*-enone system. The preparation of a 6,6,5,6,6-model for the pentacyclic core of ptilomycalin A is shown in Scheme 5.

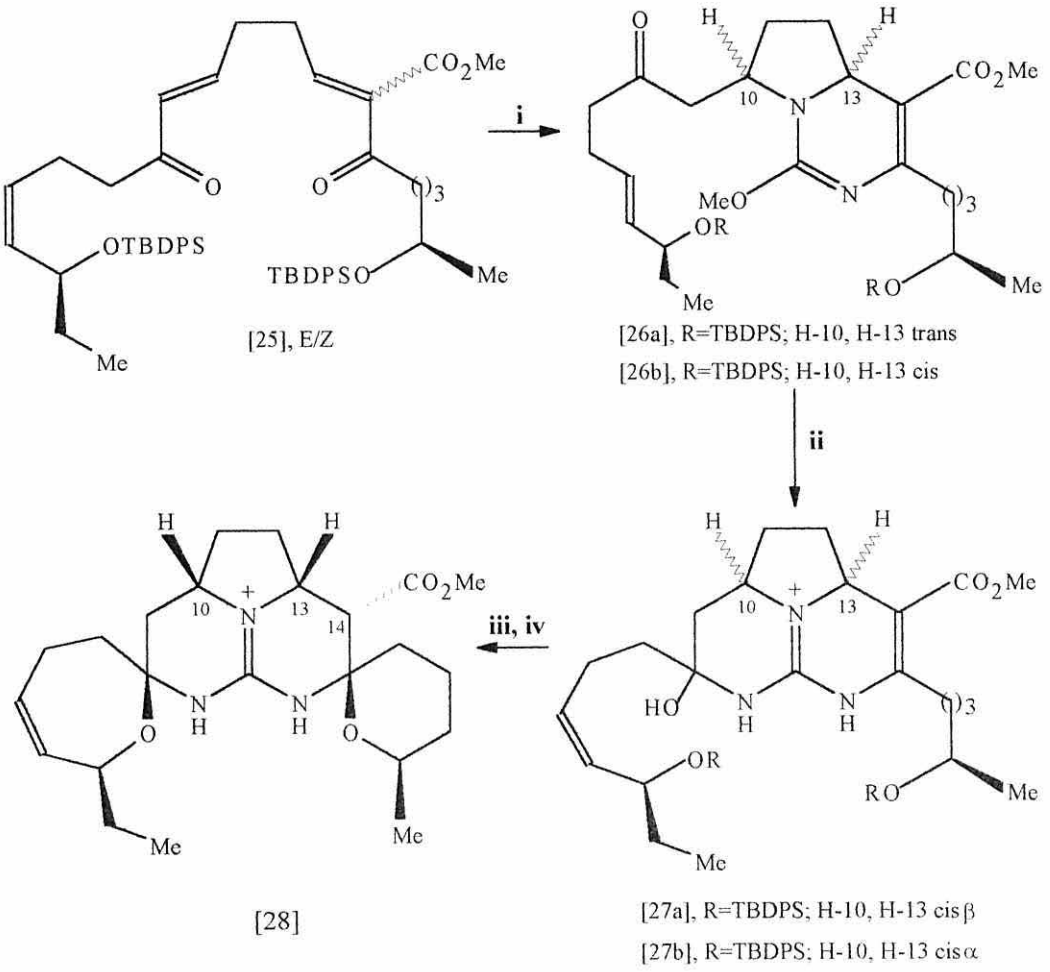
Treatment of methyl triphenylphosphonium bromide with *n*-BuLi gave methylene triphenylphosphorane *in situ*. Subsequent ring-opening of δ-valerolactone and silyl protection gave the β-ketophosphorane [19] in quantitative yield. Double Wittig reaction with succinaldehyde gave diene [20] in 45% overall yield. It was found that reaction of [20] with one equivalent of guanidine followed by deprotection/spirocyclisation and finally counter-ion exchange gave the 6,6,5,6,6-pentacyclic compound [21] as a single diastereoisomer in 25% overall yield after purification and crystallisation.[49a]



Scheme 5 *Reagents and conditions*: i) 2 equiv. $CH_2{=}PPh_3$, THF, -78°C, 1 h; ii) TBDMS-Cl, imidazole, DMF, 0-20°C, 15 h, quant.; iii) 0.4 equiv. succinaldehyde, THF, 48 h, 45% overall; iv) 1 equiv. guanidine, DMF, 0-20°C, 3 h; v) MeOH, HCl, 0-20°C, 24 h; vi) sat. $NaBF_{4(aq)}$; vii) crystallisation, 25% overall.

The modest yield of the reaction could be due to competition for polymerisation of the diene similar to that observed by Weis and Zamir.[53] The methodology was also extended to furnish an unsymmetrical 7,6,5,6,6-pentacycle.[49b]

As a continuation of the work on a tricyclic model system, in 1994 Snider published a convergent 14-step synthesis of the pentacyclic nucleus of ptilomycalin A.[54] The approach was based on the earlier findings on guanidine systems[46] and again utilised the reaction of *O*-methylisourea hydrogensulphate with a *bis*-α,β-unsaturated ketone with pendant *tert*-butyldiphenylsilyl protected hydroxyl functions. The key steps used to develop the guanidine are shown in Scheme 6.

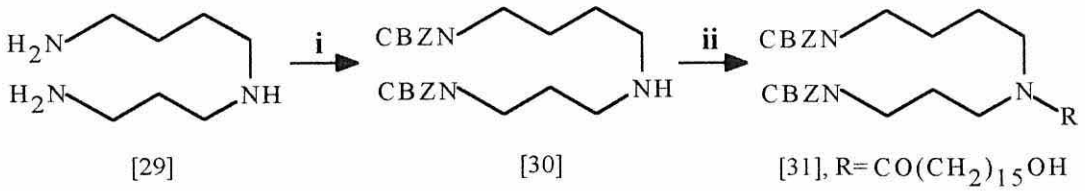


Scheme 6 *Reagents and conditions*: i) *O*-methylisourea hydrogensulphate, *i*-Pr_2EtN, DMSO, 80°C, 1.5 h, 52%, 4:1 [26a]:[26b]; ii) NH_3, NH_4OAc, *t*-BuOH, 60°C, 40 h, 72%, 1:1 [27a]:[27b]; iii) 3:7 HF: MeCN, -30°C, 3 d; iv) Et_3N, MeOH, 60°C, 20 h, ~78% from [27a], 1.3:1 α:β at C-14.

The *bis*-enone ester [25] was prepared as a 1:1 mixture of geometric isomers in 19% overall yield (10 steps) from readily available starting materials. Double Michael addition of *O*-methylisourea gave the enamine [26] as a 4:1 mixture of *cis/trans* diastereoisomers about the pyrrolidine ring. Separation of this mixture proved unnecessary since guanylation of the isourea was somewhat fortuitously accompanied by isomerisation to the two *cis* diastereoisomers [27]. Gentle desilylation followed by treatment with a triethylamine/methanol mixture gave a 1.3:1 mixture of [28] (correct stereochemistry) and the C-14 epimer. 2D-ROESY NMR spectra established the relative stereochemistry around the pentacycle.

A simple 6,6-bicyclic guanidine model [42] with pendant ester, C_{16} side-chain and spermidine unit has been reported by Hart and Grillot.[55] The synthesis was accomplished in 13 linear steps from acrylate [32], with the key step being the coupling of the requisite amido alcohol [31] with guanidinium carboxylate [40].

The spermidine portion of the model was readily accessed in a two step sequence terminating with the coupling of a 16-hydroxyhexadecanoic acid derivative with *bis*-CBZ-spermidine (Scheme 7).

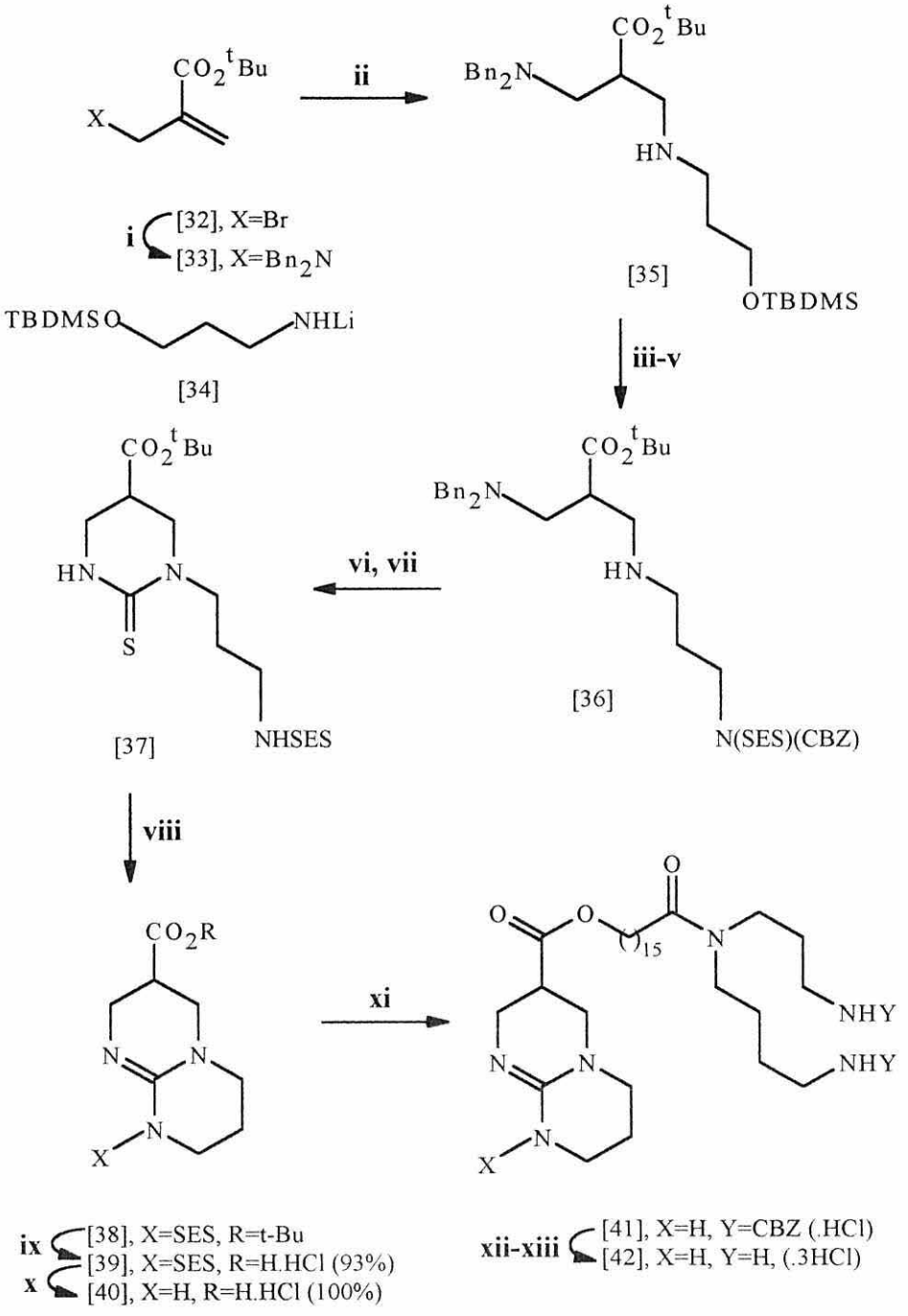


[29] [30] [31], R=$CO(CH_2)_{15}OH$

Scheme 7 *Reagents and conditions*: i) 2 equiv. 3-(carboxybenzyl)-thiazolidine-2-thione, CH_2Cl_2, 20°C, 3 h, 52%; ii) $HO_2C(CH_2)_{15}OTHP$, 1 equiv. DCC, 0.1 equiv 1-hydroxybenzotriazole, THF, 20°C, 27 h, 95%, then Dowex-50, 20°C, 8 h, 87%.

The preparation of the bicyclic guanidine moiety was initially problematic, but eventually *tert*-butyl 2-bromomethylacrylate [32] was chosen as the starting point (Scheme 8). Treatment of [32] with dibenzylamine gave unsaturated ester [33] (82%) which reacted with lithium derivative [34] yielding diamine [35] (66%). Following desilylation, Mitsunobu displacement of the hydroxyl group gave sulphonamide [36] in 94% yield. Hydrogenolysis effected removal of the carbobenzoxy and benzyl protecting groups and the resulting diamine treated with thiocarbonyldiimidazole to give thiourea [37] (51%). Alkylation (MeI) of [37] followed by treatment with Hunig's base gave the bicyclic guanidine [38] in 81% yield. This was easily manipulated by treatment with mineral acid and TBAF to give the guanidinium carboxylate [40].

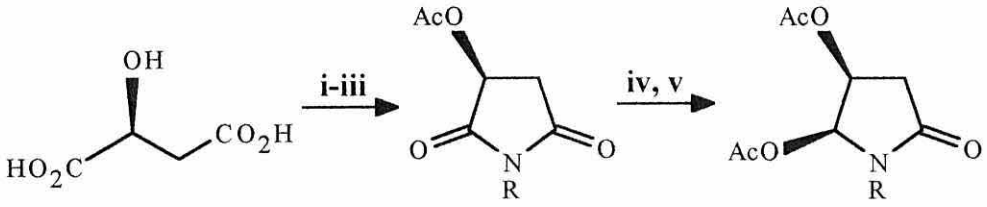
While the *bis*-CBZ derivative [41] was very stable, the free amine [42] suffered decomposition over time with loss of the ester linkage, thus precluding the biological evaluation.



Scheme 8 *Reagents and conditions*: i) Bn_2NH, K_2CO_3, MeCN, Δ 3 h, 82%; ii) THF, -78°C, 3.5 h, 66%; iii) $PhCH_2OCOCl$, Et_3N, THF, Δ, 2 h, 84%; iv) Bu_4NF, THF, 0-20°C, 2.5 h 97%; v) Ph_3P, $EtO_2CN{=}NCO_2Et$, $HN(SO_2CH_2CH_2SiMe_3)(OCOCH_2Ph)$, THF, 0-20°C, 4 h, 94%; vi) $Pd(OH)_2$, H_2, EtOH, 60psi, 16 h; vii) $(Im)_2C{=}S$, CH_2Cl_2, Δ, 18 h, 51% overall; viii) MeOH, MeI, Δ, 1 h, *i*-Pr_2EtN, CH_2Cl_2, 81%; ix) HCl, CH_2Cl_2; x) Bu_4NF, DMF, 80°C, 4 h, then HCl, H_2O; xi) [31], DMF, EDCI.HCl, DMAP, 22 h, 55%; xii) $Pd(OH)_2$, 1,4-cyclohexadiene, EtOH, 60°C, 3 h; xiii) HCl, MeOH, 70%.

One of the most recent strategies towards ptilomycalin A and related systems is that of Hiemstra and co-workers.[56] Their strategy is based on *N*-acyliminium coupling reactions of enantiopure derivatives of pyrrolidin-2-one with silyl enol ethers.[56b]

Thus, (*S*)-malic acid [43] was converted to the imides [44] in good yields by sequential treatment with acetyl chloride, an amine and acetyl chloride. The *bis* acetoxylactams [45] were accessed in a stereoselective manner by reduction with sodium borohydride in ethanol.



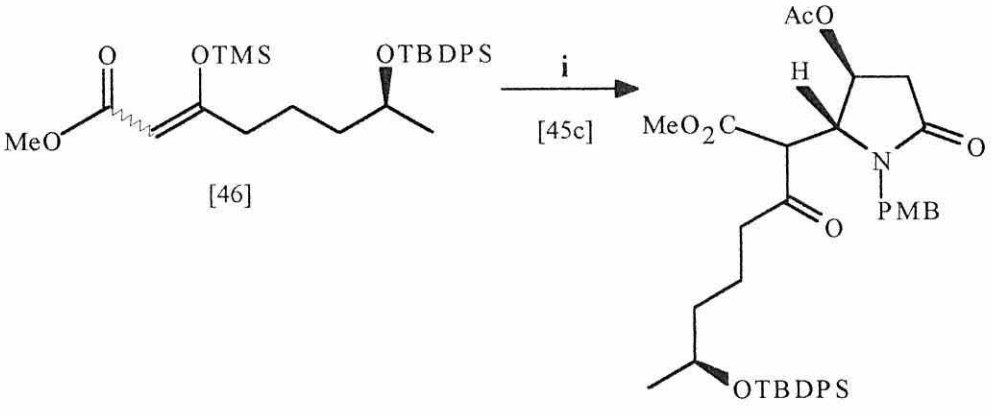
[43]

[44a], R=Bn
[44b], R=DMB
[44c], R=PMB

[45a], R=Bn
[45b], R=DMB
[45c], R=PMB

Scheme 9 *Reagents and conditions*: i) AcCl, 20°C, 18 h ([44a], Δ, 2h); ii) RNH_2, 4-18 h, THF, 20°C; iii) AcCl, Δ, 18 h, 78-93%; iv) 5 equiv. $NaBH_4$, EtOH ([44c] EtOH/THF), -35°C, 15 min, 68-85%; v) 5 equiv. Ac_2O, pyridine, 0.15 equiv. DMAP, 20°C, 3-18 h, 88-100%.

Subsequent reaction of [45c] with, for example, trimethylsilyl enol ether [46] in the presence of $^{i}Pr_2EtN$ and TMSOTf gave exclusively the *trans*-2,3-disubstituted lactam [47]. This suggested that, in principle, this approach could be geared towards an advanced intermediate in a projected synthesis of ptilomycalin A.



[46]

[47]

Scheme 10 *Reagents and conditions*: i) $^{i}Pr_2EtN$, TMSOTf, CH_2Cl_2, -78°C, 1 h, 75%.

It is interesting to note that while this work was continuing, the absolute stereochemistry of ptilomycalin A was unknown. It was not until the total synthesis was published by Overman[48] that it became apparent that the use of (*S*)-malic acid would lead to the unnatural (+)-enantiomer of ptilomycalin A.

In a later publication, the same workers reported the preparation of bicyclic guanidines using this approach. Three 2-substituted pyrrolidines [49a-c] (Table 1) were prepared *via* *N*-acyliminium ion couplings of precursor [48a-c] with three different silyl enol ethers.

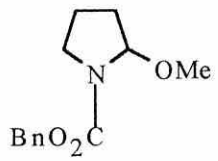
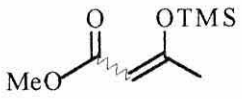
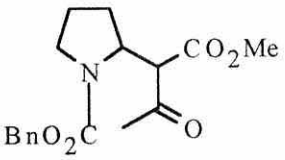
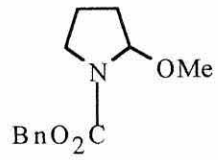
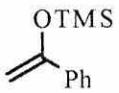
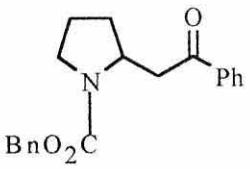
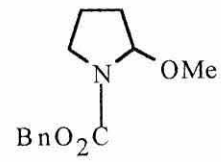
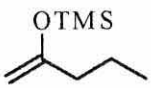
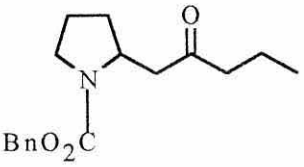
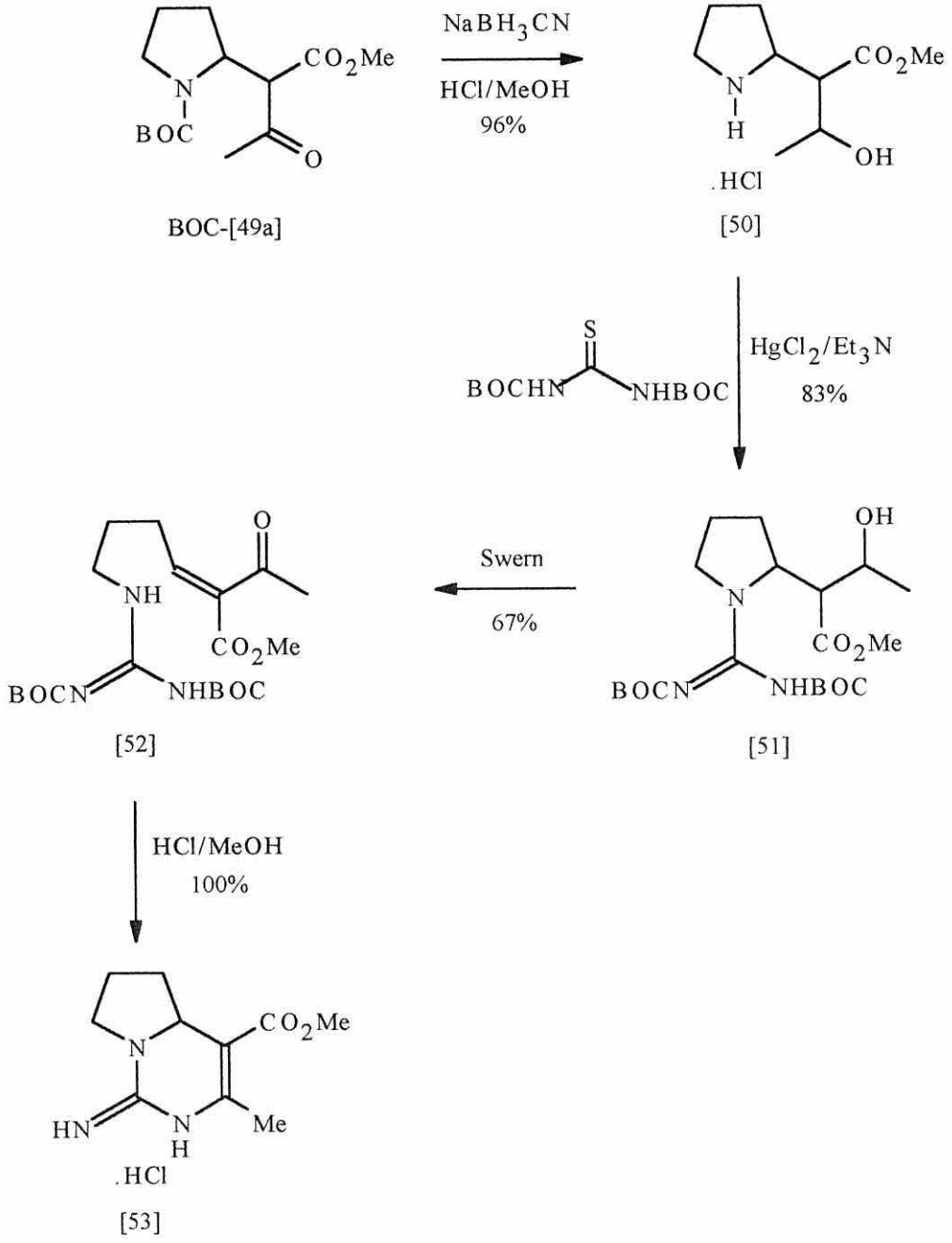
Precursor [48a-c]	Silyl enol ether	Product [49a-c]	Yield
			[49a] 100%
			[49b] 90%
			[49c] 100%

Table 1. Coupling of silyl enol ethers with [48]

Guanylation was performed on the deprotected pyrrolidines using *N,N'-bis*-(*tert*-butoxycarbonyl)-thiourea and $HgCl_2$.[57] Although guanylation of deprotected [49b] and [49c] proceeded smoothly to bicyclic guanidines [50b] and [50c] in good yield (Scheme 12), [49a] gave a tricyclic product. In order to convert pyrrolidine [49a] to the expected bicycle [53], it was found that reduction of the ketone of the *N*-BOC derivative of [49a] to alcohol [50] was required before the coupling reaction with *bis*-BOC-thiourea (Scheme 11). Swern oxidation of [51] unexpectedly led to the formation of the acyclic product *E*/*Z*-[52]. This is presumably formed by *retro*-Michael

addition in the presence of Et_3N used in the Swern oxidation. Other oxidative reagents did not lead to the formation of the desired pyrrolidine intermediate.

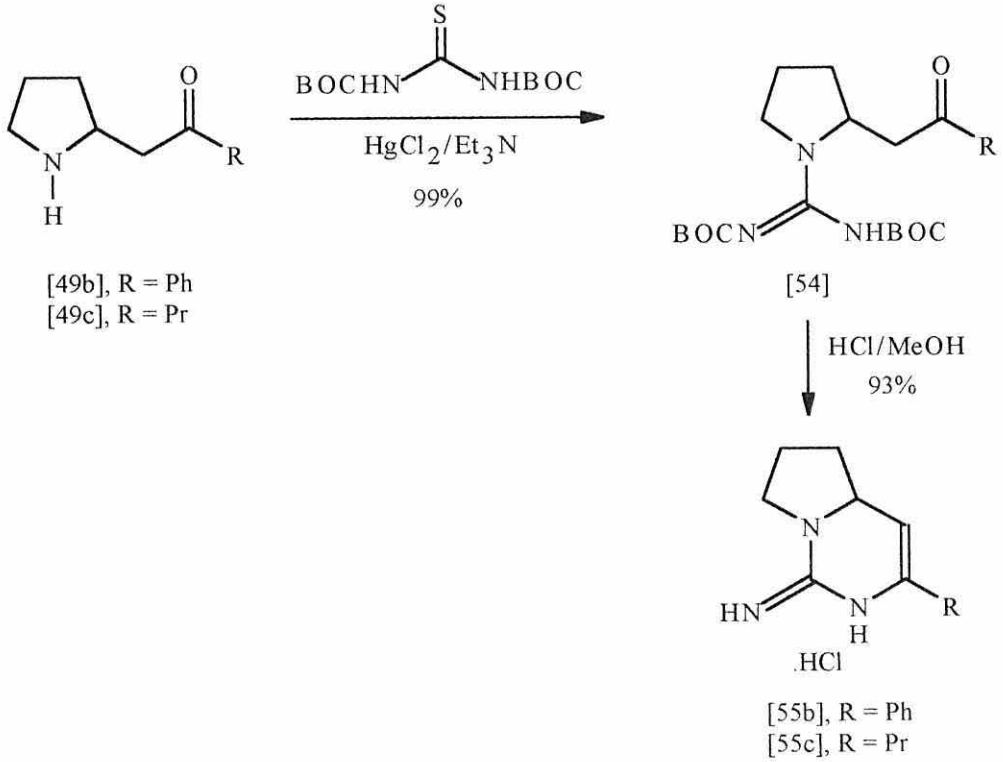


Scheme 11

However, treatment of [52] with methanolic HCl effected removal of the BOC groups with cyclisation of the six-membered ring and also regenerated the pyrrolidine by Michael addition to give the desired bicyclic guanidine [53].

It was clear that the *retro*-Michael addition encountered *via* the described route would lead to a loss of stereochemical integrity about the pyrrolidine ring in a planned extended synthesis towards ptilomycalin A. Therefore, an alternative strategy was required for this approach to be viable. Protection of the ketone function of [49a] as the dimethoxyacetal ($CH(OMe)_3$, MeOH, H_2SO_4) proceeded with concomitant removal of the BOC group. The familiar coupling with *bis*-BOC-thiourea followed by treatment with methanolic HCl gave the desired bicyclic guanidine [53] as a *ca.* 2:1 mixture of diastereoisomers.

The simple pyrrolidine derivatives [49b] and [49c] were much less problematic (Scheme 12).[56c]



Scheme 12[56c]

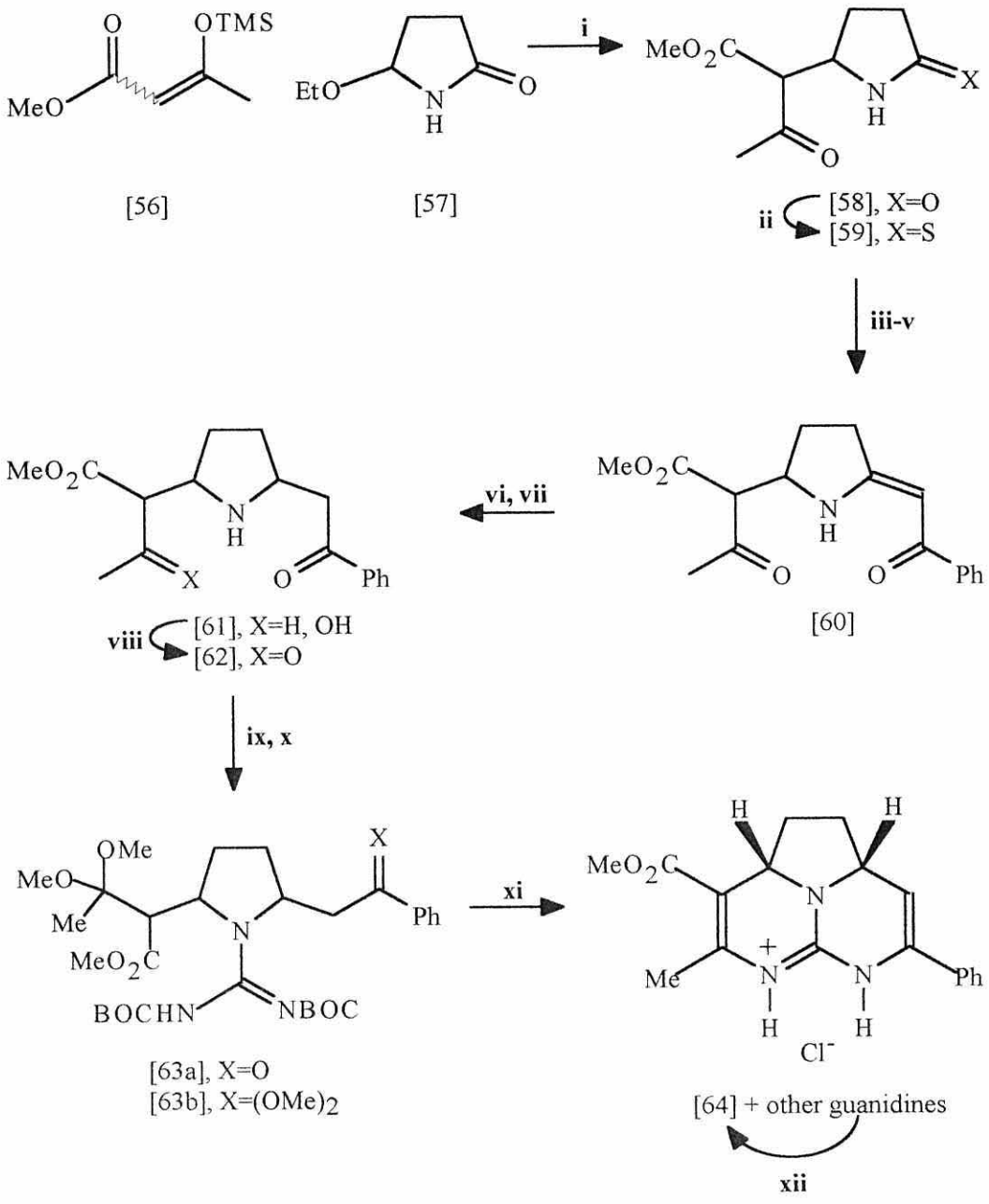
Treatment of the precursor with *bis*-BOC-thiourea in the presence of $HgCl_2$ and triethylamine gave [54] in good yield. The guanylation of [49c] proceeded with formation of [54] as a *ca.* 2:1 mixture with the *retro*-Michael product. Only a trace of this compound was observed for [49b]. Deprotection and cyclisation of [54] with

methanolic HCl gave, for [54b], the desired bicyclic guanidine [55b] as a single product. Guanidine [54c] gave this product along with the hemiaminal and an isomer of [55c] with the double bond outside the ring.

Essentially the same methodology was employed in an allied study[56a] which describes the elaboration at C-5 of the pyrrolidine to generate a tricyclic system (Scheme 13). Thus, *N*-acyliminium ion coupling of commercially available silyl enol ether [56] with ethoxylactam [57] gave 5-substituted pyrrolidine-2-one [58] as a 1:1 mixture of isomers. Lawesson's reagent was employed for the conversion of [58] to [59], with the β-ketoester unaffected.

Eschenmoser sulphide contraction with 2-bromoacetophenone gave the vinylogous amide [60] as a single geometric isomer (*Z*). Reduction of [60] with sodium cyanoborohydride unfortunately gave the over-reduced product [61]. BOC-protection of amino alcohol [61] followed by PCC oxidation gave the β-ketoester [62] as a mixture of four diastereoisomers. Protection of the β-ketoester gave a mixture of mono- and diacetals [63] and this was followed by guanylation using *bis*-BOC-thiourea and $HgCl_2$. The two six-membered rings were formed by treatment with methanolic HCl giving a mixture of guanidinium salts. Tricyclic guanidine [64] was isolated chromatographically in 33% yield from [62]. A more polar fraction, likely to be 2,5-*trans*-substituted pyrrolidines was converted to the tricyclic guanidine by reaction with ammonia and ammonium acetate to give an additional 20% of [64].

The stereochemistry of [64] was established by nOe experiments. An enhancement was observed between the two pyrrolidine bridge hydrogens, indicating a *cis* orientation. The ^{13}C NMR shifts also compared well for the analogous structures Batzelladines B and E[63] (Section 2.7).

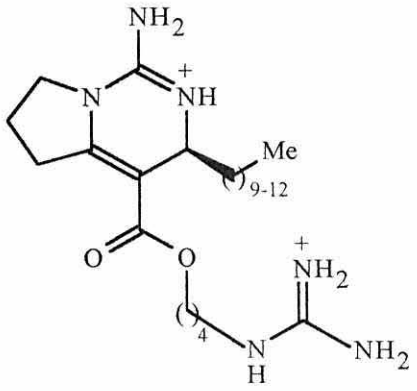


Scheme 13[56a] *Reagents and conditions*: i) 1.5 equiv. [56], 1.1 equiv. TMSOTf, CH_2Cl_2, -78°C 1h, RT 18 h, 63%; ii) 0.55 equiv. Lawesson's reagent, PhMe, 80°C, 10 min, 91%; iii) 1.2 equiv. 2-bromoacetophenone, Et_2O, RT, 18 h; iv) Et_3N, CH_2Cl_2, RT, 2 h, 83% from [59]; v) 4 equiv. PPh_3, $CHCl_3$, 60°C, 18 h, 82%; vi) $NaBH_3CN$, 3:1 AcOH/THF, 0°C, 40 min, 99%; vii) BOC_2O, DIPEA, THF, RT, 18 h, 91%; viii) 2 equiv. PCC, mol. sieves, CH_2Cl_2, RT, 3 h, 91%; ix) $CH(OMe)_3$ (excess), H_2SO_4 (cat.), MeOH, 50°C, 5 h; x) 1.05 equiv. *bis*-BOC-thiourea, 1.05 equiv. $HgCl_2$, 3.5 equiv. Et_3N, DMF, 0°C 30 min, RT 18 h; xi) HCl, MeOH, RT, 3 h, 33%; xii) NH_3, NH_4OAc, MeOH, 60°C, 3 days, 20%.

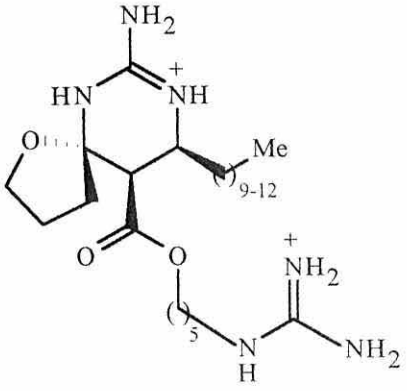
In summary, this synthetic approach has proved effective for the preparation of bicyclic and tricyclic guanidine compounds and extension of this could provide access to a number of polycyclic guanidine alkaloids. Although the project is aimed at a projected synthesis of ptilomycalin A, the structures obtained thus far are very closely allied to the batzelladine alkaloids (Section 2.7).

2.6 CRAMBINES AND CRAMBESCIDINS

Many guanidine containing natural products have been isolated from the sponge *Crambe crambe*, the first being crambines A and B [65] and [66].[58] *Crambe crambe* is a bright red encrusting sponge found off the rocky coasts of the Mediterranean. The methanol extract of the sponge showed high toxicity towards the fish *Lebistes reticulatus* and was found to inhibit the reaggregation of cells of *Ephydiatia fluviatillis*. The crambines A and B are believed to be related biogenetically to ptilomycalin A and the isolation of other compounds from *Crambe crambe* which have a similar structure to ptilomycalin A supports this belief.[61]



[65]

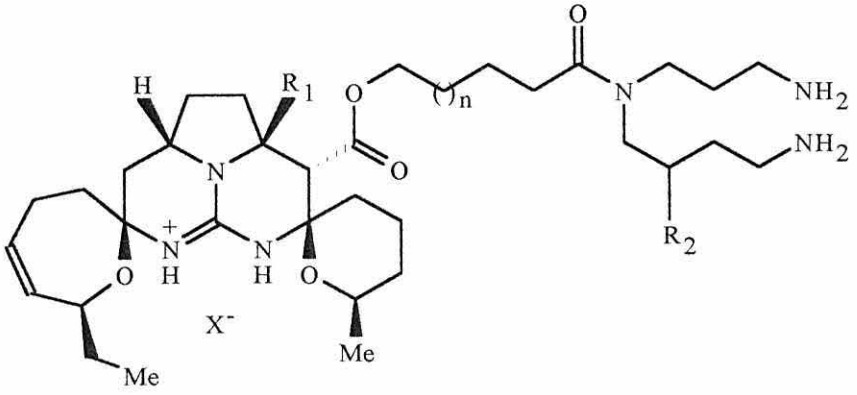


[66]

Two further ichthyotoxic alkaloids were isolated from *C. crambe* by Braekman and co-workers[59] (crambines C1 and C2) and were found to have almost identical structures which are similar to crambine B, but with the tetrahydrofuran spirocycle opened to an alcohol. The synthesis of crambines A, B, C1 and C2 has been reported by Snider and Shi[60] together with a revision of the structures of crambines B and C1.

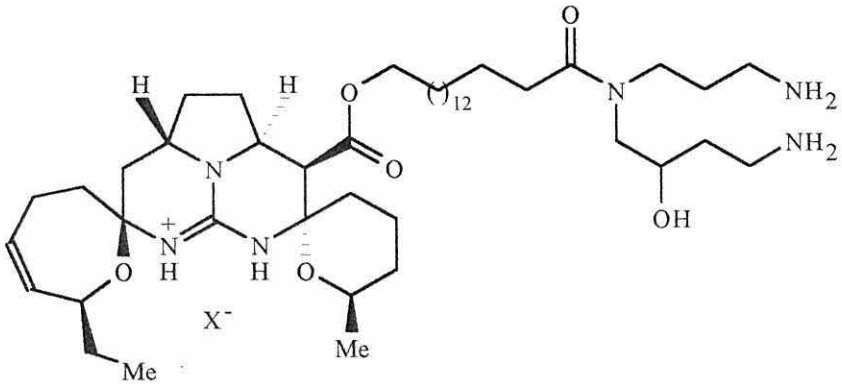
Subsequently, a family of complex pentacyclic guanidine alkaloids were isolated from *C. crambe* by Rinehart, *et al.*[61] These were named crambescidins 816 [67], 830 [68], 844 [69] and 800 [70] and were found to possess the same pentacyclic framework as ptilomycalin A, but with an additional hydroxyl group.

Also present was an unprecedented hydroxyspermidine moiety. It is interesting to note that the crambescidins have the same stereochemical configuration about the guanidine core (*syn*-spirocycles) as ptilomycalin A.



[67]: R_1=R_2=OH, n=12
[68]: R_1=R_2=OH, n=13
[69]: R_1=R_2=OH, n=14
[70]: R_1=H, R_2=OH, n=12

In a further study, 13,14,15-isocrambescidin 800 [71] was isolated and in contrast to the previous structures, it was found that the spirocyclic units of the pentacyle were *anti*.[62] The importance of this was indicated when [71] was found to be considerably less bioactive than its congeners, which suggested that the pocket formed at the guanidine core of these metabolites plays a key role in the bioactivity.

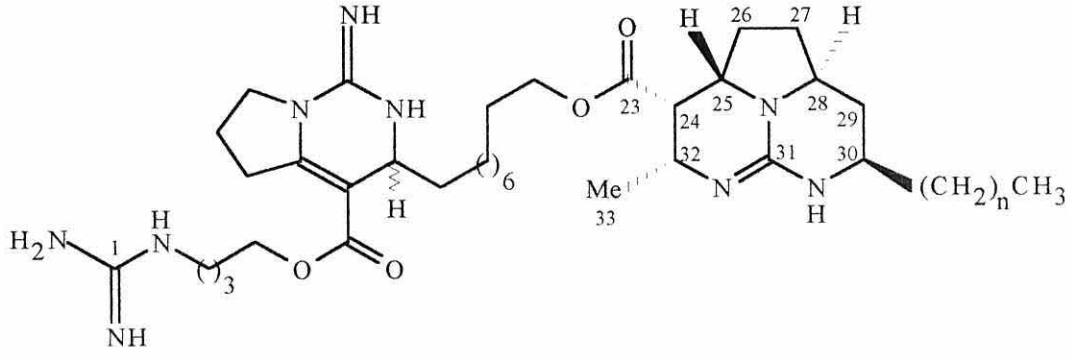


[71]

2.7 THE BATZELLADINE ALKALOIDS

2.7.1 BATZELLADINES A-E†

The first five members of a fascinating new family of guanidine alkaloids were reported in 1993 by Patil and co-workers.[63] They were isolated from the methanolic extract of the bright red encrusting sponge *Batzella* sp., collected off the Berry Islands, Bahamas. Batzelladines A [72] and B [73] were found to inhibit the binding of the HIV envelope glycoprotein gp-120 to CD4. The hallmark of acquired immune deficiency syndrome (AIDS) is the progressive decline in the number of $CD4^+$ cells making the body susceptible to opportunistic infections, such as pneumonia. The exact mechanism by which the virus depletes $CD4^+$ cells has not been established. However, it has been shown that agents which inhibit HIV replication *in vitro* also provide a therapeutic benefit *in vivo* (*e.g.* AZT). Therefore, antagonism of HIV replication constitutes a therapeutic strategy for AIDS. An obvious way to inhibit viral replication is to block the interaction of the virus with the host cell, because the virus cannot replicate without the biosynthetic apparatus of a host cell. In the case of HIV, the virus replicates *via* a high affinity interaction with the CD4 receptor on the surface of a T cell.



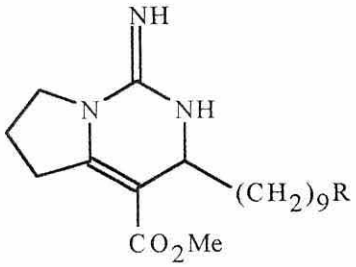
Batzelladine A [72], n = 8 (major), 9, 10.

† The structures of batzelladines A and D have been recently revised. For a discussion of the revision, see section 2.7.5. For clarity, the revised structures will be used throughout.

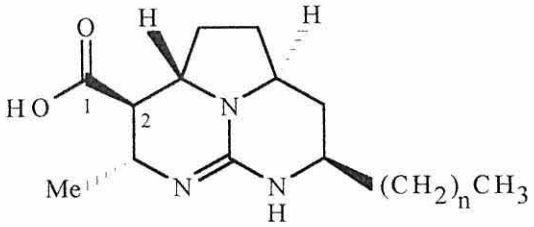
2.7.2 STRUCTURE ELUCIDATION

A total of 21 compounds including several known metabolites were isolated chromatographically in this screen. The structures of the novel alkaloids, batzelladines A-E were elucidated by the range of modern analytical methods. The conclusions for batzelladine A [72] will be described as a representative example.

The high resolution and fragmentation FABMS spectra resulting from high energy, collision induced dissociation of the $[MH]^+$ ion inferred batzelladine A to be a protonated alkaloid of formula $C_{42}H_{74}N_9O_4$ with minor higher homologues. Deuterium-exchange experiments confirmed the presence of 8 exchangeable protons. Comparison of the ^1H and ^{13}C NMR data of [72] with those of crambine A [65] allowed the C-1 to C-16 portion of the molecule to be assigned the same structure as [65]. The HOHAHA spectrum revealed a contiguous spin system from H-32 to H-30 in the tricyclic portion. Correlations were observed in the HMBC spectrum between H-25 and C-23, C-26 and C-27, and between H-32 and C-23 and C-33. This confirmed the connectivity of the ester carbon at C-24. The chemical shifts of the four methine protons H-32 (δ 3.83), H-25 (δ 3.93), H-28 (δ 3.52) and H-30 (δ 3.53) were typical of protons α to nitrogen and the 6,5,6-tricycle was constructed. The absence of correlations between C-31 and H-32, H-25, H-28 and H-30 in the HMBC and COLOC spectra was disconcerting, however the same situation occurs in ptilomycalin A.[41] Hydrolysis of batzelladine A using sodium methoxide in methanol (65°C, 16h) gave methyl ester [74a] and acid [75].



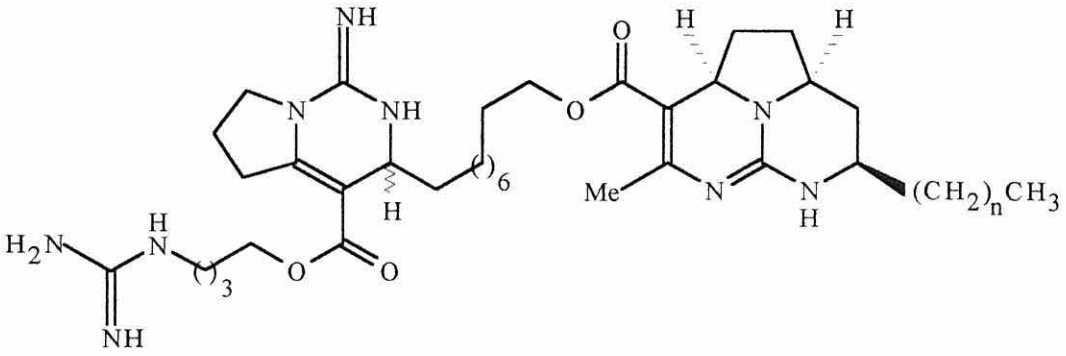
[74a], R = OH
[74b], R = H



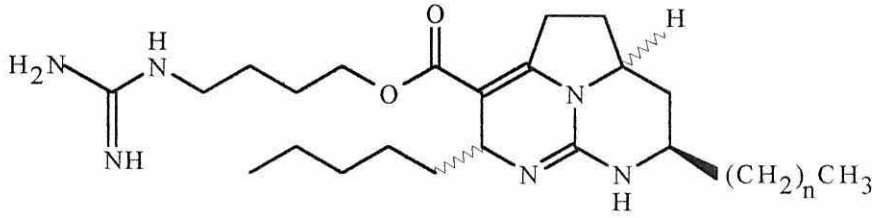
[75], n = 8 (major), 9, 10

The methyl ester [74a] was identical to the methanolysis product of crambine A [74b], but with a terminal hydroxyl group on the hydrocarbon chain. Analysis of the ^1H NMR spectrum of acid [75] revealed that epimerisation at the position α to the ester

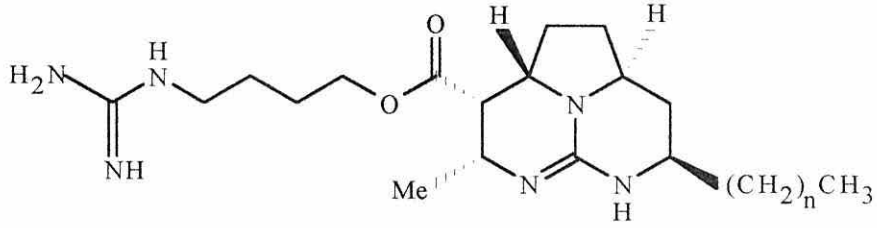
group had taken place. This was inferred by the coupling constant of the H-2 signal in [75] (δ 2.01, t, J = 10.5Hz) compared with that in [63] (δ 3.11, dd, J = 4.6, 3.6Hz).



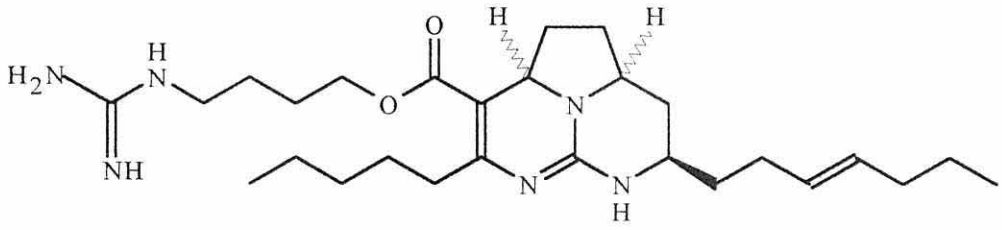
Batzelladine B [76], n = 6 (major), 7, 8.



Batzelladine C [77], n = 6 (major), 7, 8.



Batzelladine D [78], n = 8 (major), 9, 10.



Batzelladine E [79]

2.7.3 BIOLOGICAL EVALUATION

Batzelladines A-D were evaluated along with crambine A [65] for comparison in an ELISA-based assay to measure the association of soluble CD4 to immobilised recombinant gp120 according to a prescribed method.[64] Batzelladines A and B showed activity with IC_{50} values of the order of 30μM whereas batzelladines C and D and crambine A were significantly less active (Table 2). The cell-based assay measured the binding of HIV-1 glycoprotein gp120 to $CD4^+$ T cells. The decrease in activity noted for batzelladines C and D and crambine A alone suggests that both the tricyclic guanidine system and the bicyclic moiety of crambine A must be present in the same molecule for optimal activity.

Compound	gp120-CD4 ELISA IC_{50} (μM)	Cell-based assay IC_{50} (μM)
Batzelladine A [72]	29 ± 4	10
Batzelladine B [76]	31 ± 12	25
Batzelladine C [77]	>100	>100
Batzelladine D [78]	72 ± 2	>100
Crambine A [65]	>100	>100

Table 2. Inhibition of HIV-1 gp120 Binding with CD4

Additional bioassays were carried out to assess the scope of bioactivity in these compounds. Again, it was found that batzelladines A and B were effective at low concentrations against various ligand-receptor interactions (Table 3).

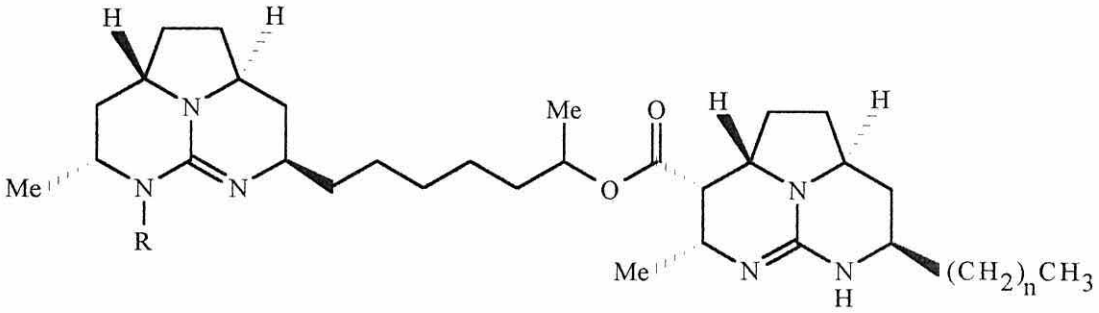
Compound	PKC[a]	IL8a[b]	IL8b[c]	CGRP[d]	Cytotoxicity[e]
Batzelladine A [72]	1.4	4.7	7.8	1.7	1.6
Batzelladine B [76]	1.5	2.6	6.5	1.7	1.8
Batzelladine C [77]	6.8	9.4	9.4	4.3	1.1
Batzelladine D [78]	11	15	14	26	0.5
Crambine A [65]	9.6	47	41	7.1	0.7

a Protein kinase C enzyme assay using rat brain enzyme and histone protein as substrate
b Binding of interleukin-8 to the non-permissive receptor (radioligand binding assay)
c Binding of interleukin-8 to the permissive receptor (radioligand binding assay)
d Binding of calcitonin gene-related peptide to porcine lung membranes (radioligand assay)
e Cytotoxicity to proliferating Vero cells (72h exposure with XTT read)

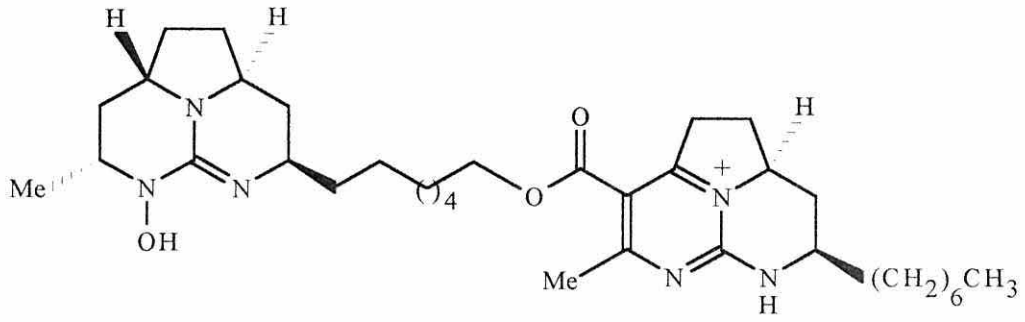
Table 3. Additional Assays of Batzelladines A-D and Crambine A

2.7.4 BATZELLADINES F-I

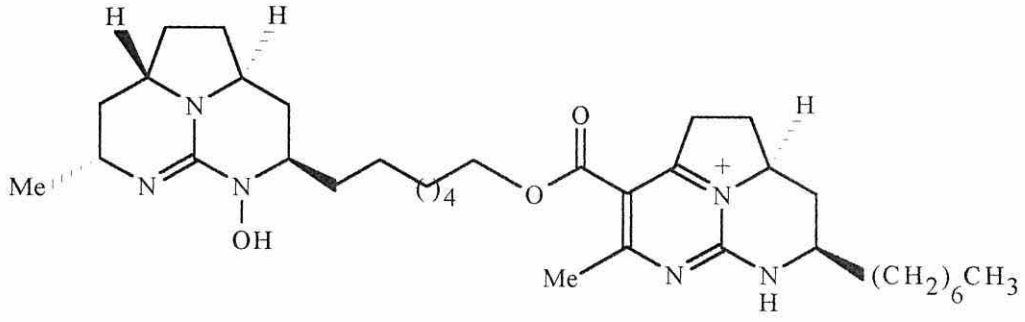
Very recently,[65] another four new guanidine alkaloids have been isolated from the sponge *Batzella* sp. collected in Discovery Bay, Jamaica. These metabolites were shown to be inducers of $p56^{lck}$-CD4 dissociation. The protein tyrosine kinase $p56^{lck}$ has been shown to interact with the cytoplasmic tail of CD4 and this interaction has been assumed necessary for antigenic activity to occur.[66]



Batzelladine F [79], R = H, n = 8 major, 9, 10.
Batzelladine G [80], R=OH, n = 8 major, 9, 10.



Batzelladine H [81]

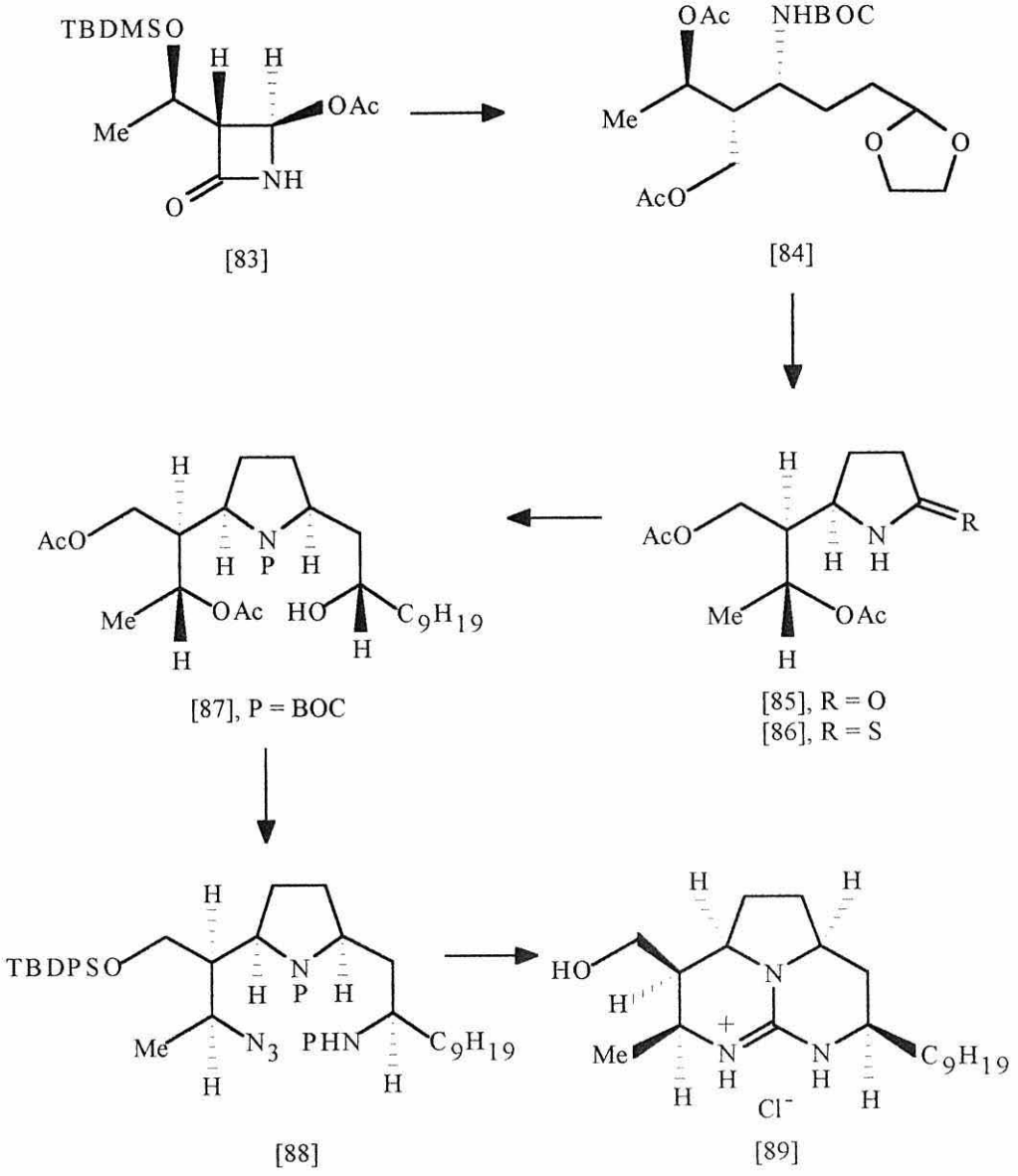


Batzelladine I [82]

2.7.5 SYNTHETIC APPROACHES TOWARDS THE BATZELLADINE METABOLITES

The unique nature of these alkaloids has made them an interesting and challenging synthetic target. The first synthesis of the tricyclic core of batzelladine A [63] was reported by Indian workers in 1995.[67] The somewhat protracted synthesis begins with optically pure β-lactam [83] to establish three of the stereocentres in the target molecule. Scheme 14 shows the key intermediates in the reaction sequence.

Thus [83] was converted to [84] by addition of a Grignard reagent at C-4 of the starting β-lactam. BOC protection of the N-H group followed by ring-opening (LiOH/MeOH), then successive reduction (DIBAL-H) and acetylation (Ac_2O/DMAP) gave [84] in 43% overall yield. A critical step in the strategy was the conversion of linear [84] to pyrrolidine-2-thione derivative [86]. It was found that this transformation could be effected *via* [85] using Jones reagent in refluxing acetone followed by treatment with Lawesson's reagent giving [86] in a satisfactory 62% yield. An Eschenmoser sulphide contraction was employed to incorporate the right-hand side-chain which on reprotection and reduction gave [87] in 47% yield. The remaining two nitrogen atoms of the guanidine moiety were incorporated using Mitsunobu conditions with hydrogen azide, giving [88] in 72% yield. Azide [88] was converted to a cyclic urea by removal of the BOC groups (TFA) and treatment with the C-1 synthon 1,1'-carbonyldiimidazole. This urea was then converted to the methyl lactim ether using dimethyl sulphate and sequential hydrogenation ($Pd/BaSO_4$) and desilylation gave tricyclic guanidine [89] in 38% yield from [88] (3.4% overall yield from [83]). The structure of [89] was assigned by ^1H NMR (COSY and NOESY) and decoupling experiments. Long-range nOe between the protons α to nitrogen gave further evidence for the stereochemical assignment of [89].



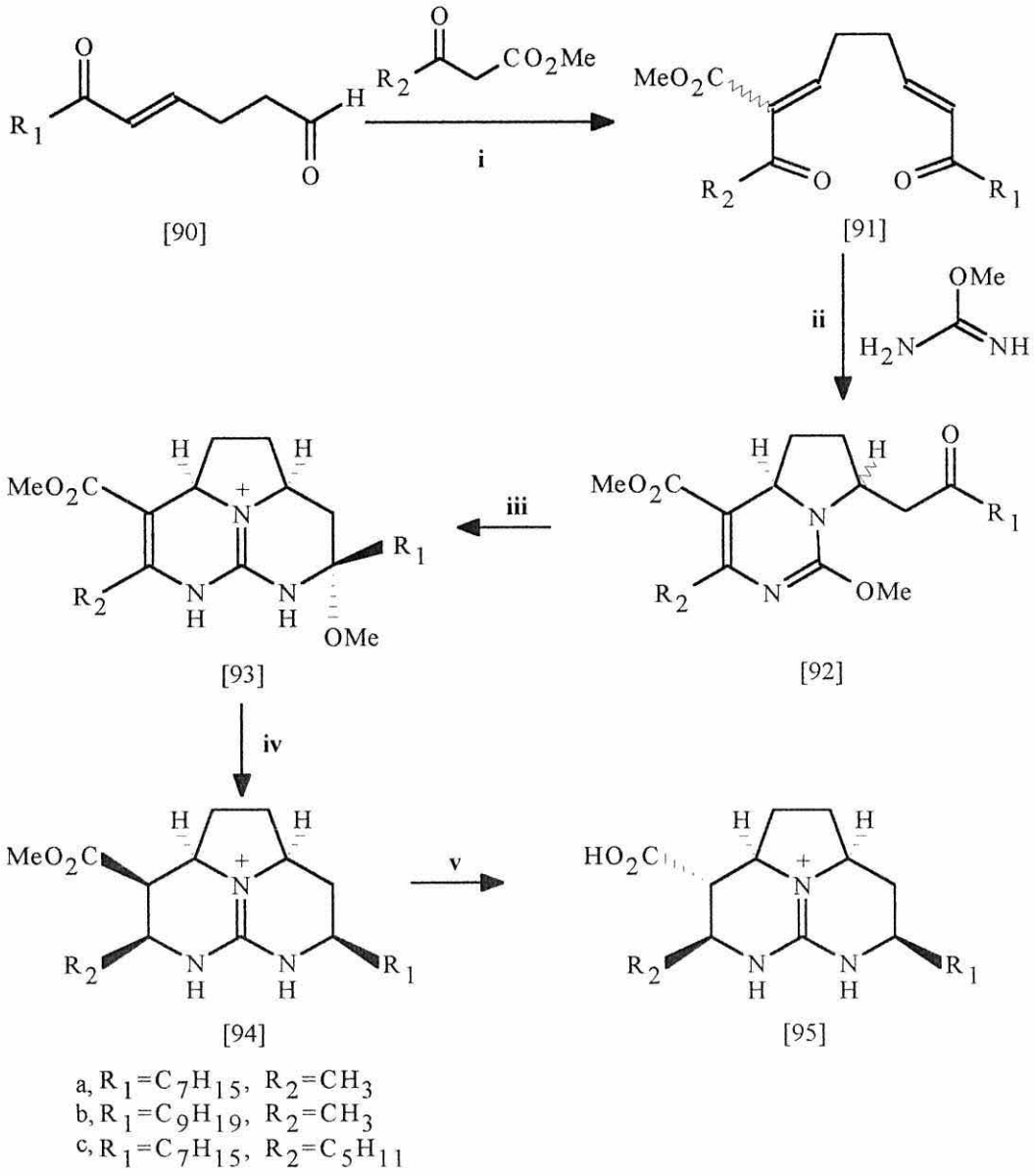
Scheme 14 Enantiospecific Synthesis of the Tricyclic Core of Batzelladine A[67]

At the time of publication, the stereochemistry of [89] was thought to be identical to that of the natural product, batzelladine A [72]. However, a report published by Snider[68] in 1996 revises the stereochemistry of the natural product. The tricyclic portions of batzelladines A and D were synthesised and compared to the natural product.

The key step in the synthesis was addition of *O*-methylisourea to a *bis*-α,β-unsaturated ketone as described previously (Scheme 6, Page 21). Aldehyde [90] was prepared by a prescribed route[46,54] and found to undergo a Knoevenagel

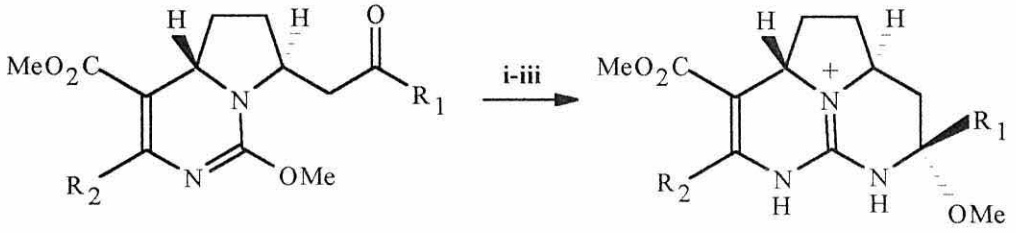
condensation with methyl acetoacetate in the presence of piperidinium acetate to give enone [91] as an equimolar mixture of geometric isomers.

Treatment of [91] with *O*-methylisourea hydrogensulphate gave a *ca.* 6:1 mixture of the *trans/cis* isomers [92]. Heating a solution of this mixture with NH_4OAc in MeOH saturated with ammonia gave tricyclic guanidine [93] as the only isolable product.



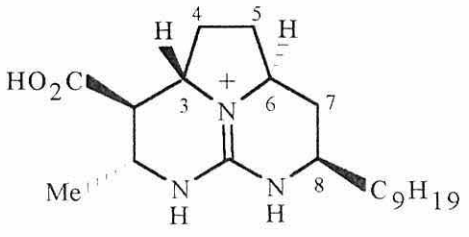
Scheme 15 *Reagents and conditions*: i) 0.2 equiv. piperidinium acetate, CH_2Cl_2, -20°C, 2 d; ii) 1.5 equiv. *O*-methylisourea hydrogensulphate, 1.8 equiv. $^iPr_2EtN$, DMSO, 75°C, 5 h, 35% from [90]; iii) NH_4OAc, $MeOH/NH_3$, 60°C, 2 d, 56%; iv) $NaCNBH_3$, MeOH, NaH_2PO_4, 65°C, 16 h, 91%; v) NaOMe, MeOH, 25°C, 12 h, >90%.

Reduction of [93] with sodium cyanoborohydride in NaH_2PO_4-buffered methanol at 65°C gave tricyclic guanidine [94] which was thought to possess the same relative stereochemistry as batzelladine A. However, comparison of the NMR data of [94] with those of batzelladine A [72] apparently suggested that they were similar, but not identical. Therefore, [94] was hydrolysed under basic conditions with concomitant epimerisation to the acid [95], for comparison with the hydrolysis product of batzelladine A [75]. Again, the two compounds were similar, but not identical. The stereochemistry of the synthetic compound [95] was elucidated by nOe experiments to be that shown (*syn* six-membered rings). This was the stereochemistry originally assigned to the tricyclic core of batzelladine A, based on comparison with known natural products. The stereochemistry of the hydrolysis product [75] was therefore assumed to be *anti*. In order to provide evidence for this conclusion, the *anti* isomer was prepared from *trans*-[92b] (Scheme 16). Reduction of *trans*-[92b] with $NaBH_4$ in isopropanol followed by ammonolysis and Dess-Martin oxidation gave tricyclic aminal [97]. Reduction again with $NaCNBH_3$ in NaH_2PO_4-buffered methanol followed by basic hydrolysis gave the desired tricyclic acid [98] which was identical to the major homologue of [75] (overleaf).



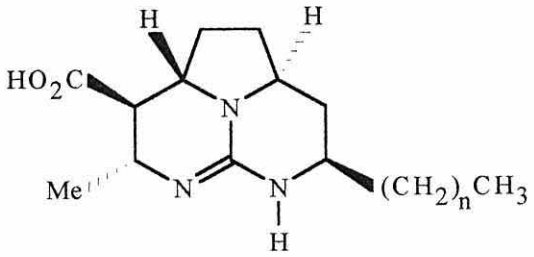
trans- [92b]

Revised structure for the hydrolysis product of batzelladine A [72]



[98] (=[75])

Scheme 16 *Reagents and conditions*: i) $NaBH_4$, ^iPrOH, 25°C; ii) NH_4OAc, $MeOH/NH_3$, 60°C, 2 d; iii) Dess-Martin, CH_2Cl_2, 25°C then MeOH, 25°C, 12 h; iv) $NaCNBH_3$, MeOH, NaH_2PO_4, 25°C, 16 h, 65°C, 5h; v) NaOH, MeOH, 25°C, 18 h.



[75], n=8 (major), 9, 10

It is interesting to note that in the case of batzelladine A, only the hydrolysis products were compared. It is reasonable to assume that detailed NMR analysis of the tricyclic compound with the ester function would provide more reliable and direct evidence for the stereochemistry.

Thus, the structure of [98] was deduced by comparison with the NMR data of the natural hydrolysis product [75]. nOe was observed between $H_{5\alpha}$ (δ 1.72) and both H_3 (δ 2.01) and H_7 (δ 3.58), and also between $H_{6\beta}$ (δ 1.57) and both H_4 (δ 3.60) and $H_{8\beta}$ (δ 1.35). Taking into account the epimerisation of the ester position during base-catalysed hydrolysis, the stereochemistry of the natural product batzelladine A [72] was concluded to be that shown in Section 2.7.1 (Page 32).

CHAPTER 3

SYNTHETIC GUANIDINIUM RECEPTORS

3.1 OVERVIEW

The strategy adopted for the synthesis of guanidinium receptor molecules depends heavily on the intended function of the host and the chemical nature of the substrate. If the lock-and-key principle is accepted, then it is clear that the receptors will need to have very specific molecular structures in order to be effective.

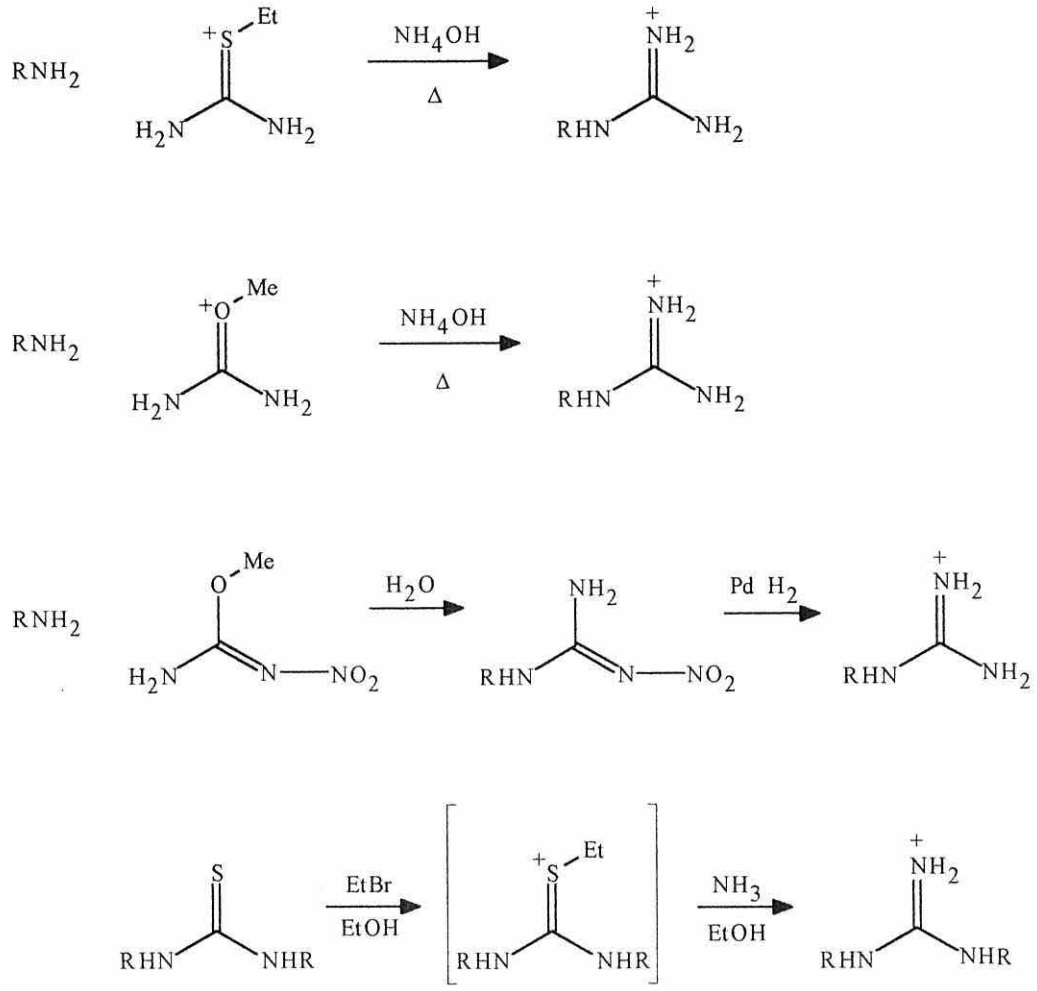
The basicity of guanidine enables it to remain protonated over a wide pH range allowing the binding of anions in a variety of aqueous environments. As may be expected, many of the synthetic receptors that have been reported have rigid frameworks, with the guanidine functions in well defined orientations. However, some research has been done towards more flexible systems, which will have the ability to adopt a number of different conformations in solution, thereby allowing for folding around a substrate.

The receptors discussed below are divided into two groups, monoguanidinium and diguanidinium receptors.[69]

3.2 SYNTHESIS OF SUBSTITUTED GUANIDINES

There are a variety of methods which can be used to prepare substituted guanidine compounds.[70] The standard methods involve the use of thioureas or isoureas where the reaction depends on the displacement of a thiol or alcohol leaving group. A summary of the four standard methods is shown in Scheme 17.

Perhaps the simplest reaction to visualise is that of an amine with *S*-ethylthiouronium bromide.[71] This gives the guanidinium compound directly, but yields are often poor, particularly with hindered amines. The second, analogous method[72] uses *O*-methylisouronium sulphate with the appropriate amine. These methods are suitable for the formation of simple monosubstituted guanidines, but if more than one guanidinium unit is required, problems arise due to electrostatic repulsion. In this case it is necessary to use 2-methyl-1-nitroisourea[70] which reacts in aqueous conditions with polyamines to give polynitroguanidines. The polynitroguanidines precipitate and can be hydrogenated under acidic conditions to form a free polyguanidine. An approach similar to the first method in which a thiourea is *S*-alkylated with ethyl bromide and the ethanethiol group eliminated *in situ* with ethanolic ammonia has also proved successful.[73]



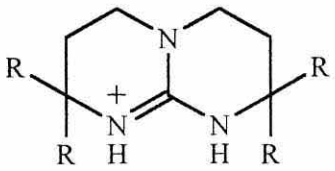
Scheme 17 Preparation of substituted guanidines.[70]

3.3 EXAMPLES OF SYNTHETIC GUANIDINIUM RECEPTORS

3.3.1 MONOGUANIDINIUM RECEPTORS

One way of imparting rigidity onto the receptor is to prepare a bicyclic guanidine and this has been exploited by a number of groups. An early example was prepared by Lehn *et al.*,[74] starting from the triamine asparagine, however the overall yield (5-10%) was rather low. The group of Schmidtchen[75] has published a number of papers on the preparation and complexing properties of bicyclic guanidines. An early communication of this group[75a] showed that the symmetrically substituted guanidines [99] were found to coordinate with a variety of guest anions such as acetate, chloride and carboxylate. The method of Lehn, *et al.*[74] was somewhat improved by Schmidtchen and Kurzmeier[75c]

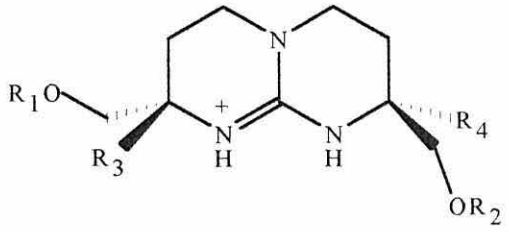
(20%) who introduce the three nitrogen atoms from two different sources in a convergent strategy which is highlighted by a high-yielding guanidinium cyclisation step. This relatively simple system is an attractive starting point for the development of abiotic receptors as it can be synthesised in chiral forms.



[99a] R= CH_3
[99b] R= $CH_2CH{=}CH_2$
[99c] R= $CH_2CH_2CH_2OH$

Direct observation of the host-guest interaction in these systems was facilitated by crystallisation of [99c].acetate. This confirmed a symmetrical bonding pattern of the acetate oxygens to the N-H groups of [99c]. In addition, a second molecule of [99c] was found to coordinate to the acetate ion *via* hydrogen-bonding to the hydroxypropyl side-chains.

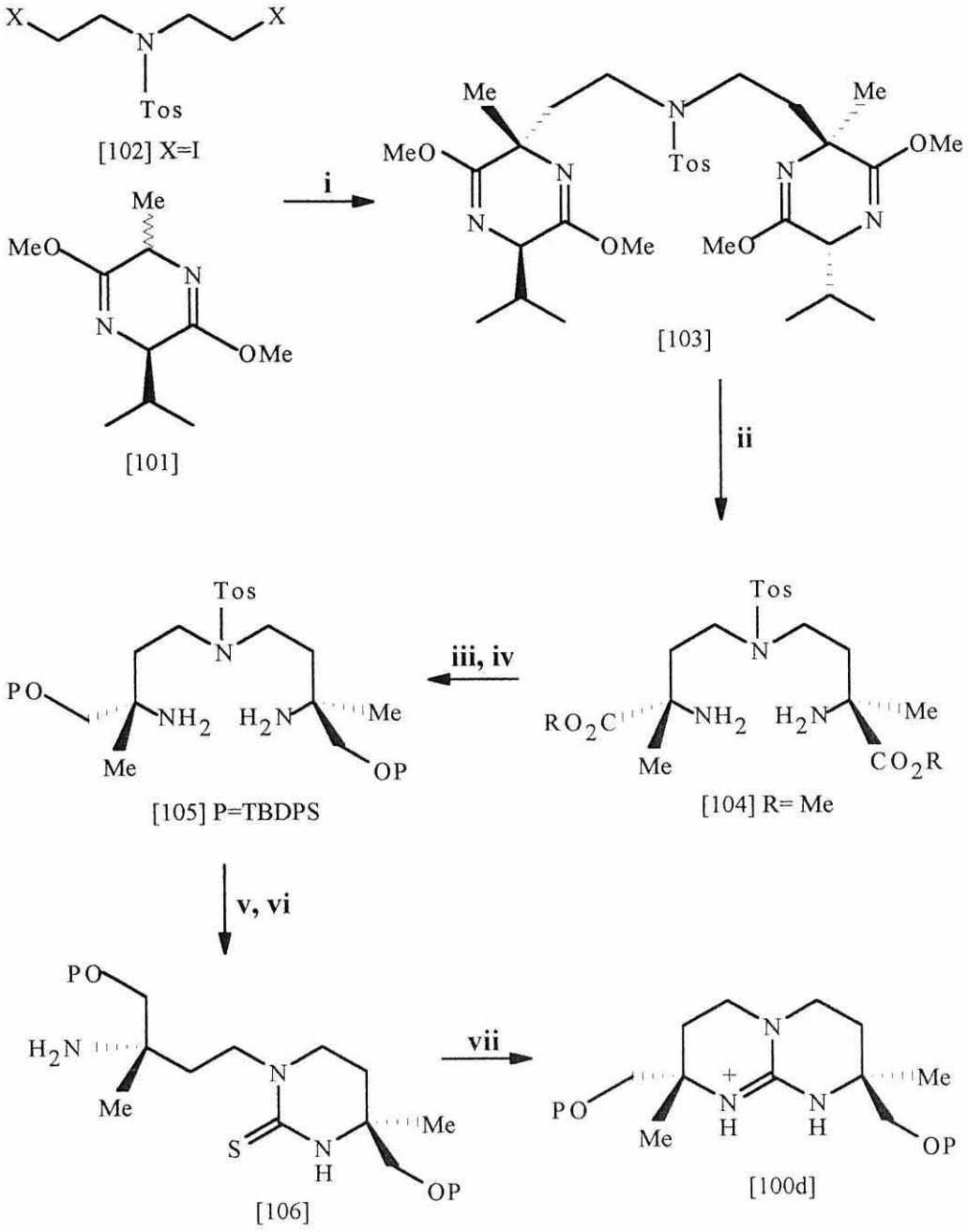
Following on from the model studies with symmetrical systems, this strategy was then extended towards chiral bicyclic guanidines [100].[75d]



[100a] $R_1{=}R_2{=}R_3{=}R_4{=}$ H
[100b] $R_1{=}R_2{=}$ TBDPS, $R_3{=}R_4{=}$ H
[100c] $R_1{=}$ TBDPS, $R_2{=}$ TBDMS, $R_3{=}R_4{=}$ H
[100d] $R_1{=}R_2{=}$ TBDPS, $R_3{=}R_4{=}$ Me

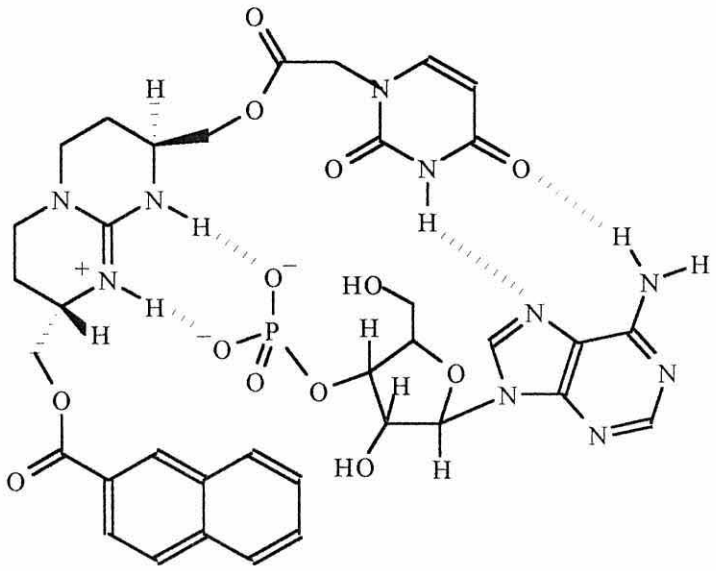
The key step in the synthesis of [100d] was asymmetric alkylation of a bislactim ether [101] according to the procedure of Schöllkopf[76] (Scheme 18). Thus, treatment of the commercially available [101] (derived from L-valine and alanine) with *n*-butyllithium

followed by quenching with diiodide [102] gave [103] in a diastereomeric excess of >95%. The *bis*-lactim ethers were cleaved under mild conditions using dilute aqueous acid at room temperature yielding amino ester [104]. Reduction to the diol by $Ca(BH_4)_2$ followed by standard protection gave silyl ether [105]. Prior to guanylation, it was necessary to remove the *N*-tosyl protecting group. This was accomplished by modification of a mild electrochemical method,[77] which left the silyl ethers intact; phenol was added as a weak acid to inhibit desilylation. Guanylation was then achieved by treatment with thiophosgene in acetonitrile giving thiourea [106]. The thiourea function was converted to the *S*-methyl thiouronium salt by methyl iodide under acidic conditions whereupon ring closure to the bicyclic guanidine [100d] could be effected by a tertiary amine such as $^{i}Pr_2EtN$. The diastereomeric purity of [100d] was estimated at >95%. The enantiomeric purity was assessed by complexation with L- and DL-acetylalanine. Carboxylate derivatives that are chiral at the α-carbon form diastereomeric complexes with chiral bicyclic guanidines. Accordingly, [100d] furnished two well separated signals when mixed with DL-acetylalanine whereas only one signal was observed for the L-acetylalanine complex.



Scheme 18 *Reagents and conditions*: i) [101], THF, -78°C, 1.01 equiv. *n*-BuLi, 30 min, then 0.4 equiv. [102], 65%; ii) [103], MeCN, 400 mol% 0.2N $HCl_{(aq)}$, 20°C, 40 h; iii) 15 equiv. $NaBH_4$, 7.5 equiv. $CaCl_2$, EtOH, -10°C, 90 min, then [104], 20°C, 2 h, $HCl_{(aq)}$ to pH 3, 70%; iv) 11.5 equiv. imidazole, 2.3 equiv. TBDPS-Cl, DMF, 20°C, then 40°C, 30 min, 71%; v) Electrolysis (Hg/Pt) at 2.5V relative to $Ag/AgNO_3$, 5 equiv. TBAB (0.2M in MeCN), 1 equiv. PhOH, 2.5h, 73%; vi) [105], MeCN, 2.75 equiv. $^{i}Pr_2EtN$, 0°C, then 1.04 equiv. $CSCl_2$, 3h; vii) AcOH, MeI, 20°C, 30 min, then $^{i}Pr_2EtN$.

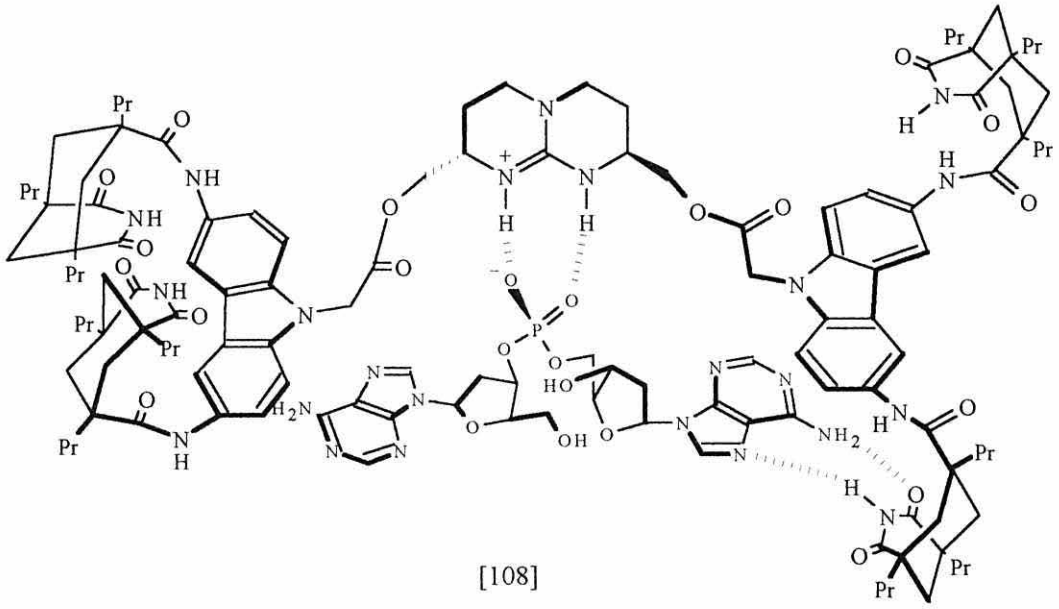
The basic bicyclic guanidine template [100] was elaborated by de Mendoza, *et al.*[79] for the selective recognition of a range of biomolecules. The first example was the preparation of a receptor for AMP nucleotides.[79a] The receptor [107] incorporates a naphthoyl residue for π-stacking and a uridine base for complementarity, thus selectively recognising 3'-AMP *via* Hoogsteen base pairing.



[107]

Figure 6. Host-guest interaction of [107] with 3'-AMP

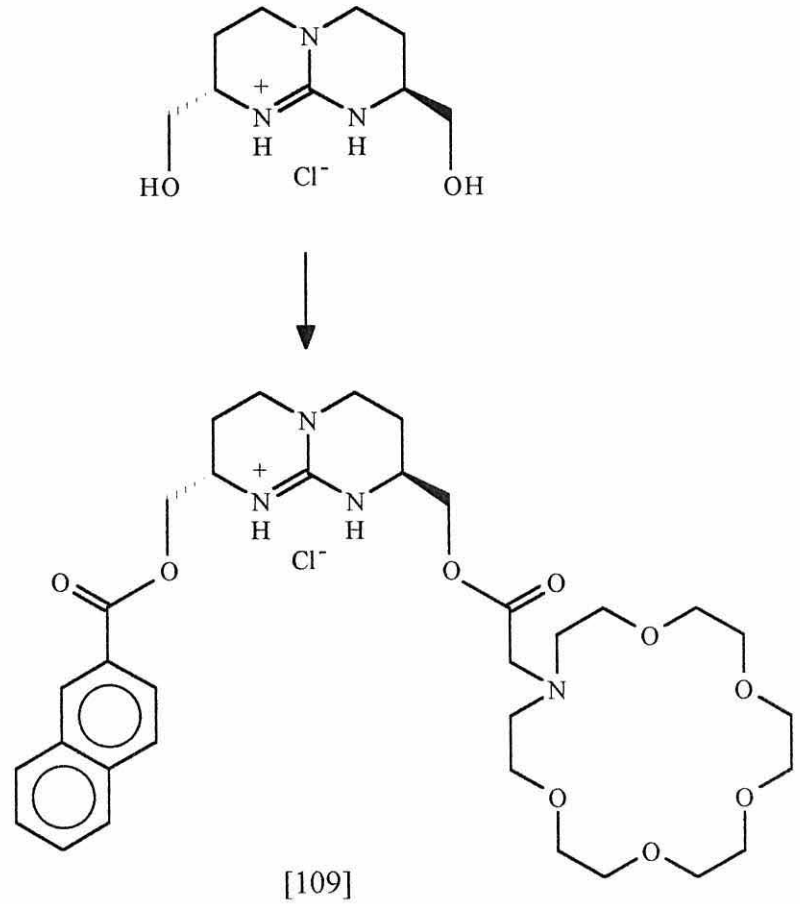
The second example was a receptor for dinucleotides, specifically deoxyadenosyladenosine (dAA). The receptor [108] was found to extract one equivalent of dAA into chlorinated solvent, but the NMR resonances were very broad, which made determination of the binding constant impossible. Therefore, the binding was studied in DMSO, with structural assignments being made on the basis of nOe enhancements. The strongest driving force for complexation is the orientation of dAA by the guanidinium salt bridge. The π-stacking of the carbazole moiety of the receptor with the purine nucleus of the dAA also stabilises the complex (Fig. 7).



[108]

Figure 7. A synthetic receptor for dAA based on Hoogsteen base pairing and ion pairing

Reported by the same research group was the design of receptor [109].[79d] This receptor was specifically designed for the recognition of amino acids and the structural features can be summarised as follows: i) non-self-complementary binding sites for carboxylate ion (the guanidinium residue) and ammonium ion (the crown ether residue); ii) an aromatic group for π-stacking with aromatic amino acids; iii) an optically active structure for enantiodifferentiation (*S*,*S* enantiomer shown). The compound was readily prepared from the basic bicyclic building block originally reported by Lehn.[74] Condensation of deprotected [100b] with 2-naphthoyl chloride gave the monosubstituted naphthoyl ester in 50% yield. Subsequent coupling with bromoacetic acid and reaction with monoazacrown ether furnished the desired receptor [109] (Scheme 19).



[109]

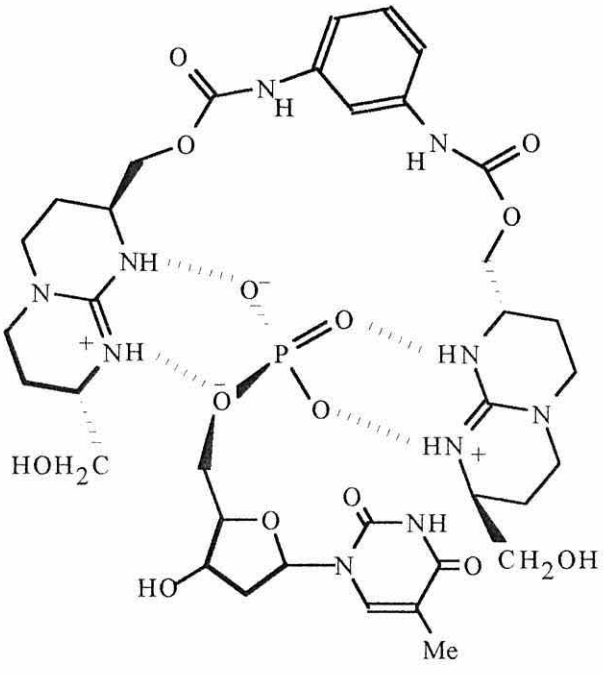
Scheme 19

The affinity of [109] for amino acids was assessed by extraction of an aqueous solution of L-Trp, L-Phe and L-Val (0.2M) with a dichloromethane solution of [109] (5.5mM). The fraction of amino acid complexed by the receptor was calculated from NMR integration. L-Trp and L-Phe (with aromatic residues) showed an efficiency of *ca.* 40% whereas L-Val (without aromatic residues) was not detected. Enantiodifferentiation was illustrated by the observation that the D-enantiomers were not extracted by the *S,S* form of the receptor. Reciprocally, use of the *R,R* enantiomer of [109] allowed extraction of the D-enantiomers to the exclusion of the corresponding L-enantiomers.

3.3.2 DIGUANIDINIUM RECEPTORS

The earliest work on diguanidinium receptors was reported by Lehn in 1979.[80] Extensive data were collected for the complexation of a series of polyguanidinium salts to phoshate and carboxylate anions. This helped to determine several guidelines for the selectivity and stability of such coordination complexes.

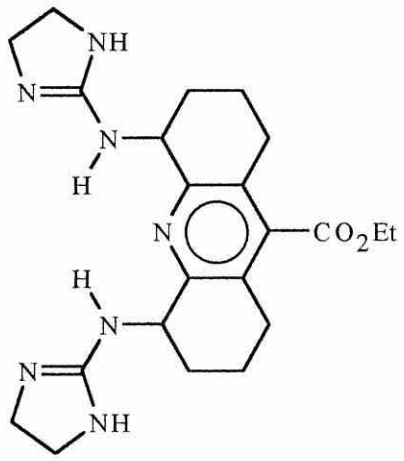
The effectiveness of a *bis*-bicyclic guanidine was tested[78] and it was found that spacing with 1,3-phenylenediisocyanate gave a *bis*-guanidinium compound which was shown to be an effective host for a variety of dicarboxylate anions such as succinate, fumarate, folate and *N*-acetylaspartate which could all be extracted from aqueous media. Note that the four secondary N-H groups of the guanidinium residues are configured into a pseudotetrahedron; it was therefore not surprising that [110] was an ideal host for tetrahedral anions such as phosphates. Furthermore, structurally well-defined adducts were observed in what appeared to be the first example of a synthetic host which could complex a mononucleotide in aqueous media (Fig. 8).



[110]

Figure 8. Host-guest interaction of thymidine-5'-phosphate with *bis*-guanidine [110]

The fourth method for the preparation of substituted guanidines described in Section 3.2 and minor variations have also proved very effective in the construction of synthetic guanidinium receptors. An interesting study was performed by Anslyn and Ariga[81] using the compound [111] based on a *bis*-aminoimidazoline system.

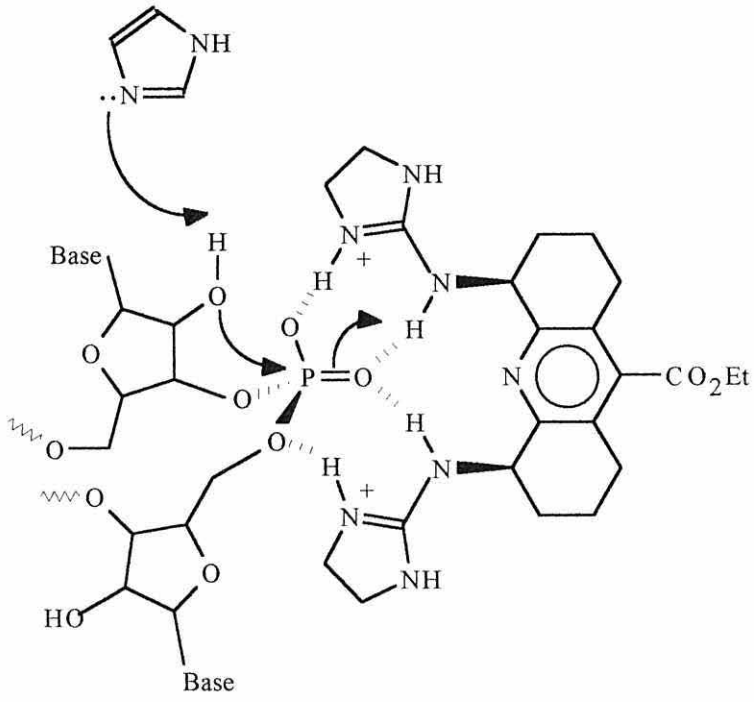


[111]

The aim was to develop a synthetic receptor to bind phosphate anion in a similar way to that seen in Staphylococcal nuclease as described earlier (Section 1.3.1, Page 4). Formerly, synthetic catalysts for RNA hydrolysis had been metal complexes and amines; unfortunately, most metal complexes have the disadvantage of being toxic.[82]

It was found that the guanidinium moieties contained within the amino-imidazoline groups of [111] form a V-shaped cleft which can accommodate the divergent oxygen lone pairs of dibenzyl phosphate. The aminoimidazoline group was chosen to alleviate the complexation of multiple phosphate anions. Indeed, it was found that in DMSO/water solution, dibenzyl phosphate formed both 1:1 and 2:1 complexes with [111]. Using a variety of techniques including gel electrophoresis and radiolabelling, Anslyn demonstrated that by using [111] in combination with imidazole itself, the rate of RNA hydrolysis was increased by 8-20 fold. As a control, imidazole and the receptor were tested independently and had little effect on the rate of reaction. This behaviour mimics the role of Staphylococcal nuclease with imidazole acting as the general base to deliver the 2'-OH to the phosphodiester linkage (Fig. 9). In addition, the aminoimidazoline groups used in these types of receptor have pK_a values equal to or greater than free guanidinium ion. This enables protonation of both guanidine moieties,

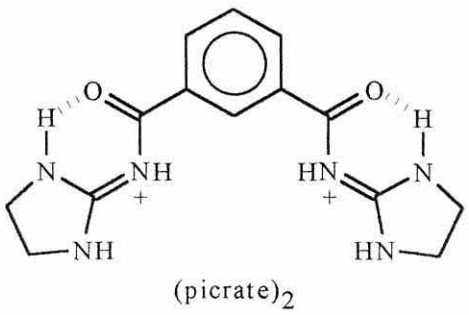
even at neutral pH, and facilitates nucleophilic addition to a bound phosphate anion for example.



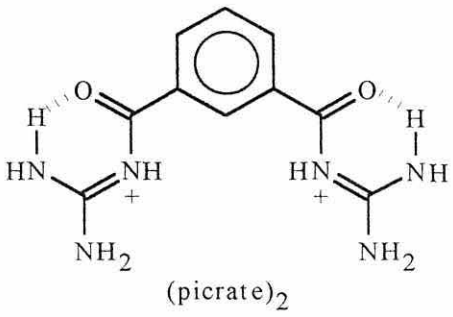
[111]

Figure 9. A water-soluble mimic for Staphylococcal nuclease

A similar type of receptor containing the imidazoline group was prepared by Hamilton *et al.*[83] It was found that in 2-(acylamino)-imidazolines, a hydrogen-bond exists between a ring N-H and the acyl C=O bonds. This has the effect of holding the molecule in the conformation shown in Fig. 10.



[112]



[113]

Figure 10. Dicationic receptors of Hamilton, *et al.*[83]

The isophthaloyl spacer was chosen to position the guanidinium residues for hydrogen-bonding to a diphosphate species. On addition of up to one mole equivalent of *tert*-butylammonium diphenylphosphate (TDPP) to the tetraphenylborate (TPB) salt of compound [112], a 1:1 adduct was formed. Addition of an excess (up to 3 equivalents) of TDPP to [113]-TPB_2, however, led to weaker association of second and third phosphate anions. It was therefore concluded that synthetic receptors of this type should be suitable for the trigonal bipyramidal phosphate intermediate as discussed earlier. It was further surmised that the reduced basicity of the acylguanidine sub-unit could enable it to function as an acid for protonation of the leaving alcohol.

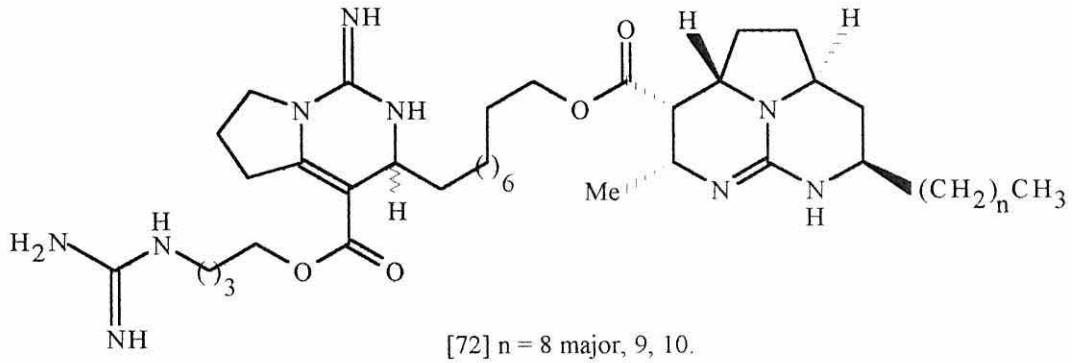
In a subsequent publication, the catalysis of phosphodiester cleavage mediated by [113] was reported.[83b] The experiment to determine the catalytic activity involved the hydrolysis of, for example, *bis*-2,4-dinitrophenyl phosphate with lutidine (2,6-dimethyl-pyridine) acting as the general base. As expected, there were significant increases in the rates of phosphodiester hydrolysis.

CHAPTER 4

SYNTHETIC APPROACH TO THE BATZELLADINE ALKALOIDS

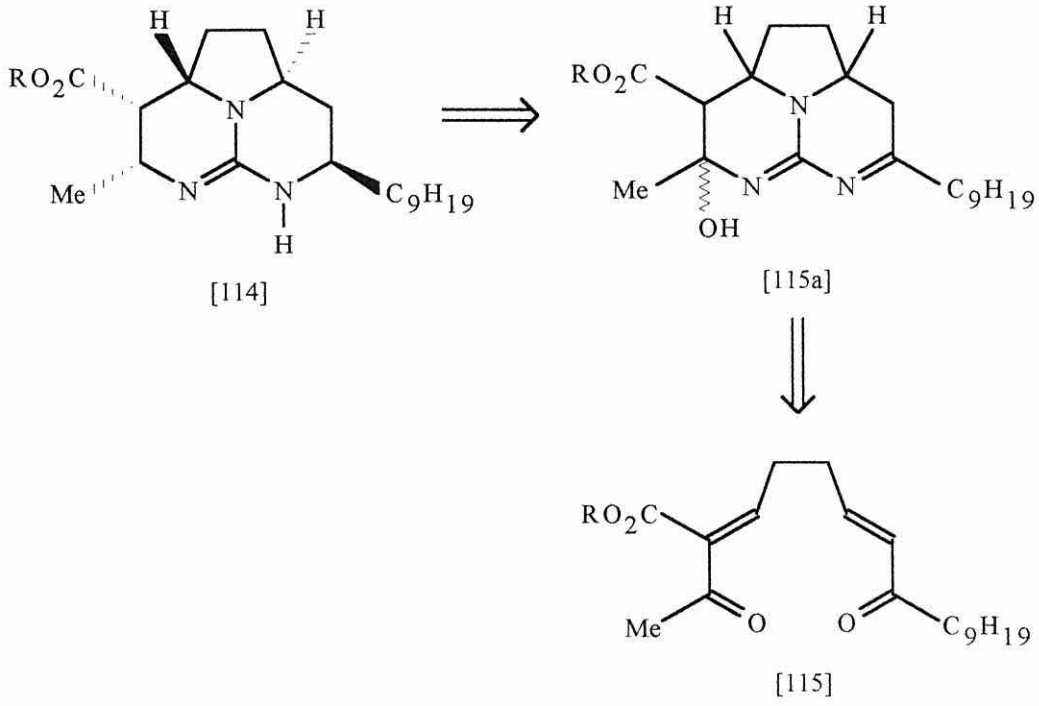
4.1 SYNTHETIC STRATEGY

As stated earlier, one of the two main aims of this project was to develop a synthetic strategy towards the tricyclic guanidinium core of batzelladine A [72]. The bicyclic moiety of [72] has the same structure as crambine A [65] and has been synthesised as a racemate and in optically pure form.[60] The novel tricyclic guanidine core was therefore deemed to be the most challenging part of the molecule and directed the initial synthetic efforts.



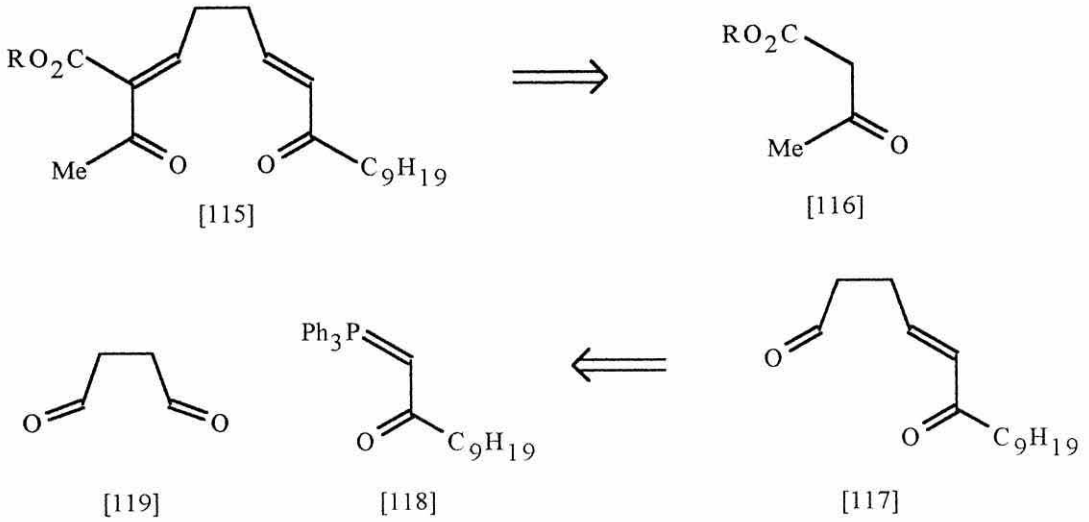
[72] n = 8 major, 9, 10.

Based on previous results within the group,[49-51] it was proposed that the tricyclic core of batzelladine A [114] could be accessed by a proposed biomimetic approach, *via* a reductive double Michael addition of guanidine to a suitably functionalised *bis*-α,β-unsaturated enone system [115]. The Michael addition of guanidine to each enone followed by ring closure at the carbonyl groups would give intermediate hemiaminal [115a]. Reduction by hydride ion equivalent should furnish tricyclic guanidine [114]. The retrosynthetic analysis for [115] is shown in Scheme 20. It appears that [115] should be accessible in reasonably short order using standard organic chemistry. The disubstituted olefin is constructable by Wittig chemistry and the trisubstituted olefin by Knoevenagel condensation.



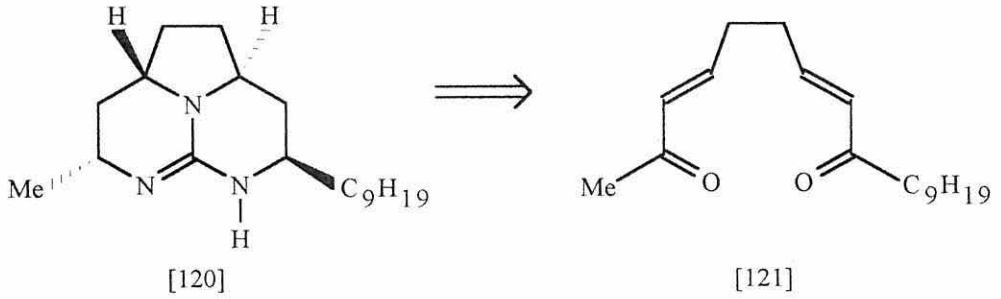
Scheme 20

A retrosynthetic analysis of [115] is shown in Scheme 21. Thus, aldehyde [117] is prepared by a Wittig reaction of phosphorane [118] with an excess of succinaldehyde [119]. The alkylation of the commercially available acetylmethylene triphenylphosphorane with alkyl halides has been reported and is described in Section 4.2. Trisubstituted olefin [115] may be accessible *via* Knoevenagel condensation[84] of aldehyde [117] with β-ketoester [116]. This would give the precursor to the tricyclic core of batzelladine A in a linear 3-step sequence from readily available starting materials.



Scheme 21

From previous work towards other natural products containing guanidine,[85] it was apparent that incorporation of the ester functionality into the cyclisation precursor may not have been a trivial step in the synthesis. It was therefore decided to investigate the preparation of a relatively simple unfunctionalised tricyclic guanidine model compound [120] in order to gauge the feasibility and stereochemical implications of this synthetic rationale. The retrosynthesis is basically the same, but with a Wittig reaction in place of the Knoevenagel reaction giving a simple *bis*-α,β-unsaturated ketone [121] (Scheme 22).



Scheme 22

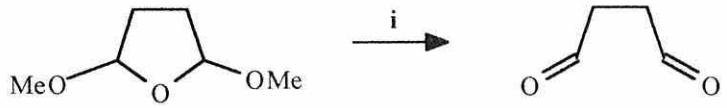
In the planned syntheses illustrated above, the guanidine will be added as the free base in unprotected form. As described in Section 1.2, guanidine is one of the strongest

organic bases known with a pK_a comparable to sodium hydroxide. It might therefore be postulated that any synthetic organic chemistry towards molecules of even moderate complexity may be fraught with danger. Despite the report by Sugino and Tanaka[52] in 1968 that free guanidine undergoes addition to alkyl acrylates giving pyrimidinediones (Section 2.5.2, Page 19), this type of reaction appears to have received rather scant attention.[86-88] Considering the rapidity of access into potentially complex heterocyclic frameworks coupled with a number of interesting results within the group, this rationale was deemed worthy of a full investigation.

4.2 SYNTHESIS OF THE TRICYCLIC MODEL [120][89]

4.2.1 PREPARATION OF THE *BIS*-α,β-UNSATURATED KETONE PRECURSOR [121]

In order to test the applicability of this methodology to the batzelladine metabolites, the first target molecule was the simple tricyclic model [120]. The starting point in the synthesis was the commercially available phosphorus ylid, acetylmethylene triphenylphosphorane [122] (Scheme 24). Succinaldehyde[90] [119] was prepared by hydrolysis of the synthetic equivalent, 2,5-dimethoxytetrahydrofuran with 0.1N hydrochloric acid (Scheme 23). It was found that succinaldehyde can polymerise over time (~weeks) even if stored at -15°C. This polymerisation was indicated by developing signals at δ 5.5 ppm in the ^{1}H NMR spectrum. Therefore, [119] was prepared and distilled immediately prior to use.



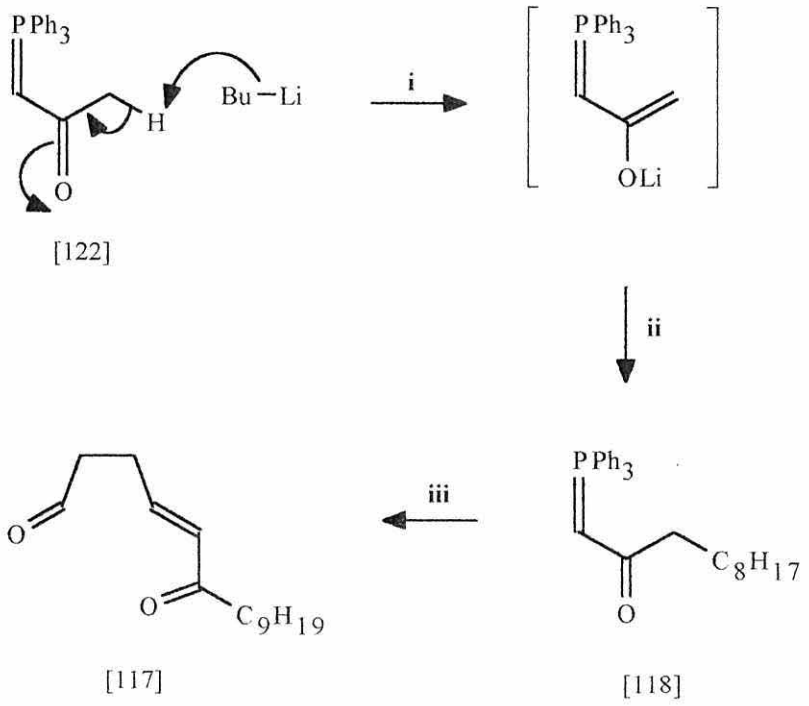
[119]

Scheme 23 *Reagents and conditions*: i) 0.1N $HCl_{(aq)}$, Δ, 2 h, 49%.

According to the procedure of Taylor and Wolf,[91] acetylmethylene triphenylphosphorane [122] can be deprotonated by a strong base and alkylated α to the carbonyl group. Thus, treatment of a THF suspension of [122] at -78°C with 1.1 equivalents of *n*-butyllithium gave a deep red solution due to the formation of the lithium enolate. This

was then trapped *in situ* with 1.4 equivalents of *n*-octyl iodide which gave, on work-up, the alkylated phosphorane [118] in quantitative yield. Also present was the slight excess of *n*-octyl iodide indicated by a triplet at δ 3.20 ppm in the ^{1}H NMR spectrum of [118]. It was not necessary to purify the phosphorane at this stage as the excess iodide was easily removed by chromatography after the following Wittig olefination.

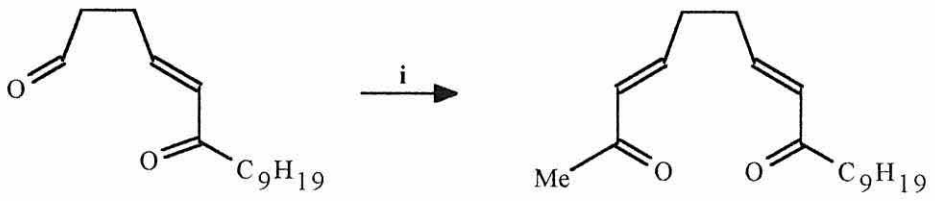
Wittig reaction of [118] with 3 equivalents of succinaldehyde (Scheme 24) gave the aldehyde [117] in 71% yield together with a small amount (*ca.* 10-15%) of the symmetrical *bis*-α,β-unsaturated ketone [124d] (Section 4.4, Page 67) as a by-product; these two compounds were easily separable by column chromatography.



Scheme 24 *Reagents and conditions*: i) 1.1 equiv. *n*-BuLi, THF, -78°C, 1 h; ii) 1.4 equiv. n-$C_8H_{17}I$, 20°C, 16 h, quant.; iii) 3.0 equiv. succinaldehyde, CH_2Cl_2, 20°C, 24 h, 71%.

Further Wittig reaction of [117] with 1.2 equivalents of acetylmethylene triphenylphosphorane [122] gave the desired *bis*-α,β-unsaturated ketone [121] in 66% yield after chromatography (Scheme 25). It was found, somewhat surprisingly, that [121] has the same R_f (silica gel TLC) as the starting aldehyde [117]. It was therefore necessary in the early runs to monitor the second Wittig reaction by ^{1}H NMR spectroscopy in order to

optimise the reaction conditions. Note that in both Wittig reactions, the geometry of the olefin is exclusively *trans* (within the limits of NMR spectroscopy).



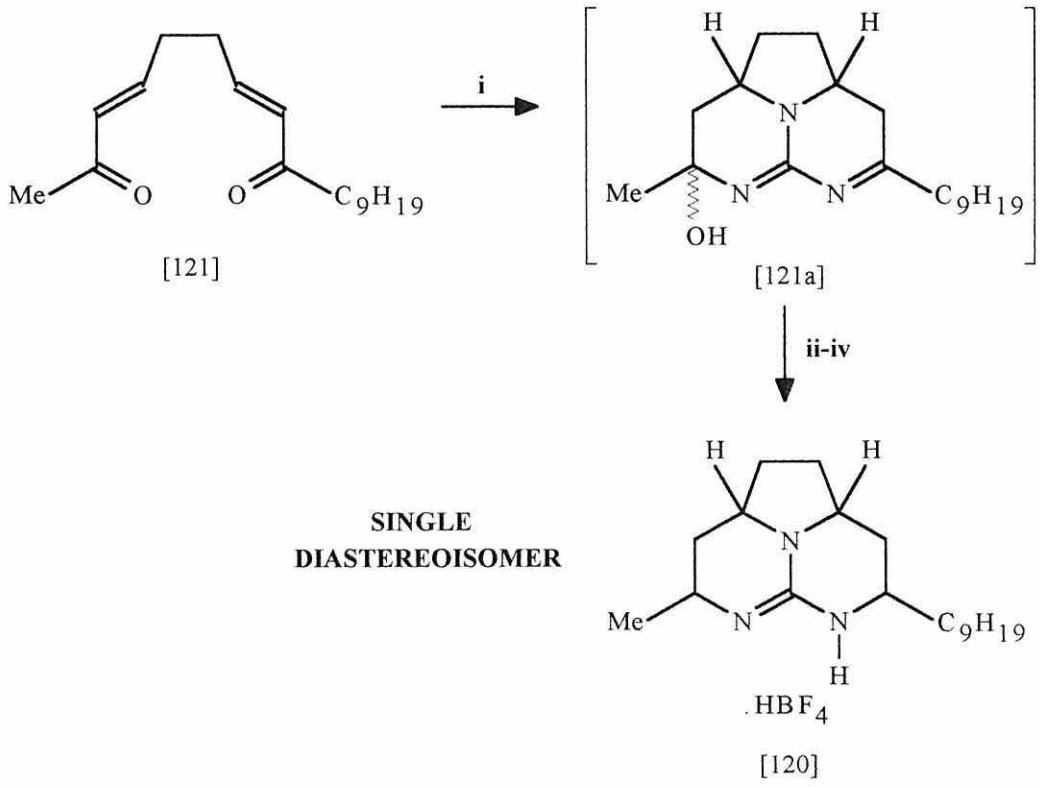
[117] [121]

Scheme 25 *Reagents and conditions*: i) 1.2 equiv. $MeCOCHPPh_3$, CH_2Cl_2, 20°C, 24 hours, 66%.

This was inferred from the magnitude of the coupling constant between the olefinic protons (J = 15.9Hz). This is a well known consequence of Wittig reactions of α-stabilised phosphorus ylids.[92]

4.2.2 REDUCTIVE GUANIDINE ADDITION-CYCLISATION

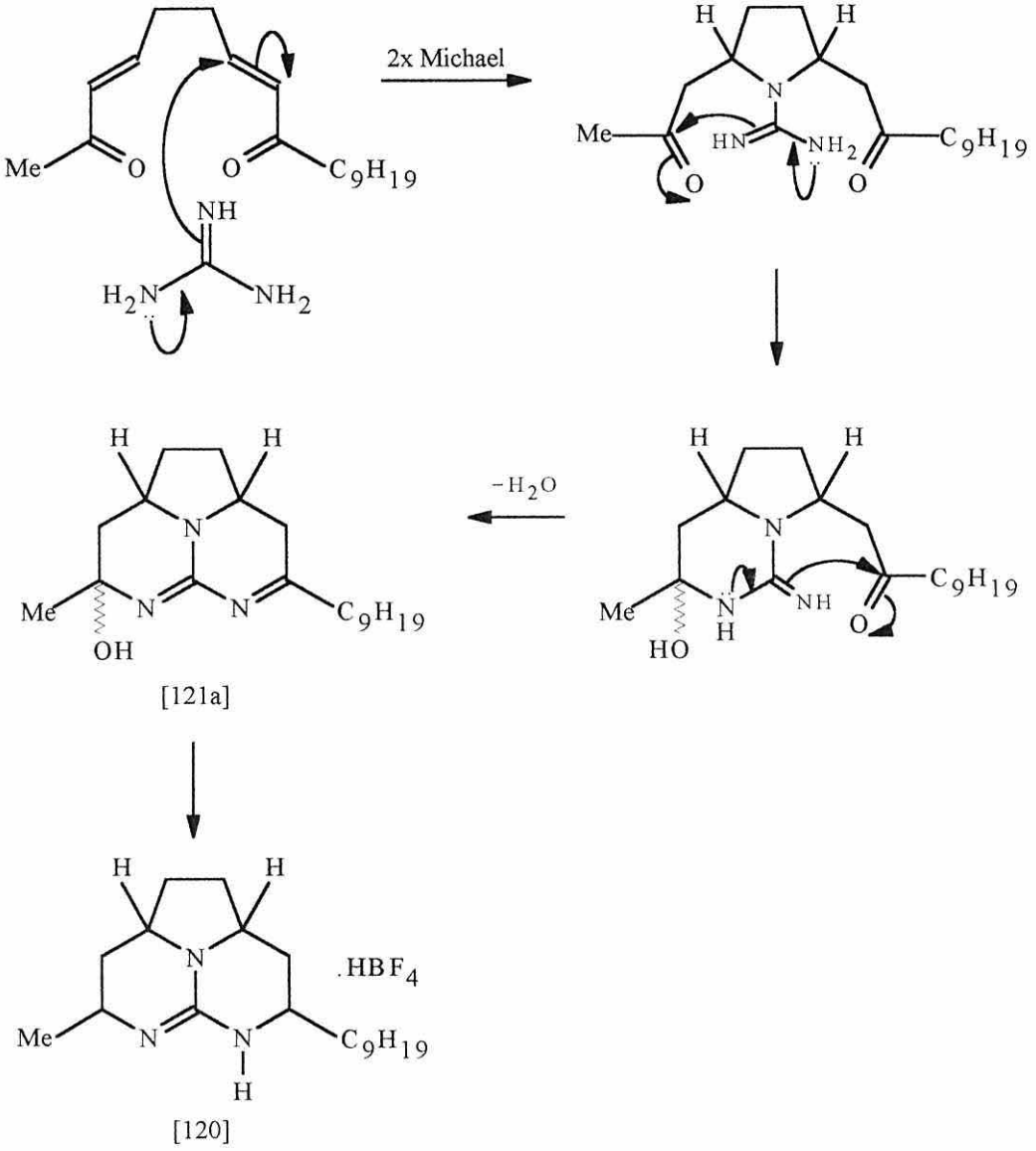
By relatively simple and reproducible chemistry, then, it proved possible to access the precursor to guanidine addition in very short order and in good overall yield (47%). Obviously the key step in the synthesis was the reductive guanidine addition-cyclisation sequence and with a multigram stock of the precursor [121], the reaction was investigated. It was found that treatment of [121] with guanidine in DMF at 0-20°C over 5 hours followed by *in situ* reduction of the presumed intermediate hemiaminal [121a] with sodium borohydride ($MeOH/H_2O$) gave tricyclic guanidine [120] as a *single diastereoisomer* in 32% overall yield from [121] after chromatography and trituration (Scheme 26). No minor isomers were detectable by ^1H or ^{13}C NMR spectroscopy of either the crude reaction mixture or the purified material. The structural and stereochemical assignment of [120] is discussed in Section 4.3.



Scheme 26 *Reagents and conditions*: i) 1 equiv. guanidine, DMF, 0-20°C, 5 h; ii) 3:1:3 DMF:H_2O:MeOH (v/v), then 6 equiv. $NaBH_4$, 0-20°C, 16 h; iii) 1M $HCl_{(aq)}$; iv) sat. $NaBF_{4(aq)}$, 32% overall.

This extremely encouraging observation was somewhat surprising as in previous work[49-51] it was found that additon of guanidine to *bis*-α,β-unsaturated ketones leads to the preferential formation of *cis*-substituted pyrrolidines with *ca.* 4:1 selectivity. Also, with 4 stereocentres and three rings being formed diastereospecifically in one synthetic step, this method is an extremely effective way of generating these tricyclic compounds.

Although the exact sequence of reactions is unknown, a mechanism for the guanidine addition-cyclisation can be visualised (Scheme 27). Double Michael addition of one nitrogen of guanidine to the β-position of the α,β-unsaturated ketone [121] establishes the pyrrolidine ring and two stereocentres. Ring closure at the two carbonyl groups then gives the assumed intermediate hemiaminal [121a]. Reduction by sodium borohydride then takes place stereospecifically from the same face as the pyrrolidine bridge hydrogens. Chelation of the boron atom to the nitrogen atoms could well be assisting the selectivity of this reduction step.



Scheme 27

4.3 DETERMINING THE STEREOCHEMISTRY OF TRICYCLE [120]

4.3.1 NOE STUDIES ON [120][93]

In order to further confirm the connectivity of the tricyclic compound a TOCSY experiment was performed (CD_3OD, 30°C). Figure 11 shows the structure and the various nOe interactions around the tricycle.

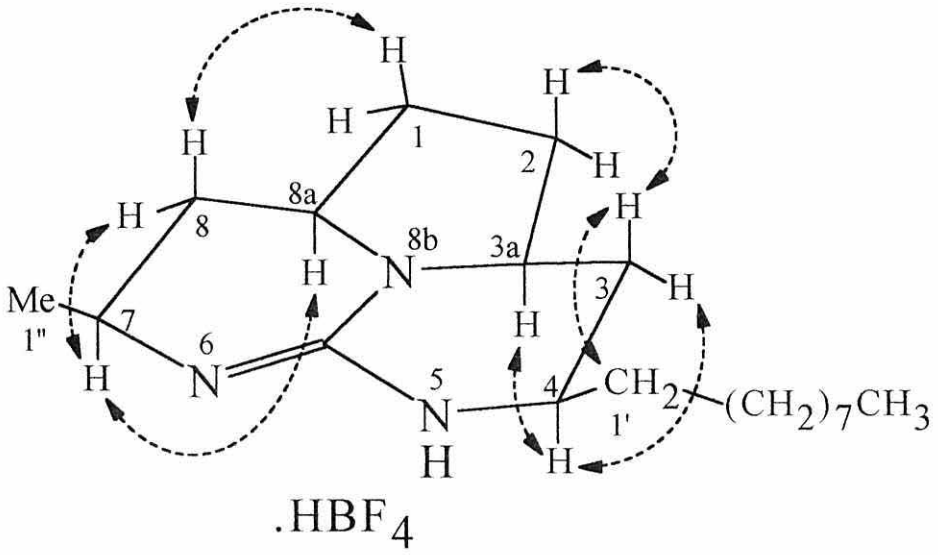


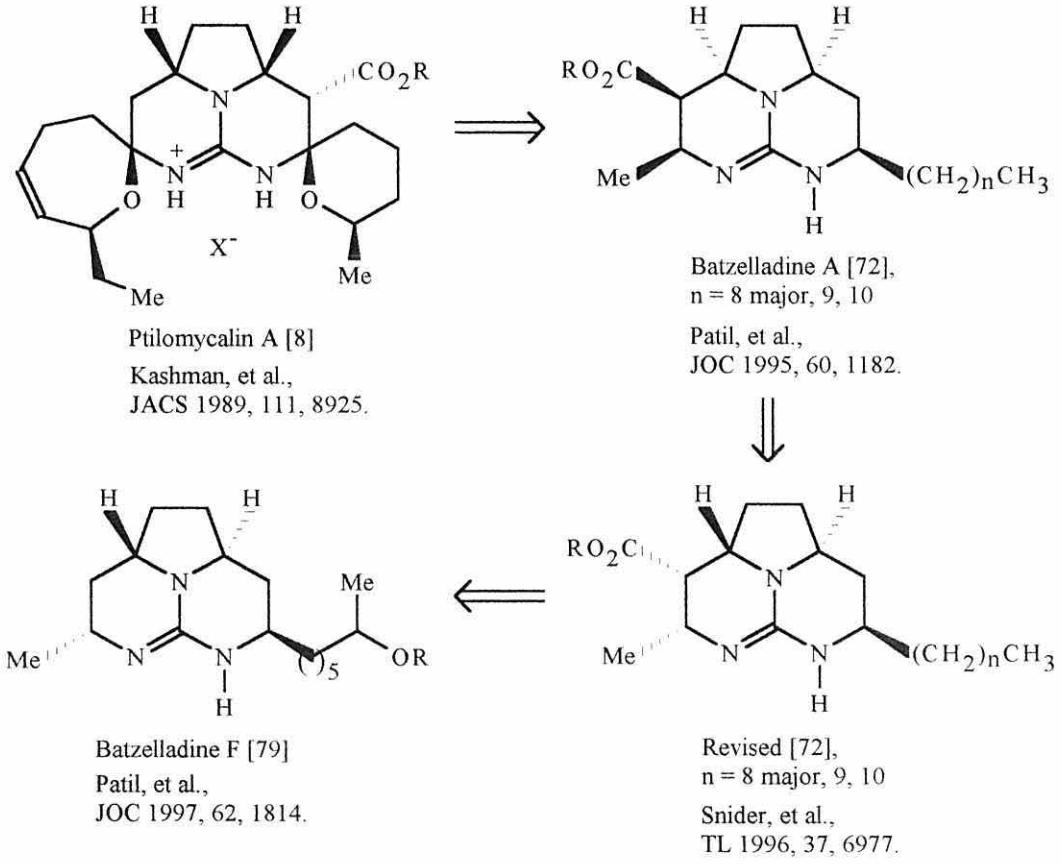
Figure 11

Irradiation of H-3a and H-8a (both at δ 3.74-3.75) led to nOe at H-4 and H-7, which indicated that H-7 and H-8a are *syn* as are H-4 and H-3a. This conclusion was supported by the magnitude of coupling constants and nOe around the ring. Thus, irradiation of the H-1' methylene pair at δ 1.56 led to an enhancement of one of the H-3 protons at 1.25 ppm. This proton signal was obscured by the methylene envelope at δ 1.25-1.35, but selective irradiation showed it to be a quartet, with three large (*ca.* 10Hz) couplings. This identified it as $H\text{-}3_{ax}$ proton, with typical *trans*-diaxial couplings to H-3a and H-4. Irradiation of H-1' also gave nOe at H-4; however, the enhanced signal appeared as a double doublet. The reason for this apparent change in multiplicity is unclear.

An nOe between Me-1' and $H\text{-}8_{ax}$ could not be seen because both protons resonated at δ ~1.26. However, irradiation of the H-1/2 multiplet at δ 1.68 enhanced both the $H\text{-}3_{ax}$ quartet, and also a second quartet at δ 1.26, which could be identified as $H\text{-}8_{ax}$. Thus it was possible to deduce that the relative stereochemistry of [120] was that shown

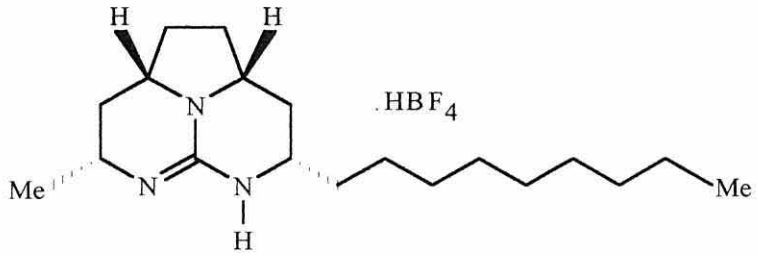
above. Unfortunately, it was not possible to observe nOe between H-3a and H-8a directly which would have confirmed the overall relative stereochemistry.

Shortly after the preparation of the tricyclic model compound [120], there was a report in the literature[65] detailing the isolation of four new members of the batzelladine family of alkaloids including batzelladine F [79]. It was somewhat surprising to find that on comparison of the spectroscopic data of [120] with [79] (Table 4), the NMR chemical shifts (CD_3OD) of respective protons and carbons are almost exactly the same. As stated, the stereochemistry of batzelladines A and D were originally assigned the *syn* configuration in line with ptilomycalin A [8] until the revision of Snider *et al.*,[68] to the *anti* configuration. On the basis of this revision, Patil *et al.*[65] assigned both tricyclic units of batzelladine F the *anti* configuration (Scheme 28), apparently without any concerted effort towards obtaining absolute proof of this. This is clearly a dangerous supposition

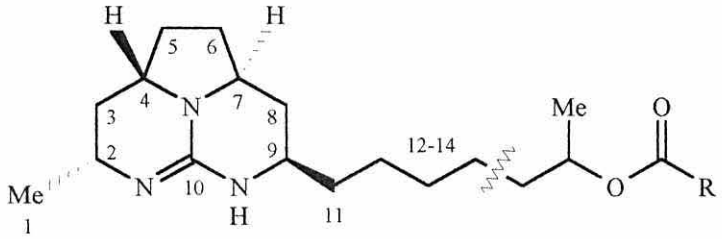


Scheme 28

and it was decided to attempt a synthesis of the left-hand-portion of batzelladine F in an attempt to overcome this shortfall (Chapter 5).



[120]



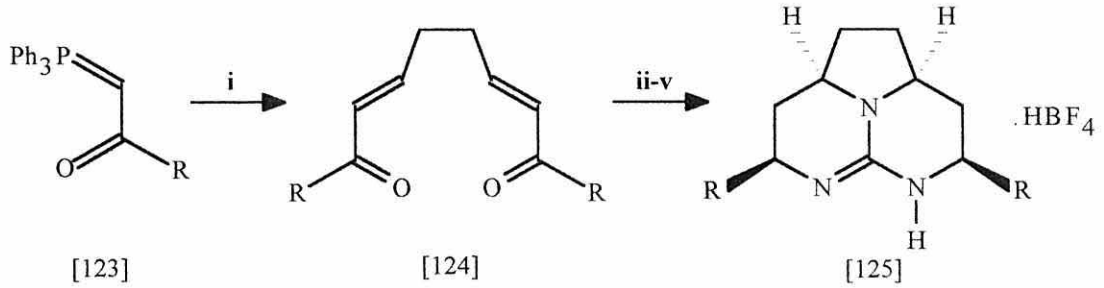
[79]

	TRICYCLIC MODEL [120]		BATZELLADINE F [79]	
POSITION	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
1	1.26	20.70	1.26	20.7
2	3.54	47.26	3.52	47.2
3α 3β	1.26 2.20	36.63	1.25 2.20	36.9
4	3.75	57.50	3.72	57.5
5α 5β	1.68 2.20	30.99	1.67 2.20	31.1
6α 6β	1.68 2.20	30.99	1.67 2.20	31.1
7	3.74	57.45	3.72	57.4
8α 8β	1.25 2.25	34.69	1.21 2.28	34.8
9	3.42	51.58	3.40	51.6
10	-	151.20	-	151.2
11	1.56	35.82	1.54 1.60	35.8
12-14	1.3-1.4	26.18 30.39	1.30	26.2 30.5

Table 4

4.4 PREPARATION OF SYMMETRICALLY SUBSTITUTED MODELS

The *syn* relationship of the two six-membered rings and therefore the *cis* stereochemistry of the pyrrolidine ring was inferred from previous work. However, crystals of [120] suitable for X-ray diffraction analysis proved elusive. In order to overcome this shortfall and further test the generality of this methodology, a series of symmetrically substituted tricyclic models were prepared (Scheme 29) with a view to obtaining crystalline material suitable for X-ray diffraction. The yields are shown in Table 5.



[123] [124] [125]

Scheme 29 *Reagents and conditions*: i) 0.4 equiv. succinaldehyde, CH_2Cl_2, 20°C, 24-48 h; ii) 1 equiv. guanidine, DMF, 0-20°C, 5 h; iii) 3:1:3 DMF:H_2O:MeOH (v/v), then 6 equiv. $NaBH_4$, 0-20°C, 16 h; iv) 1M $HCl_{(aq)}$; v) sat. $NaBF_{4(aq)}$.

Entry	Precursor [124] yield (%)	Tricycle yield [125] (%)
a (R=Me)	74	33
b (R=Ph)	68	32
c (R=C_5H_5)	54	27
d (R=C_9H_{19})	36†	22

† obtained as a by-product in the preparation of aldehyde [117]

Table 5

Despite considerable effort, these compounds proved very difficult to crystallise. Serendipitously, it was found that the diphenyl compound [125b] could be isolated as a 1:1 complex with triphenylphosphine oxide (TPPO) and a crystal structure was obtained.

The TPPO was present as an impurity from the Wittig reaction to generate the precursor [124b]. The phenyl resonances of TPPO were obscured by the phenyl resonances of both [124b] and [125b]. Calculation of the ^{1}H NMR integral showed *ca.* 5% TPPO to be present in [125b]. The ORTEP representation of the diphenyl analogue and the coordination to TPPO is shown in Figure 12.

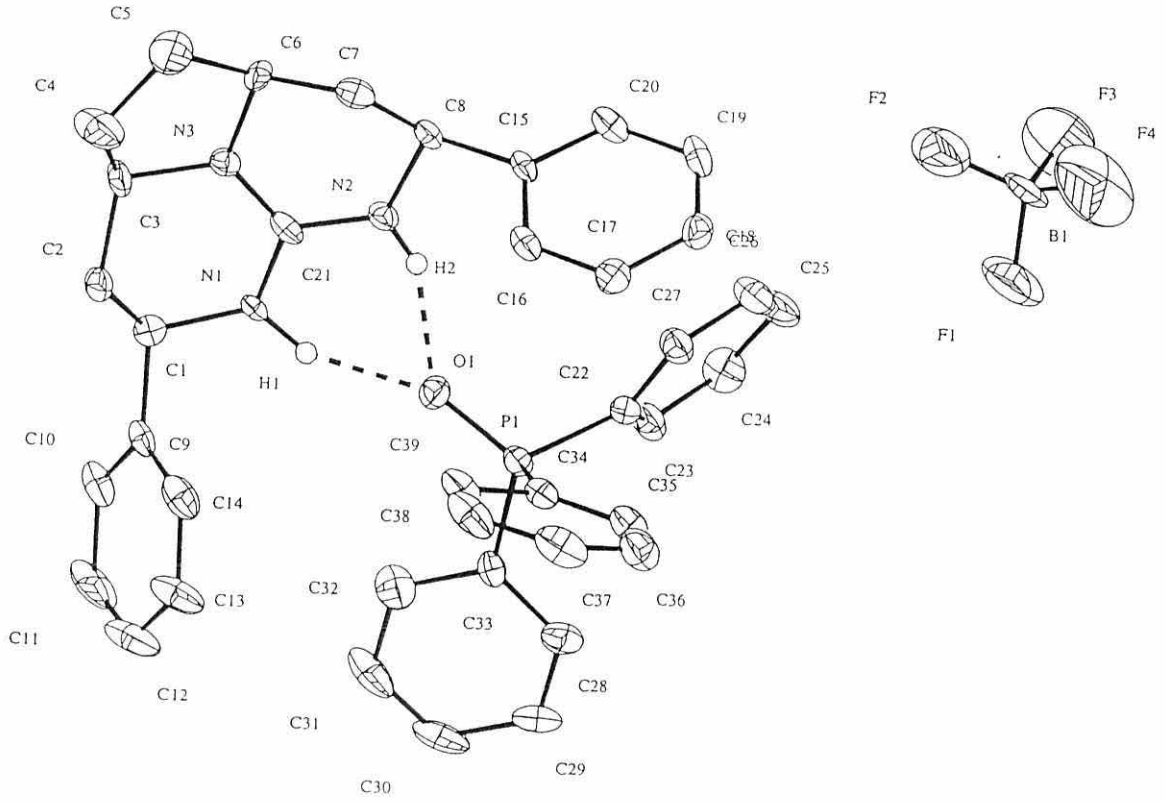


Figure 12 Crystal Structure of the Diphenyl Analogue [125b]

The details of this crystal structure revealed a unique arrangement in which the oxygen atom of triphenylphosphine oxide is coordinated to the guanidinium cation *via* two N-H···O hydrogen bonds. The interatomic distances [N(1)-H(1)....O(1)/ N(2)-H(2)....O(1)] are: N-H = 1.01(4)/0.91(5), H....O = 1.83(5)/1.97(5), N....O = 2.780(5)/ 2.817(5)Å, <N-H...O = 154(4)/154(4)°, indicating quite strong hydrogen bonding.

It is to be noted that in related guanidinium fluoroborates the guanidine N-H groups are always hydrogen bonded to the BF_4^- anions but in the case of [125b], hydrogen bonding occurs preferentially through the oxygen of triphenylphosphine oxide. In fact, triphenylphosphine oxide was initially used to modify substrate properties by complexation[94] but has been found to yield high quality crystalline complexes with a large number of organic compounds.[95]

It was found that the stereochemistry observed in [125b] is a *syn* arrangement of the two six-membered rings and consequently *cis* pyrrolidine stereochemistry. This implied that the stereochemistry of tricycle [120] was the same. At the time of this finding, this was the stereochemistry assigned to the four corresponding stereocentres of batzelladines A and D which contain the same tricyclic core with an ester substituent. The stereochemistry of the natural products was later revised[68] to the *anti* configuration. It appears, therefore, that in these simple examples, our synthetic method provides the wrong stereochemistry. However, performing the guanidine addition-reduction step with an ester functionality in the molecule could certainly have implications for the stereoselectivity.

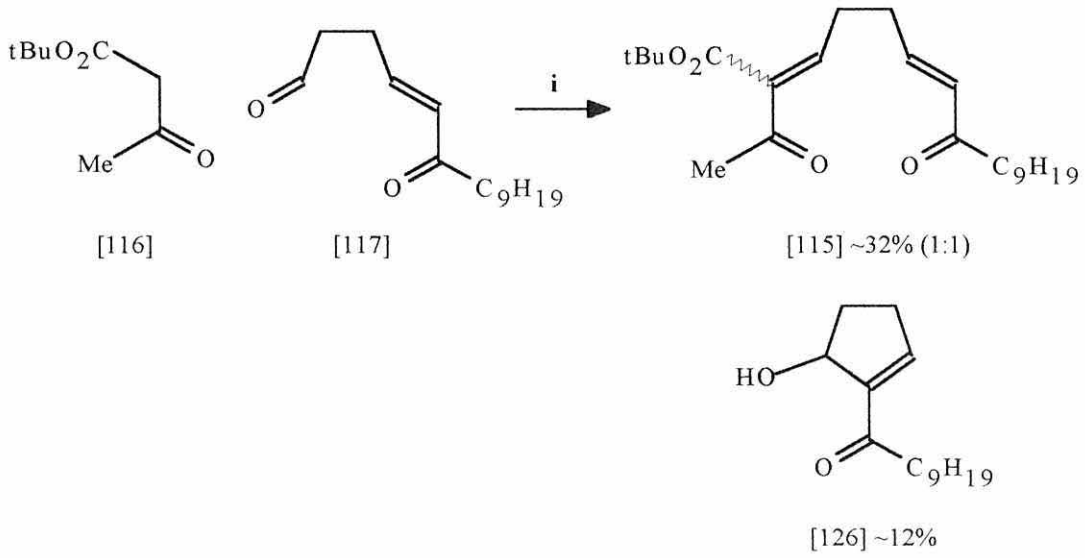
4.5 EXTENSION TOWARDS THE TRICYCLIC CORE OF BATZELLADINE A

Having established the facile nature of the proposed biomimetic approach to the tricyclic moiety of the batzelladine alkaloids, the methodology was extended in an attempt to incorporate the ester functionality found in the natural products.

4.5.1 KNOEVENAGEL CONDENSATION

A Knoevenagel condensation[84] of the previously prepared aldehyde [117] with *tert*-butyl acetoacetate [116] in the presence of piperidine or piperidine acetate was envisaged to give the ester-substituted *bis*-α,β-unsaturated ketone [115] (Scheme 30). From previous studies towards Ptilomycalin A core, we anticipated significant difficulties with the Knoevenagel condensation.

After extensive efforts it was found that the reaction could be effected by treatment of the β-ketoester [116] with piperidine at -20°C for fifteen minutes *prior* to addition to a cooled (-20°C) solution of the aldehyde [117]. The presence of a drying agent (Na_2SO_4) was also required for the reaction to proceed.



[116] [117] [115] ~32% (1:1)

[126] ~12%

Scheme 30 *Reagents and conditions*: i) 1.1 equiv. [116], 1 equiv. piperidine, Na_2SO_4, -20°C, 15 min., then 1 equiv. [117], -20°C, 4 h, 32% (*E*/*Z* 1:1).

A reaction time of 3 hours gave the required compound [115] as an approximately 1:1 mixture of geometric isomers in 32% yield with complete consumption of the aldehyde [117]. Nevertheless, the Knoevenagel condensation proved to be an extremely capricious reaction which was successful in only three runs on a small scale (*ca.* 200mg of [117]) and was not amenable to scale-up. For these reasons, only approximately 200mg of the target compound [115] were obtained.

Further chromatography of the higher polarity material afforded 12% of a Baylis-Hillman product [126] (see section 4.5.3). The remainder of the material consisted of unidentifiable decomposition products. The inconsistency of this Knoevenagel condensation was particularly baffling when a similar reaction was reported by Snider.[68]

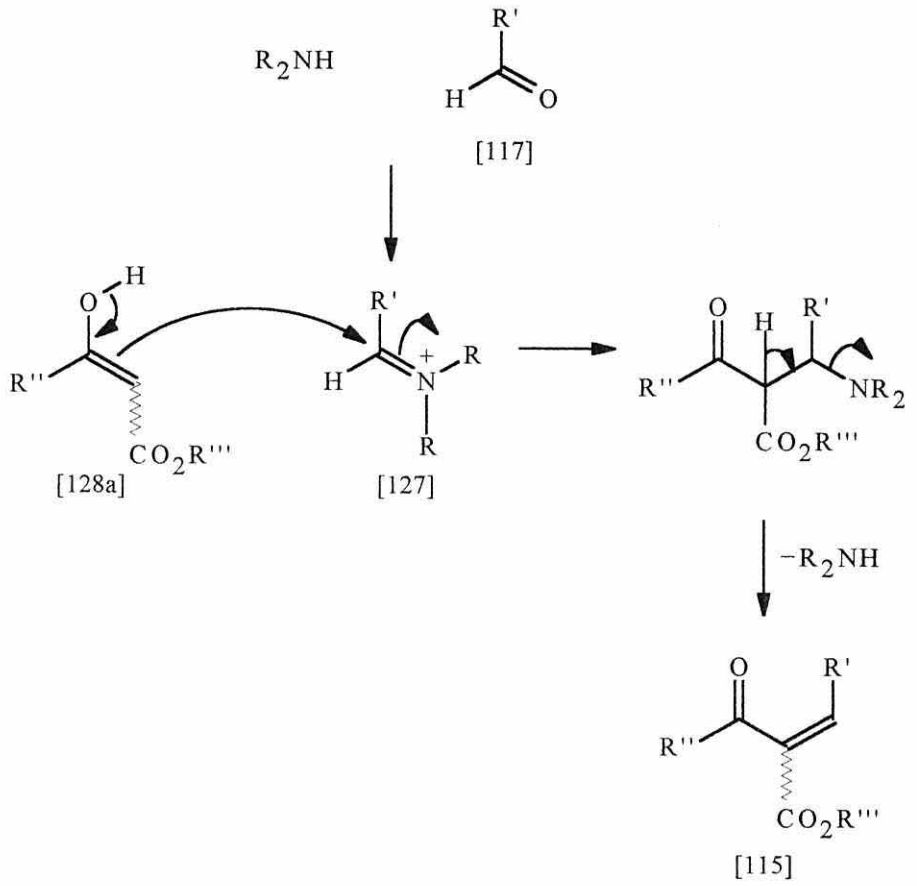
Separation of the Knoevenagel products by simple chromatography proved difficult. However, at present, isolation of a small quantity (5mg) of the less polar isomer has been accomplished. It may be possible to determine the stereochemistry about the trisubstituted double bond by nOe studies, or indeed by measuring the vicinal ^{13}C-^{1}H coupling between the olefinic proton and the carbonyl groups of the ester and ketone. This method for determining olefin stereochemistry was reported by Letcher and Acheson[96] who elucidated the geometry of a variety of trisubstituted olefins.

4.5.2 MECHANISM

Three realistic mechanisms for the Knoevenagel condensation of aldehyde [117] with β-ketoester [116] in the presence of an amine can be envisaged.

a) The Knoevenagel Mechanism

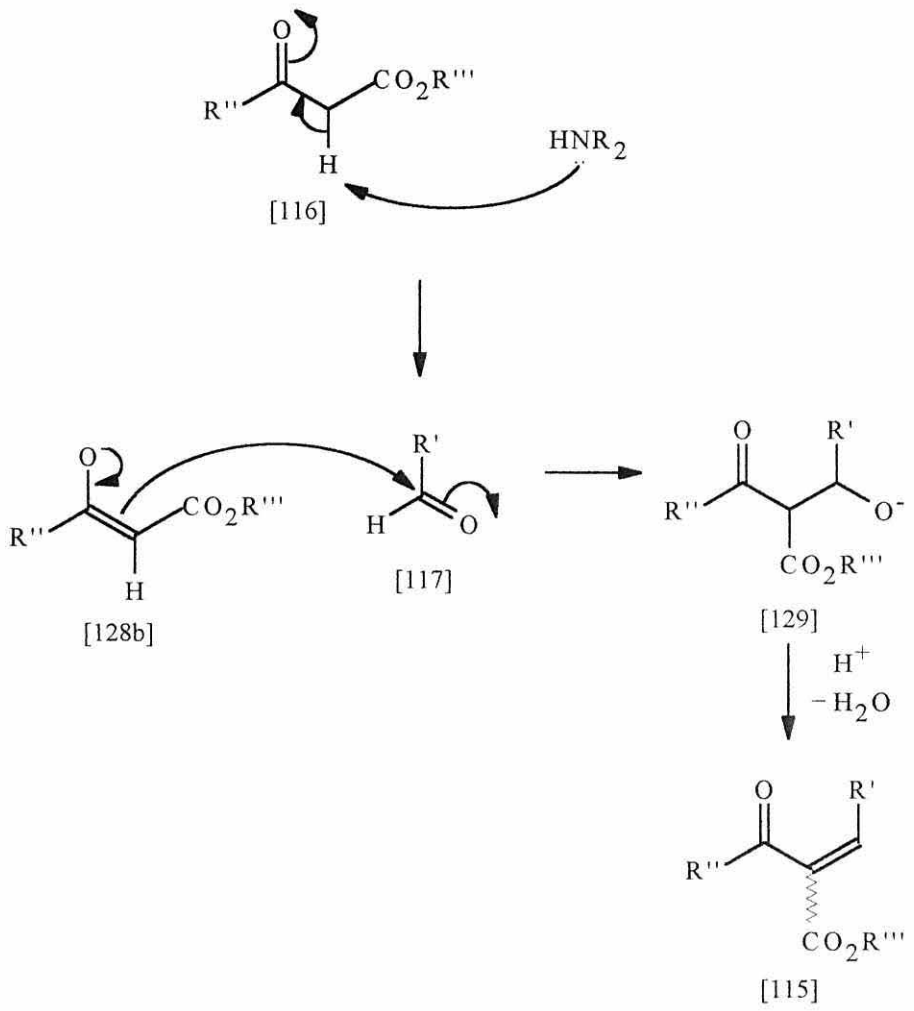
A century ago, Knoevenagel[97] himself showed that the condensation of an aldehyde with an activated methylene compound in the presence of a primary or secondary amine proceeds *via* an iminium ion pathway (Scheme 31). A condensation reaction occurs between the aldehyde [117] and the amine to give an iminium salt [127], to which the enol form of the β-ketoester [128a] then adds. Elimination of the amine gives the trisubstituted olefin [115].



Scheme 31 Knoevenagel Condensation *via* Iminium Ion

b) The Hann-Lapworth Mechanism

In the Hann-Lapworth[98] mechanism (Scheme 32), deprotonation at the activated methylene of the β-ketoester by the base occurs to give enolate [128b]. This enolate then adds directly to the aldehyde [117] to give an intermediate β-hydroxydicarbonyl compound [129]. Removal of the elements of water from [129] gives the trisubstituted olefin [115].



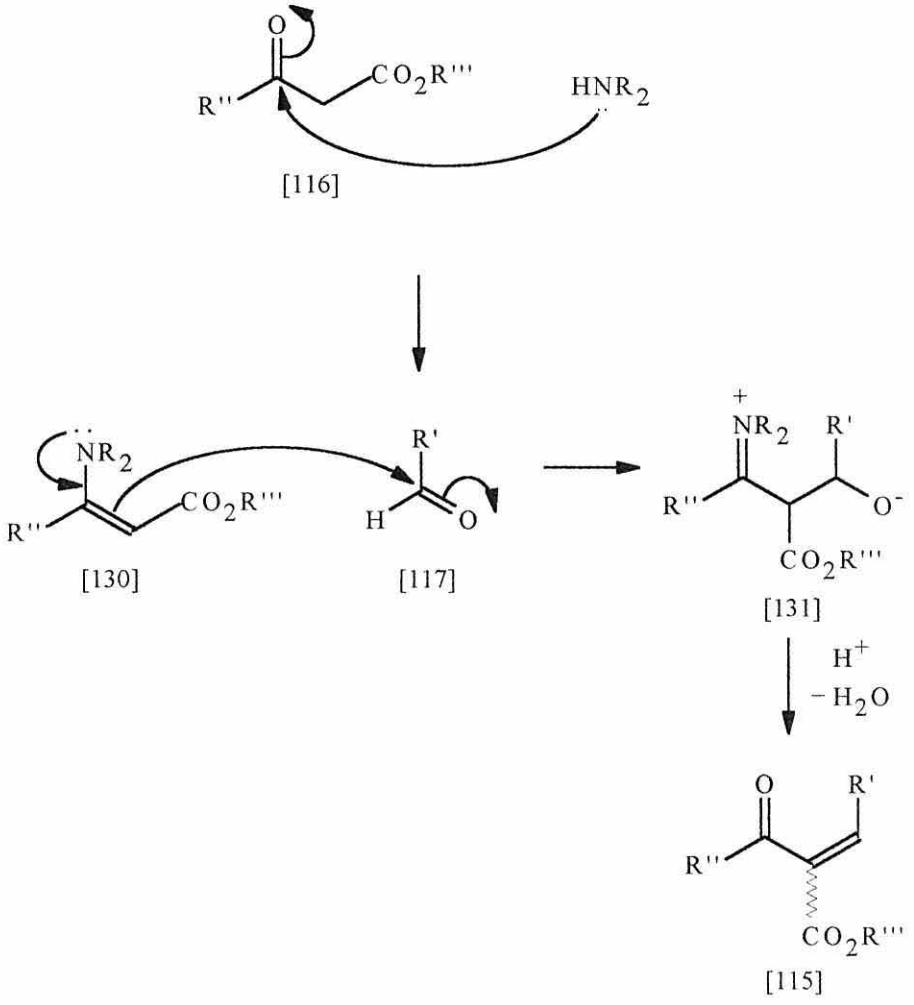
Scheme 32 The Hann-Lapworth Mechanism

In general, with a tertiary amine such as pyridine, the Knoevenagel reaction always proceeds *via* the Hann-Lapworth mechanism. With secondary and primary amines, the Knoevenagel and Hann-Lapworth mechanisms can compete and the formation of an iminium species will clearly depend on the nature of both the carbonyl compound and the amine.

c) The Enamine Mechanism

The third mechanism which can be envisaged involves the formation of an enamine [130] by addition-elimination of piperidine to the ketone group of β-ketoester [116]. Enamine [130] then adds to the aldehyde [117] to give a β-hydroxyiminium salt

[131], which on weak acidic work up would be hydrolysed to trisubstituted olefin [115] (Scheme 33). However, the typical conditions employed to generate enamines in this way (*e.g.* reflux in the presence of a drying agent) suggest that this mechanism is unlikely under the conditions used in the formation of [115].

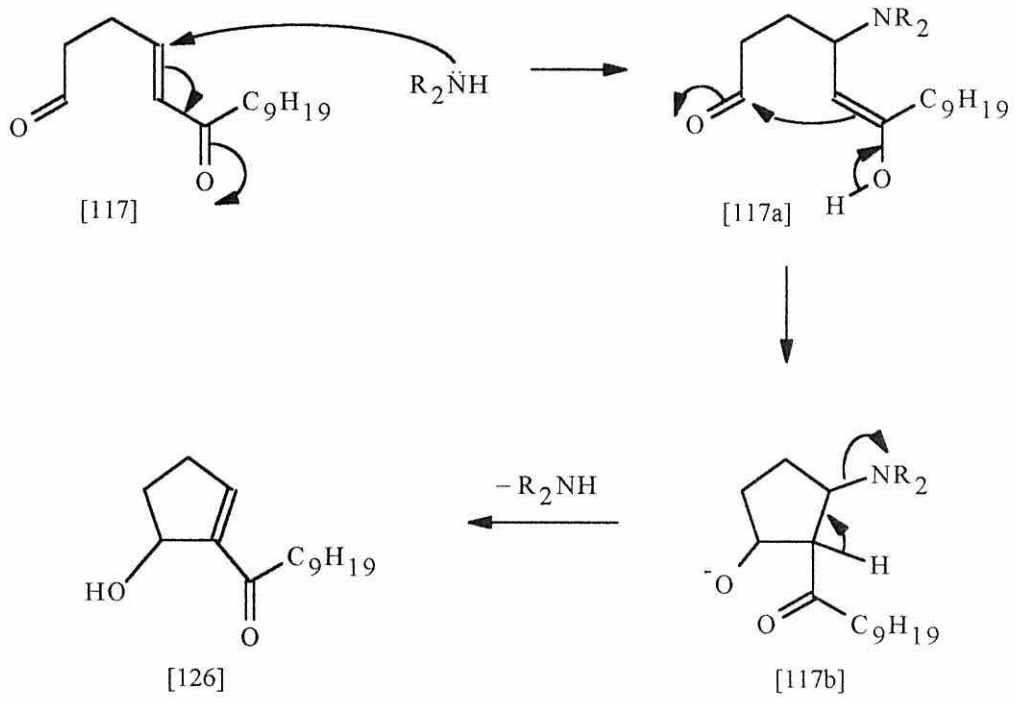


Scheme 33 Knoevenagel Condensation *via* Enamine Pathway

4.5.3 BAYLIS-HILLMAN REACTION[99a]

The Baylis-Hillman product [126] was fully characterised by 2D NMR (^{1}H COSY), ^{13}C NMR and mass spectrometry. Examples of intramolecular Baylis-Hillman reactions are scarce in the literature[99b,c] and the apparent potential of this reaction does not appear to have been fully exploited. A mechanism for the formation of [126] is proposed in Scheme 34. The first step is conjugate addition of the secondary amine to

the α,β-unsaturated ketone of the aldehyde [117]. β-aminoenol [117a] then adds intramolecularly to the aldehyde function, forming a five-membered ring [117b]. Slow elimination (regeneration) of the amine then occurs, yielding a cyclopentenol derivative [126]. The mechanism and scope of this type of intramolecular Baylis-Hillman reaction have been investigated and recently published.[100]

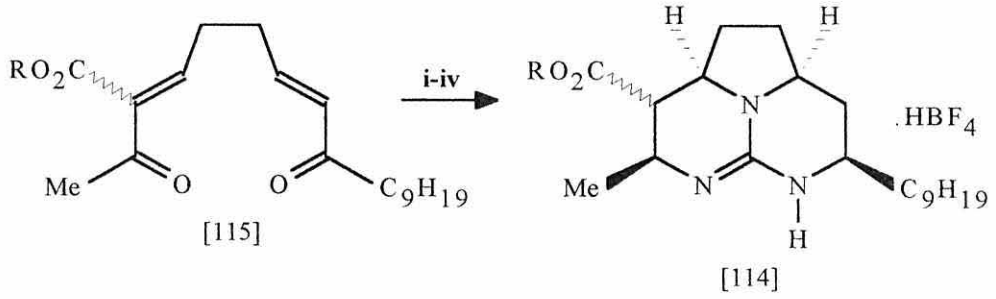


Scheme 34 Mechanism of the Baylis-Hillman Reaction

4.5.4 GUANIDINE CYCLISATION

With an authentic and pure sample of the Knoevenagel product in hand (albeit a mixture), the reductive addition of guanidine to the system was immediately investigated, as this is clearly the key step in our planned synthesis of batzelladine A. It was anticipated that the ester-substituted *bis*-α,β-unsaturated ketone would almost certainly be a more reactive Michael acceptor than in the unsubstituted case and the reaction conditions were modified accordingly.

Thus, treatment of [115] with 1 equivalent of guanidine at -30°C (with warming to -10°C) over 4 hours followed by reduction with sodium borohydride gave a brown oil, which was acidified (HCl) and subjected to counter-ion exchange with sodium tetrafluoroborate (Scheme 35). A thermospray mass spectrum showed the presence of $[M+NH_4]^+$ at m/z 424.2 (25%), $[M]^+$ at 406.3 (14%) and $[M-CO_2{}^tBu]^+$ at 306.3 (10%). This evidence showed that the desired compound was present, and importantly that the ester group had survived the guanidine addition, reduction and work-up intact. The 300MHz ^1H NMR spectrum of the crude reaction mixture was not particularly informative and indicated a significant amount of decomposition. However, an expanded 400MHz spectrum showed resonances in the expected chemical shift region (δ 3-4). The NMR spectra will undoubtedly be complex, since the starting material was a mixture of geometric isomers, therefore the product will almost certainly be a diastereomeric mixture.



Scheme 35 *Reagents and conditions*: i) 1 equiv. guanidine, DMF, -30 to -10°C, 4 h; ii) 3:1:3 DMF:H_2O:MeOH, then 6 equiv. $NaBH_4$, 0-20°C, 16 h; iii) 1M $HCl_{(aq)}$; iv), sat. $NaBF_{4(aq)}$.

4.6 ALTERNATIVE ROUTES TO [115]

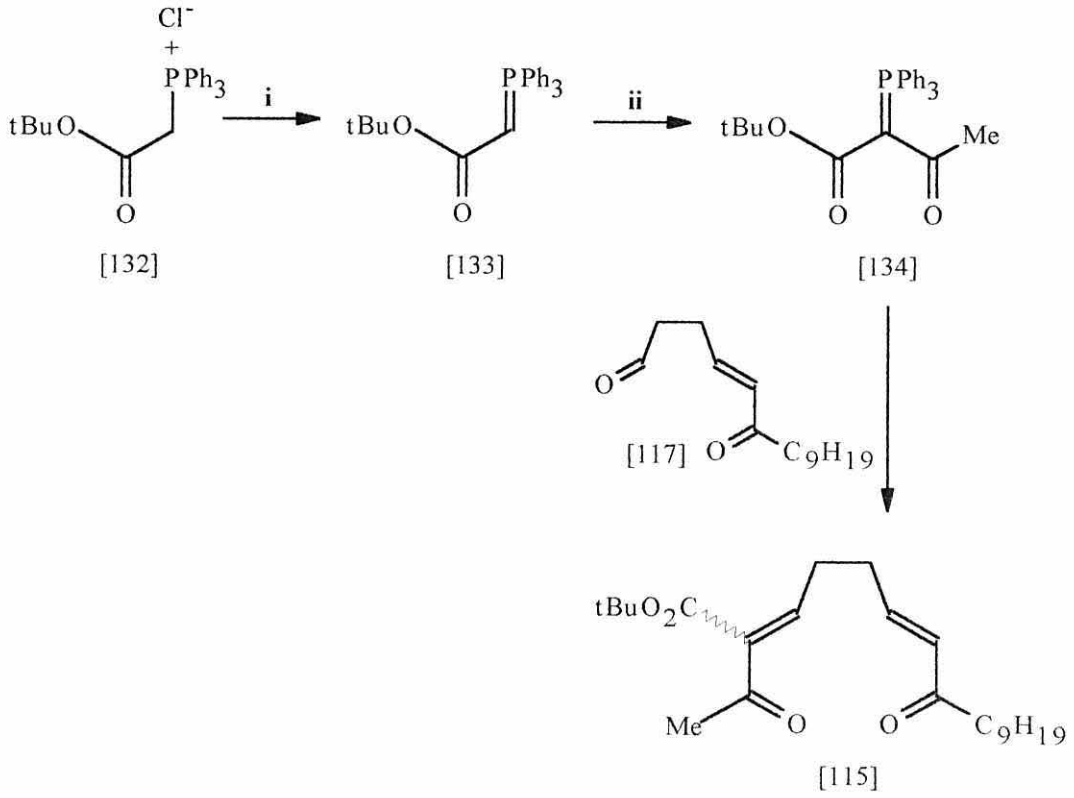
Due to the lack of material available, it was not possible to investigate this guanidine reaction further and other routes to the desired Knoevenagel product were considered.

4.6.1 WITTIG REACTION WITH A DOUBLY-STABILISED YLID

The formation of [115] *via* a simple Wittig reaction of a doubly-stabilised ylid with the aldehyde [117] was considered. The proposed reaction sequence is shown in Scheme 36. Although no examples of this type of cumulated ylid could be found in the literature, it appeared that the ylid could be prepared quite easily and the Wittig reaction was certainly worth attempting.

Treatment of the commercially available *tert*-butoxycarbonylmethylene triphenylphosphonium chloride [132] with 1.1 equivalents of aqueous sodium hydroxide gave, on work-up, ylid [133] as an oil in near quantitative yield. This ylid was then acylated with acetyl chloride in dichloromethane, followed by treatment with sodium hydroxide to give the doubly stabilised, cumulated ylid [134] as a yellow crystalline solid in 86% overall yield.

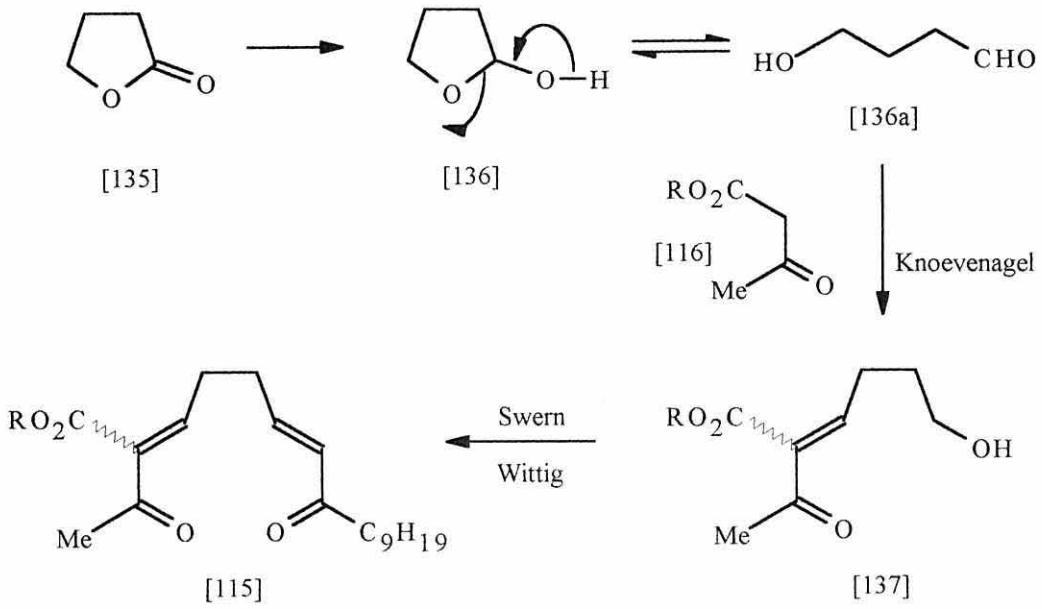
The Wittig reaction of this ylid with prescribed aldehyde [117] was then investigated. It was predicted that this doubly-stabilised ylid would be orders of magnitude less reactive than a typical acetyl ylid (*e.g.* [122]) but that it may be possible to effect a reaction with a reactive aldehyde. However, despite a number of attempts in a variety of solvents and under different reaction conditions, no reaction was observed between these materials.



Scheme 36 *Reagents and conditions*: i) 1.1 equiv. $NaOH_{(aq)}$, CH_2Cl_2, 0°C, 1 h, 98%; ii) 1.1 equiv. AcCl, CH_2Cl_2, 0-20°C, 16 h, 88%.

4.6.2 ATTEMPTED SYNTHESIS OF [115] FROM γ-BUTYROLACTONE

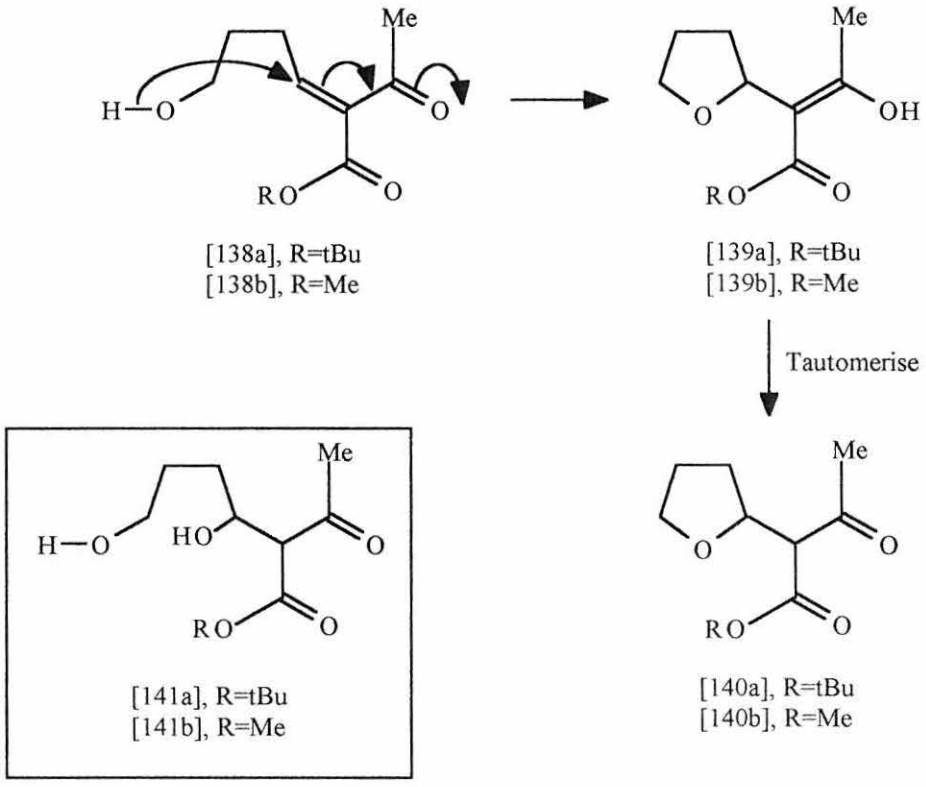
An alternative approach to the construction of [115] is to begin with the left-hand portion of the molecule and set up the trisubstituted olefin first (Scheme 37). γ-Butyrolactone [135] can be reduced to the hemiacetal [136] with diisobutylaluminium hydride. The hemiacetal exists in equilibrium with the hydroxyaldehyde [136a] which may undergo Knoevenagel condensation with a β-ketoester [116] to give a trisubstituted olefin [137]. Swern oxidation of the terminal hydroxyl function followed by Wittig reaction should then give the target molecule [115]. Again, this would provide rapid access (4 steps) to the precursor to reductive guanidine addition.



Scheme 37 Alternative route to [115] from γ-butyrolactone

The reduction of γ-butyrolactone [135] to the hemiacetal proceeded smoothly in 75% yield without the need for purification. It was found that hemiacetal [136] existed as a ~25:1 equilibrium mixture with the hydroxyaldehyde [137] in deuterated chloroform solution (10mg/ml). This ratio was calculated by comparing the integral of the anomeric proton at δ *ca.* 5.6 with the aldehyde proton at δ *ca.* 9.7. The Knoevenagel condensation of this mixture was attempted initially with *tert*-butyl acetoacetate [116] in dichloromethane with piperidine acting as a catalyst (~ 10 mol %). On consumption of the starting material, the reaction was quenched with hexane and acidified with dilute aqueous hydrochloric acid. Upon chromatography, a single product was isolated; however, no olefinic resonances were observed in the NMR spectra. It was initially thought that the compound obtained was the non-eliminated aldol product [141] (Scheme 38). However, the NMR resonances and IR absorptions for the hydroxyl groups were absent. This evidence combined with mass spectroscopic data led to the conclusion that the compound obtained was in fact 2-substituted tetrahydrofuran [140] which appears to form spontaneously after the addition of the activated methylene compound to the aldehyde. The exact mechanism of this cyclisation is unclear, but it was shown that the conditions of work-up do not affect its formation. A plausible mechanism involves

intramolecular *cyclisation* of the Knoevenagel-type product [138] to give enol [139] which on tautomerism gives 2-substituted tetrahydrofuran [140].



Scheme 38

Simple variation of the reaction conditions (base, temperature, solvent) did not lead to any of the desired product. The experiment was performed using methyl acetoacetate, but this gave the same result. The Lehnert[101] variation of the Knoevenagel condensation was first reported in 1970 and uses $TiCl_4$/pyridine/THF with an activated methylene component adding to an aldehyde. This modification did provide a trace amount of the desired Knoevenagel product [138] but still gave predominantly the tetrahydrofuran [140]. Despite a number of attempts, the amount of the desired material obtained was insufficient for practical purposes and this approach to [115] was abandoned at this juncture.

4.7 CONCLUSION

The tricyclic model system [120] was efficiently prepared in short order from readily available starting materials. This compound was formed as a single diastereoisomer and the stereochemistry determined by extensive nOe experiments and X-ray diffraction studies on a related tricyclic compound [125b]. This observed diastereospecificity demonstrates that the reductive guanidine addition-cyclisation sequence is a powerful method to rapidly access complex frameworks.

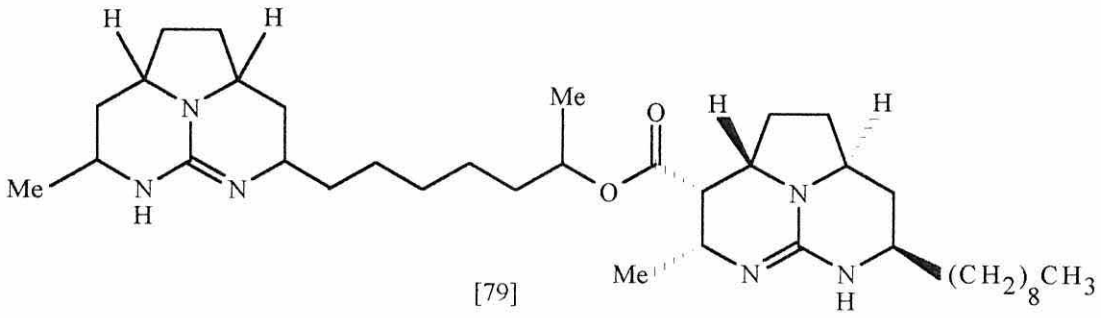
The Knoevenagel condensation of aldehyde [117] was an extremely variable reaction which was difficult to repeat. Consequently, only a small quantity of the ester-substituted *bis*-α,β-unsaturated ketone [115] was obtained. This made a detailed examination of the guanidine addition-cyclisation unrealisable. Two other routes to the target molecule [115] were investigated, but both proved fruitless.

CHAPTER 5

A SYNTHESIS OF THE LEFT-HAND PORTION OF BATZELLADINE F

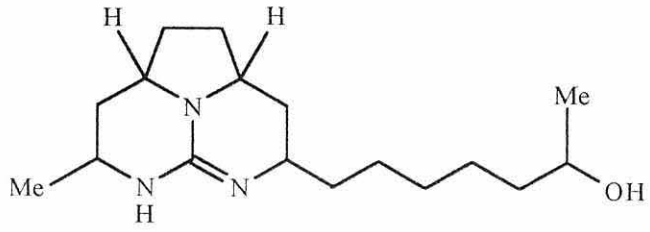
5.1 INTRODUCTION

As described in section 2.7.4 four novel guanidine metabolites have been isolated from the sponge *Batzella* sp., collected in Discovery Bay, Jamaica.[65] These were found to contain two tricyclic guanidine units tethered *via* an ester linkage, unprecedented in known natural products. The stereochemistry of the left-hand tricyclic unit has been assumed on the basis of the revision by Snider, *et al.*[68] although there appears to have been little effort directed towards proving this stereochemistry, either by X-ray crystallography or even nOe experiments.



[79]

The left-hand tricycle of batzelladine F [79] is essentially identical to the tricyclic model compound [120] described earlier. Based on these results, it was reasoned that a minor modification in the established route to elaborate the hydrocarbon side-chain should elicit the desired compound [143].

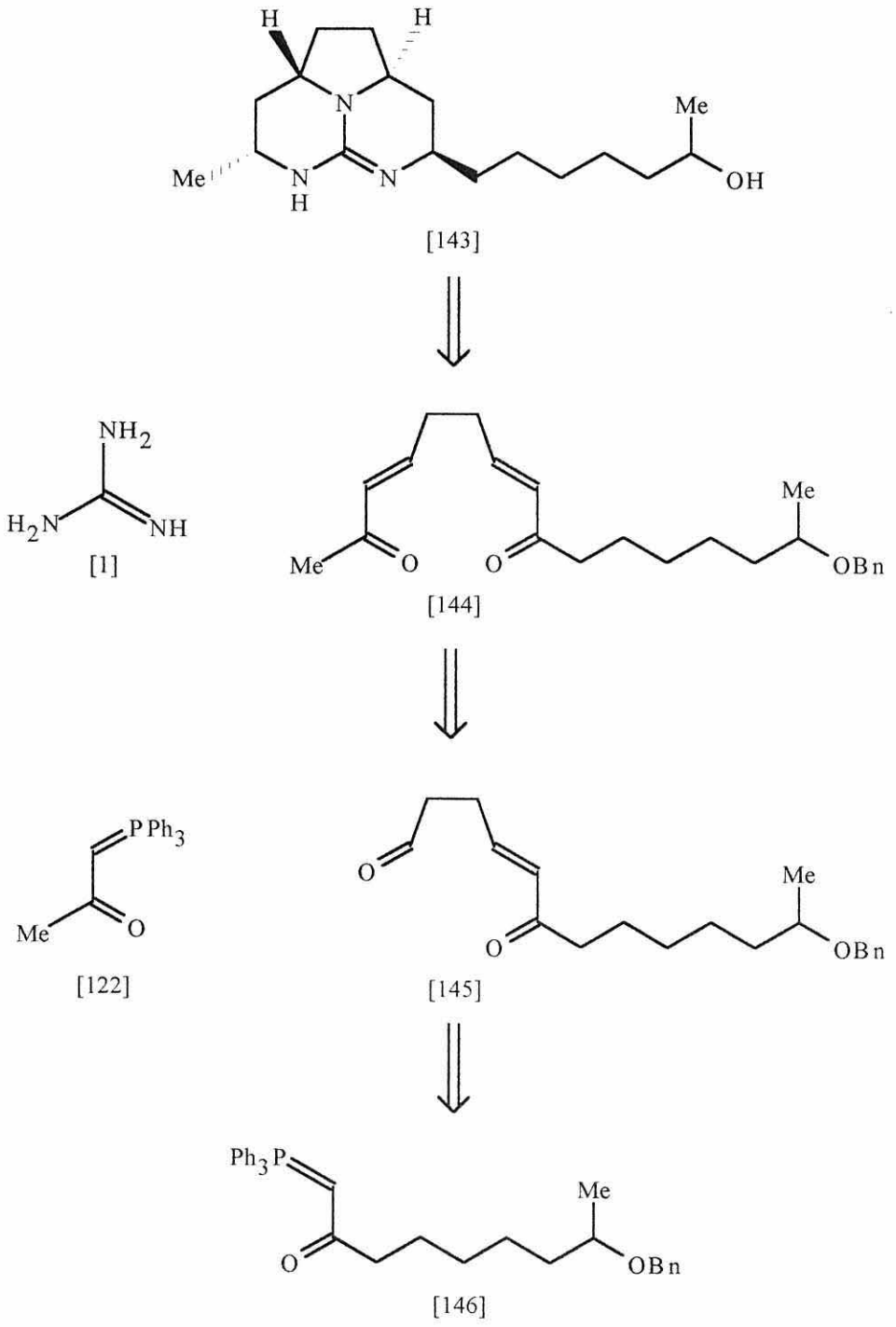


[143]

This is also the compound obtained by a proposed basic hydrolysis of the natural product. Spectroscopic comparison of the two combined with X-ray diffraction analysis (if the material can be crystallised) may give conclusive proof of the stereochemistry of the tricyclic guanidine moiety.

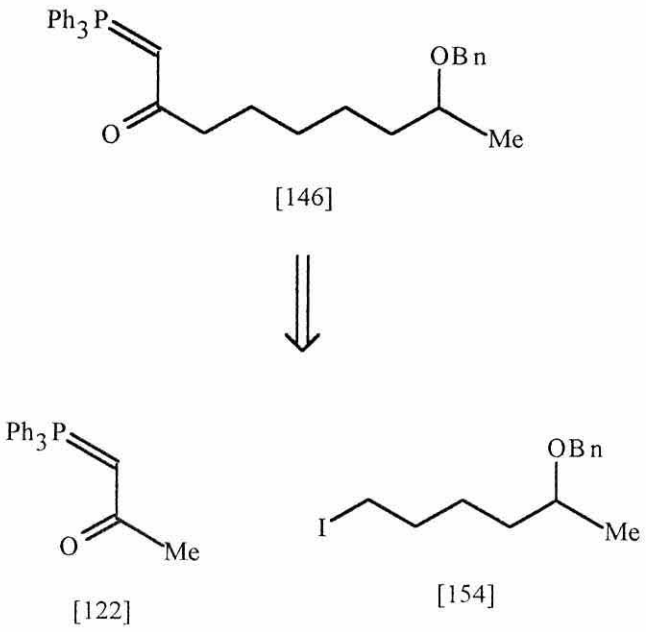
5.2 RETROSYNTHESIS

The only modification required is the incorporation of the hydroxyl function into the seven carbon side-chain. The retrosynthetic analysis employed for the simple tricyclic model [120] still holds (Scheme 39).



Scheme 39 Retrosynthesis of the left-hand tricycle of batzelladine F [143]

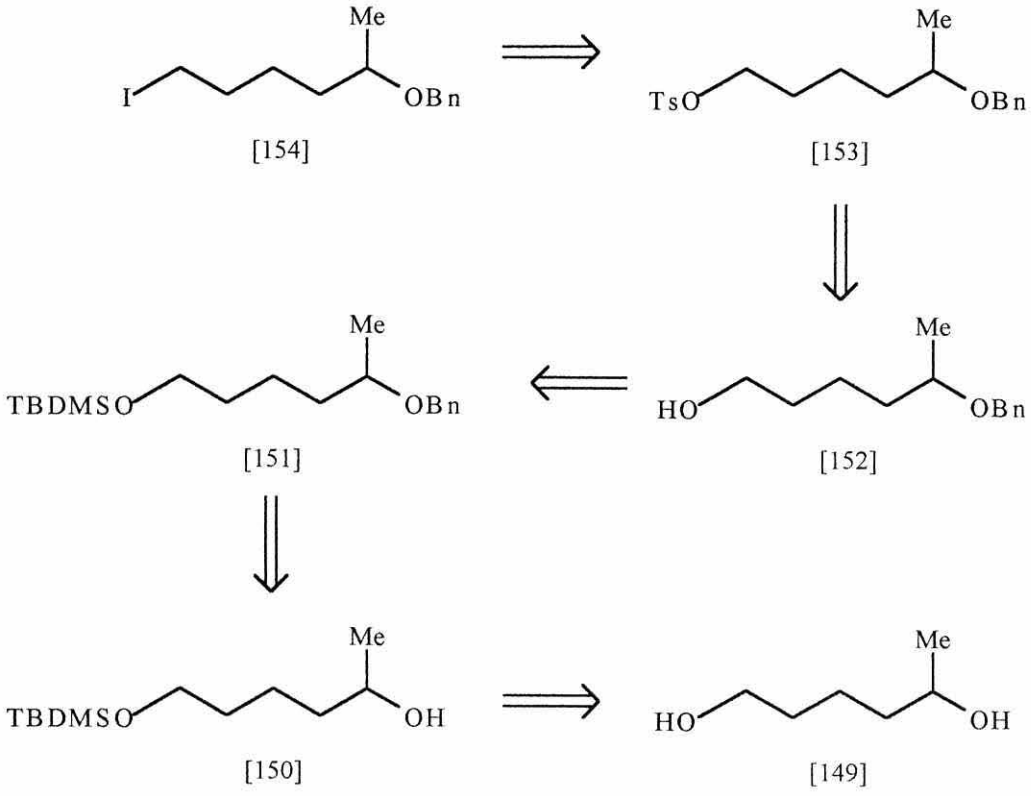
The protected hydroxyl function must therefore be incorporated into the ylid [146]. A retrosynthesis of [146] is shown in Scheme 40.



Scheme 40

The chemistry utilised in Section 4.4 will then be applied to iodide [154] which will furnish the required phosphorane [146].

The iodide [154] is accessible by a relatively trivial sequence of functional group interconversions from diol [149] (Scheme 41).

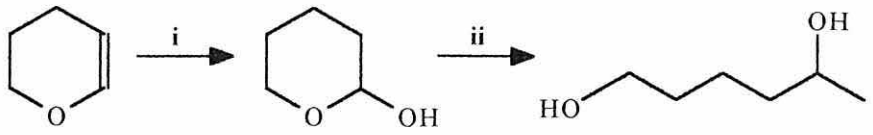


Scheme 41

5.3 SYNTHESIS OF THE PHOSPHORANE [146]

1,5-Hexanediol [149] is commercially available but is rather expensive (*ca.* £15/g). It was therefore decided to prepare [149] and this was readily accomplished in a two step sequence from 3,4-dihydropyran [147] (Scheme 42).

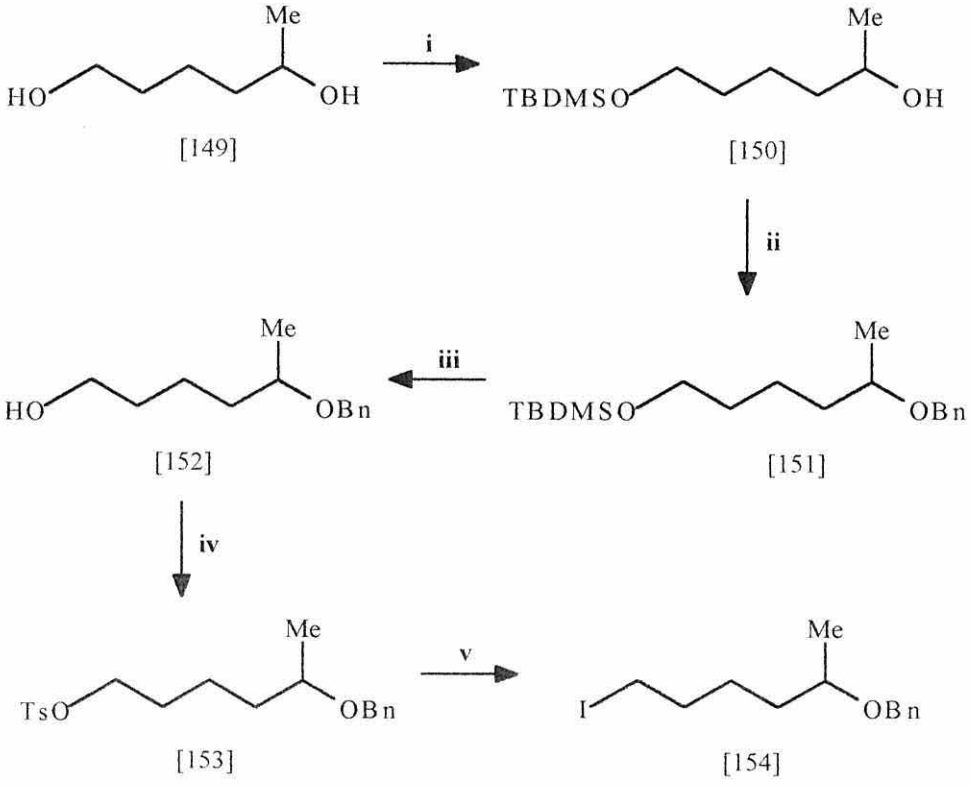
Acid hydrolysis of [147] with dilute aqueous hydrochloric acid gave the hemiacetal [148] in 75% yield.[102] This masked aldehyde was then be reacted with an excess (3 equivalents) of methylmagnesium chloride giving, on work-up, 1,5-hexanediol [149]. The yield of the Grignard reaction was only modest (60%) but these reactions could be performed on a large scale (>10g) quite easily, giving multigram quantities of [149]. The NMR and IR spectra of prepared diol [149] were identical to those of the commercially available material.



[147] [148] [149]

Scheme 42 *Reagents and conditions*: i) Excess 1M $HCl_{(aq)}$, 1 h, 75%; ii) 3 equiv. MeMgBr, THF, Δ, 6 h, 60%.

The next sequence of reactions were relatively simple functional group interconversions to manipulate [149] into the required iodide [154] (Scheme 43). Protection of the primary hydroxyl function was performed using *tert*-butyldimethylsilyl chloride and imidazole in DMF.



[149] [150] [151] [152] [153] [154]

Scheme 43 *Reagents and conditions*: i) 2 equiv. imidazole, 1 equiv. TBDMS-Cl, DMF, 0-20°C, 1 h, 92%; ii) 1.3 equiv. NaH, THF, 0°C, 10 min, then 1.4 equiv. BnBr, 0.1 equiv. TBAI, 20°C, 18 h, 71%; iii) 1.5 equiv. TBAF, THF, 0-20°C, 4 h, 80%; iv) 1.2 equiv. TsCl, pyr., 0-20°C, 18 h, 79%; v) 3 equiv. NaI, acetone, Δ, 4 h, 97%.

After a reaction time of 1 hour and disappearance of starting material (TLC), the reaction was quenched and the mixture purified by flash chromatography giving exclusively the silyl ether [150] in 92% yield.

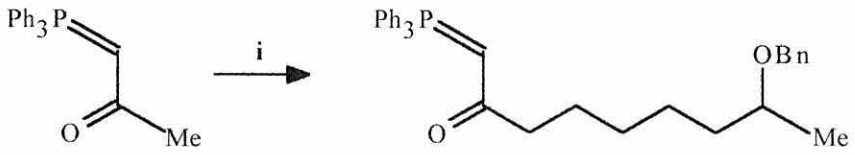
The second step was protection of the secondary hydroxyl function. The benzyl ether was chosen for stability against the subsequent FGI and the final guanidine addition. Also, eventual hydrogenation to remove it should not affect the tricyclic guanidine moiety. Treatment of silyl ether [150] with 1.3 equivalents of sodium hydride in THF followed by addition of 1.4 equivalents of benzyl bromide and 10 mol % of tetra-*n*-butyl-ammonium iodide gave, in 71% yield, the benzyl ether [151] together with recovered [150] (21%). The yield based on recovered starting material was ~90%, so that after two or three recycles, [150] was completely converted into [151]. One possible problem which was perceived with the benzylation was migration of the silyl group to the secondary hydroxyl position. However, no trace of this was seen by NMR spectroscopy in any of the runs. The benzylic protons of [151] are diastereotopic and were observed as two well separated doublets at δ 4.46 and δ 4.57 with a large coupling constant (*ca.* 11.8Hz).

Removal of the silyl group was effected cleanly with a 1.0M solution of tetra-*n*-butylammonium fluoride in THF giving alcohol [152] in 80% yield, which could be used without purification, but was purified for characterisation purposes.

In order to convert the primary hydroxyl function into the desired iodide, the procedure of Millar and Underhill[103] was initially followed. This involves formation of an iodine-triphenylphosphine complex which reacts with the alcohol and imidazole to displace directly the hydroxyl group. This method did not, however, appear to be succesful under the conditions employed. It was decided at this point to access the chosen iodide *via* the traditional tosylate route. Thus, treatment of alcohol [152] with 1.2 equivalents of tosyl chloride in pyridine gave the tosylate [153] in a satisfactory 79% yield after chromatography. Displacement of tosylate ion with 3 equivalents of sodium iodide in acetone gave the desired iodide [154], which required no further purification, in 97% yield.

The alkylation of acetylmethylene triphenylphosphorane [122] with iodide [154] (Scheme 44) was performed using exactly the same conditions as used previously (Section 4.6). Thus, treatment of [122] with 1.1 equivalents of *n*-butyllithium in THF

gave the deep-red colour due to formation of the lithium enolate *in situ*. This mixture was stirred for 0.5 hour, whereupon 1.1 equivalents of iodide [154] were added. The deep red colour turned straw yellow over a period of 4 hours signifying the consumption of the lithium enolate. The phosphorane [146] was obtained in near quantitative yield, with the slight excess of the iodide still present.

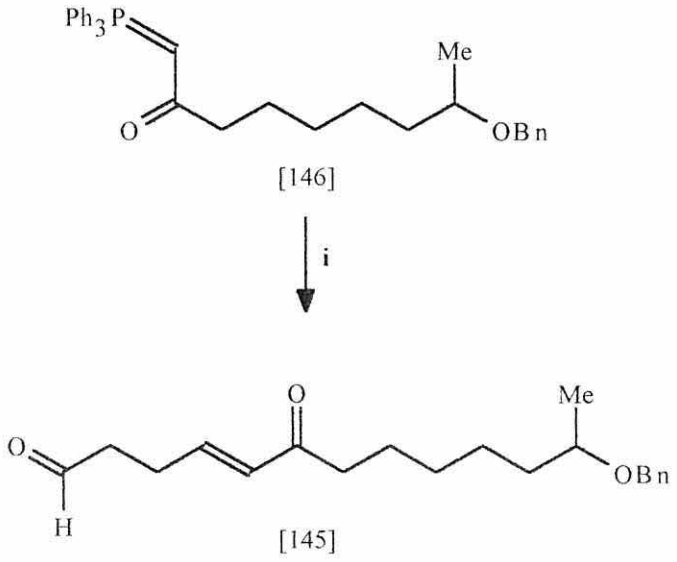


[122] [146]

Scheme 44 *Reagents and conditions*: i) 1.1 equiv. *n*-BuLi, THF, -78°C, 30 min, then 1.1 equiv. [154], -78-20°C, 4 h, quant.

5.4 SYNTHESIS OF THE *BIS*-α,β-UNSATURATED KETONE [144]

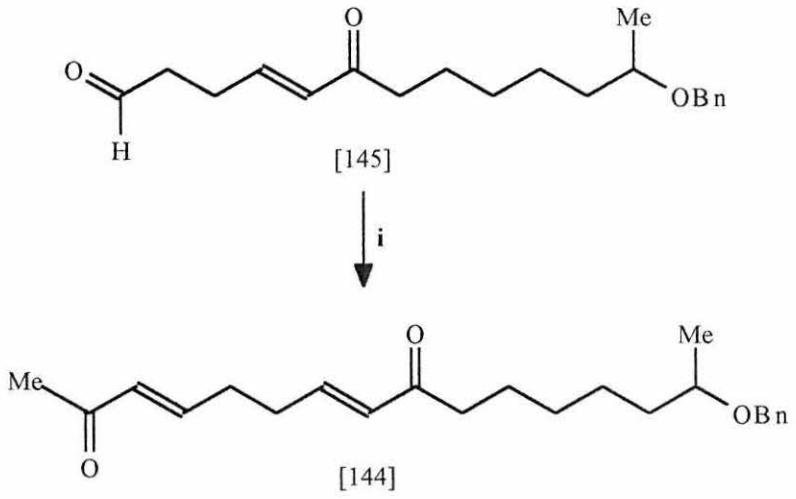
This phosphorane was used without further purification and reacted with an excess (*ca.* 10 equivalents) of succinaldehyde [119] to give the aldehyde [145] (Scheme 45).



Scheme 45 *Reagents and conditions*: i) 10 equiv. succinaldehyde, CH_2Cl_2, 20°C, 22 h, 43%.

The *trans* geometry of the new double bond was assigned from the large coupling constant (J = 16.0Hz) of the vicinal olefinic protons at δ 6.78 and 6.09. This reaction was performed only once and the yield was rather low (43% after chromatography).

The second Wittig reaction to generate the *bis*-α,β-unsaturated ketone [144] (Scheme 46) proceeded smoothly in 60% yield with complete conversion of the aldehyde [145]. The precursor to guanidine addition-cyclisation had therefore been prepared in an efficient manner, although the yields of the reactions had not been optimised.

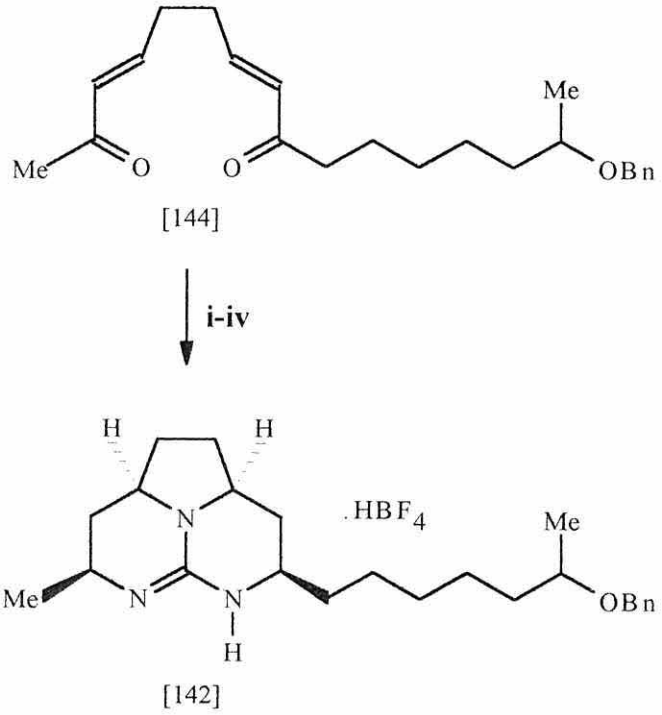


Scheme 46 *Reagents and conditions*: i) 3 equiv. $MeCOCHPPh_3$, CH_2Cl_2, 20°C, 36 h, 60%.

5.5 GUANIDINE ADDITION

The addition of guanidine was performed in exactly the same way as for [120] (Section 4.6). One equivalent of guanidine was added to a solution of [144] in DMF at 0°C. The intermediate hemiaminal was reduced using sodium borohydride with the addition of methanol and water (Scheme 47). Upon work-up, the mixture was found to contain a compound of the expected polarity (R_f = 0.23 in 5% methanol:chloroform). After counter-ion exchange with sodium tetrafluoroborate and column chromatography, this compound was isolated and found to be similar to the tricyclic model compound

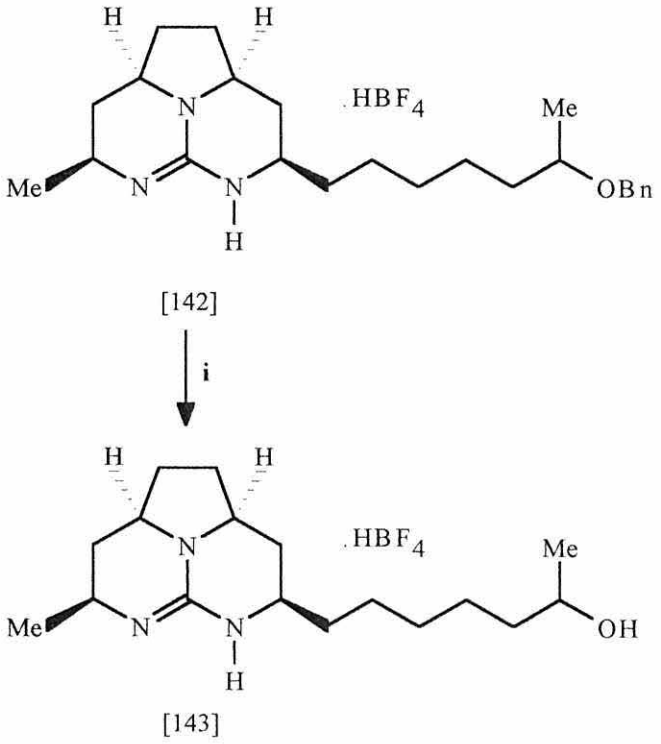
[120] by NMR spectroscopy. The benzyl ether was still intact and proton resonances at δ 3.30 and 3.62 suggested the presence of the tricyclic guanidine moiety. The ^{13}C spectrum further supported this conclusion, with key resonances at δ 55.98 and 56.06 (the pyrrolidine bridge carbons) and δ 46.13 and 50.43 (the six-membered ring carbons α to nitrogen). The singlet resonance at δ 149.19 corresponded to the guanidinium carbon.



Scheme 47 *Reagents and conditions*: i) 1 equiv. guanidine, DMF, 0-20°C, 4 h; ii) 3:1:3 DMF:H_2O/MeOH (v/v), then 6 equiv. $NaBH_4$, 0-20°C, 18 h; iii) 1M $HCl_{(aq)}$; iv) sat. $NaBF_{4(aq)}$, 37% overall.

The benzyl group in [142] was removed by hydrogenation using palladium hydroxide on charcoal (Scheme 48). The tricyclic compound [142] was dissolved in ethyl acetate and stirred briskly under a positive pressure of hydrogen gas. After 4 hours, the starting material had been consumed and a higher polarity compound had been formed (TLC). The catalyst was filtered off and the solvent evaporated, leaving a white solid. The ^1H NMR spectrum of this solid was similar to that of the starting material, but the conspicuous pair of doublets due to the benzylic protons had disappeared, indicating that the benzyl ether had been successfully cleaved, although no hydroxyl resonance was

discernible. The resonances due to the protons α to the nitrogen atoms of the guanidine were still present at δ 3.30 and 3.60, confirming that the tricyclic portion had survived the hydrogenation. Purification was attempted by chromatography, which gave a fraction which appeared to contain impurity with a trace of [143] and a fraction rich in [143]. The stereochemistries of [142] and [143] are at present assigned by analogy to the model compound [120]. Unfortunately, due to the lack of available time and material, further purification and analysis was difficult.



Scheme 48 *Reagents and conditions*: i) 1 equiv. $Pd(OH)_2$ (w/w), 1 atm H_2, EtOAc, 4 h, 67%.

5.6 CONCLUSION

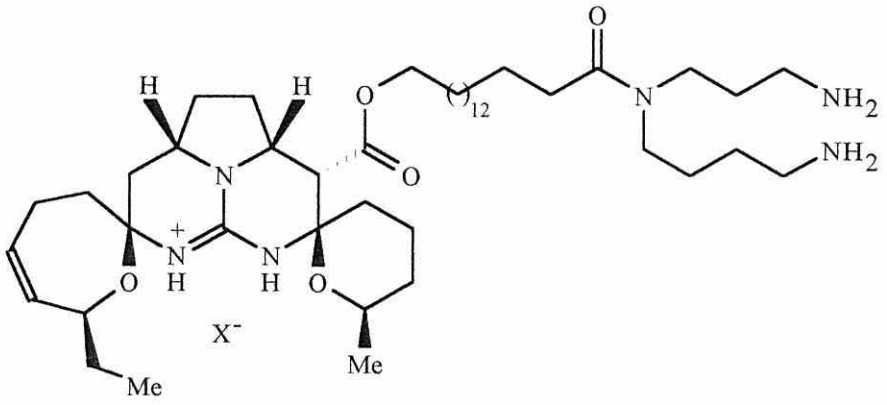
This part of the research was conducted towards the end of the project and time restraints precluded further examination of the key reactions, particularly the final hydrogenation. The preliminary indications are, however, that this is a viable route to the left-hand tricycle of batzelladine F. Further work in this area may provide definitive evidence for the stereochemistry of the tricyclic guanidine moiety found in this family of metabolites.

CHAPTER 6

TOWARDS FUNCTIONALISED ANALOGUES OF PTILOMYCALIN A

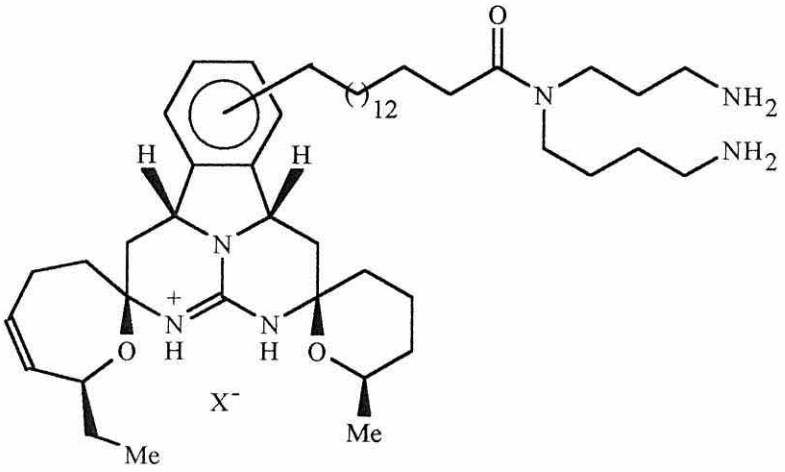
6.1 INTRODUCTION

The second aim of this project was to develop an efficient methodology for the preparation of a number of functionalised analogues of ptilomycalin A. This would hopefully give more insight into the biological mode of action of these compounds and help determine the minimal structure requirement for activity.



[8]

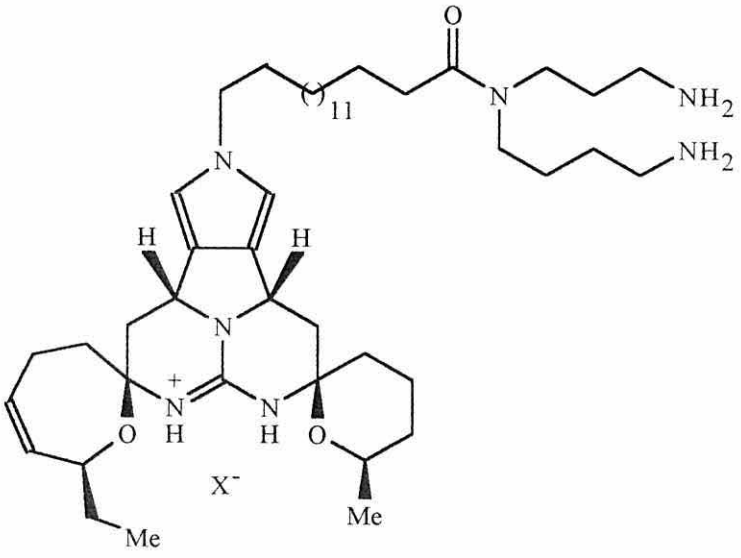
The extensive range of biological properties of [8] as discussed in Section 2.5.1 combined with the unique structural features have undoubtedly sparked the interest in this new natural product. Since the initial isolation by Kashman and Kakisawa,[41] this alkaloid has been isolated from several different sources.[43, 63] Indeed, ptilomycalin A can be considered to be the parent of an expanding class of metabolites. Ptilomycalin A can be considered to be composed of four discrete units; the guanidinium core, the ester linkage, the alkyl spacer and the spermidine amide terminus. Hart[55b] suggested that the ester linkage may impart some instability into the system and may not indeed be crucial to the bioactivity. However, the spirocyclic guanidine core, the alkyl spacer and the spermidine amide are likely to be crucial for bioactivity. These three features must therefore be incorporated into any serious synthetic strategy towards functionalised analogues. Figure 13 shows a compound which possesses these three features and could well mimic the activity of ptilomycalin A.



[155]

Figure 13. A benzo-fused analogue of ptilomycalin A

Another similar type of model compound which was immediately obvious is based on a pyrrole-fused system [156] (Fig. 14). This has the added advantage of being able to be alkylated at nitrogen with ostensibly any side-chain required.



[156]

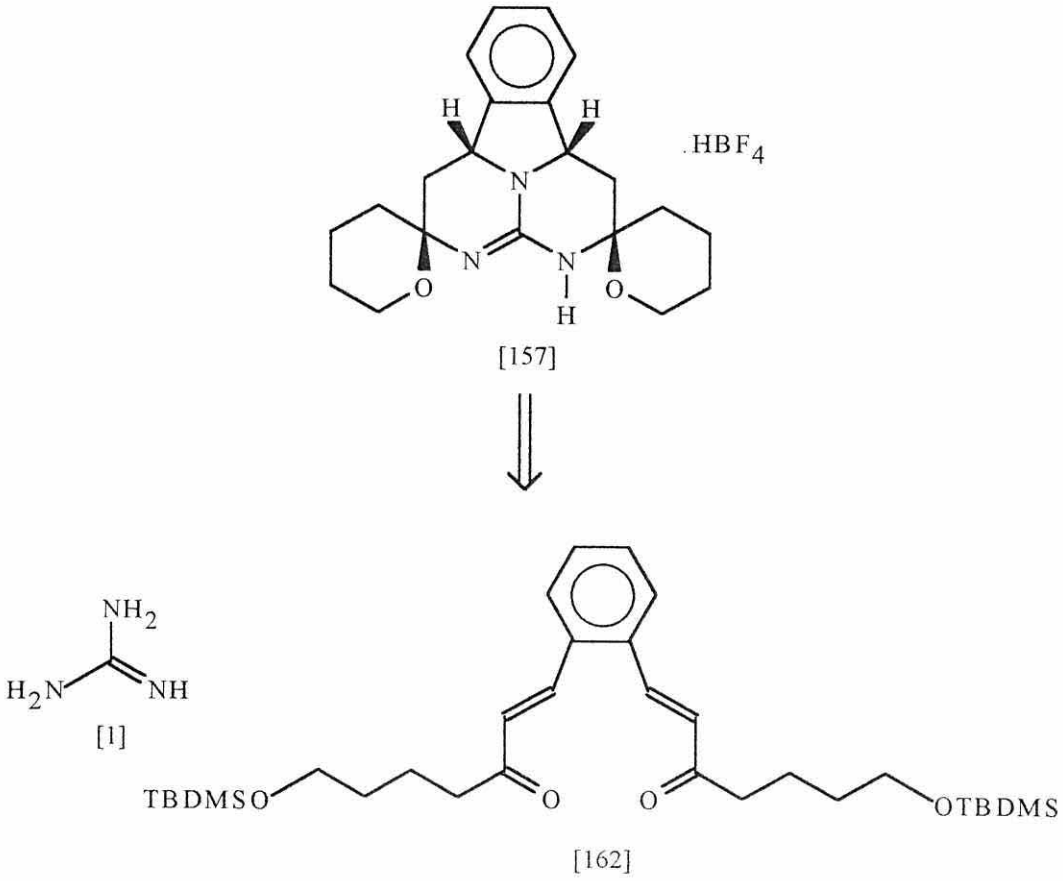
Figure 14. A pyrrole-fused analogue of ptilomycalin A

6.2 SYNTHESIS OF A SIMPLE BENZO-FUSED SYSTEM

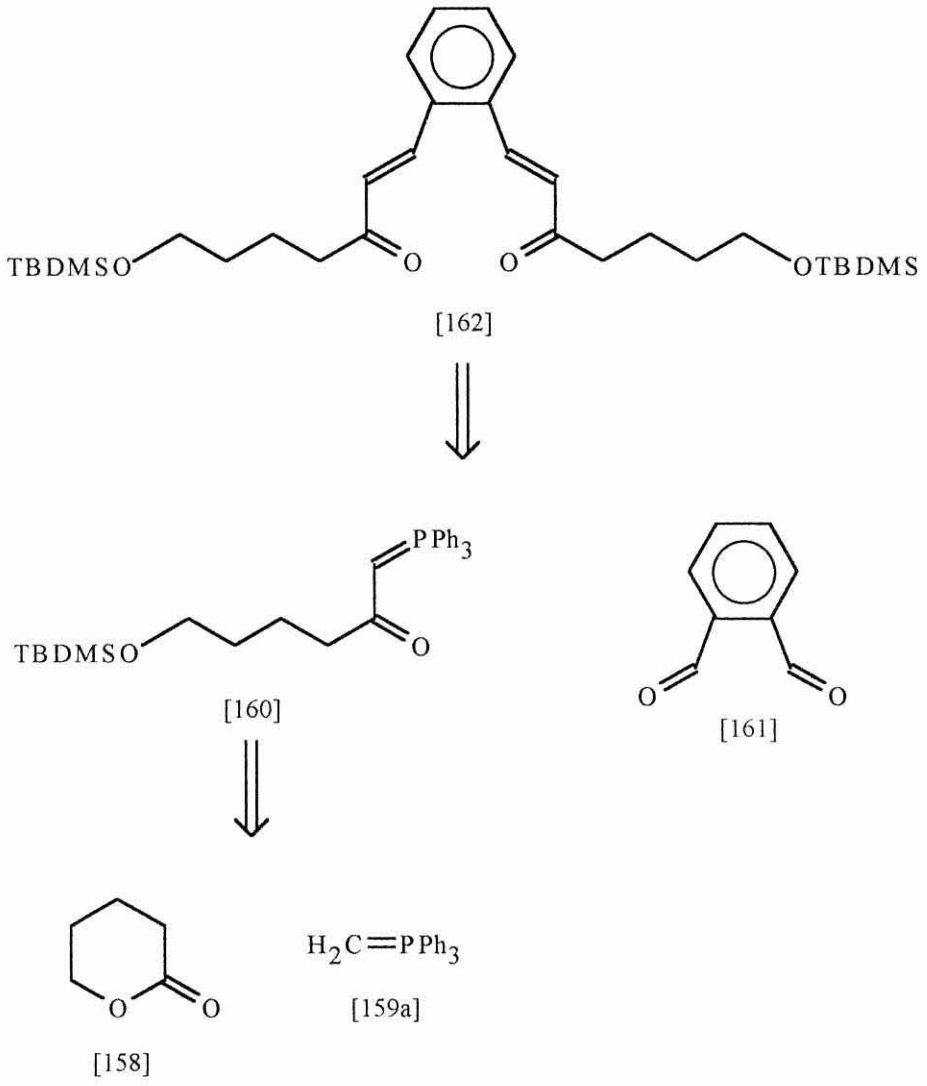
6.2.1 RETROSYNTHESIS

In order to test the feasibility of this approach, a good starting point seemed to be the simple unsubstituted benzo-fused guanidine system [157]. The retrosynthesis to the *bis*-α,β-unsaturated ketone [162] is shown in Scheme 49. This appeared to be relatively easy to prepare, using established procedures with succinaldehyde replaced by *o*-phthalic dicarboxaldehyde [161] at the Wittig step (Scheme 50).

The *tert*-butyldimethylsilyl-protected phosphorane [160] has been prepared during model studies towards ptilomycalin A[49b, 51] from δ-valerolactone [158] and methylene ylid [159a]. The guanidine addition-spirocyclisation with this type of system is also known to be facile and stereospecific towards the desired *syn* product as shown.



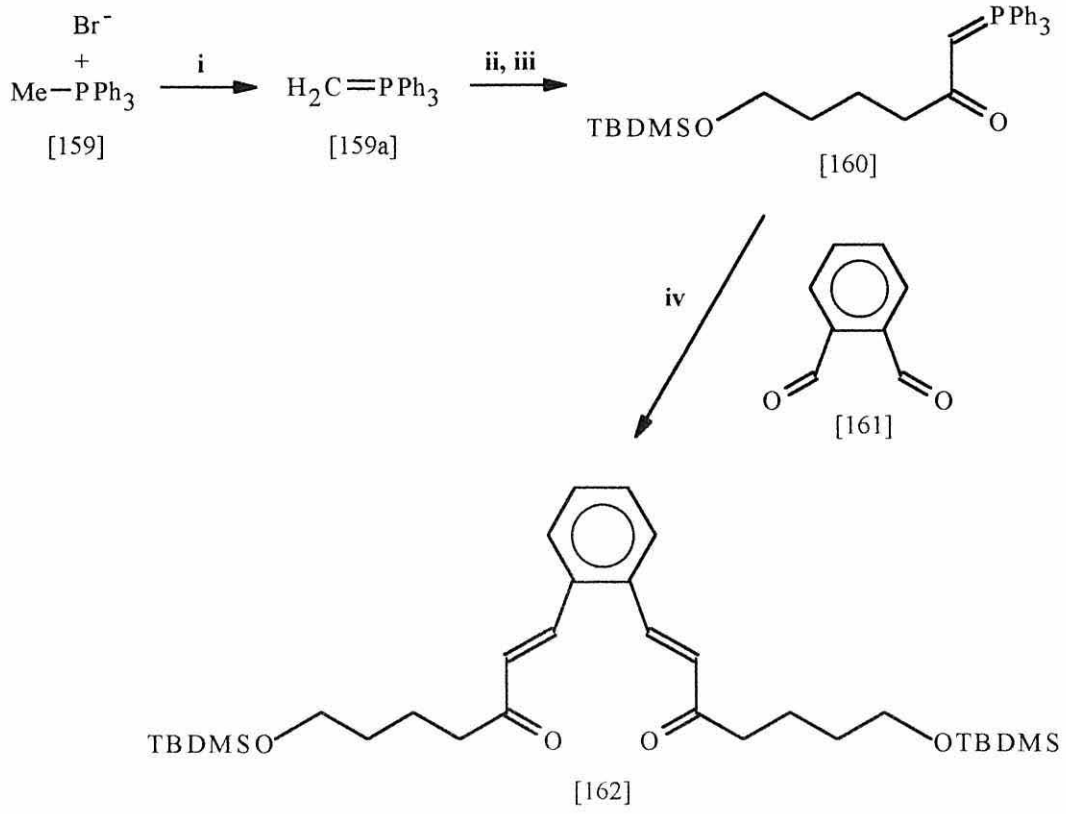
Scheme 49



Scheme 50

6.2.2 PREPARATION OF THE *BIS*-α,β-UNSATURATED KETONE [162]

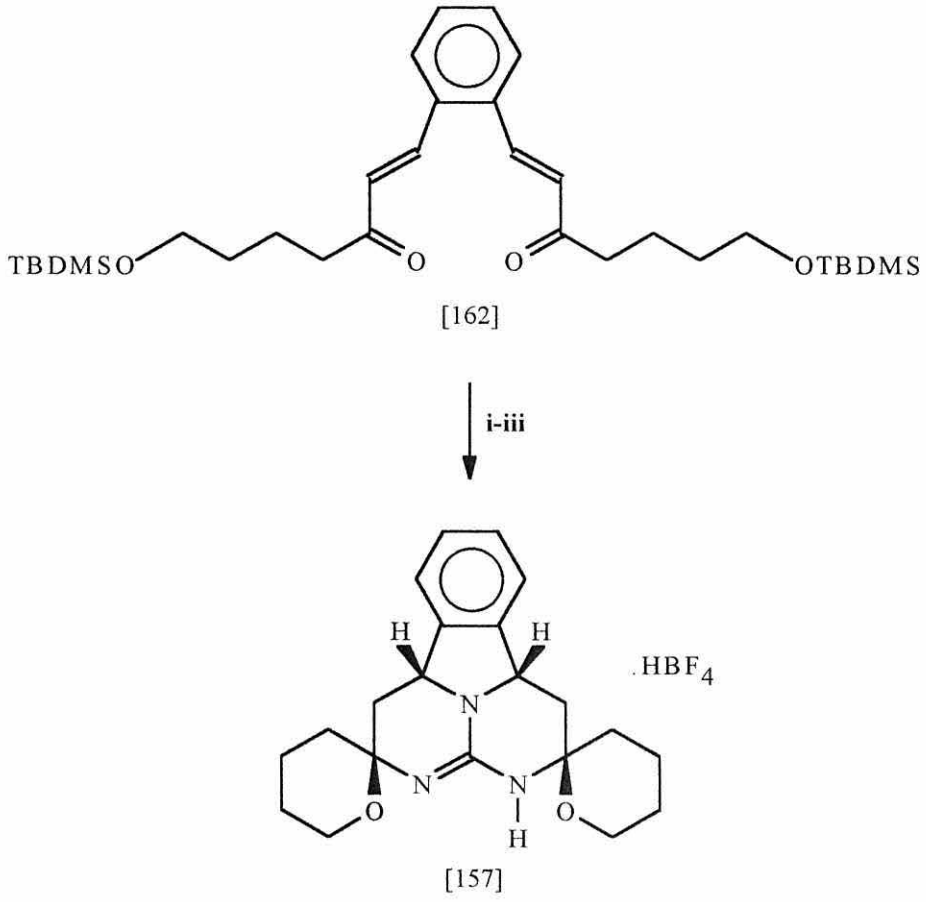
Methyltriphenylphosphonium bromide [159] was treated with *n*-butyllithium in THF to generate the corresponding methylene ylid [159a] *in situ*. Treatment with δ-valerolactone [158] and silyl protection gave the protected phosphorane [160] in 90% yield. The double Wittig reaction with *o*-phthalic dicarboxaldehyde [161] gave a satisfactory 62% yield of the desired *bis*-α,β-unsaturated ketone [162] (Scheme 51).



Scheme 51 *Reagents and conditions*: i) 1 equiv. *n*-BuLi, THF, -78°C, 0-20°C, 4 h; ii) 0.5 equiv. δ-valerolactone, -78-20°C, 1 h; iii) 0.6 equiv. imidazole, 0.55 equiv. TBDMS-Cl, DMF, 0°C, 30 min, then 20°C, 15 h, 90% overall; iv) 0.25 equiv. [161], CH_2Cl_2, 20°C, 20 h, 62%.

6.2.3 GUANIDINE ADDITION-SPIROCYCLISATION

The *bis*-α,β-unsaturated ketone [162] was then reacted with guanidine in DMF, followed by treatment with methanolic HCl. Again, the yield of the guanidine addition/spirocyclisation (Scheme 52) was somewhat modest at 35% (after purification/ crystallisation), but the compound [157] was obtained as a *single diastereoisomer* (within the limits of NMR spectroscopy, both in the crude reaction mixture and after chromatography/crystallisation). By X-ray crystallography the *syn* stereochemistry was determined (Fig. 15). This again is somewhat in contrast with similar experiments which proceed with around 4:1 stereoselectivity for the *syn* and *anti* isomers respectively[49-51] and indeed mirrors the findings in the batzelladine model studies.



Scheme 52 *Reagents and conditions*: i) 1 equiv. guanidine, DMF, 0-20°C, 5 h; ii) MeOH/HCl, 0°C, 1 h, then 20°C, 15 h; iii) sat. $NaBF_{4(aq)}$, 35% overall.

This was obviously an extremely encouraging result. Five new heterocyclic rings are formed in a single synthetic step and in a diastereospecific manner. The relative stereochemistry about the four stereocentres in the guanidine core in [157] is also the same as that observed in ptilomycalin A [8].

Some attempts have been made at electrophilically halogenating the benzene ring of [157], but as yet without success. Unfortunately, substituted derivatives of *o*-phthalic dicarboxaldehyde do not appear to be commercially available. There are a limited number of ways in which they may be prepared, but it was decided at this point to focus on the pyrrole-fused model system [156].

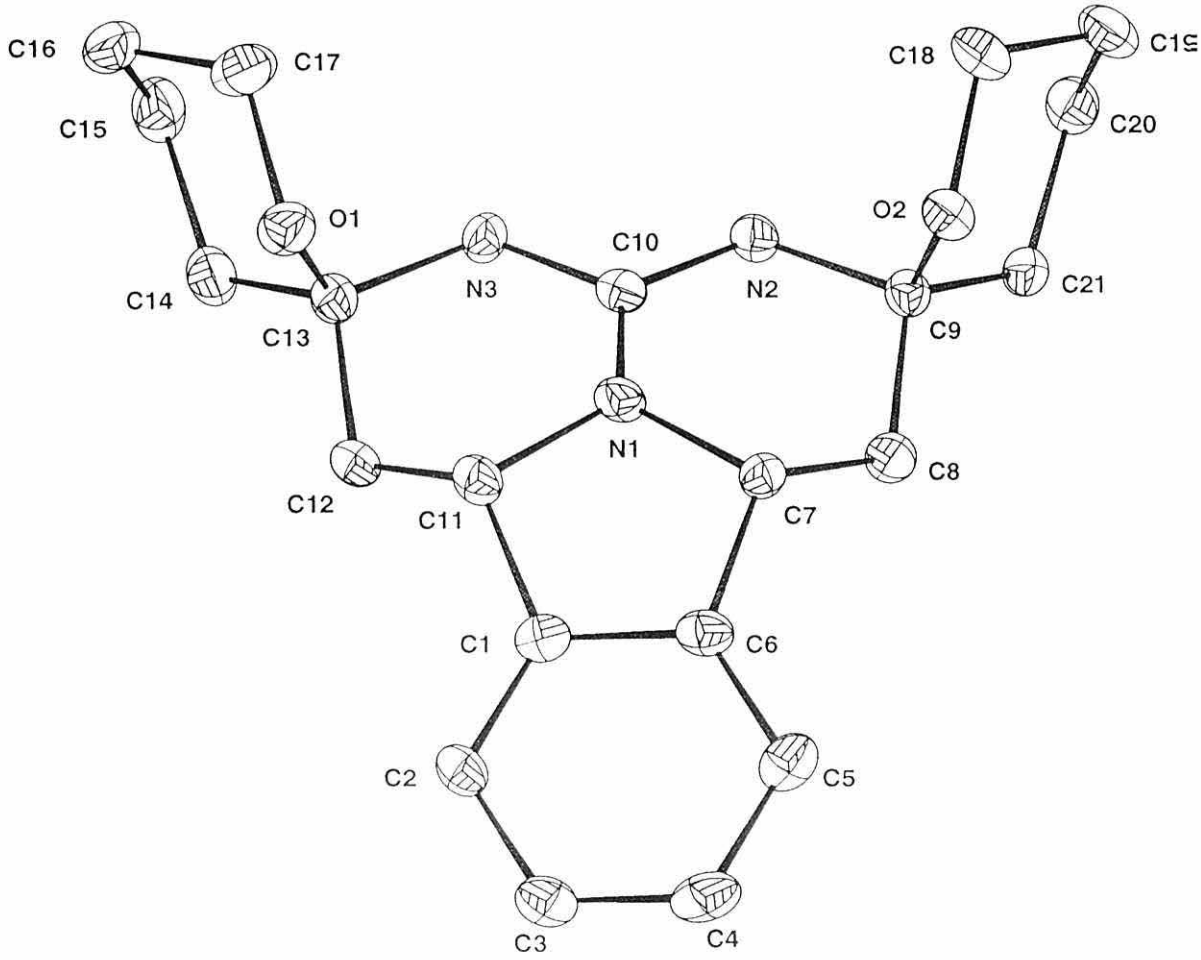
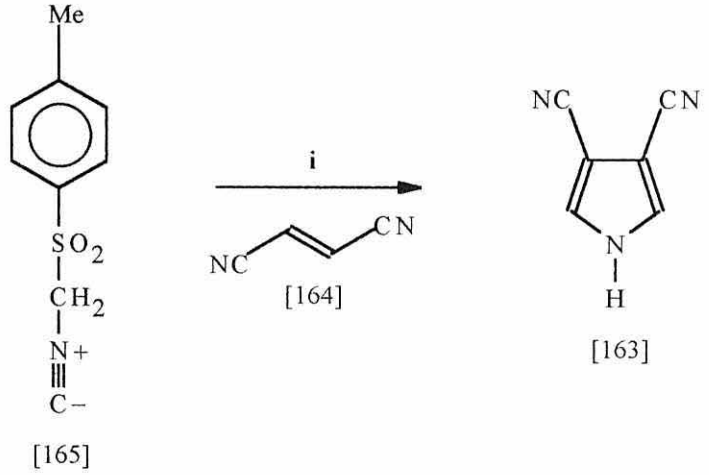


Figure 15. Crystal Structure of the Benzo-Fused Model [157]

6.3 ATTEMPTED PREPARATION OF A PYRROLE-FUSED SYSTEM

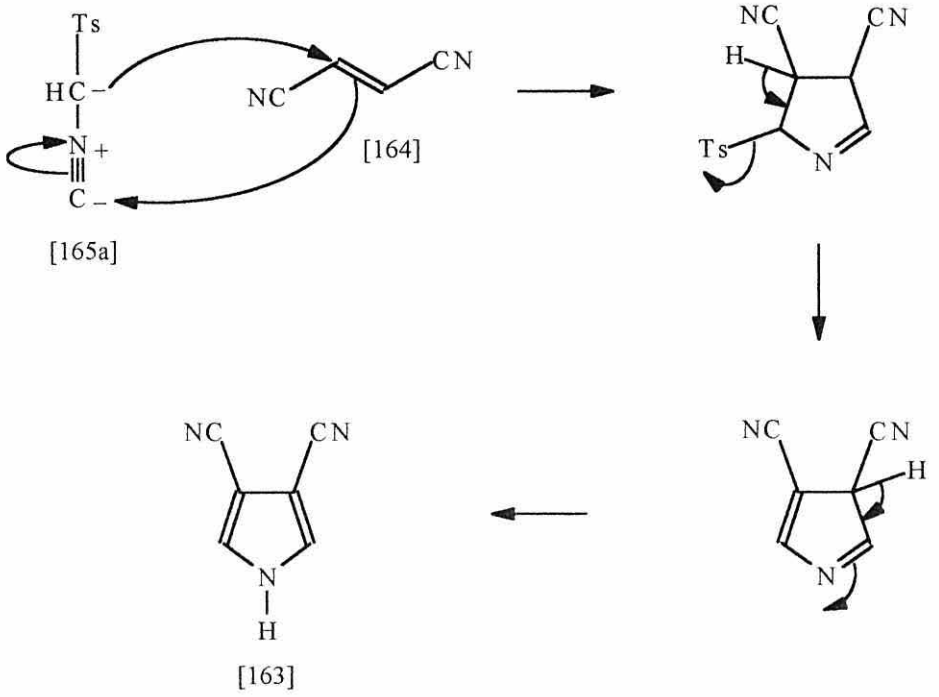
6.3.1 PREPARATION OF THE PYRROLE-3,4-DICARBOXALDEHYDE [172]

In order to prepare a pyrrole-fused system analogous to [156] by modifying the established route, it is evident that the dialdehyde required will be an *N*-alkyl-pyrrole-3,4-dicarboxaldehyde. In a report by Danikiewicz, *et al.*,[104] the preparation of pyrrole-3,4-dinitrile [163] is described from fumaronitrile [164] and *p*-toluene-sulphonylmethyl isocyanide (TOSMIC) [165] (Scheme 53).



Scheme 53 *Reagents and conditions*: i) 1 equiv. [165], 2 equiv. NaH, DMF, 0°C, 5 min, then 1.2 equiv. [164], 0°C, 15 min, 71%.

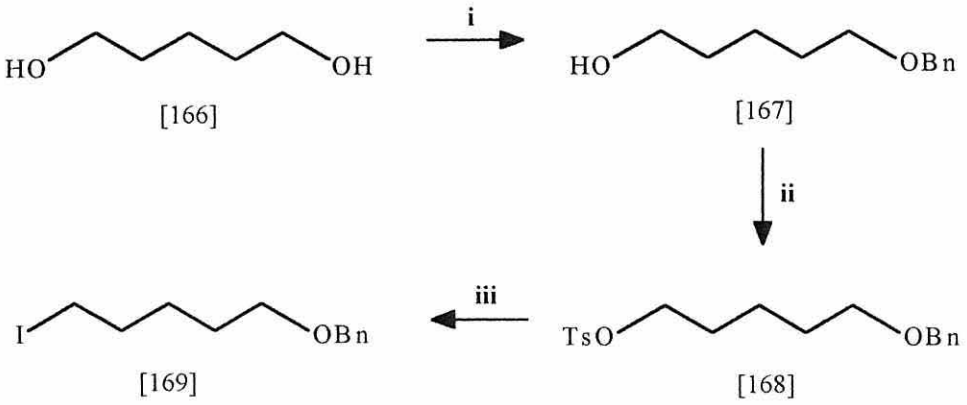
This reaction proceeds *via* a 1,3-dipolar cycloaddition with the anion of TOSMIC adding to the fumaronitrile (Scheme 54). Elimination of tosylate anion followed by tautomerisation of the 3H-pyrrole then gives pyrrole-3,4-dinitrile [163].



Scheme 54

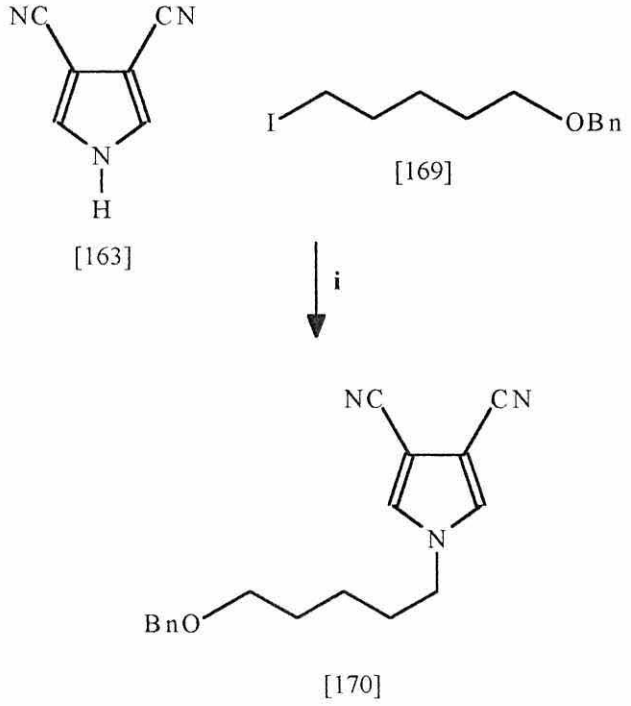
It is well known that nitrile groups can be cleanly reduced to aldehydes with diisobutylaluminium hydride, although it was necessary to block the N-H of the pyrrole prior to reduction, otherwise a complex mixture was obtained.

The side-chain chosen to alkylate the pyrrole was derived from 1,5-pentanediol [166]. A trivial sequence of functional group transformations furnished iodide [169] in reasonable overall yield (Scheme 55). Thus, 1,5-pentanediol was monobenzylated with benzyl bromide giving [167]. The benzyl group was chosen as it would survive the planned guanidine addition/spirocyclisation. The remaining primary hydroxyl function was activated by formation of the tosylate [168] and Finkelstein-type displacement with sodium iodide afforded iodide [169] in 49% overall yield from diol [166]. These reactions were also amenable to scale-up due to the economical nature of the reagents.



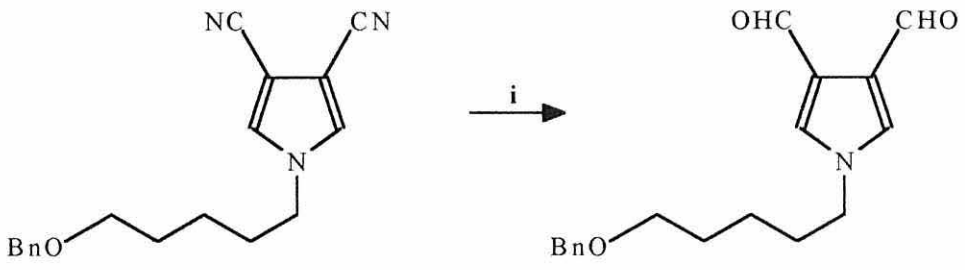
Scheme 55 *Reagents and conditions*: i) 1 equiv. NaH, THF, 0°C, 1 equiv. [166], 15 min, then 0.5 equiv. BnBr, 20°C, 20 h, 71%; ii) 1.1 equiv. TsCl, pyridine, 0-20°C 18 h, 74%; iii) 5 equiv. NaI, acetone, Δ, 4 h, 93%.

The alkylation of pyrrole-3,4-dinitrile [163] occurred smoothly in 76% yield (Scheme 56) by removal of the acidic N-H proton and quenching the resulting anion with iodide [169].



Scheme 56 *Reagents and conditions*: i) 1.2 equiv. NaH, DMF, 0°C, 5 min. then 0.9 equiv [169], 20°C, 3 h, 76%.

It was now possible to reduce the nitrile groups of the pyrrole with diisobutylaluminium hydride (Scheme 57). Treatment of a toluene solution of [170] with three equivalents of DIBAL-H (in toluene) at low temperature followed by hydrolysis with dilute aqueous sulphuric acid furnished the dialdehyde [171] in 80% yield.

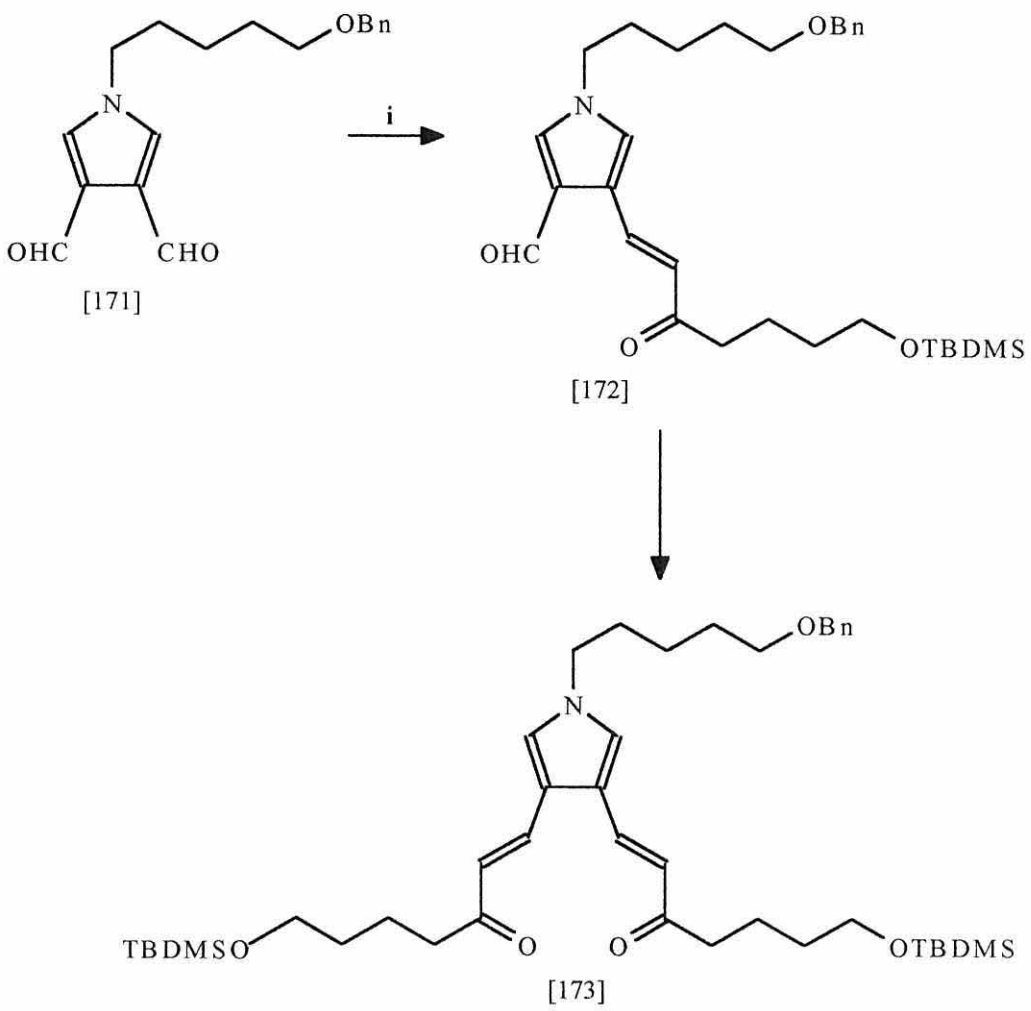


[170] [171]

Scheme 57 *Reagents and conditions*: i) 3 equiv. DIBAL-H, PhMe, -30°C, 80 min, then Et_2O, NH_4Cl, $H_2SO_{4(aq)}$, 20°C, 22 h, 80%.

6.3.2 WITTIG REACTIONS OF THE PYRROLE-3,4-DICARBOX-ALDEHYDE [172]

The desired pyrrole-3,4-dicarboxaldehyde had thus been prepared in multi-gram quantities in relatively short order. The next key step was the double Wittig reaction to generate the *bis*-α,β-unsaturated ketone. Dialdehyde [171] was treated with the prescribed α-stabilised silyl protected ylid [160]. However, this was found to react with only one of the aldehyde groups, even when an excess of ylid was present (Scheme 58). The reaction was attempted under different conditions (prolonged refluxing) and in different solvents (CH_2Cl_2, THF, DMF) but only the *mono*-olefinated product [172] was formed with no trace of the desired *bis*-α,β-unsaturated ketone [173].



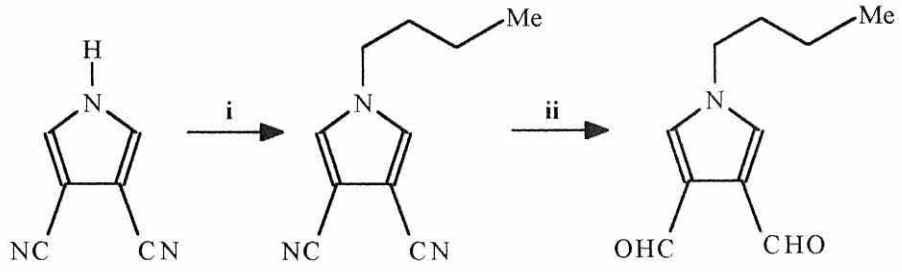
Scheme 58 *Reagents and conditions*: i) 4 equiv. [160], CH_2Cl_2, 20°C, 7 d, 69% of [172].

It is possible that this is due either to steric or electronic factors. On steric grounds, after the first Wittig reaction, the remaining aldehyde group in [172] may be too sterically hindered for further reaction. This possibility was discounted when [171] was reacted with four equivalents of the simple acetylmethylene triphenylphosphorane [122] and the same result observed. On electonic grounds, the aldehyde groups in [171] are vinylogous amides, which would thereby decrease their reactivity.

There are two immediately obvious ways to influence this reactivity. The first would be to attach an electron-withdrawing group to the nitrogen of the pyrrole, thereby negating somewhat the vinylogous effect. Alternatively, a more reactive ylid could be

used, such as a Wadsworth-Emmons or a Horner-type ylid. The second possibility seemed the easiest to implement, and it transpired that the Wadsworth-Emmons variation of the Wittig reaction would in fact lead to the desired compound.

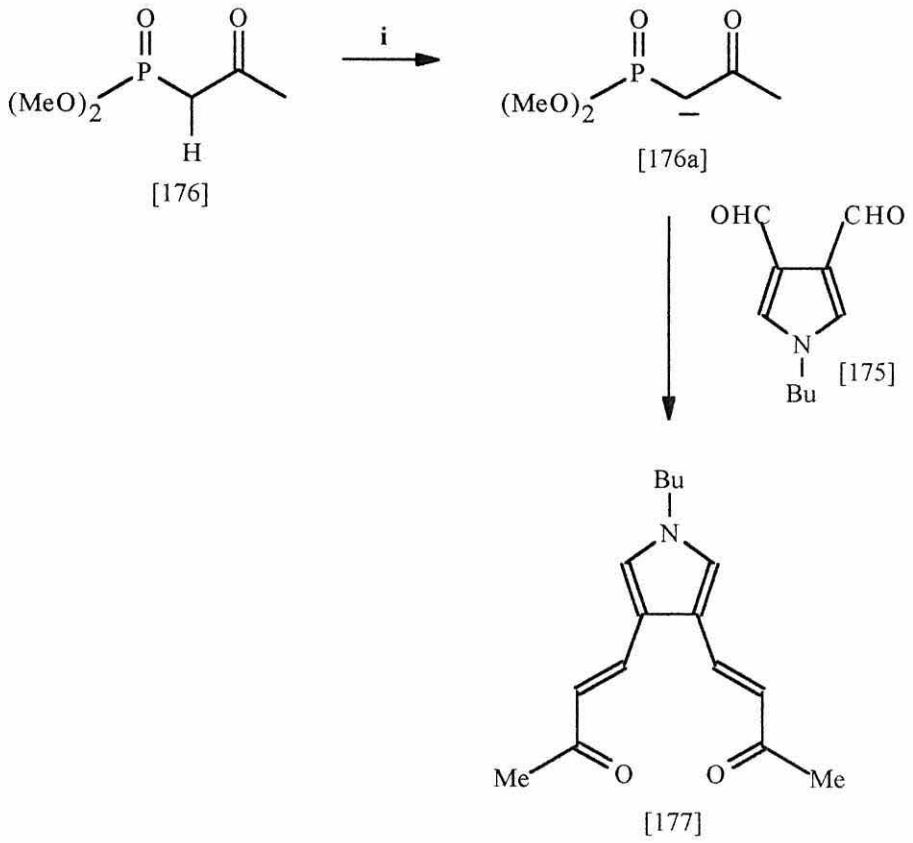
This variation of the classical Wittig reaction was reported by Wadsworth and Emmons in 1961.[105] The ylids used in this variation are activated by a P=O bond and in some cases have been shown to be several orders of magnitude more reactive than, for example, acyl ylids. In order to examine this possibility, test reactions were carried out on a simple pyrrole dicarboxaldehyde [175]. This compound was prepared by treatment of 3,4-pyrroledinitrile [163] with sodium hydride, followed by quenching with *n*-butyl iodide. The two nitrile groups of [174] were then reduced with DIBAL-H giving, on hydrolysis, the dialdehyde [175] (Scheme 59).



[163] [174] [175]

Scheme 59 *Reagents and conditions*: i) 1.2 equiv. NaH, DMF, 0°C, 5 min. then 0.9 equiv *n*BuI, 20°C, 3 h, 76%; ii) 3 equiv. DIBAL-H, PhMe, -30°C, 80 min, then Et_2O, NH_4Cl, $H_2SO_{4(aq)}$, 20°C, 22 h, 80%.

The commercially available dimethylacetylmethyl phosphonate [176] was treated with 1.1 equivalents of sodium hydride in 1,2-dimethoxyethane to generate the anion [176a]. Addition of 0.25 equivalents of aldehyde [175] followed by stirring at room temperature for 36 hours gave, after chromatography, the *bis*-α,β-unsaturated ketone [177] in 75% yield. It was found that the reaction was greatly accelerated by refluxing. However, this caused the phosphonate anion to begin reacting with the carbonyl group of the newly formed enone, such was the reactivity of this Wadsworth-Emmons ylid.



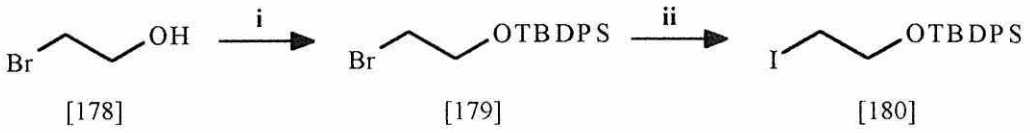
Scheme 60 *Reagents and conditions*: i) 1.1 equiv. NaH, DME, 30 min, 20°C, then 0.25 equiv. pyrrole, DME, 20°C, 36 h, 75%.

6.3.3 ATTEMPTED PREPARATION OF A SPIROCYCLIC SYSTEM

The reductive ($NaBH_4$) addition of guanidine to [177] was attempted. However, despite a number of attempts, the desired guanidine compound was not observed. Nevertheless, the spiro *N,O*-acetal units were required and such progress having been made, it was decided to attempt the guanidine cyclisation on a suitable precursor (Scheme 63). In order to construct the required precursor, it was necessary to alkylate the dimethylacetylmethyl phosphonate at the terminal carbon. It is well known that β-ketophosphonates of this sort can be alkylated exclusively at the γ-position.[106]

The halide required for the alkylation of phosphonate [176] was a 3-halopropanol derivative (Scheme 61). The hydroxyl group of 3-bromopropanol [178] was protected with *tert*-butyldiphenylsilyl chloride giving silyl ether [179]. It was subsequently found

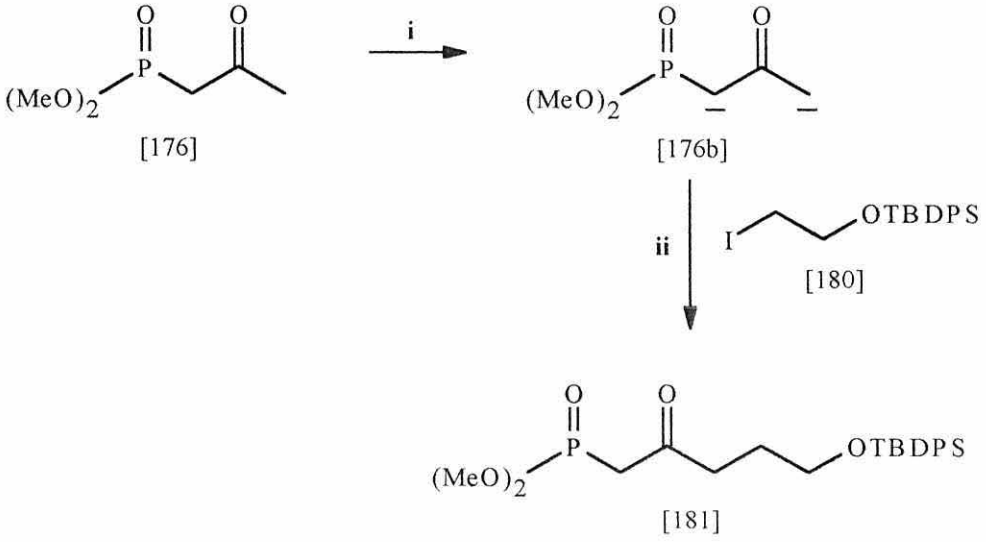
that this bromide was somewhat unreactive towards alkylation and for this reason [179] was transformed into the iodide [180] by a Finkelstein-type displacement.



[178] [179] [180]

Scheme 61 *Reagents and conditions*: i) 1 equiv. TBDPS-Cl, 1 equiv. imidazole, DMF, 0-20°C, 5h, 100%; ii) 3 equiv. NaI, acetone, Δ, 3 hours, 89%.

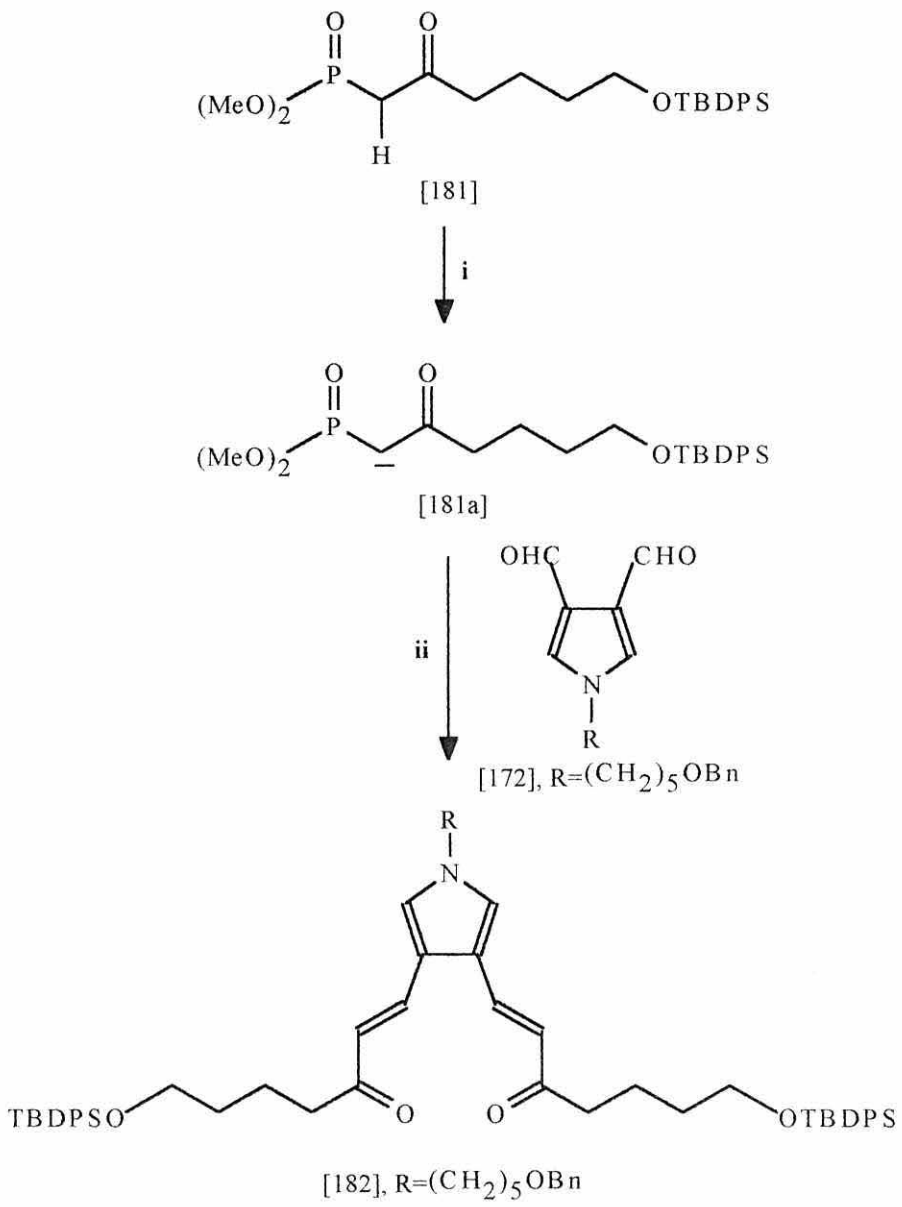
Treatment of [176] with sodium hydride followed by *n*-butyllithium gave dianion [176b]. This was quenched by iodide [180] to give exclusively the γ-alkylated phosphonate [181].



[176] [176b] [180] [181]

Scheme 62 *Reagents and conditions*: i) 1.1 equiv. NaH, THF, 20°C, 20 min, then 1.1 equiv. BuLi, 20°C, 30 min; ii) 1.5 equiv. [180], 20°C, 4 h, 70%.

The next step was the Wittig reaction of phosphonate ylid [181] with pyrrole-3,4-dicarboxaldehyde [171]. Under the same conditions as used previously, treatment of this ylid with sodium hydride in DME followed by addition of the dialdehyde gave the desired *bis*-α,β-unsaturated ketone [182] in an encouraging 73% yield (Scheme 63).



Scheme 63 *Reagents and conditions*: i) NaH, DME, 30 min; ii) 0.3 equiv. [172], 20°C, 24 h, 73%.

With the desired *bis*-α,β-unsaturated ketone [182] in hand, the crucial step was addition of guanidine and spirocyclisation. The presence of the pyrrole ring system makes [182] essentially an extended vinylogous amide and this will obviously affect the electronic characteristics of the molecule. It was felt that this would decrease the reactivity of the enones but guanidine is so reactive towards these systems that it should still undergo Michael addition followed by ring closure. However, on attempting the

guanidine addition under standard conditions, no trace of the desired hexacyclic material was observed, despite repeated attempts Guanidine was added at 0°C and the methanolic HCl was generated by mixing acetyl chloride with methanol. The starting compound was consumed, but the material obtained on work-up consisted of unidentifiable decomposition products.

6.4 CONCLUSION

The simple benzo-fused model compound [157] was prepared efficiently as a single diastereoisomer. The relative stereochemistry of the four stereocentres in [157] is identical to that found in the pentacyclic core of ptilomycalin A. The work towards a pyrrole-based system has shown that the precursors are not amenable to the guanidine addition-reduction/spirocyclisation sequence. It may therefore be logical to concentrate the future research efforts towards elaboration of the benzo-fused system [157].

CHAPTER 7

SUMMARY

7.1 SUMMARY

The aims of this research project were twofold. The primary target was the guanidine natural product batzelladine A. This novel alkaloid was found to consist of a bicyclic guanidine moiety identical to another natural product, crambine A, linked *via* an ester group to a triazaacenaphthylene heterocycle. The crambine A portion was known and had been synthesised in racemic and optically pure form.[60] The tricyclic guanidine system was therefore the subject of the initial synthetic effort. The batzelladine metabolites were found to exhibit a range of biological activity, in particular, batzelladines A and B were found to be active against the human immunodeficiency virus-type 1 (HIV-1).

The synthetic strategy was based on the double Michael addition of guanidine to an ester-substituted *bis*-α,β-unsaturated ketone. Subsequent ring closure and reduction would then lead to the formation of the tricyclic core of batzelladine A. This rationale was based on previous results within the group towards another related guanidine alkaloid, ptilomycalin A.[49-51] It was known that the formation of *cis* pyrrolidine rings is favoured over *trans* by *ca.* 4:1 using this strategy and reduction of the intermediate hemiaminal by a hydride equivalent should be diastereoselective.

In order to test the applicability of this strategy, the tricyclic model compound [120] without the ester functionality was prepared. The desired bis-α,β-unsaturated ketone precursor (3*E*,7*E*)-2,7-dioxooctadeca-3,7-diene was prepared by Wittig methodology from succinaldehyde. Addition of guanidine followed by reduction with sodium borohydride (MeOH:DMF:H_2O) led to the *diastereospecific* formation of the tricyclic guanidine compound [120]. The stereochemistry of this compound was determined by nOe experiments on [120] and X-ray diffraction analysis of a symmetrical analogue [125b]. The two six-membered rings of the triazaacenaphthylene system were found to be *syn*.[89] At the time this part of the work was being completed, the same stereochemistry had been assigned to the natural product, batzelladine A. However, a subsequent revision of the stereochemistry to *anti* suggests that the synthetic methodology employed leads to the wrong diastereoisomer.

Despite this apparent shortfall with the diasteroselection, the methodology was extended in an attempt to incorporate the desired ester functionality into the tricyclic framework. A Knoevenagel condensation of the prescribed aldehyde (4*E*)-6-

oxopentadec-4-enal [117] with *tert*-butyl acetoacetate in the presence of an amine such as piperidine gave the desired trisubstituted olefin *tert*-butyl (2*E*/*Z*,6*E*)-2-ethanoyl-8-oxoheptadeca-2,6-dienoate. After extensive efforts, the Knoevenagel reaction was found to proceed in only a moderate 32% yield, with the product formed as a 1:1 mixture of geometric isomers. The reductive addition of guanidine to this system was examined and the desired ester-substituted tricyclic guanidine compound did appear to be present by mass spectrometry, but unfortunately lack of material precluded a fuller investigation of this key reaction. Alternative routes to the ester-substituted *bis*-α,β-unsaturated ketone were analysed, but proved fruitless.

Following a report in the literature[65] towards the end of the research project, the synthetic route was modified to access the left-hand tricycle of one of the recent additions to the family, batzelladine F. This alkaloid consists of two tricyclic fragments, linked by an ester group. The stereochemistry of both triazaacenaphthylene rings in this natural product have been assigned as *anti* on the basis of the revision of batzelladines A and D.[68] By altering slightly the rationale employed for the tricyclic model [120], it was possible to access the left-hand portion of batzelladine F. Unfortunately, time restraints prevented a detailed analysis of this result and the stereochemistry of the compound [143] remained undetermined. The compound obtained is also the compound obtained *via* a proposed basic hydrolysis of batzelladine F. Spectroscopic comparison of the two, combined with X-ray diffraction analysis (if crystals can be obtained) may prove unequivocally the stereochemistry of these tricyclic systems.

The second aim of the project was to develop a route to functionalised analogues of the marine alkaloid ptilomycalin A. Previous work[49-51] had illustrated the difficulties encountered when attempting to incorporate the ester functionality into these systems. A rapid route into a series of functionalised analogues of the natural product may give insight into the biological mode of action of these compounds and help to determine the minimal structure requirement for activity.

Two such systems were investigated, the first based on a benzo-fused pentacyclic guanidine template containing two six-membered spiro *N*,*O*-acetal units similar to those found in ptilomycalin A. The desired precursor to guanidine addition-spirocyclisation was efficiently prepared from δ-valerolactone and methyltriphenylphosphonium bromide.

Double Wittig reaction of the intermediate phosphorane with *o*-phthalic dicarboxaldehyde resulted in the formation of the desired *bis*-α,β-unsaturated ketone [162]. Treatment of this compound with guanidine in DMF followed by spirocyclisation with methanolic HCl furnished the benzo-fused guanidine pentacycle [157] in 35% yield after chromatography and crystallisation as a *single diastereoisomer*. The sterochemistry was determined by X-ray diffraction analysis and found to be identical to the corresponding stereocentres in the pentacyclic core of ptilomycalin A.

The second model system was based on a pyrrole-fused pentacyclic guanidine template. By a similar methodology utilised in the formation of the benzo-fused analogue, two *bis*-α,β-unsaturated ketone precursors were prepared. The corresponding pyrrole-3,4-dicarboxaldehyde was found to be considerably less reactive towards the typical α-stabilised phosphoranes used to generate the enones. Reactions of this type succeded in affording only the mono-olefinated products. To overcome this shortfall, a Wadsworth-Emmons phoshonate ylid was used. These ylids are orders of magnitude more reactive than the corresponding α-stabilised phosphoranes and furnished the desired *bis*-α,β-unsaturated ketones in good yields (>70%). The guanidine addition was examined on the two systems but in both cases, no trace of the expected pyrrole-fused polycyclic guanidine was seen. This change in reactivity was attributed to the electronic properties of the pyrrole π-system.

7.2 FUTURE WORK

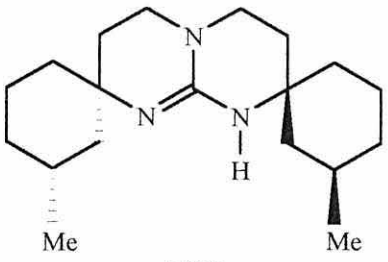
Although this research has shown that addition of guanidine in the free base form to *bis*-α,β-unsaturated ketones is a viable method of accessing reasonably complex heterocyclic frameworks, a number of challenges still remain. The addition of guanidine to an ester-substituted system has not been fully investigated and this remains the key reaction in this synthetic approach to these natural products.

An area of work which may prove fruitful is the preparation of functionalised analogues of ptilomycalin A. The pyrrole-based system does not appear to be amenable to the guanidine addition-spirocyclisation sequence, which is somewhat unfortunate, since the nitrogen seemed a very convenient site for alkylation. However, it may prove

possible to elaborate the simple unsubstituted benzo-fused model [157]. For example, halogenation of [157] followed by coupling with an alkyl spermidine derivative may furnish an extremely good mimic for ptilomycalin A. The length of the alkyl spacer could then be easily altered to enable access to a range of analogues for biological testing.

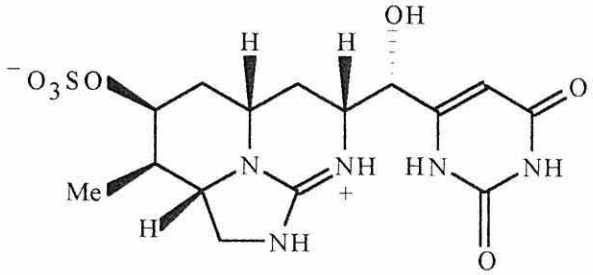
7.3 RECENT DEVELOPMENTS

Chiral C_2-symmetric guanidine bases have been shown to be effective catalysts for a variety of processes including the asymmetric nitroaldol (Henry) reaction[107] and the Michael addition of pyrrolidine to unsaturated lactones.[108] Elaboration of a synthetic route towards a tetracyclic model for ptilomycalin A has furnished [183] which has been found to catalyse the Michael addition of pyrrolidine to the α,β-unsaturated lactone 2(5*H*)-furanone.[109]



[183]

Other natural products may be accessible using this methodology. For example, current research within the group[110] is directed towards the synthesis of the marine hepatotoxin, cylindrospermopsin [5]. The complex array of functionality and stereochemistry in this molecule make it an interesting synthetic challenge.



[5]

CHAPTER 8

EXPERIMENTAL

SOLVENTS

All solvents used in reactions were dried using standard methods found in the literature.[111] In particular, diethyl ether and tetrahydrofuran were distilled from sodium wire and benzophenone; chloroform and carbon tetrachloride were distilled from phosphorus pentoxide; dichloromethane and DMF were dried over calcium hydride and freshly distilled.

CHROMATOGRAPHY

All new compounds were homogeneous 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 $EtOH/H_2SO_4$ and heating to 180°C. 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

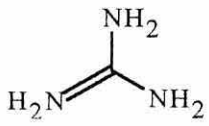
Melting points were recorded using a Gallenkamp capillary apparatus and are uncorrected. Infra-red spectra were recorded as thin films or solutions where appropriate on a Perkin-Elmer 1600 FT-IR spectrometer. Absorption frequencies are reported in wavenumber ν, whose unit is the reciprocal centimetre (cm^{-1}). Absorption intensities are reported 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. Mass spectrometric data were not collected for certain compounds due to restricted access to this service. Proton NMR spectra were run at 250MHz on a Bruker AC250 spectrometer unless otherwise stated. Carbon-13 NMR spectra were run at 62.5MHz 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 nitrogen gas. All experiments were conducted under a positive pressure of the inert gas. The solution of *n*-butyllithium in hexanes was titrated[111] against diphenylacetic acid in THF immediately before use. All yields refer to the pure isolated compound unless otherwise stated. All compounds were homogeneous by thin-layer chromatography unless otherwise stated. The term *in vacuo* refers to the reduced pressure of a Büchi rotary evaporator at water pump pressure (~15mm Hg) and/or rotary vacuum pump pressure (~1mm Hg).

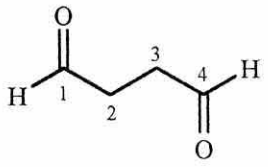
GUANIDINE [1][112]



[1]

Sodium (2.40g, 0.105mol) was washed with petrol and dissolved in methanol (200ml) at 0°C. Guanidine hydrochloride (10.0g, 0.105mol) was added to this solution and the mixture stirred at 20°C for 2 hours. The resulting residue was evaporated *in vacuo*, triturated with methanol (4 x 50ml) and filtered several times to remove solid sodium chloride. Final evaporation of the solvent *in vacuo* gave the title compound [1] as a low-melting solid in 95% yield (5.90g).

SUCCINALDEHYDE [119][90]



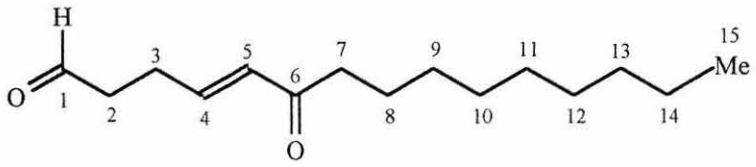
[119]

To 2,5-dimethoxytetrahydrofuran (50g, 0.38mol) was added aqueous hydrochloric acid (0.1N, 200ml) 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 (50ml) and saturated with sodium chloride. The product was extracted with ether (5 x 100ml), dried over magnesium sulphate and evaporated *in vacuo* to give a yellow oil. This oil was doubly distilled under reduced pressure using a short-path apparatus, yielding the title compound [119] as a lachrymatory colourless liquid (B.pt. 57-60°C at 15mm Hg, Lit. B.pt.[90] 62°C at 14mm Hg) in 49% yield (31.6g).

^{1}H NMR : δ 2.8 (s, $4H_{2,3}$), 9.8 (s, $2H_{1,4}$) ppm.

FT-IR : ν_{max} 2958 (s, sp^3 CH str), 1722 (s, C=O str), 1634 (w), 1456 (s), 1350 (s), 1202 (s), 978 (br, s), 820 (s) cm^{-1}.

(4*E*)-6-OXOPENTADEC-4-ENAL [117]



[117]

To a cooled (-78°C) suspension of acetylmethylene triphenylphosphorane (5.00g, 15.7mmol) in THF (200ml) was added dropwise *n*-butyllithium (13.3ml, 1.29M solution in hexanes, 17.2mmol), causing a deep red colour to develop. The mixture was stirred for 30 minutes, whereupon *n*-octyl iodide (4.57g, 19.0mmol) was added dropwise; the mixture was then allowed to stir at 20°C for 18 hours. After evaporation of the THF *in vacuo* the resulting red oil was dissolved in ethyl acetate (100ml) and washed with water (2 x 50ml). The combined aqueous phase was extracted with ethyl acetate (50ml) and the combined organics yielded, upon drying over magnesium sulphate and evaporation *in vacuo*, the crude alkylated phosphorane [118] as a red oil in quantitative yield (6.75g).

To a stirred solution of the phosphorane (6.75g, 15.7mmol) in dichloromethane (30ml) was added succinaldehyde [119] (2.7g, 31.4mmol) and the mixture was stirred at 20°C for 48 hours. The solution was washed with water (2 x 50ml) to remove excess succinaldehyde and the organic phase dried over magnesium sulphate. The solvent was evaporated *in vacuo* and most of the triphenylphosphine oxide was removed by trituration using diethyl ether:petroleum ether (1:1) and the product purified by column chromatography using 15-30% diethyl ether:petroleum ether, affording the title compound [117] as a mobile yellow oil in 67% yield (2.50g, R_f = 0.26 in 40% diethyl ether:petroleum ether). A less polar fraction was also isolated which was shown to be the symmetrical *bis*-α,β-unsaturated ketone [124d] (Page 130) in 14% yield (0.86g, R_f = 0.45 in 40% diethyl ether:petroleum ether).

^{1}H NMR : δ 0.88 (t, $3H_{15}$, J = 6.5Hz), 1.27 (m, $12H_{9\text{-}14}$), 1.60 (m, $2H_8$), 2.52 (m, $4H_{2,7}$), 2.65 (m, $2H_3$), 6.12 (d, $1H_5$, J = 15.9Hz), 6.81 (dt, $1H_4$, J = 15.9Hz, 6.6Hz), 9.81 (s, $1H_1$) ppm.

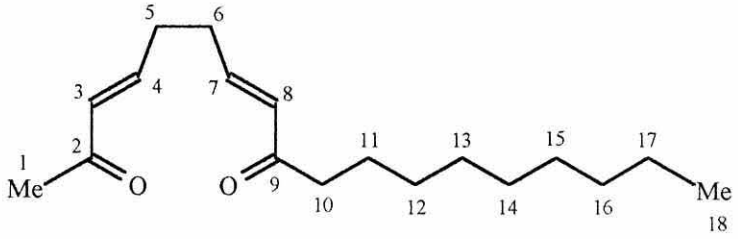
^{13}C NMR : δ 14.01 (q_{15}), 22.58 (t), 24.09 (t), 24.55 (t), 29.20 (t), 29.36 (t), 31.79 (t), 40.31 (t), 41.87 (t), 130.99 (d_5), 143.95 (d_4), 200.25 (d_1), 200.34 (s_6) ppm.

FT-IR : ν_{max} 2926 (s, sp^2 CH str), 2856 (s, sp^3 CH str), 2718 (w, (O=)CH str), 1727 (s, C=O str), 1675 (s, (C=C-)C=O str), 1631 (s,C=C str), 1456 (s), 1366 (s) cm^{-1}.

MS (CI) : m/z 257 (20% $[MH+NH_4]^+$), 256 (100% $[M+NH_4]^+$), 239 (60% $[MH]^+$), 238 (20% $[M]^+$), 221 (42% $[M-OH]^+$) daltons.

HRMS (CI) : $C_{15}H_{27}O_2$ ($[MH]^+$) requires 239.2011, found 239.2011 daltons.

(3*E*, 7*E*)-2,9-DIOXOOCTADECA-3,7-DIENE [121]



[121]

To a stirred solution of aldehyde [117] (1.66g, 6.97mmol) in dichloromethane (20ml) was added acetylmethylene triphenylphosphorane (2.67g, 8.40mmol) and the mixture stirred at 20°C for 24 hours. The solvent was evaporated *in vacuo* and triphenylphosphine oxide was removed by trituration with diethyl ether:petroleum ether (1:1) before purification by column chromatography eluting with 30-40% diethyl ether:petroleum ether giving the title compound [121] as a yellow oil in 73% yield (1.43g, R_f = 0.24 in 40% diethyl ether:petroleum ether).

^{1}H NMR : δ 0.88 (t, $3H_{18}$, J = 6.5Hz), 1.27 (bm, $12H_{12\text{-}17}$), 1.62 (m, $2H_{11}$), 2.26 (s, $3H_1$), 2.41 (m, $4H_{5,6}$), 2.53 (t, $2H_{10}$, J = 7.3Hz), 6.10 (d, $1H_8$, J = 15.9Hz), 6.16 (d, $1H_3$, J = 15.9Hz), 6.74-6.83 (m, $2H_{4,7}$) ppm.

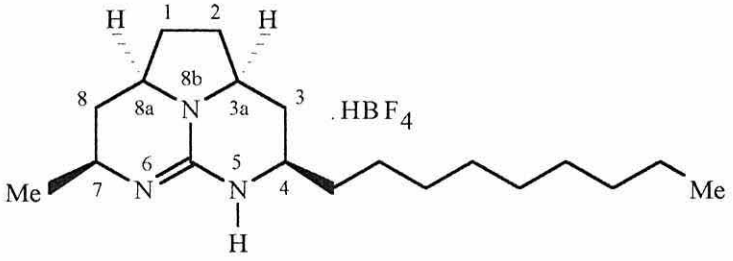
^{13}C NMR : δ 14.01 (q_{18}), 22.58 (t), 24.14 (t), 26.97 (t), 29.21 (t), 29.35 (q_1), 29.90 (t), 30.01 (t), 31.79 (t), 40.37 (t), 130.95 (d_8), 131.86 (d_3), 144.33 (d_7), 145.72 (d_4), 198.07 (s_2), 200.35 (s_9) ppm.

FT-IR : ν_{max} 3468 (m br, C=O overtone), 2928 (s, sp^2 CH str), 2852 (s, sp^3 CH str), 1690 (s, (C=C-)C=O str), 1672 (s, (C=C-)C=O str), 1630 (s, C=C str), 1455 (m), 1362 (s), 1254 (s) cm^{-1}.

MS (CI) : 296 (90% $[M+NH_4]^+$), 279 (20% $[MH]^+$), 240 (100%), 126 (42%) daltons.

HRMS (CI) : $C_{18}H_{31}O_2$ ($[MH]^+$) requires 279.2324, found 279.2324 daltons.

(±)-4*S*,3a*S*,8a*R*,7*R*-4-NONYL-7-METHYL-(1,2,3,4,7,8-HEXAHYDRO-5*H*-5,6,8b-TRIAZAACENAPHTHYLENE)-6-IUM TETRAFLUOROBORATE [120]



[120]

To a cooled (0°C) solution of [121] (550mg, 1.98mmol) in DMF (10ml) was added guanidine (117mg, 1.98mmol) as a solution in DMF (3ml) and the mixture was allowed to warm to 20°C, with stirring, over 5 hours.

Water (5ml) and methanol (15ml) were added, followed by $NaBH_4$ (451mg, 11.9mmol) at 0°C; the mixture was allowed to warm to 20°C and stirred for 16 hours. The solvent was evaporated *in vacuo*, then diluted with dichloromethane (50ml). The organic fraction was acidified with aqueous hydrochloric acid (20ml, 1N), washed with water (2 x 50ml) and brine (2 x 50ml) and the combined aqueous layers extracted with dichloromethane (20ml). The combined organic layers were then washed with saturated lithium bromide (2 x 20ml). The organic phase was dried over magnesium sulphate and evaporated *in vacuo* to give a brown oil (550mg).

Ion exchange: The crude reaction product was dissolved in dichloromethane (25ml). Saturated aqueous sodium tetrafluoroborate (25ml) was added and the mixture stirred briskly for 2 hours. The aqueous layer was extracted with dichloromethane (2 x 25ml) and the combined organics dried over magnesium sulphate and evaporated *in vacuo* giving the crude product as a brown oil (570mg).

This oil was purified by column chromatography eluting with 0-4% methanol: chloroform and trituration (dichloromethane:diethyl ether:petroleum ether) furnishing the title compound [120] as an amorphous yellow solid in 32% yield (250mg, M.pt. = 80-82°C, R_f = 0.22 in 4% methanol:chloroform).

^{1}H NMR (500MHz in CD_3OD) : δ 0.89 (t, 3H, J = 6.6Hz), 1.25 (m, H_{3ax} and H_{8ax}), 1.26 (d, 3H, J = 6.4Hz), 1.28-1.45 (m, 14H), 1.55 (m, 2H), 1.68 (m, H_{1eq} and H_{2eq}), 2.20-2.30 (m, H_{3eq}, H_{1ax}, H_{2ax} and H_{8eq}), 3.42 (ddt, H_4, J = 11.3, 3.2, and 6.6Hz), 3.54 (ddq, H_7, J = 11.1, 3.2, 6.4Hz), 3.74 (m, H_{3a} and H_{8a}), 6.55 (s, $1H_{NH}$), 6.65 (s, $1H_{NH}$) ppm.

^{13}C NMR : δ 14.14 ($q_{9'}$), 20.26 (q), 22.65 (t), 25.03 (t), 29.23 (t), 29.29 (t), 29.39 (t), 29.48 (t), 29.63 (t), 30.22 (t), 31.85 (t), 34.45 (t_3), 35.80 (t_8), 46.10 (d_4), 50.47 (d_7), 56.00[a] (d_{3a}), 56.06[a] (d_{8a}), 149.31 (s) ppm. ([a] Interchangeable assignments)

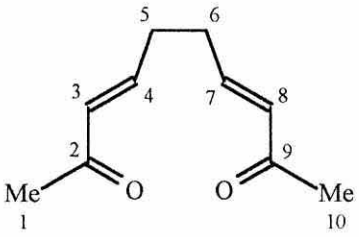
FT-IR : ν_{max} 3376 (s, N-H str), 2926 (s, sp^3 CH str), 1643 (s, C=N str), 1513 (w), 1457 (m), 1331 (w), 1062 (s br) cm^{-1}.

MS (CI) : m/z 307 (20% $[MH+H]^+$), 306 (100% $[MH]^+$) daltons.

HRMS (CI) : $C_{19}H_{36}N_3$ ($[MH]^+$) requires 306.2909, found 306.2909 daltons.

Note: No minor diastereoisomers were detectable by NMR spectroscopy either in the crude reaction mixture (before chromatography and trituration) or in the purified material.

(3*E*, 7*E*)-2,9-DIOXODECA-3,7-DIENE [124a]



[124a]

To a solution of acetylmethylene triphenylphosphorane (5.0g, 15.7mmol) in dichloromethane (20ml) was added succinaldehyde (0.54g, 6.28mmol) in dichloromethane (20ml) and the mixture stirred at 20°C for 30 hours. The solution was washed with water (2 x 50ml) to remove excess succinaldehyde and the organic phase dried over magnesium sulphate. The solvent was evaporated *in vacuo* and the resulting oil purified by column chromatography eluting with 50-100% diethyl ether:petroleum ether giving the title compound [124a] as a pale yellow oil in 74% yield (772mg, R_f = 0.11 in 40% diethyl ether:petroleum ether).

^{1}H NMR : δ 1.94 (s, $6H_{1,10}$), 2.14 (m, $4H_{5,6}$), 5.80 (d, $2H_{3,8}$, J = 15.9Hz), 6.51 (dt, $2H_{4,7}$, J = 15.9, 6.2Hz) ppm.

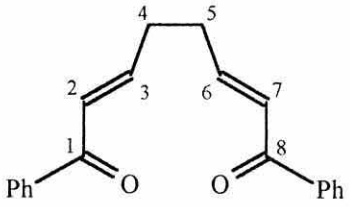
^{13}C NMR : δ 27.25 ($q_{1,10}$), 30.54 ($t_{5,6}$), 131.81 ($d_{3,8}$), 145.79 ($d_{4,7}$), 198.12 ($s_{2,9}$) ppm.

FT-IR ($CDCl_3$) : ν_{max} 3520 (br m, C=O overtone), 2925 (m, sp^3 CH str), 1670 (s, (C=C-)C=O str), 1625 (s, C=C str), 1428 (m), 1361 (s), 1255 (s), 1188 (s), 981 (s) cm^{-1}.

MS (CI) : m/z 184 (100% $[M+NH_4]^+$), 167 (20% $[MH]^+$) daltons.

HRMS (CI) : $C_{10}H_{18}NO_2$ ($[M+NH_4]^+$) requires 184.1338, found 184.1338 daltons.

(2*E*, 6*E*)-1,8-DIOXO-1,8-DIPHENYLOCTA-2,6-DIENE [124b]



[124b]

To a stirred solution of benzoylmethylene triphenylphosphorane (5.00g, 13.2mmol) in dichloromethane (50ml) was added succinaldehyde (0.45g, 5.28mmol) and the mixture stirred at 20°C for 36 hours. The solution was washed with water (2 x 50ml) to remove excess succinaldehyde and the organic phase dried over magnesium sulphate. The solvent was evaporated *in vacuo* and the resulting oil purified by column chromatography eluting with 30-50% diethyl ether:petroleum ether to give the title compound [124b] as a white solid in 68% yield (1.04g, R_f = 0.24 in 40% diethyl ether:petroleum ether).

^{1}H NMR : δ 2.60 (m, $4H_{4,5}$), 6.97 (m, $2H_{2,7}$), 7.40-7.60 (m, ~$8H_{3,6,Ph}$), 7.93 (d, $4H_{Ph}$, J = 7.4Hz) ppm.

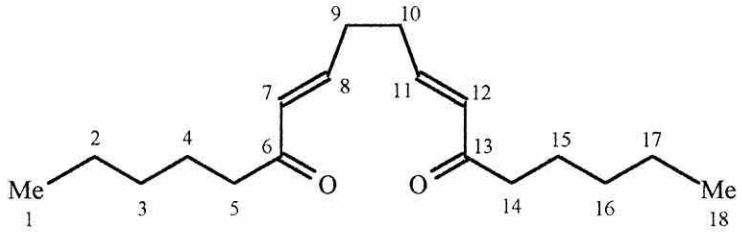
^{13}C NMR : δ 31.16 ($t_{4,5}$), 126.68 (d), 128.42 (d), 128.55 (d), 132.60 (d), 137.65 (s_{Ph}), 147.40 (d) 190.38 ($s_{1,8}$) ppm.

FT-IR : ν_{max} 3063 (m, sp^2 CH str), 2952 (s, sp^3 CH str), 2928 (s, sp^3 CH str), 1667 (s, (C=C-)C=O str), 1624 (m, C=C str), 1445 (m) cm^{-1}.

MS (CI) : m/z 308 (100% $[M+NH_4]^+$), 291 (50% $[MH]^+$), 290 (20% $[M]^+$) daltons.

HRMS (CI) : $C_{20}H_{22}NO_2$ ($[M+NH_4]^+$) requires 308.1651, found 308.1651 daltons.

(7*E*, 11*E*)-6,13-DIOXOOCTADECA-7,11-DIENE [124c]



[124c]

To a cooled (-78°C) suspension of acetylmethylene triphenylphosphorane (5.00g, 15.7mmol) in THF (200ml) was added dropwise *n*-butyllithium (8.3ml, 2.07M solution in hexanes, 17.2mmol) , causing a deep red colour to develop. The mixture was stirred for 30 minutes, whereupon *n*-butyl iodide (3.50g, 19.0mmol, 2.2ml) was added dropwise; the mixture was then allowed to stir at 20°C for 4 hours. After evaporation of the THF the resulting yellow oil was dissolved in ethyl acetate (100ml) and washed with water (2 x 50ml). The aqueous layers were extracted with ethyl acetate (50ml) which yielded, upon drying over magnesium sulphate and evaporation *in vacuo*, the phosphorane [123c] as a yellow oil in quantitative yield (6.75g). The phosphorane (6.75g, 15.7mmol) was dissolved in dichloromethane (25ml) and succinaldehyde [119] (0.54g, 6.28mmol) added; the mixture was then stirred at 20°C for 48 hours. The solution was washed with water (2 x 50ml) to remove excess succinaldehyde and the organic phase dried over magnesium sulphate. Triphenylphosphine oxide was removed by trituration using diethyl ether:petroleum ether (1:1) and the product purified by column chromatography using 20-40% diethyl ether:petroleum ether, affording the title compound [124c] as a mobile yellow oil in 70% yield (1.22g, R_f = 0.25 in 40% diethyl ether:petroleum ether).

^{1}H NMR : δ 0.86 (t, $6H_{1,18}$, J = 6.8Hz), 1.29 (m, $8H_{2,3,16,17}$), 1.60 (m, $4H_{4,15}$), 2.39 (m, $4H_{9,10}$), 2.51 (t, $4H_{5,14}$, J = 7.4Hz), 6.12 (d, $2H_{7,12}$, J = 15.8Hz), 6.80 (m, $2H_{8,11}$) ppm.

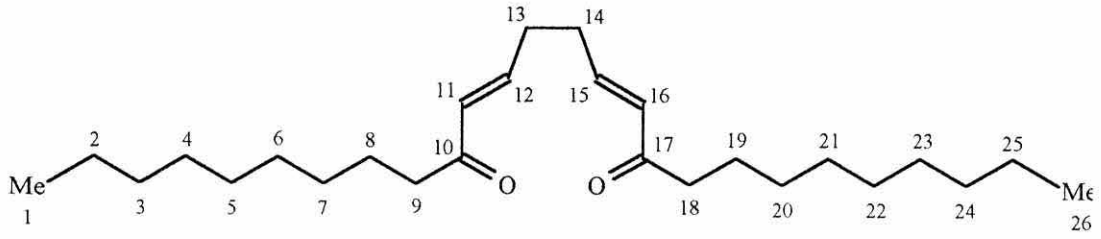
^{13}C NMR : δ 13.83 ($q_{1,18}$), 22.38 (t), 23.79 (t), 30.65 (t), 31.36 (t), 40.23 (t) 130.89 ($d_{7,12}$), 144.52 ($d_{8,11}$), 200.37 ($s_{6,13}$) ppm.

FT-IR : ν_{max} 2940 (s, sp^2 CH str), 2930 (s, sp^3 CH str), 1697 (s, C=O str), 1673 (s, C=C str), 1629 (s, C=C str), 1466 (w), 1371 (w) cm^{-1}.

MS (CI) : 296 (100% $[M+NH_4]^+$), 279 (25% $[MH]^+$) daltons.

HRMS (CI) : $C_{18}H_{31}O_2$ ($[MH]^+$) requires 279.2324, found 279.2330 daltons.

(11*E*, 15*E*)-10,17-DIOXOHEXADIDECA-11,15-DIENE [124d]



[124d]

The *bis*-α,β-unsaturated ketone [124d] was obtained as a by-product in the preparation of the aldehyde [117] as described previously (Page 121). The data for this compound are as follows:

^{1}H NMR : δ 0.89 (t, $6H_{1,26}$, J = 6.5Hz), 1.28 (bs, ~22H), 1.61 (bs, 6H), 2.41 (m, 4H), 2.53 (t, $4H_{9,18}$, J = 7.4Hz), 6.15 (d, $2H_{11,16}$, J = 15.8Hz), 6.81 (m, $2H_{12,15}$) ppm.

^{13}C NMR : δ 13.13 ($q_{1,26}$), 21.70 (t), 23.27 (t), 28.34 (t), 28.48 (t), 29.80 (t), 30.91 (t) 39.52 (t), 130.03 ($d_{11,16}$), 143.56 ($d_{12,15}$), 199.55 ($s_{10,17}$) ppm.

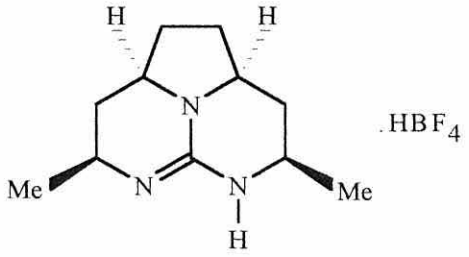
FT-IR : ν_{max} 3023 (m, sp^2 CH str), 2926 (s, sp^3 CH str), 1688 (s, (C=C)-C=O str), 1658 (m, C=C str), 1469 (s), 1411 (m), 1381 (m), 1171 (m), 984 (s) cm^{-1}.

Microanalysis : $C_{26}H_{46}O_2$ requires C=79.93, H=11.88%, found C=79.73, H=12.42%.

MS (EI) : m/z 408 (50% $[M+NH_4]^+$), 393 (25% $[M+3H]^+$), 392 (10%, $[M+2H]^+$), 391 (20% $[MH]^+$), 214 (100%), 197 (30%), 188 (55%) daltons.

HRMS (EI) : $C_{26}H_{47}O_2$ ($[MH]^+$) requires 391.3576, found 391.3576 daltons. $C_{26}H_{50}NO_2$ ($[M+NH_4]^+$) requires 408.3841, found 408.3841 daltons.

MESO-4,7-DIMETHYL-(1,2,3,4,7,8-HEXAHYDRO-5*H*-5,6,8b-TRIAZA-ACENAPHTHYLENE)-6-IUM TETRAFLUOROBORATE [125a]



[125a]

To a cooled (0°C) solution of the *bis*-α,β-unsaturated ketone [124a] (607mg, 3.66mmol) in DMF (3ml) was added guanidine (216mg, 3.66mmol) in DMF (1ml) and the mixture allowed to warm to 20°C over 4 hours. The reaction vessel was cooled to 0°C before addition of methanol (4ml), water (1ml) and sodium borohydride (834mg, 22.0mmol) and the mixture was stirred at 20°C for 12 hours. The solvent was evaporated *in vacuo*, then dichloromethane (50ml) added. The organic fraction was washed with water (2 x 50ml) and brine (2 x 50ml) and the combined aqueous layers extracted with dichloromethane (20ml). The combined organic layers were then washed with saturated aqueous lithium bromide solution (2 x 20ml). The organic phase was dried over magnesium sulphate and evaporated *in vacuo* to give a brown oil.

Ion exchange: The crude reaction product was dissolved in dichloromethane (25ml). Saturated aqueous sodium tetrafluoroborate (25ml) was added and the mixture stirred briskly for 2 hours. The aqueous layer was extracted with dichloromethane (2 x 25ml) and the combined organics dried over magnesium sulphate and evaporated *in vacuo* giving the crude product as a yellow solid and this was purified by column chromatography eluting with 0-10% methanol:chloroform which gave the title compound [125a] as a white solid in 33% yield (340mg, M.pt.=111-113°C, R_f= 0.20 in 6% methanol:chloroform).

^{1}H NMR : δ 1.23 (d, $6H_{Me}$, J = 6.2Hz), 1.60 (m, 4H), 2.10-2.32 (m, 4H), 3.51 (m, $2H_{4,7}$), 3.65 (m, $2H_{3a,8a}$), 6.01 (s, $2H_{NH}$) ppm.

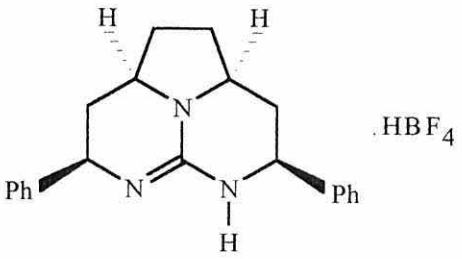
^{13}C NMR : δ 20.24 (q_{Me}), 30.11 ($t_{3,8}$), 35.63 ($t_{1,2}$), 46.05 ($d_{4,7}$), 56.00 ($d_{3a,8a}$), 149.10 (s) ppm.

FT-IR : ν_{max} 3371 (m, NH str), 2975 (w, sp^3 CH str), 2927 (w, sp^3 CH str), 1626 (s, C=N str), 1266 (s), 1070 (m, br), 738 (s), 704 (m) cm^{-1}.

MS (CI) : m/z 195 (2% $[M+2H]^+$), 194 (3% $[MH]^+$), 193 (6% $[M]^+$), 179 (6% $[MH-CH_3]^+$), 178 (35% $[M-CH_3]^+$) daltons.

HRMS (CI) : $C_{11}H_{19}N_3$ ($[M^+]$) requires 193.1579, found 193.1579 daltons.

MESO-4,7-DIPHENYL-(1,2,3,4,7,8-HEXAHYDRO-5*H*-5,6,8b-TRIAZA-ACENAPHTHYLENE)-6-IUM TETRAFLUOROBORATE [125b]



[125b]

To a cooled (0°C) solution of the *bis*-α,β-unsaturated ketone [124b] (250mg, 0.862mmol) in DMF (3ml) was added guanidine (50.9mg, 0.862mmol) in DMF (1ml) and the mixture allowed to warm to 20°C over 5 hours. A deep red colour developed after addition of guanidine. The reaction vessel was cooled to 0°C before addition of water (0.3ml), methanol (1ml) and sodium borohydride (137mg, 3.61mmol) and the mixture was stirred at 20°C for 18 hours. The solvent was evaporated *in vacuo*, then dichloromethane (50ml) added. The organic fraction was washed with water (2 x 50ml) and brine (2 x 50ml) and the combined aqueous layers extracted with dichloromethane (20ml). The combined organic layers were then washed with saturated aqueous lithium bromide solution (2 x 20ml). The organic phase was dried over magnesium sulphate and evaporated *in vacuo* to give a brown oil.

Ion exchange: The crude reaction product was dissolved in dichloromethane (25ml). Saturated aqueous sodium tetrafluoroborate (25ml) was added and the mixture stirred briskly for 2 hours. The aqueous layer was extracted with dichloromethane (2 x 25ml) and the combined organics dried over magnesium sulphate and evaporated *in vacuo* giving the crude product as a brown oil. This oil was purified by column chromatography eluting with 0-5% methanol:chloroform which, followed by trituration gave the title compound [125b] as a pale yellow solid in 32% yield (112mg, R_f= 0.23 in 5% methanol:chloroform). This solid was recrystallised from ethyl acetate and diethyl ether (1:3) to give [125b] coordinated to triphenylphosphine oxide as colourless needles (M.pt. = 90-92°C).

^{1}H NMR : δ 1.62-1.80 (m, 4H), 2.25-2.46 (m, 4H), 3.97 (m, 2H), 4.59 (dd, 2H, J=12.3, 3.9Hz), 7.29 (s, 2H), 7.30-7.60 (m, ~10H) ppm.

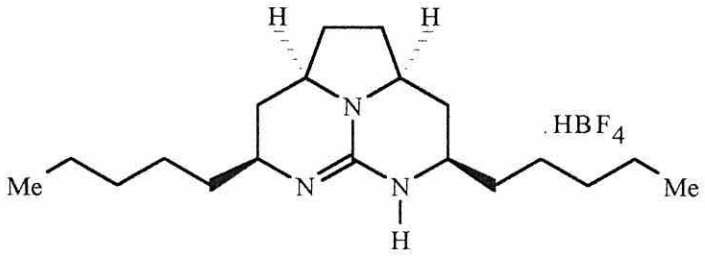
^{13}C NMR : δ 29.73 (t), 36.90 (t), 54.42 (d), 55.99 (d), 126.20 (d), 128.59 (d), 129.04 (d), 138.66 (s), 149.90 (s) ppm.

FT-IR : ν_{max} 3371 (m, NH str), 3060 (w, sp^2 CH str), 2977 (w, sp^3 CH str), 2929 (w, sp^3 CH str), 1616 (s, C=N str), 1065 (m, br), 787 (s) cm^{-1}.

MS (CI) : m/z 319 (10% $[M+2H]^+$), 318 (15% $[MH]^+$), 317 (5% $[M]^+$), 241 (35% $[MH-Ph]^+$) daltons.

HRMS (CI) : $C_{21}H_{24}N_3$ ($[MH^+]$) requires 318.1970, found 318.1970 daltons.

MESO-4,7-DIPENTYL-(1,2,3,4,7,8-HEXAHYDRO-5*H*-5,6,8b-TRIAZA-ACENAPHTHYLENE)-6-IUM TETRAFLUOROBORATE [125c]



[125c]

To a cooled (0°C) solution of the *bis*-α,β-unsaturated ketone [124c] (430mg, 1.55mmol) in DMF (2ml) was added guanidine (91.3mg, 1.55mmol) in DMF (1ml) and the mixture allowed to warm to 20°C over 5 hours. The reaction vessel was cooled to 0°C before addition of methanol (6ml), water (2ml) and sodium borohydride (353mg, 9.28mmol) and the mixture was stirred at 20°C for 16 hours. The solvent was evaporated *in vacuo*, then dichloromethane (50ml) added. The organic fraction was washed with water (2 x 50ml) and brine (2 x 50ml) and the combined aqueous layers extracted with dichloromethane (20ml). The combined organic layers were then washed with saturated aqueous lithium bromide solution (2 x 20ml). The organic phase was dried over magnesium sulphate and evaporated *in vacuo* to give a brown oil.

Ion exchange: The crude reaction product was dissolved in dichloromethane (25ml). Saturated aqueous sodium tetrafluoroborate (25ml) was added and the mixture stirred briskly for 2 hours. The aqueous layer was extracted with dichloromethane (2 x 25ml) and the combined organics dried over magnesium sulphate and evaporated *in vacuo* giving the crude product as a brown oil. This oil was purified by column chromatography eluting with 0-5% methanol:chloroform which, followed by trituration gave the title compound [125c] as a yellow solid in 27% yield (164mg, M.pt. = 84-88°C, R_f = 0.20 in 4% methanol:chloroform).

^{1}H NMR : δ 0.86 (t, $6H_{Me}$, J = 6.5Hz), 1.28-1.40 (m, 16H), 1.63 (m, 4H), 2.24 (m, 4H), 3.37 (m, $2H_{4,7}$), 3.68 (m, $2H_{3a,8a}$), 6.58 (br s, $2H_{NH}$) ppm.

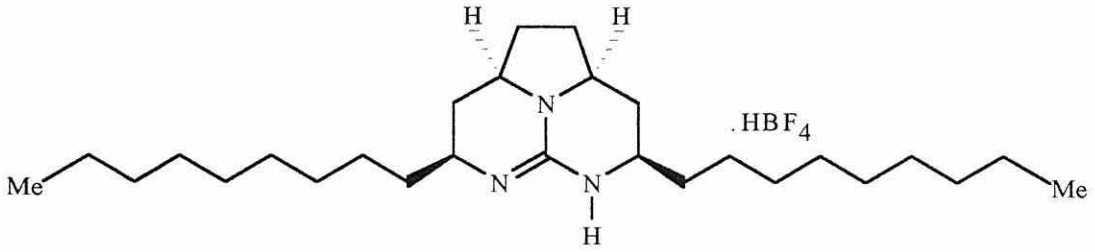
^{13}C NMR : δ 13.91 (q_{Me}), 22.37 (t), 24.59 (t), 30.22 (t), 31.44 (t), 33.56 (t), 34.34 (t), 50.49 ($d_{4,7}$), 56.04 ($d_{3a,8a}$), 149.32 (s) ppm.

FT-IR : ν_{max} 3370 (m, NH str), 2982 (m, sp^3 CH str), 2928 (s, sp^3 CH str), 1635 (s, C=N str), 1510 (m), 1456 (w), 1060 (s, br) cm^{-1}.

MS (CI) : m/z 307 (25% $[MH]^+$), 306 (100% $[M]^+$) daltons.

HRMS (CI) : $C_{19}H_{36}N_3$ ($[M]^+$) requires 306.2909, found 306.2909 daltons.

MESO-4,7-DINONYL-(1,2,3,4,7,8-HEXAHYDRO-5*H*-5,6,8b-TRIAZA-ACENAPHTHYLENE)-6-IUM TETRAFLUOROBORATE [125d]



[125d]

To a cooled (0°C) solution of the *bis*-α,β-unsaturated ketone [124d] (200mg, 0.513mmol) in DMF (1ml) was added guanidine (30.3mg, 0.513mmol) in DMF (0.1ml) and the mixture allowed to warm to 20°C over 5 hours. The reaction vessel was cooled to 0°C before addition of methanol (3ml), water (1ml) and sodium borohydride (117mg, 3.08mmol) and the mixture was stirred at 20°C for 16 hours. The solvent was evaporated *in vacuo*, then dichloromethane (50ml) added. The organic fraction was washed with water (2 x 50ml), brine (2 x 50ml) and the combined aqueous layers extracted with dichloromethane (20ml). The combined organic layers were then washed with saturated aqueous lithium bromide solution (2 x 20ml). The organic phase was dried over magnesium sulphate and evaporated *in vacuo* to give a pale yellow solid.

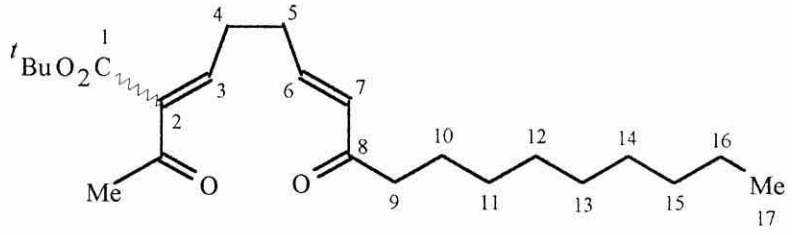
Ion exchange: The crude reaction product was dissolved in dichloromethane (25ml). Saturated aqueous sodium tetrafluoroborate (25ml) was added and the mixture stirred briskly for 2 hours. The aqueous layer was extracted with dichloromethane (2 x 25ml) and the combined organics dried over magnesium sulphate and evaporated *in vacuo* giving the crude product as a yellow solid. This solid was purified by column chromatography eluting with 0-3% methanol:chloroform which, followed by trituration gave the title compound [125d] as a yellow solid in 22% yield (57.2mg, M.pt. = 68-70°C, R_f = 0.25 in 3% methanol:chloroform).

^{1}H NMR : δ 0.87 (t, $6H_{Me}$, J = 6.4Hz), 1.18-1.33 (br s, ~32H), 2.13 (m, 4H), 2.20 (m, 4H), 3.63 (m, $2H_{4,7}$), 4.03 (m, $2H_{3a,8a}$) 6.15 (s, $2H_{NH}$) ppm.

^{13}C NMR : δ 14.03 (q_{Me}), 22.61 (t), 25.46 (t), 29.10 (t), 29.28 (t), 29.29 (t), 29.55 (t), 31.84 (t), 37.23 (t), 50.44 ($d_{4,7}$), 55.98 ($d_{3a,8a}$), 149.34 (s) ppm.

FT-IR : ν_{max} 3367 (m, NH str), 2988 (m, sp^3 CH str), 2924 (s, sp^3 CH str), 1632 (s, C=N str), 1518 (m), 1454 (w) cm^{-1}.

tert-BUTYL (2*E/Z*, 6*E*)-2-ETHANOYL-8-OXOHEPTADECA-2,6-DIENOATE [115]



[115]

The aldehyde [117] (200mg, 0.840mmol) and *tert*-butyl acetoacetate (146mg, 0.924mmol) were dissolved in dichloromethane (2.3ml and 2.0ml respectively) in separate flasks and cooled to -20°C. A small amount of anhydrous sodium sulphate (*ca.* 50mg) was added to each flask. Piperidine (72mg, 0.840mmol) was added as a solution in dichloromethane (0.7ml) to the *tert*-butyl acetoacetate and this mixture stirred for 15 minutes before being transferred to the aldehyde solution.

The reaction mixture was kept at -20°C and stirred for 4 hours before addition of hexane (10ml at -20°C) and water (10ml) containing 2 drops of acetic acid (at freezing point). The hexane layer was separated and the remaining aqueous phase extracted with dichloromethane (2 x 20ml), the combined organics dried over magnesium sulphate and evaporated *in vacuo* to give a brown oil (224mg). This oil was purified by column chromatography eluting with 8-12% ethyl acetate:hexane giving the product [115] as a 1:1 mixture of geometric isomers in 32% yield (102mg, R_f = 0.26/0.28 in 10% ethyl acetate:hexane), together with recovered *tert*-butyl acetoacetate (72mg, R_f = 0.45 in 10% ethyl acetate:hexane). Further chromatography of the baseline material eluting with 20-25% ethyl acetate:hexane gave the Baylis-Hillman product [126] in 12.5% yield (25mg, R_f = 0.25 in 20% ethyl acetate:hexane). The remaining material (24mg) consisted of unidentifiable products.

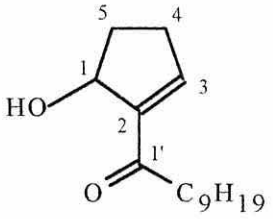
^{1}H NMR (300MHz) : δ 0.88 (t, $3H_{17}$, J = 8.7Hz), 1.2-1.3 (bs, $12H_{11\text{-}16}$), 1.50 (m, $2H_{10}$), 1.54 (s, $9H_{tBu}$), 2.3 (s, $3H_{2'}$), 2.42 (t, $2H_9$, J = 5.8 Hz), 2.52 (m, $4H_{4,5}$), 6.14 (d, H_7, J = 17.5Hz), 6.71 (t, H_3, J = 17.5Hz), 6.78 (dt, H_6, J = 17.5, 9.7Hz) ppm.

^{13}C NMR (75MHz) : δ 14.00, 22.56, 24.08, 26.97, 27.95, 28.03, 29.17, 29.33, 30.86, 31.76, 40.33, 82.53, 130.93, 144.13, 144.41, 165.43, 194.96, 200.41 ppm.

FT-IR : ν_{max} 3070 (m, sp^2 CH str), 3038 (w, sp^2 CH str), 2926 (s, sp^3 CH str), 1698 (s, (C=C-)C=O str), 1670 (s, (C=C-)C=O str), 1452 (m), 1360 (m), 1248 (s) cm^{-1}.

MS (TS) : m/z 394 (70% $[M+NH_4]^+$), 378 (100% $[M]^+$), 321 (20% $[M+NH_4\text{-}^tBuO]^+$) daltons.

2-(1'-OXODECYL)-CYCLOPENT-2-EN-1-OL [126]



[126]

^{1}H NMR (300MHz) : δ 0.87 (t, $3H_{10'}$, J = 7.8Hz), 1.2-1.3 (bs, $12H_{4'\text{-}9'}$), 1.62 (m, $2H_{3'}$), 1.82 (m, H_{5ax}), 2.30 (m, $2H_{4ax,5eq}$), 2.46 (m, H_{4eq}), 2.68 (t, $2H_{2'}$, J = 8.0Hz), 3.13 (s, H_{OH}), 5.12 (bt, H_1, J = 7.3Hz), 6.82 (t, H_3, J = 3.9Hz) ppm.

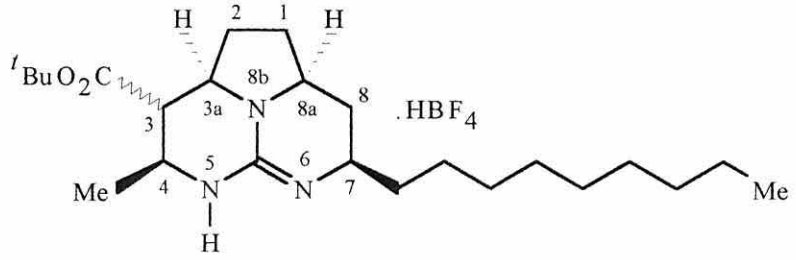
^{13}C NMR (300MHz) : δ 14.01 (q), 22.57 (t), 24.32 (t), 29.23 (t), 29.34 (t), 30.99 (t), 31.25 (t), 31.78 (t), 38.97 (t), 75.55 (d_1), 145.49 (d_3), 145.74 (s_2), 200.82 ($s_{1'}$) ppm.

FT-IR : ν_{max} 3472 (s, OH str), 2925 (s, sp^3 CH str), 2854 (m, sp^3 CH str), 1663 (s, (C=C)-C=O str), 1618 (w, C=C str) cm^{-1}.

MS (CI) : m/z 256 (100% $[M+NH_4]^+$), 239 (70% $[MH]^+$), 238 (75% $[M+NH_4\text{-}H_2O]^+$), 221 (75% $[M\text{-}H_2O]^+$) daltons.

HRMS (CI) : $C_{15}H_{30}NO_2$ ($[M+NH_4]^+$) requires 256.2277, found 256.2277 daltons.

tert-BUTYL-4-METHYL-7-NONYL-(1,2,3,4,7,8-HEXAHYDRO-5*H*-5,6, 8b-TRIAZAACENAPHTHYLENE-3-CARBOXYLATE)-6-IUM TETRAFLUOROBORATE [114]



[114]

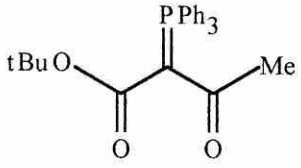
To a cooled (-30°C) solution of [115] (100mg, 0.265mmol) in DMF (10ml) was added guanidine (15.6mg, 0.265mmol) as a solution in DMF (1ml) and the mixture was allowed to warm to 0°C, with stirring, over 4 hours.

Water (0.3ml) and methanol (1ml) were added, followed by $NaBH_4$ (60.4mg, 1.59mmol) at 0°C; the mixture was allowed to warm to 20°C and stirred for 16 hours. The solvent was evaporated *in vacuo*, then diluted with dichloromethane (50ml). The organic fraction was acidified with aqueous hydrochloric acid (20ml, 1N), washed with water (2 x 50ml) and brine (2 x 50ml) and the combined aqueous layers extracted with dichloromethane (20ml). The combined organic layers were then washed with saturated lithium bromide (2 x 20ml). The organic phase was dried over magnesium sulphate and evaporated *in vacuo* to give a brown oil.

Ion exchange: The crude reaction product was dissolved in dichloromethane (10ml). Saturated aqueous sodium tetrafluoroborate (25ml) was added and the mixture stirred briskly for 2 hours. The aqueous layer was extracted with dichloromethane (2 x 10ml) and the combined organics dried over magnesium sulphate and evaporated *in vacuo* giving the crude product as a brown oil whose purification was attempted by column chromatography eluting with 0-5% methanol:chloroform giving a brown oil in 29% yield (38mg, R_f= 0.15 in 4% methanol:chloroform).

MS (TS) : m/z 424 (100% $[MH+NH_4]^+$), 406 (10% $[M]^+$) daltons.

2-ACETYL-(*tert*-BUTOXYCARBONYLMETHYLENE)-TRIPHENYLPHOSPHORANE [134]



[134]

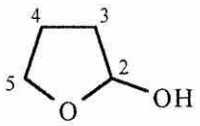
To a stirred solution of *tert*-butoxycarbonylmethyl triphenylphosphonium chloride (5.0g, 12.1mmol) in dichloromethane (50ml) was added aqueous sodium hydroxide (0.58g, 0.5M, 14.5mmol). The mixture was allowed to stir for 30 minutes before washing the organic layer with water (2 x 25ml), drying over magnesium sulphate and evaporation *in vacuo* to give phosphorane [133] as an oil in 98% yield (4.5g). This oil (4.5g, 11.9mmol) was dissolved in dichloromethane (30ml) and treated at 0°C with acetyl chloride (1.03g, 13.1mmol, 0.93ml). The mixture was stirred for 15 hours at 20°C, washed with water (4 x 50ml), the organic fractions dried over magnesium sulphate and evaporated *in vacuo* to give an orange solid which was recrystallised from dichloromethane giving the title compound [134] as yellow crystals in 88% yield (4.4g, M.pt. = 176-178°C).

^{1}H NMR : δ 1.06 (s, $9H_{tBu}$), 2.46 (s, $3H_{Me}$), 7.47 (m, $9H_{Ph}$), 7.70 (m, $6H_{Ph}$) ppm.

^{13}C NMR : δ 28.14 (q_{tBu}), 29.16 (q_{Me}), 71.21 (s_2), 78.43 (s_{tBu}), 127.04 (s_{Ph}), 128.48 (d_{Ph}), 131.45 (d_{Ph}), 132.98 (d_{Ph}), 167.66 (s), 195.08 (s) ppm.

FT-IR : ν_{max} 3050 (m, sp^2 CH str), 2929 (m, sp^3 CH str), 1960 (br w), 1886 (br w), 1813 (br w), 1764 (m, C=O str), 1661 (s, C=O str), 1543 (s, C=C str), 1484 (s, C=C str), 1439 (s, sp^2 C=C str) cm^{-1}.

2-HYDROXYTETRAHYDROFURAN [136][113]



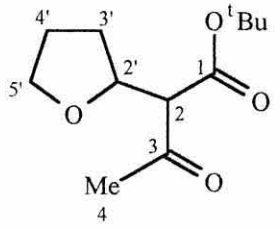
[136]

To a cooled (-20°C) solution of γ-butyrolactone (10.0g, 8.95ml, 0.116mol) in toluene (100ml) was added diisobutylaluminium hydride (127ml, 1.0M solution in toluene, 0.127mol) over 15 minutes. The mixture was stirred at -20°C for 90 minutes. Methanol (50ml) was added and the mixture warmed to 20°C, followed by addition of water (10ml). The mixture was concentrated *in vacuo* and the resulting white solid triturated with diethyl ether (5 x 50ml). The diethyl ether solution was concentrated *in vacuo* giving the title compound [136] as a colourless liquid in 75% yield (7.66g).

^{1}H NMR : δ 1.80-1.95 (m, 4H), 3.47 (br s, $1H_{OH}$), 3.86[a] (m, $1H_{5ax}$), 4.05[a] (m, $1H_{5eq}$), 5.53 (br s, $1H_2$) ppm. ([a] Interchangeable assignments)

FT-IR : ν_{max} 3398 (s, OH str), 2954 (s, sp^3 CH str), 2884 (s, sp^3 CH str), 1774 (w, C=O str), 1722 (m, C=O str), 1460 (m), 1443 (m), 1058 (s), 1033 (s), 990 (s) cm^{-1}.

tert-BUTYL 2-(2'-TETRAHYDROFURANYL) ACETOACETATE [140a]



[140a]

To a cooled (-20°C) mixture of hemiacetal [136] (1.0g, 11.36mmol) and *tert*-butyl acetoacetate (3.59g, 22.72mmol) in dichloromethane (5ml) was added piperidine (97mg, 1.136mmol, 0.11ml) and the mixture stirred for 3 hours. The reaction was quenched with hexane (10ml) and aqueous acetic acid (10ml, 1%) and extracted with hexane (2 x 25ml). The combined organic phases were dried over magnesium sulphate and evaporated *in vacuo* giving a yellow liquid. This was purified by column chromatography eluting with 15-20% diethyl ether:petroleum ether to give [140a] as a colourless liquid in 60% yield (1.55g, R_f = 0.23 in 20% ethyl acetate:petroleum ether).

^{1}H NMR : δ 1.50 (s, $9H_{tBu}$), 1.89 (m, 2H), 2.18 (m, 2H), 2.30 (s, $3H_4$), 3.51 (d, $1H_2$, J = 9.3Hz), 3.82 (m, $2H_{5'}$), 4.43 (dt, $1H_{2'}$, J = 9.3, 6.8Hz) ppm.

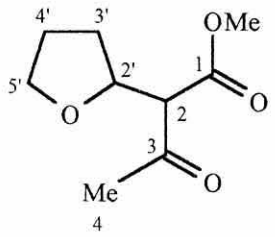
^{13}C NMR : δ 25.21 (t_3), 27.95 (q_{tBu}), 29.79 (q_{Me}), 30.35 (t_4), 52.34 (d_2), 68.17 (t_5), 77.23 ($d_{2'}$), 81.82 (s_{tBu}), 167.62 (s_1), 201.95 (s_3) ppm.

FT-IR : ν_{max} 2964 (s, sp^3 CH str), 2885 (s, sp^3 CH str), 1713 (s, C=O str), 1702 (m, C=O str), 1460 (m), 1447 (m), 1042 (s) cm^{-1}.

MS (CI) : m/z 246 (30% $[M+NH_4]^+$), 229 (5% $[MH]^+$), 190 (100%), 146 (52%) daltons.

HRMS (CI) : $C_{12}H_{24}NO_4$ ($[M+NH_4]^+$) requires 246.1705, found 246.1705 daltons.

METHYL-2-(2'-TETRAHYDROFURANYL) ACETOACETATE [140b]



[140b]

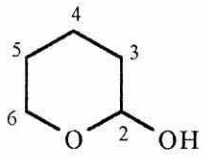
To a cooled (0°C) mixture of hemiacetal [136] (0.50g, 5.68mmol) and methyl acetoacetate (1.318g, 11.36mmol) in dichloromethane (2ml) was added piperidine (48mg, 0.568mmol, 56µl) and the mixture stirred for 3 hours. The reaction was quenched with hexane (10ml) and aqueous acetic acid (10ml, 1%) and extracted with hexane (2 x 25ml). The combined organic phases were dried over magnesium sulphate and evaporated *in vacuo* giving a yellow liquid. This was purified by column chromatography eluting with 15-25% diethyl ether:petroleum ether to give the title compound [140b] as a colourless liquid in 60% yield (1.55g, R_f = 0.20 in 20% ethyl acetate:petroleum ether)

^{1}H NMR : δ 1.91 (m, 2H), 2.19 (m, 2H), 2.31 (s, $3H_4$), 3.53 (d, $1H_2$, J = 9.4Hz), 3.72 (s, $3H_{OMe}$), 3.83 (m, $2H_{5'}$), 4.44 (dt, $1H_{2'}$, J = 9.4, 6.8Hz) ppm.

^{13}C NMR : δ 25.21 (t_3), 29.79 (q_{Me}), 30.35 (t_4), 52.34 (d_2), 64.71 (q_{OMe}), 68.17 (t_5), 77.23 ($d_{2'}$), 167.99 (s_1), 201.95 (s_3) ppm.

FT-IR : ν_{max} 2965 (s, sp^3 CH str), 2880 (s, sp^3 CH str), 1715 (s, C=O str), 1706 (m, C=O str), 1458 (m), 1443 (m), 1045 (s) cm^{-1}.

2-HYDROXYTETRAHYDROPYRAN [148][102]



[148]

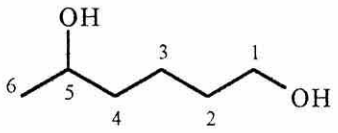
To a cooled (0°C) sample of 2,3-dihydropyran (9.22g, 10.0ml, 109.6mmol) was added aqueous hydrochloric acid (0.2N, 30ml). The mixture was stirred at 0°C for 15 minutes before warming to 20°C and stirring for a further 1 hour. The mixture was extracted with dichloromethane (4 x 50ml) and these extracts were combined and washed with saturated aqueous sodium bicarbonate solution (50ml). The organic phase was dried over magnesium sulphate and concentrated *in vacuo* giving the title compound [148] as a colourless liquid in 74% yield (8.30g).

^{1}H NMR : δ 1.45-1.58 (m, $4H_{4,5}$), 1.66-1.88 (m, $2H_3$), 3.18 (br s, $1H_{OH}$), 3.54[a] (m, $1H_{6\beta}$), 4.00[a] (m, $1H_{6\alpha}$), 4.88 (br s, $1H_2$) ppm. ([a] Interchangeable assignments)

^{13}C NMR : δ 20.34 (t_4), 25.32 (t_5), 31.97 (t_3), 63.85 (t_6), 94.43 (d_2) ppm.

FT-IR : ν_{max} 3404 (br s, OH str), 2938 (s, sp^3 CH str), 1724 (w, C=O str), 1442 (s), 1354 (s), 1275 (m), 1196 (s), 1170 (s), 1075 (s), 1014 (br s) cm^{-1}.

1,5-HEXANEDIOL [149]



[149]

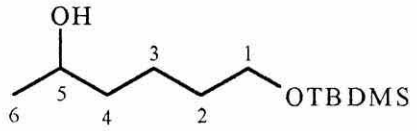
To a cooled (0°C) solution of methylmagnesium chloride in THF (32.7ml, 3.0M, 98.0mmol) was added a solution of 2-hydroxytetrahydropyran [148] (5.0g, 49.0mmol) in THF (60ml). The mixture was then allowed to warm to 20°C over 30 minutes and then refluxed for 6 hours. The reaction was quenched with saturated aqueous ammonium chloride solution (10ml), saturated with sodium chloride and extracted exhaustively with diethyl ether (7 x 50ml) to give an oil. This oil was purified by chromatography through a short plug of silica eluting with diethyl ether giving the title compound [149] as a colourless oil in 62% yield (3.58g, R_f= 0.10 in diethyl ether).

^{1}H NMR : δ 1.16 (d, $3H_6$, J = 6.2Hz), 1.43 (m, $4H_{2,3}$), 1.52 (m, $2H_4$), 2.91 (s, $2H_{OH}$), 3.60 (t, $2H_1$, J = 6.1Hz), 3.76 (m, $1H_5$) ppm.

^{13}C NMR : δ 21.84 (q_6), 23.42 (t_3), 32.34[a] (t_2), 38.67[a] (t_4), 62.26 (t_1), 67.70 (d_5) ppm. ([a]Interchangeable assignments)

FT-IR : ν_{max} 3334 (br s, OH str), 2935 (s, sp^3 CH str), 2864 (s, sp^3 CH str), 1460 (m), 1433 (m), 1133 (m), 1100 (m), 1073 (m) cm^{-1}.

1-((*tert*-BUTYLDIMETHYLSILYL)OXY)-5-HEXANOL [150]



[150]

To a stirred (0°C) solution of 1,5-hexanediol [149] (2.00g, 16.94mmol) in DMF (40ml) was added imidazole (2.30g, 33.88mmol) and *tert*-butyldimethylsilyl chloride (2.56g, 16.94mmol) and the mixture stirred for 1 hour at 20°C. The mixture was diluted with diethyl ether (100ml) and washed with water (3 x 50ml). The aqueous phase was extracted with diethyl ether (50ml) and the combined organics dried over magnesium sulphate and evaporated *in vacuo* to yield a mobile colourless oil. This oil was purified by flash chromatography eluting with 10-20% ethyl acetate:petroleum ether to give the title compound [150] as a colourless liquid in 92% yield (3.60g, R_f = 0.17 in 10% ethyl acetate:petroleum ether).

^{1}H NMR : δ 0.01 (s, $6H_{Me}$), 0.82 (s, $9H_{tBu}$), 1.13 (d, $3H_6$, J = 6.1Hz), 1.35-1.52 (m, $6H_{2-4}$), 3.61 (t, $2H_1$, J = 6.2Hz), 3.76 (m, $1H_5$) ppm. (No O-H resonance was observed)

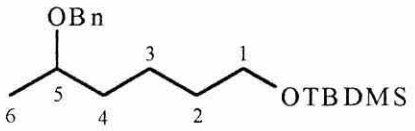
^{13}C NMR : δ -4.89 (q_{Me}), 18.29 (s_{tBu}), 21.96 (t_3), 23.34 (q_6), 25.91 (q_{tBu}), 32.66[a] (t_2), 38.95[a] (t_4), 63.06 (t_1), 67.82 (d_5) ppm. ([a] Interchangeable assignments)

FT-IR : ν_{max} 3412 (m, OH str), 2928 (s, sp^3 CH str), 2899 (s, sp^3 CH str), 1254 (m), 1096 (s) cm^{-1}.

MS (CI) : m/z 250 (8% $[M+NH_4]^+$), 234 (15% $[MH+H]^+$), 233 (100% $[MH]^+$), 231 (15% $[M-2H]^+$) daltons.

HRMS (CI) : $C_{12}H_{29}O_2Si$ ($[MH]^+$) requires 233.1937, found 233.1937.

1-((*tert*-BUTYLDIMETHYLSILYL)OXY)-5-BENZYLOXYHEXANE [151]



[151]

Sodium hydride (1.30g, 60% dispersion in mineral oil, 32.4mmol) was rigorously washed with ether and suspended in THF (100ml) at 0°C. The alcohol [150] (5.76g, 24.9mmol) was added dropwise as a solution in THF (25ml) and the effervescent mixture allowed to stir for 10 minutes at 0°C, whereupon benzyl bromide (5.97g, 34.9mmol, 4.15ml) and tetra-*n*-butylammonium iodide (0.92g, 2.49mmol) were added. The mixture was allowed to warm to 20°C, with stirring, over 18 hours. Diethyl ether (50ml) was added and the reaction mixture washed successively with water (30ml) and brine (30ml). The aqueous phase was extracted with diethyl ether (2 x 50ml) and the combined organics dried over magnesium sulphate and evaporated *in vacuo* to give a colourless oil. This oil was purified by column chromatography eluting with 0-5% diethyl ether:petroleum ether to give the title compound [151] in 71% yield (5.69g, R_f = 0.36 in 5% diethyl ether: petroleum ether) (90% yield based on recovered [150]).

^{1}H NMR : δ 0.06 (s, $6H_{Me}$), 0.91 (s, $9H_{tBu}$), 1.20 (d, $3H_6$, J = 6.1Hz), 1.44-1.50 (m, $6H_{2-4}$), 3.52 (m, $1H_5$), 3.62 (t, $2H_1$, J = 6.2Hz), 4.46 (d, $1H_{Bn}$, J = 11.8Hz), 4.57 (d, $1H_{Bn}$, J = 11.8Hz), 7.28-7.35 (m, $5H_{Ph}$) ppm.

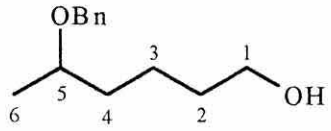
^{13}C NMR : δ -5.28 (q_{Me}), 18.37 (s_{tBu}), 19.62 (q_6), 21.89 (t), 25.99 (q_{tBu}), 32.92 (t), 36.50 (t), 63.17 (t_1), 70.32 (t_{Bn}), 74.89 (d_5), 127.35 (d_{Ph}), 127.61 (d_{Ph}), 128.29 (d_{Ph}), 139.13 (s_{Ph}) ppm.

FT-IR : ν_{max} 3063 (w, sp^2 CH str), 3029 (w, sp^2 CH str), 2929 (s, sp^3 CH str), 2856 (s, sp^3 CH str), 1495 (w), 1470 (m), 1453 (m), 1254 (m), 1097 (s) cm^{-1}.

MS (CI) : m/z 340 (35% $[M+NH_4]^+$), 324 (25% $[MH+H]^+$), 323 (100% $[MH]^+$), 108 (82%) daltons.

HRMS (CI) : $C_{19}H_{35}O_2Si$ ($[MH]^+$) requires 323.2406, found 323.2406 daltons.

5-BENZYLOXY-1-HEXANOL [152]



[152]

To a stirred (0°C) solution of silyl ether [151] (4.3g, 13.4mmol) in THF (80ml) was added tetra-*n*-butylammonium fluoride (20.1ml, 1.0M solution in THF, 20.1mmol) and the mixture allowed to warm to 20°C, with stirring, over 4 hours. The mixture was diluted with diethyl ether (200ml) and washed with water (100ml). The aqueous phase was extracted with diethyl ether (3 x 50ml) and the combined organics dried over magnesium sulphate and evaporated *in vacuo* giving a colourless oil. This oil was purified by column chromatography eluting with 40-60% diethyl ether:petroleum ether to give the title compound [152] as a colourless oil in 80% yield (2.23g, R_f = 0.12 in 40% diethyl ether:petroleum ether).

^{1}H NMR : δ 1.16 (d, $3H_6$, J = 6.2Hz), 1.39-1.54 (m, $6H_{2-4}$), 3.47 (m, $1H_5$), 3.58 (t, $2H_1$, J = 6.1Hz), 4.41 (d, $1H_{Bn}$, J = 11.7Hz), 4.54 (d, $1H_{Bn}$, J = 11.7Hz), 7.29 (m, $5H_{Ph}$) ppm. (No O-H resonance was observed)

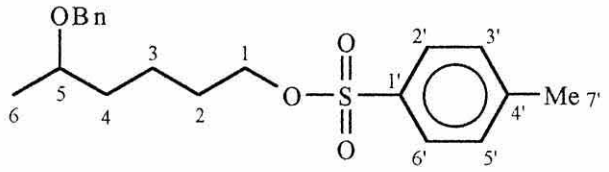
^{13}C NMR : δ 19.56 (q_6), 21.68 (t_3), 32.71[a] (t_2), 36.37[a] (t_4), 62.77 (t_1), 70.33 (t_{Bn}), 74.77 (d_5), 127.40 (d_{Ph}), 127.65 (d_{Ph}), 128.30 (d_{Ph}), 138.98 (s_{Ph}) ppm. ([a]Interchangeable assignments)

FT-IR : ν_{max} 3402 (br s, OH str), 3063 (w, sp^2 CH str), 3030 (w, sp^2 CH str), 2935 (s, sp^3 CH str), 2862 (s, sp^3 CH str), 1496 (w), 1454 (m), 1374 (m), 1066 (s, C-O str) cm^{-1}.

MS (CI) : m/z 226 (100% $[M+NH_4]^+$), 209 (75% $[MH]^+$), 108 (48%), 106 (84%), 99 (38%) daltons.

HRMS (CI) : $C_{13}H_{21}O_2$ ($[MH]^+$) requires 209.1542, found 209.1542 daltons.

5-(BENZYLOXY)HEXAN-1-*para*-TOLUENESULPHONATE [153]



[153]

To a cooled (0°C) solution of alcohol [152] (2.90g, 13.9mmol) in pyridine (15ml) was added *p*-toluenesulphonyl chloride (3.19g, 16.7mmol) and the mixture allowed to warm to 20°C and stirred for 18 hours. The reaction mixture was diluted with diethyl ether (100ml) and washed with aqueous sulphuric acid (2 x 50ml, 1%) and saturated aqueous copper sulphate solution (2 x 50ml). The organic phase was dried over magnesium sulphate and evaporated *in vacuo* to give a mobile colourless oil. This oil was purified by flash chromatography eluting with 10% diethyl ether:petroleum ether to give the title compound [153] as a colourless liquid in 79% yield (3.99g, R_f = 0.11 in 10% diethyl ether:petroleum ether).

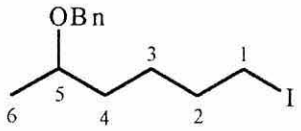
^{1}H NMR : δ 1.11 (d, $3H_6$, J = 6.1Hz), 1.37 (m, $4H_{2,3}$), 1.59 (m, $2H_4$), 2.40 (s, $3H_{7'}$), 3.40 (m, $1H_5$), 3.97 (t, $2H_1$, J = 6.4Hz), 4.36 (d, $1H_{Bn}$, J = 11.7Hz), 4.51 (d, $1H_{Bn}$, J = 11.7Hz), 7.28 (m, $7H_{Bn,3',5'}$), 7.75 (d, $2H_{2',6'}$, J = 6.6Hz) ppm.

^{13}C NMR : δ 19.49 (q_6), 21.42 (t_3), 21.61 ($q_{7'}$), 28.85 (t_4), 35.98 (t_2), 70.33 (t_1), 70.52 (t_{Bn}), 74.39 (d_5), 127.44 (d_{Ph}), 127.62 (d_{Ph}), 127.87 ($d_{3',5'}$), 128.32 (d_{Ph}), 129.79 ($d_{2',6'}$), 133.19 ($s_{4'}$), 138.90 (s_{Ph}), 144.63 ($s_{1'}$) ppm.

MS (CI) : m/z 380 (65% $[M+NH_4]^+$), 290 (35%), 210 (40%), 208 (100%), 108 (40%), 106 (30%) daltons.

HRMS (CI) : $C_{20}H_{27}O_4S$ ($[MH]^+$) requires 363.1630, found 363.1630 daltons.

5-BENZYLOXY-1-IODOHEXANE [154]



[154]

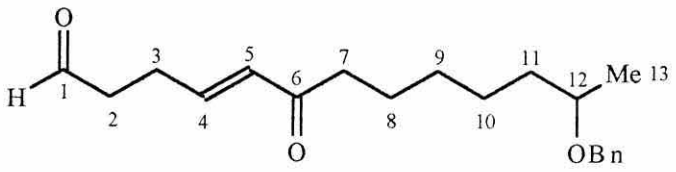
To a stirred solution of tosylate [153] (2.50g, 6.9mmol) in acetone (200ml) was added sodium iodide (3.11g, 20.7mmol) and the mixture refluxed for 4 hours. The solvent was evaporated *in vacuo* leaving an off-white solid. This solid was washed with hexane (6 x 50ml) and the resulting suspension filtered. The filtrate was concentrated *in vacuo* giving the title compound [154] as a pale yellow oil in 97% yield (2.14g) which required no further purification.

^{1}H NMR : δ 1.21 (d, $3H_6$, J = 6.1Hz), 1.49 (m, $4H_{2,4}$), 1.82 (m, $2H_3$), 3.19 (t, $2H_1$, J = 7.0Hz), 3.57 (m, $1H_5$), 4.45 (d, $1H_{Bn}$, J = 11.7Hz), 4.59 (d, $1H_{Bn}$, J = 11.7Hz), 7.29-7.36 (m, $5H_{Bn}$) ppm.

^{13}C NMR : δ 6.99 (t_1), 19.58 (q_6), 26.53 (t_2), 33.52 (t_3), 35.56 (t_4), 70.36 (t_{Bn}), 74.47 (d_5), 127.44 (d_{Ph}), 127.66 (d_{Ph}), 128.33 (d_{Ph}), 138.91 (s_{Ph}) ppm.

FT-IR : ν_{max} 3061 (m, sp^2 CH str), 2929 (s, sp^3 CH str), 2862 (s, sp^3 CH str), 1450 (m), 1361 (m) cm^{-1}.

(4*E*)-12-(BENZYLOXY)-6-OXOTRISDEC-4-ENAL [145]



[145]

To a cooled (-78°C) suspension of acetylmethylene triphenylphosphorane (1.78g, 5.61mmol) in THF (50ml) was added *n*-butyllithium (2.78ml, 2.22M solution in hexanes, 6.17mmol) dropwise. The resulting deep red solution was stirred at -78°C for 30 minutes whereupon iodide [154] (2.14g, 6.73mmol) was added dropwise as a solution in THF (10ml). The mixture was allowed to warm to 20°C with stirring over 4 hours, before evaporation of the THF *in vacuo* which gave a pale yellow oil. This oil was dissolved in ethyl acetate and washed with water (2 x 50ml) and the combined aqueous phases extracted with ethyl acetate (50ml). The combined organic phases were dried over magnesium sulphate and evaporated *in vacuo* giving phosphorane [146] as a yellow oil (*ca.* 2.9g).

To a solution of this oil in dichloromethane (10ml) was added succinaldehyde (5g, 58mmol) as a solution in dichloromethane (10ml). This mixture was stirred for 22 hours at 20°C before washing with water (2 x 50ml) to remove excess succinaldehyde which, followed by drying over magnesium sulphate and evaporation of the solvent *in vacuo* gave an oil. This oil was purified by column chromatography with 20-40% diethyl ether:petroleum ether to give the title compound [145] as a colourless oil in 43% yield (0.76g, R_f = 0.10 in 40% diethyl ether:petroleum ether).

^{1}H NMR : δ 1.17 (d, $3H_{13}$, J = 6.1Hz), 1.31[a] (m, $2H_9$), 1.40[a] (m, $2H_{10}$), 1.59 (m, $4H_{8,11}$), 2.49 (m, $4H_{2,7}$), 2.62 (m, $2H_3$), 3.47 (m, $1H_{12}$), 4.43 (d, $1H_{Bn}$, J = 11.8Hz), 4.55 (d, $1H_{Bn}$, J = 11.8Hz), 6.09 (dt, $1H_5$, J = 16.0, 1.3Hz), 6.78 (dt, $1H_4$, J = 16.0, 6.5Hz), 7.30 (m, $5H_{Bn}$), 9.77 (s, $1H_1$) ppm. ([a]Interchangeable assignments)

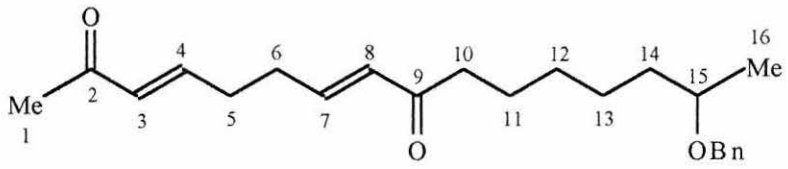
^{13}C NMR : δ 19.58 (q_{13}), 24.06 (t), 25.29 (t), 27.05 (t), 30.63 (t), 30.69 (t), 36.45 (t), 40.30 (t), 70.25 (t_{Bn}), 74.68 (d_{12}), 127.22 (d_{Bn}), 127.35 (d_{Bn}), 128.13 (d_{Bn}), 131.87 (d_5), 138.88 (s_{Bn}), 145.45 (d_4), 200.25 (d_1), 200.34 (s_5) ppm.

FT-IR : ν_{max} 3430 (w, C=O overtone), 3086 (w, sp^2 CH str), 3029 (w, sp^2 CH str), 2933 (s, sp^3 CH str), 2859 (m, sp^3 CH str), 2723 (w, CH(=O) str), 1724 (m, C=O str), 1696 m, C=O str), 1673 (s), 1629 (m), 1453 (m), 1066 (s) cm^{-1}.

MS (CI) : m/z 334 (100% $[M+NH_4]^+$), 317 (25% $[MH]^+$), 244 (40%) daltons.

HRMS (CI) : $C_{20}H_{32}O_3N$ ($[M+NH_4]^+$) requires 334.2382, found 334.2381 daltons.

(3*E*, 7*E*)-15-(BENZYLOXY)-2,9-DIOXOHEXADECA-3,7-DIENE [144]



[144]

To a solution of aldehyde [145] (360mg, 1.139mmol) in dichloromethane (5ml) was added acetylmethylene triphenylphosphorane (1.086g, 3.417mmol) and the mixture stirred at 20°C for 36 hours. The solvent was evaporated *in vacuo* giving an oil. This oil was purified by column chromatography eluting with 30-40% diethyl ether:petroleum ether giving the title compound [144] as an oil in 60% yield (242mg, R_f = 0.09 in 40% diethyl ether:petroleum ether).

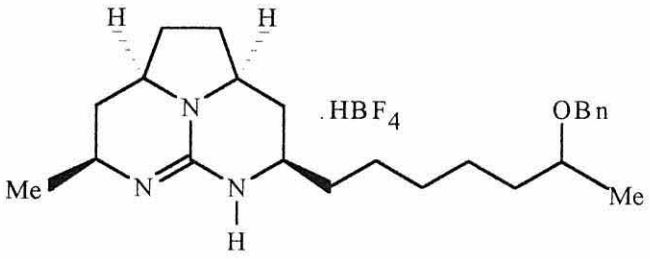
^{1}H NMR : δ 1.19 (d, $3H_{16}$, J = 6.2Hz), 1.31[a] (m, $2H_{12}$), 1.41[a] (m, $2H_{13}$), 1.60 (m, $4H_{11,14}$), 2.24 (s, $3H_1$), 2.39 (m, $4H_{5,6}$), 2.50 (t, $2H_{10}$, J = 7.3Hz), 3.49 (m, $1H_{15}$), 4.43 (d, $1H_{Bn}$, J = 11.8Hz), 4.57 (d, $1H_{Bn}$, J = 11.8Hz), 6.10 (m, $2H_{3,8}$), 6.78 (m, $2H_{4,7}$), 7.30 (m, $5H_{Bn}$) ppm.

^{13}C NMR : δ 19.58 (q_{15}), 24.06 (t), 25.29 (t), 27.05 (t), 29.28 (q_1), 30.63 (t), 30.69 (t), 36.45 (t), 40.30 (t), 70.25 (t_{Bn}), 74.73 (d_{15}), 127.32 (d_{Bn}), 127.58 (d_{Bn}), 128.26 (d_{Bn}), 130.93[b] (d_3), 131.87[b] (d_8), 139.07 (s_{Bn}), 144.45[c] (d_4), 145.80[c] (d_7),198.23 (s_2), 200.34 (s_9) ppm. ([a,b,c]Interchangeable assignments)

MS (CI) : m/z 374 (100% $[M+NH_4]^+$), 357 (15% $[MH]^+$), 292 (55%), 275 (25%) daltons.

HRMS (CI) : $C_{23}H_{33}O_3$ ($[MH]^+$) requires 357.2430, found 357.2429 daltons.

(±)-4*S*,3a*S*,8a*R*,7*R*-4-(6'-BENZYLOXY)HEPTYL-7-METHYL-(1,2,3,4,7,8-HEXAHYDRO-5*H*-5,6,8b-TRIAZAACENAPHTHYLENE)-6-IUM TETRAFLUOROBORATE [142]



[142]

To a cooled (0°C) solution of [144] (240mg, 0.674mmol) in DMF (1ml) was added guanidine (40mg, 0.674mmol) in DMF (0.5ml), which caused a deep green colour to develop and the mixture allowed to warm to 20°C over 4 hours. The mixture was cooled to 0°C before sequential addition of methanol (1.5ml), water (0.5ml) and sodium borohydride (153mg, 4.04mmol). The reaction mixture was then stirred at 20°C for 18 hours. The mixture was concentrated *in vacuo* and then diluted with dichloromethane (25ml). The organic fraction was acidified with aqueous hydrochloric acid (10ml, 1N), washed with water (2 x 25ml) and brine (2 x 25ml) and the combined aqueous layers extracted with dichloromethane (20ml). The combined organic layers were then washed with saturated lithium bromide (2 x 20ml). The organic phase was dried over magnesium sulphate and evaporated *in vacuo* to give a brown oil.

Ion exchange: The crude reaction product was dissolved in dichloromethane (25ml). Saturated aqueous sodium tetrafluoroborate (25ml) was added and the mixture stirred briskly for 2 hours. The aqueous layer was extracted with dichloromethane (2 x 25ml) and the combined organics dried over magnesium sulphate and evaporated *in vacuo* giving the crude product as a brown oil. This oil was purified by column chromatography eluting with 0-5% methanol:chloroform giving the title compound [142] as a yellow gum in 37% yield (118mg, R_f= 0.23 in 5% MeOH:$CHCl_3$).

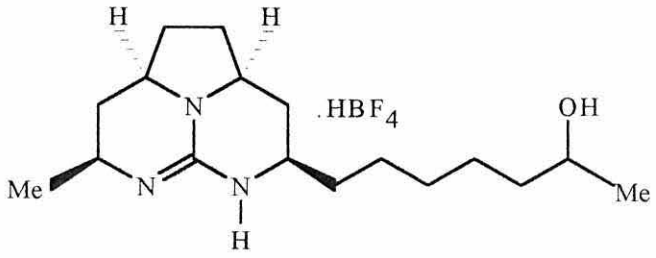
^{1}H NMR : δ 1.14 (d, $3H_{7'}$, J = 5.8Hz), 1.18-1.34 (m, ~13H), 1.40-1.60 (m, 4H), 2.17 (m, 4H), 3.30 (m, $1H_{6'}$), 3.45 (m, $2H_{4,7}$), 3.62 (m, ~$2H_{3a,8a}$), 4.40 (d, $1H_{Bn}$, J = 11.8Hz), 4.52 (d, $1H_{Bn}$, J = 11.8Hz), 6.52 (s, $1H_{NH}$), 6.63 (s, $1H_{NH}$), 7.28 (m, $5H_{Ph}$) ppm.

^{13}C NMR : δ 19.62 ($q_{7'}$), 20.22 (q), 24.90 (t), 25.27 (t), 29.32 (t), 30.17 (t), 30.23 (t), 33.59 (t), 34.38 (t), 35.70 (t), 36.48 (t), 46.13 (d_4), 50.43 (d_7), 55.98 (d_{3a}), 56.06 (d_{8a}), 70.29 (t), 74.87 (d_{Bn}), 127.33 (d_{Ph}), 127.65 (d_{Ph}), 128.28 (d_{Ph}), 139.11 (s_{Ph}), 149.19 (s) ppm.

MS (FAB) : m/z 402 (8% $[MH+NH_4]^+$), 384 (37% $[MH]^+$) daltons.

HRMS (FAB) : $C_{24}H_{38}N_3O$ ($[MH]^+$) requires 384.3015, found 384.3028 daltons.

(±)-4*S*,3a*S*,8a*R*,7*R*-4-(6'-HYDROXY)HEPTYL-7-METHYL-(1,2,3,4,7,8-HEXAHYDRO-5*H*-5,6,8b-TRIAZAACENAPHTHYLENE)-6-IUM TETRAFLUOROBORATE [143]



[143]

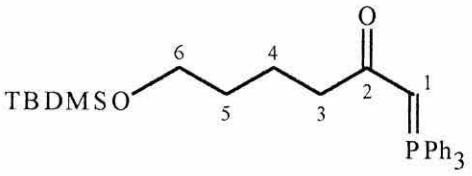
To a stirred solution of [142] (59.6mg, mmol) in ethyl acetate (2ml) was added palladium hydroxide on charcoal (60mg). The mixture was placed under a positive pressure of hydrogen and stirred briskly for 4 hours. The solution was filtered through a pad of Celite® and the residue washed with ethyl acetate (5 x 2ml). The solvent was evaporated *in vacuo* giving a white solid (60.1mg). This solid was purified by column chromatography eluting with 0-10% methanol:chloroform giving the title compound [143] as a colourless oil in 67% yield (32.1mg, R_f = 0.11 in 10% methanol:chloroform).

^{1}H NMR : δ 1.15 (d, $3H_{7'}$, J = 5.8Hz), 1.18-1.34 (m, ~13H), 1.40-1.60 (m, 4H), 2.17 (m, 4H), 3.34 (m, $1H_{6'}$), 3.47 (m, $2H_{4,7}$), 3.65 (m, ~$2H_{3a,8a}$), 6.52 (s, $1H_{NH}$), 6.62 (s, $1H_{NH}$) ppm. (No O-H resonance was observed)

MS (CI) : m/z 295 (18% $[MH+H]^+$), 294 (75% $[MH]^+$) daltons.

HRMS (CI) : $C_{17}H_{32}N_3O$ ($[MH]^+$) requires 294.2545, found 294.2545 daltons.

6-((*tert*-BUTYLDIMETHYLSILYL)OXY)-HEXANOYLMETHYLENE TRIPHENYLPHOSPHORANE [160]

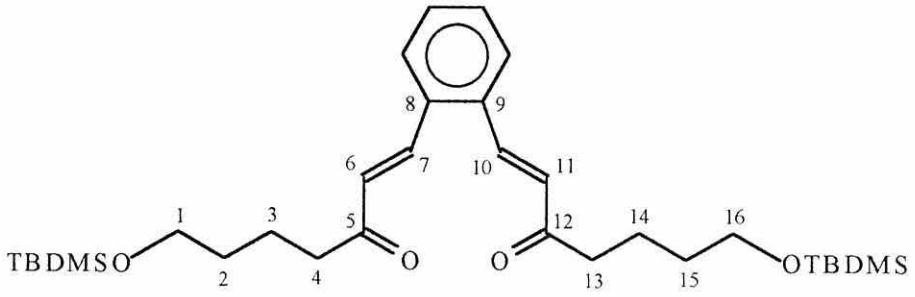


[160]

Methyltriphenylphosphonium bromide (29.3g, 82mmol) was suspended in THF (200ml) at 0°C. A solution of *n*-butyllithium in hexane (63.0ml, 1.30M solution in hexanes, 82mmol) was added portionwise and the mixture stirred for 4 hours at 20°C producing a deep red solution. The reaction vessel was cooled to -78°C and δ-valerolactone (4.10g, 41mmol, 3.80ml) added, producing a yellow precipitate. The mixture was allowed to warm to 20°C and stirred for 1 hour. The solvent was evaporated *in vacuo* giving a yellow solid which was dissolved in DMF (200ml) and cooled to 0°C before sequential treatment with imidazole (3.35g, 49.2mmol) and *tert*-butyldimethylsilyl chloride (6.79g, 45.1mmol). After 30 minutes at 0°C the mixture was allowed to warm to 20°C and stirred for 15 hours.

The mixture was treated with diethyl ether (100ml) and water (100ml) and the organic layer washed with water (3 x 50ml) and back-extracted with ethyl acetate (2 x 50ml). The combined organic layers were dried over magnesium sulphate and evaporated *in vacuo* affording the title compound [160] as a red oil in 90% yield (18.2g).

(6*E*,10*E*)-8,9-BENZO-1,16-*bis*-((*tert*-BUTYLDIMETHYLSILYL)OXY)-5,12-DIOXOHEXADECA-6,10-DIENE [162]



[162]

The phosphorane [160] (19.0g, 38.8mmol) was dissolved in dichloromethane (25ml) and *o*-phthalic dicarboxaldehyde [161] (1.30g, 9.70mmol) added. The mixture was allowed to stir at 20°C for 20 hours. The crude reaction mixture was evaporated *in vacuo* and purified by column chromatography using 20% diethyl ether:petroleum ether. The title compound [162] was isolated as a red oil in 62% yield (3.38g, R_f = 0.15 in 20% diethyl ether:petroleum ether).

^{1}H NMR : δ 0.07 (s, $12H_{Me}$), 0.91 (s, $18H_{tBu}$), 1.57 (m, $4H_{4,13}$), 1.77 (m, 4H), 2.72 (t, 4H, J = 7.2Hz), 3.67 (t, $4H_{1,16}$, J = 6.2Hz), 6.66 (d, $2H_{7,10}$, J = 15.9Hz), 7.41 (dd, $4H_{Ph}$, J = 5.5, 3.4Hz), 7.91 (d, $2H_{7,10}$, J = 15.9Hz) ppm.

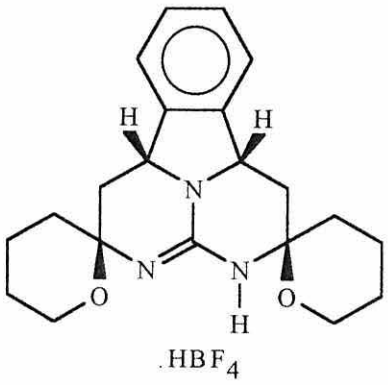
^{13}C NMR : δ -5.33 (q_{Me}), 18.25 (s_{tBu}), 20.54 (t), 25.89 (q_{tBu}), 32.20 (t), 41.00 (t), 62.80 ($t_{1,16}$), 127.65 (d), 129.33 (d), 130.10 (d), 134.61 (s_{Ph}), 138.84 ($d_{7,10}$), 199.94 ($s_{5,12}$) ppm.

FT-IR : ν_{max} 2968 (s, sp^3 CH str), 1660 (s, (C=C)-C=O str), 1609 (s, C=C str), 1048 (s) cm^{-1}.

MS (CI) : m/z 559 (100% $[MH]^+$) daltons.

HRMS (CI) : $C_{32}H_{55}O_4Si_2$ ($[MH]^+$) requires 559.3641, found 559.3640 daltons.

MESO-1',2'-BENZODISPIRO[TETRAHYDROPYRAN-2,4'-(1,2,3,4,7,8-HEXAHYDRO-5*H*-5,6,8b-TRIAZAACENAPHTHYLENE-7',2"-TETRA-HYDROPYRAN]-6-IUM TETRAFLUOROBORATE [157]



[157]

The bis-α,β-unsaturated ketone [162] (1.00g, 1.69mmol) was dissolved in DMF (5ml) at 0°C. Guanidine was added dropwise as a solution in DMF (5ml); a dark green colour developed. The mixture was allowed to warm to 20°C over 5 hours. The DMF was evaporated *in vacuo* at ambient temperature and methanol (10ml) added. Hydrogen chloride gas was bubbled steadily through the solution for 30 seconds and the solution was stirred at 20°C for 18 hours The methanol was evaporated *in vacuo* and the crude product dissolved in dichloromethane (50ml). The solution was washed with water (2 x 50ml), brine (2 x 50ml) and saturated aqueous lithium bromide solution (3 x 50ml). The organic fractions were dried over magnesium sulphate and evaporated *in vacuo* giving a brown oil (603mg).

Ion exchange: The crude reaction product was dissolved in dichloromethane (25ml). Saturated aqueous sodium tetrafluoroborate (25ml) was added and the mixture stirred briskly for 2 hours. The aqueous layer was extracted with dichloromethane (2 x 25ml) and the combined organics dried over magnesium sulphate and evaporated *in vacuo* giving the crude product as a brown solid. This solid was purified by column chromatography eluting with 0-8% methanol:chloroform, giving the title compound [157] as an orange solid in 35% yield (260mg, R_f = 0.18 in 5% methanol:chloroform). This

solid was recrystallised from chloroform and carbon tetrachloride (1:10) to give colourless needles (M.pt. = 84-85°C)

^{1}H NMR : δ 1.65 (m, 4H), 1.85 (m, 4H), 2.10 (m, 2H), 2.66 (dd, 2H, J = 13.1, 4.4Hz), 3.78 (m, 4H), 3.91 (ddd, $4H_{6,6''}$, J = 22.7, 11.5, 3.4Hz), 5.28 (dd, $2H_{3a',8a'}$, J = 12.2, 4.2Hz), 7.24 (m, $2H_{Ph}$), 7.40 (m, $2H_{Ph}$), 7.74 (s, $2H_{NH}$) ppm.

^{13}C NMR : δ 17.47 (t), 24.72 (t), 34.19 (t), 38.24 (t), 56.78 (d), 62.01 ($t_{3',8'}$), 79.74 ($s_{6,6''}$), 121.73 (d_{Ph}), 128.97 (d_{Ph}), 138.47 (s_{Ph}), 147.76 (s) ppm.

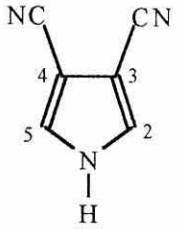
FT-IR : ν_{max} 3376 (w, NH str), 3019 (m, sp^2 CH str), 2951 (m, sp^3 CH str), 1651 (m, C=N str), 1600 (s), 1215 (s), 765 (s) cm^{-1}

Microanalysis : Found C = 48.99, H = 5.56, N = 7.87%; $C_{21}H_{26}N_3O_2BF_4.0.8CHCl_3$ requires C = 48.96, H = 5.06, N = 7.85%.

MS (EI) : 352 (40% $[M]^+$) daltons.

HRMS (EI) : $C_{21}H_{26}N_3O_2$ ($[M]^+$) requires 352.2025, found 352.2025 daltons.

PYRROLE-3,4-DINITRILE [163][104]



[163]

p-Toluenesulphonylmethyl isocyanide [165] (1g, 5.12mmol) was dissolved in DMF (20ml) at 0°C. Sodium hydride (0.42g, 60% dispersion in oil, 10.24mmol) was washed with diethyl ether and added slowly to the mixture which was stirred for 5 minutes. Fumaronitrile (0.48g, 6.14mmol) was added and the mixture stirred at 0°C for a further 15 minutes. The reaction was quenched with saturated aqueous ammonium chloride solution (50ml), washed with water (2 x 50ml), brine (50ml) and saturated aqueous lithium bromide solution (2 x 50ml). The aqueous washings were back-extracted with ethyl acetate (2 x 50ml) and the combined organic fractions dried over magnesium sulphate and evaporated *in vacuo* to give a brown oil. This oil was purified by column chromatography using 75-90% diethyl ether:petroleum ether, giving the title compound [163] as a white solid in 71% yield (0.427g, M.pt. = 210-212°C, R_f= 0.59 in diethyl ether).

^{1}H NMR : δ 7.15 (s, $2H_{2,5}$) ppm. (No N-H resonance was observed)

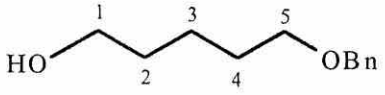
^{13}C NMR : δ 95.07 ($d_{2,5}$), 113.07 (s), 127.75 (s) ppm.

FT-IR : ν_{max} 3332 (m, NH str), 3000 (s, sp^2 CH str), 2231 (m, C≡N str), 1463 (s) cm^{-1}.

MS (EI) : m/z 117 (100% $[M]^+$), 89 (25% $[M-N_2]^+$), 51 (30%) daltons.

HRMS (EI) : $C_6H_3N_3$ ($[M^+]$) requires 117.0328, found 117.0328 daltons.

5-BENZYLOXY-1-PENTANOL [167]



[167]

Sodium hydride (3.84g, 60% dispersion in oil, 96.2mmol) was rigorously washed with diethyl ether and suspended in THF (150ml) at 0°C. Pentane-1,5-diol (10g, 96.2mmol) was added slowly to the stirred mixture and after 15 minutes at 0°C, benzyl bromide (8.23g, 48.1mmol, 5.72ml) was added dropwise and the mixture allowed to stir for 20 hours at 20°C. The reaction was quenched with isopropanol (50ml), the white solid removed by filtration and the solvent evaporated *in vacuo*. The resulting oil was dissolved in diethyl ether (100ml), washed with water (2 x 50ml) and the aqueous fractions back-extracted with diethyl ether (2 x 50ml). The combined organic fractions were dried over magnesium sulphate and evaporated *in vacuo* to give a yellow oil. This oil was purified by column chromatography using 50-75% diethyl ether:petroleum ether giving the title compound [167] as a yellow liquid in 71% yield (6.64g, R_f= 0.59 in 60% diethyl ether:petroleum ether).

^{1}H NMR : δ 1.45 (m, $2H_3$), 1.64[a] (m, $2H_2$), 1.69[a] (m, $2H_4$), 2.64 (bs, $1H_{OH}$), 3.50 (t, $2H_5$, J = 6.5Hz); 3.59 (t, $2H_1$, J = 6.4Hz); 4.52 (s, $2H_{Bn}$); 7.35 (m, $5H_{Ph}$) ppm.

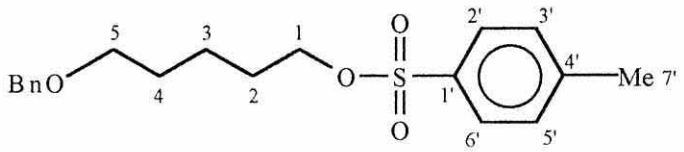
^{13}C NMR : δ 22.39 (t_3), 29.13[b] (t_2), 32.07[b] (t_4), 62.08 (t_1), 70.31 (t_{Bn}), 72.80 (t_5), 127.46 (d_{Ph}), 127.49 (d_{Ph}), 128.29 (d_{Ph}), 138.42 (s_{Ph}) ppm. ([a,b]Interchangeable assignments)

FT-IR : ν_{max} 3391 (br s, OH str), 2925 (s, sp^3 CH str), 2864 (s, sp^3 CH str), 1453 (s), 1362 (s), 1099 (s), 736 (s), 697 (s) cm^{-1}.

MS (CI) : m/z 212 (90% $[M+NH_4]^+$), 195 (100% $[MH]^+$), 108 (10% $[MH-OBn]^+$) daltons.

HRMS (CI) : $C_{12}H_{19}O_2$ ($[MH]^+$) requires 195.1385, found 195.1385 daltons.

5-(BENZYLOXY)PENTAN-1-*para*-TOLUENESULPHONATE [168]



[168]

To a solution of alcohol [167] (5.45g, 28.1mmol) in pyridine (20ml) was added *p*-toluenesulphonyl chloride (5.89g, 30.9mmol) and the mixture stirred at 20°C for 18 hours. The reaction was quenched with hexane (50ml) and washed with dilute aqueous sulphuric acid (2 x 50ml, 1M). The aqueous fractions were back-extracted with diethyl ether (2 x 50ml) and the combined organic fractions dried over magnesium sulphate and evaporated *in vacuo* to give a brown liquid. The crude product was purified by column chromatography using 25% diethyl ether:petroleum ether giving the title compound [168] as a yellow liquid in 74% yield (7.28g, R_f = 0.24 in 25% ether:petrol).

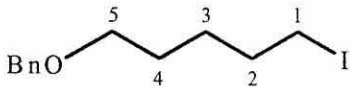
^{1}H NMR : δ 1.44 (m, $2H_3$), 1.63[a] (m, $2H_2$), 1.69[a] (m, $2H_4$), 2.46 (s, $3H_{7'}$), 3.45 (t, $2H_5$, J = 6.3Hz), 4.04 (t, $2H_1$, J = 6.4Hz), 4.45 (s, $2H_{Bn}$), 7.34 (m, $5H_{Ph}$), 7.80 (d, $4H_{2'-6'}$, J = 8.3Hz) ppm.

^{13}C NMR : δ 21.25 (t_3), 21.86 ($q_{7'}$), 28.30[b] (t_2), 28.73[b] (t_4), 69.57 (t_5), 70.22 (t_{Bn}), 72.52 (t_1), 127.18 (d), 127.25 (d), 127.51 (d), 128.03 (d), 129.54 (d), 132.80 (s), 138.27 (s), 144.38 (s) ppm. ([a,b]Interchangeable assignments)

MS (CI) : m/z 366 (100% $[M+NH_4]^+$), 349 (20% $[MH]^+$) daltons.

HRMS (CI) : $C_{19}H_{25}O_4S$ ($[MH]^+$) requires 349.1474, found 349.1474 daltons.

5-BENZYLOXY-1-IODOPENTANE [169]



[169]

To a solution of the tosylate [168] (0.63g, 1.81mmol) in acetone (200ml) was added sodium iodide (1.39g, 9.05mmol) and mixture refluxed for 4 hours. The reaction mixture was evaporated *in vacuo* giving a white solid which was triturated with hexane. The resulting solution was evaporated *in vacuo* giving a yellow oil which was purified by column chromatography using 5% diethyl ether:petroleum ether giving the title compound [169] as a yellow liquid in 93% yield (0.51g, R_f= 0.20 in 5% diethyl ether:petroleum ether).

^{1}H NMR : δ 1.55 (m, $2H_3$), 1.65[a] (m, $2H_2$), 1.88[a] (m, $2H_4$), 3.22 (t, $2H_1$, J=7.0Hz), 3.51 (t, $2H_5$, J = 6.3Hz), 4.54 (s, $2H_{Bn}$), 7.37 (m, $5H_{Ph}$) ppm.

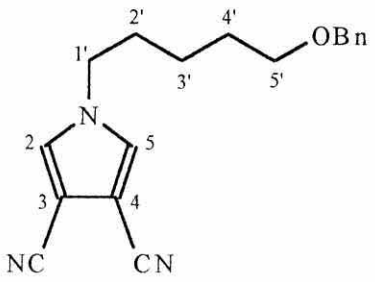
^{13}C NMR : δ 6.91 (t_1), 27.29 (t_3), 28.72[b] (t_2), 33.36[b] (t_4), 70.02 (t_{Bn}), 72.95 (t_5), 127.56 (d_{Ph}), 127.64 (d_{Ph}), 128.39 (d_{Ph}), 138.57 (s_{Ph}) ppm. ([a,b]Interchangeable assignments)

FT-IR : ν_{max} 2932 (s, sp^3 CH str), 2862 (s, sp^3 CH str), 1452 (m), 1362 (m), 1105 (s) cm^{-1}.

MS (CI) : m/z 322 (20% $[M+NH_4]^+$), 305 (5% $[MH]^+$), 198 (95% $[MH\text{-}OBn]^+$), 181 (90%), 91 (100% $[Bn]^+$) daltons.

HRMS (CI) : $C_{12}H_{18}OI$ ($[MH]^+$) requires 305.0402, found 305.0402 daltons.

N-((5'-BENZYLOXY)PENTYL)PYRROLE-3,4-DINITRILE [170]



[170]

Sodium hydride (0.26g, 60% dispersion in oil, 6.50mmol) was rigorously washed with diethyl ether and added slowly to a cooled (0°C) solution of pyrrole-3,4-dinitrile [163] (0.629g, 5.38mmol) in DMF (30ml). The mixture was stirred at 0°C for 5 minutes, whereupon the iodide [169] (1.495g, 4.92mmol) was added dropwise. The reaction was allowed to warm to 20°C and stirred for 3 hours. The reaction was then quenched with isopropanol (50ml), the resulting solid being removed by filtration and the filtrate evaporated *in vacuo* to give a brown oil. This oil was dissolved in chloroform (100ml), washed with water (50 ml), brine (50ml) and saturated aqueous lithium bromide solution (2 x 50ml). The aqueous washings were extracted with chloroform (2 x 50ml) and the combined organic fractions dried over magnesium sulphate and evaporated *in vacuo* to give a brown oil. The product was purified by column chromatography using 50-100% diethyl ether:petroleum ether to give the title compound [170] as a white solid in 76% yield (1.102g, R_f= 0.59 in diethyl ether).

^{1}H NMR : δ 1.41 (m, $2H_{3'}$), 1.62[a] (m, $2H_{2'}$), 1.80[a] (m, $2H_{4'}$), 3.46 (t, $2H_{1'}$, J = 6.4Hz), 3.94 (t, $2H_{5'}$, J = 7.0Hz), 4.46 (s, $2H_{Bn}$), 7.12 (s, $2H_{2,5}$), 7.30 (m, $5H_{Ph}$) ppm.

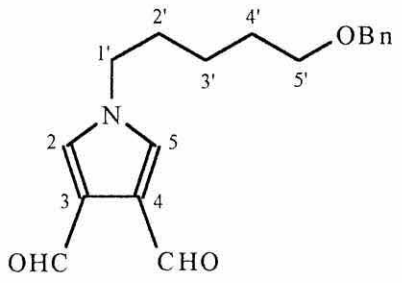
^{13}C NMR : δ 23.19 ($t_{3'}$), 28.91[b] ($t_{2'}$), 30.47[b] ($t_{4'}$), 51.15 ($t_{1'}$), 69.68 (t_{Bn}), 73.00 ($t_{5'}$), 96.42 ($d_{2,5}$), 112.94 (s), 127.70 (d_{Ph}), 128.43 (d_{Ph}), 129.11 (d_{Ph}), 138.35 (s_{Ph}) ppm. ([a,b]Inter-changeable assignments)

FT-IR : ν_{max} 3135 (s, sp^2 CH str), 3010 (s, sp^2 CH str), 2946 (s, sp^3 CH str), 2226 (s, C≡N str), 1541 (s), 1454 (s), 1361 (s), 1217 (s), 1151 (s) cm^{-1}.

MS (CI) : m/z 294 (20% $[MH]^+$), 293 (20% $[M]^+$), 91 (100%) daltons.

HRMS (CI) : $C_{18}H_{19}N_3O$ ($[M]^+$) requires 293.1528, found 293.1528 daltons.

N-((5'-BENZYLOXY)PENTYL)PYRROLE-3,4-DICARBOXALDEHYDE [171]

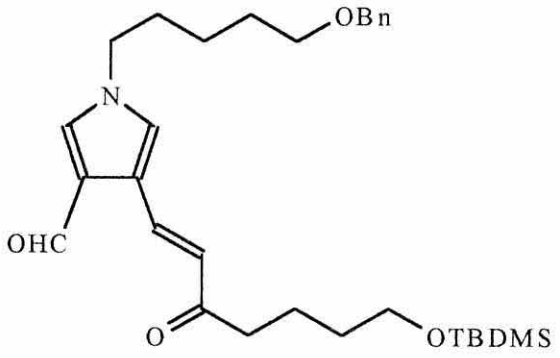


[171]

To a cooled (-45°C) solution of [170] (250mg, 0.85mmol) in toluene (10ml) was added diisobutylaluminium hydride (2.55ml, 1.0M solution in toluene, 2.55mmol) dropwise over 2 minutes. The reaction warmed to -38°C during the addition. The reaction mixture was allowed to warm to -30°C and stirred for 80 minutes. The reaction mixture was poured into a mixture of diethyl ether (20ml) and saturated aqueous ammonium chloride (20ml). Dilute aqueous sulphuric acid (1.0M) was added and the mixture stirred briskly for 22 hours. The aqueous phase was extracted with diethyl ether (2 x 50ml), dried over magnesium sulphate and evaporated *in vacuo* to give an oil. This oil was purified by column chromatography using 40% ethyl acetate:petroleum ether to give the title compound [171] as a yellow oil in 80% yield (203mg, R_f= 0.35 in 40% ethyl acetate:petroleum ether).

^{1}H NMR : δ 1.43 (m, $2H_{3'}$), 1.61[a] (m, $2H_{2'}$), 1.78[a] (m, $2H_{4'}$), 3.45 (t, $2H_{1'}$, J = 6.3Hz), 3.92 (t, $2H_{5'}$, J = 7.0Hz), 4.47 (s, $2H_{Bn}$), 7.13 (s, $2H_{2,5}$), 7.33 (m, $5H_{Ph}$), 10.12 (s, $2H_{CHO}$) ppm.
^{13}C NMR : δ 23.27 ($t_{3'}$), 28.89[b] ($t_{2'}$), 30.49[b] ($t_{4'}$), 51.16 ($t_{1'}$), 69.59 ($t_{5'}$), 73.07 (t_{Bn}), 112.74 ($s_{3,4}$), 127.70 ($d_{2,5}$), 128.44 (d_{Ph}), 128.98 (d_{Ph}), 138.25 (s_{Ph}), 186.31 (d_{CHO}) ppm. ([a,b]Interchangeable assignments)

N-((5'-BENZYLOXY)PENTYL)-3-(3"-OXO-7"-(*tert*-BUTYLDIMETHYL-SILYL)OXY)-PYRROLE-4-CARBOXALDEHYDE [172]



[172]

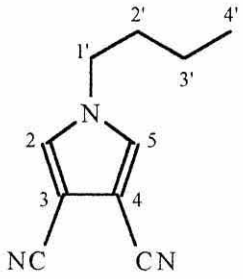
To a solution of the pyrrole dialdehyde [171] (215mg, 0.719mmol) in dichloromethane (2.5ml) was added the phosphorane [160] (1.41g, 2.88mmol) in dichloromethane (2.5ml) and the mixture allowed to stir at 20°C for 36 hours. The mixture was purified by column chromatography eluting with 30-50% diethyl ether:petroleum ether to give mono-olefinated product [172] as a brown oil in 69% yield (253mg, R_f = 0.28 in 50% diethyl ether:petroleum ether) with no trace of the desired product.

^{1}H NMR : δ 0.08 (s, $6H_{Me}$), 0.92 (s, $9H_{tBu}$), 1.43 (m, $2H_{3'}$), 1.57[a] (m, $2H_{2'}$), 1.61[a] (m, $2H_{4'}$), 1.78 (m, $4H_{5",6"}$), 2.72 (t, $2H_{4"}$, J=7.2Hz), 3.46 (t, $2H_{7"}$, J = 6.3Hz), 3.68 (t, $2H_{5'}$, J = 6.2Hz), 3.93 (t, $2H_{Bn}$, J = 7.0Hz), 4.48 (s, $2H_{Bn}$), 6.66 (d, $1H_{2"}$, J = 15.8Hz), 7.13 (s, $2H_{2,5}$), 7.33 (m, $5H_{Ph}$), 7.91 (d, $1H_{1"}$, J = 15.8Hz), 10.10 (s, $1H_{CHO}$) ppm.

([a]Interchangeable assignments)

^{13}C NMR : δ -5.31 (q_{Me}), 18.33 (s_{tBu}), 20.89 (t), 23.31 (t), 25.98 (q_{tBu}), 29.08 (t), 30.66 (t), 32.42 (t), 39.72 (t), 50.49 ($t_{1'}$), 62.94 ($t_{7"}$), 69.73 ($t_{5'}$), 72.96 (t_{Bn}), 120.46 (s_3), 124.25 (s_4), 124.67 (d), 126.35 (d), 127.63 (d_2 + d_5), 128.39 (d), 132.87 (d), 134.67 (d), 138.36 (s_{Ph}), 185.04 (d_{CHO}), 201.24 (s_{CO}) ppm.

N-BUTYLPYRROLE-3,4-DINITRILE [174]



[174]

Sodium hydride (205mg, 60% dispersion in oil, 5.12mmol) was rigorously washed with diethyl ether and added slowly to a cooled (-5°C) solution of pyrrole-3,4-dinitrile [163] (500mg, 4.27mmol) in DMF (25ml), causing the solution to effervesce. The mixture was stirred for 5 minutes and *n*-butyl iodide (0.865g, 4.70mmol) added dropwise *via* syringe. The mixture was kept at -5°C for 30 minutes then allowed to warm to 20°C and stirred for 3 hours. The reaction was quenched with isopropanol (50ml), the resulting solid removed by filtration and the filtrate evaporated *in vacuo*. The resulting oil was dissolved in chloroform (100 ml), washed with water (50ml) and saturated aqueous lithium bromide solution (2 x 50ml). The organic phase was dried over magnesium sulphate and evaporated *in vacuo* to give the crude product as a brown oil. This oil was purified by column chromatography using 50-100% diethyl ether:petroleum ether to give the title compound [174] as a light brown oil in 66% yield (484mg, R_f = 0.34 in 80% diethyl ether:petroleum ether). This reaction was not optimised.

^{1}H NMR : δ 0.96 (t, $3H_{4'}$, J = 7.3Hz), 1.31 (m, $2H_{3'}$), 1.79 (m, $2H_{2'}$), 3.96 (t, $2H_{1'}$, J = 7.2Hz), 7.18 (s, $2H_{2,5}$) ppm.

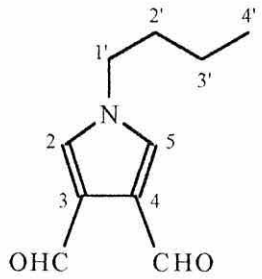
^{13}C NMR : δ 13.38 ($q_{4'}$), 19.49 ($t_{3'}$), 32.64 ($t_{2'}$), 51.03 ($t_{1'}$), 96.34 ($d_{2,5}$), 112.97 (s), 129.26 (s) ppm.

FT-IR : ν_{max} 3126 (s, sp^2 CH str), 2917 (s, sp^3 CH str), 2849 (s, sp^3 CH str), 2233 (s, C≡N str), 1651 (w, C=C str), 1544 (s), 1462 (m), 1155 (s) cm^{-1}.

MS (EI) : m/z 173 (30% $[M]^+$), 131 (100% $[M-C_3H_6]^+$), 130 (20% $[M-C_3H_7]^+$), 41 (30% $[C_3H_5]^+$) daltons.

HRMS (EI) : $C_{10}H_{11}N_3$ ($[M]^+$) requires 173.0953, found 173.0953 daltons.

N-BUTYLPYRROLE-3,4-DICARBOXALDEHYDE [175]



[175]

To a cooled (-45°C) solution of [174] (399mg, 2.31mmol) in toluene (5ml) was added diisobutylaluminium hydride (6.92ml, 1.0M solution in toluene, 6.92mmol) dropwise over 2 minutes. The reaction mixture was allowed to warm to -30°C and stirred for 80 minutes. The reaction mixture was poured into a mixture of diethyl ether (20ml) and saturated aqueous ammonium chloride (20ml). Dilute aqueous sulphuric acid (1.0M) was added and the mixture stirred briskly for 22 hours. The aqueous phase was extracted with diethyl ether (2 x 50ml), dried over magnesium sulphate and evaporated *in vacuo* to give an oil. This oil was purified by column chromatography using 40% ethyl acetate:petroleum ether to give the title compound [175] as a yellow oil in 72% yield (297mg, R_f= 0.33 in 30% ethyl acetate:petroleum ether).

^{1}H NMR : δ 0.94 (t, $3H_{4'}$, J = 7.3Hz), 1.32 (m, $2H_{3'}$), 1.80 (m, $2H_{2'}$), 3.96 (t, $2H_{1'}$, J = 7.1Hz), 7.34 (s, $2H_{2,5}$), 10.13 (s, $2H_{CHO}$) ppm.

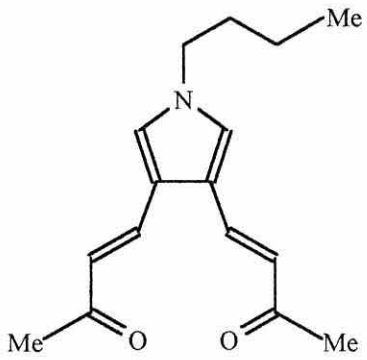
^{13}C NMR : δ 13.30 ($q_{4'}$), 19.44 ($t_{3'}$), 32.58 ($t_{2'}$), 50.35 ($t_{1'}$), 124.77 ($s_{3,4}$), 129.98 ($d_{2,5}$), 186.30 (d_{CHO}) ppm.

FT-IR : ν_{max} 3582 (br w, C=O overtone), 3116 (m, sp^2 CH str), 2917 (s, sp^3 CH str), 2845 (s, sp^3 CH str), 1681 (s, (C=C-)C=O str), 1537 (s), 1523 (s), 1461 (s) cm^{-1}.

MS (EI) : m/z 179 (70% $[M]^+$), 109 (65% $[M-C_4H_9N]^+$), 81 (65% $[M-C_4O_2H]^+$), 80 (100% $[M-C_4O_2]^+$) daltons.

HRMS (EI) : $C_{10}H_{13}NO_2$ ($[M]^+$) requires 179.0946, found 179.0946 daltons.

N-BUYTL-3,4-*bis*-(3"-OXO-BUT-1"-ENYL)-PYRROLE [177]



[177]

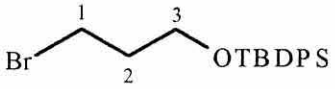
Sodium hydride (28mg, 0.704mmol, 60%) was rigorously washed with diethyl ether and suspended in DME (2ml) at 0°C. A solution of dimethylacetylmethyl phosphonate (700mg, 4.22mmol) in DME (2ml) was added dropwise, causing the solution to effervesce slightly. The mixture was allowed to warm to room temperature, with stirring, over 1 hour. A solution of [175] (126mg, 0.704mmol) in DME (1ml) was added to the reaction flask *via* syringe. A quantity of white solid developed and the mixture was stirred at 20°C for 36 hours. The solvent was removed *in vacuo* and the resulting oil dissolved in ethyl acetate (25ml) and washed with water (2 x 25ml). The aqueous washings were back-extracted with ethyl acetate (10ml) and the combined organics dried over magnesium sulphate and evaporated *in vacuo* to give a brown oil. This oil was purified by column chromatography eluting with 60-90% diethyl ether:petroleum ether giving the title compound [177] as an oil in 74% yield (137mg, R_f= 0.11 in 75% diethyl ether:petroleum ether).

^{1}H NMR : δ 0.81 (t, $3H_{4'}$, J = 7.4Hz), 1.20 (m, $2H_{3'}$), 1.62 (m, $2H_{2'}$), 2.20 (s, $6H_{4''}$), 3.74 (t, $2H_{1'}$, J = 7.1Hz), 6.31 (d, $2H_{2''}$, J = 16.1Hz), 6.89 (s, $2H_{2,5}$), 7.45 (d, $2H_{1''}$, J = 16.1Hz) ppm.

^{13}C NMR : δ 13.46 ($q_{4'}$), 19.66 ($t_{3'}$), 27.29 ($q_{4''}$), 32.93 ($t_{2'}$), 50.14 ($t_{1'}$), 119.70 ($s_{3,4}$), 123.04 ($d_{2,5}$), 124.70 ($d_{2''}$), 135.26 ($d_{1''}$), 198.13 ($s_{3''}$) ppm.

FT-IR : ν_{max} 3111 (w, sp^2 CH str), 2917 (s, sp^3 CH str), 2849 (s, sp^3 CH str), 1657 (s, (C=C-)C=O str), 1614 (s, C=C str), 1529 (s), 1462 (m) 1257 (s) cm^{-1}.

3-((*tert*-BUTYLDIPHENYLSILYL)OXY)-1-BROMOPROPANE [179][112]



[179]

To a cooled (0°C) solution of *tert*-butyldiphenylsilyl chloride (20.50g, 74.5mmol) in DMF (100ml) was added imidazole (5.07g, 74.5mmol). The resulting solution was treated with 1-bromo-3-propanol (10.36g, 74.5mmol) and stirred at 20°C for 5 hours. The solution was diluted with hexane (100ml) and washed with water (3 x 100ml). The organic phase was dried over magnesium sulphate and evaporated *in vacuo* to give the title compound [179] as a colourless liquid in 100% yield (28.01g, R_f = 0.48 in 2% diethyl ether:petroleum ether).

^{1}H NMR : δ 1.05 (s, $9H_{tBu}$), 2.06 (tt, $2H_2$, J = 6.5, 5.7Hz), 3.59 (t, $2H_1$, J = 6.5Hz), 3.78 (t, $2H_3$, J = 5.7Hz), 7.40 (m, $6H_{Ph}$), 7.66 (dd, $4H_{Ph}$, J = 7.3, 1.7Hz) ppm.

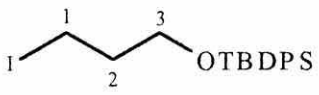
^{13}C NMR : δ 19.25 (s_{tBu}), 26.85 (q_{tBu}), 30.50 (t_1), 35.51 (t_2), 61.38 (t_3), 127.70 (d_{Ph}), 129.69 (d_{Ph}), 133.61 (s_{Ph}), 135.56 (d_{Ph}) ppm.

FT-IR : ν_{max} ($CDCl_3$) 3070 (w, sp^2 CH str), 3049 (w, sp^2 CH str), 2957 (s, sp^3 CH str), 2930 (s, sp^3 CH str), 1959 (br w), 1889 (br w), 1824 (br w), 1427 (s), 1389 (m), 1262 (m), 1112 (br s) cm^{-1}.

MS (CI) : m/z 394 (45% $[M+NH_4]^+$), 377 (90% $[MH]^+$), 350 (20%), 333 (35%), 314 (40%), 299 (70%, $[M-Br]^+$), 256 (30%), 238 (22%), 196 (20%), 74 (50%), 52 (100%) daltons.

HRMS (CI) : $C_{19}H_{26}OBrSi$ ($[MH]^+$) requires 377.0936, found 377.0936 daltons.

3-((*tert*-BUTYLDIPHENYLSILYL)OXY)-1-IODOPROPANE [180][112]



[180]

To a solution of bromide [179] (21.64g, 57.6mmol) in acetone (500ml) was added sodium iodide (25.92g, 172.8mmol) and the mixture refluxed for 3 hours. On cooling, the resulting suspension was concentrated and the residue washed with hexane (3 x 100ml). The organic filtrate was evaporated *in vacuo* to give the title compound [180] as a colourless oil in 89% yield (21.70g, $R_f = 0.38$ in 2% diethyl ether:petroleum ether).

^{1}H NMR : δ 1.08 (s, $9H_{tBu}$), 2.06 (m, $2H_2$), 3.38 (t, $2H_1$, J = 6.7Hz), 3.74 (t, $2H_3$, J = 5.4Hz), 7.47 (m, $6H_{Ph}$), 7.69 (br d, $4H_{Ph}$, J = 6.0Hz) ppm.

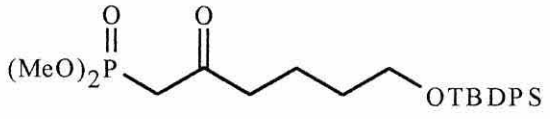
^{13}C NMR : δ 3.30 (t_1), 19.24 (s_{tBu}), 26.84 (q_{tBu}), 36.16 (t_2), 63.23 (t_3), 127.69 (d_{Ph}), 129.67 (d_{Ph}), 133.60 (s_{Ph}), 135.56 (d_{Ph}) ppm.

FT-IR ($CDCl_3$) : ν_{max} 3069 (m sp^2 CH str), 3048 (w, sp^2 CH str), 2957 (s, sp^3 CH str), 2929 (s, sp^3 CH str), 2856 (s, sp^3 CH str), 1959 (br w), 1889 (br w), 1824 (br w), 1598 (w), 1471 (m), 1427 (s), 1110 (br s) cm^{-1}.

MS (CI) : m/z 442 (50% $[M+NH_4]^+$), 425 (15% $[MH]^+$), 384 (10%), 350 (10%), 316 (10%), 275 (20%), 274 (100%), 216 (50%), 196 (90%) daltons.

HRMS (CI) : $C_{19}H_{26}OSiI$ ($[MH]^+$) requires 425.0798, found 425.0798 daltons.

DIMETHYL-(2-OXO-6-((*tert*-BUTYLDIPHENYLSILYL)OXY)-HEXYL PHOSPHONATE [181]



[181]

Sodium hydride (0.700g, 17.41mmol) was rigorously washed with diethyl ether and suspended in THF (30ml) at 0°C. Dimethylacetylmethyl phosphonate (2.628g, 15.83mmol) was added as a solution in THF (20ml) and the mixture stirred for 30 minutes, forming a white precipitate. A solution of *n*BuLi (7.9ml, 2.20M in hexanes, 17.38mmol) was added dropwise at 0°C and the mixture stirred for a further 30 minutes, before addition of iodide [180] (8.93g, 17.57mmol) as a solution in THF (10ml). The mixture was stirred for 15 hours and quenched with aqueous hydrochloric acid (1%, 40ml) and extracted with ethyl acetate (3 x 50ml) to give a pale yellow oil. This oil was purified by column chromatography eluting with diethyl ether giving the title compound [181] as a colourless oil in 51% yield (3.72g, R_f = 0.20 in diethyl ether) together with recovered [180] (4.10g, 8.08mmol).

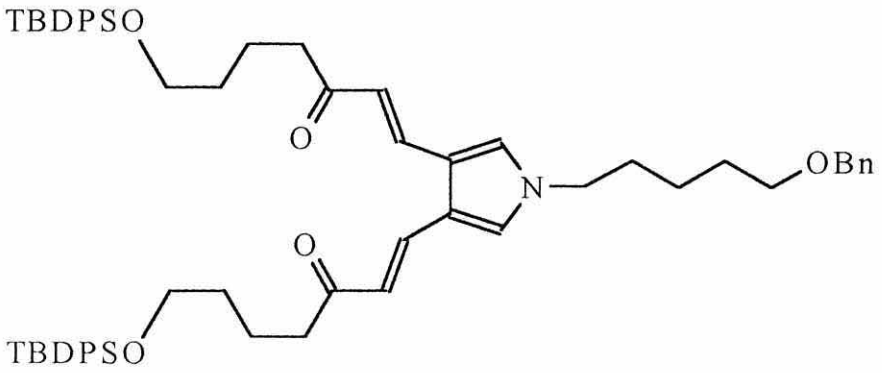
^{1}H NMR : δ 1.08 (s, $9H_{tBu}$), 1.60[a] (m, $2H_4$), 1.73[a] (m, $2H_5$), 2.64 (t, $2H_3$, J = 5.8Hz), 3.10 (d, $2H_1$, J = 22.1 Hz), 3.69 (t, $2H_6$, J = 5.5Hz), 3.81 (d, 6H, J = 11.3), 7.43 (m, 6H), 7.70 (m, 4H) ppm.

^{13}C NMR : δ 19.18 (s_{tBu}), 19.83[b] (t_4), 26.83 (q_{tBu}), 31.64[b] (t_5), 41.19 (t_3), 43.80 (t_1), 52.99 (q_{OMe}), 63.40 (t_6), 127.60 (d_{Ph}), 129.54 (d_{Ph}), 133.89 (s_{Ph}), 135.52 (d_{Ph}), 201.75 (s_2) ppm. ([a,b]Interchangeable assignments)

FT-IR : ν_{max} 2916 (s, sp^3 CH str), 2849 (sp^3 CH str), 1669 (s, C=O str), 1619 (s, P=O str), 1578 (m), 1472 (w), 1462 (m), 1448 (m), 694 (s) cm^{-1}.

MS (EI) : m/z 480 (50% $[M+NH_4]^+$), 463 (20% $[MH]^+$) daltons.

N-(5'-BENZYLOXYPENTYL)-*bis*-3,4-((1"*E*)-3"-OXO-7"-((*tert*-BUTYL-DIPHENYLSILYL)OXY)-HEPT-1"-ENYL)-PYRROLE [182]



[182]

Sodium hydride (110mg, 2.75mmol) was rigorously washed with ether and suspended in DME (5ml). The phosphonate [181] (1.15g, 2.50mmol) was added dropwise as a solution in DME (3ml). The mixture was stirred for 30 minutes forming a white precipitate before addition of the pyrrole-3,4-dicarboxaldehyde [171] (186mg, 0.625mmol) as a solution in 1,2-dimethoxyethane (2ml) before stirring for 36 hours at 20°C. The reaction mixture was diluted with ethyl acetate (25ml) and washed with water (2 x 25ml). The aqueous phase was extracted with ethyl acetate (25ml) and the combined organic fractions dried over magnesium sulphate and evaporated *in vacuo* giving a brown oil. This oil was purified by column chromatography eluting with 60-80% diethyl ether:petroleum ether giving [182] as an oil in 73% yield (118mg, R_f = 0.15 in 70% diethyl ether:petroleum ether).

^{1}H NMR : δ 1.07 (s, $18H_{tBu}$), 1.42 (m, 2H), 1.64 (m, 6H), 1.80 (m, 6H), 2.62 (t, $4H_{4"}$, J = 7.2Hz), 3.48 (t, $2H_{5"}$, J = 6.3Hz), 3.71 (t, $4H_{7"}$, J = 6.2Hz), 3.87 (t, 2H, J = 7.0Hz), 4.51 (s, 2H), 6.47 (d, 2H, J = 16.0Hz), 7.00 (s, $2H_{2,5}$), 7.3-7.4 (m, 17H), 7.64 (d, $2H_{1"}$, J = 16.0Hz), 7.68 (m, $8H_{Ph}$) ppm.

FT-IR : ν_{max} 3110 (w, sp^2 CH str), 3093 (w, sp^2 CH str), 2914 (s, sp^3 CH str), 2848 (s, sp^3 CH str), 1720 (w, C=O str), 1649 (w), 1600 (m), 1472 (m), 1111 (s) cm^{-1}.

CHAPTER 9

APPENDIX

X-RAY DIFFRACTION DATA

X-RAY DIFFRACTION DATA

	[125b].HBF_4	[157].HBF_4
Empirical formula	$C_{39}H_{39}N_3OPBF_4$	$C_{21.5}H_{28}BCl_3F_4N_3O_2$
Formula weight	683.51	553.63
T/K	293 (2)	140 (2)
Crystal system	Monoclinic	Monoclinic
Space group	C2/c	C2/c
a/Å	22.913 (5)	18.3030 (13)
b/Å	9.3242 (9)	16.4210 (9)
c/Å	33.679 (7)	17.961 (4)
β/degrees	103.56 (2)	119.300 (13)
Volume/$Å^{-3}$	6995 (2)	4707.6 (11)
Z	8	8
D_c/g cm^{-3}	1.298	1.562
Absorption coeffient/mm^{-1}	0.136	0.448
F(000)/*e*	2864	2288
Crystal size/mm	0.32 x 0.25 x 0.12	0.23 x 0.17 x 0.22
θ range for data/degrees	1.83 to 24.91	1.78 to 25.02
h_{min}, h_{max}	-25, 21	-21, 21
k_{min}, k_{max}	-7, 10	-16, 17
l_{min}, l_{max}	-36, 36	-21, 21
Reflections collected	10770	10252
Independent reflections	4914	3631
R_{int}	0.1298	0.0739
Data/restraints/parameters	4914 / 0 / 450	3631 / 0 /357
Goodness-of-fit on F^2	0.719	1.446
Final R[a] indices	R_1=0.1344	R_1=0.1482
	(0.0569)[b]	(0.1029)
	wR_2=0.1580	wR_2=0.2751
	(0.1411)[b]	(0.2602)
Largest diff. peak and hole/e $Å^{-3}$	0.841 and -0.385	0.352 and -1.188

[a] $R_1 = S(F_o\text{-}F_c/\Sigma(F_o)$; $wR_2 = [\Sigma\{w(F_o^2\text{-}F_c^2)^2\}/\Sigma\{w(F_o^2)^2\}]$; $w = 1/\sigma^2(F_o^2)$.

[b] R_1 and wR_2 values for all unique data above background; those calculated for data with $I > 2\sigma(I)$ given in parentheses.

Table 6. Crystal data, details of data collection and structure refinement for [125b] and [157]

CHAPTER 10

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