The synthesis of novel cyclic Agelasphin derivatives

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A thesis submitted in partial fulfilment of the

requirements of the Nottingham Trent University

for the degree of Doctor of Philosophy

This research programme was carried out in

collaboration with Knoll Pharmaceuticals

August 2000

Abstract

The agelasphins, α-galactosylceramides, are a group of natural products which exhibit interesting immunomodulating activity. These have served as leads for structural analogues, which are now in clinical trials as anti-cancer agents. The agelasphins and their derivatives are acyclic compounds consisting of a ceramide bound to a galactose, and have conformational freedom.

The aim of the research described herein was to synthesise conformationally restrained analogues of the agelasphins, using structures based on 3-alkyl-3,4-dihydroxy-5-hydroxymethyl pyrrolidinone. Two synthetic strategies were followed. Extensive attempts to prepare the target pyrrolidinones from acyclic precursors were unsuccessful. Elaboration of pyroglutamate derivatives, however, led to 3-(α-hydroxyalkyl)-3-hydroxy-5-hydroxymethylpyrrolidinones, but no 3,4-dihydroxy analogues were obtained.

Coupling of these to glucose or galactose by a variety of methods lead to agelasphin analogues, which were subjected to biological assay.

Acknowledgements

I would like to thank Dr. Ian Coutts (TNTU) and Dr. Rob Steele (Knoll) for their advice and guidance throughout my Ph.D, and Mick Wood for NMR spectra. I am also grateful for sponsorship and facilities provided by Knoll Pharmaceuticals, in support of my research.

Finally, I would like to sincerely thank my parents for all their support over the last four years.

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Acronyms and Abbreviations.

Ac	acetyl
Ar	aryl
AIBN	azoisobutyronitrile
Bu	butyl
^{i}Bu	*iso*-butyl
^{t}Bu	*tert*-butyl
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
Bz	benzoyl
DAST	diethylaminosulfur trifluoride
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	1,3-dicyclohexylcarbodiimide
DCM	dichloromethane
DIBAL-H	diisobutylaluminium hydride
DMAP	4-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMF	dimethylformamide
Et	ethyl
LAH	lithium aluminium hydride
LDA	lithium diisopropylamide
LiHMDS	lithium hexamethyldisilazane
MCPBA	*m*-chloroperbenzoic acid
Me	methyl
Ms	methanesulfonyl (mesyl)
NMO	4-methylmorpholine *N*-oxide
Ph	phenyl
PPTS	pyridinium toluene-*p*-sulfonate
PTSA	toluene-*p*-sulfonic acid
rt	room temperature
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimetylsilyl
Tf	trifluoromethylsulfonyl (triflyl)
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMS	trimethylsilyl
Tr	trityl
Ts	*p*-toluenesulfonyl
Z	benzyloxycarbonyl
~~	bond of unspecified stereochemistry (not a mixture)

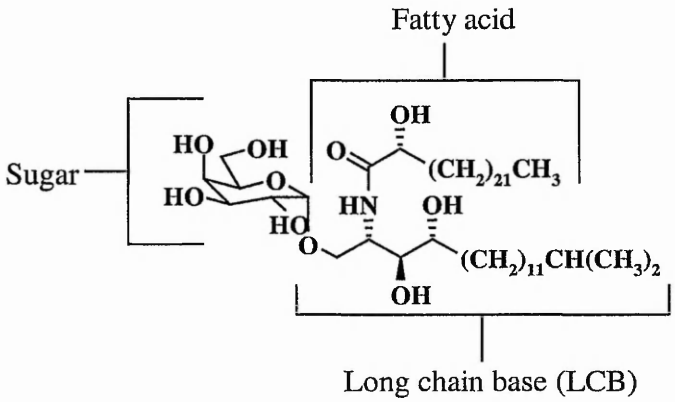
1. Introduction

1. Introduction.

The agelasphins are a group of natural products first isolated by the Kirin Brewery Company in 1993, which have been shown to be potent immunostimulating agents with possible applications in the treatment of cancer. The total synthesis of these compounds and their analogues provides a challenging target.

1.1 The agelasphins

The agelasphins, first isolated by Natori[1] *et al* from the marine sponge *Agelas mauritianus*, are α-galactosyl ceramides, with the structure of a representative example, AGL-9b **1**, being shown below. The compounds consist of a galactose unit, linked *via* an α-glycosidic bond to a ceramide moiety. The ceramide portion of the molecule itself is made up of two components, a fatty acid, and a long chain base (the amine sphingosine), linked by an amide bond.



Agelasphin 9b

1

The structure of AGL-9b, the most active of the natural products, provided a lead compound for the Kirin group to investigate further[2]. The compound had shown interesting biological activity, as it was found to inhibit cancer growth by immunostimulating activity.

1.2 Introduction to cancer and chemotherapy

1.2.1 Cancer and chemotherapy. Cancer is a group of diseases, characterised by uncontrolled cell growth, with a tendency to spread to other tissue. The cells are no longer controlled by the influences that regulate normal, healthy cell growth. Clinical differentiation of the types of tumour is by the tissue or organ of origin, such as skin, colon, or prostate cancers.

As tumour cells originate from within the body, they are not generally recognised by the immune system as foreign, and so can escape destruction. It is also this similarity to healthy tissue that causes the unpleasant side effects of many current anti-cancer therapies, such as nausea, hair loss and bone marrow depletion. This is due to the destruction of healthy fast growing tissue, such as bone marrow, as well as the tumour target.

Most anti-cancer drugs in present use rely on their cytotoxicity for their effect. The specificity of the compounds is dependent on the rapid growth of cancer cells, resulting in their absorbing more of the compound than healthy tissue, so causing a higher toxic effect.

The most common types of compound used in cancer therapy are alkylating agents, (such as nitrogen mustards, cyclophosphamide), intercalating agents (cisplatin), hormone modulators (such as tamoxifen), or antibiotics (mitomycin-C). Numerous other compounds have been found to have tumour cell killing ability, but suffer from

being highly toxic to healthy cells, and are unsuitable for clinical use. The lack of specificity towards cancer cells is the main drawback of the majority of cancer treatments.

Metastasis is an important process in the spread of tumours into healthy tissue. Inhibition of this by any means would be an important step in controlling the disease.

1.2.2 Immunomodulating agents. These are a broad structural range of compounds, many of them natural products, which exhibit an effect on the immune system. The compounds are collectively known as biological response modifiers (BRM), and these form two classes of compounds, immunostimulators and immunosupppressants.

The area of immunosuppressive agents is more advanced than that of immunostimulators, with immunosuppressants common in clinical use. The compounds are used to prevent rejection in transplant operations, and are also valuable in the treatment of autoimmune diseases, such as rheumatoid arthritis and Crohn's disease.

The immunosuppressants in use are either microbial secondary metabolites, such as cyclosporin, rapamycin and tacrolimus, or synthetic material, exemplified by leflunomide or brequinar sodium. Examples of immunostimulants in common use are fewer, with several of the compounds, such as OK-432 and Lentinan being used for cancer treatment only in Japan.

It is suggested that various polypeptides of the immune system (cytokines) are involved in the mechanism of immunostimulation, at least in part[3]. This is supported by the suppression of tumours by interleukin-2 (IL-2), and the involvement of interleukins in the regression of several diseases.

A muramyldipeptide (MDP) analogue romurtide [MDP-Lys(18)N^2-(*N*-acetylmuramoyl)-L-alanyl-D-isoglutaminyl)-N^6-stearoyl-L-lysine] has been shown[3] to

be a potent agent for the induction of several cytokines, and also to stimulate resistance to bacterial and mycotic infections in laboratory animals. Additionally, the compound enhanced the recovery from leucopenic state of mice, following X-irradiation or cyclophosphamide treatment.

Human trials of romurtide have also shown interesting results. Lung cancer patients given a low dose of the immunomodulating agent following treatment by cisplatin, vindesine or mitomycin-C showed a rapid recovery of white blood cell and platelet counts[3].

Work by Yoshida[4] *et al* supports the therapeutic use of a BRM for the treatment of immune system dysfunction, following treatment for head or neck cancer. Patients were dosed with a BRM following either surgery, chemotherapy or radiotherapy, and the survival period was studied in comparison with a control group. The BRM-treated group showed a significantly longer survival time than the control patients, and Yoshida suggests that these compounds may prove beneficial for aiding the recovery of head and neck cancer patients with depressed immunological function.

Another study, by Aoyagi[5] and co-workers, supports the possible use of BRMs in the combination treatment of cancer. The bacterial extract OK-432 (picibanil) from a strain of *Streptococcus pyogenes*, was tested for its ability to increase oestrogen and progesterone receptor levels in MCF-7 breast cancer cells. The compound caused a two-fold increase in oestrogen receptor levels, but had no effect on progesterone receptor levels. In addition, the compound caused a dose dependent inhibition of DNA synthesis in the MCF-7 cells. In combination with tamoxifen, an antioestrogen, OK-432, demonstrated an additive inhibitory effect on the growth of MCF-7 cells.

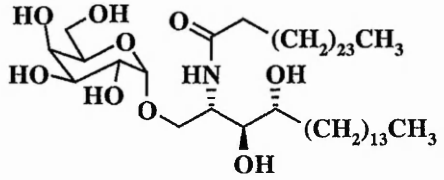
A study reported by Kirkwood[6] and co-workers evaluated the immunomodulating activity of picibanil at several different doses, in patients with

metastatic cancer. The patients treated with the compound originally had depressed cytokine production, which OK-432 reversed for interferon gamma (INF-gamma), but other cytokine levels were unchanged.

1.3 Biological activity of the agelasphins and synthetic analogues

The activity of the agelasphins has been shown to be due to their ability to stimulate 'natural killer' (NK) cells to attack and destroy cancerous tissue[7]. This was a mechanism suggested by Morita[2] *et al*, in the initial paper reporting the synthesis of agelasphin analogues. The compounds were screened *in vitro*, using a syngenic mixed leukocyte reaction (MLR) assay, based on mouse spleen cells (as responder cells), with dendritic cells (stimulator cells). Dendritic cells pre-treated with active compounds were found to stimulate the proliferation of spleen cells, contrasting with inactive compounds, which gave little stimulating effect.

Studies involving KRN7000, **2**, a synthetic analogue of the agelasphins, have suggested that the cell-killing effect of the compound is due to activation of liver associated NK cells[8].



2

KRN7000

In a study by Natori[9] *et al*, KRN7000 was found to inhibit strongly the development of lung metastasis of B16 tumour cells, and liver metastasis of colon26 cells in mice. Additionally, the compound caused an increase in the serum levels of IL-

2 and INF-gamma. It was also suggested that the biological activity of the compound could be due to an activating effect on dendritic cells.

Another publication[10] on the biology of KRN7000 also reports antimetastatic activity against mouse colon26 and EL-4 liver cells. In addition, it is also reported that the compound enhances the antigen-presenting ability of dendritic cells from murine spleens.

A study, carried out by Nakagawa[8] and co-workers, looked at the effect of KRN7000 on colon26 cells in mice, where a 80 % cure rate was observed, compared to 20 % with IL-12 Both compounds increased survival periods, and KRN7000 was found to inhibit tumour growth in the liver, and cause regression of established nodules. It was also suggested that tumour-specific immunity had developed, and that liver-associated NK cells were primarily responsible for the killing of tumour cells in the liver.

A further biological effect of agelasphin analogues has also been reported by the Kirin group. In studies published by Motoki[11] *et al*, mice pre-dosed with an α-galactosylceramide (AGL-517) were irradiated with X-rays, and the peripheral blood levels of platelets and white blood cells examined. Mice pre-treated with an α-galactosylceramide had higher counts of both platelets and white blood cells. This result suggested that the compound was having a radioprotective effect, helping to prevent bone marrow death due to irradiation.

Work by Inoue[12] and co-workers demonstrated that mice pre-dosed with AGL-517 had increased survival rates, when subjected to a lethal dose of radiation. When administered within 2 hours of lethal irradiation, a single dose of the compound was found to result in long-term survival of mice, without bone marrow transplantation.

The radioprotective effect of the compounds is suggested as being due, at least partly, to the co-operative effect of cytokines induced by the α-galactosylceramide. This mechanism is proposed by Inoue *et al*, after finding that increased levels of cytokines were present in α-galactosylceramide-dosed mice after lethal irradiation.

To test the theory, irradiated mice were given a mixture of cytokines that matched those found in the α-galactosylceramide dosed mice. This resulted in increased survival of the mice (60 % achieved a 50 day survival period), confirming that the cytokines were the main factor influencing the survival time.

The results of studies by Motoki[13] and co-workers suggested that KRN7000 may be useful for the treatment of cancer in combination with radiotherapy. This was based on an investigation in which Meth A or colon26 infected mice were dosed with KRN7000, and were then irradiated (high dose (20/30Gy) local X-ray, or low dose (3Gy) fractionated whole-body). This resulted in greater anti-tumour activity than either treatment alone achieved, as well as a 60 % cure rate. The effect of rechallenging the cured mice with more tumour cells suggested that tumour-specific immunity had developed.

Systemic administration of KRN7000 was also found to generate high levels of IL-2 and INF-gamma[13]. These compounds assist *in vivo* in the induction of tumour-specific cytotoxic lymphocytes, which are vital in preventing tumour recurrence and metastasis.

1.4 Advantages of immunomodulating agents in cancer treatment

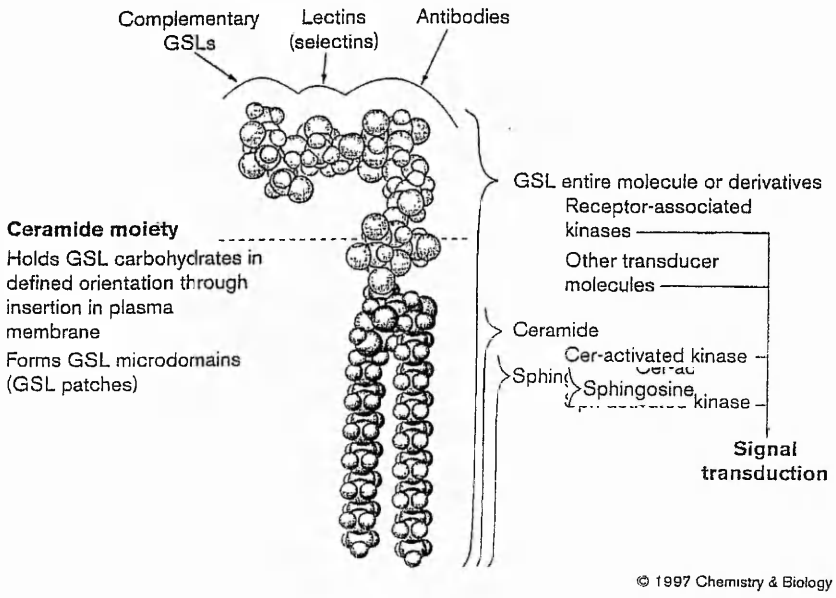
A significant advantage of using an immunomodulating agent for the treatment of cancer is the lack of direct cytotoxic action, and the accompanying side effects. Agelasphin 9b has no cytotoxic effect on tumour cells[14], the anti-tumour activity of the

compound being derived from the host immune system; this is in contrast with many anti-cancer agents, and makes its mode of action particularly attractive.

1.5 Galactosyl ceramides

1.5.1 Structure of galactosyl ceramides. Galactosyl ceramides (GalCer), members of the glycosphingolipid family, are biologically important molecules found in virtually all eukaryotic cells[15]. Due to the possible variations in the type, linkage and number of sugar residues present, as well as differences in the ceramide, over 300 different glycosphingolipids have been identified from natural sources[16].

1.5.2 Location of galactosyl ceramides in cells. Glycosphingolipids form a minor but important constituent of the lipid bilayer of cell walls[17]. The molecules are located in the cell wall with the hydrophilic sugar moiety exposed on the outer surface. The molecule is held in position in the membrane by the hydrophobic ceramide[16].



1.5.3 Biological role of galactosyl ceramides.

Galactosyl ceramides and cancer. Glycosphingolipids are known to be shed from tumour cells, and act as immunosuppressors in the host[15]. Electron microscopy studies have also shown that the glycosphingolipids are concentrated in patches on the cell membrane[18] , and it has been suggested that it is a high concentration of these patches which leads to antigenicity (reaction with antibodies) or immunogenicity (host immune response). Although present in normal cells, glycosphingolipid patches do not react with antibodies until above a certain concentration[18]. It is only on cancerous cells that the surface concentration is high enough to elicit an antibody response.

An increased concentration of glycosphingolipids in the membrane could also affect cell adhesion, and, importantly with tumour cells, the metastatic and invasive potential. The importance of glycosphingolipids in metastasis was investigated by Otsuji[19] *et al* in a study of B16 melanoma cells. The cells show a high concentration of GM3 ganglioside, a trisaccharide glycosphingolipid. It was found that metastasis of the

melanoma could be blocked by liposomes containing GM3, or by monoclonal antibodies raised against GM3.

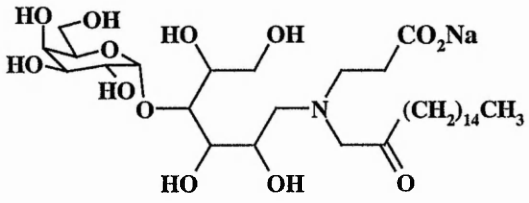
Determining the mode of action of the agelasphins and related compounds is difficult, due to the ubiquitous nature of glycosphingolipids within cells. The compounds are implicated in many important cell processes, including cell recognition, antigenic specificity, immunosuppressive activity, adhesion, and cell transduction[20]. Interference with one or more of these processes is likely to be responsible for the interesting biology of these compounds.

Galactosyl ceramides and HIV. The importance of glycosphingolipids, in particular galactosyl ceramide, in the infection of cells by HIV has been the subject of a recent investigation. As a surface binding receptor, galactosyl ceramide has been implicated in the infection of neural cells with HIV. The receptor site has been shown by Bhat *et al*[21] to bind to the gp120 domain of the HIV viral envelope, an important step in the infection of certain cells by HIV.

In another publication, Harouse[22] *et al* report the inhibition of HIV uptake in two neural cell lines, by antibodies to galactosyl ceramide. Recombinant HIV gp120 surface protein was also found to bind to galactosyl ceramide, but not other glycolipids, such as glucosyl ceramide, G_{M1} or G_{D1a}. This suggests that galactosyl ceramide, or a closely related compound, is involved in the entry of HIV into neural cells.

In a more recent study, Fantini[23] and co-workers studied the inhibition of HIV-1 entry into neural and colonic cells by synthetic galactosyl ceramide analogues. The galactosyl ceramide moiety had been shown to bind to the V3 region of the surface glycoprotein gp120, a region of the glycoprotein which may be important for entry of the virus into cells.

The soluble analogue CA52(n15) **3** was synthesised, in addition to some other analogues, and its activity as an inhibitor of HIV-1 entry into cells investigated. The compounds were first pre-screened, using an assay designed to determine binding to the V3 domain, with the most active compounds then being screened for anti-HIV activity.



3

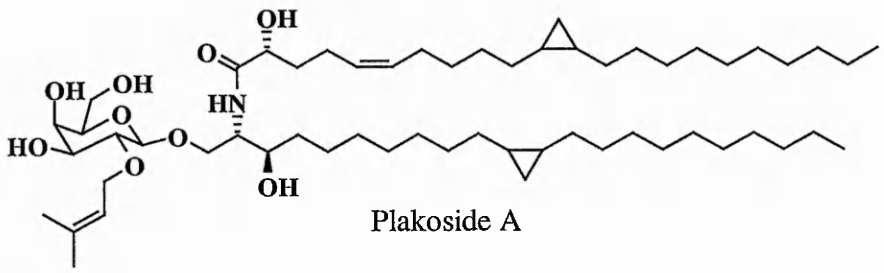
CA52(n15)

The galactosyl ceramide analogue CA52(n15) was found to be the most active, inhibiting HIV-1-induced cell fusion, and also blocking entry of HIV into $CD4^+$ and $CD4^-$ cells. The activity was closely related to its affinity for the V3 region, determined in the previous assay.

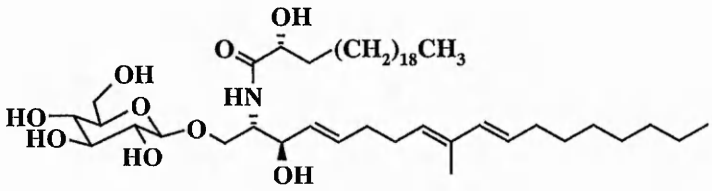
The compounds are suggested as being of possible use as anti-HIV agents, due to their ability to block HIV infection of some cell lines. However, this is only one of several routes of infection by the virus.

1.6 Natural products similar to agelasphins

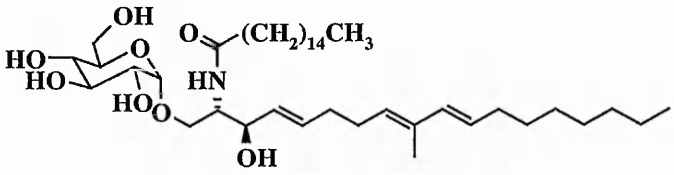
Other compounds similar to the glycosphingolipids discussed above have been isolated from marine sources, usually sponges, and these also show biological activity. Examples are the immunosuppressive plakosides[24], the ophidiacerebrosides[25], cytotoxic *in vitro* against L1210 leukaemia cells, and more recently, thraustochytrosides[26], which have yet to have their biological activity studied. All of these compounds are glycosphingolipids, closely related to the agelasphins.



Plakoside A



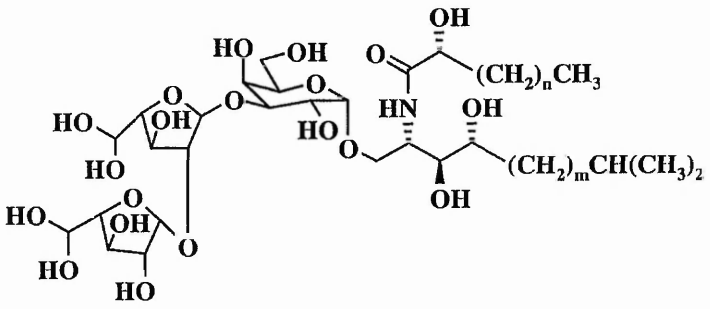
Ophidiacerebroside C



Thraustochytroside A

Other structurally similar compounds include gangliosides described by Yamada[27] *et al*, isolated from *Holothuria pervilax*, which showed neuritogenic activity. Duran[28] *et al* isolated two compounds, phallusides, from *Phallusia fumigata*, which were found to be inactive against four tumour cells lines that were tested.

Agelagalastatin **4**, isolated by Pettit[29] and co-workers from *Agelas sp*, was found to have *in vitro* activity against various cancer cell lines.



Agelagalastatin (m = 10 or 11, n = 20 or 21)

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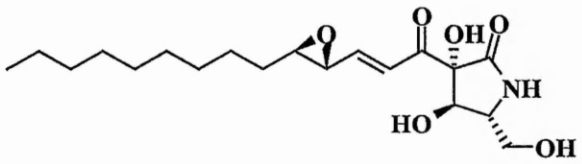
There is also a fungal metabolite, the substituted pyrrolidinone pramanicin **5**, which shows antifungal activity against pathogenic fungal infections, and which has several structural features in common with the compounds targeted in the study that follows.

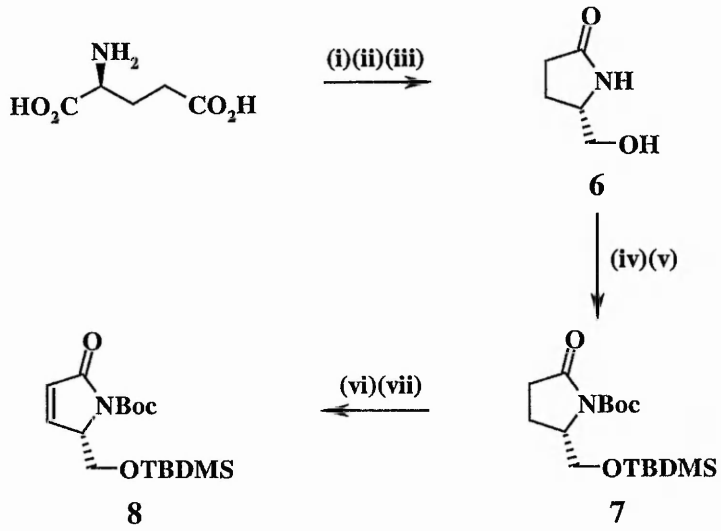


(+)-Pramanicin

5

The synthesis of pramanicin has recently been described by Barrett[30] *et al*, starting from glutamic acid. This was first converted to 5-hydroxymethylpyrrolidinone **6**, which was then diprotected as the *N*-Boc, *O*-TBDMS compound **7**, **Scheme 1.1**. Introduction of unsaturation into the molecule was then achieved, using a selenation/oxidation procedure, resulting in **8**.

Scheme 1.1



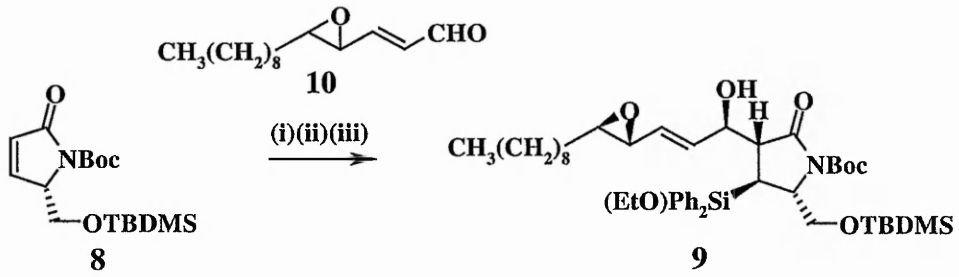
i) water, reflux. (ii) MeOH, $SOCl_2$, 25 °C. (iii) $NaBH_4$, EtOH, 25 °C.

(iv) TBDMS-Cl, Et_3N, DMF, 25 °C. (v) Boc_2O, DMAP, DCM, 25 °C.

(vi) LDA, PhSeBr, THF, -78 °C (vii) H_2O_2, pyridine.

A silyl group was then introduced, functioning as a masked alcohol, and the enolate of this intermediate was then reacted with the aldehyde **10**, **Scheme 1.2**. Tandem addition of the enolate to the aldehyde leads to the secondary alcohol **9**.

Scheme 1.2

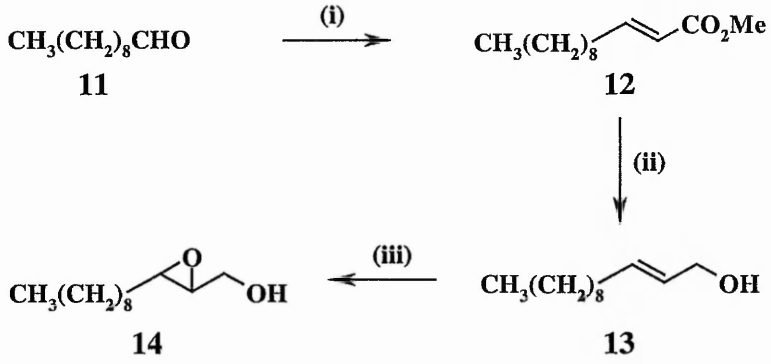


i) $(Et_2N)Ph_2SiLi$, Et_2Zn, -78 °C. (ii) **10**, THF, -78 °C. (iii) EtOH, NH_4Cl.

The aldehyde **10** had to be synthesised from decanal **11**, the product being obtained in seven steps.

The synthesis started with a Wadsworth-Emmons reaction on the aldehyde, to give the methyl ester **12**, **Scheme 1.3**. Reduction of ester **12** to the alcohol **13** was accomplished using DIBAL-H, with epoxidation of the alkene of **13** then being carried out with MCPBA, to give the epoxide **14**.

Scheme 1.3

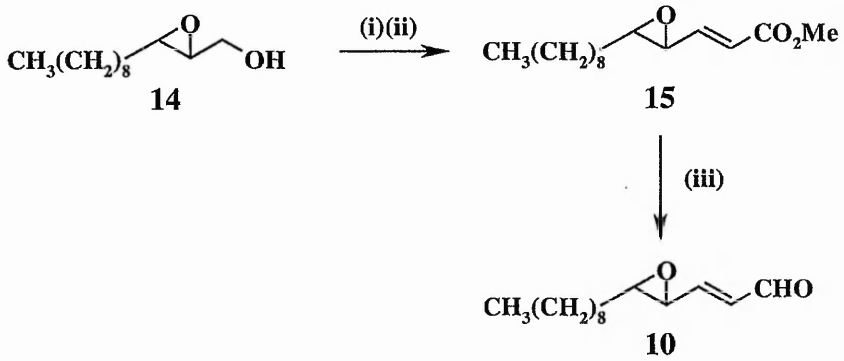


i) $(EtO)_2P(O)CH_2CO_2Me$, NaH, THF, -78 to 25 °C (ii) DIBAL-H, Et_2O, -78 to 25 °C.

(iii) MCPBA, DCM, 25 °C.

Dess-Martin oxidation of the alcohol **14** to the aldehyde left a suitable substrate for a second homologation using the Wadsworth-Emmons procedure, **Scheme 1.4**. This gave the methyl ester **15**, which was finally reduced to the aldehyde **10**, using DIBAL.

Scheme 1.4

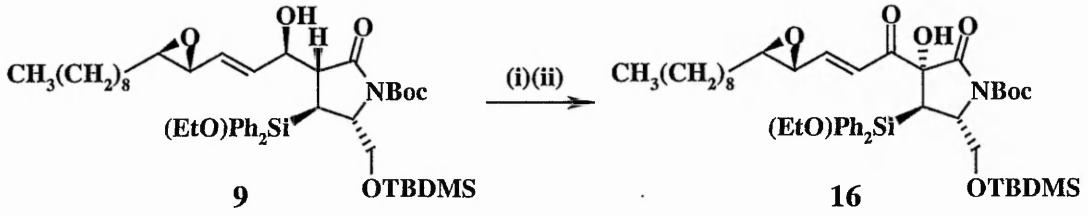


(i) Dess-Martin periodinane, DCM, 25°C.

(ii) $(EtO)_2P(O)CH_2CO_2Me$, NaH, THF, -78 to 25 °C (iii) DIBAL-H, Et_2O, -78 to 25 °C.

Two oxidations were then performed on the compound, **Scheme 1.5**, converting the secondary alcohol of **9** to the ketone, and the formation of the tertiary alcohol at the junction of the pyrrolidinone and the alkyl chain. This results in the intermediate **16**, which is ready to be deprotected to pramanicin.

Scheme 1.5

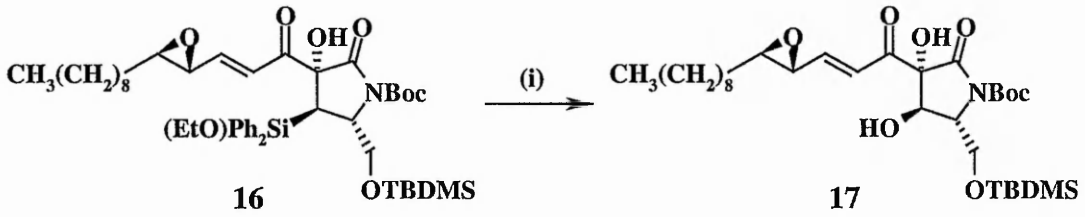


i) Dess-Martin periodinane, DCM, 25 °C.

(ii) dimethyldioxirane, $Ni(acac)_2$, acetone, water, 0 °C.

The silane moiety of **16** was then oxidised using MCPBA, to give the diol **17**, **Scheme 1.6**.

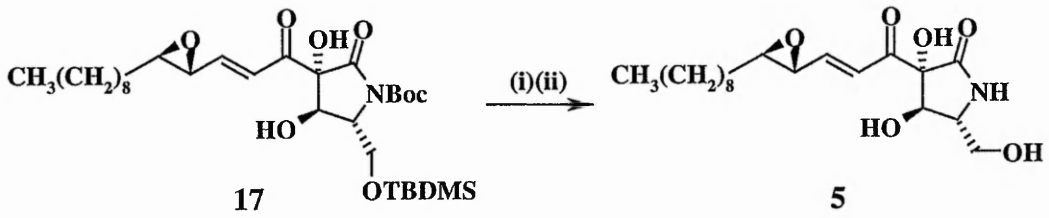
Scheme 1.6



i) MCPBA, KHF_2, DMF, 0 to 25 °C.

Finally, the two remaining protecting groups were removed, the Boc group with TFA, and then the silyl ether with fluorosilicic acid, **Scheme 1.7**. The use of these reaction conditions for cleavage of the silyl ether was found to be necessary, as the standard conditions of TBAF resulted in extensive decomposition. This left the target compound **5**, which was spectroscopically identical to authentic material.

Scheme 1.7

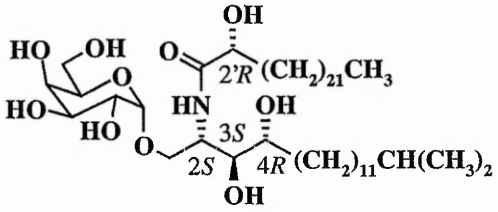


i) TFA, DCM, 0 to 25 °C. (ii) H_2SiF_6, MeCN, 0 to 25 °C.

1.7 Total synthesis of the agelasphins

To investigate further the activity of these natural products, synthetic pathways to the compounds were required. Extraction from the natural source (a sea sponge) could produce milligram quantities, enough for elucidation of the structure and initial biological screening, but insufficient for further trials.

1.7.1 Total synthesis of AGL-9b. Akimoto[31] *et al* developed a total synthesis of AGL-9b, the most active of the isolated compounds, as a confirmation of the structure. This was a challenging synthesis, due to the stereochemistry present in the molecule. The stereochemistry required was assumed to be the same as that found in other natural 4-hydroxylated cerebrosides, and the synthesis was designed to generate this (2*S*, 3*S*, 4*R*, 2'*R*) configuration.



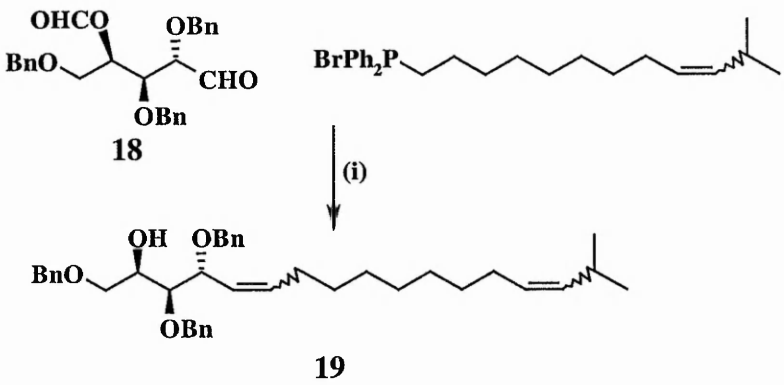
1

AGL-9b

The synthesis began with an aldehyde **18**, previously described by Koike[32] and co-workers, which was reacted with a Wittig reagent, which also had to be prepared.

Generation of the ylid with BuLi, and its subsequent reaction with the aldehyde **18** resulted in the alkene **19**, with concomitant hydrolysis of the formate ester, **Scheme 1.8**.

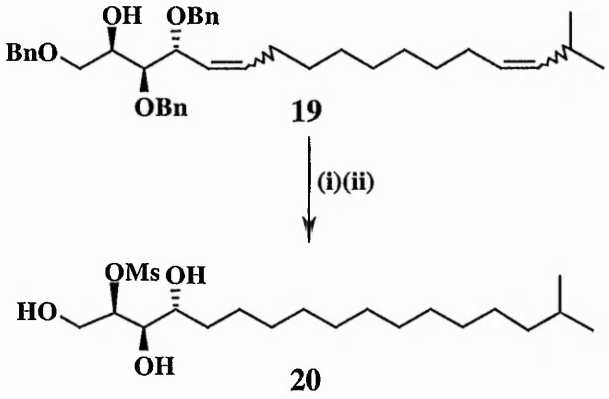
Scheme 1.8



i) ^{n}BuLi, THF, 20 °C.

This gave a mixture of geometrically isomeric alcohols **19**, which were then mesylated under standard conditions, and the alkenes reduced, using palladium catalysed hydrogenation, **Scheme 1.9**. This also resulted in the removal of the benzyl protecting groups from the hydroxyl functions, to give the triol **20**.

Scheme 1.9

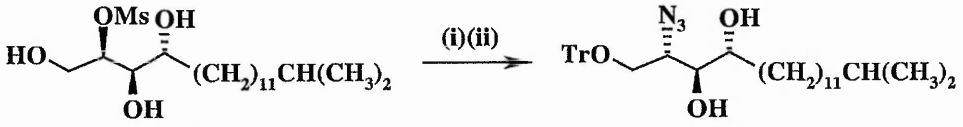


i) MsCl, pyridine, 20 °C. (ii) H_2, Pd/C, THF, 20 °C.

The mesylate group of **20** was then displaced, with inversion of the stereocentre, to give the azide, a masked amine, **Scheme 1.10**. Selective protection of the primary

alcohol moiety was then achieved, using the sterically hindered trityl (Tr) protecting group.

Scheme 1.10

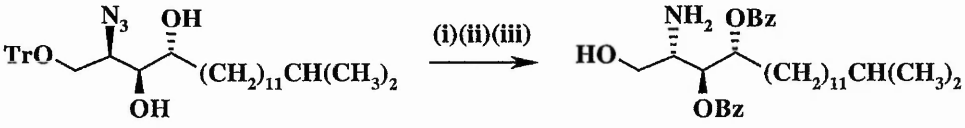


20 **21**

i) NaN_3, DMF, 100 °C. (ii) TrCl, pyridine, 50 °C.

The remaining two secondary alcohols could then be orthogonally protected, by benzoylation of **21**, **Scheme 1.11**. The trityl group was then deprotected, under acidic conditions, using toluene-*p*-sulfonic acid. This left the primary alcohol, which was then subjected to catalytic hydrogenation, to reduce the azide to the corresponding amine **22**.

Scheme 1.11



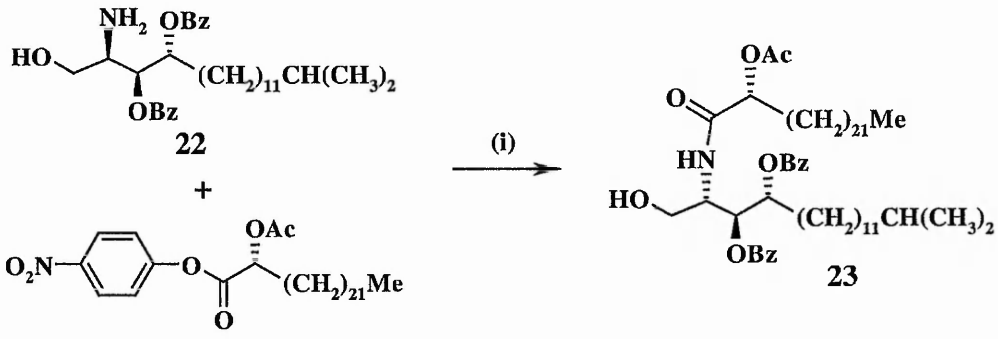
21 **22**

i) BzCl, DMAP, pyridine, 20 °C. (ii) PTSA, DCM, MeOH, 20 °C.

(iii) H_2, Pd/C, THF, 20 °C.

Reaction of the amine moiety of **22** with the nitrophenyl ester of (*R*)-2-acetoxytetracosanoic acid, **Scheme 1.12**, resulted in the ceramide **23**.

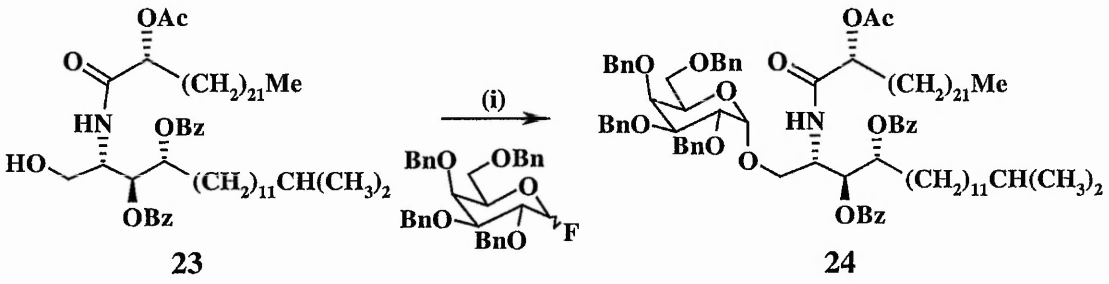
Scheme 1.12



i) THF, 20 °C.

The α-galactoside was then generated, using Mukaiyama's glycosylation conditions[33]. The primary alcohol **23** was reacted with a fluoro-activated, protected sugar, in the presence of tin(II) chloride and silver perchlorate, in anhydrous conditions, **Scheme 1.13**.

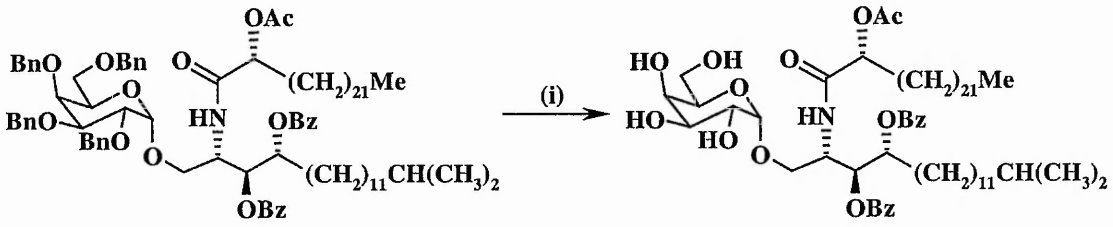
Scheme 1.13



i) $SnCl_2$, $AgClO_4$, MS-4Å, THF, 20 °C.

The final two steps of the synthesis were the deprotection of the hydroxyl functions, **Scheme 1.14**. The sugar benzyl groups of **24** were removed first, by catalytic hydrogenation.

Scheme 1.14

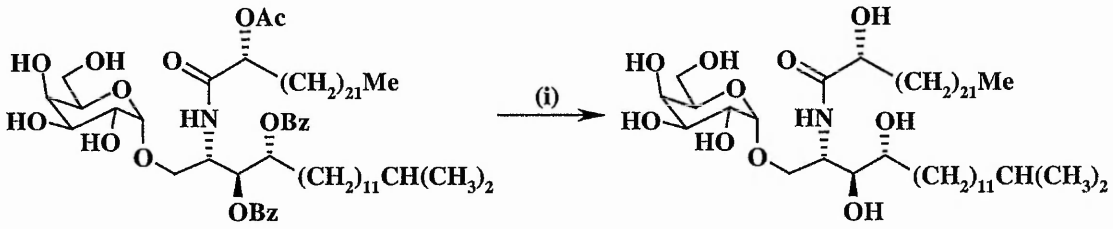


24 **25**

i) H_2, Pd/C, EtOAc, 20 °C.

Finally, the remaining benzoyl and acetyl esters of **25** were removed by sodium methoxide, **Scheme 1.15**. This gave the synthetic AGL-9b **1**, which was found to be spectroscopically identical to the natural compound. The synthesis confirmed the stereochemistry of the natural product as being (2*S*, 3*S*, 4*R*, 2'*R*).

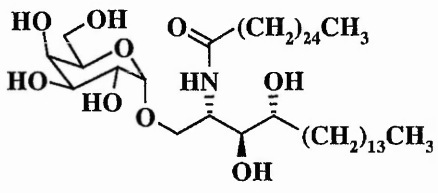
Scheme 1.15



25 **1**

i) NaOMe, MeOH, 20 °C.

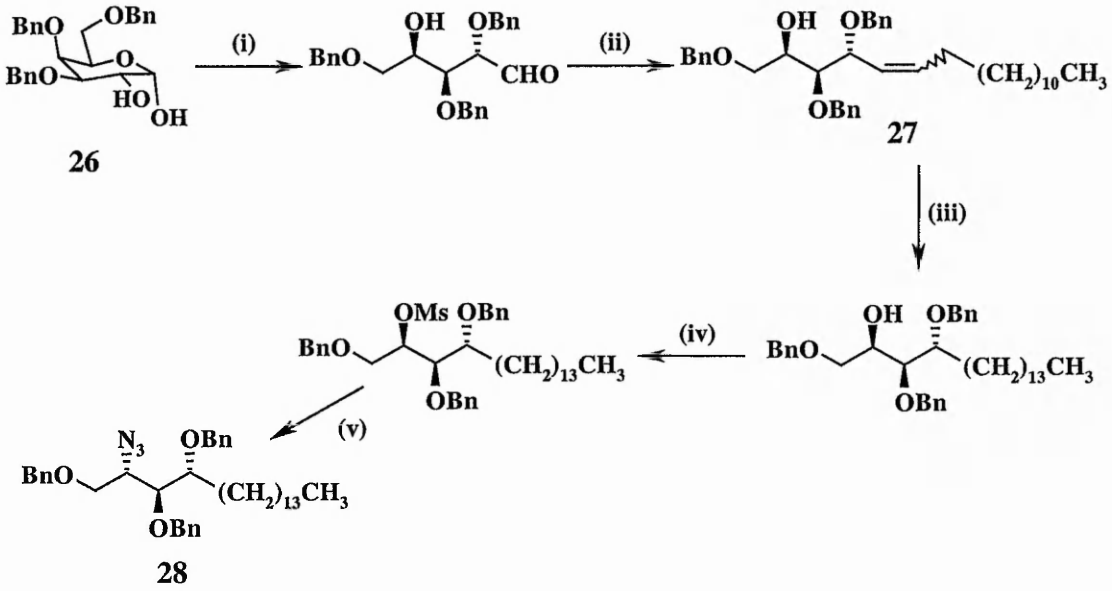
1.7.2 Synthesis of KRN7000. Although important for confirming the structure of the naturally occurring AGL-9b, the synthetic route is too complicated to be useful for a potential drug candidate.



2

The synthesis of the analogue KRN7000 follows a slightly different route[2], starting from a tri-protected galactose derivative **26**. The first step was to ring-open the sugar, followed by reaction with a Wittig reagent, giving the protected polyhydroxylated compound **27**, **Scheme 1.16**. Following double bond reduction, the free alcohol moiety was converted to the mesylate, which then underwent displacement, with inversion of the stereocentre, to give the azide **28**.

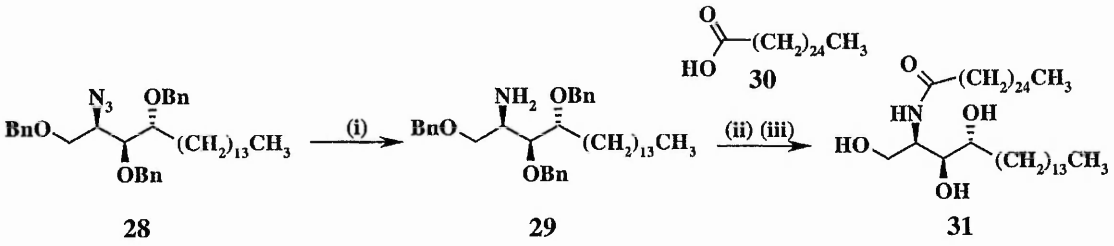
Scheme 1.16



i) $NaIO_4$, aq EtOH, -5 to 20 °C. (ii) $CH_3(CH_2)_{12}PPh_3Br$, ^nBuLi, THF, -10 to 20 °C. (iii) H_2, Pd/C, THF, 20 °C. (iv) MsCl, pyridine, 0 to 20 °C. (v) NaN_3, DMF, 100 °C.

The azide **28** was then reduced, **Scheme 1.17**, using catalytic hydrogenation, to form the amine **29**, which was then reacted with the acid **30**, to form the amide bond of the resulting ceramide **31**. The benzyl protecting groups were then removed by catalytic hydrogenation, using more vigorous conditions than were required to reduce the azide in a previous step.

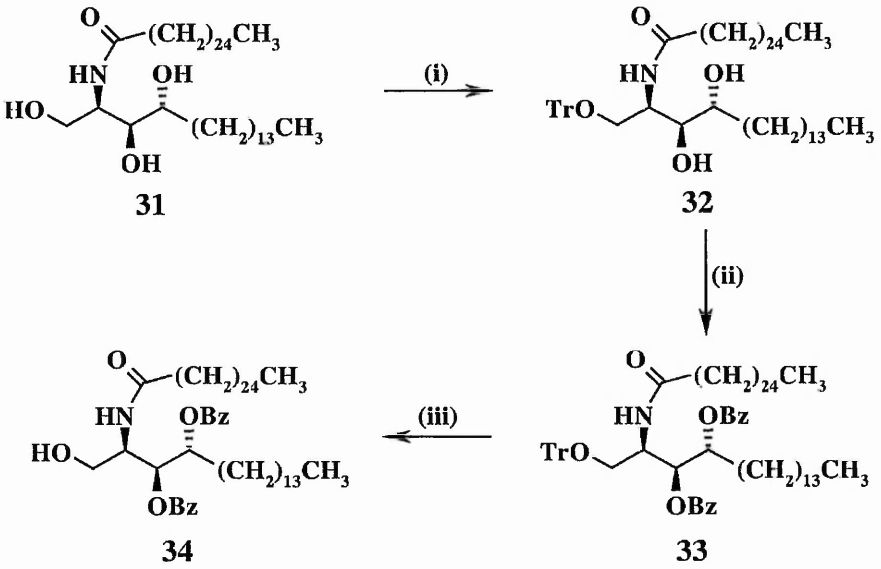
Scheme 1.17



i) H_2, Pd/C, THF, 20 °C. (ii) **30**, 2-chloro-1-methylpyridinium iodide, Bu_3N, DCM, reflux. (iii) H_2, Pd/C, 1-PrOH, 40 °C.

The solitary primary alcohol of **31** was selectively protected by the trityl group, and the remaining secondary alcohols of **32** protected with benzoyl groups, **Scheme 1.18**. This was achieved by treatment with benzoyl chloride and pyridine, giving **33**. The trityl group was then selectively removed, using catalytic toluene-*p*-sulfonic acid in aqueous methanol, to give the alcohol **34**.

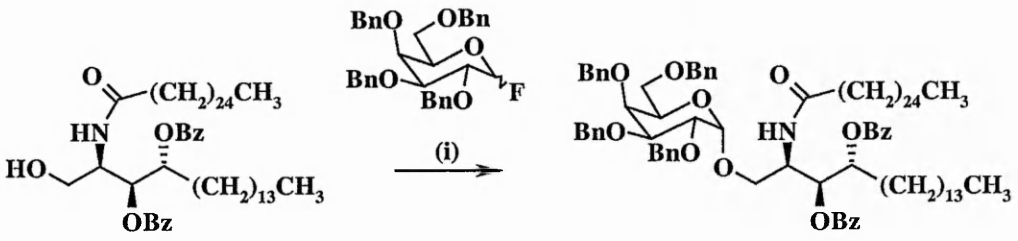
Scheme 1.18



(i) TrCl, DMAP, pyridine, 40 °C. (ii) BzCl, DMAP, pyridine, 20 °C. (iii) PTSA, H_2O/MeOH, DCM, 20 °C.

This protecting group manipulation left the primary alcohol of **34** free to undergo glycosylation, with the secondary alcohols protected. Glycosylation was achieved using conditions described in the synthesis of AGL-9b, with tetrabenzyl galactosyl fluoride, and tin (II) chloride/silver perchlorate as the coupling catalysts, **Scheme 1.19**.

Scheme 1.19

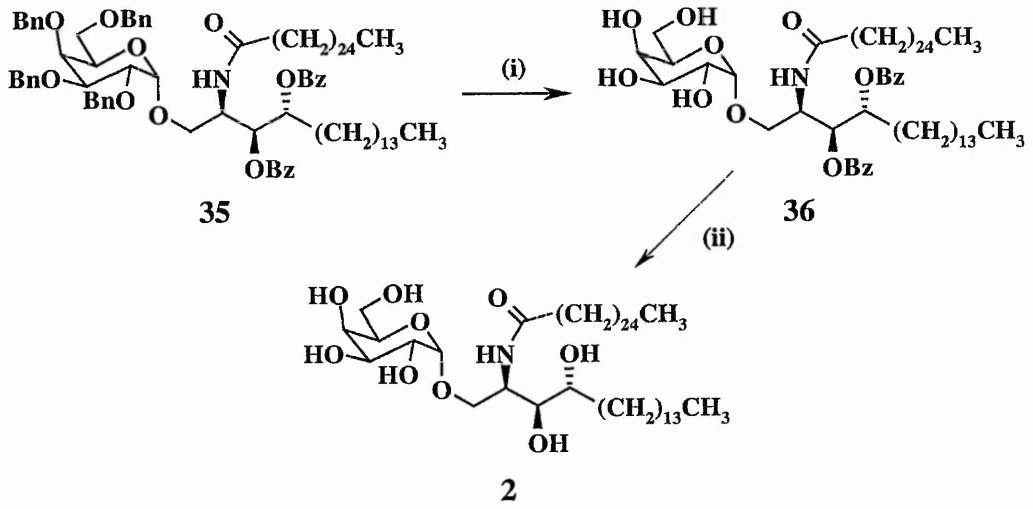


34 **35**

i) $SnCl_2$, $AgClO_4$, MS-4Å, THF, -10 to 20 °C.

Finally, deprotection of the numerous hydroxyl groups of **35** was achieved in two steps, **Scheme 1.20**, to give KRN7000 **2**. The benzyl groups of the sugar moiety were removed by catalytic hydrogenation, followed by deprotection of the secondary alcohols of the ceramide, using sodium methoxide to cleave off the benzoyl groups of **36**.

Scheme 1.20



i) H_2, Pd, EtOAc, 20 °C. (ii) NaOMe, MeOH, THF, 20 °C.

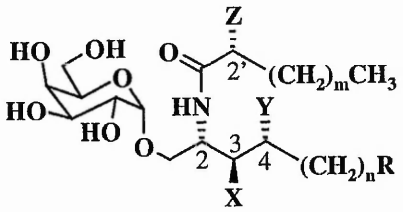
A more recent publication by Morita[34] *et al* describes the synthesis of KRN7000 from D-lyxose, in 14 steps. This follows a similar approach to that already described, but with alterations to some of the reaction conditions, giving a 16 % overall yield.

1.8 Structure-activity relationship studies on the agelasphins, leading to KRN7000

A detailed structure-activity relationship study was carried out by the Kirin group, in an attempt to enhance the activity of the natural products. This has resulted in several publications, which address different aspects of the agelasphin structure and its effect on immunostimulating activity.

Initially, a group of 18 compounds was synthesised[2], where the compounds retained the α-galactosyl moiety of the natural product, with the other structural features being investigated. The compounds studied by Morita *et al* are shown below in **Table 1.1**.

Table 1.1



Compound	X	Y	Z	R	m	n
AGL-502	OH	OH	OH	$CH(CH_3)_2$	21	11
AGL-519	OH	OH	OH	CH_3	21	12
AGL-509	OH	OH	OH	CH_3	21	6
AGL-510	OH	OH	OH	CH_3	21	10
AGL-512	OH	OH	OH	CH_3	21	13
AGL-548	OH	OH	OH	CH_3	23	13
AGL-549	OH	OH	OH	CH_3	23	14
AGL-550	OH	OH	OH	CH_3	23	15
AGL-525	OH	OH	H	CH_3	21	13
AGL-506	OH	H	OH	CH_3	21	13
AGL-514	OH	H	H	CH_3	21	13
AGL-535	H	H	H	CH_3	21	13
AGL-517	OH	H	H	CH_3	11	13
AGL-536	OH	H	H	CH_3	15	13
AGL-544	OH	H	H	CH_3	17	13
AGL-543	OH	H	H	CH_3	19	13
AGL-578	OH	H	OH	CH_3	23	13
AGL-582	OH	OH	H	CH_3	23	13

(AGL-502 is synthetic AGL-9b)

The compounds were first screened for biological activity by a tumour growth inhibition assay, where mice subcutaneously inoculated with B16 cells were treated with the compounds, both on their own, and also in combination with mitomycin C.

The role of the isopropyl terminus of the AGL-9b long chain base was investigated, by comparison with AGL-519, which lacks the methyl side chain. From the results, the substitution of the isopropyl moiety with a straight chain has a negligible effect on the tumour growth inhibition ability of the compound.

The effect of the long chain base length on activity was studied by comparison between AGL-509, AGL-510 and AGL-512 (C-11, 12, and 18, respectively), with a C'-24 fatty acid chain. AGL-512 showed the highest tumour growth inhibition ratio of the

selection, suggesting the C-18 long chain base to be preferable. Further studies with compounds AGL-548, AGL-549 and AGL-550 (C-18, 19 and 20 respectively, C'-26 fatty acid chain) had activities virtually identical to that of AGL-512, showing C-18 to be the optimum chain length.

The role of the three hydroxyl groups of AGL-512 was explored in four compounds, AGL-525 (2'-deoxy), AGL-506 (4-deoxy), AGL-514 (2',4-dideoxy) and AGL-535 (2',3,4-trideoxy). AGL-535 showed very little lymphocyte proliferation (LP) stimulating activity, and a lower tumour inhibiting ability, compared with AGL-512. The other three compounds, AGL-525, AGL-514 and AGL-506 all showed comparable activity to AGL-512. This suggests that possibly it is only the 3-hydroxyl that is essential for the biological activity of the compounds.

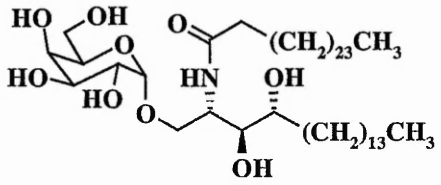
Analogues of AGL-514 were then studied, with a C-18 long chain base, but differing fatty acid chain lengths, viz. the compounds AGL-517 (C'-14), AGL-536 (C'-18), AGL-544 (C'-20), AGL-543 (C'-22). All these compounds demonstrated potent tumour suppressing activity, but AGL-514 was chosen at this stage as the most suitable compound for further investigation.

To confirm the biological activity of the compounds could be transferred to human use, the LP stimulating activity of the compounds was studied using human umbilical cord blood. AGL-514 and AGL-525 were studied, and it was found that AGL-525 was the more potent compound. This led to further investigations into the role of the hydroxyl groups in the antitumour activity. More analogues were synthesised, using a C'-26 fatty acid chain, which had been found to be slightly more potent than the C'-24, in the case of AGL-548 (C'-26, 2',4-dideoxy).

The compounds studied were AGL-578 (C'-26, 4-deoxy) and AGL-582 (C'-26, 2'-deoxy). In the assays, both AGL-548 and AGL-582 were more potent stimulators of

LP in human umbilical cord blood than AGL-578. AGL-548 and AGL-582 demonstrated similar LP stimulating activity using human peripheral blood. They also showed similar anti-tumour activity against *in vivo* B16 cells in mice. In a choice between the two compounds, AGL-582 was chosen as the candidate for clinical evaluation, and given the designation KRN7000.

The compound has now reached phase I clinical trials, as a novel immunomodulating agent for the treatment of certain types of cancer.

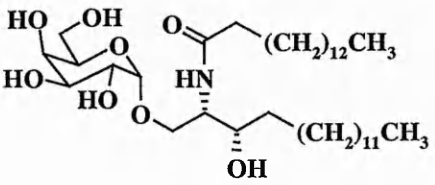


2

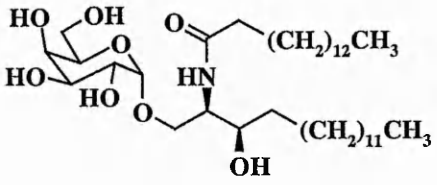
KRN7000

The compound differs from AGL-9b by the absence of the 2'-alcohol α to the amide carbonyl, and extended unbranched alkyl chains for both the long chain base and fatty acid.

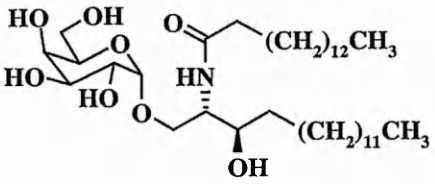
The activity of different diastereomers of the ceramide portion of the molecule was studied by Motoki[14] *et al*, where four diastereomers were synthesised. The compounds were compared by their tumour growth inhibition activity in B16-bearing mice. The most active of the four α-galactosyl ceramides in the study was the (2*S*, 3*R*) compound AGL-558, which exhibits the stereochemistry found in the naturally occurring agelasphins.



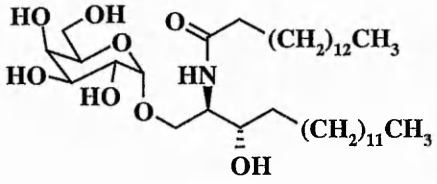
AGL-555 (2*S*, 3*S*)



AGL-556 (2*R*, 3*R*)



AGL-558 (2*S*, 3*R*)

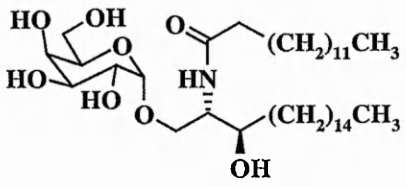


AGL-559 (2*R*, 3*S*)

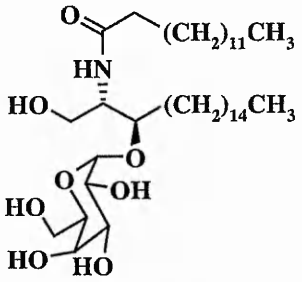
The same publication[14] also explored the influence of the sugar moiety, and its mode of attachment, to the immunostimulating activity of the compounds. The compounds were tested for their spleen cell proliferation enhancing ability in the MLR assay. The results of the assay suggested that the galactosyl compounds were preferred, with the α-galactosyl compound being the most active. Additionally, although of lower potency than the galactosyl analogue, the α-glucosyl compound was also more active than the β-glucosyl compound, showing a general preference for α-linked compounds.

The preference for α-linked compounds was reinforced by a later publication by Kobayashi[7] and co-workers, where α-galactosyl ceramides were found to have the highest activity *in vitro* for enhancement of NK cell activity.

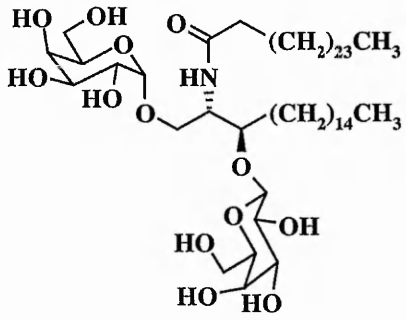
The activity of 3-glycosylated ceramides was also investigated, by Sakai[35] *et al.* Analogues of AGL-506 were studied, with both 3-α- and 3-β-galactosyl derivatives synthesised, as well as the mono α-galactosyl-3-ceramide. These compounds were to study the importance of the position of the galactose moiety, and of the 3-hydroxyl group of the ceramide.



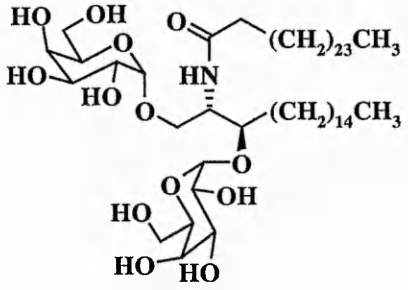
AGL-517



AGL-553



AGL-529



AGL-533

The α-linked AGL-529 showed less spleen cell stimulating activity than the 3-hydroxyl analogue AGL-517, and the mono galactosyl-3-ceramide AGL-553 was inactive. From the results, Sakai *et al* concluded that α-linked galactose was essential at the 1-position of the ceramide, and not the 3-position.

A more recent study by Iijima[36] *et al* , further investigated the structure-activity relationship of the hydroxyls on synthetic agelasphin analogues. It was found that the 3-hydroxyl group of the ceramide plays a critical role in the enhancement of the antigen presenting function of dendritic cells. Additionally, the sugar moiety was investigated, and it was found that the 2"-hydroxyl of the sugar, as well as the presence of the pyranose form, were important to the enhancement of antigen presenting function of DC cells.

Some molecular modelling studies[36], using data from a selection of active and inactive compounds, also agreed with the importance of the sugar 2"-hydroxyl and particularly the ceramide 3-hydroxyl on the ceramide portion of the molecule. The

molecular modelling studies also suggested that a receptor-like mechanism involving these functionalities may be responsible for the biological activity of the compounds.

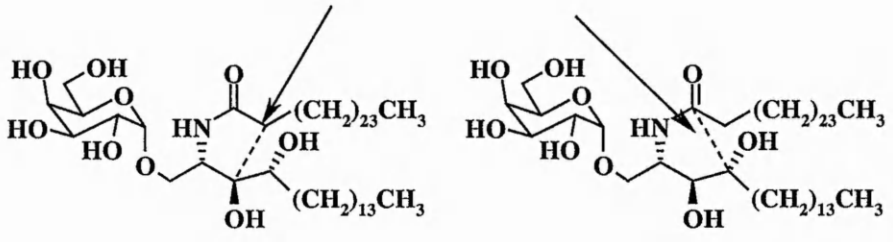
1.9 Target synthetic compounds

1.9.1 Identification of target structures. It is plausible that the hydrophobic alkyl side chains adopt a roughly parallel arrangement in the active conformation of the agelasphins, perhaps within a lipid membrane. Therefore it is possible that the biological activity could be enhanced by increasing the structural rigidity of such a conformation.

The synthesis of heterocyclic analogues may provide a useful probe into the biological activity of the agelasphins, by providing such conformationally restrained molecules. Holding the various central portions of the molecule rigidly in space may give an increase in activity, if the correct conformer is synthesised.

By the addition of an extra bond, creating a multifunctional heterocycle **Scheme 1.21**, the basic agelasphin structure can be modified to a substituted pyrrolidinone, with increased rigidity compared to the parent compound. It is the synthesis of such heterocycles that will be discussed in the following chapters.

Scheme 1.21



Thus, the target compounds are pyrrolidinone derivatives, with structural features based upon the findings of Morita[2] *et al*, and the optimised structure of

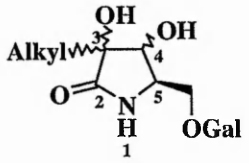
KRN7000. The structural requirements of the pyrrolidinone derivatives can be summarised as follows.

1. A fixed centre of known chirality at the 5-position of the ring, with a methylene linking unit to a sugar moiety, preferably α-galactose.

2. Hydroxyl substituents at C-3 and/or C-4. Ideally, these alcohols would need to be produced as a series of enantiomers, as all possible combinations of the alcohol substituents are potentially interesting.

3. A long (at least 10 carbons) unbranched alkyl chain is required on the 3-carbon of the ring, replacing the proposed parallel pair of alkyl chains, in both of the possible stereoisomers.

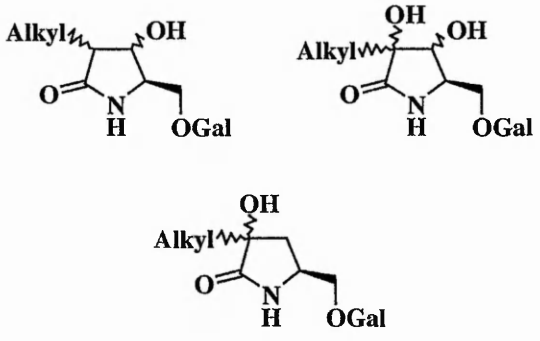
1.9.2 Target compounds for synthesis. The structural requirements of a target cyclic analogue can be summarised as **37**:



(Where Alkyl = C 10 or longer alkyl chain)

37

This gives a series of structures as target compounds, some of which are summarised below. The stereocentres are unspecified, apart from that of the 5-methylene link to the α-galactosyl moiety.



This type of compound is a challenging synthetic target, as it contains a large number of functional groups within a relatively small molecule. This is further complicated by the combination of hydrophobic alkyl chain, hydrophilic sugar moiety, and multiple stereogenic centres.

2. Synthesis from acyclic fragments, concentrating on 4-hydroxypyrrolidinones

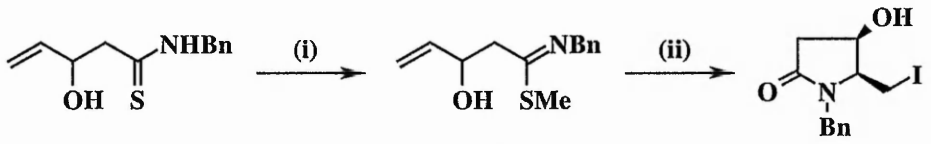
2. Synthesis from acyclic fragments, concentrating on 4-hydroxypyrrolidinones.

2.1 Introduction

Formation of the pyrrolidinone ring by a free radical cyclisation can generate compounds with a substitution pattern difficult to achieve by other methods. This is most important with respect to the 4-hydroxypyrrolidinones, as the methods described by Takahata[37] *et al*, Roberson and Woerpel[38], Bryans[39] and co-workers, or Bachi and Melman[40] have the potential to make these otherwise inaccessible compounds available.

Takahata[37] *et al* describes the synthesis of 4-hydroxypyrrolidinones **39**, by iodolactamisation of unsaturated γ,δ-unsaturated thioimidates **38**, **Scheme 2.1**. The reaction is described as exhibiting high 1,2-asymmetric induction, particularly with polar substituents, such as hydroxyl, at the allylic position. The example shown is reported to give a 12 : 1 mixture of diastereomers, with the isomer shown predominating.

Scheme 2.1

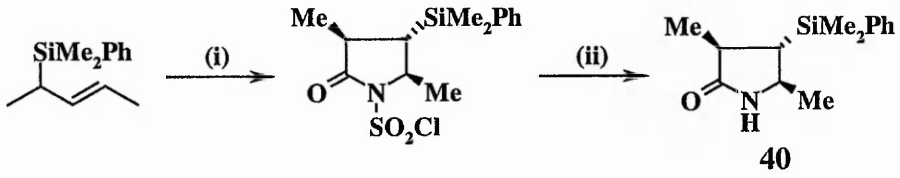


38 **39**

i) MeI, K_2CO_3, acetone, 20 °C. (ii) I_2, THF, 5 °C.

Roberson and Woerpel[38] synthesised 4-hydroxypyrrolidinones *via* a [3+2] annulation, **Scheme 2.2**, using allyl silanes with chlorosulfonylisocyanate. High stereoselectivity was achieved in the cyclisation, giving a 95 : 5 mixture of two diastereomers, the major isomer **40** being shown.

Scheme 2.2

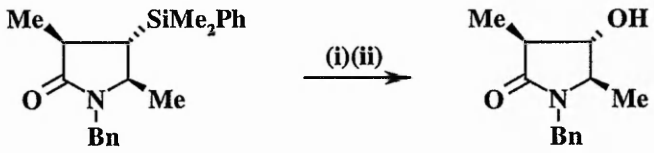


40

i) $ClSO_2NCO$, toluene, 25 °C. (ii) Red-Al, -78 to 20 °C.

Fleming/Tamao oxidation[41] was then used to convert the silyl moiety to the alcohol, **Scheme 2.3**. The oxidation was carried out on the *N*-benzyl derivative of **40**, to facilitate the isolation of the product **41**.

Scheme 2.3

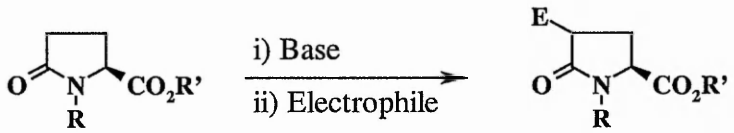


41

i) HBF_4. (ii) KF, $KHCO_3$, H_2O_2.

Substitution of the 4-position is difficult to achieve using pyroglutamic acid derivatives, as these compounds have the highest reactivity at the 3-carbon, α to the amide carbonyl moiety. Substitution of these compounds is usually achieved by anion generation, **Scheme 2.4**, which occurs at the 3-position.

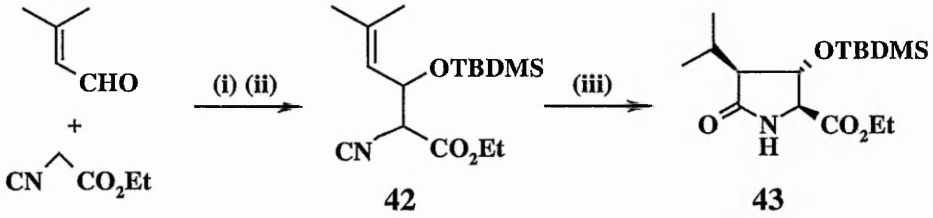
Scheme 2.4



2.2 Free radical cyclisation

After an initial literature search, the first route to be explored was the synthesis of the substituted pyrrolidinone **43** from **42**, *via* a free radical cyclisation mechanism. This synthesis, **Scheme 2.5**, had been reported by Bachi and Melman[40], and had the advantage of forming the 3-alkyl-4-*tert*-butyldimethylsilyloxypyrrolidinone **43**, as a mixture of diastereomers, which were reported to be easily separated.

Scheme 2.5



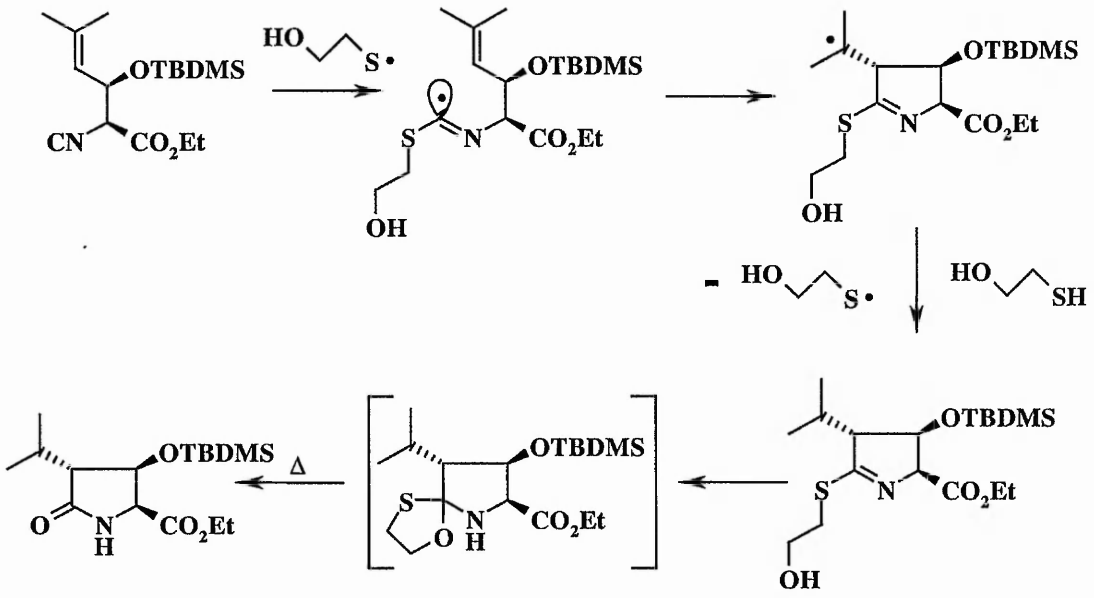
i) ^{n}BuLi, THF, -78 °C. (ii) TBDMS-OTf, THF, -78 °C.

(iii) $HOCH_2CH_2SH$, AIBN, toluene, 100 °C.

At the time this was one of the few syntheses of a 4-hydroxypyrrolidinone that had been found in the literature, whereas there are several examples of the synthesis of 3-hydroxypyrrolidinones.

The cyclisation reaction to form the 5-membered ring is reported as proceeding *via* a free radical mechanism, **Scheme 2.6**; the reaction is initiated by the AIBN.

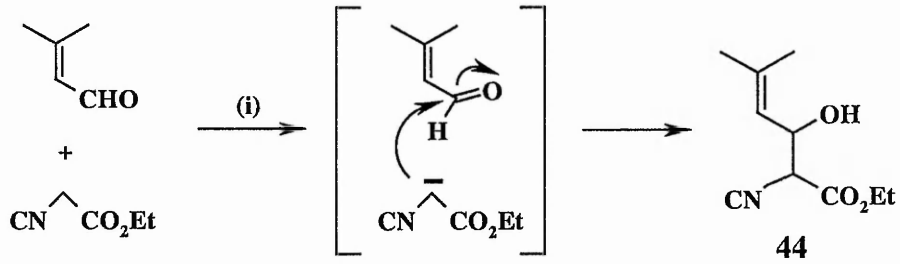
Scheme 2.6



The first synthetic step was the formation of the allylic alcohol **44**. The reaction described in the literature was the 1,2-addition of the anion of ethyl isocyanoacetate to 3-methyl butenal, **Scheme 2.7**, which was reported to give the alcohol **44** in good yield.

The reaction was carried out according to the published method, but with the addition of 1,10-phenanthroline to the reaction. The use of 1,10-phenanthroline as an indicator in anion reactions has been reported[42], where the compound forms a dark red complex when a slight excess of base is present in the reaction mixture. This allows accurate addition of a stoichiometric amount of butyl lithium, lowering the possibility of side reactions occurring due to excess butyl lithium.

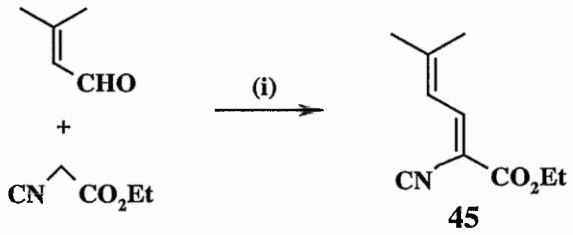
Scheme 2.7



i) 1 eq. ^{n}BuLi, THF, 1,10-phenanthroline, -78 °C.

In our hands, however, the expected product was not isolated, and even after several attempts none of the desired material could be recovered. Instead, it was observed that under the conditions described in the literature, with or without the 1,10-phenanthroline, a further elimination step was occurring. This resulted in the isolation of the α,β-unsaturated ester **45**, **Scheme 2.8**, as the only significant product, and even this was obtained only in low yield.

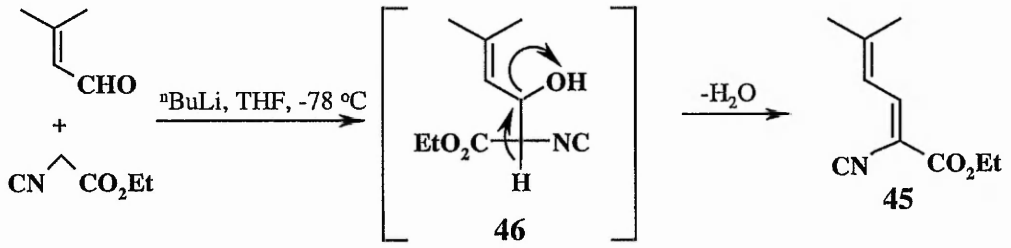
Scheme 2.8



i) ^{n}BuLi, THF, -78 °C.

By studying the structure of the product isolated, it appears that the alkoxide anion does form as a result of the 1,2-addition. However, the anion must be unstable even at -78 °C, as it spontaneously dehydrates **46** to the more stable α,β-unsaturated ester **45**, **Scheme 2.9**, rather than generate the desired alcohol on an aqueous quench.

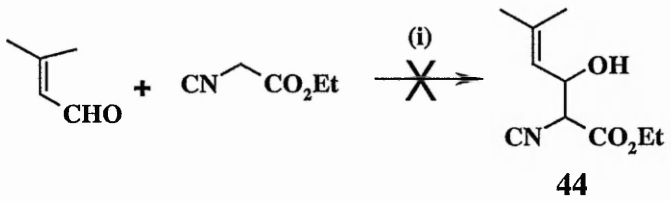
Scheme 2.9



This dehydration should not really be unexpected, as the unsaturation pattern present in the target allylic alcohol is perfectly set up to eliminate water in a dehydration.

A variety of changes to the original reaction conditions were investigated, but these were all unsuccessful as a means of obtaining the target alcohol. Firstly, the use of a weaker base, potassium carbonate, for the reaction was studied, in case it was the strong lithium base encouraging the dehydration, **Scheme 2.10**. This modification was found to be unsuccessful, as only traces of the diene were identified among the decomposition products. The reaction was carried out both at room temperature and at reflux, and also at reflux in the presence of tetrabutyl ammonium bromide, as a phase transfer catalyst.

Scheme 2.10

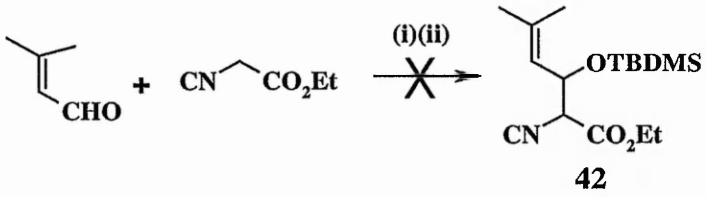


i) K_2CO_3, THF, reflux.

In retrospect, the use of a refluxing solvent was inappropriate, as isonitriles are known to undergo thermal rearrangement when refluxed.

The trapping of the alkoxide anion by reaction with TBDMS-triflate, in a one-pot procedure, was then studied, **Scheme 2.11**. This would avoid the isolation of the allylic alcohol, by converting it directly to the silyl ether **42** from the alkoxide anion. The possibility of dehydration occurring on work-up would thus be eliminated. This reaction was also found to be unsuccessful.

Scheme 2.11

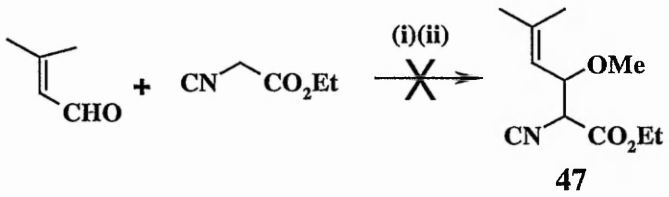


42

i) ^{n}BuLi, THF, -78 °C. (ii) TBDMS-Cl, THF, -78 °C.

An alternative strategy was to trap the alkoxide anion as the methyl ether, using methyl iodide, **Scheme 2.12**. The methyl iodide, a powerful alkylating agent, should be highly reactive towards an alkoxide ion, to give the methyl ether **47**. This reaction also failed to give an identifiable product other than the diene.

Scheme 2.12

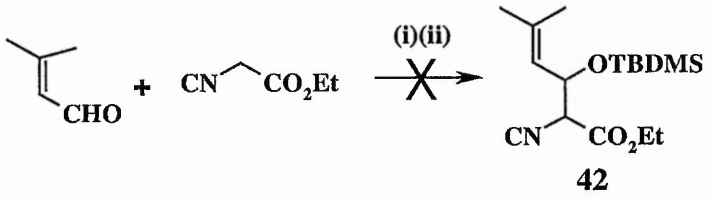


47

i) LiHMDS, THF, -78 °C. (ii) MeI, THF, -78 °C.

The use of a stronger base (^{n}BuLi) was also studied, followed by *in situ* treatment of the anion with the less reactive silylating agent TBDMS chloride, **Scheme 2.13**. This also gave the same results as the previous experiments, with small quantities of the diene being the only identifiable product.

Scheme 2.13



42

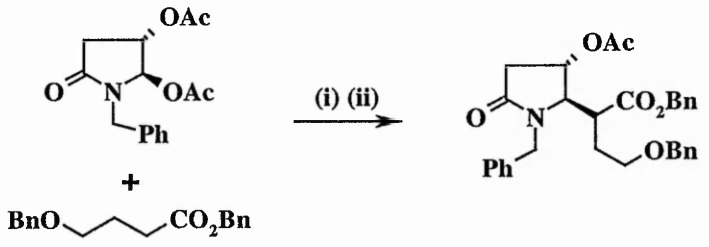
i) ^{n}BuLi, THF, -78 °C. (ii) TBDMS-Cl, THF, -78 °C.

Although a very attractive synthetic scheme, the route had to be abandoned. The initial reaction, reported to occur in good yield could not be repeated, and the intermediate allylic alcohol is vital for the success of the synthesis.

2.3 Use of acyliminium ions

A possible alternative synthesis of a 4-hydroxypyrrolidinone was provided by the work of Pilli[43] *et al*, in which the reaction of a variety of esters and silyl derivatives with an acyliminium species (generated by use of a boron Lewis acid), is used in the synthesis of 5-alkyl-4-acetoxy-2-pyrrolidinones, **Scheme 2.14**.

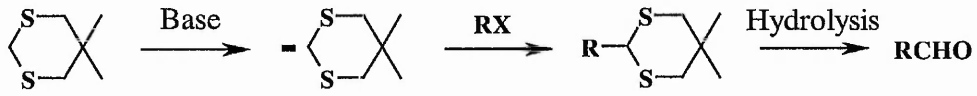
Scheme 2.14



i) LDA, THF, -78 °C. (ii) TMS-OTf.

The synthetic work described in the literature involves the introduction of functionalised side chains, which are not required for the compounds targeted in this study. The use of a simple formaldehyde synthon would be more suitable, with a dithiane **48** providing a possible source of an aldehyde moiety, **Scheme 2.15**.

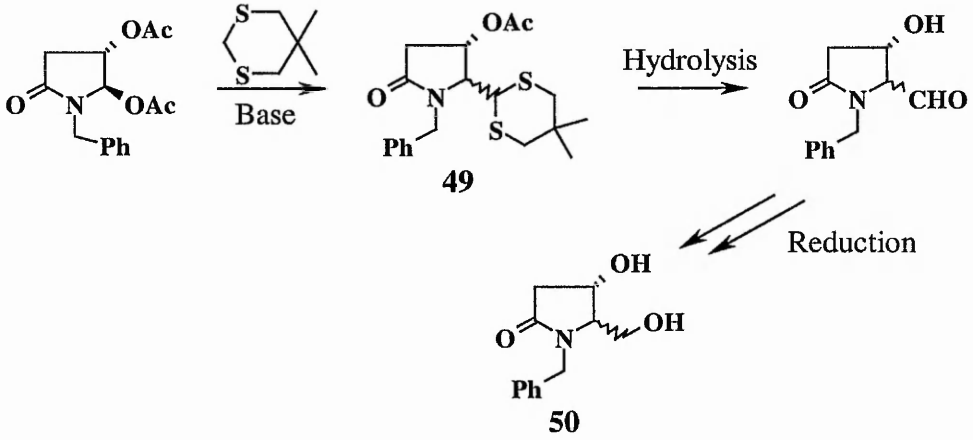
Scheme 2.15



48

This is an example of using an umpoled synthon, where the normal reactivity of the group to be introduced is reversed. The synthetic equivalent of the formyl synthon is a nucleophilic anion, obtained by treatment of a thioacetal with a strong base. The reaction of this with an electrophile would give an intermediate **49**, **Scheme 2.16**. Hydrolysis of the thioacetal would result in the aldehyde, which could then be reduced to the primary alcohol, giving the 5-hydroxymethyl-2-pyrrolidinone **50**.

Scheme 2.16



49

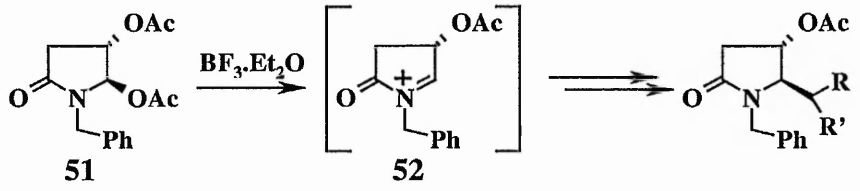
50

To test the practicality of the reaction scheme, a simple anion, generated from *tert*-butyl acetate, would be used to react with the acyliminium species **52**, **Scheme 2.17**. This would be a simple test of the reactivity of the species towards nucleophiles, and give a product easily identifiable by NMR.

A further change to the conditions described in the literature was the use of boron trifluoride etherate as the Lewis acid catalyst, although this was reported to be less effective than di-*n*-butylboron triflate, the catalyst used in most of the studies.

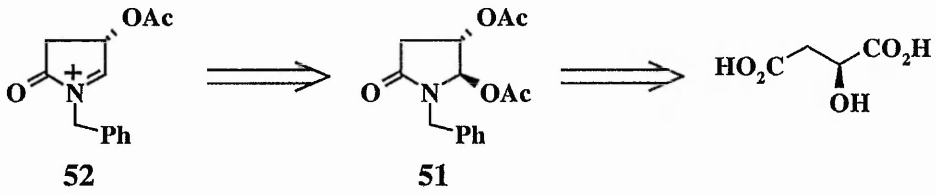
However, the latter catalyst is difficult to prepare, and the commercially available material was reported to give non-reproducible results.

Scheme 2.17



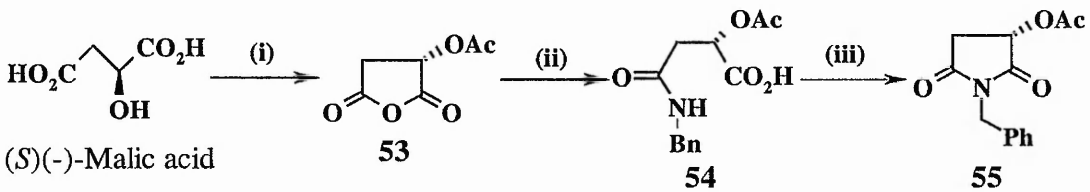
The acyliminium species **52** is generated from the 1-benzyl-*trans*-4, 5-diacetoxy-2-pyrrolidinone **51**, **Scheme 2.18**, which is synthesised from commercial malic acid.

Scheme 2.18



Pilli *et al* prepared the 1-benzyl-*trans*-2,3-diacetoxypyrrolidinone according to the method reported by Koot[44] and co-workers, in which (*S*)-(-)-malic acid was converted to the cyclic anhydride **53**, by first forming the acid chloride, which on heating resulted in the thermodynamically stable cyclic anhydride, **Scheme 2.19**. Reaction of the anhydride **53** with benzylamine gave ring opening to the amide **54**, which then underwent ring closure to the imide **55** on treatment with acetyl chloride.

Scheme 2.19



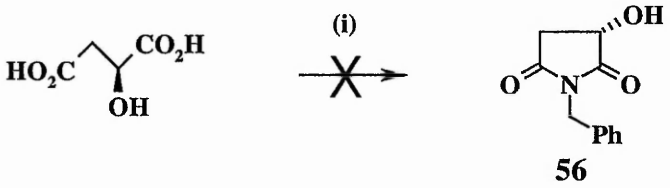
(*S*)(-)-Malic acid

i) AcCl, reflux. (ii) $BnNH_2$, THF, 20 °C. (iii) AcCl, reflux.

Slight modification of the last two steps was found to be necessary, to obtain the imide in a good yield. The procedure of Koot is carried out in three steps, with neat acetyl chloride for two of the steps. This synthesis of this fairly simple compound was complicated by a lack of detailed experimental procedures. Although a similar compound had been described by both Klaver[45] *et al* and Chamberlin and Chung[46] (*N-iso*-propyl and N-H compounds respectively), these preparations were similar, in using neat reagents without work-up in between steps.

Following these literature procedures resulted in a low yield of impure imide, with a similar result being obtained using the procedure of Bernardi[47] *et al* who reports that the reaction between malic acid and benzylamine in refluxing xylene gives a precursor to **55**, hydroxy lactam **56**, in 70% yield. This method was investigated, **Scheme 2.20**, but was also found to give a low yield of the impure amide.

Scheme 2.20



56

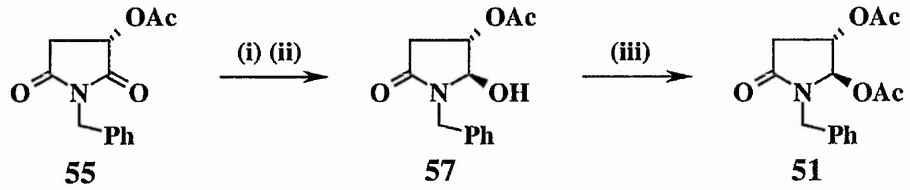
i) $BnNH_2$, xylene, reflux.

The original conditions of Koot were then modified. It was found necessary to isolate and recrystallise the acyclic amide **54**, and then to react this with acetyl chloride in DCM, instead of using neat acetyl chloride for the final cyclisation. These alterations dramatically improved the final yield of the lactam **55** to 52%.

The next step was the chemo- and stereoselective reduction of the amide carbonyl moiety adjacent to the acetoxy group. It had been reported in the literature that sodium borohydride treatment of the imide resulted in the *trans* alcohol **57**, **Scheme**

2.21. The selectivity of the reduction can be explained by the co-ordinating effects of the amide and ester groups. This co-ordination to the borohydride anion results in both chemoselectivity and stereoselectivity. The alcohol is then esterified, using acetic anhydride, to give the *trans*-compound **51.** This was found to give the diester in 16% yield from the monoester.

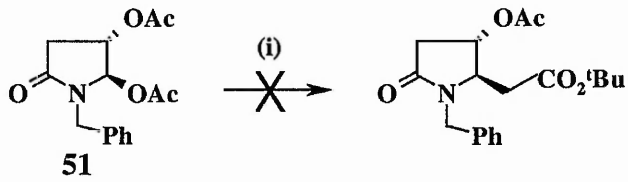
Scheme 2.21



i) $NaBH_4$, EtOH, -23 °C. (ii) HCl aq., 20 °C. (iii) Ac_2O, DMAP, Et_3N, DCM, 20 °C.

A simple trial reaction, using *tert*-butyl acetate, was then used to investigate the reactivity of the acyliminium species towards an anion, **Scheme 2.22.** The pyrrolidinone **51** was treated with boron trifluoride etherate, and then a solution of anion added, at low temperature. Addition of the nucleophile was not observed.

Scheme 2.22



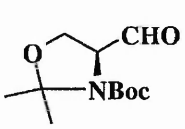
i) $BF_3.Et_2O$, $AcOBu^t$, LiHMDS or LDA, THF, -60 °C.

This lack of reaction with a simple anion suggested that a carbonyl synthon would also fail to react. The reaction described in the literature was sensitive to both the attacking species, and the complex boron catalyst being used. The changes to both of these altered the reactivity to such an extent that the reaction would no longer occur.

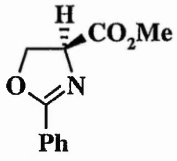
2.4 Synthesis from serine derivatives

2.4.1 Synthesis from serine. This offers an alternative route to the 4-hydroxypyrrolidinones, by using serine as a chiral starting material. The amine and alcohol functions require protecting, which can be achieved either by using two separate groups, or a single protecting group for both alcohol and amine functions.

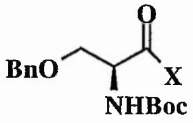
The single protecting group can be an oxazolidine **58**, such as Garner's aldehyde, or an oxazoline **59**, as used by Huang[48] *et al.* The use of two separate protecting groups is less common, with the use of *O*-benzyl-*N*-Boc (or Fmoc) serine **60** being a novel approach.



58



59



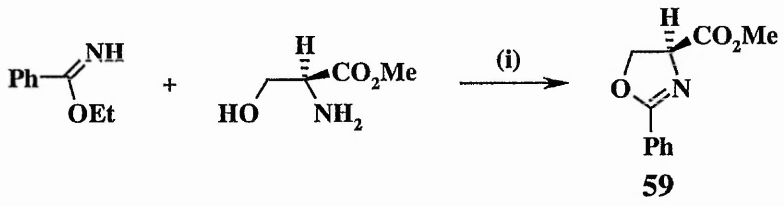
60

The protected compound is then used as a substrate for reactions with enolate anions, or Wittig reagents. Further manipulations are then required to get to the target compounds, with a key step being the cyclisation to the pyrrolidinone.

2.4.2 Oxazole protected serine. The synthesis of polyfunctional pyrrolidinones was also attempted from protected serine derivatives. The use of serine as the starting material has the advantage of beginning with enantiomerically pure material, which can then be used to direct the stereochemical outcome of later synthetic steps.

The synthesis described by Huang[48] *et al* began with the formation of the oxazoline **59**, formed *via* a condensation between serine methyl ester and ethyl benzimidate **Scheme 2.23**. This simultaneously protects the alcohol and amine functions of the molecule, the protecting group being easily removed at the end of the synthesis.

Scheme 2.23



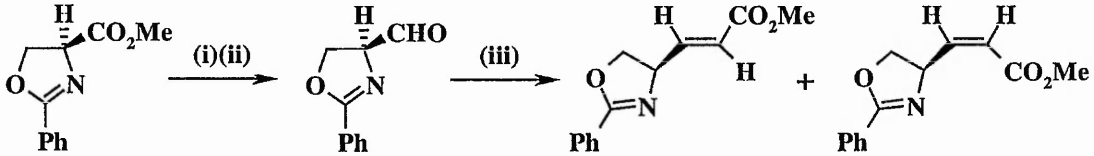
59

i) Et_3N, DCM, reflux.

The ester moiety of **59** is then reduced to the aldehyde **61**, using the sterically hindered hydride source diisobutylaluminium hydride (DIBAL-H), to prevent over-reduction to the alcohol. The aldehyde is not isolated, but is instead reacted *in situ* with a Wittig reagent obtained from commercially available (carbomethoxymethylene) triphenylphosphorane.

Wittig reagents are generated by deprotonation of a phosphonium salt by base. Instead of separately forming the Wittig reagent and adding it to the aldehyde, the DIBAL-H is quenched with methanol, resulting in *in situ* formation of lithium methoxide, to which is added the phosphonium salt, thereby generating the Wittig reagent. This conveniently achieves the one-pot synthesis of the substituted alkenes **62**, **Scheme 2.24**, without the need to isolate the aldehyde **61**.

Scheme 2.24



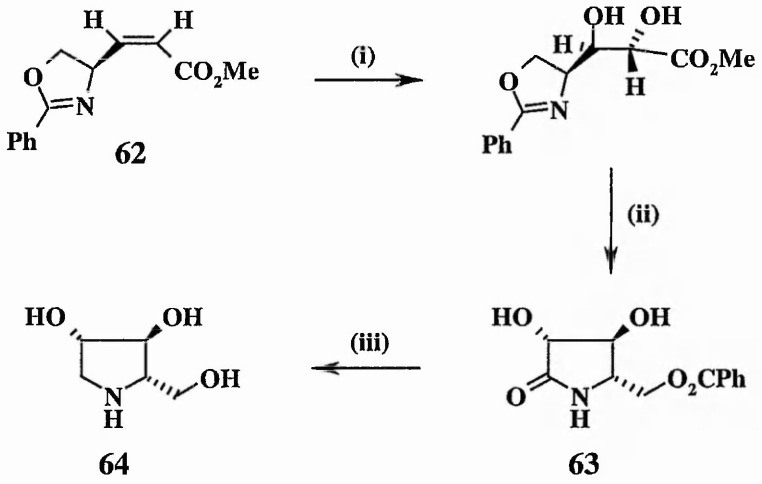
59 **61** **62**

i) DIBAL-H, toluene, -78 °C. (ii) MeOH. (iii) Ph_3PCHCO_2Me, toluene, -78 to 20 °C.

After separation of the geometric isomers by flash column chromatography, treatment of the individual alkenes with osmium tetroxide resulted in diols, **Scheme 2.25**, as separable diastereomers. The oxazole was then cleaved, which also resulted in

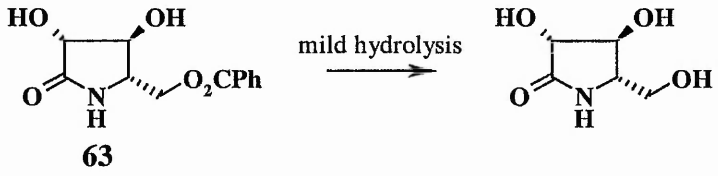
cyclisation to the pyrrolidinone **63**, closely related to our target molecules. Finally, the benzoate moiety and the amide carbonyl were reduced, using borane-tetrahydrofuran, to give the 3,4-*bis*-hydroxy-5-(hydroxymethyl)pyrrolidine **64**.

Scheme 2.25



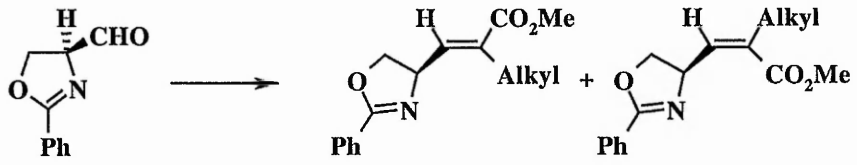
i) OsO_4, NMO, acetone, 20 °C. (ii) HCl aq., MeOH, 20 °C. (iii) BH_3-THF, .

It was intended to omit the final reduction step, to leave the amide carbonyl intact, but to hydrolyse the benzoate ester of **63** under as mild conditions as possible.



The literature method was followed, using a modification that would allow the introduction of an alkyl moiety onto the ring. The initial steps (**Scheme 2.24**) were carried out to obtain the oxazole **61**. From here, it was intended to modify the method by substituting the Wittig reagent originally used with one possessing a long alkyl chain, **Scheme 2.26**. The synthesis would then be continued as in the literature, but with an additional alkyl substituent on the alkene **65**.

Scheme 2.26

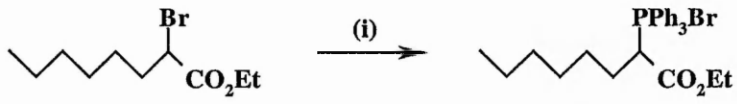


61 **65**

It was first necessary to attempt the synthesis of the phosphonium salt which would then be used to generate the Wittig reagent. There is little evidence in the literature for the synthesis of phosphonium salts possessing a long alkyl chain (>6 carbons), and none is commercially available.

The α-bromooctanoate ester **66** was chosen as a model compound, a direct synthesis of the phosphonium salt **67**, **Scheme 2.27**, being attempted with triphenylphosphine. The identification and purification of the product proved difficult, due to the presence of excess triphenylphosphine.

Scheme 2.27

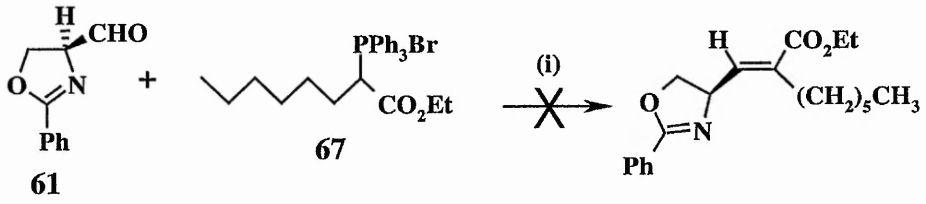


66 **67**

i) PPh_3, toluene, 20 °C.

Reaction of the aldehyde **61** with the Wittig reagent generated *in situ* by the addition of the phosphonium salt **67** to the reaction mixture failed to yield a product, **Scheme 2.28.**

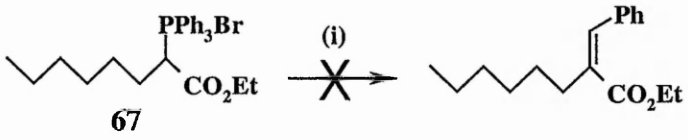
Scheme 2.28



i) LiOMe, toluene, -78 to 20 °C.

Formation of the Wittig reagent from the phosphonium salt **67** was investigated, as failure to actually generate the reagent was probably the reason for lack of reaction. The phosphonium salt was treated with freshly prepared sodium methoxide, **Scheme 2.29**, as this would provide a similar base to that being generated in the literature method. However, no product could be identified, when benzaldehyde was added to the reaction mixture. A second experiment using sodium hydride as the base was also unsuccessful.

Scheme 2.29



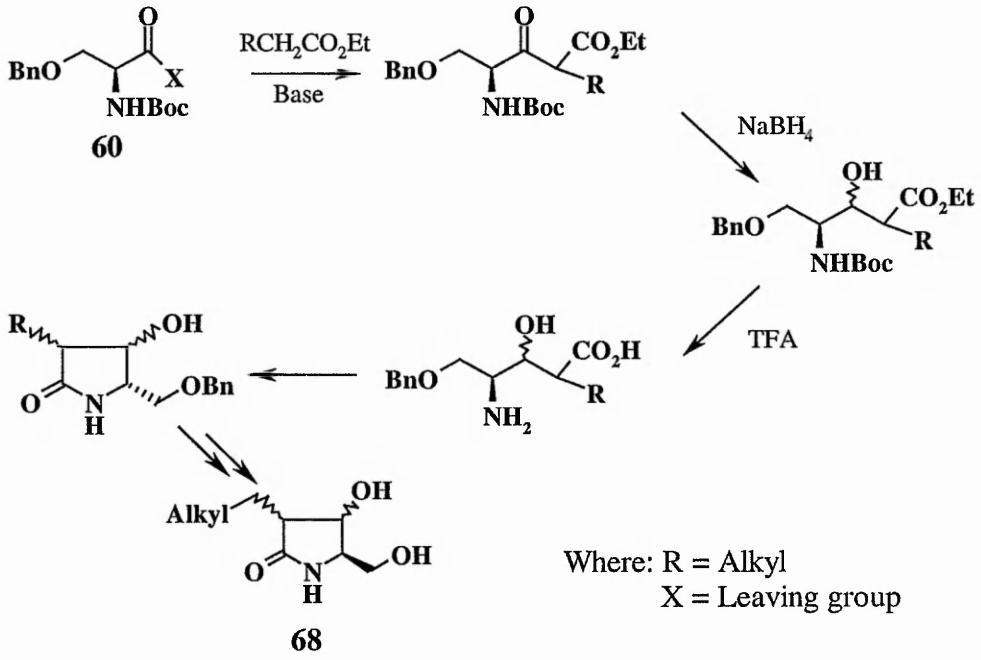
i) NaOMe, PhCHO, MeOH, 0 to 20 °C or NaH, PhCHO, DMF, 20 °C.

This result was not encouraging, and long alkyl chain Wittig reagents are uncommon in the literature. One example is reported by the Kirin group[2], where a Wittig reagent is used to introduce one of the alkyl chains into the molecule. A second example is provided by Kokotos[49] *et al*, where the reagent was prepared by refluxing a long chain alkyl iodide (C-15 chain) with triphenylphosphine in acetonitrile, a similar method to that described in Scheme 2.29.

The failure of the simple test reaction (Scheme 2.29) also suggested that the phosphonium salt had not been formed, as first thought. This considerable obstacle prevented any further progress with this route.

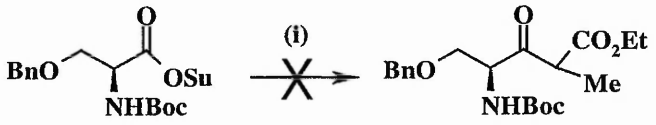
2.4.2 Separately diprotected serine. This synthetic strategy potentially allows access to the otherwise inaccessible 4-hydroxy compound **68**. The following scheme provides a general outline of the proposed route, **Scheme 2.30**.

Scheme 2.30



Initial attempts utilised commercially available *N*-Boc-*O*-benzylserine succinimide ester **69** as the starting material, **Scheme 2.31**. This was reacted with the anion generated from ethyl propionate, under various conditions. The products **70** were obtained in very low yield, in the few experiments where a product could be isolated. The succinimide ester appeared to be insufficiently reactive to yield a product with the anions being used.

Scheme 2.31

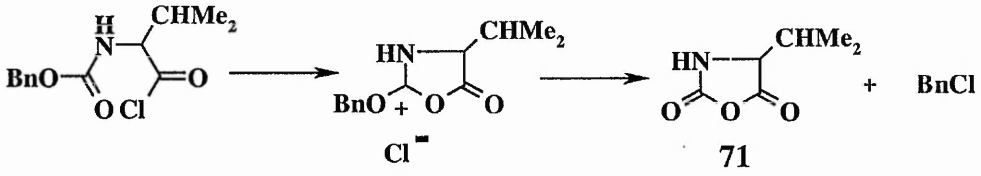


69 **70**

i) LiHMDS, $EtCO_2Et$, THF, -78 °C, or NaH, $EtCO_2Et$, THF, 0 °C.

An alternative approach was required, using a more reactive substrate for the anion to react with. A possible solution was the formation of an acid chloride from the *N,O*-protected aminoacid. The use of the Fmoc moiety for *N*-protection is essential, as treatment of the Boc protected analogue with thionyl chloride would result in a cyclisation occurring, **Scheme 2.32**, to generate the Leuchs anhydride **71**; this also occurs with Fmoc protected aminoacid chlorides, but at a slower rate[50].

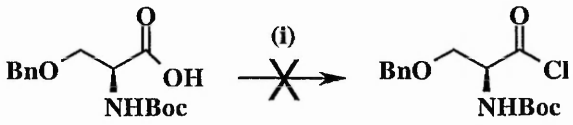
Scheme 2.32



Cl⁻ **71**

This was probably the result observed when unsuccessful attempts were made to form the acid chloride **73** of the Boc protected serine **72**, **Scheme 2.33**.

Scheme 2.33

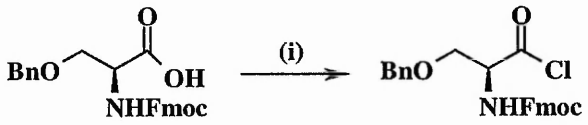


72 **73**

i) $(COCl)_2$, DCM, 0 to 20 °C.

The Fmoc acid chloride **75** has been described[51], and is synthesised from the commercially available di-protected aminoacid **74** by treatment with thionyl chloride, **Scheme 2.34**.

Scheme 2.34

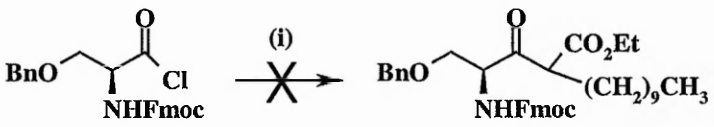


74 **75**

i) $SOCl_2$, DCM, 0 to 20 °C.

The reaction of the acid chloride with the anion generated from ethyl laurate was then examined, **Scheme 2.35**. After several attempts, little product **76** could be isolated, and it was found that starting material was being isolated, with some other products which appeared to be the result of decomposition. It is possible that the combination of bulky anion and bulky substrate decreases the reactivity drastically. Lowering the reaction temperature in an attempt to stop the decomposition slowed the reaction excessively, and raising the temperature resulted in further decomposition.

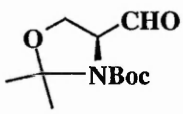
Scheme 2.35



75 **76**

i) LiHMDS, $CH_3(CH_2)_{10}CO_2Et$, THF, -78 °C.

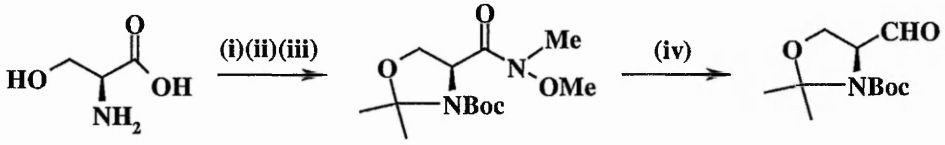
2.4.3 Attempted synthesis from Garner aldehyde. The synthesis of Garner aldehyde[52] **58** was then undertaken, as this could provide a useful alternative to the acid chlorides and activated esters previously investigated. Garner aldehyde has been used as a chiral starting material in several synthetic routes[53, 54, 55, 56].



58

The synthesis of **58** using Campbell's modification[57] of the original method[58] was undertaken, **Scheme 2.36**. Serine **77**, was *N*-Boc protected and converted to the Weinreb amide[59], followed by reaction with dimethoxypropane, to give **78**. The amide **78** was then reduced, using lithium aluminium hydride, to Garner aldehyde **58**.

Scheme 2.36

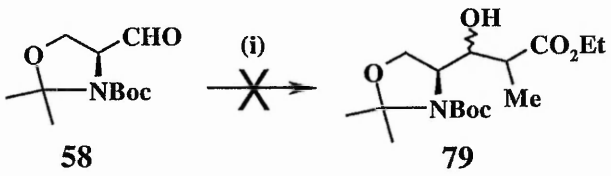


77 **78** **58**

i) Boc_2O, NaOH aq., dioxane, 0 to 20 °C. (ii) MeNH(OMe).HCl, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, *N*-methylmorpholine, DCM, -15 °C. (iii) 2,2-dimethoxypropane, $BF_3.Et_2O$, acetone, 20 °C. (iv) LAH, THF, 0 °C.

The reaction of Garner aldehyde **58** with the anion of ethyl propionate was then investigated, **Scheme 2.37**, as a comparison to the earlier work with serine succinimide esters. The reaction failed to form an identifiable product, with starting material being recovered. This would suggest that the aldehyde is unreactive towards this simple enolate anion.

Scheme 2.37

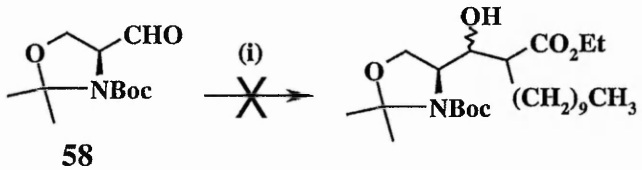


58 **79**

i) LiHMDS, $EtCO_2Et$, THF, -78 °C.

A similar reaction was carried out using the anion generated from ethyl laurate, **Scheme 2.38**, as if successful this would introduce a suitable long alkyl chain into the molecule. However, the reaction again failed to yield an identifiable product. Decomposition of some of the Garner aldehyde was observed, the remainder being recovered unchanged after several hours under the reaction conditions.

Scheme 2.38

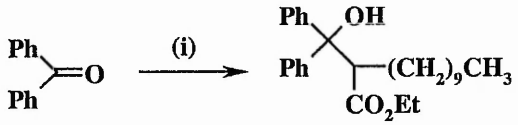


58

i) LiHMDS, $CH_3(CH_2)_{10}CO_2Et$, THF, -78 °C.

As a test of the reactivity of the anion being used for the reaction, the anion of ethyl laurate was generated, and used to attack benzophenone, **Scheme 2.39**. In this simple test system, a good yield of the alcohol **80** was obtained in a very clean reaction. This result would suggest that the Garner aldehyde is a rather hindered or deactivated substrate for nucleophilic attack, when using a bulky nucleophile.

Scheme 2.39



80

i) LiHMDS, $CH_3(CH_2)_{10}CO_2Et$, THF, -78 °C.

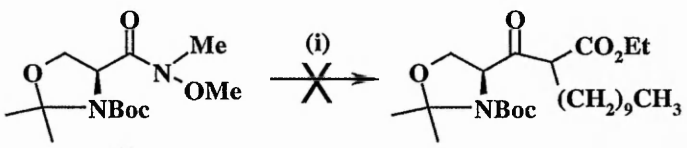
So far, no examples have been found in the literature showing a successful reaction of Garner aldehyde with an enolate anion. The published work mainly involves cycloadditions[52, 60] and addition of Grignard and active alkyl metal reagents[61].

These findings were not encouraging for the continuation of the work with Garner aldehyde. A lack of literature support for the reaction also would suggest that it may be problematic. The demonstration of the reactivity of the anion being used to attack the aldehyde implies a fundamental problem with the reaction under investigation.

The Weinreb amide precursor **78** to Garner aldehyde was also investigated as a substrate for anion reactions. Weinreb amides are reactive toward alkyl magnesium and lithium reagents, which give the ketone exclusively on work-up; this selectivity is attributed to the complexing of the metal ion by the amide, preventing multiple addition of the reagent.

Similar reaction conditions were used, with the anion being generated from ethyl laurate, and the Weinreb amide **78** was then added to the anion solution, **Scheme 2.40**.

Scheme 2.40



78

i) LiHMDS, $CH_3(CH_2)_{10}CO_2Et$, THF, -78 to 20 °C.

Unfortunately, the reaction failed, as the desired product was not isolated. A small amount of the substrate had decomposed, with the remainder being recovered, a result which suggests that this particular set of conditions is not suitable. Also, the literature does not support this reaction, as there is no evidence for the succesful reaction of an enolate anion with a Weinreb amide.

3. *N-tert*-Butyloxycarbonyl pyroglutamic acid derivatives

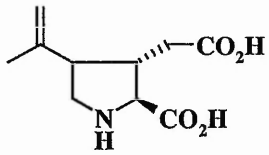
3. *N-tert*-Butyloxycarbonyl pyroglutamic acid derivatives.

3.1 Introduction

3.1.1 Chirally pure materials. The next synthetic strategy investigated was the use of pyroglutamic acid as a chiral building block. This has the advantage of being commercially available in both *R*- and *S*- forms, which are enantiomerically pure. The use of a chirally pure starting material has significant advantages, as it circumvents the problem of generating chirality from achiral compounds, avoiding the associated difficulties with chiral purity at such an early stage in the synthesis.

3.1.2 Introduction to the chemistry.

Protected pyroglutamic acids as synthetic intermediates. Pyroglutamic acid (2-pyrrolidinone-5-carboxylic acid) is a popular material in the recent literature with which to begin the synthesis of a variety of complex molecules. Many natural products have the pyroglutamate moiety as a component, and some of these have already been mentioned. Examples in the recent literature where pyroglutamate derivatives are key intermediates include the synthesis of kainic acid[62] and analogues[63], and the work on pramanicin[30, 64] (see Section 1.6), as well as the natural product calyculin[65, 66].

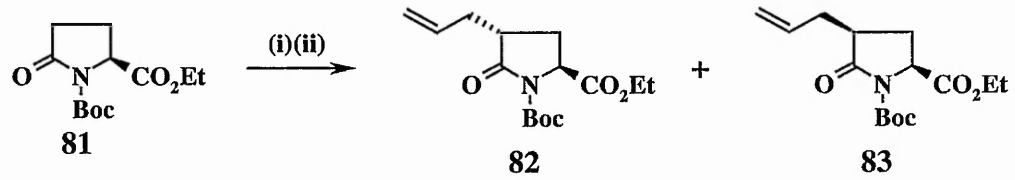


(-)-(α)-Kainic acid

The most common method of introducing functionality onto the pyrrolidinone is by the formation of an anion at the 3-position, with subsequent reaction with a suitable electrophile. Ezquerra[67] and co-workers report the stereoselective addition of electrophiles to anions of protected pyrrolidinones. An example is the reaction between

the anion of ethyl *N*-Boc-pyrrolidinone-5-carboxylate **81** and allyl bromide, **Scheme 3.1**. This gives the 3-allyl pyrrolidinone as a 2 : 1 mixture of two diastereomers **82** and **83**, respectively.

Scheme 3.1

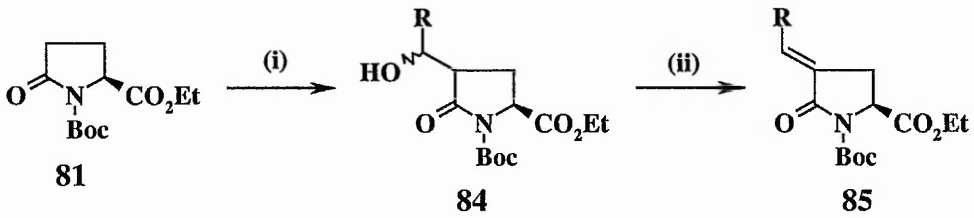


i) LiHMDS, THF, -78 °C. (ii) $BrCH_2CHCH_2$, THF, -78 °C.

The stereochemistry of the products obtained from the reaction between an *N,O*-acetal protected pyrrolidinone enolate anion and electrophiles will be discussed further in chapter 6.

Ezquerra[68] *et al* and Dikshit and Bajpai[69] have described the reaction of pyrrolidinone enolate anions with aldehydes and ketones, in the presence of Lewis acid catalysts. Ezquerra[68] *et al* report the reaction of ethyl *N*-Boc pyrrolidinone-5-carboxylate **81** with aldehydes and ketones, **Scheme 3.2**, using boron trifluoride etherate as the catalyst. This gives a mixture of diastereomers **84**, which can be dehydrated to the enone **85**, using methanesulfonyl chloride.

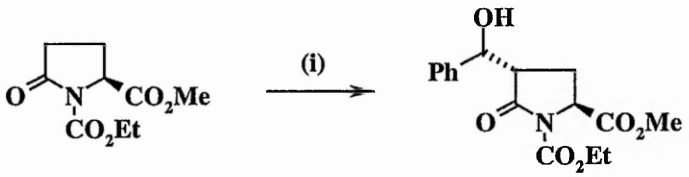
Scheme 3.2



i) RCHO, $BF_3.Et_2O$, LiHMDS, THF, -78 °C. (ii) MsCl, Et_3N, DCM, 20 °C.

The synthesis described by Dikshit and Bajpai[69] is similar, but uses a slightly different substrate, and titanium tetrachloride as the catalyst, **Scheme 3.3**. The alcohols are reported as being isolated as single diastereomers, identified by NMR.

Scheme 3.3



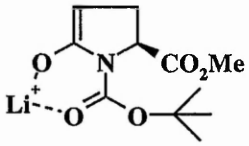
i) PhCHO, $TiCl_4$, DCM, -78 °C.

3.1.3 Chemistry of pyroglutamic acid derivatives.

Protecting group strategies. Many of these synthetic pathways rely on the ability of 2-pyrrolidinone-5-carboxylate derivatives to form nucleophilic anions at the 3-carbon. This requires protection of the acid moiety, which can be achieved in a variety of ways, as well as blocking of the amide. The acid is either protected as an ester, or reduced to the alcohol, which can be protected as an ether (usually a silyl ether).

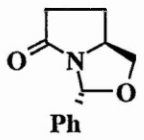
Amide protection can be achieved by using a urethane moiety, particularly a Boc group. As well as giving orthogonal protection, this also helps to activate the 3-position of the pyrrolidinone to anion formation.

It has also been suggested[70] that the carbamate moiety stabilises the formation of the lithium enolate **86**, by co-ordination of the lithium cation with the oxygen atoms of the two carbonyl groups.

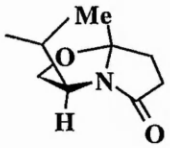


86

An alternative protecting strategy is the formation of an *N*,*O*-acetal, giving a bicyclic structure **87**, similar to Meyers' bicyclic amide[71] **88**. This method of protection will be described in more detail in chapter 6.



87



88

Choice of protecting groups. There are several options, as the stability towards various reagents has to be considered, as well as the requirement of selective deprotection. Both *N*- and *O*-protection need to be stable to lithium bases, as the generation of anions will be crucial for the introduction of further functionality onto the ring. The other requirement of the protection is that the two groups can be selectively removed without causing ring cleavage.

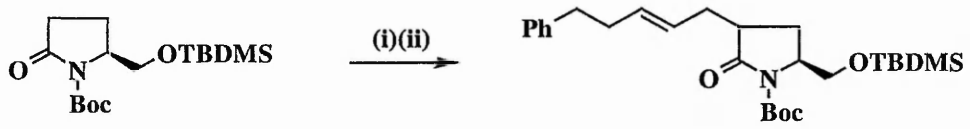
The ability to separately deprotect the primary alcohol while leaving the amide protection intact was considered as being useful later in the synthesis, where the free amide NH may interfere with glycosylation reactions. This was the reason for initially choosing the two separate protecting groups in preference to the bicyclic *N*,*O*-acetal, which exhibits anion chemistry similar to that of the diprotected compounds.

Of the options available for *O*-protection, the silyl ethers appeared to offer the best solution. The *tert*-butyldimethylsilyl (TBDMS) group was chosen, as this is stable to both acid and base conditions, but can be easily and selectively cleaved by fluoride ions[72], a consequence of the Si-F bond energy being higher than that of the Si-C bond. Additionally, the steric bulk of the group may assist in giving some degree of stereoselectivity to subsequent reactions.

This left the choice of suitable *N*-protection. Following literature precedent[73, 74, 75], the *tert*-butyloxycarbonyl (Boc) group was chosen.

The TBDMS ether **89** has been shown by Hon[74] *et al* to successfully generate an enolate ion, which will react with allyl bromides, **Scheme 3.4**. This results in the 3-allyl substituted pyrrolidinone **90**.

Scheme 3.4



89 **90**

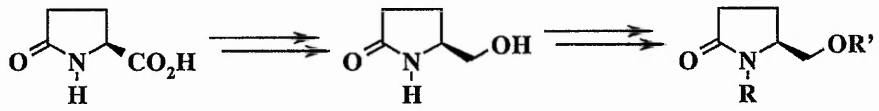
i) LDA, THF, -78 °C. (ii) $Br(CH_2)CHCH(CH_2)_2Ph$, -78 °C

Other protecting group options were also considered at this point. However, only *N*-Boc and the *N,O*-acetal had been commonly used in previous studies, and it appeared that the carbamate or acetal was necessary for generation of the anion at the 3-carbon. The benzyloxycarbonyl (Z) group was also considered, with its increased stability compared to Boc, but this was at first considered to be unnecessary.

3.2 Synthesis of *N-tert*-Butyloxycarbonyl pyroglutamic acid derivatives

3.2.1 Synthesis of 5-hydroxymethylpyrrolidinone. The first problem associated with use of pyroglutamic acid **91** was its conversion to 5-hydroxymethylpyrrolidinone **92**, **Scheme 3.5**, and then finding a suitable means of orthogonal protection **93** of the alcohol and amide functions. Although (*S*)-5-hydroxymethylpyrrolidinone is a commercially available substance, its high cost made its synthesis worthwhile, as the starting material, (*S*)-pyroglutamic acid, is relatively cheap, and the synthesis in two steps at first appears to be straightforward, but was found to be more of a challenge than may be expected from such a simple compound.

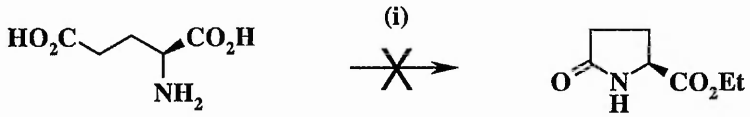
Scheme 3.5



91 **92** **93**

The most cited method for the preparation of **92** begins with the synthesis of ethyl pyrrolidin-2-one-5-carboxylate **94** from glutamic acid, using thionyl chloride[76]. This activates the two acid moieties as the acid chlorides, **Scheme 3.6**, with ensuing *in situ* cyclisation by intramolecular amide bond formation, and esterification of the remaining acid by the solvent. However, in our hands, after several attempts following the literature procedure, the reaction was found to yield only starting material.

Scheme 3.6

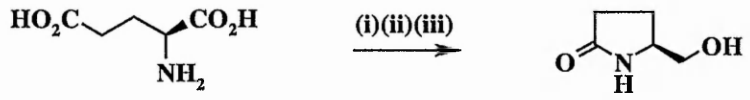


L-Glutamic acid **94**

i) $SOCl_2$, EtOH, 0 to 20 °C.

A recent publication by Barrett[30] *et al* (also see Section 1.6), suggests that the reaction should be carried out in three stages, **Scheme 3.7**.

Scheme 3.7.



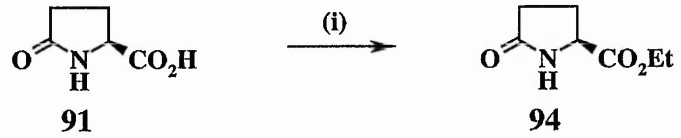
92

i) H_2O, reflux. (ii) MeOH, $SOCl_2$(cat.). (iii) $NaBH_4$, MeOH.

An alternative synthesis of ethyl pyrrolidin-2-one-5-carboxylate **94** has been reported by Pawelczak[77] and co-workers, **Scheme 3.8**. The triethylamine salt of the pyroglutamic acid **91** is formed in dimethylformamide, and this is then esterified by

reaction with bromoethane, the reaction being complete after a period of at least 24 hours at room temperature. This method was found to give the ester **94** reliably and in good (52-65%) yield.

Scheme 3.8

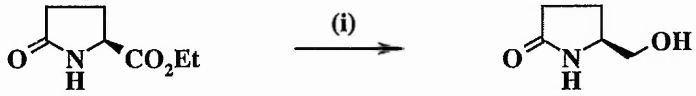


91 **94**

i) EtBr, Et_3N, DMF, 20 °C.

The next step was the reduction of the ester **94** to the corresponding alcohol, using sodium borohydride[76], **Scheme 3.9**. Sodium borohydride is the most suitable reducing agent for this particular substrate, due to its clean reduction of the ester, coupled with inactivity towards the amide moiety.

Scheme 3.9



94 **92**

i) $NaBH_4$, MeOH, THF, 5 °C.

In the original method the excess sodium borohydride was quenched with glacial acetic acid, and the crude product purified by elution from proton form ion exchange resin. This resulted in a large volume of aqueous solution to concentrate, a time consuming procedure, with the product still requiring flash column chromatography, and even then giving a product containing boron impurities.

The work-up was improved by first quenching the reaction with concentrated hydrochloric acid, until an acidic mixture was obtained. This was diluted with methanol and filtered through celite, removing many of the inorganic by-products. Evaporation of

the filtrate *in vacuo* removed a large proportion of the borate residues, as the earlier addition of methanol had converted these to volatile trimethyl borate.

Finally, the syrupy residue was subjected to flash column chromatography, to give the alcohol in good yield and excellent purity, which could be further improved by recrystallisation from acetone. These modified procedures were adapted from methods of reducing similar compounds[78,79], but had not been applied to 5-hydroxymethylpyrrolidin-2-one. A very similar set of modified conditions for this reduction has subsequently been published by Barrett[30] *et al*, as part of a synthesis of pramanicin.

3.2.2 Protection of 5-hydroxymethylpyrrolidinone. The next objective in the synthetic strategy was to protect both the amide and alcohol moieties of the heterocycle.

The protected derivative **89** was obtained in two steps, in good yield **Scheme 3.10**. The alcohol **92** is protected first, as the TBDMS ether **95**, followed by reaction of the amide with di-*tert*-butyl dicarbonate (Boc_2O), giving the *N*-Boc amide **89**.

Scheme 3.10

92 **95** **89**

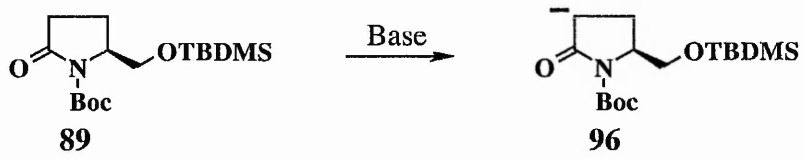
i) TBDMS-Cl, imidazole, DMF, 20 °C. (ii) Boc_2O, DMAP, DCM, 20 °C.

The protection is carried out in this order to prevent reaction of the more nucleophilic free alcohol with the di-*tert*-butyl dicarbonate, which would give the ester. A very similar synthesis of the same material has been previously reported by Ackermann[79] *et al.*

3.3 Anion chemistry of *N*-Boc pyroglutamic acid derivatives

This di-protection strategy gives a useful and easily accessible intermediate, which was then used to investigate the formation of anions **96** at the 3-position of the ring. The generation and reactivity of the anion **96** was investigated, **Scheme 3.11**, as this provides a useful method for the introduction of an alkyl substituent onto the ring.

Scheme 3.11

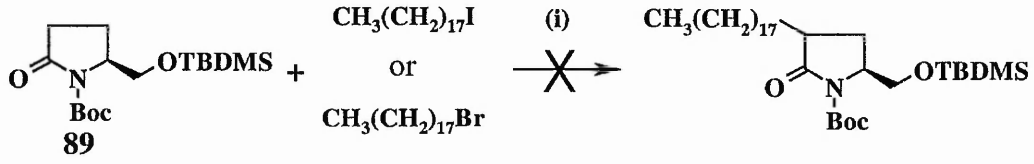


89 **96**

3.3.1 Reaction with simple electrophiles. The reactivity of the anion **96** towards a selection of electrophiles was investigated, the selection consisting of an alkyl halide two mesylates, and a ketone. However, all these simple compounds failed to react with the anion **96**.

The octadecyl iodide was prepared by a Finklestein reaction on octadecyl chloride, using sodium iodide in THF. Unfortunately, the anion of **89** failed to yield any product when treated with a solution of alkyl halide, **Scheme 3.12**.

Scheme 3.12

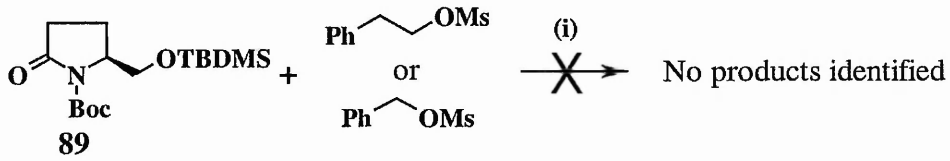


i) LiHMDS, THF, -78 °C.

Two mesylates were prepared from the corresponding alcohols, by treatment with methanesulfonyl chloride. The benzyl compound was prepared first, then the 2-phenylethanol derivative, in case the benzyl moiety from the previous compound was

affecting the reactivity. Both of the compounds failed to react with the anion of **89**, **Scheme 3.13**.

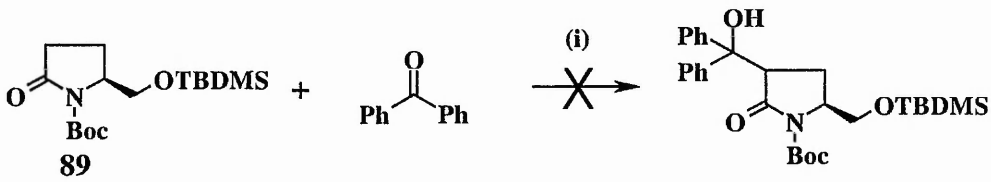
Scheme 3.13



i) LiHMDS, THF, -78 °C.

The reaction of the anion **96** with a ketone was studied, **Scheme 3.14**, using benzophenone as the substrate. Once again, the reaction failed, with only the starting material being identified.

Scheme 3.14

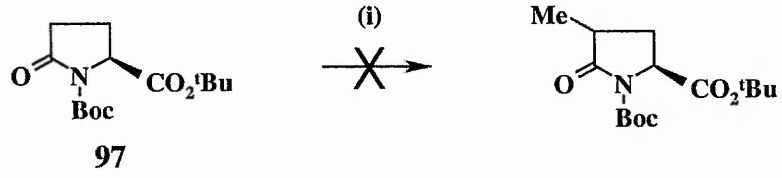


i) LiHMDS, THF, -78 °C.

The use of simple aromatic compounds such as benzophenone in the anion reactions was primarily for ease of identification of possible products. The protected pyrrolidinone has poor chromophoric groups, and is difficult to visualise on TLC plates. Thus the use of UV absorbing compounds greatly aided the identification of possible products.

The failure of these reactions is supported by work of Baldwin[80] *et al*, where *tert*-butyl-*N*-Boc-pyrrolidin-2-one-5-carboxylate **97** failed to react with methyl iodide, **Scheme 3.15**, but did react with benzyl bromide and propionaldehyde.

Scheme 3.15

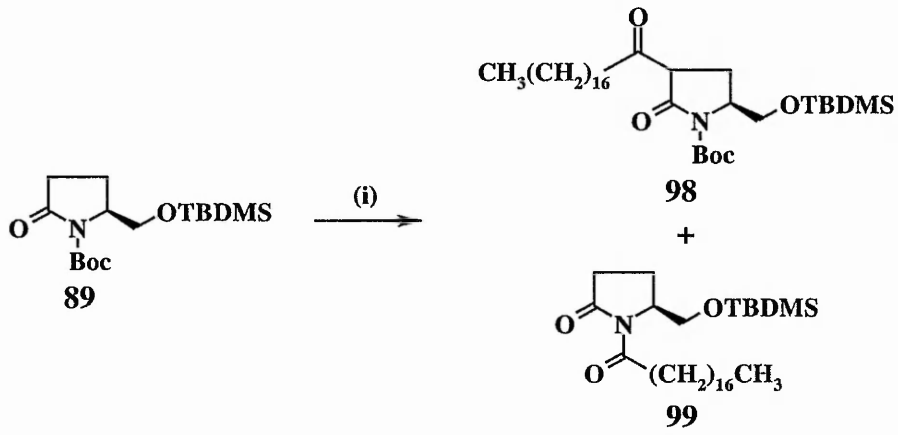


97

i) LDA, MeI, THF, -78 °C.

Stearoyl chloride was found to react, giving some of the desired product **98** in low yield, **Scheme 3.16**. However, there was a problem with *in situ* deprotection of the amide by traces of hydrochloric acid present in the acid chloride. This may have resulted in some *N*-acylation **99**, giving a mixture of products that were difficult to separate by flash chromatography, and which were identified from their mass spectra.

Scheme 3.16

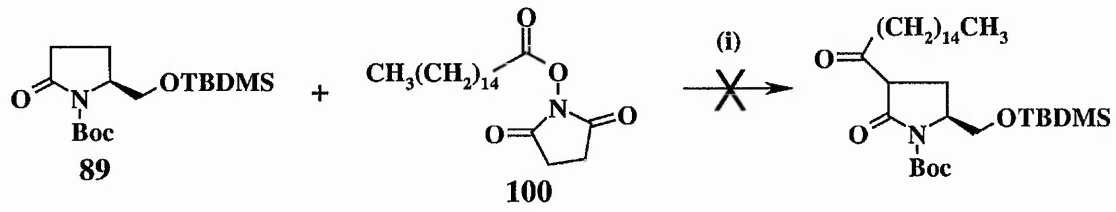


89 **98** **99**

i) $CH_3(CH_2)_{16}COCl$, LiHMDS, THF, -78 to 0 °C

To avoid this deprotection by hydrogen chloride, an activated ester, the *O*-succinimide ester of stearic acid **100**, was synthesised by reaction of stearic acid with *N*-hydroxysuccinimide in the presence of DCC. This ester again failed to react with the anion of **89**, **Scheme 3.17**, as no products were identified.

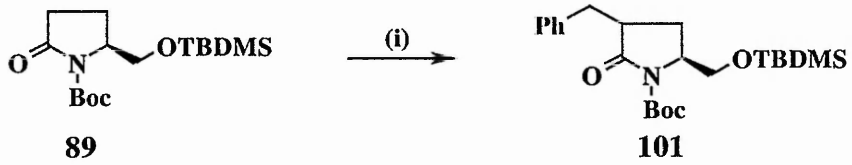
Scheme 3.17



i) LiHMDS, THF, -78 °C.

It was found that the pyrrolidinone anion **96** would react with benzyl bromide, to give the 3-benzyl derivative **101** in reasonable yield, **Scheme 3.18**. Although not in itself a particularly useful compound, it at least provided a 3-substituted compound that could be used as a model for further work.

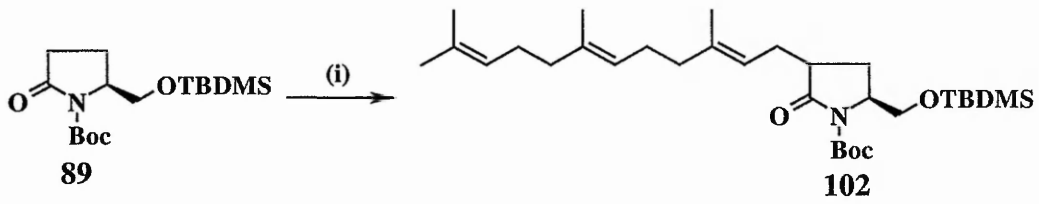
Scheme 3.18



i) LiHMDS, BnBr, THF, -78 °C

It was also found that the anion generated from the protected pyrrolidinone **89** will react with allyl bromides. Commercially available *E,E*-farnesyl bromide reacted successfully with the substrate, to give the 3-substituted product **102** in a moderate yield, **Scheme 3.19**.

Scheme 3.19

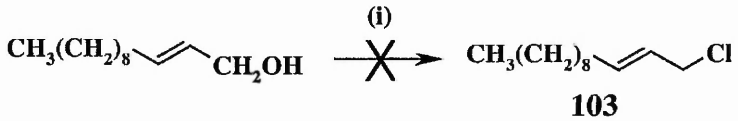


i) LiHMDS, *E,E*-farnesyl bromide, THF, -78 °C.

This was not a useful compound itself, but it demonstrated that a long chain allyl bromide might provide a means of introducing the 3-alkyl substituent onto the pyrrolidinone nucleus. To take advantage of this reaction, the synthesis of a suitable long alkyl chain allyl bromide was found to be necessary, as suitable compounds are not commercially available.

The conversion of commercially available *E*-dodec-2-en-1-ol to the halide was studied. The first synthesis attempted was that of the allyl chloride **103**, **Scheme 3.20.** The use of methanesulfonyl chloride with lithium chloride and collidine was reported[81] to be a mild method of generating allyl chlorides, but unfortunately it failed to give any of the desired product.

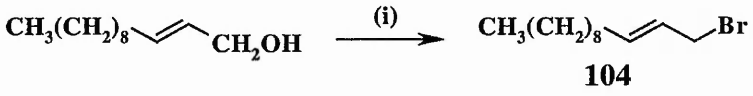
Scheme 3.20



i) $MeSO_2Cl$, LiCl, collidine, DMF, 0 °C.

An alternative method, **Scheme 3.21**, using carbon tetrabromide and triphenylphosphine[82], was, however, successful in producing the desired allyl bromide **104**, in good yield.

Scheme 3.21

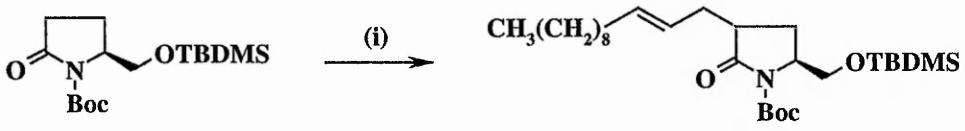


104

i) CBr_4, PPh_3, DCM, 20 °C.

Reaction of the allyl bromide **104** with the anion of the protected pyrrolidinone **89** gave the desired 3-allyl substituted derivative **105**, **Scheme 3.22**, although in a low yield.

Scheme 3.22

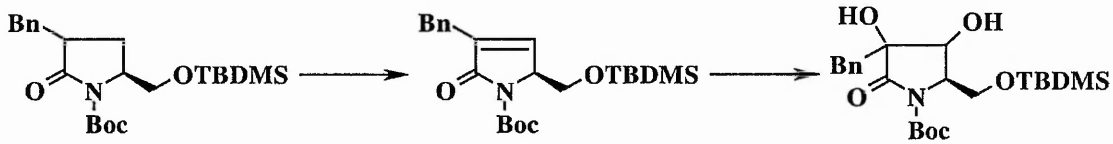


89 **105**

i) **104**, LiHMDS, THF, -78 °C.

3.3.2 Unsaturation of the pyrrolidinone. The next synthetic challenge was the introduction of unsaturation into the pyrrolidinone ring, to give **106**, **Scheme 3.23**, to allow the required diol **107** to then be formed. The 3-benzyl pyrrolidinone **101** was chosen as a model compound to investigate the chemistry, with good structural similarity to the target intermediates.

Scheme 3.23

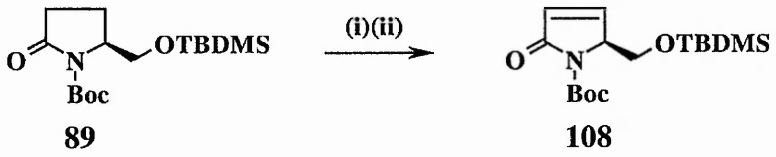


101 **106** **107**

A number of groups [83-86] have shown that protected pyrrolidinones such as **89** will react with phenylselenium chloride or bromide, giving a phenylselenyl intermediate. On oxidative work-up, this intermediate undergoes elimination, to form

the α,β-unsaturated amide **108**. This transformation is exemplified by the work of Barrett[30] and co-workers, **Scheme 3.24**, as part of the synthesis of pramanicin.

Scheme 3.24

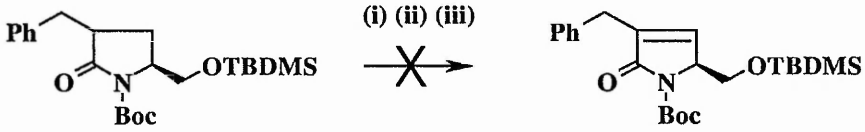


89 **108**

(i) LDA, PhSeBr, THF, -78 °C (ii) H_2O_2, pyridine.

This chemistry, however, had not been reported to have been carried out on compounds with a bulky 3-substituent, and it was subsequently found that the reaction with **101** was unsuccessful, **Scheme 3.25**.

Scheme 3.25

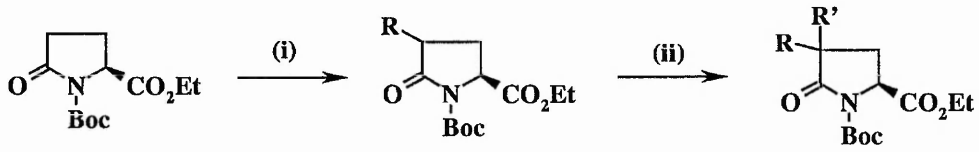


101

i) LDA, THF, -78 °C. (ii) PhSeCl. (iii) H_2O_2, 0 °C.

It was later found in the literature[87] that generation of an anion at the 3-carbon when already substituted requires more vigorous conditions, compared to those required for an unsubstituted analogue, **Scheme 3.26**. This, or steric hindrance caused by the bulky benzyl moiety at the 3-position, could have been responsible for the reaction's failure.

Scheme 3.26

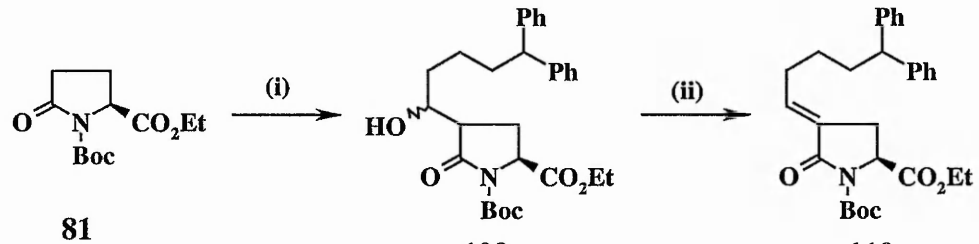


i) LiHMDS, RBr, THF, -78 °C. (ii) LiHMDS, R'Br, -78 to 20 °C.

(Where R and R'= Bn or allyl)

3.3.3 Reaction with aldehydes. It has recently been established by Ezquerra[68] *et al* that boron trifluoride etherate acts as a unique catalyst for the reaction between the carbanion of **81**, and an aldehyde or ketone, **Scheme 3.27**. The mixture of diastereomers formed **109** was then dehydrated to the enone **110**, using methanesulfonyl chloride.

Scheme 3.27



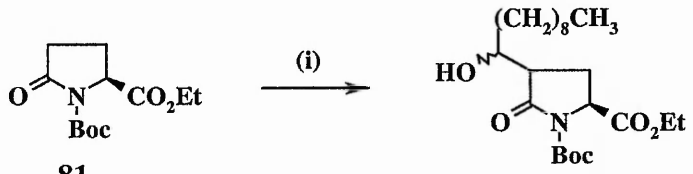
81 **109** **110**

i) $(Ph)_2CH(CH_2)_3CHO$, $BF_3.Et_2O$, LiHMDS, THF, -78 °C.

(ii) MsCl, Et_3N, DCM, 20 °C.

Previous attempts at this reaction had been made using 1-*N*-Boc-5-(*tert*-butyldimethylsilyloxymethyl)-pyrrolidin-2-one, but without the catalyst these had proved to be unsuccessful, as described earlier in Scheme 3.14. The original method of Ezquerra *et al* was modified by the use of decyl aldehyde, **Scheme 3.28**, which resulted in the successful synthesis of the useful intermediate compound **111**. Although not strictly a target molecule, a compound with a hydroxyl on the alkyl chain would still be worth testing, as an interesting analogue of the target compounds.

Scheme 3.28

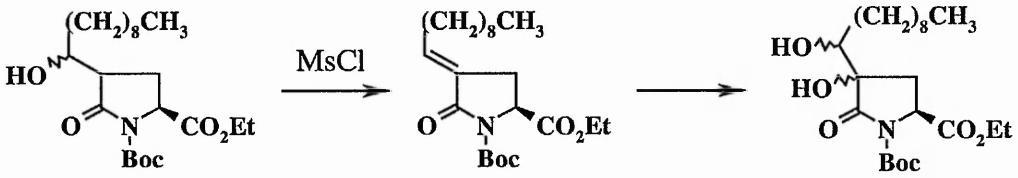


81 **111**

i) decanal, $BF_3.Et_2O$, LiHMDS, THF, -78 °C.

Dehydration of the alcohol **111** to the enone **112**, followed by dihydroxylation would result in compounds with a hydroxyl at the 3-position of the pyrrolidinone ring **113**, **Scheme 3.29**.

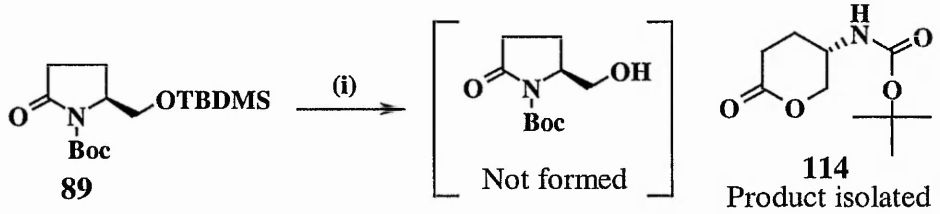
Scheme 3.29



111 **112** **113**

3.3.4 Deprotection problems. In an investigation of conditions for cleavage of the silyl ether of **89** with tetrabutylammonium fluoride[72] (TBAF), the product isolated had anomalous spectroscopic properties, more consistent with the structure **114**, **Scheme 3.30**. The deprotection was being investigated as a model for later work, which would involve more complicated, and possibly scarce, polyfunctional intermediates.

Scheme 3.30

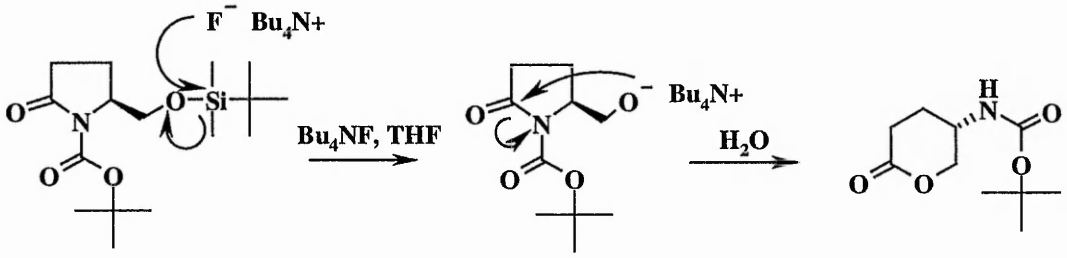


89 **114** Product isolated

i) TBAF, THF, 20 °C.

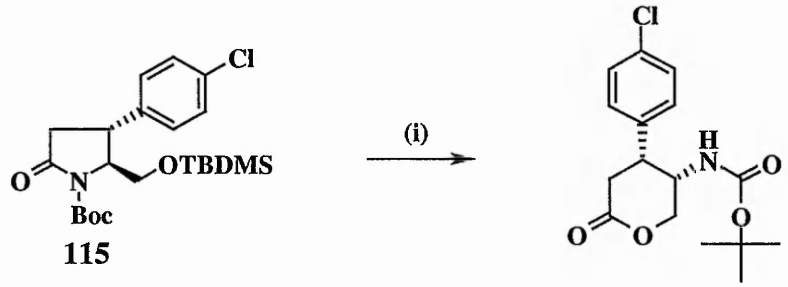
It appeared that the silyl ether had been cleaved, but the resulting alkoxide anion had carried out an intramolecular nucleophilic attack on the amide moiety, **Scheme 3.31**, with the lactone **114** being generated.

Scheme 3.31



A similar rearrangement has been observed by Herdeis[88] *et al*, during the deprotection of a 4-phenyl substituted pyrrolinone **115**, **Scheme 3.32**.

Scheme 3.32

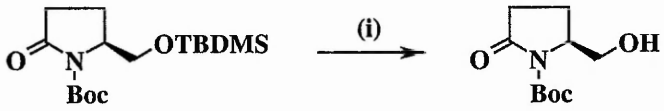


115

i) TBAF, THF, 20 °C.

An alternative method[89] for the deprotection of the intermediate **89** was investigated on a small scale, **Scheme 3.33**. The target compound **116** was obtained[90], albeit in low yield, and the structure was confirmed by NMR and HPLC/MS. It has also been reported that deprotection of the ether occurs with TBAF, when acetic acid was added to the reaction mixture[88], but this reaction was located some time after this synthetic approach had been abandoned.

Scheme 3.33



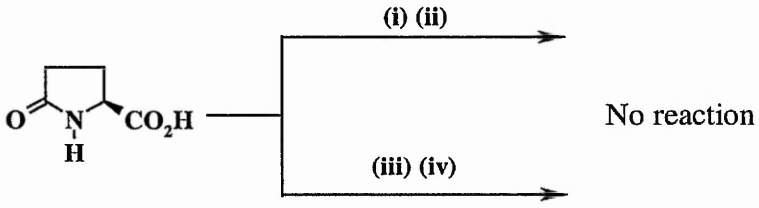
89 **116**

i) PTSA, MeOH, 20 °C.

The synthesis of the *N*-Boc alcohol **116** was attempted, as this compound would allow the mechanism of the observed rearrangement to be investigated. This may be important for later work, where the alkoxide anion may need to be generated to allow coupling to a sugar.

Several methods were studied for the synthesis of the *N*-Boc alcohol, and all were unsuccessful. Initial studies concentrated on the synthesis of *N*-Boc pyroglutamic acid[91] as this could then be reduced to the alcohol. The first obstacle was the formation of a salt of pyroglutamic acid soluble in DCM, as the free acid is virtually insoluble in this solvent. Attempts were made to form both the triethylamine and the tetramethylguanidine salts of pyroglutamic acid, and to react these with di-*tert*-butyl dicarbonate to give the *N*-Boc protected compound, **Scheme 3.34**. Both methods failed to give any product.

Scheme 3.34

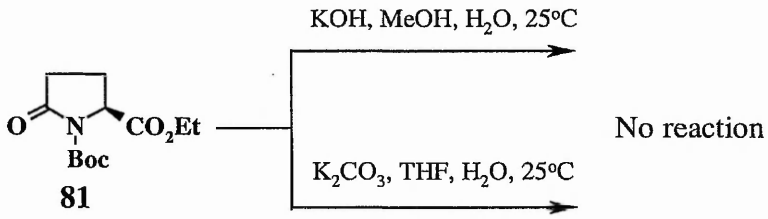


i) Et_3N, DCM, 20 °C. (ii) Boc_2O, DMAP, 20 °C.

(iii) tetramethylguanidine, DCM, 20 °C. (iv) Boc_2O, DMAP, 20 °C.

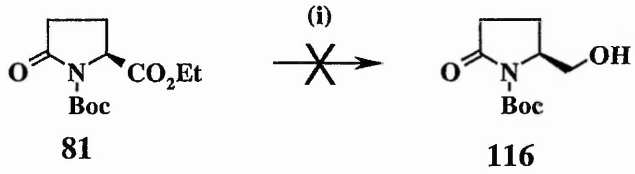
Hydrolysis of the ethyl ester **81** was then attempted, using aqueous potassium hydroxide in methanol, or aqueous potassium carbonate in THF, **Scheme 3.35**, both unsuccessfully.

Scheme 3.35



The reduction of the ester **81** to the alcohol **116** was also attempted, **Scheme 3.36**, using lithium borohydride, a more reactive hydride source than sodium borohydride. These conditions failed to give any product that could be isolated.

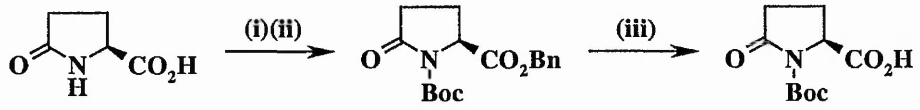
Scheme 3.36



i) $LiBH_4$, THF, 20 °C.

A synthesis of the Boc protected acid **118** has also been reported by August[92] *et al*, by first forming the benzyl ester of pyroglutamic acid **117**, **Scheme 3.37**. The amide is then Boc protected under standard conditions, and finally the benzyl ester cleaved by catalytic hydrogenolysis, to give *N*-Boc pyrrolidinone-5-carboxylic acid **118**.

Scheme 3.37



91 **117** **118**

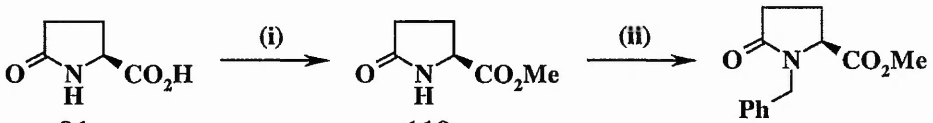
i) BnCl, Et_3N, 20 °C. (ii) Boc_2O, DMAP, MeCN, 0 °C.

(iii) H_2, 10 % Pd/C, EtOAc, 20 °C.

3.3.5 Alternative protecting groups. Revision of the *N*-protecting group was required before further chemistry could be investigated, as the rearrangement observed when removing the TBDMS moiety was unacceptable for use in further studies.

Initially, two alternative *N*-protecting groups were studied, to attempt to confirm the requirement for a urethane moiety on the amide. The *N*-benzyl methyl ester **119** previously reported by Flynn[93] and co-workers, **Scheme 3.38**, was synthesised.

Scheme 3.38

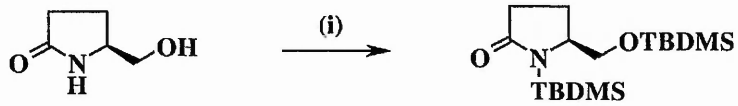


91 **119** **120**

i) oxalyl chloride, MeOH, 20 °C. (ii) BnBr, NaH, DMF, 20 °C.

The *N,O*-disilyl derivative **121**, was also synthesised, **Scheme 3.39**.

Scheme 3.39



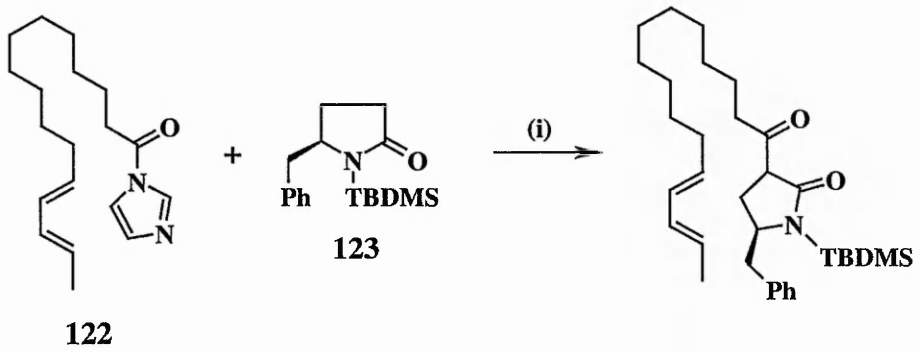
92 **121**

i) TBDMS-Cl, Et_3N, DCM, 20 °C.

Similar compounds have been reported to form anions at the 3-carbon, to give 3-substituted compounds. Harkin[94] *et al* describes the reaction of the *N*-silyl

pyrrolidinone **123** with the acyl imidazole **122**, **Scheme 3.40**, to give the 3-acyl derivative.

Scheme 3.40

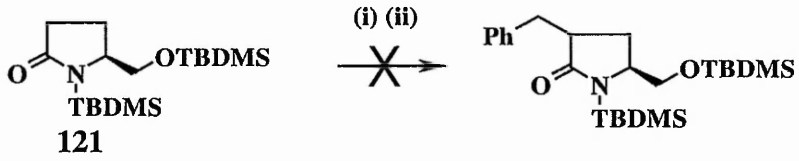


122

i) LDA, THF, -78 °C.

The *N*-silyl compound **121** was found to be unreactive towards benzyl bromide, under the same conditions that had successfully alkylated the corresponding *N*-Boc compound, **Scheme 3.41**. Substitution of LiHMDS with the stronger base lithium tetramethylpiperidide failed to give any improvement.

Scheme 3.41



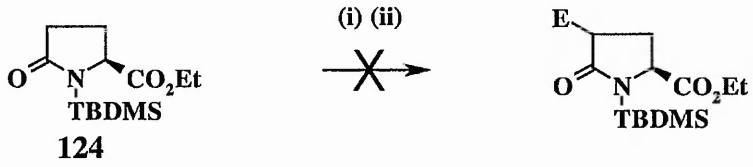
121

i) ^{n}BuLi, tetramethylpiperidine, THF, -78 °C, or LiHMDS, THF, -78 °C.

(ii) BnBr, THF, -78 to 20 °C.

The failure of this reaction is in agreement with the work of Baldwin[80] *et al*, where ethyl *N*-TBDMS-pyrrolidin-2-one-5-carboxylate **124** failed to generate an anion which would react with an electrophile, **Scheme 3.42**.

Scheme 3.42

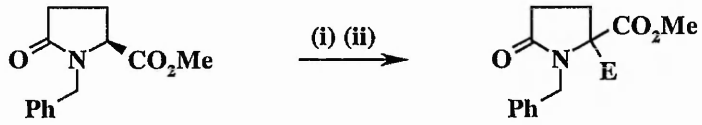


124

i) LDA or LiHMDS, THF. (ii) Electrophile (E).

The reaction of the anion of the *N*-benzyl compound **120** has been reported by Braña[70] and co-workers, but the compound gives the 5-substituted compound **125** instead of the 3-isomer, **Scheme 3.43**.

Scheme 3.43



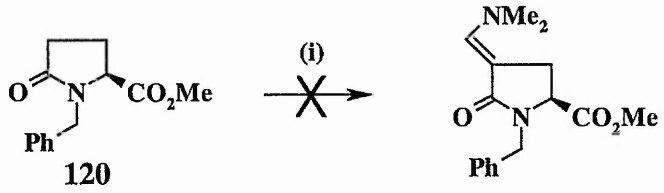
120 **125**

i) LiHMDS, THF, -78 °C. (ii) E^+, -78 °C.

This is an interesting change in reactivity, which demonstrates the importance of the nature of the *N*-protecting group on the anion chemistry of the pyrrolidinone. This subtle effect on the reactivity continues to be a feature of the following chapters, where what would at first appear to be a minor change to the protected heterocycle elicits a noticeable change in the reactivity of the anion.

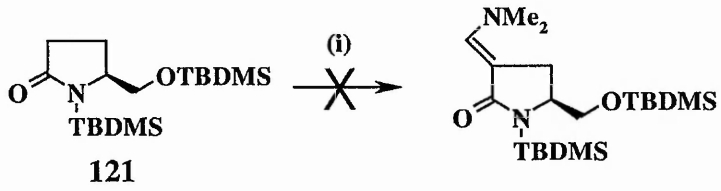
Not only did the *N*-benzyl and *N*-silyl compounds fail to generate an active anion, they also failed to react with Bredereck's reagent (see chapter 5), using standard conditions that had been successful with *N*-Boc compounds **Scheme 3.44** and **Scheme 3.45**. This reaction is dependent on the presence of an acidic methylene group in the substrate, which within these compounds has been shown to be absent, by the failure to generate a 3-enolate anion.

Scheme 3.44



i) Bredereck's reagent, DME, reflux.

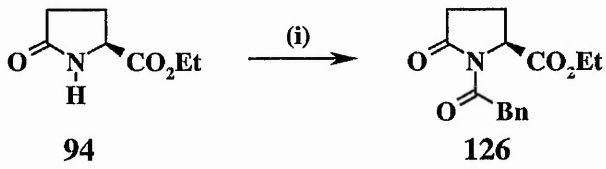
Scheme 3.45



i) Bredereck's reagent, DME, reflux.

The *N*-phenylacetyl derivative **126** was synthesised, **Scheme 3.46**, although in very poor yield. This compound was synthesised both to further investigate the effect of the amide protecting group on the reactivity of the heterocycle, and its potential to be removed enzymatically[95]. The very low yield of the compound prevented any studies being carried, due to a lack of material.

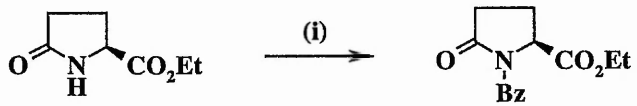
Scheme 3.46



i) BnCOCl, LiHMDS, THF, hexane, -100 °C.

Synthesis of *N*-benzoyl ethyl pyroglutamate **127** was attempted, **Scheme 3.47**, but the reaction was unsuccessful. The product obtained was extremely impure, even after a short reaction time. Use of an alternative base was also investigated, but this was found to give the same result.

Scheme 3.47



94 **127**

i) BzCl, LiHMDS, THF, -78 °C, or BzCl, NaH, DMF, 0 °C.

The difficulty in synthesising these two compounds led to a search for an alternative protecting group strategy. The earlier results with the *N*-silyl and *N*-benzyl compounds suggested that the amide protection plays an essential role in the ability to generate anions at the 3-carbon of the pyrrolidinone.

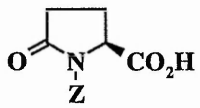
The literature also suggests that the amide protection is important, as the examples found use either Boc, Z, or an *N,O*-acetal, which is also tolerated for anion generation. The following chapters describe further synthetic work based on these findings.

4. *N*-Benzyloxycarbonyl pyroglutamic acid derivatives

4. *N*-Benzyloxycarbonyl pyroglutamic acid derivatives

4.1 Introduction

After the problems encountered with the *N*-Boc protecting group, the benzyloxycarbonyl (Z or Cbz) group was chosen as a possible alternative. The *N*-Z-pyroglutamic acid **128** is commercially available in both enantiomers.

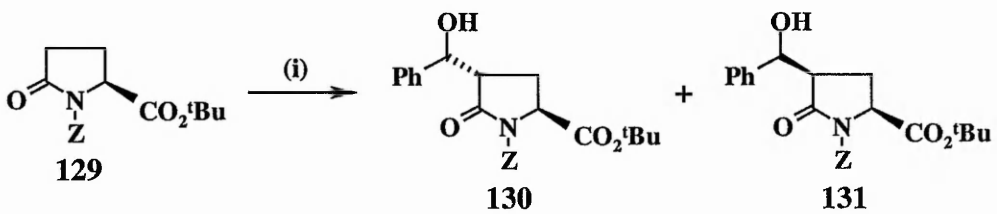


128

As it had been established that a urethane protecting group was necessary for the reactivity of the 3-position of the ring, the Z group provided a suitable alternative to Boc. The Z moiety is more stable than the Boc group to acidic and basic conditions, but can be removed by hydrogenation.

The use of Z-protected pyroglutamate derivatives is less common than for Boc compounds. An example of anion chemistry using *N*-Z-pyrrolidinones is the work of Dikshit and Panday[96]. The anion of *tert*-butyl *N*-Z-pyrrolidinone-5-carboxylate **129** was generated, and then reacted with a selection of aldehydes, mainly benzylic, to give a mixture of diastereomers, **Scheme 4.1**. In the example shown, a 3 : 1 mixture of **130** and **131** was isolated.

Scheme 4.1



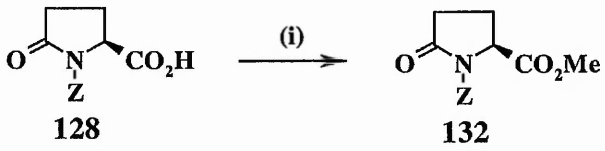
i) LiHMDS, THF, -78 °C. (ii) PhCHO, -78 °C.

This finding is in contrast to the other urethane protected pyrrolidinones (Boc and ethyl carbamate), where the reaction of the pyrrolidinone anion with an aldehyde would occur only with a catalyst.

4.2 Synthesis of the protected nucleus

It was initially attempted to form the methyl ester **132**, and then reduce this to the alcohol. However, formation of the ester under standard conditions[97], **Scheme 4.2**, gave only a poor yield after chromatography.

Scheme 4.2

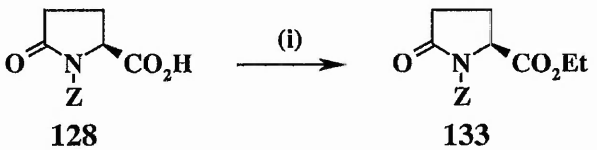


128 **132**

i) oxalyl chloride, MeOH, 20 °C.

The alternative ethyl ester was then synthesised using bromoethane, under the same conditions used for the esterification of pyroglutamic acid, **Scheme 4.3**. This resulted in the desired product **133**, in good yield.

Scheme 4.3



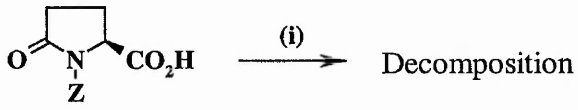
128 **133**

i) EtBr, Et_3N, DMF, 20 °C.

Direct reduction of the acid **128** to the alcohol was attempted using borane-THF[98], **Scheme 4.4**, but this was found to decompose the starting material. Borane would usually be the reagent of choice for the selective reduction of an acid to the alcohol, but in this example it is unsuccessful. The failure is possibly due to the strong

co-ordinating ability of the heterocycle, particularly with the urethane protected amide and its associated electron density.

Scheme 4.4

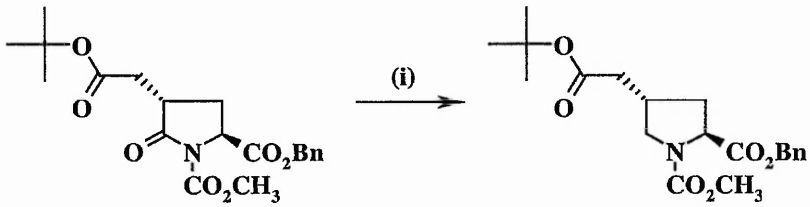


128

i) BH_3.THF, THF, 0 °C.

In retrospect, this reaction was unlikely to give the desired alcohol, as it is highly likely that the amide would be reduced as well. The reduction of a similar substrate **134**, using borane-dimethylsulfide, **Scheme 4.5**, has been reported by Langlois and Rojas[99], as part of a synthesis of (2*S*, 4*S*) 2-carboxy-4-pyrrolidineacetic acid.

Scheme 4.5

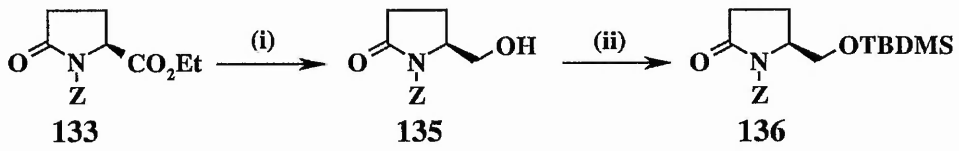


134

i) $BH_3.Me_2S$, THF.

The ethyl ester **133** was successfully reduced by sodium borohydride to the alcohol **135**, in good yield, the same conditions found to be successful with ethyl pyroglutamate. Protection of the alcohol **135** as the TBDMS ether **136** was then achieved, **Scheme 4.6**, using TBDMS chloride as the silylating agent, under standard conditions.

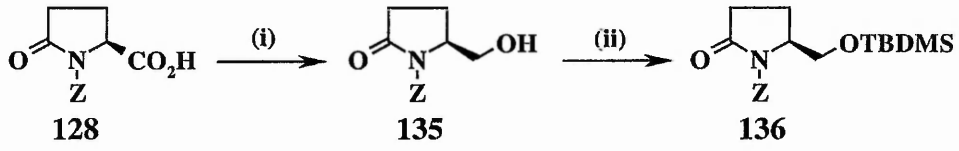
Scheme 4.6



i) $NaBH_4$, MeOH, THF, 0 °C. (ii) TBDMS-Cl, imidazole, DMF, 20 °C.

An alternative route to the same compound has been reported by Altmann[100], **Scheme 4.7**, where the chloroformate ester is formed and then reduced to the alcohol **135**, using sodium boroydride. The silyl ether formation is then carried out under the same conditions as previously described in Scheme 4.6.

Scheme 4.7

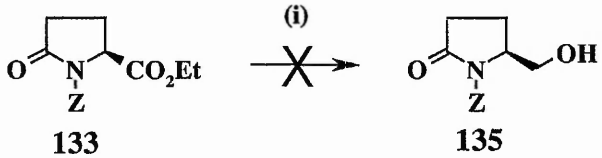


i) (a) EtOC(O)Cl, NMM, THF, 5 °C. (b) $NaBH_4$.

(ii) TBDMS-Cl, imidazole, DMF, 20 °C.

Synthesis of the alcohol **135** from the ester **133**, using DIBAL-H as the reducing agent, was studied as an alternative to the use of sodium borohydride, **Scheme 4.8**. However, surprisingly, DIBAL-H was found to be completely unreactive towards the molecule, with unchanged starting material being recovered.

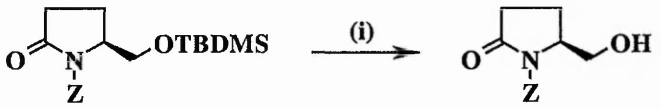
Scheme 4.8



i) DIBAL-H, THF, 0 to 20 °C.

The selective removal of the silyl ether was then investigated, **Scheme 4.9**, using TBAF as the fluoride source. This was found to successfully cleave the ether **136** to the alcohol **135**, without the rearrangement that was observed previously with the Boc protecting group.

Scheme 4.9



136 **135**

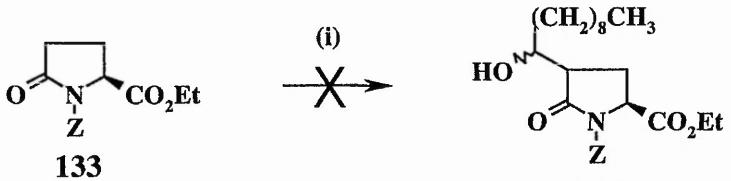
i) TBAF, THF, 20 °C.

The successful deprotection was confirmed by reapplying the TBDMS group to the alcohol, giving material identical to the initial silyl ether **136**.

4.3 Anion chemistry of *N*-Z pyroglutamic acid derivatives

The reaction between the *N*-Z ethyl ester **133** and decyl aldehyde was then investigated, to generate an analogue **137** of the previous *N*-Boc compound **111**. Identical conditions were used, **Scheme 4.10**, but the material isolated from the reaction was a complex mixture.

Scheme 4.10



133 **137**

i) decanal, $BF_3.Et_2O$, LiHMDS, THF, -78 °C

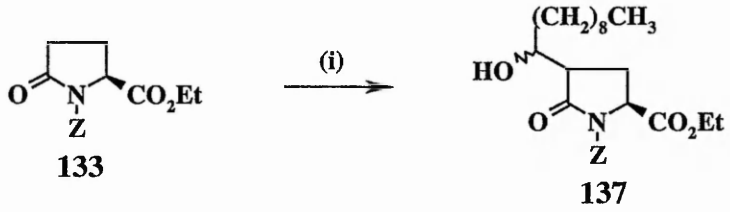
From the result of this reaction, it can be observed that the effect exerted by the urethane on the reactivity of the pyrrolidinone ring is very subtle, as even changing the

urethane from *tert*-butyl to benzyl has a dramatic effect. It may also be expected that the Z-protected compound would give a cleaner product in this reaction, as the urethane should be more stable than the Boc protected example investigated earlier.

After several further attempts at the reaction, the same complex mixture of products was still being generated. The reaction temperature had been more carefully controlled during reagent addition, more dilute reagents had been utilised, and the reaction carefully monitored by TLC. None of these changes to the method succeeded in improving the reaction.

A dramatic improvement was, however, obtained when the reaction was carried out at -100 °C, **Scheme 4.11**, instead of -78 °C, as previously. The internal temperature was carefully controlled, maintaining the internal temperature below -90 °C during addition of the reagents. It was found that after one hour, quenching the reaction resulted in approximately 50% yield of the desired alcohol **137**.

Scheme 4.11



133 **137**

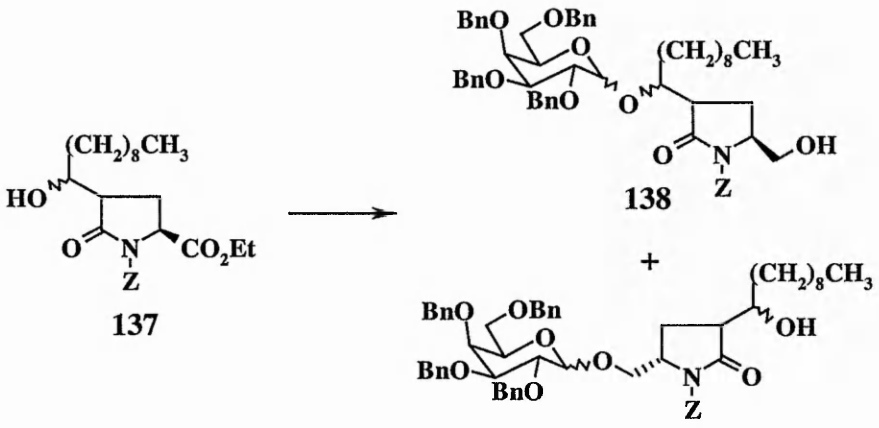
i) decanal, $BF_3.Et_2O$, LiHMDS, THF, -100 °C.

The alcohol was isolated as a single product by TLC, unlike the previous synthesis of the *N*-Boc analogue, where the product was a separable mixture of two diastereomers. However, the ^1H NMR of the pure product shows the possible presence of a minor diastereomer, which is not visible by TLC.

4.4 Protection of the secondary alcohol

The protection of the secondary alcohol of **137** was then investigated. This protection may have been necessary, as the presence of an unprotected secondary alcohol could be undesirable for the sugar coupling reaction in a later synthetic step, **Scheme 4.12**, where it may form an unwanted by-product **138**.

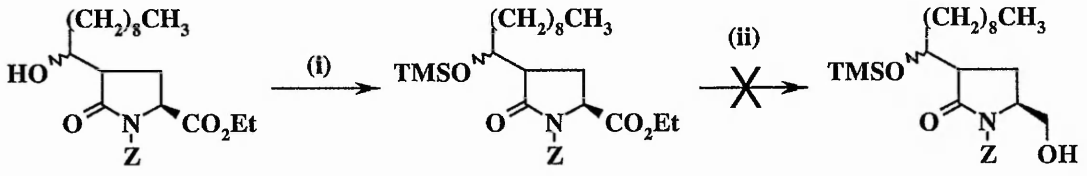
Scheme 4.12



Protection of the alcohol **137** was first attempted using trimethylsilyl chloride (TMS-Cl). The TMS protecting group was chosen due to the hindered nature of the alcohol being protected, where the TBDMS group could be too bulky to react.

The TMS ether **139** was successfully formed, but the attempted reduction of the ester to the alcohol **140** using sodium borohydride, failed, **Scheme 4.13**. This was probably due to the lability of the TMS group in the basic conditions encountered in the reduction step.

Scheme 4.13

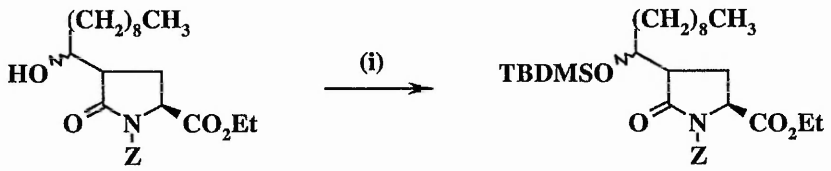


137 **139** **140**

i) TMS-Cl, Et_3N, DCM, 0 °C. (ii) $NaBH_4$, MeOH, THF, 20 °C.

The TBDMS ether was then investigated as protection for the secondary alcohol, which should be more stable than the TMS ether during the reduction of the ester. TBDMS chloride failed to react with the secondary alcohol, but when the more reactive TBDMS triflate[101] was used the reaction was successful, **Scheme 4.14**, with the desired silyl ether **141** being recovered in good yield.

Scheme 4.14



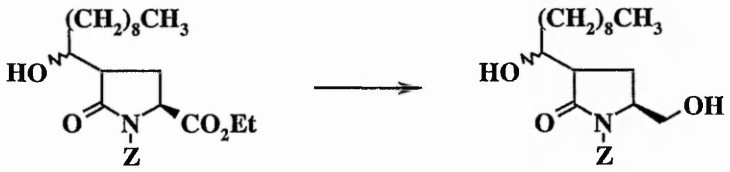
137 **141**

i) TBDMS-OTf, 2,6-lutidine, DCM, -20 °C.

4.5 Attempted reduction of the ester moiety

The reduction of the ester moiety of **137** to the alcohol **142** was attempted, **Scheme 4.15**, using a variety of reaction conditions. A literature search revealed several possible alternatives to the standard reduction method[102], but all use either sodium or lithium borohydride as the reducing agent.

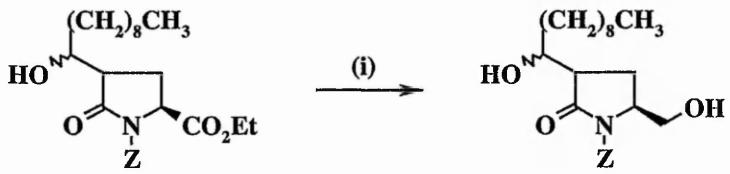
Scheme 4.15



Apart from one, all the literature examples found describe the reduction of simple pyroglutamate esters[78,103-105], without any substitution on the ring. From the single example of Pickering[106] *et al* it appears that reduction of the ester with a functionalised ring is more difficult than would be expected. This agrees with the results of the attempted reduction of **128**, and of previous attempts with similar compounds.

A summary of the conditions investigated for the reduction of **137** is shown in the following table.

Table 4.1

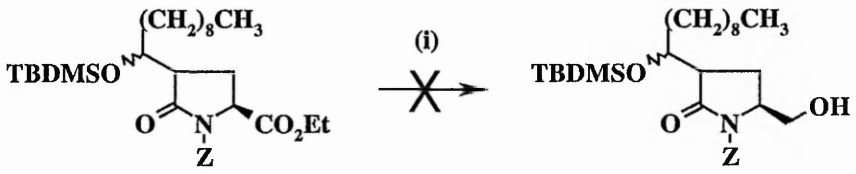


i) reducing agent, solvent, 20 °C. (See **Table 4.1**)

Reducing Agent	Solvent	Result
$NaBH_4$	EtOH	Possible product, NMR inconclusive
$NaBH_4$	THF / MeOH	Possible product?
$LiBH_4$	THF	Mixture obtained, structure uncertain
$LiBH_4$	THF / MeOH	Starting material recovered

Attempts at reduction were made using the silyl ether derivative **141**, but these reactions failed to give any product that could be identified, **Scheme 4.16**. This could be due to partial removal of the silyl ether, resulting in a complex mixture.

Scheme 4.16

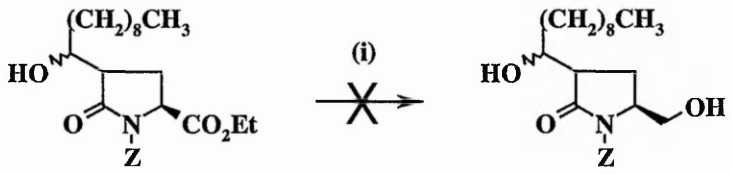


141

i) $NaBH_4$, MeOH, THF, 20 °C.

Reduction of the ester **137** with sodium borohydride was then investigated, and it was found that a trace of the alcohol **142** could be isolated after several attempts, but in poor yield and purity, **Scheme 4.17**. This suggests the problem with the reaction could have been due to hindrance from the bulky TBDMS ether. However, the NMR spectrum of the product obtained was difficult to interpret due to impurities, and the reduction may not have actually occurred, with the possibility of the material isolated being impure starting material.

Scheme 4.17



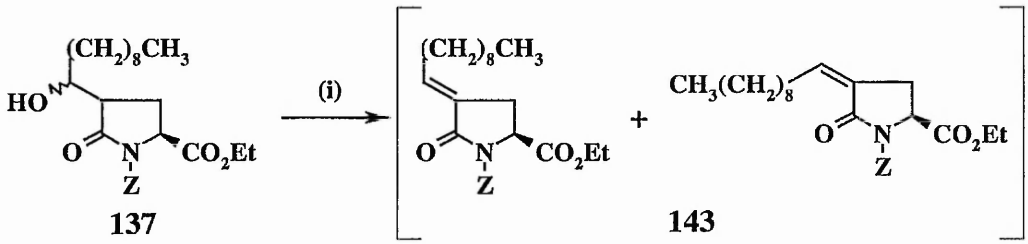
137 **142**

i) $NaBH_4$, MeOH, THF, 20 °C.

4.6 Dehydration reactions

It had been reported by Ezquerra[68] *et al* that lower homologues of alcohol **137** readily dehydrate to the enone, when treated with methanesulfonyl chloride. This was found to occur with **137**, **Scheme 4.18**, a mixture of two enones **143** being isolated in low yield (24%) after flash column chromatography. This appeared to be an impure mixture of two geometric isomers (observable by NMR).

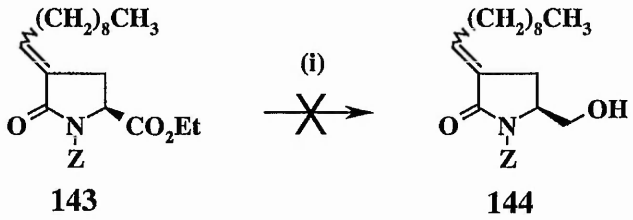
Scheme 4.18



i) MsCl, Et_3N, DCM, 20 °C.

The reduction of the ester moiety of alkene **143** was attempted, using sodium borohydride **Scheme 4.19**, but unfortunately this failed to give the alcohol **144**.

Scheme 4.19



i) $NaBH_4$, MeOH, THF, 20 °C.

Overall, the Z protected intermediates initially provided a useful alternative to the Boc compounds. However, although advanced intermediates could be synthesised, the reduction of the ester moiety of these compounds proved excessively difficult for a synthetically useful step.

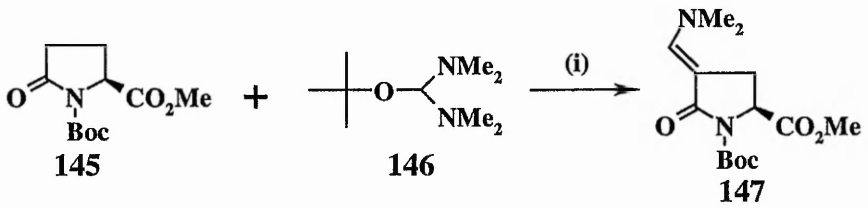
5. Pyroglutamic acid enaminone derivatives

5. Pyroglutamic acid enaminone derivatives.

5.1 Introduction

5.1.1 Bredereck's reagent. The use of Bredereck's reagent[107] (*tert*-butoxybis(dimethylamino)methane) **146** for the functionalisation of pyrrolidinones has been described several times in the literature[108-111]. Bredereck's reagent is an aminomethylenating (formylating) reagent, which will react with acidic methylene and amino groups, and will formylate pyrrolidinones at the 3-position, **Scheme 5.1**.

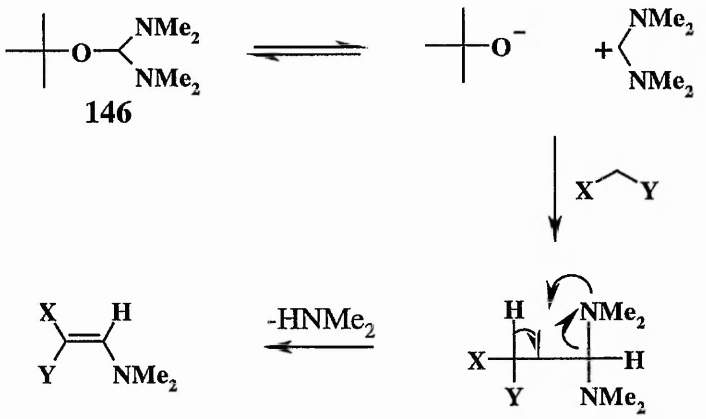
Scheme 5.1



i) DME, reflux.

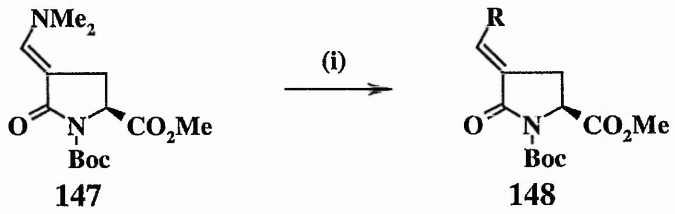
In the first step of the reaction with an acidic CH_2, the *tert*-butoxybis(dimethylamino) methane dissociates, to give a basic alkoxide ion, **Scheme 5.2**. This alkoxide anion forms an equilibrium with the acidic methylene moiety, and the resulting carbanion reacts with the formamidinium anion, with a final elimination of dimethylamine to reach the product.

Scheme 5.2



5.1.2 Reaction of enaminones. The enaminone **147** has been shown by Moody and Young[112] to be attacked by simple Grignard reagents, **Scheme 5.3**, giving 1,4-addition, resulting in an alkyl substituted alkene **148** at the 3-carbon of the pyrrolidinone.

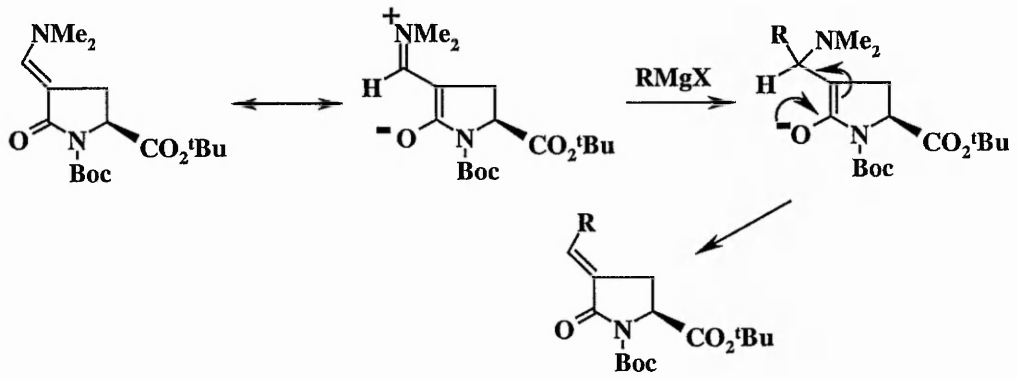
Scheme 5.3



i) RMgCl, THF, -78 °C. (Where R = Ph or Me)

It is suggested by Moody and Young that the reaction with Grignard reagents occurs with the imine form of the enaminone, **Scheme 5.4**, with the subsequent elimination of dimethylamine.

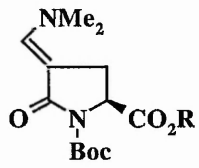
Scheme 5.4



5.2 Synthesis of enaminones

The enaminones were synthesised with methyl[110] **147**, ethyl **149**, *tert*-butyl[109] **150** and benzyl **151** esters as protection for the carboxylic acid, **Scheme 5.5**. The bulky *tert*-butyl and benzyl esters were chosen, as these should be stable to Grignard reagents used in the next step. The original work[108] utilised the *tert*-butyl ester, but benzyl esters provide an alternative that should be simple to remove by catalytic hydrogenation. Methyl[110] and ethyl esters would be easily reduced to the corresponding alcohol, and these provide another option.

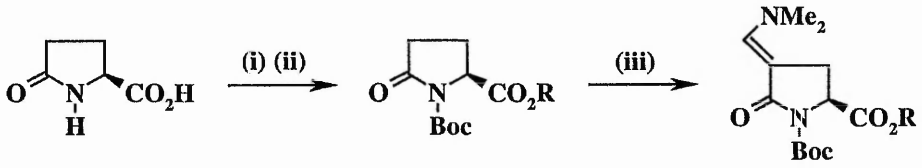
Scheme 5.5



Compound	R
147	Me
149	Et
150	tBu
151	Bn

The N-Boc protected ethyl **149** and benzyl esters **151** were both synthesised *via* the same method, **Scheme 5.6**, and then treated with Bredereck's reagent, to obtain the enaminones.

Scheme 5.6



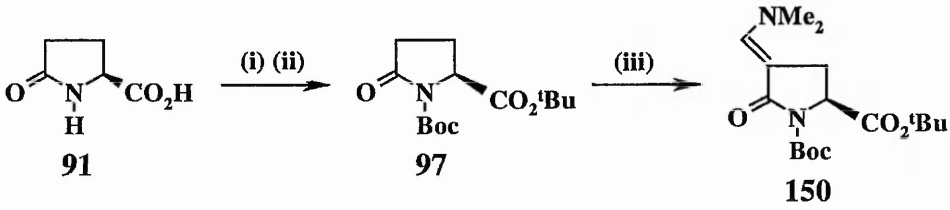
149 R = Et
151 R = Bn

i) BnBr or EtBr, Et_3N, DMF, 20 °C. (ii) Boc_2O, DMAP, DCM, 20 °C.

(iii) Bredereck's reagent, DME, reflux.

The *tert*-butyl ester protected compound **150** was synthesised using the method described by August[92] *et al*, **Scheme 5.7**. The *tert*-butyl ester of pyroglutamic acid **97** was formed using perchloric acid and *tert*-butyl acetate[113], followed by Boc protection of the amide moiety, using standard conditions. Finally, reaction of the di-protected intermediate with Bredereck's reagent gave the *tert*-butyl ester protected enaminone **150**.

Scheme 5.7



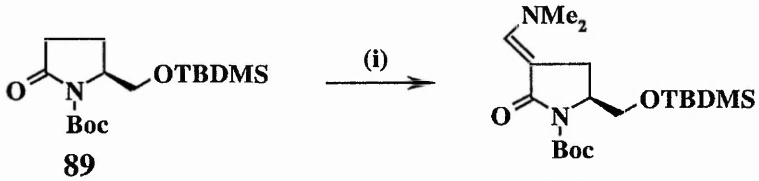
91 **97** **150**

i) $HClO_4$, $AcOBu^t$, 20 °C. (ii) Boc_2O, DMAP, DCM, 20 °C.

(iii) Bredereck's reagent, DME, reflux.

The new *N*-Boc protected silyl ether **152** was also prepared, using the same method as for the esters, **Scheme 5.8**. This compound has the advantage of a protected 5-hydroxymethyl function, which would give direct access to the alcohol on deprotection. Additionally, the silyl ether moiety improves the solubility of the compound in ethereal solvents, which is an advantage for the next synthetic step.

Scheme 5.8

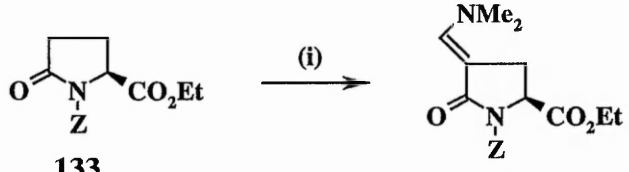


89 **152**

i) Bredereck's reagent, DME, reflux.

The enaminone route was modified at this stage, due to the complications with the *N*-Boc protection of the pyrrolidinone. It was attempted to synthesise the Z-protected analogues of previous compounds, and to investigate these further. The ethyl ester **133** was successfully converted to the novel enaminone **153** in good yield, **Scheme 5.9**, by use of Bredereck's reagent. The benzyl ester analogue has previously been prepared by Danishefsky[114] and co-workers.

Scheme 5.9

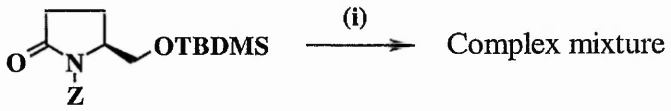


133 **153**

i) Bredereck's reagent, DME, reflux.

The reaction between 1-*N*-Z-5-(*tert*-butyldimethylsilyloxymethyl)-pyrroldin-2-one **136** and Bredereck's reagent was then investigated, **Scheme 5.10**, unfortunately without success. The reaction gave a complex mixture, mainly the result of decomposition, and a product could not be isolated.

Scheme 5.10



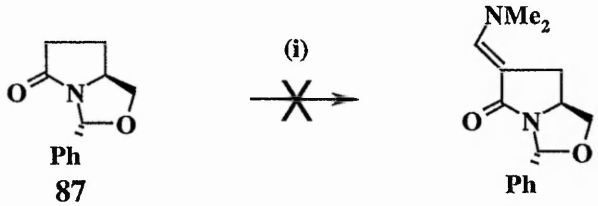
136

i) Bredereck's reagent, DME, reflux.

This was an unfortunate result, as it was hoped that the enaminone derivative of *N*-Z-protected silyl ether **133** would possess increased solubility in ethereal solvents, as the ethyl ester analogue **153** was virtually insoluble in ethers. This was a serious problem for the next synthetic step (as in Scheme 5.12), where reaction of the enaminone with a Grignard reagent requires ethereal solvents. The insoluble nature of the Z-protected compound precluded further use.

The final investigation involving Bredereck's reagent was the reaction to form the enaminone derivative of the bicyclic lactam **87** (described in the next chapter), a reaction having no literature precedent, **Scheme 5.11**. The result was the decomposition of the starting material, without the enaminone **154** being detected.

Scheme 5.11



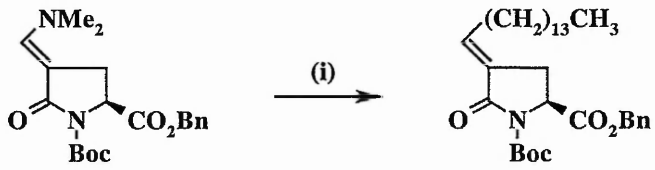
87 **154**

i) Bredereck's reagent, DME, reflux.

5.3 Reaction of enaminones with Grignards

The ability to introduce a long alkyl chain by means of a Grignard reagent was a significant advantage of the enaminones, compared to the problems encountered with the anion chemistry of previous chapters. The reaction of the enaminones with Grignard reagents, already described in the literature[109,112], was extended to utilise a long alkyl chain Grignard (commercially available tetradecylmagnesium chloride), **Scheme 5.12**. This successfully introduced the 14 carbon chain into the substrate, in a stereoselective reaction (only the E-isomer was isolated, which was observable by NMR), giving the novel enone **155**.

Scheme 5.12

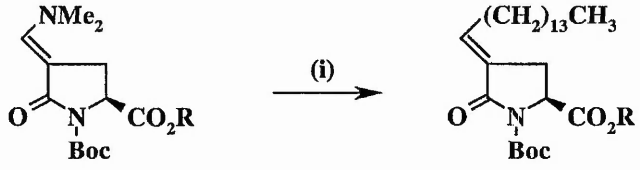


151 **155**

i) $CH_3(CH_2)_{13}MgCl$, Et_2O, 0 °C.

This reaction was also successful for the ethyl ester **149**, and the *tert*-butyl ester **150**, **Scheme 5.13**, to give the corresponding enones **156** and **157** respectively.

Scheme 5.13



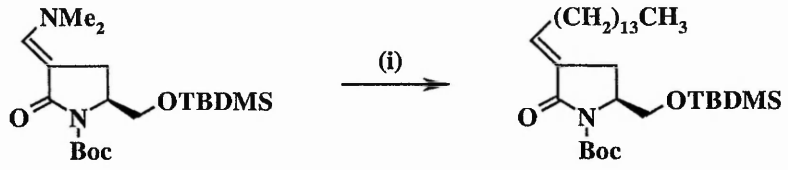
149 R = Et
150 R = ^{t}Bu

156 R = Et
157 R = ^{t}Bu

i) $CH_3(CH_2)_{13}MgCl$, Et_2O, 0 °C.

The silyl ether protected compound **152** was also found to successfully react with tetradecylmagnesium chloride, to give the corresponding enone **158**, **Scheme 5.14**.

Scheme 5.14



152 **158**

i) $CH_3(CH_2)_{13}MgCl$, Et_2O, 0 °C.

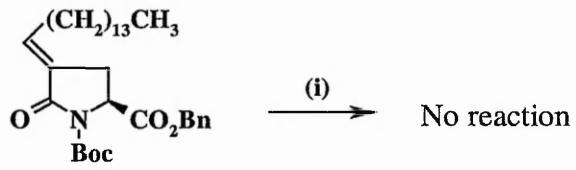
5.4 Reactions of the enones

The chemistry of the enone was then investigated, as these were useful intermediates, which have the potential to form diols when subjected to suitable conditions. Although this would give pyrrolidinones with only one hydroxyl moiety on the ring at the 3-position, these could lead to useful analogues for biological evaluation.

Asymmetric dihydroxylation of the enone of **155** was attempted, using the AD-mix-α reagent described by Sharpless[115] *et al.* The reagent comprises potassium osmate and a chiral phosphine ligand. This is reported to convert alkenes to *cis*-diols, in excellent yield and very high (>95%) enantiomeric excess. The chiral phosphine ligand complexes with the catalytic quantity of osmium tetroxide which is generated, leading to stereo control over the final product of the dihydroxylation.

However, treatment of the enone **155** with AD-mix-α following the literature procedure, **Scheme 5.15**, failed to give any reaction, even after a long reaction time.

Scheme 5.15



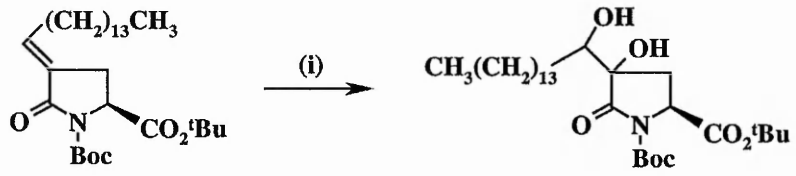
155

i) AD-mix-α, $MeSO_2NH_2$, H_2O, ^tBuOH, 0 °C.

It is possible that the reaction is failing due to steric crowding of the enaminone moiety, as this could prevent the large osmium tetroxide / ligand complex from approaching close enough to the C=C bond to react. Alternatively, the unusual character of the double bond in the molecule could be responsible, as it is an enone, not a simple alkene. The reported use of AD-mix-α has been for the conversion of fairly simple alkene systems, where excellent results had been obtained. The molecule being studied here is a more complicated system, with major steric and electronic differences.

An attempt was then made to react the enaminone **155** with a catalytic quantity of osmium tetroxide, using standard dihydroxylation conditions[116]. In contrast to using AD-mix-α, this was found to be successful, with a mixture of two diastereomers being produced, **Scheme 5.16**. These were readily separable by flash column chromatography, to give the two diastereomers **159** in good yield, and approximately 1:1 ratio. It appears that the enhanced reactivity of the osmium tetroxide is necessary to effect the transformation.

Scheme 5.16

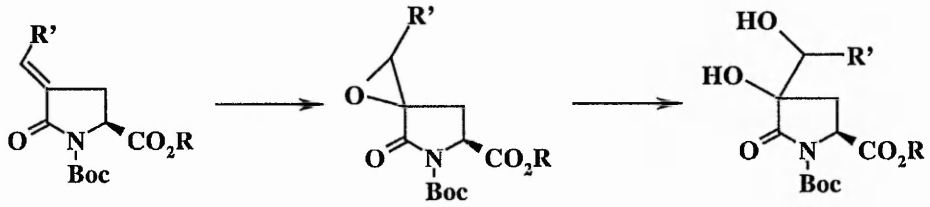


156 **159**

i) OsO_4, NMO, acetone, water, 0 °C.

An alternative method of introducing a diol into the molecule was attempted, by first forming an epoxide **160**, which could then be opened by hydrolysis to give a diol **161**, **Scheme 5.17**. Sharpless asymmetric epoxidation was also envisaged as a means of giving chiral control to the reaction.

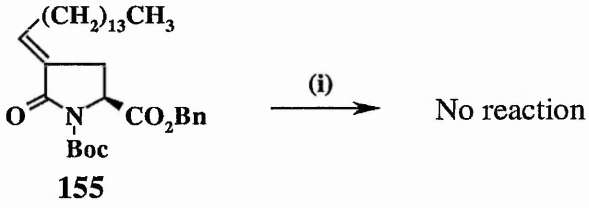
Scheme 5.17



(Where R and R' = Alkyl)

Two methods were investigated to attempt the synthesis of the epoxide, **Scheme 5.18**, and both were unsuccessful. *meta*-Chloroperoxybenzoic (MCPBA) acid in neutral conditions was reported by Fringuelli[117] and co-workers to give a *trans*-diol from a selection of simple alkenes such as cyclohexene, but failed here. Treatment of the enaminone **155** with alkaline hydrogen peroxide[118,119] was also unsuccessful.

Scheme 5.18



i) MCPBA, DCM, 20 °C, or H_2O_2, NaOH, H_2O, 20 °C.

A similar failure to form an epoxide from an enone has been reported[75]. It appears that the C=C bond present in the enaminone does not behave in a predictable way, hence reagents that react readily with α,β-unsaturated systems and unconjugated C=C bonds do not necessarily react with the enone system. This serves as another example of the difficulty of predicting chemistry of substituted pyrrolidinone derivatives.

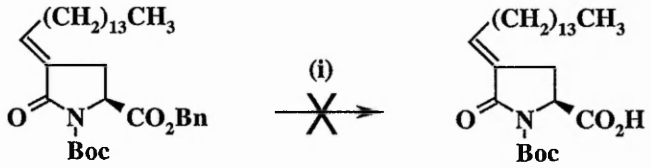
The reactivity of the exocyclic double bond towards epoxidation is also different from that which may be expected. A study of the epoxidation of the alkene derived

from the *N,O*-acetal protected pyrrolidinone[120] showed that reaction with lithium *tert*-butylhydroperoxide was necessary to give the epoxide. Alternative reagents which had failed to yield the epoxide were alkaline hydrogen peroxide, sodium perborate and dimethyl dioxirane.

5.5 Removal of the ester

It was then attempted to remove the benzyl ester of **155**, to enable reduction of the acid to the alcohol to occur. Catalytic transfer hydrogenation was attempted, using a palladium catalyst with cyclohexadiene as the hydrogen donor, **Scheme 5.19**. This proved to be unsuccessful, even though the method had been reported as being useful for the removal of benzyl protecting groups[121].

Scheme 5.19

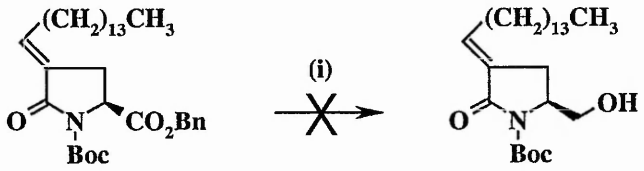


155

i) Pd/C, 1,4-cyclohexadiene, EtOH, 20 °C.

The benzyl ester **155** was also treated with sodium borohydride, **Scheme 5.20**, to attempt to produce the alcohol **162** directly, and this was again unsuccessful after several attempts. The difficulty of reducing the ester appears to be a common problem with substituted pyrrolidinones, as has been found with compounds discussed in the preceding chapters. The susceptibility of the ester to reduction would seem to vary, as does the overall stability of the molecule to the reducing conditions.

Scheme 5.20



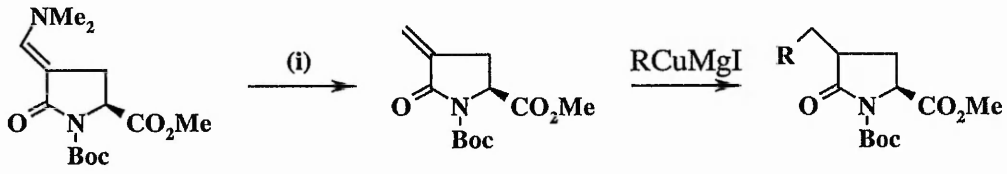
155 **162**

i) $NaBH_4$, MeOH, THF, 20 °C.

5.6 Use of organocuprates

An alternative synthetic route from the enaminones was also possible. Moody and Young[109] had described the DIBAL-H reduction of the enaminone **147** to the enone **163**, **Scheme 5.21**, and this transformation was found to work in good yield. It was envisaged that this could then be used as a substrate for 1,4-addition by an alkyl cuprate (Gilman reagent, RCuMgI).

Scheme 5.21

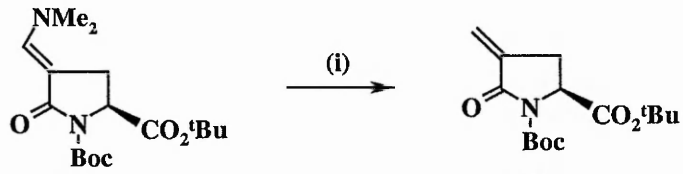


147 **163**

i) DIBAL-H, toluene, -78 °C. (Where R = alkyl)

The reduction of the enaminone moiety of **150** was successfully achieved, to give the new enone **164** in good yield, **Scheme 5.22**.

Scheme 5.22

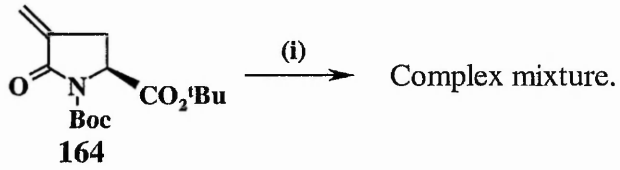


150 **164**

i) DIBAL-H, toluene, -78 °C.

The generation of an alkyl Gilman reagent was attempted, by reaction of decyl bromide with lithium metal to form the alkyl lithium species, followed by introduction of cuprous iodide into the mixture. This was then reacted with the substrate **164**, which resulted in a complex mixture of products, **Scheme 5.23**.

Scheme 5.23

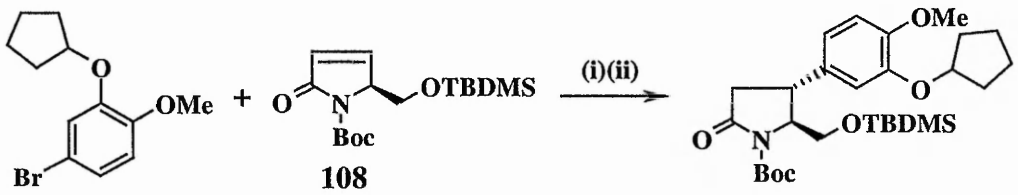


164

i) $(CH_3(CH_2)_9)_2CuLi$, THF, -78 °C.

The literature unfortunately does not support the formation of a long alkyl chain Gilman reagent, as a reagent with an alkyl chain longer than 8 carbons has not been reported. A similar reaction has been described by Diaz[122] *et al*, where a Gilman reagent is reacted with the enone **108**, **Scheme 5.24**, to give a stereoselective addition to the 4-carbon of the pyrrolidinone.

Scheme 5.24



108

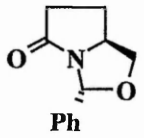
i) ^{n}BuLi, $CuBr.SMe_2$, THF, -35 °C. (ii) TMS-Cl, -78 °C.

6. Synthesis using *N*,*O*-acetal protected pyrrolidinones

6. Synthesis using *N,O*-acetal protected pyrrolidinones

6.1 Introduction

A very popular method of protecting the pyrrolidinone moiety is the use of a single group, an *N,O*-acetal, which simultaneously blocks both the amide and alcohol of the pyroglutaminol in a single step, giving a bicyclic lactam **87**. The protecting group is easily introduced, and can be removed by treatment with a catalytic quantity of acid, mild conditions unlikely to affect other functionality.

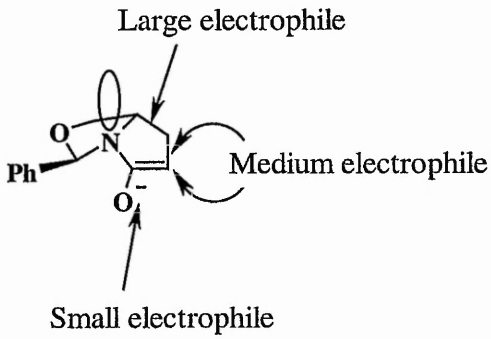


87

The *N,O*-acetal protected pyrrolidinone **87** has been used frequently[83,84,116] for the synthesis of complex molecules based on the pyroglutaminol moiety. The protected nucleus is a useful compound, in that it can be substituted at the 3-carbon, by alkylation of the anion, in a similar way to *N*-urethane protected compounds.

The *N,O*-acetal also has the effect of exerting some control on the stereochemistry of the alkylation reactions, due to the steric differences in the two faces of the protected pyrrolidinone ring. This effect is, however, fairly limited, and is suggested by Zhang[123] *et al* as being the result of competing steric and electronic effects acting upon the attacking electrophile, **Scheme 6.1**.

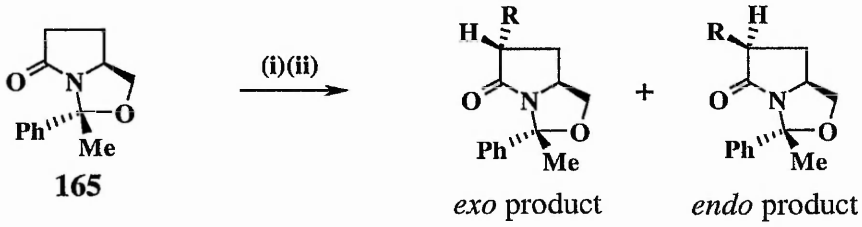
Scheme 6.1



A recent study by Bailey[124] and co-workers has further explored the stereoselectivity of the reaction between anions of the lactam **87** and electrophiles.

The study involved the synthesis of a new bicyclic lactam **165** with a methyl group at the 2-position. It was found that the stereochemical outcome of the reaction between the anion of **165** and electrophiles had been influenced by the change to the hemiaminal moiety, **Scheme 6.2**.

Scheme 6.2



165 *exo* product *endo* product

i) LDA, THF, -78 °C. (ii) RX, -78 °C.

(Where *exo*: *endo* is 1 :2.8 for RX = BnBr, up to 1 : 15.3 where RX = TsCl)

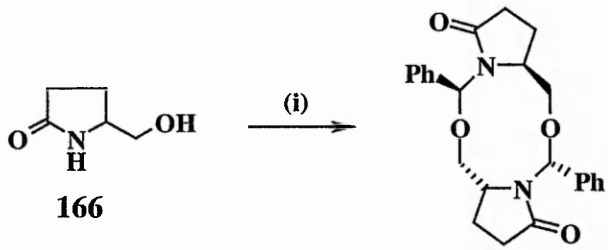
It was found that the lactam **165** was alkylated preferentially on the *endo* face, giving a diastereoselective reaction with several electrophiles. This is suggested as being due to the methyl moiety affecting the conformation of the lactam, resulting in a less convex structure which allows the nitrogen lone pair to exert a greater directing effect on attacking electrophiles.

With the original lactam **87**, the stereoselectivity of the alkylation product is proposed as being due to a combination of two effects[123], where the directing effect of the nitrogen lone pair favours *endo* products, whereas the *exo* product is favoured on steric grounds, due to the less hindered convex *exo* face of the molecule. The relative importance of these two factors depends on the size of the attacking electrophile.

It is suggested that the stereochemistry of the product is dependent upon the structure of the hemiaminal protecting group, due to the steric crowding of the *exo* face of the lactam exerted by the methyl group.

The formation of the protected nucleus can also result in an improved diastereomeric purity of the material. Thottathil[125] *et al* has shown that an enantiomeric mix of the two pyroglutaminols **166** under the protection conditions results in dimerisation, to give **167**, **Scheme 6.3**. This insoluble solid dimer is a 1:1 combination of the two enantiomers, instead of the oily *N,O*-acetal monomer **87** which forms with enantiomerically pure material. This allows an easy removal of traces of the wrong enantiomer by chromatography.

Scheme 6.3

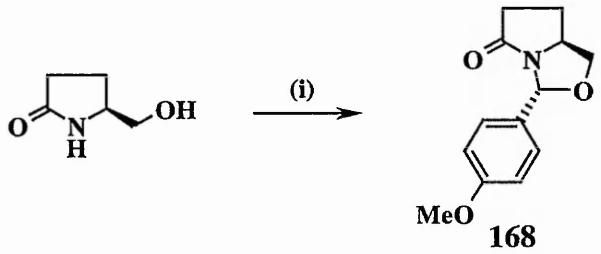


166 **167**

i) PhCHO, PTSA, toluene, Dean and Stark.

A simple analogue of the lactam has also been reported by Nagasaka and Imai[116]. Replacement of benzaldehyde with anisaldehyde in the protection step, **Scheme 6.4**, gives the bicyclic lactam **168** which has a high tendency to crystallise.

Scheme 6.4

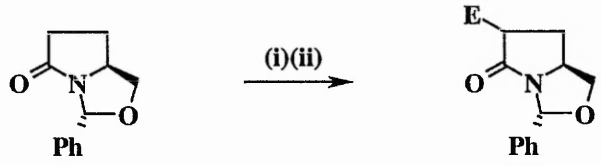


168

i) anisaldehyde, PTSA, toluene, Dean and Stark.

The bicyclic lactam **87** has been used extensively in the recent literature, as a versatile chiral synthon. The reaction of the lactam anion with a variety of electrophiles has been used for derivatisation of the pyrrolidinone ring, at the carbon adjacent to the carbonyl moiety, **Scheme 6.5**.

Scheme 6.5

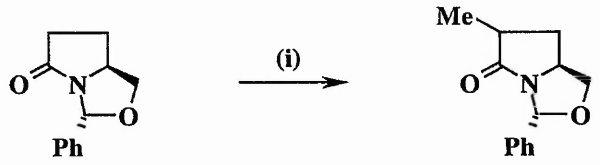


87

i) base. (ii) electrophile.

However, the electrophiles described in the literature are generally small, with benzyl being one of the largest groups used. The simple alkylation of the lactam has been used many times, for example by Uno[84] *et al*, in the synthesis of lactacystin, where the pyrrolidinone ring is methylated to give **169**, **Scheme 6.6**.

Scheme 6.6

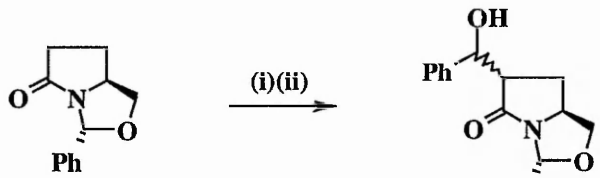


87 **169**

i) LDA, MeI, THF, -78 °C.

The literature, however, contains only two examples of the reaction of the lactam anion with aldehydes[83,126], **Scheme 6.7**. This reaction is the key step in the synthesis of the target compounds from the lactam, as it introduces the 3-alkyl substituent, to give **170**, with the possibility of generating a diol in a later step.

Scheme 6.7



87 **170**

i) LDA, THF, -78 °C. (ii) PhCHO, -78 °C.

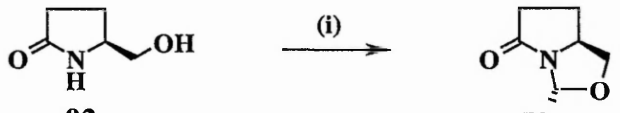
The reaction is analogous to that described for the *N*-Boc and *N*-Cbz compounds of previous chapters, following the modified procedure of Ezquerra[68] *et al.* However, the variation in reactivity between the *N*-Boc and *N*-Cbz compounds suggests that simply transferring the reaction conditions from one substrate to another does not always give a successful reaction.

6.2 Acetal synthesis

The 5-hydroxymethylpyrrolidinone was successfully protected by formation of the acetal, using the method described by Thottathil[127] *et al*, **Scheme 6.8**. The *N,O*-

acetal **87** is formed by using azeotropic removal of the water generated in the reaction, to drive the equilibrium towards the product. The reaction requires the use of freshly purified benzaldehyde, and it was found that mechanical stirring is essential for success, as the pyroglutaminol is insufficiently dispersed when using a magnetic stirrer.

Scheme 6.8



92 **87**

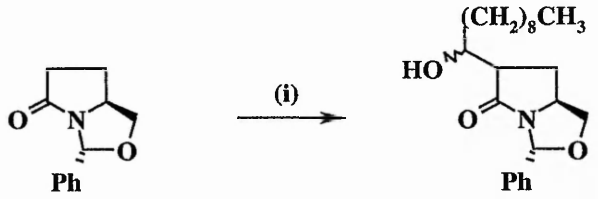
i) PhCHO, PTSA, toluene, Dean and Stark.

6.3 Anion reactions of the lactam

Following some previous work by Baldwin[83] and co-workers, and Beard[126] *et al*, as well as the results obtained with other protected pyroglutamate moieties, reaction of the anion of lactam **87** with an aldehyde should yield the 3-alkyl substituted secondary alcohol, as mentioned in Section 6.1.

This was indeed found to occur, **Scheme 6.9**, as reaction of the anion of **87** with decanal resulted in the novel compound **171**. However, using the standard reaction conditions used in previous experiments, the product was obtained in low yield, with a high recovery of starting material.

Scheme 6.9

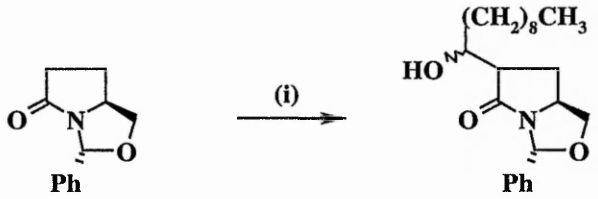


87 **171**

i) decanal, LiHMDS, THF, -78 °C.

The reaction was repeated in the presence of boron trifluoride etherate, the conditions of Ezquerra[68] *et al*, **Scheme 6.10**, in an attempt to improve the yield. This change resulted in increased decomposition, and the final product isolated was a mixture of diastereomers, from the NMR spectra of the sample.

Scheme 6.10

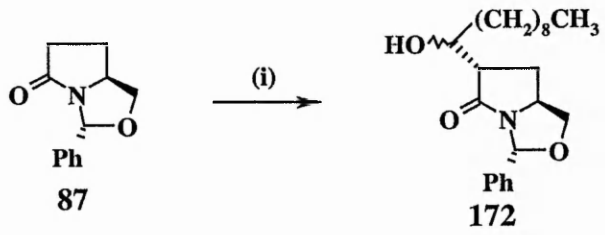


87 **171**

i) decanal, $BF_3.Et_2O$, LiHMDS, THF, -78 °C.

It was also attempted to form the anion by use of lithium tetramethylpiperidide[128], a sterically hindered base, as an alternative to lithium hexamethyldisilazane. Subsequent reaction of the anion of **87** with decanal occurred, giving the *exo* product **172** in moderate yield (20%) as a single diastereomer, **Scheme 6.11**. This alternative procedure failed to give an improvement over the original conditions.

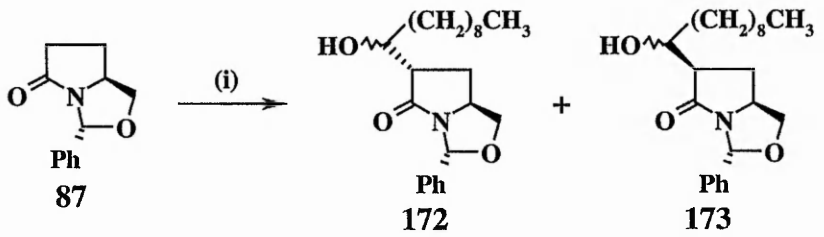
Scheme 6.11



i) lithium tetramethylpiperidide, decanal, THF, -78 °C.

The base was changed back to lithium hexamethyldisilazane, and it was found that by allowing the reaction temperature to warm to -45 °C, the yield of the reaction improved (50% overall from **87**). Also, the reaction was complete within an hour, whereas at -78 °C, at least two hours were required, with a lower yield of isolated product. The change in temperature also resulted in the isolation of a second diastereomer (the *endo* product) **173** (~20%), as well as the original diastereomer **172** (~30%), **Scheme 6.12**.

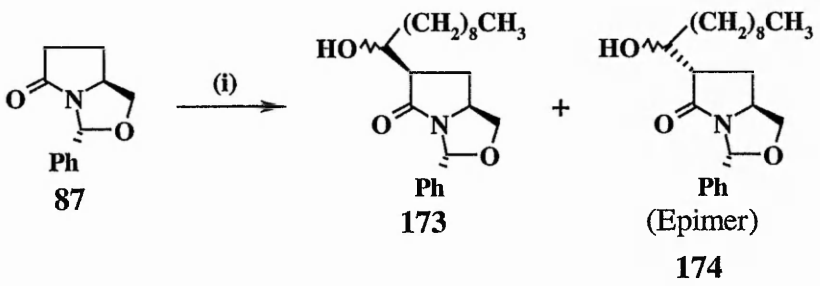
Scheme 6.12



i) LiHMDS, decanal, THF, -45 °C.

An interesting result was obtained when the reaction was allowed to warm to -35 °C, **Scheme 6.13**, as this resulted in an epimer of **172** being the major product, **174**, with **173** isolated as the minor product, and none of **172** recovered. The difference was at the carbon of the secondary alcohol, which could be seen by NMR.

Scheme 6.13



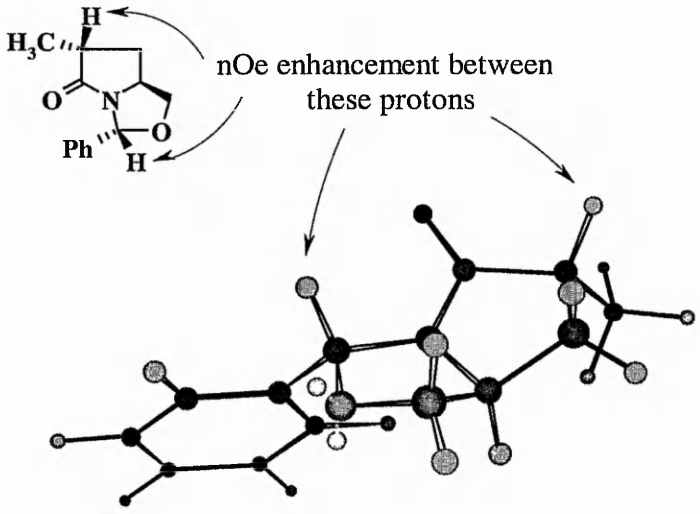
i) LiHMDS, decanal, THF, -35 °C.

These reactions once again demonstrate the powerful effect that apparently small changes to the reaction conditions can have on the final outcome. In these examples, in a similar but opposite way to the Z-protected pyrrolidinones (Chapter 4), the reaction temperature is an important variable in deciding the final product, and if, indeed, the reaction will occur at all.

The structure of the compounds isolated was deduced from NMR experiments, where the protons were first assigned using two dimensional ^{1}H-^{1}H COSY spectra. An experiment was then carried out to identify the chiral centre at C-3 of the pyrrolidinone.

This was achieved by looking for a positive nuclear Overhauser effect between the proton of the benzylic C-H, and the proton on C-3. If a positive n.O.e. enhancement was observed, the two protons were both on the same face of the lactam, which would be *exo* compound **172**. This is shown in **Scheme 6.14**, using the 3-methyl substituted lactam **169** as an example. (Three dimensional structure generated by Chemdraw Pro 3D)

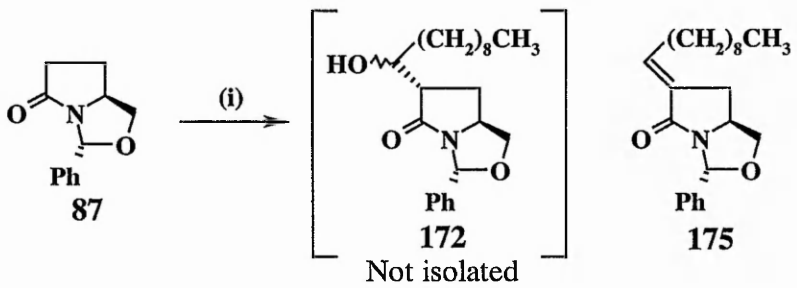
Scheme 6.14



6.4 Dehydration reactions

A further investigation into the formation of the enolate anion of **87** and its subsequent reaction with decanal was made. It was attempted to generate the anion of **87** using sodium hydride as the base, but the expected product **172** was not isolated. Instead, it appeared that an *in situ* dehydration of the secondary alcohol **172** had occurred, **Scheme 6.15**, with the enone **175** being formed as a result, although in low yield.

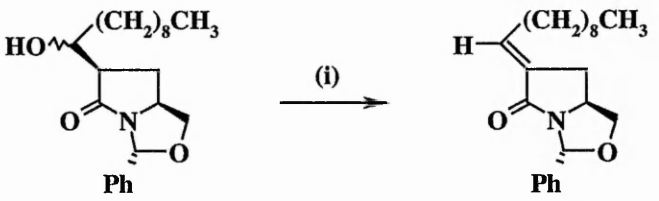
Scheme 6.15



i) NaH, decanal, THF, 0 °C.

Dehydration of the intermediate secondary alcohol **172** or **173**, using sodium hydride to cause the elimination reaction, also gives the same alkene **175** as produced in the previous reaction, **Scheme 6.16**.

Scheme 6.16



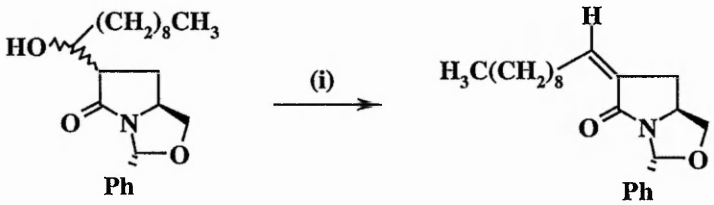
173 **175**

i) NaH, THF, 20 °C.

From this reaction, it may be deduced that the alcohol is formed first, by reaction of the enolate anion with the aldehyde, and this then dehydrates in a second step, due to the presence of excess base. It is also interesting to note that the use of LiHMDS does not cause dehydration to a noticeable extent, as the enone **175** is not usually isolated as a by-product when using this base.

Dehydration of the intermediates **172** or **173** with methanesulfonyl chloride and triethylamine, the conditions reported by Ezquerra[68] and co-workers, appears to give a different product. This seems to be the other geometrical isomer **176** by NMR, **Scheme 6.17**, but the reaction unfortunately does not give a very clean product.

Scheme 6.17

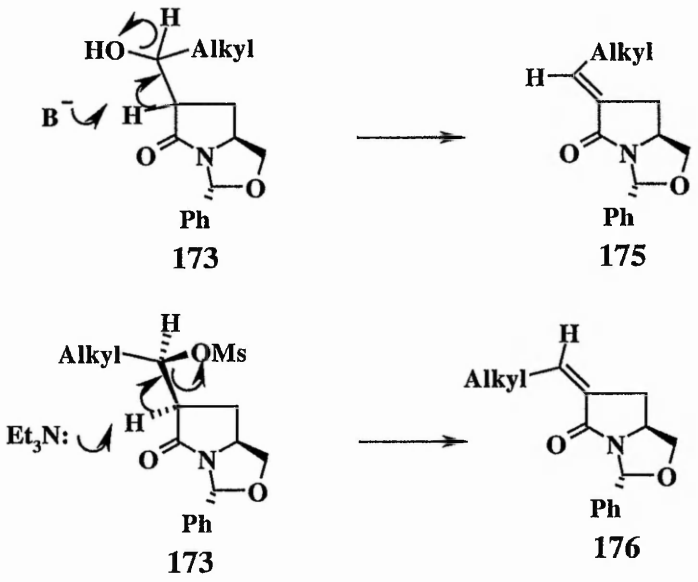


172 or **173** **176**

i) MsCl, Et_3N, DCM, 20 °C.

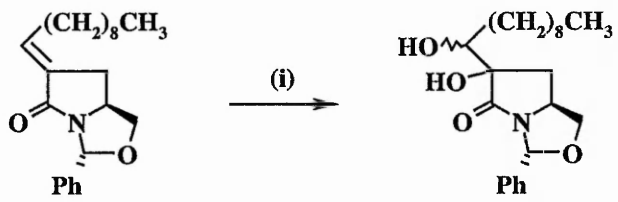
The difference in product from the two methods of dehydration would suggest that a different elimination mechanism is operating in each case, **Scheme 6.18**. This may explain the formation of different geometric isomers from the same starting material, by use of a different dehydrating agent. With sodium hydride, the small anion favours an E_2 mechanism. For the elimination of the mesylate, an E_1 mechanism may be more appropriate, as triethylamine is a bulky base, trying to extract a sterically hindered proton.

Scheme 6.18



The alkene **175** was successfully converted to the two new diols **177** and **178**, using a catalytic quantity of osmium tetroxide. The reaction gave a mixture of the two *cis*-diols in excellent yield, **Scheme 6.19**, separable by column chromatography.

Scheme 6.19



175 **177** and **178**

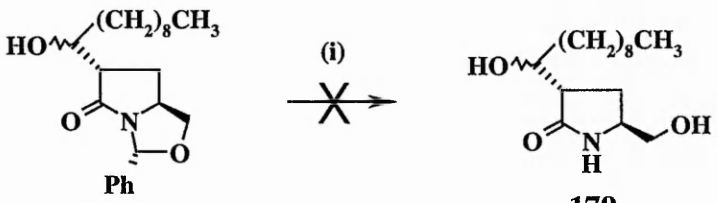
i) OsO_4, NMO, acetone, water, 0 to 20 °C

Compounds **177** and **178** are thus advanced intermediates to one of our target structures, with an additional hydroxyl group on the alkyl chain.

6.5 Deprotection of the lactam

There are several reported procedures for the deprotection of the lactam, where the *N,O*-acetal is cleaved using acid catalysed hydrolysis. Several of these methods were investigated before suitable conditions were found. The popular reagent trifluoroacetic acid[120,129] reacted slowly with the substituted pyroglutaminol **172**, **Scheme 6.20**, but was found to result in extensive decomposition of the material, instead of clean deprotection, and the target compound **179** could not be isolated.

Scheme 6.20



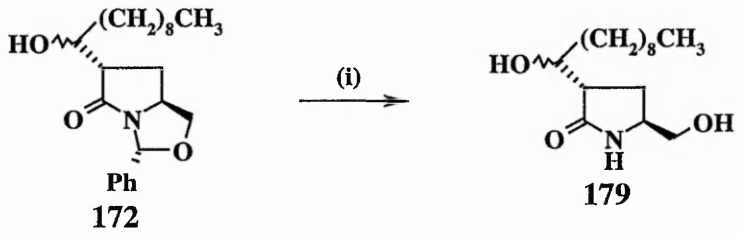
172 **179**

i) TFA, DCM, 20 °C.

Toluene-*p*-sulfonic acid was found to be the reagent of choice for the transformation, using a slightly modified literature procedure[130]. A 16 hour reflux in

aqueous methanol with a catalytic quantity of acid resulted in clean deprotection to the advanced intermediate **179**, **Scheme 6.21**.

Scheme 6.21

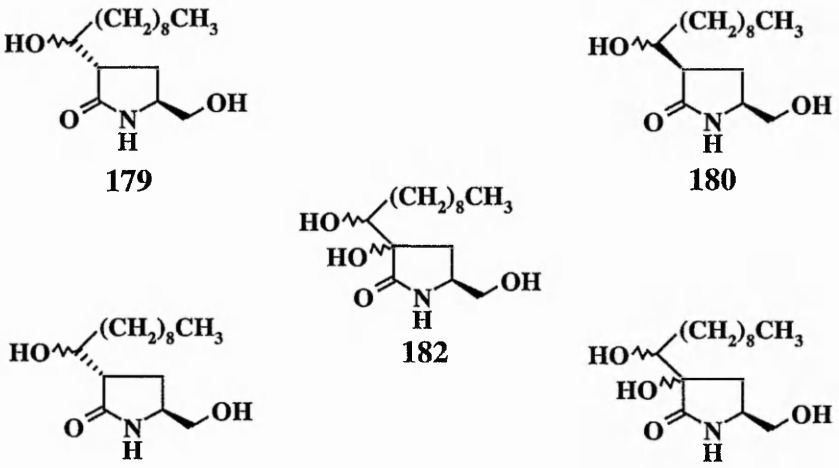


172 **179**

i) PTSA, MeOH, H_2O, reflux, 16h.

The other diastereomers were also successfully deprotected under the same conditions, in excellent yield. This gave a selection of novel 3-alkyl pyrrolidinones, **Scheme 6.22**, three mono-hydroxylated diastereomers, and two diols. These target intermediates were then used in further reactions, where a sugar is put in position on the primary alcohol, work discussed in Chapter 7.

Scheme 6.22



179 **180** **182**

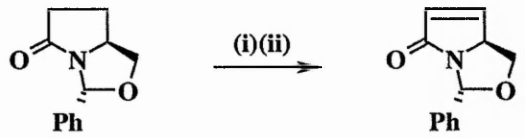
(Epimer at C-OH) **181**

(Diastereomer) **183**

6.6 Attempted introduction of unsaturation into the pyrrolidinone

The formation of the unsaturated lactam **184** was attempted, using phenylselenyl chloride, followed by hydrogen peroxide oxidation, in a one-pot reaction[131], **Scheme 6.23.** The first attempt at this reaction was unsuccessful, due to some hydrogen peroxide remaining in the crude material after work-up, which subsequently destroyed the product.

Scheme 6.23

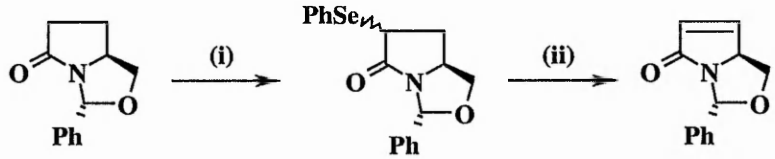


87 **184**

i) LiHMDS, PhSeCl, THF, -78 °C. (ii) H_2O_2, 0 to 20 °C.

A second attempt at the reaction was made, using an alternative reaction procedure reported by Herdeis[132] *et al.* The phenylselenyl derivative **185** was worked up before exposure to hydrogen peroxide, **Scheme 6.24.** This gave a crude product that appeared to be almost identical to the starting material by TLC.

Scheme 6.24



87 **185** **184**

i) LiHMDS, PhSeCl, THF, -78 °C. (ii) H_2O_2, EtOAc, 0 to 20 °C.

The failure of these reactions is unexpected, as the procedure is common in the literature, with many examples using the *N*-Boc protected or *N,O*-acetal pyrrolidinones. In many of these synthetic strategies, the introduction of unsaturation into the

pyrrolidinone is a fundamental step in the reaction, which must perform at least adequately to allow the remainder of the synthesis to be completed.

7. Activated sugars and sugar coupling reactions

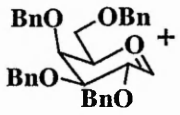
7. Activated sugars and sugar coupling reactions

7.1 Introduction.

7.1.1 Introduction to sugar chemistry. The coupling of the sugar moiety to the pyroglutaminol could be achieved by several different methods, to form the *O*-glycoside. There is considerable literature devoted to carbohydrate chemistry, and the synthesis of complicated polysaccharide structures. These complex molecules have become challenging and attractive targets for modern synthetic chemistry.

Many of the compounds synthesised, such as blood group antigen factors, are scarce natural products, where the more abundant synthetic material allows investigation of their biological effects. This is useful where isolation of the same compound from natural sources would either be too difficult, or would suffer from purity problems.

The introduction of a sugar moiety requires the presence of a good leaving group, such as a halide or trichloroimidate at the anomeric carbon. This encourages the formation of the oxonium ion **186**, which is the active species in the glycoside bond formation. This species is highly susceptible to nucleophilic attack by alcohols, but also makes the reaction highly water sensitive.

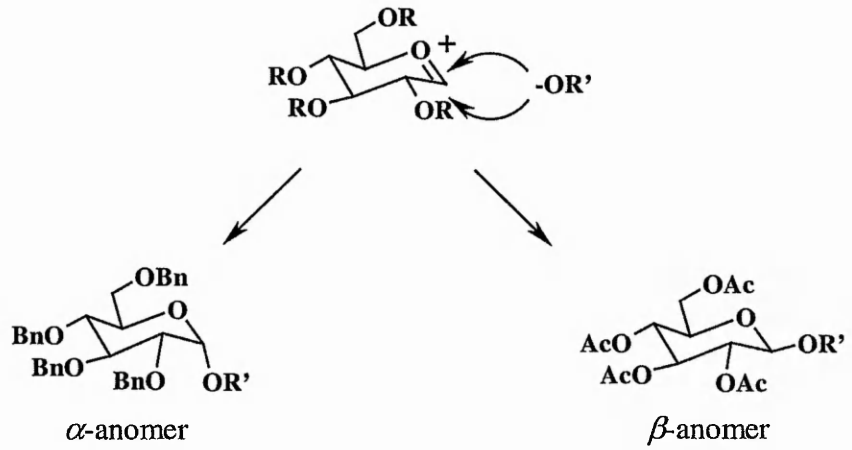


186

The reactivity of the oxonium ion can also be tuned by the protecting group strategy. This occurs due to neighbouring group participation in the formation and stability of the oxonium ion, as well as a directing effect on the attacking nucleophile. Benzyl protected 3-hydroxyl groups tend to direct the final glycoside to being the α- or

trans anomer. Acetyl protecting groups direct the final product towards being the β- or *cis* anomer.

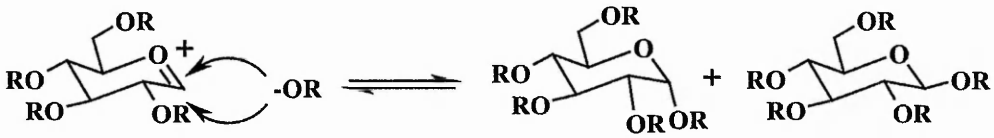
Scheme 7.1



α-anomer β-anomer

However, the formation of the oxonium ion, and the glycoside bond formation are both equilibrium processes. As a result of this, the product of the reaction is usually a mixture of the two possible products, **Scheme 7.2**, but with one anomer predominating.

Scheme 7.2



α-anomer β-anomer

The sugar coupling methods are generally applied between sugars, in the synthesis of polysaccharides, and considerably fewer methods are described for reaction with other classes of compound. Although the reactions should still occur in the same way, more complex molecules may interfere with the reaction, particularly by the possibility of complexing with the Lewis acid catalysts commonly used as coupling reagents.

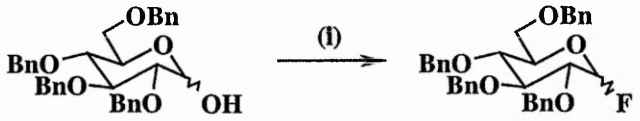
7.1.2 Activating groups and glycosidic bond formation. The field of sugar chemistry is vast and still rapidly growing, as more techniques and synthetic strategies are developed. Some of the more popular methods for the synthesis of activated glycoside moieties, and their subsequent reaction with a glycoside acceptor will be briefly discussed.

7.2 Activated sugars and glycosylation reactions

7.2.1 Fluorine activation. There are numerous methods for the generation of *O*-glycosidic bonds, many being carefully tuned in reactivity to suit a particular synthesis. The total synthesis of AGL-9b, and of the analogues generated by Morita[2] *et al*, utilised the tetrabenzyl protected fluorosugar as the glycosyl donor. In the presence of a Lewis acid catalyst and silver perchlorate, this will react with an alcohol to give the *O*-glycosidic bond.

As this method had been successful with the acyclic analogues of Morita, it was investigated for the cyclic analogues. Glucose was first chosen as the sugar, as 2,3,4,6-tetra-*O*-benzylglucopyranoside **187** is commercially available as a starting material. Synthesis of the fluoro-sugar **188** was investigated, following literature methods[133]. The protected sugar is treated with diethylaminosulfur trifluoride[134] (DAST), which results in the fluorination of the free hydroxyl moiety, **Scheme 7.3**.

Scheme 7.3



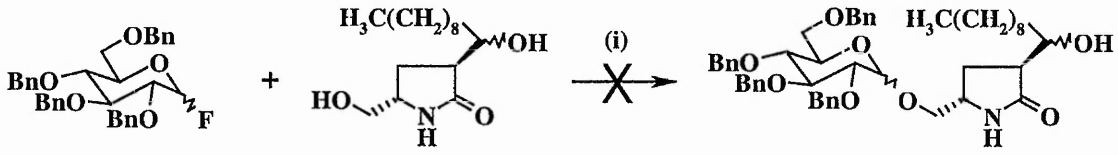
187 **188**

i) DAST, THF, -30 to 20 °C.

DAST is a highly reactive fluorinating agent, but can be safely handled in standard Pyrex glassware. This is a significant advantage over alternative fluorinating agents, such as HF / pyridine complex, which require Teflon reaction vessels for safe use.

The coupling of the activated sugar **188** to the advanced intermediate **179** was investigated, using boron trifluoride etherate as the Lewis acid coupling reagent[133], **Scheme 7.4**. This was an alternative procedure to that described by Morita[2] *et al*, but was reported to be a common method of glycosidic bond formation. However, the reaction was found to be unsuccessful, with only starting material being recovered.

Scheme 7.4

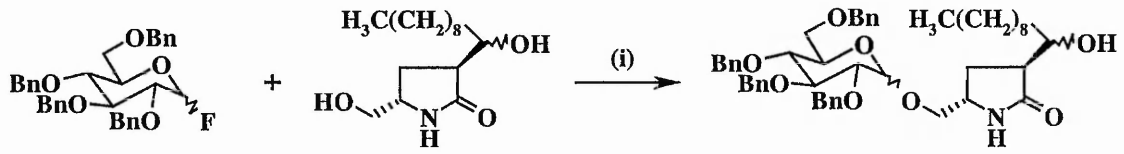


188 **179**

i) $BF_3.Et_2O$, THF, 0 to 20 °C.

A coupling reaction was attempted between the substituted pyroglutaminol **179** and the fluoro activated glucose moiety **188**, **Scheme 7.5**. This had been shown to be an effective coupling procedure when used by Morita[2] *et al* for the synthesis of KRN7000, and similar compounds.

Scheme 7.5



188 **179** **189**

i) $AgClO_4$, $SnCl_2$, MS-4Å, THF, 20 °C.

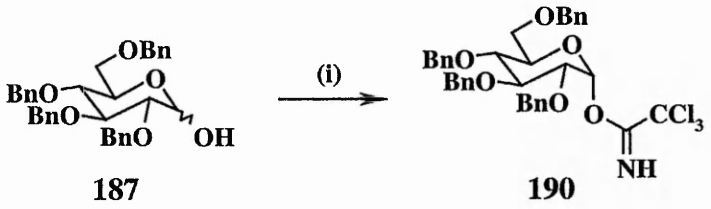
In a small scale experiment, the target compound **189** was isolated in a reasonable yield (41%). The synthesis could not be repeated, possibly due to the anhydrous catalysts being affected by moisture after opening for the first reaction. An additional problem was the reliable synthesis of the fluorosugar, which had proved difficult to synthesise consistently, even following literature methods.

7.2.2 Trichloroimidate derivatives. In view of the difficulties, an alternative activating group was sought. The trichloroimidates are a popular choice in many synthetic routes [135-137], and the glucose derivative **190** was investigated first, as a trial for future work with galactose.

188 **190**

The synthetic method of Schmidt and Michel[138] for the synthesis of trichloroimidates was followed, **Scheme 7.6**, starting from 2,3,4,6-tetra-*O*-benzylglucopyranoside **187**, but this was found to give poor results (~20% yield), with no improvement on using THF as the solvent.

Scheme 7.6

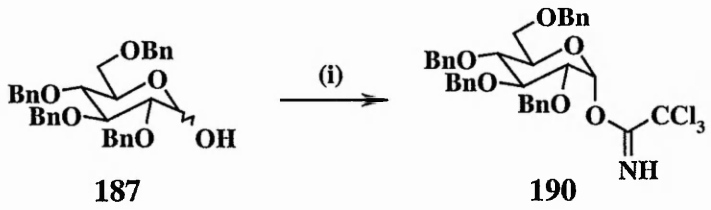


187 **190**

i) NaH, Cl_3CCN, DCM, 20 °C.

An alternative method[137] using DBU, a strongly hindered amine base, gave a significant improvement, with the trichloroimidate **190** being isolated in 75% yield and as a single anomer after chromatography, **Scheme 7.7**. The trichloroimidate was freshly prepared for each reaction, as the compound was found to decompose to starting material on standing for several days.

Scheme 7.7

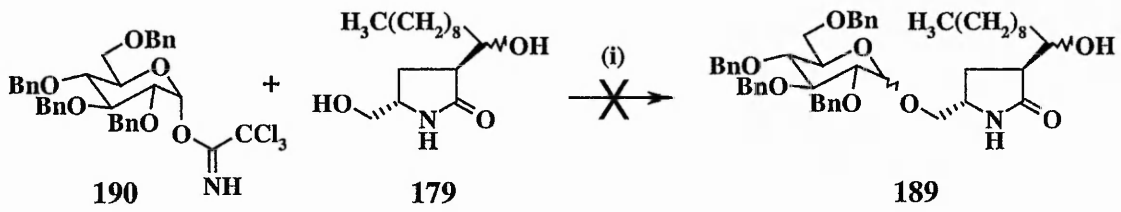


187 **190**

i) DBU, Cl_3CCN, DCM, 20 °C.

Reaction of the substituted pyroglutaminol **179** with the trichloroimidate derivative **190** was then studied. Following the work of Schmidt and Michel[138], using boron trifluoride etherate as the catalyst, a low yield of the target compound was isolated, **Scheme 7.8**. The reaction conditions appeared to be causing decomposition of the substrates, resulting in a low yield of product, with considerable by-product formation.

Scheme 7.8

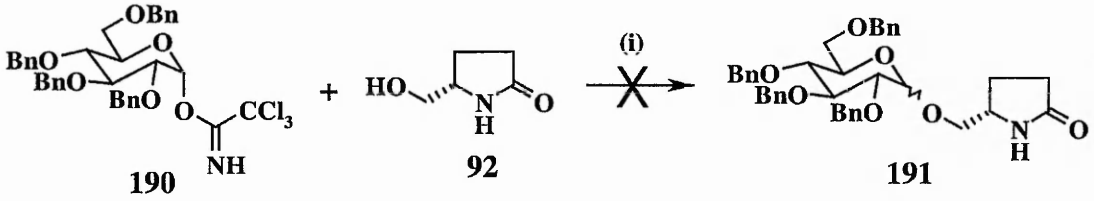


i) $BF_3.Et_2O$ (catalytic), DCM, 20 °C.

The reaction between the simple pyroglutaminol **92** and the trichloroimidate **190** was then studied as a model system, to investigate the effect of fewer co-ordinating sites for the Lewis acid on one of the substrates. This may be one of the reasons for the poor reaction between the substrates, as the catalyst may have become co-ordinated to one of the three possible alternative sites on the pyroglutaminol other than the primary alcohol.

However, repeating the experiment using the simple pyroglutaminol **92** still resulted in only a trace of the target compound **191** being isolated, **Scheme 7.9**.

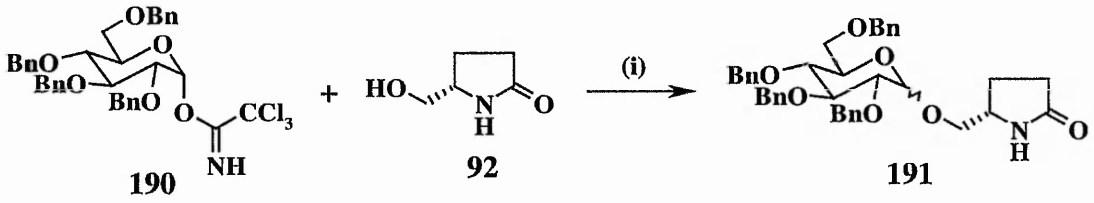
Scheme 7.9



i) $BF_3.Et_2O$ (catalytic), DCM, 20 °C.

The alternative coupling conditions described by Schmidt and Michel[138] were then studied. Replacing boron trifluoride etherate with toluene-*p*-sulfonic acid as the catalyst resulted in the formation of the product glycoside **191** in moderate yield, **Scheme 7.10**.

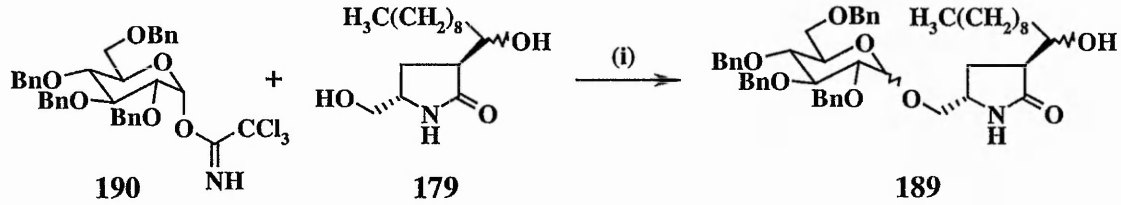
Scheme 7.10



i) PTSA (catalytic), DCM, 20 °C.

Following the encouraging result with the simple pyroglutaminol, the experiment was repeated using the substituted pyroglutaminol **179**, **Scheme 7.11**. This resulted in an initial 68% yield of **189**, when carried out on a small scale. A repeat of the reaction on a slightly increased scale unexpectedly resulted in a lower yield (33%), but the product was spectroscopically identical to the original sample.

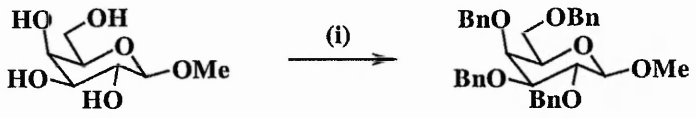
Scheme 7.11



i) PTSA (catalytic), DCM, 20 °C.

7.2.3 Galactose compounds. The benzyl protection of methyl galactopyranoside **192** was undertaken, using conditions described by Austin[139] *et al*, **Scheme 7.12**. This resulted in a clean reaction, albeit with a moderately violent delayed initiation, and resulted in the fully protected compound **193** in good yield.

Scheme 7.12

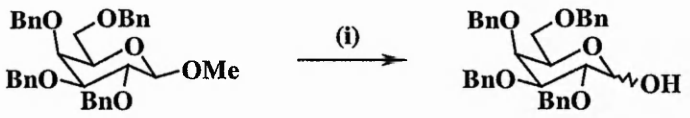


192 **193**

i) BnBr, NaH, Bu_4NI, DMF, 20 °C.

The removal[139] of the methyl protecting group from the anomeric hydroxyl group, **Scheme 7.13**, could be achieved only in moderate yield (30%) with high recovery of the starting material, even after five days refluxing. This acid catalysed hydrolysis is particularly slow, in comparison with tetrabenzyl glucose, where the reaction is reported[140] as being complete in 24 hours.

Scheme 7.13

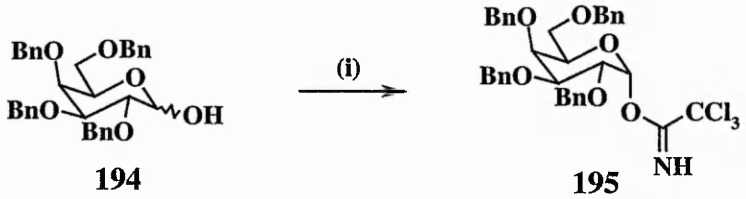


193 **194**

i) H_2SO_4, 1,4-dioxane, reflux.

The formation of the trichloroimidate from **194** was carried out using the conditions used for the glucose analogue, **Scheme 7.14**. These were again successful, resulting in excellent yields of the trichloroimidate **195**.

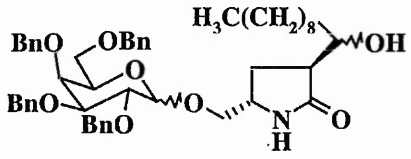
Scheme 7.14



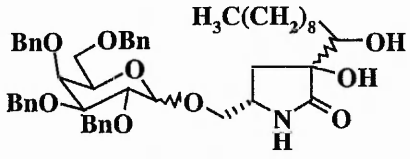
194 **195**

i) DBU, Cl_3CCN, DCM, 20 °C.

The glycosylation of the substituted pyroglutaminols with the galactose trichloroimidate **195** was then studied. It was found that reaction conditions effective with glucose were also successful with the galactose analogue. The diols did require the addition of some ethyl acetate to the reaction mixture, to improve the solubility of the pyroglutaminol. This resulted in the synthesis of three glycosylated pyroglutaminols, with galactose as the sugar, two of which (the two diols) were diastereomers.



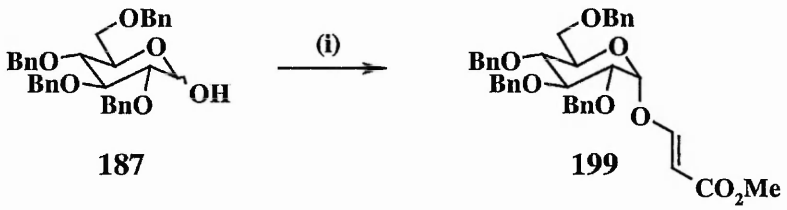
196



197 and **198**
(Two separate diastereomers)

7.2.3 Alternative activating groups. Alternatives to the trichloroimidate were also studied. The activated sugar **199** was synthesised, following the method of Osa[141] *et al.* Treatment of 2,3,4,6-tetra-*O*-benzylglucopyranoside **187** with tributylphosphine and methyl propiolate resulted in **199**, a colourless syrup, recovered in 70% yield, **Scheme 7.15**.

Scheme 7.15

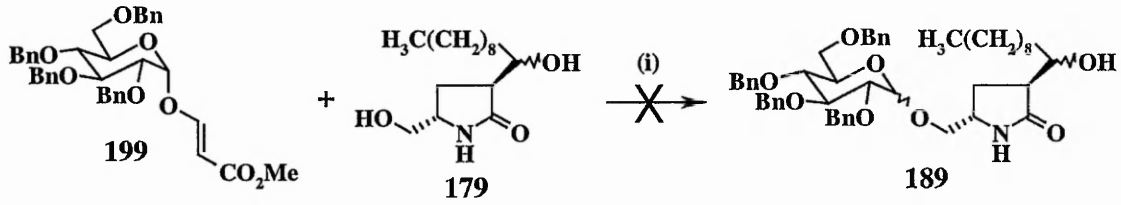


187 **199**

i) methyl propiolate, Bu_3P, DCM, 20 °C.

Unfortunately, the attempted coupling of the activated sugar with the pyroglutaminol failed, **Scheme 7.16**, as no product was identified.

Scheme 7.16



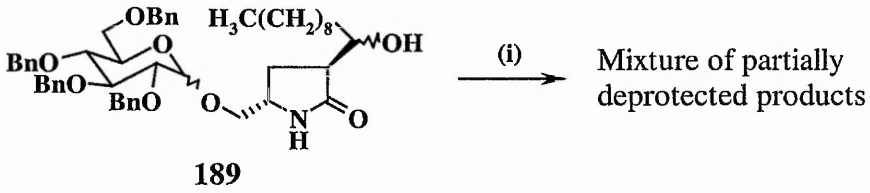
i) TMS-OTf (catalytic), DCM, MS-4Å, -50 °C.

7.3 Deprotection of the sugar

The next objective was the removal of the sugar protecting groups, to give the fully deprotected final compound. The glucose compounds were investigated first, with deprotection of **189** attempted using transfer hydrogenation[121]. This is a mild alternative procedure to catalytic hydrogenation, using a hydrogen donor molecule, in this example 1,4-cyclohexadiene.

However, these conditions achieved only incomplete removal of the benzyl ethers of the sugar, resulting in a mixture, **Scheme 7.18**. This method appeared unsuitable, as only complete removal of all four benzyl groups was acceptable.

Scheme 7.18

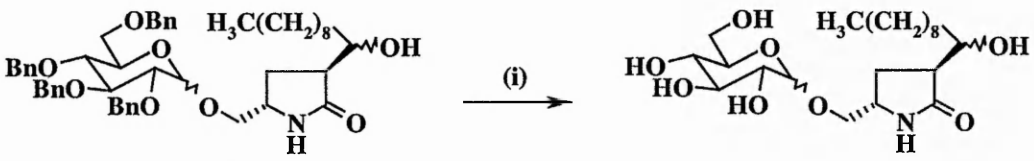


i) 10% Pd/C, 1,4-cyclohexadiene, EtOH, 20 °C.

Atmospheric pressure catalytic hydrogenation, using palladium on charcoal as catalyst, was then attempted. This gave the target compound **200** after 4 hours at room temperature, in 65% yield, **Scheme 7.19**. The low yield was probably due to the large excess of catalyst required, with some of the compound remaining on the catalyst even

after thorough washing. Washing with ethyl acetate improved the yield, with 90% of one compound being recovered.

Scheme 7.19



189 **200**

i) 10% Pd/C, H_2, EtOH, atmospheric pressure, 20 °C.

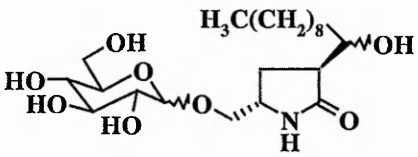
A repeat of the experiment was made, using less catalyst. This greatly lowered the rate of deprotection, with incomplete removal of the benzyl groups being observed after 24 hours. However, addition of further catalyst resulted in the reaction going to completion.

On changing the sugar moiety from glucose to galactose, the reaction times necessary for complete deprotection were drastically increased. Galactose compounds were found to require at least two days to obtain full deprotection, using the same conditions that were effective with the glucose analogues.

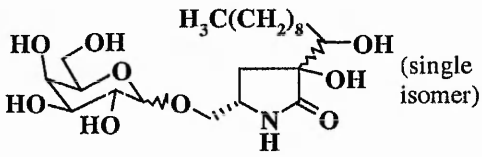
It is also interesting to note that the FAB mass spectrum and NMR of the deprotected compounds **201** and **203** show evidence of incomplete deprotection. These two compounds have additional peaks indicating M+96 (C_7H_{12}), which could be explained by reduction of one of the benzene rings, to give a methylcyclohexyl derivative.

Compound **202** also appears to have a protecting group to some extent intact, as the NMR seems to show aromatic protons, and again the mass spectrum shows peaks that could be attributed to a partially benzylated compound.

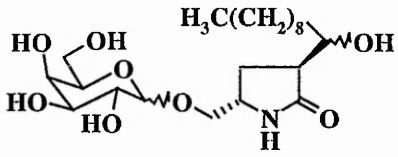
The three compounds synthesised in a suitable quantity for biological investigation were close structural analogues, **201**, **202** and **203**, the latter two compounds being two diastereomers, close to the original target compounds.



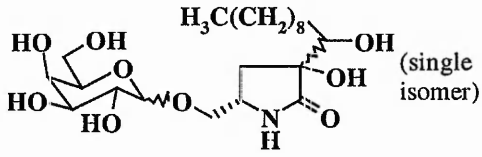
200



202



201



203

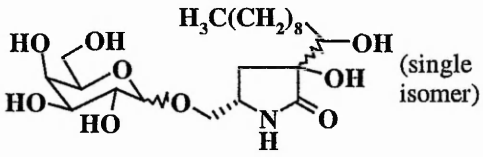
Unfortunately, the glucosyl pyroglutaminol **200** was obtained in inadequate quantities to be available for biological study. However, the work of Morita[2] *et al* suggests that the galactose compounds should be the most active. The two diastereomers **202** and **203** were evaluated for activity as immunostimulating agents by Knoll Pharmaceuticals

8. Biological results

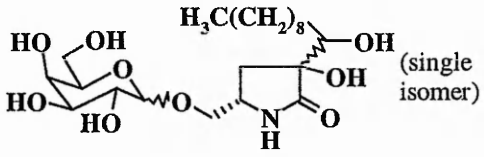
8. Biological results

8.1 Introduction.

Two compounds were tested for their stimulating activity in the mixed lymphocyte response (MLR) assay, by Knoll Pharmaceuticals. The compounds studied were the two separate diastereomers **202** and **203**, which possess a galactosyl moiety with an undetermined anomeric stereochemistry.



202



203

8.2 Conclusions.

Both **202** and **203** lacked believable efficacy as modulators of human mixed lymphocyte response.

These two compounds were not the predicted optimum structure for activity as immunostimulating agents, but the nearest to the ideal structures that could be produced in the time available. It is therefore not entirely unexpected that they fail to show the intended immunomodulating effect.

9. Conclusions and further work

9. Conclusions and further work

9.1 Conclusion.

A group of compounds were synthesised, closely related to the target molecules. The successful synthesis of these compounds was achieved after several changes to the original synthetic strategy throughout the project. Two of the compounds finally obtained were subjected to biological evaluation by Knoll Pharmaceuticals. Unfortunately, these compounds failed to show activity as immunostimulants in the MLR assay for immunomodulating activity.

It is possible that closer analogues of the target compounds may show activity, particularly if a receptor type mechanism is responsible for the activity of the natural products.

9.2 Suggestions for future work.

The synthesis of 4-hydroxyl compounds *via* serine or other acyclic precursors, as discussed in chapter 2, may be worth further investigation. In particular, the method of Huang[48] and co-workers would still be attractive, if a suitable Wittig reagent could be produced.

Alternatively, the di-protected serine method could be worth further study. Alternative methods of activating the acid moiety, such as other active esters, or Weinreb amides, may be worth further investigation.

Moving on to glycosylated compounds, the synthesis of α-linked compounds by use of fluorine activation of the sugar may be worth renewed investigation. A reliable method of synthesising the fluorosugar is, however, fundamental to this route.

Another important area for further study could focus on the final deprotection step, the removal of the four benzyl protecting groups. This proved to be problematical, with partial deprotection occurring with some of the compounds. Following the work of Morita[2] and co-workers on the synthesis of KRN7000 and analogues, hydrogenation at a higher temperature may be more effective. An alternative to this would be the use of high pressure to assist in the deprotection.

10. Experimental methods

10. Experimental methods.

10.1 General information

Dry solvents were prepared using standard methods as described in 'Purification of Laboratory Chemicals' (3rd Ed., D D Perrin, W L Armarego, Pergamon Press, New York, NY.). THF, toluene and dioxane were dried by distillation from sodium benzophenone ketyl immediately prior to use. Ethyl acetate and petroleum, b. p. 40-60 °C, were distilled before use. Flash column chromatography was carried out using Fluka silica gel 60, and TLC analysis performed on aluminium backed silica gel plates (Whatman). Reactions were carried out under nitrogen in flame dried glassware, using standard septum cap techniques, unless otherwise stated.

NMR spectra were recorded on a JEOL machine at 270MHz, or a Bruker 250MHz or 360MHz machine, where stated, using TMS as the internal standard, coupling constants recorded in Hertz. Infra-red spectra were obtained on a Perkin Elmer spectrophotometer, using either thin film (sodium chloride plates) or KBr discs. Melting points were determined using a Gallankemp melting point apparatus, and are uncorrected.

Commercially available reagents were used as purchased.

10.2 Experimental procedures

10.2.1. Acyclic compounds

2-Isocyano-5-methyl-hexa-2,4-dieneoic acid ethyl ester **45**

A 1 M solution of lithium hexamethyldisilazide in THF (3 ml, 3 mmol) was added slowly to a cold (-78 °C), stirred solution of ethyl isocyanoacetate (0.33 ml, 3 mmol) in dry THF (15 ml). After stirring for 5 minutes, 3-methyl-2-butenal (0.25 ml, 3 mmol) was

added, and after 20 min at -78 °C, the reaction was quenched with 4 M aqueous acetic acid (0.75 ml) and allowed to warm. The mixture was poured into saturated sodium hydrogencarbonate solution (20 ml), and separated, and the aqueous fraction was extracted with petroleum (4 x 20 ml). The organic phases were combined, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by flash column chromatography (2% ethyl acetate/petroleum). This yielded the title ester as a pale yellow oil (176 mg, 33%). ν_{max} (film)/cm^{-1} 2962m (C-H), 2934m (C-H), 2112m (CN), 1724vs (C=O), 1624s (C=C), 1262vs (C-O); δ_H 1.3 (3 H, t, CH_2CH_3), 2.0 (6 H, t, $C(CH_3)_2$), 4.3 (2 H, q, CH_2CH_3), 6.3 (1 H, d, C-4 C=CH), 7.6 (1 H, d, C-3 C=CH); δ_C 14.2 (CH_2CH_3), 19.9 (*cis*-CH_3), 27.3 (*trans*-CH_3), 62.2 (CH_2CH_3), 101.2 (C-2 C=C), 120.1 (C-4 C=C), 136.0 (C-5 $C(CH_3)_2$), 154.1 (C=O).

3-Acetoxy-1-benzyl-2,5-dioxopyrrolidine[44] **55**

Malic acid (10 g, 75 mmol) was suspended in acetyl chloride (22 ml, 300 mmol), and heated under reflux for 2 hours The mixture was cooled and concentrated *in vacuo*, giving compound **53** as a yellow oil, which was diluted with dry THF (50 ml), and cooled to 0 °C. A solution of benzylamine (22 ml, 194 mmol) in dry THF (25 ml) was then added to the cold, stirred solution, over 10 minutes. The mixture was then allowed to warm to 20 °C, resulting in formation of a white precipitate. After 2 days, the mixture was diluted with diethyl ether (100 ml), and the resulting solid compound **54** filtered off, and washed with a portion of ether, and air dried. The solid was suspended in DCM (200 ml), and the stirred suspension cooled to 0 °C. A solution of acetyl chloride (17 ml, 225 mmol) in DCM (100 ml) was added, and the mixture allowed to warm to 20 °C. After standing for 16 hours, the mixture was concentrated *in vacuo*, and the residue taken up in

DCM (200ml), and filtered. The filtrate was concentrated *in vacuo*, to give a brown oil, which was purified by flash column chromatography (25% ethyl acetate/petroleum). The target compound was obtained as a pale yellow oil, (9.66 g, 52%), characterised by IR and NMR (the material is described by Koot[44] *et al* as a crystalline solid, m.p. 58-60 °C). ν_{max} (film)/cm^{-1} 1790m (C=O), 1715vs (C=O); δ_H 2.1 (3 H, s, C*H*$_3$), 2.6 (1 H, d.d., C-3 C*H*$_2$), 3.1 (1 H, q, C-3 C*H*$_2$), 4.7 (1 H, d, PhC*H*$_2$), 5.4 (1 H, q, C-4 C*H*O), 7.3 (5 H, Ar*H*); δ_C 20.5 (C*H*$_3$), 35.7 (C-3 *C*H$_2$), 42.7 (Ph*C*H$_2$), 67.4 (C-4 *C*H), 128.1 (Ar), 128.8 (Ar), 128.9 (Ar), 135.1 (Ar), 169.8 (CH$_3$*C*O$_2$), 172.8 (*C*ON), 173.1 (*C*ON).

3-Acetoxy-1-benzyl-2-hydroxy-5-oxopyrrolidine **57**

Sodium borohydride (1.48 g, 39 mmol) was added to a cold (-23 °C) stirred solution of compound **53** (4.82 g, 19.5 mmol) in dry ethanol (40 ml). After stirring for one hour, the reaction was quenched by addition of 2% HCl (aq), until pH 2 was reached. The mixture was neutralised with saturated sodium hydrogencarbonate solution, and allowed to warm to 20 °C. The mixture was extracted with DCM (5 x 50 ml), and the combined extracts dried ($MgSO_4$) and concentrated *in vacuo*. A pale yellow residue was obtained, which solidified on standing, to yield the title pyrrolidinone (3.88 g, 80 %), m.p. 132-137 °C. This material was used without further purification. ν_{max} (film)/cm^{-1} 3147s (OH), 1732vs (C=O), 1662vs (C=O); δ_H 2.1 (3 H, s, C*H*$_3$), 2.7 (2 H, m, C-3 C*H*$_2$), 4.2 (1 H, d, *J* 14.8, PhC*H*$_2$), 4.9 (1 H, d, *J* 14.8, Ph*C*H$_2$), 5.1 (1 H, m, C-4 C*H*, C-5 C*H*OH), 7.2 (5 H, m, Ar); δ_C 20.7 (C*H*$_3$), 35.5 (C-3 *C*H$_2$), 67.7 (C-4 *C*H), 80.8 (C-5 *C*HOH), 127.8 (Ar), 127.9 (Ar), 128.2 (Ar), 128.5 (Ar), 128.8 (Ar), 135.9 (Ar), 170.3 (*C*O$_2$), 170.6 (*C*ON).

2,3-diacetoxy-1-benzyl-pyrrolidin-2-one **51**

Acetic anhydride (2.41 ml, 25 mmol) was added to a cold (0 °C) stirred solution of the ester compound **57** (crude material) with 4-dimethylaminopyridine (240 mg, 0.19 mmol), and triethylamine (2.43 ml, 18 mmol) in DCM (60 ml). The mixture was allowed to warm to 20 °C, and was left to stir for 1 hour The mixture was diluted with DCM (100 ml), and washed with 10% HCl (aq) (2 x 50 ml), saturated sodium hydrogencarbonate solution (2 x 50 ml), and water (3 x 50 ml), and finally dried ($MgSO_4$) and concentrated *in vacuo*. This resulted in a pale brown oil, which was subjected to flash column chromatography (25% ethyl acetate/petroleum). Starting material (1.6 g, 33%) was recovered, as well as the title compound (930 mg, 16%), both as colourless oils. ν_{max} (film)/cm^{-1} 1747s (C=O), 1717vs (C=O); δ_H 1.9 (3 H, s, CH_3), 2.1 (3 H, s, CH_3), 2.7 (2 H, m, C-3 CH_2), 4.2 (1 H, d, PhCH_2), 4.7 (1 H, d, PhCH_2), 5.3 (1 H, m, C-4 C*H*), 6.3 (1 H, d, C-5 C*H*), 7.2 (5 H, m, Ar); δ_C 20.5 (*C*H_3), 33.9 (C-3 *C*H_2), 44.7 (Ph*C*H_2), 66.0 (C-4 *C*H), 86.1 (C-5 *C*H), 127.7 (Ar), 127.9 (Ar), 128.4 (Ar), 128.6 (Ar), 128.8 (Ar), 135.8 (Ar), 169.8 (*C*O_2), 170.1 (*C*O_2), 171.5 (*C*ON). The spectral data are in agreement with the literature values[44].

Methyl 2-phenyl-4,5-dihydro-oxazole-4-carboxylate[48] **59**

L-Serine methyl ester hydrochloride (5.0 g, 32 mmol) was dissolved, with stirring, in a mixture of triethylamine (5.6 ml, 40 mmol) and dry DCM (70 ml). Ethyl benzimidate hydrochloride (6.0 g, 32.2 mmol) was added to the solution, which was then heated under reflux for 4 h, resulting in the formation of an off-white solid. The mixture was cooled and left to stand for 16 hours, before the mixture was washed with saturated sodium hydrogencarbonate solution (1 x 30 ml). The organic phase was dried ($MgSO_4$)

and concentrated *in vacuo*, to give a red oil, which was purified by flash column chromatography on silica (50% ethyl acetate/petroleum). The title compound was obtained as a colourless oil (4.81 g, 73%), with spectral data consistent with the reported literature.

1-(Ethoxycarbonyl)-heptyltriphenylphosphonium bromide **67**

Ethyl 2-bromooctanoate (1.68 g, 7.4 mmol) and triphenylphosphine (1.94 g, 7.4 mol) were heated under reflux in toluene (10 ml) for 2 hours. The mixture was allowed to cool, and was then concentrated *in vacuo*, resulting in a yellow oil. The oil was triturated with ether, to give a colourless low melting solid, which was filtered and air dried (3.8 g, 100%).

N-*Fmoc-2-amino-3-benzyloxypropionyl chloride* **75**

The compound was prepared from *N*-Fmoc-*O*-benzyl serine by reaction with thionyl chloride, according to the method of Carpino[51] *et al.*

N-tert-*butyloxycarbonyl serine*

The compound was prepared from L-serine by reaction with di-*tert*-butyldicarbonate, according to the method of Campbell[57] *et al.*

*(S)-3-(*tert-*Butyloxycarbonyl)*-N-*methoxy-2,2,*N-*trimethyloxaxolidine-4-carboxamide* **78**

The compound was prepared in two steps from Boc-serine, using *N,O*-dimethylhydroxylamine hydrochloride and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, following the method of Campbell[57] *et al.*

Ethyl 2-(hydroxydiphenylmethyl)-dodecanoate **80**

A 1 M solution of lithium hexamethyldisilazide in THF (6.0 ml, 6.0 mmol) was added slowly to a cold (-78 °C), stirred solution of ethyl laurate (1.6 ml, 6.0 mmol) in dry THF (40 ml) and pentane (1 ml). Addition was at a rate that maintained the reaction temperature below -60 °C. Following the addition, the yellow mixture was left to stir at -78 °C. After 20 minutes, a solution of benzophenone (1.0 g, 5.5 mmol) in dry THF (2 ml) was added dropwise. Following addition, the mixture was left to stir for 3 hours at -78 °C. The reaction was quenched with saturated ammonium chloride solution (50 ml), and the mixture extracted with diethyl ether (4 x 50 ml), and the combined organic fractions dried ($MgSO_4$), and concentrated *in vacuo*. The gave a golden oily residue which solidified slowly on standing. The material was recrystallised from ethanol, to give colourless solid, m.p. 55-57 °C (790 mg, 35%). ν_{max} (KBr)/cm^{-1} 3474m, (O-H), 1708vs (C=O); δ_H 0.7 (3 H, t, alkyl C*H*$_3$), 0.9 (3 H, t, $CO_2CH_2CH_3$), 1.1 (18 H, m, alkyl C*H*$_2$), 1.7 (2 H, m, C-3 C*H*$_2$), 3.4 (1 H, m, C-2 CH_2CHCO_2), 3.8 (2 H, q, CO_2CH_2), 4.5 (1 H, s, OH) (D_2O exchangeable), 6.9 (1 H, m, Ar*H*), 7.1 (1 H, m, Ar*H*), 7.3 (1 H, d.d., O*H*), 7.4 (2 H, d.d., Ar*H*); δ_C 13.9 ($CO_2CH_2CH_3$), 14.1 (alkyl *C*H$_3$), 22.7 (C-3 *C*H$_2$), 25.6 (alkyl *C*H$_2$), 27.7 (alkyl *C*H$_2$), 27.8 (alkyl *C*H$_2$), 29.3 (alkyl *C*H$_2$), 29.4 (alkyl *C*H$_2$), 29.5 (alkyl *C*H$_2$), 29.6 (alkyl *C*H$_2$), 31.9 (alkyl *C*H$_2$), 53.0 (CO_2CH_2), 60.7 (C-3 *C*H), 78.4 (Ph_2C(OH)), 125.2 (Ar), 125.4 (Ar), 126.6 (Ar), 126.8 (Ar), 128.1 (Ar), 128.2 (Ar), 128.3 (Ar), 130.1 (Ar), 144.3 (Ar), 147.4 (Ar), 177.1 (C=O).

10.2.2. *N-tert*-Butyloxycarbonyl pyroglutamic acid derivatives

Ethyl (*5*S)*-pyrrolidin-2-one-5-carboxylate* **94**

(5*S*)-(+)-Pyrrolidin-2-one-5-carboxylic acid (101.35 g, 0.78 mol) was dissolved in dry DMF (300 ml) with triethylamine (109 ml, 0.78 mol). Bromoethane (65 ml, 0.86 mol) was added to the stirred solution, resulting in a pale yellow mixture, which was left to stir at 20 °C for 2 days. The mixture was then filtered, and the solid washed with diethyl ether (500 ml). The combined filtrates were concentrated *in vacuo*, and the residue diluted with diethyl ether (500 ml). The resulting solid was filtered off, and the filtrate concentrated *in vacuo*. This gave a brown oily residue purified by Claisen distillation (b.p. 250 °C / 30 mm / Hg), to give a pale golden oil (80.0 g, 65%). The pure compound is reported[102] as being an oily residue (b.p. 55 °C / 0.1 mm / Hg), or a low melting solid (Literature[76] m.p. 48-50 °C). δ_H 1.3 (3 H, t, *J* 7.2, CH_2CH_3), 2.2 (1 H, m, C-4 CH_2), 2.4 (3 H, m, C-3 CH_2, C-4 CH_2), 4.2 (3 H, q, *J* 7.2, CH_2CH_3, C-5 $CHCO_2$), 6.5 (1 H, s, NH).

(*5*S)*-5-Hydroxymethyl-pyrrolidin-2-one* **92**

Ethyl (5*S*)-pyrrolidin-2-one-5-carboxylate compound **94** (123.1 g, 0.78 mol) was dissolved in a mixture of dry THF (250 ml) and dry methanol (250 ml), and cooled to 0 °C. To the stirred solution was added sodium borohydride (59 g, 1.56 mol), in small portions over 1 h, resulting in vigorous effervescence. Following addition, the mixture was left to stir at 0 °C for 1 h, before being cautiously diluted with methanol (200 ml), added slowly. Slow addition of concentrated hydrochloric acid (100 ml) resulted in a milky white mixture, which was filtered through celite, the residue being washed with 1:1 methanol/THF (200 ml). The filtrate was concentrated *in vacuo*, giving a colourless oil (115 g), which was purified by dry flash column chromatography (20% methanol/ethyl

acetate), the chromatography being repeated using the same conditions. After the second chromatographic separation, a pale yellow waxy solid was obtained (59.13g). Recrystallisation of this from acetone yielded (5*S*)-5-hydroxymethylpyrrolidin-2-one as a colourless crystalline solid (39.74 g, 44%) m.p. 84-86 °C (Literature[78] m.p. 72-73 °C). ν_{max} (KBr)/cm^{-1} 3204vs (O-H), 1662vs (C=O); δ_H 1.8 (1 H, m, C-4 C*H*$_2$), 2.2 (1 H, m, C-4 C*H*$_2$), 2.4 (2 H, m, CHC*H*$_2$OH), 3.5 (1 H, q, C-3 C*H*$_2$), 3.7 (1 H, d.d., C-3 C*H*$_2$), 3.8 (1 H, m, C-5 C*H*CH$_2$OH), 4.0 (1 H, s, O*H*), 7.3 (1 H, s, N*H*); δ_C 22.6 (C-4 *C*H$_2$), 30.3 (C-3 *C*H$_2$), 56.5 (C-5 *C*H), 65.9 (*C*H$_2$OH), 179.4 (*C*=O).

(5*S*) 5-(tert-*Butyldimethyl-silyloxymethyl)-pyrrolidin-2-one*[79] **95**

tert-Butyldimethylsilyl chloride (12.07 g, 80 mmol) was added to a stirred solution of (*S*)-5-hydroxymethyl-pyrrolidin-2-one compound **92** (7.68 g, 66 mmol) and imidazole (11.37 g, 167 mmol) in dry DMF (150 ml) at 20 °C. After dissolution of the solid, the colourless solution was left to stand for 16 hours. The mixture was concentrated *in vacuo*, and the resulting oil diluted with ethyl acetate (200 ml). The solution was washed with brine (3 x 100 ml), dried ($MgSO_4$), and concentrated *in vacuo*. This gave the title compound as a golden oil (9.39 g, 62%), used without further purification. ν_{max} (film)/cm^{-1} 3210br (NH), 1740vs (C=O), 1690vs (C=O); δ_H 0.8 (14 H, s, SiC(C*H*$_3$)$_3$), 1.7 (1 H, m, C-4 C*H*$_2$), 2.1 (1 H, m, C-4 C*H*$_2$) 2.2 (2 H, m, CHC*H*$_2$OSi), 3.4 (1 H, t, C-3 C*H*$_2$), 3.6 (1 H, d, C-3 C*H*$_2$), 3.7 (1 H, m, C-5 C*H*CH$_2$O), 5.9 (1 H, s, N*H*); δ_C 22.7 (C-4 *C*H$_2$), 25.6 (SiC(*C*H$_3$)$_3$), 29.8 (C-3 *C*H$_2$), 55.8 (C-5 *C*H$_2$), 66.9 (*C*H$_2$OSi), 117.7 (*C*(CH$_3$)$_3$), 178.0 (C=O).

N-tert-*Butyloxycarbonyl-5-*(tert-*butyldimethylsilyloxymethyl)-pyrrolidin-2-one*[79] **89**

Di-*tert*-butyldicarbonate (9.84 g, 45 mmol) was added to a stirred solution of 5-(*tert*-butyldimethylsilyloxymethyl)-pyrrolidin-2-one compound **95** (9.39 g, 41.0 mmol) and dimethylaminopyridine (250 mg, 2.0 mmol) in DCM (200 ml) at 20 °C. The solution was left to stand for 3 days, before addition of a further portion of di-*tert*-butyldicarbonate (2 g, 9.2 mmol). After 24 hours, the mixture was concentrated *in vacuo*, and the residue was purified by flash column chromatography (40% ethyl acetate/petroleum). This gave a colourless oil (10.4 g, 77%). ν_{max} (film)/cm^{-1} 1798vs (C=O),1753vs (C=O), 1712vs (C=O); δ_H 0.8 (9 H, s, SiC(CH_3)$_3$), 1.5 (9 H, s, $CO_2C(CH_3)_3$), 2.0 (2 H, d.d., C-4 CH_2), 2.3 (1 H, d.d., C-3 CH_2), 2.6 (1 H, d.d., C-3 CH_2), 3.8 (1 H, d.d., C-5 $CHCH_2O$), 4.1 (2 H, m, $CHCH_2OSi$); δ_C 14.2 (Si$C(CH_3)_3$), 18.1(C-4 CH_2), 21.2 ($CO_2C(CH_3)_3$), 25.7 (SiC(CH_3)$_3$), 32.4 (C-3 CH_2), 58.9 (C-5 $CH(CH_2)_2$), 64.3 (CH_2OSi), 82.7 ($C(CH_3)_3$), 150.1 ($NCO_2C(CH_3)_3$), 174.9 (CON).

Benzyl methanesulfonate

Methanesulfonyl chloride (0.3 ml, 4.4 mmol) was added to a cold (0 °C) stirred solution of benzyl alcohol (0.38 ml, 3.6 mmol) and triethylamine (0.6 ml, 4.4 mmol) in dry THF (10 ml), resulting in a white precipitate. After stirring for 1 hour, the supernatant was removed *via* syringe, and then added slowly to a cold (-78 °C), stirred solution of anion generated from compound **89**. The reaction was continued following the procedure described for compound **98**.

3-Phenylpropyl methanesulfonate

Methanesulfonyl chloride (0.57 ml, 7.34 mmol) was added to a cold (0 °C) stirred solution of 3-phenylpropanol (1.0 ml, 7.34 mmol) and triethylamine (1.02 ml, 7.34 mmol) in dry THF (50 ml), resulting in a white precipitate. After stirring for 1 hour, there was some alcohol still visible by TLC. A further 2 drops of methanesulfonyl chloride were then added, and after 10 minutes, no further alcohol was visible by TLC. The supernatant was removed *via* syringe, and then added slowly to a cold (-78 °C), stirred solution of anion generated from compound **89**. The reaction was continued following the procedure described for compound **98**.

Octadecyl iodide

A solution of octadecyl bromide (0.5 g, 1.5 mmol) in dry acetone (5 ml) was added to a solution of sodium iodide (263 mg, 1.57 mmol) in acetone (5 ml). After 1 hour at 20 °C, a white precipitate had formed, which was removed by filtration. The filtrate was concentrated *in vacuo*, to give a yellow waxy solid, which was triturated with dry diethyl ether. This resulted in the iodide as a low melting colourless solid (190 mg, 33%). (Literature[142] m.p. 33-35 °C).

N-tert-*Butyloxycarbonyl-5-(*tert-*butyldimethylsilyloxymethyl)-3-hexadecanoyl-pyrrolidin-2-one* **98**

A 1 M solution of lithium hexamethyldisilazide in THF (3.1 ml, 3.1 mmol) was added slowly to a cold (-78 °C), stirred solution of diprotected pyrrolidin-2-one compound **89** (1.0 g, 3.04 mmol) in dry THF (100 ml). After 0.5 hour at -65 °C, a solution of stearoyl chloride (1.03 ml, 3.04 mmol) in dry THF (15 ml) was added over 10 minutes, with the reaction temperature being maintained below -60 °C. Following

addition, the mixture was left to stir at -78 °C for 1 hour. The reaction was quenched with saturated ammonium chloride solution (100 ml), and the mixture separated. The aqueous layer was extracted with ethyl acetate (3 x 75 ml), and the combined organic fractions dried ($MgSO_4$) and concentrated *in vacuo*. The white solid residue was purified by flash column chromatography (10% ethyl acetate/petroleum), resulting in the title pyrrolidinone being obtained as a colourless oil (380 mg, 20%). ν_{max} (film)/cm^{-1} 1781vs (C=O), 1741vs (C=O), 1715vs (C=O), 1679s (C=O); δ_H 0.9 (9 H, s, $SiC(CH_3)_3$), 1.3 (30 H, s, alkyl CH_2), 1.5 (2 H, s, alkyl CH_2), 1.6 (9 H, s, $CO_2C(CH_3)_3$), 2.4 (2 H, t, C-4 CH_2), 2.5 (1 H, m, $COCH_2CH_2$), 2.8 (1 H, t, C-3 CH), 3.7 (2 H, m, CH_2OSi), 4.1 (1 H, m, C-5 CH); δ_C 14.1 (alkyl CH_3), 22.7 (C-4 CH_2), 25.0 ($CO_2C(CH_3)_3$), 25.8 ($SiC(CH_3)_3$), 26.7 (alkyl CH_2), 28.1 (alkyl CH_2), 29.1 (alkyl CH_2), 29.3 (alkyl CH_2), 29.4 (alkyl CH_2), 29.5 (alkyl CH_2), 29.6 (alkyl CH_2), 29.7 (alkyl CH_2), 31.9 (alkyl CH_2), 34.2 (C-3 CH_2), 55.0 (C-5 CH), 63.8 (CH_2OSi), 84.2 ($C(CH_3)_3$), 161.2 ($NCO_2C(CH_3)_3$), 172.5 (CON).

N-tert-*Butyloxycarbonyl-3-benzyl-5-(*tert-*butyldimethylsilyloxymethyl)-pyrrolidin-2-one*

101

A 1 M solution of lithium hexamethyldisilazide in THF (3.04 ml, 3.04 mmol) was added slowly to a cold (-78 °C), stirred solution diprotected pyrrolidin-2-one (compound **89**) (1.0 g, 3.04 mmol) in dry THF (50 ml). After 0.5 hour at -78 °C, benzyl bromide (0.35 ml, 2.89 mmol) was added, and the pale yellow mixture was left to stir for 4 h at -78 °C. The reaction was quenched with saturated ammonium chloride solution (50 ml), and the mixture separated. The aqueous layer was extracted with ethyl acetate (3 x 40 ml), and the combined organic fractions washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The brown oily residue was purified by flash column chromatography (20% ethyl acetate/petroleum), resulting the title compound, a colourless oil (590 mg, 46%).

ν_{max} (film)/cm^{-1} 3026m (Ar C-H), 1787s (C=O), 1747vs (C=O), 1712vs (C=O), 1603vs (C=O); δ_H 0.8 (9 H, s, SiC(C*H*$_3$)$_3$), 1.5 (9 H, s, CO$_2$C(C*H*$_3$)$_3$), 1.8 (1 H, m, C-4 C*H*$_2$), 2.0 (1 H, m, C-4 C*H*$_2$), 2.5 (1 H, t, C-3 C*H*), 3.1 (1 H, m, PhC*H*$_2$), 3.3 (1 H, d, CHC*H*$_2$OSi), 3.6 (1 H, d, CHC*H*$_2$OSi), 3.8 (1 H, m, C-5 C*H*CH$_2$O), 4.0 (5 H, m, Ar); δ_C 14.2 (Si*C*(CH$_3$)$_3$), 18.2 (C-4 *C*H$_2$), 25.6 (CO$_2$C(*C*H$_3$)$_3$), 28.1 (SiC(*C*H$_3$)$_3$), 37.1 (Ph*C*H$_2$), 44.4 (C-5 *C*H(CH$_2$)$_2$), 56.9 (C-3 *C*H), 64.0 (*C*H$_2$OSi), 82.8 (*C*(CH$_3$)$_3$), 126.3 (Ar), 128.5 (Ar), 139.3 (Ar), 150.2 (N*C*O$_2$C(CH$_3$)$_3$), 175.8 (*C*ON). *m/z* (CI) 420 (M$^+$, 38 %).

N-tert-*Butyloxycarbonyl-3-farnesyl-5-(*tert-*butyldimethylsilyloxymethyl)-pyrrolidin-2-one*

102

A 1 M solution of lithium hexamethyldisilazide in THF (1.52 ml, 1.52 mmol) was added slowly to a cold (-78 °C), stirred solution of diprotected pyrrolidin-2-one (compound **89**) (500 mg, 1.52 mmol) in dry THF (25 ml). After 0.5 hour at -78 °C, farnesyl bromide (0.35 ml, 2.89 mmol) was added to the pale yellow mixture. Following addition, the mixture was left to stir for 2 hours at -78 °C, and 3 hours at 20 °C. The reaction was quenched with saturated ammonium chloride solution (30 ml), and the mixture separated. The aqueous layer was extracted with ethyl acetate (3 x 40 ml), and the combined organic fractions washed with brine, dried (MgSO$_4$), and concentrated *in vacuo*. The brown oily residue was purified by flash column chromatography (10% ethyl acetate/petroleum), resulting in the title compound being obtained as a pale yellow oil (260 mg, 32%). ν_{max} (film)/cm^{-1} 1788vs (C=O), 1752vs (C=O), 1712vs (C=O); δ_H 0.9 (9 H, s, SiC(C*H*$_3$)$_3$), 1.5 (9 H, s, CO$_2$C(C*H*$_3$)$_3$), 1.6 (10 H, s, C=CC*H*$_3$), 2.0 (10 H, m, C=CHC*H*$_2$), 2.5 (2 H, m, C-4 C*H*$_2$), 2.8 (1 H, m, C-3 C*H*), 3.6 (1 H, m, CHC*H*$_2$OSi), 3.8 (1 H, d, CHC*H*$_2$OSi), 4.1 (1 H, m, C-5 C*H*CH$_2$O), 5.1 (3 H, t, C=C*H*CH$_2$); δ_C 16.0

(Si*C*$(CH_3)_3$), 16.2 (C=C*C*H_3), 17.7 (C=C*C*H_3), 25.7 (C-4 *C*H_2), 25.8 (C=C*C*H_3), 26.6 (farnesyl *C*H_2), 26.7 (farnesyl *C*H_2), 27.8 (farnesyl *C*H_2), 28.1 (SiC(*C*$H_3)_3$), 29.3 (CO_2C(*C*$H_3)_3$), 34.3 (C=C(CH_3)*C*H_2), 39.8 (C=C(CH_3)*C*H_2), 42.9 (C-5 *C*H($CH_2)_2$), 57.0 (C-3 *C*H), 64.0 (*C*H_2OSi), 82.8 (*C*$(CH_3)_3$), 120.7 (CH_2*C*=C), 124.4 (CH_2*C*=C), 135.1 (C=*C*(CH_3)CH_2), 137.6 (C=*C*(CH_3)CH_2), 150.1 (N*C*O_2C($CH_3)_3$), 176.2 (*C*ON). *m/z* (CI) 534 (M^+, 52 %).

trans-*1-Bromo-dodec-2-ene* **104**

Triphenylphosphine (1.13 g, 4.28 mmol) was added in portions to a stirred solution of *trans*-dodec-2-ene-1-ol (0.72 g, 3.89 mmol) and carbon tetrabromide (1.42 g, 4.28 mmol) in DCM (5 ml) at 20 °C. This produced effervescence, and the resulting pale brown solution was left to stir at 20 °C for 16 hours. The solution was concentrated *in vacuo*, and the residue purified by flash column chromatography (15% ethyl acetate/petroleum), to yield the target compound as a colourless oil (800 mg, 83%). ν_{max} (film)/cm^{-1} 3020m (C-H), 1660s (C=C); δ_H 0.9 (3 H, t, C*H*$_3$), 1.3 (14 H, s, C*H*$_2$), 2.0 (2 H, m, CHC*H*$_2$CH_2), 3.9 (2 H, d, CHC*H*$_2$Br), 5.7 (2 H, m, C=C*H*); δ_C 14.1 (*C*H_3), 22.7 (*C*H_2CH_3), 28.8 (*C*H_2), 29.1 (*C*H_2), 29.3 (*C*H_2), 29.5 (*C*H_2), 31.9 (*C*H_2), 32.0 (*C*H_2), 33.7 (C*H*$_2$Br), 126.2 (C=*C*CH_2Br), 136.8 (*C*=CCH_2Br).

N-tert-*Butyloxycarbonyl-3-dodec-2-enyl-5-*(tert-*butyldimethylsilyloxymethyl)-pyrrolidin-2-one* **105**

A 1 M solution of lithium hexamethyldisilazide in THF (1.1 ml, 1.1 mmol) was added slowly to a cold (-78 °C), stirred solution of diprotected pyrrolidin-2-one (compound **89**) (329 mg, 1.0 mmol) in dry THF (20 ml). After 0.5 hour at -78 °C, a solution of *trans*-1-bromo-dodec-2-ene compound **104** (271 mg, 1.1 mmol) in dry THF (1 ml) was added to the pale yellow mixture. Following addition, the mixture was left to stir for 2 hours at -78 °C, and 3 hours at 20 °C. The reaction was quenched with saturated ammonium chloride solution (25 ml), and the mixture separated. The aqueous layer was extracted with ethyl acetate (3 x 20 ml), and the combined organic fractions washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The yellow oily residue was purified by flash column chromatography (10% ethyl acetate/petroleum), resulting in isolation of the carbamate compound **102** as a colourless oil (90 mg, 18%). ν_{max} (film)/cm^{-1} 1790vs (C=O), 1753vs (C=O), 1714vs (C=O); δ_H 0.9 (14 H, s, SiC(CH_3)$_3$, CH_2CH_3), 1.3 (16 H, s, alkyl CH_2), 1.6 (9 H, s, COC(CH_3)$_3$), 1.8 (1 H, m, C-4 CH_2), 2.0 (2 H, m, C=CCH_2CH), 2.1 (1 H, m, C-4 CH_2), 2.6 (1 H, m, C-3 CH), 2.8 (0.5 H, m, C-3 CH, isomer), 3.7 (1 H, d.d, CHCH_2OSi), 3.8 (1 H, d.d, CHCH_2OSi), 4.1 (1 H, m, C-5 CHCH_2O), 5.4 (2 H, m, C=C*H*); δ_C 14.2 (Si*C*(CH_3)$_3$), 22.7 (C-4 *C*H_2), 25.9 (alkyl *C*H_2), 27.7 (alkyl *C*H_2), 28.1 (COC(*C*H_3)$_3$), 29.1 (SiC(*C*H_3)$_3$), 29.3 (alkyl *C*H_2), 29.5 (alkyl *C*H_2), 29.6 (alkyl *C*H_2), 31.9 (alkyl *C*H_2), 32.6 (alkyl *C*H_2), 34.2 (alkyl *C*H_2), 42.6 (C-5 *C*H(CH_2)$_2$), 57.0 (C-3 *C*H), 64.0 (*C*H_2OSi), 82.7 (*C*(CH_3)$_3$), 126.4 (*C*=C), 133.3 (*C*=C), 150.3 (N*C*O_2C(CH_3)$_3$), 175.2 (*C*ON).

Ethyl N-(tert-*butyloxycarbonyl)-pyrrolidin-2-one-5-carboxylate*[93] **81**

Di-*tert*-butyldicarbonate (6.02 g, 28 mmol) and dimethylaminopyridine (140 mg, 1.15 mmol) were added to a stirred solution of ethyl (5*S*)-pyrrolidin-2-one-5-carboxylate compound **94** (3.79 g, 23 mmol) in DCM (100 ml) at 20 °C. This gave a pale yellow solution, which was left to stand at 20 °C for 2 days. The solution was concentrated *in vacuo*, giving a yellow oil. Purification by flash column chromatography (50% ethyl acetate/petroleum) yielded the title compound as a yellow oil (5.13 g, 87%). ν_{max} (film)/cm^{-1} 1793vs (C=O), 1748vs (C=O), 1716vs (C=O); δ_H 1.3 (3 H, t, CH_2CH_3), 1.5 (9 H, s, $C(CH_3)_3$), 2.0 (1 H, m, C-4 CH_2), 2.4 (3 H, m, C-3 CH_2, C-4 CH_2), 4.2 (2 H, q, CH_2CH_3), 4.6 (1 H, d, C-5 $CHCO_2$); δ_C 14.2 (CH_2CH_3), 21.6 (C-4), 27.4 ($C(CH_3)_3$), 31.2 (C-3), 59.0 (C-5), 61.7 (CH_2CH_3), 83.6 ($C(CH_3)_3$), 149.7 ($NCO_2C(CH_3)_3$), 171.4 (CO_2CH_2), 173.4 (CON).

*Ethyl 3-(1-hydroxydecyl)-1-*N-*(*tert-*butyloxycarbonyl)-pyrrolidin-2-one-5-carboxylate* **111**

A 1 M solution of lithium hexamethyldisilazide in THF (4.8 ml, 4.8 mmol) was added slowly to a cold (-78 °C), stirred solution of diprotected pyrrolidinone (compound **89**) (628 mg, 2.4 mmol) in dry THF (50 ml). Addition was at a rate that maintained the reaction temperature below -60 °C. Following the addition, the yellow mixture was left to stir at -78 °C. After 1 hour, a solution of boron trifluoride etherate (0.56 ml, 4.4 mmol) and decanal (0.83 ml, 4.4 mmol) in dry THF (1 ml) was added dropwise. Following addition, the mixture was left to stir for 1 hour at -78 °C. The reaction was quenched with saturated ammonium chloride solution (50 ml), and the mixture extracted with diethyl ether (4 x 50 ml), and the combined organic fractions dried ($MgSO_4$), and concentrated *in vacuo*. The golden oily residue was purified by flash column

chromatography (40% ethyl acetate/petroleum), resulting in isolation of a pale golden oil (540 mg, 69%), a mixture of isomers R_f 0.58 and R_f 0.52 , and a single isomer R_f 0.52 (50 mg, 7%), which gave a colourless solid on standing, m.p. 70-74 °C. (Single isomer) ν_{max} (film)/cm^{-1} 3500m, br (O-H), 3496m (OH), 1787vs (C=O), 1748vs (C=O); δ_H 0.9 (3 H, t, alkyl C*H*$_3$), 1.2 (19 H, m, alkyl C*H*$_2$, $CO_2CH_2CH_3$), 1.4 (9 H, s, C(C*H*$_3$)$_3$), 1.6 (2 H, m, CH(OH)C*H*$_2$), 2.4 (1 H, m, C-3 C*H*), 2.5 (1 H, m, C-4 C*H*$_2$), 2.6 (1 H, m, C-4 C*H*$_2$), 3.8 (1 H, m, C*H*(OH)), 4.1 (1 H, br. s, O*H*) (D_2O exchangeable), 4.2 (2 H, q, CO_2CH_2), 4.4 (1 H, t, C-5 C*H*CO_2); δ_C 14.1 (*C*H$_3$), 22.7 (C-4 CH$_2$), 24.8 (alkyl *C*H$_2$), 25.0 (alkyl *C*H$_2$), 27.8 (C(*C*H$_3$)$_3$), 29.3 (alkyl *C*H$_2$), 29.6 (alkyl *C*H$_2$), 31.9 (alkyl *C*H$_2$), 34.5 (alkyl *C*H$_2$), 47.3 (C-3 *C*HCH(OH)), 57.5 (C-5 *C*HCO_2), 61.8 (CO_2CH_2), 72.1 (*C*HOH), 84.3 (*C*(CH$_3$)$_3$), 155.3 (N*C*O_2C(CH$_3$)$_3$), 169.6 (*C*O_2CH$_2$), 170.2 (*C*O_2CH$_2$), 175.9 (*C*ON).

(6-Oxo-tetrahydropyran-3-yl)-carbamic acid tert-*butyl ester* **114**

A 1 M solution of tetrabutylammonium fluoride in THF (1.1 ml, 1.1 mmol) was added to a stirred solution of diprotected pyrrolidinone (compound **89**) (329 mg, 1.0 mmol) in dry THF (5 ml) at 20 °C. This gave a pale pink solution, which was left to stir for 2 hours. The solution was then concentrated *in vacuo*, and the residue taken up in DCM (25 ml). The solution was washed with saturated ammonium chloride solution (1 x 20 ml), dried ($MgSO_4$), and concentrated *in vacuo*, giving the title ester as a golden oil (160mg, 70%). ν_{max} (film)/cm^{-1} 3232s (NH), 1742vs (C=O), 1698vs (C=O), 1277vs (C-O); δ_H 1.5 (9 H, s, C(C*H*$_3$)$_3$), 1.8 (1 H, m, C-3 C*H*$_2$), 2.3 (1 H, m, C-2 C*H*$_3$), 2.4 (2 H, m, C-2 C*H*$_2$), 3.9 (2 H, m, C-4 C*H*$_2$, C-5 C*H*$_2$), 4.2 (1 H, d, C-5 C*H*$_2$), 5.9 (1 H, br. s, N*H*); δ_C 23.1 (C-3 *C*H$_2$), 27.7 (C(*C*H$_3$)$_3$), 29.3 (C-4 CH$_2$), 52.7 (C-5 CH), 69.5 (C-6 CH$_2$), 82.9 (C(CH$_3$)$_3$), 153.2 (NH*C*O_2), 177.6 (C-1 *C*O_2).

N-tert-*Butyloxycarbonyl-5-hydroxymethylpyrrolidin-2-one*[90] **116**

Toluene-*p*-sulfonic acid (570 mg, 3.0 mmol) was added to a stirred solution of diprotected pyrrolidinone compound **89** (1.0 g, 3.0 mmol) in methanol (6 ml) at 20 °C. After 2 hours, the solution was made slightly basic by addition of saturated sodium hydrogencarbonate solution. The mixture was then concentrated *in vacuo*, and the residue taken up in water (10 ml), and extracted with DCM (5 x 10ml). The combined extracts were dried ($MgSO_4$), and concentrated *in vacuo*. This gave a colourless oil, which was purified by flash column chromatography (ethyl acetate). This yielded a colourless oil, which solidified on standing (70 mg, 10%), m.p. 92-95 °C (Literature[90] m.p. 98-99 °C) . ν_{max} (KBr)/cm^{-1} 3440m, br (O-H), 1778vs (C=O), 1690vs (C=O); δ_H 1.6 (10 H, s, $COC(CH_3)_3$), 2.0 (1 H, m, C-4 CH_2), 2.2 (1 H, m, C-4 CH_2), 2.4 (1 H, m, C-3 CH_2), 2.6 (1 H, t, *OH*) (D_2O exchangeable), 2.7 (1 H, quint., C-3 CH_2), 3.7 (1 H, m, $CHCH_2OH$), 3.9 (1 H, m, $CHCH_2OH$), 4.3 (1 H, m, C-5 $CHCH_2O$); δ_C 20.9 (C-4 CH_2), 28.1 ($COC(CH_3)_3$), 32.0 (C-3 CH_2), 59.4 (C-5 $CH(CH_2)_2$), 64.7 (CH_2OH), 83.4 ($C(CH_3)_3$), 150.8 ($NCO_2C(CH_3)_3$), 174.8 (*C*ON). *m/z* (EI) 216.1 ($M+H^+$, 20 %).

Methyl pyrrolidin-2-one-5-carboxylate[110] **119**

Oxalyl chloride (0.96 ml, 1.0 mmol) was added dropwise to an ice cold stirred solution of *S*-(+)-pyroglutamic acid (1.29 g,10 mmol) in dry methanol (50 ml). The mixture was then allowed to warm to 20 °C, and left to stir for 1 hour. The colourless solution was then concentrated *in vacuo*, and the residue taken up in DCM (30 ml). The solution was washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*, to give the title compound as a colourless oil (1.24 g, 87%), used without further purification. ν_{max} (film)/cm^{-1} 3238m, br. (N-H), 1743vs (C=O), 1698vs (C=O); δ_H 2.2 (1 H, m, C-4 CH_2),

2.4 (2 H, m, C-3 CH_2), 2.5 (1 H, m, C-4 CH_2), 3.8 (3 H, s, CH_3), 4.3 (1 H, m, C-5 $CHCO_2$), 6.7 (1 H, s, br., NH).

Methyl N-*benzyl*-*pyrrolidin-2-one-5-carboxylate* **120**

Sodium hydride, 60% dispersion in oil, (690 mg, 18 mmol) was added to an ice cold stirred solution of methyl L-pyroglutamate compound **119** (1.24 g, 8.67 mmol) in dry DMF (50 ml). After 1 hour at 0 °C, the grey suspension was allowed to warm to 20 °C, and benzyl bromide (1.06 ml, 8.76 mmol) added. The mixture was stirred at 20 °C for 16 hours, before quenching with ice cold water (50 ml). The mixture was extracted with ethyl acetate (5 x 20 ml), and the combined extracts washed (brine), dried ($MgSO_4$), and concentrated *in vacuo*. This gave a yellow oily residue (1.93 g), which was purified by flash column chromatography (50% ethyl acetate/petroleum), to give the title compound as a colourless oil (870 mg, 43%). ν_{max} (film)/cm^{-1} 3030m (Ar C-H), 1741vs (C=O), 1697vs (C=O); δ_H 2.4 (4 H, m, C-3 CH_2, C-4 CH_2), 3.7 (3 H, s, CO_2CH_3), 4.0 (2 H, m, $PhCH_2$), 5.0 (1 H, d, C-5 $CHCH_2O$), 7.2 (5 H, m, ArH); δ_C 22.8 (C-4 CH_2), 29.5 (C-3 CH_2), 45.6 ($PhCH_2$), 52.4 (CO_2CH_3), 58.7 (C-5 $CHCO_2$), 127.8 (Ar), 128.5 (Ar), 128.7 (Ar), 135.8 (Ar), 172.3 (CO_2CH_3), 175.0 (CON)

N-(tert-*Butyldimethylsilyl)-5-(*tert-*butyldimethylsilyloxymethyl)-pyrrolidin-2-one* **121**

Triethylamine (1.81 ml, 13 mmol) and *tert*-butyldimethylsilyl chloride (1.96 g, 13 mmol) were added to a stirred solution of (5*S*)-5-hydroxymethyl-pyrrolidin-2-one compound **92** (680 mg, 5.9 mmol) in DCM (10 ml) at 20 °C. This resulted in a white cloudy mixture, which went pale brown on standing. After 2 days, the mixture was concentrated *in vacuo*, and the residue triturated with ice cold, dry ether. The mixture was filtered, and the filtrate concentrated *in vacuo*. The residue was purified by flash column chromatography (50% ethyl acetate/petroleum) to give the title compound as a colourless oil (1.05 g, 56%). ν_{max} (film)/cm^{-1} 1742vs (C=O), 1692vs (C=O), 1665s (C=O); δ_H 0.1 (6 H, s, SiCH_3), 0.8 (20 H, d, SiC(CH_3)$_3$), 1.6 (2 H, br. s, C-4 CH_2), 2.0 (1 H, m, C-3 CH_2), 2.2 (1 H, m, C-3 CH_2), 2.4 (1 H, m, C-5 C*H*CH$_2$O), 3.3 (1 H, m, CHCH_2OSi), 3.5 (1 H, m, CHCH_2OSi); δ_C -4.5 (Si*C*H$_3$), -3.6 (Si*C*H$_3$), 14.2 (Si*C*(CH$_3$)$_3$), 18.0 (C-4 *C*H$_2$), 19.3 (SiC(*C*H$_3$)$_3$), 21.1(SiC(*C*H$_3$)$_3$), 55.8 (C-3 *C*H$_2$) , 59.9 (C-5 *C*H(CH$_2$)$_2$), 65.7 (*C*H$_2$OSi), 184.2 (*C*ON).

Ethyl N-*phenylacetyl-pyrrolidin-2-one-5-carboxylate* **124**

A solution of ethyl pyrrolidin-2-one-5-carboxylate compound **94** in dry THF (20 ml) and hexane (2 ml) was cooled to -100 °C. A 1 M solution of lithium hexamethyldisilazide in THF (7.0 ml, 7.0 mmol) was then added slowly, maintaining the internal reaction temperature below -90 °C. Following addition, the mixture was left to stir at -100 °C for 1 hour, before adding phenylacetyl chloride (0.84 ml, 0.64 mmol). After a further 1 hour at -100 °C, the reaction was quenched by pouring into saturated ammonium chloride solution (30 ml), and stirring for 1 hour. The mixture was then extracted with ethyl acetate (3 x 20 ml), and the combined extracts washed with 2M

sodium hydroxide (2 x 30 ml), water (1 x 30 ml), and finally brine (1 x 30 ml). The organic phase was dried ($MgSO_4$), and concentrated *in vacuo*, giving a waxy yellow residue. Purification of this material by flash column chromatography (50% DCM/chloroform) resulted in the title compound as a pale yellow oil (140 mg, 8%). ν_{max} (film)/cm^{-1} 1747vs (C=O), 1700vs (C=O); δ_H 1.2 (3 H, t, *J* 6.9, CH_3), 2.1 (1 H, m, C-4 CH_2), 2.3 (1 H, m, C-4 CH_2), 2.6 (1 H, m, C-3 CH_2), 2.8 (1 H, m, C-3 CH_2),), 4.2 (2 H, q, *J* 6.9, CH_2CH_3), 4.3 (2 H, d, $PhCH_2$), 4.8 (1 H, d.d., C-5 $CHCO_2$), 7.3 (5 H, m, Ar*H*); δ_C 14.1 (CH_3), 21.3 (C-4 CH_2), 32.0 (C-3 CH_2), 42.7 ($PhCH_2$), 58.2 (C-5 $CHCO_2$), 61.8 (CO_2CH_2), 127.0 (Ar), 128.4 (Ar), 129.7 (Ar), 133.7 (Ar), 169.2 (NCOBn), 171.9 ($CHCO_2$), 174.4 (C-2 CON).

10.2.3. *N*-Benzyloxycarbonyl pyroglutamic acid derivatives

Methyl N-*(benzyloxycarbonyl)-pyrrolidin-2-one-5-carboxylate* **132**

Oxalyl chloride (0.35 ml, 4.0 mmol) was added dropwise to an ice cold stirred solution of 1-*N*-Z-pyrrolidin-2-one-5-carboxylic acid (Nova) (1.0 g, 3.8 mmol) in dry methanol (40 ml). The colourless solution was allowed to warm to 20 °C, and was left to stir for 1 hour. The solution was concentrated *in vacuo*, and the residue dissolved in diethyl ether (100 ml). The ethereal solution was washed with saturated sodium hydrogencarbonate solution (2 x 40 ml), dried ($MgSO_4$), and concentrated *in vacuo*. The colourless oily residue was purified by flash column chromatography (60% ethyl acetate/petroleum), to give the title compound as a colourless oil (160 mg, 15%). ν_{max} (film)/cm^{-1} 3034m (Ar C-H), 1797vs (C=O), 1748vs (C=O), 1722 (C=O); δ_H 2.1 (1 H, m, C-4 CH_2), 2.5 (3 H, m, C-3 CH_2, C-4 CH_2), 3.7 (3 H, s, CO_2CH_3), 4.7 (1 H, d, $CHCO_2Me$), 5.3 (2 H, q, CH_2Ph), 7.4 (5 H, m, Ar*H*); δ_C 21.8 (C-4 CH_2), 31.0 (C-3 CH_2),

52.7 (CO_2CH_3), 58.6 (C-5 $CHCO_2$), 68.4 ($PhCH_2$), 128.2 (Ar), 128.5 (Ar), 128.6 (Ar), 135.0 (Ar), 171.5 (CO_2CH_3), 172.8 (CON).

Ethyl N-*(benzyloxycarbonyl)-pyrrolidin-2-one-5-carboxylate* **133**

Bromoethane (0.31 ml, 4.2 mmol) was added to a stirred solution of 1-*N*-Z-pyrrolidin-2-one-5-carboxylic acid (Nova) (1.0 g, 3.8 mmol), and triethylamine (0.53 ml, 3.8 mmol) in dry DMF (15 ml) at 20 °C. The mixture was stirred for 1 hour, and left to stand in a stoppered flask for 3 days. The mixture was concentrated *in vacuo*, and the residue diluted with dry diethyl ether (50 ml). The resulting precipitate was filtered, and washed with dry diethyl ether (30 ml). The filtrate was concentrated *in vacuo*, to give a pale golden oil, which was purified by flash column chromatography (50% ethyl acetate/petroleum). This gave the title compound as a colourless oil (880 mg, 80%). ν_{max} (film)/cm^{-1} 3034m (Ar C-H), 1799vs (C=O), 1738vs (C=O); δ_H 1.2 (3 H, t, CH_2CH_3), 2.0 (1 H, m, C-4 CH_2), 2.5 (3 H, m, C-3 CH_2, C-4 CH_2), 4.2 (2 H, q, CH_2CH_3), 4.7 (1 H, d.d. $CHCO_2Et$), 5.3 (2 H, q, CH_2Ph), 7.4 (5 H, m, Ar*H*); δ_C 14.0 (CH_2CH_3), 21.9 (C-4 CH_2), 22.3 (C-3 CH_2), 31.0 (C-3 CH_2), 58.8 (C-5 $CHCO_2$), 61.8 (CO_2CH_2), 68.3 ($PhCH_2$), 128.2 (Ar), 128.5 (Ar), 128.6 (Ar), 135.0 (Ar), 150.9 (NCO_2CH_2Ph), 171.0 (CO_2CH_2), 172.9 (CON).

N-*(Benzyloxycarbonyl)-5-hydroxymethyl-pyrrolidin-2-one* **135**

Sodium borohydride (150 mg, 3.92 mmol) was added to a stirred solution of ethyl ester compound **133** (570 mg, 1.96 mmol) in dry THF (25 ml) at 20 °C. Dry methanol (5 ml) was then added to the mixture, resulting in slow dissolution of the solid. After stirring at 20 °C for 16 hours, the colourless solution was concentrated *in vacuo*, giving a

white solid residue. The solid was dissolved in a minimum quantity of water, and acidified to pH 2, using 2M hydrochloric acid. The mixture was then extracted with diethyl ether (4 x 40 ml), and the combined extracts washed (brine), dried ($MgSO_4$), and concentrated *in vacuo*. This gave the title alcohol as a colourless oil, used without further purification (380 mg, 78%). ν_{max} (film)/cm^{-1} 3334m, br (O-H), 3032m (Ar C-H), 1701vs (C=O), 1534m (C-O); (d^6acetone) δ_H 1.6 (1 H, m, C-4 C*H*$_2$), 2.9 (1 H, s, O*H*) (D_2O exchangeable), 3.6 (4 H, m, C-3 C*H*$_2$, C-4 C*H*$_2$, C-5 C*H*$(CH_2)_2$), 5.0 (2 H, d, C*H*$_2$Ph), 7.4 (5 H, m, Ar*H*); δ_C 14.5 (C-4 *C*H_2), 51.8 (C-3 *C*H_2), 54.0 (C-5 *C*HCO_2), 66.4 (*C*H_2OH), 66.5 (Ph*C*H_2), 128.5 (Ar), 128.6 (Ar), 129.2 (Ar).

N-*(Benzyloxycarbonyl)-5-(*tert-*butyldimethylsilyloxymethyl)-pyrrolidin-2-one* **136**

Imidazole (260 mg, 3.82mmol) was added to a stirred solution of the previous carbamate compound **135** (380 mg, 1.53 mmol) in dry DMF at 20 °C. After 5 minutes, *tert*-butyldimethylsilyl chloride (460 mg, 3.06 mmol) was added, and the solution left to stir at 20 °C for 16 hours. The colourless solution was concentrated *in vacuo* and the oily residue purified by flash column chromatography (20% ethyl acetate/petroleum). This yielded the title compound as a colourless oil (410 mg, 74%). ν_{max} (film)/cm^{-1} 3334, 3033m (Ar C-H), 1726vs (C=O); δ_H 0.9 (9 H, s, SiC(C*H*$_3)_3$), 1.6 (5 H, m), 3.6 (6 H, s), 4.9 (1 H, d, C*H*CH_2Si), 5.1 (2 H, s, C*H*$_2$Ph), 7.4 (5 H, s, Ar*H*); δ_C 18.3 (C-4 *C*H_2), 25.9 (SiC(*C*$H_3)_3$), 52.3 (C-3 *C*H_2), 62.9 (C-5 *C*HCO_2), 64.7 (*C*H_2OSi), 66.5 (Ph*C*H_2), 128.1 (Ar), 128.5 (Ar), 136.7 (Ar), 156.0 (N*C*O_2CH_2Ph).

Reaction of N-*(Benzyloxycarbonyl)-5-(*tert-*butyldimethylsilyloxymethyl)-pyrrolidin-2-one with tetrabutylammonium fluoride.*

A 1 M THF solution of tetrabutylammonium fluoride (0.61 ml, 0.61 mmol) was added to a solution of silyl ether compound **136** (200 mg, 0.55 mmol) in dry THF (20 ml). The colourless solution was left to stir at 20 °C for 30 minutes, and was then concentrated *in vacuo.* The oily residue was taken up in 50% ether/ethyl acetate (70 ml), and the solution washed with saturated ammonium chloride solution, and the organic phase dried ($MgSO_4$), and concentrated *in vacuo.* This gave an off-white solid, m.p. 87-91 °C, which appeared to be the alcohol by IR, although impure. The material was dissolved in dry DMF (5 ml), imidazole (75 mg, 1.1 mmol), and *tert*-butyldimethylsilyl chloride (135 mg, 0.88 mmol) was added, and the solution left to stir at 20 °C for 16 hours. The colourless solution was concentrated *in vacuo* and the oily residue purified by flash column chromatography (20% ethyl acetate/petroleum). This yielded the title compound as a colourless oil (60 mg, 40%), with spectral data identical to the authentic material, compound **136.**

*Ethyl 3-(1-hydroxydecyl)-*N*-(benzyloxycarbonyl)-pyrrolidin-2-one-5-carboxylate* **137**

A 1 M solution of lithium hexamethyldisilazide in THF (24 ml, 24 mmol) was added slowly to a cold (-110 °C, ethanol/liquid nitrogen slush), stirred solution of ethyl *N*-Z-pyrrolidin-2-one-5-carboxylate (compound **133**) (3.0 g, 10 mmol) in dry THF (120 ml) and hexane (12 ml). Addition was at a rate that maintained the reaction temperature below -95 °C. Following the addition, the yellow mixture was left to stir at -110 °C. After 1.5 hours, a solution of boron trifluoride etherate (2.9 ml, 22 mmol) and decanal (4.26 ml, 22 mmol) in dry THF (15 ml) was added slowly over 10 minutes, again maintaining the temperature below -95 °C. Following addition, the mixture was left to

stir for 1 hour at -110 °C. The reaction was quenched with saturated ammonium chloride solution (150 ml), and the mixture extracted with diethyl ether (4 x 100 ml), and the combined organic fractions dried (Na_2SO_4), and concentrated *in vacuo*. The golden oily residue was purified by flash column chromatography (20% ethyl acetate/petroleum), to give a pale golden oil (3.06 g, 69%). ν_{max} (film)/cm^{-1} 3500m, br (O-H), 3034m (Ar C-H), 1794vs (C=O), 1743vs (C=O); δ_H 0.9 (3 H, t, alkyl C*H*$_3$), 1.2 (18 H, m, alkyl C*H*$_2$, $CO_2CH_2CH_3$), 1.5 (2 H, m, CH(OH)C*H*$_2$CH$_2$), 2.1 (2 H, m, C-4 C*H*$_2$), 2.7 (1 H, m, C-3 C*H*CHOH), 3.7 (1 H, m, C*H*OH), 4.2 (2 H, m, CO_2CH_2), 4.6 (1 H, d, C-5 C*H*CO_2), 5.3 (2 H, m, PhC*H*$_2$), 7.3 (5 H, m, Ar*H*); δ_C 14.0 ($CO_2CH_2CH_3$), 14.1 (alkyl *C*H$_3$), 22.7 (alkyl *C*H$_2$), 24.8 (alkyl *C*H$_2$), 24.9 (C-4 *C*H$_2$), 25.8 (alkyl *C*H$_2$), 29.1 (alkyl *C*H$_2$), 29.2 (alkyl *C*H$_2$), 29.5 (alkyl *C*H$_2$), 29.7 (alkyl *C*H$_2$), 31.9 (alkyl *C*H$_2$), 34.2 (alkyl *C*H$_2$), 46.3 (C-3 *C*H), 57.0 (C-5 *C*HCO_2), 61.9 (CO_2CH_2), 68.4 (Ph*C*H$_2$), 72.1 (*C*HOH), 128.1 (Ar), 128.2 (Ar), 128.3 (Ar), 128.6 (Ar), 128.7 (Ar), 134.8 (Ar), 150.6 (N*C*O_2CH_2Ph), 170.6 (*C*O_2CH_2), 175.8 (*C*ON). *m/z* (EI) 448 ($M+H^+$, 20 %).

Ethyl 3-(1-trimethylsilyloxydecyl)-N-*(benzyloxycarbonyl)-pyrrolidin-2-one-5-carboxylate* **139**

A solution of alcohol compound **137** (459 mg, 1.03 mmol) in dry DCM (4 ml) was purged with nitrogen. Triethylamine (0.14 ml, 1.03 mmol) and chlorotrimethylsilane (0.13 ml, 1.03 mmol) were then added, resulting in a cloudy white mixture which was left at 20 °C for 24 hours. The mixture was then concentrated *in vacuo*, the residue taken up in dry ether (2 ml), and the mixture filtered, washing the white solid with ether (10 ml). The filtrate was concentrated *in vacuo*, giving the title silyl ether as a pale pink oil (470 mg, 88%). ν_{max} (film)/cm^{-1} 1799vs, (C=O), 1749vs (C=O), 1726vs (C=O), 842s (Si-O);

δ_H 0.1 (9 H, s, $Si(CH_3)_3$) 0.9 (6 H, t, CH_2CH_3), 1.3 (18 H, m, alkyl CH_2, $CO_2CH_2CH_3$), 1.4 (2 H, m, $CH(OSi)CH_2CH_2$), 2.1 (1 H, m, C-4 CH_2),), 2.3 (1 H, m, C-4 CH_2), 2.9 (1 H, m, C-3 CHCHOSi), 4.1 (1 H, m, CHOSi) 4.2 (2 H, m, CO_2CH_2), 4.6 (1 H, d, C-5 $CHCO_2$), 5.3 (2 H, m, $PhCH_2$), 7.3 (5 H, m, ArH); δ_C 1.6 ($Si(CH_3)_3$), 13.7 ($CO_2CH_2CH_3$), 13.8 (alkyl CH_3), 22.4 (C-4 CH_2), 23.7 (alkyl CH_2), 26.0 (alkyl CH_2), 28.9 (alkyl CH_2), 29.1 (alkyl CH_2), 29.3 (alkyl CH_2), 29.4 (alkyl CH_2), 31.5 (alkyl CH_2), 32.5 (alkyl CH_2), 47.2 (C-3 CH), 56.9 (C-5 $CHCO_2$), 61.4 (CO_2CH_2), 67.8 ($PhCH_2$), 71.6 (CHOSi), 127.7 (Ar), 127.9 (Ar), 128.0 (Ar), 128.1 (Ar), 128.2 (Ar), 128.3 (Ar), 134.7 (Ar), 150.7 (NCO_2CH_2Ph), 170.9 (CO_2CH_2), 172.2 (CON).

*Ethyl 3-(1-*tert*-butyldimethylsilyloxydecyl)-*N*-(benzyloxycarbonyl)-pyrrolidin-2-one-5-carboxylate* **141**

2,6-Lutidine (0.72 ml, 6.2 mmol) followed by *tert*-butyldimethylsilyl triflate (0.58 ml, 2.07 mmol) were added to a cold (-40 °C) stirred solution of alcohol compound **137** (460 mg, 1.08 mmol) in dry DCM (12 ml). After 2 hours at -40 °C, the mixture was warmed to 0 °C, and stirring continued for a further 20 minutes. Water (4 ml) was added to quench the reaction, and after 20 minutes, the mixture was diluted with DCM (30 ml), and washed with brine (2 x 20 ml). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo*, giving a colourless oil, purified by flash column chromatography (10% ethyl acetate/petroleum). This gave the title compound as a colourless oil (250 mg, 43%). ν_{max} (film)/cm^{-1} 1798vs, (C=O), 1747vs (C=O), 1720vs (C=O), 836s (Si-O); δ_H 0.1 (6 H, s, $Si(CH_3)_2$), 0.8 (9 H, s, $SiC(CH_3)_3$), 0.9 (6 H, t, CH_2CH_3), 1.3 (16 H, m, alkyl CH_2, $CO_2CH_2CH_3$), 1.4 (2 H, m, $CH(OSi)CH_2CH_2$), 2.0 (1 H, m, C-4 CH_2),), 2.3 (1 H, m, C-4 CH_2), 2.9 (1 H, m, C-3 CHCHOSi), 4.0 (1 H, m, CHOSi) 4.1 (2 H, m, CO_2CH_2),

4.6 (1 H, d, C-5 C*H*CO_2), 5.3 (2 H, m, PhC*H*$_2$), 7.3 (5 H, m, Ar*H*); δ_C -4.6 (Si(*C*H_3)$_2$), 14.0 ($CO_2CH_2CH_3$, alkyl *C*H_3), 17.9 (Si*C*$(CH_3)_3$), 22.7 (C-4 *C*H_2), 23.8 (alkyl *C*H_2), 25.6 (alkyl *C*H_2), 25.7 (alkyl *C*H_2), 26.2 (alkyl *C*H_2), 29.3 (alkyl *C*H_2), 29.6 (alkyl *C*H_2), 31.9 (alkyl *C*H_2), 32.7 (alkyl *C*H_2), 47.5 (C-3 *C*H), 57.3 (C-5 *C*HCO_2), 61.7 (CO_2*C*H_2), 68.3 (Ph*C*H_2), 71.9 (*C*HOSi), 128.0 (Ar), 128.4 (Ar), 128.6 (Ar), 134.2 (Ar), 150.5 (N*C*O_2CH_2Ph), 171.3 (*C*O_2CH_2), 172.5 (*C*ON).

Ethyl N-(*benzyloxycarbonyl)-3-decylidene-pyrrolidin-2-one-5-carboxylate* **143**

Methanesulfonyl chloride (0.2 ml, 2.7 mmol) was added to a cold (0 °C), stirred solution of alcohol compound **137** (1.07 g, 2.4 mmol) and triethylamine (5 ml, 36 mmol) in dry DCM (30 ml). The mixture was allowed to warm to 20 °C following addition, and was left to stand for 16 hours. The brown solution was poured into water (100 ml), and left to stir for 1 hour. The mixture was then extracted with DCM (3 x 50 ml), and the combined extracts washed with 2 M hydrochloric acid (1 x 50 ml) and brine (1 x 50 ml), dried ($MgSO_4$), and concentrated *in vacuo*. The brown oily residue was purified by flash column chromatography (25% ethyl acetate/petroleum). This gave the title compound as a colourless oil (250 mg, 24%), possibly a mixture of two isomers by TLC. ν_{max} (film)/cm^{-1} 1792s (C=O), 1742 (C=O); δ_H 0.9 (3 H, t, alkyl C*H*$_3$), 1.3 (18 H, m, alkyl C*H*$_2$, CO_2CH_2C*H*$_3$), 1.6 (2 H, m, C=CHC*H*$_2$CH$_2$), 2.2 (2 H, m, C-4 C*H*$_2$), 4.1 (2 H, q, CO_2C*H*$_2$), 4.6 (1 H, d, C-5 C*H*CO_2), 5.3 (2 H, m, PhC*H*$_2$), 6.8 (1 H, t, C=C*H*CH_2), 7.3 (5 H, m, Ar*H*); δ_C 13.9 ($CO_2CH_2CH_3$), 14.1 (alkyl *C*H_3), 22.7 (C-4 *C*H_2), 25.9 (C=C*C*H_2), 28.1 (alkyl *C*H_3), 29.3 (alkyl *C*H_3), 29.4 (alkyl *C*H_3), 29.5 (alkyl *C*H_3), 29.6 (alkyl *C*H_3), 31.9 (alkyl *C*H_3), 55.8 (C-5 *C*HCO_2), 61.8 (CO_2*C*H_2), 68.3 (Ph*C*H_2), 127.9 (Ar), 128.1 (Ar), 128.4 (Ar), 128.6 (Ar), 135.9 (C-3 *C*=CHCH_2), 140.5 (C=*C*CH_2).

10.2.4. Pyroglutamic acid enaminone derivatives

tert-*Butyl pyrrolidin-2-one-5-carboxylate*[113]

A 70% solution of perchloric acid in water (0.96 ml, 11 mmol) was added dropwise to a stirred suspension of (5*S*)-(+)-pyrrolidin-2-one-5-carboxylic acid (4.0 g, 31 mmol) in *tert*-butyl acetate (62 ml, 0.46 mol) at 20 °C. The reaction vessel was stoppered, and the colourless solution left to stand for 2 days. The mixture was then poured into saturated sodium hydrogencarbonate solution (200 ml), and solid sodium hydrogencarbonate added until effervescence ceased. The mixture was then extracted with diethyl ether (4 x 100 ml), and the combined organic extracts dried ($MgSO_4$), and concentrated *in vacuo*. This yielded a colourless oil which solidified on standing, giving a white solid, the title compound, without further purification (3.77 g, 66%) m.p. 108-110 °C [Literature[113] m.p. 91-92 °C, or 118-119 °C[92]]. ν_{max} (KBr)/cm^{-1} 3267m (N-H), 1736vs (C=O), 1679vs (C=O); δ_H 1.5 (9 H, s, C(CH_3)$_3$), 2.2 (1 H, m, C-4 CH_2), 2.4 (3 H, m, C-3 CH_2, C-4 CH_2), 4.1 (1 H, m, C-5 $CHCO_2$), 6.2 (1 H, s, N*H*); δ_C 24.9 (C-4 CH_2), 28.0 (C(CH_3)$_3$), 29.3 (C-3 CH_2), 56.0 (C-5 $CHCO_2$), 82.4 ($C(CH_3)_3$), 171.1 ($CO_2C(CH_3)_3$), 177.7 (*C*ON).

tert-*Butyl* N-(tert-*butyloxycarbonyl)-pyrrolidin-2-one-5-carboxylate*[92,93] **97**

Di-*tert*-butyldicarbonate (5.24 g, 24 mmol) and dimethylaminopyridine (122 mg, 1.0 mmol) were added to a stirred solution of *tert*-butyl pyrrolidin-2-one-5-carboxylate in DCM (50 ml) at 20 °C. The mixture was stirred until all the solids had dissolved, to give a pale yellow solution. After standing at 20 °C for 24 hours, the solution was concentrated *in vacuo*, giving a brown oil (6.74 g). Purification by flash column chromatography (20% DCM/ethyl acetate) yielded the title compound as an off-white

solid (5.63 g, 98%) m.p. 57-58 °C [Literature[92] m.p. 54 -56 °C]. ν_{max} (KBr)/cm^{-1} 1795vs (C=O), 1739vs (C=O); δ_H 1.5 (18 H, d, C(C*H*$_3$)$_3$), 2.0 (1 H, m, C-4 C*H*$_2$), 2.2 (1 H, m, C-4 C*H*$_2$), 2.5 (2 H, m, C-3 C*H*$_2$), 4.5 (1 H, d, C-5 C*H*CO$_2$); δ_C 21.7 (C-4 *C*H$_2$), 28.0 (C(*C*H$_3$)$_3$), 31.2 (C-3 *C*H$_2$), 59.6 (C-5 *C*HCO$_2$), 82.3 (*C*(CH$_3$)$_3$), 83.3 (*C*(CH$_3$)$_3$), 149.3 (N*C*O$_2$C(CH$_3$)$_3$), 170.4 (*C*O$_2$C(CH$_3$)$_3$), 173.6 (*C*ON).

Benzyl N-(tert-*butyloxycarbonyl)-pyrrolidin-2-one-5-carboxylate*

Di-*tert*-butyldicarbonate (3.06 g, 14.9 mmol) and dimethylaminopyridine (90 mg, 0.7 mmol) were added to a stirred solution of benzyl pyrrolidin-2-one-5-carboxylate in DCM (30 ml) at 20 °C. The mixture was stirred until all the solids had dissolved, to give a pale yellow solution. After standing at 20 °C for 2 days, the solution was concentrated *in vacuo*, giving a brown oil (5.38 g). Purification by flash column chromatography (20% DCM/ethyl acetate) yielded the title compound as a pale golden oil (4.43 g, 98%). The material is reported[92] as being a crystalline solid (m.p. 72-74 °C). ν_{max} (film)/cm^{-1} 3033m (Ar C-H), 1790vs (C=O), 1748vs (CO), 1700vs (C=O); δ_H 1.4 (9 H, s, C(C*H*$_3$)$_3$), 2.3 (2 H, m, C-4 C*H*$_2$), 2.5 (2 H, m, C-3 C*H*$_2$), 4.6 (1 H, d, C-5 C*H*CO$_2$), 5.2 (2 H, d, PhC*H*$_2$), 7.5 (5 H, s, Ar*H*); δ_C 21.5 (C-4 *C*H$_2$), 27.8 (C(*C*H$_3$)$_3$), 31.1 (C-3 *C*H$_2$), 58.9 (C-5 *C*HCO$_2$), 67.3 (Ph*C*H$_2$), 83.6 (*C*(CH$_3$)$_3$), 128.5 (Ar), 128.6 (Ar), 128.7 (Ar), 135.0 (Ar), 149.2 (N*C*O$_2$C(CH$_3$)$_3$), 171.2 (*C*O$_2$CH$_2$), 173.3 (*C*ON).

Ethyl N-(tert-*butyloxycarbonyl)-3-(dimethylaminomethylene)-pyrrolidin-2-one-5-carboxylate* **149**

Ethyl *N*-Boc-pyrrolidin-2-one-5-carboxylate compound **94** (3.0 g, 11.7 mmol) was heated under reflux in dimethoxyethane (20 ml) with *tert*-butoxybis(dimethylamino)

methane (3.6 ml, 17.5 mmol). After heating for 7 hours, the colourless solution was left to stand for 16 hours, and then heated for a further 7 hours. The mixture was concentrated *in vacuo*, giving a brown oil which solidified on trituration with cyclohexane. The resulting off-white solid was filtered, and washed with cyclohexane (10 ml), to give the title compound, used without further purification (2.69 g, 74%) m.p. 82-84 °C. ν_{max} (KBr)/cm^{-1} 1764vs (C=O), 1743vs (C=O), 1681s (C=O), 1618vs (C=N); δ_H 1.3 (3 H, t, CH_2CH_3), 1.5 (9 H, s, $C(CH_3)_3$), 2.9 (1 H, m, C-4 CH_2), 3.0 (6 H, s, $N(CH_3)_2$), 3.2 (1 H, t, C-4 CH_2), 4.2 (2 H, q, $CO_2CH_2CH_3$), 4.5 (1 H, d.d., C-5 $CHCO_2$), 7.1 (1 H, s, C=C*H*NMe$_2$); δ_C 14.2 (CH_2CH_3), 26.1 (C-4 CH_2), 28.1 ($C(CH_3)_3$), 42.0 ($N(CH_3)_2$), 56.0 (C-5 $CHCO_2$), 61.9 (CO_2CH_2), 88.2 (C-3 C=CH), 90.7 ($C(CH_3)_3$), 146.4 (C=CHNMe$_2$), 150.1 ($NCO_2C(CH_3)_3$), 171.5 (CO_2CH_2), 172.7 (CON).

Benzyl N-(tert-*butyloxycarbonyl)-3-(dimethylaminomethylene)-pyrrolidin-2-one-5-carboxylate* **151**

Benzyl *N*-(*tert*-butyloxycarbonyl)-pyrrolidin-2-one-5-carboxylate (4.43 g, 14.5 mmol) was heated under reflux in dimethoxyethane (40 ml) with *tert*-butoxybis(dimethylamino)methane (4.5 ml, 22 mmol). After heating for 4.5 hours, the brown solution was left to stand at 20 °C for 16 hours, before concentration *in vacuo*. The resulting brown oil, purified by flash column chromatography (ethyl acetate), gave the title compound as a brown oil (5.02 g, 96%). ν_{max} (film)/cm^{-1} 3032m (Ar C-H), 1768vs (C=O), 1737vs (C=O), 1698vs (C=O), 1623s (C=N); δ_H 1.4 (9 H, s, $C(CH_3)_3$), 2.9 (1 H, m, C-4 CH_2), 3.0 (6 H, s, $N(CH_3)_2$), 3.2 (1 H, t, C-4 CH_2), 4.6 (1 H, d.d., C-5 $CHCO_2$), 5.2 (2 H, q, $PhCH_2$), 7.1 (1 H, s, C=C*H*NMe$_2$), 7.4 (5 H, s, Ar*H*); δ_C 26.3 (C-4 CH_2), 28.0 ($C(CH_3)_3$), 42.0 ($N(CH_3)_2$), 56.1 (C-5 $CHCO_2$), 67.0 ($PhCH_2$), 82.3 (C-3

C=CH), 90.9 (*C*(CH_3)$_3$), 128.4 (Ar), 128.6 (Ar), 128.7 (Ar), 135.4 (Ar), 146.4 (C=*C*HNMe$_2$), 150.2 (N*C*O$_2$C(CH_3)$_3$), 171.0 (*C*O$_2$CH$_2$), 171.9 (*C*ON).

tert-*Butyl* N-(tert-*butyloxycarbonyl)-3-(dimethylaminomethylene)-pyrrolidin-2-one-5-carboxylate* **150**

Pyrrolidinone compound **97** (3.0 g, 10.5 mmol) was heated under reflux in dimethoxyethane (10 ml) with *tert*-butoxybis(dimethylamino)methane (3.3 ml, 16 mmol). After heating for 6 hours, the brown solution was concentrated *in vacuo*. This gave a brown oil, which solidified on trituration with ice cold diethyl ether. The resulting solid was filtered, and washed with diethyl ether (5 ml). Addition of cyclohexane to the filtrate produced more solid, which was filtered and combined with the first crop. This gave the title compound as a white solid, without further purification (2.28 g, 64%) m.p. 131-133 °C [Literature[92] m.p. 126-127 °C). ν_{max} (KBr)/cm^{-1} 3462m (N-H), 1759vs (C=O), 1738vs (C=O), 1686vs (C=O), 1633s (C=N); δ_H 1.5 (9 H, s, C(C*H*$_3$)$_3$), 1.6 (9 H, s, C(C*H*$_3$)$_3$), 2.6 (1 H, d, C-4 C*H*$_2$), 3.0 (6 H, s, N(C*H*$_3$)$_2$) 3.2 (1 H, t, C-4 C*H*$_2$), 4.5 (1 H, d, C-5 C*H*CO$_2$), 7.1 (1 H, s, C=CH); δ_C 26.3 (C-4 *C*H$_2$), 28.0 (C(*C*H$_3$)$_3$), 28.1 (C(*C*H$_3$)$_3$), 42.0 (N(*C*H$_3$)$_2$), 56.6 (C-5 *C*HCO$_2$), 81.8 (C-3 *C*=CH), 82.0 (*C*(CH$_3$)$_3$), 91.3 (*C*(CH$_3$)$_3$), 146.3 (C=*C*HNMe$_2$), 171.2 (*C*O$_2$C(CH$_3$)$_3$).

N-(tert-*butyloxycarbonyl)-3-(dimethylaminomethylene)-5-(*tert-*butyldimethyl-silyloxymethyl)-pyrrolidin-2-one* **152**

Diprotected pyrrolidinone compound **89** (0.5 g, 1.52 mmol) was heated under reflux in dimethoxyethane (5 ml) with *tert*-butoxybis(dimethylamino)methane (0.48 ml, 2.28 mmol). After heating for 4 hours, the mixture was left to stand for 16 hours, and

was then concentrated *in vacuo*. The oily residue was purified by flash column chromatography (25% ethyl acetate/petroleum). The title compound was obtained as a yellow oil (160 mg, 27%). ν_{max} (film)/cm^{-1} 1741vs (C=O), 1701s (C=O), 1624vs (C=N); δ_H 0.8 (9 H, Si(C*H*$_3$)$_3$), 1.5 (9 H, s, C(C*H*$_3$)$_3$), 2.9 (2 H, m, C-4 C*H*$_2$), 3.0 (6 H, s, N(C*H*$_3$)$_2$), 3.5 (1 H, t, CHC*H*$_2$Si), 3.7 (1 H, d.d., C-5 C*H*CO$_2$), 4.1 (1 H, m CHC*H*$_2$Si), 7.1 (1 H, s, C=C*H*NMe$_2$); δ_C 14.2 (Si*C*(CH$_3$)$_3$), 18.2 (C-4 *C*H$_2$), 25.8 (C(*C*H$_3$)$_3$), 28.0 (C(*C*H$_3$)$_3$), 41.9 (N(*C*H$_3$)$_2$), 55.5 (C-5 *C*HCH$_2$), 63.7 (CH*C*H$_2$Si), 81.5 (C-3 *C*=CH), 93.5 (*C*(CH$_3$)$_3$), 146.2 (C=*C*HNMe$_2$), 151.1 (N*C*O$_2$C(CH$_3$)$_3$), 170.6 (*C*ON).

Ethyl N-(*benzyloxycarbonyl)-3-(dimethylaminomethylene)-pyrrolidin-2-one-5-carboxylate* **153**

Ethyl *N*-Z-pyrrolidin-2-one-5-carboxylate compound **133** (250 mg, 0.86 mmol) was heated under reflux in dimethoxyethane (5 ml) with *tert*-butoxybis(dimethylamino) methane (0.3 ml, 1.3 mmol). After heating for 7 hours, the brown solution was left to stand for 16 hours, before concentration *in vacuo*. The resulting brown oil was triturated with cyclohexane, to give the title compound after filtration as a colourless solid (220 mg, 74%) m.p. 128-130 °C. ν_{max} (KBr)/cm^{-1} 3034m (Ar C-H), 1764vs (C=O), 1743vs (C=O), 1681s (C=O), 1618vs (C=N); δ_H 1.3 (3 H, t, CH$_2$C*H*$_3$), 2.9 (1 H, m, C-4 C*H*$_2$), 3.0 (6 H, s, N(C*H*$_3$)$_2$), 3.2 (1 H, t, C-4 C*H*$_2$), 4.2 (2 H, q, CO$_2$C*H*$_2$CH$_3$), 4.6 (1 H, d.d., C-5 C*H*CO$_2$), 5.3 (2 H, q, C*H*$_2$Ph), 7.1 (1 H, s, C=C*H*NMe$_2$), 7.4 (5 H, m, Ar*H*); δ_C 14.2 (CH$_2$*C*H$_3$), 24.1 (C-4 *C*H$_2$), 42.0 (N(*C*H$_3$)$_2$), 57.5 (C-5 *C*HCO$_2$), 61.9 (CO$_2$*C*H$_2$), 68.3 (Ph*C*H$_2$), 88.5 (C-3 *C*=CH), 128.2 (Ar), 128.4 (Ar), 128.6 (Ar), 135.1 (Ar), 146.4 (C=*C*HNMe$_2$), 150.1 (N*C*O$_2$C(CH$_3$)$_3$), 171.3 (*C*O$_2$CH$_2$), 172.7 (*C*ON).

Methyl N-(tert-*butyloxycarbonyl)-pyrrolidin-2-one-5-carboxylate*[110] **145**

Di-*tert*-butyldicarbonate (330 mg, 1.5 mmol) was added to a stirred solution of methyl pyrrolidin-2-one carboxylate compound **119** (180 mg, 1.3 mmol) and dimethylaminopyridine (20 mg, catalytic) in DCM (10 ml) at 20 °C. After standing for 24 hours, the mixture was concentrated *in vacuo*, and the residue was purified by flash column chromatography (20% DCM/ethyl acetate). This gave a colourless oil which solidified on trituration with cold ether, giving a colourless solid, m.p. 98-99 °C, (171 mg, 56%). ν_{max} (KBr)/cm^{-1} 1760vs (C=O), 1740vs (C=O), 1704vs (C=O); δ_H 1.5 (9 H, s, C(C*H*$_3$)$_3$), 2.1 (1 H, m, C-4 C*H*$_2$), 2.3 - 2.8 (3 H, br. m, C-3 C*H*$_2$, C-4 C*H*$_2$), 3.8 (3 H, s, CO$_2$C*H*$_3$), 4.6 (1 H, d.d., C-5 C*H*CO$_2$).

Methyl N-(tert-*butyloxycarbonyl)-3-(dimethylaminomethylene)-pyrrolidin-2-one-5-carboxylate*[110] **147**

Methyl ester compound **145** (1.0 g, 4.12 mmol) was heated under reflux in dimethoxyethane (15 ml) with *tert*-butoxybis(dimethylamino)methane (1.3 ml, 6.2 mmol). After heating for 14 hours, the mixture was left to stand for 16 hours, and was then concentrated *in vacuo*. The resulting white solid was filtered, and washed with diethyl ether (5 ml). This gave the title compound without further purification (940 mg, 77%) m.p. 130-132 °C (Literature[110] m.p. 129 °C). ν_{max} (KBr)/cm^{-1} 1766vs (C=O), 1745vs (C=O), 1681s (C=O), 1616vs (C=N); δ_H 1.5 (9 H, s, C(C*H*$_3$)$_3$), 2.9 (1 H, m, C-4 C*H*$_2$), 3.0 (6 H, s, N(C*H*$_3$)$_2$), 3.2 (1 H, t, C-4 C*H*$_2$), 3.7 (3 H, s, CO$_2$C*H*$_3$), 4.5 (1 H, d.d., C-5 C*H*CO$_2$), 7.1 (1 H, s, C=C*H*NMe$_2$); δ_C 26.3 (C-4 *C*H$_2$), 28.1 (C(*C*H$_3$)$_3$), 42.0 (N(*C*H$_3$)$_2$), 56.0 (C-5 *C*HCO$_2$), 88.3 (C-3 *C*=CH), 90.9 (*C*(CH$_3$)$_3$), 146.5 (C=*C*HNMe$_2$), 150.1 (N*C*O$_2$C(CH$_3$)$_3$), 169.5 (*C*O$_2$CH$_3$), 172.7 (*C*ON).

Benzyl N-(tert-*butyloxycarbonyl)-3-(tetradecylidene)-pyrrolidin-2-one-5-carboxylate* **155**

A 1M solution of tetradecylmagnesium chloride in THF (Aldrich) (5.88 ml, 5.88 mmol) was added to an ice cold stirred solution of enaminone compound **151** (2.0 g, 5.35 mmol) in dry THF (50 ml). The resulting yellow mixture was left to stir at 0 °C for 3.5 hours. The reaction was quenched with saturated ammonium chloride solution (40 ml), and the mixture separated. The aqueous phase was extracted with ethyl acetate (3 x 30 ml), and the combined organic phases washed (brine), dried ($MgSO_4$), and concentrated *in vacuo*. The yellow oily residue was purified by flash column chromatography (30% ethyl acetate/petroleum). The title compound was obtained as a golden oil (1.49 g, 53%). ν_{max} (film)/cm^{-1} 3032m (Ar C-H), 1785vs (C=O), 1743vs (C=O), 1678vs (C=O); δ_H 0.9 (3 H, t, CH_2CH_3), 1.3 (24 H, s, alkyl CH_2), 1.4 (2 H, s, $C{=}CHCH_2$), 2.1 (1 H, m, C-4 CH_2), 3.6 (1 H, m, C-4 CH_2), 4.6 (1 H, d.d., C-5 $CHCO_2$), 5.1 (2 H, q, $PhCH_2$), 6.7 (1 H, s, $C{=}CHCH_2$), 7.3 (5 H, m, Ar*H*); δ_C 14.4 (CH_3), 22.7 (C-4 CH_2), 25.7 (alkyl CH_2), 27.9 (alkyl CH_2), 28.1 ($C(CH_3)_3$), 28.2 (alkyl CH_2), 28.3 (alkyl CH_2), 29.3 (alkyl CH_2), 29.4 (alkyl CH_2), 29.5 (alkyl CH_2), 29.6 (alkyl CH_2), 29.7 (alkyl CH_2), 31.9 (alkyl CH_2), 55.9 (C-5 $CHCO_2$), 67.3 ($PhCH_2$), 83.5 (C-3 $C{=}CH$), 128.2 (Ar), 128.6 (Ar), 139.8 ($C{=}CH$), 171.5 (*C*ON).

Ethyl N-(tert-*butyloxycarbonyl)-3-(tetradecylidene)-pyrrolidin-2-one-5-carboxylate* **156**

A 1M solution of tetradecylmagnesium chloride in THF (Aldrich) (7.1 ml, 7.1 mmol) was added to an ice cold stirred solution of enaminone compound **149** (2.0 g, 6.4 mmol) in dry ether (50 ml). The resulting yellow mixture was left to stir at 0 °C for 3 hours, and 20 °C for 2 days. The reaction was quenched with saturated ammonium chloride solution (75 ml), and the mixture separated. The aqueous phase was extracted

with ether (3 x 30 ml), and the combined organic phases washed (brine), dried ($MgSO_4$), and concentrated *in vacuo*. The yellow oily residue was purified by flash column chromatography (20% ethyl acetate/petroleum). The title compound was obtained as a colourless oil (320 g, 10%). ν_{max} (film)/cm^{-1} 1787m (C=O), 1743s (C=O); δ_H 0.9 (3 H, t, alkyl C*H*$_3$), 1.3 (24 H, s, alkyl C*H*$_2$), 1.5 (9 H, s, C(C*H*$_3$)$_3$), 2.1 (2 H, m, C=CHC*H*$_2$), 2.6 (1 H, m, C-4 C*H*$_2$), 2.9 (1 H, m, C-4 C*H*$_2$), 4.1 (2 H, q, CO_2CH_2), 4.6 (1 H, m., C-5 C*H*CO$_2$), 6.7 (1 H, m, C=C*H*CH$_2$); δ_C 14.1 (*C*H$_3$), 14.2 (*C*H$_3$), 22.7 (C-4 *C*H$_2$), 25.7 (alkyl *C*H$_2$), 25.8 (alkyl *C*H$_2$), 27.9 (C(*C*H$_3$)$_3$), 28.2 (C(*C*H$_3$)$_3$), 29.3 (alkyl *C*H$_2$), 29.4 (alkyl *C*H$_2$), 29.5 (alkyl *C*H$_2$), 29.6 (alkyl *C*H$_2$), 29.7 (alkyl *C*H$_2$), 31.9 (alkyl *C*H$_2$), 32.8 (alkyl *C*H$_2$), 55.9 (C-5 *C*HCO$_2$), 61.6 (CO_2CH_2), 82.1 (*C*(CH$_3$)$_3$), 128.7 (C-3 *C*=CH), 139.7 (C=*C*H), 150.1 (NCO$_2$C(CH$_3$)$_3$), 166.2 (*C*ON), 170.3 (CO_2CH_2).

tert-*Butyl* N-(tert-*butyloxycarbonyl)-3-(tetradecylidene)-pyrrolidin-2-one-5-carboxylate* **157**

A 1M solution of tetradecylmagnesium chloride in THF (Aldrich) (6.48 ml, 6.48 mmol) was added to an ice cold stirred solution of enaminone compound **150** (2.0 g, 5.84 mmol) in dry ether (50 ml). The resulting yellow mixture was left to stir at 0 °C for 3 hours. The reaction was quenched with saturated ammonium chloride solution (40 ml), and the mixture separated. The aqueous phase was extracted with ethyl acetate (3 x 30 ml), and the combined organic phases washed (brine), dried ($MgSO_4$), and concentrated *in vacuo*. The yellow oily residue was purified by flash column chromatography (20% ethyl acetate/petroleum). The title compound was obtained as a white solid (1.0 g, 35%), m.p. 59-61 °C. ν_{max} (KBr)/cm^{-1} 1784s (C=O), 1743s (C=O), 1720s (C=O); δ_H 0.9 (3 H, t, CH$_2$C*H*$_3$), 1.3 (24 H, s, alkyl C*H*$_2$), 1.4 (9 H, s, C(C*H*$_3$)$_3$), 1.5 (9 H, s, C(C*H*$_3$)$_3$), 2.1 (2 H, m, C=CHC*H*$_2$), 2.5 (1 H, d, C-4 C*H*$_2$), 2.9 (1 H, m, C-4 C*H*$_2$), 4.5 (1 H, d.d., C-5

C*H*CO_2), 6.7 (1 H, m, C=C*H*CH_2); δ_C 14.4 (*C*H_3), 22.7 (C-4 *C*H_2), 25.7 (alkyl *C*H_2), 25.8 (alkyl *C*H_2), 27.9 (C(*C*$H_3)_3$), 28.2 (C(*C*$H_3)_3$), 29.3 (alkyl *C*H_2), 29.4 (alkyl *C*H_2), 29.5 (alkyl *C*H_2), 29.7 (alkyl *C*H_2), 31.9 (alkyl *C*H_2), 56.5 (C-5 *C*HCO_2), 82.2 (*C*$(CH_3)_3$), 83.2 (*C*$(CH_3)_3$), 128.6 (C-3 *C*=CH), 139.3 (C=*C*H), 150.1 (N*C*O_2C$(CH_3)_3$), 166.2 (*C*ON), 170.3 (*C*O_2C$(CH_3)_3$).

N-(tert-*butyloxycarbonyl)-3-(tetradecylidene)-5-(*tert-*butyldimethylsilyloxy-methyl)-pyrrolidin-2-one* **158**

Using the method described for compound **155**, a golden oil was obtained. Purification of the crude material by flash column chromatography (10% ethyl acetate/petroleum) resulted in the title compound as a pale yellow oil (50 mg, 20%). ν_{max} (film)/cm^{-1} 1779m (C=O), 1715vs (C=O), 1678s (C=O); δ_H 0.8 (9 H, s, SiC(C*H*$_3)_3$), 0.9 (3 H, t, CH_2C*H*$_3$), 1. (24 H, s, alkyl C*H*$_2$), 1.4 (9 H, s, CO_2C(C*H*$_3)_3$), 2.1 (1 H, m, C-4 C*H*$_2$), 2.6 (1 H, m, C-4 C*H*$_2$), 3.7 (2 H, m, C*H*$_2$OSi), 4.1 (1 H, m, C-5 C*H*CH_2), 6.6 (1 H, m, C=C*H*); δ_C 14.5 SiC(C$H_3)_3$), 22.7 (C-4 *C*H_2), 25.7 (SiC(*C*$H_3)_3$), 28.1 (CO_2C(*C*$H_3)_3$), 29.4 (alkyl *C*H_2), 29.5 (alkyl *C*H_2), 29.6 (alkyl *C*H_2), 29.7 (alkyl *C*H_2), 31.9 (alkyl *C*H_2), 55.7 (C-5 *C*HCH_2), 65.3 (*C*H_2OSi), 82.4 (CO_2*C*$(CH_3)_3$), 130.6 (C-3 *C*=CH), 137.5 (C=*C*H), 167.5 (*C*ON). *m/z* (CI) 538 ($M+H^+$, 100 %).

tert-*Butyl* N-(tert-*butyloxycarbonyl)-3-hydroxy-3-(1-hydroxytetradecyl)-pyrrolidin-2-one-5-carboxylate* **159**

N-Methyl morpholine-*N*-oxide, 50 wt% in water, (0.2 ml, 1.04 mmol) and osmium tetroxide, 4 wt% in water, (0.02 ml, 0.03 mmol), were added to an ice-cold stirred solution of alkene compound **157** (340 mg, 0.69 mmol) in acetone (3 ml) and

water (0.5 ml). A further portion of acetone (5 ml) was added to redissolve an off-white precipitate that formed, to give a pale yellow solution. After stirring at 0 °C for 10 hours, and at 20 °C for 16 hours, the reaction was quenched by addition of saturated sodium hydrogen sulfite solution (20 ml). The mixture was stirred for 20 minutes, before being extracted with ethyl acetate (4 x 20 ml). The combined extracts were dried (Na_2SO_4) and concentrated *in vacuo*, to give a pale yellow oil (370 mg). Purification by flash column chromatography (40% ethyl acetate/petroleum) yielded a mixture of two products R_f 0.62 and R_f 0.54 (220 mg, 17%) and a single isomer B R_f 0.54 (50 mg, 4%), a white solid, m.p. 74-75 °C. Isomer B ν_{max} (KBr)/cm^{-1} 3463m, br (O-H), 1792vs (C=O), 1743vs (C=O); δ_H 0.9 (3 H, t, alkyl C*H*$_3$), 1.3 (22 H, s, alkyl C*H*$_2$), 1.5 (18 H, d, C(C*H*$_3$)$_3$), 1.9 (1 H, d.d., C-4 C*H*$_2$), 2.4 (1 H, q, C-4 C*H*$_2$), 3.4 (2 H, s, O*H*) (D_2O exchangeable), 3.8 (1 H, d, C*H*OH), 4.5 (1 H, t, C-5 C*H*CO$_2$); δ_C 14.1 (*C*H$_3$), 22.7 (C-4 *C*H$_2$), 25.7 (alkyl *C*H$_2$), 27.8 (alkyl *C*H$_2$), 27.9 (alkyl *C*H$_2$), 28.4 (C(*C*H$_3$)$_3$), 29.5 (C(*C*H$_3$)$_3$), 29.6 (alkyl *C*H$_2$), 29.7 (alkyl *C*H$_2$), 31.5 (alkyl *C*H$_2$), 31.9 (alkyl *C*H$_2$), 56.5 (C-5 *C*HCO$_2$), 73.4 (*C*HOH), 82.7 (*C*(CH$_3$)$_3$), 84.3 (*C*(CH$_3$)$_3$), 149.0 (N*C*O$_2$C(CH$_3$)$_3$), 169.8 (*C*O$_2$C(CH$_3$)$_3$), 175.4 (*C*ON).

Methyl N-(tert-*butyloxycarbonyl)-3-methylene-pyrrolidin-2-one-5-carboxylate* **163**

A 1.5 M solution of DIBAL-H in toluene (0.81 ml, 1.22 mmol) was added to a stirred solution of enaminone compound **147** (182 mg, 0.61 mmol) in dry THF (20 ml) at -78 °C. After 1 hour, the reaction was quenched by addition of saturated ammonium chloride solution (5 ml), and the mixture was allowed to warm to room temperature. The mixture was diluted with saturated ammonium chloride solution (20 ml) and extracted with ethyl acetate (3 x 20 ml). The combined extracts were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The colourless oily residue (130 mg) was purified by flash column chromatography (10% DCM/ethyl acetate), resulting in a colourless oil

being obtained (100 mg, 64%). ν_{max} (film)/cm^{-1} 1787vs (C=O), 1748vs (C=O), 1720vs (C=O), 1664s (C=O); δ_H 1.5 (9 H, s, C(C*H*$_3$)$_3$), 2.7 (1 H, d, C-4 C*H*$_2$), 3.1 (1 H, m, C-4 C*H*$_2$), 3.8 (3 H, s, CO$_2$C*H*$_3$), 4.6 (1 H, d, C-5 C*H*CO$_2$), 5.5 (1 H, t, C=C*H*$_2$), 6.2 (1 H, t, C=C*H*$_2$); δ_C 27.9 (C(*C*H$_3$)$_3$), 52.7 (CO$_2$*C*H$_3$), 55.7 (C-5 *C*HCO$_2$), 83.9 (*C*(CH$_3$)$_3$), 121.0 (C=*C*H$_2$), 136.5 (C-3 *C*=CH$_2$), 149.8 (N*C*O$_2$C(CH$_3$)$_3$), 165.4 (*C*ON), 171.6 (*C*O$_2$CH$_3$).

tert-*Butyl* N-(tert-*butyloxycarbonyl)-3-methylene-pyrrolidin-2-one-5-carboxylate* **164**

A 1.5 M solution in toluene of DIBAL-H (3.92 ml, 5.88 mmol) was added to a stirred solution of enaminone compound **150** (1.0 g, 2.94 mmol) in dry THF (20 ml) at -78 °C. After 1.5 hours, the reaction was quenched by addition of saturated ammonium chloride solution (50 ml), and the mixture was allowed to warm to room temperature. The mixture was extracted with ethyl acetate (3 x 30 ml), the combined extracts washed with brine, dried (MgSO$_4$), and concentrated *in vacuo*. The colourless oily residue (700 mg) was purified by flash column chromatography (40% ethyl acetate/petroleum), to give a colourless oil (440 mg, 50%). ν_{max} (film)/cm^{-1} 1789vs (C=O), 1741vs (C=O), 1720vs (C=O); δ_H 1.5 (9 H, s, C(C*H*$_3$)$_3$), 1.6 (9 H, s, C(C*H*$_3$)$_3$), 2.6 (1 H, m., C-4 C*H*$_2$), 3.0 (1 H, m, C-4 C*H*$_2$), 4.5 (1 H, d, C-5 C*H*CO$_2$), 5.5 (1 H, t, C=C*H*$_2$), 6.2 (1 H, t, C=C*H*$_2$); δ_C 27.9 (C(*C*H$_3$)$_3$), 28.0 (C(*C*H$_3$)$_3$), 56.4 (C-5 *C*HCO$_2$), 82.4 (*C*(CH$_3$)$_3$), 83.5 (*C*(CH$_3$)$_3$), 120.5 (C=*C*H$_2$), 136.9 (C-3 *C*=CH$_2$), 149.9 (N*C*O$_2$C(CH$_3$)$_3$), 165.3 (*C*ON), 170.1 (*C*O$_2$C(CH$_3$)$_3$).

10.2.5. *N,O*-Acetal protected pyrrolidinones

(2R, 5S)-2-phenyl-1-aza-3-oxabicyclo[3.3.0]octan-8-one[127] **87**

(5*S*)-5-Hydroxymethyl-pyrrolidin-2-one compound **92** (20 g, 0.17 mol) was suspended in dry toluene (140 ml), with toluene-*p*-sulfonic acid (370 mg, 1.95 mmol) and benzaldehyde (23 g, 0.21 mol). The mixture was stirred vigorously, using an overhead stirrer, while being heated to reflux under Dean and Stark conditions. After 19 hours, the mixture was allowed to cool, before being diluted with ethyl acetate (100 ml). The mixture was washed with saturated sodium hydrogensulfite solution (3 x 150 ml), and brine (2 x 150 ml). The organic phase was dried ($MgSO_4$), and concentrated *in vacuo*, to give a golden brown oil (25 g). This material was purified by dry flash column chromatography (gradient elution, 30 to 50% ethyl acetate/petroleum). This resulted in the title compound being obtained as a pale golden oil (13.79 g, 50 %). ν_{max} (film)/cm^{-1} 3051m (Ar C-H), 1706vs (C=O); δ_H 1.9 (1 H, m, C-6 CH_2), 2.4 (1 H, m, C-6 CH_2), 2.6 (1 H, m, C-7 CH_2), 2.8 (1 H, m, C-7 CH_2), 3.5 (1 H, t, *J* 7.8, C-4 CH_2), 4.1 (1 H, m, C-5 C*H*), 4.2 (1 H, t, *J* 8.1, C-4 CH_2), 6.4 (1 H, s, C-2 C*H*), 7.4 (5 H, m, Ar*H*); δ_C 22.3 (C-6 *C*H_2), 33.4 (C-7 *C*H_2), 58.8 (C-5 *C*H), 71.6 (C-4 *C*H_2), 87.1 (C-2 Ph*C*H), 125.9 (Ar), 128.5 (Ar), 138.8 (Ar), 178.1 (C=O).

(2R, 5S, 7S)-2-phenyl-1-aza-7-(1-hydroxydecyl)-3-oxabicyclo[3.3.0]octan-8-one **172**

n-Butyl lithium, 1.6 M in hexane, (1.38 ml, 2.2 mmol) was added dropwise to a stirred solution of tetramethylpiperidine (0.37 ml, 2.2 mmol), in dry THF (15 ml) at -78 °C. After 20 minutes, dropwise addition of a solution bicyclic lactam compound **84** (200 mg, 1.0 mmol) in dry THF (1 ml) resulted in a pale yellow solution. After a further 20 minutes at -78 °C, a solution of decanal (0.38 ml 2.0 mmol) in dry THF (0.75 ml) was

added dropwise to the mixture, at a rate that maintained the internal temperature of the reaction below -65 °C. Following addition, the yellow mixture was left to stir at -78 °C for a further 0.5 hour, before pouring into saturated ammonium chloride solution (25 ml). The mixture was separated, and the aqueous layer extracted with ether (3 x 25 ml). The combined organic fractions were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. This gave a pale golden oil (540 mg), which was purified by flash column chromatography (30% ethyl acetate/petroleum), yielding the title compound as a colourless solid, R_f 0.27 (50% ethyl acetate/petroleum) (80 mg, 20%) m.p. 57-59 °C. ν_{max} (KBr)/cm^{-1} 3381m, br (O-H), 3031m (Ar C-H), 1679vs (C=O); δ_H 0.8 (3 H, t, *J* 6.4, alkyl C*H*$_3$), 1.3 (12 H, m, alkyl C*H*$_2$), 1.5 (4 H, m, alkyl C*H*$_2$), 2.1 (2 H, m, C-6 C*H*$_2$), 2.7 (1 H, q, *J* 8.1, C-7 C*H*), 3.4 (2 H, t, *J* 7.9, C-4 C*H*$_2$, O*H*), 3.8 (1 H, m, C*H*OH), 4.0 (1 H, m, C-5 C*H*), 4.2 (1 H, t, *J* 6.2, C-4 C*H*$_2$), 6.3 (1 H, s, C-2 C*H*), 7.3 (3 H, m, Ar*H*), 7.5 (2 H, m, Ar*H*); δ_C 14.1 (*C*H$_3$), 22.7 (alkyl *C*H$_2$), 24.2 (alkyl *C*H$_2$), 25.2 (alkyl *C*H$_2$), 29.3 (alkyl *C*H$_2$), 29.6 (alkyl *C*H$_2$), 31.9 (alkyl *C*H$_2$), 34.8 (alkyl *C*H$_2$), 48.8 (C-7 *C*H), 57.2 (C-5 *C*H), 70.6 (*C*HOH), 72.9 (C-4 *C*H$_2$), 87.6 (C-2 *C*H), 125.8 (Ar), 128.5 (Ar), 128.6 (Ar), 138.7 (Ar), 180.7 (C=O).

(2R, 5S, 7R) and (2R, 5S, 7S)-2-phenyl-1-aza-7-(1-hydroxydecyl)-3-oxabicyclo [3.3.0] octan-8-one **172** and **173**

A 1 M solution of lithium hexamethyldisilazide in THF (47 ml, 47 mmol) was added slowly to a cold (-50 °C), stirred solution of bicyclic lactam compound **87** (4.3 g, 21 mmol), in dry THF, with the temperature being maintained below -30 °C during the addition. The resulting yellow solution was left to stir at -50 °C for 1 hour. A solution of decanal (8.0 ml, 21 mmol) in dry THF (10 ml) was then added slowly to the mixture. After a further 30 minutes, the yellow mixture was poured (cold) into saturated ammonium chloride solution (100 ml). The mixture was extracted with ethyl acetate (4 x 100 ml), and the combined extracts washed (brine), and dried ($MgSO_4$). Concentration *in vacuo* yielded a yellow oil, purified by flash column chromatography (25% ethyl acetate/petroleum). This yielded compound **173**, R_f 0.40 (50% ethyl acetate/petroleum) (0.92 g, 12%), a colourless solid, m.p. 85-87 °C. Also isolated was compound **172**, R_f 0.27 (50% ethyl acetate/petroleum), (2.86 g, 38%), a colourless solid, m.p. 57-58 °C. *(2R, 5S, 7R)-2-phenyl-1-aza-7-(1-hydroxydecyl)-3-oxabicyclo [3.3.0] octan-8-one* **173** ν_{max} (KBr)/cm^{-1} 3399m, br. (O-H), 3052m (Ar C-H), 1682vs (C=O); δ_H 0.8 (3 H, t, *J* 6.2, alkyl C*H*$_3$), 1.3 (16 H, s, alkyl C*H*$_2$), 1.5 (2 H, m, CH(OH)C*H*$_2$), 2.0 (1 H, m, C-6 C*H*$_2$), 2.4 (2 H, m, C-6 C*H*$_2$), 2.8 (1 H, m, C-7 C*H*), 3.4 (1 H, t, *J* 8.0, C-4 C*H*$_2$), 4.1 (2 H, m, C-4 C*H*$_2$, C*H*OH), 4.3 (1 H, t, *J* 6.3, C-4 C*H*$_2$), 6.3 (1 H, s, C-2 C*H*), 7.3 (3 H, m, Ar*H*), 7.5 (2 H, m, Ar*H*); δ_C 14.1 (CH_3), 21.8 (C-6 *C*H_2), 22.7 (alkyl *C*H_2), 26.2 (alkyl *C*H_2), 29.1 (alkyl *C*H_2), 29.3 (alkyl *C*H_2), 31.9 (alkyl *C*H_2), 34.5 (alkyl *C*H_2), 50.7 (C-7 *C*H), 57.8 (C-5 *C*H), 70.7 (*C*HOH), 71.4 (C-4 *C*H_2), 87.5 (C-2 *C*H), 125.9 (Ar), 128.4 (Ar), 128.5 (Ar), 138.8 (Ar), 179.7 (C=O). *m/z* (EI) 360.2 ($M+H^+$, 100 %).

(2R, 5S, 7S)-2-phenyl-1-aza-7-(1-hydroxydecyl)-3-oxabicyclo [3.3.0] octan-8-one **172** ν_{max} (KBr)/cm^{-1} 3381m, br. (O-H), 3031m (Ar C-H), 1679vs (C=O); δ_H 0.8 (3 H, t, *J* 6.4, alkyl C*H*$_3$), 1.3 (12 H, m, alkyl C*H*$_2$), 1.5 (4 H, m, alkyl C*H*$_2$), 2.1 (2 H, m, C-6 C*H*$_2$), 2.7 (1 H, q, *J* 8.1, C-7 C*H*), 3.4 (2 H, t, *J* 7.9, C-4 C*H*$_2$, OH), 3.8 (1 H, m, C*H*OH), 4.0 (1 H, m, C-5 C*H*), 4.2 (1 H, t, *J* 6.2, C-4 C*H*$_2$), 6.3 (1 H, s, C-2 C*H*), 7.3 (3 H, m, Ar*H*), 7.5 (2 H, m, Ar*H*); δ_C 14.1 (CH$_3$), 22.7 (alkyl *C*H$_2$), 24.2 (alkyl *C*H$_2$), 25.2 (alkyl *C*H$_2$), 29.3 (alkyl *C*H$_2$), 29.6 (alkyl *C*H$_2$), 31.9 (alkyl *C*H$_2$), 34.8 (alkyl *C*H$_2$), 48.8 (C-7 *C*H), 57.2 (C-5 *C*H), 70.6 (*C*HOH), 72.9 (C-4 *C*H$_2$), 87.6 (C-2 *C*H), 125.8 (Ar), 128.5 (Ar), 128.6 (Ar), 138.7 (Ar), 180.7 (C=O).

(2R, 5S, 7S) and (2R, 5S, 7R)-2-phenyl-1-aza-7-(1-hydroxydecyl)-3-oxabicyclo[3.3.0] octan-8-one **173** and **174**

A 1 M solution of lithium hexamethyldisilazide in THF (47 ml, 47 mmol) was added slowly to a cold (-50 °C), stirred solution of bicyclic lactam compound **87** (4.3 g, 21 mmol) in dry THF (100 ml). After 1 hour at -50 °C, a solution of decanal (8.0 ml, 42 mmol) in THF (10 ml) was added dropwise to the pale yellow mixture. Following addition, the mixture was left to stir for 40 minutes at -30 °C. The reaction was quenched with saturated ammonium chloride solution (100 ml), and the mixture extracted with ethyl acetate (4 x 50 ml). The combined organic fractions were dried ($MgSO_4$), and concentrated *in vacuo*, giving a colourless oily residue. This was purified by flash column chromatography (25% ethyl acetate/petroleum), yielding compound **174**, a low melting colourless solid R_f 0.42 (30% ethyl acetate/petroleum) (1.95 g, 26%), m.p. 34-35 °C, and compound **173**, a colourless oil that formed a colourless solid on standing R_f 0.27 (30% ethyl acetate/petroleum) (1.08 g, 14%), m.p. 87-89 °C. *(2R, 5S, 7S)-2-phenyl-1-aza-7-(1-hydroxydecyl)-3-oxabicyclo [3.3.0] octan-8-one* **174** ν_{max} (KBr)/cm^{-1} 3399m, br.

(O-H), 3052m (Ar C-H), 1682vs (C=O); δ_H 0.8 (3 H, t, *J* 6.2, alkyl C*H*$_3$), 1.3 (16 H, s, alkyl C*H*$_2$), 1.5 (2 H, m, CH(OH)C*H*$_2$), 2.0 (1 H, m, C-6 C*H*$_2$), 2.4 (2 H, m, C-6 C*H*$_2$), 2.8 (1 H, m, C-7 C*H*), 3.4 (1 H, t, *J* 4.6, C-4 C*H*$_2$), 4.1 (2 H, m, C-4 C*H*$_2$, C*H*OH), 4.3 (1 H, t, *J* 6.6, C-4 C*H*$_2$), 6.3 (1 H, t, C-2 C*H*), 7.3 (3 H, m, Ar*H*), 7.5 (2 H, m, Ar*H*); δ_C 14.1 (CH_3), 21.8 (C-6 *C*H$_2$), 22.7 (alkyl *C*H$_2$), 26.2 (alkyl *C*H$_2$), 29.1 (alkyl *C*H$_2$), 29.3 (alkyl *C*H$_2$), 31.9 (alkyl *C*H$_2$), 34.5 (alkyl *C*H$_2$), 50.7 (C-7 *C*H), 57.8 (C-5 *C*H), 70.7 (*C*HOH), 71.4 (C-4 *C*H$_2$), 87.5 (C-2 *C*H), 125.9 (Ar), 128.4 (Ar), 128.5 (Ar), 138.8 (Ar), 179.7 (C=O).

(2R, 5S, 7R)-2-phenyl-1-aza-7-(1-hydroxydecyl)-3-oxabicyclo [3.3.0] octan-8-one **173** ν_{max} (KBr)/cm^{-1} 3381m, br. (O-H), 3031m (Ar C-H), 1679vs (C=O); δ_H 0.8 (3 H, t, *J* 6.2, alkyl C*H*$_3$), 1.3 (12 H, m, alkyl C*H*$_2$), 1.5 (4 H, m, alkyl C*H*$_2$), 2.1 (2 H, m, C-6 C*H*$_2$), 2.7 (1 H, m, C-7 C*H*), 3.4 (2 H, t, *J* 8.0, C-4 C*H*$_2$, OH), 3.8 (1 H, m, C*H*OH), 4.0 (1 H, m, C-5 C*H*), 4.2 (1 H, t, *J* 6.3, C-4 C*H*$_2$), 6.3 (1 H, s, C-2 C*H*), 7.3 (3 H, m, Ar*H*), 7.5 (2 H, m, Ar*H*); δ_C 14.1 (CH_3), 22.7 (alkyl *C*H$_2$), 24.2 (alkyl *C*H$_2$), 25.2 (alkyl *C*H$_2$), 29.3 (alkyl *C*H$_2$), 29.6 (alkyl *C*H$_2$), 31.9 (alkyl *C*H$_2$), 34.8 (alkyl *C*H$_2$), 48.8 (C-7 *C*H), 57.2 (C-5 *C*H), 70.6 (*C*HOH), 72.9 (C-4 *C*H$_2$), 87.6 (C-2 *C*H), 125.8 (Ar), 128.5 (Ar), 128.6 (Ar), 138.7 (Ar), 180.7 (C=O).

E-*(2R, 5S)-7-Decylidine-2-phenyl-1-aza-3-oxabicyclo[3.3.0]octan-8-one* **175**

Sodium hydride, 60% dispersion in oil, (1.44 g, 36 mmol) was added in portions to an ice cold stirred solution of bicyclic lactam compound **87** (6.0 g, 30 mmol) in dry THF (200 ml). After addition of the solid, the off-white suspension was left to stir for 0.5 hour. Decanal (11.2 ml, 60 mmol) was added slowly to the mixture, resulting in a yellow suspension, after some effervescence. The mixture was stirred for 2 hours, before

quenching with water (100 ml). The layers were separated, and the aqueous layer extracted with ethyl acetate (3 x 200 ml). The combined organic fractions were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. This gave a yellow oil, which was purified by flash column chromatography (10% ethyl acetate/petroleum), to give the title compound as a colourless oil R_f 0.7 (50% ethyl acetate/petroleum) (4.78 g, 47%). ν_{max} (film)/cm^{-1} 1739vs (C=O), 1706vs (C=O), 1673vs (C=O); δ_H 0.9 (3 H, t, *J* 6.2, alkyl C*H*$_3$), 1.3 (16 H, m, alkyl C*H*$_2$), 1.5 (2 H, m, alkyl C*H*$_2$), 2.1 (2 H, q, *J* 7.3, C=CC*H*$_2$), 2.6 (1 H, d, *J* 17.3, C-6 C*H*$_2$), 2.9 (1 H, d.d., *J* 8.0, 17.3, C-6 C*H*$_2$), 3.3 (1 H, t, *J* 8.8, C-4 C*H*$_2$), 4.0 (1 H, q, *J* 8.5, C-5 C*H*), 4.3 (1 H, t, *J* 6.5, C-4 C*H*$_2$), 6.4 (1 H, s, C-2 C*H*), 6.6 (1 H, m, C=C*H*CH$_2$), 7.3 (3 H, m, Ar*H*), 7.4 (2 H, d, Ar*H*); δ_C 14.2 (*C*H$_3$), 22.7 (alkyl *C*H$_2$), 25.3 (C=CH*C*H$_2$), 28.3 (alkyl *C*H$_2$), 29.1 (alkyl *C*H$_2$), 29.3 (alkyl *C*H$_2$), 29.5 (alkyl *C*H$_2$), 31.9 (alkyl *C*H$_2$), 55.4 (C-5 *C*H), 71.3 (C-4 *C*H$_2$), 87.6 (C-2 *C*H), 125.9 (Ar), 128.4 (Ar), 131.3 (Ar), 137.6 (C=*C*), 139.2 (Ar), 172.1 (C=O).

Z-(2R,5S)-7-Decylidine-2-phenyl-1-aza-3-oxabicyclo[3.3.0]octan-8-one **176**

Methanesulfonyl chloride (0.09 ml, 1.1 mmol) was added to a cold (0 °C), stirred solution of alcohol compound **172** (360 mg, 1 mmol) and triethylamine (2.1 ml, 15 mmol) in dry DCM (10 ml). The mixture was allowed to warm to 20 °C following addition, and was left to stand for 16 hours. The brown solution was poured into water (25 ml), and left to stir for 1 hour. The mixture was then extracted with DCM (3 x 20 ml), and the combined extracts washed with 2 M hydrochloric acid (2 x 30 ml) and brine (1 x 30 ml), dried ($MgSO_4$), and concentrated *in vacuo*. The golden oily residue was purified by flash column chromatography (30% ethyl acetate/petroleum). This gave the title compound as a colourless oil R_f 0.5 (50% ethyl acetate/petroleum) (180 mg, 50%),

which appeared to be a single isomer. ν_{max} (film)/cm^{-1} 1738vs (C=O), 1705vs (C=O); δ_H 0.9 (3 H, t, *J* 6.2, alkyl C*H*$_3$), 1.3 (20 H, m, alkyl C*H*$_2$), 1.7 (2 H, m, alkyl C*H*$_2$), 2.1 (2 H, m, C=CHC*H*$_2$), 2.4 (1 H, m, C-6 C*H*$_2$), 3.0 (1 H, m, C-6 C*H*$_2$), 3.4 (1 H, t, *J* 8.3, C-4 C*H*$_2$), 4.1 (1 H, m, C-4 C*H*$_2$), 4.3 (1 H, t, *J* 6.3, C-5 C*H*), 5.0 (1 H, m, C=C*H*CH$_2$), 6.3 (1 H, s, C-2 C*H*), 7.3 (5 H, m, Ar*H*); δ_C 14.1 (*C*H$_3$), 21.9 (C-6 *C*H$_2$), 22.7 (alkyl *C*H$_2$), 25.2 (C=CH*C*H$_2$), 29.2 (alkyl *C*H$_2$), 29.4 (alkyl *C*H$_2$), 29.5 (alkyl *C*H$_2$), 31.8 (alkyl *C*H$_2$), 31.9 (alkyl *C*H$_2$), 33.7 (alkyl *C*H$_2$), 57.4 (C-5 *C*H), 71.4 (C-4 *C*H$_2$), 87.4 (C-2 *C*H), 125.8 (Ar), 125.9 (Ar), 128.4 (Ar), 128.6 (Ar), 128.9 (Ar), 138.5 (C=*C*), 176.7 (C=O).

Isomers of *(2R,5S)-7-Hydroxy-7-(1-hydroxydecyl)-2-phenyl-1-aza-3-oxabicyclo [3.3.0] octan-8-one* **177** and **178**

Osmium tetroxide, 4 wt% in water, (2 ml, 0.63 mmol), was added to an ice cold stirred solution of alkene compound **175** (4.78 g, 14 mmol) and *N*-methylmorpholine-*N*-oxide, 50 wt% in water, (3.4 ml 14 mmol) in acetone (150 ml) and water (40 ml). The resulting pale yellow solution was stirred at 0 °C for 8 hours, and at 20 °C for 16 hours. The reaction was quenched by the addition of saturated sodium hydrogen sulfite solution (300 ml). After 20 minutes, the mixture was extracted with ethyl acetate (4 x 150 ml), and the combined extracts washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The resulting brown oil was purified by flash column chromatography (40-50% ethyl acetate/petroleum). Compound **178** (diastereomer), R_f 0.40, a colourless oil which gave a colourless solid on standing (1.97 g, 38%) m.p. 49-52 °C, and compound **177**, R_f 0.12, a colourless oil (2.31 g, 44%) were obtained. Compound **178** (diastereomer) ν_{max} (KBr)/cm^{-1} 3428m, br (O-H), 1737vs (C=O), 1689vs (C=O); δ_H 0.9 (3 H, t, *J* 6.2, alkyl C*H*$_3$), 1.3 (14 H, s, alkyl C*H*$_2$), 1.6 (3 H, br., alkyl C*H*$_2$, C-6 C*H*$_2$), 2.3 (1 H, d.d., *J* 5.9,

13.5, C-6 CH_2), 3.5 (2 H, m, C-4 CH_2, O*H*), 3.8 (1 H, d, *J* 9.9, C-5 C*H*), 4.5 (3 H, m, C*H*OH, C-4 CH_2, O*H*), 6.2 (1 H, s, C-2 C*H*), 7.4 (5 H, m, Ar*H*); δ_C 14.1 (CH_3), 22.7 (alkyl CH_2), 25.7 (alkyl CH_2), 29.2 (alkyl CH_2), 29.3 (alkyl CH_2), 29.6 (alkyl CH_2), 31.9 (alkyl CH_2), 36.0 (alkyl CH_2), 56.2 (C-5 *C*H), 72.1 (C-4 CH_2), 74.3 (*C*HOH), 82.0 (C-7 *C*OH), 86.2 (C-2 *C*H), 126.0 (Ar), 128.6 (Ar), 129.0 (Ar), 137.0 (Ar), 178.1 (C=O).

Compound **177** ν_{max} (film)/cm^{-1} 3427m, br (O-H), 1738vs (C=O), 1694vs (C=O); δ_H 0.9 (3 H, t, *J* 6.3, alkyl CH_3), 1.3 (14 H, s, alkyl CH_2), 1.6 (3 H, br., alkyl CH_2, C-6 CH_2), 1.9 (1 H, d.d., *J* 5.9, 13.9, C-6 CH_2), 2.7 (1 H, q, *J* 7.3, C-6 CH_2), 3.0 (1 H, s, O*H*), 3.6 (1 H, t, *J* 8.2, C-4 CH_2), 3.7 (1 H, m, C-5 C*H*), 3.9 (2 H, m, C*H*OH, O*H*), 4.3 (1 H, t, *J* 2.0, C-4 CH_2), 6.3 (1 H, s, C-2 C*H*), 7.4 (5 H, m, Ar*H*); δ_C 14.1 (CH_3), 22.7 (alkyl CH_2), 26.1 (alkyl CH_2), 29.3 (alkyl CH_2), 29.5 (alkyl CH_2), 29.6 (alkyl CH_2), 30.5 (alkyl CH_2), 31.9 (alkyl CH_2), 33.9 (alkyl CH_2), 55.2 (C-5 *C*H), 72.5 (C-4 CH_2), 75.1 (*C*HOH), 83.4 (C-7 *C*OH), 87.0 (C-2 *C*H), 126.0 (Ar), 128.9 (Ar), 138.1 (Ar), 177.4 (C=O).

3-Hydroxy-3-(1-hydroxydecyl)-5-hydroxymethyl-pyrrolidin-2-one **182**

Diol compound **177** (2.31 g, 6.16 mmol) was dissolved in methanol (30 ml) and water (10 ml) with toluene-*p*-sulfonic acid (120 mg, 0.60 mmol). The colourless solution was refluxed for 5 hours, allowed to stand for 16 hours, and refluxed for a further 7 hours. After standing for 16 hours, the colourless solution was concentrated *in vacuo*, and dried azeotropically with toluene (2 x 20 ml). The crude material was purified by flash column chromatography (10% methanol/DCM). This gave the title triol as a colourless oil (1.23 g, 70%), which on standing gave a colourless solid, m.p. 88-90 °C. ν_{max} (KBr)/cm^{-1} 3390m, br (N-H), 1688vs (C=O); δ_H 0.9 (3 H, t, *J* 6.2, alkyl CH_3), 1.3 (14 H, s, alkyl CH_2), 1.6 (2 H, br., alkyl CH_2), 1.8 (1 H, q, *J* 3.3, C-4 CH_2), 2.3 (1 H, m, C-4 CH_2), 3.6 (4

H, br., C*H*$_2$OH, C-5 C*H*CH$_2$, C*H*OH), 4.0 (1 H, s, O*H*), 4.2 (1 H, s, O*H*), 6.8 (1 H, s, N*H*); (d^4 methanol), δ_C 14.1 (*C*H$_3$), 22.8 (alkyl *C*H$_2$), 26.3 (C-4 *C*H$_2$), 29.4 (alkyl *C*H$_2$), 29.7 (alkyl *C*H$_2$), 30.5 (alkyl *C*H$_2$), 32.0 (alkyl *C*H$_2$), 33.0 (alkyl *C*H$_2$), 53.0 (C-5 *C*H), 64.9 (*C*H$_2$OH), 74.7 (*C*HOH), 178.7 (C=O).

3-Hydroxy-3-(1-hydroxydecyl)-5-hydroxymethyl-pyrrolidin-2-one **183**

Diol compound **178** (1.37 g, 6.16 mmol) was dissolved in methanol (15 ml) and water (5 ml) with toluene-*p*-sulfonic acid (70 mg, 0.4 mmol). The colourless solution was refluxed for 5 hours, allowed to stand for 16 hours, and refluxed for a further 7 hours. The colourless solution was concentrated *in vacuo*, and dried azeotropically with toluene (2 x 20 ml). The crude material was purified by flash column chromatography (10% methanol/DCM). This gave the title triol as a colourless oil (850 mg, 81%), which gave a colourless solid on standing m.p. 105-107 °C. ν_{max} (KBr)/cm^{-1} 3442m (O-H), 3337m (O-H), br (N-H), 1707vs (C=O), 1666vs (C=O); δ_H (d^4 methanol) 0.9 (3 H, t, *J* 6.3, alkyl C*H*$_3$), 1.3 (14 H, s, alkyl C*H*$_2$), 1.6 (2 H, br., alkyl C*H*$_2$), 2.9 (1 H, m, C-4 C*H*$_2$), 3.3 (1 H, m, C-4 C*H*$_2$), 3.5 (1 H, m, C-5 C*H*CH$_2$), 3.6 (1 H, m, C*H*OH), 3.7 (2 H, m, C*H*$_2$OH); (d^4 methanol), δ_C 14.5 (*C*H$_3$), 23.8 (alkyl *C*H$_2$), 27.5 (C-4 *C*H$_2$), 30.5 (alkyl *C*H$_2$), 30.8 (alkyl *C*H$_2$), 32.6 33.1 (alkyl *C*H$_2$), 33.8 (alkyl *C*H$_2$), 54.1 (C-5 *C*H), 65.6 (*C*H$_2$OH), 76.0 (*C*HOH), 80.1 (C-3 *C*OH), 179.6 (C=O).

(3S,5S)-3-(1-hydroxydecyl)-5-hydroxymethyl-pyrrolidin-2-one **179**

Pyrrolidinone compound **172** (950 mg, 2.7 mmol) was dissolved in methanol (12 ml) and water (4 ml), with toluene-*p*-sulfonic acid (20 mg, catalytic). The colourless solution was heated under reflux for 7 hours, left to stand for 16 hours, and heated under

reflux for a further 7 hours. The solution was then concentrated *in vacuo*, and dried azeotropically with toluene (2 x 10 ml). This gave a colourless oil, which was purified by flash column chromatography (10% methanol/DCM), to give the title diol as a colourless solid (610 mg, 85%), m.p. 81-83 °C. ν_{max} (KBr)/cm^{-1} 3353m (N-H), 3362m, (O-H), 1645vs (C=O); δ_H 0.9 (3 H, t, *J* 6.3, alkyl C*H*$_3$), 1.3 (12 H, s, alkyl C*H*$_2$), 1.4 (2 H, m, alkyl C*H*$_2$), 1.9 (2 H, m, C-4 C*H*$_2$), 2.5 (1 H, q, *J* 9.0, C-3 C*H*), 3.5 (1 H, m, C-5 C*H*CH$_2$), 3.7 (3 H, m, C*H*$_2$OH, C*H*OH), 4.0 (1 H, s, O*H*), 4.9 (1 H, s, O*H*), 7.3 (1 H, s, N*H*); δ_C 14.1 (*C*H$_3$), 22.7 (alkyl *C*H$_2$), 25.0 (C-4 *C*H$_2$), 26.7 (alkyl *C*H$_2$), 29.4 (alkyl *C*H$_2$), 29.6 (alkyl *C*H$_2$), 29.7 (alkyl *C*H$_2$), 32.0 (alkyl *C*H$_2$), 34.8 (alkyl *C*H$_2$), 45.0 (C-5 *C*H), 54.3 (C-3 *C*H), 65.5 (*C*H$_2$OH), 73.4 (*C*H(OH)CH$_2$), (C-2 *C*H), 181.2 (C=O).

*(3*R*,5*S*)-3-(1-hydroxydecyl)-5-hydroxymethyl-pyrrolidin-2-one* **180**

Pyrrolidinone compound **173** (590 mg, 1.6 mmol) was dissolved in methanol (10 ml) and water (2.5 ml), with toluene-*p*-sulfonic acid (30 mg, catalytic). The colourless solution was heated under reflux for 6 hours. The solution was then concentrated *in vacuo*, and dried azeotropically with toluene (2 x 10 ml). This gave a colourless oil, which was purified by flash column chromatography (10% methanol/DCM), giving the title compound (400 mg, 92%) as a colourless solid, m.p. 73-75 °C. ν_{max} (KBr)/cm^{-1} 3325m, br. (O-H), 1681vs (C=O); (d^6 acetone) δ_H 0.9 (3 H, t, *J* 6.3, alkyl C*H*$_3$), 1.3 (16 H, s, alkyl C*H*$_2$), 1.5 (2 H, m, alkyl C*H*$_2$), 2.2 (1 H, m, C-4 C*H*$_2$), 2.4 (1 H, m, C-4 C*H*$_2$), 2.9 (1 H, d, *J* 9.8, O*H*), 3.4 (1 H, m, C-5 C*H*CH$_2$), 3.6 (3 H, br. m, C*H*$_2$OH, C*H*OH), 4.0 (1 H, s, O*H*), 6.8 (1 H, s, N*H*); (d^6 acetone) δ_C 14.4 (*C*H$_3$), 23.3 (alkyl *C*H$_2$), 23.8 (alkyl *C*H$_2$), 26.9 (C-4 *C*H$_2$), 29.0 (alkyl *C*H$_2$), 29.3 (alkyl *C*H$_2$), 29.6 (alkyl *C*H$_2$), 29.9 (alkyl

CH_2), 32.6 (alkyl CH_2), 36.1 (CH(OH)CH_2), 47.0 (C-5 *C*H), 54.9 (C-3 *C*H), 66.4 (*C*H(OH)CH_2), 69.9 (CH_2OH), 180.7 (C=O).

(3S,5S)-3-(1-hydroxydecyl)-5-hydroxymethyl-pyrrolidin-2-one **181**

Pyrrolidinone compound **174** (1.51 g, 4.2 mmol) was dissolved in methanol (15 ml) and water (5 ml), with toluene-*p*-sulfonic acid (80 mg, 0.4 mmol). The colourless solution was heated under reflux for 5 hours. The solution was then concentrated *in vacuo*, and dried azeotropically with toluene (2 x 10 ml). This gave a colourless oil, which solidified on standing for 16 hours. Purification of the crude material by flash column chromatography (10% methanol/DCM) resulted in diol compound **181** as a colourless oil (1.14 g, 100%), solidifying on standing m.p. 72-74 °C. ν_{max} (KBr)/cm^{-1} 3448m (N-H), 3342m, br. (O-H), 1677vs (C=O); (d^6 acetone) δ_H 0.9 (3 H, t, *J* 6.9, alkyl C*H*$_3$), 1.3 (16 H, s, alkyl C*H*$_2$), 1.5 (2 H, m, alkyl C*H*$_2$), 2.4 (1 H, q, *J* 9.9, C-4 C*H*$_2$), 2.4 (1 H, m, C-4 C*H*$_2$), 2.5 (1 H, m, C-3 C*H*), 2.9 (1 H, s, O*H*), 3.4 (1 H, m, C-5 C*H*CH_2), 3.6 (3 H, m, C*H*$_2$OH, C*H*OH), 4.1 (1 H, s, O*H*), 7.1 (1 H, s, N*H*); (d^6 acetone) δ_C 14.4 (*C*H_3), 23.3 (alkyl *C*H_2), 25.7 (C-4 *C*H_2), 27.4 (alkyl *C*H_2), 29.0 (alkyl *C*H_2), 32.6 (alkyl *C*H_2), 35.7 (CH(OH)*C*H_2), 46.5 (C-5 *C*H), 55.1 (C-3 *C*H), 65.8 (*C*H(OH)CH_2), 73.8 (CH_2OH), 180.5 (C=O). *m/z* (EI) 272.2 ($M+H^+$, 100 %).

10.2.6. Sugar chemistry

2,3,4,6-Tetra-O-benzylglucopyranose fluoride[134] **188**

Diethylaminosufur trifluoride (360 mg, 2.2 mmol) was added dropwise to a cold (-30 °C) stirred solution of 2,3,4,6-tetra-*O*-benzylglucopyranose (1.0 g, 1.9 mmol) in dry THF (40 ml). Following addition, the colourless solution was allowed to warm to 20 °C

for 0.5 hour. The solution was again cooled to -30 °C, and quenched by dropwise addition of methanol (6 ml). Following the addition, the solution was allowed to warm to 20 °C. The mixture was then diluted with saturated sodium hydrogencarbonate solution (50 ml), and extracted with diethyl ether (4 x 20 ml). The combined extracts were dried ($MgSO_4$) and concentrated *in vacuo*, giving a golden oily residue (950 mg). Purification by flash column chromatography (DCM), yielded the title compound as a colourless oil (620 mg, 60%). ν_{max} (film)/cm^{-1} 3030m (Ar C-H), 1103s (C-O); δ_H 3.6 (5 H, br,C-3, C-4, C-5, C*H*OBn, C-7 CH_2OBn), 4.7 (9 H, br, PhCH_2, C-6 C*H*CH_2), 5.2 (1/2 H, d,C-2 C*H*F), 5.4 (1/2 H, d,C-2 C*H*F), 7.4 (20 H, m, Ar*H*); δ_C 68.3 (C-6 *C*HCH_2), 73.6 (C-4 *C*HOBn), 74.4 (C-7 *C*H_2OBn), 74.8 (C-5 *C*HOBn), 75.0 (C-3 *C*HOBn), 81.3 (Ph*C*H_2), 81.6 (Ph*C*H_2), 83.4 (Ph*C*H_2), 83.5 (Ph*C*H_2), 108.3 (C-2 *C*HF), 111.4 (C-2 *C*HF), 127.7 (Ar), 127.8 (Ar), 127.9 (Ar), 128.0 (Ar), 128.1 (Ar), 128.2 (Ar), 128.4 (Ar), 137.7 (Ar), 137.8 (Ar), 138.2 (Ar). *m/z* (FAB) 565.3 ($M+Na^+$, 100 %).

*3-(1-Hydroxydecyl)-5-(tetra-*O*-benzyglucosyl)oxymethyl-pyrrolidin-2-one* **189**

(Fluorosugar method)

Anhydrous tin (II) chloride (85 mg, 0.45 mmol), silver perchlorate (86 mg, 0.41 mmol) and powdered 4Å molecular sieves (250 mg) were added to a stirred solution of pyrrolidinone compound **179** (50 mg, 0.18 mmol) in dry THF (5 ml) at 20 °C. This resulted in a white suspension, which was left to stir at 20 °C for 0.5 hour. The mixture was cooled to -10 °C (ice/salt bath), and a solution of 2,3,4,6-tetra-*O*-benzylglucopyranose fluoride compound **188** (100 mg, 0.18 mmol) in dry THF (1 ml) added. The mixture was allowed to warm to 20 °C, and left to stir for 2 hours. The mixture was then filtered through celite, rinsing with a small volume of acetone. The filtrate was concentrated *in vacuo*, giving a colourless syrup. Purification of the crude

material by flash column chromatography (50% ethyl acetate/petroleum) resulted in a waxy white solid being obtained (59 mg, 41%). ν_{max} (film)/cm^{-1} 3428m (OH), 3212m (NH), 3030 (Ar C-H), 1618s (C=O); δ_H 0.9 (3 H, t, C*H*$_3$), 1.3 (14 H, s, alkyl C*H*$_2$), 1.4 (2 H, m, CH(OH)C*H*$_2$), 1.8 (2 H, m, C-4 C*H*$_2$), 2.4 (1 H, q, C-3 C*H*), 3.4 (2 H, d, C*H*$_2$OGlu), 3.5-3.6 (5 H, br. m, C-5 C*H*CH$_2$, C*H*OH, Glu C*H*), 3.7 (2 H, m, Glu C*H*), 4.4-4.6 (4 H, br. m, PhC*H*$_2$), 4.7-4.9 (4 H, br.m, PhC*H*$_2$), 6.4 (1H, br. s, Glu C-1 C*H*), 7.2 (20 H, m, Ar*H*). δ_C 14.1 (*C*H$_3$), 22.7 (alkyl *C*H$_2$), 24.9 (alkyl *C*H$_2$), 26.6 (C-4 *C*H$_2$), 29.3 (alkyl *C*H$_2$), 29.6 (alkyl *C*H$_2$), 29.7 (alkyl *C*H$_2$), 31.9 (alkyl *C*H$_2$), 34.9 (alkyl *C*H$_2$), 43.7 (C-3 *C*H), 51.5 (C-5 *C*H), 68.5 (*C*HOBn), 70.7 (*C*HOBn), 72.8 (*C*HOBn), 73.1 (*C*H$_2$OBn), 73.5(*C*H(OH)CH$_2$), 75.0(PhCH$_2$O), 75.2(PhCH$_2$O), 75.7 (PhCH$_2$O), 79.9 (PhCH$_2$O), 81.9 (PhCH$_2$O), 98.1 (Glu C-1 *C*H), 127.7 (Ar), 127.8 (Ar), 127.9 (Ar), 128.0 (Ar), 128.1 (Ar), 128.2 (Ar), 128.4 (Ar), 128.6 (Ar), 137.7 (Ar), 137.9 (Ar), 138.0 (Ar), 138.6 (Ar), 179.8 (*C*=O).

2,3,4,6-tetra-O-*Benzylglucopyranose trichloroimidate*[137] **190**

DBU (1.4 ml, 9.3 mmol) was added dropwise to a stirred solution of 2,3,4,6-tetra-*O*-benzylglucopyranose (5.0 g, 9.3 mmol), in dry DCM (50 ml) at 20 °C. After stirring for 5 minutes, freshly distilled trichloroacetonitrile (3.25 ml, 32 mmol) was added dropwise to the colourless solution. This gave a dark brown solution after 0.5 hour. After 1.5 hours, the solution was concentrated *in vacuo*, giving a black residue. Purification of the residue by flash column chromatography (40% ether/petroleum) yielded a pale yellow syrup (5.72 g, 90%). ν_{max} (film)/cm^{-1} 3340m (N-H), 3030m (ArC-H), 1670s (C=N); δ_H 3.7-3.8 (4 H, br. m, C*H*(OBn)), 4.0 (2 H, br. m, C*H*OBn), 4.6-5.0 (10 H, br. m, PhC*H*$_2$), 6.5 (1 H, d, C-1 C*H*), 7.3 (20 H, m, Ar*H*), 8.6 (1 H, s, C=N*H*); δ_C

68.0 (*C*HOBn), 72.8 (*C*HOBn), 73.1 (*C*H_2OBn), 73.5 (*C*HOBn), 75.3 (*C*HOBn), 75.6 (Ph*C*H_2O), 76.3 (Ph*C*H_2O), 76.4 (Ph*C*H_2O), 76.5 (Ph*C*H_2O), 91.2 (*C*Cl_3), 94.4 (Glu C-1 *C*H), 127.5 (Ar), 127.6 (Ar), 127.7 (Ar), 128.0 (Ar), 128.1 (Ar), 128.2 (Ar), 128.3 (Ar), 128.4 (Ar), 137.8 (Ar), 137.9 (Ar), 138.0 (Ar), 138.6 (Ar), 161.3 (*C*=NH).

3-(1-Hydroxydecyl)-5-(tetra-O-*benzyglucosyl)oxymethyl-pyrrolidin-2-one* **189** (Boron trifluoride etherate method)

Boron trifluoride etherate (0.06 ml, 0.5 mmol) was added to a stirred solution of 2,3,4,6-tetra-*O*-benzylglucopyranose trichloroimidate compound **190** (170 mg, 0.25 mmol) and pyrrolidinone compound **178** (50 mg, 0.19 mmol) in dry DCM (2 ml) at 20 °C. After 16 hours, the mixture was concentrated *in vacuo*, and the residue triturated with methanol (2 ml) and evapourated *in vacuo*. The residue was purified by flash column chromatography (50% ethyl acetate/petroleum). This yielded a colourless syrup (30 mg, 20%). The spectral data were consistent with those of compound **189** prepared using compound **188**.

5-(tetra-O-*Benzyglucosyl)oxymethyl-pyrrolidin-2-one* **191**

Toluene-*p*-sulfonic acid (10 mg, catalytic) was added to a stirred solution of 2,3,4,6-tetra-*O*-benzylglucopyranose trichloroimidate compound **190** (200 mg, 0.28 mmol) and pyrrolidinone compound **92** (36 mg, 0.28 mmol) in dry DCM (2 ml) at 20 °C. After 5 days, purification of the mixture by flash column chromatography (ethyl acetate) yielded a colourless syrup (80 mg, 45%). ν_{max} (film)/cm^{-1} 3209m (NH), 3030m (Ar C-H), 1694s (C=O); δ_H 1.7 (1 H, m, C-4 C*H*$_2$), 2.2 (1 H, m, C-4 C*H*$_2$), 2.4 (2 H, q, C-3 C*H*$_2$), 3.4 (2 H, d, C*H*$_2$OGlu), 3.5-3.6 (5 H, br. m, C-5 C*H*CH$_2$, C*H*OH, Glu C*H*), 3.7 (2

H, m, Glu C*H*), 4.4-4.6 (4 H, br. m, PhCH_2), 4.7-4.9 (4 H, br.m, PhCH_2), 6.3 (1H, br. s, Glu C-1 C*H*), 7.2 (20 H, m, ArH). δ_C 23.1 (C-4 *C*H_2), 29.6 (C-3 *C*H_2), 53.4 (C-5 *C*H), 68.4 (*C*HOBn), 70.7 (*C*HOBn), 73.0 (*C*HOBn), 73.4 (*C*HOBn), 73.6 (*C*H_2OGlu), 75.1 (*C*H_2OBn), 75.8 (Ph*C*H_2O), 76.6 (Ph*C*H_2O), 79.9 (Ph*C*H_2O), 81.9 (Ph*C*H_2O), 97.8 (Glu C-1 *C*H), 127.6 (Ar), 127.8 (Ar), 127.9 (Ar), 128.0 (Ar), 128.1 (Ar), 128.2 (Ar), 128.4 (Ar), 128.6 (Ar), 137.7 (Ar), 137.8 (Ar), 137.9 (Ar), 138.0 (Ar), 138.1 (Ar), 138.6 (Ar), 177.6 (C=O).

3-(1-Hydroxydecyl)-5-(tetra-O-*benzyglucosyl)oxymethyl-pyrrolidin-2-one* **189** (Toluene-*p*-sulfonic acid method)

Toluene-*p*-sulfonic acid (10 mg, catalytic) was added to a stirred solution of 2,3,4,6-tetra-*O*-benzylglucopyranose trichloroimidate compound **190** (200 mg, 0.28 mmol) and pyrrolidinone compound **178** (76 mg, 0.28 mmol) in dry DCM (2 ml) at 20 °C. After 16 hours, the mixture was concentrated *in vacuo*, and the residue purified by flash column chromatography (50% ethyl acetate/petroleum). This yielded a colourless syrup (150 mg, 68%). *m/z* (FAB) 794.6 (M^+, 100 %). The spectral data were consistent with those of compound **189** produced by the previous methods.

Methyl-2,3,4,6-tetra-O-*benzylgalactopyranose*[139] **193**

Sodium hydride, 60% dispersion in oil, (21 g, 0.52 mol), was added in portions to a stirred solution of methyl-β-D-galactopyranoside (25 g, 0.13 mol), and tetrabutyl ammonium iodide (100 g, 0.27 mol), in dry DMF (300 ml) at 20 °C. After 0.5 hour, benzyl bromide (63 ml, 0.52 mol) was added slowly, followed by delayed effervescence. The gave a pale yellow mixture, which was left to stand for 2 hours. The reaction was

then quenched by addition of water (500 ml), added cautiously at first. The mixture was left to stir for 1 hour, then the pale yellow solid which had separated out was filtered and washed with water (3 x 250 ml), and petroleum (250 ml). The crude product was recrystallised from 60-80 petroleum, to yield the title compound as a white solid (40.36 g, 56%) m.p. 81-83 °C (Literature[139] 80-81 °C). ν_{max} (KBr)/cm^{-1} 3446m (O-H), 3028m (Ar C-H), 1108s (C-O); δ_H 3.5 (8 H, m, OC*H*$_3$, C*H*OBn), 3.7 (1 H, t, C*H*OBn), 3.8 (1 H, d, C*H*OMe), 4.3 (1 H, d, C*H*$_2$Ph), 4.4 (2 H, d, C*H*$_2$Ph), 4.6 (2 H, d, C*H*$_2$Ph), 4.7 (2 H, m, C*H*$_2$Ph), 4.9 (2 H, t, C*H*$_2$Ph), 7.3 (20 H, m, Ar*H*); δ_C 57.0 (O*C*H$_3$), 68.9 (C-4 *C*HOBn), 69.5 (C-3 *C*HOBn), 72.9 (C-4 *C*HOBn), 73.4 (C-6 *C*H$_2$OBn), 74.5 (C-2 *C*HOBn), 75.1 (Ph*C*H$_2$), 78.7 (Ph*C*H$_2$), 80.0 (Ph*C*H$_2$), 83.1 (Ph*C*H$_2$), 99.5 (C-1 *C*H(OH)), 127.5 (Ar), 127.9 (Ar), 128.1 (Ar), 128.2 (Ar), 128.3 (Ar), 128.4 (Ar)), 128.5 (Ar), 137.7 (Ar), 137.8 (Ar), 138.2 (Ar).

2,3,4,6-tetra-O-*benzylgalactopyranose*[139] **194**

Methyl 2,3,4,6-tetra-*O*-benzylgalactopyranose compound **193** was dissolved in dioxane (280 ml), water (72 ml), and 10 N sulphuric acid (7.8 ml), and the solution heated to reflux for 5 days. The pale yellow solution was then neutralised with calcium carbonate, and the mixture filtered through celite. The filtrate was concentrated *in vacuo*, and the oily residue purified by chromatography on alumina (10% ether/toluene). The title compound was obtained as a colourless syrup (6.72 g, 36%), as well as recovered starting material (10.32 g, 52%). ν_{max} (film)/cm^{-1} 3416m (O-H), 3029m (Ar C-H), 1098s (C-O); δ_H 3.4-4.1 (6 H,br. m, C*H*OBn, C*H*$_2$OBn), 4.4-4.9 (8 H, br. m, CH$_2$Ph), 5.2 (1 H, s, C-1), 7.3 (20 H, m, Ar*H*); δ_C 68.9 (C-5 *C*HOBn), 69.5 (C-3 *C*HOBn), 72.9 (C-4 *C*HOBn), 73.5 (C-6 *C*H$_2$OBn), 74.5 (C-2 *C*HOBn), 75.1 (Ph*C*H$_2$), 78.7 (Ph*C*H$_2$), 80.7

(Ph*C*H_2), 82.2 (Ph*C*H_2), 91.9 (C-1 *C*H(OH)), 97.8 (C-1 *C*H(OH)), 125.3 (Ar), 127.5 (Ar), 127.9 (Ar), 128.1 (Ar), 128.2 (Ar), 128.3 (Ar), 128.4 (Ar), 128.5 (Ar), 137.7 (Ar), 137.8 (Ar), 138.2 (Ar), 138.4 (Ar), 138.5 (Ar), 138.6 (Ar).

2,3,4,6-tetra-O-*Benzylgalactopyranose trichloroimidate* **195**

DBU (0.23 ml, 1.5 mmol) was added dropwise to a stirred solution of 2,3,4,6-tetra-*O*-benzylgalactopyranose compound **194** (810 mg, 1.5 mmol), in dry DCM (10 ml) at 20 °C. After stirring for 20 minutes, freshly distilled trichloroacetonitrile (0.53 ml, 5.25 mmol) was added dropwise to the colourless solution. This gave a dark brown solution after 0.5 hour. After 2 hours, the solution was concentrated *in vacuo*, giving a black residue. Purification of the residue by flash column chromatography (40% ether/petroleum) yielded a pale yellow syrup (1.03 g, 100%). ν_{max} (film)/cm^{-1} 3339m (N-H), 3030m (Ar C-H), 1669s (C=N); δ_H 3.6 (2 H, br. C*H*OBn), 4.0 (2 H, br. m, C*H*OBn), 4.2 (2 H, br. m. C*H*OBn), 4.4-4.8 (8 H, br. m, CH_2Ph), 6.6 (H, d, C-2 C*H*O), 7.3 (20 H, m, Ar*H*), 8.5 (1 H, s, C=N*H*); δ_C 68.3 (*C*HOBn), 72.1 (*C*HOBn), 72.9 (*C*H_2OBn), 73.0 (*C*HOBn), 73.4 (*C*HOBn), 73.5 (Ph*C*H_2O), 74.6(Ph*C*H_2O), 74.9 (Ph*C*H_2O), 75.9 (Ph*C*H_2O), 95.2 (C-1 *C*HO), 127.4 (Ar), 127.5 (Ar), 127.6 (Ar), 127.7 (Ar), 127.8 (Ar), 128.0 (Ar), 128.1 (Ar), 128.2 (Ar), 128.3 (Ar), 128.4 (Ar), 137.8 (Ar), 137.9 (Ar), 138.0 (Ar), 138.5 (Ar), 163.2 (*C*=N).

*3-(1-Hydroxydecyl)-5-(tetra-*O*-benzygalactosyl)oxymethyl-pyrrolidin-2-one* **196**

Using the same reaction procedure as described for compound **191**, using pyrroldinone compound **181**, and trichloroimidate compound **195**, the title compound was isolated as a colourless oil (1.37 g, 41%), following flash column chromatography (50% ethyl acetate/petroleum). ν_{max} (film)/cm^{-1} 3398m (NH), 3208m (OH), 3030m (Ar C-H), 1738vs (C=O), 1682vs (C=O); δ_H 0.8 (3 H, t, alkyl C*H*$_3$), 1.3 (14 H, s, alkyl C*H*$_2$), 1.4 (2 H, m, CH(OH)C*H*$_2$), 2.1 (1 H, br. m, C-4 C*H*$_2$), 2.3 (1 H, br. m, C-4 C*H*$_2$), 3.3 (1 H, t, C-3 C*H*), 3.5 (2 H, m, C*H*$_2$OGal), 3.5-3.9 (5 H, br. m, C-5 C*H*CH$_2$, Gal C*H*), 4.3-4.6 (4 H, br. m, PhC*H*$_2$), 4.7-4.9 (4 H, br. m, PhC*H*$_2$), 5.9 (0.5 H, s, Gal C-1 C*H*), 6.7 (0.5 H, s, Gal C-1 C*H*), 7.3 (20 H, m, Ar*H*).

*3-Hydroxy-3-(1-hydroxydecyl)-5-(tetra-*O*-benzyglucosyl)oxymethyl-pyrrolidin-2-one* **197**

Using the same reaction procedure as described for compound **196**, using pyrroldinone compound **183** and trichloroimidate compound **195**, the title compound was isolated as a colourless oil (0.91 g, 38%). ν_{max} (film)/cm^{-1} 3312m br., (O-H), 1738vs (C=O), 1694vs (C=O); δ_H 0.9 (3 H, t, alkyl C*H*$_3$), 1.3 (12 H, s, alkyl C*H*$_2$), 1.6 (2 H, br., CH(OH)C*H*$_2$), 2.1 (1 H, m, C-4 C*H*$_2$), 3.3 (1 H, m, C-4 C*H*$_2$), 3.5-4.1 (8 H, br., C-5 C*H*, Gal C*H*), 4.3-4.9 (8 H, br. m, PhC*H*$_2$), 6.1 (0.5 H, s, Gal C-1 C*H*), 6.9 (0.5 H, s, Gal C-1 C*H*), 7.3 (20 H, m, Ar*H*).

*3-Hydroxy-3-(1-hydroxydecyl)-5-(tetra-*O*-benzyglucosyl)oxymethyl-pyrrolidin-2-one* (isomer) **198**

Using the same reaction procedure as described for compound **196**, using pyrroldinone compound **182** and trichloroimidate compound **195**, the title compound was

isolated as a colourless oil (0.91 g, 38%). ν_{max} (film)/cm^{-1} 3324m (OH), 1738vs (C=O), 1696vs (C=O); δ_H 0.9 (3 H, t, alkyl CH_3), 1.3 (12 H, s, alkyl CH_2), 1.5 (2 H, m, CH(OH)CH_2), 2.2 (1 H, m, C-4 CH_2), 3.5 (1 H, m, C-4 CH_2), 3.6 (2 H, m, Gal C*H*), 3.9 (3 H, m, Gal C*H*), 4.0 (1 H, d.d., C-5 C*H*), 4.3-4.9 (8 H, br. m, PhCH_2), 6.2 (0.5 H, s, Gal C-1 C*H*), 6.8 (0.5 H, s Gal C-1 C*H*,), 7.3 (20 H, m, Ar*H*); δ_C 14.1 (*C*H_3), 22.7 (alkyl *C*H_2), 26.0 (C-4 *C*H_2), 29.3 (alkyl *C*H_2), 29.6 (alkyl *C*H_2), 29.7 (alkyl *C*H_2), 31.9 (alkyl *C*H_2), 32.7 (alkyl *C*H_2), 50.9 (C-5 *C*H), 69.3 (*C*HOBn), 70.1 (*C*HOBn), 73.2 (*C*HOBn), 73.4 (*C*HOBn), 73.8 (PhCH_2), 74.6 (PhCH_2), 74.8 (PhCH_2), 76.0 (PhCH_2), 78.9 (PhCH_2), 98.5 (Gal C-1 C*H*), 127.5 (Ar), 127.6 (Ar), 127.7 (Ar), 127.9 (Ar), 128.1 (Ar), 128.3 (Ar), 128.4 (Ar), 137.7 (Ar), 138.2 (Ar), 138.6 (Ar), 178.0 (C=O).

*Methyl 3-*O*-(2,3,4,6-tetra-*O*-benzylglucopyranosyl)-2-propenoate*[141] **199**

2,3,4,6-tetra-*O*-Benzylglucopyranose was dried *in vacuo* for 10 minutes, before being dissolved in dry DCM (1 ml). Methyl propiolate (0.09 ml, 1 mmol) was then added to the solution, at 20 °C, followed by tri-*n*-butyl phosphine (0.04 ml, 0.15 mmol), which resulted in an exothermic reaction. The resulting black mixture was allowed to stir at 20 °C for 24 hours, and was then concentrated *in vacuo*. The residue was purified by flash column chromatography (30% ethyl acetate/petroleum) yielded a pale yellow syrup (220 mg, 70%). The spectral data was in agreement with the literature values.

3-(1-Hydroxydecyl)-5-(glucosyl)oxymethyl-pyrrolidin-2-one **200**

10% Palladium on carbon (50 mg) was added to a stirred solution of pyrrolidinone compound **189** (130 mg, 0.16 mmol) in dry ethanol (4 ml) at 20 °C. The mixture was stirred under an atmosphere of hydrogen (balloon pressure) for 2 days. The mixture was

filtered through celite, and the filtrate concentrated *in vacuo*, to give a white solid as the title compound (66 mg, 95%), m.p. 78-80 °C. %). ν_{max} (KBr)/cm^{-1} 3350m br. (OH), 1692s (C=O), 1666s (C=O), 1650s (C=O); δ_H (d^4 methanol) 0.9 (3 H, t, alkyl CH_3), 1.2 (12 H, s, alkyl CH_2), 1.5 (2 H, m, CH(OH)CH_2), 1.9 (1 H, br., C-4 CH_2), 2.1 (1 H, br., C-4 CH_2), 2.6 (1 H, br., C-3 C*H*), 3.2-3.4 (2 H, br., Glu C*H*), 3.5 (2 H, br., Glu C*H*), 3.8 (2 H, br., Glu C*H*), 4.2 (1 H, d.d., C-5 C*H*CH_2); *m/z* (FAB) 434.2 (M^+, 100 %).

3-(1-Hydroxydecyl)-5-(galactosyl)oxymethyl-pyrrolidin-2-one **201**

10% Palladium on carbon (1.8 g, 1.7 mmol Pd) was added to a stirred solution of pyrrolidinone compound **196** (1.37 g, 1.73 mmol) in dry ethanol (50 ml) and ethyl acetate (5 ml) at 20 °C. The mixture was stirred under an atmosphere of hydrogen (balloon pressure) for 5 days. The mixture was filtered through celite, and the filtrate concentrated *in vacuo*, to give a colourless solid as the title compound (430 mg, 57%), m.p. 142-143 °C. ν_{max} (KBr)/cm^{-1} 3403m (O-H), 1668vs (C=O); (d^6 DMSO) δ_H 0.9 (3 H, t, CH_3), 1.2 (14 H, s, alkyl CH_2), 1.4 (2 H, m, CH(OH)CH_2), 2.1 (1 H, m, C-4 CH_2), 2.4 (1 H, m, C-4 CH_2), 3.3-3.7 (6 H, br.m, Gal C*H*), 3.9 (1 H, d.d., C-5 C*H*CH_2), 4.4-4.9 (2 H, br. m, PhCH_2), 8.0 (1 H, m, Gal C-2 C*H*). *m/z* (high resolution FAB) found 434.2767 ($M+H^+$), ($C_{21}H_{39}NO_8$ requires *M*, 433.2676). Also found 530.3721 (($M+CH_2C_6H_{12}$)+H^+), ($C_{28}H_{51}NO_8$ requires 529.3615). (Found C, 57.4; H, 8.6; N, 2.95%. $C_{21}H_{39}NO_8$.0.5 H_2O requires C, 57.0; H, 9.0; N, 3.2%) (Karl Fischer analysis shows 2.4% H_2O);

3-Hydroxy-3-(1-hydroxydecyl)-5-(galactosyl)oxymethyl-pyrrolidin-2-one **202**

Using the same procedure as described for compound **201**, compound **198** was hydrogenated, to obtain the title compound as a colourless glassy solid (230 mg, 83%), m.p. 62-65 °C. ν_{max} (KBr)/cm^{-1} 3402vs (O-H), 1691vs (C=O); (d^6 DMSO) δ_H 0.9 (3 H, t, alkyl C*H*$_3$), 1.2 (12 H, s, alkyl C*H*$_2$), 1.5 (2 H, m, CH(OH)C*H*$_2$), 2.4 (2 H, m, C-4 C*H*$_2$), 3.3-3.9 (8 H, br. m, Gal C*H*), 4.0 (1 H, m, C-5 C*H*), 4.5-4.8 (4 H, br. m, PhC*H*$_2$), 7.2-7.5 (4 H, br. m, Ar*H*), 7.9 (1 H, m,). *m/z* (high resolution FAB) found 450.2712 (M+H$^+$), ($C_{21}H_{40}NO_9$ requires *M*, 449.2625). Also found 546.3648 ((M+$CH_2C_6H_{11}$)+H$^+$) ($C_{28}H_{52}NO_9$ requires *M*, 535.3564). (Found C, 54.8; H, 8.4; N, 2.8%. $C_{21}H_{39}NO_9$ requires C, 56.1; H, 8.7; N, 3.1%.);

3-Hydroxy-3-(1-hydroxydecyl)-5-(galactosyl)oxymethyl-pyrrolidin-2-one **203**

Using the same procedure as described for compound **201**, compound **197** was hydrogenated, to obtain the title compound as a colourless solid (390 mg, 77%), m.p. 77-79 °C. ν_{max} (KBr)/cm^{-1} 3404vs (O-H), 1685vs (C=O); (d^6 DMSO) δ_H 0.9 (3 H, t, C*H*$_3$), 1.2 (14 H, s, alkyl C*H*$_2$), 1.5 (2 H, m, CH(OH)C*H*$_2$), 1.7 (1 H, m, C-4 C*H*$_2$), 1.9 (1 H, m, C-4 C*H*$_2$), 3.3-3.7 (6 H, br.m, Gal C*H*), 3.9 (1 H, d.d., C-5 C*H*CH$_2$), 4.4-4.8 (1 H, m, PhC*H*$_2$), 8.0 (1 H, t, Gal C-2 C*H*). *m/z* (high resolution FAB) found 450.2712 (M+H$^+$), ($C_{21}H_{39}NO_9$ requires *M*, 449.2625). Also found 546.3648 ((M+$CH_2C_6H_{12}$)+H$^+$) ($C_{28}H_{52}NO_9$ requires *M*, 545.3564). (Found C, 54.8; H, 8.4; N, 2.8%. $C_{21}H_{39}NO_9$.0.5 H_2O requires C, 55.0; H, 8.7; N, 3.1%.) (Karl Fischer analysis shows 3.6% H_2O);

10.3 MLR assay protocol.

Responder cells for the mixed lymphocyte culture are prepared from the peripheral blood of a human donor. The blood is collected into CPT Vacutainer tubes (Becton Dickinson) and spun in a 25 °C centrifuge for 20 minutes at 2600 RPM (within the 1500-1800 RCF range recommended by manufacturer). The cell interface is resuspended by tube inversion and the plasma fraction diluted at least three fold in medium (RPMI 1640 supplemented with 10 % fetal bovine serum, 5 x 10^{-5} M β-mercaptoethanol and penicillin / streptomycin) prior to pelleting of lymphocytes. The cells are washed three times (1000 RPM x 10 minutes) and resuspended at 4 x 10^6 per ml in medium.

The Epstein Barr virus transformed JY cell line is used as a stimulator for these studies. These cells are suspended at 5 x 10^6 per ml in medium warmed to 37 °C. Mitomycin C is added to a final concentration of 25 µg per ml. The tube is wrapped with aluminium foil to prevent exposure to light and the cells are incubated for 30 minutes in a 5 % CO_2 incubator. The stimulator cells are washed three times in medium and resuspended at 2 x 10^6 per ml.

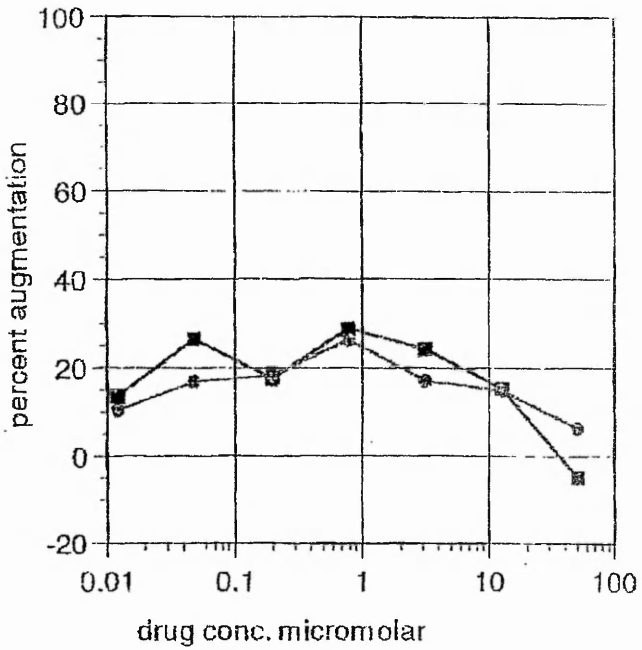
The mixture of 1 part responder cells with 1 part stimulator cells results in a suspension which is 2X the final concentration of the cells.

The culture is set up in a 96 well U bottom microtiter tissue culture plate. Compounds are serially diluted in a medium to which 1 % DMSO has been added. The final volume of diluted compound is 0.1 ml per well. With the addition of 0.1 ml of the 2X suspension of responder and stimulator cells to each well, the working concentration of cells and compound is achieved (1 x 10^5 responders plus 5 x 10^4 stimulators in a final volume of 0.2 ml medium supplemented with 0.5 % DMSO). The culture is harvested with a Betaplate harvester (Wallac) and assayed by Betaplate reader (Wallac).

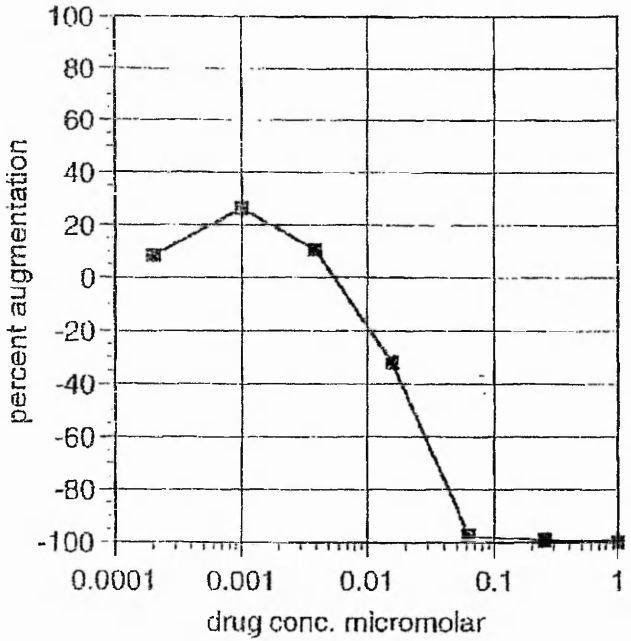
10.4 Results.

The compounds **201** and **203**, RR455 and RR456 respectively, were assayed in a concentration range from 50 μmol to 12 nmol with serial 4 fold dilution's. The pan kinase inhibitor staurosporine was included as an experimental control. Staurosporine is a potent inhibitor of the mixed lymphocyte reaction. Both **201** and **203** had little or no effect in the assay. As can be seen in the graph, a maximum augmentation of approximately 30 % was seen for both compounds at an 0.78 μmol concentration. The addition of staurosporine and other tyrosine kinase inhibitors has also resulted in augmented rather than suppressed responses when added at sub-efficacious concentrations. The staurosporine data were also represented as percent augmentation rather than inhibition to highlight this fact.

Immunomodulation of Human MLR by RR455 and RR456



Immunomodulation of Human MLR by Staurosporine



11. References

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