

STUDIES ON N-HYDROXYGUANIDINE DERIVATIVES,
POTENTIAL NITRIC OXIDE DONOR DRUGS

Neil D. Anderson

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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A thesis presented for the degree of

Doctor of Philosophy

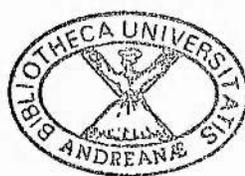
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Dedicated to Mum, Dad, Jenni and Katy for all your love and support.

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Finally I must thank my Parents and my two sisters, Jenni and Katy, for their continued support through the last few years.

GLOSSARY OF ABBREVIATIONS

L-Arg	Arginine
bp	Boiling point
CI	Chemical ionisation
m-CPBA	<i>m</i> -Chloroperbenzoic acid
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
DMAP	4-Dimethylaminopyridine
DMD	<i>N,N</i> -Dimethylformamide
DMSO	Dimethyl sulfoxide
EDRF	Endothelium-derived relaxing factor
EDTA	Ethylenediaminetetraacetic acid
EI	Electron impact mass spectrometry
ES ⁻	Negative ion electrospray mass spectrometry
FT-IR	Fourier transform infra-red spectrophotometry
cGMP	Cyclic guanosine monophosphate
GSNO	<i>S</i> -Nitrosoglutathione
GTN	Glycerol trinitrate
GTP	Guanosine triphosphate
HPLC	High performance liquid chromatography
IR	Infra-red spectrophotometry
mp	Melting point
NALA	<i>Nγ</i> -Allyl-L-arginine
NCPA	<i>Nγ</i> -Cyclopropyl-L-arginine
NHMA	<i>Nγ</i> -Hydroxy- <i>Nγ</i> -methyl-L-arginine
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NOHA	<i>Nω</i> -hydroxy-L-arginine

NOS	Nitric oxide synthase
bNOS	Recombinant constitutive brain nitric oxide synthase
cNOS	Constitutive nitric oxide synthase
iNOS	Inducible nitric oxide synthase
mNOS	Murine macrophage nitric oxide synthase
P450	Cytochrome P450 reductase
ppm	Parts per million
SNAP	<i>S</i> -Nitrosoacetylpenicillamine
THF	Tetrahydrofuran
tlc	Thin layer chromatography
UV	Ultra violet spectrophotometry

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ABSTRACT

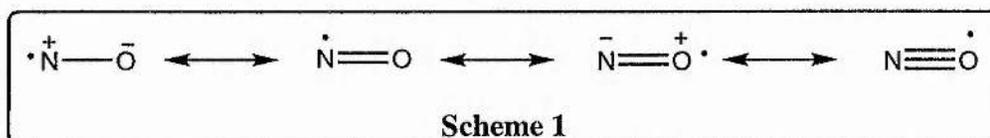
The ability of substituted *N*-hydroxyguanidines to act as nitric oxide donor drugs has been previously established. To further investigate the potential of this class of compound as nitric donor drugs a number of new *N*-hydroxyguanidines were prepared. The compounds were synthesised and then subjected to a number of chemical and biological tests. A number of the examples prepared were indeed shown to be oxidised under chemical conditions to give nitric oxide. The nitric oxide was tested for using the Greiss test. One example, 1-benzyl-1-methyl-2-hydroxyguanidine showed biological activity and was found to be a vasodilator. In addition an X-ray crystal structure of this compound was obtained which gave a further insight into the conformation of this type compound and related analogues. A number of ureas were also prepared for use in the biological and chemical testing. Under chemical conditions the ureas were not oxidised thus indicating that the hydroxyguanidine functionality was required for nitric oxide generation.

O-Substituted *N*-hydroxyguanidines were also prepared in an effort to examine the possibility of selectively targeting the molecule to the site of action for nitric oxide release. These compounds were found to produce a small amount of nitric oxide under chemical oxidation, therefore confirming their potential as potential nitric oxide donor compounds. Acid catalysed decomposition of *O*-THP-1,1,-dibenzyl-2-hydroxyguanidine was studied. The reaction was carried out at various pHs and was monitored using HPLC. It was found that the THP protecting group could be removed to give the free *N*-hydroxyguanidine. More easily removed *O*-substituents are probably required to produce successful prodrugs. However the reaction only gave a reasonable rate at a very low pH ($t_{1/2} = 122$ s at pH 0). A number of *O*-substituted hydroxylamines were then prepared in an attempt to increase the range of *O*-substituted *N*-hydroxyguanidines that could be obtained.

Chapter One
INTRODUCTION

1.0 NITRIC OXIDE

Nitric oxide is a colourless gas with b.p. $-151.8\text{ }^{\circ}\text{C}$ and m.p. $-163.6\text{ }^{\circ}\text{C}$. Nitric oxide is a simple odd-electron species but its structure has been the subject of a lot of interest and debate. In valence bond terms it is best represented by the canonical forms given in **Scheme 1**.¹

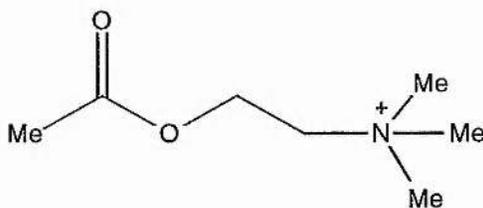


The presence of the unpaired electron effectively reduces the bond order to approximately 2.5 unlike NO^+ where the bond order is 3. The geometrical location of the odd electron and also the virtually unchanged bond order in the dimer explains the reluctance of nitric oxide to dimerize. The odd electron leads to nitric oxide being paramagnetic and also explains its use as a free radical trap in gas-phase reactions. It is also an efficient quencher of excited singlet states.

Lab preparations involve the reduction of nitric acid, using copper or nitrous acid with either iodine or ascorbic acid. Industrially, nitric oxide can be prepared by the catalytic oxidation of ammonia with a palladium/ rhodium catalyst, however the majority of nitric oxide produced is further oxidised to nitrogen dioxide and then reacted with water to give nitric acid.¹ Thermodynamically the direct formation of nitric oxide from nitrogen and oxygen is unfavourable and G° for the reaction is 173 kJ mol^{-1} . However its production can be achieved to a small extent at high temperatures for example during lightning discharge and more importantly in the internal combustion engine. This nitric oxide, in the absence of a catalytic converter, is oxidised in the air to nitrogen dioxide which is an air pollutant (this is often ascribed to NO_x) in the industrialised world. When nitric oxide is found in the atmosphere it can lead to major photochemical smog, for example in Los Angeles. There is also concern regarding the role of nitric oxide emitted from the engines of supersonic aircraft in the depletion of the ozone layer.²

1.1 BIOLOGICAL ACTIVITY OF NO

In 1980 Robert Furchgott and John Zawadzki of Down State Medical centre, New York reported strange findings.³ While studying the action of acetylcholine (1) on pre-contracted artery rings they found that, contrary to previous work, acetylcholine did not always induce muscle relaxation and thus artery dilation. This occurred when the endothelial cells had inadvertently been removed or damaged. This result lead them to report that acetylcholine does not act directly upon the muscle cells but on the endothelium, which then produces a secondary messenger. This messenger passes through the endothelium to the surrounding muscles to stimulate guanylate cyclase which is responsible for smooth muscle relaxation.

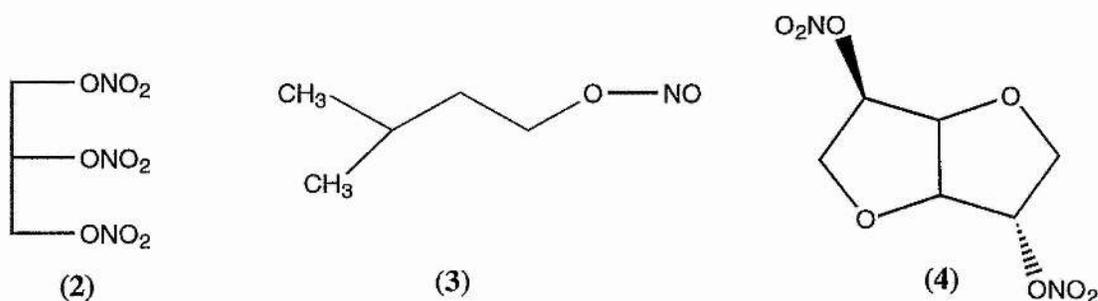


(1)

The chemical identity of the secondary messenger, which became known as the endothelium-derived relaxing factor (EDRF), was the subject of intense speculation. In 1987 Moncada and co-workers,⁴ at the Wellcome research laboratories, and Ignarro and co-workers⁵ published independent findings which identified nitric oxide as the EDRF. Both groups had found that in a bioassay the EDRF and nitric oxide behaved in an identical manner. The identification of NO as the EDRF explained two earlier results. Firstly, the lifetime of the EDRF was prolonged on addition of the enzyme superoxide dismutase (SOD). Superoxide reacts with nitric oxide and thus when SOD was added it destroys the superoxide leaving the nitric oxide unreacted. Secondly, if haemoglobin was added, the action of the EDRF was destroyed due to the strong binding of haemoglobin to nitric oxide.

The identification of nitric oxide as the EDRF has been questioned. Myers and co-workers, suggested that the EDRF was in fact *S*-nitrosocysteine.⁶ However, this is doubtful as it is impossible to obtain *S*-nitrosocysteine in pure form. The search for an alternative EDRF is motivated by the mistrust that a gas is the answer. However nitric oxide has all the necessary requirements; it is small and therefore mobile, it is soluble in both water and lipid, as a radical it is highly reactive but it is stable in isolation and finally another gas, ethene, is also effective as a messenger molecule. It has been reported that nitrosated thiol groups on proteins provide a vasodilator that is more stable than nitric oxide itself, so it may be possible that nitric oxide is stored in the form of an *S*-nitrosothiol.⁷

It has long been known that a number of NO-donating compounds act as vasodilators (substances that enlarge the blood vessels) and these are used in the treatment of diseases where increased blood flow will relieve the symptoms, for example angina pectoris. Treatments for these diseases include the use of glyceryl trinitrate (2) (GTN), amyl nitrite (3) and isosorbide dinitrate (4).



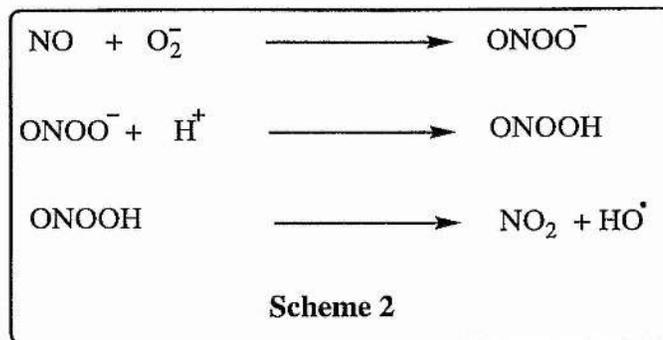
Until it was discovered that nitric oxide was the EDRF the mode of action of these compounds was unknown. However, it is now known that these compounds work by bypassing the NO-generating system in the endothelium and deliver nitric oxide directly to the muscle cells in the walls of the artery.

1.1.1 Platelet Aggregation and Adhesion

Nitric oxide is also involved in platelet aggregation and adhesion.⁸ This process prevents excessive bleeding when a blood vessel has been damaged. NO and prostacyclin act synergistically to inhibit platelet aggregation and to disaggregate platelets, but there is no parallel synergism in platelet adhesion. However myocardial infarction (heart attack) may be caused by an abnormal clotting in a coronary vessel coated with atherosclerotic plaque (a coating on the inside of a blood vessel containing quantities of cholesterol).

1.1.2 Immune Response

It was discovered that activated macrophages in culture produce nitrite and nitrate in the supernatant liquid.⁹ Macrophages are part of the body's immune response to invasion by foreign particles. They move around the body and upon encountering a foreign microbe are stimulated to engulf and kill it. It has been shown that the macrophage requires L-arginine for this destructive activity, which is also accompanied by the formation of citrulline and nitrite.¹⁰ Marletta suggested, taking into account these observations, that the arginine to nitric oxide pathway occurs in activated macrophages as well as endothelial cells (**Section 1.2**). It has also been suggested that the cytotoxic agent produced by the macrophages and responsible for foreign cell death is nitric oxide however, there is no direct evidence concerning the mechanism for NO involvement. It could be that as a radical species, nitric oxide is sufficiently destructive to the lipid cell membrane but there is no experimental evidence to either prove or disprove this theory. An alternative mechanism has been suggested (**Scheme 2**).¹¹ Firstly NO reacts with superoxide to give peroxynitrite (ONOO^-), which is a weak acid and will become protonated at normal physiological pH. This could then fragment to give nitrogen dioxide and a hydroxyl radical. Hydroxy radicals have already been shown to be more destructive towards lipid membranes and DNA.



A further alternative is that NO reacts with an enzyme iron-sulfur centre, which is required for metabolic activity and produces an iron-sulfur cluster nitrosyl and this disrupts the enzyme activity.

1.1.3 Septic Shock

There seems to be a rather curious and incompatible combination of beneficial effects and the cytotoxic effects of nitric oxide. This dual role does however explain a life threatening condition known as septic shock.² Here when the body suffers from a massive infection there will be a lot of macrophage activity. In addition to having massive cytotoxicity towards the invading cells the nitric oxide produced will bring about massive hypotension and septic shock.

1.1.4 Nitric Oxide in the Brain

Nitric oxide is produced by the post synaptic neurone when the glutamate receptors are activated.² The nitric oxide then appears to diffuse back to the presynaptic neurone thus acting as a feedback mechanism. It also diffuses to the surrounding neurones on reaching the presynaptic neurone. This could be one way in which memory is established although at the moment there is no direct evidence of this. It has been suggested that over production of nitric oxide could be involved with the process of

senility.² This example contrasts the very different roles that nitric oxide does play within the body.

Nitric oxide is also partially responsible for brain damage occurring after a stroke. The enzyme nitric oxide synthase is involved in the biosynthesis of nitric oxide from arginine involving NADPH and dioxygen (**Section 1.2**). When the brain is starved of oxygen, for example during a stroke, nitric oxide synthase is deprived of one of its substrates. As a result the concentration of NOS becomes elevated to overcome the drop in activity. Then upon reoxygenation there is a sudden burst of nitric oxide production which is one of the causes of brain damage. If a drug could be designed that would inhibit the NOS then some of this reoxygenation injury could be avoided. Recent reports have shown that schizophrenia also involves a disturbance within the arginine-nitric oxide pathway in the brain.¹²

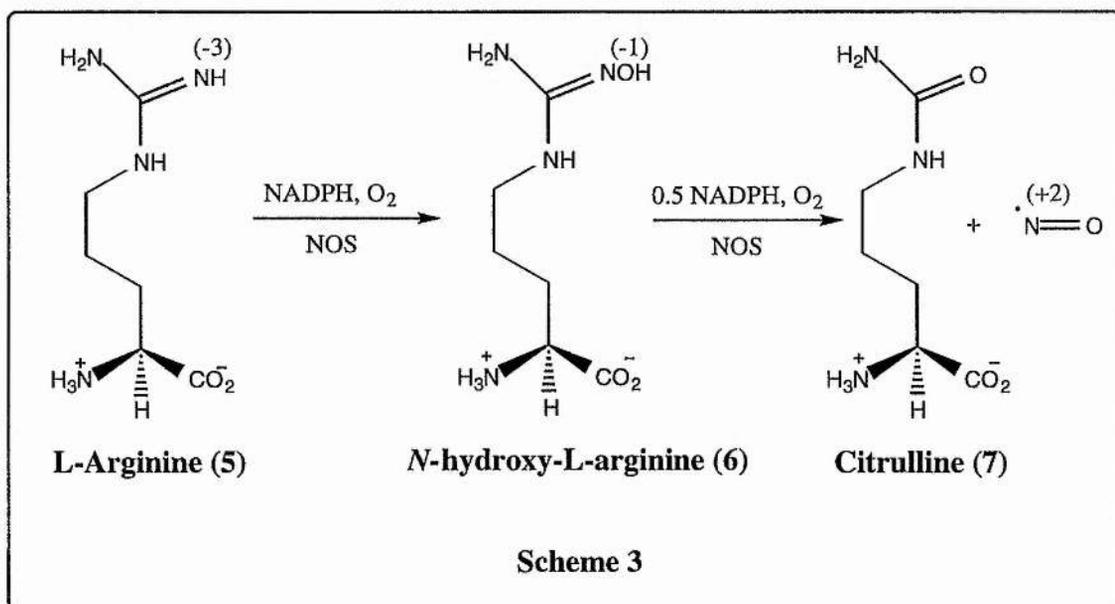
1.1.5 Diabetes and other conditions

Nitric oxide appears in so many areas of human physiology that an abnormal nitric oxide metabolism is present in a number and variety of diseased states. Nitric oxide destroys the β -cells of the pancreas when macrophages invade the Islets of Langerhans to produce the condition known as insulinitis.¹³ When the β -cells of the sufferer can no longer produce insulin the patient suffers from diabetes. Arthritis and inflammation in general are associated with elevated production of NO.¹⁴ The injury associated with paraquat poisoning appears also to arise from an over stimulation of nitric oxide synthase, resulting in high levels of nitrite in lung tissue.¹⁵ These three examples surely represent a more general phenomenon.

1.2 BIOSYNTHESIS

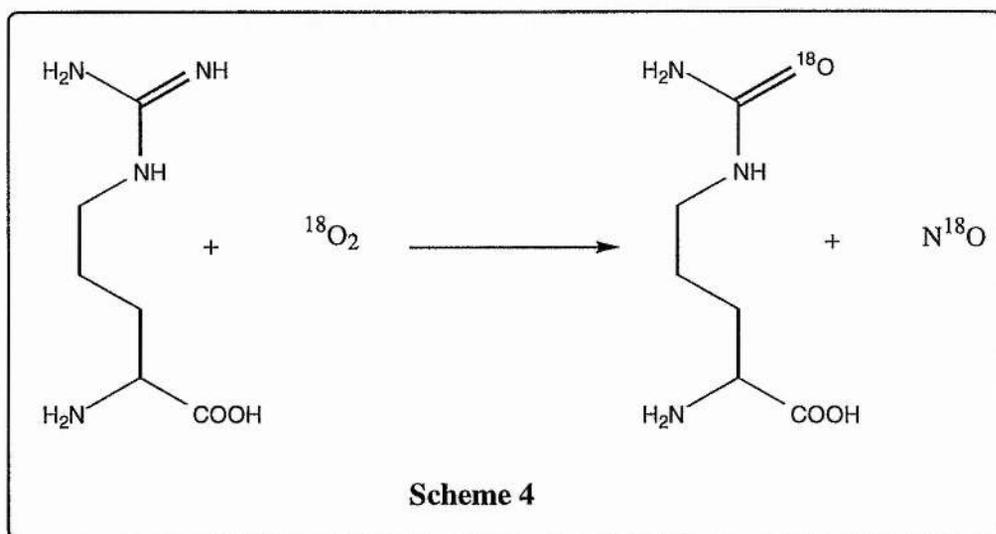
1.2.1 Introduction

NO is produced *in vivo* by the oxidation of the guanidine function of L-arginine (5) in a process that involves two monooxygenations and proceeds via the stable biosynthetic intermediate, *N*^ω-hydroxy-L-arginine (NOHA) (6) (Scheme 3). The products of the reaction are L-citrulline (7) and nitric oxide. The family of enzymes involved in the process are nitric oxide synthases (NOS) of which there are 2 distinct classes.



The two monooxygenation steps probably involve the reductive activation of the dioxygen species at a heme centre in the enzyme with the required electrons coming from nicotinamide adenine dinucleotide phosphate (NADPH). Overall the reaction requires 1.5 equivalents of NADPH and 2 equivalents of dioxygen and is a five electron oxidation of the terminal guanidino N atom. Each monooxygenation requires four electrons to reduce the dioxygen and these electrons are partially derived from both the substrate and the NADPH. In the first oxygenation two electrons come from NADPH and the other two

from the substrate. A two electron oxidation from N(-3)_{ARG} to N(-1)_{NOHA} occurs. In the second oxygenation NADPH contributes a single electron and the substrate undergoes a three electron oxidation from N(-1)_{NOHA} to N(+2)_{NO}. The use of ¹⁸O₂ resulted in both the products, citrulline and nitric oxide, containing an ¹⁸O label (**Scheme 4**).¹⁶



Since the initial discovery of NO biosynthesis it has become apparent just how widely the arginine-NO pathway is distributed in mammals and how many biological processes it regulates. This led to the proposal that there must be more than one isoform of the enzyme.^{17,18} There are now three NOS isoforms characterised which correspond to the different tissue/cell types in which the NO synthase was first discovered. The three isoforms are endothelial NOS (eNOS),¹⁹ neuronal NOS (nNOS),^{19,20} and inducible NOS (iNOS).²¹ Two, eNOS and nNOS, are constitutively expressed in cells where as iNOS is not normally present in resting cells but is produced in macrophages in response to bacterial products and immunoactive cytokines.

NOS isoforms in mammalian systems are the product of three distinct genes which are composed of 26 exons for iNOS,²² and eNOS²³ or 29 exons for nNOS.²⁴ The NOSs are cytosolic enzymes, with the exception of eNOS, which are active as homodimers, with each subunit containing one equivalent of heme.²⁵ The three isoforms have very similar biochemical properties and catalyse the same reaction but clearly show

differences in the primary structure and molecular weight. However the main difference between the constitutive and inducible isoforms is in the method of regulation.

The regulation factor of NOS activity appears to be the presence of Ca^{2+} /calmodulin system. It appears that calmodulin binding is a switch that allows electron transfer from the reductase domain into the oxygenase domain of the enzyme, thus allowing enzyme activity. The two constitutive isoforms are Ca^{2+} /calmodulin dependent and are therefore activated by hormones or neurotransmitters that cause a change in the intracellular concentration of Ca^{2+} ions. These isoforms produce a low concentration of NO as a signal for regulatory purposes, mediated, for example by the interaction of NO with guanylate cyclase. It is interesting to note, however, that whereas nNOS shows a dependence on calcium ions guanylate cyclase is inhibited by free Ca^{2+} . It has been suggested that this forms the basis of a control mechanism where the release of NO in a cell would affect guanylate cyclase in the neighbouring target cells while at the same time minimising the activity of the enzyme in the cell producing the NO.¹⁷ Recently it has been shown that there may be Ca^{2+} /calmodulin independent mechanisms which regulate constitutive NOS activity.²⁶

In contrast the activity of iNOS is independent of the Ca^{2+} concentration, a characteristic that arises from its tight binding with calmodulin at normal physiological Ca^{2+} concentrations. Once it has been expressed it means in effect that this isoform is active all the time and provides NO at high concentrations as part of the cytotoxic/cytostatic immune response to invading pathogens and tumour cells. There are, however, regulation methods for this isoform and these include, regulation of the transcription that expresses the iNOS gene;²⁷ control of the availability of the substrate arginine via arginase activity;²⁸ and control of the biosynthesis of the essential cofactors for example H₄B.

The nitric oxide biosynthetic pathway is not restricted to mammalian systems and has been shown to be a fundamental process in birds,²⁹ fish,³⁰ invertebrates,³¹ plants,³² fungi³³ and bacteria.³⁴ It is also known that some denitrifying bacteria can produce NO by reduction of nitrite and then further reduction to nitrous oxide by nitrite and nitric

oxide reducing enzymes³⁵ or by oxidative metabolism of ammonia.³⁶

1.2.2 Mechanism of transformations

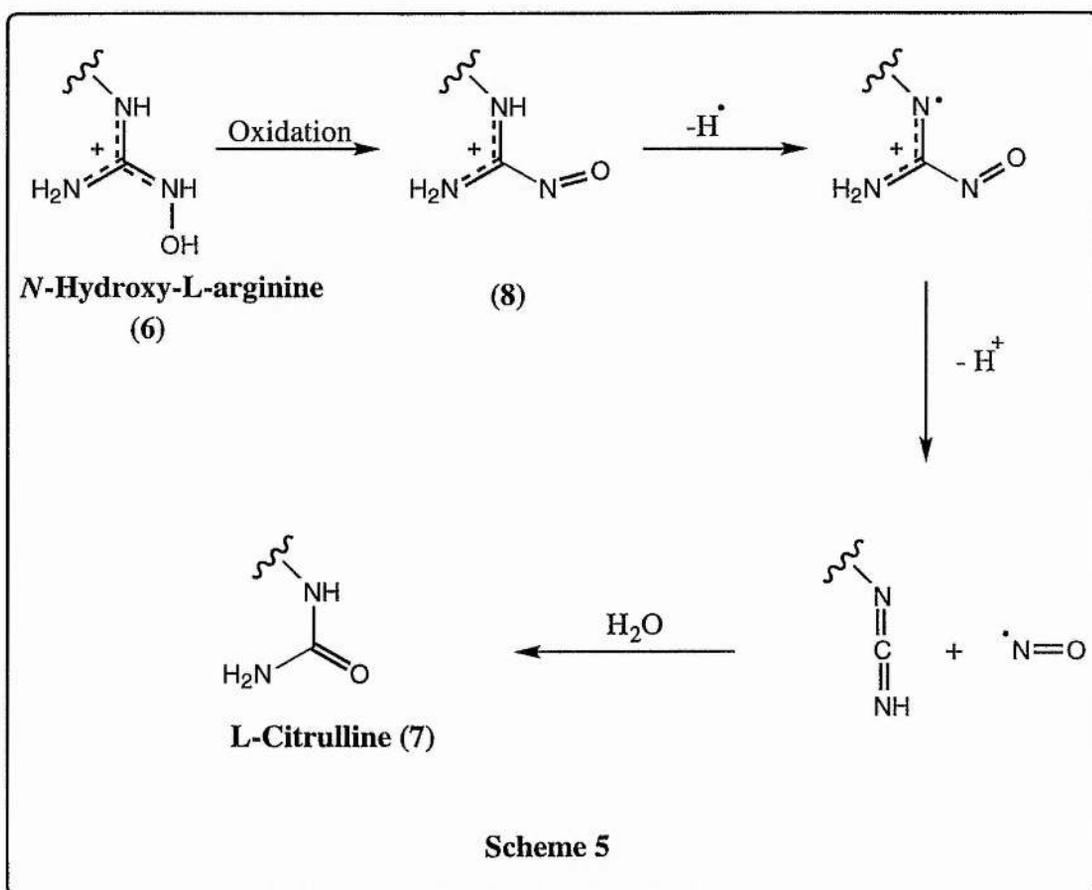
There are two very different mechanisms proposed for the two monooxygenations, although both are assumed to be mediated by the activation of O₂ at the heme centre of the enzyme. Several issues including the precise mechanistic role of the heme centre, the protonation state of both arginine and *N*-hydroxyarginine during the reaction and also the timing of the possible proton transfers are still unresolved.

1.2.2.1 Monooxygenation I

Monooxygenation I occurs mechanistically in the same manner as cytochrome P450 *N*-oxidations.^{37,38} It is probably, although it has not been proved beyond doubt, mediated by a transient, high-valent oxoiron porphyrin complex and may start by a single electron oxidation of the guanidine substrate followed by a recombination of this radical with the iron-bound oxygen. Although there is more known about this mechanism than the second monooxygenation, for which there actually appears to be no biological precedent, there are many unanswered questions about the fine mechanistic details. It maybe that substrate binding regulates the kinetics of the electron entry which facilitates the reductive activation of dioxygen. There is evidence to support this theory in particular the observation that substrate binding does not facilitate heme iron reduction but does influence the flux of electrons through the NOS redox centres.^{39,40} The position of the substrate with respect to the active site perferryl oxygen and the protonation state of the guanidine will have a major impact with respect to the actual mechanistic detail but little is known about the active site as of yet. The other major matter to be resolved is the role of the H₄B in catalysis. It is assumed that the hydroxylation step is catalysed by the action of heme without the biopterin being involved as a redox active cofactor and this is generally accepted.⁴¹

1.2.2.2 Monooxygenation II

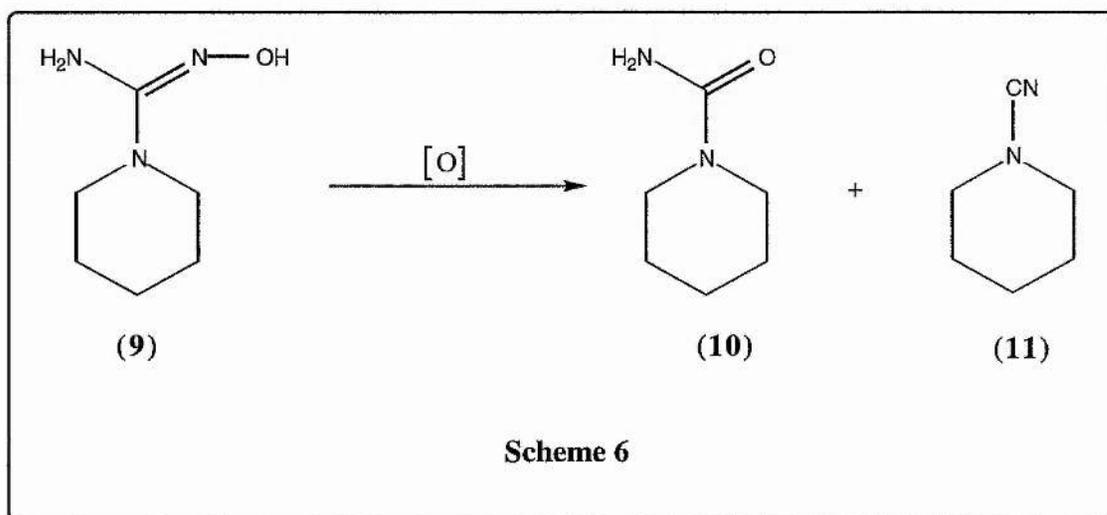
The mechanism of NOS monooxygenation II is not well understood and has been the subject of extensive speculation. As with monooxygenation I it has been suggested recently that the mechanism involves a heme-mediated monooxygenation rather than direct involvement of H₄B as a redox active cofactor. Initially it was proposed that the *N*-hydroxyarginine (6) was oxidised to a *C*-nitroso derivative (8) (Scheme 5).^{9,42}



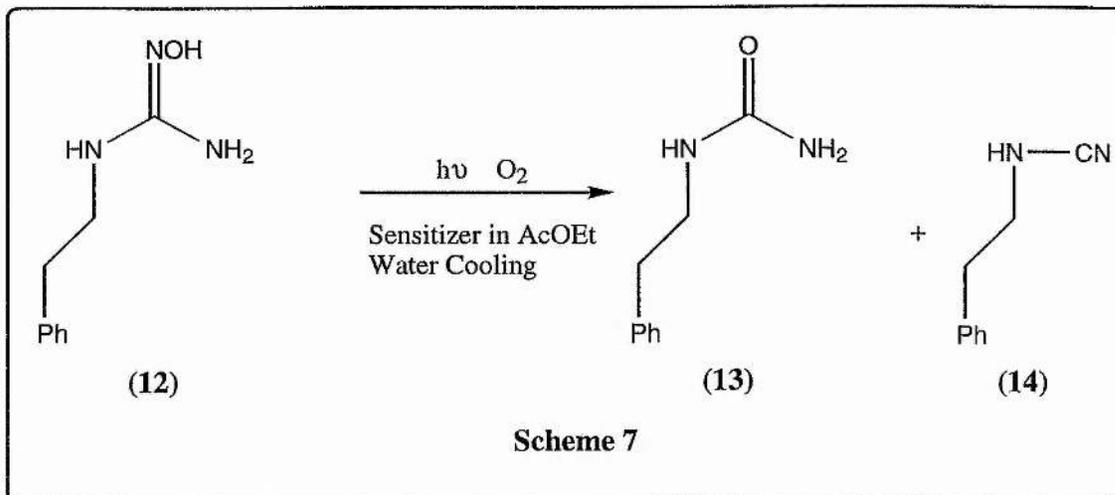
Various groups have attempted to try and probe the mechanism of the second oxidation. Fukuto and co-workers oxidised *N*-(*N*-hydroxyamidino)piperidine (NHAP) (9) using various chemical oxidation agents including Ag(I), PbO₂ and Fe(III)/H₂O₂.⁴³ Oxidation was observed, producing either NO or N₂O. The N₂O is formed from the

dimerisation of HNO to give hyponitrous acid which then decomposes to give water and N₂O in aqueous solution.

Feldman also reported a chemical model for the oxidation of *N*-hydroxy-*L*-arginine (9) by nitric oxide synthase.⁴⁴ *N*-Hydroxyguanidines were reacted with a range of oxidants; *m*-chloroperbenzoic acid (mCPBA), monoperoxyphthalic acid (magnesium salt, hexahydrate) and ozone. This confirmed the results of Fukuto and co-workers that suggested that either NO or HNO could be the oxidation product dependent on the oxidant. Both groups looked at the reaction of *N*-(*N*-hydroxyamidino)piperidine (NHAP) (9), but Feldman detected piperidinecarboxamide (PCA) (10) while Fukuto found no trace of PCA but detected large quantities of 1-piperidinecarbonitrile (PCN) (11) (Scheme 6).

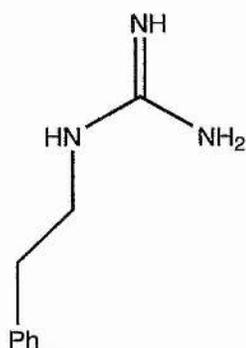


Ishikawa and co-workers examined the photo-sensitised oxygenation of phenethylguanidoxime (phenethylhydroxyguanidine) (12) as a possible chemical model for the biological oxidation of *N*-hydroxyarginine (6) to *L*-citrulline (7) (Scheme 3).⁴⁵ The singlet oxygen (¹O₂) ene reaction is a powerful method for the allylic oxidation of alkenes. The authors thought that the oxime bond (C=N-OH) would act as the ene to give a nitrosohydroperoxide which is structurally related to the peroxyheme complex (Scheme 7).



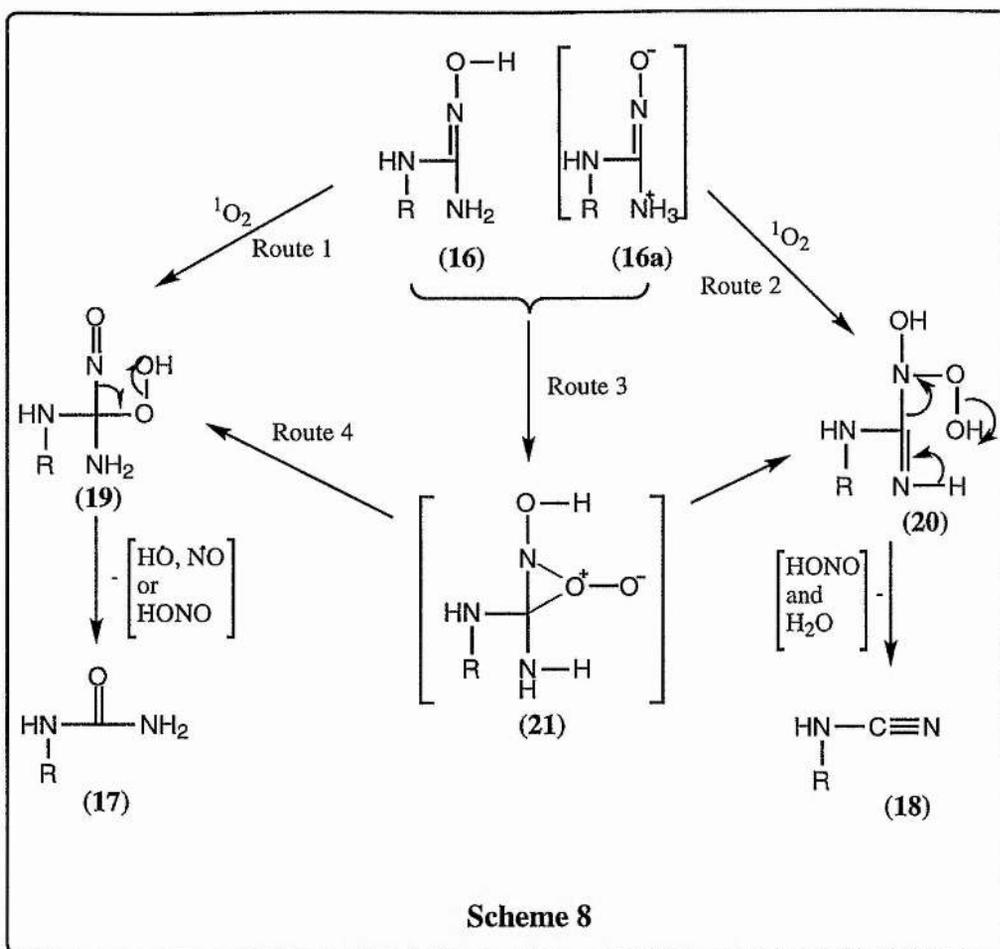
A solution of (12) in ethyl acetate was irradiated with a high pressure mercury lamp (400W) for 1 hour through a pyrex filter, while O_2 was bubbled through the solution under water-cooling with rose bengal (RB) as the sensitizer. The rose bengal was removed from the mixture with decolourising charcoal and the products obtained by preparative tlc which gave the expected phenethylurea (13) and phenethylcyanamide (14) in 58% and 4% isolated yields respectively. When reactions were repeated omitting one or more of the components there was no reaction. The use of tetraphenylporphine as a sensitizer led to a very similar product distribution. Thus the C=N-OH of the hydroxyguanidine appears to be cleaved by 1O_2 to yield the expected urea.

The formation of the cyanamide would also suggest a second possible mechanism in which the amidino (N=C-NH) bond could act as the ene. However the authors observed no reaction with phenethylguanidine (15) with 1O_2 . This suggests that the whole hydroxyguanidine function [RNHC(NH₂)=NOH] and not the limited function of either the amidino or oxime bond is essential for the photooxidative production of the urea and the cyanamide.



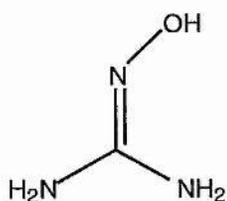
(15)

It is, therefore, reasonable to assume that a guanidoxime (**16**) would be oxygenated to yield a urea (**17**) and cyanamide (**18**) (Scheme 8). In the photooxygenation the *C*- and *N*- hydroperoxides (**19**) and (**20**) play a crucial role because they can spontaneously degrade into (**17**) and (**18**) with the loss of NO or its equivalent. Compounds (**19**) and (**20**) could be formed either by direct reaction of the ene (**16**) with $^1\text{O}_2$ (Routes 1 or 2) or by hydrogen transfer triggered by ring opening of the perepoxide (**21**), derived from a 1,2-cycloaddition of $^1\text{O}_2$ to (**16**) (Route 3). The fact that more of (**17**) is produced compared to (**18**) suggests that there is more of the intermediate (**19**) than (**20**). The reason (**18**) could be produced preferentially is the potential zwitterionic character of a guanadoxime (**16a**), with the more nucleophilic carbon of the $\text{C}=\text{N}-\text{OH}$ being attacked by the electrophilic $^1\text{O}_2$. In the route through to the cycloadduct (**21**), the facile transfer of the more acidic hydrogen on the hydroxy group rather than that on the amino group could also lead predominantly to the formation of (**19**) (Route 4).



These reactions produce either nitric oxide or nitrous acid. However, under the reaction conditions these must be oxidised to NO_2 , which is easily hydrolysable to NO_2^- or NO_3^- . Therefore analysis of the products, using the Griess test and other methods, led to both NO_2^- and NO_3^- being found.⁴⁶ These results mean that it is possible to consider the oxygenation reaction as one possible chemical model for the NOS-catalysed oxidation of NOHA to citrulline and nitric oxide.

The reaction between nitric oxide and *N*-hydroxyguanidine (**22**) under anaerobic conditions was examined.⁴⁷



(22)

N-Hydroxyguanidine (22) in buffer (pH 7.4) reacted with 2 equivalents of nitric oxide (in the reaction headspace) to give N_2O as the only detectable gaseous product. Control experiments lacking either *N*-hydroxyguanidine or nitric oxide failed to produce N_2O . These results suggest that although slow under anaerobic conditions, *N*-hydroxyguanidine (22) does reduce nitric oxide to N_2O . Further analysis of the reaction mixture showed that less than 0.01 moles of NO_2^- were formed per mole of N_2O . Experiments under aerobic conditions showed an increase in the rate of N_2O production. The authors then ran similar tests under both anaerobic and aerobic conditions and again found that the rate of reduction of nitric oxide in the system containing oxygen was quicker. The reaction was repeated with phenethylhydroxyguanidine (12). Reduction of nitric oxide to N_2O proved that the reaction was general for substituted hydroxyguanidines. The products of the reaction were phenethylurea (PEU) (13) and phenethylcyanamide (PECA) (14). A typical 24 hour run would yield PEU (13), PECA (14) and N_2O in 42, 12, 60% yield respectively. It was also observed that PECA (14) is not a precursor to PEU (13) as PECA (14) is stable under aqueous conditions.

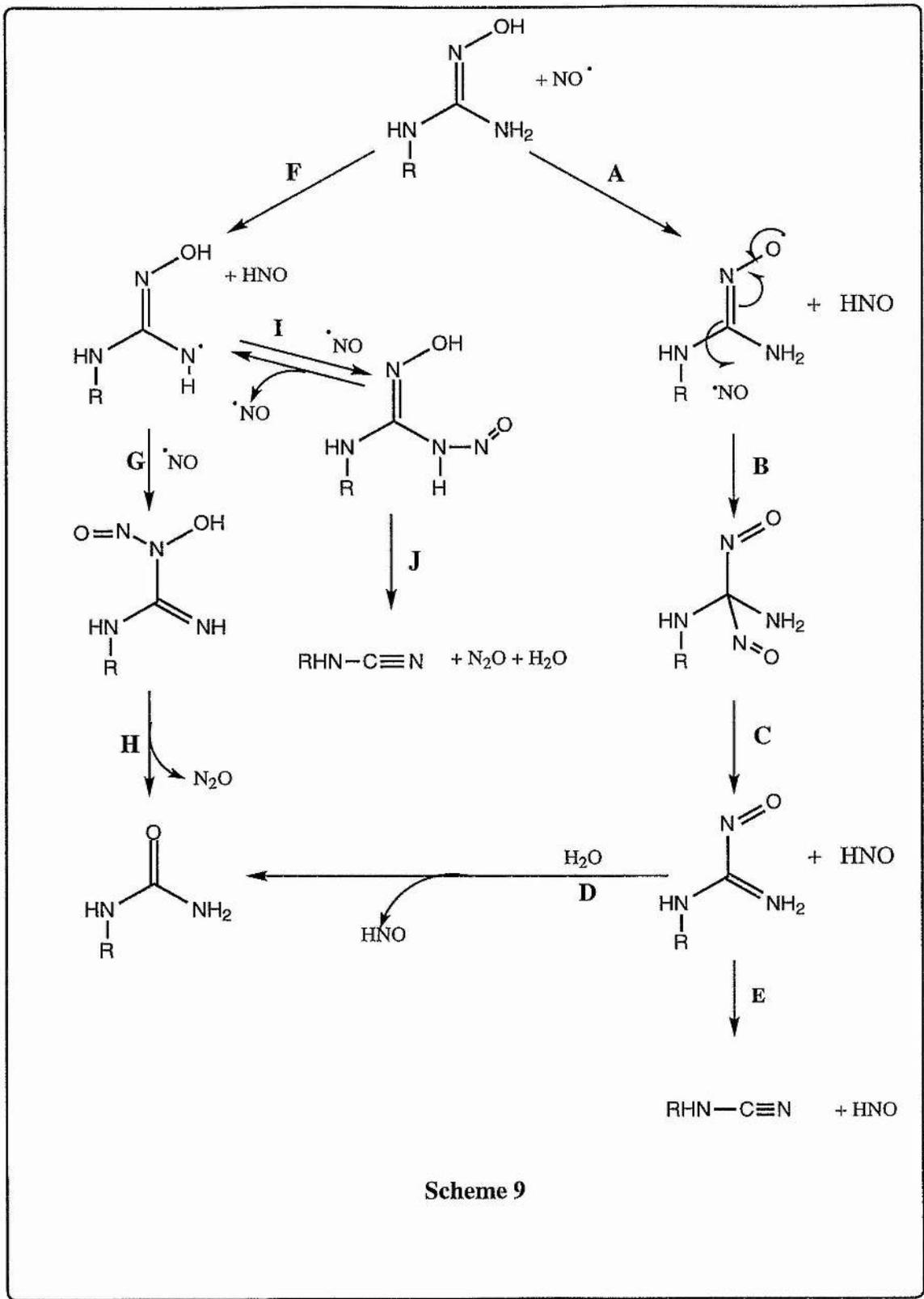
Nitrous oxide (N_2O) can be formed from the dimerisation-dehydration of nitroxyl (HNO) (Equation 1) thus N_2O detection can be used as an indication of nitroxyl generation.



However mechanisms have been proposed without the intermediacy of nitroxyl.⁴¹ The two products from the reaction, PECA (14) and PEU (13), have the

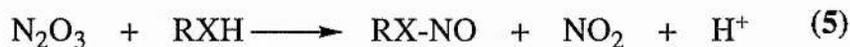
same carbon oxidation state as the carbon in the *N*-hydroxyguanidine (**22**). This suggests that oxidation has occurred at the hydroxy-substituted nitrogen. The mechanisms given (**Scheme 9**) propose that nitric oxide is initially reduced by the *N*-hydroxyguanidine (**22**) to give nitroxyl which can then dimerise and yield some N₂O. Although not considered to be a good H-abstractor it has been shown that nitric oxide will abstract a hydrogen from hydroxylamine and alkyl hydroxylamines under basic conditions.^{48,49}

Mechanism **A-B-C-D** and **A-B-C-E** (**Scheme 9**) require the nitric oxide to act as a radical trap for nitroxyl to form the dimer intermediate and all the N₂O which is produced to have come from dimerisation-dehydration of nitroxyl. Mechanisms **F-G-H** and **F-I-J** involve the H-abstraction step as the first step. Subsequent trapping of nitric oxide by the nitrogen centred species results in a *N*-nitroso compound which decomposes to give N₂O directly. Depending on whether H-abstraction occurs from the oxygen or nitrogen the product is either the urea or cyanamide.



Scheme 9

The observed acceleration with O₂ is likely to be greater than indicated as nitric oxide is unstable in the presence of O₂. This means that the aerobic reaction contains less nitric oxide than the anaerobic reaction. The reason for O₂ enhancement could be that H-abstraction by nitric oxide is slow (either **A** or **B** in **Scheme 9**) and the oxidation products from reaction of nitric oxide with O₂, e.g. ONOO[•] or NO₂ are better H-abstractors or alternatively addition of O₂ may lead to the production of N₂O₃ (**Equations 2-4**) which may then act as the nitrosating agent (**Equation 5**) degrading via either **D**, **H** or **J** to give either the cyanamide or the urea.



X=N, C or O

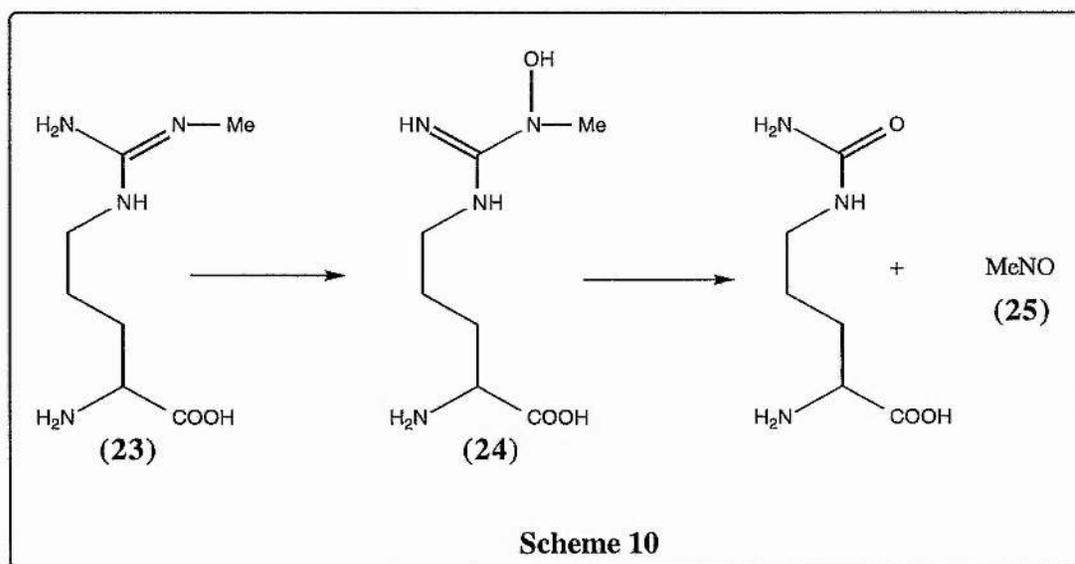
Although the mechanisms discussed are unproven they do give a reasonable explanation for the generation of other vasoactive species from the interaction of nitric oxide with *N*-hydroxyguanidines. It has been shown that *N*-hydroxyguanidines can reduce nitric oxide to produce nitroxyl and/or an nitric oxide-adduct as the product. The pharmacological activity of the reaction products may be due to either nitric oxide directly or an nitric oxide releasing intermediate. The possible *N*-nitrosated species which result from the trapping of the nitric oxide by a nitrogen centred radical (Step **G** or **I** **Scheme 9**) may lead to the re-release of nitric oxide since compounds with an analogous structure, *N*-nitrosoureas, release nitric oxide by thermal homolytic cleavage of the N-N bond.⁵⁰ Thus the apparent lifetime of nitric oxide may be prolonged by temporary "sequestration" by the oxidised *N*-hydroxyguanidine intermediate. The biological activity associated with this reaction cannot be due to N₂O as it has been shown that N₂O has no vasorelaxing properties. All this indicates that nitric oxide is capable of acting as an

oxidising agent for easily oxidised substrates that may result in the generation of other biologically active species with pharmacokinetic properties.

1.2.3 Inhibitors of NOS

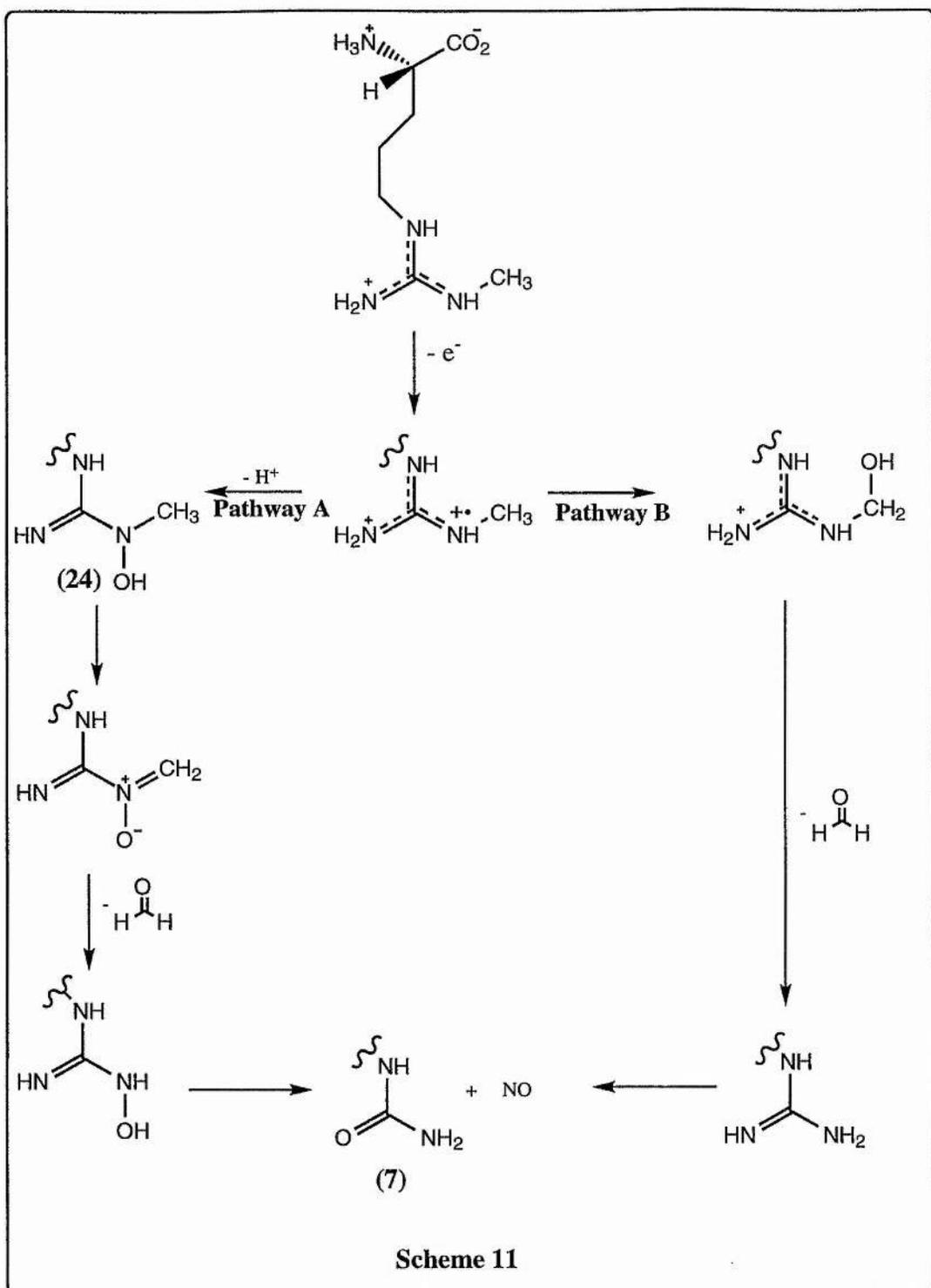
Although the NOS enzymes have a very high degree of substrate specificity, they will also transform certain arginine derivatives that act as mechanism-based inhibitors, for example *N* γ -methyl-L-arginine (NMA) (**23**). Feldman and co-workers reported work that established *N* γ -methyl-L-arginine (L-NMA) (**23**) as an irreversible inhibitor of murine macrophage nitric oxide synthase (mNOS) and recombinant constitutive brain nitric oxide synthase (bNOS).⁵¹

They originally suggested that the first step of the biosynthesis was essentially the same as for arginine i.e. an *N*-hydroxylation occurs to give *N* γ -hydroxy-*N* γ -methyl-L-arginine (**24**) (Scheme 10). This is then further oxidised to yield citrulline (**7**) and nitrosomethane radical (**25**). However the nitrosomethane radical was not observed.



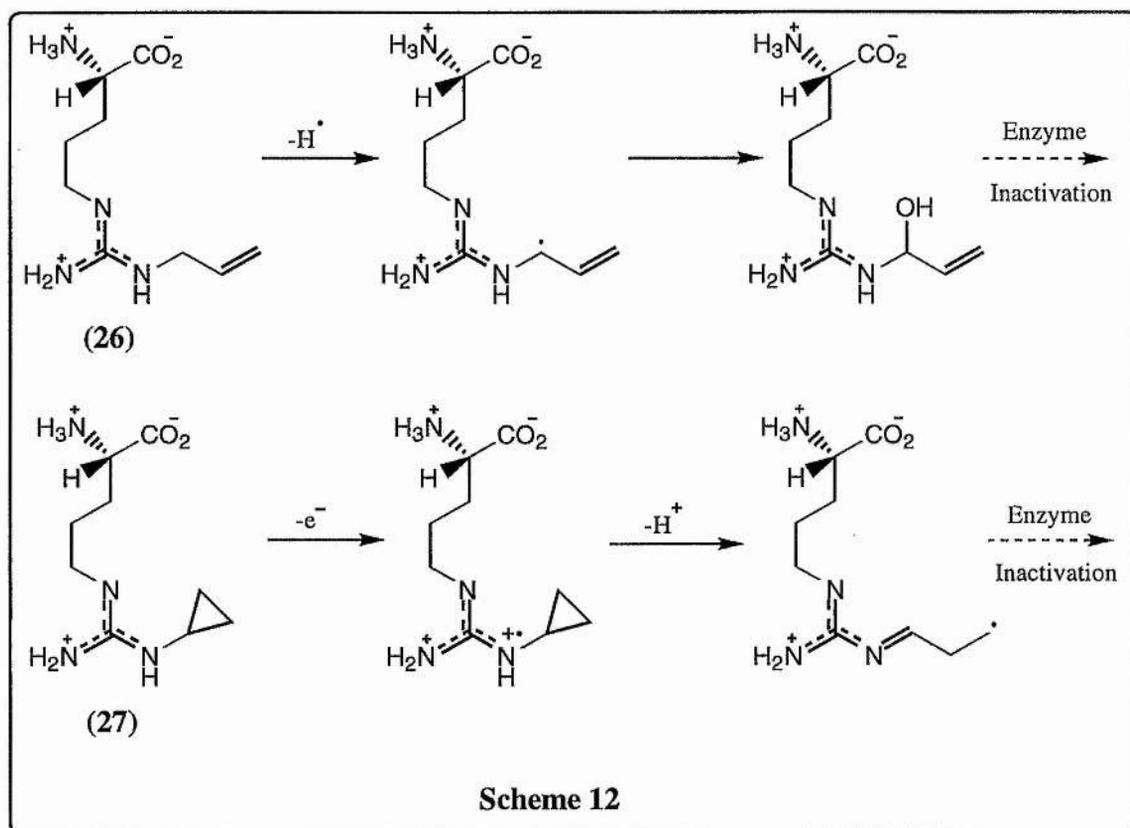
The mechanism also fails to explain the observation that formaldehyde was formed when NMA was the substrate for NOS.^{52,53} It was thus suggested that the first step of the process was a single electron oxidation to give a guanidinium radical

(Scheme 11). It was then assumed that oxygen rebound and deprotonation of the latter, similar to that of NOS monooxygenation I, then occurs to give the *N* γ -hydroxy-*N* γ -methyl-L-arginine (NHMA) (24) (Pathway A). Deprotonation and then the loss of formaldehyde would yield NOHA (6) which could then be oxidised to citrulline (7) and nitric oxide. A competing *C*-deprotonation (Pathway B) of the guanidinium radical followed by oxygen rebound could also occur. Here loss of formaldehyde from the intermediate would give the arginine, via α -amino radical and carbinolamine intermediates, which could then be oxidised to nitric oxide and citrulline (7) by the standard NOS pathway. Interestingly NHMA (24), arginine and formaldehyde have all been detected during the enzymatic reaction.



Other NOS inhibitors synthesised to try and further probe the *N*-dealkylation pathway and the mechanism of NOS inhibition include *N* γ -allyl-L-arginine (NALA) (26) and *N* γ -cyclopropyl-L-arginine (NCPA) (27).⁵⁴ These 2 examples were chosen for

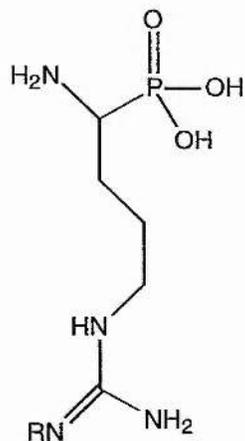
specific reasons. In the case of NALA (26) the hydrogen abstraction at the α -carbon should be promoted due to the ease of oxidation of an allylic methylene (Scheme 12). A single electron transfer from the guanidinium nitrogen in NCPA (27), should lead to a rapid rearrangement of the nitrogen centred radical to give a carbon radical which should irreversibly inhibit the enzyme (Scheme 12).



Further studies have shown that major loss of the heme chromophore accompanies enzyme inactivation.⁵⁵ There was not one major heme adduct that could be detected in these studies and it would seem that multiple modes of inactivation are possible. Radiolabelling indicates that a significant amount of NOS modification occurs when derivatives derived from *N*-methylguanidine, but lacking the amino acid side chain, are used. This is consistent with formation of the nitrosomethane radical cation.

Recent work by Cowart has concentrated on the synthesis of a number of phosphorus containing amino acid analogues as possible inhibitors.⁵⁶ They have found

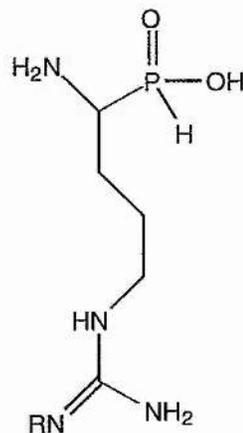
that L-NMA (**23**) displays a low selectivity for inhibition of nNOS versus iNOS. This led them to synthesise analogues with an enhanced potency and selectivity for nNOS to allow them to probe the role of nNOS in CNS pathophysiology.



(28a) R= H

(28b) R= Me

(28c) R=NO₂



(29a) R= H

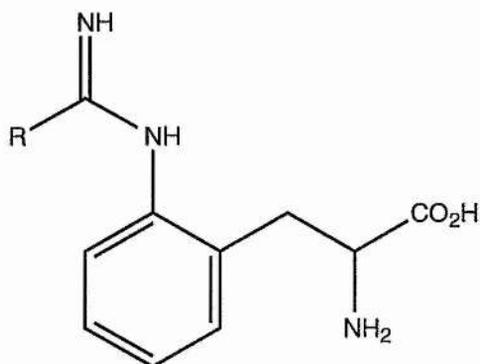
(29b) R= Me

(29c) R=NO₂

The ability of the phosphorus analogues to inhibit brain derived NOS (nNOS), or the inducible NOS from the macrophages (iNOS) was measured using the method of Bredt and Synder.⁵⁷ NOS activity was measured by the inhibition of the conversion of 10 mM [³H]-L-arginine to [³H]-L-citrulline. The nNOS was obtained from rat brain cytosol and the iNOS from lipopolysaccharide-induced RAW macrophage cytosol. The 3 α -amino- ϵ -guanidino phosphonic acids (**28a-c**) which contain phosphorus in oxidation state (v) were inactive against NOS at concentrations up to 100 mM. More success was obtained with the phosphonous acids (**29a-c**) where the phosphorus is in oxidation state (III). Two of the compounds (**29b**) and (**29c**) demonstrated inhibitory activity toward nNOS. This could be because at physiological pH the phosphonous acid contains one negative charge and exists as a zwitterion much like natural amino acid analogues where as the phosphonic acid has two negative charges at physiological pH.⁵⁸

More recently the synthesis of conformationally restricted L-arginine analogues

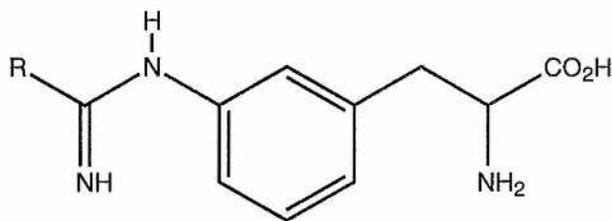
(30) and (31) and the arginine based inhibitors *N* γ -methyl-L-arginine (L-NMA) (23) and *N* γ -iminomethyl-L-ornithine (L-NIO) (32) was reported.⁵⁹



(30a) R = NH₂

(30b) R = NHCH₃

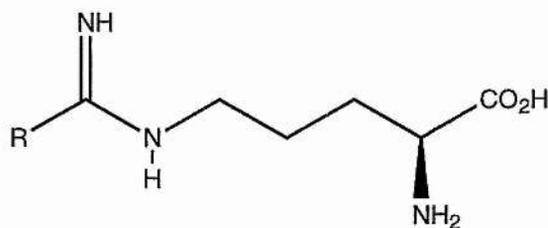
(30c) R = CH₃



(31a) R = NH₂

(31b) R = NHCH₃

(31c) R = CH₃



(23) R = NHCH₃

(32) R = CH₃

Previously it has been reported that there is diversion in the active sites of the isoforms of NOS and that it should be possible to design selective inhibitors for the different isoforms.⁶⁰ The series of conformationally restricted arginine based analogues (30a-c) and (31a-c) was synthesised and compared with the known inhibitors L-NMA (23) and L-NIO (32), which have already been shown to have no selectivity between the various isoforms.

The inhibition was determined by monitoring the conversion of L-[¹⁴C]-arginine to L-[¹⁴C]-citrulline using the method described by McMillan and co-workers.⁶¹ The results of this testing are given below (Table 1). The compounds (30a-c) and (31a-c) were all prepared as racemic mixtures although previous work has shown that the L-enantiomer of both substrates and inhibitors is required for binding at the active site. This would suggest that the inhibitory potency of the inhibitors would be increased if the pure L-enantiomers were synthesised, although this assumes that the D-isomers lack any appreciable inhibitory properties.

Table 1:- Inhibition of Human NOS by Arginine Based Inhibitors and Conformationally Restricted analogues

Compound	R	K _i , (μM) ¹		
		iNOS	eNOS	nNOS
L-arginine	NH ₂	2.20 ²	0.90 ²	1.60 ²
L-NMA (23)	NHCH ₃	0.86	0.40	0.84
L-NIO (32)	CH ₃	0.34	0.81	0.23
30a	NH ₂	2.60	0.25	0.37
30b	NHCH ₃	0.60	0.27	0.41
30c	CH ₃	1.90	1.60	1.30
31a	NH ₂	100	46	44
31b	NHCH ₃	96	8.8	8.4
31c	CH ₃	82	39	29

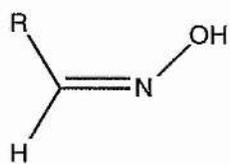
NOTE:- (1) Inhibition constants were obtained by measuring percentage inhibition at a single time point with at least 3 concentrations of inhibitor. Values have a standard deviation of $\leq 10\%$ (n=3). (2) Value represents K_m for L-arginine in μM concentration units

Compound (30a) was found to be a potent NOS inhibitor and the observed binding affinity for the isoforms is similar to that for L-arginine and since the measured

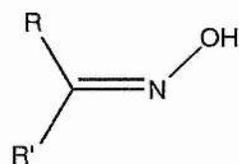
binding constant for L-arginine (**6**) is approximately the same as its K_m value then the observed values for (**30a**) are directly comparable to those for L-arginine. Therefore by introducing a phenyl ring a maximum four-fold effect was seen on binding affinity. Compound (**30b**) inhibited all three isoforms of NOS comparable to L-NMA, and also inhibited NOS-mediated relaxation of rat aortic rings in whole tissue preparations.^{62,63} Both eNOS ($IC_{50}=2.76 \mu M$) and iNOS ($IC_{50}=4.46 \mu M$) were inhibited in a dose-dependent manner which would suggest that the restriction of the L-arginine backbone chain with a phenyl group does not affect its transport into the cells. Compound (**30c**) also inhibited all 3 isoforms although with slightly less potency than the comparable arginine compound, L-NIO (**32**). The meta-substituted analogues were weaker NOS inhibitors although (**31b**) did show selectivity for both the constitutive isoforms (eNOS and nNOS). It is known that arginine binds in an orientation where the alkyl chain is folded in such a manner that allows spatial alignment of the amino acid and terminal guanidine moieties for potent binding.

1.3 P450 Oxidation

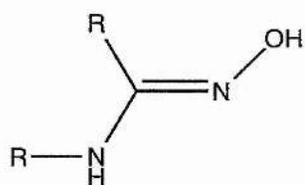
Work has shown that the P450 enzymes from rat liver would oxidise NOHA (**6**) to citrulline (**7**) and oxides of nitrogen including nitric oxide.⁶⁴ It was also found that a range of other compounds that would produce oxides of nitrogen including alkyl- and aryl-aldoximes (**33**), ketoximes (**34**), amidoximes (**35**) and guanidoximes (**36**) (**Figure 1**). These compounds all contain the grouping $C=N(OH)$. The results suggested that compounds containing a $C=N(OH)$ could act as precursors of NO and be exogenous equivalents to NOHA (**6**) for nitric oxide formation. It was concluded that the P450-catalysed oxidative cleavage reaction to give oxides of nitrogen was general for any class of compound containing the $C=N(OH)$ grouping.



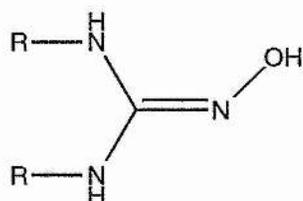
(33)



(34)



(35)



(36)

Figure 1

1.4 NO PRODUCING DRUGS

There have been many varied approaches to the design of NO delivery drugs, although much of the research so far has concentrated on their potential for antithrombotic applications. A fundamental problem is the inherent instability of compounds such as *N*-hydroxyguanidines for oral administration and therefore a range of compounds have been examined.

1.4.1 *S*-NITROSO THIOLS

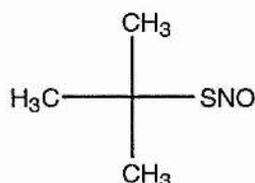
For a number of years there has been a search for alternatives to GTN (2) which is used in the treatment of diseases such as angina and other such cardiovascular conditions. Alternatives have included nitrates, notably isosorbide di- and tri-nitrates and alkyl nitrites. Further study examined *S*-nitrosothiols as alternatives. There have also been suggestions that this class of compound may be involved in the storage and transport of NO *in vivo* and this would make them ideal drug candidates.

S-Nitrosothiols can be synthesised by the electrophilic nitrosation of thiols (Equation 6) where XNO is a carrier of NO⁺ for example, H₂NO₂⁺, ClNO, BrNO, N₂O₃, N₂O₄, RONO or R₂NNO.⁶⁵ If the thiol is water soluble then the simplest method is via the use of sodium nitrite in dilute aqueous acid. Non-aqueous soluble thiols can be treated with sodium nitrite in methanol/aqueous hydrochloric acid,⁶⁶ sodium nitrite in acetic acid,⁶⁷ or *tert*-butyl nitrite in a number of organic solvents.^{68,69}

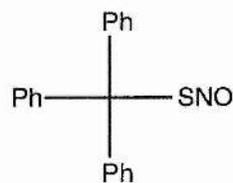


Until recently there were only a few examples of *S*-nitrosothiols known due to their instability. The stable well characterised examples include *S*-nitroso-*tert*-butyl thiol (37) and the corresponding triphenyl derivative (38). Recently the increased interest in these compounds has led to the synthesis of further stable examples including *S*-

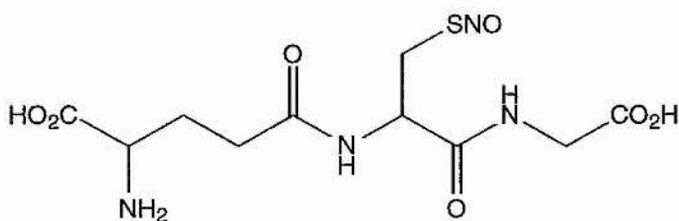
nitrosogluthathione (39), *S*-nitrosoacetylpenicilamine (40), *S*-nitrosocaptopril (41), *S*-nitrosothioglucose (42).



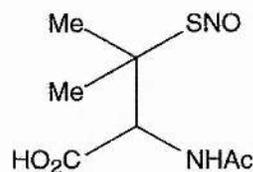
S-nitroso-*tert*-butyl thiol (37)



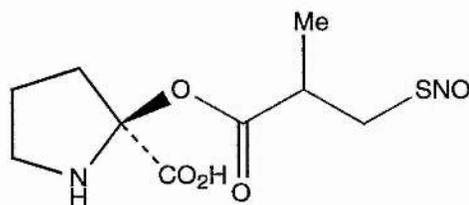
S-nitroso-triphenyl thiol (38)



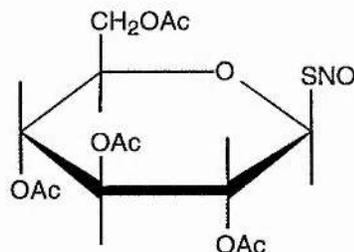
S-nitrosogluthathione (39)



S-nitrosoacetylpenicilamine (40)



S-nitrosocaptopril (41)



S-nitrosothioglucose (42)

It has been shown that *S*-nitrosogluthathione (39) (GSNO) releases nitric oxide when irradiated at either 340 or 545 nm and that the cytotoxic effect of GSNO (39) on leukaemia cells is enhanced upon radiation.⁷⁰ This could mean that GSNO (39) and other nitrosothiols might be useful as photochemotherapeutic agents.

Biologically the properties of nitrosothiols are more significant. It has been shown that many effect vasodilatation and also inhibit platelet aggregation, giving them the same properties as nitric oxide itself. The most interesting question is whether nitric oxide formation from the nitrosothiols accounts for their biological properties. Under biological conditions GSNO (39), *S*-nitrosoacetylpenicilamine (SNAP) (40), *S*-

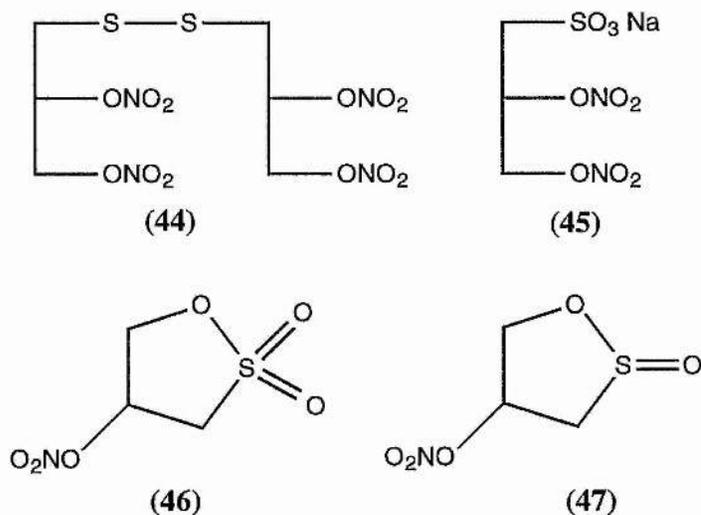
nitrosothioglucose (42) and *S*-nitrosocaptopril (41) have been shown to have biologically important properties.^{71,72} It has been shown that nitrosothiols release nitric oxide at pH 7.4 in the presence of Cu²⁺ ions, however it has been shown that the actual active species involved in the breakdown of the nitrosothiols to nitric oxide is Cu⁺.⁹ There is still debate as to whether the complete breakdown of the nitrosothiol is required to give the nitric oxide. Due to the similarities in properties between nitrosothiols and nitric oxide it could be suggested that this is the case. It has been shown that for *S*-nitrosocysteine (43) the formation of cyclic GMP (a precursor for vasodilation) is inhibited by the presence of a Cu⁺ chelator, bathocuprione sulfonate.⁷³ This suggests that nitric oxide formation is essential for the biological properties of these compounds. However another group has shown that bronchodilation in guinea pigs is brought about by intact GSNO (39) and not by the formation of nitric oxide. This is because the effects were not inhibited by haemoglobin which traps nitric oxide.⁷⁴ This fundamental question clearly needs to be resolved.

Nitrosothiols may play an important role in the *in vivo* storage of nitric oxide. The rapid nitric oxide group exchange may be important given the wide reactivity range of RSNO compounds towards nitric oxide release. For example stable GSNO (39) is readily converted to a more reactive *S*-nitrosocysteine (43) by the presence of cysteine.

However these compounds do work *in vivo* and there is no doubting that their biological properties may give them widespread clinical uses in years to come. It has been shown, in preclinical trials, that GSNO (39) is very good at inhibiting platelet aggregation following coronary angioplasty,⁷⁵ and also in the treatment of preeclampsia, a high blood pressure condition that affects pregnant women.⁷⁶ The potential of *S*-nitrosoglucose (42) and related compounds in the treatment of a number of diseases has also been studied. Glyco-*S*-nitrosothiols also outperform SNAP (35) in aqueous solubility and stability, with and without EDTA and in the presence or absence of Cu²⁺.⁷⁷

1.4.2 NITRITE ESTERS

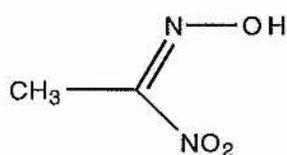
Thatcher and co-workers have synthesised four novel sulfur containing nitrate esters in which the sulfur is β to the nitrite ester (**44-46**).⁷⁸



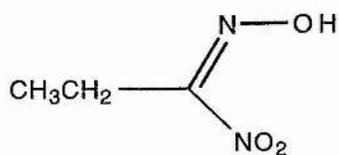
The compounds are designed to be vasodilators or function as NO prodrugs based upon the action of GTN (**2**). Three out of the four compounds, the two mononitrites (**46,47**) and the dinitrate (**45**) were shown to activate GCase at a higher level than GTN which is used in the treatment of angina pectoris. These preliminary results on the activation of soluble guanylate cyclase and relaxation of smooth muscle suggests that these compounds may be a new class of nitrate esters with potential therapeutic significance.

1.4.3 NITROLIC ACIDS

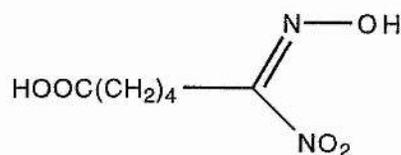
Rehse and co-workers investigated the antithrombotic and vasodilatory activities of five nitrolic acids (**48-52**).⁷⁹



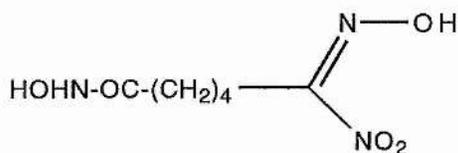
(48)



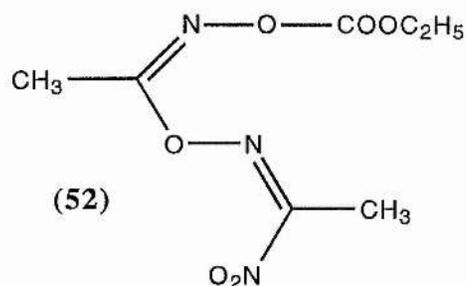
(49)



(50)



(51)



(52)

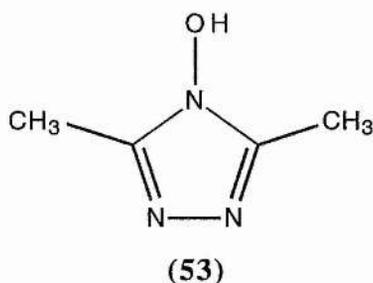
These compounds were shown to release nitroxyl (HNO), which in turn breaks down to form nitric oxide. They are activated by the electron withdrawing nitro group and HNO was found to be released from both the hydroxyimino and nitro group. Inhibition of thrombus formation in rats was found to be 69% and 46% in arterioles and venules respectively after oral administration. Only a 10% drop in blood pressure was observed in these experiments, indicating that antithrombotic and vasodilatory effects can be dissociated, which is an important consideration in the design of nitric oxide donor drugs.

1.4.4 1,3,4-TRIAZOL-1-OLES, OXATRIAZOLES AND NITROSOHYDRAZINES

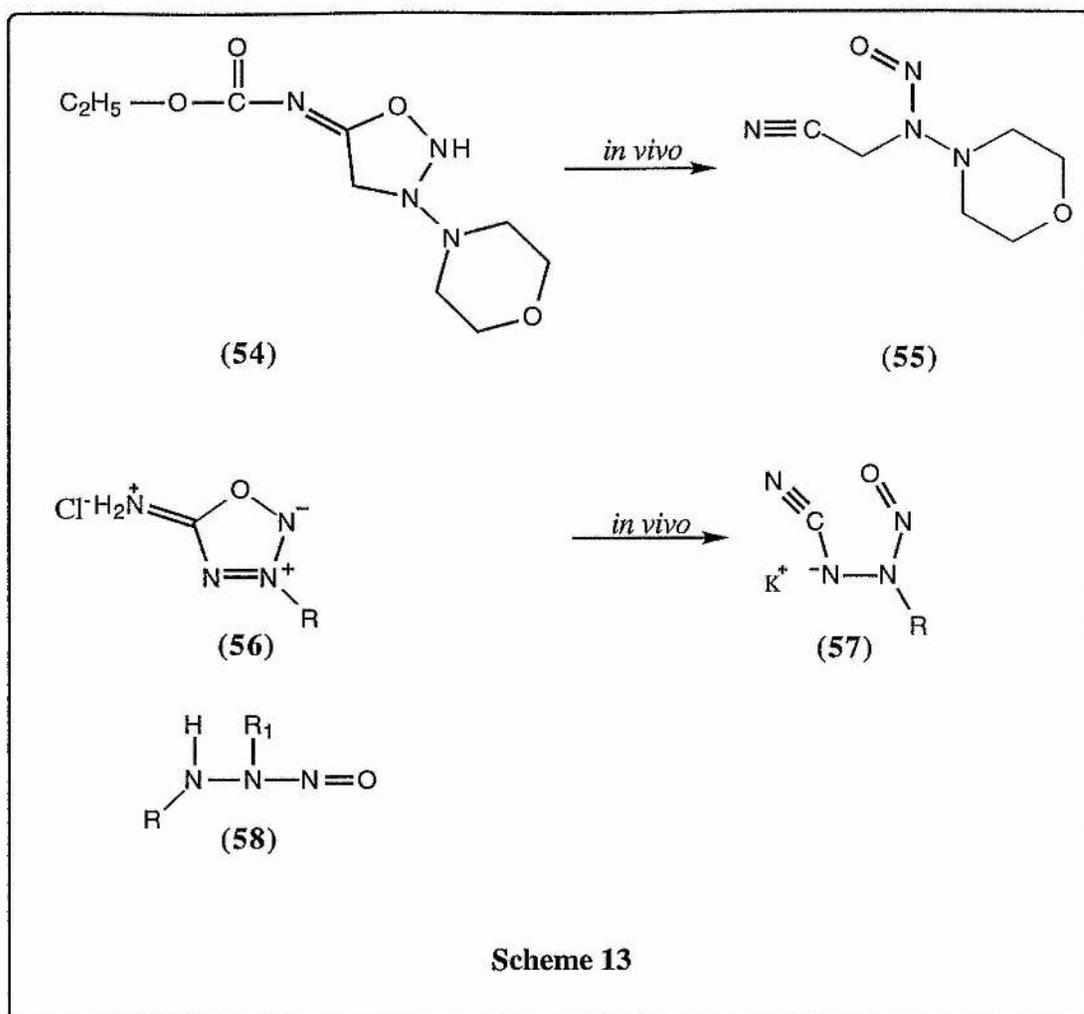
Investigation of 1,3,4-triazol-1-oles, oxatriazoles and nitrosohydrazines was based on the success of the nitrolic acids as nitric oxide donors. It was believed that the use of alternative electron withdrawing groups to the nitro group may also yield valuable results.

Five 1,3,4-triazol-1-oles were synthesised and screened for their antithrombotic

properties.⁸⁰ The screening was performed on rats and resulted in a small but significant drop in blood pressure, indicating that an intermediate capable of giving rise to nitric oxide was produced in significant quantity. Although the evidence was strong it was not entirely conclusive. The compounds were administered orally and so their pharmacokinetic viability was also determined. One compound (**53**) was found to have significant activity relative to the others tested. This activity may be due to its small size and lipophilicity.

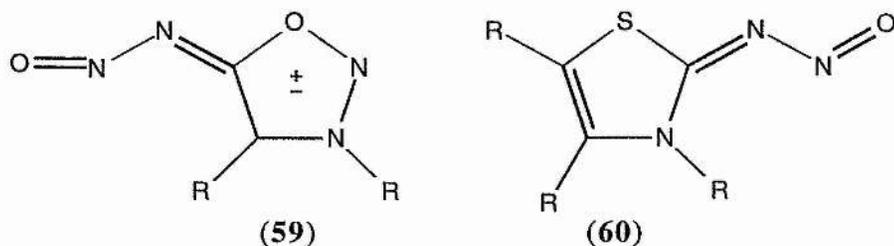


The related mesoionic oxatriazoles and noncyclic nitrosohydrazine derivatives have also been found to inhibit thrombus formation.⁸¹ These compounds are also thought to act by the evolution of nitric oxide. The ring opening reaction of the oxatriazolimine (**54**) exposes the nitrosohydrazine structure (**55**) which is thought to be responsible for nitric oxide production. Molsidonine (**56**), another nitric oxide donor drug, produces a similar intermediate (**57**) following enzymatic hydrolysis (**Scheme 13**). A series of acyclic nitrosohydrazines (**58**) were also studied. Active compounds were identified from both the oxatriazolimines and nitrosohydrazines. There were marked differences between the *in vitro* and *in vivo* results, which strongly suggested an enzyme mediated pathway and the importance of absorbability and stability of the drug in the gastrointestinal tract.

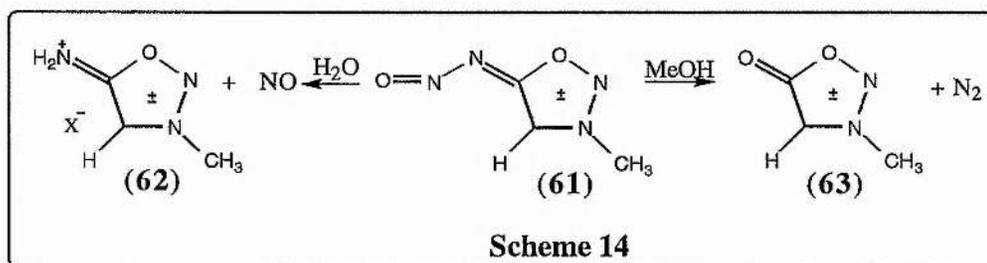


1.4.5 NITROSOSYNDNONE-5-IMINES AND THIAZOLE-2-NITROSIMINES

Extensive studies have been carried out on the use of nitrosimines as potential NO donor drugs.⁸² Initial studies concentrated on the chemical reactivity of these compounds to gain an insight into their pharmacological properties.



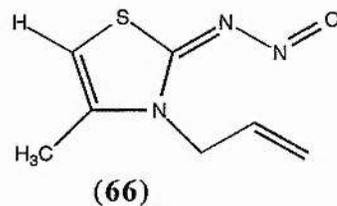
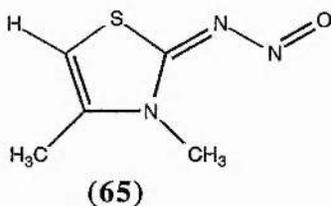
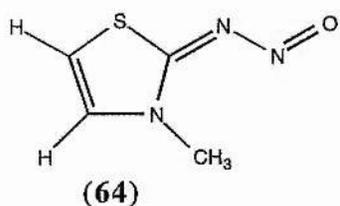
Both nitrososydnone-5-imines (**59**) and thiazole-2-nitrosimines (**60**) undergo a photolytic cleavage of the C=N-NO bond.⁸³ This reaction gives rise to the NO radical which is believed to be responsible for many physiological processes. In this study nitric oxide production was found to be dependent on the solvent. The nitrososydnoimines (**61**) were found to decompose photolytically in water to give exclusively the syndone imine (**62**) and nitric oxide. The reaction rate was enhanced when the reaction was performed under nitrogen instead of air. In methanol, however, formation of both compound (**62**) and the syndone compound (**63**) occurred giving rise to nitric oxide and nitrogen respectively (Scheme 14).



Similar solvent effects were observed in the decomposition of thiazole-2-nitrosimines (**60**). This illustrates the complex nature of these compounds and suggests that when designing drugs, great care must be taken to determine which reactions occur *in vivo* and to what extent and to assess the biological impact of the by-products.

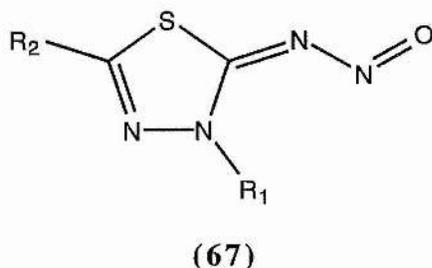
This work was followed up by screening 24 thiazole-2-nitrosimines, in both cell free and *in vivo* systems, for antithrombotic properties.⁸¹ Inhibition of platelet aggregation was assessed using the *Born*-test (inhibition of collagen induced platelet aggregation in human plasma). All the compounds that were tested by *in vitro* methods,

where cleavage of the C=N-NO bond was achieved by visible light, exhibited some inhibition properties. The variation observed was approximately within one order of magnitude. There was, however, no observable logical relationship between the nature and position of the substituents and the activity observed. Further complications occurred when the three most potent compounds were tested *in vivo*. This was done by testing their inhibition of laser induced thrombus formation in rats. Out of the compounds tested one showed no activity at all (64), and the other two compounds (65,66) were equipotent *in vivo* despite showing a considerable disparity in their *in vitro* rates with (65) showing a rate much lower than that of (66).



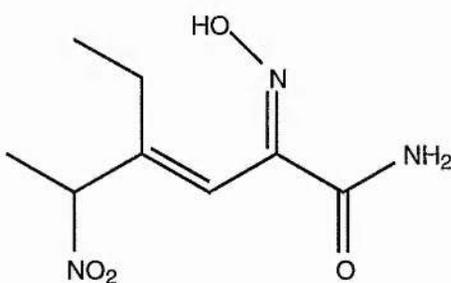
These results are not entirely surprising considering that the mechanisms are vastly different between *in vivo* and *in vitro* testing. The enzymatic interactions involved in the *in vivo* pathways are likely to mean that the process is more selective than the *in vitro* process. This illustrates that sometimes simplified models of biological systems are not accurate.

The potential of thiadiazole nitrosimines (67) was also investigated.⁸⁴ These compounds, however, showed poor activity due to their chemical stability.



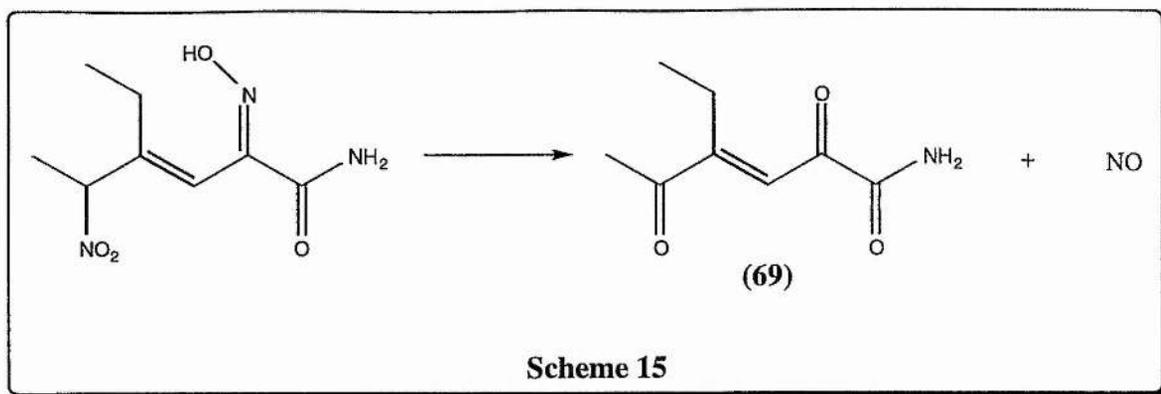
1.4.6 FK 409

Recent work has been done by Fontecave and co-workers involving FK 409 (68). FK 409 (68) was isolated from the fermentation broth of *Streptomyces griseoporeus* as a semifermentation product. They showed that FK 409 (68) rapidly decomposes in aqueous alkaline solution at 37 °C releasing nitric oxide but is unaffected in acid solution (pH 2.0, $k = 9.28 \times 10^{-5} \text{ min}^{-1}$; pH 8.0, $k = 2.93 \times 10^{-2} \text{ min}^{-1}$).^{85,86} Measurement of the of release of nitric oxide was carried out using various methods including chemiluminescence analysis, ESR spectroscopy (using carboxy-PTIO) and nitrite analysis using the Griess test.⁴⁶ The rate of spontaneous nitric oxide release was approximately parallel with that of the decomposition in aqueous solution.



(68)

This compound has a potent pharmacological action, which has been attributed to the increase in concentration of intracellular cGMP levels. Although there are several reports about the pharmacological actions of FK 409 (68), the mechanism of nitric oxide release has not yet been elucidated. Fontecave proposed that the FK 409 may decompose into (69) and nitric oxide although the mechanism of formation is unclear (Scheme 15).

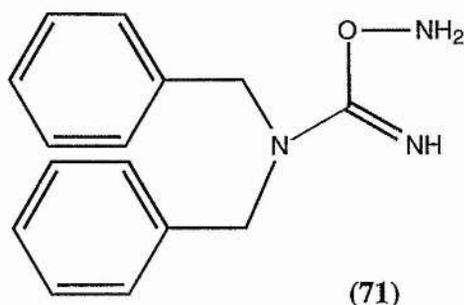
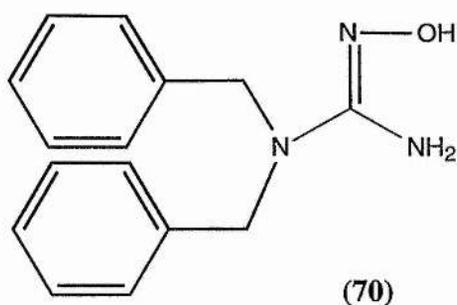


1.5 N-HYDROXYGUANIDINES AND RELATED DERIVATIVES

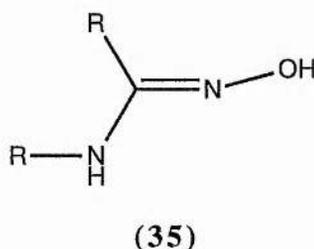
As reported previously *N*-hydroxyguanidines (**36**) have been shown to release nitric oxide following the action of P450 enzymes and for this reason have become a focus of work in the area of nitric oxide donor drugs. There are, however, a number of other applications of these compounds (**Section 2.7.2**)

1.5.1 Nitric Oxide Donating Properties

Recent work at St Andrews investigated the use of *N*-hydroxyguanidines as nitric oxide donor drugs.⁸⁷ It was found that they were difficult to synthesise without contamination from the corresponding urea and that subsequent separation from the urea was difficult. 1,1-Dibenzyl-2-hydroxyguanidine (**70**) was the only hydroxyguanidine obtained in a microanalytically pure form. The urea by-product appears to be formed via the unstable aminoxyformamidine (**71**) or by hydrolysis of the cyanamide intermediate.



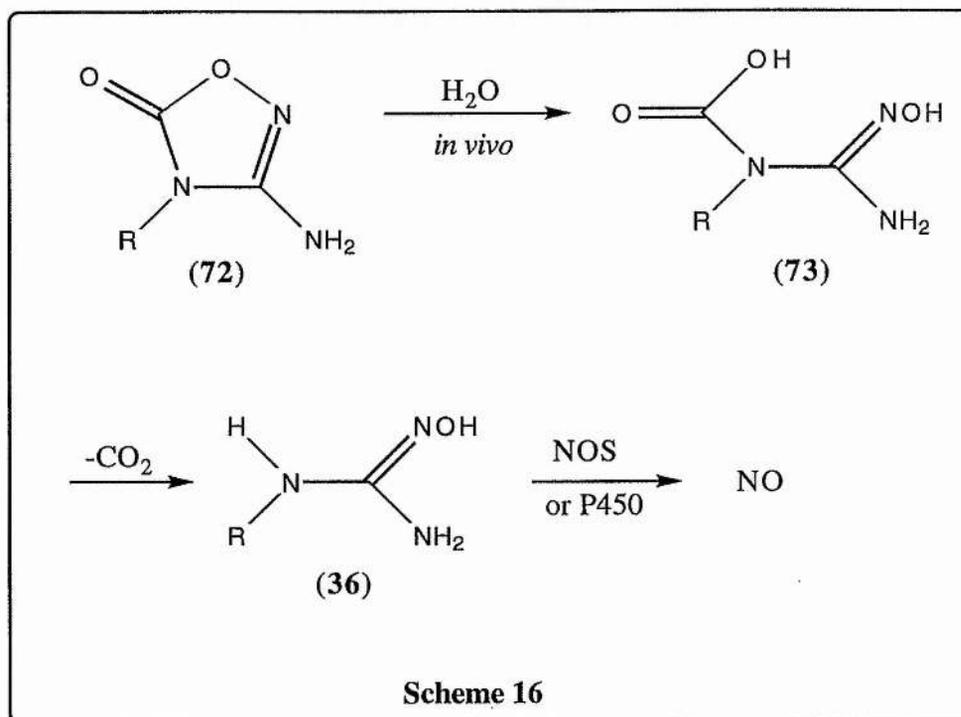
More success was achieved when the compounds were made via the thiourea derivatives, in particular aminoiminomethanesulfonic acids. The compounds that were produced, although still contaminated with the corresponding urea were purer. It was shown that hydroxyamidines (**35**) were easier to synthesise and handle and were structurally quite close to hydroxyguanidines.



Both hydroxyguanidines (**36**) and hydroxyamidines (**35**) are physiologically active as vasodilators and have also been shown to produce nitric oxide under physiological conditions. The hydroxyguanidines (**36**) were found to be active at lower concentrations and they also reached a maximum activity over a smaller concentration range. The hydroxyamidines (**35**) were found to be less active than hydroxyguanidines and had a wider activity range. However the concentrations at which they relaxed the artery was not determined as using these compounds at the high concentrations required may have damaged the artery.

There have been a number of potential vasoactive compounds designed around the *N*-hydroxyguanidine template. The use of 3-amino-1,2,4-oxadiazol-5-ones (**72**) as possible *N*-hydroxyguanidine prodrugs has been reported.⁸⁸ These cyclic compounds undergo hydrolysis *in vivo* to yield the free *N*-hydroxyguanidine derivative (**73**) which

in turn lose carbon dioxide to yield the free *N*-hydroxyguanidine (36) (Scheme 16). This free *N*-hydroxyguanidine (36) can then undergo reaction with NOS or P450 enzymes to yield nitric oxide. Biological testing has shown that these compounds have significant antithrombotic activity.



The authors submitted compounds (72a-s) (Table 2) to an *in vivo* thrombosis model.⁸⁹

TABLE 2:- Antithrombotic effects of type 72 1,2,4-oxadiazol-5-ones or acetylsalicylic acid (ASA). 2h after p.o administration of 60 mg/kg to rats. Statistics: Man-Whitney U-Test: n.s = not significant

Compound	R	Inhibition of thrombus formation in	
		arterioles % \pm s (p ²)	venules % \pm s (p ²)
72a	Methyl	6 \pm 3 (0.01)	6 \pm 3 (0.05)
72b	<i>n</i> -butyl	10 \pm 2 (0.002)	5 \pm 3 (0.02)
72c	<i>n</i> -pentyl	15 \pm 2 (0.002)	15 \pm 4 (0.002)
72d	<i>n</i> -hexyl	13 \pm 2 (0.002)	7 \pm 4 (0.02)
72e	<i>n</i> -octyl	5 \pm 4 (0.05)	n.s
72f	benzyl	17 \pm 3 (0.002)	12 \pm 2 (0.002)
72g	cyclohexylmethyl	6 \pm 3 (0.01)	6 \pm 3 (0.05)
72h	4-chlorobenzyl	8 \pm 2 (0.01)	4 \pm 2 (0.1)
72i	4-nitrobenzyl	8 \pm 2 (0.01)	7 \pm 2 (0.002)
72j	2-phenethyl	16 \pm 2 (0.002)	12 \pm 3 (0.002)
72k	3-phenylpropyl	17 \pm 3 (0.002)	17 \pm 5 (0.002)
72l	3-cyclohexylpropyl	n.s	n.s
72m	a	11 \pm 4 (0.01)	11 \pm 3 (0.002)
72n	1-naphthylmethyl	9 \pm 2 (0.002)	4 \pm 2 (0.02)
72o	cinnamyl	6 \pm 2 (0.01)	7 \pm 3 (0.1)
72p	b	13 \pm 2 (0.002)	9 \pm 3 (0.002)
72q	3-aminopropyl	8 \pm 2 (0.01)	7 \pm 3 (0.01)
72r	c	n.s	n.s
72s	1,6-hexane-bis	5 \pm 2 (0.05)	n.s

a = 3-phthalimidylpropyl: b = 5-ethoxycarbonylbutyl:

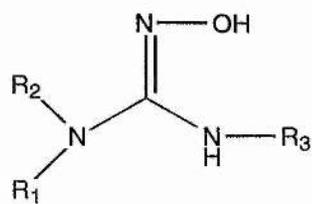
c = (CH₂)₃-CH(NHAc)-COOCH₃

The results for **72c** and **72d** reflect their affinity for cell membranes and their possible penetration into the cells where the expected metabolism could occur. The authors have shown in previous studies that arylalkyl substituents exhibit “membranotropic” properties.⁹⁰ To test this theory in this case they synthesised **72f, h, i, j, k, m, n** and **o**. The maximum effects were found for the compounds with simple aromatic groups attached i.e. **72f, j** and **k**. The derivative **72c** was prepared as a *N*-hydroxyguanidine analogue, however no significant activity was observed. The introduction of the ethoxycarbonyl group **72p** was reasonably well tolerated and a significant activity was observed. However introduction of a basic group at a similar distance from the oxadiazolone decreased the thrombotic activity. Consequently **72r** was synthesised, which contained an acylamine group, as a prodrug for *N*-hydroxyguanidine but this compound showed no antithrombotic activity.

Also, when the aromatic ring was substituted as in compounds **72h** and **i** the antithrombotic activity was reduced. The same was true for complete hydrogenation of the aromatic ring to give the corresponding cyclohexyl derivatives **72g** and **l**.

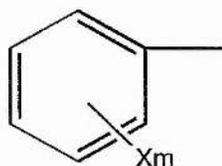
1.5.2 Other Medicinal Applications

It was reported by Cherkofsky that certain mono and disubstituted hydroxyguanidines are useful as metal chelating agents and as antidepressants.⁹¹ The author described two different series of compounds and their uses. The first series (**74**) are useful as metal chelating agents (**Figure 2**).



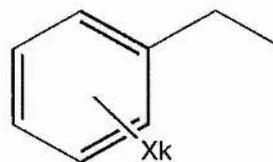
(74)

Where $R_1 =$



(a)

OR



(b)

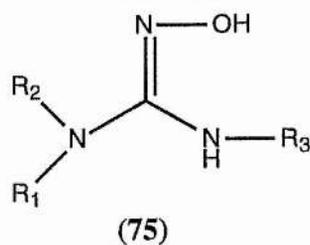
X = F, Cl, Br, CF_3 , NO_2 , C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 alkylthio or C_1-C_6 dialkylamino

m = 1 to 3

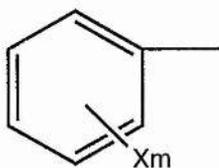
k = 0 to 3 with the proviso that no more than one NO_2 grp is present

Figure 2

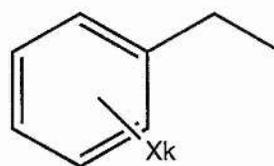
The second series of compounds (75) are useful as metal chelating agents and as antidepressants in the central nervous system of warm blooded animals (Figure 3).



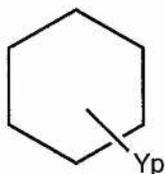
Where $R_1 =$



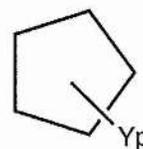
OR



OR



OR



$X = \text{F, Cl, Br, CF}_3, \text{NO}_2, \text{C}_1\text{-C}_6 \text{ alkyl, C}_1\text{-C}_6 \text{ alkoxy, C}_1\text{-C}_6 \text{ alkylthio or C}_1\text{-C}_6 \text{ dialkylamino}$

$Z = \text{F, Cl, Br, CF}_3, \text{NO}_2, \text{C}_1\text{-C}_6 \text{ alkyl, C}_1\text{-C}_6 \text{ alkoxy, C}_1\text{-C}_6 \text{ alkylthio or C}_1\text{-C}_6 \text{ dialkylamino}$

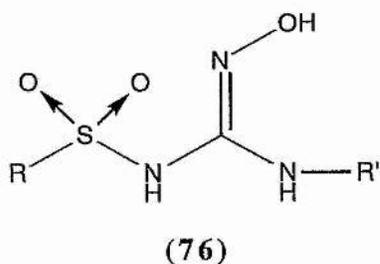
$k = 0 \text{ to } 3 \text{ with the proviso that there are no more than } 1 \text{ NO}_2$

$Y = \text{F, Cl, Br or C}_1\text{-C}_6 \text{ alkyl}$

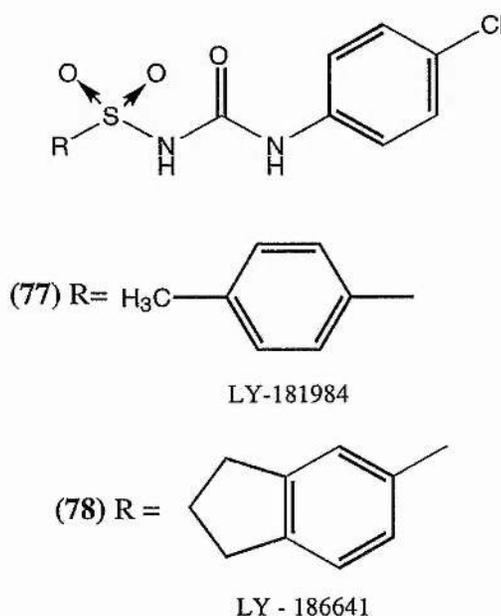
$p = 0 \text{ to } 3$

Figure 3

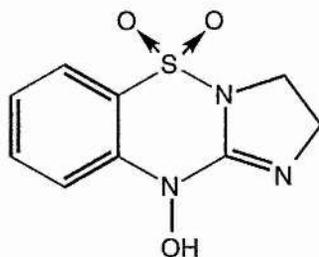
Recently the synthesis of a series of sulfonyl-*N*-hydroxyguanidines (76) as potential antitumour agents was reported.⁹²



The compounds were designed and synthesised on the basis of the lead compound LY-181984 (77) which is a sulfonyleurea and constitutes an important class of therapeutic agents in medicinal chemistry. LY-181984 and its related compound LY-186641 (78) have shown a wide range of activity in several solid tumour models and LY-186641 is in extensive clinical trials based on its pre-clinical activity and lack of toxicity to proliferating normal tissues.^{93 94} These compounds were discovered by utilising *in vivo* tumour screening models to overcome the poor correlation between cytotoxicity and antitumour activity. It has been shown that the sulfonyleureas accumulate in the cell mitochondria suggesting that it might be the target sight for the antitumour activity. The problem however with these compounds is that they are susceptible to hydrolysis under physiological conditions.⁹⁵

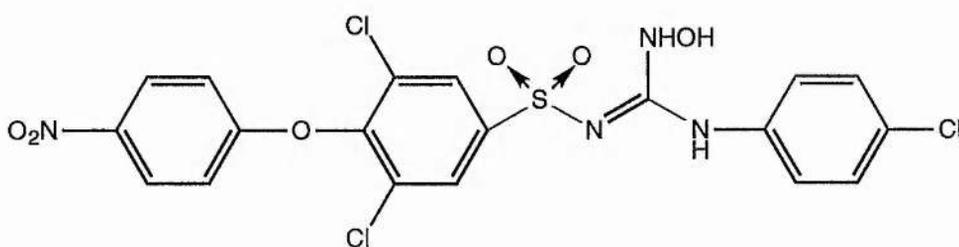


Recently Chern and co-workers reported that 2,10-dihydro-10-hydroxy-3*H*-imidazo[1,2-*b*][1,2,4]benzothiadiazine 6,6-dioxide (**79**), which contains a built-in sulfonylhydroxyguanidine functionality, showed activity against several tumour lines.⁹⁶ The hydroxyguanidine functional group combines the imino group of guanidine with the hydroxylamino group of hydroxyurea which has been reported to exhibit potent antiviral and anticancer activity by inhibition of ribonucleotide reductase.⁹⁷

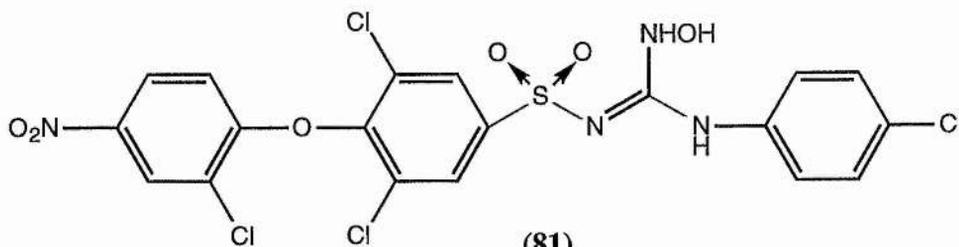


(79)

Although all the sulfonyl-*N*-hydroxyguanidines (**76**) tested showed an equal or greater cytotoxic activity when compared to LY-181984 (**77**), *N*-(4-chlorophenyl)-*N'*-[3,5-dichloro-4-(4-nitro-phenoxy)phenyl]sulfonyl-*N''*-hydroxyguanidine (**80**) and *N*-(4-chlorophenyl)-*N'*-[[3,5-dichloro-4-(2-chloro-4-nitrophenoxy)phenyl]sulfonyl]-*N''*-hydroxyguanidine (**81**) were particularly promising.



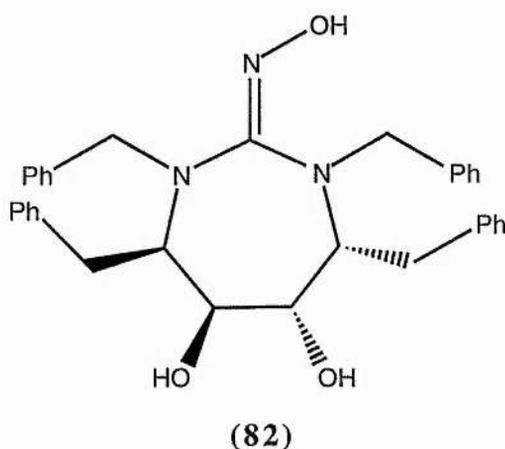
(80)



(81)

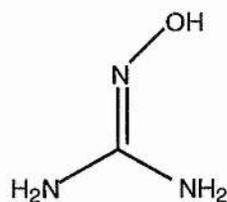
One compound (**81**) gave enhanced activity against COLO 205, KB and TSGH 8302 and was therefore examined against solid tumour lines in a murine K-1735/M2 melanoma xenograft model along with LY-181984. The tumour growth inhibition was 64.7 and 70.7 % for LY-181984 and (**81**) respectively 20 days after tumour transplantation, in addition to this it seemed that (**81**) was less toxic. As yet the pharmacological mechanism has been determined.

Jadhav and co-workers recently reported the synthesis of a 7-membered cyclic hydroxyguanidine (**82**) as possible HIV protease inhibitor.



The compound was synthesised and then tested as an inhibitor and found to have an inhibition constant of 42 nM for HIV Protease. This value is about 10-fold higher than for the corresponding cyclic urea and could be further optimised by the variation of the substituents.

Recent work by a number of groups has shown that the parent hydroxyguanidine (HOG) (**22**) exhibits cytotoxicity in human leukaemia cell line⁹⁸ and antitumour activity *in vivo* against a variety of experimental murine tumours via an unknown mechanism.⁹⁹

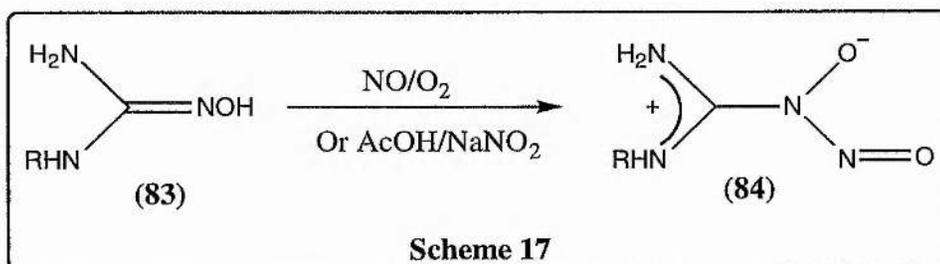


(22)

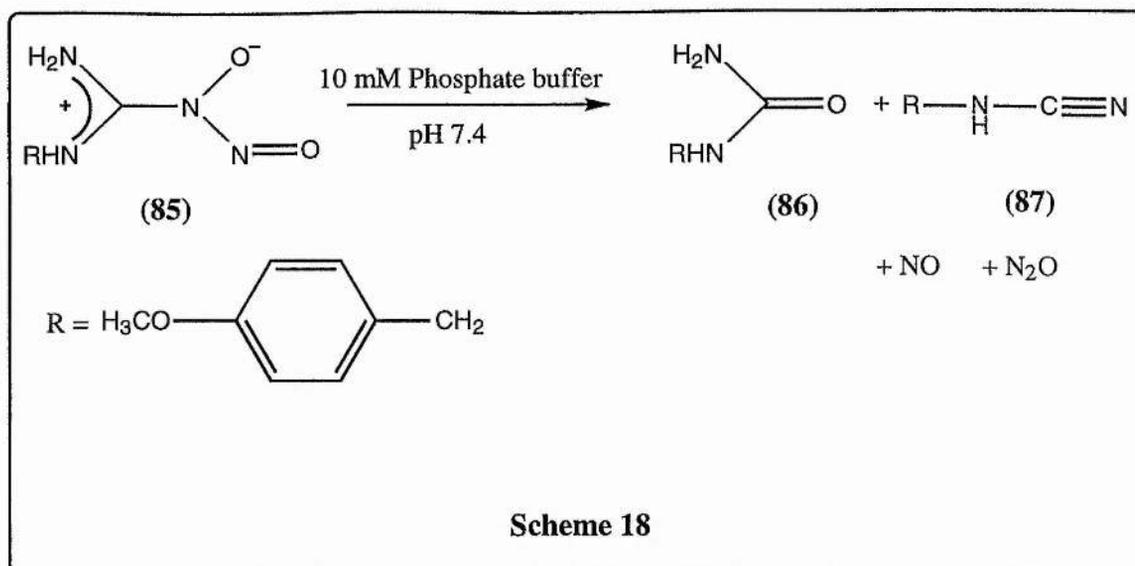
The cytostatic effects of hydroxyguanidine (22) on cell growth do correlate with a key enzyme in DNA biosynthesis, ribonucleotide reductase, via a different mechanism to structurally similar compounds, for example hydroxyurea.¹⁰⁰ Nitric oxide can also induce cytostasis in macrophages by scavenging the tyrosyl radical of ribonucleotide reductase¹⁰¹ and thus causing oxidative damage which may be responsible for necrotic and apoptotic cell death.¹⁰² Everett and co-workers proposed that the elimination of nitric oxide from HOG represents a possible mechanistic basis for both the cytotoxic and cytostatic effects of HOG that have both been observed *in vitro* and *in vivo*.¹⁰³ This means that oxidative denitrification of HOG must be possible via NOS-independent pathways since cytotoxicity and cytostasis have both been observed in leukaemia cells which lack both isoforms of NOS. The authors probed the release of nitric oxide from HOG and found that a one electron oxidation causes a fast and efficient denitrification of HOG and release of NO. It was also found that oxygen participated in the reaction mechanism. It did not prevent elimination of NO but it may have, promoted the formation of more damaging nitrogen oxides, for example ONOO⁻, which may account for the aerobic toxicity. Clearly an enzyme that could perform a one-electron oxidation of HOG could produce NO. It has been shown, for example, that peroxidases catalyse the oxidative denitrification of HOG.⁹² HOG induces cytostasis and apoptosis in tumour cells, which are devoid of NOS, via mechanisms that involve the oxidative release of NO.¹⁰⁴ The investigation of the oxidative denitrification of HOG has provided a mechanistic basis for the HOG-induced NO production *in vitro* and supports the notion that *N*-hydroxyguanidines (36) may be a potential controllable NO source.

Recent work has shown that mono substituted *N*-hydroxyguanidines (83) can be

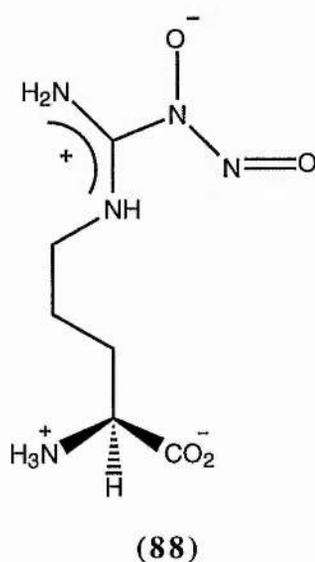
converted to zwitterionic diazeniumdiolates (**84**) which hydrolytically dissociate at physiological pH to give both nitric oxide and nitrous oxide.¹⁰⁵ The diazeniumdiolates are produced from the corresponding *N*-hydroxyguanidine (**83**) using either NO/O₂ or acetic acid/sodium nitrite (**Scheme 17**).



The authors became interested in these compounds after it had been recently reported that the formation of a 'potential intercellular nitric oxide carrier' occurred when *N*-hydroxy-L-arginine was exposed to aerobic nitric oxide solutions.¹⁰⁶ The intermediate was UV-active and longer lived as a vasodilator than nitric oxide itself. At pH 7.4 the *p*-methoxybenzyl diazeniumdiolate (**85**) derivative decomposes to give *N*-(*p*-methoxybenzyl)urea (**86**) and a small amount of *N*-(*p*-methoxybenzyl)cyanamide (**87**) and 0.5 moles of nitrous oxide were produced along with 0.3 moles of nitric oxide per mole of (**85**) dissociated (**Scheme 18**). The diazeniumdiolate also has a ultraviolet maxima at 320 nm.



The authors data suggested that if *N* γ -hydroxy-L-arginine (**6**) could encounter suitable nitrosating conditions *in vivo* the corresponding diazeniumdiolate (**88**) would constitute a naturally occurring spontaneous nitric oxide donor. Two natural products, which contain a diazeniumdiolate functionality, dopastin and alanosine, release nitric oxide only when exposed to a one electron oxidation.^{107,108} Also the nitric oxide generating properties of these compounds may give them potential as prodrugs in the treatment of clinical disorders where there is a deficiency of biosynthetic NO.¹⁰⁹

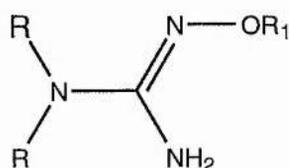


Chapter 2

Synthesis and Properties of *N*-Hydroxyguanidines

2.1 AIMS

The introduction has given a review of the biological importance of nitric oxide and the possibilities in this area of research. This project is concerned with the synthesis of a number of *O*-substituted *N*-hydroxyguanidines (**89**).



(89)

The major challenge in using nitric oxide delivery drugs as therapeutic agents is selectively directing the drug and thus the nitric oxide to the required site of action. This is especially important because of the variety of biological roles played by nitric oxide.

N-Hydroxyguanidines can act as a convenient source of nitric oxide because the action of non-specific oxidases e.g. P450 will release nitric oxide.⁶⁴ It is also possible that nitric oxide synthase (NOS) could act upon these compounds and release nitric oxide. It should then be possible to introduce selectivity by chemically blocking the hydroxyl group of these compounds which means that nitric oxide release can only occur after this group has been removed either by an enzyme or by physiological action. Thus careful manipulation of this group might lead to a nitric oxide prodrug.

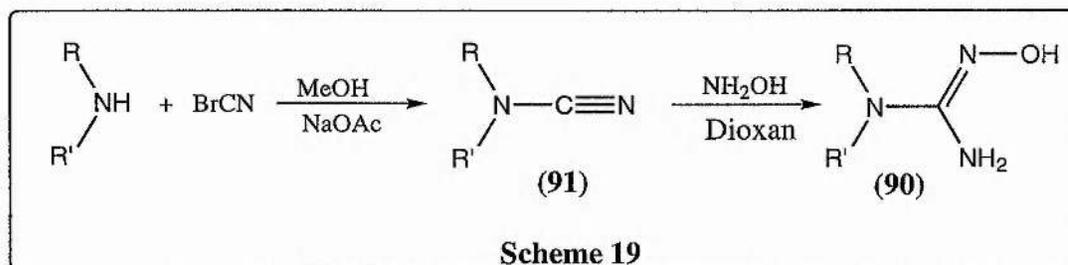
In order to study these compounds the free *N*-hydroxyguanidines first need to be synthesised and tested to ensure that they are indeed nitric oxide donors.

2.2 LITERATURE SYNTHESIS

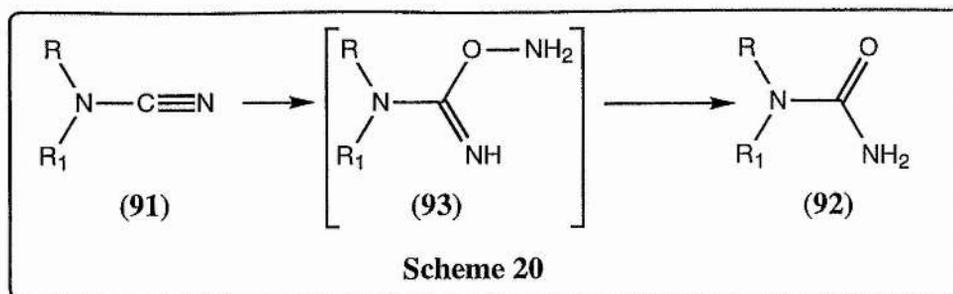
N-Hydroxyguanidines are inherently unstable and there are very few reported literature syntheses of these compounds.

2.2.1 Synthesis Via Cyanamides

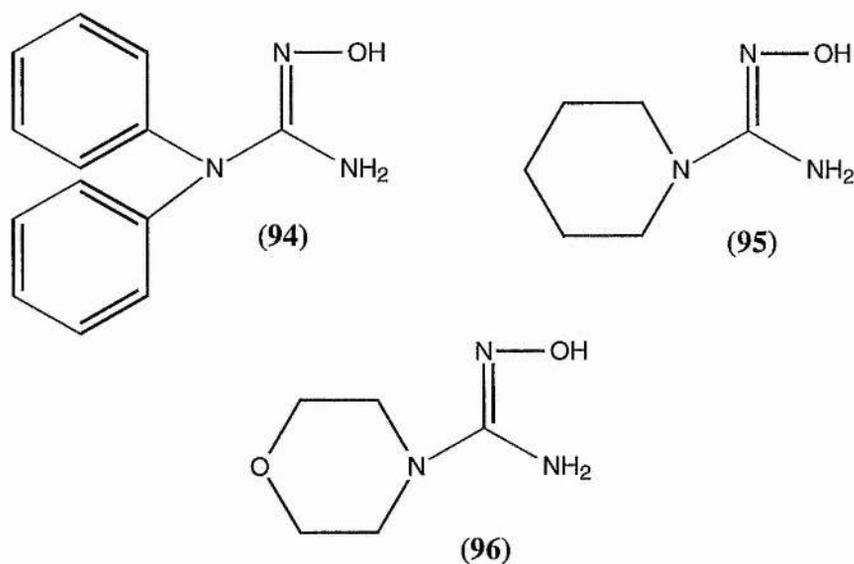
Braun and co-workers in 1903 and then subsequently Belzecki in 1976 reported the synthesis of *N,N'*-disubstituted hydroxyguanidines (**90**) from the corresponding amine via the intermediate cyanamide (**91**) (Scheme 19).^{110,111}



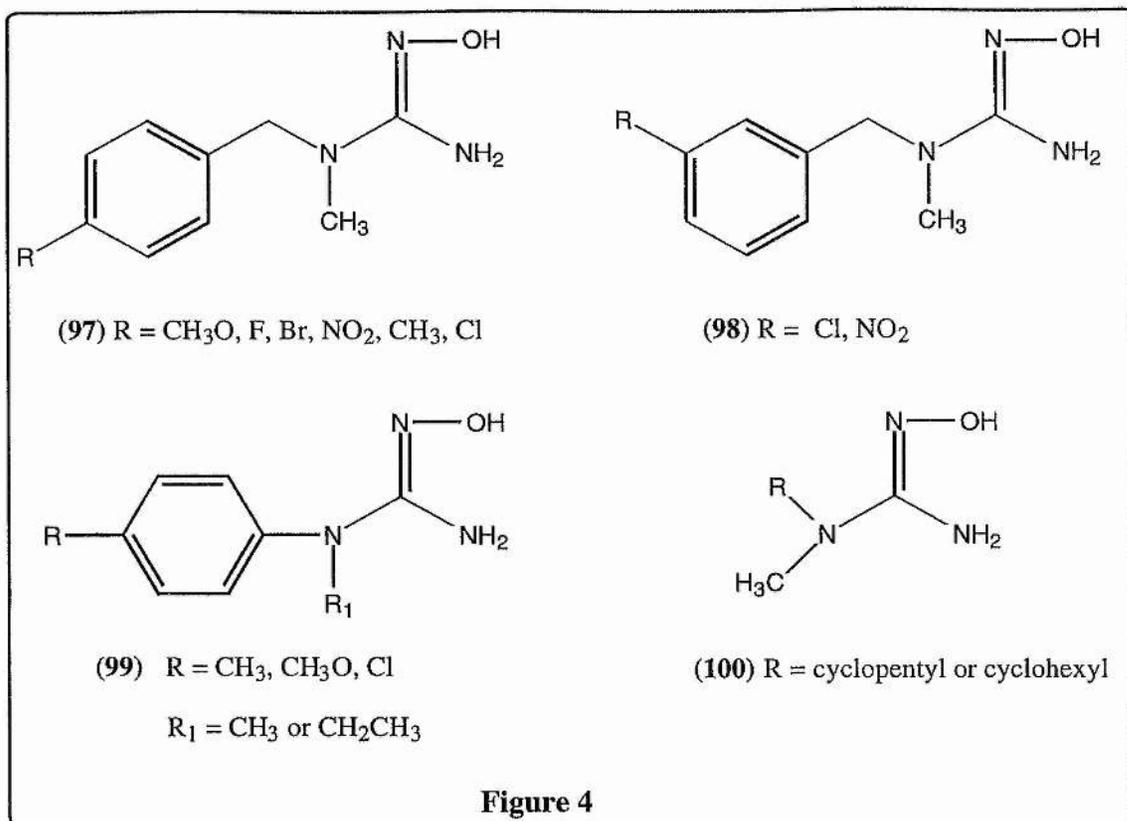
Reaction of the amine with cyanogen bromide and sodium acetate in methanol gave the disubstituted cyanamide (**91**). Subsequent reaction with free hydroxylamine in dioxan, under dry conditions, then afforded the substituted *N,N'*-disubstituted hydroxyguanidines (**90**) in reasonable yields after recrystallisation. Belzecki found that dependent on the solvent either the *N,N'*-disubstituted hydroxyguanidines (**90**) or the corresponding urea (**92**) was formed. The urea (**92**) is formed via the corresponding aminooxyformamidine (**93**) which results from attack of the hydroxylamine oxygen, rather than the nitrogen, on the cyanamide (**91**) (Scheme 20). Alternatively the urea can be formed by reaction of the cyanamide with any water present in the reaction. It appeared that urea was the major product when the reaction was carried out in a proton donating solvent such as ethanol.



A range of examples including a diphenyl derivative (94) and the cyclic piperidine (95) and morpholine (96) derivatives were prepared by this route.



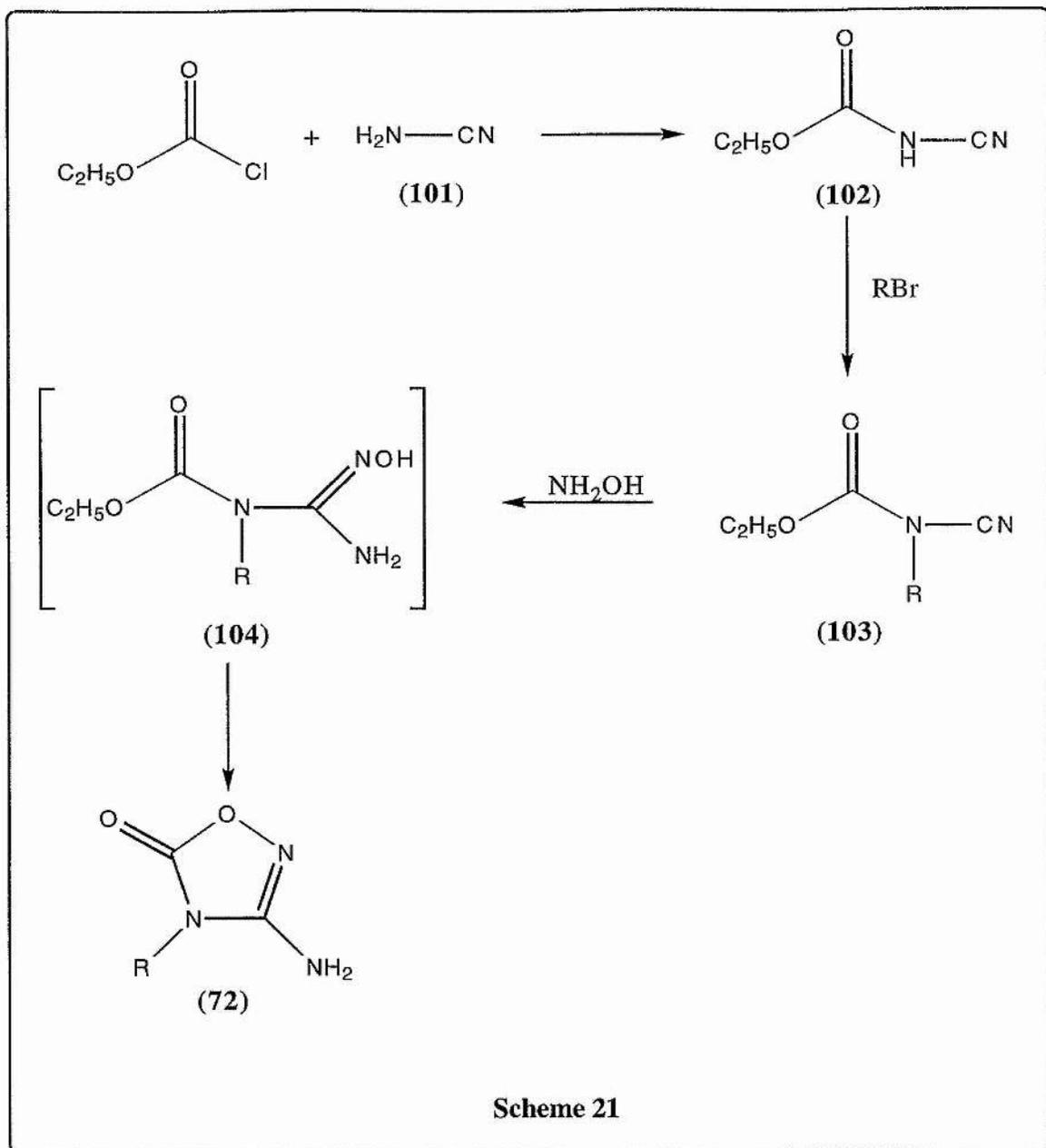
A similar method was also used by Cherkofsky who synthesised a range of mono and disubstituted hydroxyguanidines for the treatment of depression.^{91,112} A range of cyanamides were synthesised and then used to prepare the corresponding hydroxyguanidines (Figure 4, 97-100).



The *N*-hydroxyguanidines (**97-100**) were synthesised in one of two ways. The first method involved stirring the disubstituted cyanamide (**91**) in methanol with either one equivalent of hydroxylamine hydrochloride or a slight excess followed by addition of potassium hydroxide. The reaction was heated under reflux for two hours. Work up of the reaction mixture yielded the desired product as either the free hydroxyguanidine or as the hydrochloride salt. The authors found for some of the cyanamides that the use of methanol yielded a high proportion of the isomeric aminooxyformamide (**93**) and thus the urea (**92**). This problem was overcome by using dioxan as the solvent. The second method used involved reacting the cyanamide (**91**) with an equimolar equivalent of hydroxylamine hydrochloride in refluxing methanol with a catalytic amount of potassium hydroxide. After work up and recrystallisation the hydroxyguanidines were obtained as their hydrochloride salts.

The use of 3-amino-1,2,4-oxadiazol-5-ones (**72**) as possible *N*-hydroxyguanidine prodrugs has been reported.⁸⁸ These compounds were synthesised

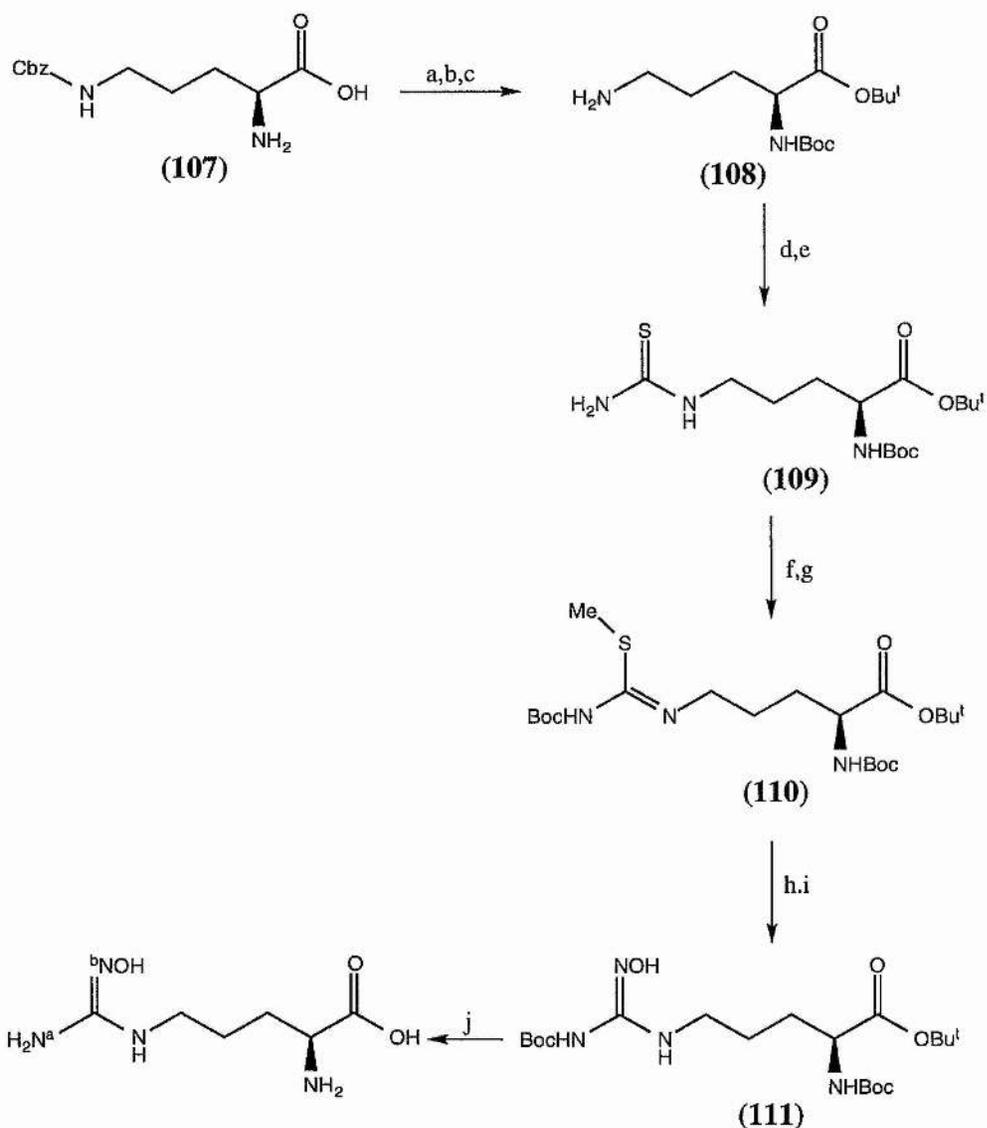
from cyanamide (**101**) by firstly reacting with ethyl chloroformate to give the *N*-cyanourethane (**102**) (**Scheme 21**). The crude *N*-cyanourethane (**102**) was then reacted with suitable alkyl bromides to give a variety of *N*-alkylated products (**103**). Addition of hydroxylamine to this *N*-alkylated cyanourethane gave the hydroxyguanidine (**104**) which cyclised in polar solvents to give the oxadiazolone (**72**). The hydroxyguanidine (**104**) derivative was thought to be too unstable to be isolated so there was no attempt to do so. The oxadiazolones were obtained in yields of up to 86 %.



A range of derivatives were synthesised although the limitation was the availability of the starting bromide. The examples that were synthesised and tested for antithrombotic activity are given in **Table 2 (Section 1.5.1)**.

2.2.2 Synthesis Via Thioureas

Feldman reported the synthesis of N^{ω} -hydroxy-L-arginine (**6**), the intermediate in the oxidation of L-arginine (**5**) to citrulline (**7**) and nitric oxide, and its ^{15}N labelled analogues (**105**) (**106**) from an enantiomerically pure starting material, L-ornithine via the intermediate thiourea (**Scheme 22**). The labelled compounds were prepared so that they could then be used in the elucidation of the mechanism of NOS, the enzyme that converts L-arginine (**5**) into citrulline (**7**) and nitric oxide (**Scheme 3**).¹¹³



(6); a=b=14

(105); a=14, b=15

(106); a=15, b=14

Reagents and conditions

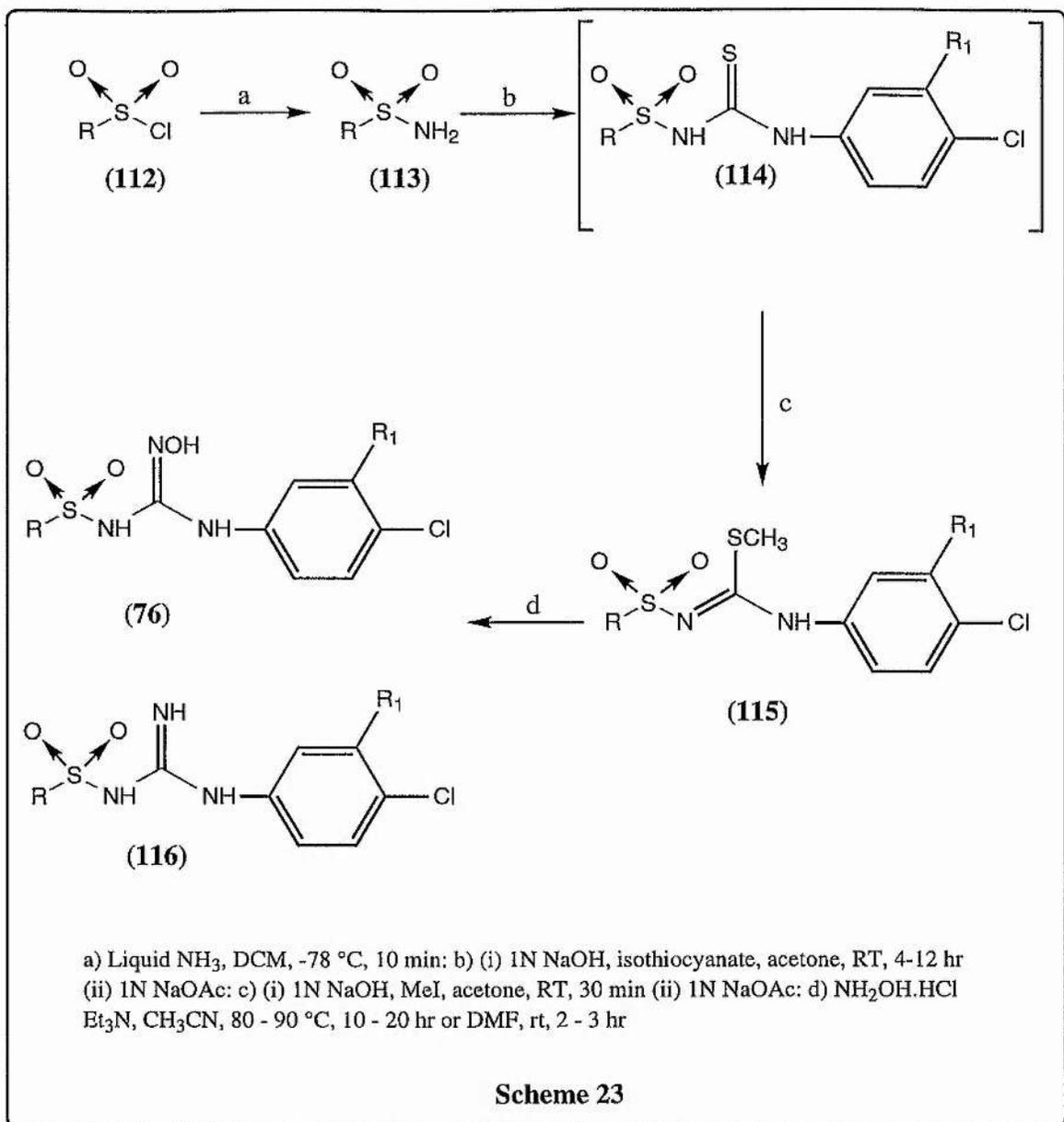
a) (BOC)₂O, dioxan, sat NaHCO₃, 23 °C, 24hr; b) *O*-tert-butyl-*N,N*-diisopropylisourea; c) H₂, Pd on C, MeOH; d) thiophosgene, CaCO₃, H₂O, CHCl₃, 23 °C e) NH₃, MeOH, 0 °C; f) MeI, CH₃CN, 23 °C, 16h; g) (BOC)₂O, dioxan, sat. NaHCO₃, 23°C ; h) *O*-benzylhydroxylamine, TEA, AgNO₃, CH₃CN, 0 °C, 2h i) 10% Pd-C (20 wt %), MeOH, H₂ (1atm), 30mins; j) HCl/ dioxan, 23 °C, 20h

Scheme 22

The synthesis started with the conversion of (107) into the fully protected L-ornithine derivative (108) in 3 steps, using modified literature procedures.¹¹⁴ The acid

was esterified using *O*-*t*-butyldiisopropylisourea and the CBZ group removed by hydrogenation. Addition of (108) in CHCl₃ to a suspension of thiophosgene in aqueous CaCO₃ rapidly afforded the isothiocyanate which was dissolved in methanol and treated with excess aqueous ammonia to give the thiourea (109). The thiourea, (109), was then activated by methylation with methyl iodide. Subsequent BOC protection of the free amine of the thiourea was achieved under the same conditions as an earlier step to give the protected *S*-alkylated compound (110). Reaction of (110) with *O*-benzylhydroxylamine and triethylamine (TEA) in the presence of AgNO₃ displaced the activated sulfur and yielded the *O*-benzyl substituted *N*-hydroxyarginine (111). The benzyl group was then removed by hydrogenation using 10% Pd-C as the catalyst. Removal of the BOC groups and hydrolysis of the *t*-butyl ester was achieved in one step using HCl/ dioxan at room temperature. The analytically pure L-*N*-hydroxyarginine compound precipitated out of solution. The labelled compounds were prepared in an identical manner using labelled precursors.

Recently the synthesis of a series of sulfonyl-*N*-hydroxyguanidines (76) as potential antitumour agents was reported.⁹² The compounds were synthesised from sulfonyl chloride (112) which was reacted with liquid ammonia to give the sulfonamide derivative (113). Reaction of this compound with the required isothiocyanate gave the thiourea (114) (Scheme 23). Sulfonylthioureas are easily attacked by nucleophiles and the thioureas were therefore not isolated but were treated *in situ* with methyl iodide to give the psuedothiourea derivative (115). Reaction of the psuedothiourea (115) with hydroxylamine hydrochloride yielded the target compounds (76) and the guanidine by-product (116). Due to the closeness of the two products by tlc, intensive column chromatography was required to separate them, thus explaining the low yield of the target compounds.



2.3 SYNTHESIS OF CYANAMIDES

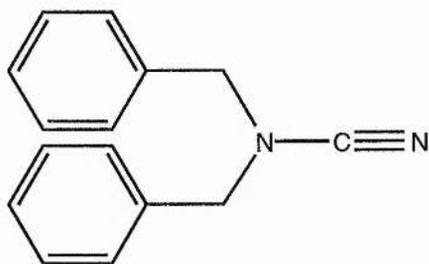
For the synthesis of the free *N*-hydroxyguanidines it was decided to use the cyanamide synthetic route as employed by Belzecki.¹¹¹ Therefore it was first necessary to prepare the cyanamide starting materials. This was accomplished by reaction of the appropriate amine with cyanogen bromide. Nucleophilic attack of the amine nitrogen on the cyanide carbon occurs, eliminating bromide ion. The identity of the product cyanamide is then readily confirmed by its characteristic spectroscopic data. In the i.r. spectrum there is a strong stretch at approximately 2200 cm^{-1} which is due to the carbon nitrogen triple bond stretch. In the ^{13}C NMR spectrum the cyanamide carbon gives a characteristic resonance at 115-120 ppm. The work concentrated on disubstituted derivatives because it has been previously shown that the monosubstituted derivatives are more unstable.⁸⁷

2.3.1. Alkyl Disubstituted Cyanamides

The synthesis was carried out by stirring the amine in methanol at $0\text{ }^{\circ}\text{C}$ and adding sodium acetate. A solution of cyanogen bromide was then added dropwise to the suspension and the reaction stirred at $0\text{ }^{\circ}\text{C}$ for 3 hours before being stirred at room temperature for 2-18 hours. The methanol was then removed and the residue partitioned between water and dichloromethane. The organic layer was then washed with dilute hydrochloric acid and then a saturated brine solution. Removal of the solvent under reduced pressure yielded the product as either an oil or a solid which could be recrystallised from ethanol or methanol.

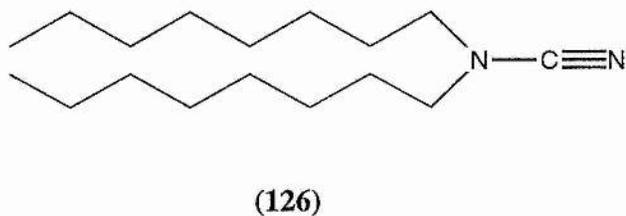
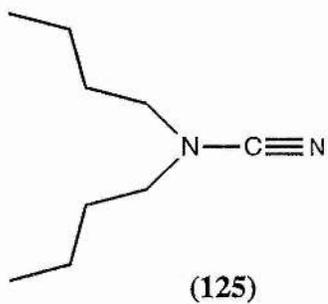
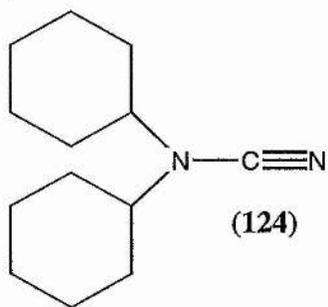
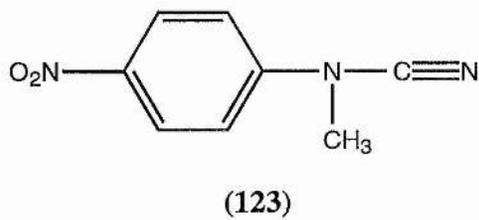
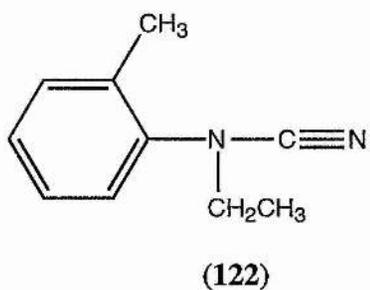
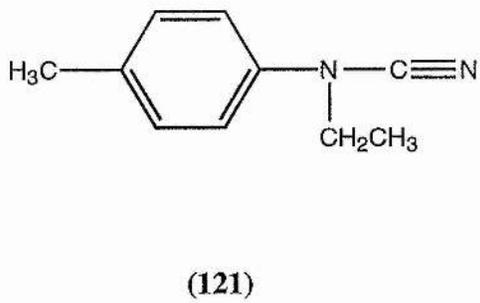
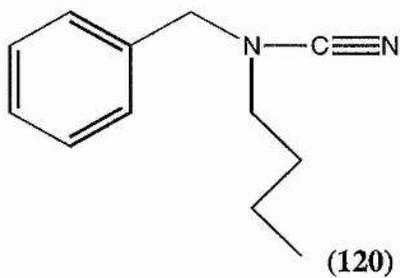
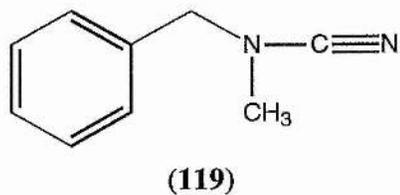
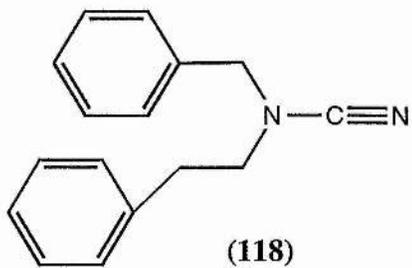
The first example synthesised was *N,N*-dibenzylcyanamide (**117**) as extensive work has already been done on this compound and the corresponding hydroxyguanidine. Dibenzylamine was reacted with cyanogen bromide to give the product (**117**) after recrystallisation from methanol as a white solid in 72% yield. The solid gave the characteristic ^{13}C peak at 118.38 ppm and the CN stretch in the i.r. at 2200 cm^{-1} . The

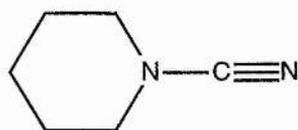
mass spectrum also indicated the presence of the molecular ion, M^+ , at 222 and the melting point of 50-52 °C agreed well with the literature value reported by Traube of 54 °C.¹¹⁵



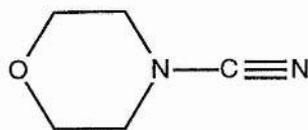
(117)

After the synthesis of this compound had been successfully completed the synthesis of a range of other derivatives was attempted using this methodology. The derivatives (118-128) were chosen to give a variety of substituents on the nitrogen including substituted aromatic rings, aliphatic chains and cyclic groups.





(127)



(128)

All of these compounds were successfully synthesised and the results are summarised below (Table 3).

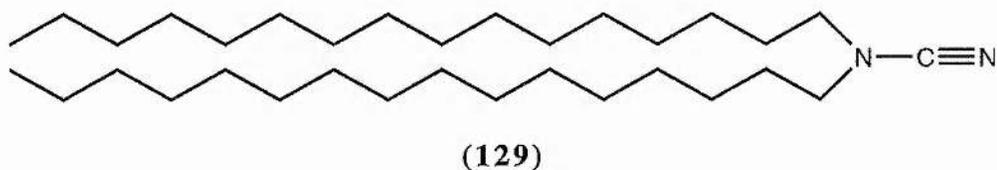
Table 3:- Cyanamide Derivatives

Derivative	Yield / %	m.p	¹³ C CN resonance/ ppm	i.r. CN stretch/ cm ⁻¹
(118)	70	43-44	118.3	2200
(119)	84	oil	118.78	2160
(120)	74	oil	117.97	2200
(121)	81	oil	113.61	2225
(122)	69	oil	115.57	2200
(123)	70	130-132	-	2100
(124)	69	44-46	116.88	2200
(125)	85	oil	118.48	2225
(126)	70	oil	118.02	2175
(127)	85	oil	118.66	2225
(128)	66	oil	117.12	2150

Although *N*-methyl-*N*-(*p*-nitrophenyl)cyanamide (123) was obtained as a yellow solid the NMR data originally suggested that the reaction had not occurred as there was a small NH peak in the ¹H NMR and no visible CN peak in the ¹³C NMR. However the mass spectrum gave the expected M⁺ peak for the product at 177 and the i.r. stretch for

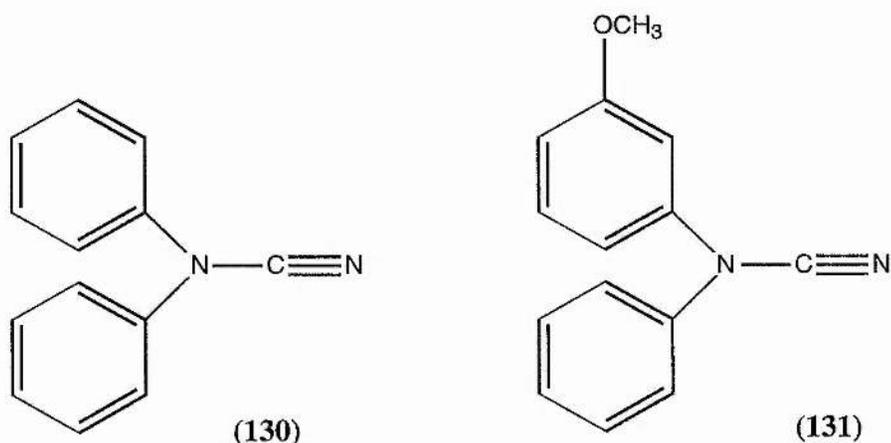
CN was observed at 2100 cm^{-1} , with no visible NH stretching present. A better, overnight, ^{13}C NMR spectrum indicated that the CN resonance may be present at 126.18 ppm .

The synthesis of *N,N*-distearylcyanamide (**129**) a longer chain aliphatic analogue of (**125**) and (**126**) was also attempted. Here the starting amine was stirred in methanol and sodium acetate and cyanogen bromide added. The reaction was stirred at $0\text{ }^{\circ}\text{C}$ for 3 hours and then room temperature for a further six hours. However upon work-up the product obtained was insoluble in organic solvents and water and it was unable to be characterised so no further work was done on this amine



2.3.2 Aromatic Substituted Cyanamides

The syntheses of two diphenyl derivatives, *N,N*-diphenylcyanamide (**130**) and *N*-(3-methoxyphenyl)-*N*-phenylcyanamide (**131**) were investigated.

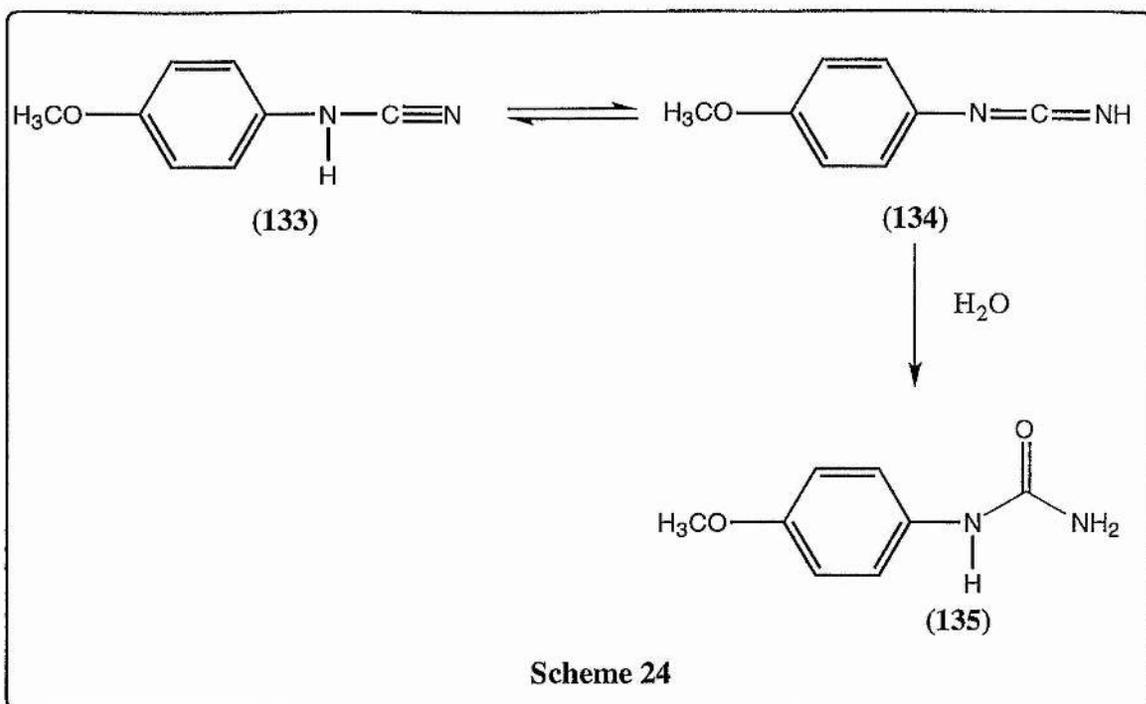


The synthesis of *N,N*-diphenylcyanamide (**130**) was first attempted using the standard methodology. Thus diphenylamine was reacted with cyanogen bromide in

methanol in the presence of sodium acetate for 5 hours. When the reaction was worked up a solid product was obtained, however the ^{13}C NMR spectrum showed no evidence of the CN group. This result was confirmed by the absence of the CN stretch in the i.r. and the presence of a NH stretch at 3300 cm^{-1} suggesting that the product isolated was the starting amine. The mass spectrum confirmed this result with the M^+ peak at 169 rather than the expected peak at 194. The reaction was then repeated with the reaction mixture being heated for 14 hours and then stirred overnight at room temperature. Again a solid product was obtained but all the data suggested that this product was the starting amine rather than the desired cyanamide product. The desired cyanamide (**130**) was described in the literature and it was discovered that due to the unreactive nature of the amine the target compound had been synthesised by heating the amine and the cyanogen bromide under pressure in a sealed vessel. The amine is less reactive because it is an aromatic amine therefore less nucleophilic. An attempt to repeat this reaction did indeed give a product which showed a peak in the ^{13}C NMR spectrum at 118.31 ppm. However it was also clear that a number of other products including the amine were present and it was decided to attempt the synthesis using another derivative.

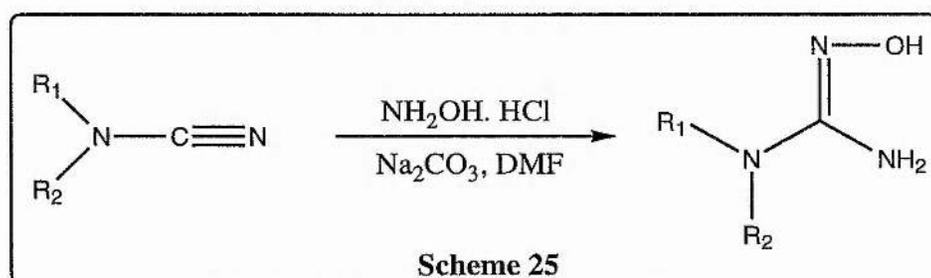
The synthesis of *N*-(3-methoxyphenyl)-*N*-phenylcyanamide (**131**) was thus attempted by reacting *N*-(3-methoxyphenyl)-*N*-phenylamine with cyanogen bromide and sodium acetate in methanol. This amine was chosen as the presence of the electron donating substituent should increase the nucleophilicity of the nitrogen. Again the reaction was worked up in the usual method and an oil was obtained, however ^{13}C NMR and i.r. indicated that this oil was the starting amine rather than the desired cyanamide. It was decided to leave this work on these two derivatives at this point to concentrate on a number of other examples.

It should be possible to complete the synthesis of these two cyanamides (**130**) (**131**) by reacting the amine together with cyanogen bromide in a sealed vessel at higher temperature for a longer period of time.



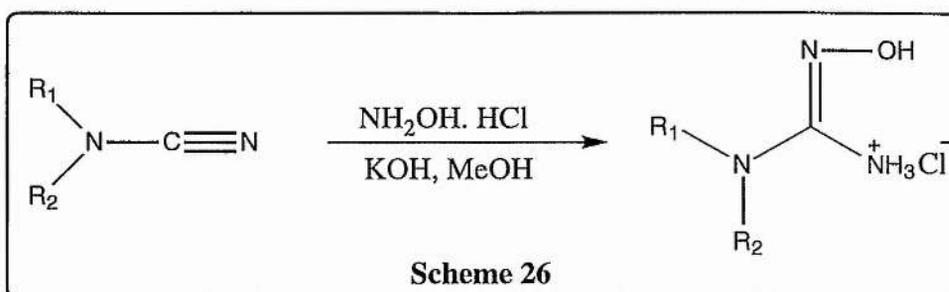
2.4 SYNTHESIS OF *N*-HYDROXYGUANIDINES

With a range of starting cyanamides successfully synthesised the next step was the synthesis of the corresponding *N*-hydroxyguanidines. The *N*-hydroxyguanidines could be synthesised in one of two ways. The first method was reaction of the cyanamide with hydroxylamine hydrochloride and sodium carbonate in purified DMF (**Scheme 25**). The reaction was heated at 60-80 °C before being filtered hot to remove the inorganic salts. The salts were then washed with a small quantity of warm DMF. Concentration of the solution under reduced pressure gave the hydroxyguanidine as either an oil or a solid. For the solid examples a recrystallisation then gave the product pure. However the oils were first added to a vigorously stirred ice cold solution of water to induce crystallisation. Recrystallisation of the solid obtained then yield the desired pure hydroxyguanidine. A number of problems were experienced with this reaction. Firstly if the reaction was heated at above 80 °C, or under reflux, then the amount of by-products produced increased and purification became impossible. Secondly the DMF needed to be dried and purified before use. This was accomplished by distilling off magnesium sulfate under reduced pressure. The DMF was then used within two or three days of this purification.



The second method of preparation involved reacting the cyanamide with hydroxylamine hydrochloride and a catalytic amount of potassium hydroxide in refluxing methanol (**Scheme 26**). In this case the reaction was refluxed for 12 hours, cooled and

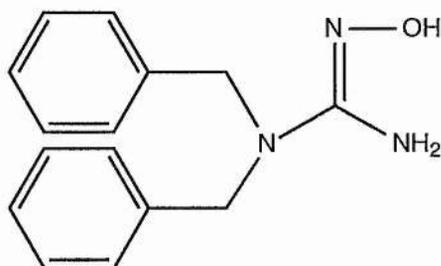
then diluted with diethyl ether. At this point a solid formed which was then filtered off and recrystallised to give the desired hydroxyguanidine as the hydrochloride salt.



The disappearance of the cyanamide peak at 115-120 ppm in the ^{13}C NMR spectrum and the appearance of the peak due to the $C=NOH$ group at 155-160 ppm confirmed that the reaction had occurred as expected. This was also confirmed by the presence of NH_2 and OH stretching in the i.r. spectrum and the absence of the CN stretch at 2200 cm^{-1} .

2.4.1 Alkyl Disubstituted Derivatives

1,1-Dibenzyl-2-hydroxyguanidine (**70**) was first synthesised within the chemistry department at the University of St Andrews.¹¹⁶ Subsequent work established that the compound did release nitric oxide under biological conditions and following the action of chemical oxidants and that the compound (**70**) is a vasodilator.



(70)

The synthesis of this compound was repeated by reacting the cyanamide with hydroxylamine hydrochloride in the presence of sodium carbonate in DMF. The reaction was heated for 1.25 hours before working the reaction up as previously described. The oil that was obtained was then added to a small amount of ice-cold water with vigorous stirring and after a period of up to 30 minutes it became a solid. This solid was recrystallised from ethanol to give the product in 65%. Initially the recrystallisation was attempted from methanol however it was found that the product crystallised from solution too quickly and ethanol was used to slow the crystallisation down and therefore improve the crystalline structure of the compound. However this did not work and the solid that was obtained was unsuitable for X-ray crystallography. The solid was pure by microanalysis and the ^{13}C NMR spectrum showed that the CN peak at been replaced by the C=NOH peak at 157.24 ppm. The melting point of 119-120 °C also agreed well with the literature value of 120-121 °C.⁸⁷

The synthesis of other examples was then attempted from the corresponding cyanamides (**118-128**). However the synthesis of these proved to be problematic and only a small number of examples were successfully obtained (**136-140**) (**Table 4**).

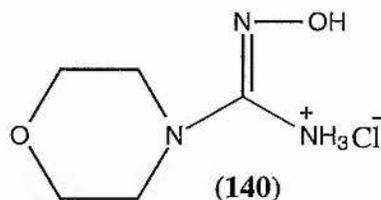
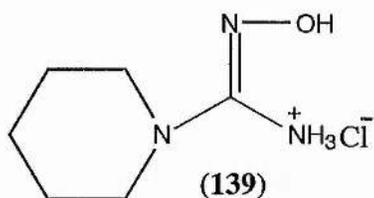
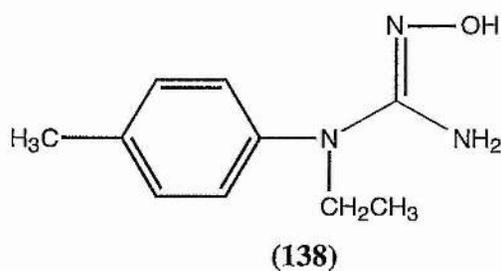
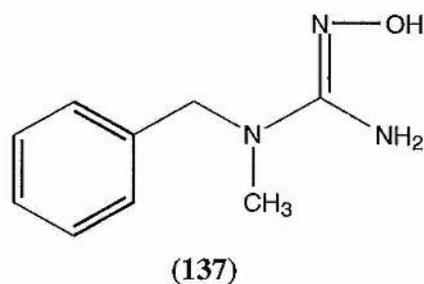
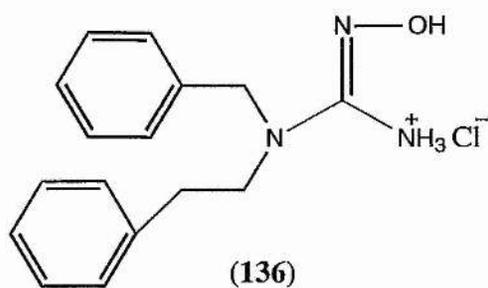


Table 4:- Hydroxyguanidine Derivatives

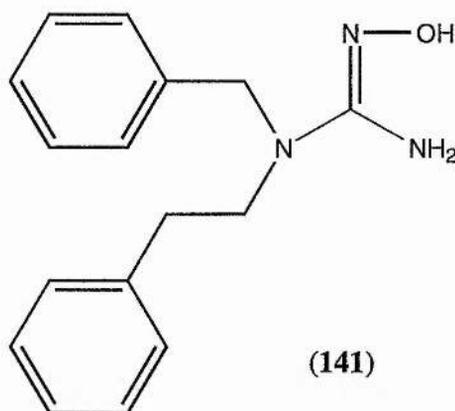
Cyanamide	Hydroxy guanidine	Route	Yield / %	m.p / °C	¹³ C Peak
(118)	(136)	B	53	240-242	160.42
(119)	(137)	A	48	88	157.52
(121)	(138)	A	61	144-146	154.73
(127)	(139)	B	30	270-271	160.42
(128)	(140)	B	64	214-216	155.92

Route A:- NH₂OH, Na₂CO₃, DMF

Route B:- NH₂OH, KOH, MeOH

The synthesis of 1-benzyl-1-(2-phenethyl)-2-hydroxyguanidine (**141**) using similar conditions to those used in the synthesis (**70**) proved to be less successful and

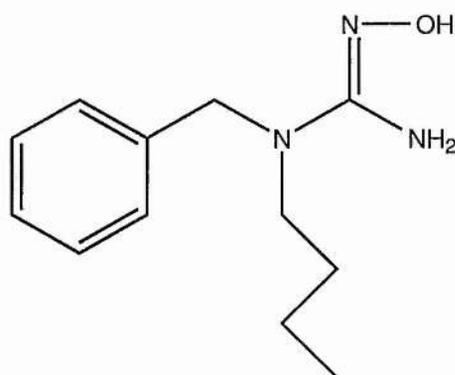
when the compound was added to water an oil was formed. This oil was then extracted into ethyl acetate and the solvent then removed under reduced pressure to yield an oil, which was seen by both ^1H and ^{13}C NMR spectra to be a mixture of compounds with some of the desired compound present. A number of attempts at purification were carried out including recrystallisation from ethanol and flash column chromatography with ethanol as the elutant. However, recrystallisation yielded the impure oil again and the column chromatography failed to yield any of the desired compound.



Due to these problems it was decided to try and synthesise this compound as the hydrochloride salt. Thus 1-benzyl-1-(2-phenethyl)-2-hydroxyguanidine hydrochloride (**136**) was synthesised by reaction of the corresponding cyanamide (**118**) with hydroxylamine hydrochloride in refluxing methanol and in the presence of a catalytic amount of potassium hydroxide. The ^1H and ^{13}C NMR spectra both indicated that there was only one product present with the $\text{C}=\text{NOH}$ signal at 160.42 ppm. When the compound was submitted for microanalysis it indicated that the compound was impure and was maybe not one compound, but a simple flame test on the compound indicated that the compound was probably contaminated with a potassium salt. Removal of this salt proved to be very difficult and despite numerous attempts the compound could not be purified to give a microanalytically pure solid.

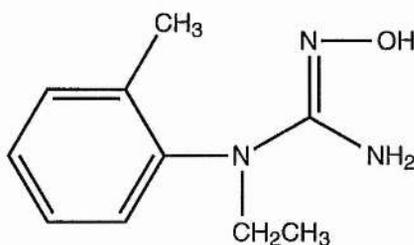
The synthesis of 1-benzyl-1-butyl-2-hydroxyguanidine (**142**) from the corresponding cyanamide (**120**) was attempted using the same procedure that was used

in the synthesis of (70). However for this compound an oil was obtained and although the hydroxyguanidine was present the oil could not be purified to give the desired product.



(142)

When the synthesis of 1-ethyl-1-(*o*-tolyl)-2-hydroxyguanidine (143), an isomer of 1-ethyl-1-(*p*-tolyl)-2-hydroxyguanidine (138), was attempted the reaction did not work.

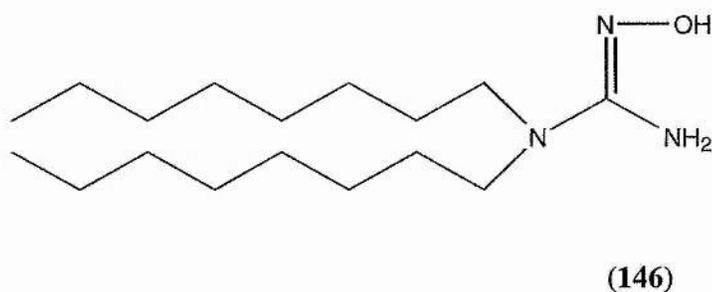
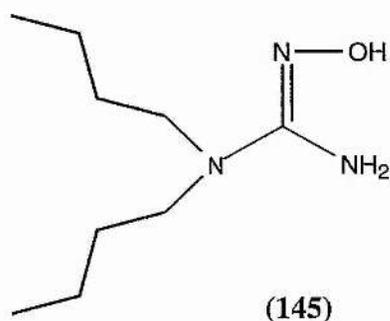


(143)

The reaction was carried out in the same way as the synthesis of 1-ethyl-1-(*p*-tolyl)-2-hydroxyguanidine (138) had been successfully completed, however an oil was obtained that was could not be purified. This is probably because of the sterically hindered nature of the cyanamide and also it may be that the cyanamide is not as reactive due to the different substitution.

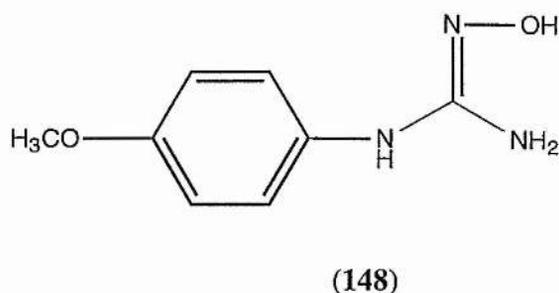
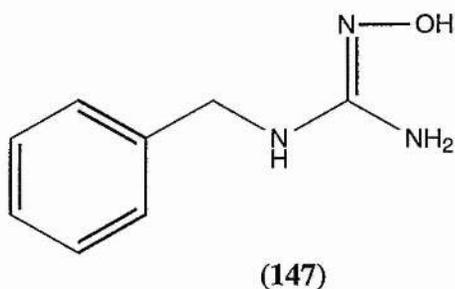
Preparation of 1-methyl-1-(*p*-nitrophenyl)-2-hydroxyguanidine (**144**) from the corresponding cyanamide (**123**) resulted in an unusual observation. The reaction was carried out using the standard method and when the DMF was removed a brown solid was obtained which looked to be correct by ^1H and ^{13}C NMR spectra and the i.r. spectrum showed that there was no CN stretch. When the brown solid was recrystallised from ethanol a crystalline green solid was then obtained. The ^1H NMR spectrum of this compound proved to be strange because it appeared that upon recrystallisation the molecule seemed to have reacted with two moles of DMF. The mass spectrum of the green solid indeed confirmed this result with the M^+ peak at 356 and not as would be expected for the correct product at 210. As it was suspected that the nitro group would affect the biological activity of this compound and in light of this strange result further investigation of this compound was not attempted.

The synthesis of both 1,1-dibutyl-2-hydroxyguanidine (**145**) and 1,1-dioctyl-2-hydroxyguanidine (**146**) proved to be unsuccessful. The synthesis was carried out as before and in both cases an impure oil that contained some of the desired product was obtained but as in previous examples purification of these compounds proved to be impossible.



2.4.2 Monosubstituted Analogues

The syntheses of 1-benzyl-2-hydroxyguanidine (**147**) and 1-(*p*-methoxyphenyl)-2-hydroxyguanidine (**148**) were attempted from *N*-benzylcyanamide (**132**) and *N*-(*p*-methoxyphenyl)cyanamide (**133**) respectively.

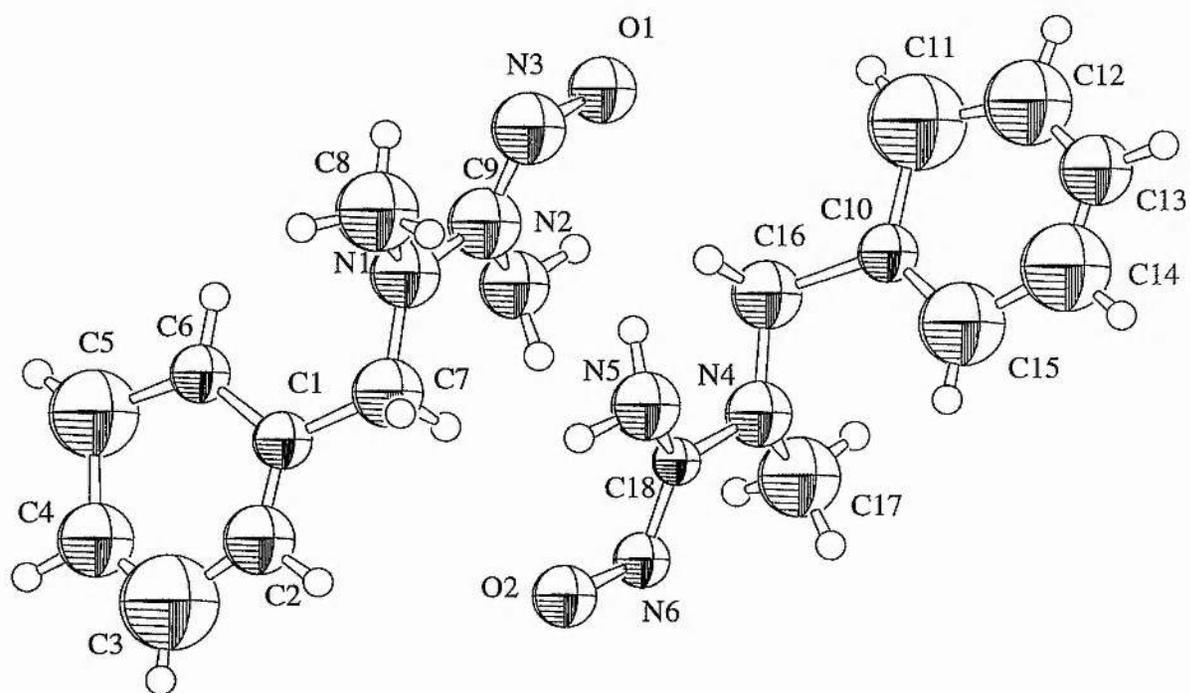


These were carried out using the second method described because it had been shown in previous work that monosubstituted analogues were unstable and could not be isolated from all the by-products.⁸⁷ The cyanamide was heated under reflux in methanol with one equivalent of hydroxylamine and catalytic potassium hydroxide for 14 hours. The two reactions were then cooled to room temperature and poured onto a large excess of diethyl ether and for one, 1-(*p*-methoxyphenyl)-hydroxyguanidine (**148**), a solid was obtained. An attempt to filter this solid failed as the compound was clearly hygroscopic and upon absorbing atmospheric water disappeared. The filtrate of this reaction also failed to show any trace of the expected product. The other reaction, 1-benzyl-2-hydroxyguanidine (**147**), yielded an oil which could not be separated from the methanol/diethyl ether solution. These two reactions would seem to confirm earlier observation that the monosubstituted analogues are more unstable and therefore not so easy to access.

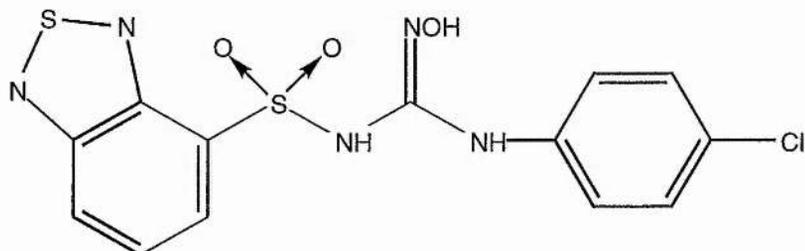
2.4.3 Crystal Structure of 1-benzyl-1-methyl-2-hydroxyguanidine (**137**)

1-Benzyl-1-methyl-2-hydroxyguanidine (**137**) was the only example to crystallise in a suitable form for X-ray crystallography. The X-ray crystal structure (APPENDIX 1) showed that the compound existed in a dimeric form in the unit cell (Figure 5)

FIGURE 5:- Crystal structure of 1-benzyl-1-methyl-2-hydroxyguanidine (**137**)

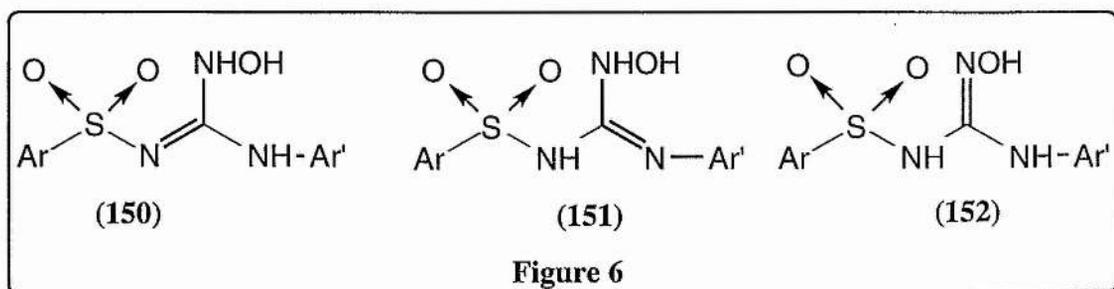


Chern and co-workers have reported the synthesis of substituted sulfonyl-*N*-hydroxyguanidines (**Section 2.7.1**). The authors reported the crystal structure of one of these compounds, *N*-(4-chlorophenyl)-*N'*-[benzo[2,1,3]thiadiazol-4-yl]sulfonyl-*N''*-hydroxyguanidine (**149**) and compared with theoretical calculations.⁸

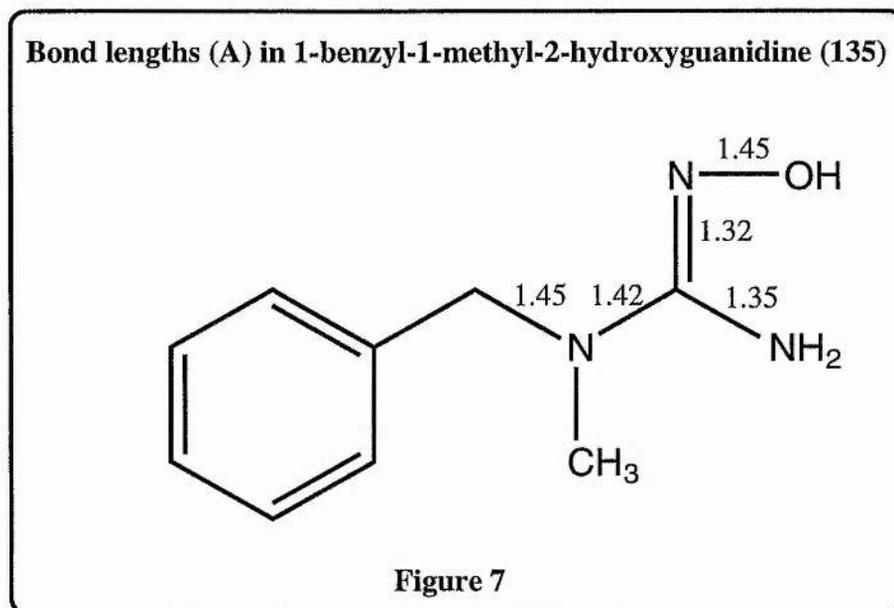


(149)

There are three possible tautomers of the hydroxyguanidine group (**Figure 6**, **150-152**). The NMR spectrum of (**149**) was consistent with tautomers (**150**) and (**152**) but not (**151**). The crystal structure showed that the distances between the central carbon of the hydroxyguanidine group and the 3 nitrogens was neither a pure single N-C bond or a pure double bond. This would suggest some form of coexistence of the three forms and/or a degree of delocalisation between these three bonds. However from the crystal structure it was possible to determine the location of the proton density and along with the NMR data it would suggest that tautomer (**150**) is the most favoured form for this sort of compound. This result was confirmed by computer modelling using MOPAC with PM3 force field parameters.¹¹⁷ The modelling showed that favoured tautomer (**150**) had an energy difference of 1.8 kcal/mol over the other favoured tautomer (**152**).



The crystal structure of 1-benzyl-1-methyl-2-hydroxyguanidine (**137**) showed that despite the possible cis and trans isomers of the C=N bond only the trans isomer was present in the crystal structure. This has been assumed for these compounds but until now had never been proven without doubt. The bond lengths from the X-ray data are given (**Figure 7**). It can be seen from the full data (**Appendix 1**) that the bond lengths for the aromatic ring are unreliable and therefore the data needs to be improved.



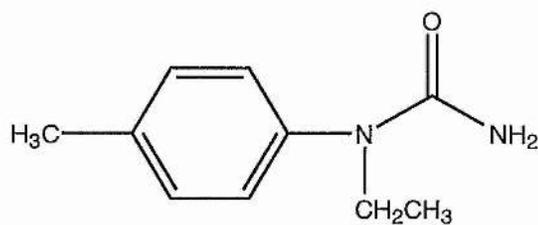
However the data given (**Figure 7**) does imply that the double bond character is found predominantly between the two terminal nitrogens, N2 and N3, and the guanidino carbon, C9. There is, as expected, no double bond character between the quaternary nitrogen N1 and the guanidino carbon C9 which has a bond length of 1.42 Å compared to the C9-N2 and C9-N3 bond lengths of 1.35 and 1.32 Å respectively. The crystal structure also showed that the C-O bond was a single bond. These results in some respects agree with Chern's which show that there are 3 forms of these compounds but that 2 are favoured and that there is a degree of delocalisation over all the bonds. As already stated some of the bond lengths in the aromatic ring are inaccurate but this is because the X-ray data was obtained at room temperature which resulted in decomposition of the crystals. The solution would be to carry out the X-ray diffraction at

low temperature. This was not undertaken as the original attempt at obtaining the crystal structure had degraded the crystals and difficulty was experienced when trying to obtain a new batch of suitable crystal.

2.5 UREA SYNTHESIS

The synthesis of some of the related urea derivatives was also undertaken. These derivatives were required for a number of reasons. Firstly they were required as standards in later work on the decomposition of the *O*-substituted analogues of the hydroxyguanidines, as they could be the product of hydrolysis rather than the hydroxyguanidine. Secondly they were also necessary for biological testing to ensure that the guanidino functionality of the *N*-hydroxyguanidine was required for the biological activity and that the cyanamide or urea were not the active species.

Two alternative routes were investigated, using *N*-ethyl-*N*-(*p*-tolyl)urea (**153**) as the initial example.

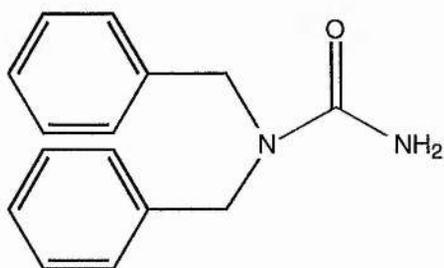


(153)

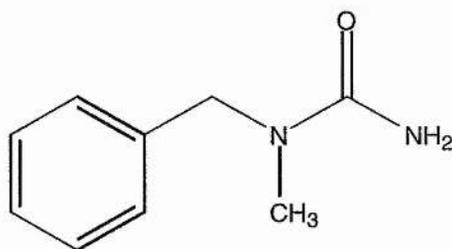
N-Ethyl-*N*-(*p*-tolyl)cyanamide (**121**) was thus hydrolysed under both basic (2M NaOH) and acidic conditions (2M, H₂SO₄) in ethanol. After stirring at room temperature for 18 hours the product was extracted and examined. The acid catalysed reaction gave a product that appeared to be pure by ¹H NMR spectroscopy and showed a characteristic urea C=O resonance at 160 ppm in the ¹³C NMR spectrum. Mass spectrometry gave a molecular ion at 178 confirming the identity of the product. The base catalysed reaction appeared to give a mixture of products with a peak in the mass spectrum at 160 which

corresponds to the cyanamide. As a result of these observations further reactions were carried out using acid conditions.

N,N-Dibenzylurea (**154**) was then synthesised by reacting *N,N*-dibenzylcyanamide (**117**) in ethanol and aqueous sulfuric acid for sixteen hours at room temperature. After work-up the reaction yielded an off-white solid which was recrystallised from ethanol to give a white crystalline solid with a melting point of 122-124 °C which agreed well with the literature value of 125 °C. The C=O resonance was found in the ¹³C NMR spectrum at 160.32 ppm and the M⁺ was found at 222. *N*-Benzyl-*N*-methylurea (**155**) was also synthesised using this method and the crude product was obtained as a solid in 34% yield. Recrystallisation from ethanol yielded the product as a microanalytically pure white solid with a M⁺ in the mass spectrum at 164. The ¹³C NMR spectrum showed the C=O resonance at 159.83 ppm.



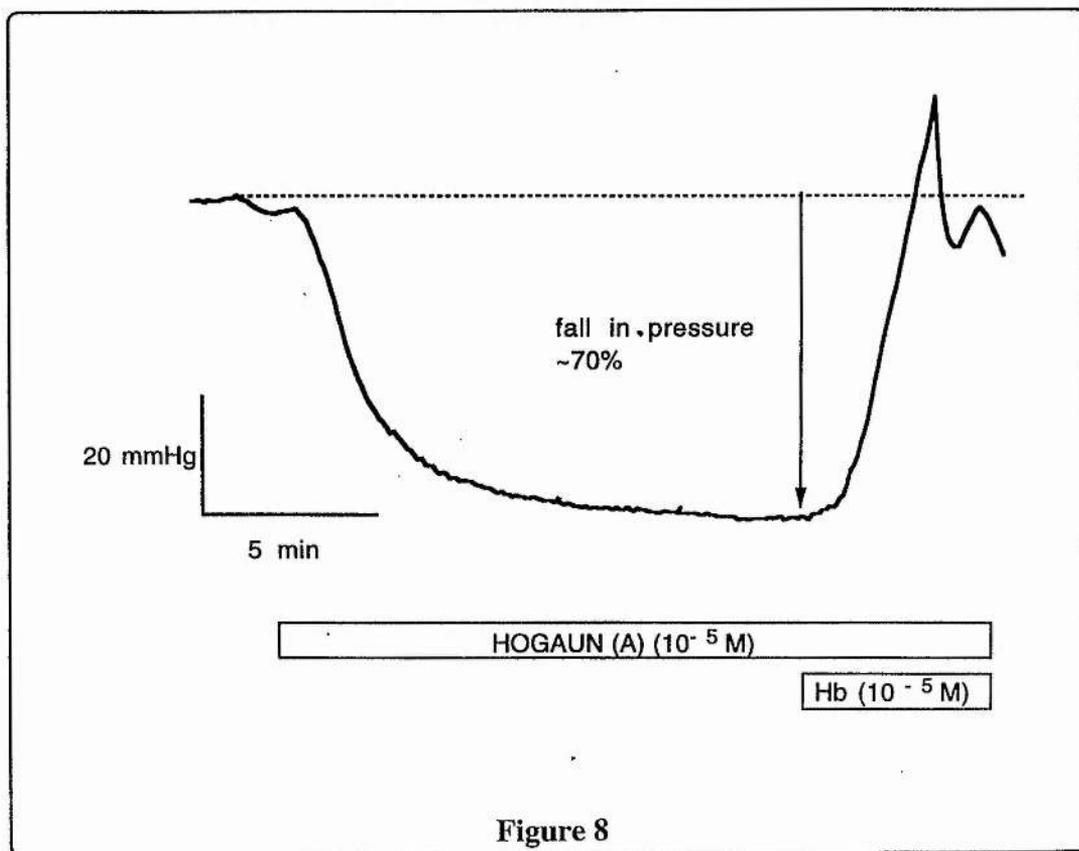
(154)



(155)

2.6 BIOLOGICAL TESTING OF 1-BENZYL-1-METHYL-2-HYDROXYGUANIDINE (137)

Following the procedure of Flitney 1-benzyl-1-methyl-2-hydroxyguanidine (137) was found to have vasodilatory activity.¹¹⁸ This work was carried out by Ian Megson at the University of Edinburgh. When added to the perfused artery the 10^{-5} M solution caused a fall in pressure of approximately 71% (Figure 8). When used at similar concentrations it is known that some *S*-nitrosothiols give 100% relaxation of the artery. This is a positive result as it indicates that 1-benzyl-1-methyl-2-hydroxyguanidine (137) is a reasonable vasodilator at these concentrations.



It was shown that this relaxation was directly due to nitric oxide because when the known nitric oxide scavenger haemoglobin was added the effect was reversed thus

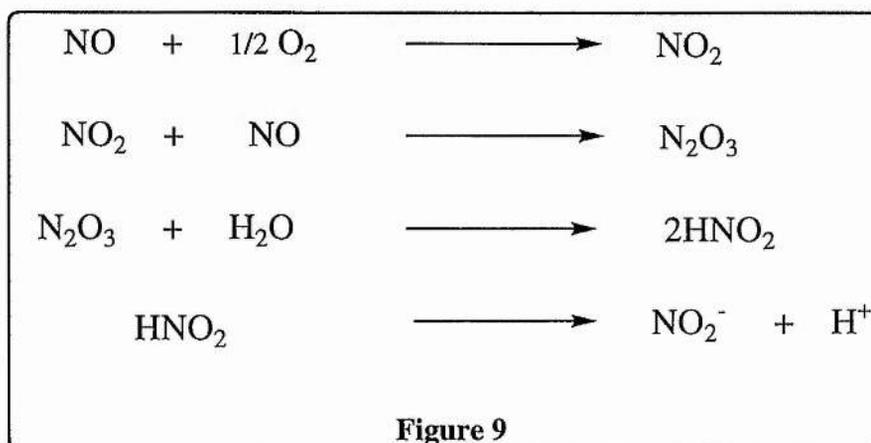
confirming the role of nitric oxide. Haemoglobin works because it traps the nitric oxide and forms a stable complex.

2.7 CHEMICAL OXIDATION OF *N*-HYDROXYGUANIDINES

2.7.1 Introduction

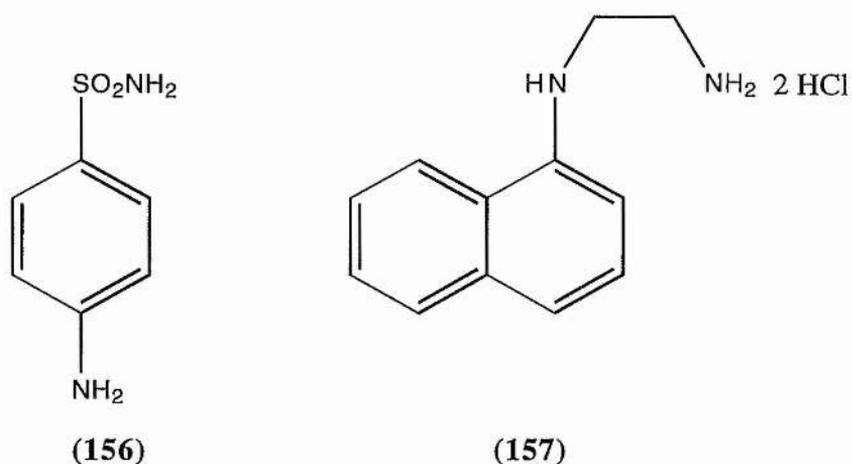
Previous work carried out on *N*-hydroxyguanidines and related derivatives have shown that these compounds can be oxidised by a range of oxidising agents for example hydrogen peroxide or *m*-chloroperbenzoic acid (mCPBA).⁶⁴ Previous work within the department has also shown that 1,1-dibenzyl-2-hydroxyguanidine (**70**) can be oxidised by hydrogen peroxide to give a near quantitative yield of nitrite, which was detected using the Griess test.⁸⁷ The nitrite is derived from nitric oxide and it is therefore possible to measure the nitric oxide release from these compounds. It was observed that two equivalents of the oxidant were required to carry out this oxidation.

The Griess test is used because it is known that the nitric oxide released from the *N,N*-hydroxyguanidines will react with oxygen as shown to give nitrous acid, (**Figure 9**), which then dissociates in aqueous solution to give nitrite (NO_2^-). This nitrite can then be detected using the Greiss test.

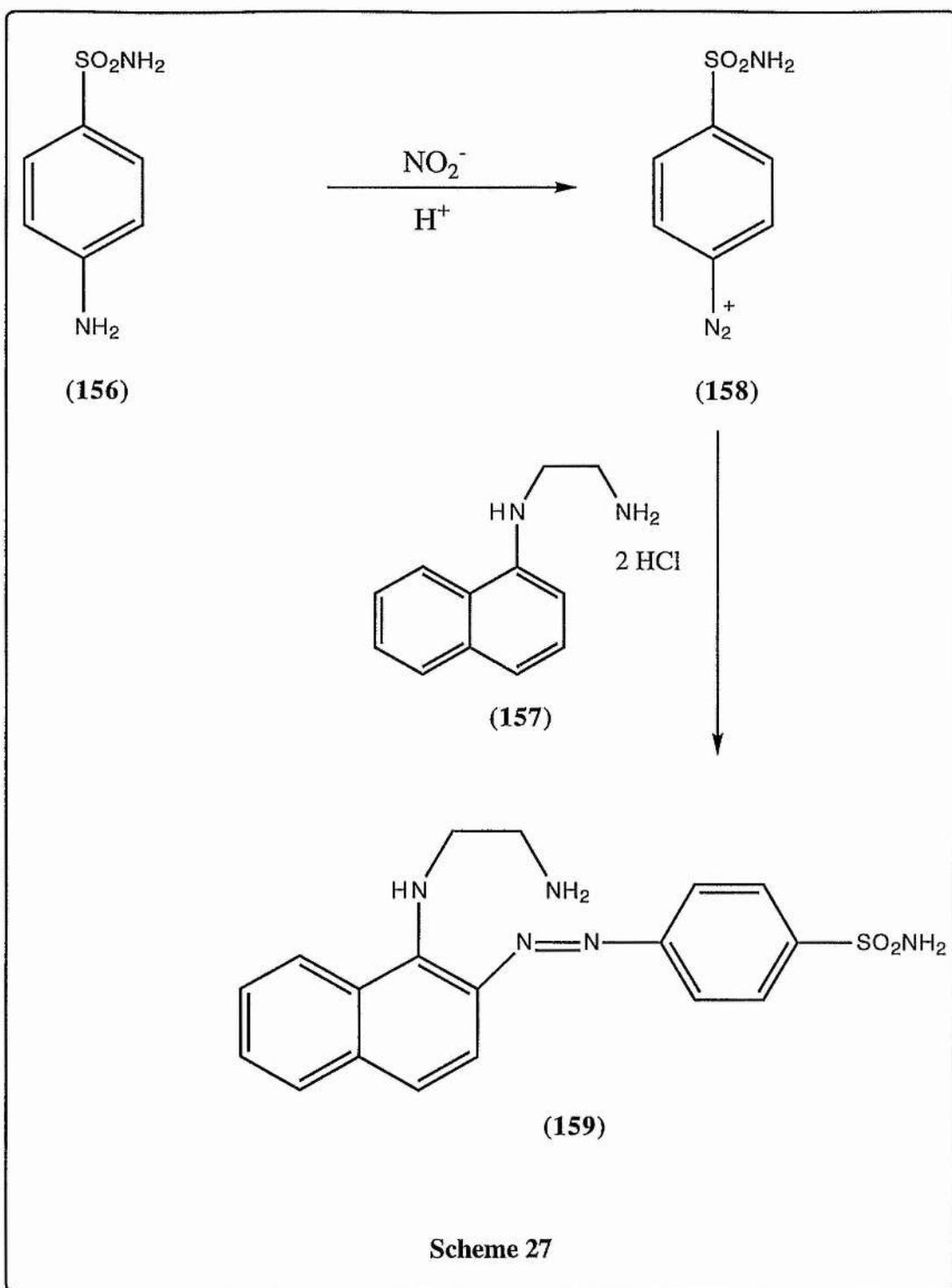


2.7.2 Griess Test

The Griess test involves the formation of an azo dye between sulfanilamide (156) and *N*-(1-naphthyl)ethylenediamine dihydrochloride (157).⁴⁶



The NO_2^- produces nitrous acid under the acidic conditions of the test. The sulfanilamide (156) is then converted to the diazonium ion (158). Finally the diazonium ion (158) couples with the *N*-(1-naphthyl)ethylenediamine (157) to give the azo dye (159) (Scheme 27). This azo dye is highly conjugated and is responsible for the purple colour associated with this test. The absorbance of the solution is directly related to the concentration of the nitrite that is present in the solution.



The two solutions used in the Griess test are sulfanilamide (156) in aqueous solution containing 2% v/v hydrochloric acid and the *N*-(1-naphthyl)-ethylenediamine dihydrochloride (157) in aqueous solution containing 1% v/v hydrochloric acid. The

first step in this work was to obtain a nitrite calibration curve by measuring the absorbance of the standard nitrite solution. A $110 \times 10^{-6} \text{ mol dm}^{-3}$ was used as stock solution and was diluted to give a range of concentrations. The Greiss test was carried out by removing 1 cm^3 of the standard nitrite solution and adding 0.5 cm^3 of sulfanilamide (**156**) solution followed 5 minutes later by 0.5 cm^3 of the *N*-(1-naphthyl)ethylenediamine dihydrochloride (**157**) solution. The colour was then allowed to develop for 15 minutes before the absorbance was measured at 550 nm. The nitrite calibration curve was then plotted and was used to work out the concentration of the nitrite in the reaction solutions.

2.7.2.1 Oxidation of 1-benzyl-1-methyl-2-hydroxyguanidine (**137**)

For the oxidation reactions a 0.1 mmol dm^{-3} solution of 1-benzyl-1-methyl-2-hydroxyguanidine (**137**) was employed, to give suitable concentrations of nitrite for the Griess test. Initially reactions were carried out in aqueous solution however this caused problems with the solubility of the oxidising agents, so a solution of water/acetonitrile (85/15%) was used to solve this problem. The solution of 1-benzyl-1-methyl-2-hydroxyguanidine (**137**) was placed in a water bath at $37 \text{ }^\circ\text{C}$ and the oxidising agent was added. The reaction was then tested for nitrite after various time intervals using the Greiss test.

It had been reported that hydrogen peroxide gave the best results for the oxidation of these compounds, so it was decided to try this reagent first.⁸⁷ An aliquot of 0.2 cm^3 of 30% hydrogen peroxide was added, which gave a sufficient excess to give complete reaction (**Table 5**).

Table 5:- Oxidation of (137) by hydrogen peroxide

Time/ min	Absorbance	Conc. of nitrite/ $\times 10^{-6} \text{ mol dm}^{-3}$	% Oxidation
30	0.0195	0.5	1
60	0.0299	1.0	2
90	0.278	1.0	2

The final concentration of nitrite should be $55 \times 10^{-6} \text{ mol dm}^{-3}$, giving an absorbance of 1.46. Therefore it can be seen that only a very low yield of nitrite, 2% after sixty minutes, was obtained using hydrogen peroxide.

Taking into account these results it was decided to attempt the oxidation using an excess (0.01g) of mCPBA and the results are given below (Table 6). In this case after 30 minutes of reaction there was a 27% yield of nitrite.

Table 6:- Oxidation of (137) by mCPBA

Time/ min	Conc of nitrite / $\times 10^{-6} \text{ mol dm}^{-3}$	% Oxidation
30	15	27
60	14	25.5
90	11.5	21

A third oxidation using an excess (0.012g) of oxone as the oxidant was carried out and the results are given below (Table 7).

Table 7:- Oxidation of (137) by oxone

Time/ min	Conc of nitrite $\times 10^{-6} \text{ mol dm}^{-3}$	% Oxidation
30	1	2
60	1.5	3
90	1.5	3
120	2	4

After 120 minutes of reaction the yield of nitrite in this case was only 4%.

A comparison of the maximum % oxidation for the 3 oxidants (**Table 8**) reveals that mCPBA gives the highest degree of oxidation.

Table 8:- Comparisons of oxidation reactions

Oxidant	Max Oxidation / %
H ₂ O ₂	2
mCPBA	27
Oxone	4

It would seem that oxone is not very effective at oxidising the 1-benzyl-1-methyl-2-hydroxyguanidine (137) and that despite reports that suggested 100% oxidation occurred with hydrogen peroxide this was not the case.⁸⁷ What is interesting is that a microanalytically pure compound would be expected to give 100% oxidation but this is clearly not occurring for some reason. However it may be that it was the presence of an impurity in the compounds that were previously tested that helped the oxidations occur which is not present in the compounds tested. As the results appeared to suggest that mCPBA was the best oxidising agent it was decided to use this oxidising agent in all further oxidations.

2.7.2.2 Oxidation of 1,1-dibenzyl-2-hydroxyguanidine (70)

A 0.1 mmol dm⁻³ solution of 1,1-dibenzyl-2-hydroxyguanidine (70) in water/acetonitrile (85/15%) was oxidised with an excess (0.01 g) of mCPBA and the results are shown below (Table 9), giving 18% oxidation after 60 minutes..

Table 9:- Oxidation of (70) by mCPBA

Time/ min	% Oxidation
30	16
60	18
90	18

2.7.2.3. Oxidation of 1-ethyl-1-(*p*-tolyl)-2-hydroxyguanidine (138)

A 0.1 mmol dm⁻³ solution of 1-ethyl-1-(*p*-tolyl)-2-hydroxyguanidine (138) was oxidised using an excess (0.01 g) of mCPBA (Table 10).

Table 10:- Oxidation of (138) by mCPBA

Time/ min	Conc. of nitrite/ x10 ⁻⁶ mol l ⁻¹
30	5.5
60	6
90	5.5

After 60 minutes there was only 6% oxidation of 1-ethyl-1-(*p*-tolyl)-2-hydroxyguanidine (138).

2.7.3.4 Oxidation of 1-morpholino-2-hydroxyguanidine hydrochloride (140)

The oxidation of a 0.1 mmol dm⁻³ solution of 1-morpholino-2-hydroxyguanidine hydrochloride (140) was carried out using an excess (0.01 g) of mCPBA. The results are below (Table 11)

Table 11:- Oxidation of (140) by mCPBA

Time/ min	% Oxidation
30	6
60	7
90	6

The % oxidation of 1-morpholino-2-hydroxyguanidine hydrochloride (138) after 60 minutes is 7%

2.8 CONCLUSIONS

The synthesis of the substituted *N*-hydroxyguanidines proved to be more difficult than originally envisaged. This is probably due to the inherent instability of these compounds. The range of *N*-hydroxyguanidines synthesised (Section 2.4) was small compared to the number of substituted cyanamides (Section 2.3) that were successfully prepared. However one compound, 1-benzyl-1-methyl-2-hydroxyguanidine (137) proved particularly useful in a number of areas. As reported the compound was obtained in a crystal form suitable for X-ray structural analysis (Section 2.4.3). The data, while not perfect, showed that the double bond character was mainly between the terminal nitrogens as had previously been assumed but never determined. The compound

was also tested under biological conditions and was found, as expected, to be a vasodilator. However the concentration of the compound that was required for vasodilation is quite high and may not be suitable for *in vivo* use. Previous work has shown that 1,1-dibenzyl-2-hydroxyguanidine (**70**) was a vasodilator at similar concentrations, and it can therefore be assumed that the other free *N*-hydroxyguanidines are also likely to be vasodilators.

The chemical oxidation of these compounds gave unexpected results. Previous reports suggested that the chemical oxidation of both 1,1-dibenzyl-2-hydroxyguanidine (**70**) and 1-benzyl-1-methyl-2-hydroxyguanidine (**137**) with hydrogen peroxide gave a near quantitative yield of nitric oxide, as measured via the Greiss test.⁸⁷ However the work undertaken in this project showed only 2% oxidation with hydrogen peroxide. The previous work also indicated less success with *m*-chloroperbenzoic acid (mCPBA). However oxidation of 1-benzyl-1-methyl-2-hydroxyguanidine (**137**) by mCPBA indicated a 27% oxidation. However it may be that the previous work, which used a non microanalytically pure sample of 1-benzyl-1-methyl-2-hydroxyguanidine (**137**), gave a false result and that the impurity that was present was responsible for the oxidation. However the major impurity was likely to be the corresponding urea and when the chemical oxidation was attempted using 1-benzyl-1-methylurea (**155**) there was no indication of any oxidation as expected.

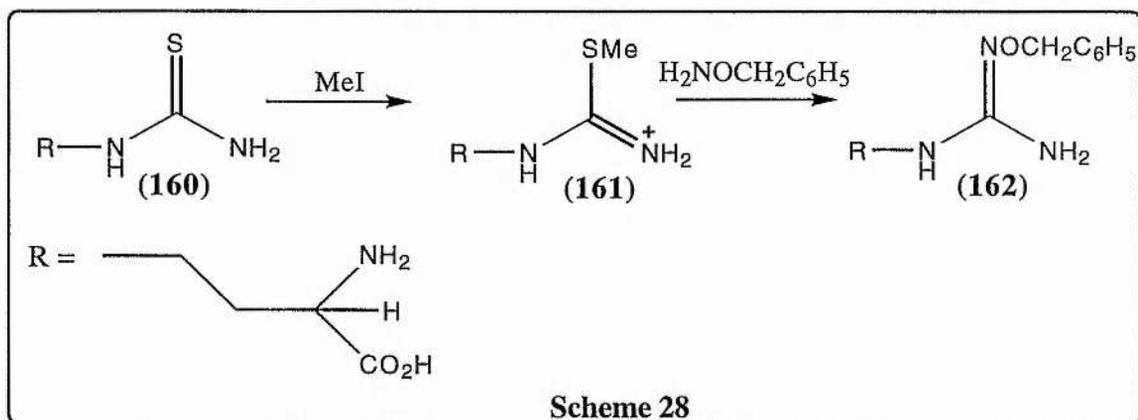
The instability of the products meant that the range of derivatives synthesised was not as great as hoped but the results obtained from the testing of the derivatives obtained confirms their potential as nitric oxide donor drugs.

Chapter 3

**Synthesis and Studies on
O-Substituted
N-Hydroxyguanidines**

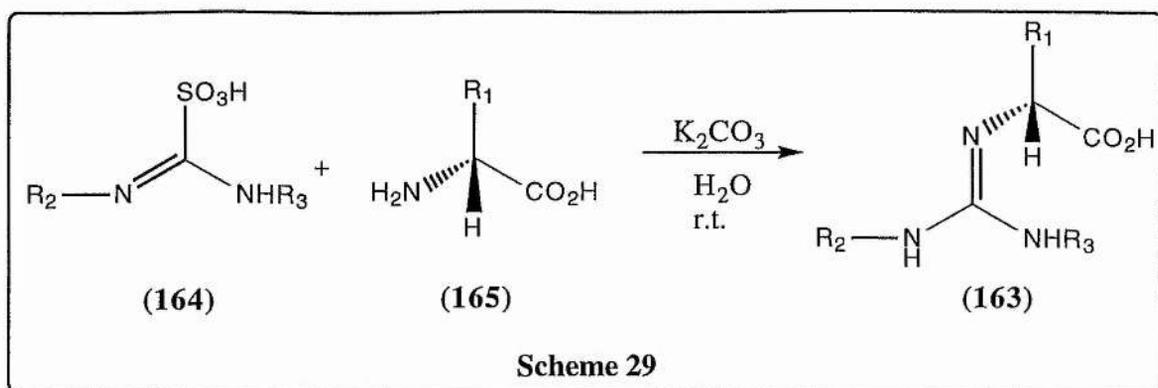
3.1 INTRODUCTION

It was decided to approach the synthesis of the *O*-substituted *N*-hydroxyguanidines using a different synthetic methodology to that employed for the unsubstituted derivatives. The use of activated sulfur leaving groups has been reported in the synthesis of *N*-hydroxy-L-arginine (**Scheme 28**).¹¹³ In this synthesis the thiourea derivative (**160**) was alkylated with methyl iodide to give the methylated sulfur derivative (**161**) that was then displaced with *O*-benzylhydroxylamine in the presence of a silver salt to give the *O*-benzyl *N*-hydroxy-L-arginine derivative (**162**) (**Scheme 27**).

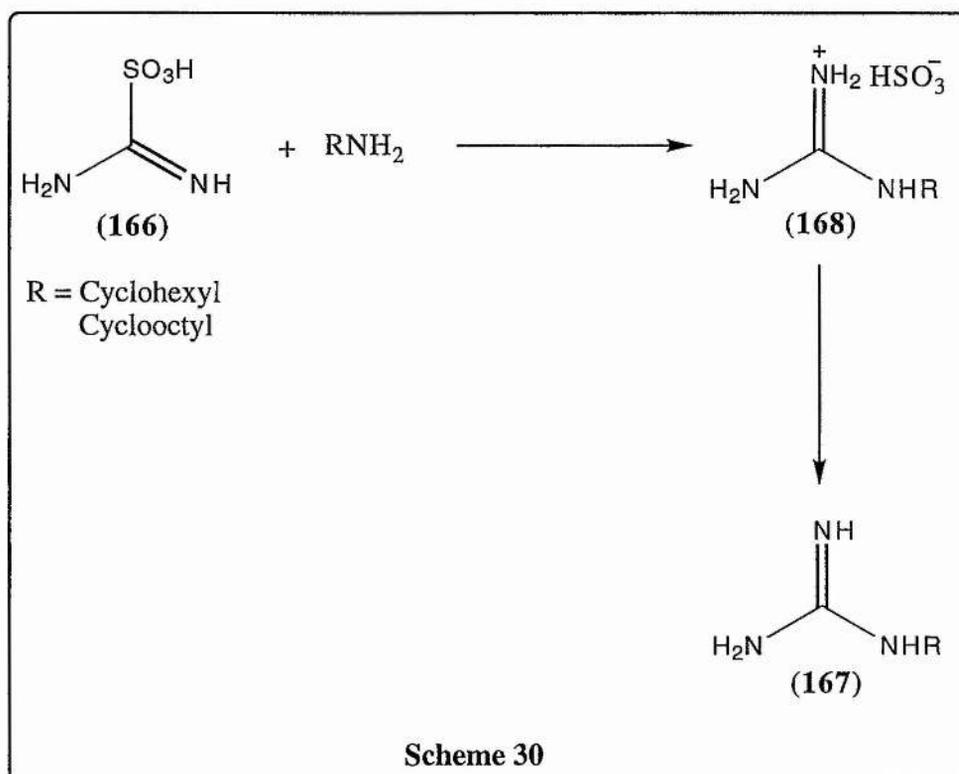


The problem with this synthesis is that the last step, the replacement of the activated sulfur group requires the presence of a silver salt which is generally toxic and the by-product is smelly. Methyl iodide, used earlier in the synthesis, is also toxic. The activated sulfur species are not very stable and are probably best formed in situ and then reacted on without isolation.

There have been a number of milder methods reported for this conversion. Miller reported the synthesis of guanidino acids (**163**) (**Scheme 29**).¹¹⁹ Reaction of the desired aminoiminomethanesulfonic acid (**164**) with an amino acid derivative (**165**) under aqueous conditions with base at room temperature gave the desired guanidino acids (**163**) in good yields.



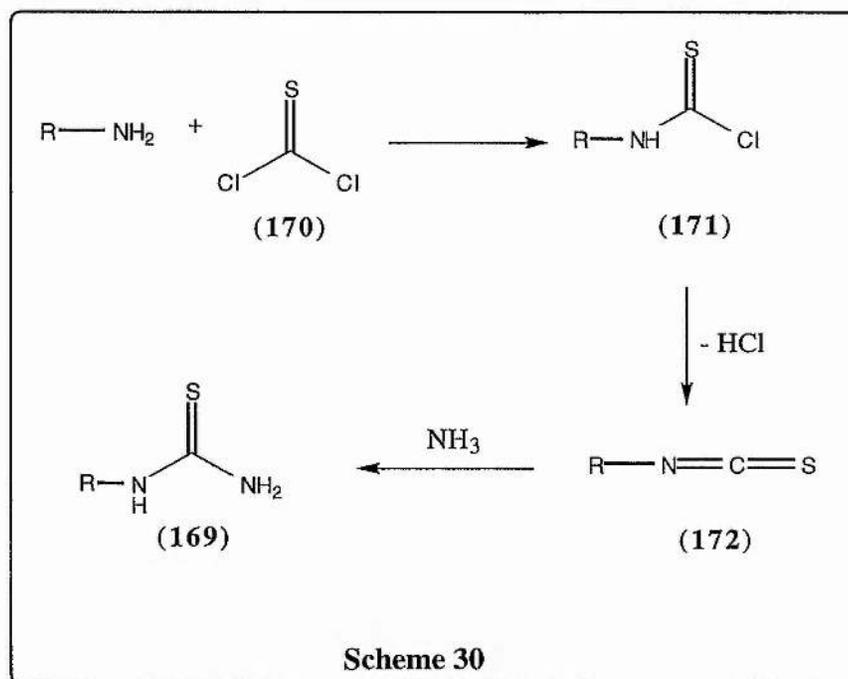
The conversion of aminoiminomethanesulfonic acid (**166**) to monosubstituted guanidines (**167**) has also been reported.¹²⁰ The authors reacted the sulfonic acid (**166**) with a primary amine in absolute methanol to give the product as the bisulfite salt (**168**) which was then neutralised to give the monosubstituted guanidine (**167**) (**Scheme 30**)



It was decided that aminoiminomethanesulfonic acids were a good starting point in the synthesis of *O*-substituted *N*-hydroxyguanidines. To synthesise these compounds the intermediate thiourea was required, which could then be oxidised to give the corresponding aminoiminomethanesulfonic acid.

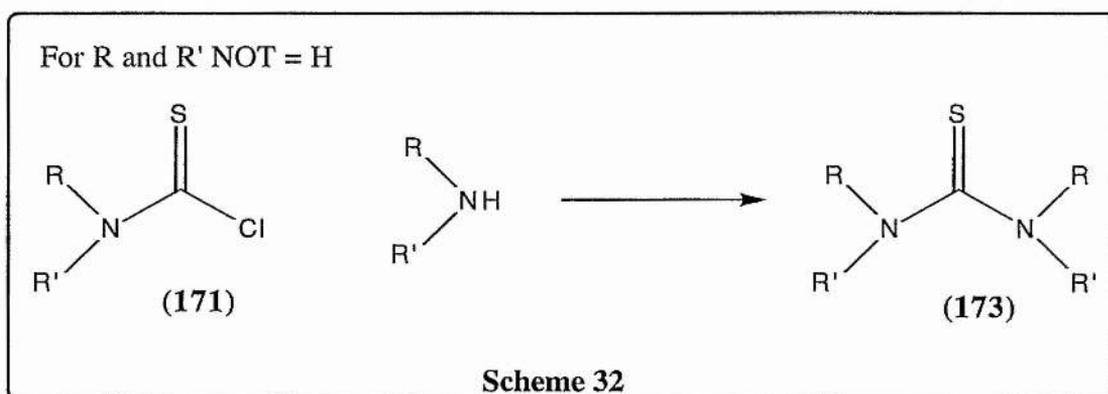
3.2 THIOUREA SYNTHESIS

One of the most convenient syntheses of thioureas (**169**) is the reaction of thiophosgene (**170**) with an amine (**Scheme 31**). The first step is reaction of the amine and thiophosgene to give the intermediate thiocarbonyl chloride (**171**) which is unstable and reacts to give the corresponding isothiocyanate (**172**). This isothiocyanate (**172**) is then reacted with ammonia to give the thiourea (**169**).



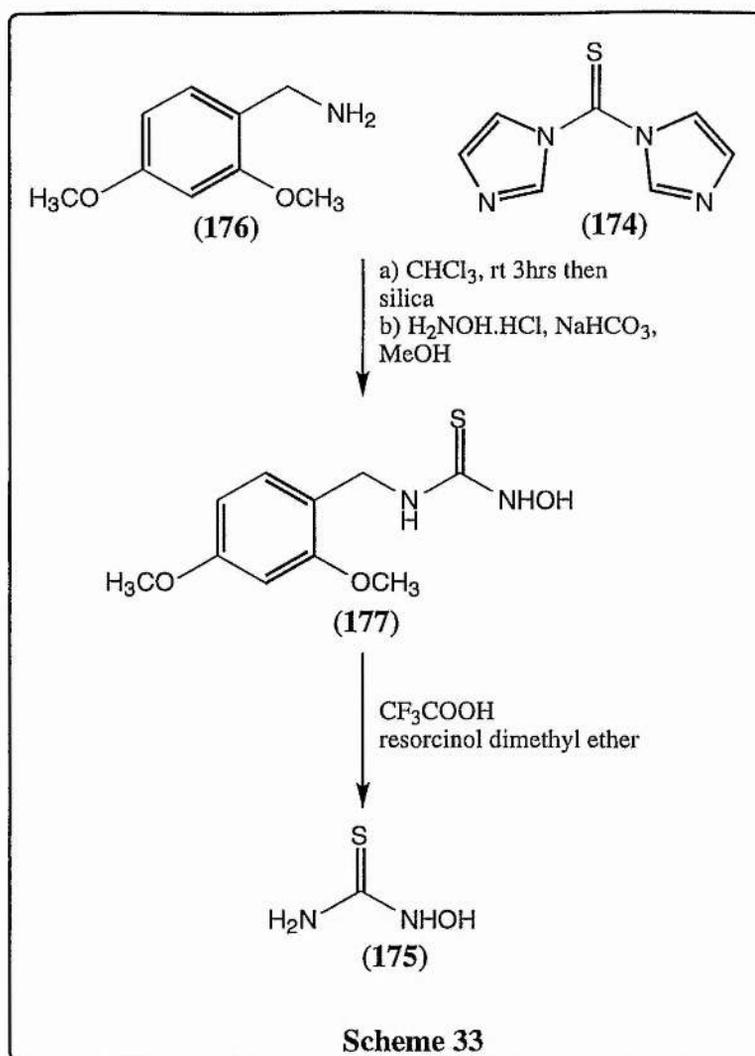
There are, however, problems associated with this synthesis when the amine that is being used is secondary rather than primary.¹²¹ With a secondary amine often the *N,N'*-tetrasubstituted thiourea (**173**) is obtained rather than the desired *N,N*-disubstituted thiourea (**169**). This occurs because the thiocarbonyl chloride (**171**), is

more stable and also because the secondary amine is more nucleophilic. Thus the thiocarbamyl chloride (**171**) can react with another mole of the amine forming the *N,N'*-tetrasubstituted thiourea (**173**) (**Scheme 32**). Formation of the *N,N'*-disubstituted thiourea, as a byproduct, can also occur with primary amines if the thiocarbamyl chloride (**171**) that is formed is stable

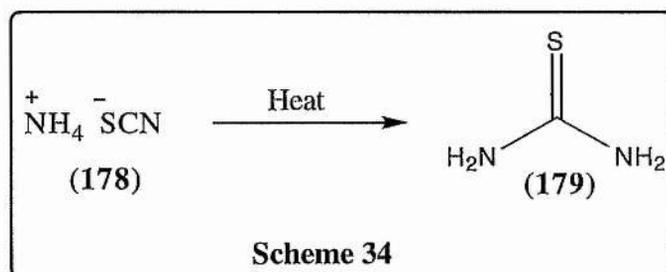


As thiophosgene (**170**) is toxic alternatives to this compound have been sought. One recent example is 1,1-thiocarbonyldiimidazole (**174**) which was used by Stammer and co-workers in the synthesis of *N*-hydroxyurea (**175**).¹²²

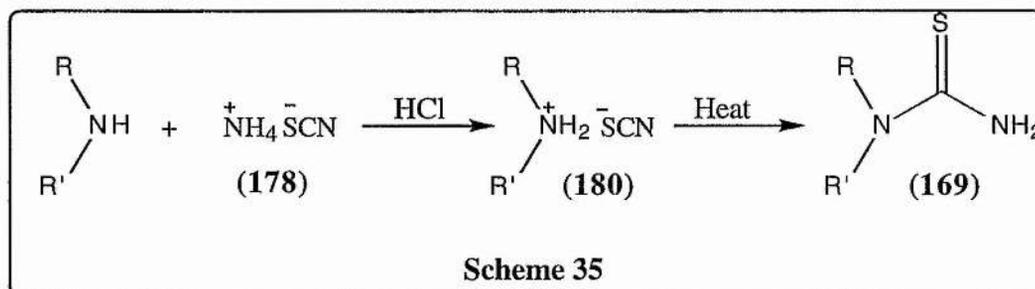
Reaction of 2,4-dimethoxybenzylamine (**176**) with *N,N'*-dithiocarbonyldiimidazole (**174**) in dry chloroform followed by column chromatography, yielded the desired isothiocyanate which was then reacted with hydroxylamine hydrochloride in the presence of sodium hydrogen carbonate to give *N*-(2,4-dimethoxybenzyl)-*N'*-hydroxythiourea (**177**) (**Scheme 33**). Removal of the 2,4-dimethoxybenzyl protecting group was then achieved using trifluoroacetic acid to give the target *N*-hydroxythiourea (**175**) in 34% overall yield.



Another alternative method for thiourea synthesis involves the use of ammonium thiocyanate (178). Heating ammonium thiocyanate (178) at 160 °C for a number of hours is known to produce thiourea (179) (Scheme 34).



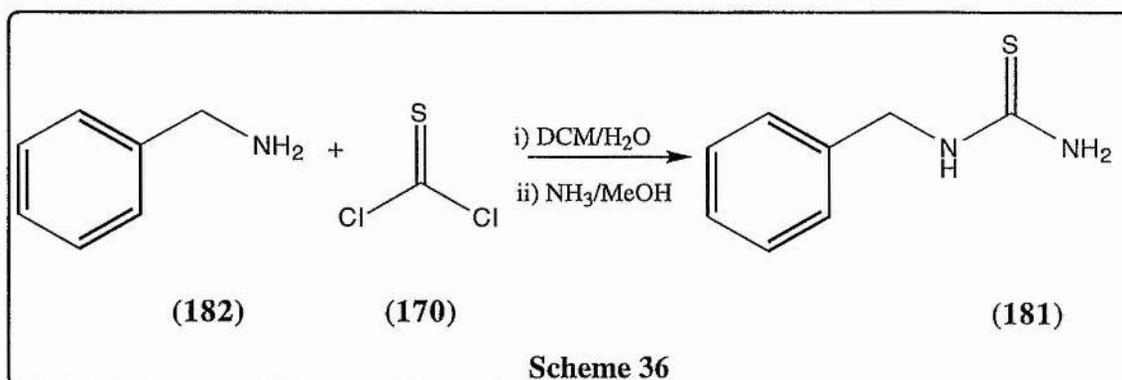
Various groups have reported the extension of this work for the synthesis of a range *N,N*-disubstituted thioureas and *N*-substituted thioureas using this methodology.^{123,124,125} The reaction occurs because when the amine and ammonium thiocyanate (**178**) are added together the primary or secondary ammonium ion (**180**) is formed which then rearranges upon heating to give the desired thiourea (**169**) (**Scheme 35**).



The reaction is carried out either in an inert organic solvent which has been saturated with dry hydrogen chloride gas prior to addition of the amine and ammonium thiocyanate, or in aqueous hydrochloric acid. The reaction is suitable for both aliphatic and aromatic amines.

3.2.1 *N*-Benzylthiourea (181)

The first target compound was *N*-benzylthiourea (**181**). Its synthesis was undertaken using the reaction of thiophosgene (**170**) and benzylamine (**182**) (Scheme 36).



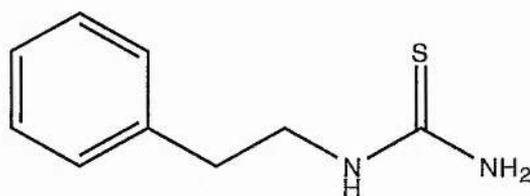
Benzylamine (**182**) and an excess of thiophosgene (**170**) were stirred together in a suspension of dichloromethane and water in a two phase reaction. After stirring for two hours at room temperature the layers were separated and the organic layer was washed with water. The organic layer was concentrated under reduced pressure to give an orange oil which was dissolved in methanol and aqueous ammonia added. The reaction was stirred at 0 °C for three hours and then concentrated under reduced pressure. Recrystallisation of the crude solid from ethanol to gave the product (**181**) as a white solid in 20% yield. The melting point of 159-161 °C corresponded well with the literature value of 161-162 °C,¹²⁶ and the microanalysis indicated that the product was pure. The characteristic C=S peak in the ¹³C NMR spectrum was found at 183.71 ppm.

3.2.2 Other Monosubstituted Examples

It was decided to synthesise the other thioureas using the methodology of Passing. This method was used because it was an easy and convenient synthesis of both primary and secondary thioureas unlike the thiophosgene method (Section 3.1). The

reaction also involved the reaction of the readily available, cheap and non toxic ammonium thiocyanate. The inert solvent that was used for the reaction was chlorobenzene which was saturated with dry hydrogen chloride gas for thirty minutes before reaction. To this cloudy solution was added the amine followed by the ammonium thiocyanate (**178**) which resulted in a thick suspension. This suspension was then heated under reflux for 8-10 hours. On cooling the product crystallised from solution. The crude solid product was then stirred in water to remove the inorganic salts, filtered and dried.

N-(2-Phenylethyl)thiourea (**183**) was synthesised using this procedure from phenethylamine. The product was obtained as a white solid in 51% yield.

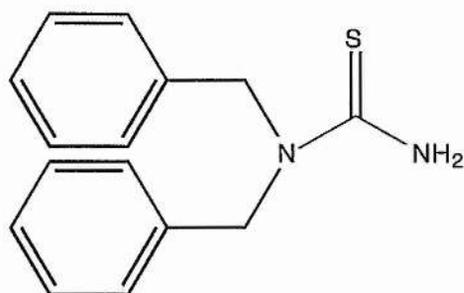


(**183**)

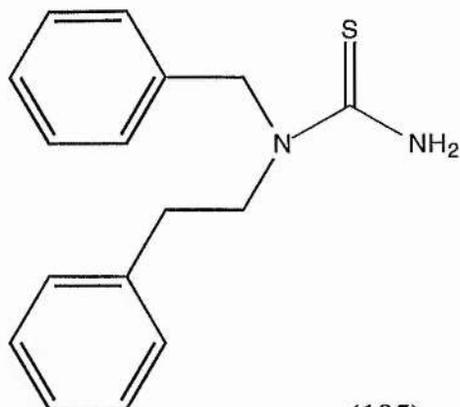
The structure was confirmed by the molecular ion at 180 in the mass spectrum and also the presence of the C=S peak in the ^{13}C NMR spectrum at 183.51 ppm. The compound also gave a correct microanalysis.

3.2.3 Disubstituted analogues

Two disubstituted analogues, *N,N*-dibenzylthiourea (**184**) and *N*-benzyl-*N*-(2-phenethyl)thiourea (**185**), were also successfully prepared using this procedure in 59% and 76% yields respectively. The major difference was that the preparation of *N*-benzyl-*N*-(2-phenethyl)thiourea (**185**) was carried out in aqueous hydrochloric acid. This was because an earlier attempt using chlorobenzene had given no product which may have been because the product was soluble in chlorobenzene and so an attempt was made using the aqueous acid solution.



(184)

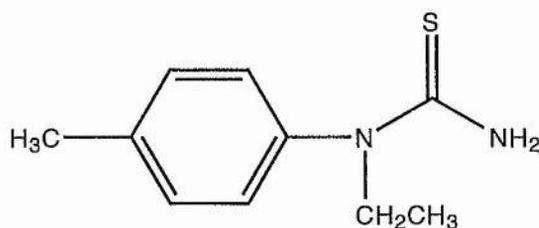


(185)

N,N-Dibenzylthiourea (**184**) was obtained as a white solid and the molecular ion in the mass spectrum was found at 256 and the ^{13}C NMR spectrum showed the C=S peak at 182.84 ppm. The i.r. spectra also showed a C=S stretch at 1510 cm^{-1} and the melting point of $134\text{--}135\text{ }^\circ\text{C}$ was in agreement with the literature value of $134\text{--}136\text{ }^\circ\text{C}$.¹²⁷

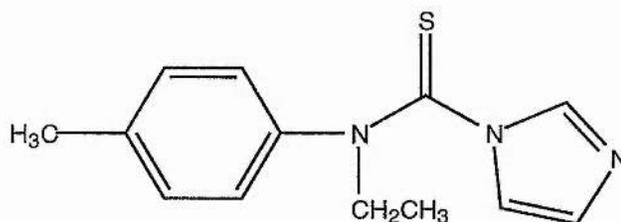
N-Benzyl-*N*-(2-phenethyl)thiourea (**185**) was obtained as a solid which gave the expected molecular ion peak, M^+ , at 270 and the C=S stretch in the ^{13}C NMR spectrum at 184.56 ppm. The i.r. also showed the C=S stretch at 1490 cm^{-1} .

The synthesis of *N*-ethyl-*N*-(*p*-tolyl)thiourea (**186**) was attempted using this method. However when the reaction was attempted in refluxing chlorobenzene the starting amine was isolated and this was also true when the reaction was attempted in aqueous acid. This seemed to confirm that the aromatic amines are less basic and also less nucleophilic and therefore do not react with the ammonium thiocyanate. A similar problem was seen when the synthesis of *N,N*-diphenylcyanamide (**130**) was attempted from the corresponding amine and cyanogen bromide.



(186)

It was then decided to attempt the synthesis using the thiophosgene analogue, *N,N'*-thiocarbonyldiimidazole (**174**). The reaction was carried out by reacting *N*-ethyl-*N*-(*p*-tolyl)amine with *N,N'*-thiocarbonyldiimidazole (**174**) in THF for an hour. The reaction mixture was then concentrated under reduced pressure and aqueous ammonia solution added and the reaction stirred overnight at room temperature. Removal of the solvent under reduced pressure yielded an orange oil which was shown by tlc to be at least two products which were separated using column chromatography. However the NMR of the product indicated that the product that was isolated still contained an imidazole group and this was confirmed by the mass spectrum which showed the molecular ion at 245 and not the expected 194. This suggested that the second step of the reaction had not worked and that the product isolated was the reaction intermediate (**187**).



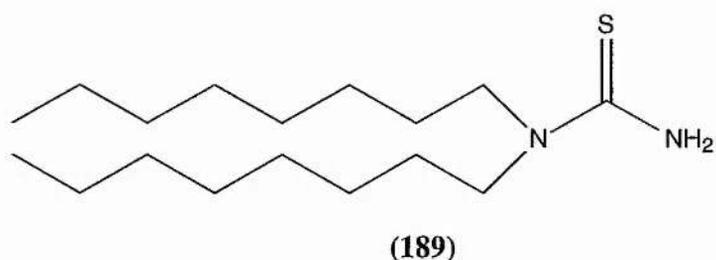
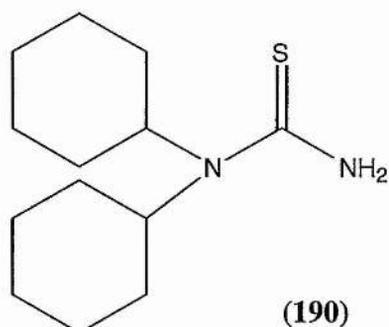
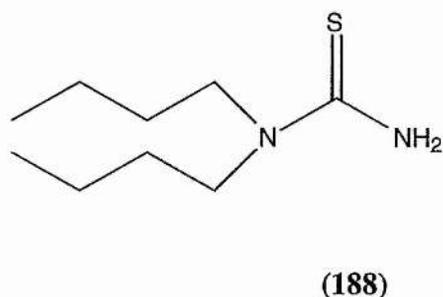
(187)

Another attempt was made at this reaction except when the aqueous ammonia was added the solution was heated for six hours and then stirred overnight at room temperature. Similarly after column chromatography the product obtained still contained an imidazole group and the mass spectrum confirmed that (**187**) rather than the desired product had been formed. The synthesis was then attempted using thiophosgene (**170**). Here the amine was stirred in DCM and a suspension of thiophosgene in water was added and the reaction stirred at room temperature for three hours. Separation of the organic layer and subsequent concentration of this layer under reduced pressure yielded an orange oil which was dissolved in methanol and aqueous methanol was added and the

reaction then stirred at room temperature overnight. Concentration of the reaction under reduced pressure yielded an orange oil which was shown by NMR spectroscopy to be mainly the starting amine. The explanation for the unreactive nature of these compounds has been discussed and the less reactive nature of these compounds has proved to be a limiting factor in the number of derivatives that can be synthesised.

3.2.4 Other Examples

The synthesis of a number of examples were attempted, including *N,N*-dioctylthiourea (**188**), *N,N*-dibutylthiourea (**189**) and *N,N*-dicyclohexylthiourea (**190**) using the reaction of the corresponding amine and ammonium thiocyanate.

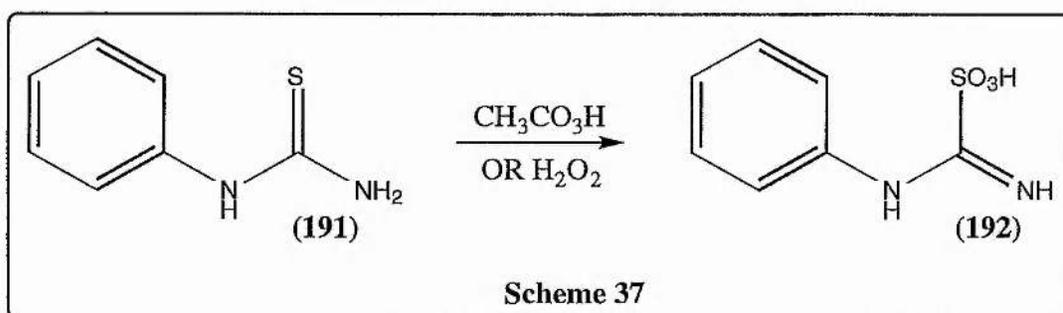


The synthesis of the two dialiphatic derivatives, *N,N*-dibutylthiourea (**188**) and *N,N*-dioctylthiourea (**189**), could not be achieved using this route and were not attempted using either of the other routes. The problem here must be that the amines are less nucleophilic and more soluble in the organic solvent. However the synthesis of

N,N-dicyclohexylthiourea (**190**) was successfully achieved by heating the corresponding amine in chlorobenzene in the presence of ammonium thiocyanate. The desired product was obtained as a white solid in 77% yield. The presence of the product was confirmed by the ^{13}C NMR spectrum which showed the presence of the C=S peak at 182.1 ppm

3.3 AMINOIMINOMETHANESULFONIC ACID SYNTHESIS

As previously discussed it was decided that the use of aminoiminomethanesulfonic acids would prove useful as an alternative to the use of the alkylated sulfur derivatives (See Section 3.1). Aminoiminomethanesulfonic acids can be synthesised from the corresponding thiourea by oxidation. It has been reported that this oxidation can be carried out using hydrogen peroxide in either methanol or water in the presence of a catalyst, sodium molybdate dihydrate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$).¹²⁸ The use of this catalyst was first reported by Maryanoff and co-workers who had attempted to oxidise *N*-phenylthiourea (**191**) to the corresponding *N*-phenylaminoiminomethanesulfonic acid (**192**) in the absence of a catalyst (Scheme 37).¹²⁹

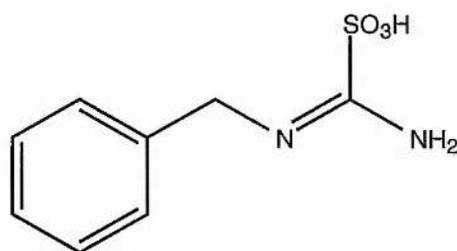


The authors used the reported literature procedure which employed freshly prepared peracetic acid or hydrogen peroxide to carry out the oxidation.^{130,131} However they found that the oxidation was slow in comparison to the decomposition of the intermediates and a number of products were formed. The yield was improved by

increasing the rate of oxidation in comparison to the rate of decomposition of the intermediates. It has long been known that the metal peroxo d^0 complexes catalyse oxidation by hydrogen peroxide and that molybdenum catalysts have been used in the oxidation of sulfur containing groups. Marynoff found that sodium molybdate acted as a catalyst in the oxidation of *N*-phenylthiourea (**191**) giving the desired *N*-phenylaminoiminomethanesulfonic acid (**192**) in good yield.

3.3.1 Monosubstituted Derivatives

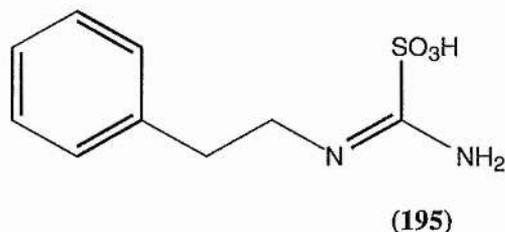
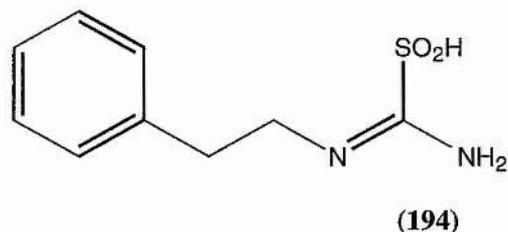
The synthesis of *N*-benzylaminoiminomethanesulfonic acid (**193**) was successfully undertaken using a modification of the above literature method. *N*-Benzylthiourea (**181**) and sodium molybdate were stirred in water at 0 °C and hydrogen peroxide was added dropwise to this suspension. After addition of the hydrogen peroxide was complete the reaction was removed from the ice bath and an exothermic reaction then occurred. When the exotherm was complete the reaction was cooled back down to 0 °C and the solid filtered and dried. *N*-Benzylaminoiminomethanesulfonic acid (**193**) was obtained in 85% yield.



(193)

The product gave a resonance in the ^{13}C NMR spectrum at 165.96 ppm compared to that for the thiocarbonyl group at 183.71 ppm. The melting point of 163-165 °C also agreed well with the literature value of 168 °C and the negative ion electrospray mass spectrum gave the necessary molecular ion, $[\text{M}-\text{H}]^-$, at 213.

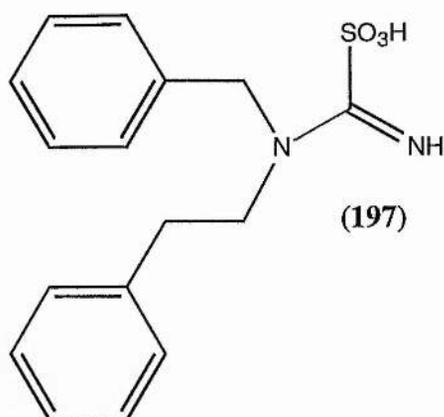
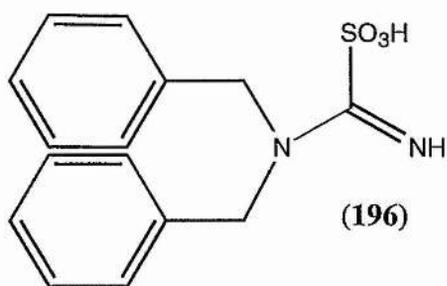
When the oxidation was initially carried out on *N*-(2-phenethyl)thiourea (**183**) a product was obtained which appeared to be pure by NMR spectroscopy. However the negative ion electrospray mass spectrum gave the molecular ion, $[M-H]^-$, at 212 rather than the expected 228. It, therefore, seemed that complete oxidation had not occurred and that the intermediate *N*-(2-phenethyl)aminoiminomethanesulfonic acid (**194**) rather than *N*-(2-phenethyl)aminoiminomethanesulfonic acid (**195**) had been obtained.



However this problem was overcome by using a greater excess of hydrogen peroxide which ensured that the oxidation went to completion. When the reaction was repeated a solid was obtained in 59% yield which was seen by negative ion electrospray to be the product with the $[M-H]^-$ ion at 228.

3.3.2 Disubstituted Derivatives

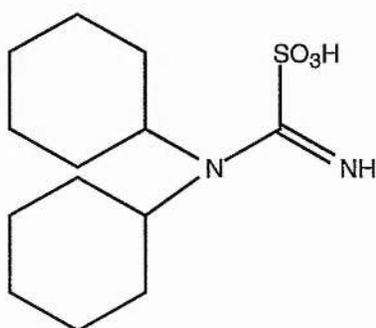
The syntheses of *N,N*-dibenzylaminoiminomethanesulfonic acid (**196**) and *N*-benzyl-*N*-(2-phenethyl)aminoiminomethanesulfonic acid (**197**) were also completed using this method in 55% and 57% yields respectively.



The structure of *N,N*-dibenzylaminoiminomethanesulfonic acid (**196**) was confirmed by the presence of the peak in the ^{13}C NMR spectrum at 167.69 ppm and the presence in the mass spectrum of the molecular ion, $[\text{M}-\text{H}]^-$ at 303.

N-Benzyl-*N*-(2-phenethyl)aminoiminomethanesulfonic acid (**197**) also gave the expected $[\text{M}-\text{H}]^-$ ion at 317 in the mass spectrum and a peak in the ^{13}C NMR spectrum at 168.20 ppm.

The synthesis of *N,N*-dicyclohexylaminoiminomethanesulfonic acid (**198**) was carried out from *N,N*-dicyclohexylthiourea (**198**). The product was obtained as a solid in 47% yield and the ^{13}C NMR spectrum showed the desired C-SO₃H peak at 167.90 ppm and the mass spectrum found the $[\text{M}-\text{H}]^-$ ion at 287.



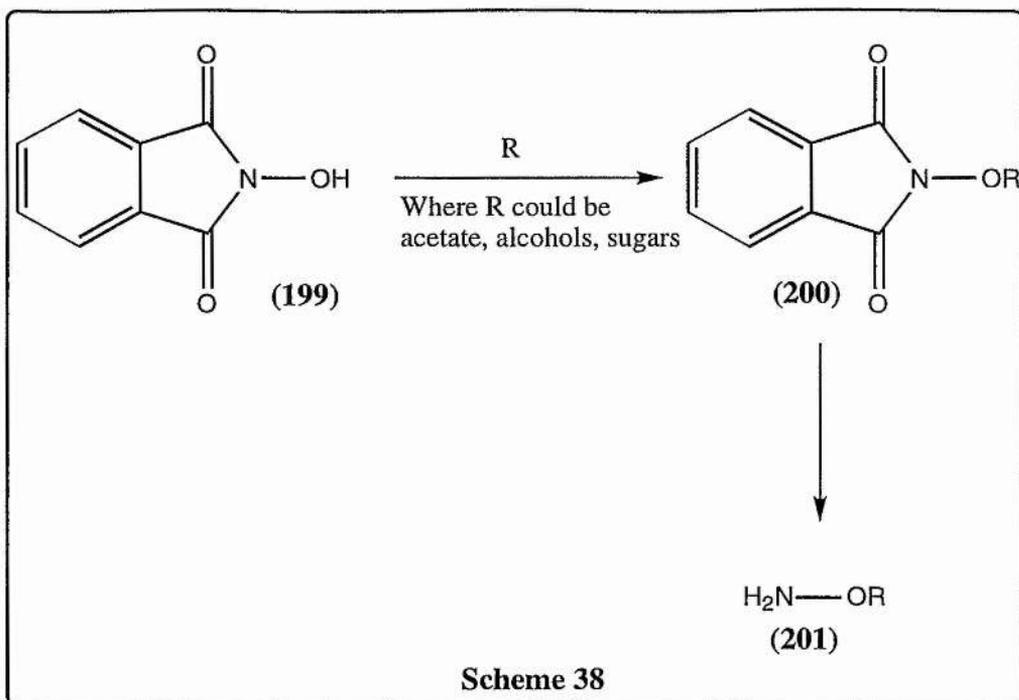
(198)

3.4 SYNTHESIS OF *O*-SUBSTITUTED HYDROXYLAMINES

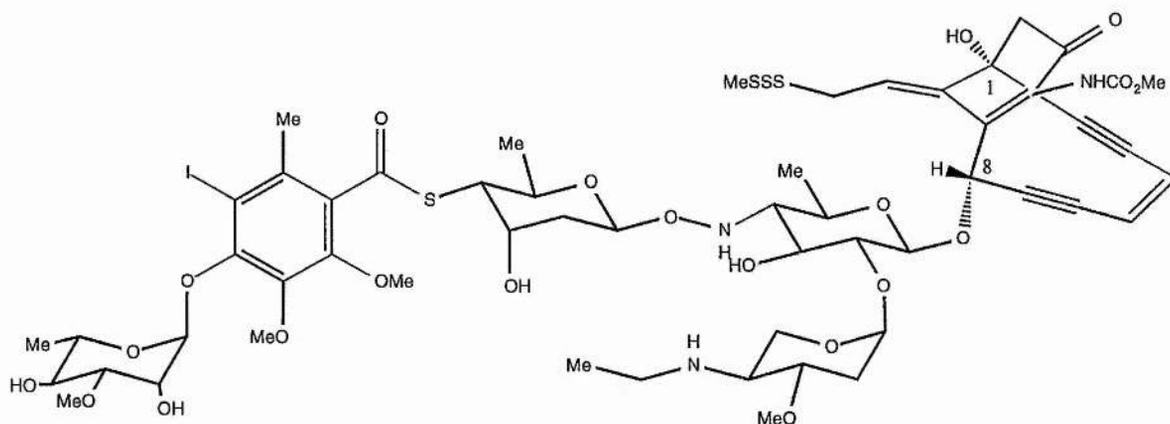
In order to prepare the *O*-substituted *N*-hydroxyguanidines it was decided to employ *O*-substituted hydroxylamines as nucleophiles to react with either the aminoiminomethanesulfonic acids or the cyanamides. Only a few *O*-substituted hydroxylamines are commercially available, e.g. benzyl, methyl, and so other examples had to be synthesised.

The route to the *O*-substituted hydroxylamines requires initial *N*-protection, reaction at oxygen and then deprotection to reveal the free amino group. Two *N*-protecting groups have been examined in this work, the phthalamide group and the acetohydroxamic acid ethyl ester.

N-Hydroxyphthalimide (**199**) is commercially available and substituents can be attached to the oxygen either via a Mitsunobu type coupling or a nucleophilic displacement from a carbon centred electrophile to give the *O*-substituted *N*-hydroxyphthalimide (**200**). Removal of the phthalimide group can then be carried out using hydrazine hydrate to yield the desired *O*-substituted hydroxylamine (**201**) (**Scheme 38**). However it is clear that the use of hydrazine limits this reaction to hydrazine stable substituents on the oxygen atom.

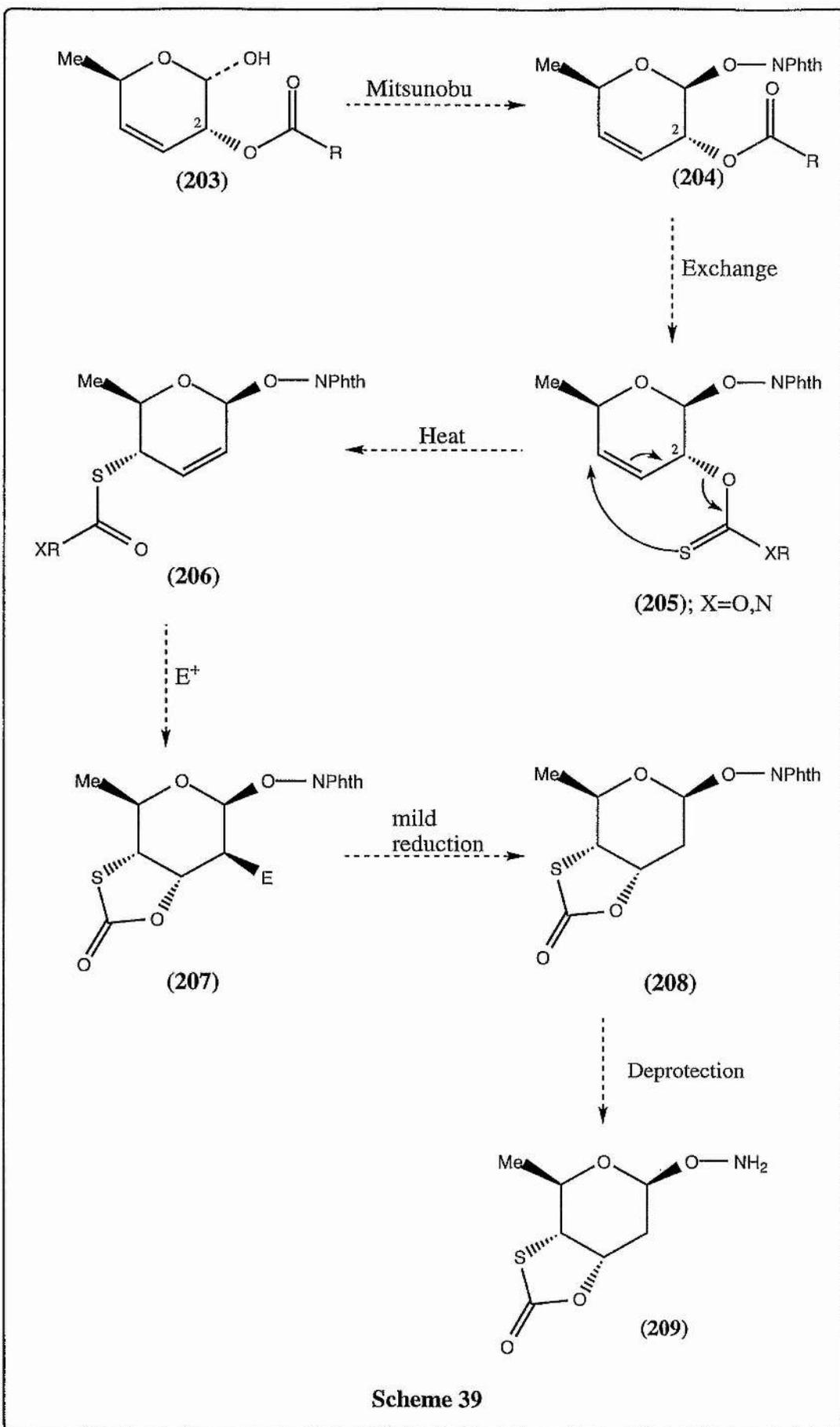


The use of the phthalimide methodology was employed by Nicolaou in his synthesis of calicheamicin γ_1 (202).¹³² Calicheamicin is a minor groove binder that causes double-stranded scission of DNA at oligopyrimidine runs. It is an interesting natural product because it cleaves the DNA selectively. The unusual feature of calicheamicin is the highly unusual oligosaccharide on C-8 which includes a hydroxylamine glycosidic linkage and an iodinated, hexasubstituted thiobenzoate. The oligosaccharide fragment serves as a recognition and delivery system and it binds the molecule to a remarkably specific sequence in the minor groove of duplex DNA at 5'-TCCT and 5'-TTTT.



(202)

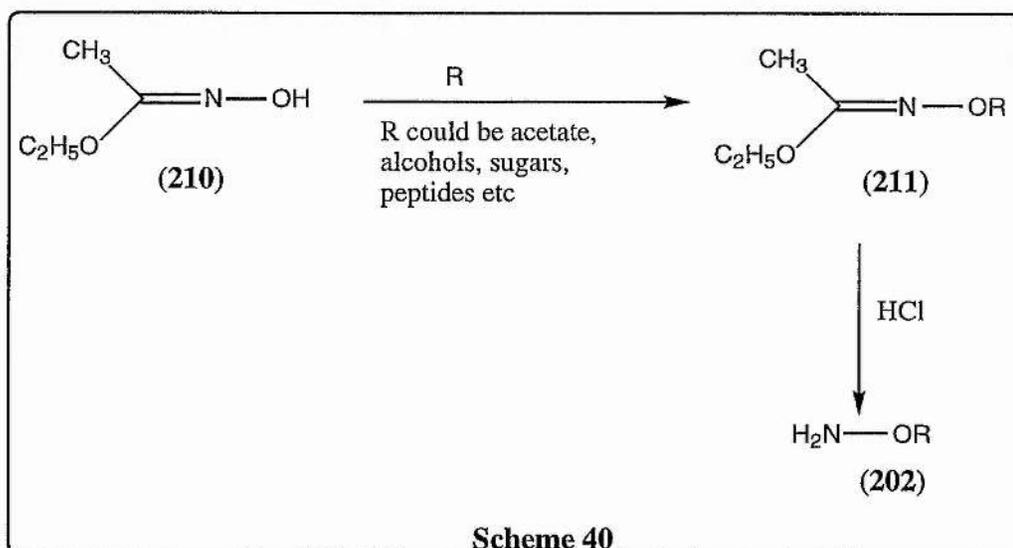
The authors felt that the ring in the centre of the molecule would prove to be the most difficult part of the synthesis due to the array and complex nature of the functionality including the unusual hydroxylamino glycosidic linkage, the sulfur atom at the 4-position, the 2,6-dideoxy positions and by far the most challenging problem, the 2-deoxy- β -glycosidic linkage. The production of such linkages employ a participating group in the 2 position (e.g. an ester or thiophenyl) and then reductive removal of the group at the 2 position which in the presence of the N-O bond, which is sensitive to reduction, would not be synthetically possible. The authors developed new methodology which involved the generation of the unsaturated lactol (**203**) (**Scheme 39**). The ester in position 2 helps in the stereoselective incorporation of the β -hydroxylamino glycoside through a Mitsunobu reaction with *N*-hydroxyphthalimide (**200**) to give (**204**). With the glycosidic linkage installed the remaining functionality of the ring can be built up. The exchange of the ester for a thionocarbonate or carbamate (**205**) would allow deoxygenation of position-2 and introduction of the sulfur at position-4 by a [3,3]-thermal sigmatropic rearrangement to yield (**206**). Reaction of this with an electrophile allows oxygenation at position-3 which is delivered stereoselectively in an intramolecular reaction to yield a cyclic thiocarbonate (**207**). Mild reduction then removes the electrophile and yield the protected target ring (**208**) which can be deprotected to give the target molecule (**209**)



Scheme 39

The most important thing about the phthalimide protecting group is the number of steps and range of conditions under which it remains stable, as can be seen in the synthesis of calicheamicin γ 1 (**202**). The group can then be selectively removed by reaction with hydrazine hydrate in ethanol at room temperature.

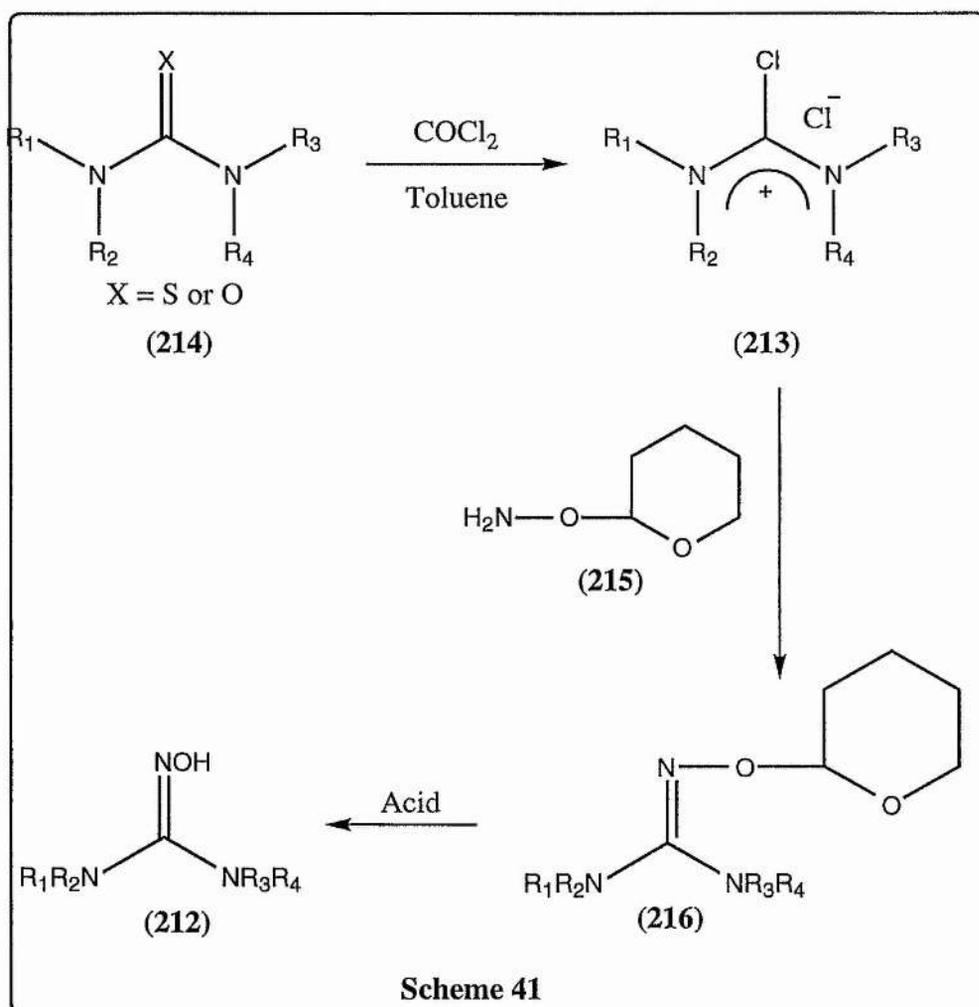
The other potential route involves the use of acetoxyhydroxamic acid ethyl ester (**210**), which is also a commercially available, as the hydroxylamine equivalent. Again it can be envisaged that reaction of this compound either via a Mitsunobu coupling or by a nucleophilic displacement of a leaving group from a carbon centred electrophile would yield the *O*-substituted acetoxyhydroxamic acid ethyl ester (**211**) (Scheme 40). Removal of the nitrogen protecting group is carried out by using hydrogen chloride gas and one equivalent of water.



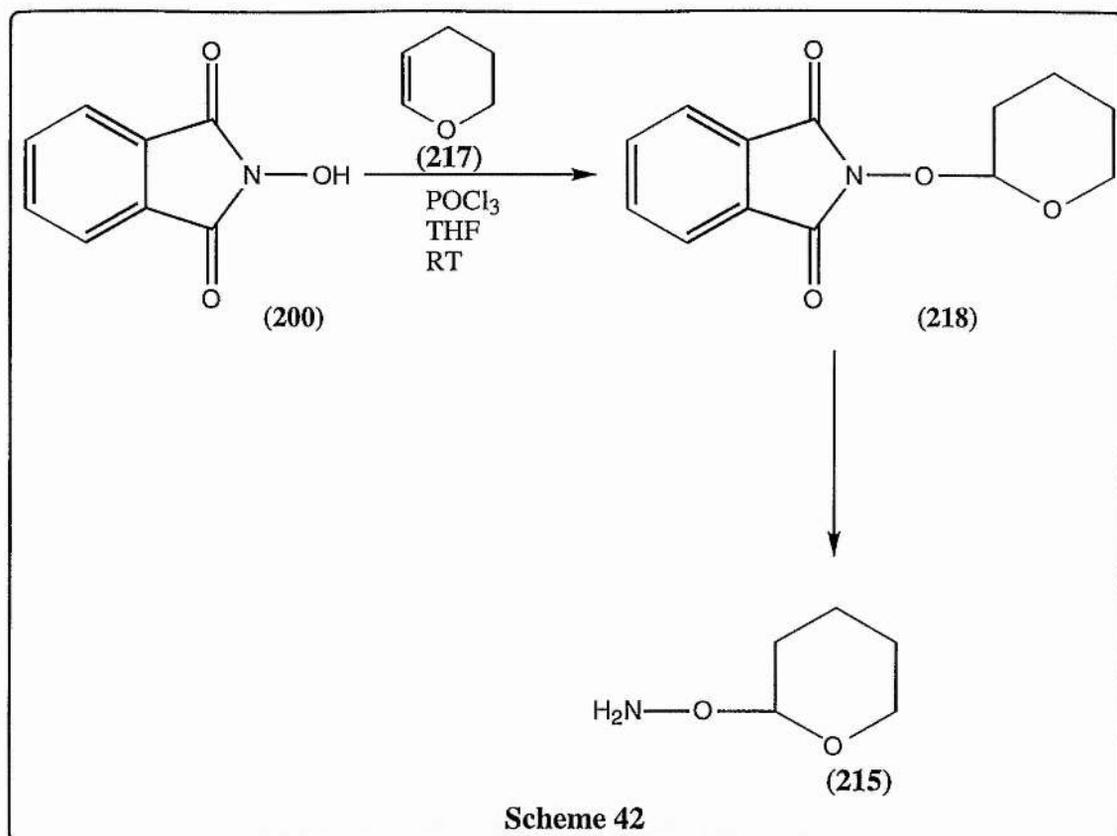
3.4.1 *O*-(Tetrahydro-2-pyranyl)hydroxylamine

Zinman reported the preparation of 1,1,3-trisubstituted hydroxyguanidines (**212**) from *C*-chloroformamidinium chlorides (**213**), which were synthesised from either the corresponding urea or thiourea (**214**). Reaction of the *C*-chloroformamidinium chloride (**213**) with *O*-tetrahydropyranyloxyhydroxylamine (**215**) yielded the *O*-THP protected

1,1,3-trisubstituted hydroxyguanidine (**216**). Removal of the protecting group by acid hydrolysis yielded the 1,1,3-trisubstituted hydroxyguanidines (**212**) (**Scheme 41**).¹³³



The synthesis of *O*-tetrahydropyranylhydroxylamine (**215**) was first reported by Warrenner and Cain in 1976.¹³⁴ They were preparing 1-hydroxy-2-thiouracil and required a synthesis of *O*-tetrahydropyranylhydroxylamine (**215**). Reaction of *N*-hydroxyphthalimide (**200**) with dihydro-2H-pyran (**217**) in THF with phosphoryloxychloride acting as a catalyst yielded *O*-tetrahydropyranyl-*N*-hydroxyphthalimide (**218**) (**Scheme 42**). The *O*-tetrahydropyranyl-*N*-hydroxyphthalimide (**218**) was then reacted with hydrazine hydrate in toluene to give the desired *O*-(tetrahydropyranyl)hydroxylamine (**215**) as a clear oil.



When the first step of the synthesis of *O*-tetrahydropyranyl-*N*-hydroxyphthalimide (**218**) was attempted instead of the expected white solid a black solid was obtained. Examination by tlc showed a number of compounds so an attempt to purify by silica column chromatography using 4:1 ethyl acetate/ petroleum ether as the eluant was made. A white solid was obtained from the column however analysis by NMR spectroscopy showed no protons due to the tetrahydropyranyl group. One of the advantages of the tetrahydropyranyl protecting group is that it can be removed under mild acidic conditions so it appears that the silica used on the column was acidic enough to remove the protecting group.

An alternative catalyst for the reaction was then sought. There are various reports of catalysts for the tetrahydropyranylation of alcohols. The use of iodotrimethylsilane as a catalyst in the protection of a number of primary and secondary alcohols with THP has been reported.¹³⁵ The alcohol and dihydropyran are stirred in dichloromethane with a

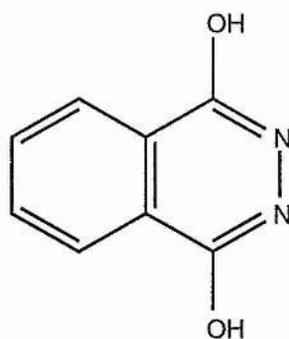
catalytic amount of iodotrimethylsilane (or allyltrimethylsilane or hexamethyldisilane and a few crystals of iodine). The corresponding tetrahydropyranyl protected alcohols are obtained in excellent yields within 20 to 30 minutes. The use of triphenylphosphine bromide as a catalyst has also been reported, however its use is restricted to tertiary alcohols.¹³⁶ Two groups reported that pyridinium *p*-toluenesulfonate (**219**) will act as a catalyst for the tetrahydropyranalation of alcohols.^{137,138} Both groups had problems when they tried to protect their acid sensitive alcohols using *p*-toluenesulfonic acid as the catalyst but pyridinium *p*-toluenesulfonate (**219**) is a milder reagent and is useful where there are acid sensitive groups present in the molecule.

The catalyst was prepared by stirring *p*-toluenesulfonic acid in an excess of pyridine at room temperature. Removal of the excess pyridine yields a solid which is then recrystallised from acetone to give the product (**219**) as a white solid in 74% yield. The microanalysis of the compound showed that the compound was pure and the melting point of 117-118 °C agrees well with the literature value of 120 °C.¹³⁸

This catalyst was then used in the tetrahydropyranylation of *N*-hydroxyphthalimide to produce *O*-tetrahydropyranyl-*N*-hydroxyphthalimide (**218**). The reaction was carried out in dry dichloromethane at room temperature. The white solid that was obtained after recrystallisation from ethyl acetate was found to be correct by NMR spectroscopy and had a melting point of 119-121 °C which agreed with the literature value of 123 °C.¹³⁹

The *O*-tetrahydropyranyl-*N*-hydroxyphthalimide (**218**) was then reacted with hydrazine hydrate in refluxing toluene for 5-6 hours. The reaction was filtered to remove the by-product (**220**), and the solvent removed under reduced pressure to obtain a yellow oil. Excess hydrazine hydrate was then removed by extraction into water. The yellow oil that was obtained was found to be correct by NMR spectroscopy. However the yield of this reaction was quite low, <20%, so an alternative route was sought. The reaction was tried again in toluene this time at room temperature. The reaction was followed by tlc and after 12 hours the reaction appeared to be complete. Again the by-product was removed by filtration and the filtrate was concentrated under reduced

pressure to yield a clear liquid. The clear liquid was weighed, redissolved in toluene, washed with water and reweighed. After this it was noted that the majority of the product was not recovered suggesting that the product obtained was very soluble in water. This meant that washing with water to remove the excess hydrazine hydrate could not be carried out without losing significant amounts of the product in the aqueous layer.



(220)

Due to its unstable, explosive nature it is not possible to remove excess hydrazine by distillation. To overcome this problem a deficiency of hydrazine hydrate was therefore used rather than an excess. The reaction was thus carried out in ethanol at room temperature to yield a yellow residue which was distilled under reduced pressure using a Kugelrohr apparatus. The *O*-tetrahydropyranylhydroxylamine (215) was then obtained as a white solid in 36% yield. The NMR spectrum of the compound showed that all the resonances relating to the phthalimide had disappeared and there was a resonance in the ^1H spectrum due to the NH_2 at 5.47 ppm. The mass spectrum also confirmed the structure with a peak at 118 corresponding to the $[\text{M}+\text{H}]^+$ ion. As the yields of the reaction were variable, in an attempt to optimise the conditions five reactions were carried out in either toluene or ethanol, at various temperatures and for different periods of time.

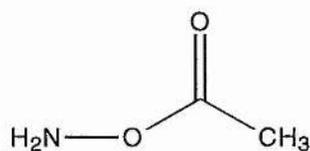
Table 12:- Conditions and results of the optimisation of the deprotection of (215)

Reaction number	Solvent	Temp.	Reaction Time	% Yield
1	Ethanol	RT	16 hours	34
2	Toluene	RT	16 hours	43
3	Ethanol	Reflux	4 hours	66
4	Toluene	Reflux	4 hours	51
5	Ethanol	RT	4 hours	38

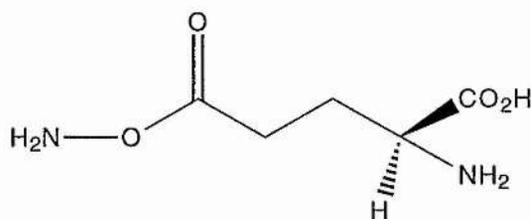
It can be seen that the best results (**Table 12**) were obtained when the reaction was carried out in refluxing ethanol for 4 hours and the worst when the reaction was carried out in ethanol at room temperature. The reaction was then tried on a large scale using refluxing ethanol and *O*-(tetrahydro-2-pyranyl)hydroxylamine (**215**) was obtained in a 69% yield. In addition to this increased yield the reaction was now reproducible and similar yields could be obtained repeatedly.

3.4.2 *O*-Acetylhydroxylamine

The preparation of *O*-acetyl hydroxylamine (**221**) would act as a simple model synthesis for the preparation of more complicated peptide derivatives for example γ -L-glutamyl hydroxylamine (**222**) which was a longer term synthetic target.

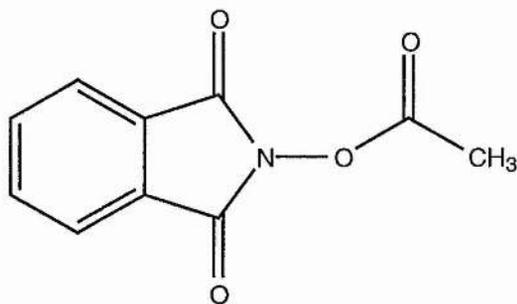


(221)



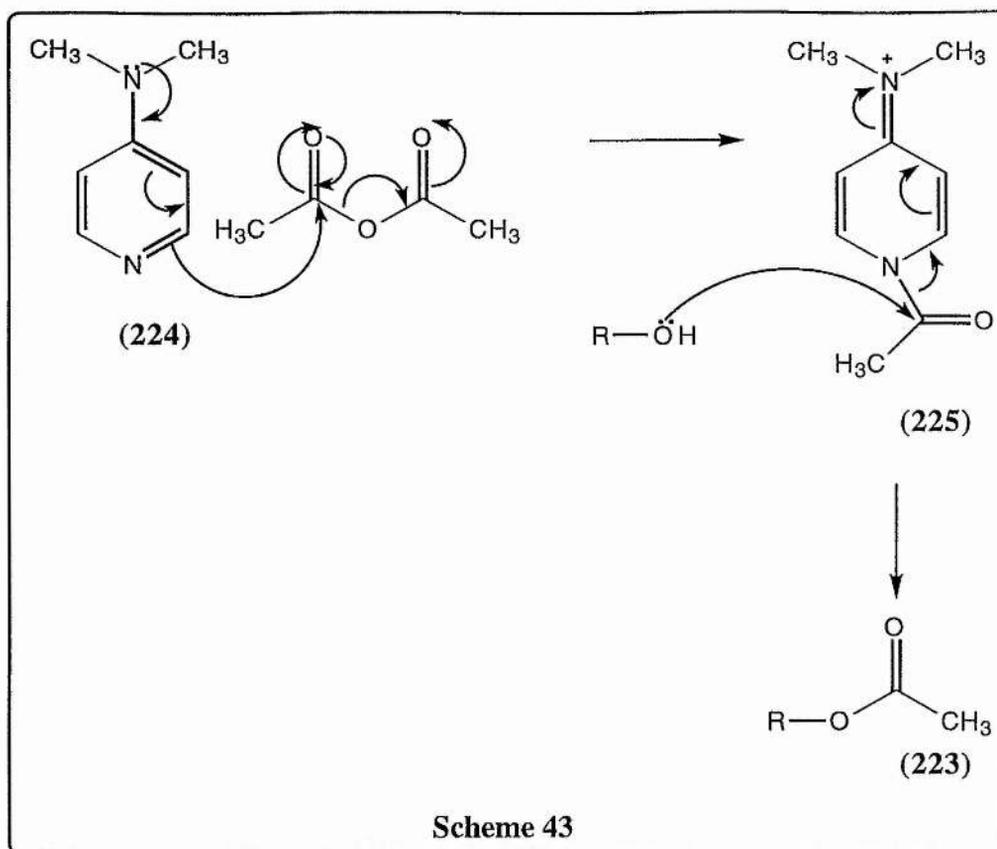
(222)

Synthesis of *O*-acetyl-*N*-hydroxyphthalimide (**223**) was initially carried out in a very similar manner to the preparation of *O*-(tetrahydro-2-pyranyl)-*N*-hydroxyphthalimide (**218**).



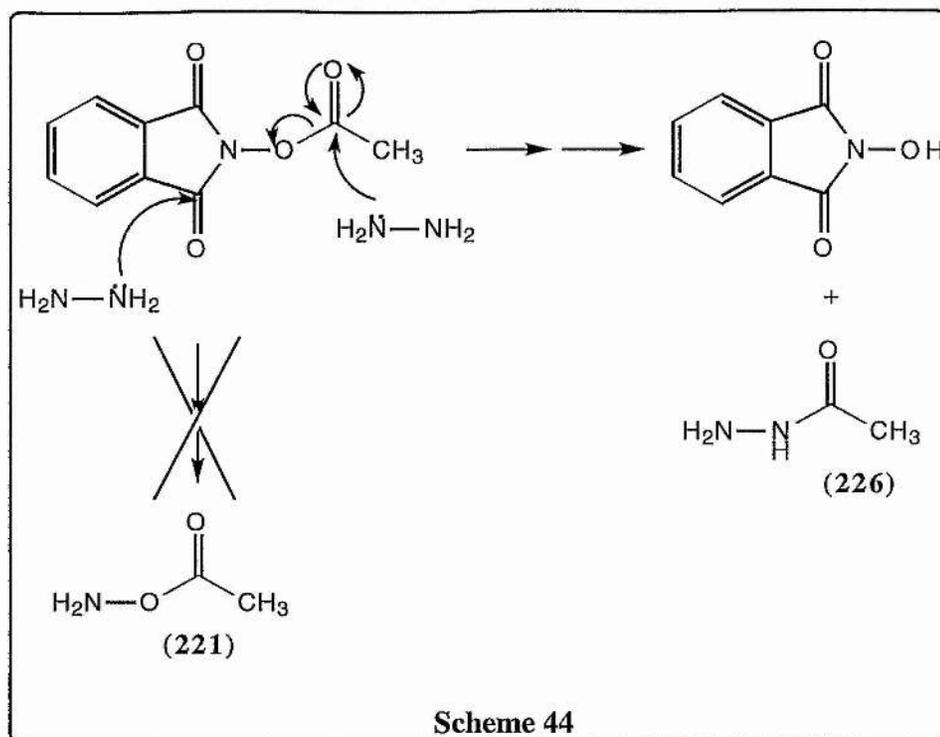
(**223**)

The reaction was carried out using *N*-hydroxyphthalimide (**200**) and an excess of both acetic anhydride and pyridine in dry DCM as the solvent. The yields for the reaction were variable and ranged from 30 to 50% and the product that was obtained was difficult to purify. In an attempt to improve the yield the reaction was carried out in the presence of a catalytic amount of DMAP (2,2-dimethylaminopyridine) (**224**). In this reaction the DMAP (**224**) acts as a nucleophilic catalyst and initially reacts with the acetic anhydride to give an more electrophilic species (**225**) which then reacts with the *N*-hydroxyphthalimide (**200**) (Scheme 43).

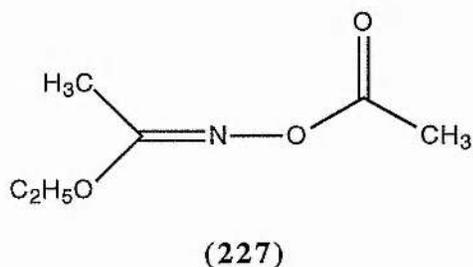


Scheme 43

N-Hydroxyphthalimide (**199**), triethylamine, acetic anhydride and DMAP (**224**) were stirred in dry THF at room temperature for 18 hours. Work up gave the product as a white solid in 60% yield. The product had a cleaner NMR spectrum than previous attempts and contained a new resonance in the ^{13}C NMR spectrum at 162.10 ppm which was due to the acetate carbonyl. The mass spectrum of this solid also contained the desired M^+ ion at 205. The deprotection of the compound was then attempted. It was a concern that the hydrazine might attack at the acetyl carbonyl rather than the phthalimide carbonyl thus giving acetyl hydrazine (**226**) rather than the desired *O*-acetyl hydroxylamine (**221**) (Scheme 44)



Indeed when this reaction was carried out the product obtained was a white solid, which when analysed by NMR spectroscopy and mass spectrometry was found to be *N*-hydroxyphthalimide (**199**). This confirmed that the acetyl carbonyl was indeed more susceptible to attack by hydrazine than the phthalimide carbonyl. It was decided for this reason to try and synthesise *O*-acetyl hydroxylamine (**221**) via the *O*-acetyl acetohydroxamic acid ethyl ester (**227**) instead.

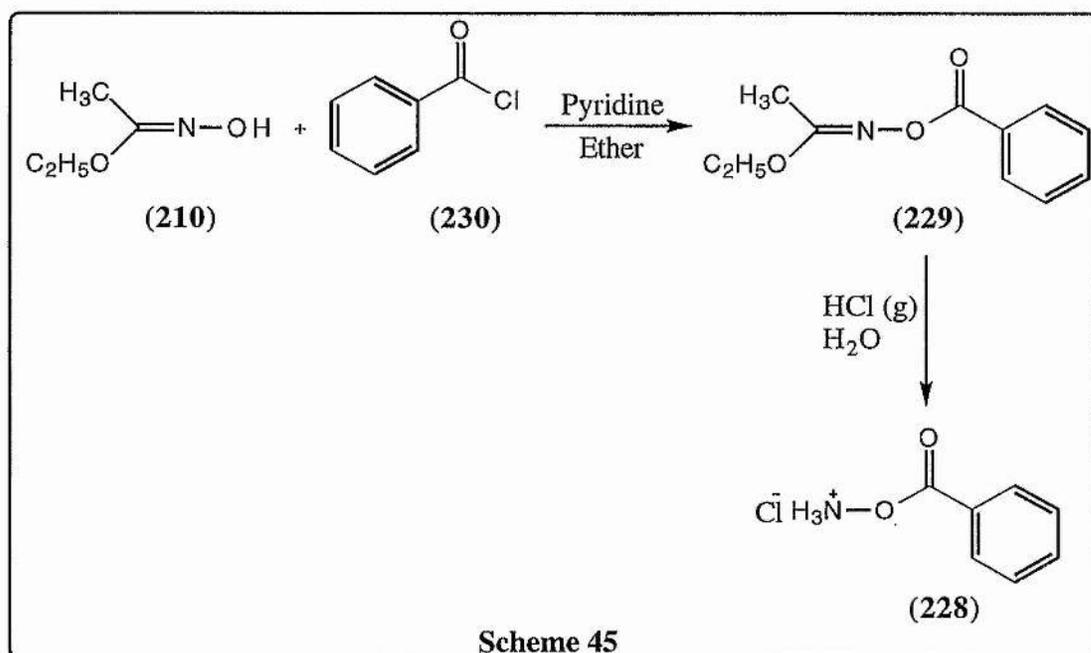


However the synthesis of the intermediate (**227**) proved to be very difficult. The synthesis was only attempted twice and in each case the product obtained was shown by NMR spectroscopy and tlc to be 2 products. The reaction was carried out by the

dropwise addition of a solution of freshly distilled acetyl chloride in ether to a solution of acetohydroxamic acid ethyl ester (**210**) and pyridine in ether. After stirring at room temperature for four hours in the first experiment and sixteen hours in the second the reaction was worked up. In both cases the dark yellow oil was a mixture of at least 2 components. However in both reactions there was a good indication that reaction had gone to some extent, because in the ^{13}C NMR spectrum there was a peak at 165 ppm which is consistent with a $\text{C}=\text{NO}$. However purification to obtain the desired product proved to be impossible.

3.4.3 *O*-Benzoylhydroxylamine Hydrochloride

The synthesis of *O*-benzoyl-hydroxylamine hydrochloride (**228**) from the intermediate *O*-benzoylacetohydroxamic acid ethyl ester (**229**) has been reported in the literature (Scheme 45).¹⁴⁰



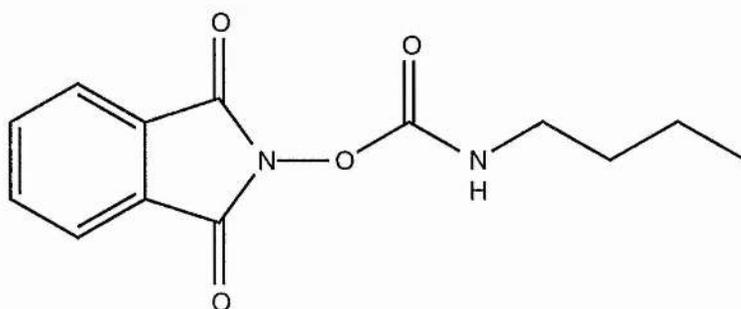
Acetohydroxamic acid ethyl ester (**210**) was therefore reacted with benzoyl chloride (**230**) in the presence of pyridine in dry ether at room temperature for 3 hours.

Removal of the pyridinium chloride and then concentration of the filtrate yielded a white solid. Recrystallisation of this solid from petroleum ether (40-60 °C) gave *O*-benzoyl acetohydroxamic acid ethyl ester (**229**) in 58% yield (**Scheme 45**). The solid was found to be microanalytically pure and the mass spectrum showed the expected M^+ ion at 208. The melting point of 72-74 °C was close to the literature value of 77-79 °C.¹⁴⁰

The *O*-benzoyl acetohydroxamic acid ethyl ester (**229**) was then deprotected using hydrogen chloride gas and one molar equivalent of water. Dry HCl gas was bubbled into a solution of *O*-benzoyl acetohydroxamic acid ethyl ester (**229**) in dioxan and the *O*-benzoyl hydroxylamine hydrochloride (**228**) crystallised out of solution. The compound was then characterised and the NMR spectrum showed no resonances due to the acetohydroxamic acid ethyl ester protecting group suggesting that it had been removed. The mass spectrum showed the expected molecular ion at 174 and the melting point of 106-108 °C, agreed well with the literature data of 110-112 °C.¹⁴⁰

3.4.4 *O*-Urethane Derivatives

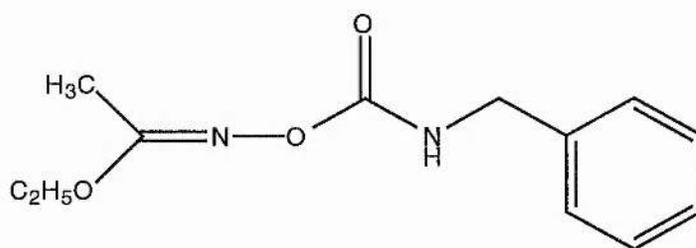
As a comparison with the deprotection of *N*-acetyl-phthalimide (**223**) it was decided to synthesise *O*-butylcarbonyl-*N*-hydroxyphthalimide (**231**). Due to the resonance effect of the O and N atoms the carbonyl in the urethane derivative is much less reactive than the carbonyl in the acetyl derivative.



(231)

O-Butylcarbamyl-*N*-hydroxyphthalimide (**231**) was easily prepared by the reaction of *N*-hydroxyphthalimide (**199**) and *n*-butyl isocyanate in dry THF. The solvent was removed under reduced pressure when the tlc indicated that the two starting materials had disappeared. The product was obtained as an orange oil and NMR spectroscopy indicated that the reaction had indeed occurred due to the presence in the ¹³C NMR spectrum of the carbonyl peak at 164.26 ppm. The deprotection of *O*-butylcarbamyl-*N*-hydroxyphthalimide (**231**) was then attempted by refluxing with hydrazine hydrate in ethanol for four hours. A dark oil was obtained which, when examined by tlc, was shown to contain a number of products. It proved to be impossible to separate these products using column chromatography and only the starting material, *N*-hydroxyphthalimide (**199**), was isolated.

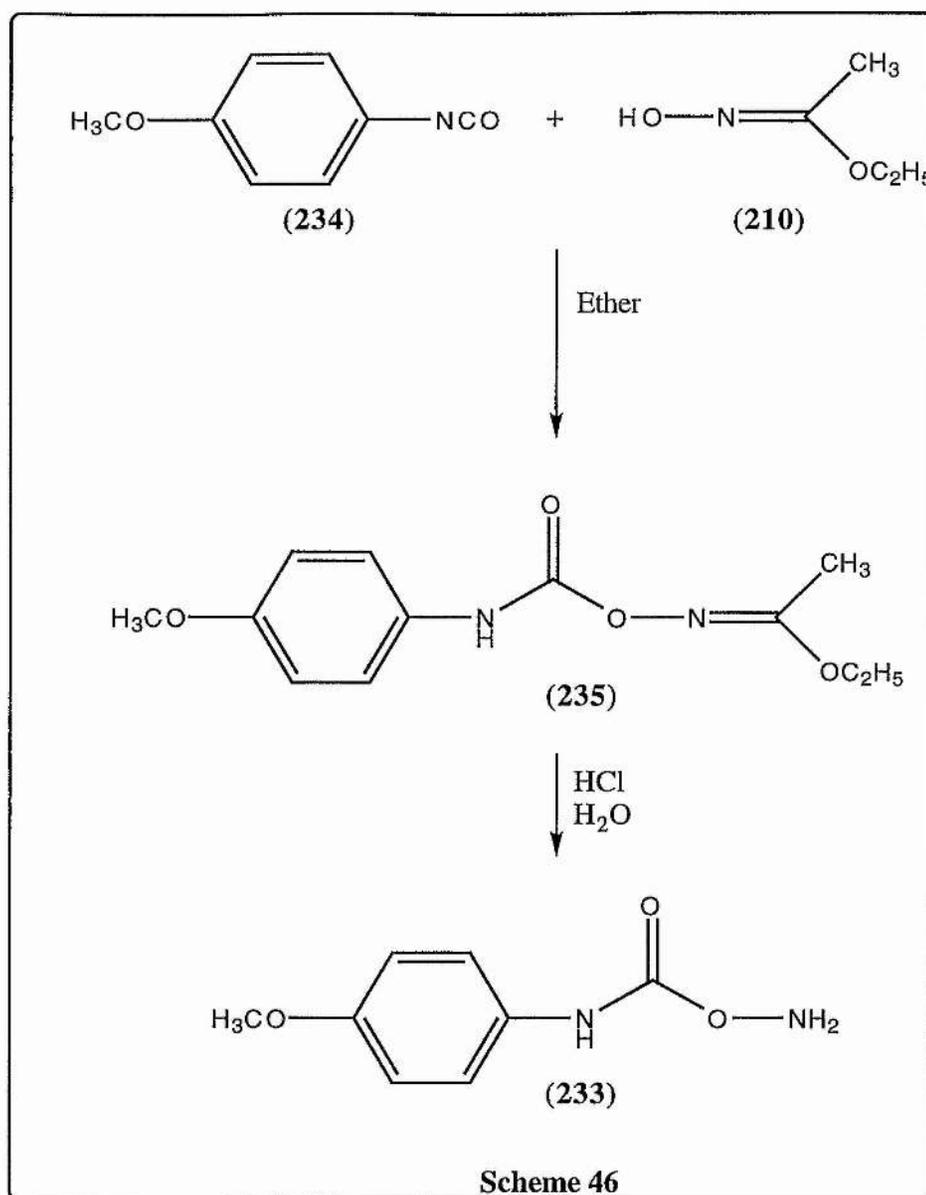
The preparation of these compounds was then attempted via the acetohydroxamic acid ethyl ester derivatives. In the synthesis via the phthalimide derivative an aliphatic isocyanate was used to give groups which would be easier to identify on the NMR spectra. However in this case it was important not to have aliphatic groups as they were already present on the acetohydroxamic acid ethyl ester and so it was decided to work with benzyl isocyanate and synthesise *O*-benzylcarbamyl acetohydroxamic acid ethyl ester (**232**).



(232)

Acetohydroxamic acid ethyl ester (**210**) was stirred in dry ether and benzyl isocyanate added dropwise to this solution. The reaction was stirred at room temperature for 3 hours and then the solvent removed under reduced pressure to yield a dark oil which was shown by tlc to contain some product but also some of both starting materials.

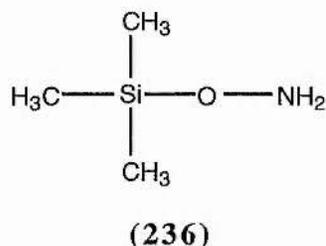
However the synthesis of *O*-(*p*-methoxyphenyl)hydroxylamine (**233**) was achieved using *p*-methoxyphenyl isocyanate (**234**) and acetohydroxamic acid ethyl ester (**210**). This reaction occurs more readily because the isocyanate is more reactive due to the presence of the electron withdrawing *p*-methoxyphenyl substituent. The isocyanate (**234**) and the acetohydroxamic acid ethyl ester (**210**) were heated at reflux for an hour and then stirred for 18 hours at room temperature before being worked up to give the crude *p*-methoxyphenyl acetohydroxamic acid ethyl ester (**235**) (**Scheme 46**). This compound was stable enough to be purified by column chromatography and give the purified product as a white solid in 40% yield. The NMR spectral data was consistent with the expected product with the carbonyl resonance found in the ¹³C NMR spectrum at 149.51 ppm.



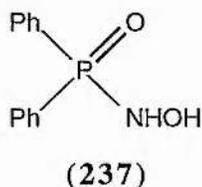
The deprotection was then achieved by cooling a solution of *O*-(4-methoxyphenylcarbonyl)acetohydroxamic acid ethyl ester (**235**) in dioxan and water to 0 °C and bubbling dry hydrogen chloride gas through for thirty minutes. The reaction was diluted with diethyl ether and the precipitated solid was filtered and washed with diethyl ether and dried to give the product (**233**) as a white solid in 95% yield. The ¹H and ¹³C NMR spectra both confirmed the absence of the acetohydroxamic acid ethyl ester resonances and the presence of the expected resonances due to the methoxyphenyl group.

3.4.5 *O*-Trimethylsilylhydroxylamine

The advantage of a trimethylsilane protecting group is that removal is normally carried out using TBAF (tetra butyl ammonium fluoride, $\text{Bu}_4\text{N}^+ \text{F}^-$). There are at least two syntheses of *O*-trimethylsilylhydroxylamine (**236**) in the literature with the first reported in 1983.¹⁴¹

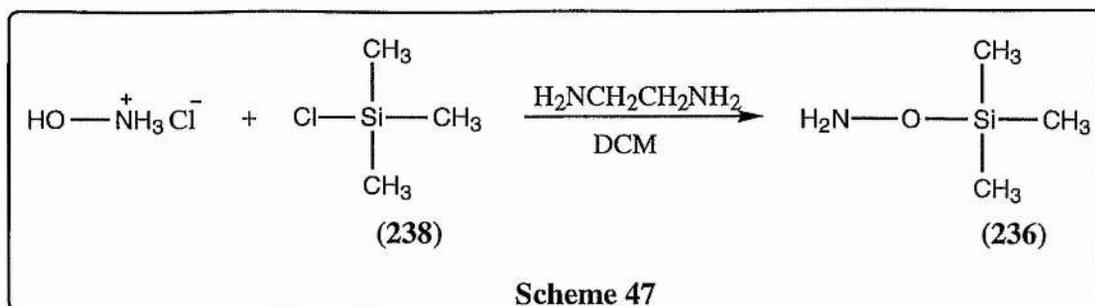


The *O*-trimethylsilylhydroxylamine (**236**) was used as an intermediate in the synthesis of *N*-(diphenylphosphinoyl)hydroxylamine (**237**).



The *O*-trimethylsilylhydroxylamine (**236**) was prepared by reaction of finely ground hydroxylamine hydrochloride and a mixture of 1,1,1,3,3,3-hexamethyldisilazane and tripropylamine. The reaction was stirred at room temperature for 24 hours and then filtered under nitrogen to remove the solid (Pr_3NHCl). Distillation of the filtrate yielded the desired *O*-trimethylsilylhydroxylamine (**236**) in 72% yield. The problem with this synthesis would be the distillation as the product and by-products all have very similar boiling points and the distillation required specialist apparatus.

Zwierzak and co-workers reported the synthesis *O*-trimethylsilylhydroxylamine (**236**) via an alternative route (**Scheme 47**).¹⁴²

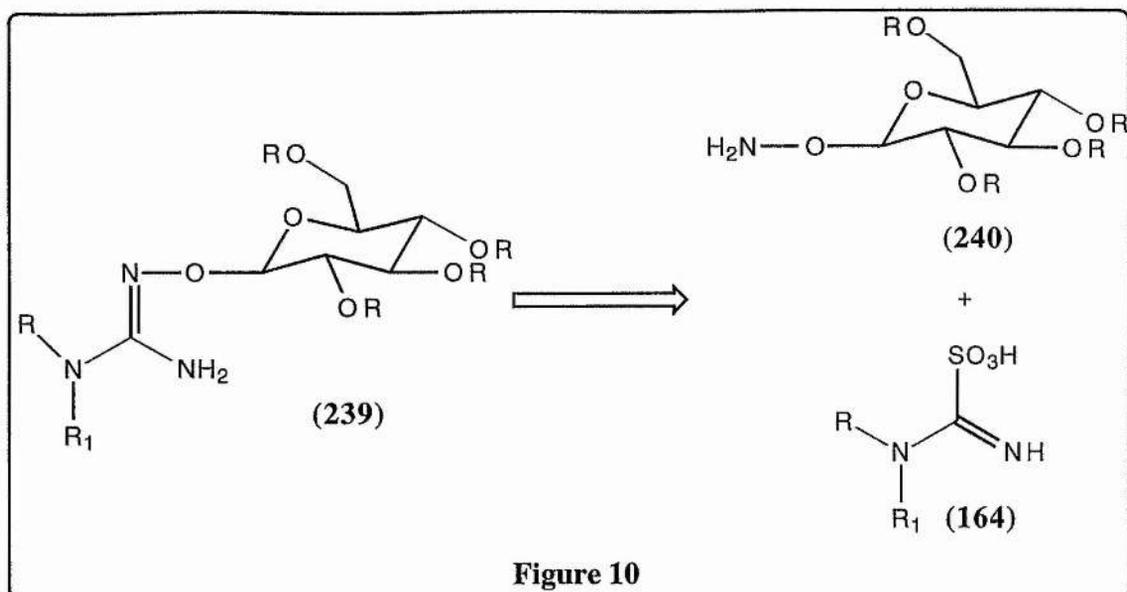


They prepared the *O*-trimethylsilylhydroxylamine (236) by vigorously stirring finely powdered hydroxylamine hydrochloride in ethylenediamine in dichloromethane. This mixture was stirred at room temperature for 6 hours until there were two clearly defined liquid phases present. Chlorotrimethylsilane (238) was added dropwise over a period of an hour. After reflux was complete the reaction was stirred at room temperature for 24 hours. Removal of the salt and evaporation of the solvent yielded a clear liquid that was distilled to give the *O*-trimethylsilylhydroxylamine (236) as a clear liquid (b.p. 98-100 °C) in 58% yield. The advantage of this reaction is that the by-products have boiling points which are different enough from the product that the distillation becomes simpler.

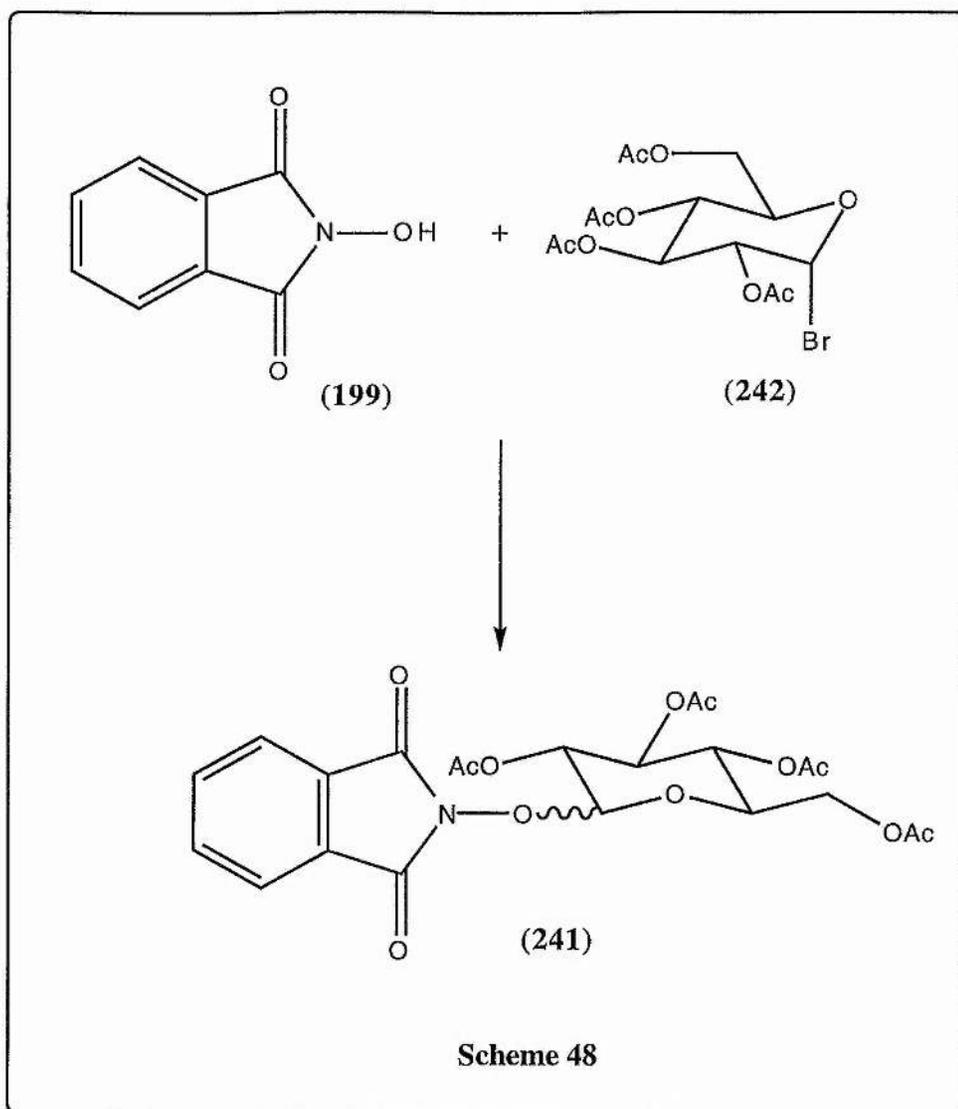
This reaction was attempted using the above method and the clear liquid was obtained in three different fractions in 56% yield. The b.p. of this liquid, 98-100 °C, was in good agreement with the literature value of 98-100 °C under an identical vacuum.

3.4.6 *O*-Glycosylhydroxylamine

One of the longer term aims of the project was the synthesis of *N*-substituted hydroxyguanidines (239) with a sugar moiety attached to the oxygen via a glycosidic linkage. These could be synthesised from *O*-glycosylhydroxylamines (240) and the desired aminoiminomethanesulfonic acid (164) (Figure 10).



Therefore the synthesis of *O*-(tetra-*O*-acetyl-D-glucopyranose)-*N*-hydroxyphthalimide (241) was undertaken. Initially this synthesis was attempted from 2,3,4,6-tetra-*O*-acetyl-1-bromo- α -D-glucopyranose (242) and *N*-hydroxyphthalimide (199) (Scheme 48)

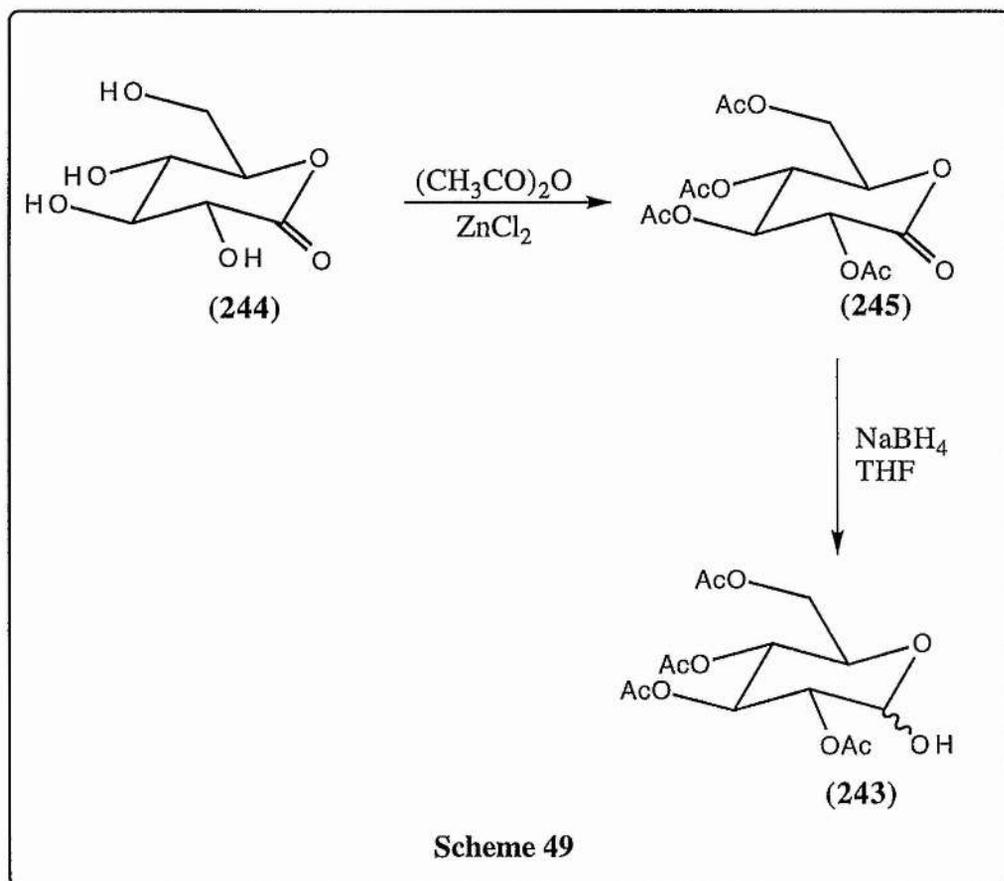


2,3,4,6-Tetra-*O*-acetyl-1-bromo- α -D-glucopyranose (**242**) was synthesised from D-glucose which was dissolved in acetic anhydride and hydrobromic acid in acetic acid was added. The reaction was then stirred at room temperature before further excess hydrobromic acid in acetic acid was added and the reaction stirred overnight. Work up of the reaction yielded a white solid which was further purified by recrystallisation from ether. The melting point of the solid, 87-88 °C, agreed well with the literature value of 88-89 °C.¹⁴³

N-Hydroxyphthalimide (**199**) was then dissolved in dry THF and sodium hydride was added in small portions. To the dark solution that was produced was added

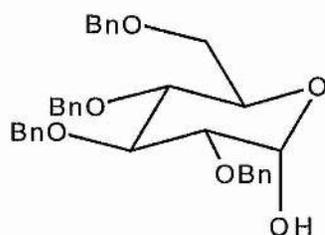
2,3,4,6-tetra-*O*-acetyl-1-bromo- α -D-glucopyranose (**242**) before the reaction was stirred overnight at room temperature. The reaction was worked up by first adding water, to quench any unreacted sodium hydride and then concentrating under reduced pressure. The residue that was obtained was redissolved in water and extracted with ethyl acetate. The ethyl acetate was removed under reduced pressure to yield a white solid. This white solid was shown to be 2,3,4,6-tetra-*O*-acetyl-1-bromo- α -D-glucopyranose (**242**) by the ^1H and ^{13}C NMR spectroscopy showing that the reaction had been unsuccessful.

The synthesis of the protected phthalimide derivative (**241**) was then attempted using a Mitsunobu coupling of *N*-hydroxyphthalimide (**199**) and 2,3,4,6-tetra-*O*-acetyl- α/β -D-glucopyranose (**243**). The 2,3,4,6-tetra-*O*-acetyl- α/β -D-glucopyranose (**243**) was synthesised in a simple two step procedure from D-glucono- γ -lactone (**244**) (Scheme 49).



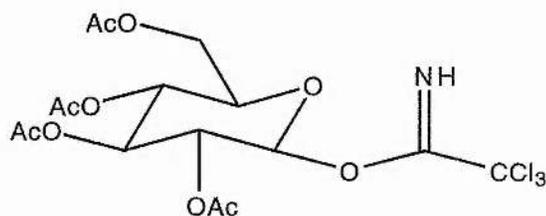
D-Glucono- γ -lactone (**244**) was acetylated using acetic anhydride and zinc chloride. The reaction was stirred at room temperature for 40 minutes and then poured onto ice, extraction of the aqueous layer with dichloromethane and subsequent vigorous removal of the solvent yielded 2,3,4,6-tetra-*O*-acetyl-D-glucono- γ -lactone (**245**) as a oil in quantitative yield. The NMR spectra showed the compound to be correct with a resonance in the ^{13}C spectrum for C-1 (lactone) at 164.59 ppm and the mass spectrum showed the M^+ ion at 347. Reduction of the ketone functionality occurred by dissolving (**245**) in THF and cooling the solution to 0 °C and adding a solution of sodium borohydride in water. The reaction was stirred at 0 °C for three hours before DOWEX 50W ion exchange resin was added. Removal of the resin by filtration and concentration of the filtrate under reduced pressure yielded an oil. This oil was washed with methanol to give the product, 2,3,4,6-tetra-*O*-acetyl- α/β -glucopyranose (**243**) as an oil in 58% yield. The ^{13}C NMR spectrum showed the disappearance of the C-1 resonance at 164.59 and the presence of new C-1 resonances at 90.15 and 95.54 ppm corresponding to the α and β anomers respectively.

The Mitsunobu coupling of (**243**) was then attempted by dissolving (**243**) in dry THF and adding *N*-hydroxyphthalamide (**199**) and triphenylphosphine. To the resulting suspension diethyl azodicarboxylate was added and the reaction stirred for twenty hours at room temperature. Concentration of the reaction yielded an oil which was purified by column chromatography to yield a number of compounds. When the NMR spectra of these compounds were checked it was seen that these materials were starting materials indicating that reaction had not occurred. Other attempts were made at this reaction including one using 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (**246**) instead of (**243**). In this reaction methanol was added to the crude reaction product and a solid precipitated which was purified by column chromatography and found by NMR spectroscopy to be the starting material. As it was clear that this reaction was not occurring an alternative method of coupling the protected glucose to *N*-hydroxyphthalimide (**199**) was sought.



(246)

Work within the group has shown that 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl trichloroacetimidate (247) can be coupled with hydroxyl groups in the presence of boron trifluoride etherate.¹⁴⁴

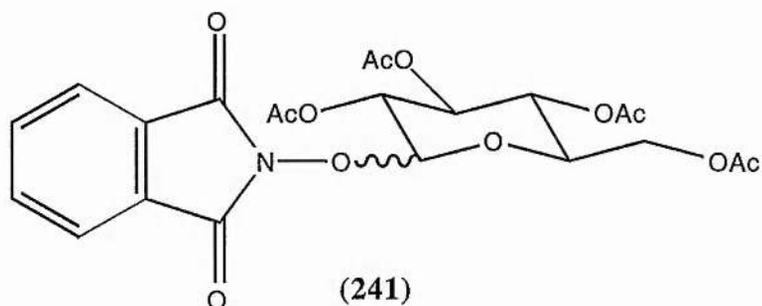


(247)

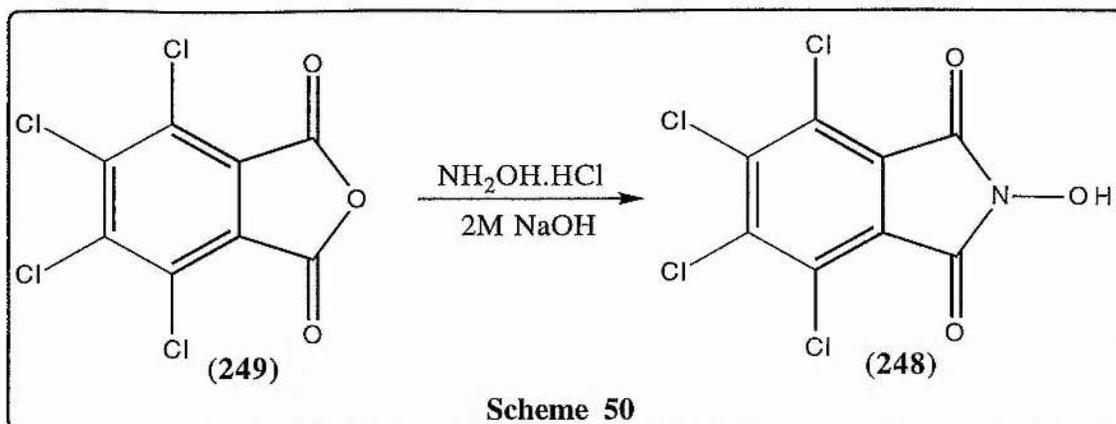
2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranose (243) and trichloroacetonitrile were stirred in DCM and glowing hot potassium carbonate was added. The reaction was stirred for twenty five hours at room temperature and then the inorganic salts were removed by filtration and the reaction concentrated under reduced pressure to give an oil which was purified by column chromatography to yield 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl trichloroacetimidate (247) in 43% yield. The ¹H NMR spectrum showed the expected doublet due to H-1 at 6.55 and the resonance due to the NH at 8.7 ppm and the ¹³C NMR spectrum showed resonances at 92.88 and 160.87 due to the C-1 and C=NH respectively.

2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranosyl trichloroacetimidate (247) and *N*-hydroxyphthalimide (199) were then stirred in dry DCM and cooled to -15 °C. To the suspension was added boron trifluoride diethyl etherate and the reaction was stirred for 75 minutes before it was recooled and further, excess boron trifluoride diethyl etherate

was added and the reaction stirred overnight at room temperature. The reaction was then worked up and an oil obtained which was stirred in methanol until a solid precipitated. The solid was filtered and gave the desired product, *O*-(tetra-*O*-acetyl- α -D-glucopyranose)-*N*-hydroxyphthalimide (**241**) in 29% yield. The mass spectrum of this solid showed the molecular ion corresponding to $[M+Na]^+$ at 516 and the ^{13}C NMR spectrum showed a resonance corresponding to C-1 at 103.91 ppm. The major problem with this reaction is the low yield of the coupling reaction and also the low yield of the 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl trichloroacetimidate (**247**). Further work is required to optimise this reaction to provide synthetically useful amounts of the material.



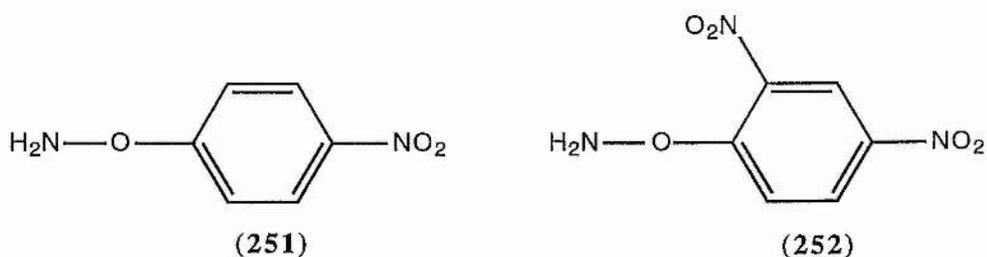
The removal of the phthalimide group proved to be difficult so it was decided to look at an alternative protecting group, 2,3,4,6-tetrachloro-*N*-hydroxyphthalimide (**248**). The desired compound was synthesised by reacting tetrachlorophthalic anhydride (**249**) and hydroxylamine in refluxing 2M aqueous sodium hydroxide (Scheme 50). The reaction was heated under reflux for eight hours and then stirred at room temperature overnight before partial concentration under reduced pressure yielded the product in 56% yield.



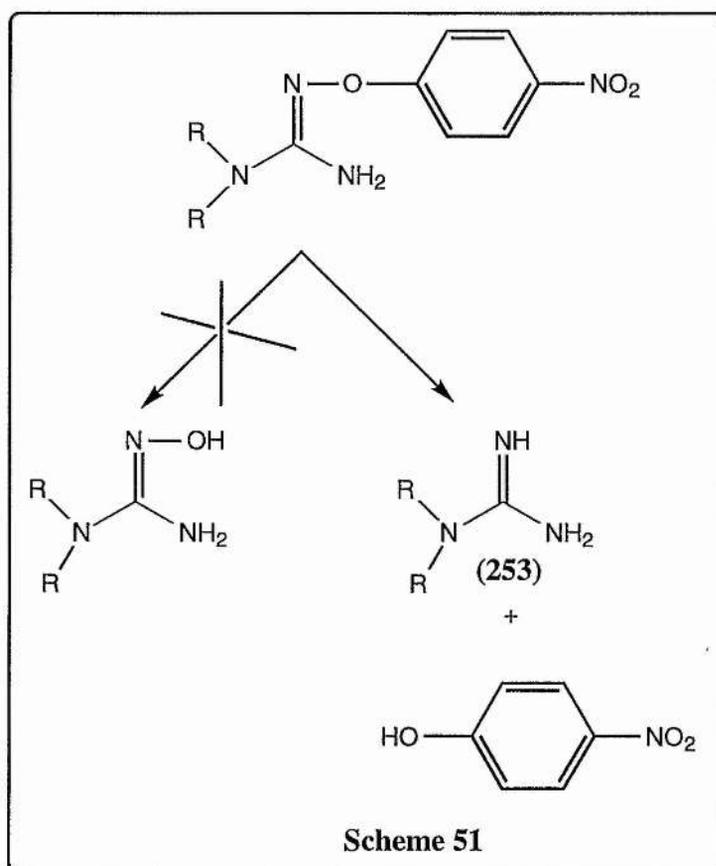
However before it was required to use this protecting group the deprotection of *O*-(tetra-*O*-acetyl- α -D-glucopyranose)-*N*-hydroxyphthalimide (**241**) was successfully achieved using hydrazine hydrate in refluxing THF. The reaction was heated under reflux for ten minutes and then cooled to room temperature and diluted with sodium bicarbonate solution. Extraction with diethyl ether and then concentration under reduced pressure yielded the crude *O*-(tetra-*O*-acetyl-D-glucopyranose)hydroxylamine (**250**). The solid was contaminated with some of the phthalazine (**220**) by-product, however it was decided to react this product on in a crude form to see whether or not the next step would occur.

3.4.7 *O*-Mono and Disubstituted Nitrophenylhydroxylamines

The syntheses of *O*-(4-nitrophenyl)hydroxylamine (**251**) and *O*-(2,4-dinitrophenyl)hydroxylamine (**252**) were attempted.

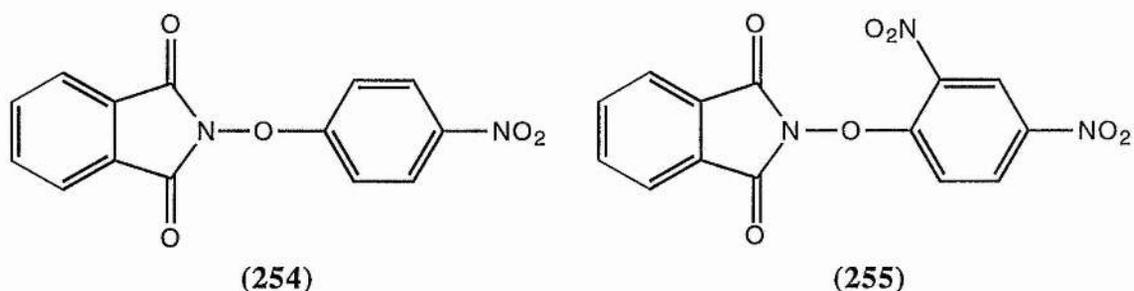


The *O*-protected *N*-hydroxyguanidine derivatives of (251) and (252) should give N-O bond cleavage to give the guanidine derivative (253), which is in fact undesirable (Scheme 51). However it would be useful to know how these derivatives behaved in comparison to other example especially in light of the difficulties with the attempted removal of the benzyl group from *O*-benzyl *N*-hydroxyguanidines (Section 3.5.4). The *O*-benzyl derivatives gave N-O bond cleavage despite the fact that the benzyl group is a poorer leaving group than either of the nitro derivatives.

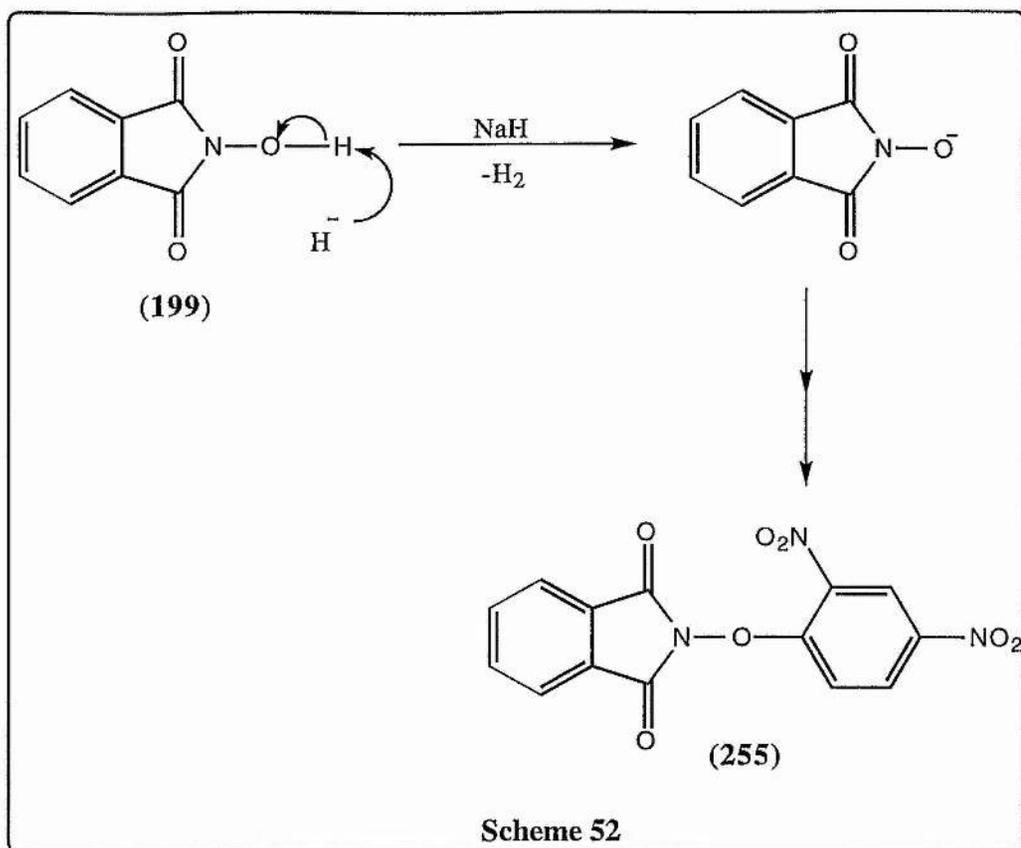


It was envisaged that these compounds could be synthesised from *N*-hydroxyphthalimide (199) and the corresponding fluoronitrobenzene. Initial attempts involved the attempted synthesis of *O*-(4-nitrophenyl)-*N*-hydroxyphthalimide (254). *N*-Hydroxyphthalimide (199) was stirred in dry DCM and 4-fluoronitrobenzene and pyridine added, after sixteen hours at room temperature the reaction was checked by tlc and found not to have occurred. The reaction was then heated at between 40 and 60 °C

for twelve hours and then stirred for seven days at room temperature. After work up an orange solid was obtained which was checked by NMR spectroscopy and tlc. The NMR spectra showed that the solid was a mixture of compounds and this was confirmed by tlc. The 4-fluoronitrobenzene may not have been electrophilic enough and it was decided to use the 2,4-dinitrofluorobenzene derivative instead.



The preparation of *O*-(2,4-dinitrophenyl)-*N*-hydroxyphthalimide (**255**) was initially attempted using the method that had been successful in preparing *O*-acetyl-*N*-hydroxyphthalimide (**223**), the addition of catalytic DMAP. *N*-Hydroxyphthalimide (**199**), 2,4-dinitrofluorobenzene, triethylamine and a catalytic amount of DMAP were stirred in dry THF. The reaction was stirred at room temperature for twenty four hours and then worked up in an identical manner to the preparation of *O*-acetyl-*N*-hydroxyphthalimide (**223**). The solid was then characterised by tlc and NMR spectroscopy and both of these showed that the solid was a mixture of two compounds with the expected phthalimide protons at 7.9-8.1 ppm being absent from the ^1H spectrum. It was then thought that *N*-hydroxyphthalimide might not be nucleophilic enough to attack the 2,4-dinitrofluorobenzene so it was decided that it might be possible to improve its nucleophilic character by initially deprotonating the *N*-hydroxyphthalimide (**199**) using sodium hydride, this then should react with the 2,4-dinitrofluorobenzene to give the desired substituted phthalimide (**Scheme 52**).



When the reaction was attempted, *N*-hydroxyphthalimide (**199**) was stirred in dry THF and sodium hydride was added in small portions. When evolution of hydrogen was complete 2,4-dinitrofluorobenzene was added. The reaction was then stirred at room temperature for forty eight hours and worked up. A yellow solid was obtained, however the NMR spectra of this solid suggested that it was a mixture with at least two compounds present. A subsequent attempt where the reaction was refluxed for six hours also failed.

It was then decided to take a different approach, and attempt a Mitsunobu coupling between *N*-hydroxyphthalimide (**199**) and 2,4-dinitrophenol. *N*-Hydroxyphthalimide (**199**) was dissolved in cold THF and 2,4-dinitrophenol and triphenylphosphine were then added. Diethyl azodicarboxylate was then added dropwise over a period of 10 minutes. The resulting solution was stirred at room temperature for 30 minutes and then worked up. The NMR spectra of the crude product showed that a

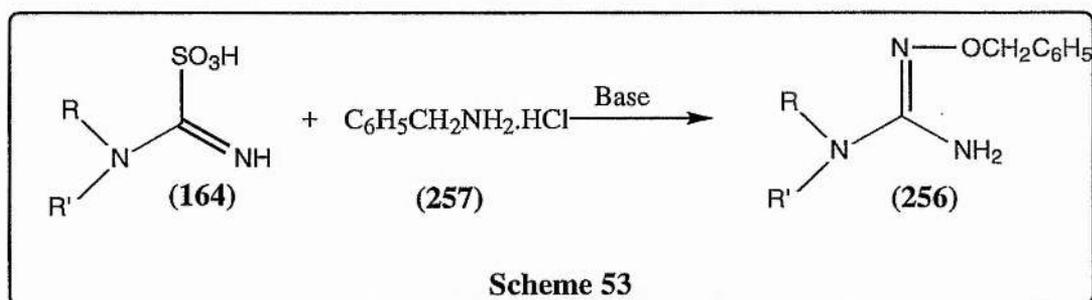
number of products were present, these were mainly the byproducts from the reaction and this was confirmed by tlc. However attempts to purify this compound by column chromatography failed. All the by products were isolated but the desired product appeared to be left on the column.

In light of this failure it was decided to leave the synthesis of these two hydroxylamines and concentrate on the other derivatives.

3.5 SYNTHESIS OF *O*-SUBSTITUTED *N*-HYDROXYGUANIDINES

3.5.1 Synthesis Of *O*-Benzyl *N*-Hydroxyguanidines

The synthesis of *O*-benzyl substituted hydroxyguanidines (**256**) was attempted first because *O*-benzylhydroxylamine hydrochloride (**257**) is a commercially available compound allowing the optimisation of the reaction conditions without using material that had required a multistep synthesis to obtain. The initial synthetic procedure examined was the reaction of *O*-benzylhydroxylamine hydrochloride (**257**) with the substituted aminoiminomethanesulfonic acids (**164**) (Scheme 53)

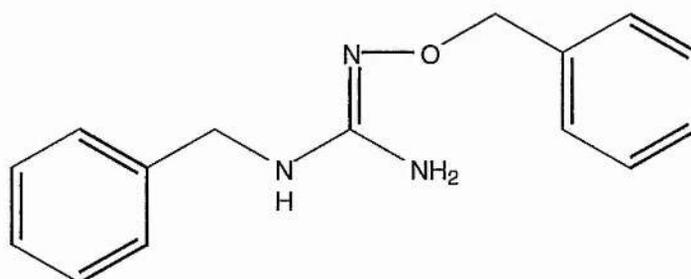


The first attempts at the reaction employed triethylamine as the base and acetonitrile as the solvent. The reaction was stirred at room temperature for twenty four hours and then it was concentrated under reduced pressure to yield an oil. Extraction of the product into diethyl ether gave an oil which was shown by NMR spectroscopy to be inconsistent with the expected product.

The reactions were then carried out by stirring the potassium carbonate and *O*-benzylhydroxylamine hydrochloride in water until a solution was obtained and then the aminoiminomethanesulfonic acid added in small portions over a ten minute period. The reaction was stirred at room temperature for twenty four hours and then the product was filtered off if it was solid, or extracted into ethyl acetate if it was not quite solid.

3.5.1.2 Monosubstituted Derivatives

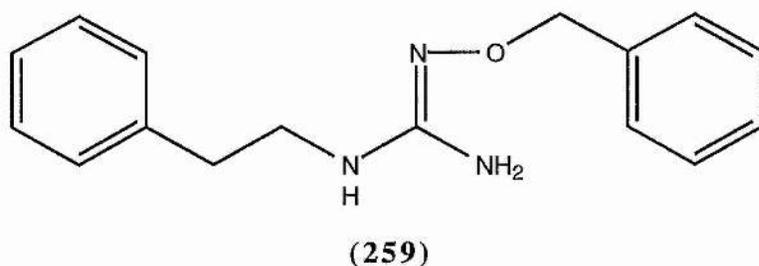
O-Benzyl-1-benzyl-2-hydroxyguanidine (**258**) was synthesised using this procedure. The reaction was stirred at room temperature for eight hours and then the white solid that was present was filtered off and dried under vacuum to give the product in 48% yield.



(258)

The white solid was microanalytically pure and showed a new CH₂ resonance due to the *O*-benzyl group at 4.7 ppm in the ¹H NMR spectrum and 74.27 ppm in the ¹³C NMR spectrum. The resonance due to the C of the guanidino group was observed at 154.95 ppm in the ¹³C NMR spectrum, which was different from the quaternary carbon resonance at 165.96 ppm in the ¹³C NMR spectrum of the starting material. The mass spectrum of the compound gave the expected M⁺ ion at 255.

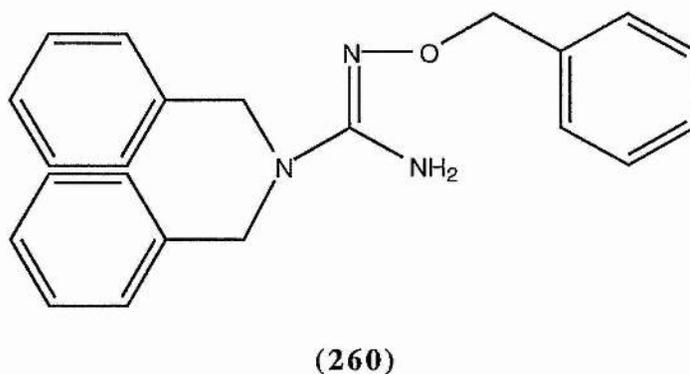
Initially there were problems with the synthesis of *O*-benzyl-1-(2-phenethyl)-2-hydroxyguanidine (**259**).



This was probably caused by the problems described for the oxidation step and it is known that the SO_2H group is not as good a leaving group as SO_3H and it may have been that the reaction was slower. However once the correct sulfonic acid had been obtained the reaction of *N*-(2-phenethyl)aminoiminomethanesulfonic acid (**195**) proceeded as previously described. However instead of a solid being obtained a sticky mass was found in the bottom of the flask. This mass was extracted into ethyl acetate and the product was subsequently obtained as a colourless oil in 46% yield. The ^1H and ^{13}C NMR spectra showed the resonances for the CH_2 of the benzyl group at 4.70 ppm and 72.17 ppm respectively. The mass spectrum of the oil also gave the desired M^+ ion at 269.

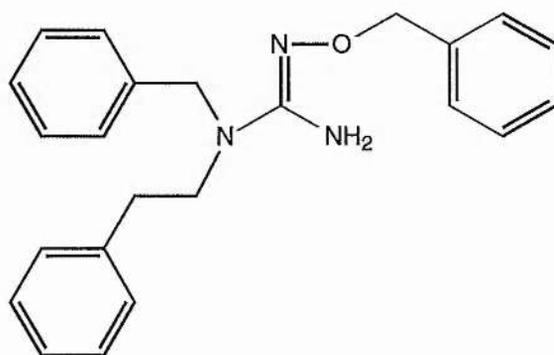
3.5.1.2 Disubstituted Derivatives

The synthesis of *O*-benzyl-1,1-dibenzyl-2-hydroxyguanidine (**260**) was achieved in 89% yield. The solid that was obtained was purified by recrystallising the crude solid from ethyl acetate.



The product was found to be microanalytically pure and gave the correct M^+ ion at 345 in the mass spectrum. The ^{13}C NMR spectrum also showed a peak at 75.97 ppm due to the CH_2 of the benzyl group and a peak at 157.63 ppm due to the guanidino carbon.

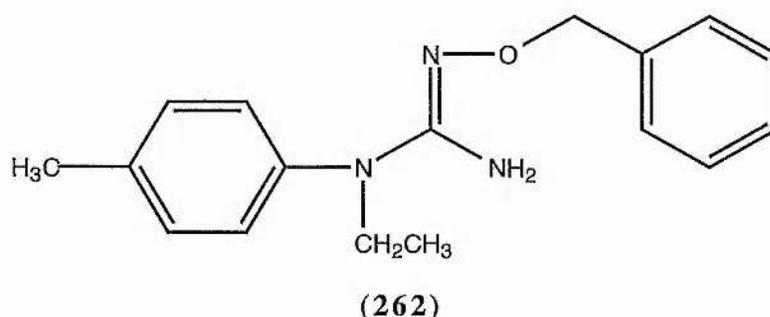
Various attempts have been made to synthesise *O*-benzyl-1-benzyl-1-(2-phenethyl)-2-hydroxyguanidine (**261**) from the corresponding sulfonic acid (**197**). Initially standard reaction conditions were used, *O*-benzylhydroxylamine hydrochloride and potassium carbonate were dissolved in water and the sulfonic acid added in small portions over a period of 10 minutes. The reaction was stirred for 14-18 hours at room temperature. Normally a solid is formed that can be filtered off however in this case a thick oil was produced, which was extracted into ethyl acetate. Concentration of the ethyl acetate under reduced pressure to give an orange oil. NMR showed that this oil was probably a mixture of two components, although the second component was only a minor impurity. Numerous attempts were made to try and either crystallise the product or the impurity from this oil, however they were all unsuccessful. Separation was attempted by column chromatography using 3:1 ethyl acetate / petroleum ether as the eluant, however when this was attempted none of the desired product (**261**) was obtained.



(261)

3.5.1.3. Attempted Synthesis of *O*-Benzyl Derivatives From The Corresponding Cyanamides

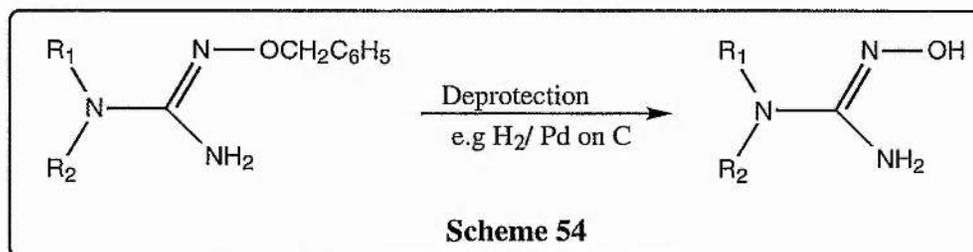
In light of the difficulty in synthesising *O*-benzyl-*N*-benzyl-*N*-(2-phenethyl)-2-hydroxyguanidine (**261**) from the sulfonic acid it was decided to attempt the synthesis from the corresponding cyanamide (**118**). *N*-Benzyl-*N*-(2-phenethyl)cyanamide (**118**) was stirred in DMF and *O*-benzylhydroxylamine hydrochloride (**257**) and sodium carbonate added. The reaction was then heated under reflux for 2 hours before the reaction mixture was filtered hot and the inorganic salts washed with warm DMF. The combined organic extracts were concentrated under reduced pressure to yield an oil which showed the presence of the *O*-benzyl group by ^1H NMR and ^{13}C NMR. When an attempt to purify the compound using distillation was undertaken a clear oil was obtained which when examined by NMR was found to be *O*-benzylhydroxylamine. The distillation residue was also examined and found to contain none of the desired product. At this point it was assumed that the distillation must have caused a degradation of the compound back to the starting material. However when the synthesis of *O*-benzyl-1-ethyl-1-(*p*-tolyl)-2-hydroxyguanidine (**262**) was attempted a similar result was achieved.



However in this reaction the purification was attempted using column chromatography. These two reactions would suggest that the reaction via the cyanamide does not occur which seems a strange result and at present limits the synthesis of *O*-substituted *N*-hydroxyguanidines to derivatives where the aminoiminomethanesulfonic acid can be synthesised.

3.5.1.4 Deprotection of *O*-benzyl *N*-hydroxyguanidines

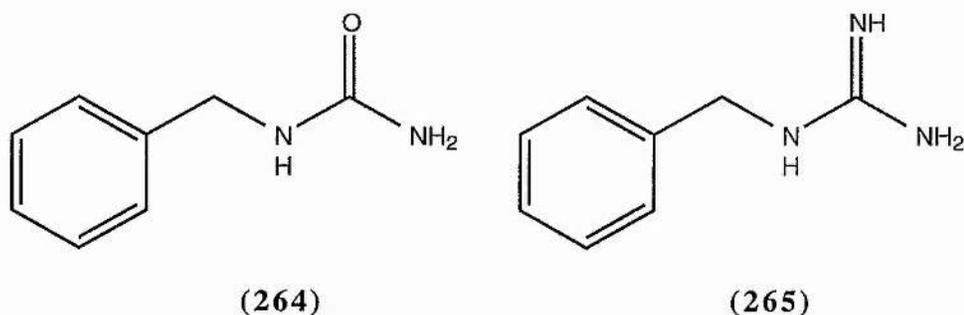
Originally it was envisaged that one possible route to the free *N*-hydroxyguanidines was by deprotection of the *O*-benzyl substituted derivatives (Scheme 54)



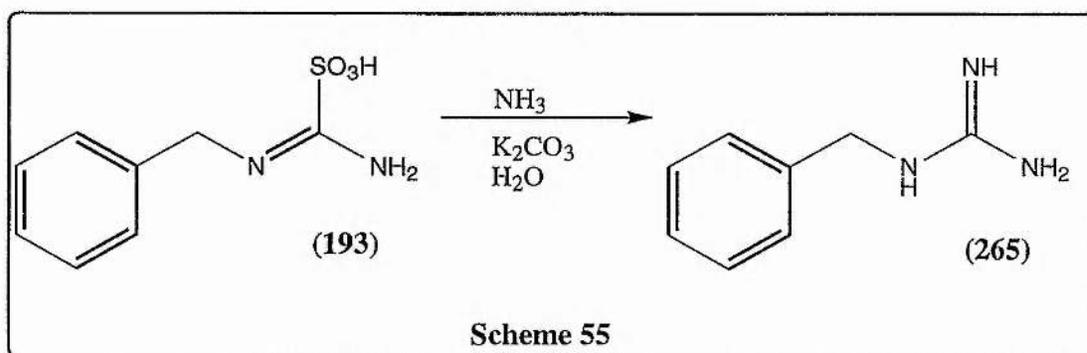
The obvious method to remove the benzyl group was reaction with hydrogen gas with a palladium on carbon catalyst. Initial attempts using 5% palladium on carbon proved to be unsuccessful with a mixture of compounds being obtained. This was also the case when 10% palladium on carbon was employed.

Although there was the potential for the added complication of the removal of the *N*-benzyl group, experiments have been carried out under more vigorous conditions. The deprotection of *O*-benzyl-1-benzyl-2-hydroxyguanidine (**258**) was attempted using ethanol with a catalytic amount of acetic acid and 10% palladium on charcoal in non-catalytic amount (1:1 catalyst to compound). The reaction was stirred under an atmosphere of hydrogen gas for sixty one hours. The reaction was monitored by tlc and worked up when the reactant spot had disappeared. The product was obtained as an off white solid. The NMR spectrum of the product showed that the peaks due the CH₂ of the *O*-benzyl group had disappeared from both the ¹H and ¹³C spectra. The tlc also showed that the product was only one compound and the R_f value was different from the starting material. However, when the mass spectrum was obtained it showed that the principal molecular ion M⁺ was at 149 and not at 165 as would be expected if the compound was 1-benzyl-2-hydroxyguanidine (**263**). There were 2 other possible

products that could of been obtained from the reaction. The first was benzylurea (264) which could be formed by attack of water at the carbon but this compound has a molecular weight of 150. The second product could arise from the hydrogen adding across the N-O bond rather than the C-O bond to give *N*-benzylguanidine (265). This reaction could be catalysed by the acetic acid.



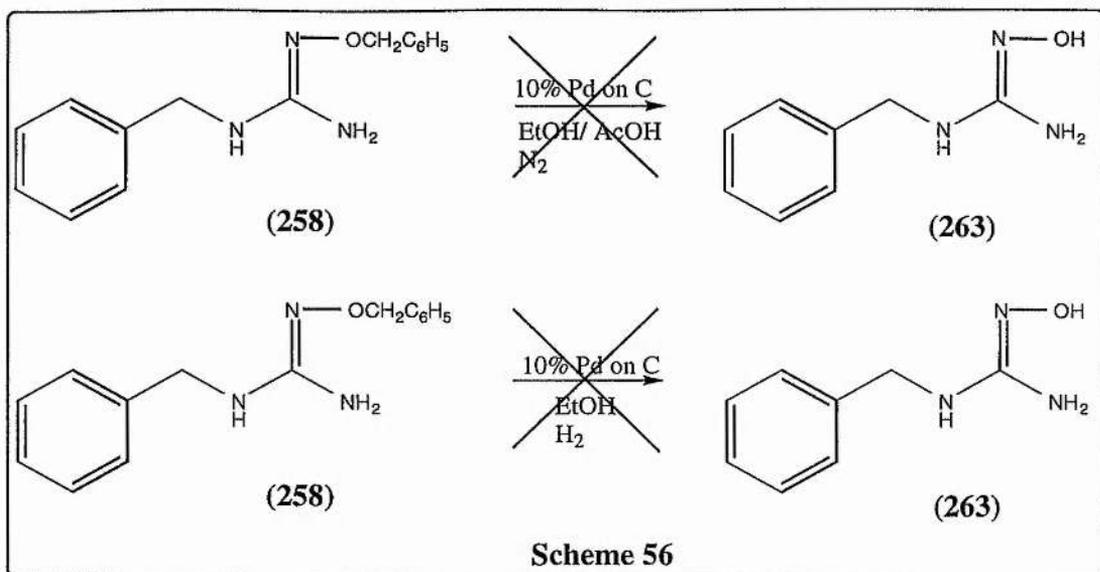
It was decided to investigate this N-O bond cleavage as there appears to be no literature precedent for this reaction for *N*-hydroxyguanidines. The first problem was to obtain *N*-benzylguanidine (265) but as the compound is not commercially available a synthesis had to be found. The most logical method was to try reacting aqueous ammonia with *N*-benzylaminoiminomethanesulfonic acid (193) (Scheme 55).



The reaction was carried out in water with potassium carbonate as a base. After stirring at room temperature for 24 hours the solvent was removed and the residue was stirred in ethanol. The insoluble salts were then filtered and the filtrate reduced under reduced pressure to give a white solid. The ^1H NMR spectrum of the product was

inconclusive as there is very little present in the molecule, however the mass spectrum indicated that the expected M^+ ion at 149 was present. The authentic compound was then compared to the compound obtained from the hydrogenation and there was found to be good agreement in the data and the tlc showed that both compounds had the same R_f value.

At the same time it was decided to try a number of control experiments. The first was an attempted acid hydrolysis of *O*-benzyl-1-benzyl-2-hydroxyguanidine (**258**) (Scheme 56). The protected hydroxyguanidine (**258**) was dissolved in ethanol and a few drops of acetic acid were then added. Palladium on charcoal was then added before the flask was purged with nitrogen and stirred under an nitrogen atmosphere for forty eight hours. The reaction was then worked up and a yellow oil was obtained. The NMR spectrum showed that the *O*-benzyl group was still present in the compound. It was also shown by tlc that the product, which was a single spot, had the same R_f value as the starting material. The second experiment was a hydrogenation without the presence of the acetic acid. The reaction was stirred at room temperature for forty eight hours and then worked up. Again both the NMR spectrum and tlc suggested that the yellow oil that was obtained was starting material. These controlled experiments would suggest that the hydrogenation does not occur without the presence of the acetic acid but that in the presence of the acid the N-O bond is cleaved rather than the C-O bond.



These deprotections were also been attempted on *O*-benzyl-1-phenethyl-2-hydroxyguanidine (**259**) and *O*-benzyl-1,1-dibenzyl-2-hydroxyguanidine (**260**) and again the free *N*-hydroxyguanidine was not obtained.

The deprotection of *O*-benzyl-1-benzyl-hydroxyguanidine (**258**) has been attempted in a number of other solvents. Attempts have been made in ethyl acetate and trifluoroethanol but again these reactions appear to be incomplete. There are literature reports where palladium hydroxide on carbon has been used as a catalyst for the removal of benzyl groups where all attempts with palladium on carbon have failed. This catalyst was used in an attempt to remove the benzyl group from *O*-benzyl-1-phenethyl-hydroxyguanidine (**259**) however again there was very little evidence that the benzyl group had been removed.

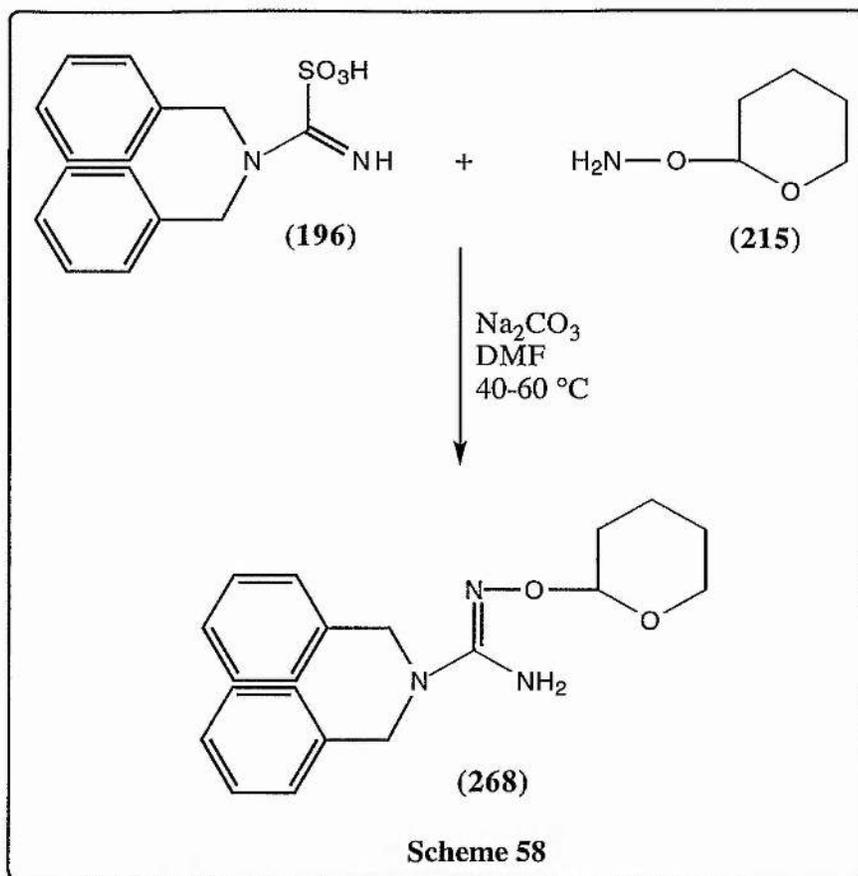
Jung and co-workers reported a deprotection of alkyl ethers using trimethylsilyliodide (**266**) (Scheme 57).¹⁴⁵

the mass spectra of the compound showed an M^+ ion at 149 which was consistent with the formation of the *N,N*-dibenzylguanidine species.

It was decided since the synthesis of the free *N*-hydroxyguanidines had been completed by other methods that there was no requirement to do any further work on this deprotection reaction. However the results seem to be inconsistent with the work of Feldman (Section 2.2.2).¹¹³ He reported the synthesis of *N*-hydroxy-L-arginine, where the last step of his synthesis was removal of the *O*-benzyl protecting group using catalytic hydrogenation. This step was reported to give a 30% yield of the desired product. There was no mention of any problem with N-O bond cleavage.

3.5.2 SYNTHESIS OF *O*-THP *N*-HYDROXYGUANIDINES

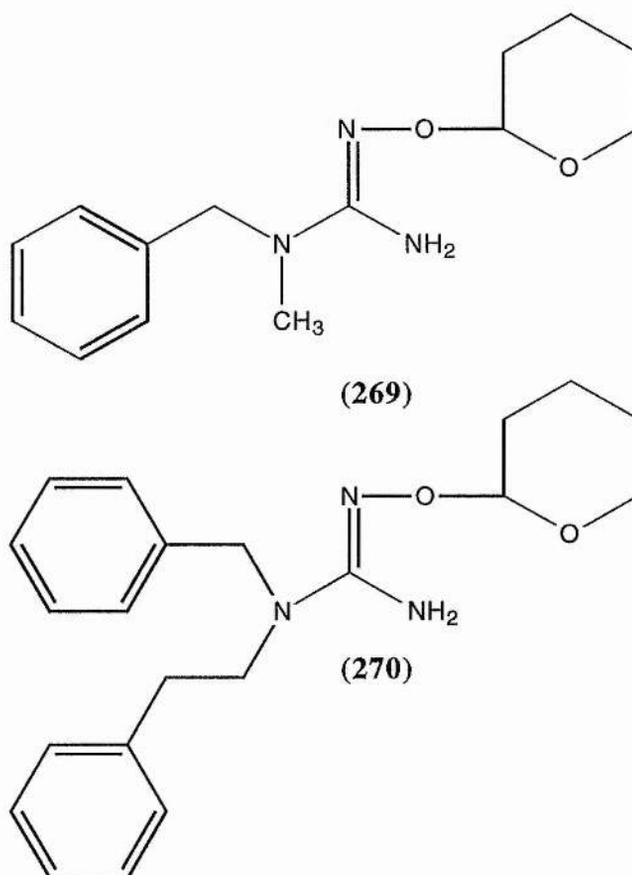
The preparation of *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**) was attempted by reacting *N,N*-dibenzylaminoiminomethanesulfonic acid (**196**) with *O*-THP-hydroxylamine (**215**) together in dry DMF (Scheme 58). The sulfonic acid, *O*-THP-hydroxylamine (**215**) and sodium carbonate were stirred together in dry DMF and then stirred at 40-60 °C for seven hours followed by fourteen hours at room temperature. On work up a solid was obtained however the purification of this compound was impossible. A number of recrystallisations were tried and all failed and a small portion was passed down a short silica column using ethyl acetate as the eluant and another solid obtained. However the NMR spectra showed the absence of the THP group and suggested that the column had cleaved this group from the molecule. It is known that THP groups are acid sensitive and it seems that silica is acidic enough to remove the group.



The synthesis of *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**) was then attempted using identical methodology to that which had been successful in the synthesis of the *O*-benzyl derivatives (**Section 3.5.1**). *O*-THP-Hydroxylamine (**215**) and potassium carbonate were dissolved in water and *N,N*-dibenzylaminoiminomethanesulfonic acid (**196**) was added over ten minutes. The reaction was stirred overnight at room temperature and then the solid that was present was filtered off and dried. The ^{13}C NMR spectrum showed that there was some of the expected product present because there was a C=N resonance at 158 ppm. However the purification of this compound proved to be very difficult and various techniques were tried including a small scale reverse phase silica purification using a Sep-pack. However although this did indeed give the desired product in a 1% yield it was not microanalytically pure and clearly the yield was less than desirable.

After a number of attempts at the purification it was found that the pure compound could be obtained by heating the crude mixture in refluxing diethyl ether and then the insoluble by-product could be removed by filtration. The pure product then crystallised from the filtrate. The white solid was found to be microanalytically pure and the ^{13}C NMR spectrum contained all the expected peaks including the peak at 158.05 ppm due to the guanidino carbon. The desired molecular ion, M^+ was also observed in the mass spectrum at 340. Although the yield of the reaction was only 28% this was still a distinct improvement over previous attempts.

The synthesis of other derivatives including *O*-THP-1-benzyl-1-methyl-2-hydroxyguanidine (**269**) and *O*-THP-1-benzyl-1-(2-phenethyl)-2-hydroxyguanidine (**270**) was then attempted.



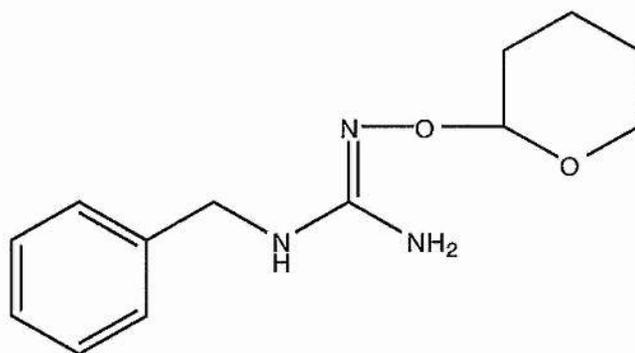
For *O*-THP-1-benzyl-1-methyl-2-hydroxyguanidine (**269**) the reaction was carried out as described before using *N*-benzyl-*N*-methylaminoiminomethanesulfonic

acid, *O*-THP-hydroxylamine (**215**) and potassium carbonate in water. Purification of the product was attempted by heating in refluxing diethyl ether and subsequent removal of the insoluble by-products. However the ^1H NMR spectrum of the white solid which was obtained showed the absence of the THP group and the spectrum was very similar to that obtained for 1-benzyl-1-methyl-2-hydroxyguanidine (**137**). When the ^{13}C NMR spectrum was obtained of the white solid it was confirmed that the product was the hydroxyguanidine because there was a peak at 156 ppm due to the C=N. This is an interesting result because for 1-benzyl-1-methyl-2-hydroxyguanidine (**137**) to have been obtained the *O*-THP-hydroxylamine must have reacted to give the desired product (**269**) which was then hydrolysed to give the free hydroxyguanidine (**137**). This confirms a previous result with *O*-THP-1-benzyl-1-methyl-2-hydroxyguanidine (**269**) which was seen to decompose when an attempt to purify the reaction by washing with dilute acid was undertaken. The *O*-THP-1-benzyl-1-methyl-2-hydroxyguanidine (**269**) had been seen to be present in the reaction mixture before this acid wash and after the wash the product was shown by ^1H and ^{13}C NMR to be 1-benzyl-1-methyl-2-hydroxyguanidine (**137**). This seems to suggest that *O*-THP-1-benzyl-1-methyl-2-hydroxyguanidine (**269**) is less stable than the dibenzyl substituted analogue and that purification may only be possible using reverse phase HPLC.

The synthesis of *O*-THP-1-benzyl-1-(2-phenethyl)-2-hydroxyguanidine (**270**) has proved equally unsuccessful and again the problem is with the purification which proved totally unsuccessful even using the method that worked for *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**). The synthesis of this compound was also attempted via the *N*-benzyl-*N*-(2-phenethyl)cyanamide (**118**). *N*-Benzyl-*N*-(2-phenethyl)cyanamide (**118**) was added to a solution of *O*-THP-hydroxylamine (**215**) in DMF. Sodium carbonate was then added to this solution before the reaction was heated under reflux for periods of time ranging from four to twelve hours. In every case when the inorganic salts were removed and the DMF concentrated under reduced pressure an oil was obtained which was added to water in an attempt to crystallise the product. However no crystallisation occurred and the oil was extracted back into ethyl acetate. Removal of the

ethyl acetate yielded an oil which was shown in the NMR spectrum to contain no THP group. This suggested that reaction had not occurred and the oil obtained was the cyanamide starting material.

The situation was the same with *O*-THP-1-benzyl-2-hydroxyguanidine (**271**). The reaction was carried out and it was shown by NMR spectroscopy that the crude product contained some of desired product. However purification of the product was not possible.

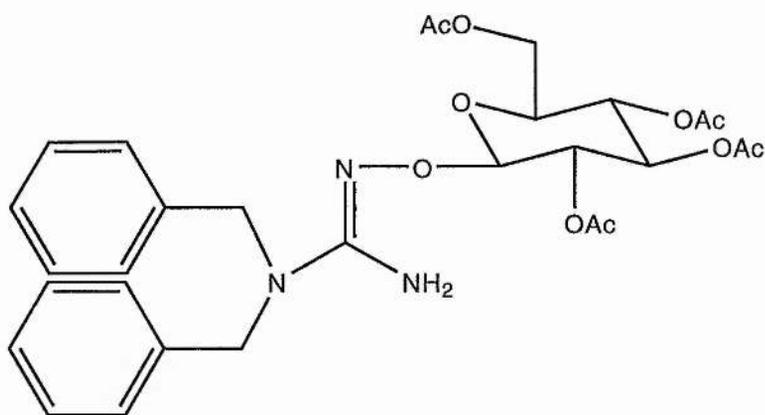


(271)

The problems in purification might be solved by the use of preparative HPLC because work done on these compounds (**Chapter 4**) has shown that they are stable and can be separated from possible by-products.

3.5.3 SYNTHESIS OF *O*-GLYCOSYL *N*-HYDROXYGUANIDINES

Due to lack of time only the synthesis of *O*-(tetra-*O*-acetyl-D-glucopyranose)-1,1-dibenzyl-2-hydroxyguanidine (**272**) was attempted.



(272)

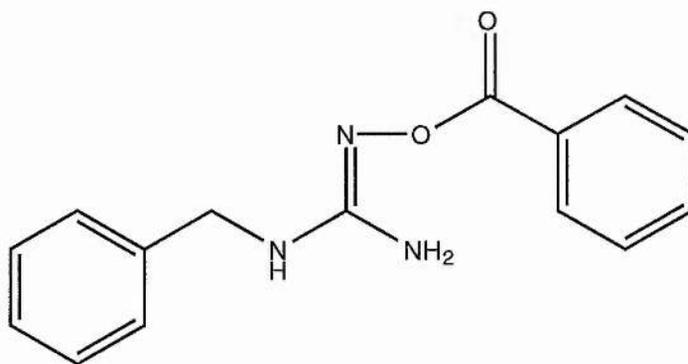
The synthesis of the compound was attempted by reacting the corresponding *N,N*-dibenzylaminoiminomethanesulfonic acid (**196**) with *O*-(tetra-*O*-acetyl-*D*-glucopyranose)hydroxylamine (**250**) in the presence of potassium carbonate in water. The reaction was stirred overnight at room temperature and then the aqueous layer was extracted with dichloromethane. Concentration of the dichloromethane yielded an oil which was purified by flash column chromatography to give a product that was one spot by tlc.

The NMR spectra of the product were then obtained and the ^1H spectrum showed all the required peaks in the right proportions indicating that reaction had indeed occurred. The ^{13}C NMR spectrum showed the resonance for C-1 of the glucose at 105.13 as expected and also the presence of a resonance at 159.45 ppm due to the guanidino group of the hydroxyguanidine. The mass spectrum of the product also found the molecular ion, $[\text{M}+\text{Na}]^+$, at 618. No further work was possible with these derivatives and it was not possible to obtain the intermediates in larger quantities or to purify the very small amount of *O*-(tetra-*O*-acetyl-*D*-glucosepyranosyl)-1,1-dibenzyl-2-hydroxyguanidine (**272**) any further.

3.5.4 SYNTHESIS OF OTHER *O*-SUBSTITUTED *N*-HYDROXYGUANIDINES

Although other *O*-substituted hydroxylamines were synthesised there was very little further work done with these compounds.

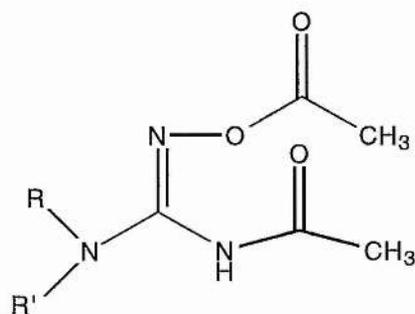
The synthesis of *O*-benzoyl-1-benzyl-2-hydroxyguanidine (**273**) was attempted. The synthesis was attempted from *N*-benzylaminoiminomethanesulfonic acid (**193**) and *O*-benzoylhydroxylamine hydrochloride (**228**) in the presence of potassium carbonate under aqueous conditions. Extraction of the aqueous layer with ethyl acetate and subsequent concentration of the ethyl acetate yielded a dark oil. The NMR spectrum of this dark oil indicated that it was not the expected product as there was no benzoyl group present and ¹³C NMR indicated that the product was most probably the *N*-benzylurea.



(273)

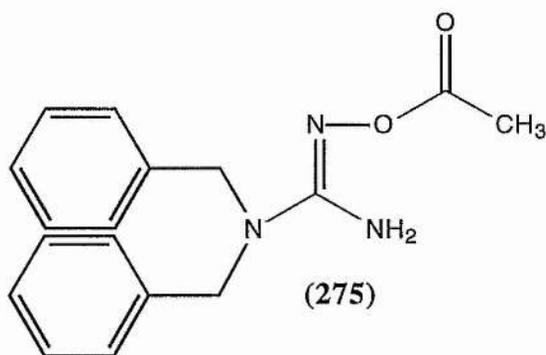
3.5.5 DIRECT ACYLATION OF 1,1-DIBENZYL-2-HYDROXYGUANIDINE

In light of the failure to produce *O*-acetylhydroxylamine it was decided to attempt the direct acylation of 1,1-dibenzyl-2-hydroxyguanidine (**70**). Although, previous reports had suggested that an attempt at acylating an *N*-hydroxyguanidine would lead to full acylation of both the oxygen and the free nitrogen and give a diacylated species (**274**) it was decided to attempt this reaction.¹⁴⁶

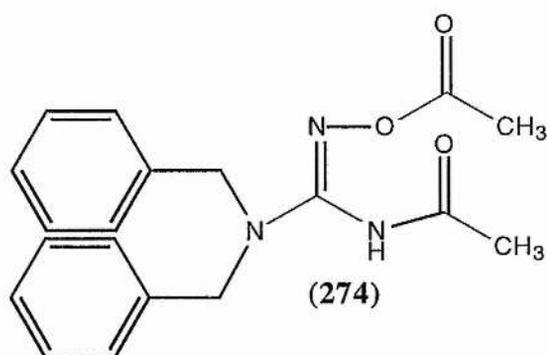


(274)

In an attempt to prevent this diacylation from occurring one molar equivalent of the acylating agent, in this case acetic anhydride, was used. The 1,1-dibenzyl-2-hydroxyguanidine (70) was stirred in pyridine and acetic anhydride added, the reaction was stirred for eighteen hours at room temperature before the pyridine and acetic acid were removed under reduced pressure. The NMR spectrum indicated that there was a mixture of products which were probably the mono (275) and diacylated (274) products so it was decided not to pursue this work any further



(275)



(274)

3.6 CHEMICAL OXIDATION OF *O*-THP-1,1-DIBENZYL-2-HYDROXYGUANIDINE (268) AND *O*-BENZYL-1,1-DIBENZYL-2-HYDROXYGUANIDINE (260)

Two of the *O*-substituted *N*-hydroxyguanidines, *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (268) and *O*-benzyl-1,1-dibenzyl-2-hydroxyguanidine (260) were subjected to chemical oxidation and the amount of nitric oxide released measured using the Greiss test (See Section 2.7 for discussion).

3.6.1 *O*-Benzyl-1,1-dibenzyl-2-hydroxyguanidine (260)

A solution of *O*-benzyl-1,1-dibenzyl-2-hydroxyguanidine (0.1 mmol dm^{-3}) (260) in water/acetonitrile (85/15%) as the solvent was placed in a water bath at 37°C and an excess (0.01 g) of mCPBA was added and the amount of nitrite released was measured at various intervals (Table 13)

Table 13:-oxidation of (260) by mCPBA

Time/ min	Conc. of nitrite/ $\times 10^{-6} \text{ mol dm}^{-3}$	% nitrite
30	3	6
60	4	7
90	4	7

The results show that there is clearly some release of nitrite occurring which is a slightly unexpected result, although there only 7% oxidation as the oxygen is protected by a benzyl group it would be thought that oxidation to nitric oxide would be impossible. However this result implies that the benzyl group must be removed, to a limited extent

under the reaction conditions. The 1,1-dibenzyl-2-hydroxyguanidine (**70**) thus produced then oxidises as previously observed.

3.6.2 *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**)

A solution of 0.1 mmol dm⁻³ solution of *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**) in water/acetonitrile (85/15%) as the solvent was placed in a water bath at 37 °C and an excess of mCPBA (0.01 g) was added and the nitrite concentration measured at various time periods (**Table 14**).

Table 14:-oxidation of (**268**) by mCPBA

Time/ min	Conc of nitrite / x10 ⁻⁶ mol dm ⁻³	% oxidation
30	7	13
60	7.5	14
90	7.5	14

It can be seen that *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**) does indeed give a positive test for nitrite. This is not entirely unexpected as the THP group is acid sensitive and the conditions are acidic enough to hydrolyse the THP group and yield 1,1-dibenzyl-2-hydroxyguanidine (**70**) which has already been shown to oxidise. Although not quantitative 14% oxidation is observed which is close to the 18% obtained for pure 1,1-dibenzyl-2-hydroxyguanidine (**70**).

3.7 CONCLUSIONS

More was achieved in this area of the project, with a number of *O*-substituted hydroxyguanidines being synthesised. One of the limiting factors was the successful preparation of the required substituted hydroxylamine and then subsequent reaction to give the *O*-substituted hydroxyguanidines. In particular a number of substituted hydroxylamines including, *O*-benzoylhydroxylamine hydrochloride (**228**) and *O*-trimethylsilylhydroxylamine (**236**) were prepared but their subsequent reaction could not be achieved using the various methods attempted. Also it was surprising that the synthesis of the *O*-substituted derivatives from the corresponding cyanamides was unsuccessful. It could be that the *O*-substitution of the hydroxylamine makes the nitrogen less nucleophilic and therefore makes the reaction less likely. This problem means that the synthesis is restricted to derivatives where the corresponding thiourea and aminoiminomethanesulfonic acid can be obtained. The synthesis of some thioureas was not achieved, especially the less nucleophilic aromatic amines.

The preparation of an *O*-glycosylhydroxylamine was achieved, however at the moment the low yields during the preparation are a limiting factor and a consistent methodology for its synthesis needs to be found. However the subsequent reaction of *O*-(tetra-*O*-acetyl-*D*-glucopyranose)hydroxylamine (**250**) and *N,N*-dibenzylaminoiminomethanesulfonic acid (**196**) was successful and indicated that if reasonable quantities of the substituted hydroxylamine can be obtained then the synthesis of *O*-(tetra-*O*-acetyl-*D*-glucopyranose)-1,1-dibenzyl-2-hydroxyguanidine (**272**) and other derivatives will be possible. These may be very interesting pro-drugs especially if it can be shown that the glucose can be cleaved off enzymatically.

The major success of this work was the synthesis of a number of *O*-benzyl substituted hydroxyguanidines and *O*-THP substituted hydroxyguanidines. The synthesis of the *O*-benzyl derivatives was easily achieved and a number of disubstituted derivatives and one mono substituted derivative were prepared from the corresponding aminoiminomethanesulfonic acid and *O*-benzyl hydroxylamine. However in an attempt

to obtain a number of the free *N*-hydroxyguanidines the removal of the *O*-benzyl group was attempted by various methods. Removal of the group was not possible and when the reaction was attempted using catalytic hydrogenation N-O bond cleavage was observed. This is a very unusual result especially as previous reports had suggested that removal of this group should have occurred.¹¹³

A number of *O*-THP derivatives were obtained in a impure form, however it has been shown (**Chapter 4**) that it should be possible to purify these products using preparative HPLC. Chemical oxidation of *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**) indicated that some nitric oxide was obtained.

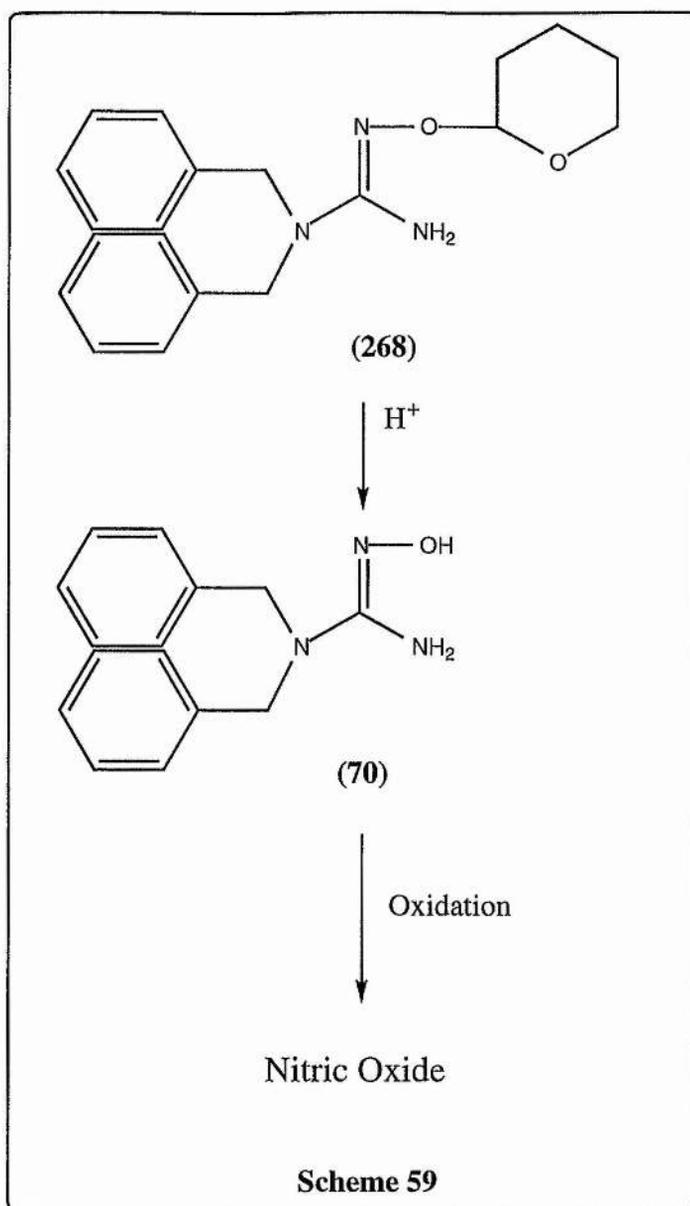
The results from the work undertaken in this area indicate that it should be possible to design an appropriate *O*-substituent that could be used on a prodrug and thus allow selective targeting of the nitric oxide.

Chapter 4

Studies on the Acid Catalysed Decomposition of *O*-THP-1,1-dibenzyl-2- hydroxyguanidine

4.0 INTRODUCTION

The THP group was used in the synthetic part of the project for a number of reasons. Firstly the group is very acid sensitive and should therefore be removed under acidic conditions and would therefore act as a prodrug because the free *N*-hydroxyguanidine would then be released. The THP group was also a simple model for the more interesting and probably more useful sugar derivatives. It was thus necessary to examine the acid catalysed decomposition of *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**) to demonstrate that the corresponding *N*-hydroxyguanidine, 1,1-dibenzyl-2-hydroxyguanidine (**70**), was indeed formed. There was some circumstantial evidence for this from earlier synthetic experiments (**Section 3.6**) but it needed to be clearly demonstrated. Also the rate of reaction and its variation with pH was of interest, as a basis for the design of future molecules as nitric oxide donor pro-drugs. The proposed pathway for the production of nitric oxide *in vivo* from *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**) is given (**Scheme 59**).

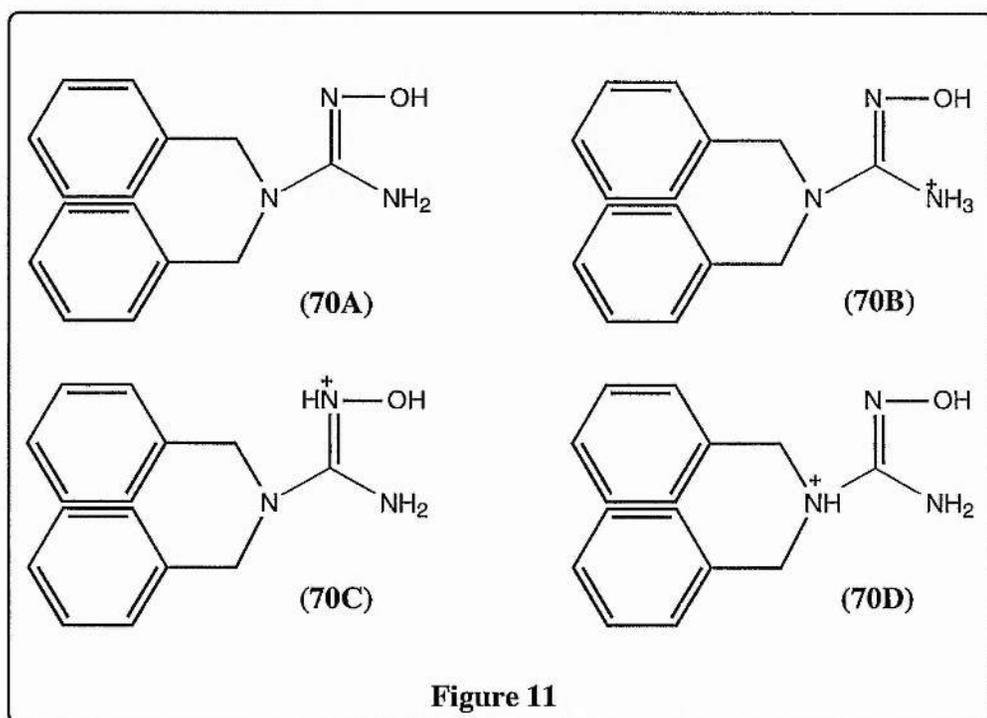


4.1 ANALYSIS OF THE DECOMPOSITION REACTION

In order to monitor the hydrolysis of *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (268) a suitable analytical technique to measure the concentration of either the reactant or the product was required. Many of the well used methods were unsuitable for example the NMR of the product and reactant were too similar and would be too difficult to analyse, the compounds were not volatile enough for GC analysis and tlc is not a quantitative method. For these reasons it was therefore decided to investigate the use of

HPLC. In order to be able to do this the first step was to show that the two possible products of the reaction, 1,1-dibenzyl-2-hydroxyguanidine (**70**) and *N,N*-dibenzylurea (**154**) could be separated from *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**). The HPLC analysis was carried out using a CECIL instruments HPLC and a reverse phase silica column. Initially a Phenomenex JUPITER column and 80/20 acetonitrile/water solvent system was used, however it could be seen that the trace of 1,1-dibenzyl-2-hydroxyguanidine (**70**) contained a number of peaks (**Figure 11**).

It was thought that under the conditions employed protonation of (**70**) could take place giving various protonated forms of the compound (**Figure 11, 70A-D**), which were separable by HPLC.



The trace for *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**) (**Figure 12**) also showed that it was not a single peak again due to protonation and the presence of a variety of protonated forms of the molecule being present.

Figure 11

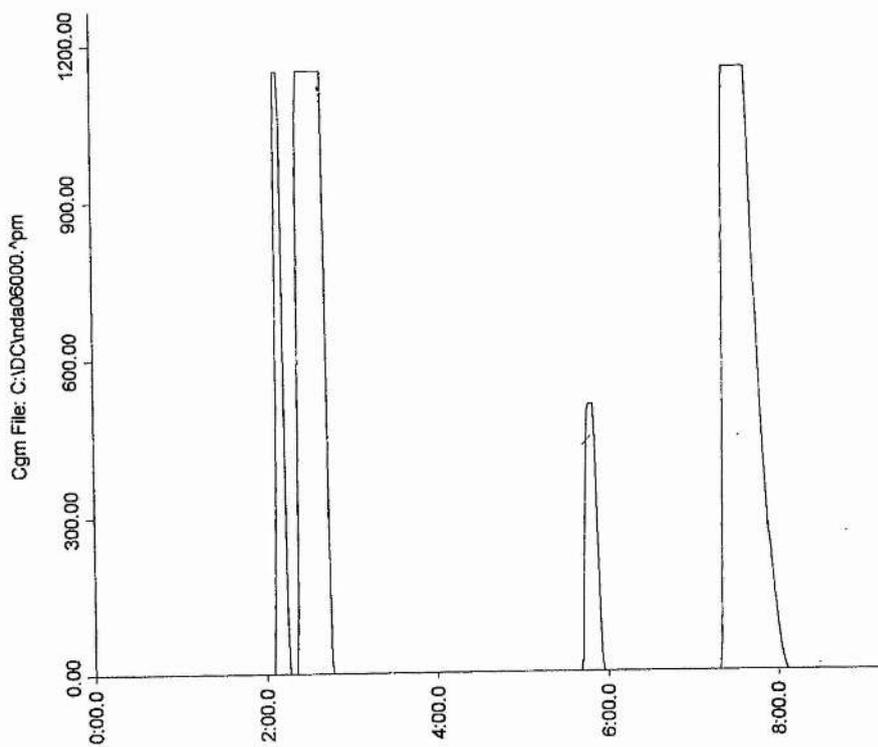
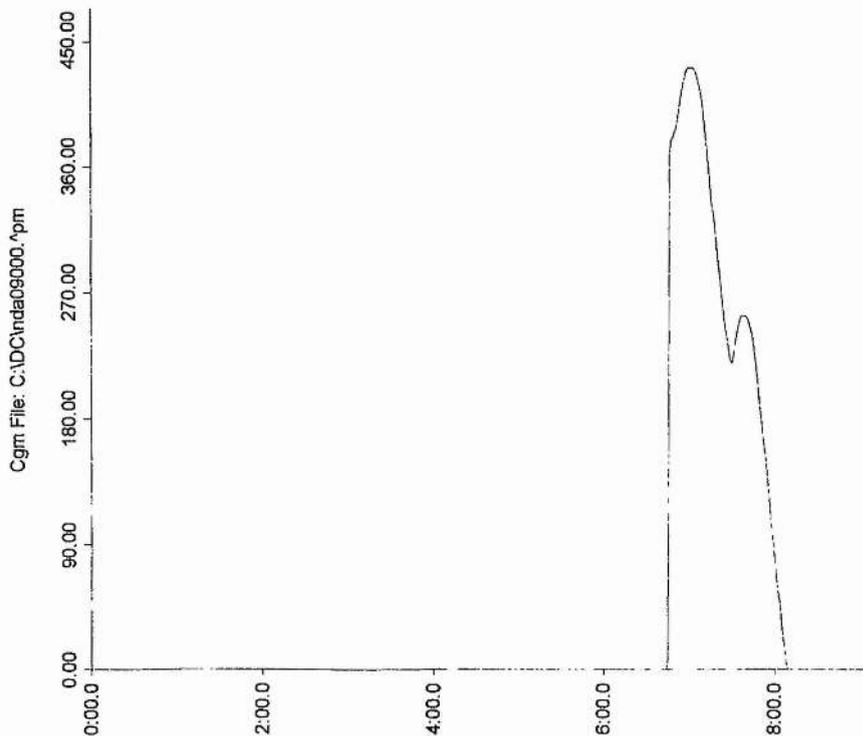


Figure 12



In order to remove this problem it was decided to use a basic solvent system because obviously the HPLC could not be run under acidic conditions as this would cause hydrolysis of the THP derivative (**268**). Initially 1% triethylamine was added to the solvent system. The three standards were then run using 50/50 acetonitrile/water with 1% triethylamine. Both 1,1-dibenzyl-2-hydroxyguanidine (**70**) and *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**) did indeed give single peaks with retention times of 4.1 and 8.1 minutes respectively.

N,N-Dibenzylurea (**154**) which had given a single peak using the original solvent system became two peaks with retention times of 2.25 and 4.45 minutes however as it was clear that the products could be separated it was decided to attempt an initial reaction.

O-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**) was added to a solution of 1M hydrochloride acid and the reaction was monitored by removing aliquots after 5, 20, 40 and 60 minutes reaction. The samples were quenched by adding excess triethylamine and then acetonitrile was added to this solution to solubilise it. The solutions were then examined by HPLC. It was immediately clear that the amount of the THP derivative (**268**) was decreasing and after 60 minutes there was no trace of it present (**Figure 13+14**).

Figure 13: 5 minutes reaction

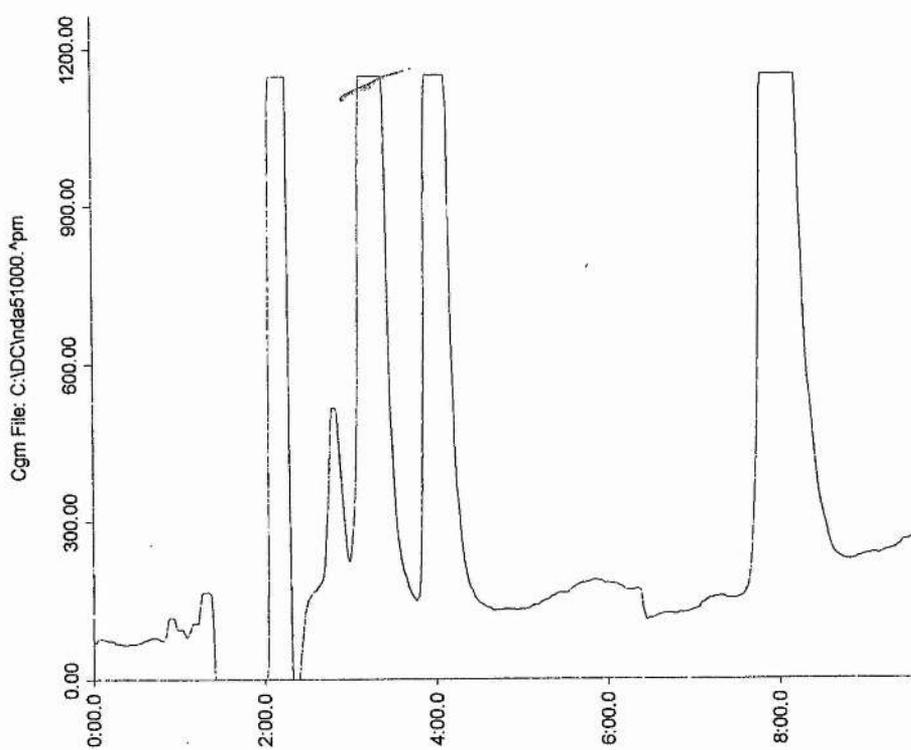
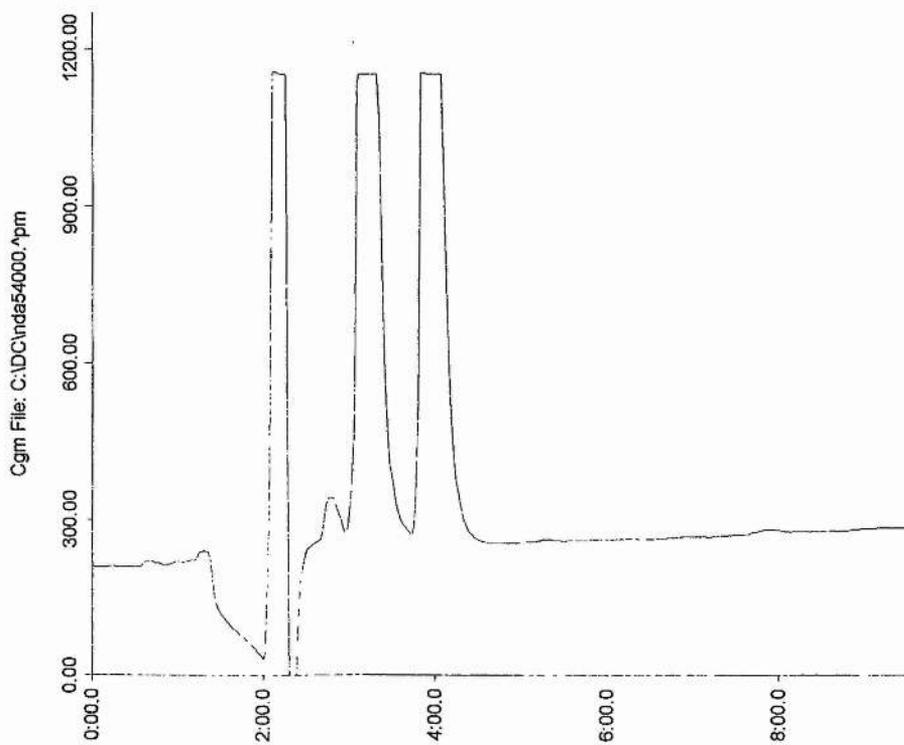


Figure 14:- 60 minutes reaction



The remaining reaction mixture was then concentrated under reduced pressure and the white solid that was obtained was analysed by NMR spectroscopy. The ^1H NMR spectrum did not reveal much except to suggest, contrary to the HPLC trace, that there was only a single product. The ^{13}C NMR spectrum showed a resonance at 161.81 ppm indicating that the product was a salt of the desired 1,1-dibenzyl-2-hydroxyguanidine (**70**). Both the ^{13}C and ^1H NMR spectra were also identical to the NMR spectra obtained for the authentic 1,1-dibenzyl-2-hydroxyguanidine (**70**). Further evidence that this was indeed the case was obtained when the white solid was oxidised. As described previously (**Section 2.7**) the Griess test can be used to show the presence of nitrite which is derived from nitric oxide. A solution of the decomposition product (0.1 mol dm^{-3}) was incubated at 37°C and an excess of mCPBA was added. The reaction was tested for nitrite after various periods of time (**Table 15**)

Table 15:- Oxidation of decomposition reaction product.

Time / min	Conc of nitrite / $\times 10^{-6} \text{ mol dm}^{-3}$	% Oxidation
30	6	11
60	7.5	14
90	9.5	17
120	7.5	14

The maximum % oxidation (100% oxidation, concentration of nitrite = $55 \times 10^{-6} \text{ mol dm}^{-3}$) after 90 minutes of 17% compares favourably to the 18% observed for the authentic 1,1-dibenzyl-2-hydroxyguanidine (**70**).

This reaction confirmed that it was possible to protect the oxygen of an *N*-hydroxyguanidine with the THP group and then remove this under acidic conditions to give the free *N*-hydroxyguanidine. This free *N*-hydroxyguanidine could then be oxidised using mCPBA to give a positive Griess test, indicating that nitric oxide had been

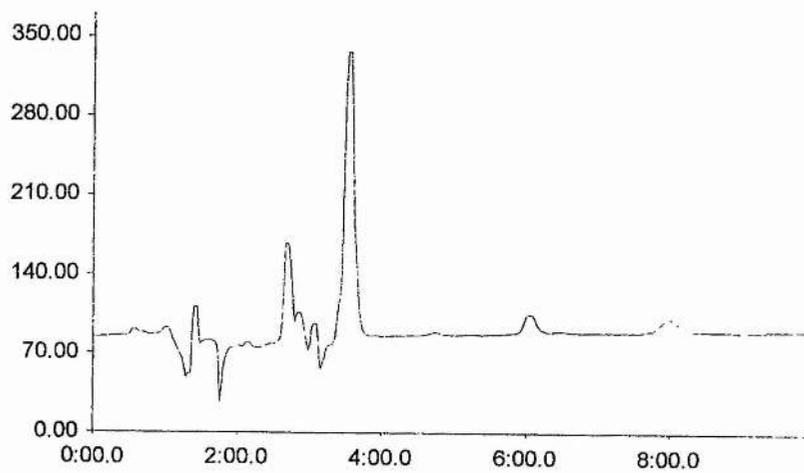
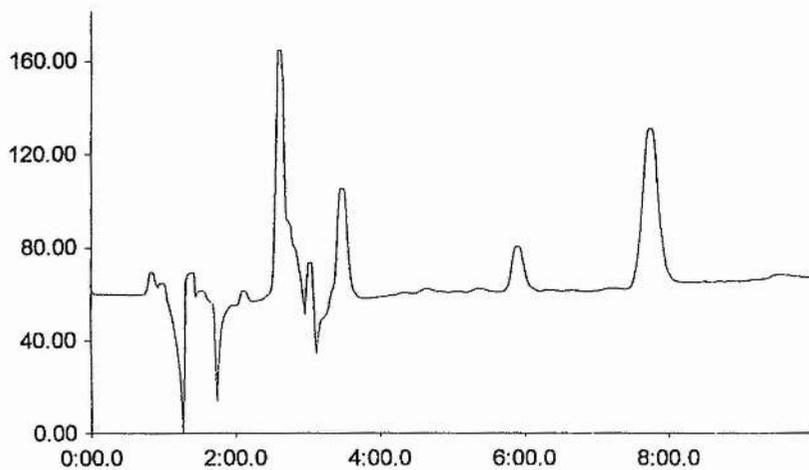
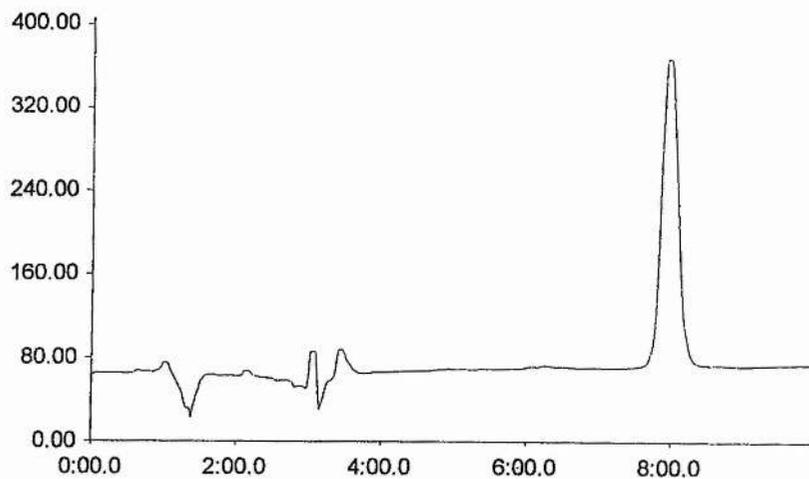
produced (**Scheme 59**). It was thus decided to carry out a kinetic study on the reaction and examine the ease of hydrolysis.

4.2.KINETIC STUDIES OF HYDROLYSIS REACTION

It was decided to examine the reaction over a range of pH values and determine the pH profile of the reaction. Reactions were run at 37 °C i.e. physiological temperature. In the preliminary study, the reactions had been quenched by the addition of triethylamine. However on close analysis it was observed that this did not completely stop the reaction, and the amount of the *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**) observed by HPLC was still slowly decreasing. As an alternative it was decided to quench the reaction with a molar equivalent of aqueous 1M sodium hydroxide. Unfortunately this had an effect on the HPLC traces, splitting the peak due to *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**) into two separate peaks. It was also observed that tailing and broadening of the peaks was becoming a problem. An alternative reverse phase column was then investigated, which is recommended for use at high pH. The column used was a Phenomenex LUNA C₁₈ column. Eventually suitable conditions were found that gave good peak separation. These were 60/40 acetonitrile/water with 1% triethylamine which gave a single peak for *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**) at approximately 8 minutes.

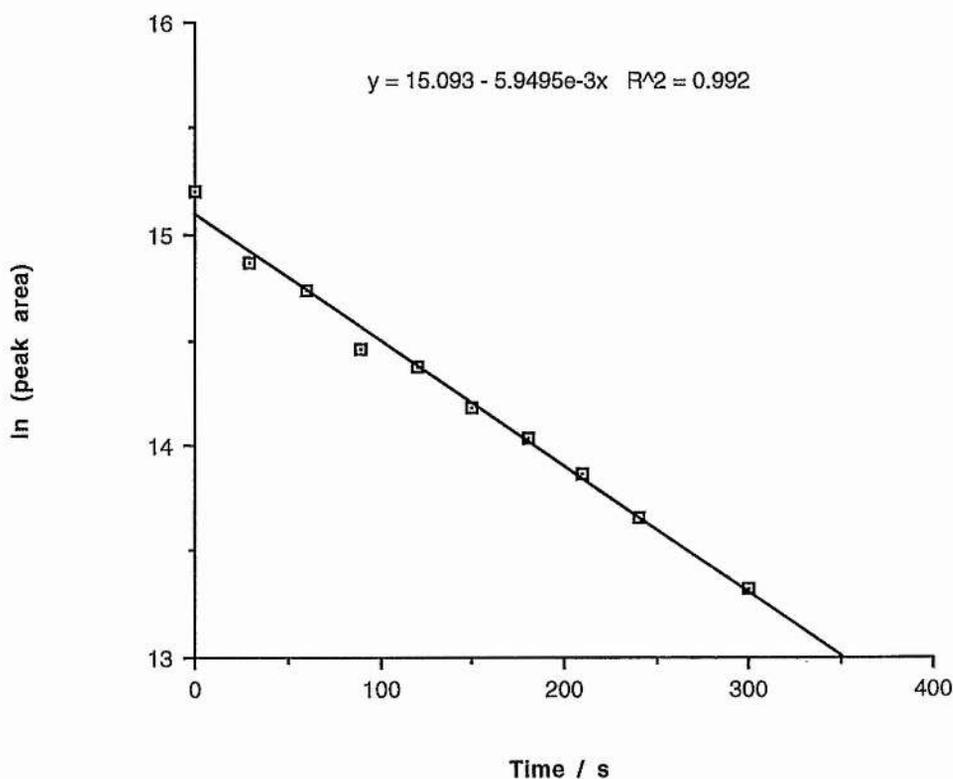
Using the new analysis conditions the hydrolysis of *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**) at pH 0 and 37 °C was examined. Aliquots (0.02 cm³) were removed after various times and quenched with an equal volume of 1M sodium hydroxide. The aliquot was then diluted with acetonitrile and analysed by HPLC. The peak at approximately 8 minutes was seen to disappear over a period of 20 minutes (**Figure 15**).

Figure 15



Using the computer software the area of the peak, which was a measure of the concentration of the *O*-THP-1,1-dibenzyl-2-hydroxyguanidine present, was calculated. A plot of ln of the area of peak against time (**Graph 1**) gave a straight line confirming that the acid catalysed hydrolysis was pseudo first order. Linear regression was used to give the best fit line and to calculate the errors, in addition all the graphs gave fairly good straight line. The gradient was $5.948 \times 10^{-3} \text{ s}^{-1}$ which is the observed first order rate constant, k_{Obs} .

Graph 1:- Plot of ln (THP peak area) against time for acid catalysed decomposition of *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**) at pH 0



From the rate of reaction it is possible to calculate half life as follows:

$$t_{1/2} = \frac{\ln 2}{k_{\text{obs}}}$$

$$\Rightarrow t_{1/2} = \frac{0.6932}{k_{\text{obs}}}$$

For this reaction the $t_{1/2}$ is equal to 116 seconds.

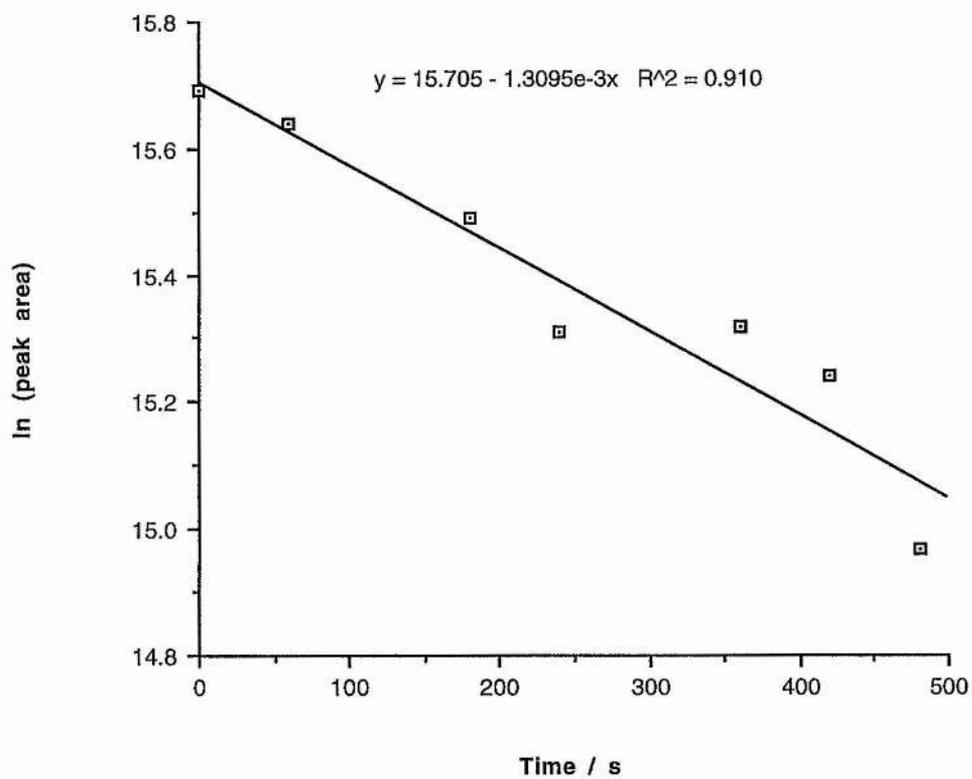
The reaction was repeated in order to check that the results were reproducible. Again the reaction gave a straight line graph with a gradient, k_{obs} , of $5.366 \times 10^{-3} \text{ s}^{-1}$, the $t_{1/2}$ is equal to 129 seconds. Averaging this values out gives the average values shown (Table 16).

Table 16:- Average kinetic values for decomposition reaction at pH 0

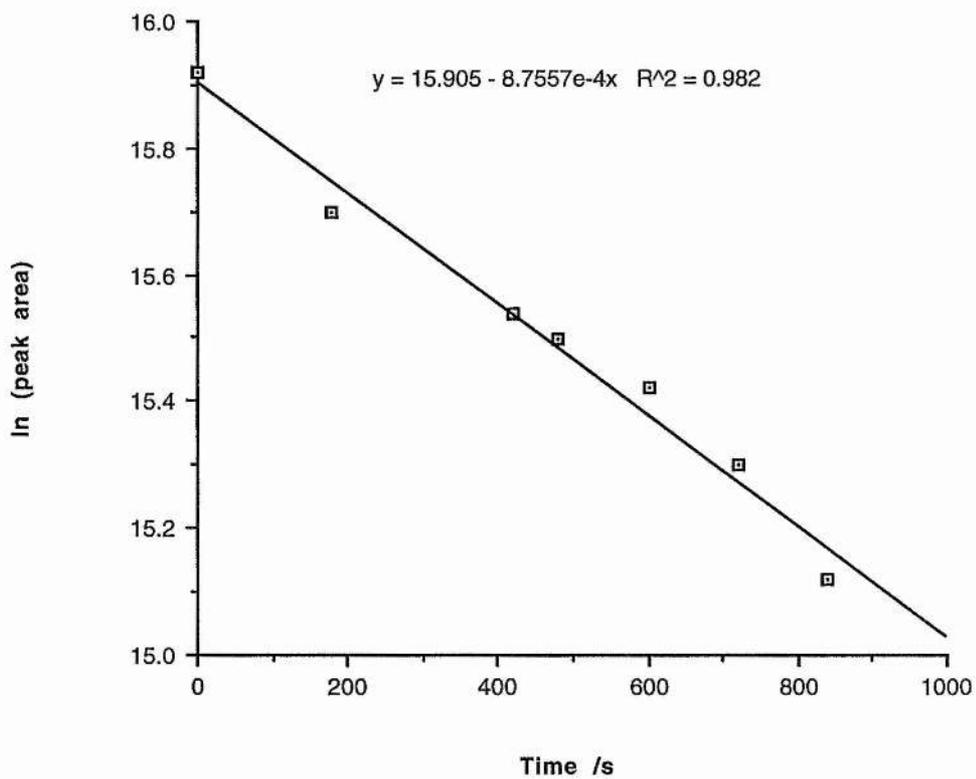
Parameter	Average value	Error
k_{obs}	$5.66 \times 10^{-3} \text{ s}^{-1}$	$\pm 0.29 (5\%)$
$t_{1/2}$	122 s	$\pm 6.5 (5\%)$

The reaction was then examined at pH 0.5, 1, 1.5 and 2 (Graphs 2-5) and the kinetic parameters calculated for these reactions. The reactions at pH 1.5 and 2 were quenched by addition of an equal volume of triethylamine as it had been observed that upon addition of an equal volume of sodium hydroxide that multiple peaks were seen in the HPLC trace. The values for k_{obs} and $t_{1/2}$ for these reactions were calculated along with the errors associated with those values (Table 17). The errors were calculated by using the linear regression of the straight line.

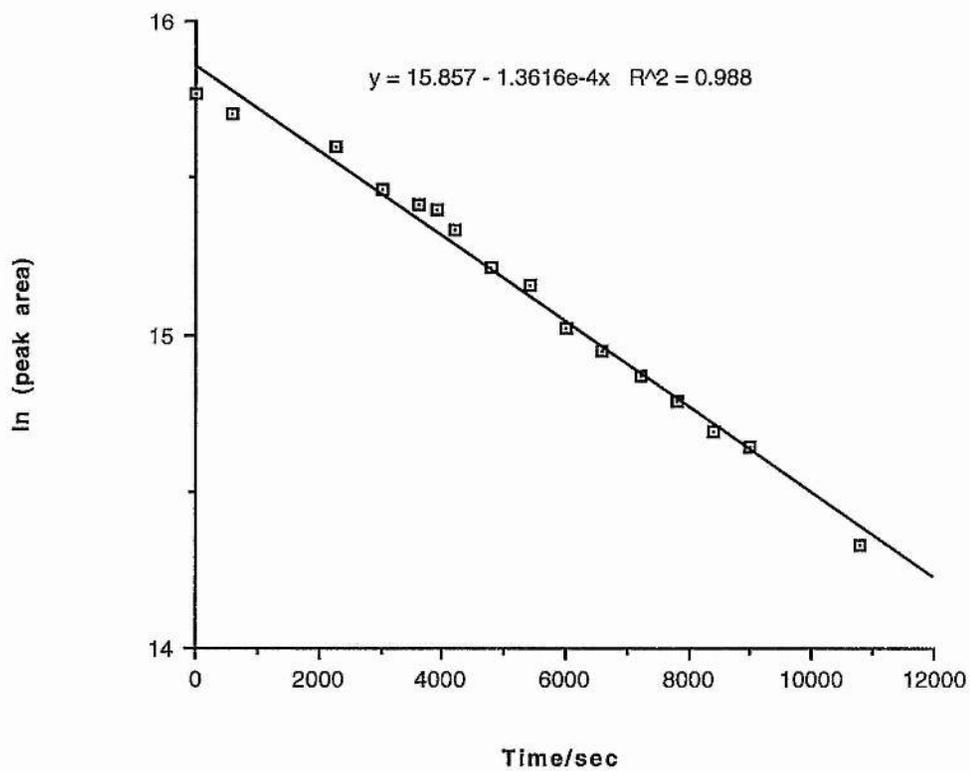
Graph 2:- plot of \ln (peak area) against time for the acid catalysed decomposition of (268) at pH 0.5



Graph 3:- Plot of $\ln(\text{peak area})$ against time for the acid catalysed decomposition of (268) at pH 1



Graph 4:- Plot of ln (peak area) against time for the acid catalysed decomposition of (268) at pH 1.5



Graph 5:- Plot of $\ln(\text{peak area})$ against time for the acid catalysed decomposition of (268) at pH 2.0

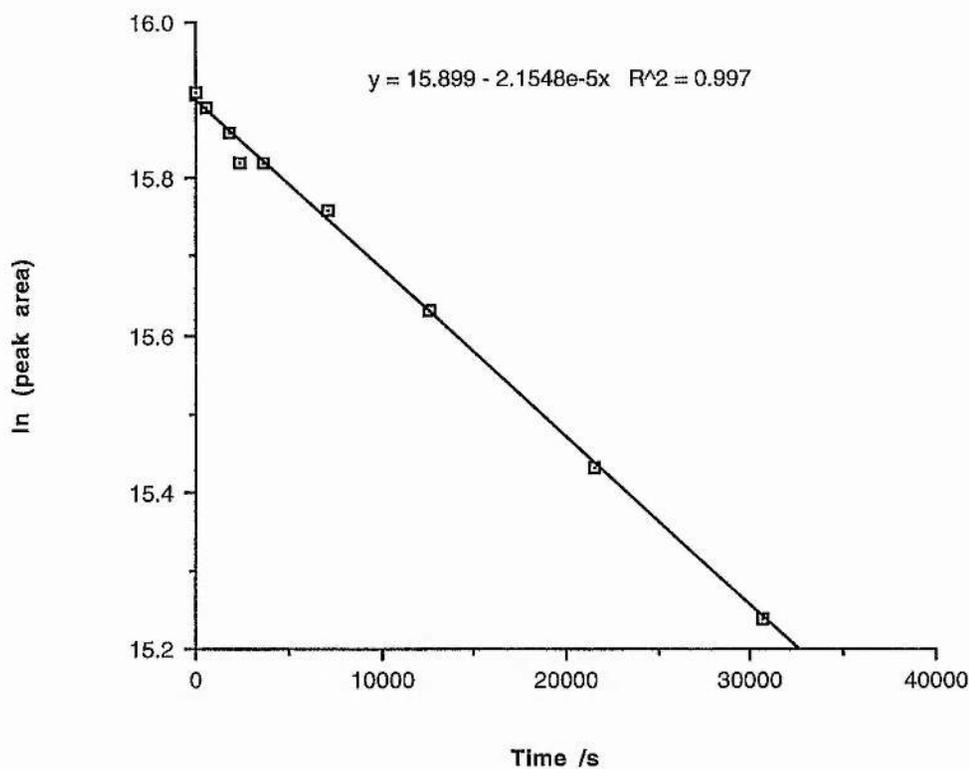


Table 17:- Calculated values of k_{obs} and $t_{1/2}$ for the pH

pH	Concentration of H^+ / mol dm^3	k_{obs} / s^{-1}	$t_{1/2}$ / s
0	1	$(5.66 \pm 0.29) \times 10^{-3}$	122 ± 6.5
0.5	0.316	$(1.31 \pm 0.12) \times 10^{-3}$	530 ± 84
1	0.1	$(8.71 \pm 0.17) \times 10^{-4}$	796 ± 16
1.5	0.0316	$(1.36 \pm 0.02) \times 10^{-4}$	5100 ± 60
2.0	0.01	$(2.16 \pm 0.06) \times 10^{-5}$	3200 ± 970

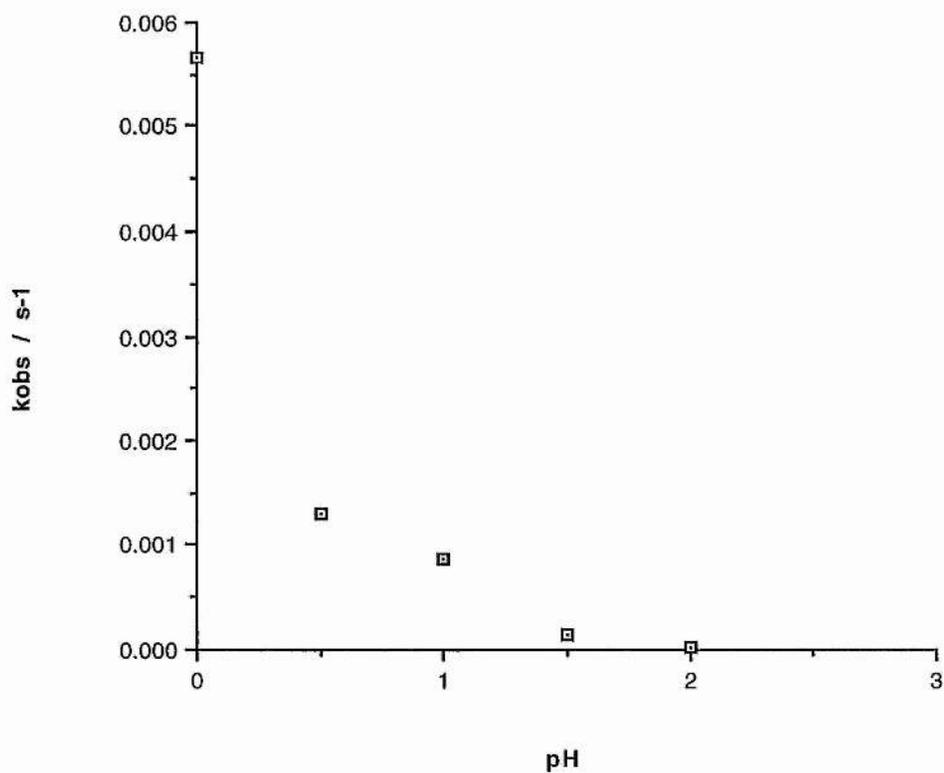
A pH profile of the reaction (**Graph 6**) indicates that k_{obs} decreases as pH decreases. This would indicate that k_{obs} is proportional to $[\text{H}^+]$. A plot of the concentration against k_{obs} can then be made (**Graph 7**). From the plot (**Graph 7**) it can be seen that this gives a straight line which indicates that, as suspected, that the reaction is first order in H^+ . This would also suggest that the rate equation can be expressed as shown:-

$$\text{Rate} = k_{\text{obs}} [268]$$

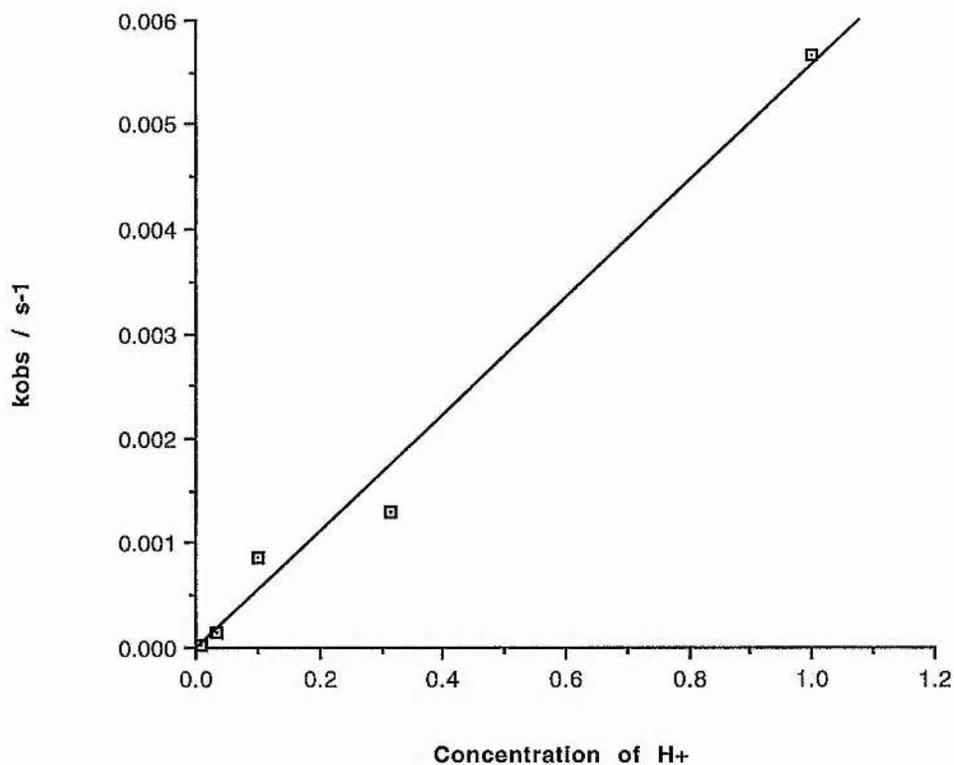
$$\text{Rate} = k_2 [\text{H}^+][268]$$

This implies that the reaction involves specific acid catalysis rather than general acid catalysis. It is likely that the oxygen attached to nitrogen is protonated as the first step of the reaction followed by cleavage of the C-O bond facilitated by the oxygen in the six membered ring.

Graph 6:- Plot of pH against k_{obs} for the acid catalysed decomposition of *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**)

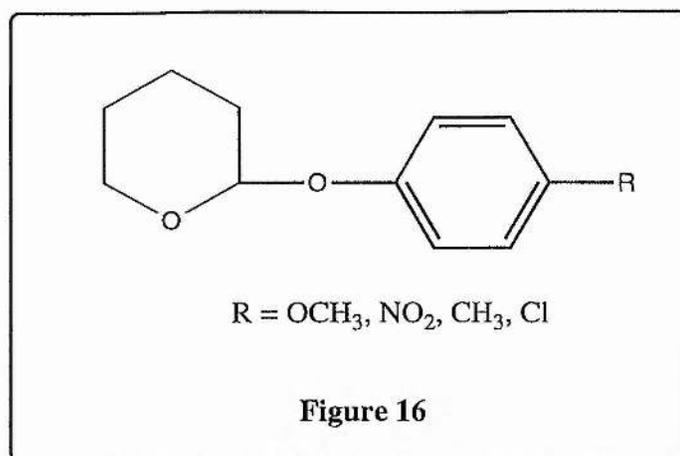


Graph 7:- Plot of concentration against rate of reaction for the acid catalysed decomposition of *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**)



This result was also confirmed when a reaction was carried out at pH 3. The reaction indicated that even after 7 days at 37 °C there had only been a 5 % decrease in the concentration of *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**).

Work has been carried out by Fife to examine the rates of acid catalysed hydrolysis of a series of 2-alkoxy and 2-aryloxytetrahydropyrans (**Figure 16**) in a 50% dioxane-water mixture.¹⁴⁷

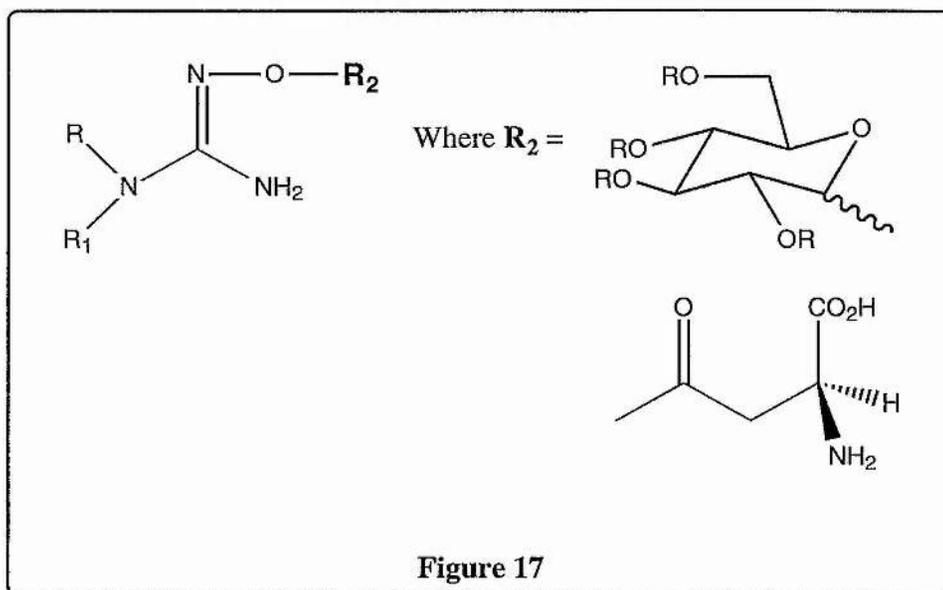


It was found that reaction rate was dependent on the nature of the substituent on the aryl group and that the reaction rate for the hydrolysis of 2-(*p*-nitrophenoxy)-tetrahydropyran was pH dependent. The pH profile indicated that at low pH reaction rate was fast, however at higher pH the reaction rate plateaued which is very similar to the results obtained from our studies. At pH 1.3 the half life of the reaction was 9 mins for the 2-(*p*-methoxyphenoxy)-tetrahydropyran. From our studies it can be estimated that at pH 1.3 the hydrolysis of **(268)** would have a half-life of 46 minutes. This would indicate that the acid catalysed decomposition of **(268)** is slower than that of aromatic compounds presumably because of the *N*-hydroxyguanidine is a poorer leaving group.

4.3 CONCLUSION

The results of the kinetic work indicate that the decomposition reaction *O*-THP-1,1-dibenzyl-2-hydroxyguanidine **(268)** is first order and dependent on the concentration of H⁺ ions. The work also shows that the rate of reaction at pH 3 is negligible which is a disappointing result because it means that the THP derivatives may be too stable to act as an efficient prodrugs. However the work has shown that it is possible to protect the hydroxyl group of an *N*-hydroxyguanidine and then remove the protecting group and yield the free nitric oxide donor drug which can be oxidised to give nitric oxide.

The results of this study mean that further work will have to be done to find a less stable protecting group or it may be that removal of the protecting group could occur by enzymatic action. Suitable alternatives for the THP group might include glycosidic groups or peptides (**Figure 17**).



These groups have an advantage because it is possible that both of them could be removed by enzymatic action rather than relying on an acid catalysed decomposition.

Chapter 5

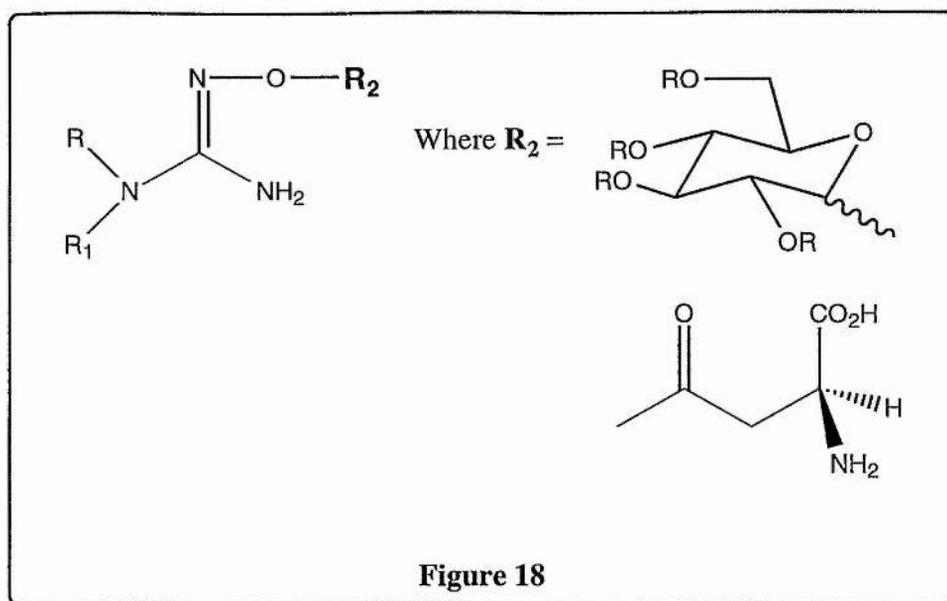
**CONCLUSIONS AND
FUTURE WORK**

It has been shown by the work undertaken that *N*-hydroxyguanidines remain a potential source of nitric oxide donor drugs. However their biological usefulness is limited by their inherent chemical instability and the inability to find a suitable, versatile and reliable method for their synthesis and subsequent purification. It maybe that HPLC may will prove to be the most suitable and convenient method to obtain useful amounts of pure *N*-hydroxyguanidines. Two disubstituted derivatives, 1,1-dibenzyl (**70**) and 1-benzyl-1-methyl (**137**) were obtained pure and subsequent chemical and biological testing confirmed their potential.

The synthesis of *O*-substituted analogues proved easier and more productive. The synthesis of a number of *O*-substituted hydroxyguanidines was achieved from the corresponding *O*-substituted hydroxylamines. The major problem was the transformation of the hydroxylamine into the *O*-substituted hydroxyguanidine, the reaction with the cyanamide did not occur and the range of aminoiminomethanesulfonic acids was limited due to problems with the synthesis of the thioureas. However if this synthetic problem could be solved then a significant range of *O*-substituted hydroxyguanidines could be synthesised.

The work done with *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (**268**) showed that the compound could be hydrolysed under acidic conditions to give the free 1,1-dibenzyl-2-hydroxyguanidine (**70**) which could then be chemically oxidised to give nitric oxide. The hydrolysis is fast at low pH ($t_{1/2} = 122$ s at pH 0) but does not appear to occur appreciably at pH 2 or above. However it does show that the group can be cleaved to give free *N*-hydroxyguanidine.

Thus the principle of preparing pro-drugs by protection of the oxygen has been demonstrated. The next step will be to design alternative *O*-substituents which can be removed under milder, more physiological like conditions, The most appropriate strategy maybe to target enzymatic removal of the substituent. Potential groups would be *O*-glycosyl or peptide like substituents (**Figure 18**), which could be cleaved by glycosidases or proteases respectively.



More work also needs to be undertaken to probe the release of the nitric oxide from these compounds. It may be that the oxidation can be carried out either by P450 enzymes or by nitric oxide synthase or both. Again once more has been elucidated about the release of nitric oxide from these compounds the design of more suitable *N*-substituents can be addressed to prepare derivatives which are more water soluble and will have better pharmacokinetic properties.

However all the results of the work presented indicate that it may be possible to achieve the synthesis of a medically useful *N*-hydroxyguanidine derivative. This derivative could be targeted to the desired site of action by design of a suitable *O*-substituent to be removed by enzymatic action, which is required before release of nitric oxide is possible.

Chapter 6

Experimental

6.1 General

Melting points were recorded using a Gallenkamp melting point apparatus and are uncorrected.

Elemental analyses were carried out in the departmental microanalytical laboratory.

Optical rotations were measured at room temperature using an Optical Activity Ltd. AA 1000 polarimeter and 10 cm path-length cells.

NMR spectra were recorded on a Varian 2000 f.t spectrometer (^1H , 300 MHz; ^{13}C , 75.42 MHz) and a Varian Gemini f.t spectrometer (^1H 200 MHz; ^{13}C 50.31 MHz). ^1H NMR spectra are referenced on chloroform, TMS, d^4 -methanol or d^6 -DMSO; ^{13}C NMR are referenced on chloroform, TMS, d^4 -methanol or d^6 -DMSO. NMR spectra are described in parts per million downfield shift from TMS and are reported consecutively as position (δ_{H} or δ_{C}), relative integral, multiplicity (s-singlet, d-doublet, t-triplet, q-quartet, m-multiplet, dd-doublet of doublets and br-broad), coupling constant ($J_{\text{x,y}}$ Hz if applicable) and assignment.

I.R. spectra were taken on a Perkin-Elmer series 1420 i.r. spectrometer or a Perkin Elmer Paragon 1000 FT-i.r. The samples were prepared as nujol mulls or thin films and run between sodium chloride plates. Absorption maxima are given in wavenumbers (cm^{-1}) relative to a polystyrene standard.

Low and high resolution mass spectra were recorded on an A.E.I MS-902 spectrometer using electron impact at 70 eV or were obtained on an E.P.S.R.C service basis based at the University of Swansea using a VG ZAB E. Low and high resolution CI mass spectra were recorded on a VG Autospec using *isobutane* as the ionising gas. Low resolution electropray mass spectra were also obtained using a VG platform.

Flash column chromatography was performed according to the procedure of Still using sorbisil C₆₀ (40-60 mm mesh) silica gel.¹⁴⁸

Analytical thin layer chromatography was carried out on 0.25mm precoated silica gel plates (Whatman PE SIL G/UV₂₅₄) and spots were visualised by UV fluorescence, iodine vapour or dipping in 5% sulfuric acid in aqueous ethanol followed by charring.

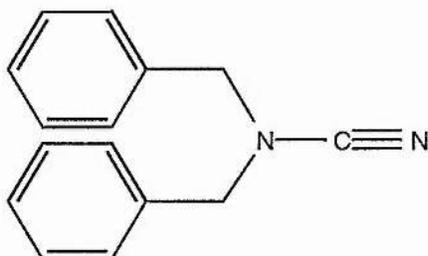
Solvents, where required to be dry, were dried as follows using the methods of Perrin and Armarego.¹⁴⁹ Dichloromethane, and pyridine were refluxed over, then distilled from calcium hydride; diethyl ether and tetrahydrofuran were refluxed over, then distilled from sodium/benzophenone; methanol, ethanol and butanol were refluxed over, then distilled from magnesium/iodine (Grignard reaction). Dimethylformamide was distilled from magnesium sulfate under reduced pressure.

UV readings were taken using a UVICON 932 spectrophotometer and 1 cm path length plastic cuvettes.

HPLC work was carried out on a CECIL instruments CE 1200 High Performance Variable Length Monitor and a CE 1100 Liquid Chromatography Pump. A Phenomenex LUNA C₁₈ 5 μ (150 x 4.6 mm) column or a Phenomenex JUPITER C₁₈ 5 μ (150 x 4.6 mm) column was used. The data were examined using an Icycle 486 PC and Datacontrol software package. Solvents were purified by filtration. Deionised water was obtained using a Milli-Q-RG ultrapure water purification system. The solvents were then degassed for 15 minutes using an Ultrawave sonic bath.

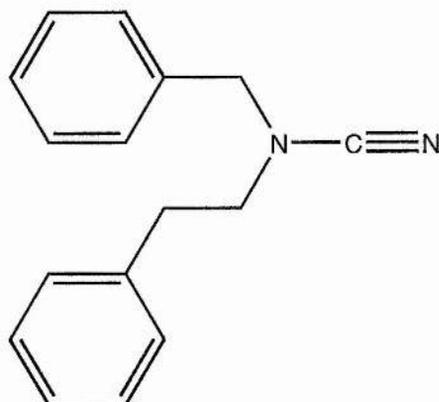
6.2 COMPOUNDS SYNTHESISED

N,N-Dibenzylcyanamide (117)



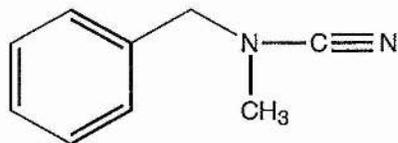
Cyanogen bromide (2 g, 20 mmol) was dissolved in dry methanol (10 cm³) and added dropwise to a cooled suspension of *N,N*-dibenzylamine (3.94 g, 20 mmol) and anhydrous sodium acetate (2.98 g, 30 mmol) in methanol (20 cm³). The resultant suspension was stirred for 2.5 hours in the ice/water bath and then at room temperature for a further 3 hours. The solution was concentrated under reduced pressure to give a solid residue which was dissolved in water and extracted with DCM (3 x 20 cm³). The combined organic extracts were washed with HCl (2M, 20 cm³) and brine (20 cm³), dried (MgSO₄), filtered and concentrated under reduced pressure to yield a yellow solid which was recrystallised from methanol to yield the product (**117**) as a cream solid (3.22 g, 72%), m.p. 50-52 °C (lit.,¹¹⁵ 54 °C); (Found: C, 81.13; H, 6.28; N, 12.50; Calc. for C₁₅H₁₄N₂: C, 81.5; H, 6.35; N, 12.60%); ν_{\max} (nujol)/cm⁻¹ 2200 (CN), 725 and 750 (aromatics); δ_{H} (200 MHz, C²HCl₃) 4.1 (4H, s., CH₂ x2), 7.29-7.42 (10H, m., aromatics); δ_{C} (75.42 MHz, C²HCl₃) 54.14 (CH₂ x2), 118.38 (CN), 129.19, 129.32 and 129.47 (aromatics), 134.38 (quat. aromatic); *m/z* (EI) 222 (*M*⁺, 19%), 131 (14, [*M*-C₆H₅CH₂]⁺), 91 (100, [C₆H₅CH₂]⁺), 77 (8, [C₆H₅]⁺), 65 (20, [C₅H₅]⁺)

***N*-Benzyl-*N*-(2-phenylethyl)cyanamide (118)**



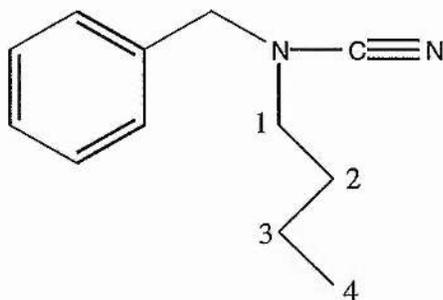
This compound was prepared in an identical manner to *N,N*-dibenzylcyanamide (**117**) using *N*-benzyl-2-phenylethylamine (4.22 g, 4.19 cm³, 20 mmol) to give the product (**118**) as a solid (3.70 g, 70%); m.p. 43-44 °C; (Found: C, 81.43 ; H, 6.90; N, 11.68: Calc. for C₁₆H₁₆N₂: C, 81.32; H, 6.82; N, 11.85%); ν_{\max} (nujol)/cm⁻¹ 2200 (CN), 765 and 705 (aromatic); δ_{H} (300 MHz, C²HCl₃) 2.94 (2H, t., *J* 7.5, PhCH₂CH₂), 3.17 (2H, t., *J* 7.4, CH₂CH₂N), 4.13 (2H, s., PhCH₂N) 7.17-7.35 (10H, m., aromatics); δ_{C} (50.31 MHz, C²HCl₃) 34.54 (PhCH₂CH₂), 52.15 and 56.67 (CH₂CH₂N and PhCH₂N), 118.3 (CN), 127.36, 128.89, 129.07, 129.22, 129.31 and 129.41 (aromatics), 135.09 and 138.04 (quat. aromatics); *m/z* (EI) 236 (*M*⁺, 15%), 145 (9, [*M*-C₆H₅CH₂]⁺), 91 (100, [C₆H₅CH₂]⁺), 77 (10, [C₆H₅]⁺), 65 (22, [C₅H₅]⁺)

N-Benzyl-*N*-methylcyanamide (119)



This compound was prepared in an identical manner to *N,N*-dibenzylcyanamide (**117**) using *N*-benzylmethylamine (2.42 g, 2.58 cm³, 20 mmol) to give the product (**119**) as a pale yellow oil (2.44 g, 84%); ν_{\max} /cm⁻¹ 2160 (CN), 720 and 690 (aromatic); δ_{H} (300 MHz, C²HCl₃) 2.76 (3H, s., CH₃), 4.15 (2H, s., PhCH₂), 7.33-7.39 (5H, m., aromatics); δ_{C} (50.31 MHz, C²HCl₃) 37.60 (CH₃), 56.92 (CH₂), 118.78 (CN), 128.34, 128.55 and 128.85 (aromatics), 134.29 (quat. aromatic); *m/z* (EI) 146 (*M*⁺, 59%), 91 (100, [C₆H₅CH₂]⁺), 77 (7, [C₆H₅]⁺), 65 (27, [C₅H₅]⁺), 51 (12, [C₄H₃]⁺)

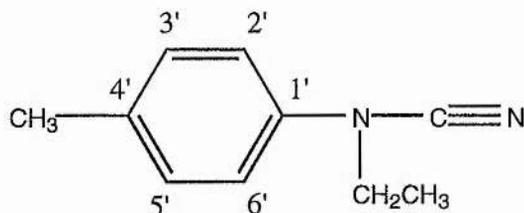
N-Benzyl-*N*-butylcyanamide (120)



This compound was prepared in an identical manner to *N,N*-dibenzylcyanamide (**117**) using *N*-butylbenzylamine (1.63 g, 10 mmol) giving the product (**120**) as an oil (1.40 g, 74%); ν_{\max} /cm⁻¹ 2200 (CN); δ_{H} (300 MHz, C²HCl₃) 0.87 (3H, t., *J* 7.5, CH₃), 1.30-1.38 (2H, sextet, *J* 7.0., CH₂-3), 1.58-1.63 (2H, quintet, *J* 7.0., CH₂-2), 2.9 (2H, t., *J*

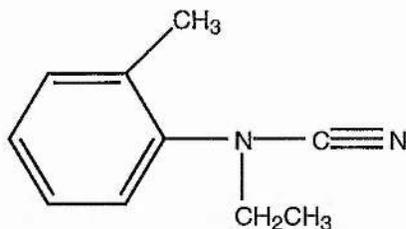
7.4, CH_2 -1), 4.16 (2H, s., ArCH_2), 7.30-7.37 (5H, m., aromatics); δ_{C} (75.42 MHz, C^2HCl_3) 13.32 (C-4), 19.39 (C-3), 29.19 (C-2), 49.83 (C-1), 55.6 (ArCH_2), 117.97 (CN), 128.29, 128.42 and 128.8 (aromatics), 134.84 (quat. aromatic)

***N*-Ethyl-*N*-(*p*-tolyl)cyanamide (121)**



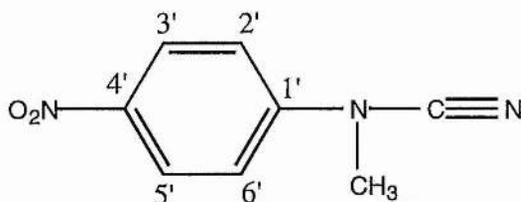
This compound was prepared in an identical manner to *N,N*-dibenzylcyanamide (**117**) using *N*-ethyl-*p*-toluidine (1.35 g, 10 mmol) giving the product (**121**) as an oil (1.29 g, 81%); $\nu_{\text{max}}/\text{cm}^{-1}$ 2225 (CN), 800, 775 and 650 (aromatics); δ_{H} (300 MHz, C^2HCl_3) 1.41 (3H, t., J 7.2, CH_2CH_3), 2.3 (3H, s., CH_3Ar), 3.58 (2H, q., J 7.2, CH_2CH_3), 6.97 (2H, d., J 8.7, H -2',6'), 7.13 (2H, d., J 8.7, H -3',5'); δ_{C} (75.42 MHz, C^2HCl_3) 12.52 (CH_2CH_3), 20.35 (CH_3Ar), 44.17 (CH_3CH_2), 113.61 (CN), 115.94 (C-2',6'), 130.12 (C-3',5'), 133.18 (C-4') 137.47 (C-1'); m/z (EI) 160 (M^+ , 72%), 145 (20, $[\text{M}-\text{CH}_3]^+$), 132 (82, $[\text{M}-\text{C}_2\text{H}_4]^+$), 91 (100, $[\text{CH}_3\text{C}_6\text{H}_4]^+$), 77 (29, $[\text{C}_6\text{H}_5]^+$), 65 (19, $[\text{C}_5\text{H}_5]^+$), 51 (11, $[\text{C}_4\text{H}_3]^+$)

***N*-Ethyl-*N*-(*o*-tolyl)cyanamide (122)**



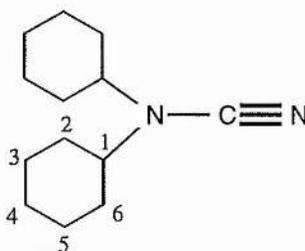
This compound was prepared in an identical manner to *N,N*-dibenzylcyanamide (**117**) using *N*-ethyl-*o*-toluidine (1.35 g, 10 mmol) giving the product (**122**) as an oil (1.11 g, 69 %); $\nu_{\max}/\text{cm}^{-1}$ 2200 (CN), 750 and 650 (aromatics); δ_{H} (300 MHz, C^2HCl_3) 1.35 (3H, t., J 7.2, CH_2CH_3), 2.19 (3H, s., CH_3Ar), 3.21-3.28 (2H, q., J 7.2, CH_2CH_3), 6.66-6.73 (4H, m., aromatics); δ_{C} (75.42 MHz, C^2HCl_3) 12.99 (CH_3CH_2), 17.63 (CH_3Ar), 48.64 (CH_3CH_2), 115.57 (CN), 124.79, 127.23, 127.69 and 131.75 (aromatics), 134.03 ($\text{C}-2'$), 139.18 ($\text{C}-1'$); m/z (EI) 160 (M^+ , 100%), 145 (20, $[\text{M}-\text{CH}_3]^+$), 132 (89, $[\text{M}-\text{C}_2\text{H}_4]^+$), 91 (33, $[\text{CH}_3\text{C}_6\text{H}_4]^+$), 77 (26, $[\text{C}_6\text{H}_5]^+$), 65 (22, $[\text{C}_5\text{H}_5]^+$), 51 (15, $[\text{C}_4\text{H}_3]^+$)

***N*-Methyl-*N*-(*p*-nitrophenyl)cyanamide (123)**



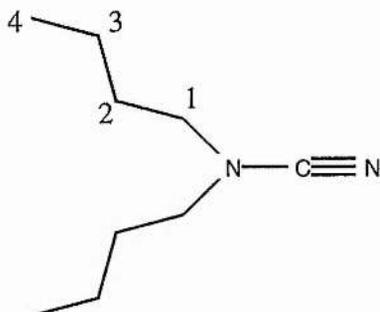
This compound was prepared in an identical manner to *N,N*-benzylcyanamide (**117**) using *N*-benzyl-4-nitroaniline (15.21 g, 100 mmol) giving the product (**123**) as a yellow solid (12.4 g, 70%), m.p. 130-132 °C; δ_{H} (300 MHz, C^2HCl_3) 2.95 (3H, s., CH_3), 6.52 (2H, d., J 9.3, H -2',6'), 8.09 (2H, d., J 9, H -3',5'); δ_{C} (75.42 MHz, C^2HCl_3) 30.69 (CH_3), 118.24 (C -2',6'), 126.18 (CN), 126.90 (C -3',5'), 138.43 (C -1'), 154.66 (C -4'); m/z (EI) 177 (M^+ , 28%), 149 (11, $[M-\text{CN}]^+$), 77 (54, $[\text{C}_6\text{H}_5]^+$)

***N,N*-Dicyclohexylcyanamide (124)**



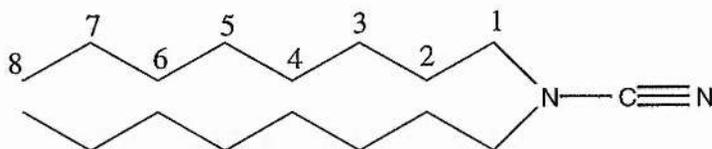
This compound was prepared in an identical manner to *N,N*-dibenzylcyanamide (**117**) using *N,N*-dicyclohexylamine (3.63 g, 20 mmol) giving the product (**124**) as a solid (2.85 g, 69%) m.p. 44-46 °C; ν_{max} (nujol)/ cm^{-1} 2200 (CN); δ_{H} (300 MHz, $\text{C}^2\text{H}_3\text{O}^2\text{H}$) 1.10-1.95 (10H, m., CH_2 -2,3,4,5,6), 2.88 (2H, m., CH -1); δ_{C} (75.42 MHz, $\text{C}^2\text{H}_3\text{O}^2\text{H}$) 26.19 and 26.27 (C -3,4,5), 33.06 (C -2,6), 59.37 (C -1), 116.88 (CN); m/z (EI) 206 (M^+ , 71%), 124 ($[M-\text{C}_6\text{H}_{10}]^+$, 81), 83 ($[\text{C}_6\text{H}_{11}]^+$, 100)

N,N-Dibutylcyanamide (125)



This compound was prepared in an identical manner to *N,N*-dibenzylcyanamide (117) using *N,N*-dibutylamine (2.59 g, 3.38 cm³, 20 mmol) giving the product (125) as a pale yellow oil (2.62 g, 85%); $\nu_{\max}/\text{cm}^{-1}$ 2225 (CN); δ_{H} (200 MHz, C²HCl₃) 0.93 (3H, t., *J* 7.4, CH₃), 1.32-1.40 (2H, m., CH₂-3), 1.58 (2H, quintet, *J* 7.2, CH₂-2), 2.97 (2H, t., *J* 7.2 Hz, CH₂-1); δ_{C} (50.31 MHz, C²HCl₃) 14.17 (C-4), 20.18 (C-3), 30.17 (C-2), 51.68 (C-1), 118.48 (CN); *m/z* (EI) 154 (M⁺, 34%)

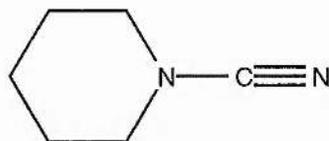
N,N-Dioctylcyanamide (126)



This compound was prepared in an identical manner to *N,N*-dibenzylcyanamide (117) using *N,N*-dioctylamine (4.83 g, 6 cm³, 20 mmol) giving the product (126) as an oil (3.56 g, 70 %); $\nu_{\max}/\text{cm}^{-1}$ 2225 (CN); δ_{H} (300 MHz, C²HCl₃) 0.85 (3H, t., *J* 7.2, CH₃) 1.3 (10H, s., CH₂-3,4,5,6,7), 1.6 (2H, m., CH₂CH₂NCN), 2.9 (2H, t., *J* 7.2, CH₂CH₂NCN); δ_{C} (74.43 MHz, C²HCl₃) 13.94 (C-1), 22.48, 26.37, 26.79, 27.55,

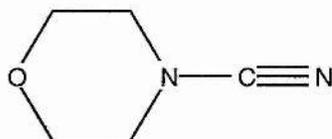
29.03 and 31.64 (CH₂), 51.45 (C-8) and 118.02 (CN); *m/z* (EI) 268 (M⁺, 15%)

N-Piperidylcyanamide (127)



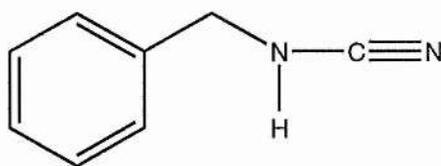
This compound was prepared in an identical manner to *N,N*-dibenzylcyanamide (**117**) using piperidine (8.52 g, 9.89 cm³, 100 mmol) to give the product (**127**) as a yellow oil (9.40 g, 85%); $\nu_{\max}/\text{cm}^{-1}$ 2225 (CN); δ_{H} (300 MHz, C²HCl₃) 1.53-1.61 (6H, m., CH₂-3,4,5) and 3.14-3.17 (4H, m., CH₂-2,6); δ_{C} (50.31 MHz, C²HCl₃) 22.87 (C-4), 24.42 (C-3,5), 50.06 (C-2,6) and 118.66 (CN); *m/z* (EI) 110 (M⁺, 83%), 82 (12, [M-CN]⁺), 69 (55, [C₅H₉]⁺), 55 (60, [C₄H₇]⁺)

N-Morpholinocyanamide (128)



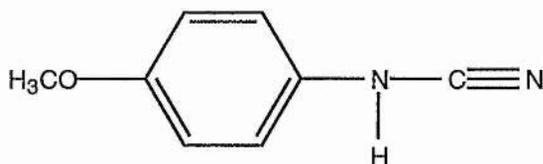
This product was prepared in an identical manner to *N,N*-dibenzylcyanamide (**117**) using morpholine (1.74 g, 20 mmol) to give the product (**128**) as a yellow oil (1.49 g, 66%); $\nu_{\max}/\text{cm}^{-1}$ 2200 (CN); δ_{H} (300 MHz, C²HCl₃) 3.19 (2H, t., *J* 4.8, CH₂N), 3.7 (2H, t., *J* 4.8, CH₂O); δ_{C} (75.42 MHz, C²HCl₃) 48.59 (CH₂N), 65.4 (CH₂O), 117.12 (CN); *m/z* (EI) 112 (M⁺, 67%)

***N*-Benzylcyanamide (132)**



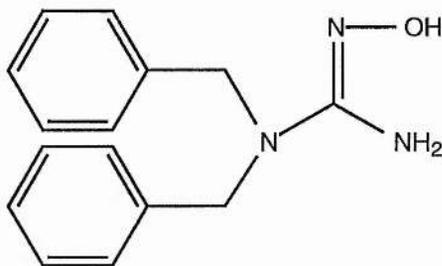
This compound was prepared in an identical manner to *N,N*-dibenzylcyanamide (**117**) using benzylamine (2.64 g, 20 mmol) to give the product (**132**) as a yellow oil (1.65 g, 62%); ν_{\max} (nujol)/ cm^{-1} 2225 (CN), 950, 850 and 800 (aromatics); δ_{H} (200MHz, C^2HCl_3) 4.1 (2H, s., CH_2), 4.7 (1H, br. s., NH), 7.3 (5H, s., aromatics); δ_{C} (50.31 MHz, C^2HCl_3) 50.30 (CH_2), 117.17 (CN), 128.32, 128.83 and 129.38 (aromatics), 136.90 (quat. aromatic)

***N*-(*p*-Methoxyphenyl)cyanamide (133)**



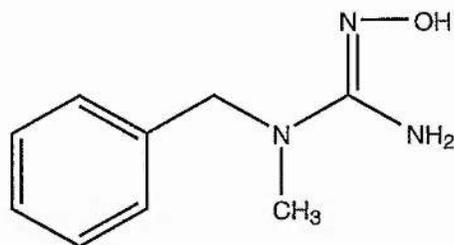
This compound was prepared in an identical manner to *N,N*-dibenzylcyanamide (**117**) using *p*-anisidine (2.74 g, 22 mmol) to give the product (**133**) as a solid (2.27 g, 77%), m.p. 60-62 °C; ν_{\max} (nujol)/ cm^{-1} 2220 (CN), 840 and 790 (aromatics); δ_{H} (200MHz, C^2HCl_3) 3.8 (3H, s., CH_3O), 6.05 (1H, br. s., NH), 6.8-7.0 (4H, m., aromatics);

1,1-Dibenzyl-2-hydroxyguanidine (70)



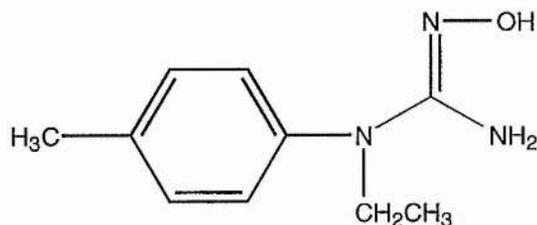
N,N-Dibenzylcyanamide (**117**) (0.44 g, 2 mmol) and hydroxylamine hydrochloride (0.21 g, 3 mmol) were stirred in dry DMF (10 cm³). Sodium carbonate (0.59 g, 5.6 mmol) was then added to this suspension slowly over a period of 10 minutes. The resultant suspension was heated at 60-80 °C for 1.25 hrs and then filtered. The inorganic salts were washed with a small quantity of warm DMF (2 x 5 cm³) and the combined washings and filtrate concentrated under reduced pressure to yield a yellow oil. The yellow oil was added dropwise to ice cold water (10 cm³) and a solid was obtained. This solid was filtered and recrystallised from ethanol to give the product (**70**) as a white solid (0.33 g, 65%), m.p. 119-120 °C (lit.,⁸⁷ 120-121 °C); (Found: C, 70.30; H, 6.59; N, 16.27; Calc. for C₁₅H₁₇N₃O: C, 70.57; H, 6.71; N, 16.46%); ν_{\max} (nujol)/cm⁻¹ 3475 and 3425 (NH₂), 3250 (OH), 950, 900 and 750 (aromatics); δ_{H} (300 MHz, C²HCl₃) 4.35 (4H, s., CH₂ x2), 4.4 (2H, s., NH₂), 7.2-7.35 (10H, m., aromatics); δ_{C} (75.44 MHz, C²HCl₃) 50.79 (CH₂ x2), 127.31, 127.47 and 128.70 (aromatics), 138.04 (quat. aromatic), 157.24 (C=NOH)); *m/z* (CI) 256 ([*M*+H]⁺, 100%)

1-Benzyl-1-methyl-2-hydroxyguanidine (137)



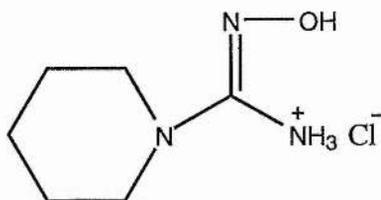
This compound was prepared in an identical manner to 1,1-dibenzyl-2-hydroxyguanidine (70) using *N*-benzyl-*N*-methylcyanamide (119) (0.29 g, 2 mmol) to give the product (137), after recrystallisation from ethanol, as white crystals (0.17 g, 48%), m.p. 88 °C (lit.,⁹¹ 89-90 °C); (Found: C, 60.48; H, 7.42; N, 23.50; Calc. for C₉H₁₃N₃O: C, 60.32; H, 7.42; N, 23.50%); ν_{\max} (nujol)/cm⁻¹ 3450 (NH₂), 2800 (OH), 990 and 880 (aromatics); δ_{H} (200 MHz, C²HCl₃) 2.74 (3H, s., CH₃), 4.30 (2H, s., CH₂Ph), 7.24-7.33 (5H, m., aromatics); δ_{C} (50.31 MHz, C²HCl₃) 36.17 (CH₃), 54.62 (CH₂), 127.28, 127.37 and 128.76 (aromatics), 138.43 (quat. aromatic), 157.52 (C=NOH)); *m/z* (CI) 180 ([M+H]⁺, 100%)

1-Ethyl-1-(*p*-toluidine)-2-hydroxyguanidine (138)



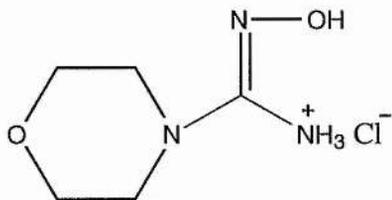
This compound was prepared in an identical manner to 1,1-dibenzyl-2-hydroxyguanidine (**70**) using *N*-ethyl-*N*-(*p*-toluidine)cyanamide (**121**) (3.2 g, 20 mmol) to give the product as a solid (2.36 g, 61%), m.p. 144-146 °C; (Found: C, 62.03; H, 7.98; N, 21.98; Calc. for C₁₀H₁₅N₃O: C, 62.15; H, 7.82; N, 21.74%); ν_{\max} (nujol)/cm⁻¹ 3475 and 3375 (NH₂), 3250 (OH), 900, 825 and 800 (aromatics); δ_{H} (300 MHz, C²HCl₃) 1.10 (3H, t., *J* 7.2, CH₃CH₂), 2.34 (3H, s., CH₃Ar), 3.60 (2H, q., *J* 7.2, CH₃CH₂), 7.10 (2H, d., *J* 8.4, *H*-2',6'), 7.17 (2H, d., *J* 8.4 Hz, *H*-3',5'); δ_{C} (75.42 MHz, C²HCl₃) 12.83 (CH₃CH₂), 20.81 (CH₃Ar), 44.85 (CH₂), 127.06 (*C*-2',6'), 130.14 (*C*-3',5'), 135.79 (*C*-4'), 140.58 (*C*-1'), 154.73 (*C*=NOH); *m/z* (CI) 194 ([*M*+H]⁺, 100%)

1-Piperidyl-2-hydroxyguanidine hydrochloride (**139**)



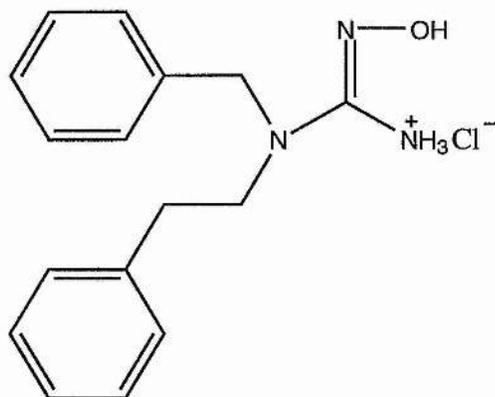
N-Piperidylcyanamide (**127**) (2.20g, 20 mmol) and hydroxylamine hydrochloride (1.38 g, 20 mmol) were stirred in dry methanol (40 cm³) and catalytic potassium hydroxide (0.2 g) added. The reaction was heated under reflux for 13 hours, cooled and poured onto diethyl ether (600 cm³). The white precipitate was collected by filtration and dried to give the product (**139**) as a white solid (2.03 g, 57%). A small amount was recrystallised from methanol/diethyl ether to give a white solid, m.p 200-202 °C (decomp.); ν_{\max} (nujol)/cm⁻¹ 3450 and 3350 (NH₂), 3100 (OH); δ_{H} (300 MHz, ²H₂O) 1.5 (6H, s., CH₂-3,4,5), 3.35 (4H, 2., CH₂-2,6); δ_{C} (75.42 MHz, ²H₂O) 19.69 (CH₂-4), 21.27 (CH₂-3,5), 43.37 (CH₂-2,6), 155.17 (C=NOH); m/z (CI) 145 ([*M*-Cl]⁺, 20%)

1-Morpholino-2-hydroxyguanidine hydrochloride (140)



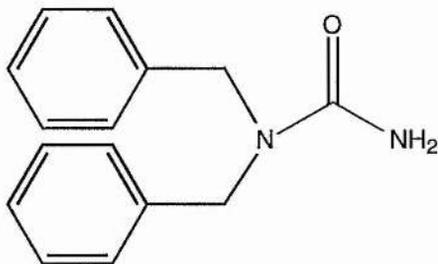
This compound was prepared in an identical manner to 1-piperidyl-2-hydroxyguanidine hydrochloride (**139**) using *N*-morpholinocyanamide (**128**) (1.12 g, 10 mmol) to give the product (**140**) as a white solid (1.17 g, 64%). A small amount was recrystallised from methanol/diethyl ether to give the pure product as a white solid, m.p. 214-216 °C (decomp.); (Found: C, 32.83; H, 6.58; N, 22.81; Calc. for C₅H₁₂N₃O₂Cl: C, 33.06; H, 6.66; N, 23.13%); ν_{\max} (nujol)/cm⁻¹ 3475 and 3400 (NH₂), 3200 (OH); δ_{H} (200 MHz, ²H₂O) 3.37 (2H, t., *J* 5.0, CH₂N), 3.73 (2H, t., *J* 5.0, CH₂O); δ_{C} (50.31 MHz, ²H₂O) 41.82 (CH₂N), 62.12 (CH₂O), 155.92 (C=NOH); *m/z* (CI) 147[M-Cl]⁺, 60%

1-Benzyl-1-(2-phenylethyl)-2-hydroxyguanidine hydrochloride (136)



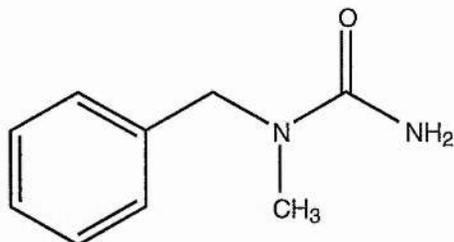
This compound was prepared in an identical manner to 1-piperidyl-2-hydroxyguanidine hydrochloride (**139**) using *N*-benzyl-*N*-(2-phenylethyl)cyanamide (**118**) (1.98 g, 10 mmol) to give the product (**136**), after recrystallisation from methanol/diethyl ether as a white solid (8.1 g, 30%), m.p. 270-271 (Decomp.); ν_{\max} (nujol)/ cm^{-1} 3400 and 3300 (NH_2), 3100 (OH), 970, 830 (aromatics); δ_{H} (300 MHz, $\text{C}^2\text{H}_3\text{O}^2\text{H}$) 2.90 (2H, t., J 7.4, PhCH_2CH_2), 3.56 (2H, t., J 7.4, $\text{CH}_2\text{CH}_2\text{N}$), 4.39 (2H, s., PhCH_2N), 7.18-7.40 (10H, m., aromatics); δ_{C} (75.43 MHz, $\text{C}^2\text{H}_3\text{O}^2\text{H}$) 34.01 (PhCH_2CH_2), 50.93 and 52.90 ($\text{CH}_2\text{CH}_2\text{N}$ and PhCH_2N), 128.06, 128.44, 129.36, 129.94, 130.11 and 130.18 (aromatics), 136.09 and 139.16 (quat. aromatics), 160.42 ($\text{C}=\text{NOH}$); m/z (CI) 246 ($[\text{M}-\text{Cl}]^+$, 45%)

***N,N*-Dibenzylurea (154)**



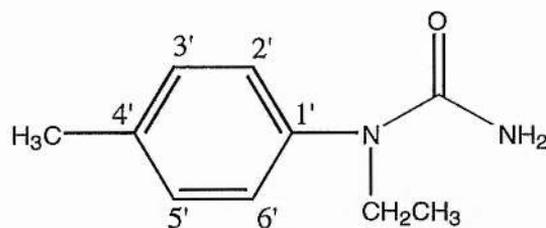
N,N-Dibenzylcyanamide (**117**) (2.22g, 10 mmol) was dissolved in ethanol (10 cm³) and sulfuric acid (2M, 10 cm³) was added. The solution was stirred overnight at room temperature and then concentrated under reduced pressure. The residue was partitioned between water (10 cm³) and ethyl acetate (10 cm³) and the aqueous layer was washed with ethyl acetate (2 x 10 cm³). The combined organic extracts were then washed with brine (20 cm³), dried (MgSO₄), filtered and concentrated under reduced pressure to yield the crude product as an off-white solid (1.56 g, 65%). A small portion of this compound was recrystallised from ethanol to give the product (**154**) as a white crystalline solid, m.p. 122-124 °C (lit.,¹⁵⁰ 125 °C); ν_{\max} (nujol)/cm⁻¹ 3410 (NH₂), 1630 (C=O), 900, 730 (aromatics); δ_{H} (200 MHz, C²HCl₃) 4.5 (4H, s., CH₂), 7.20-7.35 (10H, m., aromatics); δ_{C} (50.31 MHz, C²HCl₃) 50.65 (CH₂), 127.56, 127.78 and 128.14 (aromatics), 137.22 (quat. aromatics), 160.35 (C=O); *m/z* (EI) 222 (*M*⁺, 14%), 131 (5, [*M*-CH₂C₆H₅]⁺), 91 (100, [C₆H₅CH₂]⁺)

***N*-Benzyl-*N*-methylurea (155)**



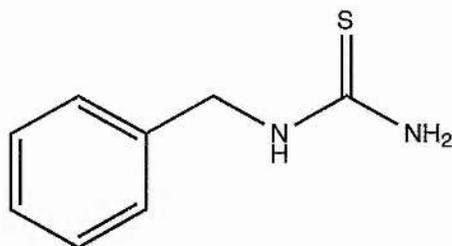
This compound was prepared in an identical manner to *N,N*-dibenzylurea (**154**) using *N*-benzyl-*N*-methylcyanamide (**119**) (1.46 g, 10 mmol) to give the crude product as a solid (0.56 g, 34%). A small portion was recrystallised from ethanol to afford the product (**155**) as a white solid, m.p. 132-134 °C (lit.,⁸⁷ 130-132 °C); (Found: C, 65.60; H, 7.28; N, 16.81; Calc. for C₉H₁₂N₂O: C, 65.83; H, 7.37; N, 17.06 %); ν_{\max} (nujol)/cm⁻¹ 3400 (NH₂), 1600 (C=O), 950, 730 (aromatics); δ_{H} (200 MHz, C²HCl₃) 2.92 (3H, s., CH₃), 4.5 (2H, s., ArCH₂), 7.23-7.73 (5H, m., aromatics); δ_{C} (50.31 MHz, C²HCl₃) 35.28 (CH₃), 52.84 (CH₂), 127.71, 127.98 and 129.27 (aromatics), 137.71 (quat. aromatic), 159.83 (C=O); *m/z* (EI) 164 (M⁺, 100%), 147 (9, [M-OH]⁺), 120 (49, [M-C(=O)NH₂]⁺), 106 (55, [C₆H₅CH₂NH]⁺), 91 (87, [C₆H₅CH₂]⁺), 77 (17, [C₆H₅]⁺), 65 (22, [C₅H₅]⁺)

***N*-Ethyl-*N*-(*p*-tolyl)urea (153)**



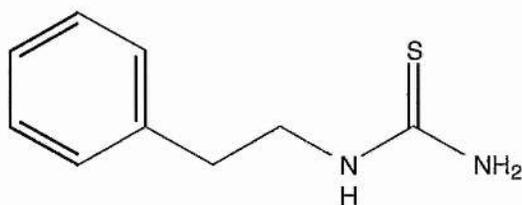
This compound was prepared in an identical manner to *N,N*-dibenzylurea (**154**) using *N*-ethyl-*N*-(*p*-tolyl)cyanamide (**121**) (1.6 g, 10 mmol) to give the product (**153**) as an oil (0.7 g, 39%), $\nu_{\max}/\text{cm}^{-1}$ 3350 (NH₂), 1650 (C=O), 890, 700 (aromatics); δ_{H} (300 MHz, C²HCl₃) 1.4 (3H, t., *J* 7.5, CH₃CH₂), 2.3 (3H, s., CH₃Ar), 3.6 (2H, q., *J* 7.5, CH₃CH₂), 6.9 (2H, d., *J* 8, *H*-2',6'), 7.15 (2H, d., *J* 8.2, *H*-3',5'); δ_{C} (50.31 MHz, C²HCl₃) 12.49 (CH₃CH₂), 20.33 (CH₃Ar), 44.14 (CH₃CH₂), 115.92 (C-2'6'), 130.12 (C-3',5'), 133.16 and 137.45 (quat. aromatics); *m/z* (EI) 178 (*M*⁺, 7%), 160 (100, [*M*-OH₂]⁺), 145 (39, [(*M*-OH₂)-CH₃]⁺), 132 (55, [(*M*-OH₂)-CH₂CH₃]⁺), 91 (67, [CH₂C₆H₅]⁺), 77 (24, [C₆H₅]⁺), 65 (22, [C₅H₅]⁺), 51 (13, [C₄H₃]⁺)

N-Benzylthiourea (**181**)



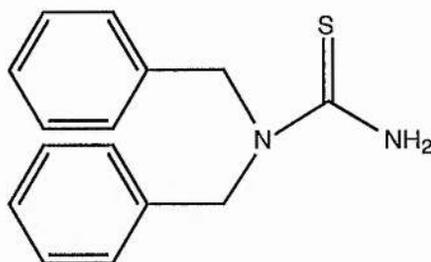
Benzylamine (2.0 g, 18.6 mmol) was dissolved in dichloromethane (40 cm³) and added to a vigorously stirred suspension of thiophosgene (3.2 cm³, 42 mmol) in water (60 cm³). The reaction was stirred for 2 hours at room temperature. The resulting suspension was separated and the organic layer washed with water (50 cm³), dried (MgSO₄) and concentrated under reduced pressure to give an orange oil. The orange oil was then dissolved in methanol (40 cm³), cooled to 0 °C and aqueous ammonia (1.6 cm³ of a 32% solution) slowly added. The reaction mixture was stirred at 0 °C for a further 3 hours and then concentrated under reduced pressure to give a pale orange solid. This was recrystallised from ethanol to give the product (**181**) as a white solid (0.59 g, 20%), m.p. 159-161 °C (lit.,¹²⁶ 161-162 °C); (Found: C, 57.65; H, 5.88; N, 16.52. Calc. for C₈H₁₀N₂S: C, 57.80; H, 6.06; N, 16.85%); ν_{\max} (nujol)/cm⁻¹ 3390 (NH), 3200 (NH₂), 1500 (C=S), 720, 700 (aromatics); δ_{H} (200 MHz, C²H₃O²H) 4.7 (2H, s., PhCH₂), 7.21-7.34 (5H, m., aromatics); δ_{C} (50.3 MHz, d⁶-DMSO) 47.79 (CH₂), 127.23, 127.63 and 128.59 (aromatics), 139.49 (quat. aromatics), 183.71 (C=S); *m/z* (EI) 166 (*M*⁺, 100%), 149 (5, [PhCH₂NCS]⁺), 106 (47, [PhCH₂NH]⁺), 91 (85, [C₆H₅CH₂]⁺)

***N*-(2-phenylethyl)thiourea (183)**



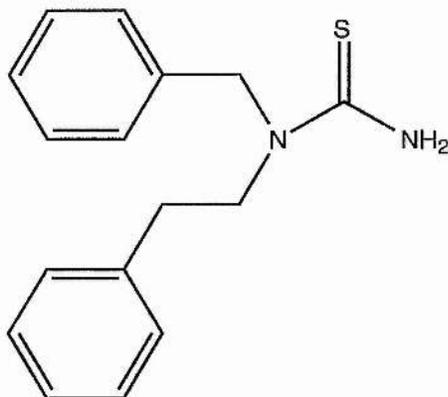
This compound was prepared in an identical manner to *N,N*-dibenzylthiourea (**181**) using 2-phenethylamine (24.2 g, 200 mmol) to give the product (**183**) as a white solid (18.5 g, 51%), m.p. 126-128 °C; (Found: C, 60.31; H, 6.72; N, 15.36. Calc. for $C_9H_{12}N_2S$: C, 59.97; H, 6.71; N, 15.54%); ν_{\max} (nujol)/ cm^{-1} 3400 and 3350 (NH_2), 3100 (NH), 1340 (C=S) 730 (aromatic); δ_H (200 MHz, $C^2H_3O^2H$), 2.87 (2H, t., J 7.4, $PhCH_2$), 3.71 (2H, t., J 7.4, CH_2NH), 7.14-7.34 (5H, m., aromatics), 7.6 (1H, s., NH); δ_C (75.42 MHz, $C^2H_3O^2H$) 35.03 ($PhCH_2$), 45.57 (CH_2NH), 126.50, 128.71 and 128.98 (aromatics), 139.61 (quat. aromatics), 183.51 (C=S); m/z (EI) 180 (M^+ , 74%), 120 (14, [$M-H_2NCS$] $^+$), 104 (100, [$M-C_6H_5$] $^+$), 91 (32, [$C_6H_5CH_2$] $^+$)

***N,N*-Dibenzylthiourea (184)**



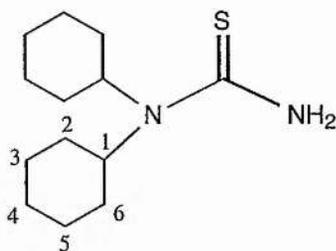
Chlorobenzene (45 cm³) was saturated with dry HCl gas. Dibenzylamine (12 g, 11.7 cm³, 60 mmol) was then added to the resultant cloudy solution, followed by ammonium thiocyanate (4.62 g, 60 mmol). The resultant white suspension was heated under reflux for 8.5 hours then cooled. The white solid was filtered off, washed with water and dried to give the product (**184**) as a white solid (9.07 g, 59%), m.p. 134-135 °C (lit.,¹²⁷ 134-136 °C); ν_{\max} (nujol)/ cm⁻¹ 3250 (NH₂), 1510 (C=S) 760, 700 and 640 (aromatics); δ_{H} (200 MHz, C²HCl₃) 4.9 (4H, s., PhCH₂ x2), 5.7 (2H, s., NH₂), 7.3 (10H, m., aromatics); δ_{C} (75.42 MHz, C²HCl₃) 50.41 (CH₂), 127.36, 127.56 and 128.83 (aromatics), 137.14 (quat. aromatic), 182.84 (C=S); m/z (EI) 256 (*M*⁺, 23%), 197 (28, [*M*-HNCS]⁺), 91 (100, [C₆H₅CH₂]⁺)

***N*-Benzyl-*N*-(2-phenylethyl)thiourea (185)**



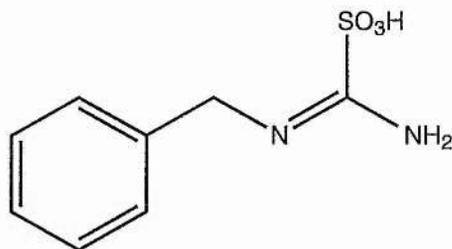
N-Benzyl-2-phenylethylamine (4.2 g, 4.2 cm³, 20 mmol) was added an aqueous solution of HCl (2M, 50 cm³). Ammonium thiocyanate (0.76 g, 20 mmol) was added and the suspension heated under reflux for 7 hours. The solid was filtered giving the product (**185**) as a pale solid (4.08 g, 76%), m.p. 260-262 °C ; ν_{\max} (nujol)/cm⁻¹ 3200 (NH₂), 1510 (C=S), 710 and 640 (aromatics); δ_{H} (300 MHz, C²H₃O²H) 3.06 (2H, t., *J* 8.2, PhCH₂CH₂), 4.20 (2H, t., *J* 8.2, PhCH₂CH₂N), 4.24 (2H, s., PhCH₂N), 7.26-7.55 (10H, m., aromatics); δ_{C} (75.42 MHz, C²H₃O²H) 33.16 (PhCH₂CH₂), 49.69 and 52.33 (PhCH₂CH₂N and PhCH₂N), 128.34, 129.86, 130.06, 130.36, 130.77 and 131.18 (aromatics), 132.61 and 137.90 (quat. aromatic), 184.5 (C=S); *m/z* (EI) 270 (*M*⁺, 24%), 212 (9, [*M*-SCN]⁺), 120 (100, [PhCH₂CH₂NH]⁺), 105 (16, [PhCH₂CH₂]⁺), 91 (94, [PhCH₂]⁺)

***N,N*-Dicyclohexylthiourea (190)**



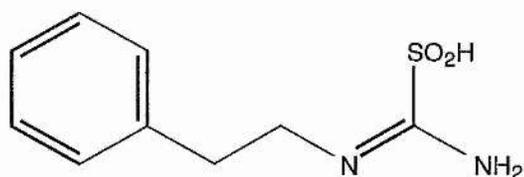
This compound was prepared in an identical manner to *N,N*-dibenzylthiourea (**184**) using dicyclohexylamine (3.63 g, 20 mmol). The reaction was heated under reflux for 6.5 hours. The product (**190**) was obtained as a white solid (3.71 g, 77%), m.p. 226-225 °C ; ν_{\max} (nujol)/ cm^{-1} 3300 (NH_2), 1490 ($\text{C}=\text{S}$); δ_{H} (300MHz, $\text{C}^2\text{H}_3\text{O}^2\text{H}$) 1.2-1.7 (6H, m., CH_2 -4,5,6), 1.80-1.95 (2H, m., CH_2 -3), 2.05-2.10 (2H, m., CH_2 -2), 3.1-3.25 (1H, m., CH -5); δ_{C} (75.42 MHz, $\text{C}^2\text{H}_3\text{O}^2\text{H}$) 25.43 (C -3,5), 26.08 (C -4), 30.45 (C -2,6), 54.45 (C -1), 182.1 ($\text{C}=\text{S}$); m/z (EI) 240 (M^+ , 40%)

N-Benzylaminoiminomethanesulfonic acid (**193**)



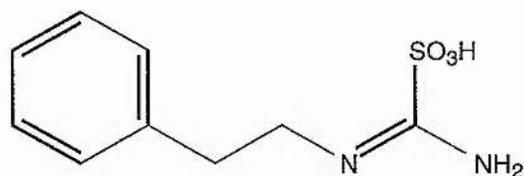
N-Benzylthiourea (**181**) (8.3 g, 50 mmol) and sodium molybdate dihydrate (181 mg, 0.75 mmol) were stirred in water (100 cm³). The resulting suspension was cooled to 0 °C and hydrogen peroxide (100 vol, 16 cm³) added at such a rate that the temperature did not exceed 0 °C. After addition was complete the ice bath was removed and the reaction allowed to warm. Once exotherm was complete the reaction was recooled to 0 °C and the resulting white precipitate was filtered off and dried under vacuum to yield the product (**193**) as a white solid (9.13 g, 85%), m.p. 163-165 °C (decomp.) (lit.,¹³¹ 168 °C (decomp.)); (Found: C, 45.26; H 4.85; N, 13.19; Calc. for C₈H₁₀N₂SO₃: C, 44.85; H, 4.70; N, 13.08%); ν_{\max} (nujol)/cm⁻¹ 3320 and 3300 (NH₂), 1240 (RSO₃⁻), 740 and 690 (aromatic); δ_{H} (200 MHz, d⁶-DMSO) 4.51 (2H, s., PhCH₂), 7.31-7.40 (5H, m., aromatics), 9.4 (2H, s., NH₂); δ_{C} (50.31 MHz, d⁶-DMSO) 51.10 (PhCH₂), 127.54, 127.72 and 128.82 (aromatics), 136.75 (quat. aromatic), 165.96 (C-SO₃H); *m/z* (ES⁻) 213 ([*M*-H]⁻, 100%)

***N*-Phenylethylaminoiminomethanesulfonic acid (194)**



This compound was prepared in an identical manner to *N*-benzylaminoiminomethanesulfonic acid (**193**) using *N*-phenylethylthiourea (**183**) (5.41 g, 30 mmol) to give the product (**194**) as a solid (2.18 g, 54%); m.p. 119-120 °C; ν_{\max} (nujol)/ cm^{-1} 3200 (NH_2), 1600 ($\text{C}=\text{N}$) 1050 (R-SO-OH) 810 and 680 (aromatics); δ_{H} (200 MHz, $\text{C}^2\text{H}_3\text{O}^2\text{H}$) 2.95 (2H, t., J 7.4, PhCH_2), 3.57 (2H, t., J 7.4, CH_2NH), 7.20-7.36 (5H, m., aromatics); δ_{C} (50.31 MHz, $\text{d}^6\text{-DMSO}$) 33.70 (PhCH_2), 43.27 (CH_2N), 126.77, 128.67 and 128.94, 129.09 (aromatics), 138.31 (quat. aromatic), 177.59 ($\text{C-SO}_2\text{H}$); m/z (ES^-) 211 ($[\text{M-H}]^-$, 100%)

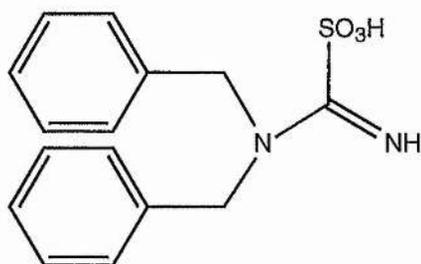
***N*-Phenethylaminoiminomethanesulfonic acid (195)**



This compound was prepared in an similar manner to *N*-benzylaminoiminomethanesulfonic acid (**193**) using *N*-phenethylthiourea (**183**) (30 mmol, 5.41 g) and a greater excess of hydrogen peroxide (10.2 cm^3) to give the product (**195**) as a solid (4.07 g, 59%), m.p. 135-137 °C; (Found: C, 47.69; H, 5.40; N,

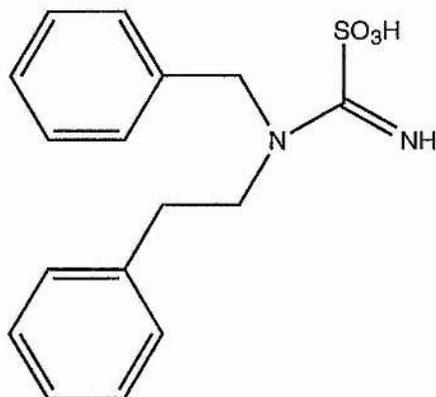
12.10. Calc. for $C_9H_{12}N_2SO_3$: C, 47.36; H, 5.30; N, 12.27%; ν_{\max} (nujol)/ cm^{-1} 3100 (NH₂) 1600 (C=N) 1250 (R-SO₃⁻); δ_H (200 MHz, C²H₃O²H) 2.95 (2H, t., *J* 7.3, PhCH₂), 3.58 (2H, t., *J* 7.4, CH₂NH), 7.19-7.36 (5H, m., aromatics); δ_C (50.31 MHz, C²H₃O²H) 34.02 (PhCH₂), 45.50 (CH₂NH), 127.04, 128.65 and 128.86 (aromatics), 138.00 (quat. aromatic), 185.03 (C-SO₃H); *m/z* (ES⁻) 227 ([*M*-H]⁻, 100%)

***N,N*-Dibenzylaminoiminomethanesulfonic acid (196)**



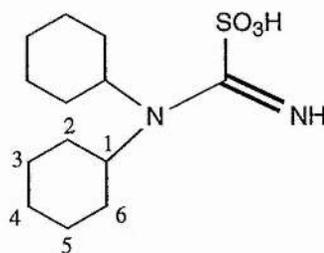
This was prepared in an identical manner to *N*-benzylaminoiminomethanesulfonic acid (**181**) using *N,N*-dibenzylthiourea (**184**) (2.56 g, 10 mmol) to give the product (**196**) as a yellow solid (1.68 g, 55%), m.p. 118-119 °C (decomp.); (Found: C, 58.68; H, 5.01; N, 9.01. Calc. for $C_{15}H_{16}N_2SO_3$: C, 59.19; H, 5.30; N, 9.20%); ν_{\max} (nujol)/ cm^{-1} 3340, 3250 and 3200 (NH+NH₂), 1240 (RSO₃⁻), 800 and 700 (aromatic); δ_H (300 MHz, C²H₃O²H) 4.6 (2H, s., PhCH₂), 5.2 (2H, s., PhCH₂), 7.37 (10H, s., aromatics); δ_C (75.42 MHz, C²H₃O²H) 52.34 (PhCH₂), 56.00 (PhCH₂), 127.97, 129.53, 129.82, 129.96, 130.09 and 130.35 (aromatics), 133.32 and 135.22 (quat. aromatics), 167.89 (C=SO₃H); *m/z* (ES⁻) 303 ([*M*-H]⁻, 100%)

***N*-Benzyl-*N*-(2-phenylethyl)aminoiminomethanesulfonic acid (197)**



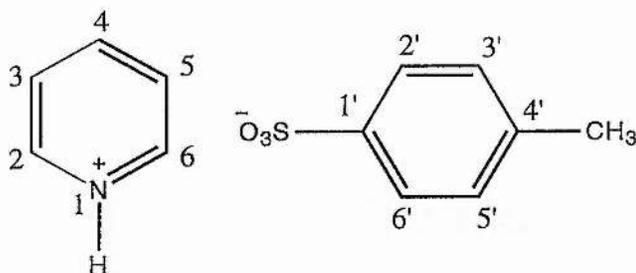
This compound was prepared in an identical manner to *N*-benzylaminoiminomethanesulfonic acid (**181**) using *N*-benzyl-*N*-(2-phenylethyl)thiourea (**185**) (2.70 g, 10 mmol) to yield the product (**197**) as a solid (1.81 g, 57%), m.p. 254 °C (decomp.); ν_{\max} (nujol)/ cm^{-1} 3250 (NH), 1600 (CN), 1250 (SO_3H), 880 and 770 (aromatics); δ_{H} (300 MHz, $\text{C}^2\text{H}_3\text{O}^2\text{H}$) 3.03 (2H, t., J 7.8, PhCH_2CH_2), 3.27 (2H, t., J 7.8, $\text{CH}_2\text{CH}_2\text{N}$), 4.25 (2H, s., PhCH_2N), 7.28-7.56 (10H, m., aromatics); δ_{C} (75.42 MHz, d^6 -DMSO) 31.99 (PhCH_2CH_2), 48.35 and 50.80 ($\text{CH}_2\text{CH}_2\text{N}$ and PhCH_2N), 127.58, 129.32, 129.41, 129.52, 129.81 and 130.63 (aromatics), 132.43 and 137.68 (quat aromatics), 166.90 ($\text{C-SO}_3\text{H}$); m/z (ES^-) 317 ($[\text{M-H}]^-$, 100%)

***N,N*-Dicyclohexylaminoiminomethanesulfonic acid (198)**



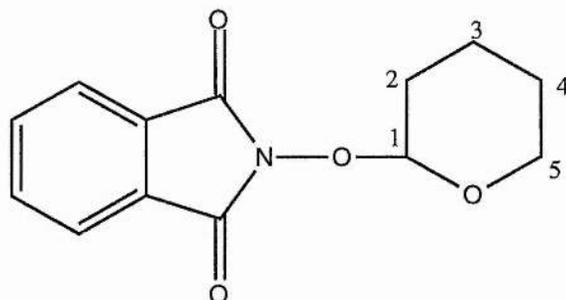
This compound was prepared in an identical manner to *N*-benzylaminoiminomethanesulfonic acid (**181**) using *N,N*-dicyclohexylthiourea (**190**) (2.40 g, 10 mmol). The product (**198**) was obtained as a white solid (1.35 g, 47%), m.p. >290 °C; ν_{\max} (nujol)/ cm^{-1} 3200 (NH), 1260 (SO_3H); δ_{H} (300MHz, $\text{C}^2\text{H}_3\text{O}^2\text{H}$) 1.2-1.7 (6H, m., CH_2 -3,4,5), 1.85 (2H, m., CH_2 -2), 2.05 (2H, m., CH_2 -6), 3.15 (1H, m., CH -5); δ_{C} (75.42 MHz, $\text{C}^2\text{H}_3\text{O}^2\text{H}$) 25.45 (C-3,5), 26.10 (C-4), 30.49 (C-2,5), 54.47 (C-1); m/z (ES^-) 287 ($[\text{M}-\text{H}]^-$, 100%)

Pyridinium *p*-toluenesulfonate (219)



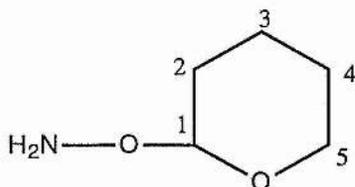
p-Toluenesulfonic acid monohydrate (22.8 g, 120 mmol) was added to pyridine (48.4 cm³, 300 mmol) with stirring. After the exotherm had ceased, the excess pyridine was removed under reduced pressure to yield a white solid in quantitative yield. The white solid was then recrystallised from acetone to give the product (**219**) as a white solid (22.3 g, 74%), m.p. 117-118 °C (lit.,¹³⁸ 120 °C); (Found: C, 57.44; H, 5.25; N, 5.78%. Calc. for C₁₂H₁₃NSO₃: C, 57.35; H, 5.27; N, 5.57%); ν_{\max} (nujol)/cm⁻¹ 3450 (NH), 1200 (SO₃H), 750 and 675 (aromatics); δ_{H} (300 MHz, C²HCl₃) 2.23 (3H, s., CH₃), 7.15 (2H, d., *J* 5.4, CH-3',5'), 7.78 (2H, d., *J* 5.4, CH-2',6'), 7.92 (2H, d.d., *J* 3.6 and 5.3, CH-3,5), 8.4 (1H, t., *J* 5.3, CH-4), 8.98 (2H, d., *J* 3.6, CH-2,6); δ_{C} (75.42 MHz, C²HCl₃) 21.33 (CH₃), 126.00 (C-2',6'), 127.51 and 129.05 (C-3,5 and C-3'5'), 140.39 (C-4'), 142.04 (C-2,6), 142.35 (C-1'), 146.32 (C-4)

***O*-Tetrahydropyranyl-*N*-hydroxyphthalimide (218)**



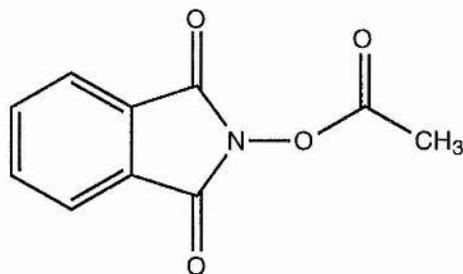
N-Hydroxyphthalimide (1.63 g, 10 mmol) was dissolved in dry dichloromethane (30 cm³). Dihydropyran (1.26 g, 15 mmol) and pyridinium *p*-toluenesulfonate (**219**) (0.25 g, 0.1 mmol) were then added. The reaction was stirred for 16 hours at room temperature under an atmosphere of nitrogen. The resultant solution was then diluted with diethyl ether (250 cm³) and washed with saturated brine solution (3 x 70 cm³). The organic layer was then dried, and concentrated under reduced pressure to yield a yellow solid (2.10 g, 97%). The yellow solid was recrystallised from ethyl acetate to give the product (**219**) as a white solid (0.85 g, 40%), m.p. 119-121 °C (lit.,¹³⁹ 123 °C), (Found: C, 63.72; H, 5.34; N, 5.66%; Calc. for C₁₃H₁₃NO₄: C, 63.55; H, 5.34; N, 5.66%); ν_{\max} (nujol)/cm⁻¹ 1700 (C=O) 1010, 920 (O-C-O), 810 and 690 (aromatics); δ_{H} (300 MHz, C²HCl₃) 1.69-2.13 (6H, m., CH₂-2,3,4), 3.64-3.70 (1H, m., CH-5), 4.47-4.52 (1H, m., CH-5), 5.35 (1H, m., CH-1), 7.73-7.83 (4H, m., aromatics); δ_{C} (75.42 MHz, C²HCl₃) 17.65, 24.9, 27.75 (C-2,3,4), 62.30 (C-5), 103.17 (C-1), 123.54, 134.50 (aromatics), 129.34 (quat. aromatic), 163.98 (C=O); *m/z* (CI) 248 ([M+H]⁺, 21%), 148 (100, [C₈H₂NO₂]⁺), 85 (100, [C₅H₉O]⁺)

O-Tetrahydropyranylamine (215)



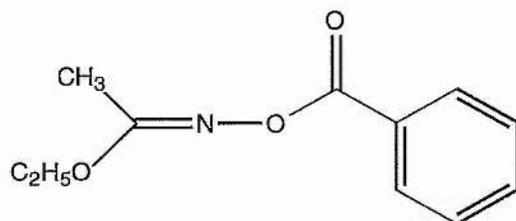
O-Tetrahydropyranyl-*N*-hydroxyphthalimide (**218**) (17.29 g, 70 mmol) was dissolved in ethanol (15 cm³) and hydrazine hydrate was added (3.68 g, 69 mmol). The reaction was heated under reflux for 4 hours and then cooled to room temperature. The by-product was removed by filtration and the filtrate concentrated under reduced pressure to yield a yellow residue. The yellow residue was then distilled under reduced pressure using the Kugelrohr apparatus to give the product (**215**) as a white solid (5.41 g, 69%), m.p. 35-36 °C; ν_{\max} (nujol)/cm⁻¹ 3200 (NH₂) 1020 and 950 (O-C-O); δ_{H} (300 MHz, C²HCl₃) 1.53-1.73 (6H, m, CH₂-2,3,4), 3.58 (1H, m., CH-5), 3.90 (1H, m., CH-5), 4.7 (1H, m., CH-1), 5.47 (2H, s., NH₂); δ_{C} (75.42 MHz, C²HCl₃) 19.72, 25.39, 28.96 (C-2,3,4), 62.66 (C-5), 102.73 (C-1); m/z (CI) 118 ([M+H]⁺, 100%), 85 (60, [C₅H₉O]⁺)

O-Acetyl-*N*-hydroxyphthalimide (223)



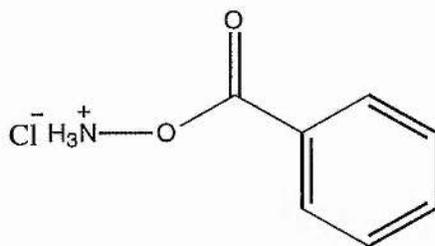
N-Hydroxyphthalimide (1.63 g, 10 mmol) was dissolved in dry THF (10 cm³). Acetic anhydride (1.4 cm³, 15 mmol), *N,N*-dimethyl- π -aminopyridine (DMAP) (0.12 g, 1 mmol) and triethylamine (2.09 cm³, 15 mmol) were added. The reaction was stirred for 18 hours at room temperature under an atmosphere of nitrogen. The reaction was then partitioned between diethyl ether (20 cm³) and 2M HCl (20 cm³), separated and the aqueous layer was further extracted with diethyl ether (2 x 20 cm³). The combined organic layers were then washed with saturated sodium hydrogen carbonate solution (20 cm³), dried (MgSO₄), filtered and concentrated under reduced pressure to give the product (**223**) as a white solid (1.21 g, 59%), m.p. 160-162 °C; ν_{\max} (nujol)/cm⁻¹ 1800, 1760 (C=O), 710, 580 (aromatics); δ_{H} (300 MHz, d⁶-DMSO) 1.27 (3H, s., CH₃), 7.28-7.91 (4H, m., aromatics); δ_{C} (50.3 MHz, d⁶-DMSO) 17.45 (CH₃), 124.27 and 135.79 (aromatics), 128.43 (quat. aromatic), 162.10 (CH₃CO) and 167.60 (C=O); *m/z* (EI) 205 (*M*⁺, 28%), 163 (56, [M-CH₂CO]⁺)

O-Benzoyl acetohydroxamic acid ethyl ester (229)



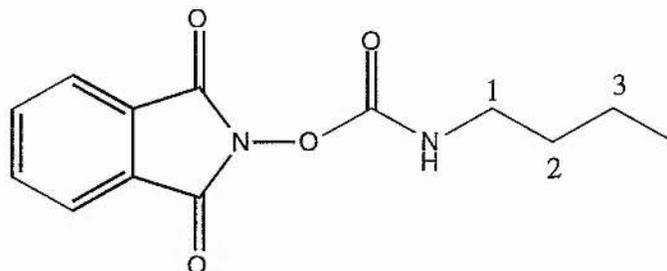
Acetohydroxamic acid ethyl ester (2.6 g, 25 mmol) was dissolved in dry diethyl ether (20 cm³) and cooled in an ice bath. Pyridine (1.95 g, 25 mmol) was added before a solution of freshly distilled benzoyl chloride (3.5 g, 25 mmol) in diethyl ether (10 cm³) was added dropwise. The reaction was warmed to room temperature and stirred for 3 hours. The pyridinium chloride was removed by filtration and the filtrate was dried (MgSO₄), filtered and concentrated under reduced pressure to yield a white solid. Recrystallisation from petroleum ether (40-60 °C) gave the product (**229**) as a white solid (3.03 g, 58%), m.p. 72-74 °C (lit.,¹⁴⁰ 77-79 °C); (Found: C, 63.77; H, 6.11; N, 6.71; Calc. for C₁₁H₁₃NO₃: C, 63.76; H, 6.32; N, 6.76%); ν_{\max} (nujol)/cm⁻¹ 1750 (C=O), 975 and 725 (aromatics); δ_{H} (300 MHz, C²HCl₃) 1.35 (3H, m., CH₃CH₂O), 2.14 (3H, s., CH₃), 4.27 (2H, q., *J* 7.2, CH₃CH₂O), 7.4 (2H, m., CH-3,5), 7.6 (1H, m., CH-4), 8.0 (2H, m., CH-2,6); δ_{C} (75.42 MHz, C²HCl₃) 14.10 and 15.05 (CH₃CH₂O and CH₃), 63.75 (CH₃CH₂O), 128.52, 129.44 (C-3',5' and C-4'), 129.3 (C-1'), 133.13 (C-2',6'), 164.01 (C=N), 169.66 (C=O); *m/z* (CI) 208 ([*M*+*H*]⁺, 96%)

***O*-Benzoylhydroxylamine hydrochloride (228)**



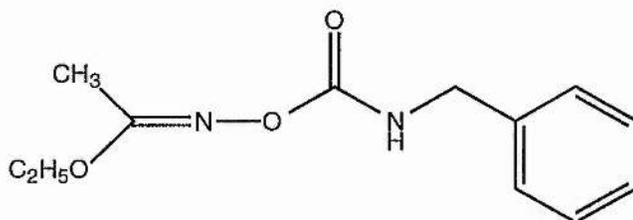
O-Benzoylacetohydroxamic acid ethyl ester (**229**) (1.05 g, 5 mmol) was stirred in dioxan (10 cm³) and water (0.9 cm³, 5 mmol) was added. Dry HCl gas was then bubbled through the solution and a white solid precipitated from solution. The suspension was stirred for 30 minutes before dry diethyl ether (10 cm³) was added. The solid was removed by filtration and washed repeatedly with diethyl ether to give the product (**228**) as a white solid (0.72 g, 83%), m.p. 106-108 °C (lit.,¹² 110-112 °C); ν_{\max} (nujol)/cm⁻¹ 3380 and 3370 (NH₂), 1760 (C=O), 690 and 680 (aromatics); δ_{H} (200 MHz, ²H₂O) 7.43 (2H, m., CH-3,5), 7.6 (1H, m., CH-4), 8.0 (2H, m., CH-2,6); δ_{C} (75.42 MHz, ²H₂O) 125.94, 126.64 (C-2,6 and C-5,3), 130.67 (C-1), 132.12 (C-4), 162.27 (C=O); m/z (CI) 138 ([M-Cl]⁺, 82%)

***O*-ButylcarbamyI-*N*-hydroxyphthalimide (231)**



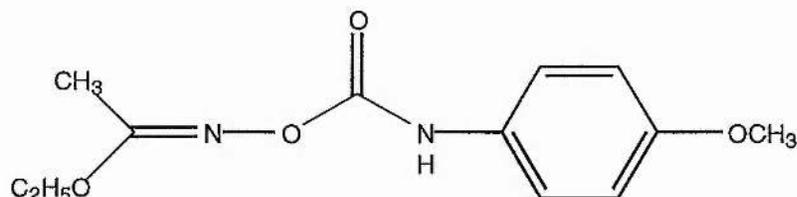
N-Hydroxyphthalimide (1.63 g, 10 mmol) was stirred in dry THF (10 cm³) and butyl isocyanate (0.9 g, 1.02 cm³, 10 mmol) was added. The reaction was monitored by tlc (1:1 ethyl acetate/petroleum ether (40-60 °C)). After 45 minutes reaction was complete and the reaction mixture was concentrated under reduced pressure to yield a orange solid (**231**) (2.28 g, 87%), m.p. 154-155 °C; ν_{\max} (nujol)/cm⁻¹ 1750 (C=O), 1075, 975 (O-C-O), 825 and 700 (aromatics); δ_{H} (300 MHz, C²H₃O²H) 0.96 (3H, t., *J* 7.3, CH₃), 1.37 (2H, m., CH₂-3), 1.53 (2H, m., CH₂-2), 3.2 (2H, t., *J* 6.6, CH₂-1), 7.8 (4H, m., aromatics); δ_{C} (75.42 MHz, C²H₃O²H) 13.98 (CH₃), 20.77 (C-3), 32.62 (C-2), 42.39 (C-1), 124.18 and 124.81 (aromatics), 135.67 and 136.28 (quat. aromatics)

***O*-(Benzylcarbamyl) acetohydroxamic acid ethyl ester (232)**



Benzyl isocyanate (1.33 g, 1.23 cm³, 10 mmol) in dry ether (10 cm³) was added dropwise to a solution of acetohydroxamic acid ethyl ester (1.03 g, 10 mmol) in ether (10 cm³). The reaction was stirred at room temperature for 3 hours and then concentrated under reduced pressure to give the product (**232**) as an oil (1.25 g, 53%); δ_{H} (300 MHz, C²HCl₃) 1.20 (3H, m., CH₃CH₂O), 2.09 (3H, s., CH₃), 3.98 (2H., m., CH₂Ph), 4.30 (2H, q, *J* 7, CH₃CH₂O), 7.2 (5H., m., aromatics); δ_{C} (75.42 MHz, C²HCl₃) 13.99 and 15.02 (CH₃CH₂O and CH₃), 44.89 (CH₂Ph), 63.29 (CH₃CH₂O), 127.50, 127.57 and 128.73 (aromatics), 138.26 (quat. aromatic), 207.15 (C=N); *m/z* (CI) 237 (9, [M+H]⁺)

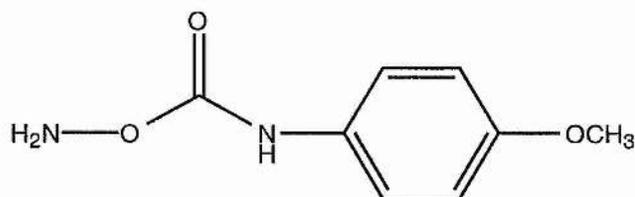
***O*-(4-Methoxyphenylcarbamyl) acetohydroxamic acid ethyl ester (235)**



Acetohydroxamic acid ethyl ester (1.03 g, 10 mmol) was dissolved in sodium dried toluene (10 cm³) and *p*-methoxyphenyl isocyanate (1.49 g, 10 mmol) was added. The mixture was stirred under nitrogen at room temperature for an hour before it was heated

at reflux for an hour and then stirred at room temperature for 12 hours. The resultant solution was concentrated under reduced pressure to yield a yellow oil with a trace of solid present. The oil was dissolved in ethyl acetate and the solid removed by filtration. The filtrate was concentrated under reduced pressure to give a yellow oil which became solid on standing. This solid was purified by silica column chromatography (elutant 3:1 petroleum ether (40-60 °C)/ethyl acetate) to give the product (**235**) as a white solid (1.0 g, 40 %), m.p. 76.5 °C; ν_{\max} (nujol)/ cm^{-1} 3180 (NH), 1680 (C=O), 950 and 790 (aromatics); δ_{H} (200 MHz, $\text{C}^2\text{H}_3\text{O}^2\text{H}$) 1.3 (3H, t., $\text{CH}_3\text{CH}_2\text{O}$), 2.1 (2H, m., $\text{CH}_3\text{CH}_2\text{O}$), 2.3 (3H, s., OCH_3), 4.2 (3H, m., CH_3), 7.2 (4H, 2 x d., aromatics); δ_{C} (75.42 MHz, $\text{C}^2\text{H}_3\text{O}^2\text{H}$) 10.93 ($\text{CH}_3\text{CH}_2\text{O}$), 17.55 (OCH_3), 26.46 (CH_3CH_2), 60.33 (OCH_3), 126.43 and 126.50 (aromatics), 130.70 and 131.43 (quat. aromatics), 149.51 (C=N) and 163.68 (C=O)

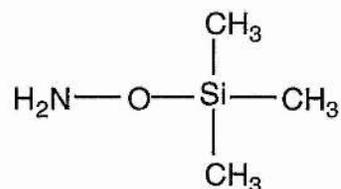
***O*-(4-Methoxyphenylcarbonyl)hydroxylamine (233)**



O-(Methoxyphenylcarbonyl)acetohydroxamic acid ethyl ester (**235**) (3 mmol, 0.76g) was stirred in dioxan (10 cm^3) and water (3 mmol, 0.06 cm^3) added. The reaction was cooled to 0 °C and dry HCl gas was bubbled through the solution and a white precipitate was formed. After stirring at 0 °C for 30 minutes the suspension was diluted with dry diethyl ether (10 cm^3) and filtered. The collected solid was repeatedly washed with diethyl ether to give the product (**233**) as a white solid (0.52 g, 95%); m.p. 119-120 °C; ν_{\max} (nujol)/ cm^{-1} 3180 (NH), 1740 (C=O), 900, 840 (aromatics); δ_{H} (200 MHz,

C^2HCl_3) 2.3 (2H, s., NH_2), 3.34 (1H, s., NH), 4.98 (OCH_3), 7.25 (4H, d.d., aromatics); δ_C (75.42 MHz, C^2HCl_3) 15.54 (OCH_3), 120.51, 130.89 (aromatics), 135.98 (quat. aromatic)

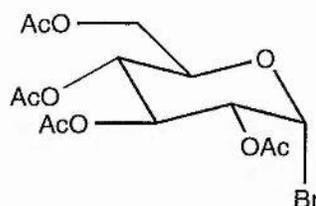
O-Trimethylsilylhydroxylamine (236)



Powdered hydroxylamine hydrochloride (35 g, 0.5 mol) was added slowly (Care reaction is exothermic) to a stirred solution of ethylenediamine (30 g, 0.5 mol) in dichloromethane (150 cm³). This mixture was stirred at room temperature for 6 hours until 2 clearly defined liquid phases were present. Chlorotrimethylsilane (65 cm³, 0.5 mol) was then added dropwise over an hour. Once reflux was complete the reaction was stirred for 24 hours at room temperature. The resulting solid (ethylenediamine chloride) was filtered off and washed with portions of dichloromethane. The filtrate was then evaporated at reduced pressure and the residue distilled to give the product (**236**) as a colourless liquid, b.p. 98 -100 °C; δ_H (300 MHz, C^2HCl_3) 0.1 (9H, s., $Si(CH_3)_3$), 5.00 (2H, s., ONH_2)

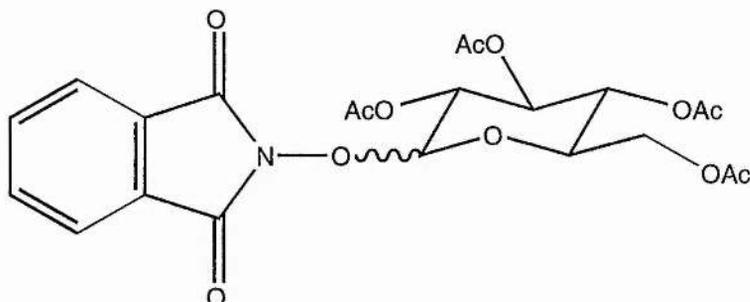
2,3,4,6-Tetra-*O*-acetyl-1-bromo- α -D-glucopyranose

(α -D-Acetobromoglucose) (242)



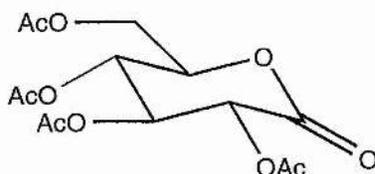
D-Glucose (9 g, 50 mmol) was dissolved in acetic anhydride (35 cm³) and hydrobromic acid in acetic acid (45% w/v, 14 cm³, 77 mmol) was added dropwise. After complete addition the reaction was stirred at room temperature for an hour before further hydrobromic acid (42 cm³, 231 mmol) was added. The reaction was then stirred for 18 hours at room temperature before being poured, carefully, onto ice-cold water (200 cm³). The homogenous solution was extracted with dichloromethane (2 x 200cm³). The combined organic extracts were then carefully poured onto ice cold saturated sodium hydrogen carbonate solution (200 cm³). The mixture was separated and the organic layer washed with sodium hydrogen carbonate solution (3 x 200 cm³), dried (MgSO₄), filtered and concentrated under reduced pressure to give a thick oil. Crystallisation was induced by adding ether to the oil and leaving at -18 °C for a number of hours. The solid obtained was filtered and recrystallised to give the product as a solid (8.87 g, 40%), m.p. 87-88 °C (lit.,¹⁵¹ 88-89 °C); [α]_D +196.3 ° (c 2.42 in CHCl₃) (lit.,¹⁵² +197.84 ° (c 2.42 in CHCl₃)); ν_{\max} (nujol)/cm⁻¹ 1720 (CO); δ_{H} (200 MHz, C²HCl₃) 2.04 (12H, 4 x s., COOCH₃), 4.09 (1H, m., *H*-5), 4.29 (2H, m., *H*-6^a,6^b), 4.81 (1H, dd., *J*_{1,2} 4, *J*_{2,3} 10,*H*-2), 5.14 (1H, t., *J*_{3,4}=*J*_{4,5} 10, *H*-4), 5.53 (1H, t., *J*_{2,3}=*J*_{3,4} 10, *H*-3), 6.59 (1H, d., *J*_{1,2} 4, *H*-1); δ_{C} (75.42 MHz, C²HCl₃) 21.01 and 21.11 (CH₃), 61.39 (*C*-6), 67.6 (*C*-4), 70.59 (*C*-2), 71.00 (*C*-5), 72.57 (*C*-3), 86.56 (*C*-1), 169.91, 170.23, 170.29 and 170.94 (*C*=O); *m/z* (CI) 430 and 428 ([*M*+NH₄]⁺, 18%)

Attempted preparation of *O*-(2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranose)-*N*-hydroxyphthalimide (241) from acetobromo- α -D-glucose (242)



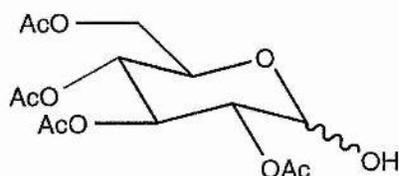
N-Hydroxyphthalimide (0.82 g, 5 mmol) was dissolved in dry THF (15 cm³) and then sodium hydride (0.2 g of a 60% suspension in oil) was added in small portions. To the resultant dark solution acetobromo- α -D-glucose (**242**) (2.06 g, 5 mmol) was added and the reaction stirred overnight at room temperature. After this period water was added carefully until evolution of gas ceased. The residue was then concentrated under reduced pressure and the resultant solid was partitioned between ethyl acetate (20 cm³) and water (20 cm³). The aqueous layer was washed with ethyl acetate (2 x 20 cm³) and the combined organic extracts were washed with brine (2 x 20 cm³), dried (MgSO₄), filtered and concentrated under reduced pressure to yield a white solid (1.32 g, 26%). Both the ¹H and ¹³C NMR spectra of the product were consistent with the product being a mixture of starting materials.

2,3,4,6-Tetra-*O*-acetyl-D-glucono- γ -lactone (245)



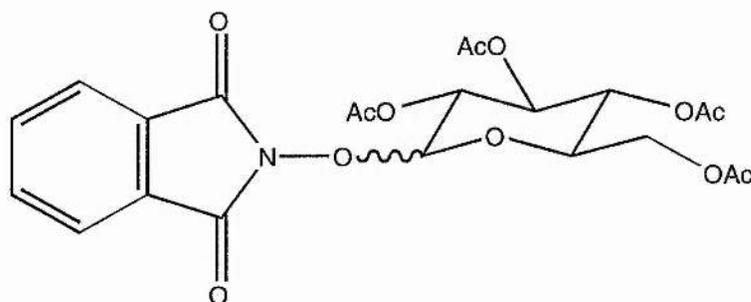
Zinc chloride (8.91 g, 65 mmol) was dissolved in acetic anhydride (90 cm³) and stirred under nitrogen. To the suspension D-glucono- γ -lactone (17.83 g, 100 mmol) was added slowly. The reaction was stirred at room temperature for 40 minutes and then poured onto crushed ice (300 cm³). After shaking, the homogenous solution was extracted with dichloromethane (2 x 250 cm³). The combined organic extracts were washed with ice-cold water (2 x 250 cm³), dried (MgSO₄) and concentrated under reduced pressure to give the product (**245**) as a gold oil (34.6 g, 100%); $[\alpha]_D^{20}$ 77.2° (c 2.0 in CHCl₃) (lit.,¹⁵³ +79.7° (c 2.0 in CHCl₃); ν_{\max} (nujol)/cm⁻¹ 2950 (CH), 1730 (CO); δ_H (300 MHz, C²HCl₃) 1.99-2.08 (12H, 4s., 4 x CH₃), 4.18 (1H, d.d., $J_{5,6a}$ 2.6, $J_{6a,6b}$ 12.8, *H*-6a), 4.32 (1H, d.d., $J_{5,6b}$ 3.8, $J_{6a,6b}$ 12.8, *H*-6b), 4.53-4.58 (1H, m., *H*-5), 5.07 (1H, d., $J_{2,3}$ 9.0, *H*-2), 5.3 (1H, t., $J_{3,4}=J_{4,5}$ 9.0, *H*-4), 5.48 (1H, t., $J_{2,3}=J_{3,4}$ 9.0, *H*-3); δ_C (75.42 MHz, C²HCl₃) 20.11, 20.18, 20.24 and 21.80 (4 x CH₃), 61.18 (*C*-6), 66.27 (*C*-4), 69.99 (*C*-3), 70.10 (*C*-5), 75.60 (*C*-2), 164.59 (*C*-1), 169.13, 169.34, 169.84 and 170.20 (4 x C=O); m/z (EI) 347 (*M*⁺, 22%)

2,3,4,6-Tetra-*O*-acetyl- α/β -D-glucopyranose (243)



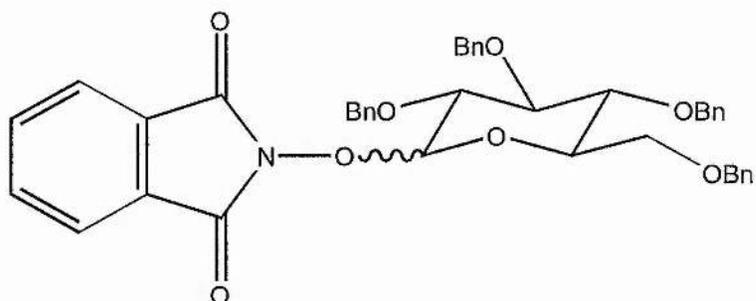
2,3,4,6-Tetra-*O*-acetyl-D-glucono- γ -lactone (**245**) (100 mmol, 34.6 g) was dissolved in THF (150 cm³) and cooled to 0 °C. Sodium borohydride (50 mmol, 1.91 g) in ice-cold water (30 cm³) was then added dropwise to the resultant solution. The reaction mixture was stirred at 0 °C for 3 hours before DOWEX 50W H⁺ resin (15 g) was added. The suspension was filtered and the filtrate concentrated under reduced pressure to give an oily solid which was washed with methanol to yield the product (**243**) as a colourless syrup. (20.16 g, 58 %), $[\alpha]_D +11.1^\circ$ (c 1.0 in CHCl₃) (lit.,¹⁵⁴ +9.8° (c 0.816 in CHCl₃); ν_{\max} (nujol)/cm⁻¹ 3300 (OH) and 1750 (CO); δ_H (200 MHz, C²HCl₃) 2.00-2.15 (12H, 4s., 4 x CH₃), 3.7 (1H, m., *H*-5), 4.10 (2H, m., *H*-6a,6b), 4.75-5.70 (4H, m., *H*-1,2,3,4); δ_C (75.42 MHz, C²HCl₃) 20.47 and 20.58 (CH₃), 61.41 (C-6 β), 61.91 (C-6 α), 67.17 (C-4 α), 67.71 (C-4 β), 68.46 (C-2 α), 69.82 (C-5 α), 70.19 (C-2 β), 71.04 (C-3 α), 72.03 (C-5 β), 72.19 (C-3 β), 90.15 (C-1 α), 95.54 (C-1 β), 169.80, 170.33, 170.40 and 170.99 (4 x C=O); *m/z* (CI) 366 ([M+NH₄]⁺, 95%), 331 (100, [M-OH]⁺), 271 (8, [M-C₂H₅O₃]⁺), 211 (3, [M-C₄H₉O₅]⁺)

Attempted preparation of *O*-(2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranose)-*N*-hydroxyphthalimide (241) from 2,3,4,6-Tetra-*O*-acetyl- α/β -D-glucopyranose (243)



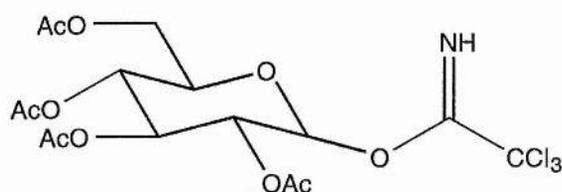
2,3,4,6-Tetra-*O*-acetyl- α/β -D-glucopyranose (243) (1.42 g, 4.1 mmol), *N*-hydroxyphthalimide (0.69 g, 4.2 mmol) and triphenylphosphine (1.1 g, 4.2 mmol) were stirred in dry THF (15 cm³) under a nitrogen atmosphere. To this suspension diethyl azodicarboxylate (DEAD) (0.73 g, 4.2 mmol) was added. The reaction was stirred for 20 hours at room temperature. After this time the reaction was concentrated under reduced pressure to yield a yellow oil. Purification of the crude oil was attempted by column chromatography (silica, toluene: ether, 9:1), however the ¹H NMR spectrum of the isolated fractions showed them to be starting materials.

Attempted preparation of *O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranose-*N*-hydroxyphthalimide



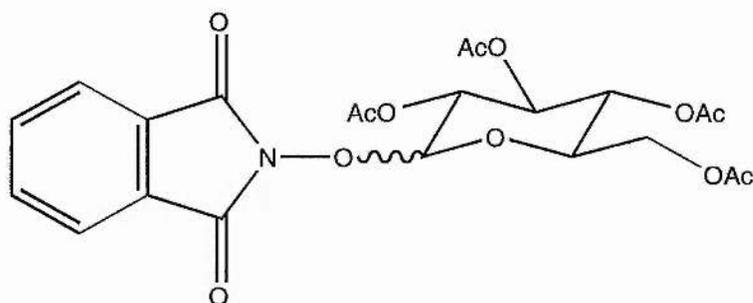
2,3,4,6-Tetra-*O*-benzyl-D-glucopyranose (2.7 g, 5 mmol), *N*-hydroxyphthalimide (0.82 g, 5 mmol) and triphenylphosphine (1.31 g, 5 mmol) were dissolved in THF (15 cm³). To the resultant solution DEAD (0.96 g, 0.87 cm³, 5.5 mmol) was added, the reaction was slightly exothermic. The reaction was stirred at room temperature for 46 hours and then concentrated under reduced pressure to yield an oil. Methanol was added to the oil and a solid precipitate was obtained, which was filtered off and dried. An attempt to purify this white solid by column chromatography (silica, ethyl acetate: petroleum ether, 1:3) yielded *N*-hydroxyphthalimide and 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose. The filtrate was concentrated under reduced pressure to yield an oil. An attempt to purify the oil by column chromatography (silica, ethyl acetate : petroleum ether, 1:1) yielded no target compound.

2,3,4,6-Tetra-*O*-acetyl- β -D-glucopyranosyl trichloroacetimidate (247)



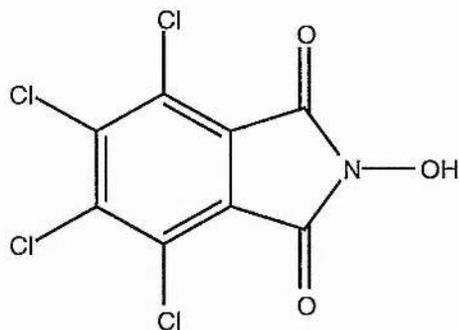
2,3,4,6-Tetra-*O*-acetyl- α/β -D-glucopyranose (**243**) (58 mmol, 20.19 g) and trichloroacetonitrile (29 cm³) were stirred together in dry dichloromethane (100 cm³). To this solution was added hot potassium carbonate (22.5 g). The reaction was stirred at room temperature for 25 hours. The reaction mixture was then filtered to remove the inorganic salts and the filtrate concentrated under reduced pressure to yield an oil. Purification by column chromatography (silica, 2:3 ethyl acetate/petroleum ether) gave the product (**247**) as a white solid (11.89 g, 43%), m.p. 69-71 °C (lit. (α),¹⁵⁵ oil, (β),¹⁵⁵ 154-155 °C); [α]_D +44.7 ° (c 1.0 in CHCl₃) (lit. (α),¹⁵⁵ +61.5 ° (c 1.0 in CHCl₃), (β),¹⁵⁵ +8.3 (c 1.0 in CHCl₃)); δ _H (200 MHz, C²HCl₃) 2.10 (12H, 4s., 4 x CH₃), 4.05-4.30 (3H, m., *H*-5,6a,6b), 5.10-5.20 (2H, m., *H*-3,4), 5.60 (1H, t., *J*, *H*-2), 6.55 (1H, d., *J* 8.0, *H*-1), 8.7 (1H, s., *NH*); δ _C (75.42 MHz, C²HCl₃) 14.03 (CCl₃), 20.27, 20.41, 20.50 and 20.87 (4 x CH₃), 60.28, 61.29 (*C*-5,6), 67.72 (*C*-4), 69.80 (*C*-3), 69.96 (*C*-2), 92.88 (*C*-1), 160.87 (*C*=NH), 169.58, 169.92, 170.07 and 170.63 (4 x C=O)

***O*-(Tetra-*O*-acetyl- α -D-glucopyranose)-*N*-hydroxyphthalimide (241)**



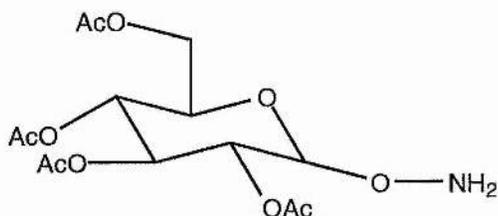
N-Hydroxyphthalimide (1.86 g, 3.8 mmol) and 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl trichloroacetimidate (**247**) (0.62 g, 3.8 mmol) were stirred in DCM (10 cm³) and cooled to -15 °C. Boron trifluoride diethyl etherate (0.76 cm³, 15.2 mmol) was added and the reaction stirred for 75 minutes. After this time the reaction was recooled to -15 °C and further boron trifluoride diethyl etherate (0.76 cm³) was added to the solution. The reaction was then stirred overnight at room temperature. The resultant solution was added to saturated bicarbonate solution (15 cm³) and ethyl acetate (15 cm³). The aqueous layer was washed with ethyl acetate (2 x 20 cm³). The combined organic extracts were washed with brine (2 x 20 cm³), dried (MgSO₄), filtered and concentrated under reduced pressure to yield an oil. This oil was stirred in methanol and the solid obtained was filtered and dried to give the product (**241**) as a white solid (0.53 g, 29%), m.p. 168-170 °C; ν_{\max} (nujol)/cm⁻¹ 1870 (CO), 710 and 690 (aromatics); δ_{H} (200 MHz, d⁶-DMSO) 1.94-2.47 (12H, 4s., CH₃), 3.94-4.02 (2H, m., *H*-6a and *H*-5), 4.22 (1H, d.d., *J*_{5,6b} 4.8, *J*_{6a,6b} 12.2, *H*-6b), 5.03 (2H, m., *H*-2 and *H*-4), 5.38 (2H, m., *H*-3 and *H*-1), 7.86 (4H, s., aromatics); δ_{C} (75.42 MHz, d⁶-DMSO) 19.74, 19.81 and 19.91 (CH₃), 61.04 (*C*-6), 67.39 (*C*-4), 68.87 (*C*-2), 70.56 (*C*-5), 71.12 (*C*-3), 103.91 (*C*-1), 123.09 and 134.67 (aromatics), 127.98 (quat. aromatics), 161.98 (aromatic C=O), 168.88, 169.25 and 169.60 (C=O); *m/z* (ES⁺) 516 ([*M*+Na]⁺, 100%)

3,4,5,6-Tetrachloro-*N*-hydroxyphthalimide (248)



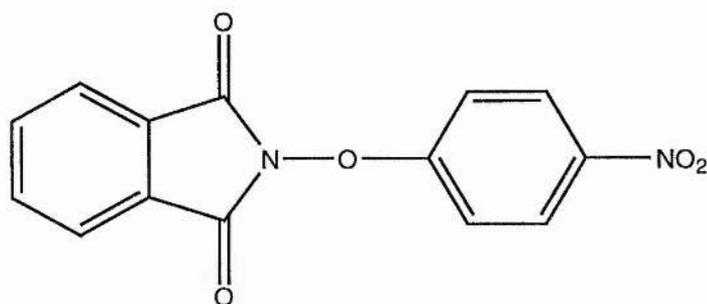
Tetrachlorophthalic anhydride (5.71 g, 20 mmol) was stirred in 2M sodium hydroxide solution (20 cm³) and hydroxylamine hydrochloride (1.39g, 20 mmol) added. The reaction was heated at reflux for 8 hours and then stirred overnight at room temperature. Partial concentration under reduced pressure of the reaction solution yielded a solid which was removed by filtration and dried under vacuum to yield the product as a solid (2.21 g, 37%), m.p >300; ν_{\max} (nujol)/cm⁻¹ 3300 (OH), 1570 (CO), 650, 605 (aromatics); δ_C (75.42 MHz, ²H₂O), 109.87, 128.25 and 133.84 (aromatics), 168.58 (C=O)

***O*-(Tetra-*O*-acetyl-*D*-glucopyranose)hydroxylamine (250)**



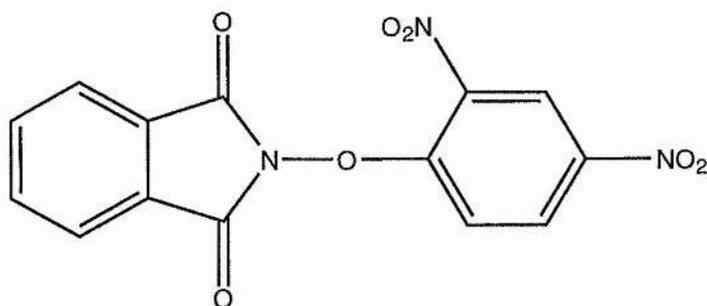
N-(Tetra-*O*-acetyl- α -*D*-glucopyranose)phthalimide (**241**) (0.49 g, 1 mmol) was stirred in THF (10 cm³) and hydrazine hydrate added (0.1 cm³). The reaction mixture was heated under reflux for 10 minutes and then cooled to room temperature, when the by-product phthalazine precipitated from the solution. The suspension was diluted with aqueous sodium bicarbonate solution (5%, 10 cm³) and washed with diethyl ether (3 x 15 cm³). The combined organic extracts were washed with water (2 x 15 cm³), dried (MgSO₄), filtered and concentrated under reduced pressure to yield an off-white solid (**250**) (0.25g, 69 %); ν_{\max} (nujol)/cm⁻¹ 3450 and 3400 (NH₂), 1670 (C=O); δ_{H} (300 MHz, C²HCl₃) 1.99-2.08 (12H, 4s., 4 x CH₃), 3.72 (1H, m., *H*-5), 4.15 (1H, d.d., *J*_{5,6a} 2, *J*_{6a,6b} 12.4, *H*-6a), 4.30 (1H, d.d., *J*_{5,6b} 4.3, *J*_{6a,6b} 12.4, *H*-6b), 4.70 (1H, d., *J* 8.2, *H*-1), 5.02-5.27 (3H, m., *H*-2,3,4); δ_{C} (75.42 MHz, C²HCl₃) 21.05, 21.09, 21.17 and 21.22 (CH₃), 62.21 (*C*-6), 68.61 (*C*-4), 70.06 (*C*-3), 72.23 (*C*-5), 73.35 (*C*-2), 103.88 (*C*-1), 169.77, 170.02, 170.70 and 171.20 (C=O); *m/z* (ES⁺) 386 ([*M*+Na]⁺, 100%)

Attempted preparation of *O*-(4-nitrophenyl)-*N*-hydroxyphthalimide (254)



N-Hydroxyphthalimide (1.63 g, 10 mmol), 4-nitrofluorobenzene (1.38 g, 10 mmol) and pyridine (0.79 g, 10 mmol) were stirred together in dry DCM (15 cm³) for sixteen hours. After this time tlc indicated that reaction had not occurred and the reaction was then heated at between 40 and 60 °C for twelve hours and then stirred at room temperature for seven days. The reaction was concentrated under reduced pressure and the residue partitioned between water (15 cm³) and ethyl acetate (15 cm³). The aqueous layer was washed with ethyl acetate (2 x 15 cm³) and the combined organic extracts were washed with brine (15 cm³), dried (MgSO₄), filtered and concentrated under reduced pressure to yield an orange solid. The NMR spectrum of this compound showed that it was a mixture of compounds but that the major product was starting material.

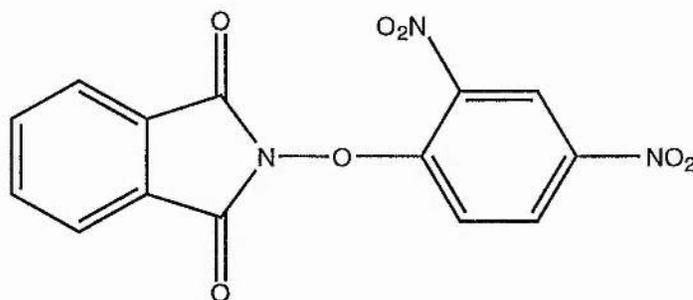
Attempted preparation of *O*-(2,4-dinitrophenyl)-*N*-hydroxyphthalimide (255)



N-Hydroxyphthalimide (1.63 g, 10 mmol), 2,4-dinitrofluorobenzene (1.86 g, 10 mmol),

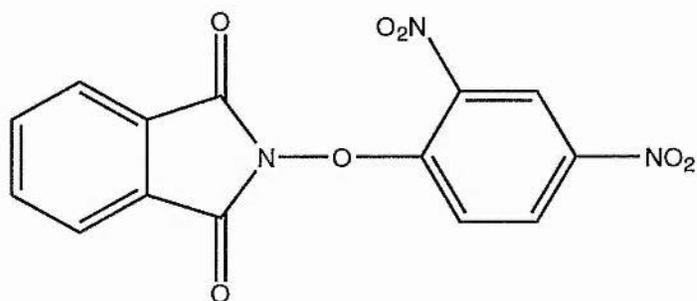
DMAP (0.12 g, 1 mmol) and triethylamine (2.09 cm³, 15 mmol) were stirred together in THF (10 cm³). The reaction was stirred at room temperature for 24 hours and then partitioned between diethyl ether (15 cm³) and 2M HCl (15 cm³). The aqueous layer was washed with diethyl ether (2 x 15 cm³) and the combined organic layers were washed with saturated hydrogen carbonate solution (15 cm³), dried (MgSO₄), filtered and concentrated under reduced pressure to yield a yellow solid. The NMR spectrum indicated that the desired product had not been obtained.

Attempted preparation of *O*-(2,4-dinitrophenyl)-*N*-hydroxyphthalimide (255)



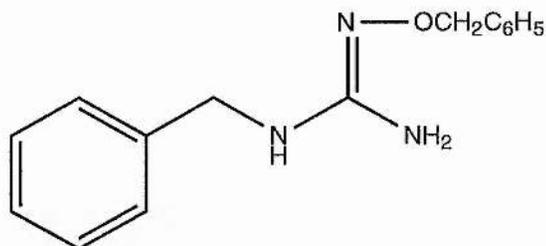
N-Hydroxyphthalimide (2.45 g, 15 mmol) was stirred in dry DCM (15 cm³) and sodium hydride (0.6 g) was added. Once gas evolution had ceased 2,4-dinitrofluorobenzene (2.79 g, 15 mmol) was added and the solution heated under reflux for 5 hours and then the solid residue was removed by filtration. Concentration of the filtrate under reduced pressure yielded an orange solid. The NMR spectrum of this solid indicated that the compound was a mixture of compounds with some of the desired product present. However all attempts to purify this compound failed.

Attempted preparation of *O*-(2,4-dinitrophenyl)-*N*-hydroxyphthalimide
(255)



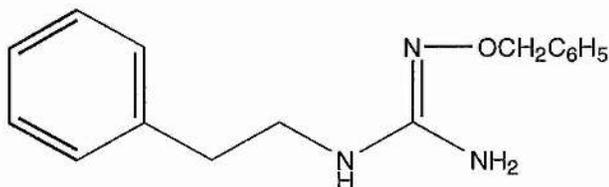
2,4-Dinitrophenol (0.93 g, 5 mmol) was stirred in dry THF (15 cm³) at 0 °C and *N*-hydroxyphthalimide (0.82 g, 5 mmol) and triphenylphosphine (1.46 g, 5.7 mmol) added. Diethyl azodicarboxylate (0.89 cm³, 5.7 mmol) was added to the suspension over a period of 10 minutes. The reaction was then stirred at room temperature for 30 minutes and then concentrated under reduced pressure to yield a dark oil. Subsequent column chromatography (silica, 3/1 ethyl acetate/ diethyl ether) yielded a number of products. The NMR spectrum of these products showed that there was none of the desired compound present.

***O*-Benzyl-1-benzyl-2-hydroxyguanidine (258)**



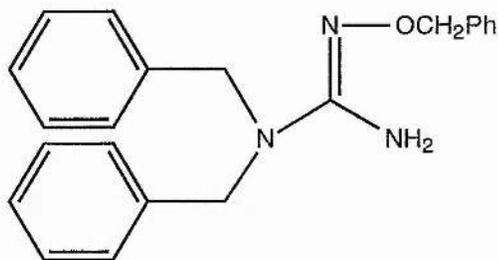
Potassium carbonate (2.76 g, 20 mmol) was added to water (30 cm³), followed by *O*-benzylhydroxylamine hydrochloride (1.60 g, 10 mmol). *N*-Benzylaminoiminomethanesulfonic acid (**193**) (2.14 g, 10 mmol) was added to the solution in small quantities. Once addition was complete the reaction was stirred at room temperature for 8 hours. The resultant suspension was filtered and the solid dried under vacuum to give the product (**258**) as a white solid (1.22 g, 48%), m.p. 84-86 °C; (Found: C, 70.35; H, 6.95; N, 16.63. Calc. for C₁₅H₁₇N₃O: C, 70.57; H, 6.71; N, 16.46%); ν_{\max} (nujol)/cm⁻¹ 3450 (NH) 3100, 3060 (NH₂), 760 and 700 (aromatics); δ_{H} (200 MHz, d⁶-DMSO) 4.1 (2H, s., PhCH₂NH), 4.7 (2H, s., OCH₂Ph), 5.0 (2H, s., NH₂), 7.2 (10H, m., aromatics); δ_{C} (50.3 MHz, d⁶-DMSO) 44.57 (PhCH₂NH), 74.27 (OCH₂Ph), 127.74, 128.16, 128.74, 129.09, 129.17 and 129.48 (aromatics), 140.74 (quat. aromatic), 154.95 (C=N); m/z (EI) 255 (*M*⁺, 11%), 164 (38, [*M*-PhCH₂]⁺), 133 (79, [*M*-NH₂]⁺), 91 (99, [PhCH₂]⁺), 77 (26, [C₆H₅]⁺)

***O*-Benzyl-1-phenylethyl-2-hydroxyguanidine (259)**



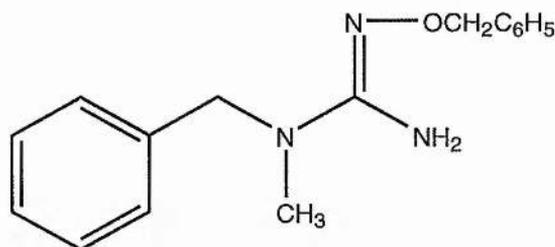
This was prepared in a manner identical to *O*-benzyl-1-benzyl-2-hydroxyguanidine (258) using *N*-phenylethylaminoiminomethanesulfonic acid (195) (10 mmol, 2.28 g) to give the product (259) as an oil (1.23 g, 46%); ν_{\max} (nujol)/ cm^{-1} 3450, 3020 and 3010 (NH+NH₂), 740 and 690 (aromatics); δ_{H} (300 MHz, C²HCl₃) 2.65 (2H, m., PhCH₂CH₂), 3.10 (2H, m, CH₂CH₂NH), 4.70 (2H, s., OCH₂Ph), 7.10 (10H, m., aromatics); δ_{C} (75.42 MHz, C²HCl₃) 32.99 (PhCH₂CH₂), 39.89 (CH₂CH₂NH), 72.17 (OCH₂Ph), 124.36, 124.57, 124.94, 125.17, 125.28 and 125.77 (aromatics), 135.24 (quat. aromatic), 152.13 (C=N); m/z (EI) 269 (*M*⁺, 29%), 178 (39, [*M*-CH₂C₆H₅]⁺), 147 (96, [*M*-NOCH₂C₆H₅]⁺), 105 (93, [C₆H₅CH₂CH₂]⁺), 91 (100, [C₆H₅CH₂]⁺), 77 (25, [C₆H₅]⁺)

***O*-Benzyl-1,1-dibenzyl-2-hydroxyguanidine (260)**



This was prepared in an identical manner to *O*-benzyl-1-benzyl-2-hydroxyguanidine (**258**) using *N,N*-dibenzylaminoiminomethanesulfonic acid (**196**) (3.04 g, 10 mmol) to give the product (**260**) as a solid (3.08 g, 89%), m.p. 63-64 °C, (Found: C, 76.73; H, 6.79; N, 12.09; Calc. for C₂₂H₁₇N₃O: C, 76.49; H, 6.71; N, 12.16%); ν_{\max} (nujol)/cm⁻¹ 3030 and 3000 (NH₂), 750 and 670 (aromatics); δ_{H} (200 MHz, C²HCl₃) 4.3 (6H, m., 2 x PhCH₂ and NH₂), 5.0 (2H, s., OCH₂Ph), 7.4 (15H, m., aromatics); δ_{C} (50.3 MHz, C²HCl₃) 51.05 (PhCH₂), 75.97 (OCH₂Ph), 127.74, 128.16, 128.74, 129.09, 129.17 and 129.84 (aromatics), 138.57 and 139.02 (quat. aromatics), 157.63 (C=N); *m/z* (EI) 345 (M⁺, 17%), 254 (38, [M-CH₂C₆H₅]⁺), 223 (56, [M-NHOCH₂C₆H₅]⁺), 196 (49, [(C₆H₅CH₂)₂N]⁺), 106 (79, [C₆H₅CH₂NH]⁺), 91 (100, [C₆H₅CH₂]⁺)

***O*-Benzyl-1-benzyl-1-methyl-2-hydroxyguanidine**

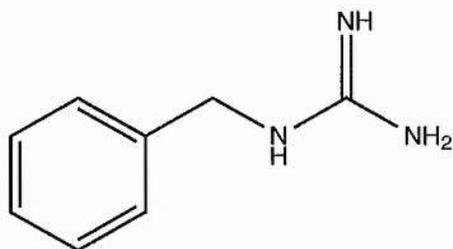


This compound was prepared in an identical manner to *O*-benzyl-1-benzyl-2-hydroxyguanidine using *N,N*-benzylmethylaminoiminomethanesulfonic acid (0.91 g, 4 mmol) to give the crude product as a pale yellow oil. This oil was purified by column chromatography (silica, 3/1 ethyl acetate/petroleum ether (40-60 °C)) to give the product as an oil (0.24g, 11.2%); ν_{\max} (nujol)/ cm^{-1} 3450 (NH_2), 970 and 890 (aromatics); δ_{H} (200 MHz, C^2HCl_3) 2.78 (3H, s., CH_3), 4.34 (2H, s., PhCH_2N), 4.38 (2H, s., NH_2), 4.94 (2H, s., OCH_2Ph), 7.3 (10H, m., aromatics); δ_{C} (75.42 MHz, C^2HCl_3) 35.58 (CH_3), 54.07 (PhCH_2N), (OCH_2Ph), 127.22, 127.63, 128.04, 128.22, 128.41 and 128.61 (aromatics), 138.16 and 138.39 (quat. aromatics), 157.30 ($\text{C}=\text{NO}$); m/z (EI) 269 (M^+ , 100%)

General Hydrogenation Procedure

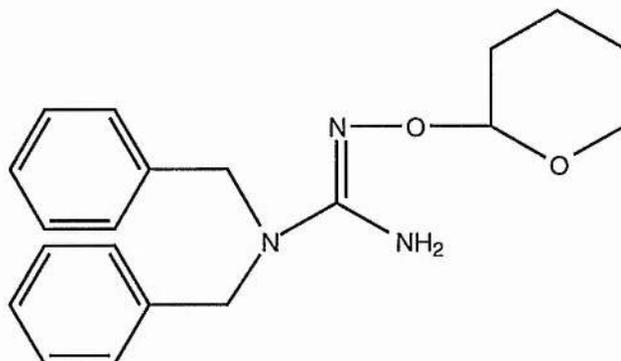
The *O*-benzyl *N*-hydroxyguanidine was dissolved in solvent (ethanol, methanol, ethyl acetate or trifluoroethanol). If required acetic acid was added to the reaction mixture. The catalyst (10% palladium on carbon) was then added to the reaction flask. The flask was fitted with a septum, purged with hydrogen gas and stirred under an atmosphere of hydrogen for 16 to 29 hours. The reaction was then filtered through celite to remove the catalyst and the filtrate concentrated under reduced pressure to yield the product.

1-Benzylguanidine (265)



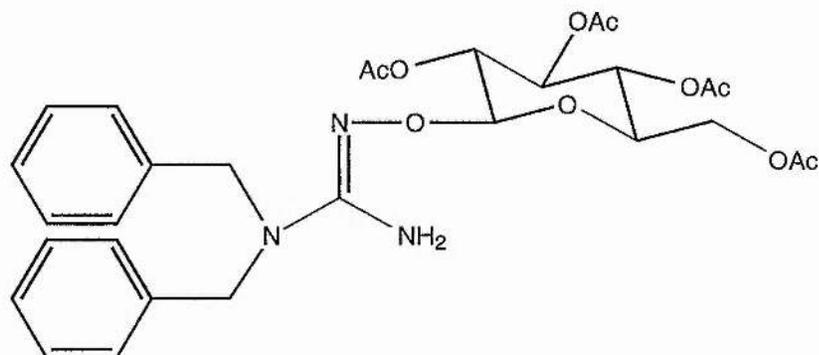
Potassium carbonate (2.06 g, 15 mmol) was added to water (30 cm³) followed by aqueous ammonia (32% solution, 100 mmol, 6 cm³). *N*-Benzylaminoiminomethanesulfonic acid (**193**) (3.21 g, 15 mmol) was then added to the solution in small quantities. After addition was complete the reaction was stirred at room temperature for 24 hours. The solvent was then removed under reduced pressure to yield a yellow solid. This solid was stirred in ethanol and the inorganic salts were removed by filtration. The solvent was removed at reduced pressure to give the product (**265**) as a white solid (1.83 g, 82%), m.p. 159-161 °C; δ_{H} (200 MHz, ²H₂O) 4.3 (2H, s., CH₂), 7.2 (5H, m., aromatics); δ_{C} (75.42 MHz, ²H₂O) 43.58 (CH₂), 127.18, 127.26 and 128.57 (aromatics), 138.21 (quat. aromatic), 157.93 (C=NH); *m/z* (EI) 149 (*M*⁺, 35%), 148 (40, [*M*-H]⁺), 106 (100, [*M*-C(=NH)NH₂]⁺), 91 (50, [C₆H₅CH₂]⁺), 77 (27, [C₆H₅]⁺)

***O*-Tetrahydropyranyl-1,1-dibenzyl-2-hydroxyguanidine (268)**



O-Tetrahydropyranyl-hydroxylamine (0.59 g, 5 mmol) and potassium carbonate (4.15 g, 30 mmol) were dissolved in water (30 cm³). To the resulting solution *N,N*-dibenzylaminoiminomethanesulfonic acid (**196**) (1.52 g, 5 mmol) was added. The reaction was stirred for 18 hours at room temperature and filtered. The crude product was obtained as an off-white solid. This solid was dissolved in hot diethyl ether and the insoluble residue removed by filtration. The filtrate was cooled and the solid collected by filtration to yield the product (**268**) as a white solid (0.45 g, 28%), m.p. 99-101 °C; (Found: C, 70.77; H, 7.22; N, 12.06; Calc. for C₂₀H₂₅N₃O₂: C, 70.77; H, 7.42; N, 12.38%); ν_{\max} (nujol)/cm⁻¹ 3390 and 3380 (NH₂), 1010, 960 (O-C-O), 850, 830 and 680 (aromatics); δ_{H} (200 MHz, C²H₃Cl) 1.57-1.84 (6H, m., CH₂-2,3,4), 3.56-3.64 (1H, m., CH₂-5) 3.92-4.00 (1H, m., CH₂-5), 4.37 (4H, pair of d., *J* 10, ArCH₂), 4.46 (2H, s., NH₂), 5.00-5.02 (1H, m., CH-1), 7.21-7.36 (10H, m., aromatics); δ_{C} (50.31 MHz, C²H₃Cl) 21.30, 25.85 and 30.11 (C-2,3,4), 51.23 (ArCH₂), 64.20 (C-5), 101.57 (C-1), 127.723, 128.01 and 129.03 (aromatics), 138.54 (quat. aromatics), 158.05 (C=NOH); *m/z* (CI) 340 (M⁺, 12%), 249 (59, [M-C₆H₅CH₂]⁺), 223 (17, [M-C₅H₁₁NO₂]⁺), 118 (45, [C₅H₁₂NO₂]⁺)

***O*-(Tetra-*O*-acetyl-*D*-glucopyranose)-1,1-dibenzyl-2-hydroxyguanidine
(272)**



O-(Tetra-*O*-acetyl-*D*-glucopyranosyl)hydroxylamine (**250**) (0.22 g, 0.6 mmol) and potassium carbonate (0.21 g, 1.5 mmol) were stirred in water (5 cm³). To this solution *N,N*-dibenzylaminoiminomethanesulfonic acid (**196**) (0.18 g, 0.6 mmol) was added and the reaction stirred overnight at room temperature. The aqueous layer was diluted with water (15 cm³) and then covered with dichloromethane (15 cm³). The aqueous layer was washed further with dichloromethane (2 x 15 cm³). The combined organic extracts were washed with brine (2 x 15 cm³), dried (MgSO₄), filtered and concentrated under reduced pressure to yield an oil (**272**) (0.09 g, 25%); δ_{H} (300 MHz, C²H₃Cl) 1.90-2.14 (12H, 4s., 4 x CH₃), 3.70 (1H, m., *H*-5), 4.03-4.50 (5H, m., *H*-2,3,4,6), 5.00-5.24 (5H, m., *H*-1 and CH₂ x2), 7.10-7.38 (10H, m., aromatics); δ_{C} (75.42 MHz, C²H₃Cl) 20.43, 20.47 and 20.54 (CH₃), 50.37 (ArCH₂), 61.73 (*C*-6), 68.11 (*C*-4), 69.59 (*C*-2), 72.33 (*C*-5), 72.40 (*C*-3), 105.13 (*C*-1), 127.17, 127.64, 128.71, 128.90, 129.00, 129.22 (aromatics), 134.40 (quat aromatic), 159.45 (*C*=NOH), 169.44, 169.68, 170.26 and 170.76 (*C*=O); *m/z* (ES⁺) 618 ([*M*+Na]⁺, 100%)

5.3 CHEMICAL OXIDATION REACTIONS

6.3.1 Griess Reagent

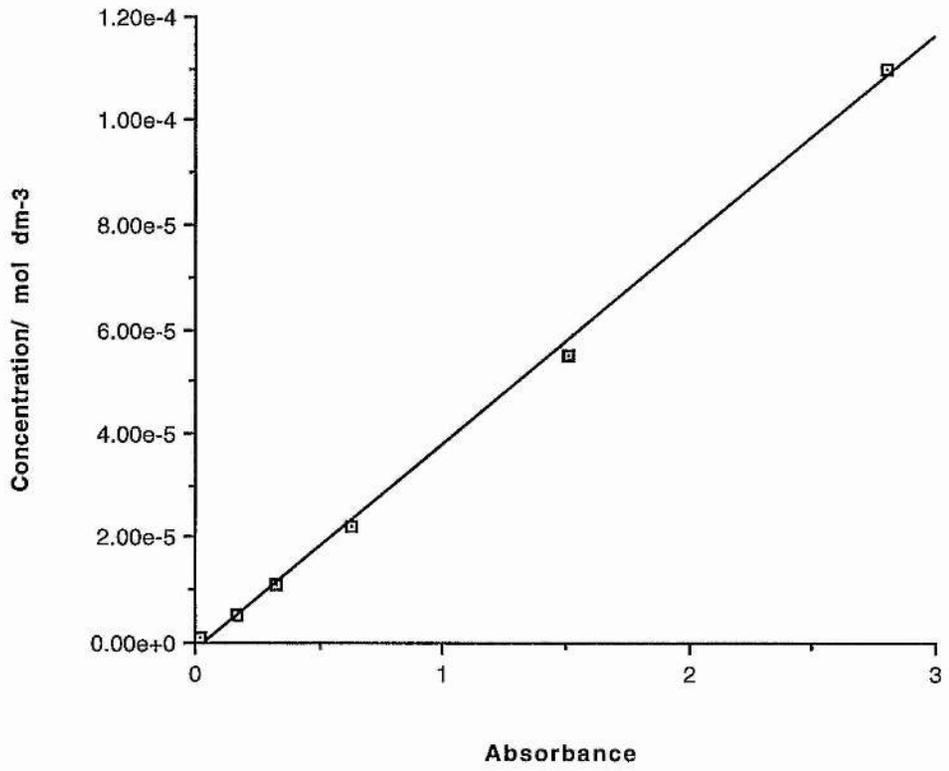
The two solutions were prepared as follows.⁴⁶ Sulfanilamide (2.5 g) was dissolved in water (500 cm³) containing 2% v/v concentrated hydrochloric acid. *N*-(1-Naphthyl)ethylenediamine dihydrochloride (1.5 g) was dissolved in water (500 cm³) containing 1% v/v concentrated hydrochloric acid. The two solutions were stored separately and used as required.

6.3.2 Nitrite Calibration Curve

The stock nitrite solution was prepared by dissolving sodium nitrite (0.0038 g, 0.055 mmol) in water (500 cm³). This solution was then diluted to the required concentrations. The Griess test was carried out by withdrawing a sample of the nitrite solution (1 cm³) and adding the sulfanilamide solution (0.5 cm³) followed, 5 minutes later, by the *N*-(1-naphthyl)ethylenediamine dihydrochloride solution (0.5 cm³). The solution was shaken thoroughly and the optical density was measured at 550 nm after 15 minutes (**Table 18**) (**Graph 8**).

Table 18:- Nitrite Calibration Data

Concentration/ 10 ⁻⁴ mol dm ⁻³	Absorbance
1.1	2.8052
0.55	1.5115
0.22	0.6270
0.011	0.3208
0.0055	0.1629
0.0011	0.0254



Graph 8:- Nitrite Calibration Curve

6.4 DECOMPOSITION STUDIES OF *O*-THP-1,1-DIBENZYL-2-HYDROXYGUANIDINE (268)

6.4.1 HPLC Analysis

The HPLC analysis was carried out using a Phenomenex LUNA column and 60/40 acetonitrile/water with 1% triethylamine as the eluant. Each HPLC was run for 10 minutes using a flow rate of $1 \text{ cm}^3 \text{ min}^{-1}$ and UV detection was employed at a wavelength of 240 nm. All organic solvents used were HPLC grade and water used was purified as described. The organic solvents were filtered and then the solvent system was degassed by placing in a sonic bath for 20 minutes.

6.4.2 Reactions at pH 0

A 1M volumetric solution of hydrochloric acid was obtained from concentrated hydrochloric acid (3 cm^3 in 100 cm^3 of distilled water). A small portion (25 cm^3) was placed in a round bottom flask and incubated at $37 \text{ }^\circ\text{C}$ ($\pm 0.1 \text{ }^\circ\text{C}$) in a water bath. *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (268) (0.0454 g, 0.125 mmol) was dissolved in acetonitrile (1 cm^3) and added to the solution. After various periods of time a small aliquot (0.2 cm^3) of the reaction mixture was removed and quenched with 1M sodium hydroxide (0.2 cm^3) solution. The aliquots were then diluted with acetonitrile (0.2 cm^3) and analysed by HPLC as described (Section 6.4.1). The peak due to the *O*-THP-1,1-dibenzyl-2-hydroxyguanidine (268) had a retention time of 7 minutes 50 seconds and was seen to totally disappear over a twenty minute period. The area of the peak was calculated using the computer software (Table 19)

Table 19:- Results of acid catalysed hydrolysis of (268) at pH 0.

Time / s	Area of peak
0	3991000
30	2859000
60	2513000
90	1900000
120	1738000
150	1441000
180	1254000
210	1053000
240	859700
270	1000000
300	616100
360	500600
420	350200
480	315400
540	269500
600	226800
1200	0

6.4.2 Reaction at pH 0.5

The hydrolysis was carried out using the same procedure as described for the hydrolysis of (268) at pH 0. The reaction was carried out in 0.32M hydrochloric acid (32 cm³ of 1M hydrochloric acid in 100 cm³). Again the reaction was quenched by adding an equal volume of 1M sodium hydroxide solution and then diluting with acetonitrile. The areas of the peak at 8.50 minutes are given (Table 20).

Table 20:- Results of acid catalysed hydrolysis of (268) at pH 0.5

Time / s	Peak area
0	6484000
60	6169000
120	6460000
180	5319000
240	4467000
300	3771000
480	3166000
540	3100000
600	3159000
720	3128000
840	2187000
960	2181000
1080	1951000
1200	1541000
1800	792000
2400	0

6.3.3 Reaction at pH 1

The hydrolysis was carried out as previously described using a potassium chloride/hydrochloric acid buffer.¹⁵⁶ The buffer was made up from 200 mmol dm³ potassium chloride solution (25 cm³) and 200 mmol dm³ hydrochloric acid (67 cm³). The pH was adjusted to pH 1 and then the solution was diluted (100 cm³). The aliquots were quenched using an equal volume of 1M sodium hydroxide. The HPLC analysis was carried out as before and the area of the peak at 8.25 minutes was calculated (**Table 21**)

Table 21:- Results of the acid catalysed hydrolysis of (268) at pH 1

Time / s	Area of peak
0	7871000
200	6783000
420	5594000
480	5308000
600	4778000
720	4302000
840	3873000
1200	3100000
1500	2754000
1800	2320000
2100	1832000
2400	1561000
2700	1356000
3000	1144000
3300	1012000
3600	919000
3900	762100
4200	542000
4500	0

6.3.4 Reaction at pH 1.5

The hydrolysis was carried out as previously described using a potassium chloride/hydrochloric acid buffer. The buffer was made up from 200 mmol dm³ potassium chloride solution (25 cm³) and 200 mmol dm³ hydrochloric acid (20.7 cm³). The pH was adjusted to pH 1.5 and then the solution was diluted (100 cm³). The reaction was run and aliquots removed and quenched with an equal volume of triethylamine. The HPLC analysis was then carried out before and the area of the peak at 8.30 minutes calculated (Table 22).

Table 22:- Results from the acid hydrolysis of (268) at pH 1.5

Time / s	Peak area
0	7066000
600	6599000
2280	5927000
2400	3848000
3000	5202000
3600	4922000
3900	4880000
4200	4528000
4800	4027000
5400	3845000
6000	3346000
6600	3098122
7200	2871000
7800	2639000
8400	2401000
9000	2287000
10800	1674000
14400	482300
15000	0

6.3.5 Reactions at pH 2

The hydrolysis was carried out as described previously using a potassium chloride/hydrochloric acid buffer. The buffer was made up from 200 mmol dm³ potassium chloride solution (25 cm³) and 200 mmol dm³ hydrochloric acid (6.5 cm³). The pH was adjusted to pH 2 and then the solution was diluted (100 cm³). The reaction was run and aliquots were removed and quenched with an equal volume of triethylamine. The HPLC analysis were run as before and the areas of the peak at 8:30 minutes was calculated (Table 23)

Table 23:- Results of the acid catalysed hydrolysis of (268) at pH 2

Time / s	Peak area
0	8103000
600	7926000
1200	7906000
1800	7712000
2400	7456000
3600	7416000
4800	7829000
6000	7797000
7200	6997000
8400	7266000
12600	6159000
14400	6474000
21600	5046000
23400	5483000
27000	5421000
30600	4140000
102600	2591000
115200	2294000
216000	0

6.5 BIOLOGICAL TESTING OF 1-BENZYL-1-METHYL-2-HYDROXYGUANIDINE (137)

Isolated segments of a rat tail artery (male Wistar, 325-355 g, terminated by cervical dislocation) were perfused internally with oxygenated Krebs solution (composition, mM: NaCl 118, KCl 4.7, NaHCO₃ 25, NaH₂PO₄ 1.15, CaCl₂ 2.5, MgCl₂ 1.1, glucose 5.6, gassed with 95% O₂/5% CO₂ to maintain pH7.4, at 32 °C), initially at low flow rate which was increased gradually over the next 10-20 mins to reach a maximum final rate of 2 cm³ min⁻¹. The preparation was allowed to stabilise for 20 to 30 mins after which it was precontracted with Krebs solution and phenylephrine HCl (5 x 10⁻⁶ M) (agonist-induced pressure = 70 mmHg). A solution of 1-benzyl-1-methyl-2-hydroxyguanidine (137) was then prepared using the internal perfusate as the solvent. This solution was then internally perfused for 10 minutes during which time the pressure dropped to 20 mmHg indicating a 70% artery dilation.

Chapter 7
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REFERENCES

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APPENDIX 1

Experimental

Data Collection

A colorless plate crystal of $C_9H_{13}N_3O$ having approximate dimensions of 0.40 x 0.20 x 0.10 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC7S diffractometer with graphite monochromated Mo-K α radiation.

Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 18 carefully centered reflections in the range $6.38 < 2\theta < 12.53^\circ$ corresponded to a primitive orthorhombic cell with dimensions:

$$a = 11.367(6) \text{ \AA}$$

$$b = 7.728(7) \text{ \AA}$$

$$c = 22.651(6) \text{ \AA}$$

$$V = 1989(1) \text{ \AA}^3$$

For $Z = 8$ and F.W. = 179.22, the calculated density is 1.20 g/cm³. Based on the systematic absences of:

$$0kl: l \neq 2n$$

$$h0l: h \neq 2n$$

packing considerations, a statistical analysis of intensity distribution, and the successful solution and refinement of the structure, the space group was determined to be:

$$Pca2_1 \text{ (\#29)}$$

The data were collected at a temperature of $20 \pm 1^\circ\text{C}$ using the ω - 2θ scan technique to a maximum 2θ value of 50.0° . Omega scans of several intense reflections, made prior to data collection, had an average width at half-height of 0.32° with a take-off angle of 6.0° . Scans of $(1.78 + 0.35 \tan \theta)^\circ$ were made at a speed of $16.0^\circ/\text{min}$ (in omega). The weak reflections ($I < 15.0\sigma(I)$) were rescanned (maximum of 4 scans) and the counts were accumulated to ensure good counting statistics. Stationary background counts were recorded on each side of the reflection. The ratio of peak counting time to background counting time was 2:1. The diameter of the incident beam collimator was 1.0 mm and the crystal to detector distance was 235 mm. The computer-controlled slits were set to 9.0 mm (horizontal) and 13.0 mm (vertical).

Data Reduction

Of the 2055 reflections which were collected, 2054 were unique ($R_{int} = 0.266$). The intensities of three representative reflection were measured after every 150 reflections. No decay correction was applied.

The linear absorption coefficient, μ , for Mo-K α radiation is 0.8 cm^{-1} . An empirical absorption correction based on azimuthal scans of several reflections was applied which resulted in transmission factors ranging from 0.94 to 1.00. The data were corrected for Lorentz and polarization effects.

Structure Solution and Refinement

The structure was solved by direct methods¹ and expanded using Fourier techniques². The non-hydrogen atoms were refined isotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement³ was based on 661 observed reflections ($I > 3.00\sigma(I)$) and 104 variable parameters and converged (largest parameter shift was 0.04 times its esd) with unweighted and weighted agreement factors of:

$$R = \Sigma||Fo| - |Fc||/\Sigma|Fo| = 0.091$$

$$R_w = \sqrt{(\Sigma w(|Fo| - |Fc|)^2/\Sigma wFo^2)} = 0.054$$

The standard deviation of an observation of unit weight⁴ was 3.90. The weighting scheme was based on counting statistics and included a factor ($p = 0.001$) to downweight the intense reflections. Plots of $\Sigma w(|Fo| - |Fc|)^2$ versus $|Fo|$, reflection order in data collection, $\sin \theta/\lambda$ and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.33 and $-0.26 e^-/\text{\AA}^3$, respectively.

Neutral atom scattering factors were taken from Cromer and Waber⁵. Anomalous dispersion effects were included in F_{calc} ⁶; the values for $\Delta f'$ and $\Delta f''$ were those of Creagh and McAuley⁷. The values for the mass attenuation coefficients are those of Creagh and Hubbel⁸. All calculations were performed using the teXsan⁹ crystallographic software package of Molecular Structure Corporation.

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(3) Least-Squares:

Function minimized: $\Sigma w(|Fo| - |Fc|)^2$

$$\text{where } w = \frac{1}{\sigma^2(Fo)} = \frac{4Fo^2}{\sigma^2(Fo^2)}$$

$$\sigma^2(Fo^2) = \frac{S^2(C+R^2B)+(pFo^2)^2}{Lp^2}$$

S = Scan rate

C = Total integrated peak count

R = Ratio of scan time to background counting time

B = Total background count

Lp = Lorentz-polarization factor

p = p-factor

(4) Standard deviation of an observation of unit weight:

$$\sqrt{\sum w(|F_o| - |F_c|)^2 / (N_o - N_v)}$$

where: N_o = number of observations

N_v = number of variables

(5) Cromer, D. T. & Waber, J. T.; "International Tables for X-ray Crystallography", Vol. IV, The Kynoch Press, Birmingham, England, Table 2.2 A (1974).

(6) Ibers, J. A. & Hamilton, W. C.; Acta Crystallogr., 17, 781 (1964).

(7) Creagh, D. C. & McAuley, W.J. ; "International Tables for Crystallography", Vol C, (A.J.C. Wilson, ed.), Kluwer Academic Publishers, Boston, Table 4.2.6.8, pages 219-222 (1992).

(8) Creagh, D. C. & Hubbell, J.H.; "International Tables for Crystallography", Vol C, (A.J.C. Wilson, ed.), Kluwer Academic Publishers, Boston, Table 4.2.4.3, pages 200-206 (1992).

(9) teXsan: Crystal Structure Analysis Package, Molecular Structure Corporation (1985 & 1992).

EXPERIMENTAL DETAILS

A. Crystal Data

Empirical Formula	$C_9H_{13}N_3O$
Formula Weight	179.22
Crystal Color, Habit	colorless, plate
Crystal Dimensions	0.40 X 0.20 X 0.10 mm
Crystal System	orthorhombic
Lattice Type	Primitive
No. of Reflections Used for Unit	
Cell Determination (2θ range)	18 (6.4 - 12.5°)
Omega Scan Peak Width at Half-height	0.32°
Lattice Parameters	$a = 11.367(6)\text{\AA}$ $b = 7.728(7)\text{\AA}$ $c = 22.651(6)\text{\AA}$
	$V = 1989(1)\text{\AA}^3$
Space Group	$Pca2_1$ (#29)
Z value	8
D_{calc}	1.196 g/cm ³
F_{000}	768.00
$\mu(\text{MoK}\alpha)$	0.82 cm ⁻¹

B. Intensity Measurements

Diffractometer	Rigaku AFC7S
Radiation	MoK α ($\lambda = 0.71069\text{\AA}$)

	graphite monochromated
Attenuator	Zr foil (factor = 8.53)
Take-off Angle	6.0°
Detector Aperture	9.0 mm horizontal 13.0 mm vertical
Crystal to Detector Distance	235 mm
Temperature	20.0°C
Scan Type	ω -2 θ
Scan Rate	16.0°/min (in ω) (up to 4 scans)
Scan Width	$(1.78 + 0.35 \tan \theta)^\circ$
$2\theta_{max}$	50.0°
No. of Reflections Measured	Total: 2055 Unique: 2054 ($R_{int} = 0.266$)
Corrections	Lorentz-polarization Absorption (trans. factors: 0.9382 - 1.0000)

C. Structure Solution and Refinement

Structure Solution	Direct Methods (SIR92)
Refinement	Full-matrix least-squares
Function Minimized	$\Sigma w(F_o - F_c)^2$
Least Squares Weights	$\frac{1}{\sigma^2(F_o)} = \frac{4F_o^2}{\sigma^2(F_o^2)}$
p-factor	0.0010
Anomalous Dispersion	All non-hydrogen atoms
No. Observations ($I > 3.00\sigma(I)$)	661
No. Variables	104
Reflection/Parameter Ratio	6.36
Residuals: R; Rw	0.091 ; 0.054
Goodness of Fit Indicator	3.90

Max Shift/Error in Final Cycle	0.04
Maximum peak in Final Diff. Map	$0.33 e^-/\text{\AA}^3$
Minimum peak in Final Diff. Map	$-0.26 e^-/\text{\AA}^3$

Table 1. Atomic coordinates and B_{iso}/B_{eq}

atom	x	y	z	B_{eq}
O(1)	0.731(1)	0.661(2)	0.2258	5.3(3)
O(2)	0.523(1)	-0.156(2)	0.1587(5)	4.7(3)
N(1)	0.704(2)	0.359(2)	0.1084(10)	5.5(4)
N(2)	0.814(1)	0.361(2)	0.1956(9)	5.5(4)
N(3)	0.679(2)	0.592(2)	0.1722(9)	6.3(5)
N(4)	0.543(1)	0.144(2)	0.2740(9)	5.1(4)
N(5)	0.440(1)	0.139(2)	0.1853(9)	5.5(4)
N(6)	0.568(1)	-0.089(2)	0.2130(8)	3.7(4)
C(1)	0.790(2)	0.101(2)	0.060(1)	4.0(5)
C(2)	0.800(2)	-0.055(3)	0.049(1)	5.8(6)
C(3)	0.873(3)	-0.151(4)	0.015(1)	11(1)
C(4)	0.943(2)	-0.050(3)	-0.011(1)	6.6(7)
C(5)	0.966(3)	0.151(4)	-0.007(1)	9.2(8)
C(6)	0.871(2)	0.209(2)	0.031(1)	4.1(5)
C(7)	0.712(2)	0.173(3)	0.101(1)	5.7(5)
C(8)	0.612(2)	0.445(3)	0.071(1)	7.7(7)
C(9)	0.737(2)	0.447(3)	0.161(1)	6.1(6)
C(10)	0.448(2)	0.393(2)	0.325(1)	4.1(5)
C(11)	0.449(3)	0.599(4)	0.341(2)	11.1(10)
C(12)	0.355(2)	0.626(4)	0.383(1)	8.7(8)
C(13)	0.278(2)	0.516(3)	0.404(1)	6.0(7)
C(14)	0.292(3)	0.364(4)	0.391(1)	9.3(8)
C(15)	0.372(2)	0.278(3)	0.350(1)	8.5(7)
C(16)	0.545(2)	0.331(3)	0.278(1)	5.7(5)

Table 1. Atomic coordinates and B_{iso}/B_{eq} (continued)

atom	x	y	z	B_{eq}
C(17)	0.622(2)	0.057(3)	0.315(1)	7.8(7)
C(18)	0.522(2)	0.063(3)	0.2220(10)	2.7(4)
H(1)	0.7440	-0.1244	0.0697	7.0766
H(2)	0.8717	-0.2744	0.0102	13.5265
H(3)	0.9937	-0.1064	-0.0382	7.8944
H(4)	1.0261	0.2163	-0.0256	10.6809
H(5)	0.8639	0.3294	0.0374	4.9473
H(6)	0.7326	0.1260	0.1385	6.8775
H(7)	0.6353	0.1333	0.0905	6.8775
H(8)	0.6158	0.5663	0.0766	9.2521
H(9)	0.6235	0.4163	0.0312	9.2521
H(10)	0.5357	0.4050	0.0835	9.2521
H(11)	0.5010	0.6832	0.3269	13.1952
H(12)	0.3488	0.7405	0.3972	10.3451
H(13)	0.2141	0.5521	0.4281	7.2062
H(14)	0.2404	0.2867	0.4106	11.3105
H(15)	0.3708	0.1586	0.3417	10.0631
H(16)	0.6211	0.3672	0.2920	6.7870
H(17)	0.5297	0.3807	0.2414	6.7870
H(18)	0.5898	-0.0523	0.3263	9.1286
H(19)	0.6967	0.0374	0.2967	9.1286
H(20)	0.6334	0.1254	0.3494	9.1286
H(21)	0.8227	0.4216	0.2319	6.6606
H(22)	0.7860	0.2473	0.2033	6.6606

Table 1. Atomic coordinates and B_{iso}/B_{eq} (continued)

atom	x	y	z	B_{eq}
H(23)	0.4420	0.0850	0.1477	6.6240
H(24)	0.4566	0.2582	0.1815	6.6240

$$B_{eq} = \frac{8}{3}\pi^2(U_{11}(aa^*)^2 + U_{22}(bb^*)^2 + U_{33}(cc^*)^2 + 2U_{12}aa^*bb^* \cos \gamma + 2U_{13}aa^*cc^* \cos \beta + 2U_{23}bb^*cc^* \cos \alpha)$$

Table 2. Bond Lengths(Å)

atom	atom	distance	atom	atom	distance
O(1)	N(3)	1.45(2)	O(2)	N(6)	1.43(2)
N(1)	C(7)	1.45(2)	N(1)	C(8)	1.50(3)
N(1)	C(9)	1.42(3)	N(2)	C(9)	1.35(2)
N(3)	C(9)	1.32(3)	N(4)	C(16)	1.45(2)
N(4)	C(17)	1.46(3)	N(4)	C(18)	1.36(2)
N(5)	C(18)	1.38(2)	N(6)	C(18)	1.30(2)
C(1)	C(2)	1.24(3)	C(1)	C(6)	1.40(3)
C(1)	C(7)	1.41(3)	C(2)	C(3)	1.35(3)
C(3)	C(4)	1.27(3)	C(4)	C(5)	1.57(3)
C(5)	C(6)	1.46(4)	C(10)	C(11)	1.63(4)
C(10)	C(15)	1.36(3)	C(10)	C(16)	1.60(3)
C(11)	C(12)	1.43(4)	C(12)	C(13)	1.31(3)
C(13)	C(14)	1.22(3)	C(14)	C(15)	1.45(4)

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Table 3. Bond Lengths(Å)

atom	atom	distance	atom	atom	distance
N(2)	H(21)	0.95	N(2)	H(22)	0.95
N(5)	H(23)	0.95	N(5)	H(24)	0.95
C(2)	H(1)	0.96	C(3)	H(2)	0.96
C(4)	H(3)	0.95	C(5)	H(4)	0.95
C(6)	H(5)	0.95	C(7)	H(6)	0.96
C(7)	H(7)	0.95	C(8)	H(8)	0.94
C(8)	H(9)	0.94	C(8)	H(10)	0.96
C(11)	H(11)	0.94	C(12)	H(12)	0.95
C(13)	H(13)	0.95	C(14)	H(14)	0.95
C(15)	H(15)	0.94	C(16)	H(16)	0.96
C(16)	H(17)	0.94	C(17)	H(18)	0.95
C(17)	H(19)	0.96	C(17)	H(20)	0.95

Table 4. Bond Angles(°)

atom	atom	atom	angle	atom	atom	atom	angle
C(7)	N(1)	C(8)	114(1)	C(7)	N(1)	C(9)	123(1)
C(8)	N(1)	C(9)	116(1)	O(1)	N(3)	C(9)	106(1)
C(16)	N(4)	C(17)	114(1)	C(16)	N(4)	C(18)	121(1)
C(17)	N(4)	C(18)	116(1)	O(2)	N(6)	C(18)	108(1)
C(2)	C(1)	C(6)	115(1)	C(2)	C(1)	C(7)	124(2)
C(6)	C(1)	C(7)	119(1)	C(1)	C(2)	C(3)	134(2)
C(2)	C(3)	C(4)	108(2)	C(3)	C(4)	C(5)	133(2)
C(4)	C(5)	C(6)	102(2)	C(1)	C(6)	C(5)	125(1)
N(1)	C(7)	C(1)	120(2)	N(1)	C(9)	N(2)	115(2)
N(1)	C(9)	N(3)	116(2)	N(2)	C(9)	N(3)	128(2)
C(11)	C(10)	C(15)	123(2)	C(11)	C(10)	C(16)	115(1)
C(15)	C(10)	C(16)	121(2)	C(10)	C(11)	C(12)	106(2)
C(11)	C(12)	C(13)	129(2)	C(12)	C(13)	C(14)	116(2)
C(13)	C(14)	C(15)	132(2)	C(10)	C(15)	C(14)	111(2)
N(4)	C(16)	C(10)	109(1)	N(4)	C(18)	N(5)	116(1)
N(4)	C(18)	N(6)	118(1)	N(5)	C(18)	N(6)	123(1)

Table 5. Bond Angles(°)

atom	atom	atom	angle	atom	atom	atom	angle
C(9)	N(2)	H(21)	109.3	C(9)	N(2)	H(22)	110.1
H(21)	N(2)	H(22)	109.5	C(18)	N(5)	H(23)	109.9
C(18)	N(5)	H(24)	109.5	H(23)	N(5)	H(24)	110.0
C(1)	C(2)	H(1)	113.0	C(3)	C(2)	H(1)	112.4
C(2)	C(3)	H(2)	127.0	C(4)	C(3)	H(2)	125.0
C(3)	C(4)	H(3)	113.3	C(5)	C(4)	H(3)	112.9
C(4)	C(5)	H(4)	128.6	C(6)	C(5)	H(4)	129.0
C(1)	C(6)	H(5)	117.5	C(5)	C(6)	H(5)	117.2
N(1)	C(7)	H(6)	106.6	N(1)	C(7)	H(7)	107.0
C(1)	C(7)	H(6)	106.4	C(1)	C(7)	H(7)	106.9
H(6)	C(7)	H(7)	109.0	N(1)	C(8)	H(8)	109.4
N(1)	C(8)	H(9)	109.5	N(1)	C(8)	H(10)	108.7
H(8)	C(8)	H(9)	110.6	H(8)	C(8)	H(10)	109.2
H(9)	C(8)	H(10)	109.3	C(10)	C(11)	H(11)	127.0
C(12)	C(11)	H(11)	126.5	C(11)	C(12)	H(12)	114.8
C(13)	C(12)	H(12)	115.3	C(12)	C(13)	H(13)	122.1
C(14)	C(13)	H(13)	121.6	C(13)	C(14)	H(14)	113.8
C(15)	C(14)	H(14)	113.5	C(10)	C(15)	H(15)	124.2
C(14)	C(15)	H(15)	124.7	N(4)	C(16)	H(16)	109.0
N(4)	C(16)	H(17)	109.9	C(10)	C(16)	H(16)	108.8
C(10)	C(16)	H(17)	109.9	H(16)	C(16)	H(17)	109.7
N(4)	C(17)	H(18)	110.0	N(4)	C(17)	H(19)	109.3
N(4)	C(17)	H(20)	110.3	H(18)	C(17)	H(19)	108.7
H(18)	C(17)	H(20)	109.7	H(19)	C(17)	H(20)	108.9

Table 5. Bond Angles($^{\circ}$) (continued)

atom	atom	atom	angle	atom	atom	atom	angle
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Table 6. Torsion Angles(°)

atom	atom	atom	atom	angle	atom	atom	atom	atom	angle
O(1)	N(3)	C(9)	N(1)	-177(1)	O(1)	N(3)	C(9)	N(2)	10(3)
O(2)	N(6)	C(18)	N(4)	173(1)	O(2)	N(6)	C(18)	N(5)	2(2)
N(1)	C(7)	C(1)	C(2)	-177(1)	N(1)	C(7)	C(1)	C(6)	7(3)
N(2)	C(9)	N(1)	C(7)	22(3)	N(2)	C(9)	N(1)	C(8)	175(1)
N(3)	C(9)	N(1)	C(7)	-150(2)	N(3)	C(9)	N(1)	C(8)	2(3)
N(4)	C(16)	C(10)	C(11)	173(1)	N(4)	C(16)	C(10)	C(15)	-5(3)
N(5)	C(18)	N(4)	C(16)	-37(2)	N(5)	C(18)	N(4)	C(17)	174(1)
N(6)	C(18)	N(4)	C(16)	151(1)	N(6)	C(18)	N(4)	C(17)	2(2)
C(1)	C(2)	C(3)	C(4)	0(4)	C(1)	C(6)	C(5)	C(4)	4(3)
C(1)	C(7)	N(1)	C(8)	92(2)	C(1)	C(7)	N(1)	C(9)	-114(2)
C(2)	C(1)	C(6)	C(5)	-2(3)	C(2)	C(3)	C(4)	C(5)	4(4)
C(3)	C(2)	C(1)	C(6)	0(4)	C(3)	C(2)	C(1)	C(7)	-175(3)
C(3)	C(4)	C(5)	C(6)	-6(4)	C(5)	C(6)	C(1)	C(7)	172(2)
C(10)	C(11)	C(12)	C(13)	-3(4)	C(10)	C(15)	C(14)	C(13)	3(4)
C(10)	C(16)	N(4)	C(17)	-89(2)	C(10)	C(16)	N(4)	C(18)	121(1)
C(11)	C(10)	C(15)	C(14)	1(3)	C(11)	C(12)	C(13)	C(14)	7(4)
C(12)	C(11)	C(10)	C(15)	-1(3)	C(12)	C(11)	C(10)	C(16)	179(2)
C(12)	C(13)	C(14)	C(15)	-7(4)	C(14)	C(15)	C(10)	C(16)	-179(1)

Table 7. Non-bonded Contacts out to 3.30 Å

atom	atom	distance	ADC	atom	atom	distance	ADC
O(1)	N(6)	2.69(2)	56501	O(1)	N(5)	2.98(2)	56504
O(1)	O(2)	3.14(1)	56501	O(1)	C(15)	3.27(3)	56504
O(2)	N(3)	2.66(2)	54501	O(2)	N(2)	2.98(2)	45504
N(3)	N(6)	2.92(2)	56501				

The ADC (atom designator code) specifies the position of an atom in a crystal. The 5-digit number shown in the table is a composite of three one-digit numbers and one two-digit number: TA (first digit) + TB (second digit) + TC (third digit) + SN (last two digits). TA, TB and TC are the crystal lattice translation digits along cell edges a, b and c. A translation digit of 5 indicates the origin unit cell. If TA = 4, this indicates a translation of one unit cell length along the a-axis in the negative direction. Each translation digit can range in value from 1 to 9 and thus ± 4 lattice translations from the origin (TA=5, TB=5, TC=5) can be represented.

The SN, or symmetry operator number, refers to the number of the symmetry operator used to generate the coordinates of the target atom. A list of symmetry operators relevant to this structure are given below.

For a given intermolecular contact, the first atom (origin atom) is located in the origin unit cell and its position can be generated using the identity operator (SN=1). Thus, the ADC for an origin atom is always 55501. The position of the second atom (target atom) can be generated using the ADC and the coordinates of the atom in the parameter table. For example, an ADC of 47502 refers to the target atom moved through symmetry operator two, then translated -1 cell translations along the a axis, +2 cell translations along the b axis, and 0 cell translations along the c axis.

An ADC of 1 indicates an intermolecular contact between two fragments (eg. cation and anion) that reside in the same asymmetric unit.

Symmetry Operators:

(1)	X,	Y,	Z	(2)	-X,	-Y,	1/2+Z
(3)	1/2-X,	Y,	1/2+Z	(4)	1/2+X,	-Y,	Z

