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Guanidine glycomimetics

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Abbreviations

Å	Ångström
App.	Apparent
Bn	Benzyl
Br.	Broad
Boc	*tert*-Butoxycarbonyl
DCM	Dichloromethane
DEPT	Distortionless enhancement by polarisation transfer
DMAP	4-Dimethylaminopyridine
DMDO	2, 2-Dimethyldioxirane
DMF	N, N-Dimethylformamide
FTIR	Fourier transform infra-red
h	Hour(s)
HRMS	High resolution mass spectrometry
HPLC	High Performance Liquid Chromatography
IMS	Industrial Methylated Spirits
Mpt	Melting point
Ms	Mesyl
NMR	Nuclear magnetic resonance
Ph	Phenyl
PG	Protecting group
PNP	p-NitroPhenol
RBF	Round bottomed flask

rt	Room temperature
TBDMS	*tert*-butyldimethylsilyl
THF	Tetrahydrofuran
TFA	Trifluoroacetic acid
Tris	2,4,6-Triisopropylbenzenesulfonate
Z	Benzyloxycarbonyl

Contents

Abstract

This thesis describes the investigation of methodologies towards the synthesis and purification of 5-, 6- and 7- membered guanidine heterocycles. Methods include intermolecular epoxide ring opening reactions using guanidine and the intramolecular cyclization reactions of *N*-Boc-allylguanidines and *N*-Boc-homoallylguanidines in both epoxide ring opening reactions and palladium mediated cyclisations. The possible utility of these heterocycles as glycosidase inhibitors was investigated using enzymatic assays.

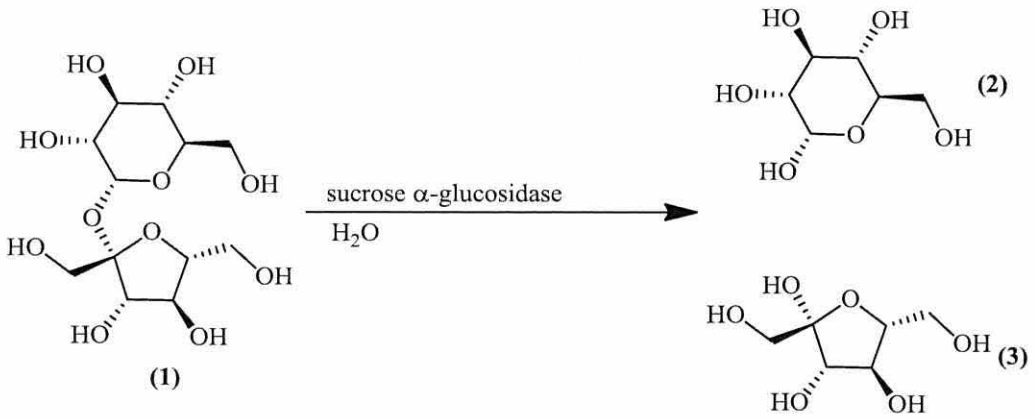
Introduction

Glycosidases and glycomimetics

Enzymes act as catalysts for the chemistry underpinning the processes vital for life. Due to this, a large part of the ongoing search for therapeutics for the treatment of diseases is devoted to molecules that can modify and inhibit the action of enzymes. This thesis concerns the development of synthetic methods for the preparation of compounds that act on a class of enzymes known as glycosidases.

Glycosidases are a wide ranging group of enzymes that catalyse the hydrolysis of glycosidic bonds between found in saccharides or between a carbohydrate and a non-carbohydrate moiety in glycoconjugates.

An example of a glycosidase is sucrose α-glucosidase (EC 3.2.1.48), this sucrase catalyses the hydrolysis of the disaccharide sucrose **1** to give the monosaccharides glucose **2** and fructose **3** (Scheme 1)[1].



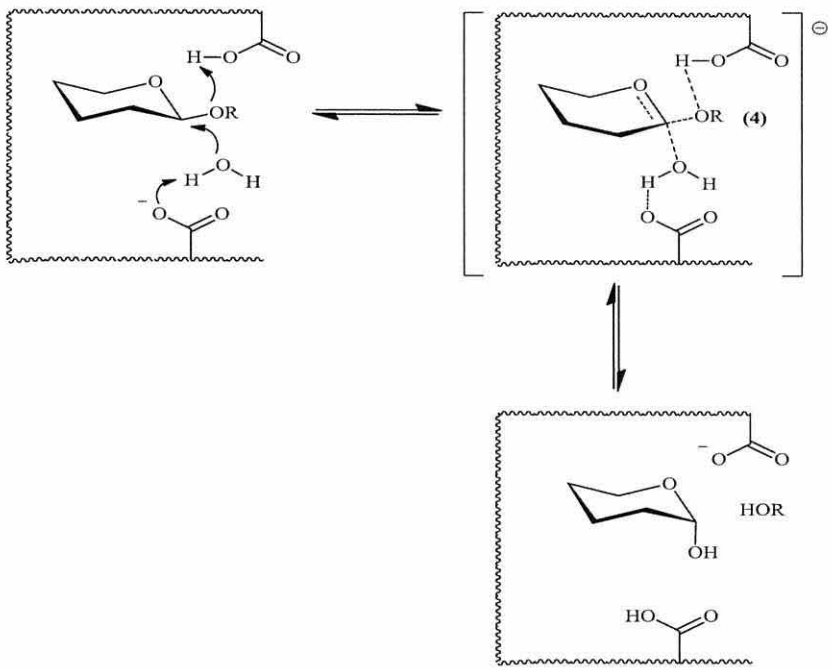
Scheme 1: Hydrolysis of sucrose.

To date more than two thousand glycosidases have been identified and classified into over a hundred different families[2]. Glycosidases can be divided into exo-glycosidases, which

hydrolyse bonds at the non-reducing terminal and endo-glycosidases which break interal glycosidic bonds[3]. The glycosidase catalysed hydrolysis of the glycosidic bond can take place through one of two mechanisms leading to two possible stereochemical outcomes; inversion or retention of configuration at the anomeric carbon. In both mechanisms the cleavage of the glycosidic bond is carried out by two carboxylic acid residues in the active site with the addition of a water molecule[4].

Hydrolysis by inversion

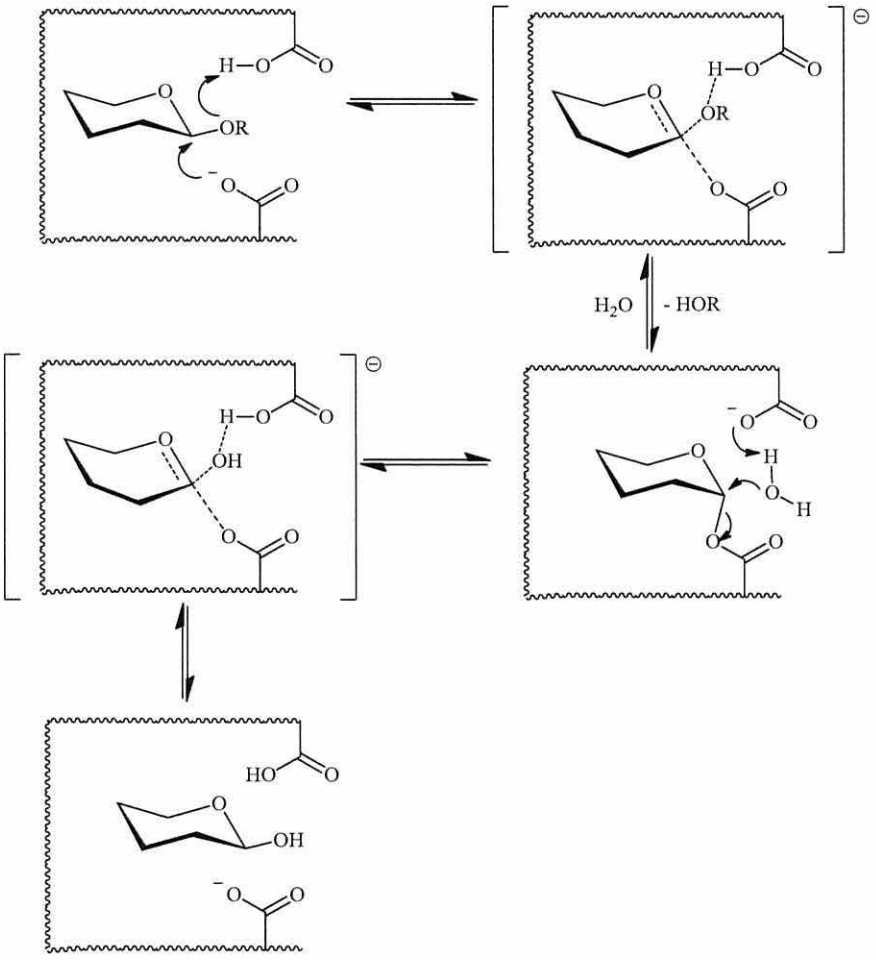
In inverting glycosidases the two amino acid residues are positioned approximately 9.0 and 9.5 Å apart for α- and β-glycosidases respectively[5]. The reaction takes place in a single step with one of the amino acid residues acting as a base binding with a proton of the water molecule that in turn binds with the substrates anomeric carbon. The glycosidic oxygen then binds with the acidic proton of the second amino acid residue giving a oxacarbenium ion-like transition state **4**. The substrate's glycosidic bond is then cleaved and the products are released (Scheme 2).



Scheme 2: Proposed mechanism for the hydrolysis of a glycosidic bond by a glycosidase enzyme leading to inversion[4]

Hydrolysis by retention

Hydrolysis with net retention of configuration is most commonly achieved via a two step mechanism involving two inversions with each going through a oxacarbenium ion-like transition state. Reaction occurs with acid/base and nucleophilic assistance provided by two amino acid side chains, located 4.8 and 5.3 Å apart for α- and β-glycosidases respectively[5]. In the first step one residue plays the role of a nucleophile, attacking the anomeric centre to displace the aglycon and form a glycosyl enzyme intermediate. At the same time the other residue functions as an acid catalyst and protonates the glycosidic oxygen as the bond cleaves. In the second step the glycosyl enzyme is hydrolysed by water, with the other residue now acting as a base catalyst deprotonating the water molecule as it attacks (Scheme 3).



Scheme 3: Proposed mechanism for the hydrolysis of a glycosidic bond by a glycosidase enzyme leading to retention[4]

Glycomimetics as therapeutics

Glycosidases are responsible for a number of processes including the digestion of polysaccharides and complex carbohydrates for metabolism, biosynthesis of glycoproteins and catabolism of glycoconjugates[6]. These processes have far reaching consequences when it comes to health and disease.

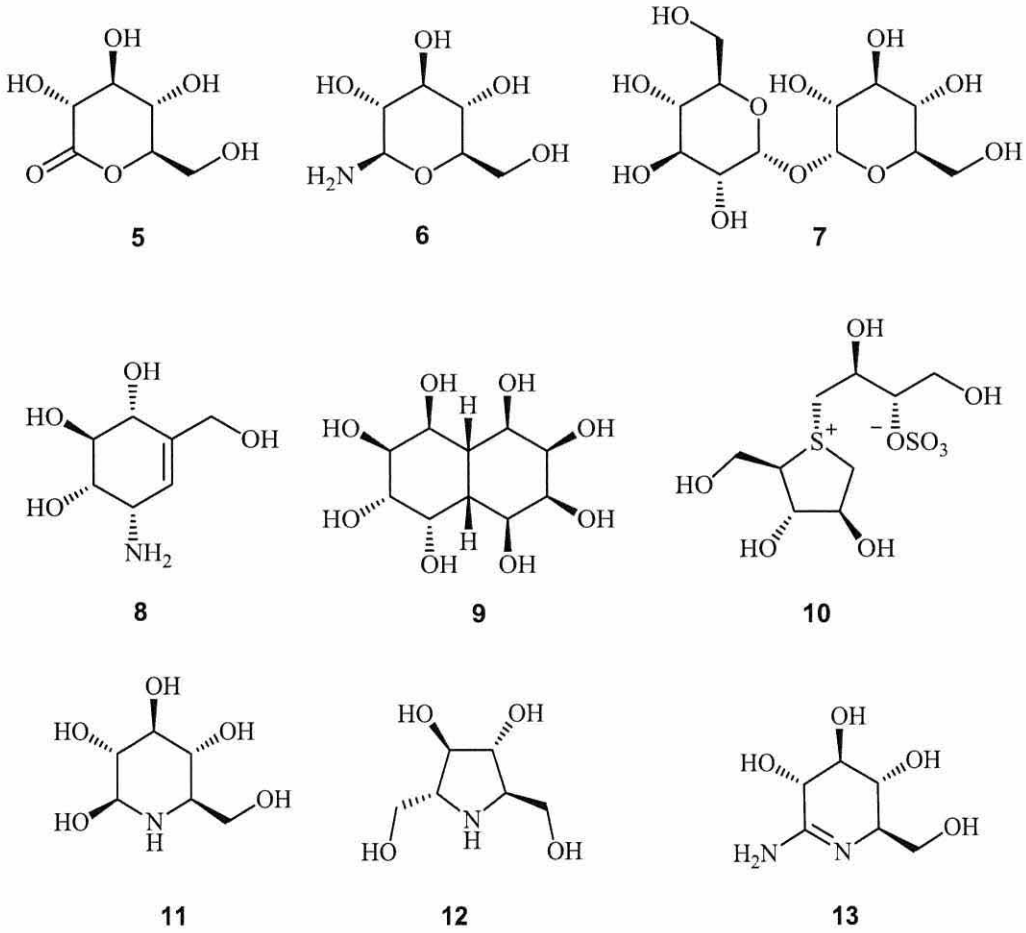
The digestion of carbohydrates has an important role to play in the management in diseases such as type 2 diabetes mellitus[7]. By inhibiting the action of the digestive glycosidases, the amount of glucose being released through digestion and then entering the bloodstream can be reduced leading to easier management of blood glucose levels.

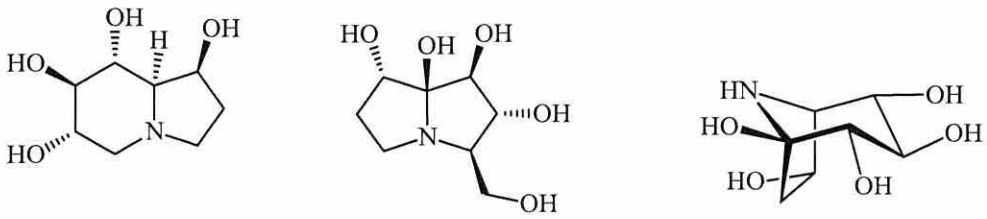
Glycosidases are also responsible for the control of glycoprotein production. This process is important because the *N*-linked oligiosaccaride chains found in glycoproteins on cell surfaces have been found to be involved in a large range of cell-cell interactions, recognition, differentiation, and also infection processes by various pathogens[8,9]. These roles make the glycosidases involved in the processing of glycoproteins useful targets for the treatment of diseases such as cancer[10] and viral infections[11].

The catabolism of substrates in the lysosomes of cells is vital for their proper function. Some individuals are born with genetic disorders that prevent the proper function of some enzymes within their lysosomes, leading to an increased amount of substrates being held in the lysosomes because of this retarded degradation. This group of disorders is referred to as Lysosomal Storage Diseases (LSD), which includes Tay-Sachs disease, Pompe disease, Gaucher disease and Fabry disease. There is interest towards developing glycosidase inhibitors as treatments for these diseases, as they may be able to help balance synthesis and degradation at the defective step thus helping alleviate the build up and storage of substrates within the lysosome. This form of treatment is termed substrate deprivation. More recently there are indications that some inhibitors can interact with defective mutant enzymes and enhance their activity by improving folding. This emerging treatment is known as pharmacological chaperone therapy[12].

Considerable research has been undertaken towards the development of molecules capable of inhibiting and modulating the activity of glycosidases. These molecules are often referred to as glycomimetics.

It was with the discovery of the first natural glucose analogue nojirimycin **11** (figure 1) in 1966 from the cultured broths of several strains of *Streptomyces*[13] that widespread interest in glycomimetics truly began. Nojirimycin was found to be a potent inhibitor of α- and β-glucosidases[14]. In subsequent years a wide range of compounds including, aldonolactones **5**[15], glycosylamines **6**[16], disaccharides **7**[17], carbasugars[18,19] such as conduritols **8**[20] and polyols **9**[21], thiosugars **10**[22,23,24], and iminosugars[25,26,] such as polyhydroxylated piperidines **11**, pyrrolidines **12**, amidine containing heterocycles **13**, indolizidines **14**, pyrrolizidines **15** and nortropanes **16** have shown to posses glycomimetic properties allowing them to inhibit and modify the activity of glycosidases (Figure 1).





14 **15** **16**

Figure 1: The range of compounds that found to act as glycomimetics.

Many compounds that have shown to inhibit glycosidases have been further biologically tested, developed and commercialised as therapeutics. Examples include drugs for the treatment of type 2 diabetes mellitus such as acarbose **17**[27,28] an inhibitor of α-glucosidase and α-amylase, and miglitol **18**[29,30] an α-glucosidase inhibitor. Miglustat **19** is a ceramide glucosyltransferase inhibitor used in the treatment of type 1 Gaucher disease[31], there has also been some indication that Miglustat's therapeutic effect is partially due to its activity as a chaperone for the mutated acid β-glucosidase, increasing its activity and decreasing the accumulation of glucosylceramide[32]. Treatments for influenza such oseltamivir (Tamiflu) **20** and Zanamivir **21**, both of these compounds inhibit viral neuraminidase an enzyme found on the surface of influenza virus that allows it to be released from the host cell[33] (Figure 2).

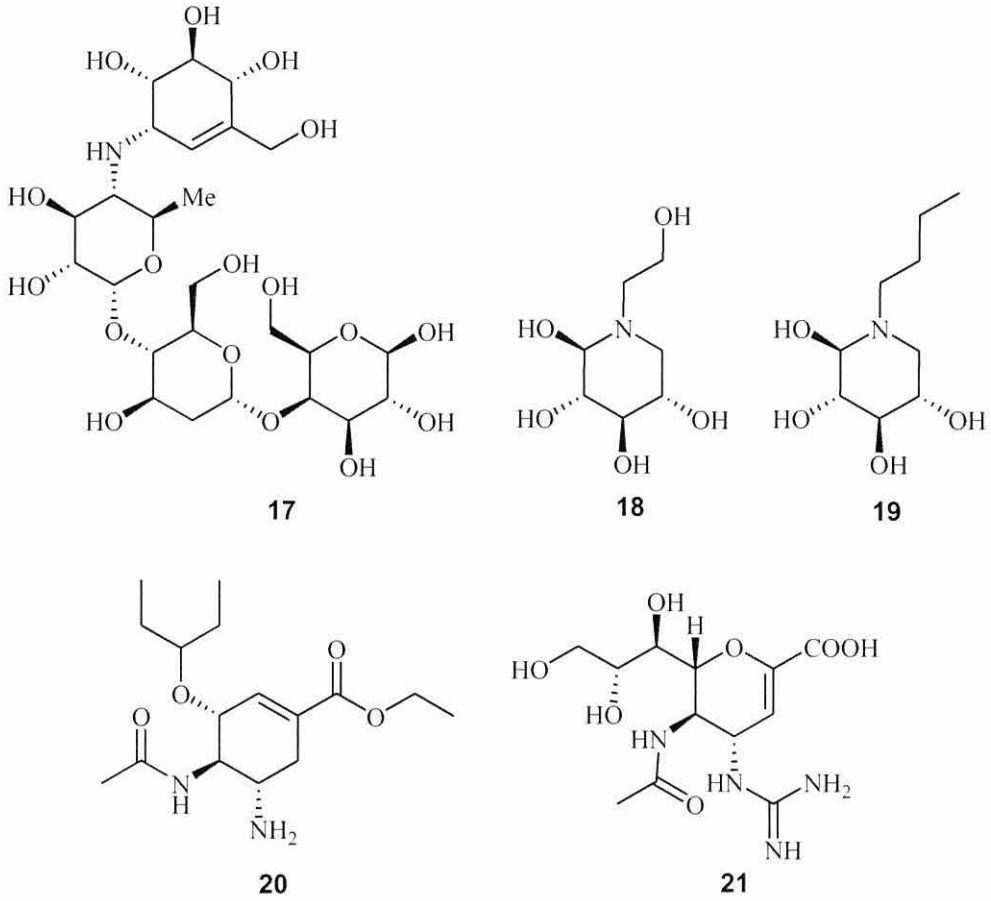


Figure 2: Glycomimetics currently marketed as therapeutics.

Guanidine containing glycomimetics

As stated previously the research undertaken for this projects was to investigate synthetic methods towards the preparation of compounds that act on glycosidases. The main focus of this was to investigate the synthesis of guanidine based heterocycles.

Guanidine and its chemistry

Guanidine **23** (Figure 3) is a colourless crystalline solid that is readily soluble in water and alcohols.

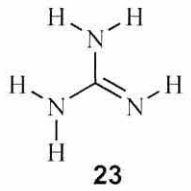
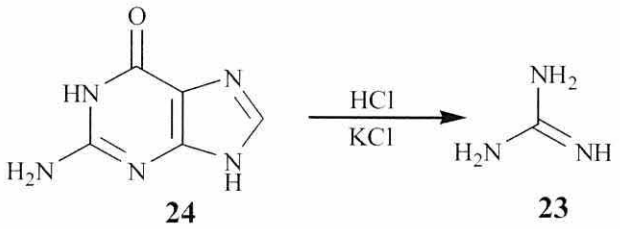


Figure 3: The guanidine functional group

It was first prepared in 1861 by A Strecker by the hydrolysis of guanine **24** with HCl and KCl[34]. (Scheme 4)



Scheme 4: Preparation of guanidine **23** from guanine **24**.

Guanidine is one of the strongest organic bases known with a pK_a of 13.7 in water allowing it to remain protonated over a wide pH range[35]. This is because once protonated on

the imino nitrogen the charge of the guanidinium cation is delocalized giving it greater stabilization through resonance (Figure 4).

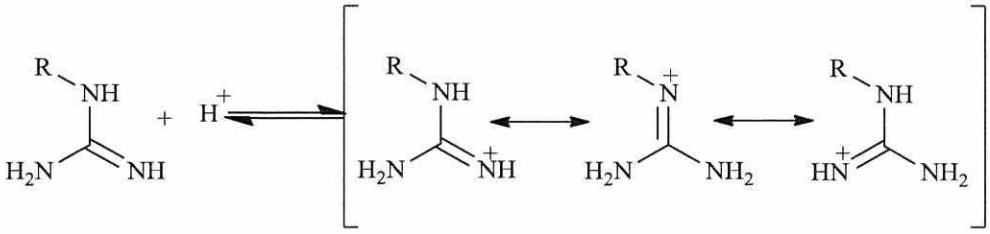


Figure 4: The three resonance structures of the guanidinium cation

This delocalisation is possible because three nitrogen atoms and the central carbon atom of guanidine have a co-planer arrangement at 120° from each other and with a bond length of 1.33 Å, which is a result of the partial double bond character of each of the C-N bonds[36]. This ability is an important property of guanidine as the presence of any proton sources (e.g. amino acids) leads to the formation of the protonated form allowing excellent hydrogen-bond mediation with anionic substrates such as carboxylates[37]. (Figure 5)

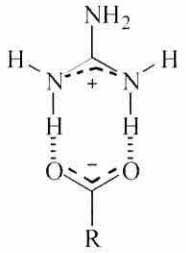
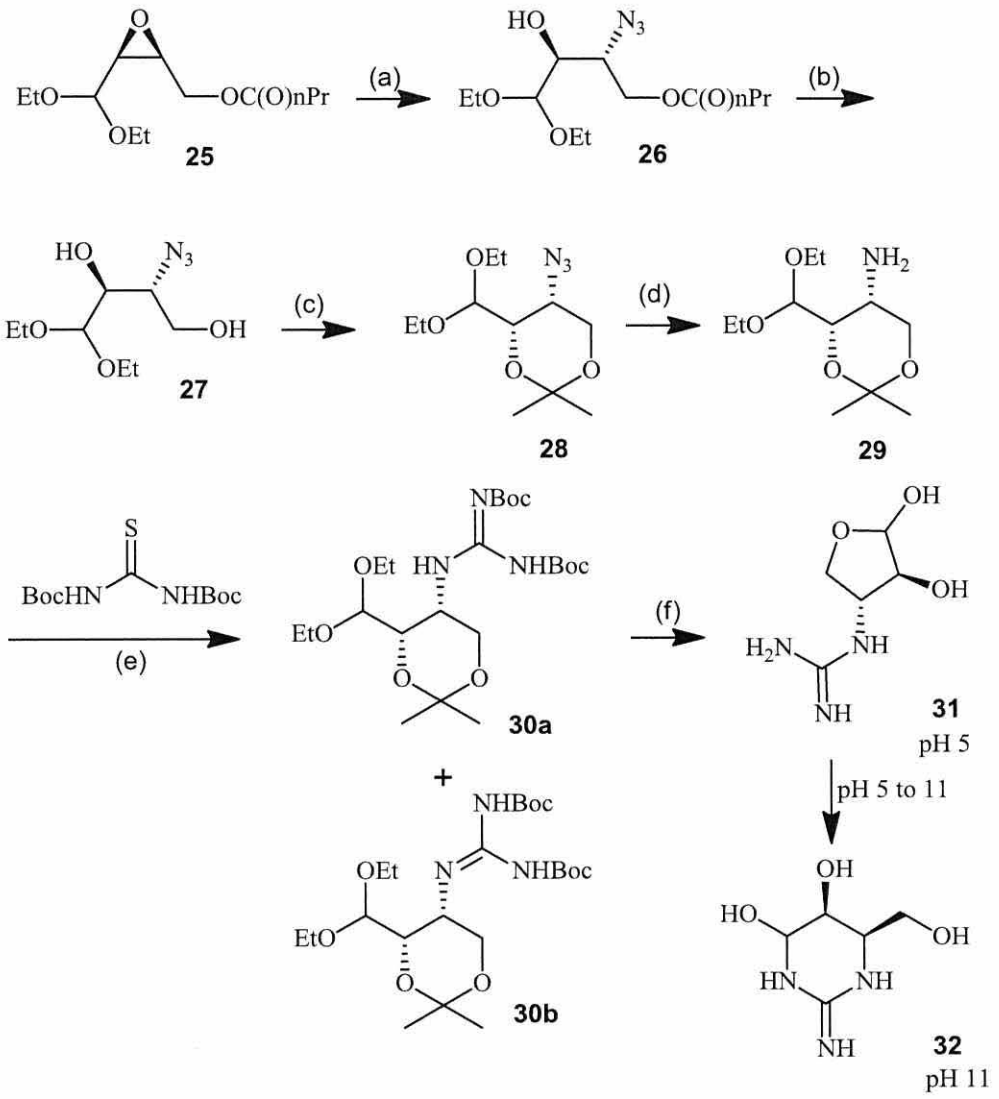


Figure 5: Binding of carboxylate anions to guanidine motif.

Synthesis of guanidine based glycometimics

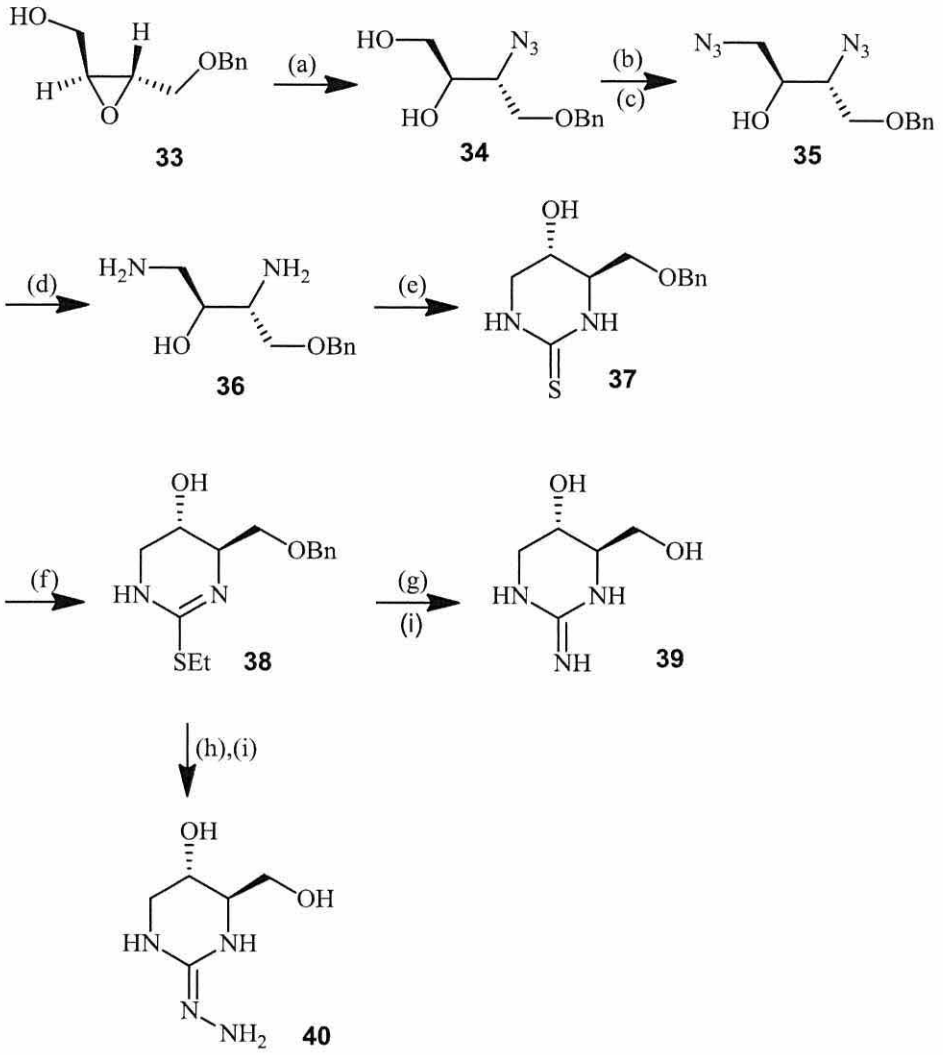
Previous literature reports of the preparation of guanidine heterocycles as glycosidase inhibitors are relatively few. The first reported example found was work by Fotsch and co-worker[38]; in their synthesis epoxide **25** was regioselectively ring opened to give 2-hydroxy-3-azido product **26**, which was then hydrolysed using K_2CO_3 in methanol to give the diol **27**. This was then protected as the 1,3-acetonide **28** and the azide reduced with palladium on charcoal to give amine **29**. This was converted to the *bis*-Boc derivative **30** in a mixture of tautomers **30a** and **30b** on treatment with N,N`-*bis*-*tert*-butoxycarbonyl thiourea. The Boc and acetal protecting groups were removed by treatment with trifluroacetic acid and water to give the guanidine **31** as a mixture of α- and β-furanose anomers, as observed on ^{1}H NMR. It was found through NMR studies that increasing the pH from the initial 5 to 11 gave the 6-membered guanidine **32**. Enzymatic assays using coffee bean α-galactosidase (EC 3.2.1.22) showed an increase in inhibition with an increased pH. Inhibition at pH 7.5 showed an IC_{50} of 1.3 mM and when the assay was adjusted to pH 10.7 the IC_{50} dropped to 480 μM giving strong evidence that the 6-membered **32** form is the primary source of inhibition (Scheme 5).



Scheme 5: (a) NaN_3, NH_4Cl, $EtOH/H_2O$, 20 h, 57%; (b) K_2CO_3, MeOH, 1 h, 20 °C, 81%) c) $(CH_3)_2C(OMe)_2$, PPTS, DMF, 32 h, 20 °C, 97%; (d) H_2 (1 atm), Pd/C, 82%; (e) 1.1 eq $HgCl_2$, 4 eq Et_3N, DMF, 71%; (f)1:1CH_2Cl_2/TFA, 10% H_2O, 48 h, rt, 57%

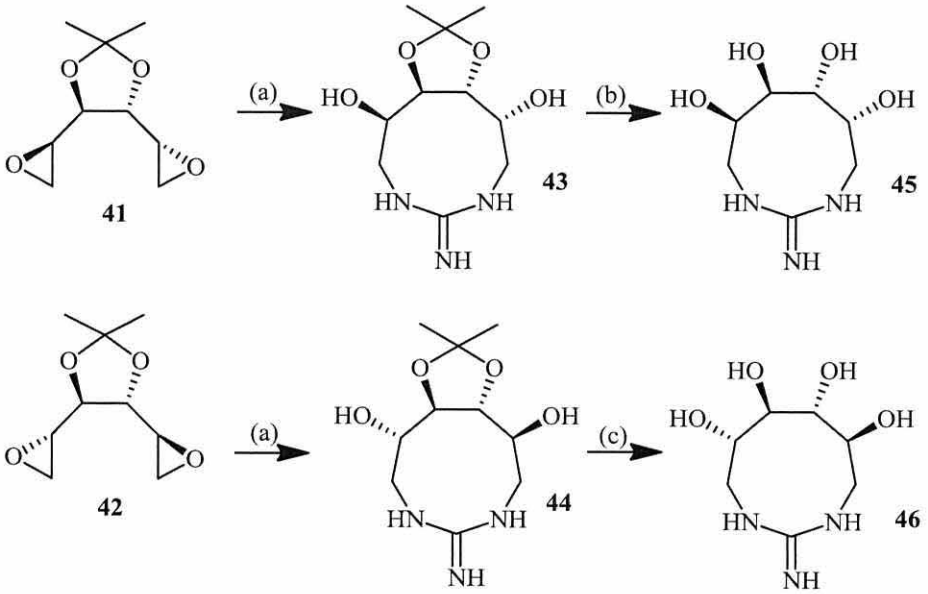
A similar investigation was carried out by B. Ganem and co-worker[39] who prepared guanidine **39** and aminoguanidine **40** beginning with the regioselective opening of epoxide S,S-epoxide **33** with $Ti(OiPr)_2(N_3)_2$ giving the azido-l,2-diol **34** as the major product (6:1 ratio to the azido-l,3-diol). Selective mesylation of the primary alcohol followed by displacment with NaN_3 gave the diazide **35** which on reduction with H_2 and palladium on charcoal gave the diamine **36** with little loss of the benzyl protecting group. This was successfully cyclised to give the thiourea **37** with 1,1'-thiocarbonyl-diimidazole. The S-ethylisothiourea **38** was then formed by treatment with ethyl iodode and converted into the

required guanidine by treatment with ammonium propanoate and subsequent deprotection to give guanidine **39.** A similar sequence of reactions utilizing hydrazine also gave the aminoguanidine **40** (Scheme 6).



Scheme 6: (a) 1.2 eq. $Ti(iPrO)_4$, 2.4 eq. $TMSN_3$, benzene, 75°C, 15 min, 55-65%; (b) MsC1, Et_3N,CH_2Cl_2, 0°C, 3 h; (c) NaN_3, DMF, 60°C, 18 h; (d) 10% Pd/C, H_2,CH_3OH, rt, 18 h, 80-90%; (e),1'thiocarbonyldiimidazole,CH_2Cl_2, rt, 16 h, 40-50%; (f) EtI, EtOH, rt, 2 d, 75%; (g) 2 eq. $NH_4O_2CCH_2CH_3$, 130°C, 4 h; (h) NH_2NH_2, rt, 15 min; (i) Na, NH_3, -78°C to -33°C,

Of particular relevance to this study, Merrer *et al.*[40] prepared two C_2-symmetric guanidino-sugar analogues/mimics utilizing a ring opening cyclization of *bis*-epoxides derived from D-mannitol **41** and L-iditol **42** with guanidine leading to the heterocycles **43** and **44** in 97% yield. Deprotection of these with aqueous trifluroacetic acid or hydrochloric acid in methanol gave heterocycles **45** and **46** in good overall yields (Scheme 7).



Scheme 7: (a) Guanidine, EtOH, Δ, 97%; (b) HCl/MeOH, 70%; (c) TFA. H_2O, 70%.

These guanidino-sugars were tested with enzymatic assays and found to be weak inhibitors of some glycosidases. No further assays to determine IC_{50} were carried out (Table 1).

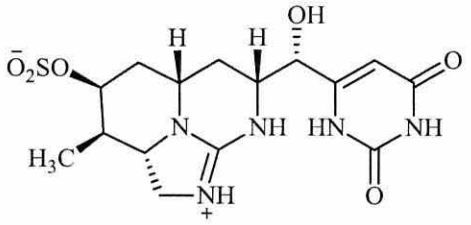
Enzyme	% inhibition[a] by	
	45	**46**
α-D-glucosidase (EC 3.2.1.20)[b]	24	36
β-D-glucosidase (EC 3.2.1.21)[c]	7	34
α-D-mannosidase (EC 3.2.1.24)[d]	10	41
α-L-fucosidase (EC 3.2.1.51)[e]	21	31

Table 1[40]

[a] Inhibition determined at 1mM concentration of inhibitor
[b] *Bacillus stearothermophilus*, pH 6.8.
[c] Almond, pH 5.0.
[d] Jack beans, pH 4.5.
[e] Bovine kidney, pH 5.5.

Aims and previous work.

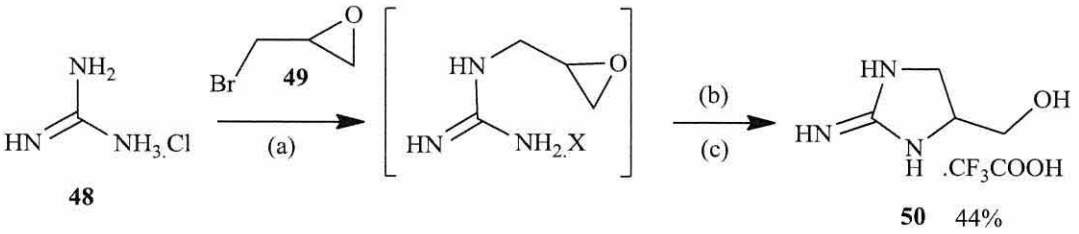
The Murphy group's long term interest in developing guanidine heterocycles stems from an interest in the synthesis of guanidine containing natural products[41,42]. It was through the study of new methods for preparing guanidine based polycyclic natural products such as Cylindrospermopsin[43] **47** (Figure 6) that it was found that epoxide ring opening reactions using guanidine could be useful synthetic strategies for preparing functionalized guanidine heterocycles[44,45,46].



47

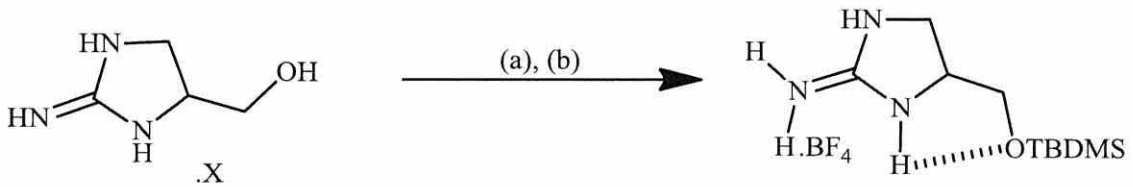
Figure 6: Cylindrospermopsin

Initial investigations focused on the reaction of guanidine hydrochloride **48** with epibromohydrin **49** in the presence of the base potassium *tert*-butoxide. It was found that this led to the formation of the 5-exo-tet cyclised product **50** in 44% yield (Scheme 8). However this reaction suffered from considerable amounts of decomposition and the formation of dimeric and polymeric derivatives, making purification difficult[46].



Scheme 8: (a) 1 eq *tert*-BuOK, tert-BuOH, rt, 16 h; (b) *tert*-BuOK, 60 °C, 24 h; (c) CF_3COOH, MeOH.

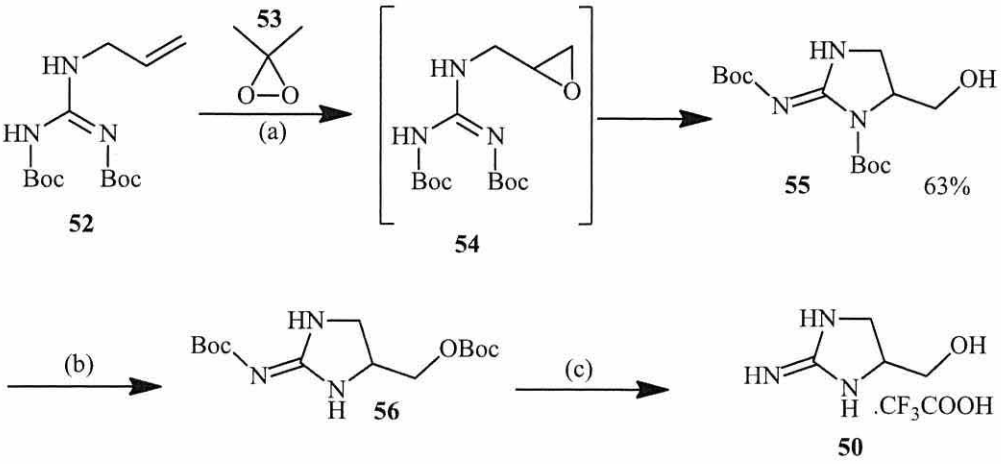
Attempted purification of **50** by flash chromatography was found to be difficult due to the product's polar nature and presence of a mixture of counter-ions. In an attempt to aid purification, silylation of the molecule's alcohol group was attempted; however this was unsuccessful and gave poor yields (5 - 33%) of the protected compound **51**. These low yields were explained as being due to the low stability of the silyl ether due to a neighbouring group participation of the guanidine in accelerating its hydrolysis. A similar process was observed by Elliott and Long[47] and it was concluded that the silyl group was being cleaved during chromatography due to this anchimeric assistance[46] (Scheme 9).



50 **51**

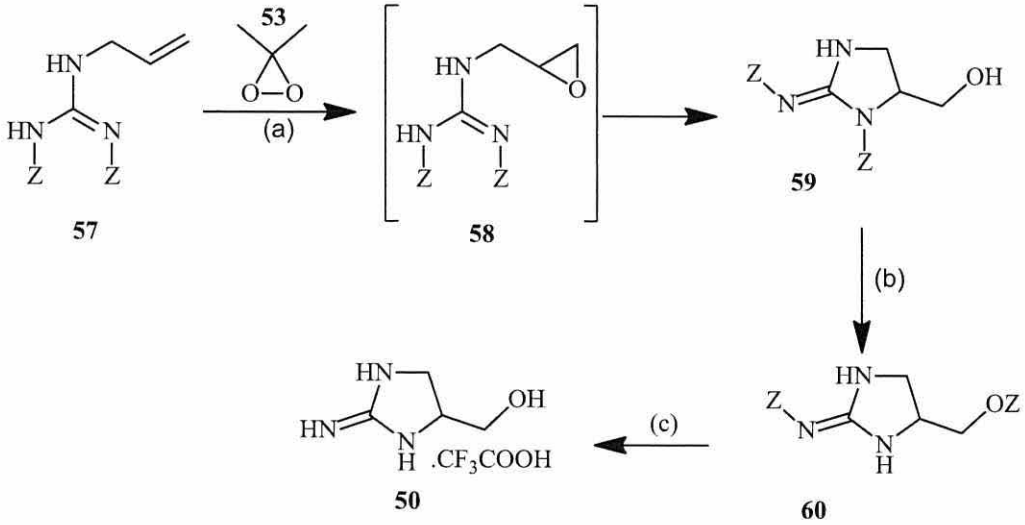
Scheme 9: (a) Dry DMF, 0 °C, imidazole, TBDMSCl, 0 °C, rt, overnight (b) $NaBF_4$ (sat solution)

An alternate method was then investigated which involved the epoxidation of the protected *N*-allyl-*N'*,*N''*-*bis*-Boc-guanidine **52** with dimethyl dioxirane (DMDO) **53** at low temperature which produced the cyclised intermediate **55** in 63% yield. Interestingly on standing over silica gel or on chromatography the compound undergoes selective rearrangement to give the isomeric *O*-protected guanidine **56** in quantitative yield. Reaction of either of these two derivatives with trifluoroacetic acid leads to the removal of the *Boc*-protecting groups to give the desired compound **50** in quantitative yield (Scheme 10).



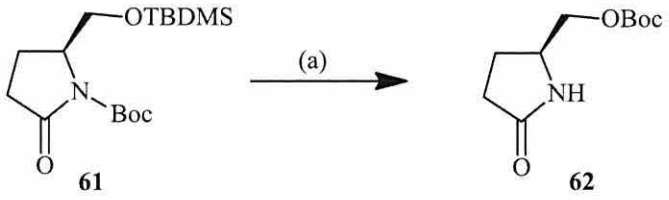
Scheme 10: (a) Acetone, -20°C, rt 48 h; (b) SiO_2; (c) TFA, MeOH 1 h.

A similar process was observed with the *bis*-Z-protected guanidine analogue **57**, it was found that, by NMR observation the proposed intermediate product **59** was formed as observed by NMR. However on purification by silica gel chromatography, the rearranged compound **60** was the only product isolated[48]. (Scheme 11)



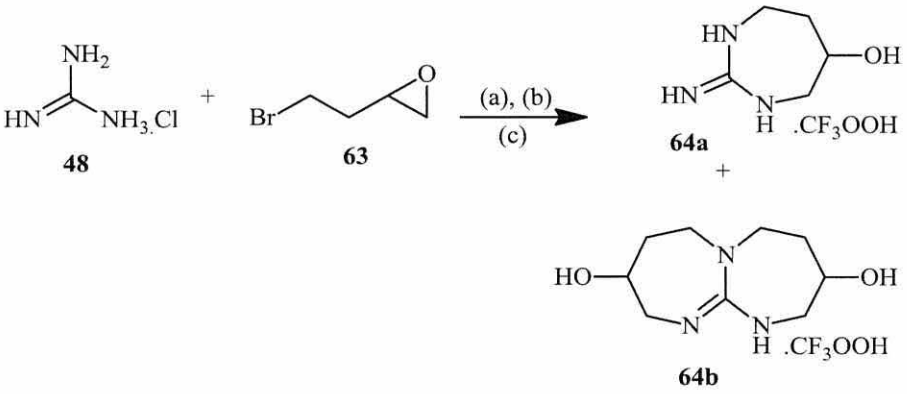
Scheme 11: (a) Acetone, -20°C, rt 48 h; (b) SiO_2; (c) TFA, MeOH 1 h.

L. Bunch and co-workers reported a similar N-O Boc migration upon the cleavage of *O*-silyl ether in pyroglutaminol **61** giving the rearranged product **62**[49] (Scheme 12).



Scheme 12: 1.6 eq TBAF, THF, rt, 30 mins.

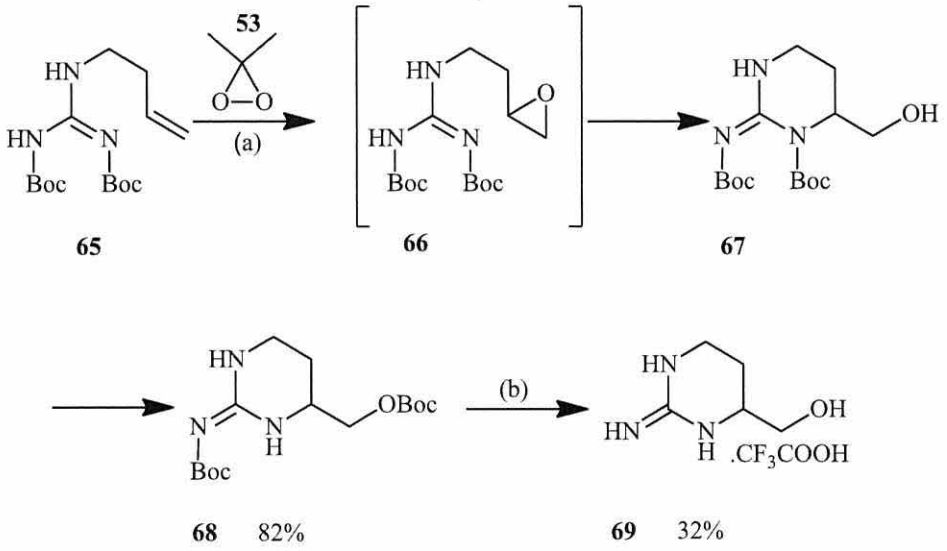
The reaction between bromoepoxide **63** with guanidine **48** was also investigated and it was found that this led to the formation of the 7-endo-tet cyclised product **64a** and variable amounts of the bicyclic compound **64b**, which was composed of a mixture of the racemic RR/SS mixture and the meso-heterocycles in equivalent amounts (Scheme 13).



Scheme 13: (a)1 eq *tert*-BuOK, *tert*-BuOH, 16 h, rt; (b) 1 eq *tert*-BuOK, 60 °C, 24 h. c) TFA, MeOH.

The conditions reported in Scheme 10 gave the two products **64a** and **64b** in a 70:30 ratio with the moncyclic product **64a** being isolated in 43% yield[44]. It was found that, by adding a further equivalent of **63** the ratio was 35:65 in favour of the bicyclic product **64b.** The addition of another equivalent of **63** led to an increase to a 25:75 ratio in favour of the bicyclic product but this was accompanied with an increase in unwanted by-products and a diminished overall yield as chromatographic separation was found to be difficult.

In a similar sequence, *N*-homoallyl-*N',N''-bis*-Boc-guanidine **65** was also treated with DMDO **53**, which led to the formation of the rearranged 6-membered guanidine **68** also in this example the *N*-Boc protecting group had migrated to the hydroxyl group. The structure of **66** was determined by x-ray crystallography, and NMR studies indicated that the epoxide **66** was initially formed. This slowly cyclised to give the guanidine **67** which on purification on silica gel rearranged to give 6-membered heterocycle **68**[48] (Scheme 14).



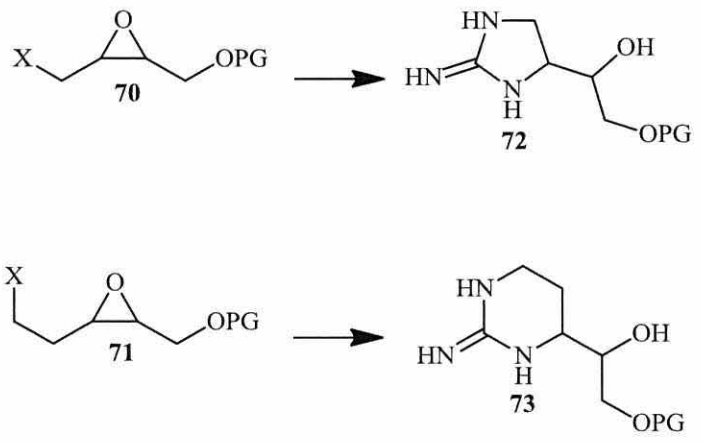
Scheme 14: (a) acetone, -20°C, rt 10 days; (b) TFA, MeOH

The overall conclusions from these initial studies were that the cyclisation of the protected and unprotected guanidines were largely successful and led to predictable structures. Despite problems with rearrangements, the chemistry was expected to provide a viable route to several interesting heterocycles.

Aims

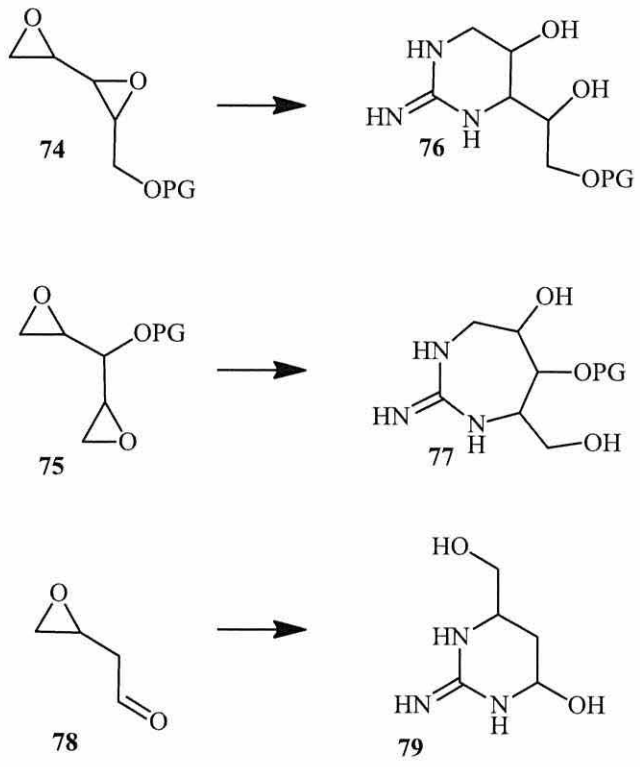
The aims of this study were to expand upon the initial work on the ring opening of epoxides with guanidine and to prepare a range of guanidine containing heterocycles which may have potential applications as glycosidase inhibitors. The biological activities of these could then be investigated in collaboration with Phytoquest (previously Molecular Nature) the industrial sponsor.

It is easy to envisage several functionalised epoxides which will be suitable precursors for ring opening studies. For example the generalised epoxides **70** and **71** will give access to dihydroxylated 5- and 6-membered systems **72** and **73**, with the potential to access further homologous heterocycles using similar chemistry (Scheme 15).



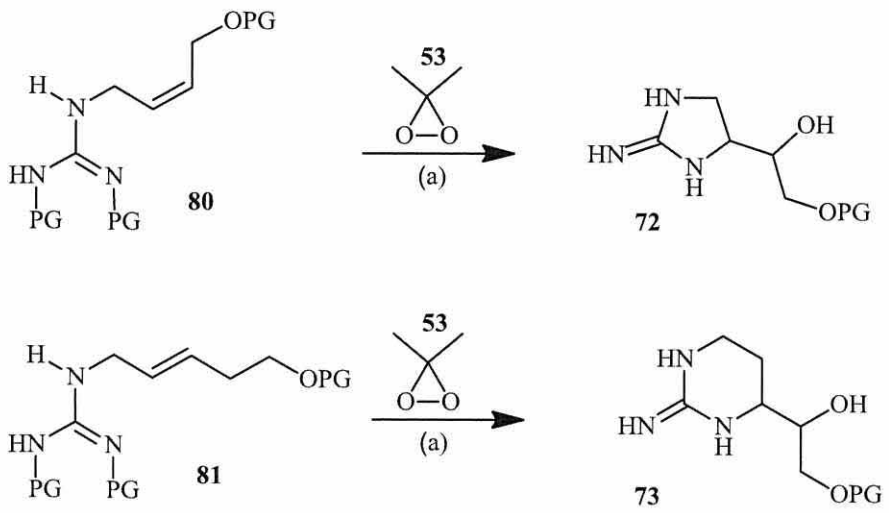
Scheme 15: X = Leaving group, PG = Protecting group,

The use of the *bis*-epoxides such as **74** and **75** might also allow access to other further 6- and 7-membered guanidine heterocycles **76** and **77.** While the use of aldehyde containing substrates **78** may enable access to hemi-aminal containing heterocycles **79**. (Scheme 16)



Scheme 16: PG = Protecting group,

An alternate strategy to produce 5- and 6-membered guanidines **72** and **73** will also be investigated. Thus treating alkenes substituted guanidines **80** and **81** with DMDO **43** is proposed to lead to **72** and **73** respectively (Scheme 17)



Scheme 17: (a) acetone, -20°C, rt

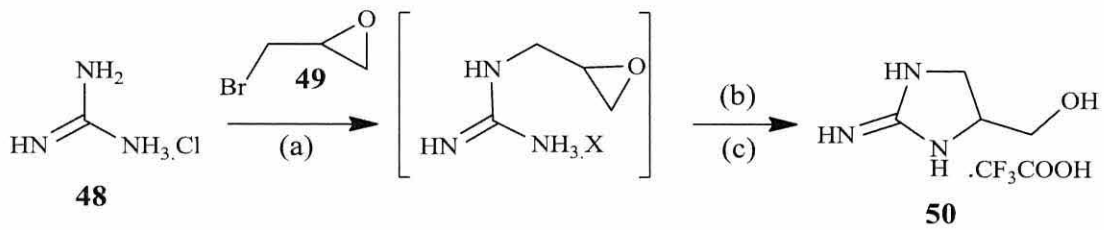
PG = Protecting group

Results and Discussion

Synthesis of (2-iminoimidazolidin-4-yl)methanol (50)

As part of the initial study we wished to prepare a high purity sample of the 5-membered guanidine **50**[44] to be used in biological assays as preliminary testing had shown this to be a weak but specific inhibitor of β-galactosidase[44].

The synthesis of **50** involved the addition of epibromohydrin **49** to a solution of guanidine generated in *tert*-butanol by treating guanidine hydrochloride. The desired 5-membered guanidine heterocycle **50** was isolated in 45% yield after flash chromatography (Scheme 18).



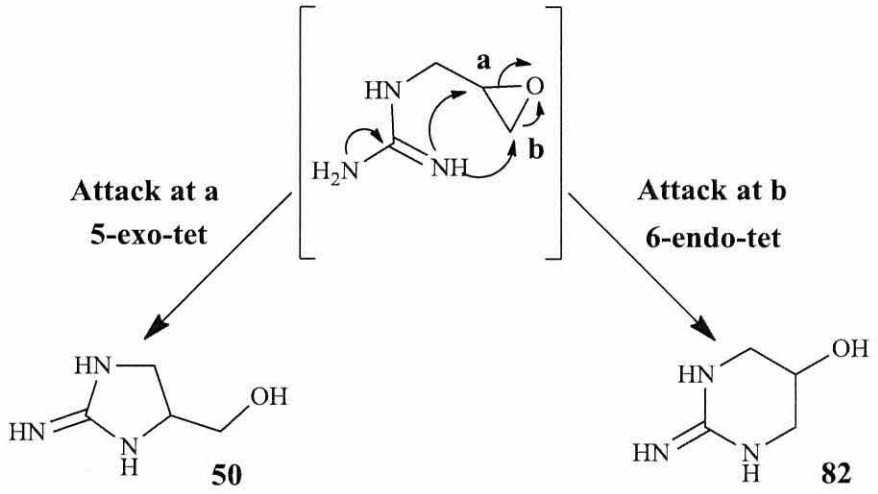
Scheme 18: (a) 1.1 eq *tert*-BuOK, *tert*-BuOH, rt, 62 h;

(b) 1.1 eq *tert*-BuOK, 60 °C, 24 h; (c) CF_3COOH, MeOH

Analysis by NMR spectroscopy showed that the reaction was successful. The ^{1}H NMR spectrum showed 5 signals. The signals at δ 3.54 (1H, dd, J = 6.0, 10.1 Hz) and δ 3.76 (1H, app t, J = 10.1 Hz) corresponded to the two protons in the ring CH_2 group. The next two signals at δ 3.57 (1H, dd, J = 5.2, 12.1 Hz) ppm and δ 3.65 (1H, dd, J = 4.3, 12.1 Hz) corresponded to the two protons on the CH_2 group adjacent to the OH. The final signal at δ 4.30 (1H, m) corresponded to the ring CH proton. ^{1}H NMR spectroscopy also indicated the presence of impurities in the sample. These were seen as broad low lying signals, which were difficult to resolve and assign because of overlap between themselves those and of the target

molecule.

A sample was further purified using preparative HPLC chromatography. Proton spectroscopy of the purified fraction gave the expected signals of **50**, however 3 additional signals were present which from which COSY $^1H - ^1H$ NMR spectroscopy were shown to be a single impurity. The additional signals were at δ 3.28 (2H, dd, J = 12.3, 3.8 Hz), δ 3.43 (2H, dd, J = 12.3, 3.8 Hz) and δ 4.20 (1H, multiplet) ppm. likely identity to belong to the supposedly disfavoured 6-endo-tet isomer **82** formed by the attack of the guanidine on the epoxide carbon **b** rather than carbon **a** (Scheme 19).



Scheme 19: The two epoxide ring opening reactions leading to **50** and **82**

The reinvestigation of the ^1H NMR spectrum of the crude sample obtained before chromatography allowed to estimate the ratio of 6-endo-tet isomer **82** and desired 5-exo-tet heterocycle **51** formed in the reaction by comparing the integration of the 6-endo-tet heterocycle's α-carbon signal at δ 4.20 ppm to the 5-exo-tet's heterocycle's CH signal at δ 4.12. This indicated that the 6-endo-tet isomer made up approximately 11% of the cyclisation products. (Figure 8)

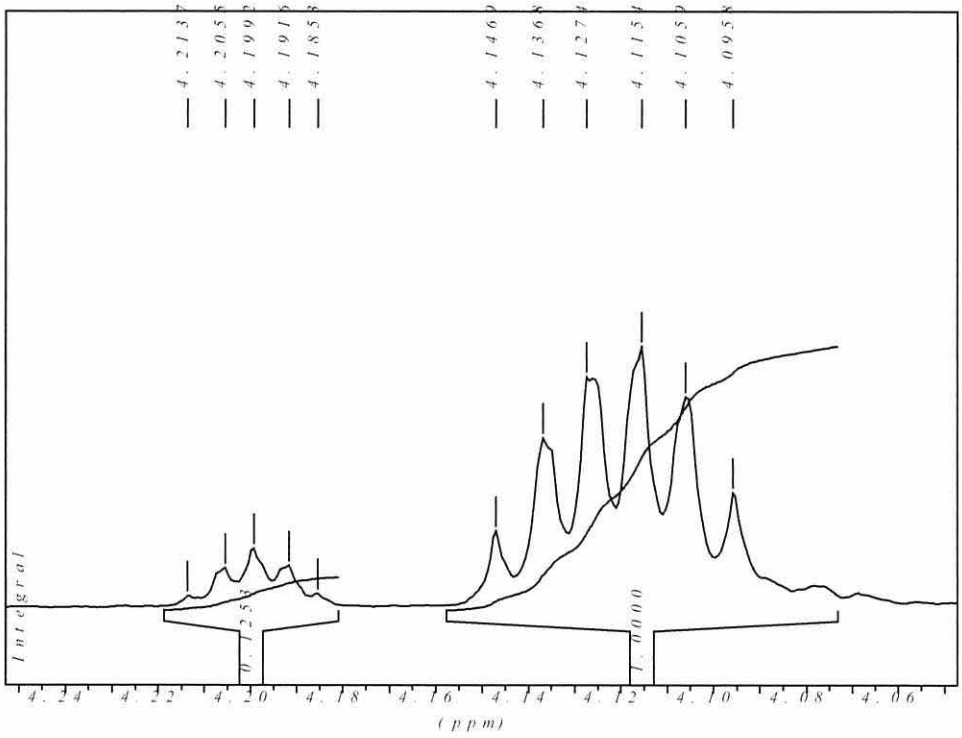
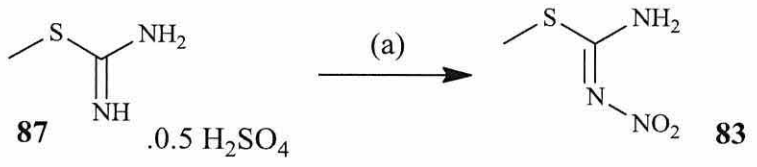


Figure 8: Comparison of ring CH's of **50** and **82** to give approximate ratios.

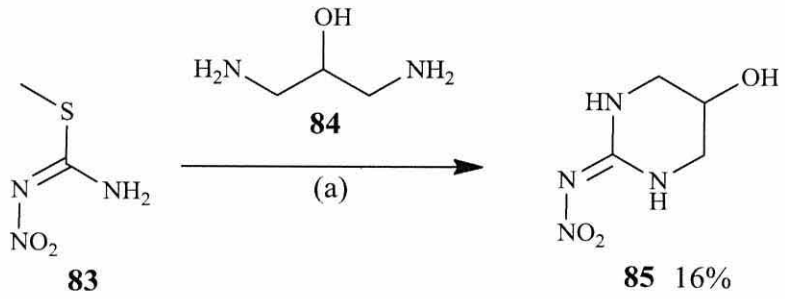
To confirm the structure of **82**, a pure sample was prepared using a literature method reported by Hafner and Evans. The authors reported that the reaction of 2-methyl-1-nitro-2-thiopseudourea **83** with 1,3-diaminopropan-2-ol **84** gave 5-hydroxy-2-nitrimino-1,3-diazacyclohexane **85**[50], which could be hydrogenated in aqueous acetic acid to give 6-membered guanidine **86** as an acetate salt.[51].

2-Methyl-1-nitro-2-thiopseudourea **83** is not commercially available but was easily prepared in 96% yield by the nitration of 2-methyl-2-thiopseudourea sulfate **87** with a 1:3 mixture of 69% nitric and 98% sulfuric acid at – 10 °C[52]. The observed melting point of 159 – 161 °C was in good agreement with the literature value of 160 – 161 °C (Scheme 20).



Scheme 20: (a) 1/3 69% nitric acid /98% sulfuric acid, – 5 °C, 0.5 h.

Reaction of 2-methyl-1-nitro-2-thiopseudourea **83** with 1,3-diaminopropan-2-ol **84** was effected by refluxing a mixture of the two in ethanol for 3 h. The solution was then cooled to room temperature and the resulting solid was isolated by filtration to give **85** in a low 16% yield (Scheme 21).



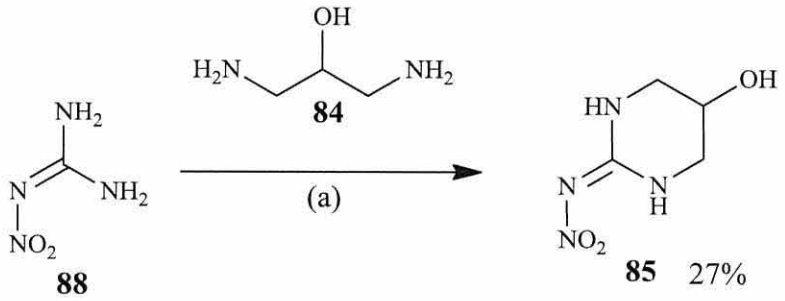
Scheme 21: (a) Ethanol, reflux, 2 h

The melting point of 230-3 °C was in good agreement with the literature value of 233-5 °C[51], and in addition HRMS showed an ion at a mass of 160.0589 Daltons which was in agreement with the predicted mass of 160.0591 Daltons.

Whilst the reaction in principal provided the desired product, obvious problems were the low yield of **85** and the putrid smelling methanethiol evolved in the reaction. The mother liquor from the reaction was analysed by TLC and found to have very little further **85** present but contained a large amount of unreacted diamine **84**, that the yield may be improved by allowing a longer period of reflux.

An alternative method for the synthesis of **85** had been reported by McKay[53] and involved using nitroguanidine **88** instead of 2-methyl-1-nitro-2-thiopseudourea **83**. This method has the advantage that **88** is commercially available and that no methanethiol is evolved in the reaction. Thus nitroguanidine **88** was refluxed with 1 equivalent of 1,3-

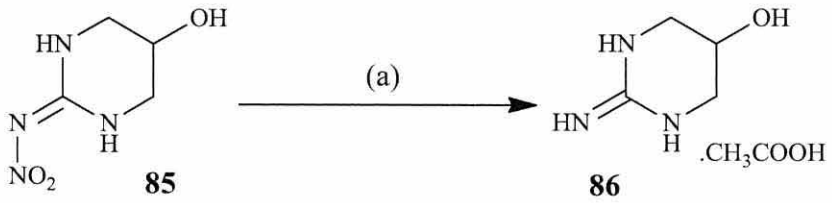
diaminopropan-2-ol **84** in water for 2 h before being cooled to 5 °C to precipitate the product. After filtration and drying the heterocycle **85** was obtained in a 27% yield (Scheme 22).



Scheme 22: (a)Water, 70 °C, 2 h.

The melting point, HRMS and NMR spectroscopy of the product were compared with those of the previously made sample and found to be a match.

The hydrogenation of **85** was carried out using 5% palladium on charcoal in 15% aqueous acetic acid and stirring for 3 days under hydrogen at normal atmospheric pressure. Initially **85** remained as a suspension in the reaction but as the reaction proceeded, it gradually dissolved into the acid solution (Scheme 23).



Scheme 23: (a) H_2 (1 atm), Pd/C, 15% aqueous acetic acid 72 h

After final purification by column chromatography on silica, the desired product **86** as a white solid in 75% yield. The removal of the nitro group was confirmed by HRMS, which showed a molecular ion mass of 116.0817 Daltons that compared favourably with the required 116.0818 Daltons. The ^{1}H NMR spectrum of **86** was compared with that of the impurity **82** generated in the cyclisation reaction. (Table 2)

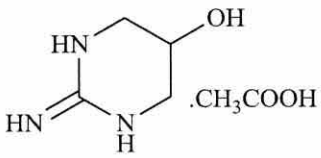
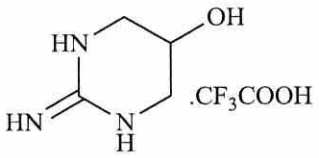
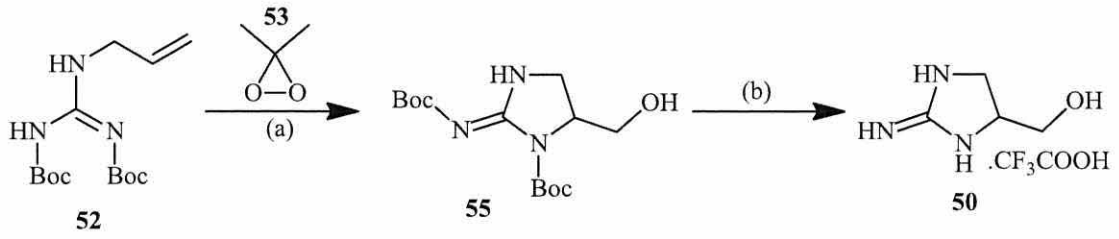
^{1}H NMR Signal	**Compound 86**	**Impurity 82**
		
C*H*H	δ 3.21	δ 3.21
CH*H*	δ 3.35	δ 3.33
C*H*OH	δ 4.22	δ 4.20

Table 2: Comparison of ^{1}H NMR signals of impurity **82** and compound **86**

It is apparent from this data that the impurity is suspected 6-membered compound **82**. This makes the previously obtained biological data[44] uncertain in that it might be impurity **86** that is responsible for the observed activity and not the 5-membered heterocycle **50.** Thus a pure sample of the 5-membered guanidine **50** and of the 6-membered salt **82/86** have to be investigated independently making an alternative synthesis of heterocycle **50** necessary.

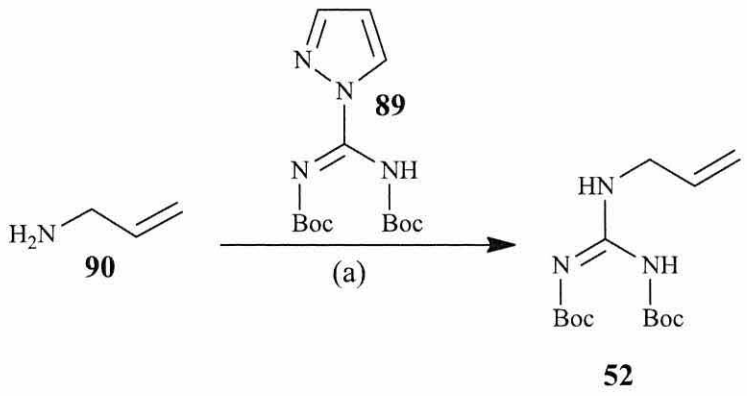
Synthesis of 5-membered Heterocycle (50)

Synthesis of the desired uncontaminated heterocycle via the DMDO epoxidation of allyl-*N'N''-bis*-(*tert*-butoxycarbonyl)-guanidine **52** (see Scheme 22) should be possible and should also offer the opportunity to see if any 6-membered guanidine heterocycles are formed in this reaction (Scheme 24).



Scheme 24: (a) Acetone, -20°C, rt 48 h; (b) TFA, MeOH 1 h

The precursor **52** was prepared using the method reported by Drake *et al*[54], where *N,N'-bis-(tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamide **89** is treated with allylamine **90** in dry acetonitrile for 16 h. After chromatography allyl-*N'N''-bis*-(*tert*-butoxycarbonyl)-guanidine **52** was obtained in 95% yield (Scheme 25).

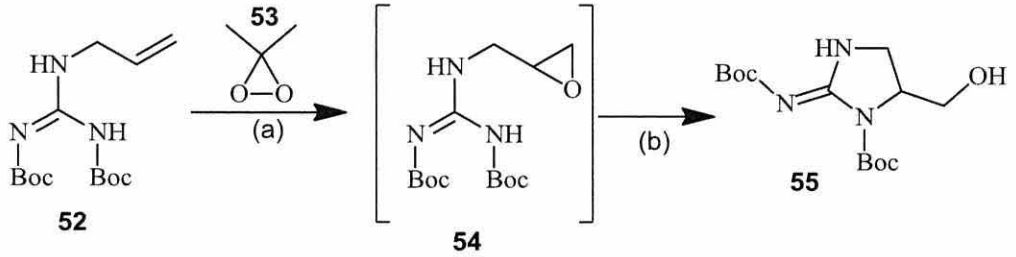


Scheme 25: CH_3CN, rt, 16 h.

The ^{1}H NMR spectrum of **52** displayed 8 signals, the first two were the Boc methyl singlets at δ 1.49 (s, 9H) and δ 1.50 (s, 9H) ppm. Further signals at δ 4.07 (2H, t, J = 5.4 Hz) ppm, correspond to the CH_2 adjacent to the guanidine nitrogen. Signals at δ 5.13 (1H, d, J =

10.4) and 5.23 (1H, d, J = 17.4 Hz) ppm correspond to the terminal protons of the alkene, whilst the other alkene signal was observed at δ 5.88 (1H, ddt, J = 17.4, 10.4, 5.7 Hz) ppm. A futher 2 signals were observed at δ 8.39 (1H, broad) and 11.51 (1H, broad) ppm and correspond to the N-H protons on the guanidine. ^{13}C NMR spectrum showed 10 signals, the first two at δ 28.1 and 28.3 ppm for the due to the methyl carbons of the two Boc groups. The next at δ 43.3 ppm corresponds to the allyl CH_2 with the signals at δ 79.4 and 83.2 ppm corresponding to the quaternary carbons of the Boc groups. The signals at δ 116.8 ppm (CH_2) and 113.3 (CH) ppm are from the alkene portion of the molecule whilst the three signals at δ 153.3 (C), 156.0 (C) and 163.5 (C) ppm are from the carbonyl and guanidine quaternary carbons.

The allyl substituted Guanidine **52** was then dissolved in acetone and cooled to -20 °C and treated with a solution of DMDO **53** before being slowly warmed to room temperature and stirred for 96 h (Scheme 26).

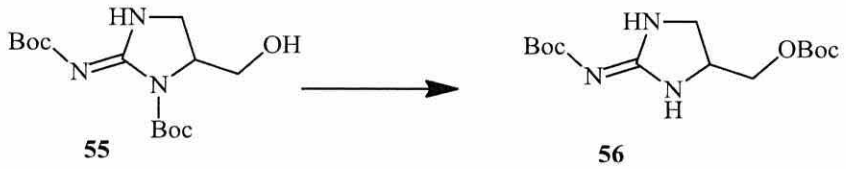


Scheme 26: -20 °C, dry acetone; (b) rt, 96 h

Solvent was removed at room temperature under vacuum giving a white solid, which was analysed by NMR spectroscopy. The ^{1}H NMR spectrum showed 5 significant signals, the first two at δ 1.49 (s, 9H) and 1.54 (s, 9H) ppm correspond to the methyl protons of the two Boc groups. The next signal at δ 3.68 – 3.74 (2H, multiplet) ppm corresponded to the protons of the CH_2 next to the OH. The ring CH_2 protons showed as a signal at δ 3.79 – 3.88 (2H, multiplet) ppm and the ring CH appeared as a signal at δ 4.24 (1H, multiplet). The ^{13}C NMR spectrum showed 9 signals, the first at δ 27.9 ppm corresponds to the carbons of the methyl groups present in the two Boc groups. The ring CH carbon corresponded to the signal at δ 57.6 ppm, the ring CH_2 carbon corresponded to the signal at δ 62.2 ppm and the CH_2 carbon next to the alcohol oxygen corresponded to the signal at δ 65.7 ppm. The signals for the Boc terminal carbons were present at δ 80.5 and δ 83.2 ppm. The carbonyl carbons of the Boc

groups and the quatenary carbon of the guanidine corresponded to signals at δ 150.8, 152.7 and 155.5 ppm.

With heterocycle **55** in hand, an experiment was carried out to determine the effects of prolonged exposure to silica gel would have on the compound. This was of interest as rearrangement on silica gel chromatography had been previously observed. Thus, crude **55** was dissolved in chloroform, silica gel was added and the suspension was then stirred at room temperature for 24 h. The silica was removed by filtration, washed with chloroform followed by ethyl acetate and the filtrate was evaporated to dryness under vacuum giving a crude solid. This was then recrystallised from chloroform to give **56** as colourless crystals in 63% yield (Scheme 27).



Scheme 27: SiO_2, $CHCl_3$, 24 hrs

The ^{1}H NMR spectrum showed 6 signals, the first two at δ 1.44 (9H, s, 3 x CH_3) and 1.47 (9H, s, 3 x CH_3) ppm correspond to the methyl protons of the two Boc groups. The next signals at δ 3.41 (1H, dd, J = 6.0, 9.8 Hz), and δ 3.73 (1H, app t, J = 9.8 Hz) corresponded to the ring CH_2 protons. The next signal at δ 4.09 (2H, m) corresponded to the protons of the CH_2 next to the OH. The final signal at δ 4.17 (1H, m) corresponds to the ring CH protons. The ^{13}C NMR spectrum showed 10 signals, the first two at δ 27.6 and 28.2 ppm corresponded to the carbons of the methyl groups present in the two Boc groups, the next signals at δ 55.8 and δ 63.7 ppm corresponded to the ring CH and CH_2 carbons respectivly. The next signal at δ 67.4 ppm corresponded to the CH_2 next to the OH and teh signals at δ 78.9 and 82.8 ppm corresponded to the tertiary carbons of the Boc groups. The last 3 signals at δ 150.4, 152.4 and 159.2 ppm corresponded to the carbonyl carbons of the Boc groups and the guanidinum carbon.

The migration of the Boc group was confirmed by X-ray crystallography, which showed a Boc group had migrated to the oxygen (Figure 9).

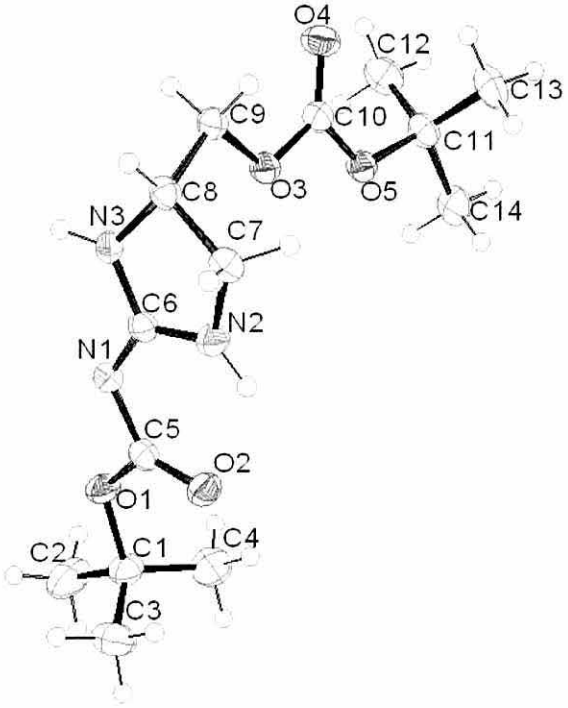
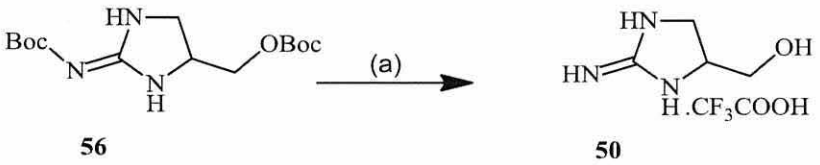


Figure 9: X-ray crystal structure of **56**

Deprotection of **56** was accomplished by treatment with excess trifluroacetic acid in dichlomethane for 4 h. After evaporation the required guanidine **50** was obtained in 77% yield (Scheme 28).



Scheme 28: TFA, DCM, 4 h, rt

The ^{1}H NMR spectrum confirmed that the desired **50** had been isolated without contamination by **82** (Figure 10).

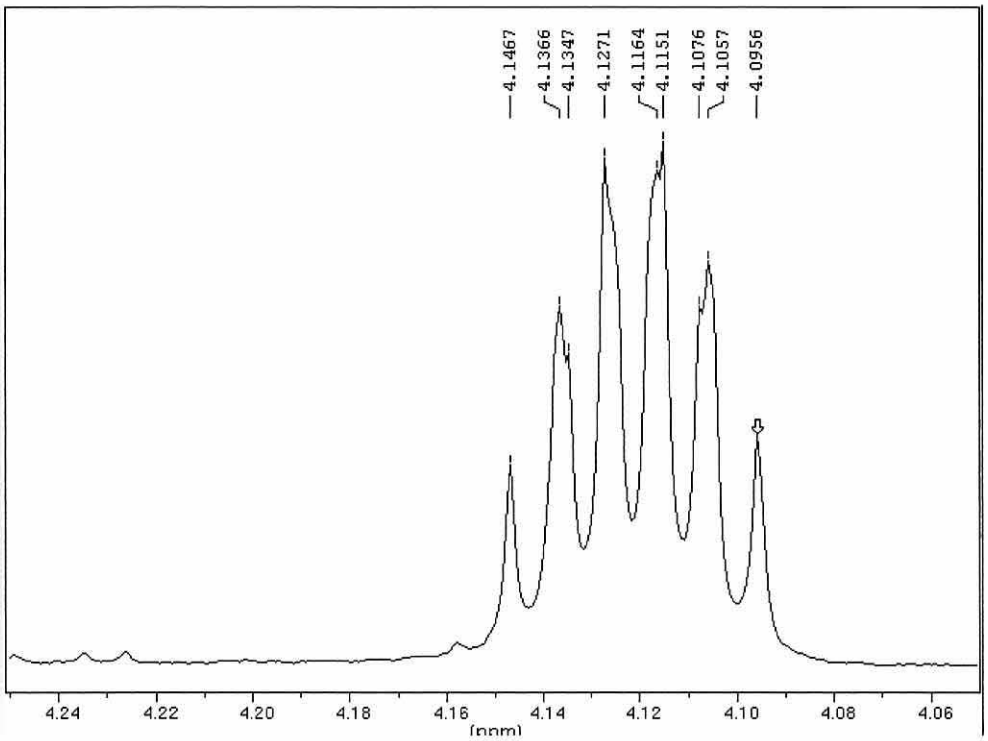
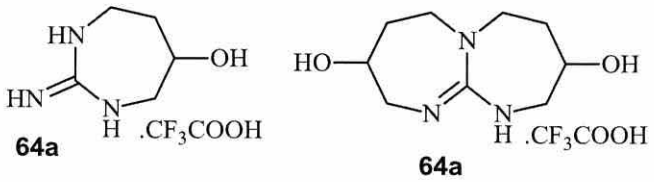


Figure 10 : NMR of **50** prepared via intramolecular route showing the absence of impurity **82**

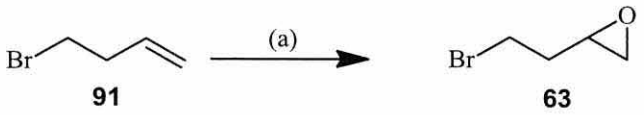
Synthesis of 2-imino-1,3-diazepan-5-ol (64a) and 2,3,4,5,7,8,9,10-octahydro-1H-[1,3]diazepino[1,2-a][1,3]diazepine-3,9-diol (64b) trifluroacetic acid salts.

The 7-membered guanidine **64a** had been previously studied and found to be a specific inhibitor of β-galactosidase (Bovine liver) (IC_{50} = 49 μg mL^{-1})[44] and it was thus desirable to prepare a sample for comparison and further investigation. In addition it would be interesting to prepare a sample of the bicyclic guanidine **64b** as this may also have interesting activity (Scheme 29).



Scheme 29

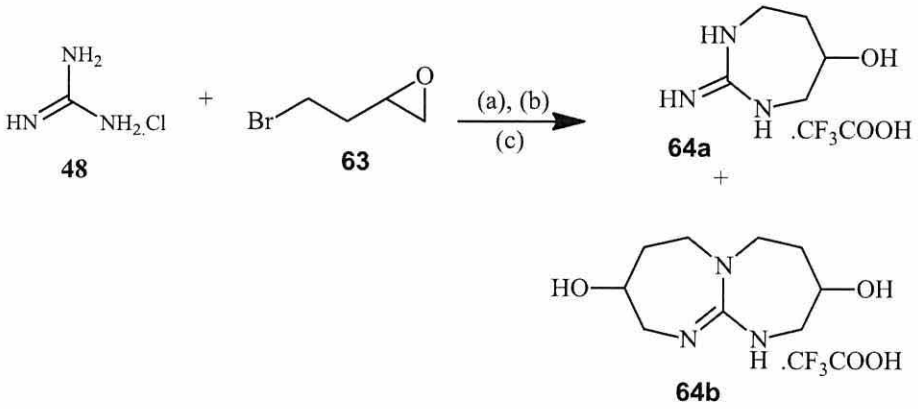
The precursor epoxide **63** for this synthesis was prepared[55] from 4-bromobut-1-ene **91** to give **63** in 52% yield as an oil (Scheme 30).



Scheme 30: (a) 2.5 eq *m*CPBA, DCM, 56 h, rt

It was decide to prepare a mixture of the two heterocycles in which the bicyclic compound **64b** would be the major product as the separation of two compounds is fairly straight forward. Thus **63** was treated with guanidine hydrochloride **48** and potassium *tert*-butoxide (1.1 eq). This mixture was stirred for 56 h at room temperature before more potassium *tert*-butoxide was added followed by a further equivalent of 2-(2-

bromoethyl)oxirane **63** to give a crude product after quenching with TFA and removal of solvents (Scheme 31).



Scheme 31: (a) *t*-BuOH, guanidine.HCl, *t*-BuOH, stir 56 h rt;

(b)1 eq of **63**, *t*-BuOK, 60°C 28 h; (c) TFA, MeOH.

The crude product was analysed by ^{1}H and ^{13}C NMR spectroscopy, which confirmed that the desired 7-membered **64a** and the bicyclic **64b** heterocycles were present. The ^{13}C NMR spectrum of the crude mixture allowed the approximate ratio of monocyclic **64a** and bicyclic **64b** products formed in the reaction to be determined by comparing the closely related signals in the produced by the mono **64a** and the paired signals due to the diastereoisomers of the bicyclic **64b** as shown in Figure 11.

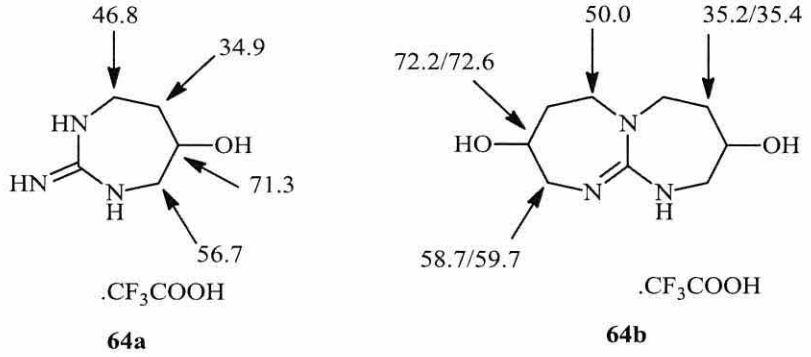


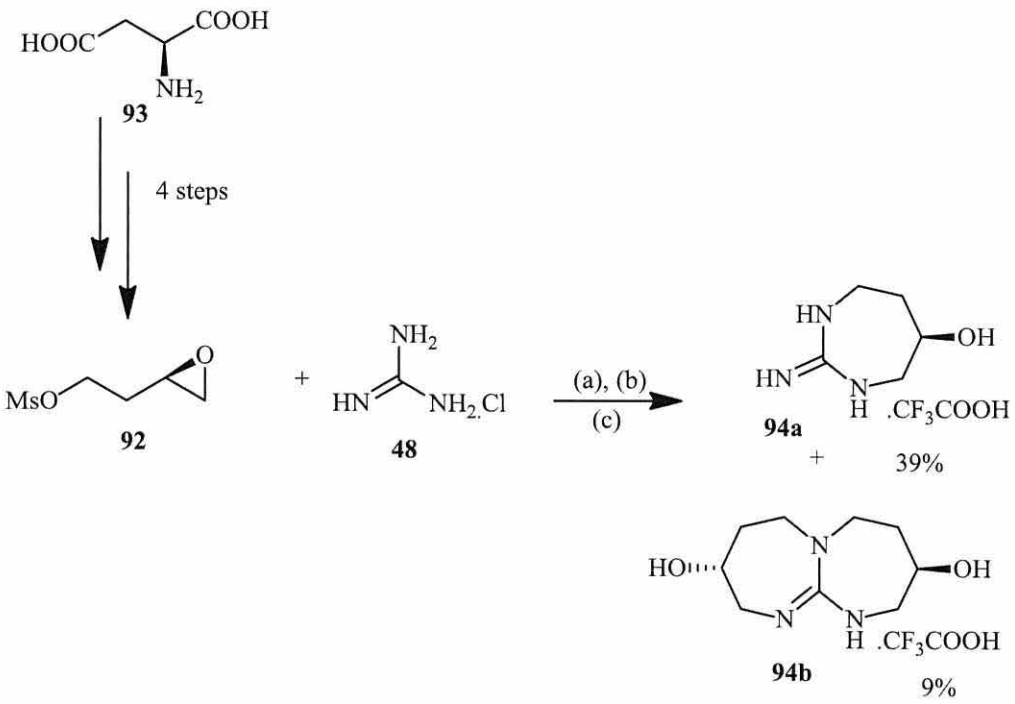
Figure 11: Comparison of ^{13}C NMR shifts of monocyclic **64a** and bicyclic **64b**

This indicated that the two products were present in an approximate ratio of 60% of the mono product **64a** to 40% of the bicyclic product **64b**.

. The two main products were isolated and analysed by ^{1}H and ^{13}C NMR spectroscopy. The ^{1}H NMR spectrum of the 7-membered heterocycle **64a** showed 3 signals. The first signal, had a range of δ 1.87-2.24 (2H, multiplet) ppm, this broad signal appeared to be two overlapping signals corresponding to the two protons of the CH_2 at the β position to the OH. The second signal appeared as the range of δ 3.39-3.66 (4H, multiplet) ppm corresponding to the four protons present in the two CH_2 groups next to the two NH groups. The last signal at 4.56 (1H, multiplet) ppm corresponded to the CH group next to the OH. The ^{13}C NMR spectrum showed 5 signals at δ 34.9, 46.8, 56.7, 71.3 and 158.2 ppm and are assigned in Figure 11.

The ^{1}H NMR spectrum of the bicyclic heterocycle **64b** gave three main 3 signals. The first at δ 2.00-2.25 (2H, multiplet) ppm, corresponded to the two overlapping signals protons of the CH_2 at the β position to the OH. The second signal in the range of δ 3.56-3.86 (4H, multiplet) ppm corresponded to the four protons present in the two CH_2 groups adjacent to the two NH groups. The last signal at δ 4.55 (1H, multiplet) ppm corresponded to the CH at the α position to the OH. The ^{13}C NMR spectrum had 8 signals at 35.2, 35.4, 50.0, 58.7, 59.7, 72.2, 72.6 and 158.4 ppm and are assigned in Figure 11.

The above procedure was also carried out using epoxide **92** that had been previously prepared from L-aspartic acid **93** as reported by Volkmann[56]. As before epoxide **92** was then added in two portions to guanidine prepared *in situ* by treating guanidine hydrochloride **49** with potassium *tert*-butoxide in *tert*-butanol giving the monocycle **94a** in 39% yield and the bicyclic heterocycle **94b** in 9% yield (Scheme 32).

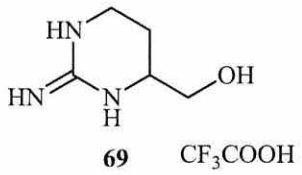


Scheme 32: (a) *t*-BuOH, guanidine.HCl, *t*-BuOH, stir 18 h rt;

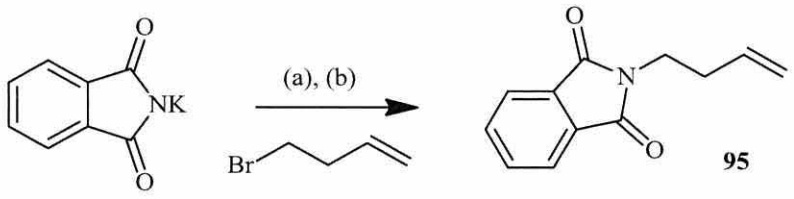
(b)1 eq of **92**, *t*-BuOK, 60°C 24 h; (c) TFA, MeOH.

Synthesis of (2-iminohexahydropyrimidin-4-yl)methanol (69)

One compound of considerable interest is the previously prepared[48] 6-membered guanidine **67** this can been seen as an analogue of the pyrano sugars and might exhibit interesting biological properties

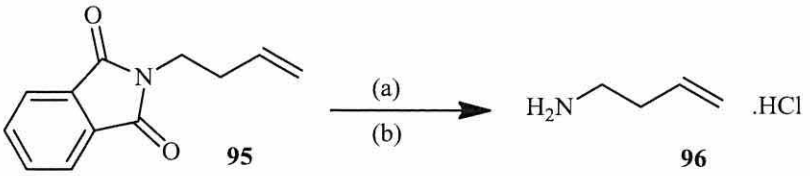


The required precursor **65** was prepared from the hydrochloride salt of the homoallylamine **96**, which itself was prepared via Gabriel synthesis[57]. Thus, reaction of 4-bromobut-1-ene with potassium phthalimide in DMF gave the required *N*-(but-3-enyl) phthalimide **95** in 83% yield (Scheme 33).



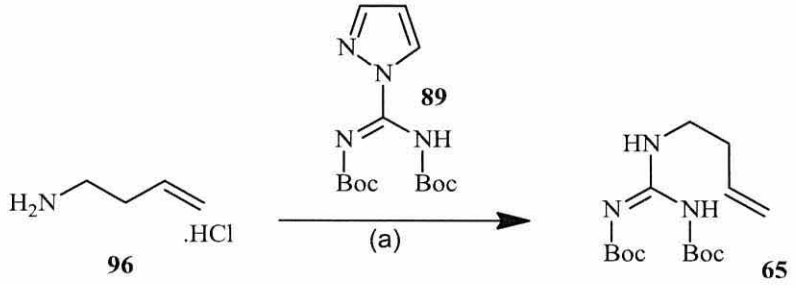
Scheme 33: (a) DMF stir 20 h, rt; (b) Reflux 8 h

The crude N-(but-3-enyl) phthalimide **95** was then converted to but-3-enylamine hydrochloride **96** using the Ing-Manske procedure[58], in which the *N*-(but-3-enyl) phthalimide **95** was heated under reflux with hydrazine monohydrate (1.3 eq) for 4-5 h in IMS. The crude material was analysed using ^{1}H and ^{13}C NMR spectroscopy, which showed the isolated solid was the desired but-3-en-1-amine hydrochloride **96** in 92% yield (Scheme 34).



Scheme 34: (a) $H_2N_2.H_2O$, Reflux 4 h ; (b) conc HCl.

The but-3-en-1-amine hydrochloride **96** was then reacted with *N,N'-bis-(tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamide **89** in the presence of triethylamine (3.1 eq) in dry acetonitrile overnight under Argon. After chromatography in the desired *N*-homoallyl-*N'N''-bis-*(*tert*-butoxycarbonyl)-guanidine **65** was obtained in 82% yield. (Scheme 35)



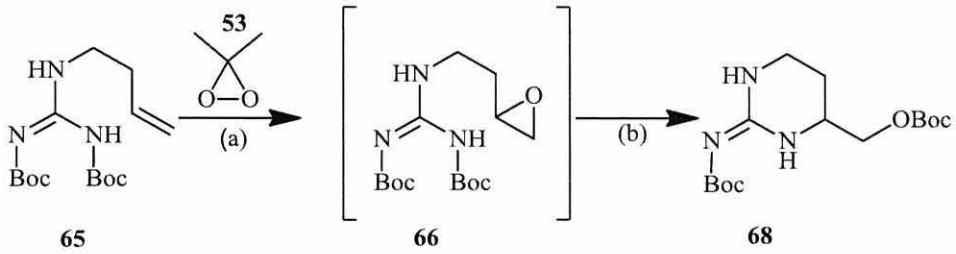
Scheme 35: (a) 3.1 eq Et_3N, acetonitrile, rt, overnight

The presence of the desired product **65** was confirmed using ^1H and ^{13}C NMR spectroscopy. The ^1H NMR spectrum showed 8 signals. The first two at δ 1.50 (9H, singlet) and 1.52 (9H, singlet) ppm, represent the protons found in the two Boc groups. The next signal at δ 2.33 (2H, dt, J = 6.8, 7.0 Hz) ppm was attributed to the protons of the β-methylene carbon of the homoallyl group whilst the protons of the α-methylene carbon of homoallyl appear further downfield at δ 3.51 (2H, triplet, J = 7.0 Hz) ppm. The two δ-protons which form the terminus of the alkene appear at δ 5.16 (2H, multiplet) ppm. The proton on the CH of the alkene group appears at δ 5.80 (1H, ddt, J = 17.1, 10.4, 6.8 Hz) ppm and finally, the N-H protons on the guanidine appear as broad signals at δ 8.36 and δ 11.50 ppm.

The ^{13}C NMR spectrum showed 11 signals, the first two at δ 28,0 and 28,3 ppm corresponded to the six CH_3 present in the Boc groups. The next signal at δ 33.1 ppm is attributed to the β-methylene carbon of the homoallyl group and at δ 40.0 ppm is the signal

due to the α-methylene carbon of the homoallyl. Appearing next, at δ 79.2 and 82.9 ppm are the quaternary carbons of the Boc groups. At δ 117.5 ppm is the δ-methylene of homoallyl while at δ 134.7 ppm is a signal attributed to the CH carbon of homoallyl. The two carbonyls of the Boc groups appear at δ 153.2 and 156.1 ppm while the guanidine carbon appears most downfield at δ 163.6 ppm.

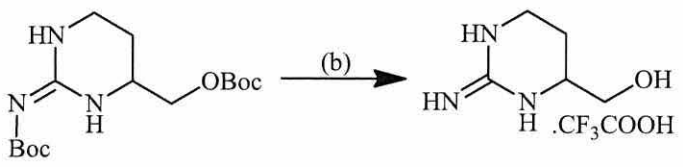
Compound **65** was treated with an excess of DMDO **53** to give the desired *tert*-butyl-[(2*Z*)-2-(*tert*-butoxycarbonyl)hexahydropyrimidin-4-yl] ethyl carbonate **68** as a solid in 82% yield (Scheme 36).



Scheme 36: (a) acetone, – 25 °C, 4 h; (b) rt, 3 days.

^{1}H and ^{13}C NMR spectroscopy was used to confirm that the isolated material was the desired product. The ^{1}H NMR spectrum showed 8 signals, the first two signals at δ 1.50 (s, 9H)) and δ 1.52 (s, 9H) ppm correspond to the *t*-Bu of the Boc groups. The signals at δ 1.86 (m, 1H,) and δ 2.01 (m, 1H) ppm corresponded to the protons of CH_2 at the the 5-position of the ring whilst the 4-CH_2 was a multiplet at 3.48 (m, 2H.) The 6-CH signal was observed at δ 3.83 (m, 1H) whilst the $\underline{CH_2}$OBoc was a observed at δ 4.05 (1H, dd, J = 6.75, 11.0 Hz) and 4.26 (1H, dd, J = 4.88, 11.0 Hz) ppm The ^{13}C NMR spectrum had the required 11 signals with characteristic signals at δ 25.8, 40.4 and 71.7 ppm corresponds to the three CH_2 carbons and δ 52.6 ppm for the ring CH. Quaternary signals at δ 156.3, 157.2 and 160.6 ppm correspond to the two Boc protecting groups and the guanidine carbon.

Guanidine **68** was then deprotected by treatment with excess trifluroacetic acid in chloroform to give the required product in 66% yield (Scheme 37).



68 **69**

Scheme 37: (a) TFA, $CHCl_3$, rt, 24 h

The ^{1}H NMR spectrum showed 7 signals, the first two signals at δ 1.75 (1H, m) and 2.0 (1H, m) ppm corresponded to the protons of the ring CH_2 at the β position to the ring NH. The signals at δ 3.32 (1H, m) and 3.41 (1H, dt, J = 12.6, 5.1 Hz) ppm corresponded to the N-CH_2 ring methylene. The signal at δ 3.48 (1H, dd, J = 10.1, 7.25 Hz) corresponded to the ring CH. The last two signals at δ 3.52 (1H, m) and 3.68 (1H, dd, J = 10.1, 4.05 Hz) ppm corresponded to the protons of the CH_2 next to the OH. The ^{13}C NMR spectrum showed the expected 5 signals, the first at δ 23.3 corresponded to the carbon β to the ring NH. The next at δ 38.1 and 52.0 ppm corresponded to the ring CH_2 and CH respectively. The signal at δ 65.0 corresponded to the CH_2 next to the OH and the last signal at δ 65.0 corresponded to the guanidine carbon.

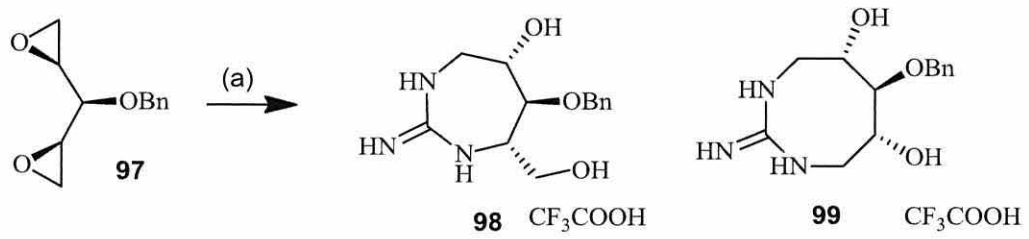
Section Conclusion

The synthesis of previously prepared heterocycles **50**, **64a**, **64b**, **94a**, **94b** and **69** was successful. During the synthesis of **50** it was found that the guanidine mediated cyclisation of epoxide **49** also gave the disfavoured 6-endo-tet product **82** (Scheme 19). To confirm this, the heterocycle was synthesised using an alternative method giving its acetate salt **86** (Scheme 23). As **50** and **82** could not be separated by chromatography it was decided to prepare **50** via the DMDO epoxidation of allyl-*N'N''-bis*-(*tert*-butoxycarbonyl)-guanidine **52** (Scheme 24) and intermediate **56** the result of a N-O Boc migration was isolated and characterized by x-ray diffraction (Figure 9). Deprotection of **56** with trifluroacetic acid gave **50** as a pure sample with no indication of **82** in its ^{1}H NMR spectrum (Figure 10).

On completion of these syntheses and purification by preparative HPLC, 8 compounds were available for testing, the original target heterocycles **50**, **64a**, **64b**, **65a**, **65b** and **69**, the 6-membered heterocycle **86** and the nitro-guanidine intermediate **85**.

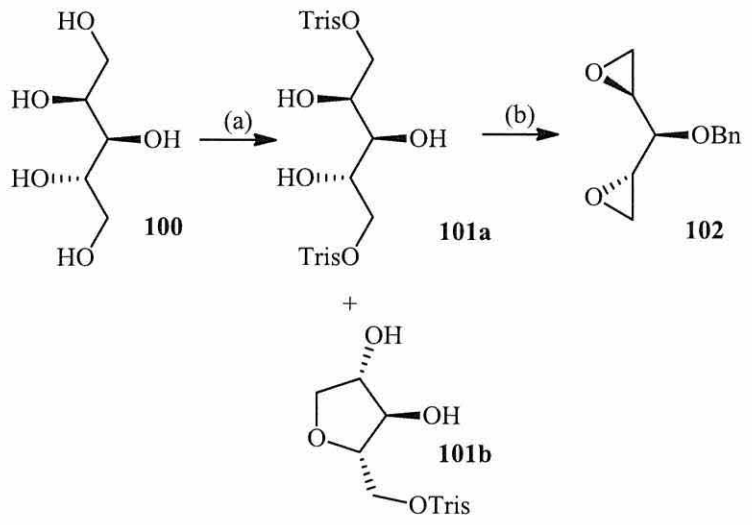
Attempted investigation of guanidine mediated heterocyclisation of *bis*-epoxides

One of the goals of the study was to investigate the use of *bis*-epoxide as the electophiles in the guanidine mediated heterocyclisation. For example the *meso*-epoxide **97** on treatment with guanidine would be expected to undergo ring opening at one of the terminal positions followed by either a 7-exo or 8-endo cyclisation leading to either **98** or **99** respectively (Scheme 38).



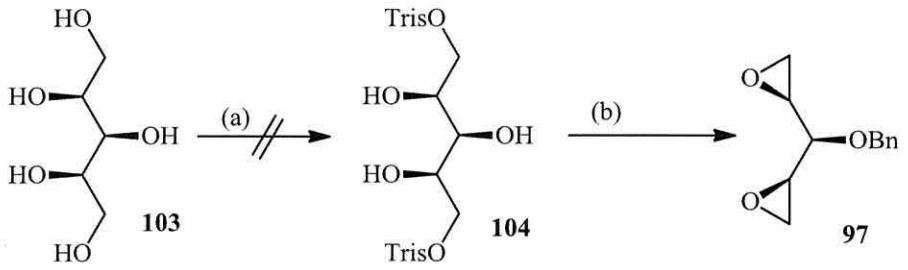
Scheme 38: (a) guanidine, *t*BuOH, (b) MeOH, TFA.

On inspection of the literature for a method to prepare *bis*-epoxide **102** a report by Leung and co workers to form a *bis*-epoxide **99** from L-arabitol **100** in two steps[59] was found. The first step involved the reaction of the L-arabitol **100** with 2,4,6-triisopropylbenzene sulfonyl chloride (TrisCl) in pyridine with DMAP over 24 hours at 0 °C. This was reported to generate a di tris-arabitol **101a** together with a small amount of the cyclisation product **101b**. Di tris-protected arabitol **101a** was then reacted with NaH in DMF/THF giving the *bis*-epoxide **102** following an intramolecular cyclisation subsequent addition of benzyl bromide gave benzyl ether protected **102** (Scheme 39).



Scheme 39: (a) TrisCl, DMAP, pyridine 0 °C 24 h, (b) NaH, DMF 0 °C, 1 h; BnBr, NaH, rt, 24 h

Using this method, we attempted to prepare the *bis*-epoxide **97** from xylitol **103** *via* the protected intermediate **104** (Scheme 40).

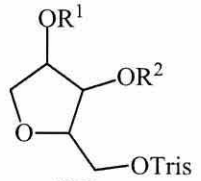


Scheme 40: (a) TrisCl, DMAP, pyridine 0 °C 24 h, (b) NaH, DMF 0 °C, 1 h; BnBr, NaH, rt, 24 h

Initial studies we disappointing with no product being detected by NMR spectroscopy of the crude reaction mixture. Varying the conditions by increasing the equivalents of TrisCl ranging from 2.2 eq to 3.2 eq at varying temperatures (-5 °C to rt) and reaction time (16-72 hours) were tested but did not yield any of the desired product.

Mass spectrometry of the crude reaction mixture gave a molecular ion at 689.31 Daltons ($M+Na^+$), which closely corresponds to the expected mass of furan **105** ($M+Na^+$ =

689.32 Daltons) (Figure 12).



105

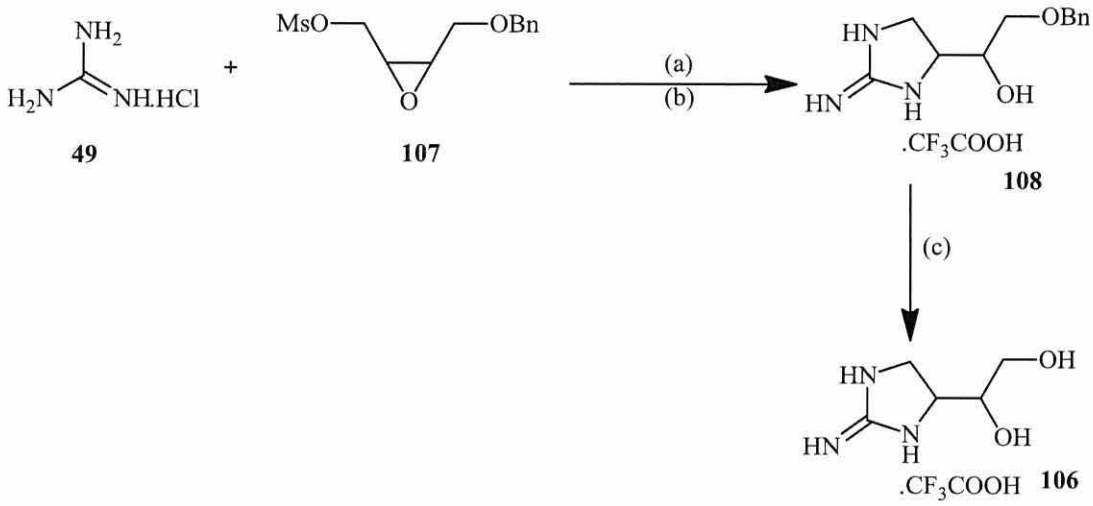
Figure 12: R^1 or R^2 = Tris or H

It was noted in the original work of Leung[59] that a similar by-product **91b** was observed in the L-arabitol series, which Leung limited by performing the reaction at 0 °C but this did not appear to be successful with xylitol even at – 5 °C.

Since we were unable to prepare the intermediate compound **101a** this avenue of investigation was abandoned.

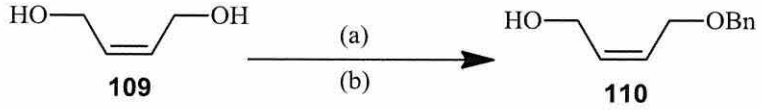
Synthesis of furanoide sugar analogues (103)

Focusing on the glycosidase inhibitor pathways, guanidine **106** which could be considered an analogue of a furanoide hexose. The idea was to use a mesylated epoxide **107** which on cyclisation with guanidine would lead to **108**, which on deprotection should give **106** (Scheme 41).



Scheme 41: (a)*tert*-BuOK, *tert*-BuOH, (b) TFA, MeOH. (c) H_2 pd/C

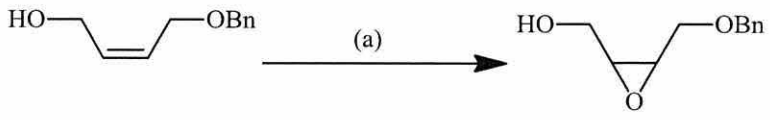
The epoxide **107** was initially prepared by benzylation of (Z)-but-2-ene-1,4-diol **109** utilising and excess of NaH (3.3 eq) with 0,4 eq. of benzyl bromide in THF. To minimise the formation of any dibenzylated by-product. This reaction gave a respectable 53% yield after chromatography but also the dibenzylated product was also formed in 17% yield. The synthesis of this compound **110** has been reported in a yield of 92%[60] using NaH (1.1 eq) and benzyl bromide (1.1 eq) in the presence of tetrabutylammonium iodide (0.5 eq) in a high dilution of THF. We repeated this reaction and in our hands this gave a 76 % yield of **110** (Scheme 42).



Scheme 42: (a) NaH, BnBr, But_4NI, THF, rt, 18 h (b) 60°C 6 h.

^{1}H and ^{13}C NMR spectroscopy was used to confirm that the product was the desired compound.

Epoxidation of **110** was accomplished using with *m*CPBA (2.5 eq) in DCM to give epoxide **111** in 60% yield (Scheme 43).

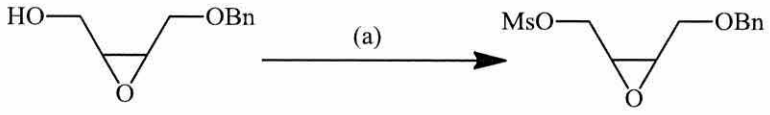


110 **111**

Scheme 43: (a) *m*CPBA (2.5 eq), DCM, rt, 12 h

Evidence for the formation of **111** was given by the disappearance of the alkene protons present in the spectrum of **110** and the appearance of a complex signal at δ 3.29 – 3.43 (2H, m) ppm corresponding to the two protons on the epoxide ring. Similarly the presence of the two methyne signals at δ 55.9 and 56.3 ppm in the ^{13}C NMR spectrum was evidence of the formation of an epoxide.

Mesylation of **111** was accomplished by treatment with methansulfonyl chloride, triethylamine and a catalytic amount of DMAP in DCM leading to the epoxide **107** in 72% yield (Scheme 44).

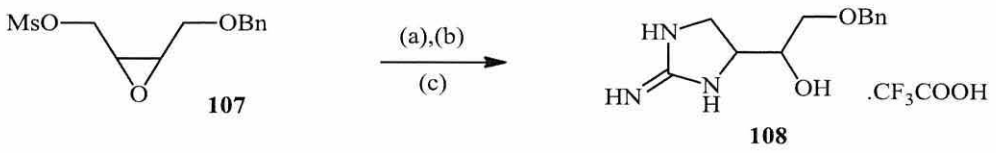


111 **107**

Scheme 44: (a) MsCl, DMAP, DCM 18 h

The structure was confirmed by ^{1}H NMR as the broad singlet of the OH present in **111** had disappeared and been replaced by a signal at δ 3.05 (3H, s) corresponding to the methyl of the mesylate group.

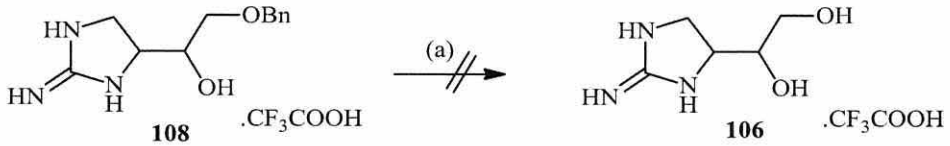
With the epoxide **107** in hand the reaction with guanidine was investigated. Thus, epoxide **107** was reacted with guanidine **43** in a similar fashion as discussed earlier to give derivative **108** in 51% yield (Scheme 45).



Scheme 45: (a) guanidine, 1.1 eq *t*-BuOK, *t*-BuOH, rt, 20 h; (b) 1.1 eq *t*-BuOK, 60 °C, 20 h; (c) 3.3 eq TFA, MeOH

Analysis of the ^{1}H NMR spectrum was showed overlapping signals between δ 3.50 – 4.70 ppm, which were in a similar region to those observed for the 5-membered heterocycle **50**. The ^{13}C spectrum gave the expected 9 signals with the ring methylene at δ 46.9 ppm and the ring methyne at δ 66.2 ppm. The C<u>H</u>OH signal was observed at δ 71.6 whilst a further two signals at δ 72.3 and 73.6 ppm corresponded to the remaining 2 methylene carbons. The four signals δ 128.4, 128.9, 129.0 and 129.5 ppm were due to the aromatic ring whilst the guanidine signal was observed at δ 160.2 ppm. Analysis by mass spectrometry suggests the presence of the guanidine **108** as an accurate mass of 236.1363 Daltons was observed with an expected mass of 236.1399 Daltons.

The final stage of the synthesis was the removal of the benzyl group which was envisaged as being easily accomplished using hydrogenation over palladium on charcoal. (Scheme 46)



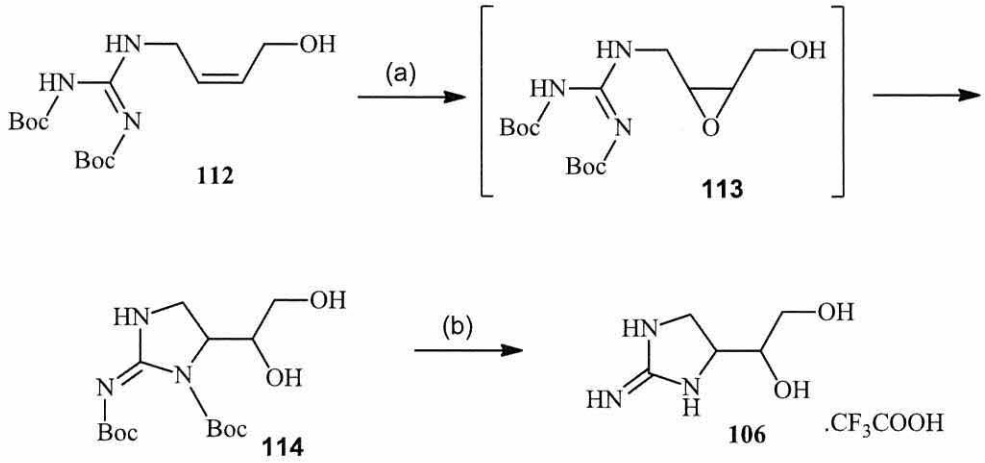
Scheme 46: (a) H_2, 5% Pd/C, ethanol.

Deprotection of heterocycle **108** was attempted using catalytic hydrogenation but analysis of the crude reaction product by ^{1}H and ^{13}C gave complex spectra which were obviously composed of a mixture of several compounds.

Previous work[46] by the group indicated that isolation of some guanidine heterocycles after hydrogenation has been problematic in the past and it was decided to abandon this approach for synthesising the guanidine **106**.

Alternative synthetic route to 106.

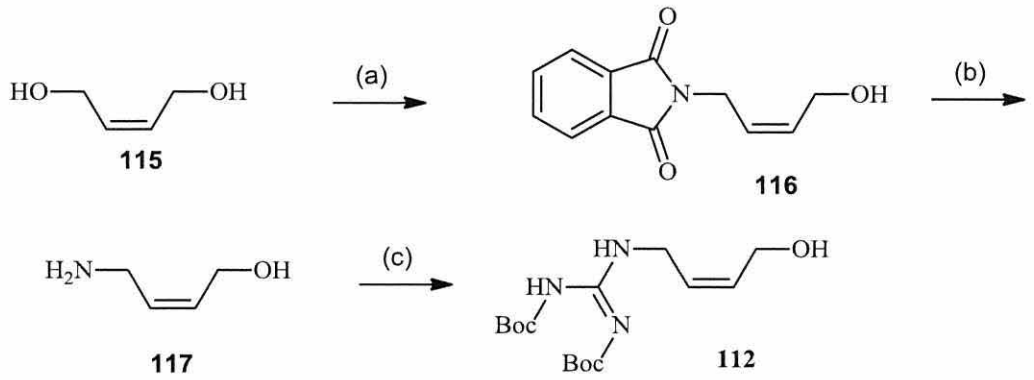
To avoid the difficulties in the deprotection step an alternate synthesis was proposed. This approach relies upon the epoxidation of the protected guanidine **112** which on treatment with DMDO should lead to the intermediate epoxide **113** followed by cyclisation to give the 5-membered product **114**. Acid mediated deprotection should then lead to the required compound **106** (Scheme 47).



Scheme 47: (a) DMDO, Acetone, (b) TFA, MeOH.

Alkene **112** was prepared via a two step process from (Z)-but-2-ene-1,4-diol **115**. The first step required a monosubstitution of one of the hydroxyls with phthalimide under Mitsunobu conditions. Thus **115** was treated with phthalimide, 1.1 eq of DEAD and 1.5 eq triphenylphosphine in dry THF. After 15 h, trituration and chromatography gave the required

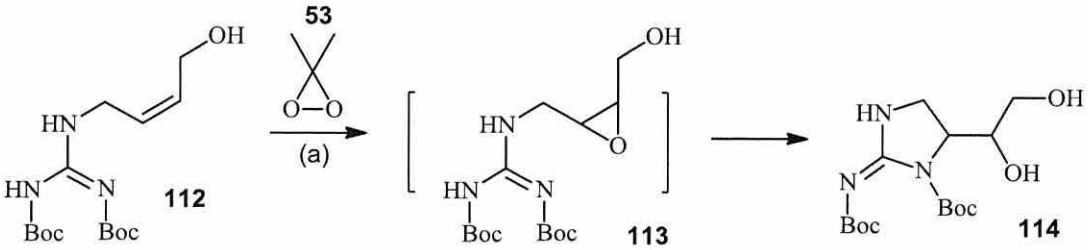
phthalimide **116** in 67% yield. The phthalimide **116** was then converted to the amine **117** *via* the Ing-Minsk procedure[58] using hydrazine monohydrate in refluxing ethanol. After filtration to remove the by-product a solution of amine was obtained which was treated with the guanilating agent **89** to give the desired product **112** in 56% yield (Scheme 48).



Scheme 48: (a) 1eq phthalimide, 1.1 eq DEAD, 1.5 eq PPh_3, THF, 0°C to rt, 15 h. (b) 1.3 eq $H_2N_2.H_2O$, Reflux 2 h, (c) 1.1 eq *N,N'-bis-(tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamide **89**, rt 72 h.

The structure of **112** was identified using the ^{1}H NMR spectrum, which gave signals at δ 1.48 (9H, s) and 1.49 (9H, s) ppm for the Boc *t*-butyl groups and a signal at δ 4.05 (2H, app. t, J = 6.6 Hz) ppm for the protons of the CH_2 next to the guanidine NH. The signal at δ 4.23 (2H. d, J = 6.9 Hz) ppm corresponded to protons of the CH_2 next to the OH whilst the alkene protons were at δ 5.44 (1H, dt, J = 10.7, 7.6 Hz) and 5.84 (1H, broad dt, J = 10.7, 6.9 Hz). The two NH protons of the guanidine were found at δ 8.52 (1H, broad s) and 11.39 (1H broad s). The ^{13}C NMR spectrum gave the required 11 signals and HRMS gave a molecular ion mass 330.2022 Daltons that matched the theoretical mass of 330.2023 Daltons.

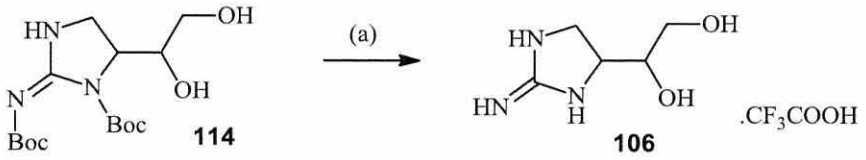
With the guanidine **112** in hand the epoxidation was attempted by reaction with DMDO in acetone at – 30°C. Again the intermediary of the epoxide **113** is assumed which should cyclise to give guanidine **114** (Scheme 49).



Scheme 49: (a) acetone, -30 °C 1.5 h, then rt, 14 h.

The possibility that the compound undergoes a similar Boc group migration to heterocycle **55** was investigated by dissolving **114** in dichloromethane and stirring in presence of SiO_2 for 48 h. NMR spectroscopy showed no change in signals indicating there was no Boc migration induced by SiO_2.

Deprotection of **114** was achieved using TFA in chloroform for 48 h which after purification by column chromatography gave **106** in 59% yield (Scheme 50).



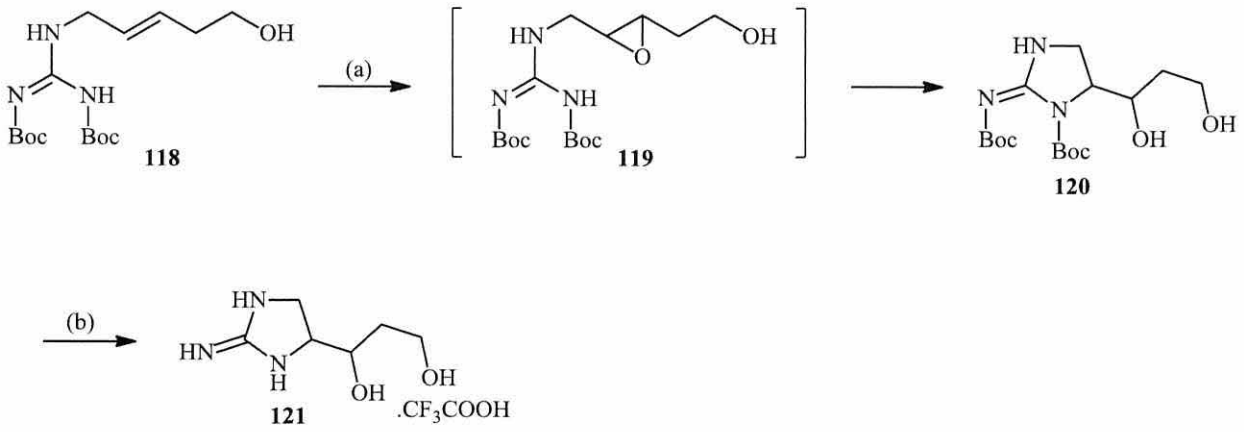
Scheme 50: TFA, $CHCl_3$, rt 48 h

The overlapping of signals made structural analysis by ^{1}H NMR spectroscopy difficult but the disappearance of the signals at δ 1.48 and 1.49 ppm confirmed the removal of the Boc groups. HRMS gave a molecular ion mass of 146.0926 Daltons, which is a good match the expected of 146.0924 Daltons.

In preparation for biological testing a sample of **106** was further purified using preparative HPLC chromatography.

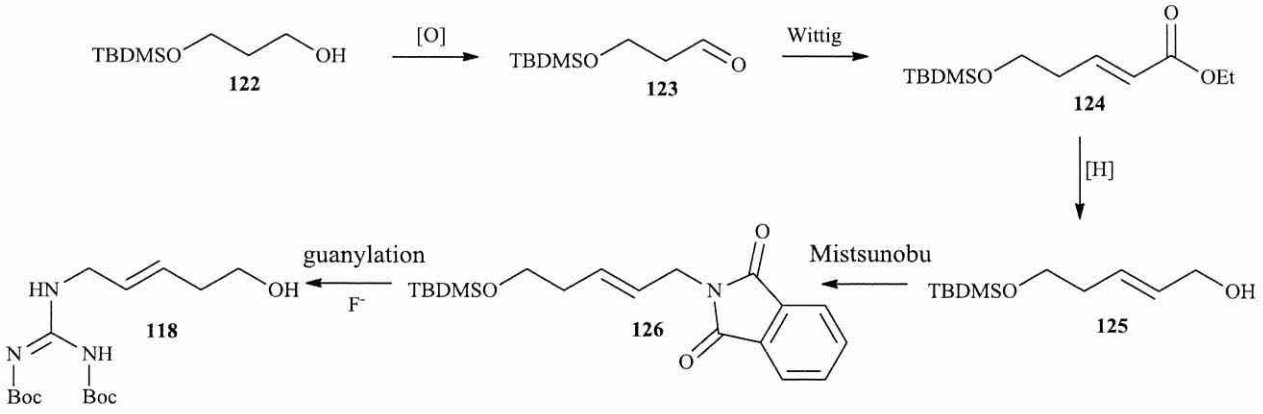
Synthesis of 1-(2-iminoimidazolidin-4-yl)ethane-1,2-diol trifluoroacetate

The successful synthesis of 5-membered heterocycle **106** it was decided to investigate the cyclisation of **118** and deprotection to give guanidine heterocycle **121** under similar conditions (Scheme 51).



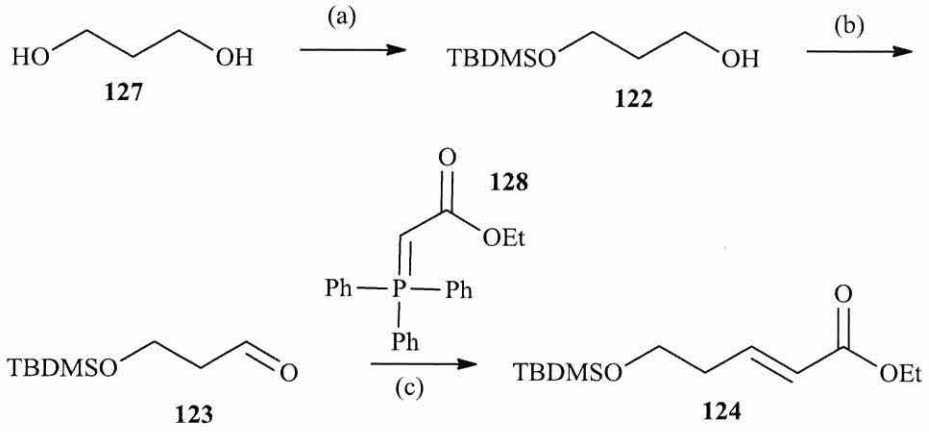
Scheme 51: (a) DMDO, Acetone, (b) TFA, MeOH.

Alkene **118** was prepared over multiple steps from Silyl protected alcohol **122** using Wittig chain extension methodology (Scheme 52).



Scheme 52: Synthesis of alkene **118**.

Silyl protected alcohol **122** was prepared in 82% yield by a literature method[61] involving the monosilylation of propane-1,3-diol **127**, which was then oxidised to the aldehyde **123** under Swern conditions. NMR analysis of the crude aldehyde gave a diagonostic signal at δ 9.81 (1H, t, 1.9 Hz). Wittig reaction was achieved by reaction with carboethoxymethylenetriphenylphosphorane **128** in dichloromethane to give **124** as an oil (Scheme 53).

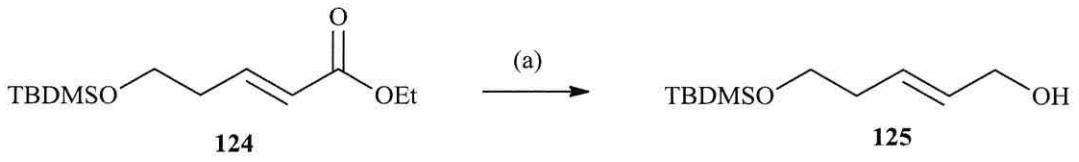


Scheme 53: (a) 1 eq NaH, 0 °C, 1.5 h, 1.1 eq TBDMCl, 1.5 h (b) Oxalyl chloride, DMSO, Et_3N, (c), dichloromethane, rt, 14 h.

Analysis of the crude spectrum of **124** indicated the presence of the *E*-alkene as evidenced by signals at δ 5.84 (1H, dt, J 15.6, 1.4 Hz) and δ 6.94 (1H dt, J = 15.6, 7.1 Hz) with a large *trans*- coupling constant of 15.6 Hz. The minor Z-isomer was also present with signals at δ 6.32 (1H, dt, J 11.4, 1.3 Hz) with a smaller *cis*- coupling constant of 11.4 Hz. The relative ratios of these two isomers was estimated to be 94:6 by the integration of these signals. It was possible to separate the Z-isomer by careful column chromatography to give the E-alkene **124** in 79 % yield. Attempts were made to improve the selectivity of this reaction by carrying out reactions at 0 °C and – 5 °C but no great effect on the E:Z ratio was observed.

Ester **124** was reduced to the alcohol **125** by treatment with DIBAL in dichloromethane at -40 °C. Analysis by TLC indicated that the reaction had gone to

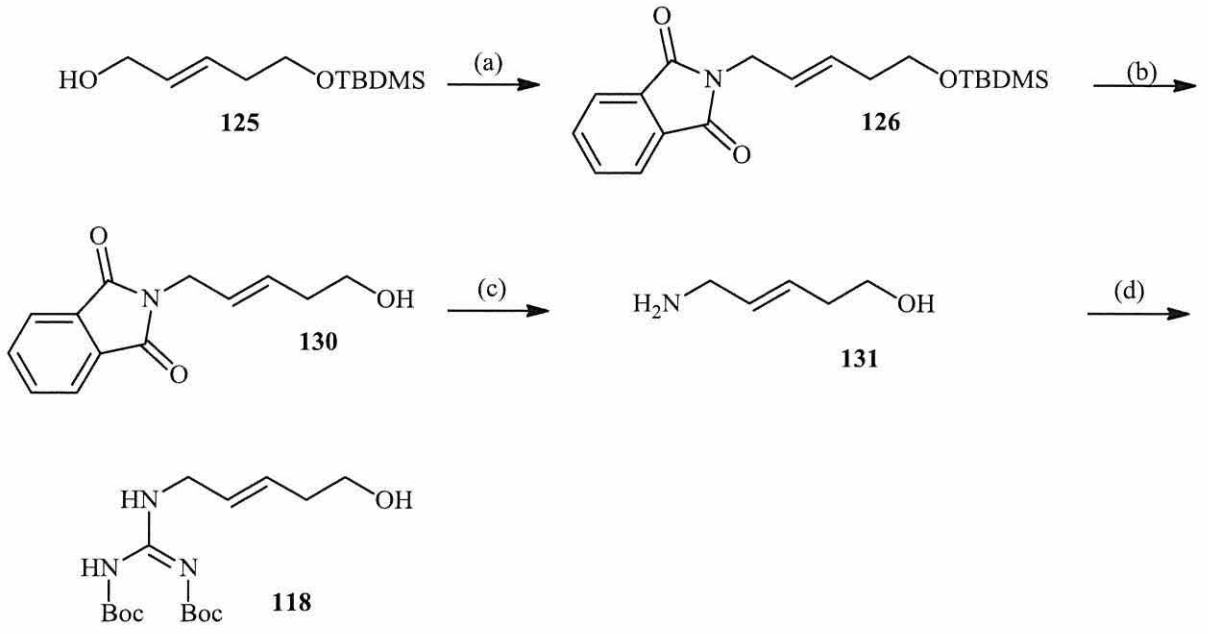
completion in approximately 15 minutes and after work-up the alcohol **125** was obtained as a clear oil in 98% yield (Scheme 54).



Scheme 54: (a) DIBAL, DCM, – 40 °C

Analysis of the ^{1}H NMR spectrum indicated that the ethyl ester proton signals were no longer present and a new signals at δ 2.18 (1H, br s) and δ 4.04 (2H, d, J 3.5 Hz) corresponding to the hydroxyl and new methylene groups respectively, were present. IR spectroscopy gave a band at 3356 cm^{-1} corresponding to the hydroxyl group.

The alcohol **125** was treated with phthalimide in the presence of DEAD and triphenylphosphine to give the phthalimide **126** in 48% yield. The silyl group was then removed with TBAF in THF at room temperature to give alcohol **130.** Reaction of this with hydrazine hydrate followed by guanylation under standard conditions gave the guanidine **118** (Scheme 55).

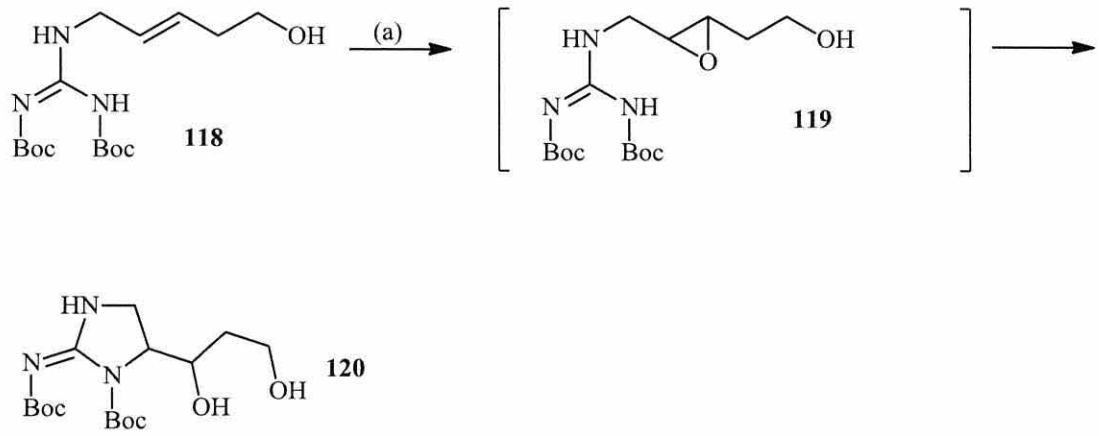


Scheme 55: (a) 1.5 eq phthalimide, 1.5 eq DEAD, 2 eq PPh_3, dichloromethane, 0°C, 1 h.

(b) 1.2 eq TBAF, THF, rt, 20 h. (c) 1.2 eq $H_2N_2.H_2O$, Reflux 2 h,

(d) 2.2 eq *N,N'-bis-(tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamide **89**, rt 84 h

Epoxidation of **118** was then carried out using standard conditions with DMDO in acetone (Scheme 56).



Scheme 56: (a) DMDO, acetone, - 30°C to rt, 14 h.

The successful epoxidation and cyclisation of **118** to give **120** was confirmed by ^{1}H NMR, which showed a complex set of signals that would be expected. Key ^{1}H NMR signals are two multiplets at δ 1.55 – 1.62 (1H, m, C*H*H) and 1.64 – 1.71 (1H, m, C*H*H) ppm, which

correspond to the CH_2 found adjacent to the OH groups in the 1,3 diol. The signal at δ 4.02 (1H, m, C*H*) ppm also indicates that a ring has been formed. The ^{13}C NMR also showed the expected 11 signals and mass spectrometry gave a mass of 260.3 Daltons, which is consistent with the target molecule's ($[M+H]^+$.

Section Conclusion

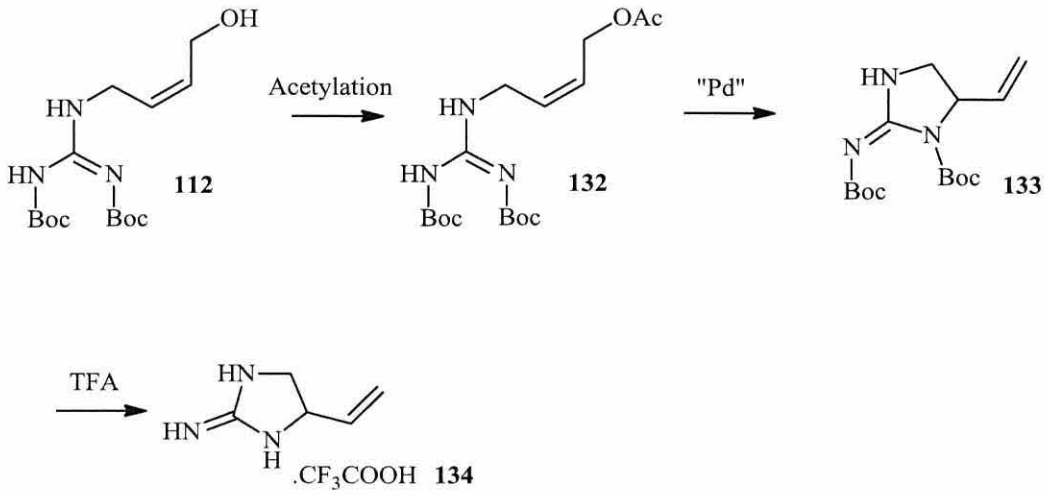
The synthesis of guanidine heterocycles **98/99** was unsuccessful due to the inability to prepare *bis*-epoxide **104** from xylitol, instead the proposed heterocycle **105** was the only product detected.

The initial attempt of preparing **106** using a intermolecular cyclisation route was unsuccessful due to difficulties in the benzyl ether Deprotection step. An alternative route using an intramolecular cyclisation from **112** treated with DMDO was successful and the desired heterocycle **106** was isolated after the deprotection of Boc protected intermediate **114**.

Protected guanidine heterocycle **120** was also synthesised using the same cyclisation methodology and would be available for biological testing after deprotection.

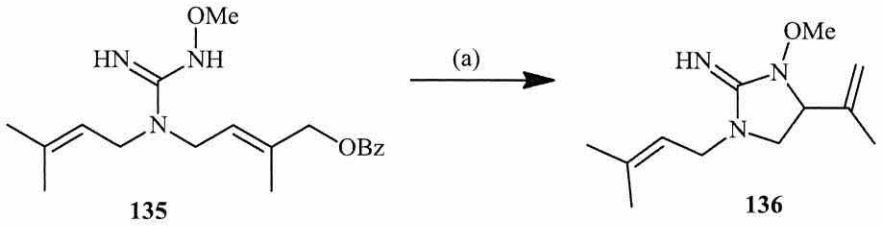
Pd initiated cyclisations

Intermediate **112** offered another avenue for the synthesis of cyclic guanidines by palladium cyclisation. It was envisaged that acetylation of the terminal alcohol followed by a palladium catalysed cyclisation would lead to the guanidine **133**, producing heterocycle **134**[62] after deprotection (Scheme 57).



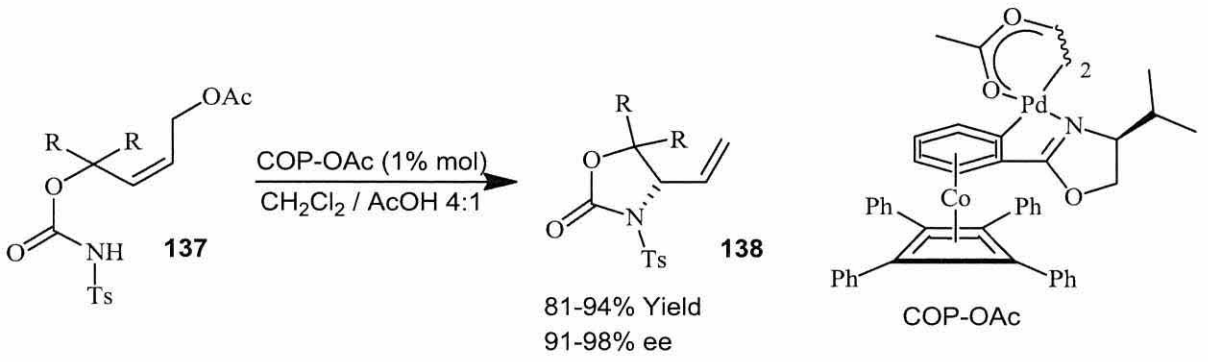
Scheme 57: Proposed palladium catalysed cyclisation

On inspection of the literature Buchi *et al* had reported the cyclisation of the *N*-methoxy-guanidine **135** which led to the 5-membered heterocycle **136** in 81% yield (Scheme 58).[63]



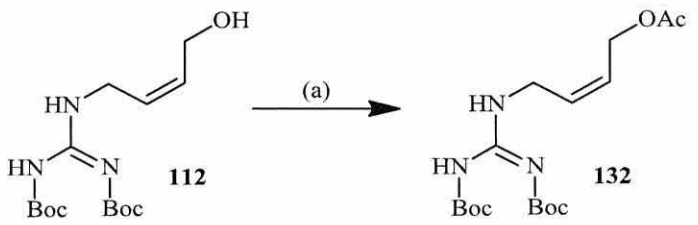
Scheme 58: (a) $Pd(PPh_3)_4$, Et_3N, MeCN, 50 °C, 3 h

A report by Overman of high yielding palladium catalysed enantioselective cyclisation of amideines **137** is of considerable interest as it describes the access to a range of 4-vinyloxazolidin-2-ones **138**, which can be seen as analogues of guanidine **134** (Scheme 59).[64]



Scheme 59: Synthesis of 4-vinyloxazolidin-2-ones by Overman group[64].

Guanidine **112** was thus acetylated by treatment with acetic anhydride, pyridine and a catalytic amount of DMAP in dichloromethane to give the desired acetate **132** was obtained in 71% yield. (Scheme 60)

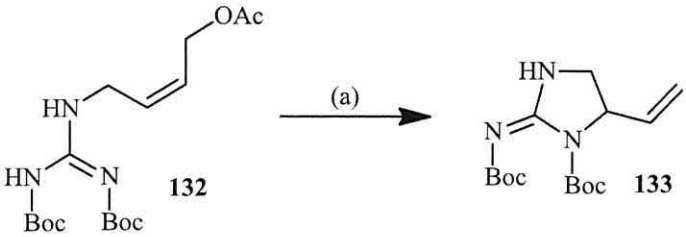


Scheme 60: (a) 1.5 eq acetic anhydride, 3 eq pyridine, 0.3 eq DMAP, CH_2Cl_2, 0 °C, 1 h

The ^{1}H NMR spectrum of **118** gave a diagnostic signal at δ 2.03 (3H, s) for the acetate methyl whilst in the ^{13}C NMR signals at δ 20.9 (CH_3) and 169.7 (C=O) ppm confirmed this supposition. High resolution mass spectrometry gave a molecular mass of 372.2135 Daltons which is in close agreement with the expected mass of 372.2129 Daltons.

Cyclisation of **132** was attempted under similar conditions to those reported by Buchi for compound **135** i.e. a solution of $Pd(PPh_3)_4$. Monitoring of the reaction by TLC demonstrated, that after 2 hours all of **132** had been consumed. After evaporation and column

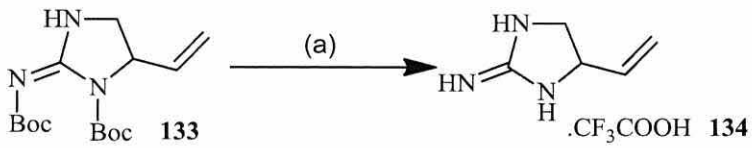
chromatography the desired compound **133** had been formed in 84% yield as an off white solid (Scheme 61).



Scheme 61: (a) $Pd(PPh_3)_4$, Et_3N, THF, 55 °C, 2 h

Analysis of the data for **133** gave a distinctive ABX pattern for the alkene protons at δ 5.13 (1H, d, 10.1 Hz, C*H*), 5.17 (1H, d, 18.0 Hz, C*H*) 5.77 (1H, ddd, 18.0 10.1, 7.55 Hz, C*H*). Signals at δ 3.49 (dd, 1 H, *J* = 13.0, 4.1 Hz) δ 3.93 (1 H, dd, J = 13.0, 9.45 Hz) corresponding to the protons of the ring methylene and a signal at δ 4.53 (m, 1H) for the ring methyne proton. The ^{13}C NMR spectrum showed the expected 10 signals, the key signals being at δ 53.8 (ring CH_2), δ 58.9 (ring *C*H) ppm and δ 116.1 (alkene CH_2), 136.3 (alkene *C*H) ppm.

Guanidine **133** was deprotected using TFA in dichloromethane to give **134**[62] in 91% yield (Scheme 62).



Scheme 62: (a) TFA, CH_2Cl_2 0 °C, rt 48 h

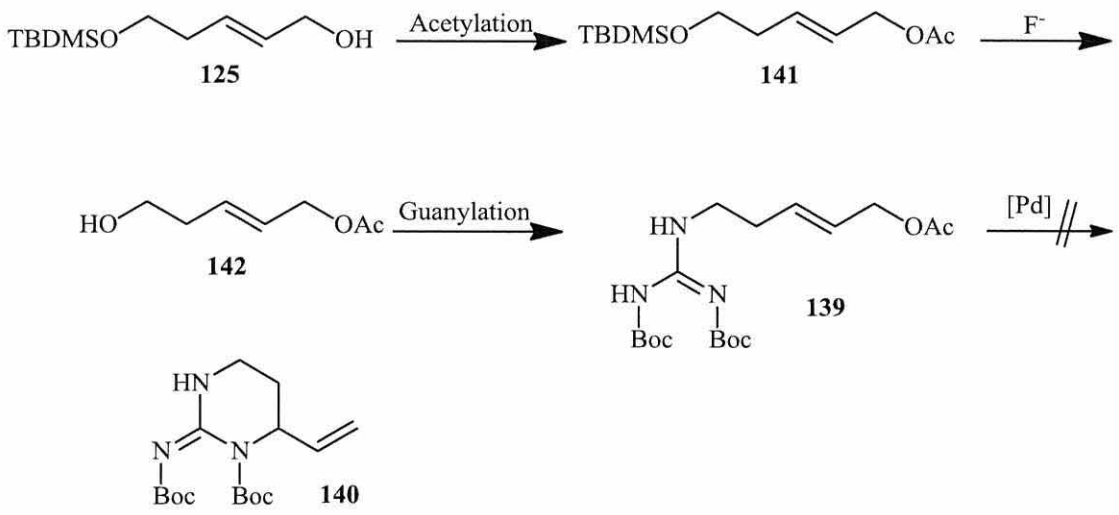
The ^1H NMR spectrum showed that the signals corresponding to the Boc groups were now absent indicating a successful deprotection. The remaining signals were seen at δ 3.32 (2H, m) corresponding to the ring CH_2 protons, δ 3.81 (1H, m), corresponding to the ring CH and the last two signals at δ 5.38 (2H, m), and 5.89 (1H, m) ppm correspond to the alkene protons. The ^{13}C NMR spectrum also showed the expected 5 signals at δ 41.5 (ring CH_2), δ

53.8 (ring CH), δ 121.1 (alkene CH_2), δ 130.8 (alkene CH) and the guanidine carbon at δ 154.1 ppm.

In preparation for biological testing a sample of **133** was further purified using preparative HPLC chromatography.

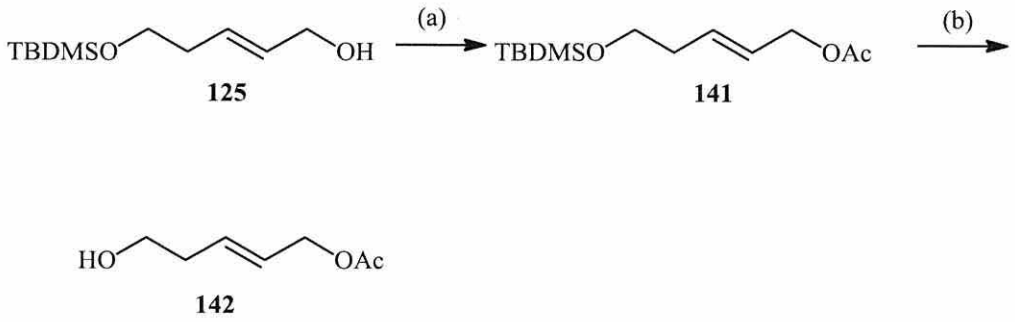
Synthesis of *tert*-butyl 2-((*tert*-butoxycarbonyl)imino)-6-vinyltetrahydropyrimidine-1(2H)-carboxylate

The success of this methodology opened an avenue for the synthesis of further more complex heterocycles. It was envisaged that synthesis of the intermediate **139** should offer access to the 6 membered hetrocycle **140**. As shown access to **139** will be possible via the alcohol **125** prepared previously (Scheme 63).



Scheme 63: Synthesis of 6-membered guanidine **132**

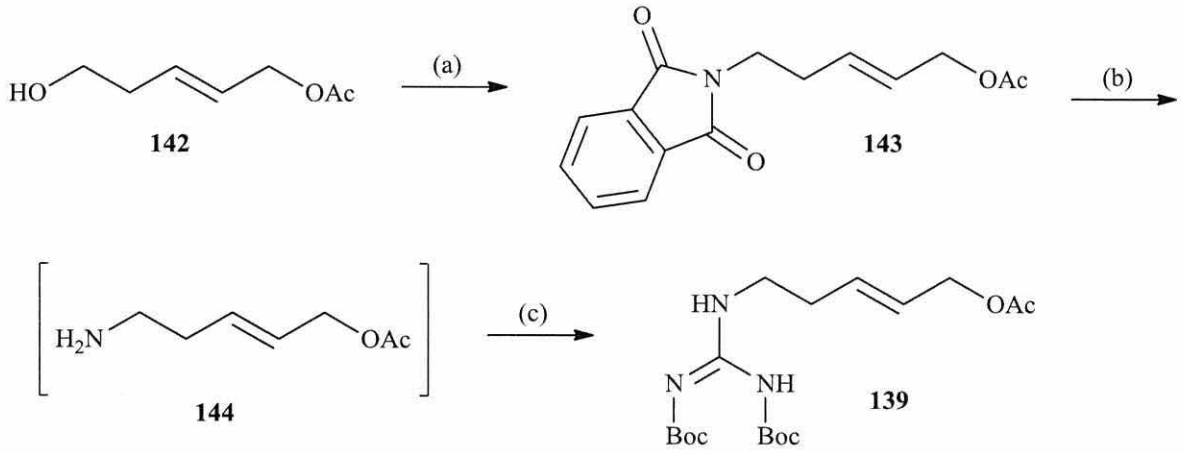
The allyl alcohol in **125** was then protected using acetic anhydride in pyridine and the silyl group removed with TBAF in THF to give the homo-allyl alcohol **142** in 91% and 90 % yields respectively (Scheme 64).



Scheme 64: (a) 3 eq acetic anhydride, 3 eq pyridine, 0.3 eq DMAP, CH_2Cl_2, 0 °C, 36 h

(b) 2 eq TBAF, THF, 0 °C, 20 h.

The guanylation of **142** was achieved via a three stage process the first of which was the formation of the phthalimide **143** directly from the alcohol under Mitsunobu conditions. Thus alcohol **142** was reacted with phthalimide in the presence of DEAD and triphenylphosphine to give **143** as an oil in 58% yield. The phthalimide protecting group was then removed by refluxing with hydrazine to give the intermediate amine alcohol **144.** This was not isolated but reacted with the guanilating agent **89** to give **139** in 52 % overall yield from **142** (Scheme 65).



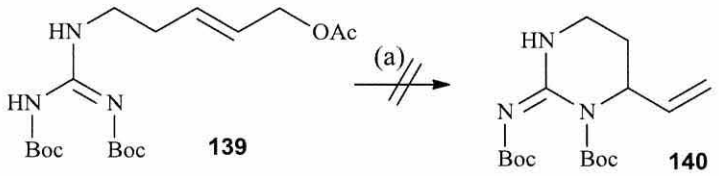
Scheme 65: (a) 1.5eq phthalimide, 2.4 eq DEAD, 1.5 eq PPh_3, THF, 0°C to rt, 16 h. (b) 1.3 eq $H_2N_2.H_2O$, Reflux 4 h, (c) 1.1 eq *N,N'-bis-(tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamide **89**, rt 84 h

The guanidine **139** was identified by its ^{1}H NMR spectrum, which gave signals at δ 1.47 (9H, s) and 1.49 (9H, s) ppm for the Boc *t*-butyl groups, the signal at δ 2.04 (3H, s CH_3) corresponded to the acetate protons and the signal at δ 3.47 (2H, dt, J = 6.6 Hz) ppm for the

protons of the CH_2 next to the guanidine NH. The signal at δ 4.50 (2H. d, J = 6.9 Hz) ppm corresponded to protons of the CH_2 next to the OAc whilst the alkene protons appeared δ 5.63 – 5.75 (2H, m, 2 x CH). The two NH protons of the guanidine were found at δ 8.33 (1H, broad s) and 11.46 (1H broad s). The ^{13}C NMR spectrum gave the required 14 signals, key signals being the acetate CH_3 at δ 20.9 ppm, the Boc signals at δ 28.0 (^{t}Bu), 28.2 (^{t}Bu), 39.9 (CH_2), 79.3 (C), 83.3 (C), 153.3 (C), 155.9 (C) ppm, the guanidine carbon at δ 163.5 ppm and the acetate carbonyl carbon at δ 171.1 ppm.

With **139** in hand the palladium catalysed cyclisation was investigated. As with the cyclisation of **132**, the Pd – catalyst was prepared *in situ* before the acetate **139** and triethylamine were added. The reaction mixture was monitored by TLC but after 3 hours no new spot that could be attributed to the cyclised product could be seen and the acetate starting material **139** spot was seen to remain. The solution was stirred at 55 °C for a further 3 hours with no apparent change in the TLC.

A sample was taken and analysed by ^1H NMR spectrometry, the crude its ^1H NMR showed no evidence of the expected ABX pattern expected from the cyclised product **140** and still showed acetate **139** present. The reaction was attempted 2 further times but with the same result of the acetate not undergoing cyclisation. This investigation was reluctantly abandoned because of the lack of time and starting material (Scheme 66).



Scheme 66: (a) $Pd(PPh_3)_4$, Et_3N, THF, 55 °C, 6 h

Section conclusions

Our investigation of using Pd cyclisation was a partial success with the successful synthesis of **134**. However, cyclisation of the one carbon extended homologue **139** to produce the six membered vinyl heterocycle **140** could not be achieved.

Another possible avenue of producing a six membered vinyl heterocycle would be to attempt the cyclisation with the Z-isomer **145**, as the different configuration of the double bond may allow greater success (Figure 13).

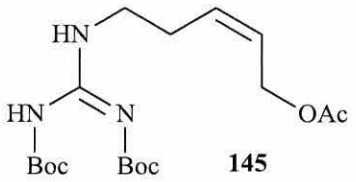
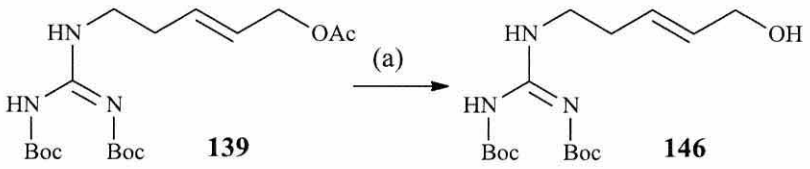


Figure 13: Z-isomer **145**, possible intermediate for cyclisation

Attempted synthesis of (Z)-tert-butyl 2-((tert-butoxycarbonyl)imino)-6-(1,2-dihydroxyethyl)tetrahydropyrimidine-1(2H)-carboxylate (148)

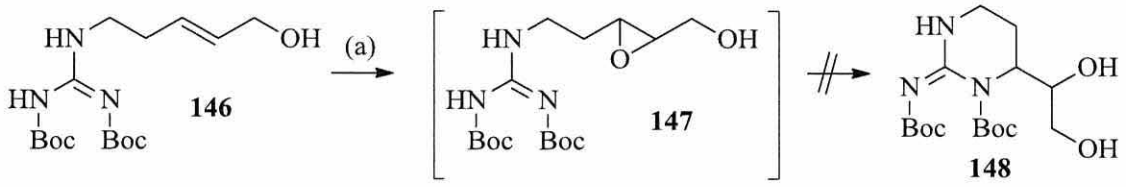
The failure of the cyclisation of **139** was disappointing however the alcohol **146** is a possible substrate for epoxidation based cyclisation. The alcohol was prepared in 89% yield by deprotection of the acetate **139** with anhydrous potassium carbonate in methanol at room temperature (Scheme 67).



Scheme 67: (a) K_2CO_3, Methanol, rt, 72 h.

The successful synthesis of **146** was confirmed by NMR, which showed the loss of the acetate signals seen in **139** and IR spectrometry, which showed the expected OH absorption at 3332 cm^{-1}.

To the alcohol **146** a fresh solution of DMDO in acetone was added at -30 °C, the solution was stirred at this temperature for a further 1 hour before being allowed to warm to room temperature and then stirred for a further 14 hours (Scheme 68).

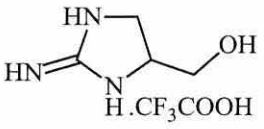


Scheme 68: (a) acetone, -30 °C, 1 h, then rt, 17 h

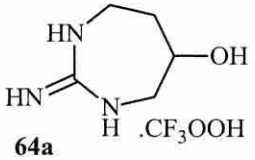
The reaction mixture was analysed by NMR spectroscopy, which showed signals consistent with **137** and but no signals that would be expected to be consistent exclusively of **139** were observed. The reaction was repeated at -30 °C for 2 hours before stirring at room temperature but again analysis of the reaction showed only the starting material **146** was present.

Biological testing

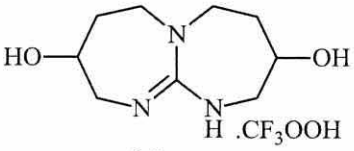
Biological testing of the guanidine **50, 64a, 64b, 69, 85, 86, 94a, 94b** and **134** was performed at Summit Plc Wales (now Phytoquest[tm]) using enzymatic assays to assess the inhibition of the activity of a range of glycosidase enzymes of the selected compounds.



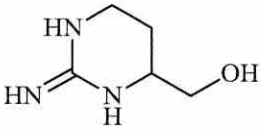
50



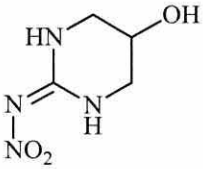
64a



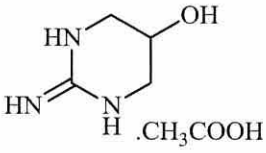
64b



69 CF_3COOH

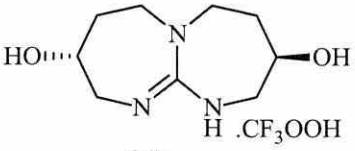


85

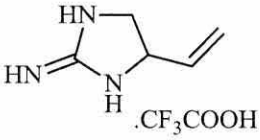


86

94a



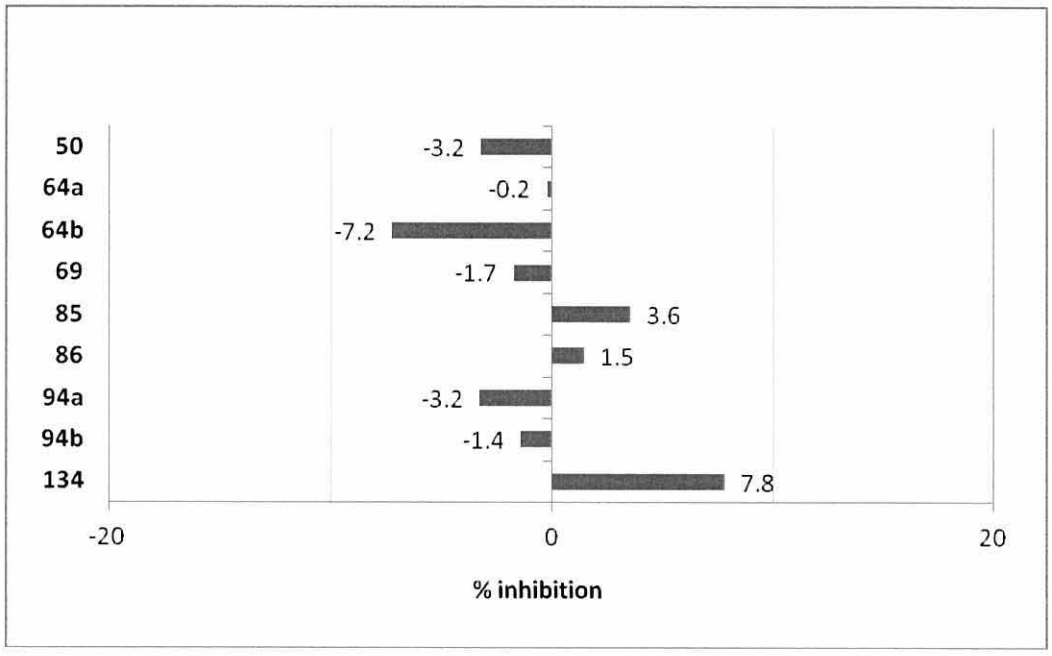
94b



134

Change in activity of α-D-glucosidase from *Saccharomyces cerevisiae*

All of the compounds tested showed little effect on the activity of α-D-glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20). The greatest inhibition of 7.8% was shown by vinyl guanidine **134.** The racemic bicyclic guanidine **64b** showed a similar activation of 7.2% but the R,R stereoisomer **94b** showed only 1.4% activation suggesting if any activation is taking place it's as a result of other stereoisomers of the bicyclic compound (Graph 1).

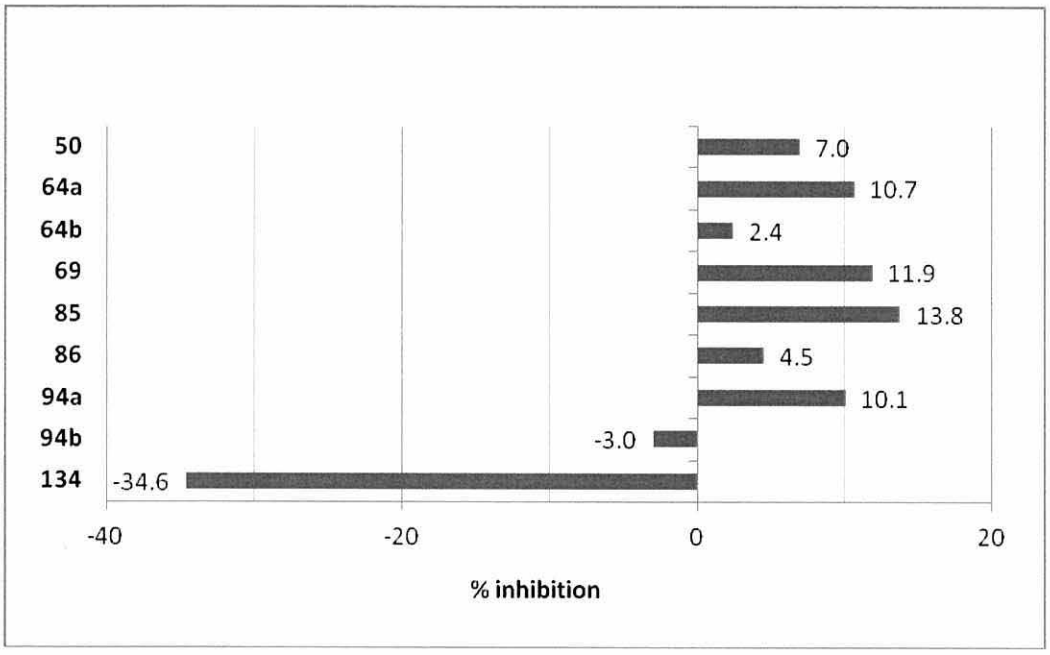


Graph 1: Change in activity of α-D-glucosidase from *Saccharomyces cerevisiae*

Change in activity of α-D-glucosidase from *Bacillus stearothermophilus*

The most notable result of testing on α-D-glucosidase from *Bacillus stearothermophilus* (EC 3.2.1.20) showed a 34.6% activation of the enzyme by **134**. The other compounds tested showed little activity with the majority showing a small amount of inhibition (<15%) with the strongest inhibition of 13.8% being shown by nitro guanidine **85**. It's non nitro counterpart **86** shows little inhibition at 4.5% indicates that the nitro group may be increasing binding (Graph 2).

The activation by the enzyme by **134** shows a strong contrast to the activity of the other compounds and may be an indication that the alkene group may have a significant effect on activity.

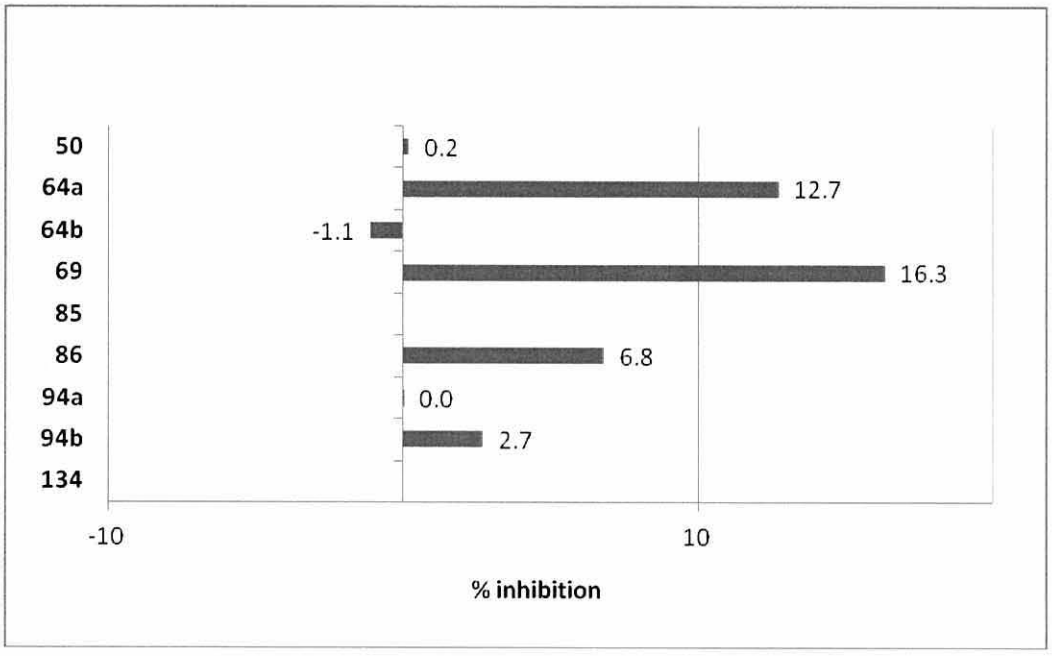


Graph 2: Change in activity of α-D-glucosidase from *Bacillus stearothermophilus*

Change in activity of α-D-glucosidase from Rice

When testing on α-D-glucosidase from Rice (EC 3.2.1.20) six membered heterocycle **69** showed the most inhibition at 16.3% with racemic **64a** showing slightly less at 12.7% interstingly the R enantiomer **94a** showed no inhibition suggesting the activity may be due to the S enantiomer exclusively. Preparation of the S enatiomer to test this hypothesis would be of interest in future work (Graph 3).

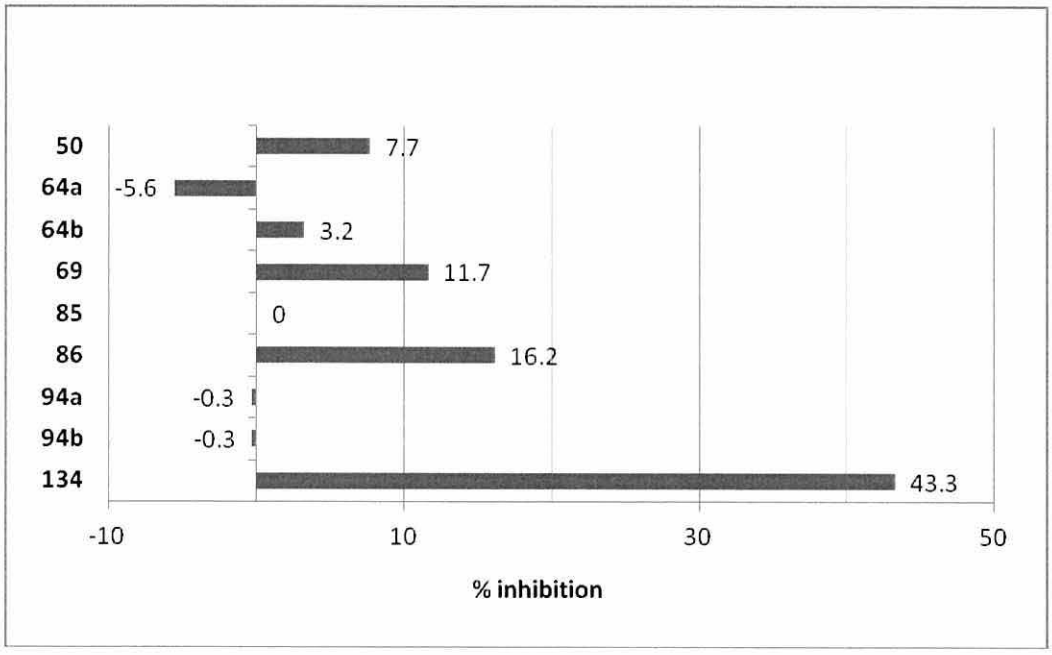
Compounds **85** and **134** were not tested.



Graph 3: Change in activity of α-D-glucosidase from Rice

Change in activity of β-D-glucosidase from Almond

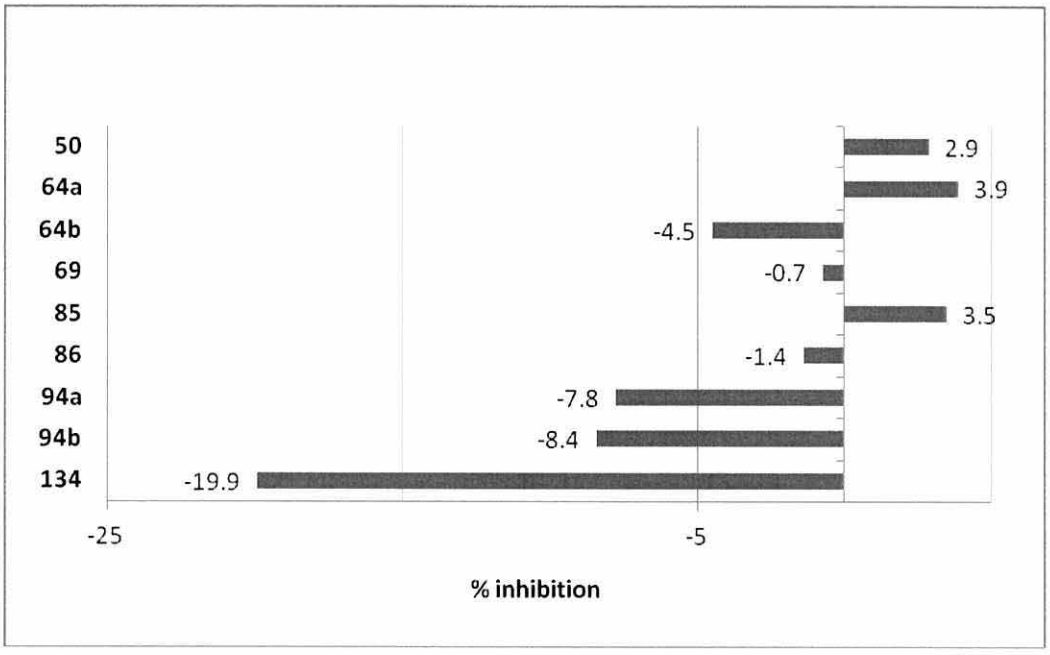
When tested with β-D-glucosidase from Almond (EC 3.2.1.21) vinyl guanidine **134** showed the strongest inhibition at 43.3%. Six membered heterocycle **86** showed 16.2% inhibition but its nitro derivative **85** had no inhibition indicating that in this case of the β-D-glucosidase the nitro group may have been preventing binding (Graph 4).



Graph 4: Change in activity of β-D-glucosidase from Almond

Change in activity of α-D-galactosidase from Green coffee bean

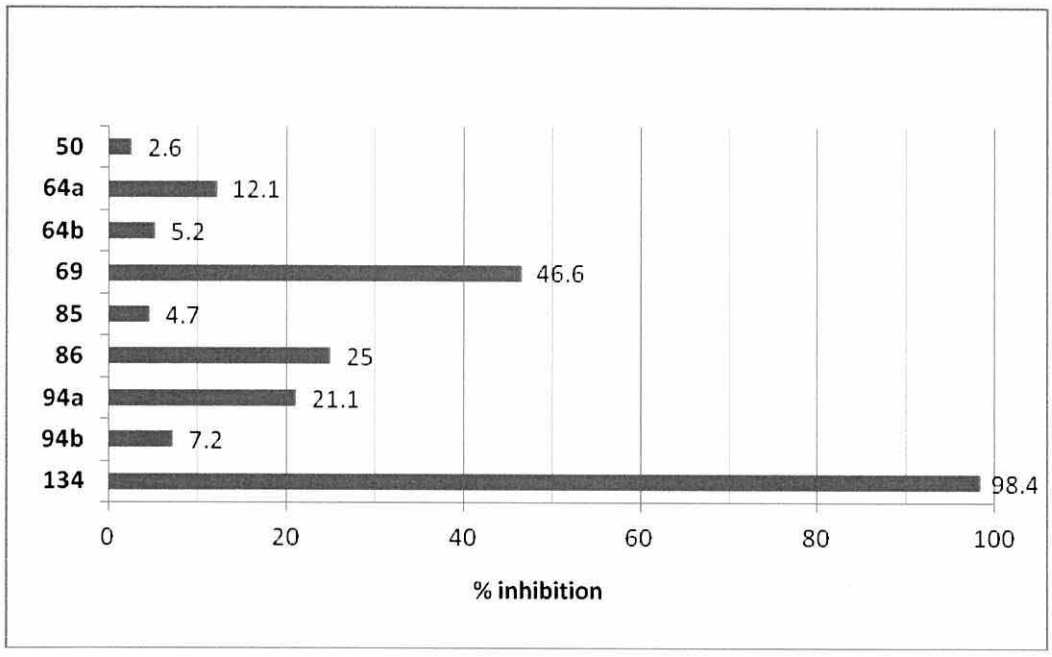
Many of the compounds showed activation of α-D-galactosidase from Green coffee bean (EC 3.2.1.22) with again the strongest effect being from **134** increasing activity by 19.9%. The R Seven membered heterocycle **94a** and R,R bicyclic **94b** also increased activity by 7.8% and 8.4% respectively. Interestingly the racemic seven membered **64a** showed weak inhibition suggesting again that the different enantiomers are differing in their interaction with the enzyme (Graph 5).



Graph 5: Change in activity of α-D-galactosidase from Green coffee bean

Change in activity of β-D-galactosidase from Bovine liver

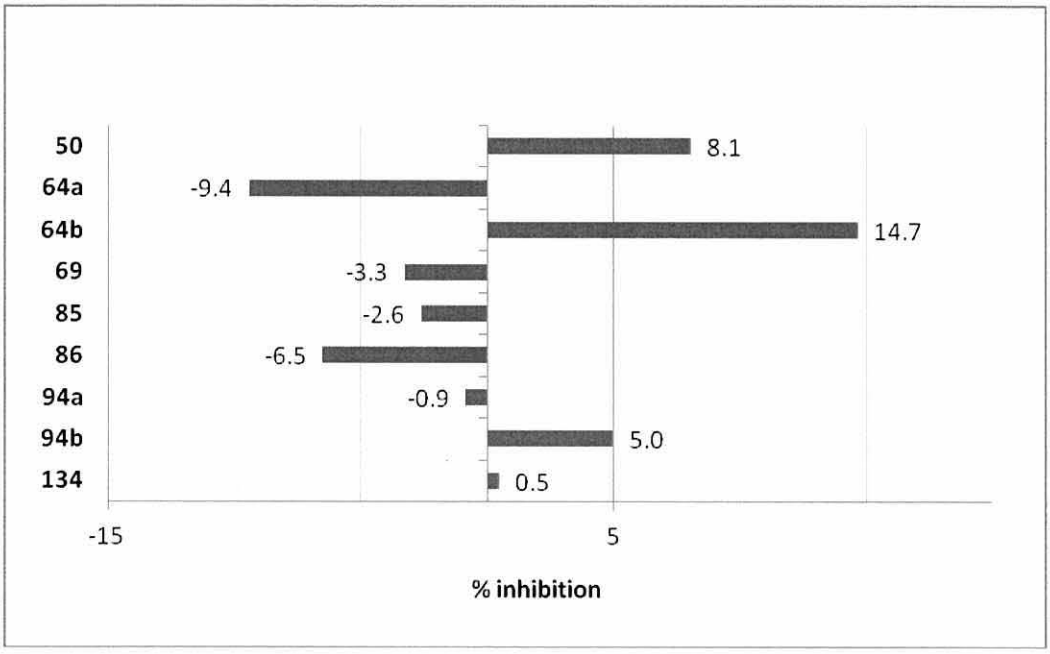
Previous work[44] had shown inhibition of β-D-galactosidase from Bovine liver (EC 3.2.1.23) by some guanidine heterocycles. All compounds tested showed some inhibition with the strongest inhibition by **134** inhibiting the enzyme by 98.4% and being found to have a IC_{50} of 68 μg mL^{-1}. Other compounds that showed a notable amount of inhibition were **69**, **86** and **94a**, which showed inhibitions of 46.6%, 25% and 21.1% respectively. Again there was a difference of inhibition shown regarding the R enantiomer **94a** (21.1% inhibition) and the racemic mixture **64a** (12.2% inhibition) indicating the differing activity due to stereochemistry (Graph 6).



Graph 6: Change in activity of β-D-galactosidase from Bovine liver.

Change in activity of α-D-mannosidase from Jack bean

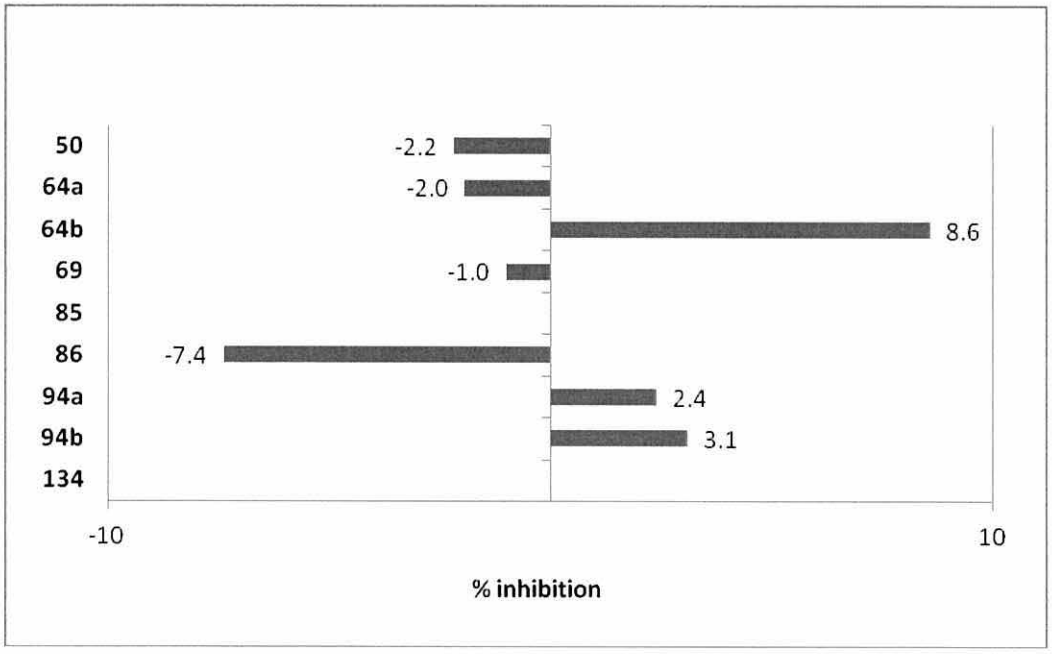
The compounds tested showed weak and varied effects on α-D-mannosidase from Jack bean (EC 3.2.1.24). The strongest inhibition was shown by bicyclic **64b** (14.7%) and **50** with an inhibition of 8.1%. The greatest activation were shown by **64a** (9.4%) and **86** (6.5%). Overall the compounds had little effect on the activity of the enzyme (Graph 7).



Graph 7: Change in activity of α-D-mannosidase from Jack bean

Change in activity of β-D-mannosidase from *Cellulomonas fimi*

β-D-mannosidase from *Cellulomonas fimi* (EC 3.2.1.25) showed little change in activity when tested. The strongest inhibition was by **64b** with an inhibition of 8.6% and the strongest activation was shown by **86** with an enhancement of activity of 7.4% (Graph 8). Compounds **85** and **134** were not tested.

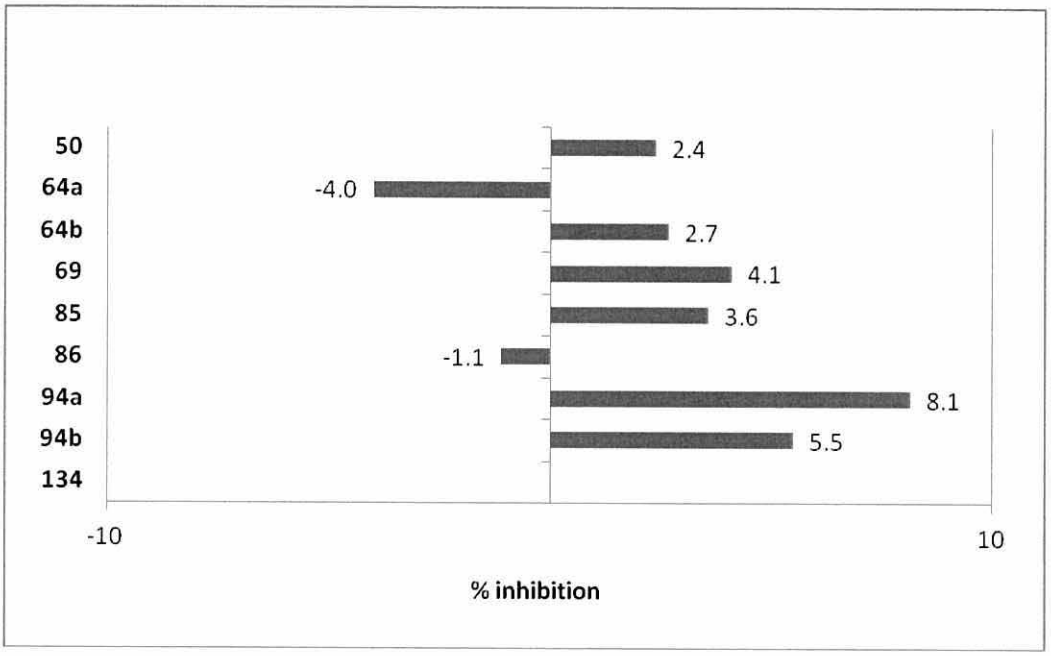


Graph 8: Change in activity of β-D-mannosidase from *Cellulomonas fimi*

Change in activity of Naringinase from *Penicillium decumbens*

When the compounds were tested Naringinase from *Penicillium decumbens* (EC 3.2.1.40) little change in activity was observed. All compounds tested gave less than 10% inhibition or activation with the strongest activity shown by R seven membered heterocycle **94a** (Graph 9).

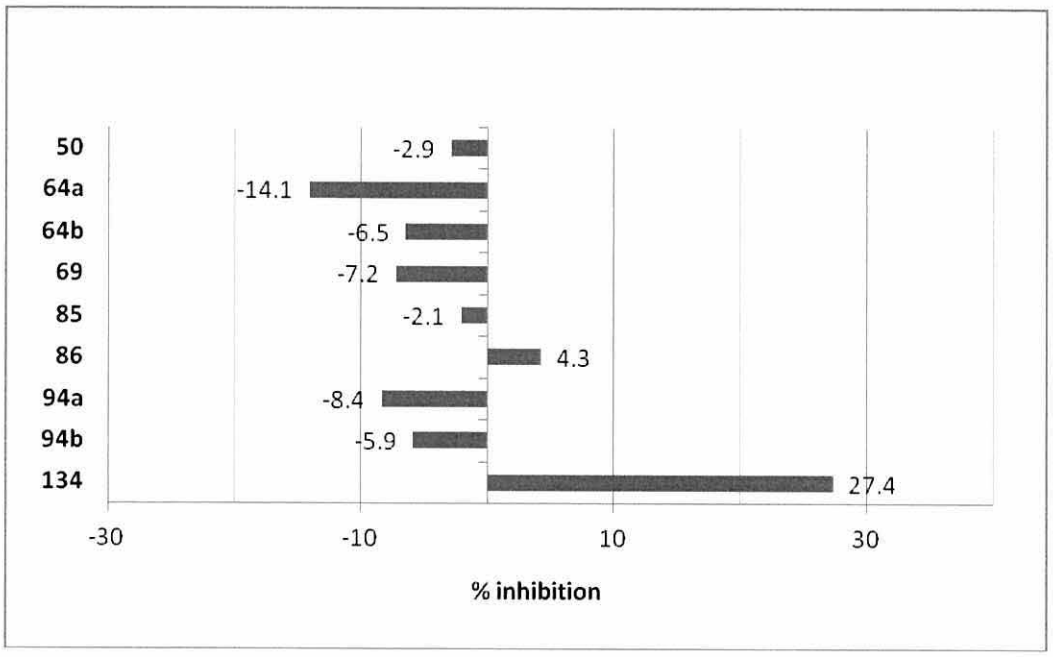
Compound **134** was not tested.



Graph 9: Change in activity of Naringinase from *Penicillium decumbens*

Change in activity of N-acetyl-β-D-glucosaminidase from Bovine kidney

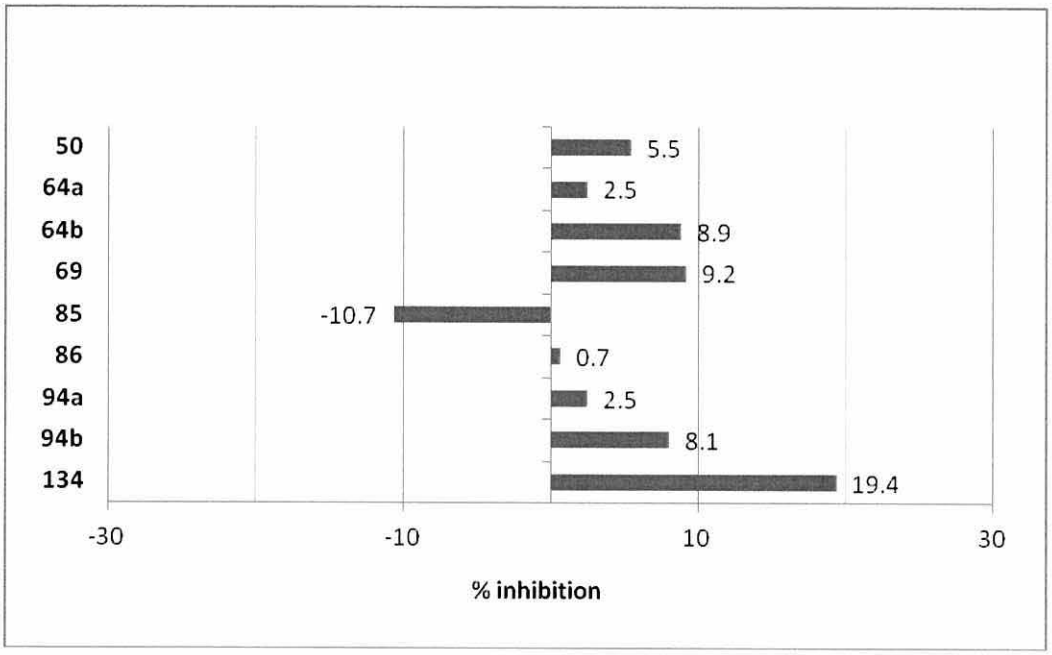
Testing on N-acetyl-β-D-glucosaminidase from Bovine kidney (EC 3.2.1.52) showed the most inhibition from compound **134** inhibiting enzyme activity by 27.4%. The other compounds tested showed little effect on the enzyme with some activation seen by 7 membered **64a** and **64b** enhancing enzyme activity by 14.1% and 8.4% respectively (Graph 10).



Graph 10: Change in activity of N-acetyl-β-D-glucosaminidase from Bovine kidney

Change in activity of N-acetyl-β-D-glucosaminidase from Jack bean

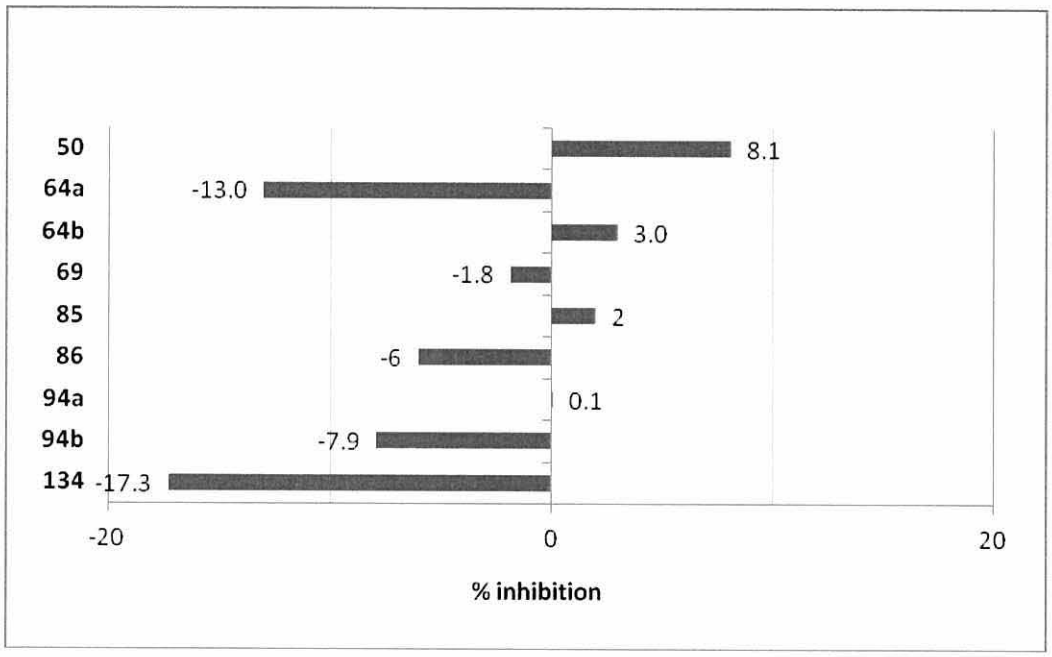
When tested with of N-acetyl-β-D-glucosaminidase from Jack bean (EC 3.2.1.52) the compound that showed the greatest effect was once again **134** showing an inhibition of 19.4%. The other compounds showed weak inhibition <10% except for nitro guanidine **85** which showed a weak activation of 10.7% (Graph 11).



Graph 11: Change in activity of N-acetyl-β-D-glucosaminidase from Jack bean

Change in activity of N-acetyl-β-D-hexosaminidase from *Aspergillus oryzae*

N-acetyl-β-D-hexosaminidase from *Aspergillus oryzae* (EC 3.2.1.52) when tested showed some mild activation (17.3%) when exposed to **134**. Some activation (-13%) was also observed with **64a**, interestingly the R enantiomer **94** showed no change in the activity indicating that the S enantiomer maybe effecting the enzyme exclusively. The other compounds tested showed very weak activation > 8% except for **50** which showed some weak inhibition of 8.1% (Graph 12).

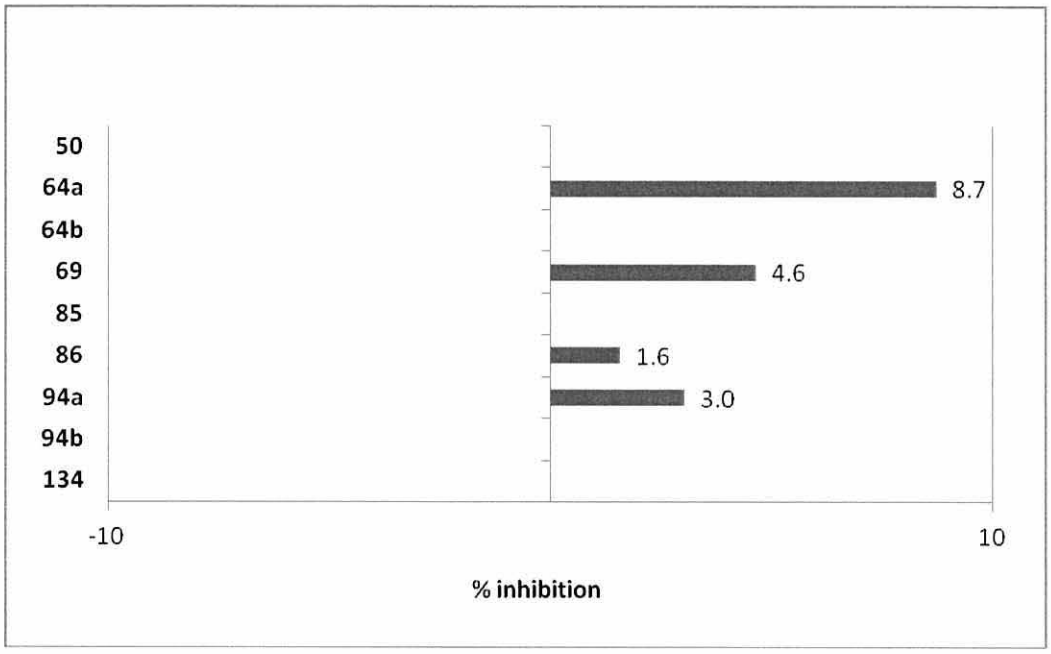


Graph 12: Change in activity of N-acetyl-β-D-hexosaminidase from *Aspergillus oryzae*

Change in activity of Amyloglucosidase from *Aspergillus niger*

While only of the compounds prepared were tested it appears that guanidine heterocycles has little effect on Amyloglucosidase from *Aspergillus niger* (3.2.1.3) activity. When tested with the compounds available some weak inhibition of <10% was observed (Graph 13).

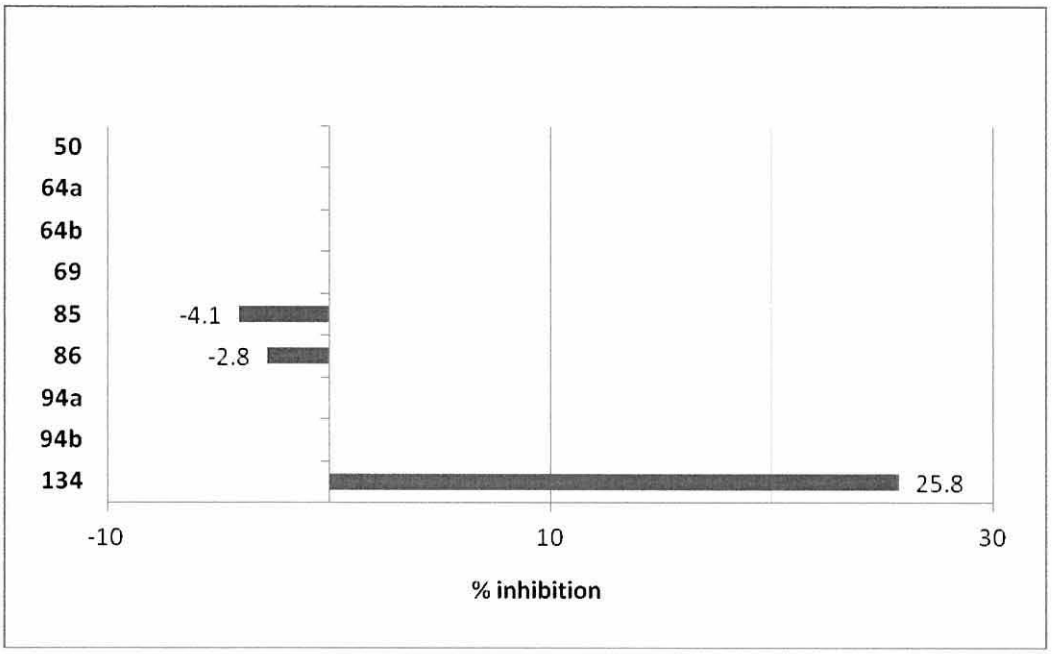
Compound **50**, **64b**, **85**, **94b** and **134** were not tested.



Graph 13: Change in activity of Amyloglucosidase from *Aspergillus niger*

Change in activity of β-glucuronidase from Bovine liver

Due to availability only three of the compounds were tested for activity with β-glucuronidase from Bovine liver (3.2.1.31). Again **134** showed the greatest inhibition of 25.8% while **85** and **86** showed weak activation of <5% (Graph 14)



Graph 14: Change in activity of β-glucuronidase from Bovine liver

Summary of results from enzymatic assays

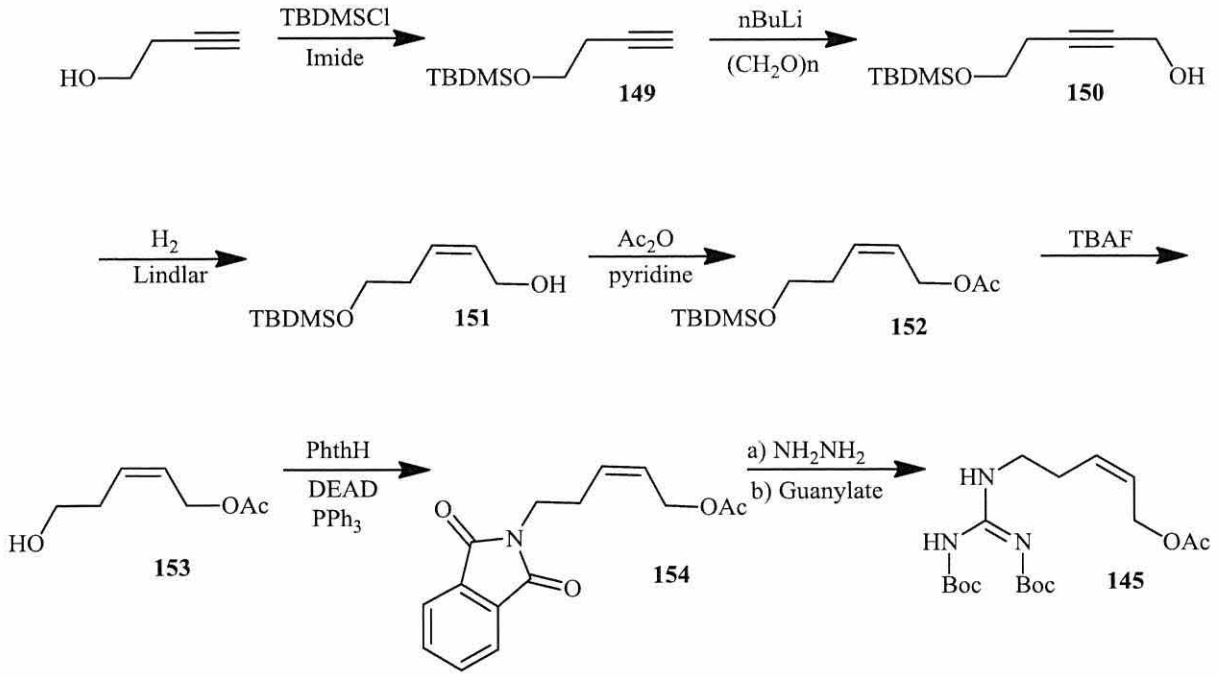
The enzymatic testing showed little inhibition (< 25%) in most of the compounds with most inhibition being seen against β-D-Galactosidase from bovine liver, which was not surprising considering the results from previous work by the group. The newly synthesised five membered vinyl **134** showed the strongest inhibition against this enzyme showing 98% inhibition at 1 mg/mL concentration and showing a IC_{50} of 68 μg mL^{-1}.

. There was also some interesting increase in some enzyme activity suggesting that some of these compounds can act as mild enzymatic activators the strongest activation was by compound **134** on α-D-galactosidase from *Bacillus sterothermophilus*, which increased the enzymes activity by 34.6%.

The results also showed an interesting difference between the effects of the racemic mixures **64a** and **64b** compared to the pure enantiomers **94a** and **94b**. This observation is not altogether surprising because of the known stereoselective nature of enzymes and would suggest that developing stereospecific syntheses would be worthwhile venture in any future work.

Overall Conclusions and future work

In this project a number of cyclisation methods have been utilised to prepare guanidine heterocycles. These methods such as epoxidation followed by intra- and intermolecular cyclisation and Pd mediated cyclisation have shown to be useful in preparing 5-, 6- and 7-membered guanidine heterocycles with alcohol and vinyl functional groups. The heterocycles with a vinyl side chain may be especially useful as intermediates can be used to generate larger and more complex molecules. It is interesting to note that the *E*-alkenes **131** and **137** and failed to cyclise with both DMDO and Pd mediated cyclisations yielding starting material. A possible alternative route would be to produce the Z-alkene **145**, synthesis would begin with 3-but-yn-1-ol being first silylated to give **149.** This would be converted to the alcohol **150** by metallation with n-BuLi and subsequent reaction with paraformaldehyde. Acetylation with pyridine and acetic anhydride followed by deprotection with TBAF would give alcohol **153**. Reaction of alcohol **153** with phthalimide in the presence of PPh_3 and DEAD would give protected amine **154**. Reaction of **154** with hydrazine hydrate to remove the phthalimide protecting group and sequential treatment with triethylamine and the guanylating agent **89** would yield the guanidine **145** (Scheme 69).



Scheme 69: Proposed Synthesis of *E*-alkene intermediate

Experimental

REAGENTS

Reagents were obtained from commercial suppliers and were used without further purification. Rochelle salt[65] was prepared by dissolving KOH (10.66 g, 0.27 mol) and NaOH (14.92 g, 0.27 mol) in water (100 mL) before tartaric acid (40.00 g, 0.27 mol) was added.

SOLVENTS

All solvents used in reactions were purified using methods described in the literature[66]. Diethyl ether and tetrahydrofuran were distilled from benzophenone and sodium wire, whilst dichloromethane was dried over CaH and freshly distilled. DMF, chloroform and methanol were purchased as dry from Aldrich. Petrol refers to the fraction distilled between boiling range of 40-60 °C.

CHROMATOGRAPHY

TLC was performed on glass plates coated with kieselgel 60 F254 (Art. 5554; Merck) with eluent specified in each case. The eluent percentage refers to a solution of the more polar solvent in the least polar solvent. Compounds were visualised using ultraviolet light and/or iodine or stained using polyphosphomolybdic acid (PMA) in EtOH or vanillin in EtOH/H_2SO_4, with heating.[67,68] Column chromatography was performed using Merck 7736 silica gel (particle size 40 – 63 μm) under medium pressure with the eluent specified in each case.

ANALYTICAL METHODS

Melting points were recorded with a Gallenkamp MF370 apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer 1600 FTIR spectrometer as thin film or solution as appropriate with the solvent quoted in each case. Absorption

frequencies are reported in wavenumber ν, whose unit is the reciprocal centimetre (cm^{-1}). Electron impact (EI) and chemical ionisation (CI) were recorded on a VG Masslab Model 12/253 spectrometer and high resolution mass spectra (HRMS) on a VG Analytical ZAB-E spectrometer at the EPSRC Mass Spectrometry Service Centre at Swansea. Mass measurements are reported in Daltons. Routine NMR samples were performed on a Bruker AC250 spectrometer, ^{1}H at 250 MHz and ^{13}C at 62.5 MHz, with samples made up in $CDCl_3$ solvent unless stated otherwise. Detailed spectra of products were performed on a Bruker Avance-500 spectrometer, ^{1}H at 500 MHz and ^{13}C at 125 MHz, made up in $CDCl_3$ solvent unless stated otherwise. Chemical shifts are reported as δ values (ppm) relative to tetramethylsilane as an internal standard. Spin couplings are denoted as J values (Hz), whilst splitting patterns are reported as singlet (s), doublet (d), triplet (t), quartet (q), pentet (pent) multiplet (m), broad (br) or any combination of these.

MISCELLANEOUS

All non-aqueous reactions were performed using oven dried glassware (250 °C) and were conducted under a positive atmosphere of argon. All new compounds were homogeneous by TLC (unless otherwise stated). Solids were purified by either recrystallisation or chromatography, whilst liquids and oils were purified either by chromatography or distillation. The term 'dried' refers to the treatment of a solution of the compound with anhydrous magnesium sulfate. The term under vacuum refers to the reduced pressure of a Büchi rotary evaporator, at water pump pressure (14 mm Hg) at 30-50 °C, or at 1 mm Hg at 25 °C for higher boiling solvents. All yields quoted are for the purified compounds (unless otherwise stated).

Biological testing methodology

Biological testing of the guanidine **50, 64a, 64b, 69, 85, 86, 94a, 94b** and **134** was performed at Summit Plc Wales (now Phytoquest^{tm}) using enzymatic assays to assess the inhibition of the activity of a range of glycosidase enzymes of the selected compounds.

Reagents

All enzymes and substrates were bought from Sigma, with the exception of b-mannosidase and glucose detection reagent which were purchased from Megazyme. Disodium hydrogen phosphate, citric acid, sodium hydroxide and glycine were purchased from Merck, and amylopectin was bought from Sigma (Fluka).

Reagent preparation

Enzyme solutions were made using 0.2 M MacIlvaine (citrate-phosphate) buffers, at the pH values recommended by the manufacturer. PNP-pyranoside substrates were made as 5 mM solutions, using a buffer solution appropriate to the enzyme used in the reaction. 0.1% (w/v) amylopectin solution was made using the same type of buffer, at the pH recommended for amyloglucosidase. Glucose detection reagent was made according to the manufacturer's instructions.

Enzyme concentrations used in the assays were determined using serial dilutions of each enzyme solution (from 1 U mL^{-1}) in incubations of 5 – 20 minutes at 27 ^{o}C. Concentrations were chosen which produced absorbance readings of 0.3 – 1.2 units after 10 minutes, although in some cases, 20 minutes were required. Linearity of the reaction at chosen enzyme concentrations was confirmed using a series of incubation times. Linearity of the time course of the reaction between the glucose detection reagent and glucose was confirmed using a glucose standard.

Glycine solution (0.4 M) was adjusted to pH 10.4 using concentrated sodium hydroxide solution, added dropwise.

Assay method

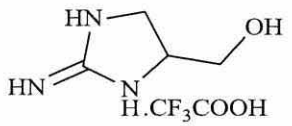
The incubation mixture for assays using PNP-pyranoside substrates consisted of 10 ul of enzyme solution, 10 ul of 1 mg mL^{-1} test compound solution (or dH_2O for control reactions), and 50 ul of 5 mM substrate solution. Reactions were incubated at 27 – 29 °C for 10 or 20

minutes, after which the reactions were stopped by the addition of 70 ul of glycine solution. Absorbance at 405 nm was read against control blanks.

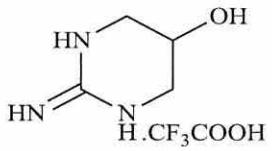
Amyloglucosidase assays were carried out using a reaction mixture consisting of 10 ul of enzyme solution, 10 ul of 1 mg mL^{-1} test compound solution (or dH_2O for control reactions) and 50 ul of 0.1% (w/v) amylopectin solution. Reactions were incubated for 10 minutes at 37 ^{o}C, after which 100 µl of glucose detection reagent was added. The reaction was incubated for a further 10 minutes at 37 ^{o}C, and the absorbance at 500 nm was read against control blanks.

Assays were carried out in triplicate per test compound, and average absorbance values were calculated using values from the three replicates. Inhibition was calculated as the percentage difference between mean absorbance in the control and test reactions.

(2-Iminoimidazolidin-4-yl)methanol trifluroacetate 50 and hexahydro-2-iminopyrimidin-5-ol trifluroacetate 82[45]



50



82

Guanidine hydrochloride (1.68 g, 17.5 mmol, 1.2 eq) was added to dry *tert*-butanol (30 mL) and the mixture was stirred for 10 minutes. The solution was cooled to 5 °C, potassium *tert*-butoxide (1.8 g, 16.1 mmol, 1.1 eq) was added and the suspension was allowed to warm to rt with stirring. After 40 min epibromohydrin (1.26 mL, 14.6 mmol) was added slowly and the mixture was stirred at rt for 62 h. Further potassium *tert*-butoxide (1.8 g, 16.1 mmol) was added and the stirred mixture was heated to 60 °C for 24 h. The resultant white slurry was cooled (0 °C) and TFA (5 mL) in methanol (15 mL) was added slowly and the mixture stirred to rt over 30 min. After evaporation the crude product was purified by column chromatography on silica, eluting with a graduated solvent system of 100% chloroform to 100% methanol giving a mixture of the desired product **50** and **82** (1.87 g, 50% yield) as an 11:1 mixture which eluted in the 25% methanol in chloroform fractions. (**Rf.** 0.12 in 20% methanol/chloroform)

50[45]

δ_H (CD_3OD) 3.54 (1 H, dd, J = 6.0, 10.1, CH*H*), 3.57 (1 H, dd, J = 5.2, 12.1, C*H*H), 3.65 (1H, dd, J = 4.3, 12.1, CH_2), 3.76 (1 H, t, J = 10.1, CH_2), 4.13 (1 H, m, C*H*).

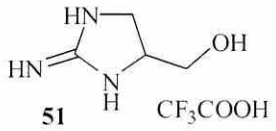
δ_C (CD_3OD) 46.2 (CH_2), 58.2 (CH_2), 64.0 (*C*H), 160.2 (*C*=N).

ν_{max} 3302 (br, O-H) 1550 (C=N) cm^{-1}.

82 (partial data)

δ_H (CD_3OD) 3.28 (2H, dd, J = 12.3, 3.8, 2x HC*H*), 3.43 (2H, dd, J = 12.3, 3.8 2x HC*H*). 4.22 (1H, m, C*H*).

(2-Imino-imidazolidin-4-yl)-methanol trifluoro-acetic acid salt 51



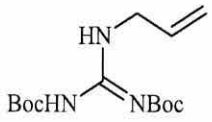
51

Protected guanidine **56** (134 mg, 0.425 mmol) was dissolved in dichloromethane (5 mL) and trifluroacetic acid (5 mL) was added dropwise. The solution was stirred for 4 h then the solvent removed under vacuum. Excess trifluroacetic acid was removed by adding dichlomethane followed by re-evaporation under vacuum three times to give **51** (96 mg, 0.419 mmols, 98% yield). The product displayed identical data to that previously prepared (page 86).

δ_H (CD_3OD) 3.54 (1 H, dd, J = 6.0, 10.1, CH*H*), 3.57 (1 H, dd, J = 5.2, 12.1, C*H*H), 3.65 (1 H, dd, *J* 4.3, 12.1, CH_2), 3.76 (1 H, dd, *J* 10.1, 10.1 CH_2), 4.13 (1 H, m, C*H*)

δ_C (CD_3OD) 46.2 (*C*H_2), 58.2 (*C*H_2), 64.0 (*C*H), 160.2 (*C*=N).

Allyl-*N'N''-bis*-(*tert*-butoxycarbonyl)-guanidine 52[54]



52

N,N'-bis-(tert-butoxycarbonyl)-1*H*-pyrazole-1-carboxamide **89** (2 g, 6.44 mmol) and allylamine **90** (0.87 mL, 0.66 g, 11.69 mmol, 1.8 eq) were dissolved in dry acetonitrile (10 mL) and left to stir at room temperature for 16 h. Solvent was removed under vacuum and purification by silica gel chromatography eluting with 5% ether in petrol gave the desired product **52** as a white solid (1.83 g, 95% yield).

Rf. 0.52 in 30% ethyl acetate : petrol.

Mpt 87-89 °C

δ_H 1.49 (9H, s, 3 x CH_3), 1.50 (9H, s, 3 x CH_3), 4.07 (2H, t, J = 5.4, CH_2), 5.16 (1H, d, J = 10.4, C*H*H), 5.23 (1H, d, J = 17.4, CH*H*), 5.88 (1H, ddt, J= 17.4, 10.4, 5.7, C*H*), 8.39 (1H, broad, N*H*), 11.51 (1H, broad, N*H*)

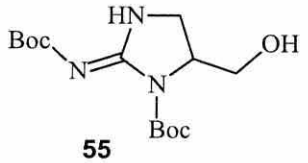
δ_C 28.0 (3 x CH_3), 28.3 (3 x CH_3), 43.2 (CH_2), 79.3 (*C*), 83.1 (*C*), 116.7 (CH_2), 133.3 (*C*H), 153.2 (C), 156.0 (C), 163.5 (*C*).

DMDO 53[48]

53

A mixture of sodium hydrogen carbonate (58 g, 700 mmol, 79.4 equiv) in 3:4 acetone: water (95 mL: 127 mL) was cooled in an ice bath before portion wise addition of Oxone® (6 x 12 g portions, 54.6 mmol) at 15 min intervals. The product solution was distilled under full-water aspirator vacuum into a liquid N_2 cooled collection flask until no further foaming was evident in the reaction vessel. After 90 minutes the remaining Oxone® (4 x 12 g, 18.2 mmol) was added to the solution and distillation commenced for another 90 minutes. The resulting yellow solution was dried with K_2CO_3, filtered and used immediately.

2-*tert*-Butoxycarbonylimino-5-hydroxymethyl-imidazolidine-1-carboxylic acid tert-butyl ester 55

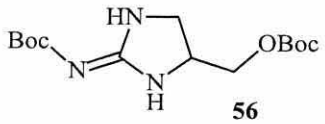


55

A 250 mL RBF containing allyl-*N'N''-bis*(*tert*-butoxycarbonyl)-Guanidine **52** as a powder (0.404 g, 1.35 mmol) was cooled (approx -35 °C) and a cooled (-30 °C) freshly prepared solution of dried acetone/DMDO (~100 mL) was added. The solution was stirred under argon for 2 hours at -25 °C, left to warm up to room temperature overnight and stirred for 6 days. The solvent was removed under vacuum at room temperature giving the desired product **56** as a white solid. (0.46 g) that was used in the next preparation without further purification.

Mpt	113-115 °C
δ_H	1.49 (9H, s, 3 x CH_3), 1.54 (9H, s, 3 x CH_3), 3.68 – 3.74 (2H, m, CH_2), 3.79-3.88 (2H, m, *CHH*), 4.24 (1H, m, *CH*)
δ_C	27.9 (6 x CH_3), 57.6 (*C*H), 62.1(CH_2), 65.7 (CH_2), 80.5 (*C*), 83.2 (*C*), 150.8 (*C*), 152.7 (*C*), 155.5 (*C*)
ν_{max}	3346 (s, NH), 2984, 2975 (s, CH) 1753, 1743 (s, C=O), 1600 (s, C=N), 1140(s, CO) cm^{-1}

Carbonic acid 2-*tert*-butoxycarbonylimino-imidazolidin-4-ylmethyl ester *tert*-butyl ester 56.



Guanidine **55** (110 mg, 0.35 mmol) was dissolved in chloroform (20 mL) and silica (1 g) was added. The suspension was stirred at room temperature for 72 h then filtered through a layer of sand on cotton wool which was washed with portions of chloroform and ethyl acetate. Evaporation of the solvent gave the product **56** (70 mg, 64% yield) which was recrystallised from chloroform by slow evaporation to give crystals suitable for X-ray analysis (Appendix).

Mpt 92-94 °C

δ_H 1.44 (9H, s, 3 x C*H*$_3$), 1.47 (9H, s, 3 x C*H*$_3$) 3.41 (1H, dd, J = 6.0, 9.8, C*H*), 3.73 (1H, app t, J = 9.8, C*H*), 4.09 (2H, app t, J = 5.1, CH_2), 4.17 (1H, m, C*H*).

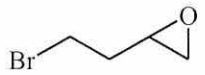
δ_C 27.6 (3 x CH_3), 28.2 (3 x CH_3), 55.8 (*C*H), 63.7 (CH_2), 67.4 (CH_2), 78.9 (*C*), 82.8 (*C*), 150.4 (C), 152.4 (C), 159.2 (C)

HRMS (EI) ([M+H]$^+$) *m/z*:

Found 316.1871, $C_{14}H_{26}N_3O_5$ requires 316.1867 Daltons.

X-ray crystallography **Appendix**

2-(2-Bromoethyl)oxirane 63[55]



63

A solution of 4-bromobut-1-ene **91** (14.3 g, 106.7 mmol) in dichloromethane (100 mL) was cooled (0 °C) and added to a cooled (0 °C) solution of *m*CPBA (35 g, 271.0 mmol, 2.5 eq) in dichloromethane (50 mL) then slowly warmed room temperature and stirred for 56 h. The resulting mixture was then filtered through a pad of $MgSO_4$ and silica and the filtrate washed with sodium metabisulfite (sat. aq., 4 x 50 mL), sodium bicarbonate (sat. aq., 4 x 50 mL) and water (100 mL). After drying ($MgSO_4$), filtration and evaporated under reduced pressure 2-(2-bromoethyl)oxirane **63** (8.3 g, 52%) was obtained as an oil and was used in the next step without further purification.

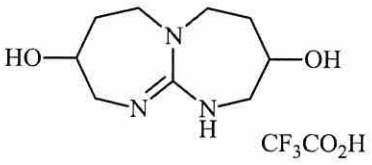
δ_H 2.09 (2 H, m, C*H*$_2$), 2.56 (1 H, m, C*H*), 2.82 (1 H, m, C*H*) 3.07 (1 H, m, C*H*), 3.50 (2 H, t, J = 6.7, C*H*$_2$)

δ_C 29.0 (*C*H$_2$), 35.7 (*C*H$_2$), 47.0 (*C*H$_2$), 50.1 (*C*H)

ν_{max} 2993 (CH), 1264 (CO) cm^{-1}

2-Imino-[1,3]diazepan-5-ol trifluoroacetate salt 64a and 2,3,4,5,6,7,8,9-Octahydro-1H-1,5a,10-triaza-heptalene-3,8-diol trifluoroacetate salt 64b.

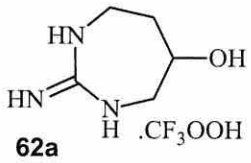
64a



64b

Guanidine hydrochloride (1.68 g, 17.5 mmol, 1.2 eq) was added to dry *tert*-butanol (24 mL) and the solution was cooled (5 °C). Potassium *tert*-butoxide (1.8 g, 16.1 mmol, 1.1 eq) was added and the suspension was stirred for 40 minutes. 2-(2-bromoethyl)oxirane **63** (2.18 g, 14.6 mmol) was then added drop wise over 5 min and the mixture stirred at room temperature for 62 h. A further portion of potassium *tert*-butoxide (1.8 g, 16.1 mmol, 1.1 eq) was added and the mixture heated to 60 °C and stirred for 28 h. The resultant white slurry was then cooled to 0°C and TFA (5 mL) in dry methanol (15 mL) was slowly added and the mixture warmed to room temperature and stirred for 30 min. After evaporation a mixture of methanol and chloroform (20 mL : 30 mL) was then added to the resultant solid the slurry obtained was stirred for 15 minutes. The slurry was then filtered through a pad of celite, which was washed with further methanol chloroform mix (10 mL : 15 mL) solution and the combined filtrates concentrated under reduced pressure to give the crude product as an oil. Purification by column chromatography using a gradient solvent system of 100% chloroform to 100% methanol. **2-Imino-[1,3]diazepan-5-ol trifluoroacetate salt 64a** (1.23g, 35%) and **2,3,4,5,6,7,8,9-Octahydro-1H-1,5a,10-triaza-heptalene-3,8-diol trifluoroacetate salt 64b** (0.21g, 5%) were detected by TLC in the 50% methanol in chloroform and the 30% methanol in chloroform fractions respectively.

2-Imino-[1,3]diazepan-5-ol trifluoroacetate salt 64a



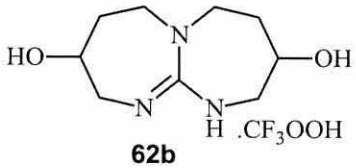
62a

R_f 0.12 (20% MeOH:$CHCl_3$)

δ_H (CD_3OD) 1.87-2.24 (2H, m, CH_2), 3.39-3.66 (4H, m, 2 x CH_2), 4.56 (1H, m, 1 x CH)

δ_C (CD_3OD) 36.9 (CH_2), 48.8 (CH_2), 58.7 (CH_2), 73.3 (CH), 156.3 (C=N).

2,3,4,5,6,7,8,9-Octahydro-1H-1,5a,10-triaza-heptalene-3,8-diol trifluoroacetate salt 64b



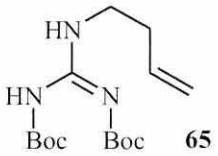
62b

R_f 0.18 (20% MeOH:$CHCl_3$)

δ_H (CD_3OD) 2.00-2.25 (4H, m, 2 x CH_2), 3.56-3.86 (8H, m, 4 x CH_2), 4.55 (2H, m, 2 x CH).

δ_C (CD_3OD). 35.1/35.3 (2 x CH_2), 49.6 (CH_2), 58.7/59.8 (CH_2), 71.4 (CH), 155.8 (C).

***N,N'-bis*-Boc-*N"*-but-3-enyl-guanidine 65**[48]



N,N'-bis-(tert-butoxycarbonyl)-1*H*-pyrazole-1-carboxamide (4.0 g, 13 mmol) and but-3-en-1-amine hydrochloride **93** (4.86 g, 45.2 mmols) were dissolved in dry acetonitrile (50 mL) and triethylamine (3.6 mL, 49.1 mmol) was added drop wise and the mixture stirred overnight. The mixture was evaporated onto silica gel (ca 20 g) and purified by column chromatography, eluting with 10% EtOAc in petrol to give **65** as a white solid (3.31 g, 80%)

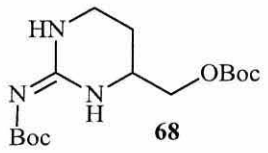
R_f 0.51 in 30:70 EtOAc : petrol).

Mpt. 81-83 °C

δ_H 1.50 (9H, s, 3xCH_3), 1.52 (9H, s, 3xCH_3), 2.33 (2H, dt, *J* 6.8, 7.0, C*H*$_2$), 3.51 (2H, t, 7.0), 5.16 (2H, m, C*HH*), 5.80 (1H, ddt, *J* 17.1, 10.4, 6.8, C*H*), 8.36 (1H, broad, NH) 11.50 (1H, broad, NH)

δ_C 28.0 (3 x *C*H_3), 28.3 (3 x *C*H_3), 33.1 (CH_2), 40.0 (CH_2), 79.2 (*C*), 82.9 (*C*), 117.5 (CH_2), 134.7 (*C*H), 153.2 (C), 156.1 (C), 163.6 (*C*)

Carbonic acid 2-tert-butoxycarbonylimino-hexahydro-pyrimidin-4-ylmethyl ester tert-butyl ester 68 [48]



68

Solid guanidine **65** (1.02 g, 33 mmol) was placed in a 250 mL RBF and cooled to – 25°C for 30 minutes, whereupon a pre-cooled (-30 °C) solution of freshly prepared DMDO in acetone (~100 mL) was added and the solution stirred for 4 hours at – 25°C. The reaction was allowed to reach room temperature overnight then stirred for 3 days. After evaporation and column chromatography eluting with EtOAc and 10% MeOH in EtOAc the compound **68** (0.88 g) was isolated in 82% yield as a pale yellow waxy solid.

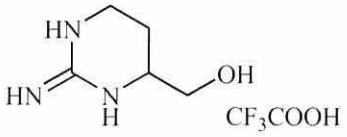
R_f 0.90 in 40% MeOH in EtOAc.

Mpt 160 -162 °C (lit. Mpt.[48], Mpt 160 –161°C)

δ_H (CD_3OD). 1.50 (9H, s, 3 x CH_3) 1.52 (9H, s, 3 x CH_3), 1.86 (1H, m), 2.01 (1H, m, CHH), 3.48(1H, m, CHH), 3.83 (1H, m, CHH), 4.05 (1H, dd, J = 6.75, 11.0, CHH), 4.26 (1H, dd, 4.88 Hz, J = 11, CH).

δ_C (CD_3OD) 25.8 (CH_2), 30.5 (3 x CH_3), 31.2 (3 x CH_3) 40.4 (CH_2), 52.6 (CH), 71.7 (CH_2), 82.9 (*C*), 86.0 (*C*), 156.3 (*C*), 157.2 (C), 160.6 (*C*)

6-(hydroxymethethyl)-1,4,5,6-tetrahydropyrimidin-2-aminium trifluoroacetate 69 [48]



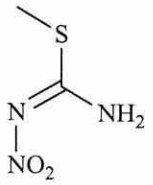
69

68 (38.2 mg, 0.116 mmol) was dissolved in chloroform (4 mL), trifluoroacetic acid (1 mL) was added and the solution was stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure and the resulting residue evaporated three times with chloroform to remove residual TFA. Purification by column chromatography on silica gel eluting with dichlomethane followed by 10% methanol in dichloromethane gave the desired product **69** (18.5 mg, 0.076 mmol, 66%) as a glass.

δ_H (CD_3OD) 1.75 (1H, m), 2.0 (1H, m), 3.32 (1H, m), 3.41 (1H, dt, J = 12.6, 5.1 Hz), 3.48 (1H, dd, J = 10.1, 7.25 Hz), 3.52 (1H, m), 3.68 (1H, dd, J = 10.1, 4.05 Hz).

δ_C (CD_3OD) 23.3 (CH_2), 38.1 (CH_2), 52.0 (CH), 65.0 (CH_2), 155.8 (C)

2-Methyl-1-nitro-2-thiopseudourea 83[52]



83

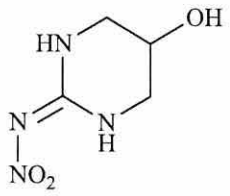
To a stirring solution of concentrated nitric acid (69%. 5 mL) and concentrated sulphuric acid (98%, 40 mL) cooled to – 10 °C, 2-Methyl-2-thiopseudourea sulphate **87** (5g, 17.9 mmol) was added portion wise over 10 minutes. The resulting yellow solution was stirred for 30 minutes at – 5 °C before being poured into ice (250 g) giving a white precipitate. After all the ice had melted the white slurry was filtered, the solid washed with water (40 mL) and left to air dry on the funnel. The crude solid was recrystallised from a mixture of ethanol (30 mL) and water (60 mL) giving the product **83** as a light white solid (2.32 g, 96 % yield).

Mpt = 159 – 161 °C, lit mpt[52] = 160 - 161 °C.

δ_H (CD_3OD) 2.46 (3H, s, CH_3)

δ_C (CD_3OD) 13.8 (CH_3), 174.1 (C)

5-Hydroxy-2-nitrimino-1, 3-diazacyclohexane 85[50]



85

2-Methyl-1-nitro-2-thiopseudourea **83** (2.50 g, 18.5 mmol) was added to a solution of 1,3-diaminopropan-2-ol **84** (1.67 g, 18.5 mmol) in ethanol (50 mL) and the mixture heated to reflux which results in the solid present dissolving. After 1 h at reflux a white precipitate began to form and the mixture was refluxed for a further 1 h. After being cooled to room temperature the precipitate was isolated by filtration, washed with ethanol (10 mL) and dried under vacuum with phosphorus pentoxide to constant weight. The guanidine **85** was obtained as a white solid (0.473 g, 16 % yield)

Mpt 230 – 233 °C, lit mpt[50] = 233 - 235 °C

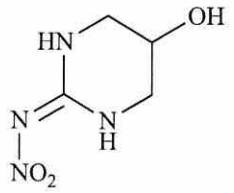
δ_H (D_2O). 3.42 – 3.46 (2H, dd, *J* 12.9, 2.85, 2x HC*H*), 3.52 – 3.57 (2H, dd, *J* 12.9, 2.85, 2x *H*CH), 4.38 (1H, pentet, *J* 2.85 Hz, C*H*)

δ_C (D_2O) 44.2 (CH_2), 58.1 (*C*H)

HRMS (EI) (M+) m/z:

Found 160.0589, $C_4H_8N_4O_3$ requires 160.0591 Daltons.

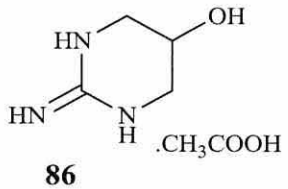
5-hydroxy-2-nitrimino-1, 3-diazacyclohexane from **nitroguanidine 85**[50]



85

Nitroguanidine **88** (1.50 g, 16.6 mmol) was added to a solution of 1,3-diaminopropan-2-ol **84** (2.30 g, 16.6 mmol) in water (60 mL) and the mixture was stirred at 70 °C for 2 h. The reaction mixture was then cooled to room temperature and stirred for a further 12 h to give a white precipitate. The reaction mixture was cooled to 0 °C and the precipitate collected by filtration and washed with chilled water (15 mL). The solid was then air-dried then dried under vacuum with phosphorus pentoxide to constant weight. The guanidine **85** was obtained as a white solid (0.52 g, 27 % yield). Data for **85** was identical to the sample prepared previously (page 99)

Hexahydro-2-iminopyrimidin-5-ol acetate 86[51]



86

85 (0.473 g, 2.9 mmol) was dissolved in aqueous acetic acid (15 %, 50 mL) and Pd/C (5%, 0.06 g) was added and the mixture stirred under H_2(balloon) for 72 h. The mixture was filtered through a Celite™ pad and the solvent removed under vacuum giving the product as a cream coloured solid. Further purification by silica chromatography eluting with 5% - 20% methanol in chloroform with a few drops of acetic acid added to the 15% - 20% methanol mixtures gave the desired product **86** as a white solid (0.388 g, 75% yield).

Mpt 61 – 62 °C

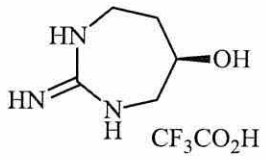
δ_H (D_2O) 3.42 – 3.46 (2H, dd, J = 12.9, 2.85 Hz, 2x HC*H*), 3.52 – 3.57, (2H, dd, J = 12.9, 2.85 Hz, 2x *H*CH), 4.38 (1H, pent, J = 2.85 Hz, C*H*).

δ_C (D_2O). 23.21 (CH_3), 43.81 (CH_2), 58.65 (*C*H), 153.61 (*C*=N), 181.49 (*C*=O)

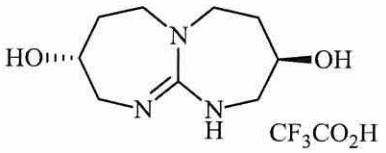
HRMS (EI) (M^+) *m/z*:

Found 116.0817, $C_4H_{10}N_3O_1$ requires 116.0818 Daltons.

***(R)*-2-Imino-[1,3]diazepan-5-ol trifluoroacetate salt 94a and *(R,R)*-2,3,4,5,6,7,8,9-Octahydro-1H-1,5a,10-triaza-heptalene-3,8-diol trifluoroacetate salt 94b**



94a



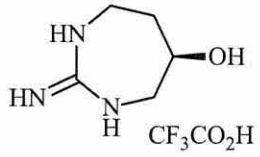
94b

Guanidine hydrochloride (1.2 g, 12.5 mmol) was added to dry *tert*-butanol (20 mL) and the solution was cooled (5 °C). Potassium *tert*-butoxide (1.35 g, 12.5 mmol) was added and the suspension was stirred for 30 minutes. Epoxide **92**[69] (2.9 g, 17.6 mmol) in dry *tert*-butanol (20 mL was then added drop wise over 5 min and the mixture stirred at room temperature for 18 h. A further portion of potassium *tert*-butoxide (1.35 g, 12.5 mmol) was added and the mixture heated to 60 °C and stirred for 24 h. The resultant white slurry was then cooled to 0 °C and TFA (5 mL) in dry methanol (15 mL) was slowly added and the mixture warmed to room temperature and stirred for 20 min. After evaporation a mixture of methanol and chloroform (20 mL : 30 mL) was then added to the resultant solid the slurry obtained was stirred for 15 minutes. The slurry was then filtered through a pad of celite, which was washed with further methanol chloroform mix (10 mL : 15 mL) solution and the combined filtrates concentrated under reduced pressure to give the crude product as an oil. Purification was effected by column chromatography using a gradient solvent system (100% $CHCl_3$, then increasing polarity incrementally by the addition of MeOH; 5%, 10%, 15%, 20%, 30%, 50%, 70% 100%). ***(R)*-2-Imino-[1,3]diazepan-5-ol trifluoroacetate salt 94a** and ***(R,R)*-2,3,4,5,6,7,8,9-Octahydro-1H-1,5a,10-triaza-heptalene-3,8-diol trifluoroacetate salt 94b** were detected by TLC in the 50% MeOH in $CHCl_3$ and the 30% MeOH in $CHCl_3$ fractions respectively.

***(R)*-2-Imino-[1,3]diazepan-5-ol trifluoroacetate salt 94a** was isolated to yield (1.13 g, 37%) and ***(R,R)*-2,3,4,5,6,7,8,9-Octahydro-1H-1,5a,10-triaza-heptalene-3,8-diol trifluoroacetate**

salt 94b was isolated to yield (0.35 g, 9%)

***(R)*-2-Imino-[1,3]diazepan-5-ol trifluoroacetate salt 94a**

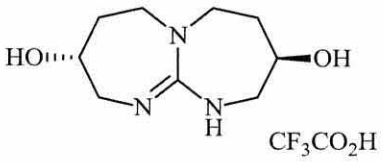


R_f 0.12 (20% MeOH:$CHCl_3$)

δ_H (CD_3OD) 2.00-2.40 (2H, m, 2 x CH), 3.30-3.45 (2H, m, CH_2), 3.50-3.70 (2H, m, CH_2), 4.50-4.55 (1H, m, CH)

δ_C (CD_3OD) 35.2 (CH_2), 47.1 (CH_2), 57.0 (CH_2), 71.7 (CH), 157.4 (C)

***(R,R)*-2,3,4,5,6,7,8,9-Octahydro-1H-1,5a,10-triaza-heptalene-3,8-diol trifluoroacetate salt 94b**

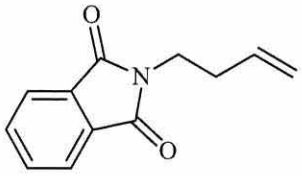


R_f 0.18 (20% MeOH:$CHCl_3$)

δ_C (CD_3OD) 3.95-2.30 (4H, m, 2 x CH_2), 3.40-3.75 (8H, m, 4 x CH_2), 4.45-4.50 (2H, m, 2 x CH)

δ_C (CD_3OD) 35.1 (CH_2), 49.3 (CH_2), 59.5 (CH_2), 71.4 (CH), 158.4 (C)

N(But-3-enyl) phthalimide 95



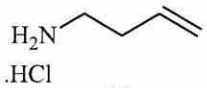
95

4-Bromobut-1-ene (10 g, 74.1 mmol) was added to a stirred solution of potassium phthalimide (14.4 g, 77.8 mmol, 1.05 eq) in DMF (60 mL). After stirring at rt for 14 h the mixture was heated under reflux for 8 h then cooled to room temperature before being poured onto ice (60 g). The aqueous layer was extracted with DCM (4 x 30 mL) and the combined extracts washed with aqueous potassium hydroxide solution (200 mL, 0.2M) and water (150 mL). The organic extracts were then dried ($MgSO_4$) and evaporated under vacuum. The resultant oil was dissolved in diethyl ether (300 mL) and washed with water (300 mL x 4) to remove excess DMF. After drying ($MgSO_4$) and evaporation **95** (12.95 g, 82%) was obtained as an off-white solid and was used in the next step without further purification.

δ_H 2.43 (2 H, dt, J = 7.1, 6.7, CH_2), 3.78 (2 H, t, J = 7.1, CH_2), 5.0 (2H, m, 2 x CH), 5.80 (1H, ddt, J = 17.1, 10.1, 7.0, CH), 7.72 (2H, m, 2x CH), 7.84 (2H, m, 2 x CH)

δ_C 32.8 (CH$_2$), 37.3 (CH$_2$), 117.5 (CHH), 123.18 (2 x CH) 133.8 (2 x CH), 134.5 (CH), 168.3 (C=O)

But-3-enylamine hydrochloride 96 Error! Bookmark not defined.



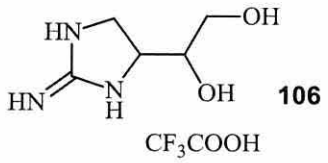
96

N-(But-3-enyl) phthalimide **92** (10 g, 49.7 mmol) was dissolved in IMS (120 mL) and hydrazine hydrate (2 mL, 64.6 mmol, 1.3 equiv) was added dropwise following which the solution heated under reflux for 4-5 hours. Further IMS (120 mL) was added as the solution thickened and reflux was continued for a further 30 min. The solution was then allowed to cool to room temperature and HCl (11.6 M, 6 mL, 69.6 mmol) was added dropwise, with stirring, over 5 minutes. The was then evaporated *in vacuo* to a paste which was dissolved in chloroform (120 mL), dried ($MgSO_4$), filtered and evaporated under vacuum to give crude **93** (4.86g, 92%) as an off-white solid.

δ_H (CD_3OD) 2.48 (2H, dt, J = 7.0, 7.0, C*H*$_2$), 3.05 (2H, t, J = 7.0, C*H*$_2$), 5.23 (1H, m, C*H*H), 5.31 (2 H, m, C*H*H), 5.87 (1H, ddt, *J* 17.1, 10.1, 6.8, C*H*)

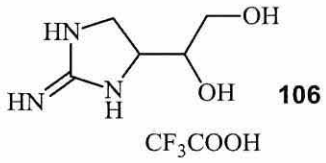
δ_C (CD_3OD) 35.2 (*C*H$_2$), 42.4 (*C*H$_2$), 121.8 (*C*HH), 137.9 (C*H*)

Attempted 1-(2-iminoimidazolidin-4-yl)ethane-1,2-diol trifluoroacetate 106



Guanidine **108** (0.6 g, 2.55 mmol) was dissolved in IMS (6 mL) and Pd/C (5%, 0.1 g) was added and the mixture stirred under H_2 (balloon) for 18 h after which tlc indicated the complete disappearance of starting material. The reaction mixture was then filtered through Celite™, which was washed with methanol (30 mL) and methanol acidified with a few drops of TFA (20 mL). The filtrate was evaporated to give a dark oil which on analysis (NMR) gave a complex spectrum indicative of decomposition. Attempted purification by column chromatography failed to give any indication of the required product.

1-(2-iminoimidazolidin-4-yl)ethane-1,2-diol 2,2,2-trifluoroacetate 106



Guanidine **114** (0.150 g, 0.44 mmol) was dissolved in chloroform (5 mL), cooled (5 °C) and trifluroacetic acid (2 mL) was added drop-wise. After slow warming to rt stirring was continues for 48 h. After evaporation under reduced pressure, the residue was co-evaporated with chloroform three times to remove excess trifluroacetic acid to give **106** (0.063 g, 55 %) as an oil.

δ_H 3.44- 3.58 (3H, m, 3 x CH), 3.61 (1H, m, CH), 3.74 (1H, app t, 9.7 Hz), 4.07 (1H, m, CH)

δ_C 45.0 (CH_2), 56.5 (CH), 62.5 (CH_2), 72.2 (CH), 153.2 (C)

HRMS (ES) (M+H) m/z:

Found 146.0926, $C_5H_{12}N_3O_2$ re quires 146.0924 Daltons

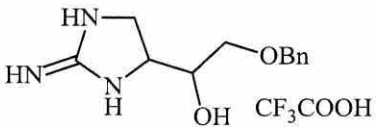
(3-((benzyloxy)methyl)oxiran-2-yl)methyl methanesulfonate 107

107

Alcohol **111** (8.8 g, 45.3 mmol) was dissolved in dry dichloromethane (100 mL), cooled (-5 °C) and triethylamine (6.86 g, 67.9 mmol, 1.5 eq) and DMAP (0.550 g, 4.5 mmol, 0.1 eq) was added. Methanesulfonyl chloride (5.19 g, 45.3 mmol, 1 eq) was then added drop-wise over 5 min and the mixture warmed slowly to rt stirred for 18 hours. The reaction mixture was then washed with HCl (aq. 1M, 50 mL), sodium bicarbonate $NaHCO_3$ (sat. aq., 2 x 50 mL) and brine (60 mL). After drying ($MgSO_4$) the solvent was removed under reduced pressure to give **107** (8.87 g, 72%) as a red oil which was used in the next step without further purification.

δ_H	3.05 (3H, s, C*H*$_3$), 3.34 (2H, m, 2 x CH), 3.65-3.74 (2h, m, CH_2), 4.27 (1H, m, C*H*H), 4.47 (1H, m, CH*H*), 4.54 (1H, d, 11.7 Hz, C*H*H), 4.59 (1H, d, 11.7 Hz, CH*H*), 7.31-7.61 (5H, m, Ph)
δ_C	37.7 (CH_3), 52.9 (CH), 54.8 (CH), 67.2 (CH_2), 68.0 (CH_2), 73.4 (CH_2), 127.8 (CH), 127.9 (CH), 128.4 (CH), 137.3 (C)
ν_{max}	3029, 2937, 2864 (CH), 1177 (CO) 1099 (CO)

2-Benzyloxy-1-(2-imino-imidazolidin-4-yl)-ethanol, trifluoroacetate salt 108



108

A suspension of guanidine hydrochloride (0.88 g, 8.8 mmol, 1.2 eq) in *tert*-butanol (15 mL) was cooled (0 °C) and potassium *tert*-butoxide (0.9 g, 8.5 mmol, 1.1 eq) was added. The resultant suspension was stirred for 25 min and then a solution of mesylate **107** (2.0 g, 7.4 mmol, 1 eq) in *tert*-butanol (10 mL) was added dropwise and the mixture stirred to rt over 20 h. Further potassium *tert*-butoxide (0.95 g, 8.9 mmol, 1.2 eq) was then added and the reaction mixture heated (60 °C) and stirred for 20 h. After cooling (0 °C) a solution of TFA (3.2 mL) in methanol (10 mL) was added dropwise and the mixture was stirred to rt for 20 mins. The reaction was evaporated to dryness and a methanol:chloroform mixture (1:2, 30 mL) was added and the slurry obtained was filtered through a celite pad which was washed with further methanol:chloroform mixture (1:2, 15 mL). After evaporation, purification by column chromatography on silica with gradient elution (initially chloroform increasing in 2% increments to chloroform:methanol 80:20) Guanidine **108** (1.05g, 41%) was isolated as a pale yellow solid which eluted in the 18:82 and 20:80 methanol:chloroform fractions.

Rf 0.13 in 20:80 methanol:chloroform.

δ_H (CD_3OD) 3.69 (1H, dd, *J* 2.9, 9.8 Hz, CH*H*), 3.74 (1H, dd, *J* = 2.6, 9.8 Hz, C*H*H), 3.94 (1H, m, C*H*), 4.06 (1H, dd, *J* 5.1, 9.8 Hz, CH*H*), 4.10 (1H, dd, *J* 5.4, 9.5 Hz, C*H*H), 4.28 (dt, *J* 2.2, 4.8 Hz, C*H*), 5.57 (2H, dt, *J* 12.0, 19.2 Hz), 7.39 (5H, m, Ph)

δ_C (CD_3OD) 46.9 (CH_2), 66.2 (CH), 71.6 (CH), 72.3 (CH_2), 73.6 (CH_2), 128.4, (CH), 128.9 (CH), 129.0 (CH), 129.5 (CH) 160.2 (C)

HRMS (ESI) ($[M+H]^+$) *m/z*

Found 236.1363, $C_{12}H_{18}N_3O_2$ requires 236.1399 Daltons

(Z)-4-(benzyloxy)but-2-en-1-ol 110 [60]

110

In a 1 L flask NaH (4.4 g, 110 mmol, 1.1 eq)) was added with stirring to cooled (0 °C) dry THF (400 mL) and (*Z*)-but-2-ene-1,4-diol **109** (8.8 g, 100 mmol) in dry THF (200 mL) was added slowly over 30 min. After a further 60 minutes, benzyl bromide (18.9 g, 110 mmol, 1.1 eq) in dry THF (100 mL) was then added drop wise over 20 min followed by *tetra-N*-butylammonium iodide (18.5 g, 50 mmol, 0.5 eq). The reaction mixture was stirred at rt for 16 h and then at 60 °C for 6 h. After cooling water (~100 mL) and ethyl acetate (200 mL) were added and the mixture was washed with sodium bicarbonate solution (sat. 3 x 50 mL) and brine (2 x 50 mL. The organic layer was then dried ($MgSO_4$) filtered and evaporated under reduced pressure leaving a dark yellow oil. Purification using flash chromatography with a graduated solvent (100% petrol – 50% ethyl acetate in petrol) gave **110** (13.53 g, 76 mmol, 76%) as a light yellow oil which eluted in the 20-25% fractions.

δ_H	2.06 (1H, OH), 4.09 (2H, d, *J* 5.6, C*H*$_2$OBn), 2.14 (2H, d, *J* 5.7, C*H*$_2$OH), 4.53 (2H, s, OC*H*$_2$Ph), 5.78 (2H, m, 2 x C*H*), 7.36 (5H, m, Ph)
δ_C	58.5 (*C*H$_2$), 65.7 (CH$_2$), 72.5 (O*C*H$_2$Ph), 127.8 (CH), 127.9 (CH), 128.0 (CH), 128.5 (CH), 132.5 (CH), 137.9 (CH)

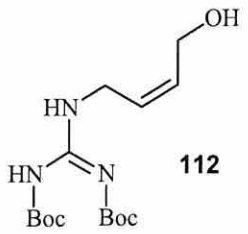
(3-((benzyloxy)methyl)oxiran-2-yl)methanol 111

111

A solution of alcohol **110** (13.5 g, 75.8 mmol) dichloromethane (250 mL) was cooled (0 °C) and *m*CPBA (46.72 g, 189.5 mmol) was added portionwise. The reaction warmed slowly to rt and stirred for 12 h before being filtered through a small pad of $MgSO_4$ and silica gel. The filtrate was was washed with sodium thiosulfate solution (sat. aq., 100 mL), sodium bicarbonate (sat. aq., 100 mL), then dried ($MgSO_4$) and evaporated under reduced pressure to give **111** (8.83g, 60%) as an oil which was used in the next step without further purification.

δ_H	2.95 (1H, br s, OH), 3.29 – 3.43 (2H, m, 2 x C*H*), 3.77 – 3.84 (4H, m, 2 x CH_2), 4.63 (1H, d, *J* = 11.6, CH), 4.73, (1H, d, *J* = 11.6, CH), 7.46 (5H, Ph).
δ_C	55.9 (*C*H), 56.3 (*C*H), 60.2 (*C*H_2), 67.8 (*C*H_2), 73.5 (*C*H_2), 127.6 (*C*H), 127.8 (*C*H), 127.9 (*C*H), 137.8 (*C*)
ν_{max}	3424 (br, OH), 2864 (CH) 1097 (CO) cm^{-1}

N'N"-bis-Boc-N"'-(4-Hydroxy-but-2-enyl)-guanidine 112



112

Phthalimide **116** (1.20 g, 5.0 mmol) was dissolved in IMS (20 mL) and a solution of hydrazine monohydrate (85% in water, 0.33 g, 1.1 eq, 5.5 mmol) was added and the reaction mixture heated under reflux for 2 h. The milky white suspension was cooled (0 °C) and filtered and the filter pad washed with chilled IMS (5 mL). The filtrate solution was allowed to warm to room temperature and *N,N'-bis-(tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamide (1.70 g, 1.1 eq, 5.5 mmol) was added and the solution stirred at room temperature for 72 h. After evaporation purification by column chromatography on silica gel using gradient elution with ethyl acetate:petrol (10-30% ethyl acetate in petrol). The guanidine **112** (0.93 g, 2.8 mmol) eluted in the 20-30% fractions as a white solid in 56% yield.

Rf 0.43 in 50:50 Ethyl acetate:petrol.

Mpt 70-73 °C

δ_H 1.48 (9H, s, 3 x CH_3), 1.49 (9H, s, 3 x CH_3), 3.25 (1H br s, OH) 4.05 (2H, t, *J* 6.6, CH_2), 4.23 (2H. d, *J* 6.9, CH_2), 5.44 (1H, dt, *J* 10.7, 7.6, *CH*), 5.84 (1H, br dt, *J* 10.7, 6.9, *CH*), 8.52 (1H, br s), 11.39 (1H br s)

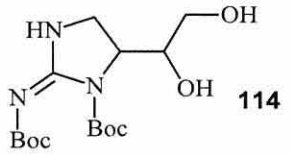
δ_C 28.0 (3 x CH_3), 28.2 (3 x CH_3), 37.7 (CH_2), 57.9 (CH_2), 79.6 (*C*), 83.3 (*C*), 126.4 (*C*H), 132.2 (*C*H), 153.2 (C), 156.11 (C), 162.9 (C)

ν_{max} 3584 (OH) 3319 (NH), 2975, 2934 (CH), 1739 (C=O), 1722 (C=O) 1643 (C=N)

HRMS (ES) (M+H) m/z

Found 330.2022, $C_{15}H_{28}N_3O_5$ requires 330.2023 Daltons

(Z)-tert-butyl-2-((tert-butoxycarbonyl)imino)-5-(1,2-dihydroxyethyl)imidazolidine-1-carboxylate 114



Guanidine **112** (0.331 g, 1.00 mmol) in a 250 mL RBF was cooled (-30 °C) and a cooled (-30 °C) solution of DMDO in acetone was added (as prepared on page XX). The mixture was stirred for 1.5 h before being allowed to warm to room temperature slowly. After a further 14 h, the solvent was removed under reduced pressure and the residue dissolved in dichloromethane, dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give **114** (0.359 g, 1.00 mmol) as a white solid in quantitative yield.

Rf 0.25 (2:98 methanol:ethyl acetate)

Mpt 92-94 °C

δ_H 1.48 (9H, s, 3 x C*H*$_3$), 1.49 (9H, s, 3 x C*H*$_3$) 3.67 (1H, dd, 10.1, 7.3 Hz, CH*H*), 3.82 (1H, app t, 10.1 Hz, C*H*H), 3.86 (1H, dd, 10.7, 5.4 Hz, CH), 4.12 -4.20 (3H, m, 3 x CH).

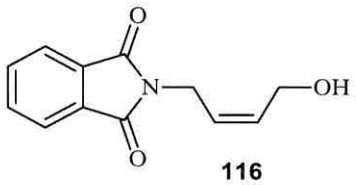
δ_C 27.7 (3 x *C*H$_3$), 28.0 (3 x *C*H$_3$),), 44.6 (CH$_2$), 56.6 (CH), 67.5 (CH$_2$), 70.4 (CH), 81.2 (C), 82.9 (C) 153.4 (C)

ν_{max} 3397 (OH, NH), 2982 (CH), 1741 (C=O), 1651 (C=N)

HRMS (ES) (M+H) m/z:

Found 346.1976, $C_{15}H_{28}N_3O_6$ requires 346.1973 Daltons

(Z)-2-(4-hydroxybut-2-en-1-yl)isoindoline-1,3-dione 116



(Z)-But-2-ene-1,4-diol **115** (10.0 g, 9.35 mL, 113.5 mmol), phthalimide (16.7 g, 113.5 mmol, 1 eq) and triphenylphosphene (44.65 g, 170.0 mmol, 1.5 eq) were dissolved in dry THF (300 mL) and the solution was cooled (0 °C). DEAD (21.74 g, 19.7 mL, 124.8 mmol, 1.1 eq) was added drop wise over 10 min and stirring continued for 1 h before being stirred to rt over 16 h. After evaporation the solid obtained was triturated with ethyl acetate:petrol (20:80, 200 mL) and the suspension obtained was stirred for 1 h. The mixture was filtered and the solid filtrate further washed with chilled ethyl acetate:petrol (40:60, 50 mL). The filtrate was evaporated under reduced pressure and the residue purified by column chromatography on silica gel eluted with 30:70 ethyl acetate:petrol to give **116** (16.51 g, 73.8 mmol) as a solid in 65% yield.

Rf 0.13 in 30:70 ethyl acetate:petrol.

Mpt 47-50 °C

δ_H 2.43 (1H, br s, OH), 4.39 (2H, d, *J* 6.9, C*H*$_2$), 4.41 (2H. d, *J* 7.9, C*H*$_2$) 5.56 (1H, br dt, *J* 10.8, 7.9, C*H*), 5.91 (1H, br dt, *J* 10.8, 6.9, C*H*), 7.70 - 7.74 (2H, m, 2 x C*H*), 7.82 – 7.85 (2H, m, 2 x C*H*)

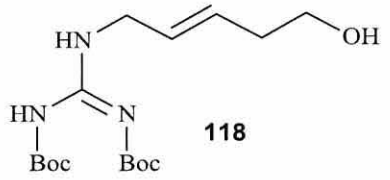
δ_C 34.4 (*C*H$_2$), 58.0 (*C*H$_2$), 123.4 (*C*H), 124.9 (*C*H), 132.0 (*C*), 133.2 (*C*H), 134.1 (*C*H), 168.2 (*C*=O).

ν_{max} 3463 (OH), 3020 (CH), 1714 (C=O)

HRMS (ES) (M+NH$_4$) m/z:

Found 235.1079, $C_{12}H_{15}N_2O_3$ requires 235.1077 Daltons

N, N'-bis-Boc-N''-(*E*-5-Hydroxy-pent-2-enyl)-guanidine 118



Phthalimide **130** (0.24 g, 1.04 mmol) was dissolved in IMS (10 mL) together with hydrazine monohydrate (0.09 mL, 1.22 mmols 1.2 eq) and the mixture heated under reflux for 2 h. After cooling the white suspension filtered through a celite pad which was washed with chilled IMS (5 mL). The filtrate solution was warmed to rt and *N,N'-bis-(tert-*butoxycarbonyl)-1*H*-pyrazole-1-carboxamide **89** (0.715 g, 2.3 mmol, 2.2 eq) was added and the solution stirred at room temperature for 84 h. Solvent was removed under reduced pressure and the residue purified by column chromatography on silica gel eluted using 10-30 % ethyl acetate in petrol to give **118** (0.196 g, 0.57 mmol, 55%) as a white solid eluting in the 20-30% ethyl acetate in petrol fractions.

Rf 0.20 in 50% ethyl acetate/petrol

δ_H 1.45 (18H, s, 6 x Me), 2.26 (2H, dt, J 6.3 Hz, C*H*$_2$), 2.87 (1H, broad s, OH) 3.61 (2H, t, J 6.3 Hz, C*H*$_2$), 3.96 (2H, t, J 5 Hz, C*H*$_2$), 5.52 – 5.63 (2H, m, 2 x C*H*), 8.3 (broad s, NH), 11.46 (1H, broad s, NH)

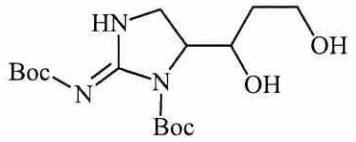
δ_C 28.1 (3 x CH_3), 28.3 (3 x CH_3), 35.7 (CH_2), 42.8 (CH_2), 61.7 (CH_2), 79.4 (C), 83.2 (C), 127.8 (CH), 129.8 (CH), 153.2 (C), 156.0 (C), 163.5 (C)

ν_{max} 3330 (N-H), 2982 (C-H), 1722 (C=N, 1618 (C=O) 1134 (C-O).

HRMS (NES) (M+H) m/z:

Found 344.2175, $C_{16}H_{30}N_3O_5$ requires 344.2180 Daltons

2-tert-Butoxycarbonylimino-5-(1,3-dihydroxy-propyl)-imidazolidine-1-carboxylic acid tert-butyl ester 120



120

Guanidine **118** (0.200 g, 0.583 mmol) was placed in a 250 mL RBF and cooled (-30 °C) for 30 mins. A pre-cooled (-30 °C) solution DMDO in acetone was then added and the reaction stirred for a further 1 h at this temperature before slowly warming to rt and stirred over 14 h. The reaction solvent was then removed under reduced pressure and the resulting residue re-dissolved in dichloromethane (20 mL), dried ($MgSO_4$), filtered the solvent removed under reduced pressure and the residue purified by column chromatography on silica gel eluted using 10-20 % ethyl acetate in petrol to give **120** (0.210 g) in quantitative yield.

Rf 0.24 in 50% ethyl acetate/petrol

δ_H 1.47 (18H, s, 6 x Me), 1.55 – 1.62 (1H, m, C*H*H), 1.64 – 1.71 (1H, m, C*H*H)

3.71 – 3.78 (3H, m, C*H* and C*H*2), 3.83 (1H, m, C*H*), 3.97 (1H, m, C*H*), 4.02 (1H, m, C*H*).

δ_C 28.0 (6 x CH_3), 34.6 (CH_2) 57.6 (*C*H), 60.4 (C*H*) 62.1(*C*H_2), 65.7 (*C*H_2), 80.5 (*C*), 83.2 (*C*), 150.8 (*C*), 152.7 (*C*), 155.5 (*C*)

MS (CI) *m/z:* Found 260.3 ($[M+H]^+$)

3-((tert-butyldimethylsilyl)oxy)propan-1-ol 122[61]

122

A stirred suspension of sodium hydride (3.16 g, 1 eq, 79.0 mmol) in dry THF (200 mL) under argon was cooled (0 °C) and dried and distilled propane-1,3-diol **127** (6.0 g, 5.7 mL, 79.0 mmol) was added *via* a syringe over 5 minutes. After stirring the reaction mixture for 1.5 h *tert*-butyldimethylchlorosilane (12.1 g, 1.1 eq, 86.9 mmol) was added portion wise over 3 minutes and the reaction mixture was stirred for 1.5 h at 0 °C. The white suspension was poured into ether (600 mL), washed with potassium carbonate solution (20% w/v aqueous, 200 mL), brine (200 mL), dried ($MgSO_4$) and evaporated under reduced pressure to give a crude oil. The oil was purified by passing through a bed of silica initially eluting with 100% petrol to remove impurities followed by 50:50 ethyl acetate : petrol to isolate the desired product **122** as a colourless oil (12.3 g, 82% yield).

Rf	0.48 (50% ethyl acetate/petrol)
δ_H	0.06 (6H, s, 2 x CH_3), 0.89 (9H, s, 3 x CH_3), 1.76 (2H, pentet, J = 5.7, CH_2), 2.77 (1H, br s, O*H*), 3.78 (2H, t, *J* 5.7, CH_2), 3.82 (2H, t, *J* 5.7, CH_2OH)
δ_C	-5.41 (2 x CH_3), 18.30 ($C(CH_3)_3$), 25.87 (3 x CH_3), 34.25 (CH_2), 62.22 (CH_2OSi), 62.76 (CH_2OH)
ν_{max}	3357 (bs,OH), 2961, 2858 (CH), 1256, 1110.

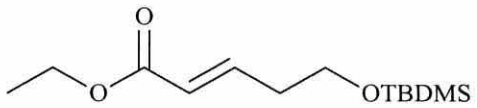
3-(tert-Butyl-dimethyl-silanyloxy)-propionaldehyde 123

123

Oxalyl chloride (8.94 g, 5.94 mL, 1.1 eq, 70.5 mmol) was dissolved in dry dichloromethane (300 mL) and the solution was cooled (– 78 °C). DMSO (11.0 g, 10.13 mL, 2.2 eq, 141.0 mmol) was added *via* syringe and the reaction mixture stirred for 20 min. Alcohol **122** (12.2 g, 64.1 mmol) in DCM (40 mL) was added *via* syringe and the reaction stirred for 2 h. Triethylamine (17.0 g, 24 mL, 2.7 eq, 173.1 mmol) was slowly added *via* syringe and the reaction mixture stirred until reaching room temperature. Sodium hydrogen carbonate solution (saturated, 200 mL) was added and the organic layer separated and the aqueous fraction further extracted with DCM (3 x 100 mL). The combined organic fractions were dried ($MgSO_4$) and evaporated under reduced pressure to approximately 200 mL and used immediately in the following reaction.

δ_H 0.04 (6H, s, 2 x CH_3), 0.85 (9H, s, 3 x CH_3), 2.57 (2H, td, J = 6.0, 1.9, CH_2), 3.96 (2H, t, J = 6.0, CH_2), 9.77 (1H, t, J = 1.9, aldehyde H)

***E*-5-(*tert*-Butyl-dimethyl-silanyloxy)-pent-2-enoic acid ethyl ester 124**[70]



124

Ethoxycarbonylmethylenetriphenylphenylphosphorane (26.8 g, 1.2 eq, 76.9 mmol) was added to a solution of the aldehyde **123** (64.1 mmol) in dichloromethane (200 mL). After stirring for 15 h, the solution was evaporated under reduced pressure to give a solid which was triturated with 25% ethyl acetate in petrol (300 mL) and the suspension filtered through a celite™ pad. The filtrate was evaporated under reduced pressure and the resulting oil purified by column chromatography on silica gel eluting with 2% ethyl acetate in petrol to give **124** (13.07 g, 79% yield from **122**).

Rf 0.71 in 50% ethyl acetate in petrol.

δ_H 0.03 (6H, s, 2 x CH_3), 0.87 (9H, s, 3 x CH_3), 1.26 (3H, t, J 7.1, CH_3), 2.39 (2H, dtd, J 6.9, 6.7, 1.4, CH_2) 3.70 (2H, t, J 6.5, CH_2), 4.16 (2H, q, J 7.1, (CH_2), 5.84 (1H, dt, J 15.6, 1.4, CH), 6.94 (1H dt, J = 15.6, 7.1, CH)

δ_C -5.37 (2 x CH_3), 14.23 (CH_3), 18.26 ($C(CH_3)_3$), 25.84 (3 x CH_3), 35.68 (CH_2), 60.11 (OCH_2), 61.55 (CH_2O), 122.94 (CH)), 145.79 (CH) 166.42 (C=O).

ν_{max} 2965 (C-H), 1725 (C=O), 1096 (C-O) cm^{-1}

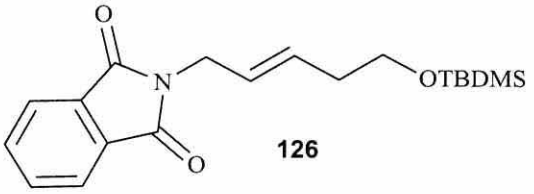
***E*-5-(tert-Butyl-dimethyl-silanyloxy)-pent-2-en-1-ol 125**

125

Ester **124** (5.4 g, 19.3 mmol) in dry dichloromethane (200 mL) was cooled (-45 °C) and a solution of DIBAL (1M in hexane, 42.5 mL, 2.2 eq, 42.5 mmol) was added drop wise over 5 min. The reaction mixture was stirred at -40 °C for 1 h then warmed slowly to room temperature. A solution of Rochelle's salt solution (saturated, 50 mL) was cautiously added followed by ether (50 mL) and the mixture was stirred until two clear layers were visible. After separation, the aqueous layer was further extracted with dichloromethane (2 x 30 mL) and the combined organic fractions were dried ($MgSO_4$) and evaporated under reduced pressure to give **125** (4.1g, 98% yield) as a colourless oil.

Rf	0.54 in 50% ethyl acetate in petrol
δ_H	0.03 (6H, s, 2 x CH_3), 0.87 (9H, s, 3 x CH_3), 2.18 (1H, bs. O*H*), 2.24 (2H, dt, J 6.8, CH_2), 3.62 (2H, t, J 6.8,CH_2O), 4.04 (2H, d, J 3.5, CH_2OH), 5.51–5.79 (2H, m, 2 x C*H*)
δ_C	-5.3 (2 x CH_3), 18.3 ($C(CH_3)_3$), 25.8 (3 x CH_3), 35.7 (CH_2), 62.7 (CH_2O), 62.5 (CH_2O) 129.0 (CH), 131.0 (CH)
ν_{max}	3356 (O-H), 2956 (C-H), 1101 (C-O)

E- 2-[5-(tert-Butyl-dimethyl-silanyloxy)-pent-2-enyl]-isoindole-1,3-dione 126



Alcohol **125** (4.0 g, 18.5 mmol) was dissolved in dry dichloromethane (150 mL), cooled (0 °C), then phthalimide (4.08g, 1.5 eq, 27.8 mmol), triphenylphosphine (9.97 g, 2 eq, 38.0 mmol) and DEAD (4.37 mL, 1.5 eq, 27.8 mmol) were added. After stirring for 1 h the solution was evaporated under reduced pressure and triturated by dissolving the minimum volume of dichloromethane then adding ether (100 mL) and petrol (50 mL) then filtering the supernatant liquid. Evaporation of the filtrate under reduced pressure and column chromatography on silica gel eluting with 5-10% ethyl acetate in petrol gave **126** (2.7 g, 42% yield) as a colourless oil.

Rf 0.23 in 10% ethyl acetate/petrol

δ_H 0.00 (6H, s, 2 x CH_3), 0.82 (9H, s, 3 x CH_3), 2.22 (2H, dt, J = 7.0, 6.6, CH_2), 3.60 (2H, t, J 6.6, CH_2O), 4.23 (2H, d, J 6.1, CH_2N), 5.56 (1H, dt, J 15.2, 7.0 Hz, *CH*), 5.71 (1H, dt, J 15.1, 7.0 Hz, *CH*), 7.70 (2H, m, ar *CH*), 7.78 (2H, m, ar *CH*)

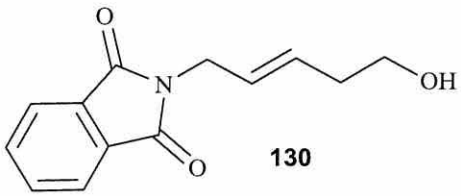
δ_C -5.4 (2 x CH_3), 18.2 ($C(CH_3)_3$), 25.8 (3 x CH_3), 35.7 (CH_2), 39.4, (CH_2), 62.5 (CH_2O), 123.2 (CH), 125.1 (CH), 131.3 (CH), 132.2 (*C*), 133.8 (*C*H), 167.9 (*C*)

ν_{max} 2955 (C-H), 1718 (C=O), 1102 (C-O) cm^{-1}.

HRMS (ES) (M+H) m/z:

Found 346.1837, $C_{19}H_{28}NO_3Si$ requires 346.1833 Daltons

***E*-2-(5-Hydroxy-pent-2-enyl)-isoindole-1,3-dione 130**



130

Silyl ether **126** (2.7 g, 7.8 mmol) was dissolved in dry THF (50 mL) and a THF solution of TBAF (1M, 9.8 mL, 1.2 eq, 9.8 mmol) was added *via* syringe and the reaction was stirred at rt for 20 h. Chloroform (120 mL) was added and the mixture washed with water (4 x 80 mL) then dried ($MgSO_4$). After evaporated under reduced pressure the residue was purified by column chromatography on silica gel eluting initially with 20-30% ethyl acetate in petrol to give recovered starting materials (0.4 g, 15%). Elution with ethyl acetate gave the desired product **130** (0.88 g, 48% yield) as a colourless crystalline solid.

Rf. 0.11 (30% ethyl acetate/petrol eluted twice)

Mpt 77-79 °C

δ_H 1.87 (1H, br s, OH), 2.29 (2H, dt, *J* 6.6, 6.3 Hz, CH_2), 3.64 (2H, t, *J* 6.3 Hz, CH_2), 4.25 (2H, d, *J* 6 Hz, CH_2), 5.63 (1H, dt, *J* 16.4, 6.0 Hz, CH), 5.73 (1H, dt, *J* 16.4, 6.9 Hz, CH), 7.7 (2H, m, 2 x CH), 7.83 (2H, m, 2 x CH).

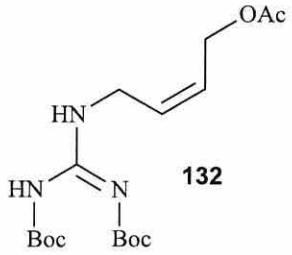
δ_C 35.5 (CH_2), 39.6 (*C*H_2), 61.6 (*C*H_2O), 123.3 (CH), 126.3 (CH), 130.9 (CH), 132.1 (*C*), 134.0 (*C*H), 168.0 (*C*).

ν_{max} 3463 (O-H), 2930 (C-H), 1711 (C=O), 1115 (C-O)

HRMS (ES) (M+NH_4) m/z:

Found 249.1235, $C_{13}H_{17}N_2O_3$ requires 249.1234 Daltons

N'N"-bis-Boc-N"'-(4-Acetoxy-but-2-enyl)-guanidine 132



Guanidine **112** (0.455 g, 1.38 mmol) was dissolved in dry dichloromethane (15 mL) together with pyridine (0.33 g, 0.34 mL, 3 eq, 4.14 mmol) and DMAP (0.05 g, 0.3 eq, 0.4 mmol) The mixture was cooled (0 °C) and acetic anhydride (0.21 g, 0.2 mL, 1.5 eq 2.07 mmol) stirred for 1 h and warmed to room temperature overnight. After removal of solvent under reduced pressure the residue was purified by column chromatography on silica gel eluting 10-20% ethyl acetate in petrol to give **132** (0.367 g) as a white solid in 71% yield.

Rf 0.59 in 50:50 ethyl acetate in petrol.

Mpt 67-71 °C

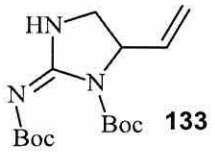
δ_H 1.46 (9H, s, 3 x CH_3), 1.47 (9H, s, 3 x CH_3), 2.03 (3H, s, CH_3) 4.10 (2H, t, *J* 5.4, CH_2), 4.63 (2H, d, *J* 5.6, CH_2), 5.64 – 5.71 (2H, m, 2 x *CH*), 8.29 (1H, br S, NH), 11.44 (1H br s, NH)

δ_C 20.9 (CH_3), 28.0 (3 x CH_3), 28.2 (3 x CH_3) 37.7 (CH_2), 57.9 (CH_2), 79.6 (*C*), 83.3 (*C*), 126.4 (*C*H), 132.2 (*C*H), 153.2 (C), 156.11 (C), 162.9 (C), 169.7 (C)

HRMS (ES) (M+H) m/z:

Found 372.2125, $C_{17}H_{30}N_3O_6$ requires 372.2126 Daltons

2-tert-Butoxycarbonylimino-5-vinyl-imidazolidine-1-carboxylic acid tert-butyl ester 133



Palladium acetate (0.0127 g, 0.057 mmol) and triphenylphosphine (0.293 g, 1.116 mmol) were combined in dry degassed THF (10 mL) under argon. The mixture was heated (50 °C) until the solids had dissolved giving a bright yellow solution. This was cooled to room temperature and a solution of **132** (0.180 g, 0.48 mmol) and triethylamine (0.121 g, 1.2 mmol) in dry THF (10 mL) was added. The reaction mixture was then heated (55 °C) for 2 h. After cooling to room temperature the solvent was removed under reduced pressure and the residue purified by column chromatography on silica eluting with 20-30% ethyl acetate in petrol to give **133** (0.130 g, 0.42 mmol) 84% yield.

R*f* 0.30 (50% EtOAc : Petrol)

δ_H 1.45 (9H, s, 3 x CH_3) 1.46 (9H, s, 3 x CH_3) 3.49 (1H, dd, 4.1, 8.9 Hz CH_2), 3.93 (1H, dd, 9.5, 12.9 Hz, C*H*), 4.53 (1H, m, C*H*), 5.13 (1H, d, 10.1 Hz, C*H*), 5.17 (1H, d, 18.0 Hz, C*H*) 5.77 (1H, ddd, 18.0 10.1, 7.55 Hz, C*H*) ppm.

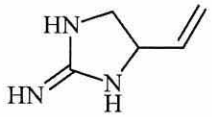
δ_C 28.0 (3 x CH_3), 28.1 (3 x CH_3), 53.8 (CH_2), 58.9 (*C*H), 80.9 (C), 83.1 (C), 116 (CH_2), 136 (*C*H), 137 (CH), 151 (*C*) ppm

ν_{max} 3332 (NH), 2978 (CH), 1758 (C=O), 1703 (C=O), 1602 (C=N), cm^{-1}

HRMS (ES) (M+H) m/z:

Found 312.1912 $C_{15}H_{26}N_3O_4$ requires 312.1915 Daltons

4-vinylimidazolidin-2-imine 2,2,2-trifluoroacetate 134

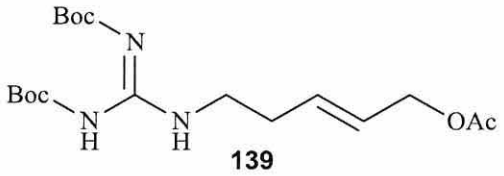


134 CF_3COOH

Guanidine **133** (0.02g, 0.06 mmol) was dissolved in dichloromethane (10 mL), cooled (5 °C) and trifluroacetic acid (2 mL) was added drop-wise. After slow warming to rt stirring was continues for 48 hours. After evaporation under reduced pressure, the residue was co-evaporated with chloroform three times to remove excess trifluroacetic acid to give **134** (0.012 g, 0.055 mmol) 91% yield as an oil.

δ_H (CD_3OD)	3.32 (2H, m), 3.81 (1H, m), 5.38 (2H, m), 5.89 (1H, m)
δ_C (CD_3OD)	41.5 (CH_2), 53.8 (CH), 121.1 (CH_2), 130.8 (CH), 154.1 (C)

(*E*)-5-((Z)-2,3-bis(tert-butoxycarbonyl)guanidino)pent-2-en-1-yl acetate 139



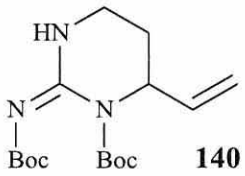
Phthalimide (0.3 g, 1.1 mmol) **130** was dissolved in IMS (10 mL), to the stirring solution hydrazine monohydrate (0.1 mL, 1.3 mmols 1.2 eq) was added and the reaction mixture was refluxed for 4 h. The white suspension was cooled to 0 °C, the solid removed by filtration and washed with chilled IMS (5 mL). The filtrate solution was allowed to warm to room temperature before *N,N'-bis-(tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamide **89** (0.746 g, 2.4 mmol, 2.2 eq) was added and the solution stirred at room temperature for 84 h. Solvent was removed by evaporation under reduced pressure and remaining residue was purified by column chromatography on silica gel eluting with 20% ethyl acetate in petrol gave **139** (0.42, 83% yield)

δ_H 1.47 (9H, s, ^{t}Bu) 1.49 (9H, s, ^{t}Bu), 2.04 (3H, s, CH_3), 2.31 (2H, pent, J = 6.6 Hz, CH_2), 3.47 (2H,dt, J = 7.0, 5.4 Hz, CH_2), 4.50 (2H, d, J = 5.7 Hz, CH_2), 8.33 (1H, broad NH), 11.46 (1H, broad s, NH)

δ_C 20.9 (CH_3), 28.0 (^{t}Bu), 28.2 (^{t}Bu), 31.6 (CH_2), 39.9 (CH_2), 63.5 (CH_2), 79.3 (C), 83.3 (C), 129.0 (CH), 132.9 (CH), 153.3 (C), 155.9 (C), 163.5 (C), 171.1 (C).

ν_{max} 3334 (N-H), 2979 (C-H), 1723 (C=N), 1640 (C=O), 1136).

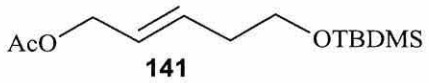
Tert-butyl 2-((tert-butoxycarbonyl)imino)-6-vinyltetrahydropyrimidine-1(2H)-carboxylate 140



140

Palladium acetate (0.018 g, 0.08 mmol) and triphenylphosphine (0.39 g, 1.48 mmol) were combined in dry degassed THF (10 mL) under argon. The mixture was heated (45 °C) until the solids had dissolved giving a bright yellow solution. This was cooled to room temperature and a solution of **139** (0.3 g, 0.77 mmol) and triethylamine (0.2 g, 1.98 mmol) in dry THF (10 mL) was added. The reaction mixture was then heated (55 °C) for 3 h. TLC (50% ethyl acetate in petrol) of the reaction mixture showed the presence of **139**. After a further 3 h at 55 °C solvent was removed under reduced pressure and crude residue was analysed (NMR). This showed signals consistent with **139** with no indication of the target molecule **140**.

Acetic acid *E*-5-(tert-butyl-dimethyl-silanyloxy)-pent-2-enyl ester 141



Alcohol **125** (4.1 g, 18.9 mmol) was dissolved in dichloromethane (80 mL), cooled (0 °C) and Pyridine (4.5 g, 4.6 mL, 56.8 mmol, 3 eq) and DMAP (0.7 g, 5.8 mmol, 0.3 eq) were added. Acetic anhydride (5.8 g, 56.8 mmol 3 eq) was then added and the reaction mixture stirred at rt for 36 h. Solvent was removed under reduced pressure and the residue purified by column chromatography on silica gel eluted using 10% ethyl acetate in petrol to give **141** (4.5 g, 17.4 mmol, 92% yield).

Rf 0.65 in 50% ethyl acetate:petrol

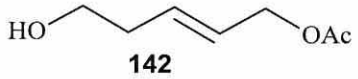
δ_H 0.04 (6H, s, 2 x CH_3), 0.88 (9H, s, 3 x CH_3), 2.05 (3H, s, CH_3), 2.27 (2H, q, *J* 6.6 Hz, CH_2), 3.65 (2H, t, *J* 6.7 Hz, CH_2), 4.51 (2H, d, *J* 6.7 Hz, CH_2), 5.63 (1H, dtd, *J* 15.5, 6.3, 1.0 Hz, CH), 5.77 (1H, dtd, *J* 15.5, 7.0, 1.0 Hz, CH)

δ_C -5.3 (2 x CH_3), 18.3 ($C(CH_3)_3$), 21.0 (CH_3) 25.9 (3 x CH_3), 35.8 (CH_2), 62.4 (CH_2), 65.1 (CH_2), 125.8 (CH), 132.7 (CH), 170.7 (C)

HRMS (NES) (M+H) m/z:

Found 259.1727, $C_{13}H_{27}NO_3Si$ requires 259.1724 Daltons

Acetic acid *E*-5-hydroxy-pent-2-enyl ester 142



142

Silyl ether **141** (2.86 g, 11 mmol) was dissolved in dry THF (10 mL) and cooled to 0 °C. A solution of TBAF in THF (1M, 22 mL, 22 mmol, 2 eq) was added *via* syringe and the reaction stirred at room temperature for 20 h. Ethyl acetate (60 mL) was added and the mixture washed with water (4 x 20 mL) and dried ($MgSO_4$). After evaporation under reduced pressure the residue was purified by column chromatography on silica gel eluting with 20-30% ethyl acetate in petrol to give **142** (1.45 g, 10.5 mmol, 90 % yield) as a colourless oil.

Rf 0.08 in 50% ethyl acetate:petrol.

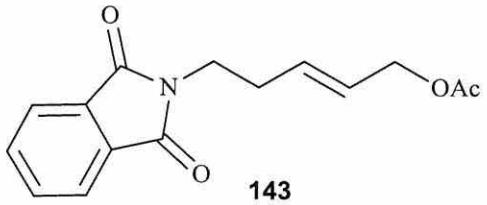
δ_H 2.01 (3H, s, CH_3), 2.15 (1H, br s, OH), 2.29 (2H, q, *J* 6.4 Hz, CH_2), 3.64 (2H, t, *J* 6.7 Hz, CH_2), 4.51 (2H, d, *J* 6.7 Hz, CH_2), 5.63 (1H, dtd, *J* 15.5, 6.3, 1.0 Hz, CH), 5.77 (1H, dtd, *J* 15.5, 7.0, 1.0 Hz, CH)

δ_C 21.0 (CH_3), 35.7 (CH_2), 60.8 (CH_2), 65.0 (CH_2), 126.6 (CH), 132.0 (CH), 171.0 (C)

HRMS (ES) ($M+NH_4$) m/z:

Found 162.1125, $C_7H_{16}NO_3$ requires 162.1125 Daltons

(*E*)-5-(1,3-dioxoisoindolin-2-yl)pent-2-en-1-yl acetate 143



143

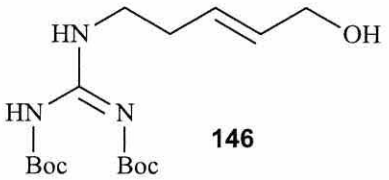
Alcohol **142** (0.23 g, 1.6 mmol) was dissolved in dry dichloromethane (30 mL), cooled (0 °C), then phthalimide (0.352 g, 1.5 eq, 2.4 mmol), triphenylphosphine (0.63 g, 1.5 eq, 2.4 mmol) and DEAD (0.37 mL, 1.5 eq, 2.4 mmol) were added. After stirring for 1 h the solution was allowed to warm to rt and stirred for 16 h. Solvent was removed by evaporation under reduced pressure and remaining residue was purified by column chromatography on silica gel eluting with 0-20% ethyl acetate in petrol gave **143** (0.301 g, 70% yield) as a white solid.

δ_H 1.96 (3H, s, CH_3), 2.42 (2H, q, *J* 6.8 Hz, CH_2), 3.72 (2H, t, *J* 6.8 Hz, CH_2), 4.42 (2H, d, *J* 6.7 Hz, CH_2), 5.58 (1H, dtd, *J* 15.5, 6.3, 1.0 Hz, CH), 5.72 (1H, dtd, *J* 15.5, 7.0, 1.0 Hz, CH) 7.68 (2H, m, 2 x CH), 7.80 (2H, m, 2 x CH)

δ_C 21.0 (CH_3), 32.7 (CH_2), 37.3 (CH_2), 63.5 (CH_2), 123.2 (2 x *C*H) 129.0 (CH), 132.9 (CH) 133.8 (2 x *C*H), 134.5 (*C*H), 168.3 (*C*), 171.0 (C).

ν_{max} 2946 (C-H), 1773 (C=N), 1714 (C=O).

***N'N"-bis-boc-N"'-(E)*-1-(5-hydroxypent-3-en-1-yl)guanidine 146**



146

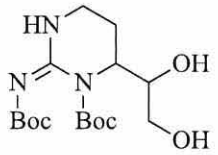
The acetate **139** (0.44 g, 1.1 mmol) was dissolved in methanol (15 mL) and potassium carbonate (0.6 g) was added. The reaction mixture was then stirred for 72 hours, TLC (50% EtOAc/petrol) indicated that the reaction had gone to completion. The solvent was removed under vacuum, the resulting residue was suspended in ethyl acetate (20 mL) and then filtered with the remaining solid being washed with ethyl acetate and dichloromethane. The filtrate was reduced under vacuum to give a clear oil. This was passed through a silica plug eluting with 50% ethyl acetate in petrol, solvent was removed under reduced pressure to give **146** (0.349 g, 89% yield) as a clear oil.

δ_H 1.47 (9H, s, ^{t}Bu) 1.49 (9H, s, ^{t}Bu), 2.31 (2H, pent, J = 6.6 Hz, CH_2), 3.47 (2H,dt, J = 7.0, 5.4 Hz, CH_2), 4.50 (2H, d, J = 5.7 Hz, CH_2), 5.58 (1H, dt, J = 16.0, 6.0 Hz, CH), 5.72 (1H, dt, J = 16.0, 6.0 Hz, CH) 8.35 (1H, broad NH), 11.48 (1H, broad s, NH)

δ_C 28.0 (^{t}Bu), 28.2 (^{t}Bu), 31.8 (CH_2), 39.9 (CH_2), 63.5 (CH_2), 79.3 (C), 83.3 (C), 129.0 (CH), 132.9 (CH), 153.3 (C), 155.9 (C), 163.5 (C), 171.1 (C).

ν_{max} 3330 (O-H), 2979 (C-H), 1722 (C=N), 1644 (C=O), 1135).

(S,Z)-tert-butyl 2-((tert-butoxycarbonyl)imino)-6-((S)-1,2-dihydroxyethyl)-tetrahydropyrimidine-1(2H)-carboxylate 148



148

Guanidine **146** (0.200 g, 0.582 mmol) was placed in a 250 mL RBF and cooled (-30 °C) for 30 mins. A pre-cooled (-30 °C) solution DMDO in acetone was then added and the reaction stirred for a further 1 h at this temperature before slowly warming to rt and stirred over 17 h. The solvent was removed under reduced pressure, the remaining residue was redissolved in DCM, dried ($MgSO_4$). The solvent was removed and the remaining residue was analyzed (NMR). This showed signals consistent with **146** with no indication of the target molecule **148**.

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