DOCTOR OF PHILOSOPHY

Studies towards the synthesis of ptilomycalin A and synthetic analogues

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STUDIES TOWARDS THE SYNTHESIS OF PTILOMYCALIN A AND SYNTHETIC ANALOGUES

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of Philosophiae Doctor

by

Christopher Gareth Moore

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For Mum, Dad and Debbie,

Diolch o galon.

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CONTENTS

ABSTRACT

This thesis describes the work performed towards the enantioselective synthesis of the marine alkaloid, ptilomycalin A and also the development of a methodology for the preparation of structural analogues of the natural product.

An attempted stereospecific synthesis of the *tert*-butyl ester derivative of the pentacyclic core of ptilomycalin A is reported. A convergent synthesis is employed in the preparation of the chiral precursors; an unsaturated aldehyde prepared starting from (*S*)-(+)-2-aminobutyric acid and a chiral β-keto ester synthesised from ethyl (*R*)-3-hydroxybutyrate. The preparation of a *bis*-α,β-unsaturated ketone double Michael acceptor *via* a somewhat problematical Knoevenagel condensation reaction between these precursors, and the attempted guanidine addition-cyclisation reaction are also described.

The Knoevenagel condensation reaction was also investigated in a further effort to optimise the reaction conditions using a related unsaturated aldehyde and a selection of β-keto esters. In addition, an alternative approach towards the ester-substituted pentacyclic core of ptilomycalin A utilising the Wadsworth-Emmons reaction is also discussed. Initial attempts being directed towards the preparation of a functionalised guanidine tricycle.

The synthesis of a chiral pentacycle is reported, having an identical stereochemistry to the pentacyclic core of ptilomycalin A. The key steps involve a Wittig reaction leading to a *bis*-α,β-unsaturated ketone, followed by the addition of guanidine to generate the pentacyclic core. The stereochemistry of the pentacycle is confirmed by means of TOCSY and NOESY experiments and by comparison with the spectroscopic data quoted for the pentacyclic cores of ptilomycalin A and 13,14,15-isocrambescidin 800.

A synthetic methodology enabling the preparation of advanced, hexacyclic analogues of ptilomycalin A is described. The analogue consists of a hexacyclic core, linked to a spermidine residue *via* a 16-carbon aliphatic chain. Two tetrahydropyranyl spiro *N,O*-acetal units are incorporated within the hexacyclic unit which possesses an identical stereochemistry to the pentacyclic nucleus of ptilomycalin A.

ABBREVIATIONS

$[\alpha]_D$	specific rotation
Å	Ångström (s)
Ac	acetyl
AcOH	acetic acid
AEHC	aminoethylhomocysteine
AIDS	acquired immune deficiency syndrome
All	allyl
Ar	aryl
Arg	arginine
Asp	aspartic acid
9-BBN-H	9-borabicyclo[3.3.1]nonane
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Boc-ON	2-(*tert*-butoxycarbonyloximino)-2-phenylacetonitrile
bp	boiling point
br	broad
Bu	butyl
CAN	ceric ammonium nitrate
cat.	catalytic
CBZ	benzyloxycarbonyl
CC-50	concentration of a cytotoxic agent required to reduce target cell viability by 50%
CD	circular dichroism
°C	degrees Celsius
CIT	citrulline
COLOC	correlated spectroscopy for long-range coupling
COSY	correlated spectroscopy
d	doublet (spectral)
DCC	*N*, *N*-dicyclohexylcarbodiimide
Δ	reflux
δ	chemical shift
DEPT	distortionless enhancement by polarisation transfer

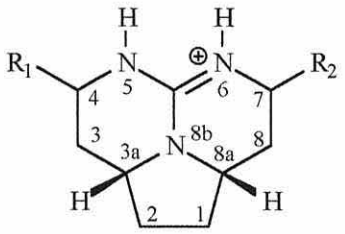
DIBAL-H	diisobutylaluminium hydride
DIPEA	diisopropylethylamine
DMAP	4-(*N*, *N*-dimethylamino)pyridine
DME	1,2-dimethoxyethane
DMF	*N*, *N*-dimethylformamide
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
E	entgegen
EDCI	1-ethyl-3-[3-(dimethylamino)-propyl]-carbodiimide hydrochloride
EI	electron impact
eqv.	equivalent (s)
Et	ethyl
FAB	fast atom bombardment
FGI	functional group interconversion
FT-IR	Fourier transform infrared spectroscopy
g	gram (s)
Glu	glutamic acid
gp	glycoprotein
h	hour (s)
HIV	human immunodeficiency virus
HMBC	heteronuclear multiple bond correlation
HMPA	hexamethylphosphoramide
HOBT	1-hydroxybenzotriazole
HOHAHA	homonuclear Hartman-Hahn spectroscopy
HRMS	high resolution mass spectroscopy
HSV	herpes simplex virus
Hz	hertz
i	iso
IC_{50}	concentration at which treatment results in 50% inhibition of target cells
IC_{90}	concentration at which treatment results in 90% inhibition of target cells

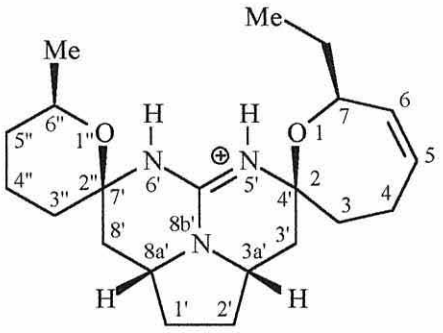
ID_{50}	concentration required for 50% inhibition of cell growth in culture
i.p.	intra peritoneal
IR	infrared
J	coupling constant
kcal	kilocalorie (1 kcal = 4.19 kJ)
kg	kilogram (s)
LD_{50}	lethal dose 50; the amount of a given toxic substance that will elicit a lethal response in 50% of test organisms
$LiAlH_4$	lithium aluminium hydride
Lit.	literature
M	moles per litre or multiplicity (spectral)
m	multiplet (spectral)
MHz	megahertz
Me	methyl
µg	microgram (s)
mg	milligram (s)
MIC	minimum inhibitory concentration
min	minute (s)
ml	millilitre (s)
µM	micromoles per litre
µm	micromole (s)
mm Hg	millimetres of mercury
mmol	millimole (s)
mol	mole (s)
MS	mass spectrometry
m/z	mass ion
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	correlated nuclear Overhauser effect spectroscopy
pdTp	deoxythymidine 3',5'-diphosphate
p	pentet (spectral)
p-TsCl	*para*-toluenesulfonyl chloride
p-TsOH	*para*-toluene sulfonic acid

PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
Ph	phenyl
pKa	acid dissociation constant
PMB	*para*-methoxybenzyl
ppm	parts per million (NMR)
PPTS	pyridinium *p*-toluenesulfonate
q	quartet (spectral)
R_f	retention factor (in chromatography)
RNA	ribonucleic acid
ROESY	through-space correlated nuclear Overhauser effect spectroscopy
rt	room temperature
s	singlet (spectral)
sat.	saturated
SNase	staphylococcal nuclease
S_N2	bimolecular nucleophilic substitution
sp.	spiculifer
STX	saxitoxin
t	triplet (spectral)
TBAF	tetra-*n*-butylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
t-Bu	*tert*-butyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl
TMSOTf	trimethylsilyltrifluoromethanesulfonate
TOCSY	total correlated spectroscopy
TTX	tetrodotoxin
Z	zusammen

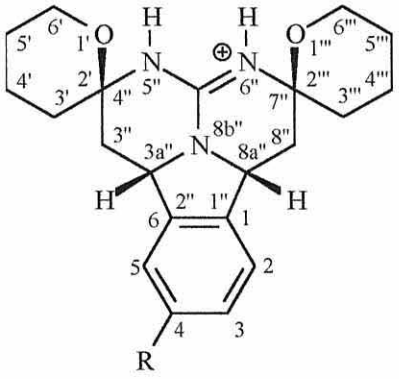
NOMENCLATURE

The guanidine compounds discussed in this thesis are named using a numbering system which is based on the acenapthylene ring system and is as follows:





In the case of compounds containing the hexacyclic, benzo-fused guanidine system, the numbering is again based on the acenapthylene ring system, but is numbered in the following manner:



All NMR spectroscopic assignments are made using the numbering systems illustrated above, unless otherwise stated.

CHAPTER 1

THE GUANIDINIUM GROUP; ITS ROLE IN NATURE AND ARTIFICIAL RECEPTORS

1.1 Structural and Basic Properties of Guanidine

In recent years compounds containing the protonated guanidine functional group have become important targets for the synthetic organic chemist due to the diverse range of biological activity that they exhibit.[1] This activity is demonstrated in their versatility as very strong bases, in their propensity to act as binding sites for anionic functional groups,[2] as active hypotensive and adrenergic neuron blocking agents[3] and in addition, their potential in asymmetric catalysis.

Guanidine **1** is one of the most basic nitrogen-containing compounds known with the conjugate acid having a pKa of 13.6 in water.[4] The high level of basicity can be attributed to the equal delocalisation of a positive charge over the three nitrogens of the protonated guanidinium ion. This results in the formation of three equivalent resonance forms of the cation which itself results in an overall stabilisation of charge (Figure 1.1).

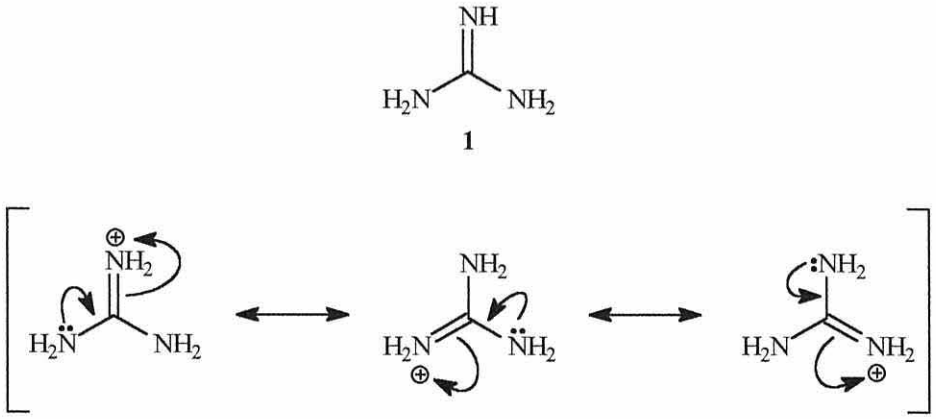


Figure 1.1 Guanidine **1** and the three resonance forms of the positively charged guanidinium cation.

The three nitrogen atoms and the central carbon atom of guanidine have a coplanar arrangement brought about by the partial double bond character of each of the C–N bonds.[5] Simple guanidinium derivatives have been studied by X-ray crystallography in order to establish molecular dimensions with the results supporting the delocalisation of the π system in the guanidine cation. The observed average value for the C to N bond distance in the methyl guanidinium cation is 1.323Å, which is comparable to that of a C=N bond at 1.29Å, and shorter than the value of a characteristic C–N bond length of 1.41Å.[6] In

addition, the three C–N bonds in the guanidinium ion are almost equal in length with an average value of 1.33Å and similarly, the three N–C–N bond angles are nearly always equal at 120°.

By virtue of its basicity, guanidine remains protonated over a wide pH range and this confers an excellent anion binding affinity in aqueous media. It is capable of absorbing carbon dioxide and water from air (typical strong base behaviour) and the cationic form is inert to decomposition.[7]

1.2 Oxoanionic Bonding

As previously discussed, compounds containing guanidine have the ability to act as binding sites for anionic functional groups.[2] Specific examples of anionic substrates that can bind to the guanidinium moiety are the oxoanionic carboxylate and phosphate groups. Oxoanionic bonding occurs through the formation of characteristic pairs of zwitterionic hydrogen bonds of the form $N–H^{\oplus}\cdots X^{\ominus}$, with the overall charge resulting in electrostatic attraction which contributes towards the binding strength. Furthermore, the parallel arrangement of the hydrogen bonds provides structural organisation, as is evident in many crystal structures of guanidinium salts[8,9] (Figure 1.2).

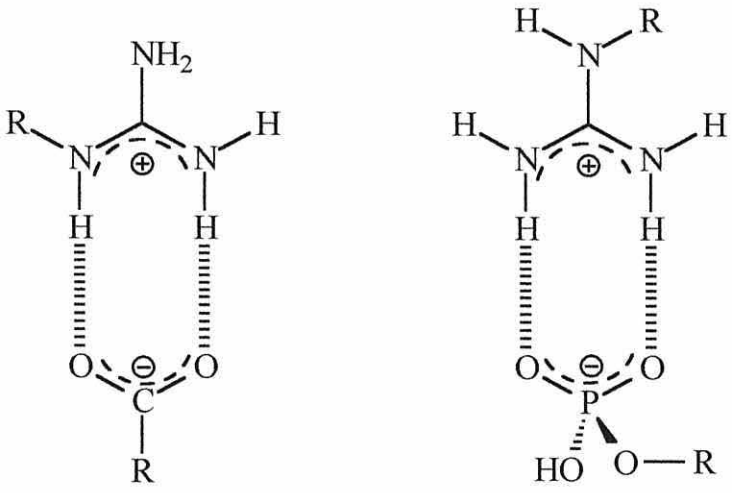


Figure 1.2 Characteristic binding patterns of oxoanions with the guanidinium moiety as found in many X-ray structures of the corresponding salts.

1.3 ARGININE AND ENZYMES

At physiological pH, the guanidine group of the naturally occurring amino acid arginine is protonated and consequently, it exists in the zwitterionic form shown below (Figure 1.3). Structural coplanarity has enabled arginine to achieve unique extended patterns of hydrogen bonding, perhaps explaining the evolutionary selection of arginine as a constituent of many proteins. In fact, the guanidyl groups of arginine residues play an important function in binding, as well as active roles at the functional sites of both enzymes and non-catalytic proteins. The hydrogen bonding patterns found in arginine side chains of proteins function in maintaining protein conformation and in the binding and recognition of anionic substrates by enzymes, receptor sites and antibodies. Functionally active arginine residues have been found in *E. coli* alkaline phosphatase, lactate dehydrogenase, D-amino acid oxidase, carboxypeptidases A and B, ribonuclease T_1, pepsin and antibody combining sites directed against haptens containing anions such as arsonate, phosphonate and carboxylates.[6,10]

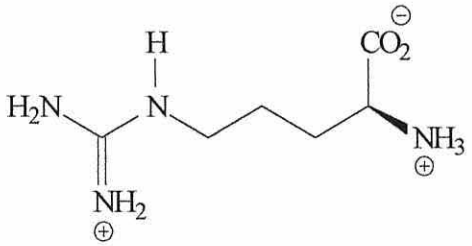


Figure 1.3 The naturally occurring amino acid L-arginine.

1.3.1 STAPHYLOCOCCAL NUCLEASE

A specific example of an enzyme where arginine demonstrates its ability to form parallel hydrogen bonds with oxoanions is staphylococcal nuclease (SNase). This feature was demonstrated through observations by Cotton *et al.* on the interactions that play an important part in the binding of the enzyme inhibitor deoxythymidine 3',5'-diphosphate

(pdTp) to the staphylococcal nuclease enzyme in the nuclease-Ca^{2+}-pdTp ternary complex.[6] Oxoanionic binding takes place through hydrogen bonds between the guanidyl side chains of arginine residues 35 and 87 and the 5'-phosphate group as shown in Figure 1.4 below.

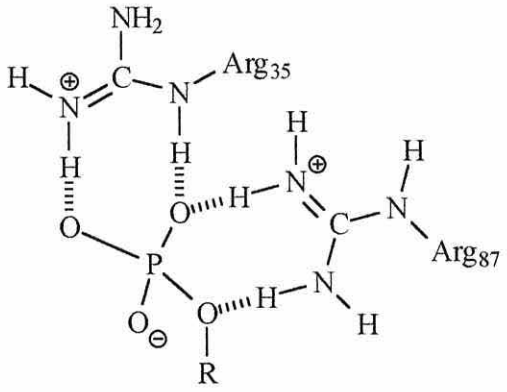


Figure 1.4 Representation of the hydrogen bonding interactions of the guanidyl side chains of arginine residues 35 and 87 with 5'-phosphate group in the staphylococcal nuclease-Ca^{2+}-pdTp ternary complex.

The two guanidyl groups of arginine residues 35 and 87 each form a pair of hydrogen bonds with two of the phosphate oxygen atoms, one being shared between two hydrogens of separate residues. The two three-atom chains, O–P–O and N–C–N are connected by O···H–N hydrogen bonds which result in the formation of an elongated hexagon. SNase functions as an extemely efficient catalyst for the hydrolysis of phosphodiester linkages in DNA and RNA, accelerating cleavage by a factor of 10^{16}, yielding 3'-mononucleotides and 3'-dinucleotides *via* the mechanism shown in Figure 1.5 overleaf.[11]

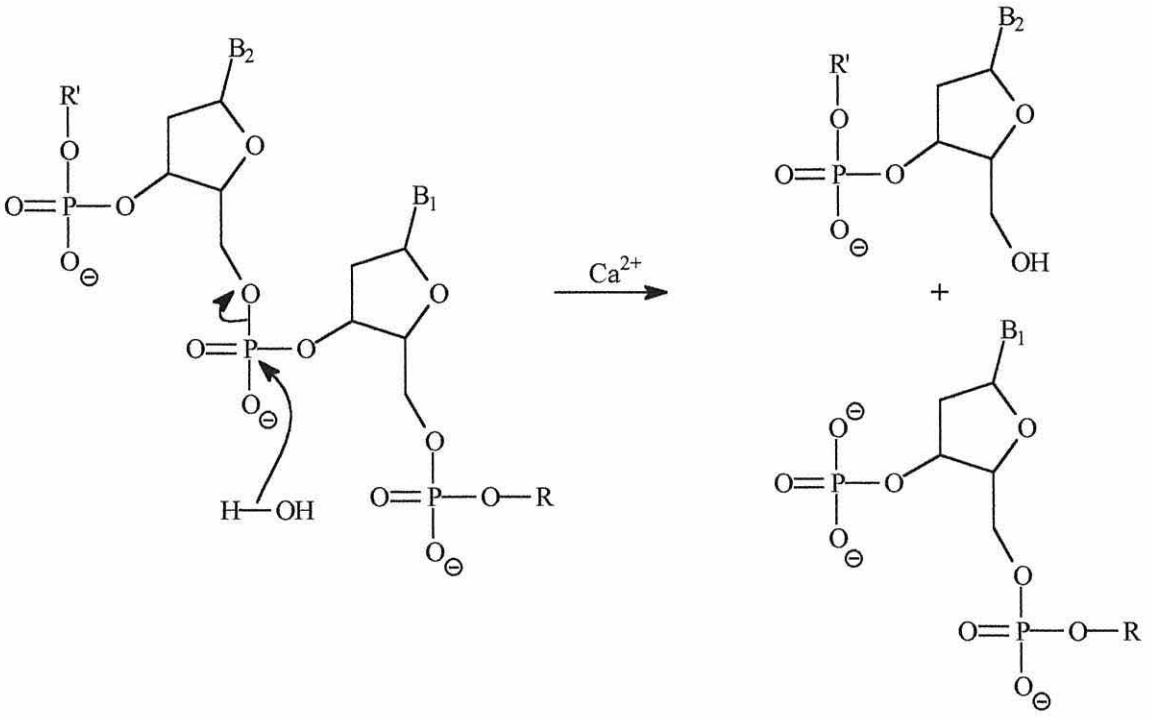
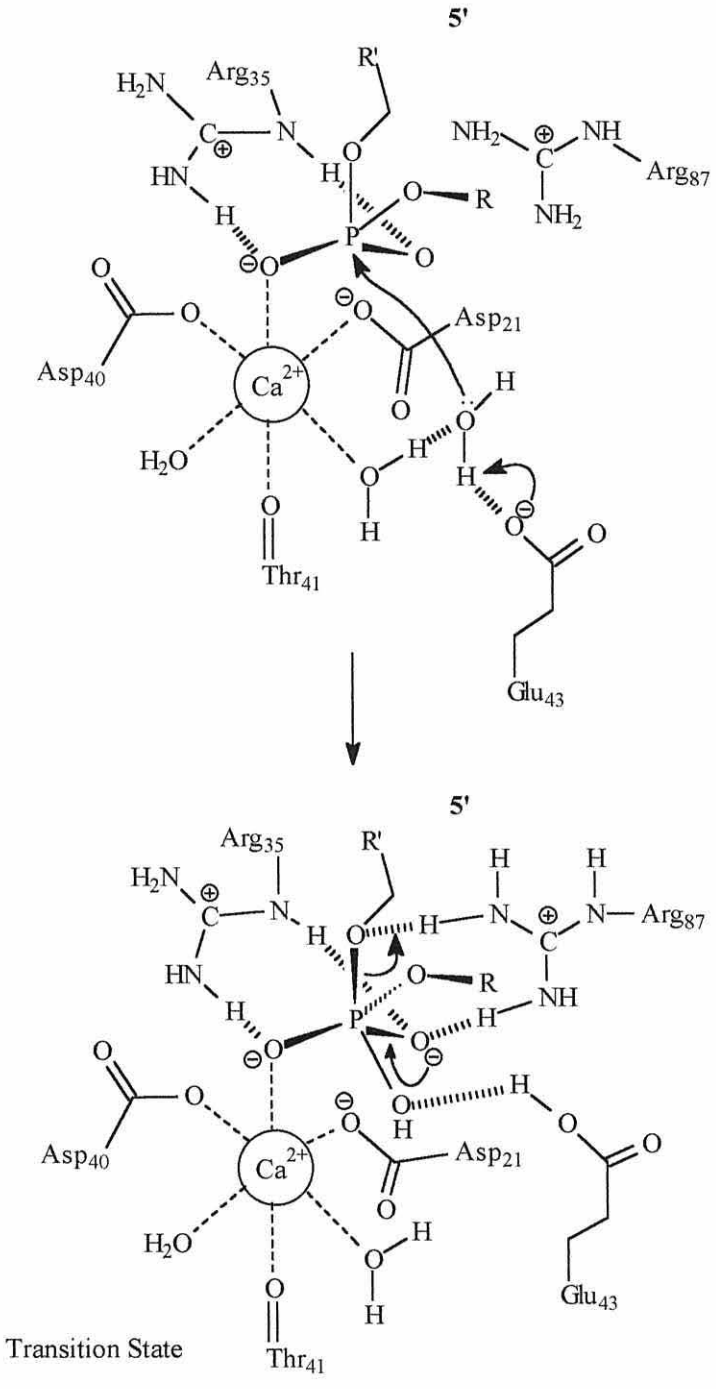


Figure 1.5 Phosphodiester cleavage catalysed by SNase. Nucleophilic substitution by water on the phosphodiester phosphorus of DNA or RNA yields the 3'-mononucleotide or the 3'-dinucleotide.

Studies on high resolution X-ray crystal structures as well as a series of mechanistic studies have resulted in a proposed mechanism for the catalytic mode of action of SNase. [12] The active site of the enzyme contains two arginine residues in conjunction with a calcium ion which results in electrophilic activation of the phosphodiester towards hydrolysis. [13] Mildvan *et al.*[11] have put forward a favourable mechanism for the enzymatic reaction where electroneutrality is preserved at the phosphodiester reaction centre throughout the course of the reaction (Scheme 1.1, page 7).

In the ground state, the phosphodiester exists as a monoanion and is neutralised by the cationic guanidinium moiety of Arg-35, with the divalent calcium being neutralised by Asp-21 and Asp-40. There are two water molecules within the active site but only one is believed to take part in hydrolysis. Glu-43 acts as a general base assisting nucleophilic attack of a water molecule which initially forms a hydrogen bond to phosphorus, thus converting the phosphodiester monoanion at the reactive centre to a phosphorane dianion. The trigonal bipyramidal transition state is stabilised through neutralisation of the developing negative charge by formation of a hydrogen bond with the second cationic guanidinium group present in Arg-87.



Scheme 1.1 A proposed reaction mechanism for staphylococcal nuclease based upon studies with liganding and arginine mutants.

The development of the second negative charge on the equatorial oxygens of the transferred nucleophile in the transition state is typical of an associative nucleophilic substitution at a phosphorus atom. It is thought that the proton required to form the 5'-hydroxyl leaving group ($H_2O^{\oplus}$–CH_2 –R') is supplied from Arg-87 which is acting as a general acid,[12] thereby completing hydrolysis.

Mechanistic studies can be performed on enzymes through structural mutations at the enzyme active site and also through kinetics. Studies on SNase involved the substitution of the active residues Glu-43, Arg-35 and Arg-87 with unnatural amino acid analogues. These analogues typically have similar structures and conformations to the constituent they are replacing.[12] Arginine, for example, can be substituted with aminoethylhomocysteine (AEHC) or citrulline (CIT), the structures of which are shown in Figure 1.6.

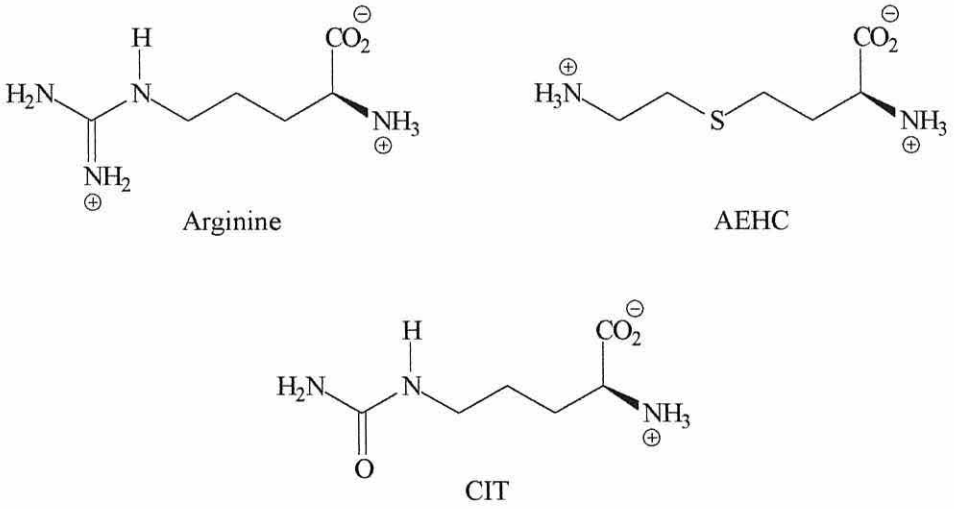


Figure 1.6 Arginine and the unnatural amino acids AEHC and CIT.

Substituting different amino acids into the active site can result in conformational changes within the enzyme, which can lead to different electrostatic interactions and hydrogen bonding patterns. The consequences of these substitutions typically decrease the rate of the enzyme's catalytic activity, or affect the way in which the enzyme responds to different pH ranges.

1.4 Synthetic Guanidinium Anion Receptors

The fact that the guanidinium group has the ability to act as a host for binding anions has led to the development of a significant number of artificial guanidinium-based receptors. The electrostatic attraction between anion and host is a result of the extremely high basicity of guanidine which guarantees protonation over a wide pH range. Examples of guanidinium-based receptors were first reported in 1978 by Lehn *et al.*,[14] who synthesised macrocyclic compounds **2** and **3** (Figure 1.7), which have the ability to complex, albeit weakly, the phosphate (PO_4^{3-}) anion.

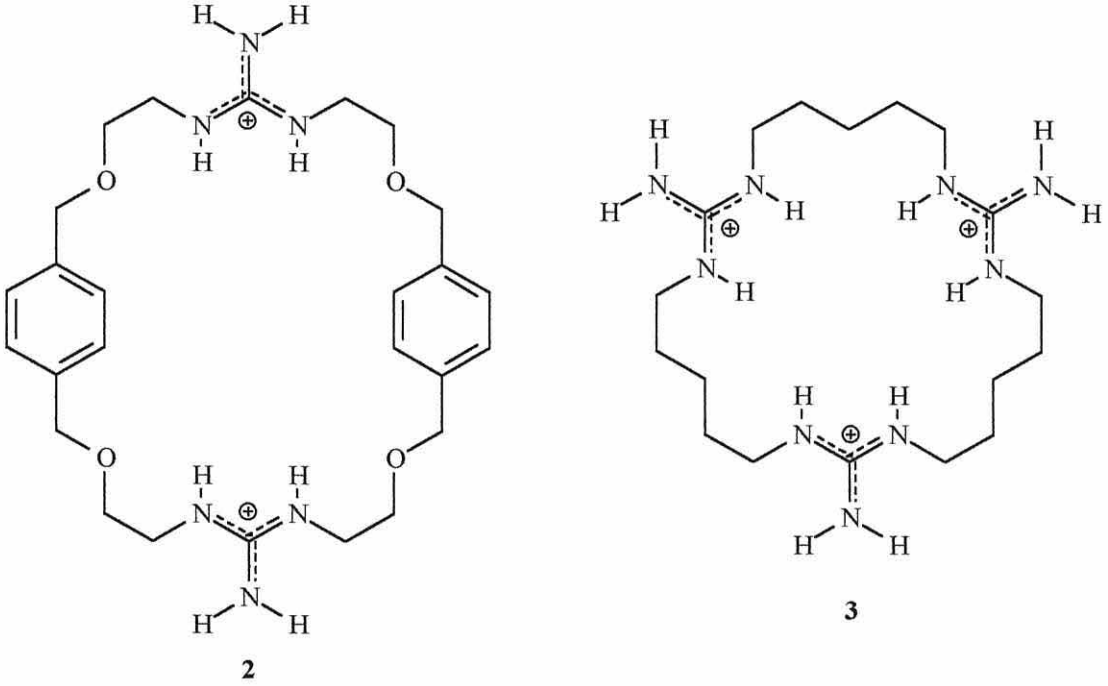


Figure 1.7 The first reported synthetic, macrocyclic guanidinium-based receptors.

Studies into receptor binding and anion recognition have been extended towards artificial hosts capable of binding anions by imitation of the natural product's binding ability.[15] This work focussed on the bicyclic guanidines **4**, **5** and **6**, which are essentially strain free, having the affinity to bind substrates by imitation of the natural host orientation and spacing (Figure 1.8). Furthermore, chiral synthetic receptors have also been prepared which can be used extensively as anchor groups for enantioselective recognition, catalysis

and specific transport of substrates across membranes.[9] The rigid, C_2 symmetric bicyclic guanidines **7**[16] and **8**,[17] are such examples, with the latter possessing the ability to form diastereomeric complexes with α-substituted carboxylates in acetonitrile (Figure 1.8).

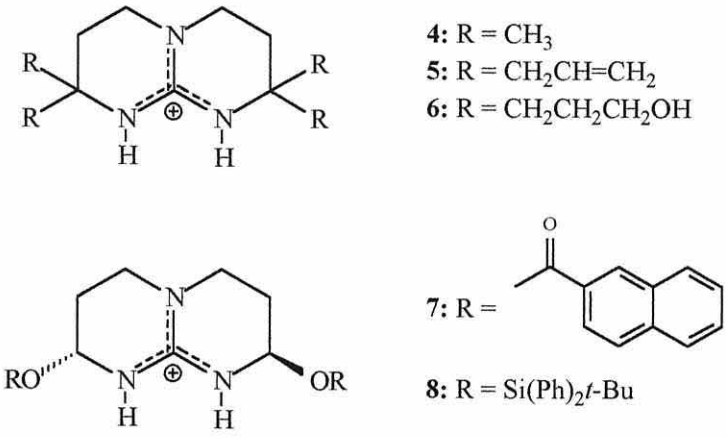


Figure 1.8 Examples of bicyclic symmetrical (**4** - **6**) and bicyclic C_2 symmetrical (**7**, **8**) guanidinium-based receptors.

The molecular recognition, transport and catalytic hydrolysis of phosphodiesters has attracted extensive attention due to potential applications in nucleotide chemistry. Molecular recognition and catalytic studies performed on *bis*-acylguanidines[18] and *bis*-alkylguanidines,[19] demonstrated that compounds of this nature have the capability to act as artificial receptors and also as catalysts in phosphodiester cleavage. Such catalytic agents may have potential in the controlled hydrolysis of DNA and RNA.

CHAPTER 2

GUANIDINE-CONTAINING SECONDARY METABOLITES OF MARINE ORIGIN

2.1 INTRODUCTION

Alkaloids are a diverse group of nitrogen containing natural products, they are classified as secondary metabolites and are compounds that are generally unique to certain species. The majority of alkaloids occur in flowering plants, for instance, the allelopathic agent, hordenine which is produced in barley (Figure 2.1). Pumilotoxin C is an alkaloid of animal origin isolated from a species of Panamanian frog and is used by the local Indians as a poison to coat the tips of their arrows for hunting. Alkaloids are also common in fungi, an example being chanoclavine I, an ergot alkaloid that was responsible for numerous deaths during the middle ages.[20]

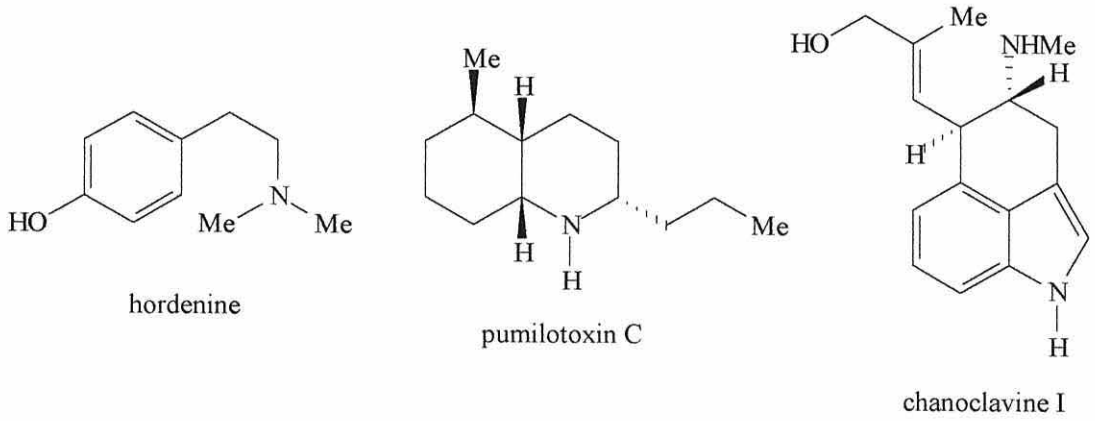


Figure 2.1 Examples of alkaloids present in plants, animals and fungi.

Studies on marine organisms have yielded a wide variety of unusual nitrogen-containing natural products, many of which are unique to a marine environment[21] and are also of pharmaceutical and biological importance.[22] Of current particular interest are guanidine-containing marine natural products. Again, they are often unique to certain organisms,[23] typically have cyclic structures whilst many demonstrate remarkable ranges of biological activity. An example of a guanidine-containing compound of marine origin is ptilocaulin **9** (Figure 2.2, page 13), which has been shown to possess antimicrobial and cytotoxic activitity.[24]

In contrast, it is interesting to note that guanidine-containing alkaloids from terrestrial sources are rare, one such example being the acylpolyamine toxin nephilatoxin-9

found in numerous spiders **10**.[25] Terrestrial aromatic systems containing the guanidine moiety are considerably more common with milletonine **11**, found in the bark of *Millettia laurenti*, being a recent example[26] (Figure 2.2).

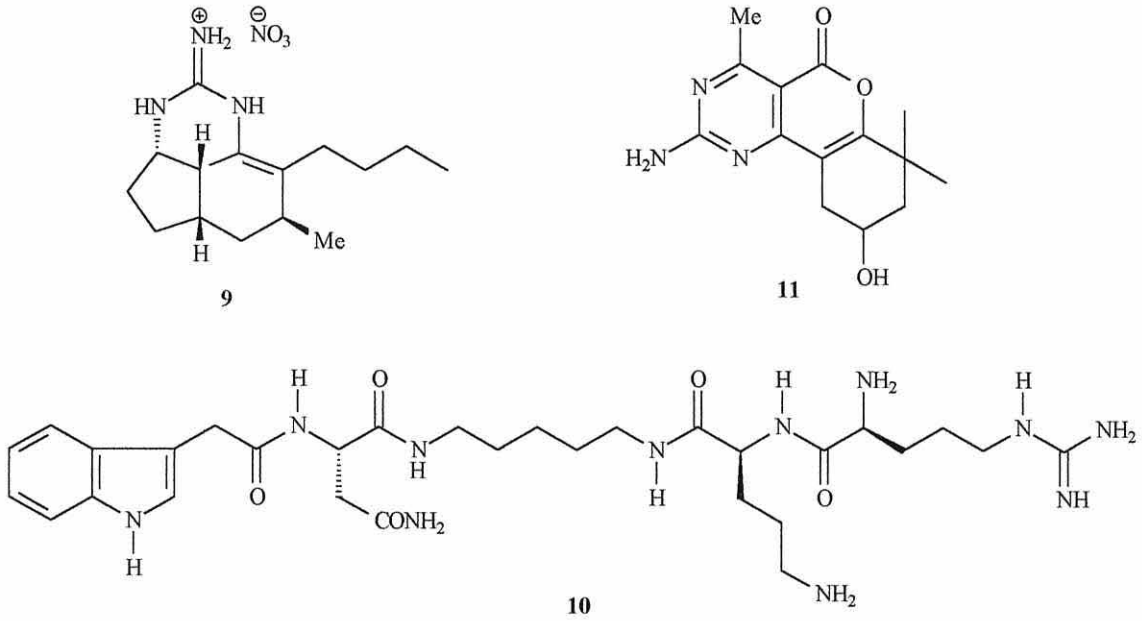


Figure 2.2 Examples of various guanidine-containing natural products.

The remainder of this chapter describes some of the more important bioactive marine natural products incorporating guanidine as a major constituent.

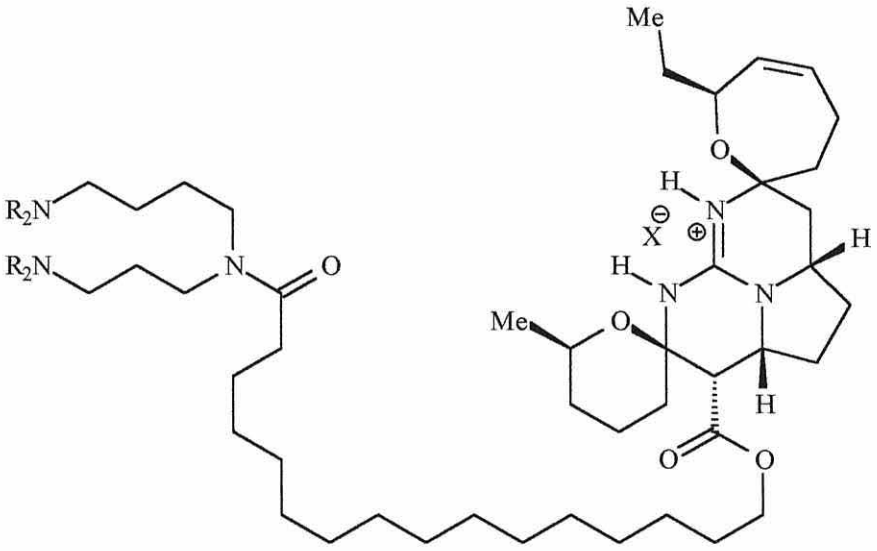
2.2 Guanidine-Containing Marine Natural Products

2.2.1 Ptilomycalin A

In 1989, during the course of screening for unusual bioactive agents from marine sponges, Kashman *et al.* isolated a novel antitumour, antiviral and antifungal compound designated ptilomycalin A **12** from the Caribbean sponge *Ptilocaulis spiculifer* and from a red *Hemimycale sp.* of the Red Sea.[27] More recently, ptilomycalin A has been isolated from another Caribbean sponge, *Batzella sp.*[28] and also from the starfish *Fromia monilis* and *Celerina heffernani* collected in the waters off the coast of New Caledonia.[29]

The structure of ptilomycalin A was determined based upon detailed ^{1}H and ^{13}C NMR experiments, along with high resolution fast atom bombardment (HR-FAB) mass

spectrometry of the *bis*-trifluoroacetyl derivative **13**. These results showed that the structure of ptilomycalin A consisted of a unique pentacyclic guanidine core linked to a spermidine residue by a 16-hydroxyhexadecanoyl chain (Figure 2.3). The presence of a guanidine moiety was confirmed by the occurrence of two N–H resonances at δ 10.22 and δ 9.87 in the proton spectrum, along with a carbon signal at δ 149.09 in the ^{13}C NMR spectrum which showed no correlation with protons in the COLOC or HMBC spectra. The stereochemistry of the natural product was determined on the basis of phase-sensitive NOESY and ROESY experiments.[30]



12: R = H, X = unspecified
13: R = $COCF_3$, X = CF_3CO_2

Figure 2.3 The structure of ptilomycalin A showing the absolute stereochemistry.

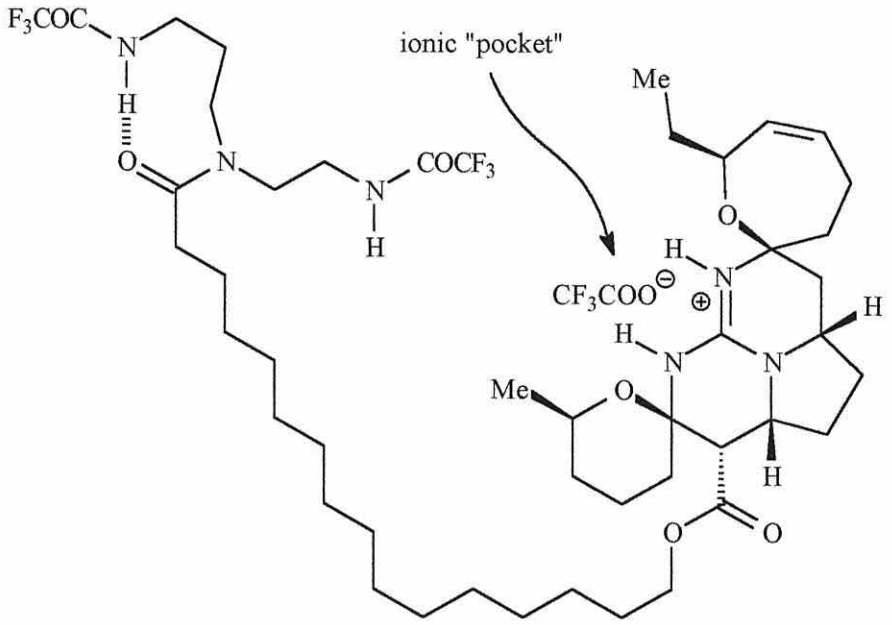
In biological tests, ptilomycalin A demonstrated a remarkable range of bioactivity which included *in vitro* cytotoxicity towards the following cancer cell lines: P388 (IC_{50} 0.1 μg/ml), L1210 (IC_{50} 0.4 μg/ml) and KB (IC_{50} 1.3 μg/ml), antifungal activity against *Candida albicans* (MIC 0.8 μg/ml), as well as antiviral activity towards the Herpes Simplex Virus (HSV) at a concentration of 0.2 μg/ml.[27] In addition, an anti-HIV assay on cells CEM 4 infected by HIV-1 showed **12** to be highly cytotoxic towards the target cells with a CC-50 of 0.11 μg/ml without cytoprotective effects at a dose of < 0.1 μg/ml.[29]

The significance of this biological testing is emphasised by the fact that in each case the bioactivity occurs at very low concentrations, as summarised in Table 2.1.

Biological Test	Activity
Cytotoxicity: P388	IC_{50} 0.1 μg/ml
L1210	IC_{50} 0.4 μg/ml
KB	IC_{50} 1.3 μg/ml
Antifungal: *Candida albicans*	MIC 0.8 μg/ml
Antiviral: HSV	0.2 μg/ml
HIV (CEM 4)	CC-50 0.11 μg/ml

Table 2.1 The biological activity of ptilomycalin A.

The exact biological role of ptilomycalin A remains unclear, although speculation surrounding the structural features present have focussed on its structural similarities to abiotic guanidine based anionic receptor molecules. Ptilomycalin A behaves as if it were non-polar, despite the fact that it contains several polar functional groups such as the guanidine moiety and the spermidine residue.[30] This physical property supports an anionic binding ability for **12**, inferring the presence of an enclosed ionic "pocket" at the central guanidine core which acts as a specific recognition site, which in turn may impart much of the biological activity of ptilomycalin A. The enclosed ionic "pocket" is a result of the stereochemistry about the pentacyclic core, where the six and seven membered rings adopt a *syn* geometry as do the two protons on the pyrrolidine ring, with requisite folding of the spermidine residue through the 16-carbon chain occurring above the pentacyclic guanidine core. This can be envisioned by considering Kashman's proposed molecular conformation of the *bis*-trifluoroacetyl derivative **13** of ptilomycalin A (Figure 2.4).



13

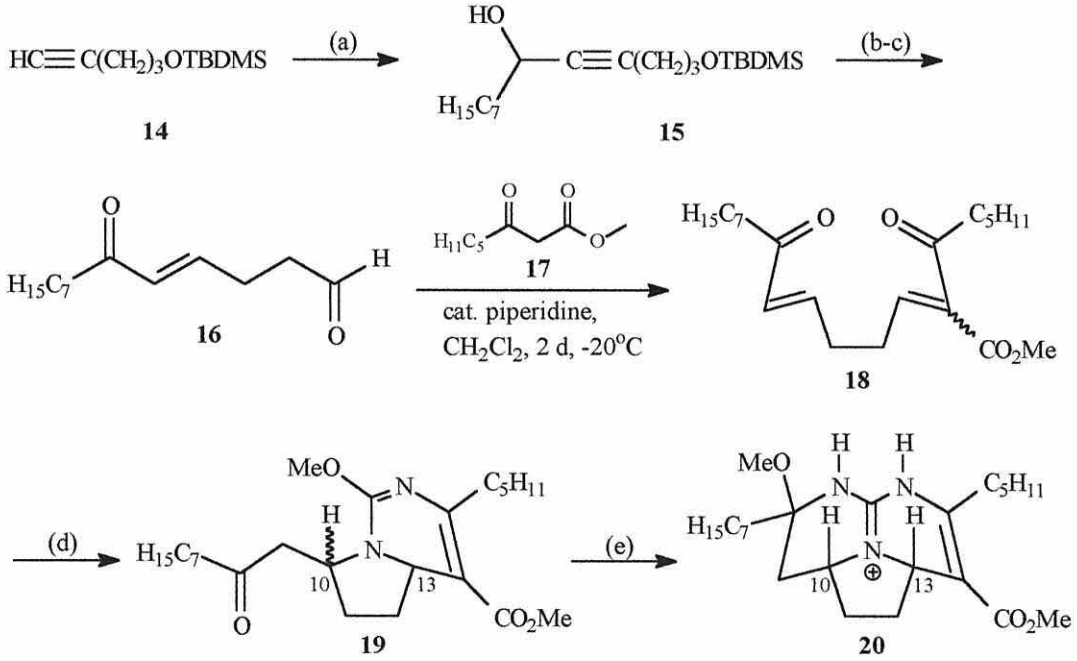
Figure 2.4 Kashman's proposed molecular conformation of ptilomycalin A.

Kashman and co-workers[30] have noted that the pentacyclic core of **12** possesses a structural similarity to the enantioselective anion receptor **7**, (see Chapter 1, Figure 1.8, page 10). In an effort to establish whether the *bis*-trifluoroacetate derivative **13** exhibited a similar enantioselective anion recognition ability, they performed an NMR study on complexes of **13** and several *N*-acetylamino acid anions. Although no evidence of any enantioselectivity was obtained, the relative ability of **13** to form complexes with *N*-acetylamino acids in organic solution was estimated to be: L-*N*-acetylmethionate ≈ L-*N*-acetylvalinate > L-*N*-acetylalanate ≈ L-*N*-acetylisoleucinate >> *N*-acetylglycinate. Kashman has proposed that the order of the series may reflect the "fit" of the anions in the cavity of ptilomycalin A. In addition, there is evidence suggesting that the spermidine residue is also involved in the binding of anionic species to the pentacyclic core of ptilomycalin A.[31]

In an alternative theory, Overman *et al.*[32] have speculated that *in vivo*, the aliphatic methylene chain of ptilomycalin A is embedded in a membrane such that anion binding is enhanced. It can thus be surmised that **12** may very well be involved in the transport of anions across cell membranes by an as yet unidentified process.

2.2.2 SYNTHETIC ACTIVITY TOWARDS PTILOMYCALIN A

Activity towards the synthesis of ptilomycalin A is currently attracting considerable attention due to its unique structure, and wide ranging biological activity. Initial attempts to synthesise ptilomycalin A were reported by Snider and Shi,[33] who undertook a model study leading to the central tricyclic portion of **12**, using a proposed biomimetic approach (Scheme 2.1). Addition of the lithium acetylide prepared from **14**, to octanal, gave propargylic alcohol **15** which was sequentially reduced, deprotected then oxidised under Swern conditions to give keto aldehyde **16**. Knoevenagel condensation between **16** and methyl-3-oxooctanoate **17** gave *bis*-enone **18** as a 1:1 mixture of stereoisomers. A double Michael addition of *O*-methylisourea to both isomers of the *bis*-enone afforded bicycle **19** in a *trans* to *cis* ratio of 3:1. Fortuitously, both stereoisomers could be converted into the desired *cis*-tricycle **20** on treatment with ammonia/ammonium acetate in methanol. Tricycle **20** was established as the *cis* isomer through the observation of a strong ROESY cross peak between H-10 and H-13, and through molecular modelling calculations which indicated that the *cis* isomer is 3 kcal/mol more stable than the corresponding *trans* isomer.



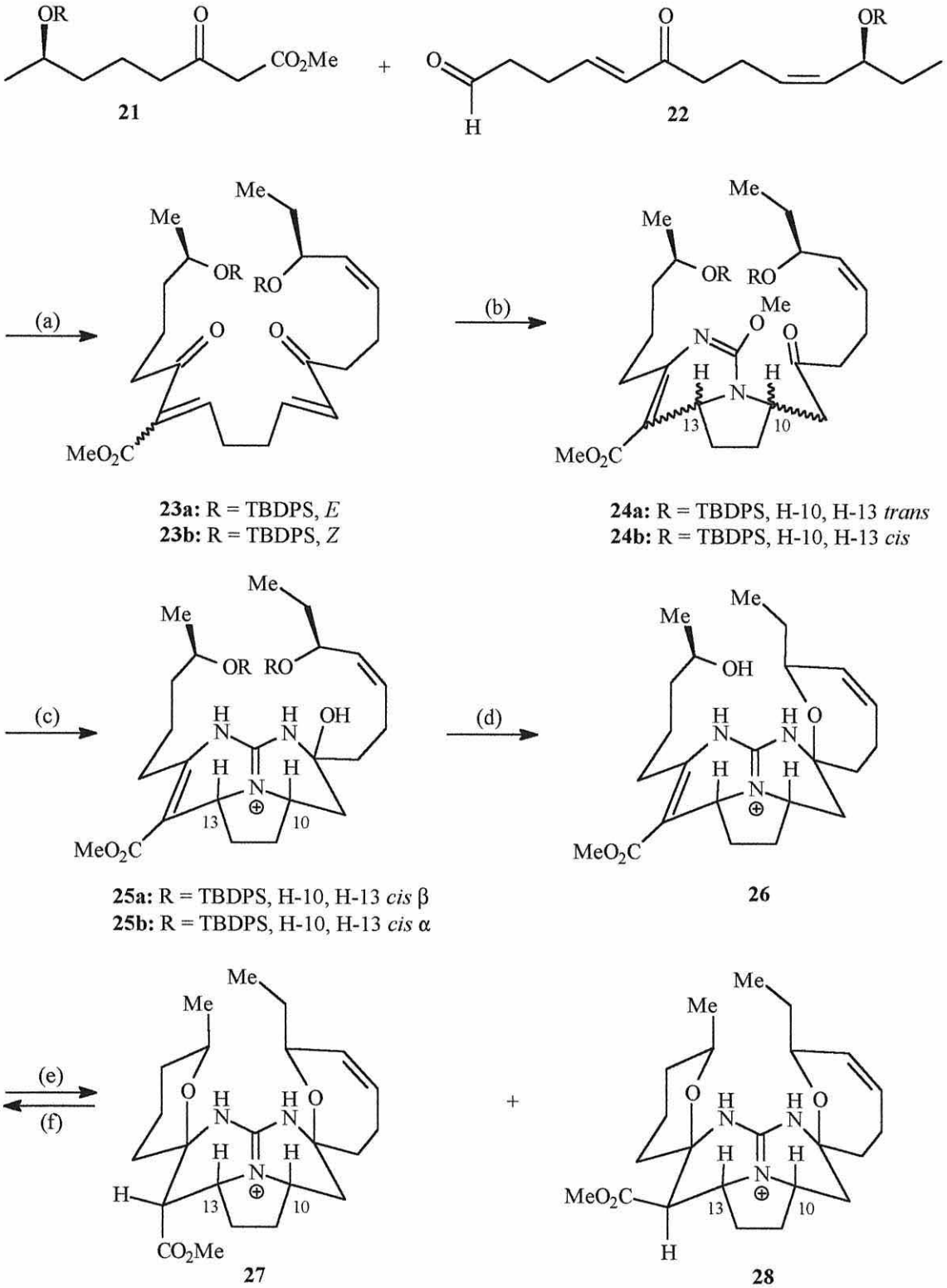
Reagents and Conditions: (a) *n*-BuLi, octanal; (b) $LiAlH_4$, THF, reflux, 5 h; (c) Swern oxidation; (d) *O*-methylisourea, $NaHCO_3$, DMF, 50 °C, 2 h; (e) NH_4OAc, NH_3, MeOH, 4 d, 60 °C.

Scheme 2.1 Snider's synthesis of the central tricyclic portion of ptilomycalin A.

With this preliminary study as a basis, Snider and Shi[34] have synthesised the pentacyclic nucleus **27** of ptilomycalin A, using a similar methodology to that employed for the preparation of their tricyclic model compound.[33] Again two key steps are involved, firstly a Knoevenagel condensation between scalemic β-keto ester **21** and unsaturated keto aldehyde **22** to give *bis*-α,β-unsaturated ketones **23a** and **23b** in 64% yield as an 1:1 ratio of stereoisomers (Scheme 2.2, page 19). Secondly, the double Michael addition of *O*-methylisourea to **23a** and **23b** afforded the corresponding isoureas as a 4:1 mixture which consisted of the two possible *trans* diastereomers **24a** and the two possible *cis* diastereomers **24b** in a 52% yield. Treatment of both geometric isomers with excess ammonium acetate in *tert*-butanol saturated with anhydrous ammonia gave an 1:1 mixture of tricycles **25a** and **25b**.

Deprotection of the silyl ethers, followed by cyclisation with triethylamine in methanol, gave the desired pentacyclic core of ptilomycalin A in 60% yield (~65% pure) as a 1.3:1 mixture of the methyl ester **27** and the diastereomer **28** possessing an equatorial methyl ester group (Scheme 2.2, page 19). Purification was achieved by means of a reversal of the previous cyclisation step where the mixture of **27** and **28** was treated with Et_3N in 1:1 $MeOH/H_2O$ at 60°C for 16 hours yielding tetracyclic alcohol **26**. Subsequent purification by means of column chromatography, and finally, cyclisation as before gave a 1.3:1 mixture of **27** and **28**. Separation of the two isomers by careful flash column chromatography gave pure **27** (34% from **25a**) and pure **28** (26% from **25a**).

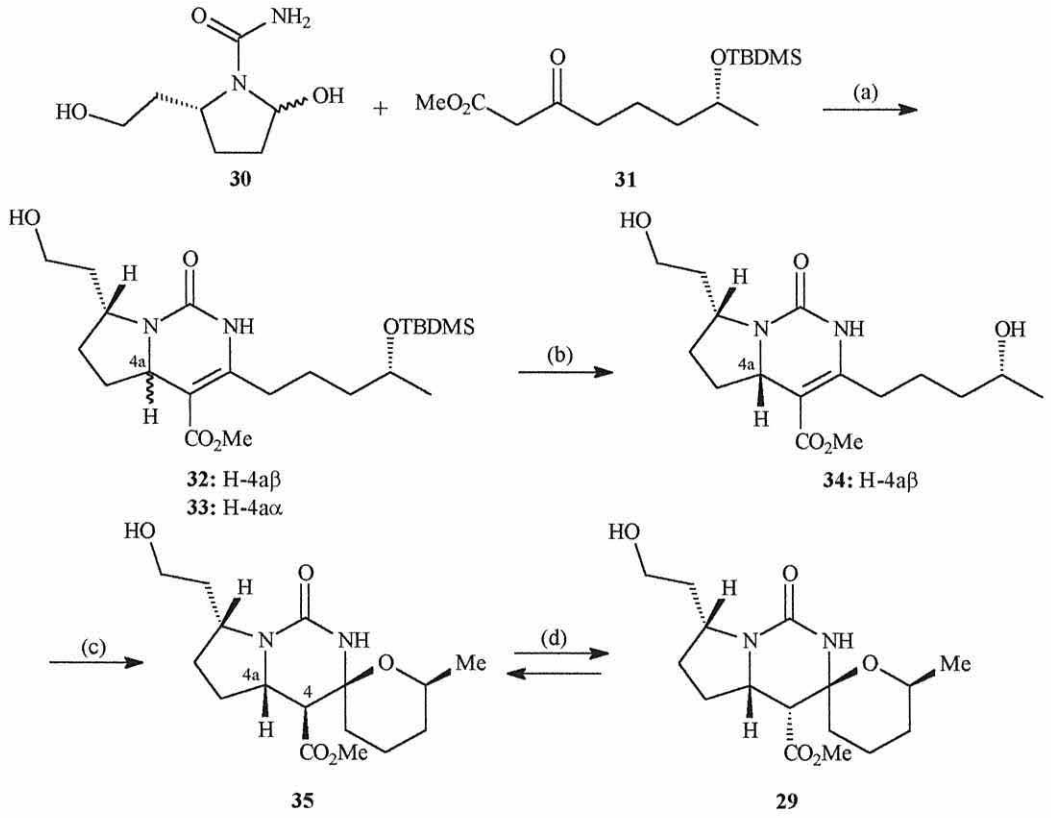
The ^1H and ^{13}C spectra of **27** were found to be virtually identical to those of the pentacyclic nucleus of ptilomycalin A. In addition, biological testing of methyl ester **27** resulted in cytotoxicity towards L1210 murine leukaemia cells, with an IC_{90} value of 2.5 μg/ml and an IC_{50} value of 1.25 μg/ml. The IC_{50} activity is therefore lower than that of ptilomycalin A (see Table 2.1, page 15), requiring a threefold increase in concentration to achieve a similar 50% inhibition of target cells. This reduced activity may be a result of the absence of the 16-hydroxyhexadecanoyl chain and spermidine residue. Interestingly, in identical tests, the equatorial methyl ester **28** possessed a greater degree of biological activity than **27**, exhibiting IC_{90} and IC_{50} values of 1.25 μg/ml and 0.5 μg/ml, respectively, the IC_{50} value being comparable with that of ptilomycalin A.



***Reagents and Conditions*:** (a) Piperidine, CH_2Cl_2, -78°C to -20°C, 20 h; (b) *O*-methylisourea, *i*-Pr_2EtN, DMSO, 80°C, 1.5 h; (c) NH_3, NH_4OAc, *t*-BuOH, 60°C, 40 h; (d) 3:7 HF/CH_3CN, -30°C, 3 d; (e) Et_3N, MeOH, 60°C, 20 h, [1.3:1/**27**:**28**]; (f) Et_3N, 1:1 H_2O/MeOH, 60°C, 16 h.

Scheme 2.2 Snider's preparation of the pentacyclic nucleus of ptilomycalin A.

In 1993, Overman and Rabinowitz reported that an intramolecular variant of the Bignelli condensation could be utilised for the preparation of an advanced tricyclic intermediate **29** of ptilomycalin A, comprising three of the five rings and five of the seven stereogenic centres[35] (Scheme 2.3). Condensation of (*R*)-urea **30** and (*R*)-β-keto ester **31** was accomplished by heating a mixture of the reagents in a sealed tube at 70 °C yielding the readily separable Bignelli products **32** and **33** in a 5:1 ratio. Silyl ether deprotection of the major isomer **32** with TBAF provided bicyclic alcohol **34**, in a 95% yield which was subsequently converted to spirotricyclic intermediate **35** in quantitative yield, *via* a cyclisation reaction catalysed by *p*-TsOH in chloroform. A coupling constant of 11.5 Hz for the H-4 methine hydrogen in the NMR spectrum of **34** indicated that it had formed as the undesired β-carbomethoxy stereoisomer, which is thus epimeric with ptilomycalin A at this site. Epimerisation to the α-stereoisomer **29** was achieved by heating a methanolic solution of **35** at 60 °C in the presence of *p*-TsOH, yielding a separable 1:2 mixture of tricycles **35** and **29**.

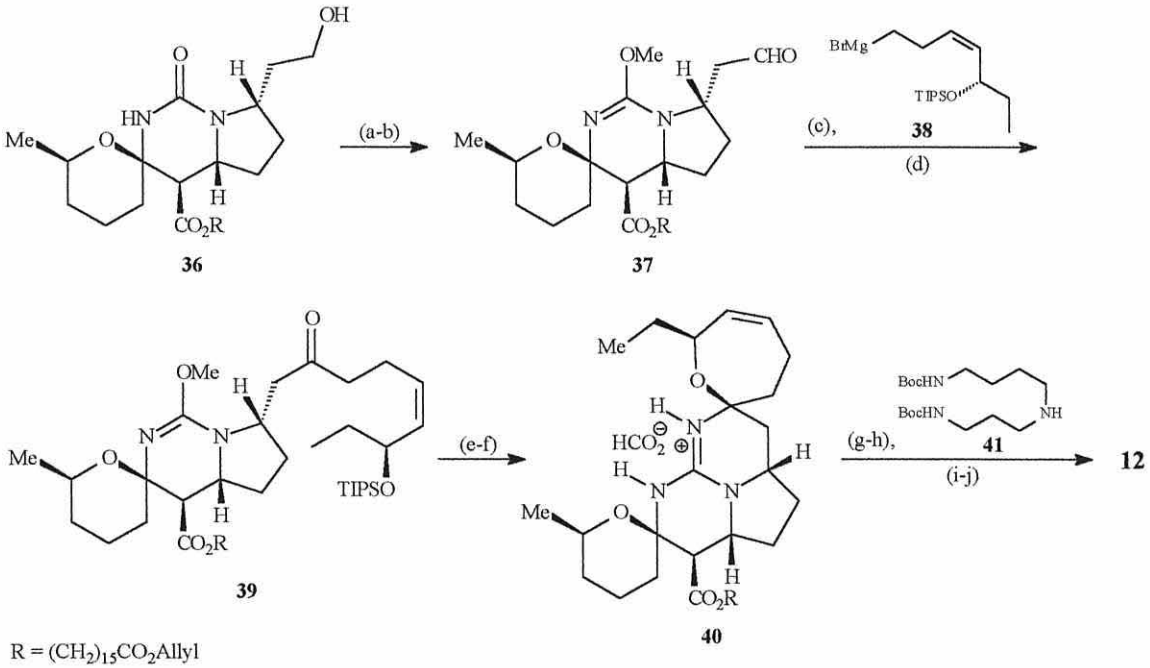


***Reagents and Conditions*:** (a) Morpholine, AcOH, CH_2Cl_2, 70 °C, [5:1/**32**:**33**]; (b) TBAF, 23 °C, 20 h; (c) *p*-TsOH, $CHCl_3$, 23 °C; (d) *p*-TsOH, MeOH, 60 °C, [1:2/**35**:**29**].

Scheme 2.3 Overman's synthesis of an advanced tricyclic intermediate of ptilomycalin A.

Further studies by Overman and co-workers[32] have led to the enantioselective total synthesis of (-)-ptilomycalin A *via* a ten step procedure starting from previously prepared alkylated tricycle **36** (Scheme 2.4). Swern oxidation of **36** followed by protection and activation of the urea moiety in preparation for guanidine formation by *O*-methylation, gave aldehyde **37**. Reaction of **37** with two equivalents of Grignard reagent **38**, followed by a second oxidation, again under Swern conditions, afforded ketone **39**. Removal of the TIPS protecting group and ammonolysis of the resulting keto alcohol, using a solution of ammonia and ammonium acetate in *tert*-butanol at 60°C in a sealed tube, gave the pentacyclic guanidinium core **40** in 51% yield, as a single diastereomer.

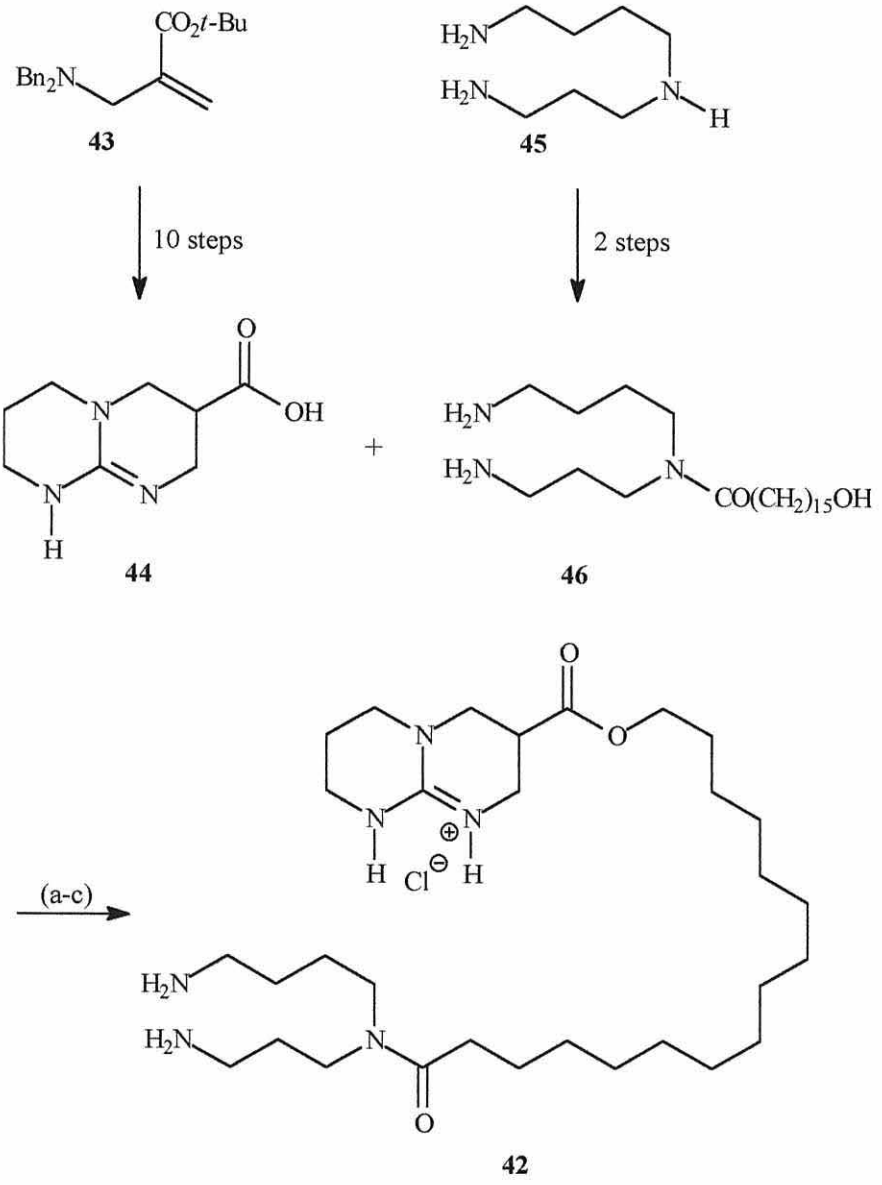
Cleavage of the allyl ester was followed by coupling to *bis*-BOC protected spermidine **41**. Subsequent epimerisation of the ester functionality, by heating in methanol in the presence of ten equivalents of triethylamine, followed by purification, gave the desired α-ester in 50% yield. Finally, removal of the BOC protecting groups with formic acid completed the convergent synthesis of (-)-ptilomycalin A. On comparison, the ^{1}H and ^{13}C spectra of synthetic **12** were identical to those of an authentic sample of ptilomycalin A. Similarly, a comparison by TLC analysis also gave consistent retention factors.



***Reagents and Conditions*:** (a) Swern oxidation; (b) MeOTf, R_3N, 23°C; (c) **38**, -78°C; (d) Swern oxidation; (e) TBAF; (f) NH_3, NH_4OAc; (g) $Pd(PPh_3)_4$, pyrrolidine, MeCN, 23°C; (h) **41**, EDCl, DMAP, CH_2Cl_2, 23°C; (i) Et_3N, MeOH, 65°C; (j) HCO_2H, 23°C.

Scheme 2.4 Overman's enantioselective total synthesis of (-)-ptilomycalin A.

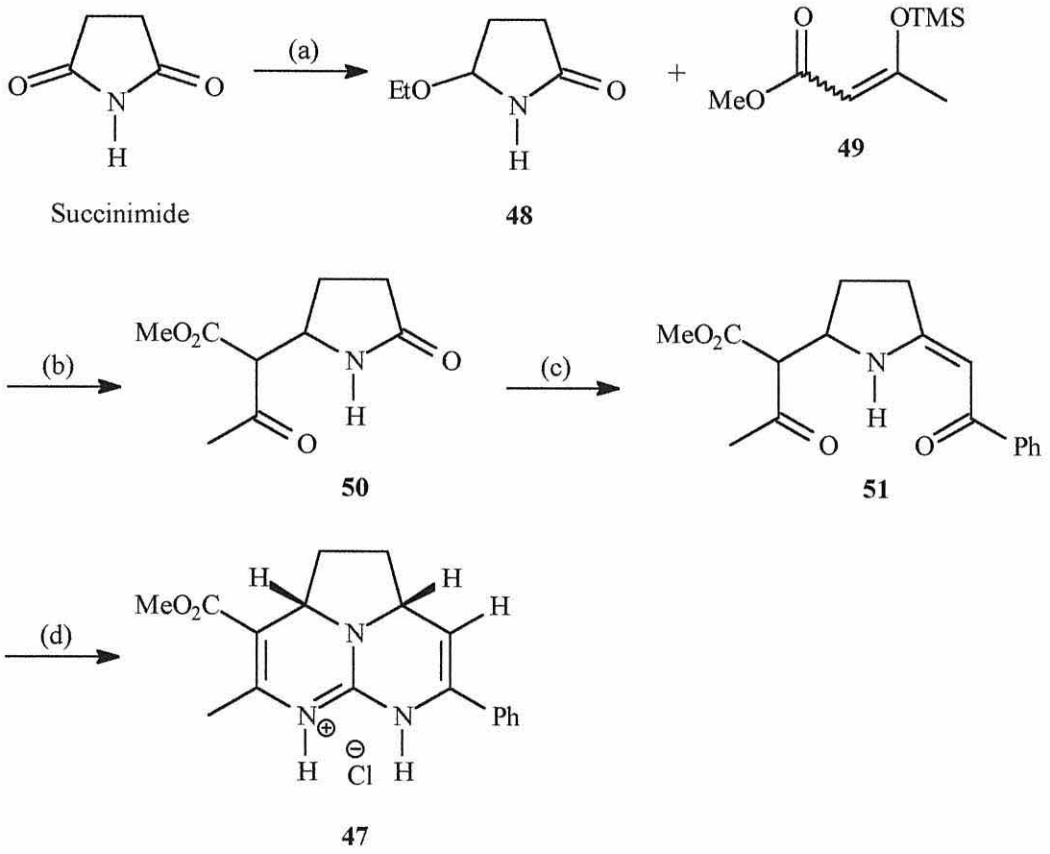
Hart and Grillot[36] have reported the synthesis of a structural analogue **42** of ptilomycalin A, which was constructed from a bicyclic guanidine attached *via* an ester linkage to the amido alcohol portion of **12**. The analogue itself was prepared starting from acrylate **43** which was initially converted to bicyclic guanidinium salt **44** *via* a ten step procedure. This was then coupled to alcohol **46** (prepared in two steps from spermidine **45**) yielding the ptilomycalin A analogue, **42**. In total, thirteen steps were required from acrylate **43**, to complete a linear sequence that proceeds in 6% overall yield (Scheme 2.5).



***Reagents and Conditions*:** (a) DCC, DMAP, DMF; (b) $Pd(OH)_2$, 1,4-cyclohexadiene, EtOH; (c) HCl, MeOH.

Scheme 2.5 The preparation of a structural analogue of ptilomycalin A.

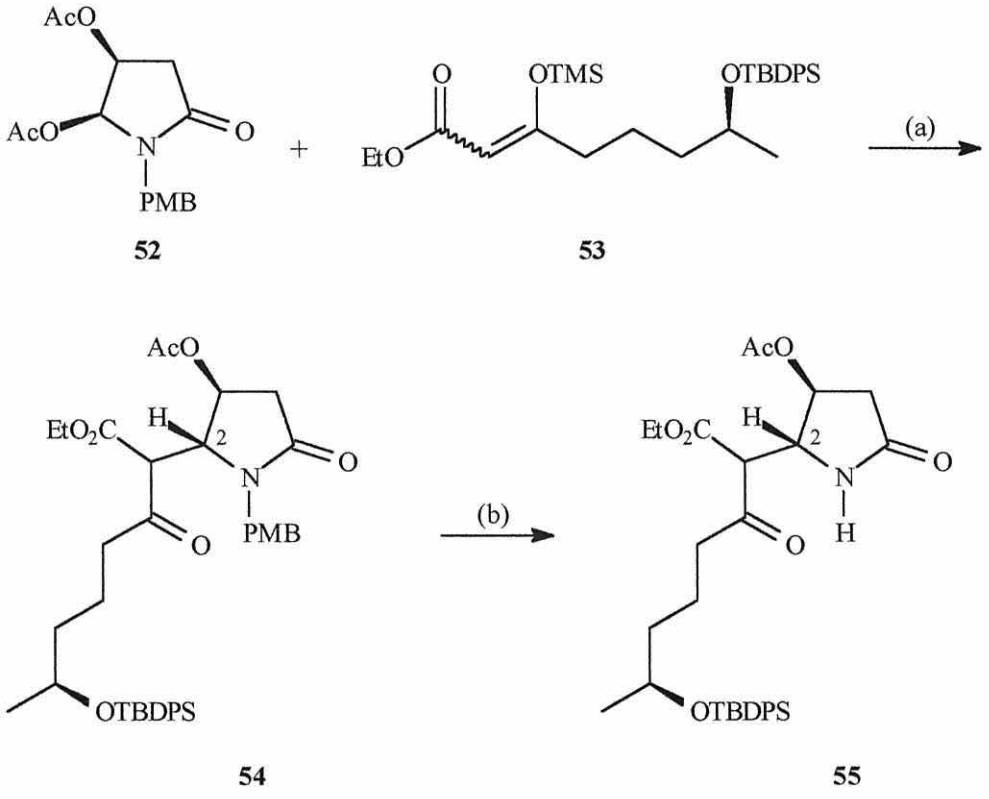
During research towards the total synthesis of ptilomycalin A, Hiemstra *et al.* [37] have investigated the synthesis of a tricyclic guanidinium model compound **47**. Their approach involved construction of the three ring system around ethoxylactam **48** readily prepared from succinimide (Scheme 2.6). The key step involves an *N*-acylinium ion coupling reaction between **48** and commercially available 3-trimethylsiloxy-2-butenoate **49** to give β-keto ester **50**, followed by an Eschenmoser coupling reaction with 2-bromoacetophenone to give the 2,5-disubstituted pyrrolidine **51**. Finally, direct guanylation with *N,N'-bis*-(*tert*-butoxycarbonyl) thiourea in the presence of mercury (II) chloride, followed by double cyclisation and dehydration on treatment with methanolic HCl, gave the tricycle as a mixture of free guanidine hydrochloride salts which were purified by column chromatography to yield the desired tricycle **47**.



***Reagents and Conditions*:** (a) $NaBH_4$, $H^{\oplus}$, EtOH; (b) TMSOTf, CH_2Cl_2, -78°C; (c) (i) Lawesson's reagent, PhMe, 80°C; (ii) 2-bromoacetophenone, Et_2O, rt; (iii) Et_3N, CH_2Cl_2, rt; (iv) PPh_3, $CHCl_3$, 60°C; (v) $NaBH_3CN$, 3:1 AcOH/THF, 0°C; (d) (i) Boc_2O, DIPEA, THF, rt; (ii) PCC, CH_2Cl_2, rt; (iii) $CH(OMe)_3$, H_2SO_4, MeOH, 50°C; (iv) *bis*-Boc-thiourea, $HgCl_2$, Et_3N, DMF, 0°C; (v) HCl, MeOH, rt.

Scheme 2.6 Hiemstra's guanidine-containing tricyclic model of ptilomycalin A.

Hiemstra[38] has further reported that enantiomerically pure *bis*-acetoxylactam **52**, derived from (*S*)-malic acid undergoes a stereoselective *N*-acylinium coupling reaction with silyl enol ether **53** to give C-2 substituted lactam **54**. Removal of the *para*-methoxybenzyl protecting group yields lactam **55**, which the author proposes as a plausible intermediate in a projected synthesis of ptilomycalin A (Scheme 2.7). A major drawback to this proposed scheme is that naturally occurring (*S*)-malic acid will result in the unnatural enantiomer of ptilomycalin A, a fact noted by Hiemstra.



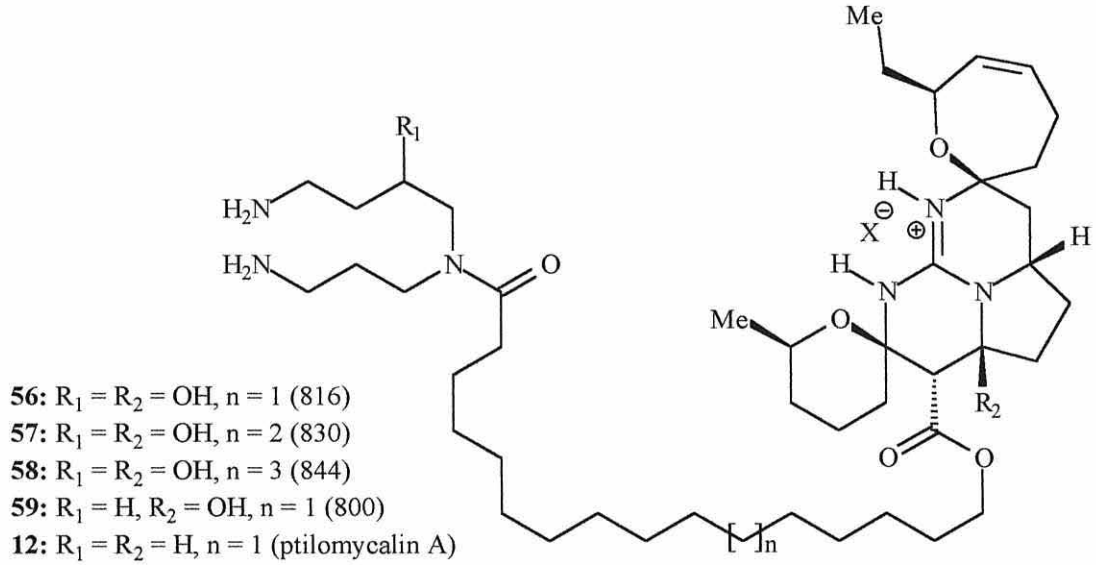
52 **53** **54** **55**

***Reagents and Conditions*:** (a) TMSOTf, DIPEA, CH_2Cl_2, -78°C to rt, 1 h; (b) 3 eqv. CAN, MeCN, H_2O.

Scheme 2.7 Hiemstra's preparation of a C-2 substituted lactam, a plausible intermediate in an anticipated synthesis of ptilomycalin A.

2.2.3 CRAMBESCIDINS

In 1991, Rinehart *et al.* reported the isolation of a family of complex pentacyclic guanidines linked by a linear ω-hydroxy fatty acid to a hydroxyspermidine unit.[39] These novel compounds named crambescidins 816 (**56**), 830 (**57**), 844 (**58**) and 800 (**59**) were extracted from the Mediterranean red encrusting sponge, *Crambe crambe*. Their structures are almost identical to ptilomycalin A **12**, having the same pentacyclic guanidine core but differing in the length of the ω-hydroxy fatty acid chain and in the nature of groups R_1 and R_2 (Figure 2.5).



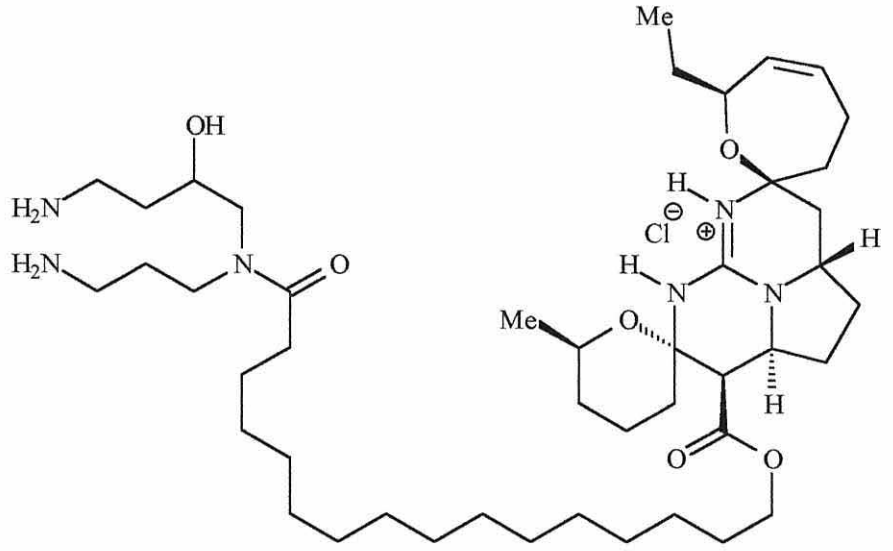
56: $R_1 = R_2 = OH$, n = 1 (816)
57: $R_1 = R_2 = OH$, n = 2 (830)
58: $R_1 = R_2 = OH$, n = 3 (844)
59: $R_1 = H$, $R_2 = OH$, n = 1 (800)
12: $R_1 = R_2 = H$, n = 1 (ptilomycalin A)

Figure 2.5 The structures of crambescidins 816, 830, 844 and 800.

In biological tests, crambescidins **56**, **57** and **59** were found to inhibit HSV-1 growth completely with diffuse cytotoxicity at 1.25 μg/well and also to be 98% effective against L1210 cell growth at a concentration of 0.1 μg/ml. Further analyses by Braekman and co-workers[40] have shown that crambescidin 816 (**56**) was active towards HCT-16 human colon carcinoma cells (IC_{50} 0.24 μg/ml). In addition, **56** was found to exert a potent Ca^{2+} antagonist effect and also inhibited the acetylcholine induced contraction of guinea pig ileum at very low concentrations.

Continued work by Rinehart[41] led to the isolation of a new crambescidin, named 13,14,15-isocrambescidin 800 (**60**), (Figure 2.6, page 26). Interestingly, this compound

was found to be substantially less cytotoxic to L1210 cancer cells (10% inhibition at 10 μg/ml) than the other crambescidins and showed no observable activity against HSV-1. Rinehart has suggested that the reason for this loss in bioactivity is due to stereochemical differences in the structure of **60** compared to the other crambescidins. Crambescidins 816 (**56**), 830 (**57**), 844 (**58**) and 800 (**59**) have a similar structure to ptilomycalin A incorporating the enclosed ionic "pocket". In contrast, **60** has an overall *anti* orientation about the two spirocycles and the pyrrolidine ring which results in the breakdown of this ionic "pocket" and consequently, may result in the loss of bioactivity (Figure 2.6).



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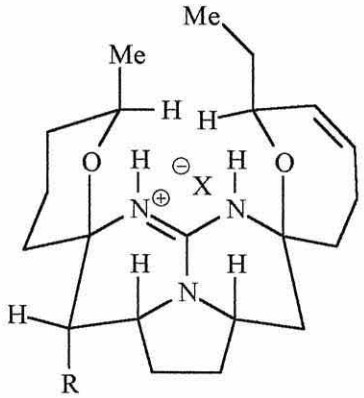
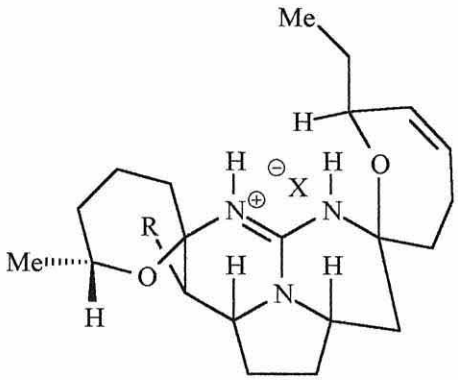


12: R = $C_{24}H_{48}N_3O_3$

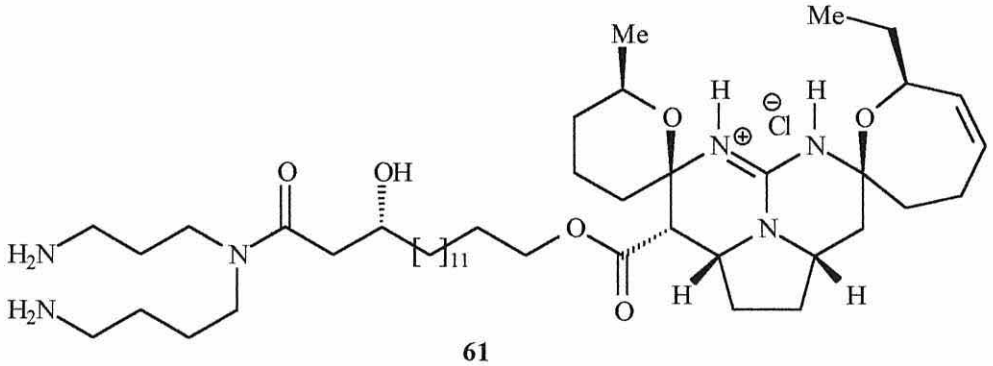


60: R = $C_{24}H_{48}N_3O_4$

Figure 2.6 The structure of 13,14,15-isocrambescidin 800 (**60**) and a comparison of its relative stereochemistry about the central guanidinium unit with that of ptilomycalin A **12**.

2.2.4 CELEROMYCALIN AND FROMIAMYCALIN

A recent publication[29] has reported the isolation of two structurally related pentacyclic guanidine alkaloids from New Caledonian starfish, celeromycalin **61** from *Celerina Heffernani* and fromiamycalin **62** from *Fromia monilis* (Figure 2.7). Ptilomycalin A was also present in *Celerina Heffernani* whereas crambescidin 800 (**59**) was isolated from both species of starfish. Compounds **61** and **62** in anti-HIV assays on cells CEM 4 infected by the HIV-1 virus showed cytotoxic activity with CC-50 values of 0.32 μg/ml and 0.11 μg/ml, respectively without cytoprotective effects at a dose of <0.1 μg/ml.



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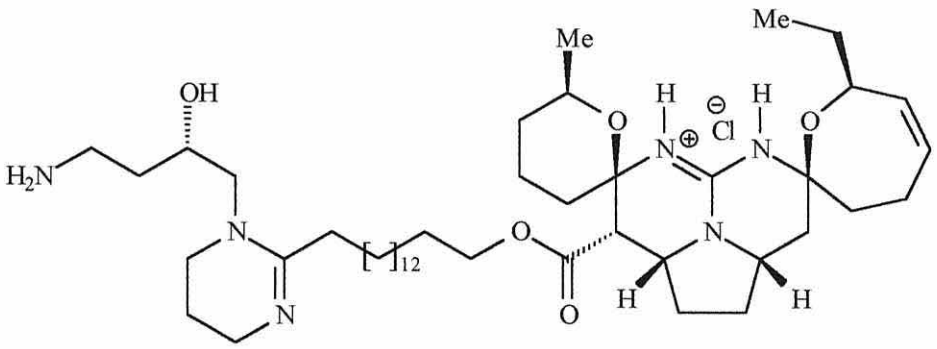
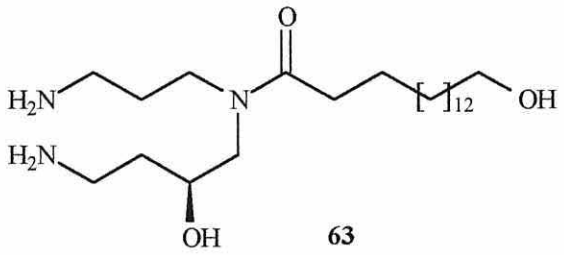


62

Figure 2.7 The structures of the guanidine alkaloids celeromycalin and fromiamycalin.

A less active compound made up of a long chain ω-hydroxyacid linked to a hydroxyspermidine residue **63** was also isolated from *Fromia monilis* (Figure 2.8, page 28). In identical assays **63** exhibited a weaker level of cytotoxicity demonstrating a CC-50 value of 2.7 μg/ml. The bioactivity results for this group of marine alkaloids strongly supports the hypothesis[30] that there is a strong relationship between biological activity and the presence of the central pentacyclic guanidinium portions of these compounds.



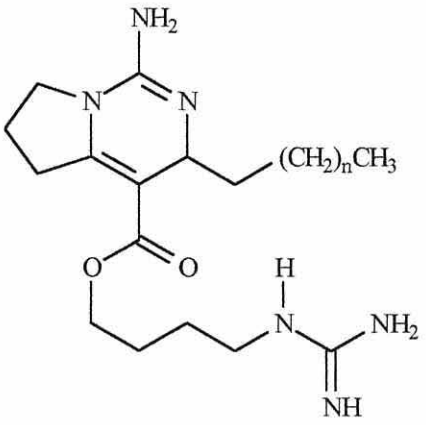
63

Figure 2.8 The modestly active alkyl substituted hydroxyspermidine isolated from *Fromia monilis*.

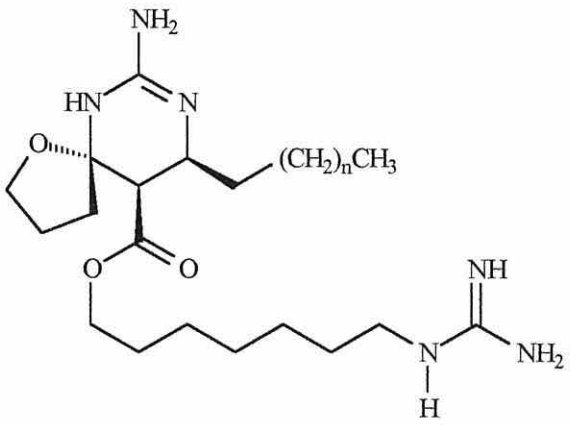
2.2.5 CRAMBESCINS

Two new *bis*-guanidine alkaloids related to ptilomycalin A were isolated by Berlinck and co-workers[42] in 1990, from the Mediterrean sponge *Crambe crambe*. The compounds named crambescin A (**64**) and crambescin B (**65**) were isolated from the organic extracts of the sponge along with homologues that varied in the length of the alkyl side chain. Further work by Berlinck *et al.*[43] resulted in the isolation of two further crambescins, namely crambescin C1 (**66**) accompanied by a series of homologues (n = 8, 10, 11) and crambescin C2 (**67**), which are biogenetically related to crambescins B. Their structures are almost identical, with the exception of **66** and **67** in which the five membered tetrahydrofuran ring has opened to the corresponding alcohol (Figure 2.9, page 29).

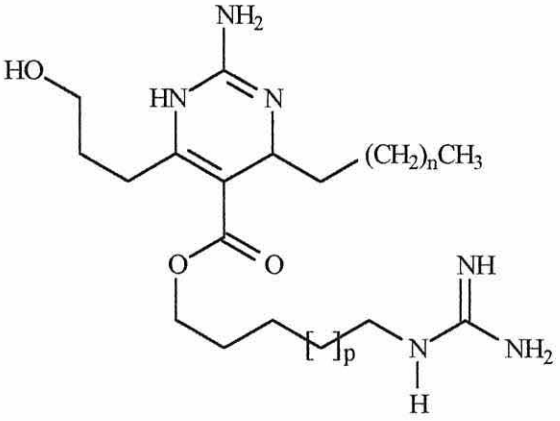
Snider[44] has developed synthetic routes towards all four crambescins [A (**64**): eight steps, 22%; B (**65**): eight steps, 19%; C1 (**66**): seven steps, 21% and C2 (**67**): seven steps, 27%] *via* proposed biomimetic syntheses from methyl acetoacetate. Investigations by Rinehart *et al.*[45] towards the crambescins have led to the unambiguous clarification of their structures as shown in Figure 2.9, page 29. Biological evaluation of crambescins A and B has shown them to be cytotoxic towards L1210 cells with IC_{50} values of less than 1 μg/ml. Synthetic samples of crambescins C1 and C2 were later shown to have comparable bioactivity.



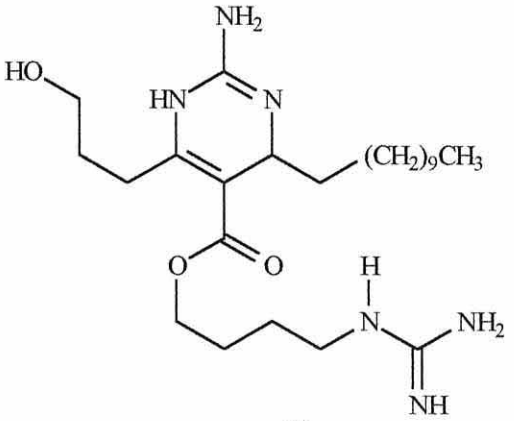
64: n = 8, 9 (major), 10, 11



65: n = 8, 9 (major), 10, 11



66: n = 8, 9 (major), 10, 11; p = 3



67

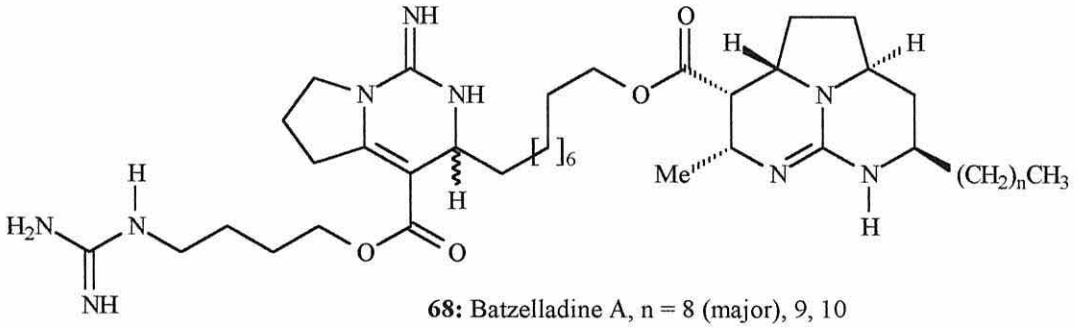
Figure 2.9 The structures of crambescins A (**64**), B (**65**), C1 (**66**) and C2 (**67**) including homologues.

2.2.6 BATZELLADINES

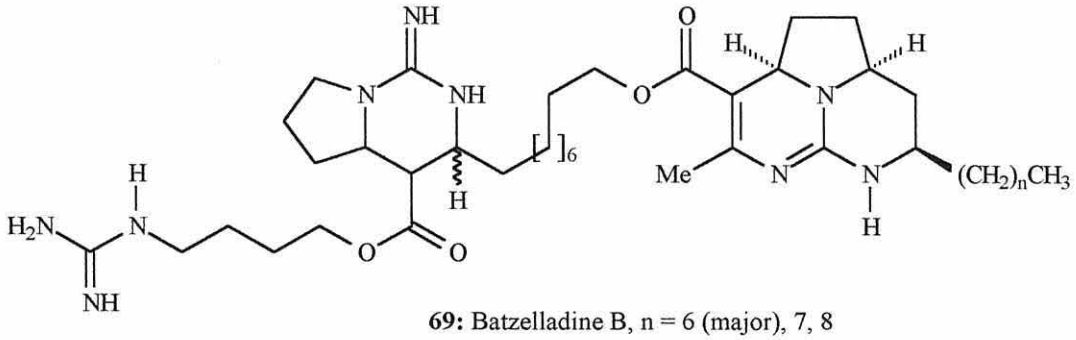
Another group of marine natural products that contain guanidine are batzelladines A-E, which were first isolated by Patil and co-workers.[28] Batzelladines A **68** and B **69** were the first natural products of low molecular weight that were shown to disrupt the AIDS infective process. This occurred through inhibition of the binding of the gp120 domain of the HIV-envelope gp160 glycoprotein to the CD4 receptor on the surface of the human T cell.[46] The batzelladines were isolated from the methanolic extract of the bright red Caribbean sponge *Batzella sp.* (which is apparently the same species earlier identified as *Ptilocaulis spiculifer*) during biochemical screening for natural products having potential

to treat AIDS. Ptilomycalin A **12**, ptilocaulin **9**, crambescin A (**64**), crambescidins 800 (**59**) and 816 (**56**) were also isolated from the same methanolic extract. In biological assays, batzelladines A and B demonstrated IC_{50} values of approximately 30 μM for the association of soluble CD4 to immobilised recombinant gp120, whereas batzelladines C **66** and D **67** showed significantly less activity to the same assay.

Batzelladines A-D, **68-71** were isolated along with higher homologues which varied in the length of the alkyl side chains, whereas batzelladine E **72** was identified as a single homologue (see Figure 2.10 and Figure 2.11, page 31). The structures of the batzelladines were elucidated through extensive analysis by means of NMR, mass spectrometry and chemical degradation, although the structures of batzelladines A and D were later revised to those shown in Figure 2.10 and Figure 2.11, by Snider and co-workers.[47] The structure of batzelladine B **69** has also been amended by Overman *et al.*,[48] to that depicted below, following their asymmetric synthesis of the tricyclic guanidine portion of this metabolite. Interestingly, batzelladines A-E all consist of a central tricyclic guanidine core coupled to a guanidine-containing side chain. Considerable efforts have been made towards the synthesis of the batzelladines, in particular the tricyclic portion.[47-49]

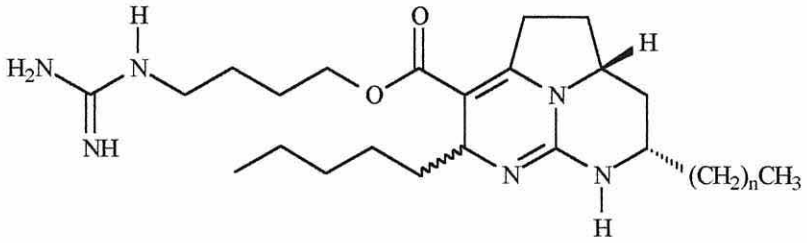


68: Batzelladine A, n = 8 (major), 9, 10

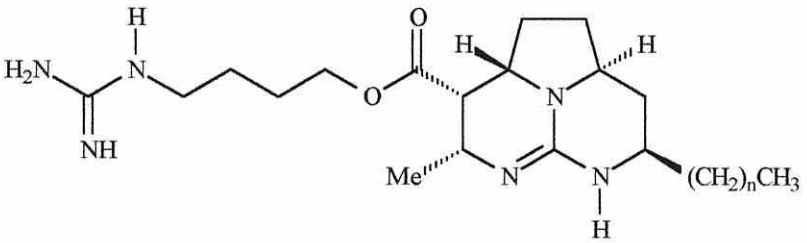


69: Batzelladine B, n = 6 (major), 7, 8

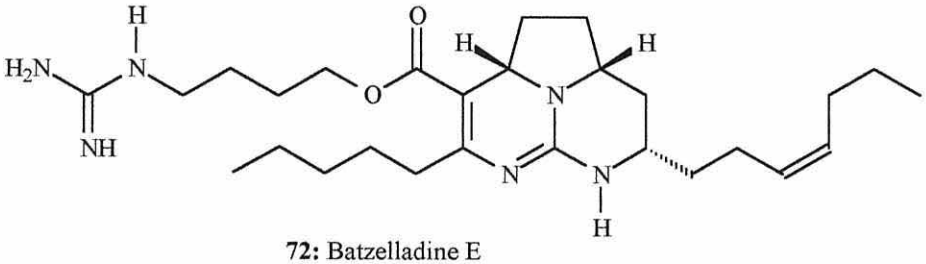
Figure 2.10 The structures of batzelladines A and B.



70: Batzelladine C, n = 6 (major), 7, 8



71: Batzelladine D, n = 8 (major), 9, 10



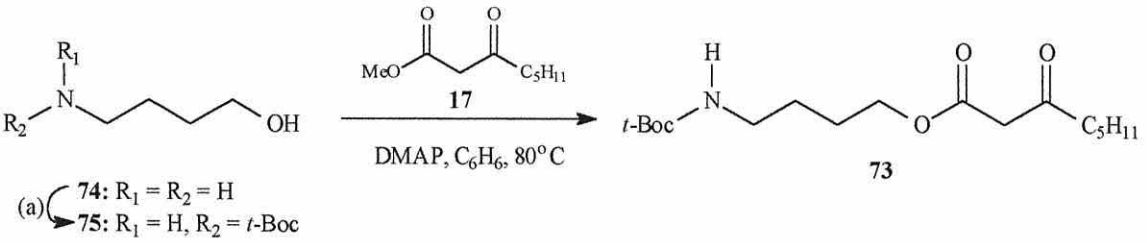
72: Batzelladine E

Figure 2.11 The structures of batzelladines C-E.

The first total synthesis of a batzelladine, namely batzelladine E **72**, albeit in racemic form, was recently reported by Snider and Chen.[50] Their synthesis involves a ten step procedure utilising a similar methodology[31] to that of their preparation of the tricyclic model **20** of ptilomycalin A (see Scheme 2.1, page 17), and resulted in a 3% overall yield of **72**, as the bis-trifluoroacetate salt. They initially synthesised the *trans* isomer of batzelladine E, but found the NMR data in the region of the side chain double bond to be inconsistent with that of the natural product. A modification of their synthesis was thus employed in order to generate the *cis* isomer of **72** which on this occasion gave consistent NMR data for the unsaturated side chain.

They initially synthesised Knoevenagel precursor **73** starting from 4-amino-1-butanol **74** which was reacted with di-*t*-butyl dicarbonate and triethylamine to give

alcohol **75** in 96 % yield. A subsequent DMAP catalysed condensation with methyl-3-oxooctanoate afforded keto ester precursor **73** (Scheme 2.8).

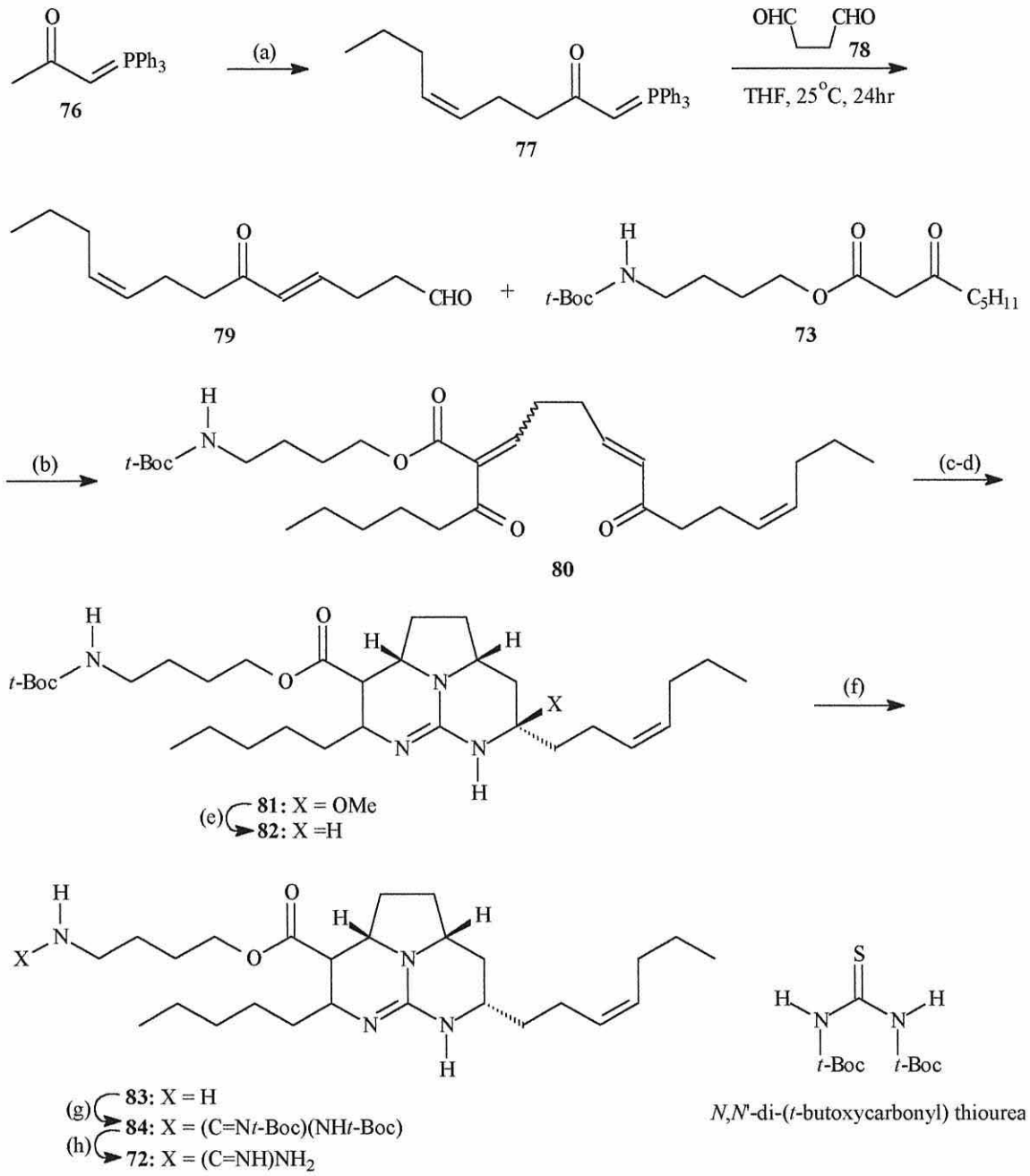


***Reagents and Conditions*:** (a) Di-*t*-butyl dicarbonate, Et_3N.

Scheme 2.8 Preparation of Knoevenagel precursor **73**.

Deprotonation of acetylmethylene triphenylphosphorane **76** with *n*-butyllithium followed by alkylation with 1-bromo-2(*Z*)-hexene gave phosphorane **77** in 64% yield (Scheme 2.9, page 33). Wittig reaction of **77** with the *bis* aldehyde, succinaldehyde **78**, afforded a 65% yield of unsaturated aldehyde **79**. The Knoevenagel condensation between precursors **73** and **79** was accomplished using a basic medium of 0.33 equivalents of piperidine and 0.30 equivalents of acetic acid, yielding *bis*-enone **80**. (This basic reaction medium was found to prevent partial isomerisation of the side chain *cis*-alkene which occured when using piperidine acetate as the reaction catalyst). Treatment of crude **80** with *O*-methylisourea in an analogous manner to the synthesis of the ptilomycalin A models, and subsequent ammonolysis gave cyclised **81** (14% yield from **79**).

The masked aminal function was reduced using sodium cyanoborohydride in NaH_2PO_4-buffered MeOH at 25°C for forty hours, giving tricycle **82**. Removal of the *t*-Boc group was achieved by brief dissolution in 1:4 TFA/CH_2Cl_2 affording **83**. The second guanidine moiety was introduced following Lipton's procedure which involved reaction of **83** with *N,N'*-di-(*t*-butoxycarbonyl) thiourea, 2-chloro-*N*-methylpyridinium iodide and triethylamine in CH_2Cl_2, resulting in a 64% yield of **84**. The synthesis of batzelladine E **72** was completed following removal of the *t*-Boc groups using a mixture of 1:1 TFA/CH_2Cl_2 (Scheme 2.9, page 33).



Reagents and Conditions: (a) *n*-BuLi, THF, -78°C, 1-bromo-2(*Z*)-hexene; (b) 0.33 eqv. piperidine, 0.30 eqv. AcOH, CH_2Cl_2, -20°C, 2 d; (c) *O*-methylisourea, DMSO, *i*-Pr_2EtN, 55°C, 4 h; (d) NH_3, NH_4OAc, *t*-BuOH, 60°C, 24 h; (e) $NaCNBH_3$, NaH_2PO_4, MeOH, 25°C; (f) 1:4 TFA/CH_2Cl_2, 25°C, 5 min; (g) Et_3N, *N,N'*-di-(*t*-butoxycarbonyl) thiourea, 2-chloro-*N*-methylpyridinium iodide, CH_2Cl_2, 25°C, 1 h; (h) 1:1 TFA/CH_2Cl_2, 25°C, 2 h.

Scheme 2.9 Completion of the synthesis of batzelladine E.

Following the isolation of these metabolites, a further four batzelladine alkaloids were isolated from the same source. These compounds, named batzelladines F-I **85-88** were obtained from the methanolic extracts of the sponge following observations during screening of their ability to induce $p56^{lck}$-CD4 dissociation.[51] (Association of $p56^{lck}$ with CD4 has been shown to result in antigenic activation). The structures of these new batzelladines (Figure 2.12) were determined *via* extensive spectroscopic and chemical analysis and through spectral comparisons with batzelladines A-E.

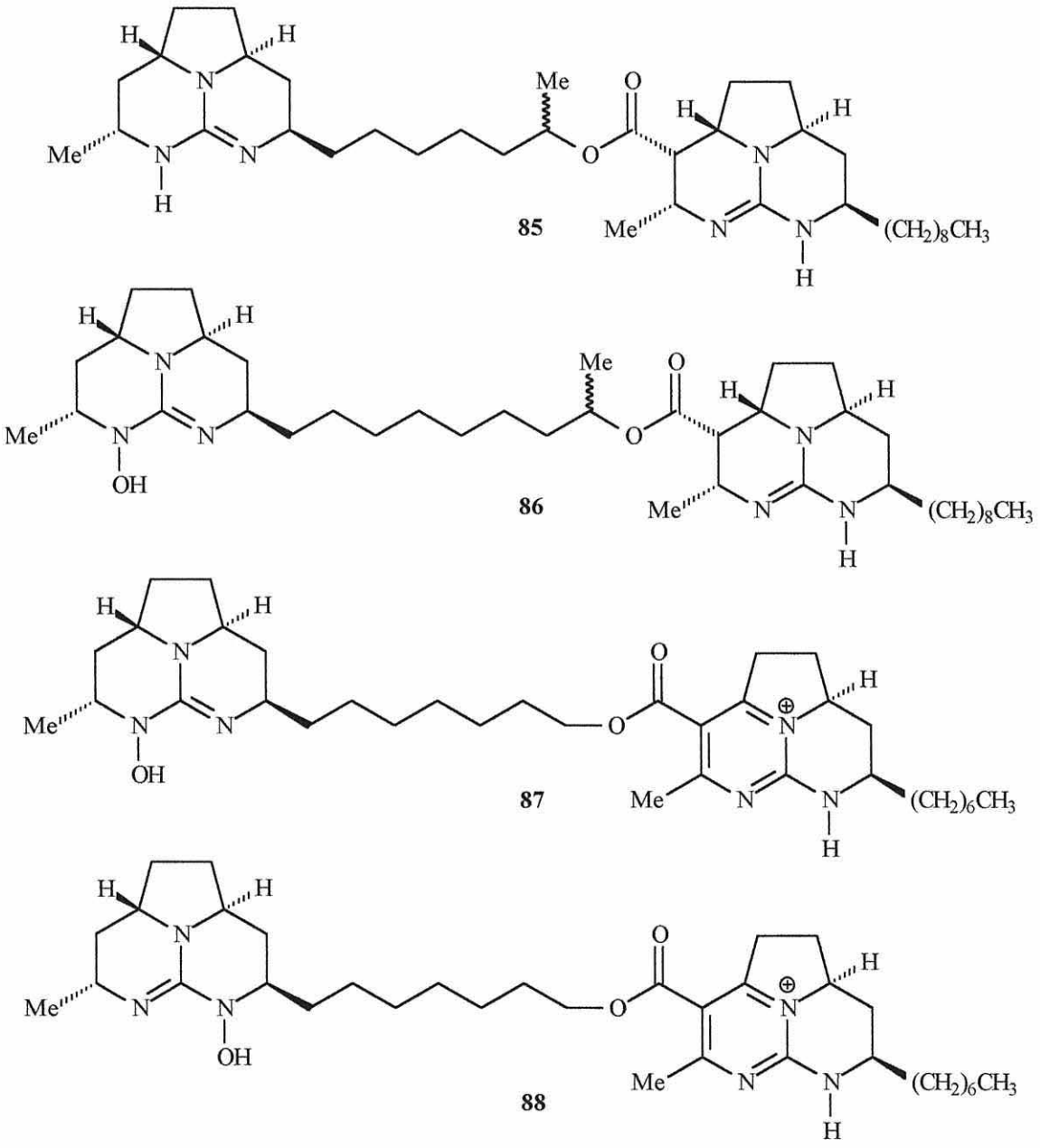


Figure 2.12 The structures of batzelladines F-I.

2.2.7 CYLINDROSPERMOPSIN

Cylindrospermopsin **89**, a potent marine hepatotoxin, was isolated from the cyanobacterium *Cylindrospermopsis raciborskii* by Moore *et al.*[52] and was shown to have been responsible for a severe outbreak of hepatoenteritis in Australia. It has more recently been found in the alga *Umezakia natans*, collected in Lake Mikata, Japan and is believed to exert its toxic effects through inhibition of cell-reduced glutathione.[53] The natural product demonstrates a novel structure containing guanidine embedded in a tricyclic system along with a high degree of functionality[54] (Figure 2.13).

By virtue of its unique structure, **89** represents a significant synthetic challenge. To date synthetic studies by Weinreb and co-workers[54] have led to the construction of a functionalised bicyclic **AB** ring model, **90**. Similarly, Snider and Harvey[55] have undertaken a ten step model study towards the synthesis of a functionalised bicyclic **AC** ring model **91**, having an identical stereochemistry to the **AC** ring system of cylindrospermopsin (Figure 2.13).

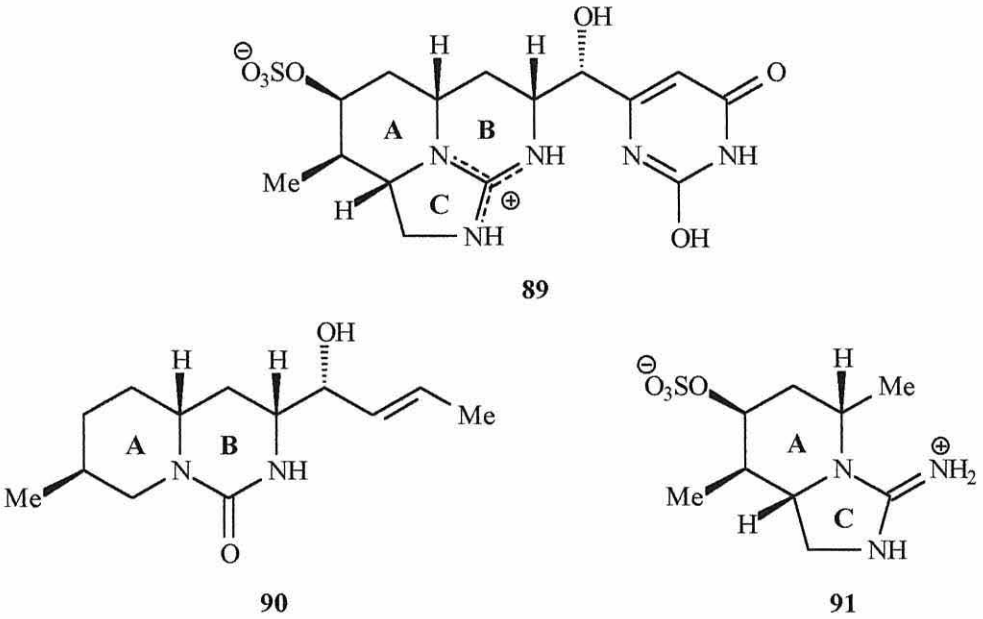
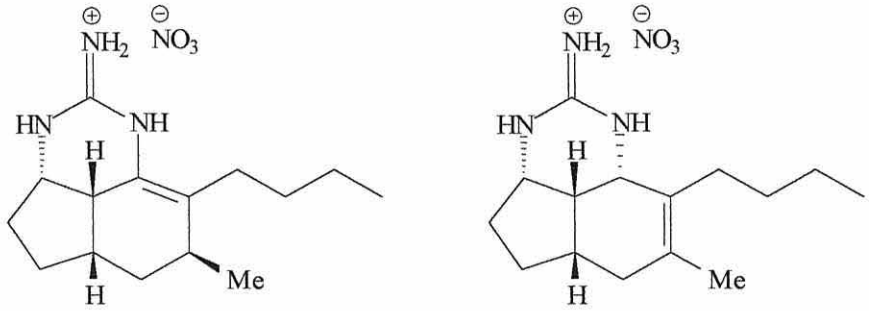


Figure 2.13 The structure of cylindrospermopsin **89** and the two synthetic bicyclic model compounds **90** and **91**.

2.2.8 PTILOCAULIN AND ISOPTILOCAULIN

The isolation of two novel isomeric, antimicrobial and cytotoxic tricyclic guanidine-containing marine natural products, ptilocaulin **9** and isoptilocaulin **92**, as their nitrate salts from the Caribbean sponge *Ptilocaulis aff. P. spiculifer* (Lamarck 1814), was reported by Rinehart *et al.*[24] in 1981 (Figure 2.14). Both compounds contain a tricyclic ring system where guanidine is fused to a substituted perhydroindene nucleus.[56] The absolute stereochemistry of ptilocaulin **9** and isoptilocaulin **92** was not reported during the initial structure determination. Ptilocaulin is the more bioactive of the two isomers showing an ID_{50} value of 0.39 μg/ml against L1210 leukaemia cells along with the following antimicrobial minimum inhibitory concentrations values: *Streptococcus pyrogenes*, 3.9 μg/ml; *S. pneumoniae*, 15.6 μg/ml; *S. faecalis*, *Staphylococcus aureus* and *Escherichia coli*, all 62.5 μg/ml.

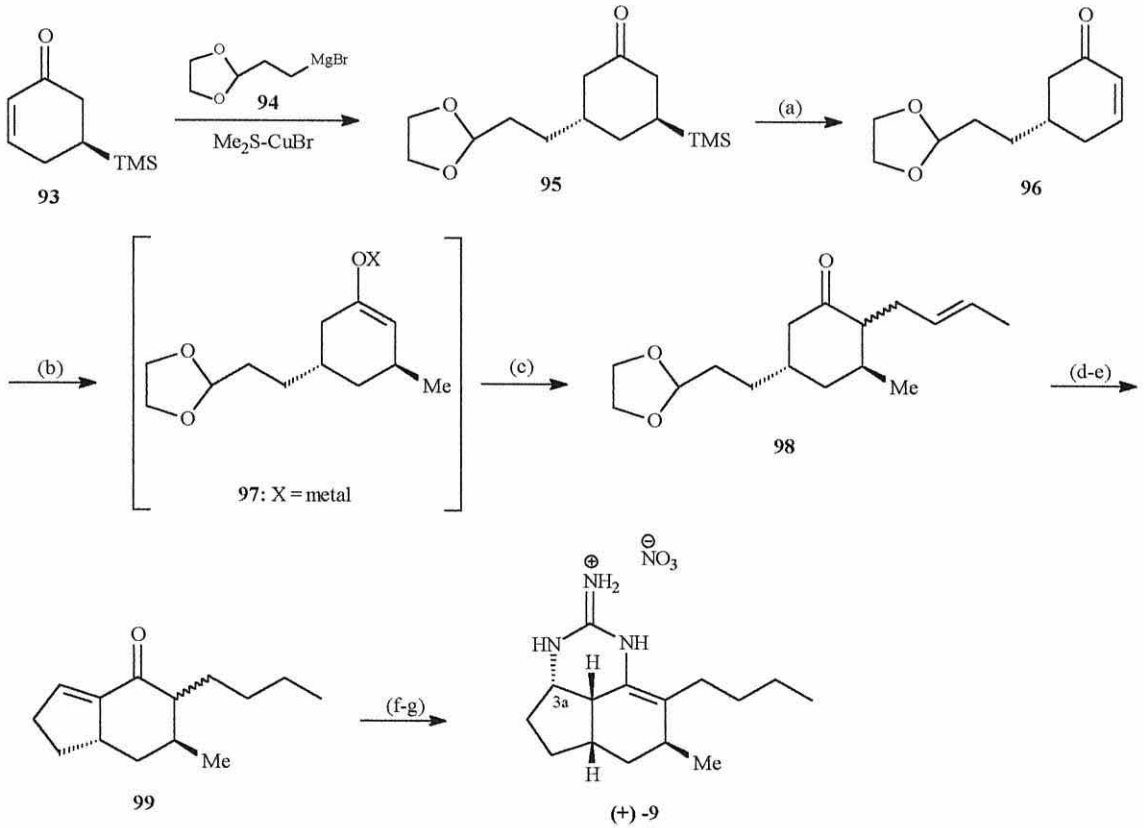


9 **92**

Figure 2.14 The isomeric marine natural products, ptilocaulin **9** and isoptilocaulin **92**.

The literature contains numerous accounts of synthetic efforts towards ptilocaulin. Snider and Faith were successful in preparing (±)-ptilocaulin in 1983[57] and optically active (-)- ptilocaulin in 1984[58] by analogous five step procedures. (-)-Ptilocaulin was found to have identical chemical properties to that of the natural product in all respects apart from the $[\alpha]_D$ measurement and the CD spectrum which were of comparable magnitude but of opposite sign, thereby establishing the absolute stereochemistry as (+)-ptilocaulin. Roush and Walts[59] have also reported the successful synthesis of the unnatural isomer of **9**.

The first synthesis of (+)-ptilocaulin, *via* a diastereocontrolled route was published by the Japanese group of Asaoka, Sakurai and Takei[60] in 1990 (Scheme 2.10). A 1,4-addition of the Grignard reagent, **94** (prepared from 3-bromo-propanal ethylene acetal) to (*S*)-(+)-5-trimethylsilyl-2-cyclohexanone, **93**, gave adduct **95** as a single diastereomer. Elimination of the trimethylsilyl group gave enone **96** which underwent a diastereoselective 1,4-addition of dimethyl cuprate to afford, after alkylation of the intermediate enolate **97** with crotyl bromide, alkene **98** in 80% yield. Hydrogenation of the double bond followed by treatment with 2M aqueous HCl/THF gave bicyclic enone **99** as a mixture of two diastereomers. On reaction of this mixture with guanidine in refluxing benzene for 24 hours (with removal of water) and subsequent treatment with dilute nitric acid, a mixture of (+)-**9** and its C-3a epimer, was obtained in 35-42% yield. Careful purification by column chromatography, followed by recrystallisation, gave pure synthetic (+)-ptilocaulin.



Reagents and Conditions : (a) $CuCl_2$, DMF, 55-60°C, 1 h; (b) Me_2CuLi, Et_2O, -5°C, 1 h; (c) Crotyl bromide, Et_2O/THF/HMPA (4:1:1), -5°C, 1.5 h; (d) H_2, Pd/C; (e) 2M aq. HCl/THF; (f) Guanidine, C_6H_6, reflux, 24 h; (g) Dilute HNO_3.

Scheme 2.10 A diastereocontrolled synthesis of (+)-ptilocaulin.

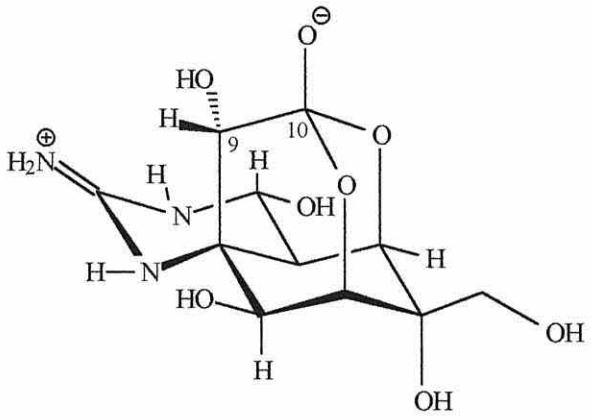
2.2.9 TETRODOTOXIN AND SAXITOXIN

Probably the most notable of marine natural products is tetrodotoxin (TTX) **100**, a highly potent neurotoxin first isolated in crystalline form by Yokoo in 1950.[61] It is found in the ovaries and livers of many species of *Tetraodontidae sp.*, the puffer fish,[62] especially the tiger puffer (*tora fugu*) and the closely related common puffer (*ma fugu*), and if ingested, usually results in fatal food poisoning in humans. Tetrodotoxin functions through selective binding with high affinity to voltage-dependent sodium channels on neuro cell membranes[62] and has been the subject of extensive chemical and pharmacological investigations.[63]

An account of the symptoms of puffer fish poisoning was recorded as early as 1774 by Captain James Cook in his journals describing his second voyage around the world on the 'Resolution'.[64] He recounts after he and Mr. Forster (the ships' naturalist) had tasted a small amount of the liver and roe of the fish; "About three to four o'clock in the morning we were seized with most extraordinary weakness in all our limbs attended with numbness and sensation like to that caused by exposing one's hands and feet to a fire having been pinched by much frost. I had almost lost the sense of feeling nor could I distinguish between light and heavy objects, a quart pot full of water and a feather was the same in my hand. We each took a vomit and after that a sweat which gave great relief. In the morning one of the pigs which had eaten the entrails was found dead."

Although TTX was initially recognised as the puffer fish toxin, Mosher *et al.*[64] have proposed that the toxin originally known as tarachatoxin, found in the skin and ova of the Western American newts of the genus *Taricha*, is actually TTX. The fact that TTX has been found in distantly related organisms has led to speculation that a symbiont such as a bacterium might be the source of the toxin.[63]

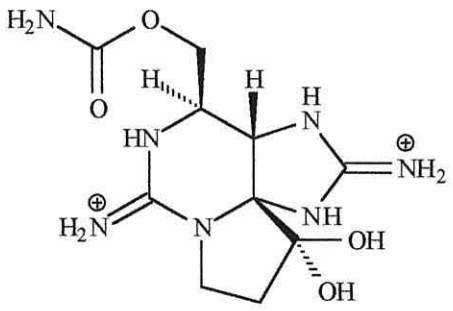
In 1964, two Japanese groups and an American group simultaneously elucidated the structure of tetrodotoxin,[65,66,67] but it was not until 1972 that the total synthesis of the racemic form of TTX was first accomplished, by Kishi and co-workers.[68] The structure itself comprises a terminal guanidine unit and a unique hemilactal function (Figure 2.15, page 39). It is thought that the guanidine moiety forms an ion pair with an anionic site on the receptor, whilst the C9-OH and C10-$O^{\ominus}$ form hydrogen bonds with other sites.[69]



100

Figure 2.15 The puffer fish neurotoxin, tetrodotoxin.

Similarly, saxitoxin (STX) **101** is an extremely toxic non-protein poison found in the Alaska butter clam, *Saxidomas giganteus*[70] and the bivalve, *Mytilius californianus.*[71] The actual source of STX is a toxic single-cell dinoflagellate, *Gonyaulax catanella* whose toxic principle is concentrated within the organs of the shellfish during feeding.[72] The toxin acts in a similar manner to TTX, selectively blocking specific sodium-channels. It was first isolated by Schantz and co-workers[73] in 1957 and the structure elucidated eighteen years later, in 1975 using X-ray crystallography[71] (Figure 2.16). Saxitoxin is a unique tricyclic, *bis*-guanidine structure which has found widespread use in the study of nerve disorders.[70] It has been successfully synthesised in enantiomeric form by Kishi[74] in 1977 and Jacobi[70] in 1984. Both TTX and STX have been responsible for fatal food poisoning in humans and are two of the most toxic non-proteinogenic poisons known, the minimum lethal dose of each for mice being approximately 8 μg/kg.



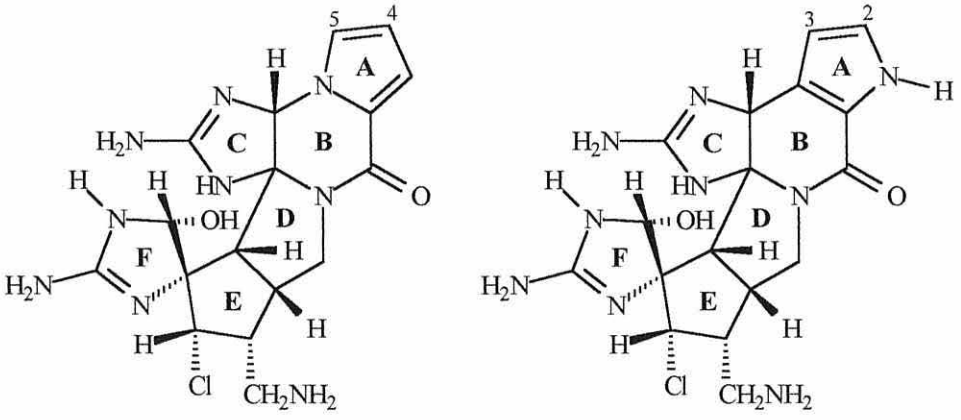
101

Figure 2.16 Saxitoxin, a lethal, non-proteinogenic poison.

2.2.10 PALAU'AMINE AND STYLOGUANIDINE

Palau'amine **102** is another example of a marine natural product containing the guanidine moiety. It constitutes a complex, hexacyclic, *bis*-guanidine structure with an unbroken chain of eight chiral centres and was isolated from the sponge *Stylotella agiminata* collected off the Western Caroline Islands.[75] The structure, as shown in Figure 2.17, was elucidated using NMR and mass spectrometry. It is a reasonably non-toxic compound [LD_{50} 13 mg/kg (i.p. mice)] but is relatively active against P-388 and A549 cancer cells. It also possesses antibiotic (*Staphylococcus aureus* and *Bacillus subtilis*) and antifungal (*Penicillium notatum*) activities. Further biological testing has shown it to possess impressive immunomodulatory activity.[75]

More recently **102** was extracted from the sponge, *Stylotella aurantium*, collected in the Yap Sea, along with the **A** ring regioisomer, styloguanidine **103** (isopalau'amine), itself a potent chitinase inhibitor. The less active 4-bromo and 4,5-dibromo derivatives of **102** as well as the 2-bromo and 2,3-dibromo derivatives of **103** have also been reported and fully characterised.[76,77] On a synthetic front, Overman has published a model synthesis of the tetracyclic core (rings **C-F**) of both regioisomers.[78]



102 **103**

Figure 2.17 The structures of the hexacyclic guanidine-containing marine alkaloids, palau'amine and styloguanidine.

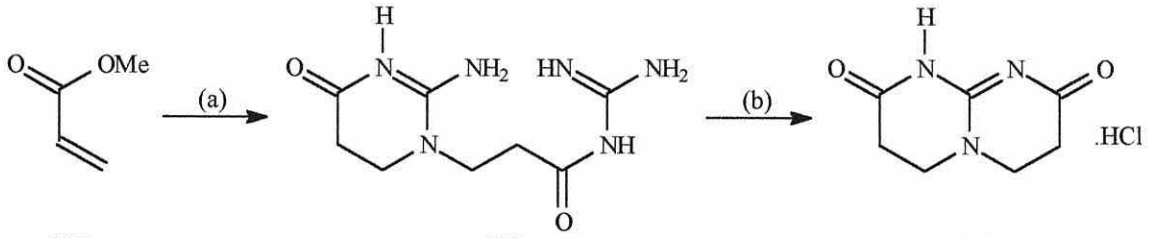
For a comprehensive account of natural guanidine derivatives the reader is referred to an excellent review by Roberto Berlinck.[46]

CHAPTER 3

AN OVERVIEW OF BIOMIMETIC STUDIES TOWARDS PTILOMYCALIN A; A SYNTHETIC STRATEGY

3.1 INTRODUCTION

In 1967, Sugino and Tanaka,[79] demonstrated the addition of guanidine to various alkyl acrylates. In one such example, a solution of guanidine in DMF was reacted with methyl acrylate **104** *via* a double Michael addition reaction to give *bis*-guanidine **105**. A subsequent 1,2-cyclisation then gave the bicyclic guanidine compound, **106** in a very respectable 82% yield (Scheme 3.1).



104 **105** **106**

***Reagents and Conditions*:** (a) Guanidine, DMF; (b) 6M HCl.

Scheme 3.1 An example of the double Michael addition of guanidine to methyl acrylate.

Murphy and Williams[80] proposed a retrosynthesis of the central pentacyclic core of ptilomycalin A **12** which illustrated the potential of a double Michael addition of guanidine **1** to *bis*-α,β-unsaturated ketone **107** (Figure 3.1, page 43). The addition of guanidine is presumed to occur *via* a biomimetic pathway, yielding the central pyrrolidine ring of **12**, with subsequent *bis*-spirocyclisation generating the desired pentacycle. The ingenuity of this strategy lies in the fact that a one step reaction results in the formation of the five heterocyclic ring systems and five of the seven chiral centres comprising the target compound.

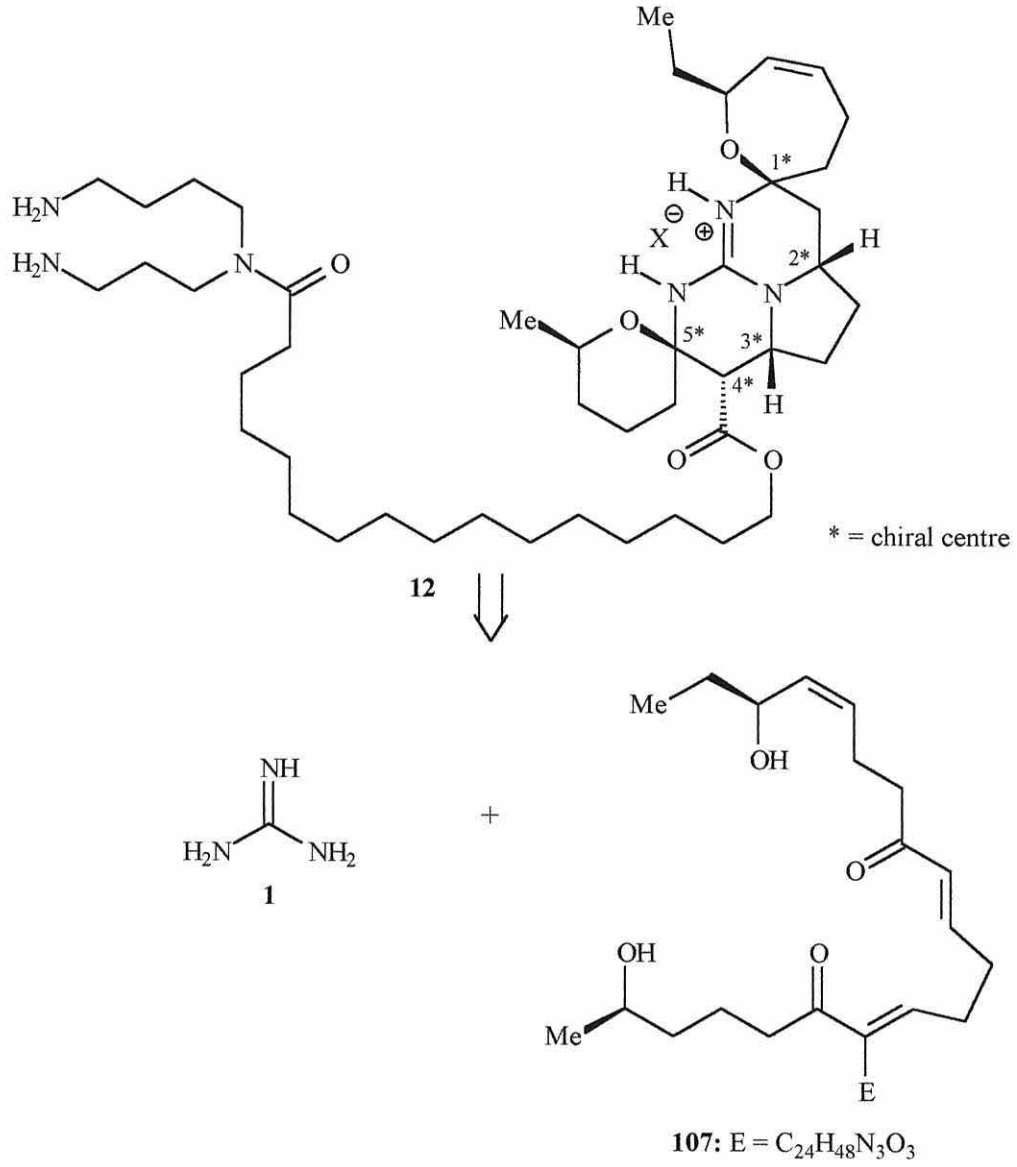


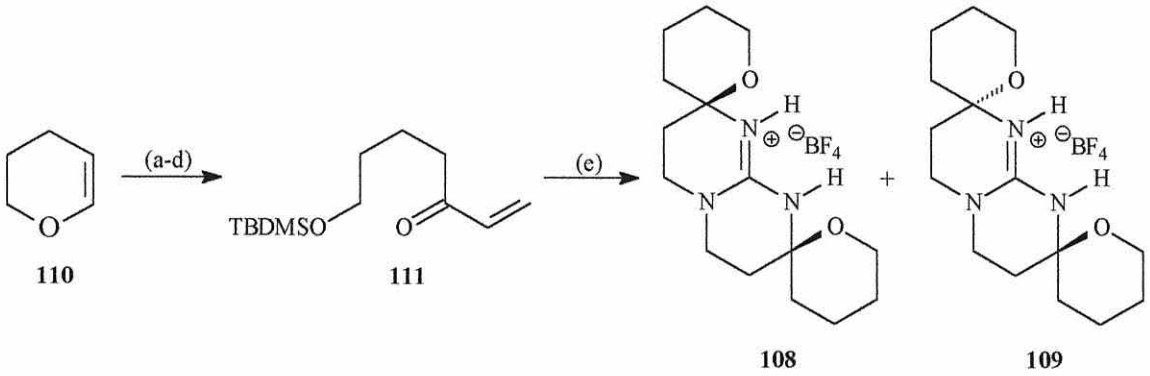
Figure 3.1 Retrosynthesis of ptilomycalin A illustrating the potential of the double Michael addition of guanidine.

3.2 Biomimetic Model Studies Towards Ptilomycalin A

To test the feasibility of the proposed addition of guanidine to a double Michael acceptor, Murphy and Williams[80] have prepared a series of polycyclic model compounds utilising the Michael addition reaction as the key synthetic step. The syntheses of these model compounds are outlined in Schemes 3.2 to 3.5.

3.2.1 PREPARATION OF A TETRACYCLIC MODEL

Tetracyclic model compounds **108** and **109** were accessed *via* a relatively straightforward methodology starting from 2,3-dihydropyran **110**. A four step procedure gave vinyl ketone **111** which was reacted with half an equivalent of guanidine in DMF over a period of four hours. Removal of the solvent followed by deprotection of the silyl ethers, cyclisation with methanolic HCl and finally, counter ion exchange, gave a 1:1 mixture of tetracycles **108** and **109** in an 80% yield (Scheme 3.2). The *syn* product **108** was separated from the mixture by crystallisation and the structures of both tetracycles were determined by means of X-ray crystallography.



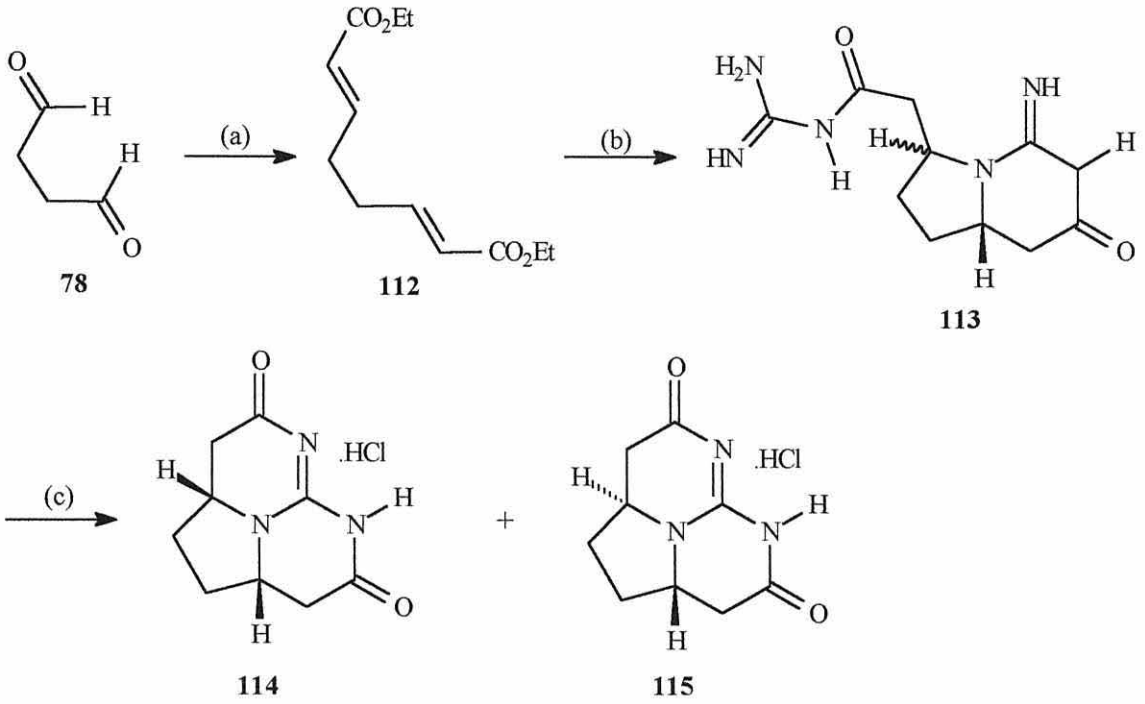
110 **111** **108** **109**

***Reagents and Conditions*:** (a) 0.1 M HCl, 5 min; (b) CH_2=CHMgBr, THF; (c) TBDMSCl, Imidazole, DMF; (d) PCC, CH_2Cl_2, celite; (e) (i) Guanidine, DMF, 4 h; (ii) MeOH, HCl, 0 °C; (iii) Saturated aq. $NaBH_4$.

Scheme 3.2 Preparation of a tetracyclic model compound.

3.2.2 PREPARATION OF A TRICYCLIC MODEL

Using similar methodology, they have also prepared a tricyclic model of the three central pyrrolidine rings of ptilomycalin A. A Wittig reaction between succinaldehyde **78** and excess carboethoxymethylene triphenylphosphorane gave the unsaturated diester **112** which was then treated with a solution of guanidine in DMF, yielding the bicyclic intermediate **113** as a 2~3:1 mixture of diastereomers (Scheme 3.3, page 45). Both diastereomers were then treated with concentrated hydrochloric acid affording tricyclic model compounds **114** and **115** in the diastereomeric ratio of 2:1. The structures of both diastereomers were confirmed through nOe observations.



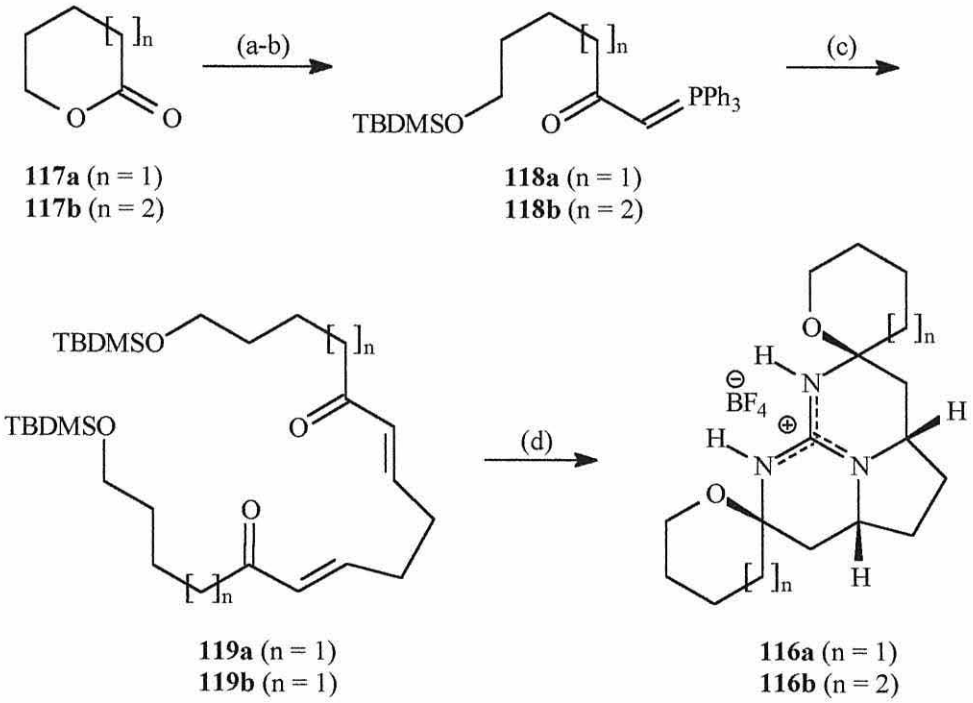
***Reagents and Conditions*:** (a) 2 eqv. $Et_2OCCH{=}PPh_3$, THF; (b) Guanidine, DMF; (c) Conc. HCl.

Scheme 3.3 Synthesis of a tricyclic model of the central pyrrolidine ring system of ptilomycalin A.

3.2.3 Synthesis of Pentacyclic Models of Ptilomycalin A

Following their success, the synthetic methodology was further developed in order to prepare a 6,6,5,6,6 (**116a**), and a 7,6,5,6,7 (**116b**) pentacyclic model of the polycyclic framework of ptilomycalin A (Scheme 3.4, page 46). An analogous sequence of reactions were employed starting from either δ-valerolactone **117a**, or caprolactone **117b**, which were reacted with two equivalents of methylene triphenylphosphorane yielding, after silyl protection, the corresponding phosphorane **118a** or **118b**. A double Wittig reaction with 0.4 equivalents of succinaldehyde gave either symmetrical *bis*-α,β-unsaturated ketone **119a** (45% from **118a**) or **119b** (20% from **118b**). The desired pentacycles were obtained as mixtures of diastereomers, after addition of one equivalent of guanidine followed by removal of the solvent, deprotection/cyclisation using methanolic HCl, and finally counter ion exchange.

Pentacycle **116a** was obtained in 32% yield as a mixture of two diastereomers in an approximate ratio of 4:1. The major diastereomer was isolated in 25% yield by trituration with diethyl ether and identified by X-ray crystallography as the 6,6,5,6,6-pentacycle **116a**, having an identical relative stereochemistry to that found in ptilomycalin A. Similarly, pentacycle **116b** was obtained as a mixture of two diastereomers, on this occasion, in an approximate ratio of 5:1. The major diastereomer was again separated by means of trituration in 25% overall yield, and was identified as the 7,6,5,6,7-pentacycle **116b**, again possessing the same relative stereochemistry as found in the pentacyclic core of ptilomycalin A.



117a (n = 1)
117b (n = 2)

118a (n = 1)
118b (n = 2)

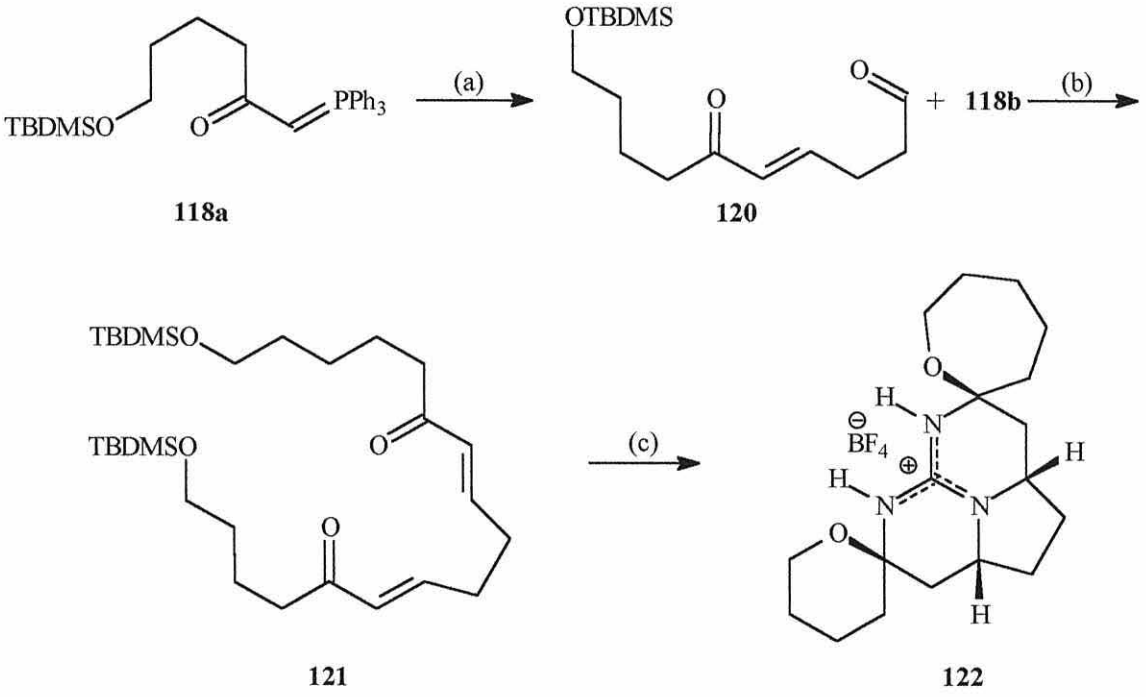
119a (n = 1)
119b (n = 1)

116a (n = 1)
116b (n = 2)

***Reagents and Conditions*:** (a) 2 eqv. CH_2=PPh_3, THF, -78°C; (b) TBDMSCl, Imidazole, DMF; (c) 0.4 eqv. succinaldehyde, THF, 48 h; (d) (i) Guanidine, DMF, 3 h; (ii) MeOH, HCl, 0°C to rt, 24 h; (iii) Saturated aq. $NaBH_4$; (iv) Trituration and crystallisation.

Scheme 3.4 The synthesis of two pentacyclic models of the polycyclic framework found in ptilomycalin A *via* an analogous sequence of reactions.

An exact 7,6,5,6,6 model of the ring system found in the central pentacyclic nucleus of ptilomycalin A has also been prepared. This was achieved following treatment of phosphorane **118a** with 10 equivalents of succinaldehyde to give aldehyde **120** which after Wittig reaction with phosphorane **118b**, afforded unsymmetrical *bis*-α,β-unsaturated ketone **121** in 37% yield (70% based upon recovered **120**). Reaction of **121** with guanidine under standard conditions gave pentacycle **122** as a mixture of two diastereomers in an approximate ratio of 4:1 (Scheme 3.5). The major product was once again isolated by trituration in 25% yield and identified on the basis of ^{1}H and ^{13}C NMR spectroscopy as the desired 7,6,5,6,6-pentacycle, **122**.

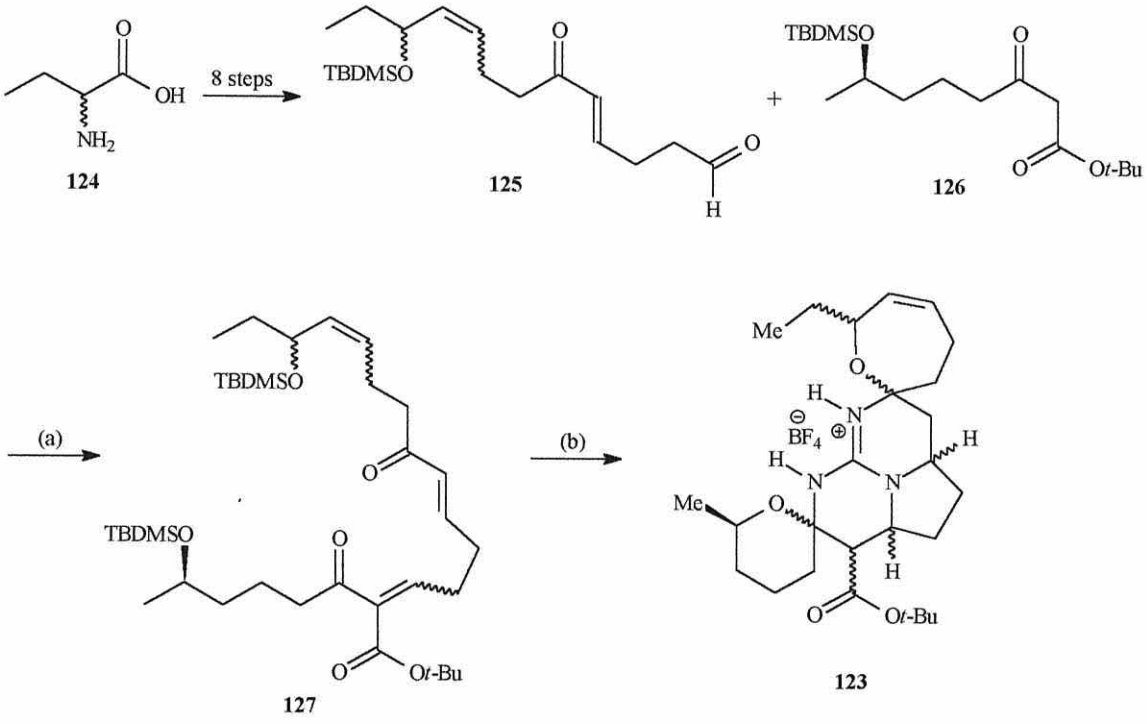


***Reagents and Conditions*:** (a) 10 eqv. succinaldehyde; (b) THF, 48 h; (c) (i) Guanidine, DMF, 3 h; (ii) MeOH, HCl, 0 °C to rt, 24 h; (iii) Saturated aq. $NaBH_4$; (iv) Trituration and crystallisation.

Scheme 3.5 An exact 7,6,5,6,6 model of the ring system found in the polycyclic frame work of ptilomycalin A.

Having successfully prepared three pentacyclic models of ptilomycalin A, Murphy and Williams next attempted the synthesis of a functionalised pentacycle, **123** incorporating the methyl and ethyl substituents as well as the double bond found in the

seven membered ring. A racemic synthesis[81] was undertaken (Scheme 3.6) starting from (±)-2-aminobutyric acid **124**, which was initially converted to unsaturated aldehyde **125**, in eight steps. Coupling of racemic aldehyde **125** to β-keto ester **126** by means of the Knoevenagel condensation reaction was found to be problematical, eventually leading to impure acyclic precursor **127** in 17% yield. The results from an attempted guanidine cyclisation under standard conditions in order to access pentacycle **123** proved inconclusive with ^{1}H NMR analysis presumably showing a complex mixture of diastereomers.



***Reagents and Conditions*:** (a) CH_2Cl_2, piperidine acetate, -78°C, then -20°C, 48 h; (b) (i) Guanidine, DMF, 0°C, 7 h; (ii) MeOH, HCl, 0°C to rt, 16 h; (iii) Saturated aq. $NaBF_4$.

Scheme 3.6 An attempted racemic synthesis of the pentacyclic core of ptilomycalin A.

3.3 X-RAY CRYSTALLOGRAPHIC STUDIES

The crystal structures of model compounds **108**, **116a** and **116b** show an interesting behaviour in the interaction of the guanidinium moieties with their fluoroborate counter ions.[82] It is known that the fluoroborate anion has the ability to interact with a guanidinium ion in a similar manner to the bidentate ligating interaction observed for carboxylate and phosphate anions. The crystal structure of the 6,6,6,6 tetracyclic model **108** (Figure 3.2), intriguingly showed that a single fluorine atom alone was involved in a strong hydrogen bonding interaction to both N–H bonds of the guanidinium moiety. This suggested that the guanidinium cavity of **108** was not large enough to accommodate the fluoroborate counter anion.

In contrast, the X-ray crystal structure of the 6,6,5,6,6 pentacyclic model compound **116a**, which corresponds more closely to the structure of ptilomycalin A demonstrated that the fluoroborate anion formed two disparate, non-symmetrical hydrogen bonding interactions with the guanidine unit, but was unable to achieve co-planarity. The 7,6,5,6,7 pentacyclic model **116b** experiences the same hydrogen bonding interactions as **116a**, however, the crystal structure indicates that co-planarity is almost achieved (Figure 3.2).

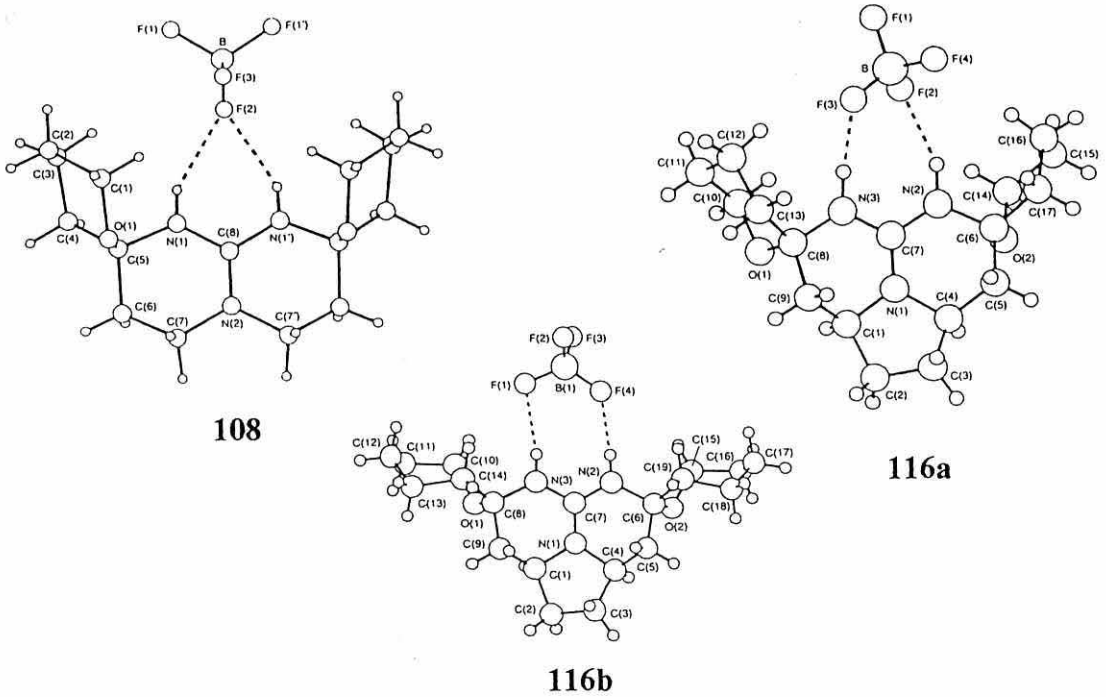


Figure 3.2 The X-ray crystal structures of tetracycle **108** and pentacycles **116a** and **116b**.

The evidence from these crystals structures supports the theory that the cavity in ptilomycalin A and related molecules, which is modelled to an extent by **116a** and **116b**, is involved in the efficient recognition of carboxylate species. Comparisons can also be made between the bond lengths in a guanidinium carboxylate,[83] (N–H···O distances: 0.909/2.007 and 1.024/1.805 Å), a guanidinium phosphate,[84] (N–H···O distances: 0.863/1.960 and 0.885/2.026 Å) and in the described model guanidinium fluoroborates (N–H···F distances: 0.86/2.03 and 0.86/2.22 Å, typically). As can be seen, the bond distances are relatively similar, thereby supporting the theory of the central cavity in ptilomycalin A being involved in a carboxylate binding process. The increase in the degree of co-planarity in the fluoroborate-guanidinium interaction in the model compounds as they approach the structure of ptilomycalin A, seems to suggest that the structure of **12** may represent an optimum host design for an as yet undetermined guest molecule.

3.4 SUMMARY

To summarise, it is apparent that the guanidinium group plays an extremely important and diverse role in nature. This diversity is demonstrated through its ability to function as a very strong organic base, its strong affinity for binding anionic substrates and its versatility in catalysis. In addition, the guanidine moiety is found in numerous marine natural products and to a lesser extent in alkaloids of terrestrial origin. The unusual polycyclic structures adopted by these guanidine-containing natural products plausibly explains their unique bioactivities. The fact that many of these alkaloids are found in unrelated species from different parts of the world invites speculation as to whether these secondary metabolites are manufactured by the organism in question or by a symbiont. Indeed, Kashman[85] has revealed that on a separate occasion he failed to isolate any nitrogen containing compounds from the sponge, *Ptilocaulis spiculifer*. This evidence might therefore support the case for symbionts such as bacteria and hence explain a theory for irreproducibility. Another factor worth consideration is that these metabolites may be produced as a response to seasonal variations or indeed, nutritional requirements.

Finally, the reaction schemes outlined in the biomimetic model studies towards ptilomycalin A demonstrates the viability of the developed synthetic methodology.

CHAPTER 4

THE ATTEMPTED SYNTHESIS OF THE PENTACYCLIC CORE OF PTILOMYCALIN A

4.1 INTRODUCTION

The main aim of this work was to attempt the total synthesis of the marine natural product, ptilomycalin A, **12**. Upon closer inspection of the structure of **12**, it can be seen that the compound consists of three main units: a pentacyclic core, a sixteen carbon spacer and a spermidine residue (Figure 4.1). It was anticipated that the methodology developed[81] for the attempted preparation of the racemic 7,6,5,6,6-pentacyclic nucleus **123** (see Chapter 3, page 48), could be adopted and modified in order to synthesise the pentacycle as a single diastereomer, having the same relative stereochemistry as **12**. Finally, attaching the spacer unit and the spermidine residue through the ester functionality, would complete the total synthesis of ptilomycalin A.

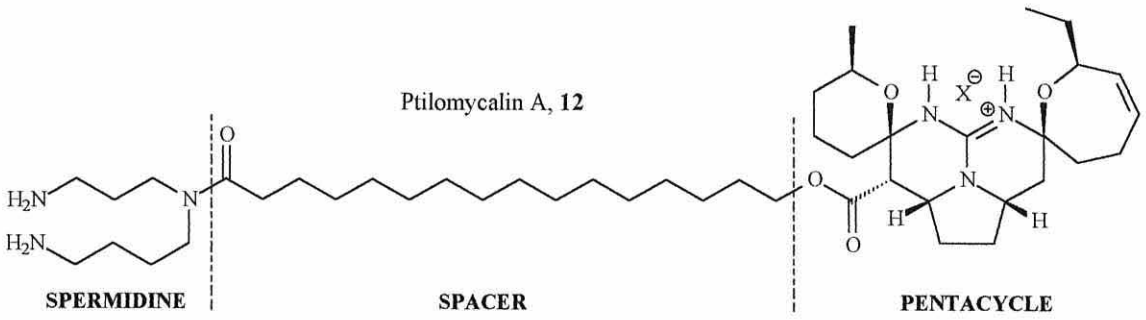


Figure 4.1 The three main structural units of ptilomycalin A.

4.2 RETROSYNTHETIC ANALYSIS

Disconnection of the spermidine and spacer units of **12** affords an ester substituted pentacycle, in this case, *tert*-butyl ester **128**. In an identical manner to that described in Chapter 3 (page 43), retrosynthesis of **128** would yield guanidine **1** and *bis*-α,β-unsaturated ketone, **129**. A subsequent Knoevenagel type disconnection at the ester substituted olefin gives chiral unsaturated aldehyde **130** and chiral β-keto ester **126** (Figure 4.2, page 53). Thus, **129** can be prepared by means of a Knoevenagel condensation reaction between synthons **130** and **126**, followed by a double Michael addition of guanidine with an ensuing cyclisation step affording pentacycle **128**.

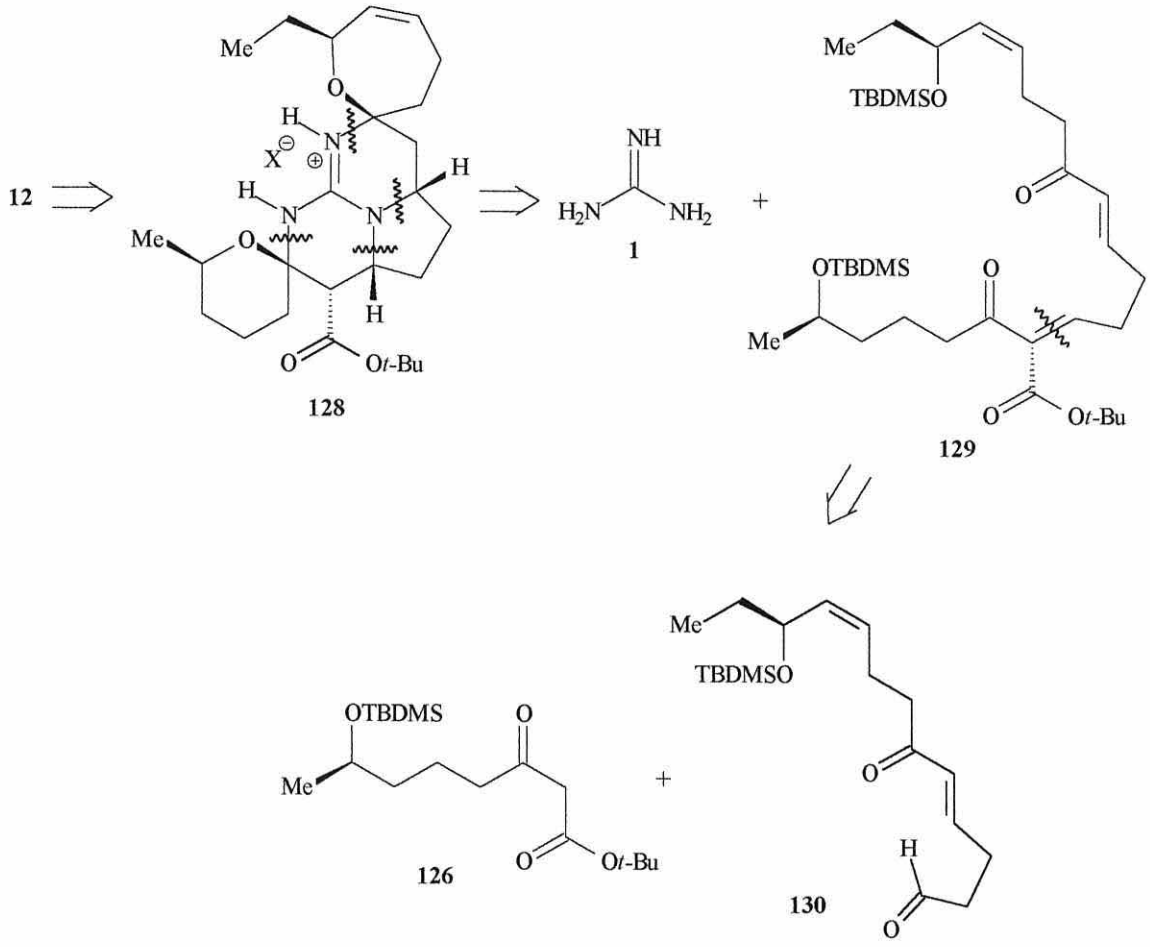


Figure 4.2 Retrosynthesis of the pentacyclic nucleus of ptilomycalin A.

Similarly retrosynthesis of chiral unsaturated aldehyde **130**, *via* a Wittig type disconnection at the *trans* double bond yields as the synthons, phosphorane **131** and succinaldehyde **78** (Figure 4.3, page 54). A further disconnection at the phosphorus-carbon double bond of **131** affords chiral unsaturated ester **132** and methylene triphenylphosphorane, **133**. Hence, the synthesis of the target aldehyde, **130** can be accomplished by way of addition of phosphorane **133** to ester **132**, followed by a subsequent Wittig reaction with *bis*-aldehyde **78**.

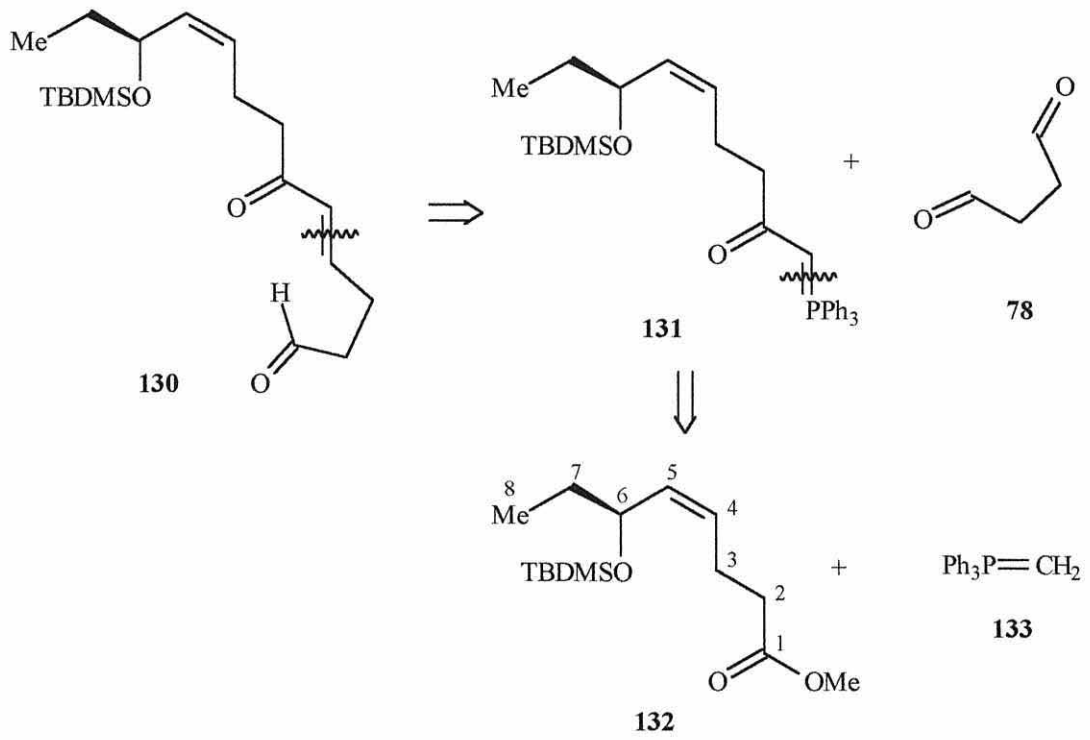


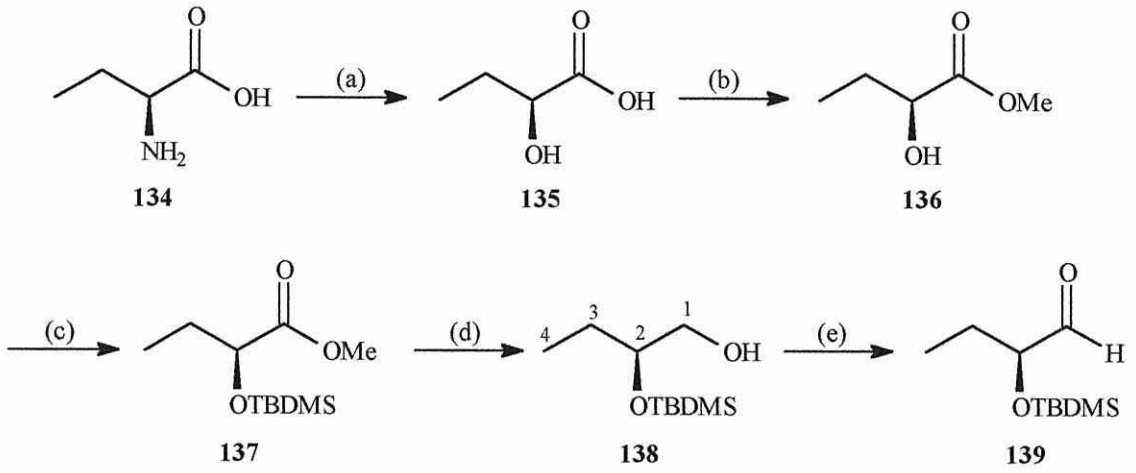
Figure 4.3 Retrosynthesis of chiral unsaturated aldehyde **130**.

4.3 Synthesis Of Chiral Unsaturated Ester **132**

Prior to undertaking the synthesis of **132**, two main issues had to be considered. Firstly, the chiral centre at C-6 and secondly, the geometry about the double bond between C-4 and C-5. In the natural product, the corresponding chiral centre has the *S* orientation and the equivalent double bond possesses a *cis* geometry. It was therefore decided to attempt the preparation of **132** starting from a chiral precursor having an *S* stereocentre and then use Wittig conditions that favour the formation of *Z*-olefins.

The unnatural amino acid (*S*)-(+)-2-aminobutyric acid, **134** was deemed to be a suitable chiral precursor for the synthesis of **132**. Thus, **134** was treated with 1 M H_2SO_4 and an aqueous solution of sodium nitrite to give the corresponding α-hydroxy acid **135** in approximately 45% yield (Scheme 4.1). The reaction proceeded *via* diazotisation where the secondary amino group reacts with nitrous acid, generated *in situ*, to give a diazonium salt. Subsequent hydrolysis yielded the required alcohol along with evolution of nitrogen

gas. The rather low yield of **135** can be explained by the tendency of this reaction to form a mixture of by-products.[86] Due to the polar nature of carboxylic acids, **135** was used in the proceeding step without further purification.



***Reagents and Conditions*:** (a) $NaNO_2$, 1M H_2SO_4; (b) AcCl, MeOH; (c) Imidazole, TBDMSCl, DMF; (d) 2.25 eqv. DIBAL-H, hexane; (e) Swern oxidation.

Scheme 4.1 Preparation of chiral Wittig precursor **139**.

The crude α-hydroxy acid was subsequently esterified at 0°C using a solution of concentrated hydrochloric acid in methanol, prepared *in situ*, by the addition of acetyl chloride to dry methanol. After warming to room temperature and stirring for 48 hours the corresponding α-hydroxy methyl ester **136** was obtained. The volatile nature of **136** made its isolation and extraction extremely difficult. Nevertheless, pure **136** was secured as a yellow oil in 23% overall yield from **134**, after flash column chromatography on silica gel and careful evaporation of the solvent at low temperature under reduced pressure.

Protection of the alcohol functionality was accomplished by reaction with *tert*-butyldimethylsilyl chloride and imidazole under standard conditions, yielding silyl ether **137** in a disappointing 47% yield. Analysis by IR and ^{1}H NMR spectroscopy confirmed the structure of **137**, with the disappearance of the broad O–H stretch (IR) and the broad singlet resonance at δ 4.20 (NMR) as were present in the starting ester. Signals corresponding to the *tert*-butyldimethylsilyl group were evident, with the three methyl resonances of the *tert*-butyl group appearing at δ 0.88 and the diastereotopic methyls attached to silicon, appearing as two singlets at δ 0.06 and δ -0.01, respectively.

The next step involved the conversion of the ester functionality to the corresponding alcohol. It is well known that esters can be converted directly to aldehydes by reduction in the presence of one equivalent of DIBAL-H at low temperature[87] (Figure 4.4). Nevertheless, a previous study using this type of reduction on a similar system,[81] resulted in low yields of the corresponding aldehyde. Instead, a two step procedure was employed to prepare the aldehyde *via* the intermediate alcohol.

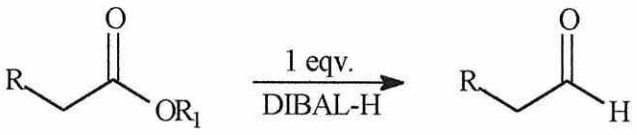


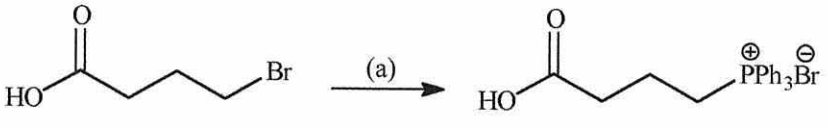
Figure 4.4 The single step reduction of an ester to the corresponding aldehyde.

Thus, reduction of **137** was achieved by reaction with 2.25 equivalents of DIBAL-H at -20°C over a period of 5 hours,[88] affording protected alcohol **138** in 72% yield. The IR spectrum confirmed the presence of the hydroxyl moiety showing a broad stretch at 3358 cm^{-1}. Further structural confirmation was provided by the proton spectrum which displayed a resonance at δ 1.91, indicative of the hydroxyl group. In addition, multiplets correlating to the diastereotopic protons at C-1, δ 3.58 and δ 3.48, were also present.

Oxidation of the alcohol under Swern conditions[89] then gave, without further purification, the desired aldehyde **139** in a yield of 93% (Scheme 4.1, page 55). The IR spectrum of the product supported the presence of an aldehyde, showing two weak bands corresponding to the aldehydic C–H stretches at 2805 cm^{-1} and 2718 cm^{-1} along with a strong carbonyl absorption at 1737 cm^{-1}. Analysis of the ^1H NMR spectrum showed a doublet resonance at δ 9.62 (J = 1.7 Hz) indicative of an aldehyde proton. The carbon spectrum confirmed that the product was indeed an aldehyde, displaying a signal for the requisite carbon at δ 204.38.

Having prepared chiral Wittig precursor **139**, the next priority was to elaborate the aldehyde moiety by reacting it with a suitable phosphonium salt, to give preferentially, the *cis* isomer of unsaturated ester **132**, as dictated by the natural product. To this end, phosphonium salt **140** was prepared by refluxing commercially available 4-bromobutyric acid **141** in the presence of 1.2 equivalents of triphenylphosphine in acetonitrile

(Scheme 4.2) using the method of Corey *et al.*[90] After 24 hours, the mixture was left to cool during which time the desired product precipitated from solution. Removal of the solvent by filtration left a crude solid which was washed with diethyl ether and dried to afford a fine white solid in a 25% yield. An improved product yield of 62% was obtained by heating the two reagents at reflux, as a more concentrated solution in acetonitrile. The proton NMR data for **140** was consistent with the literature data and the melting point (238-241 °C) was also of a comparable value to that published (234-240 °C).[91]



141 **140**

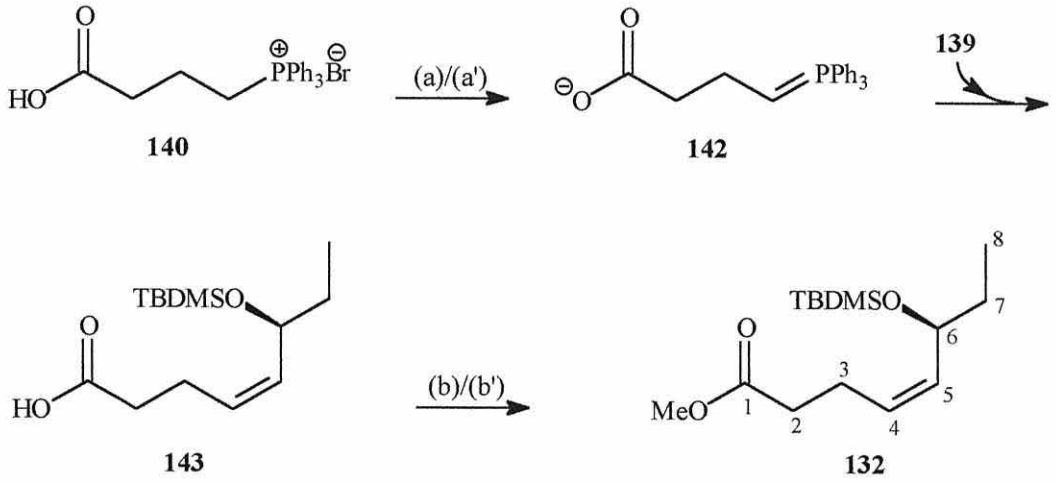
***Reagents and Conditions*:** (a) PPh_3, CH_3CN, Δ.

Scheme 4.2 Preparation of phosphonium salt **140**.

Before attempting the Wittig reaction between compounds **139** and **140** it was necessary to consider what factors, if any, could be employed to promote the formation of the *cis* geometrical isomer. Previous studies,[92, 93] have suggested that different isomeric ratios of *cis* and *trans* olefins generated through the Wittig reaction could be attributed to the influence of temperature, solvents, additives, base-derived salts and the overall stability of the ylid employed. Non-stabilised ylids normally react with aldehydes to afford *Z* alkenes, whereas stabilised ylids favour the formation of *E* alkenes. Both ylid types react by a process suggested to involve betaine and/or oxaphosphetane intermediates. The exclusion of lithium salts, low reaction temperatures and the use of polar, aprotic solvents all serve to enhance *Z* stereoselectivity.

Another factor that can affect the geometric outcome of the Wittig reaction is the presence of anionic, nucleophilic groups in the side chains of triphenylphosphonium ylids which tend to favour *E* stereoselectivity. Examples of such anionic functional groups include oxido, amino and as in this case of **140**, carboxylate groups. The enhancement of *E* stereoselectivity also depends upon the distance of the anionic group from the phosphorus atom. Studies by Maryanoff and co-workers[94] have shown that carboxy-substituted ylids will react with aliphatic aldehydes with only minor *trans* stereoselective enhancement.

Taking these factors into consideration it was decided to use sodium *bis*(trimethylsilyl) amide ($NaN[Si(CH_3)_3]_2$) as the base for generating ylid **142**, the main reason being that the exclusion of lithium and the lack of chelation in the transition state would give mainly the *cis* olefinic intermediate **143**. In addition, aprotic DMSO was used as the initial reaction solvent, whilst methyl iodide was employed to alkylate intermediate acid **143** (Scheme 4.3, steps (a)-(b)).



***Reagents and Conditions*:** (a) DMSO, $NaN[Si(CH_3)_3]_2$; (b) MeI.
(a)' THF, $NaN[Si(CH_3)_3]_2$; (b)' CH_2N_2.

Scheme 4.3 Completion of the synthesis of chiral unsaturated ester **132**.

Preliminary attempts at the Wittig reaction resulted in extremely disappointing yields of the desired ester; the results are summarised in Table 4.1 (page 59). In each reaction, phosphonium salt **140** was used to prepare the ylid. For the initial reaction (entry 1), the ylid was generated at room temperature and following stirring for thirty minutes, neat aldehyde was added, also at room temperature. After stirring for four hours, the solution was treated with freshly distilled methyl iodide and the resulting mixture left to stir for a further forty eight hours. Work-up gave a 7% overall yield of alkene, which consisted of both *cis* and *trans* isomers in the ratio of 3:1.

In an effort to further investigate this reaction, whilst conserving aldehyde **139**, a test reaction (entry 2), was undertaken using hexanal (hex) as the aldehyde but once again only a very poor yield (6%) of product was obtained. Substituting the reaction solvent with THF failed to yield any product whatsoever (entry 3). In an endeavour to determine whether any olefin could be obtained using this reaction procedure, the base was replaced

with *n*-butyllithium and different reaction conditions were employed. These included varying the temperature for ylid formation (Ylid T) and the temperature at which the aldehyde was added (Ald T), (entries 4-7), but in each case work-up failed to yield any olefinic material.

Entry	Solvent	Base	Ylid T	Ald	Ald T	Time	$Me^{\oplus}$	Yield *Z+E*	Mass *Z*
1	DMSO	§	rt	**139**	rt	4 h	CH_3I	7%	12 mg
2	DMSO	§	15°C	hex	rt	4 h	CH_3I	6%	7 mg
3	THF	§	15°C	**139**	rt	4 h	CH_3I	/	/
4	THF	*n*-BuLi	0°C	hex	-78°C	1 h	‡	/	/
5	THF	*n*-BuLi	-40°C	hex	-40°C	1 h	‡	/	/
6	THF	*n*-BuLi	rt	hex	-40°C	1 h	‡	/	/
7	THF	*n*-BuLi	rt	hex	rt	1 h	‡	/	/

***Key*:** § = $NaN[Si(CH_3)_3]_2$, Ald = aldehyde, $Me^{\oplus}$ = alkylating agent, ‡ = crude product not alkylated.

Table 4.1 Initial investigations into the Wittig reaction aimed towards the synthesis of ester **132**.

A second series of reactions were attempted using the same reaction system, (see Table 4.2, page 60), this time specifically focussing on the successful generation of the ylid prior to addition of the aldehyde. The alkylating agent was changed to ethereal diazomethane and THF was used as the reaction solvent throughout (see Scheme 4.3, page 58, steps (a')-(b')). An identical reaction procedure was adopted to that previously discussed, except that on this occasion, after work-up, the crude material was re-dissolved in diethyl ether and excess ethereal diazomethane added. Following gentle stirring for one hour, the mixture was left to evaporate and subsequently purified on silica gel.

The initial reaction (entry 1), after work-up and purification, showed a threefold increase in yield (22%) with both *cis* and *trans* isomers obtained in an approximate ratio of 3:1. Further investigations revealed that deprotonation of the phosphonium salt at room temperature and addition to the aldehyde at 0°C gave an improved yield of 31% of **132** (entry 3), in a geometric isomer ratio of 4:1 (*Z*/*E*). Increasing the ylid equivalence (Ylid E) from 1.25 to 2.5 appeared to have no effect on the overall yield of olefin (entry 4).

During the aforementioned studies, a communication by Matsumura *et al.*[95] came to hand, which led to a reappraisal of the methodology employed for this particular Wittig reaction. They reported the formation of an ylid containing a nucleophilic carboxyl moiety by heating their reaction mixture at a temperature of 70°C for one hour, in THF, before the addition of their ketone. In light of the fact that they obtained their desired unsaturated product in 63% yield as an 87:13 mixture of *Z* and *E* isomers, it was decided to attempt to duplicate this procedure. The initial attempt proved successful affording, after methylation, a 45% yield of *cis* and *trans* isomers in the ratio of 4:1 (entry 7). Separation of the individual geometric isomers was possible by careful column chromatography on silica gel.

Entry	Solvent	Ylid[a,b] E	Ylid T	Ald[c] T	Time	$Me^{\oplus}$	Yield	Mass *Z*
1	THF	1.25	rt	rt	1 h	CH_2N_2	22%	43 mg
2	THF	1.25	rt	0°C	25 min	CH_2N_2	25%	49 mg
3	THF	1.25	rt	0°C	25 min	CH_2N_2	31%	80 mg
4	THF	2.5	rt	0°C	20 min	CH_2N_2	29%	58 mg
5	THF	1.25	0°C	-25°C	30 min	CH_2N_2	18%	30 mg
6	THF	1.25	0°C	-40°C	20 min	CH_2N_2	2%	6 mg
7	THF	7	reflux	rt	30 min	CH_2N_2	45%	81 mg

***Key*:** Ylid T = temperature at which ylid was formed, Ald T = temperature at which aldehyde was added, $Me^{\oplus}$ = alkylating agent. [a] $NaN[Si(CH_3)_3]_2$ was used as the base, [b] ylid was prepared from phosphonium salt **140**, [c] aldehyde **139** was used in each reaction.

Table 4.2 Further Wittig reaction investigations towards the synthesis of ester **132**.

Evidence of the presence of the *Z* isomer of **132** was obtained by NMR spectroscopy. The proton spectrum displayed signals corresponding to the double bond, one of which appeared as a double doublet (CH-5) at δ 5.42 coupling to protons CH-4 and CH-6. An examination of the coupling constants showed, $J_{5\text{-}4}$ to be 11.0 Hz, whilst $J_{5\text{-}6}$ was calculated to be 9.1 Hz. The value of $J_{5\text{-}4}$ was within the allowed range for a *cis* olefin thus proving that (*Z*)-**132** was the major isomer. The signal for the second olefinic proton

(CH-4) appeared as a complex multiplet at δ 5.30. A resonance for the chiral proton (CH-6) was observed as a double triplet at δ 4.35 (J = 7.8, 6.4 Hz) whilst the methyl of the ester appeared as a singlet at δ 3.69. The ^{13}C NMR showed the presence of olefinic carbons with resonances at δ 135.43 and δ 126.47 corresponding to C-5 and C-4, respectively.

Analysis of the *trans* isomer of **132** (Figure 4.5), which represents the minor product, by spectroscopic means showed that the olefinic signals for protons CH-4 and CH-5, were not resolved, appearing as a complex multiplet at δ 5.55. The resonance for the methine proton (CH-6), occurred as a multiplet at δ 3.99 whilst the remaining spectrum was almost identical to that of *cis*-**132** except that all the signals were displaced slightly upfield.

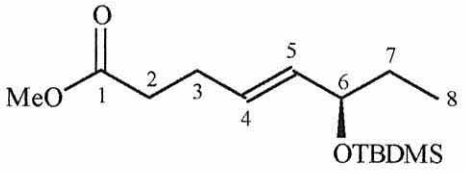


Figure 4.5 The *trans* isomer of **132**.

4.4 Synthesis Of Unsaturated Aldehyde **130**

Having successfully prepared chiral protected ester **132** the next step was its conversion to unsaturated aldehyde **130**. As previously discussed in the retrosynthetic analysis (Figure 4.3, page 54), this was to be accomplished by means of Wittig methodology. Before attempting the synthesis, it was necessary to prepare the reaction intermediates namely, succinaldehyde **78**, and methyltriphenylphosphonium iodide **144** (the precursor to phosphorane **133**). Methyltriphenylphosphonium iodide was prepared by a reaction between methyl iodide and triphenylphosphine in toluene, which proceeded *via* the S_N2 mechanism, yielding phosphonium salt **144** as a white solid (Figure 4.6, page 62).

The *bis*-aldehyde, succinaldehyde[96] **78** was prepared by heating a mixture of 2,5-dimethoxytetrahydrofuran **145** in the presence of a 1% aqueous solution of acetic acid, at reflux for 20 minutes. After cooling, the mixture was neutralised and extracted with chloroform followed by ethyl acetate. Purification of the crude product by fractional distillation under reduced pressure gave **78** as a colourless, lachrymatory oil in a 51% yield

(Figure 4.6). The nature of the product was established by ^{1}H NMR spectroscopy with the signal at δ 9.85 corresponding to the two aldehyde protons. A singlet resonance for the two methylenes was observed at δ 2.81. It was essential to prepare succinaldehyde as soon as possible before use in reactions; storing **78** at room temperature for periods longer than 24 hours resulted in the formation of a clear, 'gel like' oil believed to be the product of polymerisation, as was evident through NMR analysis.[81] Similarly, storing in a freezer (-15°C) for periods longer than two weeks also resulted in the formation of the previously described oil.

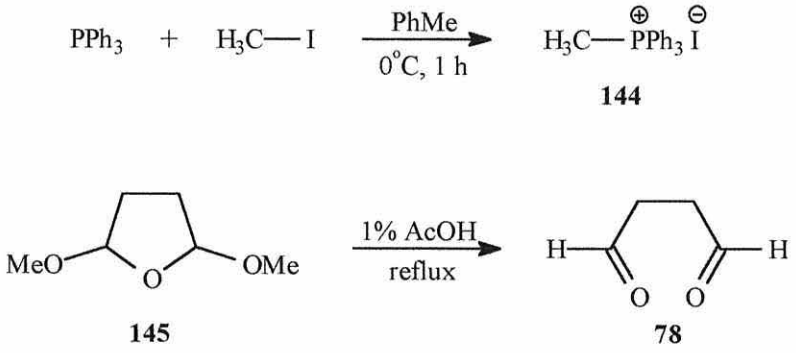
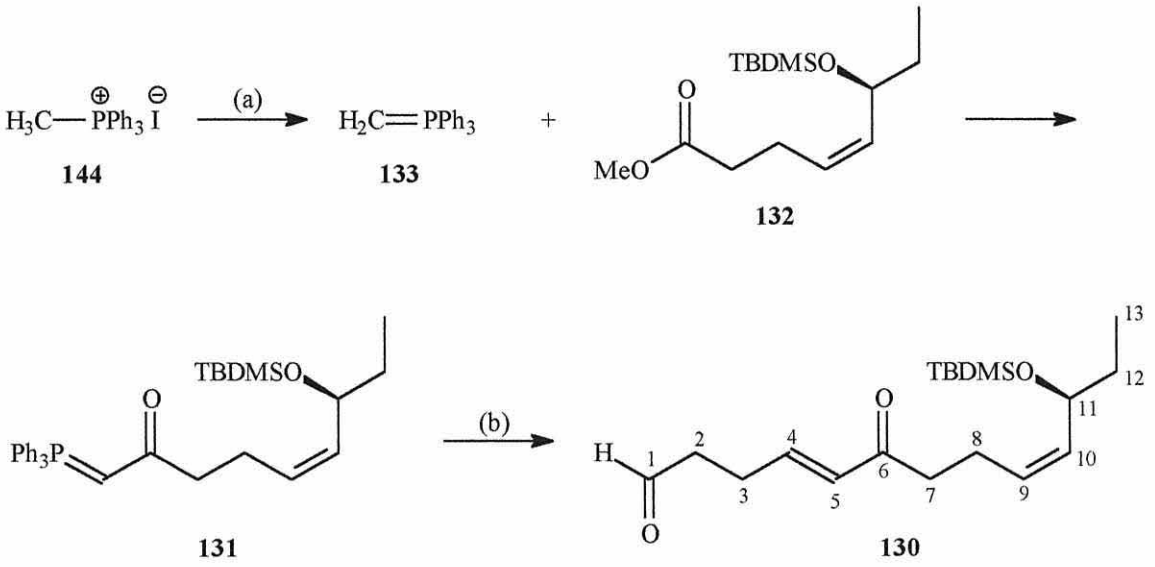


Figure 4.6 Synthesis of the intermediates, methyltriphenylphosphonium iodide **144** and succinaldehyde **78**.

The preparation of unsaturated aldehyde **130** involved two further synthetic steps, addition of phosphorane **133** to afford intermediate **131**, followed by a Wittig reaction to yield unsaturated aldehyde **130** (Scheme 4.4, page 63). Thus, deprotonation of methyltriphenylphosphonium iodide with *n*-butyllithium at 0°C gave the corresponding phosphorane **133**. After stirring for 30 minutes, the phosphorane was treated with a solution of (*Z*)-**132** in THF at -78°C, and the resulting mixture stirred for a further two hours at room temperature. The reaction mixture was subsequently quenched with water, extracted with ethyl acetate and dried, yielding a yellow oil which was used in the next step without further purification. The crude phosphorane was dissolved in THF and reacted with an excess of freshly distilled succinaldehyde **78** at room temperature over a period of 42 hours. Evaporation of the solvent followed by purification, gave the desired unsaturated aldehyde **130** in a modest 42% overall yield (Scheme 4.4, page 63).



144 133 132 131 130

***Reagents and Conditions*:** (a) *n*-BuLi, THF, 0 °C; (b) Excess **78**, THF, rt.

Scheme 4.4 Synthesis of unsaturated aldehyde **130**.

Analysis of the proton NMR spectrum confirmed the structure and olefinic geometry of **130**. The aldehyde proton appeared as a singlet resonance at δ 9.82, whilst the *trans* olefinic protons CH-5 and CH-4 appeared as a pair of double triplets at δ 6.83 and δ 6.14, respectively. The coupling constants calculated for these conjugated vinylic protons, CH-5 (J = 16.0, 6.5 Hz) and CH-4 (J = 16.0, 2.1 Hz), are within the specified range for a typical *trans* olefin. On this occasion, the protons of the *cis* double bond, CH-9 and CH-10, appeared as an unresolved multiplet between δ 5.42-5.26. A complex multiplet resonance occurring at δ 4.34 corresponded to the proton (CH-11) at the chiral centre. The carbon NMR showed resonances for the aldehyde carbon at δ 200.25 and the ketone at δ 199.15. Four downfield signals were present corresponding to the vinyl carbons at δ 144.44 (C-4), δ 135.13 (C-10), δ 130.88 (C-5) and δ 126.90 (C-9), respectively, whilst the remainder of the spectrum was consistent with the structure of **130**.

4.5 Synthesis Of *Bis*-α,β-Unsaturated Ketone **129**

Prior to attempting the synthesis of *bis*-α,β-unsaturated ketone **129**, it was necessary to prepare chiral β-keto ester **126** which had been previously identified as a

suitable precursor for the Knoevenagel condensation (Figure 4.2, page 53 and Chapter 3, page 48). Retrosynthetic analysis of **126** gave chiral iodide **146** and *tert*-butylacetoacetate **147**. A series of functional group interconversions starting from the iodide led to ethyl (*R*)-3-hydroxybutyrate **148** as a commercially available chiral precursor (Figure 4.7).

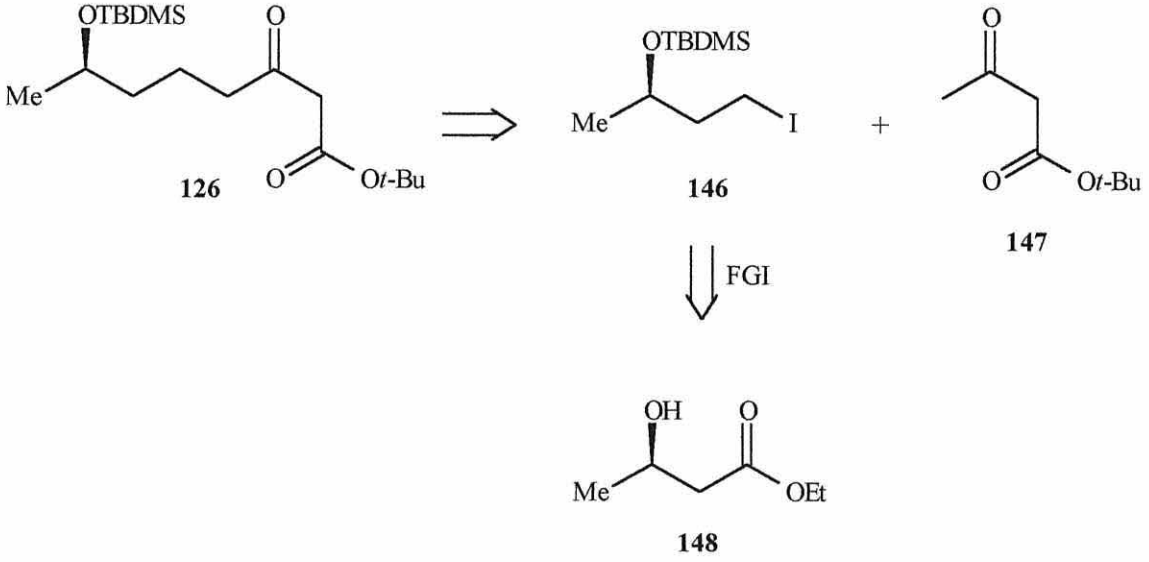
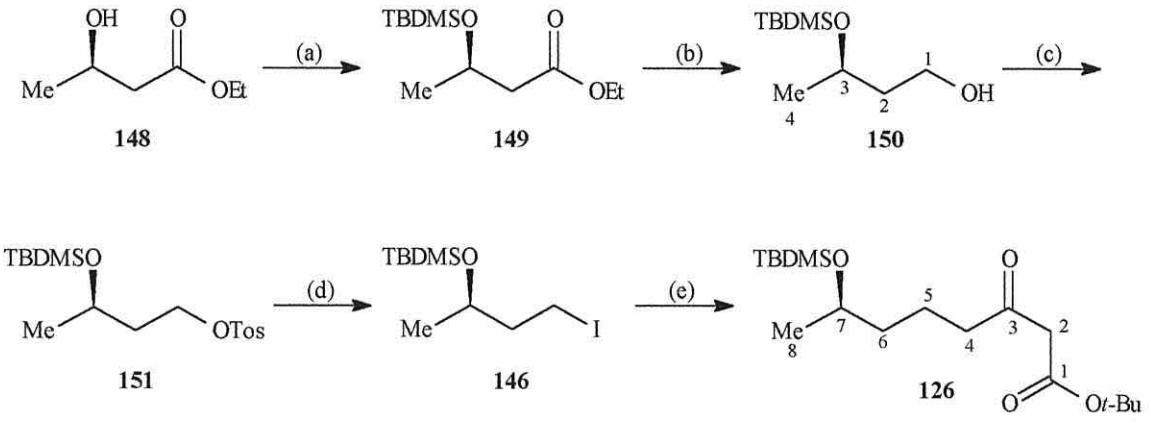


Figure 4.7 Retrosynthesis of chiral β-keto ester **126**.

Thus, **148** was initially protected as the silyl ether **149** in 82% yield by reaction with *tert*-butyldimethylsilyl chloride under standard conditions (Scheme 4.5, page 65). The ester functionality was subsequently reduced, as previously discussed (see Scheme 4.1, page 55), using 2.25 equivalents of DIBAL-H, giving the corresponding alcohol **150** in a 73% yield. Initial confirmation of a successful reduction was obtained from the IR spectrum which showed a broad stretch at 3345 cm^{-1} corresponding to a hydroxyl group. Furthermore, there was no evidence of the carboxyl band at 1739 cm^{-1}, as was present in the starting material.

The proton and carbon spectra confirmed the structure of **150** as that shown in Scheme 4.5. The hydrogen at the chiral centre (CH-3) appeared as a complex multiplet at δ 4.11 whilst a broad triplet at δ 2.59 was identified as the O–H resonance coupling (J = 5.0 Hz) to the adjacent methylene (CH_2-1). In addition, the signals corresponding to the ethyl group of the starting ester (δ 4.12, CH_2 and δ 1.27, CH_3) were no longer present. Similarly, structural confirmation was obtained through interpretation of the ^{13}C NMR spectrum which showed a resonance for the methylene attached to the hydroxyl group at δ 60.40 with no indication of the starting ester carboxyl group (δ 171.66 in **149**).



***Reagents and Conditions*:** (a) Imidazole, TBDMSCl, DMF; (b) 2.25 eqv. DIBAL-H, hexane; (c) TosCl, pyridine; (d) NaI, acetone, reflux; (e) LDA, **147**, THF.

Scheme 4.5 Synthesis of chiral β-keto ester **126**.

The next step involved the conversion of the alcohol to the corresponding tosylate, **151**. This was achieved by treating a solution of *para*-toluenesulfonyl chloride in dry pyridine with a solution of **150**, also in dry pyridine, at 0 °C. After stirring for 16 hours, extraction and purification gave the desired tosylate as a colourless oil in 62% yield. Analysis of the proton NMR confirmed the presence of aromatic C–H signals, displaying two doublet resonances (4H) at δ 7.80 and δ 7.35, respectively. The methylene attached to the tosyl residue appeared as a triplet at δ 4.11 and the methyl substituent on the aromatic ring occurred as a singlet at δ 2.46. Substantiation of the successful formation of **151** was given by the mass spectrum (CI) which displayed a peak at 359 Daltons corresponding to the $[M+H]^+$ ion.

Finkelstein conditions[97] were employed to displace the tosyl group in order to form the corresponding iodide **146**. This was accomplished by heating a solution of 5.5 equivalents of sodium iodide and tosylate **151** in acetone, at reflux, over a period of 4 hours which resulted in the formation of a white suspension. Filtration and washing of the remaining solid with diethyl ether followed by evaporation gave a crude oil which was subsequently triturated with hexane. Evaporation of the solvent gave, without further purification, the expected iodide in an excellent yield of 95%.

Interpretation of the ^1H NMR spectrum showed a complex multiplet at δ 3.90 indicating the methine at the chiral centre whilst the methylene adjacent to the iodine

appeared as a multiplet resonance at δ 3.24. The same methylene group appeared as a highfield resonance at δ 3.69 in the carbon NMR of **146**. This highfield shift is characteristic of compounds where a carbon is bonded to an electronegative atom such as iodine and occurs due to deshielding of the carbon atom.

The synthesis of chiral β-keto ester **126** was completed following the addition of the dianion of *tert*-butylacetoacetate (Figure 4.8) to chiral iodide **146**. To this end, LDA was prepared by treating a solution of diisopropylamine in THF with *n*-butyllithium at 0°C and stirred over a period of 30 minutes. Formation of the dianion was accomplished by the addition of *tert*-butylacetoacetate to 2.3 equivalents of the freshly prepared LDA solution. After stirring for 1 hour at 0°C, iodide **146** was slowly added in a dropwise manner, at this temperature, to the dianion which underwent addition to the iodide at the least stabilised position (C-4), resulting in the generation of the desired β-keto ester, **126**.

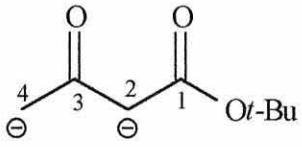


Figure 4.8 The dianion of *tert*-butyl acetoacetate.

Evidence of the successful preparation of **126** was given by the IR spectrum which showed two strong carbonyl absorptions at 1735 cm^{-1} and 1717 cm^{-1}. The IR spectrum also indicated that the product existed exclusively in the keto form, showing no sign of a corresponding enolic O–H stretch (see Figure 4.9, page 67). Further confirmation of the structure of **126** was provided by the proton and carbon NMR spectra. The ^1H NMR spectrum showed a multiplet at δ 3.79 corresponding to the proton at the chiral centre, (CH-7). A singlet resonance was observed at δ 3.34 for the methylene (CH_2-2) in between the two carbonyls and a triplet signal (J = 7.2 Hz) at δ 2.53 corresponded to the methylene (CH_2-4) adjacent to the ketone. The terminal methyl (CH_3-8) appeared as a doublet at δ 1.12 (J = 6.1 Hz). Two quaternary signals were observed in the carbon spectrum, at δ 203.15 (C-3) and δ 164.44 (C-1), which corresponded to the ketone and ester carbonyls, respectively. The resonance at δ 81.75 was indicative of a *tert*-butyl quaternary carbon whilst the signal at δ 68.23 correlated with the carbon (C-7) at the chiral centre. The remaining resonances in the carbon spectrum were consistent with the structure of **126**.

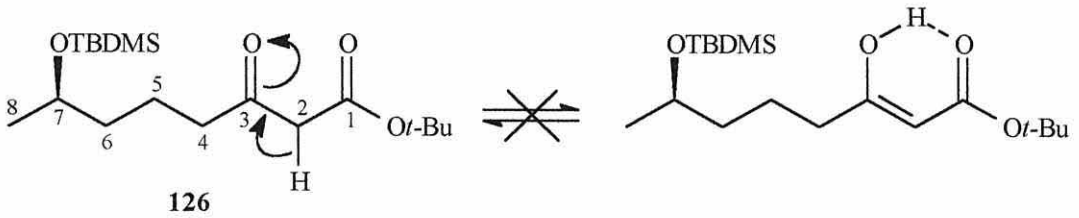


Figure 4.9 β-Keto ester **126** and its theoretical enol tautomer.

4.5.1 THE KNOEVENAGEL CONDENSATION

As previously discussed, it was envisaged that *bis*-α,β-unsaturated ketone **129**, could be prepared by coupling intermediates **130** and **126**, by means of the Knoevenagel condensation reaction (Figure 4.10). The aforementioned reaction has been shown to be of particular use in the condensation of aldehydes or ketones with compounds of the form Z-CH_2-Z', were Z and Z' are electron withdrawing groups,[98] an example being β-keto ester **126**. The direct attachment of an electron withdrawing group, such as a nitrile or cyano group, activates the adjacent methylene and in the majority of cases, two such groups are required to provide sufficient activation[99] for the reaction to occur.

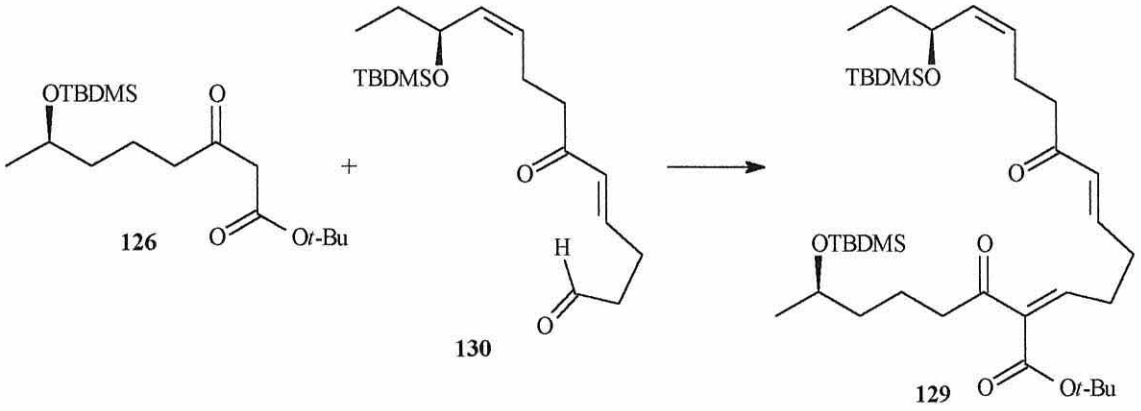
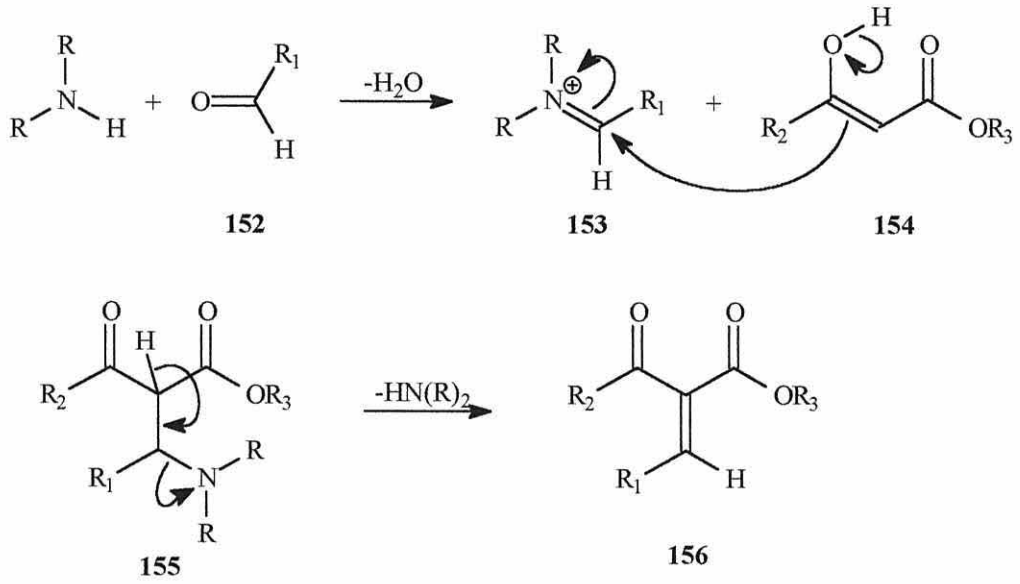


Figure 4.10 The Knoevenagel condensation reaction.

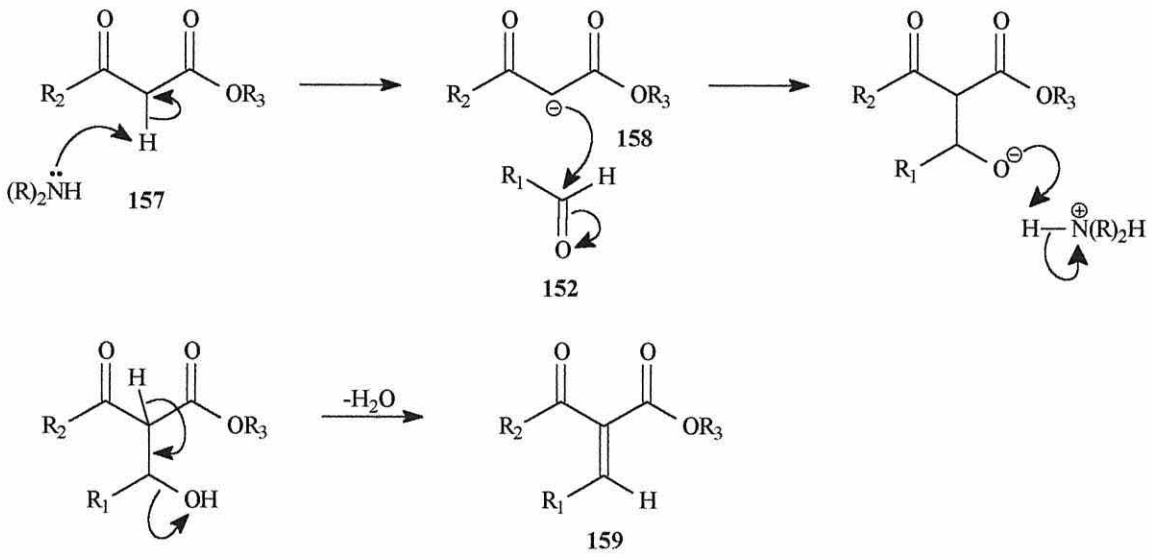
Knoevenagel condensation reactions are typically base-catalysed, the basic catalysts are usually ammonia and ammonium salts and primary, secondary or tertiary amines and their corresponding salts. Other catalysts employed in this reaction include weakly basic ion exchange resins, potassium fluoride or caesium fluoride [100,101] and titanium tetrachloride in the presence of a base.[102]

Although the exact mechanistic pathway of the Knoevenagel condensation remains unclear, several possible routes have been proposed. Knoevenagel himself, proposed that the role of the basic amine was to combine with an aldehyde such as **152**, to form an iminium salt **153** which then undergoes an addition reaction with the enolic form, **154** of the corresponding β-keto ester. The unsaturated product **156** results after elimination of the amine from intermediate **155** (Scheme 4.6).



Scheme 4.6 Knoevenagel's proposed mechanism for his eponymous reaction.

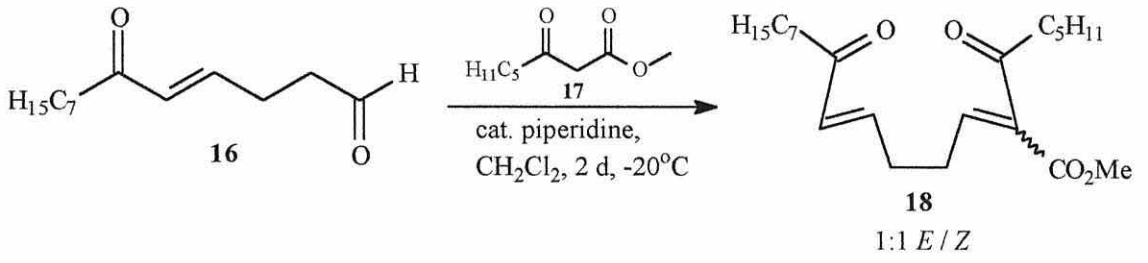
A second method, put forward by Hann and Lapworth,[103] suggested that the base abstracts a proton from the active methylene component in the β-keto ester **157**, forming a carbanion **158** which then adds to the aldehyde, **152**. Subsequent base hydrolysis followed by elimination of the elements of water, yields the unsaturated, trisubstituted olefin **159** (Scheme 4.7, page 69). Both reaction mechanisms are discussed in detail in an informative review by G. Jones.[99]



Scheme 4.7 The Hann and Lapworth[103] mechanism for the Knoevenagel condensation.

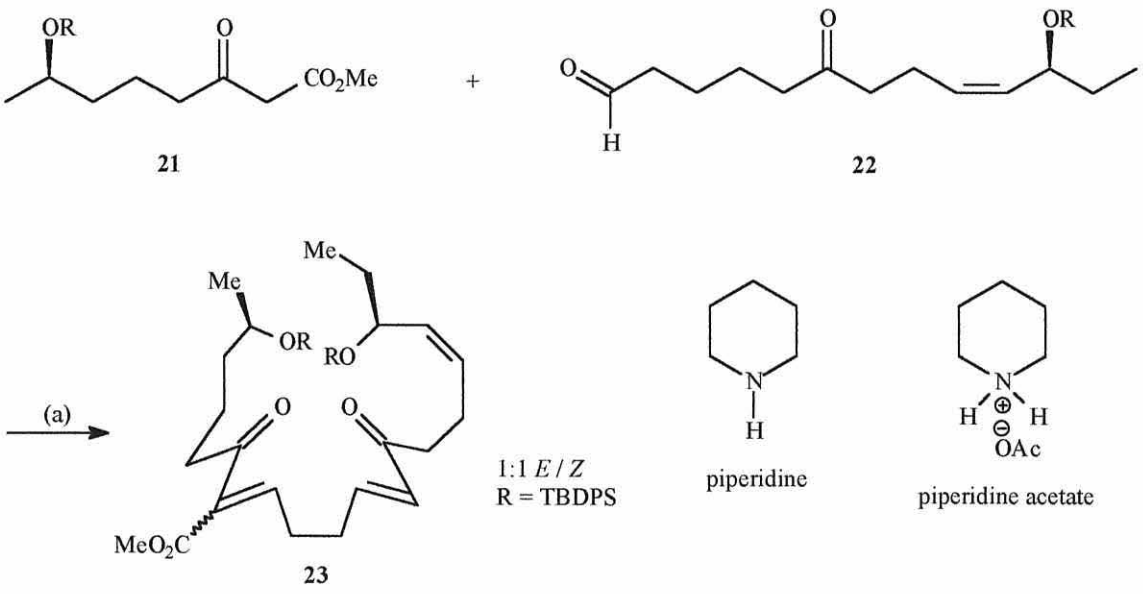
It is impossible to say with any certainty which of these two mechanisms might be occurring in the case of the reaction outlined in Figure 4.10, page 67. The mechanism proposed by Knoevenagel (Scheme 4.6, page 68) is disfavoured, as β-keto ester **126** was found to exist solely as the keto form. Furthermore, on a separate occasion, Lapworth was quoted[104] as favouring an alternative method to the one he proposed with Hann, one which was more akin to the Knoevenagel mechanism.

A review of the work undertaken by Snider and Shi[34] towards the synthesis of ptilomycalin A showed two examples where the Knoevenagel reaction was utilised in the synthesis, in high yields, of *bis*-α,β-unsaturated ketones (see Chapter 2, pages 17-19). In their synthesis of the tricyclic model of **12** they effect a condensation reaction between aldehyde **16** and β-keto ester **17** to give unsaturated ketone **18** in 61% yield (89% yield based on recovered aldehyde), using a catalytic amount of the secondary amine, piperidine in dichloromethane over a period of two days at a temperature of -20 °C (Scheme 4.8).



Scheme 4.8 An example of a Knoevenagel condensation reaction as reported by Snider and Shi.[34]

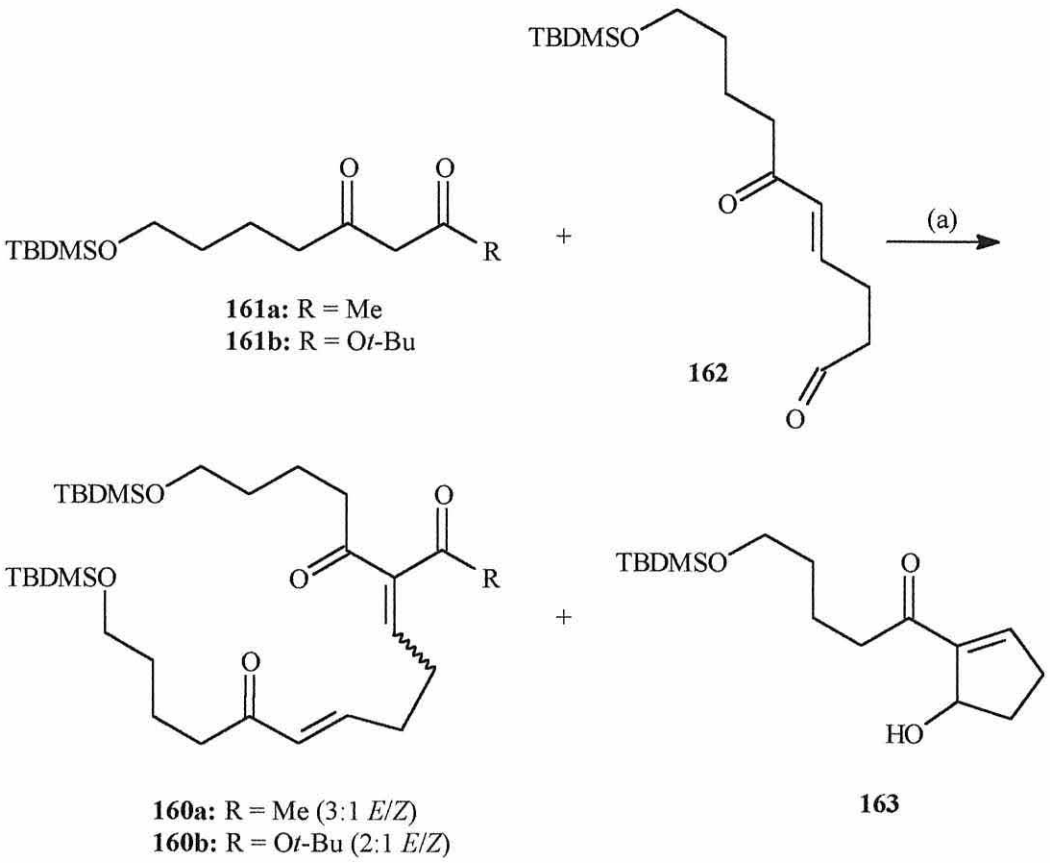
A similar methodology was employed in Snider's preparation of the pentacyclic nucleus of ptilomycalin A.[34] On this occasion, the desired product **23** was obtained in 64% yield (86% yield based upon recovered aldehyde **22**, and 94% yield on recovered β-keto ester **21**), (Scheme 4.9). For this reaction, a catalytic amount of either piperidine or piperidine acetate was employed as the base with dichloromethane as solvent. The reaction itself was performed at -78 °C and warmed to -20 °C over a period of twenty hours with work-up, followed by purification, giving the desired *bis*-α,β-unsaturated ketone **23**.



***Reagents and Conditions*:** (a) CH_2Cl_2, piperidine or piperidine acetate, -78 °C to -20 °C, 20 h.

Scheme 4.9 A second example of a high yielding Knoevenagel condensation reaction as demonstrated by Snider and Shi.[34]

In contrast, an investigation by Williams[81] into the Knoevenagel reaction on similar systems to that shown in Scheme 4.9 above, concluded that the condensation was not as straightforward a procedure as it appeared. It was only with considerable time and effort that the acyclic precursors **160a** and **160b** were obtained in maximum yields of 40% and 26%, respectively, from the reactions between β-keto esters **161a** and **161b** with aldehyde **162** (Scheme 4.10, page 71). In the case of **160a** no unreacted aldehyde was recovered, but for **160b**, 20% recovery of the starting aldehyde was possible.



161a: R = Me
161b: R = O*t*-Bu

162

160a: R = Me (3:1 *E*/*Z*)
160b: R = O*t*-Bu (2:1 *E*/*Z*)

163

***Reagents and Conditions*:** (a) CH_2Cl_2, piperidine acetate, -78°C to -20°C, 24 h.

Scheme 4.10 Williams' synthesis of *bis*-α,β-unsaturated ketones **160a** and **160b** and the Baylis-Hillman by-product **163** from the reaction between **161a** and aldehyde **162**.

During the purification of **160a**, a compound was consistently isolated in a 15-40% yield which was identified through NMR spectroscopy as the cyclopentenol, **163** (Scheme 4.10). The cyclic by-product resulted from a base catalysed rearrangement of the aldehyde precursor *via* a process similar to that of the Baylis-Hillman reaction.[105] Cyclisation probably occured by way of a two step procedure, involving a base catalysed 1,4-intramolecular aldol addition followed by elimination of the base to yield **163**.

With this in mind, it was decided to attempt the Knoevenagel condensation reaction between aldehyde **130** and β-keto ester **126**, using the secondary amine, morpholine, as the catalytic base. Morpholine was chosen as the base as it had been previously demonstrated to successfully effect the Knoevenagel condensation reaction[81] whilst limiting the formation of the unwanted Baylis-Hillman by-product.

To this end the Knoevenagel reaction (see Figure 4.10, page 67) was initially attempted by dissolving a mixture of **130** and 1.2 equivalents of **126** together, in dichloromethane (entry 1, Table 4.3). A small amount of anhydrous sodium sulfate was added to ensure a dry reaction media. After cooling to -20°C, morpholine was added and the reaction mixture stirred at this temperature for one hour and then at -15°C for a further 24 hours. TLC analysis of the reaction mixture showed that virtually no reaction had taken place, hence, the mixture was stirred at approximately 3°C for another 24 hours. Analysis by TLC showed that the product was now forming and the mixture was left to stir for a total of 168 hours at this temperature before work-up. It was difficult to assess to what extent the aldehyde was being consumed, as the cyclised by-product had been shown to possess a very similar retention factor on TLC.[81] Work-up and purification yielded 13% of the desired *bis*-α,β-unsaturated ketone **129** (22% yield based upon recovered aldehyde).

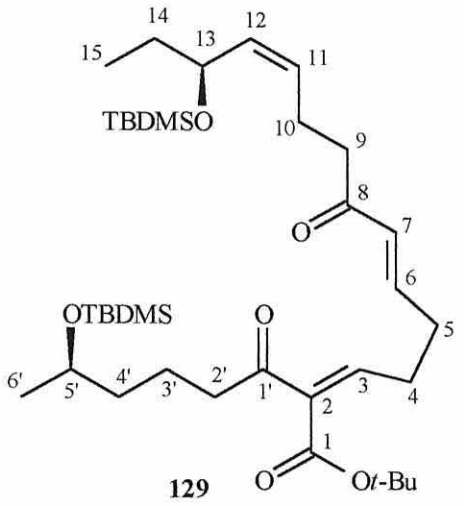
Entry	Base	Duration	Temp.	Eqv. **126**	Yield	Yield[a]	Yield[b]
1	morpholine	168 h	-20°C to 3°C	1.2	13%	22%	§
2	morpholine	120 h	0°C to 3°C	1.5	0%	§	§
3	morpholine	6 h	-20°C	1.2	22%	29%	60%

***Key*:** § = no precursor recovered. [a] % yield based upon recovered **130**, [b] % yield based upon recovered **126**.

Table 4.3 Investigations into the Knoevenagel condensation reaction.

The reaction was attempted a second time using 1.5 equivalents of **126**, the base was added at 0°C and the resulting mixture stirred at 3°C for 120 hours (entry 2). On this occasion, work-up failed to yield any product whatsoever. In addition, no aldehyde was recovered, instead, a low running compound (R_f = 0.21 in 40% diethyl ether/petrol) was isolated in a 15% yield and was presumed to be the cyclised Baylis-Hillman by-product. In the case of entry 3, a mixture of **130** and **126** was again dissolved in dichloromethane and cooled to -20°C. The reaction mixture was subsequently treated with morpholine and stirred at -20°C for a total of six hours. Work-up, followed by purification gave the desired trisubstituted olefin **129** (Figure 4.11, page 73) in a somewhat improved yield of 22%.

Due to the small amount of **129** obtained (23 mg), it was only possible to obtain a proton NMR spectrum for analysis. On comparison to the ^{1}H NMR spectrum of aldehyde **130**, it was apparent that the olefinic signals for the double bonds between C-6 (δ 6.89) and C-7 (δ 6.09) and C-11 and C-12 (δ 5.33, multiplet) were present. A triplet resonance at δ 6.67 (J = 7.4 Hz) was identified as the trisubstituted double bond proton at C-3 coupling to the methylene protons at C-4. The protons at the chiral centres, CH-13 and CH-5', were observed as a quartet at δ 4.33 (J = 13.4, 6.3 Hz) and as a double quartet at δ 3.80 (J = 11.9, 6.1, 2.1 Hz), respectively. Further confirmation of the structure was given by the presence of resonances corresponding to those expected for the *tert*-butyldimethylsilyl protecting groups. The low yield of **129** made it difficult to determine with any accuracy the exact ratio of geometric isomers. (TLC analysis had indicated the presence of two isomers).



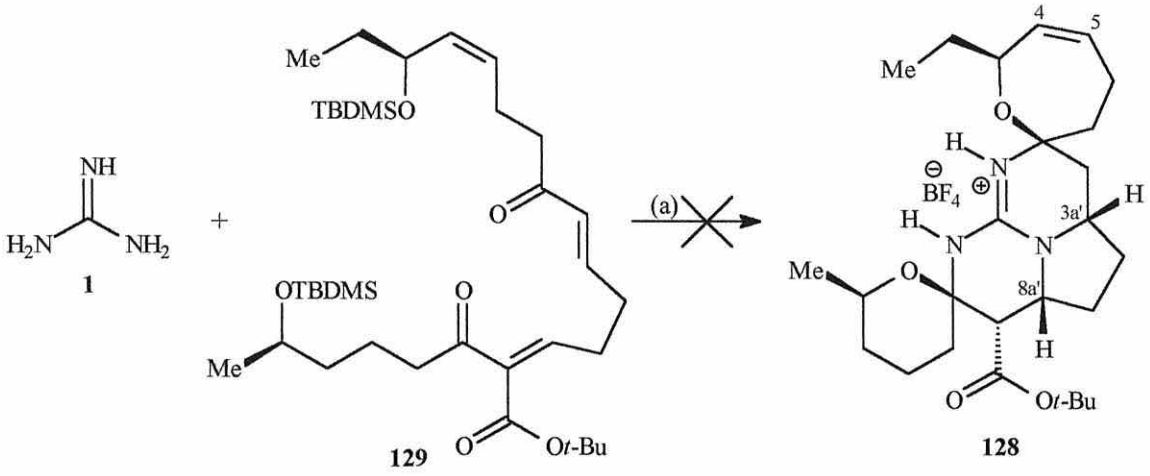
129

Figure 4.11 Knoevenagel reaction product **129**.

4.5.2 GUANIDINE CYCLISATION

At this juncture, it was decided to attempt the double Michael addition of guanidine to **129** and effect the *bis* spirocyclisation, thereby generating the functionalised pentacycle **128** (Scheme 4.11, page 74). The standard conditions for the reaction were adopted, which involved the addition of a solution of freshly prepared guanidine in DMF to *bis*-α,β-unsaturated ketone **129**, also in DMF at 0 °C. After stirring for seven hours at this

temperature, the reaction was quenched with water, treated with a solution of methanolic HCl and left to stir overnight. Following extraction of the product and counterion exchange (effected by vigorous stirring of a dichloromethane solution of the crude material in the presence of a saturated sodium tetrafluoroborate solution), the crude product was purified on silica gel yielding three separate fractions. Analysis of the fractions by proton NMR spectroscopy indicated that the expected reaction had not taken place. This was apparent as the expected resonances corresponding to the double bond between carbons 4 and 5 and the signals indicative of the pyrrolidine protons, 3a' and 8a' (typically at δ 5.20), were not present. This reaction could not be repeated as no further supplies of the *bis*-α,β-unsaturated ketone **129**, were available. Thus, it was decided to adopt an alternative synthetic strategy, due to the low yields of reaction precursors and the prohibitive cost of repeating these reactions.



***Reagents and Conditions*:** (a) (i) Guanidine, DMF, 0 °C, 7 h; (ii) Methanolic HCl; (iii) CH_2Cl_2, saturated $NaBF_4$.

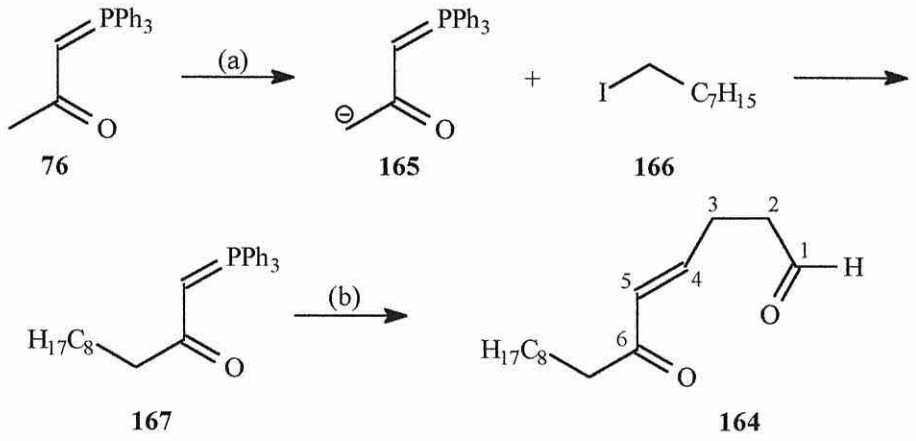
Scheme 4.11 The attempted guanidine cyclisation reaction to access pentacycle **128**.

4.6 Further Studies On The Knoevenagel Condensation Reaction

As previously discussed, Snider and Shi[34] have reported product yields in the magnitude of 60-64% for the Knoevenagel reaction along with the recovery of unreacted starting materials. It was therefore both bewildering and frustrating when the reaction could not be repeated and in each case, the majority of the starting aldehyde was not recovered. In an attempt to determine the optimum conditions for the Knoevenagel

reaction, a study was undertaken to ascertain whether electronic effects within the β-keto ester could have any effect on the outcome of the reaction. In a previous study towards the batzelladine alkaloids,[49d] aldehyde **164** was prepared, and was deemed to be a suitable aldehyde for use in this investigation (Scheme 4.12).

Aldehyde **164** was synthesised in two steps starting from acetylmethylene triphenylphosphorane **76**. Deprotonation of the α-methyl group of **76** using *n*-butyllithium at -78°C gave the corresponding enolate **165** as a deep red coloured solution. Following stirring for thirty minutes, the enolate was quenched with *n*-octyl iodide **166**, to yield, after extraction, phosphorane **167**. The crude phosphorane was dissolved in THF and treated with three equivalents of freshly prepared succinaldehyde which after stirring for 72 hours, was extracted and purified, affording aldehyde **164** in 48% overall yield (Scheme 4.12).



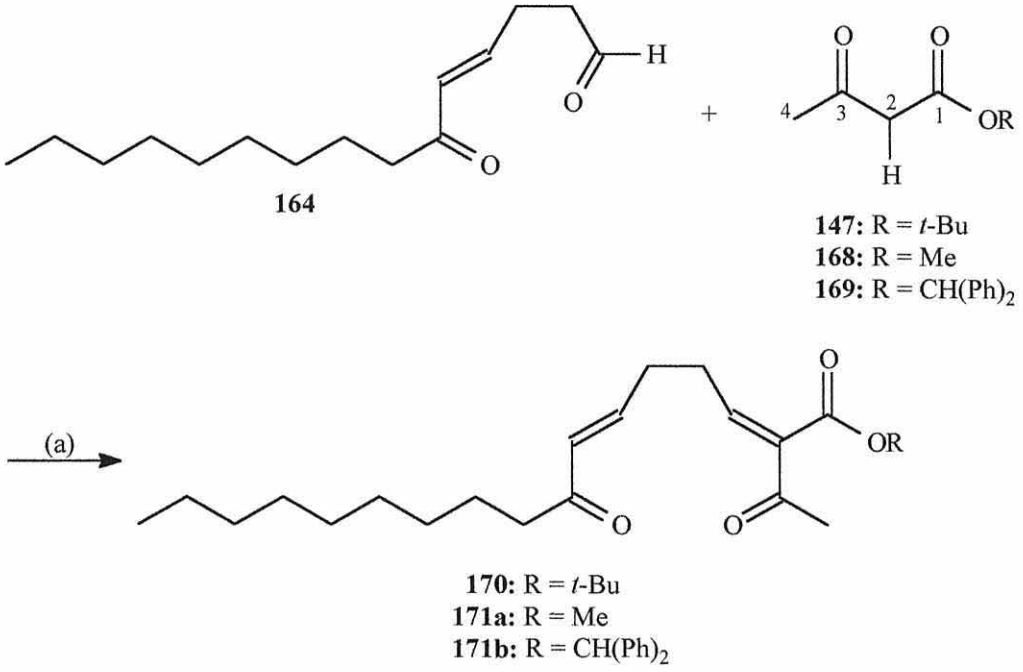
***Reagents and Conditions*:** (a) *n*-BuLi, THF, -78°C; (b) 3 eqv. succinaldehyde, THF, 72 h.

Scheme 4.12 Synthesis of unsaturated aldehyde **164** for use in the Knoevenagel reaction studies.

The structure of **164** was confirmed by NMR analysis and proved, as expected, to be the desired *trans* isomer. The olefinic protons appeared as resonances at δ 6.81 (CH-4, dt) and δ 6.13 (CH-5, d), and for each signal the coupling constant was calculated at 15.9 Hz, which is within the normal range for a *trans* alkene. A resonance corresponding to the aldehyde functionality was observed at δ 9.81 and the remainder of the signals were consistent with those expected for the product.

It was intended to react unsaturated aldehyde **164** with a series of β-keto esters under the exact conditions adopted by Snider and Shi,[34] in order to generate a Knoevenagel

product as shown in Scheme 4.13 below. Three β-keto esters were selected for the study which differed in the electronic nature of the R group. In theory, the greater the electron withdrawing nature of the ester group, the more acidic the protons at C-2 will be. It was anticipated that increased proton acidity would encourage the Knoevenagel condensation.



164

147: R = *t*-Bu
168: R = Me
169: R = $CH(Ph)_2$

170: R = *t*-Bu
171a: R = Me
171b: R = $CH(Ph)_2$

***Reagents and Conditions*:** (a) Base, CH_2Cl_2, -78°C to -20°C, (or -20°C).

Scheme 4.13 The attempted synthesis of the trisubstituted olefin.

For the initial reaction (entry 1, Table 4.4, page 77), aldehyde **164** was dissolved in dichloromethane, treated with *tert*-butylacetoacetate **147**, and cooled to -78°C. A solution of piperidine in dichloromethane was added slowly at -78°C and the resulting mixture allowed to warm to -20°C. After stirring for 22 hours, the reaction mixture was quenched and extracted. An attempted purification yielded several fractions which were analysed by proton NMR spectroscopy. Examination of the spectra indicated the formation of a trace amount of the desired product **170**, contaminated with unidentified by-products, which could not be separated, even after further purification on silica gel. The by-products were not characterised but appeared to be a mixture of the previously discussed cyclised Baylis-Hillman product along with other polymerisation/decomposition products.

Substituting the base for 2,6-dimethylpiperidine, a hindered base, whose steric bulk was expected to prevent the aldol addition step of the Baylis-Hillman reaction (entry 2),

failed to yield the condensation product even after stirring the reaction mixture for a total of 160 hours. These reaction conditions resulted in the total decomposition of the starting aldehyde.

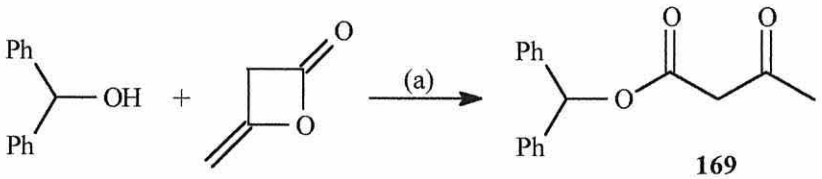
Entry	Base	R	Temperature	Duration	Yield
1	piperidine	*t*-Bu	-78°C to -20°C	22 h	Trace[a]
2	2,6-dimethyl piperidine	*t*-Bu	-78°C to -20°C	160 h	0%
3	piperidine	Me	-78°C to -20°C	22 h	Trace[a]
4	piperidine	$CH(Ph)_2$	-78°C to -20°C	2 h	Trace[a]
5	piperidine acetate	$CH(Ph)_2$	-78°C to -20°C	2 h	Trace[a]
6	piperidine	$CH(Ph)_2$	-20°C	5 h	0%

Table 4.4 Investigations into the Knoevenagel condensation employing the reaction conditions reported by Snider and Shi.[34] [a] NMR indicated the presence of a trace amount of product, in typically 2%-3% yield, contaminated with an inseparable by-product(s).

The three electron donating methyls of the *tert*-butyl group in **147** increase the electron density within the ester group which probably decreases the overall acidity of the C-2 methylene protons. In contrast, methylacetoacetate **168** has only one such electron donating group influencing the electron density of the ester group and would consequently be expected to be more reactive in the Knoevenagel reaction. Hence, aldehyde **164** was reacted with methylacetoacetate (entry 3), under identical conditions to those described for entry 1. Unfortunately, only a trace amount of impure product **171a** was obtained, again contaminated with inseparable by-products.

The methylene group of benzhydryl 3-oxobutanoate **169**, was identified as having C-2 methylene protons of increased acidity due to the electron withdrawing effects of the two aromatic rings. Thus, β-keto ester **169**, was synthesised following the procedure of Nudelman and co-workers[106] and obtained in a satisfactory 83% yield (Scheme 4.14, page 78). Confirmation of the structure was obtained through NMR analysis where the proton adjacent to the two phenyl groups appeared as singlet resonance at δ 6.95. The methylene group was identified as a singlet appearing at δ 3.57, whilst the terminal methyl resonance

occurred as a singlet at δ 2.24. A multiplet between the range δ 7.41-7.30 integrating to ten, corresponded to the C–H resonances of the aromatic rings.



169

Benzhydrol Diketene

***Reagents and Conditions*:** (a) THF, DMAP, 20°C, 30 min.

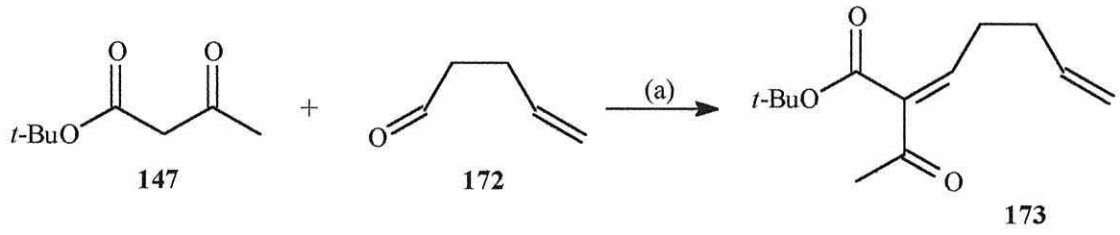
Scheme 4.14 Preparation of benzhydryl 3-oxobutanoate, **169**.

Having successfully prepared **169**, it was subsequently reacted with aldehyde **164** under identical conditions to those described for entries 1-3, with the exception that the reaction was quenched following stirring for two hours (entry 4). The TLC after two hours had indicated the formation of a product (R_f = 0.08 in 30% diethyl ether/petrol) running just below the aldehyde (R_f = 0.11 in 30% diethyl ether/petrol). These products were isolated following purification by column chromatography and subsequently analysed by proton NMR spectroscopy. The lower running fraction contained a trace amount of the desired product **171b** contaminated with the familiar, inseparable impurity. The ^{1}H NMR spectrum of the higher running fraction, which was expected to be unreacted aldehyde, was not consistent with that of pure **164** and therefore, must be a decomposition/polymerisation product. An almost identical result was obtained when the base was exchanged for piperidine acetate (entry 5). Again proton NMR analysis showed only a trace of contaminated product which could not be further purified.

In the case of entry 6, a different procedure was adopted where aldehyde **164** and β-keto ester **169** were dissolved in dichloromethane at -20°C in separate flasks. A solution of piperidine in dichloromethane was added to **169**, which was then left to stir for fifteen minutes after which the contents of the flask were transferred to the flask containing the aldehyde, whilst maintaining the temperature at -20°C. After five hours the reaction mixture was quenched, extracted and purified, however, on this occasion, a small amount of starting aldehyde was recovered, but no trace of any product was evident.

On comparison to the work of Snider and Shi,[34] it was hoped that the reaction could be repeated in a relatively straightforward manner whilst yielding satisfactory amounts of the desired products. It was therefore somewhat disappointing and surprising that no significant yield of any condensation product could be obtained. Consequently, with no apparent success, this specific investigation into the Knoevenagel reaction was discontinued.

During a recent study to develop a new methodology towards the synthesis of the batzelladine alkaloids, a specific Knoevenagel condensation reaction step was shown to proceed in an excellent 85% yield.[107] The simple aliphatic aldehyde **172** was reacted with *tert*-butylacetoacetate **147**, using the procedure reported by Lehnert[102] in 1974, with the product being diene **173**, as shown in Scheme 4.15 below.



***Reagents and Conditions*:** (a) CCl_4, $TiCl_4$, 0 °C, THF, pyridine/THF, 48 h.

Scheme 4.15 The Knoevenagel condensation reaction using Lehnert's[102] procedure.

As these reaction conditions represented an alternative method for the synthesis of *bis*-α,β-unsaturated ketone **170**, an identical procedure was adopted for the reaction between aldehyde **164** and acetate **147** (see Scheme 4.13, page 76). To this end, titanium tetrachloride was added to carbon tetrachloride at 0 °C and the resulting yellow solution transferred to a separate flask containing anhydrous THF, previously cooled to 0 °C, yielding a yellow precipitate of [tetrachloro*bis*(tetrahydrofuran)titanium)]. A mixture of **164** and *tert*-butylacetoacetate **147**, in THF was added to the complex, again at 0 °C, giving a red coloured solution which after stirring for ten minutes was treated with a solution of pyridine in THF, very slowly, over a period of 1.5 hours, whilst maintaining a constant 0 °C. The resulting mixture was left to stir at room temperature for sixteen hours, then quenched with water and extracted to afford a crude oil. Unfortunately, the proton NMR spectrum of this oil showed no olefinic signals whatsoever and was subsequently found to

be a mixture consisting of unreacted *tert*-butylacetoacetate **147** and an unidentified decomposition product.

4.7 An Alternative Approach To A Functionalised Pentacycle

Having gained little success with the Knoevenagel approach, an alternative strategy to a functionalised polycyclic framework was devised. On this occasion, a retrosynthesis of *bis*-α,β-unsaturated ester **129**, disconnecting at the trisubstituted olefin, gave the previously prepared aldehyde **130** along with phosphonate **174**. A further disconnection between the methine carbon and the ketone group of **174** yielded a second phosphonate **175** and protected ester **176** as synthons (Figure 4.12).

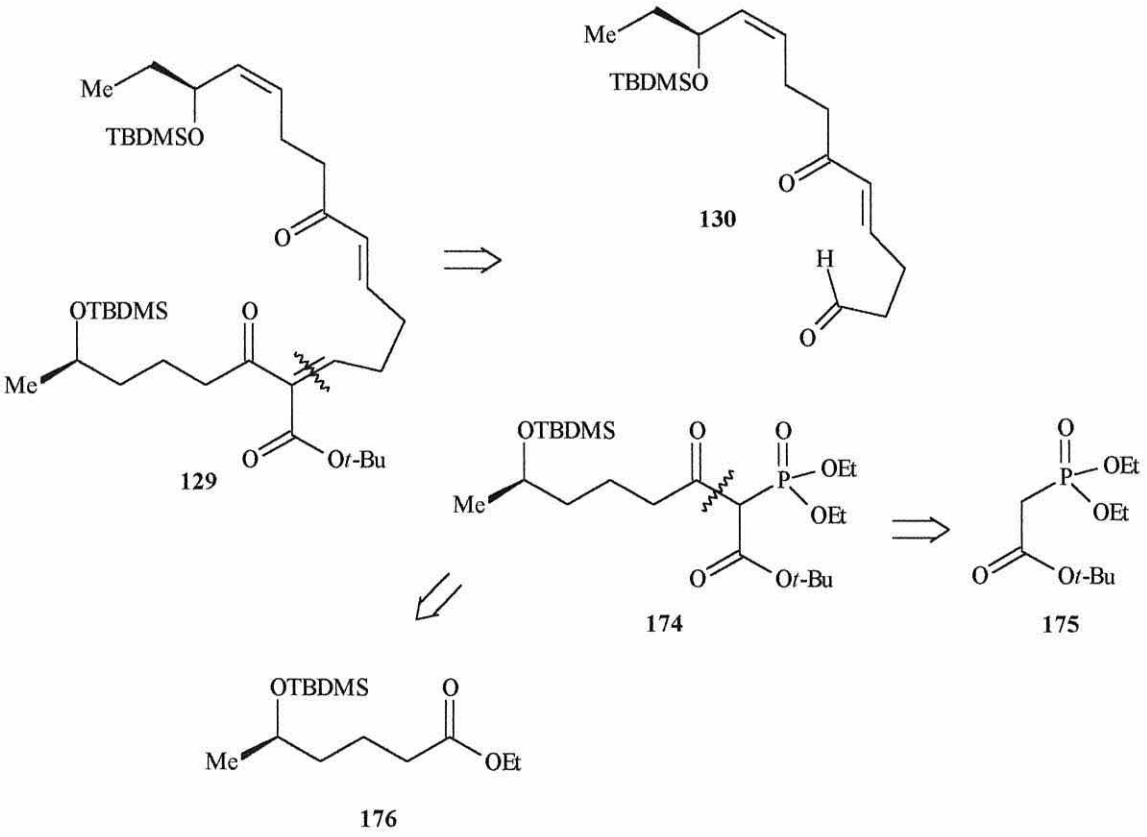
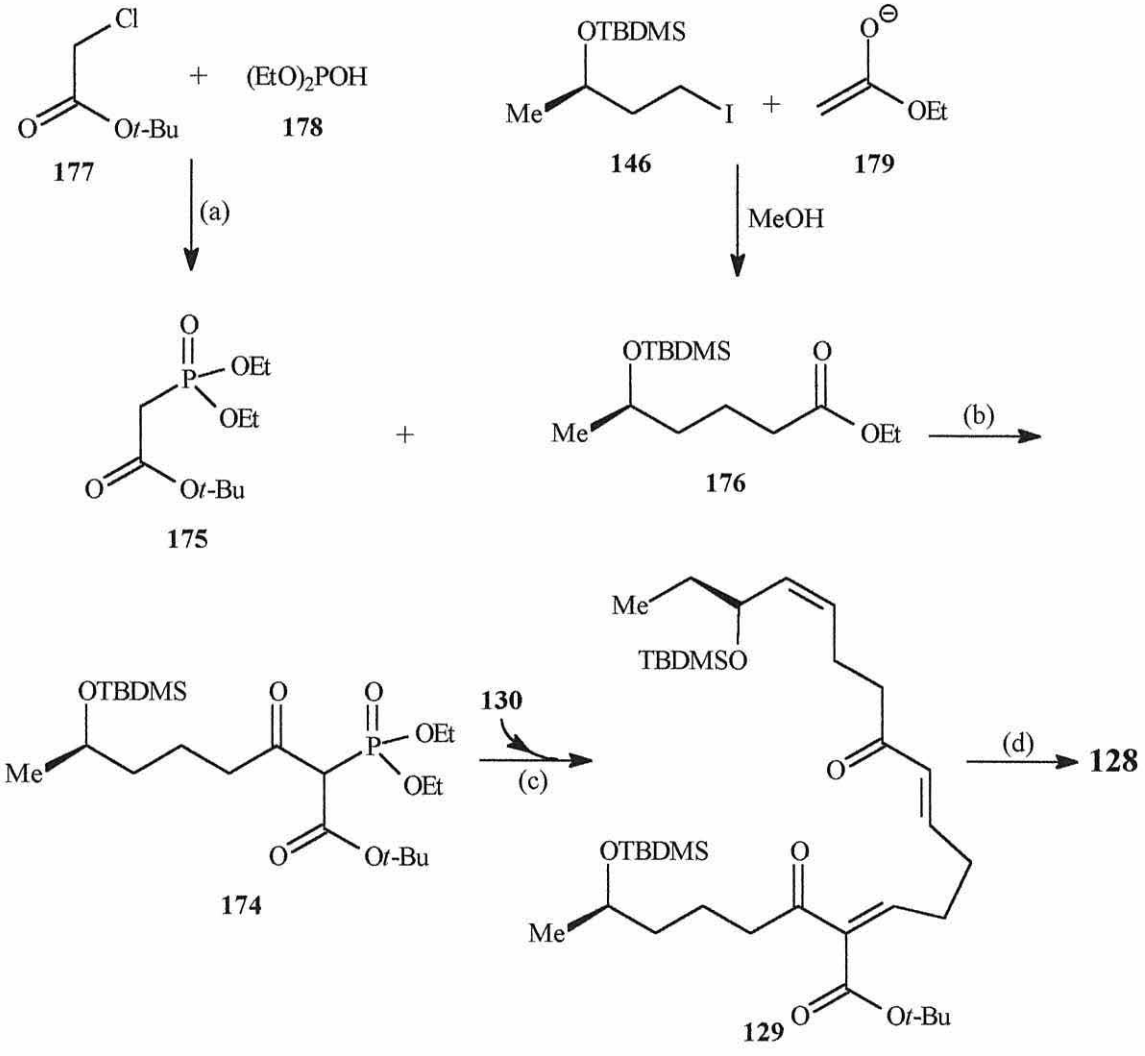


Figure 4.12 An alternative retrosynthesis of *bis*-α,β-unsaturated ester **129**.

It was anticipated that the convergent synthesis of **129** could be accomplished following the preparation of the two precursors, aldehyde **130** and keto phosphonate **174**

(Scheme 4.16). A reaction between *tert*-butylchloroacetate **177** and diethyl phosphite **178** would enable the preparation of phosphonate **175**, whilst protected ester **176** can be synthesised in a single step by the reaction of known chiral iodide **146** with the enolate **179**, of ethyl acetate. Addition of the carbanion of **175** to ester **176** would give phosphonate **174** which can then be reacted with unsaturated aldehyde **130**, by means of the Wadsworth-Emmons[108] variation of the Wittig reaction, to afford the desired *bis*-α,β-unsaturated ketone **129**. Finally, the double Michael addition of guanidine and subsequent *bis*-spirocyclisation would furnish the desired ester-substituted pentacycle **128**.

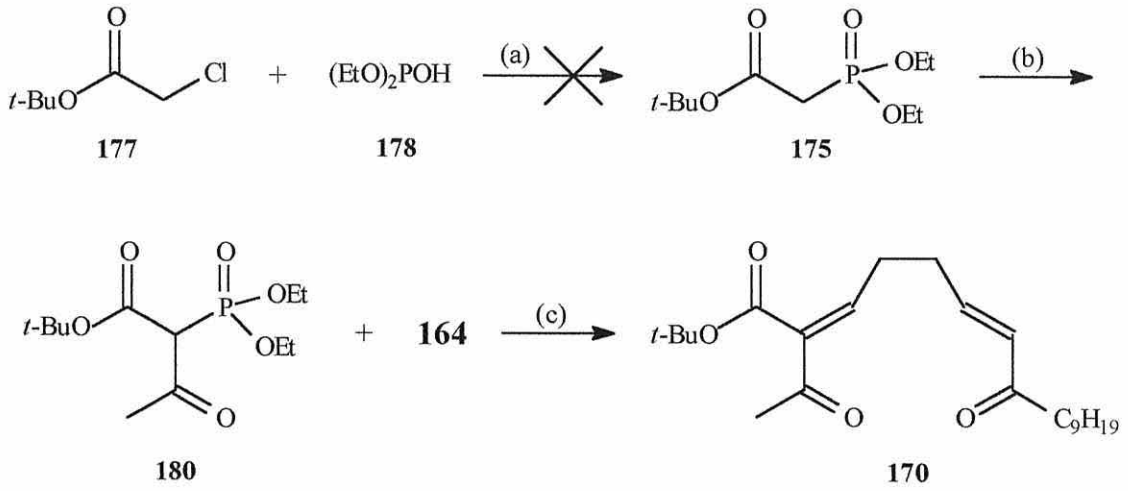


***Reagents and Conditions*:** (a) NaH, *t*-BuOH, 60°C, 2 h, **177**, 10°C, 1 h; (b) NaH, THF, 0°C, 3 h; (c) NaH, Et_2O, 0°C, **130**, 16 h; (d) (i) Guanidine, DMF, 0°C, 7 h; (ii) Methanolic HCl; (iii) CH_2Cl_2, sat. $NaBF_4$.

Scheme 4.16 The convergent synthesis of *bis*-α,β-unsaturated ester **129**.

Initial development of the proposed synthesis was aimed towards the preparation of the trisubstituted olefin **170** (Scheme 4.17) whose synthesis was originally attempted during the Knoevenagel reaction studies (see Scheme 4.13, page 76). The reasoning behind this decision being that supplies of aldehyde precursor **164** were readily accessible. In addition, a successful preparation of **170** would open up an alternative approach to the synthesis of the batzelladine alkaloids.

Following the procedure of Magerlein and Kagan,[109] diethylphosphite was added to a suspension of sodium hydride in *tert*-butyl alcohol forming a gelatinous solid, dissolution of which was achieved by heating the reaction mixture at 60°C for two hours. The resulting solution was cooled to 10°C, after which *tert*-butylchloroacetate **177** was slowly added yielding a suspension which was stirred at this temperature for one hour. Filtration, followed by distillation gave an extremely disappointing yield (4%) of the desired phosphonate **175**, with the majority of the distillate identified as being a mixture of unreacted starting materials.

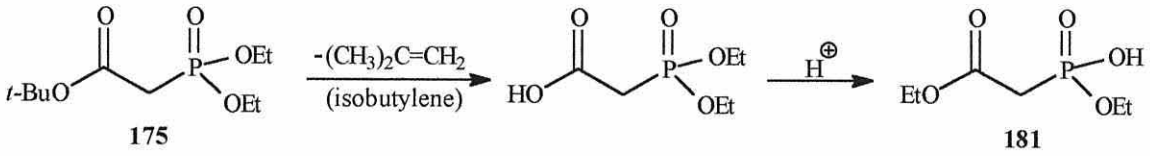


***Reagents and Conditions*:** (a) NaH, *t*-BuOH, 60°C, 2 h, then **177**, 10°C, 1 h; (b) NaH, THF, 0°C, AcCl, 3 h; (c) NaH, Et_2O, 0°C, 16 h.

Scheme 4.17 The proposed synthesis of trisubstituted olefin **170**.

A second attempt at the reaction was made using potassium *tert*-butoxide as the base. Following the addition of **177** to diethylphosphite, the mixture was left to stir at room temperature overnight, after which the solvent was evaporated leaving a white coloured suspension which was filtered through celite to remove any particulate matter.

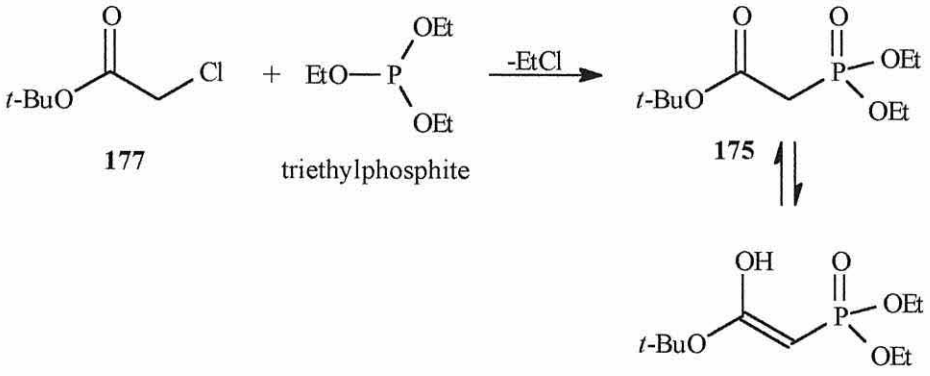
Distillation of the filtrate yielded three fractions, which as in the case of the previous reaction, were found to be a mixture of unreacted starting reagents, but on this occasion contaminated with an unidentified by-product. It was surprising that this reaction could not be repeated, although, at high temperatures, above 140°C, elimination of isobutylene followed by transesterification of one of the ester groups in **175** can occur to yield ethyl ester **181** (Scheme 4.18).



175 **181**

Scheme 4.18 The transesterified product **181** derived from phosphonate **175**.

A survey of the literature revealed a further three potential methods[110] for the synthesis of phosphonate **175**. For reasons of simplicity, the method of Kenner *et al.*[110a] was adopted (Scheme 4.19). Thus, a mixture of **177** and triethylphosphite, under a nitrogen atmosphere was heated at 100°C for ten minutes and then carefully at 140°C for a further three hours. Distillation under reduced pressure effected removal of any unreacted starting materials, leaving the desired phosphonate as a yellow oil in a satisfactory 73% yield. The reaction itself proceeded *via* the Arbuzov rearrangement with elimination of ethyl chloride affording a 95% mixture (within the limits of ^{13}C NMR) of the desired phosphonate and its corresponding enol.

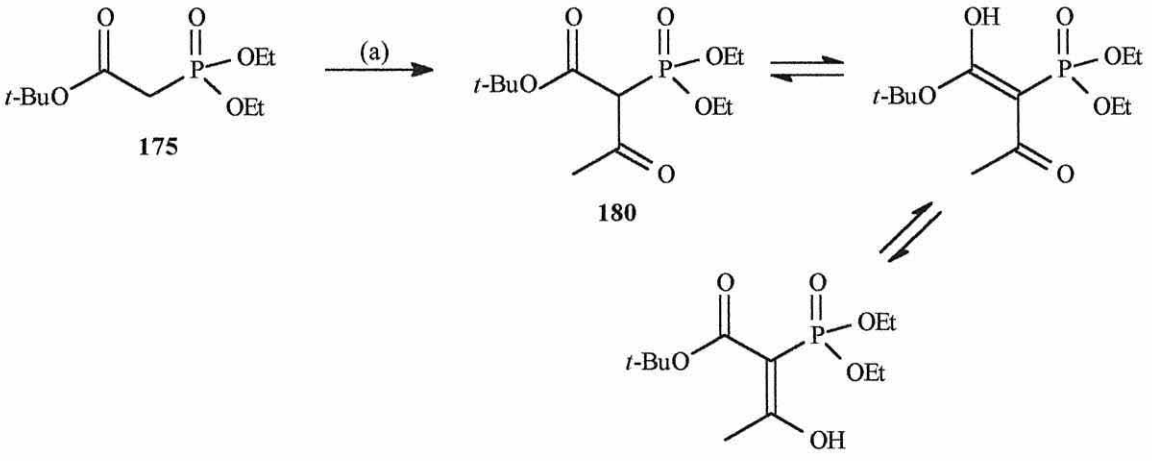


177 triethylphosphite **175**

Scheme 4.19 Preparation of phosphonate **175** and its corresponding enol *via* the Arbuzov rearrangement.

Analysis of the IR spectrum of **175** confirmed the presence of the ester carbonyl and also indicated the presence of the enolic form, showing a broad O–H stretch at 3484 cm^{-1}. The proton NMR spectrum demonstrated a double quartet resonance at δ 4.17 corresponding to the ethyl methylene protons coupling to phosphorus (J_{P-H} = 8.0 Hz). The methylene adjacent to the ester carbonyl was observed as a doublet at δ 2.28 (J_{P-H} = 21.4 Hz). A singlet resonance at δ 1.47 was identified as the three methyls of the *tert*-butyl group whilst the two remaining methyl groups occurred as a triplet, integrating to six, at δ 1.35. Further confirmation of the existence of **175** was given by the mass spectrum, which showed a peak at 253 Daltons corresponding to the $[M+H]^+$ mass ion.

Having prepared the phosphonate, the next step in the proposed synthesis was acylation of the methylene carbon of **175** to give keto phosphonate **180**. It was envisaged that formation of the enolate followed by treatment with acetyl chloride would yield **180**. Examples of similar reactions in the literature were scarce, although one such procedure was reported by Sutherland and co-workers.[111] Using their described procedure, **175** was dissolved in THF and treated with two equivalents of sodium hydride in small portions. A solution of freshly distilled acetyl chloride, also in THF, was added carefully and the mixture stirred for three hours. Extraction, followed by purification on silica gel, gave the desired keto phosphonate as an equilibrium mixture with its two enolic forms (Scheme 4.20). Keto phosphonate **180** was obtained in 33% yield, which compared favourably with the 25% yield of acylated product, as quoted by Sutherland and co-workers.[111]



***Reagents and Conditions*:** (a) NaH, THF, 0 °C, AcCl, 3 h.

Scheme 4.20 Keto phosphonate **180** and its two possible enolic forms.

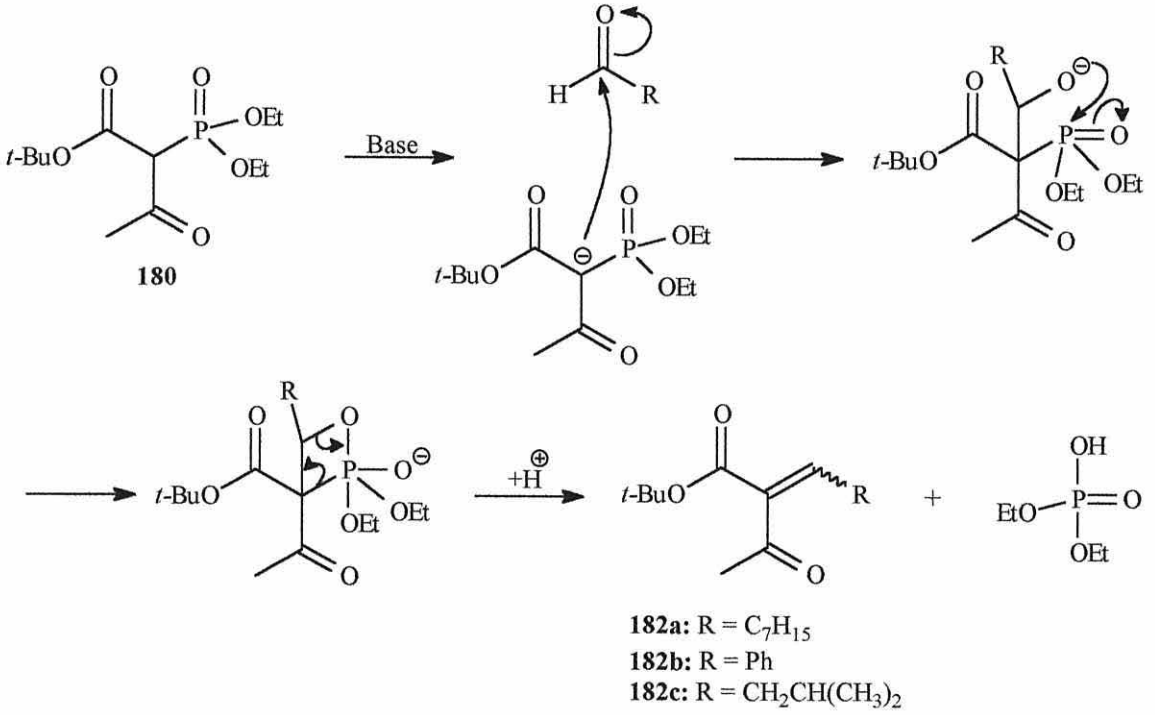
Evidence of the successful formation of the keto phosphonate was provided on the basis of ^{1}H and ^{13}C NMR spectroscopy, with the resonances for the major tautomer of **180** interpreted below. A multiplet integrating to four at δ 4.13 was identified as the ethyl methylenes with the adjacent methyls occurring as an individual triplet at δ 2.41, (6 H, $J = 7.0$ Hz). The methine proton α to the ester carbonyl appeared as a doublet coupling to the adjacent phosphorus atom, resonating at δ 2.41($J_{P\text{-}H} = 2.8$ Hz), whilst a singlet at δ 1.49 corresponded to the *tert*-butyl group. Analysis of the carbon spectrum showed the presence of two carbonyl groups, the ketone at δ 196.69 and the ester carbonyl at δ 163.13. The methine carbon resonance was observed at δ 63.76 and the methyl adjacent to the ketone appeared at δ 22.33. The remainder of the signals were consistent with the expected product. (The carbon NMR also showed resonances due to the enolic forms but only the signals corresponding to the major tautomer are described). The low yield of **180** can be explained by considering the stability of phosphonate precursor **175**. Upon deprotonation the negative charge of the resulting carbanion can delocalise into the ester group, thereby, stabilising the charge and decreasing the overall nucleophilicity of the carbanion.

Prior to attempting the Wadsworth-Emmons reaction between keto phosphonate **180** and aldehyde **164**, it was decided to first develop reliable and reproducible reaction conditions. To this end, keto phosphonate **180** was reacted with a series of aldehydes under varying conditions, the results being summarised in Table 4.5.

Entry	Aldehyde	R	Eqv.	Base	Eqv.	Solvent	Yield
1	Octanal	C_7H_{15}	1.1	NaH	1.1	Et_2O	0%
2	Benzaldehyde	Ph	1.5	KO*t*-Bu	1.1	THF	0%
3	Isovaleraldehyde	$CH_2CH(CH_3)_2$	1	KO*t*-Bu	1.4	THF	1%
4	Isovaleraldehyde	$CH_2CH(CH_3)_2$	1	KO*t*-Bu	0.9	THF	0%
5	Isovaleraldehyde	$CH_2CH(CH_3)_2$	1	LDA	1.1	THF	0%
6	Isovaleraldehyde	$CH_2CH(CH_3)_2$	1.2	NaH	1	DME	1%
7	Benzaldehyde	Ph	1.2	NaH	1	DME	0%
8[a]	Isovaleraldehyde	$CH_2CH(CH_3)_2$	3	piperidine	0.3	CH_2Cl_2	6%

Table 4.5 Investigations into the Wadsworth-Emmons reaction.[a] Knoevenagel reaction.

An initial effort towards olefin **182a** was attempted by the reaction between **180** and the aliphatic aldehyde, octanal, using sodium hydride as base (entry 1, Table 4.5). Sodium hydride was suspended in diethyl ether at 0 °C and treated with a solution of **180** in THF in order to generate the corresponding carbanion. After stirring for thirty minutes, octanal was added and the resulting mixture left to stir at room temperature for a further sixteen hours. At this stage, addition of the anion to the aldehyde carbonyl should occur, which, after formation of the four membered transition state, fragments to yield the desired olefin and diethyl phosphate as the by-product (Scheme 4.21). Unfortunately, after work-up, analysis by proton NMR spectroscopy of the crude material recovered failed to show any evidence of the expected alkene **182a**.

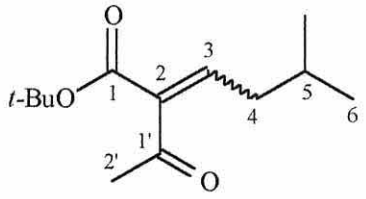


182a: R = C_7H_{15}
182b: R = Ph
182c: R = $CH_2CH(CH_3)_2$

Scheme 4.21 The mechanism of the Wadsworth-Emmons reaction.

A second attempt was made using potassium *tert*-butoxide (1.5 equivalents) and benzaldehyde with THF as the solvent (entry 2, Table 4.5). The reaction mixture was stirred at 0 °C for one hour and then at reflux for a further four hours. After work-up, a crude yellow oil was obtained, the proton NMR of which again showed no evidence of the desired olefin **182b**. In the case of entry 3, isovaleraldehyde was added to the keto phosphonate at room temperature and then heated at reflux for two hours. Following

work-up and purification, the expected olefinic product was isolated in an extremely disappointing 1% yield (Figure 4.13). On this occasion, the ^{1}H NMR spectrum showed a triplet resonance (J = 7.8 Hz) corresponding to the alkene proton at δ 6.80. The methylene protons at C-4 were identified as a triplet (J = 7.0 Hz) at δ 2.19, whilst the methine proton at C-5 appeared as a complex multiplet at δ 1.82. The carbon NMR confirmed the presence of the double bond, showing two signals which were identified as C-2 and C-3 at δ 146.31 and δ 145.78, respectively. The remainder of the carbon spectrum was consistent with the structure of trisubstituted olefin **182c**.

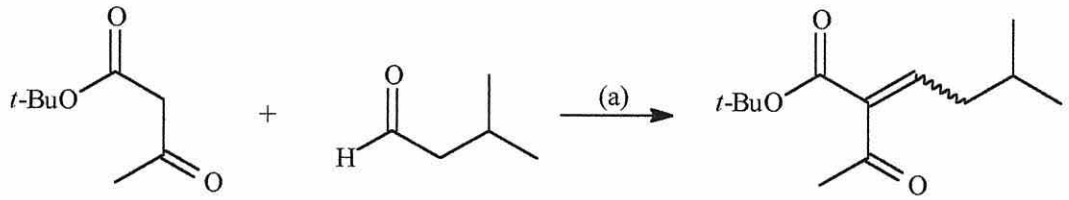


182c

Figure 4.13 The Wadsworth-Emmons reaction product **182c**.

A further reaction attempt was made (entry 4), on this occasion using 0.9 equivalents of base. The reaction mixture was heated at reflux for nine hours and then at room temperature for another fourteen hours. Following work-up and purification no trace of the expected alkene was obtained. Changing the base to LDA (entry 5) again failed to result in the formation of any of the desired product whatsoever.

A report by Sakai *et al.*[112] demonstrated a similar reaction to that described above using the base sodium hydride in 1,2-dimethoxyethane (DME) at reflux. These conditions were adopted for entry 6, where a mixture of base, keto phosphonate and aldehyde were heated at reflux for five hours. After cooling, the resulting solution was quenched, extracted and purified, unfortunately yielding only a trace amount of the desired olefin. Identical conditions were employed in the case of entry 7, this time using benzaldehyde, but once again no olefinic material was obtained. The olefin in the case of entry 8, was synthesised under Knoevenagel reaction conditions where *tert*-butylacetoacetate and isovaleraldehyde were reacted to form unsaturated product **182c** (Scheme 4.22, page 88). A poor yield of the alkene was obtained (6%) which was nevertheless useful in confirming the exact nature of the product resulting from entry 3.



147 isovaleraldehyde **182c**

***Reagents and Conditions*:** (a) Piperidine, CH_2Cl_2, -78 °C to -15 °C, 24 h.

Scheme 4.22 Preparation of trisubstituted olefin **182c** *via* the Knoevenagel reaction.

With no positive results from this investigation, the proposed scheme towards pentacycle **128** was not pursued any further. The Wadsworth-Emmons approach was adopted ahead of a Wittig approach as phosphonates are generally more reactive than their corresponding phosphoranes and phosphonium salts. Previous work[113] had shown that phosphorane **183** (Figure 4.14, page 89), which represents the Wittig equivalent of keto phosphonate **180**, was inactive towards the Wittig reaction. It was anticipated that the overall stability of keto phosphonate **180** would lead to a decrease in reactivity but not to the actual extent observed.

The inactivity of **180** was probably a result of the stabilisation of the carbanion generated after deprotonation of the methine carbon, as was the case in the previous reaction step. However, on this occasion, the negative charge was doubly stabilised through delocalisation into the ester group (1), or the keto group (2), (Figure 4.14, page 89). This again has the effect of decreasing the nucleophilicity of the carbanion thus further decreasing the probability of a reaction occurring.

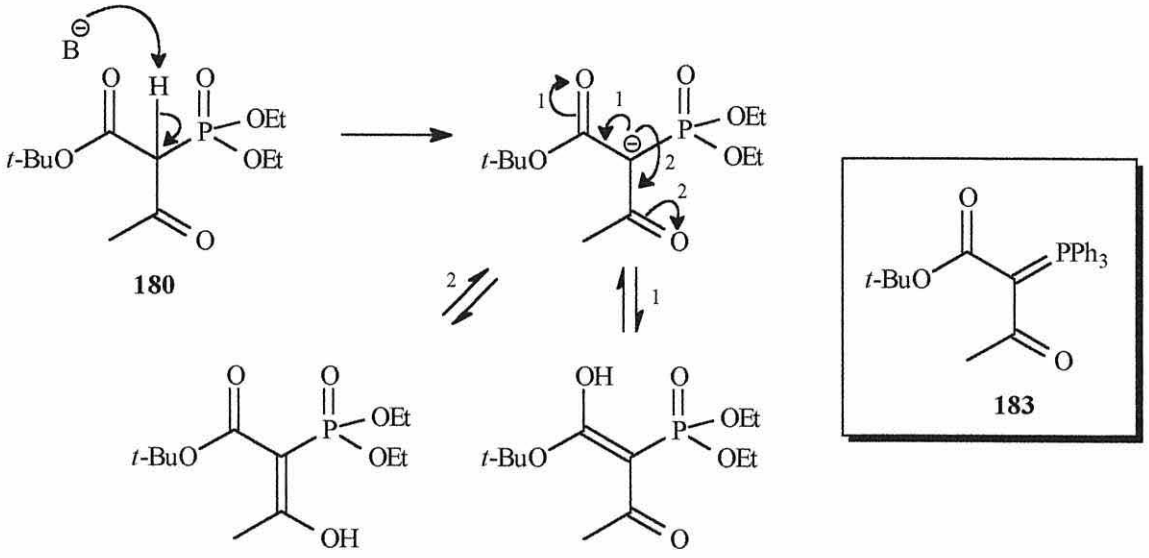


Figure 4.14 Diagrammatic representation of the double stabilisation of the phosphono enolate. The insert shows the corresponding, less active phosphorane **183**.

4.8 CONCLUSION

In this chapter, the attempted synthesis of the pentacyclic core of ptilomycalin A has been discussed. Although unsuccessful, the pentacyclic precursor, *bis*-α,β-unsaturated ketone **129** was prepared, albeit in rather low yield. The small quantity of **129** available prevented a study of the guanidine cyclisation step and the determination of the diastereoselectivity of the reaction. The effectiveness and reproducibility of the Knoevenagel condensation reaction in the preparation of *bis*-α,β-unsaturated systems such as **129** remains questionable, especially on comparison with the results reported by Snider and Shi.[34] The fact that the investigations undertaken failed to address the problems, or indeed, yield a definite outcome, further serve to complicate an already bewildering issue.

It was particularly disappointing that the Wadsworth-Emmons approach towards **129** failed to result in a successful synthesis of the *bis*-α,β-unsaturated ketone. Although problems were anticipated during the planning stage, it was hoped that they could be overcome during the synthetic stage. This lack of success also prevented the intended study of the guanidine cyclisation to the ester substituted pentacyclic nucleus **128**, of ptilomycalin A.

CHAPTER 5

SYNTHESIS OF A CHIRAL PENTACYCLIC MODEL OF PTILOMYCALIN A

5.1. INTRODUCTION

Having been unable to prepare the C-14 ester substituted pentacycle **128**, efforts were instead concentrated towards the synthesis of a chiral pentacyclic model lacking the C-14 ester group, as depicted in Figure 5.1 below. The preparation of **184** would allow an investigation into the double Michael addition of guanidine to a *bis*-α,β-unsaturated ketone precursor and also the determination of whether cyclisation would result in the induction of the correct relative stereochemistry about the six and seven membered rings of the pentacyclic core. That is, the formation of the *syn* diastereomer of **184** due to the *cis* orientation of the bonds to oxygen at C-8 and C-15 and also the pyrrolidine protons at C-10 and C-13. In addition, it was essential to maintain the correct stereochemistry about the ethyl substituent at C-3 and the methyl substituent at C-19 (Figure 5.1). The unambiguous determination of the relative stereochemistry of the pentacycle can be accomplished by means of 1D and 2D NMR spectroscopy.

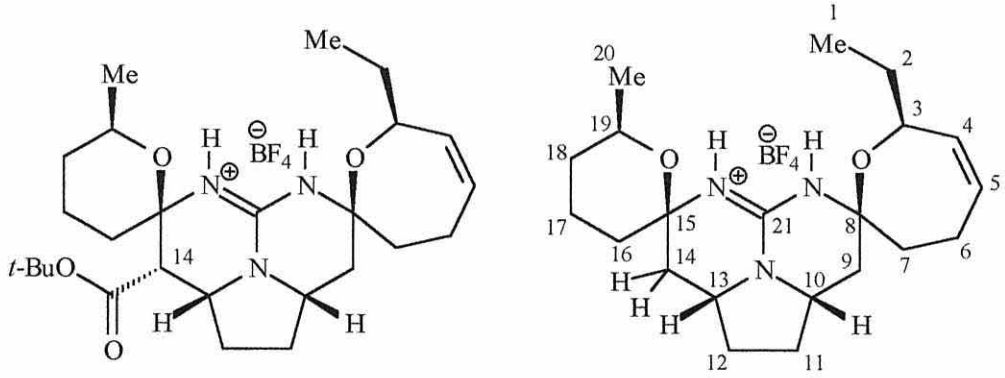


Figure 5.1 A comparison of the pentacycles **128** and **184** showing the difference in substitution at C-14.

As previously described in Chapter 2 (Section 2.2.2, page 18), the pentacyclic core of ptilomycalin A was shown to possess a degree of biological activity; it was therefore necessary to determine whether pentacycle **184** also demonstrated any bioactivity. The biological activity of pentacycle **184** is discussed in Chapter 6, pages 140-141.

5.2. RETROSYNTHESIS

In a similar manner to that previously shown for the ester substituted pentacycle **128** in Chapter 4 (see Figure 4.2, page 53), a disconnection removing guanidine from **184** led on this occasion to *bis*-α,β-unsaturated ketone **185**. A subsequent disconnection at the *trans* double bond as indicated in Figure 5.2, afforded chiral phosphorane **186** and known aldehyde **22**.[34] It was expected that a Wittig reaction between synthons **186** and **22** would lead to **185** which, after double Michael addition with guanidine, followed by cyclisation, would yield the desired pentacyclic model of ptilomycalin A.

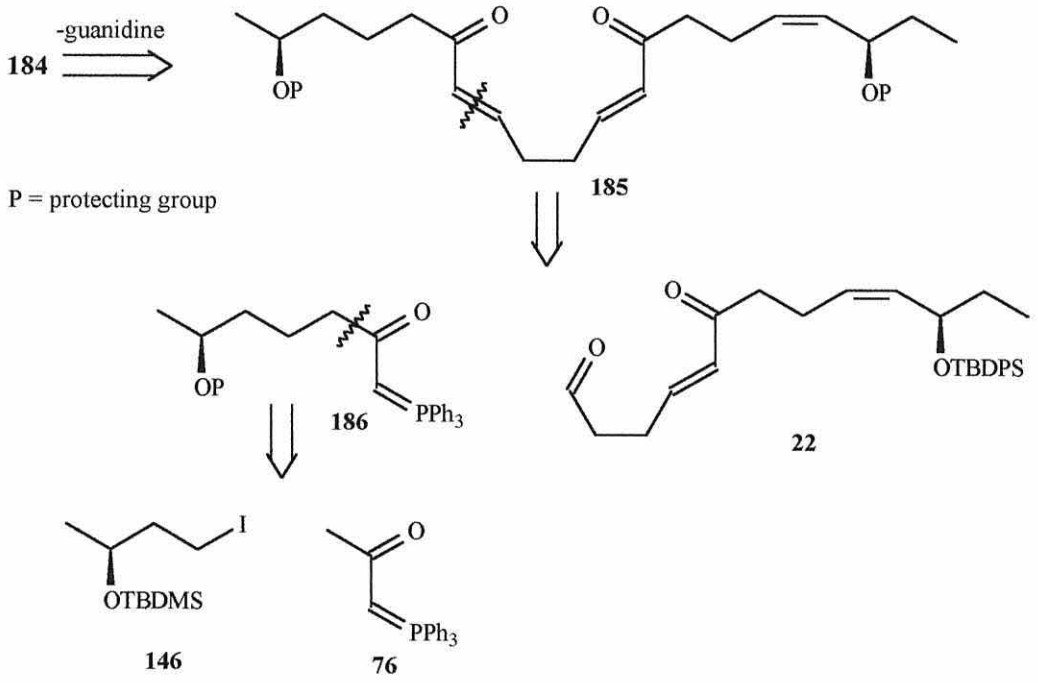
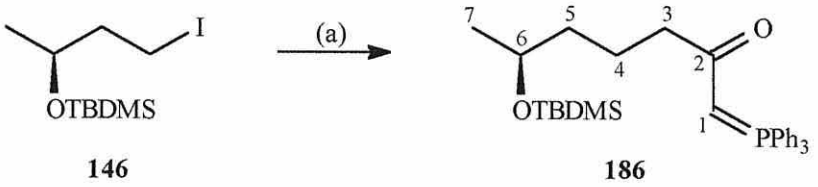


Figure 5.2 Retrosynthetic analysis of pentacycle **184**.

5.3. PREPARATION OF CHIRAL PHOSPHORANE **186**

Chiral phosphorane **186** was prepared by reaction of the anion of acetylmethylene triphenylphosphorane **76** with the known chiral iodide **146**. The anion of **76** was generated by reaction with *n*-butyllithium at -78°C giving a deep red coloured solution, which was then stirred for one hour at -55°C. The mixture was again cooled to -78°C, treated with a solution of **146** in THF and after stirring overnight at room temperature, the mixture was extracted and purified to afford **186** in a moderate 51 % yield (Scheme 5.1, page 93).



146 **186**

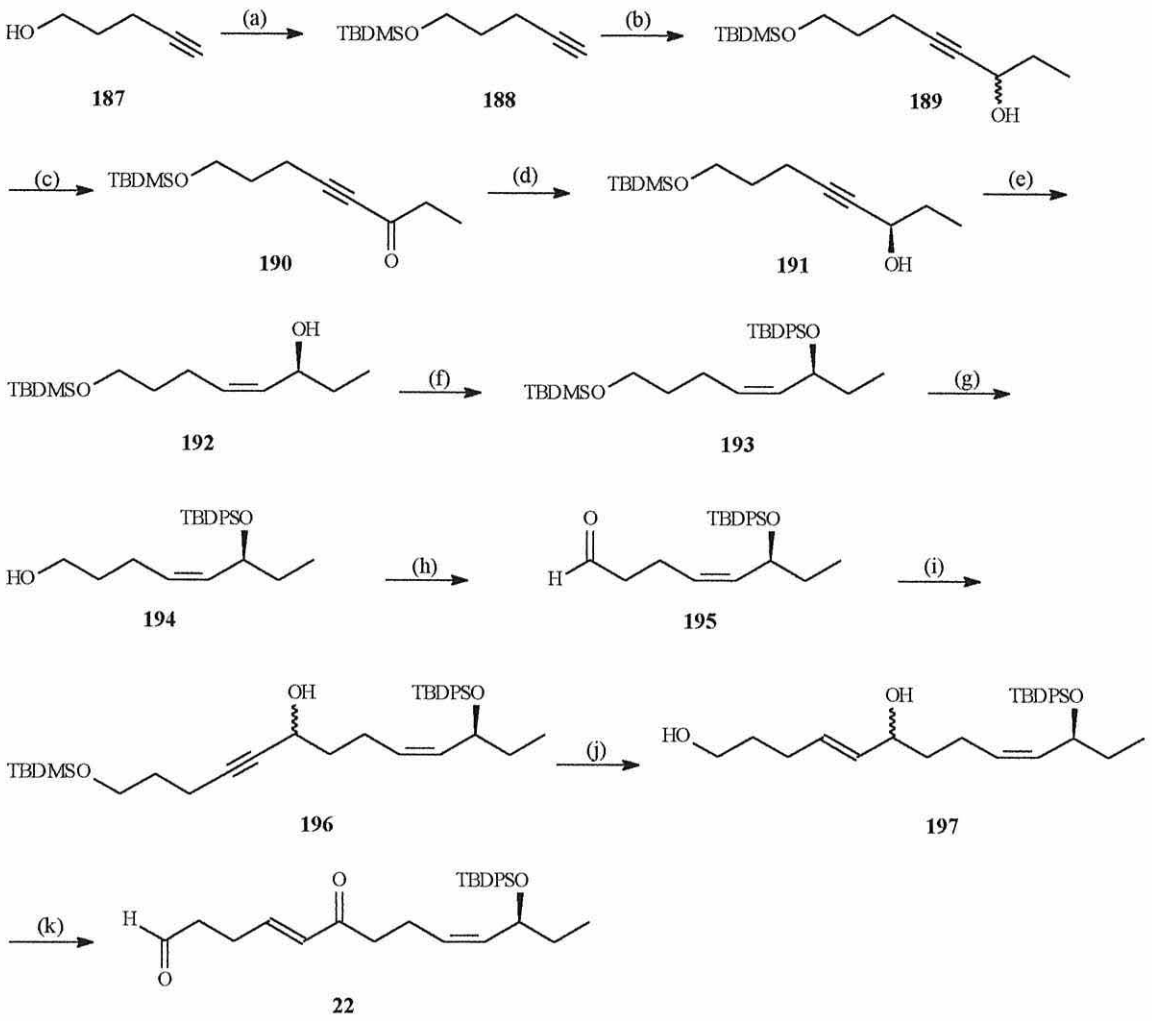
***Reagents and Conditions*:** (a) **76**, THF, *n*-BuLi, -78°C.

Scheme 5.1 Synthesis of chiral phosphorane **186**.

The IR spectrum of **186** indicated the presence of aromatic C–H stretches showing a weak band at 3059 cm^{-1}. Aliphatic C–H stretches were observed at 2956 cm^{-1} and 2855 cm^{-1}, whilst the carbonyl stretch appeared as a strong band at 1727 cm^{-1}. Analysis of the proton NMR spectrum showed a complex multiplet between the range δ 7.72 - δ 7.42, integrating to fifteen, which corresponded to the three phenyl groups. A resonance at δ 4.13 was attributed to the methine proton at C-6, whilst the terminal methyl signal appeared as a doublet (J = 7.2 Hz) at δ 1.29. The resonance at δ 3.66 was identified as the vinyl proton at C-1, adjacent to the carbonyl group. The carbon NMR spectrum further confirmed the structure of the phosphorane, demonstrating a signal at δ 193.95 which was consistent with the carbon atom of the carbonyl group at C-2. The carbon at position C-1 appeared as a doublet ($J_{C\text{-}P}$ = 106.7 Hz), at δ 51.03, due to coupling between the carbon and the adjacent phosphorus atom.

5.4. Synthesis Of Chiral Protected Aldehyde **22**

The methodology developed by Snider and Shi[34] was adopted in order to prepare chiral protected aldehyde **22**. The synthesis (Scheme 5.2, page 94) is briefly described and expanded upon where problems were encountered or adaptations made. Starting from commercially available 4-pentyn-1-ol **187**, the alcohol moiety was protected as the *tert*-butyldimethyl silyl ether **188** according to the procedure of Marshall.[114] The acetylide of **188** was prepared by deprotonation with *n*-butyllithium at -78°C and subsequently treated with propan-1-al to give the alkynol **189**, in 92% yield. This was then converted to the corresponding ketone **190** by means of a Swern oxidation, in a yield of 94%.



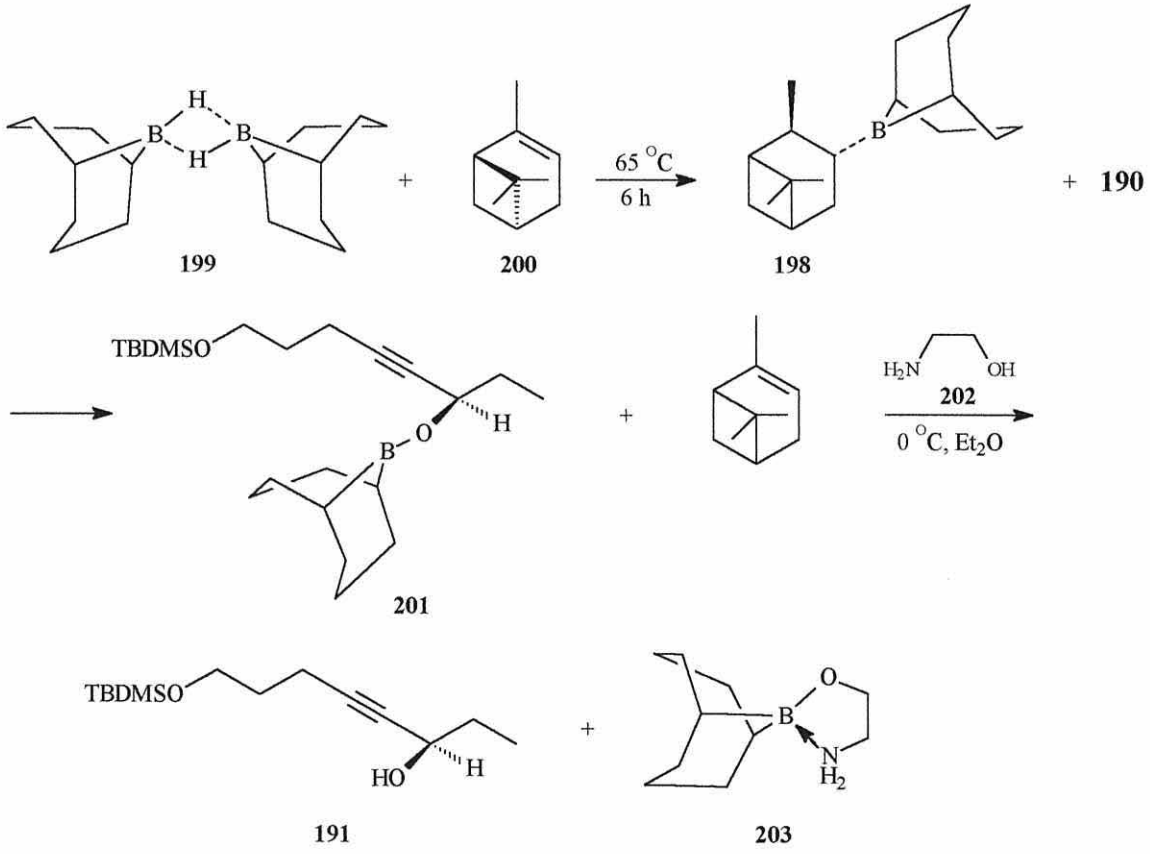
***Reagents and Conditions*:** (a) Et_3N, TBDMSCl, DMAP, CH_2Cl_2; (b) *n*-BuLi, THF, DMPU, -78 °C, propan-1-al; (c) Swern oxidation; (d) 9-BBN-H, (-)-α-pinene, THF; (e) H_2, quinoline, Lindlar catalyst; (f) Et_3N, TBDPSCl, DMAP, CH_2Cl_2; (g) PPTS, EtOH; (h) Swern oxidation; (i) *n*-BuLi, DMPU, -78 °C, acetylide from **188**; (j) $LiAlH_4$, THF, reflux; (k) Swern oxidation.

Scheme 5.2 Preparation of chiral protected aldehyde **22**.

Problems were encountered during step (d), the asymmetric reduction of prochiral ketone **190** to the corresponding (*S*)-alcohol **191**. Low yields of **191** were obtained (*ca* 25%-55%) and complications were encountered whilst attempting purification. This prevented an accurate assessment of the extent of reduction and in addition, whether any chirality had been induced. These initial reaction attempts were made using commercially available 9-BBN (0.5 M solution in THF). On substituting for solid 9-BBN-H dimer applying a similar reaction procedure to that reported by Brown,[115] thereby giving a more

concentrated solution of reagent, greatly improved reaction yields were obtained. The alcohol **191** was eventually prepared in 84% yield having a specific rotation measured at -3.6 (c = 1.0, $CHCl_3$). The obvious discrepancy in this value on comparison to that quoted by Snider[34] ($[\alpha]_D$ = -6.0 (c = 0.45, $CHCl_3$)) was due a trace amount of impurity being present which was probably derived from the borane and could not be removed, even after repeated column chromatography.

The asymmetry was induced by way of addition of the chiral reducing agent *B*-3-pinanyl-9-borabicyclo[3.3.1]nonane (Alpine-borane) **198** (formed by heating a mixture of 9-BBN-H dimer **199** and (*S*)-(-)-α-pinene **200**) to ketone **190** yielding the corresponding, chiral borinate ester **201** (Scheme 5.3). Upon completion of the reaction, acetaldehyde was added to destroy excess reagent whilst the free alcohol **191** was liberated from the borinate ester by an exchange reaction with ethanolamine **202**. At 0 °C, the 9-BBN-ethanolamine adduct **203** precipitated from the reaction media and was removed by filtration. The corresponding *R* enantiomer can be prepared using (*R*)-(+)-α-pinene.



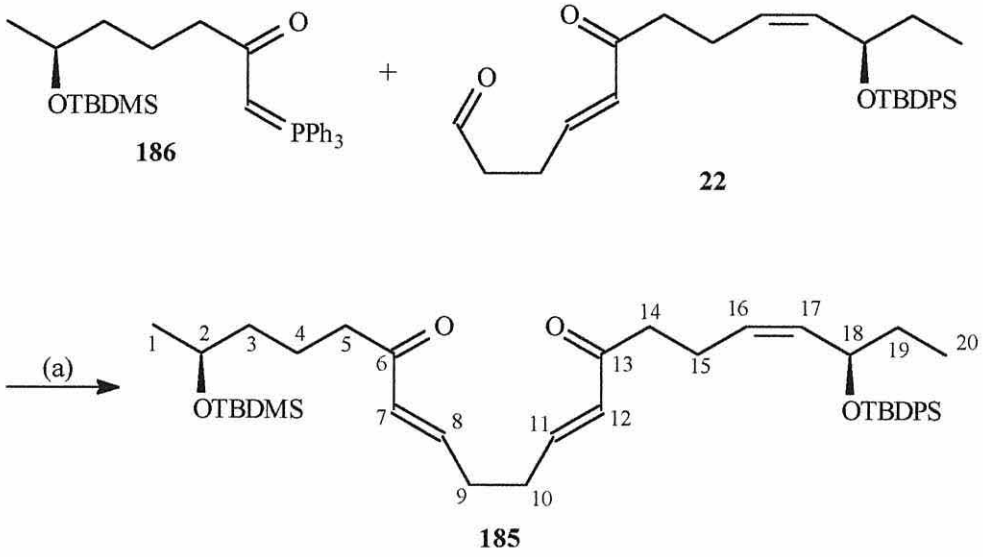
Scheme 5.3 Schematic representation of the asymmetric reduction of prochiral ketone **190**.

The chiral alcohol was hydrogenated using Lindlar catalyst (palladium on calcium carbonate poisoned with lead) whilst stirring in hexane under an atmosphere of hydrogen. Removal of the solid residue by filtration, followed by purification on silica, yielded the corresponding allylic alcohol **192** in an 88% yield (see Scheme 5.2, page 94). The optical rotation of **192** was measured at +12.3 (c = 1.1, $CHCl_3$) which on this occasion was almost identical with the rotation reported by Snider[34] ($[\alpha]_D$ = +12.9 (c = 0.45, $CHCl_3$)). Protection of the secondary alcohol gave the *tert*-butyldiphenylsilyl ether **193**, which was then subjected to selective cleavage of the *tert*-butyldimethylsilyl ether affording primary alcohol **194**. Swern oxidation of the alcohol moiety gave the corresponding aldehyde **195** in 93% yield which was then reacted with the acetylide of **188**, using an identical procedure to that previously described (see pages 93-94), affording the desired unsaturated alcohol, **196**.

Deprotection of the *tert*-butyldimethylsilyl ether and reduction of the triple bond was accomplished in a single step by heating **196**, at reflux, in the presence of a solution of lithium aluminium hydride in THF. After four hours, the cooled mixture was quenched, extracted and purified on silica, affording diene **197** in a disappointing 57% yield (65% yield based upon recovered starting material). All efforts to improve the yield of **197** proved unsuccessful. The diene was subsequently oxidised under standard Swern conditions to give the desired aldehyde **22** in 98% yield. This eleven step synthesis represents a 21% overall yield of chiral aldehyde **22** starting from alkyne **187**.

5.5. Preparation Of *Bis*-α,β-unsaturated Ketone **185**

The synthesis of *bis*-α,β-unsaturated ketone **185** was carried out in a single step by means of the Wittig reaction, whereby a solution of **186** in dichloromethane was slowly added to a solution of chiral aldehyde **22**, also in dichloromethane at 0°C. After stirring at room temperature for 36 hours, the solvent was evaporated and the resulting crude material purified on silica gel, affording **185** in a satisfactory 73% yield (Scheme 5.4, page 97). As phosphorane **186** represents a stabilised Wittig reagent, the condensation reaction therefore resulted solely in the formation of the required *trans* double bond between C-7 and C-8 in the *bis*-α,β-unsaturated ketone.



***Reagents and Conditions*:** (a) CH_2Cl_2, 36 h.

Scheme 5.4 Preparation of *bis*-α,β-unsaturated ketone **185**, *via* a Wittig reaction between stabilised phosphorane **186** and chiral aldehyde **22**.

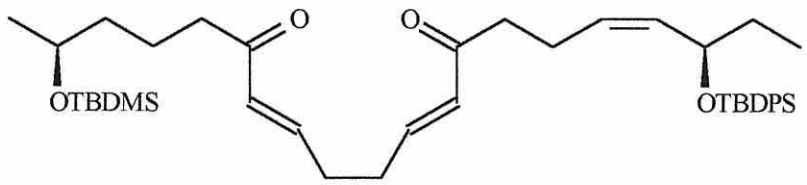
Structural evidence for **185** was provided by proton and carbon NMR spectroscopy. The ^1H NMR demonstrated two multiplets between the ranges δ 7.69-7.66 (4H) and δ 7.42-7.32 (6H) corresponding to the aromatic C–H resonances of the *tert*-butyldiphenylsilyl protecting group. Resonances due to the four protons of the two *trans* double bonds appeared at δ 6.81 (CH-11), δ 6.67 (CH-8), δ 6.14 (CH-7) and δ 6.01 (CH-12). The coupling constants for the protons CH-7 and CH-12 were both calculated at 15.8 Hz, thereby confirming the *trans* nature of both double bonds. In contrast, the resonances for the *cis* double bond occurred upfield at δ 5.44 (dt, J = 10.9, 1.6 Hz), CH-16 and at δ 5.18 (dt, J = 10.9, 7.4 Hz), CH-17. The lower values of these coupling constants are typical of *Z* olefins of this type. In addition, the proton spectrum showed signals at δ 4.38 and δ 4.14 which were identified as the resonances of the chiral methine protons adjacent to the protecting groups, CH-18 and CH-2, respectively.

The carbon NMR showed carbonyl resonances at δ 199.88 (C-13) and δ 198.62 (C-6). Signals for the two carbons adjacent to the silyl ethers appeared at δ 70.54 (C-18) and δ 68.36 (C-2). The resonances for the phenyl and olefinic carbons appeared as overlapping signals between the range δ 144.61-127.27, whilst the remaining signals were consistent with those expected for the product.

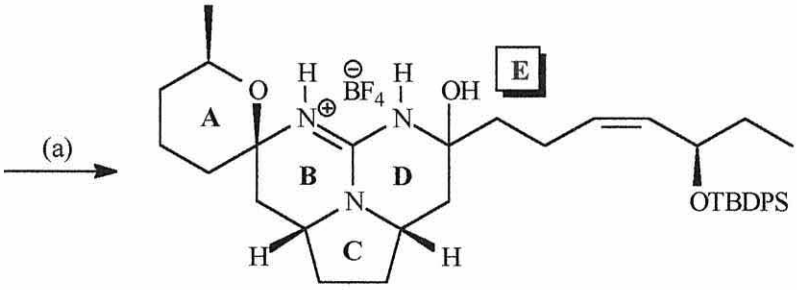
5.6. SYNTHESIS OF PENTACYCLIC MODEL **184**

Following the successful construction of *bis*-α,β-unsaturated ketone **185**, it was now possible to attempt the synthesis of the guanidine pentacycle **184**. Using a similar procedure to that described for the ester-substituted pentacycle in Chapter 4 (section 4.5.2, pages 73-74), a solution of guanidine in DMF was added to precursor **185**, also in DMF, at 0 °C. After stirring at this temperature for six hours, analysis by TLC indicated complete consumption of **185**. Thus, the DMF was evaporated and the remaining crude material re-dissolved in methanol, treated with a solution of methanolic HCl (acetyl chloride in methanol) and the resultant mixture left to stir overnight. Work-up, followed by counterion exchange using a saturated solution of $NaBF_4$, gave a brown solid which was purified by column chromatography. A fraction, thought to be the desired pentacycle, was isolated as a solid in 62% yield.

Analysis of this material by ^{1}H NMR spectroscopy showed the presence of protons in the aromatic region of the spectrum, whilst the remaining resonances seemed to be consistent with those expected for the product. From this information, it was assumed that the pentacycle had successfully formed, but was possibly contaminated with *tert*-butyldiphenylsilanol, a by-product of the alcohol deprotection stage. In an effort to remove the impurity the solid was re-extracted using dichloromethane and then sequentially washed with water, saturated lithium bromide solution and brine. Removal of the solvent, followed by counterion exchange and purification on silica gel, again gave a solid material whose proton NMR appeared identical to that previously described. Further detailed analysis of the spectrum suggested that the presence of these aromatic resonances were due to incomplete cyclisation where the *tert*-butyldiphenylsilyl ether remained uncleaved. The nature of the product was presumed to be tetracyclic compound **204** (Scheme 5.5, page 99), however, as detailed spectral analysis proved difficult this was merely an assumption. It was therefore suspected that, following the double Michael addition of guanidine, the methanolic HCl solution cleaved the *tert*-butyldimethylsilyl ether, but left the *tert*-butyldiphenylsilyl group in place. Thus, formation of the six-membered ring (**A**) occurred but cyclisation of the seven-membered ring (**E**) remained obstructed (Scheme 5.5).



186



204

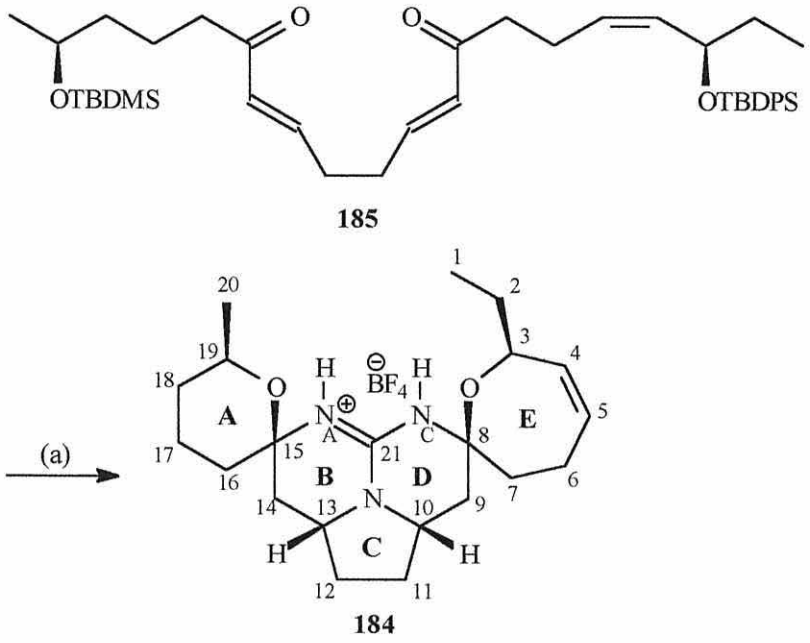
***Reagents and Conditions*:** (a) (i) DMF, guanidine, 0°C; (ii) methanolic HCl; (iii) CH_2Cl_2, sat. $NaBF_4$.

Scheme 5.5 Structure of the presumed pentacycle **204**. **E** indicates where the seven-membered ring should have formed.

In an effort to determine whether the seven-membered ring could be cyclised independently after removal of the *tert*-butyldiphenylsilyl group, tetracycle **204** was dissolved in THF and treated with one equivalent of tetrabutylammonium fluoride (TBAF). After stirring overnight the reaction mixture was extracted and purified yielding a pale brown solid. The proton NMR spectrum was found to be identical to that of **204** indicating that deprotection had not occurred.

In a further attempt to effect cyclisation, **204** was again dissolved in THF and on this occasion, treated with five equivalents of TBAF. The resulting mixture was heated at 25-30°C for three hours and then stirred at ambient temperature for a further 64 hours. After work-up and purification, a solid material was recovered whose ^{1}H NMR spectrum no longer demonstrated the aromatic C–H resonances. With preliminary evidence in hand of the successful formation of pentacycle **184**, an attempt was made at its preparation without isolating the intermediate tetracycle. Thus, *bis*-α,β-unsaturated ketone **185** was treated with guanidine in the normal manner and following work-up, was treated with a solution of methanolic HCl and left to stir overnight. A second work-up resulted in the

isolation of a crude oil which was re-dissolved in THF and treated with five equivalents of TBAF in an identical manner to that previously described. Following work-up, counterion exchange and purification, the desired pentacycle **184** was isolated in a moderate 18% yield, as a pale brown solid (Scheme 5.6).

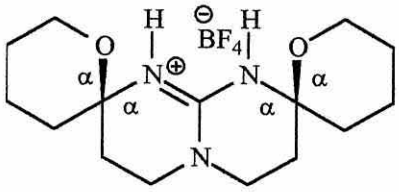


***Reagents and Conditions*:** (a) (i) Guanidine, DMF, 0°C; (ii) Methanolic HCl; (iii) THF, TBAF, 25-30°C then rt; (iv) CH_2Cl_2, sat. $NaBF_4$.

Scheme 5.6 The synthesis of pentacyclic model **184**.

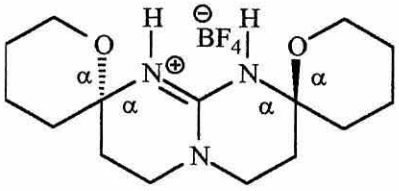
The ^{13}C NMR spectrum of **184** showed twenty one different carbon environments which corresponded with the total number of carbons present in the pentacycle. Previous investigations by Williams[81] had shown that the 6,6,6,6-tetracycles, **108** and **109** (see Chapter 3, page 44) formed in a 1:1 equilibrium mixture of *syn* (**108**) and *anti* (**109**) diastereomers. Similarly, the 6,6,5,6,6-pentacycles, **116a** (see Chapter 3, page 46) and **206** (Figure 5.3) were also formed as a diastereomeric mixture consisting of a major (*syn*-**116a**) and a minor (*anti*-**206**) diastereomer in the ratio, 4:1. The fact that diastereomeric mixtures were present was evident from the carbon NMR spectra which in both cases demonstrated resonances attributable to both diastereomers, the exact ratios being determined from the respective proton spectra.

The structures of both tetracyclic diastereomers and pentacycle **116a** were confirmed as shown in Figure 5.3, by means of X-ray crystallography[80] (see Chapter 3, page 49), whereas the structure of the minor pentacyclic isomer **206** was established through molecular modelling studies and by spectral comparisons with TFA-ptilomycalin A (that is, the *bis*-trifluoroacetate derivative of **12**)[30] and 13,14,15-isocrambescidin 800 (**60**).[41] These X-ray structures indicated that the bonds to the spirocyclic oxygens were axially orientated (denoted by the symbol α in Figure 5.3) with respect to the guanidine-containing portions. In a similar manner, the C–N bonds are also mutually axial with respect to the six-membered spirocyclic rings (Figure 5.3).

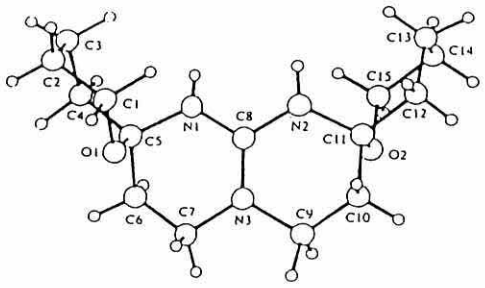


α = bond axially orientated

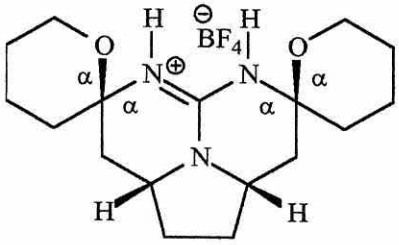
108 (*syn*)



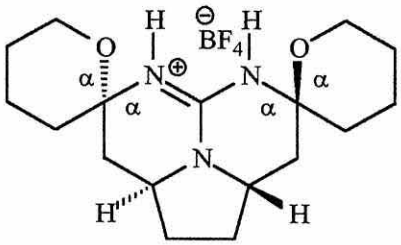
109 (*anti*)



109 (*anti*)



116a (*syn*)



206 (*anti*)

Figure 5.3 The structures and spatial orientations of the 6,6,6,6-tetracyclic and 6,6,5,6,6-pentacyclic model compounds. The X-ray crystallographic structure of tetracyclic diastereomer **109** indicates the mutually axial orientation of the oxygen and nitrogen atoms in their respective six-membered rings.

Further evidence that compounds of this nature formed as a mixture of two diastereomers, each of which possessed the axial structural arrangement, was provided following the synthesis of the 7,6,5,6,7-pentacyclic model **116b** (see Chapter 3, page 46) and its epimeric *anti* diastereomer, in the ratio, 4:1. Once again, an X-ray structure of the major diastereomer confirmed the mutual axial orientation of the heterocyclic rings.

During these studies, polycycles having ring systems equatorially orientated were never isolated from the reaction mixtures. This indicated that the axial arrangement of heteroatoms represented a lower energy state than the corresponding equatorial orientation and is hence, the preferred orientation of these polycyclic systems. As these compounds formed as an approximate 4:1 mixture of diastereomers it became apparent that addition of guanidine to the *bis*-α,β-unsaturated ketones was irreversible, but that spirocyclisation was reversible, a feature demonstrated by Williams.[81]

In contrast to the examples described by Williams,[81] the carbon NMR spectrum of pentacycle **184** did not show any resonances that could be ascribed to the presence of any minor diastereomers. One possible reason for this could stem from the scale of the reaction; as this was small, the isolation of limited quantities of minor isomers may have presented a problem. However, considerations of the stereochemistry and energetics of the polycyclic system may offer another explanation as to why only a single diastereomer was recovered.

On closer inspection it was apparent that there were four possible diastereomers that could potentially form as a result of spirocyclisation of tricyclic pyrrolidine intermediate **207** (Figure 5.4, page 104), the stereochemistry of which was imposed during the guanidine addition step. It has already been established that from an energetic point of view the preferred orientation of the oxygen and nitrogen atoms in polycyclic systems of this type was mutually axial. In addition, the *syn*/*anti* ratio of the central pyrrolidine system was always found to be in the region of 4:1. Hence, the four potential diastereomers **184**, **208**, **209** and **210**, as shown in Figure 5.4 (page 104), could occur in the theoretical ratio, 4:4:1:1.

Of these, pentacycle **184** possess an identical relative stereochemistry to the natural product, ptilomycalin A, where the methyl and ethyl substituents exist in the energetically and sterically favoured equatorial positions. This configuration therefore represents the

most energetically stable product of the four possible diastereomers. From an energetic perspective, the accompanying *syn*-orientated product, **208**, represents the least stable of the four possible configurations. This instability arises as a consequence of the methyl and ethyl substituents of the oxacycles being forced into sterically unfavoured axial positions (Figure 5.4, page 104). As there was no direct evidence for the existence of **208**, it was proposed that these steric interactions prevented the effective cyclisation of the tricyclic intermediate **207** to this structure **208**, and hence this explained why pentacycle **208** was not formed during this reaction.

It is reasonable to assume that the minor *anti* diastereomers **209** and **210**, would energetically represent an intermediate position between the two extremes, in which each configuration possesses one high energy, axially orientated spirocyclic substituent (Figure 5.4, page 104). The reason that neither of these diastereomers were isolated may have been due to precisely the same energetic factors as were apparent for pentacycle **208**.

In fact, the relative stereochemistry of **209** is identical to the pentacyclic core of 13,14,15-isocrambescidin 800 (**60**).[41] That **60** can be isolated as a stable product is probably due to the anomeric effect, where the *anti* configuration has resulted in the methyl group preferentially adopting an axial orientation in favour of the lower energy equatorial position. The anomeric effect is usually small and can result as a consequence of an electronic bonding effect or from minimisation of dipole-dipole repulsion.

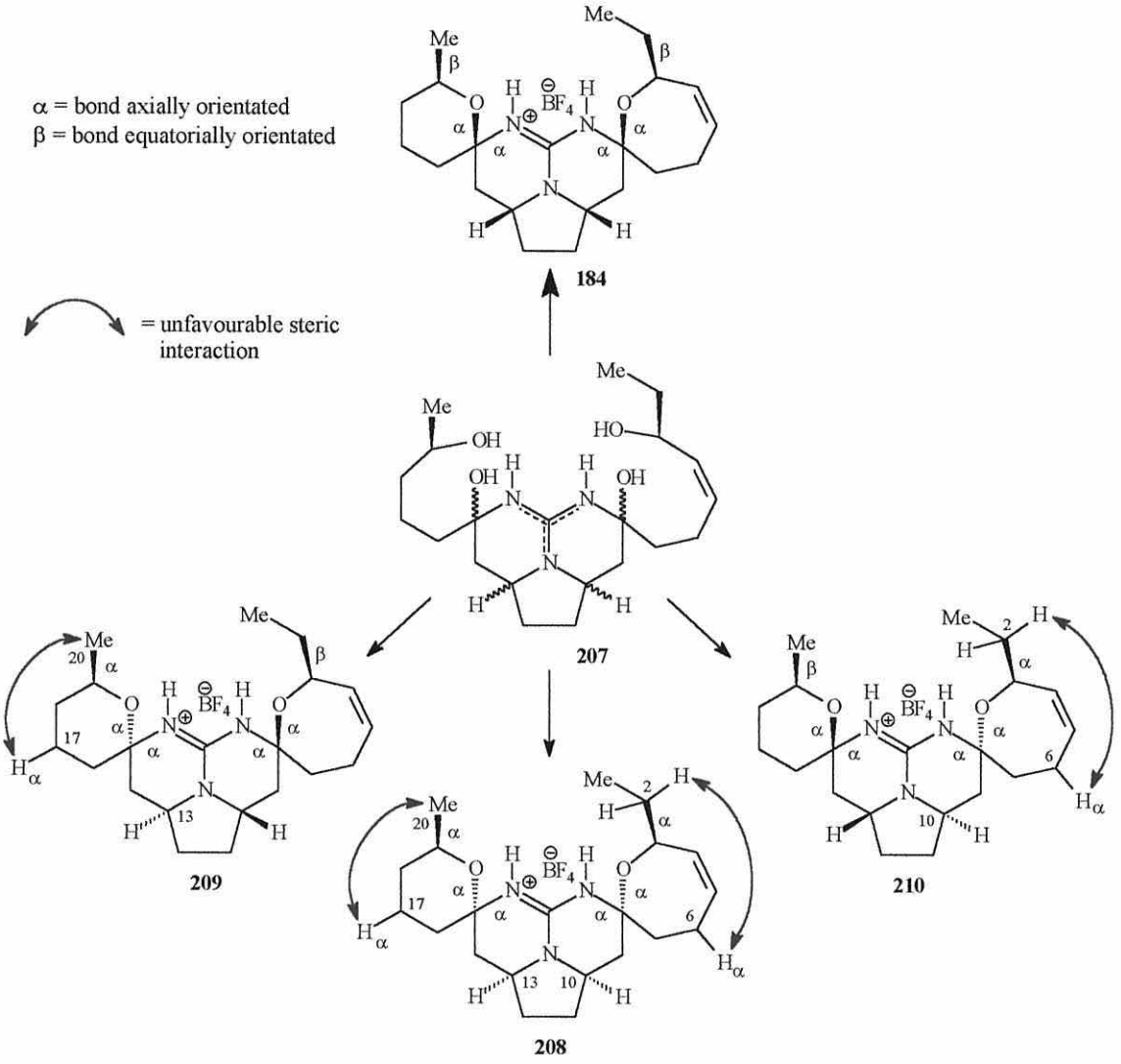
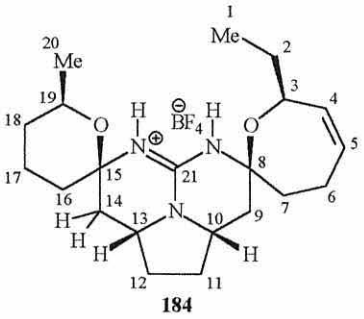


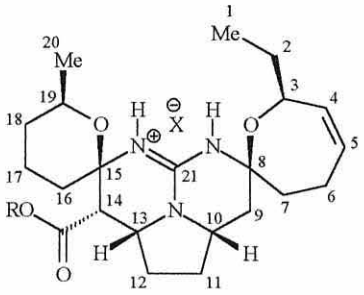
Figure 5.4 A diagrammatic representation of the unfavoured steric interactions that exist in the three possible diastereomers, **208**, **209** and **210**, of stable pentacycle **184**.

5.6.1. SPECTROSCOPIC EVIDENCE

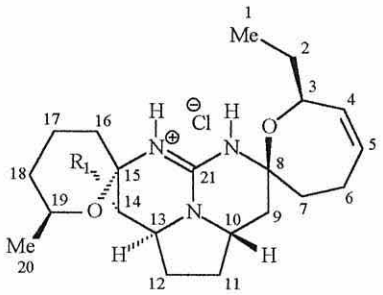
In this section, spectroscopic evidence is provided supporting the successful preparation of the *syn* diastereomer of pentacycle **184**. For simplicity, a generic numbering system is adopted for **184**, where the whole unit is regarded as a single carbon chain as shown overleaf. In addition, the evidence is also supported by a comparison of the proton and carbon spectra of pentacycle **184** with those of the pentacyclic cores of TFA-ptilomycalin A **13**,[30] and 13,14,15-isocrambescidin 800 (**60**),[41] see Tables 5.1 and 5.2.



184



13: R = $C_{23}H_{48}N_3O$
X = CF_3CO_2



60: R_1 = $C_{24}H_{48}N_3O_4$

	Pentacycle **184** (500MHz, $CDCl_3$)			TFA-ptilomycalin A[30] (500MHz, $CDCl_3$)			13,14,15-isocrambescidin 800[41] (500MHz, CD_3OD)		
Position	$^1H(\delta)$	M	*J* (Hz)	$^1H(\delta)$	M	*J* (Hz)	$^1H(\delta)$	M	*J* (Hz)
1	0.83	t	7.2	0.79	t	7.5	0.90	t	7.0
2α	1.52	m	-	1.51	m	-	1.45	m	-
2β	1.42	m	-	1.42	tq	14.0, 7.5	1.51	m	-
3	4.47	br d	-	4.44	m	-	4.45	br s	-
4	5.49	dt	11.0, 2.8	5.47	dt	11.0, 2.5	5.50	br d	-
5	5.66	ddt	11.4, 7.7, 2.4	5.65	ddt	11.0, 8.0, 2.5	5.68	br t	-
6α	2.16	m	-	2.15	m	-	2.12	m	-
6β	2.32	m	-	2.35	br t	14.0	2.35	m	-
7α	2.55	br t	13.7	2.58	br t	14.0	1.94	m	-
7β	1.87	dd	14.3, 5.7	1.86	dd	14.0, 6.0	2.38	m	-
9α	1.36	t	12.4	1.40	t	12.5	1.45	t	13.0
9β	2.59	dd	12.7, 5.7	2.55	dd	12.5, 6.5	2.56	dd	13.0, 2.3
10	4.06	m	-	4.00	tt	12.5, 6.5	3.74	m	-
11α	1.69	m	-	1.60	m	-	1.68	m	-
11β	2.19	dd	14.0, 5.7	2.19	m	-	2.24	m	-
12α	1.70	m	-	1.79	ddt	5.0, 3.5, 13	1.65	m	-
12β	2.32	m	-	2.25	m	-	2.29	m	-
13	4.06	m	-	4.29	dt	10.0, 5.0	4.00	m	-

Table 5.1 Continued overleaf:

	Pentacycle **184** (500MHz, $CDCl_3$)			TFA-ptilomycalin A[30] (500MHz, $CDCl_3$)			13,14,15-isocrambescidin 800[41] (500MHz, CD_3OD)		
Position	$^1H(\delta)$	M	*J*(Hz)	$^1H(\delta)$	M	*J*(Hz)	$^1H(\delta)$	M	*J*(Hz)
14α	1.49	t	12.7	*a*	-	-	*a*	-	-
14β	2.28	m	-	2.94	d	5.0	3.80	d	3.4
16α	1.72	m	-	1.68	m	-	1.52	m	-
16β	1.72	m	-	1.68	m	-	1.80	m	-
17α	2.06	m	-	2.15	m	-	1.75	m	-
17β	1.72	m	-	1.68	m	-	1.75	m	-
18α	1.62	m	-	1.65	m	-	1.15	m	-
18β	1.20	m	-	1.18	dq	5.0, 12.5	1.60	m	-
19	3.81	ddq	12.6, 6.5,2.8	3.84	ddq	12.5, 6.5, 2.5	4.02	m	-
20	1.06	d	6.4	1.04	d	6.5	1.13	d	6.5
N_AH	7.89	br s	-	10.22	br s	-	*b*	-	-
N_CH	7.83	br s	-	9.87	br s	-	*b*	-	-

Table 5.1 A comparison of the ^1H NMR signals of the pentacyclic nuclei of **184**, TFA-ptilomycalin A **13** and 13,14,15-isocrambescidin 800 (**60**). [a] Inappropriate comparison, [b] literature chemical shift value not quoted.

From Table 5.1 above, it can be seen that there is an excellent correlation between the chemical shifts (δ), multiplicities (M) and coupling constants (*J*) of pentacycle **184** and the pentacyclic nucleus of TFA-ptilomycalin A **13**. A signal at δ 1.49 was identified as the proton at position 14α which is not present in **13**. In addition, the resonance at position 14β (δ 2.28) is shifted upfield by 0.66 ppm compared to the same resonance in **13** (δ 2.94), the reason being due to the presence of the adjacent ester group in **13**. There is also a good correlation between **184** and the pentacyclic core of 13,14,15-isocrambescidin 800 (**60**), although differences are observed in the signals corresponding to the protons of the respective six-membered rings. These differences can be explained by considering the contrasting spacial orientations of the two rings in question due to the *syn* and *anti* relationships. The large difference between the chemical shifts for the two N–H protons is due to the nature of the counterion and also the concentration of the NMR solutions.

	Pentacycle **184** (62.5 MHz, $CDCl_3$)	TFA-ptilomycalin A[30] **13** (125 MHz, $CDCl_3$)	13,14,15-isocrambescidin 800[41] (**60**) (125 MHz, CD_3OD)[a]
Carbon	δ	δ	δ
1	10.21	10.21	10.7
2	29.21	29.19	30.2
3	70.94	70.89	71.9
4	133.61	133.69	134.0
5	129.77	129.96	131.2
6	23.72	23.74	24.9
7	36.46	36.22	39.0
8	84.06	83.86	86.6
9	37.07	36.89	38.0
10	53.50	54.06	54.6
11	30.04	30.65	30.8
12	29.84	26.81	29.6
13	51.97	52.13	54.2
14	39.80	50.10	42.5
15	80.46	80.82	84.4
16	32.19	31.68	33.6
17	17.91	18.01	20.9
18	33.56	32.06	32.9
19	67.17	67.12	70.0
20	21.66	21.56	22.3
21	147.68	149.09	150.1

Table 5.2 A comparison of the ^{13}C NMR signals of the pentacyclic nuclei of **184**, TFA-ptilomycalin A **13** and 13,14,15-isocrambescidin 800 (**60**). [a] Literature data quoted to one decimal place.

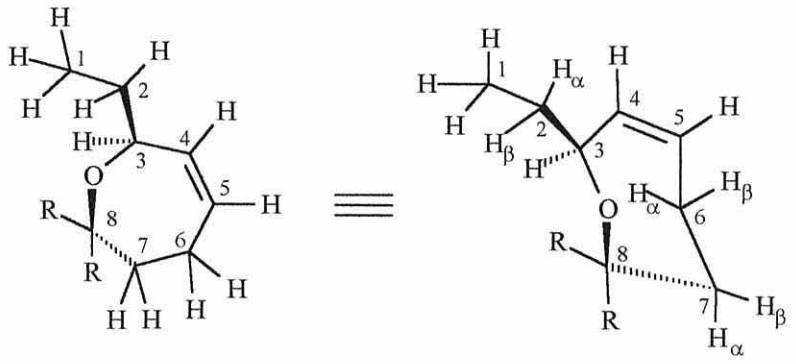
The ^{13}C NMR spectra of the pentacyclic cores of TFA-ptilomycalin A and pentacycle **184** show a good signal correlation, thus supporting the structural parallels. The obvious discrepancy in chemical shifts occurs at C-14 where the nature of the substituent differs. There is also a good correlation with the resonances of the pentacyclic nucleus of

13,14,15-isocrambescidin 800 (**60**), which reflects the almost identical structures of the two polycyclic nuclei. In **60** there is a small difference in the chemical shift at C-15 (~4 ppm) compared to the same chemical shift in pentacycle **184** and TFA-ptilomycalin A **13**. This difference in chemical shift further supports the structural feature of the *syn/anti* relationship between the three pentacycles.

5.6.2 TOCSY ANALYSIS

Analysis of the TOCSY (HOHAHA) spectrum established that pentacycle **184** could be grouped into the following three separate assemblies: (i) CH_3-1 to CH_2-7, (ii) CH_2-9 to CH_2-14 and (iii) CH_2-16 to CH_3-20. Proton correlations within each of the three spin system assemblies are tabulated in Tables 5.3 to 5.5.

5.6.2.1 TOCSY CORRELATION - SPIN SYSTEM (i) CH_3-1 TO CH_2-7



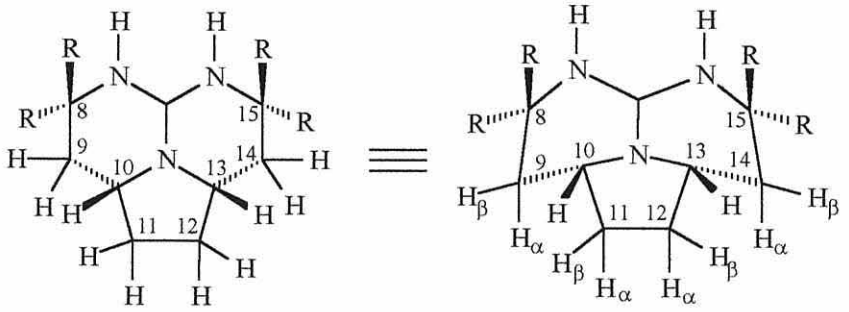
α - axial, β - equatorial

		TOCSY couplings, Spin System (i) CH_3-1 to CH_2-7									
H	δ	Me-1	H-2α	H-2β	H-3	H-4	H-5	H-6α	H-6β	H-7α	H-7β
Me-1	0.83		√	√	√	√	√	√	§	√	√
H-2α	1.52	√		√	√	§	§	√	√	§	‡
H-2β	1.42	√	√		√	§	§	√	√	§	‡
H-3	4.47	√	√	√		√	√	§	§	√	√
H-4	5.49	√	§	§	√		√	√	√	√	√
H-5	5.65	√	§	§	√	√		√	√	√	√
H-6α	2.16	§	‡	§	§	√	√		√	√	√
H-6β	2.32	§	√	√	§	√	√	√		√	√
H-7α	2.55	√	§	§	√	√	√	√	√		§
H-7β	1.87	√	‡	‡	√	√	√	√	√	√	

Key: √ = coupling observed, § = small coupling observed, ‡ = no observed coupling.

Table 5.3 TOCSY correlations for spin system (i) CH_3-1 to CH_2-7.

5.6.2.2 TOCSY CORRELATION - SPIN SYSTEM (ii) CH_2-9 TO CH_2-14



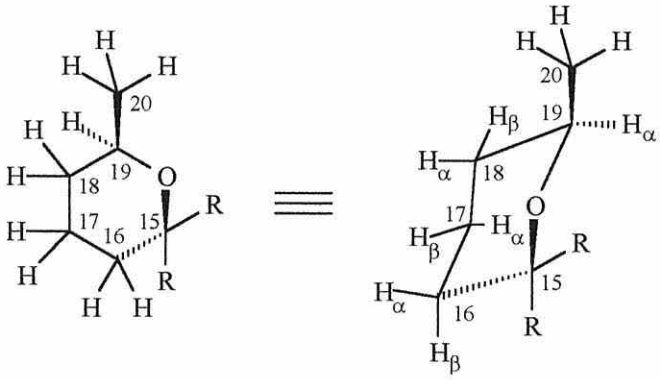
α - axial, β - equatorial

		TOCSY couplings, Spin System (ii) CH_2-9 to CH_2-14									
H	δ	H-9α	H-9β	H-10	H-11α	H-11β	H-12α	H-12β	H-13	H-14α	H-14β
H-9α	1.36		√	√	√	√	√	√	√	√	√
H-9β	2.59	√		√	√	√	√	√	√	√	√
H-10	4.06	√	√		√	√	√	√	√	√	√
H-11α	1.69	√	√	√		√	√	√	√	√	√
H-11β	2.19	√	√	√	√		√	√	√	√	√
H-12α	1.70	√	√	√	√	√		√	√	√	√
H-12β	2.32	√	√	√	√	√	√		√	√	√
H-13	4.06	√	√	√	√	√	√	√		√	√
H-14α	1.49	§	§	√	√	√	√	√	√		√
H-14β	2.28	√	√	√	√	√	√	√	√	√	

Key: √ = coupling observed, § = small coupling observed.

Table 5.4 TOCSY correlations for spin system (ii) CH_2-9 to CH_2-14

5.6.2.3 TOCSY CORRELATION - SPIN SYSTEM (iii) CH_2-16 TO CH_3-20



α - axial, β - equatorial

		TOCSY couplings, Spin System (iii) CH_2-16 to CH_3-20							
H	δ	H-16α	H-16β	H-17α	H-17β	H-18α	H-18β	H-19	Me-20
H-16α	1.72		√	√	√	√	√	√	√
H-16β	1.72	√		√	√	√	√	√	√
H-17α	2.06	√	√		√	√	√	§	√
H-17β	1.72	√	√	√		√	√	√	√
H-18α	1.62	√	√	√	√		√	√	√
H-18β	1.20	√	√	√	√	√		√	√
H-19	3.84	√	√	√	√	√	√		√
Me-20	1.04	√	√	√	√	√	√	√	

***Key*:** √ = coupling observed, § = small coupling observed.

Table 5.5 TOCSY correlations for spin system (iii) CH_2-16 to CH_3-20

Tables 5.3 to 5.5 (pages 109-111) show that the protons within each assembly correlate well with one another. Couplings were only observed within each assembly but not between separate assemblies. In some cases the couplings observed are small (§) or the expected couplings are not present (‡), a fact which can be explained by considering the spatial differences between the protons in question. For example, in spin system (i), (see Table 5.3) there is no observed coupling between protons H-2α and H-7β which are spatially distant to one another within the pentacycle and hence the coupling is not seen.

5.6.3 Nuclear Overhauser Effect Correlations

An NOE experiment was conducted in order to establish the absolute stereochemistry of pentacycle **184** and also to prove that it existed as a single diastereomer. On this occasion, pentacycle **184** is compared with Kashman's NOESY analysis of TFA-ptilomycalin A **13**[30] and with Rinehart's NOESY/ROESY experiments on 13,14,15-isocrambescidin 800 (**60**).[41] In each case, the relevant NOE's are compared in Table 5.6 below and contrasted in Table 5.7 (page 113).

Selected NOE's[30]	TFA-ptilomycalin A[30] **13** (500MHz, $CDCl_3$)	13,14,15-isocrambescidin 800[41] (**60**) (500MHz, $CDOD_3$)	Pentacycle **184** (500MHz, $CDCl_3$)
H-3 - H-7α	√	√	√
H-7α - N_AH	√	*a*	*b*
H-3 - N_AH	√	√	*b*
H-3 - H-5	√	√	√
H-4 - H-6β	√	√	√
H-9α - H-10	√	x	√
H-9β - H-10	√	√	√
H-10 - H-11β	√	√	√
H-10 - H-13	√	x	√
H-12β - H-13	√	x	√
H-13 - H-14α	√	√	√
CH_3-1 - H-10	√	√	√
CH_3-1 - H-13	√	x	√
H-19 - H-18α	√	x	x
H-19 - H-17α	√	x	x
H-17α - N_CH	√	*a*	*b*
N_CH - H-19	√	*a*	*b*
H-19 - CH_3-1	√	x	√

***Key*:** √ = NOE present, x = NOE not present.

Table 5.6 NOESY correlations between **184**, TFA-ptilomycalin A **13**, and 13,14,15-isocrambescidin 800 (**60**). [a] No data for comparison, [b] downfield NOE's not recorded.

Selected NOE's[30]	13,14,15-isocrambescidin 800[41] (**60**) (500MHz, $CDOD_3$)	Pentacycle **184** (500MHz, $CDCl_3$)
H-9α - H-11α	√	x
H-11α - H-13	√	x
H-14α - H-13	√	√
H-19α - H-13	√	x
H-19α - H-14α	√	x
H-13 - H-14β[a]	x	√

***Key*:** √ = NOE present, x = NOE not present.

Table 5.7 Selected NOESY correlations between **184** and 13,14,15-isocrambescidin 800 (**60**).[41]

[a] This specific NOE has been selected from the NOESY spectrum of **184**.

From Table 5.6 (page 112) it can be seen that there is a good correlation between the NOE's recorded by Kashman *et al.*[30] for TFA-ptilomycalin A with those displayed in the NOESY spectrum of pentacyclic model **184** (see Figure 5.5, page 114). This suggests that the two structures have an identical stereochemistry about the polycyclic framework. In a similar manner, NOE correlations can be made between the seven-membered rings of **13**, **184** and 13,14,15-isocrambescidin 800 (**60**), again suggesting an equivalent stereochemistry. The presence of the NOE's between CH-19 and CH_3-1 and CH-10 and CH-13 confirm the *syn* orientatation of the spirocyclic rings and the two pyrrolidine protons (CH-10 and CH-13) of the pentacyclic model. These NOE's are not present in 13,14,15-isocrambescidin 800 which indicates the opposite (*anti*) stereochemistry of the pentacyclic guanidine moiety. In addition, Rinehart[41] states that the observed NOE's between CH-19 and CH-13 and between CH-19 and CH-14 (Table 5.7) can only occur if the configuration is inverted at C-15, as was the case in 13,14,15-isocrambescidin 800 (**60**). These NOE's are not present in **184**, hence the stereochemistry at C-15 must be identical to that found in TFA-ptilomycalin A and accordingly in the natural product **12**.

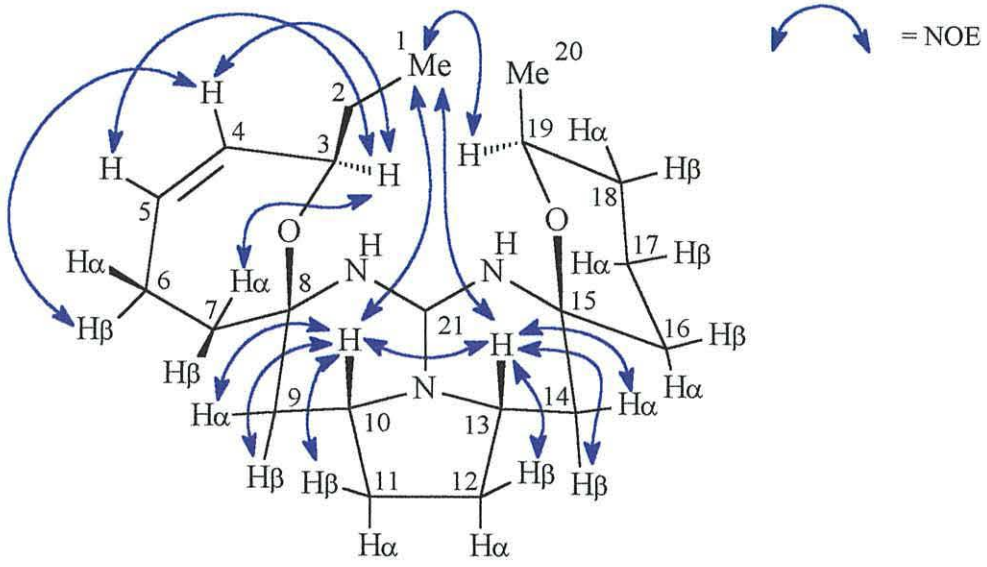


Figure 5.5 Diagram showing the NOE's present in **184** which confirm the overall *syn* stereochemistry found in the pentacyclic model compound.

5.7 CONCLUSION

This chapter detailed the preparation of a biologically active pentacyclic model **184**, of ptilomycalin A. The model compound was synthesised *via* a convergent route where the two precursors were reacted together at the penultimate stage to yield *bis*-α,β-unsaturated ketone **185**. The precursor, chiral phosphorane **186** was prepared from commercially available ethyl (*R*)-3-hydroxybutyrate, whilst chiral unsaturated aldehyde **22** was synthesised using known chemistry. The double Michael addition of guanidine to **185** was successfully accomplished with a rather problematical deprotection and cyclisation yielding the desired pentacyclic model.

Unfortunately, the pentacycle could not be obtained in crystalline form, hence, the stereochemistry of **184** was determined by means of one and two dimensional NMR experiments. The stereochemistry of **184** was unambiguously assigned as the *syn* diastereomer on the basis of these experiments and in addition, by comparison with the NOESY/ROESY data reported for TFA-ptilomycalin A **13** and 13,14,15-isocrambescidin 800 (**60**).

CHAPTER 6

PREPARATION OF A SYNTHETIC ANALOGUE OF PTILOMYCALIN A

6.1 INTRODUCTION

The second aim of this project was to develop an efficient approach towards the preparation of synthetic analogues of ptilomycalin A. Biological testing of these analogues could help determine their mode of action, as well as providing information on the minimal structural and functional requirements for activity. In addition, the effects of altering the substitution patterns, functionality and counter ion could all be investigated.

As was previously discussed, ptilomycalin A consists of a guanidine-containing polycyclic core, a spacer unit and a spermidine residue (Figure 6.1). Hence, the design of an analogue of ptilomycalin A should incorporate these three structural units in the correct sequence. The only previous example of a ptilomycalin A analogue **41** that obeys the above criteria was reported by Hart and Grillot,[36,116] where a bicyclic guanidine is attached *via* an ester linkage to a spermidine bound spacer unit as shown in Figure 6.1.

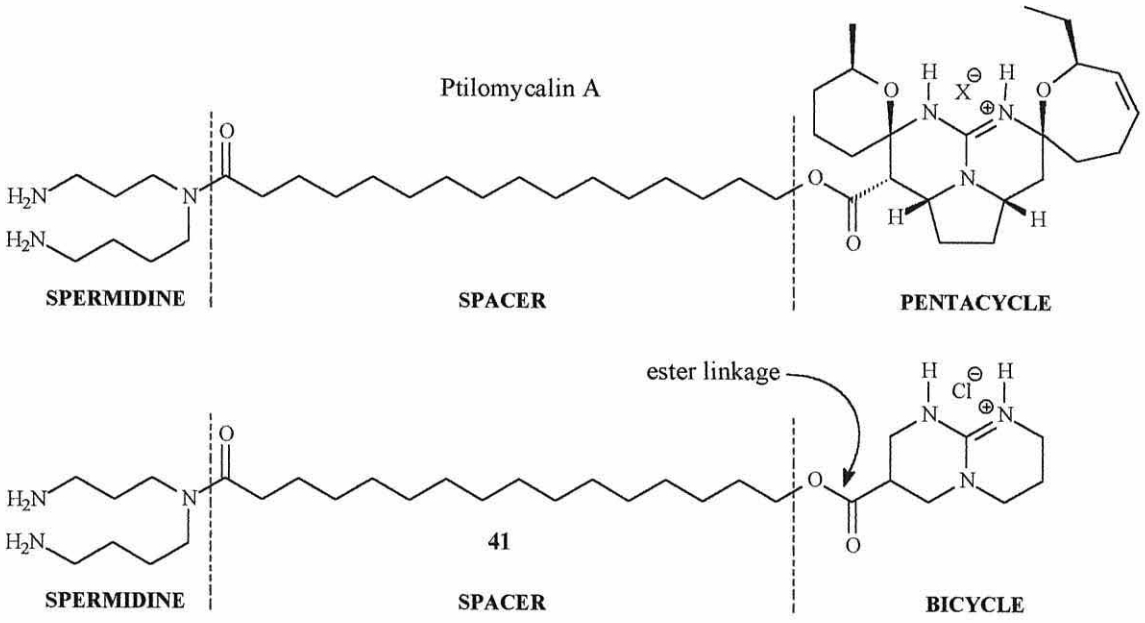
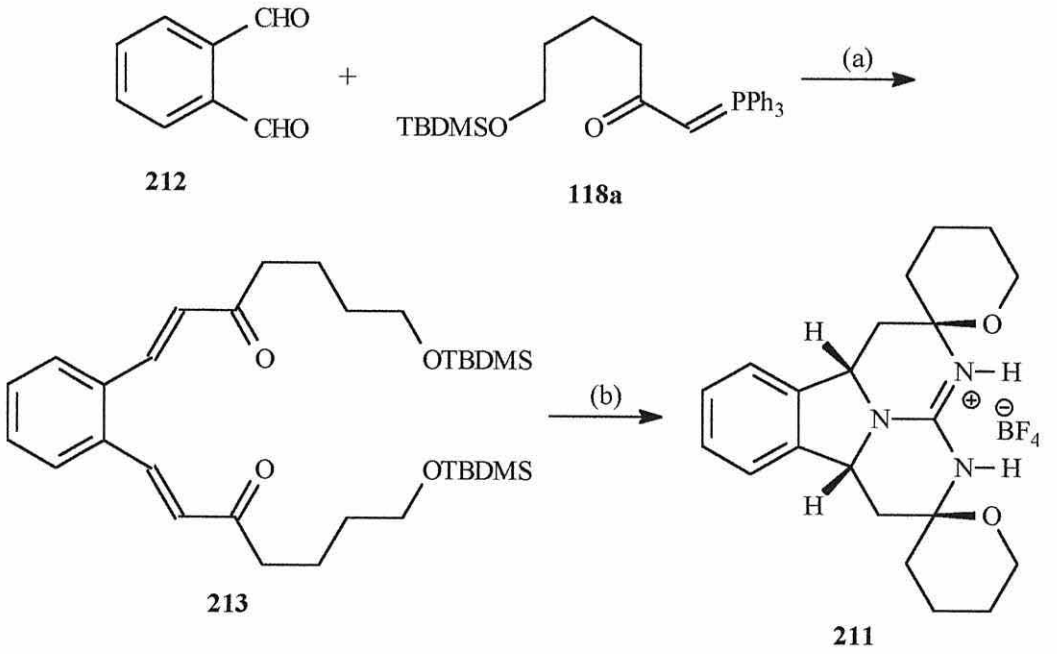


Figure 6.1 Hart and Grillot's bicyclic analogue **41**; a comparable structure to ptilomycalin A.

Unfortunately, Hart and Grillot[36] reported that storing amine **41** over a period of a few weeks resulted in cleavage of the ester linkage through an unidentified process which prevented a biological evaluation of the model compound. The structures of the decomposition products were not determined although NMR spectroscopic analysis

revealed that the signal due to the methylene group adjacent to the ester oxygen was no longer present. This observation led the authors to speculate that the role of the spiro *N,O*-acetal in **12** was to sterically protect the ester linkage from hydrolysis or ammonolysis.[36] If this is actually the case, then inclusion of the ester group in any ptilomycalin A analogue might not be essential as it may be imparting a degree of instability into the system.

A previous study[113] towards the preparation of compounds that mimic the pentacyclic core of ptilomycalin was successful in generating benzo-fused hexacycle **211**. The synthesis of **211** was accomplished *via* a simple two step procedure starting from commercially available *o*-phthalic dicarboxaldehyde **212**. A double Wittig reaction between **212** (0.25 equivalents) and the known protected phosphorane **118a**, (see Chapter 3, page 46), gave *bis*-α,β-unsaturated ketone **213** in 62% yield. Subsequent guanidine cyclisation under standard conditions followed by counter ion exchange gave the desired hexacycle in an overall yield of 35% (Scheme 6.1).



***Reagents and Conditions*:** (a) CH_2Cl_2, 20°C, 20 h; (b) (i) DMF, guanidine, 0-20°C, 5 h; (ii) MeOH/HCl, 0°C, 1 h, then 20°C, 15 h; (iii) Saturated aq. $NaBF_4$; (iv) Trituration and crystallisation.

Scheme 6.1 Preparation of benzo-fused guanidine hexacycle **211**.[113]

Fortuitously, the isolation of the hexacycle in crystalline form was possible with X-ray and NMR investigations proving that it had formed as a single diastereomer with no evidence of the presence of any minor diastereomers. The hexacyclic model possesses an identical stereochemistry to that found in the pentacyclic core of ptilomycalin A and would therefore be expected to bind anions in a similar manner. It was surmised that hexacycle **211** could be utilised as the polycyclic unit for the synthesis of a structural analogue of ptilomycalin A. Attaching the spermidine bound spacer directly to the aryl portion of **211** would yield a plausible analogue, **214**, of the natural product, whilst dispensing with the potentially unstable ester group. In addition, analogue **214** possesses all the aforementioned structural requirements (Figure 6.2).

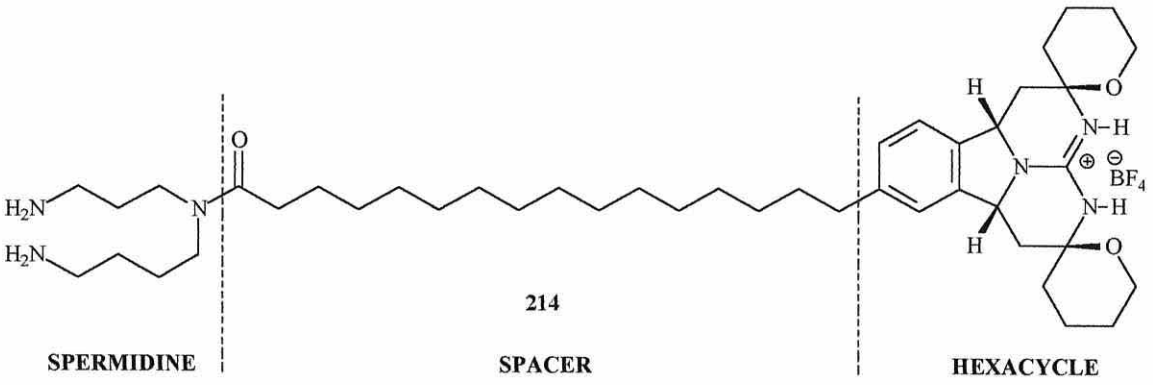
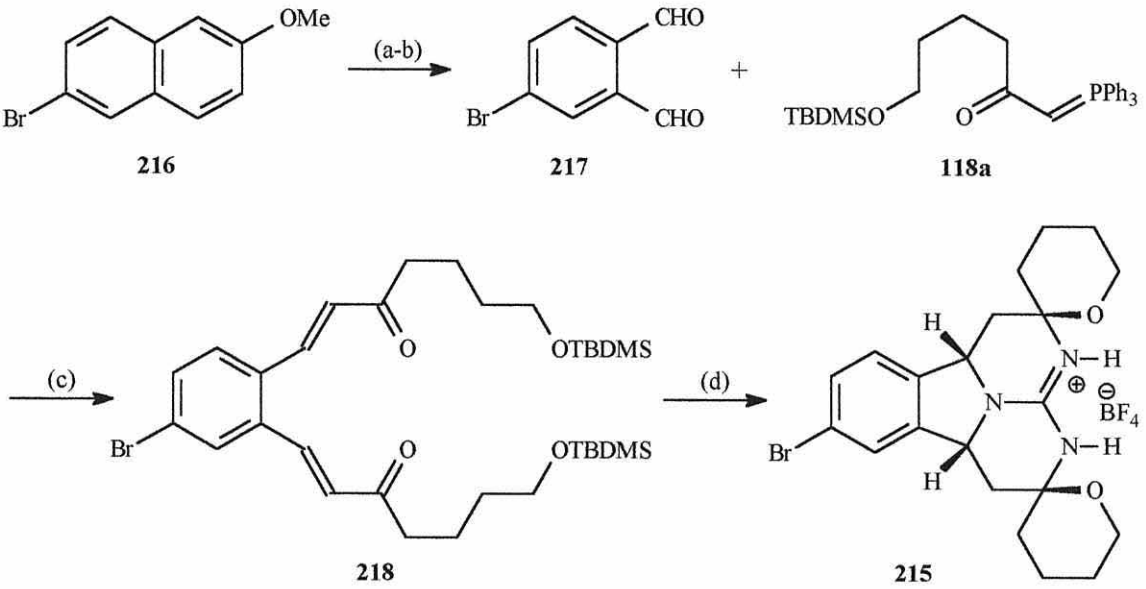


Figure 6.2 Structure of the proposed synthetic analogue of ptilomycalin A, **214**.

Further synthetic studies[117] led to the formation of the corresponding halogenated benzo-fused hexacycle **215** (Scheme 6.2, page 119). The synthesis was accomplished in four steps starting from commercially available 2-bromo-6-methoxynaphthalene, **216**. Ozonolysis of **216** followed by reductive cleavage and acid hydrolysis of any intermediates (Scheme 6.2, steps [a-b]), gave dialdehyde **217** in a disappointing overall yield of 16%. As was the case for hexacycle **211**, a double Wittig reaction between **217** and phosphorane **118a** led to the *bis*-α,β-unsaturated ketone, **218** which was subsequently reacted with guanidine to afford, after counter ion exchange, the desired brominated hexacycle **215** (14% yield from **218**). On this occasion, the hexacycle was not obtained in crystalline form, however, the proton and carbon NMR spectra indicated the presence of a single hexacyclic diastereomer which was assumed to possess the same relative stereochemistry as that found in hexacycle **211** and ptilomycalin A.



***Reagents and Conditions*:** (a) 50% MeOH/CH_2Cl_2, O_3, then $S(CH_2CH_2COONa)_2$; (b) HCl (aq.) reflux; (c) CH_2Cl_2, rt, 3 d; (d) (i) DMF, guanidine, 0-20°C, 5 h; (ii) MeOH/HCl, 0°C, 1 h, then 20°C, 15 h; (iii) Saturated aq. $NaBF_4$.

Scheme 6.2 Synthesis of halogenated benzo-fused guanidine hexacycle **215**.[117]

Brominated hexacycle **215** represents a suitable starting material for the preparation of the ptilomycalin A model compound **214**, (see Figure 6.2, page 118). It was envisaged that the analogue could be prepared by coupling the halogenated hexacycle to a spermidine bound spacer chain by means of the Suzuki[118] or Sonogashira[119] reactions. Hydrogenation of the resulting alkene (Suzuki product) or alkyne (Sonogashira product) would then afford the desired structural analogue.

6.2 Retrosynthetic Analysis

A retrosynthesis of hexacyclic analogue **214** reveals a relatively straightforward synthetic procedure where the three structural units are added sequentially. As shown in Figure 6.3 (page 120), a disconnection **[A]** between the spacer chain and the hexacyclic core leads to the brominated polycycle **215** and protected amide **219**. A second disconnection **[B]** across the amide bond yields *bis*-protected spermidine **220** and the free spacer unit as the alkynic carboxylic acid **221**. During the synthesis, it is imperative to selectively protect the primary amino groups of spermidine **45**, (see Chapter 2, page 22) to ensure that the secondary amino moiety forms the amide link with carboxylic acid **221**.

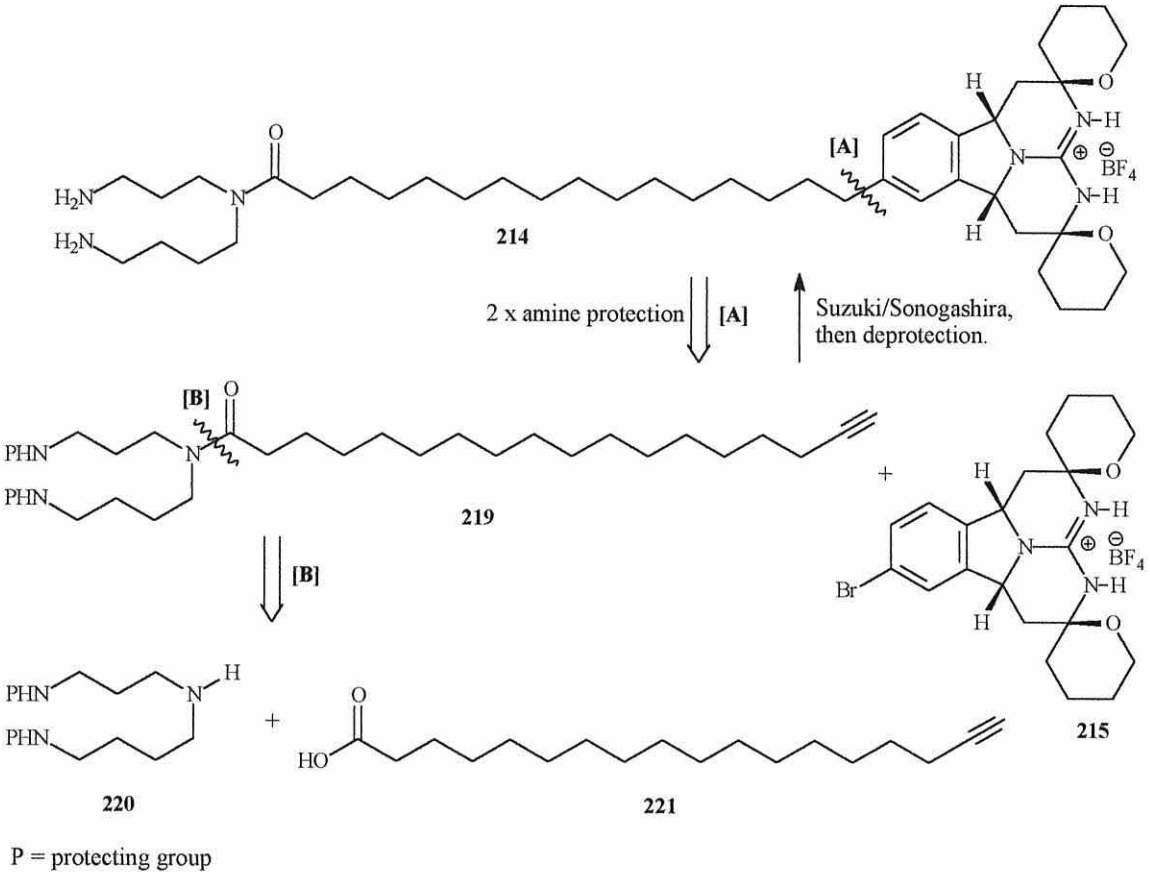
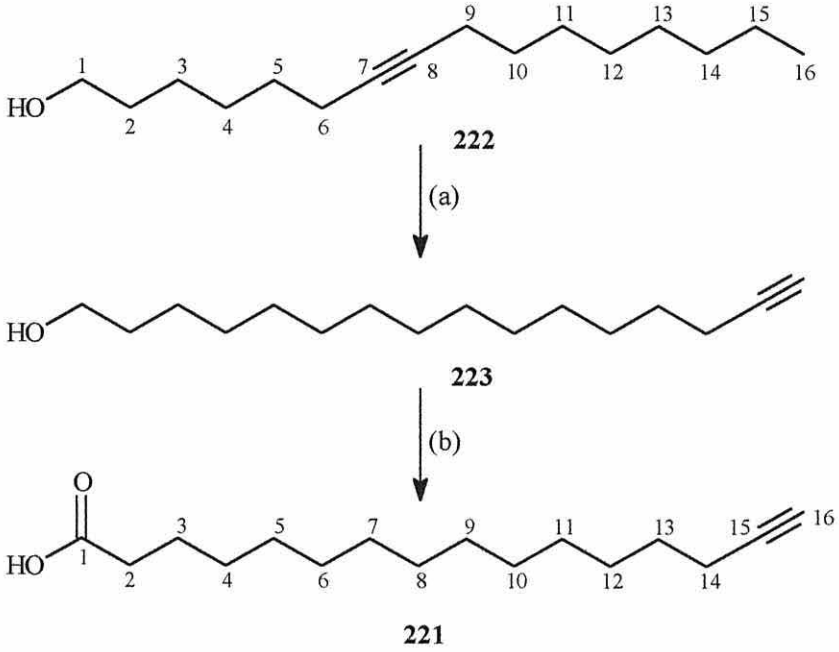


Figure 6.3 Retrosynthesis of the proposed ptilomycalin A analogue, **214**.

6.3 Preliminary Synthetic Efforts Towards Analogue **214**

6.3.1 Preparation Of Alkynic Carboxylic Acid **221**

In 1975, Brown and Yamashita[120] reported that a triple bond in any position of a straight chain hydrocarbon or acetylinic alcohol could be isomerised exclusively to the free terminus of the chain, when treated with a sufficiently strong base. The reaction, termed the "Zipper reaction", consequently provides a solution for the preparation of compounds containing remote functionalisation in long hydrocarbon chains. Commercially available 7-hexadecyn-1-ol, **222**, was identified as being a suitable starting material for the preparation of carboxylic acid **221**. The triple bond at C-7 was successfully isomerised to the free terminus at C-16 by means of the aforementioned reaction, using the synthetic procedure outlined by Abrams and Shaw[121] (Scheme 6.3, page 121).



***Reagents and Conditions*:** (a) Lithium, 1,3-diaminopropane, 70°C, KO*t*-Bu, Et_2O; (b) PDC, DMF.

Scheme 6.3 Synthesis of alkynic carboxylic acid **221**.

Following work-up and purification on silica gel, isomerised alcohol **223** was obtained in a 72% yield, (97% purity within NMR limits; impurity being unreacted starting material). The infrared spectrum of **223** immediately showed the presence of a terminal alkyne demonstrating a band at 3287 cm^{-1}. Confirmation of the structure of **223** was given by the proton NMR spectrum which showed a triplet resonance at δ 1.94 (J = 2.6 Hz) which was identified as the terminal alkyne proton.

The migration of the triple bond from an internal position to the terminus is known to occur through a process of random and reversible 1,3-proton transfers between intermediate acetylenes and allenes.[122,123] Evidence exists suggesting that the mechanism for isomerisation may involve a series of both concerted and non-concerted 1,3-prototropic shifts. In addition, the rates of isomerism are variable, depending on base strength, reaction conditions and the presence of other functional groups within the structure. Stabilisation of the relatively electron deficient acetylene group is achieved when adjacent electron donating groups are present; consequently destabilisation occurs in the presence of electron withdrawing groups.[123] In the case of **222**, the terminal hydroxyl group will

prevent isomerisation towards C-1, thereby forcing migration of the triple bond to the opposite terminus. Upon formation of the terminal acetylene, it is immediately trapped as the acetylide salt, thus preventing further isomerisation.

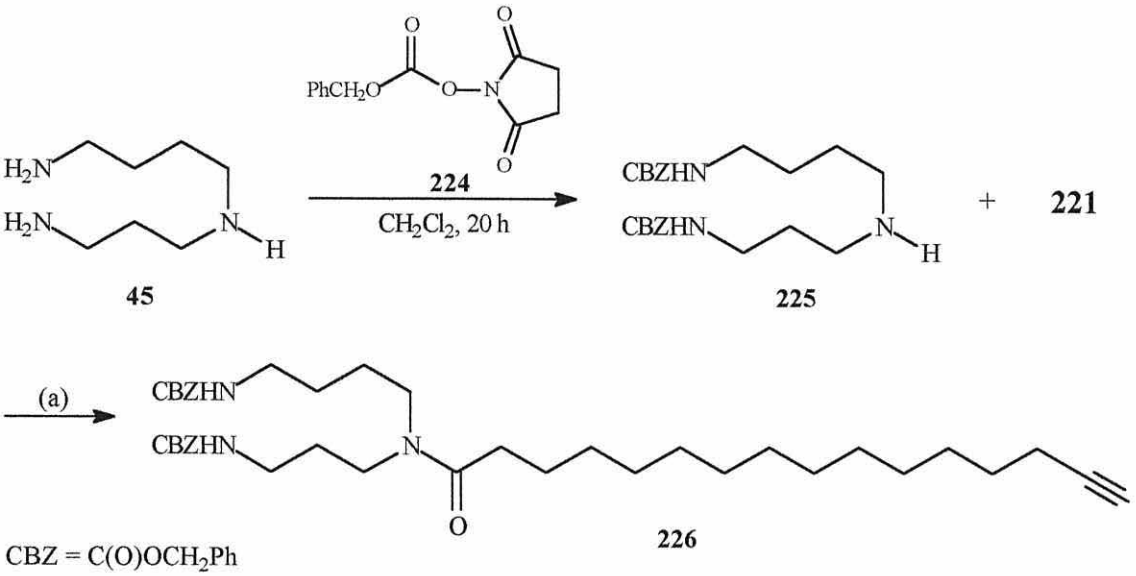
The hydroxyl group of **223** was oxidised to the corresponding carboxylic acid using PDC[124] as the oxidising agent. This was achieved by treating a solution of the primary alcohol in DMF with PDC followed by stirring for sixteen hours. The reaction was subsequently quenched with water and after work-up, the desired acid **221** was isolated as a light brown solid in a highly satisfactory yield of 87%.

6.3.2 Synthesis Of *Bis*-Protected Amide **226**

As previously stated, prior to coupling spermidine to carboxylic acid **221**, it was necessary to protect the two primary amino groups with an appropriate protecting group. Spermidine **45** is one of a class of ubiquitous, naturally occurring polyamines [125] which also includes putrescine [$H_2N(CH_2)_4NH_2$] and spermine [$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$]. They are biomolecules of considerable importance, having a high affinity for DNA and in addition, play a major role in cellular growth and differentiation.[126] Furthermore, rapidly proliferating tumour cells have been found to contain elevated concentrations of these polyamines.[127]

Using a procedure similar to that described by Hart and Grillot,[36] the two primary amino groups of spermidine were selectively protected upon treatment with two equivalents of *N*-(benzyloxycarbamoyloxy) succinimide, **224**, giving carbamate **225** in a moderate 40% yield (Scheme 6.4, page 123). The spectral data for **225** was found to be consistent with that reported by Hart and Grillot.[36]

Amide **226** was prepared by treating a dichloromethane solution of carboxylic acid **221** with carbamate **225** using DCC as the coupling agent; HOBT was also added to the reaction in order to prevent DCC rearrangement. After stirring for 24 hours, the reaction mixture was cooled to 0°C to precipitate the dicyclohexyl urea by-product which was then removed by filtration. Proton NMR analysis showed the presence of remaining by-product, hence, further purification by column chromatography was required, eventually affording amide **226** as a dense yellow oil in an 86% yield (Scheme 6.4).



***Reagents and Conditions*:** (a) CH_2Cl_2, DCC, HOBT, 24 h.

Scheme 6.4 Preparation of protected amide **226**.

The proton NMR spectrum of **226** demonstrated a multiplet resonance between the range δ 7.39-7.28 integrating to ten, which corresponded to the ten aromatic hydrogens. A broad singlet at δ 6.82 was identified as the protons of the two protected amine groups, whilst the two methylenes adjacent to the phenyl groups appeared as a singlet at δ 5.04. The multiplet between δ 3.29-3.20 was attributed to the two methylenes next to the CBZ protected amine groups, whilst the multiplet between δ 3.09-2.99 coincided with the methylenes juxtaposed to the amide nitrogen. Further confirmation of the presence of the amide bond was given by the occurrence of a triplet resonance at δ 2.23 (J = 7.1 Hz), corresponding to the methylene adjacent to the amide carbonyl. The remaining proton NMR signals as well as those from the carbon NMR spectrum were consistent with the structure of **226**. The advantage of using the CBZ protecting group is that it can be removed at the same time as reduction of the double bond, formed during the ensuing Suzuki cross-coupling reaction.

6.3.3 Attempted Suzuki Cross-Coupling

As previously discussed in Section 6.1 (page 119), it was intended to make use of the Suzuki[118] or Sonogashira reaction[119] to couple amide **226** to halogenated, benzo-fused

guanidine system **215**. Initial couplings were attempted using the Suzuki reaction which is an example of a palladium catalysed cross-coupling reaction between an organoborane compound (R-M) and an organic halide (R'-X), and is an extremely useful method for the generation of carbon-carbon bonds (Figure 6.3). The formation of the organoborane compound is achieved by reaction of an alkene or alkyne with a hydroborating agent such as catecholborane, **227** (1,3,2-benzodioxaborazole).[128]

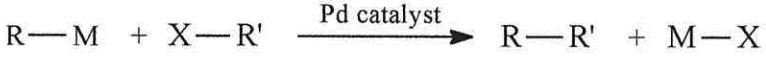
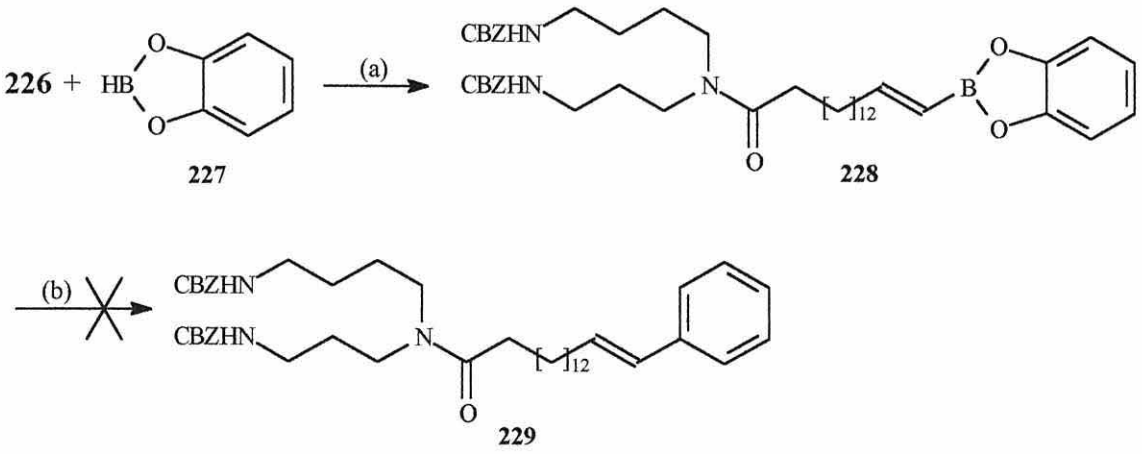


Figure 6.3 A general equation for the Suzuki reaction.

As supplies of hexacycle **215** were limited, the viability of the cross-coupling reaction was first tested on a model system between amide **226** and either bromo- or iodobenzene. Employing the one-pot procedure described by Suzuki and co-workers,[129] amide **226** was dissolved in dry benzene and treated with a catalytic amount of tetrakis(triphenylphosphine) palladium (0), [$Pd(PPh_3)_4$], followed by catecholborane, **227** (Scheme 6.5). This step was used to form the intermediate organoborane **228**, which after stirring for 22 hours, was treated with one equivalent of bromobenzene and an aqueous solution of sodium hydroxide (3M), and then heated at reflux for two hours. Subsequent cooling and work-up gave a crude oil whose proton NMR spectrum failed to show any indication of the olefinic resonances of the expected cross-coupled product **229**.

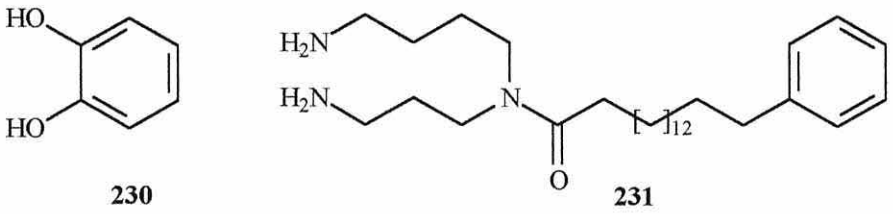


***Reagents and Conditions*:** (a) $Pd(PPh_3)_4$, C_6H_6; (b) ArX (X = Br or I), NaOH (3M) or Na_2CO_3 (3M).

Scheme 6.5 The attempted Suzuki cross-coupling reaction using a model system.

The reaction was attempted on a second occasion and after formation of the organoborane, one equivalent of iodobenzene, followed by three equivalents of aqueous 3M sodium carbonate solution, were added. The mixture was heated at reflux for three hours, cooled to room temperature and then quenched with water but again, work-up, followed by purification on silica gel failed to yield the desired olefin. At this point it remained unclear as to which of the reaction stages might not be working, either (a) formation of organoborane **228**, or (b) reductive elimination to alkene **229**. It was therefore decided to attempt to prepare and subsequently isolate the intermediate organoborane.

To this end, amide **226** and catecholborane were heated together at reflux for two hours and then allowed to cool to room temperature. The resulting product was absorbed onto silica and purified by column chromatography. Two fractions were obtained which were identified, by way of NMR spectroscopy, as catechol **230** and unreacted amide **226**. Catechol was formed as a result of hydrolysis under acidic conditions of either the organoborane or alternatively, unreacted catecholborane (Figure 6.4). An attempt to isolate the organoborane by distillation also proved unsuccessful, resulting in decomposition of the starting materials. This was a very disappointing outcome as Suzuki product **229** could have been simultaneously reduced and deprotected *via* hydrogenation, to yield the desired product model compound, **231** (Figure 6.4).



230 **231**

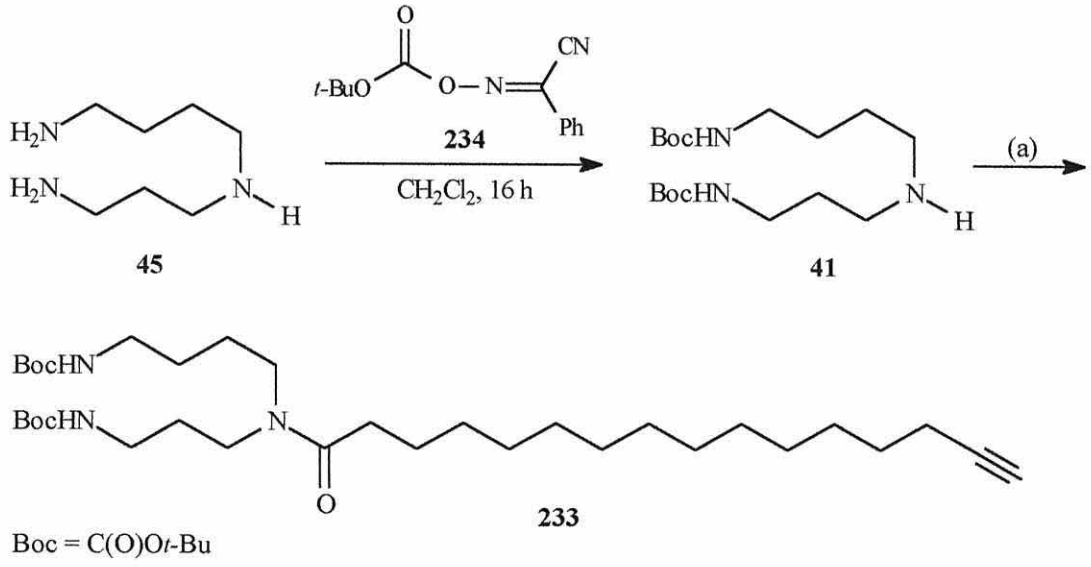
Figure 6.4 The hydrolysis product catechol, **230** and the desired model compound **231**.

6.3.4 SYNTHESIS OF *BIS*-BOC-PROTECTED AMIDE **233**

Having failed to synthesise Suzuki model compound **231** it was decided to attempt the cross-coupling reaction between the corresponding *bis*-Boc protected amide **233** and

an aryl halide (Scheme 6.6). The advantage being that the NMR spectrum of the product would on this occasion be simpler to interpret as the presence of aromatic protons would be indicative of the Suzuki product with no influence from the protecting groups, as in the case of **229**. To this end, *bis*-Boc protected amide **233** was prepared using an almost identical procedure to that described for CBZ-protected amide **229**.

Protection of spermidine was accomplished following treatment with two equivalents of a dichloromethane solution of 2-(*tert*-butoxycarbonyloximino)-2-phenylacetonitrile (Boc-ON) **234**, which after stirring overnight gave N^1,N^8-Boc_2-spermidine **41**, in a 47% yield (Scheme 6.6). The melting point was found to be in good agreement with that quoted in the literature,[130] (melting point = 84-86°C, literature melting point = 85-86°C). Reaction of **41** with carboxylic acid **221** then furnished, after purification, the desired amide as a yellow oil in a satisfactory 78% yield. The proton and carbon NMR spectra of amide **233** were very similar to those of amide **229**, differing only in the resonances of the protecting groups. In the proton NMR spectrum, the six methyls of the two Boc groups were observed as a broad singlet at δ 1.27. Similarly, the ^{13}C NMR spectrum demonstrated an overlapping resonance at δ 28.39 which corresponded to both Boc groups.

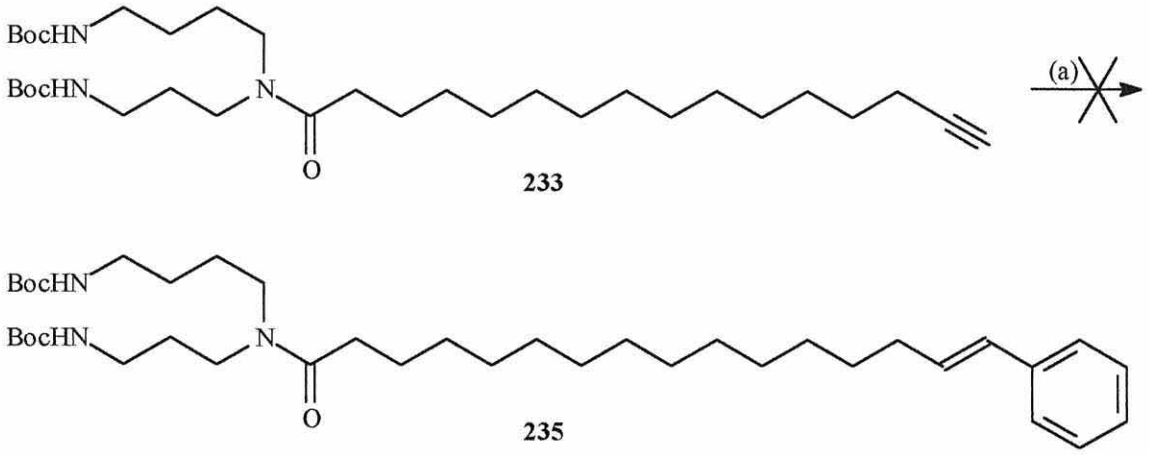


***Reagents and Conditions*:** (a) CH_2Cl_2, **221**, DCC, HOBT, 24 h.

Scheme 6.6 The synthesis of *bis*-Boc protected amide **233**.

6.3.5 ATTEMPTED MODEL CROSS-COUPLING

The cross-coupling reaction was again attempted on a model system in an effort to determine whether an alkene could be prepared. A similar procedure to that previously described in Section 6.3.3 (pages 123-125) was employed in an attempt to prepare alkene **235**. On this occasion, following stirring overnight a further portion of catalyst was added along with three equivalents of an aqueous 3M sodium carbonate solution and three equivalents of iodobenzene (Scheme 6.7). The resulting mixture was heated at reflux over a period of three hours before work-up and purification by column chromatography. In all, two fractions were recovered which were identified through ^{1}H NMR spectroscopy as unreacted starting materials, that is, amide **233** and iodobenzene. Further attempts at this reaction proved unsuccessful hence, an alternative coupling procedure was pursued.



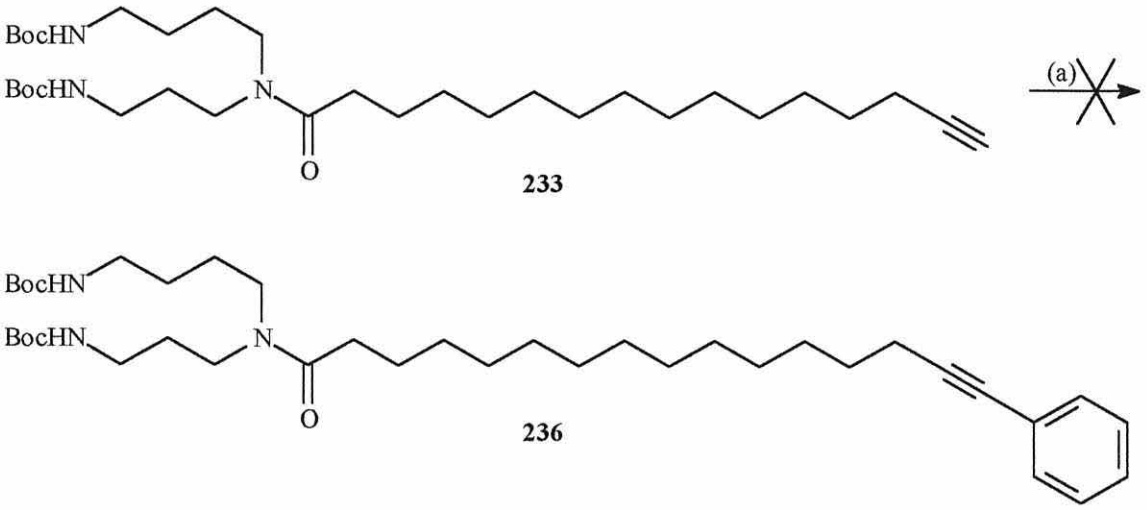
***Reagents and Conditions*:** (a) C_6H_6, $Pd(PPh_3)_4$, catecholborane, rt, 22h, then $Pd(PPh_3)_4$, aq. Na_2CO_3 (3M), iodobenzene, reflux, 3 h.

Scheme 6.7 The attempted synthesis of model Suzuki product **235**.

6.4. AN ALTERNATIVE COUPLING REACTION

An alternative method for coupling an aryl halide to a terminal alkyne has been reported by Lau and co-workers.[131] Their procedure describes the cross-coupling of a protected acetylene to various substituted aryl halides using palladium(II) acetate as the catalyst. In an effort to determine whether their procedure could be utilised to facilitate

the preparation of hexacyclic analogue **214**, an attempt was made to couple amide **233** to bromobenzene (Scheme 6.8). Using Lau's[131] approach, a mixture consisting of bromobenzene, triethylamine, toluene, triphenylphosphine, palladium(II) acetate and protected amide **233** was heated at reflux for five hours. After cooling, the mixture was extracted with dichloromethane and purified by column chromatography yielding three separate fractions. Analysis of each fraction by proton NMR spectroscopy revealed the presence of unreacted amide **233** and bromobenzene, but no trace of the desired cross-coupled product, **236**.



233

236

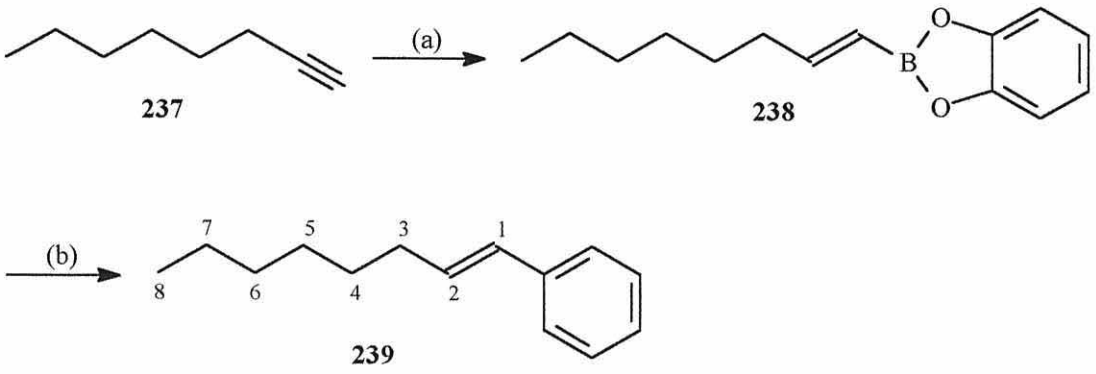
***Reagents and Conditions*:** (a) Bromobenzene, Et_3N, PhMe, PPh_3, $Pd(OAc)_2$, reflux, 5 h.

Scheme 6.8 An alternative attempt to generate a cross-coupled product using the procedure of Lau and co-workers.[131]

6.5 Further Model Suzuki Reactions

As Suzuki reactions are well known in the literature,[118] it was both disappointing and bewildering that no cross-coupled products could be obtained from the attempted model system reactions. In another report by Suzuki and co-workers,[132] they describe the synthesis of conjugated, palladium cross-coupled products in high yields using relatively strong bases such as sodium alkoxide, phenoxide and hydroxide. It was therefore decided to utilise their procedure in an effort to determine whether a simple cross-coupled alkene could be generated by a Suzuki reaction between 1-octyne **237** and iodobenzene (Scheme 6.9, page 129).

Thus, an equimolar mixture of **237** and catecholborane was heated at reflux for two hours. Removal of the reaction solvent and any unreacted alkyne by distillation left a black oil which was diluted with benzene and treated with $Pd(PPh_3)_4$ catalyst followed by iodobenzene. A solution of sodium ethoxide (2M) in ethanol was added after thirty minutes and the resulting mixture heated at reflux for a further two hours. After cooling to room temperature, aqueous NaOH (3M) solution was added in order to hydrolyse the intermediate boronic ester **238**. Work-up proved to be extremely difficult, with incomplete separation of the aqueous and organic phases resulting in a low yield (28%) of recovered olefinic product, **239** (Scheme 6.9).



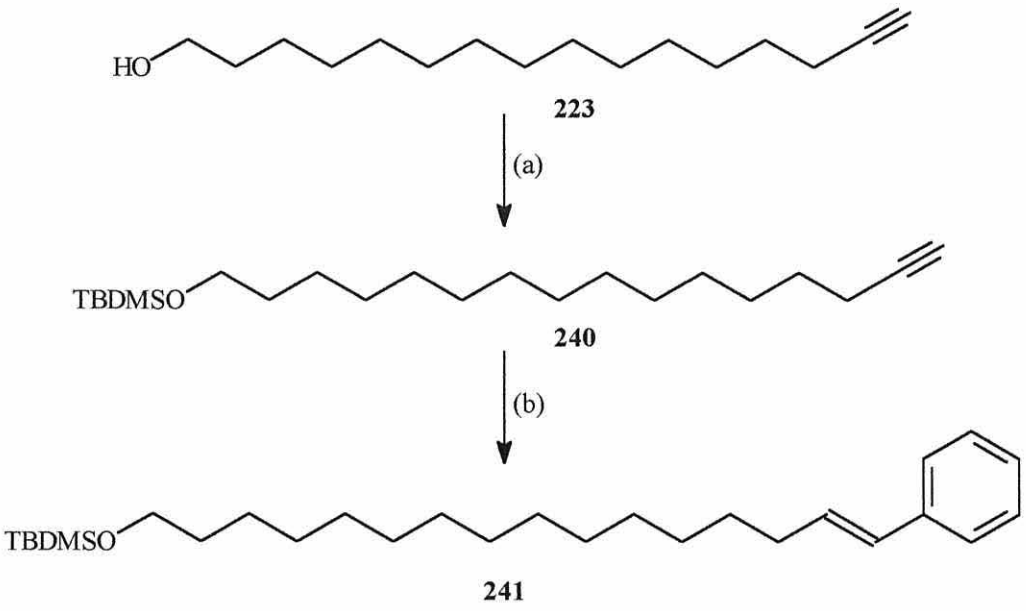
***Reagents and Conditions*:** (a) Catecholborane, reflux, 2 h; (b) C_6H_6, $Pd(PPh_3)_4$, iodobenzene, 2M NaOEt/EtOH, reflux, 2 h, then 3M NaOH, rt, 16 h.

Scheme 6.9 Synthesis of the simple cross-coupled alkene, **239**.

Evidence of the successful preparation of **239** was provided by proton NMR spectroscopy, the spectrum of which demonstrated a complex multiplet between the range, δ 7.45-7.13 which corresponds to the five protons of the aromatic ring. A doublet at δ 6.40 (J = 17.0 Hz) and a double triplet at δ 6.26 (J = 16.9, 7.8 Hz) indicated the olefinic protons at C-1 and C-2, respectively. The coupling constants indicated that the product formed exclusively as the *trans* isomer; no evidence of the *cis* isomer was present in the NMR spectrum. A resonance at δ 2.22 was due to the methylene adjacent to the double bond whilst the terminal methyl group occurred as a triplet at δ 0.92 (J = 7.0 Hz).

This procedure was also employed in order to cross-couple the previously prepared 16-carbon alcohol **223**, (see Section 6.3.1, page 121) to iodobenzene. Prior to attempting

this reaction, the alcohol was protected as the corresponding silyl ether **240**, under standard conditions, in an excellent 98% yield. On this occasion the cross-coupled, model alkene **241**, was successfully synthesised, albeit in a fair yield of 35% (Scheme 6.10).

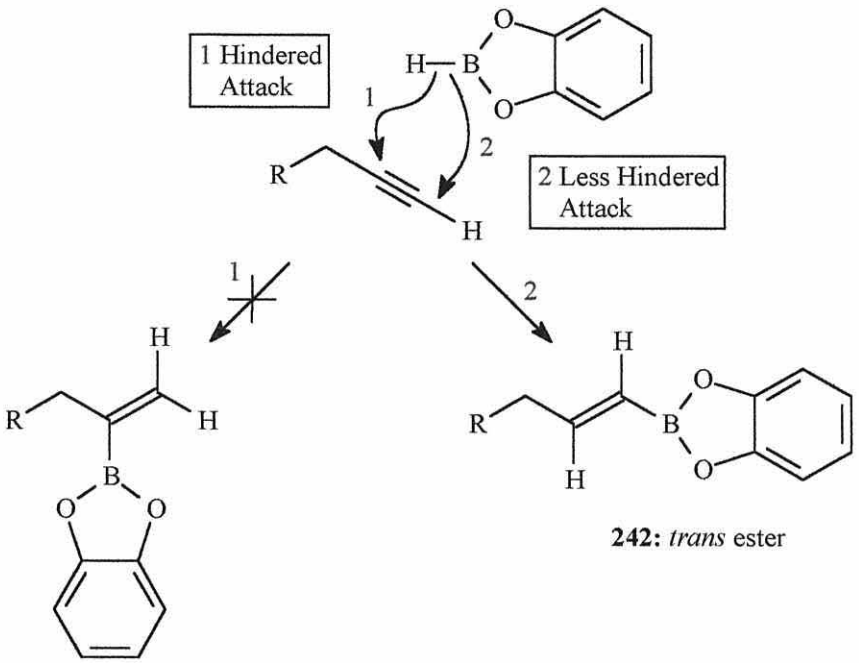


***Reagents and Conditions*:** (a) DMF, imidazole, TBDMSCl, 2.5 h; (b) Catecholborane, reflux, 2 h, then rt, 16 h, then C_6H_6, Pd $(PPh_3)_4$, iodobenzene, 2M NaOEt/EtOH, reflux, 2 h, then 3M $NaOH/H_2O$, rt, 20 h.

Scheme 6.10 Preparation of model compound **241** *via* the Suzuki reaction.

The proton and carbon NMR spectra of model alkene **241** demonstrated all of the expected resonances. The olefinic protons appeared as a doublet at δ 6.34 (J = 16.3 Hz) and a double triplet at δ 6.18 (J = 16.1, 5.5 Hz). Furthermore, the values of the coupling constants were again consistent with those of a *trans*-olefin and once again, there was no evidence of a *cis*-geometrical isomer in either the ^1H or the ^{13}C NMR spectra.

The addition of catecholborane to alkynes normally proceeds in a *cis* anti-Markovnikov manner[118] from the less hindered side of the triple bond. Thus addition of B–H across the triple bond results in the formation of a *trans*-1-alkenylboronic ester **242** and not the *geminal*-1-alkenylboronic ester **243** which would be the expected product of the characteristic Markovnikov addition at the least substituted carbon atom (Figure 6.5, page 131).



243: *geminal* ester

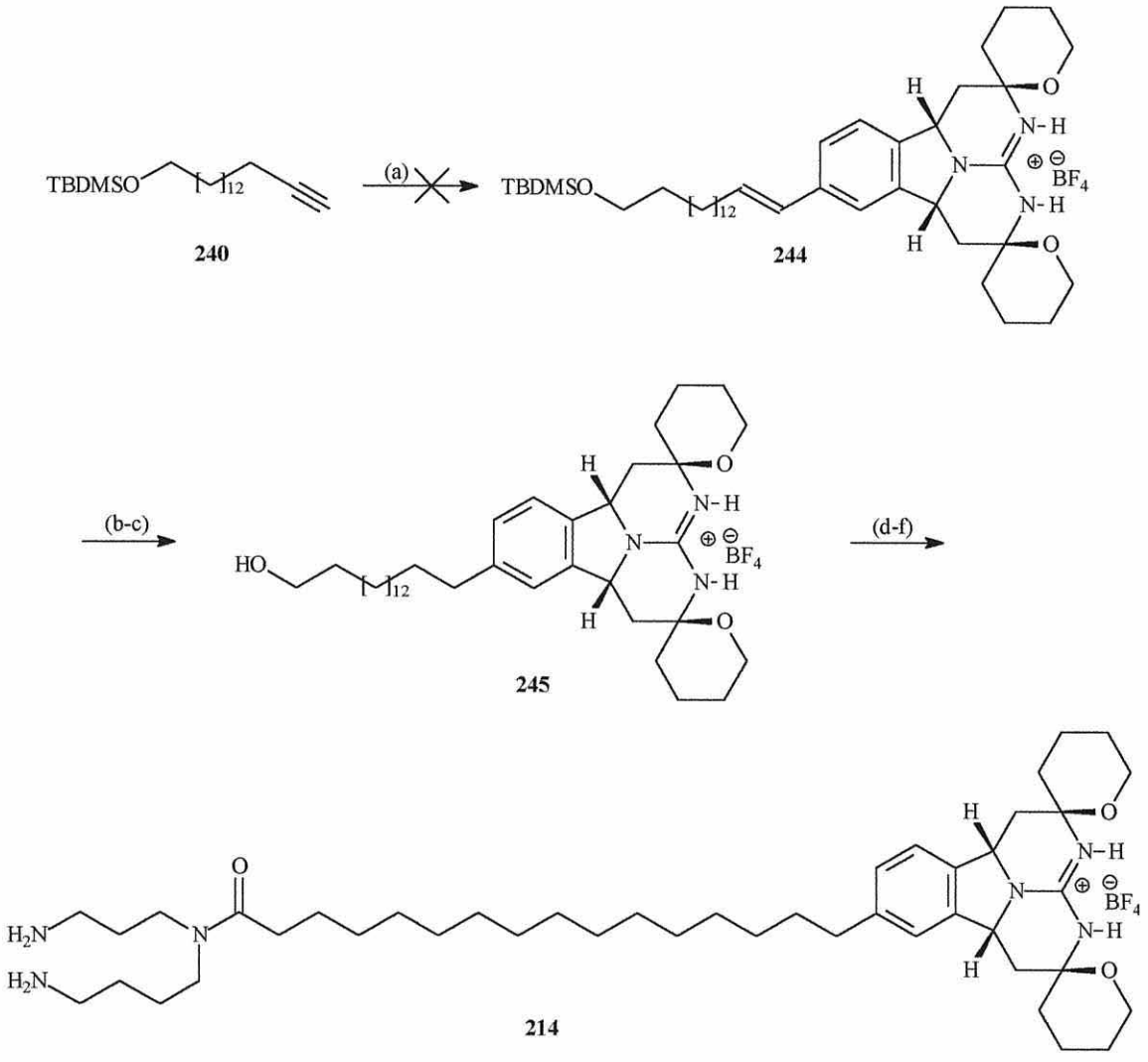
242: *trans* ester

Figure 6.5 Diagrammatic representation showing the favoured *cis* anti-Markovnikov addition and the resulting *trans* product, **242**.

6.6 Attempted Suzuki Coupling To Hexacycle **215**

Having successfully generated an olefinic product from the Suzuki cross-coupling reaction, it became evident that an alternative approach was available for the preparation of analogue **214**. This procedure involved cross-coupling protected alkyne **240** to brominated hexacycle **215** and then attaching the spermidine residue by way of established methodology. It was therefore anticipated that alkene **244** could be prepared using identical Suzuki reaction conditions to those outlined for the synthesis of alkenes **239** and **241**. Palladium catalysed hydrogenation of the resulting alkene double bond and subsequent TBAF mediated deprotection of the silyl ether would afford intermediate alcohol **245**. Subsequent oxidation of the alcohol to the corresponding carboxylic acid followed by DCC coupling of *bis*-Boc protected spermidine **41** and finally, amine deprotection would yield the desired hexacyclic, ptilomycalin A analogue **214** (Scheme 6.11, page 132).

Unfortunately, purification of the crude material from the Suzuki reaction (Scheme 6.11, step [a]), failed to produce the required cross-coupled alkene **244**. This was extremely disappointing as neither starting material was recovered as had been the case in previous reactions of this type. It therefore appears that benzo-fused hexacycle **215** can not tolerate the rather harsh, basic reaction conditions employed for this Suzuki reaction and must therefore be decomposing *via* an (as yet) unidentified process.



***Reagents and Conditions*:** (a) Catecholborane, reflux, 2 h, then rt, 16 h, then C_6H_6, Pd $(PPh_3)_4$, **215**, 2M NaOEt/EtOH, reflux, 2 h, then 3M $NaOH/H_2O$, rt, 16 h; (b) EtOAc, Pd/C, H_2, 10 min; (c) TBAF, THF; (d) DMF, PDC, rt, 24 h; (e) CH_2Cl_2, **41**, DCC, HOBT, rt, 24 h; (f) HCOOH, rt, 2 h.

Scheme 6.11 An alternative synthesis of ptilomycalin A analogue, **214**.

6.7 An Alternative Strategy Towards Analogue **214**

Having been unable to prepare hexacyclic analogue **214** by way of the previously discussed routes, it became apparent that an alternative synthetic strategy was required. A potential synthetic strategy involves coupling protected 16-carbon spacer **240** to 2-bromo-6-methoxynaphthalene, **216** (see Section 6.1, page 118), by way of the established Suzuki methodology. Following hydrogenation of the alkene double bond the hexacyclic core could then be fashioned using a similar procedure to that outlined in the synthesis of brominated hexacycle **215** (see Scheme 6.2, page 119). Finally, deprotection of the silyl ether followed by addition and subsequent deprotection of *bis*-Boc spermidine, **41** would yield the desired hexacyclic analogue, **214**.

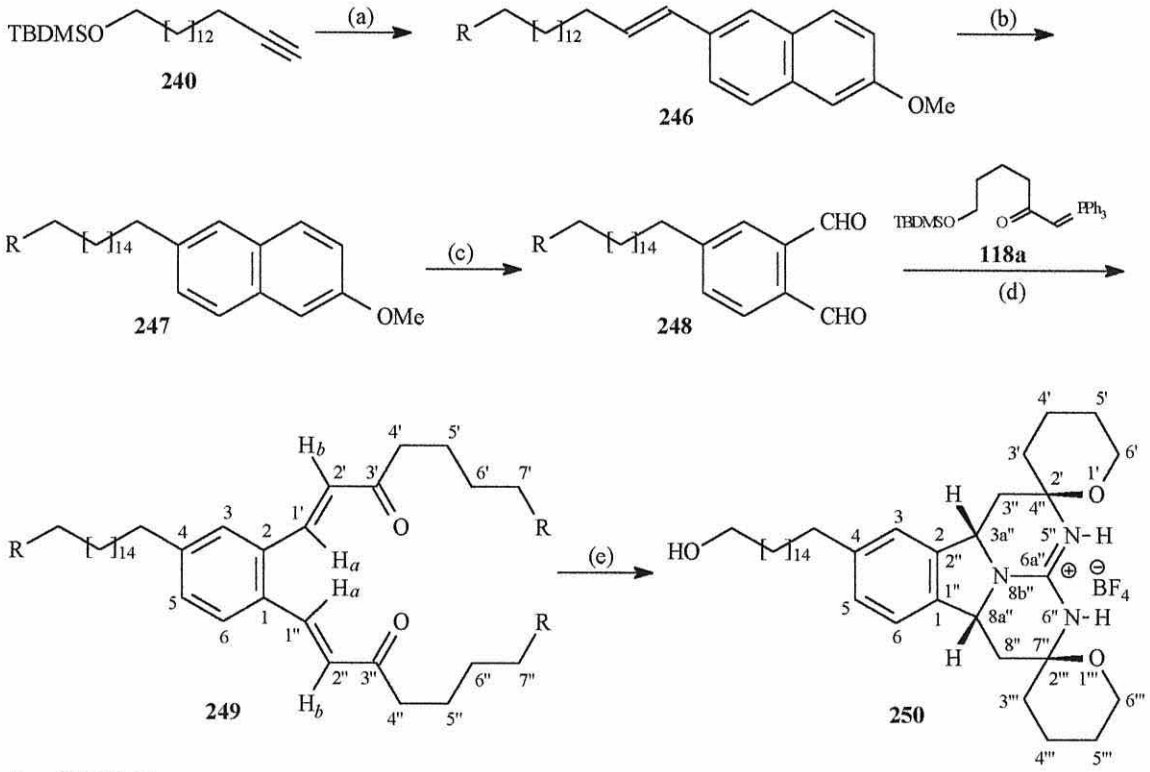
6.7.1 Preparation Of Benzo-Fused Guanidine System **250**

The synthesis of benzo-fused hexacycle **250** was carried out in five steps starting from the previously prepared protected alkyne **240**. The first step involved the synthesis of cross-coupled alkene **246**, which was obtained, after work-up and purification, as a fine white solid in a satisfactory 78% yield (Scheme 6.12, page 134). Proton and carbon NMR spectral analysis confirmed the structure of the product which as expected, formed exclusively as the *trans* isomer.

It seemed appropriate at this stage to hydrogenate the potentially reactive olefinic bond of **246**. Hence, the alkene was dissolved in ethyl acetate, treated with palladium on activated carbon and stirred vigorously under an atmosphere of hydrogen over a ten minute period. Subsequent filtration of the reaction mixture through celite and evaporation of the solvent afforded the hydrogenated product, **247**, in an excellent 91% yield. The proton and carbon NMR spectra were virtually identical to those of alkene **246**, except that the vinylic protons were no longer evident.

Oxidation to dialdehyde **248** was achieved by means of ozonolysis of the methoxy-substituted ring of naphthalene **247**, using a similar procedure to that described by Pappas *et al.*[133] In their research, they demonstrated that an electron withdrawing methoxy group encourages ozone attack on the methoxy-substituted ring of naphthalenes,

although information was not available for disubstituted naphthalenes such as **247**. To this end, a dichloromethane solution of **247** was cooled to -78°C and a stream of ozonised oxygen bubbled into the solution, until a faint blue colour was observed. Reductive cleavage of the intermediate ozonide with triphenylphosphine followed by work-up and purification gave the required dialdehyde **248** as a pale yellow oil in a disappointing 22% yield (53% based on recovered **247**), (Scheme 6.12). Despite considerable effort, this rather low yield could not be improved upon, with the reaction itself proving to be extremely inconsistent. Nevertheless, further dialdehyde was obtained by recycling the recovered starting material in repeated ozonolysis reactions; up to three recycles were possible leading to a 40% overall yield.



R = OTBDMS

***Reagents and Conditions*:** (a) Catecholborane, reflux, 3 h, then rt, 16 h, then C_6H_6, Pd $(PPh_3)_4$, **216**, 2.5 h, then 2M NaOEt/EtOH, reflux, 2 h, then 3M $NaOH/H_2O$, rt, 16 h; (b) EtOAc, Pd/C, H_2, 10 min; (c) CH_2Cl_2, O_3, -78°C then PPh_3 5 h; (d) CH_2Cl_2, 4 eqv. **118a**, rt, 48 h; (e) (i) Guanidine, DMF, 0°C-rt, 7 h; (ii) Methanolic HCl, 0°C-rt, 14 h; (iii) Sat. $NaBF_4$ solution.

Scheme 6.12 The preparation of benzo-fused guanidine system **250**.

Interpretation of the ^{1}H NMR spectrum of dialdehyde **248** showed two singlet resonances at δ 10.58 and δ 10.18 which were identified as the two aldehydic protons. The three remaining aromatic protons coincided with the signals at δ 7.91 (d, J = 7.8 Hz), δ 7.79 (s) and δ 7.58 (d, J = 7.8 Hz). The methylene adjacent to the silyl ether protecting group appeared as a triplet (J = 6.6 Hz) at δ 3.60 whilst the methylene group next to the aromatic ring also appeared as a triplet resonance at δ 2.76 (J = 7.6 Hz). The remainder of the signals were consistent with the fourteen methylenes of the aliphatic chain and the five methyls of the protecting groups. Similarly, the carbon spectrum demonstrated two aldehyde carbons at δ 192.60 and δ 192.07 respectively, and in addition, six aromatic carbon resonances were also observed. Furthermore, the mass spectrum showed a peak at 489 Daltons which represents the protonated mass ion ($[M+H]^+$).

The next stage of the synthesis involved the preparation of *bis*-α,β-unsaturated ketone **249**. Using a similar procedure to that described for the synthesis of brominated hexacycle **215**, (see Scheme 6.2, page 119), a mixture of dialdehyde **248** and four equivalents of known phosphorane[81] **118a**, in dichloromethane, were stirred together. After two days, TLC analysis indicated complete reaction hence, the solvent was removed and the remaining material immediately purified on silica gel, affording the desired product as a yellow oil in 70% yield (Scheme 6.12, page 134).

As compound **118a** represents a stabilised phosphorane, the resultant product of the Wittig reaction ought to have a *trans* orientation about the double bonds. Indeed, this was found to be the case with the proton NMR spectrum demonstrating a pair of overlapping doublets at δ 7.91 (J = 16.0 Hz) and δ 7.90 (J = 16.0 Hz) representing the two H_b protons. Similarly a second pair of doublets, again overlapping, at δ 6.65 (J = 16.0 Hz) and δ 6.64 (J = 16.0 Hz) were interpreted as being due to the two H_a protons. The high coupling constant values indicated that the protons in question were certainly *trans* to one another. In addition, the ^{1}H NMR spectrum demonstrated a double triplet at δ 3.66 (J = 6.3, 1.3 Hz) which corresponded to the methylene protons adjacent to the silyl protecting groups, CH_2-7' and CH_2-7", whilst the resonance at δ 3.61 (t, J = 6.6 Hz) coincided with the methylene protons adjacent to the silyl protecting group at the end of the 16-carbon spacer.

The benzo-fused hexacycle **250** was accessed in a modest 25% yield following the addition of guanidine to *bis*-α,β-unsaturated ketone **249** and treatment with a solution of

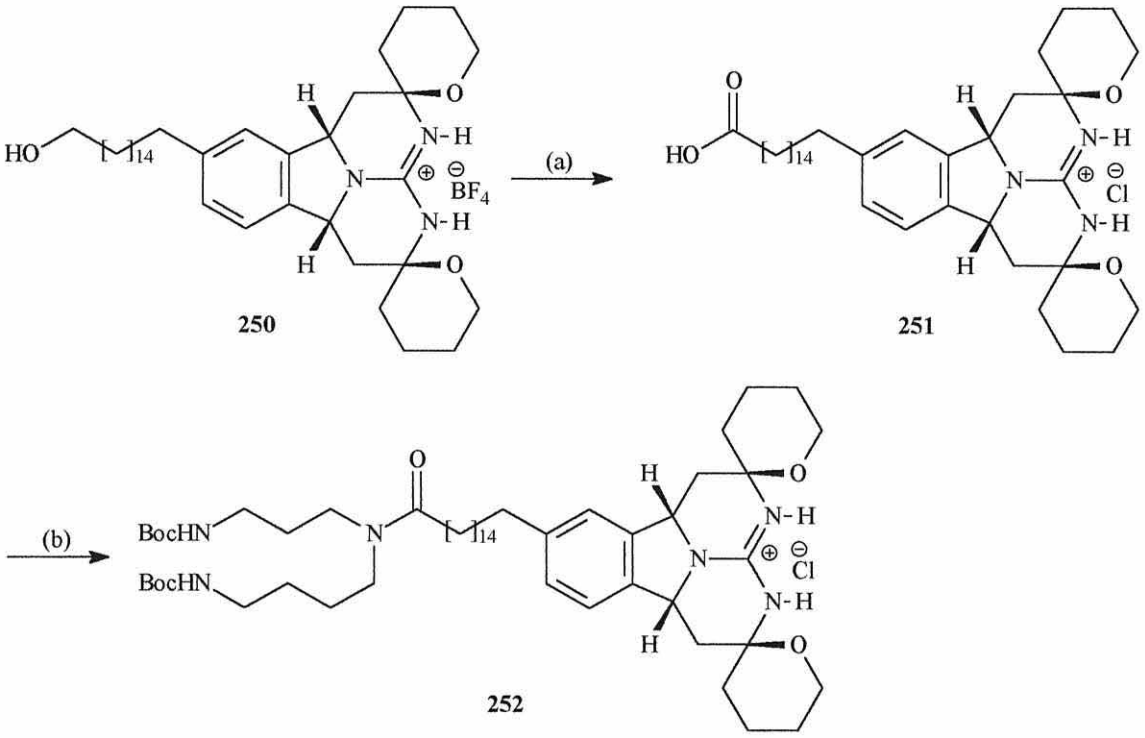
methanolic HCl under standard conditions (Scheme 6.12, page 134). Unfortunately, hexacycle **250** could not be obtained in crystalline form thus, the relative stereochemistry could not be confirmed, although it should follow that hexacycle **250** exists exclusively as the *syn* diastereomer as was found to be the case for hexacycle **211**, (see Section 6.1, pages 117-118). Analysis of the proton and carbon NMR spectra indicated that **250** had indeed formed as a single diastereomer. This was particularly apparent in the ^{13}C NMR spectrum which only demonstrated a single series of resonances. If diastereomers had been present, these resonances would have been appended with a series of minor signals attributable to one or more diastereomers.

The ^{1}H NMR spectrum of **250** demonstrated significant correlations in the hexacyclic region to those of unsubstituted benzo-fused hexacycle **211**. A broad singlet at δ 7.72 corresponded to the two guanidine N-H protons, whose chemical shift is dependent upon the concentration of the NMR solution as well as the nature of the counter ion. The pyrrolidine protons, CH-3a" and CH-8a", appeared as a doublet of doublets at δ 5.22 (J = 11.6, 3.9 Hz). The axial (α) and equatorial (β) protons of the methylene groups adjacent to oxygen in the oxacyclic rings appeared as individual resonances; the signal at δ 3.89 corresponded to the CHα-6' and CHα-6''' protons whilst the resonance at δ 3.76 correlated to the CHβ-6' and CHβ-6''' protons respectively. A triplet at δ 3.64 (J = 6.5 Hz) was identified as the resonance of the methylene group bonded to the terminal hydroxyl. In the carbon NMR spectrum the guanidine carbon (C-6a") occurred as a signal at δ 147.77, whilst the quaternary ring junction carbons, C-4" and C-7" appeared as an overlapping resonance at δ 79.73. Two further resonances at δ 56.69 (C-3a") and δ 56.59 (C-8a") were due to the carbons of the five-membered pyrrolidine ring whilst the remaining signals were found to be consistent with those expected for the hexacyclic product.

6.7.2 Addition Of *Bis*-Boc Spermidine

Having prepared the hexacyclic core with the spacer unit attached, the final step in the synthesis of analogue **214** was to attach the spermidine residue. This was to be accomplished using an analogous procedure to that employed in the preparation of amides **226** and **233**. Prior to attempting the condensation, it was necessary to oxidise the terminal

hydroxyl group of **250** to the corresponding carboxylic acid. This was achieved by way of PDC oxidation, affording acid **251** in a 55% yield as the hydrochloride salt (Scheme 6.13). Carboxylic acid **251** was subsequently coupled to *bis*-Boc spermidine, **41** using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) as the coupling agent instead of DCC, as was demonstrated by Overman and co-workers[32] in their total synthesis of ptilomycalin A. One major advantage of using EDCI for amide formation is that the urea by-product of coupling is highly water soluble and can be easily removed during work-up.



***Reagents and Conditions*:** (a) PDC, DMF, 24 h; (b) CH_2Cl_2, **41**, HOBT, EDCI, rt, 18 h.

Scheme 6.13 Preparation of hexacyclic *bis*-Boc spermidine adduct **252**.

Amide **252** was obtained as a solid in a highly satisfactory 88% yield, whilst the structure was confirmed by way of NMR spectroscopy. The proton NMR demonstrated two broad singlets at δ 5.42 and δ 4.67 which were due to the N-H protons adjacent to the Boc protecting groups. The signals for the methyl protons of the *tert*-butyl groups were present as two distinct singlets at δ 1.42 and δ 1.41 (each corresponding to nine protons). The ^{13}C NMR spectrum verified the presence of the amide bond, displaying a typical amide carbonyl signal at δ 155.99. Resonances for the quaternary carbons present in the Boc

groups appeared at δ 79.14 and δ 78.79 whilst the six methyl carbons appeared as an overlapping signal at δ 28.38. The mass spectrum (FAB) further aided in establishing the successful formation of **252**, showing a peak at 935 Daltons which corresponded to the ($[M+H]^+$) mass ion.

6.7.3 DEPROTECTION

The final step in the synthesis of analogue **214** involves the removal of the Boc protecting groups. This is normally achieved following exposure to an aqueous solution of hydrochloric acid, but in the case of **252** care must be taken in order to prevent amide hydrolysis. In his synthesis of ptilomycalin A, Overman[32] reports the use of formic acid to deprotect the amine groups. Using this procedure, amide **252** was stirred in the presence of formic acid (98-100%) for 18 hours after which the reaction mixture was diluted with chloroform and extracted with a solution of 50% NaOH (2M)/saturated sodium chloride solution. Following evaporation of solvent, a solid was recovered whose proton and carbon NMR seemed to indicate successful deprotection. Contradictory evidence was given upon analysis of the mass spectrum which demonstrated the presence of a mixture of compounds. A peak occurring at 735 Daltons correlated to the ($[M+H]^+$) mass ion of the desired product **214**. However, the presence of strong ions at higher *m/z*, in particular the peaks at 763 and 791 Daltons showed a pattern where the successive difference in mass units equalled 28 Daltons; this value being indicative of a carbonyl (C=O) group. This fact suggests that these particular mass ions represent a mono-formylated (763 Daltons: $[M+CHO]^+$) and a di-formylated (791 Daltons: $[M+2(CHO)]^+$) product (Figure 6.6).

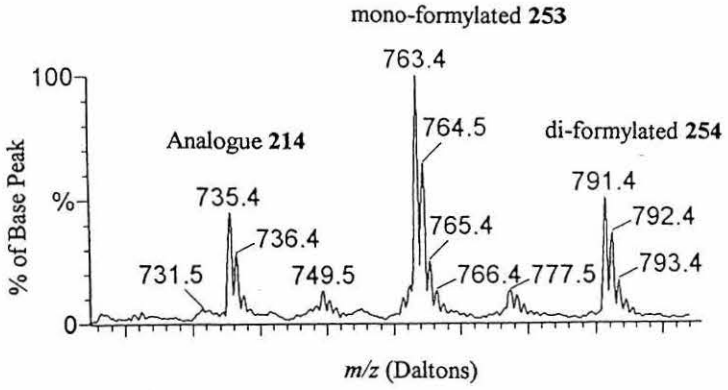
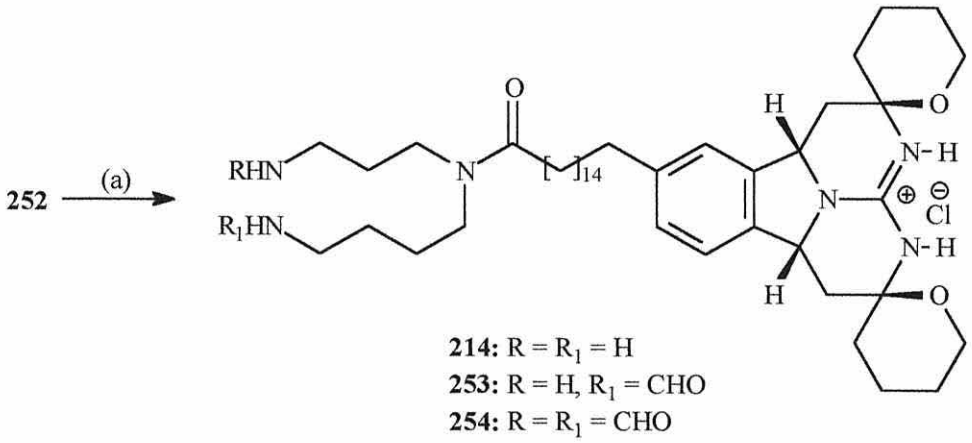


Figure 6.6 The mass spectrum (electrospray) of the mixed product of amine deprotection.

It is known in the literature[134] that formic acid can be utilised in the *N*-formylation of amines. Hence, in the case of **252**, formic acid initially cleaves both Boc groups yielding analogue **214** which in turn undergoes *mono* and *bis* *N*-formylation of the liberated amines in the presence of excess reagent, resulting in the formation of the undesired products, **253** and **254** (Scheme 6.14). This undesired formylation should be reversed during extraction with the 50% NaOH (2M)/saturated sodium chloride solution, thereby regenerating the free amines. Unfortunately, the formyl groups could not be removed even after repeated basic extractions.



214: R = R_1 = H
253: R = H, R_1 = CHO
254: R = R_1 = CHO

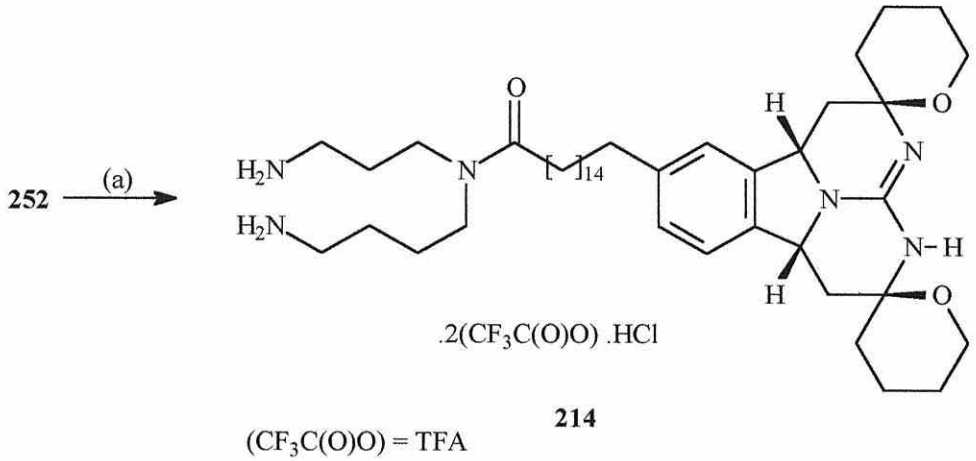
***Reagents and Conditions*:** (a) HCOOH, 18 h, then 50% NaOH (2M)/saturated sodium chloride solution.

Scheme 6.14 The attempted amine deprotection reaction and the resulting products.

Overman and co-workers[135] have recently reported similar Boc deprotections using hydrochloric acid (3M). Adopting their procedure, amide **252** was dissolved in methanol, treated with 3M HCl and left to stir at room temperature over four hours. Disappointingly, work-up led only to recovery of the starting material with no traces of the desired product. The reaction was attempted a second time; on this occasion the reaction mixture was stirred over a period of sixteen hours, but these conditions resulted in decomposition of the starting material.

A further effort at deprotection was made by treating a chloroform solution of protected amide **252** with trifluoroacetic acid (99%). The reaction was monitored over a period of one hour using NMR spectroscopy, with a decrease in the height of the resonances of the methyls of the two Boc groups at δ 1.42 and δ 1.41, respectively,

indicating successful ester cleavage. Excess TFA was removed by azeotroping the reaction product with chloroform under reduced pressure. The analogue **214** was recovered in quantitative yield as the *bis*-trifluoroacetate hydrochloride salt, as shown in Scheme 6.15 below. The mass spectrum confirmed the presence of analogue **214**, demonstrating a peak at 735 Daltons which corresponds to the ($[M+H]^+$) mass ion.



214

$(CF_3C(O)O)$ = TFA

***Reagents and Conditions*:** (a) $CHCl_3$, TFA, rt, 1 h.

Scheme 6.15 The preparation of ptilomycalin A analogue, **214**.

6.8 BIOLOGICAL TESTING

Pentacyclic model compound **184** (see Chapter 5), hexacycle **211** [113] (see page 117), hexacyclic analogue **214** and hexacyclic intermediate **250** were tested for biological activity against a range of cancer cell lines, Table 6.1, page 141. Pentacyclic unit **184** was found to be active towards all the cancer cell lines tested. The IC_{50} values are comparatively high for the first three cancer cell lines indicating that an increased concentration of material is required to achieve 50% inhibition of the target cells. In contrast, the pentacycle is relatively active against the P388 cancer cell line (IC_{50} 2.93 μg/ml), but less active than ptilomycalin A (IC_{50} 0.11 μg/ml), implying that the presence of the 16-carbon spacer and spermidine residue are necessary for biological activity at low substrate concentration. This was further confirmed from the activity results of hexacycle unit **211** which was found to be even less active against the same cancer cell lines.

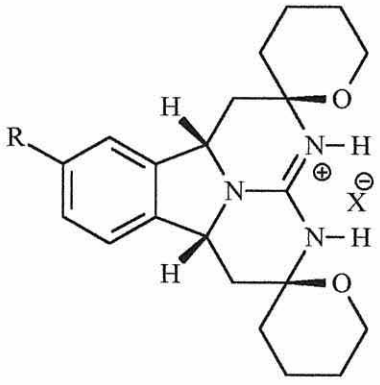
Biological Test	Activity (IC_{50} in μg/ml)				
	184	**211**[113]	**214**	**250**	Ptilomycalin A[27]
Cytotoxicity: K562	12.30	24.93	0.52	1.10	*a*
A2780	24.44	22.06	0.92	4.16	*a*
H-460	10.36	21.36	0.52	1.00	*a*
P388	2.93	9.11	0.69	2.70	0.11

***Key*:** K562: Human, chronic, myelogenous leukaemia; A2780: Human, ovarian carcinoma; H-460: Human, large cell carcinoma, lung. High DT-Diaphorase; P388: Mouse, lymphoid neoplasm.

Table 6.1 The cytotoxic activities of pentacyclic model **184**, hexacyclic unit **211**,[113] hexacyclic analogue **214**, hexacyclic intermediate **250** and ptilomycalin A[27]. [a] No data for comparison.

In contrast, hexacyclic analogue **214** showed an excellent range of activity towards the cancer cell lines tested, with the activity against the P388 cell line approaching that of ptilomycalin A. Hexacyclic intermediate **250** also demonstrated significant activity towards the same cancer cell lines. This data supports the hypothesis that inclusion of the ester linkage is not necessary for biological activity. Furthermore, the data also infers that the presence of the 16-carbon spacer chain and spermidine residue are essential for the compounds to demonstrate significant biological activities.

The role of the carbon spacer chain was further supported by recent biological testing data for an analogous series of polycyclic compounds **255**, **256**, **257** and **258** (Figure 6.7), which contain a hexacyclic unit attached to a 14-carbon spacer *via* an ether linkage.[136]



211: R = H, X = BF_4
255: R = $O(CH_2)_{14}OH$, X = BF_4
256: R = $O(CH_2)_{13}C(O)OH$, X = Cl
257: R = $O(CH_2)_{13}(O)N((CH_2)_3NHBoc)(CH_2)_4NHBoc$, X = Cl
258: R = $O(CH_2)_{13}(O)N((CH_2)_3NH_2)(CH_2)_4NH_2$, X = Cl

Figure 6.7 An analogous series of hexacyclic, biologically active guanidinium compounds.

The data indicated that these compounds were highly active against the cancer cell lines tested (excluding acid **256**), but not as active as corresponding hexacycles, **214** and **250**, which contain 16-carbon spacer chains, Table 6.2. This suggests that the number of carbons present in the spacer chain is another important factor that can affect the overall biological activity of this class of compounds.

Biological Test	Activity (IC_{50} in μg/ml)			
	255	**256**	**257**	**258**
Cytotoxicity: K562	1.29	40.09	10.26	7.28
A2780	1.42	12.06	6.81	11.02
H-460	3.57	10.27	1.25	5.87
P388	1.98	NT	2.34	4.25

***Key*:** NT = Non Toxic.

Table 6.2 The anti-cancer activities of hexacyclic guanidinium compounds **255**, **256**, **257** and **258**.

6.9 CONCLUSION

The successful synthesis of a novel, hexacyclic analogue, **214** of ptilomycalin A has been outlined in this chapter. Although the analogue could not be prepared *via* the originally proposed scheme it was possible to adapt the methodology in order to achieve the goal. The make-up of the hexacyclic analogue consists of the three required structural units, that is, a polycylic core linked to a spermidine residue through a 16-carbon spacer. Biological testing demonstrated that analogue **214** as well as intermediate **250** were potent anti-cancer agents. Unfortunately, conclusive evidence to verify that the hexacycle had formed exclusively as the *syn* isomer could not be obtained; ideally a crystal structure would be required. Nevertheless, it is reasonable to assume that the hexacycle exists solely as the *syn* diastereomer in an identical manner to unsubstituted hexacycle **211**. Confirmation of the structure could be obtained by means of relevant 2D NMR experiments such as COSY and NOESY. The hexacyclic analogue was obtained *via* a total of 8 steps starting from 2-bromo-6-methoxynaphthalene, **216**, in an overall yield of 2%.

CHAPTER 7

SUMMARY

7.1 SUMMARY

The main aim of this research project was to develop a route towards the stereoselective total synthesis of the marine natural product, ptilomycalin A. This novel metabolite was first isolated in 1989, and in biological testing, demonstrated a remarkable range of bioactivity which included antiviral (HSV and HIV), cytotoxic and antifungal activity. The structure of ptilomycalin A consists of a unique pentacyclic guanidinium sub-unit attached to a spermidine residue *via* a 16-hydroxyhexadecanoyl chain. More recently, structural relations of ptilomycalin A have been isolated from various species of marine fauna, which include the biologically active crambescidins (816, 830, 844 and 800), 13,14,15-isocrambescidin 800 and the cytotoxic agents celeromycalin and fromiamycalin.

Previous research[80] had demonstrated that guanidine-containing polycycles could be accessed following the double Michael addition of the free-base form of guanidine to a suitable *bis*-α,β-unsaturated ketone. Addition is presumed to occur *via* a biomimetic pathway with subsequent acid mediated deprotection and *bis*-spirocyclisation affording the desired guanidinium polycycles, typically in approximate *syn* to *anti* ratios of 4:1. The *bis*-α,β-unsaturated ketones were synthesised using the Wittig reaction, whereas the ester functionalised versions were prepared by means of the Knoevenagel condensation reaction.

Initial efforts towards ptilomycalin A were focussed on the preparation of the chiral pentacyclic core of the natural product incorporating an ester functionality at C-3', thereby allowing the subsequent coupling of the 16-hydroxyhexadecanoyl chain and spermidine residue. The strategy adopted for the synthesis was based upon a previously attempted racemic synthesis[81] of the pentacyclic core. The Knoevenagel precursors (*S*)-6-oxo-11-[(*tert*-butyldimethylsilyl)-oxy]-(4*E*,9*Z*)-tridecadieneal and (*R*)-*tert*-butyl 7-[(*tert*-butyldimethylsilyl)oxy]-3-oxooctanoate were prepared stereospecifically from the chiral starting reagents, (*S*)-(+)-2-aminobutyric acid and ethyl (*R*)-3-hydroxybutyrate, respectively. Wittig conditions favouring the formation of *cis* double bonds were employed to generate the *Z*-geometry between C-9 and C-10 in the unsaturated aldehyde, which coincide with the double bond in the seven-membered ring of the natural product.

A Knoevenagel condensation, in the presence of a catalytic amount of morpholine, was used to prepare the acyclic product, *tert*-butyl (13*S*, 5'*R*),(2*E*/*Z*, 6*E*, 11*Z*)-2-{5'-[(*tert*-

butyldimethylsilyl)oxy]-1'-oxo-hexanoyl}-[(13-*tert*-butyldimethylsilyl)oxy]-8-oxopentadeca-2,6,11-trienoate, in rather unsatisfactory yields. The small overall quantity of product obtained prevented a complete spectroscopic analysis. Unfortunately, the attempted double Michael addition of guanidine to the *bis*-α,β-unsaturated ketone did not yield the desired pentacyclic core, the result being decomposition of the starting material. This synthetic approach was not pursued any further due to lack of precursors and the prohibitive cost of the chiral starting reagents employed.

In direct contrast to the poor yields obtained from the Knoevenagel step, a report in the literature described satisfactory yields of condensed products attained from similar reaction systems.[34] In light of this, an investigation into the conditions employed for the Knoevenagel reaction was undertaken using a model system consisting of an unsaturated aldehyde and various β-keto esters. The results of this investigation were extremely disappointing with only small quantities of impure product being obtained. An alternative reaction procedure as described by Lehnert[102] was also attempted, but once again, the desired model *bis*-α,β-unsaturated ketone was not obtained. With no success from these studies a different approach to an ester functionalised pentacycle was sought.

It was anticipated that the previously discussed acyclic precursor could be prepared by way of a Wadsworth-Emmons reaction between a chiral substituted keto phosphonate and (*S*)-6-oxo-11-[(*tert*-butyldimethylsilyl)-oxy]-(4*E*,9*Z*)-tridecadieneal. Before attempting this procedure, it was decided to first test the proposed synthesis using a model system which would enable the preparation of guanidine tricycles. Following the synthesis of the model precursor, *tert*-butyl 2-(diethoxyphosphoryl)-3-oxobutanoate was reacted under a variety of Wadsworth Emmons reaction conditions with several trivial aldehydes, but with no success. The probable reason for the lack of reactivity observed was due to the stabilisation and resulting decreased nucleophilicity of the keto phosphonate carbanion intermediate, with the negative charge being doubly stabilised by delocalisation into the keto or ester moieties. The reaction was not attempted using Wittig methodology, as the corresponding keto phosphorane had already been shown to be inactive in similar reaction systems.[113]

The successful synthesis of a guanidine-containing chiral pentacycle possessing an identical stereochemistry to the polycyclic core of ptilomycalin A was achieved, the only

structural difference being the lack of the ester functionality, thus proving the applicability of the guanidine addition step. In testing, the pentacyclic nucleus of ptilomycalin A was shown to possess a degree of biological activity, hence, it was of interest to ascertain whether the polycyclic model also demonstrated any significant biological activity. On this occasion, the acyclic *bis*-α,β-unsaturated ketone was prepared by the Wittig reaction between the unsaturated aldehyde, (*S*)-6-oxo-11-[(*tert*-butyldiphenylsilyl)oxy]-(4*E*,9*Z*)-tridecadienal and the stabilised phosphorane, (*R*)-6-[(*tert*-butyldimethylsilyl)oxy]-1-(triphenylphosphanylidene)-heptan-2-one. Addition of guanidine followed by a somewhat problematical deprotection/spirocyclisation step afforded the desired chiral pentacycle in a typical yield for this reaction.

Interestingly, the pentacycle was isolated as a single diastereomer, in contrast to the typical 4:1 (*syn*/*anti*) ratio, possessing an identical overall stereochemistry and structural orientation as ptilomycalin A. There are two possible reasons for this, firstly, the small scale of the reaction presenting difficulties when attempting isolation of the diastereomeric mixture or, secondly, and more likely, the energetics and steric interactions that exist within the polycycle favouring the formation of a single diastereomer. The *syn* orientation of the pyrrolidine protons and the oxacyclic rings within the polycycle represents the lowest energy state for this compound. The overall stereochemistry of the pentacycle was unambiguously assigned by means of 1D and 2D NMR experiments. In addition, comparisons were made with the spectroscopic data quoted for ptilomycalin A and the *anti* pentacyclic natural product, 13,14,15-isocrambescidin 800 to support these observations. In biological testing the pentacycle was found to be moderately active towards a range of cancer cell lines.

The secondary aim of this project involved the development of a synthetic methodology to prepare advanced hexacyclic analogues of ptilomycalin A. It was essential to incorporate the three structural units present in ptilomycalin A in the proposed analogue, that is, the guanidinium polycycle, the spacer and spermidine residue. The synthetic strategy adopted for this study was based on a previous investigation[113] towards a benzo-fused hexacyclic mimic of the central guanidine core of ptilomycalin A. Interestingly, this compound was also isolated as a single diastereomer having an identical stereochemistry to that found in the natural product.

The initial approach involved coupling a spermidine residue, attached to a 16-hexadecanoyl spacer, to a brominated benzo-fused hexacycle by means of the Suzuki reaction, however, the desired product was not obtained. Attempted model Suzuki cross-coupling reactions between the spermidine bound spacer and various aryl halides were also unsuccessful. In contrast, the cross-coupling of the spacer chain to 2-bromo-6-methoxynaphthalene was achieved.

Subsequent ozonolysis of the Suzuki product, followed by a double Wittig reaction with a phosphorane prepared from δ-valerolactone, gave the intermediate benzo-fused *bis*-α,β-unsaturated ketone, in a satisfactory yield. Addition of guanidine gave, after desilylation and cyclisation, the alkylated hexacycle presumably as a single diastereomer, as was observed for the simple model hexacycle. The terminal hydroxy group was oxidised and coupled to *bis*-Boc-spermidine, with a somewhat problematical deprotection step finally yielding the desired hexacyclic, ptilomycalin A analogue.

The ptilomycalin A analogue was found to be a potent, biologically active compound demonstrating an impressive range of anti-cancer activity.

7.2 Future Work

Further investigations into the Knoevenagel reaction and the successful preparation of chiral *bis*-α,β-unsaturated ketone **129** remains the key challenge in the synthesis of ptilomycalin A. If this acyclic material can be obtained in significant quantities, then the addition of guanidine to access the ester substituted pentacycle **128** should prove relatively straightforward. Attaching the 16-hexadecanoyl chain and spermidine residue *via* the ester linkage would complete the synthesis of the target compound.

The optimisation of reaction yields involved in the preparation of the functionalised hexacyclic analogue requires further study, in particular, the irreproducibility encountered during the ozonolysis step. Furthermore, it is anticipated that by adapting the methodology employed to synthesise the analogue, numerous functionalised hexacycles of varying spacer length could be prepared. This would enable a comparison of biological activities and aid in determining the minimal structural requirement for activity.

7.3 RECENT DEVELOPMENTS

Recently, Murphy and co-workers[137] have reported the efficient synthesis of a novel, chiral C_2 symmetric guanidine base, **255** (Figure 7.1). This was achieved by modification of the synthetic procedure developed towards the preparation of the tetracyclic model compounds of ptilomycalin A, **108** and **109** (see Chapter 3, page 44). Tetracycle **255** was found to catalyse the conjugate Michael addition of pyrrolidine to the unsaturated lactone, 2(*5H*)-furanone. This compound along with related structures may have potential as asymmetric catalysts.

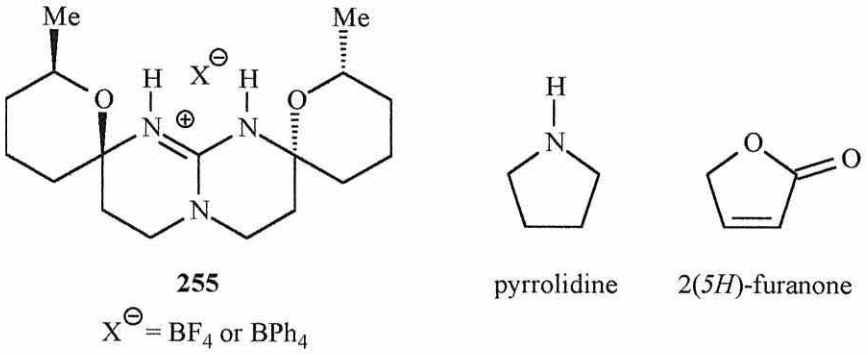


Figure 7.1 The chiral C_2 symmetric tetracyclic guanidine base **255** and the reagents employed in the catalysed Michael addition reaction.

A study towards the preparation of the guanidine-containing, marine hepatotoxin, cylindrospermopsin **89** is currently on-going.[138] To date, synthetic efforts have been directed towards the functionalised **AB** ring model **256** of the natural product (Figure 7.2).

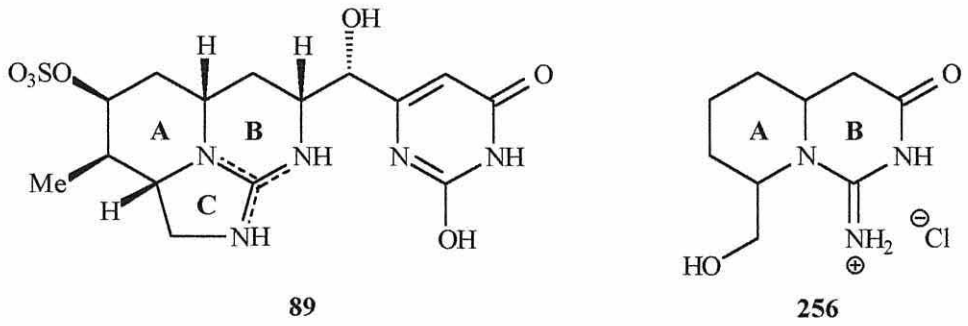


Figure 7.2 Cylindrospermopsin **89** and the structure of the **AB** model compound.

CHAPTER 8

EXPERIMENTAL

Reagents and Solvents

Reagents in each case were obtained from commercial suppliers and were used without further purification unless otherwise stated. Methyl iodide and triethylamine were distilled from calcium hydride before use. Reaction solvents were dried prior to use through standard literature procedures.[139] In particular, diethyl ether and tetrahydrofuran were distilled from sodium wire and benzophenone; dichloromethane, DMF and hexane were dried by distillation over calcium hydride. Dry methanol was distilled from magnesium and iodine.

Chromatography

The extent of reaction and purity of compounds was assessed by thin layer chromatography (TLC), and was carried out on precoated Kieselgel 60 F254 (Art. 5554; Merck) plates. Compounds were made visual using ultraviolet light and/or by staining with phosphomolybdic acid or permanganate,[139] followed by heating to 180°C. Column chromatography was conducted using BDH Silica Gel (particle size 40-63 μm) with the eluting solvent system specified in each case.

Analytical Methods

Melting points were recorded using a Gallenkamp MF370 capillary apparatus and are uncorrected. Infra-red analyses were conducted as thin films, solutions or as KBr discs where appropriate, using a Perkin Elmer 1600 Series FT-IR spectrometer. The band positions are reported in wavenumbers (ν) whose unit is the reciprocal centimetre (cm^{-1}). Band intensities are reported semiquantitatively as strong (s), medium (m), weak (w) and broad (br).

Proton NMR spectra were recorded on a Bruker AC250 spectrometer at 250MHz using deuteriochloroform as solvent, unless otherwise stated. Similarly ^{13}C NMR and ^{13}C DEPT spectra were recorded on the same instrumentation at 62.5MHz, in deuteriochloroform, unless otherwise stated. Chemical shifts are reported in δ values

relative to the internal standard, tetramethylsilane. Proton spin couplings are denoted as *J* values quoted in Hz with the splitting patterns defined as singlets (s), doublets (d), triplets (t), quartets (q), apparent pentents (app. p) and multiplets (m), or any combination of these.

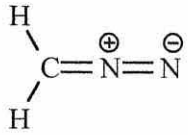
All mass spectra were run at the EPSRC Mass Spectrometry Service Centre at the University of Wales, Swansea. Low resolution mass spectra were recorded on a VG Biotech Quattro II triple quadrupole mass spectrometer using chemical ionisation (CI), with ammonia as reagent gas, or by means of electron impact (EI). Accurate mass spectra (HRMS) were recorded on a VG ZAB-E mass spectrometer whereas fast atom bombardment (FAB) spectra were run on a VG Autospec mass spectrometer using caesium ion bombardment at 25kV energy. Mass measurements are reported in Daltons, with Br and Cl referring to the isotopes ^{79}Br and ^{35}Cl, respectively.

Microanalyses were run on all solid compounds when available using a Carbo-Erba Model 1106 CHN analyser. Specific rotations [α] were determined using a PolAAr 2001 polarimeter, (cell path length, $l = 1$), with the temperature (t), concentration (c) and solvent recorded in each case.

MISCELLANEOUS

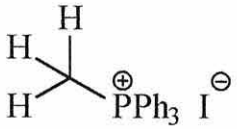
All non-aqueous reactions were performed in oven-dried glassware (200-250°C) under a positive pressure of argon or nitrogen gas. The yields quoted refer to isolated products purified on silica gel unless otherwise noted. The molarity of *n*-butyllithium solutions in hexane was determined prior to use by titration[139] against diphenylacetic acid in tetrahydrofuran. The term '*in vacuo*' refers to the removal of solvent under the reduced pressure of a rotary evaporator at water pump pressure (14-15 mmHg) or 'house' vacuum pump pressure (*ca* 1.4 mmHg). Correlation of individual ^{13}C NMR methylene resonances in the spectra of compounds containing the 16 carbon aliphatic chain were not possible using available techniques. Thus, in each case all the methylene chemical shifts are listed, followed by the number of corresponding methylenes.

Ethereal Diazomethane



Potassium hydroxide (2.5 g, 45 mmol) was dissolved in water (4 ml), treated with ethanol (12.5 ml) and heated to a temperature between 60-65°C. Diazald (6.36 g, 29.7 mmol) was dissolved in ether (65 ml) and added slowly through a dropping funnel to the heated basic solution. The ethereal diazomethane distillate was collected in a closed vessel at ice/salt temperature.

Methyltriphenylphosphonium Iodide,[140] **144**



Triphenylphosphine (23 g, 88.3 mmol) was dissolved in toluene (15 ml) with gentle heating and the solution then cooled to 0°C. Methyl iodide (11.4 g, 80 mmol, 5 ml) was added slowly in a dropwise manner resulting in the formation of a white suspension that was left to stir for 1 hour. The solid precipitate was collected by filtration, washed with dry diethyl ether and the product dried *in vacuo* over phosphorus pentoxide, to yield the title phosphonium salt (30.5 g, 85%) as powdery, white solid.

Melting point = 181-183°C. [Lit. = 184.5-185.5°C].[140]

IR ($CHCl_3$) ν_{max} **:** 3021 (w), 2941 (m), 2340 (w), 1439 (w), 1027 (w), 998 (w), 928 (w), 899 (m), 849 (w) cm^{-1}.

^{1}H NMR δ : 7.77 (15H, m, CH), 3.22 (2H, d, $J_{H\text{-}P}$ = 12.5 Hz, CH_3) ppm.

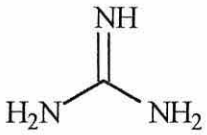
Succinaldehyde,[96,141] **78**

A mixture of 2,5-dimethoxytetrahydrofuran (10.93 g, 82.8 mmol) and an 1% aqueous solution of acetic acid (22 ml) was heated at reflux for 20 minutes. After cooling to room temperature, an aqueous solution of sodium bicarbonate was added until pH 8 and the mixture then saturated with sodium chloride. The resulting solution was extracted with chloroform (50 ml) followed by ethyl acetate (3x50 ml), the combined organic fractions dried over anhydrous magnesium sulfate and the solvent evaporated leaving a crude oil. This oil was purified by vacuum distillation (55°C @ 20 mmHg) yielding succinaldehyde (3.63 g, 51%) as a colourless, lachrymatory oil.

IR (neat) ν_{max} **:** 2958 (s), 1722 (s), 1634 (w), 1456 (s), 1350 (s), 1202 (s), 978 (br s), 820 (s) cm^{-1}.

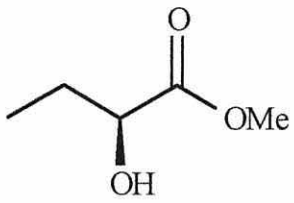
^{1}H NMR δ : 9.85 (2H, s, CH), 2.81 (4H, s, CH_2) ppm.

Guanidine,[80] **1**



A static nitrogen atmosphere was maintained throughout this experimental procedure. Sodium methoxide was prepared *in situ* by the addition of small portions of sodium (2.4 g, 104.4 mmol) washed with petrol, to a flask containing dry methanol (200 ml). Once evolution of hydrogen had ceased, guanidine hydrochloride (10.0 g, 104.4 mmol) was added and the solution left to stir for 16 hours. The resulting mixture was filtered to remove solid sodium chloride, washed with dry methanol (20 ml) and the solvent evaporated. The product was dissolved in dry methanol (30 ml) and filtered a second time. Concentration of the solvent gave free guanidine (5.86 g, 95%) as a pale yellow solid.

Methyl-(*S*)-2-hydroxybutanoate,[41] **136**



(*S*)-(+)-2-Aminobutyric acid (10 g, 97 mmol) was dissolved in sulfuric acid (1 M, 200 ml), cooled to 0°C and a solution of sodium nitrite (11 g, 159 mmol) in water (40 ml) added in a dropwise manner. Following addition, the mixture was allowed to stir at room temperature for 24 hours before being saturated with sodium chloride (*ca* 50 g). The resulting solution was extracted with ethyl acetate (4x80ml), the combined organic fractions dried over anhydrous magnesium sulfate and concentrated *in vacuo* to give crude (*S*)-2-hydroxybutanoic acid as a yellow oil.

Acetyl chloride (11.2 g, 143 mmol, 10.2 ml) was added slowly to methanol (100 ml) at 0°C and the resulting solution allowed to stir for ten minutes prior to addition of the crude α-hydroxy acid. The mixture was allowed to stir at room temperature for 48 hours, after which careful evaporation of the solvent gave a yellow oil, which was purified by column chromatography on silica gel (eluent: dichloromethane) yielding the title compound (2.68 g, 23%) as a yellow oil.

TLC : $R_f = 0.48$ in 60% diethyl ether/petrol.

IR (neat) ν_{max} **:** 3453 (br), 2925 (m), 2850 (w), 1734 (s), 1654 (m), 1230 (br), 1136 (m) cm^{-1}.

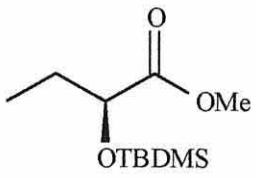
^{1}H NMR δ : 4.58 (1H, br s, OH), 4.20 (1H, dd, $J = 6.6$, 4.5 Hz, CH), 3.82 (3H, s, CH_3), 1.86 (1H, m, CH), 1.73 (1H, m, CH), 0.97 (3H, t, $J = 7.4$ Hz, CH_3) ppm.

^{13}C NMR δ : 175.54 (C), 71.49 (CH), 52.34 (CH_3), 27.33 (CH_2), 8.86 (CH_3) ppm.

MS (CI) *m/z* : 136 (45% $[M+NH_4]^+$), 104 (8%), 78 (21%), 74 (18%), 58 (14%), 46 (32%) Daltons.

HRMS (CI) *m/z* : found: 136.0974, $C_5H_{14}O_3N$ ($[M+NH_4]^+$) requires: 136.0974 Daltons.

Methyl (*S*)-2-[(*tert*-butyldimethylsilyl)oxy]-butanoate, **137**



A solution of imidazole (6.33 g, 93 mmol) in DMF (60 ml) was cooled to 0°C and methyl-(*S*)-2-hydroxybutanoate (3.44 g, 29 mmol) added. The resulting mixture was stirred for ten minutes at room temperature then again cooled to 0°C, treated with *tert*-butyldimethylsilyl chloride (7.84 g, 52 mmol) and left to stir for 48 hours. The reaction mixture was extracted with hexane (3x60 ml), and the combined organic fractions washed with a 2% aqueous acetic acid solution (50 ml) followed by water (2x40 ml), then dried over anhydrous magnesium sulfate. Evaporation of the solvent gave a crude oil which was purified on silica gel (gradient elution: 0-10% diethyl ether/petrol) yielding the desired protected ester (3.17 g, 47%) as a colourless oil.

TLC : R_f = 0.63 in 10% diethyl ether/petrol.

IR (neat) ν_{max} **:** 2954 (s), 2889 (s), 2859 (s), 1757 (s), 1464 (s), 1361 (m), 1255 (s), 1201 (s), 1145 (s), 1073 (m), 1022 (s), 935 (w), 842 (br s), 780 (s), 723 (w) cm^{-1}.

^{1}H NMR δ : 4.06 (1H, dd, J = 7.0, 5.1 Hz, CH), 3.64 (3H, s, CH_3), 1.65 (2H, m, CH_2), 0.89 (3H, t, J = 7.4 Hz, CH_3), 0.88 (9H, s, CH_3), 0.06 (3H, s, CH_3), -0.01 (3H, s, CH_3) ppm.

^{13}C NMR δ : 174.24 (C), 73.35 (CH), 51.68 (CH_3), 28.37 (CH_2), 25.70 (3xCH_3), 18.33 (C), 9.59 (CH_3), -5.00 (CH_3), -5.35 (CH_3) ppm.

MS (CI) *m/z* : 250 (100% $[M+NH_4]^+$), 233 (60% $[M+H]^+$), 217 (15%), 175 (21%), 132 (35%), 106 (16%), 74 (24%), 58 (30%), 52 (92%), 44 (43%), 36 (49%) Daltons.

HRMS (CI) *m/z* : found: 233.1573, $C_{11}H_{25}O_3Si$ ($[M+H]^+$) requires: 233.1573 Daltons.

$[\alpha]_D^{24}$ = -4.2 (c = 0.3, $CHCl_3$).

(*S*)-2-[(*tert*-Butyldimethylsilyl)oxy]-butan-1-ol, **138**

(*S*)-methyl-[(*tert*-butyldimethylsilyl)oxy]-butyrate (2.12 g, 9 mmol) was dissolved in hexane (100ml), cooled to -78°C and treated with diisobutylaluminium hydride (1 M solution in hexanes, 20 ml, 20 mmol) in a dropwise manner. The solution was warmed to -20°C and stirred for five hours before being treated with a mixture of 50% methanol/benzene (10 ml). The resulting mixture was diluted with hexane (50 ml) followed by an aqueous solution of saturated ammonium chloride (50 ml) and then filtered *in vacuo*. Extraction of the organic phase with water (3x50 ml) followed by drying and evaporation of the solvent gave a clear oil which was purified on silica gel (eluent: 10% diethyl ether/petrol) giving the title compound **138** (1.34 g, 72%), as a clear oil.

TLC : R_f = 0.30 in 15% diethyl ether/petrol.

IR (neat) ν_{max} **:** 3358 (br), 2955 (s), 2858 (s), 1464 (s), 1361 (w), 1256 (s), 1050 (br s), 938 (m), 836 (s), 775 (s), 665 (w) cm^{-1}.

^{1}H NMR δ : 3.69 (1H, m, CH), 3.58 (1H, m, CH), 3.48 (1H, m, CH), 1.91 (1H, br t, J = 7.8 Hz, OH), 1.54 (2H, dq, J = 7.3, 6.3 Hz, CH_2), 0.93 (9H, s, CH_3), 0.90 (3H, t, J = 7.5 Hz, CH_3), 0.11 (6H, s, CH_3) ppm.

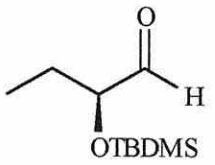
^{13}C NMR δ : 74.09 (CH), 65.85 (CH_2), 26.71 (CH_2), 25.83 (3xCH_3), 18.09 (C), 9.70 (CH_3), -4.48 (CH_3), -4.62 (CH_3) ppm.

MS (CI) *m/z* : 222 (13% $[M+NH_4]^+$), 205 (30% $[M+H]^+$), 187 (7% $[M-OH]^+$), 132 (19%), 100 (12%), 98 (13%), 74 (28%), 72 (18%), 58 (28%), 52 (100%), 46 (29%), 44 (41%), 36 (53%) Daltons.

HRMS (CI) *m/z* : found: 205.1624, $C_{10}H_{25}O_2Si$ ($[M+H]^+$) requires: 205.1624 Daltons.

$[\alpha]_D^{25}$ = +6.2 (c = 1.2, $CHCl_3$).

(*S*)-2-[(*tert*-Butyldimethylsilyl)oxy]-butanal, **139**



Oxalyl chloride (1.0 g, 7.85 mmol, 0.7 ml) in dry dichloromethane (100 ml) was cooled to -78°C and treated with dimethyl sulfoxide (1.1 g, 14.2 mmol, 1 ml) to give an effervescent mixture which was left to stir for 10 minutes at -78°C. (*S*)-2-[(*tert*-butyldimethylsilyl)oxy]-butan-1-ol (1.0 g, 4.9 mmol) was added and the mixture stirred at -60°C for ten minutes. The solution was again cooled to -78°C and triethylamine (2.97 g, 29.4 mmol, 4 ml) added. The resulting mixture was stirred for 2 hours at room temperature before being extracted with hexane (2x50 ml). The combined organic fractions were sequentially washed with water (2x40 ml), a 7% aqueous solution of acetic acid (2x40 ml) and brine (2x40 ml). Drying over anhydrous magnesium sulfate and concentration *in vacuo* gave the desired product (0.92 g, 93%) as a yellow oil.

TLC : R_f = 0.60 in 5% diethyl ether/petrol.

IR (neat) ν_{max} **:** 2953 (s), 2931 (s), 2888 (s), 2858 (s), 2805 (w), 2718 (w), 1737 (s), 1464 (m), 1362 (w), 1254 (s), 1138 (br), 1005 (m), 939 (w), 838 (s), 778 (s), 669 (w) cm^{-1}.

^{1}H NMR δ : 9.62 (1H, d, J = 1.7 Hz, CH), 3.93 (1H, ddd, J = 7.0, 5.5, 1.7 Hz, CH), 1.77 (2H, m, CH_2), 0.98 (3H, t, J = 7.4 Hz, CH_3), 0.95 (9H, s, CH_3), 0.11 (3H, s, CH_3), 0.10 (3H, s, CH_3) ppm.

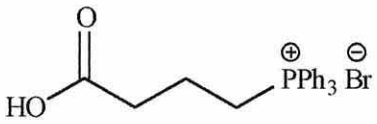
^{13}C NMR δ : 204.38 (CH), 78.65 (CH), 25.75 (CH_2), 25.66 (3xCH_3), 18.12 (C), 9.04 (CH_3), -4.76 (CH_3), -5.03 (CH_3) ppm.

MS (CI) *m/z* : 220 (100% $[M+NH_4]^+$), 203 (40% $[M+H]^+$), 202 (45% $[M]^+$), 187 (27% $[M-CH_3]^+$), 173 (11% $[M-CHO]^+$), 145 (40 % $[M-(t\text{-Bu})]^+$), 132 (40 % [M-TBDMSOH]$^+$), 91 (13%), 74 (23%), 52 (17%), 46 (16%), 44 (10%) Daltons.

HRMS (CI) *m/z* : found: 220.1732, $C_{10}H_{26}O_2Si$ ($[M+NH_4]^+$) requires: 220.1733 Daltons.

$[\alpha]_D^{23}$ = -11.5 (c = 1.0, $CHCl_3$).

(3-Carboxypropyl)triphenylphosphonium Bromide,[90] **140**



Triphenylphosphine (9.41 g, 36 mmol) was dissolved in dry acetonitrile (15 ml) with gentle heating. The resulting solution was treated with a solution of 4-bromo butyric acid (5.00 g, 30 mmol) in acetonitrile (25 ml) and heated under reflux for 24 hours. After cooling to room temperature, a solid precipitated which was removed by filtration, washed with diethyl ether and dried over phosphorus pentoxide to give the required phosphonium salt (7.90 g, 62%), as a powdery white solid.

Melting point = 238-241°C. [Lit. = 234-240°C].[91]

IR (KBr disc) ν_{max} **:** 3925 (br), 3050 (br), 2891, (m), 1716 (s), 1440 (m), 1400 (m), 1147 (s), 1112 (s), 1018 (m), 995 (m), 790 (m), 754 (s), 726 (s), 693 (s) cm^{-1}.

^{1}H NMR ($CD_3OD/CDCl_3$) δ : 8.03 (15H, m, CH), 3.66 (2H, m, CH_2), 2.78 (2H, t, J = 7.5 Hz, CH_2), 2.13 (2H, m, CH_2) ppm.

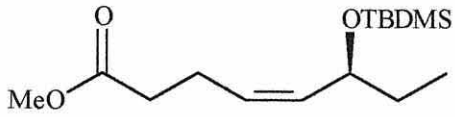
^{13}C NMR ($CD_3OD/CDCl_3$) δ : 174.38 (C), 135.36 (3xCH), 133.40 (3xCH), 133.24 (3xCH), 130.63 (3xCH), 130.43 (3xCH), 117.61 (d, $J_{C\text{-}P}$ = 85.6 Hz, 3xC), 33.31 (d, $J_{C\text{-}P}$ = 35.5 Hz, CH_2), 20.29 (CH_2), 17.93 (CH_2) ppm.

MS (ES) *m/z* : 349 (100% $[M\text{-}Br]^+$) Daltons.

HRMS (ES) *m/z* : found: 349.1357, $C_{22}H_{22}O_2P$ ($[M\text{-}Br]^+$) requires: 349.1357 Daltons.

Microanalysis : found: C, 61.2%, H, 5.8%, $C_{22}H_{22}O_2BrP$ requires: C, 61.6%, H, 5.2%.

(*S*)-Methyl-(*E*),(*Z*)-6-[(*tert*-butyldimethylsilyl)-oxy]-oct-4-enoate, **132**



(3-Carboxypropyl)triphenylphosphonium bromide (2.02 g, 4.7 mmol, 7 equivalents) was suspended in THF (4.6 ml) and sodium *bis*(trimethylsilyl) amide (1.56 M solution in THF, 6.02 ml, 9.4 mmol) added dropwise at room temperature to give a bright orange solution which was then heated under reflux for 1 hour. The solution was allowed to cool to room temperature before transferring (*via* syringe) to a solution of **139** (0.19 g, 0.94 mmol) in THF (3.2 ml). After stirring for 30 minutes the mixture was quenched with an 1% aqueous acetic acid solution (53 ml) and extracted with diethyl ether (5x35 ml). The combined organic fractions were then washed with brine (3x50 ml), dried over anhydrous magnesium sulfate and the solvent evaporated to give a crude, yellow oil.

The oil was dissolved in diethyl ether (5 ml), cooled to 0°C and treated with excess ethereal diazomethane solution (*ca* 6 ml). This solution was allowed to stir for 1 hour and left to evaporate overnight to give a crude semi-crystalline product which was purified by column chromatography on silica gel (gradient elution: 1-4% diethyl ether/petrol) yielding the title ester (0.12 g, 45%) as a 4:1 mixture of geometric isomers (0.08 g *cis* isomer and 0.04 g of mixed *cis* and *trans* isomers). The mixed isomers were found to be separable by careful flash column chromatography on silica gel (eluent: 0.5% diethyl ether/petrol).

Data for the *cis* (*Z*) isomer:

TLC : $R_f = 0.29$ in 5% diethyl ether/petrol.

IR (neat) ν_{max} **:** 2956 (s), 2930 (s), 2856 (m), 1743 (s), 1462 (w), 1437 (w), 1361 (w), 1253 (s), 1166 (m), 1082 (m), 1048 (m), 860 (w), 836 (s), 777 (m) cm^{-1}.

^{1}H NMR δ : 5.42 (1H, dd, $J = 11.0, 9.1$ Hz, CH), 5.30 (1H, m, CH), 4.35 (1H, dt, $J = 7.8, 6.4$ Hz, CH), 3.69 (3H, s, CH_3), 2.45 (4H, m, CH_2), 1.56-1.41 (2H, m, CH_2), 0.91 (9H, m, 3xCH_3), 0.90 (3H, t, $J = 7.4$ Hz, CH_3), 0.06 (3H, s, CH_3), 0.03 (3H, s, CH_3) ppm.

^{13}C NMR δ : 173.28 (C), 135.43 (CH), 126.47 (CH), 69.99 (CH), 51.37 (CH_3), 33.95 (CH_2), 31.27 (CH_2), 25.79 (3xCH_3), 23.31 (CH_2), 18.13 (C), 9.53 (CH_3) -4.42 (CH_3), -4.82 (CH_3) ppm.

MS (CI) *m/z* : 304 (5% $[M+NH_4]^+$), 287 (8% $[M+H]^+$), 229 (48% [M-(*t*-Bu)]$^+$), 155 (100% [M-TBDMSO]$^+$) Daltons.

HRMS (CI) *m/z* : found: 287.2042, $C_{15}H_{31}O_3Si$ ($[M+H]^+$) requires: 287.2042 Daltons.

$[\alpha]_D^{25}$ (*cis* isomer) = +19.8 (c = 0.4, $CHCl_3$).

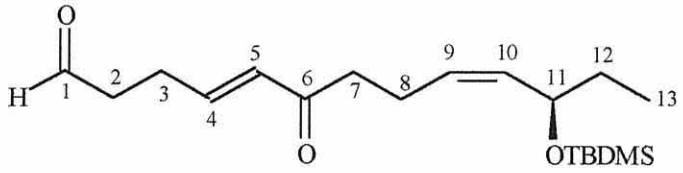
Data for the *trans* (*E*) isomer:

TLC : R_f = 0.23 in 5% diethyl ether/petrol.

^{1}H NMR δ : 5.55 (2H, m, CH), 3.99 (1H, m, CH), 3.70 (3H, s, CH_3), 2.39 (4H, m, CH_2), 1.55-1.38 (2H, m, CH_2), 0.90 (9H, m, CH_3), 0.89 (3H, t, J = 7.4 Hz, CH_3), 0.07 (3H, s, CH_3), 0.05 (3H, s, CH_3) ppm.

^{13}C NMR δ : 173.28 (C), 134.80 (CH), 127.76 (CH), 74.54 (CH), 51.37 (CH_3), 33.85 (CH_2), 31.10 (CH_2), 27.38 (CH_2), 25.79 (3xCH_3), 18.13 (C), 9.64 (CH_3), -4.42 (CH_3), -4.82 (CH_3) ppm.

(*S*)-6-Oxo-11-[(*tert*-butyldimethylsilyl)-oxy]-(4*E*,9*Z*)-tridecadieneal, **130**



Methyltriphenylphosphonium iodide (0.32 g, 0.78 mmol) was suspended in THF (10 ml), cooled to 0°C and treated with *n*-butyllithium (2.0 M, 0.51 ml, 0.86 mmol) with stirring to give an orange coloured solution. After stirring at room temperature for 30 minutes the solution was cooled to -78°C and (*Z*)-**132** (0.11 g, 0.39 mmol) added. The resulting pale yellow mixture was left to stir at room temperature for two hours after which time the solution was quenched with water (20 ml), extracted with ethyl acetate (4x25 ml), the combined organic fractions washed with brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo* to give a crude yellow oil. The crude oil was dissolved in THF (15 ml) and treated with freshly distilled succinaldehyde (0.53 g, 6.2 mmol). After stirring for 42 hours the solvent was evaporated and the crude material purified by column chromatography on silica gel (eluent: 25% diethyl ether/petrol) yielding (0.06 g, 42%) of the desired product.

TLC : $R_f = 0.23$ in 35% diethyl ether/petrol.

IR ($CDCl_3$) ν_{max} **:** 2956 (s), 2929 (s), 2856 (s), 1727 (s), 1698 (m), 1674 (s), 1631 (m), 1409 (m), 1253 (s), 1080 (br s), 1007 (m), 979 (m), 836 (m), 727 (w), 668 (w) cm^{-1}.

^{1}H NMR δ : 9.82 (1H, s, CH-1), 6.83 (1H, dt, J = 16.0, 6.5 Hz, CH-4), 6.14 (1H, dt, J = 16.0, 2.1 Hz, CH-5), 5.42-5.26 (2H, m, CH-9, CH-10), 4.34 (1H, m, CH-11), 2.68-2.40 (6H, m, CH_2-2, CH_2-3, CH_2-7), 2.34 (2H, m, CH_2-8), 1.51-1.27 (2H, m, CH_2-12), 0.89 (9H, s, CH_3), 0.87 (3H, t, J = 7.5 Hz, CH_3-13), 0.06 (3H, s, CH_3), 0.03 (3H, s, CH_3) ppm.

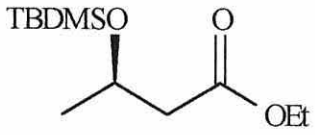
^{13}C NMR δ : 200.25 (CH-1), 199.15 (C-6), 144.44 (CH-4), 135.13 (CH-10), 130.88 (CH-5), 126.90 (CH-9), 70.02 (CH-11), 41.88 (CH_2), 39.97 (CH_2), 31.29 (CH_2), 25.84 (3xCH_3), 24.60 (CH_2), 22.25 (CH_2), 18.21 (C), 9.81 (CH_3-13), -4.36 (CH_3), -4.76 (CH_3) ppm.

MS (CI) *m/z* : 356 (10% $[M+NH_4]^+$), 338 (5% $[M]^+$), 281 (10% $[M-(t\text{-Bu})]^+$), 207 (100% $[M\text{-TBDMSO}]^+$) Daltons.

HRMS (CI) *m/z* : found: 356.2621, $C_{19}H_{38}NO_3Si$ ($[M+NH_4]^+$) requires: 356.2621 Daltons.

$[\alpha]_D^{25} = +16.6$ (c = 1.1, $CHCl_3$).

Ethyl (*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]butanoate,[142] **149**



Imidazole (3.25 g, 47.8 mmol) was dissolved in DMF (25 ml) and the resulting solution cooled to 0°C before being treated with ethyl (*R*)-3-hydroxybutyrate (2.50 g, 18.4 mmol). The mixture was warmed to room temperature for 10 minutes then cooled to 0°C and *tert*-butyldimethylsilyl chloride (3.60 g, 23.9 mmol) added. After stirring at room temperature for 4 hours the mixture was extracted with hexane (3x40 ml), the combined organic fractions washed with an aqueous 2% acetic acid solution (40 ml) and water (3x40 ml). The combined organic fractions were dried and the solvent evaporated giving a crude oil which was purified by column chromatography on silica gel (eluent: 5% diethyl ether/petrol) yielding the title compound **149** (3.39 g, 82%) as an oil.

TLC : R_f = 0.57 in 10% diethyl ether/petrol.

IR (neat) ν_{max} **:** 2932 (s), 2897 (s), 1739 (s), 1471 (m), 1376 (br s), 1301 (br s), 1255 (br s), 1184 (br s), 1140 (br s), 1085 (br s), 1003 (s), 940 (m) cm^{-1}.

^{1}H NMR δ : 4.28 (1H, m, CH), 4.12 (2H, dq, *J* = 7.1, 1.8 Hz, CH_2), 2.48 (1H, dd, *J* = 14.4, 7.5 Hz, CH), 2.36 (1H, dd, *J* = 14.5, 5.4 Hz, CH), 1.27 (3H, t, *J* = 7.2 Hz, CH_3), 1.20 (3H, d, *J* = 6.1 Hz, CH_3), 0.87 (9H, s, CH_3), 0.07 (3H, s, CH_3), 0.05 (3H, s, CH_3) ppm.

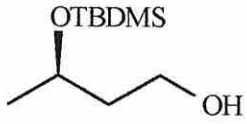
^{13}C NMR δ : 171.66 (C), 65.85 (CH), 60.23 (CH_2), 44.95 (CH_2), 25.71 (3xCH_3), 23.91 (CH_3), 17.93 (C), 14.18 (CH_3), -4.53 (CH_3), -4.76 (CH_3) ppm.

MS (CI) *m/z* : 247 (100% $[M+H]^+$), 189 (10% $[M-(t\text{-Bu})]^+$) Daltons.

HRMS (CI) *m/z* : found: 247.1729, $C_{12}H_{27}O_3Si$ ($[M+H]^+$) requires: 247.1729 Daltons.

$[\alpha]_D^{25}$ = -27.0 (c = 0.37, $CHCl_3$). [Lit., $[\alpha]_D^{18}$ = -26.9 (c = 1.0, $CHCl_3$)].[142]

(*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]butan-1-ol,[142] **150**



A solution of **149** (3.32 g, 15.2 mmol) in dry hexane (50 ml) was cooled to -78°C, treated with diisobutylaluminium hydride (1 M solution in hexanes, 34.2 ml, 34.2 mmol) in a dropwise manner and stirred for eight hours at -20°C. After warming to 0°C, methanol (2 ml) was added and the resulting mixture treated with hexane (40 ml) followed by an aqueous saturated solution of ammonium chloride (30 ml). The resulting precipitated solid was filtered and washed with hexane after which the organic fraction was separated from the filtrate, washed with water (4x50 ml), dried over anhydrous magnesium sulfate and the solvent evaporated to give the desired product (2.26 g, 73%) as a yellow oil.

TLC : R_f = 0.15 in 10% diethyl ether/petrol.

IR (neat) ν_{max} **:** 3346 (br s), 2930 (s), 2858 (s), 1472 (s), 1375 (m), 1255 (s), 1129 (s), 1028 (s), 940 (m), 837 (s), 775 (s), 718 (w), 658 (w) cm^{-1}.

^{1}H NMR δ : 4.11 (1H, m, CH), 3.85 (1H, m, CH), 3.75 (1H, m, CH), 2.59 (1H, br t, J = 5.0 Hz, OH), 1.86-1.80 (1H, m, CH), 1.79-1.57 (1H, m, CH), 1.21 (3H, d, J = 6.2 Hz, CH_3), 0.90 (9H, s, 3xCH_3), 0.10 (3H, s, CH_3), 0.09 (3H, s, CH_3) ppm.

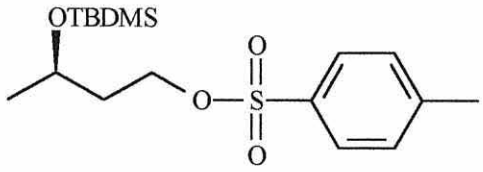
^{13}C NMR δ : 68.29 (CH), 60.40 (CH_2), 40.47 (CH_2), 25.78 (3xCH_3), 23.42 (CH_3), 17.92 (C), -4.38 (CH_3), -4.54 (CH_3) ppm.

MS (CI) *m/z* : 205 (100% $[M+H]^+$), 202 (2%), 132 (5%), 92 (12%), 91 (10%) Daltons.

HRMS (CI) *m/z* : found: 205.1624, $C_{10}H_{25}O_2Si$ ($[M+H]^+$) requires: 205.1624 Daltons.

$[\alpha]_D^{30}$ = -17.8 (c = 0.41, $CHCl_3$). [Lit., $[\alpha]_D^{18}$ = -30.1 (c = 1.1, $CHCl_3$)].[142]

(*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]butan-1-*para*-toluene Sulfonate,[143] **151**



Para-toluenesulfonyl chloride (2.27 g, 11.88 mmol) was dissolved in dry pyridine (5 ml) and added dropwise to a solution of **150** (2.21 g, 10.8 mmol) in dry pyridine (5 ml) at 0°C. The resulting mixture was stirred at room temperature for 16 hours and then extracted with hexane (40 ml). The organic layer was washed with an aqueous solution of sulfuric acid (2 M, 2x25 ml) followed by water (2x40 ml). Drying and concentration of the solvent *in vacuo* gave a crude oil which was purified by column chromatography on silica gel (eluent: 3% diethyl ether/petrol) to give the desired tosylate (2.41 g, 62%) as a colourless oil.

TLC : R_f = 0.15 in 5% diethyl ether/petrol.

IR (neat) ν_{max} **:** 2955 (s), 2856 (s), 1598 (m), 1471 (m), 1362 (br s), 1255 (s), 1178 (s), 1097 (m), 1047 (m), 1006 (m), 938 (m), 891 (m), 837 (m), 776 (m), 655 (m) cm^{-1}.

^{1}H NMR δ : 7.80 (2H, d, J = 8.3 Hz, CH), 7.35 (2H, d, J = 8.3 Hz, CH), 4.11 (2H, t, J = 6.2 Hz, CH_2), 3.91 (1H, m, CH), 2.46 (3H, s, CH_3), 1.74 (2H, m, CH_2), 1.11 (3H, d, J = 6.2 Hz, CH_3), 0.82 (9H, s, CH_3), 0.03 (3H, s, CH_3), -0.02 (3H, s, CH_3) ppm.

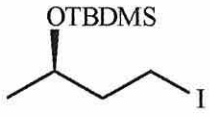
^{13}C NMR δ : 144.68 (C), 133.04 (C), 129.81 (2xCH), 127.90 (2xCH), 67.88 (CH_2), 64.62 (CH), 38.48 (CH_2), 25.73 (3xCH_3), 23.86 (CH_3), 21.61 (CH_3), 17.89 (C), -4.37 (CH_3), -5.07 (CH_3) ppm.

MS (CI) *m/z* : 359 (100% $[M+H]^+$), 246 (8%), 227 (4% $[M\text{-}TBDMSO]^+$), 205 (40%), 52 (12%) Daltons.

HRMS (CI) *m/z* : found: 359.1712, $C_{17}H_{31}O_4SiS$ ($[M+H]^+$) requires: 359.1712 Daltons.

$[\alpha]_D^{26}$ = -22.6 (c = 0.49, $CHCl_3$).

(*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-1-iodobutane, **146**



Tosylate **151** (2.23 g, 6.23 mmol) was dissolved in acetone (95 ml), treated with sodium iodide (5.20 g, 34.3 mmol) and heated at reflux for 4 hours. The resulting suspension was filtered and the remaining solid washed with diethyl ether (100 ml). The solvent was evaporated leaving a crude oil which was triturated with hexane (5x30 ml). The combined organic fractions were dried and the solvent concentrated *in vacuo* yielding without further purification, the title compound (1.87 g, 95%) as a clear oil.

TLC : R_f = 0.73 in 5% diethyl ether/petrol.

IR (neat) ν_{max} **:** 2955 (s), 2929 (s), 2892 (m), 2856 (s), 1472 (m), 1374 (m), 1255 (s), 1178 (s), 1147 (m), 1127 (s), 1063 (s), 967 (s), 871 (w), 835 (s), 775 (s), 709 (w) cm^{-1}.

^{1}H NMR δ : 3.90 (1H, m, CH), 3.24 (2H, m, CH_2), 1.97-1.88 (2H, m, CH_2), 1.17 (3H, d, J = 6.1 Hz, CH_3), 0.90 (9H, s, CH_3), 0.11 (3H, s, CH_3), 0.09 (3H, s, CH_3) ppm.

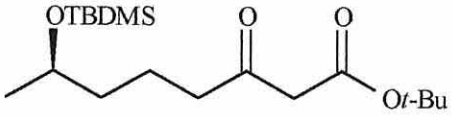
^{13}C NMR δ : 68.23 (CH), 43.24 (CH_2), 25.84 (3xCH_3), 23.45 (CH_3), 17.99 (C), 3.55 (CH_2), -4.24 (CH_3), -4.62 (CH_3) ppm.

MS (CI) *m/z* : 315 (45% $[M+H]^+$), 272 (10%), 189 (36%), 187 (64% $[M-I]^+$), 132 (100% $[TBDMSOH]^+$), 92 (42%), 91 (21%) Daltons.

HRMS (CI) *m/z* : found: 315.0641, $C_{10}H_{24}IOSi$ ($[M+H]^+$), requires: 315.0641 Daltons.

$[\alpha]_D^{27}$ = -46.3 (c = 0.41, $CHCl_3$). [Lit., $[\alpha]_D^{24}$ = -49.6 (c = 1.3, $CHCl_3$)].[143]

(*R*)-*tert*-Butyl 7-[(*tert*-butyldimethylsilyl)oxy]-3-oxooctanoate, **126**



Diisopropylamine (0.97 g, 9.57 mmol, 1.34 ml) was added to THF (50 ml) with stirring and cooled to 0°C. The resulting solution was treated with *n*-butyllithium (2.44 M, 3.92 ml, 9.57 mmol) and stirred for 30 minutes at 0°C after which *tert*-butylacetoacetate (0.66 g, 4.15 mmol, 0.69 ml) was added and the mixture left to stir for 1 hour at the same temperature. Chiral iodide **146** (1.0 g, 3.19 mmol) was added and the resulting mixture warmed to room temperature and stirred for a further 2 hours. The solution was diluted with hexane (45 ml) followed by treatment with saturated aqueous ammonium chloride solution (15 ml) and water (45 ml). The organic layer was separated and washed with brine (2x45 ml) and the solvent dried and concentrated *in vacuo* to give a crude yellow oil which was purified on silica gel (eluent: 3% diethyl ether/petrol) giving the desired product **126**, as a pale yellow oil (0.82 g, 75%).

TLC : R_f = 0.58 in 20% diethyl ether/petrol.

IR (neat) ν_{max} **:** 2930 (s), 2856 (s), 1735 (s), 1717 (s), 1642 (br m), 1462 (m), 1406 (w), 1369 (s), 1318 (m), 1253 (s), 1147 (br s), 1032 (m), 836 (s), 775 (s) cm^{-1}.

^{1}H NMR δ : 3.79 (1H, m, CH), 3.34 (2H, s, CH_2), 2.53 (2H, t, J = 7.2 Hz, CH_2), 1.72-1.53 (2H, m, CH_2), 1.48 (9H, s, CH_3), 1.45-1.22 (2H, m, CH_2), 1.12 (3H, d, J = 6.1 Hz, CH_3), 0.89 (9H, s, CH_3), 0.05 (6H, s, CH_3) ppm.

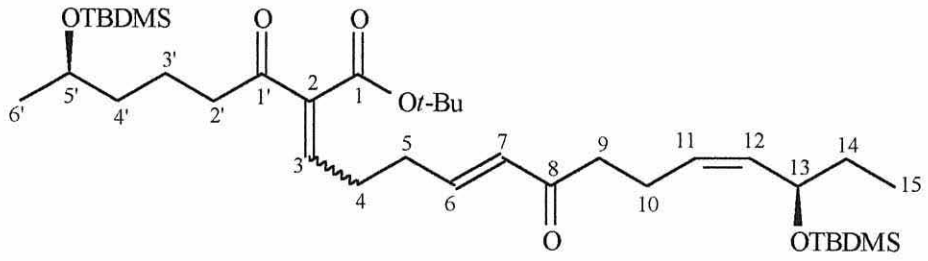
^{13}C NMR δ : 203.15 (C), 166.44 (C), 81.75 (C), 68.23 (CH), 50.53 (CH_2), 42.87 (CH_2), 38.84 (CH_2), 27.92 (3xCH_3), 25.84 (3xCH_3), 23.66 (CH_3), 19.68 (CH_2), 18.04 (C), -4.45 (CH_3), -4.78 (CH_3) ppm.

MS (CI) *m/z* : 345 (14% $[M+H]^+$), 289 (39%), 245 (58%), 157 (17%), 132 (23% $[TBDMSOH]^+$), 113 (100%), 58 (24%), 52 (82%), 44 (65%), 36 (50%) Daltons.

HRMS (CI) *m/z* : found: 345.2461, $C_{18}H_{37}O_4Si$ ($[M+H]^+$) requires: 345.2461 Daltons.

$[\alpha]_D^{27}$ = -12.7 (c = 0.37, $CHCl_3$).

tert-Butyl (13*S*, 5'*R*),(2*E*/*Z*, 6*E*, 11*Z*)-2-{5'-[(*tert*-butyldimethylsilyl)oxy]-1'-oxo-hexanoyl}-[(13-*tert*-butyldimethylsilyl)oxy]-8-oxopentadeca-2,6,11-trienoate, **129**



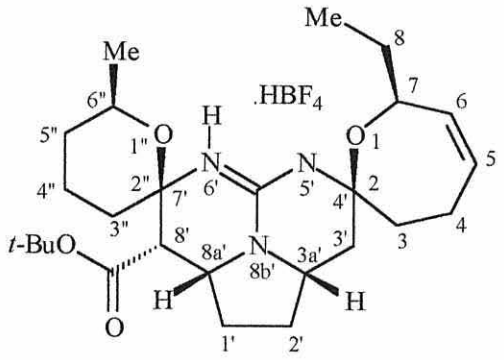
Aldehyde **130** (0.055 g, 0.16 mmol), β-keto ester **126** (0.071 g, 0.19 mmol) and sodium sulfate (*ca* 0.5 g) were mixed together with stirring in dry dichloromethane (0.5 ml) for ten minutes at -20°C. Morpholine (0.006 g, 0.064 mmol, 0.006 ml) was added and the mixture stirred for six hours at -20°C. The mixture was diluted with hexane (5 ml), washed with an aqueous 2% acetic acid solution (2x15 ml) followed by water (2x15 ml), and the combined organic fractions extracted with hexane (2x30 ml). The organic layer was dried over anhydrous magnesium sulfate and the solvent evaporated to yield a crude orange oil. An attempted purification on silica gel (gradient elution: 5-30% diethyl ether/petrol) gave a low yield of the impure product (0.023 g, 22%) as an oil.

Assignable data:

TLC : R_f = 0.63 in 40% diethyl ether/petrol.

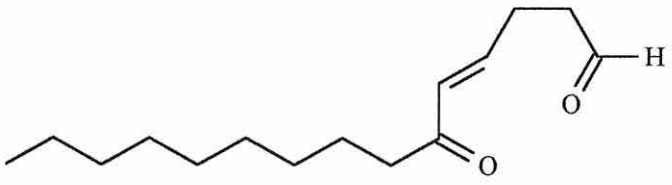
^{1}H NMR δ : 6.89 (1H, m, CH-6), 6.67 (1H, t, J = 7.4 Hz, CH-3), 6.09 (1H, dd, J = 16.2, 4.0 Hz, CH-7), 5.33 (2H, m, CH-11, CH-12), 4.33 (1H, q, J = 13.4, 6.3 Hz, CH-13), 3.80 (1H, dq, J = 11.9, 6.1, 2.1 Hz, CH-5'), 2.61 (2H, br t, J = 6.0 Hz, CH_2), 2.47 (2H, m, CH_2), 2.36 (2H, br t, J = 7.7 Hz, CH_2), 1.72-1.18 (19H, m, 5xCH_2, *t*-Bu), 1.13 (3H, dd, J = 6.1, 1.7 Hz, CH_3-6'), 0.88 (21H, s, CH_3-15, 6xCH_3), 0.05 (12H, s, 4xCH_3) ppm.

Attempted Synthesis of *tert*-Butyl (2*S*,7*S*,3a'*S*,7'*R*,8'*R*,8a'*R*,6"*R*)-dispiro[7-ethyl-5*Z*-oxepene-2,4'-(1',2',3',4',7',8'-hexahydro-5*H*-5',6',8b'-triazaacenaphthylene-8'-carboxylate)-6"-methyl-7',2"-tetrahydropyran]-6'-ium Tetrafluoroborate, **128**



Guanidine (0.002 g, 0.035 mmol) was dissolved in DMF (0.5 ml) and stirred for 30 minutes before adding to a solution of **129** in DMF (2 ml) at 0°C. After stirring at this temperature for 7 hours, water (1 ml) was added followed by methanolic HCl (2 ml, [acetyl chloride (0.1 ml) in methanol (1.9 ml)]) and the resulting mixture left to stir overnight at room temperature. The reaction was diluted with water (30 ml), extracted with CH_2Cl_2 (4x20 ml) and the combined organic fractions washed with water (2x30 ml), saturated lithium bromide solution (40 ml) and water (40 ml). After concentration of the organic fraction (*ca* 10 ml remaining solvent), saturated sodium tetrafluoroborate solution was added (10 ml) and the mixture stirred vigorously for 2 hours. The mixture was diluted with CH_2Cl_2 (30 ml), the organic fraction separated, dried over anhydrous magnesium sulfate and the solvent evaporated to give a yellow solid. An attempted purification by flash column chromatography on silica gel (gradient elution: 0-10% MeOH/$CHCl_3$) failed to yield the desired pentacyclic product.

(4*E*)-6-Oxopentadec-4-enal, **164**



Acetylmethylene triphenylphosphorane (5.0 g, 15.7 mmol) was dissolved in THF (200 ml), cooled to -78°C and treated in a dropwise manner with *n*-butyllithium (2.35 M, 7.35 ml, 17.3 mmol) giving a deep red coloured solution. After stirring for 30 minutes at -60°C, *n*-octyl iodide (4.57 g, 19.0 mmol, 3.45 ml) was added and the mixture left to stir for a further 20 hours at room temperature. The solvent was evaporated giving a crude red oil which was dissolved in ethyl acetate (100 ml) and washed with water (2x50 ml). The aqueous washes were recombined and extracted with ethyl acetate (50 ml). After drying the combined organic fractions over anhydrous magnesium sulfate, the solvent was concentrated *in vacuo*, affording the crude alkylated phosphorane as a red oil in a quantitative yield (6.75 g).

To a stirred solution of the crude phosphorane (6.75 g, 15.7 mmol) in dichloromethane (30 ml) was added freshly distilled succinaldehyde (4.05 g, 47.1 mmol), and the resulting mixture left to stir for 72 hours at room temperature. The solvent was evaporated yielding an oil which was dissolved in diethyl ether (30 ml) and washed with water (3x250 ml containing 3.4 ml of 1 M HCl). Each aqueous washing was back-extracted with diethyl ether (50 ml), the combined organic fractions dried over anhydrous magnesium sulfate and the solvent evaporated. Purification by column chromatography on silica gel (gradient elution: 15-40% diethyl ether/petrol) gave the desired product **164**, (1.81 g, 48%) as a yellow oil.

TLC : R_f = 0.27 in 40% diethyl ether/petrol.

IR (neat) ν_{max} **:** 2926 (s), 2856 (s), 2718 (w), 1727 (s), 1675 (s), 1631 (s), 1366 (s) cm^{-1}.

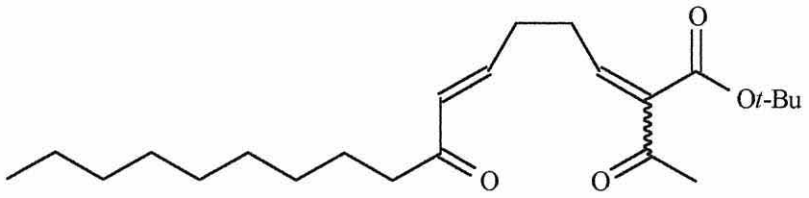
^{1}H NMR δ : 9.81 (1H, s, CH), 6.81 (1H, dt, J = 15.9, 6.6 Hz, CH), 6.13 (1H, d, J = 15.9 Hz, CH), 2.65 (2H, m, CH_2), 2.55 (4H, m, CH_2), 1.61 (2H, m, CH_2), 1.27 (12H, br s, CH_2), 0.88 (3H, t, J = 7.0 Hz, CH_3) ppm.

^{13}C NMR δ : 200.34 (CH), 200.25 (C), 143.95 (CH), 130.99 (CH), 41.87 (CH_2), 40.31 (CH_2), 31.79, 29.36, 29.20, 24.55, 24.09, 22.58 (8xCH_2), 14.01 (CH_3) ppm.

MS (CI) *m/z* : 256 (100% $[M+NH_4]^+$), 239 (36% $[M+H]^+$), 238 (17% $[M]^+$), 223 (14% $[M-CH_3]^+$), 221 (12%), 214 (13%), 188 (11%) Daltons.

HRMS (CI) *m/z* : found: 239.2011, $C_{15}H_{27}O_2$ ($[M+H]^+$) requires: 239.2011 Daltons.

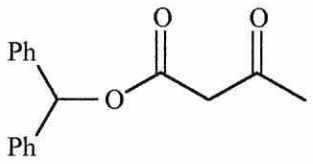
Attempted Synthesis of *tert*-Butyl (2*E*/*Z*,6*E*)-2-ethanoyl-8-oxoheptadec-2,6-dienoate, **170**



(i) A solution of aldehyde **164** (0.48 g, 2.02 mmol) in CH_2Cl_2 (6 ml) was treated with *tert*-butylacetoacetate (0.34 g, 2.16 mmol, 0.36 ml) and cooled to -78°C. Piperidine (0.025 g, 0.29 mmol, 0.03 ml) in CH_2Cl_2 (15 ml) was added slowly *via* a dropping funnel and the resulting mixture allowed to warm to -20°C over a period of 30 minutes and then stirred at this temperature for a further 22 hours. The reaction mixture was diluted with hexane (80 ml) and washed with aqueous 2% AcOH (10 ml) followed by water (2x10 ml). The combined aqueous washes were back extracted with a mixture of 1:1 CH_2Cl_2/hexane (2x40 ml). Both organic fractions were combined, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. An attempted purification on silica gel (gradient elution: 30-50% diethyl ether/petrol) failed to produce the desired product.

(ii) Titanium tetrachloride (1.0 M in heptane, 0.11 mmol, 0.5 ml) was added to carbon tetrachloride (2 ml) at 0°C and the resulting yellow solution transferred to a flask containing THF (20 ml) also at 0°C, resulting in a yellow suspension of [tetrachloro*bis*(tetrahydrofuran) titanium]. In a second flask aldehyde **164** (0.53 g, 2.24 mmol) and *tert*-butylacetoacetate (0.35 g, 2.24 mmol) were dissolved in THF (1 ml) and added to the titanium complex at 0°C, giving a red coloured solution. After stirring for 10 minutes, pyridine (0.8 ml) in THF (2 ml) was added, very slowly over a period of 1.5 hours whilst maintaining the temperature at 0°C. A brown coloured mixture formed which was left to stir for 16 hours at room temperature. Water (10 ml) and diethyl ether (20 ml) were added, after which the mixture was extracted with aqueous copper (II) sulfate solution (3x20 ml). The combined aqueous washes were back extracted with diethyl ether (2x25 ml) and the organic fractions washed with saturated sodium chloride solution (20 ml), dried over anhydrous magnesium sulfate and the solvent evaporated. ^{1}H NMR analysis of the crude material obtained showed no evidence of the desired product.

Benzhydryl 3-Oxobutanoate, **169**



Diketene [stabilised with copper sulfate, (1.83 g , 21.7 mmol, 1.68 ml)] was carefully added to a solution of benzhydrol (2.00 g, 10.90 mmol) and DMAP (0.11 g, 0.87 mmol) in THF (22 ml) at 0°C. The resulting orange mixture was stirred at 20°C for 30 minutes after which the solvent was evaporated under reduced pressure. Purification of the residue by flash chromatography on silica gel (eluent: 20% diethyl ether/hexane) gave the pure acetate (2.09 g, 83%) as a powdery, white solid.

TLC : R_f = 0.30 in 30% diethyl ether/hexane.

Melting point = 50-51°C. [Lit = 46-47°C].[106]

IR ($CHCl_3$) ν_{max} **:** 3038 (w), 1743 (s), 1717 (s), 1496 (m), 1452 (w), 1360 (w), 1316 (m), 1148 (m), 1080 (w), 1028 (m), 969 (w), 928 (w), 700 (s) cm^{-1}.

^{1}H NMR δ : 7.41-7.30 (10H, m, CH), 6.95 (1H, s, CH), 3.57 (2H, s, CH_2), 2.24 (3H, s, CH_3) ppm.

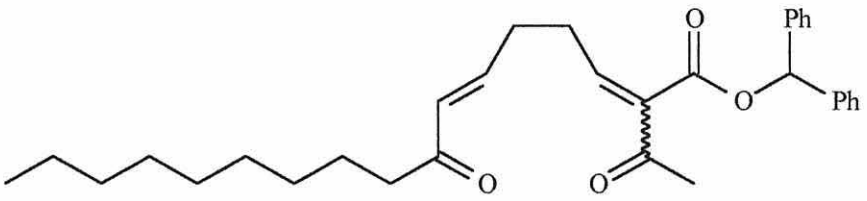
^{13}C NMR δ : 200.27 (C), 166.17 (C), 139.53 (2xC), 128.58 (4xCH), 128.14 (2xCH), 127.17 (4xCH), 77.97 (CH), 50.30 (CH_2), 30.17 (CH_3) ppm

MS (EI) *m/z* : 268 (2% $[M]^+$), 224 (15%), 183 (37% $[M\text{-}CH_3C(O)CH_2C(O)]^+$), 168 (17%), 167 (78% $[M\text{-}CH_3C(O)CH_2C(O)O]^+$), 166 (58%), 165 (100%), 152 (31%), 105 (45%), 103 (27%), 77 (32% $[C_6H_5]^+$), 51 (20%), 44 (21%), 43 (98% $[CH_3CO]^+$), 39 (22%) Daltons.

HRMS (EI) *m/z* : found: 268.1099, $C_{17}H_{16}O_3$ ($[M]^+$) requires: 268.1099 Daltons.

Microanalysis : found : C, 76.1%, H, 5.9%, $C_{17}H_{16}O_3$ requires: C, 76.1%, H, 6.0%.

Attempted Synthesis of Benzhydryl (2*E*/*Z*, 6*E*)-2-Ethanoyl-8-oxoheptadeca-2,6-dienoate, **171b**



Benzhydryl 3-oxobutanoate (0.25 g, 0.92 mmol) and aldehyde **164** (0.20 g, 0.84 mmol), in separate flasks were dissolved in CH_2Cl_2 (2.0 ml and 2.3 ml, respectively) and both cooled to -20°C. A small amount of anhydrous magnesium sulfate was added to the acetate which was subsequently treated with a solution of piperidine (0.07 g, 0.84 mmol, 0.08 ml) in CH_2Cl_2 (1 ml) and stirred for 15 minutes. The acetate/piperidine mixture was transferred to the flask containing the aldehyde and stirred for 5 hours at -20°C. Hexane (10 ml) cooled to -20°C was added, followed by ice water (10 ml) containing 1 drop of acetic acid. The organic layer was separated and the remaining aqueous phase extracted with CH_2Cl_2 (2x20 ml). Combination of the organic phases followed by drying over anhydrous magnesium sulfate and evaporation gave a brown oil. Attempted purification of the oil by column chromatography on silica gel (gradient elution: 8-20% ethyl acetate/petrol) failed to yield the desired product. Evidence of the product (*ca* 2-3% yield) was present in the proton NMR spectrum, but it could not be separated from the impurities.

tert-Butoxycarbonylmethyldiethylphosphonate,[110a] **175**

175

A mixture of *tert*-butylchloroacetate (4.50 g, 29.8 mmol, 4.27 ml) and triethylphosphite (4.95 g, 29.8 mmol, 5.11 ml), under a nitrogen atmosphere, were heated at 100°C for 10 minutes and then at 140°C for another 3 hours. The reaction mixture was distilled at reduced pressure (8 mmHg), using a Kugelrohr, to remove any unreacted starting material, leaving the product as a pale yellow oil (5.58 g, 73%).

TLC : R_f = 0.15 in 75% diethyl ether/petrol.

Boiling Point = 125-131°C at 8 mmHg. [Lit. = 77-86°C at 0.1 mm and 82-82.5°C at 0.05 mm].[109]

IR (neat) ν_{max} **:** 3484 (br, enol OH), 2981 (s), 2933 (m), 1728 (s), 1479 (w), 1394 (m), 1369 (m), 1289 (s), 1258 (s), 1167 (s), 1116 (s), 1027 (s), 972 (br), 883 (w), 830 (w), 699 (w) cm^{-1}.

^{1}H NMR δ : 4.17 (4H, dq, $J_{P\text{-}H}$ = 8.0 Hz, CH_2), 2.88 (2H, d, J = 21.4 Hz, CH_2), 1.77 (1H, br s, enol OH), 1.47 (9H, s, CH_3), 1.35 (6H, t, J = 7.1 Hz, CH_3) ppm.

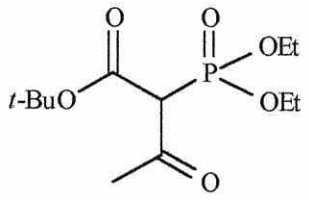
^{13}C NMR δ : 164.81 (d, $J_{C\text{-}P}$ = 6.4 Hz, C), 81.91 (C), 62.45^a (CH_2), 62.35^a (CH_2), 35.48 (d, $J_{C\text{-}P}$ = 132.2 Hz, CH_2), 27.82 (3xCH_3), 16.29^b (CH_3), 16.19^b (CH_3) ppm.

MS (CI) *m/z* : 253 (23% $[M+H]^+$), 214 (69%), 197 (38%), 195 (18% $[M-(t\text{-Bu})]^+$), 170 (51%), 156 (18%), 153 (100%), 74 (39% $[t\text{-BuOH}]^+$), 58 (64%) Daltons.

HRMS (CI) *m/z* : found: 253.1197, $C_{10}H_{22}O_5P$ ($[M+H]^+$) requires: 253.1205 Daltons.

^{a,b} Interchangeable assignments.

tert-Butyl 2-(Diethoxyphosphoryl)-3-oxobutanoate, **180**



To a solution of phosphonate **175** (1.00 g, 3.97 mmol) in THF (5 ml), cooled to 0 °C was added sodium hydride (60% dispersion in mineral oil, 0.24 g, 5.96 mmol), in small portions. After effervescence had ceased, freshly distilled acetyl chloride (0.28 ml) was slowly added and the resulting pale yellow oil stirred for 14 hours. The reaction mixture was quenched with water (20 ml) followed by 2 M H_2SO_4 (2.5 ml) and extracted with diethyl ether (3x30 ml), the combined organic fractions dried over anhydrous magnesium sulfate and the solvent concentrated *in vacuo* yielding a crude oil. Purification on silica gel (gradient elution: 0-50% diethyl ether/petrol) gave the title phosphonate **180** in a disappointing yield (0.39 g, 33%), as a mixture of enolic forms.

TLC : $R_f = 0.43$ in 75% diethyl ether/petrol.

IR (neat) ν_{max} **:** 3483 (br, enol OH), 2980 (s), 2919 (m), 1703 (br), 1589 (br), 1478 (m), 1368 (s), 1252 (br), 1169 (br), 1026 (br), 978 (s), 801 (w), 773 (w) cm^{-1}.

^{1}H NMR δ : 4.13 (4H, m CH_2), 2.41 (1H, d, $J_{P\text{-}H} = 2.8$ Hz, CH), 1.50 (3H, s, CH_3), 1.49 (9H, s, CH_3), 1.32 (6H, t, $J = 7.0$ Hz, CH_3) ppm.

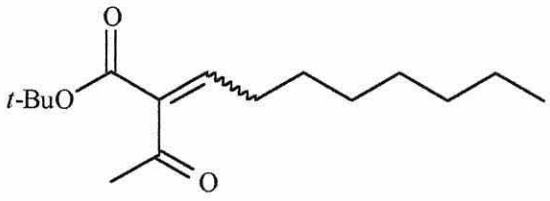
^{13}C NMR (major resonances)[a] δ : 196.69 (C), 163.13 (C), 80.45 (C), 63.76 (CH), 62.35 (CH_2), 62.27 (CH_2), 28.16 (3xCH_3), 22.33 (CH_3), 16.12 (2xCH_3) ppm.

MS (CI) *m/z* : 295 (14% $[M+H]^+$), 239 (21%), 195 (27%), 156 (27%), 139 (47%), 120 (26%) Daltons.

HRMS (CI) *m/z* : found: 295.1310, $C_{12}H_{24}O_6P$ ($[M+H]^+$) requires: 295.1311 Daltons.

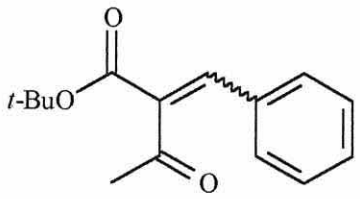
[a] Major resonances refer to those of highest intensity, the remaining signals being due to the enolic isomers present.

Attempted Synthesis of *tert*-Butyl (2*E*/*Z*)-2-Ethanoyl-dec-2-enoate, **182a**



Sodium hydride (60% dispersion in mineral oil, 0.015 g, 0.374 mmol) was washed with diethyl ether (2x1 ml), dried and suspended in diethyl ether (2 ml). After cooling to 0°C, phosphonate **180** (0.100 g, 0.340 mmol) in diethyl ether (1 ml) was added and the resulting mixture stirred at 0°C for 30 minutes. Octanal (0.049 g, 0.374 mmol, 0.058 ml) was added and the reaction mixture stirred for a further 20 hours. The reaction mixture was quenched with water (15 ml), extracted with diethyl ether (3x25 ml), the combined organic fractions washed with water (30 ml), dried over anhydrous magnesium sulfate and the solvent evaporated to yield a yellow oil. ^{1}H NMR analysis of the crude material recovered showed no evidence of olefinic protons hence, the desired product was deemed not to have formed.

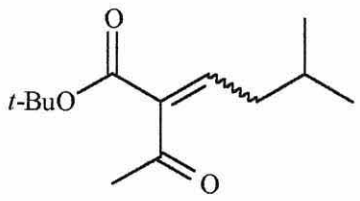
Attempted Synthesis of *tert*-Butyl (2*E*/*Z*)-2-Ethanoyl-3-phenylprop-2-enoate, **182b**



(i) Phosphonate **180** (0.100 g, 0.340 mmol) in THF (1 ml) was added to a suspension of potassium *tert*-butoxide (0.041 g, 0.374 mmol) in THF (2 ml) at 0 °C and the resulting mixture stirred at this temperature for 1 hour. Benzaldehyde (0.054 g, 0.510 mmol, 0.050 ml) was added and the reaction mixture heated at 70 °C for 4 hours and then at room temperature for a further 12 hours. The reaction mixture was quenched with water (15 ml), extracted with diethyl ether (3x25 ml) and the combined organic fractions washed with water (30 ml). Drying of the solvent over anhydrous magnesium sulfate followed by evaporation yielded a yellow oil whose proton NMR spectrum showed no evidence of olefinic protons, hence the desired reaction had not occurred.

(ii) Sodium hydride (95%, 0.017 g, 0.680 mmol) was suspended in DME (1 ml) and treated with a mixture of phosphonate **180** (0.200 g, 0.680 mmol) and benzaldehyde (0.089 g, 0.840 mmol, 0.11 ml) in DME (1 ml). After effervescence had ceased the mixture was heated at reflux for 6 hours and then at 100 °C for a further 15 hours. The reaction mixture was cooled to room temperature, quenched with water (10 ml) and acidified with 10% HCl solution. The resulting mixture was extracted with diethyl ether (3x20 ml) and the combined organic fractions washed with aqueous saturated sodium chloride solution (30 ml), dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure leaving a pale yellow oil. Purification of the oil on silica gel (gradient elution: 0-50% diethyl ether/petrol) failed to afford title model compound **182b**.

tert-Butyl (2*E*/*Z*)-2-Ethanoyl-5-methylhex-2-enoate, **182c**



(i) A suspension of potassium *tert*-butoxide (0.09 g, 0.79 mmol) in THF (4 ml) was cooled to 0°C and treated with phosphonate **180** (0.16 g, 0.56 mmol) in THF (1 ml). After stirring for 30 minutes isovaleraldehyde (0.03 g, 0.37 mmol, 0.04 ml) was added to the reaction mixture which was then heated at reflux for 2 hours. The mixture was allowed to cool to room temperature, treated with 2 M H_2SO_4 (3 ml) and extracted with diethyl ether (3x15 ml). The organic fractions were combined, washed with water (30 ml), dried over anhydrous magnesium sulfate and concentrated *in vacuo*. Purification of the remaining oil on silica gel (gradient elution: 0-50% diethyl ether/petrol) gave the title product in very low yield (0.001 g, 1%).

(ii) Isovaleraldehyde (0.82 g, 9.49 mmol, 1.02 ml) was added to a solution of *tert*-butylacetoacetate (0.50 g, 3.17 mmol, 0.52 ml) in CH_2Cl_2 (7 ml) at -78°C. After stirring for 15 minutes, piperidine (0.08 g, 0.99 mmol, 0.09 ml) was added and the mixture stirred at -15°C for a further 22 hours. The reaction mixture was diluted with hexane (25 ml), washed with aqueous 2% AcOH (30 ml) and water (30 ml). Drying of the solvent over anhydrous magnesium sulfate followed by evaporation gave an oil which was purified by means of column chromatography on silica gel (gradient elution: 0-1% diethyl ether/petrol) giving the title compound (0.14 g, 6%) as a fruity smelling oil.

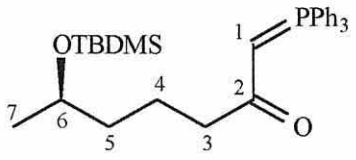
TLC : R_f = 0.50 in 20% diethyl ether/petrol.

IR (neat) ν_{max} **:** 2959 (s), 2885 (m), 1720 (s), 1631 (s), 1460 (m), 1393 (s), 1369 (s), 1252 (s), 1155 (s), 1053 (m), 961 (m), 846 (m), 734 (w) cm^{-1}.

^{1}H NMR δ : 6.80 (1H, t, J = 7.8 Hz, CH), 2.32 (3H, s, CH_3), 2.19 (2H, t, J = 7.0 Hz, CH_2), 1.82 (1H, m, CH), 1.57 (9H, s, CH_3), 0.98 (6H, d, J = 6.3 Hz, CH_3) ppm.

^{13}C NMR δ : 201.82 (C), 164.38 (C), 146.31 (C), 145.73 (CH), 81.88 (C), 38.52 (CH_3), 38.01 (CH_2), 28.26 (CH), 28.11 (3xCH_3), 22.49 (CH_3), 22.39 (CH_3) ppm.

(*R*)-6-[(*tert*-Butyldimethylsilyl)oxy]-1-(triphenylphosphanylidene)-heptan-2-one, **186**



Acetylmethylene triphenylphosphorane (3.80 g, 11.94 mmol) was suspended in THF (70 ml), cooled to -78°C and *n*-butyllithium (2.33 M, 11.94 mmol, 5.12 ml) slowly added. The deep red solution was warmed to -55°C and stirred for 1 hour before re-cooling to -78°C. At this stage, iodide **146** (2.50 g, 7.96 mmol) in THF (15 ml) was added and the mixture left to stir for 16 hours, whilst warming to ambient temperature. The solvent was removed under reduced pressure leaving a crude oil which was purified by flash column chromatography on silica gel (gradient elution: 70-85% ethyl acetate/petrol) to give the title compound (2.03 g, 51%) as a dense orange oil.

TLC : R_f = 0.13 in 70% ethyl acetate/petrol.

IR ($CHCl_3$) ν_{max} **:** 3059 (w), 2956 (s), 2855 (m), 1727 (w), 1525 (s), 1482 (m), 1438 (s), 1400 (s), 1254 (m), 1107 (m), 1028 (w), 874 (w), 836 (m), 693 (s) cm^{-1}.

^{1}H NMR δ : 7.72-7.42 (15H, m, CH), 4.13 (1H, q, J = 14.3, 7.2 Hz, CH-6), 3.66 (1H, br s, CH-1), 2.31 (2H, br t, J = 7.4 Hz, CH_2-3), 1.69 (2H, m, CH_2-5), 1.50 (2H, m, CH_2-4), 1.29 (3H, d, J = 7.2 Hz, CH_3-7), 0.89 (9H, s, CH_3), 0.05 (6H, s, CH_3) ppm.

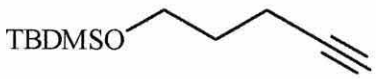
^{13}C NMR δ : 193.95 (C-2), 133.13, 132.97, 132.16, 132.01, 131.91, 128.86, 128.67, 128.59, 128.40 (15xCH), 128.10, 126.66 (3xC), 68.82 (CH-6), 51.03 (d, $J_{C\text{-}P}$ = 106.9 Hz, CH-1), 41.68 (d, $J_{C\text{-}P}$ = 14.38 Hz, CH_2-3), 39.80 (CH_2-5), 25.97 (3xCH_3), 23.81 (CH_3-7), 23.73 (CH_2-4), 18.19 (C), -4.41 (CH_3), -4.63 (CH_3) ppm.

MS (CI) *m/z* : 505 (11% $[M+H]^+$), 279 (24%), 263 (100%), 245 (44%), 187 (41%), 113 (45%) Daltons.

HRMS (CI) *m/z* : found: 505.2691, $C_{31}H_{42}O_2PSi$ ($[M+H]^+$) requires: 505.2692 Daltons.

$[\alpha]_D^{22}$ = -2.1 (c = 1.0, $CHCl_3$).

5-[(*tert*-Butyldimethylsilyl)oxy]-pent-1-yne,[114] **188**



A solution of 4-pentyn-1-ol (3.45 g, 41 mmol) in dry dichloromethane (50 ml) was treated with triethylamine (9.29 g, 92 mmol, 12.8 ml) in a dropwise manner giving a yellow coloured solution. The mixture was cooled to 0°C and *tert*-butyldimethylsilyl chloride (6.9 g, 46 mmol) added along with a catalytic amount of DMAP (*ca* 100 mg). After stirring for 24 hours at room temperature, the solution was quenched with a mixture of 2% HCl (50 ml) in CH_2Cl_2 (50 ml), the separated organic fraction washed with water (3x50 ml) and the combined aqueous fractions back-extracted with CH_2Cl_2 (4x40 ml). The organic washes were dried over anhydrous magnesium sulfate and concentrated *in vacuo* to yield the title product (7.63 g, 94%), as a red oil.

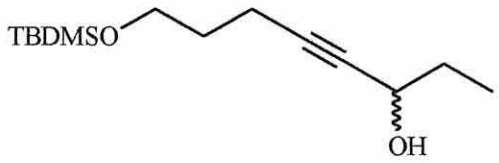
TLC : $R_f = 0.61$ in 10% ethyl acetate/hexane.

IR (neat) ν_{max} **:** 3313 (m), 2954 (s), 2929 (m), 2886 (w), 2857 (m), 2120 (w), 1604 (br), 1472 (m), 1388 (w), 1256 (m), 1107 (s), 979 (m), 835 (s), 776 (s), 629 (m) cm^{-1}.

^{1}H NMR δ : 3.67 (2H, t, $J = 6.0$ Hz, CH_2), 2.25 (2H, dt, $J = 7.1$, 2.7 Hz, CH_2), 1.90 (1H, t, $J = 2.7$ Hz, CH), 1.70 (2H, m, CH_2), 0.87 (9H, s, CH_3), 0.03 (6H, s, CH_3) ppm.

^{13}C NMR δ : 84.26 (C), 68.21 (CH), 61.41 (CH_2), 31.49 (CH_2), 25.91 (3xCH_3), 18.32 (C), 14.82 (CH_2), -3.59 (CH_3), -3.98 (CH_3) ppm.

(±)-8-[(*tert*-Butyldimethylsilyl)-oxy]-4-octyn-3-ol,[34] **189**



Alkyne **188** (7.0 g, 35.0 mmol) was dissolved in THF (80 ml), cooled to -78°C and treated with *n*-butyllithium (2.13 M, 19.9 ml, 40.3 mmol). The solution was warmed to room temperature for 5 minutes and DMPU (6.9 ml) added. After stirring for a further 5 minutes, the mixture was again cooled to -78°C and a solution of propanal (2.23 g, 38.5 mmol) in THF (20 ml) added in a dropwise manner. The resulting mixture was allowed to warm to room temperature and stirred for 2 hours, after which saturated NH_4Cl solution (60 ml) and brine (20 ml) were added. Following stirring for 5 minutes, the organic layer was separated and the aqueous layer extracted with 50% hexane/ethyl acetate mixture (3x50 ml). The combined organic fractions were washed with brine (2x50 ml), dried over anhydrous magnesium sulfate and the solvent evaporated to afford a crude oil which was purified by column chromatography on silica gel (eluent: 20% ethyl acetate/petrol) giving the desired alcohol (8.21 g, 92%).

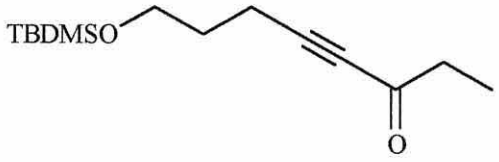
TLC : R_f = 0.21 in 10% ethyl acetate/petrol.

IR (neat) ν_{max} **:** 3392 (br), 2930 (s), 2857 (m), 2243 (w), 1464 (m), 1255 (m), 1106 (br), 962 (m), 835 (s), 776 (s), 662 (m) cm^{-1}.

^{1}H NMR δ : 4.31 (1H, tt, J = 6.3, 1.9 Hz, CH), 3.71 (2H, t, J = 6.1 Hz, CH_2), 2.32 (2H, dt, J = 7.1, 2.0 Hz, CH_2), 1.67 (4H, m, CH_2), 0.94 (3H, t, J = 7.4 Hz, CH_3), 0.87 (9H, s, CH_3), 0.08 (6H, s, CH_3) ppm.

^{13}C NMR δ : 85.10 (C), 81.18 (C), 63.96 (CH), 61.56 (CH_2), 31.70 (CH_2), 31.17 (CH_2), 25.91 (3xCH_3), 18.31 (C), 15.07 (CH_2), 9.43 (CH_3), -5.43 (2xCH_3) ppm.

8-[(*tert*-Butyldimethylsilyl)-oxy]-4-octyn-3-one, **190**



A solution of oxalyl chloride (4.0 g, 31.2 mmol, 2.80 ml) in dry dichloromethane (100 ml) was cooled to -78°C and treated with dimethyl sulfoxide (4.4 g, 56.7 mmol, 3.98 ml) giving an effervescent mixture which was left to stir for 10 minutes at -78°C. Alcohol **189** (5.0 g, 19.5 mmol) was added and the mixture stirred at -60°C for ten minutes. The solution was again cooled to -78°C and triethylamine (11.9 g, 117.0 mmol, 15.9 ml) added. The resulting mixture was allowed to stir at room temperature for 2 hours before being extracted with hexane (2x50 ml) and the combined organic fractions washed sequentially with water (2x60 ml), a 7% aqueous solution of acetic acid (2x40 ml) and brine (2x50 ml). Drying of the solvent over anhydrous magnesium sulfate followed by concentration *in vacuo* gave a crude material which was purified on silica gel (eluent: 5% ethyl acetate/petrol) yielding the desired ketone (4.67 g, 94%) as a yellow oil.

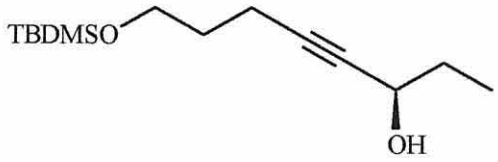
TLC : $R_f = 0.42$ in 10% ethyl acetate/hexane.

IR (neat) ν_{max} **:** 2956 (s), 2931(s), 2857 (s), 2211 (m), 1681 (s), 1472 (s), 1349 (w), 1256 (m), 1175 (m), 1175 (m), 1106 (m), 961 (m), 836 (s), 777 (m) cm^{-1}.

^{1}H NMR δ : 3.71 (2H, t, J = 5.9 Hz, CH_2), 2.58 (2H, q, J = 14.8, 7.5 Hz, CH_2), 2.48 (2H, t, J = 7.1 Hz, CH_2), 1.79 (2H, app. p, J = 6.5 Hz, CH_2), 1.16 (3H, t, J = 7.4 Hz, CH_3), 0.92 (9H, s, CH_3), 0.08 (6H, s, CH_3) ppm.

^{13}C NMR δ : 188.81 (C), 93.87 (C), 80.82 (C), 61.16 (CH_2), 38.75 (CH_2), 30.74 (CH_2), 25.85 (3xCH_3), 18.19 (C), 15.37 (CH_2), 8.06 (CH_3), -5.43 (2xCH_3) ppm.

(*S*)-8-[(*tert*-Butyldimethylsilyl)-oxy]-4-octyn-3-ol,[34,115] **191**



9-BBN-H dimer (4.23 g, 34.7 mmol) and (*S*)-(-)-α-pinene (5.18 g, 38.1 mmol, 6.1 ml) were heated at 65°C for 6 hours. The mixture was cooled to 0°C, treated with protected ketone **190** (5.65 g, 22.0 mmol) in a dropwise manner and the resulting solution left to stir for 65 hours. Excess acetaldehyde (2 ml) was added and after stirring for 30 minutes, the solution was treated with cold ether (20 ml) followed by ethanolamine (2.48 g, 41 mmol, 2.5 ml). After stirring for 10 minutes at 0°C, a solid precipitated, which was removed by filtration through celite and the residue washed with cold diethyl ether (30 ml). The filtrate was washed with brine (3x20 ml), dried over anhydrous magnesium sulfate and the solvent evaporated to give a crude yellow oil. Careful column chromatography on silica gel (gradient elution: 2-30% ethyl acetate/petrol) gave the desired chiral alcohol (4.74 g, 84%) as a yellow oil.

TLC : R_f = 0.24 in 10% ethyl acetate/petrol.

IR (neat) ν_{max} **:** 3392 (br), 2930 (s), 2857 (m), 2243 (w), 1464 (m), 1255 (m), 1106 (br), 962 (m), 835 (s), 776 (s), 662 (m) cm^{-1}.

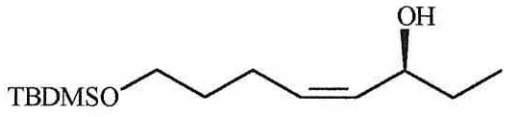
^{1}H NMR δ : 4.27 (1H, tt, *J* = 6.3, 1.9 Hz, CH), 3.71 (2H, t, *J* = 6.1 Hz, CH_2), 2.32 (2H, dt, *J* = 7.1, 2.0 Hz, CH_2), 1.67 (4H, m, CH_2), 0.94 (3H, t, *J* = 7.4 Hz, CH_3), 0.87 (9H, s, CH_3), 0.08 (6H, s, CH_3) ppm.

^{13}C NMR δ : 85.10 (C), 81.18 (C), 63.96 (CH), 61.56 (CH_2), 31.70 (CH_2), 31.17 (CH_2), 25.91 (3xCH_3), 18.31 (C), 15.07 (CH_2), 9.43 (CH_3), -5.43 (2xCH_3) ppm.

$[\alpha]_D^{25}$ = -3.6 (c = 1.0, $CHCl_3$).[a] [Lit., $[\alpha]_D$ = -6.0 (c = 0.45, $CHCl_3$)].[34]

[a] The large difference in the specific rotation value of the product on comparison to that quoted in the literature was due to the presence of a trace amount of impurity, probably derived from the borane, which could not be removed even after repeated column chromatography.

(*Z*)-(*S*)-8-[(*tert*-Butyldimethylsilyl)oxy]-4-octen-3-ol,[34] **192**



Alcohol **191** (0.8 g, 3.13 mmol) was dissolved in hexane (6 ml) and treated with quinoline (0.04 g, 0.29 mmol, 0.034 ml) followed by palladium on calcium carbonate, poisoned with lead (0.07 g, Lindlar catalyst, Aldrich 20,573-7). The resulting suspension was stirred vigorously under an atmosphere of hydrogen for 1 hour. Diethyl ether (15 ml) was added and the reaction mixture filtered through celite and the residue washed with further diethyl ether (20 ml). Purification by column chromatography on silica gel (eluent: 10% ethyl acetate/hexane) gave the desired *cis* isomer as a colourless oil (0.71 g, 88%).

TLC : $R_f = 0.21$ in 10% ethyl acetate/hexane.

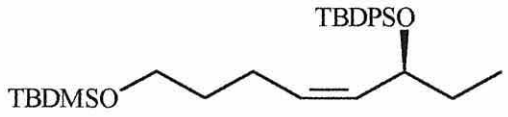
IR (neat) ν_{max} **:** 3352 (br), 3028 (w), 2963 (s), 2929 (s), 2857 (s), 1657 (w), 1463 (m), 1388 (m), 1360 (w), 1255 (s), 1102 (s), 1006 (m), 963 (m), 836 (s), 775 (s), 662 (w) cm^{-1}.

^{1}H NMR δ : 5.56-5.38 (2H, m, CH), 4.37 (1H, q, $J = 14.4$, 6.7 Hz, CH), 3.65 (2H, t, $J = 6.0$ Hz, CH_2), 2.27 (1H, m, CH), 2.11 (1H, m, CH), 1.89 (1H, br s, OH), 1.69-1.41 (4H, m, CH_2), 0.91 (9H, s, CH_3), 0.88 (3H, t, $J = 7.5$ Hz, CH_3), 0.07 (6H, s, CH_3) ppm.

^{13}C NMR δ : 133.32 (CH), 131.50 (CH), 68.60 (CH), 62.01 (CH_2), 32.35 (CH_2), 30.10 (CH_2), 25.90 (3xCH_3), 23.77 (CH_2), 18.27 (C), 9.71 (CH_3), -5.32 (2xCH_3) ppm.

$[\alpha]_D^{24}$ = +12.3 (c = 1.1, $CHCl_3$). [Lit., $[\alpha]_D$ = +12.9 (c = 1.5, $CHCl_3$)].[34]

(*Z*)-(*S*)-1-[(*tert*-Butyldimethylsilyl)oxy)]-6-[(*tert*-butyldiphenylsilyl)oxy]-4-octene,[34] **193**



To a solution of **192** (4.46 g, 17.3 mmol) in CH_2Cl_2 (25 ml) at room temperature was added imidazole (1.76 g, 26 mmol), *tert*-butyldiphenylchlorosilane (5.21 g, 19.03 mmol) and a catalytic amount of DMAP (0.06 g, 0.45 mmol). After stirring overnight, hexane (80 ml) was added and the resulting precipitate removed by filtration. The organic filtrate was dried over anhydrous magnesium sulfate and concentrated *in vacuo* to give an oil which was purified by column chromatography on silica gel (eluent: 1% diethyl ether/petrol) yielding the title compound (7.38 g, 86%) as a colourless oil.

TLC : R_f = 0.67 in 10% ethyl acetate/petrol.

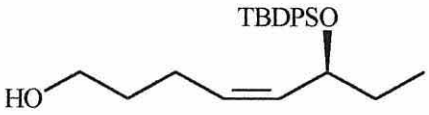
IR (neat) ν_{max} **:** 3070 (w), 3049 (w), 3012 (w), 2956 (s), 2930 (s), 2892 (m), 2857 (s), 1472 (m), 1462 (m), 1428 (m), 1389 (w), 1360 (w), 1255 (m), 1106 (s), 1056 (m), 1006 (m), 939 (w), 836 (s), 775 (m), 738 (w), 701 (s), 613 (m) cm^{-1}.

^{1}H NMR δ : 7.70-7.62 (4H, m, CH), 7.42-7.34 (6H, m, CH), 5.43 (1H, m, CH), 5.23 (1H, dt, J = 11.1, 7.1 Hz, CH), 4.38 (1H, m, CH), 3.44 (2H, dt, J = 6.6, 2.0 Hz, CH_2), 1.76-1.42 (4H, m, CH_2), 1.39-1.25 (2H, m, CH_2), 1.06 (9H, s, CH_3), 0.87 (9H, s, CH_3), 0.79 (3H, t, J = 7.5 Hz, CH_3), 0.01 (6H, s, CH_3) ppm.

^{13}C NMR δ : 135.96 (2xCH), 135.87 (2xCH), 134.69 (C), 134.51 (C), 133.01 (CH), 129.39 (CH), 129.32 (CH), 129.23 (CH), 127.41 (2xCH), 127.28 (2xCH), 70.72 (CH), 62.68 (CH_2), 32.66 (CH_2), 31.20 (CH_2), 27.00 ($3xCH_3$), 25.93 ($3xCH_3$), 24.00 (CH_2), 19.31 (C), 18.29 (C), 9.27 (CH_3), -5.30 ($2xCH_3$) ppm.

$[\alpha]_D^{24}$ = +19.8 (c = 0.9, $CHCl_3$). [Lit., $[\alpha]_D$ = +18.3 (c = 0.9, $CHCl_3$)].[34]

(*Z*)-(*S*)-6-[(*tert*-Butyldiphenylsilyl)oxy]-4-octen-1-ol,[34] **194**



A solution of **193** (1.97 g, 3.97 mmol) in absolute ethanol (15 ml) was treated with PPTS (0.32 g, 1.27 mmol) and left to stir at room temperature for 48 hours. The solvent was concentrated and the residue purified on silica gel (gradient elution: 1-5% ethyl acetate/petrol) yielding the title compound (1.31 g, 86%).

TLC : R_f = 0.05 in 4% ethyl acetate/petrol.

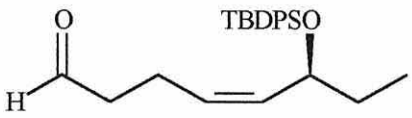
IR (neat) ν_{max} **:** 3329 (br), 3070 (m), 3048 (w), 3018 (w), 2958 (s), 2931 (s), 2857 (s), 1658 (w), 1589 (w), 1472 (m), 1465 (m), 1428 (s), 1390 (w), 1360 (w), 1111 (s), 1057 (s), 821 (s), 740 (s), 702 (s), 612 (s) cm^{-1}.

^{1}H NMR δ : 7.73-7.62 (4H, m, CH), 7.48-7.30 (6H, m, CH), 5.47 (1H, m, CH), 5.26 (1H, dt, *J* = 13.2, 6.3 Hz, CH), 4.39 (1H, m, CH), 3.44 (2H, t, *J* = 6.3 Hz, CH_2), 1.75-1.30 (6H, m, CH_2), 1.00 (9H, s, CH_3), 0.75 (3H, t, *J* = 7.5 Hz, CH_3) ppm.

^{13}C NMR δ : 136.01 (2xCH), 135.91 (2xCH), 134.55 (2xC), 133.45 (CH), 129.45 (CH), 129.36 (CH), 128.84 (CH), 127.45 (2xCH), 127.29 (2xCH), 70.68 (CH), 62.34 (CH_2), 32.35 (CH_2), 31.21 (CH_2), 26.97 (3xCH_3), 23.82 (CH_2), 19.30 (C), 9.26 (CH_3) ppm.

$[\alpha]_D^{24}$ = +18.8 (c = 1.0, $CHCl_3$). [Lit., $[\alpha]_D$ = +19.9 (c = 0.9, $CHCl_3$)].[34]

(*Z*)-(*S*)-6-[(*tert*-Butyldiphenylsilyl)oxy]-4-octen-1-al, **195**



A solution of oxalyl chloride (1.89 g, 17.90 mmol, 1.32 ml) in dry dichloromethane (70 ml) was cooled to -78°C and treated with dimethyl sulfoxide (2.10 g, 26.9 mmol, 1.9 ml) to give an effervescent mixture which was left to stir for 10 minutes at -78°C. Alcohol **194** (3.56 g, 9.30 mmol) was added and the mixture stirred at -60°C for ten minutes. The solution was again cooled to -78°C and triethylamine (5.62 g, 55.60 mmol, 7.6 ml) added. The resulting mixture was allowed to stir at room temperature for 2 hours before being extracted with hexane (2x60 ml) and the combined organic fractions washed sequentially with water (2x80 ml), a 7% aqueous solution of acetic acid (2x80 ml) and brine (2x80 ml), followed by drying over anhydrous magnesium sulfate and concentration *in vacuo* to yield the aldehyde (3.27 g, 93%) as a yellow oil which was used without further purification in the proceeding step, (see page 189).

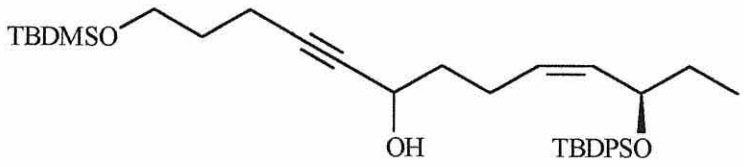
TLC : $R_f = 0.29$ in 5% diethyl ether/petrol.

IR (neat) ν_{max} **:** 3070 (m), 3063 (m), 3028 (m), 3960 (s), 2930 (s), 2908 (m), 2856 (s), 2718 (w), 1727 (s), 1589 (w), 1472 (m), 1427 (s), 1389 (m), 1360 (m), 1260 (w), 1111 (s), 1081 (s), 1053 (s), 1010 (m), 945 (w), 822 (m), 741 (m), 703 (s), 613 (m) cm^{-1}.

^{1}H NMR δ : 9.56 (1H, s, CH), 7.71-7.59 (4H, m, CH), 7.47-7.29 (6H, m, CH), 5.48 (1H, m, CH), 5.16 (1H, dt, J = 12.5, 7.0 Hz, CH), 4.38 (1H, m, CH), 2.13 (2H, m, CH_2), 1.89 (2H, m, CH_2), 1.67-1.39 (2H, m, CH_2), 1.04 (9H, s, CH_3), 0.81 (3H, t, J = 7.8 Hz, CH_3) ppm.

^{13}C NMR δ : 201.46 (CH), 135.92 (2xCH), 135.80 (2xCH), 134.28 (2xC), 134.14 (CH), 129.48 (CH), 129.39 (CH), 127.45 (2xCH), 127.28 (2xCH), 127.04 (CH), 70.47 (CH), 43.30 (CH_2), 31.06 (CH_2), 26.91 (3xCH_3), 20.16 (CH_2), 19.24 (C), 9.26 (CH_3) ppm.

(*Z*)-(11*S*)-1-[(*tert*-Butyldimethylsilyl)oxy]-11-[(*tert*-butyldiphenylsilyl)oxy]-9-tridecen-4-yn-6-ol,[34] **196**



A solution of alkyne **188** (0.6 g, 3.03 mmol) in THF (7 ml) was cooled to -78°C, treated with *n*-butyllithium (2.4 M, 3.03 mmol, 1.25 ml) and stirred for 10 minutes. The mixture was warmed to room temperature for 5 minutes and DMPU (0.6 ml) added. After cooling to -78°C, aldehyde **195** (1.05 g, 2.75 mmol) in THF (1.5 ml) was added in a dropwise manner and the resulting mixture stirred for 1 hour at this temperature and finally for another 1.5 hours, whilst warming to room temperature. The solution was diluted with hexane (50 ml) before washing with saturated aqueous ammonium chloride solution (80 ml) and brine (2x50 ml). Drying of the solvent over anhydrous magnesium sulfate, followed by concentration under reduced pressure and purification by column chromatography on silica gel (gradient elution: 0-10% ethyl acetate/petrol), afforded the title product (1.29 g, 81%) as a colourless oil.

TLC : R_f = 0.19 in 10% ethyl acetate/petrol.

IR (neat) ν_{max} **:** 3427 (br), 3070 (m), 3050 (w), 2955 (s), 2929 (s), 2890 (s), 2860 (s), 1656 (w), 1589 (w), 1472 (s), 1463 (s), 1428 (s), 1389 (m), 1360 (m), 1255 (s), 1188 (w), 1106 (br), 975 (m), 836 (s), 777 (s), 740 (s), 702 (s), 612 (m) cm^{-1}.

^{1}H NMR δ : 7.74-7.64 (4H, m, CH), 7.48-7.32 (6H, m, CH), 5.46 (1H, m, CH), 5.22 (1H, m, CH), 4.41 (1H, m, CH), 4.12 (1H, m, CH), 3.65 (2H, t, J = 6.0 Hz, CH_2), 2.26 (2H, br t, J = 7.2 Hz, CH_2), 1.83-1.59 (4H, m, CH_2), 1.58-1.36 (4H, m, CH_2), 1.06 (9H, s, CH_3), 0.91 (9H, s, CH_3), 0.81 (3H, dt, J = 7.5, 1.7 Hz, CH_3), 0.06 (6H, s, CH_3) ppm.

^{13}C NMR δ : 136.00 (2xCH), 135.91 (2xCH), 134.55 (2xC), 133.79 (0.5xCH^a), 133.59 (0.5xCH^a), 129.45 (CH), 129.38 (CH), 128.41 (0.5xCH^a), 128.32 (0.5xCH^a), 127.47 (2xCH), 127.32 (2xCH), 85.13 (0.5xC^a), 85.03 (0.5xC^a), 81.08 (C), 70.70 (CH), 62.14 (0.5xCH^a), 61.91 (0.5xCH^a), 61.57 (CH_2), 37.88 (0.5xCH_2^a), 37.68 (0.5xCH_2^a), 31.72

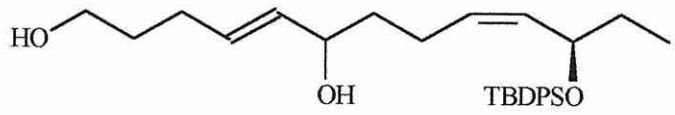
(CH_2), 31.25 (CH_2), 27.00 ($3xCH_3$), 25.94 ($3xCH_3$), 23.50 (CH_2), 19.31 (C), 18.33 (C), 15.27 ($0.5xCH_2$[a]), 15.09 ($0.5xCH_2$[a]), 9.29 (CH_3), -5.31 ($2xCH_3$) ppm.

MS (CI) *m/z* : 596 (23% $[M+NH_4]^+$), 340 (83%), 323 (55% $[M\text{-}TBDPSO]^+$), 305 (19%), 191 (100%), 132 (8% $[TBDMSOH]^+$) Daltons.

HRMS (CI) *m/z* : found: 596.3955, $C_{35}H_{58}NO_3Si_2$ ($[M+NH_4]^+$) requires: 596.3955 Daltons.

[a] Specific resonances are denoted as 0.5xC, 0.5xCH or $0.5xCH_2$ due to a mixture of *Z* and *E* isomers being present.

(4*E*, 9*Z*)-(11*S*)-[(*tert*-Butyldiphenylsilyl)oxy]-4,9-tridecadiene-1,6-diol,[34] **197**



To a stirred solution of alkyne **196** (0.86 g, 1.49 mmol) in THF (2 ml) was added $LiAlH_4$ (1 M solution in THF, 1.59 mmol, 1.59 ml) in THF (8 ml) at room temperature and the resulting mixture heated at reflux for 4 hours. The solution was cooled to 0 °C, treated with hexane (60 ml) followed by saturated NH_4Cl solution (40 ml) and saturated Na_2SO_4 solution (40 ml). The mixture was filtered to remove solid material and the organic fraction separated, washed with brine (2x60 ml), dried over anhydrous magnesium sulfate and evaporated leaving a colourless oil. This oil was purified by column chromatography on silica gel (eluent: 60% ethyl acetate/hexane) to give the title product **197** (0.39 g, 57%, [65% based on recovered starting material]), as a colourless oil.

TLC : $R_f = 0.33$ in 60% ethyl acetate/hexane.

IR (neat) ν_{max} **:** 3334 (br), 3070 (m), 3050 (m), 2932 (s), 2857 (s), 1664 (w), 1589 (w), 1473 (s), 1460 (s), 1427 (s), 1340 (m), 1360 (m), 1111 (s), 1055 (s), 970 (s), 822 (s), 739 (s), 705 (s), 613 (s) cm^{-1}.

^{1}H NMR δ : 7.77-7.67 (4H, m, CH), 7.49-7.33 (6H, m, CH), 5.65-5.36 (3H, m, CH), 5.26-5.17 (1H, m, CH), 4.41 (1H, m, CH), 3.84 (1H, m, CH), 3.62 (2H, dt, $J = 6.4$, 2.6 Hz, CH_2), 2.09 (2H, q, $J = 14.0$, 6.9 Hz, CH_2), 1.76-1.53 (6H, m, CH_2), 1.52-1.10 (2H, m, CH_2), 1.06 (9H, s, CH_3), 0.81 (3H, t, $J = 7.4$ Hz, CH_3) ppm.

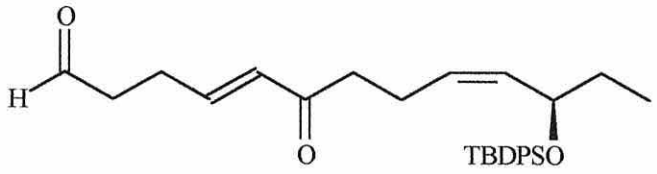
^{13}C NMR δ : 136.02 (2xCH), 135.91 (2xCH), 134.58 (2xC), 133.40 (CH), 133.33 (0.5xCH^a), 133.25 (0.5xCH^a), 131.07 (0.5xCH^a), 130.96 (0.5xCH^a), 129.46 (CH), 129.34 (CH), 128.90 (CH), 127.46 (2xCH), 127.30 (2xCH), 72.30 (0.5xCH^a), 72.14 (0.5xCH^a), 70.70 (CH), 62.21 (CH_2), 37.00 (0.5xCH_2^a), 36.84 (0.5xCH_2^a), 31.99 (CH_2), 31.23 (CH_2), 28.47 (CH_2), 26.97 (3xCH_3), 23.71 (0.5xCH_2^a), 23.62 (0.5xCH_2^a), 19.29 (C), 9.28 (CH_3) ppm.

MS (CI) *m/z* : 484 (4% $[M+NH_4]^+$), 467 (8% $[M+H]^+$), 466 (16% $[M+NH_4-H_2O]^+$), 274 (30%), 228 (32%), 211 (37% $[M-TBDPSO]^+$), 210 (100%), 175 (20%) Daltons.

HRMS (CI) *m/z* : found: 484.3247, $C_{29}H_{46}NO_3Si$ ($[M+NH_4]^+$) requires: 484.3247 Daltons.

[a] Specific resonances are denoted as 0.5xCH or 0.5xCH_2 due to a mixture of *Z* and *E* isomers being present.

(*S*)-6-Oxo-11-[(*tert*-butyldiphenylsilyl)oxy]-(4*E*,9*Z*)-tridecadienal, **22**

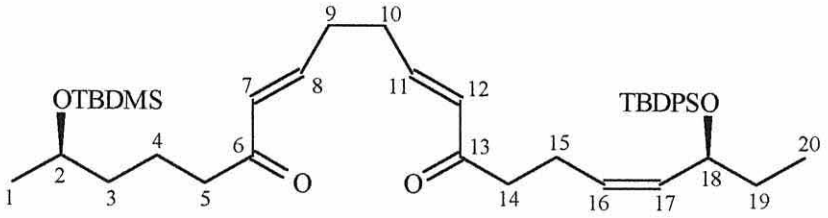


A solution of oxalyl chloride (0.19 g, 1.49 mmol, 0.13 ml) in dry dichloromethane (7 ml) was cooled to -78°C and treated with dimethyl sulfoxide (0.24 g, 3.11 mmol, 0.22 ml) to give an effervescent mixture which was left to stir for 10 minutes at -78°C. Alcohol **197** (0.19 g, 0.41 mmol) in dichloromethane (1 ml) was added and the mixture stirred at -60°C for ten minutes. The solution was again cooled to -78°C and triethylamine (0.68 g, 6.75 mmol, 0.94 ml) added. The resulting mixture was stirred at room temperature for 2 hours, before being extracted with hexane (2x15 ml) and the combined organic fractions washed sequentially with water (2x20 ml), an aqueous 5% solution of acetic acid (2x10 ml) and brine (2x20 ml). Subsequent drying of the solvent over anhydrous magnesium sulfate and concentration *in vacuo* gave, without further purification, the desired aldehyde (0.18 g, 98%) as a pale yellow oil.

TLC : R_f = 0.39 in 30% ethyl acetate/hexane.

^{1}H NMR δ : 9.82 (1H, s, CH), 7.72-7.66 (4H, m, CH), 7.48-7.30 (6H, m, CH), 6.68 (1H, dt, *J* = 15.9, 6.6 Hz, CH), 6.00 (1H, dt, *J* = 15.9, 1.6 Hz, CH), 5.44 (1H, m, CH), 5.18 (1H, m, CH), 4.38 (1H, m, CH), 2.65 (2H, m, CH_2), 2.54 (2H, dt, *J* = 6.5, 1.6 Hz, CH_2), 2.23 (2H, m, CH_2), 1.91 (2H, m, CH_2), 1.66-1.41 (2H, m, CH_2), 1.05 (9H, s, CH_3), 0.81 (3H, t, *J* = 7.4 Hz, CH_3) ppm.

(2*R*,18*S*), (7*E*,11*E*,16*Z*)-2-[(*tert*-Butyldimethylsilyl)oxy]-18-[(*tert*-butyldiphenylsilyl)oxy]-eicosa-7,11,16-triene-6,13-dione, **185**



A solution of phosphorane **186** (0.24 g, 0.47 mmol) in CH_2Cl_2 (1 ml) was added dropwise to a cooled (0°C) mixture of aldehyde **22** (0.15 g, 0.31 mmol) in CH_2Cl_2 (1 ml) and left to stir for 36 hours at room temperature. The solvent was evaporated and the remaining material purified on silica (gradient elution: 3-20% ethyl acetate/petrol) yielding the required *bis*-α,β-unsaturated ketone as a pale yellow oil (0.16 g, 73%).

TLC : R_f = 0.59 in 20% ethyl acetate/petrol.

IR (neat) ν_{max} **:** 3068 (w), 3007 (w), 2961 (s), 2930 (s), 2891 (m), 2856 (s), 1697 (s), 1675 (s), 1630 (s), 1472 (m), 1428 (m), 1361 (m), 1254 (m), 1110 (s), 1046 (m), 983 (m), 910 (w), 836 (s), 775 (m), 735 (s), 703 (s) cm^{-1}.

^{1}H NMR δ : 7.69-7.66 (4H, m, CH), 7.42-7.32 (6H, m, CH), 6.81 (1H, m, CH-11), 6.67 (1H, m, CH-8), 6.14 (1H, d, J = 15.8 Hz, CH-7), 6.01 (1H, d, J = 15.8 Hz, CH-12), 5.44 (1H, dt, J = 10.9, 1.6 Hz, CH-17), 5.18 (1H, dt, J = 10.9, 7.4 Hz, CH-16), 4.38 (1H, m, CH-18), 4.14 (1H, q, J = 14.3, 7.2 Hz, CH-2), 2.54 (2H, t, J = 7.2 Hz, CH_2-10), 2.38 (4H, m, CH_2-9, CH_2-5), 2.22 (2H, t, J = 6.4 Hz, CH_2-15), 1.92 (2H, m, CH_2-14), 1.69-1.49 (4H, m, CH_2-3, CH_2-19), 1.48-1.36 (2H, m, CH_2-4), 1.13 (3H, d, J = 6.1 Hz, CH_3-1), 1.05 (9H, s, CH_3), 0.89 (9H, s, CH_3), 0.80 (3H, t, J = 7.4 Hz, CH_3-20), 0.06 (6H, s, CH_3) ppm.

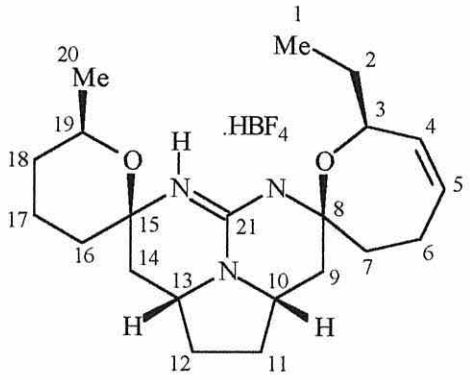
^{13}C NMR δ : 199.88 (C), 198.62 (C), 144.61 (CH), 144.48 (CH), 136.00 (2xCH), 135.85 (2xCH), 134.44 (C), 134.13 (C), 133.77 (CH), 130.84 (CH), 130.74 (CH), 129.47 (CH), 129.33 (CH), 127.72 (CH), 127.45 (2xCH), 127.27 (2xCH), 70.54 (CH), 68.36 (CH), 40.47 (CH_2), 39.75 (CH_2), 39.10 (CH_2), 31.13 (CH_2), 30.70 (2xCH_2), 26.94 (3xCH_3), 25.88 (3xCH_3), 23.71 (CH_3), 21.94 (CH_2), 20.38 (CH_2), 19.29 (C), 18.31 (C), 9.25 (CH_3), -4.39 (CH_3), -4.74 (CH_3) ppm.

MS (CI) *m/z* : 706 (75% $[M+NH_4]^+$), 438 (49%), 433 (100%), 297 (52%) Daltons.

HRMS (CI) *m/z* : found: 706.4687, $C_{42}H_{68}NO_4Si_2$ ($[M+NH_4]^+$) requires: 706.4687 Daltons.

$[\alpha]_D^{26}$ = -9.4 (c = 0.9, $CHCl_3$).

(2*S*,7*S*,3a'*S*,7'*R*,8a'*R*,6"*R*)-Dispiro[7-ethyl-5*Z*-oxepene-2,4'-(1',2',3',4',7',8'-hexahydro-5*H*-5',6',8b'-triazaacenaphthylene)-6"-methyl-7',2"-tetrahydropyran]-6'-ium Tetrafluoroborate, **184**[a]



[a] The pentacycle has been named systematically according to the numbering system outlined on page iv, but for NMR purposes has been numbered here using the generic system described in Chapter 5, page 104.

Guanidine (0.028 g, 0.48 mmol) was dissolved in DMF (1 ml) at 0°C and left to stir for 30 minutes before adding to a solution of the protected *bis*-α,β-unsaturated ketone **185** (0.30 g, 0.44 mmol) in DMF (3 ml), also at 0°C. The resulting green mixture was left to stir at 0°C for 6 hours eventually turning black. The reaction mixture was quenched with water (3.5 ml), treated with a solution of methanolic HCl (10 ml, [acetyl chloride (0.5 ml) in methanol (9.5 ml)]) at 0°C and stirred for 16 hours whilst warming to ambient temperature.

Water (40 ml) was added, the mixture extracted with CH_2Cl_2 (4x40 ml) and the combined organic fractions washed sequentially with water (2x50 ml), saturated lithium bromide solution (50 ml) and water (60 ml). After drying the solvent layer over anhydrous magnesium sulfate and concentration *in vacuo*, the resulting dense brown oil was dissolved in THF (1 ml) and treated with tetrabutylammonium fluoride (1 M in THF, 2.2 mmol, 2.2 ml). This mixture was then stirred at 25-30°C for three hours and at ambient temperature for a further 64 hours, after which the solution was diluted with water (10 ml), transferred to a separating funnel and extracted with CH_2Cl_2 (3x20 ml). The combined organic fractions were washed with water (25 ml), dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure.

The remaining brown oil was dissolved in methanol (1 ml) before being treated, at 0°C with a solution of methanolic HCl (8 ml, [acetyl chloride (0.4 ml) in methanol (7.6 ml)]). After stirring for 4 hours the mixture was again quenched with water (10 ml), extracted with CH_2Cl_2 (6x20 ml) and the combined organic fractions washed with water (50 ml). Concentration of the solvent gave a light brown oil which was dissolved in CH_2Cl_2 (2 ml) and stirred vigorously for 3 hours in the presence of an aqueous solution of saturated sodium tetrafluoroborate (3 ml). The organic layer was separated, dried over anhydrous magnesium sulfate and concentrated *in vacuo* giving a brown oil which was purified by column chromatography on silica gel (gradient elution: 0-5% MeOH/CH_2Cl_2) yielding the title product (0.036 g, 18%) as a semi-solid.

TLC : R_f = 0.10 in 3% methanol/dichloromethane.

IR ($CDCl_3$) ν_{max} **:** 3224 (w), 2975 (m), 2933 (s), 2868 (w), 1656 (s), 1604 (s), 1445 (w), 1342 (w), 1236 (w), 1066 (s), 1023 (s) cm^{-1}.

^{1}H NMR ($CDCl_3$, 500 MHZ) **δ :** 7.89 (1H, br s, NH), 7.83 (1H, br s, NH), 5.66 (1H, ddt, *J* = 11.4, 7.7, 2.4 Hz, CH-5), 5.49 (1H, dt, *J* = 11.0, 2.8, CH-4), 4.47 (1H, br d, *J* = 9.8 Hz, CH-3), 4.06 (2H, m, CH-10, CH-13), 3.81 (1H, ddq, *J* = 12.6, 6.5, 2.8 Hz, CH-19), 2.59 (1H, dd, *J* = 12.7, 5.7 Hz, CH-9β), 2.55 (1H, br t, *J* = 13.7 Hz, CH-7α), 2.32 (2H, CH-6β, CH-12β) 2.28 (1H, m, CH-14β), 2.19 (1H, dd, *J* = 14.0, 5.7 Hz, CH-11β), 2.16 (1H, m, CH-6α), 2.06 (1H, m, CH-17α), 1.87 (1H, dd, *J* = 14.3, 5.7 Hz, CH-7β), 1.72 (3H, m, CH-16α, CH-16β, CH-17β), 1.70 (1H, m, CH-12α), 1.69 (1H, m, CH-11α), 1.62 (1H, m, CH-18α), 1.52 (1H, m, CH-2α), 1.49 (1H, t, *J* = 12.7 Hz, CH-14α), 1.42 (1H, m, CH-2β), 1.36 (1H, t, *J* = 12.4 Hz, CH-9α), 1.20 (1H, m, CH-18β), 1.06 (3H, *J* = 6.4 Hz, CH_3-20), 0.83 (3H, t, *J* = 7.2 Hz, CH_3-1) ppm.

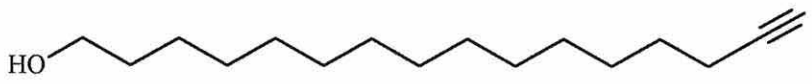
^{13}C NMR δ : 147.68 (C-21), 133.61 (CH-4), 129.77 (CH-5), 84.06 (C-8), 80.46 (C-15), 70.94 (C-3), 67.17 (C-19), 53.50 (CH-10), 51.97 (CH-13), 39.80 (CH_2-14), 37.08 (CH_2-9), 36.46 (CH_2-7), 33.56 (CH_2-18), 32.19 (CH_2-16), 30.04 (CH_2-11), 29.84 (CH_2-12), 29.21 (CH_2-2), 23.72 (CH_2-6), 21.65 (CH_3-20), 17.91 (CH_2-17), 10.21 (CH_3-1) ppm.

MS (CI) *m/z* : 360 (5% $[M+H]^+$), 45 (54%) Daltons.

HRMS (EI) *m/z* : found: 359.2566, $C_{21}H_{33}N_3O_2$ ($[M]^+$) requires: 359.2573 Daltons.

$[\alpha]_D^{22}$ = -47.7 (c = 0.2, $CHCl_3$).

15-Hexadecyn-1-ol, **223**



A 100 ml, three-necked, round-bottomed flask was equipped with a thermometer, a condenser fitted with a drying tube containing potassium hydroxide pellets and a rubber septum inlet. Lithium (0.55 g, 78 mmol) was added to the flask previously charged with 1,3-diaminopropane (32.9 g, 444 mmol, 37 ml) and the resulting mixture stirred for 30 minutes before heating to 70°C with continuous stirring until the blue colouration discharged (*ca* 3 hours). The solution was cooled to room temperature and potassium *tert*-butoxide (5.82 g, 52 mmol) added giving an orange mixture which was stirred for 20 minutes after which 7-hexadecyn-1-ol (3.09 g, 13 mmol) in dry diethyl ether (5 ml) was slowly added. After stirring for a further 2 hours the mixture was cooled to 0°C and poured onto ice/water (*ca* 40 g) and extracted with diethyl ether (5x50 ml). The combined organic fractions were then washed with 10% HCl (100 ml) and brine (100 ml), followed by drying over anhydrous magnesium sulfate and concentration *in vacuo* to give a crude solid which was purified on silica gel (eluent: 20% diethyl ether/petrol) yielding the desired product (2.23 g, 72%, [97% purity]), as a white, flaky solid.

TLC : R_f = 0.23 in 30% diethyl ether/petrol.

Melting point = 50-51°C. [Lit. = 49-50.5°C].[144]

IR ($CHCl_3$) ν_{max} **:** 3307 (br), 3287 (m), 2928 (s), 2854 (s), 2114 (w), 1462 (m), 1352 (w), 1122 (w), 1053 (m), 1026 (w), 922 (w), 629 (s) cm^{-1}.

^{1}H NMR δ : 3.63 (2H, t, J = 6.5 Hz, CH_2O), 2.18 (2H, dt, J = 6.9, 2.6 Hz, CH_2), 1.94 (1H, t, J = 2.6 Hz, CH), 1.71-1.26 (24H, m, CH_2) ppm.

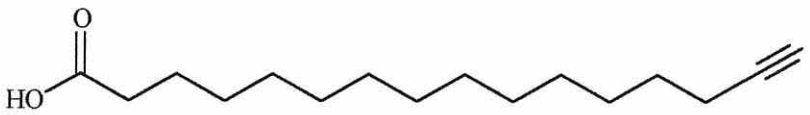
^{13}C NMR δ : 84.80 (C), 68.02 (CH), 63.02 (CH_2), 32.77, 29.60, 29.50, 29.43, 29.10, 28.89, 28.75, 28.48, 25.73, 18.38 (13xCH_2) ppm.

MS (CI) *m/z* : 239 (9%$[M+H]^+$), 238 ($[M]^+$), 221 (7%$[M-OH]^+$), 109 (57%), 95 (96%), 81 (100%), 67 (91%), 55 (88%), 41 (50%) Daltons.

HRMS (CI) *m/z* : found: 239.2375, $C_{16}H_{31}O$ ($[M+H]^+$) requires: 239.2375 Daltons.

Microanalysis : found: C, 80.9%, H, 12.9%, $C_{16}H_{30}O$ requires: C, 80.6%, H, 12.7%.

15-Hexadecyn-1-oic Acid,[144] **221**



Hexadec-15-yn-1-ol (2.0 g, 8.4 mmol) was dissolved in DMF (35 ml) and pyridinium dichromate (11.0 g, 29.4 mmol) was added and the mixture stirred for 16 hours. The resulting solution was poured into cold water (350 ml) and extracted with diethyl ether (4x100 ml). The combined organic fractions were washed with water (100 ml), dried over anhydrous magnesium sulfate and the solvent evaporated to give 2.29 g of a brown solid. The crude material was dissolved in diethyl ether (20 ml), washed with 1 M HCl (2x20 ml) followed by brine (20 ml). The aqueous layer was back extracted with diethyl ether (30 ml) and the combined organic fractions dried over anhydrous magnesium sulfate and the solvent concentrated *in vacuo* affording the title product **221** (1.83 g, 87%) as a light brown coloured solid.

Melting point = 52-54°C. [Lit. = 51.4-53°C].[144]

IR ($CHCl_3$) ν_{max} **:** 3281 (m), 3115 (br), 2919 (s), 2851 (s), 2112 (w), 1705 (s), 1471 (m), 1410 (w), 1314 (m), 1270 (m), 1247 (m), 1060 (m), 918 (br), 637 (m) cm^{-1}.

^{1}H NMR δ : 2.36 (2H, t, J = 7.4 Hz, CH_2), 2.19 (2H, dt, J = 7.0, 2.5 Hz, CH_2), 1.95 (1H, br s, CH), 1.69-1.27 (22H, m, CH_2) ppm.

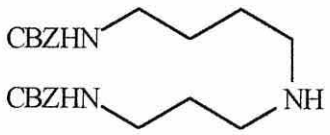
^{13}C NMR δ : 180.34 (C), 84.74 (C), 68.03 (CH), 34.07 (CH_2), 29.55, 29.45, 29.40, 29.21, 29.08, 29.04, 28.74, 28.47, 24.64, 18.36 (12xCH_2) ppm.

MS (CI) *m/z* : 270 (100% $[M+NH_4]^+$), 252 (3% $[M]^+$), 95 (7%), 80 (11%), 52 (15%) Daltons.

HRMS (CI) *m/z* : found: 270.2433, $C_{16}H_{32}NO_2$ ($[M+NH_4]^+$) requires: 270.2433 Daltons.

Microanalysis : found: C, 76.5%, H, 11.4%, $C_{16}H_{28}O_2$ requires: C, 76.2%, H, 11.2%.

[4-(3-Benzyloxycarbonylamino-propylamino)butyl]carbamic Acid Benzyl Ester, **225**



N-(benzyloxycarbomoyloxy) succinimide (6.87 g, 27.6 mmol) in CH_2Cl_2 (20 ml) was added to a stirred solution of spermidine (2.0 g, 13.8 mmol) in CH_2Cl_2 (60 ml) and the resulting mixture stirred for 20 hours. The mixture was quenched with water (20 ml) and stirred for 5 minutes, before being washed with saturated sodium hydrogen carbonate solution (50 ml) and water (50 ml). After drying over anhydrous magnesium sulfate and evaporation of the solvent, the resultant crude material was purified by flash column chromatography on silica gel (eluent: 50% EtOAc/hexane followed by 10% MeOH/CH_2Cl_2) to give the title product (1.93 g, 40%) as a white solid.

TLC : R_f = 0.16 in 10% methanol/dichloromethane.

Melting point = 104-106°C. [Lit. = 103-105°C].[36]

IR (Nujol®) ν_{max} **:** 3326 (s), 3028 (w), 2853 (s), 1686 (s), 1530 (s), 1331 (m), 1265 (s), 1233 (m), 1138 (m), 1035 (m), 1000 (m), 751 (m), 696 (m), 629 (w) cm^{-1}.

^{1}H NMR δ : 7.42-7.32 (10H, m, CH), 5.51 (1H, br s, NH), 5.18 (1H, br s, NH), 5.08 (4H, s, 2xCH_2O), 3.32-3.24 (2H, m, $CH_2N(H)CO$), 3.20-3.12 (2H, m, $CH_2N(H)CO$), 2.66 (2H, t, J = 7.0 Hz, CH_2N), 2.58 (2H, t, J = 6.5 Hz, CH_2N), 1.60-1.48 (7H, m, 3xCH_2, NH) ppm.

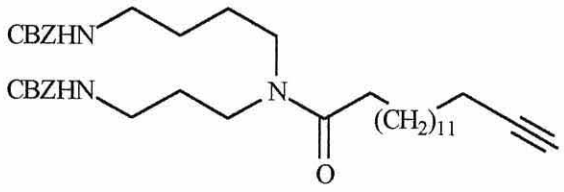
^{13}C NMR δ : 156.51 (C), 156.34 (C), 136.71 (2xC), 128.44 (6xCH), 128.03 (4xCH), 66.50 (2xCH_2), 49.24 (CH_2), 47.64 (CH_2), 40.87 (CH_2), 39.81 (CH_2), 29.44 (CH_2), 27.73 (CH_2), 27.17 (CH_2) ppm.

MS (CI) *m/z* : 414 (8% $[M+H]^+$), 280 (15%), 198 (21%), 172 (60%), 115 (34%), 106 (41%), 101 (51%), 70 (100%), 44 (33%) Daltons.

HRMS (CI) *m/z* : found: 414.2393, $C_{23}H_{32}N_3O_4$ ($[M+H]^+$) requires: 414.2393 Daltons.

Microanalysis : found: C, 67.0%, H, 7.8%, N, 10.4%, $C_{23}H_{31}N_3O_4$ requires: C, 66.8%, H, 7.6%, N, 10.2%.

{4-[(3-Benzyloxycarbonylaminopropyl)-hexadec-15-ynoyl-amino]butyl}carbamic Acid Benzyl Ester, **226**



Hexadec-15-ynoic acid (0.97 g, 3.96 mmol) was dissolved in CH_2Cl_2 (15 ml) with stirring and to this solution were sequentially added, DCC (0.88 g, 4.25 mmol), HOBT (0.57 g, 4.25 mmol) and protected amine **225** (1.76 g, 4.25 mmol). After stirring for 24 hours at room temperature the solution was cooled to 0°C for 1 hour and the resulting precipitate removed by filtration and washed with ethyl acetate (25 ml). This procedure was repeated twice and the combined washes were dried over anhydrous magnesium sulfate and the solvent concentrated *in vacuo* to yield a crude material which was purified on silica gel (gradient elution: 45-70% ethyl acetate/petrol) yielding the desired product (2.15 g, 86%) as a dense yellow oil.

TLC : R_f = 0.30 in 50% ethyl acetate/petrol.

IR ($CHCl_3$) ν_{max} **:** 3306 (w), 2929 (s), 2854 (m), 1711 (s), 1620 (m), 1514 (m), 1453 (w) cm^{-1}.

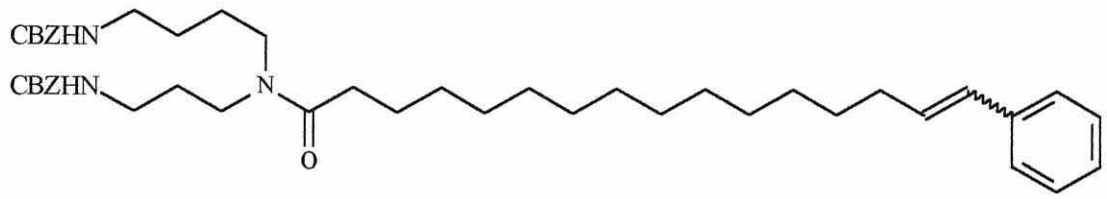
^{1}H NMR (DMSO-d_6 at 363K) **δ :** 7.39-7.28 (10H, m, CH), 6.82 (2H, br s, 2xNH), 5.04 (4H, s, CH_2O), 3.29-3.20 (4H, m, $CH_2N(H)CO$), 3.09-2.99 (4H, m, CH_2N), 2.23 (2H, t, J = 7.1 Hz, CH_2CO), 2.15 (2H, dt, J = 7.0, 2.7 Hz, $CH_2C{\equiv}C$), 1.69-1.63 (1H, m, CH), 1.51-1.39 (8H, m, CH_2), 1.27 (20H, s, CH_2) ppm.

^{13}C NMR δ : 173.65 (C), 156.63 (2xC), 136.82 (C), 136.60 (C), 128.46, 128.38, 127.98, 127.91 (10xCH), 84.73 (C), 68.22 (CH), 66.54 (CH_2), 66.31 (CH_2), 47.34, 42.21, 40.35, 37.71, 33.04, 29.58, 29.49, 29.08, 28.72, 28.46, 27.78, 27.39, 26.01, 25.56, 18.36 (20xCH_2) ppm.

MS (CI) *m/z* : 649 (4% $[M+2H]^+$), 648 (9% $[M+H]^+$), 540 (20%), 432 (22%), 126 (100%), 108 (81% $[C_6H_5CH_2OH]^+$) Daltons.

HRMS (CI) *m/z* : found: 648.4380, $C_{39}H_{58}N_3O_5$ ($[M+H]^+$) requires: 648.4377 Daltons.

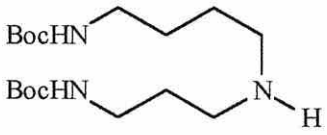
Attempted Synthesis of (*E*/*Z*){4-[(3-Benzyloxycarbonylaminopropyl)-(16-phenylhexadec-15-enoyl)amino]butyl}carbamic Acid Benzyl Ester, **229**



A solution of amide **226** (0.20 g, 0.31 mmol) in benzene (1 ml) was treated with $Pd(PPh_3)_4$ (0.006 g, 0.002 mmol) and catecholborane (1 M in THF, 0.31 mmol, 0.31 ml). After stirring for 22 hours, bromobenzene (0.05 g, 0.31 mmol, 0.03 ml) and an aqueous NaOH solution (3 M, 0.31 ml) were added. The resulting mixture was heated at reflux for two hours, then stirred at room temperature for a further 18 hours.

The reaction mixture was quenched with water (20 ml), extracted with ethyl acetate (3x20 ml) and the combined organic fractions washed with 10% aqueous Na_2CO_3 solution (2x20 ml) followed by water (2x20 ml). Drying of the combined organic fractions over anhydrous magnesium sulfate and removal of the solvent *in vacuo*, gave a brown oil. An attempted purification on silica gel (gradient elution: 10-50% ethyl acetate/petrol) failed to yield the desired product.

N^1,N^8-*bis*-Boc-Spermidine, **41**



Spermidine (1.0 g, 6.7 mmol) was dissolved in dichloromethane (15 ml) with stirring and 2-(*tert*-butoxycarbonyloximino)-2-phenylacetonitrile (3.3 g, 13.4 mmol) in CH_2Cl_2 (15 ml) was added slowly, in a dropwise manner. After stirring for 16 hours, the solvent was removed *in vacuo* and the crude mixture purified on silica gel (eluent: 50% ethyl acetate/petrol followed by 10% MeOH/CH_2Cl_2) to give 1.28 g of a solid which was recrystallised from 10% ethyl acetate/hexane to afford the title product (1.08 g, 47%) as a fine white solid.

TLC : R_f = 0.20 in 10% methanol/dichloromethane.

Melting point = 84-86°C.

IR ($CHCl_3$) ν_{max} **:** 3452 (m), 2978 (m), 2934 (w), 1708 (s), 1511 (s), 1502 (m), 1367 (m), 1249 (m), 1170 (m) cm^{-1}.

^{1}H NMR δ : 5.18 (1H, br s, NH), 4.85 (1H, br s, NH), 3.19 (4H, m, CH_2), 2.54 (4H, m, CH_2), 1.78-1.49 (7H, m, 3xCH_2, NH), 1.45 (18H, s, CH_3) ppm.

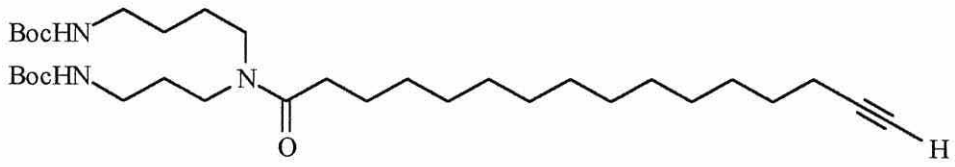
^{13}C NMR δ : 156.15 (C), 156.03 (C), 78.98 (2xC), 49.34 (CH_2), 47.58 (CH_2), 40.38 (CH_2), 39.11 (CH_2), 29.73 (CH_2), 28.42 (6xCH_3), 27.78 (CH_2), 27.18 (CH_2) ppm.

MS (CI) *m/z* : 346 (100% $[M+H]^+$), 272 (34% $[M-O$*t*$-Bu]^+$), 216 (10%), 198 (27%), 172 (12%) Daltons.

HRMS (CI) *m/z* : found: 346.2706, $C_{17}H_{36}N_3O_4$ ($[M+H]^+$) requires: 346.2706 Daltons.

Microanalysis : found: C, 58.8%, H, 10.5%, N, 12.3%, $C_{17}H_{35}N_3O_4$ requires: C, 59.1%, H, 10.2%, N, 12.2%.

{4-[(3-*tert*-Butoxycarbonylaminopropyl)-hexadec-15-ynoyl-amino]butyl}-carbamic Acid *tert*-Butyl Ester, **233**



Hexadec-15-ynoic acid (0.10 g, 0.40 mmol) was dissolved in CH_2Cl_2 (2 ml) with stirring and to this solution were sequentially added, DCC (0.09 g, 0.44 mmol), HOBT (0.06 g, 0.44 mmol) and *bis*-protected spermidine **41** (0.15 g, 0.44 mmol). After stirring for 24 hours at room temperature the solution was cooled to 0°C for 1 hour and the resultant precipitate removed by filtration and washed with EtOAc (20 ml). The filtrate was washed with 0.5 M HCl (2x20 ml) followed by saturated sodium chloride solution (2x20 ml), dried over anhydrous magnesium sulfate and the solvent concentrated *in vacuo* to yield a crude oil which was purified on silica gel (gradient elution: 10-50% ethyl acetate/petrol) giving the title product (0.18 g, 78%) as a yellow oil.

TLC : $R_f = 0.40$ in 50% ethyl acetate/petrol.

IR ($CHCl_3$) ν_{max} **:** 3473 (w), 3318 (w), 3980 (w), 2929 (s), 2855 (m), 1707 (s), 1620 (m), 1547 (w), 1511 (s), 1463 (w), 1444 (w), 1367 (s), 1168 (s), 669 (m) cm^{-1}.

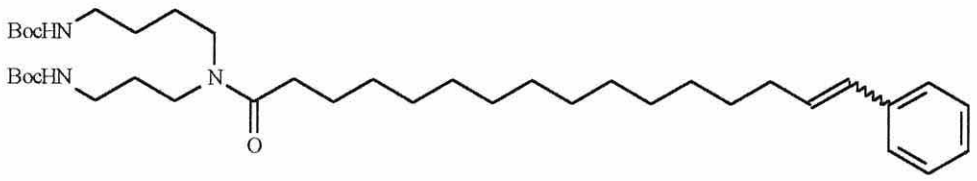
^{1}H NMR δ : 5.43 (1H, br s, NH), 4.63 (1H, br s, NH), 3.40 (2H, t, J = 6.5 Hz, CH_2), 3.32-3.09 (4H, m, CH_2), 3.05 (2H, q, J = 12.3, 6.4 Hz, CH_2), 2.29 (2H, t, J = 7.2 Hz, CH_2), 2.19 (2H, dt, J = 6.9, 2.6 Hz, CH_2), 1.95 (1H, t, J = 2.6 Hz, CH), 1.78-1.49 (10H, m, CH_2), 1.45 (9H, s, CH_3), 1.43 (9H, s, CH_3), 1.27 (18H, br s, CH_2) ppm.

^{13}C NMR δ : 173.64 (C), 156.42 (C), 156.05 (C), 83.77 (C), 79.44 (C), 79.38 (C), 68.01 (CH), 49.26 (CH_2), 47.39 (CH_2), 42.47 (CH_2), 40.00 (CH_2), 37.4, 34.14, 33.14, 29.60, 29.51, 29.10, 28.75, 28.45, 28.03, 27.65, 26.15, 25.60, 24.69, 20.29, 18.38 (16xCH_2), 28.39 (6xCH_3) ppm.

MS (CI) *m/z* : 580 (100% $[M+H]^+$), 566 (7%), 524 (9%), 480 (6%), 170 (8%), 70 (64%) Daltons.

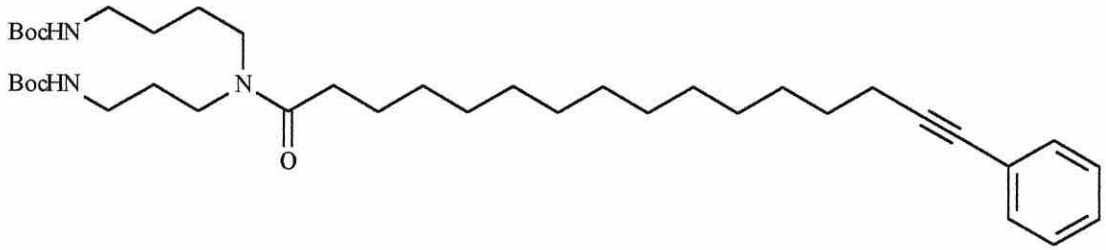
HRMS (CI) *m/z* : found: 580.4690, $C_{33}H_{62}N_3O_5$ ($[M+H]^+$) requires: 580.4689 Daltons.

Attempted Synthesis of (*E*/*Z*)-{4-[(3-*tert*-Butoxycarbonylaminopropyl)-(16-phenylhexadec-15-enoyl)amino]butyl}-carbamic Acid *tert*-Butyl Ester, **235**



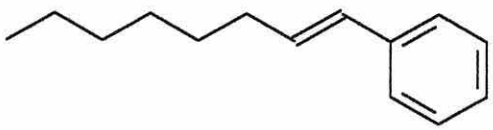
Amide **233** (0.08 g, 0.13 mmol) in benzene (0.5 ml) was treated with $Pd(PPh_3)_4$ (5 mg) and catecholborane (1 M in THF, 0.13 mmol, 0.13 ml) and left to stir overnight. Further $Pd(PPh_3)_4$ (5 mg) was added to the reaction mixture along with aqueous Na_2CO_3 solution (3 M, 0.13 ml), iodobenzene (0.08 g, 0.39 mmol, 0.04 ml) and the mixture then heated at reflux for 3 hours. The resulting crude material was extracted with ethyl acetate (3x20 ml) and the combined organic fractions washed with water (2x20 ml). Drying of the solvent over anhydrous magnesium sulfate followed by concentration *in vacuo* gave a crude solid. An attempted purification on silica gel (gradient elution: 10-50% ethyl acetate/petrol) failed to yield the desired product.

Attempted Synthesis of {4-[(3-*tert*-Butoxycarbonylaminopropyl)-(16-phenylhexadec-15-ynoyl)amino]butyl}-carbamic Acid *tert*-Butyl Ester, **236**



A 10 ml flask was charged sequentially with bromobenzene (0.11 g, 0.69 mmol, 0.07 ml), triethylamine (2 ml), toluene (1 ml), triphenylphosphine (0.02 g, 0.07 mmol), palladium(II) acetate (0.01 g, 0.04 mmol), a solution of amide **233** (0.20 g, 0.35 mmol) in toluene (1 ml) and heated at reflux for 5 hours. After cooling to room temperature, the reaction mixture was extracted with dichloromethane (3x20 ml), the combined organic fractions washed with water, dried over anhydrous magnesium sulfate and the solvent evaporated yielding a black oil. Attempted purification by column chromatography on silica gel (gradient elution: 10-50% ethyl acetate/petrol) gave three fractions, none of which, after ^{1}H NMR analysis, were consistent with the desired product.

(*E*)-1-Phenyloct-1-ene, **239**



Catecholborane (1 M in THF, 4.55 mmol, 4.55 ml) was added to 1-octyne (0.5 g, 4.55 mmol) and heated at reflux for 2 hours. After cooling, the solvent and unreacted 1-octyne (bp = 127-128°C) were removed by distillation at atmospheric pressure and the resulting black oil dissolved in benzene (14 ml) and treated with $Pd(PPh_3)_4$ (10 mg) and iodobenzene (0.93 g, 4.56 mmol, 0.54 ml). After stirring for 30 minutes, NaOEt (2 M in ethanol, 4.5 ml) was added and the mixture heated again at reflux for 2 hours. An aqueous solution of NaOH (3 M, 4.5 ml) was added at room temperature and the mixture stirred for a further 16 hours.

An attempted extraction of the reaction mixture with 1:1 benzene/hexane (30 ml) proved difficult leaving an emulsion which only partially separated over a period of 2 hours. Approximately 50% of the organic phase was separated which was then subsequently dried over anhydrous magnesium sulfate and concentrated *in vacuo* yielding an orange oil. Purification on silica gel (eluent: petrol) afforded the title product **239** as a colourless oil (0.23 g, 28%[a]).

[a] Low yield possibly due to incomplete separation of phases.

TLC : R_f = 0.61 in petrol.

IR (neat) ν_{max} **:** 3024 (m), 2955 (s), 2925 (s), 2854 (s), 1598 (w), 1493 (m), 1466 (m), 1377 (w), 1100 (w), 1039 (w), 962 (s), 741 (s), 692 (s) cm^{-1}.

^{1}H NMR δ : 7.45-7.13 (5H, m, CH), 6.40 (1H, d, J = 17.0 Hz, CH), 6.26 (1H, dt, J = 16.9, 7.8 Hz, CH), 2.22 (2H, q, J = 15.6, 7.8 Hz, CH_2), 1.54-1.19 (8H, br m, CH_2), 0.92 (3H, t, J = 7.0 Hz, CH_3) ppm.

^{13}C NMR δ : 137.86 (C), 131.33 (CH), 129.58 (CH), 128.35 (2xCH), 126.62 (CH), 125.80 (2xCH), 32.96 (CH_2), 31.67 (CH_2), 29.26 (CH_2), 28.82 (CH_2), 22.54 (CH_2), 14.01 (CH_3) ppm.

1-[(*tert*-Butyldimethylsilyl)oxy]-hexadec-15-yne, **240**

A solution of hexadec-15-yn-1-ol **223** (0.70 g, 2.94 mmol) in DMF (7 ml) was treated with imidazole (0.40 g, 5.88 mmol) followed by *tert*-butyldimethylsilyl chloride (0.44 g, 2.94 mmol) and the resulting mixture stirred for 2.5 hours. The reaction was diluted with hexane (30 ml), extracted with diethyl ether (2x30 ml) and the combined organic fractions washed with water (2x40 ml). Drying of the solvent over anhydrous magnesium sulfate followed by concentration *in vacuo* gave a yellow oil which was purified by column chromatography on silica gel (eluent: 10% diethyl ether/petrol) yielding the required protected alkyne (1.02 g, 98%) as a colourless oil.

TLC : $R_f = 0.80$ in 10% diethyl ether/petrol.

IR (neat) ν_{max} **:** 3313 (m), 2926 (s), 2654 (s), 2119 (w), 1463 (m), 1254 (m), 1100 (s), 836 (s), 775 (s), 629 (m) cm^{-1}.

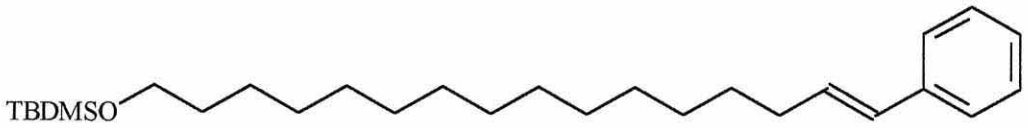
^{1}H NMR δ : 3.61 (2H, t, $J = 6.5$ Hz, CH_2), 2.19 (2H, dt, $J = 6.9$, 2.6 Hz, CH_2), 1.95 (1H, t, $J = 2.6$ Hz, CH), 1.62-1.18 (24H, m, CH_2), 0.91 (9H, s, CH_3), 0.06 (6H, s, CH_3) ppm.

^{13}C NMR δ : 84.88 (C), 68.01 (CH), 63.33 (CH_2), 32.89, 29.51, 28.77, 28.50 (11xCH_2), 25.98 (3xCH_3), 25.80 (CH_2), 20.02 (C), 18.39 (CH_2), -5.27 (2xCH_3) ppm.

MS (CI) *m/z* : 370 (28% $[M+NH_4]^+$), 353 (39% $[M+H]^+$), 132 (100% $[TBDMSOH]^+$), 91 (41%) Daltons.

HRMS (CI) *m/z* : found: 353.3243, $C_{22}H_{45}OSi$ ($[M+H]^+$) requires: 353.3240 Daltons.

(*E*)-1-[(*tert*-Butyldimethylsilyl)oxy]-16-phenylhexadec-15-ene, **241**



A solution of 1-[(*tert*-butyldimethylsilyl)oxy]-hexadec-15-yne (0.33 g, 0.94 mmol) in catecholborane (1.0 M solution THF, 0.94 mmol, 0.94 ml) was heated at reflux under a positive pressure of nitrogen for 2 hours. The resulting mixture was left to stir for a further 16 hours at room temperature after which the solvent was evaporated leaving a black oil. This oil was dissolved in benzene (4 ml) and treated with $Pd(PPh_3)_4$ (10 mg) followed by iodobenzene (0.58 g, 2.85 mmol, 0.32 ml) and the solution stirred for 30 minutes. Sodium ethoxide (2 M in ethanol, 0.94 ml) was added and the reaction heated at reflux for 2 hours. After cooling, aqueous sodium hydroxide solution (3 M, 0.94 ml) was added and the mixture left to stir for a further 20 hours. The resulting mixture was stirred over celite (5 g) for 10 minutes before being filtered through a pad of celite. Drying of the solvent over anhydrous magnesium sulfate, followed by evaporation and purification on silica gel (eluent: 1% ethyl acetate/petrol) gave the title compound (0.14 g, 35%) as a colourless oil.

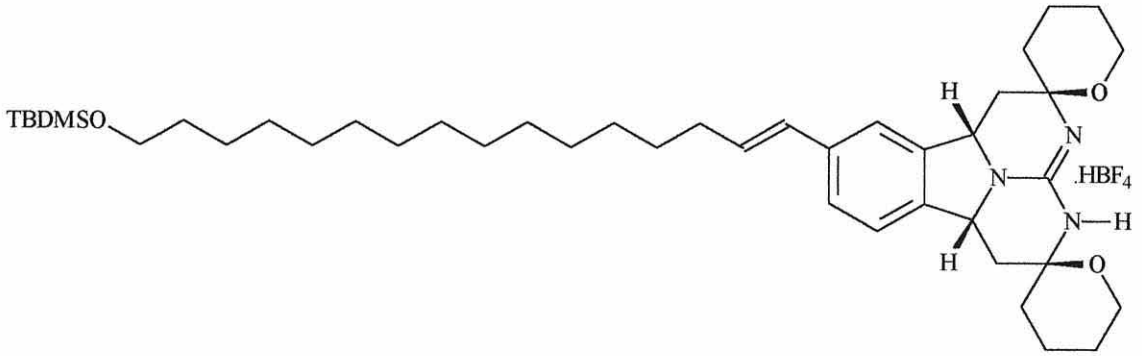
TLC : $R_f = 0.48$ in 2% ethyl acetate/petrol.

IR (neat) ν_{max} **:** 3028 (w), 2926 (s), 2853 (s), 1601 (w), 1462 (m), 1388 (s), 1360 (w), 1254 (m), 1100 (m), 836 (s), 775 (m), 699 (w) cm^{-1}.

^{1}H NMR δ : 7.34-7.09 (5H, m, CH), 6.34 (1H, d, $J = 16.3$ Hz, CH), 6.18 (1H, dt, $J = 16.1$, 5.5 Hz, CH), 3.56 (2H, t, $J = 7.8$ Hz, CH_2), 2.15 (2H, q, $J = 16.4$, 5.6 Hz, CH_2), 1.50-1.34 (4H, m, CH_2), 1.22 (20H, br s, CH_2), 0.87 (9H, s, CH_3), 0.01 (6H, s, CH_3) ppm.

^{13}C NMR δ : 137.95 (C), 131.25 (CH), 129.63 (CH), 128.42 (2xCH), 126.69 (CH), 125.87 (CH), 125.48 (CH), 63.34 (CH_2), 32.88, 32.34, 29.63, 29.44, 29.10, 28.49 (11xCH_2), 25.98 (3xCH_3), 25.79 (CH_2), 18.38 (CH_2), 18.13 (C), -5.26 (2xCH_3) ppm.

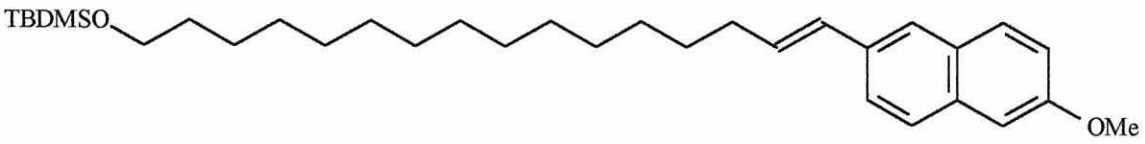
Attempted Synthesis of (2'*R*,3a"*S*,7"*S*,8a"*R*)- / (2'*S*,3a"*R*,7"*R*,8a"*S*), (1*E*)-1,2-Benzo-4-{16-[(*tert*-butyldimethylsilyl)oxy]-hexadec-1-enyl}-dispiro[tetrahydropyran-2',4"-(1",2",3",4",7",8"-hexahydro-5"*H*-5",6",8b"-triazaacenaphthylene)-7",2"-tetrahydropyran]-6"-ium Tetrafluoroborate, **244**



Catecholborane (1 M in THF, 0.42 mmol, 0.42 ml) was added to alkyne **240** (0.15 g, 0.42 mmol) at 0°C, the mixture heated at reflux for 2 hours and then stirred at room temperature for a further 16 hours. The solvent was removed under reduced pressure and the resulting black oil dissolved in benzene (2 ml) and treated with $Pd(PPh_3)_4$ (10 mg) followed by hexacycle **215** (0.07 g, 0.14 mmol). After stirring for 30 minutes NaOEt (2 M in ethanol, 4.5 ml) was added and the mixture heated at reflux for 2 hours. An aqueous solution of NaOH (3 M, 4.5 ml) was added at room temperature and the mixture stirred for another 16 hours.

Extraction of the reaction mixture with dichloromethane (2x20 ml) followed by drying of the solvent over anhydrous magnesium sulfate and evaporation gave a black oil. An attempted purification by column chromatography on silica gel (gradient elution: 0-10% chloroform/methanol) gave four fractions, none of which after ^1H NMR analysis were consistent with the desired product.

(15*E*)-1-[(*tert*-Butyldimethylsilyl]oxy)-16-(6-methoxynaphthalen-2-yl)-hexadec-15-ene, **246**



1-[(*tert*-butyldimethylsilyl)oxy]-hexadec-15-yne (0.98 g, 2.78 mmol) was cooled to 0°C and treated with catecholborane (1 M in THF, 2.78 mmol, 2.78 ml). The mixture was heated at reflux for 2.5 hours and then left to stir for a further 15 hours at room temperature, after which the solvent was removed *in vacuo*. The resulting black oil was dissolved in benzene (10 ml), treated with $Pd(PPh_3)_4$ (70 mg) followed by 2-bromo-6-methoxynaphthalene (0.44 g, 1.85 mmol) and left to stir for 2.5 hours. A solution of sodium ethoxide (2 M in ethanol, 2.66 ml) was added and the mixture heated at reflux for 3 hours. After cooling to room temperature, an aqueous solution of sodium hydroxide (2 M, 2.66 ml) was added and the resulting mixture stirred for a further 16 hours. The reaction mixture was filtered through a pad of anhydrous magnesium sulfate layered over celite and washed thoroughly with dichloromethane (100 ml). Evaporation of the solvent followed by purification on silica gel (gradient elution: 0-2% ethyl acetate/petrol) yielded the desired alkene **246** (0.73 g, 78%) as a fine white solid.

TLC : $R_f = 0.24$ in 2% ethyl acetate/petrol.

Melting point = 57-59°C.

IR ($CHCl_3$) ν_{max} **:** 2926 (s), 2853 (s), 1630 (m), 1602 (m), 1463 (m), 1390 (m), 1255 (m), 1177 (w), 1100 (br), 1034 (m), 961 (w), 836 (s) cm^{-1}.

^{1}H NMR δ : 7.72-7.55 (4H, m, CH), 7.16-7.11 (2H, m, CH), 6.53 (1H, d, J = 15.9 Hz, CH), 6.32 (1H, dt, J = 15.8, 6.7 Hz, CH), 3.93 (3H, s, CH_3), 3.63 (2H, t, J = 6.5 Hz, CH_2), 2.27 (2H, q, J = 13.7, 6.8 Hz, CH_2), 1.63-1.48 (4H, m, CH_2), 1.30 (20H, m, CH_2), 0.93 (9H, s, CH_3), 0.08 (6H, s, CH_3) ppm.

^{13}C NMR δ : 133.70 (C), 133.37 (C), 130.67 (CH), 129.78 (CH), 129.31 (CH), 129.14 (2xC), 126.89 (CH), 125.11 (CH), 124.15 (CH), 118.77 (CH), 105.81 (CH), 63.35 (CH_2),

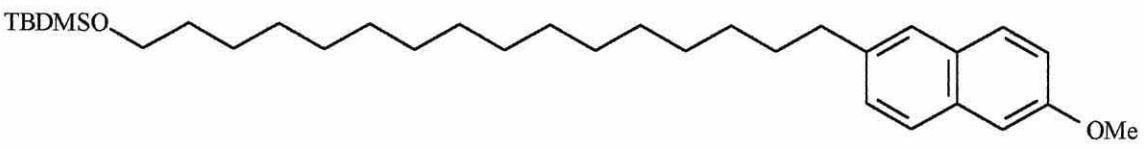
55.25 (CH_3), 33.19, 32.91, 29.86, 29.59, 29.48, 29.30 (12xCH$_2$), 26.00 (3xCH$_3$), 25.82 (CH_2), 18.40 (C), -5.24 (2xCH_3) ppm.

MS (EI) *m/z* : 510 (16% $[M]^+$), 453 (41% $[M-(t\text{-Bu})]^+$), 438 (47%), 197 (52%), 171 (51%), 165 (53%), 75 (100%), 41 (49%) Daltons.

HRMS (EI) *m/z* : found: 510.3892, $C_{33}H_{54}O_2Si$ ($[M]^+$) requires: 510.3893 Daltons.

Microanalysis : found: C, 77.7%, H, 10.9%, $C_{33}H_{54}O_2Si$ requires: C, 77.6%, H, 10.7%.

1-[(*tert*-Butyldimethylsilyl]oxy)-16-(6-methoxynaphthalen-2-yl)-hexadecane, **247**



Alkene **246** (2.59 g, 5.08 mmol) was dissolved in ethyl acetate (30 ml) with stirring, before being treated with palladium on activated carbon (2.20 g, Aldrich 20,569-9). The resulting suspension was stirred vigorously under a hydrogen atmosphere for 10 minutes, after which the reaction mixture was diluted with ethyl acetate (30 ml) and filtered through celite. The solvent was dried over anhydrous magnesium sulfate and evaporated to give the title product (2.37 g, 91%) as a fine white solid.

TLC : R_f = 0.48 in 2% ethyl acetate/petrol.

Melting point = 44-46°C.

IR ($CHCl_3$) ν_{max} **:** 3055 (w), 3004 (w), 2922 (s), 2949 (s), 1608 (m), 1462 (m), 1391 (m), 1265 (m), 1257 (m), 1061 (w), 1102 (s), 1029 (m), 837 (s), 775 (s) cm^{-1}.

^{1}H NMR δ : 7.64 (1H, d, J = 5.5 Hz, CH), 7.59 (1H, d, J = 5.5 Hz, CH), 7.54 (1H, br s, CH), 7.25 (1H, m, CH), 7.06 (2H, m, CH), 3.92 (3H, s, CH_3), 3.61 (2H, t, J = 6.6 Hz, CH_2), 2.74 (2H, t, J = 7.5 Hz, CH_2), 1.64 (2H, m, CH_2), 1.46 (2H, m, CH_2), 1.26 (24H, m, CH_2), 0.91 (9H, s, CH_3), 0.06 (6H, s, CH_3) ppm.

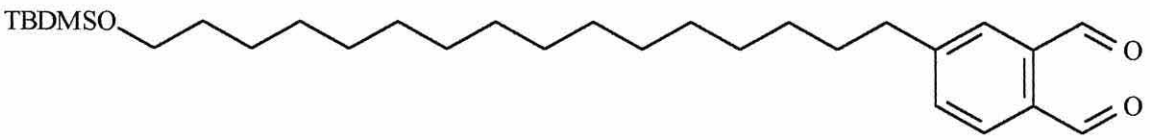
^{13}C NMR δ : 138.14 (C), 132.87 (C), 129.13 (2xC), 128.88 (CH), 127.94 (CH), 126.59 (CH), 126.14 (CH), 118.35 (CH), 105.61 (CH), 63.36 (CH_2), 55.26 (CH_3), 35.94, 32.92, 31.52, 29.70, 29.48, 29.39 (14xCH_2), 26.01 (3xCH_3), 25.83 (CH_2), 18.40 (C), -5.23 (2xCH_3) ppm.

MS (CI) *m/z* : 530 (23% $[M+NH_4]^+$), 513 (87% $[M+H]^+$), 512 (5% $[M]^+$), 455 (12% [M-(*t*-Bu)]$^+$), 158 (28%), 132 (41% [TBDMSOH]$^+$), 92 (78%), 91 (100%), 74 (69%) Daltons.

HRMS (EI) *m/z* : found: 512.4020, $C_{33}H_{56}O_2Si$ ($[M]^+$) requires: 512.4050 Daltons.

Microanalysis : found: C, 76.9%, H, 10.7%, $C_{33}H_{56}O_2Si$ requires: C, 77.3%, H, 11.0%.

4-{[(16-*tert*-Butyldimethylsilyl)oxy]-hexadecyl}-benzene-1,2-dicarbaldehyde, **248**



A three-necked flask fitted with a silica drying tube and an ozone inlet was charged with a solution of **247** (2.25 g, 4.39 mmol) in dichloromethane (100 ml) under an atmosphere of nitrogen. The reaction mixture was cooled to -78°C and a stream of ozonised oxygen bubbled through the solution until a faint blue colour was observed (*ca* 12 minutes). Triphenylphosphine (2.30 g, 8.78 mmol) was added at -78°C and the resulting solution left to stir whilst warming to room temperature over a period of five hours. Evaporation of the solvent followed by trituration of the crude product with a mixture of diethyl ether/petrol gave an oil which was purified on silica gel (gradient elution: 0-2% ethyl acetate/petrol) to give the required dialdehyde (0.48 g, 22% [53% yield based on recovered starting material]) as a pale yellow oil at room temperature.

TLC : $R_f = 0.22$ in 2% ethyl acetate/petrol.

IR (neat) ν_{max} **:** 2924 (s), 2853 (s), 1774 (w), 1698 (s), 1600 (s), 1569 (m), 1463 (s), 1254 (s), 1202 (s), 1100 (s), 1006 (w), 836 (s), 776 (s) cm^{-1}.

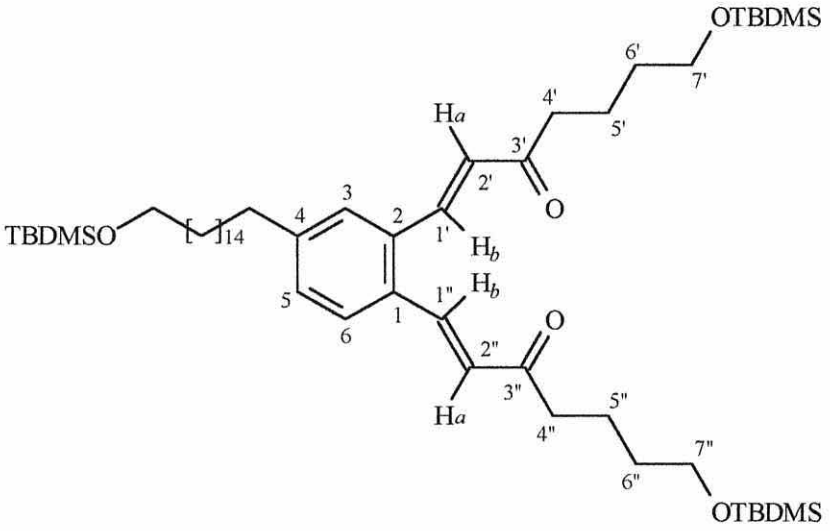
^{1}H NMR δ : 10.58 (1H, s, CH), 10.18 (1H, s, CH), 7.91 (1H, d, $J = 7.8$ Hz, CH), 7.79 (1H, s, CH), 7.58 (1H, d, $J = 7.8$ Hz, CH), 3.60 (2H, t, $J = 6.6$ Hz, CH_2), 2.76 (2H, t, $J = 7.6$ Hz, CH_2), 1.68-1.37 (4H, m, CH_2), 1.26 (24H, m, CH_2), 0.90 (9H, s, CH_3), 0.06 (6H, s, CH_3) ppm.

^{13}C NMR δ : 192.60 (CH), 192.07 (CH), 149.97 (C), 136.47 (C), 134.17 (C), 133.60 (CH), 131.73 (CH), 130.81 (CH), 63.32 (CH_2), 35.92 (CH_2), 32.86 (CH_2), 30.87 (CH_2), 29.63, 29.49, 29.43, 29.37, 29.18 (11xCH_2), 25.96 (3xCH_3), 25.78 (CH_2), 18.35 (C), -5.28 (2xCH_3) ppm.

MS (CI) *m/z* : 489 (5% $[M+H]^+$), 357 (14%), 159 (17%), 132 (80% $[TBDMSOH]^+$), 92 (68%), 74 (48%), 69 (24%) Daltons.

HRMS (CI) *m/z* : found: 489.3766, $C_{30}H_{53}O_3Si$ ($[M+H]^+$) requires: 489.3764 Daltons.

7'-[(*tert*-Butyldimethylsilyl)oxy]-1'-{4-(16-[(*tert*-butyldimethylsilyl)oxy]-hexadecyl)-1-(7"-[(*tert*-butyldimethylsilyl)oxy]-3"-oxohept-1"-enyl)-phenyl}-hept-1'-en-3'-one, **249**



Dialdehyde **248** (0.64 g, 1.31 mmol) was dissolved in dichloromethane (5 ml) and added to a solution of phosphorane **118a** (2.42 g, 5.25 mmol) in dichloromethane (18 ml) in a dropwise manner. The resulting orange solution was left to stir for 48 hours after which the solvent was removed. Purification of the residue on silica gel (gradient elution: 4-15% diethyl ether/petrol) gave the title compound as a yellow oil (0.84 g, 70%).

TLC : $R_f = 0.08$ in 10% diethyl ether/petrol.

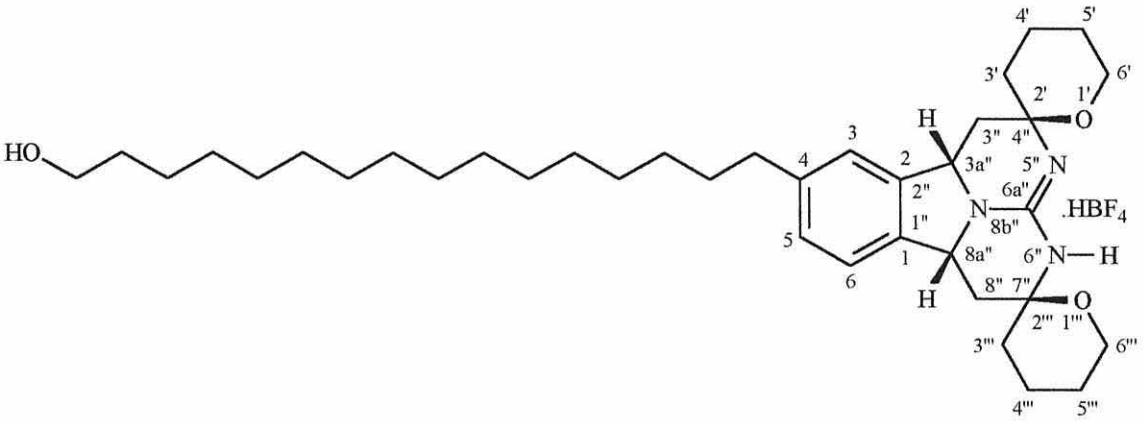
IR (neat) ν_{max} **:** 3025 (w), 2927 (s), 2854 (s), 1692 (m), 1666 (m), 1612 (m), 1600 (m), 1470 (m), 1462 (m), 1360 (w), 1254 (m), 1102 (s), 975 (m), 836 (s), 775 (s) cm^{-1}.

^{1}H NMR δ : 7.91 (1H, d, J = 16.0 Hz, CH_b), 7.90 (1H, d, J = 16.0 Hz, CH_b), 7.53 (1H, d, J = 8.1 Hz, CH), 7.40 (1H, s, CH), 7.24 (1H, d, J = 8.1 Hz, CH), 6.65 (1H, d, J = 15.9 Hz, CH_a), 6.64 (1H, d, J = 15.9 Hz, CH_a), 3.66 (4H, dt, J = 6.3, 1.3 Hz, CH_2), 3.61 (2H, t, J = 6.6 Hz, CH_2), 2.78-2.59 (6H, m, CH_2), 1.85-1.68 (4H, m, CH_2), 1.69-1.44 (8H, m, CH_2), 1.27 (24H, m, CH_2), 0.91 (27H, s, CH_3), 0.07 (12H, s, CH_3), 0.06 (6H, s, CH_3) ppm.

^{13}C NMR δ : 199.81 (C-3', C-3"), 145.51 (C), 139.06 (CH_b), 138.75 (CH_b), 134.76 (C), 132.15 (C), 130.41 (CH), 129.33 (CH), 128.54 (CH), 127.64 (2xCH_a), 63.32 (CH_2), 62.85 (2xCH_2), 41.03 (CH_2), 40.98 (CH_2), 35.80, 32.89, 32.32, 31.21, 29.67, 29.45, 29.30, 25.80, 20.66 (19xCH_2), 25.97 (9xCH_3), 20.31 (C), 18.32 (2xC), -5.29 (6xCH_3) ppm.

MS (CI) *m/z* : 914 (100% $[M+2H]^+$), 913 (6% $[M+H]^+$), 912 (5% $[M]^+$), 855 (18% $[M-(t\text{-Bu})]^+$), 699 (53%), 685 (80%), 567 (72%), 471 (68%), 132 (60% $[TBDMSOH]^+$) Daltons.

(2'*R*,3a"*S*,7"*S*,8a"*R*)- / (2'*S*,3a"*R*,7"*R*,8a"*S*)-1,2-Benzo-4-(16-hydroxyhexadecyl)-dispiro[tetrahydropyran-2',4"-(1",2",3",4",7",8"-hexahydro-5"*H*-5",6",8b"-triazaacenaphthylene)-7",2"-tetrahydropyran]-6"-ium Tetrafluoroborate, **250**



The protected *bis*-α,β-unsaturated ketone **249** (0.27 g, 0.30 mmol) was dissolved in DMF (2 ml) and stirred at 0°C for 30 minutes, after which a solution of guanidine (0.02 g, 0.33 mmol) in DMF (1 ml) was added slowly, in a dropwise manner. After stirring at 0°C for 4 hours, the reaction mixture was warmed to room temperature and left to stir for a further 3 hours. The resulting green solution was again cooled to 0°C and treated with a solution of methanolic HCl (10 ml, [acetyl chloride (0.2 ml) in methanol (9.8 ml)]), and stirred for 14 hours before quenching with water (20 ml).

The mixture was extracted with CH_2Cl_2 (5x25 ml) and the combined organic fractions washed sequentially with water (50ml), saturated lithium bromide solution (50 ml) and saturated sodium chloride solution (50 ml). The solvent was concentrated and the resulting oil dissolved in 1:1 MeOH/CH_2Cl_2 (8 ml), treated with an aqueous solution of saturated sodium tetrafluoroborate (3 ml) and stirred vigorously for 3 hours. Extraction of the mixture with CH_2Cl_2 (2x20 ml) followed by drying and evaporation of the solvent gave a brown oil which was purified on silica gel (gradient elution: 0-5% MeOH/CH_2Cl_2) yielding the title product (0.05 g, 25%) as a brown semi-solid.

TLC : R_f = 0.10 in 4% methanol/dichloromethane.

IR ($CDCl_3$) ν_{max} **:** 3370 (br), 3234 (br), 2924 (s), 2852 (s), 1650 (m), 1606 (s), 1332 (w), 1276 (w), 1222 (w), 1064 (s), 1036 (s), 997 (w) cm^{-1}.

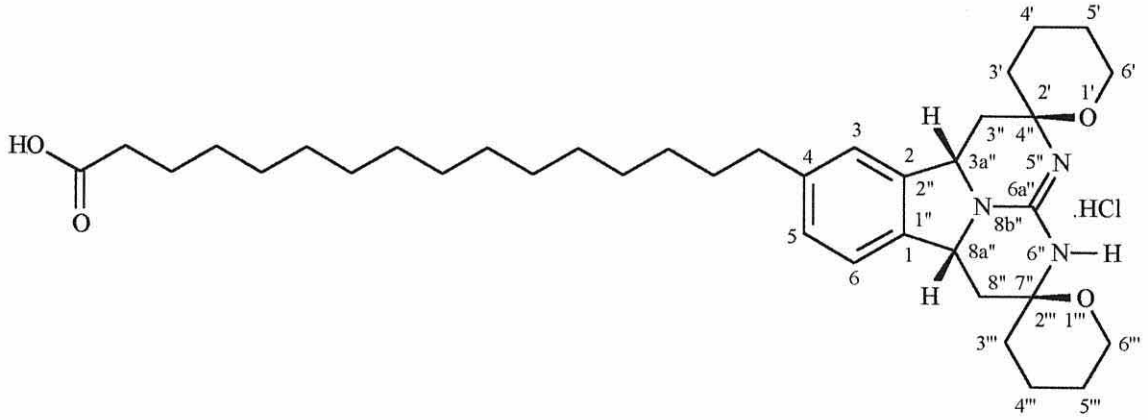
^{1}H NMR δ : 7.72 (2H, br s, NH), 7.20 (1H, d, *J* = 7.7 Hz, CH), 7.13 (1H, d, *J* = 7.9 Hz, CH), 7.04 (1H, s, CH), 5.22 (2H, dd, *J* = 11.6, 3.9 Hz, CH-3a", CH-8a"), 3.89 (2H, ddd, *J* = 22.9, 10.8, 2.9 Hz, CHα-6', CHα-6'"), 3.76 (2H, br d, *J* = 11.4 Hz, CHβ-6', CHβ-6'"), 3.64 (2H, t, *J* = 6.5 Hz, CH_2), 2.63 (4H, m, CH_2), 2.17-2.00 (2H, m, CH_2), 1.94-1.48 (16H, m, CH_2), 1.26 (24H, br s, CH_2) ppm.

^{13}C NMR δ : 147.77 (C-6a"), 144.77 (C-4), 138.55 (C-2"), 135.73 (C-1"), 129.14 (CH-5), 121.53 (CH-3), 121.47 (CH-6), 79.73 (C-2', C-7"), 63.01 (CH_2), 61.95 (CH_2-6', CH_2-6'"), 56.69 (CH-8a"), 56.59 (CH-3a"), 38.26 (CH_2-8"), 38.16 (CH_2-3"), 35.84 (CH_2), 34.20 (CH_2-3', CH_2-3'"), 32.76, 31.56, 29.64, 29.43, 29.23, 25.73, 24.56, 17.54 (18xCH_2) ppm.

MS (FAB) *m/z* : 594 (100% $[M+H]^+$), 367 (5% $[M-(CH_2)_{15}OH]^+$) Daltons.

HRMS (FAB) *m/z* : found: 594.4611, $C_{37}H_{60}N_3O_3$ ($[M+H]^+$) requires: 594.4635 Daltons.

(2'*R*,3a"*S*,7"*S*,8a"*R*)- / (2'*S*,3a"*R*,7"*R*,8a"*S*)-1,2-Benzo-4-(hexdecyl-16-carboxylic acid)-dispiro[tetrahydropyran-2',4"-(1",2",3",4",7",8"-hexahydro-5"*H*-5",6",8b"-triazaacenaphthylene)-7",2"-tetrahydropyran]-6"-ium}-hexadecanoic Acid Chloride, **251**



Hexacyclic alcohol **250** (0.08 g, 0.12 mmol) was dissolved in DMF (1 ml) and stirred for 10 minutes before being treated with pyridinium dichromate (0.26 g, 0.71 mmol). After stirring for 24 hours, the reaction mixture was poured into water (10 ml) and extracted with CH_2Cl_2 (8x20 ml). The combined organic fractions were washed with 2 M HCl (2x50 ml) followed by saturated sodium chloride solution (2x50 ml). Drying of the solvent over anhydrous magnesium sulfate and concentration *in vacuo* gave a black oil which was purified by column chromatography on silica gel (gradient elution: 0-5% MeOH/CH_2Cl_2) yielding the title acid **251** (0.04 g, 55%).

TLC : $R_f = 0.11$ in 4% methanol/dichloromethane.

IR ($CDCl_3$) ν_{max} **:** 3422 (br), 3228 (br), 3174 (w), 3014 (w), 2926 (s), 2853 (m), 1710 (m), 1649 (s), 1609 (s), 1442 (w), 1332 (w), 1275 (w), 1222 (w), 1176 (w), 1101 (w), 1060 (w), 1040 (m), 1021 (w), 996 (w) cm^{-1}.

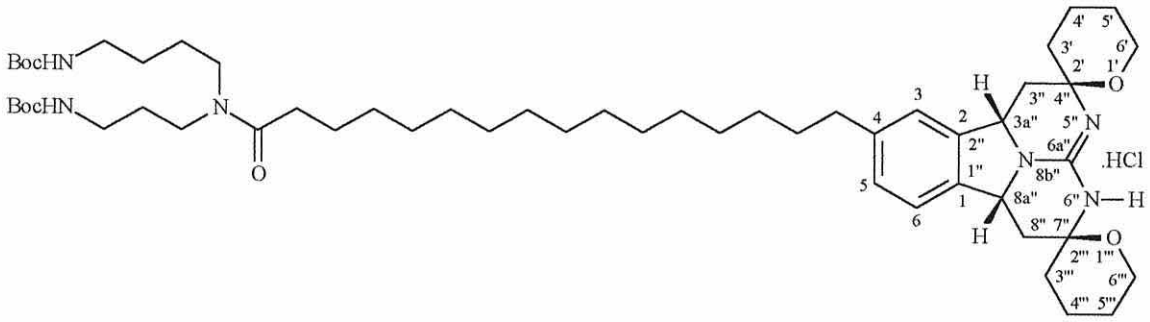
^{1}H NMR δ : 9.87 (2H, br s, NH), 7.18 (1H, d, J = 7.9 Hz, CH), 7.12 (1H, d, J = 7.8 Hz, CH), 7.02 (1H, s, CH), 5.17 (2H, dd, J = 12.4, 4.6 Hz, CH-3a", CH-8a"), 3.96 (2H, ddd, J = 21.7, 10.8, 2.3 Hz, CHα-6', CHα-6'"), 3.75 (2H, br d, J = 10.8 Hz, CHβ-6', CHβ-6'"), 2.59 (4H, m, CH_2), 2.32 (4H, br t, J = 7.7 Hz, CH_2), 2.05-1.47 (14H, m, CH_2), 1.25 (24H, br s, CH_2) ppm.

^{13}C NMR δ : 175.97 (C), 148.59 (C-6a"), 144.07 (C-4), 138.94 (C-2"), 136.12 (C-1"), 128.98 (CH-5), 121.55 (CH-3, CH-6), 79.45 (C-2', C-7"), 61.93 (CH_2-6', CH_2-6'"), 56.44 (CH-8a"), 56.23 (CH-3a"), 38.52 (CH_2-3", CH_2-8"), 35.82 (CH_2), 34.52 (CH_2-3', CH_2-3'"), 33.98, 31.52, 29.56, 29.38, 29.20, 29.04, 24.97, 24.72, 18.30 (18xCH_2) ppm.

MS (CI) *m/z* : 608 (23% $[M+H]^+$), 564 (3%) Daltons.

HRMS (CI) *m/z* : found: 608.4427, $C_{37}H_{58}N_3O_4$ ($[M+H]^+$) requires: 608.4427 Daltons.

(2'*R*,3a"*S*,7"*S*,8a"*R*)- / (2'*S*,3a"*R*,7"*R*,8a"*S*)-(4{(3-*tert*-Butoxycarbonylaminopropyl)-4-[16-hexadecanoyl]amino}butyl)-{1,2-benzodispiro[tetrahydropyran-2',4"-(1",2",3",4",7",8"-hexahydro-5"*H*-5",6",8b"-triazaacenaphthylene)-7",2"-tetrahydropyran]-6"-ium}-carbamic Acid *tert*-Butyl Ester Chloride, **252**



A solution of hexacyclic acid **251** (0.042 g, 0.065 mmol) in CH_2Cl_2 (1 ml) was treated sequentially with *bis*-protected spermidine **41** (0.027 g, 0.078 mmol) in CH_2Cl_2 (0.5 ml), HOBT (0.010 g, 0.072 mmol) in CH_2Cl_2 (0.25 ml) and EDCl (0.014 g, 0.072 mmol) also in CH_2Cl_2 (0.5 ml). The reaction mixture was left to stir for 18 hours before being washed with water (3x15 ml) followed by extraction of the combined aqueous layers with CH_2Cl_2 (25 ml). The organic fraction was then washed with 0.5 M HCl (2x20 ml) followed by saturated sodium chloride solution (2x20 ml), dried over anhydrous magnesium sulfate and concentrated *in vacuo*. Purification of the semi-solid obtained on silica gel (gradient elution: 0-10% MeOH/CH_2Cl_2) gave the title compound (0.055 g, 88%) as a brown solid.

TLC : R_f = 0.11 in 4% methanol/dichloromethane.

IR ($CDCl_3$) ν_{max} **:** 3455 (br), 3223 (br), 3122 (w), 2928 (s), 2854 (m), 1704 (s), 1648 (s), 1610 (s), 1511 (m), 1502 (m), 1462 (w), 1443 (w), 1366 (m), 1332 (w), 1275 (m), 1250 (m), 1222 (w), 1171 (s), 1102 (w), 1060 (w), 1040 (m), 1020 (w), 996 (w) cm^{-1}.

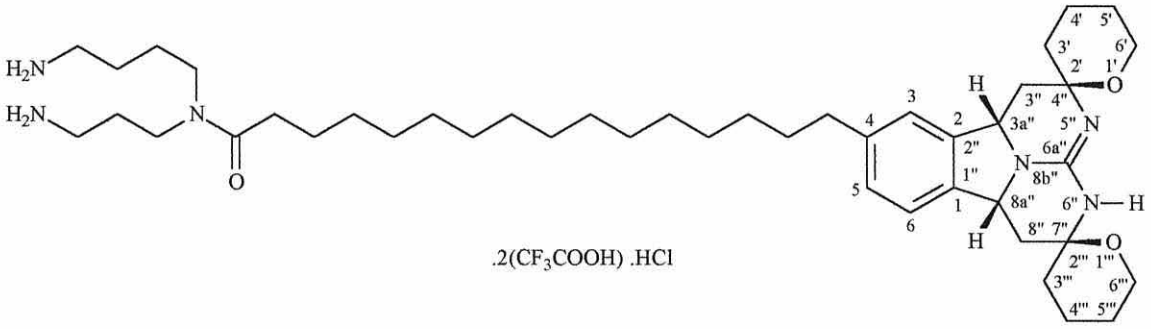
^{1}H NMR δ : 10.01 (2H, br s, NH), 7.18 (1H, d, J = 8.1 Hz, CH), 7.12 (1H, d, J = 7.6 Hz, CH), 7.01 (1H, s, CH), 5.42 (1H, br s, NH), 5.15 (2H, dd, J = 6.6, 2.1 Hz, CH-3a", CH-8a"), 4.67 (1H, br s, NH), 3.90 (2H, ddd, J = 22.8, 12.3, 2.8 Hz, CHα-6', CHα-6'"), 3.74 (2H, br d, J = 12.1 Hz, CHβ-6', CHβ-6'"), 3.37 (2H, t, J = 7.0 Hz, CH_2), 3.33-2.98 (6H, m, CH_2), 2.59 (4H, m, CH_2), 2.27 (4H, t, J = 7.2 Hz, CH_2), 1.92-1.73 (6H, m, CH_2), 1.72-1.49 (8H, m, CH_2), 1.42 (9H, s, CH_3), 1.41 (9H, s, CH_3), 1.23 (30H, br s, CH_2) ppm.

^{13}C NMR δ : 173.56 (C), 172.78 (C), 155.99 (C), 148.59 (C-6a"), 144.09 (C-4), 138.98 (C-2"), 136.15 (C-1"), 128.98 (CH-5), 121.54 (CH-3), 121.46 (CH-6), 79.44 (C-2', C-7"), 79.14 (C), 78.79 (C), 61.92 (CH_2-6', CH_2-6'''), 56.42 (CH-8a"), 56.31 (CH-3a"), 47.38 (CH_2), 45.35 (CH_2), 42.28 (CH_2), 39.93 (CH_2), 38.64 (CH_2), 38.55, 37.16, 35.84, 34.55, 33.11, 31.56, 29.63, 29.50, 29.25, 27.93, 27.62, 27.44, 26.12, 25.57, 24.97, 18.30 (25xCH_2), 28.38 (6xCH_3) ppm.

MS (CI) *m/z* : 935 (20% $[M+H]^+$), 835 (35% $[M+H-(t\text{-Boc})]^+$), 735 (67% $[M+2H-2(t\text{-Boc})]^+$), 717 (68%), 608 (20%), 346 (100%) Daltons.

HRMS (FAB) *m/z* : found: 935.6949, $C_{54}H_{91}N_6O_7$ ($[M+H]^+$) requires: 935.6949 Daltons.

(2'*R*,3a"*S*,7"*S*,8a"*R*)- / (2'*S*,3a"*R*,7"*R*,8a"*S*)-4-{(16-Hexadecanoic acid)-(4-aminobutyl)-3(aminopropyl)amide}-1,2-benzodispiro[tetrahydropyran-2',4"-(1",2",3",4",7",8"-hexahydro-5"*H*-5",6",8b"-triazaacenaphthylene)-7",2"-tetrahydropyran]-6"-ium Chloride, *bis*-Trifluoroacetate, **214**



A solution of protected hexacycle **252** (0.006 g, 0.006 mmol) in $CHCl_3$ (0.3 ml) was treated with trifluoroacetic acid (0.65 mmol, 0.05 ml) and gently agitated for one hour. The solvent and excess TFA were removed *in vacuo* and the resulting material azeotroped with $CHCl_3$ (4x0.3 ml), affording the desired hexacycle in quantitative yield (0.006 g, 100%).

^{1}H NMR δ : 9.04 (2H, br s, NH), 7.19 (1H, d, J = 8.0 Hz, CH), 7.13 (1H, d, J = 7.7 Hz, CH), 7.03 (1H, s, CH), 5.20 (2H, br d, J = 8.6 Hz, CH-3a", CH-8a"), 3.99-3.61 (4H, m, CHα-6', CHβ-6', CHα-6"',CHβ-6"'), 3.58 (8H, m, CH_2), 2.58 (4H, m, CH_2), 2.42 (4H, m, CH_2), 1.99-1.73 (6H, m, CH_2), 1.72-1.52 (12H, m, 4xCH_2, 2xNH_2), 1.23 (30H, br s, CH_2) ppm.

MS (ES) *m/z* : 735 (100% $[M+H]^+$), 608 (21%), 507 (42%), 450 (22%) Daltons.

HRMS (ES) *m/z* : found: 735.5896, $C_{44}H_{75}N_6O_3$ ($[M+H]^+$) requires: 735.9001 Daltons.

CHAPTER 9

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