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Synthetic studies towards analogues of ptilomycalin A

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SYNTHETIC STUDIES TOWARDS ANALOGUES OF PTILOMYCALIN A

• PRIFYSGOL CYMRU •
UNIVERSITY OF WALES
BANGOR

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

by

Spencer Maurice Jones

January 2004

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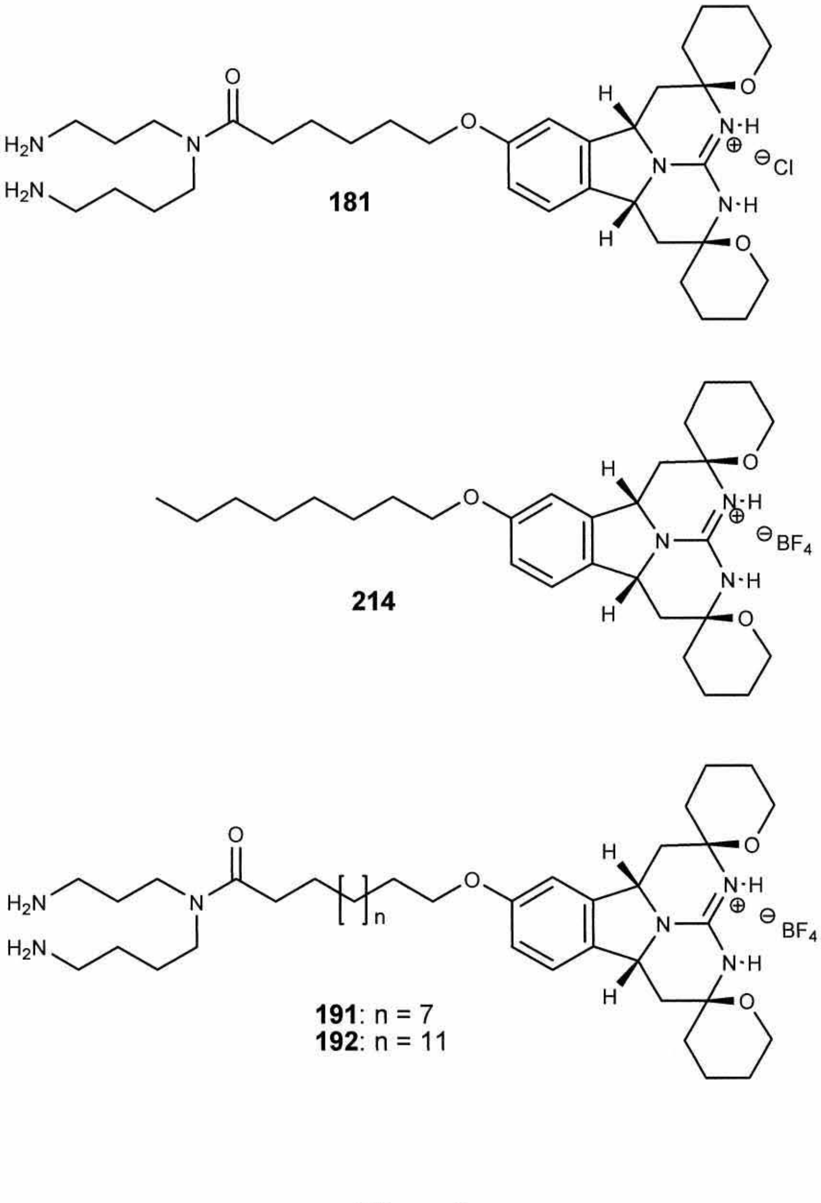
Abstract

The thesis describes three synthetic approaches to analogues of ptilomycalin A and studies their biological activity.

The analogue **181** was prepared by a linear synthetic route and this compound was found to be, as expected, biologically largely inactive. A convergent synthetic approach was applied to the synthesis of the analogues **214**, **191** and **192**.



181

214

191: n = 7
192: n = 11

Figure i.

ABBREVIATIONS

$[\alpha]_d$	specific rotation
Å	Angstrom
Ac	acetyl
AcOH	acetic acid
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
Bu	butyl
cat.	catalytic
CBZ	benzyloxycarbonyl
CC-50	concentration of a cytotoxic agent required to reduce target cell viability by 50 %
CD	circular dichroism
CIT	citrulline
COSY	correlated spectroscopy
d	doublet

DEAD	diethyl azodicarboxylate
DIAD	diisopropyl dicarboxylate
DCC	*N,N*-dicyclohexylcarbodiimide
δ	chemical shift (relative to TMS)
DEPT	distortionless enhancement by polarization transfer
DIBAL-H	diisobutylalumium hydride
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 sulfxide
DNA	deoxyribonucleic acid
E	entgegen
EDCl	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
Glu	glutamic acid
gp	glycoprotein
h	hour (s)
HIV	human immunodeficiency virus

HOBT	1-hydroxybenzotriazole
HRMS	high resolution mass spectroscopy
HSV	herpes simplex virus
Hz	hertz
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
IR	infrared
J	coupling constant
kg	kilogram (s)
LD_{50}	lethal dose 50
$LiAlH_4$	lithium aluminium hydride
lit.	literature
M	moles per litre
m	multiplet
Me	methyl
min	minute (s)
mmol	millimole (s)
mol	mole (s)
MS	mass spectrometry
m/z	mass ion
NMR	nuclear magnetic resonance

NOESY	correlated nuclear Overhauser effect spectroscopy
pdTp	deoxythymidine 3',5'-diphosphate
p-TsCl	*para*-toluenesulfonyl chloride
p-TsOH	*para*-toluene sulfonic acid
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
Ph	phenyl
pKa	acid dissociation constant
ppm	parts per million
q	quartet
R*f*	retention factor
RNA	ribonucleic acid
ROESY	through-space correlated nuclear Overhauser effect spectroscopy
rt	room temperature
s	singlet
SNase	staphylococcal nuclease
sp.	spiculifer
t	triplet
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
t-Bu	*tert*-butyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl

TLC	thin layer chromatography
TMS	trimethylsilyl
TrocCl	2,2,2-trichloroethyloxycarbonyl chloride
Ts	Tosyl

NOMENCLATURE

For the compounds reported in this thesis that contain the hexacyclic benzo-fused guanidine system, the ring numbering is based on the acenapthylene ring system as shown below in Figure 1.1.

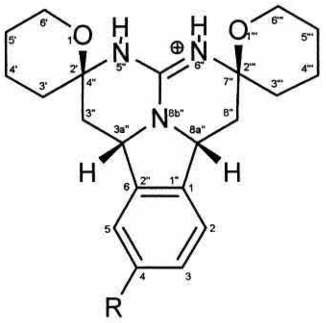


Figure 1.1: Ring system showing numbering nomenclature.

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

CHAPTER 1

THE CHEMISTRY OF GUANIDINE

1.1 STRUCTURAL AND BASIC PROPERTIES OF GUANIDINE

Guanidine **1** is one of the most basic neutral nitrogen containing organic compounds known, with a pKa of 13.6 in water.[1] This level of basicity arises from the equal spread of the positive across the three nitrogens of the protonated guanidine or guanidinium ion by the virtue of its three possible resonance structures.

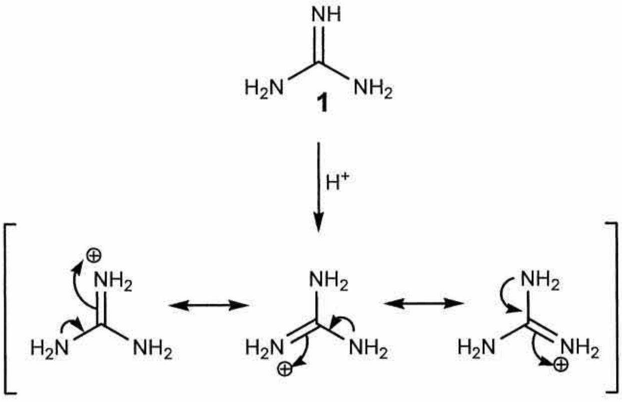
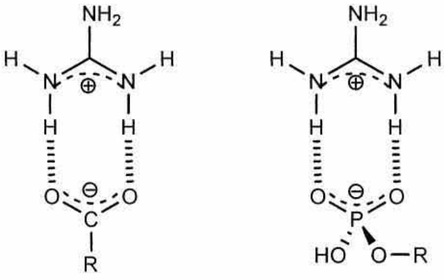


Figure 1.2: The three resonance structures of the guanidinium cation.

The three nitrogen atoms and the central carbon atom of guanidine have a co-planar arrangement brought about by the partial double bond character of each of the C-N bonds.[2] The dimensions of simple guanidine derivatives have been studied by X-ray crystallography and has shown the C-N single bond length in an alkyl guanidine to be typically shorter than the usual C-N bond length. In general the three C-N bonds in the guanidine unit are almost equal in length at an average of 1.33 Å, which is comparable to that of a C=N bond at 1.29 Å, and shorter than the value of the characteristic C-N bond length of 1.41 Å.[3] The three bond angles are nearly always equal at 120°.

Its basicity, lends itself to remain protonated over a wide pH range and this confers an excellent anion binding affinity in aqueous medium. It readily absorbs carbon dioxide and water from air and the cationic form is inert to decomposition.[4]

Mediation of the hydrogen-bond of guanidinium ions with anionic substrates such as carboxylates **2** and phosphates **3** is of considerable interest in bioorganic chemistry.[5] Oxoanionic bonding occurs through the formation of characteristic pairs of zwitterionic hydrogen bonds of the form $N\text{-}H^{+}\cdots\cdots X^{-}$, with the overall charge resulting in electrostatic attraction which contributes towards the binding strength. The parallel arrangement of the hydrogen bonds provides structural organisation, as is evident in many crystal structures of guanidine salts.[6,7]

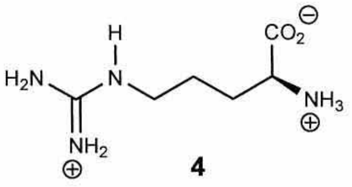


2 **3**

Figure 1.3: Binding patterns of oxoanions with the guanidine motif.

1.2 ARGININE

In the biological system, the guanidine group of the naturally occurring amino acid arginine is in the protonated zwitterionic form. Nature offers a number of examples where the arginine moiety in proteins has an important function in the maintenance of protein conformation and in the binding and recognition of the 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, ribonuclease T1, pepsin, carboxypeptidase's A and B and antibody combining sites directed against hapten containing anions such as arsonate, phosphonate and carboxylates.



4

Figure 1.4: The remarkable amino acid L-arginine.

1.3 STAPHYLOCOCCAL NUCLEASE

A particular instance of an enzyme where arginine demonstrates its ability to form parallel hydrogen bonds with oxoanions is staphylococcal nuclease (SNase). This trait is demonstrated through explanation by Cotton *et al.* on the interactions that play an key 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. 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 1.5.

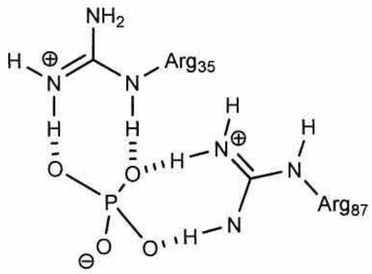


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Figure 1.5: Staphylococcal Nuclease

The two guanidyl groups of the arginine residues 35 and 87 each form a pair of hydrogen bonds with two of the phosphate oxygen atoms, one being shared between the two hydrogen atoms 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 extremely effective 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 .

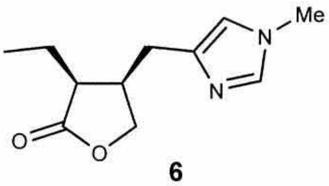
Mechanistic and high resolution X-ray crystal structures studies have resulted in a proposed mechanism for the catalytic mode of action for SNase. The active site of the enzyme contains two arginine residues in conjunction with a calcium ion which results in electrophilic activation of the phosphodiester towards hydrolysis.

CHAPTER 2

GUANIDINE-CONTAINING NATURAL PRODUCTS

2.1 ALKALOIDS

Alkaloid chemistry has developed considerably since 1818 when Meissner described all basic compounds as alkaloids. Hesse later suggested a new definition for alkaloids; nitrogen containing compounds derived from plants or animals. This definition is not all inclusive and actually excludes DNA, RNA as alkaloids. Alkaloids occur mainly in flowering plants, for instance, in 1875 two researchers independently discovered an alkaloid in the leaves of Brazilian species, *P. jaborandi* and *P. microphyllus*, and the Paraguay species *P. pennatifolius*.[8] The alkaloid pilocarpine **6**, is a histidine derivative that is used in the treatment for glaucoma.[9] Pilocarpine made the transition from Amazonian indigenous tribal use, into modern medicine based upon natural chemicals found in the plant. In 1876, the isolated pilocarpine alkaloid was introduced into conventional ophthalmology for the treatment of glaucoma.[10]



6

Figure 2.1: Pilocarpine **6**

The bark extract from cinchona, was used as the sole treatment for malaria for nearly two centuries, until quinine **7** and cinchonine where isolated as pure alkaloids in 1820.

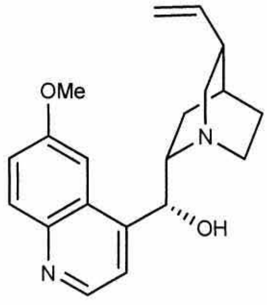


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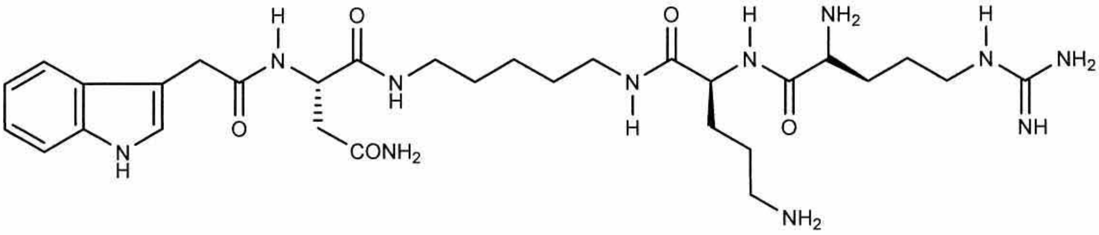
Figure 2.2: Quinine

The number of known alkaloids is constantly growing mainly due to their wide ranging medicinal properties. Studies on marine organisms have led to the discovery of many novel unusual and medicinally interesting alkaloids.[11] Within this area of discovery guanidine-containing marine natural products have become a growing and interesting field of research. Particularly interesting are that they are often unique to the organism source, they typically have cyclic structures and possess wide ranging biologically activity.

Guanidine is present in many marine organisms, such as fish and plants, of particular note sea sponges, of which the most interesting will be discussed later in this chapter. To contrast the vast number of guanidine-containing marine natural products, terrestrial sources are few, one such example being the acylpolyamine toxin nephilatoxin **8** found in numerous spiders.[12]

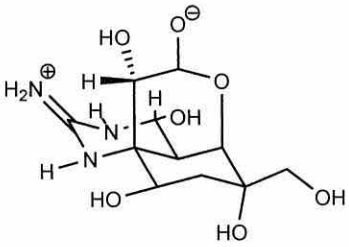


8

Figure 2.3: A terrestrial guanidine containing natural product.

2.2 TETRODOTOXIN

By virtue of its association with potential health hazards, tetrodotoxin **9** is probably one of the best known marine toxins. Tetrodotoxin is known to be a neurotoxin, acting as a highly specific sodium channel blocker of excitable cell membranes.[13] The most well known example of this potent toxin is in the liver and ovaries of puffer fish, particularly the tiger puffer (*Tora fugu*) and the common puffer fish (*Ma fugu*). Poisoning occurs mainly in parts of Asia, where the fish is consumed.[14]



9

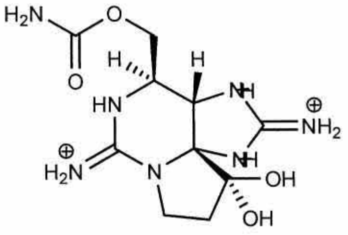
Figure 2.4: The fascinating structure of tetrodotoxin.

The potent neurotoxin was first isolated from the Californian newt *Taricha torosa* in 1934[15] and further investigations have found it in the skin and eggs of the Costa Rican frog *Atelopus chiriauiensis*, also in many other organisms such as the fish *Gobius criniger*,[16] which uses the toxin to paralyse its prey.

Workers from the Yokoo group, where the first to isolate the toxin in a crystalline form in 1950,[17] this in turn led to the elucidation of the structure of **9** simultaneously by three research groups in 1964,[18,19,20] but the total synthesis of **9** was not reported until 1972 by Kishi and co-workers.[21]

2.3 SAXITOXIN

Saxitoxin **10**, the paralysing shellfish poison found in the Alaska butter clam *saxidomas giganteus* and the Californian mussel *Mytilius californianus* after the molluscs ingest toxic single-cell marine dinoflagellate red plankton *Gonyaulax catenella*.[22] The toxin acts by selectively blocking specific sodium channels.[23] It was firstly isolated by the Schantz group[24] in 1957, and the structure was elucidated using x-ray diffraction techniques on salts of saxitoxin.[25] Total synthesis was reported 2 years later in 1977 by Kishi *et al.*[26] and by Jacobi in 1984.[27] Saxitoxin **10** has found widespread use in the study of nerve disorders.[28]

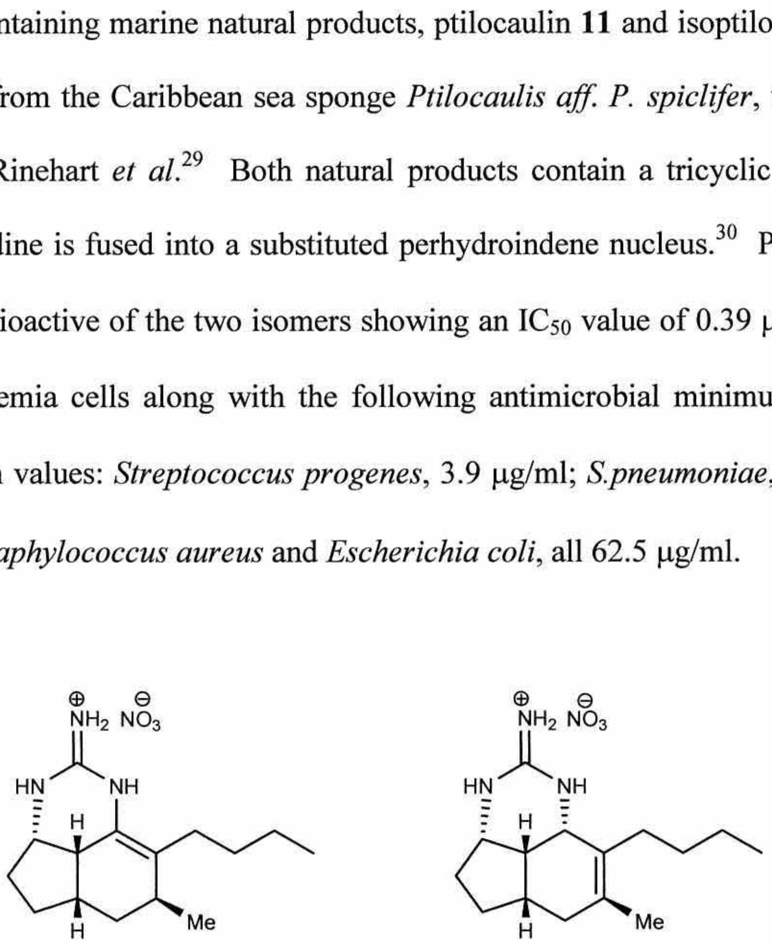


10

Figure 2.5: Saxitoxin

2.4 PTILOCAULIN AND ISOPTILOCAULIN

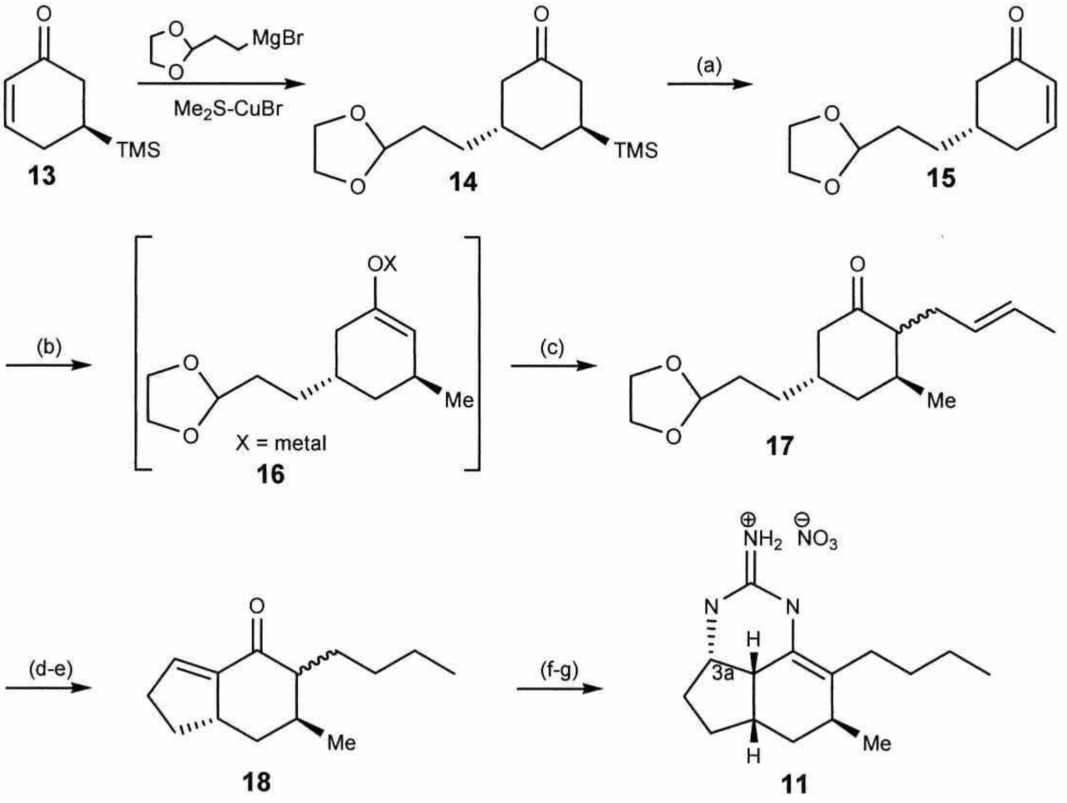
The isolation of two novel isomeric, antimicrobial and cytotoxic tricyclic guanidine-containing marine natural products, ptilocaulin **11** and isoptilocaulin **12**, as nitrate salts from the Caribbean sea sponge *Ptilocaulis aff. P. spiclifer*, was reported in 1981 by Rinehart *et al.*[29] Both natural products contain a tricyclic ring system where guanidine is fused into a substituted perhydroindene nucleus.[30] Ptilocaulin **11** is the more bioactive of the two isomers showing an IC_{50} value of 0.39 μg/ml against L1210 leukaemia cells along with the following antimicrobial minimum inhibitory concentration values: *Streptococcus progenes*, 3.9 μg/ml; *S.pneumoniae*, 15.6 μg/ml; *S.faecalis*, *Staphylococcus aureus* and *Escherichia coli*, all 62.5 μg/ml.



11 **12**

Figure 2.6: Isomeric marine natural products ptilocaulin **11** and isoptilocaulin **12**.

The absolute configuration of ptilocaulin **11** was established by the production of synthetic (-)-Ptilocaulin by Snider and Faith in 1984 by analogous five step procedures.[31] (-)-Ptilocaulin was found to have identical chemical properties to those of the natural product, in all respects apart from the [α]D measurement and the CD spectrum which were of the same magnitude but of opposite sign, establishing the absolute stereochemistry as (+)-Ptilocaulin. The first synthesis of (+)-Ptilocaulin was published in 1990, by Asaoka, Sakurai and Takei.[32] This synthesis was *via* a diastereocontrolled route. A 1,4-addition of Grignard reagent to (*S*)-(+)-5-trimethylsilyl-2-cyclohexanone **13**, gave adduct as a single diastereomer **14**. Elimination of the trimethyl group gave enone **15** which underwent a diastereoselective 1,4-addition of dimethyl cuprate **16** to afford, after alkylation of the intermediate enolate with crotyl bromide, alkene **17** in 80 % yield. Hydrogenation of the double bond followed by treatment with 2M aqueous HCl / THF gave bicyclic enone **18** as a mixture of two diastereomers. On reaction of this mixture with guanidine in refluxing benzene for 24 hours, the water was removed and subsequently treated with dilute nitric acid, a mixture of (+)-ptilocaulin **11** and its C-3a epimer, was obtained in 35-42 % yield. Column chromatography, followed by recrystallisation, gave pure synthetic (+)-ptilocaulin **11**.


MgBr
Me2S-CuBr
TMS
13
14
15
(a)
(b)
(c)
(d-e)
(f-g)
OX
X = metal
16
17
Me
18
11
NH2
NO3
N
H
3a

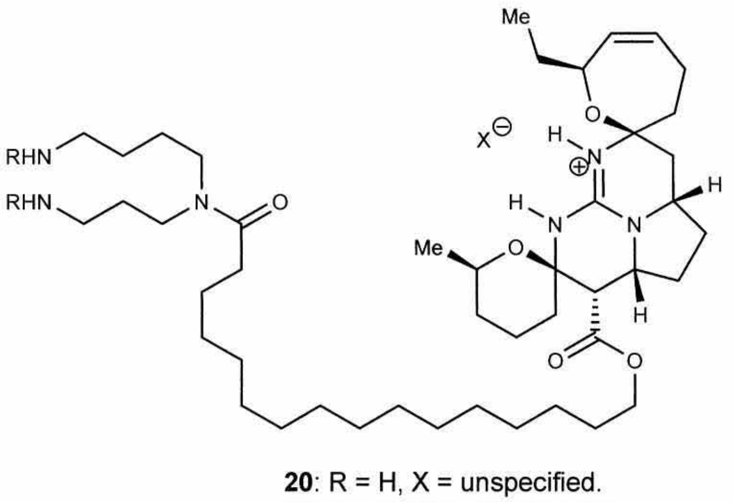
Reagents and Conditions: (a) $CuCl_2$, DMF, 55-60 °C, 1 h; (b) Me_2CuLi, Et_2O, -5 °C, 1 h; (c) Crotyl bromide, Et_2O/THF/HMPA (4:1:1), -5 °C, 1.5 h; (d) H_2, Pd/C; (e) 2M *aq.* HCl/THF; (f) Guanidine, C_6H_6, reflux, 24 h; (g) Dilute HNO_3.

Scheme 2.1

2.5 PTILOMYCALIN A

In 1989, a novel antitumour, antiviral and antifungal compound ptilomycalin A **20**, was isolated during the course of screening for unusual bioactive agents from marine sponges, by the Kashman group. Ptilomycalin A **20** was isolated from the Caribbean sea sponge *Ptilocaulis spiculifer* and also from the sponge *Hemimycale sp.* from the Red Sea.[33] Subsequently, ptilomycalin A **20** has also been isolated from the starfish *Fromia monilis* and *Celerina heffernani* collected in the water off the coast of New Caledonia, and also a second Caribbean sponge, *Batzella sp.*[34]

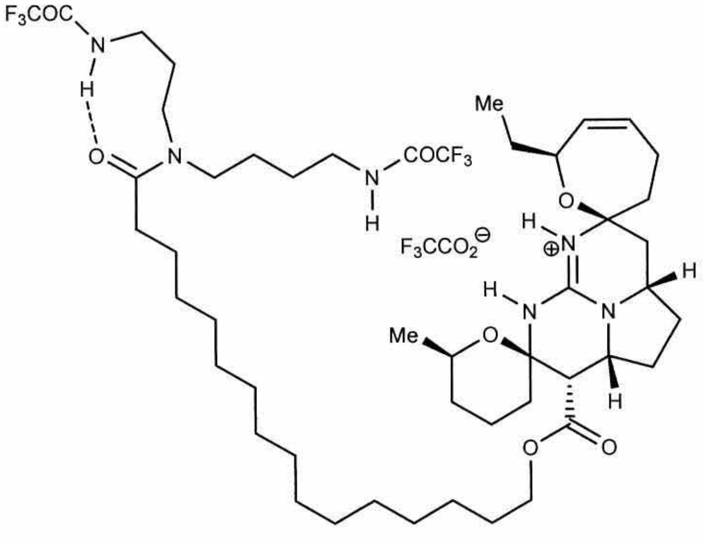
The structure of ptilomycalin A **20** was inferred from detailed ^1H and ^{13}C NMR experiments, along with high resolution mass spectrometry of the *bis*-trifluoroacetyl derivative **21**. These results showed that the structure of ptilomycalin A consisted of a novel pentacyclic guanidinium core tethered to a spermidine residue by a 16 carbon chain. The guanidine moiety was established by the presence of two N-H signals at 10.22 and 9.87 respectively, along with a carbon signal at 149.09 in the ^{13}C NMR spectrum. The stereochemistry of the biologically active alkaloid was confirmed using phase-sensitive NOESY and ROESY experiments.[35]



20: R = H, X = unspecified.
21: R = $COCF_3$, X = CF_3CO_2

Figure 2.7: The structure of Ptilomycalin A showing stereochemistry.

The exact conformation of ptilomycalin A **20** has led to much interesting debate within the literature. The presence of polar moieties such as a guanidinium cation and a spermidine unit should make ptilomycalin A **20** a particularly polar substance, but the contrary was found in that it was soluble in chloroform. With these facts the structure of the *bis*-(trifluoroacetyl) ptilomycalin A **21** was proposed to contain the counter ion encapsulated between the guanidinium and the spermidine moieties, whilst in solution.[36]



21

Figure 2.8: Kashman's proposed encapsulated ptilomycalin A **21** structure.

In contrast to this Overman and Murphy simultaneously proposed an alternative postulation that *in vivo*, the tether of the ptilomycalin A is embedded in a membrane in a manner that might enhance anion binding, or act to encapsulate the anion and enable passage through the membrane.[37]

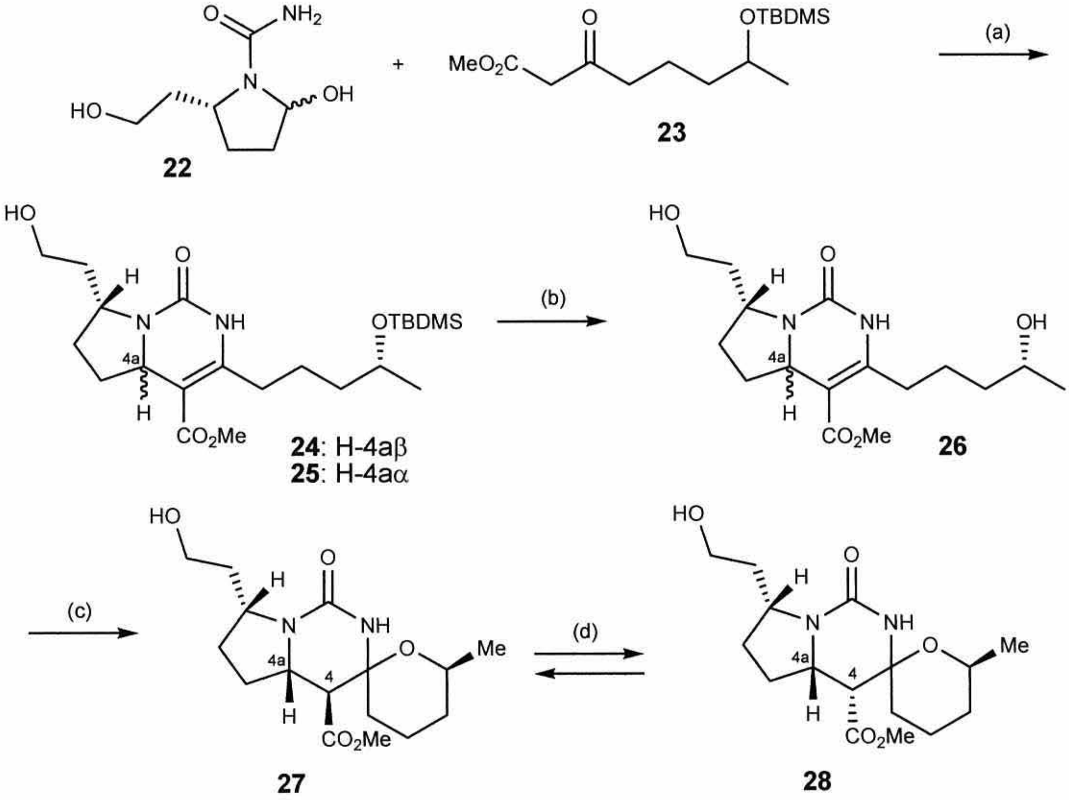
Interestingly, the pentacyclic core of ptilomycalin A **20** closely resembles several synthetic guanidine host molecules, including an example which has been employed as an enantioselective anion receptor. An NMR study was performed to investigate whether ptilomycalin A exhibited molecular recognition. Complexes of *bis*-trifluoroacetyl ptilomycalin A, and several *N*-acetylamino acid anions were used, but no enantioselectivity was obtained. During these experiments, it was observed that there was preference for certain amino acid counter ions. When a $CDCl_3$ solution containing ptilomycalin A/L-*N*-acetylalanate was shaken with an aqueous solution of L-*N*-acetylmethionate, the 1-*N*-acetylalanate in the organic layer was completely replaced with l-N-acetylmethionate. A general relative ability to exchange was suggested. L-*N*-acetylmethionate = l-*N*-acetylvalinate > L-*N*-acetylalanate = L-*N*-acetylisoleucinate >> *N*-acetylgycinate.[38] Steric reasons were suggested for this selectivity, in that some amino acids had a better "fit" in the pocket.

Ptilomycalin A has displayed a wide range of biological activity, notably it was shown to be highly cytotoxic towards cells CEM 4 infected by HIV-1 with a CC-50 of 0.11 μg/ml without cytoprotective effects at a dose of <0.1 μg/ml.[39] In addition it displayed *in vitro* cytoxicity towards several cancer cell lines: P388 (IC_{50} 0.1 μg/ml), L1210 (IC_{50} 0.4 mg/ml) and KB (IC_{50} 1.3 μg/ml), antifungal activity against *Candida albicans* (MIC 0.8 μg/ml), as well antiviral activity towards the Herpes Simplex Virus (HSV) at a concentration of 0.2 μg/ml.[40]

The mode of action of ptilomycalin A **20** in these processes is still unclear, however it has been speculated that it is involved in anion transport as it has structural similarities to abiotic guanidine based anionic receptor molecules.

2.6 THE OVERMAN SYNTHESIS

In 1993, Overman and co-workers reported the enantioselective total synthesis of (-)-ptilomycalin A,[41] based upon methodology which utilised an intramolecular variant of the Bigelli condensation to construct the central pyrrolidine. He reported that condensation of (*R*)-urea **22** and (*R*)-β-ketoester **23**, to afford urea derivatives **24** and **25** in a 5:1 ratio. The major product **24** was deprotected about the silyl ethers with TBAF gave bicyclic alcohol **26** which was subsequently converted to the spirotricyclic intermediate **27** *via* a cyclisation reaction catalysed by *p*-TsOH. A coupling constant of 11.5 Hz for the H-4 methine hydrogen in the NMR spectrum of **26** 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 **28** was achieved by heating a methanolic solution of **27** at 60 °C in the presence of *p*-TsOH, yielding a separable 1:2 mixture of tricycles **27** and **28**.

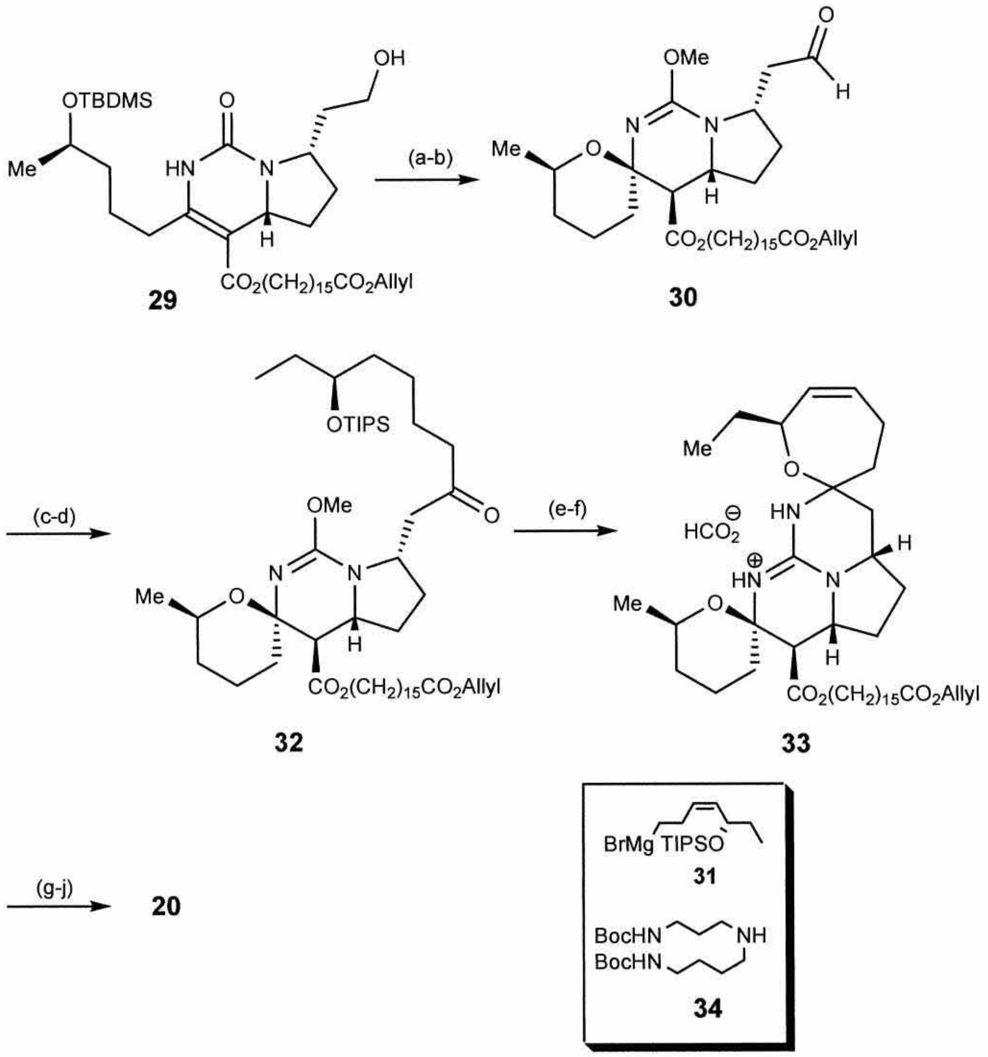


Reagents and Conditions: (a) Morpholine, AcOH, CH_2Cl_2, 70 °C, [5:1/**24**:**25**]; (b) TBAF, 23 °C, 20 h; (c) *p*-TsOH, $CHCl_3$ 23 °C; (d) *p*-TsOH, MeOH, 60 °C, [1:2/**27**:**28**].

Scheme 2.2

Further studies by Overman[42] have led to the enantioselective total synthesis of (-)-ptilomycalin A **20** *via* a ten step procedure using the methodology shown in Scheme 2.3. Swern oxidation of **29** followed by protection and activation of the urea moiety in preparation for guanidine formation by *O*-methyation gave the pseudourea aldehyde **30** in 67 % overall yield. Treatment with 2 equivalents of Grignard reagent **31**, followed by a second oxidation, again under Swern conditions, afforded ketone **32**. The TIPS protecting group was cleaved, using a solution of ammonia and ammonium acetate in *t*-BuOH to give the pentacyclic guanidine core **33** as a single diastereomer.

Cleavage of the allyl ester using $Pd(Ph_3P)_4$ and pyrrolidine gave the resulting acid, which was coupled using EDCl to the *bis*-Boc protected spermidine **34**. The ester was epimerised by heating in a solution of Et_3N in methanol. The Boc protecting groups were cleaved using formic acid to afford (-)-ptilomycalin A **20**.



OTBDMS
OH
Me
HN
N
H
CO2(CH2)15CO2Allyl
29
(a-b)
OMe
N
N
O
H
30
(c-d)
OTIPS
32
(e-f)
HCO2
HN
33
(g-j)
20
BrMg TIPSO
31
BocHN
NH
34

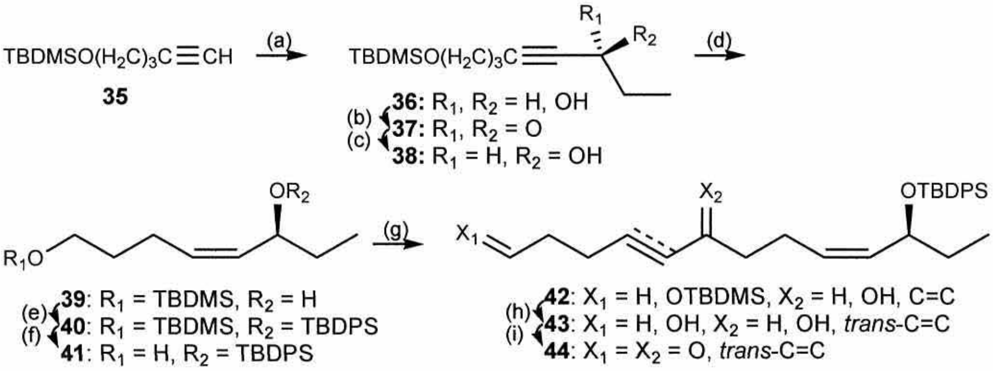
Reagents and Conditions: (a) Swern oxidation; (b) MeOTf, R_3N, 23 °C; (c) **31**, -78 °C; (d) Swern oxidation; (e) TBAF; (f) NH_3, NH_4OAc; (g) $Pd(PPh_3)_4$, pyrrolidine, MeCN, 23 °C; (h) **34**, EDCl, DMAP, DCM, 23 °C; (i) Et_3N, MeOH, 65 °C; (j) HCO_2H, 23 °C.

Scheme 2.3

2.7 THE SNIDER SYNTHESIS

Snider and co-workers also developed a method for the synthesis of ptilomycalin A **20** using a supposed biomimetic approach to the molecule.[43]

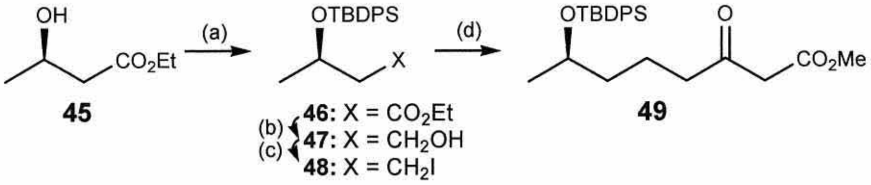
In his convergent synthesis the keto aldehyde **44** was prepared in nine steps from the acetylene **35**. Addition of the lithium acetylide prepared from **35** to propanal gave propargyl alcohol **36**, which was converted to the (*S*)-isomer **38** by a two-step sequence. Swern oxidation gave ketone **37**, asymmetric reduction with *B*-3-pinanyl-9-BBN by Midland's procedure afforded (*R*)-propargyl alcohol **38** in 93 % ee. Reduction of **38** over Lindlar catalyst, protection using *tert*-butyldiphenylsilyl chloride, and cleavage of the *tert*-butyldimethylsilyl ether afforded *cis*-allylic silyl ether **41**. Swern oxidation of **41** afforded an aldehyde that was treated with the lithium acetylide prepared from **35** to provide propargyl alcohol **42** (92 %). LAH reduced the propargyl alcohol and cleaved the silyl ether affording diol **43** (85 %). Swern oxidation provided keto aldehyde **44** (96 %).


TBDMSO(H2C)3C≡CH
(a)
35
TBDMSO(H2C)3C≡
R1
R2
(d)
36: R1, R2 = H, OH
(b)
37: R1, R2 = O
(c)
38: R1 = H, R2 = OH
OR2
R1O
(g)
39: R1 = TBDMS, R2 = H
(e)
40: R1 = TBDMS, R2 = TBDPS
(f)
41: R1 = H, R2 = TBDPS
X2
OTBDPS
X1
42: X1 = H, OTBDMS, X2 = H, OH, C=C
(h)
43: X1 = H, OH, X2 = H, OH, trans-C=C
(i)
44: X1 = X2 = O, trans-C=C

Reagents and Conditions: (a) BuLi, THF/DMPU, -78 °C, CH_3CH_2CHO; (b) Swern ox; (c) 9-BBN, a-pinene, rt, 30 h; (d) H_2, Lindlar catalyst, rt, 1 h; (e) TBDPSiCl, DMAP, Et_3N, DCM, rt, 20 h; (f) PPTS, EtOH, rt, 40 h; (g) Swern ox, acetylide prepared from **35**, *n*-BuLi, THF/DMPU, -78 °C; (h) LAH, THF, reflux, 4 h; (i) Swern ox.

Scheme 2.4

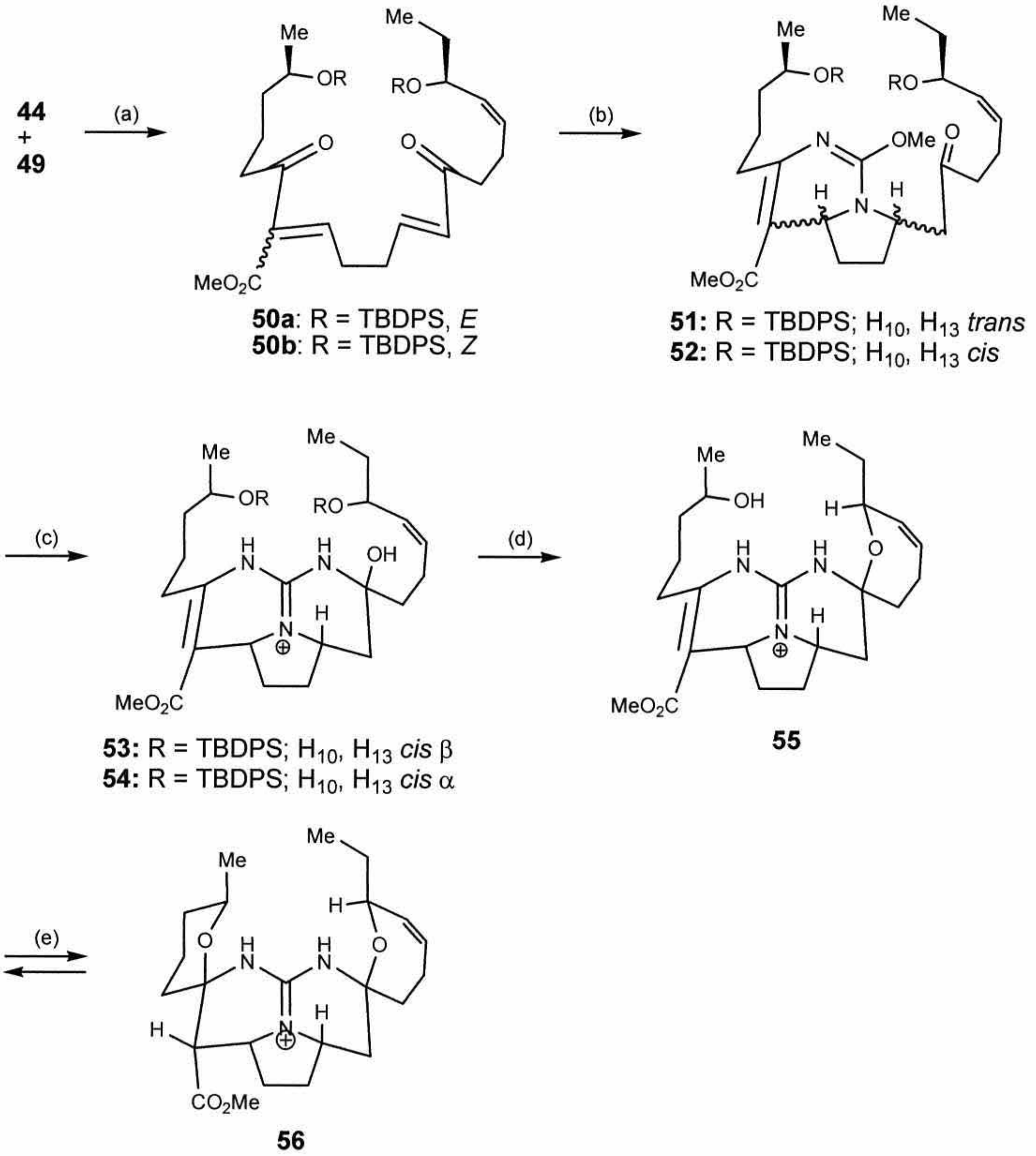
Following this, the β-keto ester was prepared in five steps. Thus the *R*-alcohol **45** was converted to **48** by *tert*-butyl-diphenylsilylation, DIBAL-H reduction, tosylation, and iodide displacement. Alkylation of the dianion of methyl acetoacetate with **48** afforded **49**.



Reagents and Conditions: (a) TBDPSiCI, imidazole, DMF, rt, 3 h; (b) DIBAL-H, hexane, 12 h, -20 °C; (c) TsCI, pyridine, -20 °C , 12 h; Nal, acetone, reflux, 1 h; (d) 2 equiv of LDA then methyl acetoacetate, THF, 0 °C, then **48,** rt, 2 h.

Scheme 2.5

With the two fragments in hand, these are converted to the *bis*-enone ester **50** in 64 % yield as a 1:1 mixture of stereoisomers by Knoevenagel condensation in dichloromethane containing a catalytic amount of piperidine at -78 to -20 °C, 20 h. Heating *bis*-eneone **50** with *O*-methyl-isoureido sulfate and *i*-Pr_2EtN in DMSO at 80 °C for 1.5 h gave a 4:1 mixture of the two *trans*-diastereomers **51** and the two *cis*-diastereomers **52**. **51** and **52** are converted to a 1:1 mixture of **53** and **54** on treatment with excess NH_40Ac in anhydrous *t*-BuOH saturated with anhydrous NH_3 for 40 h at 60 °C in a sealed tube. Deprotection of the *tert*-butyldiphenylsilyl ethers was accomplished by treatment with a 1:2 mixture of 50 % aqueous hydrofluoric acid and acetonitrile for 3 d at -30 °C. Treatment of the crude mixture with Et_3N in MeOH at 60 °C for 16 h led to a mixture of the methyl ester of the pentacyclic core of ptilomycalin A **56** and the diastereomer with an axial methyl ester.



50a: R = TBDPS, *E*
50b: R = TBDPS, *Z*

51: R = TBDPS; H_{10}, H_{13} *trans*
52: R = TBDPS; H_{10}, H_{13} *cis*

53: R = TBDPS; H_{10}, H_{13} *cis* β
54: R = TBDPS; H_{10}, H_{13} *cis* α

55

56

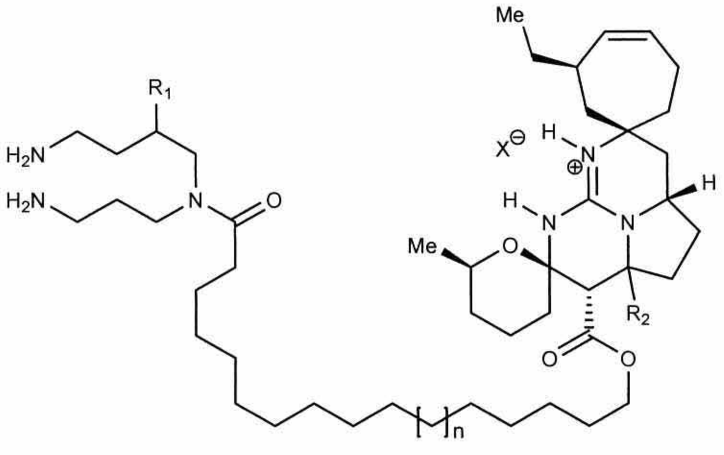
Reagents and Conditions: (a) Piperidine, DCM, -78 °C, -20 °C, 20 h; (b) *0*-methylisourea, *i*-Pr_2EtN, DMSO, 80 °C, 1.5 h; (c) NH_3, NH_4OAc, *t*-BuOH, 60 °C, 40 h; (d) 3:7 HF:CH_3CN, -30 °C, 72 h; (e) Et_3N, MeOH, 60 °C, 20 h; (f) Et_3N, 1:l H_2O-MeOH, 60 °C, 16 h.

Scheme 2.6

2.8 CRAMBESCIDINS

In 1991 Rinehart *et al.* reported the isolation of novel compounds, crambescidins 800 **57**, 816 **58**, 830 **59** and 844 **60**, which were extracted from the

Mediterranean red encrusting sponge, *Crambe crambe*.[44] These compounds were a family of complex pentacyclic guanidines linked *via* a linear ω-hydroxy fatty acid and an ester linkage to a hydroxyspermidine residue, which is nearly identical to ptilomycalin A varying only with respect to the two groups R_1, R_2 and the length of the linker chain.

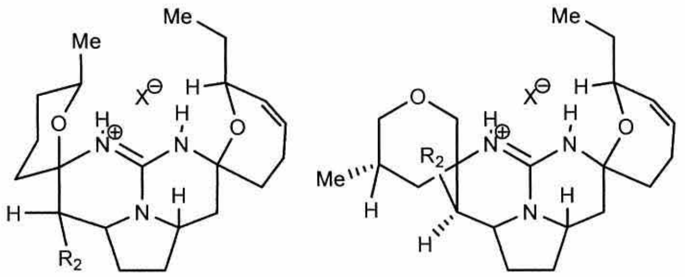


20: $R_1 = R_2 = H$, n = 1
57: $R_1 = H$, $R_2 = OH$, n = 1
58: $R_1 = R_2 = OH$, n=1
59: $R_1 = R_2 = OH$, n=2
60: $R_1 = R_2 = OH$, n=3

Figure 2.8

In biological tests, the crambescidins were found to inhibit HSV-1 growth completely with diffuse cytotoxicity of 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[45] have shown that crambescidin **58** was active towards HCT-16 human colon carcinoma cells (IC_{50} 0.24 μg/ml). In addition, **58** 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.

Further work by Rinehart led to the isolation of a new crambescidin, named 13,14,15-isocrambescidin 800 **61**.[46] This compound was found to have diminished efficacy compared to other crambescicidins towards L1210 cancer cell lines and also showed no activity verses HSV-1. Rinehart suggested that the reason for this loss of biological action is due to stereochemical differences in the structure compared to the other isolated crambescidins. 13,14,15-Isocrambescidin 800 **61** has an overall *anti* configuration about the two spirocycles and the pyrrolidine ring which results in the loss of the ionic "pocket" and consequently, may be the significant factor in the loss of the associated bioactivity.



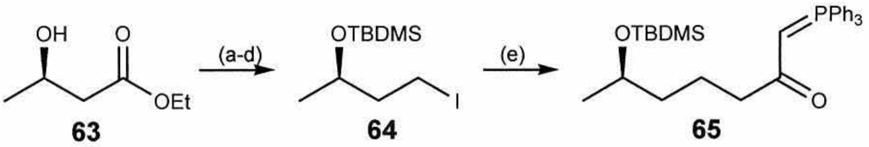
57: $R_2 = C_{24}H_{48}N_3O_3$ **61**: $R_2 = C_{24}H_{48}N_3O_4$

Figure 2.9

2.9 CRAMBESCIDIN 359 TOTAL SYNTHESIS

Crambescidin 359 **62** was isolated from marine sponge *Monanchora unguiculata* by the Braekman group.[47] The structure of **62** consists of a pentacyclic guanidine unit, similar to the related natural product ptilomycalin A **20**. It differs from **20** in that it has no pendent spermidine group *via* a ω-hydroxy fatty chain and ester linkage.

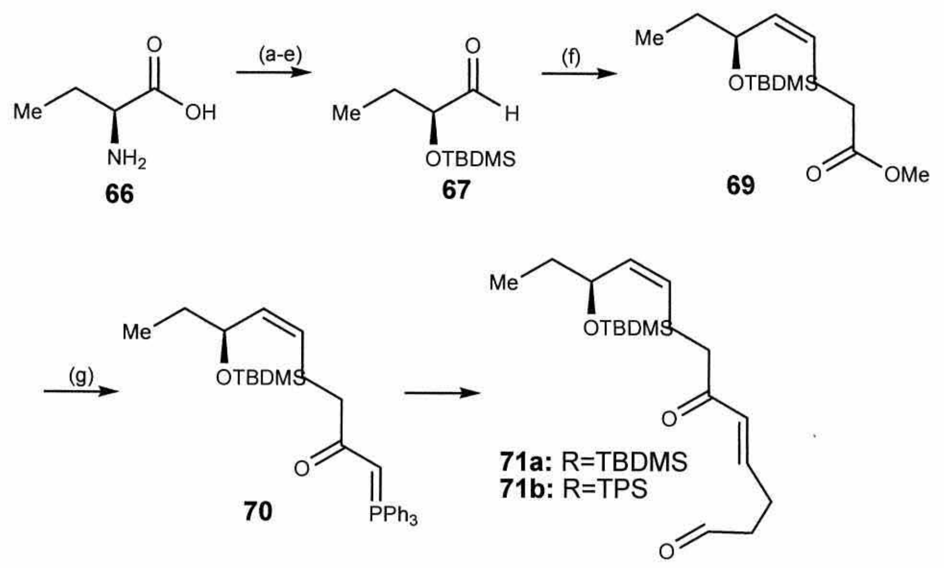
Murphy reported the synthesis of crambescidin 359 **62** *via* a biometric approach.[48] This involved the preparation of ylid **65**, which was obtained in five steps from ethyl- (*R*)-3-hydroxybutyrate **63**. Thus **63** was silyl protected, following which the ester function was reduced to the alcohol and then converted to iodide **64** *via* the corresponding tosylate. This was then treated with the lithium anion of acetylmethylene triphenylphosphorane to afford **65**.



Reagents and Conditions: (a) TBDMSCl, DMF, imidazole, 95 %; (b) DIBAL-H, hexane, -78 to -0 °C, 8 h, 79 %; (c) TosCl, pyridine, 0 °C to rt, 16 h; 78 %; (d) NaI, acetone, reflux, 4 h, 95 %; (e) $CH_3COCHPPh_3$, *n*-BuLi, -78°C, then **64**, warm to rt, 51 %.

Scheme 2.7

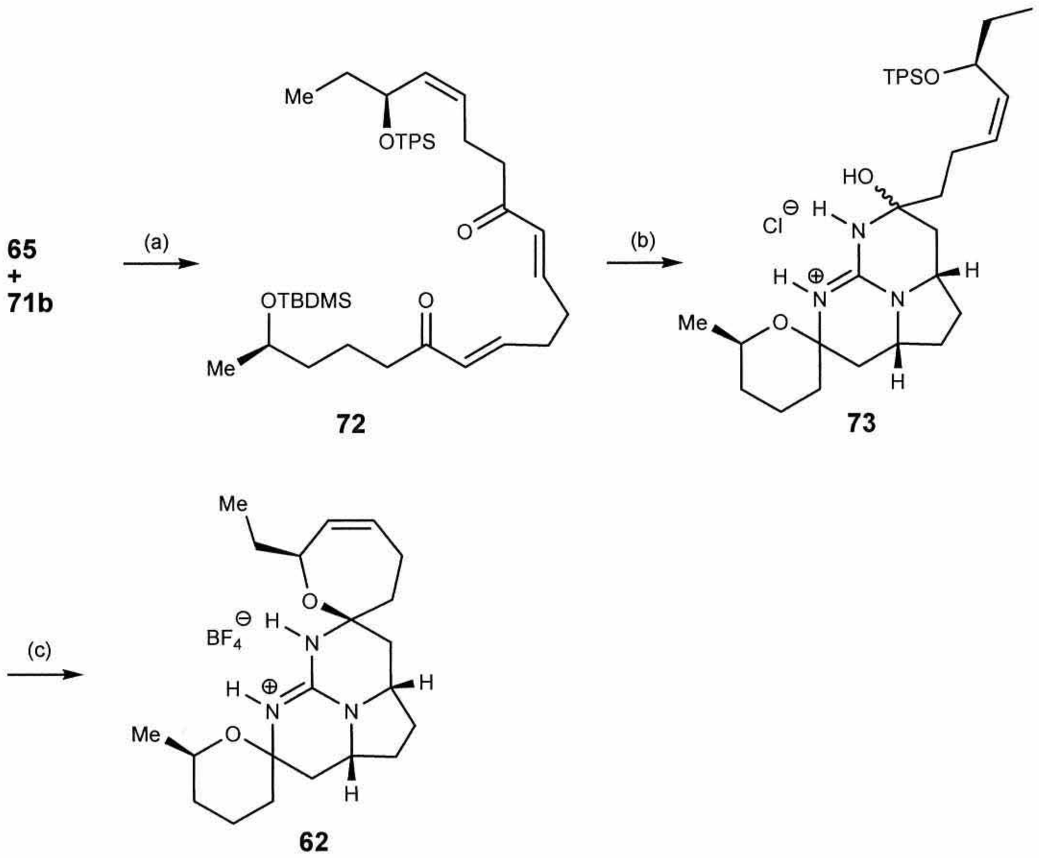
In the next stage of the synthesis, the aldehyde **71a** was prepared in 5 steps from (*S*)-2-aminobutyric acid **66**. Thus **66** was diazotised in the presence of sodium nitrite and 1 M H_2SO_4 to give the corresponding hydroxy acid which was then esterified using HCl in methanol. Silyl protection of the alcohol function was followed by DIBAL-H reduction and Swern oxidation of the resulting alcohol to give aldehyde **67**. Wittig reaction of **67** with the ylid generated from 3-carboxypropyltriphenylphosphonium bromide **68**, followed by esterification with diazomethane gave the *cis*-alkene **69**. *Cis*-**69** was treated with methylenetriphenylphosphorane leading to the stabilized phosphorane **70** which on reaction with an excess of freshly prepared succinaldehyde gave the desired aldehyde **71a**.



Reagents and Conditions: (a) $NaNO_2$, H_2SO_4 0 °C to rt, 24 h; (b) MeOH, HCl, 48 h (23 %, two steps); (c) TBDMSCl, imidazole, DMF, 0 °C to rt, 48 h, 47 %; (d) DIBAL-H, -78 °C to rt, 5 h, 72 %; (e) Swern , 93 %; (f) (i) $MeO_2C(CH_2)_2PPh_3^+$ Br^- **68**, NaHMDS, THF, reflux, 30 min, (ii) **67**, rt, 30 min, (iii) CH_2N_2, Et_2O (45 %, 4:1, *Z*:*E*); (g) (i) 2 equiv. CH_2PPh_3, THF, -78 °C to rt, 2 h, (ii) succinaldehyde, THF, 42 h (42 %, two steps).

Scheme 2.8

The synthesis of the required *bis*-enone **72** was accomplished in 73 % yield by reaction of ylid **7** with aldehyde **71b** in DCM. Addition of guanidine to **72** in DMF, at 0°C. After dilution with water and addition of methanolic HCl, work-up afforded tetracycle **73**. This was then treated with TBAF in THF which afforded acid mediated cyclisation, which after counter ion exchange gave the desired pentacycle **62**·BF_4.



Reagents and Conditions: (a) DCM, 0 °C to rt, 36 h, 73 %; (b) (i) guanidine, DMF, 0 °C, 6 h, (ii) H_2O, MeOH, HCl, 0 °C to rt, 16 h; (c) (i) THF, TBAF, 25–30 °C, 3 h, then rt, 64 h, (ii) MeOH, HCl, 0 °C, 4 h, then $NaBF_4$ (sat., aq.), DCM.

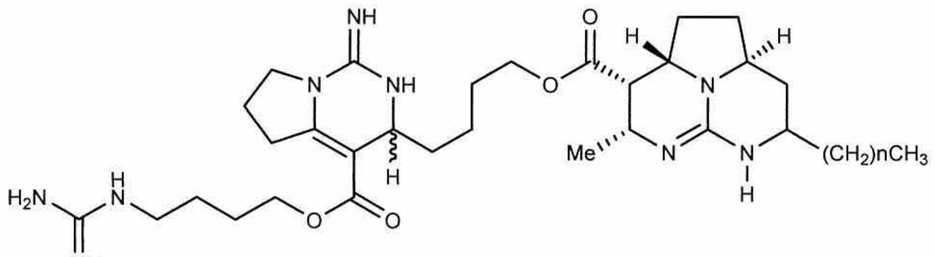
Scheme 2.9

2.10 BATZELLADINES

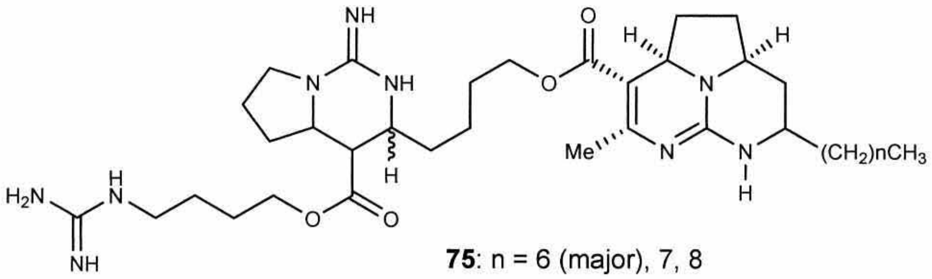
Patil and co-workers first isolated a very interesting guanidine containing marine natural products, named batzelladines A-E, from the Caribbean sponge *Batzella sp.*[49] These guanidines have been shown to disrupt the AIDS infective process; this occurs through inhibition of the binding of the gp120 domain of the HIV-envelope gp160 glycoprotein to the CD4 receptor on the surface on the surface of the human T cell. Batzelladines were also isolated from *Ptilocaulis spiculifer*. Ptilomycalin A **20**, ptilocaulin **11** and crambescidins 800 **57** and 816 **58** were also isolated from the same methanolic extract. Batzelladines A **74** and B **75** have IC_{50}

values of 30 μM for the association of soluble CD4 to immobilised recombinant gp120, batzelladines C **76** and D **77** have shown less activity to the same assay.

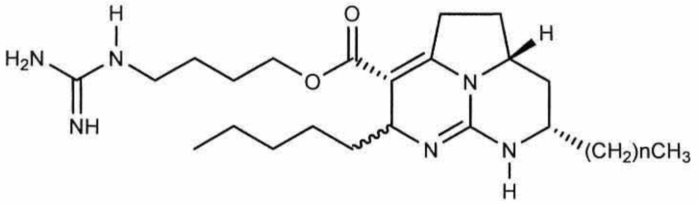
Batzelladines A-D, were isolated along with higher homologues which varied in the length of the alkyl side chain, whereas batzelladine E **78** was identified as a single homologue.[50] The structures elucidate through extensive analysis by means of NMR, mass spectrometry and chemical degradation. Batzelladines A-E all consist of a tricyclic guanidine core coupled to a guanidine-containing side chain. Considerable efforts have been made towards the synthesis of batzelladines, in particular the tricyclic guanidine moieties.[51,52]



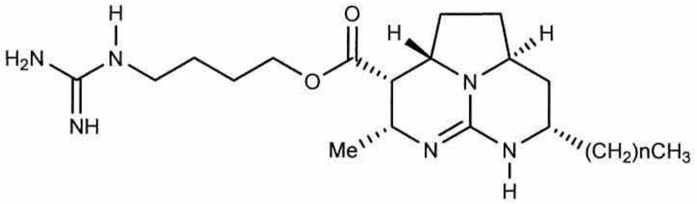
74: n = 8 (major), 9, 10



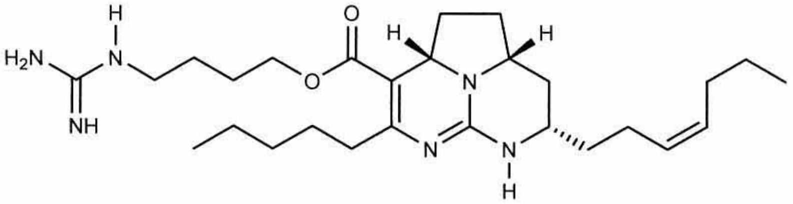
75: n = 6 (major), 7, 8



76: n = 6 (major), 7, 8



77: n = 8 (major), 9, 10

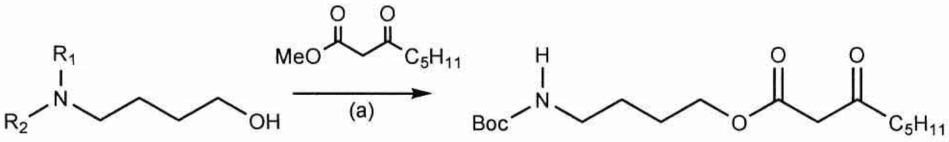


78

Figure 2.10

The first total synthesis of a batzelladine, batzelladine E **78**, was reported by Snider and Chen, in 1998.[53] Their synthesis involved a ten step procedure and resulted in a 3 % overall yield of **78**, as the *bis*-trifluoroacetate salt.

They initially synthesised Knoevenagel precursor **81** from 4-amino-1-butanol **79** which was reacted with di-*t*-butyl dicarbonate and triethylamine to give alcohol **80**. A DMAP catalysed condensation with methyl 3-oxo-octanoate afforded keto ester precursor **81**.



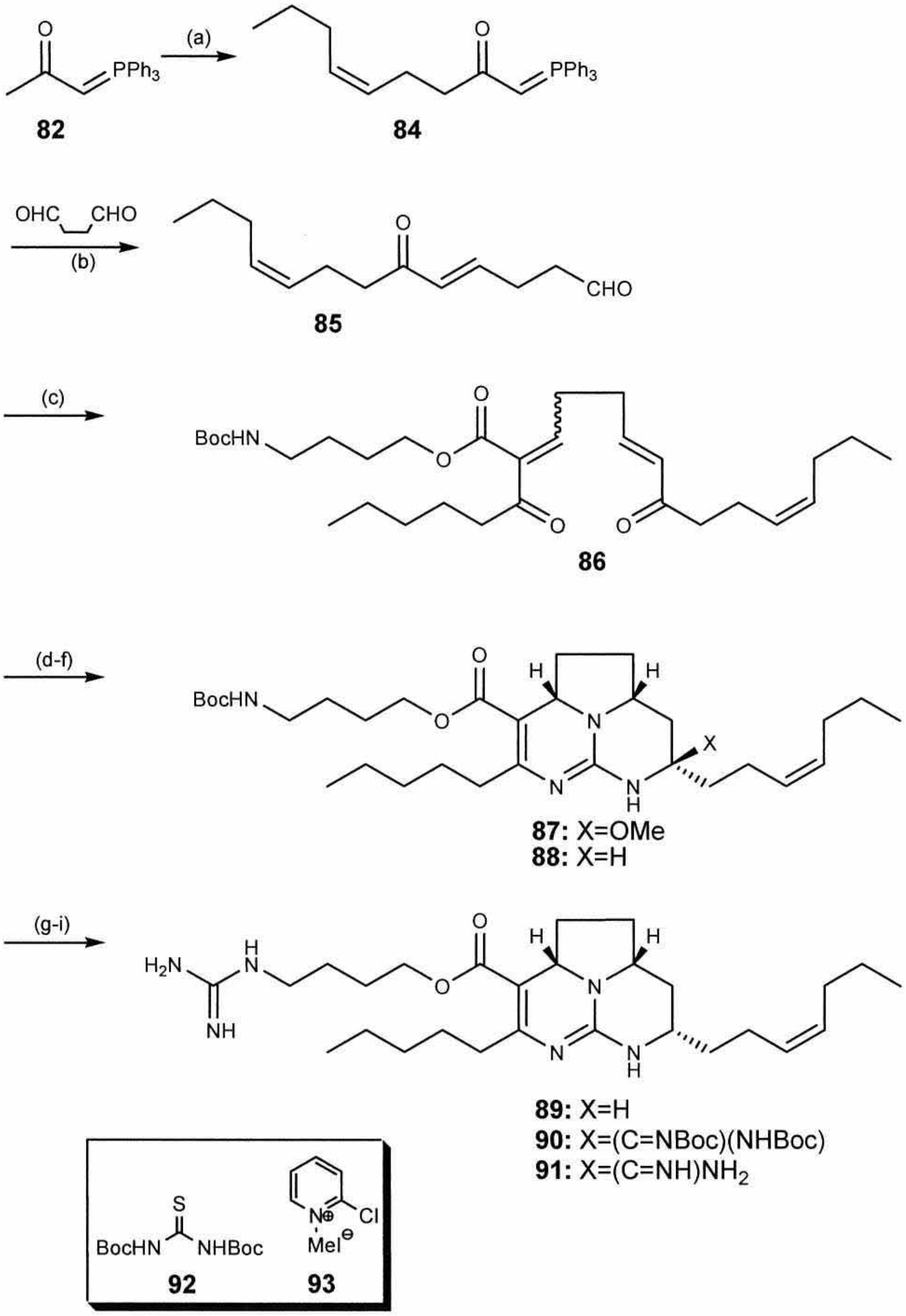
79: R$_1$ = R$_2$ = H
80: R$_1$ = H, R$_2$ = Boc

81

Reagents and Conditions: Di-*t*-butyl dicarbonate, Et$_3$N, DMAP, benzene, 80 °C.

Scheme 2.10

Acetylmethylene triphenylphosphorane **82** was deprotonated with *n*-BuLi and alkylation with 1-bromo-2*Z*-hexene **83** afforded **84**, as described by Murphy for the saturated analogue. Condensation of **84** with succinaldehyde gave **85**. The Knoevenagel condensation between **85** and **81** gave unsaturated aldehyde **86** which was treated with *O*-methylisourea and subjected to ammonolysis to give tricycle **87**. Reduction of **87** with sodium cyanoborohyride in NaH$_2$PO$_4$-buffered MeOH afforded **88** which was deprotected with 1:4 TFA/DCM gave **89**. The synthesis of batzelladine E **91** was completed by guanylation of the terminal NH$_2$ using Lipton's procedure and deprotection with 1:1 TFA/DCM.


82
84
OHC CHO
(a)
(b)
85
(c)
86
(d-f)
87: X=OMe
88: X=H
(g-i)
89: X=H
90: X=(C=NBoc)(NHBoc)
91: X=(C=NH)NH2
92
93

Reagents and Conditions: (a) *n*-BuLi, THF, -78 °C, 1-bromo-2*Z*-hexene **83**; (b) THF, 25 °C, 24 h; (c) **81**, 0.33 equiv piperidine, 0.30 equiv HOAc, DCM, -20 °C, 48 h; (d) *o*-methylisourea, DMSO, *i*-Pr_2EtN, 55 °C, 4 h; (e) NH_3, NH_4OAc, *t*-BuOH, 60 °C, 24 h; (f) $NaCNBH_3$, NaH_2PO_4, MeOH, 25 °C; (g) 1:4 TFA/DCM, 25 °C, 5 min; (h) Et_3N, **92**, **93**, DCM, 25 °C, 1 h.

Scheme 2.11

Following the isolation of these metabolites, a further four batzelladine alkaloids were isolated from the same source. These compounds, named batzelladines F-I were obtained from the methanolic extracts of the sponge following observations during screening of their ability to induce p56lck-CD4 dissociation, (Association of p56lck with CD4 has been shown to result in antigenic activation).[54] The structures of these new batzelladines were determined *via* extensive spectroscopic and chemical analysis and through spectral comparisons with batzelladines A-E. These metabolites are interesting as the left hand guanidine tricycle has no pendant ester group.

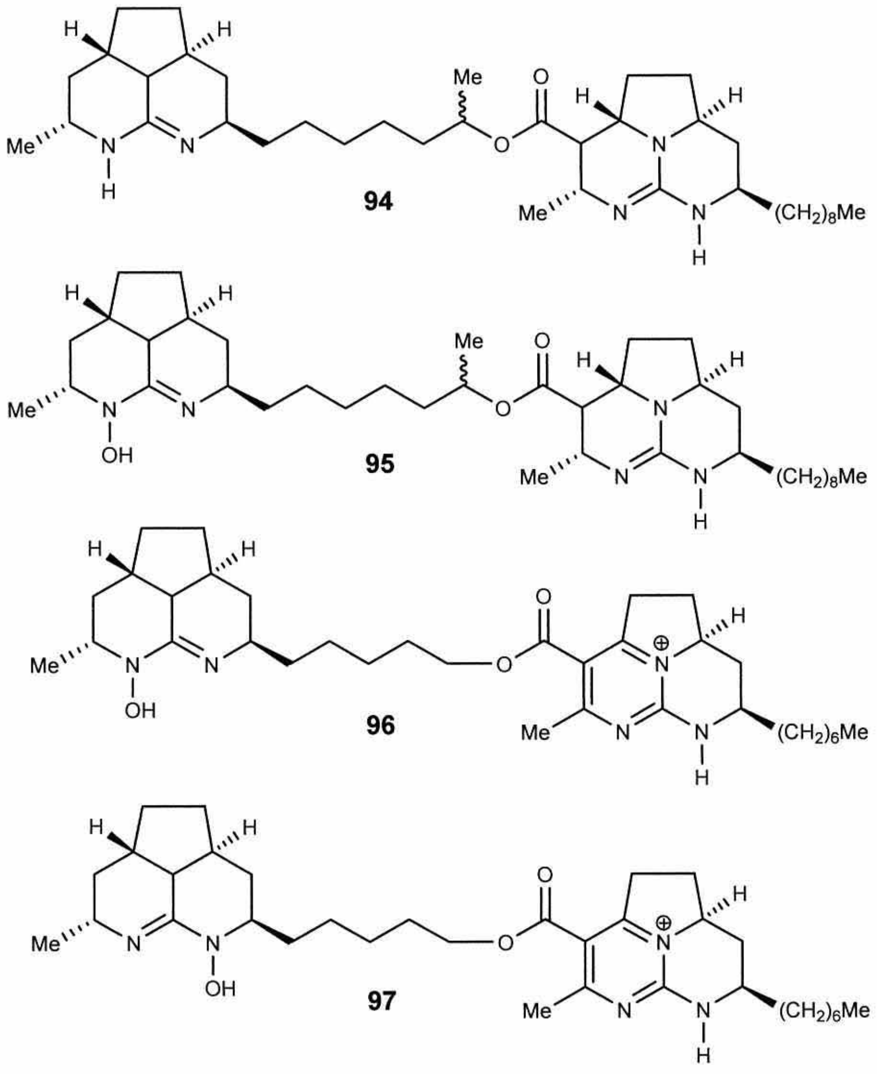
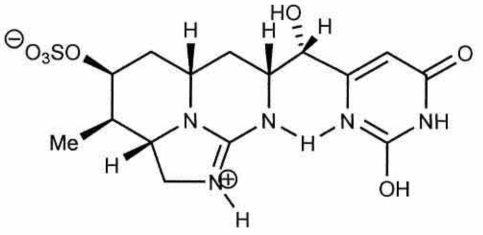


Figure 2.12 Batzelladines F-I

2.11 CYLINDROSPERMOPSIN

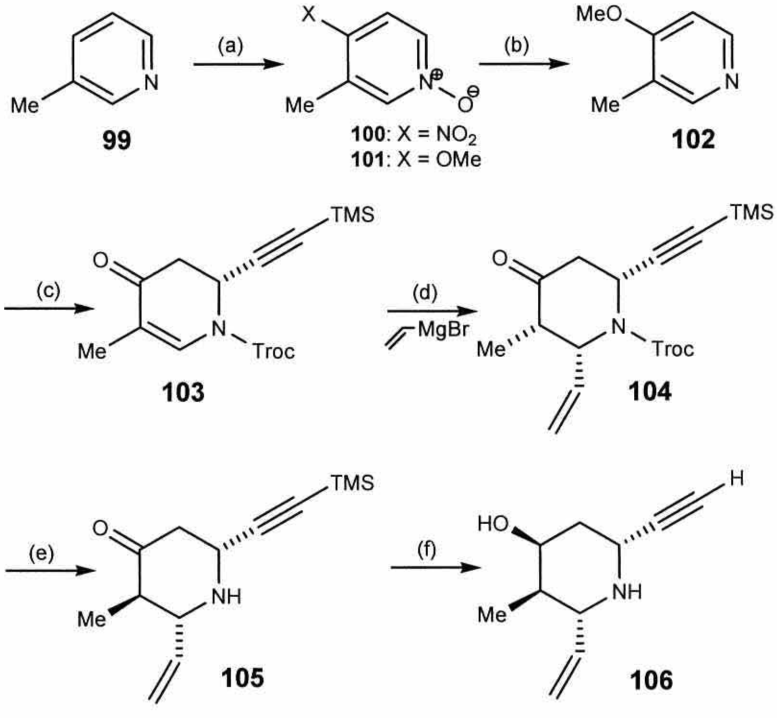
Cylindrospermopsin **98**, was isolated from the cyanobacteria *Cylindrospermosis raciborskii* by Moore *et al.* in 1992,[55] this was shown to be the cause of a severe 1979 outbreak of hepatoenteritis in Northern Queensland, Australia. It has also been extracted from Lake Mikata alga, *Umezakia natans* and exerts toxic effect through inhibition of cell-reduced glutathione.[56] The natural product has a guanidine embedded in a tricyclic system with a high degree of functionality.[57]



98

Figure 2.13

4-Methoxy-3-methylpyridine **102** was prepared from 3-methyl-4-nitropyridine *N*-oxide **100**, which was treated with K_2CO_3 to displace the nitro group providing **101**. Hydrogenolysis of **101** using H_2 over Pd/C reduced the *N*-oxide to provide **102** from **100**. Treatment of **102** with TrocCl and then trimethylsilylethynylmagnesium bromide and hydrolysis of the dihydropyridine with hydrochloric acid afforded **103**. Piperidone **104** was generated by treatment of **103** with vinylmagnesium bromide and TMSCl in the presence of a catalytic amount of $CuBr{\cdot}Me_2S$ in THF. Cleavage of the Troc group of **104** with zinc dust in acetic acid afforded **105**. Reduction of **105** with L-Selectride and basic hydrolysis cleaved the protecting group to afford **106**.

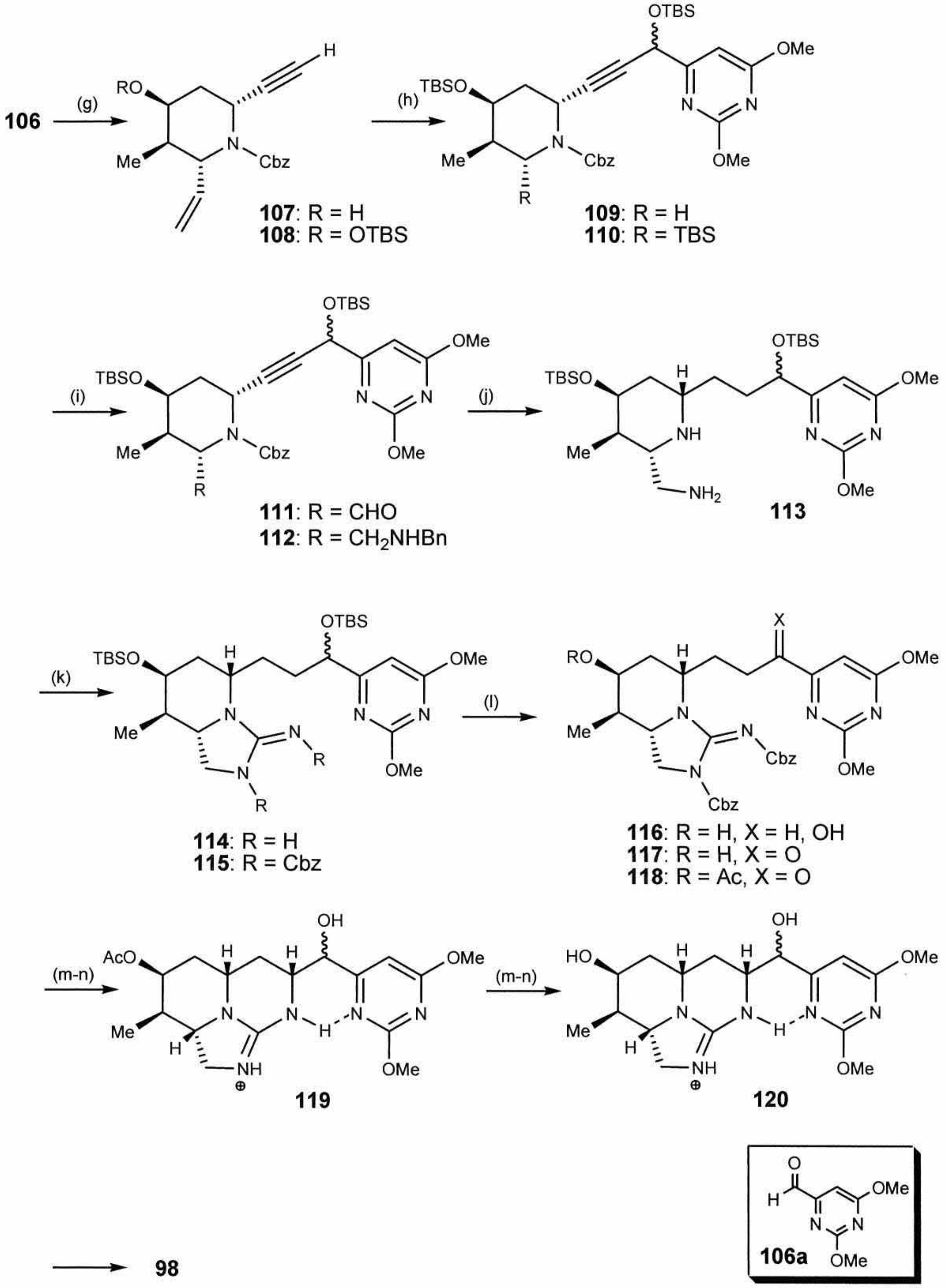


Reagents and Condition: (a) (i) H_2O_2, HOAc, reflux; (ii) HNO_3, H_2SO_4, 95 °C, 24 h; (iii) K_2CO_3, MeOH, reflux, 4 h; (b) 10 % Pd/C, 45 psi H_2, EtOH; (c) TrocCl, THF, -30 °C, $TMSC_2MgBr$, H^+; (d) $CuBr.SMe_2$, TMSCl, THF, -78 °C; (e) Zn, HOAc, 5.5 h; (f) L-Selectride, THF, -78 °C, 90 %.

Scheme 2.14

The amino and hydroxy groups of **106** were protected to give alkyne **108**, which was coupled with aldehyde **106a**. Treatment of **106** with CbzCl and Na_2CO_3 in THF afforded carbamate **107**. Protection of the alcohol of **107** with TBSCl, imidazole and a catalytic amount of DMAP in DCM provided **108**. Treatment of **108** with ethylmagnesium bromide formed the alkynylmagnesium bromide, which was treated with **203** to yield alcohol **109**. Protection of the alcohol of **109** with TBSCl, imidazole, and a catalytic amount of DMAP in DCM gave **110**. Ozonolysis of **110** cleaved the double bond, providing aldehyde **111** after reductive workup with Me_2S.

Condensation of aldehyde **111** with benzylamine in benzene, followed by reduction of the resulting imine with $NaCNBH_3$, afforded benzylamine **112**. Hydrogenation (1 atm) of **112** over 5 % Pd/C in MeOH reduced the triple bond and hydrogenolyzed the benzyl and Cbz groups to afford crude diamine **113**. Addition of 1 equiv of cyanogen bromide to **113** in dilute toluene solution gave the primary cyanamide which cyclised to form guanidine **114**. Excess cyanogen bromide led to the *bis*-cyanamide. Protection of the guanidine with CbzCl rt afforded **115** from **112**. Desilylation of **115** with TBAF at rt gave **116**, which was oxidized with MnO_2 in DCM to form ketone **117**. The alcohol was acetylated with acetic anhydride and DMAP in pyridine at rt, which gave the enol acetate **118**. Bromination of **118** with $CuBr_2$ in EtOAc at rt followed by immediate hydrogenation over 20 % $Pd(OH)_2/C$ gave **119** in 71 % yield from the mixture of acetoxy ketone **118** and the enol acetate. Hydrogenolysis of the Cbz groups afforded the free guanidine, formed the third ring. Hydrolysis of **119** in conc. HCl afforded uracil diol **120**. Reaction of **120** with 10 equiv of SO_3, DMF in anhydrous pyridine and DMF gave a mixture of cylindrospermopsin **98**.


106
(g)
107: R = H
108: R = OTBS
(h)
109: R = H
110: R = TBS
(i)
111: R = CHO
112: R = CH2NHBn
(j)
113
(k)
114: R = H
115: R = Cbz
(l)
116: R = H, X = H, OH
117: R = H, X = O
118: R = Ac, X = O
(m-n)
119
(m-n)
120
98
106a
RO
TBSO
AcO
HO
Me
Cbz
OTBS
OMe
NH
NH2
OH

Reagents and Conditions: (g) (i) CbzCl, Na_2CO_3, THF; (ii) TBDMSCl, imidazole, DMAP, DCM; (h) (i) EtMgBr, THF, 0 °C, **106a;** (ii) TBDMSCl, imidazole, DMAP, DCM; (i) (i) O_3, DMS, DCM, -78 °C. (ii) NH_2Bn, HOAc, Benzene; (iii) $NaCNBH_3$, MeOH; (j) 5 % Pd/C, H_2, MeOH; (k) (i) CNBr, Benzene; (ii) NaH, CbzCl, THF; (l) (i) TBAF, THF; (ii) MnO_2, DCM; (iii) Ac_2O, pyridine, rt; (m) (i) $CuBr_2$, EtOAc, rt, 30 min. (ii) H_2, $Pd(OH)_2/C$, MeOH; (n) (i) conc. HCl, reflux; (ii) SO_3, DMF, pyridine.

Scheme 2.15

2.12 SUMMARY CONCLUSION

It is apparent that the guanidine motif plays an important role in biological systems as it is found in many marine natural products especially sea sponges and to a lesser extent in alkaloids from terrestrial sources. The many roles played by the guanidium group are vast and varied, it has the ability to function as a very strong base, it has a strong affinity for binding anionic substrates and its versatility in catalysis.

CHAPTER 3

AIMS AND PREVIOUSLY SYNTHESISED ANALOGUES OF PTILOMYCALIN A

3.1 AIMS

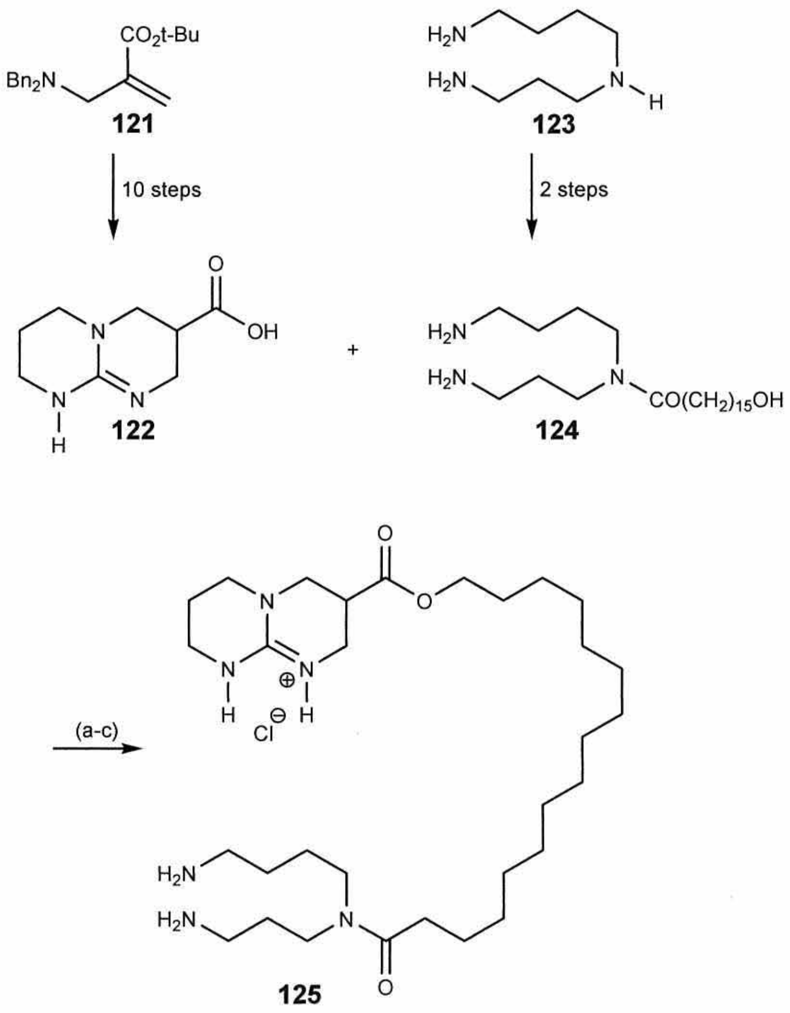
The aims of the research are to develop an effective and efficient methodology for the preparation of synthetic analogues of ptilomycalin A **20** and the crambescidins. As part of this research it is intended to design in certain methods which will allow easy modifications of these molecules to give a range of structurally modified analogues which will be evaluated using various bioassays.

This introductory section summarises the previous work done in this area, largely within our own research group.

3.2 BACKGROUND

Several ptilomycalin A analogues have been prepared to date and have shown significant and interesting biological activity. However, the synthetic routes used to access them have proved somewhat troublesome.

Hart and Grillot were the first to report the synthesis of a ptilomycalin A analogue **125**.[58] This was constructed from a bicyclic guanidine attached *via* an ester linkage to the amido alcohol portion of **124**. The analogue itself was prepared from acrylate **121** which was converted to bicyclic guanidinium salt **122** *via* a ten step route. This was then coupled to alcohol **124** (prepared in two steps from spermidine **123**) yielding the ptilomycalin A analogue **125**. In total, thirteen steps were required from acrylate **121**, to complete a linear sequence that proceeds in 6 % overall yield. Interestingly **125** was found to undergo an unidentified decomposition process over a period of a few weeks that led to cleavage of the ester linkage. Hart suggests that one possible role of the spiro *N,O*-acetal groups in ptilomycalin A might be to sterically protect the ester linkage from hydrolysis.

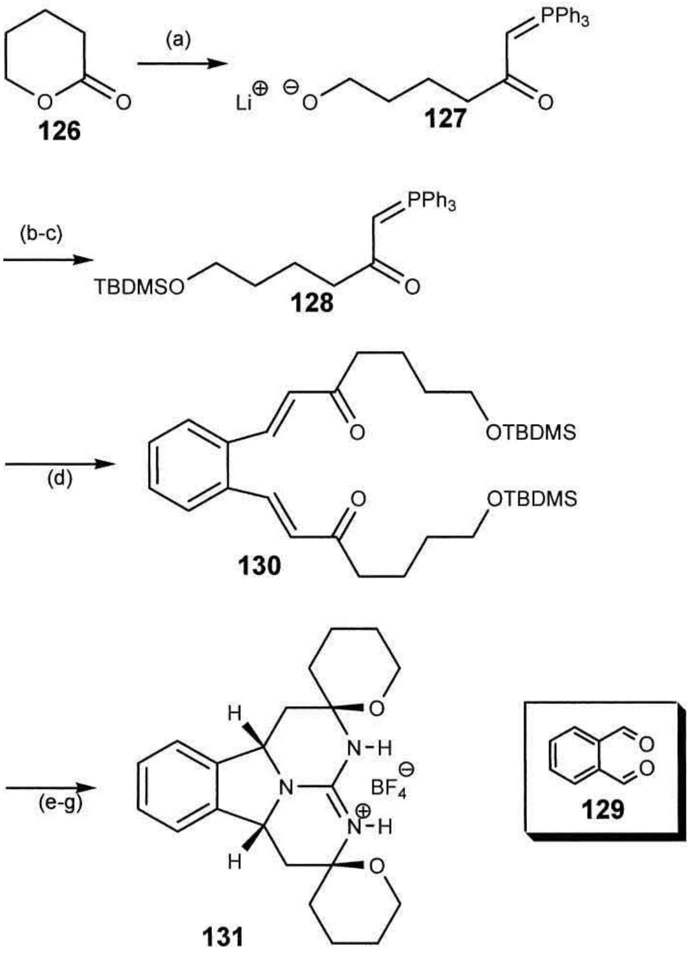

CO2t-Bu
Bn2N
121
10 steps
122
OH
+
H2N
H2N
123
2 steps
124
CO(CH2)15OH
(a-c)
Cl
125

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

Scheme 3.1

3.3 SYNTHESIS OF A BENZOFUSED PENTACYCLIC PTILOMYCALIN A ANALOGUE

The initial investigation pursued in the Murphy research group was the preparation of the benzofused analogue **131**, which was perceived as being a potentially very useful precursor for the synthesis of more complex analogues. The precursor for **131** is the *bis*-enone **130**. As with all described routes in this section this is prepared from an aromatic 1,2-dialdehyde or "phthalaldehyde" and the Wittig reagent **128**, previously reported by Black.[59] This Wittig reagent is prepared by firstly treating methyltriphenylphosphonium bromide with *n*-butyllithium in THF, generating the corresponding methylene ylid. This was then reacted with δ-valerolactone **126** to give the alkoxide ylide **127** which was silylated to give the protected phosphorane **128**. Wittig reaction of **128** with the aldehyde **129**, give the *bis*-α,β-unsaturated ketone **130** in 62 % yield. This was then treated with guanidine in DMF followed by treatment with methanolic HCl to give **131** as a crystalline product in 35 % yield. X-ray analysis of **131** indicated that it was a single diastereoisomer and detailed analysis of the crude reaction mixtures obtained from this process indicated that only a single product is formed. This is of crucial importance to the synthesis of these ptilomycalin A analogues, because if mixtures of diastereoisomers are formed then separation and purification might become problematic.

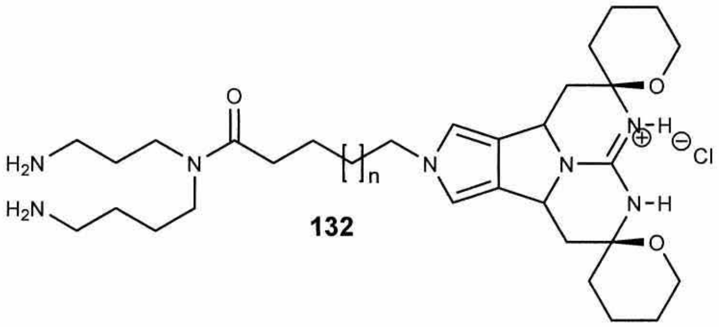

(a)
PPh3
Li⊕ ⊖O
O
126
127
(b-c)
TBDMSO
128
(d)
OTBDMS
OTBDMS
130
(e-g)
H
N-H
BF4⊖
N⊕-H
131
129

Reagents and Conditions: (a) $CH_3PPh_3^+Br^-$, THF, *n*-BuLi, -78 °C, then 0-20 °C, 4 h; (b) δ-valerolactone, -78 °C, then 20 °C, 1 h; (c) DMF, TBDMSCl, imidazole, 0 °C, 30 min, then rt, 15 h; (d) DCM, **129**, 20 °C, 20 h 62 %; (e) DMF, guanidine, 0-20 °C, 5 h; (f) methanolic HCl, 0 °C, 1 h, then 20 °C, 15 h, $NaBF_4$ (sat., aq.), 62 %.

Scheme 3.2

3.4 ATTEMPTED SYNTHESIS OF A PYRROLE LINKED PTILOMYCALIN A ANALOGUE.

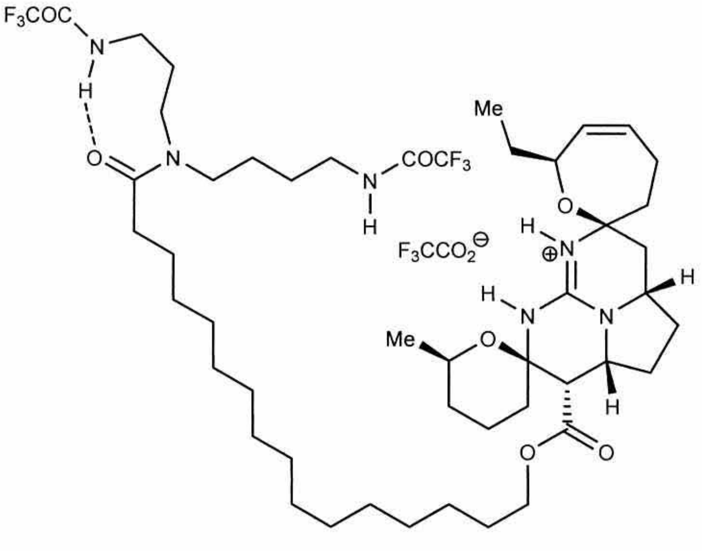
The first attempted synthesis of an actual ptilomycalin A analogue **132** was attempted by Black in 1994.[64]



132

Figure 3.1

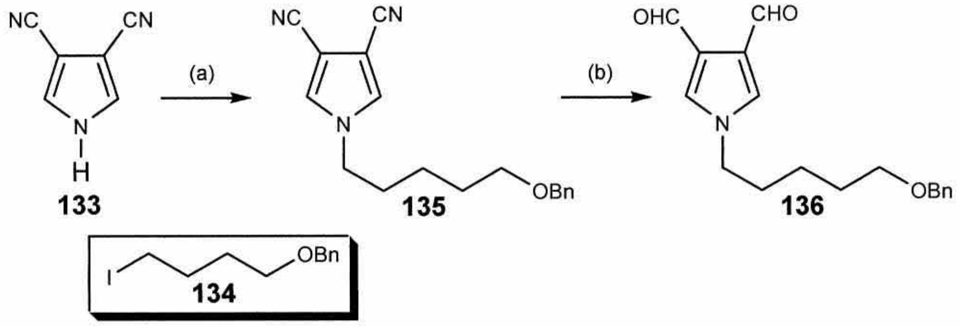
The general concept was to link together the guanidine and spermidine functions *via* a long chain alkyl group which was to be linked to the guanidine *via* a pyrrole. This molecule **132** was of interest as it is linear in nature and should be similar to a "linear" ptilomycalin A as opposed to the "encapsulated" model **21** which was proposed by Kashman.



21

Figure 3.2

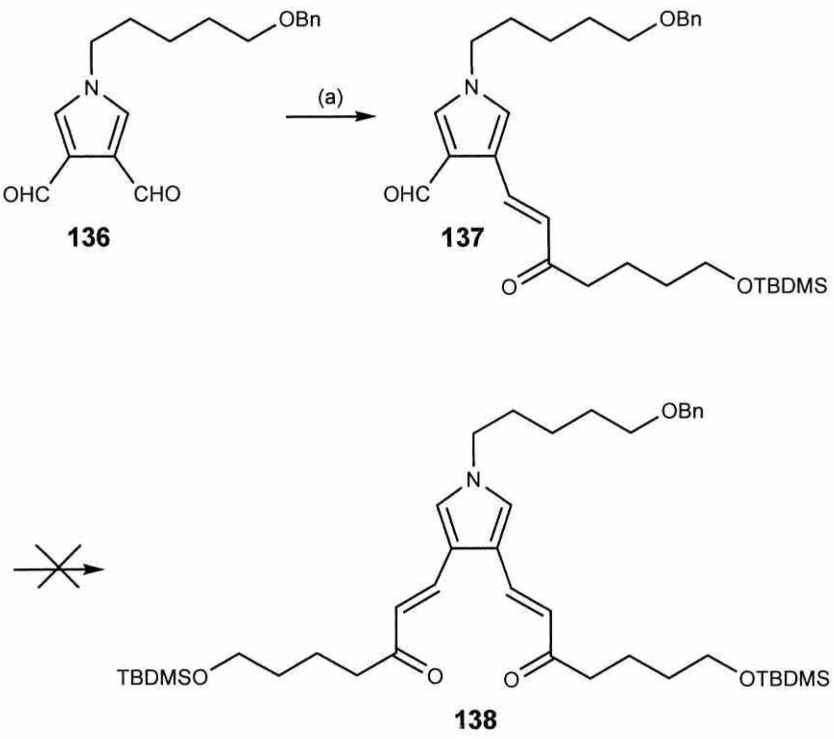
The basic synthetic route began with the known 3,4-dicyanopyrrole **133** which was alkylated with iodide **134** to give **135**. That was reduced with DIBAL-H to give the dialdehyde **136** in good yield.



Reagents and Conditions: (a) **134**, NaH, DMF, 0 °C, 5 min, then rt, 3 h, 76 %; (b) DIBAL-H, toluene, -78 °C, 2 min, then -30 °C, 80 min, 80 %.

Scheme 3.3

With this material in hand the next step was to react it with ylide **128**. However the double Wittig reaction to give **138** was not successful only the mono alkylated product **137** was obtained.

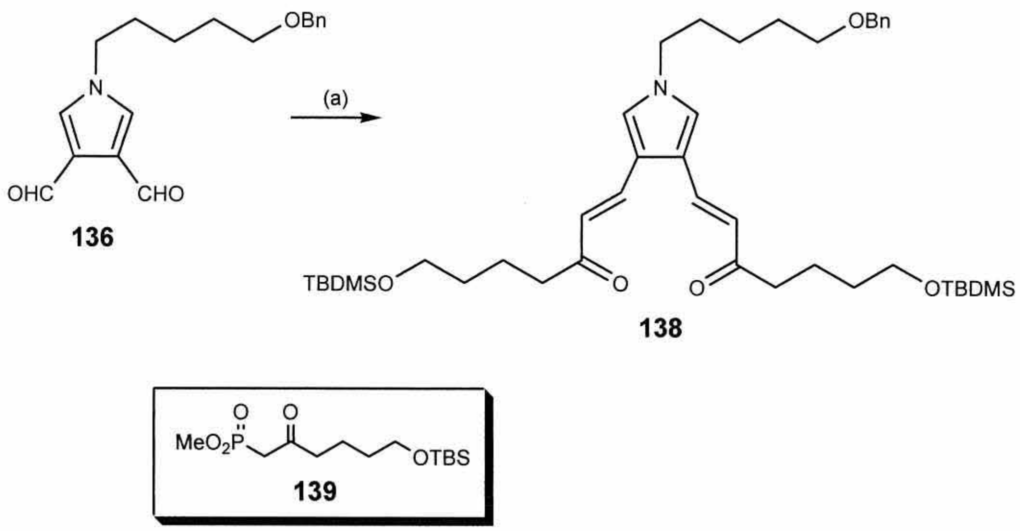

OBn
N
OHC
CHO
136
(a)
OBn
N
OHC
137
O
OTBDMS
OBn
N
TBDMSO
O
O
OTBDMS
138

Reagents and Conditions: (a) 4 equiv. **128**, DCM, 20 °C.

Scheme 3.4

This lack of reaction was probably due to either steric or electronic factors possibly that the conjugated vinylogous nature of the intermediate mono-Wittig product would lead to a lowering of the reactivity of second aldehyde function.

After this failure, reaction of the dialdehyde **136** under more forcing Wadsworth-Emmons conditions was attempted. Thus dialdehyde **136** was reacted with the phosphonate **139** to give the *bis*-enone **138** in an excellent 73 % yield.



Reagents and Conditions: (a) (i) 3 eqv., **139**, NaH, DME, 30 min; (ii) 20 °C, 24 h, 73 %.

Scheme 3.5

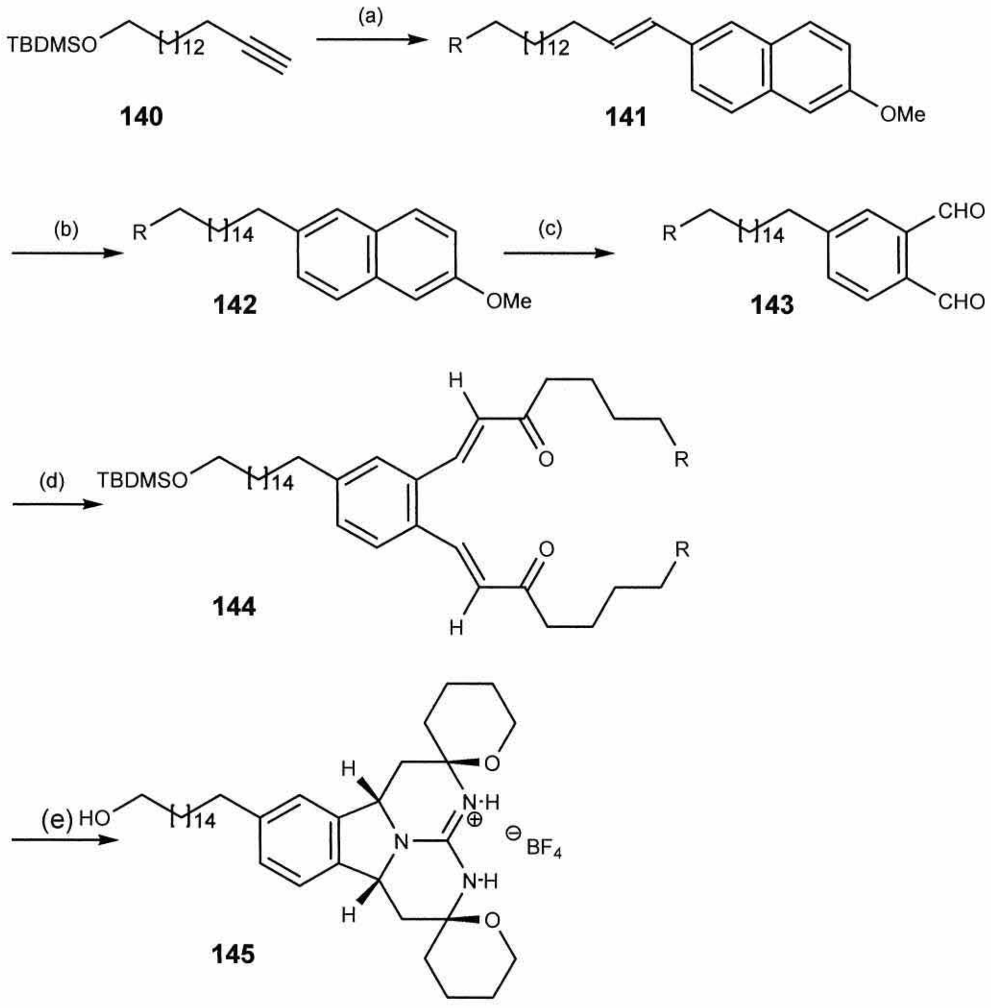
With **138** available the reaction with guanidine under the previously developed conditions was attempted several times but consistently met with failure, only decomposition being observed.[59]

3.5 PREPARATION OF AN ALKYL TETHERED PTILOMYCALIN A ANALOGUE

The lack of success of the pyrrole based methodology was attributed to the reactivity of the pyrrole heterocycle. It is plausible that the heterocycle is reactive towards guanidine which leads to decomposition.

The next investigation by Moore focused on a linker analogue based on the benzo group linked directly to an alkyl chain. The tether chain for this molecule was thus synthesised from 7-hexadecyn-1-ol **140**, by initially isomerising the C-7 alkyne bond to the C-16 by means of the "Zipper Reaction".[60] Hydroboration of **140** with catecholborane was followed by Suzuki reaction with 6-bromo-2-

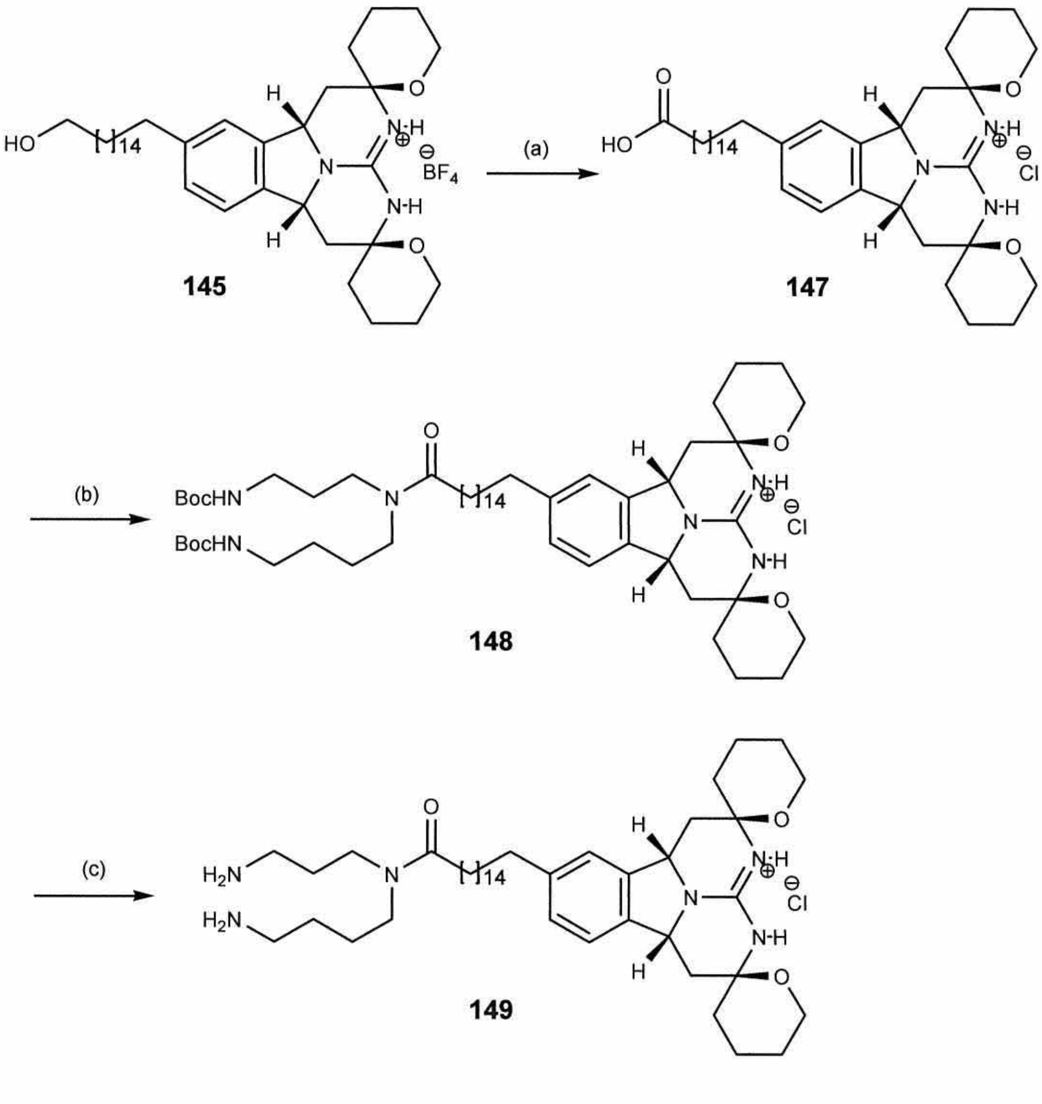
methoxynaphthalene to give alkene **141**. Hydrogenation of the double bond in **141** was followed by ozonolysis gave the dialdehyde **143**. The methoxy group was present in the molecule to direct the selectivity of the ozonolysis to the methyoxy containing ring, despite this the yields for this reaction were still generally poor. The dialdehyde was then reacted with four equivalents of phosphorane **128** for two days to give *bis*-α,β-unsaturated ketone **144**. The *bis*-enone **144** was then subjected to guanidine cyclisation protocol to give the pentacyclic product **145** again in good yield.


TBDMSO
12
140
(a)
R
12
141
OMe
(b)
R
14
142
OMe
(c)
R
14
143
CHO
CHO
(d)
TBDMSO
14
144
H
O
R
O
R
H
(e)
HO
14
145
H
N·H
⊕
N
⊖ BF4
N·H
H
O
O

Reagents and Conditions: (a) Catecholborane, reflux, 3 h, then rt, 16 h, then C_6H_6, $Pd(PPh_3)_4$, 2-bromo-6-methoxynaphthalene, 2.5 h, then 2N NaOEt/EtOH, reflux, 2 h, then 3N $NaOH/H_2O$, rt, 16 h;

(b) EtOAc, Pd/C, H_2, 10 min, 91 %; (c) DCM, O_3, -78 °C, PPh_3, 5 h, 53 %; (d) DCM, 4 eqv. **128**, rt, 48 h, 70 %; (e) (i) Guanidine, DMF, 0 °C to rt, 7 h; (ii) Methanolic HCl, 0 °C to rt, 14 h; (iii) $NaBF_4$ (sat., aq.), 25 %.

Scheme 3.6

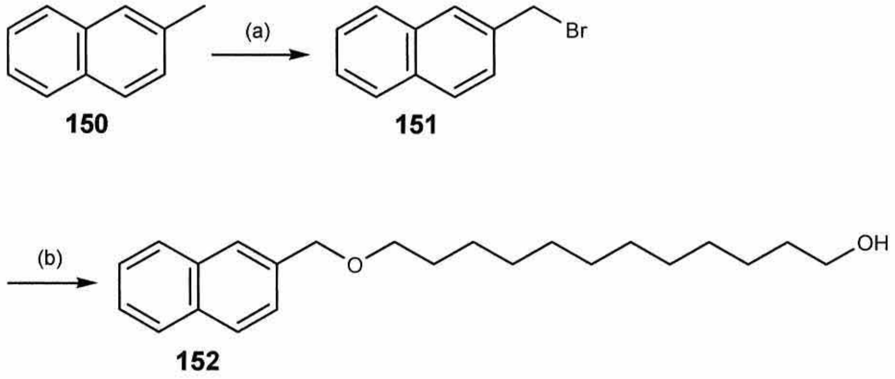

HO
14
H
N-H
BF4
N
O
145
(a)
O
HO
147
Cl
(b)
BocHN
BocHN
148
(c)
H2N
H2N
149

Reagents and Conditions: (a) PDC, DMF, rt, 24 h, 55 %; (b) **34**, EDCl, HOBT, DCM, rt, 18 h, 88 %; (c) TFA, $CHCl_3$, rt, 1 h, 100 %.

Scheme 3.7

3.6 SYNTHESIS OF AN ETHER LIKED PTILOMYCALIN A ANALOGUE.

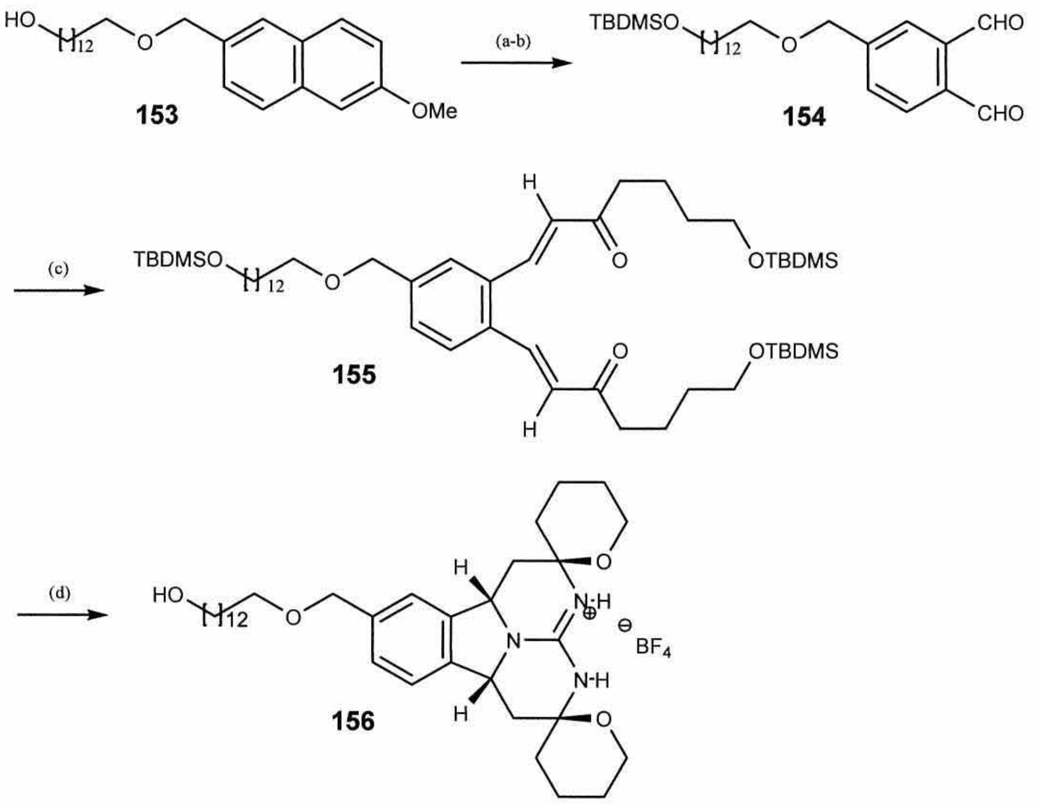
In related work, Howard-Jones prepared an analogue of ptilomycalin A in which the linker between the benzo group and the alkyl chain is an ether function. The synthesis began with the bromination of 2-methylnaphtalene in CCl_4 using NBS and AIBN. The resulting bromomethylnaphthalene was reacted with 1,12-dodecane diol in the presence of NaH in THF to give the monoalkylated product **152**.



Reagents and Conditions: (a) NBS, AIBN, CCl_4, reflux 1.5 h, 85 %; (b) $HO(CH_2)_{12}OH$, NaH, *tetra-n*-butyl ammonium iodide, DMF, THF, 64 %.

Scheme 3.8

This alcohol **152** was then protected and ozonised to give dialdehyde **154** which was reacted with four equivalents of phosphorane **128** for 48 hours to give *bis*-enone **155** in good yield. Reaction of **155** under the standard conditions gave the pentacyclic guanidine **156** in good yield.

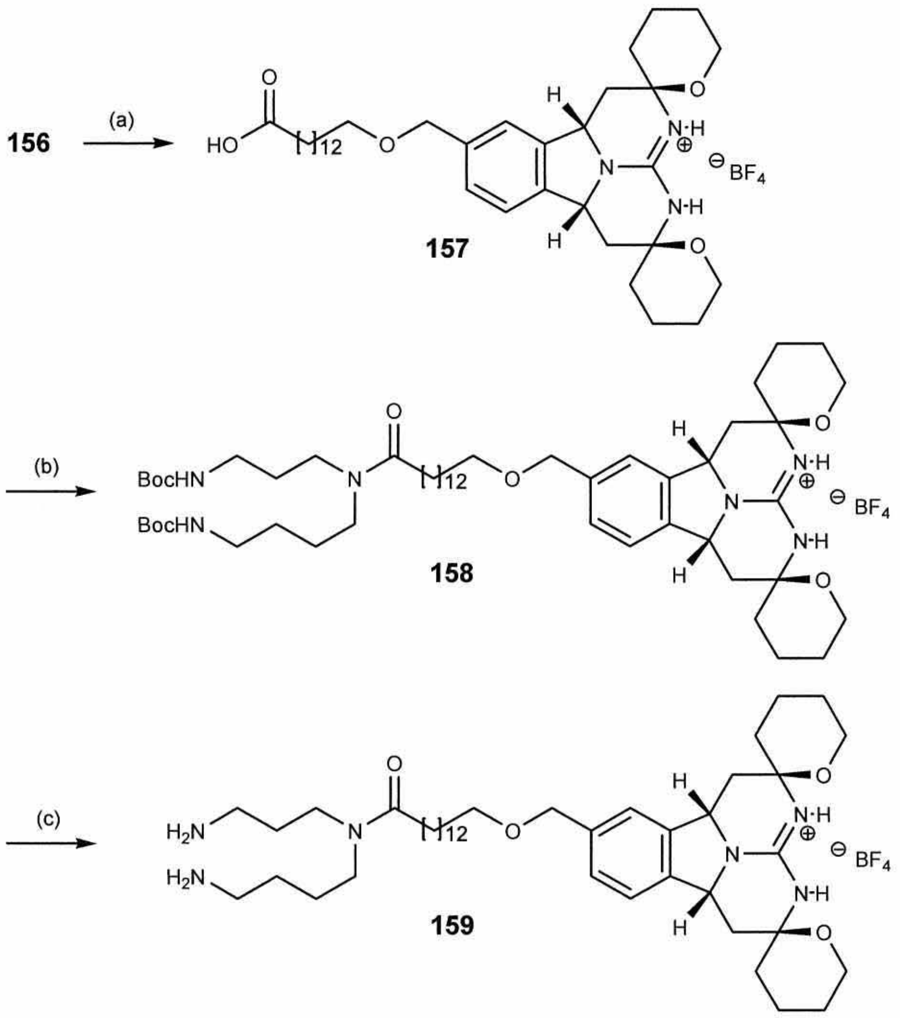


HO
12
O
153
OMe
(a-b)
TBDMSO
12
O
CHO
154
CHO
(c)
TBDMSO
12
O
H
O
OTBDMS
155
O
OTBDMS
H
(d)
HO
12
O
H
N-H
⊕
⊖
BF4
N
N-H
156
H
O
O

Reagents and Conditions: (a) TBDMSCl, imidazole, DMF, 16 h; (b) DCM, O_3, -78 °C, 1 h, then PPh_3, 1 h, 11 %; (c) DCM, 4 eqv. **73A**, reflux, 5 h, 76 %; (d) (i) Guanidine, DMF, 0 °C - rt, 12 h; (ii) Methanolic HCl, 0 °C - rt, 14 h, 20 %.

Scheme 3.9

Modification of **156** to the desired ptilomycalin A analogue **159** was accomplished using the same methodology to that employed by Moore and proceeded effectively.


156
(a)
157
(b)
158
(c)
159
BF$_4$
BocHN
H$_2$N

Reagents and Conditions: (a) PDC, DMF, 48 h, rt, 46 %; (b) **34**, HOBT, EDCl, DCM, 16 h, rt, 87 %; (c) HCl, MeOH, 4 h, rt, 100 %.

Scheme 3.10

CHAPTER 4

BIOLOGICAL ACTIVITY

4.1 BIOLOGICAL ACTIVITY

The model compounds prepared in the studies in the Murphy group were assayed against a range of cancer cell lines and the results compared with the natural products ptilomycalin A **20** (supplied by Kashman) and the synthetic crambescidin 359 **62**.

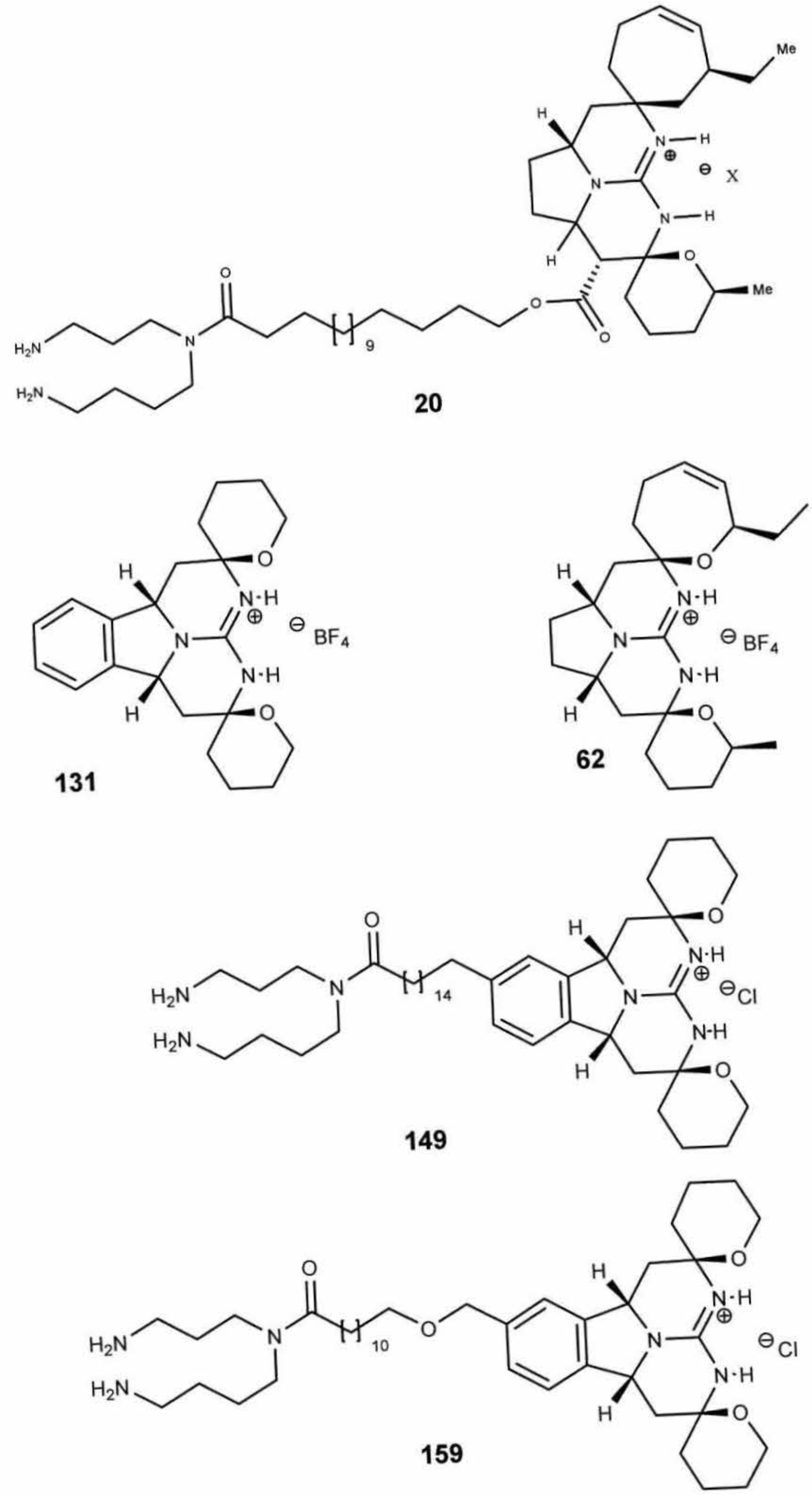

20
131
62
149
159
H₂N
N
O
9
14
10
Me
X
BF₄
Cl
N-H
H

Figure 4.1

Entry	Analogue	K562[a] IC_{50} µg/ml	A2780[b] IC_{50} µg/ml	H-460[c] IC_{50} µg/ml	P388[d] IC_{50} µg/ml
1	Benzo Analogue **131**	24.93	22.06	21.36	9.11
2	Crambescidin 359 **62**	12.30	24.44	10.36	2.93
3	Ether Analogue **159**	7.28	11.02	5.87	4.25
4	Alkyl Analogue **149**	0.52	0.92	0.52	0.69
5	Ptilomycalin A **20**	0.35	0.27	0.35	0.11

a = Human chronic myelogenous leukaemia.
b = Human ovarian carcinoma.
c = Human large cell carcinoma, lung. High DT-Diaphorase.
d = Mouse lymphoid neoplasm.
NT = Not Toxic.

Table 4.1

The initial observations are that the compounds which do not possess the spermidine side chain and the linker/spacer units (entries 1 and 2, Table 4.1) are largely devoid of activity when compared to ptilomycalin A (entry 5). This is in agreement with a report by Snider who reported that the methyl ester of the ptilomycalin A pentacycle **20** was also largely inactive in a range of screens.[43]

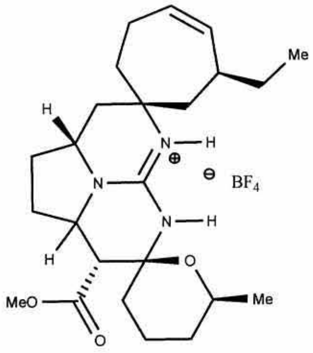


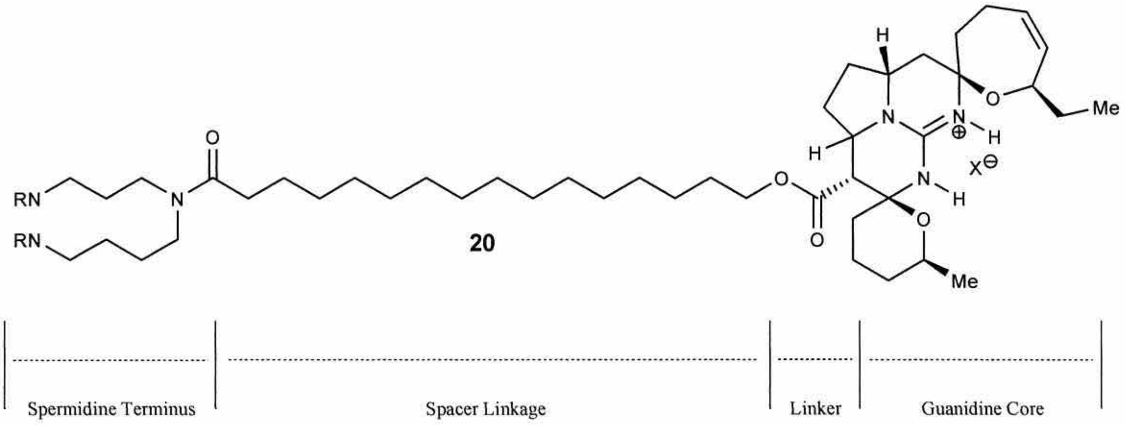
Figure 4.2

Compared to this the analogue prepared by Howard-Jones **159** displayed considerable activity (entry 4) and the analogue of Moore **149** gave activity approaching that of ptilomycalin A which is of significant importance.

4.2 CONCLUSIONS

The disadvantages of the syntheses reported to date is their linear nature. Both key synthetic routes have also depended on an ozonolysis step which has proved to be consistently low yielding.

The work performed by Moore and Howard-Jones has demonstrated that analogues of ptilomycalin A have comparable biological activity to the natural material. Conceptually ptilomycalin A can be considered to consist of four discrete parts, the guanidine core, the spermidine terminus, the linker and the spacer group as shown in Figure 4.3.


20

Spermidine Terminus
Spacer Linkage
Linker
Guanidine Core

Figure 4.3

The goal of this current work is to develop a methodology which will allow the rapid assembly of a range of synthetic mimics of ptilomycalin A containing the four basic units required for activity, however with variation in the spacer chain length. The model used in this work is shown in Figure 4.4.

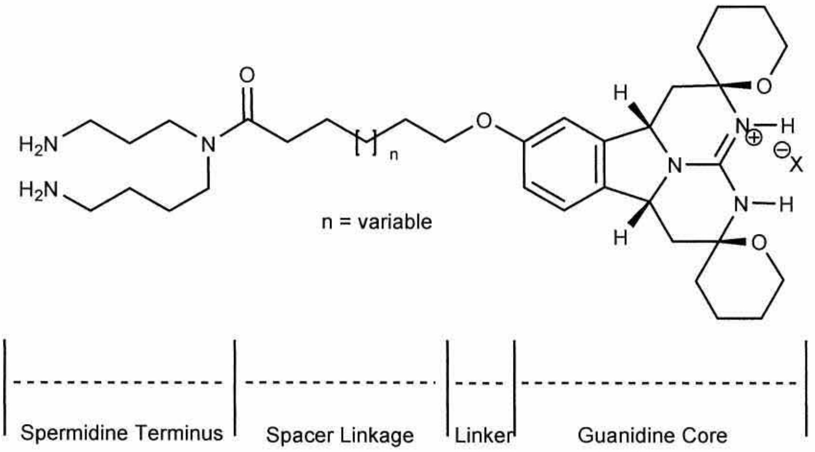

n = variable
Spermidine Terminus
Spacer Linkage
Linker
Guanidine Core

Figure 4.4

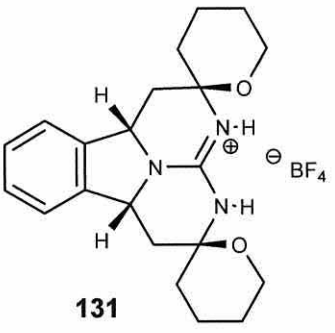
It was intended to address a key factor in this system namely the role of the spacer linkage in these molecules. It is proposed that it is highly likely that the distance between the guanidine and the spermidine functions is key to the biological activity of these systems.

CHAPTER 5

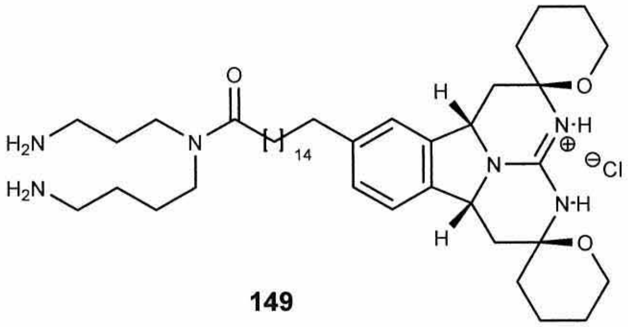
RETROSYNTHETIC ANALYSIS OF THE LINEAR MODEL

5.1 RETROSYNTHETIC ANALYSIS OF THE LINEAR MODEL

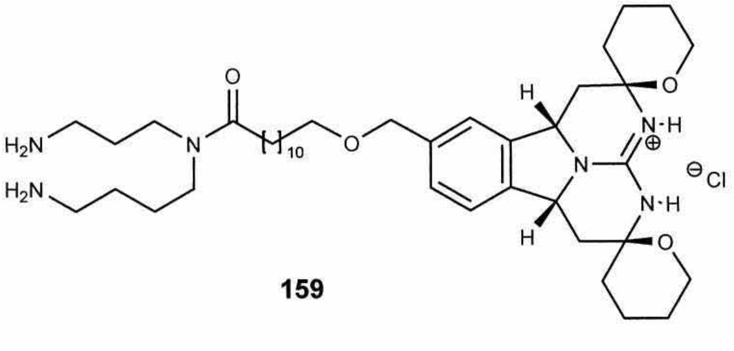
The model systems studied by Moore and Howard-Jones are obviously based upon the pentacyclic benzo-fused core **131**, previously prepared by Black.[61]


H
O
N·H
⊕
⊖ BF4
N
N·H
H
O

131


O
H2N
H2N
N
14
H
O
N·H
⊕
⊖Cl
N
N·H
H
O

149


O
H2N
H2N
N
10
O
H
O
N·H
⊕
⊖Cl
N
N·H
H
O

159

Figure 5.1

As noted previously the main difference between of analogues **149** and **159** is the linker function, which is a carbon-carbon bond in **149** and methylene ether as shown in structure **159**, the nature of the linker is obviously dictated by the methodology used to prepare them. It was our initial intention to replace the linkers already used with an ether function directly attached to the aromatic ring as shown in structure **160**, adopting a similar linear approach to that used previously. The retrosynthesis follows a similar line to those adopted before in that disconnection of the spermidine function, with the FGI of the COOH group, with protection leads to alcohol **161**. Disconnection of guanidine leads to the key intermediate the *bis*-enone **162** which, when the three pendant chains are disconnected leads to the dialdehyde **163**.

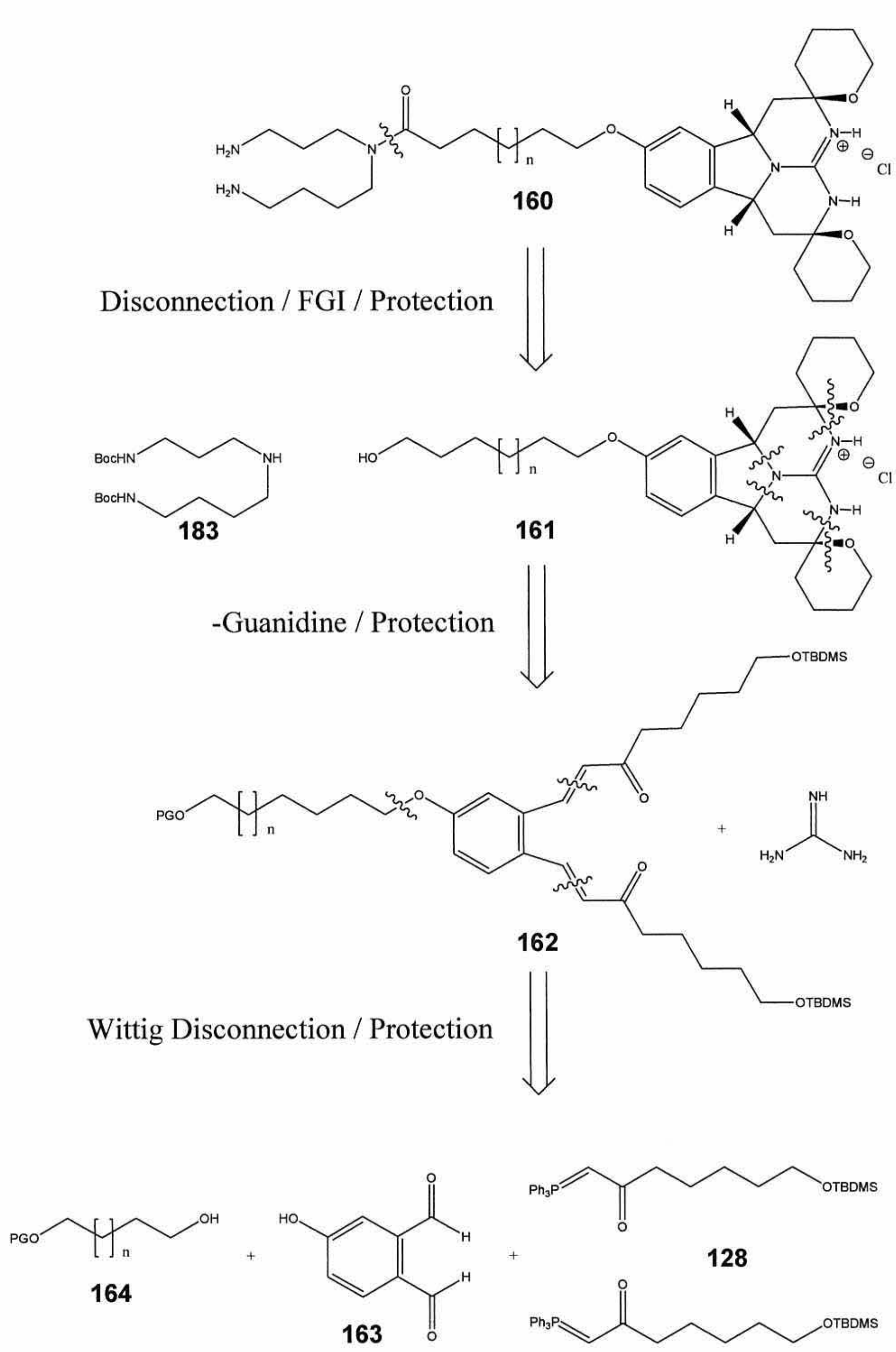
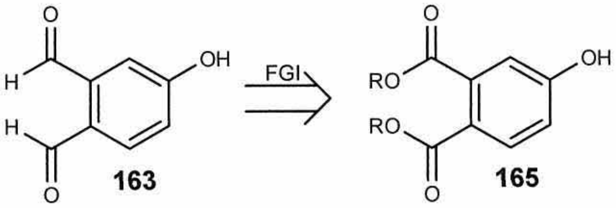

H₂N
H₂N
O
n
O
160
H
H
N
N-H
⊕
⊖
Cl
N-H
O
O
Disconnection / FGI / Protection
BocHN
NH
BocHN
183
HO
n
O
161
H
H
N-H
N
N-H
Cl
-Guanidine / Protection
OTBDMS
PGO
n
O
O
O
NH
H₂N
NH₂
+
162
OTBDMS
Wittig Disconnection / Protection
PGO
n
OH
164
HO
O
H
H
O
163
Ph₃P
O
OTBDMS
128
Ph₃P
O
OTBDMS
+
+

Figure 5.2

Thus the proposed synthetic route requires a synthesis of **163** or its synthetic equivalent. As ozonolysis routes have proved somewhat low yielding, it is proposed that it should be possible to access this synthon *via* a reductive sequence from the diester **165**.



163

165

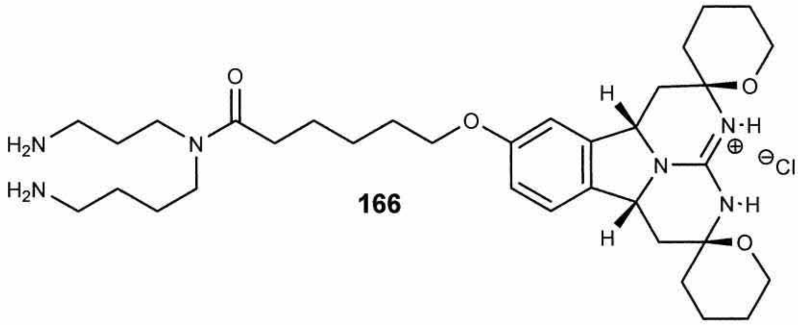
Figure 5.3

Chapter 6

Linear Synthesis of Pentacyclic Model of Ptilomycalin A

6.1 LINEAR SYNTHESIS ROUTE

Based on the retrosynthetic model it was initially wished to access a short chain length analogue of ptilomycalin A **166**. Ultimately this would be prepared from the phenol **170** and we required an efficient synthesis of this key intermediate.



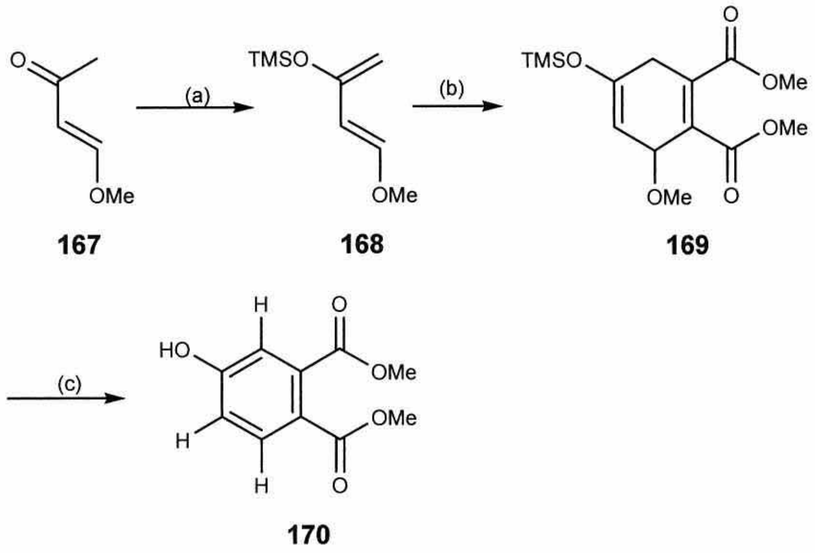
166

Figure 6.1

6.2 SYNTHESIS OF 4-HYDROXY-PHTHALIC ACID DIMETHYL ESTER

On inspection of the literature it was found that phenol **165** had previously been reported in a communication by Danishefsky and Kitahara.[62] Unfortunately only a brief description for its preparation was given with no experimental details. As Danishefsky's diene is somewhat expensive from commercial sources a large scale method for its preparation from the inexpensive ketone *trans*-4-methoxy-3-buten-2-one **167** was developed.

Thus reaction of ketone **167** with TMSCl and NEt_3:$ZnCl_2$ at 40 ˚C for 16 h led to the formation of the required diene **168** after work-up. The crude diene **168** was then treated with dimethylacetylenedicarboxylate (DMAD) at reflux in benzene for 3 h to effect a Diels-Alder reaction which is accompanied by an aromatisation. After acidic hydrolysis using methanolic HCl, purication was affected using silica gel chromatography and recrystallization to give required phenol **170** as fine yellow crystals in an excellent 89 % yield. This reaction was applicable to scale-up and was attempted it on both a small (5 g) and larger (25 g) scales.



Reagents and Conditions: (a) (i) $Et_3N.ZnCl$, Me_3SiCl, rt, 0.5 h, then 40 °C, 16 h. (ii) ether, rt, 0.5 h. (b) DMAD, reflux, 3 h. (c) Methanolic HCl, 16 h, 0 °C to rt.

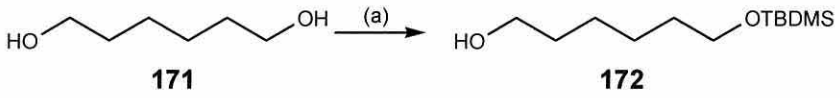
Scheme 6.1

Confirmation of the structure of **170** was obtained initially from proton NMR studies. The two methyl esters **170** appear as singlet resonances δ 3.85 and 3.89 ppm whilst the aromatic protons appear as doublets at δ 7.05 (J = 8.2 Hz) and 6.95 (J = 8.2 Hz) ppm, together with a singlet δ 6.90 ppm. Finally the phenolic proton appeared as a broad singlet resonance at δ 6.02 ppm.

In addition to this information **170** also displayed a broad absorption at 3380 cm^{-1} in the infra-red spectroscopy; indicating the presence of the phenolic function and two carbonyl absorptions at 1724 and 1710 cm^{-1}.

6.3 THE MITSUNOBU CONDENSATION

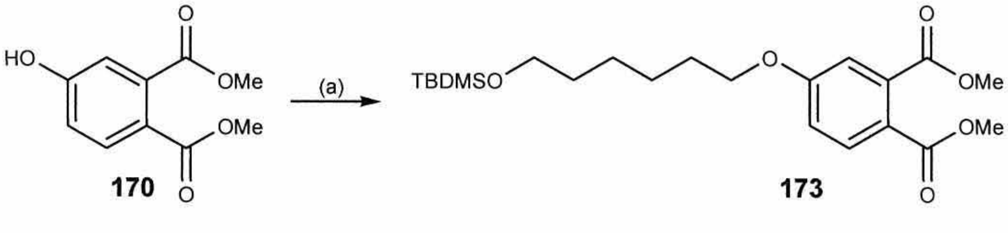
The Mitsunobu condensation reaction was chosen as a direct method for coupling the alcohol **172** with the phenol **170** as it was felt that a mild method of formation was required as this would lead to a lower likelihood of complications associated with more basic methods. Alcohol **172** was firstly prepared in 57 % yield by treatment of hexan-1,6-ol with TBDMSCl in DMF in the presence of imidazole.



Reagents and Conditions: TBDMSCl, imidazole, DMF, 0 °C, 2 h then rt, 72 h, 57 %.

Scheme 6.2

Following this phenol **170** and **172** were dissolved in dichloromethane and treated with DEAD and triphenylphosphine at 0 °C and stirred to room temperature over 16 hours. After work up and chromatography the ether **173** was obtained in a respectable 58 % yield.



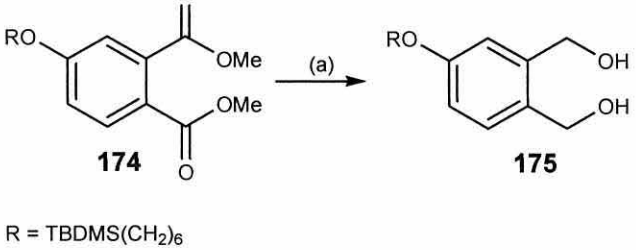
Reagents and Conditions: (a) DCM, DEAD, PPh$_3$, 0 °C to rt, 16h, 58 %.

Scheme 6.3

Evidence of the structure **173** was given initially by proton NMR. Aromatic signals were observed at δ 7.05 (d, J = 8.3 Hz), 7.00 (d, J = 2.3 Hz) and 6.96 (dd, 8.3 and 2.3) ppm and were similar in nature to the starting phenol **170**. A broad resonance at δ 1.56 integrating to 8 protons was indicative of the 4 methylenes of the alkyl chain whilst triplets at δ 4.02 (J = 5.5 Hz) and 3.62 (J = 5.5 Hz) ppm are those of the two methylenes adjacent to the ether and silyl ether respectively. The signals for the silyl protecting group were found at δ 0.90 (9H, s) and 0.06 (6H, s) ppm. High resolution mass spectrometry gave a molecular mass of 425.2360 which is in close agreement to the required 425.2359.

6.4 SYNTHESIS OF DIALDEHYDE 176.

With the synthesis of the ether **174** achieved a method for its conversion to the dialdehyde **176** was required. It was felt that a two step process which employed a DIBAL-H reduction to give the intermediate diol **175**, followed by a Swern oxidation to the dialdehyde would be advantageous as this method had been reported by Farooq to be a very efficient method for the synthesis of phthaldehydes.[63]

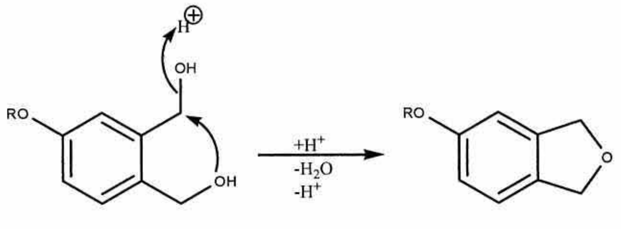


Reagents and conditions: (a) DIBAL-H, DCM, -78 °C to rt, 4.5 h.

Scheme 6.4

Thus the reduction of **174** with DIBAL-H was attempted by adding the reducing agent to the substrate dissolved in dichloromethane at -78 °C, after which the reaction was warmed to room temperature over 4.5 hours. On attempting to work up this reaction it was found that the formation of aluminium salts was highly problematic. A procedure was adopted whereby MeOH was added to the reaction as a quench and ethyl acetate was added to help to with the extraction of the product. Unfortunately this led to the formation of highly viscous gels which were difficult to work with and it was necessary to filter the reaction mixture through a silica gel pad to obtain a solution which could be separated. It was also found that the diol obtained from this step was somewhat impure and could not be purified by column chromatography without a significant drop in yield and considerable decomposition.

It was postulated that an acid catalysed cyclisation might be occurring leading to the fused heterocyclic product **178**, (Scheme 6.5) or that another undetermined, silica / acid catalysed process was occurring.



177 **178**

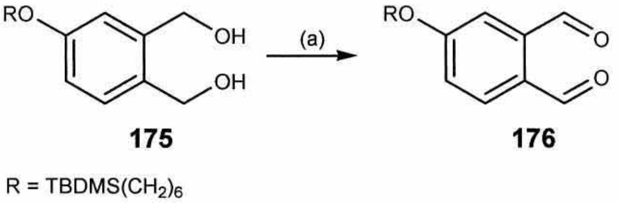
R = TBDMS$(CH_2)_6$

Scheme 6.5

Thus the wotk-up was modified by filtering the reaction after quenching through a silica pad that had been previously washed with a dilute ethereal solution of triethylamine. This modification led to more acceptable 32-49 % yield of the desired product which was used crude in the following stage of the synthesis. Despite considerable efforts, it was unable to increase the yield of this reaction.

Analysis of the crude product by proton NMR showed the presence of the aromatic ring protons at δ 7.25 (1H, d, J = 8.1 Hz), 6.92 (1H, d, 2.3) and 6.82 (1H, dd, 8.1, 2.3 Hz) ppm which again were reminiscent of the starting material. The two triplets at δ 3.96 (J = 6.1) and 3.63 (J = 6.2) ppm represent the two ether methylene protons, whilst the disappearance of the methyl ester signals and the appearance of a singlet at δ 4.54 ppm for the CH_2OH protons is proof of the reaction's success. The hydroxyl protons were present as a broad signal at δ 3.37 ppm.

Swern oxidation of the diol **175** was accomplished by addition of a solution of the diol **175** to a cooled (-78 °C) solution of oxalyl chloride and DMSO, followed by the addition of triethylamine. This gave a quantiative conversion to the dialdehyde **176**.



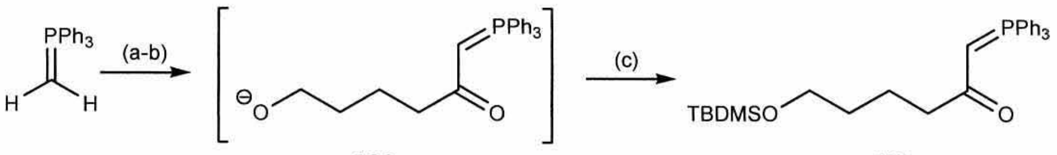
Reagents and Conditions: (a) (i) oxalyl chloride, DMSO, DCM, -78 °C, 1 h; (ii) TEA, 1 h, rt, 100 %.

Scheme 6.6

Analysis of the crude product, proton NMR showed the presence of the aromatic ring protons at δ 7.92 (d, J = 8.1 Hz), 7.44 (d, J = 2.5) and 7.20 (dd, J = 8.1 and 2.5 Hz) ppm which were similar to those in the starting material. The two triplets at δ 4.11 (J = 6.6 Hz) and 3.63 (J = 6.6 Hz) ppm represent the two ether methylenes, whilst the disappearance of the hydroxyl proton signals and the appearance of a two singlets at δ 10.61 and 10.34 ppm was indicative of the success of the reaction. High resolution mass spectrometry gave a molecular mass ion of 364.2063 which is in good agreement with the required mass of 364.2070.

6.5 SYNTHESIS OF THE *BIS*-ENONE 177

With the aldehyde in hand it was next set about the synthesis of the previously reported ylide **128** which was required for the Wittig reaction. This was prepared in a two stage reaction by treatment of δ-valerolactone with two equivalents of methylenetriphenylphosphorane in THF at -78 °C which led to the alkoxide **128a**. This reaction mixture was then evaporated and redissolved in DMF and silylated using TBDMSCl and imidazole. After work up, purification was effected by chromatography on silica gel to give **128** in typically 84-90 % yield.



128a

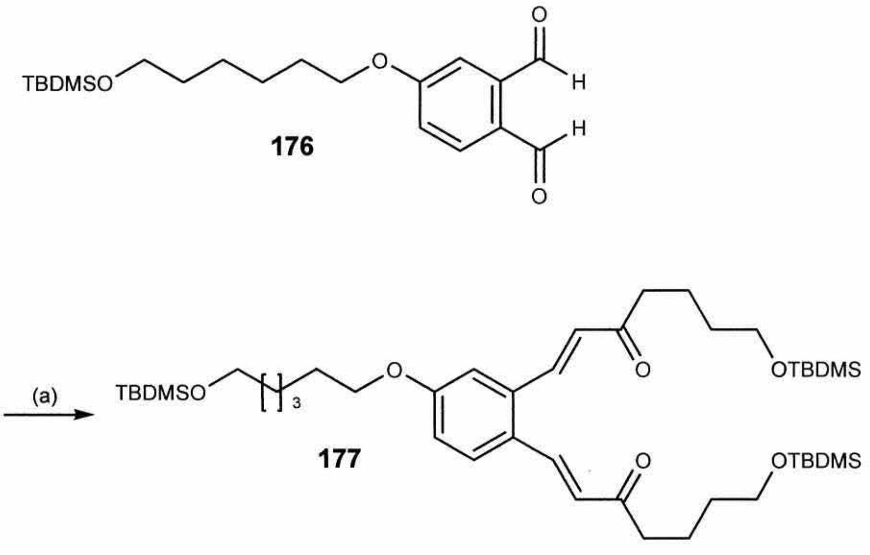
128

Reagents and conditions: (a) *n*-BuLi, THF, -78 °C-rt, 4 h; (b) δ-valerolactone, -78 °C-rt; (c) TBDMSCl, imidazole, DMF, 0 °C-rt 16 h 90 %.

Scheme 6.7

A solution of the dialdehyde **176** in dichloromethane together with four equivalents of the phosphorane **128** and stirred the resultant mixture for 72 hours.

After evaporation and chromatography the desired *bis*-enone **177** was obtained in a 61 % yield.



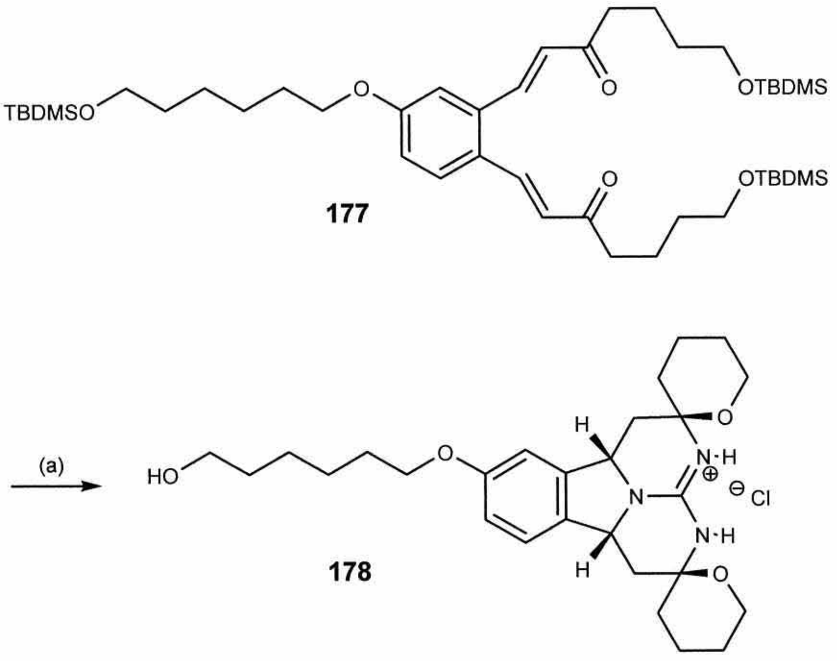
Reagents and Conditions: Phosphorane **128**, DCM, 72 h, 61 %.

Scheme 6.8

Analysis of **177** by proton NMR revealed four characteristic alkene resonances at δ 7.82, 7.78, 6.58 and 6.51 ppm with coupling constants of 15.9 and 15.8 Hz demonstrating the *trans*-nature of the α,β-unsaturated ketones. In the ^{13}C NMR also observed a signal corresponding to the carbonyl functionalities at δ 199.07 ppm. Infra-red analysis gave a strong absorption at 1694 cm^{-1} for the two ketone carbonyls and a second absorption at 1620 cm^{-1} for the conjugated alkenes. High resolution mass spectrometry gave a mass ion at 788.5263 which is in exact agreement with the mass required for this structure **177**.

6.6 REACTION OF *BIS*-ENONE 177 WITH GUANIDINE

With the *bis*-enone **177** in hand the synthesis proceeded to the key step which is the addition of the guanidine to the substrate, followed by an acid catalysed deprotection and spirocyclisation. Thus **177** was dissolved in DMF and stirred at 0 °C for ten minutes, after which a solution of guanidine was then added and the reaction stirred for 15 min then warmed to room temperature and stirred for 5 hours. At this point the reaction was cooled to 0 °C and treated with methanolic HCl and stirred for a further 16 h, which effected deprotection of the three silyl groups and the required *bis*-spirocyclisation. After purification by column chromatography using a gradient elution the required pentacycle **178** was obtained in 44 % yield as an oil.



177

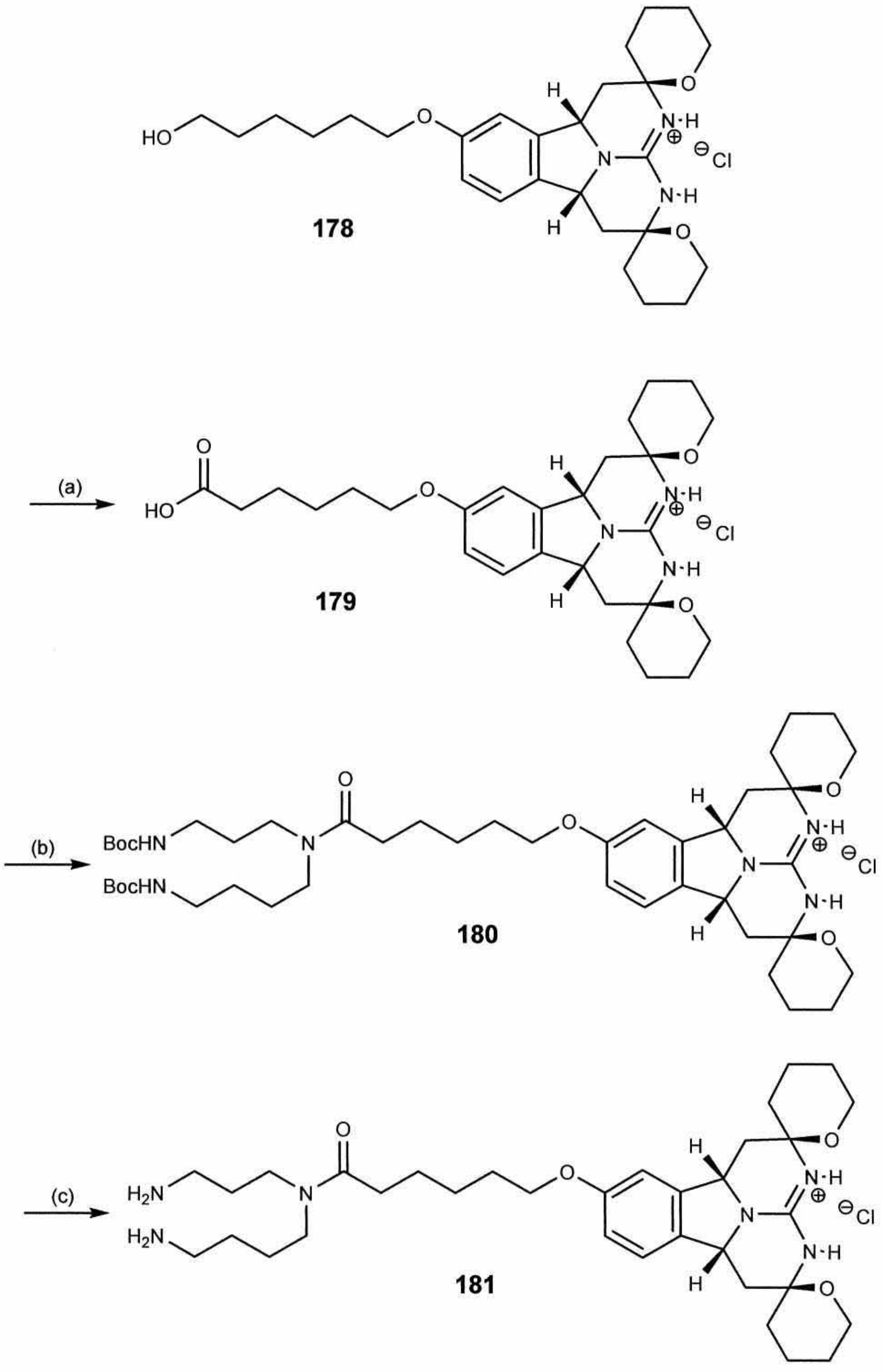
178

Reagents and Conditions: (a) (i) Guanidine, DMF, 0 °C, 15 min; then rt, 5 h; (ii) methanolic HCl, 0 °C-rt, 16 h, 44 %.

Scheme 6.9

Analysis of the product by proton NMR showed the presence of an N-H signal at δ 9.73 ppm (2H) together with characteristic signals at δ 4.19 (ddd, J = 11.6, 11.2 and 2.6 Hz) and 3.91 (br d, J = 11.6) ppm which are characteristically those of the spirocyclic methylene protons. The quaternary carbon of the guanidine was observed at δ 159.88 ppm in the ^{13}C spectrum whilst the spirocyclic carbons appeared at δ 79.56 ppm. In the IR broad bands were observed at 3400 cm^{-1} for the O-H stretch, and at 3230 cm^{-1} for the N-H and at 1658 cm^{-1} for the C=N stretch. The product gave a mass ion of 470.3013 by high resolution mass spectroscopy which corresponds very well to the expected mass of 470.3019. In addition to this, all the data indicated that the product had formed a single enantomer, as was observed in previous work by, Black,[64] Moore[65] and Howard-Jones.[66]

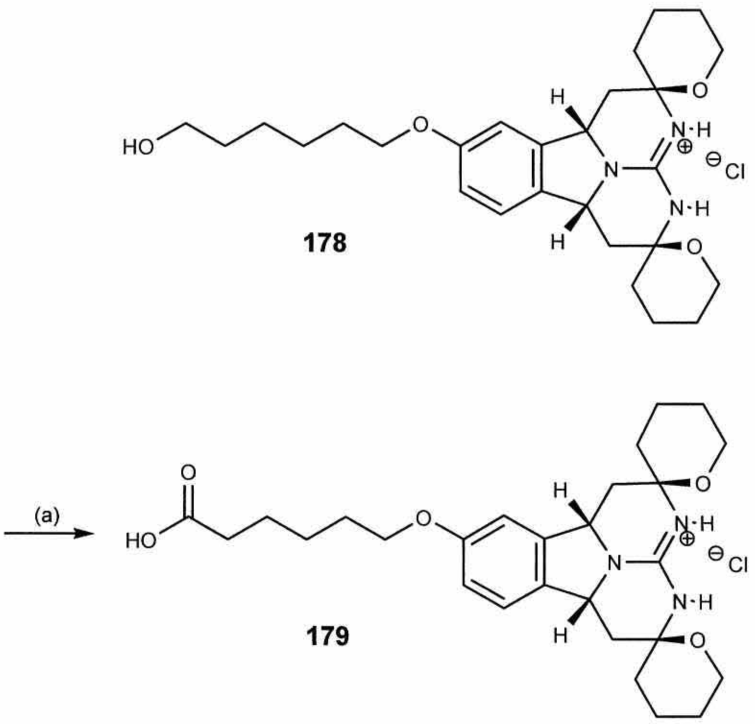
Next a spermidine function was intoducted at the terminus of the side-chain using the methodology developed by Moore and Howard-Jones. The first step of this process is the oxidation of the hydroxyl function to give the carboxylic acid, which is then coupled with *bis*-Boc spermidine and finally deprotected to give the desired analogue **181**.


178
(a)
179
(b)
180
(c)
181

Reagents and Conditions: (a) PDC, DMF, rt, 24 h, 69 %; (b) HOBT, EDCl, DCM, rt, 48 h, 49 %; (c) HCl, MeOH/H_2O, rt, 1 h, 100 %.

Scheme 6.10

Thus the alcohol **178** was dissolved in DMF and excess PDC was added after which the reaction was stirred at room temperature for 24 h. After work up and column chromatography the acid **179** was obtained in 69 % yield. Proton NMR analysis of the product was encouraging as it indicated that the triplet signal at δ 3.72 (t, J = 6.2 Hz) in the starting material which corresponds to the methylene adjacent to the alcohol had been replaced by a signal at δ 2.05 (t, J = 6.2 Hz) corresponding to a methylene adjacent to a carboxylic acid. Again, high resolution mass spectrometry gave a mass ion of 484.2815 which is close to the mass of 484.2811 required for **179**.

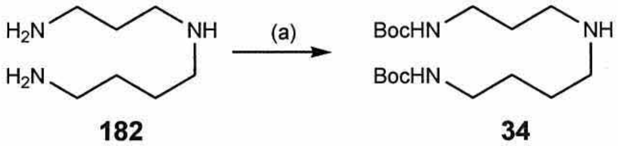


Reagents and Conditions: (a) PDC, DMF, rt, 24 h, 69 %.

Scheme 6.11

Protection of spermidine **182** was accomplished using a literature procedure, in which reaction of spermidine with equivalents of 2-(tert-butoxycarbonyloximino)-

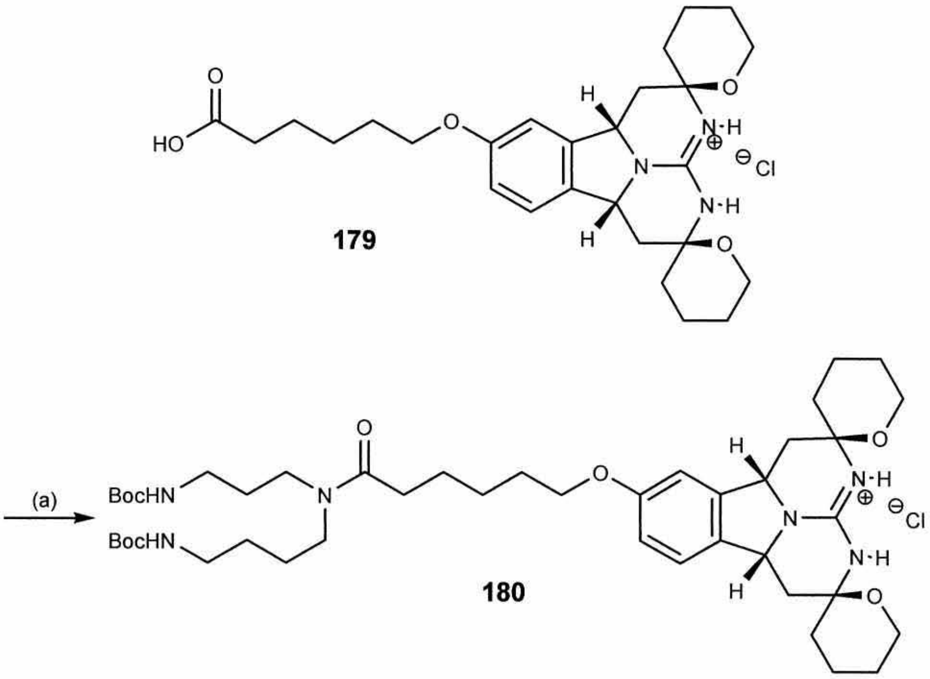
2-phenylacetonitrile (Boc-ON) in dichloromethane, gave *N,N*-1,8-Boc-spermidine **34** in a 47 % yield. The melting point was found to be in good agreement with that quoted in the literature, (m.p. = 84-86 °C; lit.mp. = 85-86 °C).[67]



Reagents and conditions: Boc-ON, DCM, 16 h, 47 %.

Scheme 6.11

The method used in the coupling of the acid **179** to the *bis*-Boc spermidine **34** was based on one described by Overman.[68] In this method acid **179** and the protected spermidine **34** were dissolved in DCM and treatment sequentially with solutions of HOBT and EDCl, then the mixture was stirred for 48 hours. After an aqueous work up and purification by column chromatography the coupled product **180** was obtained in 49 % yield.

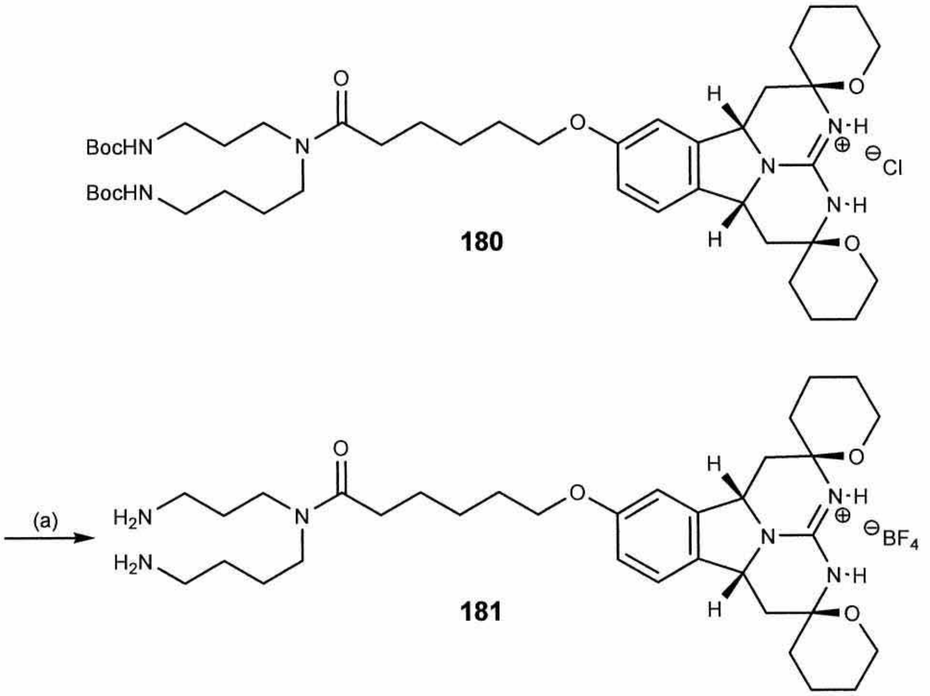


Reagents and conditions: (a) *bis*-Boc-spermidine **34**, HOBT, EDCl, DCM, rt, 48 h, 49 %.

Scheme 6.12

Analysis of **180** by proton NMR showed a broad singlet at δ 1.44 integrating to 18 hydrogens for the Boc protecting groups, together with broad singlets at δ 4.70 and 5.41 integrating to one proton each correspond to the amide NH protons. Signals in the ^{13}C NMR spectrum at δ 174.65 and 170.67 are indicative of the two carbonyl functionalities of the Boc protecting groups. High resolution mass spectrometry confirmed the structure as a mass of 811.5329 [M+H] was obtained which corresponds well with the expected value of 811.5333.

The Boc protecting groups where then removed by treatment of **180** with a solution of HCl in a methanol/water mixture. After one hour the solution was evaporated to dryness under vacuum and the resultant oil dissolved in dichloromethane and stirred for 16 hours with a saturated aqueous solution of sodium tetrafluoroborate. After separation and drying the product **181** was obtained in quantitative yield. Proton NMR demonstrated the deprotection had been successful as the t-butyl signals were not observed in the spectrum. Analysis by high resolution mass spectrometry confirmed the formation of **181** as an ion of 611.4286 was obtained which corresponds very closely to the expected [M+H] mass of 611.4285.



180

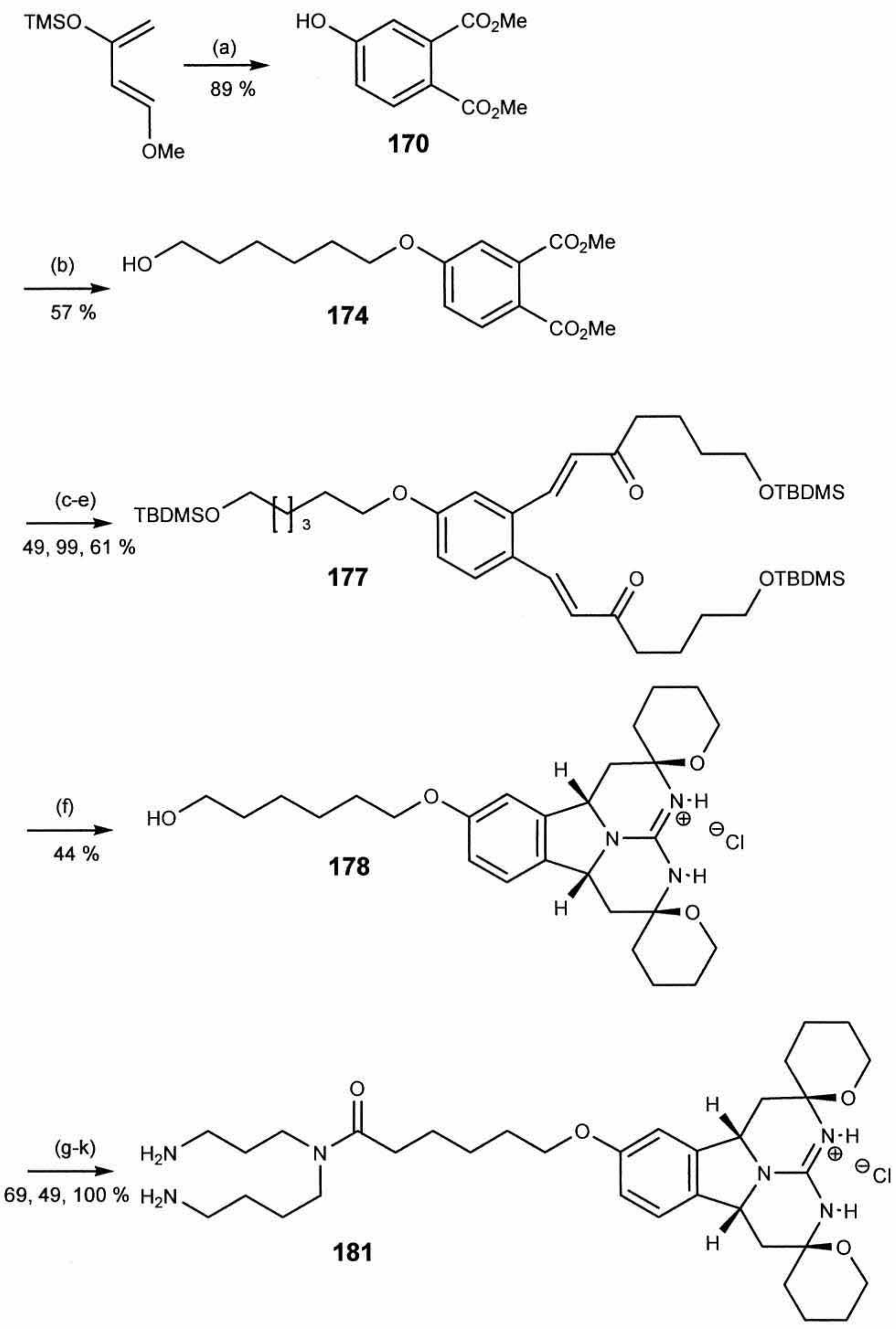
181

Reagents and Conditions: (a) (i) HCl, MeOH, rt, 1 h; (ii) DCM, $NaBF_4$ (sat., aq.),

Scheme 6.13

6.7 CONCLUSIONS

The synthetic approach to the linear molecule was successful, however the overall yield for the process proved to be somewhat low. The 9 step sequence from DMAD gave **181** in 2 % overall yield as shown in Scheme 6.14.


TMSO
OMe
(a)
89 %
HO
CO2Me
CO2Me
170
(b)
57 %
HO
O
174
(c-e)
49, 99, 61 %
TBDMSO
3
177
OTBDMS
(f)
44 %
178
H
N·H
N
Cl
(g-k)
69, 49, 100 %
H2N
181

Scheme 6.14

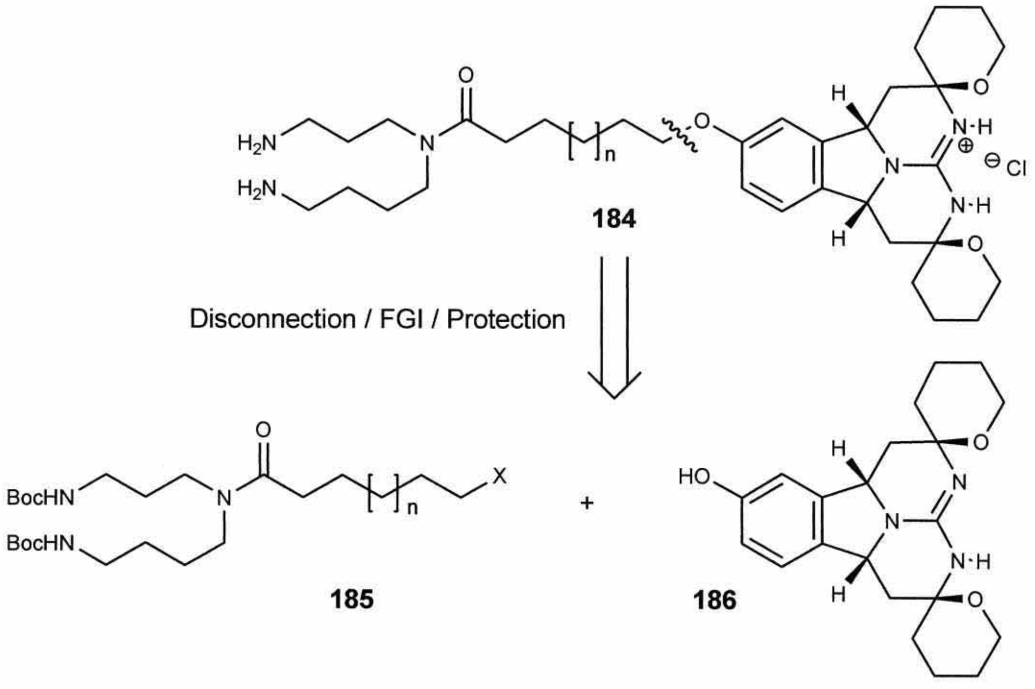
As with the work by Moore and Howard-Jones the main problem with the synthetic route to give **181** is its linear nature. Synthesis of this type is not efficient if it contains a single poor step. In our case the guanidine addition step, the DIBAL-H reduction and the Boc coupling step were problematic. It is with this in mind progression was made to our second synthetic route which is designed in a convergent manner.

CHAPTER 7

RETROSYNTHETIC ANALYSIS OF THE CONVERGENT MODEL OF PTILOMYCALIN A

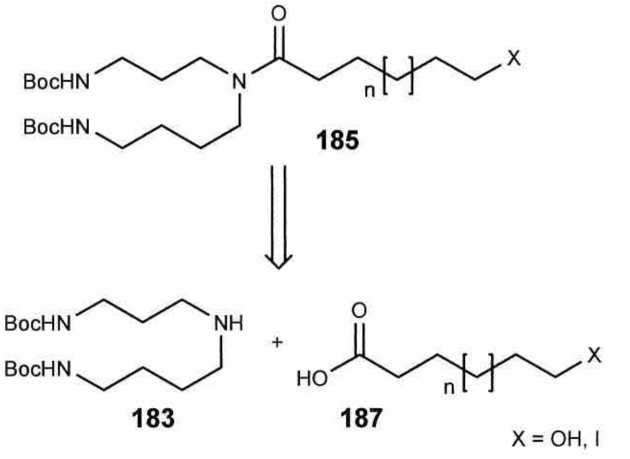
7.1 RETROSYNTHETIC ANALYSIS OF THE CONVERGENT MODEL

With the problems encountered in the previous stages of the synthesis, it was envisaged to modify our synthetic route to one that would lead to a convergent methodology. Obviously the best disconnection for **184** is to remove the linear chain and the spermidine function using the disconnection shown in Scheme 7.1 to give two the synthons **185** and **186**.


H2N
H2N
O
N
n
O
H
N·H
⊕
⊖Cl
N
N·H
H
O
184
Disconnection / FGI / Protection
BocHN
BocHN
N
O
n
X
+
HO
H
O
N
N
N·H
H
O
185
186

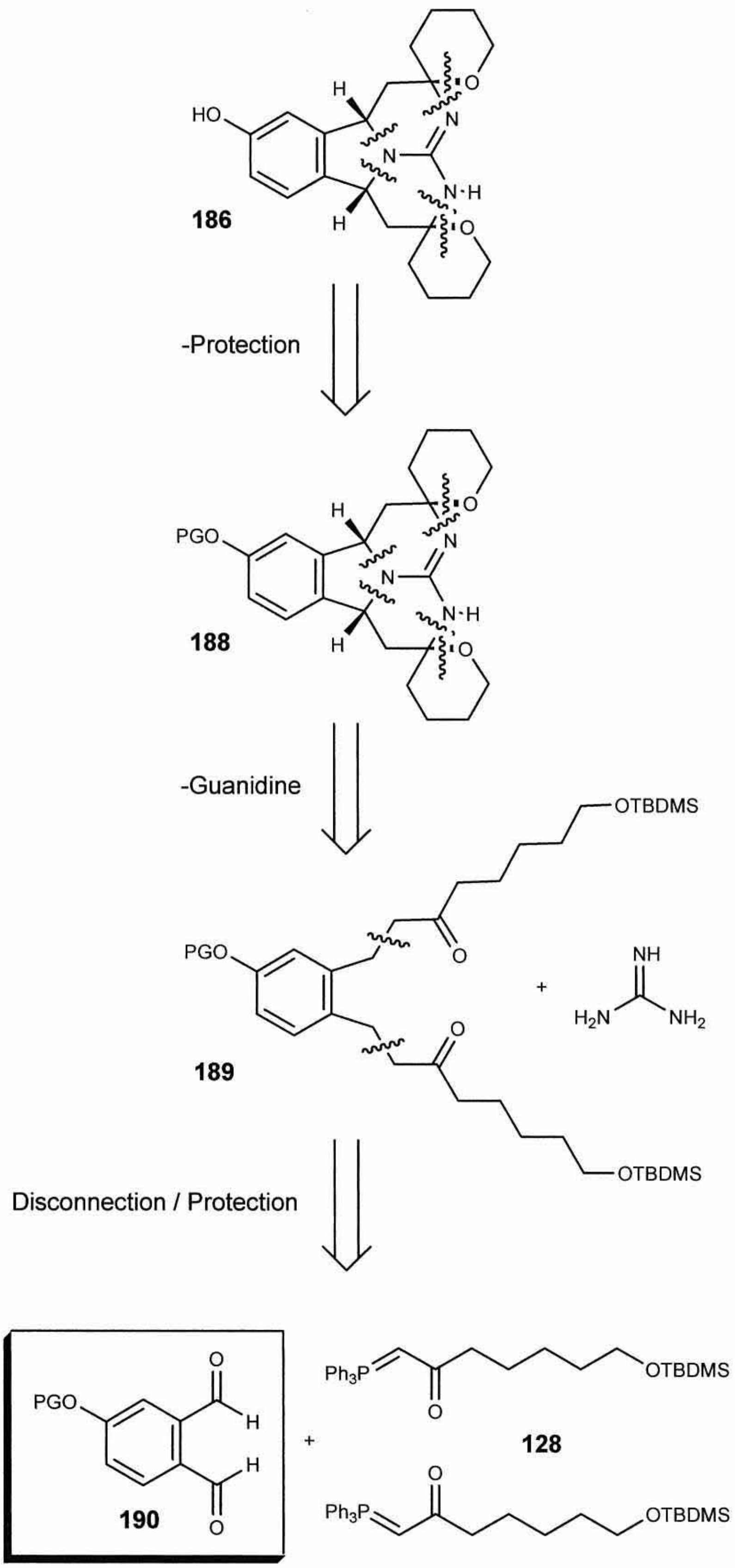
Scheme 7.1

The synthesis of the spermidine-linker unit **185** should be relatively straightforward as the methodology could be developed from the previous synthesis and should be able to adapt this to our needs. (Scheme 6.12).


O
BocHN
N
BocHN
n
X
185
NH
BocHN
BocHN
183
+
O
HO
n
X
187
X = OH, I

Scheme 7.2

A more challenging prospect is the possibly zwitterionic phenolic guanidine **186**, for which a potential retrosynthesis is shown in Scheme 7.3. Protection of the phenolic residue will lead to the guanidine **188**, which should be accessible *via* the previously developed methodology for the preparation of **181** and leads to the aldehyde **190** as the key intermediate in this synthetic route.


186
HO
H
N
N·H
O
-Protection
188
PGO
-Guanidine
OTBDMS
189
NH
H$_2$N
NH$_2$
+
Disconnection / Protection
190
Ph$_3$P
128

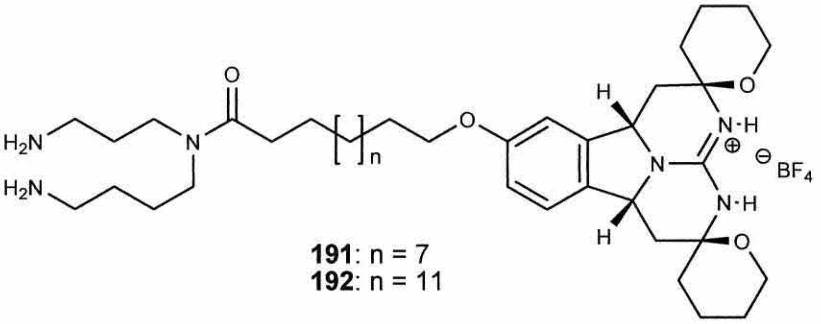
Scheme 7.3

CHAPTER 5

CONVERGENT SYNTHETIC ROUTE

8.1 CONVERGENT SYNTHETIC ROUTE

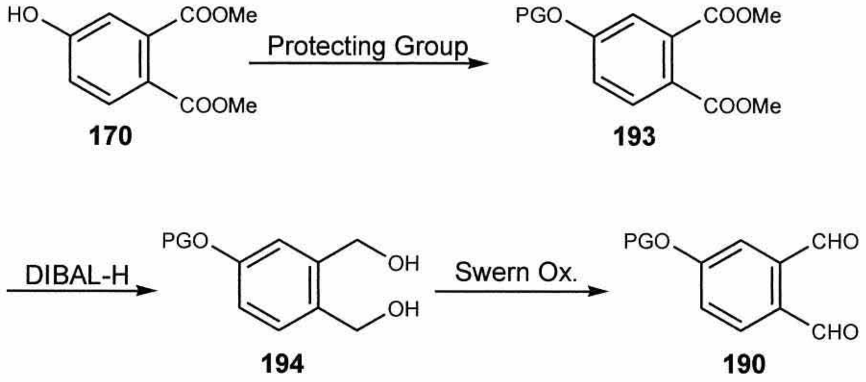
Based on the retrosynthetic model it was anticipated that different chain length analogues of ptilomycalin A **191** and **192** could be accessed. These would be prepared from the phenol **170** as discussed page 78.



191: n = 7
192: n = 11

Figure 8.1

As the phenolic diester **170** had been previously prepared it was determined that this was the best starting material to use for the synthesis of **190**. It was thus envisaged that protection, DIBAL-H reduction and Swern oxidation should lead to the key intermediate **190**. We thus began an investigation of the various protecting group strategies available.


HO
COOMe
COOMe
Protecting Group
PGO
COOMe
COOMe
DIBAL-H
PGO
OH
OH
Swern Ox.
PGO
CHO
CHO

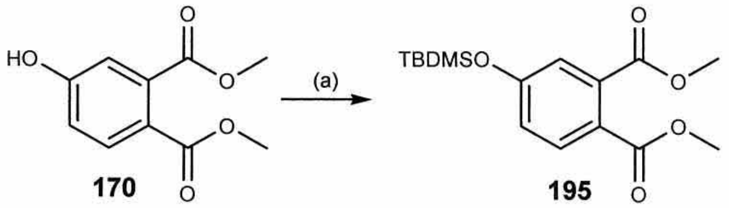
170 **193** **194** **190**

Scheme 8.1

8.2 SYNTHESIS OF THE SILYL ETHER OF 170

Initially we envisaged that a silyl ether could be the prepared protecting group as they are easily prepared and easily cleaved using mildly acidic conditions or fluoride ions.

Previously prepared phenolic diester **170** and reacted it with TBDMSCl and imidazole in DMF at -5 °C for 30 minutes, then at room temperature overnight. After work up and chromatography the protected diester **195** was obtained in 89 % yield.


O
HO
O
O
O
(a)
TBDMSO
O
O
O
O

170 **195**

Reagents and Conditions: TBDMSCl, imidazole, DMF, -5 °C, 30 min, then rt, 12 h, 89 %.

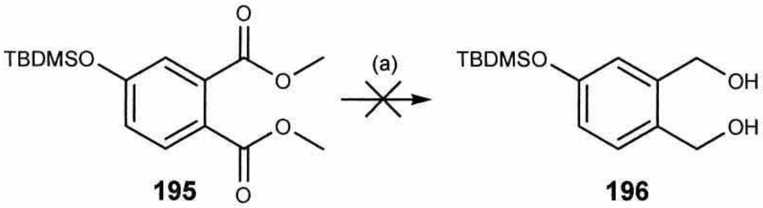
Scheme 8.2

Analysis of the product by proton NMR showed the presence of *tert*-butyl group at δ 0.89 ppm (9H) together with characteristic signals at δ 0.06 (6H) ppm which are characteristically those of the methyl of the protecting group. The *tert*-butyl groups were observed at δ 25.80 ppm and the methyl groups at δ 1.02 ppm in the ^{13}C spectrum. The product gave a mass ion of 324.1395 at high resolution mass spectrometry which corresponds very well to the expected mass of 324.1393.

With **195** in hand, DIBAL-H reduction was attempted under the conditions previously employed for substrate **174**. Thus reduction with DIBAL-H was attempted by adding the reducing agent to the substrate **195** dissolved in dichloromethane at -78 °C followed by warming to room temperature over 4.5 hours. After work-up, by filtration through silica the crude product obtained was examined by NMR and it was found that only signals corresponding to the starting material were present. The reduction was repeated, using more equivalents (4-10) of DIBAL-H in differing solvents and over extended reaction times.

Reduction of the silyl-protected diester **195** using $LiAlH_4$ was also attempted, by adding the reducing agent to the substrate at -78 °C to room temperature over 4.5 hours. After work up, the ^{1}H NMR showed no reduction of the ester groups had occurred. Similar modifications to the reaction time, temperature, and number of equivalents of the reducing agent failed to effect reduction.

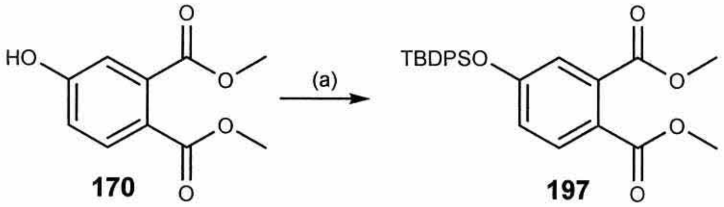
In addition reduction of **195** was attempted with RED-Al, using similar conditions described for the DIBAL-H and $LiAlH_4$ reductions. Again no reduction was observed.



(a) = DIBAL-H or $LiAlH_4$ or RedAl

Scheme 8.3

It was postulated from this work as no reaction products were observed that the TBDMS protecting group was stopping the ester functions from being reduced. The exact reason for this is unclear. We thus opted to repeat the reactions using the TBDPS protected compound **197** which was prepared in 85 % yield from the phenol **170** as shown in Scheme 8.4.



Reagents and Conditions: TBDPSCl, imidazole, DMF, -5 °C, 30 min, then rt, 15 h 85 %.

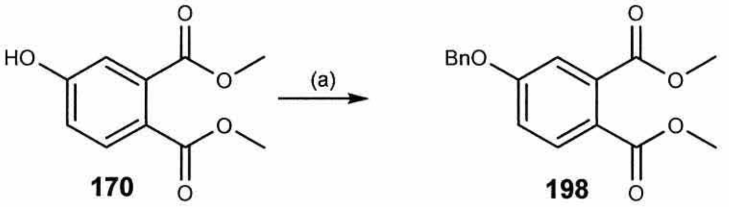
Scheme 8.4

Analysis of the ^1H NMR showed characteristic signal at δ 1.09 (9H) and signals at δ 7.42-7.39 (10H) are indicative of the phenyls of the protecting group. The product gave a molecular mass ion of 448.1706 in the high resolution mass spectrum which corresponds exactly with the expected mass ion.

Similarly to the TBDMS ether the reduction of the TBDPS ether **195** with DIBAL-H gave identical results over a range of conditions, in that only recovered starting material was obtained.

8.3 SYNTHESIS OF A BENZYL PROTECTED GUANIDINE PENTACYCLE

It was apparent that the proposed silyl protecting strategy was not a success and we thus looked at other possibilities. We postulated that a benzyl protecting group would be advantageous as this is an inert and unreactive functional group and should be readily removed under reducing conditions. Thus the diester **170** was dissolved in THF and benzyl bromide and potassium carbonate were added at room temperature. After stirring at room temperature for two hours the mixture was refluxed overnight and following aqueous work up and purification by chromatography, the benzyl ether was obtained **198** in 77 % yield.



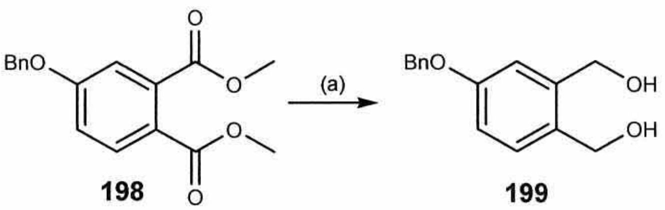
Reagents and conditions: Benzyl bromide, potassium carbonate, rt, 2 h, then reflux, 16 h, 77 %.

Scheme 8.5

Analysis of **198** by proton NMR showed a broad multiplet at δ 7.48–7.40 integrating to 5 hydrogens for the benzyl protecting group, together with the disappearance of the phenolic proton signal. Signals in the ^{13}C NMR spectrum at δ

70.34 ppm are indicative of the benzyl methylene. High resolution mass spectrometry confirmed the structure as a molecular mass ion of 300.0999 was obtained which corresponds very well with the expected value of 300.0998.

Reduction of **198** using DIBAL-H was then attempted. The substrate **170** was dissolved in dichloromethane and cooled (-78 °C) whereupon a 6.75-fold excess of DIBAL-H was added and the reaction stirred to room temperature over 4.5 hours. Analysis of the reaction mixture by TLC indicated the disappearance of the starting material and the reaction was diluted with ethyl acetate then, MeOH was added to quench the reaction. The reaction mixture was then filtered through a triethylamine washed silica gel pad and dried. This process gave a 65 % yield of the desired diol **199** which was used in the Swern oxidation without further purification.



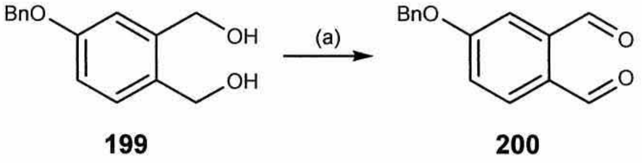
Reagents and Conditions: DIBAL-H, DCM, -78 °C, 4.5 h.

Scheme 8.6

Analysis of the crude product **199** by proton NMR indicated the presence of aromatic ring protons at δ 7.41 (5H, m) ppm for the benzyl protons, together with signals at δ 7.21 (d, J = 8.3), 6.98 (d, J = 2.5) and 6.84 (dd, J = 8.3 and 2.5) ppm which were reminiscent of the starting material. This together with the disappearance of the methyl ester signals and the appearance of a singlet at δ 4.56 and 4.53 ppm corresponding to the CH_2OH protons, and the hydroxyl protons which appear at δ

2.05 ppm, was conclusive proof of a successful transformation. This reaction was repeated on 5 occasions giving yields of 47-65 %.

The crude diol **199** was then oxidised under Swern conditions in a similar manner to that for **176**. Thus a dichloromethane solution of **199** was added to a mixture of oxalyl chloride and DMSO in DCM followed by the addition of triethylamine to give after work up a quantitative yield of the dialdehyde **199**.

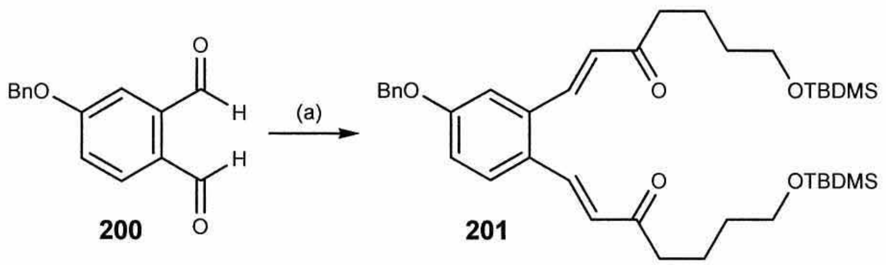


Reagents and Conditions: (a) (i) oxalyl chloride, DMSO, DCM, -78 °C; (ii) TEA, rt, 1 h, 100 %.

Scheme 8.7

Analysis of the crude product by proton NMR gave a signal at δ 7.47 (5H, br m) ppm for the benzyl protons, together with CH signals at δ 7.40 (d, J = 8.1), 7.31 (dd, J = 2.4) and 7.27 (s) ppm which were reminiscent of the starting material. In addition two aldehyde protons at δ 10.66 and 10.35 ppm are conclusive proof of a successful reaction. High resolution mass spectrometry gave a molecular ion at 240.0790 which is in very close agreement to the required mass of 240.0791. This reaction was repeated on 5 occasions and quantiative yields of 100 % were typically obtained.

A solution of the dialdehyde **200** in dichloromethane and treated it with 4 equivalents of the phosphorane **128** with stirring over 72 hours. After evaporation and chromatography the desired *bis*-enone **200** was obtained in a 62 % yield.

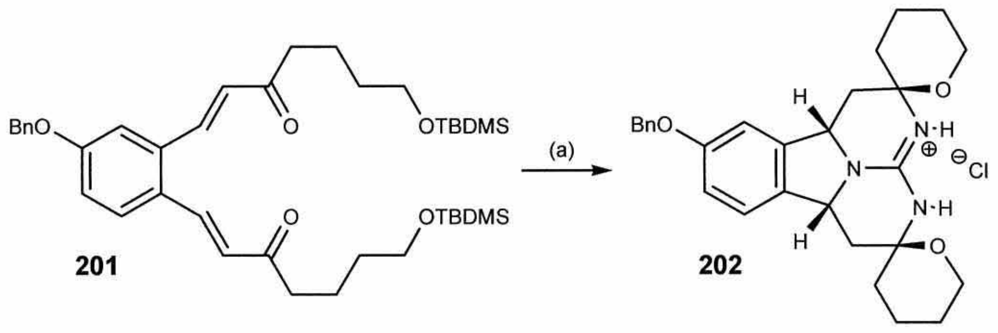


Reagents and Conditions: (a) Phosphorane **128** (4 eqv.), DCM, 72 h, 62 %.

Scheme 8.8

Analysis of **201** by proton NMR analysis revealed four characteristic alkene resonances at δ 7.92, 7.87, 6.62 and 6.60 ppm with measured coupling constant of 15.9 Hz demonstrating the *trans*-nature of the α,β-unsaturated ketones. In addition signals at δ 7.44-7.35 (5h, m) ppm for the benzyl protons and with CH signals at δ 7.33 (d, J = 8.8 Hz), 7.14 (d, J = 2.6) and 7.01 (dd, J = 8.8 and 2.6) ppm for the fused benzene ring were also present. In the ^{13}C NMR the two enone carbonyls were observed as a coincident signal at δ 199.73 ppm. High resolution mass spectrometry gave an ion at 665.4056 for $[M+H]^+$ which is in close agreement with the predicted value of 665.4057.

With the *bis*-enone **201** in hand we attempted the key step in the synthesis, which is the addition of guanidine to the substrate followed by an acid catalysed deprotection and spirocyclisation. Thus **201** was dissolved in DMF and stirred at 0 °C for ten minutes after which a solution of guanidine was added and the reaction stirred for 15 minutes then warmed to room temperature and stirred for 5 hours. At this point the reaction was cooled (0 °C) and treated with methanolic HCl and stirred for a further 16 hours. After purification by column chromatography using gradient elution the required pentacycle **202** was obtained in 41 % yield.



Reagents and Conditions: (a) (i) Guanidine, DMF, 0 °C, 10 min; then rt, 5 h; (ii) methanolic HCl, 0 °C –rt. 16 h, 41 %.

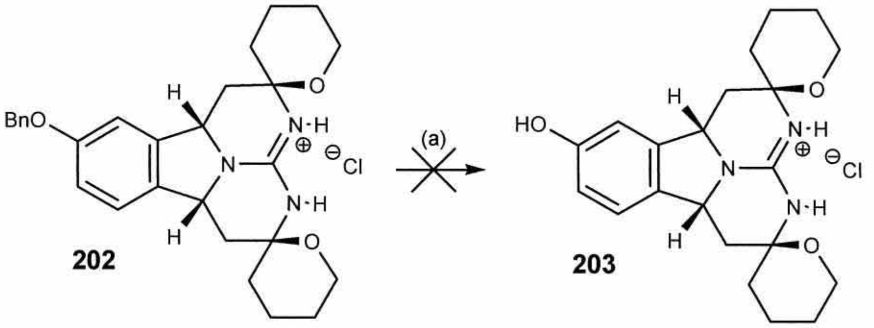
Scheme 8.9

Analysis of the product by proton NMR showed the presence of an N-H signal at 9.39 (br s) ppm together with characteristic signals at 3.94 (ddd, J = 11.8, 11.2 and 2.8) and 3.78 (ddd, J = 11.8, 11.2 and 2.8) ppm for the spirocyclic methylene protons. The quaternary carbon of the guanidine was observed at 157.37 ppm in the ^{13}C spectrum whilst the spirocyclic methylenes were observed at 80.25 ppm. The product gave a mass ion 460.2602 at high resolution, which corresponds well to the expected mass of 460.2600. In addition, the data indicated that the product had formed a single enantamer as was observed in previous work.[66]

8.4 DEPROTECTION OF THE BENZYL PROTECTED GUANIDINE PENTACYCLE

With the success in the preparation of the required protected pentacyclic precursor we next proceeded to investigate its deprotection and initially focused our efforts on hydrogenation.

A solution of **202** in dichloromethane together with a catalytic amount of Pd on activated carbon and stirred the mixture under a hydrogen atmosphere for 16 h. After filtration through a silica gel plug and evaporation of the filtrate inspection by proton NMR and TLC indicated that the starting material had been consumed, however the product was quite obviously a complex mixture of compounds. Attempting the reaction under more controlled conditions (shorter time and less hydrogen gas) also led to complete decomposition of the substrate. We also repeated the process using rhodium 5 % on activated alumina, however this was not a suitable catalyst as no reaction occurred even over a prolonged time period.



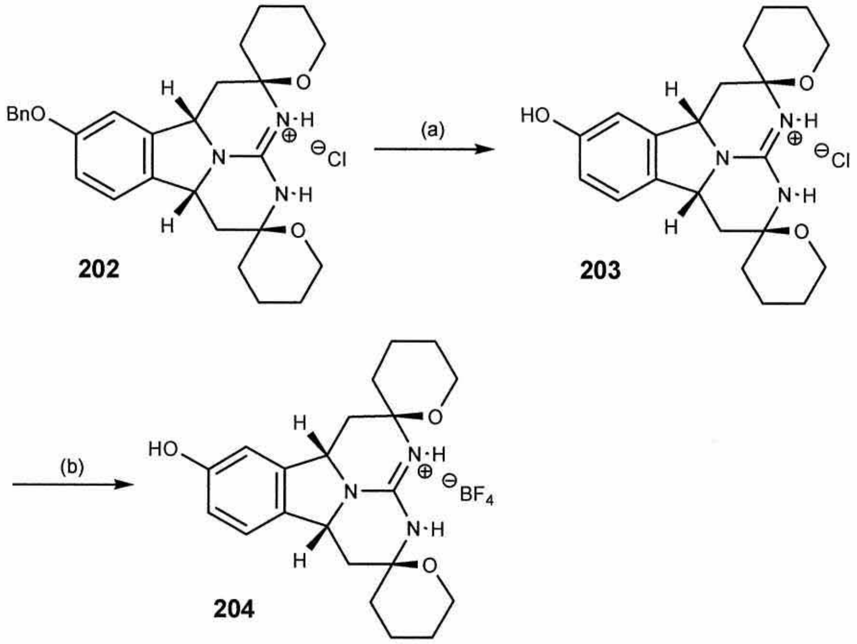
Reagents and Conditions: (a) Pd on C, H_2, DCM, rt; Rh on Al_2O_3, H_2, DCM, rt.

Scheme 8.10

The reasons for the decomposition are unclear, however it is likely that the other benzylic positions in **202**, i.e. those adjacent to the guanidine, are also undergoing hydrogenation and this is leading to the complete destruction of the pentacyclic system.

The literature was consulted and an alternative cationic method was found which employed thioanisole in the presence of trifluoroacetic acid (TFA). This method relies upon the formation of benzylic cations which are generated by protonation of the benzyl ether with the TFA, these being scavenged (removed) by the thioanisole.

Thus the benzyl protected pentacycle **202** was dissolved in TFA and thioanisole was added dropwise at 0 °C to the stirred mixture. After warming to room temperature over twelve hours the reaction was evaporated and the crude product dissolved in methanol and stirred with a saturated solution of sodium fluoroborate for 24 hours. After extractive work up and chromatography on silica gel, the phenol **204** was obtained in 48 % yield.



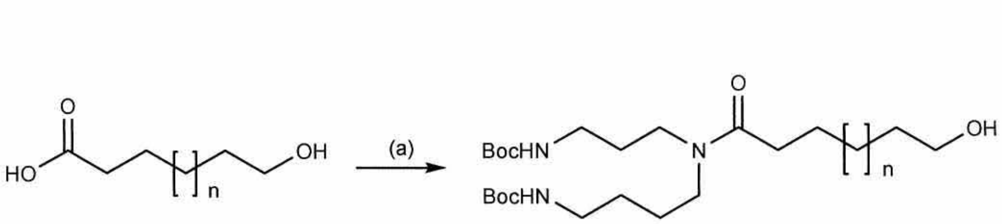
Reagents and Conditions: (a) TFA, thioanisole, 0 °C-rt, 12 h; (b) sat. BF$_4$ sol., MeOH, 24 h.

Scheme 8.11

Analysis of **204** by proton NMR showed the presence of the N-H signals at 9.20 and 9.18 ppm and a phenolic proton at 8.35 ppm with the complete absence of the signals associated with the benzyl protecting group. In addition, the signal in the ^{13}C NMR spectrum at 147.83 is indicative of the quaternary guanidium carbon. Analysis by high resolution mass spectrometry gave a molecular ion at 370.2128 which is in close agreement with the theoretical value of 370.2130.

8.5 SYNTHESIS OF THE ω-HYDROXY SPERMIDINE TETHERS

With the guanidine containing half unit in hand, we proceeded with the synthesis of the various spermidine-linker units. The previously prepared *bis*-Boc spermidine **34** was reacted in turn with the commercially available 12-carbon and 16-carbon hydroxyl acids **205** and **206** by combining the two reactants in dichloromethane and sequentially adding dichloromethane solutions of HOBT and EDCl, followed by stirring overnight. After work up and purification by column chromatography the required coupled products were formed in 78 and 75 % yields respectively.



205: n = 7
206: n = 11

207: n = 7
208: n = 11

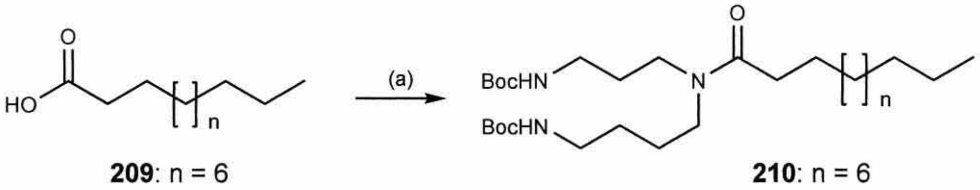
Reagents and Conditions: *bis*-Boc spermidine **34**, HOBT, EDCl, DCM, rt, 24 h, 78 % and 75 %.

Scheme 8.12

Analysis by proton NMR for **207** gave a broad singlet at 1.40 ppm integrating to 18 hydrogens for the Boc protecting groups, together with broad singlets 4.72 and 5.47 ppm integrating to one proton each corresponding to the amide NH protons and the hydroxyl proton at 2.48 ppm. High resolution mass spectrometry gave a molecular ion at 544.4315 corresponding closely to the required mass of 544.4325.

Similarly **208** gave a singlet at 1.38 (18H) ppm for the Boc protecting groups, together with broad singlets at 4.77 (1H) and 5.48 (1H) ppm integrating to one proton each corresponding to the amide NH protons, together with the hydroxyl proton at 2.39 (br s) ppm. High resolution mass spectrometry gave a molecular ion at 600.4956 corresponding closely to the required mass of 600.4951.

A spermidine linked amide **210** which does not possess a terminal hydroxyl function. The reason for the preparation of this compound is that we wished to investigate the biological activity of the spermidine linker unit which lacked the guanidine pentacycle, as this may give clues to the role of each sub-unit in contributing to the overall activity. Thus *bis*-Boc spermidine **34** was coupled with lauric acid **209** under identical conditions and obtained the amide **210** in 52 % yield.

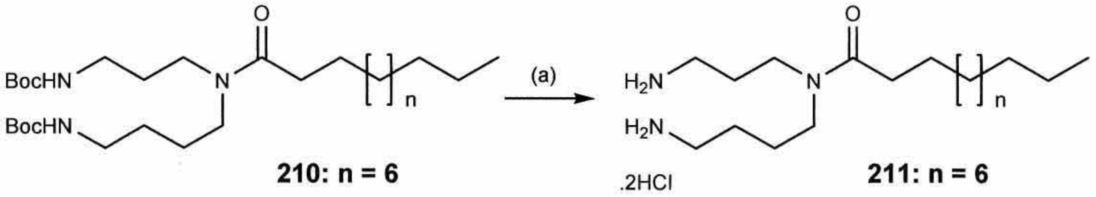

HO
O
n
(a)
BocHN
BocHN
N
O
n
209: n = 6
210: n = 6

Reagents and Conditions: (a) lauric acid, **34**, HOBT, EDCl, DCM, rt, 48 h, 52 %.

Scheme 8.13

Analysis by proton NMR showed a broad singlet at 1.44 ppm integrating to 18 hydrogens for the Boc protecting groups, together with broad singlets 5.48 (1H) and 4.72 (1H) ppm corresponding to the amide NH protons. High resolution mass spectrometry gave a molecular ion at 528.4366 corresponding closely to the required mass of 528.4376.

210 was deprotected using 3 N HCl in MeOH for 1 h, the resulting mixture was then evaporated to dryness to give **211** in quantiative yield.

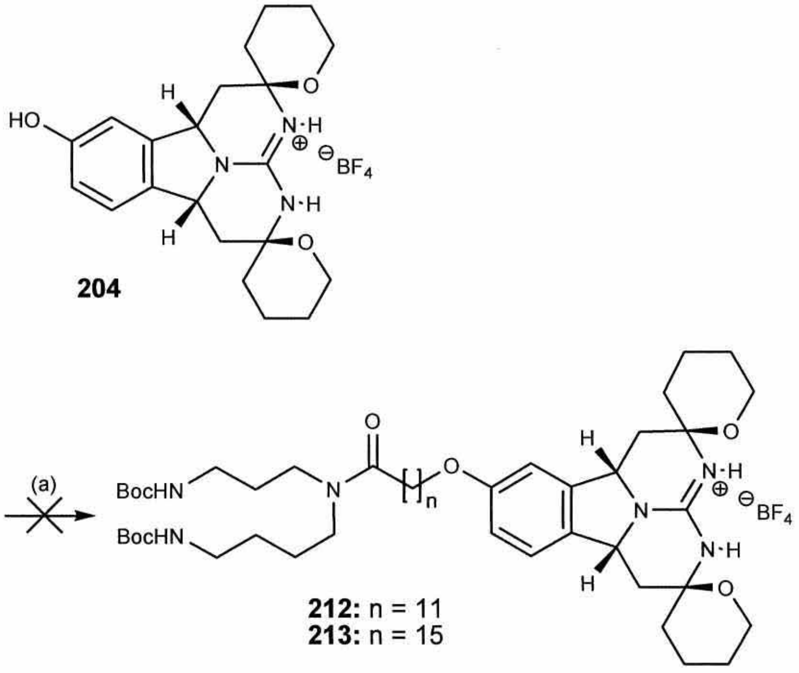

BocHN
BocHN
N
O
n
210: n = 6
(a)
H2N
H2N
.2HCl
N
O
n
211: n = 6

Reagents and Conditions: (a) HCl (3 N), MeOH, rt, 1 h.

Scheme 8.13a

8.6 ATTEMPTED COUPLING OF PHENOL 204 TO SPERMIDINE-LINKER 208

With both the phenolic unit **204** and the spermidine-linker units **207** and **208** prepared we set about the coupling of these together. We had previously developed methodology for the coupling of the phenol **170** and the alcohol **172** using DEAD and triphenylphosphine and this seemed like a very useful starting position in the synthesis. An equimolar mixture of phenol **204** and the C-16 spermidine-linker unit **208** in dichloromethane at 0 ˚C and treated it sequentially with DEAD and triphenylphosphine and stirred the mixture to room temperature over 16 hours.



204

212: n = 11
213: n = 15

Scheme 8.14

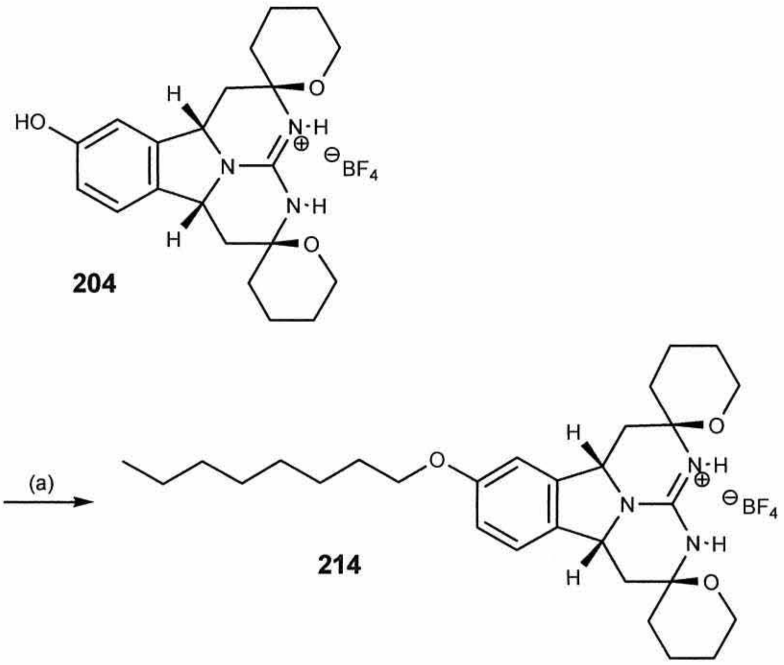
TLC monitoring of the reaction proved very difficult as the phenolic compound co-ran with some of the by-products from the reaction. On work up of the reaction it was apparent that both the phenolic compound **204** and the alcohol **208** were still present in the crude product, however there appeared to be encouraging signals and the mixture was purified by column chromatography. Unfortunately analysis of the numerous fractions obtained by both ^{1}H NMR and mass spectrometry failed to give any indication that the desired compound had been formed.

The reaction was repeated by increasing the number of equivalents of the reagents DEAD (2-5 equivalents) and triphenylphosphine (2-5 equivalents) and also modified the stoichiometry of the reaction by increasing the amount of alcohol **208** (2.5-4 equivalents) with respect to the phenol. We also employed DIAD as the coupling agent in an attempt to improve the purification. None of these measures were successful and no evidence for the production of the required product was obtained by NMR or mass spectrometry even on prolonged reaction times.

The reactions were also repeated using the shorter chain length spermidine-linker unit **205** again varying the conditions. Again no coupled product was observed.

8.7 SYNTHESIS OF A PTILOMYCALIN A MODEL

The failure of the Mitsunobu approach to the coupling of the two sub-units led us to investigate an alternative methodology. Alkylation using potassium carbonate and an alkyl iodide was envisaged as being a suitable alternative as shown in Scheme 8.15.



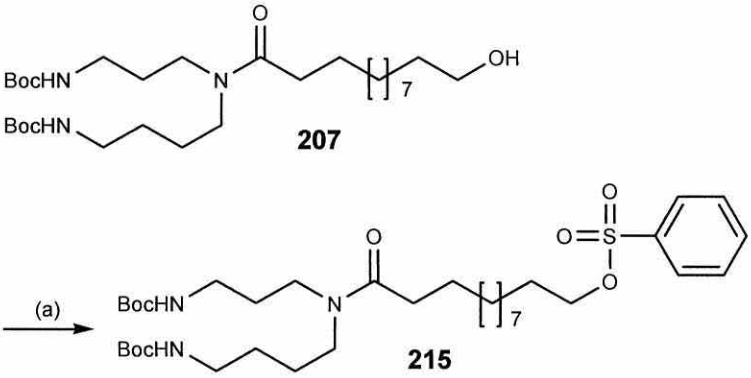
Reagents and Conditions: iodooctane, K_2CO_3, DMF, rt, 16 h, 53 %.

Scheme 8.15

After initial investigation the reaction on a model system and thus took the phenol **204** together with potassium carbonate and an excess of 1-iodooctane in DMF and stirred them at room temperature for 16 hrs. After work up and column chromatography the desired alkylated system **214** was obtained in 55 % yield. (Scheme 8.15)

Analysis of **214** by proton NMR showed the presence of an N-H signal at 9.55 (s, 2H) ppm corresponding to the guanidine protons, together with characteristic signals at 4.40 (2H, ddd, J = 12.5, 11.2, 2.5 Hz) and 4.18 (2H, br d, J = 12.5 Hz) ppm for the spirocyclic methylene protons. A signal at 1.29 (3H, t, J = 6.7 Hz) ppm corresponded to the terminal methyl group on the alkyl chain. The product also gave an ion at 482.3379 in the high resolution mass spectrum, which corresponds well to the expected mass of 482.3382.

With this result ω-iodoalkyl-spermidine units **216** and **218** where synthesised for preparation of the ptilomycalin A analogues. We thus prepared the tosylated C-12 linker unit **215** by treatment of the alcohol **207** with *p*-toluenesulphonyl chloride in pyridine over 12 hours. After evaporation and purification by column chromatography using gradient elution, the required protected spermidine-linker **215** was obtained in 78 % yield. (Scheme 8.16).

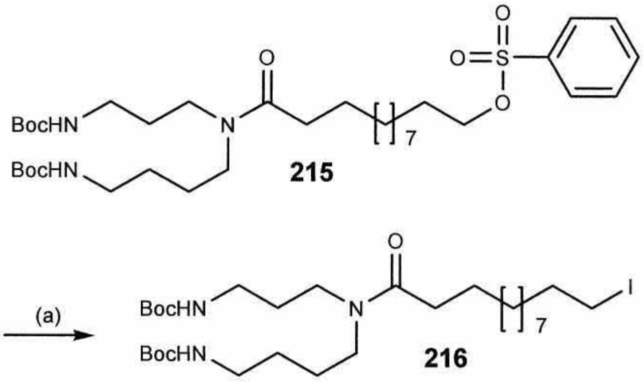


Reagents and Conditions: *p*-toluenesulphonyl chloride, pyridine, rt, 12 h, 78 %.

Scheme 8.16

Analysis of the product by proton NMR demonstrated the presence of the two signals at 7.36 (2H, d, J = 7.9 Hz) and 7.21 (2H, d, J = 7.9 Hz) ppm for the tosyl group, together with a broad singlet at d 1.42 (18H) for the Boc protecting groups. Broad singlets at 5.25 (1H) and 4.64 (1H) ppm were also observed for the amide NH protons.

Reaction of tosylate **215** with sodium iodide in acetone at reflux over four hours, followed by column chromatography on silica gave the iodide **216** in 91 % yield. (scheme 8.17).

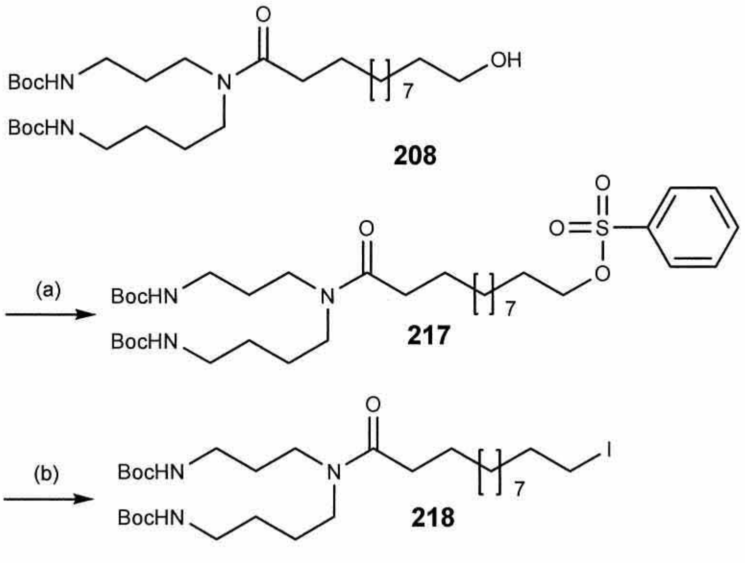


Reagents and Conditions: NaI, acetone, reflux, 4 h, 91 %.

Scheme 8.17

Analysis of **216** by proton NMR showed the presence of the broad singlet at 0.89 (18H) ppm corresponding to the Boc protecting groups, together with broad singlets at 5.42 (1H) and 4.75 (1H) ppm for the amide NH protons. LC mass spectrometry confirmed the structure as a mass of 676.3 [Na+] was obtained which corresponds exactly with expected value.

Similarly, iodide **218** was prepared from the alcohol **208** *via* the tosylate **217**, in 91 % yield.

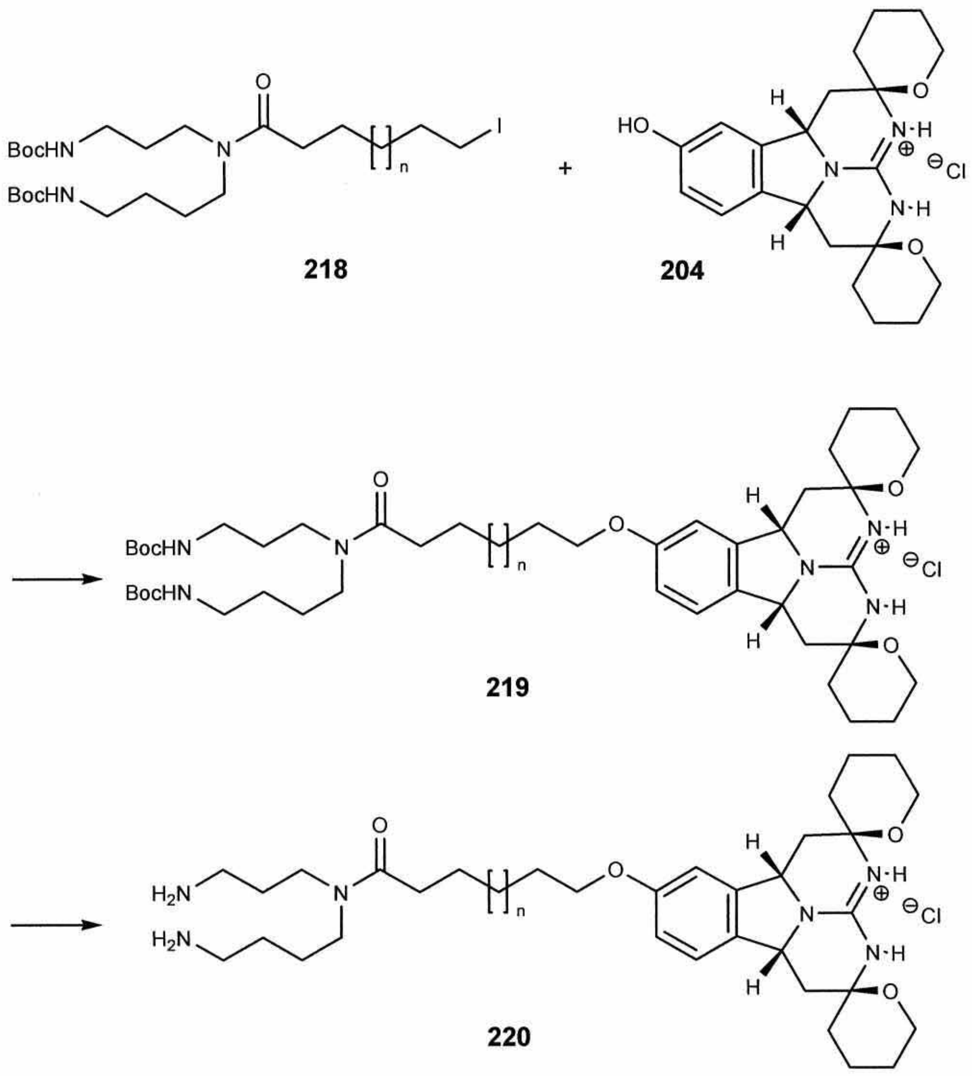


Reagents and Conditions: (a) *p*-toluenesulphonyl chloride, pyridine, rt, 12 h, 82 %; (b) NaI, acetone, reflux, 4 h, 91 %.

Scheme 8.18

8.8 COUPLING OF PHENOL 204 AND IODIDE 218

With the required sub-units in hand we next progressed to investigate the coupling of the two sub-units. Thus phenol **204** and iodide **218** were dissolved in DMF together with an excess of potassium carbonate and the mixture stirred at room temperature for 16 h, monitoring of the reaction by TLC failed to show any conversion. The reaction was heated at 40 °C for 16 h which led to the formation of a new product as indicated by tlc, after aqueous work up, the residue was purified by column chromatography to give a fraction of 80-90 % estimated (NMR) purity which gave data characteristic of the required product **219**.


BocHN
BocHN
N
O
I
n
+
HO
H
H
N
N·H
N·H
O
O
⊕
⊖Cl
218
204
219
220
H₂N
H₂N

Scheme 8.19

Analysis of **219** by proton NMR showed the presence of a singlet at δ 1.44 (18H) corresponding to the Boc protecting groups, together with doublets at δ 6.94 (1H, J = 7.7 Hz), 6.81 (1H, J = 7.7 Hz) and singlet at δ 6.44 ppm for the aromatic protons. High resolution mass spectrometry confirmed the presence of **219** as a molecular ion at 951.6895 was observed which corresponds closely with expected value of 951.6893.

The yield of this material was 80 % this represented a significant achievement as we have illustrated that the methodology is applicable to the synthesis of the required analogues.

We next attempted the deprotection of **219** using HCl (3 N) in methanol which gave **220**. Analysis of **220** by high resolution mass spectrometry confirmed the structure as a mass ion of 751.5844 was obtained which corresponds closely with expected value of 751.5827.

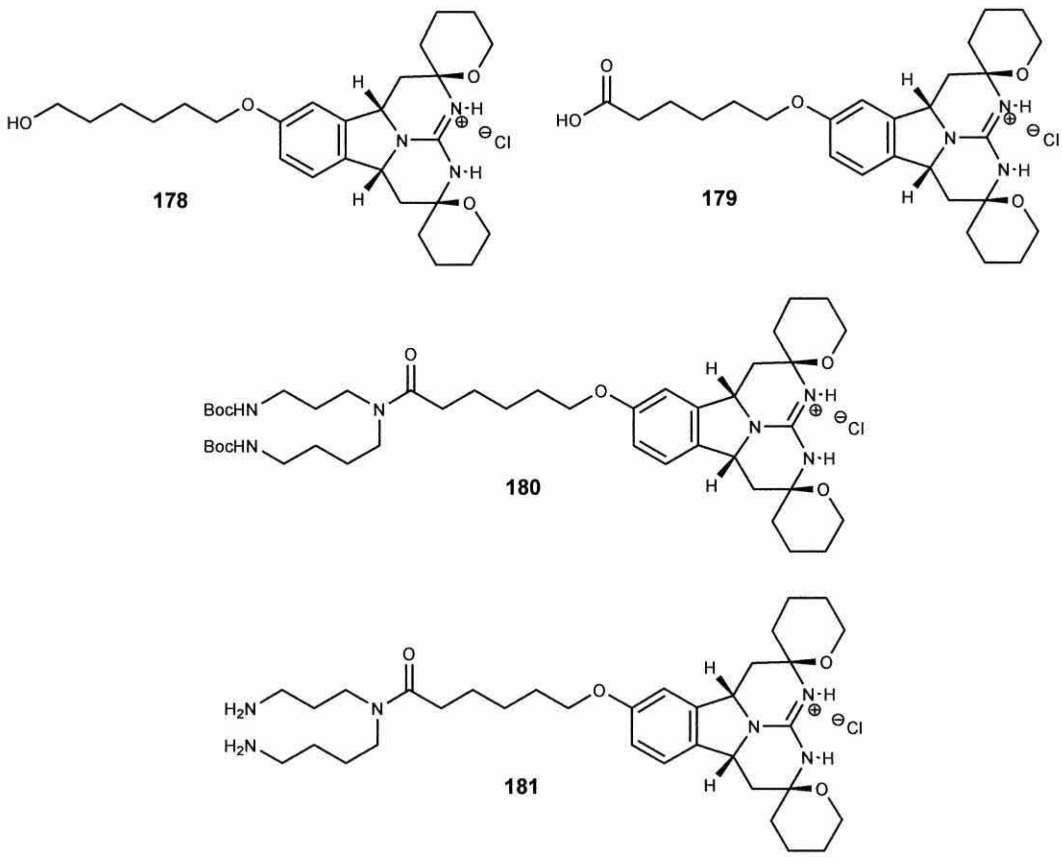
8.9 CONCLUSIONS

Our conclusion from this stage of the work is that this route appears to be a viable one. The exact nature of the reaction conditions needs to be investigated in more detail as the yield for the coupling step and the introduction of the linker function has been achieved and this should be applicable to the rapid synthesis of a range of synthesic analogues of ptilomycalin A.

8.10 BIOLOGICAL ACTIVITY

One of the original goals of this project was to investigate the biological activity of the analogues prepared and to compare these activities with the naturally occurring material and the previously prepared analogues of Moore **149** and Howard-Jones **159**.[61]

The compounds **178**, **179**, **180** and **181** were thus tested against two cell lines, A2780 (human ovarian carcinoma) and K5623 (human chronic myelogenous leukaemia), the results of which are shown in Table 8.1.[60]



178 **179** **180** **181**

Figure 8.2

Entry	Compound	A2780	K562
1	**178**	18.99	12.65
2	**179**	>50	47.99
3	**180**	>50	34.86
4	**181**	16.27	19.64

Table 8.1

The initial conclusion from these results is that the two intermediates **179** and **180** which possess a carboxylic acid and a protected spermidine are largely inactive towards either cell line. A similar observation was made by Moore and Howard-Jones[61] who tested longer chained analogues of these substances. Interestingly the alcohol **178** displayed very similar activity to the model compound **181**, as was also observed to some extent with similar analogues prepared by Moore and Howard-Jones.

We next compared the activity of the analogue **181** with those prepared previously and the natural product ptilomycalin A. These results are shown in Table 8.2.

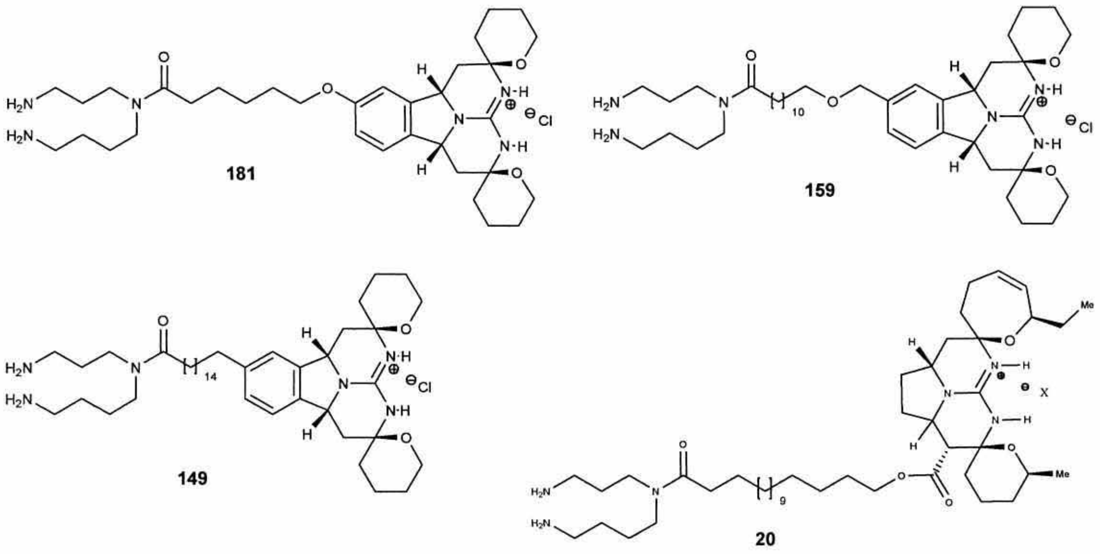


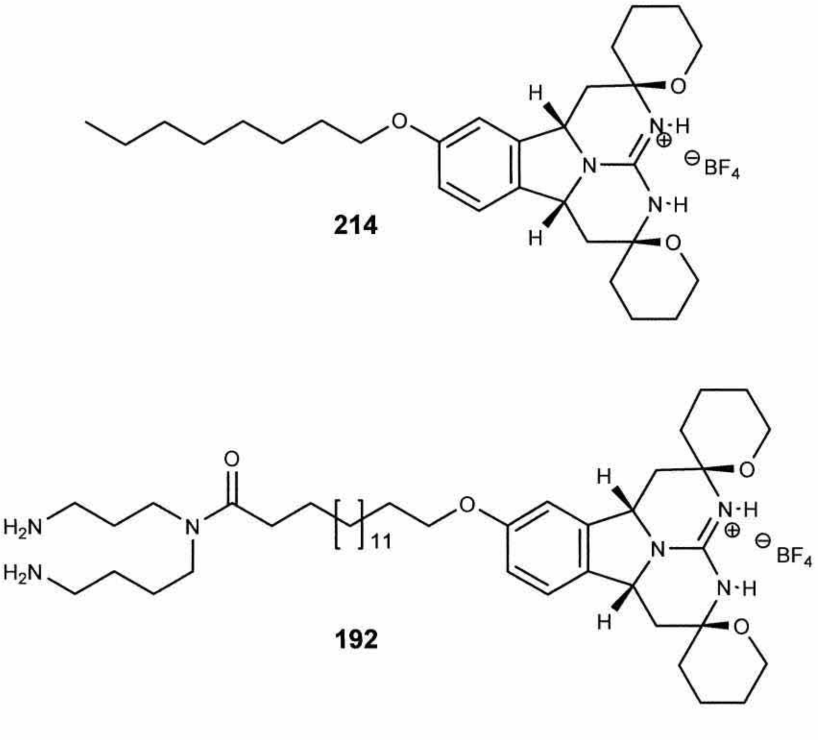
Figure 8.3

Entry	Compound	A2780	K562	Length of Spacer^a
1	**181**	16.27	19.64	7
2	**159**	11.02	7.28	14
3	**149**	0.92	0.52	16
4	**20**	0.27	0.35	18

Table 8.2

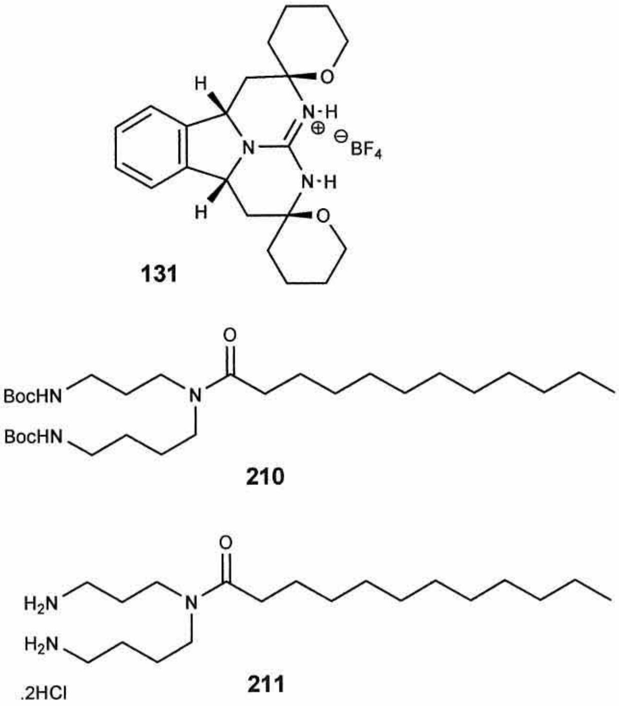
[a] The number of atoms between the fusion to the pentacycle and the amide function of the spermidine.

As can be seen the activity of the analogue **181** is less than both analogues **149**, **159** and the natural material ptilomycalin A **20**. However this is in line with our proposed theory of the origins of the activity being related to the spacing between the spermidine and the guanidine function. As can be seen there is a trend of increasing activity relating to increasing chain length. Unfortunately time constraints have left us unable to report any data for the analogues **214** or **192** prepared by the iodide coupling strategy, however this is in hand currently. Whilst the data in Table 8.2 is limited and the direct comparison of chain lengths between the analogues and ptilomycalin A **20** is not a real measure of the spacing between the two functions we feel that this offers encouragement for a further study of this area. Indeed by developing the synthesis further a range of analogues should be accessible, a larger section of data could be obtained.



Scheme 8.4

In addition to these studies we also obtained biological activity data on the compounds **210** and **211** which is shown in Table 4.3 (entries 1 and 2). Interestingly, both these analogues displayed good activity, in fact better than the analogue **181** which contains the guanidine and spermidine functions. Of possibly more interest when we took a combination of the analogues and the fairly inactive benzo-analogue **131**, we were able to increase the level of activity even further suggesting that these two might be working in a conjugate manner.


131

210

211

Figure 8.5

Entry	Compound	A2780	K562
1	**210**	13.30	11.35
2	**211**	4.80	8.49
3	**131**	22.06	24.93
4	**131/210**[a]	5.76	10.86
5	**131/211**[a]	2.80	5.76

Table 8.3

[a] 1:1 molar ratio.

.

Whilst this data is by no means comprehensive, we feel that the activities shown and the unusual observation of the increase in activity by using combinations of substrates adds merits to this and further investigations.

8.11 CONCLUSION AND FUTURE WORK

The project as a whole entailed the preparation of the ptilomycalin A analogues **181**, **191** and **192** *via* both linear and convergent routes.

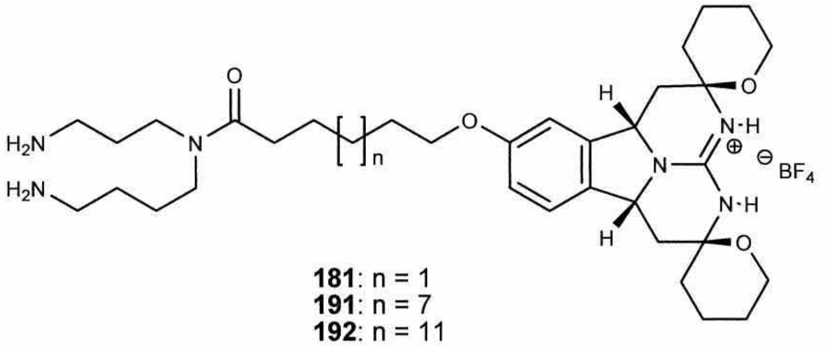


Figure 8.2

The synthesis of the short chained analogue and the observation that it has considerably lower biological activity than both the natural product and the previously prepared analogues of Moore and Howard-Jones is of considerable significance as this indicates that there is a relationship between activity and the distance of separation of the spermidine and the guanidine functional groups.

The convergent route opens up the possibility of synthesising a range of analogues with varying chain length linkers. We prepared one which closely mimics the separation distance found in ptilomycalin A and the obvious goal of further work will be to prepare several related models and to obtain a detailed set of structure activity data in order that a trend might be established.

CHAPTER 9

EXPERIMENTAL

REAGENTS

Reagents were obtained from commercial suppliers and were used without further purification unless otherwise stated. Reaction solvents were dried prior to use through literature procedures.[69] In particular, diethyl ether and tetrahydofuran were distilled from sodium wire and benzophenone; dichloromethane, DMF and hexane were dried by distillation from calcium hydride. Dry methanol was stirred with magnesium and iodine, for 5 hours then distilled.

CHROMATOGRAPHY

The extent of reaction and purity of compounds was assessed by thin layer chromatography, and was carried out on precoated 60 F254 (Art. 5554; Merck) Plates. Compounds were made visual using UV light and/or staining with PMA, permanganate or iodine. 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 semi-quantitatively as strong, medium, weak, and broad.

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.

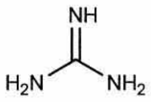
Chemical shifts are reported in δ values relative to the internal standard, tetramethylsilane. Proton spin couplings are denoted as *J* values quoted in Hz with the splitting patterns defined as singlet, doublets, triplets, quartets, apparent pentets and multiplets, or any combination of these.

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 at 25kV energy. Br and Cl refer to the isotopes ^{79}Br and ^{35}Cl, respectively.

MISCELLANEOUS

All non-aqueous reactions were performed in oven-dried glassware, 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[69] against diphenylacetic acid in THF. The term "*in vacuo*" refers to the removal of solvent under the reduced pressure of a rotary evaporator at house vacuum pump pressure (*ca* 1.4 mmHg).

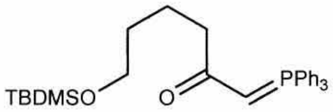
GUANIDINE



1

A static argon 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 stirred for 16 hours. The resulting mixture was filtered to remove precipitated sodium chloride and the solvent evaporated. Under argon the product was dissolved in dry methanol (30 ml) and filtered for a second time. Concentration of the solvent on high vacuum gave free guanidine (5.86 g, 95 %) as a low melting point cream solid.

6-(*TERT*-BUTYL-DIMETHYL-SILANYLOXY)-1-(TRIPHENYL-L5-PHOSPHANYLIDENE)-HEXAN-2-ONE[65]

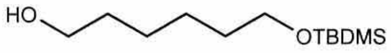


128

Methyltriphenylphosphonium bromide (7.79 g, 21.8 mmol) was suspended in THF (100 ml) and cooled to (0 ˚C). A solution of n-butyl lithium in hexane (8.7 ml, 21.8 mmol) added dropwise and the resulting mixture was stirred for ten minutes, then for four hours at room temperature producing a deep red colour.* The reaction mixture was then cooled (0 ˚C), and freshly distilled δ-valerolactone (1.09 g, 10.91 mmol) was added, and the reaction warmed to room temperature over one hour. Evaporation of the solvent gave a yellow precipitate, which was dissolved in DMF (100 ml) and cooled to (-5 ˚C) before sequential treatment with *tert*-butyldimethylsilyl chloride (1.81 g, 12.0 mmol) and imidazole (0.9 g, 13.2 mmol) in DMF (100 ml). After thirty minutes the reaction was warmed to rt and stirred for 15 hours. Water (100 ml) was then added and the reaction mixture which was extracted with ethyl acetate (3 x 50 ml), the combined extractions were dried ($MgSO_4$) and concentrated *in vacuo* to gave the crude product, which was purified by column chromatography on silica gel (eluent: 85 % ethyl acetate/petrol) yielding the title compound (4.81 g, 90 %) as a yellow viscous oil.[70]

* Note: If the deep red colour was not effected in ten minutes then *n*-BuLi (c.a. 10 %) was added.

6-(*TERT*-BUTYL-DIMETHYL-SILANYLOXY)-HEXAN-1-OL



172

Hexane-1,6-diol (50 g, 424 mmol) was dissolved in dry DMF (100 ml), cooled to 0 °C and a solution of imidazole (11.5 g, 169 mmol) in DMF (10 ml), was added following this a solution of TBDMSCl (12.7 g, 85 mmol) in DMF (60 ml) added in a dropwise manner over 2 hours. The mixture was then stirred at room temperature for 72 hours before the solution was diluted with water (500 ml), extracted with 20 % ether/petrol (2 x 200 ml) and ether (3x200 ml). The combined organic fractions where dried ($MgSO_4$) and concentrated *in vacuo* to give a yellow oil, which was purified by column chromatography on silica gel (eluent: 50 % ether/petrol) yielding the title compound (11.1 g, 57 %) as a yellow oil.

TLC : $R_f = 0.12$ in 25 % ether/petrol.

MS (CI) *m/z* : 233 (100% [M+H]).

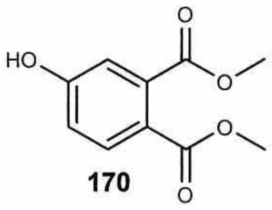
HRMS (CI) *m/z* : found 233.1939, C_{12}, H_{29}, Si, O_2, requires 233.1937.

IR (neat) ν_{max} : 3330 (br),2930 (s), 2858 (s), 1472 (m), 1462 (m), 1256 (s), 1038 (s) cm^{-1}.

^{1}H NMR δ : 1.50 (4H, m, $2CH_2$), 1.35 (4H, m, $2CH_2$), 0.88 (9H, s, $3CH_3$), 0.03 (6H, s, $2CH_2$) ppm.

^{13}C NMR δ : 63.16 (OCH_2), 62.81 (OCH_2), 32.74 ($2CH_2$), 25.94 ($3CH_3$), 25.57 ($2CH_2$), 18.33 (C), 5.30 ($2CH_3$) ppm.

4-HYDROXY-PHTHALIC ACID DIMETHYL ESTER 170[61]



170

Anhydrous, powdered zinc chloride (0.70 g, 5.22 mmol) was added to triethylamine (56 ml, 0.764 mol) and the mixture stirred until the salt was finely suspended in the amine (ca. three hours). *Trans*-4-Methoxybutene-2-one (17.55 g, 0.176 mol) in benzene (30 ml) was added followed by trimethylsilane chloride (44.2 ml, 0.475 mol). After stirring for thirty minutes at room temperature, the reaction mixture was raised to 40°C and stirring continued for 16 hours. The solution was then cooled to room temperature, ether (400 ml) was added and stirring continued for a further 30 minutes. This resulted in the formation of a precipitation which was removed from the reaction by filtration. The filtrate was concentrated *in vacuo* to give a crude black oil which was dissolved in benzene (100 ml) and to this was added dimethylacetylenedicarboxylate (17.55 g, 0.124 mol). After 15 minutes at room temperature the reaction mixture was heated to reflux for 3 hours.

After cooling (0 °C), the reaction mixture was treated with a solution of methanolic HCl [(550 ml), prepared by cautiously adding acetyl chloride (50 ml) to a stirred, cooled (0 °C) flask of methanol (500 ml)]) and stirred for 16 hours whilst warming to ambient temperature. The mixture was concentrated *in vacuo*, and purified using flash pad silica gel chromatography (gradient: 0, 15, 30, 50, 75 and 80 % ether/petrol). The combined organic fractions containing the product were dried over anhydrous magnesium sulphate and evaporated to give a crude yellow oil, which was recrystallised from 10 % ether/hexane to afford the title product (23.2 g, 89 %) as a fine pale yellow solid.

TLC : $R_f = 0.25$ in 50 % ether/petrol.

MP: 107-108 ˚C (Lit. MP: 107-108 ˚C)[62]

MS (CI) ***m/z*** **:** 211 (100 % [M+H]).

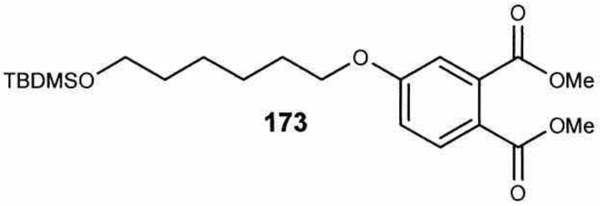
HRMS (CI) ***m/z*** **:** found 210.0603, C_{10}, H_{11}, O_5, requires 210.0606.

IR (nujol) ν_{max} **:** 3380 (br), 3020 (m), 2956 (m), 1724 (s), 1710 (s), 1614 (s), 1576 (s) cm^{-1}.

^{1}H NMR δ **:** 7.05 1H, d, J = 8.2 Hz, ArH), 6.95 (1H, d, J = 8.2 Hz, ArH), 6.90 (1H, s, ArH), 6.02 (1H, br s, OH), 3.89 (3H, s, OCH_3), 3.85 (3H, s, OCH_3) ppm.

^{13}C NMR δ **:** 169.85 (C=O), 167.46 (C=O), 159.81 (ArC), 135.56 (ArC), 131.93 (ArCH), 120.99 (ArC), 117.34 (ArCH), 115.30 (ArC), 53.03 (OCH_3), 52.57 (OCH_3) ppm.

4-[6-(*TERT*-BUTYL-DIMETHYL-SILANYLOXY)-HEXYLOXY]-PHTHALIC ACID DIMETHYL ESTER



173

4-Hydroxy-phthalic acid dimethyl ester (0.48 g, 2.29 mmol) was dissolved in DCM (10 ml) and cooled (0 ˚C), with stirring. To this solution were sequentially added 6-(*tert*-Butyl-dimethyl-silanyloxy)-hexan-1-ol (0.93 g, 4.02 mmol) dissolved in DCM (2 ml), DEAD (0.71 g, 4.10 mmol) and PPh_3 (1.41 g, 5.40 mmol). After stirring to room temperature over 16 h, the reaction mixture was quenched with water (100 ml) and extracted with DCM (100 ml). The organic fraction was washed with water (2 x 50 ml), dried ($MgSO_4$) and evaporated to yield a crude oil which was purified by chromatography (elution: 20 % ether in petrol) to give the title product (0.55g, 57 %) as a yellow oil.

TLC : $R_f = 0.15$ in 20 % ether/petrol.

MS (CI) *m/z* : 425 (100 % [M+H]), 325 (18 %), 233 (15 %), 132 (17 %).

HRMS (CI) *m/z* : found 425.2360, $C_{22}H_{37}SiO_6$ ([M+H]), requires 425.2359.

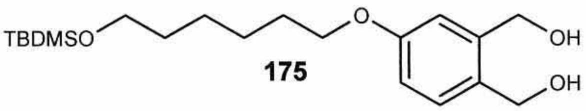
IR (neat) ν_{max} : 2960 (s), 1740 (s), 1720 (s), 1620 (s), 1576 (s), 1166 (m), 1006 (s) cm^{-1}.

^{1}H NMR δ **:** 7.05 (1H, d, J = 8.3 Hz, ArH), 7.00 (1H, d, J = 2.3 Hz, ArH), 7.00 (1H, dd, J = 8.3 and 2.3 Hz ArH), 4.02 (2H, t, J = 5.5, OCH_2), 3.92 (3H, s, OCH_3), 3.87

(3H, s, OCH_3), 3.62 (2H, t, J = 5.5, OCH_2), 1.81 (2H, m, CH_2), 1.56 (6H, m, $3CH_2$), 0.90 (9H, s, $3CH_3$), 0.06 (6H, s, $2CH_3$) ppm.

^{13}C NMR δ **:** 168.93 (C=O), 166.79 (C=O), 161.69 (ArC), 135.73 (ArC), 131.56 (ArCH), 121.77 (ArC), 116.09 (ArCH), 113.93 (ArCH), 68.39 (OCH_2), 63.04 (OCH_2), 52.70 (OCH_3), 52.30 (OCH_3), 32.68 (CH_2), 28.98 (CH_2), 25.95 ($5CH_2$), 25.73 (CH_2), 25.52 (CH_2), 18.34 (C) ppm.

{5-[6-(*TERT*-BUTYL-DIMETHYL-SILANYLOXY)-HEXYLOXY]-2-HYDROXYMETHYL-PHENYL}-METHANOL



175

4-[6-(*Tert*-butyl-dimethyl-silanyloxy)-hexyloxy]-phthalic acid dimethyl ester (1.00g, 2.36 mmol) was dissolved in DCM (2 ml), cooled (-78 ˚C), and stirred whilst DIBAL-H (1M in hexanes, 2.06 ml, 10.62 mmol) was added dropwise over 5 minutes. After stirring for thirty minutes the solution was warmed (0 ˚C) and stirring continued for 4 h. The solution was then diluted with ethyl acetate (5 ml) and stirred for 0.5 h and MeOH (30 ml) was then added. The resultant crude mixture was filtered through a silica gel pad, (which had been washed with ethyl acetate / petrol (50:50) containing 1 % TEA, which was flushed with a mixture of ethyl acetate / petrol (50:50) and 1 % TEA. Drying of the effluent over anhydrous magnesium sulphate, followed by evaporation gave the title compound (0.42g, 49 %) as a crude colourless oil, which was used immediately in the next step.

TLC : $R_f = 0.31$ in 60 % ether/petrol.

HRMS (CI) *m/z* : unstable.

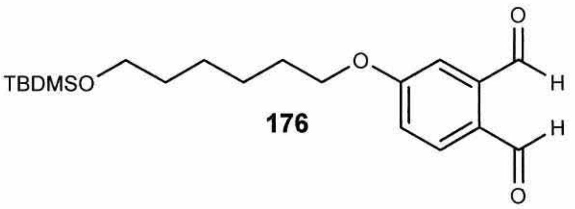
IR (neat) ν_{max} : 3322 (br), 2932 (s), 2900 (s), 1610 (s), 1578 (s), 1256 (s), 1166 (m), 1098 (s), 1006 (s) cm^{-1}.

^{1}H NMR δ : 7.25 (1H, d, J = 8.1 Hz, ArH), 6.92 (1H, d, J = 2.3 Hz, ArH), 6.82 (1H, dd, J = 8.1, 2.3 Hz ArH), 4.69 (4H, s, OCH_2), 4.54 (2H, s, CH_2OH), 3.96 (3H, t, J =

6.1, OCH_2), 3.63 (2H, t, J = 6.2, OCH_2), 3.37 (2H, br s, OH), 1.76 (2H, m, CH_2), 1.45 (6H, m, $3CH_2$), 0.90 (9H, s, $3CH_3$), 0.06 (6H, s, $2CH_3$) ppm.

^{13}C NMR δ **:** 168.20 (ArC), 136.73 (ArC), 134.01 (ArCH), 126.69 (ArC), 119.99 (ArCH), 115.72 (ArCH), 85.62 (CH_2OH), 81.73 (CH_2OH), 68.59 (OCH_2), 63.03 (OCH_2), 32.68 (CH_2), 28.95 (CH_2), 27.20 (CH_2) 25.97 (CH_2), 25.73 (CH_2), 25.53 (CH_2), 18.70 (C) ppm.

4-[6-(*TERT*-BUTYL-DIMETHYL-SILANYLOXY)-HEXYLOXY]-BENZENE-1,2-DICARBALDEHYDE



176

A solution of oxalyl chloride (0.93 ml, 10.8 mmol) in dry DCM (10 ml) was cooled (-78 °C) and dimethyl sulfoxide (1.55 ml, 21.6 mmol) was added dropwise, to give an effervescent solution which stirred for ten minutes. Alcohol (1.66 g, 4.51 mmol) in dry DCM (2 ml) was then added and the mixture stirred for one hour. Triethylamine (11.5 ml, 82.8 mmol) was then added dropwise and the reaction stirred at room temperature for one hour. The reaction was quenched with ice water (100 ml) and extracted with DCM (3 x 50 ml). After drying of the extracts ($MgSO_4$), concentration *in vacuo* gave a crude dialdehyde (1.59g, 100 %) as a yellow oil which was used immediately, in the next step.

TLC : $R_f = 0.31$ in 30 % ether/petrol.

HRMS (CI) ***m/z*** **:** found 364.2063, requires 364.2070.

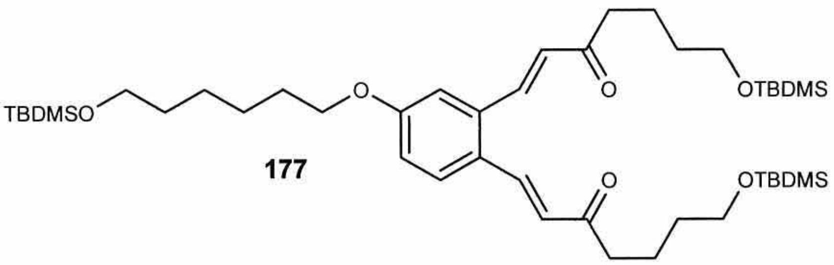
IR (neat) ν_{max} **:** 2932 (s), 2856 (s), 1768 (m), 1694 (s), 1594 (s), 1568 (s), 1254 (s), 1164 (m), 1096 (s), 1006 (s) cm^{-1}.

^{1}H NMR δ **:** 10.61 (1H, s, CHO), 10.34 (1H, s, CHO), 7.92 (1H, d, J = 8.1 Hz, ArH), 7.44 (1H, d, J = 2.5 Hz, ArH), 7.20 (1H, dd, J = 8.1, 2.5 Hz, ArH), 4.11 (2H, t, J = 6.6

Hz, OCH_2), 3.63 (2H, t, J = 6.6 Hz, OCH_2), 1.47 (8H, m, $4CH_2$), 0.90 (9H, s, $3CH_3$), 0.60 (6H, s, $2CH_3$) ppm.

^{13}C NMR δ **:** 192.07 (C=O), 191.01 (C=O), 177.50 (ArC), 138.90 (ArC), 134.60 (ArCH), 124.69 (ArC), 119.14 (ArCH), 115.26 (ArCH), 68.80 (OCH_2), 63.02 (OCH_2), 32.67 (CH_2), 28.93 (CH_2), 27.6 (CH_2), 25.96 (CH_2), 25.72 (CH_2), 25.52 (CH_2), 18.92 (C) ppm.

7-(*TERT*-BUTYL-DIMETHYL-SILANYLOXY)-1-{5-[6-(*TERT*-BUTYL-DIMETHYL-SILANYLOXY)-HEXYLOXY]-2-[7-(*TERT*-BUTYL-DIMETHYL-SILANYLOXY)-3-OXO-HEPT-1-ENYL]-PHENYL}-HEPT-1-EN-3-ONE



177

Dialdehyde **176** (0.84 g, 2.3 mmol) was dissolved in DCM (15 ml) and phosphorane **128** (2.03 g, 9.2 mmol) was added as a solution in DCM (4 ml) to the mixture and stirred for a further 72 hours. The reaction was concentrated *in vacuo* to give a yellow oil which was purified on silica gel (eluent: 10 % ether/petrol) giving the desired product, as a yellow oil (61 %, 1.11 g).

TLC : $R_f = 0.24$ in 20 % ether/petrol.

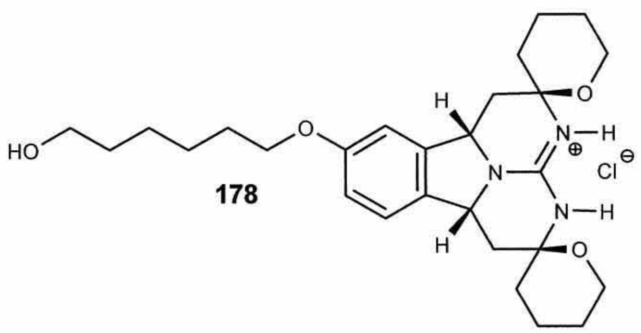
HRMS (CI) *m/z* : found 788.5263, requires 788.5263.

IR (neat) ν_{max} : 2932 (s, CH str), 1694 (s, C=O) str), 1620 (s, C=C str), 1263 (s, CO str) cm^{-1}.

^{1}H NMR δ **:** 7.82 (1H, d, J = 15.9 Hz, CH), 7.78 (1H, d, J = 15.9 Hz, CH), 7.51 (1H, d, J = 7.5 Hz, ArH), 7.21 (1H, d, J = 7.5 Hz, ArH), 7.01 (1H, s, ArH), 6.58 (1H, d, J = 15.8 Hz, CH), 6.51 (1H, d, J = 15.8 Hz, CH), 4.62 (2H, t, J = 6.4 Hz, CH_2), 3.92 (2H, t, J = 6.6 Hz, CH_2), 3.59 (4H, t, J = 6.9 Hz, 2 x CH_2), 1.72 (4H, m, 2 x CH_2), 1.40 (8H, 4 x CH_2), 0.85 (27H, s, 9 x CH_3), 0.08 (18H, s, 6 x CH_3) ppm.

^{13}C NMR δ **:** 199.07 (C=O), 160.84 (ArC), 156.33 (C=C), 138.90 (ArCH), 138.60 (ArCH), 136.69 (C=C), 129.46 (ArC), 129.14 (ArCH), 127.05 (ArCH), 116.98 (CH_2), 112.72 (CH_2), 68.10 (CH_2), 63.44 (2 x CH_2), 63.22 (CH_2), 41.19 (CH_2), 32.63 (CH_2), 32.21 (CH_2), 26.09 (9 x CH_3), 20.33 (2 x CH_2), -3.98 (3 x C) ppm.

RAC-1',2'-[3-(6-HYDROXYHEXYL)-BENZO]-ISPIRO[TETRAHYDROPYRAN-2,4'-(1,2,3,4,7,8-HEXAHYDRO-5H-5,6,8B-TRIAZAACENAPTHHYLENE-7',2''-TETRAHYDROPYRAN]-6-IUM CHLORIDE


178

Enone (1.11 g, 1.41 mmol) was dissolved in DMF (2 ml) cooled (0 °C) and stirred for ten minutes before a solution guanidine (100 mg, 1.69 mmol) was added dropwise. The resulting dark green solution stirred at 0 °C for fifteen minutes, then at room temperature for 5 hours. The reaction was then cooled (0 °C) and methanolic HCl [(10 ml), prepared by cautiously adding acetyl chloride (0.5 ml) to a stirred, cooled (0 °C) flask of methanol (9.5 ml)]) added and the reaction stirred to room temperature over 16 hours.

Water (100 ml) was added and the mixture extracted with DCM (4 x 10 ml). The combined organic fractions washed sequentially with lithium bromide solution (saturated, 100 ml), brine solution (saturated, 100 ml), dried ($MgSO_4$) and concentration *in vacuo* to give a crude solid which was purified by chromatography on silica gel (gradient elution: 0-2 % MeOH / $CHCl_3$) yielding the title product (0.313g, 44 %) as a yellow oil.

TLC : R_f = 0.05 in 2 % MeOH / $CHCl_3$.

MS (CI) ***m/z*** **:** 470 (100 %, ([M]+), 411 (12 %), 136 (30 %), 118 (42 %), 99 (48 %).

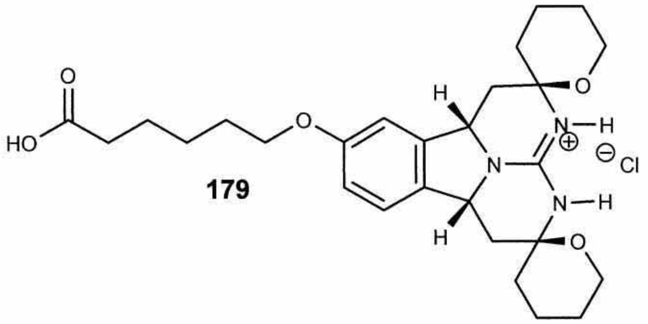
HRMS (CI) ***m/z*** **:** found 470.3013, $C_{27}H_{40}N_3O_4$ ([M]+), requires 470.3019.

IR (neat) ν_{max} **:** 3370 (br m, OH str), 3230 (m, NH str), 2910 (s, CH str), 2868 (s, CH str), 1658 (s, C=N str), 1598 (s, C=N str) cm^{-1}.

^{1}H NMR δ **:** 9.73 (2H, br s, NH), 7.08 (1H, d, J = 8.0 Hz, ArCH), 6.84 (1H, d, 8.0 Hz, ArCH), 6.68 (1H, s, ArCH), 5.11 (2H, dd, J = 11.5, 4.0 Hz, CH-3a'', CH-8a''), 4.19 (2H, ddd, J = 11.6, 11.2, 2.6 Hz, CHα-6', CHα-6'''), 3.91 (2H, br d, J = 11.6 Hz, CHβ-6', CHβ-6'''), 3.72 (2H, t, J = 6.2 Hz, CH_2OH), 3.61 (2H, t, J = 6.2 Hz, OAr), 2.55 (1H, d, J = 3.9 Hz, CH-3'') 2.50 (1H, d, J = 3.6 Hz``, CH-8''), 1.99 (1H, s, OH), 1.83-1.42 (12H, br m), 1.27 (8H, br s) ppm.

^{13}C NMR δ **:** 159.88 (C-N), 148.48 (ArC), 140.24 (ArC), 130.45 (ArC), 122.52 (ArCH), 115.03 (ArCH), 107.85 (ArCH), 79.56 ([C-2', C-4''], [C-2''', C-7''']), 68.26 (CH_2O), 62.62 (CH_2OAr), 61.94 (CH_2), 56.46 (C-3a''), 56.06 (C-8a''), 38.79 (C-3''), 34.48 (C-8''), 29.65 (CH_2), 29.31 (CH_2), 29.08 (CH_2), 26.36 (CH_2), 25.49 CH_2), 18.31 (CH_2) ppm.

RAC-1',2'-[3-(HEXYL-6-CARBOXYLIC ACID)-BENZO]-DISPIRO[TETRAHYDROPYRAN-2,4'-(1,2,3,4,7,8-HEXAHYDRO-5H-5,6,8B-TRIAZAACENAPTHHYLENE-7',2"-TETRAHYDROPYRAN]-6-IUM CHLORIDE



179

Polycyclic alcohol **178** (0.263 g, 0.52 mmol) was dissolved in DMF (4.3 ml) and stirred for 10 minutes before being treated with pyridinium dichromate (1.16 g, 3.07 mmol). After stirring for 24 hours, the reaction was poured into water (40 ml) and extracted with DCM (8 x 40 ml). The combined organic layers were washed with 2N HCl (2 x 100 ml) followed by brine solution (saturated, 2 x 100 ml). After drying ($MgSO_4$), concentration of the organic phase *in vacuo* gave a black oil which was purified by silica gel column chromatography (gradient elution: 0-5 % MeOH / DCM) yielding the title acid **179** (0.173g, 69 %).

TLC : $R_f = 0.04$ in 4 % $MeOH/CHCl_3$.

MS (CI) *m/z* : 484 (100 %), 485 (30 %), 486 (6 %), 99 (60 %).

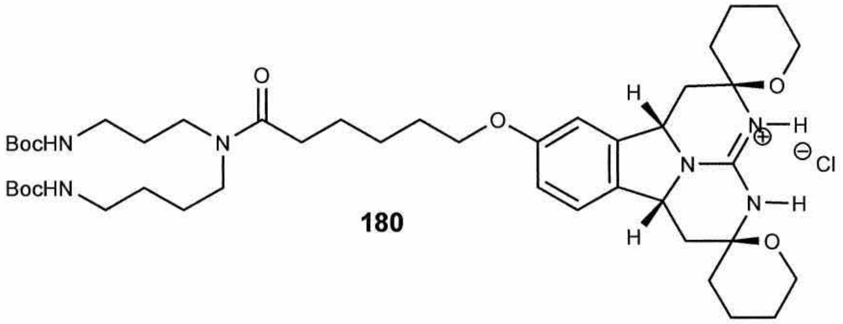
HRMS (electrospray) *m/z* : found 484.2815, $C_{27}H_{38}N_3O_5$ ([M+H]), requires 484.2811.

IR (neat) ν_{max} : 3410 (br m, OH str), 3212 (m, NH str), 2908 (s, CH str), 2861 (s, CH str), 1705 (s, C=O str), 1658 (s, C=N str), 1605 (s, C=N str) cm^{-1}.

^{1}H NMR δ **:** 9.81 (2H, br s, NH), 7.13 (1H, d, J = 8.2 Hz, ArCH), 6.89 (1H, d, J = 8.2 Hz, ArCH), 6.72 (1H, s, ArCH), 5.16 (2H, dd, J = 11.6, 2.2 Hz, CH-3a'', CH-8a''), 3.95 (2H, ddd, J = 11.6, 11.2, 2.2, CHα-6', CHα-6'''), 3.77 (2H, br d, J = 11.6 Hz, CHβ-6', CHβ-6''', 3.72 (2H, t, J = 6.2 Hz, CH_2COOH), 3.68 (2H, t, J = 6.1 Hz, OAr), 2.40 (2H, d, J = 7.0 Hz, CH-3'', CH-8''), 2.05 (2H, t, J = 6.2 Hz, CH_2), 1.81-1.47 (12H, br m), 1.26 (6H, br s) ppm.

^{13}C NMR δ **:** 166.38 (C=O), 156.98 (C-N), 148.55 (ArC), 140.32 (ArC), 130.58 (ArC), 122.55 (ArCH), 115.01 (ArCH), 107.90 (ArCH), 79.50 ([C-2', C-4''], [C-2''', C-7''']), 61.93 (CH_2OAr), 56.48 (C-3a''), 56.09 (C-8a''), 38.83 (C-3''), 38.46 (C-8''), 34.51 (CH_2), 33.86 (CH_2), 29.58 (CH_2), 28.81 (CH_2), 25.61 (CH_2), 24.96 (CH_2), 24.41 (CH_2), 18.29 (CH_2) ppm.

{4-[(3-*TERT*-BUTOXYCARBONYLAMINO-PROPYL)-(6-(*RAC*-1',2'-[3-HYDROXYBENZO]-DISPIRO[TETRAHYDROPYRAN-2,4'-(1,2,3,4,7,8-HEXAHYDRO-5H-5,6,8B-TRIAZAACENAPTHHYLENE-7',2''-TETRAHYDROPYRAN]-6-IUM CHLORIDE)HEXANOYL)-AMINO]-BUTYL}-CARBAMIC ACID TERT-BUTYL ESTER



180

A solution of the hexacyclic acid **179** (0.070 g, 0.15 mmol) in DCM (1 ml) was treated sequentially with solutions of *bis*-Boc spermidine **34** (0.063 g, 0.18 mmol) in DCM (0.5 ml), HOBT (0.040 g, 0.30 mmol) in DCM (0.25 ml) and EDCl (0.058 g, 0.30 mmol) in DCM (0.5 ml). The reaction was stirred for 48 hours, then washed with water (3 x 20 ml) and the aqueous layers back extracted with DCM (40 ml). The combined organic layers were washed with HCl (0.5 N, 2 x 20 ml) followed by brine solution (saturated, 2 x 20 ml), then stirred vigorously with a solution of sodium floroborate (saturated, 10 ml). After separation of the organic fraction was dried ($MgSO_4$) and concentrated *in vacuo*. Purification of the crude product using silica gel chromatography (gradient elution: 0-6 % MeOH / DCM), gave the title compound **180** (0.059g, 49 %) as an oil.

TLC : R_f = 0.05 in 4 % MeOH / $CHCl_3$.

MS (FAB+) ***m/z*** **:** 812 (100 %), 798 (11 %), 756 (6 %), 570 (8 %), 358 (23 %), 152 (20 %), 135 (14 %).

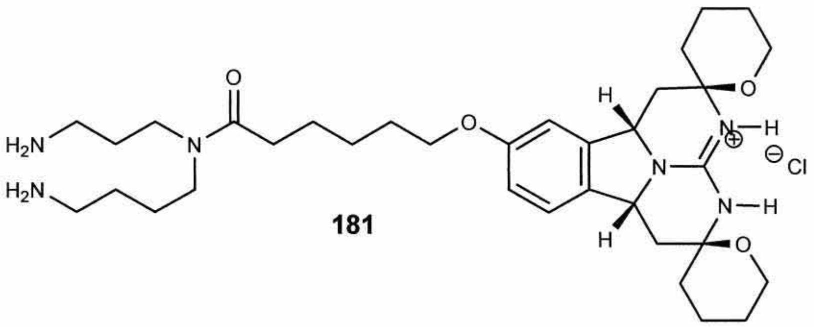
HRMS (CI) ***m/z*** **:** found 811.5329, $C_{44}H_{71}N_6O_8$ ([M+]), requires 811.5333.

IR (neat) ν_{max} : 3210 (br m, NH str), 2882 (s, CH str), 1708 (s, C=O str), 1648 (s, C=N str), 1612 (s, C=N str) cm^{-1}.

^{1}H NMR δ **:** 9.82 (2H, br s, NH), 7.14 (1H, d, J = 8.2 Hz, ArCH), 6.89 (1H, d, J = 8.2 Hz, ArCH), 6.73 (1H, s, ArCH), 5.41 (1H, br s, NHBoc), 5.17 (2H, dd, J = 5.5 Hz, CH-3a'', CH-8a''), 4.70 (1H, br s, NHBoc), 3.95 (2H, ddd, J = 12.4, 11.4, 3.3 Hz, CHα-6', CHα-6'''), 3.78 (2H, br d, J = 12.4 Hz, CHβ-6', CHβ-6''', 3.73 (2H, t, J = 6.0 Hz, CH_2CN), 3.68 (2H, t, J = 6.1 Hz, OAr), 3.39 (4H, m, CH_2), 3.31-3.05 (4H, br m), 2.40 (2H, d, J = 4.3 Hz, CH-3'', CH-8''), 1.81-1.52 (12H, br m), 1.44 (18H, br s), 1.26 (6H, br s), 0.88 (2H, m), 0.85 (4H, m) ppm.

^{13}C NMR δ **:** 174.65 (C=O), 170.67 (C=O), 156.41 (C=O), 148.23 (C=N), 140.29 (ArC), 138.97 (ArC), 138.21 (ArC), 128.43 (ArCH), 121.60 (ArCH), 120.34 (ArCH), 80.12 (2', 4'', 2''', 7''' C), 71.44 (OCH_2), 62.01 (6',6''' CH_2), 55.36 (3a'' CH), 55.30 (8a'' CH), 46.88 (CH_2), 45.20 (CH_2), 41.58 (CH_2), 40.10 (CH_2), 38.56 (3'', 8'' CH_2), 37.56 (CH_2), 34.33 (3', 3''' CH_2), 29.44(CH_2), 27.60 (CH_2), 27.55 (CH_2), 26.33 (CH_2), 25.55 (CH_2), 24.99 (5',5''' CH_2), 18.22 (4',4''' CH_2) ppm.

RAC-1',2'-[3-(6-OXY-HEXANOIC ACID (4-AMMONIUM-BUTYL)-(3-AMMONIUM-PROPYL)-AMIDE)-BENZO]-DISPIRO[TETRAHYDROPYRAN-2,4'-(1,2,3,4,7,8-HEXAHYDRO-5H-5,6,8B-TRIAZAACENAPTHHYLENE-7',2''-TETRAHYDROPYRAN]-6-IUM TRICHLORIDE



181

A solution of **180** (0.01 g, 0.012 mmol) in MeOH (0.3 ml) was treated with HCl (3 N, 0.2 ml) and gently agitated for one hour. The solvent and excess HCl were removed *in vacuo* and the resulting material azeotroped by evaporation from MeOH (3 x 0.3 ml), affording the desired hexacycle in quantiative yield (0.01 g).

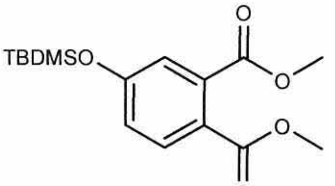
MS (ES) ***m/z*** **:** 611 (30 %), 540 (25 %), 523 (75 %), 511 (24 %), 498 (63 %), 484 (52 %), 470 (100 %).

HRMS (ES) ***m/z*** **:** found: 611.4286, $C_{34}H_{55}N_6O_4$ ([M+H])requires 611.4285

IR (neat) ν_{max} **:** 3198 (br m, NH str), 2882 (s, CH str), 1678 (s, C=O str), 1610 (s, C=N str) cm^{-1}.

^{1}H NMR δ **:** 9.80 (2H, br s, NH), 7.18 (1H, d, J = 8.2 Hz, ArCH), 6.75 (1H, d, J = 8.2 Hz, ArCH), 6.73 (1H, s, ArCH), 5.41 (2H, br s, NH_2), 5.16 (2H, dd, J = 5.5 Hz, CH-3a'', CH-8a''), 4.70 (2H, br s, NH_2), 3.94 (2H, ddd, J = 12.4, 11.4, 3.3 Hz, CHα-6', CHα-6’’’), 3.78 (2H, br d, J = 12.4 Hz, CHβ-6’, CHβ-6’’’, 3.77 (2H, t, J = 6.0 Hz, CH_2CN), 3.68 (2H, t, J = 6.1 Hz, OAr), 3.39 (4H, m, CH_2), 3.36-3.01 (4H, br m), 2.40 (2H, d, J = 4.3 Hz, CH-3’’, CH-8’’), 1.81-1.52 (12H, br m), 1.26 (6H, br s), 0.90 (2H, m), 0.79 (4H, m) ppm.

4-(*tert*-Butyl-dimethyl-silanyloxy)-phthalic acid dimethyl ester



195

The diester **170** (5.00 g, 23.8 mmol) was dissolved in DMF (15 ml) and cooled (-5 °C) before sequential treatment with *tert*-butyldimethylsilyl chloride (5.36 g, 35.7 mmol) and imidazole (3.24 g, 47.6 mmol) in DMF (10 ml). After 30 min the reaction was allowed to warm to room temperature and stirred for 12 hours. Water (150 ml) was added and the reaction mixture extracted with diethyl ether (3 x 50 ml). The combined extractions were dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product which was purified by column chromatography (eluent: 25 % ether / petrol) to give the title compound **195** (6.86g, 89 %) is a yellow solid.

MP : 107-108 °C (Lit. 105.5-106.5 °C).[61]

TLC : R_f = 0.31 in 20 % ether / petrol.

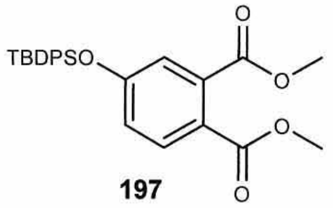
HRMS (CI) ***m/z*** **:** found 324.1393 requires 324.1395.

IR (nujol) ν_{max} **:** 3400 (br), 2992 (m), 2942 (m), 1724 (s), 1711 (s), 1615 (s), 1570 (s) cm^{-1}.

^{1}H NMR δ **:** 7.78 (1H, d, J = 7.9 Hz, ArH), 7.25 (1H, d, J = 2.3 Hz, ArH), 6.99 (1H, s, ArH), 3.95 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 0.89 (9H, s, $3CH_3$), 0.06 (6H, s, $2CH_3$) ppm.

^{13}C NMR δ **:** 172.12 (C=O), 171.52 (C=O), 159.74 (ArC), 134.89 (ArC), 132.05 (ArCH), 121.10 (ArC), 117.21 (ArCH), 115.54 (ArC), 52.89 (OCH_3), 52.51 (OCH_3), 25.80 ($3CH_3$), 1.02 ($2CH_3$) ppm.

4-(*TERT*-BUTYL-DIPHENYL-SILANYLOXY)-PHTHALIC ACID DIMETHYL ESTER



197

The diester **170** (11.35 g, 54 mmol) was dissolved in DMF (20 ml) and cooled (-5 ˚C) before sequential treatment with *tert*-butyldiphenylsilyl chloride (19.25 g, 70 mmol) and imidazole (9.59 g, 141 mmol) in DMF (10 ml). After 30 min the reaction mixture was allowed to warm to rt and stirred for 15 hours. Water (200 ml) was added and the reaction mixture extracted with diethyl ether (3 x 75 ml). After drying ($MgSO_4$) and concentration *in vacuo* the crude product was purified by column chromatography on silica gel (eluent: 25 % ether / petrol) yielding the title compound (20.56g, 85 %) as a yellow solid.

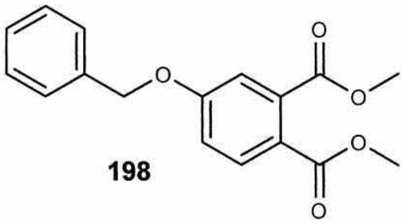
TLC : R_f = 0.26 in 20 % ether / petrol.

HRMS (CI) ***m/z*** **:** found 448.1706 requires 448.1706.

IR (nujol) ν_{max} **:** 3400 (br), 2992 (m), 2965 (m), 1731 (s), 1722 (s), 1622 (s), 1571 (s) cm^{-1}.

^{1}H NMR δ **:** 7.42-7.39 (10H, br m, ArH), 7.10 (1H, d, J = 8.0 Hz, ArH), 6.71(1H, d, J = 2.5 Hz, ArH), 6.68 (1H, s, ArH), 3.87 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 1.11 (9H, s, CH_3) ppm.

4-BENZYLOXY-PHTHALIC ACID DIMETHYL ESTER



198

Potassium carbonate (1.68 g, 12.1 mmol) was suspended in dry THF (5 ml) and **170** (1g, 5.62 mmol) added as a solution in THF (10 ml) followed by (1.92g, 11.2 mmol) dissolved in THF (5 ml). The solution was stirred for 2 hours at rt then refluxed for 16 hours. After cooling, diethyl ether (200 ml) was added and the reaction mixture filtered through a silica pad. The filtrate was dried ($MgSO_4$) and evaporated *in vacuo*. The solid obtained was recrystallised from ether / petrol to afford the title product as a fine white powder (1.10 g, 77 % yield).

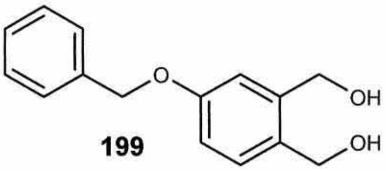
TLC : $R_f = 0.30$ in 40 % ethyl acetate / petrol.

HRMS (CI) ***m/z*** **:** 300.0999 found , 300.0998 requires.

^{1}H NMR δ **:** 7.48-7.40 (5H, br m, ArH), 7.37 (1H, d, J = 8.3 Hz, ArH), 7.19 (1H, d, J = 2.7 Hz, ArH), 7.08 (1H, dd, J = 8.3, 2.7 Hz, ArH), 5.13 (2H, s, CH_2), 3.93 (3H, s, OCH_3), 3.88 (3H, s, OCH_3) ppm.

^{13}C NMR δ **:** 168.75 (C=O), 166.79 (C=O), 161.20 (ArCO), 135.77 ($ArCCH_2$), 131.58 (ArCH), 128.91 (ArCH),128.70 (ArCH), 128.34 (ArCH), 127.49 (ArCH), 122.44 (ArCH), 16.45 (ArCH),114.47 (ArCH), 70.34 (CH_2), 52.77 (CH_3), 52.37 ppm.

(5-BENZYLOXY-2-HYDROXYMETHYL-PHENYL)-METHANOL



199

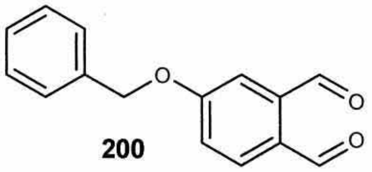
The benzyl protected dimethyl ester **198** (2.00 g, 6.67 mmol) was dissolved in DCM (2 ml), cooled (-78 °C) and DIBAL-H (1M in hexanes 9.12 ml, 45 mmol) was added dropwise over 5 minutes. After stirring for thirty minutes the solution was warmed (0 °C) and stirring continued for four hours. The solution was then diluted with ethyl acetate (10 ml) and stirred for 0.5 h whereupon MeOH (15 ml) was added. The resultant mixture was then filtered through a silica gel pad, (which had been washed with ethyl acetate / petrol (50:50) containing 1 % TEA and eluted with a mixture of ethyl acetate / petrol (50:50) and 1 % TEA. Drying of the filtrate over anhydrous magnesium sulphate, followed by evaporation *in vacuo* gave the title compound (1.05 g, 65 %) as yellow oil which was used without further purification in the next step.

TLC : R_f = 0.20 in 80 % ethyl acetate / petrol.

HRMS (CI) ***m/z*** **:** unstable.

^{1}H NMR δ **:** 7.41 (5H, br m, ArH), 7.21 (1H, d, J = 8.3 Hz, ArH), 6.98 (1H, d, J = 2.5 Hz, ArH), 6.84 (1H, dd, J = 8.3, 2.5 Hz, ArH), 5.03 (2H, s, OCH_2), 4.56 (2H, s, OCH_2), 4.53 (2H, s, OCH_2) 2.05 (2H, s, OH) ppm.

4-BENZYLOXY-BENZENE-1,2-DICARBALDEHYDE



200

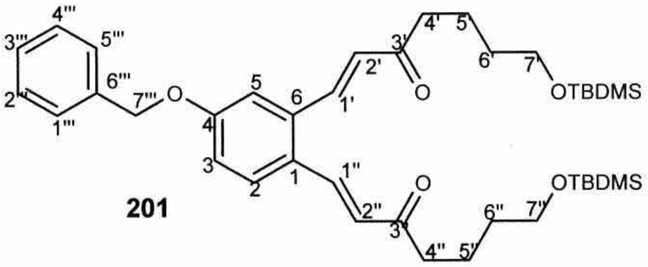
A solution of oxalyl chloride (0.73 ml, 8.34 mmol) in dry DCM (5 ml) was cooled (-78 ˚C) and dimethyl sulfoxide (1.18 ml, 16.7 mmol) was added dropwise to give an effervescent solution which was stirred for ten min. Diol **199** (1.00g, 4.10 mmol) was then added and the mixture stirred for one hour. Triethylamine (9.27 ml, 66.7 mmol) was added dropwise and the reaction was stirred at room temperature for 1 h. The reaction was quenched with ice water (100 ml) and extracted with DCM (3 x 50 ml). After drying of the extracts ($MgSO_4$) concentration *in vacuo* gave the crude product (0.98g, 100 %) as a yellow oil which was used immediately, in the next step.

TLC : $R_f = 0.32$ in 80 % ethyl acetate / petrol.

HRMS (CI) ***m/z*** **:** 240.0790 found , 240.0791 requires.

^{1}H NMR δ **:** 10.66 (1H, s, CHO), 10.35 (1H, s, CHO), 7.47 (5H, br m, ArH), 7.40 (1H, d, J = 8.1 Hz, ArH), 7.31 (1H, d, J = 2.4 Hz, ArH), 7.27 (1H, s, ArH), 5.22 (2H, s, CH_2O) ppm.

1-{5-BENZYLOXY-2-[7-(*TERT*-BUTYL-DIMETHYL-SILANYLOXY)-3-OXO-HEPT-1-ENYL]-PHENYL}-7-(*TERT*-BUTYL-DIMETHYL-SILANYLOXY)-HEPT-1-EN-3-ONE



201

Dialdehyde **200** (1.00 g, 4.17 mmol) was dissolved in DCM (20 ml) and added to a solution of phosphorane (8.29 g, 16.92 mmol) in DCM (10 ml) and the mixture refluxed for 20 h. The solvent was then concentrated *in vacuo* to furnish a crude yellow oil which was purified on silica gel (eluent: 25 % ether/petrol) giving the desired product **201**, as a yellow oil (62 %, 1.72 g).

TLC : $R_f = 0.25$ in 25 % ether / petrol.

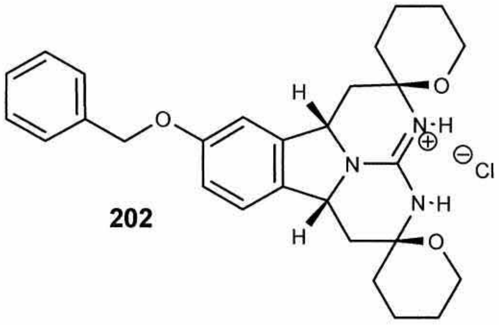
HRMS (CI) ***m/z*** **:** 665.4056 found , 665.4057 requires.

^{1}H NMR **δ :** 7.92 (1H, d, J = 15.9 Hz, CH), 7.87 (1H, d, J = 15.9 Hz, CH), 7.44-7.35 (5H, br m, ArH), 7.33 (1H, d, J = 8.8 Hz, ArH), 7.14 (1H, d, J = 2.6 Hz, ArH), 7.01 (1H, dd, J = 8.8, 2.6 Hz, ArH), 6.62 (1H, d, J = 15.9 Hz, CH), 6.60 (1H, d, J = 15.9 Hz, CH),

5.11 (2H, s, CH_2), 3.65 (2H, t, J = 5.2 Hz, CH_2), 3.59 (8H, br m, CH_2), 0.85 (27H, s, 9 x CH_3), 0.08 (18H, s, 6 x CH_3) ppm.

^{13}C NMR δ **:** 199.73 (C=O), 160.12 (ArC4), 156.33 (ArCH3'''), 138.65 (C=C1', 138.20 (ArC6), 136.51 (ArC2''', ArC4'''), 136.10 (ArCH3'''), 129.22 (ArC1), 128.70 (C=C2', C=C2''), 128.28 (ArCH$_2$), 117.10 (ArC3), 113.18 (ArC5), 70.19 (CH$_2$7'''), 62.85 (CH$_2$7'), 62.64 (CH$_2$7''), 41.05 (CH$_2$4', CH$_2$4''), 32.31 (CH$_2$6', CH$_2$6''), 32.31 (CH$_2$6', CH$_2$6''), 22.69 (6 x CH$_3$), 19.94 (CH$_2$5', CH$_2$5''), 15.27 (C(CH$_3$)$_3$), -5.31 (CH$_3$Si) ppm.

RAC-1',2'-[3-(BENZYLOXY)-BENZO]-DISPIRO[TETRAHYDROPYRAN-2,4'-(1,2,3,4,7,8-HEXAHYDRO-5H-5,6,8B-TRIAZAACENAPTHHYLENE-7',2''-TETRAHYDROPYRAN]-6-IUM CHLORIDE



202

Enone (1.72 g, 2.59 mmol) was dissolved in DMF (3 ml) cooled (0 °C) and stirred for ten minutes, whereupon a solution of guanidine (306 mg, 5.18 mmol) in DMF (2 ml) was added dropwise cooled (0 °C). The resulting dark green mixture was stirred for fifteen minutes, then warmed to room temperature and stirred for 5 hours. The reaction mixture was cooled (0 °C) then diluted with a solution of methanolic HCl [(25 ml), prepared by cautiously adding acetyl chloride (1.5 ml) to a stirred, cooled (0 °C) flask of methanol (23.5 ml)]) and stirred for 16 hours whilst warming to room temperature. Water (300 ml) was then added and the mixture extracted with DCM (4 x 20 ml). The combined organic fractions were then washed sequentially with lithium bromide solution (saturated, 200 ml), brine solution (saturated, 200 ml), dried ($MgSO_4$) and concentration *in vacuo*. The crude product was purified by silica gel chromatography (gradient elution: 0-3 % MeOH / $CHCl_3$) yielding the title product (0.526 g, 41 %) as a yellow solid.

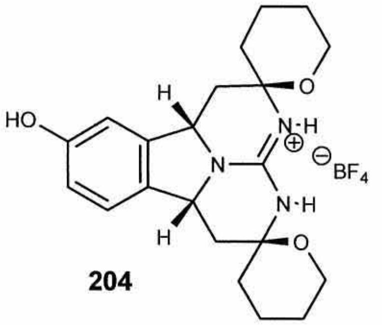
TLC : $R_f = 0.10$ in 2 % MeOH / DCM.

HRMS (CI) *m/z* : 460.2602 found , 460.2600 requires.

^{1}H NMR δ **:** 9.39 (2H, br s, N-H), 7.43-7.41 (5H, br m, ArCH), 7.38 (1H, d, 8.3 Hz, ArCH), 7.16 (1H, d, J = 1.9 Hz, ArCH), 7.00 (1H, dd, J = 8.3, 1.9), 5.24 (2H, dd, J = 11.6, 4.0 Hz, CH-3a”, CH-8a”) 5.08 (1H, s, CH$_2$), 3.94 (2H, ddd, 11.8, 11.2, 2.8 Hz, CHα-6’, CHα-6’’’), 3.78 (2H, ddd, 11.8, 11.2, 2.8 Hz, CHβ-6’, CHβ-6’’’), 2.61 (2H, m, CH$_2$), 2.17-2.04 (2H, m, CH$_2$), 1.61 (4H, br m, CH$_2$) ppm.

^{13}C NMR δ **:** 157.37 (C=N), 140.02 (ArC), 138.07 (ArC), 138.02 (ArC), 137.22 (ArC), 128.51 (ArCH), 128.06 (ArCH), 127.29 (ArCH), 127.10 (ArCH), 121.60 (ArCH), 120.74 (ArCH), 80.25 (2’, 4”, 2’’’, 7” C), 70.73 (OCH$_2$), 61.98 (6’,6’’’ CH$_2$), 56.30 (3a” CH), 56.12 (8a” CH), 38.13 (3”, 8” CH$_2$), 34.08 (3’, 3’’’ CH$_2$), 24.65 (5’,5’’’ CH$_2$), 17.83 (4’,4’’’ CH$_2$) ppm.

RAC-1',2'-[3-(HYDROXY)-BENZO]-DISPIRO[TETRAHYDROPYRAN-2,4'-(1,2,3,4,7,8-HEXAHYDRO-5H-5,6,8B-TRIAZAACENAPTHHYLENE-7',2''-TETRAHYDROPYRAN]-6-IUM FLUOROBORATE



204

The benzyl protected guanidine polycycle **202** (0.63 g, 1.27 mmol) was dissolved in TFA (4.14 ml, 24.6 mmol) cooled (0 °C) and thioanisole (0.26 ml, 2.05 mmol) was added dropwise, after stirring to rt over 12 hours, the reaction was evaporated to dryness and purified by column chromatography (gradient elution 0-5 MeOH / chloroform).

Ion Exchange. The product was dissolved in MeOH (15 ml) and stirred vigorously with floroborate solution (saturated, 10 ml) for 24 hours. Water (50 ml) was added and the mixture extracted with DCM (4 x 20 ml), the solvent was removed *in vacuo*, giving the title product **204** (0.281 g, 48 %).

TLC : R_f = 0.11 in 5 % MeOH / DCM.

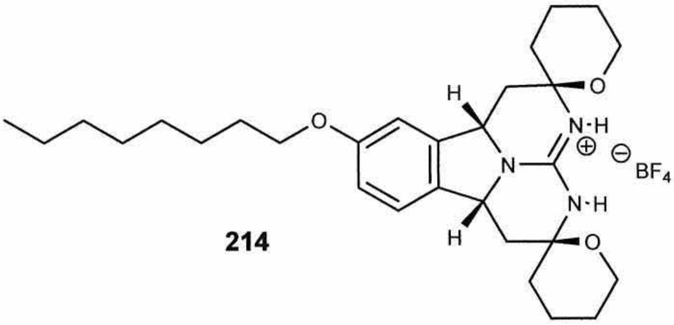
HRMS (CI) ***m/z*** **:** 370.2128 found , 370.2130 requires.

^{1}H NMR δ **:** 9.20 (1H, s, NH), 9.18 (1H, s, NH), 8.35 (1H, br s, OH), 7.00 (1H, d, 8.1 Hz, ArCH), 6.89 (1H, d, J = 8.1 Hz, ArCH), 6.60 (1H, s), 5.12 (2H, dd, J = 11.9, 4.0 Hz, CH-3a'', CH-8a''), 3.93 (2H, ddd, 11.4, 11.0, 2.8 Hz, CHα-6', CHα-6'''), 3.77

(2H, br d, 11.4 Hz, CHβ-6', CHβ-6'''), 2.88 (1H, br s, OH), 2.53 (2H, m, CH_2), 1.98-1.79 (2H, m, CH_2), 1.67 (4H, br m, CH_2) ppm.

^{13}C NMR d : 148.42 (C=N), 140.32 (ArC), 137.69 (ArC), 136.10 (ArC), 128.73 (ArCH), 122.37 (ArCH), 116.11 (ArCH), 79.47 (2', 4'', 2''', 7''' C), 61.88 (6',6''' CH_2), 56.44 (3a'' CH), 56.13 (8a'' CH), 38.06 (3'', 8'' CH_2), 34.22 (3', 3''' CH_2), 24.88 (5',5''' CH_2), 17.99 (4',4''' CH_2) ppm.

RAC-1',2'-[3-(1-OXANONYL)-BENZO]-DISPIRO[TETRAHYDROPYRAN-2,4'-(1,2,3,4,7,8-HEXAHYDRO-5H-5,6,8B-TRIAZAACENAPTHHYLENE-7',2"-TETRAHYDROPYRAN]-6-IUM CHLORIDE



214

Potassium carbonate (0.108 g, 0.789 mmol) and phenol **170** (0.12 g, 0.263 mmol) were dissolved in DMF and were stirred for 5 min. Iodooctane (0.075 g, 0.316 mmol) was then added dropwise as a solution in DMF (5 ml) and the resulting mixture stirred for 16 h. Water (30 ml) was then added and the reaction extracted with DCM (2 x 10 ml). The combined organic layers were dried ($MgSO_4$), evaporated *in vacuo* and columned on silica gel using a gradient elution (0-5 % MeOH / chloroform), affording the title compound, as a yellow solid (0.079 g, 53 %).

TLC : R_f = 0.11 in 2 % MeOH / DCM.

HRMS (CI) ***m/z*** **:** 482.3379 found , 482.3382 requires.

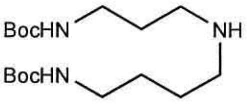
IR (neat) ν_{max} **:** 3386 (br m, OH str), 3220 (m, NH str), 2886 (s, CH str), 2868 (s, CH str), 1669 (s, C=N str), 1601 (s, C=N str) cm^{-1}.

^{1}H NMR δ **:** 9.55 (2H, br s, NH), 7.55 (1H, d, J = 8.1 Hz, ArCH), 7.31 (1H, d, 8.1 Hz, ArCH), 7.16 (1H, s, ArCH), 5.60 (2H, dd, J = 12.1, 4.2 Hz, CH-3a'', CH-8a''), 4.40

(2H, ddd, J = 12.5, 11.2, 2.5 Hz, CHα-6', CHα-6'''), 4.18 (2H, br d, J = 12.5 Hz, CHβ-6', CHβ-6'''), 3.01 (1H, d, J = 4.3 Hz, CH-3'', CH-8''), 2.97 (1H, d, J = 4.3 Hz, CH-3'', CH-8''), 2.21-1.89 (12H, br m), 1.32 (8H, br s) 1.29 (3H, t, J = 6.7 Hz, CH_3) ppm.

^{13}C NMR δ **:** 148.61 (C=N), 140.41 (ArC), 130.58 (ArC), 122.56 (ArC), 122.10 (ArCH), 114.97 (ArCH), 107.81 (ArCH), 79.50 (2', 4'', 2''', 7''' C), 68.45 (CH2), 61.93 (6',6''' CH_2), 56.49 (3a'' CH), 56.10 (8a'' CH), 38.71 (3'', 8'' CH_2), 34.45 (3', 3''' CH_2), 31.78 (CH_2), 29.43 (CH_2), 29.30 (CH_2), 25.99 (CH_2), 24.96 (5',5''' CH_2), 23.01 (CH_2), 22.64 (CH_2), 18.11 (4',4''' CH_2), 14.09 (CH_3) ppm.

[4-(3-TERT-BUTOXYCARBONYLAMINO-PROPYLAMINO)-BUTYL]-CARBAMIC ACID TERT-BUTYL ESTER (*BIS*-BOC-SPERMIDINE)



183

Spermidine (1.0 g, 6.7 mmol) was dissolved in DCM (15 ml) with stirring and 2-(tert-butoxycarbonyloximino)-2-phenylacetonitrile (3.3 g, 13.4 mmol) in DCM (15 ml) was added slowly. 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 / DCM) to afford the crude product (1.28 g) which was recrystallised from 10 % ethyl acetate / hexane to give title product (1.08 g, 47 %) as a white solid.

TLC : R*f* = 0.20 in 10 % MeOH / DCM.

Melting point = 84-86 ˚C.

IR ($CHCl_3$) vmax : 3452 (m), 2978 (m), 2934 (w), 1708 (s), 1511 (s), 1502 m(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, 3 x CH_2) 1.45 (18H, s, CH_3) ppm.

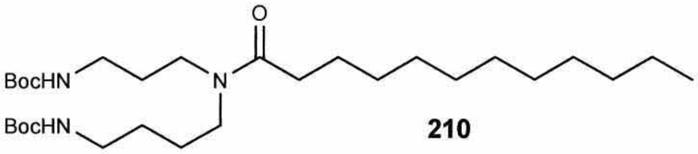
^{13}C NMR δ : 156.15 (c), 156.03 (c), 78.98 (2xC), 49.34 (CH2), 47.58 (CH2), 40.38 (CH2), 39.11 (CH2), 29.73 (CH2), 28.42 (6xCH3), 27.78 (CH2), 27.18 (CH2) ppm.

MS (CI) m/z : 346 (100 % [M+H]+, 272 (34 %[M-Ot-Bu]+), 216 (10 %, 198 (27 %), 172 (12 %).

HRMS (CI) m/z : found: 346.2706, $C_{17}H_{36}N_3O_4$ ([M+H]+) requires: 346.2706.

Microanalysis : found C, 58.8 %, H, 10.5 %, N, 12.3 %, $C_{17}H_{36}N_3O_4$ requires: C, 59.1 %, H, 10.2 %, N, 12.2 %.

{4-[(3-*TERT*-BUTOXYCARBONYLAMINO-PROPYL)-DODECANOYL-AMINO]-BUTYL}-CARBAMIC ACID *TERT*-BUTYL ESTER



210

A solution of lauric acid (250 mg, 1.2 mmol) in DCM (5 ml) was treated sequentially with solutions of *bis*-Boc spermidine **34** (490 mg, 1.4 mmol) in DCM (2 ml), HOBT (319 mg, 2.4 mmol) in DCM (1 ml) and EDCl (453 mg, 2.7 mmol) in DCM (2 ml). The reaction was stirred for 48 hours, then washed with water (3 x 20 ml) and the aqueous layers back extracted with DCM (40 ml). The combined organic layers were washed with HCl (0.5 N, 2 x 20 ml) followed by brine solution (saturated, 2 x 20 ml), then dried ($MgSO_4$) and concentrated *in vacuo*, and purified by column chromatography give title product **210** (0.34 g, 52 %) as an oil.

TLC : $R_f = 0.30$ in 50 % ethyl acetate / petrol.

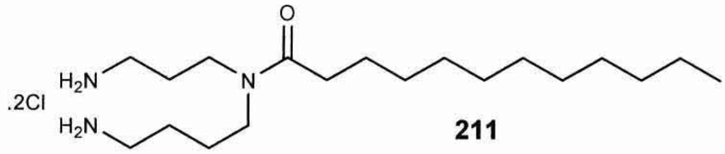
HRMS (CI) ***m/z*** **:** found 528.4366, requires 528.4376.

^{1}H NMR δ **:** 5.48 (1H, br s, NH), 4.72 (1H, br s, NH), 3.53 (2H, t, J = 6.4, CH_2), 3.32 (4H, m, CH_2), 2.84 (4H, m, CH_2), 2.28 (2H, m, CH_2), 1.63 (6H, m, 3 x CH_2), , 1.44 (18H, s, Me), 1.22 (18H, s, CH_2) 0.91 (3H, t, J = 2.1 Hz, Me) ppm.

^{13}C NMR δ **:** 171.52 (C=O), 156.81 (C=O), 156.52 (C=O), 79.20 (2 x C), 47.22 (CH_2), 45.54 (CH_2), 42.38 (CH_2), 41.12 (CH_2), 37.04 (CH_2), 34.51 (CH_2), 33.04 (CH_2), 33.04 (CH_2), 29.94 (CH_2), 29.21 (CH_2), 29.11 (CH_2), 28.55 (6 x CH_3), 27.56

(CH_2), 27.50 (CH_2), 26.02 (CH_2), 25.59 (CH_2), 25.21 (CH_2), 25.01 (CH_2), 13.45 (CH_3) ppm.

DODECANOIC ACID (4-AMINO-BUTYL)-(3-AMINO-PROPYL)-AMIDE BIS-HYDROCHLORIDE



211

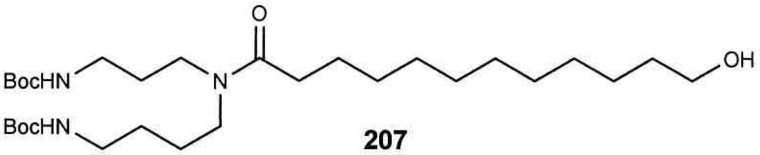
A solution of **210** (50 mg, 0.094 mmol) in MeOH (0.3 ml) was treated with HCl (3 N, 0.2 ml) and gently agitated for one hour. The solvent and excess HCl were removed *in vacuo* and the resulting material azeotroped by evaporation from MeOH (3 x 0.3 ml), affording the desired hexacycle in quantiative yield (31 mg).

TLC : R_f = 0.13 in 50 % ethyl acetate / petrol.

HRMS (CI) ***m/z*** **:** found 327.3261, requires 327.3250.

^{1}H NMR δ **:** 5.10 (1H, br s, NH), 4.55 (1H, br s, NH), 3.52 (2H, t, J = 6.6, CH_2), 3.21 (4H, m, CH_2), 2.84 (4H, m, CH_2), 2.29 (2H, m, CH_2), 1.63 (10H, m, 3 x CH_2, 2 x NH_2), , 1.44 (18H, s, Me), 1.22 (18H, s, CH_2) 0.91 (3H, t, J = 2.0 Hz, Me) ppm.

{4-[(3-*TERT*-BUTOXYCARBONYLAMINO-PROPYL)-(12-HYDROXY-DODECANOYL)-AMINO]-BUTYL}-CARBAMIC ACID *TERT*-BUTYL ESTER



207

A solution of the hydroxy acid **205** (0.260 g, 1.21 mmol) in DCM (2 ml) was treated sequentially with *bis*-Boc spermidine **34** (0.500 g, 1.45 mmol) in DCM (2 ml), HOBT (0.291 g, 2.42 mmol) in DCM (1 ml) and EDCl (0.464 g, 2.42 mmol) in DCM (1 ml). The reaction was stirred for 24 hours, diluted with DCM (50 ml) and washed with HCl (0.5 N, 2 x 20 ml), brine solution (saturated, 3 x 20 ml) and $NaHCO_3$ solution (saturated) (each aqueous wash was back extracted with DCM (10 ml) and these recombined with the main organic layers). The combined organic fractions were then dried ($MgSO_4$) and concentrated *in vacuo*. Purification of the crude product using silica gel chromatography (elution: 50 % ethyl acetate / petrol), gave the title compound (0.51 g, 78 %) as a yellow oil.

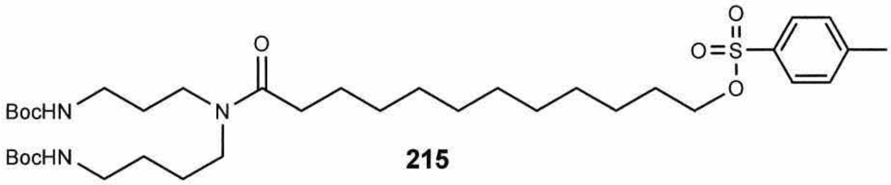
TLC : R_f = 0.18 in 2 % MeOH / DCM.

HRMS (CI) ***m/z*** **:** found 544.4315, requires 544.4325.

^{1}H NMR δ : 5.47 (1H, br s, NH), 4.72 (1H, br s, NH), 3.62 (2H, t, J = 6.4, CH2), 3.37 (4H, m, CH_2), 2.99 (4H, m, CH_2), 2.48 (1H, br s, OH), 2.28 (2H, m, CH_2), 1.63-1.47 (7H, m, 3 x CH_2, NH) 1.40 (18H, s, CH_3) 1.25 (18H, s, CH_2) ppm.

^{13}C NMR δ **:** 172.93 (C=O), 156.18 (C=O), 156.04 (C=O), 78.81 (2 x C), 62.82 (CH_2OH), 47.42 (CH_2), 45.54 (CH_2), 42.37 (CH_2), 39.91 (CH_2), 33.04 (CH_2), 32.71 (CH_2), 29.84 (CH_2), 29.45 (CH_2), 29.37 (CH_2), 28.40 (6 x Me), 27.55 (CH_2), 27.34 (CH_2), 26.10 (CH_2), 25.52 (CH_2), 25.40 (CH_2), 24.88 (CH_2) ppm.

TOLUENE-4-SULFONIC ACID 11-[(4-*TERT*-BUTOXYCARBONYLAMINO-BUTYL)-(3-*TERT*-BUTOXYCARBONYLAMINO-PROPYL)-CARBAMOYL]-UNDECYL ESTER



215

A solution of (0.337 g, 0.619 mmol) in dry pyridine (2 ml) was cooled (0 ˚C) whereupon a solution of *p*-toluenesulphonyl chloride (0.118 g, 0.619 mmol) in dry pyridine (2 ml) was added. The resulting mixture was stirred at rt for 12 hours, before the solvent was removed *in vacuo* and purified the crude mixture using silica gel chromatography (gradient elution 0-5 MeOH / chloroform), giving **215** (0.337g, 78 %) as a yellow solid.

TLC : R_f = 0.32 in 2 % MeOH / DCM .

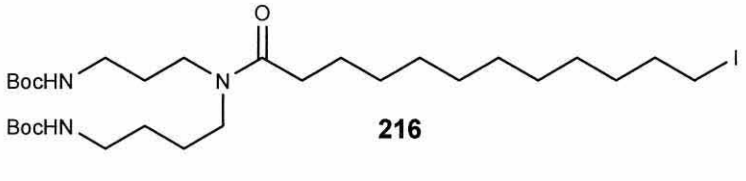
LCMS ***m/z*** **Na+ :** found 720.5, requires 720.5

^{1}H NMR δ **:** 7.36 (1H, d, J = 7.6, ArCH), 7.21 (1H, d, J = 7.6, ArCH), 5.25 (1H, br s, NH), 4.64 (1H, br s, NH), 3.64 (2H, t, J = 6.4, CH_2), 3.38 (4H, t, J = 6.4, CH_2), 3.31 (4H, m, CH_2), 2.45 (3H, s, CH_3), 2.32 (2H, t, J = 11.0, CH_2), 1.61-1.44 (7H, m, 3 x CH_2, NH) 1.42 (18H, s, CH_3) 1.23 (18H, s, CH_2) ppm.

^{13}C NMR δ **:** 172.92 (C=O), 156.25 (C=O), 156.07 (C=O), 144.10 (ArC), 138.70 (ArC), 130.11 (ArCH), 130.02 (ArCH), 129.78 (ArCH), 129.68 (ArCH), 78.75 (2 x C), 62.83 (CH_2OH), 47.39 (CH_2), 45.55 (CH_2), 42.32 (CH_2), 39.85 (CH_2), 33.05 (CH_2), 32.69 (CH_2), 29.82 (CH_2), 29.46 (CH_2), 29.39 (CH_2), 28.25 (6 x CH_3), 27.56

(CH_2), 27.35 (CH_2), 25.97 (CH_2), 25.55 (CH_2), 25.32 (CH_2), 25.01 (CH_2), 24.29 (CH_3) ppm.

{4-[(3-*TERT*-BUTOXYCARBONYLAMINO-PROPYL)-(12-IODO-DODECANOYL)-AMINO]-BUTYL}-CARBAMIC ACID *TERT*-BUTYL ESTER



216

A solution of the tosylate **215** (0.005 g, 0.0071 mmol) and sodium iodide (0.005g, 0.033) were dissolved in acetone (2 ml) and heated under reflux for four hours. The resulting mixture was evaporated and then was purified by chromatography (gradient eluent, 0-4 MeOH / DCM) to give the title product **216** (4 mg, 91 %) as a yellow oil.

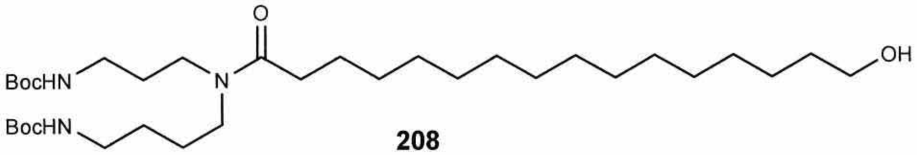
TLC : $R_f = 0.21$ in 3 % MeOH / Chloroform.

LCMS ***m/z* Na+ :** 676.3 found , 676.3 requires.

^{1}H NMR δ **:** 5.42 (1H, br s, NH), 4.75 (1H, br s, NH), 3.56 (2H, t, J = 6.7, CH_2), 3.37 (4H, m, CH_2), 3.04 (4H, m, CH_2), 2.29 (2H, m, CH_2), 1.82-1.62 (7H, m, 3 x CH_2, NH) 1.44 (18H, s, CH_3) 1.26 (18H, s, CH_2) ppm.

^{13}C NMR δ **:** 172.13 (C=O), 156.12 (C=O), 156.04 (C=O), 78.80 (2 x C), 62.82 (CH_2OH), 47.32 (CH_2), 45.75 (CH_2), 42.25 (CH_2), 40.01 (CH_2), 33.04 (CH_2), 32.70 (CH_2), 29.84 (CH_2), 29.50 (CH_2), 29.38 (CH_2), 28.31 (6 x CH_3), 27.10 (CH_2), 27.09 (CH_2), 26.09 (CH_2), 25.52 (CH_2), 25.40 (CH_2), 23.99 (CH_2) ppm.

{4-[(3-TERT-BUTOXYCARBONYLAMINO-PROPYL)-(16-HYDROXY-HEXADECANOYL)-AMINO]-BUTYL}-CARBAMIC ACID TERT-BUTYL ESTER



208

Alcohol **208** was prepared in the same manner as **207** from hydroxy acid **206** (0.66 g, 2.4 mmol), *bis*-Boc spermidine **34** (1.00 g, 2.8 mmol), HOBT (0.65 g, 4.8 mmol), EDCl (0.93 g, 4.8 mmol) giving **208** in a yield of 75 % (1.08 g).

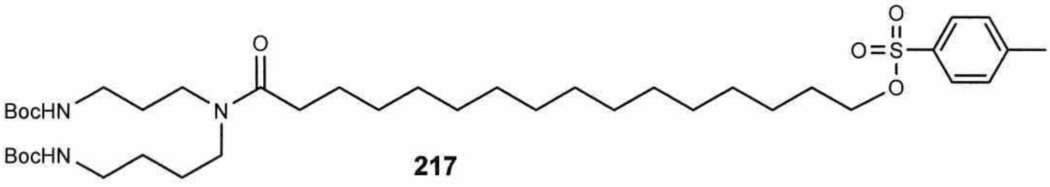
TLC : R_f = 0.22 in 2 % MeOH / DCM.

HRMS (CI) *m/z* : found 600.4956, requires 600.4951.

^{1}H NMR δ : 5.48 (1H, br s, NH), 4.77 (1H, br s, NH), 3.59 (2H, t, J = 6.7, CH_2), 3.24 (4H, m, CH_2), 2.54 (4H, m, CH_2), 2.39 (1H, br s, OH), 2.28 (2H, m, CH_2), 1.61-1.45 (7H, m, 3 x CH_2, NH) 1.38 (18H, s, CH_2) 1.23 (26H, s, CH_2) ppm.

^{13}C NMR δ : 171.18 (C=O), 156.15 (C=O), 156.01 (C=O), 78.72 (2 x C), 60.32 (OCH_2), 47.37 (CH_2), 45.32 (CH_2), 42.31 (CH_2), 39.86 (CH_2), 38.01 (CH_2), 37.15 (CH_2), 33.08 (CH_2), 29.83 (6 x CH_3), 27.82 (CH_2), 27.53 (4 x CH_2), 27.33 (3 x CH_2), 26.08 (CH_2), 25.52 (CH_2), 25.41 (CH_2), 24.87 (CH_2) ppm.

TOLUENE-4-SULFONIC ACID 15-[(4-*TERT*-BUTOXYCARBONYLAMINO-BUTYL)-(3-*TERT*-BUTOXYCARBONYLAMINO-PROPYL)-CARBAMOYL]-PENTADECYL ESTER



217

217 was prepared in the same manner as **215**, from **208** (0.137 g, 0.23 mmol) *p*-toluenesulphonyl chloride (0.044 g, 0.23 mmol) giving **217** (0.137 g, 82 %) as a yellow solid.

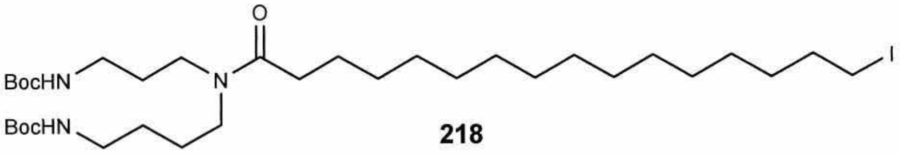
TLC : R_f = 0.33 in 2 % MeOH / DCM.

LCMS *m/z* Na+: found 776.5, requires 776.5.

^{1}H NMR δ **:** 7.34 (1H, d, J = 7.9, ArCH), 7.13 (1H, d, J = 7.9, ArCH), 5.45 (1H, br s, NH), 4.77 (1H, br s, NH), 3.60 (2H, t, J = 6.4, CH_2), 3.51 (4H, t, J = 6.7, CH_2), 3.39 (4H, m, CH_2), 2.43 (3H, s, CH_3), 2.31 (2H, m, CH_2), 1.61-1.51 (7H, m, 3 x CH_2, NH) 1.42 (18H, s, CH_3) 1.24 (26H, s, CH_2) ppm.

^{13}C NMR δ **:** 171.09 (C=O), 156.14 (C=O), 155.89 (C=O), 144.12 (ArC), 138.21 (ArC), 130.45 (ArCH), 130.23 (ArCH), 129.67 (ArCH), 129.61 (ArCH), 78.82 (2 x C), 60.25 (OCH_2), 47.40 (CH_2), 45.29 (CH_2), 42.29 (CH_2), 39.86 (CH_2), 38.03 (CH_2), 37.12 (CH_2), 32.95 (CH_2), 29.78 (6 x CH_3), 27.95 (CH_2), 27.54 (4 x CH_2), 27.36 (3 x CH_2), 26.02 (CH_2), 25.51 (CH_2), 25.45 (CH_2), 24.87 (CH_2), 24.29 (CH_3) ppm.

{4-[(3-*TERT*-BUTOXYCARBONYLAMINO-PROPYL)-(16-IODO-HEXADECANOYL)-AMINO]-BUTYL}-CARBAMIC ACID *TERT*-BUTYL ESTER



218

218 was prepared in the same manner as **216**, from **217** (0.092 g, 0.12 mmol) sodium iodide (0.094 g, 0.63 mmol) giving **217** (0.079 g, 91 %) as a oil.

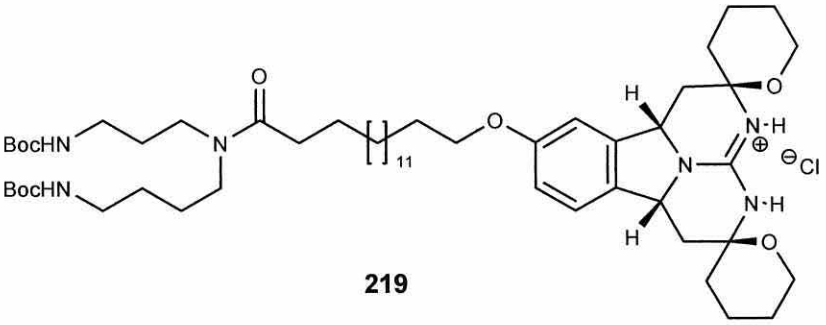
TLC : R_f = 0.29 in 2 % MeOH / DCM.

LCMS *m/z* Na+ : 732.4 found, 732.4 requires.

^{1}H NMR δ : 5.30 (1H, br s, NH), 4.89 (1H, br s, NH), 3.66 (2H, t, J = 6.4, CH_2), 3.53 (4H, t, J = 6.7, CH_2), 3.39 (4H, m, CH_2), 2.31 (3H, s, CH_3), 2.28 (2H, m, CH_2), 1.81-1.59 (7H, m, 3 x CH_2, NH) 1.43 (18H, s, CH_3) 1.25 (26H, s, CH_2) ppm.

^{13}C NMR δ : 173.66 (C=O), 156.20 (C=O), 156.04 (C=O), 79.51 (C), 79.38 (c), 63.03 (OCH_2), 47.43 (CH_2), 45.55 (CH_2), 42.36 (CH_2), 39.96 (CH_2), 37.21 (CH_2), 34.42 (CH_2), 33.19 (CH_2), 29.94 (6 x CH_3), 29.53 (CH_2), 29.48 (4 x CH_2), 29.41 (3 x CH_2), 28.46 (CH_2), 28.40 (CH_2), 27.93 (CH_2), 27.64 (CH_2), 27.43 (CH_3) ppm.

{4-[(3-*TERT*-BUTOXYCARBONYLAMINO-PROPYL)-(6-(*RAC*-1',2'-[3-HYDROXYBENZO]-DISPIRO[TETRAHYDROPYRAN-2,4'-(1,2,3,4,7,8-HEXAHYDRO-5H-5,6,8B-TRIAZAACENAPTHHYLENE-7',2''-TETRAHYDROPYRAN]-6-IUM CHLORIDE)HEXADECANOYL)-AMINO]-BUTYL}-CARBAMIC ACID TERT-BUTYL ESTER



219

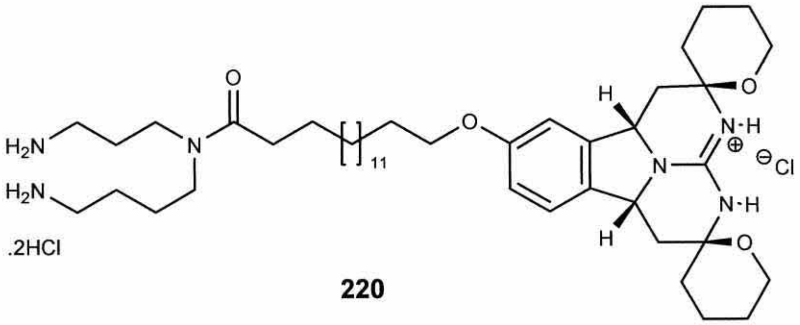
Phenol **204** (0.05 g, 0.14 mmol) and iodide **218** (0.098 g, 0.14 mmol) were dissolved in DMF (1 ml) and stirred for ten minutes whereupon potassium carbonate (0.038 g, 0.28 mmol) was added and the reaction warmed to 40 °C and stirred for 16 hours. The reaction was cooled and diluted with water (4 ml), then extracted with DCM (3 x 3 ml). The organic layers were combined and washed with LiBr solution (saturated, 2 x 10 ml) and the separated organic phase dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified using silica gel chromatography using a gradient elution (0 – 8 % MeOH / Chloroform) gave the title product (0.026 g, 20 %) in approximately 80-90 % purity (NMR).

TLC : $R_f = 0.10$ in 8 % MeOH / DCM.

HRMS (CI) *m/z* : 951.6900 found, 951.6893 requires.

^{1}H NMR δ **:** 6.94 (1H, d, J = 7.7 Hz, ArCH), 6.81 (1H, d, J = 7.7 Hz, ArCH), 6.44 (1H, s, ArCH), 5.41 (1H, br s, NHBoc), 4.97 (2H, m, CH-3a”, CH-8a”), 4.67 (1H, br s, NHBoc), 3.79 (2H, m, CHα-6’, CHα-6’’’), 3.64 (2H, br d, J = 10.8 Hz, CHβ-6’, CHβ-6’’’, 3.60 (2H, t, J = 6.0 Hz, CH$_2$CN), 3.41 (2H, t, J = 6.0 Hz, OAr), 3.36 (4H, m, CH$_2$), 3.28-3.09 (4H, br m), 2.37 (2H, d, J = 3.9 Hz, CH-3”, CH-8”), 1.84-1.55 (18H, br m), 1.44 (18H, br s), 1.23 (32H, br s), ppm.

RAC-1',2'-[3-(6-OXY-HEXADECANOIC ACID (4-AMMONIUM-BUTYL)-(3-AMMONIUM-PROPYL)-AMIDE)-BENZO]-DISPIRO[TETRAHYDROPYRAN-2,4'-(1,2,3,4,7,8-HEXAHYDRO-5H-5,6,8B-TRIAZAACENAPTHHYLENE-7',2''-TETRAHYDROPYRAN]-6-IUM TRICHLORIDE



220

219 (2 mg) was deprotected using HCl (3 N, 0.1 ml), in methanol (0.1 ml) for 24 hour. Affording title product **220** (2 mg, quantiative).

TLC : $R_f = 0.05$ in 8 % MeOH / DCM.

HRMS (CI) ***m/z*** **:** 751.5827 found, 7515844 requires.

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